Prolyl 4-Hydroxylase: Substrate Isosteres in Which an (*E*)- or (*Z*)-Alkene Replaces the Prolyl Peptide Bond

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Peptide Synthesis

General

Reagent chemicals were obtained from commercial sources (*e.g.*, Sigma–Aldrich, Acros, Combi-Blocks, Oakwood Products, Enamine, Bachem, and Novabiochem) and used without further purification.

All glassware was flame- or oven-dried, and reactions were performed under N_2(g) unless indicated otherwise. DCM and toluene were dried by passage through a column of alumina. Dimethylformamide was also dried by passage through a column of alumina and purified further by passage over an isocyanate scrubbing column. These columns were from Pure Process Technology (Nashua, NH). Other anhydrous solvents were obtained in septum-sealed bottles. Flash chromatography was performed with columns of 40–63 Å silica gel, 230–400 mesh from Silicycle (Québec City, Canada). Thin-layer chromatography (TLC) was performed on plates of EMD 250 μm silica 60-F_{254} with visualization by UV light or staining with $KMnO_4$.

The phrase "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining water-bath temperature below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr), where the term "high vacuum" refers to vacuum achieved by a mechanical belt-drive oil pump. All reported yields are unoptimized.

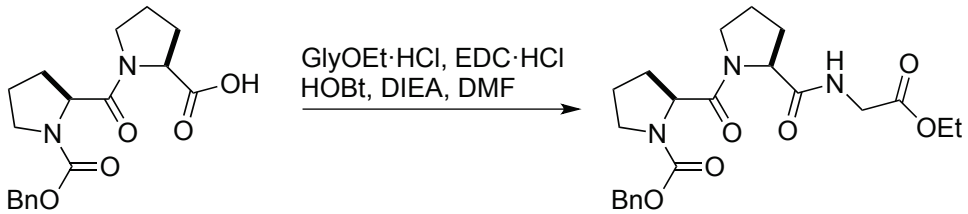
All procedures were performed in air at ambient temperature (~22 °C) and pressure (1.0 atm) unless specified otherwise.

Instrumentation

NMR spectra were acquired at ambient temperature with a DMX-400 Avance spectrometer or an Avance 500i spectrometer from Bruker (Billerica, MA) at the National Magnetic Resonance Facility at Madison (NMRFAM) and were referenced to TMS or a residual protic solvent. Some compounds exist as mixtures of rotomers that do not interconvert on the NMR timescale at ambient temperature and therefore exhibit multiple sets of NMR signals, as indicated. Electrospray ionization (ESI) and electron ionization (EI) mass spectrometry were performed with Micromass LCT® and Micromass AutoSpec® instruments, respectively, from Waters (Milford, MA) at the Mass Spectrometry Facility in the Department of Chemistry at the University of Wisconsin–Madison. X-Ray crystallography was performed at the Molecular Structure Laboratory in the Department of Chemistry at the University of Wisconsin–Madison.

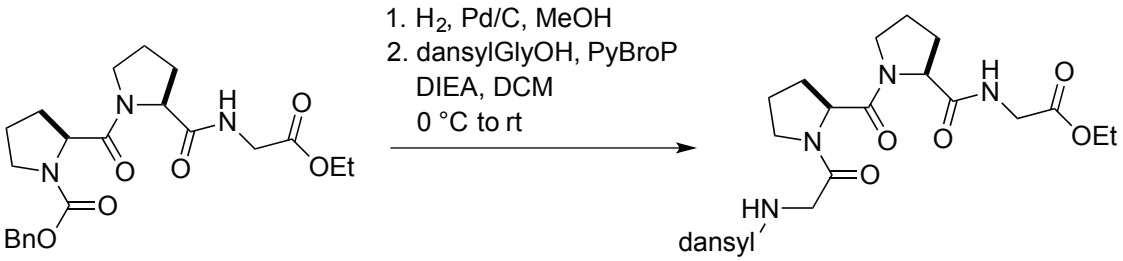
Synthesis of Tetrapeptide Substrates

N-Benzyloxycarbonyl-(2*S*)-prolyl-(2*S*)-prolylglycine Ethyl Ester (CbzProProGlyOEt)



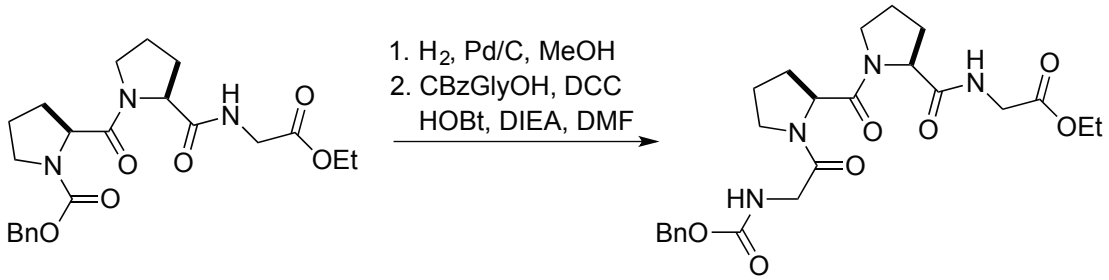
CbzProProGlyOEt was synthesized as described previously.[32] The spectral data and yield were in accord with those reported previously.

N-Dansylglycyl-(2*S*)-prolyl-(2*S*)-prolylglycine Ethyl Ester (DansylGlyProProGlyOEt, **1**)



DansylGlyProProGlyOEt (**1**) was synthesized as described previously.[32] The spectral data and yield were in accord with those reported previously. Purity: 99.90% by UPLC (retention time: 1.533 min).

N-Carboxybenzyloxyglycyl-(2*S*)-prolyl-(2*S*)-prolylglycine Ethyl Ester (CbzGlyProProGlyOEt, **2**)



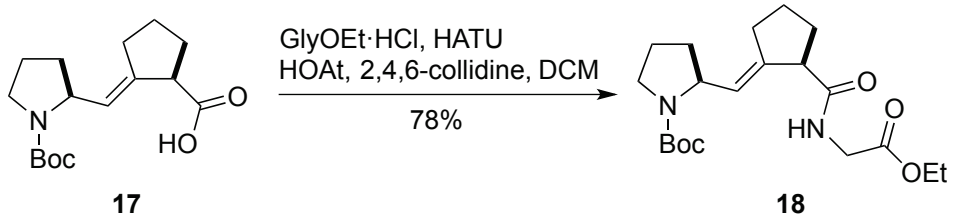
A suspension of CbzProProGlyOEt (1.08 g, 2.5 mmol) and Pd/C (10% w/w, 114 mg) in MeOH (25 mL) was stirred under an atmosphere of H_2(g) for 3 h. The mixture was filtered through a pad of Celite®, and concentrated under reduced pressure to a pale yellow viscous oil (835 mg). A portion of the oil was carried on to the subsequent coupling step. The oil (682 mg, 2.29 mmol) was dissolved in anhydrous DMF (20 mL). CbzGlyOH (436 mg, 2.09 mmol), 1-hydroxybenzotriazole hydrate (288 mg, 2.09 mmol), and *N*,*N′*-dicyclohexylcarbodiimide (430 mg, 2.09 mmol) were added, and the resulting suspension was stirred. DIEA (1.2 mL) was

added, and the reaction mixture was stirred for an additional 34 h. The reaction mixture was filtered through filter paper and concentrated under reduced pressure, after which the residue was dissolved in EtOAc (50 mL) and washed with 5% w/v $KHCO_3$ (2 × 50 mL), 5% w/v $KHSO_4$ (2 × 50 mL), and brine (1 × 50 mL). The resulting solution was dried with Na_2SO_4(s), and concentrated under reduced pressure to afford a crude yellow oil. The crude product was purified by chromatography on silica (20% v/v MeOH in EtOAc) and HPLC (30–90% v/v acetonitrile in water containing 0.1% v/v TFA) to afford CbzGlyProProGlyOEt (**2**) (282 mg, 28%) as a yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$, mixture of 3 or more rotomers, integrations are approximate, δ): 10.3 (s, 4.4 H, TFA), 8.38 (t, J = 5.6 Hz, 1.0H), 7.53–7.28 (m, 17.3H), 7.13 (m, 0.2H), 6.12–5.95 (m, 0.6H), 5.87 (m, 1.5H), 5.71 (m, 0.7H), 5.13–5.05 (m, 6.0H), 4.66–4.42 (m, 5.3H), 4.20–3.89 (m, 16.2H), 3.79–3.39 (m, 11.9H), 2.97 (s, 0.2H), 2.53–2.44 (m, 1.0H), 2.28–1.78 (m, 21.1H), 1.26–1.20 (m, 7.5H); **^{13}C NMR** (100 MHz, $CDCl_3$, mixture of 3 or more rotomers, δ): 172.3, 172.0, 171.8, 171.7, 171.6, 171.3, 171.2, 171.0, 169.6, 169.1, 168.0, 167.9, 159.4, 159.0, 158.6, 158.2, 156.7, 156.4, 136.2, 128.5 (2 signals), 128.2, 128.1, 128.0, 127.9, 116.3, 113.5, 67.3, 67.2, 67.0, 61.6, 61.4, 61.3, 61.1, 61.0, 60.1, 60.0 59.2, 58.4, 47.5, 47.3 (2 signals), 46.7, 46.5, 43.3, 43.2, 42.6, 42.5, 41.5, 41.3, 31.8, 31.7, 28.4, 28.3, 28.1, 27.7, 25.1, 24.7, 22.1, 22.0, 14.0; **ESI–EMM** (m/z): $[M + Na]^+$ calcd for $C_{24}H_{32}N_4O_7Na$, 511.2164; found, 511.2155. Purity: 96.54% by UPLC (retention time: 2.017 min).

Synthesis of *Trans* Isosteres

The syntheses of dansylGlyPro*trans*ProGlyOEt (**3**) and CbzGlyPro*trans*ProGlyOEt (**4**) were adapted from previous reports[35-39] using the route in Scheme 1.

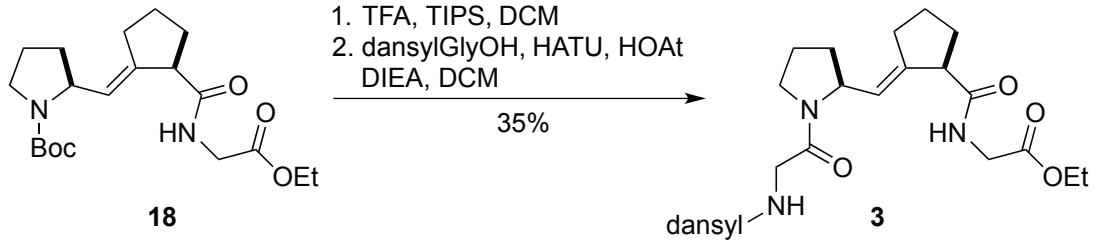
(*E*)-(*S*,*S*)-*N*-(2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl)carbonyl]glycine ethyl ester (**18**)



Acid **17** (100 mg, 0.34 mmol) was dissolved in anhydrous DCM (6.8 mL). Glycine ethyl ester hydrochloride (52 mg, 0.373 mmol), 1-hydroxy-7-azobenzotriazole (138 mg, 1.01 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (386 mg, 1.01 mmol) were added, and the resulting suspension was stirred under N_2(g). 2,4,6-Collidine (0.22 mL, 1.70 mmol) was added, and the reaction mixture was stirred for 30 min. Brine was added, after which the biphasic mixture was extracted with EtOAc. The organic extracts were combined, dried with Na_2SO_4(s), and concentrated under reduced pressure to afford a crude oil. The crude product was purified by chromatography on silica (30–100% v/v EtOAc in hexanes) to afford compound **18** (60 mg, 47%) as a pale yellow oil, which was taken on slightly crude to the next step. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 5.50 (bs, 1H), 4.38 (bs, 1H), 4.21 (app q, J = 7.6 Hz, 2H), 4.02–4.00 (m, 2H), 3.44–3.39 (m, 2H), 3.26 (bs, 1H), 2.57–2.48

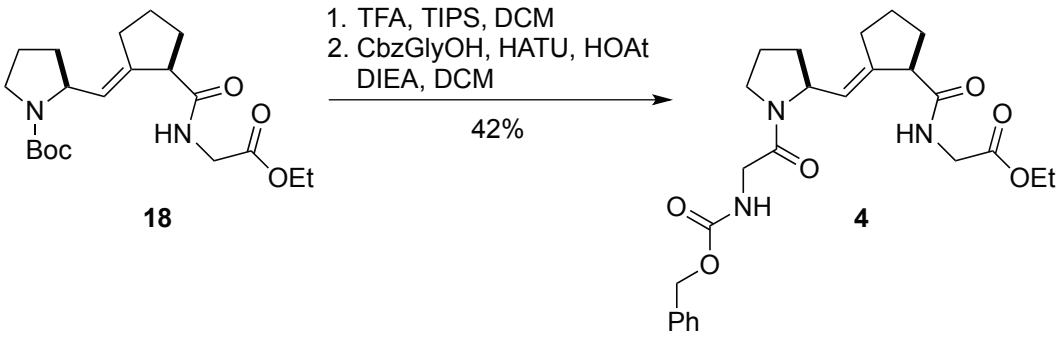
(m, 1H), 2.32–2.24 (m, 1H), 2.14–2.04 (m, 2H), 1.95–1.67 (m, 6H), 1.60 (s, 3H), 1.42 (s, 9H), 1.28 (t, J = 7.2 Hz, 3H).

DansylGlyPro*trans*ProGlyOEt (**3**)



Compound **18** (60 mg, 0.16 mmol) was dissolved in dry DCM (0.88 mL) in a vial, and triisopropylsilane (0.08 mL, 0.16 mmol) was added to the resulting solution. TFA (0.88 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was suspended in dry DCM (3.2 mL). DansylGlyOH (49 mg, 0.16 mmol), 1-hydroxy-7-azobenzotriazole (65 mg, 0.16 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (180 mg, 0.16 mmol) were added, and the reaction mixture was stirred. DIEA (0.14 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the crude product was purified by chromatography on silica (70% v/v EtOAc in hexanes) and HPLC (25–55% v/v acetonitrile in water containing 0.1% v/v TFA) to afford dansylGlyPro*trans*ProGlyOEt (**3**) (32 mg, 35%) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of 2 or more rotomers, integrations are approximate, δ): 8.55 (d, J = 9.0 Hz, 1.0H), 8.33 (dd, J = 9.0, 2.0 Hz, 1.0H), 8.22 (d, J = 7.0 Hz, 0.5H), 8.16 (d, J = 6.5 Hz, 0.5H), 7.58 (q, J = 8.0 Hz, 1.0H), 7.53–7.48 (m, 1.0H), 7.20 (dd, J = 7.5, 5.0 Hz, 0.9H), 6.98 (s, 0.1H), 6.47 (t, J = 5.0 Hz, 0.5H), 6.18 (t, J = 5.0 H, 0.5H), 5.87–5.84 (m, 0.9H), 5.32–5.27 (m, 1.0H), 4.50–4.46 (m, 0.5H), 4.22–4.12 (m, 2.4H), 4.06–3.94 (m, 2.0H), 3.66 (d, J = 4.5 Hz, 1.1H), 3.62 (d, J = 4.5 Hz, 0.4H), 3.57 (d, J = 4.0 Hz, 0.3H), 3.54 (d, J = 4.0 Hz, 0.2H), 3.49–3.38 (m, 1.0H), 3.34–3.30 (m, 0.5H), 3.25–3.15 (m, 0.9H), 3.09–3.06 (m, 0.5H), 2.89 (s, 6.0H), 2.57–2.50 (m, 0.5H), 2.36–1.96 (m, 4.2H), 1.93–1.59 (m, 13.0H), 1.43 (s, 0.6H), 1.29–1.25 (m, 5.0H). 0.93–0.82 (m, 0.5H), 0.07 (s, 0.6H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of 2 or more rotomers, δ): 174.2, 173.5 170.5, 170.3, 165.9, 165.2, 152.0, 151.9, 144.8, 144.2, 134.6, 134.5, 130.9, 130.8, 130.2 (2 signals), 130.1, 129.7, 129.5, 129.0, 128.9, 124.4 (2 signals), 123.4, 123.2, 119.6, 119.5, 115.9, 115.7, 61.9 (2 signals), 57.5, 57.0, 52.3, 52.0, 47.0, 46.0, 45.8, 45.0, 44.4, 41.8, 33.8, 31.5, 31.0, 30.7 (2 signals), 30.0, 29.4, 29.2, 25.3, 25.2, 24.7, 23.0, 14.5, 1.4; **ESI–EMM** (m/z): $[M + H]^+$ calcd for $C_{29}H_{39}N_4O_6S$, 571.2585; found, 571.2580. Purity: 99.18% by UPLC (retention time: 1.973 min).

