Conformational Changes Associated with Post-Translational Modifications of Pro\textsuperscript{143} in Skp1 of \textit{Dictyostelium} – a Dipeptide Model System

Chamini V. Karunaratne,\textsuperscript{†} Thomas K. Weldeghiorgish,\textsuperscript{†} Christopher M. West\textsuperscript{‡} and Carol M. Taylor*\textsuperscript{†}
\textsuperscript{†}Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803
\textsuperscript{‡}Department of Biochemistry & Molecular Biology, Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

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1.1 General

All reactions were performed under an atmosphere of nitrogen unless otherwise noted. Reagents were obtained from commercial sources and used directly with the following exceptions. Amines were dried and distilled from calcium hydride and stored over potassium hydroxide pellets. Methanol was distilled from magnesium turnings and stored over 4 Å molecular sieves. Methylamine hydrochloride was recrystallized from ethanol. Flash chromatography was performed using 32-63 µ silica gel from Dynamic Adsorbents Incorporated. Compounds were visualized for TLC by UV fluorescence or by staining with ethanolic solutions of anisaldehyde, ninhydrin, phosphomolybic acid, potassium mermanganate or 10% sulfuric acid. HPLC was performed on a Waters 600E multisolvent deliver system equipped with a Waters 2487 dual wavelength UV detector. For standard characterization purposes, NMR spectra were recorded on a Bruker DPX-400 or AV-400 spectrometer. Proton NMR data is reported in ppm downfield from TMS as internal standard. Most compounds exist as a mixture of rotamers about the prolyl amide bond on the time scale of the acquisition of ¹H NMR data. Signals in square parentheses refer to those of the minor rotamer. High resolution mass spectra were recorded using either an Agilent 6210 time-of-flight or Hitachi MS-8000 3DQ LC-ion trap mass spectrometer with electrospray ionization.
1.2 Synthesis of the Ac-Thr-Pro-NHMe Dipeptide (1)

**Dipeptide 1d.** N-Hydroxysuccinimide (75 mg, 0.65 mmol, 1.0 equiv.) and DCC (133 mg, 0.65 mmol, 1.0 equiv.) were added sequentially to a solution of Boc-Thr(OBn)-OH (1a) (200 mg, 0.65 mmol, 1.0 equiv.) in CH₂Cl₂ (5 mL), at 0 °C. The solution was stirred at 0 °C for 15 min, warmed to rt and stirred overnight under N₂. The suspension was filtered through a plug of cotton in a Pasteur pipet. The filtrate was concentrated to 2 mL and refrigerated 5 h. The suspension was filtered again and the filtrate concentrated. The residue was dissolved in DMF (2 mL) and cooled to 0 °C. Proline (1b) (74 mg, 0.65 mmol, 1.0 equiv.) was added, as a solid in one portion, followed by the addition of diisopropylethylamine (113 µL, 84 mg, 0.65 mmol, 1.0 equiv.). The reaction mixture was gradually warmed to rt and stirred overnight under N₂. The solution was concentrated and dissolved in water (25 mL) and washed with diethyl ether (25 mL). The aqueous layer was acidified with conc. HCl (to pH 2) and extracted with EtOAc (2 x 25 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated to give 1c that was used directly in the next step, without purification. *R*ᵣ 0.20 (4:1 CH₂Cl₂-MeOH).

The residue was dissolved in CH₂Cl₂ (5 mL) and cooled to 0 °C. N-Hydroxysuccinimide (75 mg, 0.65 mmol, 1.0 equiv.) was added, followed by the addition of DCC (133 mg, 0.65 mmol, 1.0 equiv.). The solution was stirred for 30 min at 0 °C, then gradually warmed to rt and stirred a further 6 h. The suspension was filtered through
a plug of cotton in a Pasteur pipet. The filtrate was concentrated to 2 mL and refrigerated overnight. The suspension was again filtered and the filtrate was concentrated. The residue was dissolved in DMF (3 mL) and cooled to 0 °C. Methylamine hydrochloride (44 mg, 0.65 mmol, 1.0 equiv.) was added as a solid in one portion, followed by the addition of triethylamine (225 µL, 163 mg, 1.62 mmol, 2.5 equiv.). The solution was gradually warmed to rt and stirred overnight under N₂. The mixture was diluted with EtOAc (30 mL) and washed with brine (30 mL). The aqueous layer was back-extracted with EtOAc (30 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated. Dipeptide 1d was isolated by flash chromatography, eluting with 4:1 EtOAc-hexanes (209 mg, 81% over 2 steps). $R_f$ 0.22 (4:1 EtOAc-hexanes). $^1$H NMR (CDCl₃, 400 MHz) δ 1.16 [1.21] (d, $J = 6.1$, 3H), 1.45 [1.44] (s, 9H), 1.58-1.96 (m, 4H), 2.81 (d, $J = 4.7$ Hz, 3H), 3.35-3.57 (m, 2H), 3.77-3.81 (m, 0.5H), 4.18-4.25 (m, 1.5H), 4.48-4.64 (m, 3H), 5.50 (d, $J = 6.4$ Hz, 0.5H) [5.59 (d, $J = 8.5$ Hz, 0.5H)], 6.53 (s, 1H); $^{13}$C NMR (CD₃OD, 100 MHz) δ 16.1 [15.8], 24.2 [25.1], 26.4 [26.3], 28.4 [28.5], 34.1, 46.8 [46.2], 56.1, 58.0, 71.8 [71.0], 75.0 [74.7], 80.0 [80.2], 127.8, 127.9, 128.0, 128.5, 128.6, 138.2 138.3, 155.8 [156.0], 168.6, 170.7.

**Dipeptide 1e.** Trifluoroacetic acid (1 mL) was added to a solution of Boc-Thr(OBn)-Pro-NHMe (1d) (93 mg, 0.222 mmol) in CH₂Cl₂ (2 mL) at 0 °C. The reaction mixture was gradually warmed to rt and stirred for 2 h under N₂. The solution was concentrated and then concentrated three times from CH₂Cl₂. The residue was dissolved in pyridine (1 mL) and cooled to 0 °C. Acetic anhydride (1 mL) was added, the mixture warmed to rt and stirred under N₂ overnight. The solution was concentrated and applied to a flash column, eluting first with 4:1 EtOAc-hexanes and then with 9:1 CH₂Cl₂-MeOH to give
**1e** (54 mg, 67%). $R_f$ 0.48 in 9:1 CH$_2$Cl$_2$-MeOH. $^1$H NMR (CD$_3$OD, 400 MHz) $\delta$ 1.18 [1.21] (d, $J = 6.3$ Hz, 3H), 1.75-1.90 (m, 1H), 1.95-2.05 (m, 2H), 2.03 [1.99] (s, 3H), 3.33-3.44 (m, 1.5H) [3.62-3.68 (m, 0.5H)], 4.08 (ddd, $J = 12.6$, 6.4, 3.2 Hz, 0.7H) [3.86 (dt, $J = 11.4$, 6.3 Hz, 0.3H)], 4.38 (d, $J = 3.1$ Hz, 1H), 4.43 (d, $J = 11.8$ Hz, 0.7H) [4.46 (d, $J = 12.0$ Hz, 0.3H)], 4.57 (d, $J = 11.8$ Hz, 0.7H) [4.62 (d, $J = 12.0$ Hz, 0.3H)], 4.70 (d, $J = 5.1$ Hz, 1H), [4.70-4.72 (m, 0.7H)], 7.23-7.26 (m, 5H); $^{13}$C NMR (CD$_3$OD, 100 MHz) $\delta$ 16.8 [16.6], 22.7 [22.5], 25.1, 26.6, 27.1, 48.2 [47.4], 57.3, 59.5, 72.5 [72.0], 75.8 [75.1], 128.8 [128.9], 129.1 [129.2], 129.4 [129.5], 139.8, 170.4, 173.3 [173.4], 174.0.

**Dipeptide 1.** Palladium on carbon (10%, 15 mg) was added in a single portion to a solution of Ac-Thr(Obn)-Pro-NHMe (**1e**) (17 mg, 0.05 mmol) in MeOH (2.5 mL). The reaction flask was evacuated, then opened to an atmosphere of H$_2$ and stirred overnight. The catalyst was removed by filtering through a plug of Celite® in a Pasteur pipet. The filtrate was concentrated, the brown residue was subjected to the RP-HPLC (gradient: 50%-80% acetonitrile in H$_2$O, C$_{18}$ 4.6 mm x 250 mm column 1 mL/min) ($R_f = 16$ min) to give **1** (12 mg, 94%). $R_f$ 0.24 (9:1 CH$_2$Cl$_2$-MeOH). [α]$_D$$^{25}$ -5.4 (c 0.5, MeOH). $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 1.24 [1.26] (d, $J = 5.9$ Hz, 3H), 1.24-1.27 (m, 1H), 1.91-2.07 (m, 3H), 2.14 [2.11] (s, 3H), 2.80 (s, 3H), 3.43 (dd, $J = 12.0$, 6.4 Hz, 0.5H), 3.50 (dd, $J = 12.0$, 7.0 Hz, 0.5 H), 3.62-3.81 (m, 1H), 4.15 (app. p, $J = 6.3$ Hz, 0.5 H), 4.27-4.30 (m, 3H), 4.60 (d, $J = 5.9$ Hz, 0.5 H); $^{13}$C NMR (D$_2$O, 100 MHz) $\delta$ 18.7 [18.4], 21.7 [21.5], 23.6, 25.4, 25.8, 47.4 [46.5], 57.1, 59.3, 66.8 [66.9], 169.7, 172.5, 174.8 [174.2].
Boc-Thr(Obn)-Pro-NHMe (1d)

CVK-1-175 in CDCl3 at 400 MHz
Ac-Thr(Obn)-Pro-NHMe (1e)

CVK-1-177a in CD3OD at 400 MHz
Ac-Thr(OBn)-Pro-NHMe (1e)

CVK-1-177a in CD3OD at 100 MHz
Ac-Thr-Pro-NHMe (1) – unreferenced residual H$_2$O δ 4.80 ppm

CVK-1-179 in D$_2$O at 400 MHz
Ac-Thr-Pro-NHMe (1) – unreferenced CVK-1-179 in D2O at 100 MHz
**1.3 Synthesis of the Ac-Thr-Hyp-NHMe Dipeptide (2)**

