



UNIWERSYTET OPOLSKI

Instytut Chemii

PRACA DOKTORSKA

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Właściwości konformacyjne α,β -dehydroaminokwasów z polarną grupą funkcyjną w łańcuchu bocznym

Conformational properties of the α,β -dehydroamino acids with a polar functional group in the side chain

Praca napisana pod kierunkiem

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Opole 2025

Pragnę podziękować moim Promotorom,
dr hab. Dawidowi Siodlakowi, prof. UO
oraz **dr Pawłowi Lenartowiczowi**,
za wszelką pomoc, cenne sugestie i cierpliwość podczas realizacji niniejszej
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Rodzinie i Przyjaciółom
za nieustanne wsparcie i wiarę w moje możliwości,
a w szczególności moim **Rodzicom**
za możliwość kształcenia się.

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Sieciowo-Superkomputerowe (<http://wcss.pl>)



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1. AUTOREFERAT WRAZ Z DOROBKIEM NAUKOWYM

1.1. DANE OSOBOWE I SUMARYCZNY DOROBEK AUTORA ORAZ INNE OSIĄGNIĘCIA

1.1.1. Wykształcenie

- 2019 – 2021 **Studia magisterskie** (bardzo dobry)
Uniwersytet Opolski, Wydział Chemii
Kierunek: Chemia
Tytuł pracy magisterskiej: Synteza i utlenianie wybranych pochodnych 4,5-dihydro-1,3-oksazolu
Promotor: dr hab. Dawid Siodłak, prof. UO
- 2016 – 2019 **Studia licencjackie** (bardzo dobry)
Uniwersytet Opolski, Wydział Chemii
Kierunek: Chemia
Specjalność: Chemia biologiczna
Ukończone z wyróżnieniem
- 2013 – 2016 Liceum Ogólnokształcące z Oddziałami Dwujęzycznymi im. Władysława Broniewskiego w Strzelcach Opolskich

1.1.2. Sumaryczny dorobek naukowy

- **4 Publikacje** w czasopismach z listy filadelfijskiej, w tym **3 publikacje** z zakresu pracy doktorskiej.
- **6 Wystąpień konferencyjnych** podczas konferencji o zasięgu krajowym – 1 komunikat ustny i 5 posterów.

1.1.3. Inne osiągnięcia

- **Staż naukowy** w Sieć Badawcza Łukasiewicz – Instytut Ciężkiej Syntezy Organicznej „Blachownia” w Kędzierzynie-Koźlu.
- **Inna działalność naukowa** – pomoc w realizacji prac dyplomowych.
- **Działalność popularyzująca naukę** – udział w pokazach chemicznych, przeprowadzonych w ramach Opolskiego Festiwalu Nauki oraz Opolskiego Uniwersytetu Małego Naukowca; pomoc w przeprowadzeniu licznych zajęć laboratoryjnych dla uczniów szkół.

1.2. WSKAZANIE OSIĄGNIĘCIA NAUKOWEGO STANOWIĄCEGO PODSTAWĘ POSTĘPOWANIA DOKTORSKIEGO

1.2.1. Tytuł osiągnięcia naukowego

Moim osiągnięciem naukowym, po otrzymaniu tytułu magistra, stanowiącym oryginalne rozwiązanie problemu naukowego oraz wykazującym ogólną wiedzę teoretyczną w chemii i umiejętność samodzielnego prowadzenia pracy naukowej, zgodnie z § 11, pkt 1 i 2 jednolitego tekstu regulaminu prowadzenia przewodów do uzyskania stopnia doktora nauk chemicznych w zakresie chemii na Wydziale Chemii i Farmacji Uniwersytetu Opolskiego jest cykl powiązanych tematycznie prac zatytułowany:

WŁAŚCIWOŚCI KONFORMACYJE α,β -DEHYDROAMINOKWASÓW Z POLARNĄ GRUPĄ FUNKCYJNĄ W ŁAŃCUCHU BOCZNYM

1.2.2. Wykaz jednotematycznych artykułów naukowych stanowiących osiągnięcie naukowe wraz z wartością współczynnika IF oraz liczbą punktów według listy MNiSW

Niniejsze osiągnięcie naukowe stanowi cykl trzech artykułów naukowych [D1-D3], opublikowanych w czasopismach indeksowanych w bazie Journal Citation Report. Wykaz tych artykułów znajduje się poniżej:

	Autorzy, tytuł, dane bibliograficzne	IF*	IF _{5 letni}	pkt MNiSW*
D1	Karolina Banaś , Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen <i>Cochliobolus victoriae</i> . Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine” <i>J. Agric. Food Chem.</i> 2023 , 71(30), 11642–11653, DOI:10.1021/acs.jafc.3c01387 Mój wkład w powstanie tej publikacji obejmuje przeprowadzenie badań metodami obliczeniowymi oraz zebranie i opracowanie wyników tych badań w formie tabel i rysunków, współudział w syntezie i charakterystyka związków modelowych, wykonanie pomiarów IR, opracowanie widm NMR i IR, współudział w redagowaniu manuskryptu w tym opracowanie w całości materiałów pomocniczych.	5.7	6.4	140
D2	Karolina Banaś , Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Antifungal Cyrenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine” <i>Int. J. Mol. Sci.</i> 2025 , 26(1), 340–354, DOI:10.3390/ijms26010340	4.9	5.7	140

	Mój wkład w powstanie tej publikacji obejmuje samodzielne przeprowadzenie badań metodami obliczeniowymi oraz zebranie i opracowanie wyników tych badań w formie tabel i rysunków, samodzielne przeprowadzenie syntez i charakterystyka związków modelowych, wykonanie pomiarów IR, opracowanie widm NMR i IR, współudział w redagowaniu manuskryptu w tym opracowanie w całości materiałów pomocniczych.			
D3	Karolina Banaś , Paweł Lenartowicz, Dawid Siodłak „Insight into the structure of antitubercular Callyaerins: conformational studies and synthesis of a unique dehydroamino acid, β -aminodehydroalanine” <i>Amino Acids</i> 2025 , 57(1), 41, DOI:10.1007/s00726-025-03473-2	2.4	3.1	100
	Mój wkład w powstanie tej publikacji obejmuje samodzielne przeprowadzenie badań metodami obliczeniowymi oraz zebranie i opracowanie wyników tych badań w formie tabel i rysunków, samodzielne przeprowadzenie znacznej większości syntez związków modelowych oraz współudział w pozostałych i charakterystyka produktów, opracowanie widm NMR, współudział w redagowaniu manuskryptu w tym opracowanie w całości materiałów pomocniczych.			
Sumaryczny Impact Factor (IF)		13.0		

* w roku opublikowania

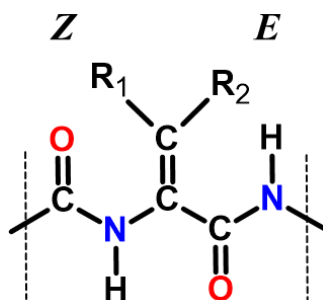
1.2.3. Wykaz skrótów

ΔAbu	dehydrobutyryna
ΔAla	dehydroalanina
$\Delta\text{Ala}(\beta\text{-Cl})$	β -chlorodehydroalanina
$\Delta\text{Ala}(\beta\text{-NHMe})$	β -aminodehydroalanina
ΔG	różnica energii Gibbsa (298.15 K, 1.0 atm)
ΔIle	dehydroizoleucyna
$\Delta(\text{Me})\text{Phe}$	<i>N</i> -Metylodehydrofenyloalanina
$\Delta\text{Ser}(\text{O-Me})$	O-metylodehydroseryna
ΔTrp	dehydrotryptofan
ΔVal	dehydrowalina
$\nu_s(\text{N-H})$	obszar drgań rozciągających wiązania N-H na widmie IR
φ	Kąt torsyjny $\angle\text{C-N-C}^\alpha\text{-C}$
ψ	Kąt torsyjny $\angle\text{N-C}^\alpha\text{-C-N}$
$\text{Ac-}\Delta\text{Ala}(\beta\text{-Cl})\text{-OMe}$	ester metylowy <i>N</i> -acetylo- β -chlorodehydroalaniny
AMOIPA	kwas 2-amino-3-(5-metoksy-2-oksoimidazolidyno-4-yliden)propionowy
$(\text{AcO})_2\text{O}$	bezwodnik octowy
Ac-Ser-NHMe	<i>N'</i> -metyloamid <i>N</i> -acetylo-seryny
$\text{Ac-(Z/E)-}\Delta\text{Ala}(\beta\text{-Cl})\text{-NHMe}$	<i>N'</i> -metyloamid <i>N</i> -acetylo-(<i>Z/E</i>)- β -chlorodehydroalaniny
$\text{Ac-(Z/E)-}\Delta\text{Ala}(\beta\text{-NHMe})\text{-NHMe}$	<i>N'</i> -metyloamid <i>N</i> -acetylo-(<i>Z/E</i>)- β -metyloaminodehydroalaniny
$\text{Ac-(Z/E)-}\Delta\text{Ala}(\beta\text{-NHMe})\text{-NMe}_2$	<i>N'N'</i> -dimetyloamid <i>N</i> -acetylo-(<i>Z/E</i>)- β -metyloaminodehydroalaniny
$\text{Ac-(Z/E)-}\Delta\text{Ser}(\text{O-Me})\text{-OMe}$	ester metylowy <i>N</i> -acetylo-(<i>Z/E</i>)-O-metylodehydroseryny
$\text{Boc-}\Delta\text{Ala}(\beta\text{-Cl})\text{-OMe}$	ester metylowy <i>tert</i> -butyloksykarbonylo- β -chlorodehydroalaniny
$\text{Boc-Gly-(Z/E)-}\Delta\text{Ala}(\beta\text{-Cl})\text{-OMe}$	ester metylowy <i>tert</i> -butyloksykarbonylo-glicylo-(<i>Z/E</i>)- β -chlorodehydroalaniny
$\text{Boc-Gly-(Z)-}\Delta\text{Ala}(\beta\text{-NHMe})\text{-OMe}$	ester metylowy <i>tert</i> -butyloksykarbonylo-glicylo-(<i>Z</i>)- β -metyloaminodehydroalaniny
$\text{Boc-Gly-(Z)-}\Delta\text{Ala}(\beta\text{-Leu-OMe})\text{-OMe}$	ester metylowy <i>tert</i> -butyloksykarbonylo-glicylo-(<i>Z</i>)- β -metyloleucylodehydroalaniny
$\text{C}^\alpha=\text{C}^\beta$	wiązanie podwójne pomiędzy atomami węgla α i β
$\text{Cbz-Gly-(Z/E)-}\Delta\text{Ala}(\beta\text{-Cl})\text{-Gly-OMe}$	ester metylowy benzyloksykarbonylo-glicylo-(<i>Z/E</i>)- β -chlorodehydroalaninylo-glicyny
CPCM	Conductor-like Polarizable Continuum Model
DAA	kwas (<i>Z</i>)-2,3-diaminoakrylowy
DCM	dichlorometan
DMF	<i>N,N</i> -dimetyloformamid
DMS	siarczan dimetylu
$\text{DMSO-}d_6$	deuterowany dimetylosulfotlenek
EDC	1-etylo-3-(3-dimetyloaminopropyl)karbodiimid
fGly	(<i>R</i>)- α -formyloglicyna
hThr	4-hydroksy-L-treonina
HYP	γ -hydroksyprolina
NBS	<i>N</i> -bromosukcynoimid
NOE NMR	spektroskopia magnetycznego rezonansu jądrowego z efektem jądrowym Overhausera
p (%)	populacja cząsteczek w temperaturze 300 K, obliczona ze wzoru: $p = 100\% \exp(-\frac{\Delta G}{RT})$, gdzie $RT = 0.595 \text{ kcal/mol}$
POCl_3	trichlorek fosforu
SCRF	samouzgodnione pole reakcji
TEA	trietyloamina

1.2.4. Omówienie celu naukowego i osiągniętych wyników

WPROWADZENIE

Dehydroaminokwasy to niekodowane aminokwasy, których charakterystycznym elementem strukturalnym jest wiązanie typu π , występujące zazwyczaj pomiędzy atomami węgla α i β α -aminokwasów (rys. 1) [1]. Obecność wiązania podwójnego C=C w strukturze α,β -dehydroaminokwasów powoduje, że różnią się one znacząco od standardowych aminokwasów. Przede wszystkim, atomy węgla α i β nie mają hybrydyzacji sp^3 , lecz hybrydyzację sp^2 . Zatem tworzone przez nie wiązania są krótsze, a kąty walencyjne pomiędzy nimi mają wyższą wartość. Ponadto, atom węgla α nie jest asymetryczny, dlatego dehydroaminokwasy nie wykazują czynności optycznej i nie występują w formie izomerów optycznych L/D. Jednak obecność wiązania podwójnego wymusza izomerię geometryczną łańcucha bocznego, stąd wyróżnia się dehydroaminokwasy *Z* i *E*. Izomer geometryczny *Z* wykazuje inne właściwości niż izomer *E*, dlatego należy je traktować jak różne związki. Przykładowo, grzybiczy depsipeptyd fomalid, zawierający w swojej strukturze resztę (*E*)-dehydrobutyryny, powoduje suchą zgniliznę upraw kapustnych. Natomiast jego izomer, izofomalid, różniący się jedynie izomerią geometryczną reszty dehydrobutyryny, zawierający (*Z*)-dehydrobutyrynę, nie wykazuje fitotoksyczności [2].



Rysunek 1. Wzór strukturalny reszty α,β -didehydro- α -aminokwasowej (w skrócie α,β -dehydroaminokwasu).

Dehydroaminokwasy powstają najczęściej w wyniku potranslacyjnej modyfikacji standardowych aminokwasów [3]. Mogą pełnić rolę produktów pośrednich w konwersji L-aminokwasów do D-aminokwasów w peptydach mikrobiologicznych [4-6]. Występują w wielu naturalnych peptydach o dużej różnorodności strukturalnej [7]. Istnieją zarówno cykliczne [8] jak i linowe [9] dehydropeptydy, wielko- [10] lub małowczątkowe [11], zawierające tylko jedną resztę dehydroaminokwasową [12] lub składające się w większości z nienasyconych aminokwasów [13] (rys. 2 i 3). Dehydropeptydy są syntezowane przez grzyby, organizmy morskie, a nawet rośliny wyższe, jednak ich główne źródło stanowią bakterie. Związki te są interesujące ze względu na swoją aktywność biologiczną. Większość dehydropeptydów wykazuje działanie antybakteryjne, jednak istnieją też takie, które charakteryzują się aktywnością przeciwrzybiczą, fitotoksyczną, przeciwnowotworową, czy też cytotoksyczną [1].

Pojawienie się dehydroaminokwasu w cząsteczce peptydu nadaje jej nietypowe właściwości konformacyjne, spowodowane obecnością wiązania podwójnego, skutkujące ograniczeniami orientacji

łańcucha bocznego [14, 15]. Okazuje się, że dehydroaminokwasy mogą przyjmować konformacje, które nie są łatwo dostępne dla ich nasyconych analogów [16]. Takie konformacje są stabilizowane przez wewnątrz- i międzycząsteczkowe wiązania wodorowe, oddziaływania dipolowe oraz sprzężenia π -elektronowe [17]. Ponadto, dehydroaminokwasy wykazują zdolność indukcji fałdowania typu II β -zgięcia [14, 15]. To właśnie te właściwości dehydroaminokwasów determinują aktywność biologiczną dehydropeptydów [1, 7]. Zbadano, że naturalne dehydropeptydy, np. azynomycyna A i B wykazują działanie antybakteryjne i przeciwnowotworowe, podczas gdy ich nasycony analog nie jest aktywny biologicznie [18]. Kolejnym przykładem jest gramicydyna S, peptyd o działaniu antybakteryjnym, złożony wyłącznie z nasyconych aminokwasów. Zsyntezowano analog gramicydyny S, w którym dwie reszty D-feniloalaniny zastąpiono resztami dehydrofeniloalaniny, co skutkowało wzmocnieniem wiązania wodorowego sąsiadującego z β -zgięciem, a w konsekwencji wzmocnieniem bioaktywnej konformacji peptydu [19]. Wykazano, że nienasycony analog gramicydyny S zachował jej aktywność biologiczną, zyskując jednocześnie większą stabilność na degradację enzymatyczną [14]. Tą ostatnią cechą dehydropeptydów potwierdzają inne badania, które wykazały większą stabilność dehydroenkefalin na hydrolizę enzymatyczną karboksypeptydazy Y w porównaniu z nasyconymi analogami [20]. Powyższe właściwości dehydroaminokwasów czynią je użytecznymi dla celów chemii medycznej do otrzymania nienasyconych analogów inhibitorów, enzymów, antybiotyków lub hormonów [21].

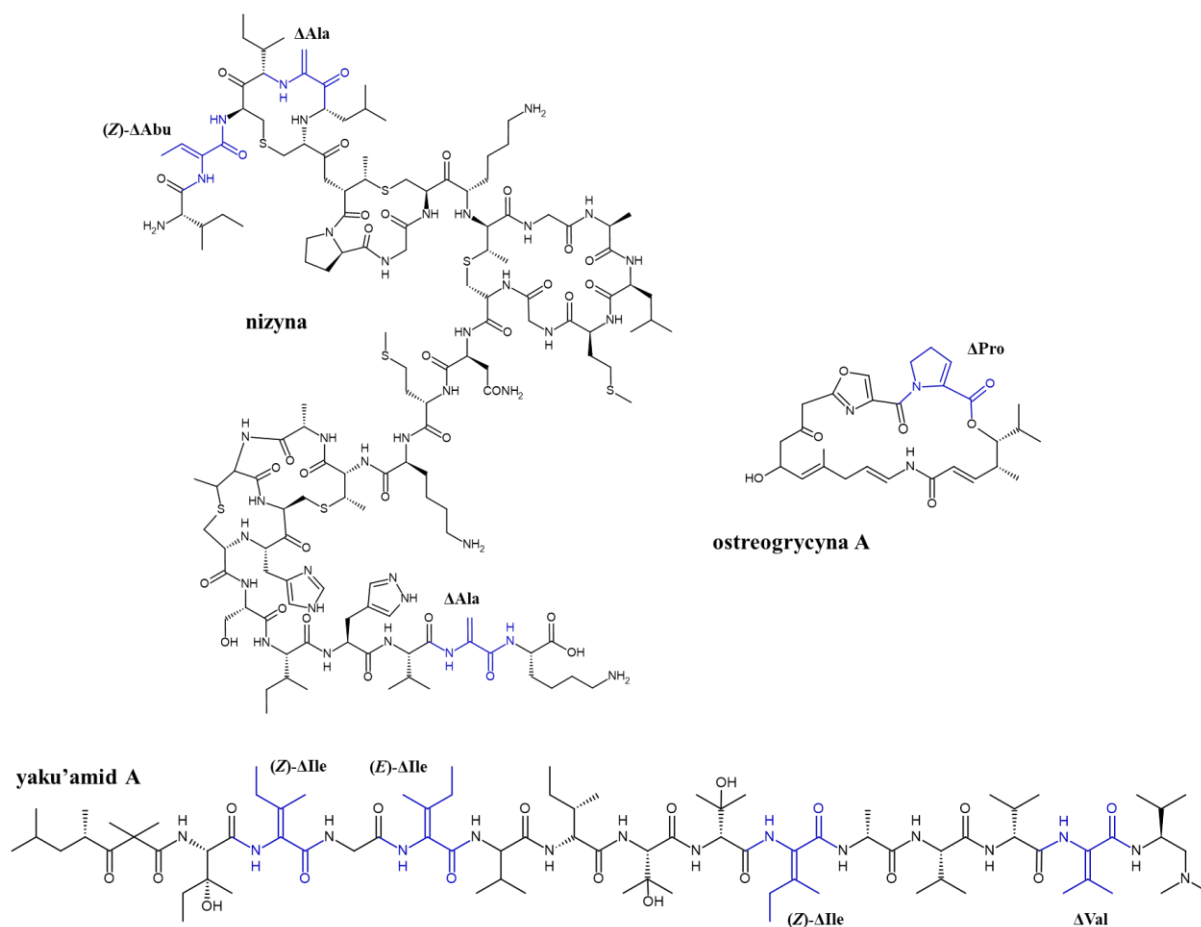
Wiązanie podwójne pomiędzy atomami węgla α i β nie tylko wpływa na właściwości konformacyjne dehydroaminokwasów, ale również na ich reaktywność chemiczną. Może bowiem ulegać reakcjom addycji, cykloaddycji, elektrocyklizacji, uwodornienia, czy też izomeryzacji *Z/E* [6, 22] i przez to modulować aktywność biologiczną dehydropeptydów.

Najczęściej spotykanymi w przyrodzie dehydroaminokwasami są powstające ze standardowych aminokwasów dehydroalanina i dehydrobutyryna. Dehydroalanina jest otrzymywana z seryny lub cysteiny, a dehydrobutyryna z treoniny w wyniku reakcji β -eliminacji [6]. Dehydroalanina (Δ Ala) to najmniejszy dehydroaminokwas, którego łańcuch boczny stanowi grupa metylenowa. Ponieważ reszta tego dehydroaminokwasu jest symetryczna, nie wykazuje on izomerii geometrycznej *Z/E*. Natomiast dehydrobutyryna (Δ Abu), której atom węgla β jest podstawiony grupą metylową, stanowi najmniejszy dehydroaminokwas, mogący występować w postaci izomeru *Z* lub *E*, spośród których ten pierwszy jest stabilniejszy [23]. Oba te dehydroaminokwasy można znaleźć w strukturze pierwszego odkrytego dehydropeptydu, nizyny (rys. 2). Nizyna to antybiotyk produkowany przez bakterie *Lactococcus lactis*, stosowany jako konserwant do żywności [24]. Dehydroalanina i dehydrobutyryna są również obecne w większości pozostałych lantibiotyków [10].

W naturalnych peptydach można znaleźć też dehydrowalinę (Δ Val) i dehydroizoleucynę (Δ Ile). Dehydrowalina ma atom węgla β podstawiony dwiema grupami metylowymi, dlatego podobnie jak dehydroalanina nie wykazuje izomerii geometrycznej *Z/E*, przeciwnie dehydroizoleucyna z podstawnikiem metylowym i etylowym przy wiązaniu podwójnym. Yaku'amid A (rys. 2) to przykład

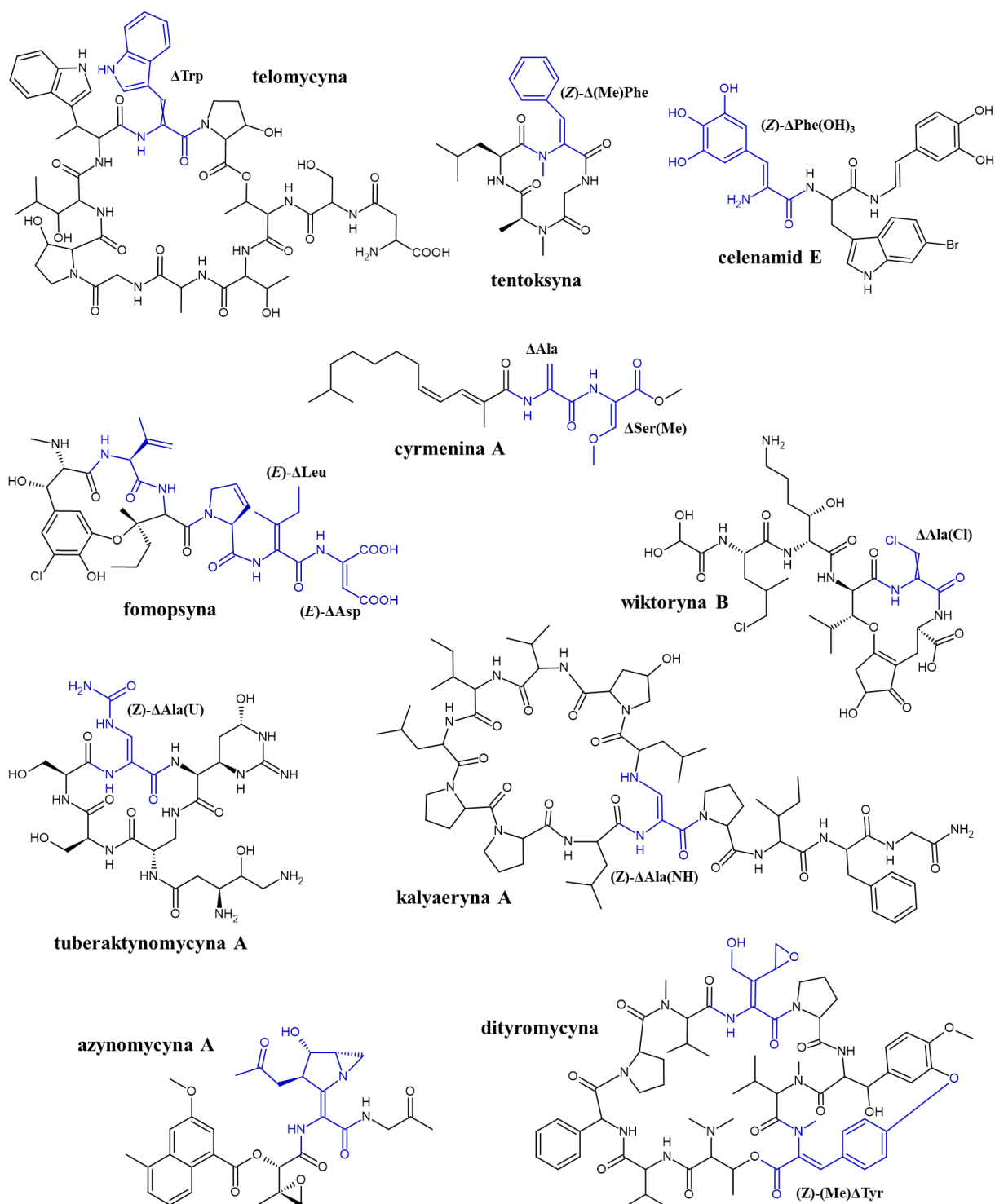
dehydropeptydu zawierającego w swojej strukturze resztę dehydrowaliny, (Z)-dehydroizoleucyny i (E)-dehydroizoleucyny. Ten liniowy peptyd został wyizolowany z gąbki morskiej *Ceratopsion sp.* i zwrócił uwagę badaczy ze względu na swoją aktywność cytotoksyczną [9].

Dehydroprolina (Δ Pro) została zidentyfikowana w jednym z antybiotyków, wyizolowanych ze *Streptomyces ostreogriseus* [8]. Ponieważ peptyd ten został wyizolowany również z innych mikroorganizmów, w literaturze istnieje wiele nazw tego samego związku. Znany jest jako ostreogrycyna A (rys. 2), mikamycyna A, stafylomycyna M₁, prystynamycyna IIA, streptogramina A oraz PA 114 A1 [25].



Rysunek 2. Wzory strukturalne nizyny, yaku'amidu A i ostreogrycyny A.

W przyrodzie istnieją również dehydroaminokwasy aromatyczne. Dehydrotryptofan (Δ Trp) można znaleźć w strukturze telomycyny (rys. 3), bakteriynego peptydu o działaniu antybiotycznym [12]. N-Metylodehydofenyloalanina (Δ (Me)Phe) jest elementem strukturalnym tentoksyny, kluczowym dla aktywności fitotoksycznej tego peptydu. Tentoksyna (rys. 3) jest syntezowana przez grzybiczy patogen z gatunku *Alternaria alternata* i jako toksyna niespecyficzna dla gospodarza, powoduje chlorozę szerokiej gamy roślin [11]. Znane są również hydroksylowe pochodne dehydrofenyloalaniny. Na przykład, antybiotyczny celenamid E (rys. 3), wyizolowany z gąbki *Cliona chilensis*, zawiera 3,4,5-trihydroksydehydrofenyloalaninę [26].



Rysunek 3. Wzory strukturalne telomycyny, tentoksyny i celenamidu E, cyrmeniny A, fomopsyny A, wiktoryny B, tuberaaktynomycyny A, kalyaeryny A, azynomycyny A i dityromycyny.

W strukturach naturalnych peptydów można znaleźć dehydroaminokwasy z polarnymi grupami funkcyjnymi w łańcuchu bocznym. Do syntezowania takich związków są zdolne grzyby i bakterie. Przykłady peptydów z polarnymi dehydroaminokwasami to cyrmenina A [27], fomopsyna A [13], wiktoryna B [28], tuberaaktynomycyna A [29], kalyaeryna A [30], azynomycyna A [18], czy dityromycyna [31] (rys. 3). Związki te wykazują zróżnicowaną aktywność biologiczną.

Poznanie właściwości konformacyjnych dehydroaminokwasów może być przydatne w projektowaniu krótkich peptydów, ponieważ znalezienie elementów strukturalnych zdolnych do przyjmowania stabilnych konformacji umożliwi projektowanie struktur i funkcji peptydów [32]. Dotychczasowe badania konformacyjne dehydroaminokwasów dotyczyły głównie związków z niepolarnym łańcuchem bocznym. Obejmowały takie reszty jak dehydrofenyloalanina, dehydroalanina, dehydrobutyryna, dehydrowalina czy dehydroleucyna [17, 32-41]. Najpowszechniej była badana dehydrofenyloalanina, ponieważ stanowi często wykorzystywany motyw w projektowaniu bioaktywnych peptydów do ograniczenia topografii farmakoforu, jakim jest pierścień fenyłowy [42, 43].

Ustalono, że te dehydroaminokwasy wykazują zdolność indukowania β -zgięcia, a to właśnie te struktury drugorzędowe są w większości przypadków odpowiedzialne za rozpoznanie lub powinowactwo w interakcjach między białkami. Dlatego też dehydropeptydy mogą znaleźć zastosowanie w chemii medycznej jako foldamery [39]. Ponadto ustalono, że wprowadzenie dehydrofenyloalaniny do łańcucha peptydowego o dłuższej sekwencji powoduje stabilizację konformacji 3_{10} -helikalnej [32]. Wyjątkowy przypadek stanowi dehydroalanina, która preferuje w pełni rozciągniętą konformację C5, wskutek krzyżowego sprzężenia π -elektronowego, obejmującego wiązanie podwójne pomiędzy atomami węgla α i β oraz sąsiednie grupy amidowe na N- i C-końcu [43]. Zbadano, że dehydroalanina sprzyja tworzeniu odwróconego γ -zgięcia [16]. Dehydrobutyryna, podobnie jak dehydroalanina, preferuje rozciągniętą konformację [44], natomiast dehydroleucyna bardziej przypomina dehydrofenyloalaninę [34]. Z przedstawionych danych wynika, że istotną rolę odgrywa tutaj rozmiar podstawnika przy atomie węgla β . Grupa metylowa jest stosunkowo niewielka, przez co rozciągnięta konformacja dehydrobutyryny jest możliwa do zachowania [44]. Inaczej jest z większymi podstawnikami w dehydrofenyloalaninie czy dehydroleucynie. Zatem w przypadku dehydroaminokwasów z niepolarnym łańcuchem bocznym to głównie zawada steryczna wpływa na właściwości konformacyjne.

CEL PRACY DOKTORSKIEJ

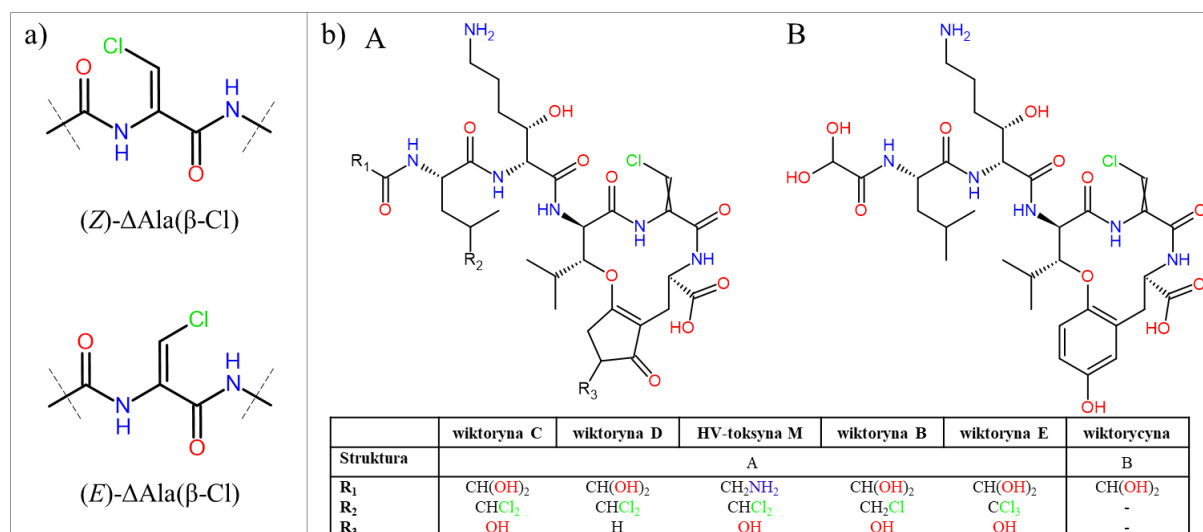
Przeprowadzone do tej pory badania konformacyjne dotyczyły zasadniczo pięciu niepolarnych dehydroaminokwasów: przede wszystkim dehydrofenyloalaniny, dehydroalaniny, dehydrobutyryny i w mniejszym stopniu dehydrowaliny i dehydroleucyny. W przyrodzie istnieją również peptydy, zawierające w swojej strukturze dehydroaminokwasy z heteroatomami w łańcuchu bocznym. Heteroatomy są zdolne do tworzenia wiązań wodorowych, oddziaływań dipolowych lub uczestniczenia w sprzężeniu π -elektronowym i wpływania przez to na przyjmowane konformacje. Zatem, w przypadku takich dehydroaminokwasów, o właściwościach konformacyjnych decyduje nie tylko rozmiar podstawników przy wiązaniu podwójnym, ale również ich właściwości elektronowe. Należy zatem oczekiwać, że prezentowane przez nie właściwości konformacyjne będą odmienne od tych wykazywanych przez niepolarne dehydroaminokwasy. Warto podkreślić, że ten typ dehydroaminokwasów nie był dotąd badany. Dlatego też celem niniejszej pracy doktorskiej było **zbadanie właściwości konformacyjnych α,β -dehydroaminokwasów z polarną grupą funkcyjną w łańcuchu bocznym.**

Ponieważ z przeglądu literaturowego wynika, że istotną rolę w modulowaniu właściwości konformacyjnych dehydroaminokwasów odgrywa natura podstawnika przy atomie węgla β , do badań wybrano związki, które mają w tej pozycji atom chloru, tlenu oraz azotu. Są to kolejno β -chlorodehydroalanina, O-metylodehydroseryna i β -aminodehydroalanina. Powyższe dehydroaminokwasy występują w naturalnych peptydach o potencjalnie użytecznych własnościach. Cel pracy był realizowany z wykorzystaniem metod obliczeniowych, poprzez stworzenie map energii potencjalnej oraz syntezę związków modelowych, a następnie analizę ich właściwości konformacyjnych, metodami spektralnymi oraz za pomocą rentgenowskiej analizy strukturalnej.

OMÓWIENIE WYNIKÓW BADAŃ

Właściwości konformacyjne β -chlorodehydroalaniny [D1]

β -Chlorodehydroalanina ($\Delta\text{Ala}(\beta\text{-Cl})$) to α,β -dehydroaminokwas, w którym atom węgla β jest podstawiony atomem chloru (rys. 4a). Jest charakterystycznym elementem strukturalnym grupy peptydów o nazwie wiktoryny (rys. 4b). Do tej pory nie została zidentyfikowana w strukturach żadnych innych związków naturalnych. Wiktoryny są syntezowane przez grzybiczy patogen z gatunku *Cochliobolus victoriae*, atakujący odmiany owsa odporne na działanie innego grzyba, *Puccinia coronata* [45]. Powodują zarazę Wiktorii (ang. Victoria Blight), niszczącą uprawy tak ważnego zboża jak owies [46], ale również i innych roślin, wrażliwych na te toksyny [47].



Rysunek 4. a) Wzory strukturalne reszty (Z)- i (E)- β -chlorodehydroalaniny. b) Struktury peptydów, wchodzących w skład toksyn o nazwie wiktoryny.

Wiktoryny są wytwarzane jako mieszanina sześciu pentapeptydów, z wiktoryną C jako głównym składnikiem [48]. Peptydy te charakteryzują się różnym stopniem podstawienia chlorem. β -Chlorodehydroalanina, jako wspólny element strukturalny wszystkich wiktoryn, wydaje się być istotna dla ich aktywności biologicznej. Poznanie właściwości konformacyjnych tego dehydroaminokwasu może być pomocne w poszukiwaniach agrochemikaliów, zwalczających fitotoksyczną aktywność wiktoryn. Do tej pory takie badania nie zostały przeprowadzone, a nawet samo określenie izomerii geometrycznej łańcucha bocznego β -chlorodehydroalaniny w wiktorynach pozostaje kwestią sporną [49, 50]. Atom chloru może wpływać na przyjmowane konformacje poprzez tworzenie zawady sterycznej, spowodowanej jego dużym rozmiarem. Dodatkowo, może stabilizować pewne konformacje za pomocą oddziaływań wewnątrz- i międzycząsteczkowych z wykorzystaniem swoich wolnych par elektronowych.

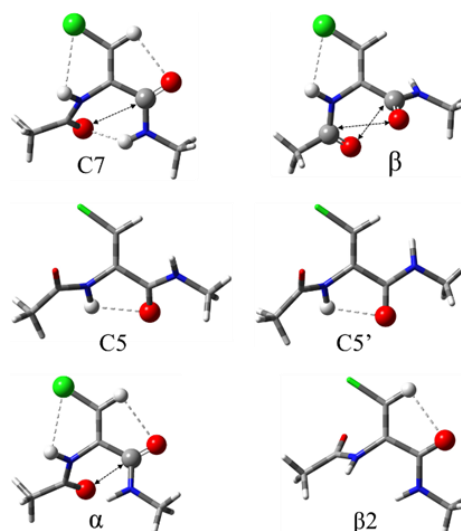
W celu określenia właściwości konformacyjnych β -chlorodehydroalaniny, w pierwszej kolejności obliczono mapy energii potencjalnych dwóch związków modelowych $\text{Ac}-(Z)-\Delta\text{Ala}(\beta\text{-Cl})-\text{NHMe}$ i $\text{Ac}-(E)-\Delta\text{Ala}(\beta\text{-Cl})-\text{NHMe}$ w trzech środowiskach (faza gazowa, chloroform, woda) (rys. 5).

Poszczególne punkty map zostały policzone przy zastosowaniu metody M06-2X/6-31+G(d,p) [51], natomiast zidentyfikowane minima lokalne zostały całkowicie zoptymalizowane przy użyciu większej bazy funkcyjnej 6-311+G(d,p). W fazie gazowej cząsteczkę uznaje się za odizolowaną od środowiska, zatem o przyjmowanych przez nią konformacjach decydują tylko oddziaływania wewnątrzcząsteczkowe.

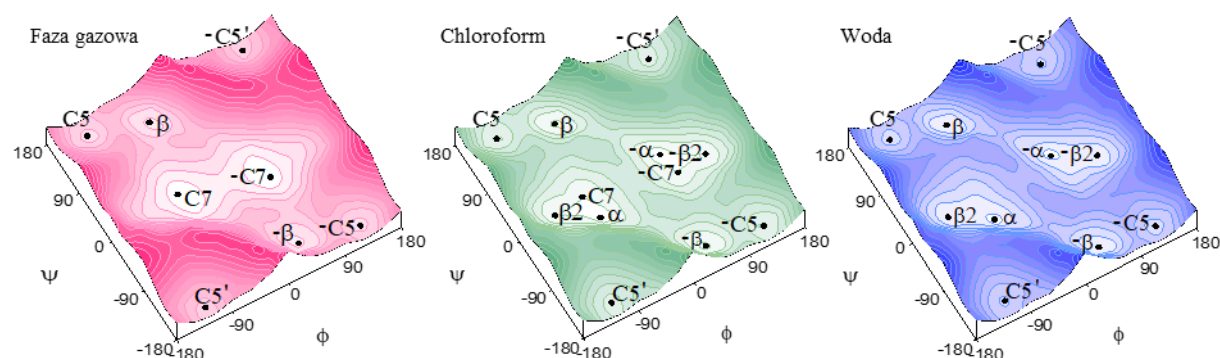
Tabela 1. Wybrane parametry konformacji Ac-(Z)- Δ Ala(β -Cl)-NHMe.

Konformacja	φ (°)	ψ (°)	ΔG (kcal·mol ⁻¹)	p (%)
<i>Faza gazowa</i>				
C7	-62.6	20.1	0.00	71.69
β	-41.4	135.4	0.65	24.09
C5	-128.2	156.7	1.92	2.85
C5'	-124.9	-165.0	2.35	1.38
<i>Chloroform</i>				
α	-61.8	-23.1	0.00	40.51
β	-42.7	138.7	0.17	30.44
$\beta 2$	-116.6	9.8	0.56	15.88
C7	-68.2	16.6	0.85	9.68
C5	-127.7	155.1	1.71	2.31
C5'	-121.0	-157.5	2.10	1.18
<i>Woda</i>				
α	-60.3	-25.0	0.00	49.58
β	-43.6	139.7	0.27	31.57
$\beta 2$	-116.4	9.4	0.65	16.54
C5	-127.6	154.5	2.09	1.47
C5'	-117.8	-153.7	2.43	0.84

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 5. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) w chloroformie z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 6. Mapy energii potencjalnej $E=f(\varphi,\psi)$ Ac-(Z)- Δ Ala(β -Cl)-NHMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

Izomer Z β -chlorodehydroalaniny w fazie gazowej, gdzie mamy do czynienia z izolowaną molekułą, przyjmuje cztery konformacje: C7, β , C5 i C5' (tabela 1, rys. 5 i 6) oraz ich lustrzane odpowiedniki o takiej samej energii, lecz przeciwnych znakach przy wartościach kątów torsyjnych. Minimum globalne w tych warunkach stanowi konformacja C7 ($\varphi, \psi = -63^\circ, 20^\circ$), przyjmowana przez prawie $\frac{3}{4}$ populacji cząsteczek. Konformacja ta stabilizowana głównie przez wiązanie wodorowe pomiędzy grupą karbonylową N-końca a grupą N-H C-końca. Dodatkowe siły stabilizujące stanowią dwa wiązania wodorowe pomiędzy grupami łańcucha bocznego i łańcucha głównego: charakterystyczne dla tego dehydroaminokwasu oddziaływanie angażujące atom chloru $N^N-H\cdots Cl$ oraz

dodatkowe $C^\beta-H\cdots O^C$ [53], a także oddziaływanie dipolowe I typu $(C=O)^N\cdots(C=O)^C$ [52]. Kolejno, prawie $\frac{1}{4}$ populacji cząsteczek przyjmuje w fazie gazowej konformację β ($\varphi, \psi = -41^\circ, 135^\circ$), stabilizowaną przede wszystkim przez oddziaływania dipolowe typu II, w które zaangażowane są obie grupy karbonylowe. Warto dodatkowo zauważyć, że w stabilizacji tej konformacji również uczestniczy wiązanie $N^N-H\cdots Cl$. Najwyższą energię mają dwie konformacje: C5 ($\varphi, \psi = -128^\circ, 157^\circ$) oraz C5' ($\varphi, \psi = -125^\circ, -165^\circ$) o przeciwnych wartościach kąta ψ , które są stabilizowane charakterystycznym dla tej konformacji wiązaniem wodorowym $N^N-H\cdots O^C$. Pojawienie się dwóch konformacji C5 jest spowodowane po pierwsze zawadą steryczną dużego atomu chloru. Po drugie, odpychaniem się wolnych par elektronowych atomu chloru i tlenu grupy karbonylowej na N-końcu, a także odpychaniem się atomów wodoru grupy N-H C-końca i $C^\beta-H$. Czynniki te uniemożliwiają przyjęcie pośredniej, koplanarnej konformacji C5 o niższej energii.

Symulacja układu w słabo polarnym otoczeniu, mimikowanym przez chloroform, stworzonym za pomocą samouzgodnionego pola reakcji (SCRF) z wykorzystaniem modelu CPCM, spowodowała zmiany na diagramie Ramachandrana w okolicach minimum globalnego. Obok konformacji C7 pojawiły się dodatkowo konformacje β_2 ($\varphi, \psi = -117^\circ, 10^\circ$) i α ($\varphi, \psi = -62^\circ, -23^\circ$), spośród których ta ostatnia charakteryzowała się najniższą energią. Podobnie jak konformacja C7, konformacja α jest stabilizowana przez oddziaływanie dipolowe $(C=O)^N\cdots(C=O)^C$ oraz dwa wiązania wodorowe spinające łańcuch główny z łańcuchem bocznym: charakterystyczne $N^N-H\cdots Cl$ oraz dodatkowe $C^\beta-H\cdots O^C$. Natomiast konformacja β_2 jest utrzymywana tylko przez $C^\beta-H\cdots O^C$. Jednak w przypadku tej konformacji pojawia się dodatkowa siła stabilizująca, jaką jest sprzężenie π -elektronowe, obejmujące wiązanie podwójne pomiędzy atomami węgla α i β oraz C-końcową grupę amidową. O zaistnieniu takiego sprzężenia świadczy koplanarność tych fragmentów strukturalnych. W naturalnym polarnym środowisku peptydów, w wodzie, zanikła całkowicie konformacja C7 na korzyść minimum globalnego, konformacji α . Porządek energetyczny oraz geometria pozostałych konformacji zostały zachowane jak w chloroformie.

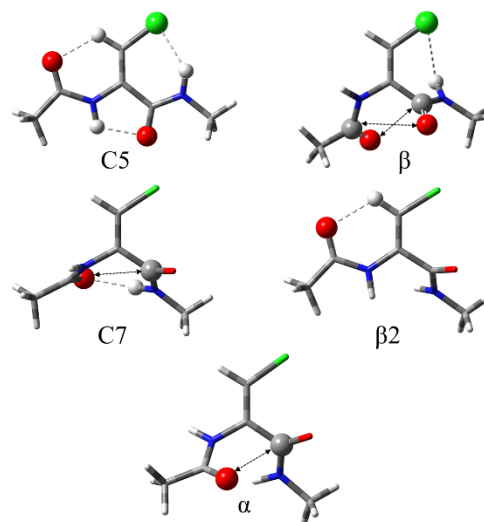
Izomer *E* β -chlorodehydroalaniny przyjmuje pięć konformacji we wszystkich trzech badanych środowiskach (tabela 2, rys. 7 i 8). Dla izolowanej cząsteczki porządek energetyczny konformacji jest następujący: C5, β , C7, β_2 , α . Minimum globalne, konformacja C5 ($\varphi, \psi = -180^\circ, 180^\circ$), jest stabilizowane przez wewnątrzcząsteczkowe wiązania wodorowe: typowe dla tej konformacji $N^N-H\cdots O^C$, charakterystyczne dla badanej reszty $N^C-H\cdots Cl$ i dodatkowe $C^\beta-H\cdots O^N$. Dodatkowo w utrzymaniu tej konformacji uczestniczy krzyżowe sprzężenie π -elektronowe, obejmujące wiązanie podwójne $C^\alpha=C^\beta$ i obie grupy amidowe, o czym świadczy koplanarność tych fragmentów strukturalnych, widoczne w wartościach kątów torsyjnych φ i ψ . Pozostałe konformacje mają energię wyższą o ponad 5 kcal/mol, więc są właściwie niedostępne. Druga w kolejności konformacja β ($\varphi, \psi = -47^\circ, 136^\circ$) jest stabilizowana głównie przez oddziaływania dipolowe II typu, w które zaangażowane są obie grupy karbonylowe. Warto znowu zauważyć, że dodatkową siłą wspierającą stanowi wiązanie wodorowe $N-H\cdots Cl$. Następną w kolejności konformacja C7 ($\varphi, \psi = -76^\circ, 70^\circ$) jest

utrzymywana przez typowe dla tej konformacji wiązanie wodorowe $N^C-H\cdots O^N$ oraz dodatkowo wspierana przez oddziaływanie dipolowe I typu. Kolejna, konformacja $\beta 2$ ($\phi, \psi = -174^\circ, 48^\circ$) jest stabilizowana przez wiązanie wodorowe $C^\beta-H\cdots O^N$ i sprzężenie π -elektronowe pomiędzy wiązaniem podwójnym $C^\alpha=C^\beta$ a N-końcową grupą amidową. Najmniej korzystna energetycznie konformacja α ($\phi, \psi = -55^\circ, -33^\circ$) jest utrzymywana tylko przez jedno oddziaływanie dipolowe $(C=O)^N\cdots(C=O)^C$.

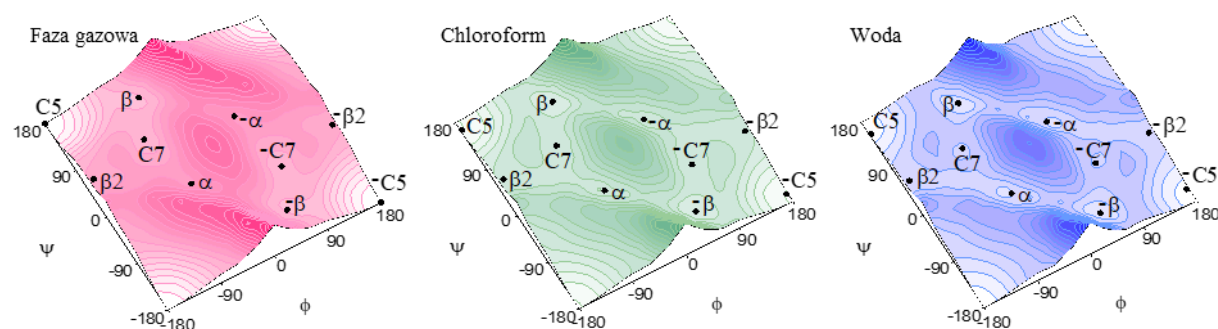
Tabela 2. Wybrane parametry konformacji Ac-(E)- Δ Ala(β -Cl)-NHMe.

Konformacja	ϕ ($^\circ$)	ψ ($^\circ$)	ΔG (kcal·mol $^{-1}$)	p (%)
<i>Faza gazowa</i>				
C5	-179.8	180.0	0.00	99.97
β	-46.9	135.6	5.25	0.01
C7	-75.8	69.7	5.45	0.01
$\beta 2$	-174.0	48.0	6.99	0.00
α	-55.4	-33.4	8.46	0.00
<i>Chloroform</i>				
C5	-177.9	165.1	0.00	91.87
β	-44.4	132.3	1.77	4.74
α	-54.5	-37.6	2.68	1.02
C7	-77.7	63.1	2.43	1.56
$\beta 2$	-172.7	53.1	2.81	0.82
<i>Woda</i>				
C5	-177.2	156.2	0.00	60.17
β	-44.0	132.2	0.73	17.57
α	-52.6	-39.6	0.71	18.21
$\beta 2$	-172.4	52.9	1.82	2.81
C7	-78.6	59.8	2.32	1.23

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



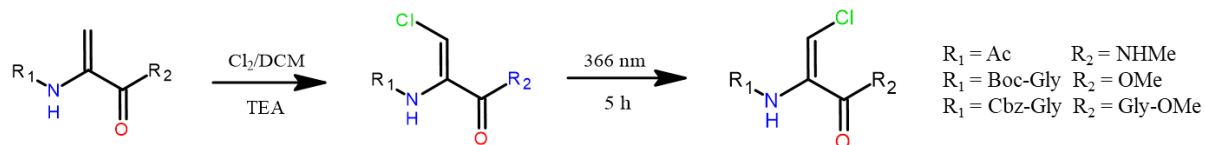
Rysunek 7. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) w chloroformie z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 8. Mapy energii potencjalnej $E=f(\phi, \psi)$ Ac-(E)- Δ Ala(β -Cl)-NHMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

W układzie z symulowanym otoczeniem dochodzi do zmniejszenia różnic energii pomiędzy poszczególnymi konformacjami. Zarówno w chloroformie, jak i w wodzie minimum globalnym pozostaje konformacja C5, jednak dostępne są również otwarte na oddziaływanie z otoczeniem konformacje β (w chloroformie i wodzie) i α (w wodzie). Geometria poszczególnych konformacji nie zmienia się znacząco w zależności od środowiska. Największe różnice występują w wartościach kąta torsyjnego ψ konformacji C5 i C7, co może być spowodowane dużym rozmiarem atomu chloru w pozycji E łańcucha bocznego dehydroaminokwasu.

W celu przeprowadzenia dalszych badań właściwości konformacyjnych reszty β -chlorodehydroalaniny zsyntezowano następujące związki: odpowiadające policzonym modelom Ac-(Z/E)- Δ Ala(β -Cl)-NHMe oraz di- i tripeptydy: Boc-Gly-(Z/E)- Δ Ala(β -Cl)-OMe i Cbz-Gly-(Z/E)- Δ Ala(β -Cl)-Gly-OMe. Synteza obejmowała trzy etapy: otrzymanie reszty dehydroalaniny, podstawienie atomu węgla w pozycji β atomem chloru oraz fotoizomeryzację (rys. 9).

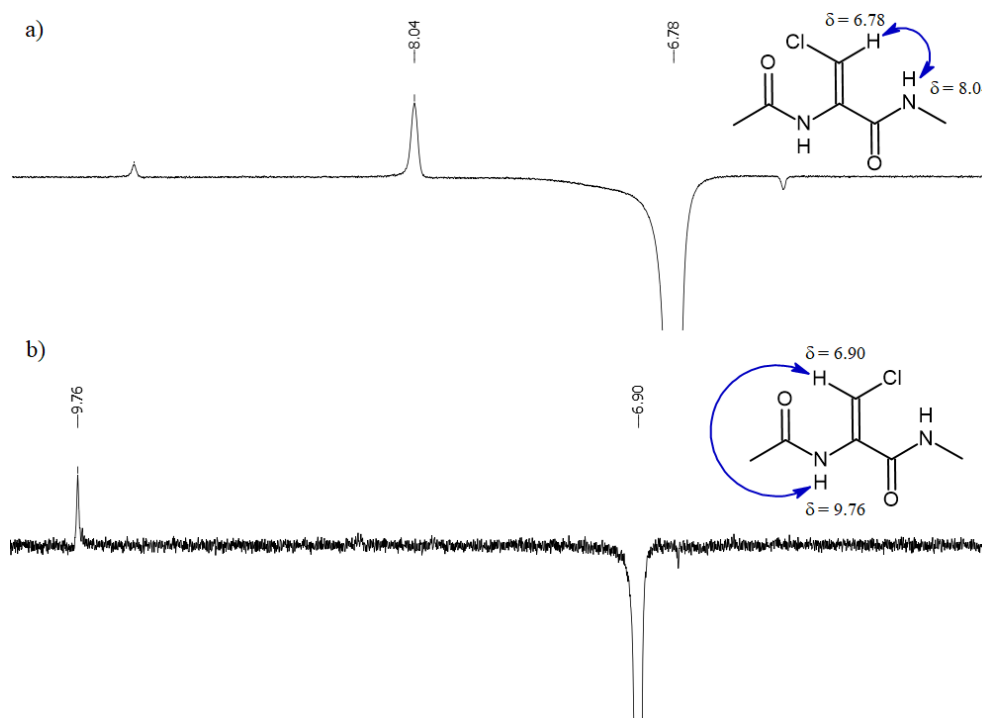


Rysunek 9. Ogólny schemat syntezy związków modelowych.

Substrat do syntezy Ac-(Z)- Δ Ala(β -Cl)-NHMe, *N*-acetylodehydroalaninę, uzyskano w reakcji kondensacji kwasu pirogronowego z acetamidem w obecności kwasu *p*-toluenosulfonowego. Otrzymany związek poddano następnie reakcji z chloromrówczanem izobutyłu, zyskując mieszaną bezwodnik, który przereagował z metyloaminą, tworząc amid metylowy *N*-acetylodehydroalaniny [54]. Boc-Gly-(Z)- Δ Ala(β -Cl)-OMe i Cbz-Gly-(Z)- Δ Ala(β -Cl)-Gly-OMe uzyskano w wyniku wieloetapowej syntezy, obejmującej otrzymanie zabezpieczonej na N-końcu Gly- Δ Ala [55, 56], estryfikację przy użyciu soli cezowej i jodku metylu [57] oraz w przypadku tripeptydu, sprzężenie z estrem metylowym glicyny za pomocą metody mieszanych bezwodników [54].

Reakcję chlorowania przeprowadzono z wykorzystaniem roztworu chloru w dichlorometanie i trietyloaminy [58-60]. W przypadku wszystkich trzech związków modelowych otrzymano izomer Z β -chlorodehydroalaniny. Dlatego związki te poddano reakcji fotoizomeryzacji, w celu uzyskania izomeru *E*. Zastosowano procedurę wykorzystaną wcześniej do dehydrofenyloalaniny [40, 61]. Procedura wymagała zoptymalizowania, ponieważ prowadziła do rozpadu substratu. Dlatego do izomeryzacji β -chlorodehydroalaniny użyto promieniowania UV o długości 366 nm w czasie 5 godzin przy intensywności 400–440 $\mu\text{W}/\text{cm}^2$, otrzymując izomer *E* zarówno w przypadku krótkiego modelu diamidowego, jak i w di- i tripeptydzie. Wydłużenie czasu reakcji, naświetlanie promieniowaniem UV o wyższej energii ($\lambda_{\text{maks}} = 254 \text{ nm}$) lub zwiększenie natężenia promieniowania powoduje rozkład substratu i zmniejszenie ogólnej wydajności. W rezultacie po raz pierwszy wykazano, że reakcję fotoizomeryzacji można z powodzeniem zastosować do reszty β -halogenodehydroaminokwasowej.

Izomerię geometryczną Z/E zsyntezowanych związków określono przy pomocy NOE NMR (rys. 10). Wzbudzony proton winylowy β -chlorodehydroalaniny silnie rezonuje z protonem grupy amidowej na C-końcu dla izomerów Z. Natomiast dla izomerów *E* jest obserwowany silny rezonans z protonem grupy amidowej na N-końcu.

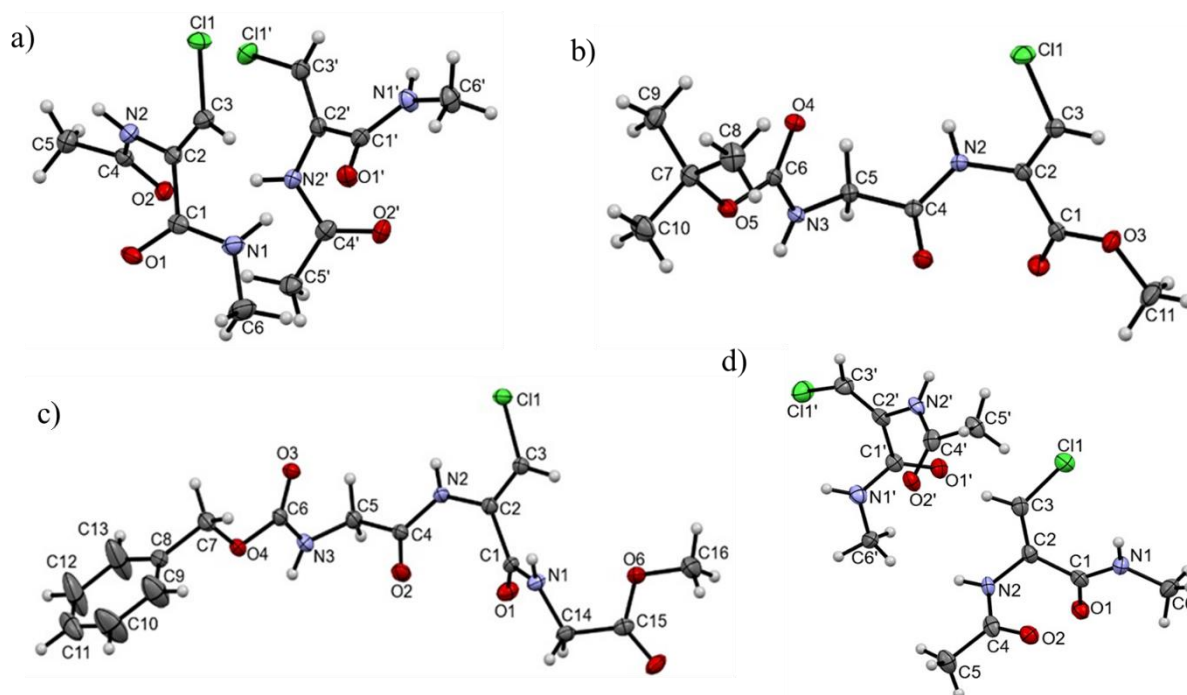


Rysunek 10. Widma różnicujące NOE NMR Ac-ΔAla-(β-Cl)-NHMe w DMSO-*d*₆ ze wzbudzonym protonem winylowym obu izomerów: (a) izomer *Z*; (b) izomer *E*.

Warto zauważyć, że początkowo przypisania izomerii geometrycznej *Z* reszcie β-chlorodehydroalaniny w wiktorynach dokonano na podstawie wartości przesunięć chemicznych protonów winylowych wzorcowych modelowych związków, izomerów *Z/E* kwasu 3-chloroakrylowego, porównanych z wartością przesunięcia chemicznego tego protonu w metylowanej HV-toksynie M [62]. W późniejszych badaniach [50] uwzględniono wpływ atomu azotu wiązania peptydowego na przesunięcie chemiczne protonu winylowego, stosując izomery *Z/E* Ac-ΔAla(β-Cl)-OMe i Boc-ΔAla(β-Cl)-OMe jako związki modelowe do badań, czyli z grupą estrową na C-końcu. Poprzez porównanie wartości przesunięć chemicznych protonu winylowego zsyntezowanych związków modelowych z wartościami przesunięć chemicznych tego protonu w wiktorynach, stwierdzono, że reszta β-chlorodehydroalaniny w wiktorynach występuje jako izomer *E*.

Należy podkreślić, że badane w tej pracy [D1] związki modelowe mają grupę amidową na C-końcu, w związku z tym są najbardziej podobne do fragmentu β-chlorodehydroalaniny w wiktorynach. Jako że to izomer *Z* tych związków jest korzystniejszy energetycznie, można przypuszczać, że reszta β-chlorodehydroalaniny w wiktorynach występuje w postaci właśnie tego izomeru. Z uzyskanych danych eksperymentalnych [D1] wynika, że wartości przesunięć chemicznych protonu winylowego dla izomerów *Z/E* zsyntezowanych związków modelowych różnią się w zależności od grupy funkcyjnej na C-końcu oraz zastosowanego rozpuszczalnika. Dlatego też przypisanie izomerii geometrycznej reszcie β-chlorodehydroalaniny w wiktorynach tylko na podstawie prostego porównania tego parametru nie jest według przedstawionych badań możliwe. Aby uzyskać jednoznaczne wyniki należałoby poddać strukturę wiktoryny rentgenowskiej analizie strukturalnej i/lub NOE NMR.

Do określenia izomerii geometrycznej *Z/E* reszty β -chlorodehydroalaniny w zsyntezowanych w ramach tej pracy [D1] związkach modelowych zastosowano obie te metody. Przypisanie izomerii dokonane na podstawie NOE NMR potwierdzono poprzez analizę struktur krystalicznych Ac-(*Z*)- Δ Ala(β -Cl)-NHMe, Ac-(*E*)- Δ Ala(β -Cl)-NHMe, Boc-Gly-(*Z*)- Δ Ala(β -Cl)-OMe oraz Cbz-Gly-(*Z*)- Δ Ala(β -Cl)-Gly-OMe. Spójność wyników obu metod potwierdza ich aplikacyjność w tego typu badaniach. Dlatego też powinny zostać wykorzystane do rozstrzygnięcia kwestii izomerii reszty β -chlorodehydroalaniny w naturalnych peptydach, wiktorynach.



Rysunek 11. Struktury molekularne: a) Ac-(*Z*)- Δ Ala(β Cl)-NHMe, b) Boc-(*Z*)- Δ Ala(β Cl)-OMe, c) Cbz-Gly-(*Z*)- Δ Ala(β Cl)-Gly-OMe i d) Ac-(*E*)- Δ Ala(β Cl)-NHMe w asymetrycznej części komórki elementarnej.

Tabela 3. Wybrane parametry badanych związków mierzone metodą rentgenowską.

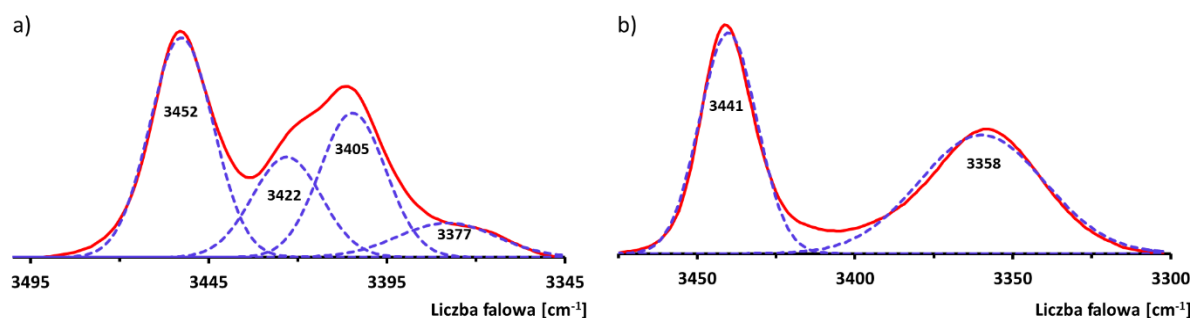
Związek modelowy	Kąt torsyjny (°)		N-H⋯Cl			C=O⋯C=O	
			Odległość (Å)		Kąt (°)	Odległość (Å)	
	φ	ψ	H⋯Cl	N⋯Cl	N-H⋯Cl	C ^N ⋯O ^C	C ^C ⋯O ^N
Ac-(Z)-ΔAla(β-Cl)-NHMe	−47.49	149.00	3.009	3.058	84.47	3.046	2.728
	−41.57	141.75	2.868	3.048	94.96	3.116	2.742
Ac-(E)-ΔAla(β-Cl)-NHMe	−49.53	134.13	2.854	3.221	106.79	3.203	2.807
	−50.04	134.30	2.918	3.256	104.80	3.220	2.833
Boc-Gly-(Z)-ΔAla(β-Cl)-OMe	−56.51	168.64	2.895	3.057	92.93	3.075	2.979
Cbz-Gly-(Z)-ΔAla(β-Cl)-Gly-OMe	−41.81	143.49	2.827	3.049	96.30	3.115	2.794

Rysunek 11 i tabela 3 przedstawiają właściwości konformacyjne β -chlorodehydroalaniny w kryształach. Małe modele diamidowe, reprezentowane przez Ac-(*Z*)- Δ Ala(β -Cl)-NHMe i Ac-(*E*)- Δ Ala(β -Cl)-NHMe, występują w sieci krystalicznej w formie dwóch konformacji β (izomer *Z*: φ , ψ = -47.5°, 149°; φ , ψ = -41.6°, 141.8°; izomer *E*: φ , ψ = -49.5°, 134.1°; φ , ψ = -50.0°, 134.3°), różniących się wartościami kątów torsyjnych φ i ψ o kilka stopni. Również w większych modelach, di- i tripeptydowym, wszystkie cząsteczki przyjmują tą samą konformację, zidentyfikowaną jako

konformacja β (dipeptyd: $\phi, \psi = -56.5^\circ, 168.6^\circ$; tripeptyd: $\phi, \psi = -41.8^\circ, 143.5^\circ$). Konformacja ta została przewidziana w wynikach obliczeń jako jedna z najkorzystniejszych energetycznie, co świadczy o dobrej korelacji eksperymentu z obliczeniami.

Odległość pomiędzy atomami węgla i tlenu sąsiednich grup karbonylowych, nieprzekraczająca 3.6 Å, umożliwia występowanie oddziaływań dipolowych II typu. Są to charakterystyczne siły stabilizujące konformację β . Analiza rentgenowska pozwoliła na identyfikację wiązania $N-H \cdots Cl$, istotnego dla badanej reszty oraz określenie jego parametrów. W izomerze *Z* jest tworzone z atomem wodoru N-końcowej grupy amidowej, natomiast w izomerze *E* z wodorem amidowym na C-końcu. Takie oddziaływanie występuje, ponieważ odległość pomiędzy atomem chloru a odpowiednim atomem wodoru jest mniejsza niż suma ich promieni van der Waalsa (2.95 Å) [63].

Zbadano również właściwości konformacyjne β -chlorodehydroalaniny w słabo polarnym rozpuszczalniku z wykorzystaniem spektroskopii w podczerwieni. Do badań zastosowano chloroformowe roztwory $Ac-(Z)-\Delta Ala(\beta-Cl)-NHMe$ i $Ac-(E)-\Delta Ala(\beta-Cl)-NHMe$.



Rysunek 12. Obszar częstości drgań rozciągających $\nu_s(N-H)$ widm FTIR a) $Ac-(Z)-\Delta Ala(\beta-Cl)-NHMe$ b) i $Ac-(E)-\Delta Ala(\beta-Cl)-NHMe$.

W obszarze drgań rozciągających $\nu_s(N-H)$ na widmie izomeru *Z* zidentyfikowano 4 pasma: dwa o większej intensywności przy 3452 cm^{-1} i 3405 cm^{-1} oraz dwa o mniejszej przy 3422 cm^{-1} i 3377 cm^{-1} (rys. 12). Związek ten ma dwie grupy N-H, zatem w roztworze chloroformu występuje w postaci dwóch konformacji. W obszarze drgań rozciągających $\nu_s(N-H)$ na widmie izomeru *E* widoczne są tylko 2 pasma: przy 3441 cm^{-1} i 3358 cm^{-1} . Zatem wszystkie cząsteczki tego związku w chloroformie przyjmują taką samą konformację.

Porównanie przeskalowanych obliczonych wartości częstości drgań z wartościami eksperymentalnymi (Tabela 7S [D1]) wskazuje współistnienie konformacji α i β dla izomeru *Z* oraz konformację C5 dla izomeru *E*. Konformacje te zostały przewidziane metodami chemii kwantowej jako najkorzystniejsze energetycznie i najliczniej obsadzone w roztworze chloroformu.

Porównując do wcześniej badanych dehydroaminokwasów strukturalnie najbardziej podobna jest dehydrobutyryna, która przy atomie węgla β w miejscu atomu chloru ma grupę metylową [35, 64]. Z porównania wynika, że pasma drgań rozciągających grupy N-H β -chlorodehydroalaniny są położone niżej. Oznacza to, że atom chloru w łańcuchu bocznym stanowi nie tylko akceptor wiązań wodorowych z wykorzystaniem swoich wolnych par elektronowych, ale również uczestniczy w zdelokalizowanym

sprężeniu krzyżowym, zwiększając gęstość elektronową grup amidowych, umożliwiając w ten sposób tworzenie przez nie silniejszych oddziaływań.

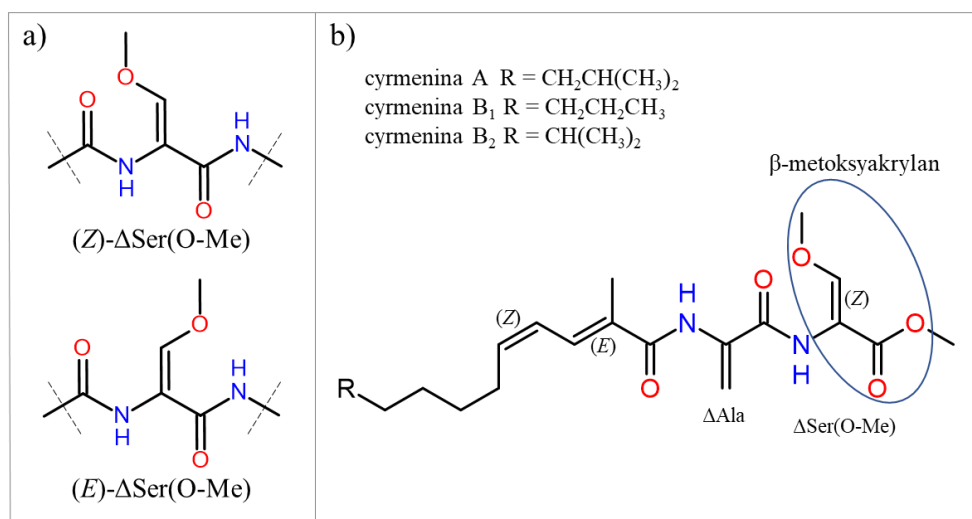
Podsumowując, w niniejszej pracy [D1] określono właściwości konformacyjne β -chlorodehydroalaniny, dehydroaminokwasu będącego elementem strukturalnym grzybiczych toksyn wiktoryn. Potencjalne konformacje, które mogą być przyjmowane przez modelowe związki diamidowe Ac-(Z)- Δ Ala(β -Cl)-NHMe i Ac-(E)- Δ Ala(β -Cl)-NHMe w trzech środowiskach (w fazie gazowej, chloroformie i wodzie), zostały wytypowane na podstawie obliczonych diagramów Ramachandrana ($E=f(\varphi, \psi)$). Izomery różnią się preferencjami konformacyjnymi. Izomer Z przyjmuje konformację helisy α ($\varphi, \psi = -61^\circ, -24^\circ$), konformację β ($\varphi, \psi = -43^\circ, 138^\circ$) i półrozciągniętą konformację β_2 ($\varphi, \psi = -116^\circ, 9^\circ$). Izomer E preferuje głównie rozciągniętą konformację C5 ($\varphi, \psi = -178^\circ, 167^\circ$), ale w polarnym środowisku przyjmuje również konformacje β ($\varphi, \psi = -45^\circ, 133^\circ$) i α ($\varphi, \psi = -54^\circ, -37^\circ$). Widma IR chloroformowych roztworów tych związków potwierdzają występowanie izomeru Z w formie mieszaniny konformacji α i β oraz izomeru E w konformacji C5. Natomiast rentgenowska analiza strukturalna następujących związków modelowych: Ac-(Z)- Δ Ala(β -Cl)-NHMe, Ac-(E)- Δ Ala(β -Cl)-NHMe, Boc-Gly-(Z)- Δ Ala(β -Cl)-OMe oraz Cbz-Gly-(Z)- Δ Ala(β -Cl)-Gly-OMe wykazała przyjmowanie konformacji β w każdym przypadku. Warto zauważyć, że konformacja ta poza oddziaływaniami dipolowymi jest stabilizowana przez wiązanie wodorowe N-H $\cdots$ Cl pomiędzy amidową grupą N-H a atomem chloru w łańcuchu bocznym, co może wpływać na obniżenie jej energii i tendencji do przyjmowania jej.

Do osiągnięć pracy należy zaliczyć opracowanie metody syntezy reszty β -chlorodehydroalaniny, w wyniku chlorowania dehydroalaniny w obecności trietyloaminy. Synteza prowadzi do otrzymania izomeru Z. Zatem do uzyskania izomeru E zastosowano reakcję fotoizomeryzacji oraz zoptymalizowano jej warunki. Izomerię geometryczną Z/E otrzymanych związków określono za pomocą NOE NMR i metody rentgenowskiej.

Stwierdzono, że proponowane wcześniej w literaturze przypisanie izomerii geometrycznej na podstawie przesunięcia chemicznego protonu winylowego nie jest wystarczająco precyzyjne. Dlatego izomeria geometryczna reszty β -chlorodehydroalaniny w wiktorynach pozostaje kwestią otwartą i wymaga dalszych badań. Określenie, który z izomerów występuje w fitotoksycznych peptydach jest istotne, ponieważ jak zbadano każdy izomer prezentuje inne właściwości konformacyjne, zatem będzie inaczej wpływał na natywną konformację całego peptydu, a tym samym na jego bioaktywność.

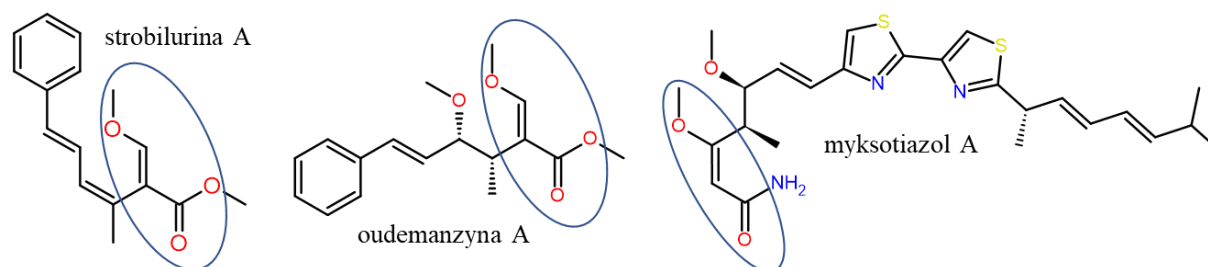
Właściwości konformacyjne *O*-metylodehydroseryny [D2]

Kolejny badany dehydroaminokwas to dehydroseryna z metylowaną grupą hydroksylową (rys. 13a). Związek ten jest elementem strukturalnym cyrmenin, przeciwgrzybiczych peptydów syntezowanych przez myksobakterie z gatunku *Cystobacter armeniacae* i *Archangium gephyra*. Cyrmeniny to *N*-acylowane dehydrodipeptydy złożone z dehydroalaniny Δ Ala i (*Z*)-*O*-metylodehydroseryny Δ Ser(*O*-Me) z odpowiednim kwasem tłuszczowym na *N*-końcu i estrem metylowym na *C*-końcu (rys. 13b) [27, 65].



Rysunek 13. a) Wzory strukturalne reszty (*Z*)- i (*E*)-*O*-metylodehydroseryny. b) Struktury przeciwgrzybiczych peptydów o nazwie cyrmeniny.

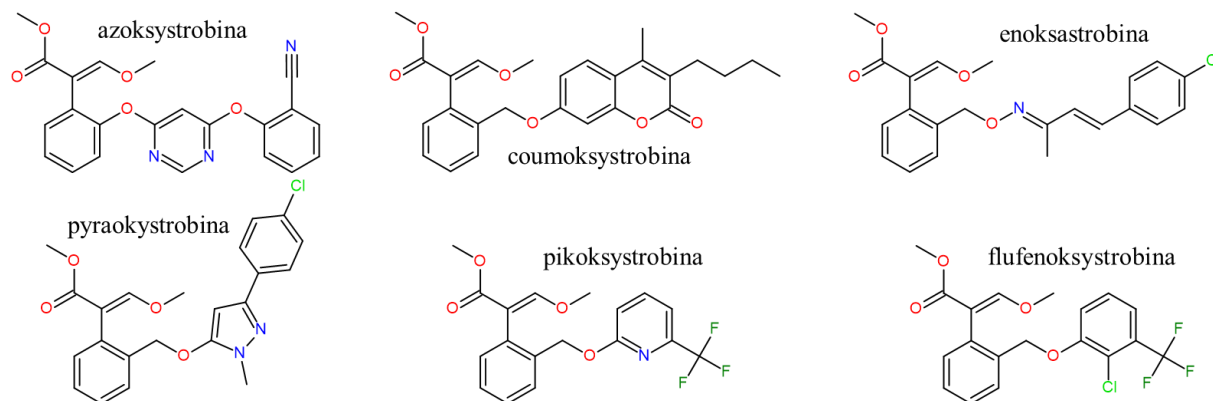
Swoją aktywność biologiczną cyrmeniny zawdzięczają specyficznemu elementowi strukturalnemu, składającemu się z grupy metoksylowej w pozycji β oraz wiązania podwójnego $C^{\alpha}=C^{\beta}$ sąsiadującego z ugrupowaniem estrowym, znanym w literaturze jako β -metoksyakrylan. Ten farmakofor został zidentyfikowany we wcześniej poznanych fungicydach – strobilurinach, oudemanzynach i myksotiazolach (rys. 14). Cechą wspólną tych wszystkich związków jest inhibicja mitochondrialnego łańcucha oddechowego poprzez blokowanie przepływu elektronów w obrębie cytochromu bc₁ [66].



Rysunek 14. Struktury naturalnych fungicydów: strobiluriny A, oudemanzyny A i myksotiazolu A, z zaznaczonym fragmentem β -metoksyakrylanowym.

Odkrycie strobiluriny, wykazującej działanie przeciwgrzybicze, zapoczątkowało badania nad syntetycznymi analogami. Poprzez zmiany w łańcuchu bocznym oraz zastosowanie bioizosterów grupy

farmakoforowej, opracowano i wprowadzono na rynek grupę związków, nazywanych fungicydami strobilurinowymi [67, 68] (rys. 15). Są one stosowane w uprawach zbóż, warzyw i owoców do zwalczania chorób grzybiczych wywoływanych przez workowce, podstawczaki, lęgowce, pleśnie, mączniaki rzekome i prawdziwe, powodujące plamistość i zarzę liści, rdzę oraz gnicie owoców [69].



Rysunek 15. Struktury wybranych syntetycznych fungicydów, zaprojektowanych na bazie strobiluriny.

Cyrmieniny, podobnie jak strobilurina A, wykazują silne działanie przeciwgrzybicze i brak aktywności antybakteryjnej. Są jednak mniej toksyczne wobec komórek ssaków [65]. Stanowią zatem potencjalne prekursory leków przeciwgrzybiczych. Do tej pory tylko w strukturze cyrmenin zidentyfikowano resztę O-metylodehydroseryny, która jest α -amino podstawionym β -metoksyakrylanem, odpowiedzialnym za działanie przeciwgrzybicze tych związków. Poznanie niezbadanych dotąd właściwości konformacyjnych O-metylodehydroseryny dostarczy informacji o geometrii β -metoksyakrylanu, co może być pomocne w lepszym zrozumieniu jego wpływu na aktywność biologiczną cyrmenin.

Wstępny obraz preferencji konformacyjnych O-metylodehydroseryny ukazały wyniki badań obliczeniowych. Zastosowano krótkie acetylowe modele obu izomerów z grupą estrową na C-końcu, aby związki modelowe lepiej imitowały fragment strukturalny cyrmenin. Podobnie jak w poprzednich badaniach [D1] wyrysowano po trzy mapy energii potencjalnej $E = f(\varphi, \psi)$ (w fazie gazowej, chloroformie i wodzie) dla każdego związku, Ac-(Z)- Δ Ser(O-Me)-OMe i Ac-(E)- Δ Ser(O-Me)-OMe.

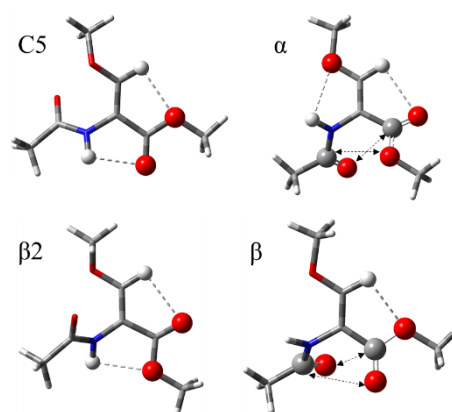
Izomer Z może przyjmować cztery konformacje, które dla izolowanej cząsteczki występują w następującym porządku energetycznym: C5 ($\varphi, \psi = -129^\circ, 178^\circ$), α ($\varphi, \psi = -54^\circ, -20^\circ$), β_2 ($\varphi, \psi = -126^\circ, -2^\circ$), β ($\varphi, \psi = -56^\circ, 164^\circ$) (tabela 4, rys. 16 i 17). Zarówno konformacja C5 jak i β_2 są stabilizowane przez wiązanie wodorowe $N-H \cdots O$, którego donorem jest N-końcowa grupa N-H. Różnią się jednak akceptorem, ponieważ w konformacji C5 jest nim karbonylowy atom tlenu grupy estrowej, a w konformacji β_2 metoksyowy atom tlenu grupy estrowej. Silniejsze wiązanie wodorowe jest tworzone przez grupę karbonylową, stąd niższa energia konformacji C5 i jej preferencyjne obsadzenie. Możliwość obrotu grupy estrowej świadczy o elastyczności konformacyjnej. Konformacja α , znajdująca się pomiędzy konformacją C5 a β_2 w porządku energetycznym, jest również stabilizowana przez wiązanie wodorowe $N-H \cdots O$, podobne do tego w konformacji β_2 , z tą różnicą, że akceptorem wiązania

jest atom tlenu grupy metoksyłowej w łańcuchu bocznym. Ta konformacja posiada dodatkowe siły wspierające w postaci dwóch oddziaływań dipolowych typu III, dlatego ma niższą energię niż konformacja $\beta 2$. Najmniej korzystna energetycznie konformacja β jest stabilizowana przez dwa oddziaływania dipolowe II typu, tworzone przez grupy karbonyłowe. Wszystkie cztery konformacje są dodatkowo wspierane przez wiązanie wodorowe $C^{\beta}-H \cdots O$, którego akceptorami są atomy tlenu grupy estrowej. Warto zauważyć niewielkie różnice energii poszczególnych konformacji.

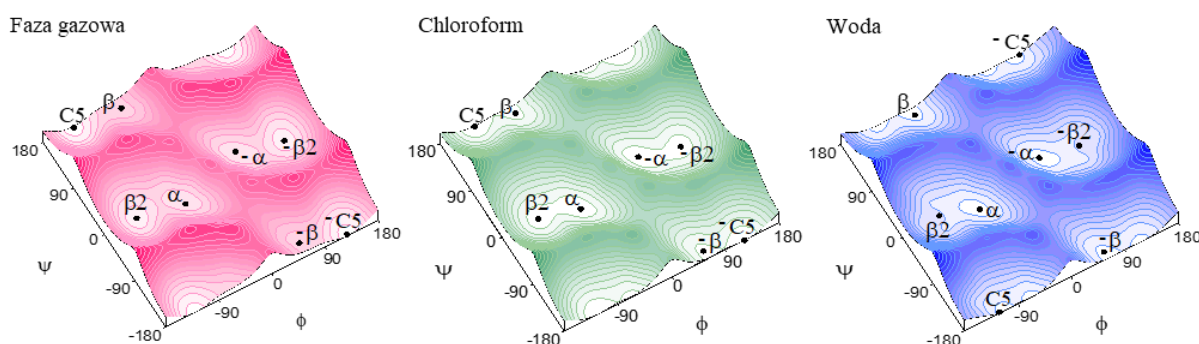
Tabela 4. Wybrane parametry konformacji Ac-(Z)- Δ Ser(O-Me)-OMe.

Konformacja	φ (°)	ψ (°)	ΔG (kcal·mol ⁻¹)	p (%)
<i>Faza gazowa</i>				
C5	-129.0	178.1	0.00	61.42
α	-53.6	-20.0	0.70	19.05
$\beta 2$	-126.4	-2.4	0.78	16.57
β	-56.3	163.9	1.81	2.96
<i>Chloroform</i>				
α	-57.9	-16.6	0.00	28.60
C5	-122.2	179.8	0.04	26.63
β	-60.7	167.2	0.04	26.52
$\beta 2$	-119.7	-0.4	0.27	18.25
<i>Woda</i>				
C5	-120.2	-179.3	0.00	33.62
β	-62.1	168.4	0.01	32.82
α	-59.2	-15.7	0.31	19.82
$\beta 2$	-117.7	0.2	0.53	13.74

Każda konformacja posiada swój lustrzany odpowiednik.
Zoptimalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 16. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) w fazie gazowej z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\cdots$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 17. Mapy energii potencjalnej $E=f(\varphi, \psi)$ Ac-(Z)- Δ Ser(O-Me)-OMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

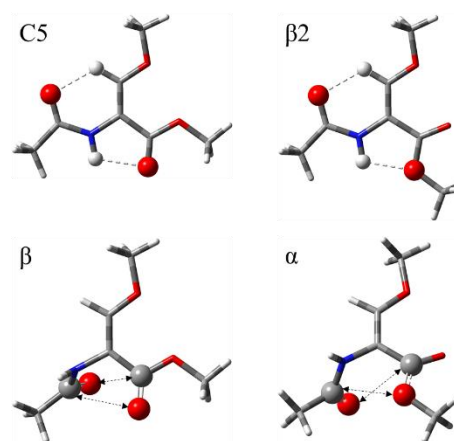
W symulowanym środowisku słabo polarnym i polarnym wszystkie cztery konformacje zostają zachowane, a różnice ich energii są jeszcze mniejsze niż dla izolowanej cząsteczki. Niewielkie zmiany geometrii konformacji polegają na tym, że N-końcowa grupa amidowa staje się bardziej prostopadła w stosunku do wiązania podwójnego $C^{\alpha}=C^{\beta}$, natomiast C-końcowa grupa estrowa bardziej równoległa. Jest to spowodowane otwarciem się grupy amidowej na oddziaływania międzycząsteczkowe oraz sprzężeniem π -elektronowym grupy estrowej ze współpłaszczyznowym wiązaniem podwójnym. To sprzężenie silnie stabilizuje koplanarność fragmentu β -metoksyakrylanowego, niezaangażowanego w tworzenie silnych oddziaływań międzycząsteczkowych.

Izomer *E* w fazie gazowej (izolowana molekula) przyjmuje tylko dwie, całkowicie rozciągnięte konformacje C5 ($\varphi, \psi = -180^\circ, 180^\circ$) i $\beta 2$ ($\varphi, \psi = -180^\circ, 0^\circ$), stabilizowane przez wiązania wodorowe N-H $\cdots$ O i C $^\beta$ -H $\cdots$ O (tabela 5, rys. 18 i 19). Analogicznie do izomeru *Z*, o różnicy energii pomiędzy tymi konformacjami decyduje akceptor wiązania wodorowego N-H $\cdots$ O. W symulowanym środowisku rozpuszczalnika, dostępne są dodatkowo konformacje β ($\varphi, \psi = -67^\circ, 173^\circ$) i α ($\varphi, \psi = -66^\circ, -9^\circ$), stabilizowane przez oddziaływania dipolowe. Podobnie jak w przypadku izomeru *Z*, C-końcowa grupa estrowa wszystkich konformacji izomeru *E* znajduje się w płaszczyźnie wiązania podwójnego C $^\alpha$ =C $^\beta$, przez co dochodzi do sprzężenia π -elektronowego, obejmującego fragment β -metoksyakrylanowy. Zarówno w fazie gazowej, jak i w chloroformie dominuje konformacja C5, natomiast w wodzie na znaczeniu zyskują otwarte konformacje β i α . Jednak różnice energii poszczególnych konformacji są dość niewielkie. Ponadto, geometrie konformacji właściwie nie zmieniają się wraz ze wzrostem polarności rozpuszczalnika.

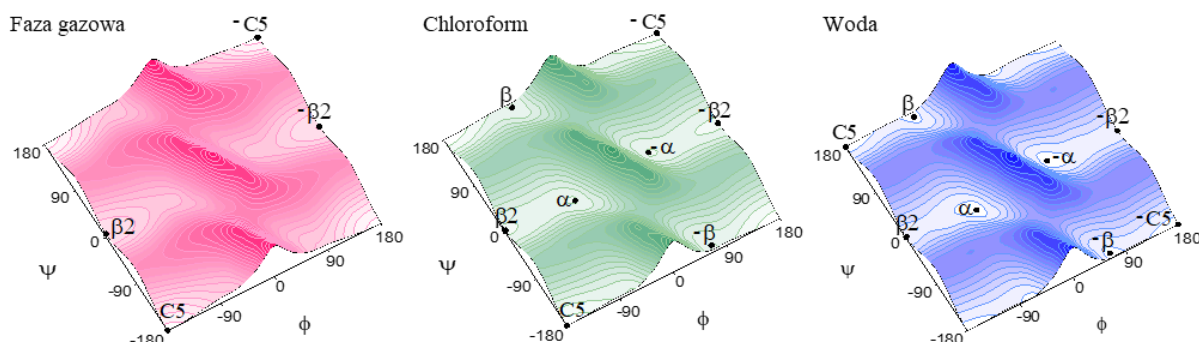
Tabela 5. Wybrane parametry konformacji Ac-(*E*)- Δ Ser(O-Me)-OMe.

Konformacja	φ ($^\circ$)	ψ ($^\circ$)	ΔG (kcal·mol $^{-1}$)	<i>p</i> (%)
<i>Faza gazowa</i>				
C5	-180.0	-180.0	0.00	94.86
$\beta 2$	-180.0	0.0	1.74	5.15
<i>Chloroform</i>				
C5	-180.0	-180.0	0.00	57.02
$\beta 2$	-180.0	0.0	0.65	19.12
β	-68.4	173.3	0.72	16.96
α	-66.8	-8.2	1.26	6.90
<i>Woda</i>				
β	-67.7	172.8	0.00	32.17
C5	-180.0	180.0	0.13	25.83
α	-66.0	-10.1	0.18	23.58
$\beta 2$	-180.0	0.0	0.33	18.42

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).

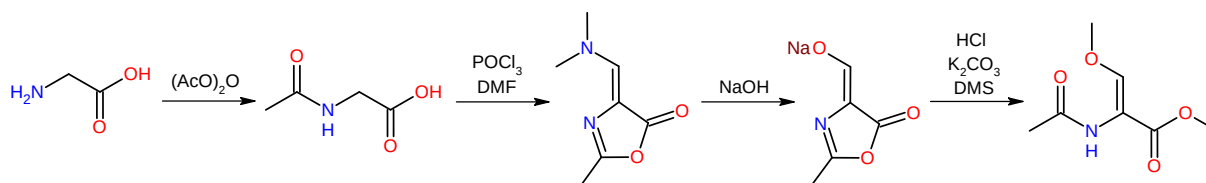


Rysunek 18. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) w fazie gazowej z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.

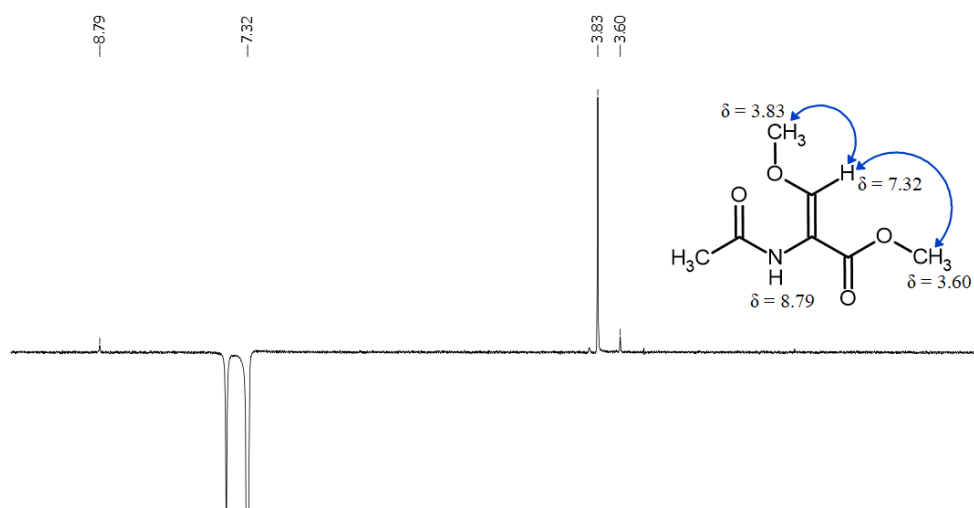


Rysunek 19. Mapy energii potencjalnej $E=f(\varphi, \psi)$ Ac-(*E*)- Δ Ser(O-Me)-OMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

Dalsze badania obejmowały przeprowadzenie czteroetapowej syntezy Ac- Δ Ser(O-Me)-OMe według procedury opisanej w patencie [70]. Substratem syntezy była glicyna, która poprzez cykliczne produkty pośrednie została zamieniona w pożądaný produkt (rys. 20). Widma różnicujące NOE NMR, otrzymane przez selektywne wzbudzenie protonu winylowego, umożliwiły zidentyfikowanie izomerii geometrycznej reszty dehydroaminokwasowej jako izomeru *Z* (rys. 21). Taki wynik jest zgodny z przewidywaniami obliczeń, według których to transoidalna jednostka β -metoksyakrylanu (izomer *Z*) jest preferowana. W celu uzyskania izomeru *E* przeprowadzono reakcję fotoizomeryzacji według wcześniej opracowanej procedury [D1], jednak substrat uległ rozpadowi do szczawianu amonu.

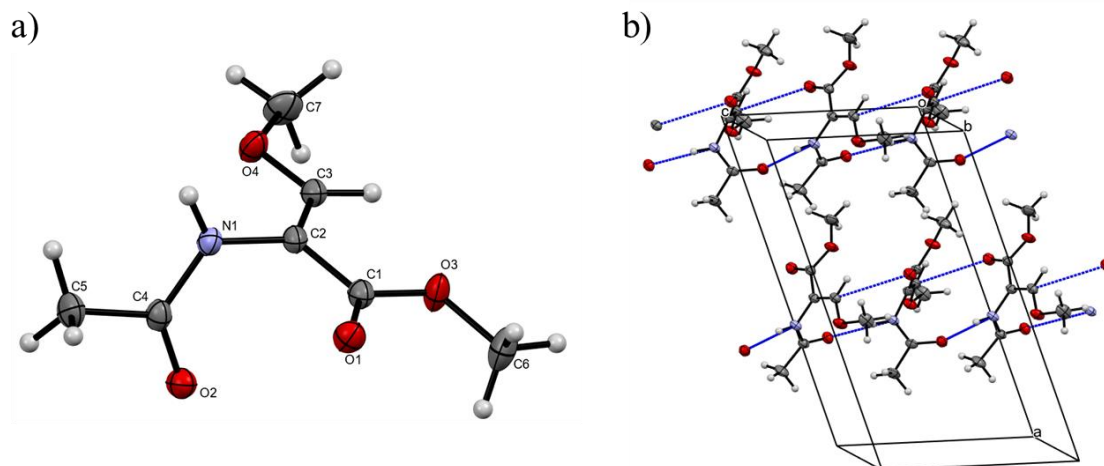


Rysunek 20. Schemat syntezy Ac-(*Z*)- Δ Ser(O-Me)-OMe.



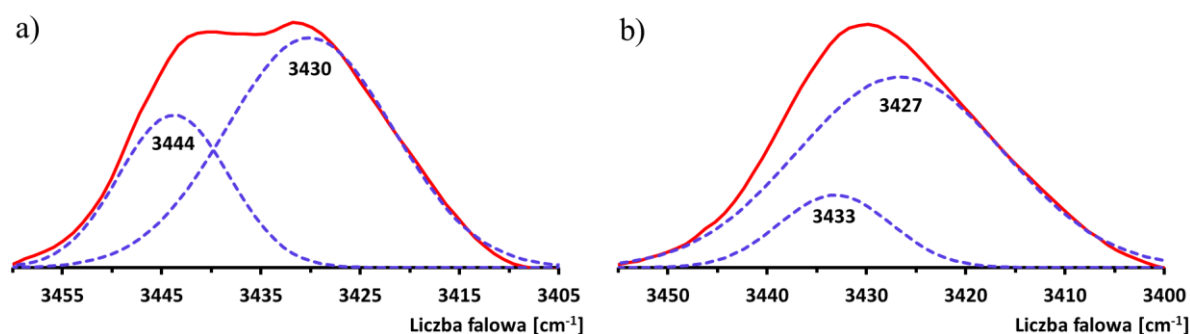
Rysunek 21. Widmo różnicujące NOE NMR Ac-(*Z*)- Δ Ser-(O-Me)-OMe w DMSO- d_6 ze wzbudzonym protonem winylowym.

Kryształ Ac-(*Z*)- Δ Ser(O-Me)-OMe poddano rentgenowskiej analizie strukturalnej, która potwierdziła położenie grupy metoksyowej w pozycji *Z* wiązania podwójnego $C^{\alpha}=C^{\beta}$ (rys. 22a). Ponadto, ustalono, że przyjmuje ona konformacje β (φ , ψ = -69° , 174°) i $-\beta$ (φ , ψ = 69° , 174°), przewidziane przez obliczenia jako najniższe energetycznie w wodzie obok konformacji C5. Fragment β -metoksyakrylanowy jest prawie płaski, co również jest zgodne z wynikami obliczeń. Ponadto, jak widać w strukturze asocjacji (rys. 22b), grupa metoksyowa nie tworzy silnych oddziaływań międzycząsteczkowych.



Rysunek 22. a) Struktura molekularna Ac-(Z)-ΔSer(Me)-OMe w asymetrycznej części komórki elementarnej. b) Oddziaływania molekularne cząsteczek Ac-(Z)-ΔSer(Me)-OMe. Wiązania wodorowe przedstawiono liniami przerywanymi. Wiązania wodorowe opierają się na sumie promieni van der Waalsa danego atomu.

Z wykorzystaniem spektroskopii w podczerwieni określono właściwości konformacyjne izomeru Z O-metylodehydroseryny w niepolarnym i słabo polarnym rozpuszczalniku. Związek ten ma tylko jedną grupę N-H, dlatego liczba pasm w obszarze drgań rozciągających $\nu_s(\text{N-H})$ na widmie IR odpowiada liczbie konformacji. Dobra rozpuszczalność związku umożliwiła pomiar w roztworze tetrachlorometanu, w którym zidentyfikowano dwa pasma, jedno przy 3444 cm^{-1} , a drugie przy 3430 cm^{-1} (rys. 23). Pasma przesunięte w stronę niższych częstotliwości można przypisać konformacji C5, ponieważ to ona tworzy najsilniejsze wiązanie wodorowe z udziałem grupy N-H, a także została przewidziana przez obliczenia jako najkorzystniejsza energetycznie (tabela S6 [D2]). Porównując eksperymentalne i obliczone częstotliwości drgań, pasmo przy 3444 cm^{-1} można przypisać konformacji β_2 .



