

Supporting Information

Facile and *E*-Selective Intramolecular Ring-Closing Metathesis Reactions in 3₁₀-Helical Peptides: a 3D-Structural Study

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Synthesis of Octapeptide 3

Boc-Ser(Al)-OH¹:

NaH (2.44 g of a 60% oil dispersion, 1.46 g, 0.061 mol) was suspended in 20 mL of DMF and cooled to 0 °C in a flask fitted with a dropping funnel. Boc-Ser-OH (5.0 g, 0.024 mol) was dissolved in 40 mL of DMF and the resulting solution was transferred to the dropping funnel. This solution was added drop-wise to the NaH solution over a period of 30 minutes. Allyl bromide (2.0 mL, 0.024 mol) was added and the flask contents warmed to room temperature and stirring was continued for 3 hours. The reaction was quenched by the slow addition of 12 mL of water. The solvents were then removed on the rotary evaporator and the residue dissolved in water (40 mL), which was washed with two 20 mL portions of ethyl acetate. The aqueous phase was acidified to pH 2 with the addition of 6 M HCl and extracted with two 40 mL portions of ethyl acetate. The organic phases were combined and dried over anhydrous magnesium sulfate. After filtration, the solvent was evaporated to yield a viscous oil (4.8 g, 82%) which was found to be of sufficient purity to be used in subsequent reactions. ¹H NMR (400 MHz, CDCl₃): δ 9.1 (1H br s), 5.85 (1H, m), 5.41 (1H, d, *J* = 8.4 Hz), 5.25 (1H, dq, *J* = 17.2, 1.5 Hz), 5.19 (1H, dq, *J* = 10.4, 1.5 Hz), 4.45 (1H, d, *J* = 8.5 Hz), 4.00 (2H, dt, *J* = 5.7, 1.5 Hz), 3.90 (1H, dd, *J* = 9.5, 3.0 Hz), 3.67 (1H, dd, *J* = 9.5, 3.6 Hz), 1.45 (9H, s). ¹³C NMR (100 MHz, CDCl₃): δ 175.96, 156.15, 134.25, 118.21, 80.77, 72.80, 69.96, 54.16, 28.70. [α]_D = +9.2° (*c* = 1.1, CH₂Cl₂). HRMS (FAB): calcd. for C₁₁H₂₀NO₅ [M+H]⁺ 246.1341, found 246.1345.

Boc-Ser(Al)-Aib-OMe:

To a solution of Boc-Ser(Al)-OH (0.318 g, .0013 mol) in 11 mL DMF was added H-Aib-OMe·HCl² (0.195 g, 0.0013 mol), HOBt·H₂O³ (0.215 g, .0014 mol), *N,N*-diisopropylethylamine

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¹ This procedure is analogous to that reported for the preparation of the corresponding benzyl ether, see: Sugano, H.; Miyoshi, M. *J. Org. Chem.* **1976**, *41*, 2352.

(DIPEA, 0.88 mL, 0.0052 mol), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 0.27 g, .0014 mol). The reaction was stirred at room temperature for 18 hours. The solvent was then removed and the residue partitioned between 25 mL of ethyl acetate and 15 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 5 mL of 5% citric acid solution, 15 mL of 5% sodium bicarbonate solution, 5 mL of 5% sodium bicarbonate solution, and 10 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield an oil (0.395 g, 90%) which was found to be of sufficient purity to be used in subsequent reactions. ¹H NMR (400 MHz, CDCl₃): δ 7.00 (1H, br s), 5.85 (1H, m), 5.40 (1H, br d), 5.24 (1H, dq, *J* = 17.3, 1.6 Hz), 5.16 (1H, dq, *J* = 10.4, 1.6 Hz), 4.17 (1H, m), 4.00 (2H, m), 3.78 (1H, dd, *J* = 9.5, 4.0 Hz), 3.69 (3H, s), 3.46 (1H, app t, *J* = 8.5 Hz), 1.51 (3H, s), 1.50 (3H, s), 1.41 (9H, s). ¹³C NMR (100 MHz, CDCl₃): δ 175.09, 170.01, 155.89, 134.38, 117.80, 80.44, 72.53, 70.00, 56.88, 53.94, 52.99, 28.66, 25.19, 25.01. [α]_D = +16.5° (*c* = 1.3, CH₂Cl₂). HRMS (FAB): calcd. for C₁₆H₂₉N₂O₆ [M+H]⁺ 345.2026, found 345.2029.

Boc-Aib-Ser(Al)-Aib-OMe:

Boc-Ser(Al)-Aib-OMe (7.5 g, 0.022 mol) was dissolved in 40 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 40 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for a period of 1.5 hours. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 54 mL of DMF and to this solution was added DIPEA (19 mL, 0.11 mol), Boc-Aib-OH⁴ (4.43 g, 0.022 mol), HOBt·H₂O (3.67 g, 0.024 mol), and EDC (4.6 g, 0.024 mol). The reaction was stirred at room temperature for 48 hours. The solvent was then removed and the residue partitioned between 200 mL of ethyl acetate and 100 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 50 mL of 5% citric acid solution, 100 mL of 5% sodium bicarbonate solution, 50 mL of 5% sodium bicarbonate solution, and 50 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a solid (7.6 g, 81% crude) which was purified by recrystallization from hot hexane/ethyl acetate (3:1, 65 mL) to yield a white solid (5.24 g, 56%). ¹H NMR (400 MHz, CD₂Cl₂): δ 7.57 (1H, br s), 7.00 (1H, d, *J* = 7.5 Hz), 5.89 (1H, m), 5.28 (1H, dq, *J* = 17.3, 1.7 Hz), 5.18 (1H, dq, *J* = 10.5, 1.7 Hz), 5.16 (1H, br s), 4.30 (1H, m), 4.01 (2H, m), 3.97 (1H, app d, *J* = 2.9 Hz), 3.66 (3H, s), 3.57 (1H, dd, *J* = 9.6, 4.4), 1.53 (3H, s), 1.50 (3H, s), 1.48 (3H, s), 1.47 (9H, s), 1.43 (3H, s). ¹³C NMR (100 MHz, CD₂Cl₂): δ 175.03, 174.15, 169.74, 155.52, 134.81, 116.94, 80.96, 72.34, 69.44, 57.22, 56.34, 53.86, 52.37, 28.41, 27.27, 25.26, 24.90, 24.01. [α]_D = -8.6° (*c* = 1.75, CH₂Cl₂). HRMS (FAB): calcd. for C₂₀H₃₆N₃O₇ [M+H]⁺ 430.2553, found 430.2572.

² Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **1999**; *121*, 6948. Leplawy, M.T.; Jones, D.S.; Kenner, G. W.; Sheppard, R.C. *Tetrahedron* **1960**; *11*, 39

³ König, W.; Geiger, R. *Chem. Ber.* **1970**; *103*, 788.

⁴ Leibfritz, D.; Haupt, E.; Dubischar, N.; Lachmann, H.; Oekonomopoulos, R.; Jung, G. *Tetrahedron* **1982**; *38*, 2165.

Boc-Aib-Aib-Ser(Al)-Aib-OMe:

Boc-Aib-Ser(Al)-Aib-OMe (0.73 g, 0.0017 mol) was dissolved in 6 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 6 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for 1 hour. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 4.2 mL of DMF and to this solution was added DIPEA (1.5 mL, 0.0085 mol), Boc-Aib-OH (.35 g, 0.0017 mol), HOBT·H₂O (0.29 g, 0.0019 mol), and EDC (0.36 g, 0.0019 mol). The reaction was stirred at room temperature for 96 hours. The solvent was removed and the residue partitioned between 75 mL of ethyl acetate and 35 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 20 mL of 5% citric acid solution, 35 mL of 5% sodium bicarbonate solution, 20 mL of 5% sodium bicarbonate solution, and 20 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a solid (0.79 g, 90%) which was of sufficient purity to use in subsequent reactions. ¹H NMR (400 MHz, CD₂Cl₂): δ 7.56 (1H, br d, *J* = 6.0 Hz), 7.50 (1H, s), 6.7 (1H, s), 5.87 (1H, m), 5.24 (1H, dq, *J* = 17.3, 1.8 Hz), 5.20 (1H, br s), 5.14 (1H, dq, *J* = 10.5, 1.8 Hz), 4.52 (1H, m), 3.97 (2H, m), 3.81 (1H, m), 3.66 (3H, s), 1.55 (3H, s), 1.51 (3H, s), 1.50 (3H, s), 1.48 (9H, s), 1.47 (3H, s), 1.42 (6H, s). ¹³C NMR (100 MHz, CD₂Cl₂): δ 175.17, 174.92, 174.27, 169.79, 155.81, 135.16, 116.42, 81.49, 71.70, 69.56, 57.28, 57.02, 56.15, 53.85, 52.30, 28.36, 27.83, 26.86, 25.26, 24.95, 23.89, 23.45. [α]_D = +7.0° (*c* = 1.12, CH₂Cl₂). HRMS (FAB): calcd. for C₂₄H₄₃N₄O₈ [M+H]⁺ 515.3081, found 515.3078.

Boc-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe:

Boc-Aib-Aib-Ser(Al)-Aib-OMe (0.64 g, 0.0012 mol) was dissolved in 6 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 6 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for 1 hour. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 3.0 mL of DMF and to this solution was added DIPEA (1.1 mL, 0.006 mol), Boc-Ser(Al)-OH (0.30 g, 0.0012 mol, dissolved in 2.1 mL DMF), HOBT·H₂O (0.23 g, 0.0013 mol), and EDC (0.29 g, 0.0013 mol). The reaction was stirred at room temperature for 25 hours. The solvent was removed and the residue partitioned between 75 mL of ethyl acetate and 30 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 20 mL of 5% citric acid solution, 30 mL of 5% sodium bicarbonate solution, 20 mL of 5% sodium bicarbonate solution, and 20 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a crude solid (0.68 g) which was purified by column chromatography (1:1 dichloromethane/EtOAc with 2→5% methanol) to afford a white solid (0.575 g, 72%). ¹H NMR (400 MHz, CD₂Cl₂): δ 7.50 (1H, s), 7.41 (1H, d, *J* = 7.7 Hz), 7.29 (1H, s), 6.71 (1H, s), 5.92 (2H, m), 5.60 (1H, d, *J* = 3.7 Hz), 5.33 (0.5 H, app q, *J* = 1.6 Hz), 5.29 (1H, m), 5.26 (1H, m), 5.24 (0.5 H, app q, *J* = 1.6 Hz), 5.14 (1H, dq, *J* = 10.5, 1.6 Hz), 4.45 (1H, m), 4.04 (5H, m), 3.78 (4H, m), 3.67 (3H, s), 1.54 (3H, s), 1.53 (3H, s), 1.51 (3H, s), 1.50 (9H, s), 1.47 (3H, s), 1.44 (3H, s), 1.43 (3H, s). ¹³C NMR (100 MHz, CD₂Cl₂): δ 175.15, 174.72, 174.50, 171.15, 169.92, 156.70, 135.46, 134.23, 118.00, 116.42, 81.43, 72.61, 71.96, 69.71, 68.92, 57.29,

56.90, 56.11, 54.40, 52.32, 28.35, 27.70, 27.49, 25.33, 24.96, 23.62, 23.35. $[\alpha]_D = +8.1^\circ$ ($c = 1.6$, CH_2Cl_2). HRMS (FAB): calcd. for $\text{C}_{30}\text{H}_{52}\text{N}_5\text{O}_{10}$ $[\text{M}+\text{H}]^+$ 642.3714, found 642.3700.

Boc-Aib-Aib-Aib-OH:

Boc-Aib-Aib-OMe⁵ (1.27 g, 0.0042 mol) was dissolved in 12 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 12 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for 1 hour. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 10.4 mL of DMF and to this solution was added DIPEA (3.6 mL, 0.021 mol), Boc-Aib-OH (0.854 g, 0.0042 mol), HOBt·H₂O (0.701 g, 0.0046 mol), and EDC (0.886 g, 0.0046 mol). The reaction was stirred at 40 °C for 72 hours. The solvent was removed and the residue partitioned between 130 mL of ethyl acetate and 50 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 30 mL of 5% citric acid solution, 50 mL of 5% sodium bicarbonate solution, 30 mL of 5% sodium bicarbonate solution, and 30 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a buff colored solid (1.15 g, 71%). This material was dissolved in 45 mL of 2:1 THF/water and treated with 0.371 g of LiOH and the resulting suspension was stirred for 3 hours. The bulk THF was removed with a rotary evaporator and the remaining aqueous phase was diluted with 36 mL of water and extracted with one 20 mL portion of diethyl ether. The organic layer was discarded and the aqueous phase was cooled to 0 °C and carefully acidified to pH 2 with portion-wise addition of 6 N HCl. The resulting turbid solution was stirred for an additional 20 minutes at 0 °C and then allowed to warm to room temperature. The solid was collected by filtration, washed with 4 mL of ice-cold water, and then transferred to a vacuum line and evacuated until the internal pressure was 10 mTorr. This material (0.956 g, 86% over one step) was found to be of sufficient purity to be used in subsequent reactions. ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.87 (1H, s), 7.62 (1H, s), 7.50 (1H, s), 7.23 (1H, s), 1.41 (9H, s), 1.32 (6H, s), 1.28 (6H, s), 1.25 (6H, s). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 176.44, 174.37, 174.22, 155.96, 79.53, 56.66, 56.31, 55.60, 28.97, 25.66, 25.57, 25.49. HRMS (FAB): calcd. for $\text{C}_{17}\text{H}_{32}\text{N}_3\text{O}_6$ $[\text{M}+\text{H}]^+$ 374.2279, found 374.2291.