CbzGlyPro*trans*ProGlyOEt (**4**)

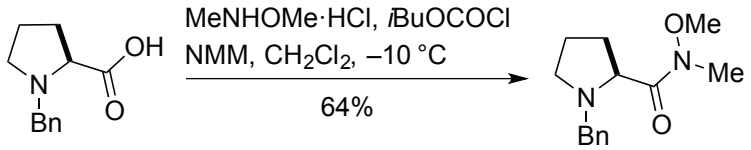


Compound **18** (87 mg, 0.23 mmol) was dissolved in dry DCM (1.3 mL) in a vial, and triisopropylsilane (0.094 mL, 0.46 mmol) was added to the resulting solution. TFA (1.3 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was suspended in dry DCM (4.6 mL). CbzGlyOH (48 mg, 0.23 mmol), 1-hydroxy-7-azobenzotriazole (94 mg, 0.69 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (262 mg, 0.69 mmol) were added, and the reaction mixture was stirred. DIEA (0.6 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, after which the crude product was purified by HPLC (30–90% v/v acetonitrile in water containing 0.1% v/v TFA) to afford CbzGlyPro*trans*ProGlyOEt (**4**) (45 mg, 42%) as a viscous yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of 2 or more rotomers, integrations are approximate, δ): 7.38–7.32 (m, 7.5H), 6.65 (t, J = 4.5 Hz, 1.0H), 6.44 (t, J = 4.5 Hz, 0.7H), 5.82–5.80 (m, 1.5H), 5.53–5.47 (m, 1.6H), 5.18–5.10 (m, 3.3H), 4.66–4.62 (m, 1.0H), 4.43–4.39 (m, 0.8H), 4.26–4.18 (m, 3.3H), 4.12 (d, J = 5.5 Hz, 0.4H), 4.09–3.88 (m, 6.4H), 3.65–3.43 (m, 4.6H), 3.33–3.28 (m, 2.1H), 2.69–2.62 (m, 1.0H), 2.55–2.48 (m, 0.8H), 2.40–2.20 (m, 2.8H), 2.17–1.70 (m, 12.4H), 1.33–1.27 (m, 4.7H); **^{13}C NMR** (100 MHz, $CDCl_3$, mixture of 2 or more rotomers, δ): 174.7, 174.1 170.0, 169.9, 167.8, 166.9, 156.5, 144.4, 143.5, 136.4, 129.9, 128.9, 128.7, 128.5, 128.1, 127.9 (2 signals), 124.5, 124.2, 68.3, 66.9, 61.5, 57.2, 56.9, 51.9, 51.7, 46.8, 45.9, 43.5, 42.8, 41.5, 33.6, 31.1, 30.7, 30.5, 29.2, 28.9, 25.0, 24.9, 24.5, 22.6, 14.1; **ESI–EMM** (m/z): $[M + H]^+$ calcd for $C_{25}H_{34}N_3O_6$, 472.2442; found, 472.2442. Purity: 97.41% by UPLC (retention time: 2.323 min).

Synthesis of *Cis* Isosteres

The alkene isosteres dansylGlyPro*cis*ProGlyOEt (**5**) and CbzGlyPro*cis*ProGlyOEt (**6**) were synthesized using the route described in Scheme 2. To avoid redundancy, only synthetic procedures and spectral data for new intermediates and the final targets are provided below.

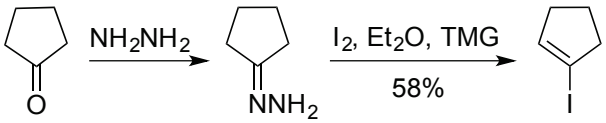
N-methoxy-*N*-methyl-1-(phenylmethyl)-(2*S*)-2-pyrrolidinecarboxamide (**20**)



19 **20**

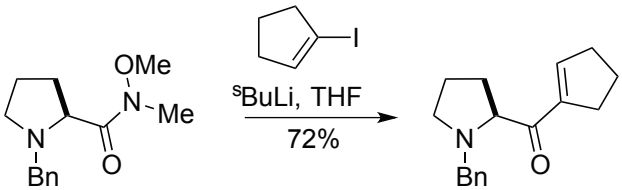
To a round-bottom flask was added compound **19** (8.1 g, 39 mmol). The flask was evacuated and purged with Ar(g). DCM (100 mL) was added, and the reaction mixture was cooled with stirring to –10 °C in an ice/NaCl bath. NMM (5.2 mL, 47 mmol) was added, after which isobutylchoroformate (7.7 mL, 59 mmol) was added dropwise. The reaction mixture was stirred for 30 min, after which *N*,*O*-dimethylhydroxylamine hydrochloride (5.8 mL, 59 mmol) was added. A second portion of NMM (9.4 mL, 86 mmol) was added, and the reaction mixture was stirred at –10 °C for 20 min. The reaction mixture was allowed to warm to ambient temperature and stirred for another 2 h, after which the mixture was diluted with DCM and washed with water and brine. The organic extract was dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica (1% v/v MeOH + 0.25% v/v NEt_3 in DCM, followed by 2% v/v MeOH + 0.5% v/v NEt_3 in DCM) to afford compound **20** (6.2 g, 64%) as a yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.35 (d, J = 7.3 Hz, 2H), 7.30 (dd, J = 7.3, 7.3 Hz, 2H), 7.24 (d, J = 7.1 Hz, 1H), 3.94 (d, J = 12.8 Hz, 1H), 3.57 (s, 3H), 3.55 (d, J = 12.8 Hz, 1H), 3.17 (s, 3H), 3.10 (ddd, J = 8.5, 8.5, 2.8 Hz, 1H), 2.44 (apparent q, J = 7.9 Hz, 1H), 2.19–2.11 (m, 1H), 1.97–1.75 (m, 4H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 175.1, 138.7, 129.5, 128.2, 127.1, 62.1, 61.3, 58.1, 53.0, 32.5, 29.2, 23.1; **ESI–EMM** (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{20}N_2O_2$, 249.1598; found, 249.1594.

1-Iodocyclopentene



1-Iodocyclopentene was synthesized as described previously.[40] The spectral data and yield were in accord with those reported previously.

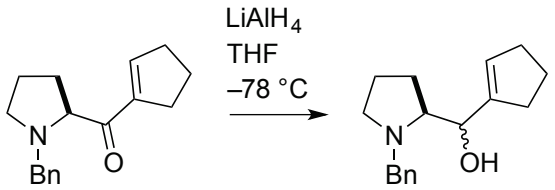
(*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanone (**21**)



20 **21**

To a round-bottom flask was added 1-iodopentene (6.1 g, 31.3 mmol) and a magnetic stir bar. The flask was evacuated and purged with Ar(g). Anhydrous THF (60 mL) was added to the flask, and the resulting solution was cooled to –40 °C in a dry ice/MeCN bath with stirring. A freshly titrated solution of ^{s}BuLi (1.02 M in cyclohexane, 30.7 mL, 31.3 mmol) was added dropwise, and the resulting solution was stirred at –40 °C for 90 min to prepare a solution of cyclopentenyllithium. In a separate flask, Weinreb amide **20** (4.6 g, 18.5 mmol) was dissolved in anhydrous THF (50 mL), and the reaction mixture was cooled to –78 °C. The solution of cyclopentenyllithium was added dropwise, and the reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl. The quenched reaction mixture was diluted with EtOAc. The resulting solution was washed with a saturated aqueous solution of NH_4Cl and with brine, dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure. The crude product was dissolved in benzene and frozen overnight, after which compound **21** was purified by chromatography on silica (25% v/v EtOAc + 0.5% v/v NEt_3 in hexanes) to afford compound **21** (1.65 g, 72%) as a brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.30–7.20 (m, 5H), 6.88 (bs, 1H), 3.86 (d, J = 12.7 Hz, 1H), 3.66 (dd, J = 9.1, 7.4 Hz, 1H), 3.39 (d, J = 12.7 Hz, 1H), 3.09–3.03 (m, 1H), 2.57–2.51 (m, 4H), 2.33 (apparent q, J = 8.2 Hz, 1H), 2.16–2.07 (m, 1H), 1.98–1.74 (m, 5H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 199.7, 144.4, 144.0, 138.7, 129.5, 128.3, 127.2, 69.6, 58.8, 53.4, 34.4, 31.3, 30.3, 23.1, 22.5; **ESI–EMM** (m/z): $[M + H]^+$ calcd for $C_{17}H_{21}NO$, 256.1696; found, 256.1703.

(*S*,*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanol (**22**)

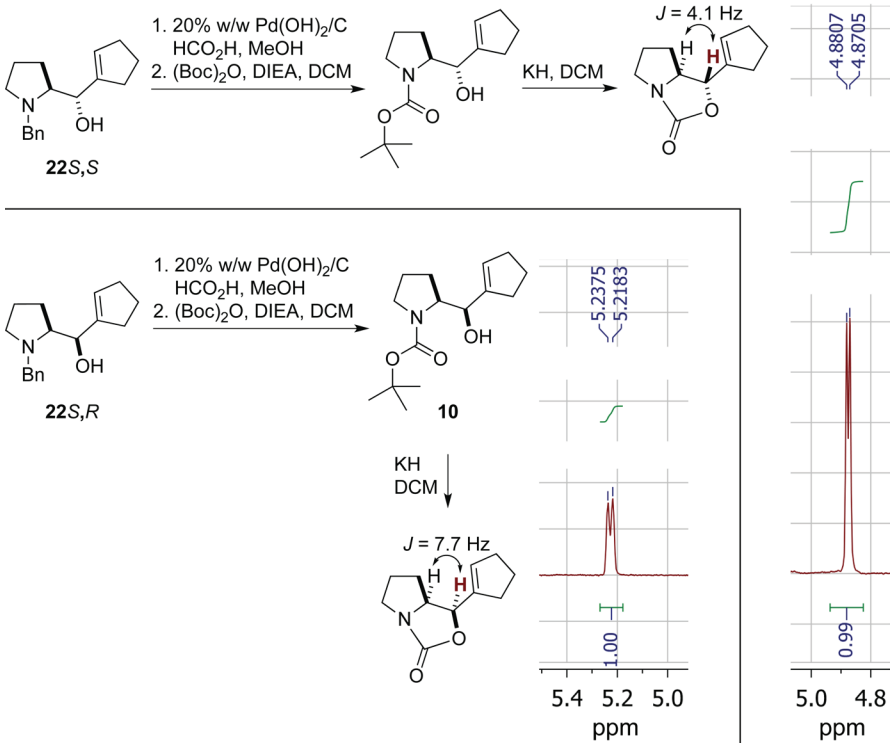


21 **22**

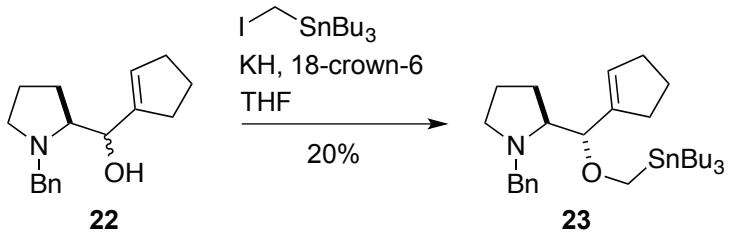
To a 250-mL round-bottom flask was added compound **21** (3.036 g, 11.89 mmol) and a magnetic stir bar. The flask was purged with Ar(g), and anhydrous THF (50 mL) was added. The reaction mixture was cooled to –78 °C in a dry ice/acetone bath. To the cooled reaction mixture was added $LiAlH_4$ (3.69 g, 97.2 mmol) in three portions. The reaction mixture was stirred for 1 h and then allowed to warm to ambient temperature. The reaction mixture was cooled to 0 °C in an ice bath, and quenched by the dropwise addition of MeOH followed by the addition of a saturated aqueous solution of NH_4Cl. The mixture was diluted with EtOAc and washed with saturated NH_4Cl and a saturated aqueous solution of sodium potassium tartrate. The combined aqueous phase was extracted thrice with CH_2Cl_2 (emulsions developed). The combined organic phase was washed with brine, dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure to afford compound **22** as a crude yellow oil (~1:1 mixture of diasteromers), which was used without further purification. Note: The desired diastereomer (*S*,*S*) can be isolated by chromatography, but separation of the diasteromers following *O*-alkylation is more facile. **22***S*,*S* **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.34–7.29 (m, 4H), 7.27–7.24 (m, 1H), 5.73 (bd, J = 1.4 Hz, 1H), 4.01 (d, J = 12.1 Hz, 1H), 3.94 (bs, 1H), 3.44 (d, J = 12.9 Hz, 1H), 3.02–2.98 (m, 1H),

2.94–2.89 (m, 1H), 2.42–2.35 (m, 2H), 2.34–2.21 (m, 3H), 2.01–1.93 (m, 1H), 1.92–1.85 (m, 2H), 1.76–1.66 (m, 4H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 146.1, 139.7, 128.9, 128.5, 127.3, 126.2, 73.1, 66.0, 61.3, 54.4, 32.3, 32.2, 29.6, 24.4, 23.7; **ESI–EMM** (*m*/*z*): $[M + H]^+$ calcd for $C_{17}H_{23}NO$, 258.1853; found, 258.1847.

Stereochemistry of Alcohol 22. To discern the stereochemistry of alcohol **22**, the two diastereomers were isolated and converted into their oxazolidinones by a route relying on debenzylation and subsequent Boc-protection as described in ref. 10. The ^{1}H NMR coupling constant between the ring protons is expected to be significantly lower for the oxazolidinone from the *S*,*S* alcohol (found, J = 4.1 Hz) than that from the *S*,*R* alcohol (found, J = 7.7 Hz). Further, the ^{1}H NMR spectra of alcohol **10** derived either from the *S*,*R* alcohol or by the route in Scheme 1 were indistinguishable, and its stereochemistry was confirmed with X-ray crystallography (*vide infra*).

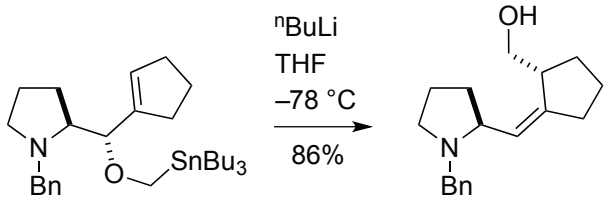


(*S*,*S*)-*N*-Benzyl-2-(cyclopent-1-enyl-tributylstannanylmethoxy-methyl)-pyrrolidine (**23**)



To a 100-mL round-bottom flask was added 230 mg KH (230 mg, 30% w/v in oil, 1.7 mmol) and a magnetic stir bar. The flask was purged with Ar(g), and dry THF (2 mL) and 18-crown-6 (710 mg, 2.69 mmol) were added. In a separate dry flask, compound **22** (257 mg, mixture of diastereomers, *S,S*:*S,R* = 1:2, 1.00 mmol) was dissolved in dry THF (2 mL), and the resulting solution was transferred to the reaction mixture by using a syringe. Additional THF (2 mL) was used for rinsing. In another dry flask, Bu_3SnCH_2I (647 mg, 1.7 mmol) was dissolved in THF (2 mL), and the resulting solution was transferred to the reaction mixture by using a syringe. Additional THF (2 mL) was used for rinsing. After stirring for 1 h, the reaction mixture was quenched by the dropwise addition of a saturated aqueous solution of NH_4Cl. The mixture was diluted with EtOAc and washed with a saturated aqueous solution of NH_4Cl and with brine. The organic phase was dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure. The desired diastereomer (*S,S*) was purified by chromatography on silica (2.5–5% v/v EtOAc in hexanes containing 0.25% v/v NEt_3) to afford compound **23** (149 mg, 20% for 2 steps) as a yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.35 (d, J = 7.1 Hz, 2H), 7.29 (dd, J = 7.2, 7.2 Hz, 2H), 7.21 (dd, J = 7.2, 7.2 Hz, 1H), 5.64 (bs, 1H), 4.49 (d, J = 13.2 Hz, 1H), 3.75 (d, J = 7.7 Hz, 1H), 3.69 (d, J = 9.8 Hz, 1H), 3.47 (d, J = 10.1 Hz, 1H), 3.38 (d, J = 13.2 Hz, 1H), 2.90–2.86 (m, 1H), 2.71 (apparent q, J = 7.8 Hz, 1H), 2.39–2.30 (m, 3H), 2.19–2.13 (m, 2H), 1.91–1.83 (m, 2H), 1.70–1.54 (m, 3H), 1.53–1.39 (m, 6H), 0.85 (t, J = 7.2 Hz, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 143.3, 140.5, 129.7, 129.3, 128.1, 126.6, 89.4, 65.0, 60.7, 58.5, 55.0, 32.1, 31.2, 29.4, 28.1, 27.6, 23.7, 22.9, 13.9, 9.0; **ESI–EMM** (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{51}NOSn$, 558.3061; found, 558.3041.