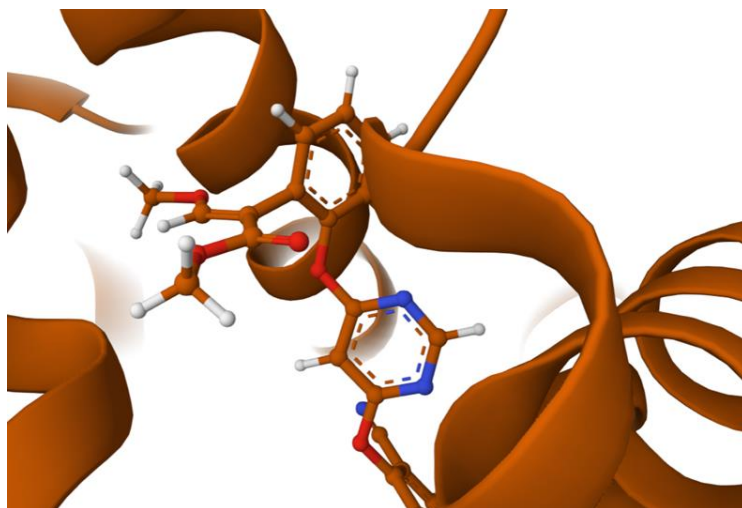
Rysunek 23. Obszar częstotliwości drgań rozciągających $\nu_s(\text{N-H})$ widm FTIR Ac-(Z)-ΔSer(O-Me)-OMe a) w tetrachlorometanie i b) w chloroformie.

Widmo zarejestrowane dla roztworu chloroformowego prezentuje jedno niesymetryczne pasmo, które po dekonwolucji podzielono na dwa z maksimum przy 3433 cm^{-1} i 3427 cm^{-1} . W tym przypadku pasmo przy niższej częstotliwości również można przypisać konformacji C5, natomiast wartość przesunięcia drugiego pasma najlepiej pasuje do konformacji α . Według wyników obliczeń obie te konformacje mają najniższą energię w chloroformie. Wartości przesunięć pasm przypisanych do konformacji C5, świadczą o umiarkowanej sile wiązania $\text{N-H}\cdots\text{O}$, osłabionego przez nie najbardziej korzystną geometrię.

Obecność grupy metoksyowej w pozycji Z łańcucha bocznego powoduje skręcenie N-końcowej grupy amidowej ($\varphi \sim -125^\circ$), przez co nie znajduje się ona w jednej płaszczyźnie z C-końcową grupą estrową. W konsekwencji, zwiększa się odległość pomiędzy grupami N-H i C=O, co osłabia tworzone przez nie wiązanie wodorowe. Podobnie jest w przypadku konformacji β_2 , z tą różnicą, że donorem wiązania wodorowego N-H $\cdots$ O jest atom tlenu grupy metoksyowej, a nie karbonylowej. To skutkuje dodatkowym osłabieniem wiązania i tłumaczy przesunięcie pasma drgań grupy N-H konformacji β_2 w stronę wyższych częstotliwości. Analogiczne wiązanie wodorowe, tworzone przez amidową grupę N-H i atom tlenu grupy metoksyowej w łańcuchu bocznym, stabilizuje konformację α . Jednak to wiązanie jest silniejsze niż w konformacji β_2 , o czym świadczy wartość przesunięcia pasma drgań grupy N-H.

Niezależnie od przyjmowanej konformacji fragment β -metoksyakrylanowy O-metylodehydroseryny jest płaski. Wartość kąta torsyjnego ψ nie odbiega o więcej niż 20° ; od 180° dla konformacji C5 i β oraz od 0° dla konformacji β_2 i α . O-metylodehydroseryna charakteryzuje się większą planarnością niż podobny dehydroaminokwas z grupą metylową przy atomie węgla β , dehydrobutyryna [71, 72]. Jest to spowodowane obecnością atomu tlenu w łańcuchu bocznym, stanowiącym mniejszą zawadę steryczną, a także uczestniczącym w krzyżowym sprzężeniu π -elektronowym z wiązaniem podwójnym C $^\alpha$ =C $^\beta$ i grupą estrową na C-końcu.

W World Protein Data Bank znajdują się struktury białek z fungicydami β -metoksyakrylanowymi jako ligandami (rys. 24) [73]. Fragment β -metoksyakrylanowy tych ligandów jest płaski, a geometria wiązania podwójnego odpowiada izomerowi Z O-metylodehydroseryny. Oznacza to, że obie te własności są kluczowe dla aktywności przeciwgrzybiczej.



Rysunek 24. Kompleks cytochromu bc₁ z azoksystrobiną (PDB DOI: 10.2210/pdb3L71/pdb).

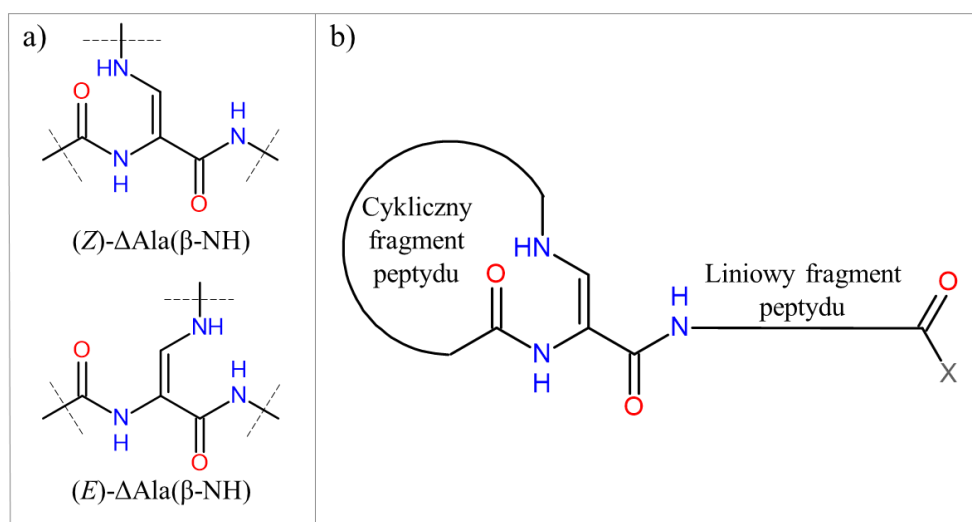
Podsumowując, niezależnie od izomerii geometrycznej Z/E, O-metylodehydroseryna może przyjmować cztery konformacje: C5, α , β_2 i β . Jednak pozycja podstawienia wiązania podwójnego C $^\alpha$ =C $^\beta$ grupą metoksyową wpływa na wartości kątów torsyjnych φ i ψ . Rentgenowska analiza strukturalna izomeru Z O-metylodehydroseryny wykazała obecność konformacji β i $-\beta$ w kryształach. Natomiast badania tego związku z wykorzystaniem spektroskopii w podczerwieni ujawniły tendencję

do przyjmowania konformacji C5 i β_2 w roztworze tetrachlorometanu oraz konformacji C5 i α w roztworze chloroformu. Uzyskane wyniki eksperymentalne potwierdzają przewidywania obliczeń chemii kwantowej.

Konformacje C5 i β_2 , stabilizowane głównie przez oddziaływania wewnątrzcząsteczkowe, są preferowane w środowisku słabo polarnym. Wraz ze wzrostem polarności środowiska chętniej przyjmowane są konformacje α i β , otwarte na oddziaływanie z otoczeniem, zasadniczo poprzez N-kończącą grupę amidową. C-kończąca grupa estrowa nie tworzy silniejszych oddziaływań wewnątrz- i międzycząsteczkowych. Natomiast we wszystkich konformacjach dąży do koplanarności z łańcuchem bocznym, kluczowej dla aktywności przeciwwgrzybiczej.

Właściwości konformacyjne β -aminodehydroalaniny [D3]

Nietypowy dehydroaminokwas z grupą aminową przy atomie węgla β (rys. 25a) stanowi element strukturalny kalyaeryn A-M i kalynorminy A (rys. 25b i tabela 6). Są to peptydy wyizolowane z gąbek morskich, wykazujące aktywność przeciwgruźliczą. W ich strukturze można wyróżnić fragment cykliczny oraz liniowy, a łącznik pomiędzy nimi stanowi właśnie β -aminodehydroalanina Δ Ala(β -NHMe) znana w literaturze również jako α -amino- β -aminoakrylamid lub kwas (Z)-2,3-diaminoakrylowy (DAA). Liniowy łańcuch składa się z samych standardowych aminokwasów, natomiast w części cyklicznej można znaleźć kilka nietypowych, na przykład γ -hydroksyprolinę (HYP) lub kwas 2-amino-3-(5-metoksy-2-oksoimidazolidyno-4-yliden)propionowy (AMOIPA) [30, 74-76].



Rysunek 25. a) Wzory strukturalne reszty (Z)- i (E)- β -aminodehydroalaniny. b) Schemat przedstawiający strukturę kalyaeryn A-M i kalynorminy A.

Tabela 6. Skład aminokwasowy kalyaeryn A-M i kalynorminy A.

Peptyd	Fragment cykliczny								Fragment liniowy					
Kalyaeryna	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	R ₁	R ₂	R ₃	R ₄	R ₅	X
A	Ile	Hyp	Val	Ile	Leu	Pro	Pro	Leu	Pro	Ile	Phe	Gly		NH ₂
B	Ile	Hyp	Ile	Ile	Leu	Pro	Pro	Leu	Pro	Ile	Ile			NH ₂
C	His	Hyp	Leu	Leu	Pro	Pro	Val		Pro	Leu	Phe	Gly		NH ₂
D	Ile	Hyp	Ile	Phe	Pro	Pro	Leu		Pro	Ile	Asn	Ala	Ile	NH ₂
E	Leu	Pro	Phe	Phe	Pro	Pro	Val		Pro	Ile	Ile	Gly		NH ₂
F	Val	Pro	Val	Phe	Pro	Pro	Leu		Pro	Ile				NH ₂
G	Leu	Pro	Phe	Phe	Pro	Pro	Leu		Pro	Pro	Phe	Gly		
H	Val	Pro	Val	Phe	Pro	Pro	Leu		Pro	Ile				NH ₂
I	Leu	Pro	Phe	Phe	Pro	Pro	Val		Pro	Leu	Phe	Gly		NH ₂
J	Phe	Pro	Leu	Phe	Pro	Pro	Val		Pro	Ile	Ile	Gly		NH ₂
K	Phe	Pro	Phe	Gly	Leu	Pro	Pro	Phe	Pro	Phe	Ile	Asp		OH
L	Ile	Hyp	Glu	Ile	Val	Pro	Pro	Leu	Pro	Leu	Phe			NH ₂
M	AMOIPA	Hyp	Leu	Leu	Pro	Pro	Val		Pro	Leu	Phe	Gly		NH ₂
Kalynormina A	Ile	Hyp	Val	Leu	Pro	Pro	Leu		Pro	Phe	Leu			OH

Podobny dehydroaminokwas, w którym wiązanie podwójne $C^{\alpha}=C^{\beta}$ jest podstawione grupą ureidową, można znaleźć w strukturach dwóch rodzin peptydów, tuberaktynomycyn i kapreomycyn [29, 77]. Te naturalne peptydy również wykazują działanie przeciwgruźlicze. Przedstawione dane wskazują, że to właśnie układ enaminowy odpowiada za taką aktywność biologiczną.

Zbadanie właściwości konformacyjnych dehydroaminokwasu z grupą aminową w łańcuchu bocznym dostarczy nowych informacji, pozwalających lepiej zrozumieć powiązanie między strukturą a aktywnością biologiczną tego elementu strukturalnego. Nietypowe właściwości konformacyjne mogą wynikać z możliwości powstania zdelokalizowanego układu π -elektronów z udziałem wolnej pary elektronowej dodatkowego atomu azotu. Ponadto, w odróżnieniu od innych badanych dehydroaminokwasów z polarną grupą funkcyjną w łańcuchu bocznym [**D1**, **D2**], grupa aminowa β -aminodehydroalaniny może być donorem wiązania wodorowego i przez to tworzyć dodatkowe oddziaływania, stabilizujące pewne konformacje.

W celu uzyskania wstępnego obrazu preferencji konformacyjnych β -aminodehydroalaniny obliczono mapy energii potencjalnej $E = f(\varphi, \psi)$ czterech związków modelowych: Ac-(Z)- Δ Ala(β -NHMe)-NHMe, Ac-(E)- Δ Ala(β -NHMe)-NHMe, Ac-(Z)- Δ Ala(β -NHMe)-NMe₂ i Ac-(E)- Δ Ala(β -NHMe)-NMe₂. Zastosowano metodę M06-2X/6-31+G(d,p), wykorzystaną wcześniej do podobnych związków [**D1**, **D2**]. Sporządzono diagramy Ramachandrana każdego związku w trzech środowiskach – w fazie gazowej, chloroformie i wodzie. Tak jak poprzednio [**D1**, **D2**], symulacji środowiska rozpuszczalnika dokonano za pomocą SCRF z wykorzystaniem CPCM. Zidentyfikowane minima lokalne zoptymalizowano całkowicie, stosując większą bazę funkcyjną 6-311+G(d,p).

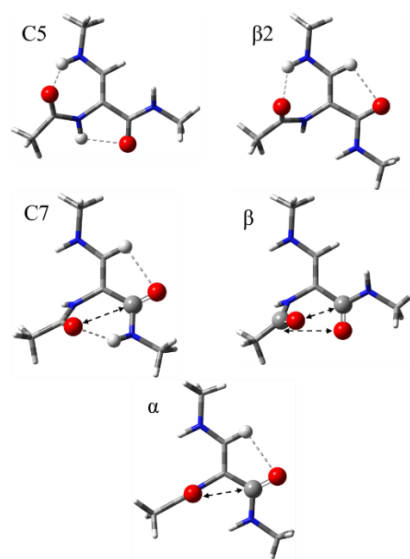
Obliczenia wykazały (tabela 7, rys. 26 i 27), że izolowana cząsteczka izomeru Z β -aminodehydroalaniny z drugorzędowym amidem na C-końcu może przyjmować cztery konformacje: C5 ($\varphi, \psi = -146^\circ, 166^\circ$), β 2 ($\varphi, \psi = -137^\circ, 12^\circ$), C7 ($\varphi, \psi = -67^\circ, 20^\circ$), β ($\varphi, \psi = -61^\circ, 157^\circ$). Najkorzystniejsza energetycznie konformacja C5 jest stabilizowana przez dwa wiązania wodorowe

N-H $\cdots$ O – jedno typowe dla tej konformacji, utworzone pomiędzy grupami amidowymi na N- i C-końcu, a drugie, charakterystyczne dla badanego dehydroaminokwasu, pomiędzy atomem tlenu grupy karbonylowej na N-końcu i grupą N-H w łańcuchu bocznym. To drugie wiązanie wodorowe uczestniczy również w stabilizowaniu drugiej dostępnej konformacji, $\beta 2$. Dodatkowo konformacja ta jest utrzymywana przez wiązanie wodorowe C $^{\beta}$ -H $\cdots$ O, tworzone przez winylowy atom wodoru oraz grupę karbonylową na C-końcu. Ostatnie dwie konformacje mają znacznie wyższą energię. Konformacja C7 jest głównie stabilizowana przez wiązanie wodorowe N-H $\cdots$ O, w które zaangażowane są grupy amidowe. Dodatkowe siły stabilizujące konformację stanowi wiązanie C $^{\beta}$ -H $\cdots$ O oraz oddziaływanie dipolowe typu I pomiędzy grupami karbonyłowymi. Konformacja β jest utrzymywana tylko przez karbonyłowe oddziaływania dipolowe typu II.

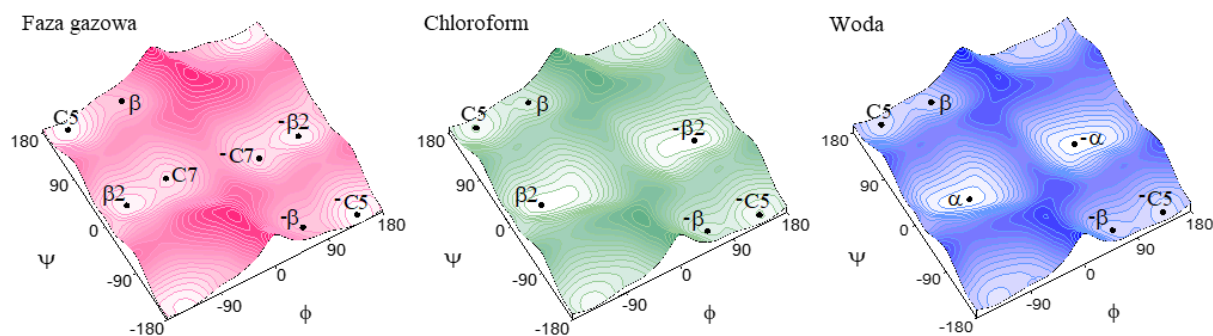
Tabela 7. Wybrane parametry konformacji Ac-(Z)- Δ Ala(β -NHMe)-NHMe.

Konformacja	φ (°)	ψ (°)	ΔG (kcal·mol $^{-1}$)	p (%)
<i>Faza gazowa</i>				
C5	-146.0	165.9	0.00	84.67
$\beta 2$	-136.6	12.3	1.10	13.44
C7	-66.8	19.6	2.28	1.83
β	-61.1	156.6	4.29	0.06
<i>Chloroform</i>				
$\beta 2$	-123.1	8.7	0.00	88.99
C5	-142.0	165.9	1.27	10.52
β	-59.4	158.2	3.09	0.50
<i>Woda</i>				
α	-89.4	-1.9	0.00	99.44
β	-60.3	159.2	3.40	0.33
C5	-140.7	166.1	3.61	0.23

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 26. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 27. Mapy energii potencjalnej $E=f(\varphi,\psi)$ Ac-(Z)- Δ Ala(β -NHMe)-NHMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

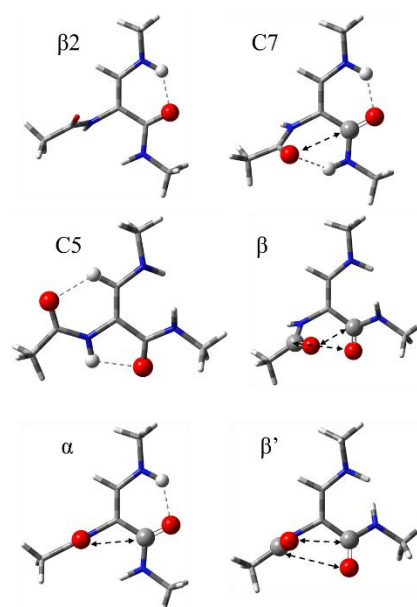
W słabo polarnym środowisku chloroformu zanika konformacja C7, a konformacje C5 i $\beta 2$ zamieniają się miejscami w porządku energetycznym. Konformacja $\beta 2$ staje się korzystniejsza, ponieważ otwiera się na oddziaływania międzycząsteczkowe poprzez rotację N-końcowej grupy

amidowej, ustawiając się bardziej prostopadle w stosunku do łańcucha bocznego. Pozostałe konformacje nie zmieniają swojej geometrii w znaczącym stopniu. W naturalnym środowisku biologicznym, w wodzie, prawie wszystkie cząsteczki przyjmują konformację α ($\varphi, \psi = -89^\circ, -2^\circ$), powstałą przez dalszy obrót N-końcowej grupy amidowej do prostopadłej pozycji względem łańcucha bocznego. Jednocześnie C-koniec dąży do koplanarności z wiązaniem podwójnym $C^\alpha=C^\beta$ i grupą aminową reszty dehydroaminokwasowej. Konformacja ta jest stabilizowana przez oddziaływanie dipolowe I typu, z udziałem grup karbonylowych oraz przez wiązanie wodorowe $C^\beta-H\cdots O$. Część N-końcowa nie jest zaangażowana w oddziaływania wewnątrzcząsteczkowe, zatem zarówno N-końcowa grupa amidowa, jak i grupa aminowa w łańcuchu bocznym mogą oddziaływać z otoczeniem. Dostępne są również konformacje β i C5, jednak ich energie są znacznie wyższe, dlatego praktycznie nie będą przyjmowane.

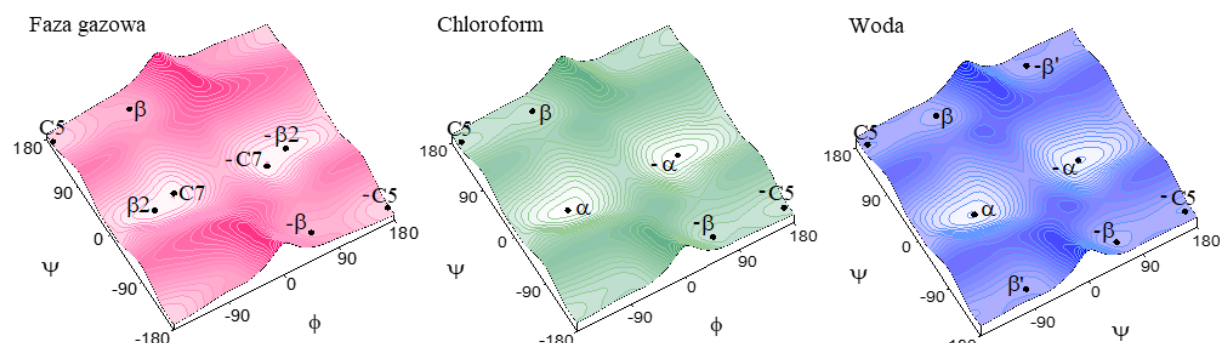
Tabela 8. Wybrane parametry konformacji Ac-(E)- Δ Ala(β -NHMe)-NHMe.

Konformacja	φ ($^\circ$)	ψ ($^\circ$)	ΔG (kcal·mol $^{-1}$)	p (%)
<i>Faza gazowa</i>				
$\beta 2$	-105.3	2.0	0.00	80.77
C7	-68.1	13.5	0.86	19.21
C5	-177.9	164.9	5.04	0.02
β	-60.7	152.1	7.60	0.00
<i>Chloroform</i>				
α	-91.2	-0.1	0.00	100.00
C5	-173.6	163.0	7.62	0.00
β	-60.4	155.0	7.95	0.00
<i>Woda</i>				
α	-87.3	-1.5	0.00	100.00
β	-59.9	155.6	7.13	0.00
β'	-92.7	-160.3	7.59	0.00
C5	-170.5	162.4	7.96	0.00

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 28. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 29. Mapy energii potencjalnej $E=f(\varphi, \psi)$ Ac-(E)- Δ Ala(β -NHMe)-NHMe otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

Dla izolowanego izomeru *E* β -aminodehydroalaniny z drugorzędowym amidem na C-końcu (tabela 8, rys. 28 i 29), podobnie jak dla izomeru *Z*, znaleziono cztery możliwe konformacje w następującym porządku energetycznym: $\beta 2$ ($\varphi, \psi = -105^\circ, 2^\circ$), *C7* ($\varphi, \psi = -68^\circ, 14^\circ$), *C5* ($\varphi, \psi = -178^\circ, 165^\circ$), β ($\varphi, \psi = -61^\circ, 152^\circ$). Dwie konformacje o najniższej energii, konformacja $\beta 2$ i *C7*, mają spośród wszystkich dostępnych konformacji N-końiec najbardziej prostopadły i C-końiec najbardziej równoległy do łańcucha bocznego. Koplanarność C-końcowej grupy amidowej z wiązaniem podwójnym $C^\alpha=C^\beta$ i grupą aminową przy węglu β umożliwia sprzężanie się elektronów π i powstanie krzyżowego układu zdelokalizowanego. Warto zauważyć, że obie te konformacje są stabilizowane poprzez wiązanie wodorowe $N-H\cdots O$, tworzone przez atom tlenu C-końcowej grupy amidowej oraz grupę N-H w łańcuchu bocznym. Konformacja *C7* jest dodatkowo utrzymana przez drugie wiązanie $N-H\cdots O$ pomiędzy grupami amidowymi, a także przez karbonyłowe oddziaływanie dipolowe I typu. Pozostałe konformacje, *C5* i β , mają znacznie wyższą energię, a szacowana populacja wskazuje, że są one praktycznie niedostępne. Konformacja *C5* jest stabilizowana przez wiązanie wodorowe $N-H\cdots O$, tworzone przez grupy amidowe oraz wiązanie $C^\beta-H\cdots O$ pomiędzy atomem tlenu N-końcowej grupy amidowej a wodorem winylowym. W części C-końcowej dochodzi do odpychania się grup N-H łańcucha bocznego i grupy amidowej, co wpływa na podwyższenie energii tej konformacji. Podobna sytuacja ma miejsce na C-końcu konformacji β , stabilizowanej przez oddziaływania dipolowe II typu, angażujące grupy karbonyłowe.

W słabo polarnym środowisku, mimikowanym przez chloroform, pojawia się konformacja α ($\varphi, \psi = -91^\circ, 0^\circ$), będąca konformacją pośrednią pomiędzy $\beta 2$ i *C7*, stabilizowaną przez to samo wiązanie wodorowe $N-H\cdots O$. Pozostałe możliwe konformacje to *C5* i β , jednak wartości ich energii są tak wysokie, że w rzeczywistości nie będą przyjmowane. W wodzie pojawia się dodatkowa konformacja, β' ($\varphi, \psi = -93^\circ, -160^\circ$), o przeciwnym znaku kąta ψ w stosunku do konformacji β . Jednak, podobnie jak w słabo polarnym środowisku, wartość energii konformacji α jest na tyle niższa od wartości energii pozostałych konformacji, że jest ona jedyną przyjmowaną konformacją.

Poza analizą prostych modeli z resztą β -aminodehydroalaniny zbadano właściwości konformacyjne związków dimetyloamidowych na C-końcu, *Ac-(Z)- Δ Ala(β -NHMe)-NMe₂* i *Ac-(E)- Δ Ala(β -NHMe)-NMe₂*. Takie modele lepiej naśladują fragment strukturalny naturalnych peptydów, kalyaeryn A-M i kalynorminy A, połączonych od strony C-końca z proliną.

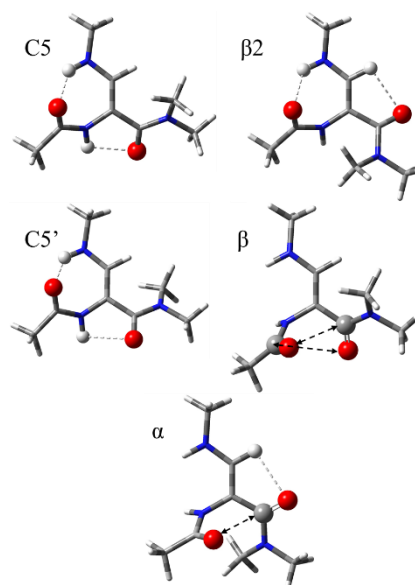
Dla izolowanej cząsteczki izomeru *Z* zidentyfikowano pięć minimów lokalnych: *C5* ($\varphi, \psi = -148^\circ, 162^\circ$), $\beta 2$ ($\varphi, \psi = -135^\circ, 162^\circ$), *C5'* ($\varphi, \psi = -144^\circ, -160^\circ$), β ($\varphi, \psi = -64^\circ, 150^\circ$) i α ($\varphi, \psi = -49^\circ, -27^\circ$) (tabela 9, rys. 30 i 31). Najniższą wartość energii ma konformacja *C5* o bardzo podobnej geometrii do tej, wykazywanej przez tą samą konformację izomeru *Z* β -aminodehydroalaniny z drugorzędowym amidem na C-końcu. Konformacja ta jest również stabilizowana przez te same wiązania wodorowe $N-H\cdots O$. Druga w porządku energetycznym jest konformacja $\beta 2$, utrzymywana przez wiązania wodorowe $N-H\cdots O$ i $C^\beta-H\cdots O$, analogicznie jak w amidzie drugorzędowym tego związku. Jednak kąt torsyjny ψ jest bardziej odchylony od płaszczyzny wiązania podwójnego $C^\alpha=C^\beta$,

co jest związane z zawadą przestrzenną, spowodowaną obecnością amidu trzeciorzędowego. Z tego samego powodu możliwa jest druga konformacja C5, o przeciwnej wartości kąta torsyjnego ψ . Jednak jej geometria jest gorsza, dlatego ma wyższą energię i raczej nie będzie przyjmowana. Ostatnie dwie konformacje, β i α , są stabilizowane przez oddziaływania dipolowe z udziałem grup karbonylowych. Konformacja α jest dodatkowo wspierana przez wiązanie wodorowe $C^\beta-H\cdots O$, utworzone pomiędzy C-końcową grupą karbonylową a winylowym atomem wodoru.

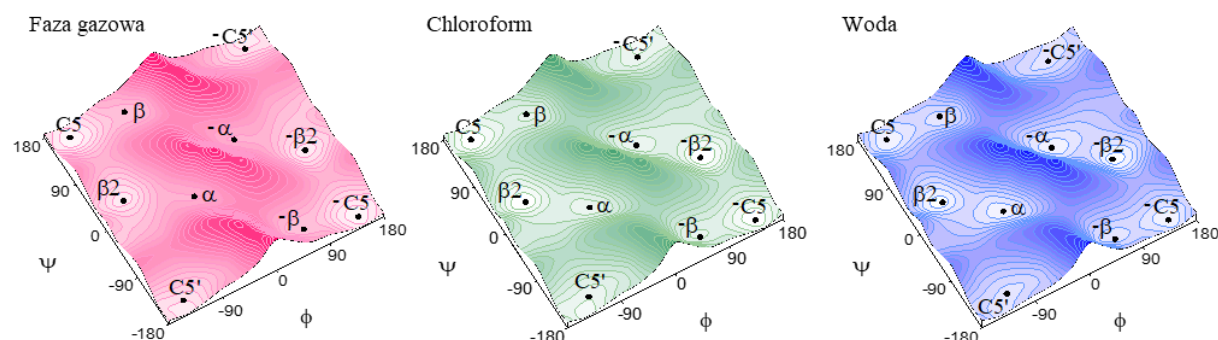
Tabela 9. Wybrane parametry konformacji Ac-(Z)- Δ Ala(β -NHMe)-NMe₂.

Konformacja	ϕ (°)	ψ (°)	ΔG (kcal·mol ⁻¹)	p (%)
<i>Faza gazowa</i>				
C5	-148.0	162.0	0.00	88.87
$\beta 2$	-135.2	29.2	1.35	9.23
C5'	-143.7	-159.9	2.43	1.50
β	-63.6	149.8	3.33	0.33
α	-48.7	-26.7	4.96	0.02
<i>Chloroform</i>				
$\beta 2$	-128.1	31.1	0.00	83.54
C5	-144.9	160.7	1.23	10.60
α	-55.1	-27.6	1.98	2.98
β	-57.8	151.4	2.19	2.10
C5'	-130.0	-154.9	2.79	0.78
<i>Woda</i>				
$\beta 2$	-125.0	30.5	0.00	38.84
α	-56.9	-28.5	0.02	37.84
β	-95.6	-157.7	0.95	7.86
C5	-144.0	160.6	1.05	6.62
C5'	-123.3	-153.4	1.70	2.23

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 30. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 31. Mapy energii potencjalnej $E=f(\phi, \psi)$ Ac-(Z)- Δ Ala(β -NHMe)-NMe₂ otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

W słabo polarnym środowisku możliwe jest pięć tych samych konformacji, z tym że dwie główne konformacje, C5 i $\beta 2$, zamieniają się miejscami w porządku energetycznym. Geometria konformacji C5' jeszcze bardziej odbiega od koplanarności, przez co rośnie jej energia. Natomiast na znaczeniu zyskuje konformacja α . W wodzie nadal najkorzystniejsza energetycznie jest konformacja $\beta 2$, chociaż konformacja α jest prawie tak samo obsadzona. Następna w kolejności jest konformacja β i na samym końcu dwie konformacje C5. Wraz ze wzrostem polarności środowiska dochodzi do odchylenia

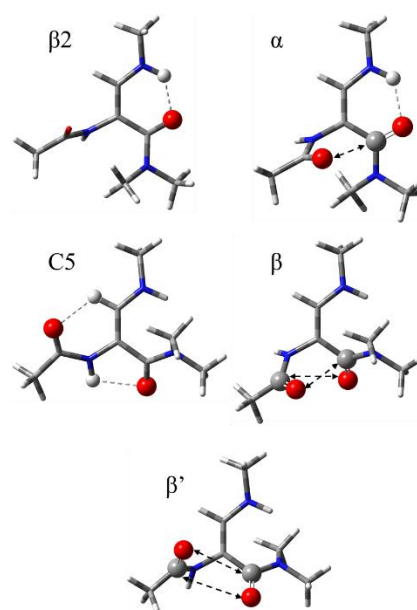
N-końcowych grup amidowych od planarności względem wiązania podwójnego $C^{\alpha}=C^{\beta}$, co umożliwia tworzenie oddziaływań międzycząsteczkowych.

Izolowana cząsteczka izomeru *E* może przyjmować cztery konformacje: $\beta 2$ ($\varphi, \psi = -114^{\circ}, 21^{\circ}$), α ($\varphi, \psi = -62^{\circ}, -18^{\circ}$), C5 ($\varphi, \psi = -171^{\circ}, 159^{\circ}$) i β ($\varphi, \psi = -61^{\circ}, 145^{\circ}$) (tabela 10, rys. 32 i 33). Najniższą energię ma konformacja $\beta 2$ i jest obsadzona przez prawie $\frac{3}{4}$ populacji cząsteczek. Pozostałe przyjmują konformację α . Obie te konformacje są stabilizowane przez wiązanie wodorowe $N-H\cdots O$, tworzone przez grupę $N-H$ łańcucha bocznego i atom tlenu C-końcowej grupy amidowej. Pozostałe konformacje, C5 i β , mają znacznie wyższą energię, dlatego praktycznie nie są obsadzone. Konformacja C5 jest stabilizowana przez dwa wewnątrzcząsteczkowe wiązania wodorowe $N-H\cdots O$ i $C^{\beta}-H\cdots O$, natomiast konformacja β przez karbonyłowe oddziaływania dipolowe II typu.

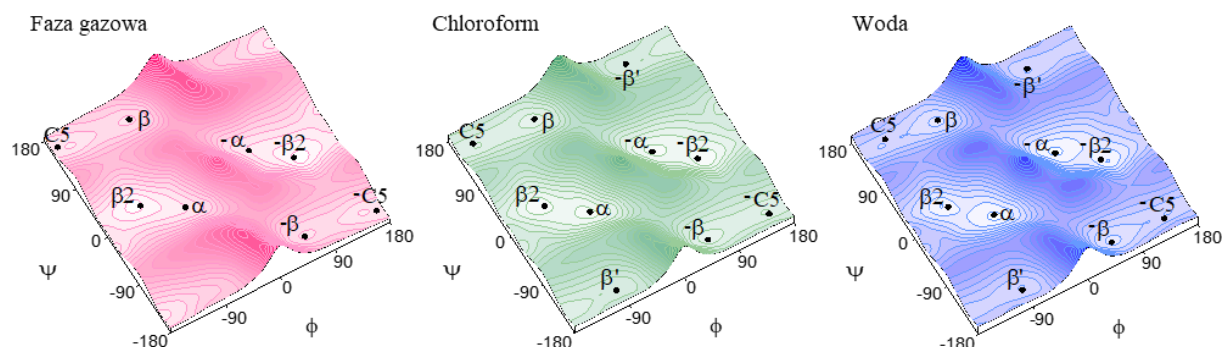
Tabela 10. Wybrane parametry konformacji Ac-(*E*)- Δ Ala(β -NHMe)-NMe₂.

Konformacja	$\varphi (^{\circ})$	$\psi (^{\circ})$	ΔG (kcal·mol ⁻¹)	<i>p</i> (%)
<i>Faza gazowa</i>				
$\beta 2$	-114.2	20.8	0.00	71.00
α	-62.3	-17.7	0.59	26.41
C5	-170.9	158.5	2.26	1.60
β	-60.8	145.3	2.54	0.99
<i>Chloroform</i>				
α	-63.0	-20.4	0.00	49.92
$\beta 2$	-113.5	20.8	0.03	47.52
β	-57.7	147.0	2.14	1.37
C5	-153.8	154.8	2.38	0.91
β'	-98.5	-157.3	3.09	0.28
<i>Woda</i>				
α	-61.8	-22.1	0.00	76.46
$\beta 2$	-113.4	20.6	0.78	20.78
β	-57.0	148.3	2.26	1.72
β'	-93.4	-155.0	2.96	0.53
C5	-139.2	155.3	2.99	0.51

Każda konformacja posiada swój lustrzany odpowiednik. Zoptymalizowano metodą M06-2X/6-311+G(d,p).



Rysunek 32. Konformacje optymalizowane metodą M06-2X/6-311+G(d,p) z wyróżnionymi najważniejszymi oddziaływaniami elektrostatycznymi ($\leftrightarrow$) [52] i wiązaniami wodorowymi ($\cdots$) [53], tworzonymi przez resztę dehydroaminokwasową.



Rysunek 33. Mapy energii potencjalnej $E=f(\varphi, \psi)$ Ac-(*E*)- Δ Ala(β -NHMe)-NMe₂ otrzymane metodą M06-2X/6-31+G(d,p) w fazie gazowej, chloroformie i wodzie. Kontury energii są naniesione co 1 kcal/mol.

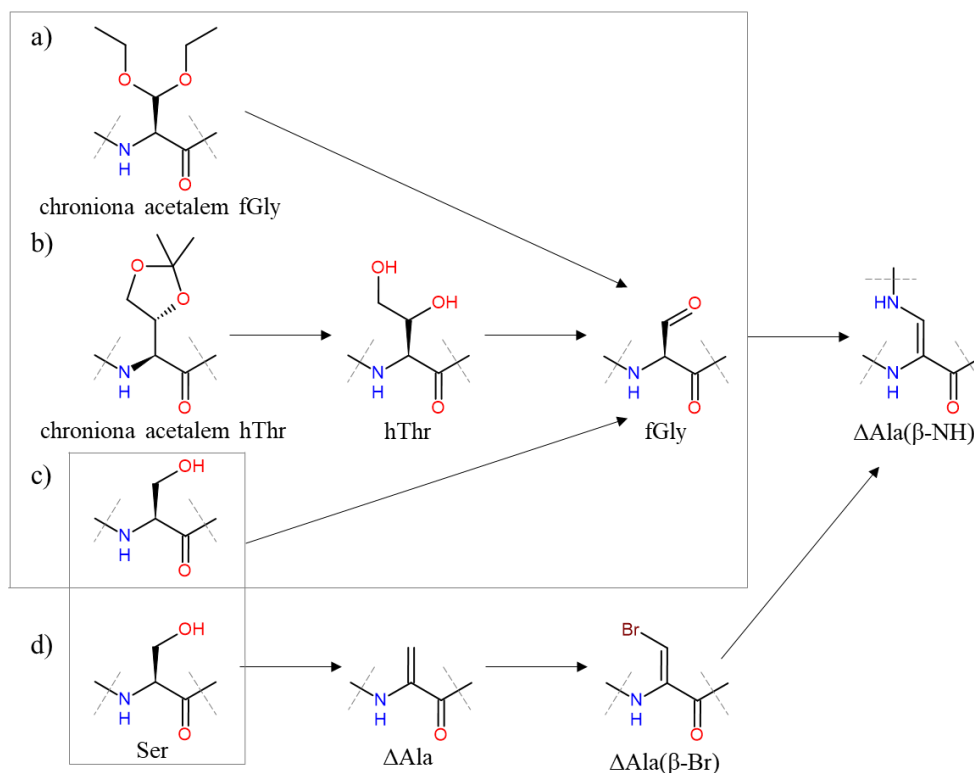
W słabo polarnym rozpuszczalniku na znaczeniu zyskuje konformacja α i to ona stanowi minimum globalne. Jednak w prawie równym stopniu obsadzona jest konformacja β_2 . Konformacje β i C5 mają energię wyższą o ponad 2 kcal, zatem podobnie jak w fazie gazowej są raczej niedostępne. Analogicznie jak dla amidu drugorzędowego wpływ środowiska umożliwia przyjmowanie konformacji β' ($\varphi, \psi = -99^\circ, -157^\circ$), jednak jest ona niekorzystna energetycznie. W polarnym rozpuszczalniku, tak jak w słabo polarnym, możliwych jest pięć konformacji w raczej niezmiennym porządku energetycznym. Jednakże wzrost polarności środowiska spowodował, że konformacja α stała się jeszcze bardziej korzystna.

Wyniki obliczeń pokazują, że niezależnie od izomeru geometrycznego Z/E i rzędowości amidu na C-końcu, reszta β -aminodehydroalaniny preferuje dwie konformacje, β_2 i α . Konformacja β_2 jest raczej przyjmowana w środowisku o niskiej polarności. Wraz ze wzrostem polarności otoczenia konformacja α zyskuje na znaczeniu. Obie te konformacje są możliwe również dla najbliższego analogicznego strukturalnie dehydroaminokwasu z niepolarnym łańcuchem bocznym, dehydrobutyryny [71]. Jednak albo nie są to preferowane konformacje, albo różnica ich energii w stosunku do pozostałych możliwych konformacji jest niewielka. Podobnie jest w przypadku wcześniej badanej β -chlorodehydroalaniny [D1] i O-metylodehydroseryny [D2].

Na stosunkowo małe wartości energii konformacji β_2 i α reszty β -aminodehydroalaniny wpływa ich stabilizacja za pomocą wewnątrzcząsteczkowego wiązania wodorowego N-H $\cdots$ O, którego donorem jest grupa N-H w łańcuchu bocznym. Jest to szczególnie widoczne w przypadku izomerów E. Konformacja β_2 izomerów Z również jest stabilizowana przez to wiązanie wodorowe, dlatego jest preferowana w środowiskach o słabej polarności. Natomiast w środowisku polarnym najchętniej jest przyjmowana konformacja α , której energia jest obniżana przez oddziaływania międzycząsteczkowe z polarnym otoczeniem. Powstaniu takich oddziaływań sprzyja ustawienie N-końcowej grupy amidowej prostopadle w stosunku do płaszczyzny wiązania podwójnego C $^\alpha$ =C $^\beta$, ponieważ daje łatwy dostęp do tej grupy cząsteczkom otoczenia. Należy również zauważyć, że w konformacjach α i β_2 łańcuch boczny i C-końcowa grupa amidowa mają tendencję do koplanarności, co można zaobserwować za pomocą kąta torsyjnego ψ , którego wartość jest bliska 0° z odchyleniem nieprzekraczającym 30° . W związku z tym w tej części cząsteczki tworzy się zdelokalizowany układ sprzężonych elektronów π , który dodatkowo stabilizuje konformacje.

Istnieje tylko kilka doniesień literaturowych, które prezentują dowody eksperymentalne na preferencje konformacyjne badanego elementu strukturalnego. Struktura rentgenowska kalynorminy A [30] przedstawia izomer Z w konformacji α ($\varphi, \psi = -49,39^\circ, -32,73^\circ$). Również blisko spokrewniony analog, dipeptyd z N-końcową grupą benzamidową i proliną na C-końcową oraz z grupą 4,6-dimetylo-2-pirymidynylo aminową, występuje w postaci izomeru Z w konformacji α ($\varphi, \psi = -62,68^\circ, -39,65^\circ$) i ($\varphi, \psi = -73,42^\circ, -44,60^\circ$) [78]. Potwierdza to uzyskane wyniki obliczeń.

Otrzymanie β -aminodehydroalaniny to kluczowy element w syntezie kalyaeryn A-M i kalynorminy A, a także dehydroaminokwasów z grupą ureidową, tuberaktynomycyn i kapreomycyn. W literaturze jest opisanych kilka sposobów syntezy tego elementu strukturalnego (rys. 34). Pierwsze podejście (rys. 34a) wykorzystuje jako substraty acetalowe pochodne (*R*)- α -formyloglicyny. Kwas 2-amino-3,3-dietoksypropionowy (dietyloacetal (*R*)- α -formyloglicyny) jest wprowadzany do łańcucha peptydowego [79, 80]. Następnie w kwaśnych warunkach zostaje zhydrolizowany do α -formyloglicyny (fGly), która w kolejnym etapie reaguje z grupą aminową, tworząc pożądany produkt [81].



Rysunek 34. Schemat przedstawiający sposoby syntezy β -aminodehydroalaniny a-c) opisane w literaturze oraz d) zaproponowany w pracy [D3].

Zaobserwowano jednak, że kwaśne warunki stosowane do odczepienia peptydu z żywicy, prowadzą do rozszczepienia wiązania peptydowego po stronie N-końca α -formyloglicyny [81]. Dzieje się tak, ponieważ protonowana grupa aldehydowa fGly zwiększa swój elektrofilowy charakter. Jest przez to podatna na nukleofilowy atak atomu tlenu N-końcowej grupy amidowej. Dochodzi do wewnątrzcząsteczkowej cyklizacji z utworzeniem pierścienia 5-hydroksyoksazolinowego, który w kolejnym etapie ulega hydrolizie kwasowej zgodnie z mechanizmem substytucji acylowej w pozycji 2 pierścienia oksazolinowego.

Drugi sposób (rys. 34b) wykorzystuje acetalową pochodną 4-hydroksy-L-treoniny (hThr) jako prekursor fGly [82]. Taki substrat podczas odczepienia peptydu z żywicy w kwaśnych warunkach ulega hydrolizie, dając hThr. Następnie hThr w reakcji z nadjodanem sodu zostaje przekształcona w fGly, w wyniku rozczepienia oksydacyjnego. W ostatnim etapie fGly reaguje z aminą w kwaśnym środowisku, tworząc β -aminodehydroalaninę.

Trzecie podejście (rys. 34c) polega na utlenieniu standardowego aminokwasu, seryny, do fGly za pomocą perjodynanu Dessa-Martina [83]. Następnie, tak jak w poprzednich metodach, fGly ulega reakcji z grupą aminową, dając pożądaný produkt. Podsumowując, wszystkie opisane w literaturze procedury wykorzystują formyloglicynę jako prekursor β -aminodehydroalaniny. Protonowana w kwaśnym środowisku grupa karbonylowa fGly staje się bardziej elektrofilowa, zatem i bardziej podatna na nukleofilowy atak aminy, w wyniku czego powstaje imina z jednoczesną eliminacją cząsteczki wody. Następnie dochodzi do tautomerizacji iminowo-enaminowej z dominującą formą enaminową, ze względu na stabilizujące krzyżowe sprzężenie π -elektronowe, zachodzące pomiędzy wiązaniem podwójnym $C^\alpha=C^\beta$ a grupami amidowymi w łańcuchu głównym.

Dwie pierwsze metody wymagają wprowadzenia do łańcucha peptydowego odpowiednio zabezpieczonego prekursora α -formyloglicyny. Trzecia metoda zakłada użycie standardowego aminokwasu, seryny, co jest prostsze i korzystniejsze. Jednak konwersja do fGly zachodzi w tym przypadku z niską wydajnością (0.7 – 47% [83]). W syntezie opisanej w pracy [D3] (rys. 34d) zastosowano odmienną metodę. Substratem również jest seryna, która zostaje poddana reakcji dehydratacji, następnie bromowaniu w pozycji β i na końcu substytucji atomu bromu grupą aminową. Dehydratacja zachodzi z dużą wydajnością i była już wcześniej stosowana w syntezie peptydów [84]. Polega na eliminacji cząsteczki wody przy użyciu karbodiimidu EDC (1-etylo-3-(3-dimetyloaminopropyl)karbodiimid). Reakcja β -bromowania zachodzi w dwóch etapach: w pierwszym następuje addycja cząsteczki bromu do wiązania podwójnego $C^\alpha=C^\beta$, w drugim dochodzi do eliminacji cząsteczki bromowodoru (dehydrohalogenacja) z wykorzystaniem aminy trzeciorzędowej [85, 86]. Istnieje również możliwość przeprowadzenia reakcji bromowania przy pomocy NBS (*N*-bromosukcynoimid) zamiast bromu cząsteczkowego [87, 88]. W ostatnim etapie syntezy amina ulega addycji Michaela do β -bromodehydroalaniny. Następnie dochodzi do odtworzenia wiązania podwójnego $C^\alpha=C^\beta$ i eliminacji bromowodoru (dehydrohalogenacja).

W ramach pracy [D3] zsyntezowany prosty model diamidowy, Ac-(Z)- Δ Ala(β -NHMe)-NHMe, poddany wcześniej badaniom z wykorzystaniem metod obliczeniowych. Jako substrat do syntezy użyto Ac-Ser-NHMe. Aby otrzymać pożądaný produkt, w ostatnim etapie zastosowano metyloaminę. W tej syntezie stosowane są warunki słabo zasadowe, a nie kwaśne. To nowe podejście stanowi alternatywę dla opisanych dotąd w literaturze [81-83].

Pokazano również, że ta metoda może zostać zastosowana do większych modeli i zsyntezowano dipeptyd, Boc-Gly-(Z)- Δ Ala(β -NHMe)-OMe. Reakcja aminowania nie zachodzi z dużą wydajnością, co jest spowodowane dużą lotnością metyloaminy. Świadczy o tym nieco wyższa wydajność syntezy trzeciego modelu, Boc-Gly-(Z)- Δ Ala(β -Leu-OMe)-OMe, w której rolę aminy pełni ester metylowy leucyny. Leucyna jest połączona z atomem węgla β -aminodehydroalaniny w kaliaerynach o silnym działaniu przeciwnowotworowym [74, 75]. Niestety, wszystkie produkty otrzymano w prawdzie w postaci krystalicznej, jednak w formie cienkich igieł na ściankach naczynia (przypominających szron), które pomimo prób analizy rentgenowskiej monokryształów nie dały wyników. Przypisania

izomerii geometrycznej zsyntezowanych związków dokonano na podstawie widm różnicujących NOE NMR (rys. 35).



Rysunek 35. Widmo różnicujące NOE NMR Boc-Gly-(Z)-ΔAla-(β-Leu-OMe)-OMe w DMSO- d_6 ze wzbudzonym protonem grupy amidowej leucyny.

Podsumowując, w pracy [D3] określono preferencje konformacyjne β-aminodehydroalaniny, dehydroaminokwasu istotnego dla właściwości przeciwegrzulicznych naturalnych peptydów. Wyniki przedstawionych badań wskazują, że grupa N-H przy atomie węgla β wpływa na właściwości konformacyjne β-aminodehydroalaniny, ponieważ tworzy wiązania wodorowe N-H $\cdots$ O z grupami amidowymi w łańcuchu głównym. Takie wiązania stabilizują głównie dwie konformacje β2 ($\varphi, \psi \sim -122^\circ, 20^\circ$) i α ($\varphi, \psi \sim -69^\circ, -16^\circ$). Niezależnie od izomeru geometrycznego Z/E i rzędowości C-końcowego amidu, istnieje tendencja do przyjmowania konformacji β2 w środowisku o niższej polarności, natomiast w środowisku polarnym preferowana jest bardziej otwarta na oddziaływania międzycząsteczkowe konformację α. Otrzymane wyniki dobrze korelują z doniesieniami literaturowymi, zgodnie z którymi w kryształach kalynorminy A reszta β-aminodehydroalaniny występuje w postaci izomeru Z i konformacji α.

Dodatkowym osiągnięciem pracy [D3] jest nowy sposób syntezy β-aminodehydroalaniny. Zaproponowana metoda pozwala na otrzymanie izomeru Z pożądanego dehydroaminokwasu ze standardowego aminokwasu, seryny, w trzech etapach: dehydratacji, β-bromowania i aminowania. Jest to podejście alternatywne do opisanych do tej pory w literaturze, obejmujące syntezę w środowisku słabo zasadowym, a także niewymagające otrzymania α-formyloglicyny, wrażliwej na kwaśne warunki.

PODSUMOWANIE

W autoreferacie przedstawiono wyniki badań opublikowane w trzech artykułach naukowych [D1-D3]. Prace dotyczą właściwości konformacyjnych trzech α,β -dehydroaminokwasów z polarnymi grupami funkcyjnymi w łańcuchu bocznym: β -chlorodehydroalaniny, O-metylodehydroseryny i β -aminodehydroalaniny. Badania prowadzono z wykorzystaniem metod obliczeniowych oraz analizy spektralnej (NMR, IR) i rentgenowskiej analizy strukturalnej zsyntezowanych związków modelowych.

W ramach niniejszej Pracy Doktorskiej uzyskano następujące osiągnięcia:

1. Określono preferencje konformacyjne β -chlorodehydroalaniny $\Delta\text{Ala}(\beta\text{-Cl})$, będącej elementem strukturalnym wiktoryn – toksyn, produkowanych przez grzybiczy patogen owsa. Preferencje te różnią się w zależności od izomerii geometrycznej łańcucha bocznego. Izomer Z ma tendencję do przyjmowania konformacji α ($\varphi, \psi \sim -61^\circ, -24^\circ$), β ($\varphi, \psi \sim -43^\circ, 138^\circ$) i $\beta 2$ ($\varphi, \psi \sim -116^\circ, 9^\circ$), podczas gdy izomer E preferuje głównie konformację C5 ($\varphi, \psi \sim -178^\circ, 167^\circ$), ale w otoczeniu wody przyjmuje również konformacje β ($\varphi, \psi \sim -45^\circ, 133^\circ$) i α ($\varphi, \psi \sim -54^\circ, -37^\circ$). W stabilizowaniu najkorzystniejszych energetycznie konformacji uczestniczy wiązanie wodorowe $\text{N-H} \cdots \text{Cl}$, tworzone przez odpowiednią grupę amidową łańcucha głównego i atom chloru w łańcuchu bocznym. Oznacza to, że takie oddziaływanie obniża energię stabilizowanej konformacji na tyle, że głównie tylko takie konformacje są przyjmowane.
2. Opracowano metodę syntezy obu izomerów geometrycznych reszty β -chlorodehydroalaniny. Izomer Z otrzymano poprzez reakcję β -chlorowania dehydroalaniny w obecności trietyloaminy, natomiast izomer E w wyniku fotoizomeryzacji (Z)- $\Delta\text{Ala}(\beta\text{-Cl})$. Reakcję fotoizomeryzacji zastosowano do β -halogenodehydroaminokwasów po raz pierwszy. Warunki tej reakcji wymagały dostosowania do właściwości stosowanych substratów.
3. Opisano doniesienia literaturowe na temat przypisania izomerii geometrycznej reszty β -chlorodehydroalaniny w wiktorynach i skonfrontowano je z wynikami własnych badań, skłaniając się ku korzystniejszemu energetycznie izomerowi Z.
4. Określono właściwości konformacyjne O-metylodehydroseryny $\Delta\text{Ser}(\text{O-Me})$, która stanowi element strukturalny cyrmenin – peptydów bakteryjnych o działaniu przeciugrzybiczym. O-metylodehydroseryna niezależnie od izomerii geometrycznej może przyjmować cztery konformacje: C5, α , $\beta 2$ i β . Utrzymywane głównie przez oddziaływania wewnątrzcząsteczkowe konformacje C5 (izomer Z: $\varphi, \psi \sim -124^\circ, 179^\circ$; izomer E: $\varphi, \psi \sim -180^\circ, 180^\circ$) i $\beta 2$ (izomer Z: $\varphi, \psi \sim -121^\circ, -2^\circ$; izomer E: $\varphi, \psi \sim -180^\circ, 0^\circ$) są preferowane w słabo polarnym środowisku. Wzrost polarności otoczenia wywołuje tendencję do przyjmowania otwartych na oddziaływania z rozpuszczalnikiem konformacji α ($\varphi, \psi \sim -61^\circ, -14^\circ$) i β ($\varphi, \psi \sim -63^\circ, 169^\circ$). W tworzenie takich oddziaływań zaangażowana jest głównie N-końcowa grupa amidowa. C-końcowa grupa estrowa dąży natomiast do koplanarności z łańcuchem bocznym, umożliwiającą wystąpienie sprzężenia

π -elektronowego, obejmującego grupę estrową, wiązanie podwójne $C^{\alpha}=C^{\beta}$ i atom tlenu grupy metoksyłowej przy atomie węgla β , która nie tworzy silnych oddziaływań międzycząsteczkowych. To właśnie ten fragment strukturalny odpowiada za aktywność przeciwgrzybiczą cyrmenin.

5. Scharakteryzowano właściwości konformacyjne β -aminodehydroalaniny $\Delta Ala(\beta-NH)$, elementu strukturalnego peptydów o działaniu przeciwgruźliczym – kalyaeryn i kalynorminy A. Wykazano, że niezależnie od izomerii geometrycznej łańcucha bocznego oraz rzędowości amidu na C-końcu preferowane są dwie konformacje: β_2 ($\varphi, \psi \sim -122^\circ, 20^\circ$) i α ($\varphi, \psi \sim -69^\circ, -16^\circ$). Istnieje tendencja do przyjmowania konformacji β_2 w środowisku słabo polarnym. Wraz ze wzrostem polarności otoczenia na znaczeniu zyskuje otwarta na oddziaływania międzycząsteczkowe konformacja α . W stabilizowaniu tych konformacji uczestniczy wiązanie wodorowe $N-H \cdots O$, tworzone pomiędzy grupą N-H w łańcuchu bocznym a atomem tlenu odpowiedniej grupy amidowej łańcucha głównego. Można również zaobserwować koplanarność struktury po stronie C-końca dehydroaminokwasu, świadczące o zdelokalizowanym układzie sprzężonych elektronów π , obejmującym C-końcową grupę amidową, wiązanie podwójne $C^{\alpha}=C^{\beta}$ oraz atom azotu w łańcuchu bocznym. Takie sprzężenie dodatkowo stabilizuje przyjmowane konformacje.
6. Zaproponowano nową ścieżkę syntezy (Z)- β -aminodehydroalaniny z zastosowaniem seryny jako substratu, którą poddaje się kolejno reakcji: dehydratacji, β -bromowania i aminowania. Takie podejście stanowi alternatywę dla rozwiązań opisanych do tej pory w literaturze, ponieważ nie wymaga otrzymywania α -formyloglicyny jako produktu pośredniego oraz zapewnia słabo zasadowe środowisko prowadzenia reakcji.

Badania stanowiące podstawę niniejszej Pracy Doktorskiej pozwoliły na znaczne wzbogacenie stanu wiedzy na temat preferencji konformacyjnych α, β -dehydroaminokwasów, których atom węgla β jest podstawiony takimi grupami jak atom chloru, grupa metoksyłowa, czy grupa aminowa. Polarne grupy funkcyjne w łańcuchu bocznym mogą tworzyć oddziaływania wewnątrzcząsteczkowe oraz uczestniczyć w sprzężeniu π -elektronowym i stabilizować przez to pewne konformacje. Dlatego też właściwości konformacyjne takich dehydroaminokwasów są odmienne od tych z niepolarnym łańcuchem bocznym. Ze względu na interesujące właściwości biologiczne badanych dehydroaminokwasów, otrzymane wyniki mogą być przydatne w projektowaniu nowych leków lub agrochemikaliów.

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3. STRESZCZENIE PRACY W JĘZYKU POLSKIM

α,β -Dehydroaminokwasy to niekodowane aminokwasy pojawiające się w naturalnych peptydach o interesujących właściwościach biologicznych. Jak powszechnie wiadomo, o aktywności związków decydują ich właściwości konformacyjne. Do tej pory zbadano i opisano preferencje konformacyjne dehydroaminokwasów z niepolarnym łańcuchem bocznym, w szczególności dehydrofenyloalaniny Δ Phe, dehydroalaniny Δ Ala, dehydrobutyryny Δ Abu, dehydrowaliny Δ Val i dehydroleucyny Δ Leu. W przyrodzie istnieją również dehydroaminokwasy z polarnymi grupami funkcyjnymi, dlatego w ramach niniejszej Pracy Doktorskiej zbadano właściwości konformacyjne: β -chlorodehydroalaniny Δ Ala(β -Cl), O-metylodehydroseryny Δ Ser(O-Me) i β -aminodehydroalaniny Δ Ala(β -NH).

Wstępny obraz preferencji konformacyjnych uzyskano na podstawie wyników badań z wykorzystaniem metod obliczeniowych. Dla obu izomerów geometrycznych *Z/E* każdego badanego dehydroaminokwasu sporządzono mapy energii potencjalnej $E = f(\phi, \psi)$ w trzech środowiskach (fazie gazowej, chloroformie i wodzie). Następnie znaleziono możliwe konformacje, identyfikując minima lokalne. W kolejnym etapie zsyntezowano związki modelowe, które poddano rentgenowskiej analizie strukturalnej i badaniom spektroskopowym (NMR, IR), pozwalającym na przypisanie izomerii geometrycznej *Z/E* łańcucha bocznego oraz zidentyfikowanie konformacji przyjmowanych w roztworze i strukturze krystalicznej.

Ustalono, że polarne grupy funkcyjne w łańcuchu bocznym, takie jak atom chloru, grupa metoksylova czy grupa aminowa, wpływają na właściwości konformacyjne dehydroaminokwasów poprzez tworzenie wiązań wodorowych oraz uczestniczenie w sprzężeniu π -elektronowym i stabilizowanie przez to pewnych konformacji. β -Chlorodehydroalanina, będąca elementem strukturalnym wiktoryn, posiada różne preferencje konformacyjne w zależności od izomerii łańcucha bocznego. Izomer *Z* ma tendencję do przyjmowania konformacji α , β i β_2 , natomiast izomer *E* występuje głównie w konformacji C5. W środowisku wody może również przyjmować konformacje β i α . Obecna w cyrmeninach O-metylodehydroseryna preferuje konformacje C5 i β_2 w słabo polarnym środowisku, natomiast w otoczeniu polarnego rozpuszczalnika chętnie przyjmuje konformacje α i β . Element strukturalny kalyaeryn i kalynorminy A, β -aminodehydroalanina, wykazuje tendencję do przyjmowania konformacji β_2 w słabo polarnym oraz konformacji α w polarnym środowisku.

W Pracy zaproponowano również nowe metody syntezy β -chlorodehydroalaniny i β -aminodehydroalaniny. Pierwszy z nich otrzymano poprzez chlorowanie dehydroalaniny, drugi w wyniku aminowania β -bromodehydroalaniny. Uzyskane wyniki badań pozwoliły na lepsze zrozumienie zależności pomiędzy strukturą analizowanych dehydroaminokwasów a ich właściwościami. Otrzymane informacje mogą zostać wykorzystane w medycynie do projektowania nowych leków lub dla celów rolniczych w pracach nad środkami ochrony roślin.

4. STRESZCZENIE PRACY W JĘZYKU ANGIELSKIM

α,β -Dehydroamino acids are noncoded amino acids found in natural peptides with interesting biological properties. It is well known that the activity of compounds is determined by their conformational properties. To date, the conformational preferences of dehydroamino acids with nonpolar side chains have been studied and described, in particular dehydrophenylalanine Δ Phe, dehydroalanine Δ Ala, dehydrobutyrine Δ Abu, dehydrovaline Δ Val, and dehydroleucine Δ Leu. Dehydroamino acids with polar functional groups also exist in nature, so this Doctoral Thesis examined the conformational properties of β -chlorodehydroalanine Δ Ala(β -Cl), O-methyldehydroserine Δ Ser(O-Me), and β -aminodehydroalanine Δ Ala(β -NH).

A preliminary understanding of conformational preferences was obtained based on the results of computational studies. Potential energy maps $E = f(\varphi, \psi)$ were constructed for both geometric *Z/E* isomers of each studied dehydroamino acid in three environments (gas phase, chloroform, and water). Possible conformations were then found by identifying local minima. Next, model compounds were synthesized and analyzed by the X-ray structural analysis and spectroscopic studies (NMR, IR), allowing for the assignment of *Z/E* geometric isomerism to the side chain and the identification of the conformations adopted in solution and crystal structure.

It was determined that polar functional groups in the side chain, such as chlorine, methoxy, or amino, influence the conformational properties of dehydroamino acids by forming hydrogen bonds and participating in π -electron coupling, thereby stabilizing certain conformations. β -Chlorodehydroalanine, a structural element of victorins, exhibits different conformational preferences depending on the isomerism of its side chain. The *Z* isomer tends to adopt the α , β , and β_2 conformations, while the *E* isomer occurs predominantly in the C5 conformation. In aqueous environments, it can also adopt the β and α conformations. O-Methyldehydroserine, present in cyrmenins, prefers the C5 and β_2 conformations in weakly polar environments, while in polar solvents, it readily adopts the α and β conformations. The structural element of callyaerins and callynormine A, β -aminodehydroalanine, tends to adopt the β_2 conformation in weakly polar and the α conformation in polar environments.

This paper also proposes new methods for the synthesis of β -chlorodehydroalanine and β -aminodehydroalanine. The first one was obtained by chlorination of dehydroalanine, and the second one by amination of β -bromodehydroalanine. The gained research results allowed for a better understanding of the relationship between the structure of the analyzed dehydroamino acids and their properties. The obtained information can be used in medicine to design new drugs or for agricultural purposes in the development of plant protection products.

5. SZCZEGÓŁOWY WYKAZ DOROBKU NAUKOWEGO

Całość dorobku naukowego stanowią **4 publikacje naukowe**, opublikowane w czasopismach z listy filadelfijskiej, w tym **3 publikacje** wchodzące w skład rozprawy doktorskiej. Sumaryczny Impact Factor wynosi 17.5, a liczba punktów MNiSW 480. Ponadto, **6 wystąpień konferencyjnych**, zaprezentowanych podczas konferencji o zasięgu krajowym. Do dodatkowych osiągnięć należy odbycie **stażu naukowego** w jednostce badawczej poza jednostką kształcenia, pomoc w realizacji **2 prac dyplomowych** oraz **działalność popularyzująca naukę**.

a) Publikacje naukowe wchodzące w skład rozprawy doktorskiej:

- D1 Karolina Banaś**, Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen *Cochliobolus victoriae*. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine”, *J. Agric. Food Chem.* **2023**, 71(30), 11642–11653, DOI:10.1021/acs.jafc.3c01387. **IF = 5.7, pkt MNiSW = 140.**
- D2 Karolina Banaś**, Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Antifungal Cyrrmenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine”, *Int. J. Mol. Sci.* **2024**, 26(1), 340–354, DOI:10.3390/ijms26010340. **IF = 4.9, pkt MNiSW = 140.**
- D3 Karolina Banaś**, Paweł Lenartowicz, Dawid Siodłak „Insight into the structure of antitubercular Callyaerins: conformational studies and synthesis of a unique dehydroamino acid, β -aminodehydroalanine”, *Amino Acids* **2025**, 57 (1), 41, DOI:10.1007/s00726-025-03473-2. **IF = 2.4, pkt MNiSW = 100.**

b) Publikacje naukowe niewchodzące w skład rozprawy doktorskiej:

- P1 Wioletta Ochędzan-Siodłak**, Dawid Siodłak, **Karolina Banaś**, Katarzyna Halikowska, Sławomir Wierzba, Karel Doležal „Naturally Occurring Oxazole Structural Units as Ligands of Vanadium Catalysts for Ethylene-Norbornene (Co)polymerization”, *Catalysts*, **2021**, 11(8), 923–930, DOI: 10.3390/catal11080923. **IF = 4.5, pkt MNiSW = 100.**

c) Wystąpienia konferencyjne:

- K1 Karolina Banaś**, Paweł Lenartowicz, Monika Staś, Dawid Siodłak „ β -Chlorodehydroalanine. Unique dehydroamino acid from natural fungal toxin victorins”, 64. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 11–16 września 2022, Lublin, poster.
- K2 Karolina Banaś**, Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak „Conformational studies of β -chlorodehydroalanine”, Zjazd Zimowy Sekcji Młodych Polskiego Towarzystwa Chemicznego, 10 grudnia 2022, Opole, poster.

K3 Karolina Banaś, Paweł Lenartowicz, Dawid Siodłak „Izomeria geometryczna β -chlorodehydroalaniny - specyficznego elementu strukturalnego wiktoryn”, XV Interdyscyplinarna Konferencja Naukowa TYGIEL 2023, 23–26 marca 2023, Lublin, poster.

K4 Karolina Banaś, Dawid Siodłak „Conformational properties of O-methyldehydroserine – cyrmenins' pharmacophore. Theoretical studies”, 65. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 18–22 września 2023, Toruń, poster.

K5 Karolina Banaś, Dawid Siodłak „ α,β -Dehydroaminokwasy z polarnym łańcuchem bocznym. Struktura molekularna, znaczenie biologiczne, zastosowanie farmaceutyczne”, XX Ogólnopolskie Seminarium Doktorantów i Studentów „Na pograniczu Chemii, Biologii i Farmacji”, 26–29 maja 2024, Jarnołtówek, komunikat ustny.

K6 Karolina Banaś, Dawid Siodłak „Conformational properties of O-methyldehydroserine – cyrmenins' pharmacophore”, Zjazd Zimowy Sekcji Młodych Polskiego Towarzystwa Chemicznego, 14 grudnia 2024, Lublin, poster.

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f) Działalność popularyzująca naukę:

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Opole, 23.09.2025

Oświadczenie wskazujące na merytoryczny wkład w powstanie publikacji naukowej

Oświadczam, że w pracy:

- Karolina Banaś, Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen
Cochliobolus victoriae. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine”,
J. Agric. Food Chem. **2023**, 71(30), 11642 – 11653, DOI:10.1021/acs.jafc.3c01387

mój udział polegał na zaplanowaniu syntezy oraz współudziale w syntezie związków modelowych, pomocy przy interpretowaniu widm NMR, przygotowaniu opisu syntezy w tekście głównym publikacji, a także współudziale w redagowaniu manuskryptu.

- Karolina Banaś, Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Antifungal Cyrenins: Conformational Studies of Unique
Dehydroamino Acid, O-Methyldehydroserine”, *Int. J. Mol. Sci.* **2024**, 26(1), 340 – 354,
DOI:10.3390/ijms26010340

mój wkład obejmuje wsparcie podczas syntez związków modelowych oraz współudział w redagowaniu manuskryptu.

- Karolina Banaś, Paweł Lenartowicz, Dawid Siodłak “Insight into the structure of antitubercular
Callyaerins: conformational studies and synthesis of a unique dehydroamino acid,
 β -aminodehydroalanine”, *Amino Acids* **2025**, 57 (1), 41, DOI:10.1007/s00726-025-03473-2

mój wkład obejmuje zaplanowanie reakcji aminowania β -bromodehydroalaniny, syntezę di- i tripeptydu oraz współudział w redagowaniu manuskryptu.



.....
Podpis

Opole, 19 marca 2025

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Oświadczenie wskazujące na merytoryczny wkład w powstanie publikacji naukowej

Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen
Cochliobolus victoriae. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine
(tytuł publikacji naukowej)

Karolina Banaś, Paweł Lenartowicz*, Monika Staś, Błażej Dziuk, Dawid Siodłak*, *Journal of
Agricultural and Food Chemistry*, 2023 71 (30), 11642-11653
DOI: 10.1021/acs.jafc.3c01387
(dane bibliograficzne)

Oświadczam, że mój wkład w powstanie publikacji polegał na pomocy w:

- przygotowaniu plików do obliczeń kwantowo-chemicznych, nadzorowaniu ich przebiegu oraz pomocy w analizie wyników.
- Przeprowadzeniu pomiarów spektroskopii w podczerwieni, nadzorowaniu ich przebiegu oraz pomocy w analizie wyników.

Oświadczam, w niniejszym artykule naukowym szacuję swój udział procentowy w jego powstanie na 10%.

dr Monika Staś-Bobis
(podpis)

Opole, 19 marca 2025

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Oświadczenie wskazujące na merytoryczny wkład w powstanie publikacji naukowej

Insight into the Structure of Antifungal Cyrmenins: Conformational Studies of Unique
Dehydroamino Acid, O-Methyldehydroserine

(tytuł publikacji naukowej)

Karolina Banaś, Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak*,
International Journal of Molecular Science, 2025, 26(1), 340;

<https://doi.org/10.3390/ijms26010340>

(dane bibliograficzne)

Oświadczam, że mój wkład w powstanie publikacji polegał na pomocy w:

- Przeprowadzeniu pomiarów spektroskopii w podczerwieni oraz pomocy w analizie wyników.

Oświadczam, w niniejszym artykule naukowym szacuję swój udział procentowy w jego powstanie na 5%.

olr... Monika Staś-Bobis
(podpis)

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Opole, 23.09.2025

Oświadczenie wskazujące na merytoryczny wkład w powstanie publikacji naukowej

Oświadczam, że w pracach:

- Karolina Banaś, Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen *Cochliobolus victoriae*. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine”, *J. Agric. Food Chem.* **2023**, 71(30), 11642 – 11653, DOI:10.1021/acs.jafc.3c01387
- Karolina Banaś, Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Antifungal Cyrenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine”, *Int. J. Mol. Sci.* **2024**, 26(1), 340 – 354, DOI:10.3390/ijms26010340

mój udział polegał na wykonaniu części krystalograficznej, tj.: analizie rentgenostrukturalnej otrzymanych monokryształów, zdeponowaniu struktury w krystalograficznej bazie danych CSD, opisie wykonanej części eksperymentalnej, przygotowaniu tabel i rysunków ze strukturami.

Błażej Dziuk

.....
Podpis

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Opole, 23.09.2025

Oświadczenie wskazujące na merytoryczny wkład w powstanie publikacji naukowej

Oświadczam, że w pracach:

- Karolina Banaś, Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen *Cochliobolus victoriae*. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine”, *J. Agric. Food Chem.* **2023**, 71(30), 11642–11653, DOI:10.1021/acs.jafc.3c01387
- Karolina Banaś, Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak
“Insight into the Structure of Antifungal Cyrrmenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine”, *Int. J. Mol. Sci.* **2024**, 26(1), 340–354, DOI:10.3390/ijms26010340
- Karolina Banaś, Paweł Lenartowicz, Dawid Siodłak “Insight into the structure of antitubercular Callyaerins: conformational studies and synthesis of a unique dehydroamino acid, β -aminodehydroalanine”, *Amino Acids* **2025**, 57 (1), 41, DOI:10.1007/s00726-025-03473-2

mój wkład obejmuje koncepcję prac, nadzorowanie przebiegu badań, edycję wyników przy współudziale pozostałych Autorów, oraz procedowanie przygotowanych manuskryptów przez etap recenzji.

Dawid Siodłak

.....
Podpis

7. WYKAZ I KOPIE PUBLIKACJI STANOWIĄCYCH OSIĄGNIĘCIE NAUKOWE

	Autorzy, tytuł, dane bibliograficzne	IF*	IF _{5 letni}	pkt MNIŚW*
D1	Karolina Banaś , Paweł Lenartowicz, Monika Staś, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen <i>Cochliobolus victoriae</i> . Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine” <i>J. Agric. Food Chem.</i> 2023 , 71(30), 11642–11653, DOI:10.1021/acs.jafc.3c01387	5.7	6.4	140
D2	Karolina Banaś , Paweł Lenartowicz, Monika Staś-Bobis, Błażej Dziuk, Dawid Siodłak „Insight into the Structure of Antifungal Cyrrmenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine” <i>Int. J. Mol. Sci.</i> 2025 , 26(1), 340–354, DOI:10.3390/ijms26010340	4.9	5.7	140
D3	Karolina Banaś , Paweł Lenartowicz, Dawid Siodłak „Insight into the structure of antitubercular Callyaerins: conformational studies and synthesis of a unique dehydroamino acid, β -aminodehydroalanine” <i>Amino Acids</i> 2025 , 57(1), 41, DOI:10.1007/s00726-025-03473-2	2.4	3.1	100

* w roku opublikowania

D1

Insight into the Structure of Victorin, the Host-Selective Toxin from the Oat Pathogen *Cochliobolus victoriae*. Studies of the Unique Dehydroamino Acid β -Chlorodehydroalanine

Karolina Banaś, Paweł Lenartowicz,* Monika Staś, Błażej Dziuk, and Dawid Siodlak*

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ABSTRACT: Victorins, a family of peptide toxins, produced by the fungal pathogen *Cochliobolus victoriae* and responsible for disease of some oat varieties, contain a β -chlorodehydroalanine residue, $\Delta\text{Ala}(\beta\text{Cl})$. To determine the conformational properties of this unique dehydroamino acid, a series of model compounds was studied using X-ray, NMR, and FT-IR methods, supported by theoretical calculations. The $\Delta\text{Ala}(\beta\text{Cl})$ geometrical isomers differ in conformational profile. The isomer *Z* prefers the helical conformation α ($\varphi, \psi = -61^\circ, -24^\circ$), PPII type conformation β ($\varphi, \psi = -47^\circ, 136^\circ$), and semiextended conformation β_2 ($\varphi, \psi = -116^\circ, 9^\circ$) in weakly and more polar solutions. The isomer *E* prefers mainly the extended conformation C_5 ($\varphi, \psi = -177^\circ, 160^\circ$), but with an increase of the environment polarity also conformations β ($\varphi, \psi = -44^\circ, 132^\circ$) and α ($\varphi, \psi = -53^\circ, -39^\circ$). In the most stable conformations the N-H...Cl hydrogen bond (*S'*) occurs, created between the chlorine atom of the side chain and the N-H donor of the flanking amide group. The method of synthesis of the β -chlorodehydroalanine residue is proposed, by chlorination of dehydroalanine and then the photoisomerization from the isomer *Z* to *E*. The presented results indicate that the assignment of the geometrical isomer of the $\Delta\text{Ala}(\beta\text{Cl})$ residue in naturally occurring victorins still remains an open question, despite being crucial for biological activity.

KEYWORDS: conformational analysis, amino acid synthesis, Ramachandran diagrams, N-H...Cl hydrogen bond, photoisomerization, dehydroamino acids

INTRODUCTION

The oat, genus *Avena sativa*, is a commercially cultivated plant of considerable economic importance.^{1–4} The necrotrophic fungal pathogen *Cochliobolus victoriae*, producing the host-selective toxins victorins, causes disease of some oat varieties.^{5,6} Victorins are a family of peptides^{7–10} in which victorin C is the predominant compound (Figure 1).

This highly modified peptide does not possess any standard amino acid. Our interest focuses on the β -chlorodehydroalanine residue, $\Delta\text{Ala}(\beta\text{Cl})$, as it is common for all compounds of

the victorin family, but so far it was not detected in other naturally occurring compounds. As a conservative unit, one can suppose that β -chlorodehydroalanine plays an important role in the bioactivity of victorins. β -Chlorodehydroalanine belongs to dehydroamino acids, which in general constitute many naturally occurring peptides¹¹ and are classified as nonstandard amino acids. Their characteristic structural feature is the π -bond between carbon atoms α and β . The carbon–carbon double bond is responsible for the specific reactivity of the dehydroamino acid residue, for example, by bonding toxin peptide via Michael addition.¹² The presence of the $\text{C}\alpha=\text{C}\beta$ bond results in the lack of asymmetric carbon α and loss of the optical activity of dehydroamino acid residue. On the other hand, it creates the possible geometrical isomers *Z/E*, which may be of key importance for the biological action of the peptide. This is shown by the example of biologically active cyclic pentadepsipeptide phomalide and the inactive isophomalide analogue.¹³

The conformational properties of the most common dehydroamino acids—dehydroalanine,¹⁴ dehydrobutyrine,¹⁵

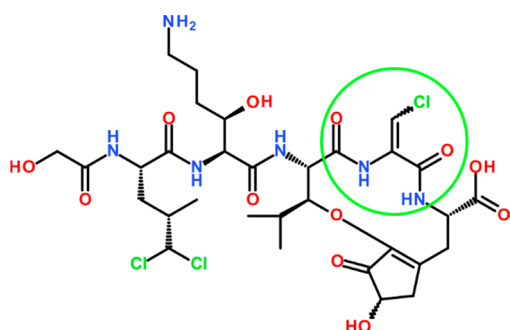


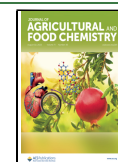
Figure 1. Structure of victorin C, the predominant compound of the family of victorins.⁷ The β -chlorodehydroalanine residue, $\Delta\text{Ala}(\beta\text{Cl})$, is marked, with flanking peptide bonds. The geometry of the $\text{C}\alpha=\text{C}\beta$ double bond is debatable (*E*⁹ or *Z*¹⁰).

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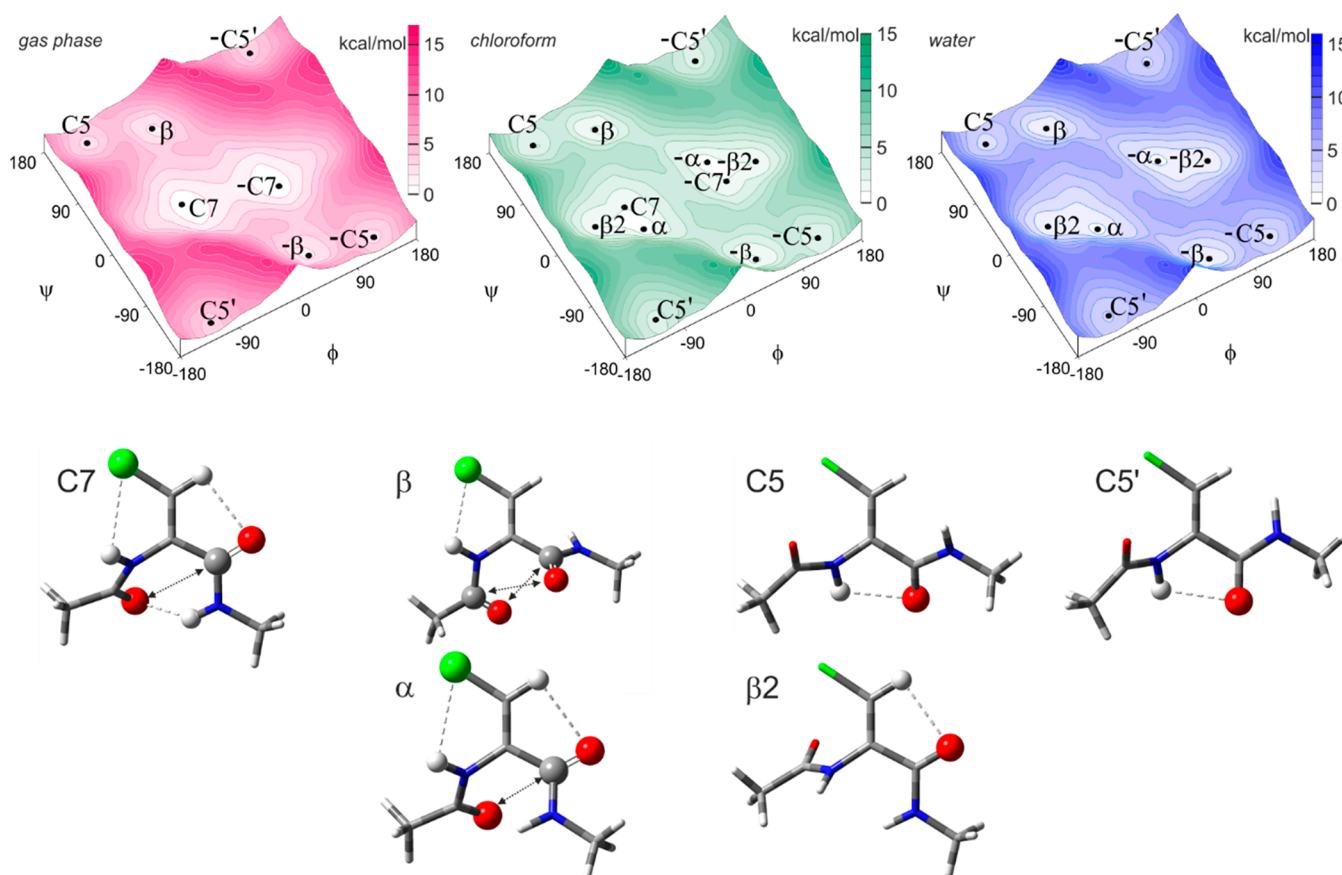
Ac-(Z)- Δ Ala(β Cl)-NHMe (1)

Figure 2. Potential energy surfaces $E = (\phi, \psi)$ of Ac-(Z)- Δ Ala(β Cl)-NHMe (1) obtained by the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environment. Energy contours are plotted every 1 kcal/mol. Below the maps are the conformations optimized at the M06-2X/6-311+G(d,p) level of theory in chloroform with the most important electrostatic interactions ($\leftrightarrow$)³⁸ and hydrogen bonds ($\cdots$)⁴¹ created within the residue.

and dehydrophenylalanine¹⁶—are well recognized. Dehydroalanine, having the simplest methyldene side chain, is prototypical for the whole family of dehydroamino acids. Dehydrobutyrine, with the β -methyl group, is the simplest dehydroamino acid, which reveals geometrical isomers Z/E. Dehydroalanine and dehydrobutyrine are the most common naturally occurring dehydroamino acids. Dehydrophenylalanine, with the β -phenyl group, mostly the isomer Z, is often used in peptide design.

The conformational properties of the β -chlorodehydroalanine were not studied before. The presence of the bulky chlorine atom in the side chain can considerably influence the conformational preferences of the amino acid residue. Not only the chlorine atom can impose a steric hindrance, but also the presence of a heteroatom in the side chain, with lone pairs of electrons, is likely to cause a specific interaction, both intramolecular in the peptide and intermolecular with the target protein or solvent. Determining the conformational properties of β -chlorodehydroalanine should provide deeper insight into the biological effects of victorins. In addition, it gives the possibility of using β -chlorodehydroalanine as a specific tool in peptide design in order to obtain desired properties.

MATERIALS AND METHODS

Computational Procedures. Analyses of short model compounds is a common approach in evaluation of the conformational properties of the selected amino acid residue.^{17,18} The model compounds Ac-(Z)- Δ Ala(β Cl)-NHMe (1) and Ac-(E)- Δ Ala(β Cl)-NHMe (2) were calculated by the DFT method using the Gaussian 16 package.¹⁹ The initial structures were prepared with GaussView5.²⁰ The configurations *trans* ($\omega_0 \approx 180^\circ$) of both amide groups were set. The potential energy surfaces $E = f(\phi, \psi)$ were obtained at M06-2X/6-31+G(d,p) level of theory,²¹ in the gas phase, and then in chloroform and water (constrained optimization). The values of the ϕ and ψ dihedral angles were changed in steps of 30° . Because of the achirality of dehydroamino acids, each structure has a mirror counterpart with the same energy but opposite torsion angles ($\phi, \psi = -\phi, -\psi$), which reduced the number of grid point structures of the maps to 91 each. The solvent effect was simulated with the self-consistent reaction field (SCRF) using the conductor-like polarizable continuum model (CPCM).

All potential energy minima localized in the maps were fully optimized by using a bigger basis set, 6-311+G(d,p). Unconstrained optimizations were followed by vibrational analysis to ensure that the resulting structures are true energy minima and to obtain the zero-point vibrational energies (ZPVEs) and Gibbs energies (298.15 K, 1.0 atm). The population of the conformations (p) was calculated at the 300 K temperature, where $RT = 0.595$ kcal/mol according to the following equations:^{22,23}

$$p_{\text{rel}} = 100\% \exp(-\Delta G/RT) \quad (1)$$

and

$$p = p_{\text{rel}} / \sum \text{conformers } p_{\text{rel}} \times 100\% \quad (2)$$

The names of the conformations are based on the Scarsdale nomenclature.²⁴

The XYZ structures of the calculated compounds, Ac-(Z)- Δ Ala(β Cl)-NHMe (1) and Ac-(E)- Δ Ala(β Cl)-NHMe (2), along with their energies are given in Table 1S.

Crystal Structure Analysis. The single crystals of the studied molecules, Ac-(Z)- Δ Ala(β Cl)-NHMe (1), Ac-(E)- Δ Ala(β Cl)-NHMe (2), Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3), Boc-(Z)- Δ Ala(β Cl)-OMe (4), and Ac- Δ Ala(β Cl₂)-NHMe (5), were collected on a Rigaku Oxford Diffraction XtaLAB SynergyR DW diffractometer equipped with a HyPix ARC 150° Hybrid Photon Counting (HPC) detector using CuK α (λ = 1.54184 Å) at 100 K. The corrections to the Lorentz and polarization factors were applied to the reflection intensities.²⁵ Data were processed using the CrysAlisPro software.²⁶ The structures were solved by direct methods using SHELXS and refined by the full-matrix least-squares methods based F² using SHELXL.^{27,28} All non-hydrogen atoms were located from difference Fourier synthesis and refined by the least-squares method in the full-matrix anisotropic approximation. The crystallographic data for compounds and details of the X-ray experiment are collected in the Supporting Information. The structure drawings in Supporting Information were prepared by using the Mercury program.²⁹ The coordinates of atoms and other parameters for structures were deposited with the Cambridge Crystallographic Data Centre: 2236959 for (1), 2236963 for (2), 2236961 for (3), 2236960 for (4), 2236962 for (5); 12 Union Road, Cambridge CB2 1EZ, UK (Fax, +44-(1223)336-033, E-mail: deposit@ccdc.cam.ac.uk).

FTIR Spectra. A Nicolet Nexus 2002 FT-IR spectrometer flushed with dry nitrogen during the measurements was used. The thickness of the KBr liquid cell was 0.01 mm. For each measurement 20 scans were accumulated with 2 cm⁻¹ resolution (spectral resolution 0.482 cm⁻¹) in the spectral range 400–4000 cm⁻¹. The Ac-(Z)- Δ Ala(β Cl)-NHMe (1) and Ac-(E)- Δ Ala(β Cl)-NHMe (2) solutions were prepared in chloroform in three different concentrations: 0.5, 1.0, and 2.0 mg/mL each. The spectral processing and peak deconvolution were conducted using the Fityk software,³⁰ applying the voigt function.

NMR Spectra. The NMR analyses were performed on a Bruker Ultrashield 400 (Bruker 2005) spectrometer with Bruker software (TopSpin Version 1.3), operating at 400 MHz for ¹H and 101 MHz for ¹³C. The spectra were recorded in (CD₃)₂SO (DMSO-*d*₆) or CDCl₃, CD₃OD, or D₂O (internal TMS standard) at room temperature. To determine the isomers, the NOE difference method was applied using the standard programs.

Synthesis—General Procedure. For the detailed synthetic procedure and characterization of each compound, please see the Supporting Information.

Chlorination of Dehydroalanine. Chlorination of dehydroalanyl residue was performed on a millimolar scale (0.3–0.6 mmol) based on a protocol adapted from the literature^{31–33} with some modifications, including the use of triethylamine as a base. The peptide substrate was dissolved in a DMF/DCM (1:8) solvent mixture. Then, a solution of chlorine gas in DCM was added dropwise to the reaction mixture to the point at which a pale yellow color appeared. The volatile components were quickly evaporated under reduced pressure. The residue was resuspended in DCM, triethylamine (2.5 equiv) was added, and the reaction mixture was stirred for 15 min. The reaction mixture was concentrated and directly purified by flash column chromatography. The desired products were crystallized from an EtOAc/hexane solvent system.

Photoisomerization of β -Chlorodehydroalanine. Photoisomerization reaction of the β -chlorodehydroalanyl residue was performed on a millimolar scale (0.04–0.2 mmol) in a quartz cuvette. The peptide substrate and benzophenone (5 equiv) were dissolved in a methanol/benzene (1:0.4) solvent mixture. The reaction mixture was stirred and illuminated with UV light (366

nm) with a power density of 400–440 μ W/cm² for five hours. Then, the reaction mixture was evaporated under reduced pressure and directly purified by flash column chromatography. The desired products were crystallized from an EtOAc/hexane solvent system.

RESULTS AND DISCUSSION

Theoretical Method. Ac-(Z)- Δ Ala(β Cl)-NHMe (1). The diamide model compound (1) containing the isomer Z of the β -chlorodehydroalanine residue was studied using theoretical methods to obtain a general view of its conformational properties. The potential energy maps with the conformations corresponding to local minima are presented in Figure 2. Selected parameters are gathered in Table 1. For the isolated

Table 1. Selected Parameters for Conformations of Ac-(Z)- Δ Ala(β Cl)-NHMe (1)^a

Conformation	φ (deg)	ψ (deg)	ΔG (kcal·mol ⁻¹)	p (%)
<i>gas phase</i>				
C7	−62.6	20.1	0.00	71.69
β	−41.4	135.4	0.65	24.09
C5	−128.2	156.7	1.92	2.85
C5′	−124.9	−165.0	2.35	1.38
<i>chloroform</i>				
α	−61.8	−23.1	0.00	40.51
β	−42.7	138.7	0.17	30.44
β 2	−116.6	9.8	0.56	15.88
C7	−68.2	16.6	0.85	9.68
C5	−127.7	155.1	1.71	2.31
C5′	−121.0	−157.5	2.10	1.18
<i>water</i>				
α	−60.3	−25.0	0.00	49.58
β	−43.6	139.7	0.27	31.57
β 2	−116.4	9.4	0.65	16.54
C5	−127.6	154.5	2.09	1.47
C5′	−117.8	−153.7	2.43	0.84

^aEach calculated conformation has its mirror counterpart. Optimized at the M06-2X/6-311+G(d,p) method (SCRF, CPCM).

molecule (gas phase, *in vacuo*), four possible conformations—C7, β , C5, and C5′—were found in the map, together with their mirror analogues, which have the same energy, but opposite signs of the torsion angles. The lowest in energy is conformation C7 (φ, ψ = −63°, 20°). The analysis of distances between the atoms within the studied residue indicates possible intramolecular interactions (Table 2S). The N^C-H...O^N hydrogen bond created between the C-terminal N-H group and the N-terminal carbonyl oxygen atom constitutes a 7-membered ring, typical for this conformation. The novelty is the N^N-H...Cl hydrogen bond.^{34,35} It is created between the N-H group of the N-terminal amide bond and the chlorine atom in the side chain and, thus, is denoted as (S′).³⁶ There is also the C ^{β} -H...O^C hydrogen bond, which is stronger in dehydroamino acids, due to the hybridization sp² of the β -carbon atom.^{14,37} It should also be noticed the dipole interaction type I of carbonyl groups.^{38–40} The concomitant intramolecular interactions are the reason why 72% of the molecular population takes conformation C7 in the gas phase. Next in the energy order is conformation β (φ, ψ = −41°, 135°). It is also stabilized, by the N^N-H...Cl hydrogen bond. Also, the dipole–dipole attraction II type between the carbonyl groups can be considered. This conformation is adopted by about 24% of the molecular population. The highest in energy

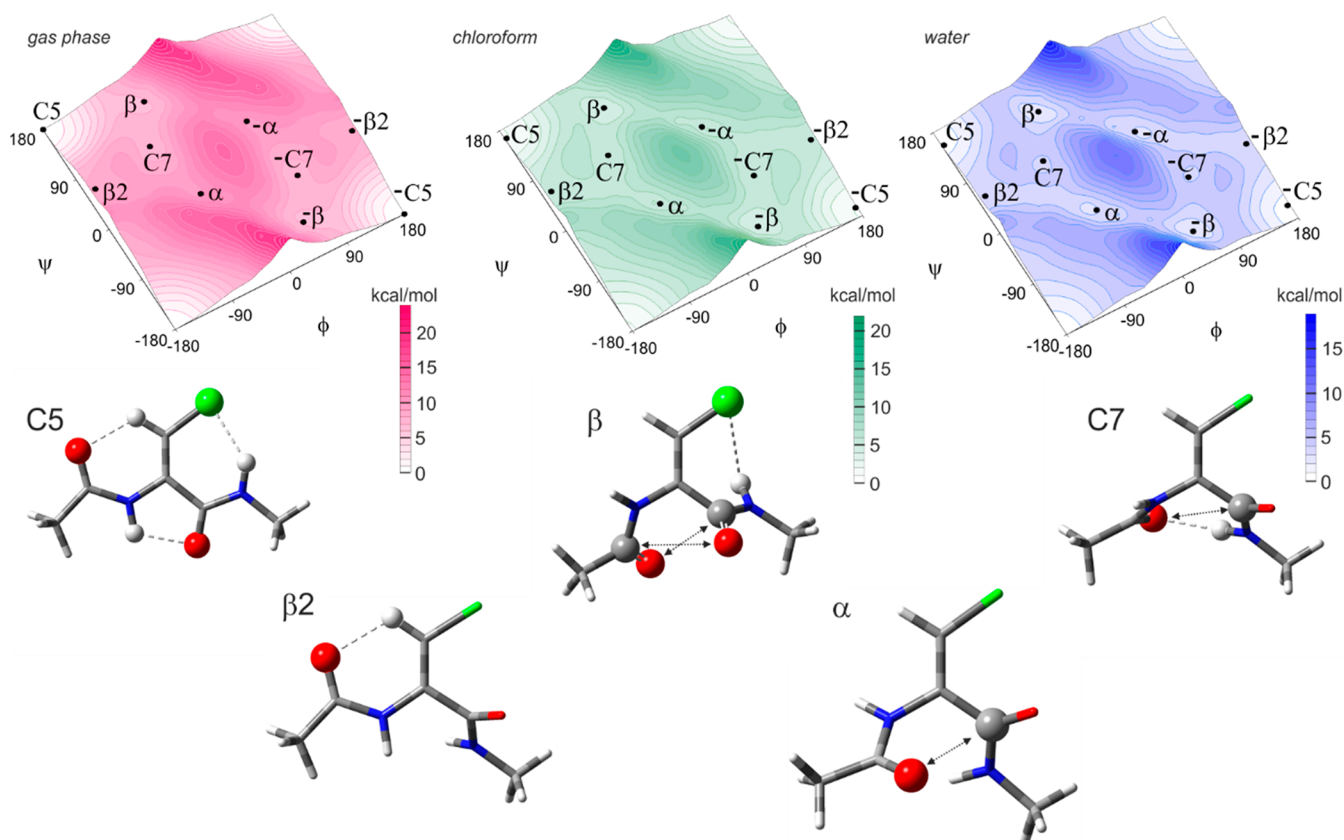
Ac-(E)- Δ Ala(β Cl)-NHMe (2)

Figure 3. Potential energy surfaces $E = (\phi, \psi)$ of Ac-(E)- Δ Ala(β Cl)-NHMe (2) obtained at the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environment. Energy contours are plotted every 1 kcal/mol. Below the maps are the conformations optimized at the M06-2X/6-311+G(d,p) level of theory in chloroform with the most important electrostatic interactions ($\leftrightarrow$)³⁸ and hydrogen bonds ($\cdots$)⁴¹ created within the residue.

are two extended conformations C5 ($\phi, \psi = -128^\circ, 157^\circ$) and C5' ($\phi, \psi = -125^\circ, -165^\circ$), which have an opposite value of the angle ψ . These conformations are stabilized by the N^N -H $\cdots$ O^C hydrogen bond, which creates a 5-membered ring formed between the N-terminal N-H group and the C-terminal oxygen atom. The formation of two extended conformations, C5 and C5', is due to the energy transition barrier caused by a steric hindrance imposed by the large chlorine atom in position Z of the dehydroamino acid side chain and a repulsion with the electronegative oxygen atom of the N-terminal amide group. Furthermore, the H $\cdots$ H repulsion from the C ^{β} -H atom and the C-terminal N-H group causes a distortion of the intramolecular N-H $\cdots$ O C5-type hydrogen bonds. This results in two nearly symmetrical positions with respect to the ψ torsional angle.

Analysis of the potential energy surface in a weakly polar environment, imitated by chloroform, showed significant changes in the potential energy valley next to conformation C7. Two conformations appear: the helical conformation α ($\phi, \psi = -62^\circ, -23^\circ$) and the semiextended conformation β 2 ($\phi, \psi = -117^\circ, 10^\circ$). Both conformations are stabilized by the C ^{β} -H $\cdots$ O^C hydrogen bond. In conformation α , the N-H $\cdots$ Cl hydrogen bond and the dipole attraction type III can be considered. In conformation β 2, the value of the torsion angle ψ indicates that the C-terminal amide group and the C α =C β double bond are in a plane; thus, the π -electron conjugation appears.

In a more polar environment, mimicked by water, conformation C7 vanishes. Conformations α and β prevail, both stabilized by the N-H $\cdots$ Cl hydrogen bond. A more polar environment does not have a major influence on the conformation geometry; changes in the values of the torsion angles ϕ and ψ do not exceed $\pm 3^\circ$. The energy order between the conformations is also preserved.

Ac-(E)- Δ Ala(β Cl)-NHMe (2). The potential energy maps of the diamide model compound (2) containing the isomer E of the β -chlorodehydroalanine residue together with the conformations corresponding to local minima are presented in Figure 3 and Table 2. Five different conformations can be found, regardless of the environment simulated: C5, β , C7, β 2, and α (and their mirror analogues).