Boc-Aib-Aib-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (1):

Boc-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (0.485 g, 0.00076 mol) was dissolved in 6 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 6 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for 1 hour. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 2.5 mL of DMF and to this solution was added DIPEA (0.65 mL, 0.0038 mol), Boc-Aib-Aib-Aib-OH (0.337 g, 0.0009 mol), HOBt·H₂O (0.15 g, 0.0010 mol), and EDC (0.188 g, 0.0010 mol). The reaction was stirred at 50 °C for 1.5 hour. The solvent was removed and the residue partitioned between 70 mL of ethyl acetate and 35 mL of 5% citric acid solution. The organic layer was removed and

⁵ Boc-Aib-Aib-OMe was prepared using an EDC coupling procedure to yield material with known physical properties, see: Wipf, P.; Li, W.; Adeyeye, C. M.; Rusnak, J. M.; Lazo, J. S. *Bioorg. Med. Chem.* **1996**, *4*, 1585-1596.

washed in succession with 20 mL of 5% citric acid solution, 35 mL of 5% sodium bicarbonate solution, 20 mL of 5% sodium bicarbonate solution, and 20 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a crude solid (0.69 g) which was purified by column chromatography (1:1 dichloromethane/EtOAc with 2→5% methanol) to afford a white solid (0.465 g, 68%). ¹H NMR (400 MHz, CD₂Cl₂): δ 7.99 (1H, d, *J* = 5.4), 7.98 (1H, s), 7.93 (1H, s), 7.62 (1H, s), 7.58 (1H, d, *J* = 7.8 Hz), 7.39 (1H, s), 6.74 (1H, s), 5.92 (2H, m), 5.38 (1H, br s), 5.31 (0.5H, aqq q, *J* = 1.6 Hz), 5.27 (1H, m), 5.24 (0.5 H, app q, *J* = 1.6 Hz), 5.14 (2H, dq, *J* = 10.5, 1.8 Hz), 4.45 (1H, m), 4.16 (1H, m), 4.05 (4H, m), 3.84 (4H, m), 3.68 (3H, s), 1.54 (6H, s), 1.53-1.49 (30H, m), 1.47 (3H, s), 1.41 (3H, s), 1.40 (3H, s). ¹³C NMR (100 MHz, CD₂Cl₂): δ 176.97, 175.97, 175.36, 175.32, 175.17, 174.96, 171.55, 170.14, 156.00, 135.59, 135.11, 116.54, 116.44, 81.67, 72.16, 72.15, 69.92, 69.04, 57.78, 57.31, 57.28, 57.17, 57.11, 56.86, 56.11, 54.83, 52.29, 28.35, 27.72, 27.60, 27.33, 27.12, 27.06, 25.30, 24.99, 23.65, 23.37, 23.28, 23.25, 23.23. [α]_D = +24.3° (*c* = 1.2, CH₂Cl₂). HRMS (FAB): calcd. for C₄₂H₇₃N₈O₁₃ [M+H]⁺ 897.5297, found 897.5278.

Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe (X = *E*-2-butenyl tether, **2**):

Boc-Aib-Aib-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (0.366 g, 0.00041 mol) was dissolved in 82 mL of dichloromethane in a nitrogen-flushed flask equipped with a water-cooled condenser. Second-generation Grubbs catalyst⁶ (0.028 g, 7 mol%) was added in a single portion and the flask was then immersed in an oil bath maintained at 50 °C. After refluxing for 0.5 hour, TLC revealed consumption of starting material and formation of a new lower-R_f product (10% methanol in 1:1 ethyl acetate/dichloromethane). The reaction was quenched by adding 0.25 mL ethyl vinyl ether directly to the flask after removing it from the oil bath. Stirring was continued for 20 minutes, after which the solvent was removed on the rotary evaporator. The residue was purified by column chromatography (1:1 dichloromethane/EtOAc with 2→6% methanol) to afford a white solid (0.331 g, 93%). ¹H NMR (400 MHz, CD₂Cl₂): δ 8.11 (1H, br s), 8.00 (1H, br s), 7.83 (1H, d, *J* = 6.9), 7.64 (1H, d, *J* = 7.8), 7.49 (1H, br s), 7.06 (1H, br s), 6.80 (1H, br s), 5.96-5.79 (2H, m, *J*_{trans} = 15.9 Hz), 5.33 ((1H, br s), 4.54 (1H, m), 4.47 (1H, dt, *J* = 2.8, 8.4 Hz), 4.17 (1H, dd, *J* = 5.1, 12.8 Hz), 4.08-4.04 (3H, m), 4.00 (1H, t, *J* = 8.9 Hz), 3.86 (1H, dd, *J* = 6.7, 12.8 Hz), 3.72 (1H, dd, *J* = 3.0, 9.0 Hz), 3.68 (3H, s), 1.56 (3H, s), 1.55 (3H, s), 1.52 (3H, s), 1.517 (6H, s), 1.51 (9H, s), 1.50 (3H, s), 1.49 (3H, s), 1.472 (3H, s), 1.468 (3H, s), 1.43 (3H, s), 1.41 (3H, s). ¹³C NMR (100 MHz, CD₂Cl₂): δ 177.06, 175.87, 175.51, 175.47, 175.28, 175.10, 171.90, 169.88, 156.02, 132.91, 130.05, 81.76, 70.70, 70.20, 69.39, 67.50, 57.70, 57.30, 57.21, 56.88, 56.14, 55.36, 54.90, 52.30, 28.36, 28.17, 27.67, 27.45, 27.16, 26.75, 25.155, 25.128, 23.71, 23.13, 22.95. [α]_D = +17.8° (*c* = 0.96, CH₂Cl₂). HRMS (FAB): calcd. for C₄₀H₆₉N₈O₁₃ [M+H]⁺ 869.4984, found 869.5002.

Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe (X = 1,4-butyl tether, **3**):

To a solution of octapeptide **2** (0.167 mg, 0.00019 mmol) in anhydrous ethanol (30 mL) was added 10% Pd/C (30 mg, 15% wt/wt). The reaction was purged with nitrogen and then placed under a hydrogen atmosphere. After 2 hours, the reaction was filtered and concentrated *in vacuo* to afford octapeptide **3** as white solid (0.168 g, 100%). ¹H NMR (400 MHz, CD₂Cl₂): δ 7.87 (1H, d, *J* = 7.9 Hz), 7.81 (1H, br s), 7.71 (1H, d, *J* = 2.8 Hz), 7.69 (1H, br s), 7.57 (1H, br s),

⁶ Benzylidene(1,3-dimesitylimidazolidin-2-ylidene)(tricyclohexylphosphine)ruthenium dichloride, CAS 246047-72-3.

7.12 (1H, br s), 6.78 (1H, br s), 5.45 (1H, br s), 4.31 (1H, q, $J = 6.3$ Hz), 4.27 (1H, m), 3.94 (1H, m), 3.88-3.78 (2H, m), 3.63 (1H, m), 3.58 (3H, s), 3.47 (1H, m), 3.39 (2H, m), 3.32 (1H, m), 1.84-1.60 (4H, m), 1.45 (3H, s), 1.44 (3H, s), 1.43 (3H, s), 1.42 (9H, s), 1.40-1.39 (30H, m), 1.37 (3H, s), 1.32 (3H, s), 1.31 (3H, s). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 176.94, 175.56, 175.52, 175.45, 175.34, 175.31, 171.85, 170.25, 156.08, 81.63, 70.85, 70.63, 69.79, 57.39, 57.21, 57.07, 56.90, 56.08, 55.86, 55.18, 52.25, 28.37, 27.62, 27.42, 27.02, 25.81, 25.54, 25.34, 24.95, 24.18, 23.20, 22.97. $[\alpha]_{\text{D}} = +36.4^\circ$ ($c = 1.4$, CH_2Cl_2). HRMS (FAB): calcd. for $\text{C}_{40}\text{H}_{71}\text{N}_8\text{O}_{13}$ $[\text{M}+\text{H}]^+$ 871.5141, found 871.5153.

Synthesis and RCM Reaction of Hexapeptide **5**

Boc-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (**5**):

Boc-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (0.54 g, 0.0008 mol) was dissolved in 7 mL of dichloromethane and the mixture was cooled to 0 °C by immersion in an ice bath. 7 mL of trifluoroacetic acid was added in small portions and the reaction was maintained in an ice bath for 1 hour. The solvents were then removed with a rotary evaporator to yield a thick viscous oil. This flask was then transferred to a high vacuum manifold and evacuated until the internal pressure was less than 100 mTorr. The residue was dissolved in 2.8 mL of DMF and to this solution was added DIPEA (0.73 mL, 0.0042 mol), Boc-Aib-OH (0.20 g, 0.0010 mol), HOBt-H₂O (0.17 g, 0.0011 mol), and EDC (0.21 g, 0.0011 mol). The reaction was stirred at 50 °C for 1 hour. The solvent was removed and the residue partitioned between 70 mL of ethyl acetate and 35 mL of 5% citric acid solution. The organic layer was removed and washed in succession with 20 mL of 5% citric acid solution, 35 mL of 5% sodium bicarbonate solution, 20 mL of 5% sodium bicarbonate solution, and 20 mL of saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and evaporated to yield a crude solid (0.66 g) which was purified by column chromatography (1:1 dichloromethane/EtOAc with 2→5% methanol) to afford a white solid (0.518 g, 85%). ^1H NMR (400 MHz, CD_2Cl_2): δ 8.10 (1H, br s), 7.58 (1H, br s), 7.51 (1H, d, $J = 7.9$ Hz), 7.26 (1H, br s), 7.14 (1H, d, $J = 5.3$ Hz), 5.89 (2H, m), 5.33 (1H, br s), 5.29 (1H, m), 5.25 (1H, m), 5.21 (1H, dq, $J = 1.7, 10.4$ Hz), 5.12 (1H, dq, $J = 1.7, 10.4$ Hz), 4.47 (1H, m), 4.06 (1H, m), 4.03-3.95 (4H, m), 3.87 (1H, dd, $J = 3.3, 9.9$ Hz), 3.85-3.77 (2H, m), 3.72 (1H, dd, $J = 3.6, 9.9$), 3.67 (3H, s), 1.57 (3H, s), 1.55 (3H, s), 1.52 (6H, s), 1.50-1.48 (15H, m), 1.48 (3H, s), 1.44 (3H, s). HRMS (FAB): calcd. for $\text{C}_{34}\text{H}_{59}\text{N}_6\text{O}_{11}$ $[\text{M}+\text{H}]^+$ 727.4242, found 727.4268.

Boc-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe (X = *E*-2-butenyl tether):

Boc-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe (0.369 g, 0.00051 mol) was dissolved in 100 mL of dichloromethane in a nitrogen-flushed flask equipped with a water-cooled condenser. Second-generation Grubbs catalyst (0.022 g, 5 mol%) was added in a single portion and the flask was then immersed in an oil bath maintained at 50 °C. After refluxing for one hour, TLC revealed consumption of starting material and formation of a new lower-R_f product (10% methanol in 1:1 dichloromethane/ethyl acetate). The reaction was quenched by addition to the refluxing mixture a suspension consisting of 183 mg P(CH₂OH)₃ and 0.200 mL Et₃N in 3.6 mL

THF.⁷ Reflux was maintained for 15 minutes, after which the solution was cooled to room temperature and stirred for an additional 20 minutes. 35 mL of water was added and the biphasic mixture was vigorously stirred for one hour. The mixture was transferred to a separatory funnel and the methylene chloride layer was removed and retained. The aqueous phase was re-extracted with 20 mL of chloroform. The combined organics were dried over sodium sulfate, filtered, and evaporated to yield a solid which was purified by column chromatography (1:1 ethyl acetate/dichloromethane with 2→8% methanol) to afford a white solid (0.252 g, 71%). ¹H NMR (400 MHz, CD₂Cl₂): δ 8.17 (1H, s), 7.53 (1H, d, *J* = 8.6 Hz), 7.45 (1H, br s), 7.14 (1H, br s), 6.92 (1H, d, *J* = 5.4 Hz), 5.93 (1H, dt, *J* = 6.5, 15.5 Hz), 5.81 (1H, dt, *J* = 6.5, 15.5 Hz), 3.79 (1H, dd, *J* = 9.5, 11.9 Hz), 3.72 (1H, dd, *J* = 3.0, 9.1 Hz), 3.68 (3H, s), 1.55 (3H, s), 1.51 (3H, s), 1.50 (3H, s), 1.48 (9H, s), 1.47 (3H, s), 1.467 (9H, m). HRMS (FAB): calcd. for C₃₂H₅₅N₆O₁₁ [M+H]⁺ 699.3929, found 699.3938.

Characterization and RCM Reaction of Heptapeptide **6**

Boc-Val-Ser(Al)-Leu-Aib-Ser(Al)-Val-Leu-OMe (**6**):