(*Z*)-(*S,S*)-[2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**24**)

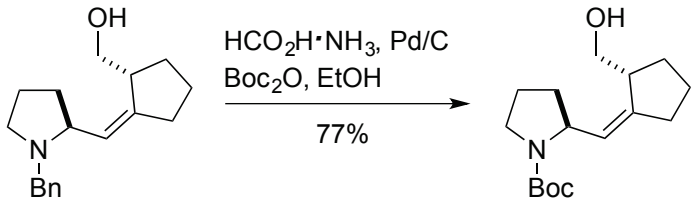


23 **24**

To a 100-mL flask was added compound **23** (2.44 g, 4.36 mmol) and a magnetic stir bar. The flask was purged with Ar(g), and anhydrous THF (27 mL) was added. The reaction mixture was cooled to –78 °C in a dry ice/acetone bath. To the cooled reaction mixture was added dropwise a freshly titrated solution of ^{n}BuLi (1.85 mL, 2.35 M in hexanes, 4.36 mmol). The reaction mixture was stirred for 1 h and then allowed to warm to ambient temperature. The reaction mixture was quenched by the addition of a saturated aqueous solution of NH_4Cl. The mixture was diluted with Et_2O and washed with a saturated aqueous solution of NH_4Cl and with brine. The organic phase was dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure. The product was purified by chromatography on silica (1% v/v NEt_3 in 1:1 EtOAc/hexanes) to afford compound **24** (1.062 g, 86.3%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.35–7.27 (m, 4H), 7.25–7.20 (m, 1H), 5.40 (dd, J = 8.7, 1.9 Hz, 1H), 3.90 (d, J = 12.6 Hz, 1H), 3.61 (dd, J = 10.6, 6.8 Hz, 1H), 3.48 (dd, J = 10.6, 7.5 Hz, 1H), 3.19 (d, J = 12.5 H, 1H), 3.22–3.15 (m, 1H), 2.92–2.82 (m, 2H), 2.39–2.23 (m, 2H), 2.19 (apparent q, J = 8.8 Hz, 1H), 2.02–1.92 (m, 1H), 1.87–1.52 (m, 7H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 146.7, 139.8, 129.0, 128.4, 127.0, 124.9,

65.2, 64.6, 57.9, 52.7, 43.5, 34.2, 31.1, 29.9, 23.8, 22.0; **ESI–EMM** (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{25}NO$, 272.2009; found, 272.2010.

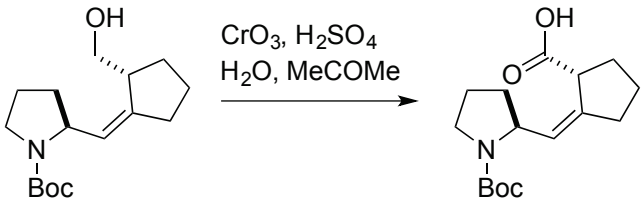
(*Z*)-(*S*,*S*)-[2-(*N*-*tert*-butyloxycarbonylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**25**)



24 **25**

To a round-bottom flask was added alcohol **24** (700 mg, 2.57 mmol), Boc_2O (1.14 g, 5.24 mmol), ammonium formate (229 mg, 3.63 mmol), and a magnetic stir bar. The flask was purged with Ar(g), and ethanol (10 mL) was added. A portion of Pd/C (10% w/w, 500 mg) was added. The reaction mixture was stirred, and the reaction progress was monitored by TLC. After approximately 30 min, the reaction mixture was filtered through a plug of Celite®, rinsed with methanol, and concentrated under reduced pressure. The product was purified by chromatography on silica (1:3 EtOAc/hexanes) to afford compound **25** (559 mg, 77%) as a colorless oil. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 5.34 (bs, 1H), 5.27 (d, J = 9.5 Hz, 1H), 4.66 (ddd, J = 7.4, 7.4, 7.4 Hz, 1H), 3.63–3.52 (m, 2H), 3.44–3.32 (m, 2H), 2.93 (bs, 1H), 2.45 (apparent quintet, J = 8.0 Hz, 1H), 2.20 (apparent quintet, J = 7.1 Hz, 1H), 2.08 (ddd, J = 13.2, 7.0, 7.0 Hz, 1H), 1.90–1.74 (m, 4H), 1.68–1.63 (m, 1H), 1.60–1.51 (m, 2H), 1.46 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 155.2, 144.8, 124.6, 79.7, 66.7, 57.2, 47.3, 43.2, 33.7, 33.4, 30.0, 28.8, 24.2, 23.9; **ESI–EMM** (*m*/*z*): $[M + H]^+$ calcd for $C_{16}H_{27}NO_3$, 282.2064; found, 282.2061.

(*Z*)-(*S*,*S*)-[2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl]carboxylic Acid (**26**)

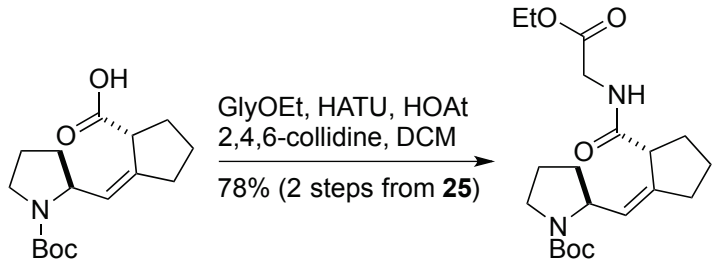


25 **26**

The Jones reagent was prepared by the addition of concentrated H_2SO_4 (1.0 mL) to CrO_3 (1.0 g). The resulting solution was cooled to 0 °C, and ice-cold water (3 mL) was added slowly with rapid stirring. In a 50-mL round-bottom flask, compound **25** (40 mg, 0.175 mmol) was dissolved in acetone (2 mL) under an atmosphere of Ar(g). The reaction flask was cooled to 0 °C, and the Jones reagent (140 μL, 0.35 mmol) was added dropwise. After 10 min, the reaction mixture was quenched by the dropwise addition of 2-propanol until the reaction mixture turned from orange to green. Brine (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The organic extracts were combined, washed with brine, dried with Na_2SO_4(s), filtered, and concentrated under reduced pressure to afford compound **26** (15 mg) which was taken on in a

crude form to the next step. **ESI–EMM** (*m*/*z*): $[M - H]^-$ calcd for $C_{16}H_{24}NO_4$, 294.1710; found, 294.1711.

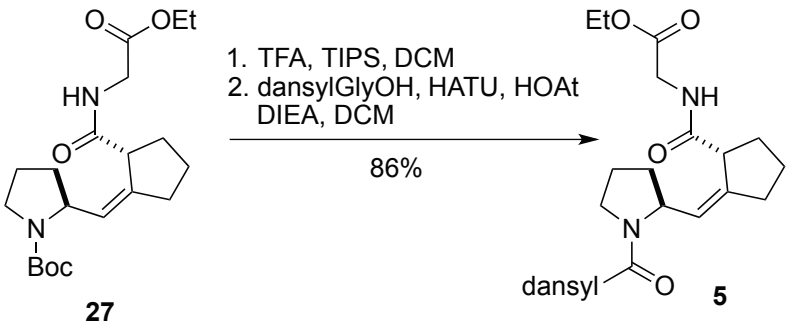
(Z)-(*S*,*S*)-*N*-(2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl)carbonyl]glycine Ethyl Ester (**27**)



26 **27**

Compound **26** (15 mg, 0.051 mmol) was dissolved in anhydrous DCM (1 mL) after which glycine ethyl ester hydrochloride (8 mg, 0.056 mmol), 1-hydroxy-7-azobenzotriazole (21 mg, 0.153 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (58 mg, 0.153 mmol) were added. The resulting suspension was stirred under N_2(g). 2,4,6-Collidine (0.034 mL, 0.255 mmol) was added, and the reaction mixture was stirred for 30 min. Brine was added, and the reaction mixture was extracted with EtOAc. The organic extracts were combined, dried with Na_2SO_4(s), and concentrated under reduced pressure to afford a crude oil. The crude product was purified by chromatography on silica (30–100% v/v EtOAc in hexanes) to afford compound **27** (15 mg, 78%) as a pale yellow oil, which was taken on in a slightly crude form to the next step. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.71 (bs, 1H), 5.42 (d, J = 9.2 Hz, 1H), 4.36 (dd, J = 16.4, 7.2 Hz, 1H), 4.20–4.07 (m, 2H), 3.99 (dd, J = 17.2, 6.4 Hz, 1H), 3.88 (dd, J = 17.2, 5.6 Hz, 1H), 3.44–3.29 (m, 3H), 2.53–2.44 (m, 1H), 2.39–2.32 (m, 1H), 2.22 (apparent quintet, J = 7.6 Hz, 1H), 2.07 (apparent sextet, J = 6.0 Hz, 1H), 1.89–1.73 (m, 3H), 1.64–1.54 (m, 5H), 1.4 (s, 9H), 1.23 (t, J = 6.8 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 173.0, 170.2, 154.9, 141.1, 127.3, 79.4, 60.7, 57.2, 47.4, 47.1, 41.6, 33.2, 33.1, 31.2, 28.5, 24.2, 23.8, 14.2.

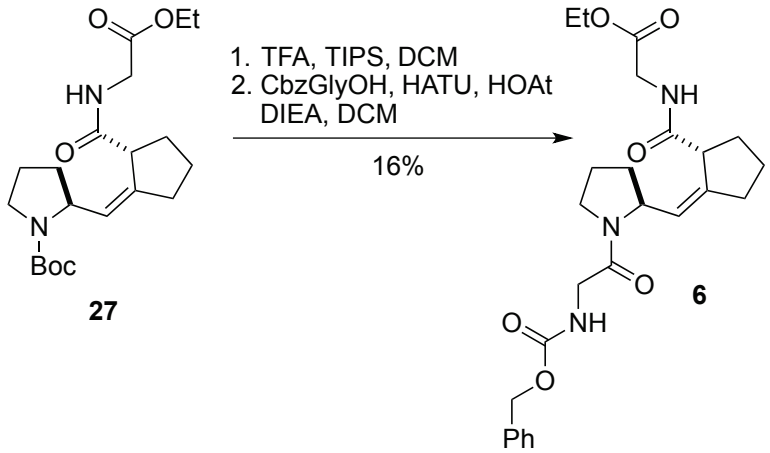
DansylGlyPro*cis*ProGlyOEt (**5**)



27 **5**

Compound **27** (38 mg, 0.1 mmol) was dissolved in dry DCM (0.56 mL) in a vial, after which triisopropylsilane (0.04 mL, 0.2 mmol) was added. TFA (0.56 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was resuspended in dry DCM (2 mL). dansylGlyOH (30 mg, 0.1 mmol), 1-hydroxy-7-azobenzotriazole (41 mg, 0.3 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (114 mg, 0.3 mmol) were added, and the reaction mixture was stirred. DIEA (0.08 mL) was added, and the reaction mixture was stirred. After 1 h, the reaction mixture was concentrated under reduced pressure, after which the crude product was purified by chromatography on silica (70% v/v EtOAc in hexanes) and HPLC 25–65% v/v acetonitrile in water containing 0.1% v/v TFA) to afford dansylGlyPro*cis*ProGlyOEt (**5**) (49 mg, 86%) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of 2 or more rotamers, integrations are approximate, δ): 8.62 (t, J = 9.0 Hz, 1.0H), 8.45 (t, J = 6.0 Hz, 0.5H), 8.32 (d, J = 7.5 Hz, 0.5H), 7.71 (q, J = 8.0 Hz, 1.0H), 7.53 (d, J = 7.5 Hz, 0.5H), 5.84 (t, J = 4.0 Hz, 0.5H), 5.26 (d, J = 9.0 Hz, 0.5H), 4.36 (q, J = 8.0 Hz, 0.6H), 4.20–4.10 (m, 1.2H), 4.05–3.98 (m, 0.2H), 3.87–3.50 (m, 7.8H), 3.41–3.04 (m, 5.1H), 2.44–2.34 (m, 0.6H), 2.27–2.18 (m, 1.1H), 2.12–2.03 (m, 0.7H), 2.00–1.81 (m, 1.7H), 1.67–1.52 (m, 1.8H), 1.43 (s, 0.2H), 1.28–1.25 (m, 4.2H), 0.89–0.82 (m, 0.5H), 0.07 (m, 0.1H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of 2 or more rotomers, δ): 177.0, 172.3, 168.2, 163.0 (q, J = 39.4 Hz, TFA) 147.7, 144.4, 137.7, 132.9, 132.4, 131.2, 130.7, 130.6, 128.7, 128.0, 126.4, 119.8, 118.1 (q, J = 286 Hz, TFA) 64.0, 61.2, 49.8, 49.0, 48.9, 47.4, 44.4, 36.1 (2 signals), 34.8, 34.7, 34.4, 33.0, 32.4 (2 signals), 32.3, 32.2, 32.1, 32.0, 31.8, 27.4, 27.1, 26.6, 25.4, 16.9, 16.8; **ESI–EMM** (m/z): $[M + H]^+$ calcd for $C_{29}H_{39}N_4O_6S$, 571.2585; found, 571.2579. Purity: 98.34% by UPLC (retention time: 2.485 min).

CbzGlyPro*cis*ProGlyOEt (**6**)



Compound **27** (15 mg, 0.039 mmol) was dissolved in dry DCM (0.22 mL) in a vial, after which triisopropylsilane (0.016 mL, 0.078 mmol) was added. TFA (0.22 mL) was added and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was resuspended in dry DCM (0.8 mL). CbzGlyOH (8.2 mg, 0.039 mmol), 1-hydroxy-7-azobenzotriazole (16 mg, 0.12 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (45 mg, 0.17 mmol) were added and the reaction mixture was stirred. DIEA

(0.034 mL) was added, and the reaction mixture was stirred. After 1 h, the reaction mixture was concentrated under reduced pressure, after which the crude product was purified by HPLC (30–90% v/v acetonitrile in water containing 0.1% v/v TFA) to afford CbzGlyPro*cis*ProGlyOEt (**6**) (3 mg, 16%) as a yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of 2 or more rotamers, integrations are approximate, δ): 8.61 (t, J = 6.0 Hz, 1.0H), 7.39–7.28 (m, 5.5 H plus $CHCl_3$), 5.66 (s, 1.0H), 5.40 (d, J = 9.0 Hz, 1.0H), 5.14–5.09 (m, 2.1H), 4.57 (app q, J = 8.0 Hz, 1.1H), 4.20–4.09 (m, 2.1H), 4.02–3.90 (m, 2.9H), 3.49–3.43 (m, 2.2H), 2.51–2.45 (m, 1.9H), 2.36–2.33 (m, 1.4H), 2.28–2.14 (m, 2.8H), 2.08–1.88 (m, 6.9H), 1.69–1.61 (m, 2.2H), 1.28–1.22 (m, 3.5H); 0.91–0.74 (m, 2.6H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 173.4, 170.0, 166.9, 156.1, 141.9, 136.3, 128.6, 128.3, 128.2, 126.1, 67.1, 61.0, 58.3, 47.4, 46.4, 43.7, 41.7, 33.2, 32.3, 31.5, 24.6, 23.9, 14.1; **ESI–EMM** (m/z): $[M + H]^+$ calcd for $C_{25}H_{34}N_3O_6$, 472.2442; found, 472.2442. Purity: 84.24% by UPLC (retention time: 2.576 min).

X-Ray Crystallography of Alcohol 10

Alcohol **10** was dissolved in hexane containing a minimal amount of EtOAc. Slow evaporation afforded crystals suitable for X-ray diffraction analysis.

Table S1. Crystal data and structure refinement for alcohol **10**.

Identification code	raines28	
Empirical formula	$C_{15}H_{25}NO_3$	
Formula weight	267.36	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	*C*2	
Unit cell dimensions	a = 18.1917(6) Å	α = 90°
	b = 6.6114(2) Å	β = 112.109(2)°
	c = 13.6374(4) Å	γ = 90°
Volume	1519.60(8) Å^3	
Z	4	
Density (calculated)	1.169 Mg/m^3	
Absorption coefficient	0.645 mm^{-1}	
F(000)	584	
Crystal size	0.25 × 0.14 × 0.13 mm^3	
θ range for data collection	3.50 to 69.46°	
Index ranges	$-21 \leq h \leq 22$, $-7 \leq k \leq 7$, $-16 \leq l \leq 15$	
Reflections collected	8550	
Independent reflections	2615 [R(int) = 0.0379]	
Completeness to θ = 69.46°	98.3%	
Absorption correction	Empirical with SADABS	
Max. and min. transmission	0.9197 and 0.8575	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2615 / 1 / 273	
Goodness-of-fit on F^2	1.012	
Final R indices [$I > 2\sigma$(I)]	$R1$ = 0.0318, $wR2$ = 0.0769	
R indices (all data)	$R1$ = 0.0354, $wR2$ = 0.0799	
Absolute structure parameter	0.09(19)	
Largest diff. peak and hole	0.135 and –0.167 e.Å^{-3}	

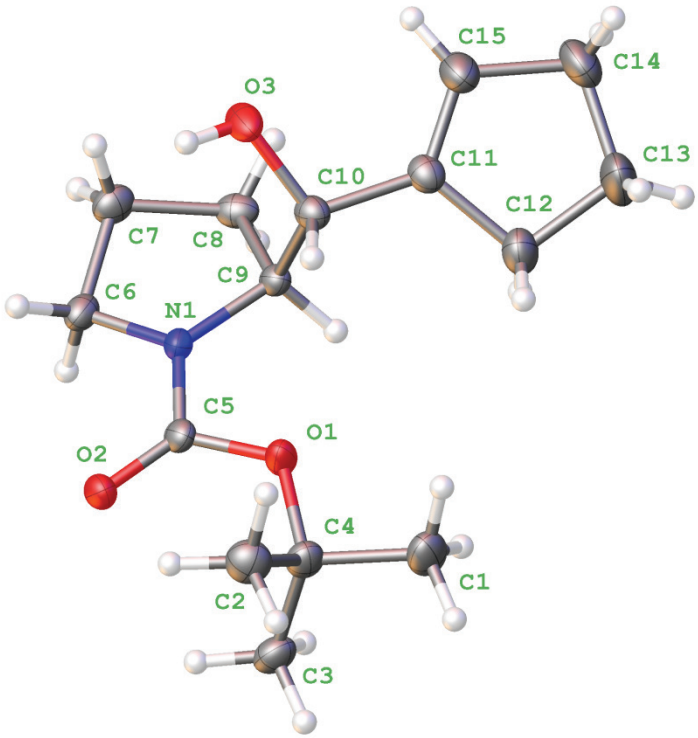


Figure S1. Molecular structure of alcohol **10**. The thermal ellipsoids are shown at the 50% probability level.

