

Supporting Information for:

Facile Synthesis of α -Tide β -Strand Peptidomimetics: Improved Assembly in Solution and on Solid Phase

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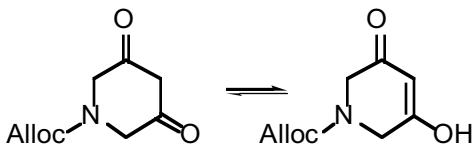
Materials and Methods

General. Reagents were obtained from commercial suppliers and used as received. Solvents were purchased from commercial suppliers in the anhydrous form or dried via distillation. Solvents were removed with a rotary evaporator at low vacuum pressure, followed by drying on high vacuum. Flash chromatography was performed according to the method of Still¹ using 60-mesh silica gel from E. Merck & Co. Thin-layer chromatography was performed using Merck silica gel 60 F254 plates. Analytical

HPLC traces were obtained using a Varian DYNAMAX-100 Å (4.6 mm x 250 mm) reverse-phase C18 column. Solvent mixtures for all chromatographic analyses included 0.1% TFA. The HPLC gradient was as follows: equilibration in 9:1 H₂O–MeCN at 1 mL·min⁻¹ flow rate, followed by ramping to 1:19 H₂O–MeCN over 15 min. This solvent mixture was then run for an additional 10 min for a total elution time of 25 min. Retention times were recorded for this gradient. Preparative HPLC purification was accomplished using a Varian DYNAMAX-100 Å (21.4 mm x 50 mm) reverse-phase C18 column. The gradient for preparative purification was as follows: equilibration in 9:1 H₂O–MeCN at 20 mL·min⁻¹ flow rate for 10 min, followed by ramping to 1:19 H₂O–MeCN over 15 min. This solvent mixture was then run for an additional 5 min for a total elution time of 20 min. A liquid chromatography-mass spectrometry (LCMS) equipped with a Zorbax SB-C18 reverse-phase column (2.1 mm ID x 5 cm) was used for identification of compounds. The LCMS gradient was as follows: equilibration in 9:1 H₂O–MeCN at 0.4 mL·min⁻¹ flow rate, followed by ramping to 1:19 H₂O–MeCN over 8 min. This solvent mixture was then run for an additional 3 min for a total elution time of 11 min.

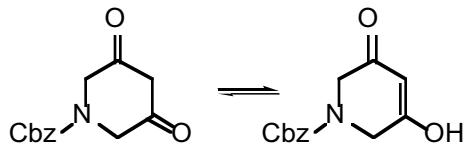
Abbreviations Used: @, the 1,2-dihydro-3(6*H*)-pyridinon-5-yl unit; DIEA, diisopropylethylamine; HATU, *N*-(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; DMF, *N,N*-dimethylformamide; CH₂Cl₂, dichloromethane; Pd(PPh₃)₄, tetrakis(triphenylphosphine)palladium(0); PyBroP, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; Et₂NH, diethylamine; MesSO₂Cl, 2-mesitylenesulfonyl chloride; TFA, trifluoroacetic acid;

NMR Methods. NMR spectra were obtained using a Bruker 500 MHz spectrometer unless otherwise indicated. Spectral data are reported as chemical shifts (multiplicity, number of hydrogens, coupling constants in Hz). ¹H NMR chemical shifts are referenced to TMS (0 ppm) in CDCl₃ or CD₃OD (3.31), and ¹³C NMR chemical shifts are referenced to CDCl₃ (77.23) or CD₃OD (49.15).



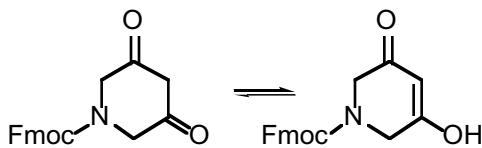
Prop-2-enyl 5-Hydroxy-3-oxo-1,2,6-trihydropyridine-1-carboxylate. This material is reported in reference,³ but a procedure is provided here for continuity. To a solution of 3,5-dimethoxypyridine (8.5 g, 61 mmol) in dry MeCN (230 mL) at -45 °C, was added NaBH₄ (4.16 g, 110 mmol) in portions over 10 min, and the resulting mixture was stirred for an additional 10 min. Allyl chloroformate (7.79 mL, 73.4 mmol) was added over 45 min while the temperature (internal thermometer) was kept at -45 to -40 °C. The reaction was allowed to proceed for an additional 15 min at -40 °C, and then 1N HCl (150 mL) was added at -40 °C. The HCl addition was followed immediately by addition of sat. NaHCO₃ (100 mL) until the pH was basic. The aq. layer was extracted with EtOAc (3 x 50 mL), and the organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude product was dissolved in THF (200 mL) and 1N HCl (200 mL). The reaction mixture was stirred for 30 min at rt and then made basic with solid NaOH at 0 °C. The aq. layer was washed once with EtOAc (50 mL), and the organic layer was extracted with 1N NaOH until the organic layer was no longer yellow. The combined aq. layers were acidified with 6N HCl at 0 °C, saturated with NaCl, and extracted three times with EtOAc (50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated to a thick oil. The enolic diketone tended to decompose on standing, so the crude product (9.5 g, 48 mmol, ca. 79%) was stabilized by addition of 1 mL AcOH. This material

was then stable for months at $-78\text{ }^{\circ}\text{C}$. An analytical sample was purified by flash chromatography using a gradient of petroleum ether–EtOAc to give the enolic diketone as an oil: ^1H NMR δ 4.20 (s, 4), 4.64 (d, 2, $J = 5.3$), 5.27 (d, 1, $J = 19$), 5.31 (d, 1, $J = 25$), 5.63 (s, 1), 5.87-6.00 (m, 1), 9.90 (br s, 1); ^{13}C NMR δ 46.56, 47.44, 66.89, 102.84, 118.31, 131.95, 154.73, 184.93, 186.86; HRMS (FAB, m/z) Calcd. for $\text{C}_9\text{H}_{11}\text{NO}_4$ ($\text{M} + \text{H}^+$): 198.0766; found: 198.0767.



Benzyl 3,5-Dioxopiperidine-1-carboxylate (Cbz@). To a solution of 3,5-dimethoxypyridine (560 mg, 4.03 mmol) in dry MeCN (15.6 mL) at $-45\text{ }^{\circ}\text{C}$, was added NaBH_4 (270 mg, 7.14 mmol) in portions over 10 min, and the resulting mixture was stirred for an additional 10 min. Benzyl chloroformate (690 μL , 4.84 mmol) was added over 20 min while the temperature (internal thermometer) was kept at -45 to $-40\text{ }^{\circ}\text{C}$. The reaction was allowed to proceed for an additional 15 min at $-40\text{ }^{\circ}\text{C}$, and then 1N HCl (5 mL) was added at $-40\text{ }^{\circ}\text{C}$. The HCl addition was followed immediately by addition of sat. NaHCO_3 (10 mL) until the pH was basic. The aq. layer was extracted with EtOAc (3 x 25 mL), and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was dissolved in THF (25 mL) and 1N HCl (25 mL). The reaction mixture was stirred for 30 min at rt and then made basic with solid Na_2CO_3 at $0\text{ }^{\circ}\text{C}$. The aq. layer was washed once with EtOAc (50 mL), and the organic layer was subsequently washed with 0.1N NaOH until the org. layer was no longer yellow. The combined aq. layers were acidified with 6N HCl at $0\text{ }^{\circ}\text{C}$, saturated with NaCl , and extracted three times with EtOAc (30 mL). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated to a white solid (470 mg, 2.01 mmol, ca. 50%). An analytical sample was purified further using preparative reverse-phase HPLC: mp 125 – 126 $^{\circ}\text{C}$

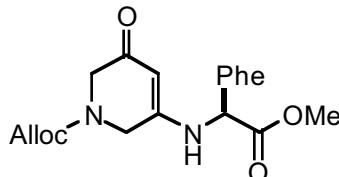
(decomp); HPLC tR 19.0 min; ^1H NMR (1:1 CDCl_3 – CD_3OD) δ 4.15 (s, 4), 51.5 (s, 2), 5.27 (bs, 2), 7.33 (m, 5); ^{13}C NMR (1:1 CDCl_3 – CD_3OD) δ 46.2, 46.9, 67.4, 101.8, 127.7, 128.1, 128.2, 136.2, 154.6; IR (film) ν_{max} 3031, 2958, 1705, 1423, 1221 cm^{-1} ; MS (ESI-neg) m/z (%) 246.1 ($\text{M} - \text{H}^-$, 100); HRMS (FAB, m/z) Calcd. for $\text{C}_{13}\text{H}_{12}\text{NO}_4$ ($\text{M} - \text{H}^-$): 246.0772; found: 246.0765.



3,5-Dioxopiperidine-1-carboxylic acid 9H-fluoren-9-ylmethyl ester (Fmoc@). To a solution of 3,5-dimethoxypyridine (3.0 g, 22 mmol) in dry MeCN (80 mL) at -45 °C was added NaBH_4 (1.5 g, 39 mmol). Fmoc-Cl (6.7g, 26 mmol) dissolved in MeCN (20 ml) was added over 45 min while the temperature (internal thermometer) was kept at -40 to -45 °C. The reaction was allowed to proceed for an additional 30 min at -40 °C, and then 1 N HCl (50 mL) was added at the same temperature. The HCl addition was followed immediately by addition of solid NaHCO_3 until the pH was basic. The aqueous layer was diluted with H_2O (20 mL), extracted with EtOAc (3 x 50 mL), and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was dissolved in THF (60 mL) and 1 N HCl (60 mL). The reaction mixture was stirred for 60 min at rt and then made basic with solid Na_2CO_3 at 0 °C. To the aqueous layer was added EtOAc (50 mL), and the organic layer was extracted with 0.1 N NaOH (5 x 50 mL). The combined aqueous layers were acidified slowly with 12 N HCl at 0 °C, saturated with NaCl, and extracted four times with EtOAc (50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to a white solid (4.7 g, 14 mmol, 65%), which is stable when stored at 0 °C. An analytical sample was purified further using preparative reverse-phase HPLC: mp 169 - 170°C (decomp); HPLC tR

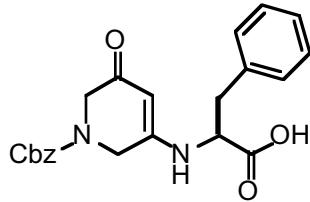
20.0 min; ^1H NMR (CD₃OD) δ 3.95 (s, 2), 4.15 (s, 2), 4.25 (t, 1, J = 6.3), 4.51 (d, 2, J = 6.3), 7.31 (dt, 2, J = 7.5, J = 7.5), 7.38 (dt, 2, J = 1.5, J = 7.5, J = 7.5), 7.55 (dd, 2, J = 7.4, J = 1.0), 7.79 (dd, 2, J = 7.4); ^{13}C NMR δ 46.4 (rot), 46.7, 47.2, 68.0, 120.1, 124.8, 127.3, 128.1, 141.3, 143.7, 155.1, 184.7, 186.4; IR (film) ν_{max} 1701, 1601, 1440, 1221 cm⁻¹; MS (ESI-neg) m/z (%) 334.1 (M⁻, 80), 156.0 (100), 112.0 (M⁻ - Fmoc, 70); Anal. Calcd for C₂₀H₁₇NO₄: C, 71.63; H, 5.11; N, 4.18. Found C, 71.29; H, 5.22; N, 3.89.

The following is a representative procedure for di-@-tide synthesis by direct condensation. Sodium acetate is omitted from this procedure when the C-terminus is unprotected:



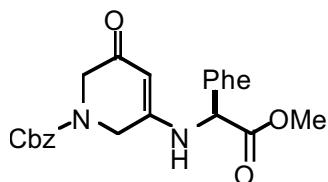
5-(1-Methoxycarbonyl-2-phenylethylamino)-3-oxo-3,6-dihydro-2H-pyridine-1-carboxylic Acid

Allyl Ester. A mixture of prop-2-enyl 5-hydroxy-3-oxo-1,2,6-trihydropyridine-1-carboxylate (8.2 g, 42 mmol), H-Phe-OMe (8.2 g, 38 mmol), and sodium acetate trihydrate (11 g, 84 mmol) in 0.38 L MeOH was stirred at 60 °C for 48 h. The solution was concentrated and the residue was re-dissolved in EtOAc (150 mL) and washed with 1 M KHSO₄ (2 x 50 mL), NaHCO₃ (3 x 50 mL), and brine (1 x 50 mL). The organic layer was dried over MgSO₄, filtered, and concentrated to provide di-@-tide Alloc-@-Phe-OMe (5.5 g, 15 mmol, 41%) as a light yellow foam. An analytical sample was purified further by preparative reverse-phase HPLC: LCMS tR 8.5 min; ^1H NMR δ 7.25 (m, 3), 7.11 (m, 2), 6.17 (bs, 1), 5.88 (m, 1), 5.25 (m, 2), 4.56 (m, 2), 4.29 (m, 3), 4.06 (s, 2), 3.71 (s, 3), 3.08 (m, 2); ^{13}C NMR δ 191.1, 171.1, 160.0, 154.7, 135.0, 132.2, 128.9, 128.5, 127.3, 117.8, 95.7, 66.5, 56.1, 52.5, 50.5, 43.9, 37.1; MS (ESI) m/z 359 (100, M + H⁺). HRMS (FAB) Calcd for C₁₉H₂₃N₂O₅ (M + H⁺): 359.1607. Found: 359.1603.



N-(1-(Benzylcarbamoyl)-1,2,5,6-tetrahydro-5-oxopyridin-3-yl)amino)-L-phenylalanine

(Cbz@Phe). A mixture of benzyl 3,5-dioxopiperidine-1-carboxylate (Cbz@) (100 mg, 405 μ mol) and phenylalanine (64.0 mg, 385 μ mol) in 6.00 mL MeOH was stirred at 60 °C for 18 h. The solution was concentrated, the residue re-dissolved in EtOAc (5.0 mL)/MeOH (0.2 mL) and washed with 1 M KHSO₄ (two 2-mL portions) and brine (one 2-mL portion). The combined organic layer was dried over MgSO₄, filtered, and concentrated. The product was further purified by flash chromatography (10% MeOH followed by 50% MeOH in CH₂Cl₂) to provide the di-@-tide Cbz-@-Phe (128 mg, 324 mmol, 80%) as a white solid. An analytical sample was purified further using preparative reverse-phase HPLC: mp 59-69 °C (decomp); HPLC tR 18.2 min; ¹H NMR δ 3.07 (dd, 1, *J* = 6.8, *J* = 7.2), 3.25 (dd, 1, *J* = 4.4, *J* = 9.5), 4.06 (s, 2), 4.12 (m, 0.5), 4.17 (s, 1), 4.30 (m, 1.5), 5.09 (d, 2, *J* = 6.8), 5.34 (s, 1), 6.08 (bs, 0.5, rot), 6.27 (bs, 0.5, rot), 7.14 (m, 2), 7.25 (m, 3), 7.30 (m, 5); ¹³C NMR δ 49.9, 56.5, 68.1, 95.1, 127.4, 127.9, 128.4, 128.6, 128.8, 129.2, 135.1, 135.6, 154.9, 162.7, 172.4, 192.9; IR (film) ν _{max} 3030, 2955, 1706, 1548, 1232 cm⁻¹; MS (ESI-neg) *m/z* 787.3 (20, M⁻, dimer), 393.1 (100, M⁻). HRMS (FAB) Calcd for C₂₂H₂₃N₂O₅ (M + H⁺): 395.1607. Found: 395.1597.