**Dipeptide 2c.** Diethylamine (800 µL) was added to a solution of Fmoc-Hyp-(O’Bu)-NHMe (2a) (65 mg, 0.15 mmol, 1.0 equiv.) in acetonitrile (3 mL). The solution was stirred at 0 °C under N₂ for 30 min, concentrated and then concentrated twice more from acetonitrile. The residue was suspended in dichloromethane (2 mL), and cooled to 0 °C. Fmoc-Thr(O’Bu)OH (2b) (71 mg, 0.18 mmol, 1.5 equiv.) was added followed by the addition of diisopropylethylamine (68 µL, 50 mg, 0.39 mmol, 2.5 equiv.) and PyBroP (108 g, 0.23 mmol, 1.5 equiv.). The solution was gradually warmed to rt and stirred overnight under N₂. The mixture was concentrated and the residue applied to a flash column, eluting with 4:1 EtOAc-hexanes to give 2c (87 mg, 98%). R_f 0.41 (4:1 EtOAc-hexanes).\(^1\) H NMR (CD₃OD, 400 MHz) \(\delta\) 1.16 (d, \(J = 8.4\) Hz, 3H), 1.19 (s, 9H), 1.23 (s, 9H), 2.03-2.18 [2.20-2.27] (m, 2H), 2.75 [2.77] (s, 3H), 3.62 (dd, \(J = 10.5, 3.5\) Hz, 1H) [3.43 (dd, \(J = 12.2, 4.2\) Hz)], 3.94 (dd, \(J = 10.5, 5.3\) Hz, 1H) [3.75 (dd, \(J = 12.2, 5.8\) Hz)], 3.98 (p, \(J = 6.0\) Hz, 1H), 4.19 (t, \(J = 6.4\) Hz, 1H), 4.39-4.42 (m, 1H), 4.44 (d, \(J = 2.3\) Hz, 2H), 4.44 (d, \(J = 2.3\) Hz, 1H), 4.54 (t, \(J = 7.4\) Hz, 1H), 7.29-7.33 (m, 2H), 7.39 (t, \(J = 7.4\) Hz, 2H) 7.65 (d, \(J = 7.4\) Hz, 2H), 7.78 (d, \(J = 7.5\) Hz, 2H);\(^{13}\) C NMR (CD₃OD, 100 MHz) \(\delta\) 19.6 [20.1], 26.3 [26.4], 28.6 [28.7], 38.6 [40.7], 47.0 [47.1], 48.5, 56.5 [55.4], 58.9 [58.5], 60.6 [60.9], 67.9 [68.0], 69.8 [69.2], 75.5 [75.3], 76.2 [75.8], 121.0, 126.2,
Dipeptide 2d. Diethylamine (2 mL) was added to a solution of 2c (119 mg, 0.21 mmol, 1 equiv.) in acetonitrile (3 mL). The solution was stirred at 0 °C under N₂ for 30 min, concentrated, and then concentrated twice more from acetonitrile. The residue was applied to a flash column, eluting first with 2:1 EtOAc-hexanes to remove Fmoc byproducts and then with 9:1 CH₂Cl₂-MeOH to isolate the ninhydrin active primary amine (R_f 0.36 in 9:1 CH₂Cl₂-MeOH) as a pale yellow oil (73 mg). This was dissolved in a mixture of pyridine (1 mL) and acetic anhydride (1 mL), stirred at rt under N₂ overnight. The red solution was concentrated and purified using flash chromatography, eluting with 9:1 CH₂Cl₂-MeOH to isolate compound 2d as a colorless foam (71 mg, 93%). R_f 0.40 (9:1 CH₂Cl₂-MeOH). ¹H NMR (CDCl₃, 400 MHz) δ 1.09 (d, J = 6.4 Hz, 3H), 1.19 (s, 9H), 1.28 (s, 9H), 2.01 (s, 3H), 2.05 (dt, J = 12.3, 8.4 Hz, 1H), 2.37 (ddd, J = 12.7, 6.1, 3.0 Hz, 1H), 2.75 [2.83], (d, J = 4.7 Hz, 3H), 3.42 (dd, J = 10.2, 6.2 Hz, 1H), 4.07-4.13 (m, 2H), 4.28 (p, J = 6.8 Hz, 1H), 4.75 (dd, J = 9.0, 2.9 Hz, 1H), 4.81 (dd, J = 7.4, 4.8 Hz, 1H), 6.62 [6.68] (d, J = 4.4 Hz, 1H), 6.93 [7.05] (d, J = 8.0 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 17.5, 23.1, 25.9, 28.0, 28.2, 36.5, 54.5, 54.6, 58.7, 68.0, 69.1, 74.2, 75.4, 169.2, 169.8, 171.7. HRMS (+ESI) calcd for C₂₀H₃₈N₃O₅ (M)⁺: 400.2806; obsd: 400.2802.

Dipeptide 2. Trifluoroacetic acid (1.5 mL) was added to a solution of Ac-Thr(O'Bu)-Hyp(O'Bu)-NHMe (2d) (130 mg, 0.325 mmol) in CH₂Cl₂ (1.5 mL) at 0 °C. The mixture was gradually warmed to rt and stirred under N₂ for two days. The solution was concentrated and then concentrated three times more from CH₂Cl₂. The brown residue
was subjected to the RP-HPLC (gradient: starting with 80% H2O in acetonitrile to 20% H2O in acetonitrile) (Rf = 4 min) to isolate 2 as a colorless oil (84 mg, 90%). Rf 0.33 (4:1 CH2Cl2:MeOH). [α]D25 -17.1 (c 0.3, MeOH). 1H NMR (D2O, 400 MHz) δ 1.23 [1.18], (d, J = 4.2 Hz, 3H), 2.06 (s, 3H), 2.08 (dd, J = 9.7, 4.3 Hz, 1H), 2.31(dd, J = 13.8, 7.6 Hz, 1H), 2.75 (s, 3H), 3.86 (dd, J = 11.6, 3.6 Hz, 1H), 3.95 (d, J = 11.6 Hz, 1H), 4.10 (app. p, J = 6.3 Hz, 1H), 4.48 (dd, J = 9.6, 7.8 Hz, 1H), 4.57-4.60 (m, 2H); 13C NMR (D2O, 100 MHz) δ 18.5, 21.5, 25.8, 37.0, 56.0, 57.2, 59.5, 67.1, 69.7, 171.0, 173.8, 174.2; HRMS (+ESI) calcd for C12H22N3O5 (M+H)+ : 288.1554; obsd: 288.1553.
Fmoc-Thr(O'Bu)-Hyp(O'Bu)-NHMe (2c)

CVK-1-145 in CD3OD at 400 MHz
Fmoc-Thr(O'Bu)-Hyp(O'Bu)-NHMe (2c)
CVK-1-145 in CD3OD at 100 MHz
Ac-Thr(O'Bu)-Hyp(O'Bu)-NHMe (2d)

CVK-1-139G in CDCl3 at 400 MHz
Ac-Thr(O'Bu)-Hyp(O'Bu)-NHMe (2d)

CVK-1-139G in CDCl₃ at 100 MHz
Ac-Thr-Hyp-NHMe (2)- unreferenced-residual H$_2$O $\delta$ 4.80 ppm

CVK-1-147 in D$_2$O at 400 MHz
Ac-Thr-Hyp-NHMe (2) - unreferenced

CVK-1-147 in D2O at 100 MHz
1.4 Synthesis of the Ac-Thr-hyp-NHMe Dipeptide (3)

**Fmoc-hyp(O\textsuperscript{t}Bu)-O\textsuperscript{t}Bu (3b).** A solution of Fmoc-Cl (1.036 g, 4.00 mmol, 1.05 equiv.) in 1,4-dioxane (1 mL) was added dropwise to a solution of *cis*-4-hydroxyproline (3a) (500 mg, 3.81 mmol, 1.00 equiv.) in 1,4-dioxane (5 mL) and 10% aqueous sodium carbonate (10 mL) at 0 °C. The mixture was gradually warmed to rt, stirred overnight, poured on to ice cold water (100 mL) and extracted with diethyl ether (2 x 60 mL). The aqueous layer was acidified by the addition of conc. HCl (pH 2) and extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried over MgSO_4_, filtered and concentrated to give Fmoc-*cis*-4-hydroxyproline as a colorless foam (1.425 g, 100%).

Isobutylene (approximately 30 mL) was condensed into a two-necked flask at -78 °C. In a separate flask a solution of *p*-toluenesulfonic acid hydrate (3.222 g, 17 mmol, 4.20 equiv.) in CH2Cl2 (20 mL) was added to Fmoc-protected *cis*-4-hydroxyproline (1.425 g, 4.03 mmol, 1.00 equiv.) in CH2Cl2 (20 mL). This mixture was added to the isobutylene over 15 min, maintaining the temperature around -78 °C. The mixture was gradually warmed to rt, stoppered, and stirred for 3 d. The reaction mixture was diluted with CH2Cl2 (10 mL), washed with sat’d aqueous NaHCO3 solution (2 x 30 mL). The combined aqueous layers were back-extracted with CH2Cl2 (40 mL). The organic layers
were combined, dried over MgSO₄, filtered and concentrated. The residue was applied to a flash column and eluted with 1:1 EtOAc-hexanes to give 3b (1.249 g, 76%). $R_f$ 0.74 (1:1 Hexane-EtOAc). $^1$H NMR (CDCl₃, 400 MHz) $\delta$ 1.18 [1.20], 1.44 [1.46], 1.92-2.07 (m, 1H), 2.36-2.49 (m, 1H), 3.36 (p, $J = 5.4$ Hz, 1H), 3.75 (dd, $J = 10.4$, 6.6 Hz, 0.5 H) 3.83 (dd, $J = 10.8$, 6.4 Hz, 0.5 H), 4.10-4.21 (m, 2H), 4.23-4.32 (m, 2H), 4.44-4.49 (m, 1H), 7.30 (t, $J = 7.4$ Hz, 2H), 7.38 (t, $J = 7.3$ Hz, 2H), 7.59 (t, $J = 7.0$ Hz, 1H), 7.64 (t, $J = 7.4$ Hz, 1H), 7.74 (d, $J = 7.4$ Hz, 1H); $^{13}$C NMR (CDCl₃, 100 MHz) $\delta$ 28.1, 28.4, 38.8 [37.8], 47.4 [47.3], 53.8 [53.2], 58.2 [58.4], 67.8 [67.5], 68.6 [69.4], 74.2, 81.5 [81.3], 120.0, 125.2, 125.6, 125.4, 127.1, 127.2, 127.7, 127.8, 141.4, 141.3, 143.7, 143.9, 144.4, 144.5, 154.6 [154.8], 171.2 [170.8].

**Fmoc-hyp(O'Bu)-OH (3c).** Trifluoroacetic acid (100 µL) was added to a solution of 3b (98 mg, 0.21 mmol) in CH₂Cl₂ (4 mL) at 0 °C. The reaction mixture was gradually warmed to rt and stirred overnight, neutralized with NaHCO₃ (500 mg), and stirred for another 15 min. The solution was filtered through a plug of cotton. The filtrate was evaporated, the residue was applied to a flash column and eluted with 9:1 EtOAc-hexanes to give 3c as a colorless foam (38 mg, 44 %). $R_f$ 0.20 (19:1 CH₂Cl₂-CH₃OH). $^1$H NMR (CDCl₃, 400 MHz) $\delta$ 1.19 (s, 9H), 2.11-2.17 (m, 1H), 2.33-2.44 (m, 1H), 3.39 (dd, $J = 10.6$, 3.8 Hz, 0.5H) 3.48 (d, $J = 11.8$ Hz, 0.5 H), 3.63-3.68 (m, 1H), 4.16-4.52 (m, 5H), 7.26-7.29 (m, 2H), 7.30-7.40 (m, 2H), 7.54-7.61 (m, 2H), 7.70 (d, $J = 7.4$ Hz, 1H), 7.75 (dd, $J = 7.4$ Hz, 2H); $^{13}$C NMR (CDCl₃, 100 MHz) $\delta$ 28.2 [28.3], 37.4 [38.4], 47.3, 53.9 [54.5], 58.2, 67.9 [68.2], 69.7 [69.1], 75.0, 75.5, 120.1, 125.3, 125.4, 127.2, 127.3, 127.9, 141.4, 141.5, 143.9, 144.2, 144.3, 154.9 [155.6], 175.5. HRMS (+ESI) calcd for C₂₄H₂₇NNaO₅ (M+Na)^+: 432.1787; obsd: 432.1784.
**Fmoc-hyp(O'Bu)-NHMe (3d).** N-Hydroxysuccinimide (18 mg, 0.16 mmol, 1 equiv.) and DCC (33 mg, 0.16 mmol, 1 equiv.) were added sequentially to a solution of 3c (65 mg, 0.16 mmol, 1 equiv.) in CH₂Cl₂ (2 mL) at 0 °C. The solution was stirred for 30 min at 0 °C, gradually warmed to rt and stirred overnight. The suspension was filtered through a plug of cotton in a Pasteur pipet. The filtrate was concentrated to 2 mL and refrigerated for 6 h. The suspension was again filtered and the filtrate was concentrated. The residue was dissolved in DMF (2 mL) and cooled to 0 °C. Methylamine hydrochloride (11 mg, 0.16 mmol, 1 equiv.) was added as a solid in one portion, followed by the addition of diisopropylethylamine (28 µL, 21 mg, 0.16 mmol, 1 equiv.). The solution was gradually warmed to rt and stirred overnight under N₂. The mixture was diluted with EtOAc (25 mL) and washed with brine (25 mL). The aqueous layer was back-extracted with EtOAc (25 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated. The product was isolated by flash chromatography, eluting with 2:1 EtOAc-hexanes to give 3d (52 mg, 77%). 