The global minimum is occupied by the extended conformation C5 ($\phi, \psi = -180^\circ, 180^\circ$). Three intramolecular hydrogen bonds play a stabilizing role: N^N-H $\cdots$ O^C, N^C-H $\cdots$ Cl, and C ^{β} -H $\cdots$ O^N. Their parameters can be found in Table S3 in Supporting Information. Additionally, the values of torsion angles ϕ and ψ indicate the flatness of the structure and, thus, the appearance of the cross-conjugate π -electron system, which overlaps the α,β -double bond and both flanking amide groups. The remaining conformations have clearly much higher energies, which exceed 5 kcal/mol. The existence of conformation β ($\phi, \psi = -47^\circ, 136^\circ$) can be explained by the dipole-dipole attraction type II between the carbonyl groups, like also the N-H $\cdots$ Cl hydrogen contact with

Table 2. Selected Parameters for Conformations of Ac-(E)- Δ Ala(β Cl)-NHMe (2)^a

Conformation	φ (deg)	ψ (deg)	ΔG (kcal·mol ⁻¹)	<i>p</i> (%)
<i>gas phase</i>				
C5	-179.8	180.0	0.00	99.97
β	-46.9	135.6	5.25	0.01
C7	-75.8	69.7	5.45	0.01
β 2	-174.0	48.0	6.99	0.00
α	-55.4	-33.4	8.46	0.00
<i>chloroform</i>				
C5	-177.9	165.1	0.00	91.87
β	-44.4	132.3	1.77	4.74
C7	-77.7	63.1	2.43	1.56
α	-54.5	-37.6	2.68	1.02
β 2	-172.7	53.1	2.81	0.82
<i>water</i>				
C5	-177.2	156.2	0.00	60.17
α	-52.6	-39.6	0.71	18.21
β	-44.0	132.2	0.73	17.57
β 2	-172.4	52.9	1.82	2.81
C7	-78.6	59.8	2.32	1.23

^aEach calculated conformation has its mirror counterpart. Optimized at the M06-2X/6-311+G(d,p) method (SCRF, CPCM).

considerably lower geometrical parameters. The conformation C7 ($\varphi, \psi = -76^\circ, 70^\circ$) is maintained by the N-H...O hydrogen

bond C7 type, formed between the C-terminal N-H group and the N-terminal carbonyl group oxygen atom, as well as by the dipole interaction type I. Conformation β 2 ($\varphi, \psi = -174^\circ, 48^\circ$) is placed in the shallow region of the map. Its main stabilization is provided by the C $^\beta$ -H...O^N hydrogen bond, whose acceptor is the oxygen atom of the N-terminal amide group. Moreover, π -electron conjugation, including the α, β -double bond and the N-terminal amide group, can be considered. Conformation α ($\varphi, \psi = -55^\circ, -33^\circ$) is stabilized by the dipole attraction O^N...C^C.

The simulated increase in the polarity of the environment shows that still the lowest energy presents conformation C5. Although the difference in energy between the conformations decreases, up to 3 kcal/mol in chloroform and less than 2.3 kcal/mol in water, most molecules (92%) occur as C5 in a weakly polar solvent, mimicked by chloroform, and about 60% occur as C5 in a natural aquatic environment.

Values of the torsion angle φ are almost unchanged in particular conformations. They are up to $\pm 5^\circ$ different for the isolated molecule than for the studied solvents. The more significant changes are in the values of the torsion angle ψ . This may be caused by the large chlorine atom in position E of the dehydroamino acid side chain.

Opposite to the gas phase, in the environment of simulated solvents, some molecules appear in conformation β and then also in conformation α . Furthermore, sterically more open conformation α changed its position in energy order. It

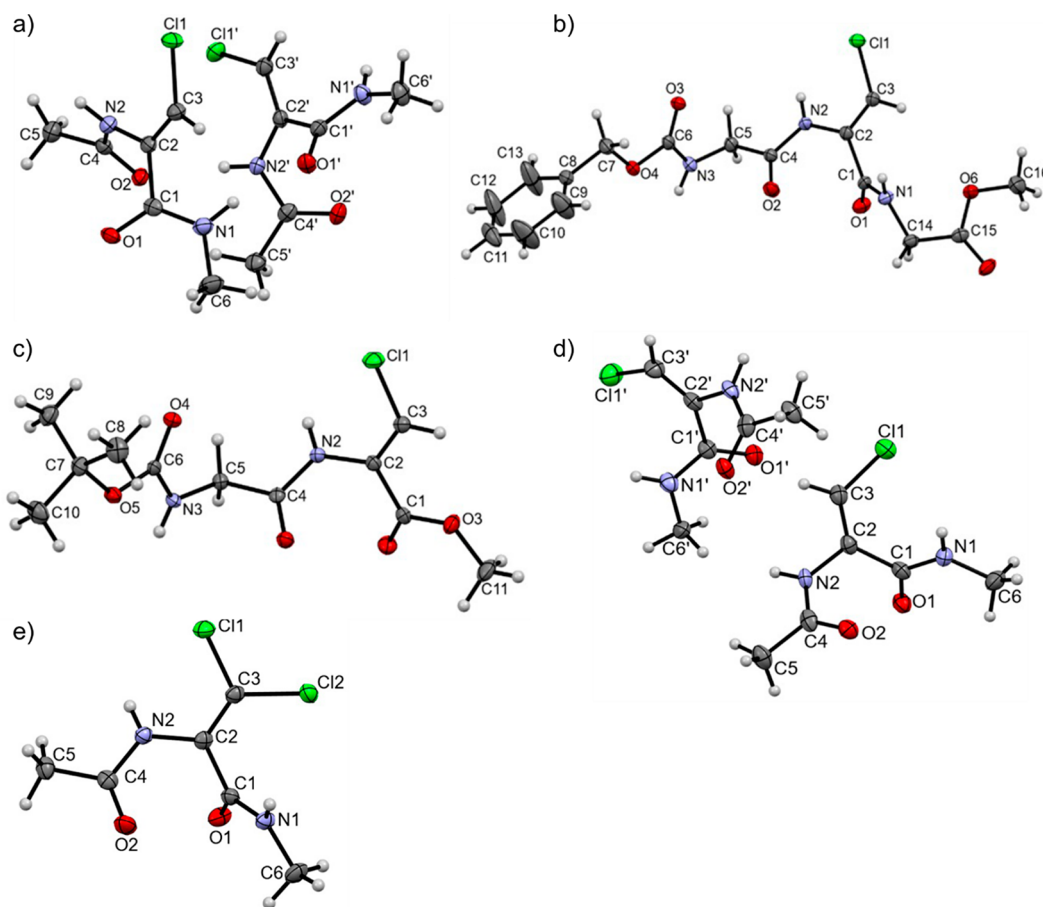


Figure 4. Molecular structures of: a) Ac-(Z)- Δ Ala(β Cl)-NHMe (1), b) Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3), c) Boc-(Z)- Δ Ala(β Cl)-OMe (4), d) Ac-(E)- Δ Ala(β Cl)-NHMe (2), and e) Ac- Δ Ala(β Cl₂)-NHMe (5) in the asymmetric part of the unit cell. Displacement ellipsoids are drawn at the 50% probability level.

Table 3. Selected Parameters for the Studied Compounds Measured by the X-ray Method

Compound	Torsion (deg)		Distance (Å)		Angle (deg)	Distance (Å)	
	φ	ψ	H...Cl	N...Cl	N-H...Cl	C ^N ...O ^C	C ^C ...O ^N
Ac-(Z)- Δ Ala(β Cl)-NHMe (1)	-47.49	149.00	3.009	3.058	84.47	3.046	2.728
	-41.57	141.75	2.868	3.048	94.96	3.116	2.742
Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3)	-41.81	143.49	2.827	3.049	96.30	3.115	2.794
Boc-(Z)- Δ Ala(β Cl)-OMe (4)	-56.51	168.64	2.895	3.057	92.93	3.075	2.979
Ac-(E)- Δ Ala(β Cl)-NHMe (2)	-49.53	134.13	2.854	3.221	106.79	3.203	2.807
	-50.04	134.30	2.918	3.256	104.80	3.220	2.833
Ac- Δ Ala(β Cl ₂)-NHMe (5)	-38.86	129.81	2.780	2.975	94.96	3.215	2.731
			2.920	3.114	96.35		

becomes privileged because of better interaction with the solvent.

Crystal Structure Analysis. The conformational properties of the Δ Ala(β Cl) residue in a polar solution were estimated using the single X-ray structures of Ac-(Z)- Δ Ala(β Cl)-NHMe (1), Ac-(E)- Δ Ala(β Cl)-NHMe (2), Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3), Boc-(Z)- Δ Ala(β Cl)-OMe (4), and Ac- Δ Ala(β Cl₂)-NHMe (5) determined at 100 K. The crystal parameters and experimental details of X-ray data collection are presented in Tables 4S and 5S. The molecular structures of (1–5) with the atomic numbering scheme are presented in Figure 4, and selected geometric parameters are presented in Table 3.

In the crystal structure of Ac-(Z)- Δ Ala(β Cl)-NHMe (1), there are two types of molecules with slightly different geometries. The conformations they adopt (φ , ψ = -47.5°, 149.0° and -41.6°, 141.75°) correspond to conformation β , one of the two most stable forms found on the conformational maps. Analysis of the crystal structure of the larger molecule, tripeptide Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3), shows that the (Z)- Δ Ala(β Cl) residue also adopts the same conformation β (φ , ψ = -41.8°, 143.5°). Conformation β (φ , ψ = -56.5°, 168.6°) can also be found for Boc-(Z)- Δ Ala(β Cl)-OMe (4) with the C-terminal ester bond, although the values of torsion angles (φ , ψ = -56.5°, 168.6°) are somehow greater than those for the residue with a C-terminal amide group. For the geometrical isomer *E*, as it is shown by Ac-(E)- Δ Ala(β Cl)-NHMe (2) that this tendency is maintained, two types of molecules with slightly different geometries were found in the crystal state and they have conformation β (φ , ψ = -49.5°, 134.1° and -50.0°, 134.3°). Finally, Ac- Δ Ala(β Cl₂)-NHMe (5), with the chlorine atoms in both positions Z and E, also reveals conformation β (φ , ψ = -38.9°, 129.8°).

In conformation β , the hydrogen atom of the N-terminal amide group is in proximity to the chlorine atom. In the studied crystal structures, the H...Cl distance varies from 2.780 to 3.009 Å and the N-H...Cl angle has a value from 96.30 to 84.47° for the (Z)- Δ Ala(β Cl) residue. In the case of the isomer (E)- Δ Ala(β Cl), there is also proximity of the hydrogen atom from the C-terminal amide group and the chlorine atom in position E. The H...Cl distance varies from 2.854 to 2.920 Å, and the N-H...Cl angle has a value from 106.79 to 94.96°. Assuming the sum of van der Waals radii of the hydrogen and chlorine atoms is 2.95 Å,⁴² it can be concluded that there is a N-H...Cl hydrogen bond, which stabilizes conformation β .

Dipole interactions of carbonyl groups II type also can be seen.³⁸ Especially, the distance between the C-terminal carbonyl carbon and the N-terminal carbonyl oxygen (C^N...O^C) from 2.728 to 2.979 Å, and thus below the sum of van der

Waals radii of the carbon and oxygen atoms, also can be perceived as a stabilizing force of conformation β .

The molecules of the studied compounds are mainly associated through N-H...O hydrogen bonds (Figures 1–5S, Table 6S). For Ac-(Z)- Δ Ala(β Cl)-NHMe (1) molecules two similar conformations, here named as β^1 (φ , ψ = -47.5°, 149.0°) and β^2 (φ , ψ = -41.6°, 141.75°), are present. The molecule with conformation β^1 (φ , ψ = -47.5°, 149.0°) creates two N-H...O hydrogen bonds with the molecule with conformation β^2 (φ , ψ = -41.6°, 141.75°). Additionally, each molecule β^1 creates with two molecules $-\beta^1$ two other N-H...O hydrogen bonds. Analogously, the molecule β^2 creates two N-H...O hydrogen bonds with two molecules $-\beta^2$ (Figure 1S). For Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (3), the molecules are placed in parallel, N-terminus to N-terminus, separately for conformations β and $-\beta$ (Figure 2S). For Boc-(Z)- Δ Ala(β Cl)-OMe (4), the molecules with conformation β are placed antiparallel between the molecules with conformation $-\beta$ (Figure 3S). For Ac-(E)- Δ Ala(β Cl)-NHMe (2), the molecules are in a linear arrangement. The molecules with conformation β are placed with the molecules with conformation $-\beta$ alternately, with the (Z)- Δ Ala(β Cl) side chains upside-down and the N-terminus to the C-terminus (Figure 4S). For Ac- Δ Ala(β Cl₂)-NHMe (5), the molecules with the opposite conformations, β and $-\beta$, and with the Δ Ala(β Cl₂) side chains upside-down, create centrosymmetric dimmers. Additionally, each molecule is joined with two other molecules with the opposite conformations (Figure 5S).

Despite various arrangements, the molecules maintain conformation β (or the opposite conformation $-\beta$), predicted by the theoretical method as one of the low-energy conformations.

Infrared Spectral Analysis. The conformational properties of the Δ Ala(β Cl) residue in a weakly polar solution were estimated using the model compounds Ac- Δ Ala(β Cl)-NHMe (1 and 2) (Figure 6S and 7S).

The ν_s (N-H) stretching mode regions of the Fourier transform infrared (FTIR) spectra for the solutions in chloroform are shown in Figure 5. The spectrum for Ac-(E)- Δ Ala(β Cl)-NHMe (2) shows two bands in the ν_s (N-H) stretching mode region, at 3441 and 3358 cm⁻¹. The analysis of theoretical frequencies (Table 7S) shows that they can be assigned respectively to the C-terminal and N-terminal amide N-H groups. The shapes of the bands are regular, which indicates that they belong to a single conformation. The relative position of the scaled frequencies fits the best to conformation C5, which is also in accordance with the population of the conformation in chloroform presented in Table 2.

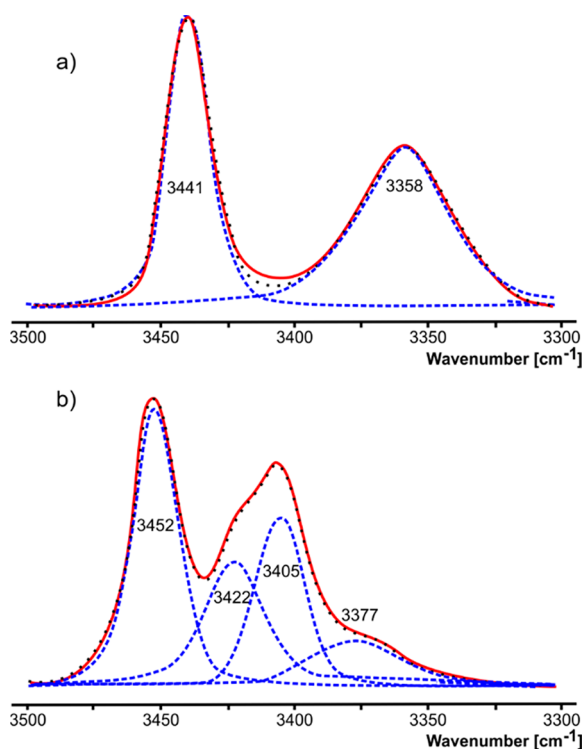


Figure 5. FTIR spectra for Ac-(Z/E)- Δ Ala-(β Cl)-NHMe in CHCl₃, region $\nu_s(\text{N-H})$: (a) isomer E (**2**), (b) isomer Z (**1**). The component bands (dashed lines) were obtained by a curve-fitting procedure.

The spectrum for Ac-(Z)- Δ Ala(β Cl)-NHMe (**1**) also shows two bands in the $\nu_s(\text{N-H})$ stretching mode region. Never-

theless, the irregularity of their shapes indicates a conformational equilibrium. Deconvolution was resolved on four bands: 3452 and 3405 cm^{-1} with a higher intensity as well as 3422 and 3377 cm^{-1} with a lower intensity. The relative position of the scaled frequencies (Table 7S) allows assuming that in a weakly polar environment conformation C7 can be excluded. Considering the differences in energy presented in Table 1, a mixture of the two conformations α and β is most likely.

It should be noted that the positions of the analyzed bands in the $\nu_s(\text{N-H})$ stretching region for the studied Ac-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-NHMe}$ and Ac-(E)- $\Delta\text{Ala}(\beta\text{Cl})\text{-NHMe}$ are considerably lower than the corresponding bands of the structurally closely related Ac-(Z)- $\Delta\text{Abu-NHMe}$ ⁴³ and Ac-(E)- $\Delta\text{Abu-NHMe}$,⁴⁴ which have the methyl group in the side chain instead of the chlorine atom. This shows the impact of the chlorine atom in the side chain. Using the lone pairs, it can not only act as the acceptor of the internal N-H $\cdots$ Cl hydrogen bonds with the N-H donors of the flanking amide groups but also participate in a delocalized cross-conjugated system.

Synthesis. The synthesis of the model compounds containing the β -chlorodehydroalanine residue, $\Delta\text{Ala}(\beta\text{Cl})$, the unsaturated fragment of victorins, was performed in three crucial steps: preparation of the dehydroalanine residue, chlorination of the carbon atom β , and photoisomerization (Figure 6).

The following methods were selected to obtain the dehydroamino acid residue. For the synthesis of the model compounds **1** and **2**, the *N*-acetyldehydroalanine was obtained by condensation of pyruvic acid with acetamide in the presence of *p*-toluenesulfonic acid. In the next step, Ac- Δ Ala-OH was converted into methylamide derivative Ac- Δ Ala-NHMe using

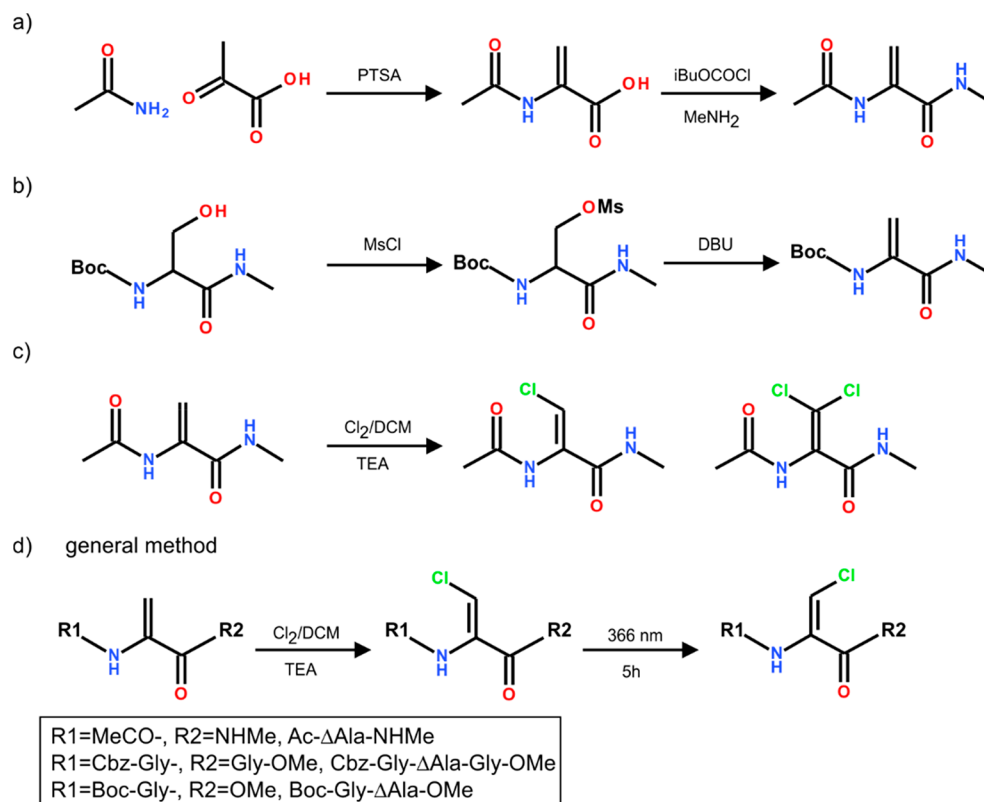


Figure 6. Synthetic procedure of the $\Delta\text{Ala}(\beta\text{Cl})$ residue.

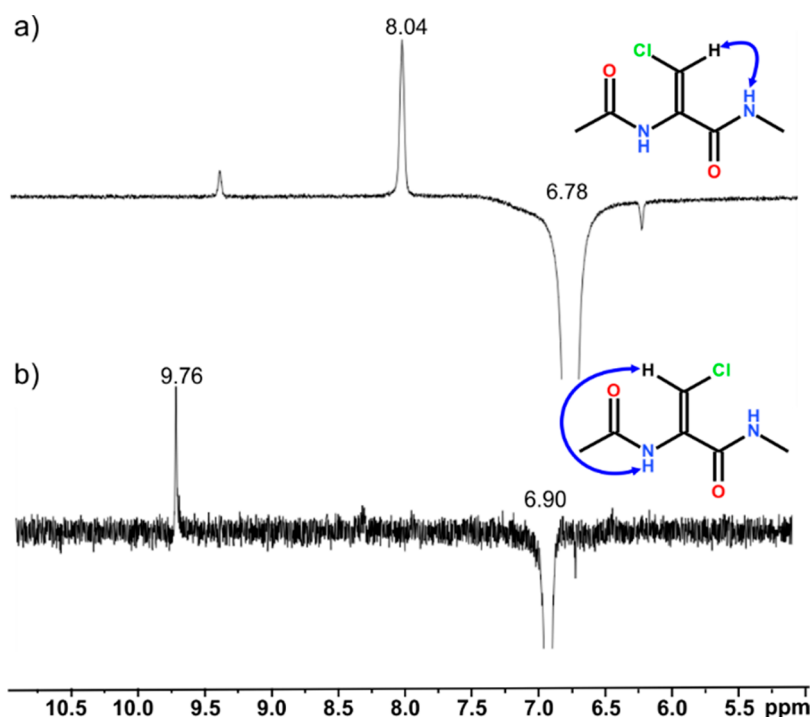


Figure 7. NOE difference NMR spectra of Ac-ΔAla(βCl)-NHMe in DMSO-*d*₆ with excitation of the vinyl proton of both isomers: (a) isomer *Z* (1); (b) isomer *E* (2).

a mixed anhydride method with isobutyl chloroformate (Figure 6a), according to the known procedure.⁴⁵

For the purpose of the synthesis of the model compounds 3 and 4, dipeptide Boc-Gly-ΔAla-OMe and tripeptide Cbz-Gly-ΔAla-Gly-OMe were obtained using a multistep procedure including preparation of an *N*-protected Gly-ΔAla fragment⁴⁶ followed by its esterification reaction with a cesium salt and methyl iodide⁴⁷ or coupling with a glycine methyl ester using a mixed anhydride protocol,^{45,48} respectively.

An attempt was also undertaken to synthesize the model compound of Boc-protected dehydroalanine, Boc-ΔAla-NHMe, to overcome the solubility problem of short acetyl derivatives in weakly polar solvents, as was done in our previous work.⁴⁹ Nevertheless, the acidic conditions of the condensation reaction are not compatible with the commonly applied amine Boc protection. Therefore, the preparation of Boc-ΔAla-NHMe required another synthetic approach involving dehydration of a β-hydroxy amino acid (serine). The Boc-Ser-NHMe was prepared (please, see Supporting Information). Dehydration using a one pot procedure with methanesulfonyl chloride/DBU was performed,⁴⁸ but a complex reaction mixture was obtained. Thus, the preparation of a methanesulfonyl serine derivative and an elimination reaction were conducted in separate steps (Figure 6b). The analysis of reaction mixtures revealed that the hydantoin derivative was the main product while the desired Boc-ΔAla-NHMe was obtained with a low yield (9%). Taking into account this obstacle, the preparation of the Boc-protected model was abandoned.

The chlorination reaction step was performed on the basis of a modified procedure^{31–33} and our experiences with the bromination of dehydroalanine derivatives.⁵⁰ The reaction was done by treating Ac-ΔAla-NHMe with a solution of chlorine in dichloromethane and then the addition of triethylamine. The isomer *Z* of Ac-ΔAla(βCl)-NHMe (1) was obtained as the

main product of the reaction. Contrary to the previously mentioned literature protocol, in which the second step of the reaction was performed in acetonitrile in the presence DABCO as organic base, the formation of the isomer *E* of the desired product was not observed in our case. Instead, the minor unsaturated product was a dehydroalanine derivative containing two chlorine atoms at position β, Ac-ΔAla(βCl₂)-NHMe (5) (Figure 6c). On the other hand, the *N*-bromosuccinimide was successfully applied to the synthesis of the β-bromodehydroalanine derivative.⁵¹ Thus, we decided to perform the chlorination with *N*-chlorosuccinimide as an alternative method, but the desired product was detected in a trace amount. Therefore, the chlorination reaction involving the Cl₂/DCM solution then triethylamine was chosen as the optimal one. The versatility of this method was shown for the synthesis of more complex structures, tripeptide (Cbz-Gly-ΔAla-Gly-OMe) and dipeptide (Boc-Gly-ΔAla-OMe) substrates, with different positions of the dehydroalanine residue in the peptide chain and different terminations of the C-end. In each case, the isomer *Z*, respectively (3) and (4), was obtained with a moderate yield of 53–71%.

To complete the preparation of β-chlorodehydroalanine model structures, the isomerization from the isomers *Z* into *E* assisted by UV irradiation was performed. The photoisomerization *Z/E* of simple dehydroamino acid residues is known.^{52,53} However, according to the best of our knowledge, the application of this reaction to the β-halogenodehydroalanyl residue is reported for the first time here. The optimal reaction conditions in this case include the irradiation of the reaction mixture with UV light with a maximum wavelength of 366 nm at 5 h with an intensity of 400–440 μW/cm² (Figure 6d). It should be noted that the extension of the reaction time, irradiation with UV light with higher energy (λ_{max} = 254 nm), or increasing the light intensity resulted in our hands in the decomposition of substrate and reduction of overall yield and

substrate recovery. Despite several attempts to optimize reaction conditions, the isomerization of Ac-(Z)- Δ Ala(β Cl)-NHMe and Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe gave the isomer *E* with a yield of 10–15%, while the methyl ester derivative, Boc-Gly-(Z)- Δ Ala(β Cl)-OMe, isomerizes slightly more easily and the product isomer *E* was obtained with a yield of 30%. We speculate that this can be the result of higher steric hindrance of the carboxamide group. On the other hand, the overall low yields of isomerization *Z/E* resulted from higher thermodynamic stability of the isomer *Z*, which is in good accordance with observations for simple dehydroamino acids.⁵⁴

NMR Spectral Analysis. Apart from the crystallographic structures of both compounds **1** and **2**, the geometries of both isomers of Ac- Δ Ala(β Cl)-NHMe were confirmed by a series of NOE difference NMR experiments, in which the vinyl and amide protons were excited during the experiment. For the isomer *Z*, a strong resonance is observed between the vinyl (δ H 6.78) and C-terminal amide (δ H 8.04) protons (Figure 7a). For the isomer *E*, strong resonance is observed between the vinyl (δ H 6.90) and, in contrast, the N-terminal amide (δ H 9.76) (Figure 7b) protons. The NOE difference NMR spectra for the studied compounds are in Supporting Information (Figures 17–20S, 24–27S, 31–33S, 37–39S).

Determination of the geometric isomer of the chlorodehydroalanine residue in victorin structures was undertaken by comparing the chemical shift of the vinyl proton of the methylated victorin M derivative to the chemical shifts of selected model chemical compounds. Initially, the geometry of the C=C double bond was established as *Z* by the comparison of the vinyl protons of both isomers of *N*-methylated β -chlorodehydroalanine Δ (Me)Ala(β Cl).^{55,56} Later, more detailed attempts for the stereochemical assignments of the chlorinated residues in victorin C were undertaken.¹⁰ The selected Δ Ala(β Cl) esters were taken as a pattern, and the geometric isomers were assigned by comparing the coupling constant C_H with the calculated values. Based on this data it was concluded that the chlorodehydroalanyl residue in victorins has *E* rather than *Z* configuration. The geometry *E* of victorins was further adopted in some articles.^{6,57,58}

In this work, we synthesized the model compounds, which seem to be structurally better matched to the unsaturated fragment of victorins, because the Δ Ala(β Cl) is flanked by secondary amide bonds. The structures of the compounds were confirmed by the X-ray method (mainly for the isomer *Z*) and the NOE difference NMR experiments (for both the isomers *Z* and *E*). The comparison of the values of the chemical shift of the vinyl protons for the model compounds (Table 4) revealed that the vinyl proton resonates in the range 6.92–7.15 and 6.58–6.98 ppm, for the isomers *Z* and *E*, respectively (Figures 14–16S, 21–23S, 29S, 30S, 34S, 36S, and 40S). The absolute value of the difference between the positions of the vinyl protons is in the range 0.03–0.39 ppm. The relative position changes with the type of solvent (Ac- Δ Ala(β Cl)-NHMe) or the type of flanking group (Cbz-Gly- Δ Ala(β Cl)-Gly-OMe, Boc-Gly- Δ Ala(β Cl)-OMe). The most significant difference (0.39 ppm in DMSO- d_6) is for the Boc-Gly- Δ Ala(β Cl)-OMe compound, in which the C-terminus is flanked by a methyl ester. The vinyl proton of the isomer *E* resonates upfield as compared to the isomer *Z* (in DMSO- d_6); however, this is opposite to that established for Ac- Δ Ala(β Cl)-OMe, where the isomer *E* resonates downfield (in CDCl₃).¹⁰ Furthermore, for the derivative of victorin M the vinyl proton

Table 4. Chemical Shift (δ ppm) Values of Vinyl Proton for the Studied Compounds

Compound/Solvent	δ [(ppm)]		$\Delta\delta$ (ppm) (<i>E</i> – <i>Z</i>)
	Isomer <i>Z</i>	Isomer <i>E</i>	
Ac- Δ Ala(β Cl)-NHMe			
DMSO- d_6	6.78	6.90	0.12
CD ₃ OD	6.85	6.81	–0.04
D ₂ O	6.92	6.58	–0.34
Cbz-Gly- Δ Ala(β Cl)-Gly-OMe			
DMSO- d_6	6.95	6.98	0.03
CD ₃ OD	7.06	-	-
Boc-Gly- Δ Ala(β Cl)-OMe			
DMSO- d_6	7.15	6.76	–0.39
Ac- Δ Ala(β Cl)-OMe ¹⁰			
CDCl ₃	6.92	7.69	0.77

of *N*-methylated Δ Ala(β Cl) resonates at δ = 7.68 ppm in CD₃OD⁵⁵ and for the product of hydrolysis of victorin C at δ = 7.52 ppm in D₂O.⁵⁹ Therefore, in our opinion, the geometry of the β -chlorodehydroalanine residue in victorins cannot be definitely assigned by a simple comparison of the chemical shifts of the vinyl protons. Summing up, the data obtained within this study clearly show that the geometrical configuration *Z/E* of the β -chlorodehydroalanyl moiety in victorins still remains an open question and the structure should be revised in this particular fragment.

In summary, the conformational preferences of the β -chlorodehydroalanine residue, Δ Ala(β Cl), the structural constituent of the family of fungal toxins victorins, were determined. Theoretical analysis, using Ramachandran diagrams ($E = f(\varphi, \psi)$) of short diamide model compounds, Ac-(*Z*)- Δ Ala(β Cl)-NHMe and Ac-(*E*)- Δ Ala(β Cl)-NHMe, in various environments (gas phase, chloroform, water), gives the overall view of possible conformations. The geometrical isomers differ in their conformational profile. The isomer *Z* prefers the helical conformation α ($\varphi, \psi = -61^\circ, -24^\circ$), PPII type conformation β ($\varphi, \psi = -47^\circ, 136^\circ$), and semiextended conformation β_2 ($\varphi, \psi = -116^\circ, 9^\circ$). The isomer *E* prefers mainly the extended conformation C5 ($\varphi, \psi = -177^\circ, 160^\circ$) but with an increase of the environment polarity also conformations β ($\varphi, \psi = -44^\circ, 132^\circ$) and α ($\varphi, \psi = -53^\circ, -39^\circ$). The experimental data and FTIR spectra of Ac-(*Z*)- Δ Ala(β Cl)-NHMe and Ac-(*E*)- Δ Ala(β Cl)-NHMe recorded in chloroform confirm the tendency of the isomer *E* toward conformation C5 and the isomer *Z* to the mixture of conformations where α , β , and β_2 are the most probable. The X-ray single crystal analyses of the following model compounds, Ac-(*Z*)- Δ Ala(β Cl)-NHMe, Cbz-Gly-(*Z*)- Δ Ala(β Cl)-Gly-OMe, Boc-Gly-(*Z*)- Δ Ala(β Cl)-OMe, Ac-(*E*)- Δ Ala(β Cl)-NHMe, and Ac- Δ Ala(β Cl₂)-NHMe, were performed, and in each case, conformation β was found. The N-H...Cl hydrogen bond created between the chlorine atom in the side chain of the Δ Ala(β Cl) residue and the N-H group of the flanking amide bonds is present in conformation β , and it seems to influence its relatively low energy.

The method of synthesis of the Δ Ala(β Cl) residue is proposed. The dehydroalanine substrate undergoes chlorination in dichloromethane in the presence of trimethylamine. The major product is the isomer *Z*. The isomer *E* can be obtained in a photoisomerization reaction. The UV wavelength ($\lambda_{\max} = 366$ nm), time (5 h), and intensity (400–440 μ W/cm²) turn out to be important.

The geometry of the the obtained isomers *Z* and *E* was determined by NMR NOE experiments supported by the X-ray method. It was found that the assignment of the geometrical isomer based on the NMR shift of the vinyl proton is not sufficiently precise to determine the geometry isomers, despite the remarkable effort described in the literature. Therefore, we suggest that the geometrical isomer of the $\Delta\text{Ala}(\beta\text{Cl})$ residue in naturally occurring victorins still remains an open question and should be revised.

The geometrical isomer of the dehydroamino acid residue is crucial for biological activity. In the mentioned example of phytotoxic phomalide, produced by the fungus *Leptosphaeria maculans* and responsible for leaf spot and stem cancer (blackleg), a disease of oilseed Brassicas (e.g., canola), the isomer *E* of the dehydrobutyrine residue with a C-terminal ester is present.⁶⁰ In contrast, the isophomalide with the isomer *Z* is biologically inactive.¹⁰ In another example, tentoxin, a selective weed killer that causes chlorosis of higher plants, the isomer *Z* of *N*-methyldehydrophenylalanine is present. In contrast, isotentoxin with the isomer *E* is biologically inactive.⁶¹ For both dehydroamino acids, the geometrical isomers have different conformational properties.^{62,63} Therefore, it can be assumed that for victorins, the host-selective toxins from the oat pathogen *Cochliobolus victoriae*, not only the presence of the β -chlorodehydroalanine residue is important but also the proper isomer *Z* or *E*. The present study shows that both the geometrical isomers differ in conformational preferences; therefore, they should have different impacts on the native conformation of victorins and, thus, on the biological activity. The proposed method of synthesis and further photoisomerization enable us to gain deeper insight into the molecular function of victorins, e.g. by comparison of the biological activity of semisynthetic isovictorins. It should be also noticed that the chlorovinyl function is a relatively reactive functional group, where the chlorine atom can be effectively changed by a nucleophile.^{32,64,65} This opens a way to apply the β -chlorodehydroalanine residue in peptide design.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.3c01387>.

Theoretical calculations: XYZ structures of the calculated compounds (1 and 2), calculated parameters of hydrogen bonds and dipole interactions in conformations 1 and 2. X-ray crystal parameters and experimental details of the studied compounds 1–5, selected geometric and hydrogen-bond parameters, and molecular interactions for 1–5. FTIR spectra for 1 and 2 in CHCl_3 and theoretical frequencies for 1 and 2. Procedure of synthesis. NMR spectra for 1–5. (PDF)

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Insight into the structure of victorin, the host-selective toxin from the oat pathogen *Cochliobolus victoriae*. Studies of unique dehydroamino acid, β -chlorodehydroalanine

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Figure 32S. ^1H NMR 1D-NOE spectra obtained by selective excitation side chain H atom (blue spectrum) of Cbz-Gly-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-Gly-OMe}$ (3) in DMSO- d_6 .	52
Figure 33S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom and the side chain H atom of Cbz-Gly-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-Gly-OMe}$ (3) in DMSO- d_6 .	53
Figure 34S. ^1H and ^{13}C NMR spectra of Cbz-Gly-(E)- $\Delta\text{Ala}(\beta\text{Cl})\text{-Gly-OMe}$ in DMSO- d_6 .	54
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Figure 38S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the side chain H atom of Boc-Gly-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-OMe}$ (4) in DMSO- d_6 .	57
Figure 39S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom and the side chain H atom of Boc-Gly-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-OMe}$ (4) in DMSO- d_6 .	58
Figure 40S. ^1H and ^{13}C NMR spectra of Boc-Gly-(E)- $\Delta\text{Ala}(\beta\text{Cl})\text{-OMe}$ in DMSO- d_6 .	59
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Table 1S. XYZ structures of the calculated compounds, Ac-(Z)- Δ Ala(β Cl)-NHMe (1) and Ac-(E)- Δ Ala(β Cl)-NHMe (2), along with their electronic energies.

Ac-(Z)- Δ Ala(β Cl)-NHMe (1)	
Gas phase	
conformation C7	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) gas phase C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.159514254 Electronic_Energy_with_ZeroPoint_Correction= -954.004264 Gibbs_Free_Energy= -954.044886 LowF= 38.8627 NImag= 0 N -0.0806596126 0.3092529903 0.1377354803 H -0.1249075957 0.706447957 1.0690642236 C 1.1882538513 0.2953964685 -0.5688564768 H 1.9928026071 0.2375275063 0.1617713898 H 1.2305637526 -0.5732085916 -1.2253188504 H 1.3132684957 1.1909014288 -1.1829139702 C -1.2211060313 0.2709138662 -0.5794856948 O -1.2458514634 0.1634900511 -1.792288385 C -2.5278811602 0.3306919596 0.1952642623 N -2.6384266034 0.015444664 1.5721199527 H -3.4517746608 -0.5380688767 1.8059697986 C -2.0335257725 0.6580312177 2.6226405811 O -1.1389958936 1.4674974261 2.479178896 C -2.5763621383 0.2872343058 3.98219631 H -1.7874522006 0.4201325093 4.7189313635 H -3.3942010291 0.9698119474 4.2250391802 H -2.9521145215 -0.7354792039 4.0177549378 C -3.6199888969 0.5364325725 -0.5402311651 H -3.5621006337 0.6593032014 -1.6112483591 Cl -5.212612271 0.5656538776 0.1256174307
conformation β	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.15847483 Electronic_Energy_with_ZeroPoint_Correction= -954.003727 Gibbs_Free_Energy= -954.043851 LowF= 53.7575 NImag= 0 N 0.0008633743 -0.0684487823 -0.0429468185 H 0.0033834265 -0.1711843786 0.9602953434 C 1.2701045443 -0.1281806765 -0.7493394431 H 1.919345853 -0.8452388718 -0.2501446087 H 1.0928003529 -0.4548004503 -1.772897558 H 1.752554818 0.8521085558 -0.7785876523 C -1.0109856081 0.6629535757 -0.5744822238 O -0.9913117676 1.1563575061 -1.6787018956 C -2.2521366123 0.7551271903 0.2914731342 N -2.8254899689 2.0168427315 0.403203092 H -3.8312358353 2.08664448 0.4544404754 C -2.0488144245 3.1508938886 0.5560529155 O -0.8516780699 3.0784704044 0.7020806186 C -2.8087182854 4.4516879704 0.4942908876 H -2.2370205468 5.2163010093 1.0146471566 H -2.911891619 4.7402132662 -0.5542100911 H -3.8055116866 4.368979975 0.9297512254 C -2.8105846957 -0.3608819499 0.757218383 H -2.4024522294 -1.3424599126 0.5711896826 Cl -4.3098833061 -0.3537057784 1.6295794044
conformation C5	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.156786227 Electronic_Energy_with_ZeroPoint_Correction= -954.001694 Gibbs_Free_Energy= -954.041825 LowF= 53.6749 NImag= 0 N -0.006052635 -0.1569366385 0.018101592 H -0.0681042737 -0.5541338979 0.9415750605 C 1.2953265048 -0.0651289038 -0.6236197985 H 2.0603852601 0.0239629425 0.1452726249 H 1.4978479815 -0.9412437258 -1.2441918899 H 1.3198892301 0.8180970551 -1.2603572873 C -1.1302564308 -0.1090567536 -0.7359781415 O -1.1138382336 0.0434042355 -1.9442117185 C -2.4510991112 -0.2231193852 -0.021968874 N -3.4801450748 -0.67714921 -0.8531910022 H -3.3216253168 -0.4779692285 -1.8338719868 C -4.3068151368 -1.7455385227 -0.5344208732 O -4.267729957 -2.304950703 0.5332600829 C -5.2828050503 -2.1085107847 -1.6295704905

	H -5.6185964917 -3.1304066605 -1.4707548626 H -6.1436410489 -1.4392594314 -1.5662998097 H -4.8442904577 -2.0086926048 -2.6234918738 C -2.6047217749 0.196881294 1.2349387623 H -1.7720654051 0.560932793 1.8187218829 Cl -4.1144505194 0.3072486811 2.0416184852
conformation C5'	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) gas phase C5' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.155098076 Electronic_Energy_with_ZeroPoint_Correction= -954.000178 Gibbs_Free_Energy= -954.041135 LowF= 28.2457 NImag= 0 N -0.0009707544 -0.107518682 -0.0304763801 H 0.0041135914 -0.2393191279 0.9668494471 C 1.2673852629 -0.062926404 -0.7385456353 H 2.00679304 -0.6228718354 -0.1691379114 H 1.1488960352 -0.5184716856 -1.7208343447 H 1.6112122762 0.9649250248 -0.8772387393 C -1.1132038758 0.3940285132 -0.6204699358 O -1.1010598339 0.8721859398 -1.7402172189 C -2.39845714 0.3688537396 0.1706723271 N -3.3853787263 1.1905727258 -0.3734507715 H -3.0164051058 1.9018170191 -0.993097559 C -4.6573997044 0.7238615755 -0.7122968222 O -5.0597464462 -0.3657068764 -0.3982207647 C -5.4714577106 1.7313403851 -1.4908739365 H -6.4231581046 1.2783818819 -1.7555440743 H -5.6431035928 2.6218532705 -0.883353576 H -4.9445042354 2.0326997605 -2.3989612613 C -2.5425957956 -0.3743051709 1.2718520485 H -1.7959435325 -1.0922518908 1.5772749123 Cl -3.8905784165 -0.3013733476 2.3310857204
Chloroform	
conformation α	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) chloroform α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.171778918 Electronic_Energy_with_ZeroPoint_Correction= -954.017304 Gibbs_Free_Energy= -954.058071 LowF= 41.2221 NImag= 0 N -0.0923550601 0.090157349 0.0394705624 H -0.1220834647 0.3269652619 1.0185655395 C 1.1868263734 -0.0547359718 -0.6318616657 H 1.9760429944 0.2374435407 0.0566587059 H 1.3418123501 -1.0897413932 -0.942613295 H 1.2251706629 0.5823752369 -1.5163365729 C -1.2393968596 -0.1874203959 -0.6020081575 O -1.2902965764 -0.5017124629 -1.7821475624 C -2.4947443941 -0.0210162501 0.2187585799 N -2.4138378182 -0.0876129742 1.622092609 H -2.9802985205 0.5628271338 2.1516678103 C -1.9846310638 -1.2330630086 2.2668148936 O -1.5128581312 -2.1665904788 1.6530815549 C -2.1558439003 -1.2139423618 3.7636632794 H -1.498068952 -1.9591363742 4.2042289288 H -1.9426356597 -0.2300778795 4.1817189195 H -3.1906218646 -1.4722405843 4.0012107549 C -3.6120369272 0.2798630965 -0.4380025967 H -3.630424167 0.395892219 -1.5112961189 Cl -5.1206400041 0.5432663878 0.3582515401
conformation β	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.171809467 Electronic_Energy_with_ZeroPoint_Correction= -954.017286 Gibbs_Free_Energy= -954.0578 LowF= 54.3536 NImag= 0 N 0.0216916882 -0.0927684112 -0.0347163912 H 0.038532259 -0.2143426445 0.9660963079 C 1.2673963459 -0.1527297134 -0.7808025849 H 2.0040170511 -0.6881930151 -0.1863551615 H 1.1081305596 -0.6825990087 -1.7195772519 H 1.6386395172 0.849826607 -1.0046502159 C -1.0883004638 0.4167152161 -0.5993010401 O -1.1536096828 0.797162518 -1.7550224377 C -2.31820761 0.4186733013 0.2830617538 N -3.1171605811 1.558516797 0.2331493162

	H -4.119843066 1.4465467324 0.2925013586 C -2.5832331599 2.8274086861 0.2197848903 O -1.3879056335 3.0116507343 0.3284872328 C -3.5824793916 3.9378323311 0.0318843889 H -3.1959944017 4.8431133078 0.4943428805 H -3.7007763652 4.1144109626 -1.039702068 H -4.5580682905 3.6911155325 0.4501779518 C -2.6620475975 -0.6981663072 0.9237307887 H -2.0884020794 -1.6108589142 0.8679710173 Cl -4.1224522359 -0.8229766272 1.8480362516
conformation $\beta 2$	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) chloroform $\beta 2$ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.170489823 Electronic_Energy_with_ZeroPoint_Correction= -954.016167 Gibbs_Free_Energy= -954.057182 LowF= 39.369 NImag= 0 N -0.0276258324 -0.0579360581 0.0271819674 H -0.0451014017 -0.1290639625 1.0325858511 C 1.2429181905 -0.0437207039 -0.6759092633 H 2.0388470248 0.1260248615 0.0453475017 H 1.4154320242 -0.9912113565 -1.1905146596 H 1.2478742308 0.7572521311 -1.415453946 C -1.1874527074 -0.1341602565 -0.6509753002 O -1.2470911314 -0.15835616 -1.8725735891 C -2.4265018517 -0.1852650586 0.2041282376 N -2.3148370011 0.0578009938 1.5915901022 H -2.1248134915 1.0037714628 1.8942462158 C -2.6094930298 -0.8880732756 2.5503022938 O -2.8618609677 -2.0393791016 2.2650866392 C -2.6079615001 -0.36858301 3.9659741285 H -2.3563423558 -1.1836713781 4.6407385099 H -1.9136396076 0.4599465816 4.1049723909 H -3.6161401289 -0.0193336002 4.2018112375 C -3.57078106 -0.5022671318 -0.3973166724 H -3.6044633281 -0.7291518689 -1.4533095033 Cl -5.0801668296 -0.5807950983 0.4167699612
conformation C7	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) chloroform C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.170082292 Electronic_Energy_with_ZeroPoint_Correction= -954.015346 Gibbs_Free_Energy= -954.056713 LowF= 19.5464 NImag= 0 N -0.026832007 0.1592312071 -0.0828054867 H -0.0160541188 0.314777635 0.9163443455 C 1.2403922663 0.0787390408 -0.7902376121 H 1.9804977865 0.6566596756 -0.241387941 H 1.5801665558 -0.9557494747 -0.8811964903 H 1.1284708596 0.4942087798 -1.7912094861 C -1.1275935464 -0.3909773335 -0.6236521348 O -1.1368453301 -0.9184836344 -1.7279067751 C -2.4048929807 -0.2991662534 0.1852070985 N -2.5559214828 0.6053493035 1.2656143429 H -3.3865421598 1.1821797891 1.238530399 C -1.8939373488 0.529495697 2.4600629032 O -0.9537887905 -0.2292224871 2.6317998053 C -2.4063938333 1.442568045 3.542901846 H -1.5580566357 1.8294790811 4.104557726 H -3.0031746408 2.2665429067 3.1548557843 H -3.0224665336 0.8485301248 4.2214469562 C -3.4349402579 -1.0189852835 -0.2575401017 H -3.3580309854 -1.6363821793 -1.1400301928 Cl -4.9827468167 -1.0081407617 0.498394104
conformation C5	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.169485309 Electronic_Energy_with_ZeroPoint_Correction= -954.014854 Gibbs_Free_Energy= -954.055353 LowF= 47.0973 NImag= 0 N 0.0269360115 -0.0369491989 -0.0094965609 H 0.0449354138 -0.0727783901 0.9974899924 C 1.2844044056 -0.0827350603 -0.7393132642 H 2.0526815089 -0.479508991 -0.0801048354 H 1.1828348466 -0.7336625051 -1.6072568587 H 1.5761862848 0.9123358925 -1.081202991 C -1.1102709662 0.3164696527 -0.6330799844

	O -1.1625251232 0.6133435811 -1.8191890773 C -2.3814933853 0.3168870623 0.1769158407 N -3.3578731952 1.1828380144 -0.3329071928 H -3.2966293109 1.3283605215 -1.3337230726 C -4.0017374242 2.1459732481 0.416762248 O -3.8398806049 2.2591730322 1.6135054168 C -4.953591423 3.0121885791 -0.3701233893 H -5.0209293574 3.986040072 0.1098645847 H -4.6509150706 3.1279432235 -1.4104700584 H -5.9404374074 2.5442207451 -0.3483036914 C -2.5639351284 -0.5344262804 1.1866337617 H -1.7848449149 -1.1984904894 1.5308196728 Cl -4.0628086468 -0.7510065964 2.0022494053
conformation C5'	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) chloroform M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.168360511 Electronic_Energy_with_ZeroPoint_Correction= -954.013751 Gibbs_Free_Energy= -954.054719 LowF= 31.4987 NImag= 0 N 0.0071765762 -0.0540448514 -0.0167856792 H 0.0209638145 -0.084828713 0.9900339375 C 1.2698782426 -0.0751628744 -0.7370855939 H 2.0398383109 -0.4658778994 -0.0760462876 H 1.1844647555 -0.7202664062 -1.6111579614 H 1.5495767354 0.9266580575 -1.0699808981 C -1.1328262821 0.278376836 -0.6523356289 O -1.1711143201 0.5866734318 -1.8343743044 C -2.4028567614 0.2704791062 0.1643819571 N -3.4234812981 1.0663055526 -0.3638475468 H -3.1118366738 1.881687204 -0.8751765745 C -4.6680265073 0.5730819986 -0.7229346942 O -5.0132385282 -0.5627565662 -0.4853072479 C -5.5588725223 1.5897417997 -1.3920759022 H -6.3606162297 1.0685607837 -1.9091242433 H -5.9903897667 2.2374940748 -0.6257114466 H -5.0066010022 2.2138661452 -2.0953108998 C -2.5200769635 -0.4752787131 1.2653225392 H -1.75413268 -1.1694335336 1.5781252675 Cl -3.8806521433 -0.4344981889 2.3177697579
Water	
conformation α	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.176427838 Electronic_Energy_with_ZeroPoint_Correction= -954.021976 Gibbs_Free_Energy= -954.062676 LowF= 41.6878 NImag= 0 N -0.1611000086 0.3355663691 -0.1762306103 H -0.3984164304 1.1619196306 0.3509965585 C 1.1904654971 0.1650173432 -0.6795066135 H 1.7868735931 1.0182925502 -0.3666515465 H 1.6317311959 -0.7497438458 -0.2803946906 H 1.1899637871 0.1052390529 -1.7690268204 C -1.1038502378 -0.5913292335 -0.4010235732 O -0.9080131986 -1.6059466119 -1.0588809473 C -2.4645603582 -0.2778857284 0.1696082456 N -2.5570661971 0.5854974477 1.2756415787 H -3.2852706 1.2876991217 1.2710897411 C -1.9444093526 0.2848303838 2.4722115365 O -1.2420147052 -0.7010111268 2.5914067425 C -2.161423646 1.2835248773 3.5771706031 H -1.3141125428 1.9730450029 3.5838863205 H -3.0763597539 1.8600094874 3.4484530371 H -2.1830658572 0.7555216915 4.5283384998 C -3.5224733565 -0.7693210606 -0.4704231317 H -3.4240177031 -1.3698579252 -1.3624087934 Cl -5.1432786316 -0.4665457358 0.0358385225
conformation β	20 Ac-(Z)-ΔAla(βCl)-NHMe (1) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.176220523 Electronic_Energy_with_ZeroPoint_Correction= -954.021786 Gibbs_Free_Energy= -954.062248 LowF= 50.4857 NImag= 0 N -0.1015352387 -0.2689422919 -0.0128895122 H -0.2136112984 -0.5941285319 0.9351231173 C 1.222300243 -0.2575267091 -0.612705465

	H 1.9607206174 -0.3834956611 0.1751903066 H 1.3266689765 -1.065284227 -1.3399984109 H 1.3943446711 0.692733941 -1.1184867725 C -1.1972052673 -0.0530418809 -0.7574545873 O -1.171786815 0.1863835244 -1.9552019868 C -2.5056132613 -0.0487065939 0.0012717046 N -3.5890924597 -0.6728358767 -0.6155864155 H -4.5055919138 -0.2588245515 -0.5108146272 C -3.4771180141 -1.8859808228 -1.2506489681 O -2.4310554628 -2.5076121862 -1.2521388348 C -4.7211438118 -2.3557313476 -1.9557552944 H -4.7371769012 -3.4433462522 -1.9593735218 H -5.6294141899 -1.9697358753 -1.4943150792 H -4.6822743551 -2.0036633516 -2.9892435083 C -2.6082634158 0.6716319562 1.1179979365 H -1.7896641231 1.2413050667 1.5313279251 Cl -4.1010027613 0.8325812328 1.9810501437
conformation $\beta 2$	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) water $\beta 2$ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.175324215 Electronic_Energy_with_ZeroPoint_Correction= -954.020958 Gibbs_Free_Energy= -954.061634 LowF= 39.3618 NImag= 0 N -0.0298507836 -0.0532750695 0.0256033313 H -0.0475391923 -0.1145165694 1.0320173887 C 1.2431152432 -0.044688107 -0.6738325743 H 2.0415544166 0.0307126287 0.0600028163 H 1.3711727124 -0.9593186115 -1.255676823 H 1.2930187259 0.8085095556 -1.3512250416 C -1.1888302771 -0.0710051177 -0.6511810191 O -1.2499679836 -0.066738443 -1.8756759071 C -2.4308159011 -0.0988493038 0.2012878388 N -2.3110888201 0.0970531837 1.5944261934 H -2.0501408544 1.0164881326 1.9260270554 C -2.6383118358 -0.8624471574 2.5244339445 O -2.9586307617 -1.9903461683 2.2040389743 C -2.586028861 -0.3948741828 3.9558916952 H -2.3675449752 -1.2449598778 4.5982361312 H -1.8456448593 0.3896687064 4.1092573938 H -3.5694193346 0.0005087544 4.2217048616 C -3.5864351447 -0.3496122638 -0.4098475777 H -3.6304021895 -0.5433095157 -1.4718691922 Cl -5.0996592831 -0.3882354494 0.4016757722
conformation C5	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.173432608 Electronic_Energy_with_ZeroPoint_Correction= -954.018863 Gibbs_Free_Energy= -954.059341 LowF= 48.0322 NImag= 0 N -0.044095742 -0.0216915465 0.0017265162 H -0.0647990298 -0.0651863199 1.0086353373 C 1.2389091941 0.1145869103 -0.6706674656 H 2.0272972428 -0.1069425127 0.0440919712 H 1.2991896923 -0.5870575354 -1.5025393673 H 1.3713262479 1.1276223476 -1.0551616728 C -1.1913429661 0.1297887949 -0.6760272631 O -1.2389093882 0.3970330919 -1.8714755682 C -2.4826260123 -0.0651860433 0.0777638218 N -3.5611913447 0.6303025698 -0.4867992942 H -3.4902532595 0.7623157213 -1.4888781739 C -4.3825164851 1.4853530626 0.2138596606 O -4.2939748325 1.6390403128 1.4162037727 C -5.425090298 2.17866239 -0.6261050981 H -5.6446870117 3.1487080564 -0.1851336797 H -5.1142725458 2.3013973154 -1.6628936166 H -6.335411171 1.5749633199 -0.6079095549 C -2.5734871721 -0.9281734045 1.0895869469 H -1.7171916518 -1.4582196406 1.4798390339 Cl -4.0578877811 -1.3737542063 1.8393185736
conformation C5'	20 Ac-(Z)- Δ Ala(β Cl)-NHMe (1) water C5' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.172825942 Electronic_Energy_with_ZeroPoint_Correction= -954.018196 Gibbs_Free_Energy= -954.058805 LowF= 43.1216 NImag= 0

	N -0.003908442 -0.0174103896 -0.0043615589 H 0.0084152073 -0.0549958728 1.002827426 C 1.2601442381 -0.0178872139 -0.7228868366 H 2.0400229735 -0.3666416873 -0.0506127545 H 1.1982146003 -0.68737514 -1.5806555675 H 1.5086018636 0.9842484661 -1.0783295799 C -1.1530642511 0.2674780394 -0.6399639871 O -1.2093763252 0.5564671892 -1.8280888869 C -2.4174156807 0.2203316461 0.1840744396 N -3.4515611193 1.0371543321 -0.2881144718 H -3.168288608 1.9278237811 -0.6767327272 C -4.6733448032 0.5528228896 -0.7139888176 O -4.996463934 -0.6095011403 -0.5826335649 C -5.5525460888 1.5962052035 -1.356497363 H -6.5459993377 1.1811599443 -1.5046516747 H -5.6103387814 2.4912607564 -0.7362152651 H -5.1333817876 1.8791097689 -2.3248229181 C -2.5252632631 -0.5930359963 1.2358706834 H -1.7491366682 -1.2916535922 1.5122373674 Cl -3.8961786508 -0.6378926242 2.2773349627
Ac-(E)-ΔAla(βCl)-NHMe (2)	
Gas phase	
conformation C5	20 Ac-(E)-ΔAla(βCl)-NHMe (2) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.161092043 Electronic_Energy_with_ZeroPoint_Correction= -954.005897 Gibbs_Free_Energy= -954.047138 LowF= 26.294 NImag= 0 N -0.0032793582 -0.0006045445 -0.0027396253 H -0.0092963423 -0.0021292609 1.002772259 C 1.2580569159 -0.0012411196 -0.7241344796 H 2.0704223613 -0.0061253993 -0.0005178526 H 1.3313671844 -0.8829756293 -1.3624285429 H 1.3362415115 0.8847297491 -1.3559884358 C -1.1559715899 0.0034359939 -0.6908150669 O -1.1751815456 0.0063526952 -1.9136926592 C -2.4850998092 0.0044066577 0.0521714756 N -3.5157698231 0.0081332042 -0.8959947181 H -3.1604178202 0.0092324793 -1.8459247917 C -4.8687743925 0.0072194409 -0.6830421297 O -5.3733873228 0.0062493369 0.4210825331 C -5.7073063154 0.0009551019 -1.9416716521 H -6.3997836527 0.8414679153 -1.8940437474 H -5.1195521983 0.0635952263 -2.8567838263 H -6.2979100541 -0.9159939099 -1.9529672946 C -2.7344495859 0.002589543 1.3690459862 H -3.7428705232 0.0039457518 1.7468901553 Cl -1.5510075901 -0.0019784455 2.6419226581
conformation β	20 Ac-(E)-ΔAla(βCl)-NHMe (2) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.15218243 Electronic_Energy_with_ZeroPoint_Correction= -953.997834 Gibbs_Free_Energy= -954.038778 LowF= 39.1947 NImag= 0 N -0.0280826819 0.0915309083 0.037849112 H -0.06047592 0.2779372447 1.0275767277 C 1.2475506272 0.1019115091 -0.6542366238 H 2.038448549 -0.1164074108 0.060798237 H 1.2427397207 -0.6614309493 -1.4315791468 H 1.4317019378 1.0717849934 -1.1227951662 C -1.1748282158 0.1760096079 -0.6623945661 O -1.2366896423 0.1753385894 -1.8751211002 C -2.4534791127 0.2639272239 0.1490680663 N -3.3706364147 1.234514824 -0.3128761677 H -4.2304291099 0.9117739145 -0.7305715432 C -2.9194169457 2.5029886088 -0.6169699431 O -1.8289371593 2.87964581 -0.2550292569 C -3.8711506736 3.3464333685 -1.427239072 H -3.65399537 4.3960744033 -1.2441058853 H -3.7013296606 3.1315926686 -2.4850216581 H -4.9145625364 3.133746937 -1.190870791 C -2.8251900599 -0.5496208747 1.1343182383 H -3.7763336046 -0.4310789907 1.6352465146 Cl -1.9181917578 -1.909919342 1.6995863115

conformation C7	20 Ac-(E)-ΔAla(βCl)-NHMe (2) gas phase C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.151119295 Electronic_Energy_with_ZeroPoint_Correction= -953.996624 Gibbs_Free_Energy= -954.038459 LowF= 19.0045 NImag= 0 N -0.0116226052 -0.1290276259 0.0771708269 H -0.0270867856 -0.1591357449 1.0893384335 C 1.2353806107 -0.0426696457 -0.6599191883 H 2.061540045 -0.0658823765 0.0477190423 H 1.3265587926 -0.8805543123 -1.3531634151 H 1.2773412565 0.8821068261 -1.2387388608 C -1.1873675901 -0.1224077248 -0.5796845788 O -1.2949781493 -0.0550364276 -1.7883301404 C -2.4114526049 -0.1832890106 0.3156745879 N -2.7135007221 0.9627835242 1.1135153656 H -3.4014230201 1.6138801879 0.7658509686 C -2.0146211381 1.2724114435 2.2482552061 O -1.1213960453 0.563146307 2.6693302837 C -2.4146172291 2.5568742758 2.9330594848 H -1.6438220617 3.3042907257 2.735905551 H -3.3775366082 2.9410606038 2.5972773595 H -2.4420985182 2.3783580325 4.0067981568 C -3.2479537067 -1.2118016651 0.3391461979 H -4.1293263433 -1.2219127745 0.9664399653 Cl -3.0264160318 -2.6352004018 -0.6024468372
conformation β2	20 Ac-(E)-ΔAla(βCl)-NHMe (2) gas phase β2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.149505838 Electronic_Energy_with_ZeroPoint_Correction= -953.994663 Gibbs_Free_Energy= -954.036003 LowF= 18.8032 NImag= 0 N 0.0645580403 -0.1463808406 -0.009786747 H 0.1592285226 -0.4116193419 0.9595242649 C 1.2673041912 -0.0242522437 -0.8186522739 H 2.0811760986 0.3337236599 -0.1906121469 H 1.5444754384 -0.9784329076 -1.2733190749 H 1.0853533318 0.6929427748 -1.6176565036 C -1.1280037372 -0.4166585896 -0.6225070529 O -1.2624786737 -0.4344678401 -1.8226447419 C -2.2741183549 -0.6157289867 0.3448471759 N -2.3429087615 0.3962445717 1.3242301868 H -1.7718864832 1.2091629618 1.1466700127 C -3.1897331848 0.4470214396 2.4092431424 O -3.9576950468 -0.445989988 2.68588313 C -3.1001838219 1.7209634265 3.2199958139 H -3.2190714751 1.4661694675 4.271525888 H -2.1641111388 2.2605847767 3.0741347001 H -3.9300411245 2.3705176781 2.9338081642 C -3.1039972925 -1.6589044282 0.2745576023 H -3.9168306376 -1.8093866049 0.964505916 Cl -2.9215711365 -2.914393871 -0.8994804971
conformation α	20 Ac-(E)-ΔAla(βCl)-NHMe (2) gas phase α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.147460081 Electronic_Energy_with_ZeroPoint_Correction= -953.992905 Gibbs_Free_Energy= -954.033653 LowF= 37.1103 NImag= 0 N -0.0213968558 -0.0206462096 -0.0057655077 H -0.0285952812 -0.0422777244 1.0007927761 C 1.2521982946 0.0136581339 -0.7011653231 H 1.9976942632 -0.505305268 -0.1011083985 H 1.1498455767 -0.4887313645 -1.662095737 H 1.5749189835 1.0425700367 -0.8815845003 C -1.1390411468 0.4122948102 -0.6438892631 O -1.1801111157 0.6551815398 -1.828556006 C -2.364001648 0.510476167 0.2361524164 N -2.1689602709 0.8699404017 1.5958788882 H -2.7719931962 0.4395144711 2.2813166063 C -1.5027489227 2.0302333586 1.9615418281 O -0.8998924406 2.6982009456 1.1583848194 C -1.5958611313 2.3722880028 3.4309994023 H -0.7312227219 2.9730948404 3.7037219716 H -2.4957933881 2.9700135925 3.5944276441 H -1.6480132484 1.4846343524 4.0626630143 C -3.5973277466 0.216806026 -0.1738269211

	H -4.4445648854 0.3288787529 0.4912309837 Cl -4.025525077 -0.4153905454 -1.7120023451
Chloroform	
conformation C5	20 Ac-(E)-ΔAla(βCl)-NHMe (2) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.169746411 Electronic_Energy_with_ZeroPoint_Correction= -954.014752 Gibbs_Free_Energy= -954.055501 LowF= 29.7346 NImag= 0 N -0.0197190252 -0.1058132276 -0.0119511207 H -0.044988163 -0.3291269759 0.9690525132 C 1.2565976444 -0.0994401283 -0.7093528708 H 2.0436802136 -0.2944465028 0.0146609422 H 1.2746271228 -0.8680779461 -1.483518 H 1.4265167804 0.870303297 -1.1782148227 C -1.1511838973 0.1366007901 -0.6822624094 O -1.1600960609 0.3591909475 -1.8889390206 C -2.4831278431 0.1071129893 0.0528276341 N -3.5140203676 0.048021428 -0.8953536198 H -3.1698665225 0.1086226625 -1.8470939723 C -4.8617730536 -0.0334475061 -0.68733004 O -5.3645461118 -0.0723635038 0.4228617756 C -5.6978630467 -0.0947485918 -1.9426876492 H -6.5112405782 0.6241821435 -1.849355345 H -5.1310184522 0.1089983905 -2.8496133827 H -6.1363870276 -1.0917969404 -2.0118840641 C -2.7266933475 0.1624566318 1.3693335846 H -3.7312500691 0.1503075423 1.7553148248 Cl -1.5337420869 0.2889306519 2.6250078267
conformation β	20 Ac-(E)-ΔAla(βCl)-NHMe (2) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.166358734 Electronic_Energy_with_ZeroPoint_Correction= -954.012118 Gibbs_Free_Energy= -954.052688 LowF= 47.3827 NImag= 0 N 0.0159344143 -0.0614983575 -0.0383449595 H 0.0247554985 -0.1719773187 0.9636297885 C 1.2672670768 -0.069077374 -0.7754398631 H 2.0642437907 -0.37852192 -0.1036328932 H 1.2070750536 -0.768435284 -1.6099980703 H 1.4889357881 0.9252179175 -1.1686385153 C -1.1389927179 0.2377046954 -0.6477621699 O -1.2384452299 0.4874265025 -1.8381642164 C -2.3771334156 0.2282586791 0.2264414912 N -3.2106779126 1.3522700134 0.090905315 H -4.2058924042 1.2091307063 0.0032421426 C -2.7089731704 2.6284888865 0.0435626942 O -1.5255480157 2.8490364376 0.2146670978 C -3.7160272084 3.7059994058 -0.2634944669 H -3.4016377997 4.6291275439 0.2185646581 H -3.7284817486 3.8645813716 -1.344243166 H -4.722227201 3.4402678319 0.0596922712 C -2.7861461329 -0.8008445586 0.9672142318 H -3.7066069131 -0.768671782 1.5340994115 Cl -1.9821277359 -2.332338995 1.0559836763
conformation α	20 Ac-(E)-ΔAla(βCl)-NHMe (2) chloroform α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.164624522 Electronic_Energy_with_ZeroPoint_Correction= -954.010348 Gibbs_Free_Energy= -954.051228 LowF= 38.1378 NImag= 0 N -0.0227839625 -0.057261555 -0.012933627 H -0.0330497985 -0.1462593625 0.9911023167 C 1.2443423897 -0.0253986848 -0.7209778154 H 2.0356159254 -0.3131291296 -0.0327550085 H 1.2243367464 -0.7229114149 -1.5588505958 H 1.4452342662 0.9762512991 -1.1074710365 C -1.1746531054 0.2090052412 -0.6577373853 O -1.2432334041 0.3962074541 -1.8600572238 C -2.3983791943 0.2196842295 0.2279605908 N -2.2448654641 0.7632098618 1.5254954419 H -2.7251698807 0.3120501976 2.2914134028 C -1.7443858866 2.0324134142 1.7218606803 O -1.3170282872 2.6930424699 0.7974312043 C -1.7792694266 2.5221182648 3.1465755872

	H -0.9481307756 3.2065662525 3.3028706791 H -2.7113931217 3.0711586025 3.2989435236 H -1.732700433 1.7064558608 3.8672746701 C -3.5694716037 -0.313179135 -0.1151658905 H -4.4239956572 -0.2708229854 0.5471048015 Cl -3.8757887828 -1.1904598591 -1.5676128164
conformation C7	20 Ac-(E)-ΔAla(βCl)-NHMe (2) chloroform C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.164378798 Electronic_Energy_with_ZeroPoint_Correction= -954.010048 Gibbs_Free_Energy= -954.051637 LowF= 24.2276 NImag= 0 N -0.0124352556 -0.0530041219 0.0570149683 H -0.0239108524 -0.0732983078 1.0705658287 C 1.2398899178 -0.0223530514 -0.6783880412 H 2.0605960481 -0.0234842536 0.0348737127 H 1.3243818381 -0.895044846 -1.3280845384 H 1.2986745686 0.8751159224 -1.2966163317 C -1.1869097608 -0.0470830416 -0.5895690312 O -1.2959845061 -0.032444081 -1.8069317414 C -2.4107408038 -0.0367510434 0.3058417219 N -2.6084979658 1.0910655984 1.1591215785 H -3.303833555 1.7717735174 0.8884800513 C -1.9216213775 1.2698090042 2.3216235388 O -1.070278574 0.4752475753 2.6949048316 C -2.2647553537 2.5064920084 3.1096252548 H -1.3852494517 3.1516475538 3.1317010975 H -3.1065598808 3.0589871084 2.6957378766 H -2.4887797066 2.2089992042 4.1338456958 C -3.3421942173 -0.9815298534 0.2834131825 H -4.2206755237 -0.9389113752 0.9130556261 Cl -3.2639019972 -2.377444836 -0.7266666687
conformation β2	20 Ac-(E)-ΔAla(βCl)-NHMe (2) chloroform β2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.163997806 Electronic_Energy_with_ZeroPoint_Correction= -954.009433 Gibbs_Free_Energy= -954.051019 LowF= 19.0846 NImag= 0 N 0.0349851546 -0.1732363132 -0.0488692946 H 0.1012247473 -0.483564788 0.9092579976 C 1.2502034671 -0.0278685159 -0.8347105688 H 2.0983970943 0.0254147397 -0.1563620045 H 1.3800465467 -0.8714237854 -1.5156091359 H 1.1989234055 0.8886580961 -1.4219968862 C -1.1769180656 -0.1390069611 -0.6418501652 O -1.3369801987 0.0631590286 -1.8310471422 C -2.3388428556 -0.318233171 0.3142959941 N -2.300844373 0.5790077873 1.3950950766 H -1.6274576166 1.3285573865 1.3217684062 C -3.120455833 0.5848285374 2.4974927384 O -3.9869898084 -0.2498739202 2.6708368874 C -2.8790673208 1.7184556305 3.4632275618 H -2.9962795516 1.3393163816 4.4769290427 H -1.8963377098 2.1742615937 3.3503777961 H -3.6427668062 2.4805760735 3.2942614329 C -3.2751256468 -1.2515743881 0.1305215558 H -4.110462581 -1.3979930224 0.7924725272 Cl -3.2060398224 -2.3827089108 -1.1839501739
Water	
conformation C5	20 Ac-(E)-ΔAla(βCl)-NHMe (2) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.172459818 Electronic_Energy_with_ZeroPoint_Correction= -954.017642 Gibbs_Free_Energy= -954.058533 LowF= 25.6187 NImag= 0 N 0.0060406824 -0.0350162675 -0.0019503024 H 0.0107109964 -0.089536617 1.0037870187 C 1.2725971428 -0.0240041891 -0.7177387387 H 2.0737655752 -0.1714680172 0.0016070446 H 1.2954521133 -0.8265126807 -1.4555104002 H 1.4146091355 0.9280608424 -1.231518205 C -1.1459933497 0.0962750801 -0.6642410101 O -1.1946899581 0.2472558977 -1.8819545754 C -2.4502984796 0.106953469 0.1151459162 N -3.4234671684 0.7756321061 -0.6414584588

	H -3.1029961271 1.0153120854 -1.5725383461 C -4.7154172983 1.0609339628 -0.3044278669 O -5.1990376596 0.7718814208 0.7786294321 C -5.5182942263 1.7516031329 -1.3787282427 H -6.0641465355 2.5776908298 -0.9244941676 H -4.9079737741 2.1228334423 -2.2002123615 H -6.2472433007 1.0393972181 -1.7701431947 C -2.7163029382 -0.4548993414 1.3013947472 H -3.6948367821 -0.4024936432 1.7458546353 Cl -1.5903043441 -1.3624642422 2.2629896507
conformation β	20 Ac-(E)- Δ Ala(β Cl)-NHMe (2) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.171095493 Electronic_Energy_with_ZeroPoint_Correction= -954.016843 Gibbs_Free_Energy= -954.057366 LowF= 47.3011 NImag= 0 N 0.0412125016 -0.1991745347 -0.1513170098 H 0.0597148914 -0.6281665046 0.761173191 C 1.242622318 -0.1957838211 -0.9680015691 H 2.0029009375 -0.7899799895 -0.4674769832 H 1.0349861183 -0.6272575548 -1.9480363211 H 1.6114346346 0.8225813544 -1.1049506713 C -1.0697638869 0.4296591781 -0.5513851865 O -1.1681806755 1.0353108864 -1.6092678649 C -2.2608003451 0.3357636069 0.379673169 N -2.9110929742 1.5523939412 0.6493686468 H -3.9208346516 1.5658950199 0.6620109 C -2.2269016148 2.7053535527 0.9304137114 O -1.0120089978 2.7165997515 1.0212637962 C -3.0764427965 3.9399728129 1.078272348 H -2.6021664888 4.6104407004 1.7918436576 H -3.1235075328 4.4414183226 0.1088685729 H -4.0919071682 3.7113352722 1.3997026585 C -2.8011257877 -0.8019271991 0.8151694113 H -3.6844682764 -0.8157923064 1.438953931 Cl -2.2469593696 -2.3842308919 0.3796951139
conformation α	20 Ac-(E)- Δ Ala(β Cl)-NHMe (2) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.17072048 Electronic_Energy_with_ZeroPoint_Correction= -954.016516 Gibbs_Free_Energy= -954.0574 LowF= 35.7979 NImag= 0 N 0.0311431741 0.1127804201 0.0364683434 H 0.0115296535 0.2495473621 1.0356939812 C 1.3064285352 0.02757178 -0.6530034626 H 2.1028790733 0.0561139166 0.0863126857 H 1.3729686568 -0.9029950624 -1.2183140096 H 1.4239430191 0.8646895541 -1.3439587587 C -1.1257660837 0.099033047 -0.6457948675 O -1.1914365937 -0.041491105 -1.8579489163 C -2.3591630461 0.2439871408 0.2143037096 N -2.2822220338 1.1581867475 1.2891467098 H -2.6887800663 0.8954787811 2.1761292547 C -1.8816305267 2.4587665258 1.0998366845 O -1.5459122084 2.8605493365 0.00070933 C -1.8381307214 3.3094376091 2.3408822739 H -0.8351068784 3.2415853206 2.7685289742 H -2.0274288834 4.3443851786 2.0643207596 H -2.5573140044 2.9860136964 3.0923810423 C -3.4623166072 -0.4854664825 0.0614156058 H -4.3280349724 -0.3441022969 0.6944059456 Cl -3.6416489591 -1.781942967 -1.0648258202
conformation $\beta 2$	20 Ac-(E)- Δ Ala(β Cl)-NHMe (2) water $\beta 2$ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.168891211 Electronic_Energy_with_ZeroPoint_Correction= -954.01431 Gibbs_Free_Energy= -954.055626 LowF= 25.2846 NImag= 0 N 0.0264306313 -0.1664739822 -0.0576812812 H 0.0845083726 -0.4687620054 0.9038854364 C 1.2496566423 -0.0215298197 -0.8314992907 H 2.0947416166 -0.0372717379 -0.1480156115 H 1.3514386325 -0.833032291 -1.5544114181 H 1.2365477678 0.9269825857 -1.3682834075 C -1.1797334521 -0.0709005475 -0.644616136

	O -1.3362683389 0.1647804949 -1.8313555145 C -2.3501256551 -0.2316647476 0.3051736891 N -2.2807507971 0.6293174851 1.4116647558 H -1.5711264689 1.3478802225 1.3697084407 C -3.0985851923 0.6304921939 2.5140538448 O -4.0030445413 -0.1723039768 2.6551987985 C -2.808568657 1.7139151605 3.5211188468 H -2.9444152256 1.3032641925 4.5201204586 H -1.8061211438 2.1278321106 3.4247676334 H -3.5361389216 2.5159974729 3.379525349 C -3.3229428312 -1.1204805127 0.0904892249 H -4.1668063061 -1.2538778826 0.7438237989 Cl -3.2942341027 -2.2177198645 -1.256493084
conformation C7	20 Ac-(E)-ΔAla(βCl)-NHMe (2) water C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -954.168567286 Electronic_Energy_with_ZeroPoint_Correction= -954.014132 Gibbs_Free_Energy= -954.054844 LowF= 33.8518 NImag= 0 N -0.0095091606 -0.0168163908 0.0478226987 H -0.0168523992 -0.024785539 1.0619250952 C 1.2425618426 -0.0109617566 -0.6893558945 H 2.0630826231 0.0226587399 0.0230068547 H 1.3332014028 -0.9097817684 -1.3016677256 H 1.2959238366 0.8611854161 -1.3429407909 C -1.185163794 -0.0251871913 -0.5930995297 O -1.2968329849 -0.0471052423 -1.8124694339 C -2.4061194601 0.018459022 0.3039513631 N -2.5610755064 1.1386150271 1.175927641 H -3.2471169704 1.8374836285 0.927132631 C -1.8825684867 1.2661644045 2.3479763446 O -1.0526732859 0.4381857028 2.7040434806 C -2.2080582475 2.4811250405 3.1742717528 H -1.2902764473 3.0507614869 3.3233317112 H -2.9623437954 3.1194149415 2.7179377156 H -2.5581366165 2.1452530799 4.1509212361 C -3.3739243396 -0.889418839 0.2714427974 H -4.2478514186 -0.8208787712 0.9051093648 Cl -3.356767564 -2.2762033499 -0.756002424

Table 2S. Structural parameters for the internal hydrogen bond X–H···A (X = N, C; A = O, Cl) and C=O ◀···▶ O=C interactions in the M06-2X/6-311+G(d,p) conformers of Ac-(Z)-ΔAla(βCl)-NHMe (**1**) in various environment.

Ac-(Z)-ΔAla(βCl)-NHMe (1)																
	C7		β			C5			C5'			α		β2		
	Gas Phase	Chloroform	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Chloroform	Water	Chloroform	Water	
N ^N -H···O ^C r H···O r N···C ∠N-H···O ∠C=O···H N ^C -H···O ^N r H···O r N···C ∠N-H···O ∠C=O···H N ^N -H···Cl r H···Cl r N···Cl ∠N-H···Cl ∠C-Cl···H C ^B -H···O ^C r H···O r C···O ∠C-H···O ∠C=O···H	Hydrogen bond															
						2.27	2.30	2.31	2.30	2.52						
						2.42	2.43	2.43	2.42	2.44						
						104.1	102.7	102.2	100.8	90.9						
						84.8	84.9	84.9	82.5	77.6						
	1.90	2.03														
	3.18	3.18														
	149.8	142.1														
	102.4	94.0														
	2.67	2.81	2.75	2.75	2.75							2.79	2.84			
	3.00	3.01	3.05	3.05	3.04							3.05	3.05			
	99.1	91.7	97.7	97.2	97.1							95.1	92.5			
	68.1	67.0	68.8	68.8	68.8							68.1	67.7			
	2.38	2.41										2.52	2.55	2.46	2.46	
	2.71	2.73										2.79	2.81	2.77	2.77	
	96.0	95.4										93.0	92.5	95.0	95.0	
	85.7	85.1										81.0	80.2	83.2	83.3	
	C=O ◀···▶ O=C dipole interaction															
3.29	3.26	2.74	2.77	2.79								3.01	3.00			
		3.18	3.17	3.18												
3.33	3.31	2.92	2.95	2.97								3.14	3.12			
4.47	4.42	3.06	3.05	3.06								3.82	3.78			
81.3	81.3	86.5	86.2	86.0								84.7	84.2			
11.3	12.0	67.0	68.5	68.9								27.8	28.7			
80.2	78.5	73.6	73.1	73.1								65.1	64.4			
163.7	157.2	93.7	91.1	90.4								123.4	120.9			
Type	I		II									III				
Data presented only for X–H···A (X = N, C; A = O, Cl) in which r H···X ≤ 2.7 Å, r H···Cl ≤ 3.3 Å and ∠X–H···A > 90° (Vargas 2002)																
Data presented only for the C=O ◀···▶ O=C contacts in which r C···O < 3.6 Å (Allen 1998)																
N, C denote the N-terminal or the C-terminal amide group																

Table 3S. Structural parameters for the internal hydrogen bond X–H···A (X = N, C; A = O, Cl) and C=O ◀···▶ O=C interactions in the M06-2X/6-311+G(d,p) conformers of Ac-(E)-ΔAla(βCl)-NHMe (2) in various environment.

Ac-(E)-ΔAla(βCl)-NHMe (2)															
	C5			β			C7			β2			α		
	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water
N ^N -H···O ^C <i>r</i> H···O <i>r</i> N···C ∠N-H···O ∠C=O···H N ^C -H···O ^N <i>r</i> H···O <i>r</i> N···C ∠N-H···O ∠C=O···H N ^C -H···Cl <i>r</i> H···Cl <i>r</i> N···Cl ∠N-H···Cl ∠C-Cl···H C ^β -H···O ^N <i>r</i> H···O <i>r</i> C···O ∠C-H···O ∠C=O···H <i>r</i> O ^N ···C ^C <i>r</i> O ^C ···C ^N <i>r</i> C ^N ···C ^C <i>r</i> O ^N ···O ^C ∠(C=O) ^N ···C ^C ∠(C=O) ^C ···C ^N ∠O ^C ···(C=O) ^N ∠O ^N ···(C=O) ^C Type	Hydrogen bond														
	1.99	2.03	2.08												
	2.37	2.37	2.38												
	112.5	111.3	109.8												
	88.9	88.0	86.2												
							2.05	2.01	2.00						
							3.27	3.24	3.23						
							140.1	144.0	145.5						
							109.3	107.1	105.6						
	2.25	2.31	2.40	2.95	2.95	2.92									
	3.06	3.07	3.07	3.22	3.22	3.21									
	137.1	130.9	123.3	96.1	96.1	97.1									
	86.2	84.5	82.2	70.5	70.5	70.8									
						</									

Table 4S. Crystal Parameters and Experimental Details of X-Ray Data Collection for the Studied Compounds.

	Ac-(Z)-ΔAla(βCl)-NHMe (1)	Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3)	Boc-(Z)-ΔAla(βCl)-OMe (4)	Ac-(E)-ΔAla(βCl)-NHMe (2)	Ac-ΔAla(βCl ₂)-NHMe (5)
Chemical formula	C ₆ H ₉ ClN ₂ O ₂	C ₁₆ H ₁₈ ClN ₃ O ₆	C ₁₁ H ₁₇ ClN ₂ O ₅	C ₆ H ₉ ClN ₂ O ₂	C ₆ H ₈ Cl ₂ N ₂ O ₂
<i>M_r</i>	176.60	383.78	292.71	176.60	211.04
Crystal system, space group	Monoclinic, <i>P</i> 2 ₁ / <i>c</i>	Triclinic, <i>P</i> $\bar{1}$	Triclinic, <i>P</i> $\bar{1}$	Triclinic, <i>P</i> $\bar{1}$	Monoclinic, <i>P</i> 2 ₁ / <i>c</i>
<i>a</i> , <i>b</i> , <i>c</i> (Å)	13.0735 (3), 14.2235 (2), 9.1292 (2)	4.9591 (1), 12.7541 (4), 14.7235 (8)	8.8797 (5), 9.3366 (5), 9.6867 (6)	8.7340 (7), 8.7933 (7), 11.7526 (10)	6.8554 (1), 15.3174 (3), 8.8543 (2)
α , β , γ (°)	90, 100.795 (2), 90	76.590 (4), 88.533 (3), 79.015 (2)	84.211 (5), 76.033 (5), 65.201 (5)	91.920 (7), 91.512 (7), 115.592 (8)	90, 99.030 (2), 90
<i>V</i> (Å ³)	1667.54 (6)	889.09 (6)	707.48 (8)	812.76 (13)	918.24 (3)
<i>Z</i>	8	2	2	4	4
μ (mm ⁻¹)	3.71	2.26	2.57	3.81	6.09
Crystal size (mm)	0.2 × 0.15 × 0.1	0.15 × 0.12 × 0.10	0.20 × 0.17 × 0.15	0.30 × 0.22 × 0.15	0.22 × 0.16 × 0.10
<i>T</i> _{min} , <i>T</i> _{max}	0.907, 1.000	0.754, 1.000	0.908, 1.000	0.694, 1.000	0.662, 1.000
No. of measured, independent and observed [<i>I</i> > 2σ(<i>I</i>)] reflections	10717, 3196, 3093	9575, 3352, 2774	7449, 2664, 2519	11162, 3119, 2954	4949, 1755, 1663
<i>R</i> _{int}	0.015	0.034	0.017	0.024	0.013
(sin θ /λ) _{max} (Å ⁻¹)	0.621	0.622	0.623	0.624	0.621
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.041, 0.100, 1.08	0.069, 0.198, 1.05	0.028, 0.073, 1.07	0.115, 0.283, 1.16	0.026, 0.069, 1.05
No. of reflections	3196	3352	2664	3119	1755
No. of parameters	217	244	184	203	115
Δρ _{max} , Δρ _{min} (e Å ⁻³)	1.22, -0.49	1.37, -0.40	0.28, -0.28	1.51, -0.74	0.25, -0.30

Table 5S. Bond distances (Å), bond angles (°), and torsion angles (°) of the studied compounds as determined by X-ray method.

Ac-(Z)-ΔAla(βCl)-NHMe (1)			
Cl1—C3	1.720 (2)	O1—C1	1.233 (3)
Cl1'—C3'	1.722 (2)	O1'—C1'	1.238 (3)
N1—C1	1.323 (3)	O2—C4	1.233 (3)
N1—C6	1.445 (3)	O2'—C4'	1.232 (3)
N1'—C1'	1.329 (3)	C1—C2	1.517 (3)
N1'—C6'	1.450 (3)	C1'—C2'	1.511 (3)
N2—C4	1.345 (3)	C2—C3	1.328 (3)
N2—C2	1.402 (3)	C2'—C3'	1.331 (3)
N2'—C4'	1.357 (3)	C4—C5	1.518 (3)
N2'—C2'	1.400 (3)	C4'—C5'	1.501 (3)
C1—N1—C6	120.8 (2)	N2—C2—C1	116.40 (18)
C1'—N1'—C6'	122.9 (2)	C3'—C2'—N2'	123.69 (19)
C4—N2—C2	120.83 (19)	C3'—C2'—C1'	118.70 (19)
C4'—N2'—C2'	121.79 (19)	N2'—C2'—C1'	116.81 (18)
O1—C1—N1	125.1 (2)	C2—C3—Cl1	122.89 (18)
O1—C1—C2	118.91 (19)	C2'—C3'—Cl1'	122.53 (18)
N1—C1—C2	115.94 (19)	O2—C4—N2	121.8 (2)
O1'—C1'—N1'	124.7 (2)	O2—C4—C5	121.4 (2)
O1'—C1'—C2'	120.36 (19)	N2—C4—C5	116.80 (19)
N1'—C1'—C2'	114.90 (18)	O2'—C4'—N2'	121.3 (2)
C3—C2—N2	123.7 (2)	O2'—C4'—C5'	122.8 (2)
C3—C2—C1	119.2 (2)	N2'—C4'—C5'	115.95 (19)
C6—N1—C1—O1	-3.7 (3)	O1'—C1'—C2'—C3'	129.0 (2)
C6—N1—C1—C2	174.4 (2)	N1'—C1'—C2'—C3'	-48.0 (3)

C6'—N1'—C1'—O1'	−3.6 (4)	O1'—C1'—C2'—N2'	−41.2 (3)
C6'—N1'—C1'—C2'	173.3 (2)	N1'—C1'—C2'—N2'	141.8 (2)
C4—N2—C2—C3	142.3 (2)	N2—C2—C3—Cl1	−7.8 (3)
C4—N2—C2—C1	−47.5 (3)	C1—C2—C3—Cl1	−177.74 (15)
O1—C1—C2—C3	137.9 (2)	N2'—C2'—C3'—Cl1'	−4.1 (3)
N1—C1—C2—C3	−40.3 (3)	C1'—C2'—C3'—Cl1'	−173.59 (15)
O1—C1—C2—N2	−32.8 (3)	C2—N2—C4—O2	−12.5 (3)
N1—C1—C2—N2	149.00 (19)	C2—N2—C4—C5	166.96 (19)
C4'—N2'—C2'—C3'	148.8 (2)	C2'—N2'—C4'—O2'	−7.8 (3)
C4'—N2'—C2'—C1'	−41.6 (3)	C2'—N2'—C4'—C5'	172.91 (19)
Boc-(Z)-ΔAla(βCl)-OMe (4)			
Cl1—C3	1.7148 (12)	O4—C6	1.2293 (14)
N2—C4	1.3600 (15)	O5—C6	1.3421 (14)
N2—C2	1.4114 (15)	O5—C7	1.4743 (14)
N3—C6	1.3415 (16)	C1—C2	1.4936 (16)
N3—C5	1.4458 (15)	C2—C3	1.3282 (17)
O1—C1	1.2030 (15)	C4—C5	1.5254 (15)
O2—C4	1.2213 (15)	C7—C8	1.5150 (18)
O3—C1	1.3370 (15)	C7—C9	1.5199 (18)
O3—C11	1.4465 (15)	C7—C10	1.5213 (18)
C4—N2—C2	121.52 (10)	O2—C4—C5	122.28 (10)
C6—N3—C5	120.71 (10)	N2—C4—C5	114.32 (10)
C1—O3—C11	115.53 (10)	N3—C5—C4	112.03 (9)
C6—O5—C7	121.78 (9)	O4—C6—N3	123.74 (11)
O1—C1—O3	124.30 (11)	O4—C6—O5	125.63 (11)
O1—C1—C2	123.86 (11)	N3—C6—O5	110.63 (10)
O3—C1—C2	111.79 (10)	O5—C7—C8	110.59 (10)
C3—C2—N2	123.85 (11)	O5—C7—C9	109.80 (10)
C3—C2—C1	118.81 (11)	C8—C7—C9	112.82 (11)
N2—C2—C1	116.99 (10)	O5—C7—C10	102.23 (10)
C2—C3—Cl1	122.86 (10)	C8—C7—C10	110.43 (11)
O2—C4—N2	123.37 (11)	C9—C7—C10	110.48 (11)
C11—O3—C1—O1	3.88 (17)	C2—N2—C4—C5	−175.85 (10)
C11—O3—C1—C2	−173.74 (10)	C6—N3—C5—C4	83.69 (13)
C4—N2—C2—C3	−130.35 (13)	O2—C4—C5—N3	37.79 (15)
C4—N2—C2—C1	56.51 (15)	N2—C4—C5—N3	−144.11 (10)
O1—C1—C2—C3	−159.77 (12)	C5—N3—C6—O4	3.78 (17)
O3—C1—C2—C3	17.86 (16)	C5—N3—C6—O5	−176.20 (9)
O1—C1—C2—N2	13.73 (17)	C7—O5—C6—O4	7.36 (17)
O3—C1—C2—N2	−168.64 (10)	C7—O5—C6—N3	−172.67 (10)
N2—C2—C3—Cl1	1.31 (18)	C6—O5—C7—C8	−64.46 (14)
C1—C2—C3—Cl1	174.34 (9)	C6—O5—C7—C9	60.67 (14)
C2—N2—C4—O2	2.23 (18)	C6—O5—C7—C10	177.96 (10)
Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3)			
Cl1—C3	1.717 (3)	O6—C15	1.330 (4)
N1—C1	1.342 (4)	O6—C16	1.451 (4)
N1—C14	1.443 (4)	C1—C2	1.506 (4)
N2—C4	1.369 (4)	C2—C3	1.336 (4)
N2—C2	1.401 (4)	C4—C5	1.520 (4)
N3—C6	1.325 (4)	C7—C8	1.504 (4)
N3—C5	1.442 (4)	C8—C9	1.350 (5)
O1—C1	1.227 (4)	C8—C13	1.367 (5)
O2—C4	1.217 (3)	C9—C10	1.397 (6)

O3—C6	1.231 (4)	C10—C11	1.341 (6)
O4—C6	1.354 (3)	C11—C12	1.337 (7)
O4—C7	1.451 (4)	C12—C13	1.388 (6)
O5—C15	1.211 (4)	C14—C15	1.511 (4)
C1—N1—C14	121.2 (3)	O3—C6—N3	125.7 (3)
C4—N2—C2	120.6 (2)	O3—C6—O4	123.1 (3)
C6—N3—C5	120.0 (2)	N3—C6—O4	111.1 (2)
C6—O4—C7	115.0 (2)	O4—C7—C8	106.9 (2)
C15—O6—C16	115.2 (3)	C9—C8—C13	117.7 (3)
O1—C1—N1	124.8 (3)	C9—C8—C7	121.8 (3)
O1—C1—C2	120.1 (2)	C13—C8—C7	120.5 (3)
N1—C1—C2	114.9 (2)	C8—C9—C10	120.7 (4)
C3—C2—N2	123.3 (3)	C11—C10—C9	120.8 (4)
C3—C2—C1	118.4 (3)	C12—C11—C10	119.0 (4)
N2—C2—C1	117.7 (2)	C11—C12—C13	120.9 (4)
C2—C3—Cl1	122.8 (2)	C8—C13—C12	120.8 (4)
O2—C4—N2	122.8 (3)	N1—C14—C15	114.1 (2)
O2—C4—C5	122.7 (3)	O5—C15—O6	122.9 (3)
N2—C4—C5	114.5 (2)	O5—C15—C14	123.7 (3)
N3—C5—C4	111.1 (2)	O6—C15—C14	113.4 (3)
C14—N1—C1—O1	5.3 (4)	C7—O4—C6—N3	−178.0 (2)
C14—N1—C1—C2	−170.2 (2)	C6—O4—C7—C8	174.3 (2)
C4—N2—C2—C3	−147.3 (3)	O4—C7—C8—C9	106.2 (4)
C4—N2—C2—C1	41.8 (4)	O4—C7—C8—C13	−74.4 (5)
O1—C1—C2—C3	−130.6 (3)	C13—C8—C9—C10	1.0 (8)
N1—C1—C2—C3	45.2 (4)	C7—C8—C9—C10	−179.6 (4)
O1—C1—C2—N2	40.8 (4)	C8—C9—C10—C11	−0.7 (9)
N1—C1—C2—N2	−143.5 (3)	C9—C10—C11—C12	1.1 (9)
N2—C2—C3—Cl1	5.1 (4)	C10—C11—C12—C13	−1.9 (9)
C1—C2—C3—Cl1	175.9 (2)	C9—C8—C13—C12	−1.8 (9)
C2—N2—C4—O2	8.5 (4)	C7—C8—C13—C12	178.9 (5)
C2—N2—C4—C5	−170.1 (2)	C11—C12—C13—C8	2.3 (10)
C6—N3—C5—C4	77.8 (3)	C1—N1—C14—C15	120.1 (3)
O2—C4—C5—N3	26.7 (4)	C16—O6—C15—O5	−2.4 (5)
N2—C4—C5—N3	−154.7 (2)	C16—O6—C15—C14	177.0 (3)
C5—N3—C6—O3	6.9 (4)	N1—C14—C15—O5	−173.1 (3)
C5—N3—C6—O4	−174.8 (2)	N1—C14—C15—O6	7.5 (4)
C7—O4—C6—O3	0.4 (4)		
Ac-AAIa(βCl₂)-NHMe (5)			
Cl1—C3	1.7240 (16)	O1—C1	1.238 (2)
Cl2—C3	1.7167 (16)	O2—C4	1.225 (2)
N1—C1	1.326 (2)	C1—C2	1.511 (2)
N1—C6	1.455 (2)	C2—C3	1.337 (2)
N2—C4	1.363 (2)	C4—C5	1.498 (2)
N2—C2	1.406 (2)		
C1—N1—C6	121.34 (14)	N2—C2—C1	116.69 (13)
C4—N2—C2	122.21 (14)	C2—C3—Cl2	123.80 (13)
O1—C1—N1	124.26 (15)	C2—C3—Cl1	121.76 (13)
O1—C1—C2	119.51 (14)	Cl2—C3—Cl1	114.34 (9)
N1—C1—C2	116.21 (14)	O2—C4—N2	121.44 (16)
C3—C2—N2	120.62 (14)	O2—C4—C5	123.50 (16)
C3—C2—C1	122.12 (15)	N2—C4—C5	115.01 (15)
C6—N1—C1—O1	2.3 (3)	N1—C1—C2—N2	−129.81 (15)

C6—N1—C1—C2	−176.04 (14)	N2—C2—C3—Cl2	−170.03 (12)
C4—N2—C2—C3	−149.61 (16)	C1—C2—C3—Cl2	1.0 (2)
C4—N2—C2—C1	38.8 (2)	N2—C2—C3—Cl1	6.0 (2)
O1—C1—C2—C3	−119.68 (18)	C1—C2—C3—Cl1	177.07 (12)
N1—C1—C2—C3	58.8 (2)	C2—N2—C4—O2	4.7 (2)
O1—C1—C2—N2	51.7 (2)	C2—N2—C4—C5	−177.87 (14)
Ac-(E)-ΔAla(βCl)-NHMe (2)			
Cl1—C3	1.738 (8)	C1'—C2'	1.513 (10)
Cl1'—C3'	1.736 (8)	C2—C3	1.320 (11)
N1—C1	1.314 (10)	C2'—C3'	1.313 (11)
N1—C6	1.443 (10)	C3—H3	0.9500
N1—H1	0.8800	C3'—H3'	0.9500
N1'—C1'	1.321 (10)	C4—C5	1.519 (11)
N1'—C6'	1.455 (10)	C4'—C5'	1.502 (11)
N1'—H1'	0.8800	C5—H5C	0.9800
N2—C4	1.340 (10)	C5—H5B	0.9800
N2—C2	1.415 (9)	C5—H5A	0.9800
N2—H2	0.8800	C5'—H5B'	0.9800
N2'—C4'	1.350 (10)	C5'—H5A'	0.9800
N2'—C2'	1.425 (9)	C5'—H5C'	0.9800
N2'—H2'	0.8800	C6—H6B	0.9800
O1—C1	1.245 (9)	C6—H6A	0.9800
O1'—C1'	1.246 (9)	C6—H6C	0.9800
O2—C4	1.236 (10)	C6'—H6A'	0.9800
O2'—C4'	1.237 (10)	C6'—H6C'	0.9800
C1—C2	1.513 (10)	C6'—H6B'	0.9800
C1—N1—C6	121.7 (7)	O2—C4—N2	122.4 (7)
C1—N1—H1	119.2	O2—C4—C5	121.5 (7)
C6—N1—H1	119.2	N2—C4—C5	116.1 (7)
C1'—N1'—C6'	119.3 (7)	O2'—C4'—N2'	120.6 (7)
C1'—N1'—H1'	120.4	O2'—C4'—C5'	121.7 (7)
C6'—N1'—H1'	120.4	N2'—C4'—C5'	117.7 (7)
C4—N2—C2	121.5 (6)	C4—C5—H5C	109.5
C4—N2—H2	119.2	C4—C5—H5B	109.5
C2—N2—H2	119.2	H5C—C5—H5B	109.5
C4'—N2'—C2'	121.7 (6)	C4—C5—H5A	109.5
C4'—N2'—H2'	119.2	H5C—C5—H5A	109.5
C2'—N2'—H2'	119.2	H5B—C5—H5A	109.5
O1—C1—N1	122.4 (7)	C4'—C5'—H5B'	109.5
O1—C1—C2	119.3 (7)	C4'—C5'—H5A'	109.5
N1—C1—C2	118.2 (7)	H5B'—C5'—H5A'	109.5
O1'—C1'—N1'	122.8 (7)	C4'—C5'—H5C'	109.5
O1'—C1'—C2'	119.3 (6)	H5B'—C5'—H5C'	109.5
N1'—C1'—C2'	117.9 (7)	H5A'—C5'—H5C'	109.5
C3—C2—N2	117.9 (7)	N1—C6—H6B	109.5
C3—C2—C1	127.4 (7)	N1—C6—H6A	109.5
N2—C2—C1	114.1 (6)	H6B—C6—H6A	109.5
C3'—C2'—N2'	118.8 (7)	N1—C6—H6C	109.5
C3'—C2'—C1'	127.8 (7)	H6B—C6—H6C	109.5
N2'—C2'—C1'	113.1 (6)	H6A—C6—H6C	109.5
C2—C3—Cl1	123.2 (6)	N1'—C6'—H6A'	109.5
C2—C3—H3	118.4	N1'—C6'—H6C'	109.5
Cl1—C3—H3	118.4	H6A'—C6'—H6C'	109.5
C2'—C3'—Cl1'	123.0 (6)	N1'—C6'—H6B'	109.5
C2'—C3'—H3'	118.5	H6A'—C6'—H6B'	109.5
Cl1'—C3'—H3'	118.5	H6C'—C6'—H6B'	109.5

C6—N1—C1—O1	−3.2 (12)	O1'—C1'—C2'—C3'	126.4 (9)
C6—N1—C1—C2	178.3 (7)	N1'—C1'—C2'—C3'	−52.5 (11)
C6'—N1'—C1'—O1'	3.4 (11)	O1'—C1'—C2'—N2'	−47.0 (9)
C6'—N1'—C1'—C2'	−177.7 (6)	N1'—C1'—C2'—N2'	134.1 (7)
C4—N2—C2—C3	−138.4 (8)	N2—C2—C3—C1	−169.5 (5)
C4—N2—C2—C1	49.7 (9)	C1—C2—C3—C1	1.1 (12)
O1—C1—C2—C3	−123.5 (9)	N2'—C2'—C3'—C1'	172.3 (5)
N1—C1—C2—C3	55.1 (11)	C1'—C2'—C3'—C1'	−0.8 (12)
O1—C1—C2—N2	47.4 (9)	C2—N2—C4—O2	6.2 (11)
N1—C1—C2—N2	−134.0 (7)	C2—N2—C4—C5	−172.2 (7)
C4'—N2'—C2'—C3'	136.4 (8)	C2'—N2'—C4'—O2'	−7.1 (11)
C4'—N2'—C2'—C1'	−49.6 (9)	C2'—N2'—C4'—C5'	171.6 (7)

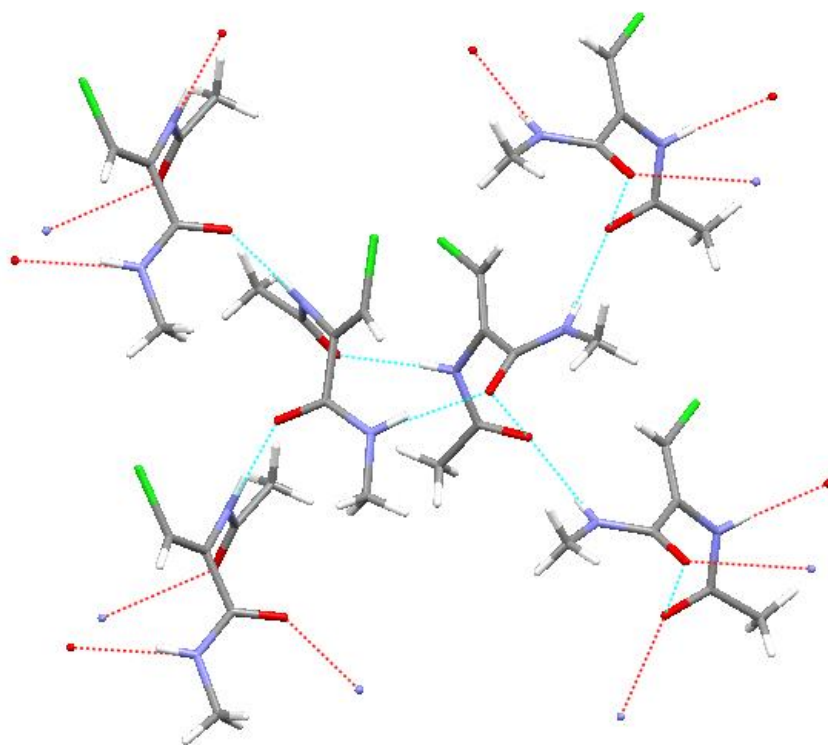


Figure 1S. Molecular interactions of the Ac-(Z)- Δ Ala(β Cl)-NHMe (**1**) molecules with visualization of the hydrogen bonds (dotted lines): Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved. Each molecule creates four N-H...O hydrogen bonds using both amide groups, and each amide plays both donor and acceptor role. Two similar conformations, let's name as β^1 ($\varphi, \psi = -47.5^\circ, 149.0^\circ$) and β^2 ($\varphi, \psi = -41.6^\circ, 141.75^\circ$) are present and the molecule with the conformation β^1 creates two N-H...O hydrogen bonds with the molecule with the conformation β^2 , one is created between the N-terminal and another between the C-terminal amide groups. In the molecule β^1 , the N-terminal amide is the acceptor and the C-terminal amide is the donor. On the contrary, in the molecule β^2 , the N-terminal amide is the donor and the C-terminal amide is the acceptor. Additionally, each molecule β^1 creates with two molecules $-\beta^1$ ($\varphi, \psi = 47.5^\circ, -149.0^\circ$) two other N-H...O hydrogen bonds, in which the N-terminal amide group of the molecule β^1 is a donor for the C-terminal amide group of the molecule $-\beta^1$. Analogously, the molecule β^2 creates two N-H...O hydrogen bonds with two molecules $-\beta^2$ ($\varphi, \psi = 41.6^\circ, -141.75^\circ$).