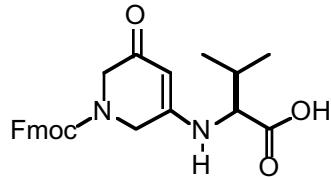


N-(1-((Benzylcarbamoyl)-1,2,5,6-tetrahydro-5-oxopyridin-3-yl)amino)-L-phenylalanine

(Cbz@PheOMe). A mixture of benzyl 3,5-dioxopiperidine-1-carboxylate (Cbz@) (0.66 g, 2.7 mmol), H-

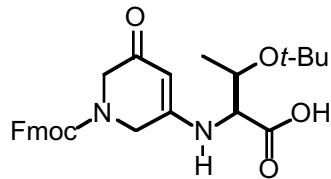
Phe-OMe (0.54 g, 2.5 mmol), and sodium acetate trihydrate (0.36 g, 2.7 mmol) in 27 mL MeOH was stirred at 60 °C for 16 h. The solution was concentrated and the residue was re-dissolved in EtOAc (50 mL) and washed with 1 M KHSO₄ (2 x 20 mL), NaHCO₃ (3 x 20 mL), and brine (1 x 20 mL). The organic layer was dried over MgSO₄, filtered, and concentrated to provide di-@-tide Cbz-@-Phe-OMe (0.35 g, 0.85 mmol, 35%) as a light yellow foam: HPLC tR 19.7 min; ¹H NMR δ 3.09 (dd, 1, *J* = 5.5, *J* = 8.5), 3.18 (dd, 1, *J* = 5.5, *J* = 8.0), 3.29 (s, 3), 4.08 (s, 2), 4.25 (bs, 2), 4.34 (q, 2, *J* = 6.0, *J* = 7.0), 5.12 (s, 2), 5.22 (s, 1), 5.56 (d, 1, *J* = 6.5), 7.06 (m, 2), 7.26 (m, 3), 7.35 (bs, 5); ¹³C NMR δ 37.1, 44.1, 50.6, 52.7, 55.9, 67.8, 96.2, 127.5, 127.9, 128.2, 128.5, 128.7, 129.1, 134.8, 135.9, 154.9, 159.3, 171.0, 191.1; MS (ESI) *m/z* 409.2 (100, M + H⁺). HRMS (FAB) Calcd for C₂₃H₂₅N₂O₅ (M + H⁺): 409.1764. Found: 409.1760.

The following is a representative procedure for the preparation of Fmoc protected di-@-tides:

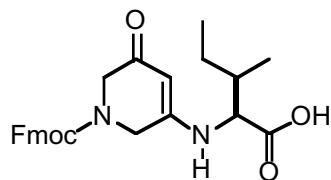


N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl)-L-valine (Fmoc@Val). A mixture of Fmoc@ (3.2, 6.5 mmol) and valine (H-Val-OH) (0.73 g, 6.2 mmol) in methanol (98 mL) was stirred at 60 °C for 16 h. The solvent was removed under reduced pressure, the resulting foam was re-dissolved in CHCl₃ and filtered, and the solution was evaporated to give the @-tide “amino acid” (2.8 g, 6.4 mmol, 98 %). An analytical sample was purified by flash chromatography using a solvent mixture of 10% methanol followed by 50% methanol in CH₂Cl₂ to provide a white solid. An analytical sample was purified further using preparative reverse-phase HPLC: mp 179°C (decomp); HPLC tR 21.1 min; ¹H

NMR δ 0.91 (bm, 6), 2.13 (bs, 1), 3.75 (bm, 1), 3.97 (bs, 4), 4.14 (bs, 2), 4.27 (bs, 2), 5.28 (s, 1), 7.22 (bs, 2), 7.32 (bm, 2), 7.47 (bs, 2), 7.68 (m, 2); ^{13}C NMR δ 18.3 (rot), 19.4, 31.2, 44.7, 47.0, 50.4, 50.7, 68.4, 94.7, 120.1, 122.2, 127.1, 127.8, 141.4, 144.5, 155.7, 163.5, 176.9, 191.3; IR (film) ν_{max} 3298, 3065, 2965, 2885, 1704, 1544, 1231 cm^{-1} ; MS (ESI) m/z (%) 435.2 (100, $\text{M} + \text{H}^+$); HRMS (FAB, m/z) Calcd. for $\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_5$ ($\text{M} + \text{H}^+$): 435.1920; found: 435.1911.

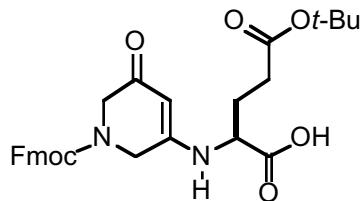


N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl)-L-threonine *t*-Butyl Ether (Fmoc@Thr[O $^{\beta}$ -*t*-Bu]). HPLC tR 21.1 min; ^1H NMR δ 1.19 (s, 9), 3.88 (m, 2), 4.05 (m, 2), 4.47-4.19 (bm, 7), 5.29 (s, 1), 6.10 (bs, 1), 7.30 (s, 2), 7.38 (s, 2), 7.54 (s, 2), 7.75 (s, 2); ^{13}C NMR δ 21.8, 28.2, 44.2, 46.9, 49.9, 61.2, 66.6, 68.3, 74.9, 94.8, 120.0, 124.9, 127.1, 127.8, 141.2, 143.5, 154.8, 163.6, 171.4, 189.6; MS (ESI) m/z (%) 493.2 (100, $\text{M} + \text{H}^+$); HRMS (FAB, m/z) Calcd. for $\text{C}_{28}\text{H}_{33}\text{N}_2\text{O}_6$ ($\text{M} + \text{H}^+$): 493.2339; found: 493.2334.



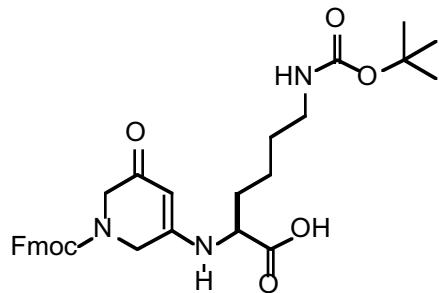
N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl)-L-isoleucine (Fmoc@Ile). HPLC tR 20.6 min; ^1H NMR δ 0.95 (bm, 6), 1.27 (bm, 1), 1.58 (bm, 1), 3.96 (bm, 1), 4.06-4.19 (bm, 3), 4.37 (bm, 2), 4.49 (bm, 2), 5.41 (s, 1), 7.28 (m, 2), 7.37 (t, 2, $J = 7.0$), 7.51 (d, 2, $J = 7.5$), 7.73 (d, 2, $J = 7.5$); ^{13}C NMR (CD_3OD) δ 14.6, 15.9, 21.0, 26.8, 38.3, 48.4, 51.4, 61.6, 121.2, 126.1, 128.3, 129.0,

142.6, 145.1, 156.4, 164.9, 165.2, 172.8, 173.1, 173.8; MS (ESI) m/z (%) 449.2 (100, $M + H^+$); HRMS (FAB, m/z) Calcd. for $C_{26}H_{29}N_2O_5$ ($M + H^+$): 449.2077; found: 449.2071.



N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl)-L-glutamic Acid *t*-Butyl Ester

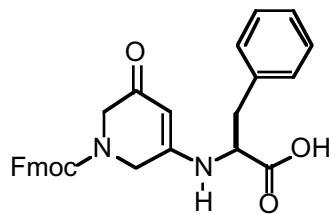
(Fmoc@Glu[O⁸-*t*-Bu]). HPLC tR 21.1 min; 1H NMR δ 1.43 (s, 9), 2.16 (bs, 2), 2.45 (bm, 2), 4.10 (bm, 2), 4.18-4.30 (bm, 3), 4.41 (bm, 3), 5.39 (s, 1), 7.30 (bm, 2), 7.39 (t, 2, $J = 7.0$), 7.55 (m, 2), 7.75 (d, 2, $J = 7.5$); ^{13}C NMR δ 27.9, 31.5, 44.0, 46.9, 50.0, 55.5, 68.3, 81.5, 120.0, 124.9, 127.1, 127.7, 141.2, 143.5, 154.1, 154.9, 162.7, 172.7, 172.9, 192.2; MS (ESI) m/z (%) 521.3 (100, $M + H^+$); HRMS (FAB, m/z) Calcd. for $C_{29}H_{33}N_2O_7$ ($M + H^+$): 521.2288; found: 521.2290.



N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl)-*t*-butylcarbamoyl-L-lysine

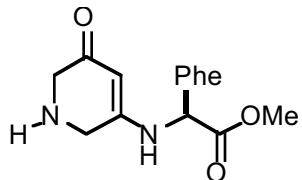
(Fmoc@Lys(N^ε-Boc)). Spectra are complicated by rotamers: HPLC tR 20.8 min; 1H NMR δ 1.40 (bs, 9), 1.88 (bs, 2), 2.95 (bs, 2), 4.04 (m, 2), 4.19-4.11 (bm, 4), 4.34 (bm, 4), 4.78 (bs, 1), 5.33 (s, 1), 6.13 (bs, 0.2, rot), 6.60 (bs, 0.2, rot), 6.78 (bs, 1), 7.33-7.26 (m, 2, rot), 7.40-7.35 (m, 2), 7.51 (d, 2, $J = 7.5$), 7.61

(d, 1, $J = 7.5$), 7.77-7.68 (m, 2); ^{13}C NMR δ 22.49, 28.59, 29.95, 30.84, 39.88, 41.16, 44.26, 50.50, 55.93, 65.29, 68.58, 79.77, 81.31, 95.06, 120.24, 124.93, 125.20, 127.26, 127.34, 127.76, 128.01, 128.52, 141.41, 141.68, 143.74, 144.54, 154.50, 155.17, 156.79, 163.10, 173.60, 192.28; MS (ESI) m/z (%) 564.3 (100, M + H $^+$), 508.3 (95, M + H $^+$ - *t*-Bu), 464.2 (15, M + H $^+$ - Boc); HRMS (FAB) Calcd for C₃₁H₃₈N₃O₇ (M + H $^+$): 564.2710. Found: 564.2708.

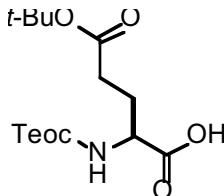


N-(1-Fluorenylmethoxycarbonyl)-5-oxo-1,2,5-trihydropyridin-3-yl-L-phenylalanine (Fmoc@Phe).
HPLC tR 20.7 min; ^1H NMR δ 3.11 (m, 1), 3.27 (m, 1), 4.08 (m, 3), 4.18 (m, 2), 4.32 (bm, 4), 5.36 (s, 1), 5.96 (bs, 1), 7.15 (m, 2), 7.21-7.29 (bm, 4), 7.37 (m, 3), 7.50 (m, 2), 8.01 (m, 2); ^{13}C NMR δ 37.09, 47.15, 56.67, 68.70, 69.89, 120.31, 125.14, 127.37, 127.73, 128.08, 128.54 (2 carbons), 129.08, 129.41, 135.23, 141.47, 143.62, 143.43, 155.21, 162.38, 172.57; IR (film) ν_{max} 3269, 3063, 1705, 1551, 1231 cm $^{-1}$; MS (ESI) m/z (%) 483.2 (100, M + H $^+$); HRMS (FAB) Calcd for C₂₉H₂₇N₂O₅ (M + H $^+$): 483.1920. Found: 483.1915.

The following is a representative procedure for deprotecting a Cbz-protected di-@-tide:

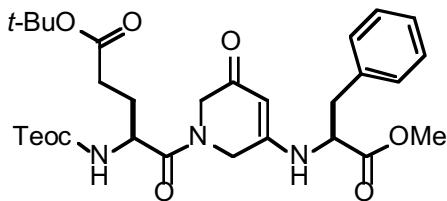


1,2,5,6-Tetrahydro-5-oxopyridin-3-ylamino-L-phenylalanine Methyl Ester (@PheOMe). To a solution of Cbz@PheOMe (22 mg, 56 μ mol) was added Pd/C (10 weight %, 10 mg) and ethanol (0.6 mL). The mixture was hydrogenated in a Parr Shaker at 25 psi H_2 pressure for 1 h, after which the solution was filtered and concentrated to provide mostly clean product as a light yellow film in essentially quantitative yield. An analytical sample was further purified by reverse-phase preparative HPLC: HPLC tR 12.5 min; 1H NMR (CD_3OD) δ 2.87 (dd, 1, J = 8.5, J = 6.0), 3.11 (dd, 1, J = 5.0, J = 9.0), 3.88 (m, 2), 3.94 (m, 2), 4.24 (dd, 1, J = 5.0, J = 3.0), 5.10 (s, 1), 7.08 (m, 5); ^{13}C NMR (CD_3OD) δ 55.8, 58.3, 61.5, 95.5, 128.1, 129.6, 130.2, 137.6, 159.7, 161.3, 173.2, 187.8; MS (ESI) m/z 261.1 (100, $M + H^+$); HRMS (FAB, m/z) Calcd. for $C_{14}H_{17}N_2O_3$ ($M + H^+$): 261.1239; found: 261.1249.

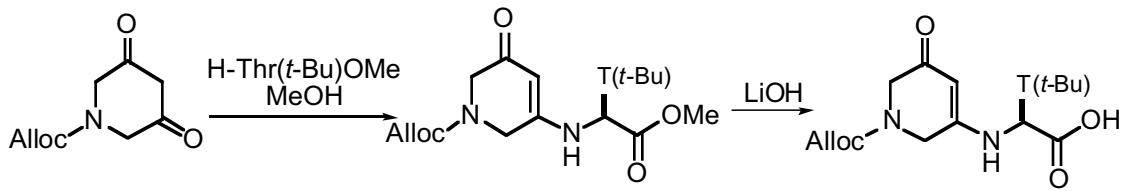


TeocGlu(O δ -t-Bu). A mixture of TeocOSu 4 (2.6 g, 11 mmol), H-Glu(O δ -t-Bu)OH (2.0 g, 9.9 mmol), and DIEA (4.3 mL, 25 mmol) in 1:1 H_2O -Dioxane (20 mL) was stirred for 16 h. The mixture was diluted with 1 N KHSO $_4$ (10 mL), the aqueous layer was extracted with ether (4 x 50 mL), the organic layer was washed with water (2 x 20 mL), dried over MgSO $_4$, filtered, and concentrated providing the Teoc-protected amino acid (2.7 g, 7.8 mmol, 85%) as a light yellow oil: 1H NMR (400 MHz) δ 0.04 (s, 9), 0.99

(t, 2, $J = 8.4, J = 8.8$), 1.45 (s, 9), 1.99 (m, 1), 2.20 (m, 1), 2.38 (m, 2), 4.16 (t, 2, $J = 8.4, J = 8.8$), 4.37 (m, 1), 5.48 (d, 1, $J = 8.0$), 8.18 (bs, 1); ^{13}C NMR δ -1.3, 17.8, 27.5, 28.2, 31.8, 53.4, 64.0, 81.4, 156.9, 172.8, 176.2; MS (ESI-neg) m/z 346.2 (100, M-), 290.1 (10, M - *t*-Bu). HRMS (FAB) Calcd for $\text{C}_{15}\text{H}_{30}\text{N}_1\text{O}_6\text{Si}$ ($\text{M} + \text{H}^+$): 348.1842. Found: 348.1842.



TeocGlu(O^δ-t-Bu)@FOMe. A mixture of @PheOMe (0.64 g, 2.4 mmol), TeocGlu(O^δ-t-Bu)OH (1.1 g 3.1 mmol), HATU (1.2 g, 3.1 mmol), and DIEA (0.82 mL, 4.7 mmol) in 1:5 CH₂Cl₂-DMF (24 mL) was stirred for 16 h. The mixture was diluted with EtOAc (30 mL), washed with sat. NaHCO₃ (3 x 20 mL), 2 M LiCl (2 x 20 mL), and the organic layer was dried over MgSO₄, filtered, and concentrated. The resulting oil was purified by flash chromatography using EtOAc providing the tri-@-tide (0.88 g, 1.5 mmol, 62%) as a light yellow film: HPLC tR 24.0 min; ¹H NMR (CD₃OD) δ 0.02 (bs, 9), 0.99 (q, 2, *J* = 9.5, *J* = 8.5), 1.48 (bs, 9), 1.73 (m, 1), 1.90 (m, 1), 2.33 (m, 2), 3.04 (m, 1), 3.21 (m, 1), 3.69 (s, 3), 4.12 (m, 2), 4.23 (m, 2), 4.41 (m, 2), 4.61 (m, 1), 5.10 (s, 0.7), 5.12 (0.3), 7.22 (m, 3), 7.28 (m, 2); ¹³C NMR δ -1.3, 18.7, 28.0, 28.5, 43.5, 51.5, 52.9, 53.1, 58.3, 58.4, 64.4, 64.5, 81.9, 82.2, 95.2, 95.7, 128.3, 128.4, 129.8, 129.9, 130.4, 137.4, 137.6, 158.8, 158.9, 164.7, 172.4, 172.5, 172.7, 172.8, 173.9, 174.4, 192.9, 194.3; MS (ESI) *m/z* 604.3 (40, M + H⁺), 576.3 (10), 548.3 (90), 520.2 (100). HRMS (FAB) Calcd for C₃₀H₄₅N₃O₈Si (M⁺): 603.2976. Found: 603.2987.