Rₙ 0.35 (4:1 EtOAc-hexanes).  

**1H NMR (CD₃OD, 400 MHz)** δ 1.14 (s, 9H), 1.82-1.98 (m, 1H), 2.21-2.36 (m, 1H), 2.68 (s, 3H), 3.14 (dd, J = 10.7, 3.4 Hz, 0.5H) [3.30-3.32 (m, 0.5H)], 3.44 (dd, J = 10.5, 4.7 Hz, 0.5H) [3.64 (dd, J = 10.8, 5.2 Hz, 0.5H)], 4.08-4.23 (m, 3H), 4.32 (d, J = 6.4 Hz, 2H), 4.40-4.49 (m, 1H), 7.27-7.37 (m, 4H), 7.53-7.63 (m, 2H), 7.77 (t, J = 6.0 Hz, 2H);  

**13C NMR (CD₃OD, 400 MHz)** δ 26.7 [26.5], 28.6, 34.9 [37.1], 40.3 [39.2], 56.0 [55.4], 61.2, 69.2 [68.5], 70.2 [70.8], 75.1, 121.1, 126.2, 128.3, 129.0, 142.7, 145.3, 145.5, 157.0, [157.1], 175.7 [175.4].

**Dipeptide 3e.** Diethylamine (800 µL) was added to a solution of Fmoc-cis-4-Hyp-(O'Bu)-NHMe 3d (52 mg, 0.12 mmol, 1.0 equiv.) in acetonitrile (3 mL). The solution
was stirred at 0 °C under N₂ for 30 min, concentrated, and then concentrated twice more from acetonitrile. The residue was suspended in dichloromethane (2 mL) and cooled to 0 °C. Fmoc-Thr(O'Bu)OH (2b) (73 mg, 0.18 mmol, 1.5 equiv.) was added, followed by the addition of diisopropylethylamine (54 µL, 400 mg, 0.31 mmol, 2.5 equiv.) and PyBroP (86 mg, 0.18 mmol, 1.5 equiv.). The solution was gradually warmed to rt and stirred overnight under N₂. The mixture was concentrated and the residue applied to a flash column eluting with 4:1 EtOAc-hexanes to give 3e (80 mg, 100%). Rᶠ 0.38 (4:1 EtOAc-hexanes). ¹H NMR (CDCl₃, 400 MHz) δ 1.14 (d, J = 7.0, 3H), 1.14 (s, 9H), 1.32 (s, 3H), 2.17-2.28 (m, 2H), 2.73 (d, J = 4.2 Hz, 3H), 3.77-3.86 (m, 2H), 4.09-4.14 (m, 2H), 4.21 (d, J = 6.8 Hz, 1H), 4.38 (d, J = 6.6 Hz, 2H), 4.59 (dd, J = 7.5, 5.2 Hz, 1H), 4.76 (dd, J = 8.4, 3.2 Hz, 1H), 5.81 [5.73] (d, J = 7.8 Hz, 1H), 7.03 (q, J = 4.4 Hz, 1H), 7.30 (t, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4, 2H), 7.58 (dd, J = 7.2, 4.4 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ 17.1 [14.3], 26.0 [25.9], 28.2, 28.3, 37.8, 47.3, 55.7, 56.5 [60.6], 59.2, 67.2, 68.7, 70.1, 74.3, 75.7, 120.2, 120.2, 125.2, 125.3, 127.2, 127.9, 141.4, 143.8, 144.0, 155.8, 169.0, 172.3. HRMS (+ESI) calcd for C₃₃H₄₅N₃NaO₆S (M + Na)⁺: 602.3206; obsd: 602.3242.

**Dipeptide 3f.** Diethylamine (2 mL) was added to a solution of 3d (119 mg, 0.21 mmol, 1 equiv.) in acetonitrile (2 mL). The solution was stirred at 0 °C under N₂ for 30 min, concentrated, and then concentrated twice more from acetonitrile. The residue was dissolved in a mixture of pyridine (1 mL) and acetic anhydride (1 mL) at 0 °C, warmed to rt and stirred under N₂ overnight. The red solution was concentrated and purified by flash chromatography, eluting with 4:1 EtOAc-hexanes to remove Fmoc byproducts and then with 9:1 CH₂Cl₂-MeOH to isolate 3f as a colorless foam (27 mg, 93%). ¹H NMR
(CDCl$_3$, 400 MHz) $\delta$ 1.10 (d, $J = 6.4$ Hz, 3H), 1.15 (s, 9H), 1.31 (s, 9H), 2.0 (s, 3H), 2.22-2.25 (m, 2H), 2.73 [2.79] (d, $J = 4.8$ Hz, 3H), 3.79 (dd, $J = 10.7$, 5.3 Hz, 1H), 3.86 (dd, $J = 10.7$, 2.6 Hz, 1H), 4.12 (dt, $J = 11.6$, 6.4 Hz, 1H), 4.21-4.25 (m, 1H), 4.74 (dd, $J = 8.2$, 4.1 Hz, 1H), 4.84 (dd, $J = 7.7$, 5.1, 1H), 6.61 (d, $J = 7.5$ Hz, 1H), 7.02, (d, $J = 8.0$ Hz, 1H); $^{13}$C NMR (CDCl$_3$, 400 MHz) $\delta$ 17.3, 23.3, 26.1, 28.2, 28.3, 37.9, 54.4, 56.6, 59.3, 68.2, 70.1, 74.4, 75.8, 169.4, 170.0, 172.3. HRMS (+ESI) calcd for C$_{20}$H$_{37}$N$_3$NaO$_5$S (M + Na)$^+$: 422.2631; obsd: 422.2709.

**Dipeptide 3.** Trifluoroacetic acid (1.0 mL) was added to a solution of Ac-Thr(tBu)-trans-4-Hyp(OtBu)-NHMe (3f) (17 mg, 0.04 mmol) in CH$_2$Cl$_2$ (1.0 mL) at 0 °C. The mixture was gradually warmed to rt and stirred overnight under N$_2$. The solution was concentrated and then concentrated three times from CH$_2$Cl$_2$. The brown residue was subjected to the RP-HPLC (gradient: 0-100% acetonitrile in H$_2$O over 60 min, C$_{18}$ 10.0 mm column 3 mL/min) ($R_T = 4$ min) to isolate 3 as a colorless oil (6 mg, 50%). $\text{Rf} 0$. [$\alpha$]$_D^{25}$ +71.7 (c 1.0, MeOH). $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 1.15 [1.23] (d, $J = 6.4$ Hz, 3H), 1.99 (dt, $J = 4.5$, 3.6 Hz, 1H), 2.04 [2.03] (s, 3H), 2.44-2.51 (m, 1H), 2.73 [2.71] (s, 3H), 3.69 (dd, $J = 11.0$, 3.5 Hz, 0.7H) [3.75 (dd, $J = 13.4$, 5.0 Hz, 0.3H), 4.03 (dd, $J = 11.1$, 5.2 Hz, 0.7H) [4.07 (dd, $J = 5.8$, 5.3 Hz, 0.3H), 4.15 (p, $J = 6.3$ Hz, 1H), 4.46 (dd, $J = 9.5$, 4.6 Hz, 1H), 4.49-4.53 (m, 1H), 4.56 (d, $J = 5.9$ Hz, 1H), 4.84-4.89 (m, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 18.4 [18.1], 21.5 [21.6], 25.8 [26.1], 36.2 [38.9], 55.1 [55.8], 56.9, 59.6 [59.9], 66.9 [67.4], 69.5 [67.9], 171.2, 174.0, 174.2. HRMS (+ESI) calcd for C$_{12}$H$_{21}$N$_3$O$_5$ (M)$^+$: 287.1481; obsd: 287.1352.
Fmoc-hyp(O'Bu)-O'Bu (3b)

CVK-2-38a in CDCl3 at 400 MHz
Fmoc-hyp(O'Bu)-O'Bu (3b)

CVK-2-38a in CDCl₃ at 100MHz
CVK-2-45b in CDCl3 at 400 MHz

Fmoc-hyp(OBu)-OH (3c)
Fmoc-hyp(O'Bu)-OH (3c)

CVK-2-45α in CDCl₃ at 100 MHz
Fmoc-hyp(O'Bu)-NHMe (3d)

CVK-2-64 in CD3OD at 400 MHz
Fmoc-hyp(O^Bu)-NHMe (3d)

CVK-2-64 in CD3OD at 100 MHz
Fmoc-Thr(O'Bu)-hyp(O'Bu)-NHMe (3e)

CVK-2-65 in CDCl3 at 400 MHz
Fmoc-Thr(O'Bu)-hyp(O'Bu)-NHMe (3e)

cvk-2-65 in CDCl₃ at 100 MHz
Ac-Thr(O'Bu)-hyp(O'Bu)-NHMe (3f)

CVK-2-72b in CDCl₃ at 400 MHz
Ac-Thr(O'Bu)-hyp(O'Bu)-NHMe (3f)

CVK-2-72b in CDCl3 at 100 MHz
Ac-Thr-hyp-NHMe (3) - unreferenced-residual H$_2$O $\delta$ 4.80 ppm

CVK-2-75 in D2O at 400 MHz

[Chemical structure image]

[Graph of NMR spectrum with peaks at δ values 2.47, 1.67, 0.93, 2.50, 3.14, and 3.00 ppm]
Ac-Thr-hyp-NHMe (3) - unreferenced

CVK-2-75 in D2O at 100 MHz
1.5 Synthesis of the GlcNAc Thioglycoside Donor

**Compound 6a.** We could not reproduce the much cited procedure of Deferrari *et al.* In our hands the major product was 2,3,4,6-tetra-O-acetylmannose. This was rectified by a lower reaction temperature (20-25 °C, *c.f.* 35-40 °C during the addition of the sodium acetate solution.

Acetic anhydride (100 mL) was placed in a 2-neck, 500 mL round bottomed flask fitted with a thermometer. Mannose (about 100 mg) was added, followed by perchloric acid (70%, 7-8 drops from a Pasteur pipet). Mannose (total of 26.4 g, 147 mmol, 1.0 equiv) was then added in small portions of a period of 2 h, keeping the reaction temperature in the range 40-45 °C. Once the addition was complete, the orange-brown solution was stirred for 1 h at rt. This leads to formation of the pentaacetate (R$_f$ 0.49,).

