SUPPLEMENTARY MATERIAL
for the manuscript entitled

Structural and Functional Characterization of a Constrained Asx-Turn Motif

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GENERAL INFORMATION

Synthesis. Reagents used in these procedures were purchased from Aldrich Chemical Co., Sigma Chemical Co., Bachem, U.S.A. or Peptide Institute. Analytical data were acquired as follows. Melting points (uncorrected) were determined on a Haake-Buchler melting point apparatus. Optical rotations were measured in a 1 dm cell at 589 nm on a Jasco DIP-181 Digital Polarimeter. Infrared spectra were recorded with a Perkin-Elmer series 1600 FTIR spectrometer. Routine nuclear magnetic resonance (NMR) spectra were obtained with a General Electric QE-300 Fourier transform instrument operating at 300 MHz for proton resonance and 75 MHz for carbon resonance. Chemical shifts are reported in δ units (ppm) relative to dimethylsulfoxide-d_6 or chloroform-d as indicated. Mass spectrometry was obtained at the University of California Riverside in either fast atom bombardment or chemical ionization mode. Thin layer chromatography was carried out on precoated silica gel 60 (F_{254}) plates from EM Separations. Compounds were visualized by UV-light, iodine vapor, ninhydrin and/or chlorine/tolidine stain reagents. High pressure liquid chromatography was carried out on a Beckman Dual Channel System operating with System Gold software.

NMR Spectroscopy. Peptide samples of *cyclo*[Asn-Add]-Thr-NHMe (**1**), N^{α}-Bu-Asn-Leu-Thr-NHMe (**2**) and N^{α}-Bu-Gln-Leu-Thr-NHMe (**3**) were analyzed at 7°C in 700 μL of 43 % methanol-d_4/water at pH 4.5 (apparent) and had concentrations of 6.7 mM, 8.3 mM and 9.7 mM, respectively. The 1D NMR spectra and required 2D TOCSY spectra were recorded on a Bruker AMX500 spectrometer operating at 500 MHz. The 1D spectra contained 16K data points with a spectral width of 5000 Hz. The TOCSY experiments were carried out using the MLEV-17 pulse sequence with a time proportional phase increment. A mixing time of 73 ms was employed. A total of 2K x 512 data points were

obtained with 16 scans in the f2 domain. The FIDs were multiplied by a phase shifted sine bell apodization function prior to Fourier transform.

The ROESY spectra were recorded on a Varian Unity Plus spectrometer operating at 600 MHz for proton resonance. A spectral width of 5000 Hz was used for *cyclo*[Asn-Add]-Thr-NHMe, and 6000 Hz was used for the other two peptides. Phase sensitive data was collected with the hypercomplex method. A mixing time of 200 ms was employed, and presaturation was carried out during a 1.3 s relaxation delay. A total of 2K x 512 data points were obtained with 28, 24 and 20 scans in the f2 domain of peptides **1**, **2** and **3**, respectively. The data were processed in a similar manner as with the TOCSY experiments.

CD spectroscopy CD spectra were recorded on a Jasco J-600 Spectropolarimeter between 260 and 190 nm. A quartz cell of 0.1 cm was used, and eight scans of each blank and sample were recorded at ambient temperature. Peptides were dissolved at a concentration of 0.5 mM in 43% aqueous methanol, or 0.05 mM in either 2% or 43% aqueous methanol. The methanol was degassed and distilled, and the water was deionized and degassed. Background spectra were subtracted from the sample spectra, and noise reduction was carried out with the Jasco System software. Baseline correction was applied on a Macintosh IIci computer using Kaleidagraph software, version 2.1.1. Molar ellipticity was calculated in units of deg cm^2 $dmol^{-1}$ with normalization for the number of amides per molecule (four).

EXPERIMENTAL SECTION

Acetylamino-(7-carboxyheptyl)-propanedioic acid diethyl ester. A suspension of 8-bromooctanoic acid (35 mmol, 7.81 g) in H_2O (20 mL) and EtOH (5 mL) was titrated with an aqueous solution of 1 N Cs_2CO_3 until the mixture was homogeneous and pH 7 was obtained. The solvent was removed under reduced pressure, and the residue was dried under reduced pressure over P_2O_5. To a chilled (0 °C) suspension of diethyl acetamidomalonate (46 mmol, 9.89 g) in EtOH (50 mL), sodium metal (46 mmol, 1.05 g) was added slowly. When the mixture was homogeneous, the dry cesium salt of 8-bromooctanoic acid was added to the EtOH solution and the mixture heated to reflux for 12 h. The reaction was allowed to cool to 20 °C and the solvent was removed under reduced pressure. The residue was suspended in AcOEt (200 mL) and extracted with saturated $NaHCO_3$ (4 x 20 mL). The chilled (0 °C) aqueous layer was

adjusted to pH 2 with 2 N $NaHSO_4$ and extracted with AcOEt (3 x 200 mL). The pooled organic layer was washed with H_2O (3 x 20 mL), 5 % aqueous sodium thiosulfate (3 x 20 mL), saturated NaCl (3 x 20 mL) and dried ($MgSO_4$). After removal of the solvent, the residue obtained was purified by silica gel chromatography ($CHCl_3$/AcOEt/AcOH : 90/10/1, R_f 0.14). The resulting pale yellow oil began to crystallize upon standing (7.50 g, 60 %): R_f 0.28 ($CHCl_3$/MeOH/AcOH : 95/5/1); mp 67.5 - 69.0 °C, ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 11.95 (br s, 1H, COOH), 8.22 (s, 1H, NH), 4.11 (q, 4H, J = 7.1 Hz, Et CH_2), 2.17 (t, 2H, J = 8.2 Hz, CH_2), 2.03 (m, 2H, CH_2), 1.89 (s, 3H, Ac CH_3), 1.45 (m, 2H, CH_2), 1.22 (s, 6H, three CH_2), 1.13 (t, 6H, J = 7.1 Hz, Et CH_3), 1.06 (m, 2H, CH_2); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 174.4, 168.9, 167.6, 66.1, 61.4, 33.6, 32.5, 28.6, 28.5, 28.3, 24.4, 22.9, 22.1, 13.8. IR ν_{max} (thin film, NaCl) 3363, 2927, 1736, 1650, 1517, 1373, 1288, 1203, 1091. CI MS m/z: 360 (MH^+); CI HRMS calcd for $C_{17}H_{30}NO_7$ (MH^+) 360.2022, found 360.2021.