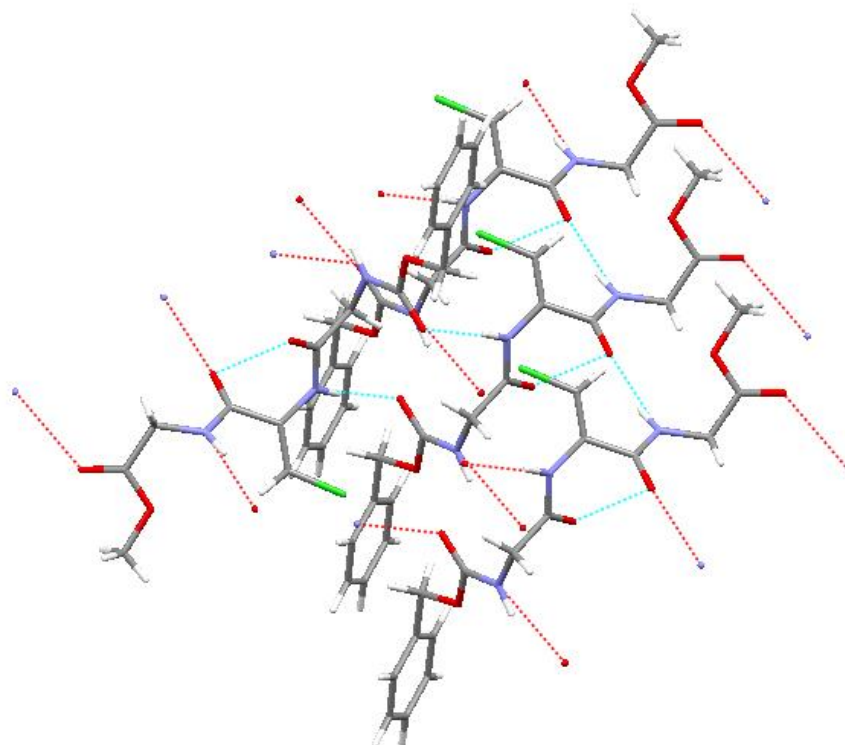


Figure 2S. Molecular interactions of the Cbz-Gly-(Z)- Δ Ala(β Cl)-Gly-OMe (**3**) molecules with visualization of the hydrogen bonds (dotted lines). Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved. The molecules with the conformation β ($\varphi, \psi = -41.8^\circ, 143.5^\circ$) are placed in parallel, N-terminus to N-terminus. Analogously, the molecules with the conformation $-\beta$ ($\varphi, \psi = 41.8^\circ, -143.5^\circ$) are placed in parallel, but separately. The (Z)- Δ Ala(β Cl) residue, using the C-terminal amide group acting both as donor and acceptor of the N-H...O hydrogen bond, is connected with parallel residues preceding and following in this molecular ladder. However, the N-terminal amide group is the donor for the urethane group of the molecule with the opposite conformation.

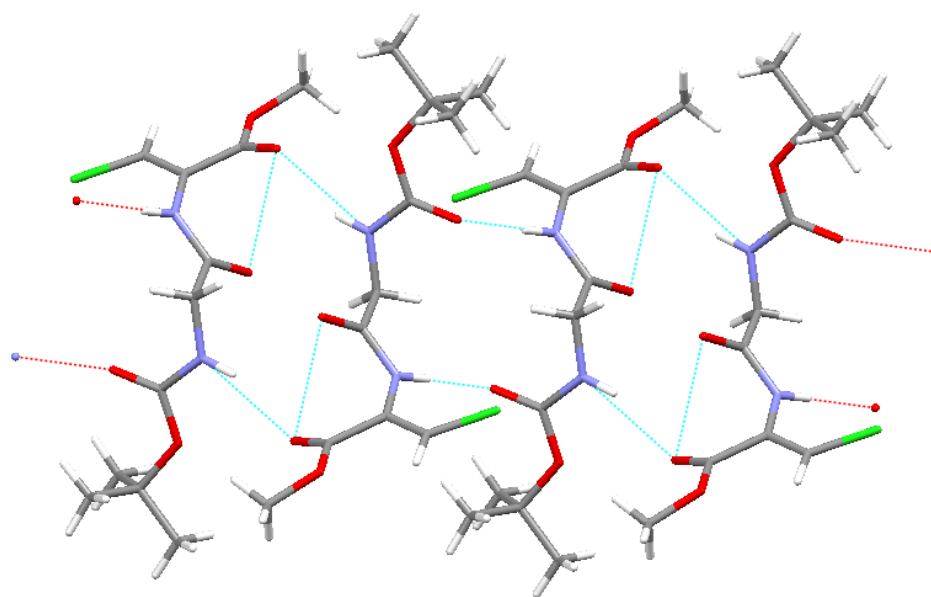


Figure 3S. Molecular interactions of the Boc-(Z)- Δ Ala(β Cl)-OMe (**4**) molecules with visualization of the hydrogen bonds (dotted lines). Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved. The molecules with the conformation β ($\varphi, \psi = -56.5^\circ, 168.6^\circ$) are placed antiparallel between the molecules with the conformation $-\beta$ ($\varphi, \psi = 56.5^\circ, -168.6^\circ$) and each molecule creates four N-H...O hydrogen bonds, two by two with the preceding and following molecules in such arrangement. The N-terminal amide groups of the Gly and (Z)- Δ Ala(β Cl) residues are donors for the C=O groups of esters and urethanes of the neighboring molecules. Additionally, the C=O group of the C-terminal ester of the (Z)- Δ Ala(β Cl) residue is bonded with the N-H group of urethane of another molecule.

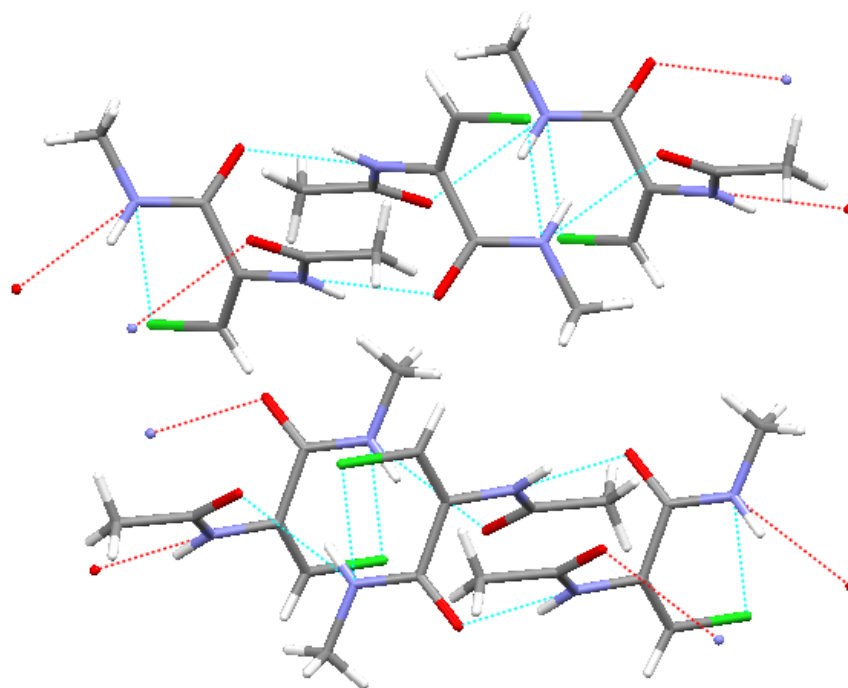


Figure 4S. Molecular interactions of the Ac-(*E*)-ΔAla(βCl)-NHMe (**2**) molecules with visualization of the hydrogen bonds (dotted lines). Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved. The molecules are in a linear arrangement. The molecules with the conformation β are placed with the molecules with the conformation $-\beta$ alternately, with the (*Z*)-ΔAla(βCl) side chains upside-down and the N-terminus to C-terminus. Each molecule creates four N-H...O hydrogen bonds, two by two with the preceding and following molecules in an arrangement.

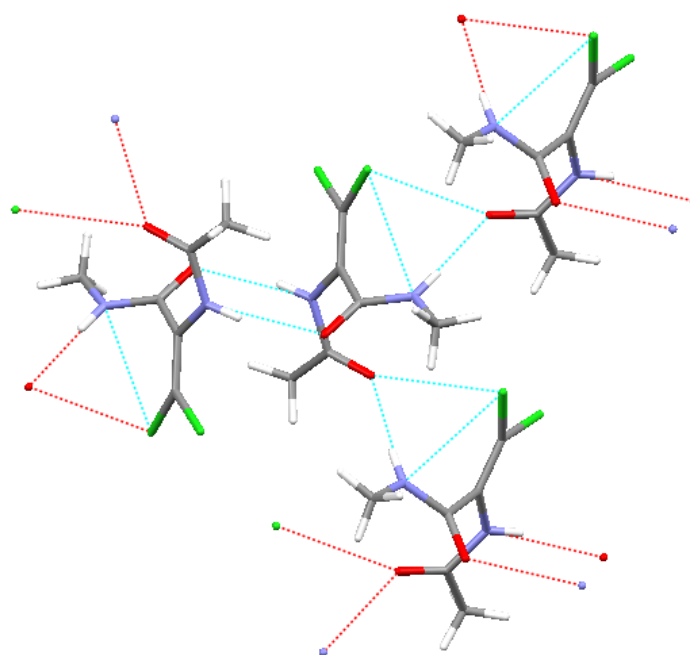


Figure 5S. Molecular interactions of the Ac-ΔAla(βCl₂)-NHMe (**5**) molecules with visualization of the hydrogen bonds (dotted lines). Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved. For Ac-ΔAla(βCl₂)-NHMe (**5**), the molecules with the opposite conformation, β and -β, and with the ΔAla(βCl₂) side chains upside-down, create centrosymmetric dimers joined by two N-H...O hydrogen bonds. Additionally, each molecule is joined with two other molecules with the opposite conformations, creating the N-H...O hydrogen bond with each one.

Table 6S. Selected hydrogen-bond parameters (Å, °) of the studied compounds as determined by X-ray method.

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
Ac-(Z)-ΔAla(βCl)-NHMe (1)				
C3'—H3'···O2 ^{ixv}	0.95	2.57	3.142 (3)	119.1
C5—H5B···O2 ^{xvi}	0.98	2.65	3.560 (3)	154.1
C5'—H5'C···O2 ^{xvii}	0.98	2.50	3.383 (3)	149.3
C5'—H5'B···O2	0.98	2.61	3.468 (3)	145.6
C6—H6A···Cl1 ^{xviii}	0.98	2.90	3.454 (3)	116.6
C6'—H6'B···Cl1 ^{xix}	0.98	2.97	3.909 (3)	161.7
N1—H1···O1'	0.92 (3)	2.01 (3)	2.923 (3)	170 (3)
N1'—H1'···O2 ^{ixv}	0.83 (3)	2.04 (3)	2.856 (3)	166 (3)
N2—H2···O1 ^{xvi}	0.90 (3)	1.92 (3)	2.791 (2)	161 (3)
N2'—H2'···O2	0.82 (3)	2.05 (3)	2.861 (2)	173 (3)
Boc-(Z)-ΔAla(βCl)-OMe (4)				
N3—H3A···O1 ⁱ	0.814 (16)	2.244 (16)	3.0211 (14)	159.7 (15)
C9—H9A···O4	0.98	2.53	3.0549 (15)	113.8
C8—H8C···O4	0.98	2.44	3.0230 (16)	118.1
C3—H3···O2 ⁱⁱ	0.95	2.39	3.1164 (15)	133.1
C5—H5A···O2 ⁱ	0.99	2.54	3.1962 (14)	123.5
N2—H2···O4 ⁱⁱⁱ	0.842 (17)	2.037 (18)	2.8770 (13)	174.7 (16)
Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3)				
N3—H3A···O5 ⁱⁱⁱ	0.88	2.09	2.909 (3)	155.4
C5—H5B···O3 ^{iv}	0.99	2.62	3.437 (4)	139.9
C7—H7A···Cl1 ^v	0.99	2.89	3.548 (3)	124.6
C14—H14B···O2 ⁱⁱⁱ	0.99	2.53	3.261 (4)	130.9
C14—H14A···O2 ⁱ	0.99	2.38	3.066 (4)	126.0
N1—H1···O1 ^{vi}	0.88 (4)	2.05 (4)	2.919 (3)	168 (3)
N2—H2···O3 ^{iv}	0.87 (4)	2.09 (4)	2.952 (3)	169 (3)
Ac-ΔAla(βCl₂)-NHMe (5)				
N1—H1···O2 ^{vii}	0.88	2.04	2.9122 (18)	172.2
N2—H2···O1 ⁱ	0.85 (2)	2.07 (2)	2.8958 (18)	163 (2)
C5—H5A···Cl2 ^{viii}	0.98	2.81	3.7197 (18)	154.4
C5—H5B···O1 ^{ix}	0.98	2.32	3.224 (2)	152.7
Ac-(E)-ΔAla(βCl)-NHMe (2)				
N1—H1···O2 ⁱ	0.88	2.09	2.914 (8)	154.9
N1'—H1'···O2 ^{ix}	0.88	2.03	2.821 (8)	148.6
N2—H2···O1 ^{xi}	0.88	2.01	2.863 (8)	162.9
N2'—H2'···O1 ^{ixii}	0.88	2.03	2.866 (8)	159.0
C3—H3···O1'	0.95	2.34	3.149 (9)	142.9
C3'—H3'···O2 ^{ixiii}	0.95	2.54	3.289 (10)	135.8
C5—H5C···O1 ^{xi}	0.98	2.64	3.486 (10)	145.0
C5—H5B···O2 ⁱⁱ	0.98	2.54	3.495 (10)	164.1
C5'—H5B'···O1 ^{ixii}	0.98	2.60	3.443 (10)	143.8
C5'—H5C'···O2 ^{ixiv}	0.98	2.54	3.512 (10)	172.3
Symmetry code(s): (i) -x+1, -y+1, -z+1; (ii) -x+1, -y, -z+1; (iii) -x, -y+1, -z+1; (iv) -x, -y+2, -z+1; (v) -x+1, -y+2, -z+1; (vi) x+1, y, z; (vii) x, -y+3/2, z-1/2; (viii) x, y, z+1; (ix) x-1, y, z; (x) -x+1, -y, -z; (xi) -x+2, -y+1, -z+1; (xii) -x+1, -y+1, -z; (xiii) x, y, z-1; (xiv) -x, -y, -z; (xv) x, -y+1/2, z-1/2; (xvi) x, -y+3/2, z-1/2; (xvii) -x+1, -y+1, -z+1; (xviii) x, y, z+1; (xix) x, -y+1/2, z+1/2.				

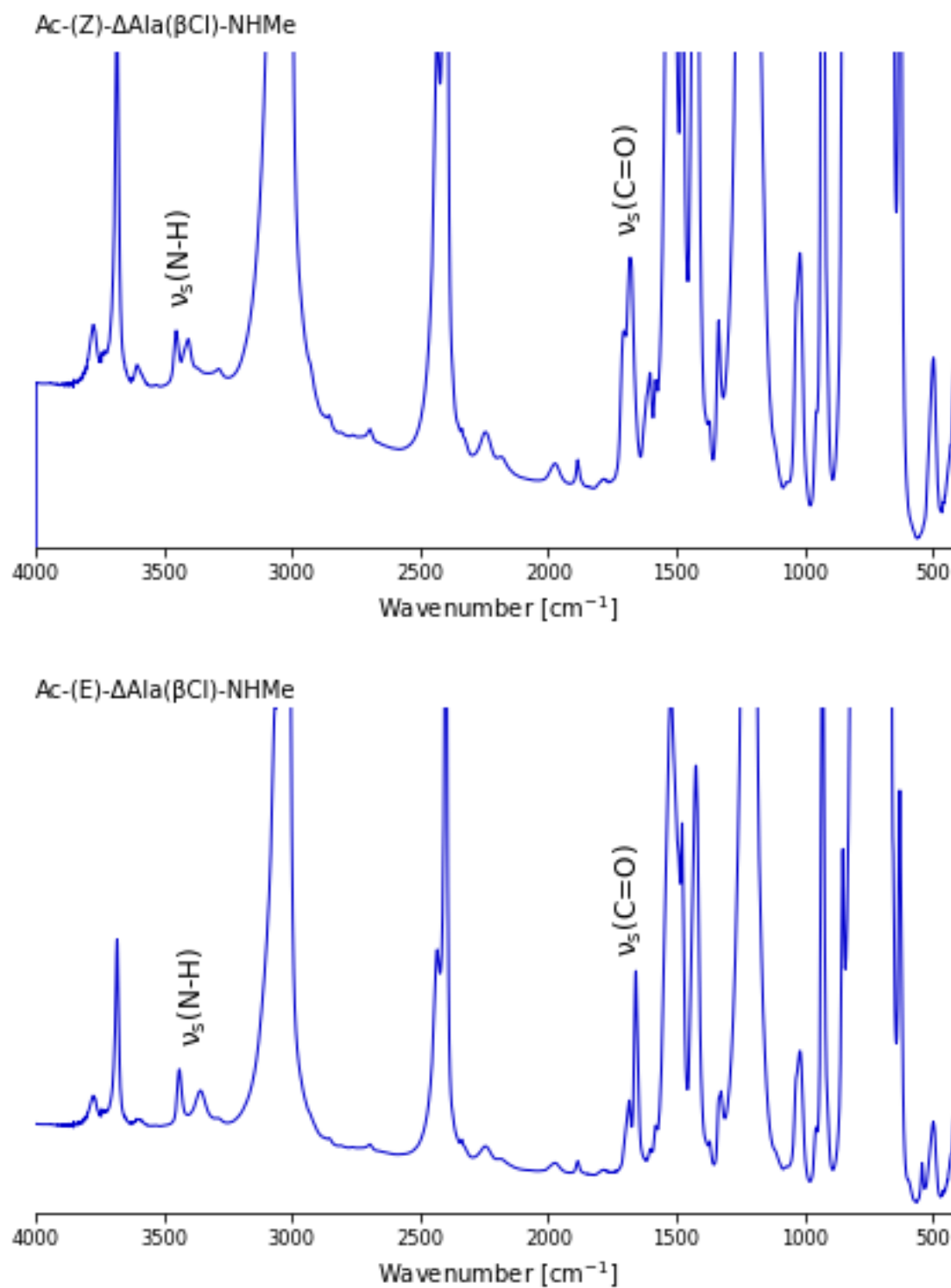


Figure 6S. FTIR spectra for the Ac-(Z)-ΔAla(βCl)-NHMe (**1**) and Ac-(E)-ΔAla(βCl)-NHMe (**2**) in CHCl₃.

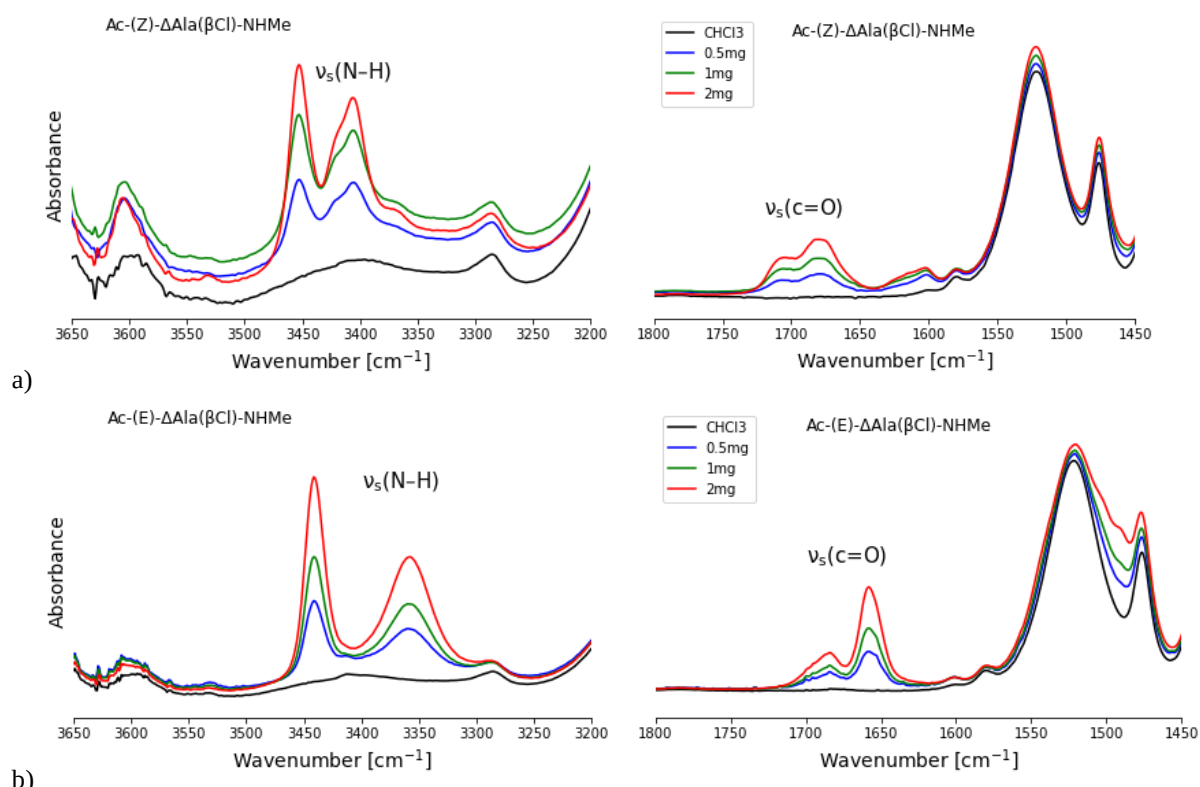


Figure 7S. FTIR spectra for the Ac-(Z)-ΔAla(βCl)-NHMe (**1**) and Ac-(E)-ΔAla(βCl)-NHMe (**2**) in CHCl₃, region ν_s(N-H) and ν_s(C=O): a) isomer Z, b) isomer E.

Table 7S. Theoretical frequencies obtained by M06-2X/6-311+G(d,p) method for the conformations of the Ac-(Z)-ΔAla(βCl)-NHMe (**1**) and Ac-(E)-ΔAla(βCl)-NHMe (**2**) in chloroform (CPCM).

Conformation			Frequencies						RMS	
Code	φ (°)	ψ (°)	Experimental		Calculated		Scaled (0.936)		I	II
			C-terminal	N-terminal	C-terminal	N-terminal	C-terminal	N-terminal		
Ac-(Z)-ΔAla(βCl)-NHMe (1)			3452	3405						
			3422	3377						
α	-61.8	-23.1			3675	3615	3440	3384	14.0	20.0
β	-42.7	138.7			3667	3636	3432	3403	17.4	13.4
β2	-116.6	9.8			3667	3626	3432	3394	16.0	14.0
C7	-68.2	16.6			3617	3621	3386	3389	48.3	27.2
C5	-127.7	155.1			3674	3603	3439	3372	24.8	12.4
C5'	-121.0	-157.5			3677	3622	3442	3390	12.8	16.7
Ac-(E)-ΔAla(βCl)-NHMe (2)			3441	3358						
C5	-177.9	165.1			3678	3584	3443	3355	2.6	
β	-44.4	132.3			3670	3651	3435	3417	42.2	
α	-54.5	-37.6			3669	3638	3434	3405	33.7	
C7	-77.7	63.1			3572	3642	3343	3409	77.8	
β2	-172.7	53.1			3655	3641	3421	3408	38.0	

Scaling factor was established by the best match of the spectra (2) with the conformation C5 (Table 2).
RMS - Root mean squared error between experimental and calculated (scaled) frequencies.

Synthesis

All reaction mixtures were mixed using a magnetic stirrer. The specific conditions are mentioned at the appropriate description of synthetic procedures. The solvents ethyl acetate (EtOAc), benzene, hexane (Hex), methanol (MeOH), *N,N*-dimethylformamide (DFM - extra dry, stored over molecular sieves) were p.a. grade and used without any further purification. Tetrahydrofuran (THF) was refluxed over sodium with benzophenone as an indicator, then distilled and stored over sodium wire. Dichloromethane (DCM) and ethyl acetate (EtOAc) were refluxed over P₂O₅ and distilled. All reactants were used as supplied (Merck Poland). The progress of the reaction was monitored by thin layer chromatography using aluminum SiO₂ plates with a fluorescent indicator (Merck). The chromatograms were additionally visualized using chlorine/*o*-tolidine method. The products were purified by means of flash column chromatography using 60H silica gel with particle size 0.040-0.063 mm (Merck). The NMR analyses were performed on the Bruker Ultrashield 400 spectrometer operating at 400 MHz for ¹H and 101 MHz for ¹³C. Samples were prepared in deuterated d₆-DMSO (99.9% at. D) or deuterated d₁-chloroform (99.8% at. D) with TMS as the internal standard.

Boc-L-Ser-NHMe Boc-L-Ser-OMe (2.107 g, 9.61 mmol) was dissolved in methanol (20 ml) and solution of methylamine in water 41% (11.85 M) (3.2 ml, 50 mmol, 5 eq.) was added. Reaction mixture was stirred for 48 h in room temperature, and then concentrated under reduced pressure. The residue was dissolved in AcOEt (150 ml), then washed with mixture of 1M HCl and brine (1:1, 20 ml). Aqueous layer then extracted with AcOEt (3x10 ml). All organic fractions were combined and dried over anhydrous magnesium sulfate. Product was crystallized from a mixture of AcOEt/Hex. Yield: 1.378 g (6.32 mmol), 66%.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 6.93 (1H, s, O=C-N-H), 5.76 (1H, d, O-C(O)-N-H), 4.19 (1H, s, O-H), 4.03 (1H, d, C^α-H), 3.83 (1H, s, CH₂), 3.68 (1H, s, CH₂), 2.83 (3H, d, CH₃), 1.45 (9H, s, C(CH₃)₃). ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 171.45 (O=C-N-H), 155.80 (O-C(O)-N-H), 80.06 (C)^{Boc}, 62.54 (C^β), 54.76 (C^α), 27.87 (CH₃)^{Boc}, 25.82 (CH₃) (Figure 1S). Melting point: 129.0-130.8°C. TLC MeOH/AcOH (1:9) R_f = 0.30, MeOH/DCM (1:9) R_f = 0.34.

Boc-L-Ser(OMs)-NHMe Boc-L-Ser-NHMe (0.2187 g, 1.00 mmol) was dissolved in anhydrous DCM (15 ml) and cooled in ice bath to -10°C. Afterwards 4-dimethylaminopyridine (DMAP) (0.0065 g, 0.053 mmol), triethylamine (0.418 ml, 3.00 mmol) and methanesulfonyl chloride (0.155 ml, 2.00 mmol) were added. Reaction solution was mixed for 80 min and warmed to room temperature. The reaction mixture was diluted with EtOAc (45 ml), washed with saturated solution of ammonium chloride (3x10 ml) and brine (10 ml) and dried with anhydrous magnesium sulfate. Product was purified by column chromatography using an increasing gradient EtOAc in Hex from 50% to 100% as eluent. Yield: 0.2630 g (0.8875 mmol), 89%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.02 (1H, d, O=C-N-H), 7.18 (1H, d, O-C(O)-N-H), 4.33 (1H, k, CH₂), 4.28 (1H, k, C^α-H), 4.23 (1H, k, CH₂), 3.16 (3H, s, (CH₃)^{Ms}), 2.60 (3H, d, CH₃), 1.39 (9H, s, C(CH₃)₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 168.46 (O=C-N-H), 155.22 (O-C(O)-N-H), 78.64 (C)^{Boc}, 69.38 (C^β), 53.38 (C^α), 36.68 (CH₃)^{Ms}, 28.15 (CH₃)^{Boc}, 25.79 (CH₃) (Figure 2S). Melting point: 131.7-132.6°C. TLC MeOH/AcOH (1:19) R_f = 0.42.

Boc-AAla-NHMe Boc-L-Ser(OMs)-NHMe (0.1403 g, 0.4734 mmol) was dissolved in DCM (8 ml) and DBU (0.08 ml, 0.5 mmol) was added. Reaction mixture was stirred for 2 h in room temperature. The reaction mixture was diluted with Hex (8 ml), and then the products were directly purified by column chromatography using an increasing gradient EtOAc in Hex from 50% to 100% as eluent. Desired product was crystallized from a mixture of AcOEt/Hex. Yield: 0.0086 (0.043 mmol), 9%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.31 (1H, d, O=C-N-H), 7.89 (1H, s, O-C(O)-N-H), 5.59 (1H, s, (*E*)-C^β-H), 5.23 (1H, s, (*Z*)-C^β-H), 2.66 (3H, d, CH₃), 1.40 (9H, s, C(CH₃)₃). ¹³C NMR (101 MHz,

DMSO- d_6) δ (ppm): 164.06 (O=C-N-H), 152.29 (O-C(O)-N-H), 135.75 (C^α), 99.42 (C^β), 79.51 (C)^{Boc}, 27.90 (CH_3)^{Boc}, 26.16 (CH_3) (Figure 3S). Melting point: 119-120°C. TLC MeOH/AcOH (1:19) R_f = 0.58. The cyclic side product was obtained in yield 0.0059 g (0.047 mmol), 10%. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.26 (1H, s, N-H), 5.47 (1H, d, (E)- C^β -H), 4.97 (1H, d, (Z)- C^β -H), 3.10 (3H, s, CH_3). ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 162.64 (O=C-N-H)^C, 154.75 (O=C-N-H)^N, 133.67 (C^α), 96.18 (C^β), 24.14 (CH_3) (Figure 4S). TLC MeOH/AcOH (1:19) R_f = 0.62. Unreacted substrate was recovered (0.0265 g, 0.0894 mmol, 19%).

Ac- Δ Ala-OH Acetamide (11.81 g, 200 mmol), *p*-toluenesulphonic acid (3.804 g, 20 mmol, 0.1 eq.) and pyruvic acid (26.42 g, 300.0 mmol, 1.5 eq) in benzene (140 ml) were heated in Dean-Stark apparatus for 4 h. The crude product was filtered off and washed with diethyl ether and ethanol. The product was recrystallized from water and dried over phosphorus(V) oxide. Yield: 2.065 g (15.99 mmol), 7%.

¹H NMR (400 MHz, DMSO- d_6) δ (ppm): 13.28 (1H, s, O=C-O-H), 9.11 (1H, s, O=C-N-H), 6.25 (1H, s, (E)- C^β -H), 5.67 (1H, s, (Z)- C^β -H), 2.03 (3H, s, CH_3). ¹³C NMR (101 MHz, DMSO- d_6) δ (ppm): 169.36 (O=C-O-H), 165.06 (O=C-N-H), 133.20 (C^α), 107.80 (C^β), 23.79 (CH_3) (Figure 5S). Melting point: 187.0-188.1°C. TLC chloroform/pyridine/AcOH (21:2:1) R_f = 0.42.

Ac- Δ Ala-NHMe Ac- Δ Ala-OH (0.258 g, 2.00 mmol) was dissolved in DMF (1 ml) then THF (1 ml) and *N*-methylmorpholine (0.231 ml, 2.1 mmol, 1.05 eq) were added. Solution was cooled in ice bath to -10°C, and isobutyl chloroformate (0.272 ml, 2.1 mmol, 1.05 eq) was added. After 10 min THF saturated with methylamine (3.7 ml, 3 mmol, 1.5 eq) was added dropwise. Reaction mixture was stirred for 3 h and warmed to room temperature. The volatile components were removed under reduced pressure and mixture was applied on column filed with ion exchange resin Dovex 2x8. The crude product was eluted with water then solvent was removed under reduced pressure. Finally, the product was purified by column chromatography using an increasing gradient of EtOAc in Hex from 50% to 100% as eluent, and crystallized from a mixture of EtOAc/Hex. Yield: 0.192 g (1.35 mmol), 34%.

¹H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.05 (1H, s, (O=C-N-H)^N), 8.27 (1H, d, (O=C-N-H)^C), 5.99 (1H, s, (E)- C^β -H), 5.32 (1H, s, (Z)- C^β -H), 2.68 (3H, d, (CH_3)^C), 2.00 (3H, s, (CH_3)^N). ¹³C NMR (101 MHz, DMSO- d_6) δ (ppm): 169.19 (O=C-N-H)^N, 164.48 (O=C-N-H)^C, 136.19 (C^α), 102.31 (C^β), 26.10 (CH_3)^C, 23.90 (CH_3)^N (Figure 6S). Melting point: 113.0-115.8°C. TLC MeOH/AcOEt (5:95) R_f = 0.33.

Ac-(Z)- Δ Ala(β Cl)-NHMe (**1**) Ac- Δ Ala-NHMe (0.083 g, 0.58 mmol) was dissolved in DMF (0.25 ml) and dichloromethane (DCM) (2 ml) was added. Afterwards chlorine, dissolved in DCM, was added to the substrate solution until the light yellow color of the reaction mixture appear. Excess chlorine was removed quickly under reduced pressure and the residue was resuspended in DCM (2 ml). Finally, triethylamine (0.202 ml, 1.46 mmol, 2.5 eq.) was added and the reaction mixture was stirred for 15 min, and then, the solvents were evaporated under reduced pressure. Products **1** and **5** were isolated by column chromatography using an increasing gradient EtOAc in Hex from 50% to 100%, then an increasing gradient of MeOH in EtOAc 1-6%. Both products were finally crystallized from a mixture of AcOEt/Hex. The yield of **1** (main product): 0.054 g (0.31 mmol), 53%.

¹H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.41 (1H, s, (O=C-N-H)^N), 8.05 (1H, d, (O=C-N-H)^C), 6.78 (1H, s, C^β -H), 2.62 (3H, d, (CH_3)^C), 1.96 (3H, s, (CH_3)^N). ¹³C NMR (101 MHz, DMSO- d_6) δ (ppm): 168.54 (O=C-N-H)^N, 162.79 (O=C-N-H)^C, 134.32 (C^α), 118.16 (C^β), 26.04 (CH_3)^C, 22.63 (CH_3)^N (Figure 7S). Melting point: 170-171°C. TLC MeOH/AcOEt (7:93) R_f = 0.17. The yield of **5** (side product) 0.020 g (0.095 mmol), 16% ¹H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.66 (1H, s, (O=C-N-H)^N), 8.32 (1H, d, (O=C-N-H)^C), 2.62 (3H, d, (CH_3)^C), 1.95 (3H, s, (CH_3)^N). ¹³C NMR (101 MHz, DMSO- d_6) δ (ppm): 168.04 (O=C-N-H)^N, 161.69 (O=C-N-H)^C, 132.12 (C^α), 114.72 (C^β), 25.94 (CH_3)^C, 22.45 (CH_3)^N (Figure 12S). TLC MeOH/AcOEt (7:93) R_f = 0.37.

Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3) The synthesis was performed according to the procedure described for compound **1** using Cbz-Gly-ΔAla-Gly-OMe (0.105 g, 0.301 mmol) as substrate. The product was purified by means of column chromatography using increasing gradient of EtOAc in Hex from 30% to 100% and crystallized from EtOAc/Hex mixture. Yield: 0.079 g (0.21 mmol), 69%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 9.58 (1H, d, (O=C-N-H)^N), 8.54 (1H, t, O=C-N-H)^C, 7.53 (1H, t, (O-C(O)-N-H)), 7.39-7.29 (5H, m, (C-H)^{bzn}), 6.95 (1H, s, C^β-H), 5.04 (2H, s, (CH₂)^{bzl}), 3.86 (2H, d, (CH₂)^{Gly,N}), 3.83 (2H, d, (CH₂)^{Gly,C}), 3.63 (3H, s, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 169.99 (O-C=O), 168.36 (O=C-N-H)^N, 162.61 (O=C-N-H)^C, 156.55 (O-C(O)-NH), 137.04 (C^{bzn}), 132.99 (C^α), 128.39 (C^{bzn,m}-H), 127.85 (C^{bzn,p}-H), 127.77 (C^{bzn,o}-H), 120.66 (C^β), 65.54 (CH₂)^{bzl}, 51.80 (CH₃), 43.39 (CH₂)^{Gly,N}, 40.97 (CH₂)^{Gly,C} (Figure 14S). Melting point: 144.2-144.9°C. TLC AcOEt R_f = 0.17.

Boc-Gly-(Z)-ΔAla(βCl)-OMe (4) The synthesis was performed according to the procedure described for compound **1** using Boc-Gly-ΔAla-OMe (0.129 g, 0.441 mmol) as substrate. The product was purified by means of column chromatography using increasing gradient of EtOAc in Hex from 10% to 80% and crystallized from EtOAc/Hex. mixture. Yield: 0.104 g (0.355 mmol), 71%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 9.68 (1H, d, O=C-N-H), 7.15 (1H, d, C^β-H), 7.04 (1H, t, O-C(O)-N-H), 3.70 (2H, d, (CH₂)^{Gly}), 3.68 (3H, s, CH₃), 1.37 (9H, d, C(CH₃)₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 168.76 (O=C-N-H), 162.76 (O=C-O), 155.81 (O-C(O)-N-H), 130.40 (C^α), 122.88 (C^β), 78.12 (C)^{Boc}, 52.48 (CH₃), 42.79 (CH₂)^{Gly}, 28.19 (CH₃)^{Boc} (Figure 19S). Melting point: 117.3-118.4°C. TLC Toluene/AcOEt (3:7) R_f = 0.50, AcOEt/Hex (4:6) R_f = 0.13.

Ac-(E)-ΔAla(βCl)-NHMe (2) Ac-(Z)-ΔAla(βCl)-NHMe (**1**) (0.0367 g, 0.208 mmol) and benzophenone (0.1895 g, 1.040 mmol, 5.0 equiv) were dissolved in methanol (2 ml) and benzene (0.8 ml) was added. Reaction mixture was mixed and illuminated simultaneously with UV light (366 nm) for 5 h with intensity 400-440 μW/cm². The volatile components was coevaporated with DCM (15 ml), residue was adsorbed on silica gel and then applied on chromatographic column. An increasing gradients EtOAc in Hex from 50% to 100%, then MeOH in EtOAc 0.5%-5% were used as eluent. The product was crystallized from a mixture of AcOEt/Hex. Yield: 0.0074 g (0.042 mM), 20%. Unreacted substrate was recovered (0.0293 g, 0.166 mmol, 80%).

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 9.76 (1H, s, (O=C-N-H)^N), 8.33 (1H, d, (O=C-N-H)^C), 6.90 (1H, s, C^β-H), 2.66 (3H, d, (CH₃)^C), 1.94 (3H, s, (CH₃)^N). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 168.95 (O=C-N-H)^N, 162.56 (O=C-N-H)^C, 135.35 (C^α), 105.78 (C^β), 25.69 (CH₃)^C, 23.13 (CH₃)^N (Figure 23S). Melting point: 130-131°C. TLC MeOH/AcOEt (6:94) R_f = 0.33.

Cbz-Gly-(E)-ΔAla(βCl)-Gly-OMe Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (**3**) (0.0153 g, 0.0399 mmol) and benzophenone (0.0364 g, 0.200 mmol, 5.0 equiv) were dissolved in methanol (1 ml) and benzene (0.4 ml) was added. Then the photoisomerization reaction was carried out in the same manner as for compound **2**. The isomers were separated by column chromatography using an increasing gradient EtOAc in Hex from 50% to 100% as eluent. Yield: 0.0023 g (0.0060 mmol), 15%. Unreacted substrate was recovered (0.0125 g, 0.0326 mmol, 82%).

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 9.80 (1H, d, (O=C-N-H)^N), 8.81 (1H, t, (O=C-N-H)^C), 7.55 (1H, t, O-C(O)-N-H), 7.40-7.29 (5H, m, (C-H)^{bzn}), 6.98 (1H, s, C^β-H), 5.03 (2H, s, (CH₂)^{bzl}), 3.93 (2H, d, (CH₂)^{Gly,N}), 3.73 (2H, d, (CH₂)^{Gly,C}), 3.65 (3H, s, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 169.64 (O-C=O), 168.71 (O=C-N-H)^N, 162.31 (O=C-N-H)^C, 156.56 (O-C(O)-NH), 137.01 (C^{bzn}), 133.55 (C^α), 128.40 (C^{bzn,m}-H), 127.86 (C^{bzn,p}-H), 127.77 (C^{bzn,o}-H), 108.54 (C^β), 65.54 (CH₂)^{bzl}, 51.78 (CH₃), 43.58 (CH₂)^{Gly,N}, 40.85 (CH₂)^{Gly,C} (Figure 28S). TLC AcOEt R_f = 0.37.

Boc-Gly-(E)-ΔAla(βCl)-OMe The photoisomerization reaction of Boc-Gly-(Z)-ΔAla(βCl)-OMe (**4**) (0.0146 g, 0.0499 mmol) was carried out in the same manner as for compound **2**. The isomers were

separated by column chromatography using an increasing gradient EtOAc in Hex from 20% to 100% as eluent. Yield: 0.0041 g (0.014 mmol), 28%. Unreacted substrate was recovered (0.0099 g, 0.034 mmol, 68%).

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 10.02 (1H, d, O=C-N-H), 7.09 (1H, t, O-C(O)-N-H), 6.77 (1H, d, C^β -H), 3.72 (3H, d, CH_3), 3.63 (2H, d, $(\text{CH}_2)^{\text{Gly}}$), 1.38 (9H, d, $(\text{CH}_3)^{\text{Boc}}$). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ (ppm): 168.65 (O=C-N-H), 162.59 (O=C-O), 155.84 (O-C(O)-N-H), 130.73 (C^α), 112.40 (C^β), 78.22 (C^{Boc}), 52.29 (CH_3), 42.99 ($(\text{CH}_2)^{\text{Gly}}$), 28.19 ($(\text{CH}_3)^{\text{Boc}}$) (Figure 29S). TLC AcOEt/Hex (4:6) R_f = 0.23.

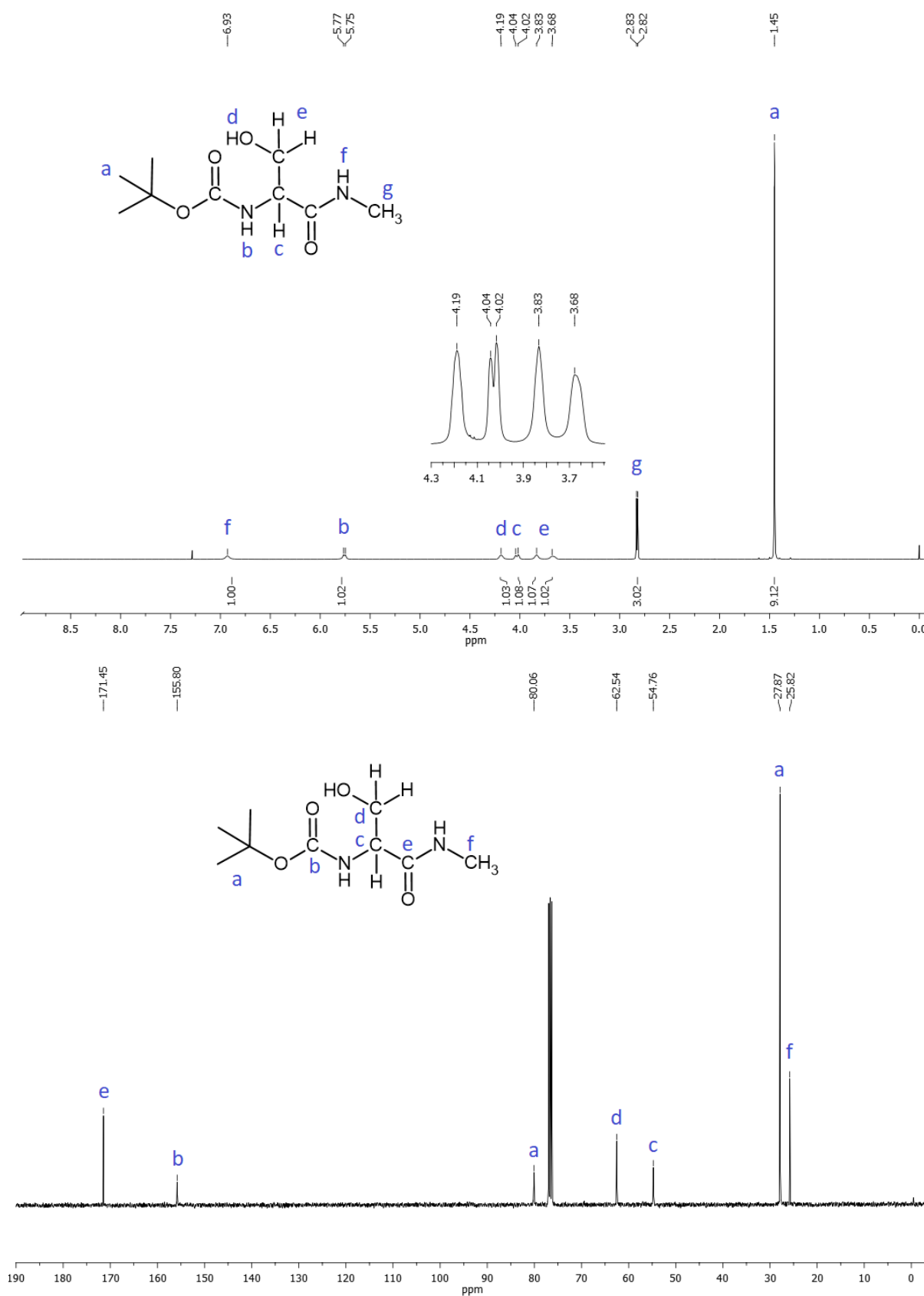


Figure 8S. ^1H and ^{13}C NMR spectra of Boc-L-Ser-NHMe in chloroform.

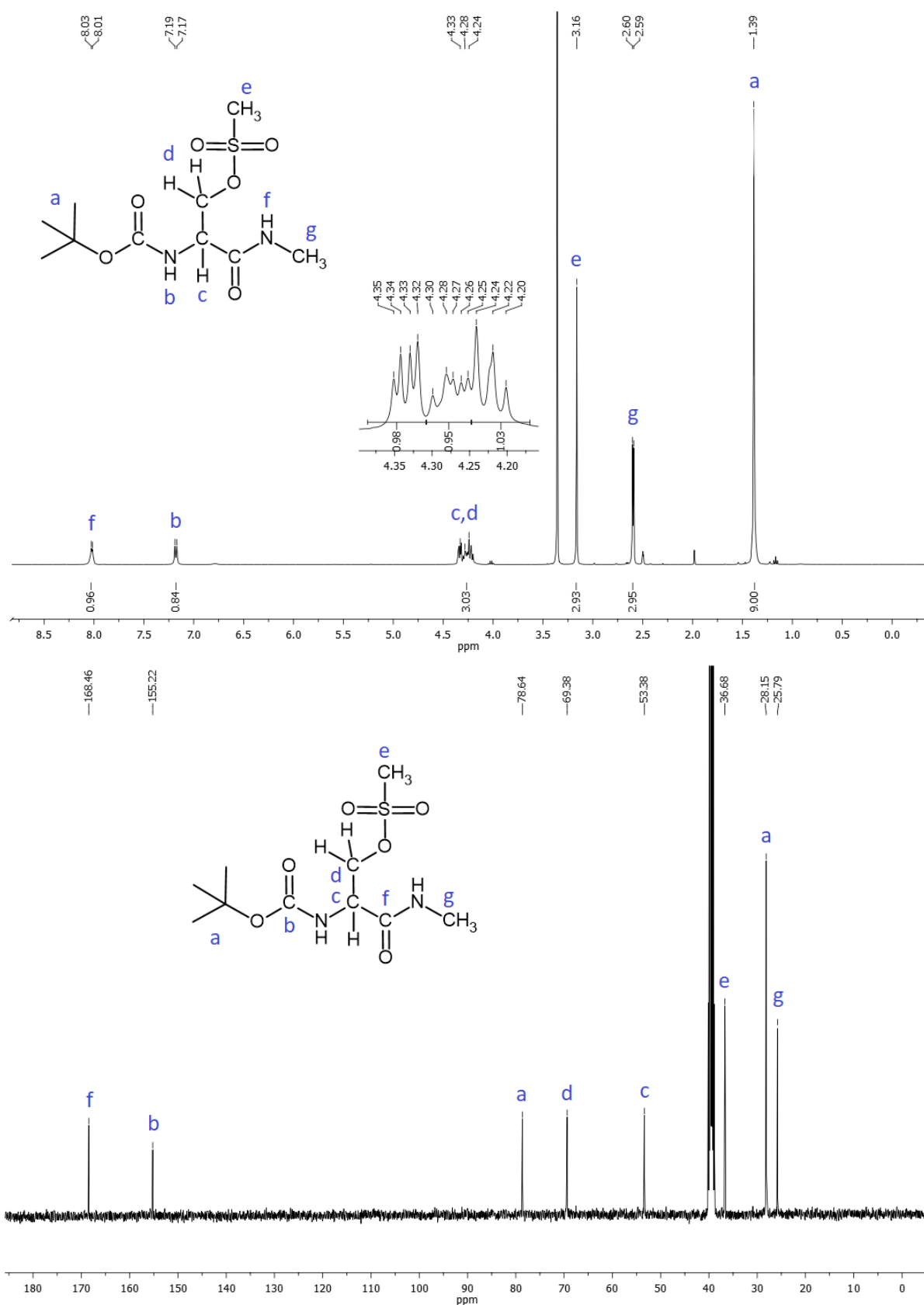


Figure 9S. ^1H and ^{13}C NMR spectra of Boc-L-Ser(OMs)-NHMe in $\text{DMSO}-d_6$.

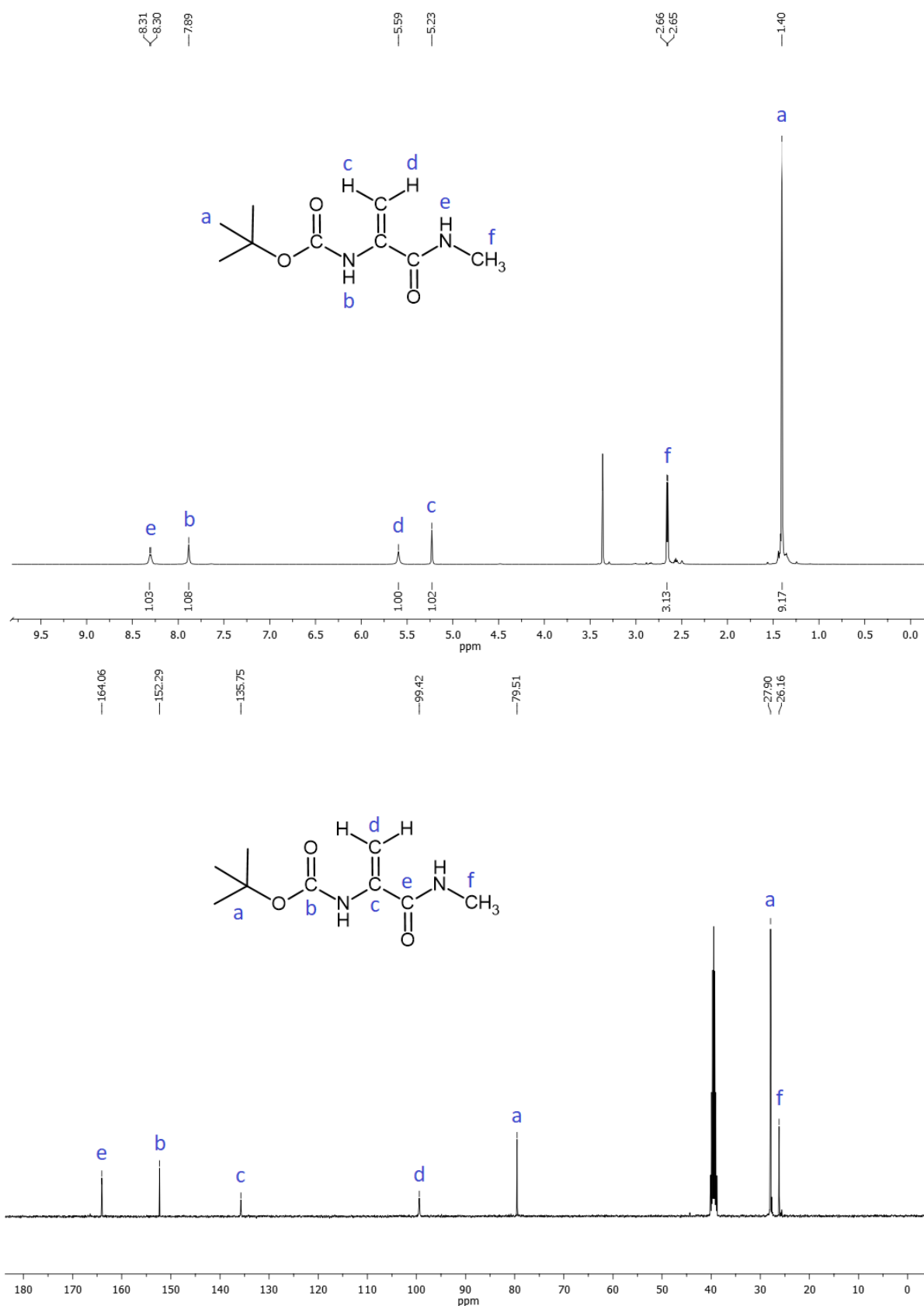


Figure 10S. ^1H and ^{13}C NMR spectra of Boc- Δ Ala-NHMe in $\text{DMSO}-d_6$.

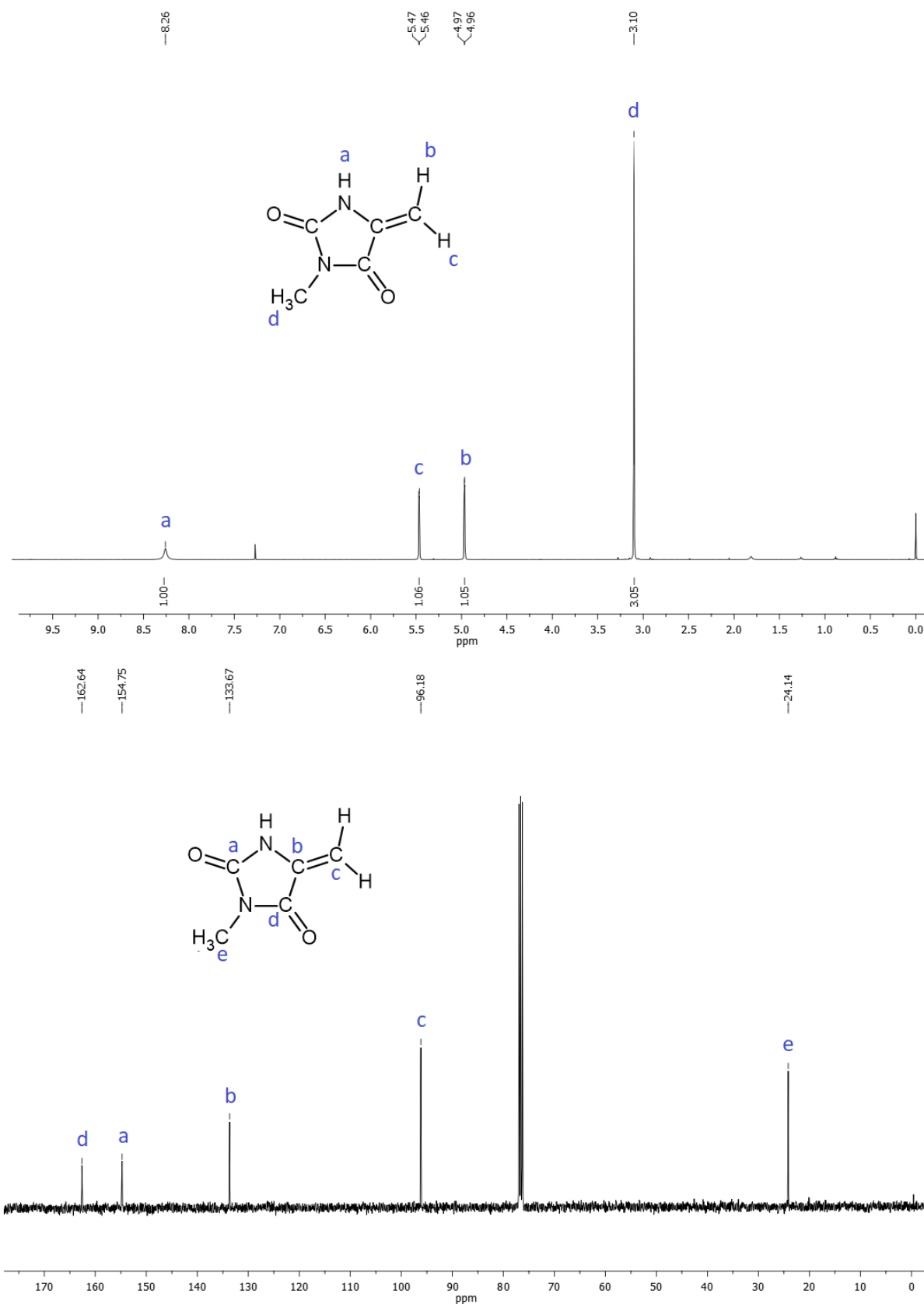


Figure 11S. ^1H and ^{13}C NMR spectra of cyclic side product Boc- Δ Ala-NHMe synthesis in CD_3Cl .

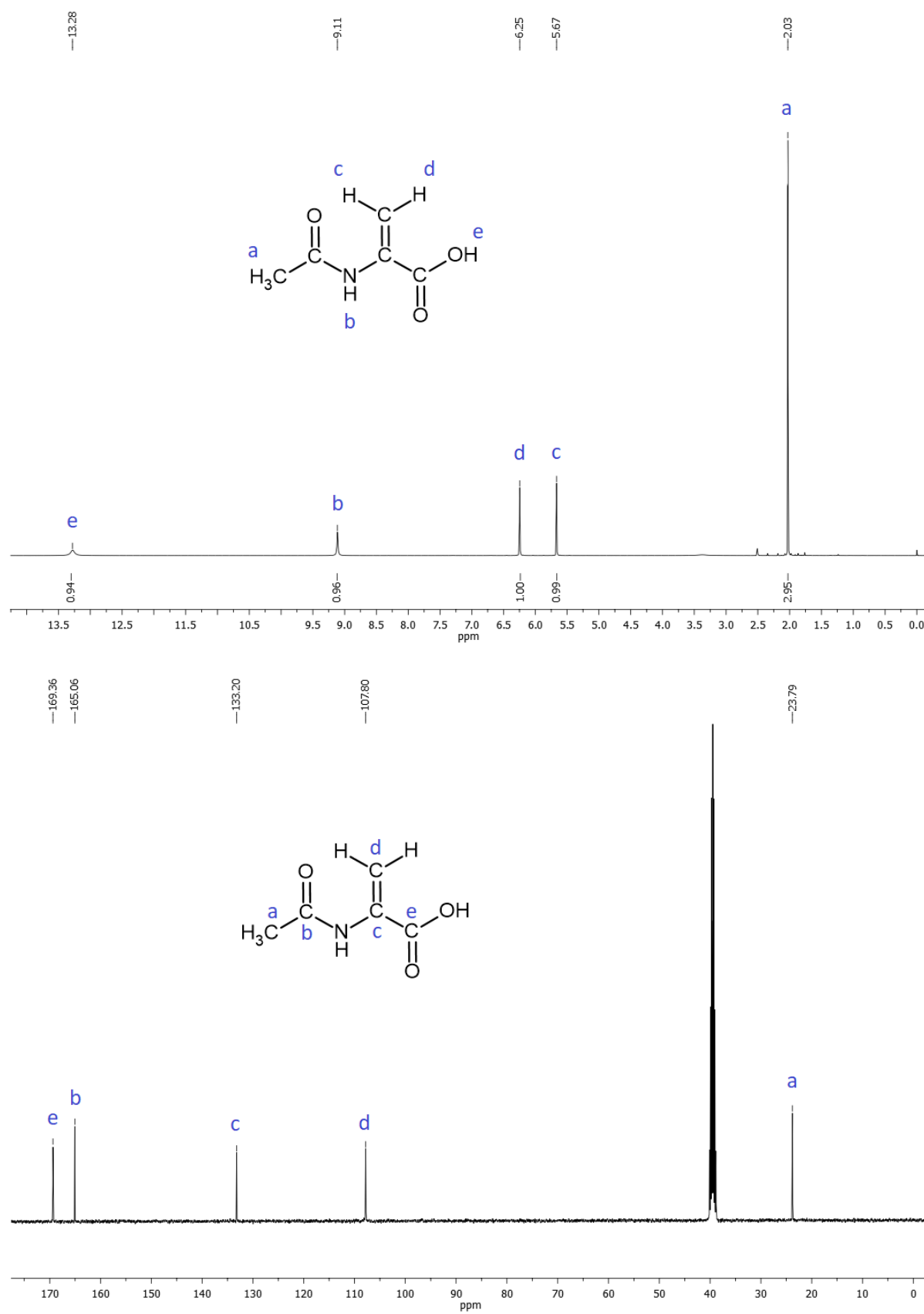


Figure 12S. ^1H and ^{13}C NMR spectra of Ac-ΔAla-OH in $\text{DMSO}-d_6$.

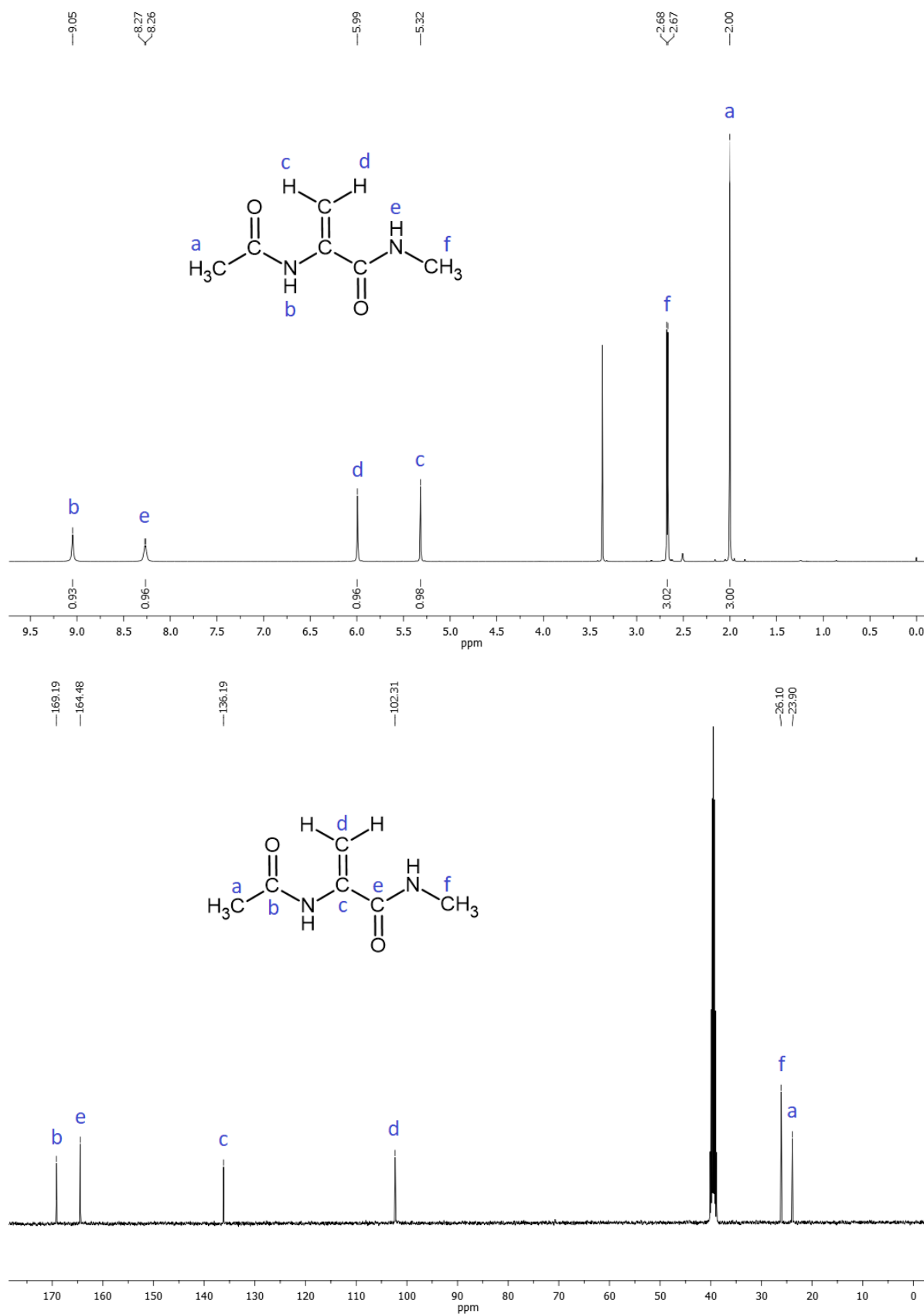


Figure 13S. ^1H and ^{13}C NMR spectra of Ac- Δ Ala-NHMe in $\text{DMSO-}d_6$.

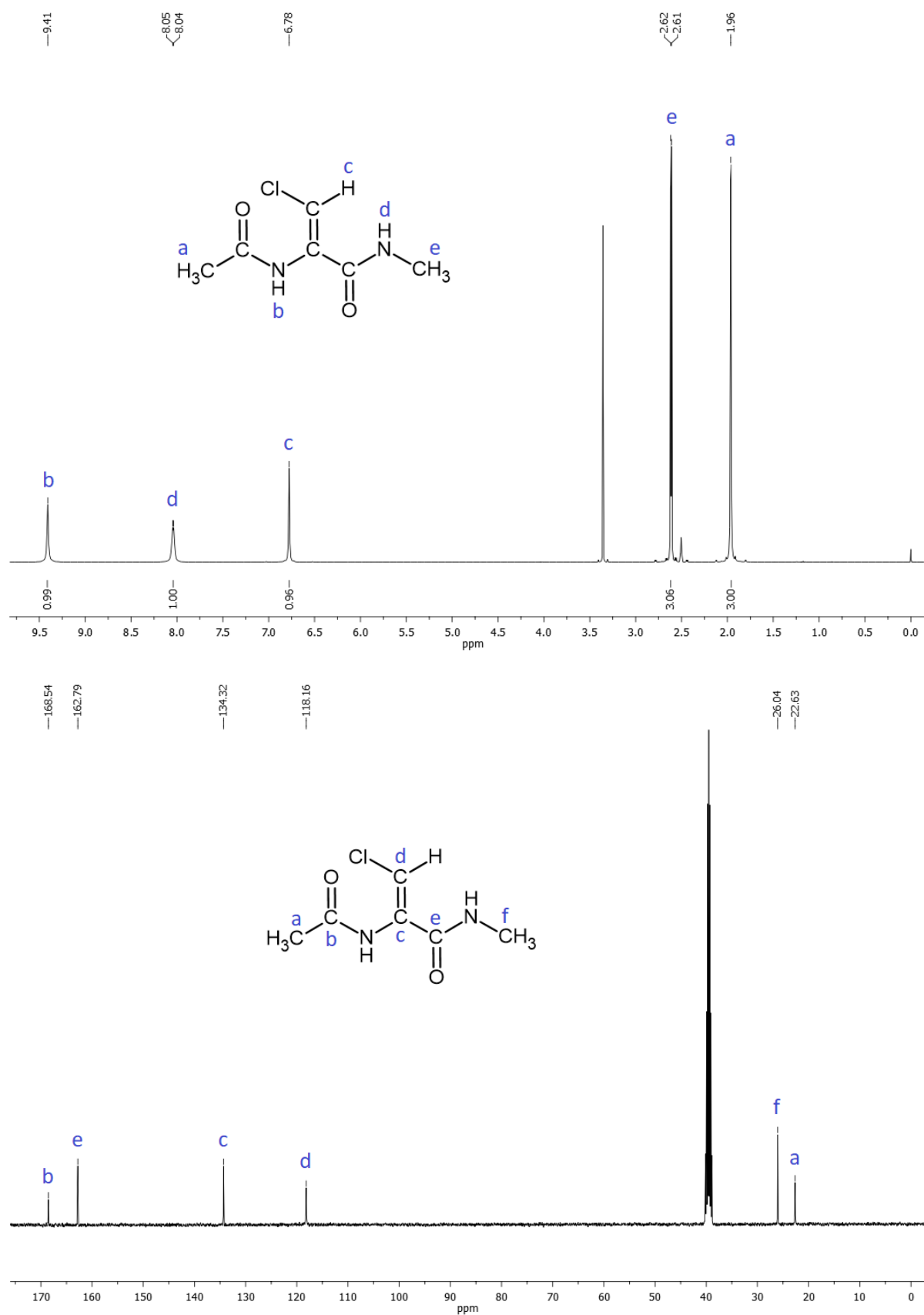


Figure 14S. ¹H and ¹³C NMR spectra of Ac-(Z)-ΔAla(βCl)-NHMe (**1**) in DMSO-*d*₆.

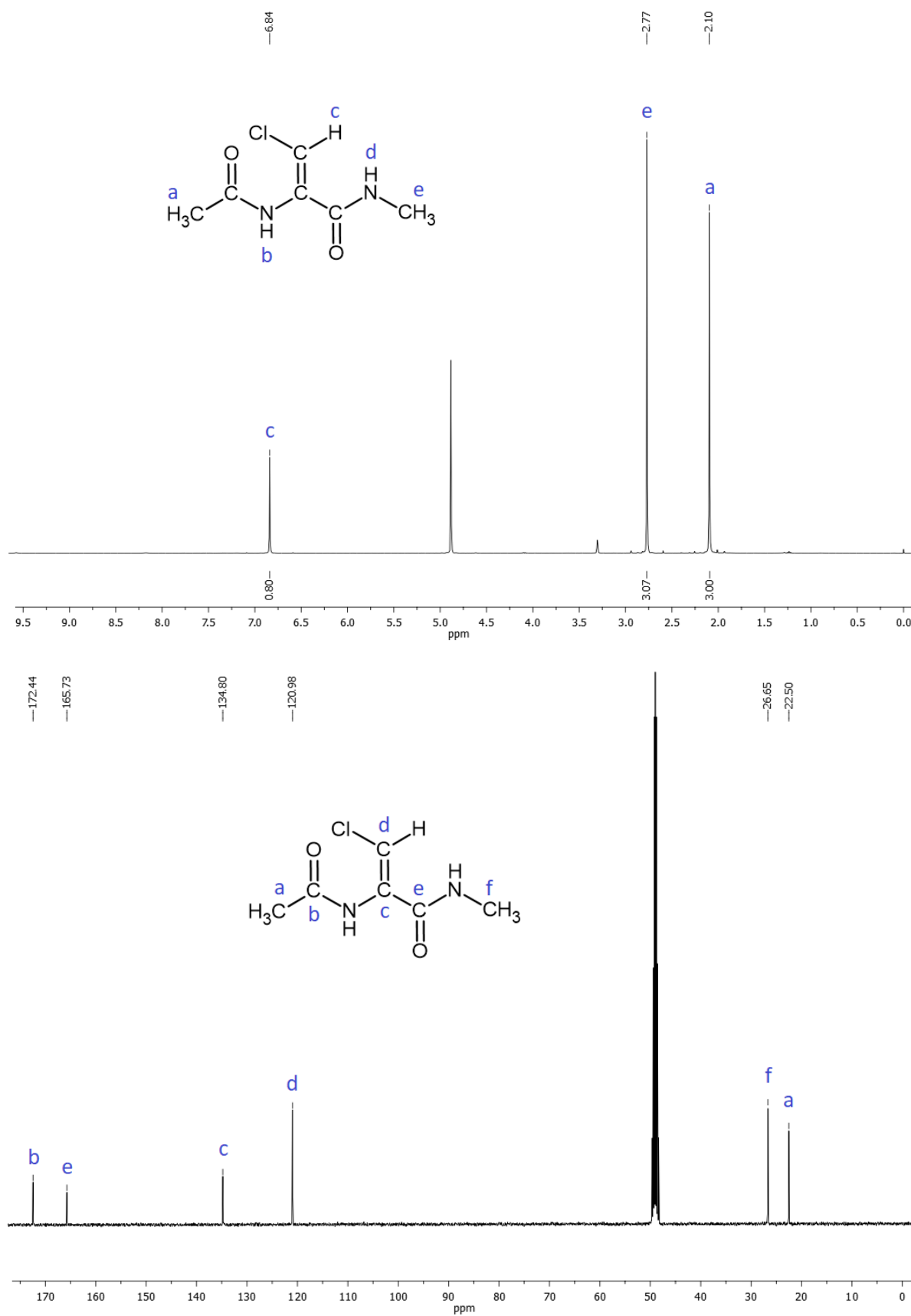


Figure 15S. ¹H and ¹³C NMR spectra of Ac-(Z)-ΔAla(βCl)-NHMe (1) in CD₃OD.

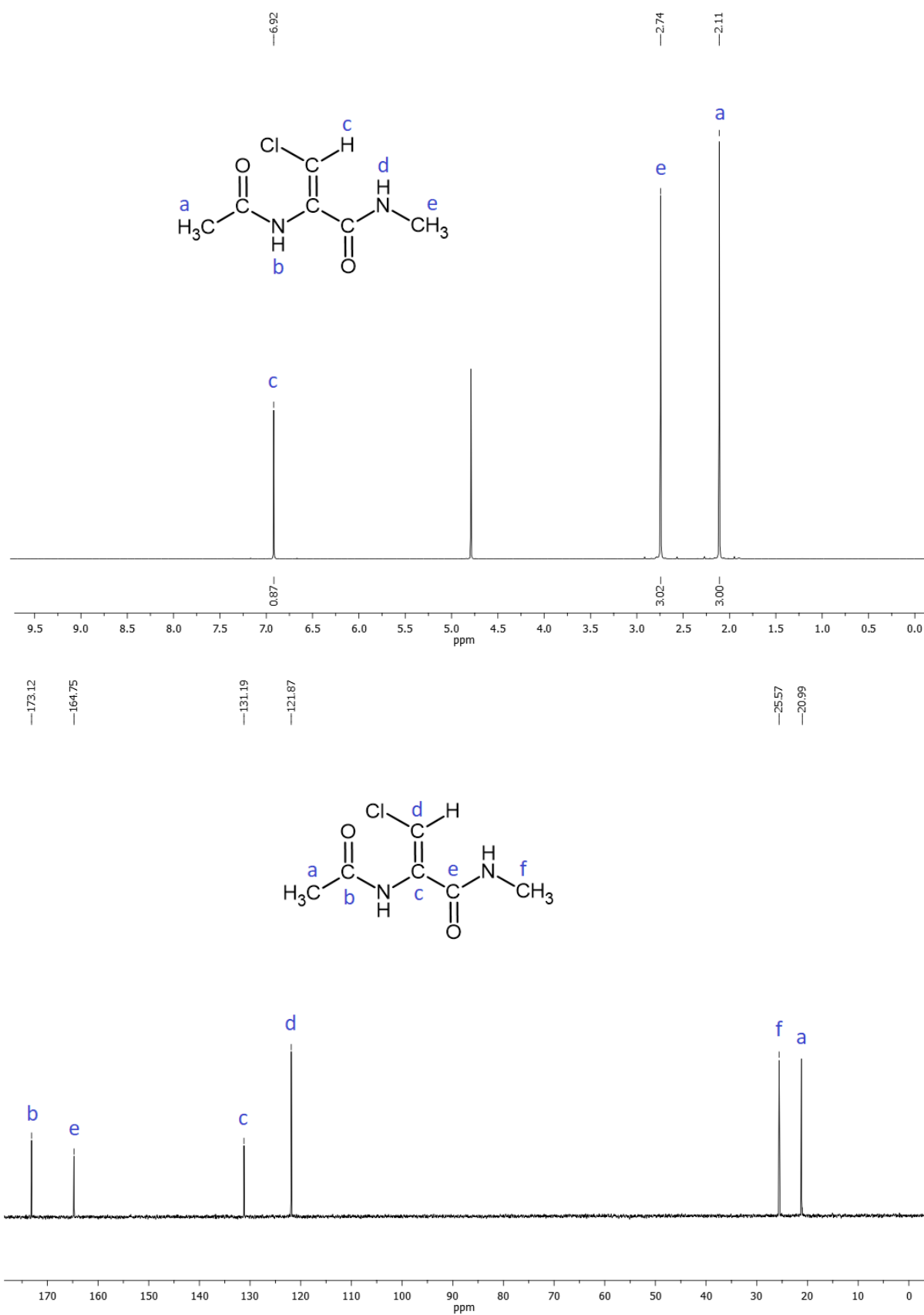


Figure 16S. ¹H and ¹³C NMR spectra of Ac-(Z)-ΔAla(βCl)-NHMe (1) in D₂O.

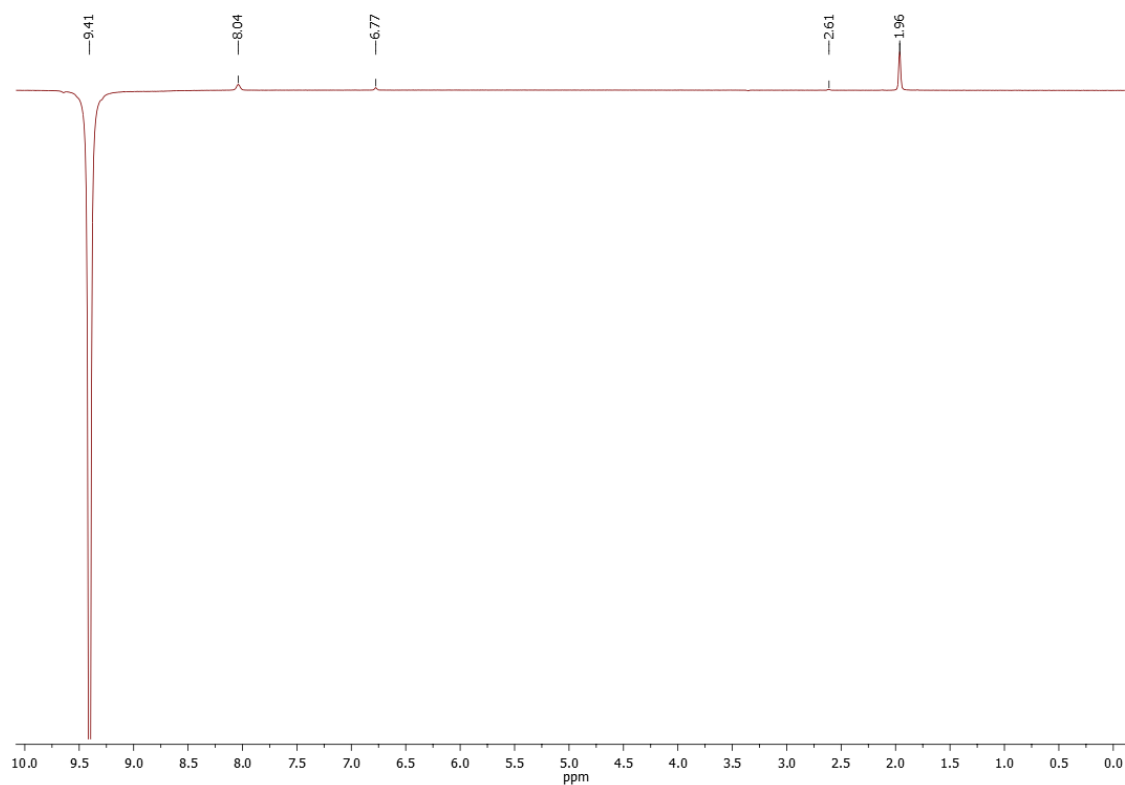


Figure 17S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom of Ac-(Z)-ΔAla(βCl)-NHMe (**1**) in DMSO-*d*₆.

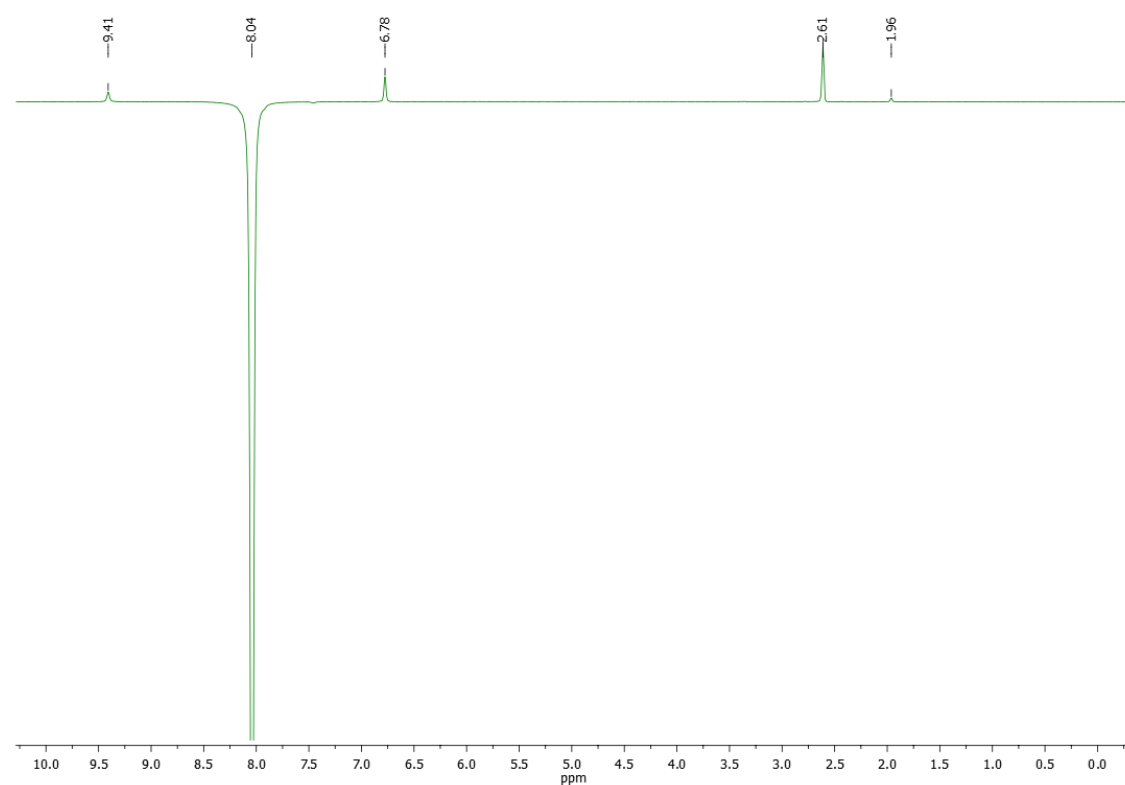


Figure 18S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the C-terminal amide H atom of Ac-(Z)-ΔAla(βCl)-NHMe (**1**) in DMSO-*d*₆.

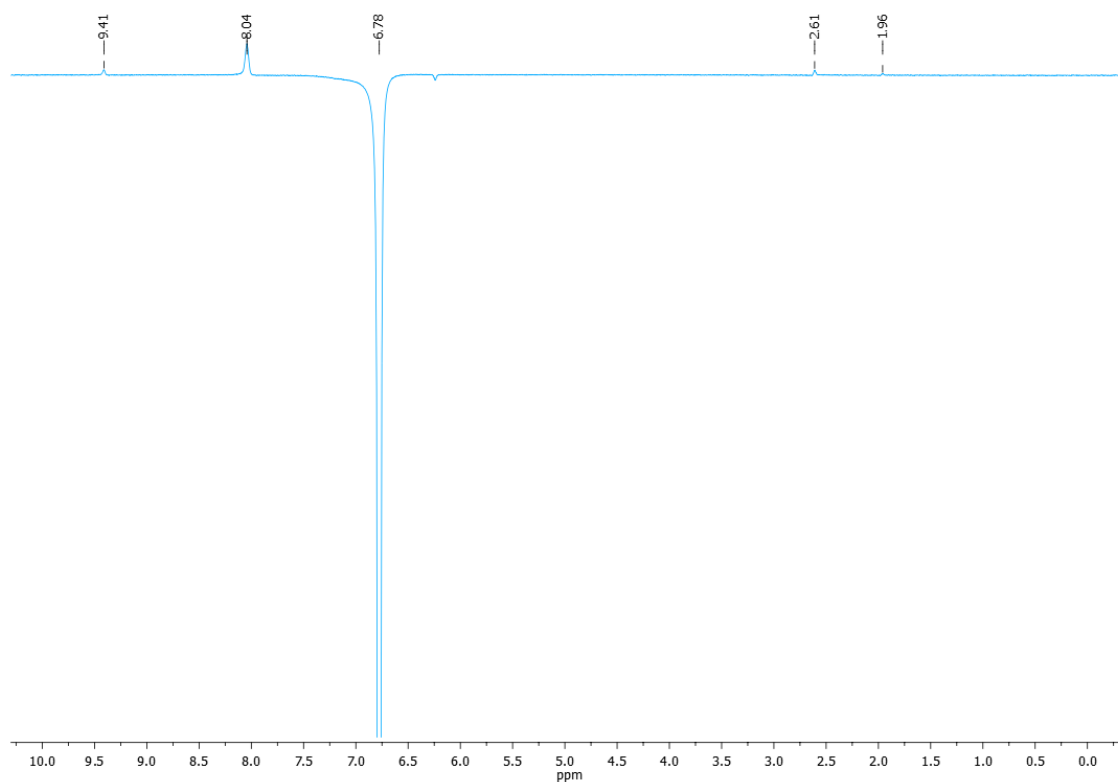


Figure 19S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the side chain H atom of Ac-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-NHMe}$ (**1**) in $\text{DMSO-}d_6$.

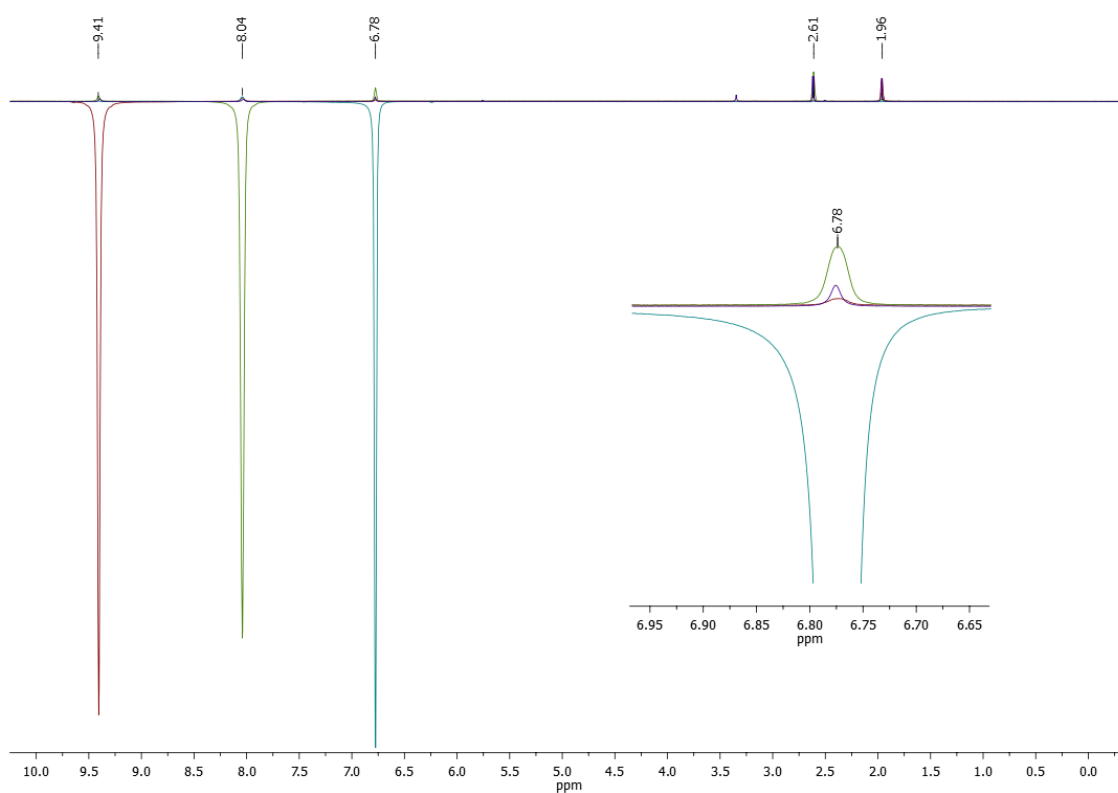


Figure 20S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom (red spectrum), the C-terminal amide H atom (green spectrum) and the side chain H atom (blue spectrum) of Ac-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-NHMe}$ (**1**) in $\text{DMSO-}d_6$.

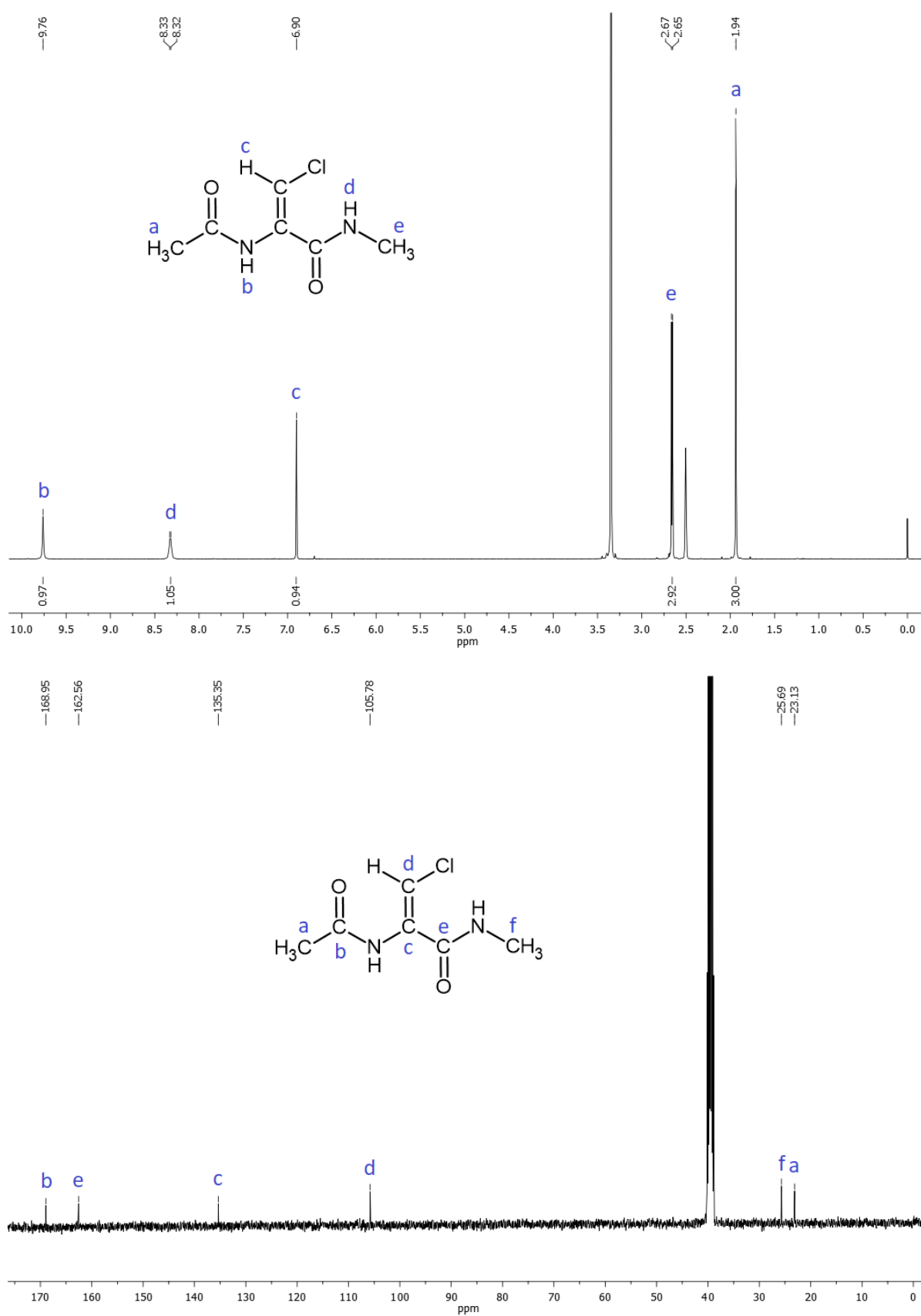


Figure 21S. ^1H and ^{13}C NMR spectra of Ac-(*E*)- $\Delta\text{Ala}(\beta\text{Cl})\text{-NHMe}$ (2) in $\text{DMSO-}d_6$.

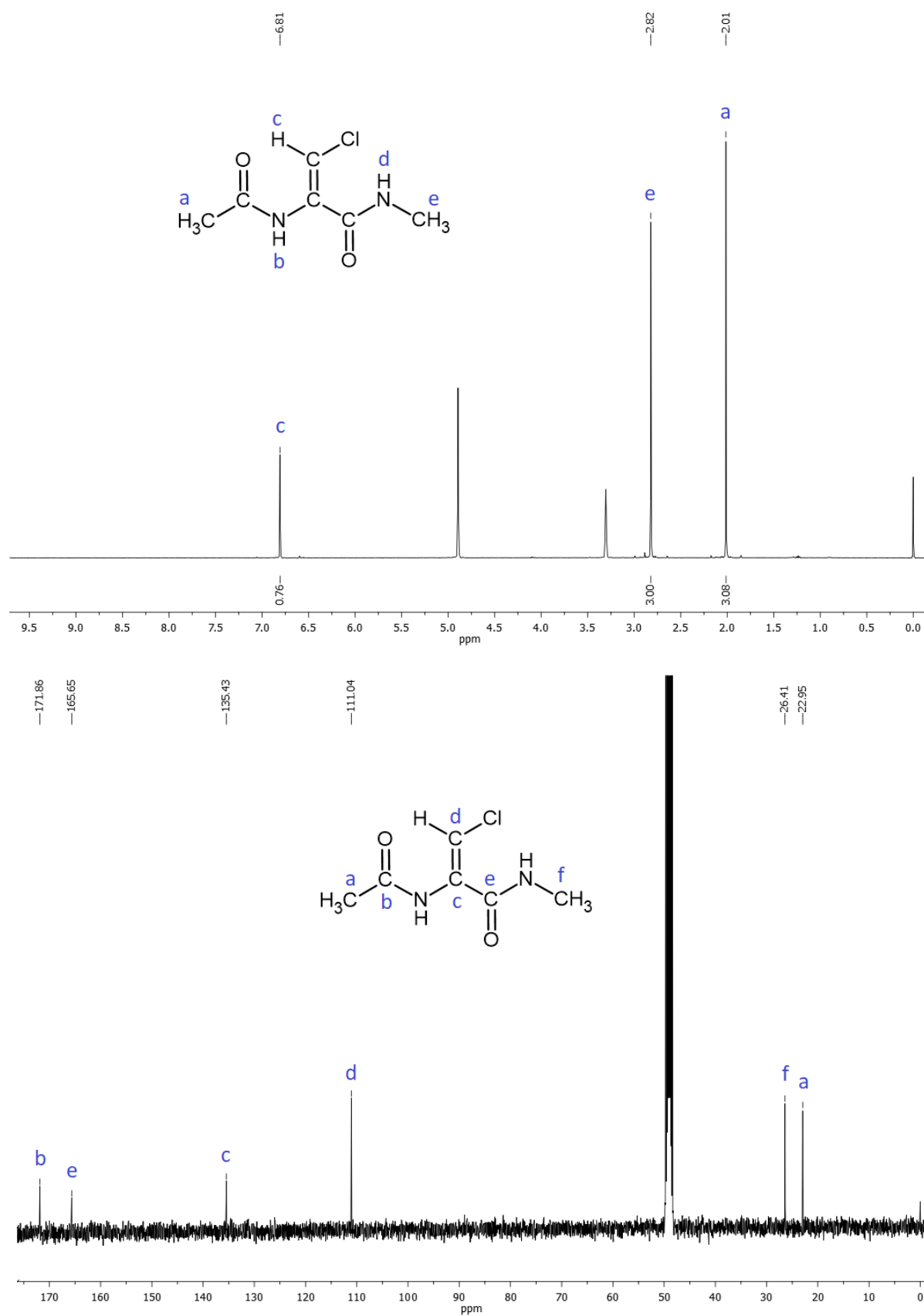


Figure 22S. ¹H and ¹³C NMR spectra of Ac-(*E*)-ΔAla(βCl)-NHMe (2) in CD₃OD.