The reaction mixture was cooled to 10 °C (ice bath) and phosphorus tribromide (20.9 mL, 59.5 g, 220 mmol, 1.5 equiv.) was added dropwise. Water (11.9 mL, 659 mmol, 4.5 equiv.) was added dropwise. This led to the formation of HBr and was quite exothermic; the reaction was cooled (ice bath) to maintain an internal temperature of 20-25 °C. Once the addition was complete, the mixture was stirred at rt for 1.5 h. This leads to formation of the anomeric bromide (R$_f$ 0.49, 1:1 EtOAc-hexanes).
The reaction mixture was cooled to 5 °C (ice bath) and a solution of sodium acetate trihydrate (79.8 g, 586 mmol, 4.0 equiv.) in water (100 mL) also at 5 °C was added dropwise. The reaction is very exothermic initially, but slows after about one quarter of the sodium acetate has been added. With cooling (ice bath) the temperature was kept at 20-25 °C and the addition completed in 30 min. The mixture was then stirred at rt for 20 min.

The mixture was poured onto ice and extracted with chloroform (3 x 120 mL). The extracts were combined and washed with ice-water (300 mL), sat’d aq. NaHCO₃ containing ice (300 mL) and ice-water (300 mL) again. The yellow-orange organic layer was dried over MgSO₄, filtered and concentrated. Diethyl ether (200 mL) was added and within a few minutes the product crystallized out of solution. The flask was left in the freezer overnight and the product collected by filtration and washed a little with cold ether, to give 1,3,4,6-tetra-O-acetylmannose as a colorless crystalline solid (9.9 g, 19% yield). \( R_f \) 0.4 (4:1 EtOAc-hexanes). \( [\alpha]_D^{25} \) – 24.3 (c 1.0, MeOH). \( ^1\)H NMR (CDCl₃, 400 MHz) \( \delta \) 2.05 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 2.18 (s, 3H), 2.47 (d, \( J = 3.9 \) Hz, 1H), 3.79 (ddd, \( J = 9.8, 4.8, 2.3 \) Hz, 1H), 4.13 (dd, \( J = 12.4, 2.2 \) Hz, 1H), 4.20 (app. t, \( J = 3.1 \) Hz, 1H), 4.30 (dd, \( J = 12.5, 5.0 \) Hz, 1H), 5.04 (dd, \( J = 9.8, 3.0 \) Hz, 1H), 5.39 (t, \( J = 9.8 \) Hz, 1H), 5.79 (d, \( J = 0.4 \) Hz, 1H); \( ^{13}\)C NMR (CDCl₃, 100 MHz) \( \delta \) 20.8, 20.9, 21.0, 21.1, 62.2, 65.4, 68.6, 73.0, 73.3, 91.9, 168.8, 169.8, 170.3, 171.0.

**Triflate 6b.** Triflic anhydride (625 µL, 1.048 g, 3.72 mmol, 2.0 equiv.) was added dropwise to a solution of 1,3,4,6-tetra-O-acetylmannose (6a) (647 mg, 1.86 mmol, 1.0 equiv.) and pyridine (376 µL, 4.65 mmol, 2.5 equiv.) in CH₂Cl₂ (10 mL) at -25 °C. Once the addition was complete, the mixture was stirred at -25 °C for 45 min, diluted with
CH₂Cl₂ (200 mL) and washed with water (400 mL), saturated aqueous NaHCO₃ (400 mL) and water (400 mL) again. The organic layer was dried over MgSO₄, filtered and concentrated to give the triflate 6b (834 mg, 93% yield) as a yellow foam. *R* 0.56 (1:1 Hexane:EtOAc). [α]D²⁵ – 14.3 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz) δ 2.07 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.17 (s, 3H), 3.86 (dd, J = 7.5, 5.2, 2.4 Hz, 1H), 4.17 (dd, J = 12.5, 2.2 Hz, 1H), 4.25 (dd, J = 12.5, 5.2 Hz, 1H), 5.16 (d, J = 2.8 Hz, 1H), 5.22 (dd, J = 10.1, 2.8 Hz, 1H), 5.30 (app. t, J = 9.9 Hz, 1H), 5.95 (s, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 20.6 (2C), 20.7, 20.8, 61.8, 64.7, 69.8, 73.6, 81.6, 89.3, 118.6 (q, JCF = 319.5 Hz), 168.2, 169.4, 170.1, 170.8. HRMS (+ESI) calcd for C₁₅H₁₉F₃NaO₁₂S (M+Na)⁺: 503.0442; obsd: 503.0444.

**Azide 6c.** Sodium azide (226 mg, 3.5 mmol, 2.0 equiv.) was added to a solution of triflate 6b (834 mg, 1.74 mmol, 1.0 equiv.) in DMF (6 mL). The solution warmed to 40 °C and stirred for 2 h under nitrogen. The mixture was cooled to rt, diluted with CH₂Cl₂ (200 mL), washed with water (300 mL) and brine (300 mL). The organic layer was dried over MgSO₄, filtered and concentrated. The residue was applied to a flash column, eluting with 1:1 EtOAc-hexanes to give the azide 6c (582 mg, 90%). *R* 0.64 (1:1 EtOAc-hexanes). [α]D²⁵ +9.2 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz) δ 2.03 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.20 (s, 3H), 3.67 (app. t, J = 9.2 Hz, 1H), 3.83 (ddd, J = 9.6, 4.2, 1.9 Hz, 1H), 4.08 (dd, J = 12.6, 1.6 Hz, 1H), 4.31 (dd, J = 12.5, 4.4 Hz, 1H), 5.05 (app. t, J = 9.6 Hz, 1H), 5.11 (app. t, J = 9.6 Hz, 1H), 5.58 (d, J = 8.6 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 20.6, 20.7, 20.8, 21.0, 61.5, 62.7, 67.9, 72.7, 72.8, 92.7, 168.6, 162.7, 169.9, 170.6. HRMS (+ESI) calcd for C₁₄H₁₉N₃NaO₉ (M+Na)⁺: 396.1019; obsd: 396.0994.
**Thioglycosides 6α and 6β.** BF$_3$.OEt$_2$ (1.44 mL, 1.654 g, 12 mmol, 15 equiv.) was added to a solution of tetraacetate 6c (290 mg, 0.777 mmol, 1.0 equiv.) and thiophenol (198 µL, 214 mg, 1.94 mmol, 2.5 equiv.) in CH$_2$Cl$_2$ (6 mL) at rt. The reaction mixture was heated at reflux for 2 h under nitrogen, cooled to rt and stirred overnight. The reaction was quenched by the dropwise addition of water (2 mL). The mixture was diluted with CH$_2$Cl$_2$ (75 mL) and washed with water (75 mL), then brine (75 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated. The residue was applied to a flash column eluting with 2:1 hexanes-EtOAc to give the thioglycoside 6 (262 mg, 80%) as a 3:1 mixture of anomers (α:β) which cannot be distinguished by TLC. $R_f$ 0.70 (1:1 EtOAc-hexanes). For clarity, data are reported separately for two isomers.

Data for α-anomer (major): $^1$H NMR (CDCl$_3$, 400 MHz) δ 2.02 (s, 3H), 2.05 (s, H), 2.10 (s, 3H), 4.03 (dd, $J = 12.4$, 2.0 Hz, 1H), 4.09 (dd, $J = 10.3$, 5.5 Hz, 1H), 4.24 (dd, $J = 12.4$, 5.2 Hz, 1H) 4.60 (ddd, $J = 10.0$, 5.2, 2.0 Hz, 1H), 5.34 (dd, $J = 10.2$, 9.5 Hz, 1H), 5.34 (dd, $J = 10.2$, 9.5 Hz, 1H), 5.65 (d, $J = 5.5$ Hz, 1H), 7.30-7.34 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 20.6, 20.7, 20.8, 61.6, 62.0, 68.6, 68.8, 72.1, 86.5, 128.1, 129.3, 132.3, 134.2, 169.7, 169.8, 170.5.

Data for β-anomer (minor): $^1$H NMR (CDCl$_3$, 400 MHz) δ 2.01 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 3.41 (app.t, $J = 9.9$ Hz, 1H), 3.71 (ddd, $J = 10.1$, 4.8, 2.4 Hz, 1H), 4.09-4.78 (m, 1H), 4.17 (dd, $J = 12.3$, 2.3 Hz, 1H), 4.24 (dd, $J = 12.3$, 4.8 Hz, 1H), 4.51 (d, $J = 10.2$ Hz, 1H), 7.30-7.34 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 20.6, 20.7, 20.8, 62.1, 62.7, 68.1, 74.5, 75.6, 85.8, 129.0, 129.2, 130.3, 132.5, 169.7, 169.8, 170.5. HRMS (+ESI) calcd for C$_{18}$H$_{21}$N$_3$NaO$_7$S (M+Na)$^+$: 446.0998; obsd: 446.0568.
1,3,4,6-tetra-O-acetyl-α-D-mannose (6a)

cvk-2-101 in CDCl3 at 400 MHz
1,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannose (6a)

cvk-2-101 in CDCl3 at 100 MHz
Triflate 6b

cvk-2-106 in CDCl3 at 100 MHz
Azide 6c

cvk-2-103 in CDCl3 at 100 MHz
Azide 6c

cvk-2-103 in CDCl3 at 100 MHz
Thioglycosides $6\alpha$ and $6\beta$

cvk-2-112 in CDCl$_3$ at 400 MHz
Thioglycosides $6\alpha$ and $6\beta$

cvk–2–112 in CDCl3 at 100 MHz
1.6 Synthesis of the Ac-Thr-[(α,1-4)GlcNAc|Hyp-NHMe Dipeptide