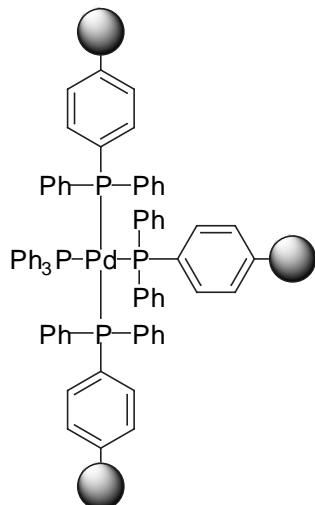


5-(2-*tert*Butoxy-1-carboxypropylamino)-3-oxo-3,6-dihydro-2*H*-pyridine-1-carboxylic Acid Allyl Ester (Alloc@Thr(O^β-*t*-Bu)).

A mixture of prop-2-enyl 5-hydroxy-3-oxo-1,2,6-trihydropyridine-1-carboxylate (Alloc@) (4.5 g, 23 mmol), H-Thr(O^β-*t*-Bu)OMe (5.2 g, 23 mmol), and NaO₂CCH₃·3H₂O (5.5 g, 46 mmol) in 0.20 L MeOH was stirred at 60 °C for 24 h. The solution was concentrated and the residue was dissolved in EtOAc (250 mL) and washed with 1 M KHSO₄ (two 75-mL portions), NaHCO₃ (three 75-mL portions), and brine (one 75-mL portion). The organic layer was dried over MgSO₄, filtered, and concentrated to provide Alloc@Thr(O^β-*t*-Bu)-OMe (4.6 g, 51%) as a light yellow foam. An analytical sample was purified further using preparative reverse-phase HPLC: HPLC tR 18.4 min; ¹H NMR δ 1.09 (s, 9), 1.23 (d, 3, *J* = 5.0), 3.73 (s, 3), 3.90 (d, 1, *J* = 9.0), 4.03 (m, 1), 4.13 (m, 1), 4.26 (q, 1, *J* = 5.5, *J* = 6.0), 4.34 (m, 2), 4.64 (bs, 2), 5.09 (s, 1), 5.23 (m, 1), 5.32 (d, 1, *J* = 17.5), 5.64 (bs, 0.3), 5.69 (m, 0.7), 5.94 (m, 1); ¹³C NMR δ 14.4, 21.9, 28.4, 51.3, 53.2, 61.0, 66.3, 66.5, 74.9, 118.9, 132.8, 155.0, 161.3, 170.4, 171.4, 192.9; IR (film) ν_{max} 2977, 2879, 1703, 1585, 1194 cm⁻¹; MS (ESI) *m/z* 369 (100, M + H⁺), 313 (10, M + H⁺ - *t*Bu). HRMS (FAB) Calcd for C₁₈H₂₉N₂O₆ (M + H⁺): 369.2026. Found: 369.2032.

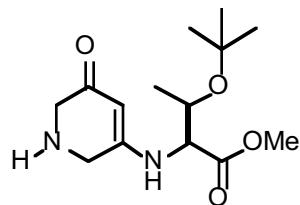
A mixture of Alloc@Thr(O^β-*t*-Bu)-OMe (1.22 g, 3.31 mmol) and LiOH (0.278 g, 6.62 mmol) in 1:1 THF-H₂O (33 mL) was stirred for 30 min. The mixture was diluted with 1 M KHSO₄ (10 mL) and extracted with EtOAc (four 50-mL portions), and the organic layer was dried over MgSO₄, filtered, and concentrated to provide Alloc@Thr(O^β-*t*-Bu) (1.13 g, 3.19 mmol, 96%) as a light yellow foam. An analytical sample was purified further using preparative reverse-phase HPLC: HPLC tR 16.3 min; ¹H NMR δ 1.17 (bs, 9), 1.26 (bs, 3), 4.11 (m, 4), 4.34 (m, 2), 4.63 (s, 2), 5.29 (m, 3), 5.92 (bs, 1), 6.39 (bs,

1); ^{13}C NMR δ 21.6, 28.2, 44.2, 49.9, 61.1, 66.5, 66.9, 75.4, 94.7, 118.2, 132.0, 154.6, 164.8, 171.2, 193.1; IR (film) ν_{max} 3296, 2975, 1704, 1543, 1232 cm^{-1} ; MS (ESI) m/z 355 (100, $\text{M} + \text{H}^+$), 299 (10, $\text{M} + \text{H}^+ - t\text{Bu}$). HRMS (FAB) Calcd for $\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_6$ ($\text{M} + \text{H}^+$): 355.1869. Found: 355.1878.



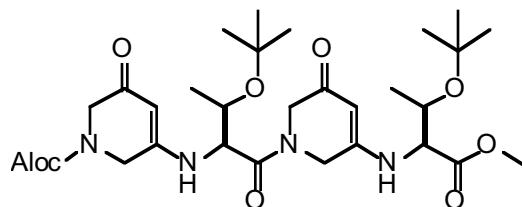
Resin-bound $\text{Pd}(\text{PPh}_3)_4$. The following procedure is modified from that reported in reference 5. A mixture of triphenylphosphine resin (Novabiochem) (7.0 g, 1.1 mmol) and $\text{PdCl}_2(\text{PPh}_3)_2$ (1.8 g, 2.6 mmol) in 70 mL CH_2Cl_2 were stirred for 1 h under N_2 . The mixture was concentrated, re-suspended in MeOH (100 mL), PPh_3 (1.4 g, 5.2 mmol) was added, and the mixture was stirred for 20 h under N_2 . Hydrazine (1.3 mL, 40 mmol) was added to the suspension and the mixture was stirred for 3 h wrapped in aluminum foil under a N_2 atmosphere. The resin was filtered, washed with DMF (3 x 10 mL) and CH_2Cl_2 (3 x 10 mL), and dried in vacuo. The resin was stored under N_2 at 0 °C wrapped in aluminum foil.

The following is a representative procedure for using resin-bound $\text{Pd}(\text{PPh}_3)_4$:

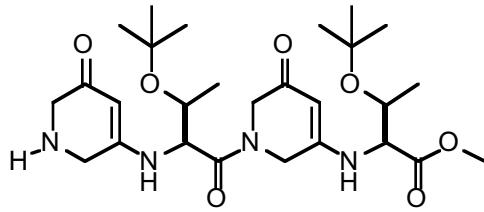


3-*tert*-Butoxy-2-(5-oxo-1,2,5,6-tetrahydro-pyridin-3-ylamino)butyric Acid Methyl Ester (@Thr(O^B-*t*-Bu)OMe). To 5-(2-*tert*-butoxy-1-methoxycarbonyl-propylamino)-3-oxo-3,6-dihydro-2*H*-pyridine-1-

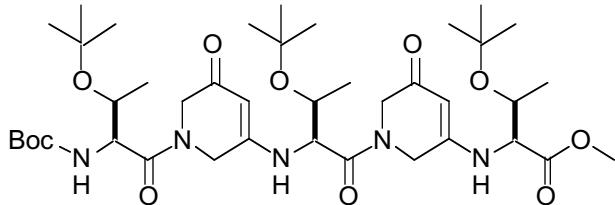
carboxylic acid allyl ester (1.43 g, 3.89 mmol) in 1:1 THF–Et₂NH (19.4 mL) was added Pd(0)-resin (972 mg, 1.17 mmol) and the resulting solution was stirred for 1 h. The mixture was filtered, the resin was washed with MeOH (5 x 5 mL) and CH₂Cl₂ (5 x 5 mL), and the organic solvents were concentrated to provide free amine @Thr(O^β-*t*-Bu)OMe as a light yellow film in essentially quantitative yield. This material was used directly without further purification: HPLC tR 13.6 min; LCMS tR 7.0 min; ¹H NMR δ 5.50 (d, 1, *J* = 9.0), 4.99 (s, 1), 4.25 (q, 1, *J* = 6.0, *J* = 6.0), 3.87 (d, 1, *J* = 9.0), 3.73 (s, 3), 3.65 (s, 2), 3.37 (s, 2), 1.23 (d, 3, *J* = 6.0), 1.13 (s, 9); MS (ESI) *m/z* 285 (80, MH⁺), 229 (100, MH⁺ - *t*-Bu).



Alloc-@-T(O^β-*t*-Bu)-@-T(O^β-*t*-Bu)OMe. A mixture of @-Thr(O^β-*t*-Bu)OMe (1.43 g, 3.89 mmol), Alloc-@-Thr(O^β-*t*-Bu) (1.52 g, 4.29 mmol), HATU (2.45 g, 6.45 mmol), and DIEA (1.32 mL, 7.58 mmol) in 39 mL DMF was stirred for 24 h. The mixture was concentrated, the crude residue was re-dissolved in EtOAc (150 mL) and washed with 1 M KHSO₄ (2 x 75 mL) and sat. NaHCO₃ (2 x 75 mL), the organic layer was dried over MgSO₄, filtered and concentrated. The resulting oil was chromatographed (5% MeOH-EtOAc followed by 10% MeOH-EtOAc) to provide the tetra-@-tide (1.55 g, 2.50 mmol, 64%) as a light yellow film: HPLC tR 19.5 min; LCMS tR 6.6 min; ¹H NMR δ 8.21 (bs, 1), 6.41 (bs, 0.5), 6.07 (bs, 0.5), 5.85 (bm, 1.5), 5.25 (m, 3), 5.17 (d, 1, *J* = 10), 5.09 (s, 1), 4.58 (d, 4, *J* = 10), 4.48 – 4.06 (bm, 8), 3.96 – 3.85 (bm, 4), 3.67 (s, 3), 1.06 (bm, 24); ¹³C NMR δ 192.1, 191.3, 189.9, 169.9, 167.8, 162.0, 161.5, 160.4, 155.0, 132.2, 117.9, 95.4, 75.1, 74.7, 67.2, 66.7, 60.9, 60.8, 57.3, 56.5, 52.6, 50.4, 49.5, 46.3, 44.4, 43.1, 28.2, 28.2, 26.3, 21.9, 21.2 20.0, 19.3; IR (film) 3074, 2977, 1727, 1556 cm⁻¹; MS (ESI) *m/z* 621 (75, MH⁺). HRMS (FAB) Calcd for C₃₁H₄₉N₄O₉ (MH⁺): 621.3500. Found: 621.3516.



@@-T(O^β-t-Bu)-@@-T(O^β-t-Bu)OMe. To Alloc-@@-T(O^β-t-Bu)-@@-T(O^β-t-Bu)OMe (828 mg, 1.34 mmol) in 1:1 THF-Et₂NH (6.70 mL) was added Pd(0)-resin (951 mg, 0.349 mmol) and the resulting solution was stirred for 1 h. The mixture was filtered, the resin was washed with MeOH (5 x 5 mL) and CH₂Cl₂ (5 x 5 mL), and the solvent was concentrated to provide the free amine in essentially quantitative yield as a light yellow foam. This material was used directly in the next reaction without further purification: HPLC tR 16.4 min; LCMS tR 5.7 min; MS (ESI) *m/z* 537 (100, MH⁺). HRMS (FAB) Calcd for C₂₇H₄₅N₄O₇ (MH⁺): 537.3288. Found: 537.3295.



Boc-T(O^β-t-Bu)-@@-T(O^β-t-Bu)-@@-T(O^β-t-Bu)OMe. A mixture of @@-T(O^β-t-Bu)-@@-T(O^β-t-Bu)OMe (828 mg, 1.34 mmol), BocThr(O^β-t-Bu)OH (405 mg, 1.47 mmol), HATU (558 mg, 1.47 mmol), and DIEA (302 μL, 1.74 mmol) in 13.4 mL DMF was stirred for 18 h. The mixture was concentrated, the resulting oil was chromatographed (5% MeOH-EtOAc followed by 10% MeOH-EtOAc), and the product was lyophilized from benzene (2 x 25 mL) to provide the penta-@@-tide (975 mg, 1.23 mmol, 92%) as a white powder: HPLC tR 22.6 min; LCMS tR 7.5 min; ¹H NMR (CD₃OD) δ 5.47 (m, 1), 5.38 (m, 1), 5.28 (m, 2), 5.02 (m, 1), 4.69 (m, 5), 4.49 (bm, 4), 4.31 (m, 4), 4.27 (m, 1), 4.22 (m, 1), 4.17 (m, 3), 4.05 (m, 2), 2.94 (s, 3), 1.58 (s, 9), 1.29 (s, 27), 1.28 (s, 9); MS (ESI) *m/z* 816 (15, MH⁺ + Na), 794 (100, MH⁺), 694 (90, MH⁺ - Boc). HRMS (FAB) Calcd for C₄₀H₆₈N₅O₁₁ (MH⁺): 794.4915. Found: 794.4912.

General Procedures for Solid Phase α -Tide Syntheses. (4-Bromomethylphenoxy)methyl polystyrene resin was obtained from NovaBiochem (ca. 0.9-1.5 mmol/g). The resin was washed in the following way: DMF (3 x) followed by CH_2Cl_2 (3 x). During washings, the resin was agitated for 2 min before the solvent was removed.

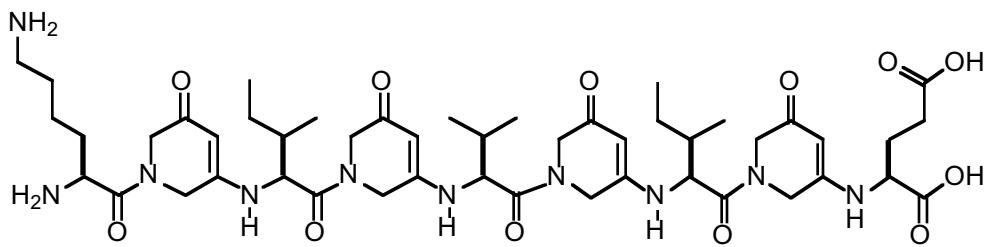
Loading of the First Di- α -tide. The first di- α -tide was loaded in analogy to known procedures for loading amino acids to this resin.²

Deprotection of the Fmoc- α -Unit. Removal of the Fmoc group was accomplished by shaking the resin in 20% piperidine in DMF for 15 min.

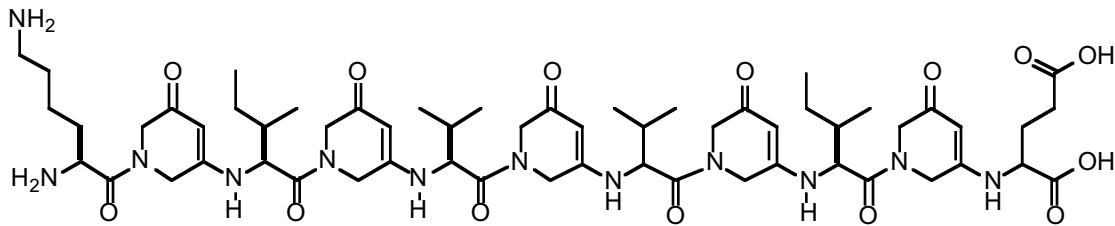
Coupling to the α -Unit. The resin-bound α -tide (0.1 g, 1.1 mmol/g) was suspended in 2 mL DMF. The desired Fmoc-amino acid or di- α -tide (2.5 eq in relation to the resin) was added to the resin, followed by HATU (2.5 eq) and DIEA (5 eq). The reaction mixture was agitated at rt for 16 h, the resin was washed and immediately Fmoc-deprotected as described above.

Cleavage From Resin. The resin was dried in vacuo for at least 16 h prior to cleavage. The resin was suspended in 1:1 CH_2Cl_2 -TFA (3 mL) and rotated in a glass vial for 2 h. The solvent was removed under reduced pressure and the product was dried for 16 h under high vacuum. The resulting resin was re-dissolved in methanol, filtered, and washed (3 x 2 mL MeOH and CH_2Cl_2). The combined organic solvents were concentrated under reduced pressure and the crude product was immediately purified using reverse-phase preparative HPLC. As noted in the text, isolated yields ranged from ca. 20-80%. Yields varied depending on the hydrophobicity and length of the sequence: high yields were obtained for shorter non-polar α -tides (penta- or hepta- α -tides) while lower yields were observed for polar and longer derivatives.

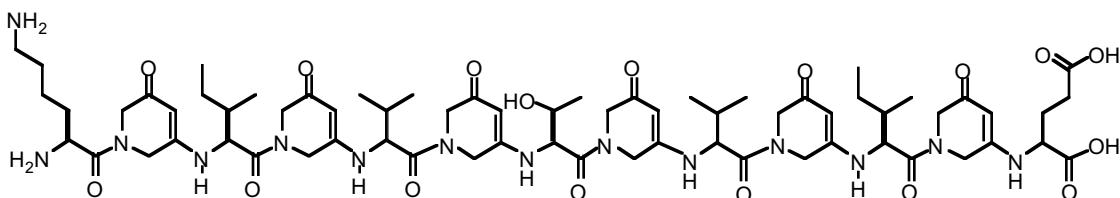
The following three α -tides were synthesized using the Fmoc di- α -tide solid phase methods described above:



K@I@V@I@E. Retention time (analytical HPLC) = 13.6 min; MS (API-ES) m/z (%) 981.7 (M^+ , 40), 491.4 ($(M + H)^{2+}$, 100); HRMS (FAB) Calcd for $C_{48}H_{73}N_{10}O_{12}$ ($M + H^+$): 981.5409. Found: 981.5406.