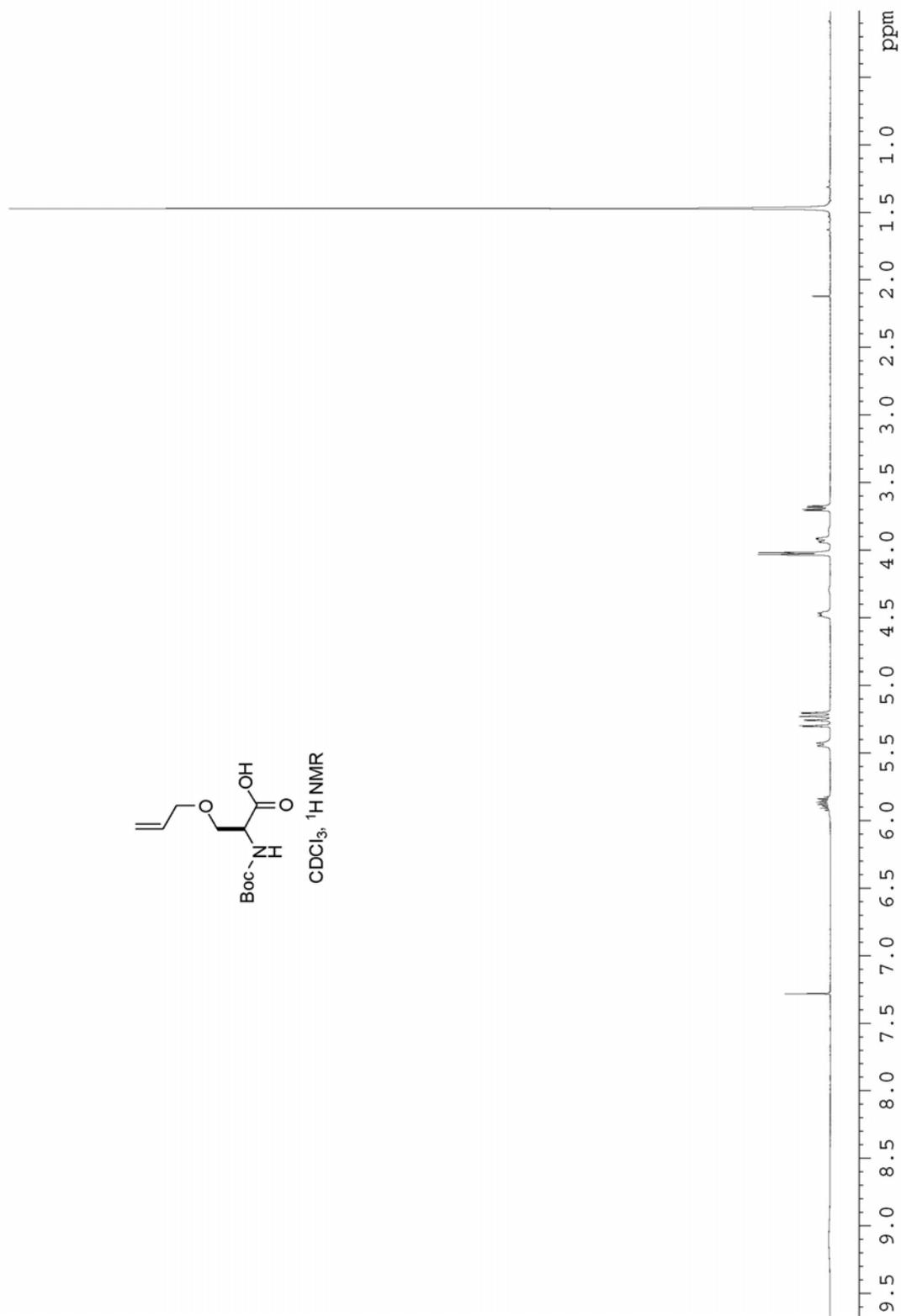
This compound was prepared using standard solution-phase DCC coupling methodology employed in an earlier study.⁸ ¹H NMR (400 MHz, CD₂Cl₂): δ 7.49-7.46 (2H, m), 7.42 (1H, d, *J* = 6.4 Hz), 7.16-7.09 (3H, m), 5.91 (2H, m), 5.31 (0.5 H, app q, *J* = 1.8 Hz), 5.29 (0.5H, app q, *J* = 1.6 Hz), 5.27 (0.5H, app q, *J* = 1.7 Hz), 5.24 (1H, m), 5.21 (0.5H, app q, *J* = 1.6 Hz), 5.18 (0.5H, app q, *J* = 1.8 Hz), 5.15 (0.5H, app q, *J* = 1.8 Hz), 5.14 (1H, br d, *J* = 2.5 Hz), 4.55 (1H, m), 4.39-4.33 (2H, m), 4.33-4.25 (2H, m), 4.17-3.95 (4H, m), 3.95-3.87 (2H, m), 3.83 (1H, dd, *J* = 2.7, 4.5 Hz), 3.78 (1H, dd, *J* = 3.6, 9.6 Hz), 3.72 (1H, dd, *J* = 3.9, 9.6 Hz), 2.38 (1H, oct, *J* = 6.6), 2.23 (1H, m), 1.81-1.57 (4H, m), 1.54 (3H, s), 1.51 (3H, s), 1.50 (9H, s), 1.09 (3H, d, *J* = 7.0 Hz), 1.05 (3H, d, *J* = 6.7 Hz), 1.00 (3H, d, *J* = 6.7 Hz), 0.98-0.91 (12H, m), 0.89 (3H, d, *J* = 6.1 Hz). HRMS (FAB): calcd. for C₄₄H₇₇N₇O₁₂ [M+Na]⁺ 918.5523, found 918.5524.

Boc-Val-Ser(X)-Leu-Aib-Ser(X)-Val-Leu-OMe (X = *E/Z*-butene tether):

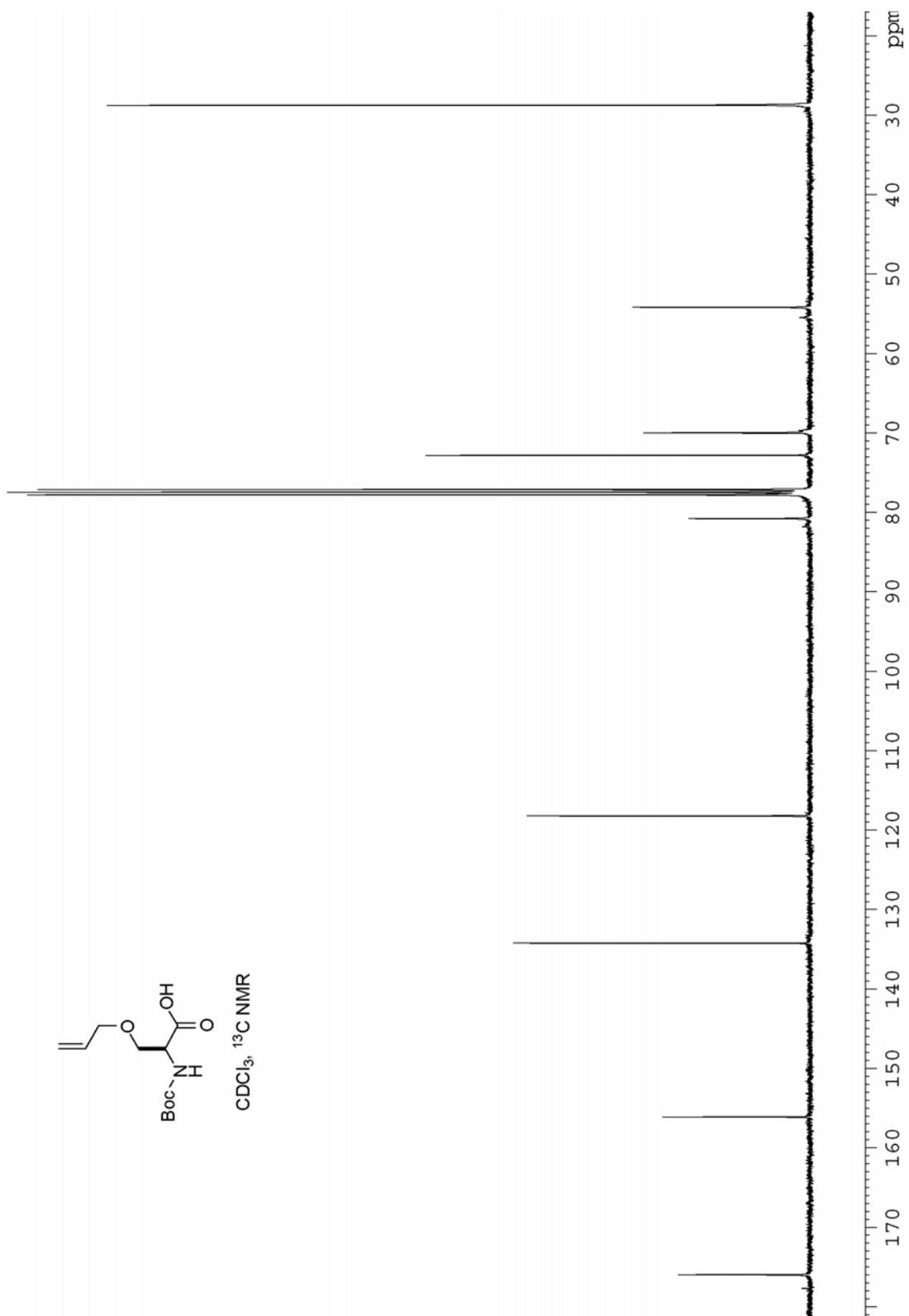
Boc-Val-Ser(Al)-Leu-Aib-Ser(Al)-Val-Leu-OMe (0.05 g, 0.0559 mmol) was dissolved in 6.2 mL of dichloromethane in a nitrogen-flushed flask equipped with a water-cooled condenser. Grubbs-2 catalyst (0.0046 g, 10 mol%) was added in a single portion and the flask was then immersed in an oil bath maintained at 50 °C. After refluxing for 3.5 hour, TLC revealed consumption of starting material and formation of a new lower-R_f product. The solvent was removed on the rotary evaporator. The residue was purified by column chromatography (EtOAc with 0→10% methanol) to afford an oil (0.048 g, 98%) which was shown to consist of a mixture of *E*- and *Z*-olefin isomers (7: 1, major compound assigned *E* on the basis of ³*J*_{trans} = 15.8 Hz). HRMS (FAB): calcd. for C₄₂H₇₃N₇O₁₂ [M+Na]⁺ 890.5209, found 890.5180.

⁷ This is a modification of a procedure designed to render the Ru by-products water soluble, see: Winkler, J. D.; Asselin, S. M.; Shepard, S.; Yuan, J. *Org. Lett.* **2004**, *6*, 3821-3824. Ferguson, M. L.; O'Leary, D. J.; Grubbs, R.H. *Org. Syn.* **2003**, *80*, 85-92

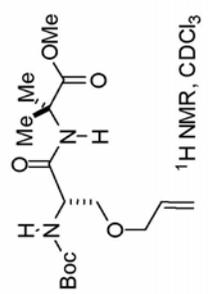
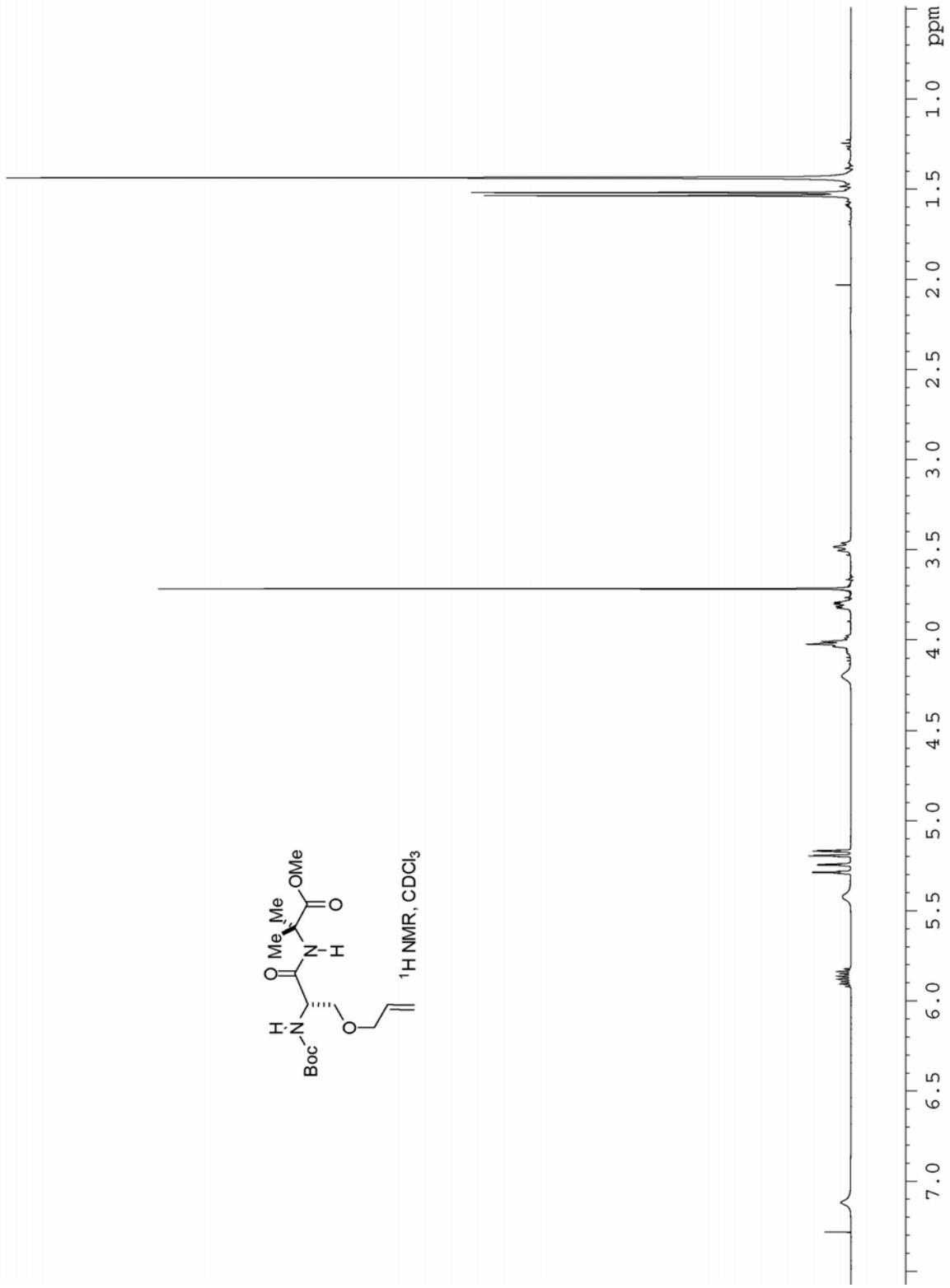
⁸ Blackwell, H. E.; Sadowsky, J. D.; Howard, R. J.; Sampson, J. N.; Chao, J. A.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 5291.