N-Ac-D,L-Add-OH. A mixture of acetylamino-(7-carboxyheptyl)-propanedioic acid diethyl ester (20 mmol, 7.09 g), 6 N HCl (200 mL) and conc. H_2SO_4 (10 mL) was heated to reflux for 12 h. To the cooled (20 °C) solution was added slowly 28.1 g of solid LiOH so that the mixture was still acidic. The solution was applied to a column of cation exchange resin (Dowex AG 50W-X8, H^+ form) which was rinsed with H_2O until the eluant reached pH 7. The compound was eluted from the resin with 1N NH_4OH and obtained as a white powder (2.60 g, 61 %) after removal of the solvent under reduced pressure. To a suspension of the solid in glacial AcOH (25 mL) at 20 °C was added acetic anhydride (18 mmol, 1.83 g). After 24 h, additional acetic anhydride (1.83 g) was added, and the mixture became homogeneous after another 24 h. The solvent was removed under reduced pressure. The residue obtained was dissolved in saturated $NaHCO_3$ (50 mL) and washed with hexanes (3 x 50 mL). The chilled (0 °C) aqueous layer was adjusted with 2 N $NaHSO_4$ to pH 2 and extracted with AcOEt (3 x 150 mL). The organic layer was washed with saturated NaCl (3 x 20 mL) and dried ($MgSO_4$). The compound was obtained as a clear, colorless oil (2.88 g, 93 %) after removal of the solvent under resuced pressure: R_f 0.27 ($CHCl_3$/MeOH/AcOH : 85/15/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 12.20 (br s, 2H, two COOH), 8.08 (d, 1H, J = 8.6 Hz, NH), 4.12 (m, 1H, CH^{α}), 2.18 (t, 2H, J = 8.6 Hz, CH_2), 1.82 (s, 3H, Ac CH_3), 1.59 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25 (s, 8H, four CH_2); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 174.5, 173.9, 169.3, 51.8, 33.6, 31.0, 28.6, 28.5, 25.3, 24.5, 22.3. IR ν_{max} (thin

film, NaCl) 3310, 3086, 2927, 2852, 1718, 1623, 1548, 1373, 1232. CI MS m/z: 260 (MH^+); CI HRMS calcd for $C_{12}H_{22}NO_5$ (MH^+) 260.1498, found 260.1493.

Resolution of *N*-Ac-D,L-Add-OH. A mixture of N-Ac-D,L-Add-OH (0.012 mol, 3.15 g) and freshly deoxygenated H_2O (75 mL) was titrated with 1 N LiOH (20 mL) until the mixture was homogeneous and maintained a pH of 7. Aqueous 0.0969 M $CoCl_2$ (1.0 mL) was added to give a final $[Co^{+2}]$ of 1 mM. Acylase I (22 mg) was added and the pH adjusted to 7.8 with additional 1 N LiOH. The reaction was allowed to proceed under nitrogen at 37 °C. The pH was adjusted with 0.1 N LiOH as needed to maintain pH 7.8, and the formation of free amine was monitored by spectrometry with a quantitative ninhydrin assay. After 10 h an additional 10 mg of Acylase I was added and no further formation of free amino acid was observed by the assay method. After a total of 21 h, the pH was adjusted to 5 with 1 N HCl and activated charcoal (0.2 g) was added. The mixture was heated to 60 °C for 10 min and the charcoal was removed by filtration. Additional 6 N HCl was added to adjust the pH to 1.5. The aqueous layer was extracted with AcOEt (3 x 25 mL) and applied to a cation exchange resin (Dowex AG 50W-X8, H^+ form). The column was rinsed with H_2O to pH 7. The compound was eluted from the resin with 1 N NH_4OH. The solvent was removed under reduced pressure and H_2O (2 x 20 mL), 0.1 N HCl (2 x 20 mL) and toluene (20 mL) were sequentially added and removed under reduced pressure. The amino acid was obtained as a white powder (0.37 g, 56 % of theoretical yield of L-Add): mp 187 - 189.5 °C, $[\alpha]_D^{25}$ +10.4° (c 0.5, 0.1 M HCl), R_f 0.46 ($BuOH/AcOH/H_2O$: 4/1/1); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 13.60 (br s, 1H, COOH), 12.15 (br s, 1H, COOH), 8.35 (br s, 3H, NH_3), 3.83 (t, 1H, J = 4.8 Hz, CH^{α}), 2.17 (t, 2H, 6.8 Hz, CH_2^{θ}), 1.75 (m, 2H, Add CH_2), 1.46 (m, 2H, Add CH_2), 1.25 (m, 8H, Add CH_2); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 174.4, 170.9, 51.9, 33.7, 29.9, 28.4, 24.5, 24.1. IR ν_{max} (KBr) 3448, 3094, 2926, 2856, 1700, 1500, 1478, 1406, 1292, 1234. CI MS m/z: 218 (MH^+); CI HRMS calcd for $C_{10}H_{20}NO_4$ (MH^+) 218.1392, found 218.1398.