K@I@V@V@I@E. Retention time (analytical HPLC) = 13.6 min; MS (API-ES) m/z (%) 1175.8 (M^+ , 15), 588.2 ($(M + H)^{2+}$, 100); HRMS (FAB) Calcd for $C_{58}H_{87}N_{12}O_{14}$ ($M + H^+$): 1175.6465. Found: 1175.6501.

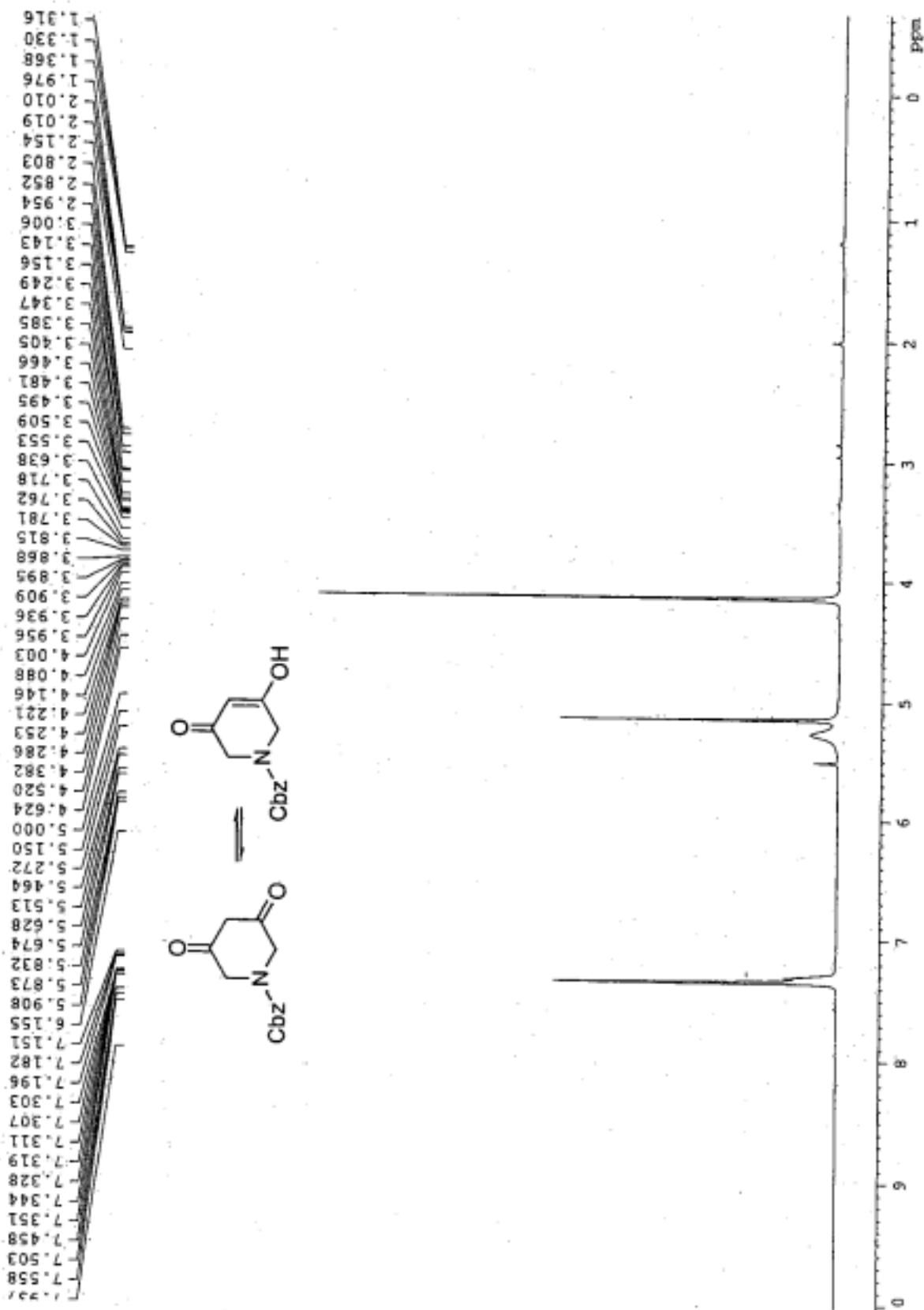


K@I@V@T@V@I@E. Retention time (analytical HPLC) = 13.4 min; MS (API-ES) m/z (%) 1371.8 (M^+ , 10), 686.6 ($(M + H)^{2+}$, 100).

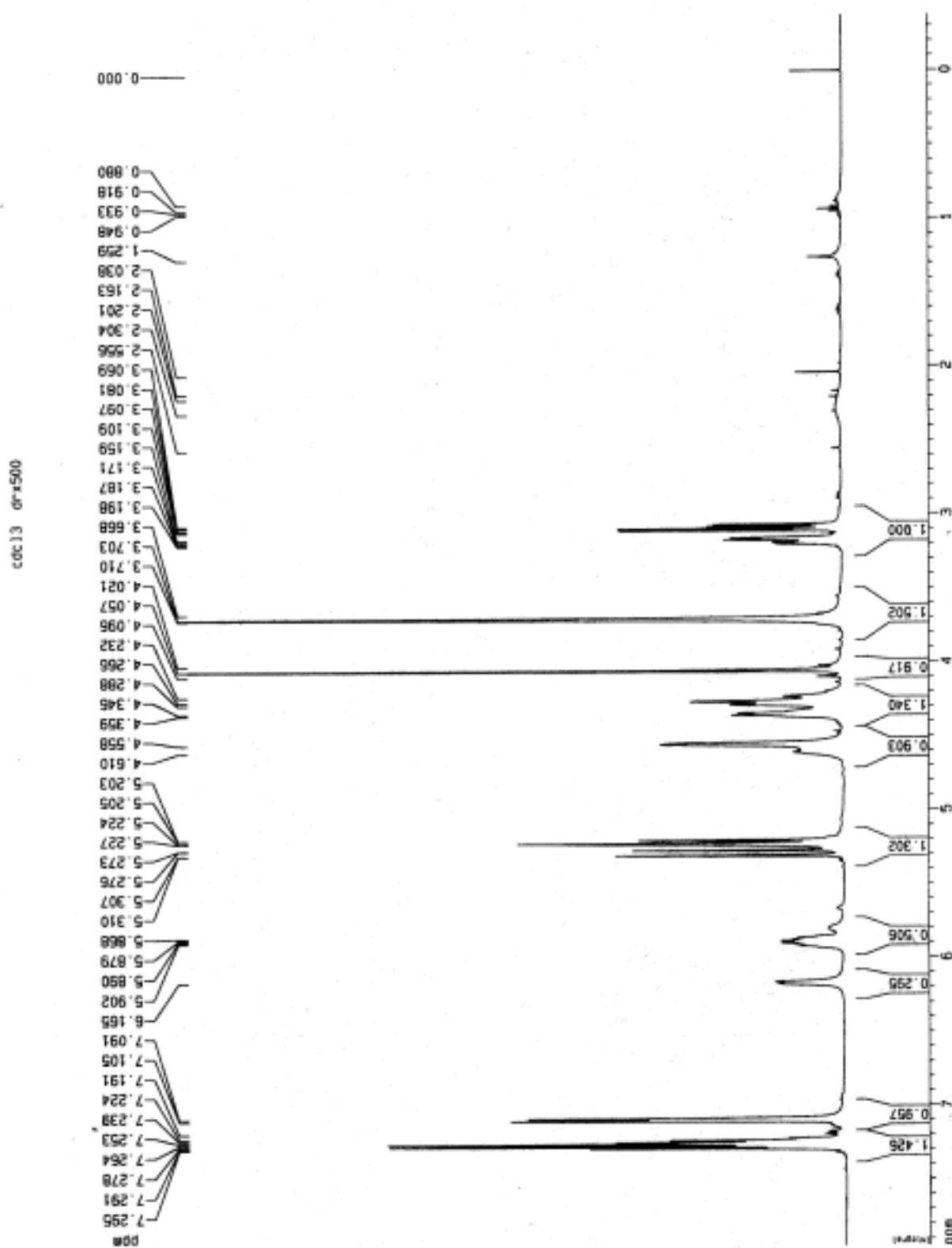
References

- 1) Still, W. C.; Kahn, M.; Mitra, S. *J. Org. Chem.* **1978**, *43*, 2923-2925.
- 2) Morales, G. A.; Corbett, J. W.; DeGrado, W. F. *J. Org. Chem.* **1998**, *63*, 1172-1177.
- 3) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 58-66.
- 4) Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346-349.
- 5) Bergbreiter, D. E.; Weatherford, D. A. *J. Org. Chem.* **1989**, *54*, 2726-2730.

¹H NMR Spectrum for Cbz@ (CDCl₃)

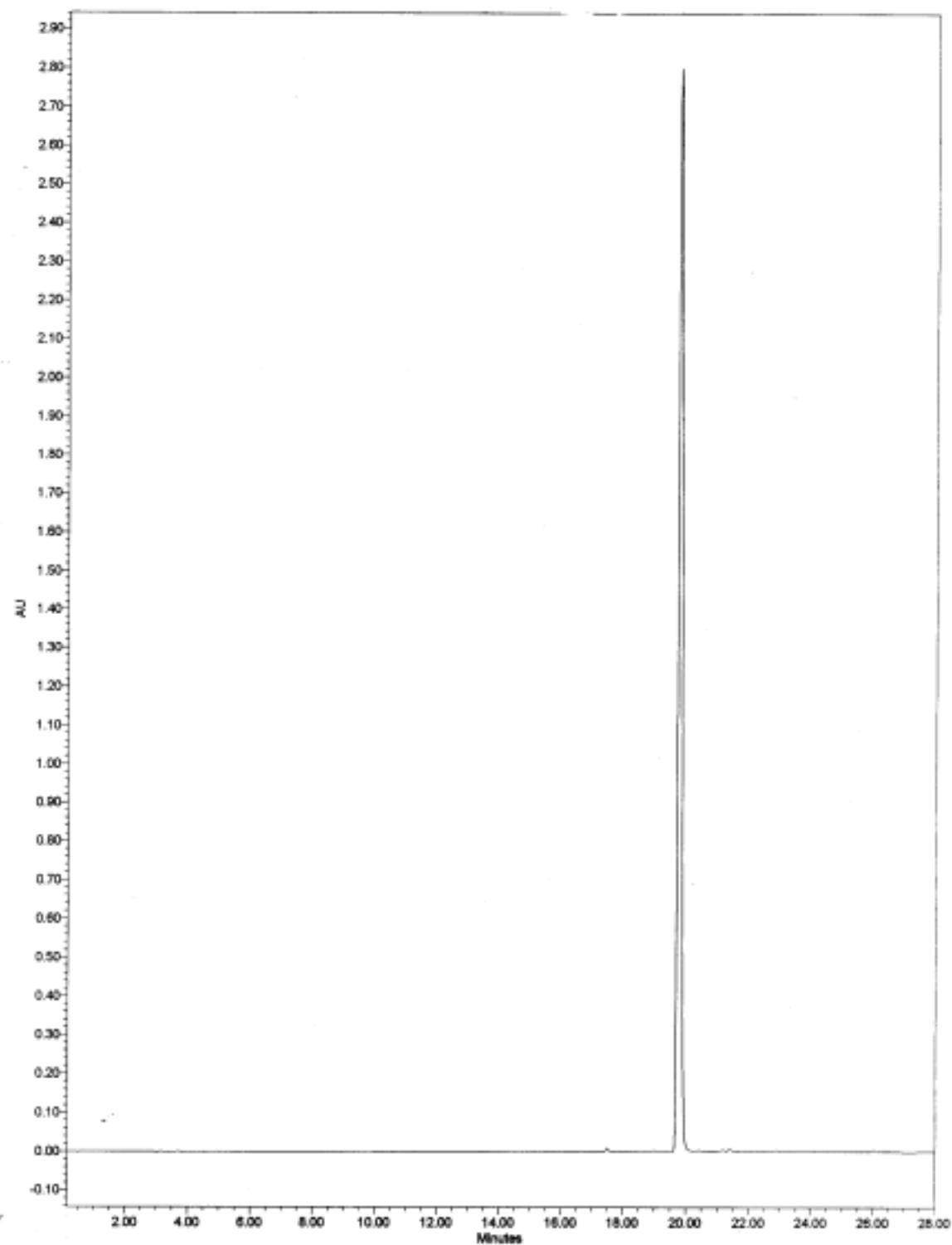


¹H NMR Spectrum for Alloc@PheOMe (CDCl₃)

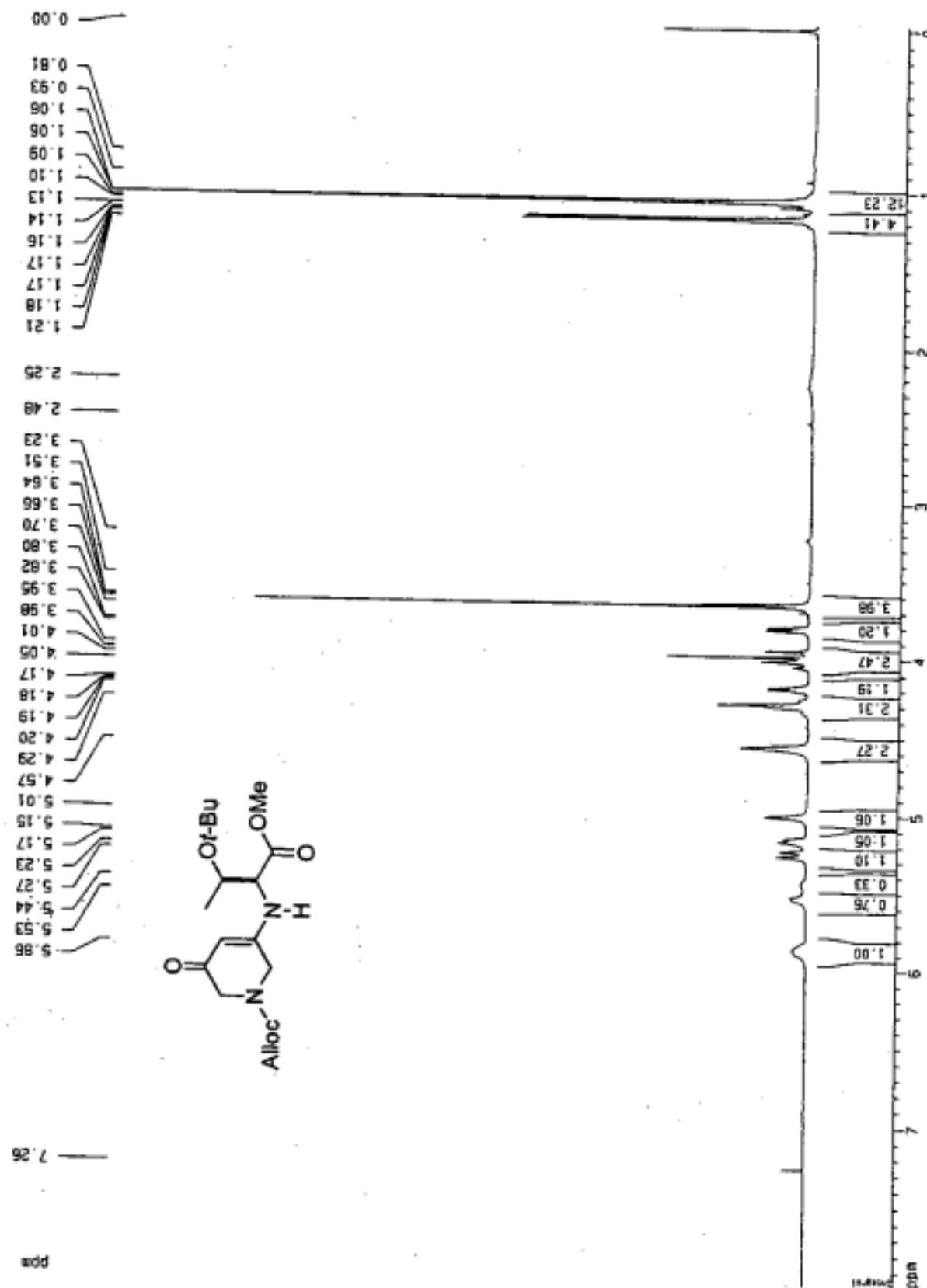


All HPLC traces are at the wavelength of maximum absorbance for the α -unit (i.e., 284 nm)