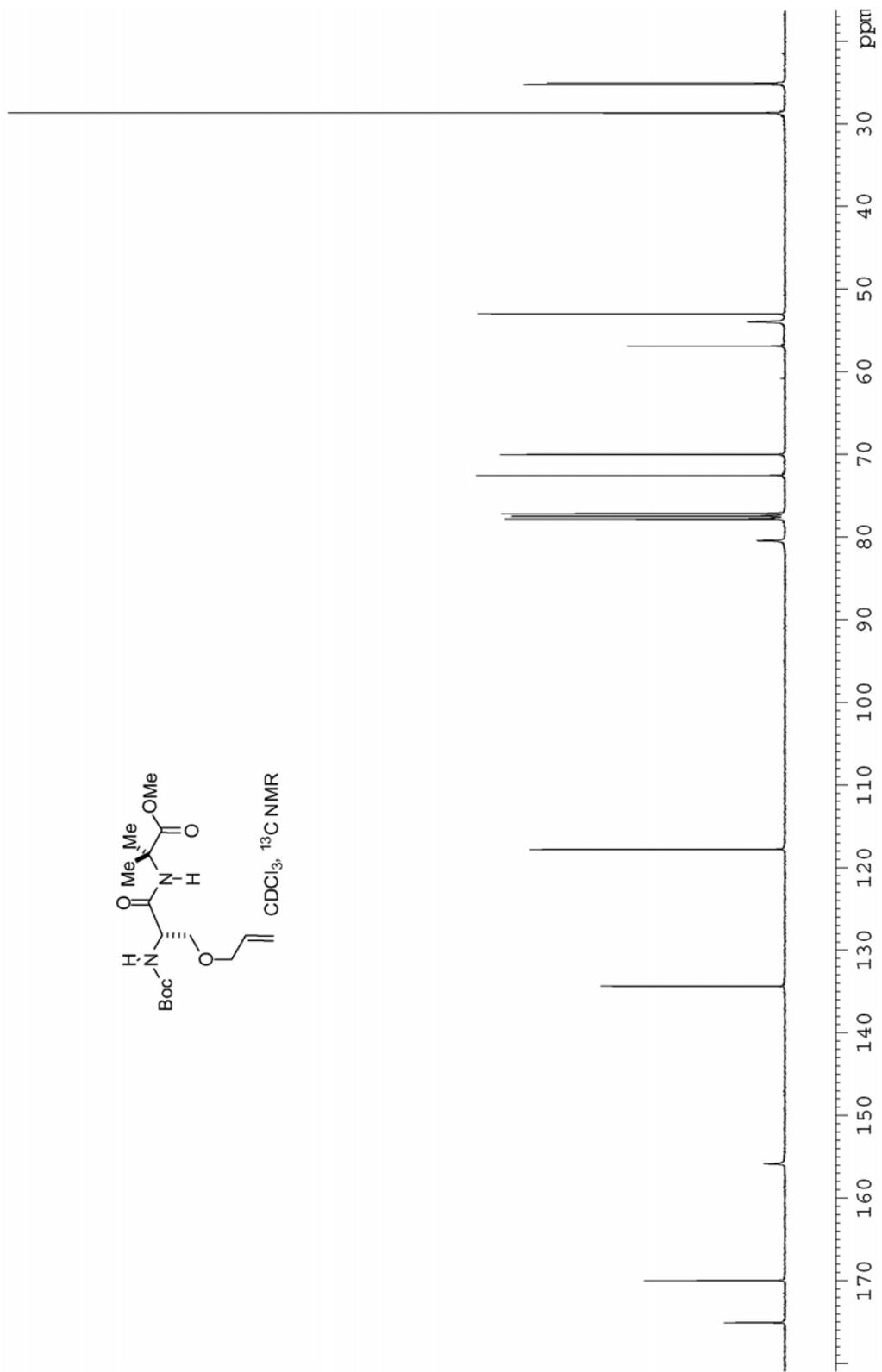
400 MHz ¹H NMR spectrum of Boc-Ser(Al)-OH in CDCl₃.



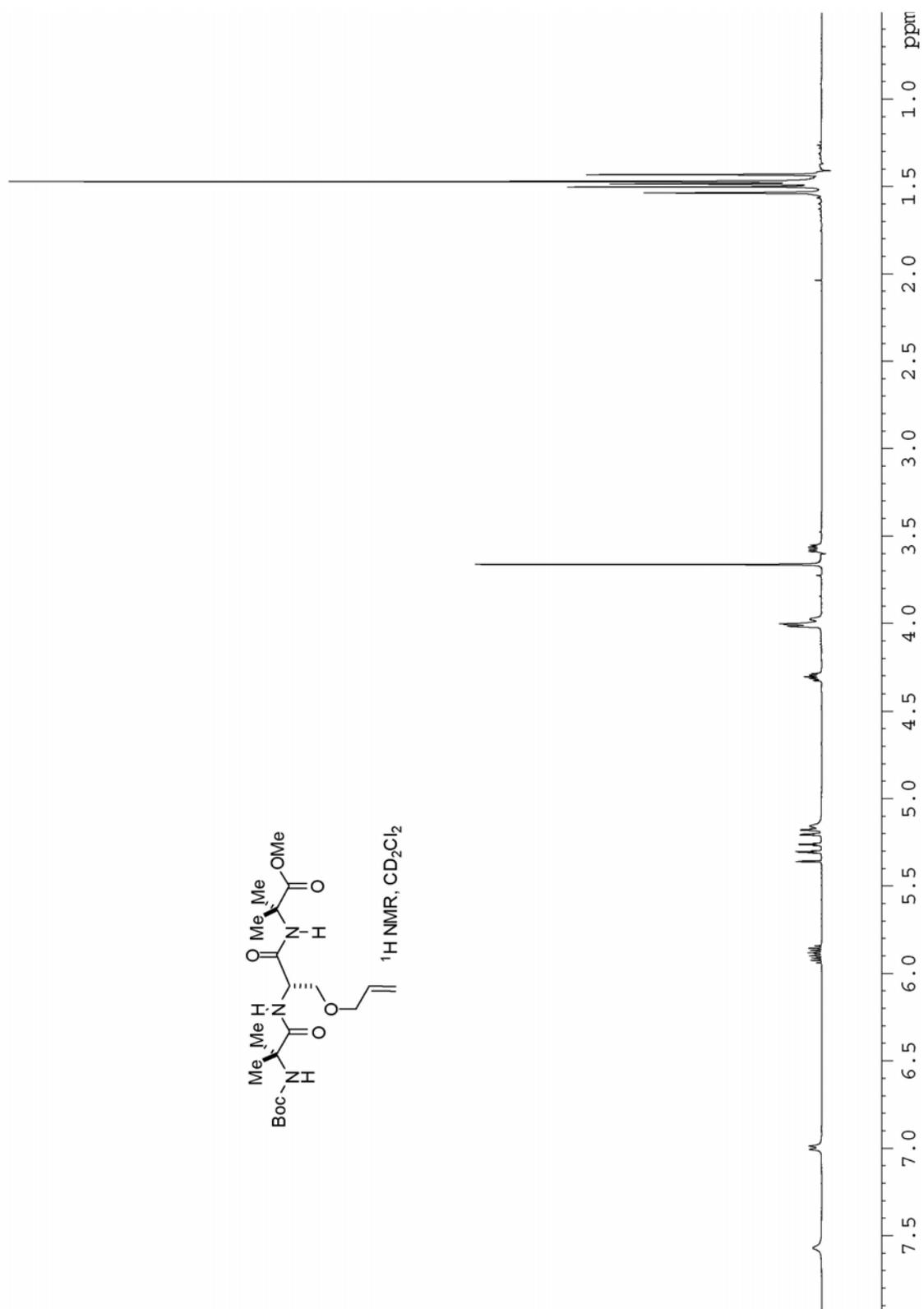
100 MHz ^{13}C NMR spectrum of Boc-Ser(Al)-OH in CDCl_3 .



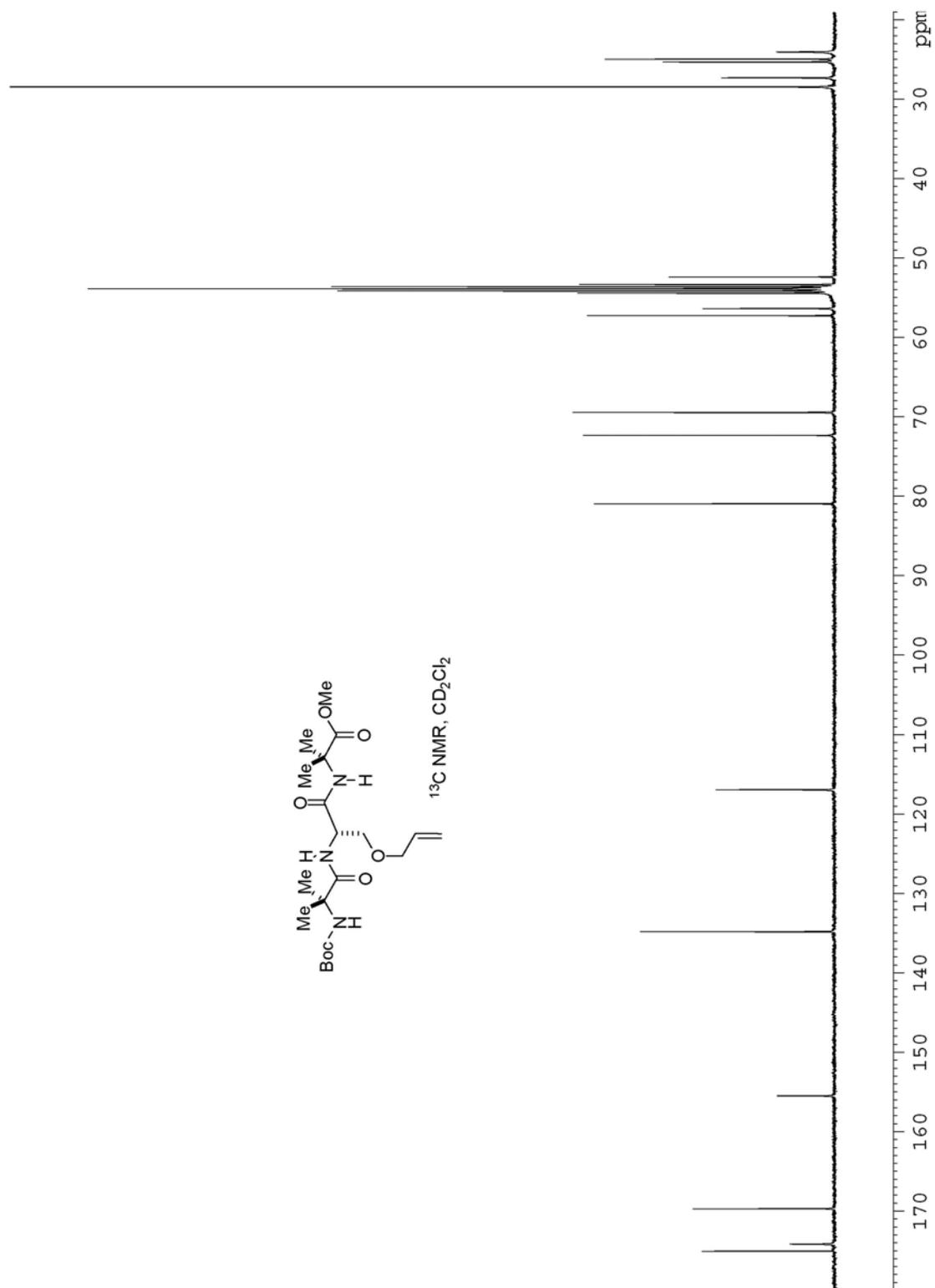
400 MHz ¹H NMR spectrum of Boc-Ser(Al)-Aib-OMe in CDCl₃.



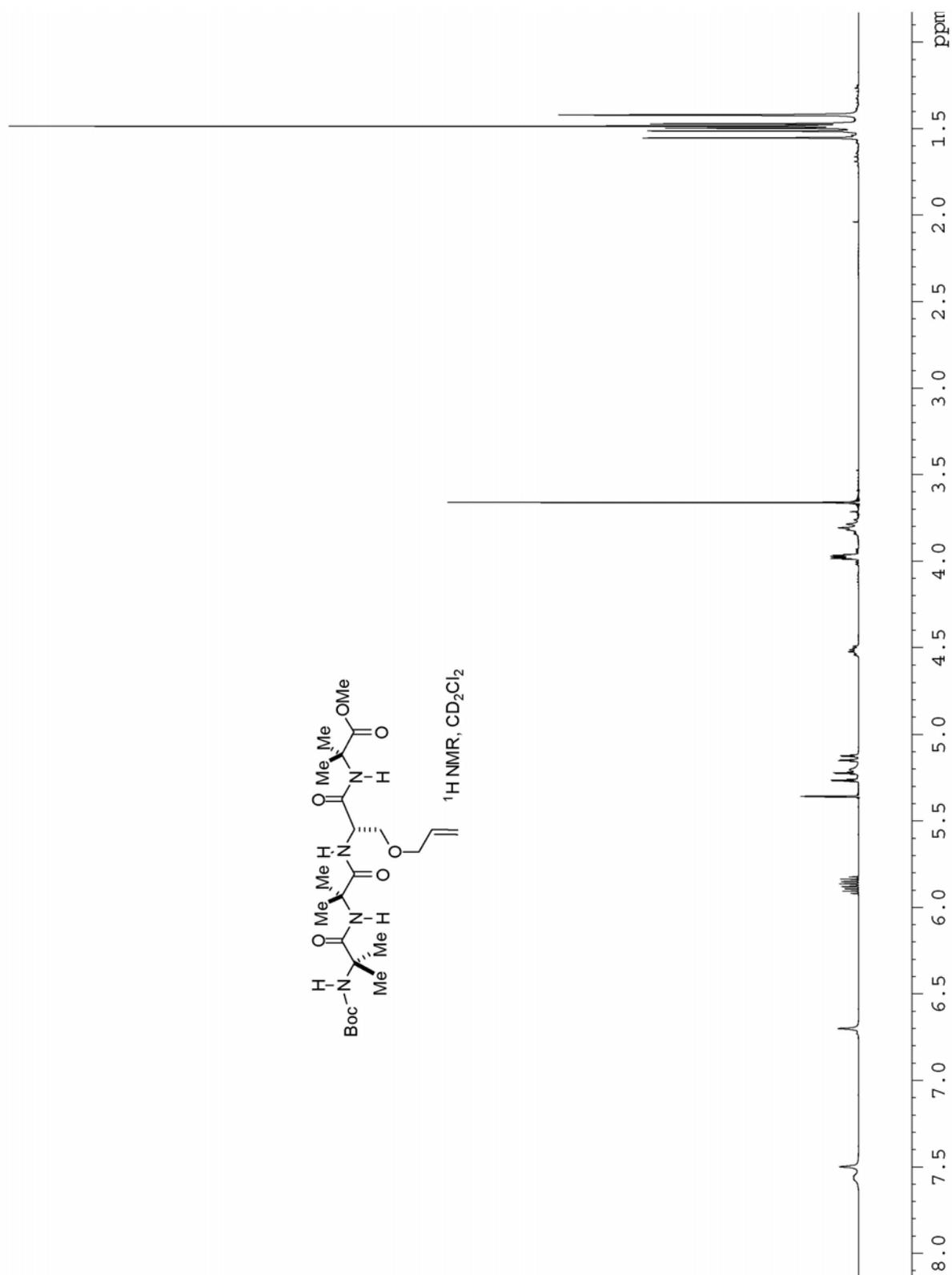
100 MHz ^{13}C NMR spectrum of Boc-Ser(Al)-Aib-OMe in CDCl_3 .