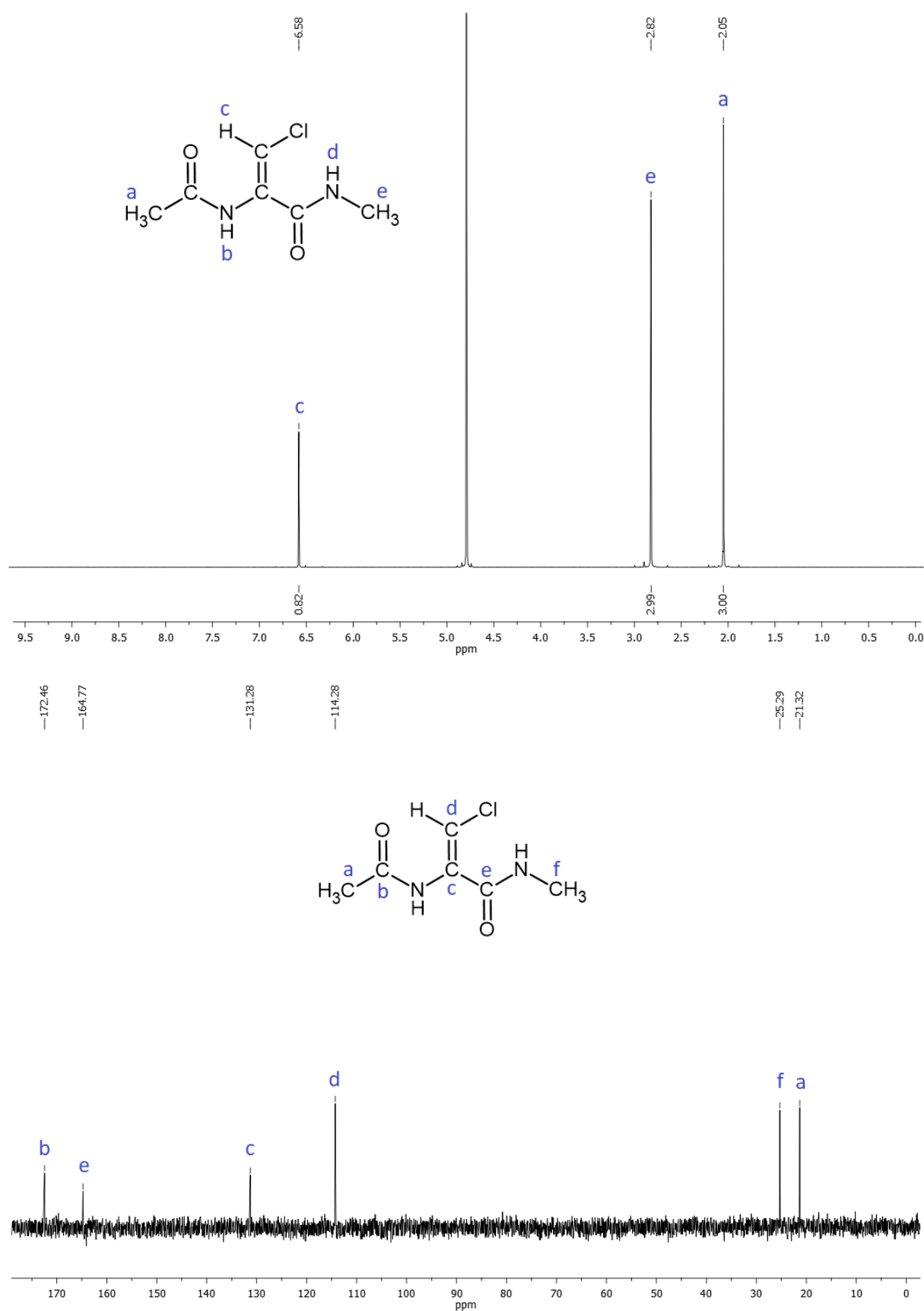


Figure 23S. ¹H and ¹³C NMR spectra of Ac-(*E*)-ΔAla(βCl)-NHMe (2) in D₂O.

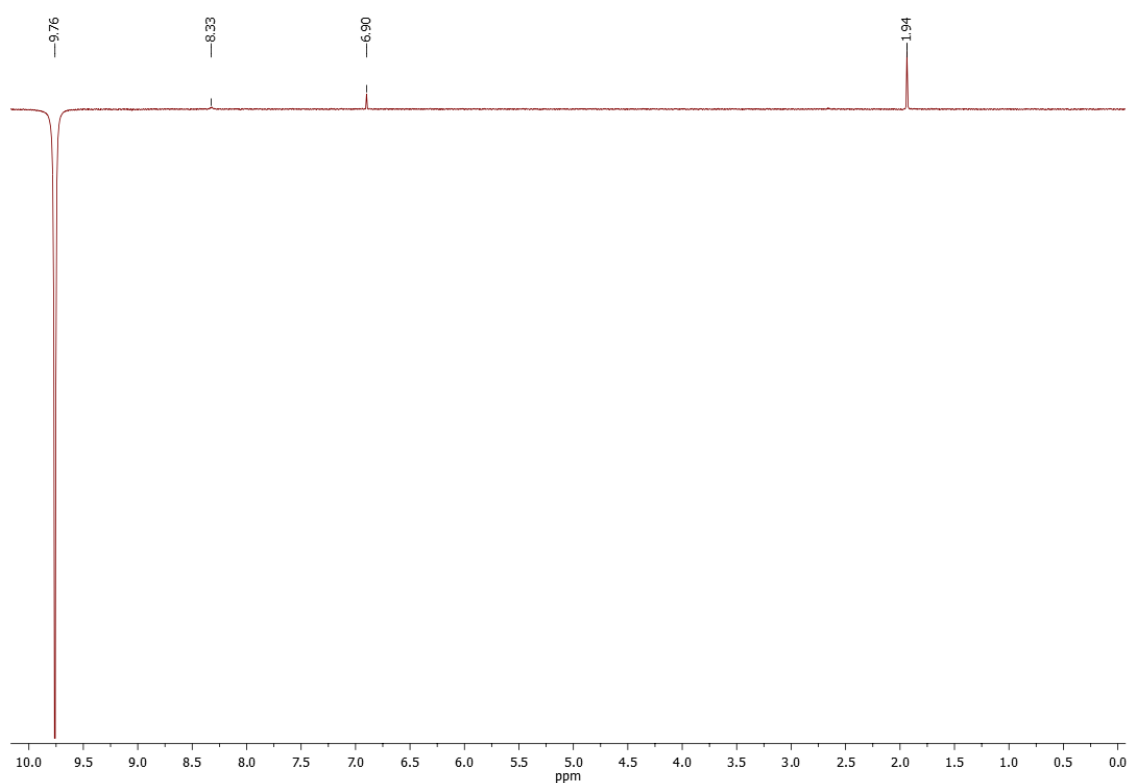


Figure 24S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom of Ac-(*E*)- Δ Ala(β Cl)-NHMe (**2**) in DMSO-*d*₆.

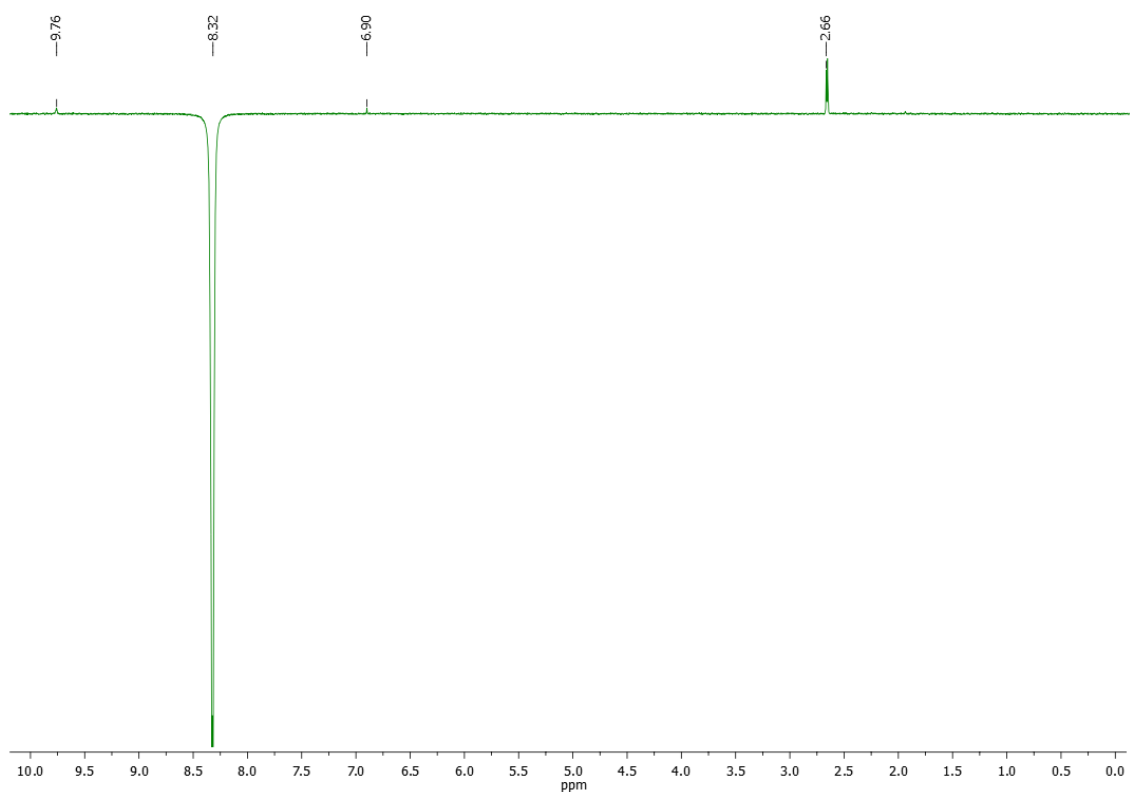


Figure 25S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the C-terminal amide H of Ac-(*E*)- Δ Ala(β Cl)-NHMe (**2**) in DMSO-*d*₆.

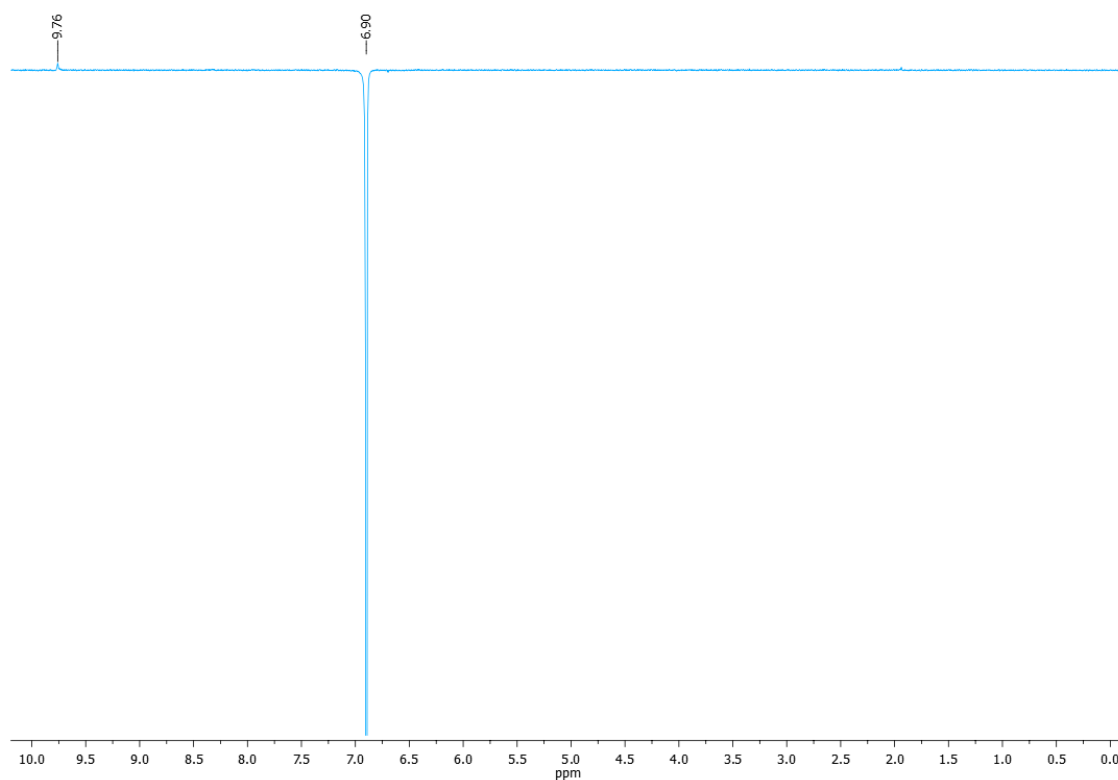


Figure 26S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the side chain H atom of Ac-(*E*)-ΔAla(βCl)-NHMe (**2**) in DMSO-*d*₆.

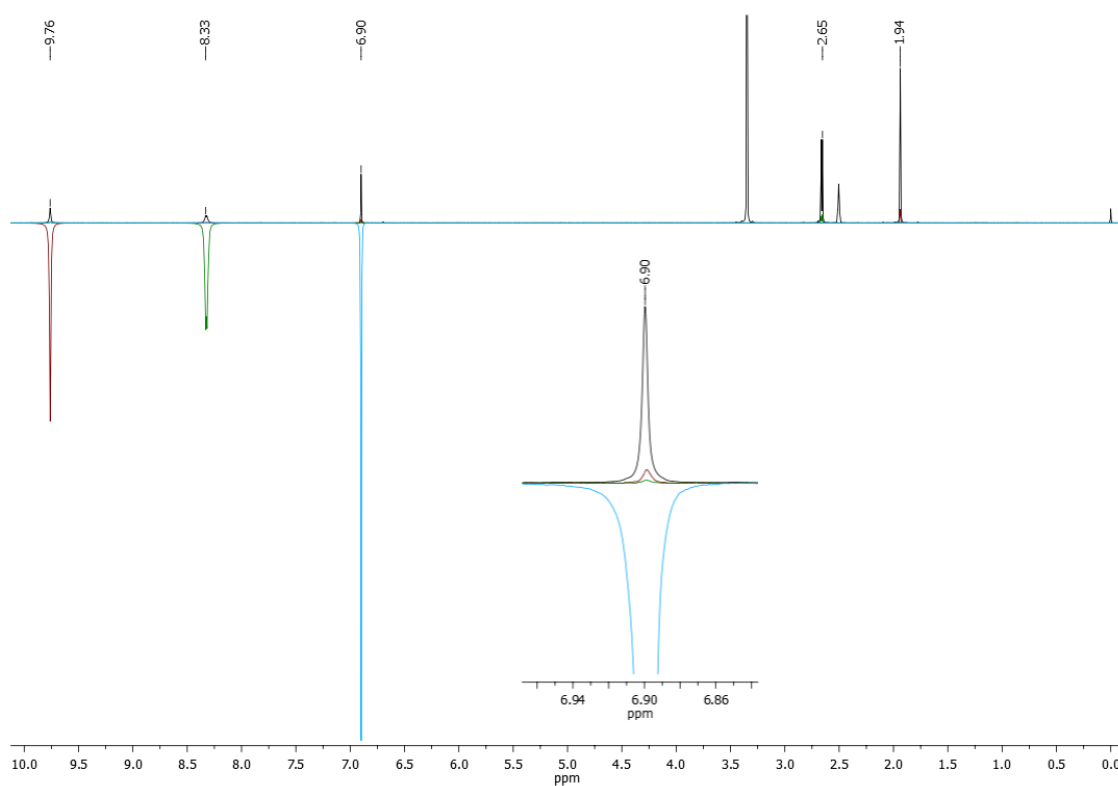


Figure 27S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom (red spectrum), the C-terminal amide H atom (green spectrum) and the side chain H atom (blue spectrum) of Ac-(*E*)-ΔAla(βCl)-NHMe (**2**) in DMSO-*d*₆.

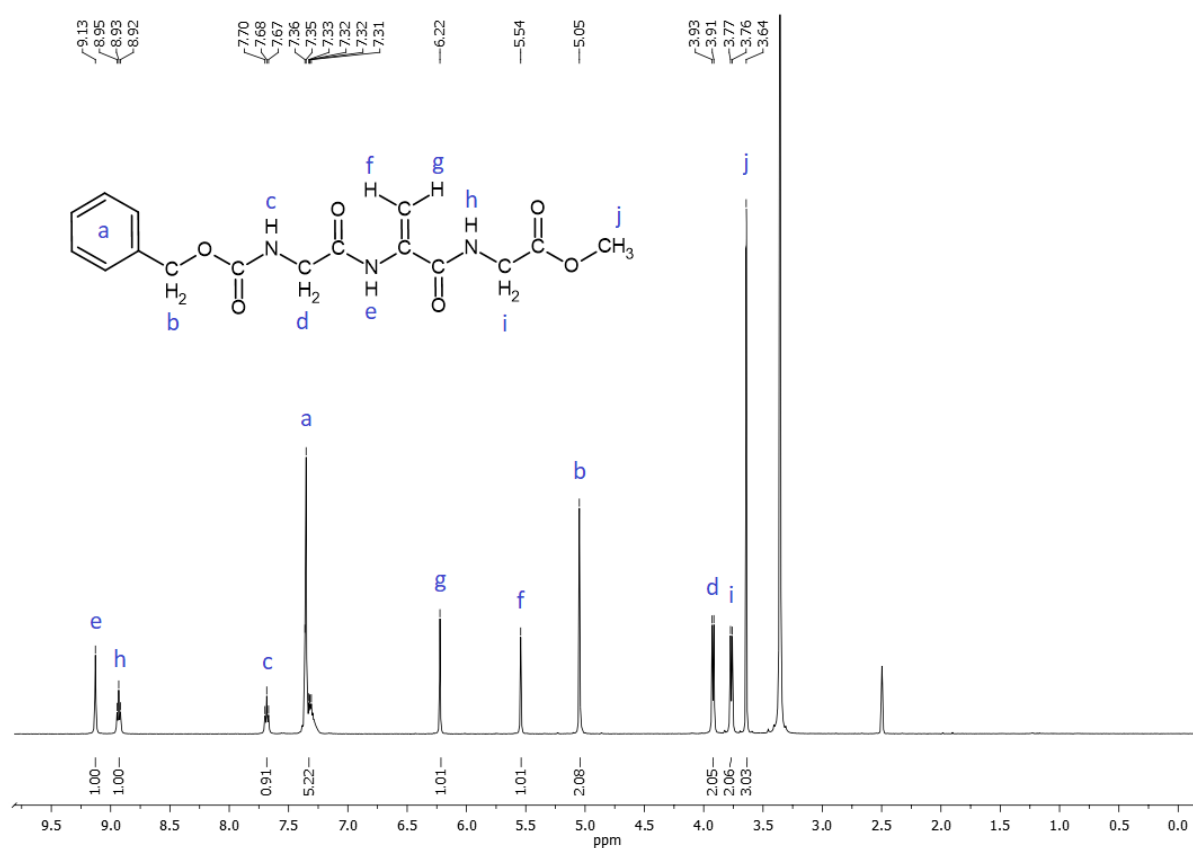


Figure 28S. ^1H NMR spectrum of Cbz-Gly- Δ Ala-Gly-OMe in $\text{DMSO}-d_6$.

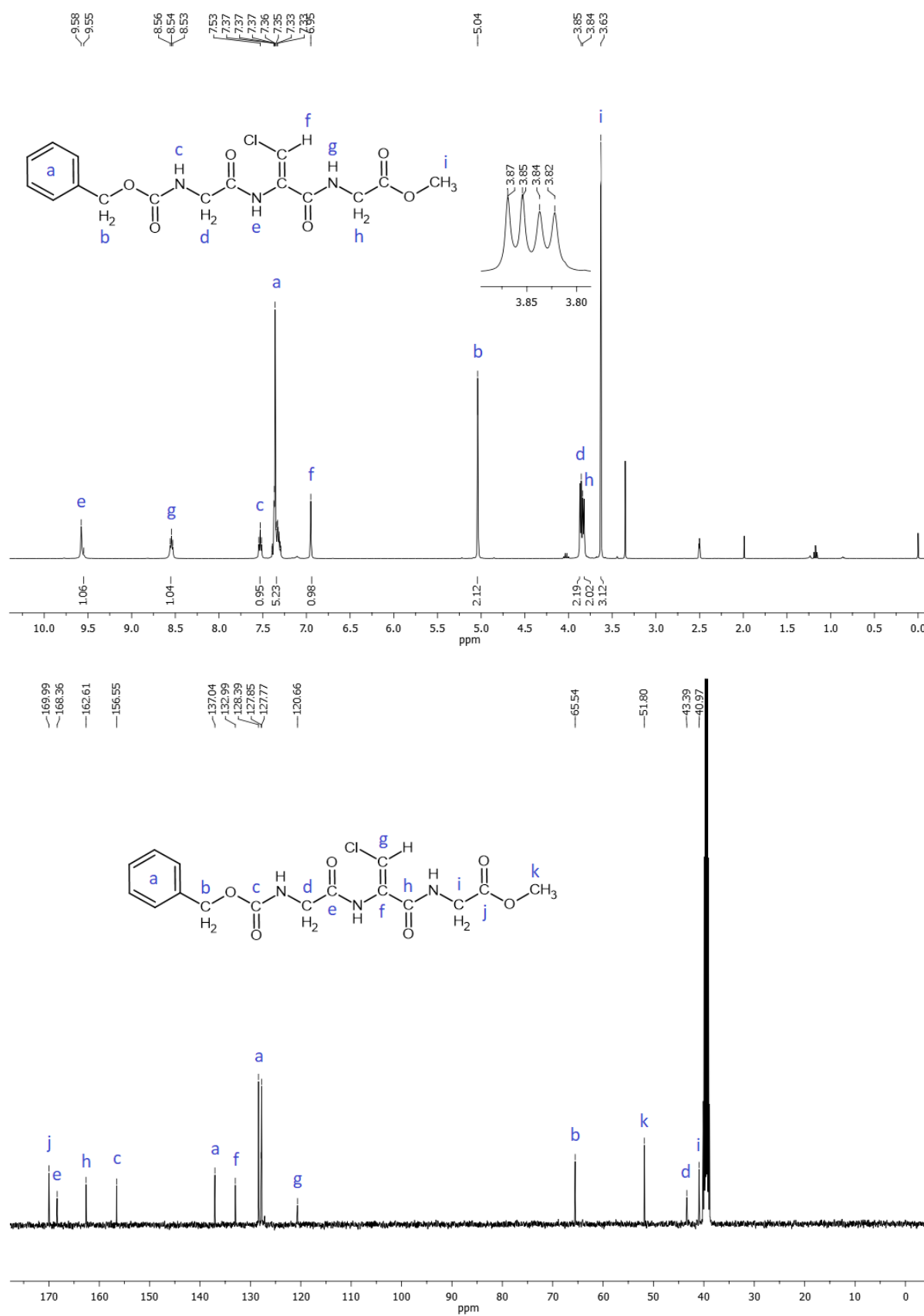


Figure 29S. ¹H and ¹³C NMR spectra of Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3) in DMSO-*d*₆.

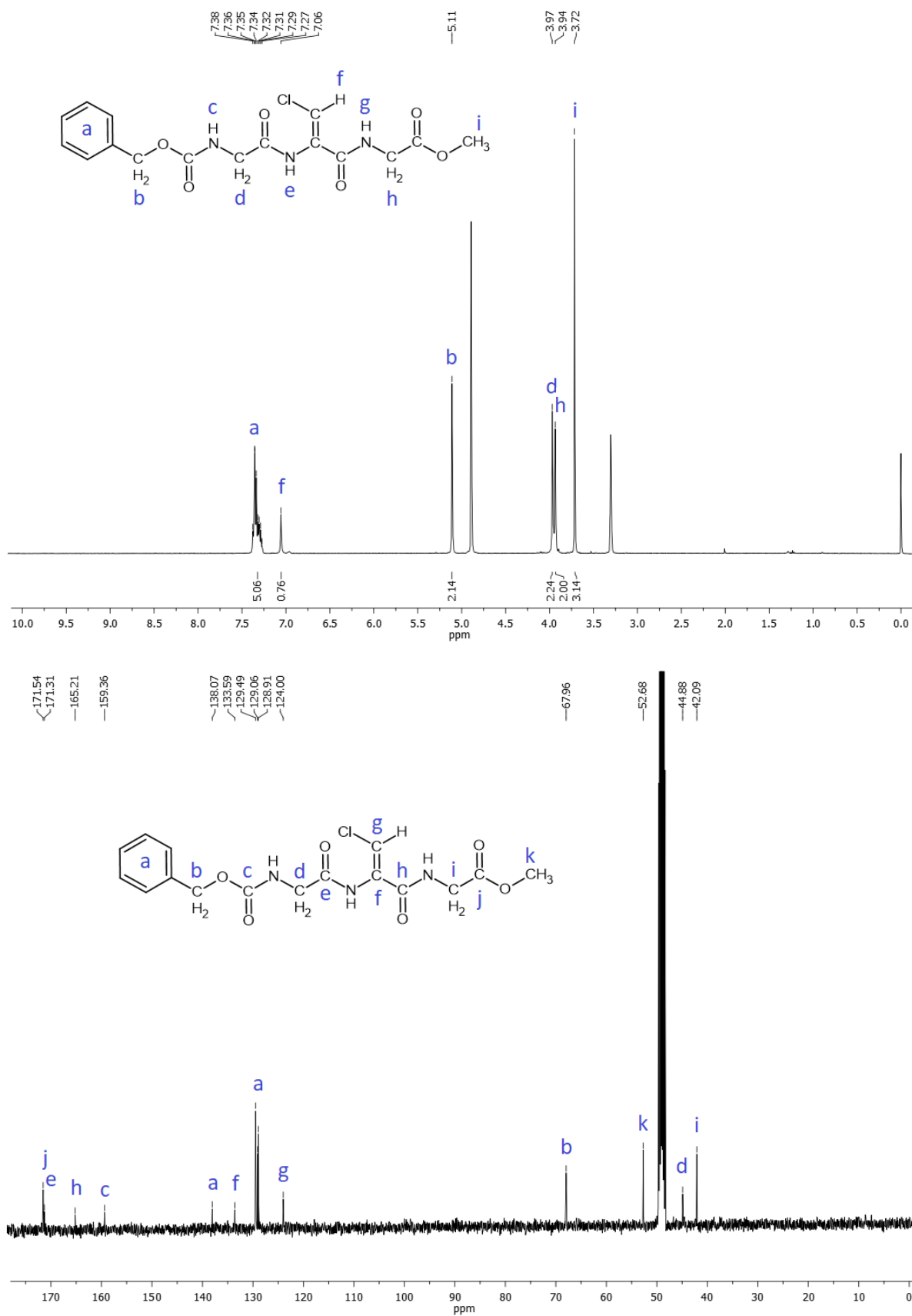


Figure 30S. ¹H and ¹³C NMR spectra of Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (3) in CD₃OD.

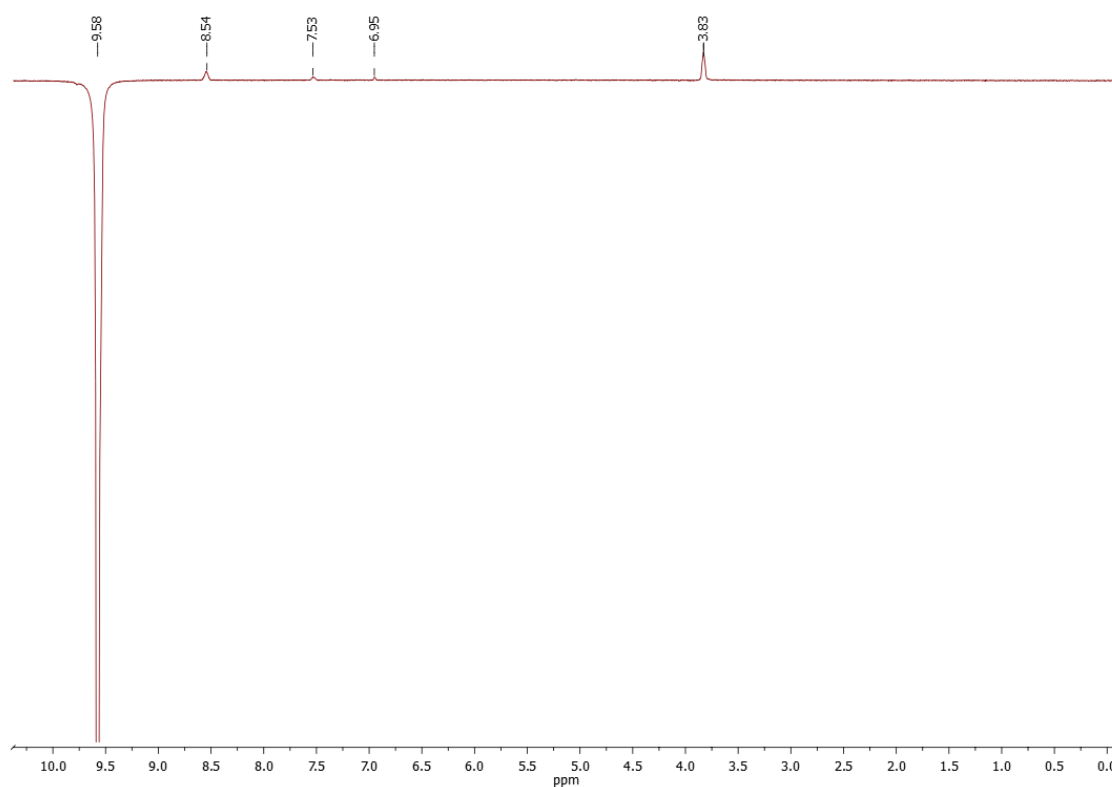


Figure 31S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H of Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (**3**) in DMSO-*d*₆.

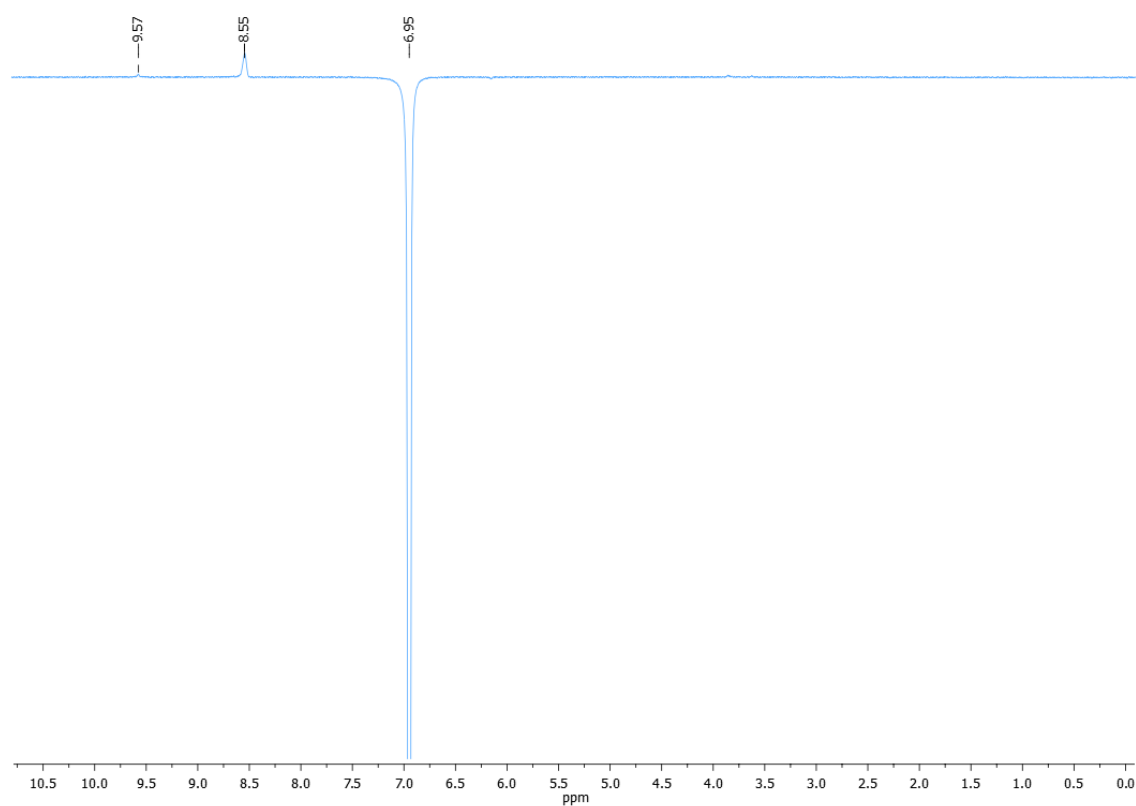


Figure 32S. ¹H NMR 1D-NOE spectra obtained by selective excitation side chain H atom (blue spectrum) of Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (**3**) in DMSO-*d*₆.

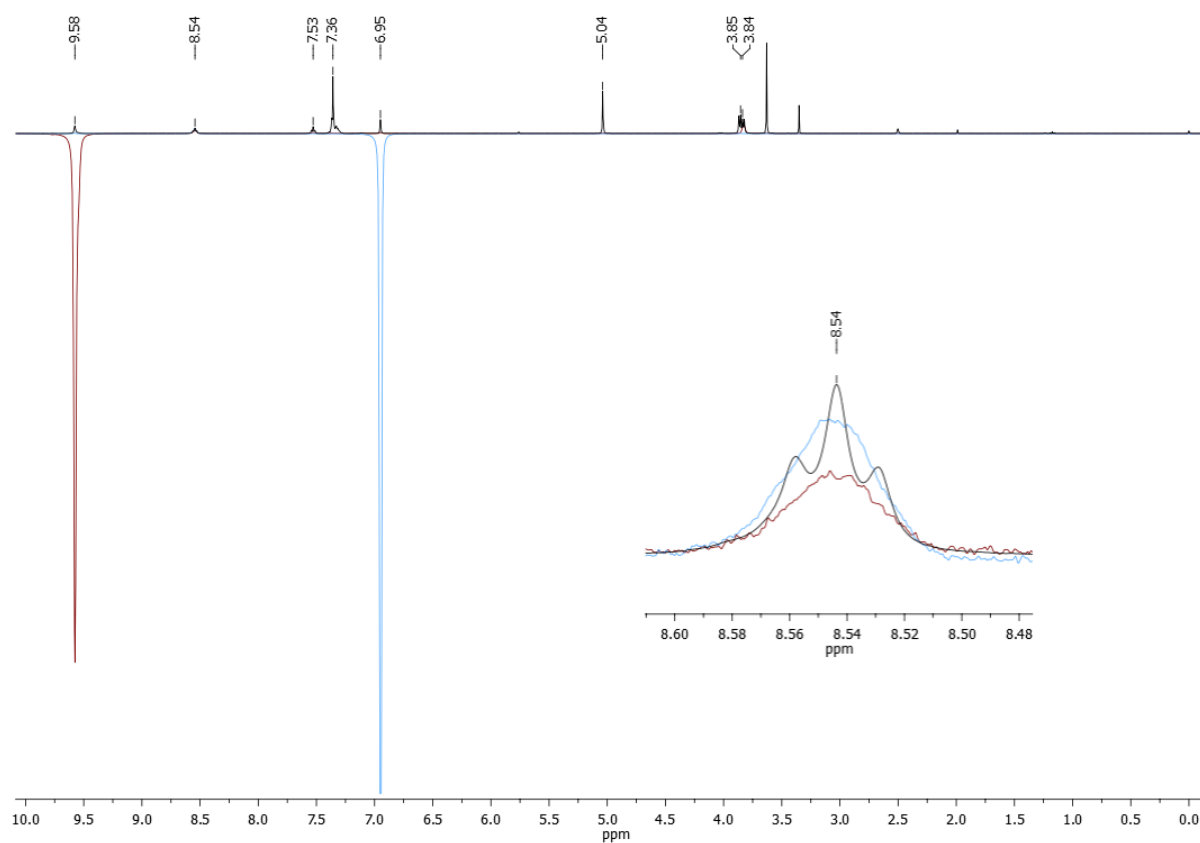


Figure 33S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom (red spectrum) and the side chain H atom (blue spectrum) of Cbz-Gly-(Z)-ΔAla(βCl)-Gly-OMe (**3**) in DMSO-*d*₆.

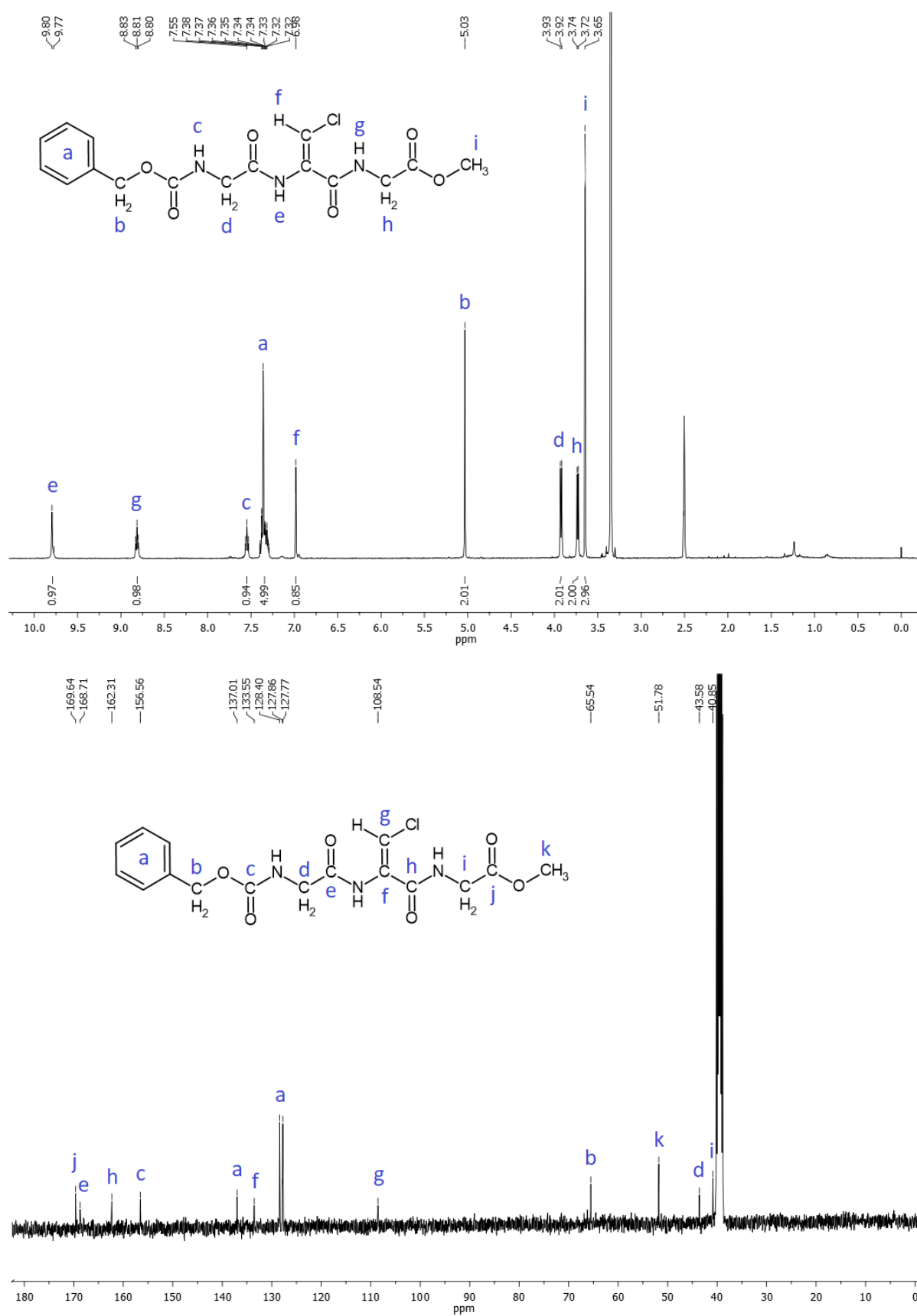


Figure 34S. ¹H and ¹³C NMR spectra of Cbz-Gly-(*E*)-ΔAla(βCl)-Gly-OMe in DMSO-*d*₆.

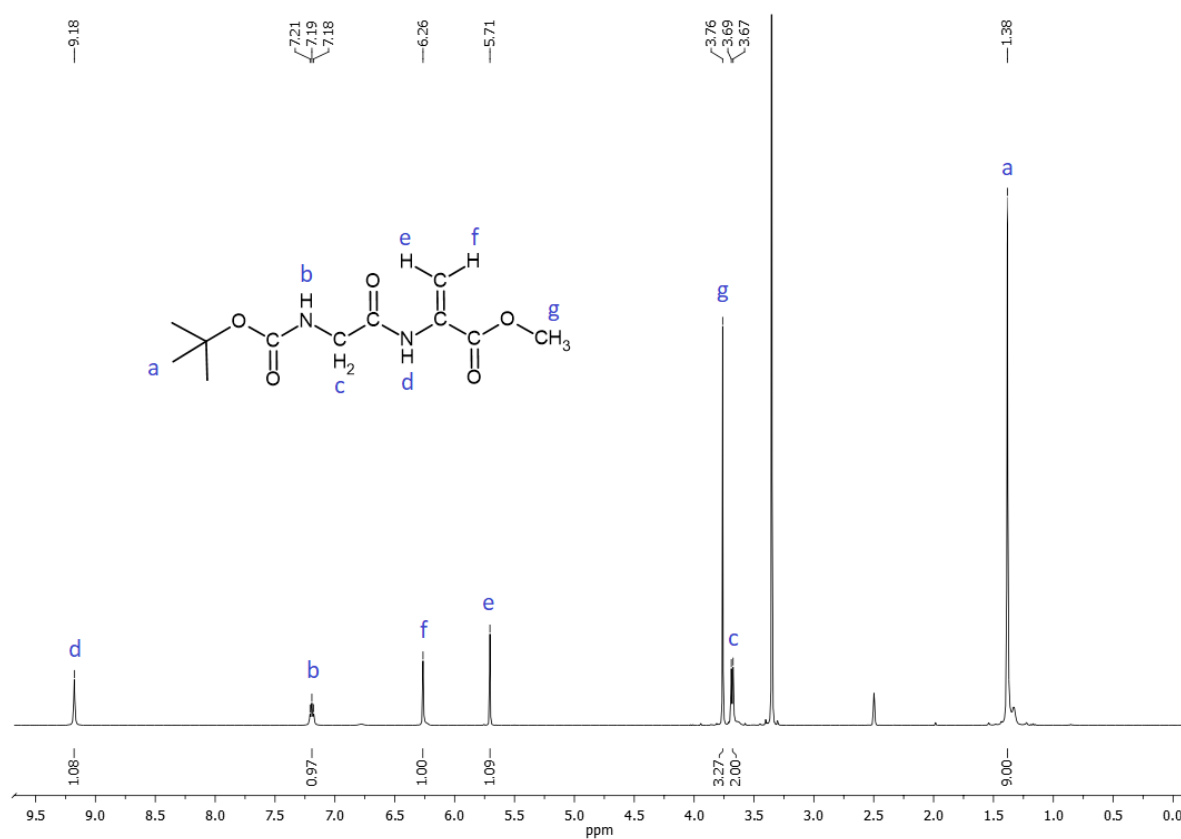


Figure 35S. ^1H spectrum of Boc-Gly-ΔAla-OMe in $\text{DMSO-}d_6$.

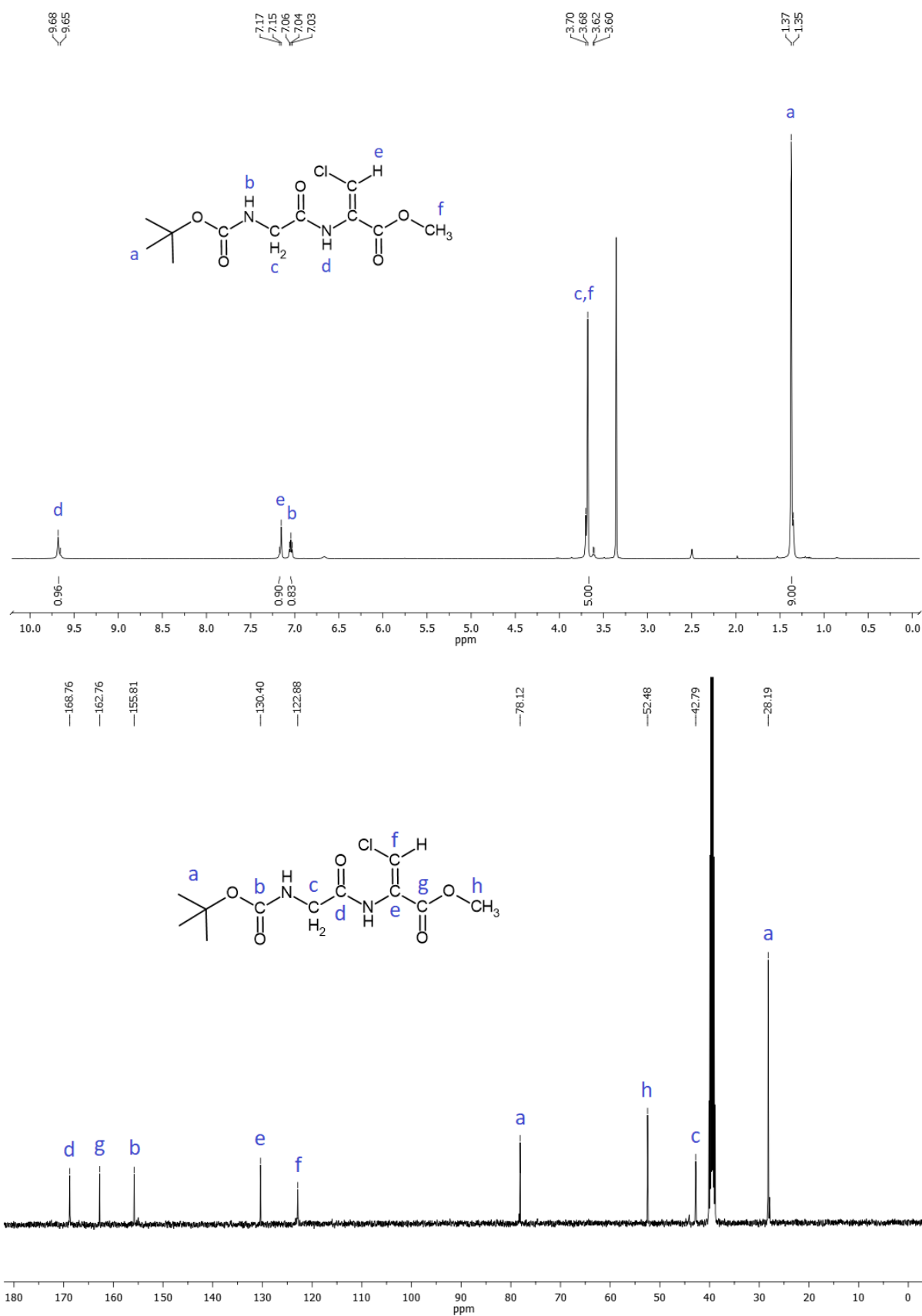


Figure 36S. ¹H and ¹³C NMR spectra of Boc-Gly-(Z)-ΔAla(βCl)-OMe (4) in DMSO-*d*₆.

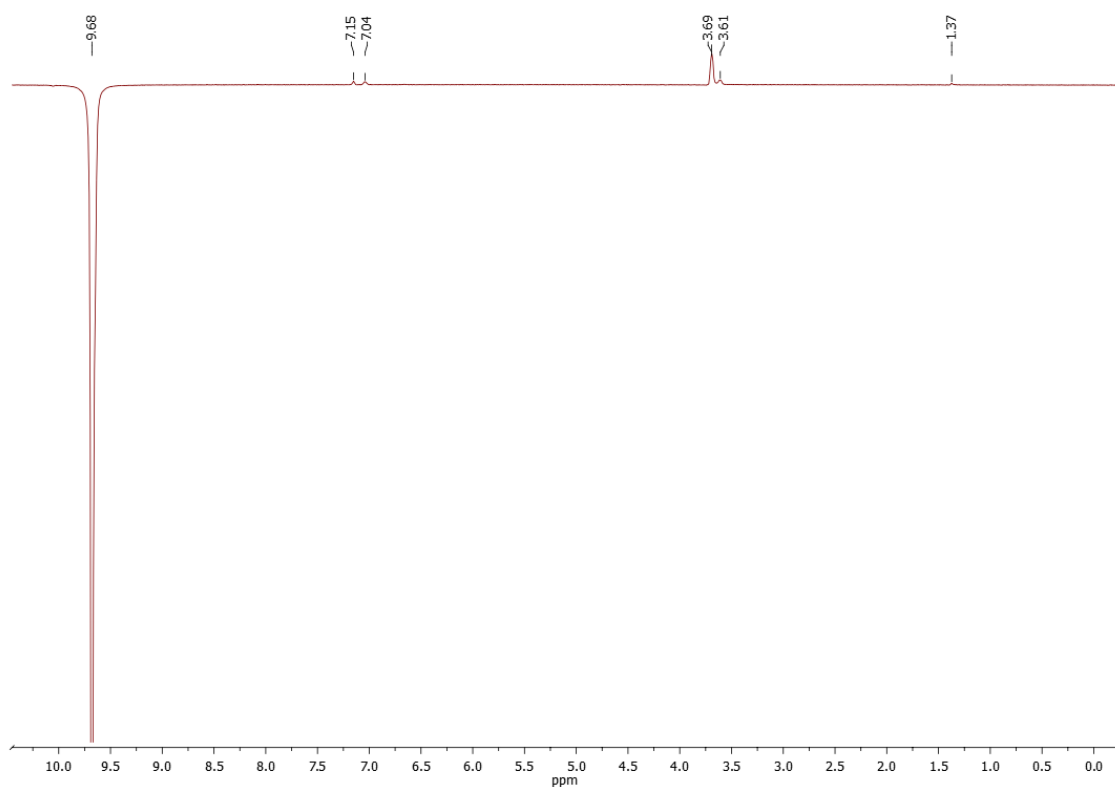


Figure 37S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom of Boc-Gly-(Z)-ΔAla(βCl)-OMe (**4**) in DMSO-*d*₆.

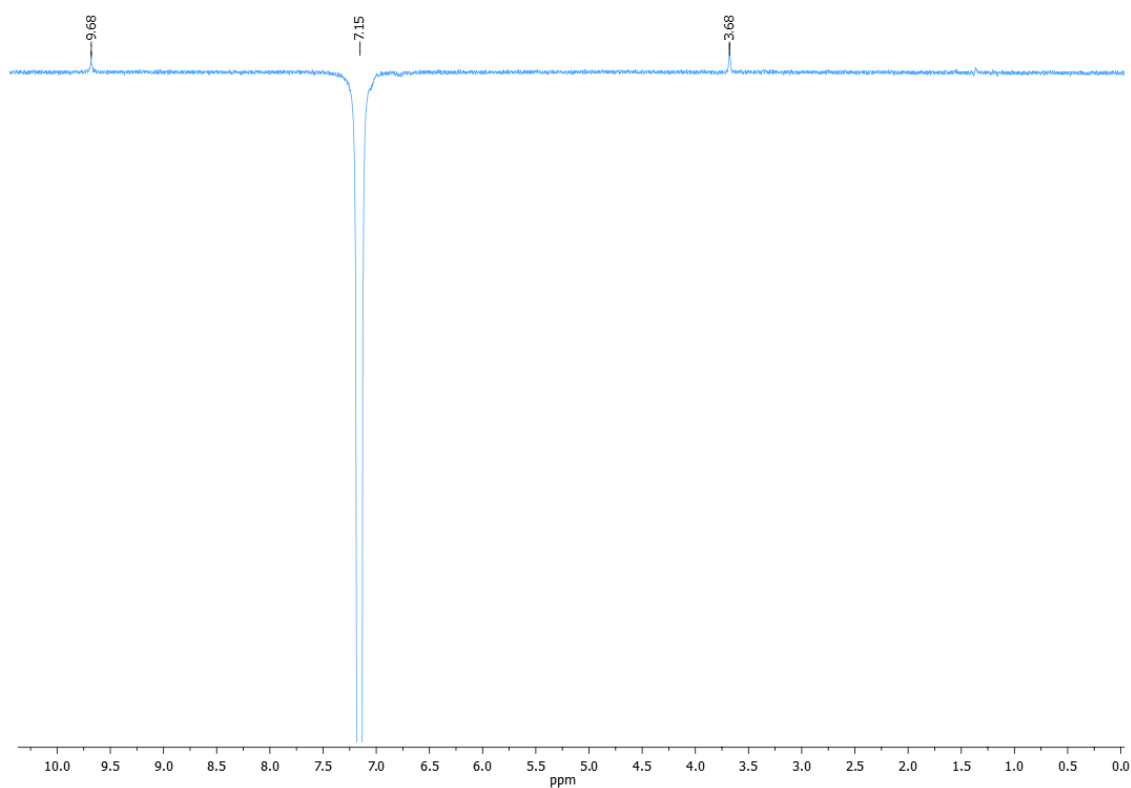


Figure 38S. ¹H NMR 1D-NOE spectra obtained by selective excitation of the side chain H atom of Boc-Gly-(Z)-ΔAla(βCl)-OMe (**4**) in DMSO-*d*₆.

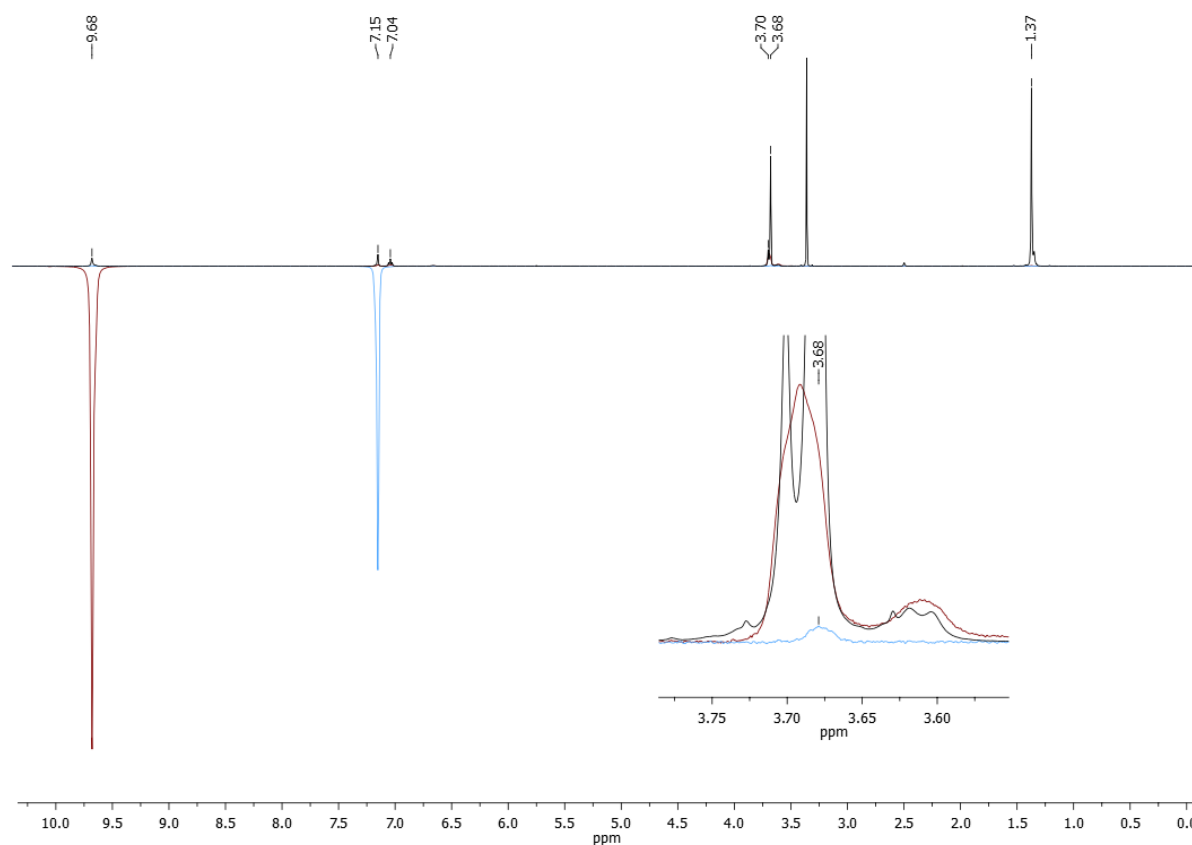


Figure 39S. ^1H NMR 1D-NOE spectra obtained by selective excitation of the N-terminal amide H atom (red spectrum) and the side chain H atom (blue spectrum) of Boc-Gly-(Z)- $\Delta\text{Ala}(\beta\text{Cl})\text{-OMe}$ (**4**) in $\text{DMSO-}d_6$.

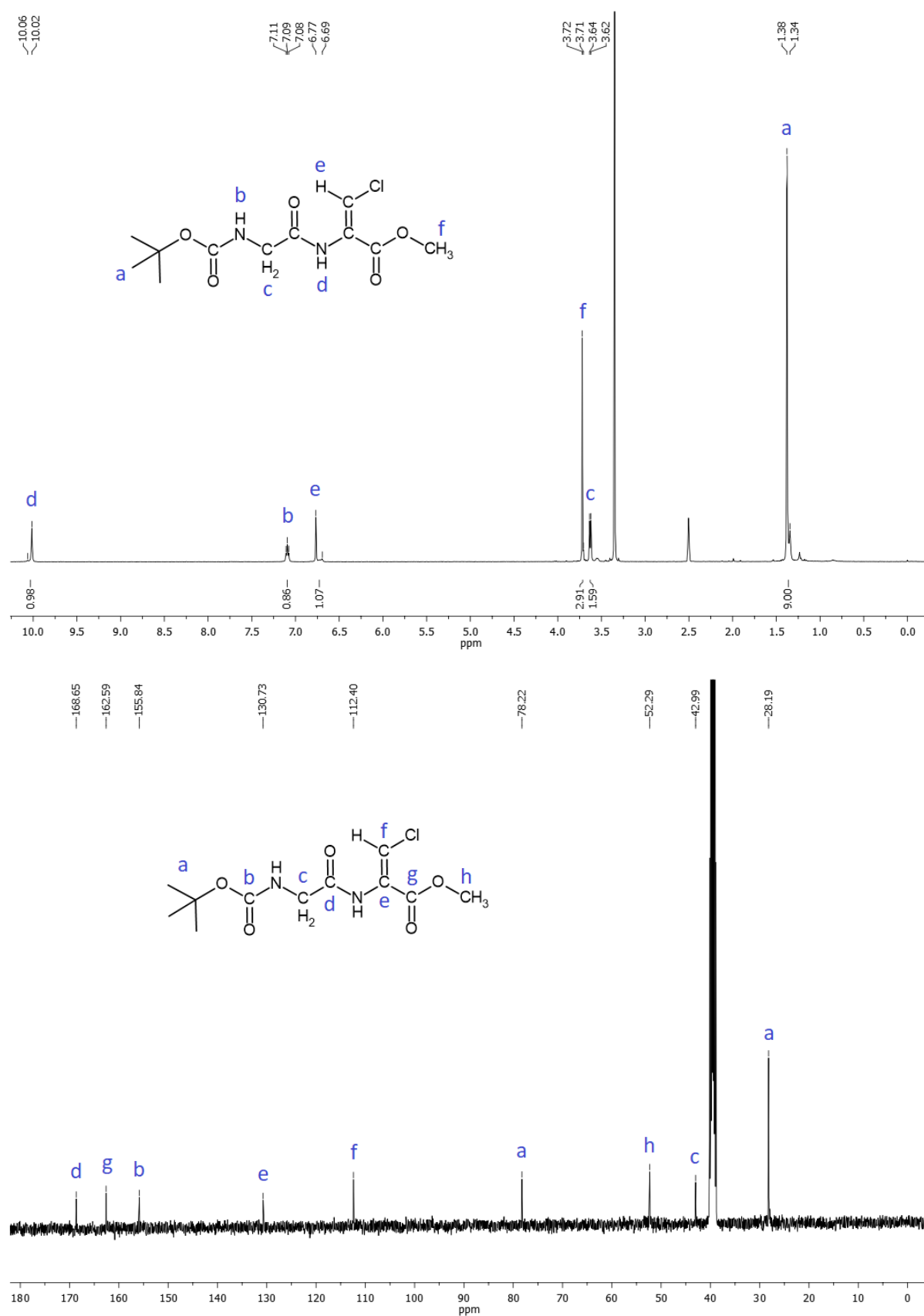


Figure 40S. ^1H and ^{13}C NMR spectra of Boc-Gly-(*E*)- Δ Ala(β Cl)-OMe in $\text{DMSO}-d_6$.

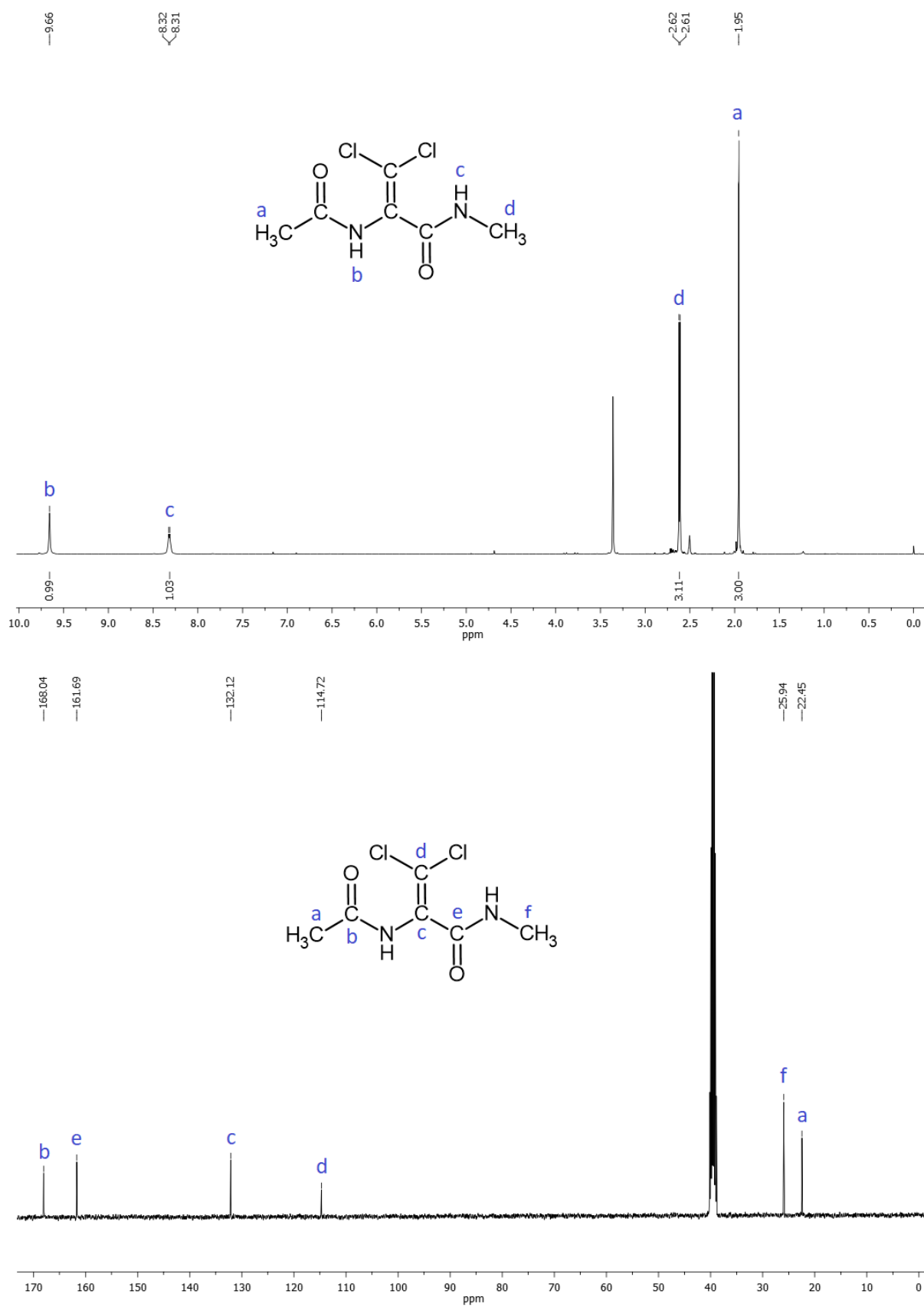


Figure 41S. ¹H and ¹³C NMR spectra of Ac-ΔAla(βCl₂)-NHMe (5) in DMSO-*d*₆.

D2



Article

Insight into the Structure of Antifungal Cyrmenins: Conformational Studies of Unique Dehydroamino Acid, O-Methyldehydroserine

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Abstract: O-Methyldehydroserine, Δ Ser(Me), is a non-standard α,β -dehydroamino acid, which occurs naturally in Cyrmenins with potential pharmaceutical application. The C-terminal part and the side chain of the Δ Ser(Me) residue constitute the β -methoxyacrylate unit, responsible for antifungal activity of Cyrmenins. The short model, Ac- Δ Ser(Me)-OMe, was analyzed considering the geometrical isomer Z (1) and E (2). The Ramachandran diagrams were created for both isomers, using quantum chemical calculations, to show possible conformations for isolated molecules (in vacuo), in weakly polar (chloroform) and polar (water) environments. The Ac-(Z)- Δ Ser(Me)-OMe (1) was synthesized and the single-crystal X-ray diffraction analysis together with FT-IR spectra were performed. The detailed analysis of the conformations of the (Z)- Δ Ser(Me) residue is presented considering the intra- and intermolecular interactions as well as their influence on the β -methoxyacrylate part. It is concluded that the β -methoxyacrylate structural motif is able to maintain a planar geometry, crucial for biological activity, regardless of the conformation adopted by O-methyldehydroserine.

Keywords: fungicide; β -methoxyacrylate; non-standard amino acids; dehydroamino acids; dehydroserine; Ramachandran diagram



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1. Introduction

Cyrmenins are naturally occurring peptides produced by *Cystobacter armeniacae* and *Archangium gephyra* (myxobacteria) [1,2]. Structurally, Cyrmenins are N-acyldehydrodipeptide methyl esters, R-CO- Δ Ala-(Z)- Δ Ser(Me)-OMe, containing two non-standard amino acid residues, dehydroalanine and (Z)-O-methyldehydroserine, (Z)- Δ Ser(Me) [3], also like the N-terminal (2E,4Z)-10-methylundecadienoic, -dodecadienoic, or -11-methyldodecadienoic acid (Figure 1). The structure of Cyrmenins is confirmed by the total synthesis [4,5].

The (Z)- Δ Ser(Me) residue contains the β -methoxyacrylate structural unit, important for biological activity as a pharmacophore (toxophore). The β -methoxyacrylate was earlier discovered in naturally occurring Strobilurins, Oudemansins, and Myxothiazols (Figure 2) [6]. All these compounds reveal antifungal activity as inhibitors of mitochondrial respiration at the cytochrome bc1 complex [7].

Isolations of Strobilurins and discovery of their antifungal activity resulted in the development of Strobilurin fungicides, known as β -methoxyacrylate fungicides such as Azoxystrobin, Coumoxystrobin, Enoxastrobin, Pyraoxystrobin, Picoxystrobin, or Flufenoxystrobin (Figure 3). They play an important role as antifungal agrochemicals, used as active agents

protecting important plants such as cereals (wheat, rice), oilseeds, vegetables (cucumbers, tomatoes), and fruits (grapes) from fungal diseases (downy mildew, blight, powdery mildew, leaf blotch, net blotch, leaf rust, brown rust, and tan spot) (Compendium of Pesticide Common Names) [8].

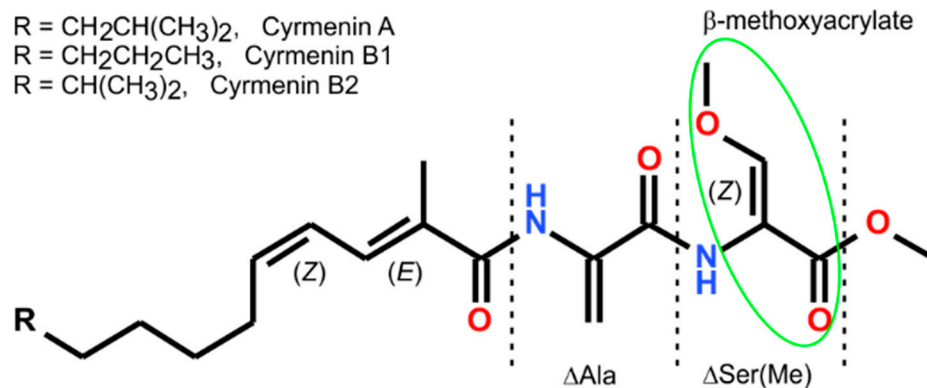


Figure 1. Structure of Cyrmenins, antifungal peptides, with marked β -methoxyacrylate unit.

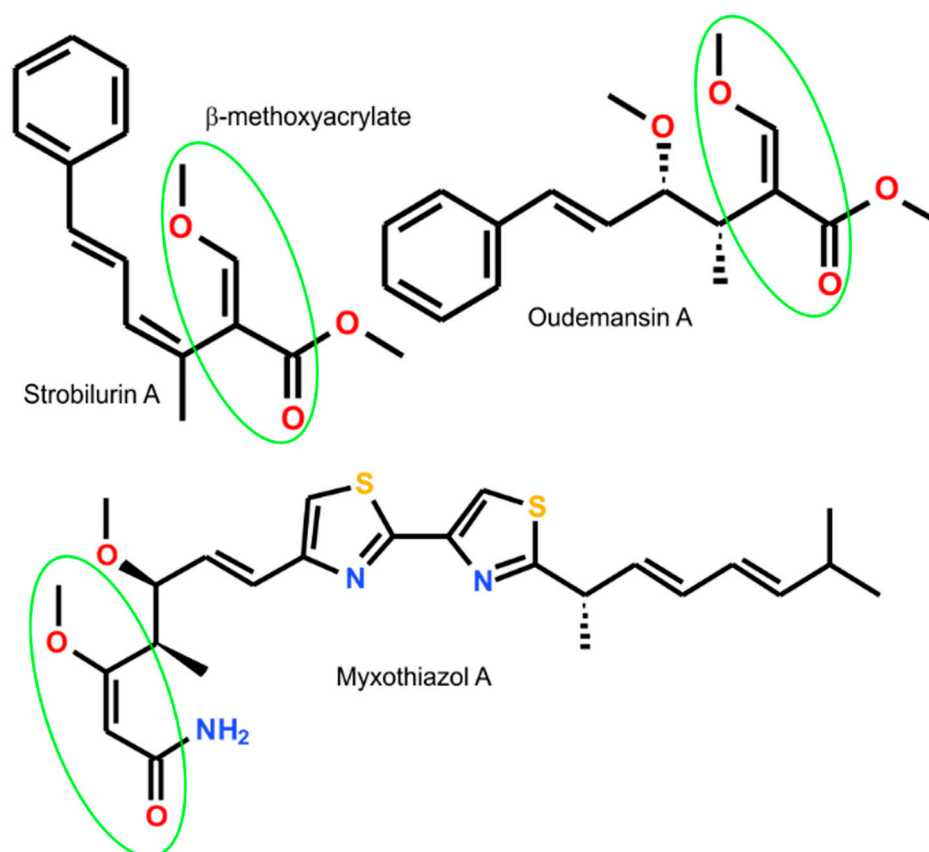


Figure 2. The structure of Strobilurin A, Oudemansin A, and Myxothiazol A, naturally occurring precursors of β -methoxyacrylate fungicides.

β -Methoxyacrylate derivatives as cytochrome bc1 inhibitors were recently reviewed [9,10]. The conclusion can be drawn that the β -methoxy group and carbonyl group at the $\text{C}=\text{C}$ double bond are in transoidal position with respect to each other. In the case of the studied $\Delta\text{Ser}(\text{Me})$ residue, it corresponds to the isomer Z.

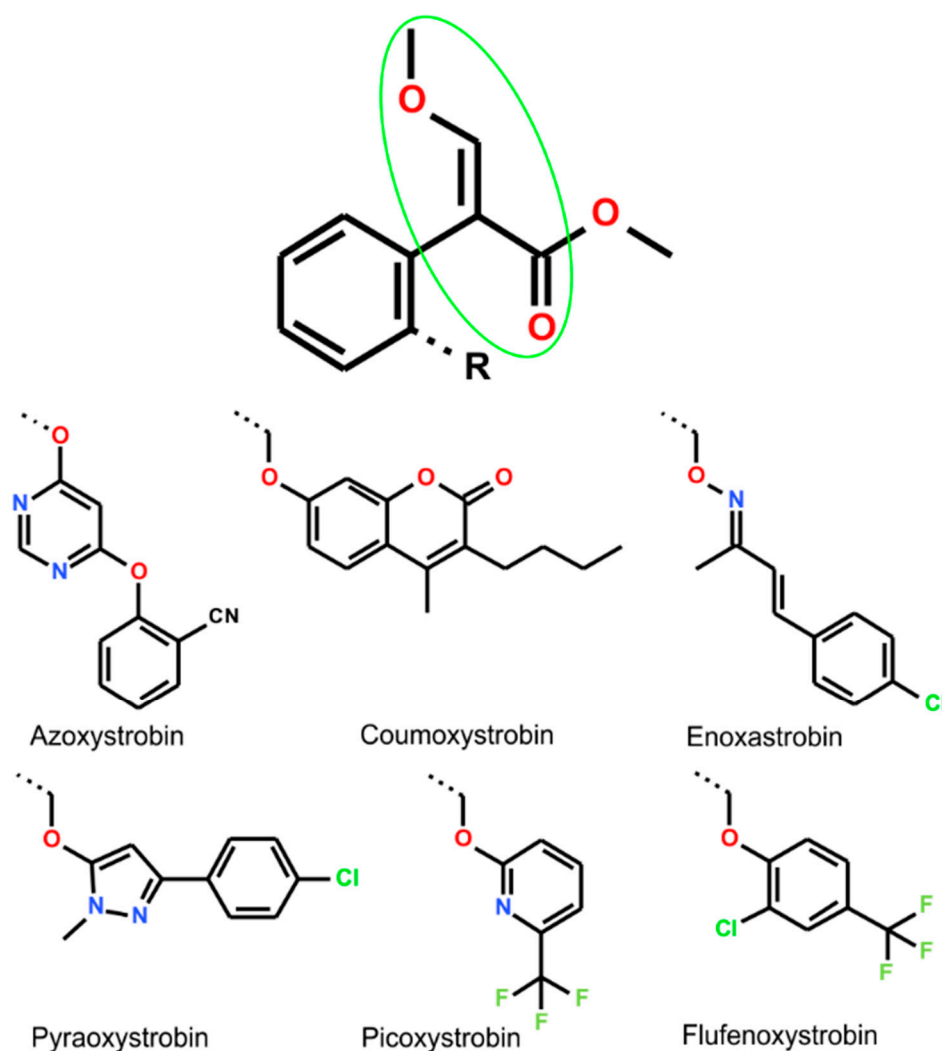


Figure 3. The structure of Azoxystrobin, Coumoxystrobin, Enoxastrobin, Pyraoxystrobin, Picoxystrobin, and Flufenoxystrobin, designed β -methoxyacrylate fungicides commonly applied as agrochemicals.

Cyrmensins, like other compounds containing a β -methoxyacrylate structural unit, also reveal an antifungal activity (yeasts and filamentous fungi), but no effect on bacteria. Their activity is similar to Strobilurin A. However, they are less toxic for animal cell cultures (mouse cells) [2]. This feature makes them promising precursors of potential antifungal pharmaceuticals.

Cyrmensins belong to peptides. For the peptides, the conformation is important because it determines the spatial arrangement of functional groups, which has a direct impact on their biological activity. The conformation of the whole peptide molecule depends on the conformation adopted by each amino acid residue. Cyrmensins are simple dehydrodipeptides. The conformational properties of dehydroalanine are well known. It has a tendency to adopt the extended conformation C5 ($\varphi, \psi \sim -180^\circ, 180^\circ$) stabilized by the internal N-H $\cdots$ O and C β -H $\cdots$ O hydrogen bonds as well as π -electron cross-conjugation expanded from the C α =C β double bond to the adjacent N- and C-terminal peptide groups, making the whole structure flat [11,12]. However, O-methyldehydroserine is a unique dehydroamino acid, found to date, only in Cyrmensins. No study on its conformational properties has been performed so far. What is important is that the O-methyldehydroserine residue contains the β -methoxyacrylate unit, created by the methoxy group at the carbon atom β and the C α =C β double bond.

The aim of the presented work is to investigate how the conformation adopted by O-methyldehydroserine residue influences the geometry of the β -methoxyacrylate unit, crucial for biological activity of Cyrmenins.

2. Results

2.1. Theoretical Method

The quantum chemistry calculations are presented first, because they give the overall picture of the conformations, which can possibly be adopted. Then, these results are compared to the experimental X-ray and FT-IR data, which give the conformational preferences of the O-methyldehydroserine residue.

A common approach to assess the conformational properties of selected amino acid residues is the analysis of short-model compounds [13]. The conformational analysis of O-methyldehydroserine residue was performed on two molecular models, Ac-(Z)- Δ Ser(Me)-OMe (1) and Ac-(E)- Δ Ser(Me)-OMe (2) (Figure 4). These models represent the geometrical isomers Z/E resulting from the presence of different substituents and the π -bond between the carbon atoms $C\alpha=C\beta$. Assuming that the N-terminal amide and C-terminal ester are typically in trans arrangement, the conformation of the molecule depends on two torsion angles: φ (C-N-C α -C) and ψ (N-C α -C-O). The potential energy surfaces $E = (\varphi, \psi)$ together with the conformations are presented in Figures 5 and 6. The selected parameters, including the values of torsion angles φ and ψ as well as the energy and the population of the conformations (p), are presented in Tables 1 and 2. The structural parameters for the internal hydrogen bond (X-H $\cdots$ A) and carbonyl interactions (C=O $\leftarrow\cdots\rightarrow$ O=C) are presented in Tables S1 and S2.

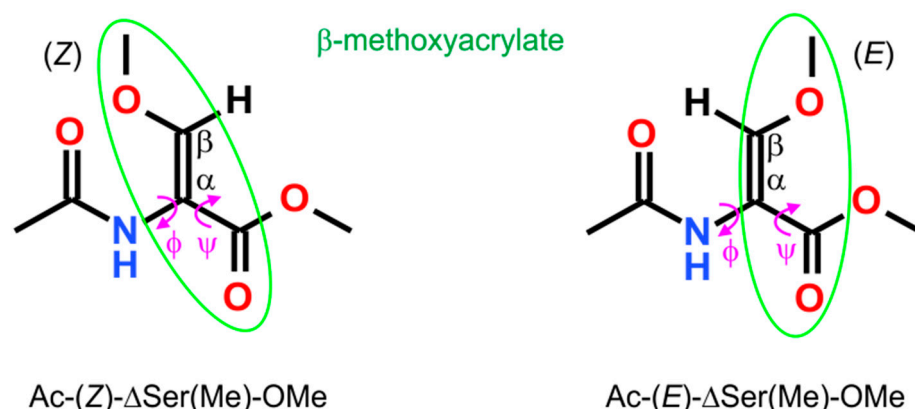


Figure 4. Two molecular models, Ac-(Z)- Δ Ser(Me)-OMe (1) and Ac-(E)- Δ Ser(Me)-OMe (2), applied in the conformational analysis of O-methyldehydroserine residue. The geometrical isomers Z/E result from the presence of different substituents and the π -bond between the carbon atoms $C\alpha=C\beta$. Two main torsion angles φ and ψ are shown together with the β -methoxyacrylate unit.

Four conformations were found, regardless of the Z/E isomers: the extended C5, semi-extended β 2, helical α , and helical polyproline II-like β . Due to the lack of chirality, each of these conformations have their mirror counterparts with the same energy but opposite sign of the torsion angle values. For clarity of description, the conformations on the left side of the maps are discussed.

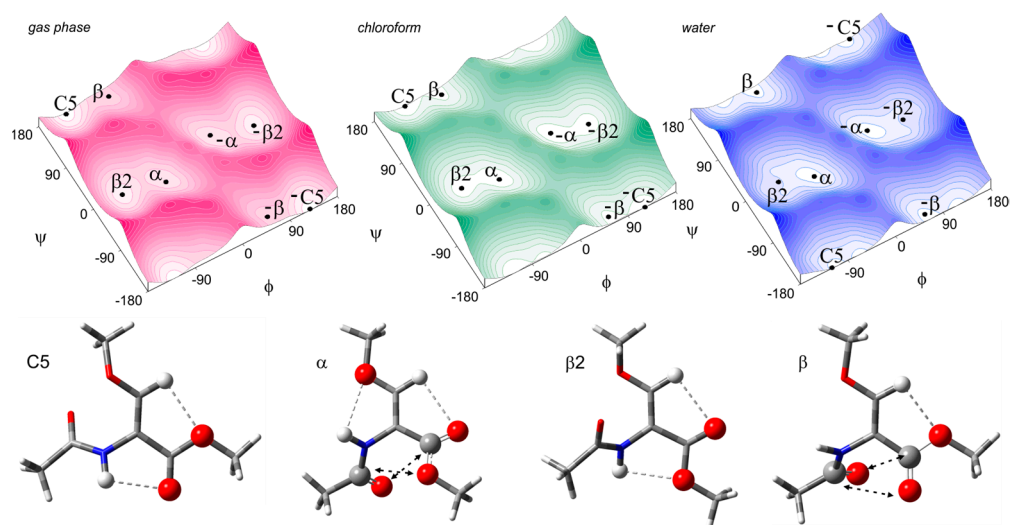
Ac-(Z)- Δ Ser(Me)-OMe (1)

Figure 5. The potential energy surfaces $E = (\phi, \psi)$ of Ac-(Z)- Δ Ser(Me)-OMe (1) calculated via the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environments. Energy contours are plotted every 1 kcal/mol. Below the maps are the conformations optimized at the M06-2X/6-311+G(d,p) level of theory with the hydrogen bonds ($\cdots$) [14] and electrostatic interactions ($\leftarrow \cdots \rightarrow$) [15] created within the residue.

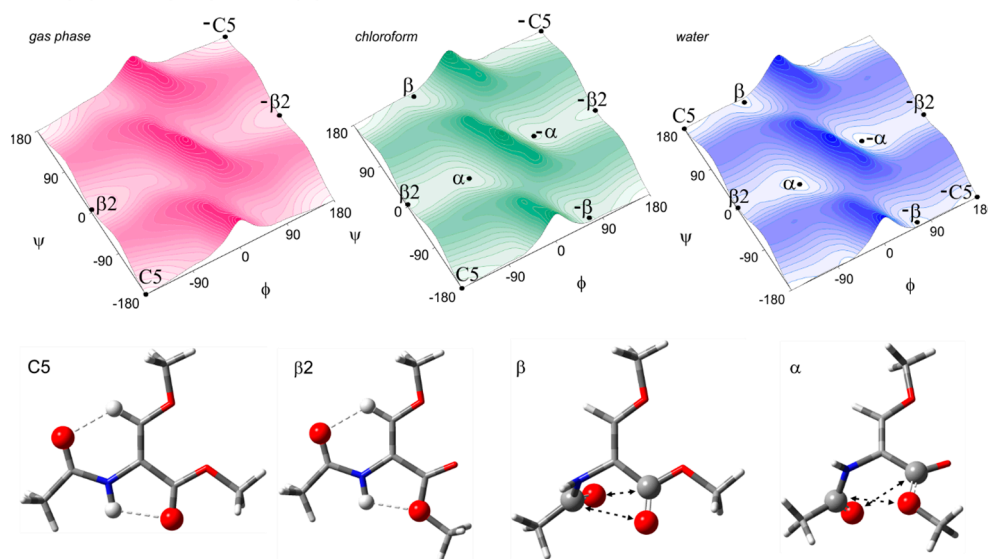
Ac-(E)- Δ Ser(Me)-OMe (2)

Figure 6. The potential energy surfaces $E = (\phi, \psi)$ of Ac-(E)- Δ Ser(Me)-OMe (2) calculated via the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environments. Energy contours are plotted every 1 kcal/mol. Below the maps are the conformations optimized at the M06-2X/6-311+G(d,p) level of theory with the hydrogen bonds ($\cdots$) [14] and electrostatic interactions ($\leftarrow \cdots \rightarrow$) [15] created within the residue.

For the isomer Z, Ac-(Z)- Δ Ser(Me)-OMe (1), for the isolated molecules, the values of the torsion angles ϕ and ψ , which determine the geometry of the residue, are as follows: C5 ($\phi, \psi = -129^\circ, 178^\circ$), β 2 ($\phi, \psi = -126^\circ, -2^\circ$), α ($\phi, \psi = -54^\circ, -20^\circ$), and β ($\phi, \psi = -56^\circ, 164^\circ$). The simulated increase in the environment polarity (chloroform, water) has rather low impact on the geometry of the conformations. The changes in the torsion angles ϕ and ψ do not exceed 9° and 5° , respectively. Nevertheless, the values of the torsion angle ϕ decrease for the extended and semi-extended conformations (C5 and

$\beta 2$) and increase for the helical conformations (α and β). This shows that the N-terminal amide group becomes more perpendicular towards the $C\alpha=C\beta$ double bond. The values of the torsion angle ψ remain almost unchanged for the conformations C5 and $\beta 2$. In the case of the helical conformations, the ψ value decreases for the conformation α but increases for the conformation β . This shows that the C-terminal ester group becomes more parallel towards the $C\alpha=C\beta$ double bond. Consequently, as the polarity of the environment increases, the β -methoxyacrylate unit becomes more flat.

Table 1. Selected Parameters for Conformations of Ac-(Z)- Δ Ser(Me)-OMe (1).

Ac-(Z)- Δ Ser(Me)-OMe (1)					
Conformation	φ [°]	ψ [°]	G [Hartrees]	Δ G [kcal]	p [%]
gas phase					
C5	−129.0	178.1	−628.79563	0.00	61.42
α	−53.6	−20.0	−628.79452	0.70	19.05
$\beta 2$	−126.4	−2.4	−628.79439	0.78	16.57
β	−56.3	163.9	−628.79276	1.81	2.96
chloroform					
α	−57.9	−16.6	−628.80850	0.00	28.60
C5	−122.2	179.8	−628.80844	0.04	26.63
β	−60.7	167.2	−628.80843	0.04	26.52
$\beta 2$	−119.7	−0.4	−628.80808	0.27	18.25
water					
C5	−120.2	−179.3	−628.81339	0.00	33.62
β	−62.1	168.4	−628.81337	0.01	32.82
α	−59.2	−15.7	−628.81289	0.31	19.82
$\beta 2$	−117.7	0.2	−628.81254	0.53	13.74

Each calculated conformation has its mirror counterpart. Optimized via the M06-2X/6-311+G(d,p) method (SCRF, CPCM). The population of the conformations (p).

Table 2. Selected Parameters for Conformations of Ac-(E)- Δ Ser(Me)-OMe (2).

Ac-(E)- Δ Ser(Me)-OMe (2)					
Conformation	φ [°]	ψ [°]	G [Hartrees]	Δ G [kcal]	p [%]
gas phase					
C5	−180.0	−180.0	−628.79370	0.00	94.86
$\beta 2$	−180.0	0.0	−628.79093	1.74	5.14
chloroform					
C5	−180.0	−180.0	−628.80438	0.00	57.02
$\beta 2$	−180.0	0.0	−628.80334	0.65	19.12
β	−68.4	173.3	−628.80323	0.72	16.96
α	−66.8	−8.2	−628.80237	1.26	6.90
water					
β	−67.7	172.8	−628.80794	0.00	32.17
C5	−180.0	180.0	−628.80773	0.13	25.83
α	−66.0	−10.1	−628.80765	0.18	23.58
$\beta 2$	−180.0	0.0	−628.80741	0.33	18.42

Each calculated conformation has its mirror counterpart. Optimized via the M06-2X/6-311+G(d,p) method (SCRF, CPCM). The population of the conformations (p).

The conformation C5 has the lowest energy for the isolated molecule. However, the energy gap to the highest-in-energy conformation β is relatively small and does not exceed 2 kcal/mol. As a result, the population has a relatively low energy value (estimated at 61%). An increase in the polarity of the environment causes a further decrease in the energy difference between the conformations. In the chloroform environment, the gap in energy does not exceed 0.3 kcal/mol, and ensures that all conformations are accessible

and populated. Also, for the water environment, the energy difference (0.5 kcal/mol) is small (Table 1).

For the isomer E, Ac-(E)- Δ Ser(Me)-OMe (2), there are also four conformations, but for the isolated molecule, only the extended and semi-extended conformations, C5 ($\varphi, \psi = -180^\circ, 180^\circ$) and β 2 ($\varphi, \psi = -180^\circ, 0^\circ$), are predicted. In the more polar environment, the helical conformations α ($\varphi, \psi = -66^\circ, -9^\circ$) and β ($\varphi, \psi = -66^\circ, 173^\circ$) appear. It should be noticed that the geometry of all conformations seems to be resistant to the increase in the polarity of the environment. For the extended conformations C5 and β 2, the studied (E)- Δ Ser(Me) residue is completely flat. For the helical conformations α and β , the N-terminal part tends to be perpendicular in relation to the C α =C β double bond. Again, the C-terminal part containing the β -methoxyacrylate unit is completely or nearly flat. The conformation C5 is the lowest in energy for the isolated molecule and almost completely populated (95%). Nevertheless, the energy gap to the conformation β 2 does not exceed 2 kcal/mol. In the low-polar environment, mimicked by the chloroform solvent, the gap in energy decreases to 1.3 kcal/mol, but the conformation C5 remains the first choice (57%). In the more polar water environment, the energy difference further decreases to 0.4 kcal/mol and the conformations with the torsion angle $\psi = 180^\circ$ and 173° , C5 and β , are favored (Table 2).

2.2. Crystal Structure Analysis

A single crystal of Ac-(Z)- Δ Ser(Me)-OMe (1) was obtained from a diethyl ether solution. The experimental details of X-ray data collection together with the selected geometric parameters are presented in Tables S3–S5. The molecular structure with the atom numbering scheme is presented in Figure 7. The X-ray diffraction analysis performed at 100 K gave indications on the dihedral angles adopted.

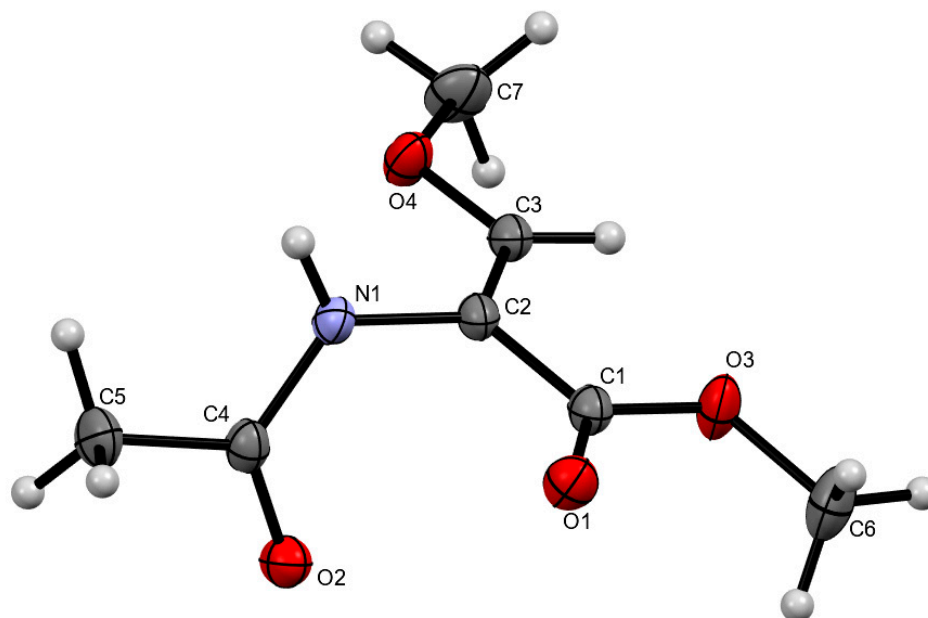


Figure 7. The molecular structure of Ac-(Z)- Δ Ser(Me)-OMe (1) in the asymmetric part of the unit cell. Displacement ellipsoids are drawn at the 50% probability level.

The conformations β ($-68.99^\circ, 174.17^\circ$) and $-\beta$ ($68.99^\circ, -174.17^\circ$) were found in the crystal. The presence of the mirror conformations is typical for dehydroamino acids, which does not have asymmetrical carbon atom alpha. These results confirm the results of quantum chemical calculations performed for the polar environment (water), for which the conformation β is amongst the lowest in energy and equally populated with the

conformation C5. The geometry of conformations is quite similar and the difference in values of torsion angles φ and ψ does not exceed 7° .

The β -methoxyacrylate structural unit is almost flat, which can be determined by co-planarity of the $C\alpha=C\beta$ π -bond and the C-terminal ester carbonyl $C=O$ group, with the absolute value of the torsion angle $C3-C2-C1-O1$ being equal to 178.98° .

The molecular interactions of the Ac-(Z)- Δ Ser(Me)-OMe (**1**) molecules (Figure 8) are determined by the N-H...O hydrogen bonds. A linear arrangement can be observed, in which the molecules adopt the conformation β and $-\beta$, alternately. Only the N-terminal amide group is involved in the N-H...O hydrogen bonds. The β -methoxyacrylate structural unit creates only weak C-H...O short contacts.

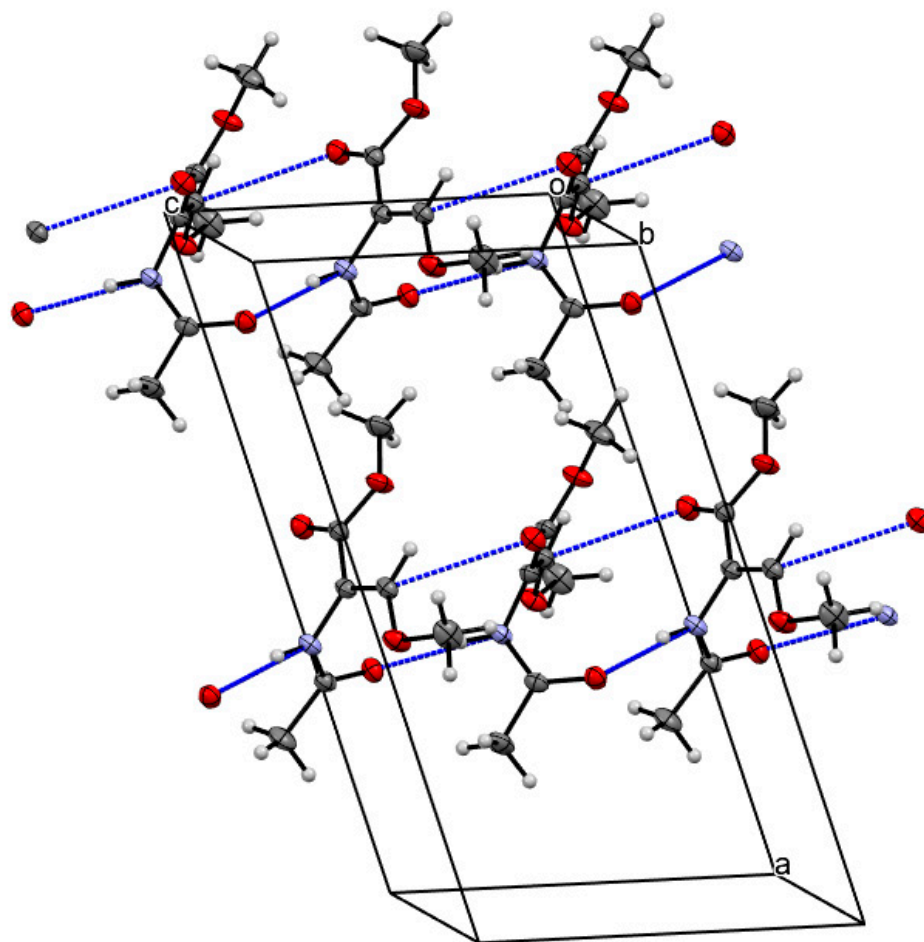


Figure 8. Molecular interactions of the Ac-(Z)- Δ Ser(Me)-OMe (**1**) molecules. The hydrogen bonds are visualized by dotted lines. Mercury 3.8 (Build RC2) (<http://www.ccdc.cam.ac.uk/mercury/>, accessed on 29 October 2024) was applied. The hydrogen bonds rely on the sum of van der Waals radii of the atom involved.

2.3. Infrared Absorption Spectroscopy

The conformational properties of the (Z)- Δ Ser(Me) residue in non-polar or weakly polar solutions were estimated using the model compound Ac-(Z)- Δ Ser(Me)-OMe (**1**). The spectra in CCl_4 and $CHCl_3$ are presented in Figures S1 and S2, respectively. The $\nu_s(N-H)$ stretching mode region was analyzed. The studied molecule has one N-H bond (N-terminal amide group). Therefore, the number of the bands in this region provides information about the number of conformations in the solution. The intensity of the bands gives information about the population of each conformation. The position of the band indicates if the N-H

group is involved in the N-H...O hydrogen bond and enables us to estimate the strength of this hydrogen bond. This information allows us to assign the bands to the conformations.

As can be seen in Figure 9, in the non-polar CCl₄ solution, the $\nu_s(\text{N-H})$ stretching mode region is occupied by two maxima. After deconvolution, the maximum of the band of higher intensity was determined as 3430 cm⁻¹, whereas another band was determined as 3444 cm⁻¹. Amongst the accessible conformations, the conformation C5 has the strongest N-H...O bond and it is predicted as the lowest in energy. Therefore, the conformation C5 can be assigned to the band at 3430 cm⁻¹. Comparison to the theoretical frequencies (Table S6) shows that the band at 3444 cm⁻¹ can be assigned to the conformation β 2.

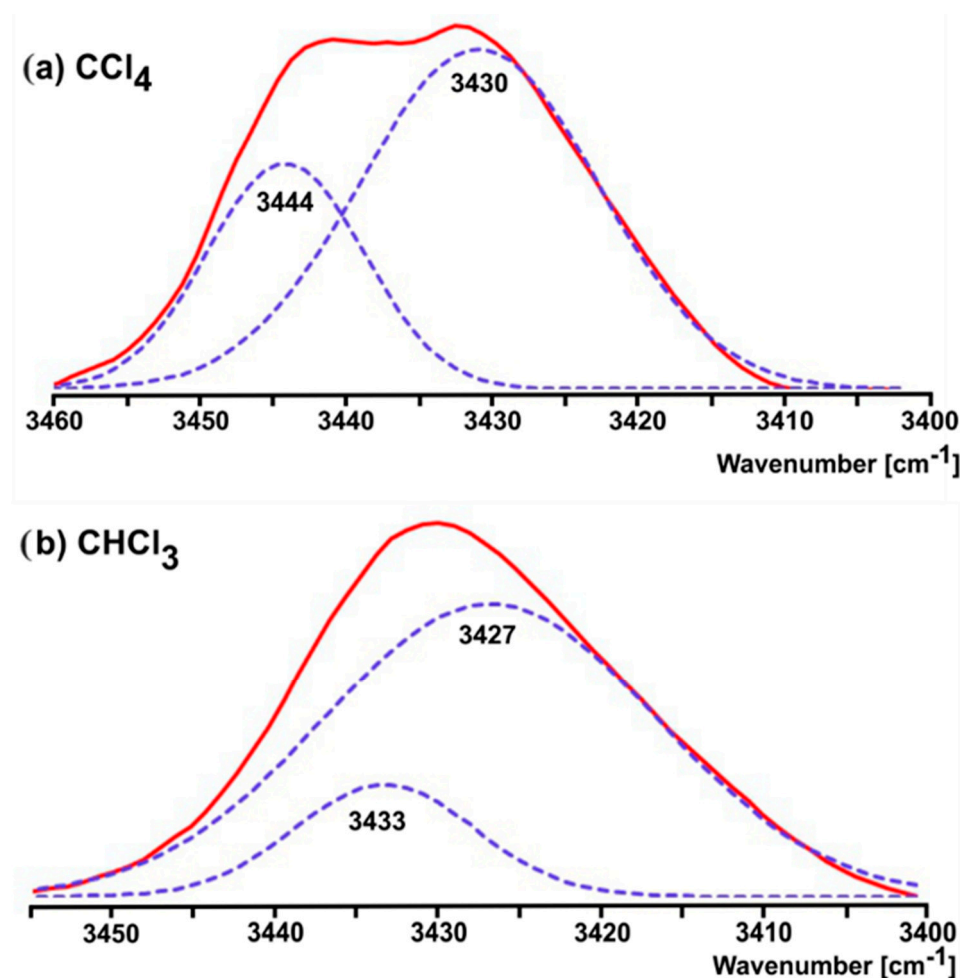


Figure 9. FTIR spectra for Ac-(Z)- Δ Ser(Me)-OMe (**1**) in region $\nu_s(\text{N-H})$: (a) CCl₄, (b) CHCl₃. The dashed lines, obtained by the curve-fitting procedure, present bands for each conformation.

The spectra recorded in the CHCl₃ solution shows in the $\nu_s(\text{N-H})$ stretching mode region the single band, but expanded towards higher wavenumber values. The deconvolution procedure fits the best when two components are applied with maxima at 3427 and 3433 cm⁻¹. Again, the intensity and the position of the band at 3427 cm⁻¹ allow us to assign this band to the conformation C5. The band at 3433 cm⁻¹, after comparison to the theoretical frequencies, fits to the conformation α . In summary, the FTIR analysis corroborates quantum chemical calculations; C5, the lowest in energy conformation, prevails in non-polar and weakly polar environments.

3. Discussion

The existence of the conformations of the Δ Ser(Me) residue as well as their relative energies, and thus order of population, can be explained by the presence of internal interactions.