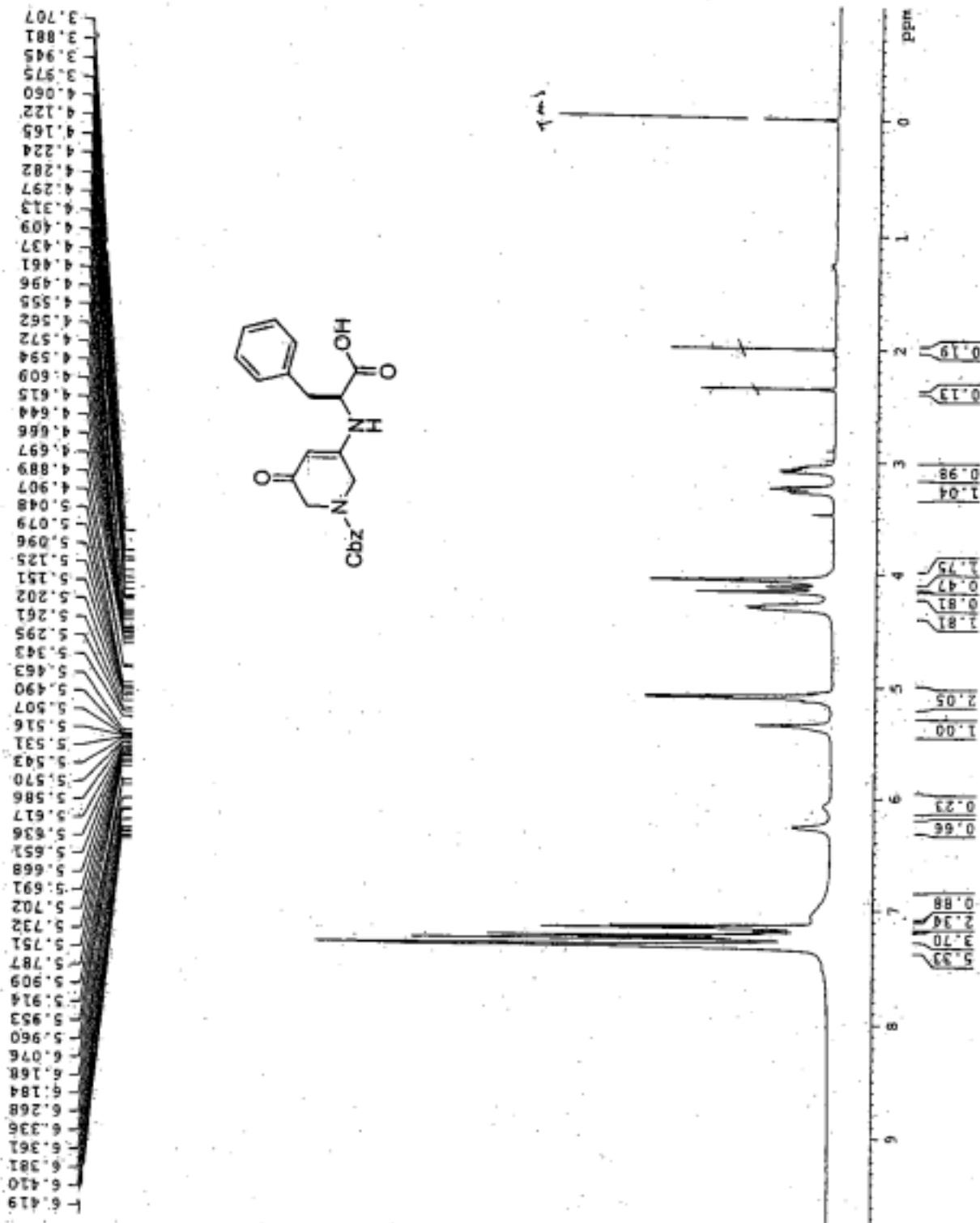
HPLC trace for Cbz α PheOMe



¹H NMR Spectrum for Alloc@Thr(O^B-t-Bu)OMe (CDCl₃)



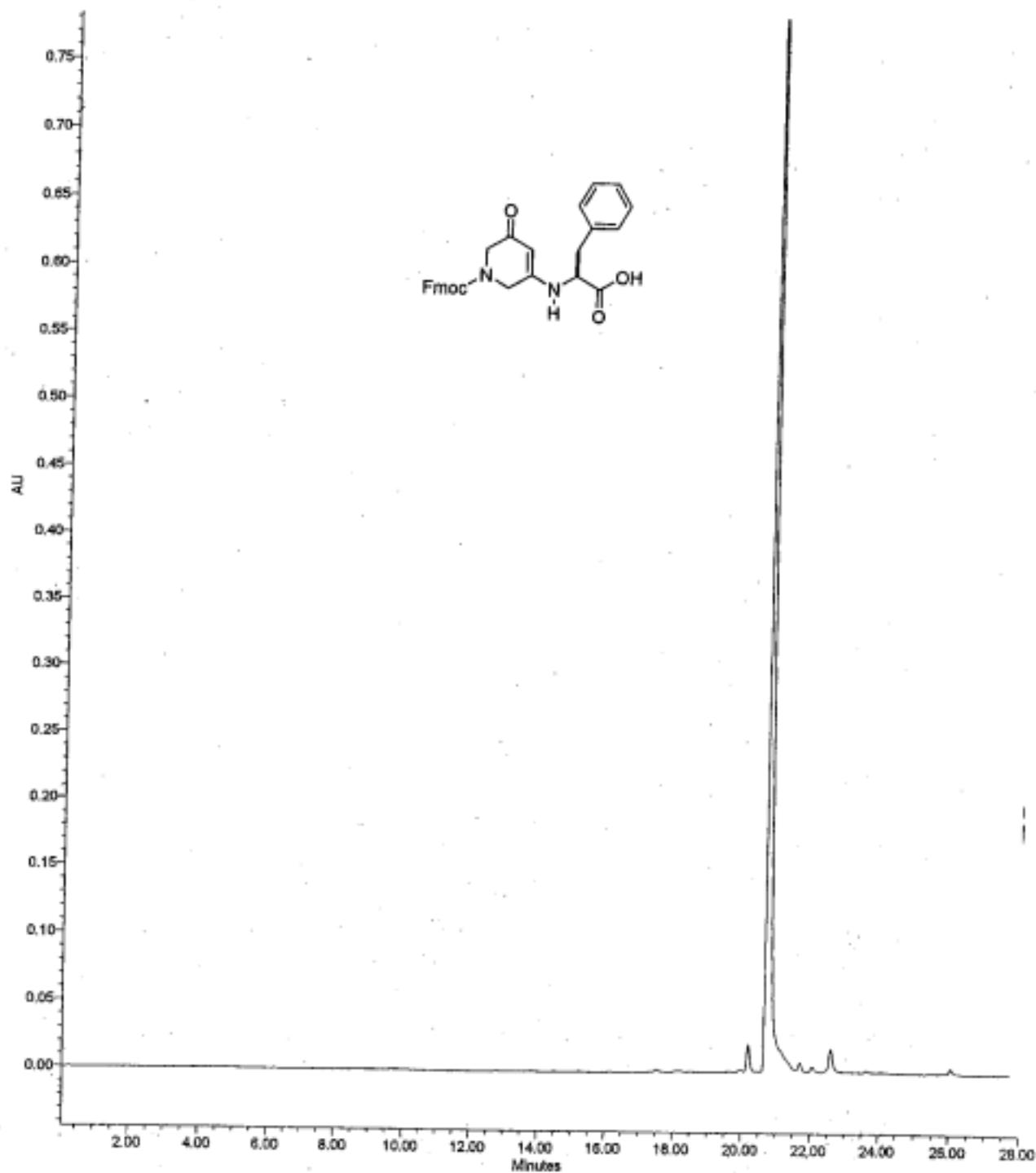
¹H NMR Spectrum for Cbz@Phe (CDCl₃)



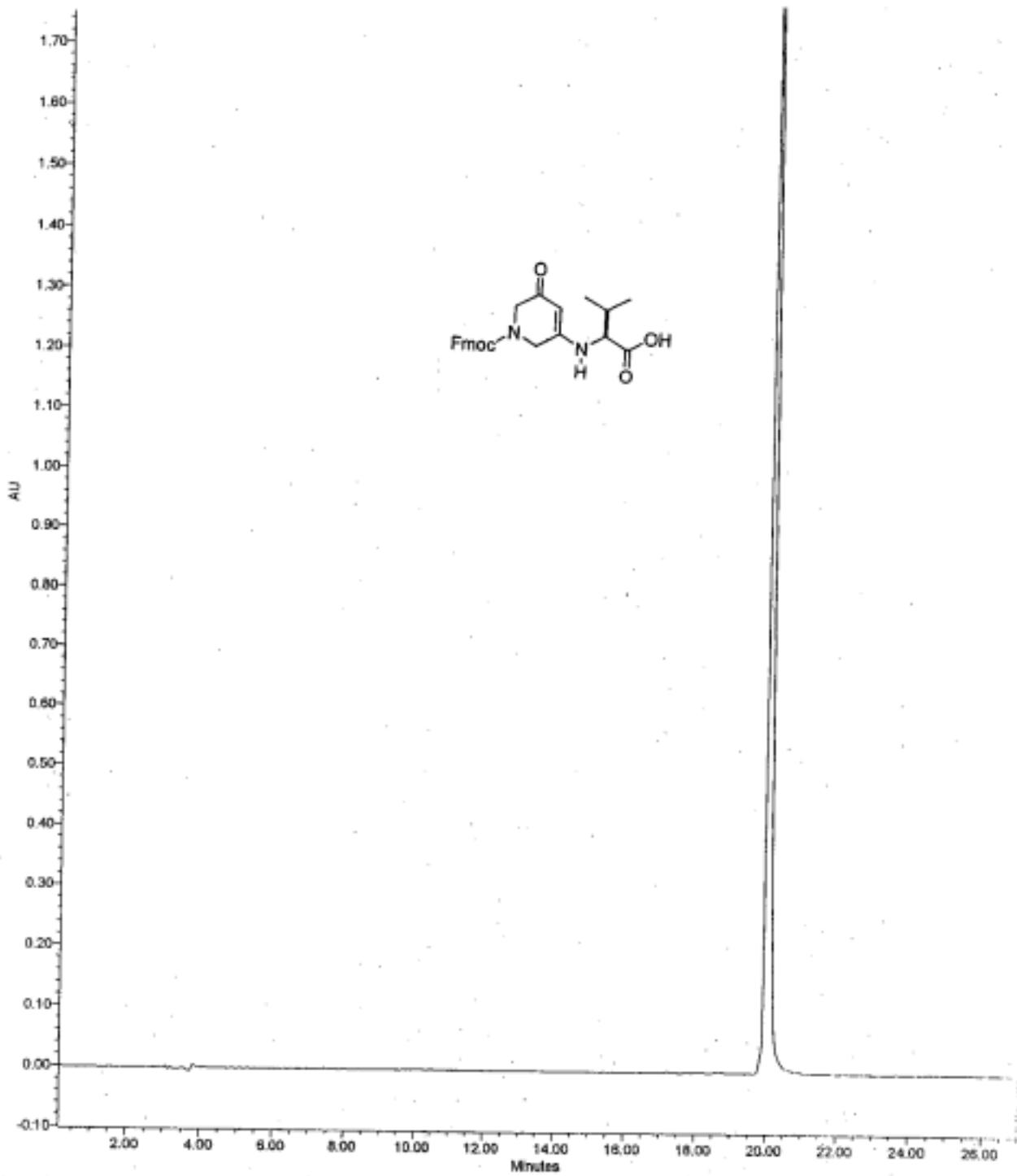
¹H NMR Spectrum for Fmoc@Phe (CDCl₃)