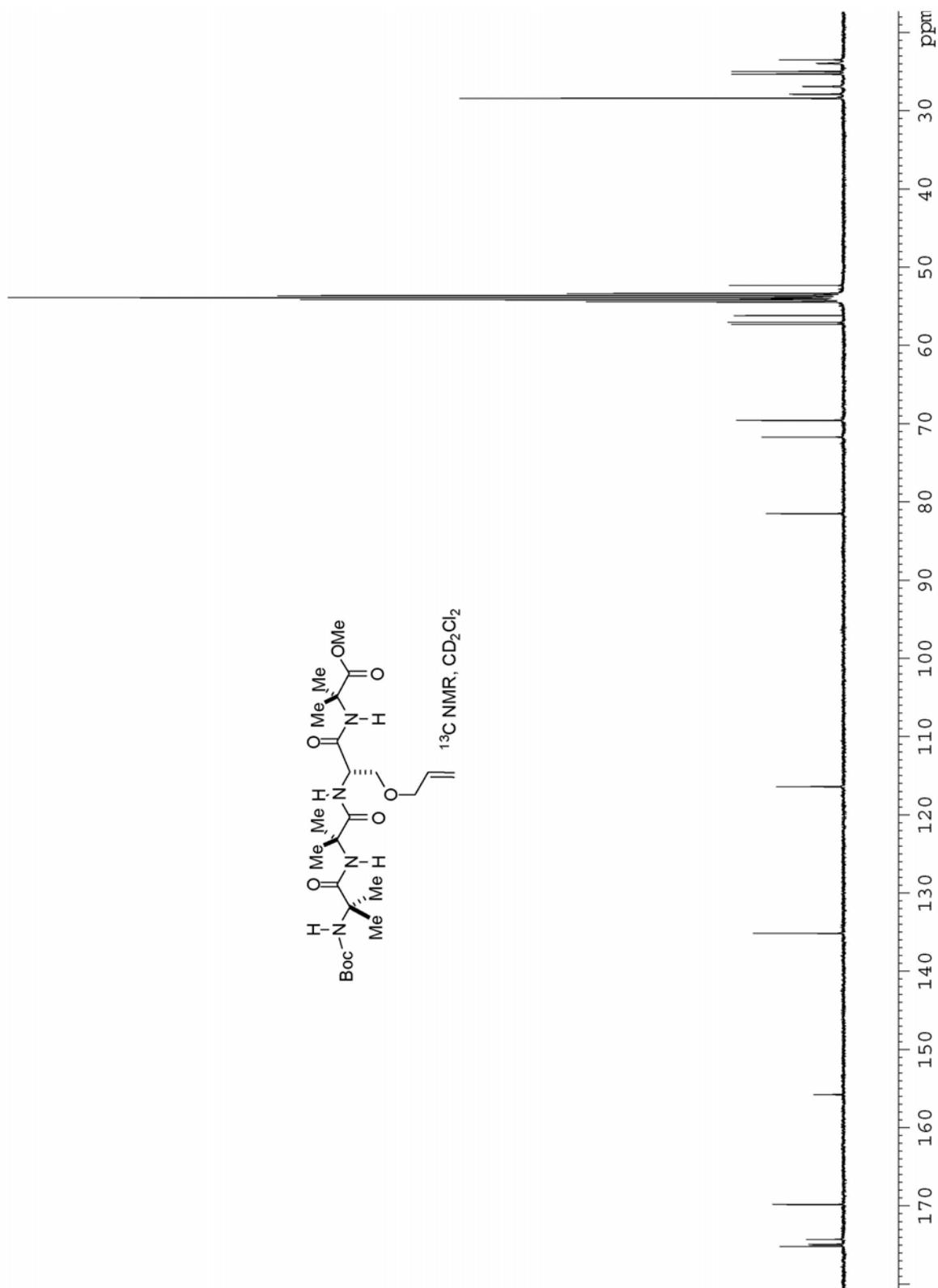
400 MHz ¹H NMR spectrum of Boc-Aib-Ser(AI)-Aib-OMe in CD₂Cl₂.



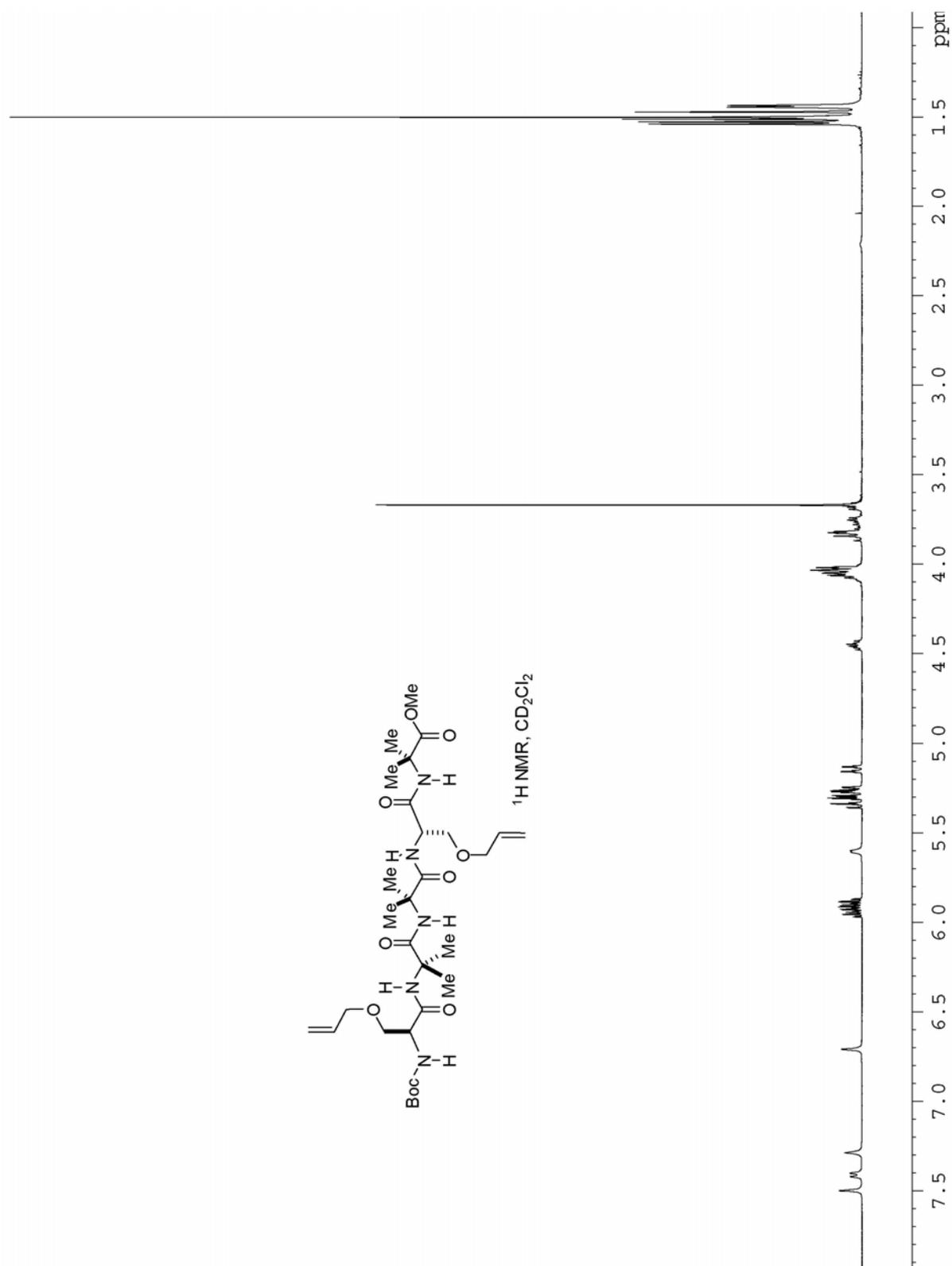
100 MHz ¹³C NMR spectrum of Boc-Aib-Ser(AI)-Aib-OMe in CD₂Cl₂.



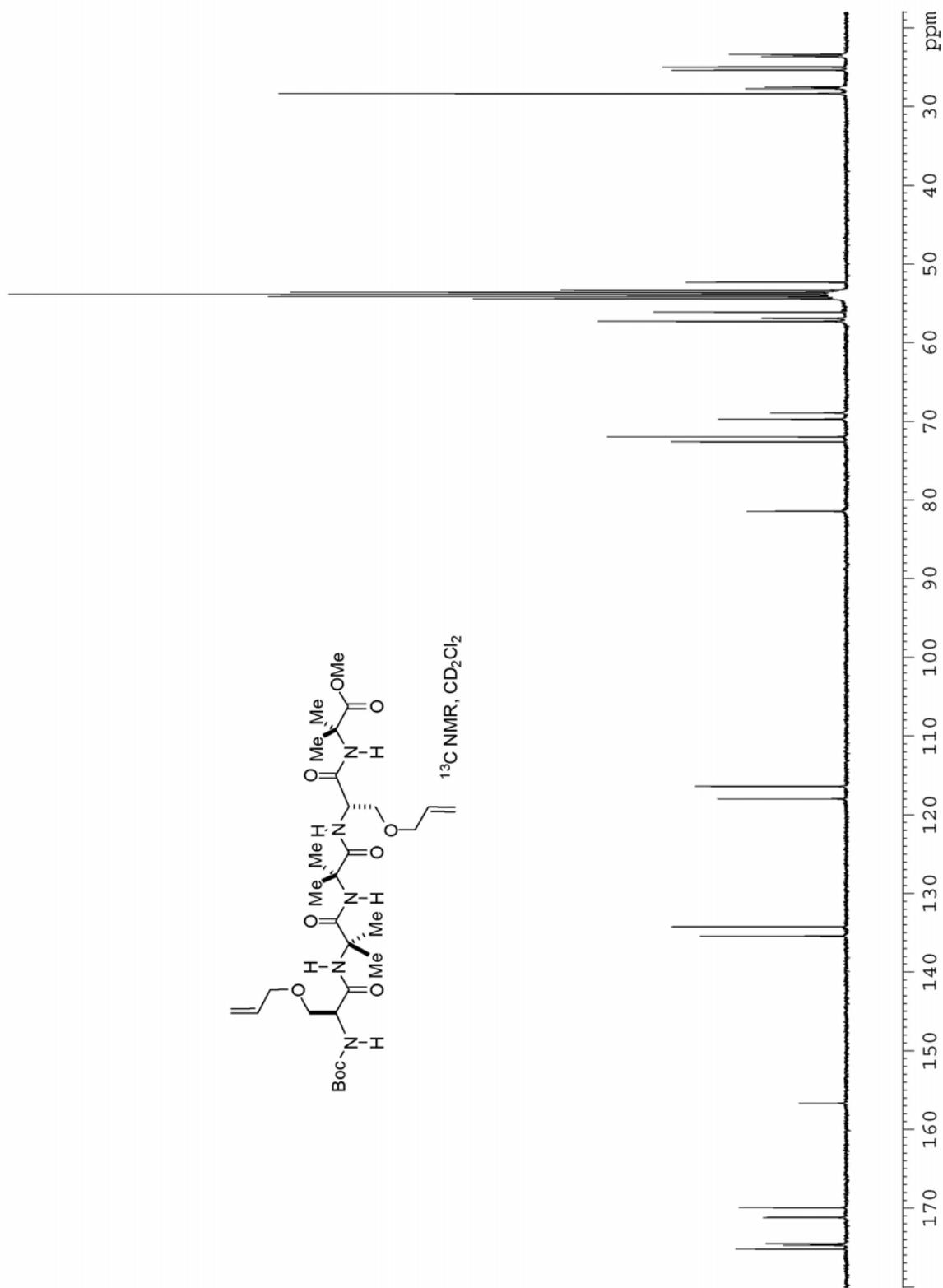
400 MHz ¹H NMR spectrum of Boc-Aib-Aib-Ser(Al)-Aib-OMe in CD₂Cl₂.



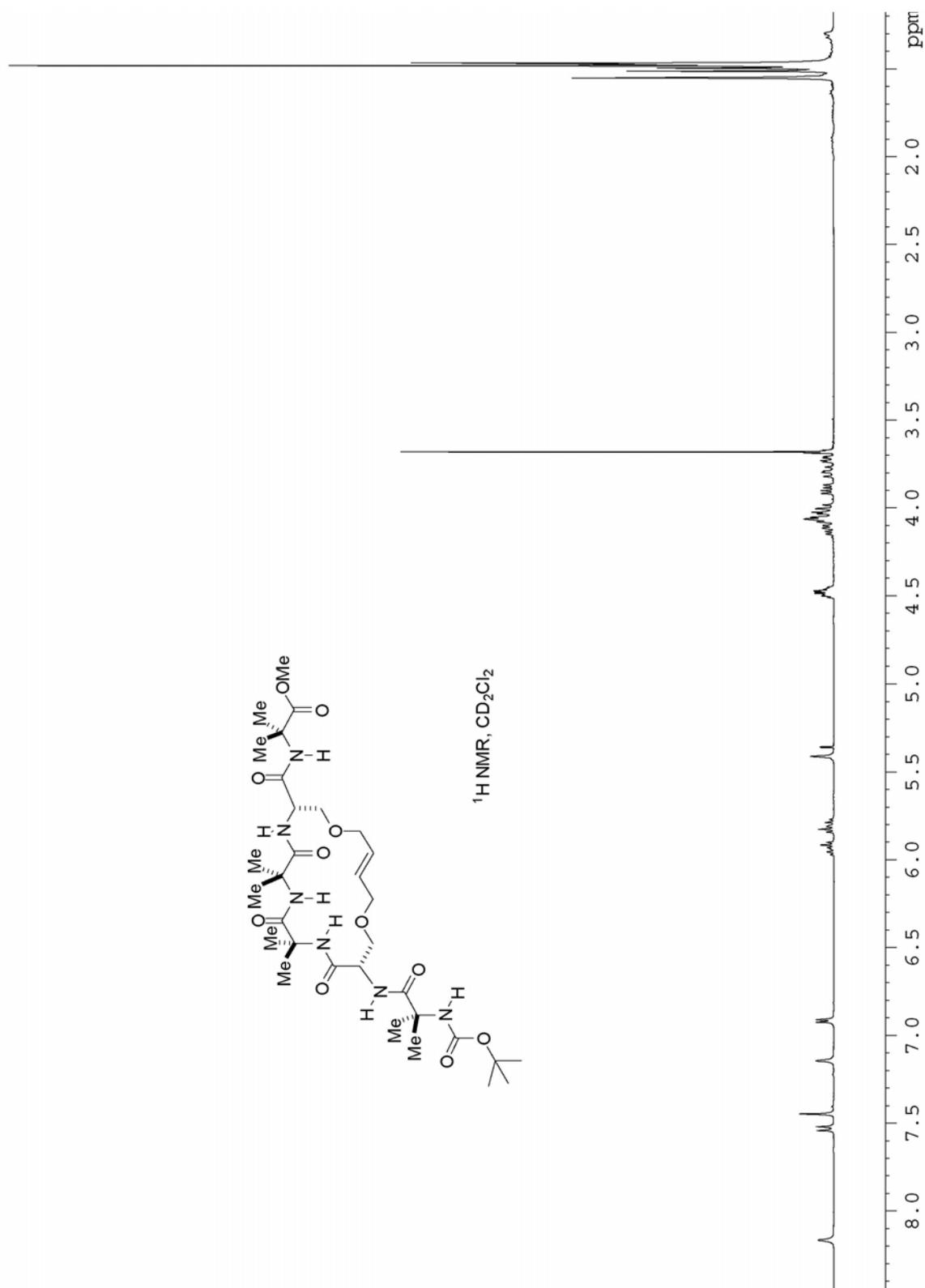
100 MHz ^{13}C NMR spectrum of Boc-Aib-Aib-Ser(Al)-Aib-OMe in CD_2Cl_2 .