For the isomer Z (1), as can be seen in Figure 5, the extended and semi-extended conformations C5 and β 2 are stabilized by the N-H...O hydrogen bonds, where the N-terminal amide group is the donor and the C-terminal ester group is the acceptor. The type of the oxygen atom, from the carbonyl or methoxy group, is important because, as the hydrogen bond acceptor, it influences the stability of the hydrogen bond. The oxygen atom from the carbonyl group is a better acceptor than that from the methoxy group, thus creating a stronger hydrogen bond. In results, the conformation C5 has lower energy, and thus, it is more populated than the conformation β 2. However, the N-H...O hydrogen bond is also found in the conformation α , where the oxygen atom of the methoxy group of the side chain is involved. Moreover, the conformation α is stabilized by carbonyl interactions, motif II [15]. This explains the second position in the energy order for the isolated molecule. The fourth conformation, β , is stabilized by carbonyl interactions, motif II. Finally, in all four conformations, the C β -H...O hydrogen bonds should be considered, where the acceptors are the oxygen atoms of the C-terminal ester bonds. The existence of the stabilizing hydrogen bond and/or carbonyl interactions in all conformations explains their relatively small energy differences.

The increase in the polarity of the environment influences the geometry of the conformations. The N-terminal amide group becomes more perpendicular towards the C α =C β . This can be explained by opening the C=O and N-H of amide towards intermolecular interactions. At the same time, the C-terminal ester group becomes more co-planar with the C α =C β bond. This indicates that this part of the Δ Ser(Me) residue does not create any stronger intermolecular interactions, so that it gains stabilization from the π -electron conjugation of the β -methoxyacrylate, C α =C β double bond, and C-terminal ester (Figure 10).

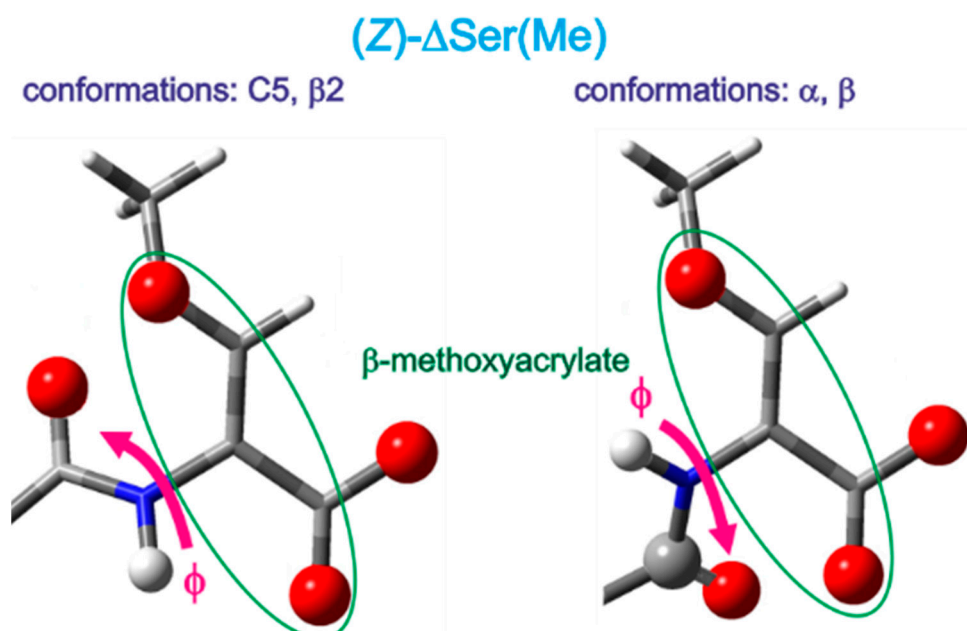


Figure 10. A schematic representation of the conformational changes in the Δ Ser(Me) residue with increasing environmental polarity.

For the isomer E (2), there is a quite similar pattern. The conformations C5 and β 2 are stabilized by the N-H...O hydrogen bonds, where the N-terminal amide group is the donor and the carbonyl or methoxy oxygen atoms from the C-terminal ester group are

acceptors, respectively. Also, the C β -H...O hydrogen bonds are present. In contrast, the conformations α and β are stabilized by carbonyl interactions (Figure 6). Nevertheless, the comparison of the energies in hartrees indicates that the transoidal β -methoxyacrylate unit (isomer Z) is predicted as the most preferable (Table 2).

The results obtained from experimental methods corroborate the quantum chemical calculations. In the case of the FT-IR method (Figure 9), the position of the band at 3430–3427 cm^{−1} assigned to the conformation C5 indicates that the strength of the N-H...O hydrogen bond is moderate. The β -methoxy group in the position Z of the side chain imposes a steric hindrance on the N-terminal amide group (torsion angle $\varphi \sim -125^\circ$). Consequently, the N-terminal amide group and C-terminal ester bond are not in a plane, which increases a distance between N-H and C=O and worsens the geometry of the hydrogen bond. The position of the band at 3444 cm^{−1} assigned to the conformation β 2 shows that the strength of the N-H...O hydrogen bond can be perceived as very weak. This can be explained by the geometry of the conformation β 2, i.e., by the steric hindrance of the β -methoxy group, as in the case of the conformation C5. Also, the donor is the oxygen atom from the C-terminal C-O methoxy group. However, the band at 3433 cm^{−1} assigned to the conformation α in the chloroform solution indicates that the N-H...O hydrogen bond between the N-terminal N-H group and C-O bond of the β -methoxy group in the position Z is stronger. The crystal structure analysis of Ac-(Z)- Δ Ser(Me)-OMe (**1**) shows that only the N-terminal amide group is involved in the N-H...O hydrogen bonds and the torsion angle φ has the highest value (-68.99°) as compared to that predicted for the chloroform and water environments. The consequence of the relatively weak intramolecular interactions is the small energy difference between the conformations. The presence of the conformation β in the crystal state can be explained by the fact that it forms stronger intermolecular N-H...O hydrogen bonds. Thus, all possible conformations of the Δ Ser(Me) residue are available.

The β -methoxyacrylate structural motif included in the O-methyldehydroserine residue has a tendency to adopt a planar geometry, regardless of the conformation adopted. The analysis of the value of the torsion angle ψ (O1-C1-C2-N1, Figure 7) shows that it does not deviate more than 20° from the planar structure (180° for the conformations C5 and β as well as 0° for the conformation β 2 and α) (Table 1). Also, a comparison to the structurally closest analog, dehydrobutyrine residue, with C-terminal ester [16] or amide [17], shows that the C-terminus is more flat for the studied Δ Ser(Me) residue, especially for the isomer Z. This can be caused by the oxygen atom in the position β of the side chain, which is able to participate in the π -electron cross-conjugation with the C α =C β double bond and C-terminal ester group, as well as a smaller steric hindrance compared to the β -methyl group of the dehydrobutyrine residue.

The World Protein Data Bank [18] shows the structures of proteins containing as ligands β -methoxyacrylate fungicide, like Azoxystrobin [19,20]. As it is presented in Figure 11, the β -methoxyacrylate unit is planar and the β -methoxy group is in the position E in relation to the ester group. This corresponds to the isomer Z for the Δ Ser(Me) residue. It indicates that both planarity of the β -methoxyacrylate unit and the position of the β -methoxy group are crucial for antifungal activity. Also, the neighboring benzene ring does not participate in a larger delocalized electron system, but instead, tends to adopt a perpendicular position. The same trends can be seen in the conformations of the Δ Ser(Me) residue in the polar environment. As can be seen, the rotation of the ester group is possible, which indicates a conformational flexibility.

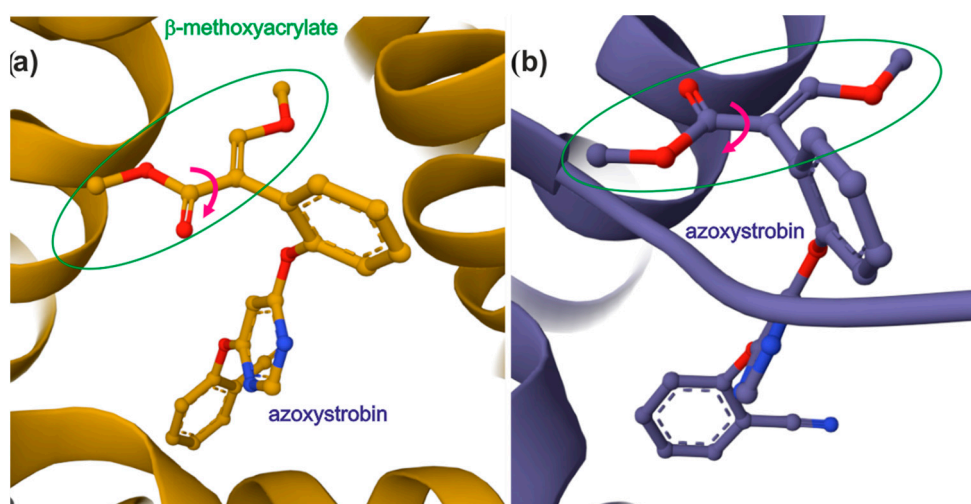


Figure 11. Cytochrome bc1 complex with Azoxystrobin: (a) ref. [18], (b) ref. [19]. β -methoxyacrylate unit circled with green oval. Possible rotation of ester group marked with pink arrow. Structures were retrieved using 3D Viewers tool accessible at PDB website (<https://www.rcsb.org>, accessed on 30 December 2024).

4. Materials and Methods

4.1. Quantum Chemical Calculations

The model compounds, Ac-(Z)- Δ Ser(Me)-OMe (**1**) and Ac-(E)- Δ Ser(Me)-OMe (**2**), were calculated by the DFT method using the Gaussian 16 package [21]. The initial structures were prepared with GaussView5 [22]. The configuration trans ($\omega_0 \approx 180^\circ$) of amide and ester groups was set. The potential energy surfaces $E = f(\varphi, \psi)$ were obtained at the M06-2X/6-31+G(d,p) level of theory [23], in the gas phase, and then in chloroform and water (constrained optimization). The values of the φ and ψ dihedral angles were changed in steps of 30° . Because of the achirality of dehydroamino acids, each structure has a mirror counterpart with the same energy, but opposite torsion angles ($\varphi, \psi = -\varphi, -\psi$). This reduces the number of grid point structures necessary to create the maps to 91 each. The solvent effect was simulated with the self-consistent reaction field (SCRF) using the conductor-like polarizable continuum model (CPCM). All potential energy minima localized in the maps were fully optimized by using a larger basis set, 6-311+G(d,p). Unconstrained optimizations were followed by a vibrational analysis to ensure that the resulting structures are true energy minima and to obtain the zero-point vibrational energies (ZPVEs) and Gibbs energies (298.15 K, 1.0 atm). The population of the conformations (p) was calculated at 300 K, where $RT = 0.595$ kcal/mol according to the following equations: $p_{rel} = 100\% \exp(-\Delta G/RT)$, $p = p_{rel} / \sum \text{conformers } p_{rel} \times 100\%$ [24,25]. The names of the conformations are based on the Scarsdale nomenclature [26].

4.2. Synthesis

Ac-(Z)- Δ Ser(Me)-OMe (**1**) was synthesized from glycine in a four-step reaction (Figure S4) according to the procedure described in a patent (WO 2012/041986). The detailed synthetic procedure is presented in the Supplementary Information. The ^1H and ^{13}C NMR spectra of the compounds are presented in Figures S4–S10. The NOE difference method was applied using the standard programs to determine the isomer Z. A photoisomerization reaction was performed using Ac-(Z)- Δ Ser(Me)-OMe (**1**) as the substrate in order to obtain the isomer E, according to a method elaborated on previously [27]. Unfortunately, the procedure led to the decomposition of the substrate to ammonium oxalate.

4.3. NMR Spectra

A Bruker Ultrashield 400 (Bruker 2005, Berlin, Germany) spectrometer was used. Bruker (TopSpin Version 1.3) software was also used, operating at 400 MHz (^1H) and 101 MHz (^{13}C). The spectra were recorded in DMSO- d_6 (internal TMS standard) at room temperature.

4.4. FTIR Spectra

A Nicolet Nexus 2002 FT-IR spectrometer (New Castle, DE, USA) was used. Measurement conditions involved a dry nitrogen atmosphere and KBr liquid cell (0.01 mm), and for each measurement, there were 20 scans with a 2 cm^{-1} resolution (spectral resolution: 0.482 cm^{-1}) in the spectral range $400\text{--}4000\text{ cm}^{-1}$. The Ac-(Z)- Δ Ser(Me)-OMe (**1**) solutions in CCl_4 and CHCl_3 (distilled over P_2O_5) were in three different concentrations: 0.5, 1.0, and 2.0 mg/mL each. The spectra processing and peak deconvolution were conducted using Fityk software (version 1.3.1) [28], applying the Voigt function.

4.5. Crystal Structure Analysis

Data from the X-ray diffraction of the single crystal for Ac-(Z)- Δ Ser(Me)-OMe (**1**) were collected on a Rigaku Oxford Diffraction XtaLAB SynergyR DW diffractometer (Tokyo, Japan) equipped with a HyPix ARC 150° Hybrid Photon Counting (HPC) detector using $\text{MoK}\alpha$ ($\lambda = 0.71073\text{ \AA}$) at 100 K. The corrections to the Lorentz and polarization factors were applied to the reflection intensities (CrysAlis CCD; Oxford Diffraction Ltd.: Abingdon, UK, (2002). CrysAlis RED; Oxford Diffraction Ltd.: Abingdon, UK, 2002). Data were processed using CrysAlisPro software (version 1.171.39.44, CrysAlis PRO; Rigaku Oxford Diffraction. Rigaku Oxford Diffraction Ltd., Yarnton, UK, 2015, 2022). The structures were solved by direct methods using SHELXS and refined by full-matrix least-squares methods based on F2 using SHELXL [29,30]. All non-hydrogen atoms were located from difference Fourier synthesis and refined by the least-squares method in the full-matrix anisotropic approximation. The crystallographic data for compounds and details of the X-ray experiment are collected in the Supplementary Information. The structure drawings in ESI were prepared by using the Mercury program [31]. The coordinates of atoms and other parameters for structures were deposited with the Cambridge Crystallographic Data Centre: 2394235 for (**1**)—12 Union Road, Cambridge CB2 1EZ, UK (Fax, +44-(1223)336-033, E-mail: deposit@ccdc.cam.ac.uk).

5. Conclusions

The O-methyldehydroserine residue, Δ Ser(Me), is able to adopt four conformations: the extended C5, semi-extended β_2 , helical α , and helical polyproline II-like conformation β . The lack of chirality of the carbon α ensures that the mirror counterparts of these conformations are equally accessible ($-\text{C5}$, $-\beta_2$, $-\alpha$, and $-\beta$) as it is presented in Ramachandran diagrams. The β -methoxy group in the position Z or E influences the value of the torsion angles φ and ψ of the main chain. The quantum chemical calculations for weakly polar (chloroform) and polar (water) environments show the following φ and ψ torsion angle values: for the isomer Z—C5 (φ , $\psi = -121^\circ$, 180°), β_2 (φ , $\psi = -119^\circ$, 0°), α (φ , $\psi = -59^\circ$, -16°), and β (φ , $\psi = -61^\circ$, 168°), and for the isomer E—C5 (φ , $\psi = -180^\circ$, 180°) and β_2 (φ , $\psi = -180^\circ$, 0°), and α (φ , $\psi = -67^\circ$, -9°) and β (φ , $\psi = -68^\circ$, 173°). The X-ray diffraction analysis performed at 100 K for a single crystal of Ac-(Z)- Δ Ser(Me)-OMe (**1**) shows the conformations β (-68.99° , 174.17°) and $-\beta$ (68.99° , -174.17°). The infrared absorption spectra for Ac-(Z)- Δ Ser(Me)-OMe (**1**) show the conformations C5 and β_2 in the non-polar CCl_4 solution as well as the conformations C5 and α in the weakly polar CHCl_3 solution. Thus, the results obtained using experimen-

tal methods corroborate those predicted by the quantum chemical calculations. It is also shown that all conformations should be accessible.

In summary, the (Z)- Δ Ser(Me) residue has a tendency to adopt in a weakly polar environment primarily the conformation C5, followed by the conformation β 2, which are mainly stabilized by intramolecular interactions. As polarity of the environment increases, the conformation α and then β should prevail as they will be stabilized by intermolecular interactions involving mainly the N-terminal amide group. The β -methoxyacrylate structural motif should maintain a planar geometry, not involved in any stronger intramolecular or intermolecular interactions. Thus, the conformation properties of the Δ Ser(Me) residue are flexible and should not affect the biological function of the β -methoxyacrylate toxophore.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms26010340/s1>.

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Insight into the structure of antifungal Cyrmenins. Conformational studies of unique dehydroamino acid, O-methyldehydroserine

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Quantum Chemical Calculations

Table S1. Structural parameters for the internal hydrogen bond (X–H···A) and carbonyl interactions (C=O ◀··· ▶ O=C) in the conformers of Ac-(Z)-ΔSer(Me)-OMe (1) in various environment calculated at M06-2X/6-311+G(d,p)

Ac-(Z)-ΔSer(Me)-OMe (1)												
Structural parameters	C5			α			β 2			β		
	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water
Hydrogen bond												
N–H···O–C												
<i>r</i> H···O	2.41			2.58			2.41					
<i>r</i> N···C	2.43			2.77			2.66					
$\angle$ N–H···O	97.7			90.2			92.9					
$\angle$ C–O···H	82.3			78.2			87.1					
C^β–H···O–C												
<i>r</i> H···O	2.41	2.38	2.37	2.53	2.53	2.53	2.51	2.49	2.49	2.42	2.40	2.40
<i>r</i> C···O	2.73	2.72	2.71	2.82	2.82	2.82	2.82	2.81	2.81	2.72	2.72	2.71
$\angle$ C–H···O	95.4	96.2	96.1	93.7	93.9	93.8	94.8	95.5	95.4	94.4	94.9	94.8
$\angle$ C–O···H	87.6	88.0	88.2	80.0	80.3	80.3	81.0	81.4	81.4	86.4	87.2	87.3
C=O ◀··· ▶ O=C dipole interactions												
<i>r</i> O ^N ···C ^C				2.96	3.02	3.04				2.94	3.02	3.04
<i>r</i> C=O ^C ···C ^N										3.10	3.13	3.15
<i>r</i> C–O ^C ···C ^N				2.99	3.01	3.02						
<i>r</i> C ^N ···C ^C				3.08	3.11	3.12				3.03	3.07	3.09
<i>r</i> O ^N ···O ^C				2.89	2.97	2.99				3.00	3.08	3.10
$\angle$ (C=O) ^N ···C ^C				84.5	82.8	82.2				82.5	80.8	80.5
$\angle$ (C=O) ^C ···C ^N										75.6	76.0	76.1
$\angle$ (C–O) ^C ···C ^N				81.2	81.4	81.5						
$\angle$ O ^C ···(C=O) ^N				73.2	76.1	77.1				74.3	76.5	76.8
$\angle$ O ^N ···(C=O) ^C										81.3	81.6	81.4
$\angle$ O ^N ···(C–O) ^C				73.9	75.1	75.4						

Data presented only for X–H···A (X = N, C; A = O) in which *r* H···X ≤ 2.7 Å and $\angle$ X–H···A > 90° [Vargas et al. *J. Phys. Chem. A* **2002**, *106*, 3213-3218].

Data presented only for the C=O ◀··· ▶ O=C contacts in which *r* C···O < 3.6 Å [Allen et al. *Acta Crystallogr. B* **1998**, *54*, 320-329].

^{N, C} denote the N-terminal or the C-terminal amide group;

Table S2. Structural parameters for the internal hydrogen bond (X–H···A) and carbonyl interactions (C=O ◀··· ▶ O=C) in the conformers of Ac-(E)-ΔAla(β-OMe)-OMe (2) in various environment calculated at M06-2X/6-311+G(d,p)

Ac-(E)-ΔAla(β-OMe)-OMe (2)										
	C5			β2			β		α	
	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Chloroform	Water	Chloroform	Water
N-H···O-C <i>r</i> H···O <i>r</i> N···C ∠N-H···O ∠C=O···H C^β-H···O=C <i>r</i> H···O <i>r</i> C···O ∠C-H···O ∠C=O···H <i>r</i> O ^N ...C ^C <i>r</i> C=O ^C ...C ^N <i>r</i> C-O ^C ...C ^N <i>r</i> C ^N ...C ^C <i>r</i> O ^N ...O ^C ∠(C=O) ^N ...C ^C ∠(C=O) ^C ...C ^N ∠(C-O) ^C ...C ^N ∠O ^C ... (C=O) ^N ∠O ^N ... (C=O) ^C ∠O ^N ... (C-O) ^C	Hydrogen bond									
	2.12	2.12	2.12	2.06	2.06	2.06				
	2.63	2.63	2.63	2.57	2.56	2.56				
	109.1	108.8	108.8	108.5	108.2	108.1				
	86.0	86.1	86.1	91.2	91.4	91.5				
	2.11	2.13	2.13	2.11	2.12	2.12				
	2.83	2.84	2.84	2.82	2.83	2.83				
	121.0	120.9	120.8	120.5	120.4	120.3				
	104.9	104.7	104.7	105.2	105.1	105.1				
	C=O ◀···▶ O=C dipole interactions									
							3.19	3.16	3.20	3.17
							3.11	3.12		
									3.01	3.02
							3.10	3.10	3.15	3.14
							3.30	3.25	3.19	3.17
							74.5	75.8	76.5	77.5
							77.9	77.8		
									83.5	82.9
							87.7	85.0	87.3	85.7
							84.3	83.2		
									77.3	77.6

Data presented only for X-H···A (X = N, C and A = O) in which *r* H···X ≤ 2.7 Å and ∠X-H···A > 90° [Vargas et al. *J. Phys. Chem. A* **2002**, 106, 3213-3218].

Data presented only for the C=O ◀···▶ O=C contacts in which *r* C···O < 3.6 Å [Allen et al. *Acta Crystallogr. B* **1998**, 54, 320-329].

^{N, C} denote the N-terminal or the C-terminal amide group

X-Ray

Table S3. Crystal Parameters and Experimental Details of X-Ray Data Collection for Ac-(Z)- Δ Ser(Me)-OMe (**1**)

	Ac-(Z)- Δ Ser(Me)-OMe (1)
Chemical formula	C ₇ H ₁₁ NO ₄
M_r	173.17
Crystal system, space group	Monoclinic, <i>Ia</i>
<i>a</i> , <i>b</i> , <i>c</i> (Å)	14.6956 (11), 8.0192 (4), 7.9532 (5)
α , β , γ (°)	90.0, 107.361 (7), 90.0
<i>V</i> (Å ³)	894.56 (10)
<i>Z</i>	4
μ (mm ⁻¹)	0.11
Crystal size (mm)	0.4 × 0.25 × 0.1
$T_{\min}$, $T_{\max}$	-
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	4800, 1526, 1504
R_{int}	0.023
$(\sin \theta/\lambda)_{\text{max}}$ (Å ⁻¹)	0.633
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, <i>S</i>	0.041, 0.119, 1.14
No. of reflections	1526
No. of parameters	112
$\Delta\rho_{\text{max}}$, $\Delta\rho_{\text{min}}$ (e Å ⁻³)	0.37, -0.27

Table S4. Selected geometric parameters (Å, °) of Ac-(Z)- Δ Ser(Me)-OMe (**1**) as determined by X-ray method

Bond length			
O1—C1	1.209 (4)	C3—H3	0.9300
O2—C4	1.228 (4)	C4—C5	1.464 (4)
O3—C1	1.317 (4)	C5—H5A	0.9600
O3—C6	1.430 (4)	C5—H5B	0.9600
O4—C3	1.335 (4)	C5—H5C	0.9600
O4—C7	1.441 (3)	C6—H6A	0.9600
N1—C4	1.361 (4)	C6—H6B	0.9600
N1—C2	1.383 (4)	C6—H6C	0.9600
N1—H1	0.8600	C7—H7A	0.9600
C1—C2	1.469 (4)	C7—H7B	0.9600
C2—C3	1.336 (4)	C7—H7C	0.9600
Bond angle			
C1—O3—C6	114.1 (2)	C4—C5—H5B	109.5
C3—O4—C7	116.0 (2)	H5A—C5—H5B	109.5
C4—N1—C2	121.5 (2)	C4—C5—H5C	109.5
C4—N1—H1	119.3	H5A—C5—H5C	109.5
C2—N1—H1	119.3	H5B—C5—H5C	109.5
O1—C1—O3	123.0 (3)	O3—C6—H6A	109.5
O1—C1—C2	126.5 (3)	O3—C6—H6B	109.5
O3—C1—C2	110.5 (2)	H6A—C6—H6B	109.5
C3—C2—N1	120.9 (3)	O3—C6—H6C	109.5
C3—C2—C1	122.5 (3)	H6A—C6—H6C	109.5
N1—C2—C1	116.5 (2)	H6B—C6—H6C	109.5
O4—C3—C2	123.2 (3)	O4—C7—H7A	109.5
O4—C3—H3	118.4	O4—C7—H7B	109.5

C2—C3—H3	118.4	H7A—C7—H7B	109.5
O2—C4—N1	124.5 (3)	O4—C7—H7C	109.5
O2—C4—C5	119.3 (3)	H7A—C7—H7C	109.5
N1—C4—C5	116.3 (3)	H7B—C7—H7C	109.5
C4—C5—H5A	109.5		
Torsion angle			
C6—O3—C1—O1	2.8 (4)	O3—C1—C2—N1	174.2 (3)
C6—O3—C1—C2	-175.9 (3)	C7—O4—C3—C2	-173.7 (3)
C4—N1—C2—C3	107.6 (3)	N1—C2—C3—O4	0.0 (4)
C4—N1—C2—C1	-69.0 (3)	C1—C2—C3—O4	176.4 (2)
O1—C1—C2—C3	179.0 (3)	C2—N1—C4—O2	-2.1 (4)
O3—C1—C2—C3	-2.4 (4)	C2—N1—C4—C5	177.8 (3)
O1—C1—C2—N1	-4.4 (4)		

Table S5. Selected hydrogen-bond parameters (Å, °) of Ac-(Z)-ΔSer(Me)-OMe (**1**) as determined by X-ray method

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
N1—H1···O2 ⁱ	0.86	1.96	2.818 (3)	171.0
C3—H3···O1 ⁱⁱ	0.93	2.55	3.128 (3)	120.3
C6—H6A···O4 ⁱⁱⁱ	0.96	2.64	3.404 (4)	137.2
C6—H6C···O2 ^{iv}	0.96	2.57	3.468 (4)	156.6
C7—H7A···O1 ⁱⁱ	0.96	2.65	3.386 (4)	133.5
C7—H7C···O2 ^v	0.96	2.41	3.251 (4)	145.6

Symmetry codes: (i) x, -y+3/2, z+1/2; (ii) x, -y+3/2, z-1/2; (iii) x-1/2, y+1/2, z-1/2; (iv) x-1/2, -y+2, z; (v) x, y-1, z.

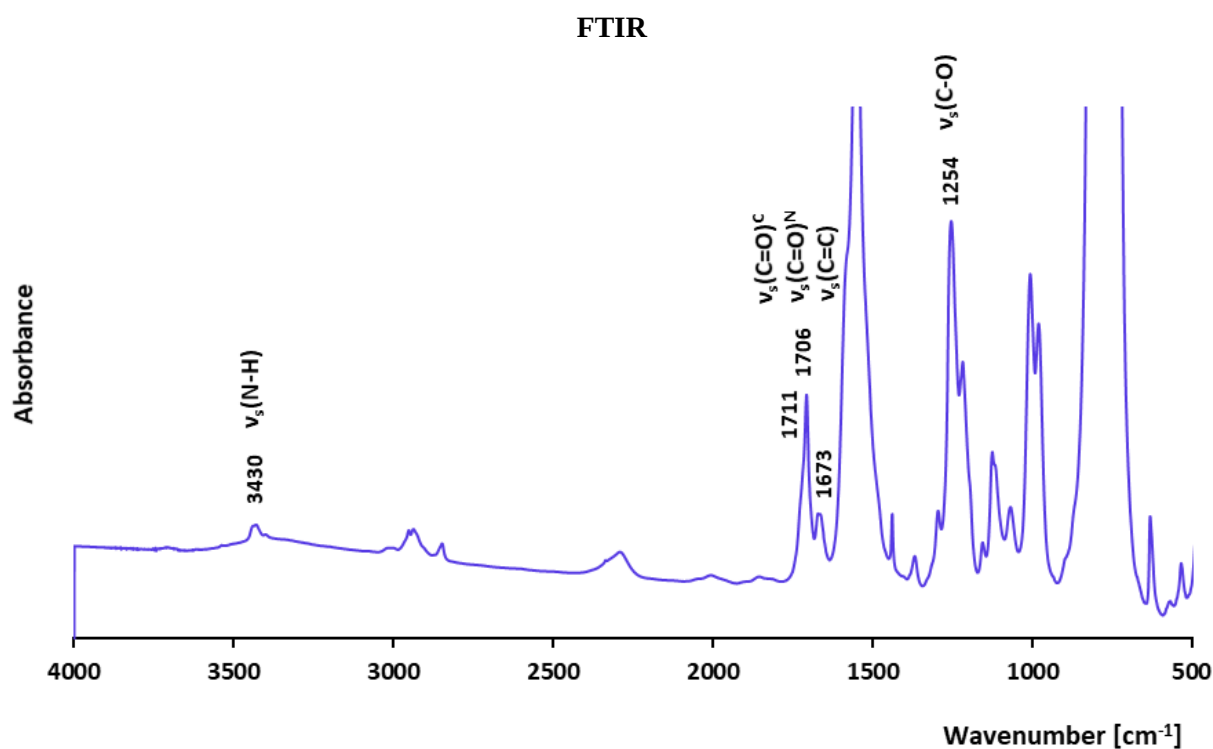


Figure S1. FTIR spectrum for Ac-(Z)- Δ Ser(Me)-OMe (**1**) in CCl_4 .

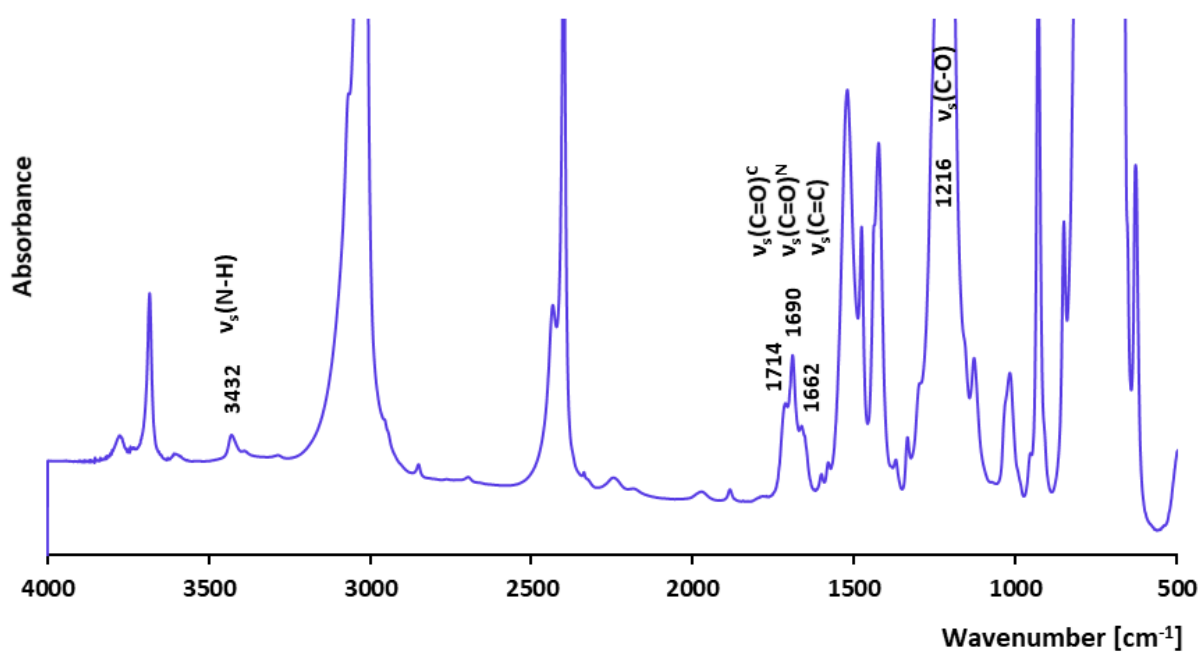
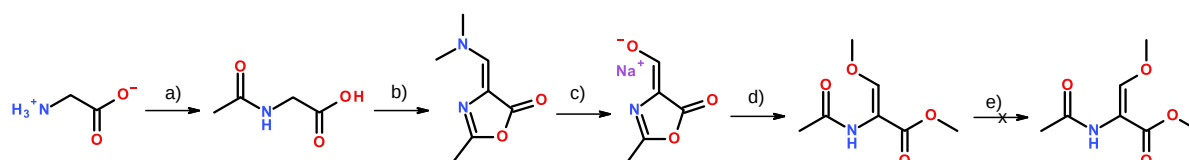


Figure S2. FTIR spectrum for Ac-(Z)- Δ Ser(Me)-OMe (**1**) in CHCl_3 .

Table S6. Theoretical frequencies obtained by M06-2X/6-311+G(d,p) method for the conformations of **A**-(Z)- Δ Ser(Me)-OMe (**1**) in region ν_s (N-H): (a) CCl₄, (b) CHCl₃

Conformation						Frequencies (cm ⁻¹)		
Code	φ (°)	ψ (°)	G (hartrees)	Δ G (kcal·mol ⁻¹)	ρ (%)	Experimental	Calculated	Scaled (0.9416)
(a) CCl ₄								
C5	-125.5	179.2	-628.80580	0.00	78.84	3430	3641	3428
β 2	-121.6	-0.9	-628.80366	1.34	8.27	3444	3657	3443
α	-56.4	-17.9	-628.80362	1.37	7.90		3652	3438
β	-58.8	165.7	-628.80318	1.64	4.99		3655	3442
(b) CHCl ₃								
α	-57.9	-16.6	-628.80850	0.00	28.60	3433	3646	3433
C5	-122.2	179.8	-628.80844	0.04	26.63	3427	3642	3429
β	-60.7	167.2	-628.80843	0.04	26.52		3651	3438
β 2	-119.7	-0.4	-628.80808	0.27	18.25		3649	3436

Synthesis

**Figure S3.** The scheme of the synthesis of spectrum of Ac-(Z)- Δ Ser(OMe)-OMe. Reaction conditions: a) Ac₂O, MeOH, 3h, reflux, (93%); b) POCl₃, DMF, 2 h, 45 °C, (46%); c) CAN, 2M NaOH, rt, overnight, (96%); d) HCl_(MeOH), MeOH, 4 h, 0 °C, (2.6%); e) benzophenone, MeOH, benzene, UV (366 nm, 400-440 μ W/cm²), 5 h intensity (no results).

Ac-Gly-OH

Acetic anhydride (10.2 ml, 108 mmol) was added dropwise to the methanolic solution of glycine (3.00 g, 40.0 mmol in 100 ml). The reaction mixture was stirred for 3 h under the reflux. Afterwards the mixture was concentrated under reduced pressure and left in the fridge overnight. White crystalline product was filtered off and washed with diethyl ether. Yield: 4.35 g (37.2 mmol), 93%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 12.51 (1H, s, O=C-O-H), 8.19 (1H, t, O=C-N-H), 3.72 (2H, d, CH₂), 1.84 (3H, s, CH₃) (Figure 3S).

Melting point: 152.5-154.2 °C. TLC MeOH/CH₂Cl₂/AcOH (4:16:1) *R*_f = 0.47.

SMILES Notation: CC(=O)NCC(=O)O

4-[(dimethylamino)methylene]-2-methyl-5(4H)-oxazolone

To the mixture of Ac-Gly-OH (1.1712 g, 10.000 mmol) and phosphorus oxychloride (2.4 ml, 25 mmol), dimethylformamide (2.0 ml, 25 mmol) was added at -4 °C. The reaction mixture was stirred for 2 h in 45 °C. The volatile compounds were removed under reduce pressure. The product was precipitated with aqueous NH₃ solution (25%, 10 ml) at about 0 °C. The precipitate was filtered off, dissolved in dichloromethane (35 ml), washed with water (2 x 10 ml), and dried over anhydrous magnesium sulphate. The solvent was then removed to give a pink crystalline product, which was recrystallized from ethanol (5 ml). Yield: 0.71 g (4.6 mmol), 46%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 7.13 (1H, s, (*E*)-H-C ^{β} =C), 3.39, 3.19 (3H, s, NCH₃), 2.14 (3H, s, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 169.36 (O=C=N), 152.96 (O=C=O), 142.89 (C ^{β}), 104.71 (C ^{α}), 45.76 (NCH₃), 14.59 (CH₃) (Figure 4S).

Melting point: 152.1-153.8°C. TLC AcOEt *R*_f = 0.30.

SMILES Notation: CN(C)\C=C1/N=C(C)OC1=O

4-(hydroxymethylene)-2-methyl-5(4H)-oxazolone, sodium salt

To the cooled mixture (ice bath 0°C) of 4-[(dimethylamino)methylene]-2-methyl-5(4H)-oxazolone (0.5204 g, 3.3755 mmol) in acetonitrile (3.0 ml) a solution of NaOH (2 M, 4.05 mmol, 2.03 ml) was added. The reaction mixture was stirred overnight at room temperature and then the volatile components were co-evaporated with toluene. The solid residue was treated with acetone (2.6 ml) and stirred for 3 h. The white solid product was collected by filtration and dried under vacuum. Yield: 0.482 g (3.23 mmol), 96%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.66 (1H, s, (*E*)-H-C^β=C), 2.00 (3H, s, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 171.56 (O-C=N), 170.37 (O-C=O), 148.96 (C^α), 108.96 (C^β), 14.57 (CH₃) (Figure 5S). Melting point: decomposition. TLC AcOEt R_f = 0.00.

SMILES Notation: [Na]O\C=C1/N=C(C)OC1=O

Ac-(Z)-ΔSer(OMe)-OMe

4-(Hydroxymethylene)-2-methyl-5(4H)-oxazolone, sodium salt (0.4577 g, 3.0702 mmol) was dissolved in methanol 2.6 ml and cooled in an ice bath. Then a methanolic solution of HCl was added dropwise (2.76 M, 4.76 mmol, 1.8 ml). The reaction mixture was stirred for 4 h maintaining the temperature at 0°C. Then the solvent was evaporated under reduced pressure. The residues was resuspended in acetone and K₂CO₃ was added (0.6449 g, 4.666 mmol). The solution was cooled in an ice bath for 30 min then dimethylsulfate (4.666 mmol, 0.44 ml) was added. The reaction mixture was stirred overnight at room temperature. The solid product was filtered off and purified by column chromatography, using an increasing gradient of methanol in ethyl acetate from 0% to 3.5% as an eluent. Desired product was crystallized from ethyl acetate. Yield: 0.0136 g (0.0785 mmol), 2.6%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.79 (1H, s, O=C-N-H), 7.32 (1H, d, C^β-H), 3.83 (3H, s, (C^β-O-CH₃), 3.60 (3H, s, O=C-O-CH₃), 1.87 (3H, s, (CH₃)^N). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 168.25 (O=C-N-H)^N, 165.34 (O=C-O), 155.374 (C^β), 107.61 (C^α), 61.59 (O-CH₃)^β, 51.27 (CH₃)^C, 22.37 (CH₃)^N (Figure 4S). Melting point: 92.4-94.5°C. TLC MeOH/AcOEt (1:19) R_f = 0.23.

SMILES Notation: O=C(C)N/C(=C\OC)C(=O)O

Photoisomerization

Ac-(Z)-ΔSer(OMe)-OMe (0.016 g, 0.092 mmol) and benzophenone (0.084, 0.46 mmol, 5 equiv) were dissolved in methanol (2 ml) and benzene was added (0.7 ml). The reaction mixture was stirred and illuminated simultaneously with UV light (366 nm) for 5 h with intensity 400-440 μW/cm². The volatile components were evaporated with DCM (15 ml). The residue was adsorbed on silica gel, applied on chromatographic column and eluted with mixture 3% MeOH in AcOEt. Unfortunately, it failed to separate the product from the substrate completely, so the fraction with the product as a main component was crystallized from a mixture of AcOEt/Hex. Yield: 0.0036 g (0.021 mmol), 23%. Unreacted substrate was recovered (0.0128 g, 0.0739 mmol, 80%). Crystallographic analysis showed that the obtained product was not Ac-(*E*)-ΔSer(OMe)-OMe, as expected, but ammonium oxalate.

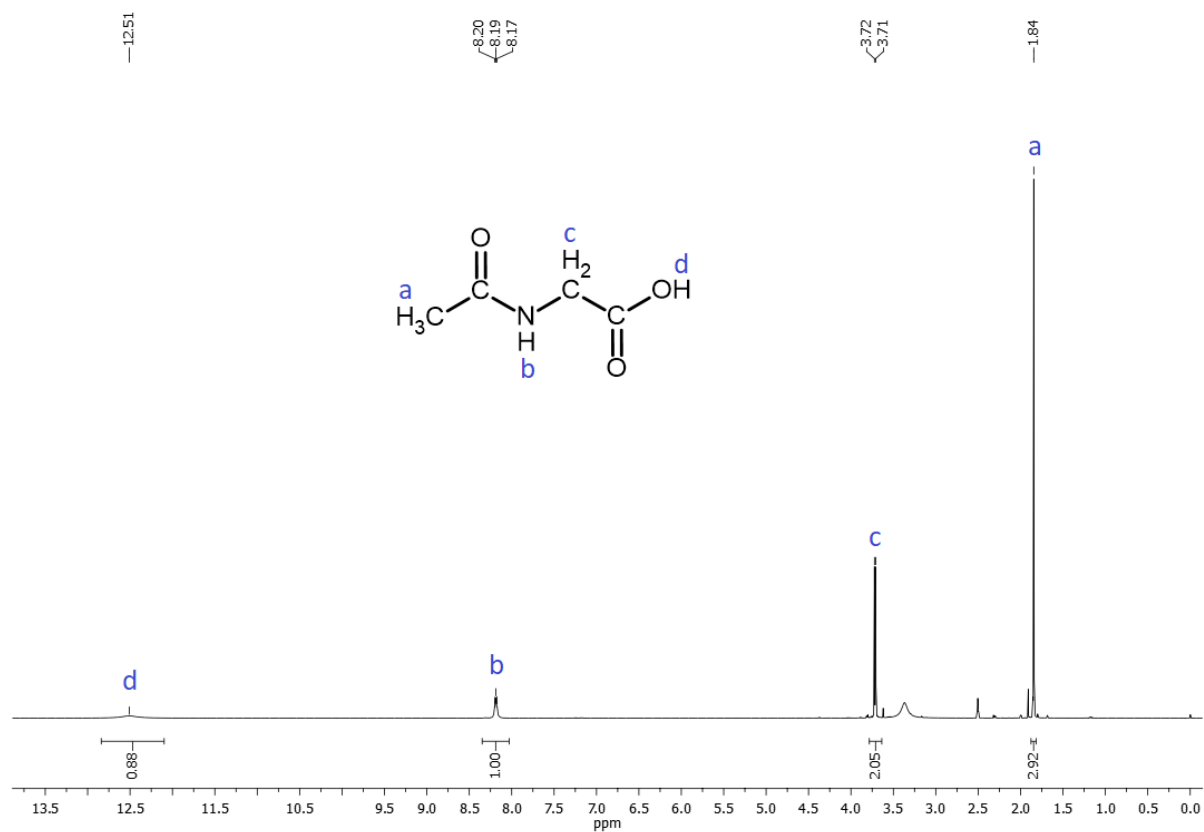


Figure S4. ^1H NMR spectrum of Ac-Gly-OH in $\text{DMSO-}d_6$.

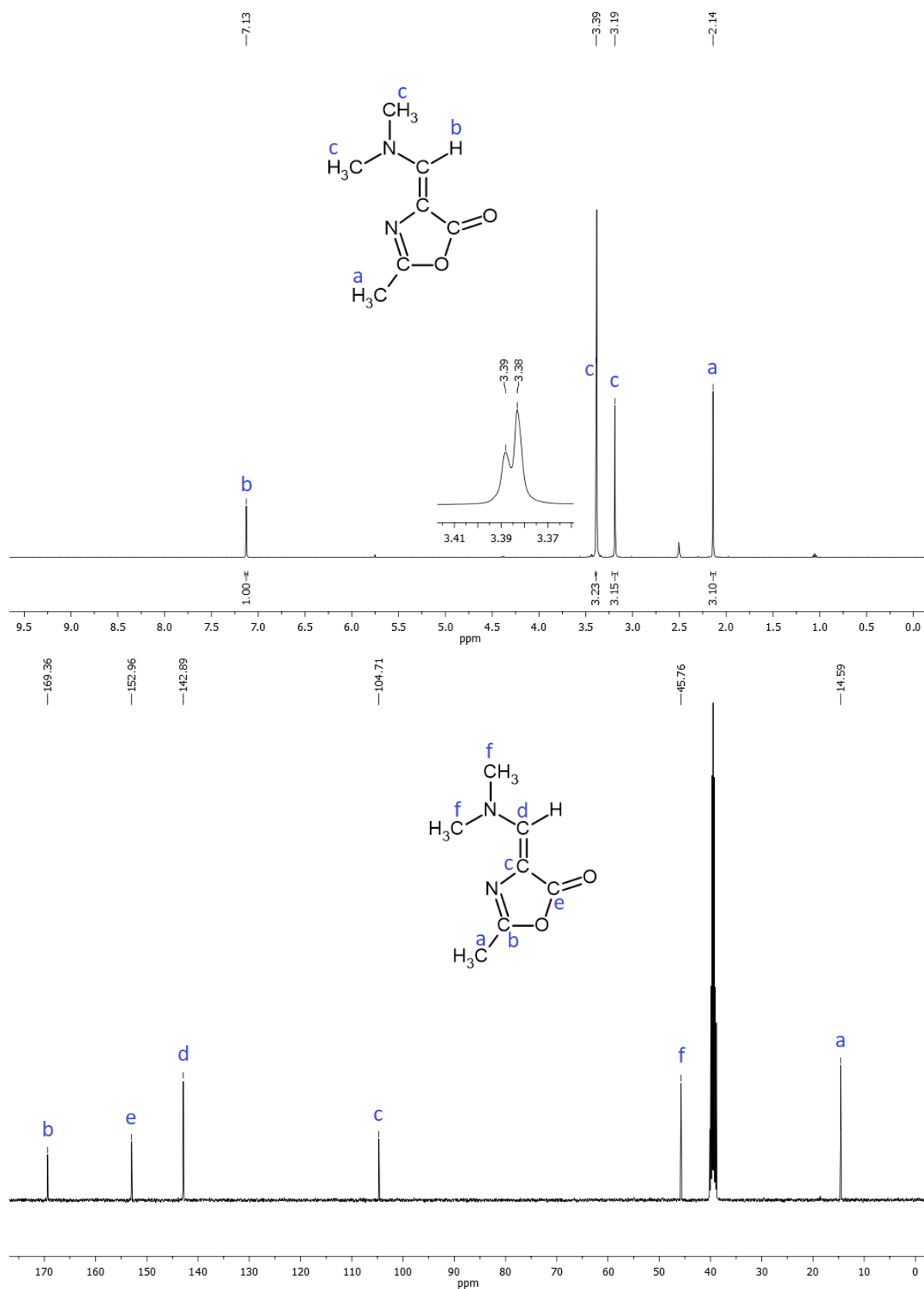


Figure S5. ^1H and ^{13}C NMR spectra of 4-[(dimethylamino)methylene]-2-methyl-5(4H)-oxazolone in $\text{DMSO}-d_6$.

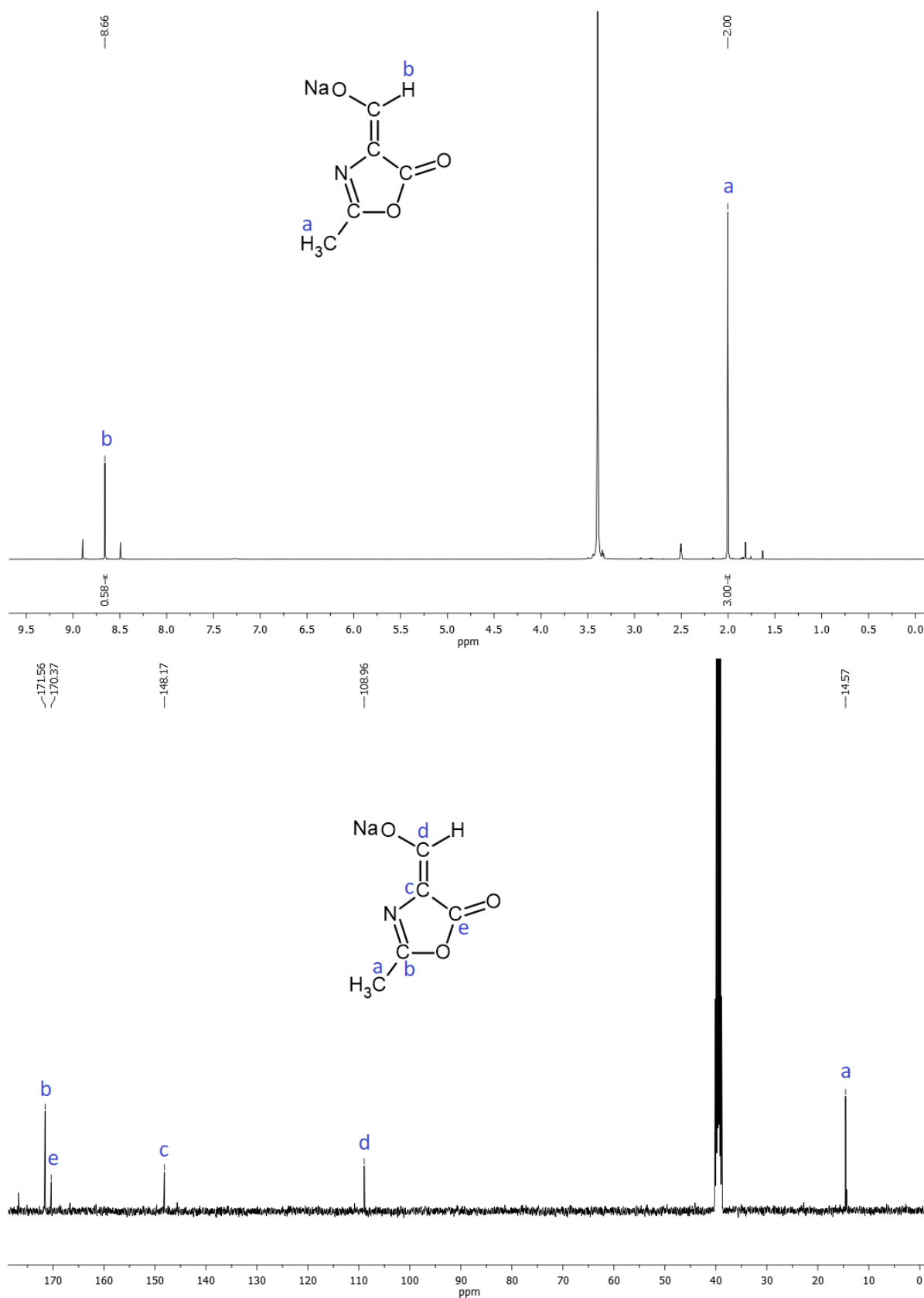


Figure 6S. ^1H and ^{13}C NMR spectra of 4-(hydroxymethylene)-2-methyl-5(4H)-oxazolone, sodium salt in $\text{DMSO-}d_6$.

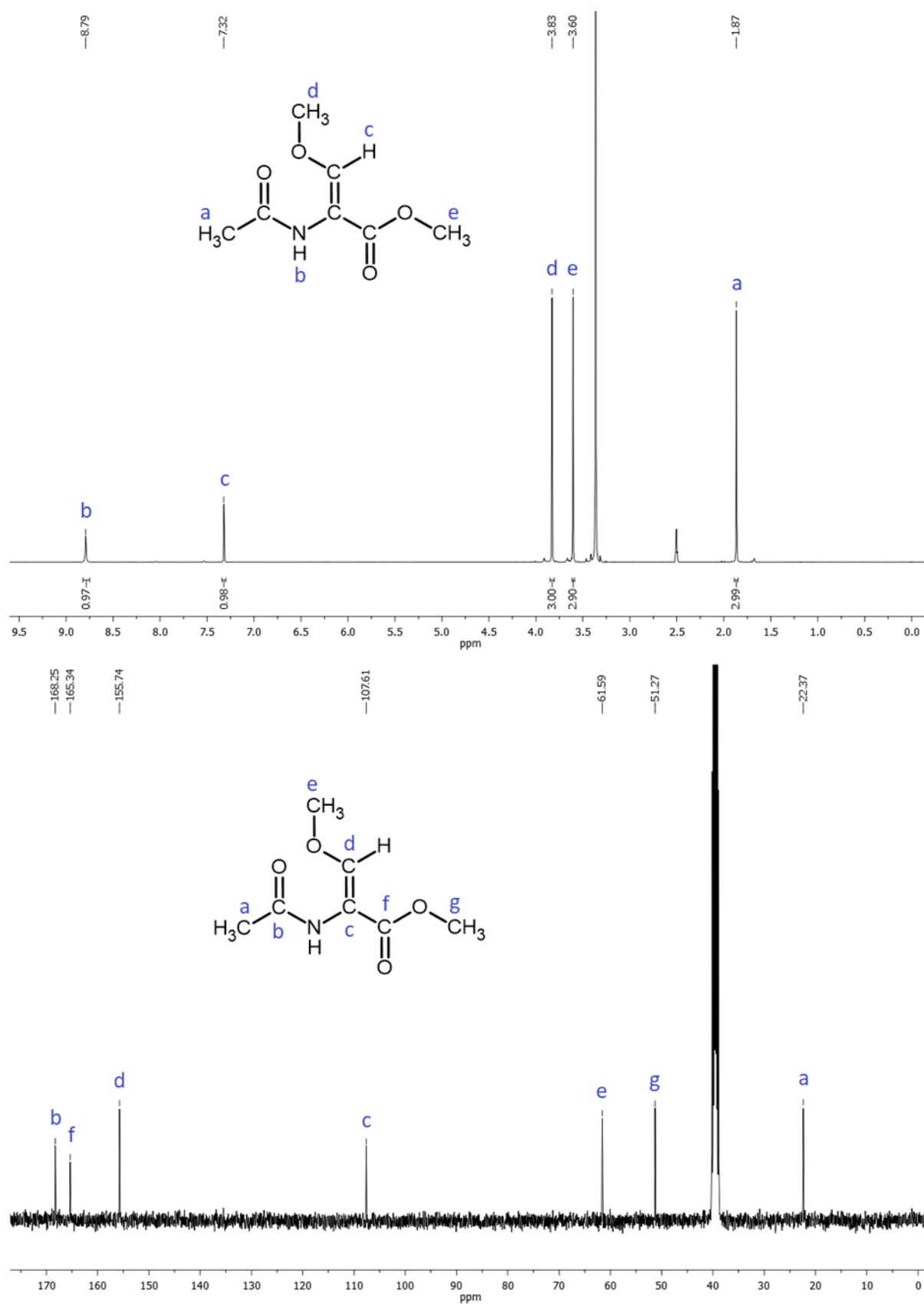


Figure S7. ¹H and ¹³C NMR spectra of Ac-(Z)-ΔSer(OMe)-OMe (1) in DMSO-*d*₆.

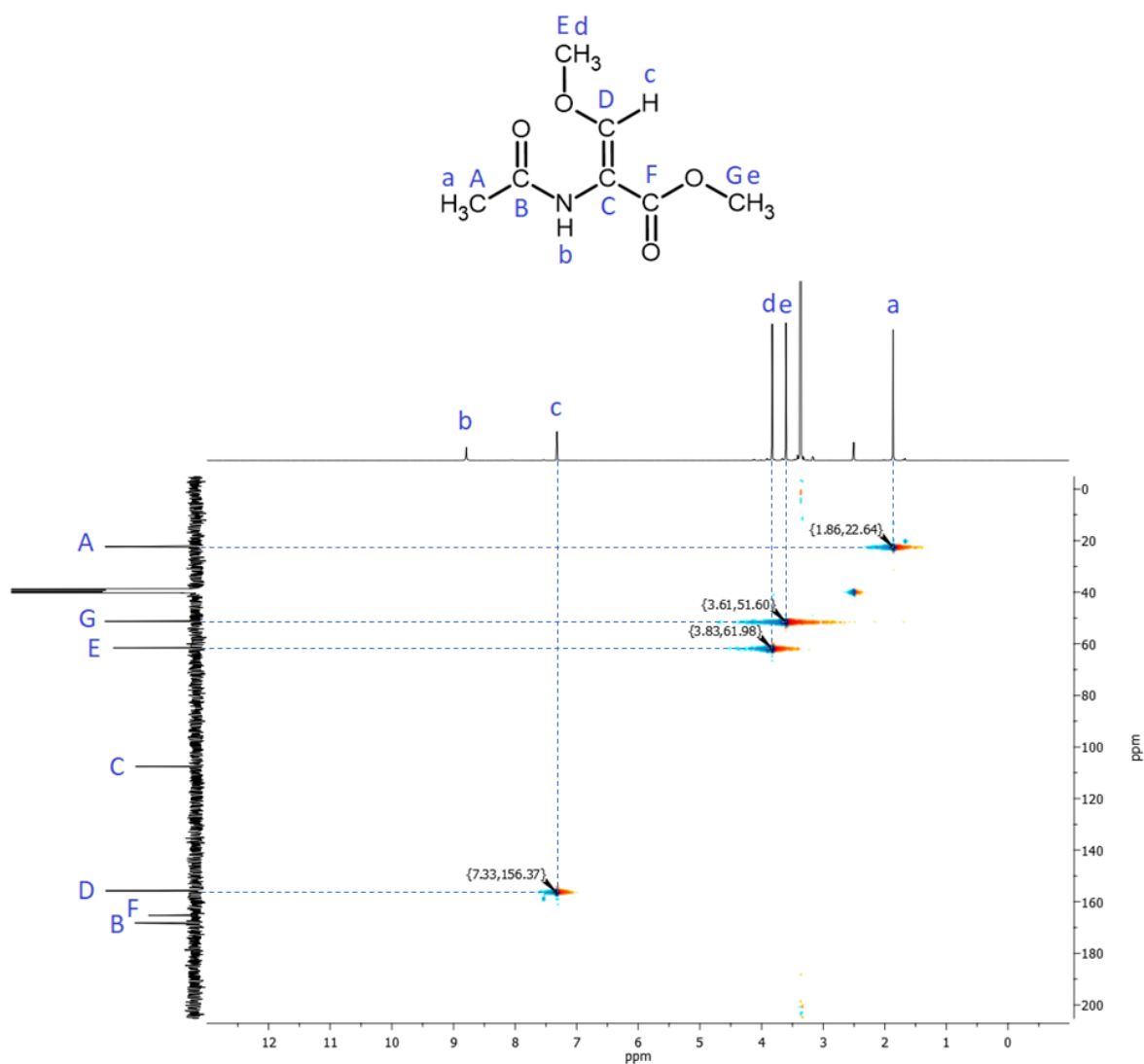


Figure S8. HSQC NMR spectrum of Ac-(Z)-ΔSer(OMe)-OMe (1) in DMSO- d_6 .

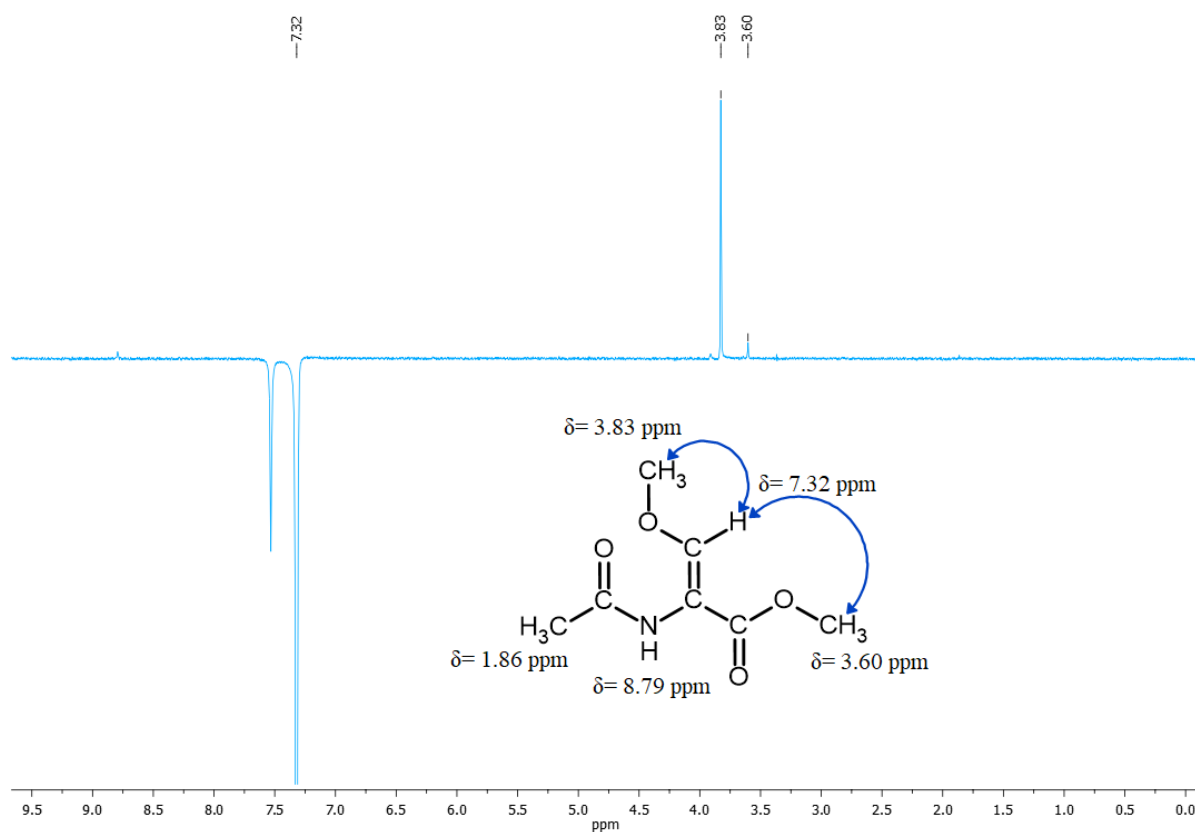


Figure S9. ¹H NMR 1D-NOE spectra obtained by selective excitation of the side chain H atom of Ac-(Z)-ΔSer(OMe) (1) in DMSO-*d*₆.

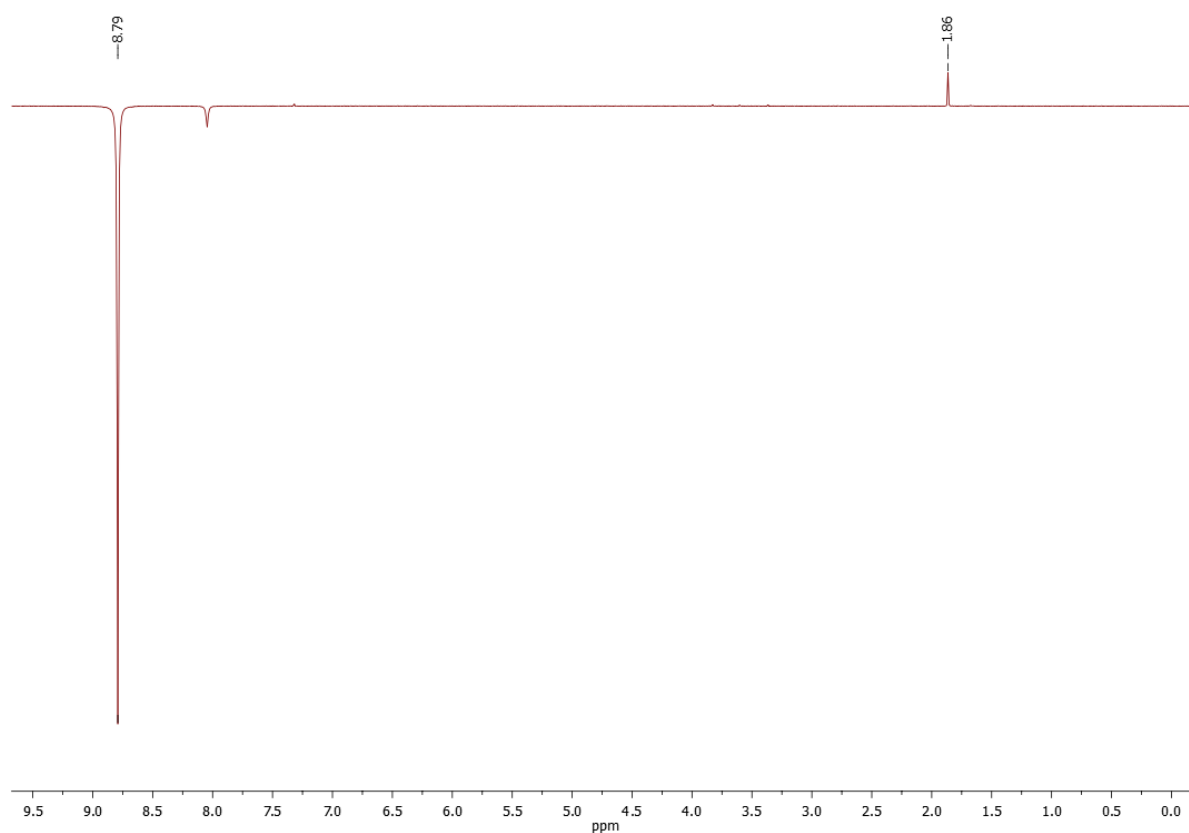


Figure S10. ¹H NMR 1D-NOE spectra obtained by selective excitation of the amide H atom of Ac-(Z)-ΔSer(OMe)-OMe (1) in DMSO-*d*₆.

D3



Insight into the structure of antitubercular Callyaerins: conformational studies and synthesis of a unique dehydroamino acid, β -aminodehydroalanine

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Abstract

β -Aminodehydroalanine, $\Delta\text{Ala}(\beta\text{-NH})$, (2,3-diaminoprop-2-enoic acid), is a unique dehydroamino acid and a central component of Callyaerins A-M and Callynormine A. The presence of this unusual structural element containing an enamine functional group may be related to the antitubercular activity of Callyaerins. According to The WHO Global Tuberculosis Report tuberculosis is the second leading cause of death worldwide caused by a single infectious agent. Therefore, it is essential to understand the molecular structure of these peptides in more detail. To investigate the conformational properties of the $\Delta\text{Ala}(\beta\text{-NH})$ residue, a series of model compounds: $\text{Ac}-(\text{Z/E})-\Delta\text{Ala}(\beta\text{-NHMe})\text{-NHMe}$, $\text{Ac}-(\text{Z/E})-\Delta\text{Ala}(\beta\text{-NHMe})\text{-NMe}_2$, $\text{Boc-Gly}-(\text{Z})-\Delta\text{Ala}(\beta\text{-NHMe})\text{-OMe}$, and $\text{Boc-Gly}-(\text{Z})-\Delta\text{Ala}(\beta\text{-Leu-OMe})\text{-OMe}$, were selected for quantum chemical calculations and/or synthesized. Two conformations, β_2 ($\phi, \psi \sim -120^\circ, 20^\circ$) and α ($\phi, \psi \sim -70^\circ, -15^\circ$) are predicted as the most preferable, regardless of the geometry of isomer (Z/E), polarity of environment, and order ($2^\circ/3^\circ$) of C-terminal amide group. The $\text{N-H}\cdots\text{O}$ hydrogen bond involving the N-H group in the β position of the side chain as a donor is a significant stabilizing factor. The Z isomer is predicted to be the most stable and has been synthesized. The following synthesis method is proposed: $\text{Ser} \rightarrow \Delta\text{Ala} \rightarrow \Delta\text{Ala}(\beta\text{-Br}) \rightarrow \Delta\text{Ala}(\beta\text{-NH})$. The advantages of the proposed method are: (i) serine as the starting substrate, (ii) mild alkaline conditions, (iii) avoidance of the reactive intermediate α -formylglycine.

Keywords β -Aminodehydroalanine · β -Aminoacrylamide · Antituberculosis · Ramachandran diagram · Dehydroamino acids · Amino acid synthesis

Introduction

Callynormine A isolated from the Kenyan marine sponge *Callyspongia abnormis* (Berer et al. 2004) together with Callyaerins A-M isolated from the Indonesian marine

sponge *Callyspongia aerizusa* (Ibrahim et al. 2008, 2010; Daletos et al. 2015) belong to a family of naturally occurring peptides, containing atypical amino acid residue, β -aminodehydroalanine, $\Delta\text{Ala}(\beta\text{-NH})$. Callyaerins have been tested on various cancer cell lines (Ibrahim et al. 2008, 2010). However, the most promising is their antituberculosis activity, especially Callyaerins A and B (Daletos et al. 2015; Podlesainski et al. 2024), which show the strongest antiTB activity and do not show cytotoxic activity against THP-1 and MRC-5 cell lines. According to The WHO Global Tuberculosis Report tuberculosis is the second leading cause of death worldwide caused by a single infectious agent (WHO 2023). Therefore, it is essential to better understand the molecular structure of these peptides in order to obtain further information on the relationship between structure and biological activity.

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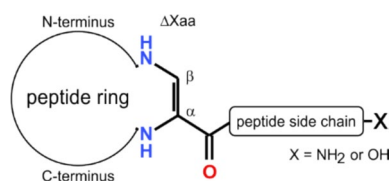


Fig. 1 Schematic diagram of the structure of Callynormine A and Callyaerins A-M, with β -aminodehydroalanine, $\Delta\text{Ala}(\beta\text{-NH}_2)$, as the central part of the molecule

The molecular structure of Callyaerins and Callynormine A consists of a cyclic backbone (6–8 amino acid residues) and a shorter side chain (2–5 amino acid residues) (Fig. 1, Table 1). The shorter, linear peptide chain consists only of standard amino acid residues. The larger cyclic chain may also contain the non-standard γ -hydroxyproline (HYP) and 2-amino-3-(5-methoxy-2-oxoimidazolidin-4-ylidene)propionic acid (AMOIPA) (Callyaerin M). In the middle of the structure there is a unique structural element that forms a link between the cyclic and linear parts of the molecule. It is called α -amido- β -aminoacrylamide (Berer et al. 2004; Ibrahim et al. 2008) or (*Z*)-2,3-diaminoacrylic acid (DAA) (Ibrahim et al. 2010; Daletos et al. 2015). In this work, this unique structural element is considered as a separate amino acid residue, (*Z*)-2,3-diaminoprop-2-enoic acid. According to amino acid nomenclature and symbolism (Moss 1984), 2,3-diaminopropanoic acid can be considered as 3-amino-alanine. Because of the unsaturation between the α and β carbon atoms, it is abbreviated as β -aminodehydroalanine, $\Delta\text{Ala}(\beta\text{-NH}_2)$, and will be referred to as such in this publication.

It is worth noting that a similar structural element, the C=C double bond and N–H group in the side chain ureido group, occurs in another well-known family of natural anti-tubercular peptides, tuberactinomycins (Laughlin and Conn 2022). This is an important premise that links this unusual structural element, the dehydroamino acid enamine system, with biological (antitubercular) activity.

The aim of this work is to describe the conformational properties of the β -aminodehydroalanine $\Delta\text{Ala}(\beta\text{-NH}_2)$ residue. To our knowledge, they have not been described so far. The presence of the $\text{C}\alpha=\text{C}\beta$ double bond causes the loss of optical activity of the studied residue. On the other hand, the lack of rotation around the $\text{C}\alpha=\text{C}\beta$ double bond causes a rigid position of the β -amino group and the appearance of the geometrical isomers *Z* and *E*. This rigidity should have a significant effect on the peptide conformation. The presence of an additional amine group at the β -carbon atom together with amide groups at the α -carbon atom may create a delocalized cross-conjugated electron system that may stabilize the structure and/or may be a specific pharmacophore responsible for the biological activity.

Table 1 List of amino acid residues in the structures of Callynormine A and Callyaerins A-M

Peptide	Peptide ring		Side peptide chain										Ref			
	<i>N</i> -terminus—C-terminus		Δ Xaa													
	R1	R2	R3	R4	R5	R6	R7	R8	C1	C2	C3	C4		C5	X	
Callyaerin	Ile	Hyp	Val	Ile	Leu	Pro	Pro	Leu	β -aminodehydroalanine, Δ Ala(β NH ₂)	Pro	Ile	Phe	Gly		NH ₂	(iii)
A	Ile	Hyp	Ile	Ile	Leu	Pro	Pro	Leu		Pro	Ile	Ile			NH ₂	(iii)
B	Ile	Hyp	Leu	Leu	Pro	Pro	Val			Pro	Leu	Phe	Gly		NH ₂	(iii)
C	His	Hyp	Leu	Leu	Pro	Pro	Val			Pro	Ile	Asn	Ala	Ile	NH ₂	(iii) (iv)
D	Ile	Hyp	Ile	Phe	Pro	Pro	Leu			Pro	Ile	Ile	Gly		NH ₂	(iii) (iv)
E	Leu	Pro	Phe	Phe	Pro	Pro	Val			Pro	Ile				NH ₂	(iii) (iv)
F	Val	Pro	Val	Phe	Pro	Pro	Leu			Phe	Pro				NH ₂	(iii) (iv)
G	Leu	Pro	Phe	Phe	Pro	Pro	Leu			Pro	Pro	Phe	Gly			(ii) (iv)
H	Val	Pro	Val	Phe	Pro	Pro	Leu			Pro	Ile				NH ₂	(iii)
I	Leu	Pro	Phe	Phe	Pro	Pro	Val			Pro	Leu	Phe	Gly		NH ₂	(iv)
J	Phe	Pro	Leu	Phe	Pro	Pro	Val			Pro	Ile	Ile	Gly		NH ₂	(iv)
K	Phe	Pro	Phe	Gly	Leu	Pro	Pro	Phe		Pro	Phe	Ile	Asp		OH	(iv)
L	Ile	Hyp	Glu	Ile	Val	Pro	Pro	Leu		Pro	Leu	Phe			NH ₂	(iv)
M	AMOIPA	Hyp	Leu	Leu	Pro	Pro	Val			Pro	Leu	Phe	Gly		NH ₂	(iv)
Callynormine A	Ile	Hyp	Val	Leu	Pro	Pro	Leu			Pro	Phe	Leu			OH	(i)

(i) (Berer et al. 2004), (ii) (Ibrahim et al. 2008), (iii) (Ibrahim et al. 2010), (iv) (Daletos et al. 2015), Hyp is γ -hydroxyproline, AMOIPA is 2-amino-3-(5-methoxy-2-oximidazolidin-4-ylidene)

(i) (Berer et al. 2004), (ii) (Ibrahim et al. 2008), (iii) (Ibrahim et al. 2010), (iv) (Daletos et al. 2015), Hyp is γ -hydroxyproline, AMOIPA is 2-amino-3-(5-methoxy-2-oxoimidazolidin-4-ylidene)propanoic acid,

Dehydroamino acids having a heteroatom (Cl, O) in the β position of the side chain can be found in naturally occurring peptides such as victorin (Kessler et al. 2020) or cyrmenins (Chakor et al. 2012). They have specific conformational properties (Banaś et al. 2023, 2025), mainly due to the presence of the electron pairs that allow conjugation with the $C\alpha=C\beta$ double bond and can serve as an acceptor for hydrogen bond interactions. However, the case of the β -aminodehydroalanine may be different, because the nitrogen atom can also act as a hydrogen bond donor.

In addition to the description of Δ Ala(β -NH₂) residue conformational properties, in this research another approach to the synthesis of β -aminodehydroalanine has been proposed. Relatively short models, analyzed and/or synthesized in this work, are presented in Fig. 2.

Materials and methods

Quantum chemical calculations

A commonly used method for obtaining conformational properties of amino acid residues is the analysis of short model compounds (Head-Gordon et al. 1991). Calculations were made for the following model compounds: Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) and Ac-(E)- Δ Ala(β -NHMe)-NHMe (2), like also Ac-(Z)- Δ Ala(β -NHMe)-NMe₂ (3) and Ac-(E)- Δ Ala(β -NHMe)-NMe₂ (4). In the first step, Ramachandran diagrams of the model compounds 1–4 were created, which describe the relationship between the values of torsion angles φ and ψ and the potential energy of the molecules (Figs. 3, 4, 5 and 6). The trans configuration ($\omega \approx 180^\circ$) of the amide group was set as the more stable. The torsion angle, which defines the position of the delta methyl group, was not constrained during geometry optimization. This position was set as the default in the initial structures. Initial structures were prepared in which the dihedral angles φ and ψ were constrained and their values were changed in steps of 30° . Since the studied dehydroamino acid residue is achiral, each structure in the map has its mirror counterpart

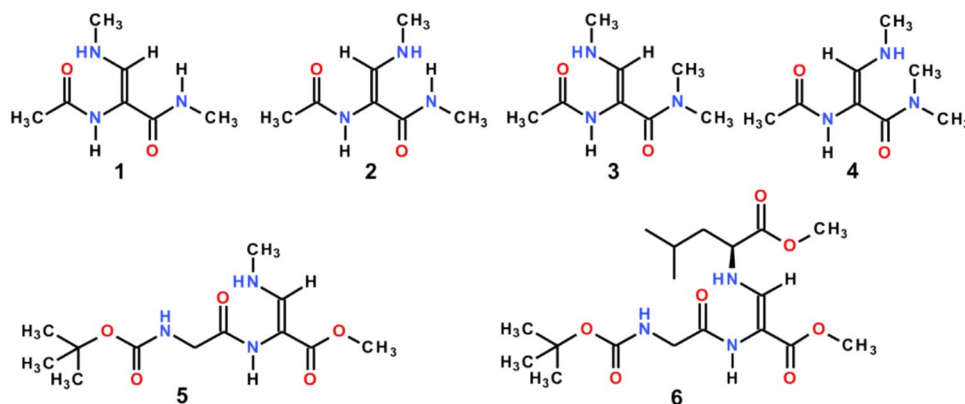
with the same energy but opposite torsion angles ($\varphi, \psi = -\varphi, -\psi$). This reduces the number of grid point structures necessary to create each map to 91. In this way, potential energy surfaces $E = f(\varphi, \psi)$ were obtained. DFT method was applied at the M06-2X/6-31+G(d,p) level of theory (Zhao and Truhlar 2008), first in the gas phase, and then in chloroform and water to generate a total of 3 maps for each molecule (constrained optimization). The solvent effect was simulated by a self-consistent reaction field (SCRF) using the conductor-like polarizable continuum model (CPCM). The potential energy minima located on the maps were fully optimized using a larger basis set, 6-311+G(d,p). After these unconstrained optimizations, vibrational analysis was performed to ensure that the resulting structures were true energy minima and to obtain the zero-point vibrational energies (ZPVEs) and Gibbs energies (298.15 K, 1.0 atm). The conformation population (p) was calculated at 300 K, where $RT = 0.595$ kcal/mol according to the following equations: $p_{\text{rel}} = 100\% \exp(-\Delta G/RT)$, $p = p_{\text{rel}} / \sum \text{conformers } p_{\text{rel}} \times 100\%$ (Hruby et al. 1997; Hudáky & Perczel 2008). The conformation names are based on the Scarsdale nomenclature (Scarsdale et al. 1983). The Gaussian 16 package (Frisch, et al. 2016) with GaussView5 (Dennington, et al. 2008) was used. To estimate the internal stabilizing forces, both hydrogen bonds and dipole interactions were analyzed. In the tables and figures, data are presented only for X–H $\cdots$ A (X=N, C; A=O, N) hydrogen bonds, where $r_{\text{H}\cdots\text{X}} \leq 2.7$ Å and $\angle \text{X–H}\cdots\text{A} > 90^\circ$ (Vargas et al. 2002). For the dipole interactions, data are presented only for C=O $\leftarrow \cdots \rightarrow$ O=C contacts, where $r_{\text{C}\cdots\text{O}} < 3.6$ Å (N, C denote the N-terminal or the C-terminal amide group) (Allen et al. 1998).

Synthesis

Ac- Δ Ala-NHMe

To Ac-Ser-NHMe (1.121 g, 7.0 mmol) methylene chloride (30 ml), EDC·HCl (1.472 g, 7.7 mmol, 1.1 equiv.) and CuCl (0.208 g, 2.1 mmol, 0.3 equiv.) were added in an argon atmosphere. The reaction mixture was stirred at

Fig. 2 The studied compounds: Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1), Ac-(E)- Δ Ala(β -NHMe)-NHMe (2), Ac-(Z)- Δ Ala(β -NHMe)-NMe₂ (3), Ac-(E)- Δ Ala(β -NHMe)-NMe₂ (4), Boc-Gly-(Z)- Δ Ala(β -NHMe)-OMe (5), Boc-Gly-(Z)- Δ Ala(β -Leu-OMe)-OMe (6). Quantum chemical calculations (1–4), synthesis (1, 5, 6)



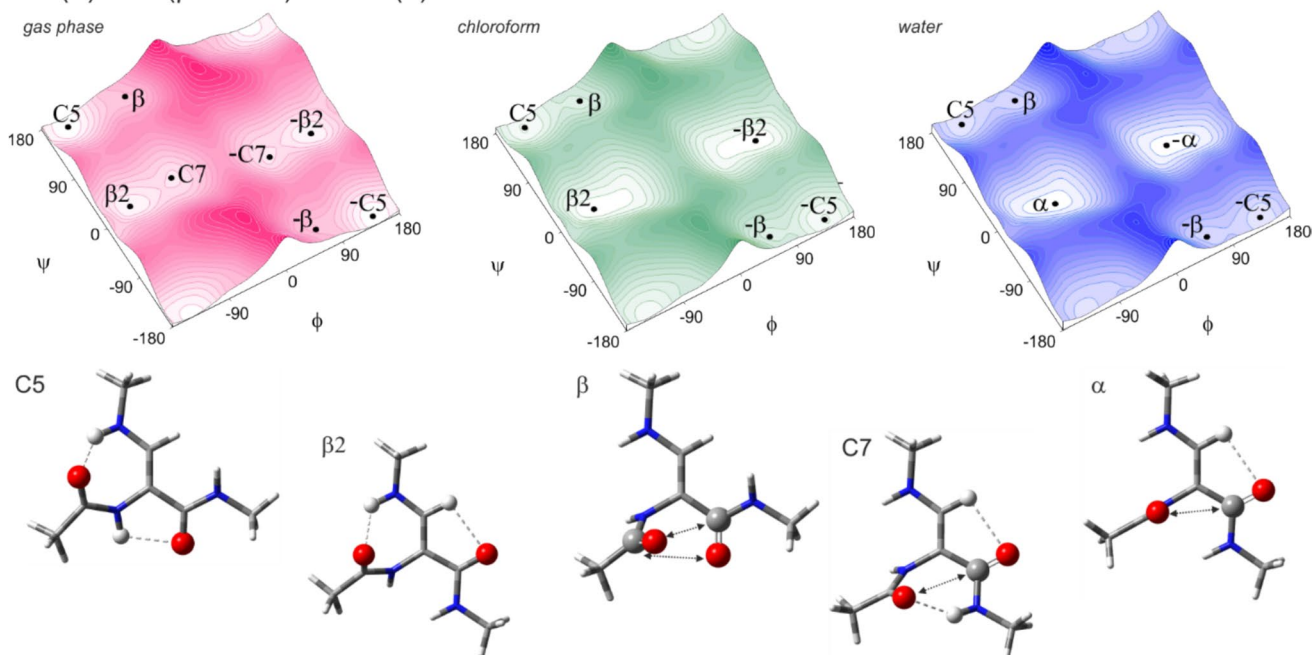
Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1)

Fig. 3 Potential energy surfaces $E=(\phi, \psi)$ of Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) calculated by the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environments. Energy con-

tours are plotted every 1 kcal/mol. Conformations optimized at the M06-2X/6-311+G(d,p) level of theory with hydrogen bonds ($\cdots$) and electrostatic interactions ($\blacktriangleleft \cdots \blacktriangleright$) formed within the studied residue

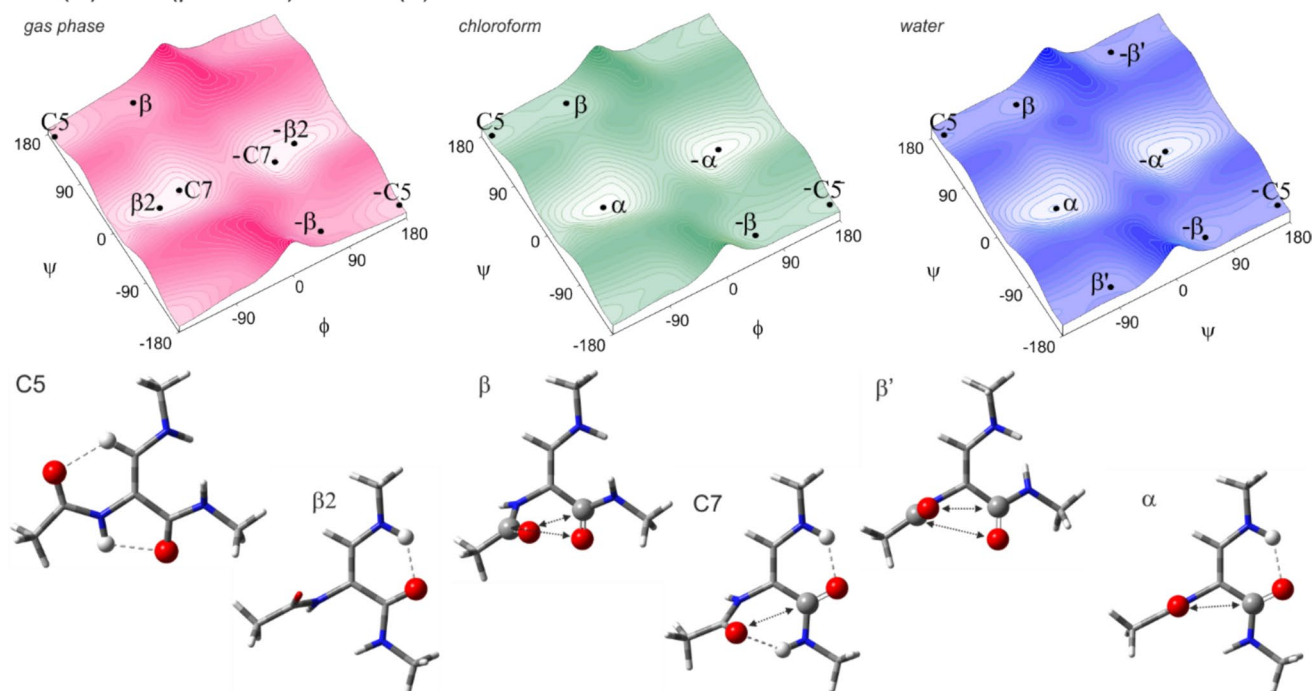
Ac-(E)- Δ Ala(β -NHMe)-NHMe (2)

Fig. 4 Potential energy surfaces $E=(\phi, \psi)$ of Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) calculated by the M06-2X/6-31+G(d,p) method in the gas phase, chloroform, and water environments. Energy con-

tours are plotted every 1 kcal/mol. Conformations optimized at the M06-2X/6-311+G(d,p) level of theory with hydrogen bonds ($\cdots$) and electrostatic interactions ($\blacktriangleleft \cdots \blacktriangleright$) formed within the studied residue

room temperature overnight. The post-reaction mixture was concentrated using a rotary evaporator and then, the product was purified by column chromatography, using a 5% MeOH/AcOEt solution as an eluent, and crystallized from ethyl acetate. Yield: 0.830 g (5.8 mmol), 74%.

^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.05 (1H, s, (N-H) N), 8.27 (1H, q, $J=4.0$ Hz (N-H) C), 5.99 (1H, s, (E)-C $^\beta$ -H), 5.32 (1H, s, (Z)-C $^\beta$ -H), 2.68 (3H, d, $J=4.8$ Hz, (CH $_3$) C), 2.00 (3H, s, (CH $_3$) N). ^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm): 169.19 (O=C) N , 164.48 (O=C) C , 136.19 (C $^\alpha$), 102.31 (C $^\beta$), 26.10 (CH $_3$) C , 23.90 (CH $_3$) N (Figs. 4S and 5S). Melting point: 112.9–115.2 °C. TLC MeOH/AcOEt (1/19) (v/v) $R_f=0.33$.

SMILES Notation: C=C(NC(=O)C)C(=O)NC.

Ac-(Z)- Δ Ala(β -Br)-NHMe

Ac- Δ Ala-NHMe (0.426 g, 3.0 mmol) was dissolved in *N,N*-dimethylformamide (1 ml), diluted with methylene chloride (5 ml) and cooled in an acetone-dry ice bath. Then, a solution of Br $_2$ (3.3 mmol, 1.1 equiv.) in methylene chloride (1 ml) was added dropwise. After 15 min triethylamine (1.25 ml, 9.0 mmol, 3 equiv.) was added. The reaction mixture was stirred at -77 °C for 1 h. Afterwards, the post-reaction mixture was concentrated using a rotary evaporator and product was purified by column chromatography, using an increasing gradient of ethyl acetate in hexane from 50 to 100%, then an increasing gradient of methanol in ethyl acetate from 1 to 10% as an eluent. Desired product was crystallized from ethyl acetate. Yield: 0.4667 g (2.1 mmol), 71%.

^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.36 (1H, s, (N-H) N), 8.06 (1H, q, $J=4.0$ Hz, (N-H) C), 6.89 (1H, s, CH), 2.60 (3H, d, $J=4.4$ Hz, (CH $_3$) C), 1.95 (3H, s, (CH $_3$) N). ^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm): 168.56 (O=C) N , 162.90 (O=C) C , 136.98 (C $^\alpha$), 108.25 (C $^\beta$), 26.12 (CH $_3$) C , 22.63 (CH $_3$) N (Fig. 6S). Melting point: 146.5–148.5 °C. TLC MeOH/AcOEt (7/93) (v/v) $R_f=0.30$.

The Z isomer was confirmed based on NOE spectra (Figs. 12–14S). The isomer E was not detected.

SMILES Notation: O=C(C)N/C(=C\Br)C(=O)NC.

Ac-(Z)- Δ Ala(β -NHMe)-NHMe

Ac-(Z)- Δ Ala(β -Br)-NHMe (0.440 g, 2.0 mmol) was dissolved in acetonitrile (30 ml). Then, K $_2$ CO $_3$ (0.275 g, 2.0 mmol) and a 6 M methanolic solution of methylamine (2.0 mmol, +1.0 mmol after 3 h) were added in an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The post-reaction mixture was concentrated using a rotary evaporator and then product was purified by column chromatography, using an increasing

gradient of methanol in ethyl acetate from 0 to 20% as an eluent. Yield: 0.139 g (0.8 mmol), 41%.

^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 8.19 (1H, s, (N-H) N), 6.98 (1H, d, $J=13.6$ Hz, CH), 6.76 (1H, q, $J=4.4$ Hz, (N-H) C), 5.55 (1H, dq, $J=13.2, 4.8$, Hz (N-H) $^\beta$), 2.76 (3H, d, $J=4.8$ Hz (CH $_3$) $^{\beta\text{-NH}}$), 2.55 (3H, d, $J=4.8$ Hz (CH $_3$) C), 1.90 (3H, s, (CH $_3$) N). ^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm): 169.23 (O=C) N , 166.68 (O=C) C , 141.96 (C $^\beta$), 99.38 (C $^\alpha$), 33.74 (CH $_3$) $^{\beta\text{-NH}}$, 25.86 (CH $_3$) C , 23.18 (CH $_3$) N (Fig. 7S). TLC MeOH/AcOEt (1/4) (v/v) $R_f=0.13$.

The Z isomer was confirmed based on NOE spectra (Figs. 15S and 16S). The isomer E was not detected.

SMILES Notation: O=C(C)N/C(=C\NC)C(=O)NC.

Boc-Gly- Δ Ala-OMe

Boc-Gly- Δ Ala-OMe was obtained using a multistep procedure as previously described (Makowski et al. 2015).

^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.2 (1H, s, (N-H) N), 7.2 (1H, t, $J=6.0$, (N-H) $^{\text{Gly}}$), 6.3 (1H, s, (E)-C $^\beta$ -H), 5.7 (1H, s, (Z)-C $^\beta$ -H), 3.8 (3H, s, (CH $_3$) C), 3.7 (2H, d, $J=6.0$, (CH $_2$) $^{\text{Gly}}$), 1.4 (9H, s, (CH $_3$) $^{\text{Boc}}$). ^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm): 169.2 (O=C) $^{\text{Gly}}$, 163.8 (O=C-O), 156.0 (O=C(O)-N-H), 132.1 (C $^\alpha$), 108.7 (C $^\beta$), 78.3 (C) $^{\text{Boc}}$, 52.8 (CH $_3$) C , 44.0 (CH $_2$) $^{\text{Gly}}$, 28.2 (CH $_3$) $^{\text{Boc}}$ (Fig. 8S). TLC toluene/AcOEt (3:7) (v/v) $R_f=0.57$.

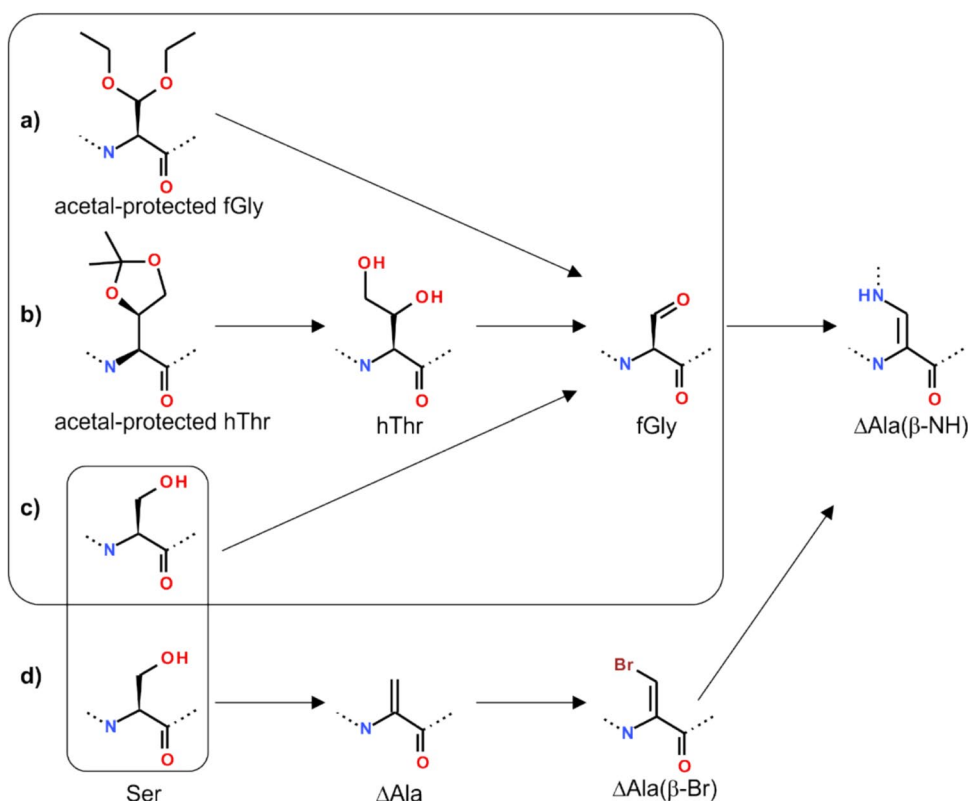
SMILES Notation: CC(C)(C)OC(=O)NCC(=O)NC(=C)C(=O)OC.

Boc-Gly-(Z)- Δ Ala(β -Br)-OMe

Boc-Gly- Δ Ala-OMe (0.258 g, 1.0 mmol) was dissolved in methylene chloride (3 ml) and cooled in an acetone-dry ice bath. Then, a solution of Br $_2$ (1.1 mmol, 1.1 equiv.) in methylene chloride (0.35 ml) was added dropwise. After 15 min triethylamine (0.70 ml, 3.0 mmol, 3 equiv.) was added. The reaction mixture was stirred at -77 °C for 1 h. Afterwards, the post-reaction mixture was concentrated using a rotary evaporator and product was purified by column chromatography, using an increasing gradient of ethyl acetate in hexane from 50 to 100%, then an increasing gradient of methanol in ethyl acetate from 1 to 5% as an eluent. Desired product was crystallized from ethyl acetate. Yield: 0.2866 g (0.85 mmol), 85%.

^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 9.6 (1H, s, (N-H) N), 7.3 (1H, s, CH), 7.0 (1H, t, $J=6.0$, (N-H) $^{\text{Gly}}$), 3.7 (2H, d, $J=6.4$, (CH $_2$) $^{\text{Gly}}$), 3.7 (3H, s, (CH $_3$) C), 1.4 (9H, s, (CH $_3$) $^{\text{Boc}}$). ^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm): 168.8 (O=C) $^{\text{Gly}}$, 162.6 (O=C-O), 155.8 (O=C(O)-N-H), 132.9 (C $^\alpha$), 113.3 (C $^\beta$), 78.1 (C) $^{\text{Boc}}$, 52.5 (CH $_3$) C , 42.8 (CH $_2$) $^{\text{Gly}}$, 28.2 (CH $_3$) $^{\text{Boc}}$ (Fig. 9S). TLC Hex/AcOEt (4:6) (v/v) $R_f=0.35$.

Fig. 7 The scheme of known (a–c) and herein described (d) the synthesis of the studied structural element, β -aminodehydroalanine: **a** 1. TFA/H₂O (95:5), rt, 2 $\times$ 20 min; 2. 1% HCOOH (v/v) in ACN, anhydrous MgSO₄ (100 equiv.), the amino group from the linear peptide rt, 10 h. (Zhang et al. 2018), **b** 1. TFA/H₂O/TIS (95:2.5:2.5 v/v), rt, 1 h; 2. NaIO₄ (1.2 equiv), water, 0 °C – rt, 20 min.; 3. HCOOH in ACN, anhydrous MgSO₄ (Yates et al. 2023); **c** DMP (3–4 equiv.), ACN, 2 h, rt / cyclisation (Podlesinski et al. 2024); **d** 1. EDC HCl (1.1 equiv.), CuCl (0.3 equiv.) in DCM; 2. Br₂ (1.1 equiv.), Et₃N (3 equiv.) in DMF; 3. R-NH₂, K₂CO₃ in ACN (this work). Abbreviations: fGly = α -formylglycine, hThr = γ -hydroxytreonine, Δ Ala = dehydroalanine ACN = acetonitrile, DCM = dichloromethane, DMF = *N,N*-dimethylformamide, TFA = trifluoroacetic acid, TIS = tri(isopropan-2-yl)silane, DMP = Dess–Martin periodinane



The Z isomer was confirmed based on NOE spectra (Figs. 17S and 18S). The isomer E was not detected.

SMILES Notation: CC(C)(C)OC(=O)NCC(=O)N/C(=C\Br)C(=O)OC.

Boc-Gly-(Z)- Δ Ala(β -NHMe)-OMe

Boc-Gly-(Z)- Δ Ala(β -Br)-OMe (0.101 g, 0.3 mmol) was dissolved in acetonitrile (5 ml). Then, K₂CO₃ (0.041 g, 0.300 mmol) and a methanolic solution of methylamine (0.300 mmol, +0.120 mmol after 3 h) were added in an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The post-reaction mixture was concentrated using a rotary evaporator and then the product was purified by column chromatography, using an increasing gradient of ethyl acetate in hexane from 60 to 100% as an eluent. Yield: 0.040 g (1.4 mmol), 47%.

The Z isomer was confirmed based on NOE spectra (Figs. 19S and 20S). The isomer E was not detected.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.36 (1H, s, (N-H)^N), 7.28 (1H, d, *J* = 14.0 Hz, CH), 6.86 (1H, t, *J* = 5.2 Hz, (N-H)^{Gly}), 6.16 (1H, m, (N-H) ^{β}), 3.63 (2H, d, *J* = 6.0 Hz, (CH₂)^{Gly}), 3.52 (3H, s, (CH₃)^C), 2.85 (3H, d, *J* = 4.4 Hz, (CH₃) ^{β -NH}), 1.39 (9H, s, (CH₃)^{Boc}). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 168.36 (O=C)^{Gly}, 166.14 (O=C-O), 155.89 (O=C(O)-N-H), 146.83 (C ^{β}), 94.51

(C ^{α}), 78.16 (C)^{Boc}, 50.28 (CH₃)^C, 43.37 (CH₂)^{Gly}, 30.99 (CH₃) ^{β -NH}, 28.22 (CH₃)^{Boc} (Figs. 10S).

SMILES Notation: CC(C)(C)OC(=O)NCC(=O)N/C(=C\NC)C(=O)OC.