Fmoc-4-O-(2-azido-3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-Hyp-OBn (4b). A solution of thioglycoside 6 (68 mg, 0.16 mmol, 1.0 equiv.) and Fmoc-Hyp-OBn (4a) (78 mg, 0.18 mmol, 1.1 equiv.) in CH₂Cl₂ (6 mL) was stirred with activated, powdered 4Å molecular sieves for 25 min at rt under nitrogen. The mixture was cooled to −78 °C, NIS (54 mg, 0.24 mmol, 1.5 equiv.) and silver triflate (21 mg, 0.09 mmol, 0.5 equiv.) were added. The mixture was allowed to reach 0 °C over 3 h, quenched with triethylamine (1 mL), diluted with EtOAc (75 mL), washed with 10% aq. Na₂S₂O₃ (75 mL), and brine (75 mL). The organic layer was dried over MgSO₄, filtered, concentrated and submitted directly to flash chromatography, eluting with 1:1 EtOAc-hexanes to give the α-O-glycoside 4b (71 mg, 59% yield). \( R_f 0.36 \) (1:1 EtOAc-hexanes). \( [\alpha]_D^{25} +49.7 \) (c 1.0, MeOH). \(^1\)H NMR (CDCl₃, 400 MHz) \( \delta 2.01 \) (s, 3H), 2.05 (s, 2H) [2.06 (s, 1H)], 2.07 (s, 1.8 H) [2.08 (s, 1.2H)], 2.15-2.25 (m, 1H), 2.50-2.55 (m, 1H), 3.22 (dd, \( J = 10.7, 3.6, 0.5H \)), 3.27 (dd, \( J = 10.7, 3.6 \) Hz, 0.5H), 3.68-3.91 (m, 2H), 3.96-4.0 (m, 0.5H), 4.01-4.10
Fmoc-[(α,1-4)GlcNAc(OAc)₄]-Hyp-OBn (4c). Zinc powder (50 mg) and saturated aqueous CuSO₄ (20 µL) were added to a solution of 4b (135 mg, 0.18 mmol) in THF (2 mL) at rt. Acetic acid (0.5 mL) and acetic anhydride (0.5 mL) were added and the mixture stirred overnight at rt. The reaction mixture was filtered and the filtrate concentrated and applied directly to a flash column, eluting with 4:1 EtOAc-hexanes to give the N-acetylated product 4c (114 mg, 83% yield). Rf 0.45 (4:1 EtOAc-hexanes). [α]D²⁵ +17.72 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz) δ 1.87 (s, 1.7H) [1.88 (s, 1.3 H), 2.02 (s, 3H), 2.04 (s, 3H), 2.05 (3H), 2.18-2.24 (m, 1H), 2.48-2.54 (m, 1H), 3.51-3.77 (m, 2H), 3.95 (ddd, J = 9.7, 5.0, 2.2 Hz, 1H), 4.02 (t, J = 6.7 Hz, 0.3 H), 4.10 (d, J = 12.2 Hz, 1H), 4.20-4.46 (m, 5.7H), 4.51-4.6 (m, 1H), 4.91 (d, J = 13.0, 3.1 Hz, 0.6H) [4.95 (dd, J = 13.0, 3.1 Hz, 0.4H)], 4.95-5.24 (m, 5H), 5.61-5.73 (m, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 20.8, 20.9, 20.9, 23.3, 37.5 [36.3], 47.3, 51.7 [52.4], 51.8 [52.1], 58.0 [58.4], 62.3, 67.4, 68.1 [68.2], 68.4, 68.7, 71.0, 76.1 [76.8], 97.3 [97.0], 120.2, 125.1, 125.3, 127.3, 128.0, 128.4, 128.6, 128.7, 128.8, 135.3, 141.5, 143.7, 144.1, 144.3, 154.8, 169.5,
Boc-Thr(OBn)-[(\(\alpha,1-4\))GlcNAc(OAc)\(_4\)]-Hyp-OBn (4d). Diethylamine (0.5 mL) was added to a solution of glycoside 4c (114 mg, 0.15 mmol, 1.0 equiv.) in dry CH\(_3\)CN (3 mL) at 0 °C. The mixture was stirred 1 h at 0 °C then concentrated. The residue was dissolved in dry CH\(_2\)Cl\(_2\) (3 mL), cooled to 0 °C. Boc-Thr(OBn)-OH (1a) (68 mg, 0.22 mmol, 1.5 equiv.), diisopropylethylamine (65 µL, 48 mg, 0.38 mmol, 2.5 equiv.) and PyBroP (103 mg, 0.22 mmol, 1.5 equiv.) were added sequentially and the mixture stirred overnight under nitrogen. The mixture was concentrated and the residue purified by flash chromatography, eluting with 4:1 EtOAc-hexanes and then with 9:1 EtOAc-hexanes to give 4d as a colorless foam (86 mg, 68% yield). \(R_f\) 0.51 (9:1 EtOAc-hexanes). 

\([\alpha]_D^{25}\) +12.4 (c 0.9, MeOH). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 1.21 (d, \(J = 6.2\) Hz, 3H), 1.4 (s, 9H), 1.95 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.08-2.14 (m, 1H), 2.42-2.45 (m, 1H), 3.80-3.82 (m, 2H), 3.88 (d, \(J = 11.2\) Hz, 1H), 3.39-3.97 (m, 1H), 4.07 (d, \(J = 12.2\) Hz, 1H), 4.11-4.20 (m, 1H), 4.32-4.35 (m, 2H), 4.42 (dd, \(J = 7.7, 5.1\) Hz, 1H), 4.50 (d, \(J = 11.5\) Hz, 1H), 4.56 (d, \(J = 11.5\) Hz, 1H), 4.73 (t, \(J = 8.1\) Hz, 1H), 4.90 (d, \(J = 3.4\) Hz, 1H), 5.06 (app. t, \(J = 8.8\) Hz, 1H), 5.15 (d, \(J = 11.5\) Hz, 1H), 5.17 (s, 2H), 5.42 (d, \(J = 7.8\) Hz, 0.6 H), 5.48-5.52 (m, 0.4H), 6.15 (d, \(J = 9.0\) Hz, 1H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 16.5, 20.8, 20.9, 21.0, 23.2, 28.6, 35.8, 51.7, 53.4, 56.6, 58.3, 62.4, 67.4, 68.6, 68.7, 71.0, 71.5, 75.6, 78.1, 80.3, 98.2, 128.0, 128.1, 128.4, 128.5, 128.6, 128.7, 128.9, 135.6, 138.4, 156.1, 169.6, 169.9, 170.7, 170.8, 171.2, 171.6. HRMS (+ESI) calcd for C\(_{42}\)H\(_{56}\)N\(_3\)O\(_{15}\) (M+H): 842.3706; obsd: 842.3698.
Ac-Thr(Obn)-[(α,1-4)GlcNac(OAc)₄]-Hyp-OBn (4e). Trifluoroacetic acid (0.5 mL) was added to a solution of 4d (42 mg, 0.05 mmol) in dry CH₂Cl₂ (2 mL) at 0 °C. The reaction mixture was gradually warmed to rt and stirred for 2 h. The mixture was concentrated, the residue dissolved in pyridine (1.5 mL) and cooled to 0 °C. Acetic anhydride (1 mL) was added. The mixture was stirred overnight under N₂. The reaction mixture was diluted with EtOAc (20 mL), washed with 1M HCl (20 mL) and brine (20 mL). The organic layer was dried over MgSO₄, filtered, concentrated and the residue purified by flash chromatography eluting with 9:1 CH₂Cl₂-MeOH to give 4e (28 mg, 72%). Rₚ 0.74 (4:1 CH₂Cl₂-MeOH). ¹H NMR (CD₃OD, 400 MHz) δ 1.16 (d, J = 3.8 Hz, 3H), 1.88 (s, 3H), 1.89 (s, 3H) [1.82 (s, 3H)], 1.90 (s, 3H), 1.93 (s, 3H) [1.94 (s, 3H), 1.97 (s, 3H)], 2.03-2.10 (m, 1H), 2.53 (dd, J = 13.5, 7.9 Hz, 1H), 3.74 (dd, J = 11.3, 3.8 Hz, 1H), 3.79 (app. t, J = 6.4 Hz, 1H), 3.95 (dd, J = 6.0, 2.1 Hz, 0.5 H), 3.98 (dd, J = 5.6, 2.1 Hz, 0.5 H), 4.01-4.06 (m, 1H), 4.13 (dd, J = 12.3, 5.7 Hz, 1H), 4.19-4.25 (m, 1H), 4.31 (d, J = 11.3 Hz, 1H), 4.40 (s, 1H), 4.47 (d, J = 11.3 Hz, 1H), 4.55 (d, J = 11.3 Hz, 1H), 4.61 (d, J = 6.7 Hz, 1H), 4.63 (app. t, J = 8.8 Hz, 1H), 4.87 (app. t, J = 9.5 Hz, 1H), 4.92 (d, J = 4.6 Hz, 1H), 5.05 (d, J = 5.3 Hz, 1H), 5.10 (s, 2H), 7.20-7.31 (m, 10H), 8.02 (d, J = 9.6 Hz, 1H), 8.17 (d, J = 7.3 Hz, 1H); ¹³C NMR (CD₃OD, 100 MHz) δ 16.9, 20.7, 20.8, 22.4, 22.7, 36.9, 52.9, 53.4, 57.9, 59.6, 63.9, 68.2, 69.8, 70.5, 72.3, 72.4, 76.6, 79.6, 78.5, 99.1, 128.8, 129.1, 129.5, 129.6, 129.8, 137.3, 140.0, 171.5, 171.7, 171.9, 172.5, 172.9, 173.5, 173.9. HRMS (+ESI) calcd for C₃₉H₄₉N₃NaO₁₄ (M+Na)+: 806.3112; obsd: 806.3123.

Ac-Thr-Hyp-[(α,1-4)GlcNac(OAc)₄]-OH (4f). Palladium on carbon (10%, 15 mg) was added in a single portion to a solution of 4e (27 mg, 0.03 mmol) in MeOH (2.5 mL). The
reaction flask was evacuated, then opened to an atmosphere of H$_2$ and stirred overnight.
The catalyst was removed by filtering through a plug of Celite® in a Pasteur pipet. The filtrate was concentrated, to give acid 4f (21 mg, 99%). $R_f$ 0.24 (4:1 CH$_2$Cl$_2$-MeOH). $[\alpha]_D^{25}$ -7.0 (c 0.5, MeOH). $^1$H NMR (CD$_3$OD, 400 MHz) $\delta$ 1.19 (d, $J = 6.2$ Hz, 3H), 1.88 (s, 3H), 1.89 (s, 3H), 1.90 (s, 3H), 1.93 (s, 3H), 1.99 (s, 3H), 2.03-2.12 (m, 1H), 2.54 (dd, $J = 13.5$, 7.7 Hz, 1H), 3.71 (dd, $J = 11.1$, 3.2 Hz, 1H), 3.89-3.92 (m, 1H), 3.95-3.99 (m, 1H), 4.05 (d, $J = 12.2$ Hz, 1H), 4.15 (dd, $J = 12.2$, 5.6 Hz, 1H), 4.27 (ddd, $J = 13.7$, 10.3, 3.6 Hz, 1H), 4.35-4.38 (m, 1H), 4.39-4.44 (m, 2H), 4.49 (app. t, $J = 8.6$ Hz, 1H), 4.89 (t, $J = 9.7$ Hz, 1H), 4.95 (d, $J = 3.5$ Hz, 1H), 5.08 (t, $J = 10.0$ Hz, 1H), 8.13 (d, $J = 9.5$ Hz, 1H), 8.15 (d, $J = 6.8$ Hz, 1H); $^{13}$C NMR (CD$_3$OD, 100 MHz) $\delta$ 20.1, 20.6, 20.7, 20.8, 22.4, 22.7, 37.2, 53.0, 54.7, 59.4, 63.8, 68.7, 69.8, 70.5, 70.6 72.4, 80.0, 99.4, 171.5, 171.9, 172.3, 172.5, 173.9. HRMS (+ESI) calcd for C$_{35}$H$_{32}$N$_4$O$_6$ (M+Na)$^+$: 604.2322; obsd: 604.2348.