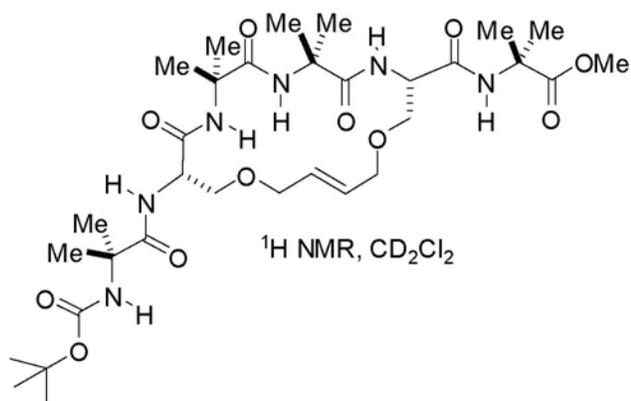
400 MHz ^1H NMR spectrum of Boc-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe in CD_2Cl_2 .



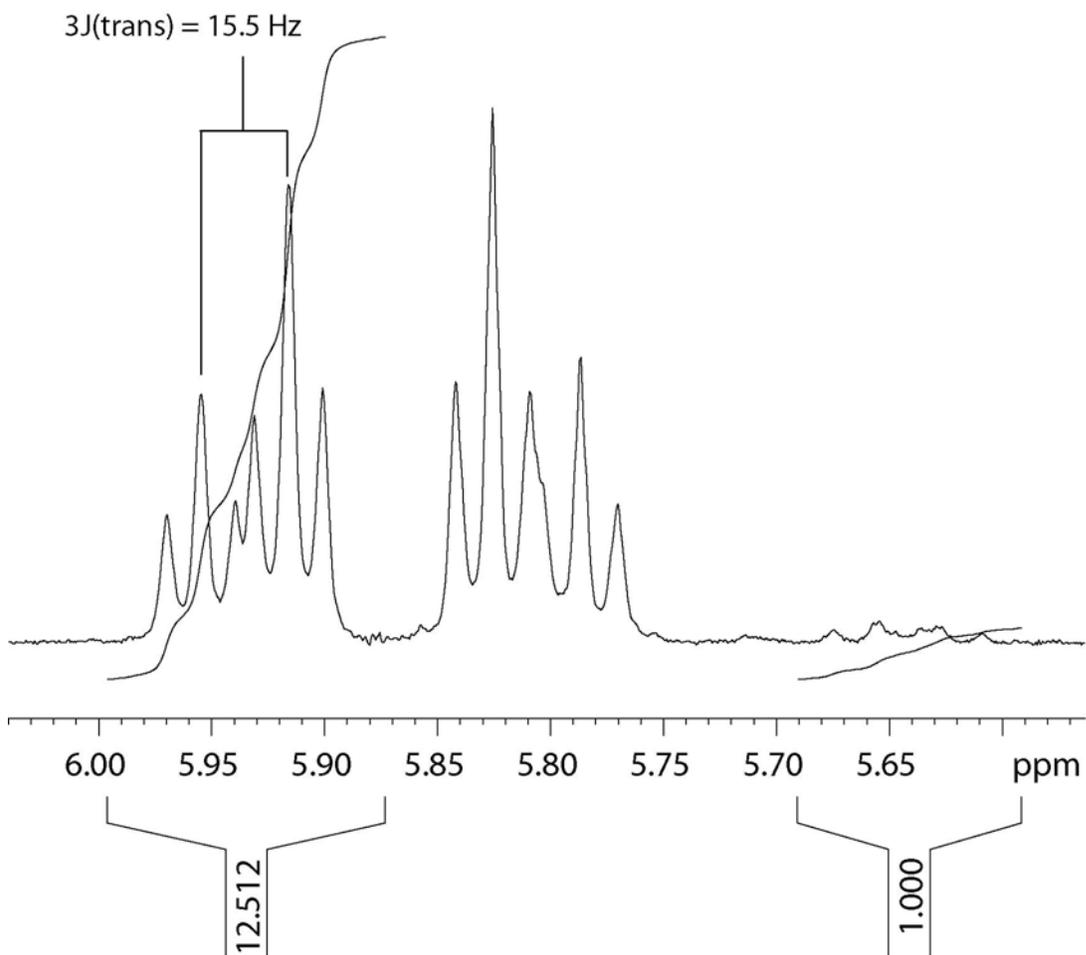
100 MHz ^{13}C NMR spectrum of Boc-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe in CD_2Cl_2 .

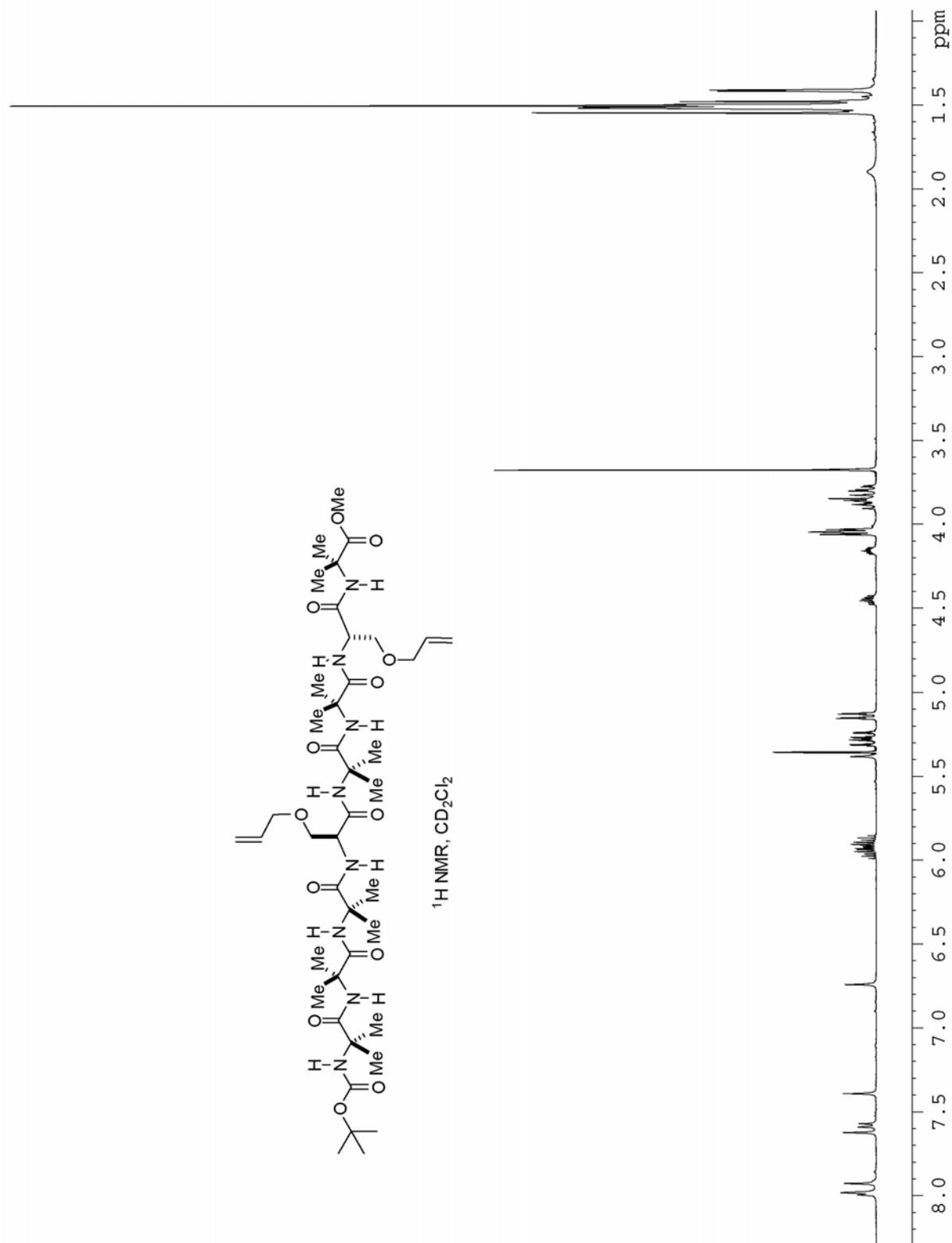


400 MHz ^1H NMR spectrum of Boc-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe [X = *E*-butene tether] in CD_2Cl_2 .