Boc-Gly-(Z)- Δ Ala(β -LeuOMe)-OMe

Boc-Gly-(Z)- Δ Ala(β -Br)-OMe 0.101 g (0.3 mmol) was dissolved in acetonitrile (7 ml) and HCl·H₂N-Leu-OMe (0.065 mg, 0.4 mmol, 1.3 equiv.), K₂CO₃ (0.083, 0.6 mmol, 2 equiv.) were added. The reaction mixture was stirred at nitrogen atmosphere. After six hours (6 h), Et₃N (0.083 mL, 0.6 mmol, 2 equiv.) was added to the mixture and reaction was continued up to 48 h. Then silica gel was added and volatile components were removed under reduced pressure. Solid residue was transferred to the top of the chromatography column and product was eluted with gradient of EtOAc in hexane from 30 to 100% then gradient of MeOH in EtOAc from 0 to 7%. Fraction with product was collected and solvents were removed under reduced pressure. The product (0.062 mg, 0.16 mmol) was obtained as colorless oil with yield of 53%.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 8.42 (1H, s, (N-H)^N), 7.34 (1H, d, *J* = 13.2 Hz, CH), 6.91 (1H, t, *J* = 4.8 Hz (N-H)^{Gly}), 6.30 (1H, dd, *J* = 11.2, 8.4 Hz N-H) ^{β}), 4.13 (1H, dt, *J* = 5.6, 8.8 Hz (CH) ^{α -Leu}), 3.63 (3H, s, (OCH₃)^{Leu}), 3.61 (2H, d, *J* = 8.0 Hz, (CH₂)^{Gly}), 3.53 (3H, s, (OCH₃)^C), 1.65

Table 2 Selected parameters of the Ac-(Z)-ΔAla(β-NHMe)-NHMe (1) conformations

Ac-(Z)-ΔAla(β-NHMe)-NHMe (1)					
Conformer	φ (°)	Ψ (°)	G (hartrees)	ΔG (kcal)	P (%)
<i>Gas Phase</i>					
C5	−146.0	165.9	−589.05130	0.00	84.67
β2	−136.6	12.3	−589.04955	1.10	13.44
C7	−66.8	19.6	−589.04766	2.28	1.83
β	−61.1	156.6	−589.04447	4.29	0.06
<i>Chloroform</i>					
β2	−123.1	8.7	−589.06634	0.00	88.99
C5	−142.0	165.9	−589.06431	1.27	10.52
β	−59.4	158.2	−589.06141	3.09	0.50
<i>Water</i>					
α	−89.4	−1.9	−589.07415	0.00	99.44
β	−60.3	159.2	−589.06873	3.40	0.33
C5	−140.7	166.1	−589.06840	3.61	0.23

Each calculated conformer has its mirror counterpart

(1H, tq, J=22.0, 6.0 Hz, (CH)^{Leu}), 1.55 (2H, dd, J=14.0, 8.8 Hz, (CH₂)^{Leu}), 1.38 (9H, s, (CH₃)^{Boc}), 0.83–0.88 (6H, dt, (CH₃)^{Leu}). ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm): 172.92 (O=C–O)^{Leu}, 168.28 (O=C)^{Gly}, 166.05 (O=C–O)^C, 155.89 (O=C(O)–N–H), 144.18 (C^β), 96.53 (C^α), 78.14 (C)^{Boc}, 52.07 (CH)^{α,Leu}, 52.07 (OCH₃)^C, 50.56 (OCH₃)^{Leu}, 43.45 (CH₂)^{Gly}, 40.71 (CH₂)^{β,Leu}, 28.25 (CH₃)^{Boc}, 23.85 (CH)^{γ,Leu}, 22.92, 21.31 (CH₃)^{δ,Leu} (Fig. 11S). TLC AcOEt R_f=0.38.

The Z isomer was confirmed based on NOE spectra (Figs. 21–23S). The isomer E was not detected.

NMR spectra

The spectra were recorded in DMSO-*d*₆ (internal TMS standard) at room temperature. Bruker Ultrashield 400 (Bruker 2005) spectrometer with Bruker (TopSpin Version 1.3) software, operating at 400 MHz (¹H) and 101 MHz (¹³C) was used.

Results and discussion

Theoretical method

To obtain a general view of the conformational properties of the β-aminodehydroalanine residue, conformational analysis was performed on simple diamide models, with *N*-terminal secondary and C-terminal secondary or tertiary amides with a methyl group limiting β-amino function. The following model compounds, Ac-(Z)-ΔAla(β-NHMe)-NHMe (1) and Ac-(E)-ΔAla(β-NHMe)-NHMe (2), like also Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3) and Ac-(E)-ΔAla(β-NHMe)-NMe₂ (4), were chosen for quantum chemical calculations. The potential energy surfaces E=(φ,ψ) together with the

Table 3 Selected parameters of the Ac-(E)-ΔAla(β-NHMe)-NHMe (2) conformations

Ac-(E)-ΔAla(β-NHMe)-NHMe (2)					
Conformer	φ (°)	ψ (°)	G (hartrees)	ΔG (kcal)	P (%)
<i>Gas Phase</i>					
β2	−105.3	2.0	−589.05287	0.00	80.77
C7	−68.1	13.5	−589.05151	0.86	19.21
C5	−177.9	164.9	−589.04484	5.04	0.02
β	−60.7	152.1	−589.04077	7.60	0.00
<i>Chloroform</i>					
α	−91.2	−0.1	−589.06955	0.00	100.00
C5	−173.6	163.0	−589.05741	7.62	0.00
β	−60.4	155.0	−589.05688	7.95	0.00
<i>Water</i>					
α	−87.3	−1.5	−589.07434	0.00	100.00
β	−59.9	155.6	−589.06298	7.13	0.00
β'	−92.7	−160.3	−589.06225	7.59	0.00
C5	−170.5	162.4	−589.06165	7.96	0.00

Each calculated conformation has its mirror counterpart

(φ,ψ=−φ,−ψ)

Optimized at the M06-2X/6–311+G(d,p) method (SCRF, CPCM)

The population of the conformations (P)

Table 4 Selected parameters of the Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3) conformations

Ac-(Z)-ΔAla(β-NHMe)-NMe ₂ (3)					
Conformer	φ (°)	ψ (°)	G (hartrees)	ΔG (kcal)	P (%)
<i>Gas Phase</i>					
C5	−148.0	162.0	−628.31604	0.00	88.87
β2	−135.2	29.2	−628.31390	1.35	9.23
C5'	−143.7	−159.9	−628.31217	2.43	1.50
β	−63.6	149.8	−628.31074	3.33	0.33
α	−48.7	−26.7	−628.30814	4.96	0.02
<i>Chloroform</i>					
β2	−128.1	31.1	−628.32946	0.00	83.54
C5	−144.9	160.7	−628.32750	1.23	10.60
α	−55.1	−27.6	−628.32630	1.98	2.98
β	−57.8	151.4	−628.32596	2.19	2.10
C5'	−130.0	−154.9	−628.32502	2.79	0.78
<i>Water</i>					
β2	−125.0	30.5	−628.33296	0.00	38.84
α	−56.9	−28.5	−628.33293	0.02	37.84
β	−95.6	−157.7	−628.33144	0.95	7.86
C5	−144.0	160.6	−628.33128	1.05	6.62
C5'	−123.3	−153.4	−628.33025	1.70	2.23

Each calculated conformation has its mirror counterpart

(φ,ψ=−φ,−ψ)

Optimized at the M06-2X/6–311+G(d,p) method (SCRF, CPCM)

The population of the conformations (P)

conformations are shown in Figs. 3, 4, 5 and 6. Selected parameters, including the torsion angles φ and ψ, as well as the energy and population of the conformations (p) are presented in Tables 2, 3, 4 and 5. The lack of chirality results in mirror conformations with the same energy but opposite

Table 5 Selected parameters of the Ac-(*E*)-ΔAla(β-NHMe)-NMe₂ (**4**) conformations

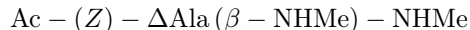
Ac-(<i>E</i>)-ΔAla(β-NHMe)-NMe ₂ (4)					
Conformer	φ (°)	ψ (°)	G (hartrees)	ΔG (kcal)	P (%)
<i>Gas Phase</i>					
β2	-114.2	20.8	-628.31323	0.00	71.00
α	-62.3	-17.7	-628.31230	0.59	26.41
C5	-170.9	158.5	-628.30963	2.26	1.60
β	-60.8	145.3	-628.30918	2.54	0.99
<i>Chloroform</i>					
α	-63.0	-20.4	-628.32675	0.00	49.92
β2	-113.5	20.8	-628.32671	0.03	47.52
β	-57.7	147.0	-628.32334	2.14	1.37
C5	-153.8	154.8	-628.32295	2.38	0.91
β'	-98.5	-157.3	-628.32183	3.09	0.28
<i>Water</i>					
α	-62.8	-22.1	-628.33223	0.00	76.46
β2	-113.4	20.6	-628.33100	0.78	20.78
β	-57.0	148.3	-628.32863	2.26	1.72
β'	-93.4	-155.0	-628.32751	2.96	0.53
C5	-139.2	155.3	-628.32747	2.99	0.51

Each calculated conformation has its mirror counterpart (φ, ψ = -φ, -ψ)

Optimized at the M06-2X/6-311+G(d,p) method (SCRF, CPCM)

The population of the conformations (P)

sign of the torsion angles values. The conformations on the left side of the maps are discussed, for clarity.



The conformational map calculated *in vacuo*, i.e. for an isolated molecule, primarily shows the restriction of rotation around torsion angles φ and ψ caused by steric hindrance between atoms. In addition, it is possible to estimate the strength of internal interactions created by the residues studied, without the influence of the environment. Figure 3 shows four conformations, found from the conformational maps *in vacuo*: C5, β2, C7, and β. Analysis of internal interactions (Table 1S) shows that the lowest energy is possessed by the extended C5 conformation (φ, ψ = -146°, 166°), stabilized by two N-H...O hydrogen bonds: one C5-membered bond characteristic for this conformation and the other involving the N-H group of the side chain as a donor. The second in energetic order is the semi-extended β2 conformation (φ, ψ = -137°, 12°), which results from the rotation of the C-terminal amide group. This conformation is stabilized by an N-H...O hydrogen bond involving the side chain amino group, as well as by a weaker C-H...O hydrogen bond between the vinyl hydrogen of the side chain and the oxygen atom of the C-terminal amide group. The remaining conformations have much higher energy. The C7 conformation (φ, ψ = -67°, 20°) is stabilized by a 7-membered N-H...O hydrogen bond between the amide groups,

a C-H...O hydrogen bond, and electrostatic dipole-dipole interactions between the carbonyl groups. The β conformation (φ, ψ = -61°, 157°) of the polyproline II helix is stabilized only by electrostatic dipole-dipole interactions.

The conformational map calculated in chloroform, simulating a weakly polar environment, i.e. the protein interior, shows only three conformations in the following energy order: β2 (φ, ψ = -123°, 9°), C5 (φ, ψ = -142°, 166°), and β (φ, ψ = -59°, 158°). The β2 conformation becomes the lowest in energy. It also undergoes the largest geometric change, the N-terminal part becomes more perpendicular to the side chain (Fig. 1S). In contrast, for the C5 and β conformations, the values of the torsion angles φ and ψ remain almost unchanged.

The conformational map calculated in water, simulating a polar environment and an obvious biological solvent, also shows three conformations in the following order: α (φ, ψ = -89°, 2°), β (φ, ψ = -60°, 159°), and C5 (φ, ψ = -141°, 166°). The lowest energy conformation is α, which results from a further perpendicular rotation of the N-terminal amide group, while the side chain with the C-terminal part becomes planar. The internal interactions are the C-H...O hydrogen bond at the C-terminal part and electrostatic dipole-dipole interactions between the carbonyl groups of amides. The N-terminal part does not form any internal interactions, and therefore both the amide and the amino group of the side chain can form interactions with the polar environment. The energy difference between the α conformation and the remaining β and C5 conformations increases and is estimated to be 3 kcal/mol. This results in a relatively large population of the lowest energy state (estimated 99%).



For the *E* geometrical isomer, Ac-(*E*)-ΔAla(β-NHMe)-NHMe (**2**), the conformational pattern is shown in Table 3 and Fig. 4. For the isolated molecule (*in vacuo*), four conformations occur: β2, C7, C5, and β. The lowest energy is found in conformations with a low torsion angle ψ: the β2 conformation (φ, ψ = -105°, 2°), followed by the C7 conformation (φ, ψ = -68°, 14°). This can be explained by the presence of an N-H...O hydrogen bond between the N-H group of the side chain and the oxygen atom of the C-terminal amide (Table 2S). Moreover, in the case of the β2 conformation, the low value of the torsion angle ψ also indicates that the side chain and the C-terminal part are planar, and thus a conjugated π-cross system is formed (supported by an internal N-H...O hydrogen bond). For the C7 conformation, a C7-type N-H...O hydrogen bond can be found, as well as a carbonyl-carbonyl dipole interaction. The remaining conformations, C5 and β, have much higher energy, and the estimated population indicates that they are almost

inaccessible. The C5 conformation ($\varphi, \psi = -178^\circ, 165^\circ$) has N–H...O as well as C–H...O hydrogen bonds. Nevertheless, the repulsion between the side chain N–H groups and the C-terminal amide seems to play a significant role. This is also seen for the β conformation ($\varphi, \psi = -61^\circ, 152^\circ$), stabilized only by electrostatic dipole–dipole interactions.

In a weakly polar environment, mimicked by chloroform, three conformations can be observed in the following energetic order: α , C5, and β . The α conformation ($\varphi, \psi = -91^\circ, 0^\circ$) is the result of the combination of the $\beta 2$ and C7 conformations (Fig. 2S), with the same main stabilizing N–H...O hydrogen bond formed by the N–H group of the side chain. The geometry of the C5 ($\varphi, \psi = -174^\circ, 163^\circ$) and β ($\varphi, \psi = -61^\circ, 152^\circ$) conformation is almost unchanged compared to the conformations of the isolated molecule. Their relative energy increases further, so the α conformation becomes the only choice in a weakly polar environment.

In a polar environment, simulated by water as a solvent, four conformations are predicted: α ($\varphi, \psi = -87^\circ, -2^\circ$), β ($\varphi, \psi = -60^\circ, 156^\circ$), β' ($\varphi, \psi = -93^\circ, 160^\circ$), and C5 ($\varphi, \psi = -171^\circ, 162^\circ$). The α conformation has the lowest energy by far. The geometry and energy of the conformations are quite similar to those in a weakly polar environment. The influence of the polar environment causes the appearance of the β' conformation, which differs in the sign of the torsion angle ψ .

$$\text{Ac} - (Z) - \Delta\text{Ala}(\beta - \text{NHMe}) - \text{NMe}_2 \quad (3)$$

The aim of the analysis of N',N' -dimethylamides (3) and (4) is to determine the conformational properties of the β -aminodehydroalanine residue with a C-terminal tertiary amide group, as in the case of the naturally occurring peptides, Callynormine A and Callyaerins A–M.

For the geometric isomer Z, calculations performed for the isolated molecule (*in vacuo*) indicate five conformations: C5, $\beta 2$, C5', β , and α (Table 4, Fig. 5). The lowest in energy is the extended C5 conformation ($\varphi, \psi = -148^\circ, 162^\circ$), which has very similar geometry compared to the analogous one (1). It is also stabilized by two N–H...O hydrogen bonds: of the C5 type in the main chain and another one involving the N–H group of the side chain as a donor. The second in energy order is the semi-extended $\beta 2$ conformation ($\varphi, \psi = -135^\circ, 29^\circ$), stabilized by N–H...O and C–H...O hydrogen bonds, both involving the side chain. The torsion angle ψ is more distorted compared to analogous conformation in (1). This is due to the steric hindrance imposed by the C-terminal tertiary amide. The steric crowding that hinders the rotation of the C-terminal amide is also responsible for the appearance of a new conformation C5' ($\varphi, \psi = -144^\circ, -160^\circ$) (Fig. 3S), which is also stabilized by two N–H...O hydrogen bonds, but with worse geometric

parameters (Table 3S). The remaining conformations, β ($\varphi, \psi = -64^\circ, 150^\circ$) and α ($\varphi, \psi = -49^\circ, -27^\circ$), are stabilized by the carbonyl–carbonyl interactions, and their relative energy is much higher. The α conformation is also stabilized by a C–H...O hydrogen bond between the vinyl hydrogen of the side chain and the C-terminal oxygen atom.

In the weakly polar environment, mimicked by chloroform, also the same five conformations can be seen. Nevertheless, the environment changes the energy order. The conformation $\beta 2$ ($\varphi, \psi = -128^\circ, 31^\circ$) becomes the lowest in energy, with the estimated population over 83%. The second is the conformation C5 ($\varphi, \psi = -145^\circ, 161^\circ$), and then the conformation α ($\varphi, \psi = -55^\circ, -28^\circ$), β ($\varphi, \psi = -58^\circ, 151^\circ$), and C5' ($\varphi, \psi = -130^\circ, -155^\circ$). The changes in torsion angles φ, ψ in comparison to isolated molecules does not exceed $\pm 7^\circ$, except the conformation C5'.

In the polar environment of water, the number and type of conformations also do not change. The $\beta 2$ conformation ($\varphi, \psi = -125^\circ, 31^\circ$) maintains the lowest energy, although the α conformation ($\varphi, \psi = -57^\circ, -29^\circ$) is almost equally occupied. The β conformation ($\varphi, \psi = -96^\circ, 158^\circ$) is next in energetic order, followed by the extended C5 ($\varphi, \psi = -144^\circ, 161^\circ$) and C5' ($\varphi, \psi = -123^\circ, -153^\circ$) conformations. In general, increasing the polarity of the environment reduces the energy gap between the conformations. The geometry of the torsion angles φ and ψ also changes, more in the N-terminal part (φ), which becomes more perpendicular to the side chain and opens the N-terminal amide group to intermolecular interactions.

$$\text{Ac} - (E) - \Delta\text{Ala}(\beta - \text{NHMe}) - \text{NMe}_2 \quad (4)$$

In the case of the E geometric isomer, Ac-(E)- $\Delta\text{Ala}(\beta - \text{NHMe}) - \text{NMe}_2$ (4), there are four conformations for the isolated molecule (*in vacuo*): $\beta 2$, α , C5, and β (Table 5, Fig. 6). The lowest energy conformation is $\beta 2$ ($\varphi, \psi = -114^\circ, 21^\circ$) followed by α ($\varphi, \psi = -60^\circ, -17^\circ$). The energy difference is relatively small and together these two conformations occupy almost the entire population of molecules. Moreover, both conformations are stabilized by a N–H...O hydrogen bond between the N–H group of the side chain and the oxygen atom of the C-terminal amide (Table 4S). The remaining conformations, C5 and β , have much higher energies and the estimated population indicates that they are almost inaccessible. The C5 conformation ($\varphi, \psi = -171^\circ, 159^\circ$) has N–H...O as well as C–H...O hydrogen bonds. The β conformation ($\varphi, \psi = -61^\circ, 152^\circ$) is stabilized only by electrostatic dipole–dipole interactions.

In a weakly polar environment, mimicked by chloroform, five conformations can be observed in the following energy order: α , $\beta 2$, β , C5, and β' . Conformations α ($\varphi, \psi = -91^\circ, 0^\circ$) and $\beta 2$ ($\varphi, \psi = -114^\circ, 21^\circ$) remain the lowest energies,

with the same main stabilizing N–H...O hydrogen bond formed using the side chain N–H group and almost the same population. Nevertheless, conformation α is predicted as a global minimum. Conformations C5 ($\phi, \psi = -154^\circ, 155^\circ$) and β ($\phi, \psi = -58^\circ, 147^\circ$) have higher energies, similar to those of the isolated molecule. Additionally, due to the influence of the environment, as in the case of the C-terminal secondary amide analogue (**2**), the β' conformation appears ($\phi, \psi = -99^\circ, 157^\circ$).

Further increase in the polarity of the environment, as mimicked by water, does not seem to have a significant effect on the number and type of conformations, as well as on their energetic order. The conformations α ($\phi, \psi = -62^\circ, -22^\circ$) and β_2 ($\phi, \psi = -113^\circ, 21^\circ$) have the lowest energy, although the α conformation is more occupied. The remaining conformations have higher energies and the energetic order is as follows: β ($\phi, \psi = -57^\circ, 148^\circ$), β' ($\phi, \psi = -93^\circ, 155^\circ$), and C5 ($\phi, \psi = -139^\circ, 155^\circ$).

The presented results obtained using quantum chemical calculations show that the studied β -aminodehydroalanine residue, regardless of the Z/E isomer or the C-terminal $2^\circ/3^\circ$ amide, tends to adopt mainly two conformations, α and β_2 . The β_2 conformation is rather preferred in low polarity environments, while increasing polarity favors the α conformation.

Both conformations, α and β_2 , are also typical for dehydroamino acids with a nonpolar side chain (Siodlak et al. 2010). This indicates that their presence is rather due to the geometry of the residue imposed by the $C\alpha=C\beta$ bond. However, they have rather higher energies or the energy gap between the α and β_2 conformations and the other conformations is relatively small. A similar pattern can be observed for the previously studied Δ Ala(β -Cl) (Banaś et al. 2023) and Δ Ser(Me) (Banaś et al. 2025) with a heteroatom in the β position (Cl or O, respectively). In the case of dehydroamino acids with a C-terminal tertiary amide, steric hindrance causes preferences mainly towards the β (H/F) conformation (Siodlak et al. 2004).

The low energies of the α and β_2 conformations of the studied Δ Ala(β -NH) residue are due to the presence of an internal N–H...O hydrogen bond, which is formed in the residue between the N–H group of the side chain, which acts as a hydrogen bond donor, and the oxygen atom of the adjacent N- or C-terminal amide group, which acts as a hydrogen bond acceptor. This is particularly evident in the case of the E isomers (**2**) and (**4**). In the case of the Z isomer, the β_2 conformation has an N–H...O hydrogen bond and therefore predominates in a low-polarity environment. In a more polar environment, intermolecular interactions are energetically more favorable, which results in the opening of the N–H group and a perpendicular position of the N-terminal part. However, an internal $C\beta$ -H...O hydrogen bond is present,

which stabilizes the α conformation. It should also be noted that in the α and β_2 conformations, the side chain and the C-terminal amide bond tend to be in the plane, which can be observed by the torsion angle ψ close to 0° with a deviation not exceeding $\pm 30^\circ$. Therefore, a delocalized system of cross-conjugated π electrons is formed in this part, which additionally stabilizes the conformations.

There are only a few literature reports that present experimental evidence for conformational preferences of the studied structural element. The X-ray structure of Callynormine A (Berer et al. 2004) shows the Z isomer and the α conformation ($\phi, \psi = -49.39^\circ, -32.73^\circ$). Also a closely related analogue, a dipeptide with an N-terminal benzamide group and a C-terminal proline with 4,6-dimethyl-2-pyrimidinylamine group, shows the Z isomer and the α conformations ($\phi, \psi = -62.68^\circ, -39.65^\circ$) and ($\phi, \psi = -73.42^\circ, -44.60^\circ$) (Djinović-Carugo et al. 1994). This confirms the presented results.

Synthesis

The formation of the β -aminodehydroalanine structural element is a key step to access the natural compounds described above and is problematic in terms of synthesis. A review of the literature shows the following synthetic approaches (Fig. 7a–c).

The first approach is based on acetal-protected precursors. In this case, 2-amino-3,3-diethoxypropionic acid ((R)- α -formylglycine diethylacetal) is introduced into the peptide chain (DeMong and Williams 2002, 2003). Then, by acid hydrolysis, it is converted into α -formylglycine (fGly), which in the next step reacts with the amino group to obtain the desired product (Zhang et al. 2018). Although the reaction progresses efficiently (yield over 80%), it was observed that the fGly-containing peptide was unstable under the acidic conditions used for peptide cleavage. Protonation of the aldehyde group of fGly increases its electrophilic character, which paves the way for nucleophilic attack by the adjacent N-terminal amide group oxygen atom, forming an oxazoline ring, which further undergoes acid hydrolysis via an acyl-type substitution at the oxazoline C2 carbon atom and ultimately leads to cleavage of the peptide chain.

Another approach with acetal protection involves introducing 1,2-diol-functionalized (acetalprotected) 4-hydroxy-L-threonine (hThr) into the peptide chain Yates et al. 2023). This is then converted to a vicinal diol by acidic cleavage. In the next step, sodium periodate is used, and oxidative cleavage produces α -formylglycine, which then reacts with the amine under acidic conditions.

In the third approach, alternatively, a standard serine residue is used as a precursor. Then, in a one-pot oxidation using Dess-Martin periodinane, α -formylglycine is formed.

Simultaneous cyclization leads to the final product (Podlesainski et al. 2024).

As can be seen, the procedures presented in the literature always involve the formation of α -formylglycine, which is probably the mechanism of formation of this type of compounds in biological systems (Krüger et al. 2019). Then fGly reacts with an amine, forming first an imine and then an enamine (a functional structural element of an β -aminodehydroamino acid). Acidic conditions are required to remove the oxygen atom in the form of a water molecule. However, fGly is susceptible to these conditions. Furthermore, in the first two methods (Fig. 7a and b), an appropriately protected, specifically synthesized α -formylglycine building block must be incorporated into the peptide chain. The third method starts directly from a serine residue, which is advantageous, but the yield is not high.

In this work, we propose a different approach to the synthesis of the target structural element, β -aminodehydroalanine, which proceeds via dehydration of serine, followed by bromination in the β -position and finally by substitution of bromine as a leaving group by an amine (Fig. 7d). The dehydration reaction gives high yields and there are examples of its application in peptide synthesis (Slootweg et al. 2015). The β -bromination reaction proceeds via the addition of two bromine atoms in the vicinal position, followed by a dehydrohalogenation reaction using a tertiary amine (Bull et al. 2007; Kishi 1975). In our hands, the use of bromine works (Lenartowicz et al. 2017). It should be noted, however, that a variant of this method using NBS has been described in the literature (Ferreira and Monteiro 2006; Ferreira et al. 2007) and higher yields have been reported. In the final step, the β -bromodehydroamino acid residue undergoes a Michael addition reaction with an amine, followed by a dehydrohalogenation reaction, which regenerates the C=C double bond.

A simple diamide model, Ac-(Z)- Δ Ala(β -NHMe)-NHMe was synthesized starting from Ac-Ser-NHMe, because it corresponds to the models used in quantum chemical calculations. In this case, methylamine was used in the final steps. To show the wider application of this method, the dipeptide model Boc-Gly-(Z)- Δ Ala(β -NHMe)-OMe was obtained. The reaction yield is rather low, mainly due to the volatility of the methylamine reagent. Better results were obtained for Boc-Gly-(Z)- Δ Ala(β -LeuOMe)-OMe using a leucine derivative (H-Leu-OMe), which is an amino acid at this site in Callyaerins. Unfortunately, all products were obtained as a frost (a solid resembling frost on glass) and therefore unsuitable for X-ray analysis of single crystals. In our hands, the total yield of these 3 steps (27%) estimated for Boc-Gly-(Z)- Δ Ala(β -LeuOMe)-OMe is comparable to the results known from the literature (Podlesainski et al. 2024).

In summary, the starting substrate is a serine residue, a standard amino acid, which is incorporated into the peptide chain under well-developed conditions. The intermediate α -formylglycine is avoided. Moderately basic conditions are also used instead of acidic ones.

Conclusions

β -Aminodehydroalanine is a unique naturally occurring dehydroamino acid residue that has been found to date in Callynormine A as well as in Callyaerins A-M, revealing potential antitubercular activity. A closely related dehydroamino acid, β -ureidodehydroalanine, occurs in Capreomycin and Tuberactinomycin, which are known for their antitubercular activity. The similar structural element found in peptides with similar activity indicates that, in general, the enamine functional group in the side chain of the amino acid residue is inherently associated with the biological properties of these peptides.

The presented studies show that the N-H group in the β position influences the conformation of the β -aminodehydroalanine residue by forming internal N-H...O hydrogen bonds with the flanking amide groups. As a result, the global minimum of the potential energy is located in the region for torsion angles φ and ψ between -137° to -55° and 30° to -30° , respectively. This region is occupied by two conformations designated as β_2 ($\varphi, \psi \sim -120^\circ, 20^\circ$) and α ($\varphi, \psi \sim -70^\circ, -15^\circ$).

Regardless of the geometric type of the Z/E isomer and the C-terminal amide (secondary/tertiary), there is a tendency that the β_2 conformation is rather favored in a lower polar environment, while an increase in the polarity of the environment favors the α conformation, which is more open to intermolecular interactions. The C-terminal tertiary amide decreases the relative energy between conformations, mainly due to steric crowding. In general, the Z isomer is more stable. The X-ray structure of Callynormin A (Berer et al. 2004) with the Z isomer and α conformations ($\varphi, \psi = -49.39^\circ, -32.73^\circ$) confirms these predictions.

Literature reports on the synthesis of β -aminodehydroalanine units show a pathway via an α -formylglycine residue and acidic conditions. Here we present an alternative 3-step synthetic route, starting from a serine residue that undergoes dehydration using EDC/CuCl, followed by subsequent bromination at the β -position and finally substitution with an amine, using mild basic conditions. In this way, a method is proposed to avoid the step of forming an α -formylglycine residue.

We believe that the presented results extend both the knowledge of the structural properties of this unique dehydroamino acid and the range of available synthetic methods.

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Author contributions K.B. prepared quantum chemical calculations, K.B. and P.L. synthesized model compounds, K.B. and D.S. wrote manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interests The authors declare no competing interests.

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Insight into the structure of antituberculosis Callyaerins. Conformational studies and synthesis of unique dehydroamino acid, β -aminodehydroalanine

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Quantum Chemical Calculations

Table 1S. Structural parameters for the internal hydrogen bond (X–H···A) and carbonyl interactions (C=O ◀···▶ O=C) in the conformers of Ac-(Z)-ΔAla(β-NHMe)-NHMe (**1**) in various environment calculated at M06-2X/6-311+G(d,p).

Ac-(Z)-ΔAla(β-NHMe)-NHMe (1)										
Structural parameters	β2		C5			β			C7	α
	Gas Phase	Chloroform	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Water
Hydrogen bond										
N ^N -H···O-C ^C										
<i>r</i> H···O			2.16	2.21	2.23					
<i>r</i> N···C			2.39	2.40	2.41					
∠N-H···O			107.4	105.0	104.0					
∠C-O···H			85.9	85.8	85.8					
N ^C -H···O-C ^N										
<i>r</i> H···O									2.08	
<i>r</i> N···C									3.15	
∠N-H···O									146.1	
∠C-O···H									91.8	
N ^β -H···O-C										
<i>r</i> H···O	2.02	2.35	1.88	1.95	1.98					
<i>r</i> N···C	3.18	3.21	3.19	3.18	3.18					
∠N-H···O	136.8	121.4	143.1	139.7	138.2					
∠C-O···H	98.3	85.0	106.1	102.0	100.3					
C ^β -H···O-C ^C										
<i>r</i> H···O	2.38	2.42							2.41	2.46
<i>r</i> C···O	2.79	2.80							2.78	2.81
∠C-H···O	100.4	98.8							97.8	97.3
∠C-O···H	82.2	82.4							83.1	82.7
C=O ◀···▶ O=C dipole interactions										
<i>r</i> O ^N ···C ^C						3.10	3.07	3.09	3.34	3.52
<i>r</i> O ^C ···C ^N						3.14	3.15	3.17		
<i>r</i> C ^N ···C ^C						3.02	3.05	3.07	3.31	3.37
<i>r</i> O ^N ···O ^C						3.41	3.29	3.27	4.49	4.45
∠(C=O) ^N ···C ^C						74.6	77.6	78.0	78.0	73.3
∠(C=O) ^C ···C ^N						73.1	74.2	74.5	10.4	21.8
∠O ^C ···(C=O) ^N						92.4	85.5	83.8	81.8	80.4
∠O ^N ···(C=O) ^C						93.7	88.9	87.4	157.7	133.0

Data presented only for X–H···A (X = N, C; A = O) in which *r* H···X ≤ 2.7 Å and ∠X–H···A > 90° [Vargas et al. *J. Phys. Chem. A* **2002**, 106, 3213-3218].

Data presented only for the C=O ◀···▶ O=C contacts in which *r* C···O < 3.6 Å [Allen et al. *Acta Crystallogr. B* **1998**, 54, 320-329].

^{N, C} denote the N-terminal or the C-terminal amide group;

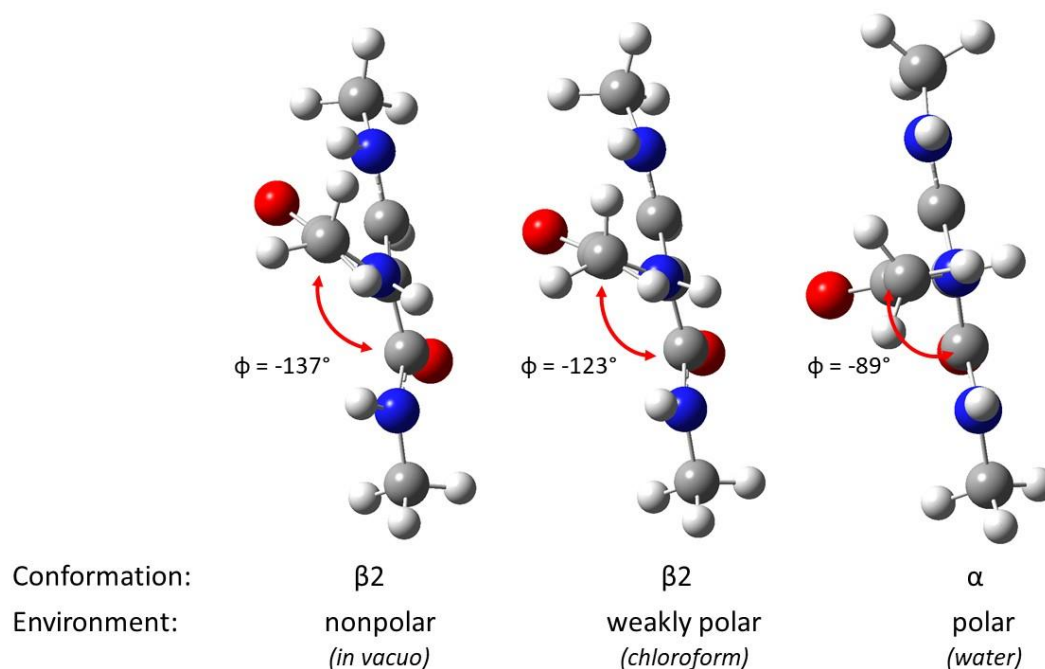


Figure 1S. Visualization of conformational changes occurring due to the increase in the polarity of the environment for Ac-(Z)- Δ Ala(β -NHMe)-NHMe (**1**). As the polarity of the environment increases, the value of the torsion angle ϕ decreases. The N-terminal amide becomes more perpendicular towards the remaining part of the molecule. The side chain and C-terminal amide maintains relatively flat, due to the C-H...O hydrogen bond as well as a π -electron cross-conjugation. The conformation $\beta 2$, which is seen for isolated molecule (*in vacuo*), and which prevails in weakly polar environment, fluently changes in the conformation α , which is more open for intermolecular interactions.

Table 2S. Structural parameters for the internal hydrogen bond (X–H···A) and carbonyl interactions (C=O ◀···▶ O=C) in the conformers of Ac-(E)-ΔAla(β-NHMe)-NHMe (**2**) in various environment calculated at M06-2X/6-311+G(d,p).

Ac-(E)-ΔAla(β-NHMe)-NHMe (2)											
Structural parameters	α		C5			β			β2	C7	β'
	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Gas Phase	Water
Hydrogen bond											
N^N-H···O-C^C											
<i>r</i> H···O			2.11	2.13	2.13						
<i>r</i> N···C			2.38	2.38	2.38						
∠N-H···O			109.6	109.2	109.0						
∠C-O···H			85.7	85.4	85.4						
N^C-H···O-C^N											
<i>r</i> H···O										2.15	
<i>r</i> N···C										3.15	
∠N-H···O										141.9	
∠C-O···H										87.8	
N^β-H···O-C											
<i>r</i> H···O	1.98	1.99							1.94	1.93	
<i>r</i> N···C	2.72	2.96							2.93	2.93	
∠N-H···O	128.1	127.6							129.3	128.9	
∠C-O···H	105.0	104.9							104.8	105.7	
C^β-H···O-C^N											
<i>r</i> H···O			2.12	2.14	2.16						
<i>r</i> C···O			2.85	2.87	2.88						
∠C-H···O			122.0	122.0	121.6						
∠C-O···H			104.2	103.5	102.9						
C=O ◀···▶ O=C dipole interactions											
<i>r</i> O ^N ···C ^C	3.54	3.48				3.04	3.05	3.04		3.29	3.60
<i>r</i> O ^C ···C ^N						3.17	3.17	3.17			3.36
<i>r</i> C ^N ···C ^C	3.38	3.34				3.00	3.03	3.04		3.27	3.33
<i>r</i> O ^N ···O ^C	4.50	4.43				3.39	3.32	3.28		4.42	3.65
∠(C=O) ^N ···C ^C	72.3	73.2				76.3	77.7	78.6		78.4	67.8
∠(C=O) ^C ···C ^N	21.2	21.4				70.8	72.4	72.8		11.8	78.2
∠O ^C ···(C=O) ^N	81.9	80.4				90.2	86.1	83.8		79.8	93.5
∠O ^N ···(C=O) ^C	134.2	133.4				96.1	91.7	89.9		151.9	82.5
Data presented only for X–H···A (X = N, C; A = O) in which <i>r</i> H···X ≤ 2.7 Å and ∠X–H···A > 90° [Vargas et al. <i>J. Phys. Chem. A</i> 2002 , 106, 3213-3218].											
Data presented only for the C=O ◀···▶ O=C contacts in which <i>r</i> C···O < 3.6 Å [Allen et al. <i>Acta Crystallogr. B</i> 1998 , 54, 320-329].											
^{N, C} denote the N-terminal or the C-terminal amide group;											

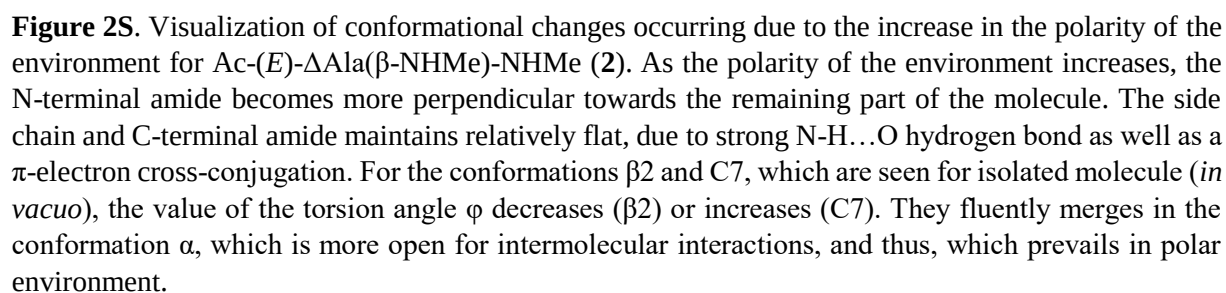


Table 3S. Structural parameters for the internal hydrogen bond (X–H···A) and carbonyl interactions (C=O ◀···▶ O=C) in the conformers of Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (**3**) in various environment calculated at M06-2X/6-311+G(d,p).

Structural parameters	Ac-(Z)-ΔAla(β-NHMe)-NMe ₂ (3)														
	β2			C5			α			β			C5'		
	Gas Phase	Chlorof orm	Water	Gas Phase	Chlorof orm	Water	Gas Phase	Chlorof orm	Water	Gas Phase	Chlorof orm	Water	Gas Phase	Chlorof orm	Water
Hydrogen bond															
N^N-H···O-C^C															
<i>r</i> H···O				2.16	2.20	2.22							2.22	2.53	2.71
<i>r</i> N···C				2.39	2.40	2.41							2.39	2.42	2.44
∠N-H···O				107.2	105.4	104.7							103.6	89.3	81.3
∠C-O···H				86.3	86.3	86.3							82.1	76.2	73.0
N^β-H···O-C															
<i>r</i> H···O	2.09	2.22	2.36	1.90	1.97	2.00							1.87	2.00	2.14
<i>r</i> N···C	3.19	3.21	3.22	3.18	3.18	3.18							3.24	3.20	3.19
∠N-H···O	136.7	127.7	121.2	142.4	138.9	137.3							142.4	134.8	127.8
∠C-O···H	98.3	90.1	85.0	104.2	100.4	98.9							109.9	100.2	93.1
C^β-H···O-C^C															
<i>r</i> H···O	2.47	2.50	2.51				2.44	2.49	2.52						
<i>r</i> C···O	2.82	2.83	2.83				2.77	2.80	2.81						
∠C-H···O	97.5	96.0	95.4				95.4	94.6	94.2						
∠C-O···H	79.3	78.9	79.2				82.0	80.7	80.0						
C=O ◀···▶ O=C dipole interactions															
<i>r</i> O ^N ···C ^C							3.05	3.08	3.09	3.11	3.02	3.04			
<i>r</i> O ^C ···C ^N										3.24	3.18	3.20			
<i>r</i> C ^N ···C ^C							3.15	3.15	3.15	3.03	3.03	3.05			
<i>r</i> O ^N ···O ^C							3.83	3.81	3.79	3.55	3.33	3.32			
∠(C=O) ^N ···C ^C							83.3	81.8	81.4	74.9	78.6	78.9			
∠(C=O) ^C ···C ^N							29.7	31.5	32.3	69.0	71.5	71.9			
∠O ^C ···(C=O) ^N							66.3	66.2	66.0	94.5	86.1	84.8			
∠O ^N ···(C=O) ^C							121.0	117.2	115.7	100.9	93.5	92.0			

Data presented only for X–H···A (X = N, C; A = O) in which *r* H···X ≤ 2.7 Å and ∠X–H···A > 90° [Vargas et al. *J. Phys. Chem. A* **2002**, 106, 3213-3218].

Data presented only for the C=O ◀···▶ O=C contacts in which *r* C···O < 3.6 Å [Allen et al. *Acta Crystallogr. B* **1998**, 54, 320-329].

^{N, C} denote the N-terminal or the C-terminal amide group;

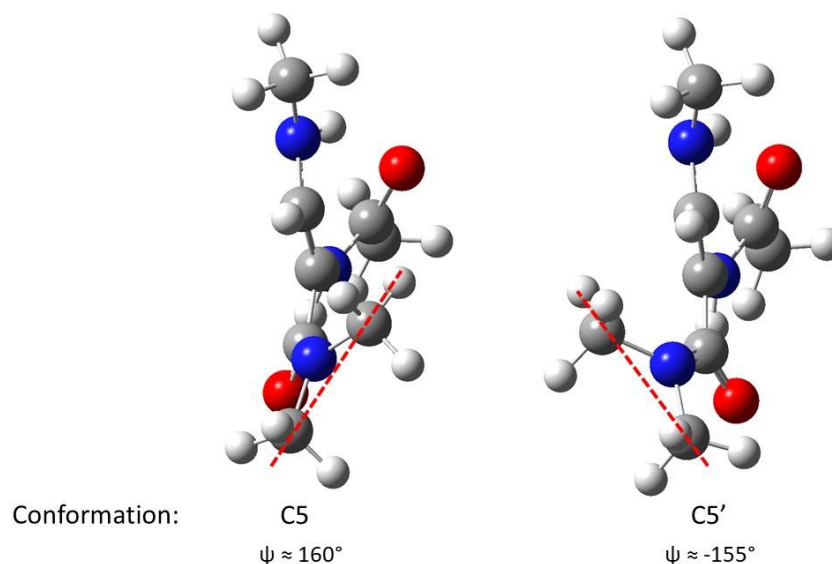


Figure 3S. Visualization of the conformational C5 and C5' for Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (**3**). The steric crowding hinders the rotation of the C-terminal amide results in the division of the extended conformation region into two parts and appearance of the new conformation C5', which is also stabilized by two N–H···O hydrogen bonds, but has a worse geometrical parameters.

Table 4S. Structural parameters for the internal hydrogen bond ($X-H\cdots A$) and carbonyl interactions ($C=O \cdots O=C$) in the conformers of Ac-(*E*)- Δ Ala(β -NHMe)-NMe₂ (**4**) in various environment calculated at M06-2X/6-311+G(d,p).

Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4)														
Structural parameters	α			β ₂			β			C5			β [*]	
	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Gas Phase	Chloroform	Water	Chloroform	Water
Hydrogen bond														
N ^N -H...O-C ^C														
r H...O										2.17	2.24	2.34		
r N...C										2.39	2.40	2.42		
∠N-H...O										107.6	104.9	100.5		
∠C-O...H										84.7	84.7	84.4		
N ^B -H...O-C														
r H...O	1.91	1.98	2.01	1.97	2.00	2.01								
r N...C	2.93	2.95	2.96	2.93	2.95	2.96								
∠N-H...O	128.2	126.4	125.6	127.7	126.3	125.8								
∠C-O...H	107.0	105.5	104.4	103.9	104.2	104.4								
C ^B -H...O-C ^N														
r H...O										2.16	2.32	2.58		
r C...O										2.86	2.92	3.01		
∠C-H...O										120.4	113.2	102.8		
∠C-O...H										101.9	96.6	88.4		
C=O ◀...▶ O=C dipole interactions														
r O ^N ...C ^C	3.17	3.16	3.15				3.01	2.98	2.97				3.71	3.62
r O ^C ...C ^N							3.25	3.22	3.21				3.40	3.34
r C ^N ...C ^C	3.19	3.18	3.17				2.99	3.00	3.01				3.38	3.35
r O ^N ...O ^C	3.99	3.95	3.92				3.48	3.35	3.30				3.77	3.59
∠(C=O) ^N ...C ^C	79.8	79.7	79.7				77.3	79.5	80.3				65.0	67.5
∠(C=O) ^C ...C ^N	27.7	29.1	29.8				67.0	68.9	69.8				78.7	79.7
∠O ^C ...(C=O) ^N	70.1	69.4	68.8				90.9	85.6	83.3				97.9	91.4
∠O ^N ...(C=O) ^C	123.2	120.8	119.5				102.5	97.0	94.4				83.2	78.7
Data presented only for X-H...A (X = N, C; A = O) in which r H...X ≤ 2.7 Å and ∠X-H...A > 90° [Vargas et al. <i>J. Phys. Chem. A</i> 2002 , 106, 3213-3218].														
Data presented only for the C=O ◀...▶ O=C contacts in which r C...O < 3.6 Å [Allen et al. <i>Acta Crystallogr. B</i> 1998 , 54, 320-329].														
N, C denote the N-terminal or the C-terminal amide group:														

Table 5S. XYZ structures of the calculated compounds, Ac-(Z)- Δ Ala(β -NHMe)-NHMe (**1**), Ac-(E)- Δ Ala(β -NHMe)-NHMe (**2**), Ac-(Z)- Δ Ala(β -NHMe)-NMe₂ (**3**), and Ac-(E)- Δ Ala(β -NHMe)-NMe₂ (**4**) along with their electronic energies.

Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1)			
gas phase			
Conformation C5	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22045781 Electronic_Energy_with_ZeroPoint_Correction= -589.00988711 Gibbs Free Energy= -589.0512966 HTot= -588.9948448 LowF= 55.1466 64.1898 82.5075 NImag= 0		
	N	-2.61008300	0.38606900 -0.32073400
	H	-2.33228400	1.08900400 -0.98565100
	C	-3.98905500	-0.07113300 -0.35257100
	H	-4.62997600	0.76529600 -0.62732000
	H	-4.13033600	-0.89114500 -1.06176300
	H	-4.27167100	-0.42769500 0.63676500
	C	-1.64207300	-0.46450500 0.13584400
	O	-1.91900300	-1.57723400 0.56373100
	C	-0.23870600	0.00982800 0.13087700
	N	0.66611300	-1.06271500 0.31866800
	H	0.21522700	-1.87800500 0.71780200
	C	1.90444100	-1.20459300 -0.21429300
	O	2.46286700	-0.33133900 -0.86575700
	C	2.58423200	-2.52609800 0.06377800
	H	3.47017600	-2.33685300 0.67156500
	H	1.94419200	-3.24391100 0.57554300
	H	2.91713300	-2.94265200 -0.88690600
	C	0.07968300	1.32601800 0.16876400
	H	-0.74308800	2.03430100 0.13475900
	N	1.30690600	1.88085200 0.32627500
	H	2.07338400	1.29598900 0.00026000
	C	1.47351900	3.31346400 0.16214800
	H	1.42635100	3.62649900 -0.88727700
	H	0.69835100	3.83958700 0.72164700
	H	2.43882600	3.61084200 0.57003900
Conformation β 2	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) gas phase β 2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21851331 Electronic_Energy_with_ZeroPoint_Correction= -589.00802681 Gibbs Free Energy= -589.0495503 HTot= -588.9929231 LowF= 57.1359 62.9262 69.0365 NImag= 0		
	N	2.46574800	0.40443000 0.04271100
	H	2.06269400	1.25402600 0.40679400
	C	3.87378500	0.15873200 0.30153400
	H	4.41504500	1.10205600 0.24148700
	H	4.03637500	-0.29646900 1.28258600
	H	4.25831800	-0.52486500 -0.45361600
	C	1.62224300	-0.67366500 -0.08726700
	O	2.05131300	-1.81476700 -0.12701400
	C	0.18537800	-0.34159000 -0.21414000
	N	-0.15703200	1.01821200 -0.45881200
	H	0.35424400	1.47774300 -1.19974400
	C	-1.15865300	1.72787500 0.13473500
	O	-1.89095000	1.25628600 0.98742200
	C	-1.32394600	3.15184700 -0.35093700
	H	-1.42153600	3.79775600 0.52100400
	H	-0.49968500	3.49837500 -0.97456700
	H	-2.25282700	3.21974000 -0.91970300
	C	-0.70303400	-1.35953900 -0.13550000
	H	-0.26762800	-2.34401800 0.01069700
	N	-2.04915100	-1.30184100 -0.24656400
	H	-2.46009900	-0.40709800 -0.00652900
	C	-2.85180700	-2.46831300 0.08257100
	H	-3.84292900	-2.36020200 -0.35691800
	H	-2.38786100	-3.35732200 -0.34527300
	H	-2.95776000	-2.60957100 1.16317800
Conformation C7	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) gas phase C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21579433 Electronic_Energy_with_ZeroPoint_Correction= -589.00557463 Gibbs Free Energy= -589.0476589 HTot= -588.9903262 LowF= 49.5237 52.8535 63.3913 NImag= 0		
	N	-2.11433300	-0.62215000 -0.04859500
	H	-2.09030700	0.28045800 0.40850400
	C	-3.30639500	-1.43230200 0.13028800
	H	-4.17917300	-0.78107100 0.11498700
	H	-3.37939600	-2.15068900 -0.68512900
	H	-3.28272600	-1.99110900 1.07031000
	C	-0.91460800	-1.25894300 -0.17610600
	O	-0.82504600	-2.47563600 -0.24175200

	C 0.28874500 -0.38632200 -0.24444800 N 0.22706500 0.99906000 -0.58742800 H 0.53163000 1.24928200 -1.51869400 C -0.35743900 1.98370500 0.15788400 O -0.89185400 1.76878200 1.22902800 C -0.32791600 3.36747200 -0.45384400 H -0.05996300 4.07940200 0.32560400 H 0.36540500 3.45756200 -1.29025000 H -1.33418900 3.61339300 -0.79805900 C 1.49661700 -0.95952100 -0.06883800 H 1.52287900 -2.03092400 0.10220000 N 2.69585300 -0.32420400 -0.13975800 H 2.65563700 0.68481500 -0.10403300 C 3.89962200 -0.94479300 0.38575200 H 4.77560900 -0.45028400 -0.03301700 H 3.95442100 -0.90044500 1.47836000 H 3.92352100 -1.99027500 0.07734800
Conformation β	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21162426 Electronic_Energy_with_ZeroPoint_Correction= -589.02383923 Gibbs Free Energy= -589.0444663 HTot= -588.9863755 LowF= 29.5141 57.6605 67.4647 NImag= 0 N 1.41394800 -1.87061300 0.28367400 H 0.82456100 -2.06098600 1.07916700 C 2.78524000 -2.34922500 0.34465800 H 2.79546800 -3.33362700 0.81065000 H 3.17981100 -2.42937000 -0.66711900 H 3.42918800 -1.66759100 0.90699500 C 1.16205800 -0.69456700 -0.37498200 O 2.02876100 -0.08832000 -0.97879000 C -0.24021500 -0.20614400 -0.34264900 N -0.41265600 1.19188800 -0.54765700 H -0.38142800 1.49846800 -1.51261800 C 0.17709100 2.05926900 0.36032000 O 0.48786200 1.70575300 1.47304400 C 0.39457600 3.46231200 -0.15465800 H 0.46556500 4.14432100 0.68932000 H 1.34042200 3.47782700 -0.70220800 H -0.40216900 3.77953100 -0.82909800 C -1.31577500 -1.00605500 -0.21868300 H -1.17884100 -2.07992800 -0.13361100 N -2.61176800 -0.58612700 -0.26946700 H -2.72700400 0.41513400 -0.18713500 C -3.68068900 -1.41662500 0.25330500 H -4.63789000 -1.04881500 -0.11486300 H -3.55308000 -2.43698800 -0.11169400 H -3.70797000 -1.43726900 1.34845600
chloroform	
Conformation β_2	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) chloroform β_2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23353593 Electronic_Energy_with_ZeroPoint_Correction= -589.00802681 Gibbs Free Energy= -589.0663397 HTot= -589.0084037 LowF= 42.2492 55.5940 60.4216 NImag= 0 N -2.45038500 0.31907200 -0.01677700 H -2.07423100 1.24732800 0.09305600 C -3.85930900 0.09673600 0.24915600 H -4.38567900 1.04477000 0.15722400 H -4.26579100 -0.60861400 -0.47548000 H -4.02036600 -0.30969800 1.25094100 C -1.58445100 -0.72739500 -0.07270400 O -1.98017300 -1.88993600 0.01342500 C -0.16378200 -0.37702800 -0.26272900 N 0.17483100 0.97366900 -0.55823900 H -0.20370100 1.37760200 -1.40475000 C 1.02959700 1.74600300 0.17273700 O 1.60063200 1.32880700 1.16857800 C 1.25182300 3.14695700 -0.34652700 H 2.27230700 3.21838900 -0.72690600 H 0.55673200 3.42825300 -1.13652400 H 1.15706800 3.84037600 0.48862300 C 0.76548700 -1.35726100 -0.14503000 H 0.39533200 -2.35284100 0.07849900 N 2.09725100 -1.23944500 -0.29858000 H 2.47729000 -0.30371500 -0.26989200 C 3.00431600 -2.30971900 0.08584100 H 3.92716800 -2.22637900 -0.48660100 H 3.24375500 -2.28022000 1.15256600 H 2.54247500 -3.26904700 -0.14608500

Conformation C5	Ac-(Z)-ΔAla(β-NHMe)-NHMe (1) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23281528 Electronic_Energy_with_ZeroPoint_Correction= -589.02278268 Gibbs Free Energy= -589.0643138 HTot= -589.0076169 LowF= 58.9640 68.0893 82.0463 NImag= 0 N 2.60414700 0.36824700 0.34278300 H 2.33305800 1.12182200 0.95318700 C 3.99429300 -0.05468900 0.35095800 H 4.60985800 0.78325000 0.67151200 H 4.15387400 -0.90018000 1.02473800 H 4.29417900 -0.35359100 -0.65300300 C 1.64313800 -0.46330300 -0.13276000 O 1.92012200 -1.57709000 -0.57871400 C 0.24132800 0.01080900 -0.13623600 N -0.67684300 -1.04845800 -0.34986500 H -0.26172900 -1.84644800 -0.81609800 C -1.88794900 -1.21065900 0.23126000 O -2.39614500 -0.36470900 0.96411600 C -2.60394800 -2.49909500 -0.09356400 H -3.52413200 -2.25814300 -0.62789300 H -2.00769900 -3.18212200 -0.69648500 H -2.87917900 -2.98330600 0.84370700 C -0.08259500 1.32763600 -0.16535700 H 0.72857500 2.04728300 -0.10956000 N -1.31198900 1.86809600 -0.33497100 H -2.08284400 1.27908900 -0.03522300 C -1.50359600 3.30255500 -0.18276200 H -2.45246000 3.58599200 -0.63566200 H -1.50764100 3.61254900 0.86713600 H -0.70690400 3.83348300 -0.70443000
Conformation β	Ac-(Z)-ΔAla(β-NHMe)-NHMe (1) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22854993 Electronic_Energy_with_ZeroPoint_Correction= -589.01907843 Gibbs Free Energy= -589.061414 HTot= -589.0036325 LowF= 34.6351 42.3699 50.5025 NImag= 0 N -1.77123300 -1.56597100 0.27662800 H -1.20975000 -1.97841800 1.00443000 C -3.19830000 -1.83633000 0.28167600 H -3.36779100 -2.80797300 0.74160500 H -3.75195200 -1.07235600 0.83365600 H -3.57134200 -1.85756200 -0.74191200 C -1.29079300 -0.45594200 -0.34875600 O -2.03072300 0.31743700 -0.94717700 C 0.17457200 -0.24356800 -0.29358100 N 0.63130100 1.08456100 -0.49806800 H 0.96723500 1.33774800 -1.41840500 C 0.23174000 2.10239000 0.32507300 O -0.33342400 1.89429900 1.38383000 C 0.53074600 3.49457500 -0.17534700 H 0.73700300 4.14118800 0.67523600 H 1.36819600 3.51702800 -0.87248500 H -0.35861300 3.87022500 -0.68678800 C 1.07328900 -1.24623700 -0.16832100 H 0.72802100 -2.27368800 -0.10836700 N 2.41959300 -1.09374400 -0.19099700 H 2.75710800 -0.14548200 -0.09422500 C 3.31403500 -2.16023100 0.22809800 H 4.30585300 -1.98161000 -0.18454100 H 3.39153000 -2.23543700 1.31696100 H 2.94831600 -3.10940800 -0.16440000
water	
Conformation α	Ac-(Z)-ΔAla(β-NHMe)-NHMe (1) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23926859 Electronic_Energy_with_ZeroPoint_Correction= -589.03020709 Gibbs Free Energy= -589.074149 HTot= -589.0144685 LowF= 47.8529 60.5293 67.5080 NImag= 0 N -2.31298500 -0.21434700 -0.25884200 H -2.14133900 0.72130900 -0.59002400 C -3.67747900 -0.67300400 -0.08478000 H -4.35241000 0.13637900 -0.35428300 H -3.88237800 -1.53559200 -0.72188800 H -3.86272100 -0.95980400 0.95220900 C -1.25948000 -1.02901900 -0.01556800 O -1.42581100 -2.19239000 0.36973400 C 0.07292400 -0.44876200 -0.23930100 N 0.20576500 0.91005200 -0.64880400 H 0.27304400 1.12232900 -1.63560200 C 0.30541100 1.94936400 0.23367300 O 0.25712300 1.78744600 1.44261600 C 0.47111500 3.31819000 -0.38087900 H 1.40016200 3.75205000 -0.00937500

	H 0.49006100 3.30143800 -1.46943300 H -0.35194300 3.94895800 -0.04307400 C 1.17749800 -1.20754600 -0.01737400 H 1.03076600 -2.23771100 0.29051100 N 2.44836100 -0.79706900 -0.12517400 H 2.61173300 0.14255300 -0.46032900 C 3.59194300 -1.68881000 -0.03383600 H 3.94698100 -1.99150600 -1.02188100 H 4.40416900 -1.19482900 0.49838200 H 3.30379500 -2.57895000 0.52411400
Conformation β	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23472512 Electronic_Energy_with_ZeroPoint_Correction= -589.02554472 Gibbs Free Energy= -589.0687325 HTot= -589.0098953 LowF= 38.9929 54.1831 63.4997 NImag= 0 N -1.99331600 -1.29171100 0.27965200 H -1.49450900 -1.82463200 0.97369400 C -3.44212800 -1.38353300 0.25723100 H -3.73745900 -2.31953400 0.72677600 H -3.90659400 -0.55106200 0.79185200 H -3.80078500 -1.37537300 -0.77205700 C -1.35400600 -0.26265500 -0.33578600 O -1.97801600 0.61561000 -0.93032200 C 0.12236200 -0.26108500 -0.27290000 N 0.77024100 0.98209500 -0.49110300 H 1.22519200 1.14251600 -1.38058900 C 0.55079700 2.06321700 0.30915000 O -0.08009900 1.98475300 1.35261700 C 1.12499800 3.36723000 -0.18807300 H 1.47426500 3.94902600 0.66291400 H 1.93869700 3.22351600 -0.89817200 H 0.32656200 3.92617200 -0.68150500 C 0.86774300 -1.38536700 -0.13199000 H 0.37625800 -2.35107900 -0.07002600 N 2.21496700 -1.43313300 -0.13509900 H 2.70611600 -0.55142600 -0.07887100 C 2.95806300 -2.63865800 0.19218200 H 3.91217500 -2.63202300 -0.33281100 H 3.14421900 -2.72656100 1.26593400 H 2.39056600 -3.50809400 -0.13853700
Conformation C5	Ac-(Z)- Δ Ala(β -NHMe)-NHMe (1) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23673757 Electronic_Energy_with_ZeroPoint_Correction= -589.02685167 Gibbs Free Energy= -589.0683968 HTot= -589.0116448 LowF= 61.5281 72.0642 81.5341 NImag= 0 N -2.60401200 0.36108400 0.34887800 H -2.33863200 1.13738100 0.93295500 C -3.99778500 -0.04953500 0.34281600 H -4.60601100 0.78786200 0.67741900 H -4.29920500 -0.32729200 -0.66708900 H -4.16666900 -0.90407500 1.00248600 C -1.64334200 -0.46459300 -0.12857000 O -1.91916900 -1.58159200 -0.57512200 C -0.24298500 0.01049800 -0.13539300 N 0.68101900 -1.04322200 -0.35777900 H 0.28144900 -1.83260200 -0.85100400 C 1.88362500 -1.21176400 0.23687700 O 2.37320600 -0.37802400 0.99847800 C 2.61586300 -2.48461400 -0.10949600 H 2.89101900 -2.98624100 0.81858700 H 2.03057600 -3.16068900 -0.73047400 H 3.53603500 -2.22263100 -0.63408000 C 0.08210600 1.32818400 -0.16215100 H -0.72554900 2.05114400 -0.10108000 N 1.31097600 1.86455200 -0.33343000 H 2.08551400 1.27258400 -0.05273600 C 1.51305900 3.29945700 -0.19101900 H 2.44773600 3.57834500 -0.67498200 H 0.70070200 3.83094500 -0.68658400 H 1.55161700 3.60950900 0.85766800
Ac-(E)- Δ Ala(β -NHMe)-NHMe (2)	
gas phase	
Conformation β 2	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) gas phase β 2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21955483 Electronic_Energy_with_ZeroPoint_Correction= -589.00963853 Gibbs Free Energy= -589.0528722 HTot= -588.9942676 LowF= 19.2262 59.5423 63.4072 NImag= 0 N -0.51139800 -2.17783400 -0.10366400 H -1.44799900 -1.81197900 -0.04790500 C -0.25400000 -3.54138600 0.31669700

	H -1.14241900 -4.14318000 0.13020600 H 0.57918500 -3.94265800 -0.25885400 H 0.00799000 -3.59815100 1.37701500 C 0.51120700 -1.27701500 -0.14804600 O 1.67836500 -1.63278900 0.03208900 C 0.15358400 0.11312500 -0.43645100 N -1.20638200 0.45292000 -0.69616100 H -1.57072400 0.31081600 -1.62711100 C -2.01289600 1.10730200 0.20704700 O -1.64839700 1.41027800 1.32028200 C -3.40137800 1.44143000 -0.30100000 H -4.12130200 1.18885400 0.47637100 H -3.45715500 2.51859700 -0.46805400 H -3.66182100 0.92529400 -1.22559900 C 1.08028500 1.10901100 -0.35404200 H 0.73473700 2.13065600 -0.49373000 N 2.39148200 0.97230100 -0.11484400 H 2.70082700 0.02086600 0.06396900 C 3.25384500 2.09311000 0.19354000 H 3.34462000 2.25612500 1.27154600 H 4.24761100 1.92154800 -0.22087000 H 2.84691500 2.99813500 -0.25973800
Conformation C7	Ac-(E)-ΔAla(β-NHMe)-NHMe (2) gas phase C7 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21988518 Electronic_Energy_with_ZeroPoint_Correction= -589.00953658 Gibbs Free Energy= -589.0515096 HTot= -588.9944428 LowF= 39.2675 56.1346 62.2953 NImag= 0 N -0.90885100 1.78928000 -0.13651600 H -1.65778900 1.25161800 0.27881900 C -0.92025900 3.22389100 0.09120200 H -1.94868900 3.57817200 0.03866400 H -0.49154000 3.48688000 1.06255600 H -0.33121600 3.71639800 -0.68114800 C 0.29673500 1.15771100 -0.21437500 O 1.35387900 1.79253300 -0.20674500 C 0.28809700 -0.30999200 -0.31656300 N -0.90421200 -1.03894000 -0.61857400 H -0.99891200 -1.41449100 -1.55025100 C -1.98565400 -1.15156200 0.20597400 O -2.04829600 -0.60120200 1.28924800 C -3.13066300 -1.98498600 -0.32859000 H -2.88913900 -2.51687900 -1.24874100 H -3.41624400 -2.70049000 0.44206800 H -3.98486200 -1.32950400 -0.50507700 C 1.44034100 -1.02243800 -0.17003100 H 1.36426400 -2.10769600 -0.18381500 N 2.68527200 -0.53832900 -0.03483700 H 2.75405100 0.47493600 -0.00656800 C 3.80343700 -1.36242500 0.37012400 H 4.71886100 -0.99758600 -0.09603300 H 3.94105500 -1.36897300 1.45587500 H 3.63782700 -2.38830400 0.03666300
Conformation C5	Ac-(E)-ΔAla(β-NHMe)-NHMe (2) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.21412530 Electronic_Energy_with_ZeroPoint_Correction= -589.00331800 Gibbs Free Energy= -589.044841 HTot= -588.9883507 LowF= 42.3235 53.4485 73.9889 NImag= 0 N 2.27350200 -0.71277100 -0.18445100 H 2.36275000 0.10287900 -0.77632500 C 3.31299700 -1.72759200 -0.26109700 H 4.26986300 -1.23685100 -0.43158500 H 3.35093300 -2.27274000 0.68062200 H 3.11968400 -2.44680000 -1.06139000 C 1.00187500 -1.10914200 0.11938100 O 0.74602300 -2.25911000 0.44443500 C -0.07654900 -0.08578300 0.02189800 N -1.34035400 -0.71519200 0.02447000 H -1.27668400 -1.71026000 0.19888000 C -2.56507500 -0.13659600 -0.08831800 O -2.73217000 1.05924800 -0.25774800 C -3.73693800 -1.08950300 0.01290400 H -4.35178200 -0.97201700 -0.87969700 H -3.44156200 -2.13338600 0.11608200 H -4.34121000 -0.79817800 0.87302600 C 0.10041600 1.25303800 -0.03720900 H -0.77628300 1.87565100 -0.16341100 N 1.31018700 1.93099300 0.02351400 H 2.00221100 1.49876800 0.62314300 C 1.23213500 3.37853300 0.17838600

	H 0.76361100 3.68034000 1.12187400 H 2.23706600 3.79674400 0.13132900 H 0.65407200 3.79580300 -0.64644900
Conformation β	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.20914682 Electronic_Energy_with_ZeroPoint_Correction= -588.99892852 Gibbs Free Energy= -589.0407657 HTot= -588.9837901 LowF= 41.3385 62.6549 71.5691 NImag= 0 N 1.11107700 1.62676200 0.28638000 H 1.33460600 1.16366900 1.15877200 C 1.11045700 3.08325400 0.31395800 H 1.97791900 3.42617600 0.87619900 H 1.17377900 3.45688000 -0.70660700 H 0.19647900 3.47876100 0.76461800 C 0.12037000 1.00215700 -0.44076600 O -0.63687500 1.61466000 -1.16710600 C 0.05568500 -0.47813200 -0.30149400 N -1.23588800 -1.04262800 -0.49827100 H -1.51276000 -1.21759800 -1.45496000 C -2.25064100 -0.64777300 0.35204100 O -2.02606300 -0.10301700 1.40903100 C -3.64725600 -0.94157300 -0.14368900 H -4.31474600 -1.02899700 0.71052500 H -3.97398700 -0.09922800 -0.75858500 H -3.68752200 -1.85004300 -0.74602100 C 1.09538300 -1.29381900 -0.03515800 H 0.87930700 -2.35607400 0.03899400 N 2.40800100 -0.96683100 0.17552600 H 2.67159600 -0.02792800 -0.09383300 C 3.42930700 -1.98838500 0.01475100 H 3.60161100 -2.25809400 -1.03317800 H 4.36488400 -1.63209600 0.44443400 H 3.13018900 -2.88406400 0.56094700
chloroform	
Conformation α	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) chloroform α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23470598 Electronic_Energy_with_ZeroPoint_Correction= -589.02537908 Gibbs Free Energy= -589.0695483 HTot= -589.009771 LowF= 25.0142 37.5870 43.4626 NImag= 0 N 0.85389600 -1.97778700 -0.17240800 H 1.71652700 -1.48669300 -0.34095700 C 0.87910900 -3.39169000 0.14734200 H 1.89098100 -3.76356300 0.00058500 H 0.57939300 -3.56724800 1.18301300 H 0.19850500 -3.94246500 -0.50362700 C -0.29965300 -1.27007400 -0.11444200 O -1.37360800 -1.82798900 0.16156600 C -0.22116000 0.16226800 -0.39720100 N 1.03857500 0.75116100 -0.71562100 H 1.31855900 0.81461300 -1.68419200 C 1.87515900 1.29173600 0.22134000 O 1.62361300 1.28263000 1.41349800 C 3.14489200 1.90934700 -0.31846400 H 3.99282500 1.48047500 0.21561600 H 3.27589300 1.76062000 -1.38956900 H 3.12239700 2.97916000 -0.10550200 C -1.31938000 0.97082100 -0.31155800 H -1.17260900 2.03229600 -0.49616500 N -2.57537600 0.60741800 -0.03349900 H -2.71512700 -0.37521900 0.17695800 C -3.65995500 1.55314000 0.14597900 H -3.36163300 2.52171700 -0.25492500 H -4.54671200 1.21507600 -0.39099300 H -3.91278200 1.67527200 1.20196200
Conformation C5	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22579500 Electronic_Energy_with_ZeroPoint_Correction= -589.01557300 Gibbs Free Energy= -589.0574091 HTot= -589.0004685 LowF= 35.6873 57.6026 74.3160 NImag= 0 N 2.25021100 -0.74317500 0.24357700 H 2.33757500 0.05968200 0.85228300 C 3.30429900 -1.74511200 0.29034000 H 4.24386300 -1.24870200 0.52363300 H 3.09909100 -2.51109900 1.04225600 H 3.38721400 -2.22940500 -0.68147800 C 0.99564300 -1.11141300 -0.12398400 O 0.73958500 -2.25115400 -0.50547300 C -0.07406800 -0.08300100 -0.03932600 N -1.34969100 -0.69836100 -0.07059900 H -1.29853600 -1.68916600 -0.27166300 C -2.56671100 -0.13282900 0.10103100

	O -2.72967200 1.06060500 0.33270200 C -3.74191100 -1.07673900 -0.01250600 H -4.38877900 -0.72529400 -0.81745500 H -3.45214400 -2.10823800 -0.20800500 H -4.31077900 -1.03114500 0.91691000 C 0.11364800 1.25792900 0.01239100 H -0.76304100 1.88512200 0.11049000 N 1.31275100 1.93427800 -0.01708500 H 2.06060400 1.47917600 -0.52519700 C 1.26109400 3.37928000 -0.21247100 H 0.61069300 3.82043100 0.54284800 H 2.26118100 3.78957100 -0.08218100 H 0.88889800 3.65558300 -1.20437600
Conformation β	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22492946 Electronic_Energy_with_ZeroPoint_Correction= -589.01503216 Gibbs Free Energy= -589.056875 HTot= -588.9998282 LowF= 45.0348 64.0913 70.1395 NImag= 0 N 1.20746100 1.59786200 0.27113600 H 1.52778400 1.10571200 1.09459300 C 1.34341000 3.04665900 0.27488600 H 2.25646400 3.30994900 0.80527600 H 1.41080800 3.40597100 -0.75095900 H 0.48934500 3.53129400 0.75486800 C 0.16586900 1.02645200 -0.40603000 O -0.59372800 1.68759600 -1.10085000 C 0.03263700 -0.44507800 -0.26808800 N -1.27728300 -0.96072700 -0.47226300 H -1.51134100 -1.33729300 -1.37992400 C -2.31144600 -0.56216900 0.32545200 O -2.13120500 0.06914700 1.35326200 C -3.69006300 -0.94421100 -0.15636600 H -4.31442800 -1.17549500 0.70464800 H -4.12148400 -0.08293600 -0.67158300 H -3.67513200 -1.79059600 -0.84261100 C 1.04026900 -1.31615200 -0.02494800 H 0.76886000 -2.36563800 0.05328800 N 2.36814500 -1.07002600 0.14171000 H 2.70088200 -0.15934600 -0.14630700 C 3.32727400 -2.16102800 0.03770600 H 3.49788000 -2.46954000 -0.99821600 H 4.27410400 -1.84602500 0.47331900 H 2.95976300 -3.01659700 0.60456500
water	
Conformation α	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23983985 Electronic_Energy_with_ZeroPoint_Correction= -589.03051785 Gibbs Free Energy= -589.0743415 HTot= -589.0149774 LowF= 24.3406 40.2747 50.7618 NImag= 0 N -0.90968700 1.91928100 -0.20013200 H -1.73133400 1.41558100 -0.49277300 C -0.99127100 3.34534900 0.04923800 H -2.02119900 3.66391400 -0.09553400 H -0.68724400 3.57799800 1.07154700 H -0.34682800 3.90022000 -0.63569500 C 0.26003700 1.25009200 -0.08134200 O 1.30180700 1.84374700 0.24955900 C 0.24252300 -0.18459700 -0.35708700 N -0.98780300 -0.81967800 -0.70141300 H -1.22304300 -0.93778600 -1.67707200 C -1.85952600 -1.31561500 0.22341600 O -1.66038400 -1.23531300 1.42618500 C -3.10773000 -1.95900600 -0.33226800 H -3.20122300 -2.95766400 0.09465700 H -3.96961600 -1.37361900 -0.00822600 H -3.10953400 -2.02717000 -1.41908000 C 1.37141300 -0.95136400 -0.26592700 H 1.27001300 -2.01295400 -0.47782300 N 2.60274600 -0.55016700 0.06146000 H 2.71442800 0.44012600 0.24823900 C 3.76160300 -1.42353400 0.04997200 H 4.42495100 -1.19059100 -0.78588800 H 4.31991500 -1.32232900 0.98112100 H 3.43023700 -2.45719100 -0.04485000
Conformation β	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.23057777 Electronic_Energy_with_ZeroPoint_Correction= -589.02089547 Gibbs Free Energy= -589.062977 HTot= -589.0056043 LowF= 43.4682 60.8782 69.5795 NImag= 0 N 1.23601700 1.58535900 0.27326900 H 1.58537600 1.08084900 1.07674700

	C 1.42753200 3.02706900 0.25816200 H 2.35502500 3.25976300 0.77706300 H 1.49953500 3.37375300 -0.77186500 H 0.59914400 3.54989800 0.74312700 C 0.18399300 1.03191000 -0.39444300 O -0.56924900 1.70967300 -1.08668200 C 0.02477400 -0.43428300 -0.25207200 N -1.29133200 -0.93271300 -0.45886700 H -1.50378200 -1.40772900 -1.32450300 C -2.33416800 -0.52473800 0.31260400 O -2.17556800 0.16290400 1.31205000 C -3.70260200 -0.95971000 -0.15181900 H -4.30451800 -1.21912200 0.71756400 H -4.17769800 -0.11512400 -0.65585700 H -3.66581000 -1.80280600 -0.84070900 C 1.02037400 -1.32500800 -0.01671600 H 0.72927200 -2.36910400 0.06467800 N 2.35168100 -1.10750800 0.13167600 H 2.70745800 -0.20593500 -0.15707800 C 3.29006000 -2.21911600 0.04793700 H 3.46666400 -2.53467000 -0.98431800 H 4.23684900 -1.91968500 0.49399300 H 2.89667800 -3.06340900 0.61374900
Conformation β^*	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) water β^* M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22943963 Electronic_Energy_with_ZeroPoint_Correction= -589.01982283 Gibbs Free Energy= -589.0622476 HTot= -589.0044834 LowF= 39.5692 58.6737 65.8971 NImag= 0 N 1.98995100 -1.15700300 -0.13940000 H 2.38817400 -0.52969900 -0.82531900 C 2.74054600 -2.36977600 0.14902100 H 3.80288000 -2.14346800 0.08553900 H 2.50926300 -2.70812300 1.15800800 H 2.49784600 -3.17383700 -0.55059200 C 0.62594700 -1.16777100 -0.03950400 O 0.02057000 -2.15472400 0.36958700 C -0.06557800 0.07578700 -0.43462600 N -1.44713700 -0.05567200 -0.75022600 H -1.69707800 -0.50836400 -1.61795100 C -2.44038800 0.12552500 0.16367900 O -2.23861900 0.55478000 1.29032500 C -3.82890400 -0.23608100 -0.30920500 H -4.51536900 0.56063800 -0.02495300 H -3.88401500 -0.40079300 -1.38446200 H -4.13768500 -1.14789000 0.20574800 C 0.46013300 1.32911200 -0.40419100 H -0.20316300 2.13550500 -0.70591000 N 1.69941200 1.73656500 -0.04856000 H 2.28048700 1.07990800 0.45554000 C 1.98049900 3.14828300 0.17828600 H 1.65171300 3.47830500 1.16744700 H 3.05156100 3.31888300 0.08489700 H 1.47067400 3.74077600 -0.58075400
Conformation C5	Ac-(E)- Δ Ala(β -NHMe)-NHMe (2) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -589.22950029 Electronic_Energy_with_ZeroPoint_Correction= -589.01952259 Gibbs Free Energy= -589.0616518 HTot= -589.0043496 LowF= 27.7899 57.5268 74.1047 NImag= 0 N -2.23717400 -0.75852300 -0.26999000 H -2.32349400 0.04151100 -0.88235800 C -3.29607700 -1.75570400 -0.30866800 H -4.22800300 -1.25921100 -0.56943800 H -3.08372000 -2.53945000 -1.03980700 H -3.39899100 -2.21536700 0.67327100 C -0.99052800 -1.11405800 0.12525000 O -0.73449400 -2.24960400 0.52713000 C 0.07388000 -0.08147600 0.05238800 N 1.35522000 -0.68781400 0.10144000 H 1.31101700 -1.67405600 0.32544400 C 2.56636700 -0.12842500 -0.11041200 O 2.72271000 1.05867200 -0.38767200 C 3.74617400 -1.06352800 0.01875500 H 4.40312300 -0.68397300 0.80262600 H 3.46269500 -2.08846500 0.25315700 H 4.30194800 -1.04866400 -0.91963100 C -0.11995200 1.26014700 0.00791300 H 0.75619600 1.89080700 -0.07169200 N -1.31600900 1.93329100 0.02176800 H -2.08470600 1.46921400 0.48872700

	C -1.28063000 3.37776500 0.22518500 H -0.60349300 3.82649300 -0.50155600 H -2.27745000 3.78190700 0.05787600 H -0.95051100 3.64971300 1.23271800
Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3)	
gas phase	
Conformation C5	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51194319 Electronic_Energy_with_ZeroPoint_Correction= -628.27304189 Gibbs Free Energy= -628.3160438 HTot= -628.2567475 LowF= 45.2981 59.3496 77.8090 NImag= 0 N -2.46229000 -0.02201300 0.13825200 C -3.76396300 -0.59221600 -0.17248100 H -4.50462400 0.20957300 -0.17507100 H -3.73114600 -1.06497000 -1.15084800 H -4.05651100 -1.34647400 0.56688200 C -1.36497800 -0.72674300 -0.29009200 O -1.47731000 -1.83723400 -0.79809600 C -0.02160200 -0.10573200 -0.20250900 N 1.00369000 -1.08008200 -0.28842000 H 0.67834700 -1.94708800 -0.70003000 C 2.21959700 -1.05207400 0.30705600 O 2.63769700 -0.09006300 0.93973700 C 3.06169000 -2.29520200 0.12876000 H 3.37639800 -2.63552500 1.11542600 H 2.54167700 -3.10065800 -0.38862600 H 3.95876200 -2.02730000 -0.43111000 C 0.16335300 1.22925300 -0.33709400 H -0.72744800 1.84923900 -0.37286900 N 1.33554200 1.89011700 -0.50541200 H 2.14348900 1.41055300 -0.11508700 C 1.35799100 3.34037700 -0.43867200 H 2.29612100 3.70413000 -0.85628100 H 0.54413300 3.74694400 -1.04111300 H 1.26189600 3.71759000 0.58586100 C -2.45480700 0.87966900 1.28175500 H -1.45456700 0.96310300 1.69860300 H -2.81277100 1.87583800 1.00305100 H -3.11911900 0.48327100 2.05681100
Conformation β 2	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) gas phase β 2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50937323 Electronic_Energy_with_ZeroPoint_Correction= -628.27072683 Gibbs Free Energy= -628.3138955 HTot= -628.2543245 LowF= 46.4478 53.8737 64.4208 NImag= 0 N 2.30253500 -0.02934800 0.07540500 C 3.68526400 -0.46427600 -0.03769900 H 4.32278900 0.41685300 -0.13098700 H 3.99866700 -1.04145100 0.83991300 H 3.79755100 -1.09375400 -0.91739800 C 1.33538300 -0.97098200 -0.22486800 O 1.62747800 -2.12584000 -0.48820100 C -0.06112700 -0.47330500 -0.28250700 N -0.26178000 0.88133700 -0.66075800 H 0.36414000 1.23455600 -1.37039200 C -1.09592600 1.77429800 -0.06473300 O -1.83446300 1.47098700 0.85939200 C -1.06973600 3.17708800 -0.62984400 H -0.93583800 3.87320900 0.19822500 H -0.28601600 3.33335000 -1.37112800 H -2.03850800 3.38721000 -1.08545700 C -1.05890200 -1.36447400 -0.08049100 H -0.74570700 -2.38299400 0.12997600 N -2.39253400 -1.14232500 -0.14282900 H -2.67195600 -0.18423600 0.03533600 C -3.31572800 -2.16124600 0.32812700 H -4.31024400 -1.95767300 -0.06761600 H -2.99804000 -3.13563500 -0.04408500 H -3.37206400 -2.20099500 1.42114400 C 2.07009000 1.01360900 1.07000400 H 2.25166400 2.01002600 0.65500600 H 1.05311800 0.96581800 1.45508800 H 2.75285600 0.86261000 1.91219100
Conformation C5'	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) gas phase C5' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50800774 Electronic_Energy_with_ZeroPoint_Correction= -628.26901084 Gibbs Free Energy= -628.3121736 HTot= -628.2527234 LowF= 40.4243 61.3793 69.7081 NImag= 0 N -2.47479000 -0.09435000 -0.06023400 C -3.66696400 -0.78509800 -0.52892400 H -4.43814000 -0.04178900 -0.73849400

	H -3.43719200 -1.33892800 -1.43591200 H -4.04329500 -1.49128100 0.22031600 C -1.29468400 -0.80227000 -0.16070200 O -1.29465900 -1.98931200 -0.46185600 C -0.00738400 -0.10259500 0.06999800 N 1.02403600 -1.02437800 0.37992900 H 0.65810700 -1.94295700 0.59521600 C 2.33440000 -0.97949300 0.02320500 O 2.86650900 -0.01145500 -0.50093700 C 3.13351200 -2.22251300 0.34552200 H 3.89777900 -1.96022100 1.07848500 H 2.52918000 -3.04148100 0.73387500 H 3.64182100 -2.54161100 -0.56437000 C 0.10776300 1.24187200 -0.05614000 H -0.79109800 1.76583200 -0.36469700 N 1.17730100 2.04321800 0.18188800 H 2.07223200 1.56248400 0.11880700 C 1.16558300 3.40376300 -0.32792900 H 1.94585700 3.97955800 0.16864200 H 1.33060300 3.44866800 -1.41021600 H 0.20597600 3.87110900 -0.10007600 C -2.73651800 0.88829900 0.98511000 H -1.84544700 1.06890100 1.58049500 H -3.08033500 1.83631400 0.55974900 H -3.52092100 0.50832000 1.64860300
Conformation β	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50418949 Electronic_Energy_with_ZeroPoint_Correction= -628.26623159 Gibbs Free Energy= -628.3107442 HTot= -628.2494832 LowF= 28.0148 44.6697 66.5366 NImag= 0 N 2.08760700 -0.58437500 0.07169300 C 3.48712000 -0.33294000 -0.23073100 H 4.06493100 -1.22668400 0.01206900 H 3.59503000 -0.10108100 -1.28765300 H 3.87402400 0.51192700 0.35028200 C 1.16494000 0.18421400 -0.59171800 O 1.48960200 1.10216200 -1.32865600 C -0.27245900 -0.17409700 -0.44659200 N -1.19042300 0.91151200 -0.53713900 H -1.38302200 1.24847400 -1.47304800 C -1.10991400 1.89293000 0.43624100 O -0.56746400 1.69342900 1.49945100 C -1.74125600 3.21362500 0.06536200 H -2.00967600 3.74743600 0.97402800 H -1.00022100 3.80133800 -0.48244300 H -2.61911600 3.08414000 -0.56906000 C -0.72544700 -1.43737000 -0.37844400 H -0.01256100 -2.25660500 -0.35195700 N -2.03982700 -1.80558500 -0.40604400 H -2.68614600 -1.04001600 -0.26912100 C -2.45109000 -3.10465200 0.09385300 H -3.46145400 -3.32210800 -0.25128100 H -1.78813300 -3.87027000 -0.31241000 H -2.42981200 -3.16894100 1.18750600 C 1.83657100 -1.16205300 1.38637500 H 0.83217900 -0.91573800 1.72170700 H 1.96975500 -2.24869100 1.37525000 H 2.54482000 -0.73670000 2.10412400
Conformation α	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) gas phase α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50148174 Electronic_Energy_with_ZeroPoint_Correction= -628.26365924 Gibbs Free Energy= -628.3081391 HTot= -628.2468965 LowF= 31.5307 40.7579 55.9202 NImag= 0 N 1.84731400 -0.92861500 0.29239700 C 2.97023000 -1.67676200 -0.24273900 H 3.80949800 -0.99517600 -0.40052500 H 3.28185200 -2.47397800 0.44136900 H 2.67991000 -2.12388500 -1.18974100 C 0.59147600 -1.25067900 -0.14152800 O 0.38043800 -2.26398400 -0.79193400 C -0.53248200 -0.32232100 0.17454200 N -0.35068700 1.07140800 0.38970600 H -0.61594600 1.45978400 1.28461800 C 0.37607300 1.87329800 -0.45994700 O 0.83668700 1.46972200 -1.50221900 C 0.57934600 3.29511800 0.02428100 H 0.55206900 3.95903400 -0.83778200 H -0.16633200 3.60884000 0.75603000 H 1.56989500 3.37300500 0.47827400

	C -1.78238000 -0.82726000 0.16028800 H -1.89739200 -1.89590900 0.01463400 N -2.92505000 -0.11013000 0.35831600 H -2.82812800 0.88659900 0.21781900 C -4.21424100 -0.68097800 0.00406800 H -5.00896400 -0.08840700 0.45627400 H -4.37446600 -0.72518300 -1.07817500 H -4.27786900 -1.69212600 0.40713200 C 2.14824700 -0.03275300 1.38988500 H 2.47745000 0.95292300 1.03951200 H 1.28697200 0.08414900 2.04494600 H 2.95580300 -0.47019100 1.98279400
chloroform	
Conformation $\beta 2$	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) chloroform $\beta 2$ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52292489 Electronic_Energy_with_ZeroPoint_Correction= -628.28484109 Gibbs Free Energy= -628.3294579 HTot= -628.2682356 LowF= 14.9580 51.1688 60.1065 NImag= 0 N 2.28724400 -0.08476500 0.10409400 C 3.67557000 -0.50722900 0.00600000 H 4.30016700 0.37657900 -0.13081100 H 3.99928400 -1.03345700 0.91066800 H 3.79628400 -1.17242100 -0.84576300 C 1.31940200 -0.99675800 -0.21637100 O 1.59914800 -2.16770700 -0.47184500 C -0.07175000 -0.49359000 -0.29930800 N -0.28470300 0.84963500 -0.71280500 H 0.24786100 1.16805200 -1.51076800 C -1.03006200 1.77400000 -0.05281900 O -1.65933300 1.50109400 0.96201400 C -1.03996200 3.16214100 -0.64576300 H -0.62425200 3.84865900 0.09361100 H -0.47063200 3.24050800 -1.57089000 H -2.07341400 3.45654800 -0.83031300 C -1.08516600 -1.36399600 -0.07264600 H -0.80625300 -2.37992200 0.18935800 N -2.40928300 -1.11711000 -0.16188300 H -2.68422200 -0.14491800 -0.11228000 C -3.38016300 -2.07473500 0.34626500 H -4.34527200 -1.89389800 -0.12484700 H -3.05776100 -3.08328000 0.08794000 H -3.49724000 -2.00718900 1.43198000 C 2.04157500 1.06155200 0.97492500 H 2.13438900 2.00801400 0.43479700 H 1.05343900 0.99930000 1.42782800 H 2.77962600 1.04769100 1.78060200
Conformation C5	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52339929 Electronic_Energy_with_ZeroPoint_Correction= -628.28483629 Gibbs Free Energy= -628.3274992 HTot= -628.2685315 LowF= 55.1060 72.2655 77.6502 NImag= 0 N -2.45597900 -0.04471100 0.16578600 C -3.77016800 -0.58715300 -0.14810800 H -4.49455500 0.22820900 -0.13356700 H -3.75448700 -1.04161400 -1.13545700 H -4.07289900 -1.34354300 0.58383500 C -1.36905800 -0.72377400 -0.29674100 O -1.48276300 -1.82566200 -0.84096800 C -0.02516800 -0.10734800 -0.20790100 N 1.01018600 -1.07267600 -0.31158500 H 0.71584500 -1.92497400 -0.77344500 C 2.20158400 -1.06219100 0.32715600 O 2.57523300 -0.12275000 1.02730400 C 3.07406500 -2.27556400 0.11414000 H 3.34291500 -2.67863200 1.09098100 H 2.59592900 -3.05147200 -0.48160200 H 3.99330000 -1.95811600 -0.38023200 C 0.16561000 1.22893700 -0.33919100 H -0.71652400 1.86087300 -0.36413600 N 1.34077700 1.87688500 -0.51790900 H 2.15601900 1.39140500 -0.15741400 C 1.38666000 3.33045400 -0.45976100 H 2.30859400 3.67821900 -0.92347200 H 0.54783800 3.74250800 -1.02126300 H 1.34438000 3.70728200 0.56708700 C -2.43725100 0.90335700 1.27388100 H -1.44414700 0.96371100 1.71058000 H -2.75143300 1.89942100 0.94940900 H -3.13306600 0.55723600 2.04312300

Conformation α	Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3) chloroform α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51993894 Electronic_Energy_with_ZeroPoint_Correction= -628.28218304 Gibbs Free Energy= -628.3262966 HTot= -628.2654502 LowF= 40.1320 48.2655 67.9129 NImag= 0 N 1.88940400 -0.86845200 0.28534600 C 3.04737900 -1.57508900 -0.23771100 H 3.90744600 -0.90390500 -0.21558500 H 3.27755900 -2.46495500 0.35804400 H 2.85345900 -1.88150300 -1.26258600 C 0.65769700 -1.21280000 -0.18461200 O 0.50420000 -2.20221600 -0.90380500 C -0.49438500 -0.34538800 0.15883800 N -0.33460900 1.04202500 0.42441500 H -0.55955800 1.39782800 1.34397500 C 0.26630500 1.89779200 -0.45479600 O 0.63734600 1.54409100 -1.56027600 C 0.46099100 3.31168000 0.04235600 H 1.52836600 3.47736500 0.20148700 H 0.12794300 4.00095500 -0.73288100 H -0.07226300 3.51796400 0.96979500 C -1.73840200 -0.87969000 0.12239500 H -1.83120500 -1.94330900 -0.06971300 N -2.89028800 -0.21369100 0.35353400 H -2.83580900 0.79576600 0.36261100 C -4.18885100 -0.80267600 0.06971900 H -4.94840800 -0.31946200 0.68276900 H -4.46635900 -0.70369900 -0.98357500 H -4.16323600 -1.86118200 0.32793400 C 2.12397300 -0.05765300 1.47052200 H 2.42296400 0.96453500 1.21704000 H 1.23862700 -0.02899300 2.10216300 H 2.92832000 -0.51863800 2.04783600
Conformation β	Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51941515 Electronic_Energy_with_ZeroPoint_Correction= -628.28248337 Gibbs Free Energy= -628.3259635 HTot= -628.2649786 LowF= 44.0253 50.3853 64.1416 NImag= 0 N 2.17369300 -0.07941400 0.06132600 C 3.47428300 0.47094100 -0.28896100 H 4.23590200 -0.28710900 -0.10088800 H 3.48635300 0.74518900 -1.34094500 H 3.70357800 1.35964700 0.30893800 C 1.07660100 0.45292700 -0.55157800 O 1.16140600 1.43587100 -1.28458900 C -0.23131600 -0.22619700 -0.36592200 N -1.38457500 0.59822300 -0.43989000 H -1.90909900 0.61495600 -1.30524000 C -1.52981600 1.66545500 0.40229300 O -0.81044900 1.82213200 1.37404200 C -2.62692900 2.63640000 0.04093600 H -3.06577400 3.03169900 0.95506700 H -2.17962500 3.46555800 -0.51216900 H -3.40076100 2.18204800 -0.57747100 C -0.36821200 -1.56773100 -0.31746600 H 0.51900600 -2.19314200 -0.34413300 N -1.54888900 -2.23968500 -0.31344500 H -2.37009600 -1.68092200 -0.12314900 C -1.61191200 -3.64268600 0.06421600 H -2.54421900 -4.07199100 -0.29963200 H -0.78556000 -4.17631500 -0.40633300 H -1.55349000 -3.78812200 1.14727300 C 2.14171800 -0.82319700 1.31502000 H 2.78429300 -0.31722900 2.04097100 H 1.13189500 -0.85380900 1.71424000 H 2.51182800 -1.84382000 1.18068200
Conformation C5'	Ac-(Z)-ΔAla(β-NHMe)-NMe₂ (3) chloroform C5' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52013497 Electronic_Energy_with_ZeroPoint_Correction= -628.28173387 Gibbs Free Energy= -628.3250192 HTot= -628.2652865 LowF= 46.2970 59.7910 65.3738 NImag= 0 N -2.47655400 -0.11807500 0.02432100 C -3.67530100 -0.76853900 0.53337800 H -4.41774800 -0.00106800 0.75614000 H -4.09462000 -1.46479800 -0.20120100 H -3.43904500 -1.31901800 1.44065500 C -1.29605500 -0.79624800 0.15890300 O -1.27375000 -1.96650900 0.53945800 C -0.02162700 -0.09275300 -0.12656100 N 1.02688000 -0.97059100 -0.50270000 H 0.72682400 -1.79558300 -1.00357900

	C 2.28291500 -1.00801600 0.01122200 O 2.70844200 -0.16337800 0.79200400 C 3.15203800 -2.14810200 -0.46293600 H 4.01897300 -1.73174800 -0.97768800 H 3.51167200 -2.68804400 0.41349000 H 2.63652000 -2.83824700 -1.12893700 C 0.11984800 1.24448500 0.03825900 H -0.75358300 1.79511500 0.37254300 N 1.21927600 2.00557800 -0.19152400 H 2.09978800 1.50679600 -0.12516400 C 1.25312700 3.37625400 0.30028200 H 2.05631000 3.91553400 -0.19974400 H 0.31104200 3.87005600 0.06052400 H 1.41366700 3.42618000 1.38197100 C -2.73211100 0.92416800 -0.96447800 H -3.00426700 1.86894800 -0.48533500 H -1.86177300 1.08021300 -1.59582600 H -3.56551700 0.60888900 -1.59863700
water	
Conformation $\beta 2$	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) water $\beta 2$ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52738268 Electronic_Energy_with_ZeroPoint_Correction= -628.28937478 Gibbs Free Energy= -628.3329593 HTot= -628.2727886 LowF= 39.7292 57.8059 60.9715 NImag= 0 N -2.28578400 -0.10445700 0.09872000 C -3.67169500 -0.53485500 -0.00330800 H -4.29697000 0.34264300 -0.17188400 H -3.78177100 -1.22298500 -0.83810900 H -4.00441100 -1.03508200 0.91257800 C -1.30865400 -1.00845700 -0.19926100 O -1.57745100 -2.19201200 -0.42770900 C 0.07790200 -0.50072500 -0.29227800 N 0.29847900 0.83673000 -0.72163000 H -0.18710200 1.13812700 -1.55569800 C 1.00174100 1.77613800 -0.03680500 O 1.57436400 1.52224700 1.01676800 C 1.03635500 3.15436400 -0.64953800 H 0.55637300 3.84536400 0.04568100 H 2.07621200 3.46166600 -0.76308000 H 0.53452300 3.20931100 -1.61430300 C 1.09957500 -1.36311500 -0.06063400 H 0.83626100 -2.37479100 0.23119800 N 2.41658000 -1.10905000 -0.17389200 H 2.69161100 -0.13825600 -0.23031200 C 3.41841100 -2.03291600 0.33579400 H 4.35256700 -1.88750200 -0.20461900 H 3.60096200 -1.89100900 1.40473200 H 3.08004900 -3.05509400 0.16812500 C -2.05593400 1.08437100 0.91559500 H -1.07937800 1.04044300 1.39485700 H -2.13178200 2.00239400 0.32666600 H -2.81357700 1.11149400 1.70191300
Conformation α	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52640549 Electronic_Energy_with_ZeroPoint_Correction= -628.28866839 Gibbs Free Energy= -628.3329346 HTot= -628.2719329 LowF= 40.2987 51.9068 62.8667 NImag= 0 N 1.89943900 -0.85464900 0.29123300 C 3.07424400 -1.53966700 -0.22439900 H 3.93128300 -0.86891900 -0.14964400 H 3.28954900 -2.45073400 0.34402600 H 2.91432500 -1.80671300 -1.26618700 C 0.67968700 -1.19744700 -0.20559700 O 0.54932600 -2.17119200 -0.95718600 C -0.48195200 -0.35127000 0.14287400 N -0.33117400 1.03331400 0.42717900 H -0.55420800 1.37842900 1.35177300 C 0.23434800 1.90710900 -0.45217900 O 0.58874200 1.57000200 -1.57251300 C 0.41870400 3.31739800 0.05423900 H 1.48672900 3.49638600 0.19395000 H 0.05878400 4.01038400 -0.70560400 H -0.09781300 3.50427300 0.99473700 C -1.72459500 -0.89513500 0.09696300 H -1.80999500 -1.95676100 -0.10939000 N -2.87841200 -0.24488300 0.33246900 H -2.83758300 0.76269000 0.40348300 C -4.18184700 -0.84754800 0.10744200 H -4.90403500 -0.42747200 0.80622400

	H -4.53771700 -0.68229000 -0.91291500 H -4.11327200 -1.92005400 0.28677500 C 2.10401400 -0.07646000 1.50600900 H 2.40059600 0.95389500 1.28811800 H 1.20513900 -0.07192800 2.11852300 H 2.89909300 -0.55001200 2.08543900
Conformation β	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52499689 Electronic_Energy_with_ZeroPoint_Correction= -628.28735649 Gibbs Free Energy= -628.3312812 HTot= -628.2706171 LowF= 44.9076 57.4865 69.1304 NImag= 0 N -2.18947100 -0.06041800 0.06175700 C -3.45326100 -0.65542000 -0.34655000 H -4.25398300 0.05850700 -0.14952100 H -3.42707500 -0.88568700 -1.40870600 H -3.65783200 -1.57660100 0.20981300 C -1.05146200 -0.50407900 -0.54650200 O -1.07149900 -1.46855600 -1.31524900 C 0.20672900 0.23762500 -0.31237400 N 1.41241700 -0.50416400 -0.42160600 H 1.98311500 -0.38895200 -1.24923200 C 1.65532300 -1.58887800 0.36416000 O 0.93225200 -1.88520000 1.30513100 C 2.86406400 -2.41155200 -0.00852600 H 3.37773900 -2.71633100 0.90195700 H 2.52051200 -3.31161500 -0.52307400 H 3.55370800 -1.87653900 -0.66023300 C 0.25694300 1.58284500 -0.16420100 H -0.66741700 2.15174500 -0.18571300 N 1.37529300 2.31802800 0.00801200 H 2.26093700 1.83366900 -0.03131300 C 1.39296000 3.76347600 -0.13973700 H 1.55229700 4.06942100 -1.17737900 H 0.44133700 4.16922800 0.20380400 H 2.18701000 4.18111500 0.47750400 C -2.23428800 0.64339100 1.33905500 H -1.24088400 0.71472900 1.77274500 H -2.65249800 1.64736500 1.22494800 H -2.87269800 0.08142000 2.02625600
Conformation C5	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52702097 Electronic_Energy_with_ZeroPoint_Correction= -628.28860577 Gibbs Free Energy= -628.3312803 HTot= -628.2722748 LowF= 54.6445 72.5861 76.6850 NImag= 0 N -2.45492300 -0.05318200 0.17301800 C -3.77181900 -0.58796700 -0.14483700 H -4.49145000 0.23119300 -0.12802800 H -3.75843000 -1.03785700 -1.13430500 H -4.07955500 -1.34413900 0.58491600 C -1.36959700 -0.72416200 -0.29612800 O -1.48223600 -1.82527400 -0.84879600 C -0.02656200 -0.10817700 -0.20626600 N 1.01333700 -1.06928400 -0.31677400 H 0.73214100 -1.91396200 -0.79998000 C 2.19733600 -1.06448200 0.33375200 O 2.55546500 -0.13516700 1.05779900 C 3.08252900 -2.26476200 0.10415900 H 3.35004800 -2.68384600 1.07463900 H 2.61518100 -3.03387800 -0.50843100 H 4.00108100 -1.92980000 -0.37999600 C 0.16517700 1.22911200 -0.33659000 H -0.71459100 1.86416800 -0.35878100 N 1.33999100 1.87361900 -0.51674600 H 2.16015200 1.38497800 -0.17453800 C 1.39484500 3.32809500 -0.46902700 H 2.30680700 3.66800700 -0.95738000 H 0.54363100 3.74006300 -1.01101300 H 1.37951100 3.70935700 0.55646800 C -2.43688200 0.91163000 1.26755400 H -1.44730600 0.96858100 1.71246500 H -2.73909000 1.90529200 0.92589000 H -3.14296300 0.58159200 2.03385700
Conformation C5'	Ac-(Z)- Δ Ala(β -NHMe)-NMe ₂ (3) water C5' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52450698 Electronic_Energy_with_ZeroPoint_Correction= -628.28637408 Gibbs Free Energy= -628.3302476 HTot= -628.2697685 LowF= 37.4812 54.3106 56.5949 NImag= 0 N -2.47740400 -0.12332300 -0.00850000 C -3.67569300 -0.75645600 -0.54031500 H -4.40659600 0.02064900 -0.76682900 H -3.43326200 -1.30141500 -1.44929100