Table S2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for alcohol **10**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(2)	1824(1)	10427(2)	4488(1)	25(1)
O(1)	1166(1)	8843(2)	2910(1)	26(1)
N(1)	2482(1)	8784(2)	3608(1)	19(1)
O(3)	2860(1)	4300(2)	3443(1)	30(1)
C(5)	1826(1)	9436(3)	3731(1)	21(1)
C(10)	2240(1)	5442(3)	2702(1)	22(1)
C(9)	2480(1)	7671(2)	2672(1)	19(1)
C(11)	2003(1)	4470(3)	1633(1)	24(1)
C(8)	3336(1)	7920(3)	2746(1)	24(1)
C(6)	3267(1)	9475(3)	4314(1)	24(1)
C(1)	–155(1)	8695(4)	1753(2)	37(1)
C(4)	378(1)	9552(3)	2819(1)	26(1)
C(2)	162(1)	8666(3)	3700(2)	33(1)
C(14)	1948(2)	2340(4)	224(2)	45(1)
C(3)	358(1)	11854(3)	2796(2)	29(1)
C(7)	3828(1)	8322(3)	3922(1)	27(1)
C(12)	1286(1)	5163(3)	708(2)	35(1)
C(15)	2361(1)	2951(3)	1365(2)	35(1)
C(13)	1198(2)	3614(5)	–142(2)	64(1)

Table S3. Bond lengths (Å) and angles (°) for alcohol **10**.

O(2)-C(5)	1.2240(19)
O(1)-C(5)	1.3542(18)
O(1)-C(4)	1.469(2)
N(1)-C(5)	1.337(2)
N(1)-C(6)	1.464(2)
N(1)-C(9)	1.4723(19)
O(3)-C(10)	1.416(2)
O(3)-H(1)	0.82(2)
C(10)-C(11)	1.501(2)
C(10)-C(9)	1.542(2)
C(10)-H(10)	0.963(17)
C(9)-C(8)	1.531(2)
C(9)-H(9)	1.011(16)
C(11)-C(15)	1.321(3)
C(11)-C(12)	1.505(2)
C(8)-C(7)	1.537(2)
C(8)-H(8B)	0.98(2)
C(8)-H(8A)	0.93(2)
C(6)-C(7)	1.522(3)
C(6)-H(6A)	1.03(2)
C(6)-H(6B)	1.007(16)
C(1)-C(4)	1.521(2)
C(1)-H(1A)	1.00(2)
C(1)-H(1B)	0.96(2)
C(1)-H(1C)	1.01(3)
C(4)-C(2)	1.515(3)
C(4)-C(3)	1.522(3)
C(2)-H(2A)	0.98(2)
C(2)-H(2B)	0.97(2)
C(2)-H(2C)	1.02(3)
C(14)-C(15)	1.506(3)
C(14)-C(13)	1.520(4)
C(14)-H(14A)	1.00(4)
C(14)-H(14B)	0.97(3)
C(3)-H(3B)	0.99(2)
C(3)-H(3A)	0.96(2)
C(3)-H(3C)	0.98(2)
C(7)-H(7A)	0.97(2)
C(7)-H(7B)	0.97(2)
C(12)-C(13)	1.509(3)
C(12)-H(12B)	0.92(2)
C(12)-H(12A)	0.96(3)
C(15)-H(15)	0.89(2)
C(13)-H(13A)	0.88(3)

C(13)-H(13B)	1.09(6)

C(5)-O(1)-C(4)	120.72(12)
C(5)-N(1)-C(6)	120.90(13)
C(5)-N(1)-C(9)	123.96(12)
C(6)-N(1)-C(9)	114.17(12)
C(10)-O(3)-H(1)	110.0(16)
O(2)-C(5)-N(1)	124.34(14)
O(2)-C(5)-O(1)	124.68(14)
N(1)-C(5)-O(1)	110.98(13)
O(3)-C(10)-C(11)	108.98(14)
O(3)-C(10)-C(9)	111.82(13)
C(11)-C(10)-C(9)	111.35(13)
O(3)-C(10)-H(10)	109.3(10)
C(11)-C(10)-H(10)	106.5(10)
C(9)-C(10)-H(10)	108.7(12)
N(1)-C(9)-C(8)	102.61(12)
N(1)-C(9)-C(10)	111.29(12)
C(8)-C(9)-C(10)	113.06(13)
N(1)-C(9)-H(9)	109.1(9)
C(8)-C(9)-H(9)	111.8(9)
C(10)-C(9)-H(9)	108.8(9)
C(15)-C(11)-C(10)	127.14(16)
C(15)-C(11)-C(12)	111.17(16)
C(10)-C(11)-C(12)	121.68(16)
C(9)-C(8)-C(7)	105.44(13)
C(9)-C(8)-H(8B)	110.3(11)
C(7)-C(8)-H(8B)	114.0(11)
C(9)-C(8)-H(8A)	110.3(12)
C(7)-C(8)-H(8A)	111.9(12)
H(8B)-C(8)-H(8A)	105.0(15)
N(1)-C(6)-C(7)	103.28(14)
N(1)-C(6)-H(6A)	108.1(10)
C(7)-C(6)-H(6A)	112.6(10)
N(1)-C(6)-H(6B)	110.8(9)
C(7)-C(6)-H(6B)	112.7(10)
H(6A)-C(6)-H(6B)	109.2(15)
C(4)-C(1)-H(1A)	110.8(11)
C(4)-C(1)-H(1B)	109.7(13)
H(1A)-C(1)-H(1B)	109.6(17)
C(4)-C(1)-H(1C)	106.8(13)
H(1A)-C(1)-H(1C)	110.1(18)
H(1B)-C(1)-H(1C)	110(2)
O(1)-C(4)-C(2)	110.24(14)
O(1)-C(4)-C(1)	102.31(14)
C(2)-C(4)-C(1)	110.54(16)

O(1)-C(4)-C(3)	109.66(15)
C(2)-C(4)-C(3)	113.01(16)
C(1)-C(4)-C(3)	110.56(16)
C(4)-C(2)-H(2A)	110.7(11)
C(4)-C(2)-H(2B)	112.6(12)
H(2A)-C(2)-H(2B)	111.7(17)
C(4)-C(2)-H(2C)	111.2(13)
H(2A)-C(2)-H(2C)	103.8(19)
H(2B)-C(2)-H(2C)	106.3(19)
C(15)-C(14)-C(13)	103.14(18)
C(15)-C(14)-H(14A)	111.9(18)
C(13)-C(14)-H(14A)	108.0(19)
C(15)-C(14)-H(14B)	111.4(15)
C(13)-C(14)-H(14B)	113.0(16)
H(14A)-C(14)-H(14B)	109(3)
C(4)-C(3)-H(3B)	108.6(12)
C(4)-C(3)-H(3A)	108.6(12)
H(3B)-C(3)-H(3A)	111.1(16)
C(4)-C(3)-H(3C)	110.9(13)
H(3B)-C(3)-H(3C)	108.6(17)
H(3A)-C(3)-H(3C)	109.2(17)
C(6)-C(7)-C(8)	104.42(13)
C(6)-C(7)-H(7A)	113.6(12)
C(8)-C(7)-H(7A)	112.3(11)
C(6)-C(7)-H(7B)	110.4(12)
C(8)-C(7)-H(7B)	109.3(12)
H(7A)-C(7)-H(7B)	106.7(16)
C(11)-C(12)-C(13)	104.18(18)
C(11)-C(12)-H(12B)	114.1(14)
C(13)-C(12)-H(12B)	109.0(15)
C(11)-C(12)-H(12A)	111.3(15)
C(13)-C(12)-H(12A)	108.8(16)
H(12B)-C(12)-H(12A)	109(2)
C(11)-C(15)-C(14)	112.62(18)
C(11)-C(15)-H(15)	120.3(13)
C(14)-C(15)-H(15)	127.1(13)
C(12)-C(13)-C(14)	107.54(18)
C(12)-C(13)-H(13A)	124(2)
C(14)-C(13)-H(13A)	117(2)
C(12)-C(13)-H(13B)	98(3)
C(14)-C(13)-H(13B)	108(3)
H(13A)-C(13)-H(13B)	98(3)

Table S4. Anisotropic displacement parameters (Å^2 × 10^3) for alcohol **10**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\, h^2 a^{*2} U^{11} + ... + 2\, h\, k\, a^*\, b^*\, U^{12}\,]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(2)	28(1)	29(1)	18(1)	–3(1)	6(1)	7(1)
O(1)	18(1)	35(1)	21(1)	–8(1)	4(1)	4(1)
N(1)	19(1)	20(1)	16(1)	–2(1)	2(1)	2(1)
O(3)	47(1)	22(1)	18(1)	4(1)	8(1)	9(1)
C(5)	23(1)	23(1)	15(1)	2(1)	5(1)	5(1)
C(10)	26(1)	21(1)	19(1)	1(1)	10(1)	3(1)
C(9)	21(1)	20(1)	15(1)	1(1)	6(1)	4(1)
C(11)	29(1)	21(1)	23(1)	0(1)	11(1)	–1(1)
C(8)	28(1)	22(1)	27(1)	6(1)	15(1)	4(1)
C(6)	22(1)	22(1)	23(1)	1(1)	2(1)	1(1)
C(1)	22(1)	50(1)	33(1)	–9(1)	5(1)	4(1)
C(4)	19(1)	34(1)	25(1)	–2(1)	7(1)	4(1)
C(2)	30(1)	37(1)	35(1)	0(1)	15(1)	1(1)
C(14)	60(2)	39(1)	33(1)	–13(1)	14(1)	5(1)
C(3)	22(1)	35(1)	29(1)	4(1)	8(1)	7(1)
C(7)	20(1)	28(1)	31(1)	2(1)	8(1)	–1(1)
C(12)	33(1)	40(1)	26(1)	–8(1)	6(1)	–1(1)
C(15)	44(1)	29(1)	28(1)	–3(1)	8(1)	8(1)
C(13)	56(2)	85(2)	35(1)	–29(1)	–2(1)	20(2)

Table S5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($Å^2 \times 10^3$) for alcohol **10**.

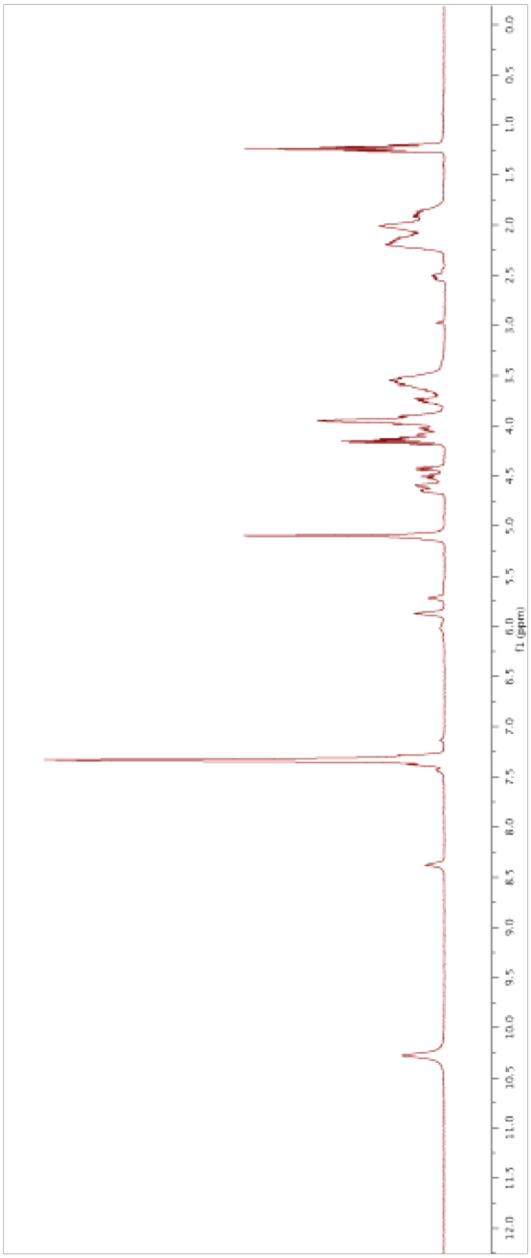
	x	*y*	*z*	*U*(eq)
H(6A)	3295(10)	11010(30)	4224(14)	19(4)
H(1)	2899(12)	4580(40)	4049(18)	39(6)
H(2A)	–404(12)	8890(30)	3560(15)	34(5)
H(10)	1781(10)	5390(30)	2891(13)	16(4)
H(6B)	3358(9)	9160(30)	5074(13)	18(4)
H(7A)	4317(11)	9040(30)	4027(14)	30(5)
H(1A)	–719(12)	9080(30)	1586(14)	35(5)
H(7B)	3977(11)	7050(40)	4291(16)	30(5)
H(8B)	3503(10)	6720(30)	2463(15)	21(4)
H(8A)	3370(11)	8980(30)	2320(15)	29(5)
H(9)	2088(9)	8330(30)	2013(12)	11(4)
H(12B)	825(14)	5200(40)	840(17)	43(6)
H(1B)	14(12)	9190(40)	1208(18)	43(6)
H(12A)	1373(14)	6470(50)	460(20)	57(8)
H(3B)	–203(12)	12300(30)	2591(15)	33(5)
H(2B)	501(11)	9160(30)	4396(16)	33(5)
H(15)	2808(13)	2440(30)	1831(16)	33(5)
H(1C)	–97(12)	7180(40)	1808(17)	43(6)
H(2C)	219(13)	7130(40)	3728(17)	47(6)
H(3A)	577(12)	12320(30)	2294(16)	31(5)
H(3C)	670(13)	12410(40)	3495(18)	41(6)
H(13A)	971(19)	3860(60)	–830(30)	86(10)
H(13B)	720(30)	2720(100)	–50(40)	180(20)
H(14A)	1789(18)	890(60)	150(20)	89(10)
H(14B)	2278(15)	2590(40)	–180(20)	65(8)

Table S6. Torsion angles (°) for alcohol **10**.