**Ac-Thr-[(\(\alpha\),1-4)GlcNAc(OAc)$_4$]Hyp-NHMe (4g).** $N$-Hydroxysuccinimide (4 mg, 0.04 mmol, 1 equiv.) and DCC (8 mg, 0.04 mmol, 1 equiv.) were added sequentially to a solution of 4f (21 mg, 0.04 mmol, 1 equiv.) in CH$_2$Cl$_2$ (2 mL) at 0 °C. The solution was stirred for 30 min at 0 °C, gradually warmed to rt and stirred overnight. The suspension was filtered through a plug of cotton in a Pasteur pipet. The filtrate was concentrated to 2 mL and refrigerated for 6 h. The suspension was filtered again and the filtrate concentrated. The residue was dissolved in CH$_3$CN (2 mL) and cooled to 0 °C. Methylamine hydrochloride (3 mg, 0.04 mmol, 1 equiv.) was added as a solid in one portion, followed by the addition of diisopropylethylamine (6 µL, 5 mg, 0.04 mmol, 1 equiv.). The solution was gradually warmed to rt and stirred overnight under N$_2$. The
mixture was concentrated and the product was isolated by flash chromatography, eluting with 4:1 CH₂Cl₂-MeOH to give 4g (14 mg, 77%). Rf 0.31 (4:1 EtOAc:hexanes). ¹H NMR (CD₃OD, 400 MHz) δ 1.18 (d, J = 6.3 Hz, 3H), 1.87 (s, 3H), 1.89 (s, 3H), 1.94 (s, 3H), 1.90 (s, 3H), 2.00 (s, 3H), 2.04 (ddd, J = 13.7, 9.3, 4.4 Hz, 1H), 2.40 (dd, J = 13.6, 7.6 Hz, 1H), 2.68 (s, 3H), 3.74 (dd, J = 11.2, 3.8 Hz, 1H), 3.96 (t, J = 6.4 Hz, 1H), 4.01 (ddd, J = 10.1, 5.0, 2.4 Hz, 1H), 4.06 (dd, J = 12.2, 2.4 Hz, 1H), 4.19 (dd, J = 9.7, 4.4 Hz, 1H), 4.22 (dd, J = 7.1, 3.8 Hz, 1H), 4.36 (d, J = 11.4 Hz, 1H), 4.39-4.41 (m, 2H), 4.45 (t, J = 8.4 Hz, 1H), 4.94 (app. t, J = 9.6 Hz, 1H), 4.94 (d, J = 3.5 Hz, 1H), 5.09 (dd, J = 10.8, 9.4 Hz, 1H); ¹³C NMR (MeOD, 100 MHz) δ 20.1, 20.7, 20.8, 22.4, 22.6, 26.4, 37.7, 52.9, 55.2, 59.2, 60.7, 63.7, 68.6, 69.6, 70.6, 72.4, 80.1, 99.7, 171.4, 172.0, 172.4, 172.6, 173.6, 173.9, 174.5. HRMS (+ESI) calcd for C₂₆H₄₀N₄NaO₁₃ (M+Na)⁺: 639.2490; obsd: 639.2463.

Ac-Thr-[(α,1-4)GlcNAc]Hyp-NHMe (4). A solution of NaOMe in MeOH (25% w/v, one drop from a 20G needle) was added to a solution of glycoside 4g (8 mg, 0.013 mmol) in MeOH (2 mL) at 0 °C. The mixture was gradually warmed to rt and stirred for 2 h. Amberlite IR-120 H⁺ resin was added to the reaction mixture and stirred for 15 min. The reaction mixture was filtered through a plug of cotton and concentrated to give the glycoside 4 (6 mg, 94%). Rf 0.30 (4:1 CH₂Cl₂:MeOH). [α]D ²⁵ +73.2 (c 1.0, MeOH). ¹H NMR (D₂O, 400 MHz) δ 1.27 (d, J = 6.3 Hz, 3H), 2.00 (s, 3H), 2.07 (s, 3H), 2.05-2.15 (m, 1H) 2.53 (dd, J = 13.5, 7.5 Hz, 1H), 2.76 (s, 3H), 3.49 (app. t, J = 9.5 Hz, 1H), 3.68-3.74 (m, 2H), 3.79 (dd, J = 12.3, 5.6 Hz, 1H), 3.84 (dd, J = 12.1, 3.2 Hz, 1H), 3.90 (dd, J = 12.3, 1.6 Hz, 1H), 3.95 (dd, J = 10.6, 3.6 Hz, 1H), 4.10 (app. p, J = 6.3 Hz, 1H), 4.17 (d, J = 12.1 Hz, 1H), 4.51 (dd, J = 9.7, 1.6 Hz, 1H), 4.57 (br. s, 1H), 4.61 (d, J = 6.3 Hz,
1H), 5.01 (d, J = 3.6 Hz, 1H); $^{13}$C NMR (D$_2$O, 100 MHz) δ 18.5, 21.6, 21.8, 25.8, 35.7, 53.0, 53.4, 57.2, 59.8, 60.6, 67.3, 69.9, 70.8, 72.5, 75.7, 95.8, 171.0, 173.7, 173.9, 174.1.

HRMS (ESI+) calcd for C$_{20}$H$_{34}$N$_4$O$_{10}$ (M+H)$^+$: 490.2275; obsd: 491.2349.
Compound 4b
crk-2-154 in CDCl3 at 400 MHz
Compound 4b

cvk-2-154 in CDCl3 at 100 MHz
Compound 4c

cv k-2-117 in CDCl3 at 400 MHz
Compound 4c

cvk-2-117 in CDCl3 at 100 MHz
Compound 4d

cvk-2-126 in CDCl3 at 100 MHz
Compound 4d

cvk-2-126 in CDCl3 at 100 MHz
Compound 4e

cvk–2–139 in MeOD at 400 MHz
Compound 4f

cvk–2–141 in MeOD at 400 MHz
Compound 4f

cvk-2-141 in MeOD at 100 MHz
Compound 4g

cvk-2-143 in MeOD at 100 MHz
Dipeptide 4

cvk-2-148 in D2O at 400 MHz
Dipeptide 4

cvk-2-148 in D2O at 100 MHz
1.7 **Synthesis of the Ac-[(α,1-4)GlcNAc]Hyp-NHMe Residue (5)**

[Chemical structure diagram]

**Ac-4-O-(2-azido-3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)Hyp-OBn (5b).** A solution of thioglycoside 6 (259 mg, 0.61 mmol, 1.0 eq.) and Ac-Hyp-OBn (5a) (177 mg, 0.67 mmol, 1.1 equiv.) in CH₂Cl₂ (3 mL) was stirred with activated 4Å molecular sieves (35 mg) for 15 min at rt under nitrogen and then the mixture was cooled to −78 °C. N-Iodosuccinimide (206 mg, 0.915 mmol, 1.5 equiv.) and silver triflate (78 mg, 0.3 mmol, 0.5 equiv.) were added. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was quenched by the addition of triethylamine (1 mL) and then diluted with EtOAc (75 mL), washed with 10% Na₂S₂O₃ (75 mL), and brine (75 mL). The organic layer was dried over MgSO₄, filtered, concentrated and the residue purified by flash chromatography eluting with 1:1 EtOAc-hexanes to give the α-O-glycoside 5b (161 mg, 46% yield). Rₑ 0.19 (4:1 EtOAc-hexanes). [α]D25 +71.7 (c 1.0, MeOH).

**¹H NMR (CDCl₃, 400 MHz) δ 2.00 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.10-2.20 (m, 0.7 H) [2.21-2.32 (m, 0.3H)], 2.48-2.54 (m, 0.7 H) [2.55-2.61 (m, 0.3H)], 3.29 (dd, J = 10.7, 3.6 Hz, 0.7H) [3.17 (dd, J = 10.7, 3.6 Hz, 0.3H)], 3.60-3.80 (m, 0.7H) [3.52-3.56 (m, 0.3H)], 3.87 (d, J = 11.1, 5.0 Hz, 1H), 4.00-4.11 (m, 2H), 4.18-4.27 (m, 1H), 4.47-4.52 (m, 0.7H) [4.43-4.46 (m, 0.3H)], 4.56-4.70 (m, 1H), 4.95 (t, J = 9.6 Hz,
1H), 5.05 (d, J = 3.7 Hz, 1H), 5.18 (s, 1.5H) [5.24 (s, 0.5H)], 5.41 (app. t, J = 9.4 Hz, 1H), 7.34-7.37 (m, 5H); 13C NMR (CDCl3, 100 MHz) δ 20.7, 20.8, 20.9, 22.4 [21.8], 35.8 [38.1], 53.2 [50.8], 57.7 [58.9], 60.7 [60.4], 62.3 [62.1], 67.3 [67.2], 68.6 [6], 68.7 [68.4], 68.6, 68.7, 70.1 [69.8], 74.0, 78.5, 98.3 [96.7], 128.4, 128.6, 128.7, 128.8, 129.0, 135.6, 169.7, 169.8, 170.1, 170.3, 170.7, 171.9, 172.0. HRMS (ESI+) calcd for C26H33N4O11 (M+H)+: 577.2140; obsd: 577.2144.

Ac-[(α,1-4)GlcNAc(OAc)4]Hyp-OBn (5c). Zinc powder (50 mg) and saturated aqueous CuSO4 (50 µL) were added to a solution of 5b (57 mg, 0.19 mmol) in THF (2 mL) at rt. Acetic acid (0.5 mL) and acetic anhydride (0.5 mL) were added and the mixture stirred overnight at rt. The reaction mixture was filtered, the filtrate concentrated and the residue purified by flash chromatography, eluting with 9:1 CH2Cl2-MeOH to give the N-acetylated product 5c (50 mg, 85% yield). Rf 0.33 (9:1 CH2Cl2-MeOH). 1H NMR (CDCl3, 400 MHz) δ 1.93 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.10 (s, 3H), 2.12-2.18 (m, 1H), 2.42-2.48 (m, 1H), 3.58 (d, J = 11.4 Hz, 1H), 3.77 (dd, J = 11.4, 4.6 Hz, 1H), 3.94 (ddd, J = 9.6, 5.1, 2.3 Hz, 1H), 4.07 (dd, J = 12.3, 2.1 Hz, 1H), 4.19 (dd, J = 12.3, 5.1 Hz, 1H), 4.28 (ddd, J = 10.3, 8.8, 3.6 Hz, 1H), 4.44-4.48 (m, 1H), 4.63 (app. t, J = 7.9 Hz, 1H), 5.02 (d, J = 3.7 Hz, 1H), 5.08 (app. t, J = 9.6 Hz, 1H), 5.11-5.21 (m, 3H), 6.02 (d, J = 8.6 Hz, 1H), 7.33-7.38 (m, 5H); 13C NMR (CDCl3, 100 MHz) δ 20.7, 20.8, 20.9, 22.4, 23.1, 35.8, 52.3, 52.7, 57.8, 62.2, 67.3, 68.2, 68.6, 70.8, 76.4, 96.3, 128.3, 128.6, 128.8, 128.9, 129.0, 135.6, 169.4, 169.9, 170.5, 170.8, 171.7, 171.8. HRMS (ESI+) calcd for C28H36N2NaO12 (M+Na)+: 615.2160; obsd: 615.2111.
**Ac-[(a,1-4)GlcNAc(OAc)₄]Hyp-NHMe (5d).** Palladium on carbon (10%, 35 mg) was added in a single portion to a solution of 5c (50 mg, 0.08 mmol) in MeOH (3.0 mL). The reaction flask was evacuated, then opened to an atmosphere of H₂ and stirred overnight. The catalyst was removed by filtering through a plug of Celite® in a Pasteur pipet. The filtrate was concentrated, to give Ac-[(a,1-4)GlcNAc(OAc)₄]Hyp-OH (40 mg, 94%). R_f 0.32 (4:1 CH₂Cl₂-MeOH). [α]_D²⁵ +41.7 (c 1.0, MeOH).