HCl·H-L-Add(All)-OH. The amino acid HCl·H-L-Add-OH (2.8 mmol, 0.70 g) was dissolved under nitrogen in freshly distilled allyl alcohol (13.8 mL) and anhydrous chlorotrimethylsilane (6.9 mmol, 0.88 mL) and allowed to react for 24

h. The mixture was added to 600 mL of hexanes/Et_2O (5 : 1) and stirred to allow crystallization. After 1 h an amorphous solid was isolated by filtration. The solid was dissolved in $CHCl_3$ (100 mL) and the solution washed with 0.5 M $NaHCO_3$ (3 x 10 mL) and H_2O (2 x 10 mL) and dried ($MgSO_4$). A pale yellow residue was obtained after removal of the solvent under reduced pressure (0.67 g, 83 %): mp 204 - 207 °C dec., $[\alpha]_D^{25}$ + 12.2 ° (c 0.5, DMF), R_f 0.51 ($CHCl_3$/MeOH/AcOH : 6/4/1); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 13.70 (br s, 1H, COOH), 8.50 (br s, 3H, NH_3), 5.87 (m, 1H, CH_2-C*H*=CH_2), 5.23 (dd, 2H, J = 18.0, 11.3 Hz, CH_2-CH=C*H*$_2$), 4.51 (d, 2H, J = 8.3 Hz, C*H*$_2$-CH=CH_2), 3.78 (m, 1 H, CH^{α}), 2.30 (t, 2H, J = 7.3 Hz, Add CH_2^{θ}), 1.77 (m, 2H, Add CH_2), 1.50 (m, 2H, Add CH_2), 1.23 (m, 8H, Add CH_2); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 172.5, 132.7, 117.6, 64.1, 51.9, 33.3, 29.8, 28.3, 28.2, 24.3, 24.1. IR ν_{max} (thin film, NaCl) 3062, 2925, 2851, 1732, 1483, 1283, 1214, 1172. FAB MS m/z: 258 (MH^+); FAB HRMS calcd for $C_{13}H_{24}NO_4$ (MH^+) 258.1705, found 258.1703.

Boc-Asn-Add(All)-OH. To a chilled (- 20 °C) solution of HCl·H-L-Add(All)-OH (2.1 mmol, 0.61 g) and HOBt (2.7 mmol, 0.37 g) in DMF (8.3 mL) was added NMM (2.1 mmol, 0.23 mL). An additional 0.040 mL of NMM was added to adjust the mixture pH to 7. The ester Boc-Asn-ONp (2.7 mmol, 0.95 g) was added and the reaction was allowed to proceed 30 min at - 20 °C. The mixture was allowed to warm to 20 °C and proceed another 18 h. After removal of the solvent under reduced pressure, the residue obtained was suspended in AcOEt (200 mL) and washed with several portions (10 mL) of 1 N $NaHSO_4$, H_2O (10 mL) and saturated NaCl (3 x 10 mL) and dried ($MgSO_4$). The residue obtained upon removal of the solvent under reduced pressure was purified by silica gel chromatography ($CHCl_3$/MeOH/AcOH : 90/10/1). The dipeptide was obtained as a white powder (0.26 g, 27 %) after the addition and removal under reduced pressure of toluene and hexanes: mp 132.0 - 133.0 °C, $[\alpha]_D^{25}$ + 10.5 ° (c 0.2, $CHCl_3$), R_f 0.26 ($CHCl_3$/MeOH/AcOH : 85/15/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 12.65 (br s, 1H, COOH), 7.85 (d, 1H, J = 8.4 Hz, Add NH), 7.06 (d, 2H, J = 105 Hz, Asn NH_2^{γ}), 6.95 (d, 1H, J = 8.4 Hz, Asn NH^{α}), 5.90 (m, 1H, CH_2-C*H*=CH_2), 5.23 (dd, 2H, J = 18.1, 11.6 Hz, CH_2-CH=C*H*$_2$), 4.63 (d, 2H, J = 5.8 Hz, C*H*$_2$-CH=CH_2), 4.26 (m, 1H, CH^{α}), 4.15 (m, 1H, CH^{α}), 2.34 (m, 4H, Asn CH_2^{β} and Add CH_2^{θ}), 1.58 (m, 4H, two Add CH_2), 1.37 (s, 9H, Boc), 1.23 (m, 8H, four Add CH_2); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 173.5, 172.5, 171.5, 171.4, 155.2, 132.8, 117.6, 78.2, 64.2, 51.7, 51.1, 37.0, 33.3, 31.1, 28.5, 28.4, 28.1, 24.9, 24.4. IR ν_{max} (thin film NaCl) 3389, 3309, 2927, 1735, 1694,

1674, 1651, 1518, 1368, 1252, 1167. FAB MS m/z: 472 (MH^+); FAB HRMS calcd for $C_{22}H_{38}N_3O_8$ (MH^+) 472.2659, found 472.2642.

Cbz-Thr(tBu)-NHMe . A suspension of the dicyclohexylammonium salt Cbz-Thr(tBu)-OH·DCHA (2.1 mmol, 1.01 g) in 150 mL of chilled (0 °C) AcOEt was washed with 1 N $NaHSO_4$ (5 x 10 mL) to generate the carboxylic acid. The solution was then washed with H_2O (10 mL), saturated NaCl (3 x 10 mL) and dried ($MgSO_4$). The oil obtained after removal of the solvent was dissolved in anhydrous THF (8 mL) and chilled (- 15 °C) under nitrogen. In four alternating portions, NMM (2.1 mmol, 0.21 g) and isobutylchloroformate (2.1 mmol, 0.29 g) were added and activation was allowed to proceed at - 15 °C for 5 min. The mixture was saturated with anhydrous monomethylamine and allowed to stir another 20 min at - 15 °C. The mixture was then allowed to warm to 20 °C and proceed for 3 h. Upon removal of the solvent under reduced pressure, the residue obtained was suspended in AcOEt (150 mL), washed with 0.5 M $NaHCO_3$ (10 mL), saturated $NaHCO_3$ (3 x 10 mL), H_2O (10 mL), 1 N $NaHSO_4$ (3 x 10 mL), saturated NaCl (3 x 10 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and hexanes was added. The methylamide was obtained as a white solid (0.61 g, 96 %) by filtration: mp 95.5 - 97.5 °C, $[\alpha]_D^{25}$ + 9.3 ° (c 0.5, EtOH), R_f 0.60 ($CHCl_3$/MeOH/AcOH : 95/5/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 7.74 (m, 1H, N*H*CH_3), 7.36 (m, 5H, arom), 6.59 (d, 1H, J = 9.3 Hz, NH), 5.05 (s, 2H, Cbz CH_2), 3.89 (m, 2H, CH^α and CH^β), 2.55 (d, 3H, J = 5.5 Hz, NHC*H*$_3$), 1.06 (s, 9H, tBu CH_3), 1.02 (d, 3H, J = 6.8 Hz, CH_3^γ); ^{13}C-NMR δ (75 MHz, $CDCl_3$) 170.0, 156.1, 128.5, 128.2, 128.0, 66.9, 66.7, 58.9, 28.2, 26.1, 22.9, 17.4. IR ν_{max} (thin film, NaCl) 3361, 2966, 1720, 1658, 1496, 1364, 1213, 1192, 1066. CI MS m/z: 323 (MH^+); CI HRMS calcd for $C_{17}H_{27}N_2O_4$ (MH^+) 323.1971, found 323.1980.