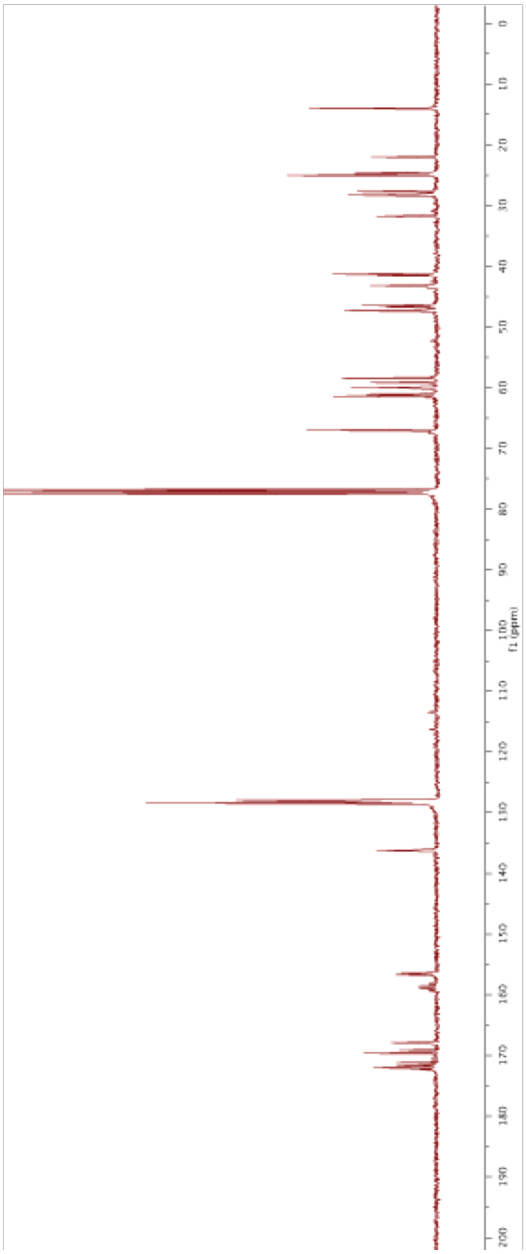
C(6)-N(1)-C(5)-O(2)	–9.1(3)
C(9)-N(1)-C(5)-O(2)	–177.14(16)
C(6)-N(1)-C(5)-O(1)	171.23(13)
C(9)-N(1)-C(5)-O(1)	3.2(2)
C(4)-O(1)-C(5)-O(2)	6.4(3)
C(4)-O(1)-C(5)-N(1)	–173.87(14)
C(5)-N(1)-C(9)-C(8)	161.74(15)
C(6)-N(1)-C(9)-C(8)	–7.05(17)
C(5)-N(1)-C(9)-C(10)	–77.06(18)
C(6)-N(1)-C(9)-C(10)	114.14(14)
O(3)-C(10)-C(9)-N(1)	–74.69(16)
C(11)-C(10)-C(9)-N(1)	163.12(13)
O(3)-C(10)-C(9)-C(8)	40.18(18)
C(11)-C(10)-C(9)-C(8)	–82.01(16)
O(3)-C(10)-C(11)-C(15)	–7.8(2)
C(9)-C(10)-C(11)-C(15)	116.0(2)
O(3)-C(10)-C(11)-C(12)	171.61(17)
C(9)-C(10)-C(11)-C(12)	–64.6(2)
N(1)-C(9)-C(8)-C(7)	24.00(17)
C(10)-C(9)-C(8)-C(7)	–95.98(16)
C(5)-N(1)-C(6)-C(7)	177.99(15)
C(9)-N(1)-C(6)-C(7)	–12.84(17)
C(5)-O(1)-C(4)-C(2)	–67.7(2)
C(5)-O(1)-C(4)-C(1)	174.73(16)
C(5)-O(1)-C(4)-C(3)	57.4(2)
N(1)-C(6)-C(7)-C(8)	27.18(17)
C(9)-C(8)-C(7)-C(6)	–32.41(18)
C(15)-C(11)-C(12)-C(13)	7.6(3)
C(10)-C(11)-C(12)-C(13)	–171.9(2)
C(10)-C(11)-C(15)-C(14)	178.91(19)
C(12)-C(11)-C(15)-C(14)	–0.5(3)
C(13)-C(14)-C(15)-C(11)	–6.7(3)
C(11)-C(12)-C(13)-C(14)	–11.4(3)
C(15)-C(14)-C(13)-C(12)	11.0(3)

NMR Spectra

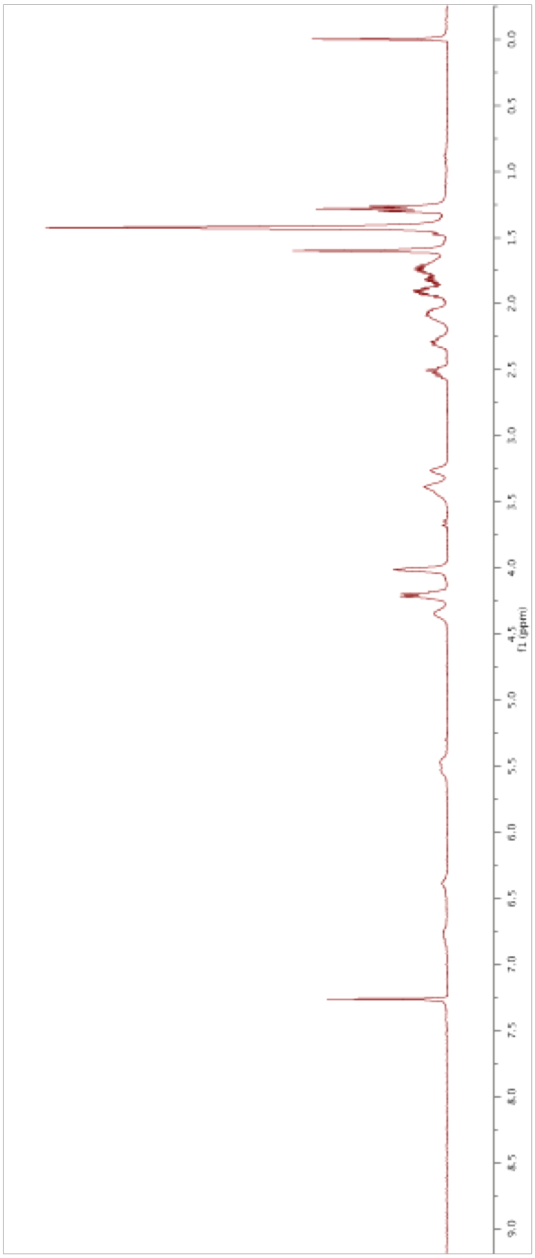
400 MHz ^{1}H NMR of CbzGPPGOEt (**2**) in $CDCl_3$



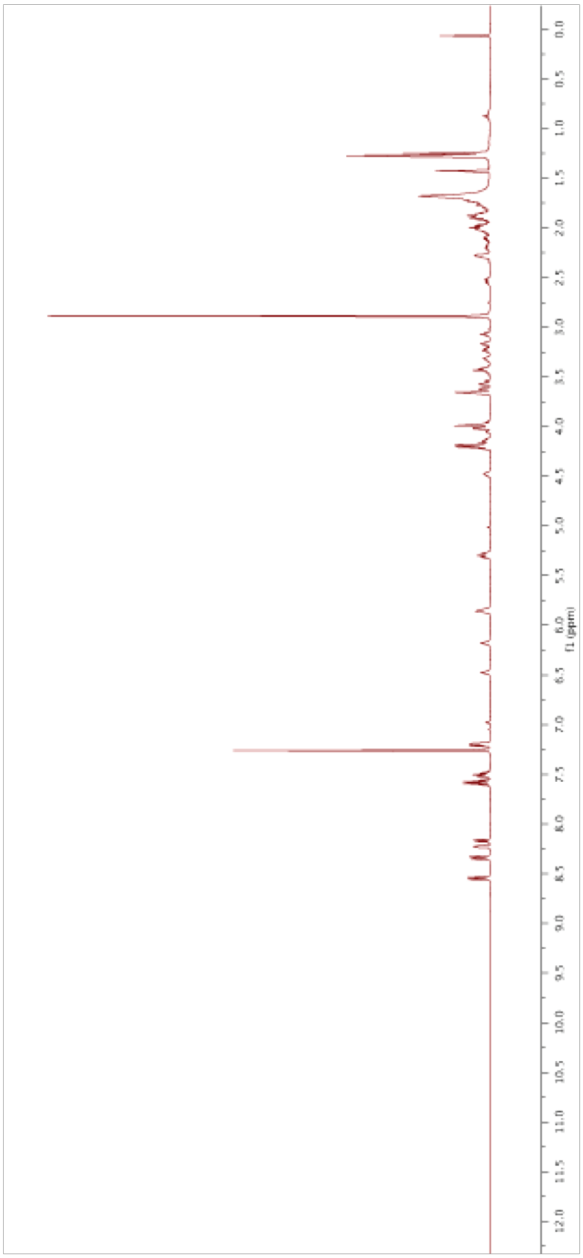
100 MHz ^{13}C NMR of CbzGPPGOEt (**2**) in $CDCl_3$



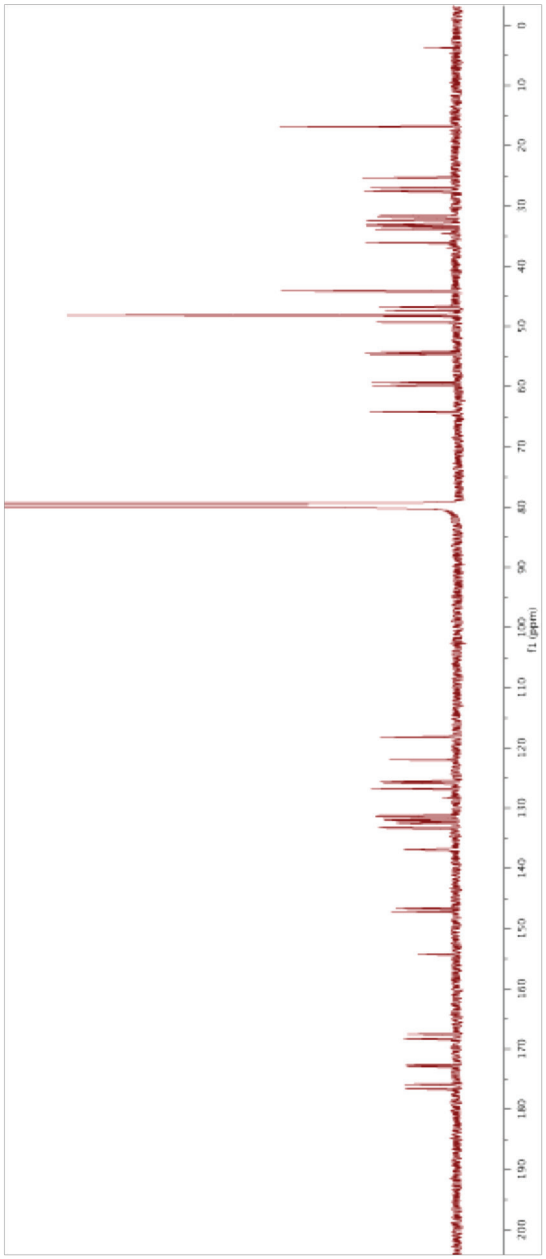
400 MHz ^{1}H NMR of (*E*)-(*S*,*S*)-*N*-(2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl)carbonyl]glycine Ethyl Ester (**18**) in $CDCl_3$



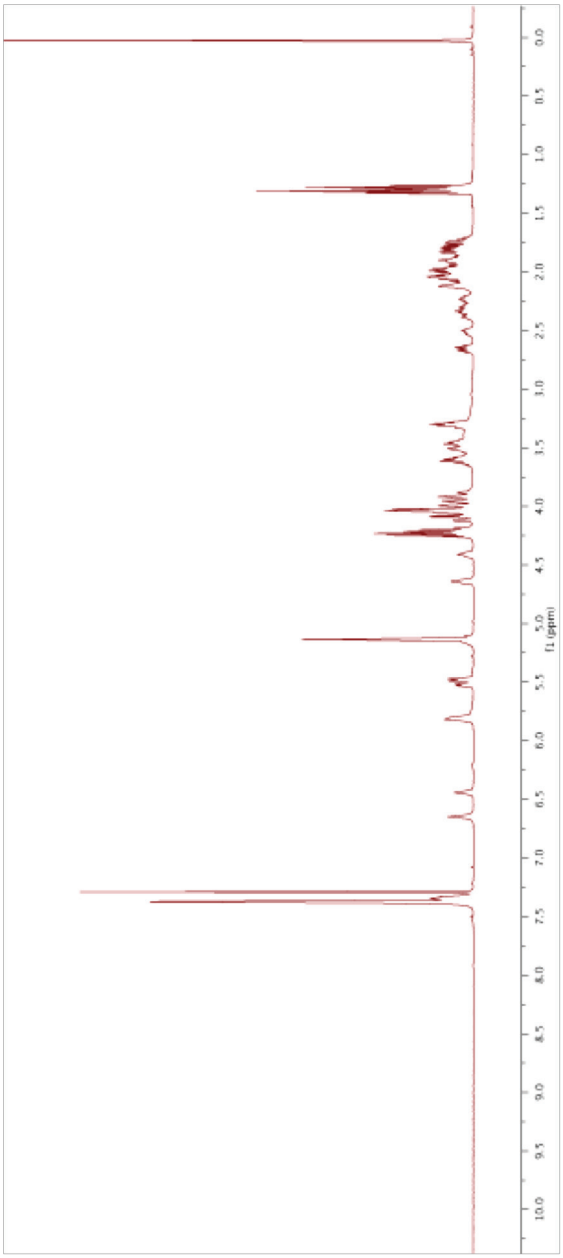
500 MHz ^{1}H NMR Spectrum of DansylGlyPro*trans*ProGlyOEt (**3**) in $CDCl_3$



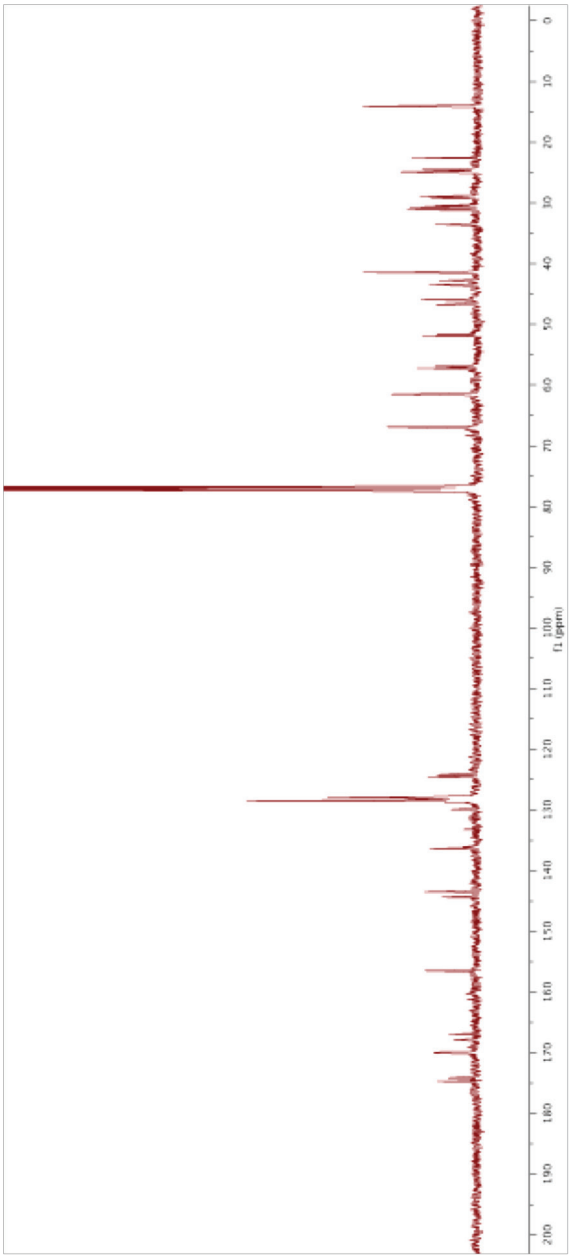
125 MHz ^{13}C NMR Spectrum of DansylGlyPro*trans*ProGlyOEt (**3**) in $CDCl_3$



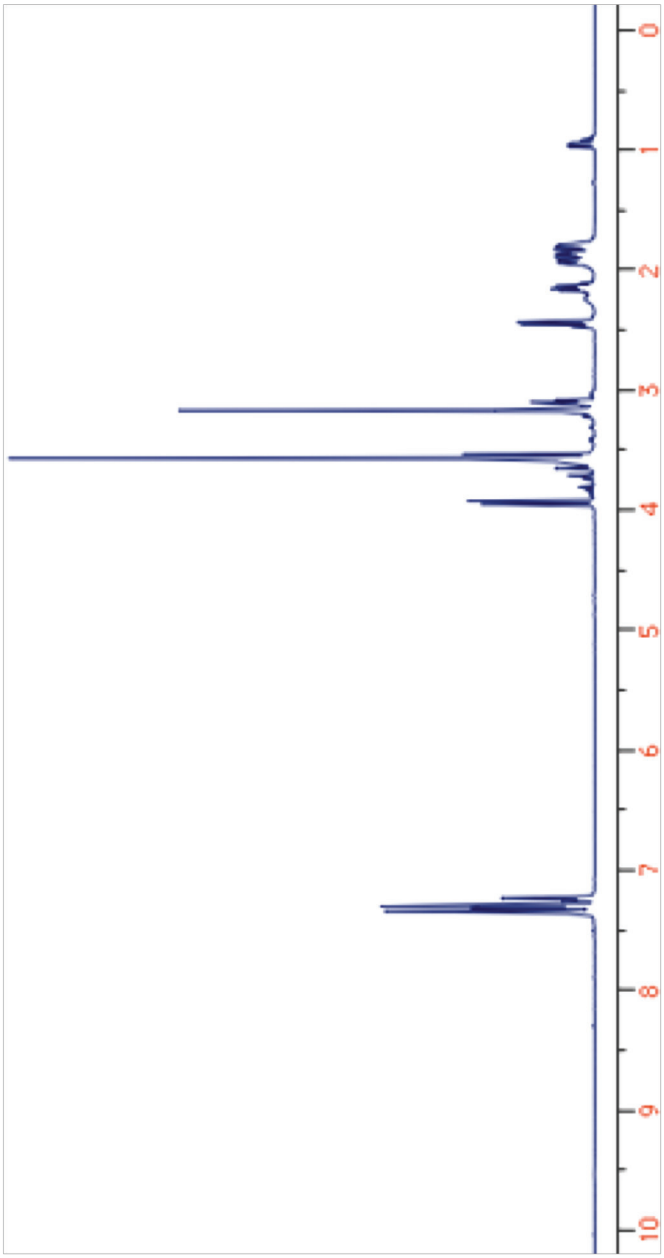
500 MHz ^{1}H NMR Spectrum of CbzGlyPro*trans*ProGlyOEt (**4**) in $CDCl_3$