N'-Hydroxysuccinimide (9.2 mg, 0.08 mmol, 1 equiv.) was added to a solution of Ac-[(a,1-4)GlcNAc(OAc)₄]Hyp-OH (40 mg, 0.08 mmol, 1 equiv.) in dry CH₂Cl₂ (2 mL), at 0 °C. The mixture was stirred for 15 min under N₂ at 0 °C. Then DCC (17 mg, 0.08 mmol, 1 equiv.) was added and the mixture gradually warmed to rt and stirred overnight. The reaction mixture was filtered and the filtrate concentrated to a volume of 0.5 mL and after storing in the freezer for 6 h, the solution was filtered and concentrated. The resulting NHS ester was dissolved in dry acetonitrile (2 mL), cooled to 0 °C and methylamine hydrochloride (5.4 mg, 0.08 mmol, 1 equiv.) was added, followed by the addition of diisopropylethylamine (14 µL, 10.3 mg, 0.08 mmol, 1 equiv.). The mixture was gradually warmed to rt, stirred overnight, concentrated, applied to a flash column and eluted with 9:1 CH₂Cl₂-MeOH. The product 5d eluted, along with free NHS. This was further purified by flash chromatography eluting with 4:1 CH₂Cl₂-MeOH (the more polar eluent reduced band broadening and afforded a better separation of the two compounds) to afford product 5d (10 mg, 24%). R_f 0.82 (4:1 CH₂Cl₂-MeOH). [α]_D²⁵ +29.5 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz) δ 1.94 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.06-2.16 (m, 1H), 2.70 (dt, J =13.2, 5.4 Hz, 1H), 2.78 (d, J = 4.9 Hz, 3H), 3.49 (dd, J = 11.0, 3.5 Hz, 1H), 3.67 (dd, J = 11.0, 5.2 Hz, 1H), 3.97 (ddd, J = 9.4,
5.0, 2.6 Hz, 1H), 4.11 (dd, J = 12.3, 2.6, 1H), 4.23 (dd, J = 12.3, 7.3 Hz, 1H), 4.29 (ddd, J = 10.3, 9.0, 3.8, 1H), 4.55 (app. p, J = 5.0 Hz, 1H), 4.63 (dd, J = 8.2, 5.2 Hz, 1H), 4.92 (d, J = 3.8 Hz, 1H), 5.10 (app. t, J = 9.5 Hz, 1H), 5.16 (app. t, J = 9.5 Hz, 1H), 5.75 (d, J = 9.0 Hz, 1H), 6.97 (app. q, J = 4.8 Hz, 1H); 13C NMR (CDCl3, 100 MHz) δ 20.8, 20.9, 22.8, 23.3, 26.5, 34.0, 52.3, 52.4, 58.3, 62.3, 68.3, 68.5, 71.1, 75.8, 96.2, 169.5, 170.2, 170.8, 170.9, 171.0, 171.9. HRMS (ESI+) calcd for C22H34N3O11 (M+H)+: 516.2188; obsd: 516.2196.

Ac-[(α,1-4)-GlcNAc]-Hyp-NHMe (5). A solution of NaOMe in MeOH (25% w/v, two drops from a 20G needle) was added to a solution of glycoside 5d (10 mg, 0.02 mmol) in dry MeOH (1.5 mL) at 0 °C. The mixture was gradually warmed to rt and stirred for 1 h. Dowex 50WX2 H+ resin (5 mg) was added to the reaction mixture and stirred for 15 min. The reaction mixture was filtered through a plug of cotton and concentrated to give the glycoside 5 (4 mg, 53%). Rf 0.20 (4:1 CH2Cl2:MeOH). [α]D25 +2.0 (c 0.2, MeOH). 1H NMR (D2O, 400 MHz) δ 2.03 (s, 2.4H) [2.01 (s, 0.6 H)], 2.05-2.18 (m, 0.8H) [2.23-2.31 (m, 0.2 H)], 2.15 (s, 2.4H) [2.05 (s, 0.6H)], 2.52 (dd, J = 13.8, 7.8 Hz, 0.7 H) [2.60-2.69 (m, 0.2 H), 2.8 (s, 2.4 H) [2.82 (s, 0.6 H)], 3.5 (app. t, J = 9.3 Hz, 1H), 3.75 (dd, J = 13.4, 6.2 Hz, 1.6 H) [3.58 (dd, J = 11.8, 6.5 Hz, 0.4H)], 3.78 (app. t, J = 4.7 Hz, 1H), 3.79-3.81 (m, 1H), 3.82 (d, J = 11.8 Hz, 1H), 3.89 (dd, J = 11.8, 2.0 Hz, 0.8H) [3.68 (J = 11.8, 4.4 Hz, 0.2 H)], 3.93 (dd, J = 10.6, 3.8 Hz, 1H), 4.50 (t, J = 8.5 Hz, 0.8H) [4.70 (t, J = 8.5 Hz, 0.2H)], 4.54 (s, 0.8H) [4.71 (s, 0.2 H)], 5.10 (d, J = 3.7 Hz, 0.8H) [5.00 (d, J = 3.7 Hz, 0.2 H); 13C NMR (CDCl3, 100 MHz) δ 21.6 [20.6], 21.7, 36.2 [37.9], 52.9 [51.4], 53.6 [53.5], 59.2, 60.6 [60.5], 70.0, 70.6 [70.5], 72.3, 74.8 [74.0], 95.0, 95.3, 173.2, 174.1, 174.2. HRMS (ESI+) calcd for C16H28N3O8 (M+H)+: 389.17871; obsd: 390.1874.
Compound 5b

cvk-3-36d in CDCl3 at 400 MHz
Compound 5b

cvk–3–36 in CDCl3 at 100 MHz
Compound 5d

cvk–3–68 in CDCl3 at 400 MHz
Compound 5d

cvk-3-68 in CDCl3 at 100 MHz
Compound 5

cvk–3-69 in D2O at 100 MHz
2. Variable Temperature NMR Experiments - Thermodynamics

Samples of 1, 2, 4, and 5 were prepared in D₂O at concentrations between 0.02 M and 0.03 M. The pH of each solution was measured and found to be 6.84, 7.46, 4.94 and 5.26 respectively. Experiments were performed over several temperatures (25-85 °C) on a Bruker 400 MHz NMR spectrometer. The ratio of trans/cis was determined by integrating well resolved ¹H NMR signals.

Table S1. Thermodynamics Data Derived from Van’t Hoff Plots.

| Equation | Slope   | Intercept | ΔH° (kJ mol⁻¹) | ΔS° (J K⁻¹ mol⁻¹) | ΔG° (298 K) (kJ K⁻¹ mol⁻¹) |
|----------|---------|-----------|----------------|-------------------|---------------------------|
| 1        | y=322.144x-0.2615 | 322.144   | -0.2615        | -2.67             | -2.17                     | -2.03                     |
| 2        | y=764.926x-0.4019   | 764.926   | -0.4019        | -6.36             | -3.34                     | -5.36                     |
| 4        | y=1086.956x-1.0473  | 1086.956  | -1.0473        | -9.04             | -8.67                     | -6.45                     |
| 5        | y=333.992x-0.0304   | 333.992   | -0.0304        | -2.78             | -0.25                     | -2.70                     |

ΔH° and ΔS° were calculated by fitting the data of the Van’t Hoff plots to the following equation:

\[ \ln K_{tc} = (-\Delta H^\circ / R)(1/T) + \Delta S^\circ / R \]

ΔG° was calculated from ΔG° = ΔH° - TΔS°

ΔH°= standard enthalpy

ΔS°= standard entropy

ΔG°=standard Gibbs free energy

R= gas constant (8.314 J K⁻¹ mol⁻¹)
Table S1. Equilibrium constant, $K_{tc}$, at Various Temperatures for the $cis\rightarrow trans$ Isomerization of the Thr-Pro Amide Bond of Ac-Thr-Pro-NHMe (1).

| T (°C) | T (K) | 1000/T | Integration of methylamide CH₃ | $K_{tc}$ | $lnK_{tc}$ |
|------|------|--------|-------------------------------|--------|-----------|
| 85   | 358  | 2.79   | $trans$ 5.4, $cis$ 2.9        | 1.9    | 0.62 ± 0.02 |
| 80   | 353  | 2.83   | $trans$ 2.2, $cis$ 1.1        | 2.0    | 0.69 ± 0.04 |
| 75   | 348  | 2.87   | $trans$ 5.7, $cis$ 2.9        | 2.0    | 0.68 ± 0.01 |
| 70   | 343  | 2.92   | $trans$ 5.0, $cis$ 2.6        | 1.9    | 0.65 ± 0.02 |
| 67   | 340  | 2.94   | -                               | -      | 0.66       |
| 65   | 338  | 2.96   | $trans$ 5.5, $cis$ 2.7        | 2.0    | 0.71 ± 0.02 |
| 60   | 333  | 3.00   | $trans$ 5.5, $cis$ 2.8        | 2.0    | 0.68 ± 0.03 |
| 55   | 328  | 3.05   | $trans$ 4.2, $cis$ 1.9        | 2.2    | 0.79 ± 0.07 |
| 50   | 323  | 3.10   | $trans$ 5.0, $cis$ 2.5        | 2.0    | 0.69 ± 0.06 |
| 45   | 318  | 3.14   | $trans$ 4.9, $cis$ 2.6        | 1.9    | 0.63 ± 0.08 |
| 40   | 313  | 3.19   | $trans$ 6.4, $cis$ 2.9        | 2.2    | 0.79 ± 0.07 |
| 35   | 308  | 3.25   | $trans$ 6.6, $cis$ 2.9        | 2.3    | 0.82 ± 0.04 |
| 30   | 303  | 3.30   | $trans$ 6.8, $cis$ 3.0        | 2.3    | 0.82 ± 0.02 |
| 25   | 298  | 3.36   | $trans$ 4.8, $cis$ 2.1        | 2.3    | 0.83 ± 0.01 |

*340 K data from fitting equation

Figure S1. Van’t Hoff Plot for the $cis\rightarrow trans$ Isomerization of Thr-Pro Amide Bond of Ac-Thr-Pro-NHMe (1) in D₂O

Ac-Thr-Pro-NHMe

![Van't Hoff Plot](image)

Linear least squares fitting of the data gives the following equation: $y = 322.1445x - 0.2615$

slope = $(-\Delta H^°/R)$

$\Delta H^° = -322.1445 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1}$

= -0.64 kcal mol⁻¹

$\Delta S^° = -0.2615 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1}$

= -0.52 cal K⁻¹ mol⁻¹

$\Delta G^° = \Delta H^° - T\Delta S$

$\Delta G^° = -0.64 \text{ kcal mol}^{-1} - (298 \times -0.52 \times 10^{-3}) \text{ kcal mol}^{-1} = -0.63 \text{ kcal mol}^{-1}$
Table S2. Equilibrium constant, \( K_{t/c} \), at Various Temperatures for the \( \text{cis} \rightarrow \text{trans} \) Isomerization of the Thr-Hyp Amide Bond of Ac-Thr-Hyp-NHMe (2).

| T (°C) | T (K) | 1000/T | Integration of Thr-H\( \gamma \) | \( K_{t/c} \) | \( \ln K_{t/c} \) |
|--------|-------|-------|-------------------------------|---------|----------------|
| 85     | 358   | 2.79  | 8.8                           | 1.6     | 5.5            | 1.70 ± 0.03 |
| 80     | 353   | 2.83  | 4.8                           | 0.8     | 6.0            | 1.79 ± 0.03 |
| 75     | 348   | 2.87  | 8.7                           | 1.4     | 6.2            | 1.82 ± 0.03 |
| 70     | 343   | 2.92  | 8.7                           | 1.4     | 6.2            | 1.82 ± 0.003 |
| 67*    | 340   | 2.94  | -                             | -       | 6.0            | 1.79         |
| 65     | 338   | 2.96  | 8.2                           | 1.3     | 6.3            | 1.84 ± 0.02 |
| 60     | 333   | 3.00  | 8.7                           | 1.3     | 6.7            | 1.90 ± 0.01 |
| 55     | 328   | 3.05  | 8.3                           | 1.2     | 6.9            | 1.93 ± 0.001 |
| 50     | 323   | 3.10  | 8.4                           | 1.2     | 7.0            | 1.95 ± 0.02 |
| 45     | 318   | 3.14  | 8.3                           | 1.1     | 7.5            | 2.01 ± 0.01 |
| 40     | 313   | 3.19  | 8.4                           | 1.1     | 7.6            | 2.03 ± 0.01 |
| 35     | 308   | 3.25  | 8.1                           | 1.0     | 8.1            | 2.09 ± 0.01 |
| 30     | 303   | 3.30  | 8.4                           | 1.0     | 8.4            | 2.13 ± 0.01 |
| 25     | 298   | 3.36  | 8.7                           | 1.0     | 8.7            | 2.16 ± 0.001 |

*340 K data from fitting of equation

Figure S2. Van’t Hoff Plot for the \( \text{cis} \rightarrow \text{trans} \) Isomerization of the Thr-Hyp Amide Bond of Ac-Thr-Hyp-NHMe (2) in D\(_2\)O.