Boc-Asn-Add(All)-Thr(tBu)-NHMe. To a solution of Cbz-Thr(tBu)-NHMe (0.62 mmol, 0.19 g) in 1 % AcOH in MeOH (5.5 mL) was added 10 % Pd/C (30 mg). H_2 was introduced at artmospheric pressure at 20 °C, and the mixture allowed to react 1 h. The catalyst was removed by filtration and the solvent removed under reduced pressure. Toluene (2 x 10 mL) was added and removed under reduced pressure. A mixture of the amino component and Boc-Asn-Add(All)-OH (0.55 mmol, 0.26 g) in DMF (2.2 mL) was chilled to - 40 °C under nitrogen. The mixture was treated with NMM (0.55 mmol, 0.061 mL) and after 5 min, HOAt

(0.55 mmol, 0.075 g) and EDC (0.57 mmol, 0.11 g) were added. After 30 min at - 40 °C, the reaction was allowed to warm to 20 °C and proceed for 18 h. The solvent was removed under reduced pressure. The residue obtained was suspended in 350 mL of AcOEt and washed with 0.5 M $NaHCO_3$ (10 mL), saturated $NaHCO_3$ (3 x 10 mL), H_2O (10 mL), 1 N $NaHSO_4$ (3 x 10 mL), saturated NaCl (3 x 10 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the tripeptide was obtained as a white powder (0.33 g, 93 %) with subsequent addition and removal under reduced pressure of hexanes: mp 155.5 - 157.5 °C, $[\alpha]_D^{25}$ - 19.5 ° (c 0.1, EtOH), R_f 0.26 ($CHCl_3$/MeOH/AcOH : 95/5/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 7.92 (d, 1H, J = 7.4 Hz, NH), 7.49 (m, 2 H, NH and N*H*CH_3), 7.08 (d, 2H, J = 114 Hz, Asn NH^{γ}), 7.00 (d, 1 H, J = 8.1 Hz, Asn NH^{α}), 5.90 (m, 1H, CH_2-C*H*=CH_2), 5.23 (dd, 2H, J = 20.7, 10.1 Hz, CH_2-CH=C*H*$_2$), 4.52 (d, 2H, J = 4.9 Hz, C*H*$_2$-CH=CH_2), 4.25 (m, 2H, two CH^{α}), 4.13 (m, 1H, CH^{α}), 3.92 (m, 1H, Thr CH^{β}), 2.57 (d, 3H, J = 4.6 Hz, NHC*H*$_3$), 2.34 (m, 4H, Asn CH_2^{β} and Add CH_2^{θ}), 1.57 (m, 4H, two Add CH_2), 1.36 (s, 9H, Boc CH_3), 1.23 (m, 8H, four Add CH_2), 1.08 (s, 9H, tBu CH_3), 0.96 (d, 3H, J = 6.1 Hz, Thr CH_3^{γ}); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 172.5, 171.5, 171.4, 170.0, 169.9, 155.5, 132.8, 117.6, 78.2, 73.5, 66.9, 64.2, 57.8, 52.7, 51.3, 37.0, 33.4, 31.8, 28.6, 28.4, 28.1, 28.0, 25.6, 24.9, 24.4, 19.6. IR ν_{max} (thin film, NaCl) 3299, 2927, 1692, 1642, 1532, 1458, 1389, 1251, 1171. FAB MS m/z: 642 (MH^+); FAB HRMS calcd for $C_{31}H_{56}N_5O_9$ (MH^+) 642.4078, found 642.4066.

Boc-Asn-Add(OH)-Thr(tBu)-NHMe. A mixture of Boc-Asn-Add(All)-Thr(tBu)-NHMe (0.49 mmol, 0.32 g) and AcOH (2.4 mmol, 0.14 mL) in anhydrous THF (5 mL) was treated with $PdCl_2(PPh_3)_2$ (0.016 mmol, 0.011 g). The heterogeneous, bright yellow mixture became homogeneous and darker upon the rapid addition of nBu_3SnH (0.52 mmol, 0.14 mL). After 30 min, a white precipitate began forming and increased as the reaction was allowed to proceed another 5 h. Additional nBu_3SnH (0.056 mmol, 0.015 mL) was added. After 1 h, the solvent was removed under reduced pressure and hexanes was added and removed. A pale yellow solid was isolated by filtration from a suspension in hexanes. The carboxylic acid was purified by recrystallization from hot AcOEt/hexanes and obtained as a white powder (0.23 g, 77 %): mp 172.0 - 175.0 °C, $[\alpha]_D^{25}$ - 13.7 ° (c 0.3, MeOH), R_f 0.10 ($CHCl_3$/MeOH/AcOH : 95/5/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 11.95 (s, 1H, COOH), 7.92 (d, 1H, J = 7.9 Hz, NH), 7.50 (m, 2H, NH and N*H*CH_3), 7.08 (d, 2H, J = 117 Hz, Asn NH_2^{γ}), 7.00 (d, 1H, J = 7.9 Hz,