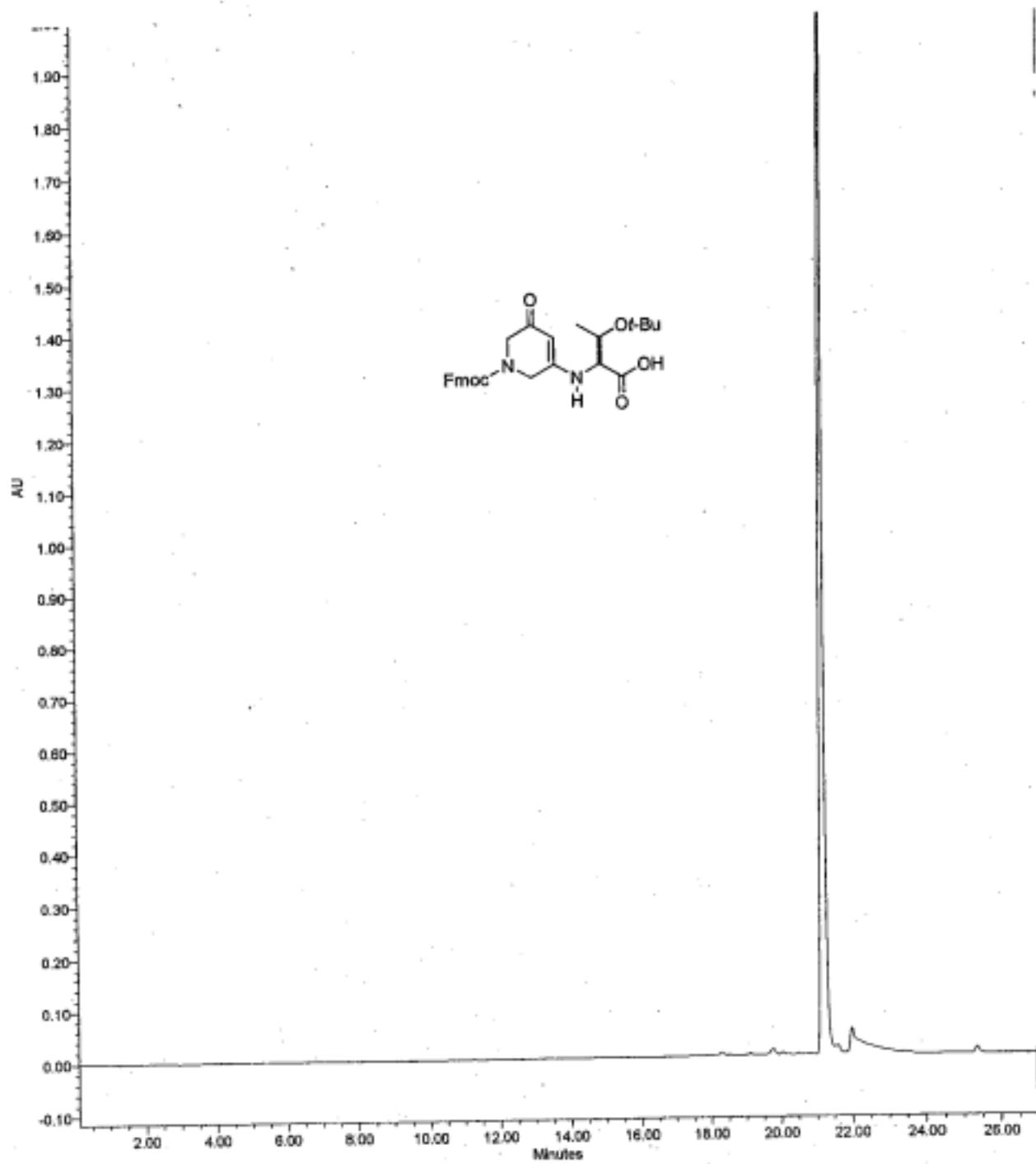
HPLC trace for Fmoc@Phe



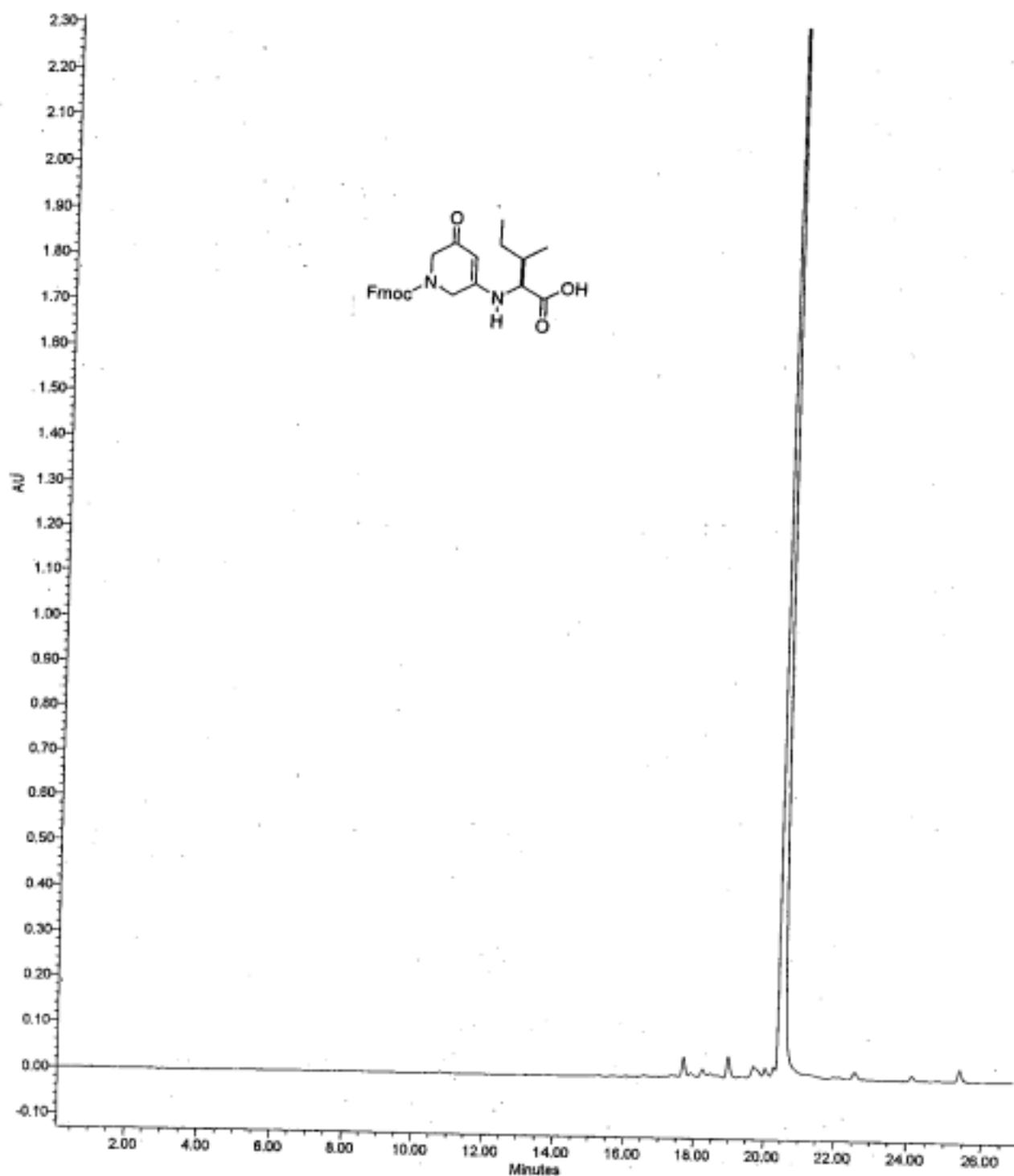
HPLC trace for Fmoc@Val



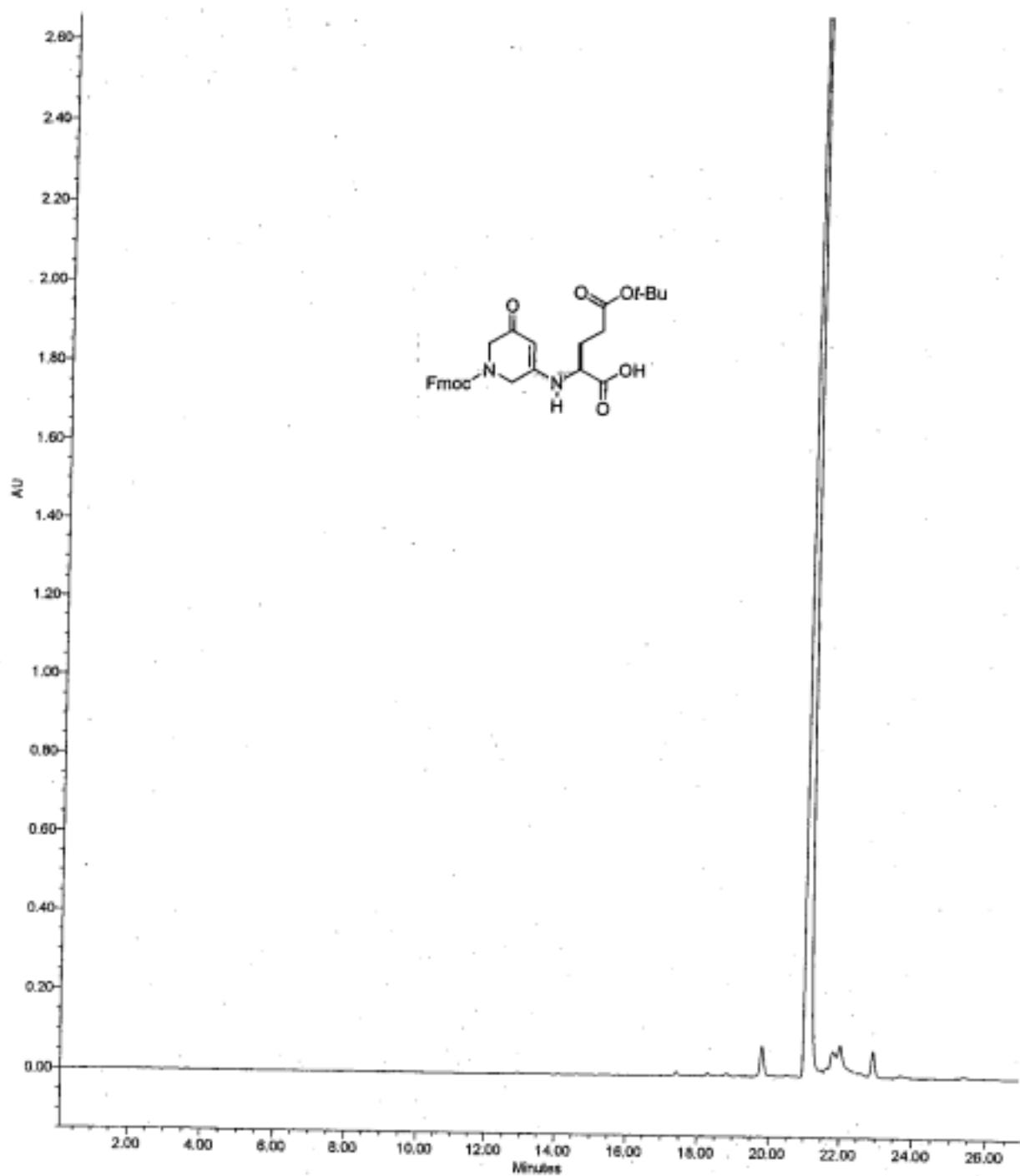
HPLC trace for Fmoc@Thr(O^β-t-Bu)



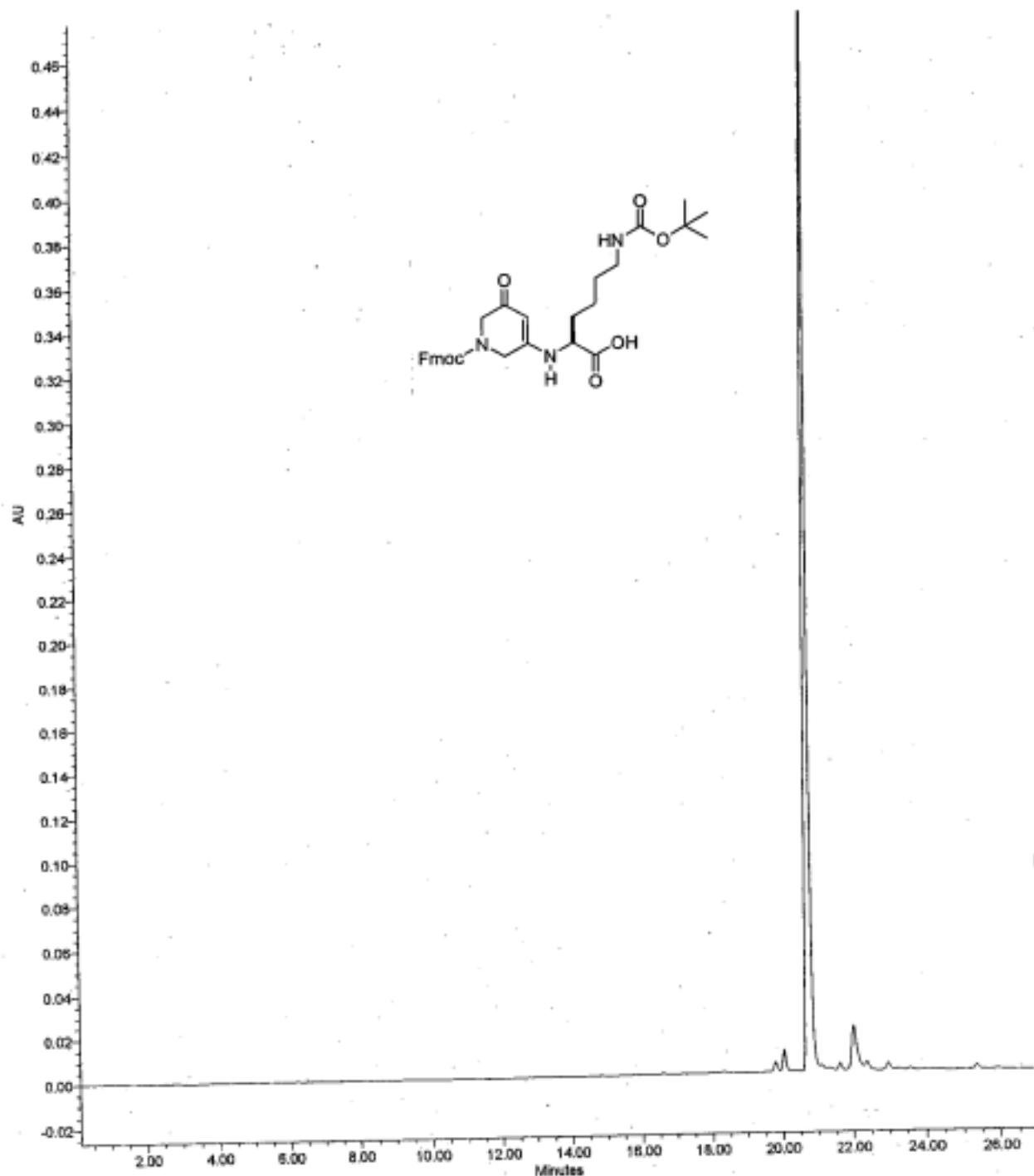
HPLC trace for Fmoc@Ile



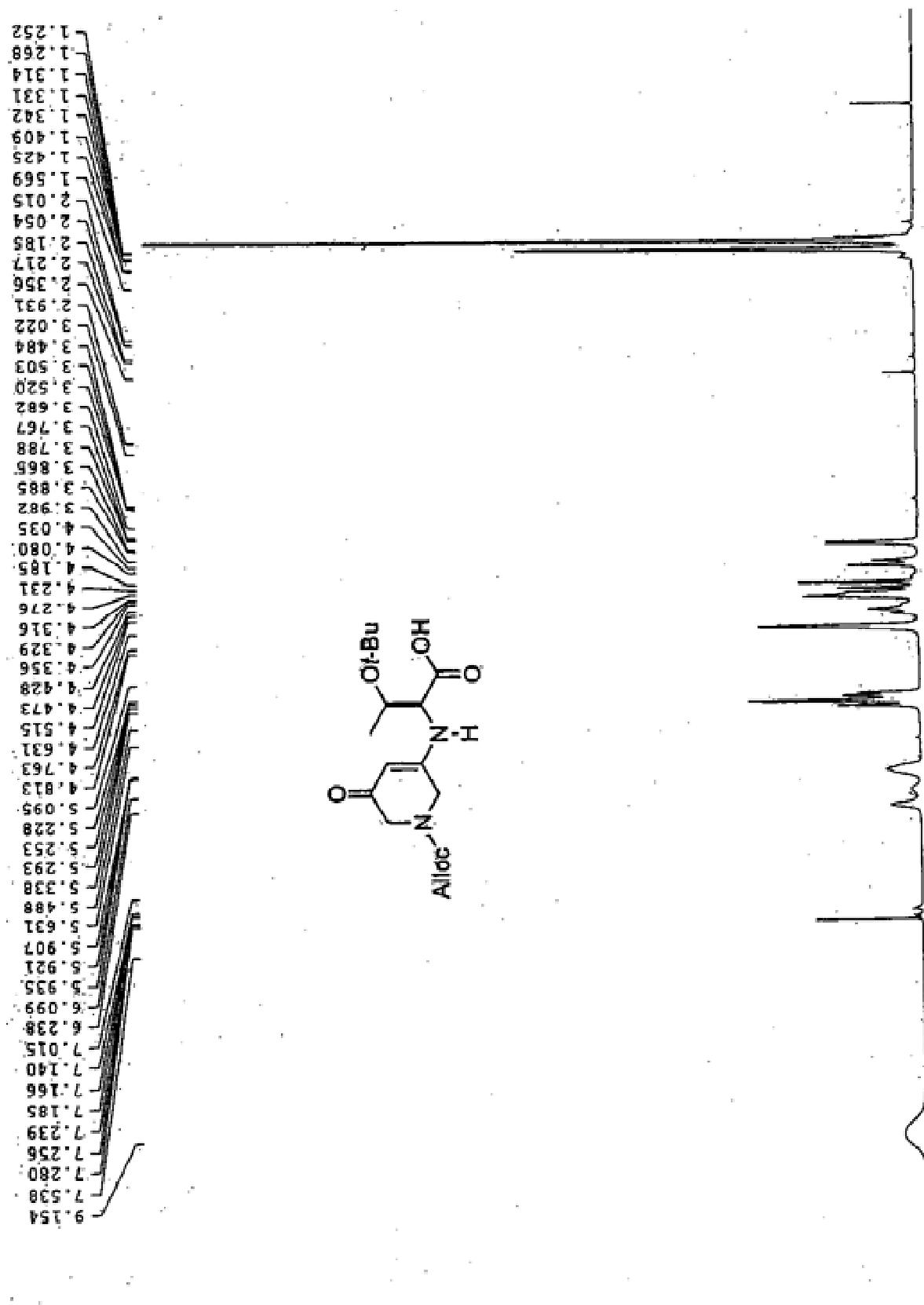
HPLC trace for Fmoc@Glu(O^δ-t-Bu)



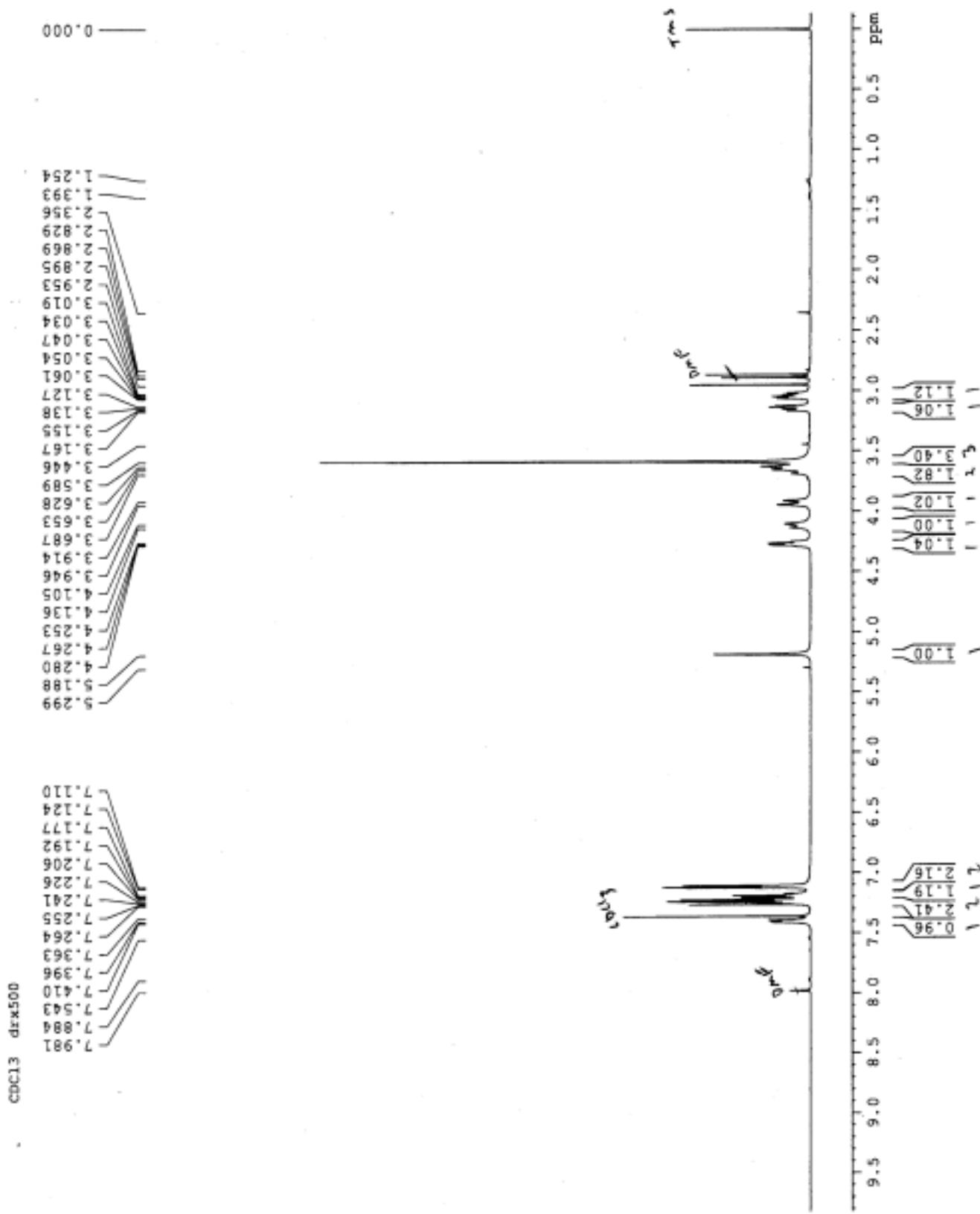
HPLC trace for Fmoc@Lys(N^ε-Boc)