	H -4.11332200 -1.45259700 0.18326100 C -1.29568700 -0.79022500 -0.15087600 O -1.26449800 -1.95252900 -0.56371800 C -0.02944000 -0.08525300 0.15904400 N 1.03158400 -0.93931500 0.55763600 H 0.78391600 -1.68241900 1.19611400 C 2.25187800 -1.02758500 -0.03011100 O 2.61091000 -0.26981900 -0.92610100 C 3.16882200 -2.09575700 0.51462700 H 4.03789700 -1.61055400 0.96155000 H 2.69441300 -2.73357100 1.25867600 H 3.51778700 -2.70516500 -0.31931700 C 0.12339200 1.24841200 -0.02816100 H -0.73688800 1.80924300 -0.37930400 N 1.23252600 1.99146500 0.19377200 H 2.10714700 1.48263300 0.19769600 C 1.31142900 3.35592100 -0.31036700 H 2.09121700 3.89227900 0.22805700 H 1.53307600 3.38883500 -1.38136900 H 0.36238100 3.86084400 -0.13063100 C -2.73418000 0.93537900 0.96288400 H -1.87116200 1.08757700 1.60490100 H -2.98722700 1.87665700 0.46730200 H -3.58075000 0.63739000 1.58703500
Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4)	
gas phase	
Conformation β ₂	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) gas phase β ₂ M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50868441 Electronic_Energy_with_ZeroPoint_Correction= -628.27009961 Gibbs Free Energy= -628.3132336 HTot= -628.2537554 LowF= 46.9703 61.2827 66.7758 NImag= 0 N 0.79344400 -1.93180100 0.00529100 C 0.72937100 -3.38418300 -0.00760300 H 0.04547500 -3.71145600 -0.78706300 H 1.72827700 -3.77598500 -0.20723900 H 0.37412700 -3.78139500 0.95034700 C -0.38103000 -1.25925400 -0.23255800 O -1.44786400 -1.86979800 -0.34003900 C -0.33038900 0.19807000 -0.40271800 N 0.88365400 0.85330700 -0.76084800 H 1.27061500 0.65785500 -1.67195700 C 1.56383800 1.72566500 0.04328000 O 1.18842300 2.02806700 1.15747000 C 2.84712400 2.27732000 -0.54196600 H 2.84624800 3.36038900 -0.42281300 H 3.68155700 1.88071900 0.03947600 H 2.99101100 2.02534800 -1.59287900 C -1.45914000 0.95284100 -0.27279300 H -1.35070700 2.03212300 -0.34937400 N -2.70650200 0.51624600 -0.04970000 H -2.81800400 -0.49171700 -0.01145000 C -3.78244300 1.38981500 0.36711600 H -3.58613700 2.40111900 0.00784800 H -4.72392700 1.04995600 -0.06504200 H -3.88612900 1.42443000 1.45572500 C 1.86169900 -1.35661400 0.81708800 H 1.52473700 -0.45255300 1.32337000 H 2.13813800 -2.08199300 1.58674300 H 2.75189000 -1.12921700 0.22257600
Conformation α	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) gas phase α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50470377 Electronic_Energy_with_ZeroPoint_Correction= -628.26678507 Gibbs Free Energy= -628.3122953 HTot= -628.250057 LowF= 24.4017 30.7825 42.6353 NImag= 0 N -1.04801200 1.61503700 0.16613300 C -1.34054300 2.89235700 -0.46150200 H -2.39936300 2.91964700 -0.72658300 H -1.11724300 3.73097000 0.20800000 H -0.73824700 2.99700900 -1.35987400 C 0.19846200 1.08428600 -0.02504000 O 1.08602000 1.76253800 -0.55272000 C 0.47044300 -0.30911200 0.36543100 N -0.57768200 -1.25004500 0.59215200 H -0.79363200 -1.54036600 1.53336400 C -1.44987000 -1.62353700 -0.39944200 O -1.35288600 -1.23397600 -1.54115200 C -2.56335700 -2.55531300 0.03832800 H -2.47377800 -2.88713400 1.07329500 H -3.51714300 -2.04196700 -0.09405400

	H -2.56190700 -3.42276700 -0.62148100 C 1.74581000 -0.79858900 0.31503700 H 1.87901500 -1.85233300 0.54952700 N 2.86715600 -0.13668800 0.00736800 H 2.73172600 0.81943300 -0.30843800 C 4.18800900 -0.72020200 0.07438300 H 4.72645000 -0.55645300 -0.86084200 H 4.77496100 -0.29752400 0.89456700 H 4.10055600 -1.79577300 0.23327100 C -1.99009400 1.17397100 1.17775400 H -2.76593700 0.51717600 0.76910100 H -1.47794700 0.66343400 1.99185200 H -2.47597900 2.05685800 1.60000300
Conformation C5	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) gas phase C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50566743 Electronic_Energy_with_ZeroPoint_Correction= -628.26676153 Gibbs Free Energy= -628.3096336 HTot= -628.2505315 LowF= 35.1388 60.9596 77.1867 NImag= 0 N -2.08351700 -0.73347300 0.13670500 C -3.12578100 -1.68532400 -0.22909600 H -3.05653300 -2.60259500 0.36611300 H -4.09594000 -1.21747000 -0.05660100 H -3.02703800 -1.95161400 -1.27890700 C -0.81071800 -1.06554600 -0.29619000 O -0.58503900 -2.12311300 -0.86592800 C 0.27651300 -0.07417800 -0.11061700 N 1.54320500 -0.71132200 -0.13281600 H 1.49548200 -1.67097900 -0.44789700 C 2.75200200 -0.17251400 0.17019000 O 2.90889500 0.99104900 0.50162500 C 3.92403700 -1.12593500 0.06823500 H 4.63176800 -0.72447600 -0.65790400 H 4.42393800 -1.15533300 1.03694400 H 3.63978700 -2.13683100 -0.22343300 C 0.12922100 1.27386100 -0.12627000 H 1.02799200 1.87652100 -0.07825100 N -1.04236100 1.98190100 -0.15870500 H -1.86306200 1.46149200 -0.44100300 C -1.00199200 3.37733700 -0.56344200 H -0.21314500 3.88719100 -0.00930600 H -0.81350900 3.50472100 -1.63533800 H -1.95046600 3.85209200 -0.31338400 C -2.26760500 -0.15810200 1.47005400 H -2.45088000 -0.95853000 2.19584600 H -1.38643200 0.40042800 1.77358400 H -3.12817000 0.51455900 1.47049100
Conformation β	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) gas phase β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.50379251 Electronic_Energy_with_ZeroPoint_Correction= -628.26563411 Gibbs Free Energy= -628.3091787 HTot= -628.2491191 LowF= 34.4285 55.3961 71.2193 NImag= 0 N -1.03083000 1.50219000 -0.07216400 C -1.24555100 2.88333600 0.33762500 H -0.42437500 3.52700400 0.00348700 H -2.17924600 3.23598000 -0.10305200 H -1.30426700 2.93880900 1.42227100 C -0.08347800 0.80641500 0.64995500 O 0.53772200 1.31575300 1.56554200 C 0.10222700 -0.63950100 0.32785700 N 1.44064600 -1.11002700 0.44486200 H 1.75110200 -1.38668900 1.36670500 C 2.39453000 -0.52474300 -0.35982900 O 2.09385500 0.14547500 -1.32405100 C 3.82571500 -0.77035600 0.05626400 H 4.46288100 -0.72040700 -0.82391900 H 4.12148200 0.02430700 0.74563800 H 3.95086900 -1.73158700 0.55600800 C -0.88781100 -1.51232300 0.06282300 H -0.61498700 -2.55603400 -0.06844400 N -2.21999000 -1.24571700 -0.09753300 H -2.50709700 -0.30970400 0.15869900 C -3.19515900 -2.30095300 0.11323900 H -3.33698000 -2.54775500 1.17145600 H -4.15287000 -1.99706300 -0.30827400 H -2.86879400 -3.19994700 -0.41216500 C -1.18229900 1.27840300 -1.50961700 H -0.80862900 0.29613200 -1.78365000 H -2.23477500 1.36308100 -1.79145400 H -0.60475100 2.02726700 -2.06129000

chloroform			
Conformation α	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) chloroform α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52054203 Electronic_Energy_with_ZeroPoint_Correction= -628.28261863 Gibbs Free Energy= -628.3267521 HTot= -628.2660501 LowF= 38.4775 49.2592 54.2525 NImag= 0		
	N	-1.03820200	1.61475700 0.17961400
	C	-1.35789400	2.90070900 -0.42073300
	H	-0.87326000	2.98166200 -1.39029400
	H	-2.43912400	2.96605500 -0.54966300
	H	-1.02497100	3.73310700 0.20893800
	C	0.18492200	1.07320100 -0.08548600
	O	1.04654800	1.73634200 -0.68538400
	C	0.46922000	-0.31259200 0.31096800
	N	-0.58308500	-1.24152400 0.56602200
	H	-0.75711000	-1.55344200 1.51016000
	C	-1.46598200	-1.62762800 -0.39700400
	O	-1.38633800	-1.24851300 -1.55399400
	C	-2.57488500	-2.54621900 0.06458900
	H	-2.61667900	-3.40281700 -0.60789900
	H	-2.44761600	-2.89370200 1.08933500
	H	-3.52311100	-2.01161700 -0.01665100
	C	1.74747200	-0.80121200 0.28747300
	H	1.88173100	-1.84517200 0.56103800
	N	2.87173900	-0.15173100 -0.03114200
	H	2.75913000	0.80500300 -0.34637400
	C	4.19684200	-0.70857000 0.15836800
	H	4.82221100	-0.48428600 -0.70584700
	H	4.67814800	-0.30811100 1.05434500
	H	4.12119000	-1.79147100 0.25861700
	C	-1.90378700	1.19464000 1.27291600
	H	-2.72505100	0.55764100 0.93062500
	H	-1.33607000	0.67072300 2.03930700
	H	-2.32969300	2.08931200 1.73137400
Conformation β_2	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) chloroform β_2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52174087 Electronic_Energy_with_ZeroPoint_Correction= -628.28351907 Gibbs Free Energy= -628.3267054 HTot= -628.2671362 LowF= 45.5280 59.4962 72.2634 NImag= 0		
	N	-0.88962300	1.89533800 0.00953200
	C	-0.91300400	3.34960600 -0.02947700
	H	-0.23160100	3.70759400 -0.79738200
	H	-1.92732700	3.67435300 -0.26464500
	H	-0.61583300	3.78357600 0.93175100
	C	0.31191400	1.27804100 -0.20049700
	O	1.35556700	1.94343200 -0.29560300
	C	0.34675200	-0.17887000 -0.36225600
	N	-0.82191100	-0.90414800 -0.74079600
	H	-1.18599100	-0.75713900 -1.67139200
	C	-1.47616200	-1.79576400 0.05193300
	O	-1.12301800	-2.05324900 1.19267300
	C	-2.70063500	-2.43514200 -0.56084600
	H	-3.57572100	-2.08761700 -0.00822400
	H	-2.82821700	-2.19930600 -1.61668200
	H	-2.63271200	-3.51545500 -0.43406300
	C	1.52036900	-0.87317700 -0.25212500
	H	1.46983400	-1.95334500 -0.36635400
	N	2.74194000	-0.38567400 -0.01306400
	H	2.81381500	0.62236500 0.06355500
	C	3.88212400	-1.22211100 0.30973300
	H	4.77658100	-0.83253800 -0.17599000
	H	4.05713600	-1.26761300 1.38766500
	H	3.70116000	-2.23283000 -0.05641500
	C	-1.98587100	1.26723600 0.74273000
	H	-2.80598300	0.97138000 0.08293300
	H	-1.63400900	0.39799100 1.29625400
	H	-2.36729100	1.98958600 1.46755100
Conformation β	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) chloroform β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51810691 Electronic_Energy_with_ZeroPoint_Correction= -628.28012281 Gibbs Free Energy= -628.3233385 HTot= -628.2636294 LowF= 41.7710 59.6817 69.8864 NImag= 0		
	N	1.08185200	1.48685900 0.06991400
	C	1.37971400	2.84312600 -0.37011800
	H	0.62990900	3.55189800 -0.00271400
	H	2.35756000	3.12661400 0.02017800
	H	1.39439000	2.88280800 -1.45671700
	C	0.09893600	0.81813600 -0.60989600
	O	-0.52935700	1.34607300 -1.52100700
	C	-0.12900800	-0.61285100 -0.26559300
	N	-1.47638200	-1.05423000 -0.37593400

	H -1.75042700 -1.59023400 -1.18697100 C -2.46088300 -0.43608900 0.33587900 O -2.21054600 0.37547800 1.21325600 C -3.87454300 -0.80496800 -0.04249200 H -4.27571900 -0.01245300 -0.67811800 H -3.93270000 -1.75028300 -0.58126600 H -4.47975600 -0.85570100 0.86100800 C 0.84299500 -1.52250400 -0.03355500 H 0.53707000 -2.55554600 0.11063600 N 2.18505500 -1.31469600 0.05921700 H 2.51802500 -0.40310500 -0.22691800 C 3.11118900 -2.42265700 -0.11958300 H 2.74564400 -3.28706700 0.43567900 H 3.23086400 -2.70456000 -1.17041100 H 4.08379500 -2.14576400 0.28454600 C 1.36727100 1.23718800 1.48052000 H 0.93233500 0.29335200 1.79539800 H 2.44627400 1.21535900 1.64956000 H 0.93426500 2.04045200 2.08476900
Conformation C5	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) chloroform C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51692901 Electronic_Energy_with_ZeroPoint_Correction= -628.27896201 Gibbs Free Energy= -628.322953 HTot= -628.2623996 LowF= 30.7401 43.5413 59.2214 NImag= 0 N -2.00062200 -0.83066300 0.21529500 C -3.04312600 -1.82692600 0.00312200 H -2.92823700 -2.67407100 0.68763200 H -4.01060000 -1.35614100 0.17924700 H -2.99765900 -2.19492800 -1.01901700 C -0.76642400 -1.11152000 -0.31029700 O -0.53222700 -2.18384100 -0.86519200 C 0.28806400 -0.07139600 -0.25421900 N 1.59021600 -0.64379500 -0.28337200 H 1.61793300 -1.58202700 -0.65977700 C 2.72373200 -0.10795000 0.22489800 O 2.77184800 1.00901300 0.72933900 C 3.95703800 -0.97535000 0.11683300 H 4.38486000 -1.08819400 1.11354000 H 3.75708600 -1.95938300 -0.30526900 H 4.69066400 -0.46005400 -0.50485900 C 0.09343400 1.26293000 -0.41012100 H 0.96956700 1.90157200 -0.42598200 N -1.08223400 1.91454000 -0.59080000 H -1.93223200 1.37077400 -0.59631300 C -1.20262900 3.33956800 -0.33872600 H -2.03988000 3.73882300 -0.90956000 H -1.35790800 3.56263400 0.72180800 H -0.29147600 3.84002800 -0.66932500 C -2.16532800 -0.04618700 1.43799600 H -2.37223000 -0.71989200 2.27524200 H -1.26102300 0.51519800 1.65774300 H -3.00592600 0.64469500 1.33693700
Conformation β'	Ac-(E)-ΔAla(β-NHMe)-NMe ₂ (4) chloroform β' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.51607721 Electronic_Energy_with_ZeroPoint_Correction= -628.27802571 Gibbs Free Energy= -628.3218277 HTot= -628.2615165 LowF= 29.7972 53.3225 62.3244 NImag= 0 N 1.93715700 -0.95344700 0.03031000 C 2.71860600 -1.97447000 0.71611300 H 2.30693000 -2.14978400 1.70720500 H 2.71376500 -2.91973900 0.16226700 H 3.74609800 -1.62043600 0.80501800 C 0.56625100 -1.06517100 0.12957800 O 0.04420600 -2.01382100 0.70508200 C -0.25330700 0.03838200 -0.42074800 N -1.59084400 -0.30603300 -0.76152000 H -1.72536300 -0.99450300 -1.48748600 C -2.65881000 -0.05707400 0.05418600 O -2.59111300 0.65695800 1.04014300 C -3.95015500 -0.72626400 -0.35685000 H -4.12453200 -1.56663800 0.31830600 H -4.76919700 -0.01888500 -0.23571800 H -3.93106800 -1.09605300 -1.38152900 C 0.06278000 1.35697800 -0.35362300 H -0.70243100 2.06116200 -0.66810000 N 1.21456200 1.93104200 0.06355900 H 1.87882900 1.32867700 0.53274500 C 1.26163000 3.34532100 0.40307700 H 0.67277700 3.90637800 -0.32264100

	H 0.86901500 3.54381200 1.40448500 H 2.29191500 3.69426100 0.35030700 C 2.58067400 -0.43339700 -1.17494300 H 1.88902700 0.17924700 -1.74622500 H 3.45215300 0.16796300 -0.90654600 H 2.91519800 -1.26733100 -1.80106400
water	
Conformation α	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) water α M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52597697 Electronic_Energy_with_ZeroPoint_Correction= -628.28817487 Gibbs Free Energy= -628.3322309 HTot= -628.2715951 LowF= 39.5036 48.9909 58.3958 NImag= 0 N -1.02422800 1.61769800 -0.19143000 C -1.35562700 2.90630200 0.39807800 H -0.98064600 3.73582000 -0.21105900 H -2.44076200 2.98653000 0.47144600 H -0.92109200 2.97815700 1.39203700 C 0.18208100 1.06490600 0.11421000 O 1.02862300 1.71561900 0.75253400 C 0.46879400 -0.31899400 -0.28474600 N -0.58645300 -1.24074400 -0.55279900 H -0.74070900 -1.56320200 -1.49738100 C -1.48149300 -1.62206500 0.39581200 O -1.41471900 -1.24039400 1.55681300 C -2.58887100 -2.53452900 -0.07678200 H -2.65028600 -3.38586800 0.60091000 H -3.53359200 -1.99062100 -0.01807900 H -2.44562200 -2.88998700 -1.09630400 C 1.74706100 -0.80806900 -0.27382800 H 1.88010100 -1.84811500 -0.56230100 N 2.87418200 -0.16337200 0.04543900 H 2.77085400 0.79474500 0.35718900 C 4.19766200 -0.70963900 -0.19163500 H 4.12817600 -1.79383800 -0.27947800 H 4.63921300 -0.31199900 -1.10884400 H 4.85214800 -0.47047800 0.64619000 C -1.85379600 1.20597900 -1.31772400 H -1.26396000 0.67177700 -2.05953100 H -2.69553100 0.58153400 -1.00476700 H -2.24890700 2.10532000 -1.79358400
Conformation β_2	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) water β_2 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52590179 Electronic_Energy_with_ZeroPoint_Correction= -628.28776009 Gibbs Free Energy= -628.330995 HTot= -628.2713666 LowF= 44.9890 59.7230 72.8982 NImag= 0 N -0.92207500 1.88252200 0.01171700 C -0.97483800 3.33603000 -0.03882900 H -0.29103200 3.70341300 -0.80007500 H -1.99201800 3.63724200 -0.29131200 H -0.70276600 3.78298000 0.92373200 C 0.28806600 1.28361800 -0.18662700 O 1.32392600 1.96706900 -0.27370000 C 0.35153500 -0.17183000 -0.34698300 N -0.80079700 -0.91971200 -0.73284700 H -1.15779900 -0.78553900 -1.66852600 C -1.44455000 -1.81934600 0.05527900 O -1.09750900 -2.06433200 1.20356500 C -2.64852000 -2.48714600 -0.56504100 H -3.53455100 -2.16648700 -0.01361200 H -2.77848100 -2.24743100 -1.61940700 H -2.55188600 -3.56630700 -0.44609900 C 1.53963900 -0.84526100 -0.24534200 H 1.50824800 -1.92458600 -0.37372600 N 2.75225200 -0.34139600 -0.00086900 H 2.81085000 0.66610200 0.08802400 C 3.91380200 -1.16304100 0.28626700 H 4.78946800 -0.75613000 -0.21886500 H 4.11582700 -1.21154800 1.35899600 H 3.73806900 -2.17354500 -0.08217300 C -2.02930500 1.23633200 0.71252200 H -2.82155000 0.92114000 0.02864400 H -1.67755300 0.37749000 1.28180300 H -2.44680200 1.95527600 1.42018900
Conformation β	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) water β M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52331703 Electronic_Energy_with_ZeroPoint_Correction= -628.28536813 Gibbs Free Energy= -628.3286312 HTot= -628.2688641 LowF= 46.3569 55.3786 68.9420 NImag= 0 N 1.11373900 1.47613700 0.07279500 C 1.44976300 2.82206100 -0.37174600 H 0.73685200 3.55718900 0.01666000

	H 2.44642800 3.06801900 -0.00498800 H 1.44128000 2.86464800 -1.45823500 C 0.10747900 0.83140000 -0.59073000 O -0.52853100 1.38276800 -1.48727100 C -0.14235900 -0.59391200 -0.25117000 N -1.49489600 -1.01871400 -0.36495600 H -1.75565700 -1.63896900 -1.11803700 C -2.48728000 -0.39868300 0.32367400 O -2.25509400 0.45897200 1.16671600 C -3.89439400 -0.81547300 -0.02744300 H -4.34822900 -0.02287600 -0.62613300 H -3.93186700 -1.74726500 -0.59030900 H -4.47020700 -0.91722500 0.89141700 C 0.81670700 -1.52583500 -0.03789200 H 0.49051800 -2.55332600 0.10246300 N 2.16036400 -1.35050600 0.03156600 H 2.51875100 -0.44803200 -0.25188200 C 3.06502600 -2.48102900 -0.11755500 H 2.67107700 -3.32992300 0.44156600 H 3.19388300 -2.77600900 -1.16314300 H 4.03663900 -2.21909400 0.29830600 C 1.44922700 1.20303600 1.46792000 H 0.98938900 0.27558700 1.79590200 H 2.53225600 1.13455800 1.59142300 H 1.07842700 2.02099400 2.09324900
Conformation β'	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) water β' M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52159869 Electronic_Energy_with_ZeroPoint_Correction= -628.28362729 Gibbs Free Energy= -628.3275085 HTot= -628.2670948 LowF= 32.4278 53.9110 58.7133 NImag= 0 N -1.88954400 -1.01682000 -0.00222500 C -2.64702200 -2.06703900 -0.67033100 H -2.24112000 -2.23989200 -1.66421800 H -2.61052900 -3.00515500 -0.10591500 H -3.68496700 -1.74386800 -0.75241800 C -0.52167400 -1.06498900 -0.12640900 O 0.03338500 -1.99609600 -0.70744200 C 0.25615500 0.07948000 0.39826800 N 1.60286200 -0.20233900 0.76532400 H 1.76565900 -0.71641000 1.61905000 C 2.66360600 -0.03628600 -0.07101000 O 2.57285300 0.49376600 -1.16928400 C 3.98794600 -0.54189300 0.45244800 H 3.94211100 -0.87219500 1.48921300 H 4.30600900 -1.37655300 -0.17462800 H 4.72840500 0.25196800 0.35568200 C -0.11584100 1.38463600 0.31124200 H 0.61992000 2.12401600 0.61652000 N -1.28681800 1.90341600 -0.10908200 H -1.94529400 1.27325900 -0.54774900 C -1.43312700 3.32106600 -0.40240600 H -1.10523700 3.56679300 -1.41608600 H -2.47807700 3.60600500 -0.28947900 H -0.84119600 3.89592900 0.30938000 C -2.54555000 -0.48532600 1.19165300 H -2.86757300 -1.31527200 1.82860000 H -1.86435000 0.14551500 1.75549700 H -3.42612400 0.09681400 0.91151500
Conformation C5	Ac-(E)- Δ Ala(β -NHMe)-NMe ₂ (4) water C5 M062X/6-311+G(d,p) Charge = 0 Multiplicity = 1 Electronic Energy= -628.52144622 Electronic_Energy_with_ZeroPoint_Correction= -628.28344092 Gibbs Free Energy= -628.3274711 HTot= -628.2669427 LowF= 20.6230 52.3886 68.8480 NImag= 0 N -1.95396400 -0.87485300 0.24315200 C -3.00366800 -1.85192300 -0.01888500 H -2.84035000 -2.77252200 0.55128800 H -3.95885600 -1.41719900 0.27552400 H -3.02588100 -2.09399400 -1.07885600 C -0.75053300 -1.08171100 -0.37538900 O -0.53258300 -2.08834800 -1.05199800 C 0.27751100 -0.02337600 -0.28954300 N 1.60281600 -0.52005100 -0.44866100 H 1.68610800 -1.33009300 -1.04760900 C 2.68656200 -0.12816400 0.26343300 O 2.66205500 0.79167200 1.07473900 C 3.96065800 -0.88742400 -0.02502700 H 4.35487300 -1.26707000 0.91813000 H 3.82109500 -1.71494600 -0.71914000 H 4.69329400 -0.19266200 -0.43848600

	C	0.04793700	1.31383600	-0.31911500
	H	0.91425700	1.96137500	-0.41136300
	N	-1.13246400	1.96996300	-0.21158700
	H	-1.97445100	1.41878800	-0.31547200
	C	-1.23215700	3.37445000	-0.58209700
	H	-1.29766600	3.51401600	-1.66504200
	H	-2.11644900	3.80573700	-0.11566900
	H	-0.35530500	3.90476000	-0.21075800
	C	-2.04286000	-0.24663600	1.56162600
	H	-1.14105800	0.32020900	1.77696500
	H	-2.90675700	0.41973600	1.60435800
	H	-2.16404500	-1.02245500	2.32384900

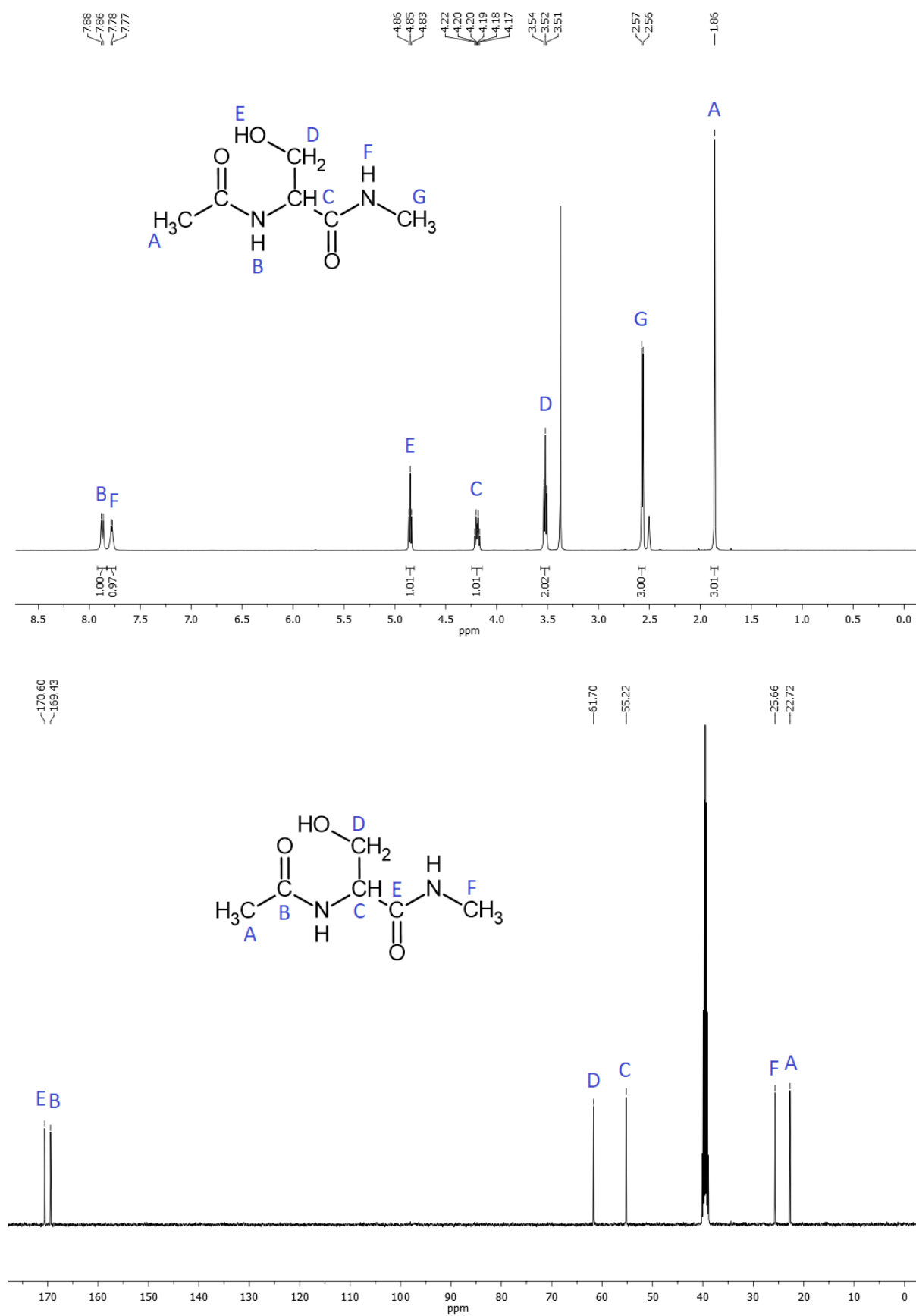


Figure 4S. ^1H and ^{13}C NMR spectra of Ac-(L)-Ser-NHMe in $\text{DMSO}-d_6$.

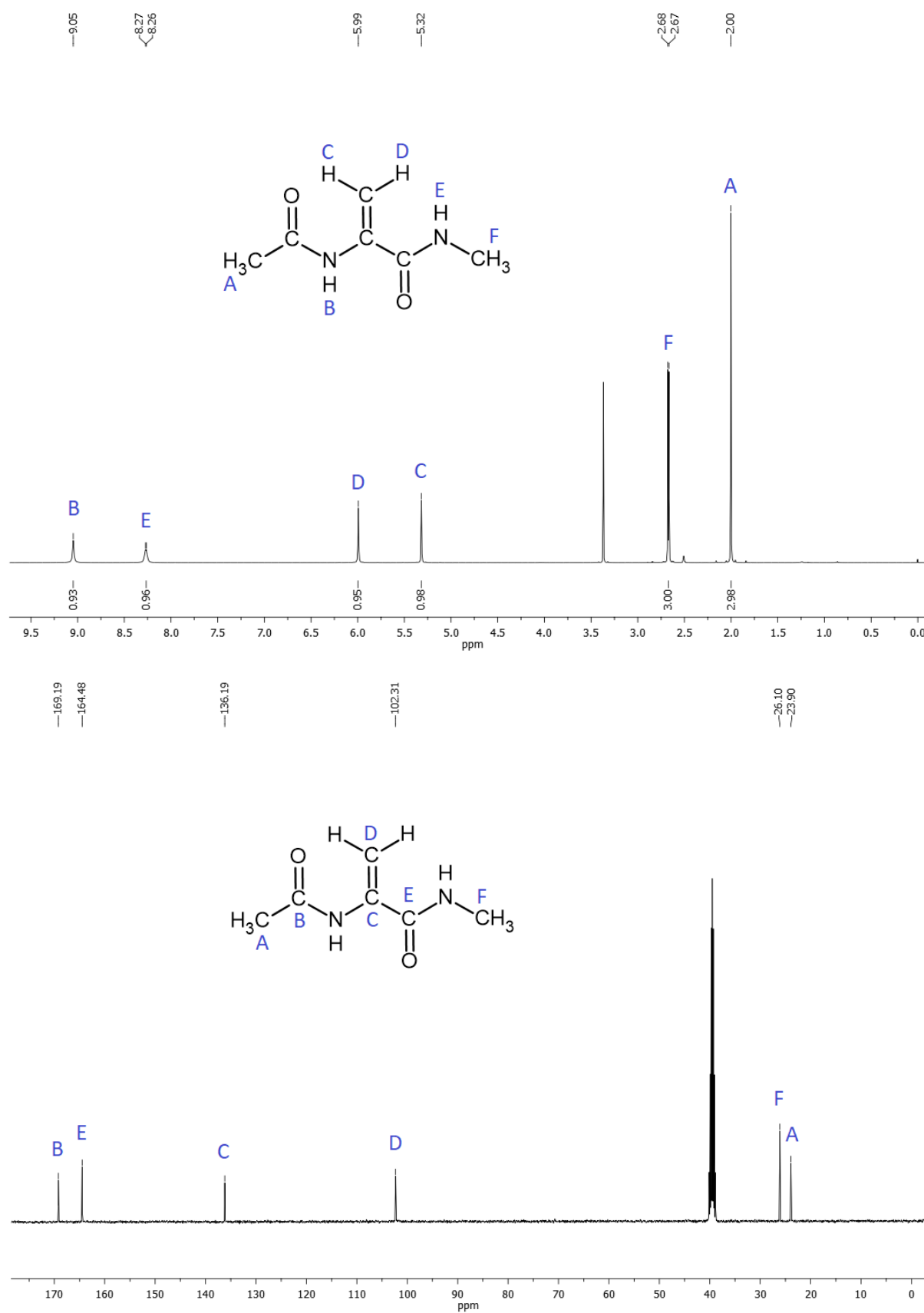


Figure 5S. ^1H and ^{13}C NMR spectra of Ac- Δ Ala-NHMe in $\text{DMSO}-d_6$.

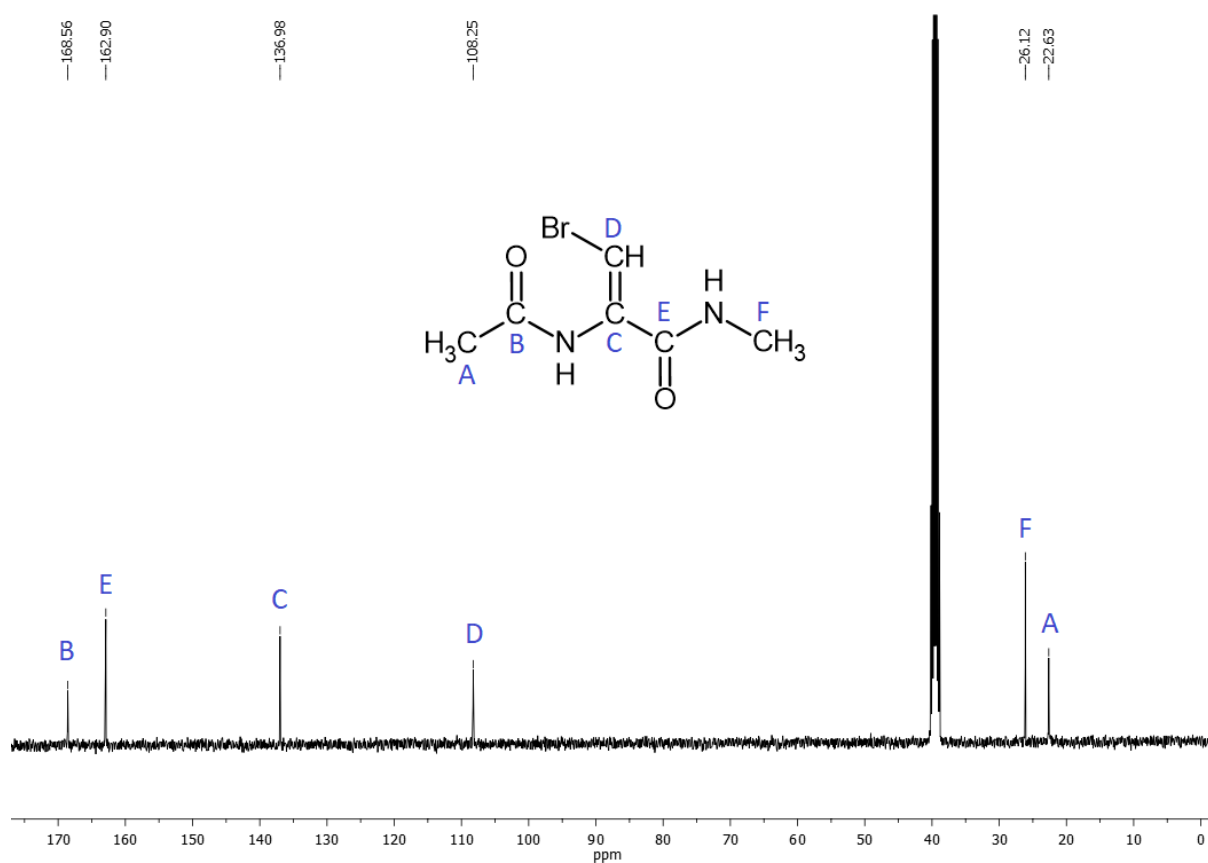
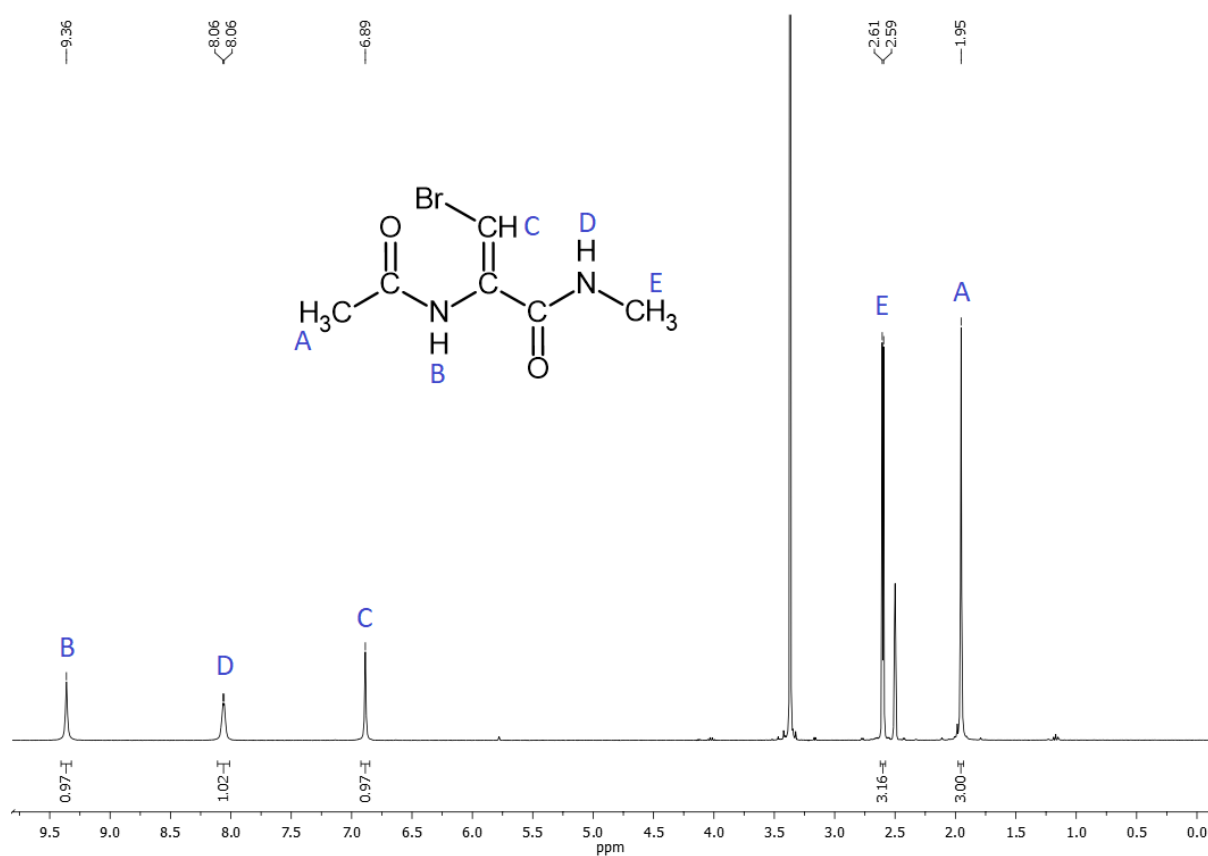
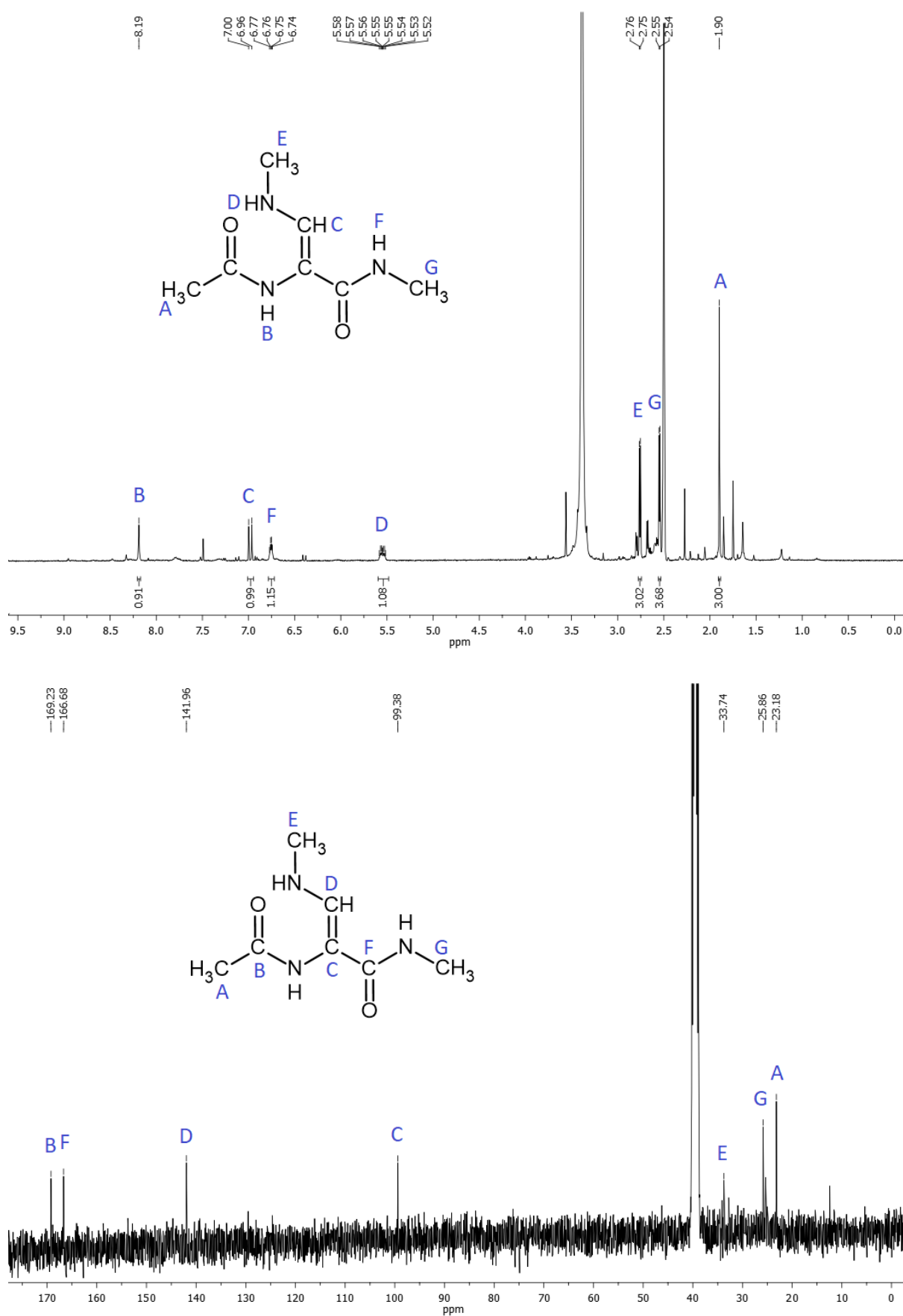


Figure 6S. ^1H and ^{13}C NMR spectra of Ac- $\Delta\text{Ala}(\beta\text{-Br})\text{-NHMe}$ in $\text{DMSO-}d_6$.**Figure 7S.** ^1H and ^{13}C NMR spectra of Ac- $\Delta\text{Ala}(\beta\text{-NHMe})\text{-NHMe}$ in $\text{DMSO-}d_6$.

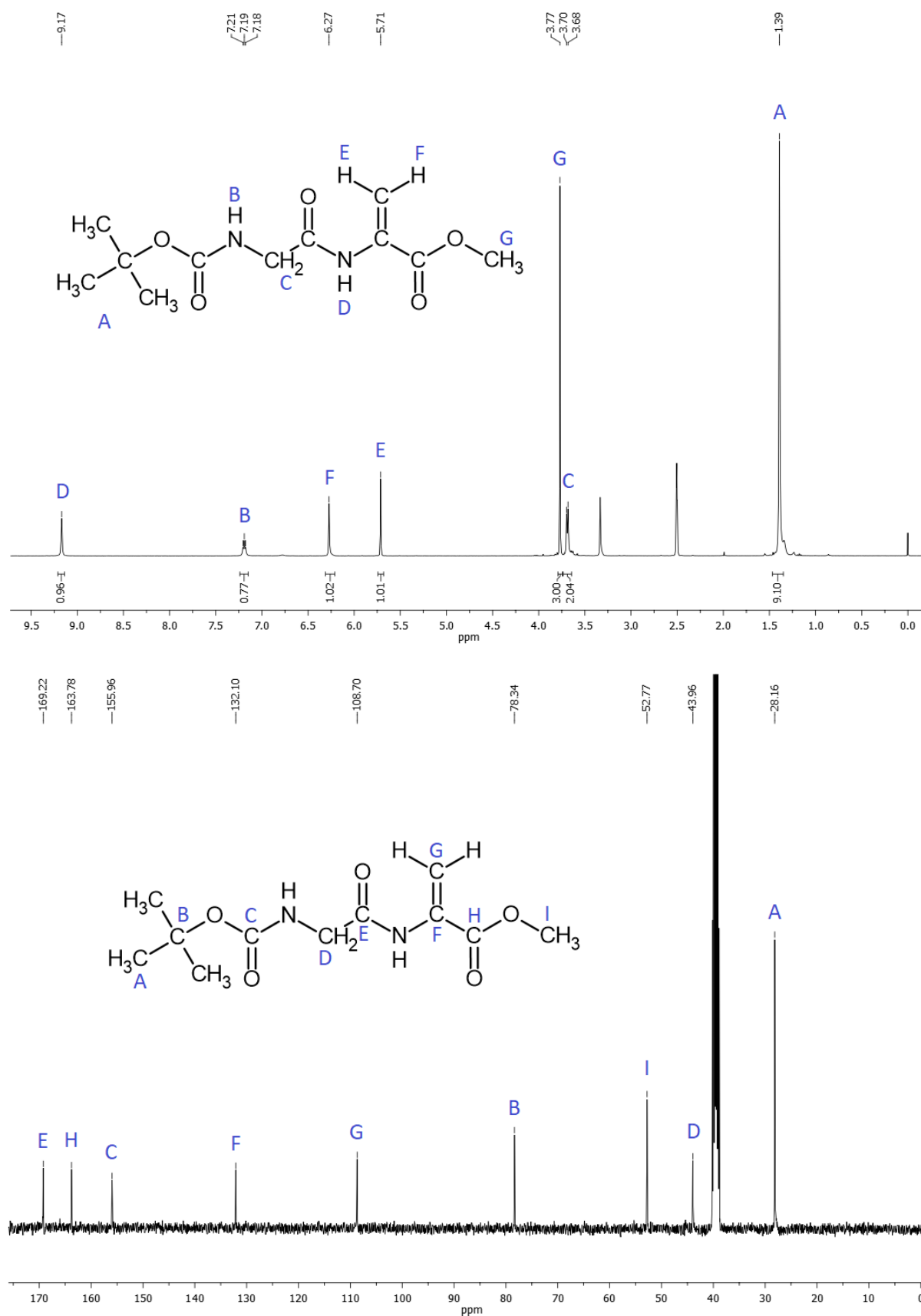


Figure 8S. ^1H and ^{13}C NMR spectra of Boc-Gly- Δ Ala-OMe in $\text{DMSO-}d_6$.

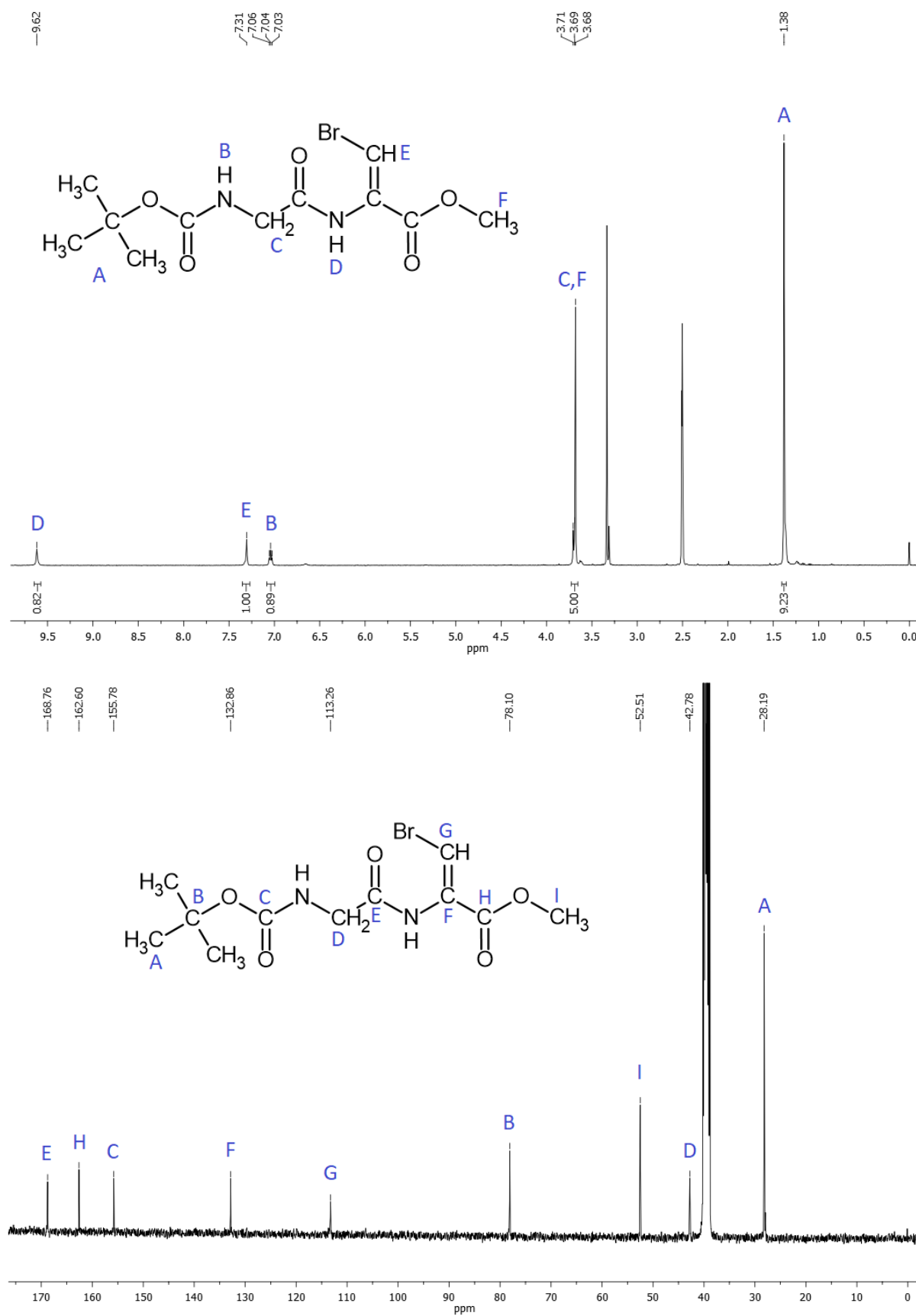


Figure 9S. ^1H and ^{13}C NMR spectra of Boc-Gly- Δ Ala(β -Br)-OMe in $\text{DMSO}-d_6$.

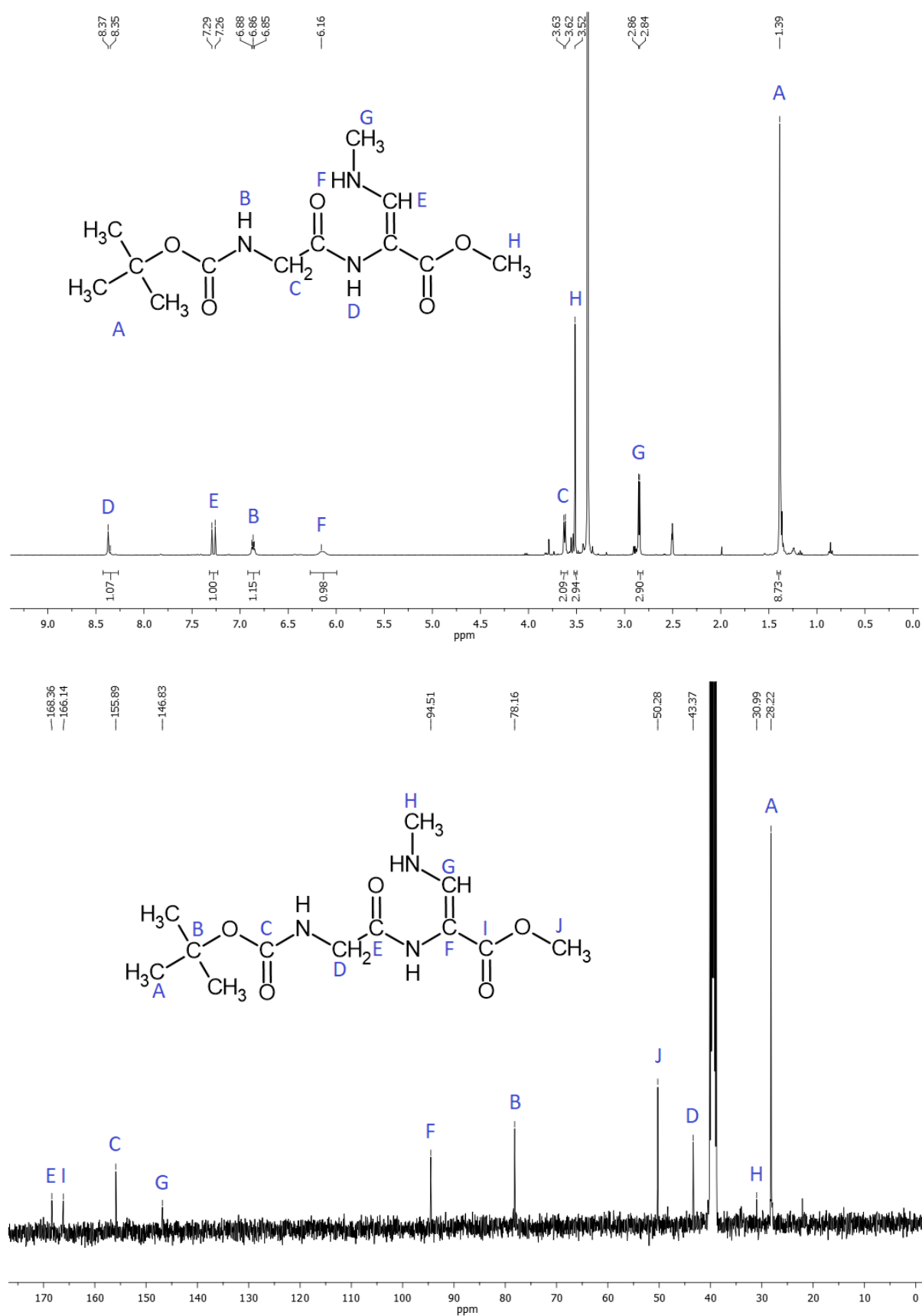


Figure 10S. ¹H and ¹³C NMR spectra of Boc-Gly-ΔAla(β-NHMe)-OMe in DMSO-*d*₆.

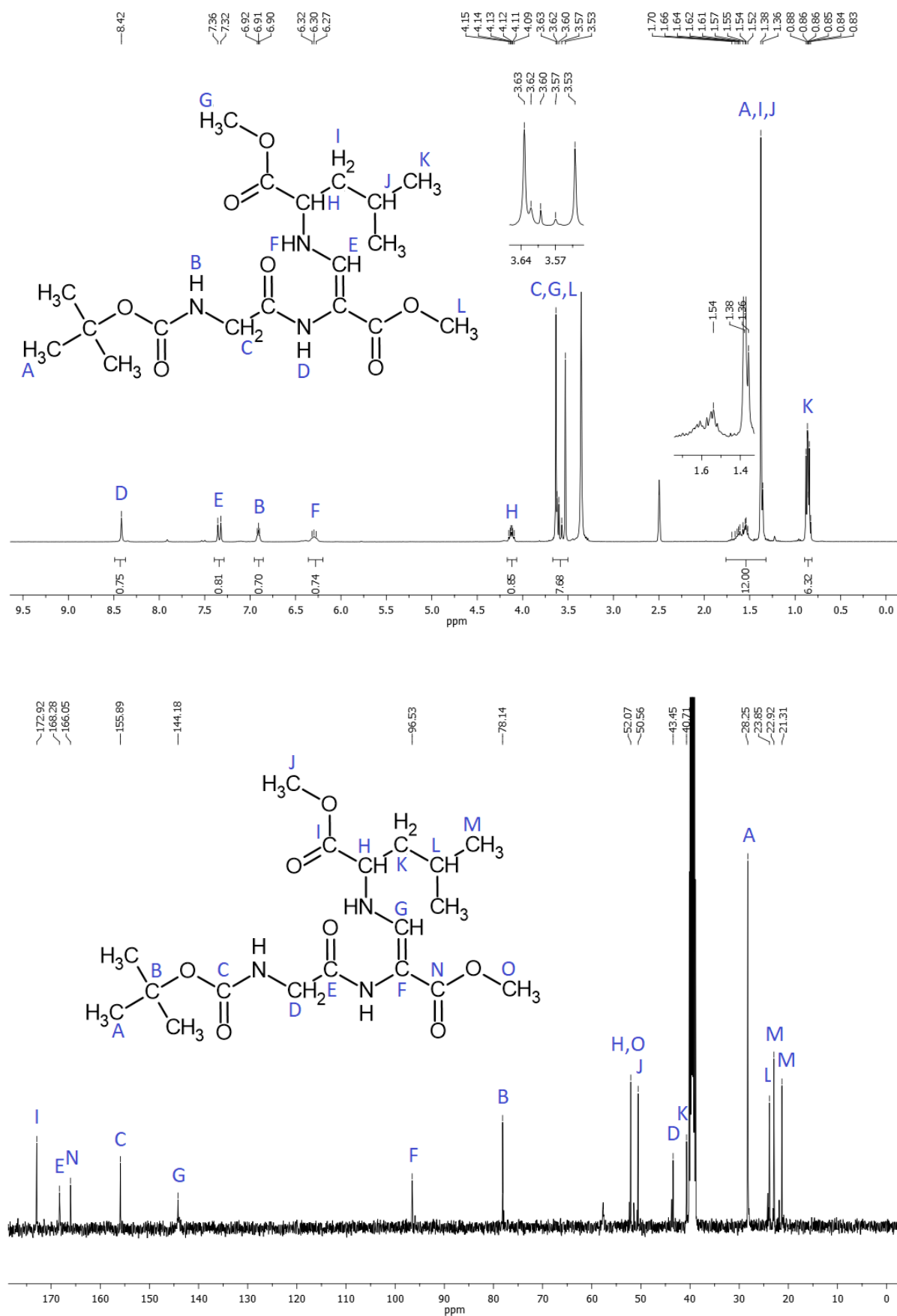


Figure 11S. ^1H and ^{13}C NMR spectra of Boc-Gly- Δ Ala(β -Leu-OMe)-OMe in $\text{DMSO-}d_6$.

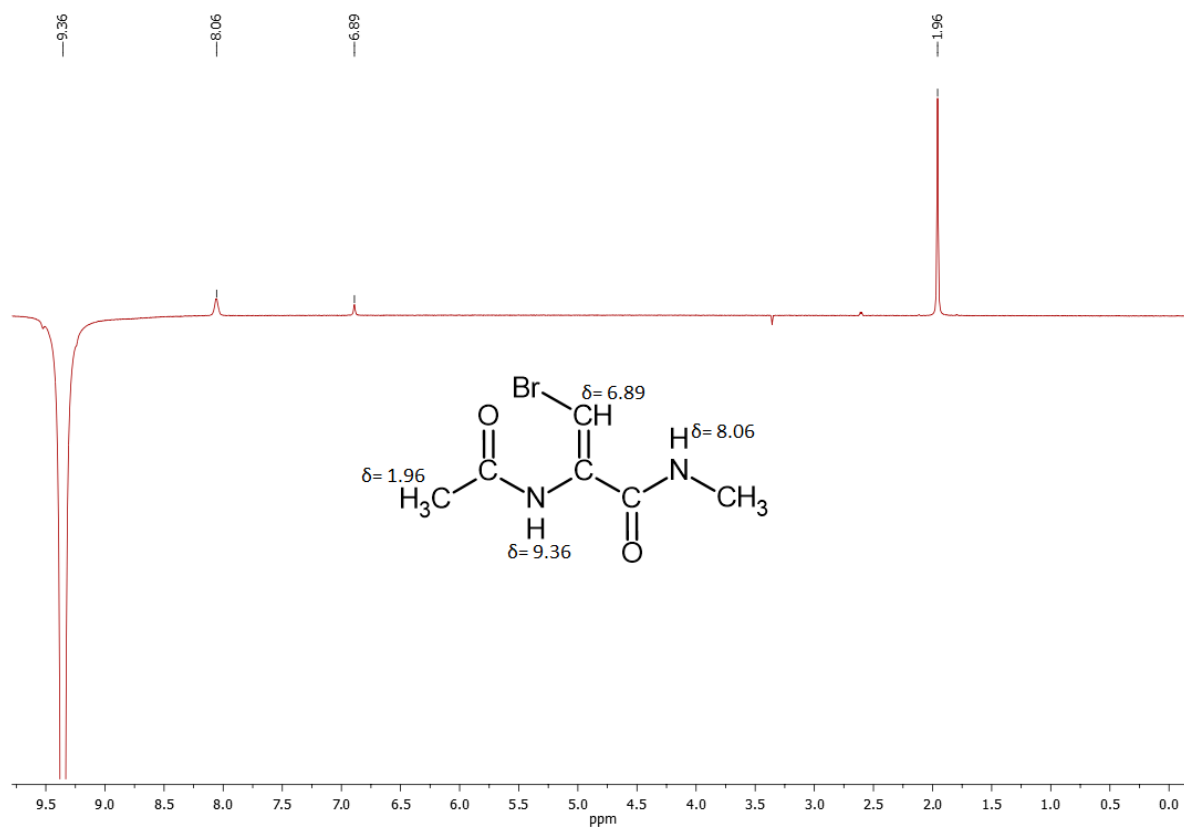


Figure 12S. ¹H NMR 1D-NOE spectrum obtained by selective excitation of the N-terminal amide H atom of Ac-ΔAla(β-Br)-NHMe in DMSO-*d*₆.

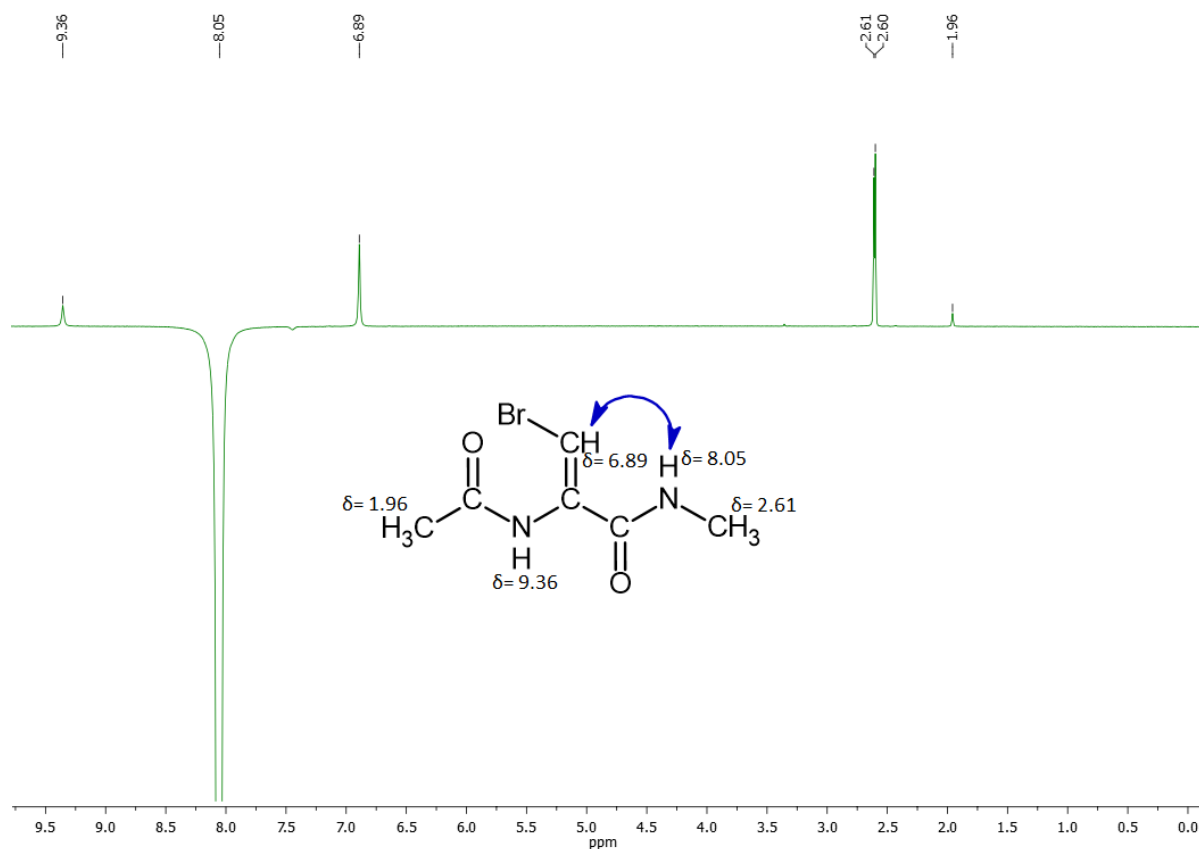


Figure 13S. ¹H NMR 1D-NOE spectrum obtained by selective excitation of the C-terminal amide H atom of Ac-ΔAla(β-Br)-NHMe in DMSO-*d*₆.

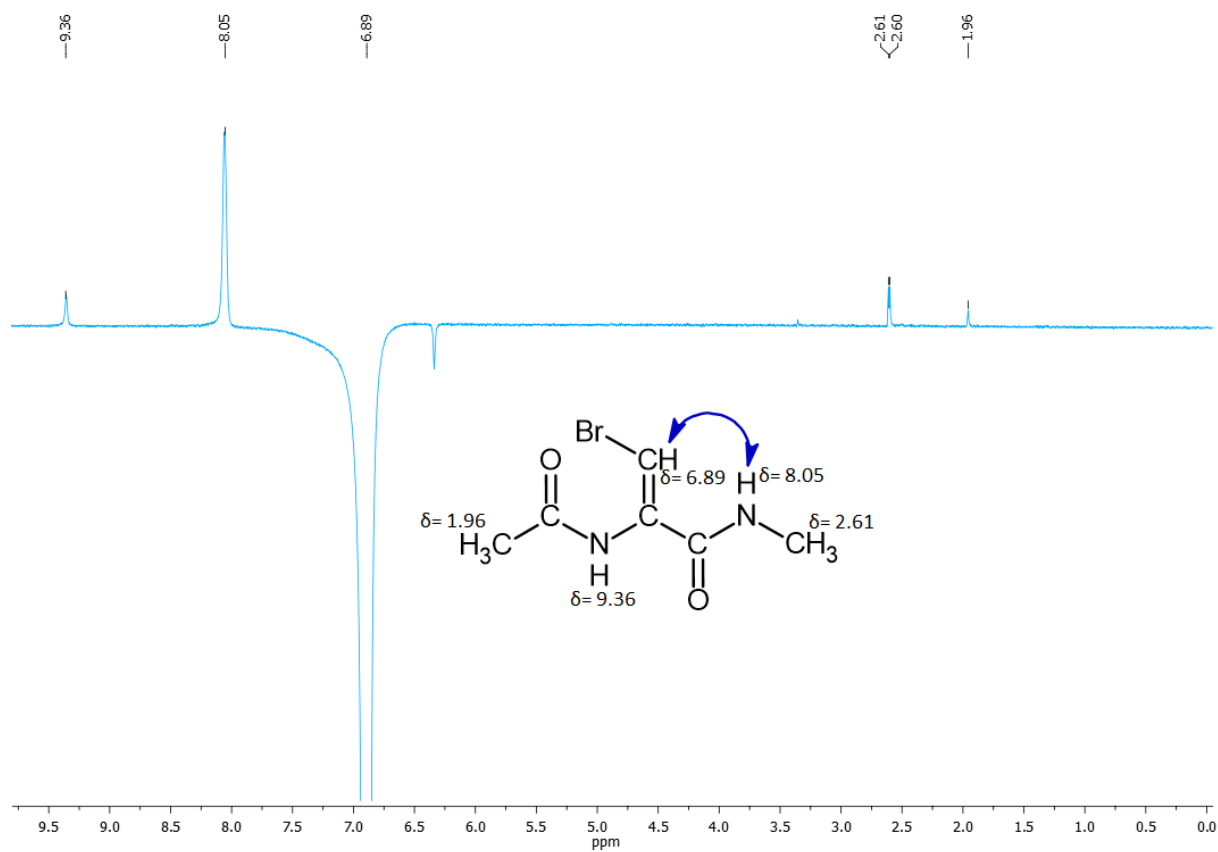


Figure 14S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (vinylic proton) of Ac- Δ Ala(β -Br)-NHMe in $\text{DMSO-}d_6$.

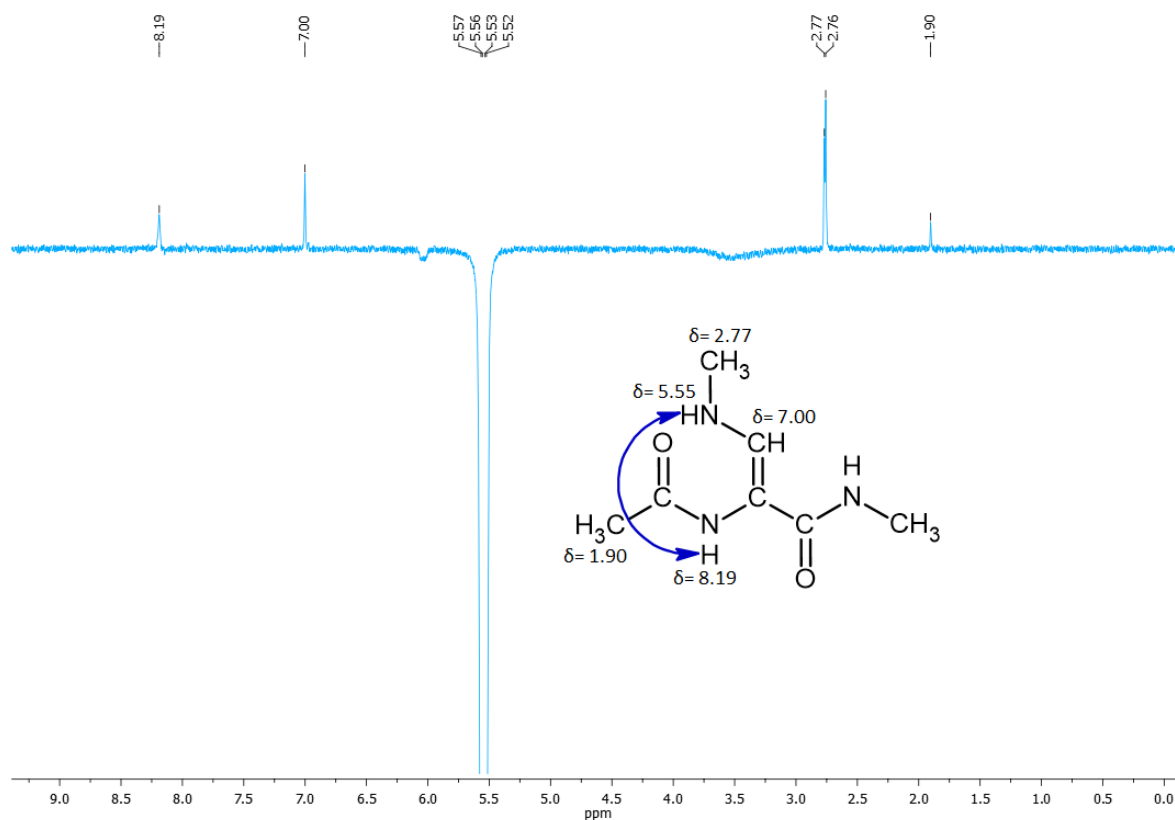


Figure 15S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (N-H group) of Ac- $\Delta\text{Ala}(\beta\text{-NHMe})\text{-NHMe}$ in $\text{DMSO-}d_6$.

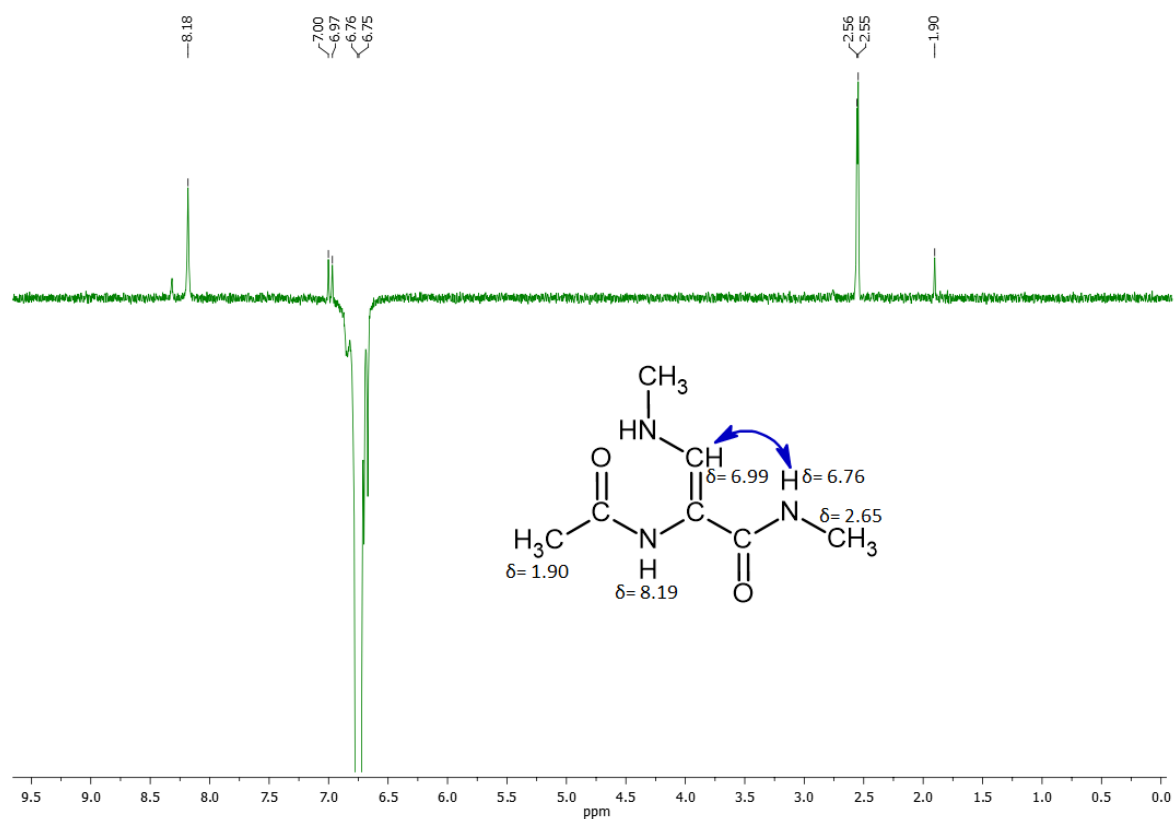


Figure 16S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the C-terminal amide H atom of Ac- $\Delta\text{Ala}(\beta\text{-NHMe})\text{-NHMe}$ in $\text{DMSO-}d_6$.

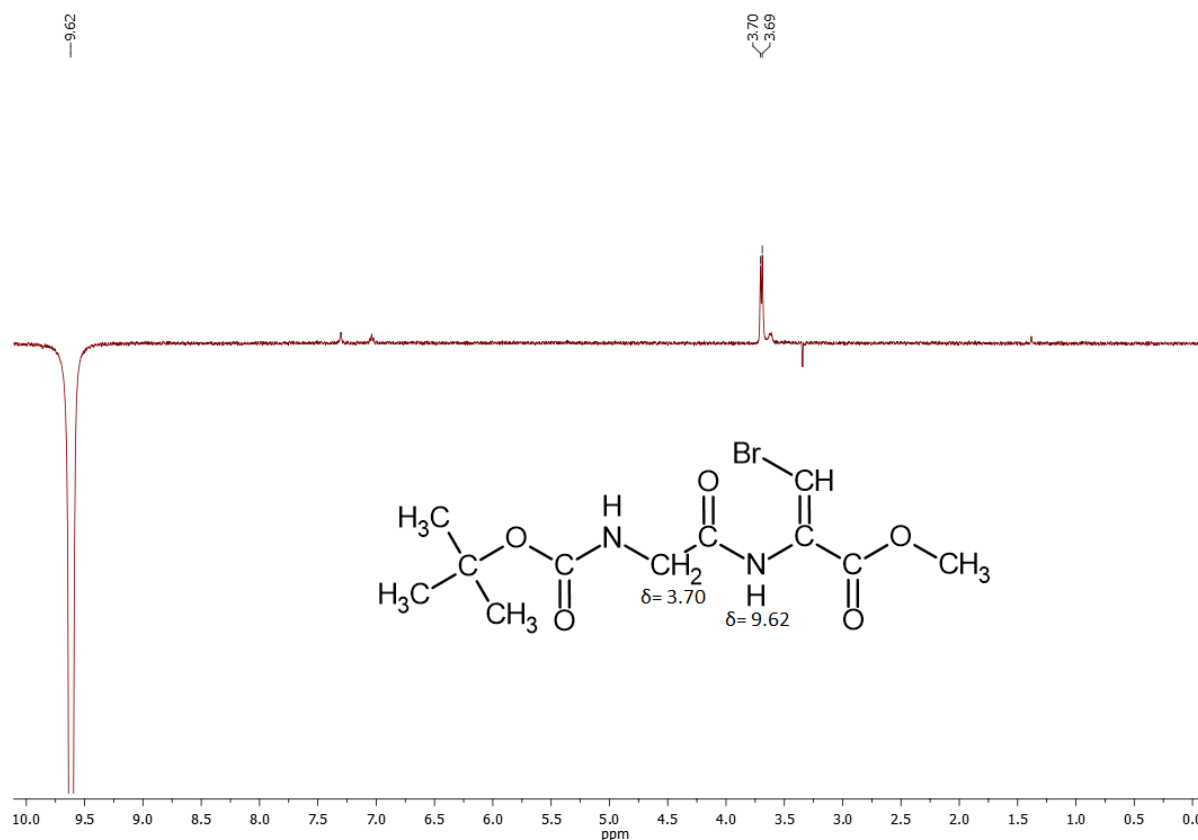


Figure 17S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the amide H atom (amide group of Δ Ala(β -Br)) of Boc-Gly- Δ Ala(β -Br)-OMe in $\text{DMSO-}d_6$.

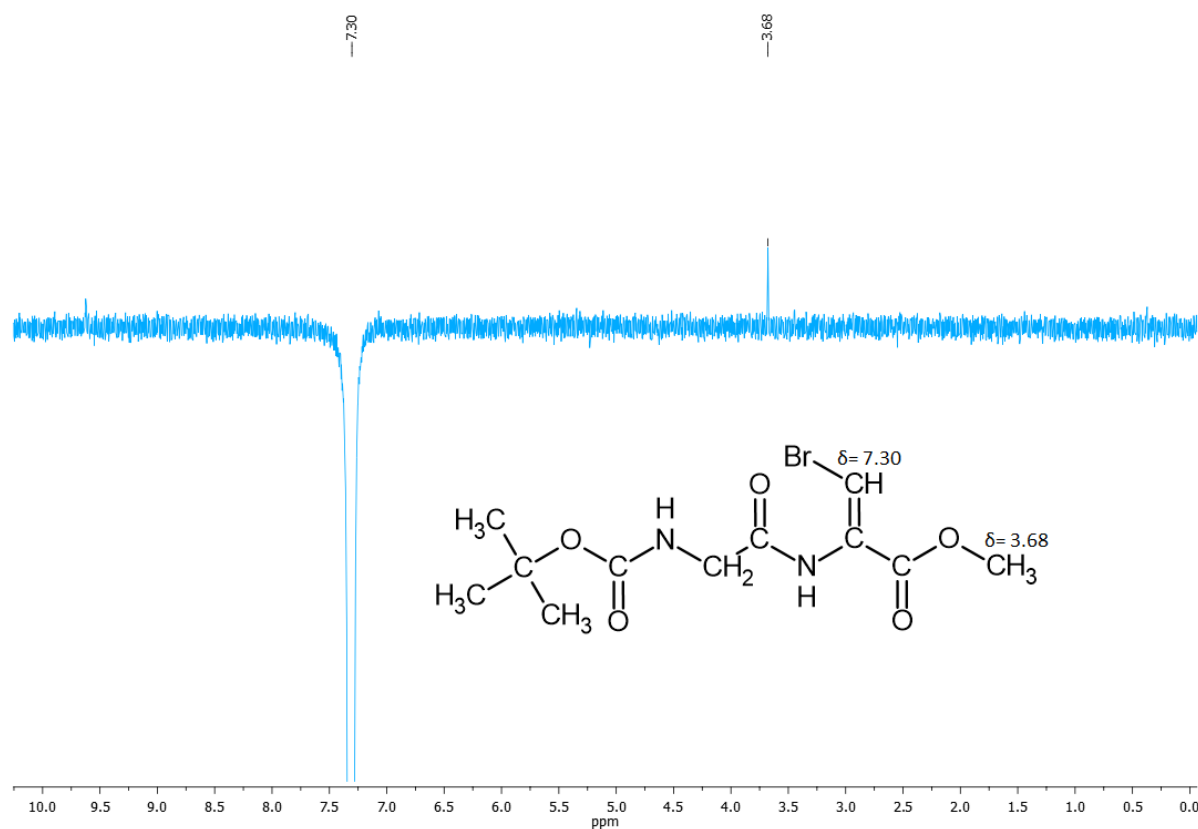


Figure 18S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (vinyl proton) of Boc-Gly- Δ Ala(β -Br)-OMe in $\text{DMSO-}d_6$.

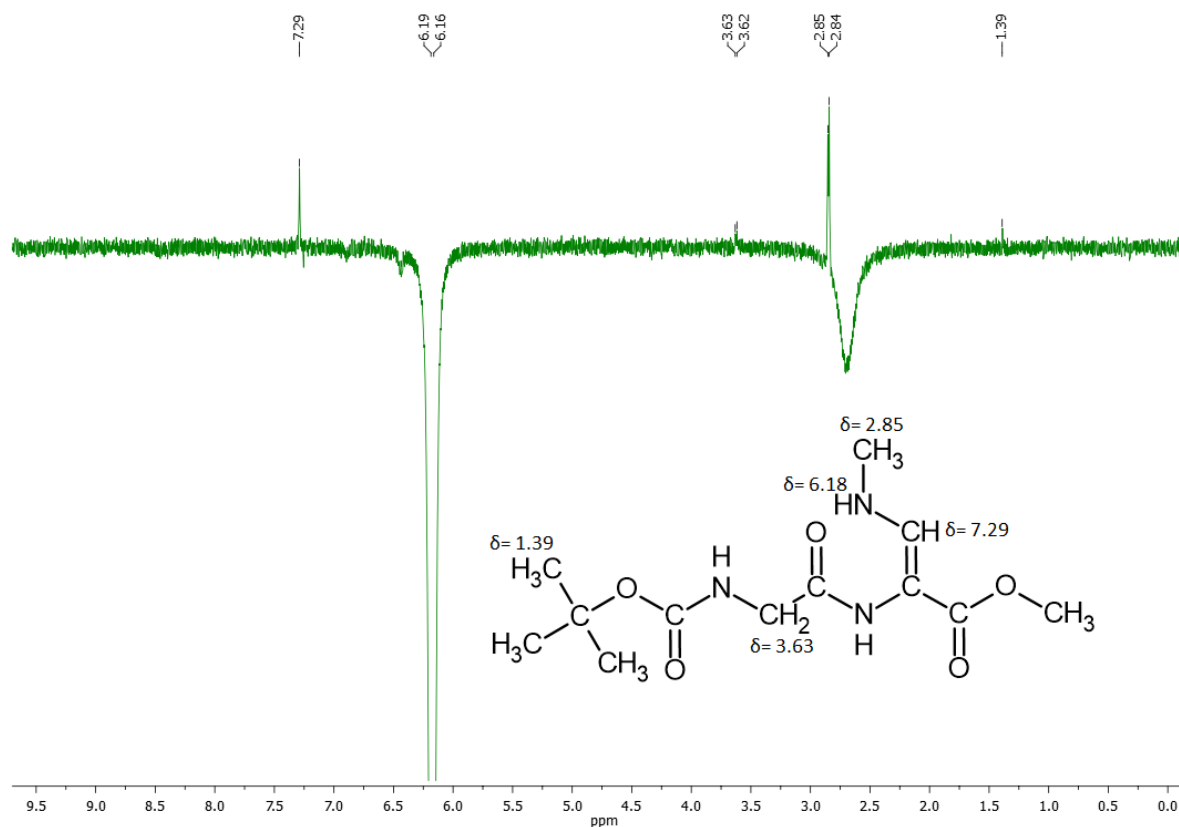


Figure 19S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (NH group) of Boc-Gly- Δ Ala(β -NHMe)-OMe in $\text{DMSO}-d_6$.

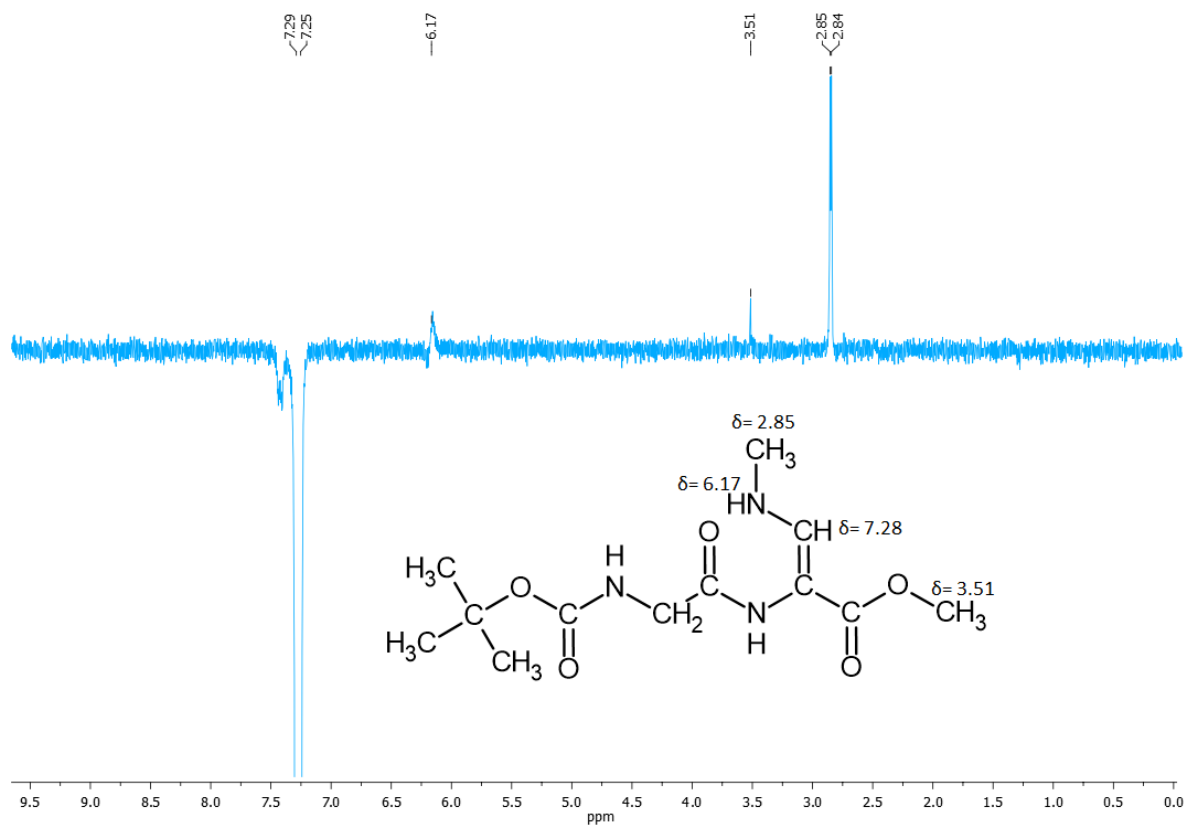


Figure 20S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (vinylic proton) of Boc-Gly- Δ Ala(β -NHMe)-OMe in $\text{DMSO}-d_6$.

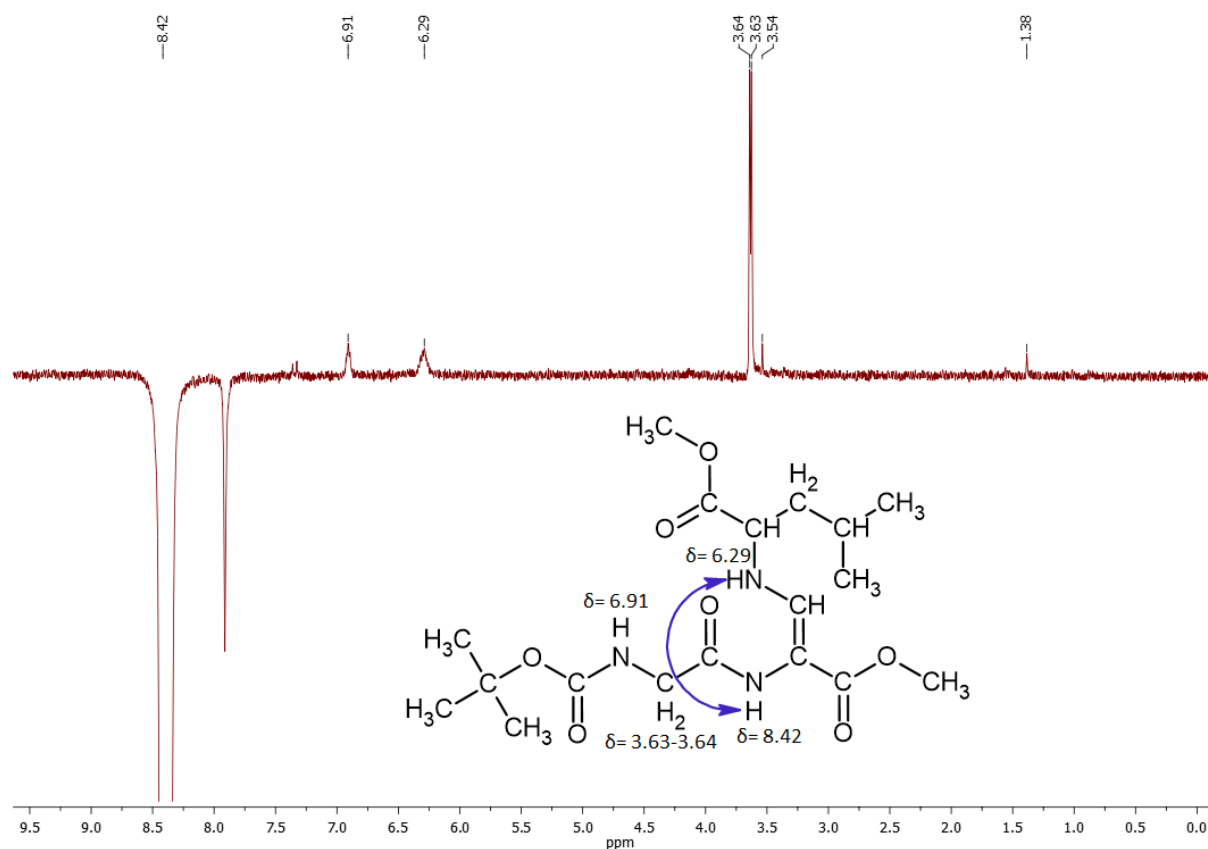


Figure 21S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the amide H atom (amide group of Δ Ala(β -Leu)) of Boc-Gly- Δ Ala(β -Leu-OMe)-OMe in $\text{DMSO}-d_6$.

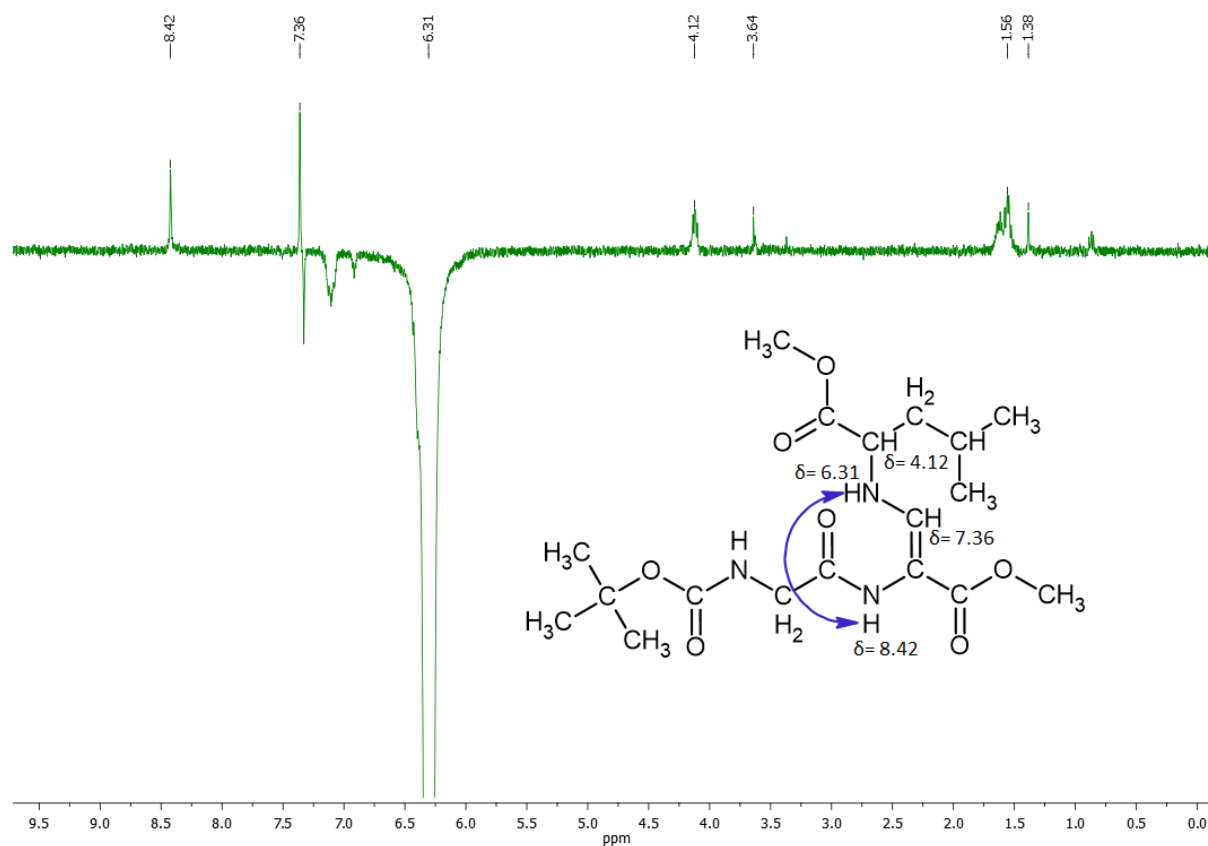


Figure 22S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (NH group) of Boc-Gly- Δ Ala(β -Leu-OMe)-OMe in $\text{DMSO}-d_6$.

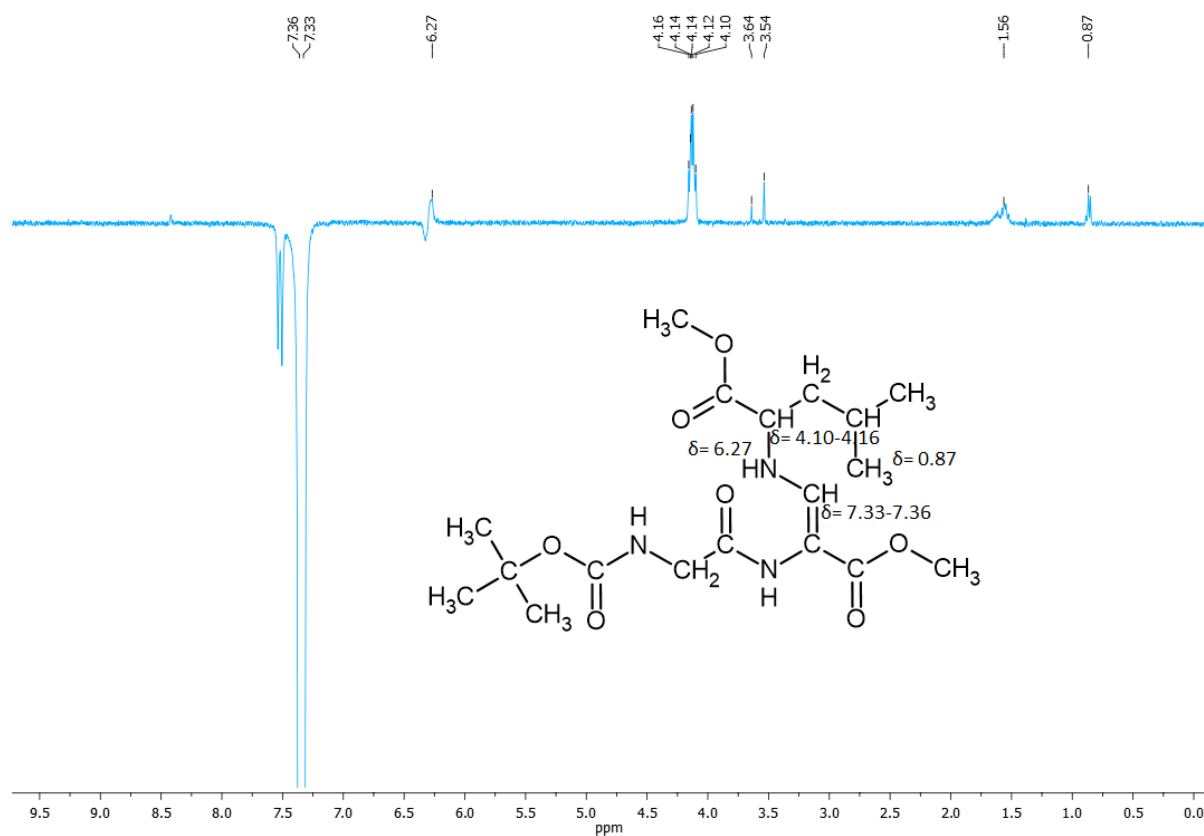


Figure 23S. ^1H NMR 1D-NOE spectrum obtained by selective excitation of the side chain H atom (vinylic proton) of Boc-Gly- Δ Ala(β -Leu-OMe)-OMe in $\text{DMSO-}d_6$.