Asn NH), 4.24 (m, 2H, two CH^{α}), 4.12 (m, 1H, CH^{α}), 3.92 (m, 1H, Thr CH^{β}), 2.58 (d, 3H, J = 4.8 Hz, $NHCH_3$), 2.35 (m, 2H, Asn CH_2^{β}), 2.17 (m, 2H, Add CH_2^{θ}), 1.53 (m, 4H, two Add CH_2), 1.36 (s, 9H, Boc CH_3), 1.22 (m, 8H, four Add CH_2), 1.07 (s, 9H, tBu CH_3), 0.96 (d, 3H, J = 6.6 Hz, Thr CH_3^{γ}); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 174.5, 174.0, 171.5, 171.4, 169.9, 155.1, 78.2, 73.5, 66.9, 57.8, 52.7, 51.3, 37.1, 33.7, 31.8, 28.6, 28.5, 28.4, 28.1, 28.0, 25.7, 25.0, 24.5, 19.6. IR ν_{max} (thin film, NaCl) 3310, 2905, 1687, 1639, 1538, 1453, 1389, 1219. FAB MS m/z: 602 (MH^+); FAB HRMS calcd for $C_{28}H_{52}N_5O_9$ (MH^+) 602.3765, found 602.3784.

Boc-Asn-Add(OPfp)-Thr(tBu)-NHMe. A chilled solution of Boc-Asn-Add(OH)-Thr(tBu)-NHMe (0.081 mmol, 0.049 g) in DMF (0.22 mL) was treated with DCC (0.12 mmol, 0.025 g). After 5 min pentafluorophenol (0.22 mmol, 0.040 g) dissolved in 0.050 mL of AcOEt was added and the reaction allowed to proceed for 30 min at - 20 °C and 18 h at 4 - 6 °C. The mixture was chilled to 0 °C and treated with additional DCC (0.044 mmol, 9.0 mg) and pentafluorophenol (0.21 mmol, 0.038 g dissolved in 0.050 mL of AcOEt) and allowed to proceed for 4 h at 20 °C. The mixture was suspended in 250 mL of $CHCl_3$, which was washed with 1 N $NaHSO_4$ (3 x 25 mL), H_2O (25 mL), 0.5 M $NaHCO_3$ (2 x 15 mL), 1 N $NaHSO_4$ (15 mL), H_2O (25 mL) and dried ($MgSO_4$). After removal of the solvent under reduced pressure, AcOEt was added and the mixture cooled to 0 °C for 1 h. The white precipitate which formed was removed by filtration and the mixture was cooled and filtered another time. The AcOEt was removed under reduced pressure, hexanes added and the pentafluorophenyl ester was isolated as a pale yellow solid (0.047 g, 75 %) by filtration: mp 145.5 - 147.5 °C, R_f 0.64 ($CHCl_3$/MeOH/AcOH : 85/15/3); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 7.37 (m, 1H, NH), 7.08 (m, 1H, NH), 6.95 (m, 1H, NH), 6.09 (br s, 2H, Asn $N^{\alpha}H$ and $N^{\gamma}H$), 5.48 (br s, 1H, Asn $N^{\gamma}H$), 4.51 (m, 1H, CH^{α}), 4.37 (m, 1H, CH^{α}), 4.25 (m, 1H, CH^{α}), 4.17 (m, 1H, Thr CH^{β}), 2.92 (m, 1H, Asn $CH^{\beta d}$), 2.82 (d, 3H, J = 5.1 Hz, $NHCH_3$), 2.66 (m, 3H, Add CH_2^{θ} and Asn $CH^{\beta u}$), 1.88 (m, 4H, Add CH_2), 1.47 (s, 9H, Boc CH_3), 1.33 (m, 8H, Add CH_2), 1.25 (s, 9H, tBu CH_3), 0.98 (d, 3H, J = 6.8 Hz, Thr CH_3^{γ}). FAB MS m/z: 768 (MH^+); FAB HRMS calcd for $C_{34}H_{51}N_5O_9F_5$ (MH^+) 768.3607, found 768.3637.

c[Asn-Add]-Thr-NHMe. The tripeptide Boc-Asn-Add(OPfp)-Thr(tBu)-NHMe (0.012 mmol, 9.0 mg) was dissolved in 6 mL of Et_2O/CH_2Cl_2 (2 : 1) which was saturated with anhydrous HCl. After 30 min the solvent was removed under reduced pressure and the residue obtained was kept under reduced pressure for 1 h. A solution of the residue in freshly distilled DMF (12 mL) was chilled to 0 °C under nitrogen and treated with a 0.29 M solution of TEA in DMF (0.029 mmol, 0.10 mL). The mixture was allowed to proceed for 30 min at 0 °C and then 2 d at 20 °C. After the solvent was removed under reduced pressure and the residue was dissolved in and lyophilized from H_2O/MeCN (9/1). The powder obtained was washed with Et_2O to remove pentafluorophenol, isolated by centrifugation and dried under a nitrogen stream. Purification of the cyclic compound was carried out by HPLC (20 → 40 % MeCN/H_2O, 0.1 % TFA over 25 min; retention time = 11.6 min). A fluffy white powder (1.5 mg, 30 %) was obtained after lyophilization of the pooled HPLC fractions: mp 247.5 - 250.0 °C dec., R_f 0.50 (BuOH/AcOH/H_2O : 4/1/1); ^{1}H-NMR δ_H (600 MHz, 43 % MeOD/H_2O, pH 4.5) 8.42 (d, 1H, J = 8.8 Hz, Asn NH), 8.10 (d, 1H, J = 8.0 Hz, Add NH), 7.81 (d, 1H, J = 8.0 Hz, Thr NH), 7.69 (d, 1H, J = 4.4 Hz, N*H*CH_3), 7.16 (d, 2H, J = 440 Hz, Asn NH_2^{γ}), 4.69 (m (buried), 1H, Asn CH^{α}), 4.24 (m, 1H, Add CH^{α}), 4.03 (m, 2H, Thr CH^{α} and CH^{β}), 2.75 (m, 1H, Asn $CH^{\beta d}$), 2.55 (d, 3H, J = 4.5 Hz, NHC*H*$_3$), 2.44 (m, 1H, Asn $CH^{\beta u}$), 2.13 (m, 1H, Add $CH^{\theta d}$), 1.92 (m, 1H, Add $CH^{\theta u}$), 1.73 (m, 1H, Add $CH^{\beta d}$) 1.49 (m, 1H, Add $CH^{\beta u}$), 1.40-1.05 (m, 10 H, five Add CH_2), 0.99 (d, 3H, J = 5.9 Hz, Thr CH_3^{γ}). IR ν_{max} (thin film, NaCl) 3256, 1624, 1543, 1407, 1201, 1141. FAB MS m/z: 428 (MH^+); FAB HRMS calcd for $C_{19}H_{34}N_5O_6$ (MH^+) 428.2509, found 428.2508.