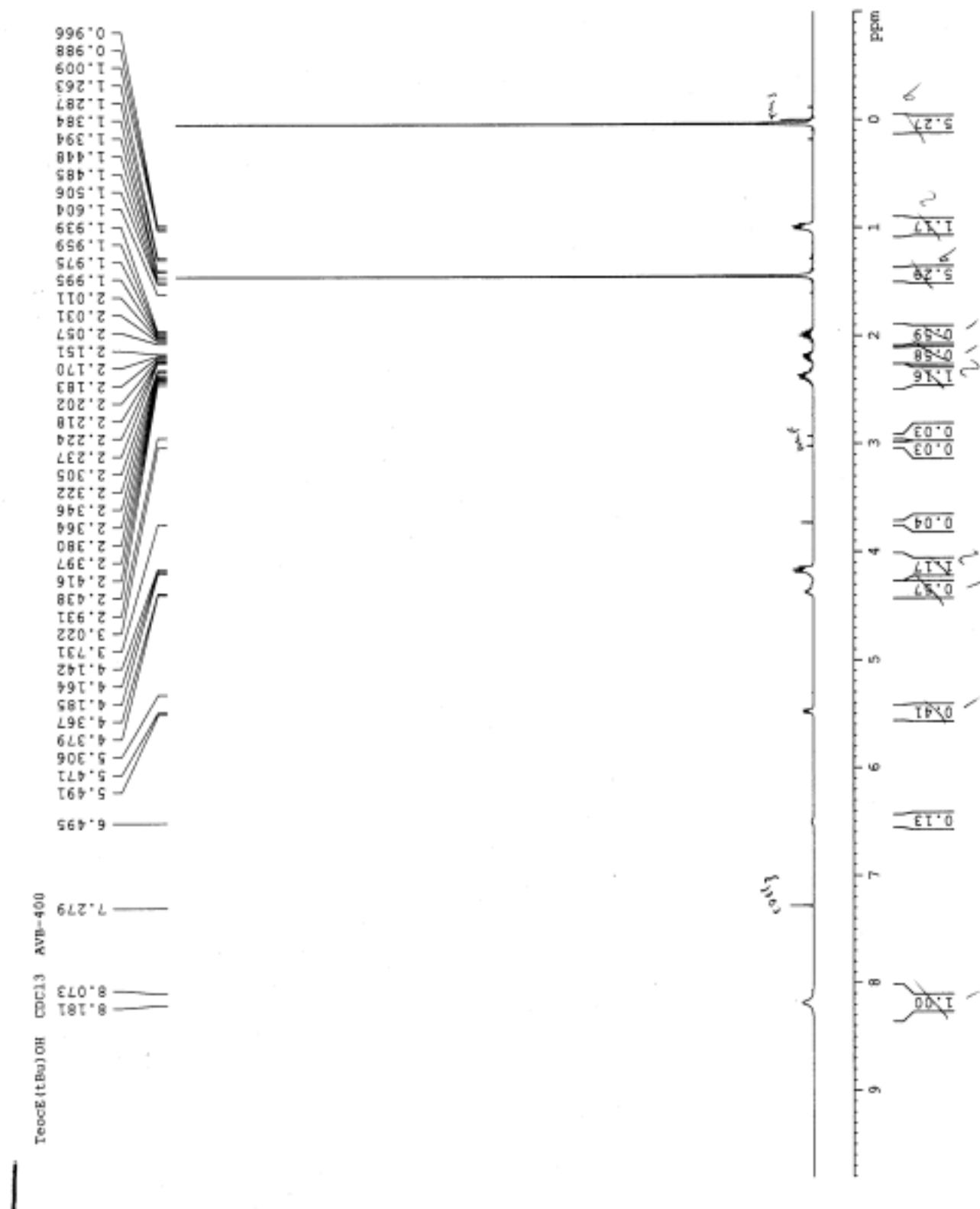
¹H NMR Spectrum for Alloc@Thr(O^β-*t*-Bu) (CDCl₃)



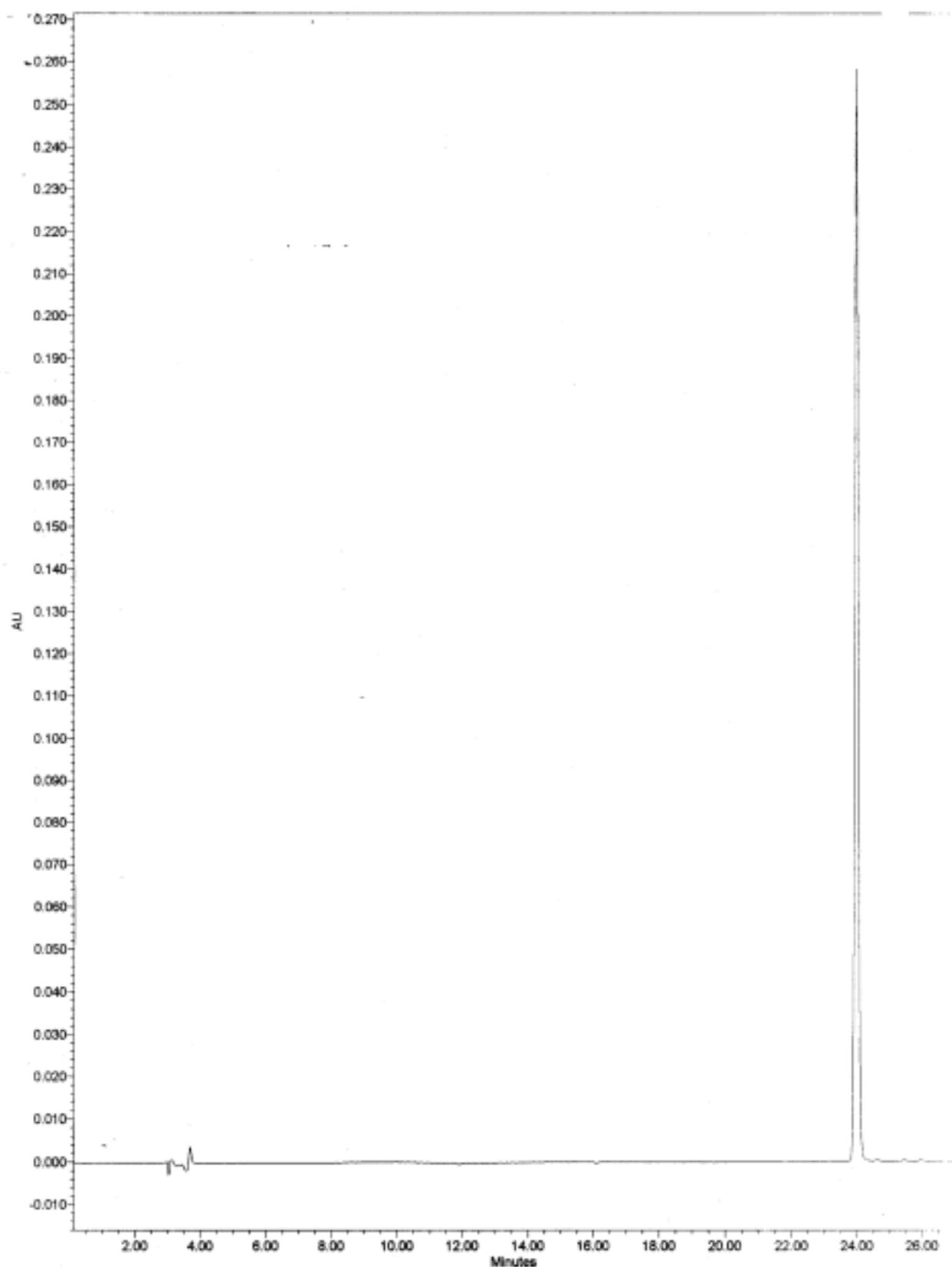
¹H NMR Spectrum for @PheOMe (CDCl₃)



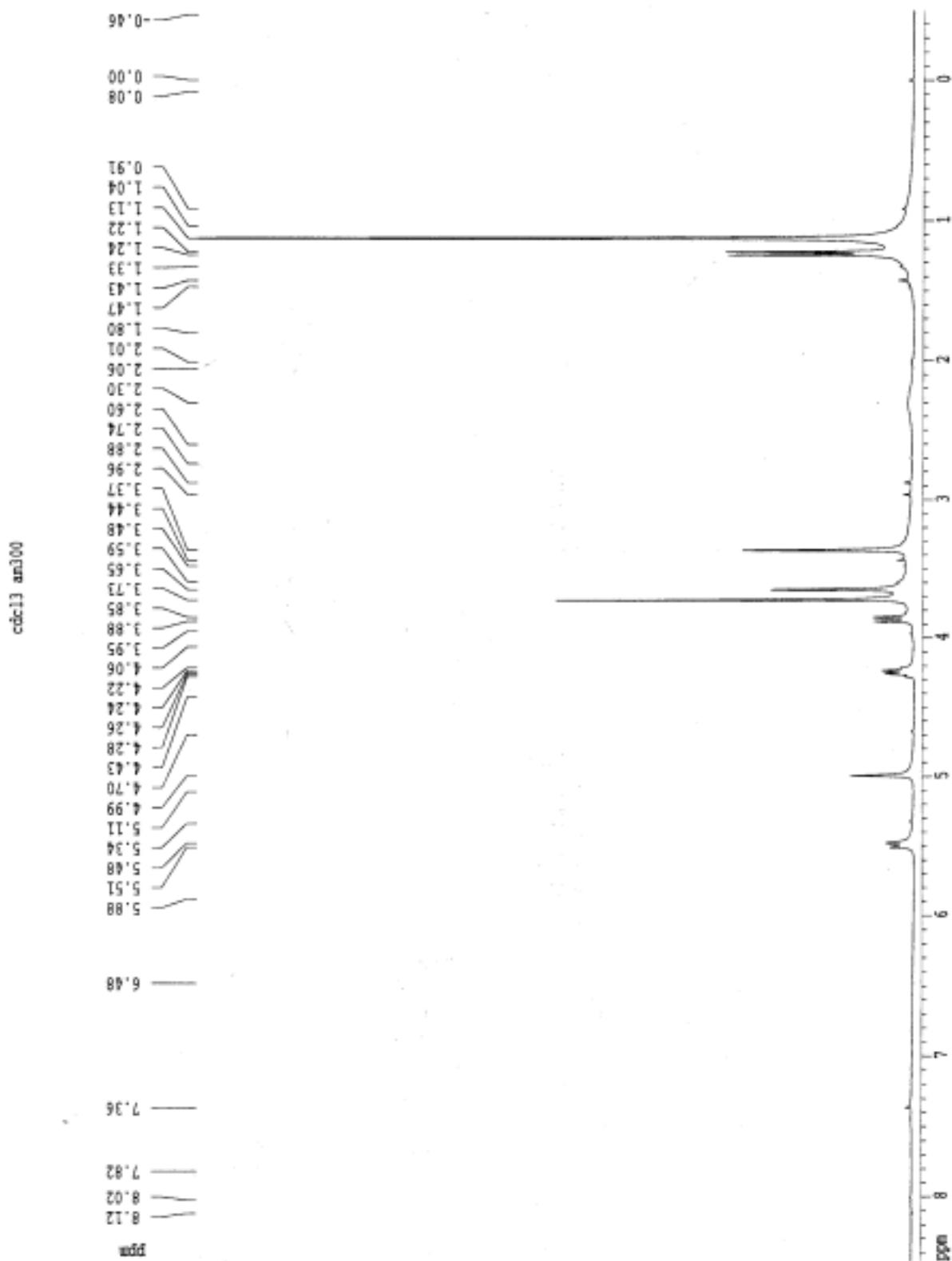
¹H NMR Spectrum for TeocGlu(O^δ-*t*-Bu) (400 MHz, CDCl₃)



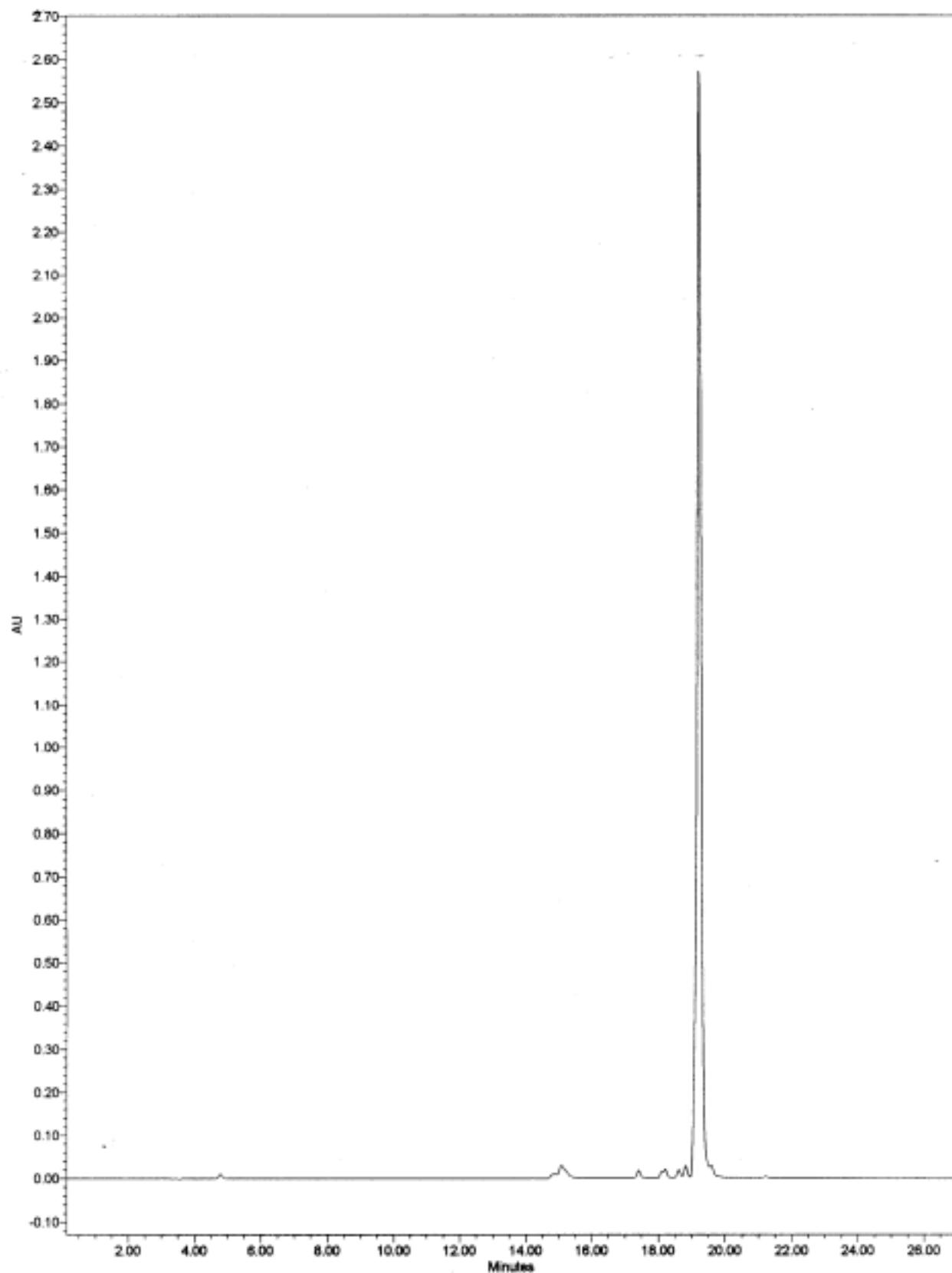
HPLC trace for TeocGlu(O^δ-*t*-Bu)@PheOMe