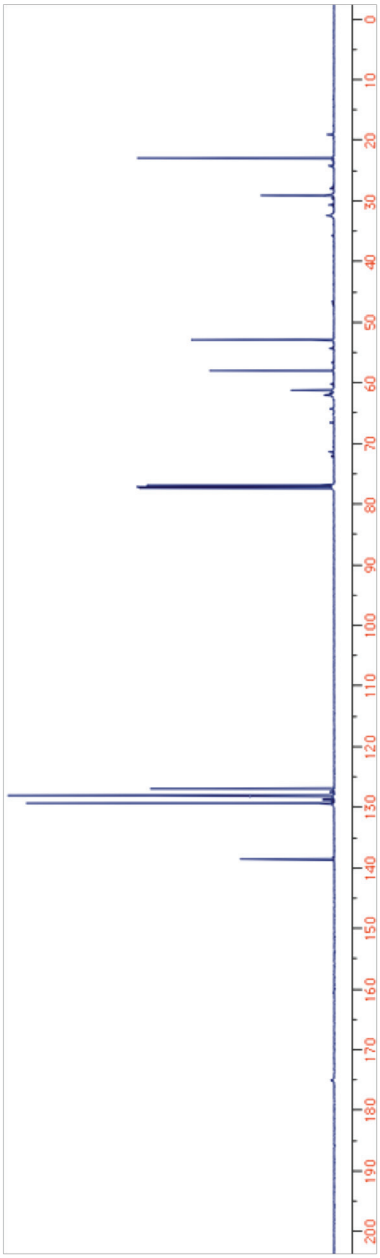
125 MHz ^{13}C Spectrum of CbzGlyPro*trans*ProGlyOEt (**4**) in $CDCl_3$



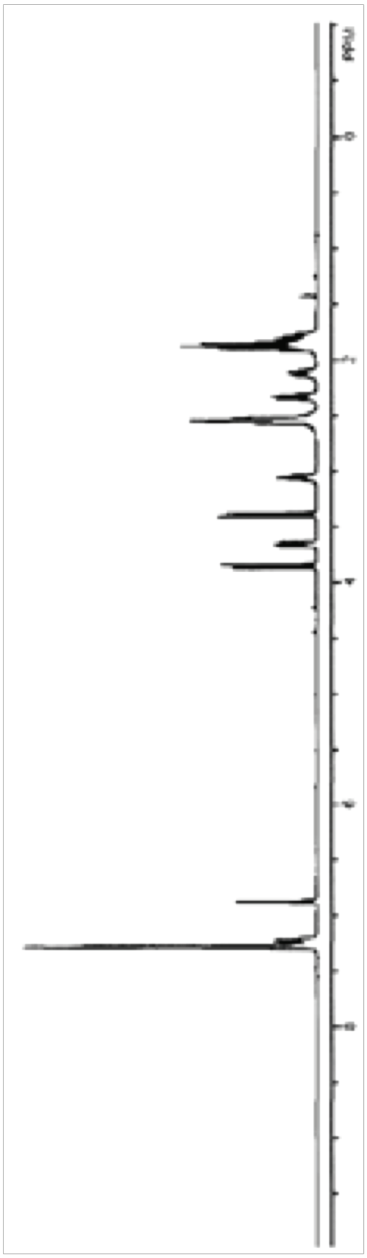
500 MHz ^{1}H NMR Spectrum of *N*-Methoxy-*N*-methyl-1-(phenylmethyl)-(2*S*)-2-pyrrolidinecarboxamide (**20**) in $CDCl_3$



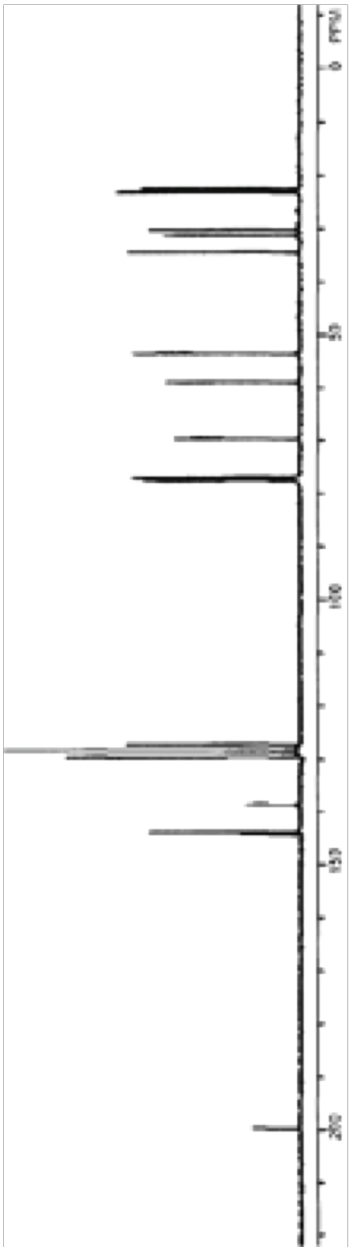
125 MHz ^{13}C NMR Spectrum of *N*-Methoxy-*N*-methyl-1-(phenylmethyl)-(2*S*)-2-pyrrolidinecarboxamide (**20**) in $CDCl_3$



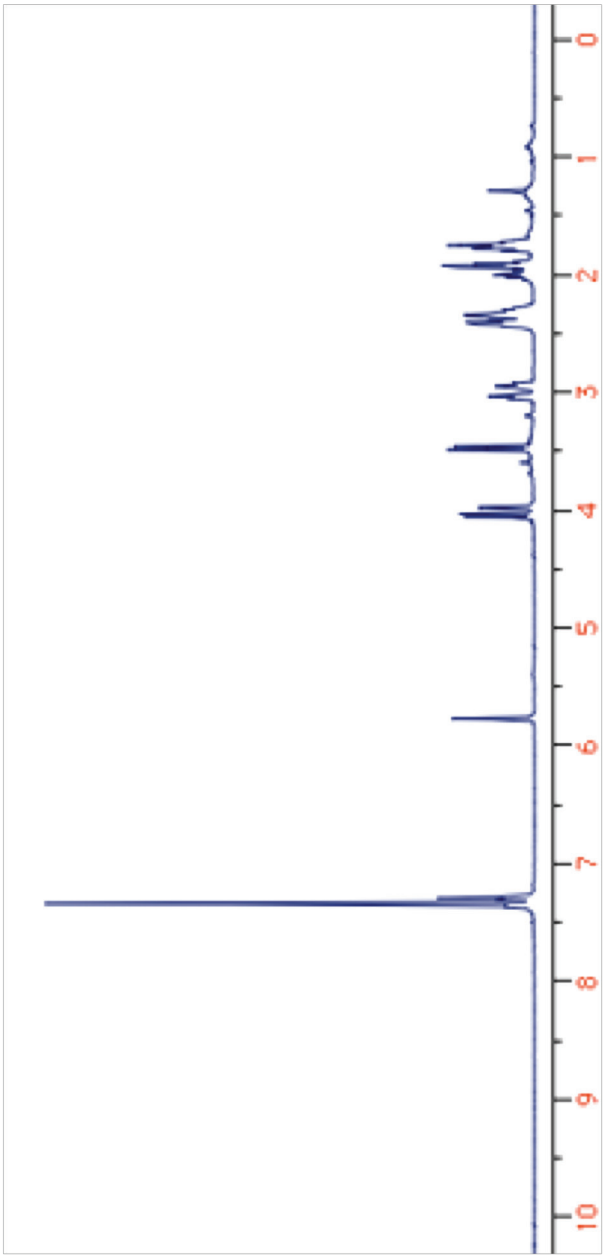
400 MHz ^{1}H NMR Spectrum of (*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanone (**21**) in $CDCl_3$



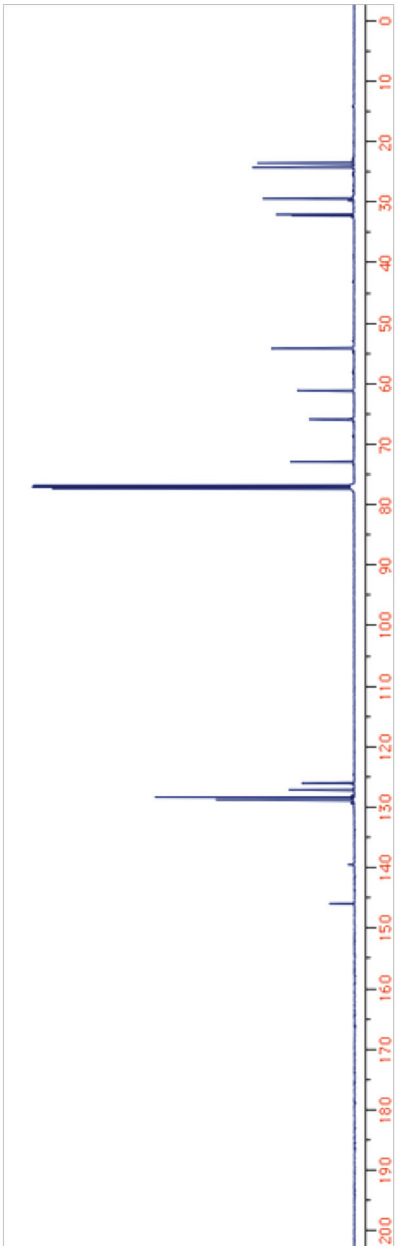
100 MHz ^{13}C NMR Spectrum of (*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanone (**21**) in $CDCl_3$



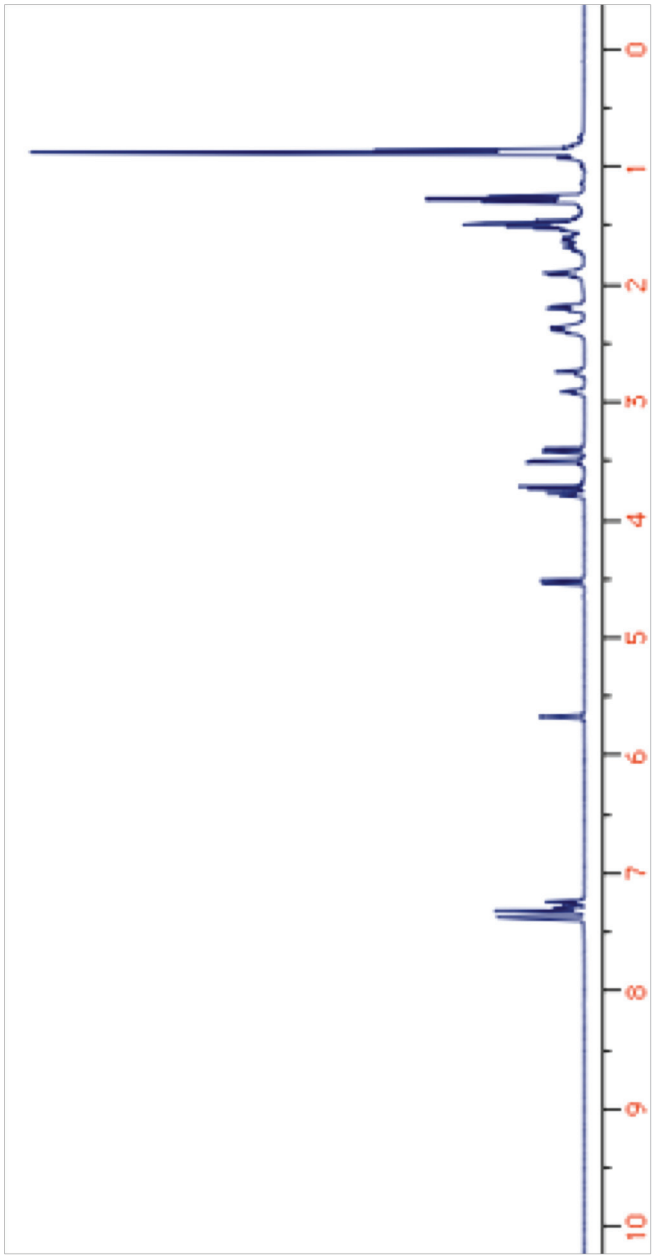
500 MHz ^{1}H NMR Spectrum of (*S*,*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanol (**22**) in $CDCl_3$



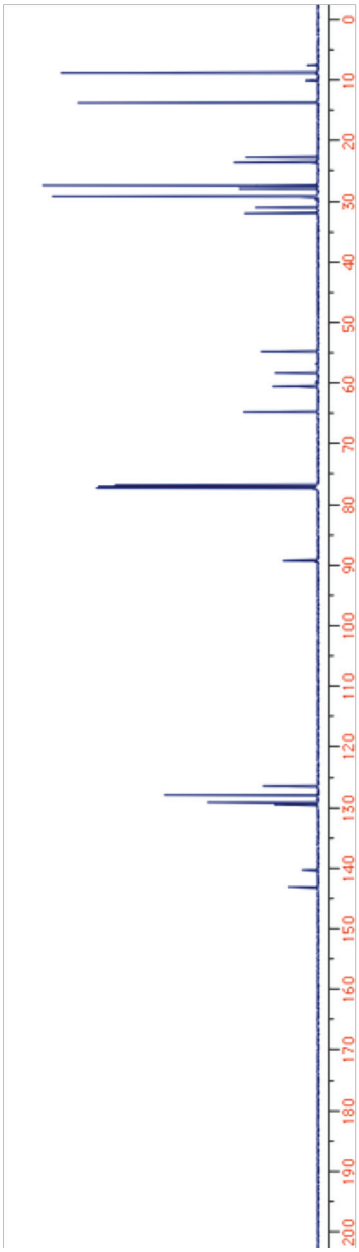
125 MHz ^{13}C NMR Spectrum of (*S*,*S*)-(*N*-Benzylpyrrolidin-2-yl)-cyclopent-1-enyl-methanol (**22**) in $CDCl_3$



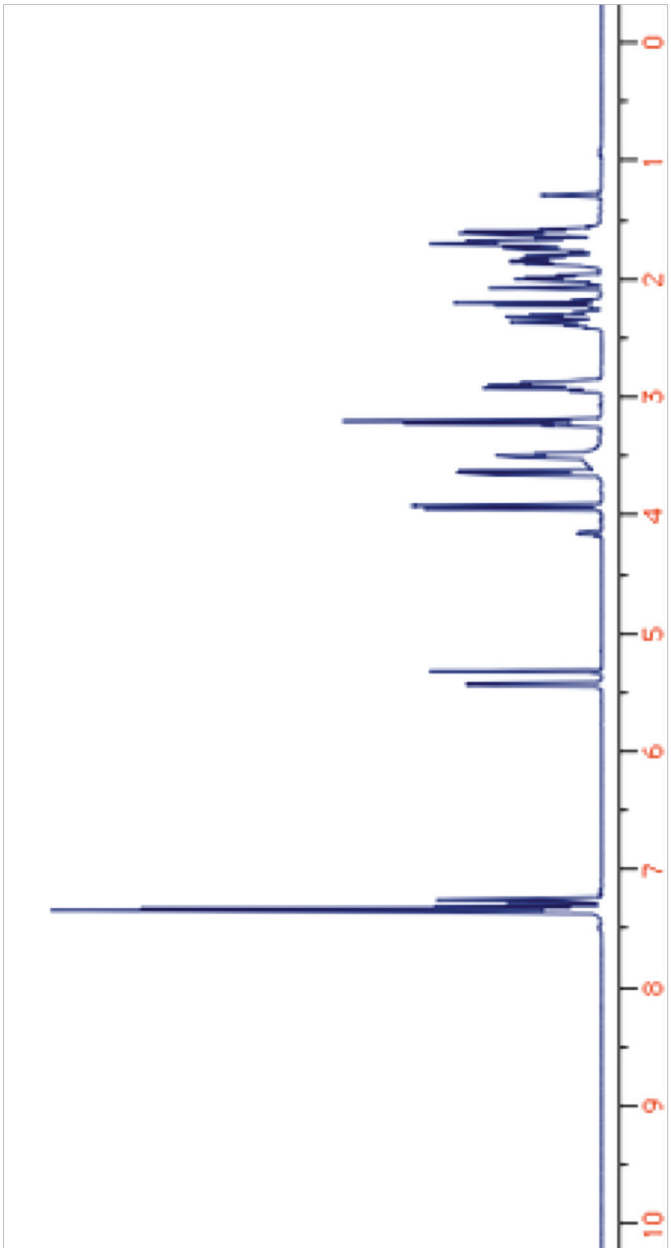
500 MHz ^{1}H NMR Spectrum of (*S*,*S*)-*N*-Benzyl-2-(cyclopent-1-enyl-tributylstannanylmethoxy-methyl)-pyrrolidine (**23**) in $CDCl_3$



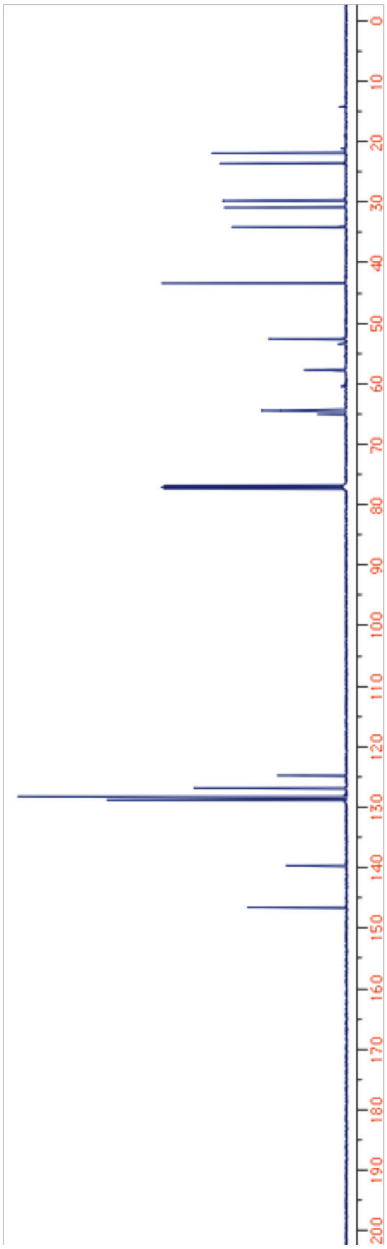
125 MHz ^{13}C NMR Spectrum of (*S*,*S*)-*N*-Benzyl-2-(cyclopent-1-enyl-tributylstannanylmethoxy-methyl)-pyrrolidine (**23**) in $CDCl_3$