***N*$^{\alpha}$-nBu-Asn-Leu-Thr-NHMe.** The amino component TFA·H-Asn-Leu-Thr-NHMe (0.021 mmol, 0.010 g) was placed under nitrogen at 20 °C and treated with a 12 % (v/v) solution of NMM in DMF (0.023 mmol, 0.021 mL), and a 33 % (v/v) solution of butyric anhydride in DMF (0.042 mmol, 0.021 mL). (For the synthesis of Boc-Asn-Leu-Thr-NHMe, see Imperiali, B. and Shannon, K.L. *Biochemistry*, **1991**, *30*, 4374-4380.) After 3 h at 20 °C, the mixture formed a solid mass which was transferred into Et_2O (2 mL), dispersed by sonication and isolated by centrifugation. The pellet was washed twice more with Et_2O by the same procedure. The butanoyl tripeptide was obtained as a white solid (0.0057 g, 63 %) which was observed to be homogeneous by reverse phase HPLC: mp 248.5 - 249.5 °C, R_f 0.62 (BuOH/AcOH/H_2O : 4/1/1); HPLC R_t 7.1 min (10 → 80 %

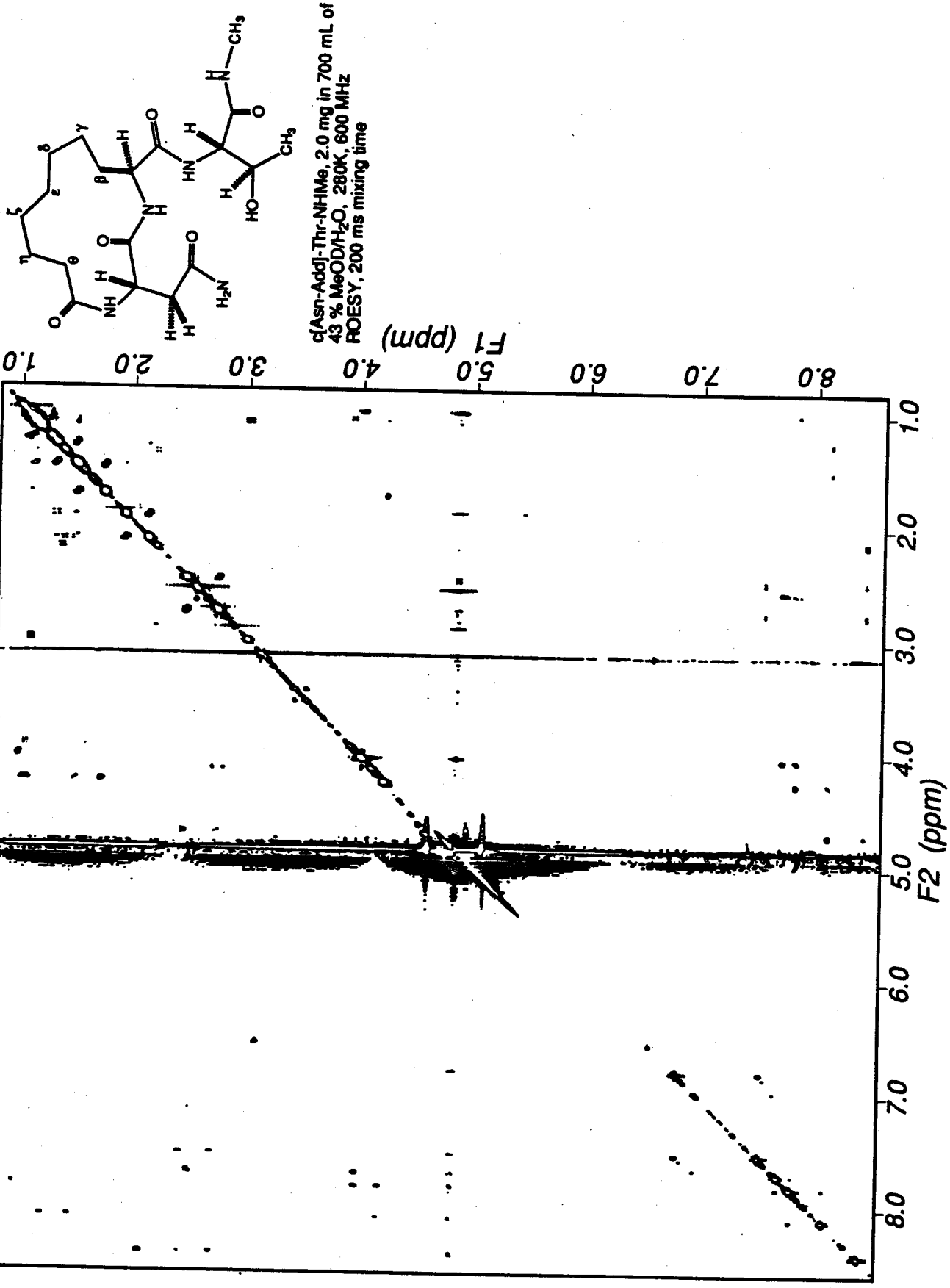
MeCN/H_2O, 0.1 % TFA over 25 min); ^{1}H-NMR δ_H (600 MHz, 43 % MeOD/H_2O, pH 4.5) 8.34 (d, 1H, J = 6.8 Hz, Leu NH), 8.23 (d, 1H, J = 7.9 Hz, Asn NH), 7.82 (d, 1H, J = 7.4 Hz, Thr NH), 7.67 (d, 1H, J = 5.3 Hz, N*H*Me), 7.20 (d, 2H, J = 426 Hz, Asn NH_2^{γ}), 4.54 (dd, 1H, J = 7.4, 6.9 Hz, Asn CH^{α}), 4.19 (dd, 1H, J = 10.7, 6.7 Hz, Leu CH^{α}), 4.04 (m, 2H, Thr CH^{α} and CH^{β}), 2.66 (dd, 1H, J = 15.5, 7.3 Hz, Asn $CH^{\beta d}$), 2.56 (d, 3H, J = 4.7 Hz, NHCH_3), 2.50 (dd, 1H, J = 16.0, 7.4 Hz, Asn $CH^{\beta u}$), 2.05 (t, 2H, J = 7.3 Hz, Bu CH_2^{α}), 1.48 (m, 3H, Leu CH_2^{β} and CH^{γ}), 1.41 (m, 2H, Bu CH_2^{β}), 0.99 (d, 3H, J = 6.0 Hz, Thr CH_3^{γ}), 0.76 (d, 3H, J = 6.0 Hz, Leu $CH_3^{\delta d}$), 0.72 (t, 3H, J = 7.4 Hz, Bu CH_3^{γ}), 0.69 (d, 3H, J = 5.5 Hz, Leu $CH_3^{\delta u}$). FAB MS m/z: 430 (MH^+); FAB HRMS calcd for $C_{19}H_{36}N_5O_6$ (MH^+) 430.2666, found 430.2646.