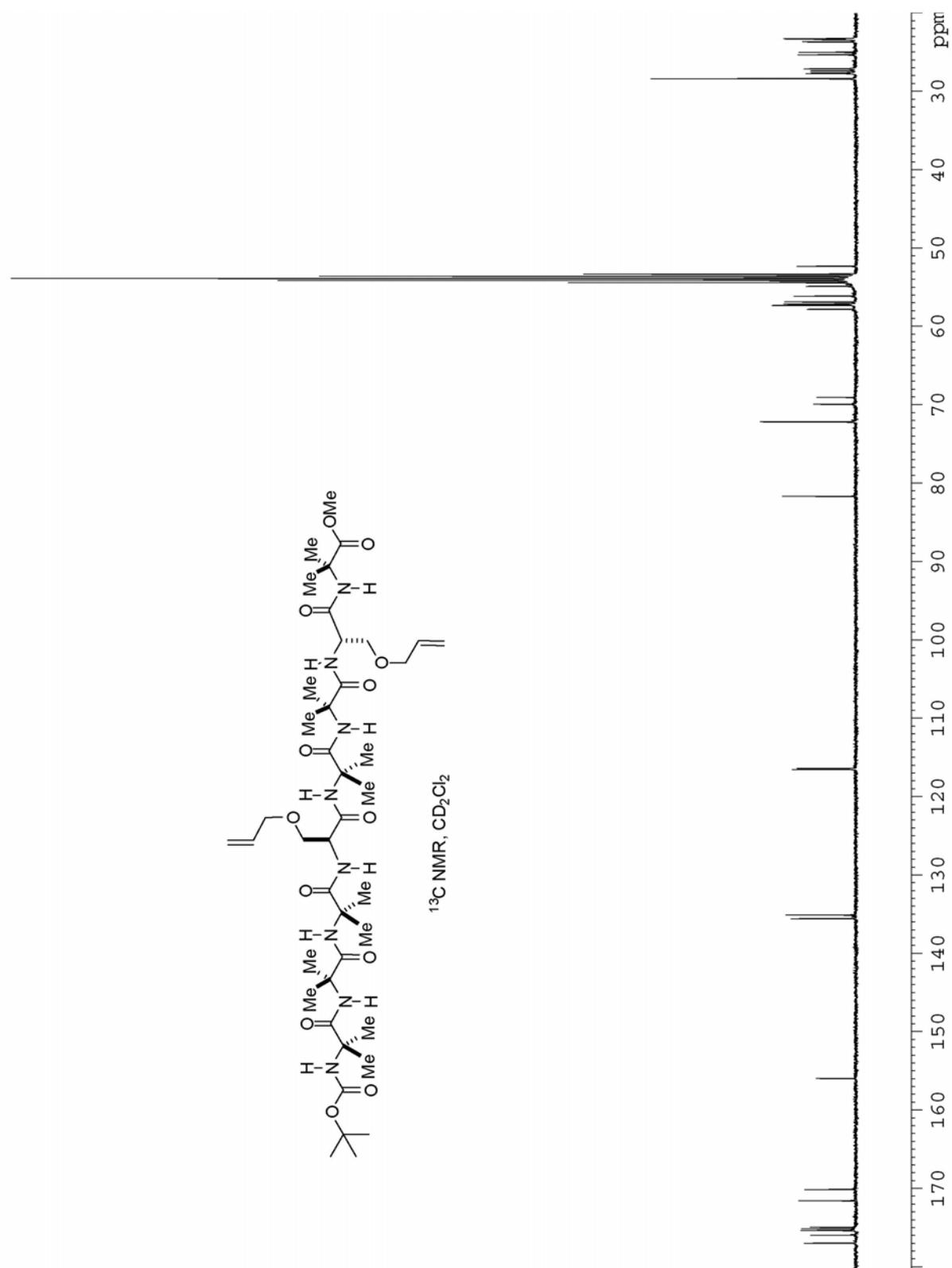


Olefin Resonances
 trans : cis ratio (12:1) estimated from proton integrals

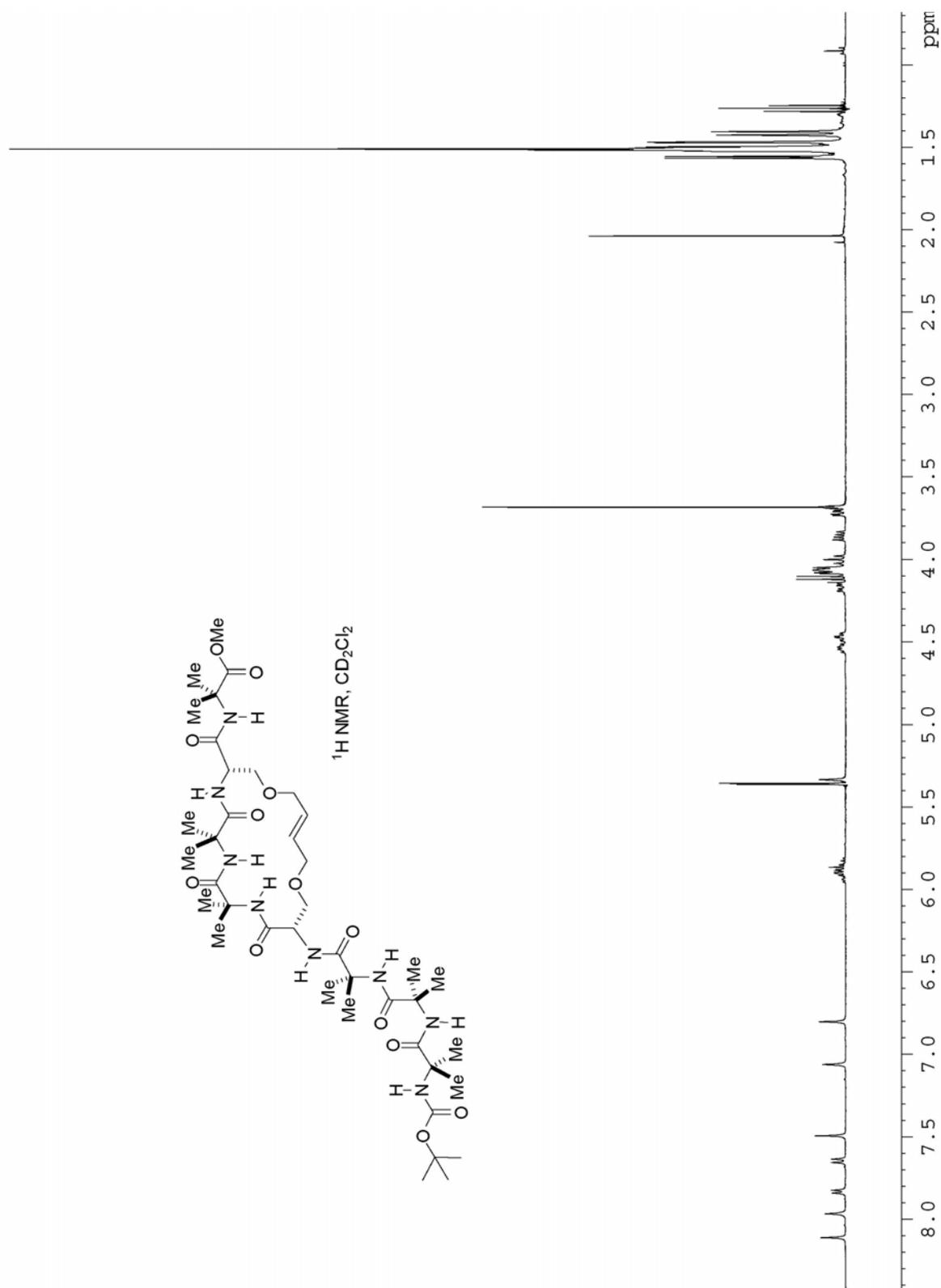




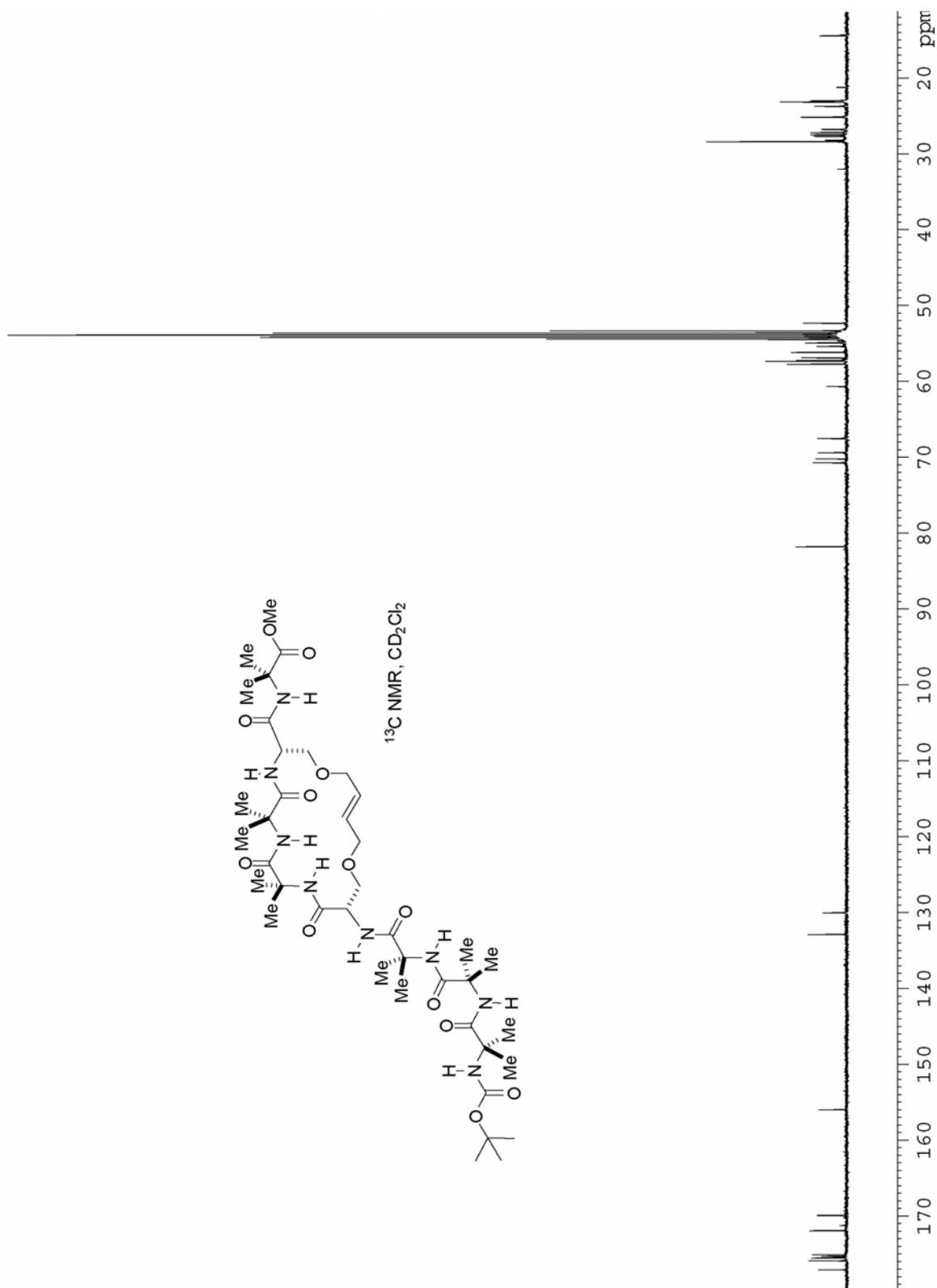
400 MHz ¹H NMR spectrum of Boc-Aib-Aib-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe in CD₂Cl₂.



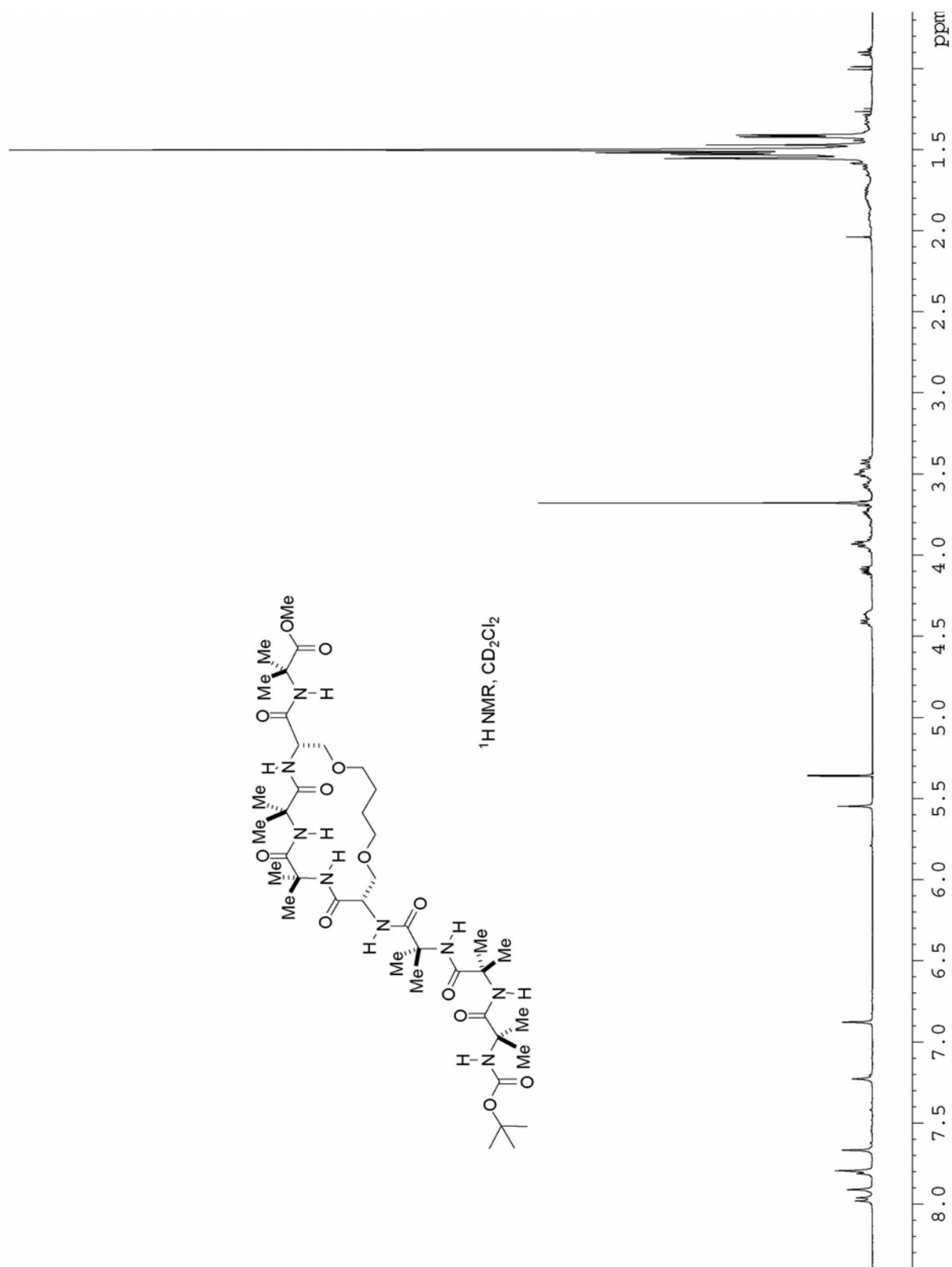
100 MHz ^{13}C NMR spectrum of Boc-Aib-Aib-Aib-Ser(Al)-Aib-Aib-Ser(Al)-Aib-OMe in CD_2Cl_2 .



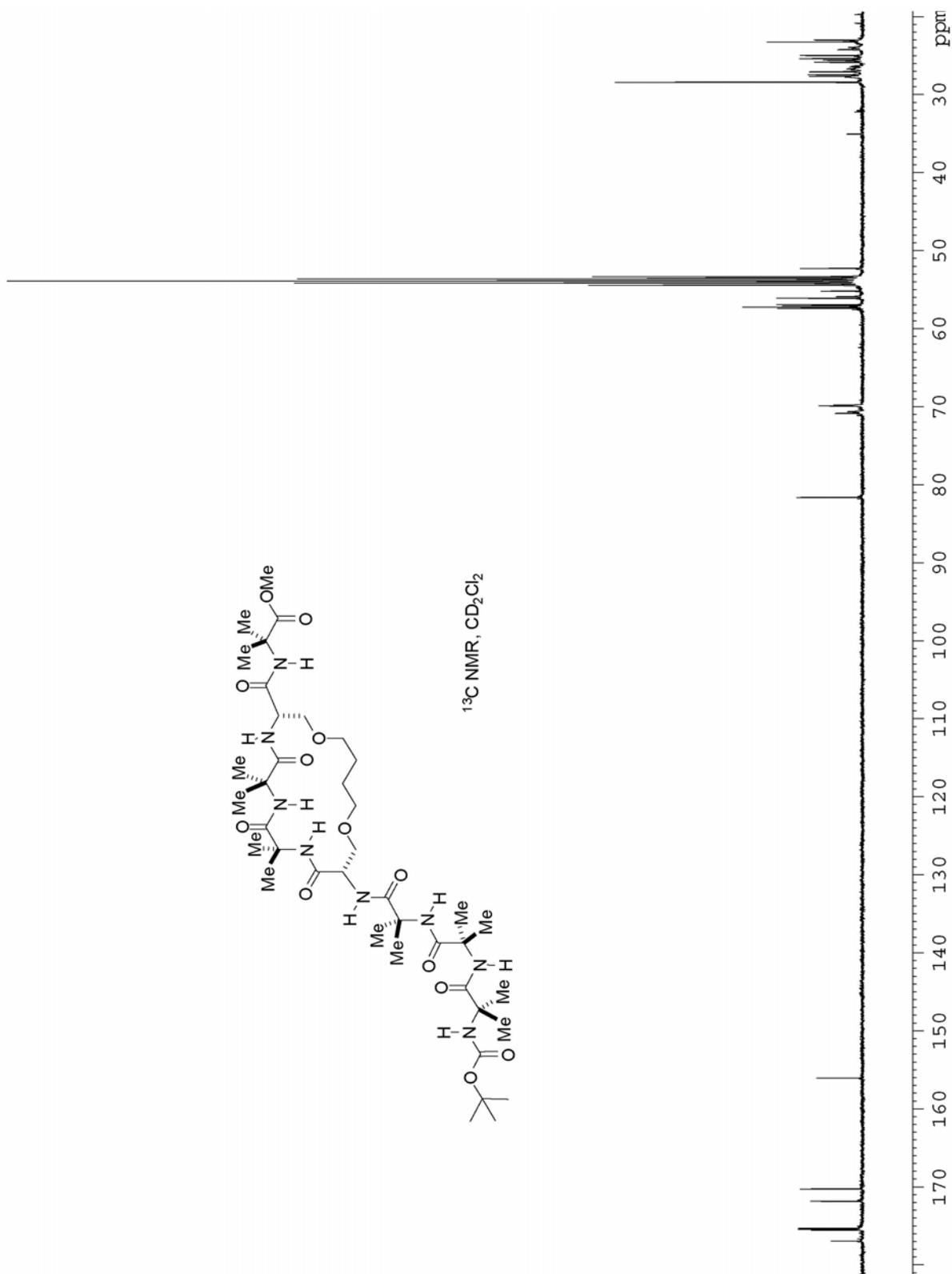
400 MHz ¹H NMR spectrum of Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe [X = *E*-butene tether] in CD₂Cl₂.