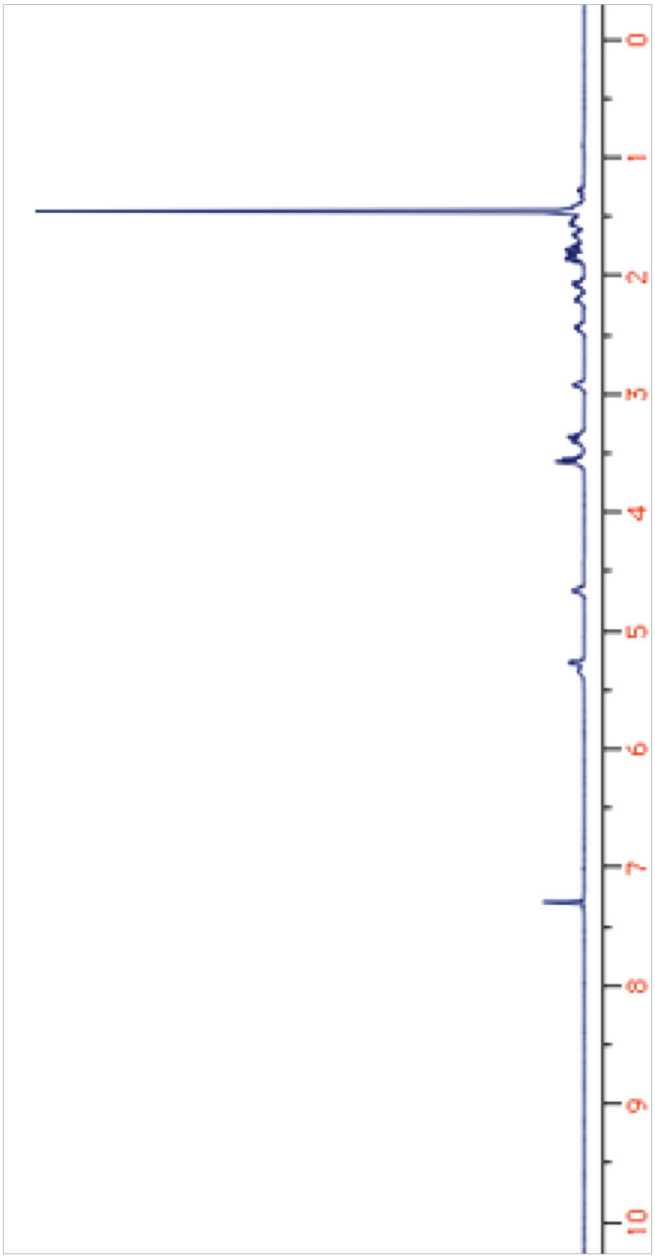
500 MHz ^{1}H NMR Spectrum of (*Z*)-(*S*,*S*)-[2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**24**) in $CDCl_3$



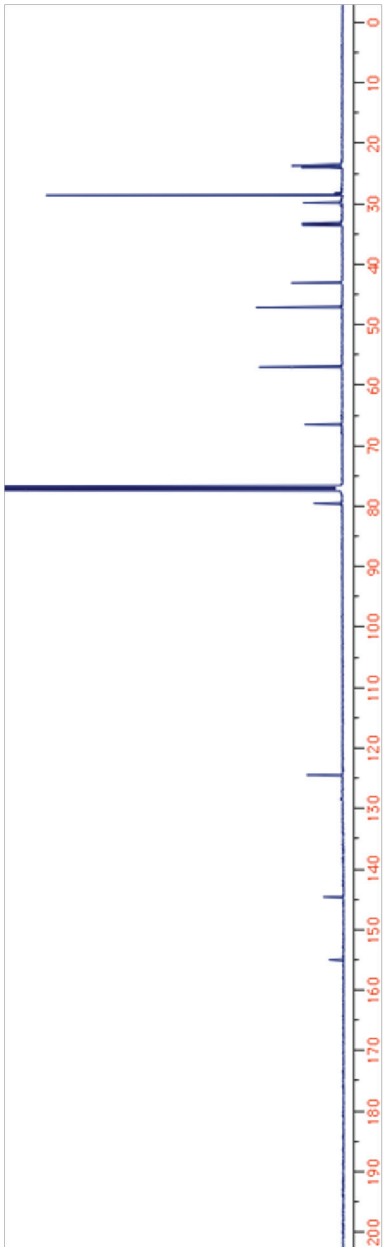
500 MHz ^{13}C NMR Spectrum of (*Z*)-(*S*,*S*)-[2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**24**) in $CDCl_3$



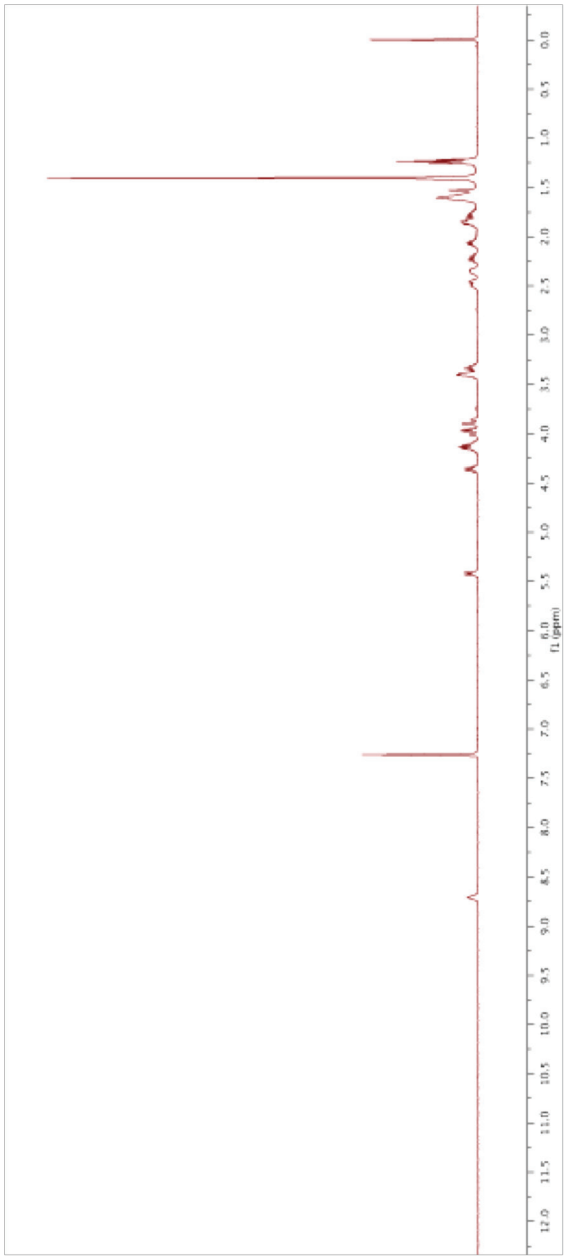
500 MHz ^{1}H NMR Spectrum of (*Z*)-(*S*,*S*)-[2-(*N*-*tert*-butyloxycarbonylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**25**) in $CDCl_3$



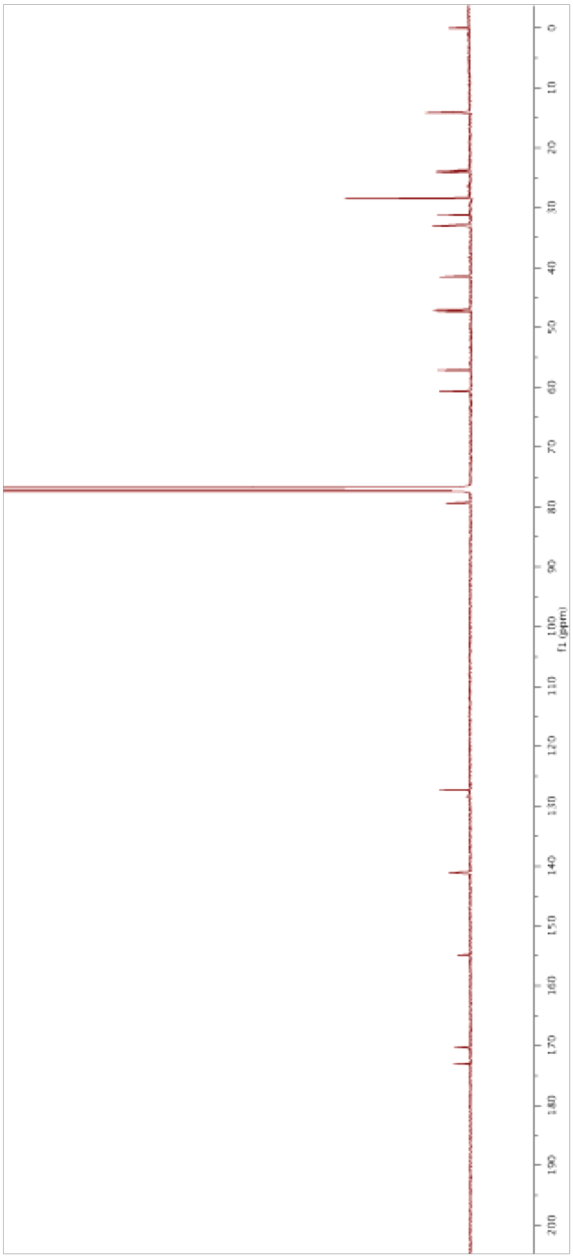
125 MHz ^{13}C NMR Spectrum of (*Z*)-(*S*,*S*)-[2-(*N*-*tert*-butyloxycarbonylpyrrolidin-2-ylmethylene)-cyclopentyl]-methanol (**25**) in $CDCl_3$



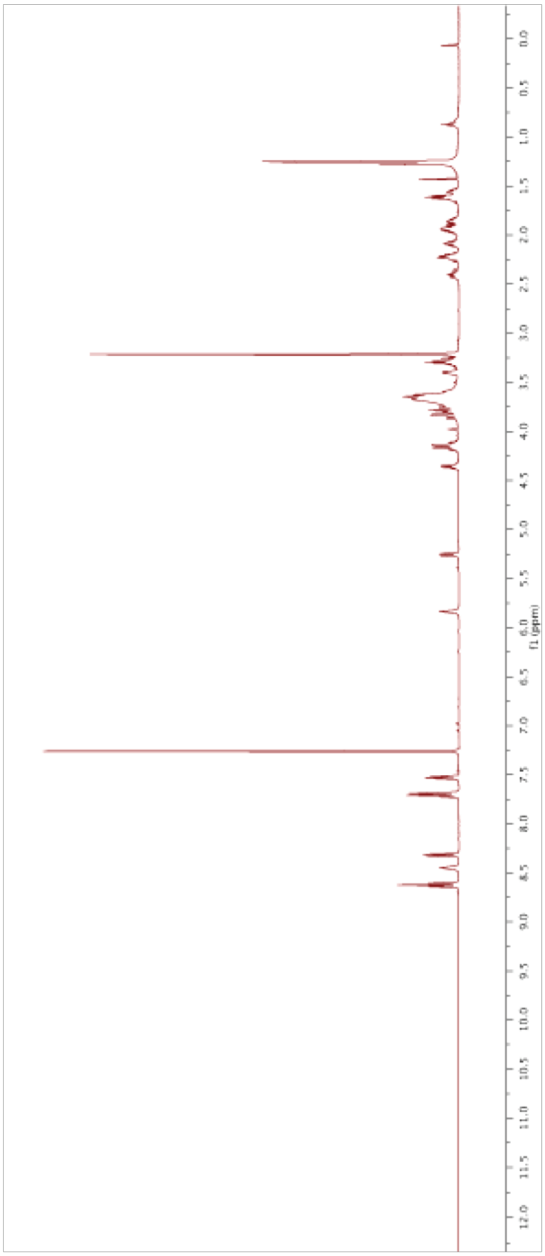
400 MHz ^{1}H NMR Spectrum of (*Z*)-(*S*,*S*)-*N*-(2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl)carbonyl]glycine Ethyl Ester (**27**) in $CDCl_3$



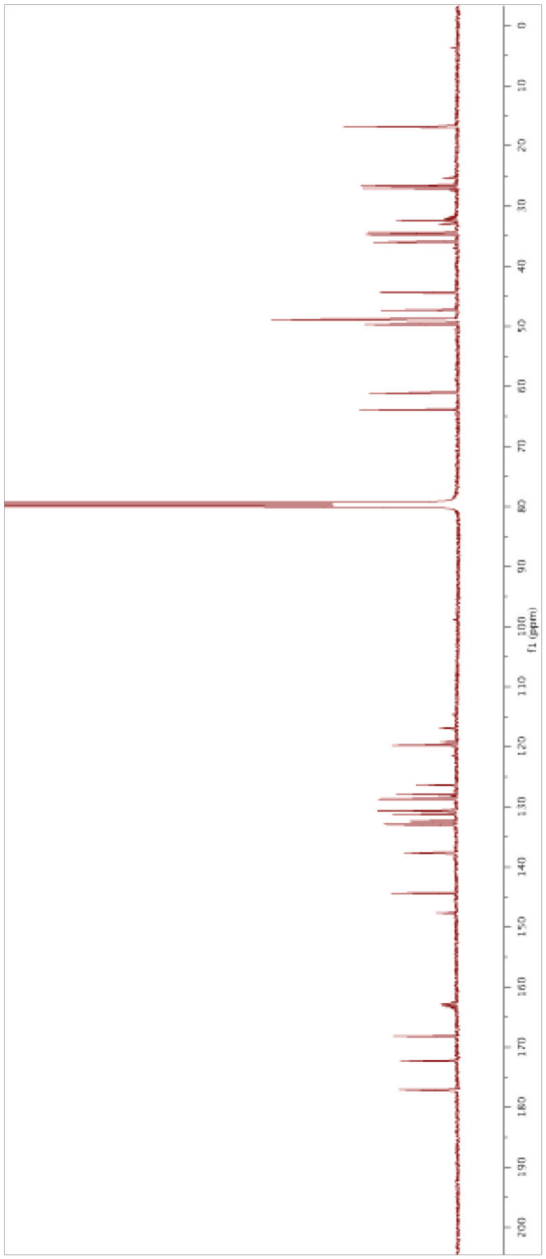
125 MHz ^{13}C NMR Spectrum of (*Z*)-(*S*,*S*)-*N*-(2-(*N*-Benzylpyrrolidin-2-ylmethylene)-cyclopentyl)carbonyl]glycine Ethyl Ester (**27**) in $CDCl_3$



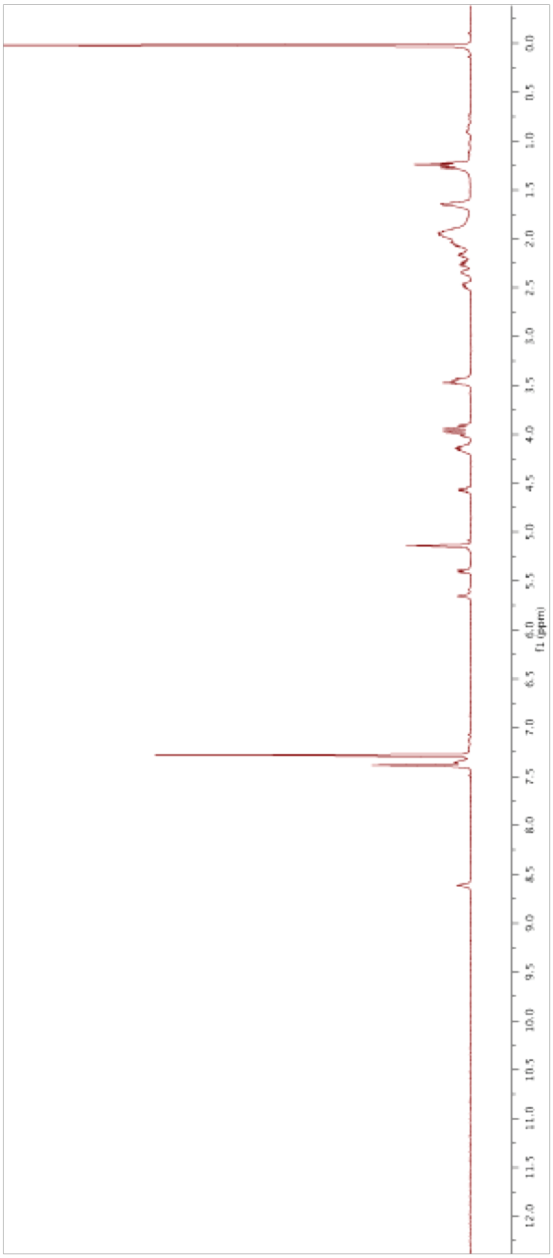
500 MHz ^{1}H NMR Spectrum of DansylGlyPro*cis*ProGlyOEt (**5**) in $CDCl_3$