Boc-Gln-Leu-Thr-NHMe. A chilled (-20 °C) solution of the amino component TFA·H-Leu-Thr-NHMe (0.21 mmol, 0.076 g) in 0.80 mL of dry DMF was treated with NMM (0.24 mmol, 0.026 mL). (*The dipeptide Boc-Leu-Thr-NHMe was synthesized in solution by an EDC/HOBt mediated coupling reaction between Boc-Leu-OH and H-Thr-NHMe.*) To this mixture, Boc-Gln-OPfp (0.29 mmol, 0.12 g) was added, and stirring was allowed to proceed for 15 min at -20 °C and 20 h at 20 °C. After removal of the DMF under reduced pressure, the residue was purified by silica gel chromatography ($CHCl_3$/MeOH/AcOH : 90/10/1) and obtained as a white solid (0.023 g, 23 %): mp 196.0 - 197.5 °C, $[\alpha]_D^{25}$ + 35.6 ° (c 0.5, $CHCl_3$), R_f 0.29 ($CHCl_3$/MeOH/AcOH : 85/15/3); ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 8.08 (d, 1H, J = 8.3 Hz, NH), 7.68 (m, 2H, two NH), 7.03 (d, 2H, J = 153 Hz, Gln NH_2^{δ}), 6.97 (d, 1H, J = 8.3 Hz, Gln NH), 4.33 (m, 1H, CH^{α}), 4.07 (m, 1H, CH^{α}), 3.94 (m, 1H, Thr CH^{α}), 3.87 (m, 1H, Thr CH^{β}), 2.56 (d, 3H, J = 4.7 Hz, NHCH_3), 2.07 (m, 2H, Gln CH_2^{γ}), 1.87 - 1.40 (m, 5H, Leu CH_2^{β}, CH^{γ} and Gln CH_2^{β}), 1.84 (s, 9H, Boc CH_3), 0.97 (d, 3H, J = 6.3 Hz, Thr CH_3^{γ}), 0.86 (d, 3H, J = 6.6 Hz, Leu $CH_3^{\delta d}$), 0.80 (d, 3H, J = 6.6 Hz, Leu $CH_3^{\delta u}$); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 174.4, 172.6, 172.5, 171.1, 155.7, 78.6, 66.9, 58.8, 54.5, 51.8, 32.0, 28.6, 26.1, 24.5, 23.7, 21.9, 20.4. IR ν_{max} (KBr) 3284, 2955, 1649, 1537, 1390, 1249, 1611. FAB MS m/z: 474 (MH^+); FAB HRMS calcd for $C_{21}H_{40}N_5O_7$ (MH^+) 474.2928, found 474.2903.

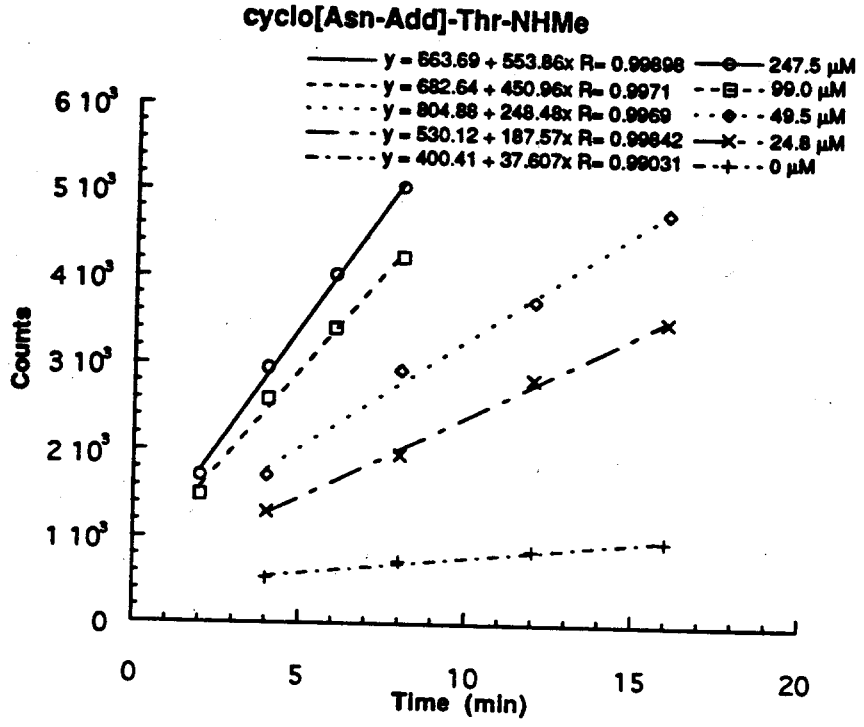
***N*$^{\alpha}$-Bu-Gln-Leu-Thr-NHMe.** A mixture of Boc-Gln-Leu-Thr-NHMe (0.046 mmol, 0.022 g) in CH_2Cl_2 (1 mL) was treated dropwise with TFA (1 mL) and the mixture allowed to stir 1 h. The solvent was removed under reduced pressure and toluene was added and removed under reduced pressure two times. The residue was treated with mixtures of NMM/DMF (12.5 % v/v, 0.046 mL, 0.052