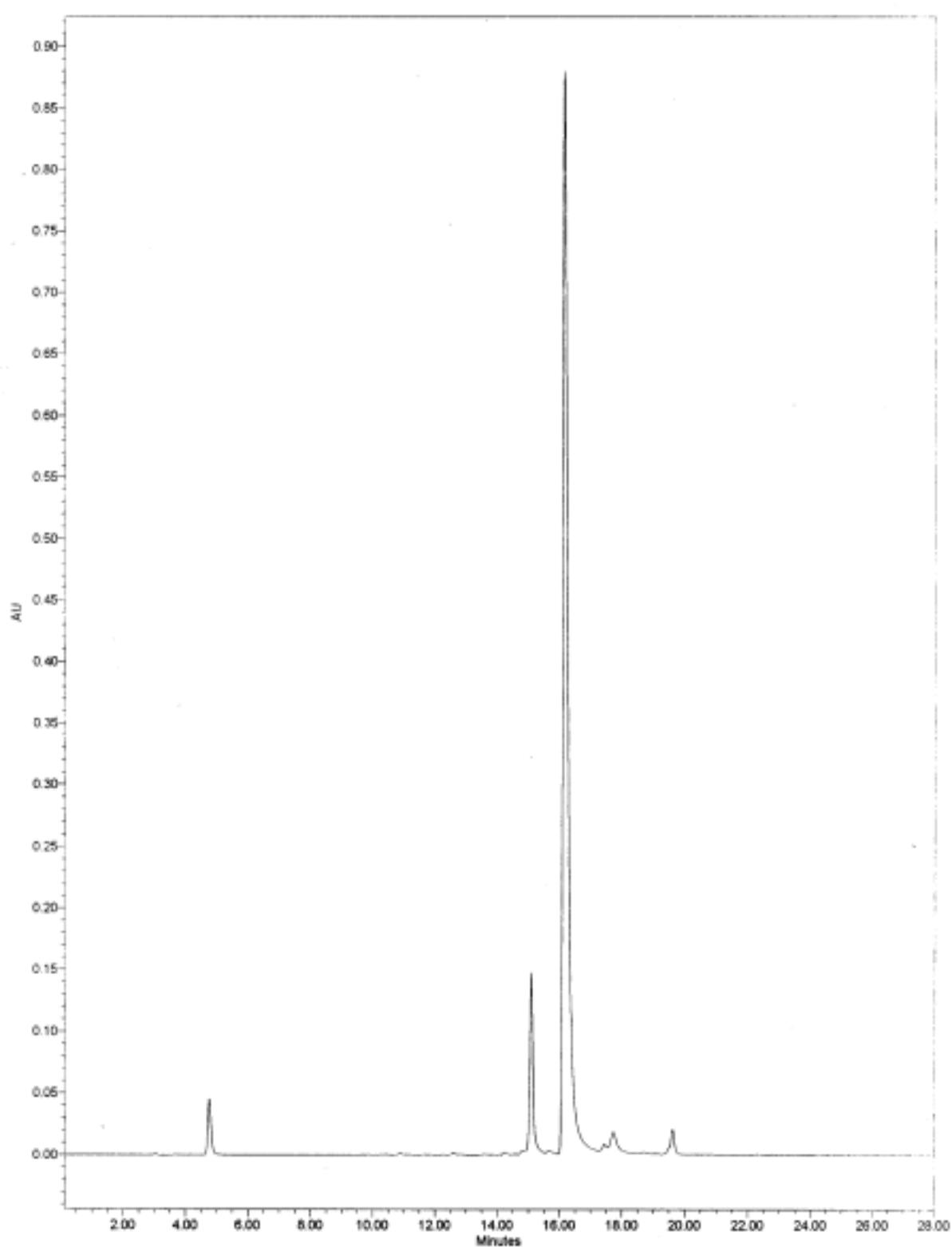
¹H NMR Spectrum for @Thr(O^B-*t*-Bu)OMe (300 MHz, CDCl₃)



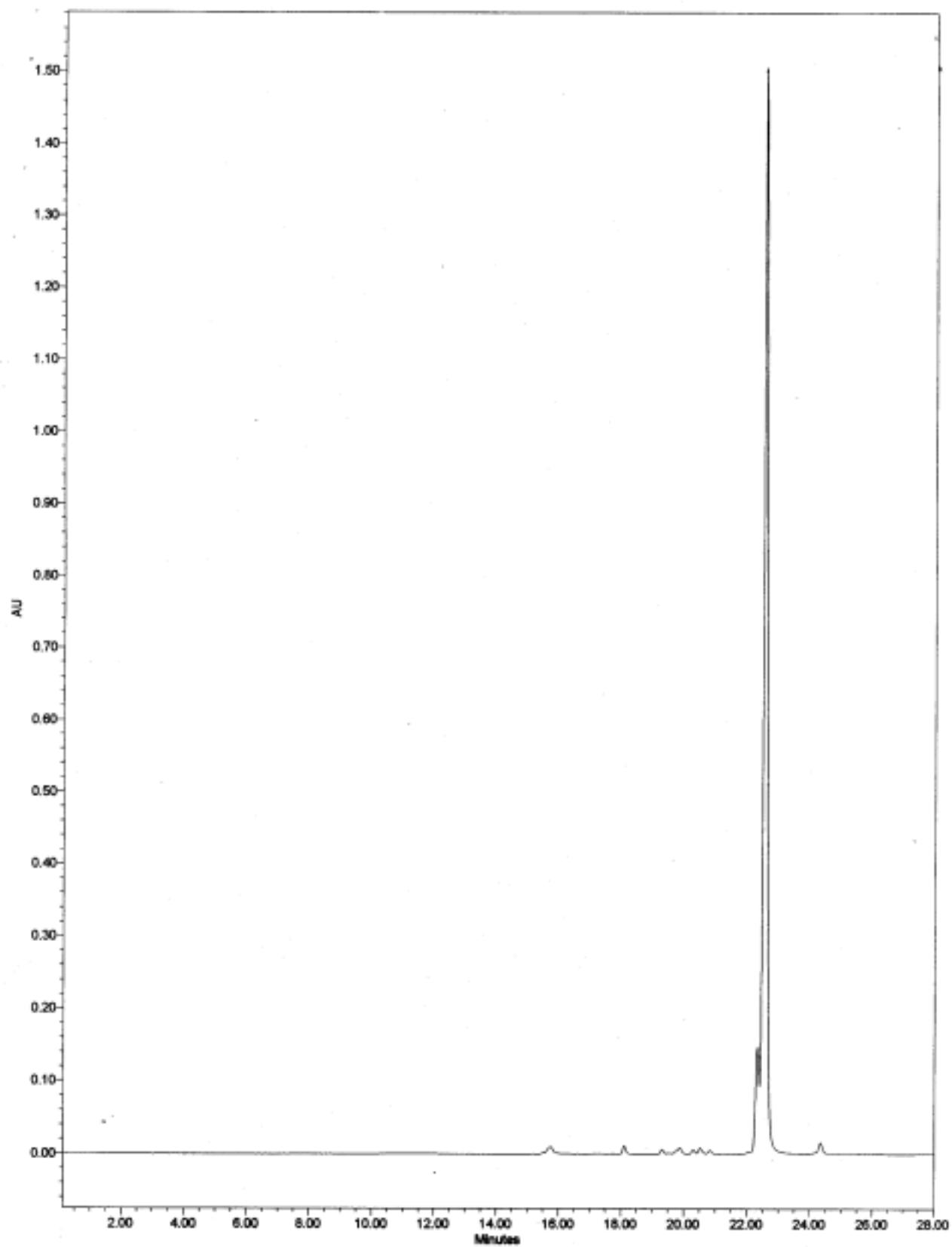
HPLC trace for Alloc@Thr(O^β-t-Bu)@Thr(O^β-t-Bu)OMe



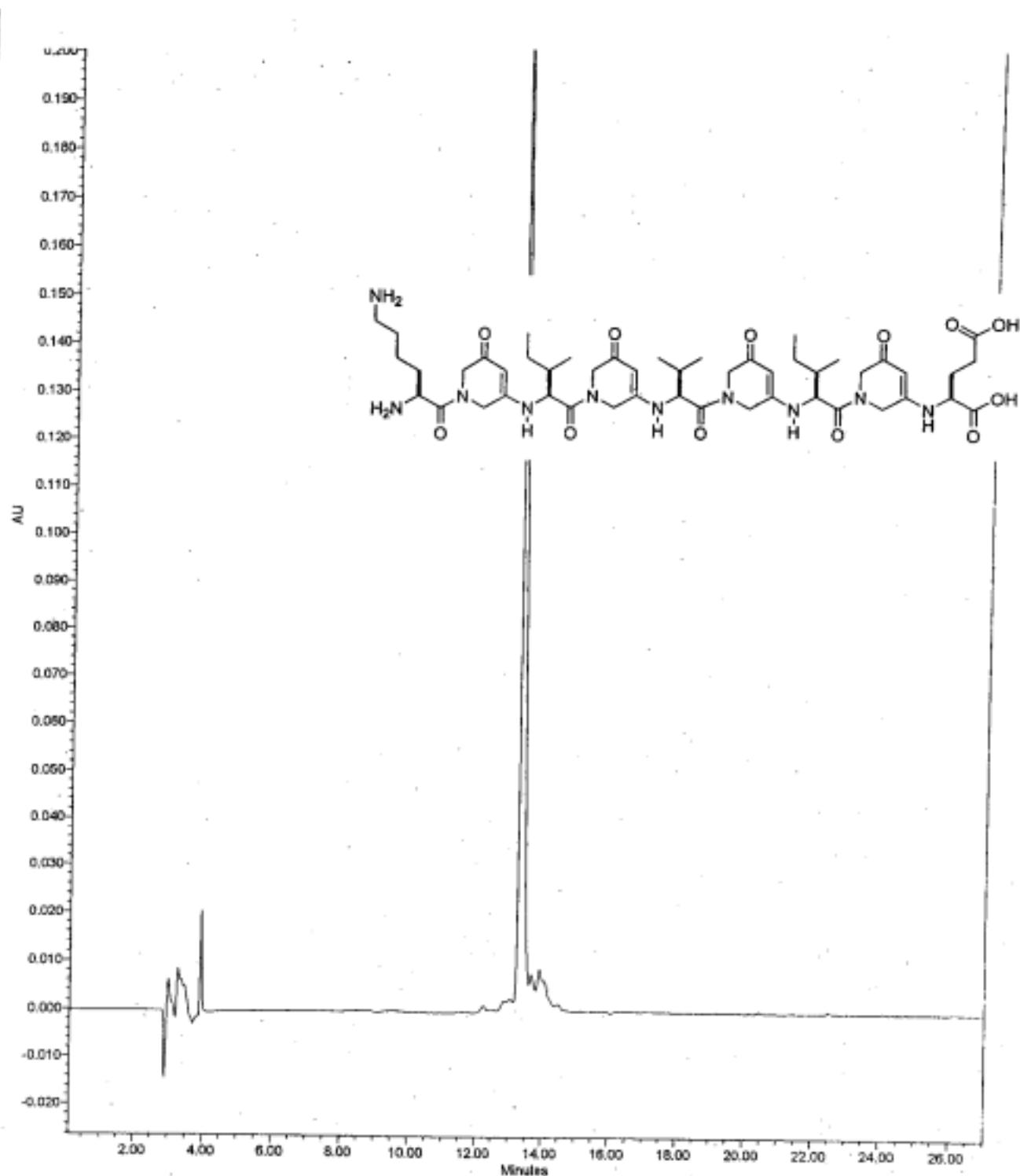
HPLC trace for $\text{Thr}(\text{O}^\beta\text{-t-Bu})@\text{Thr}(\text{O}^\beta\text{-t-Bu})\text{OMe}$ (obtained directly from a $\text{Pd}(0)$ -[resin]-catalyzed Alloc deprotection



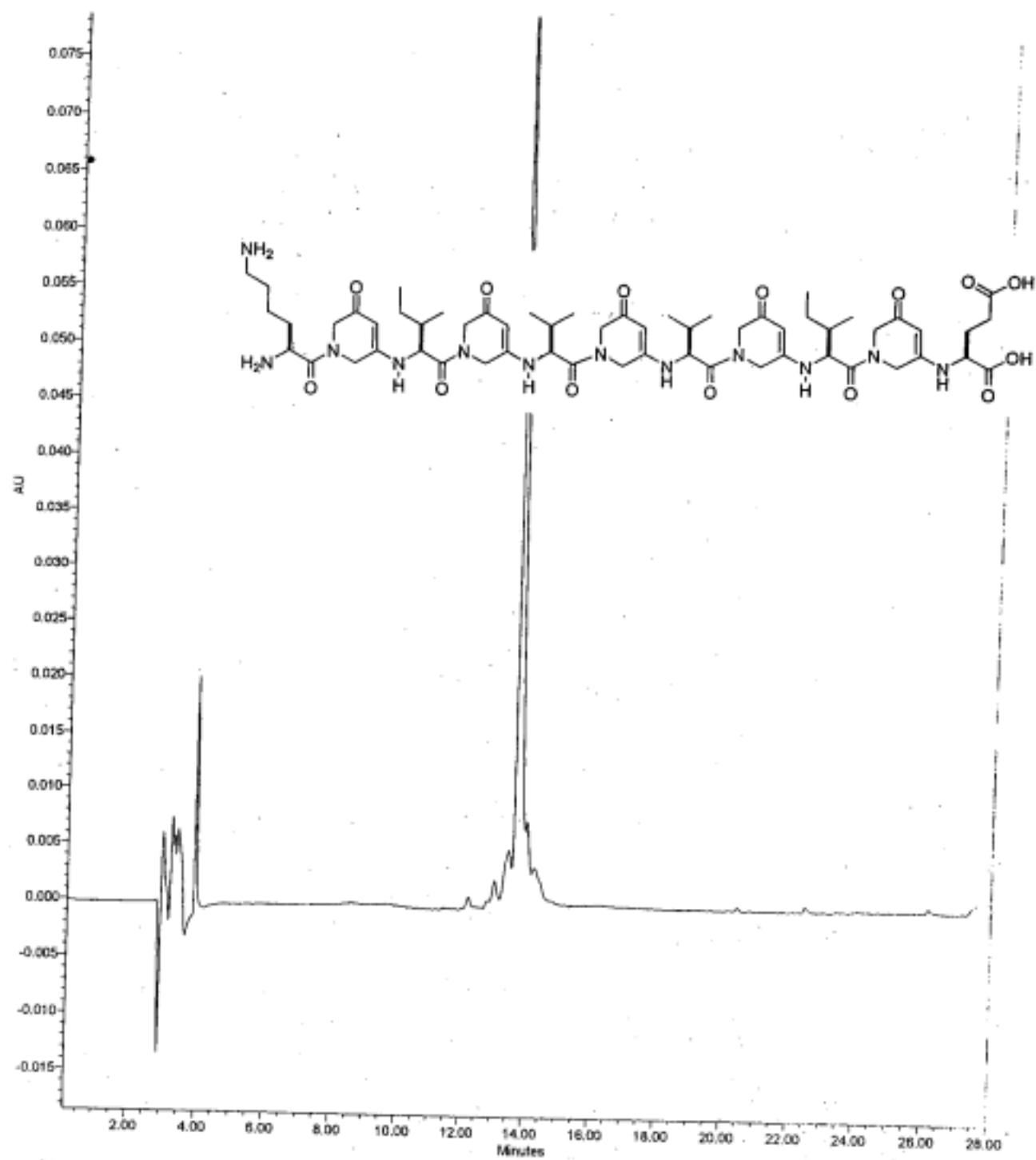
HPLC trace for BocThr(O^β-t-Bu)@Thr(O^β-t-Bu)@Thr(O^β-t-Bu)OMe



HPLC trace for K@I@V@I@E



HPLC trace for K@I@V@V@I@E



HPLC trace for K@I@V@T@V@I@E