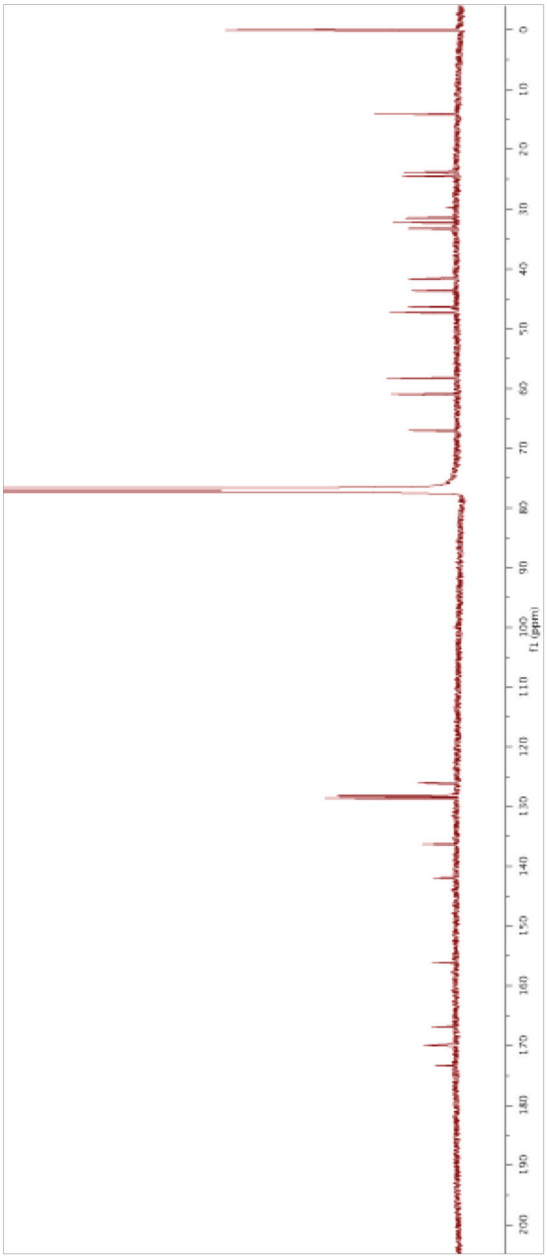
125 MHz ^{13}C NMR Spectrum of DansylGlyPro*cis*ProGlyOEt (**5**) in $CDCl_3$



500 MHz ^{1}H NMR Spectrum of CbzGlyPro*cis*ProGlyOEt (**6**) in $CDCl_3$



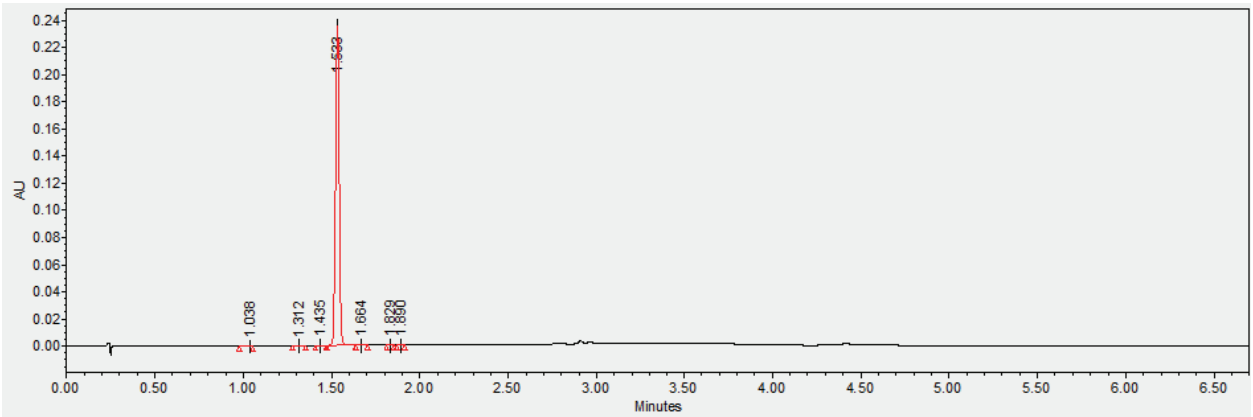
125 MHz ^{13}C NMR Spectrum of CbzGlyPro*cis*ProGlyOEt (**6**) in $CDCl_3$



Chromatograms of Compounds 1–6

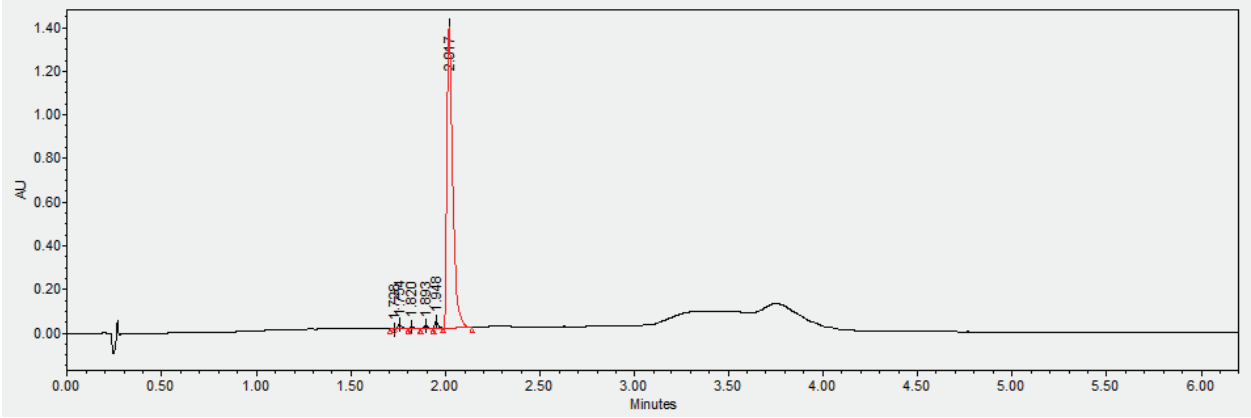
Compounds **1**–**6** were analyzed by UPLC to assess their purity. The analyses were performed with an Acquity UPLC® H-Class system from Waters that was equipped with an Acquity photodiode array detector, Acquity quaternary solvent manager, Acquity sample manager with a flow-through needle, Acquity UPLC® BEH C18 column (2.1 × 50 mm, 1.7-µm particle size) and Empower 3 software. Samples (5 µL) dissolved in water were injected into the column and eluted at 0.6 mL/min with a gradient of acetonitrile (20–68% v/v over 2.9 min for dansyl peptides; 10–90% v/v over 2.9 min for Cbz peptides) in water containing TFA (0.1% v/v). For dansyl peptides, 289 nm was used as the detection wavelength. For Cbz peptides, 218 nm was used as the detection wavelength. The broad peaks at 3–4 min are often present in blank injections and are associated with the wash sequence performed at the end of an HPLC gradient. These peaks are excluded from the integrations.

DansylGlyProProGlyOEt (**1**)



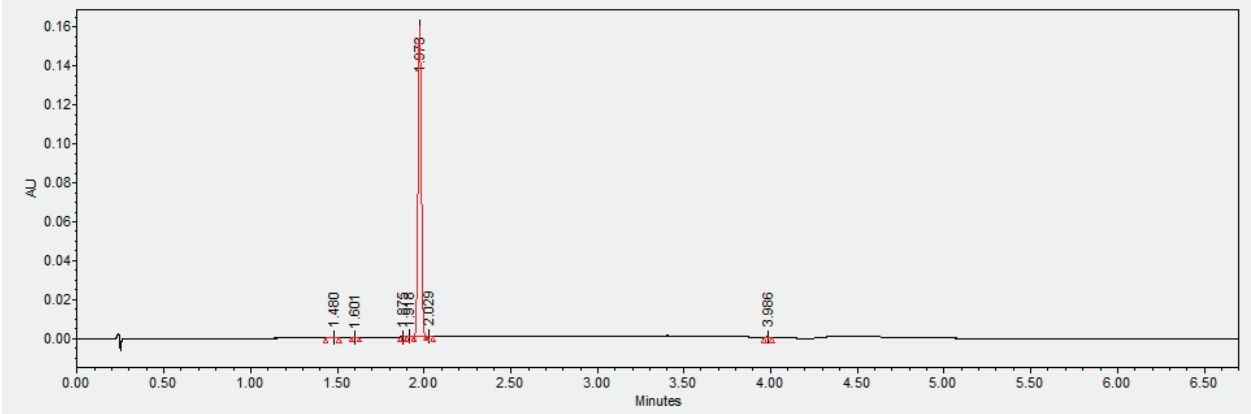
Peak	Retention Time (min)	Area (µV·s)	Area (%)	Height (µV)
1	1.038	87	0.03	44
2	1.312	41	0.01	28
3	1.435	47	0.01	35
4	**1.533**	**335060**	**99.90**	**235752**
5	1.664	71	0.02	32
6	1.829	51	0.02	42
7	1.890	47	0.01	34

CbzGlyProProGlyOEt **(2)**



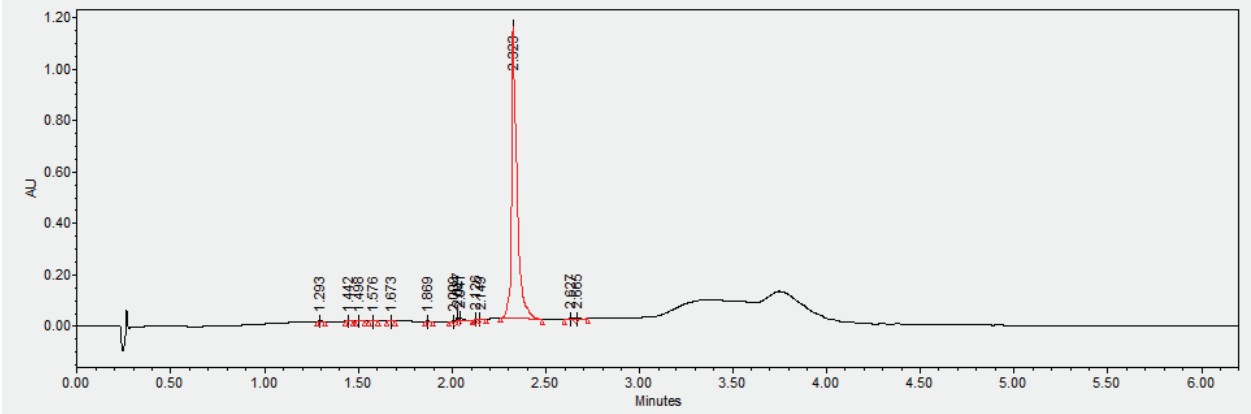
Peak	Retention Time (min)	Area (µV·s)	Area (%)	Height (µV)
1	1.728	439	0.01	744
2	1.754	29212	0.96	20800
3	1.82	13229	0.44	11425
4	1.893	23989	0.79	15200
5	1.948	38104	1.26	32938
6	**2.017**	**2930062**	**96.54**	**1382473**

DansylGlyPro*trans*ProGlyOEt **(3)**



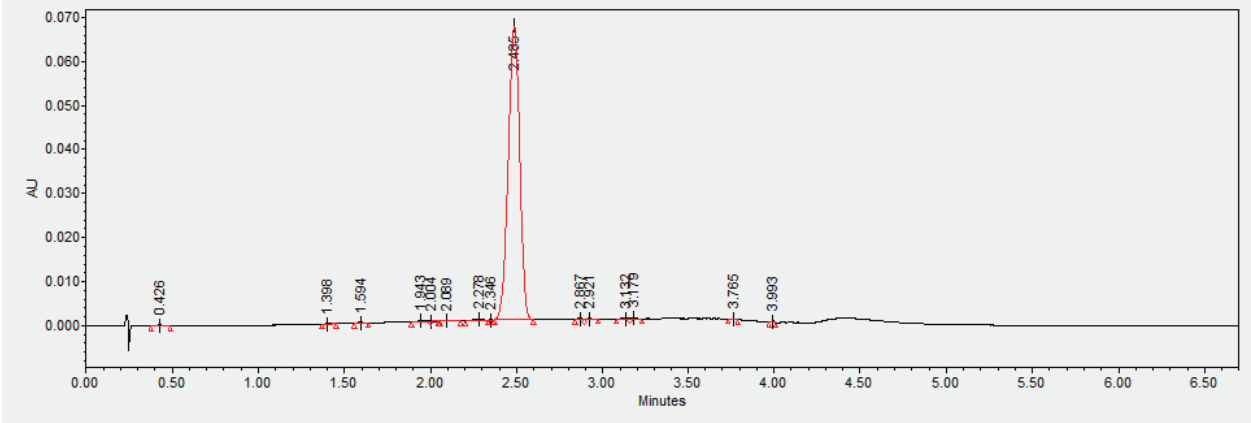
Peak	Retention Time (min)	Area (µV·s)	Area (%)	Height (µV)
1	1.480	448	0.21	284
2	1.601	103	0.05	71
3	1.875	96	0.05	93
4	1.918	333	0.16	332
5	**1.973**	**210229**	**99.18**	**159413**
6	2.029	211	0.10	209
7	3.986	553	0.26	646

CbzGlyPro*trans*ProGlyOEt **(4)**



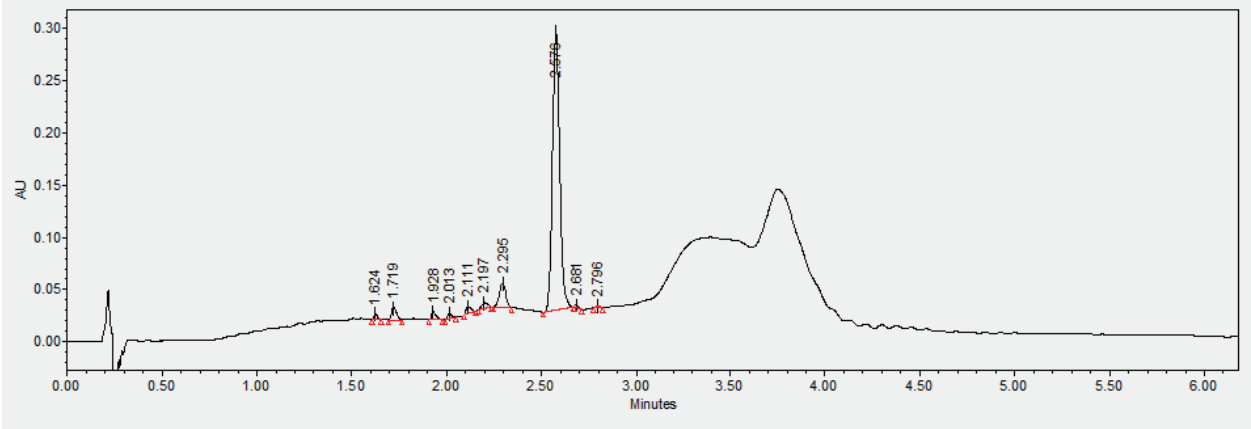
Peak	Retention Time (min)	Area (µV·s)	Area (%)	Height (µV)
1	1.293	2810	0.12	3408
2	1.442	2064	0.09	2183
3	1.498	2953	0.13	2639
4	1.576	1344	0.06	1150
5	1.673	1670	0.07	1225
6	1.869	2355	0.1	2076
7	2.009	1691	0.07	1976
8	2.027	15496	0.66	16469
9	2.041	17644	0.75	12622
10	2.126	3890	0.17	3219
11	2.149	2466	0.1	1974
12	**2.323**	**2295591**	**97.41**	**1136083**
13	2.627	4690	0.2	3286
14	2.665	2056	0.09	1320

DansylGlyPro*cis*ProGlyOEt **(5)**



Peak	Retention Time (min)	Area (μV·s)	Area (%)	Height (μV)
1	0.426	369	0.12	241
2	1.398	111	0.04	73
3	1.594	318	0.10	222
4	1.943	622	0.20	385
5	2.004	61	0.02	41
6	2.089	403	0.13	157
7	2.278	1333	0.43	393
8	2.346	113	0.04	127
9	**2.485**	**308032**	**98.34**	**66713**
10	2.867	367	0.12	238
11	2.921	301	0.10	132
12	3.132	302	0.10	135
13	3.179	481	0.15	306
14	3.765	135	0.04	123
15	3.993	298	0.10	352

CbzGlyPro*cis*ProGlyOEt **(6)**



Peak	Retention Time (min)	Area (μV·s)	Area (%)	Height (μV)
1	1.624	6708	0.79	5748
2	1.719	21346	2.53	11731
3	1.928	13352	1.58	8013
4	2.013	7427	0.88	5055
5	2.111	12284	1.45	6750
6	2.197	12457	1.47	5434
7	2.295	55179	6.53	23049
8	**2.576**	**711750**	**84.24**	**266043**
9	2.681	2446	0.29	2473
10	2.796	2007	0.24	1519