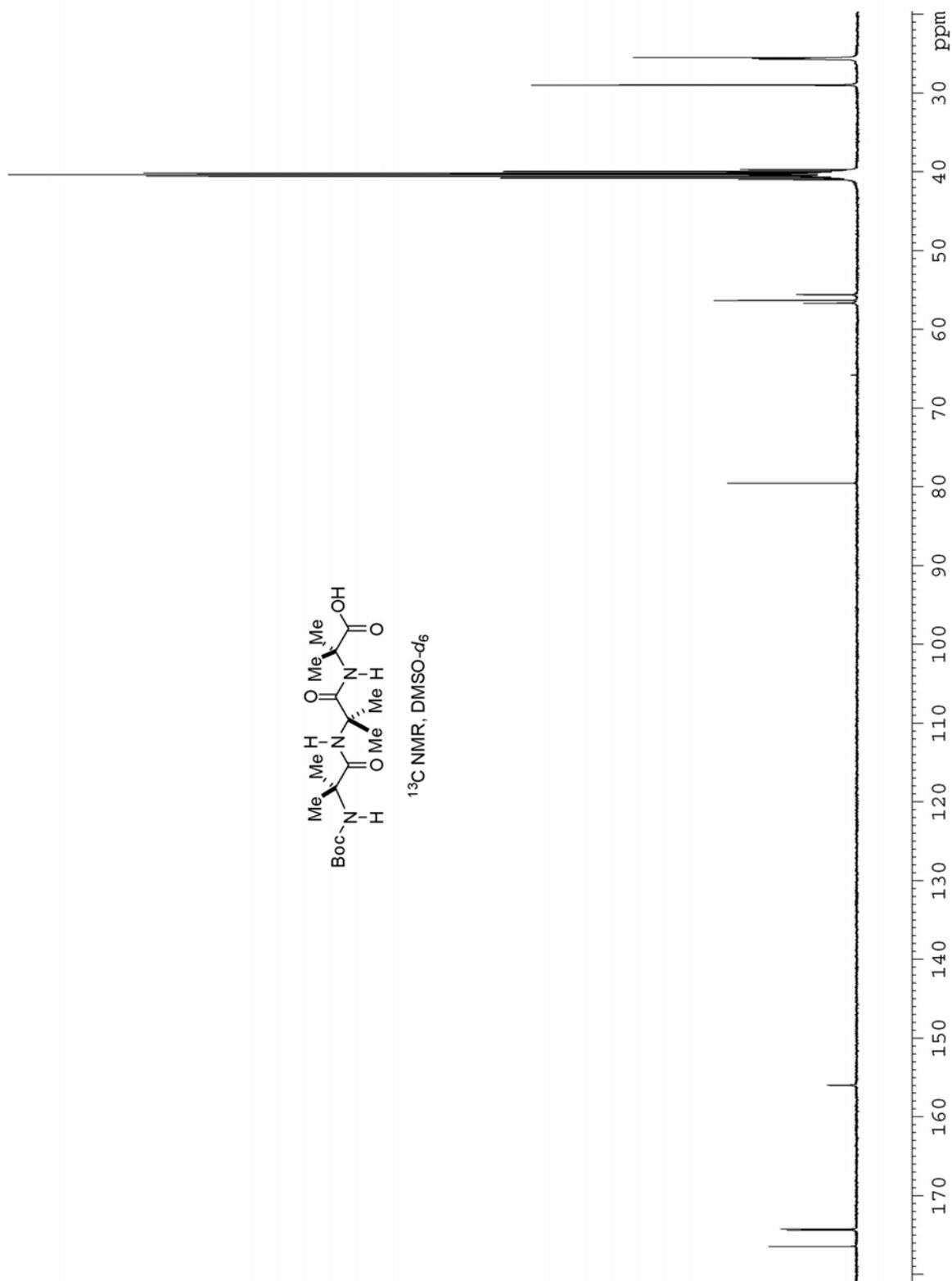
100 MHz ¹³C NMR spectrum of Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe [X = *E*-butene tether] in CD₂Cl₂.



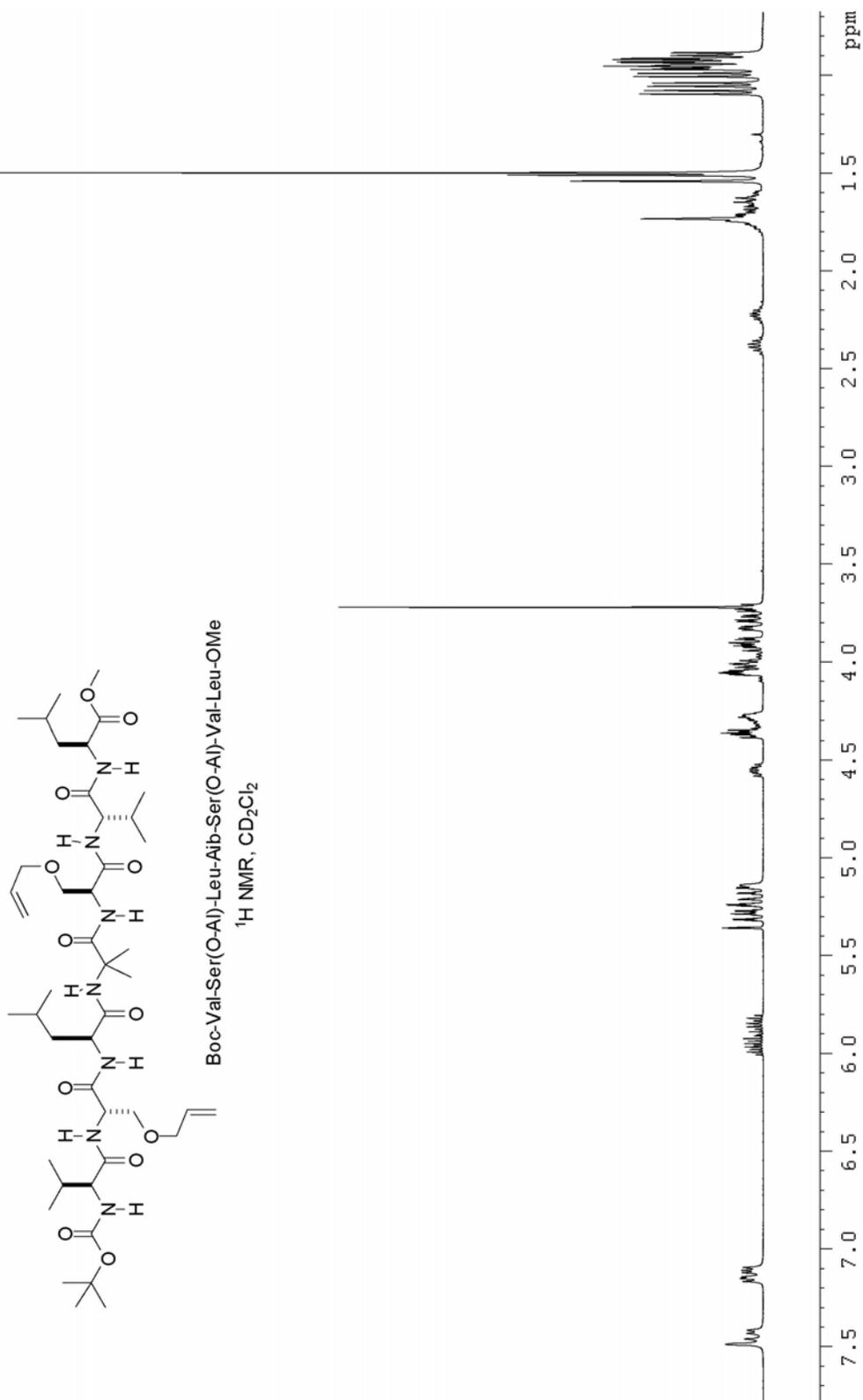
400 MHz ¹H NMR spectrum of Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe [X = 1,4-butyl tether] in CD₂Cl₂.



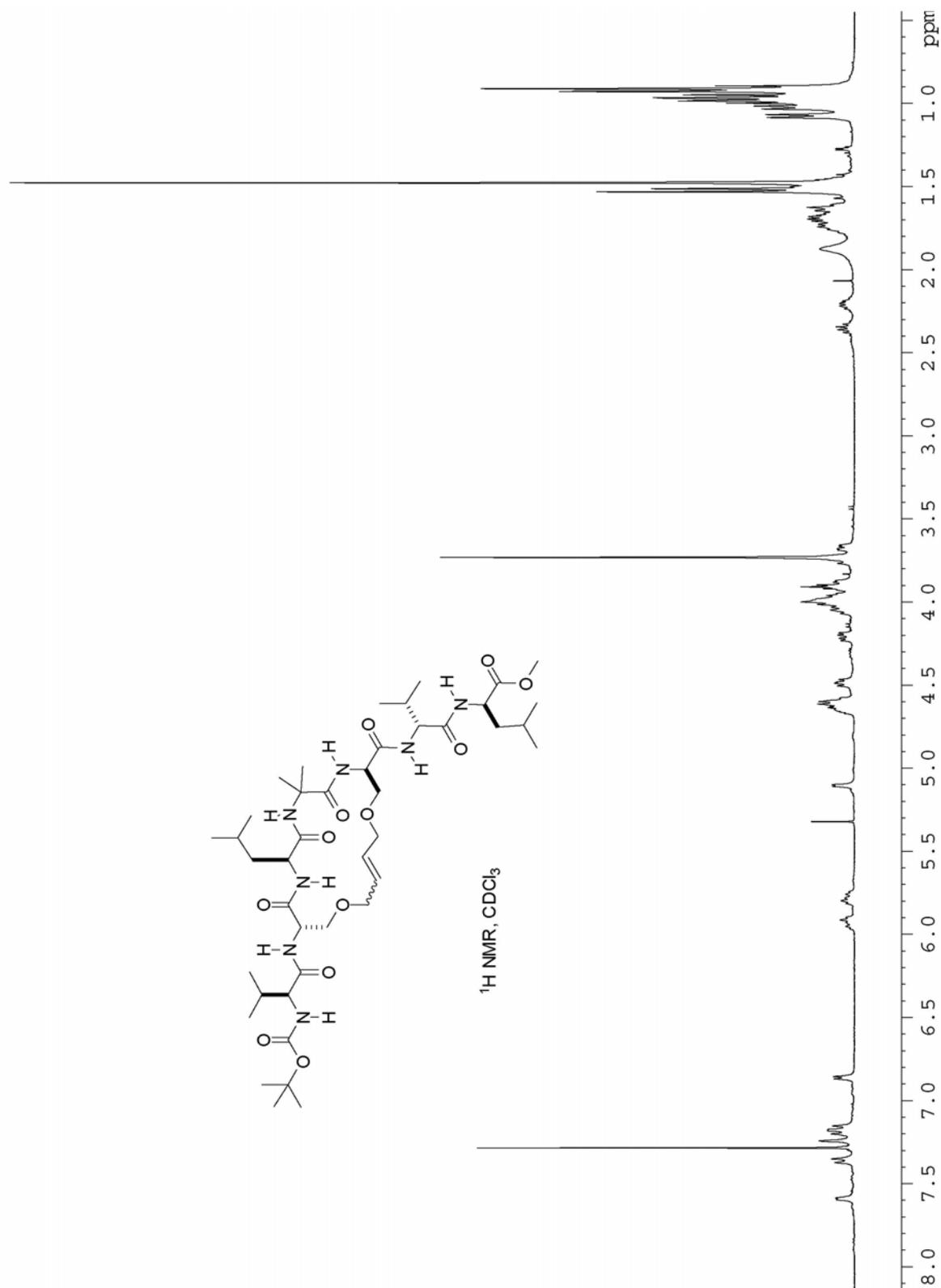
100 MHz ^{13}C NMR spectrum of Boc-Aib-Aib-Aib-Ser(X)-Aib-Aib-Ser(X)-Aib-OMe [X = 1,4-butyl tether] in CD_2Cl_2 .



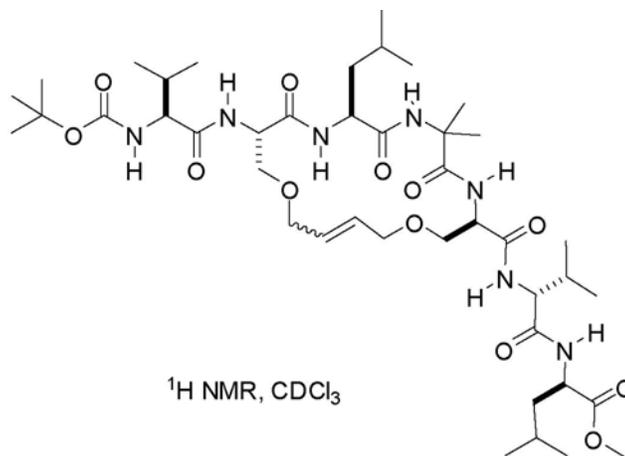
100 MHz ¹³C NMR spectrum of Boc-Aib-Aib-Aib-OH in DMSO-*d*₆.



400 MHz ¹H NMR spectrum of Boc-Val-Ser(Al)-Leu-Aib-Ser(Al)-Val-Leu-OMe in CD₂Cl₂.



400 MHz ¹H NMR spectrum of Boc-Val-Ser(X)-Leu-Aib-Ser(X)-Val-Leu-OMe [X = 2-E/Z-butene tether] in CDCl₃.



Olefin resonances
 trans : cis ratio (7:1) estimated from proton integrals