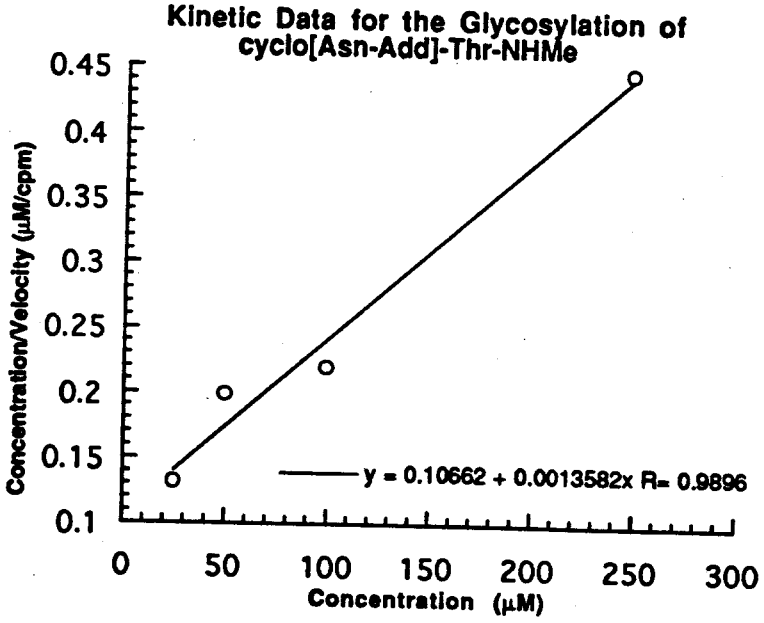
mmol) and butyric anhydride (35 % v/v, 0.046 mL, 0.098 mmol) as described for Bu-Asn-Leu-Thr-NHMe. After 4 h, the solid reaction mixture was suspended in Et_2O (2 mL) and washed by the same procedure as was described for N^{α}-Bu-Asn-Leu-Thr-NHMe. The pale yellow solid obtained was purified by chromatography using a reverse phase cartridge (Sep Pak, Waters, C_{18} packing) and a gradient elution of $0 \rightarrow 30$ % aqueous MeCN (no TFA). The compound was obtained as a white powder (0.0072g, 35 %) after lyophilization of the appropriate fractions and found to be homogeneous by reverse phase HPLC: R_f 0.48 ($BuOH/AcOH/H_2O$: 4/1/1); HPLC R_t 11.6 min ($10 \rightarrow 60$ % $MeCN/H_2O$, 0.1 % TFA over 25 min, semipreparative); mp 230.0 - 231.0 °C, ^{1}H-NMR δ_H (600 MHz, 43 % $MeOD/H_2O$, pH 4.5) 8.35 (d, 1H, J = 6.8 Hz, Leu NH), 8.16 (d, 1H, J = 6.8 Hz, Gln NH), 7.82 (m, 2H, Thr NH and N*H*CH_3), 7.16 (d, 2H, J = 443 Hz, Gln NH_2^{δ}), 4.23 (dd, 1H, J = 6.0, 4.1 Hz, Leu CH^{α}), 4.14 (dd, 1H, J = 14.8, 6.7 Hz, Gln CH^{α}), 4.06 (dd, 1H, J = 8.0, 4.6 Hz, Thr CH^{α}), 4.00 (dd, 1H, J = 6.1, 4.7 Hz, Thr CH^{β}), 2.58 (d, 3H, J = 4.7 Hz, NHC*H*$_3$), 2.17 (t, 2H, J = 7.7 Hz, Gln CH^{γ}), 2.07 (t, 2H, J = 7.4 Hz, Bu CH_2^{α}), 1.87 (dd, 1H, J = 13.8, 6.4 Hz, Gln $CH^{\beta d}$), 1.77 (dd, 1H, J = 14.1, 8.1 Hz, Gln $CH^{\beta u}$),1.50 (m, 1H, Leu $CH^{\beta d}$), 1.43 (m, 4H, Leu $CH^{\beta u}$, CH^{γ} and Bu CH_2^{β}), 0.99 (d, 3H, J = 6.2 Hz, Thr CH_3^{γ}), 0.78 (d, 3H, J = 6.1 Hz, Leu $CH_3^{\delta d}$), 0.73 (t, 3H, J = 7.4 Hz, Bu CH_3^{γ}), 0.72 (d, 3H, J = 6.6 Hz, Leu $CH_3^{\delta u}$). FAB MS m/z: 444 (MH^+); FAB HRMS calcd for $C_{20}H_{38}N_5O_6$ (MH^+) 444.2822, found 444.2826.

J8425-m12



J8425-M13





The glycosylation assays with yeast OT were run three times with similar resulting values of apparent K_m and V_{rel}. Peptide concentrations reflect corrections derived from quantitative amino acid analysis.

J 8425-m14