Linear least squares fitting of the data gives the following equation: \( y = 764.926x - 0.40186 \)

\[
\text{slope} = \left( -\frac{\Delta H^\circ}{R} \right) \\
\Rightarrow \Delta H^\circ = -764.926 \text{ K x 1.987 cal K}^{-1} \text{ mol}^{-1} \\
= -1.52 \text{ kcal mol}^{-1}
\]

\[
\text{intercept} = \frac{\Delta S^\circ}{R} \\
\Rightarrow \Delta S^\circ = -0.40186 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \\
= -0.78 \text{ cal K}^{-1} \text{ mol}^{-1}
\]

\[
\Delta G^\circ = \Delta H^\circ - T\Delta S \\
\Rightarrow \text{at 298K, } \Delta G^\circ = -1.52 \text{ kcal mol}^{-1} - (298 \times -0.78 \times 10^{-3}) \text{ kcal mol}^{-1} = -1.29 \text{ kcal mol}^{-1}
\]
Table S3. Equilibrium constant, \( K_{tc} \), at various temperatures for the cis\( \rightarrow \)trans isomerization of the Thr-Hyp amide bond of Ac-Thr-[(\( \alpha \),1-4)GlcNAc]Hyp-NHMe (4).

| \( T \) (°C) | T (K) | 1000/T | Integration of Thr-H\( \gamma \) | \( K_{tc} \) | \( \ln K_{tc} \) |
|---|---|---|---|---|---|
| 85* | 358 | 2.79 | 10.9 | 1.9 | 5.7 | 1.75 |
| 80 | 353 | 2.83 | 11.1 | 1.4 | 7.9 | 2.07 ± 0.04 |
| 75 | 348 | 2.87 | 11.4 | 1.2 | 9.5 | 2.25 ± 0.11 |
| 70 | 343 | 2.92 | 11.1 | 1.2 | 9.3 | 2.22 ± 0.05 |
| 67* | 340 | 2.94 | - | - | 7.9 | 2.06 |
| 65 | 338 | 2.96 | 7.7 | 0.9 | 8.6 | 2.15 ± 0.07 |
| 60 | 333 | 3.00 | 10.8 | 1.1 | 9.8 | 2.28 ± 0.03 |
| 55 | 328 | 3.05 | 9.3 | 1 | 9.3 | 2.23 ± 0.06 |
| 50 | 323 | 3.10 | 7.7 | 0.8 | 9.6 | 2.26 ± 0.07 |
| 45 | 318 | 3.14 | 11.6 | 1.1 | 10.5 | 2.36 ± 0.02 |
| 40 | 313 | 3.19 | 9.2 | 0.8 | 11.5 | 2.44 ± 0.02 |
| 35 | 308 | 3.25 | 11.4 | 1 | 11.4 | 2.43 ± 0.03 |
| 30 | 303 | 3.30 | 10.5 | 0.8 | 13.1 | 2.57 ± 0.06 |
| 25 | 298 | 3.36 | 11.9 | 0.9 | 13.2 | 2.58 ± 0.02 |

*This data point was eliminated due to the poor resolution of the designated \(^1\)H NMR signals.

*340 K data from fitting of equation.

Figure S3. Van’t Hoff Plot for the cis\( \rightarrow \)trans isomerization of the Thr-Hyp amide bond of Ac-Thr-[(\( \alpha \),1-4)GlcNAc]Hyp-NHMe (4) in D\(_2\)O.

\[
\text{Ac-Thr-}[\alpha-(1-4)\text{GlcNAc}]\text{Hyp-NHMe}
\]

\[
\begin{align*}
\text{Linear least squares fitting of the data gives the following equation: } & y = 871.1171x - 0.328094 \\
\text{slope} &= \left(-\Delta H^\circ/R\right) \\
\Delta H^\circ &= -871.1171 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \\
&= -1.73 \text{ kcal mol}^{-1} \\
\text{intercept} &= \Delta S^\circ/R \\
\Delta S^\circ &= -0.328094 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \\
&= -0.65 \text{ cal K}^{-1} \text{ mol}^{-1} \\
\Delta G^\circ &= \Delta H^\circ - T\Delta S \\
\text{at 298K, } \Delta G^\circ &= -1.73 \text{ kcal mol}^{-1} - (298 \times -0.65 \times 10^{-3}) \text{ kcal mol}^{-1} = -1.53 \text{ kcal mol}^{-1}
\end{align*}
\]
Table S4. Equilibrium constant, $K_{tc}$, at Various Temperatures for the cis→trans Isomerization of the Thr-Hyp Amide Bond of Ac-[α,1-4)GlcNAc]Hyp-NHMe (5).

| T (°C) | T (K) | 1000/T | Integration of methyl amide CH3 | $K_{tc}$ | $lnK_{tc}$ |
|-------|-------|--------|-------------------------------|---------|-----------|
|       |       |        | trans | cis |           |         |
| 85    | 358   | 2.79   | 1.9   | 0.6 | 3.2       | 1.14±0.15 |
| 80    | 353   | 2.83   | 2.0   | 0.8 | 2.5       | 0.97±0.03 |
| 75    | 348   | 2.87   | 2.1   | 0.8 | 2.6       | 0.96±0.04 |
| 70    | 343   | 2.92   | 2.1   | 0.8 | 2.6       | 0.94±0.08 |
| 67*   | 340   | 2.94   | -     | -   | 2.8       | 1.01     |
| 65    | 338   | 2.96   | 2.1   | 0.7 | 3.0       | 1.03±0.003 |
| 60    | 333   | 3.00   | 2.1   | 0.7 | 3.0       | 1.04±0.01 |
| 55    | 328   | 3.05   | 2.1   | 0.7 | 3.0       | 1.10±0.03 |
| 50    | 323   | 3.10   | 2.1   | 0.8 | 2.6       | 1.02±0.07 |
| 45    | 318   | 3.14   | 2.2   | 0.8 | 2.8       | 1.09±0.01 |
| 40    | 313   | 3.19   | 2.1   | 0.7 | 3.0       | 1.05±0.06 |
| 35    | 308   | 3.25   | 2.1   | 0.6 | 3.5       | 1.21±0.08 |
| 30    | 303   | 3.30   | 2.0   | 0.6 | 3.3       | 1.19±0.04 |
| 25    | 298   | 3.36   | 2.0   | 0.6 | 3.3       | 1.18±0.07 |

*340 K data from extrapolation.

Figure S4. Van’t Hoff Plot for the cis→trans Isomerization of the Thr-Hyp Amide Bond of Ac-[(α,1-4)GlcNAc]Hyp-NHMe (5) in D2O.

Linear least squares fitting of the data gives the following equation: $y = 339.9919x - 0.030411$

| slope = $(-\Delta H^°/R)$ | $\Delta H^° = -339.9919 \times 1.987 \text{cal K}^{-1}\text{mol}^{-1}$ |
| intercept = $\Delta S^°/R$ | $\Delta S^° = -0.030411 \times 1.987 \text{cal K}^{-1}\text{mol}^{-1}$ |

$\Delta G^° = \Delta H^° - T\Delta S$

$\Rightarrow$ at 298K, $\Delta G^° = -0.68 \text{kcal mol}^{-1} - (298 \times -0.07 \times 10^{-3}) \text{kcal mol}^{-1} = -0.66 \text{kcal mol}^{-1}$
3. Magnetization Transfer NMR Experiments - Kinetics

Samples of 1, 2 and 5 were prepared in D₂O at concentrations between 0.02 M and 0.03 M. Experiments were performed over several temperatures (60-80 °C) on a Varian 700 MHz NMR spectrometer with VNMRS console and HCN probe, using Varian VnmrJ 2.3 software and the presat pulse sequence from Varian’s sequence library. The temperature was calibrated using an ethylene glycol standard.

In each case, a well-resolved signal attributed to the trans rotamer [the proline Hδ multiplet (for 1), threonine Hγ doublet (for 2) and the N-methylamide NHCH₃ singlet (for 5)] was inverted using a low-power pulse that was calibrated to give maximum inverted signal intensity. The specified multiplet at δ3.61-3.81 ppm for compound 1 was inverted with a ~60 ms long inversion pulse at a power level of -13 db. The specified doublet at δ1.23 ppm for compound 2 was inverted using a 75 ms pulse at a power level of 6 db. The specified singlet at δ2.80 ppm for compound 5 was inverted with a ~60 ms long inversion pulse at a power level of -13 db. In all cases, a relaxation delay (d₁) of 20 s, acquisition time of 2.12 s, detection pulse width (pw) of 8.9 µs at detection pulse power (tpwr) of 59 db were used. In each experiment inversion transfer spectra were collected at 23-28 d₂ values between 0 s and 20 s. The number of points for each FID was 32K and 128 scans were collected at each d₂ value.

The data from the inversion transfer experiments were fitted using the CIFIT program. The initial estimates of rate, T₁, M₀ and M∞ were fed to the CIFIT mechanism file. The CIFIT program then conducts a least-squares minimization on the difference between the integration versus time curves in the data file and the curves predicted by the McConnell-Bloch equations given the initial guesses in the mechanism file. Standard errors (SE) were calculated by CIFIT.

\[ T₁ = \text{spin lattice relaxation time} \]
\[ M_0 = \text{initial magnetization} \]
\[ M_\infty = \text{equilibrium magnetization} \]

\[ k_{tc} = \text{reaction rate for } trans \rightarrow cis \text{ (reported in the CIFIT output files)} \]
\[ k_{ct} = \text{reaction rate for } cis \rightarrow trans \text{ (derived from } k_{ct} = K_{tc} \times k_{ct}) \]

\[ \Delta H^\dagger \text{ and } \Delta S^\dagger \text{ were calculated by fitting the data of the Eyring plots to the following equation:} \]
\[
\ln \left( \frac{k}{T} \right) = \left( \frac{-\Delta H^\dagger}{R} \right) \left( \frac{1}{T} \right) + \frac{\Delta S^\dagger}{R} + \ln \left( \frac{k_B}{h} \right)
\]

\[ \Delta G^\dagger \text{ was calculated from } \Delta G^\dagger = -\Delta H^\dagger - T \Delta S^\dagger \]
Table S5. Data for Eyring Analysis of Isomerization About the Thr-Pro Amide Bond of Ac-Thr-Pro-NHMe (1)

| T (K) | 1000/T (K⁻¹) | K    | \( k_{ic} \) (s⁻¹) | \( ln(k_{ic}/T) \) | \( ln(k_{ic}/T) - ln(k_B/h) \) | \( k_{ct} \) (s⁻¹) | \( ln(k_{ct}/T) \) | \( ln(k_{ct}/T) - ln(k_B/h) \) |
|-------|--------------|------|-----------------|-----------------|-----------------|-----------------|----------------|-----------------|----------------|
| 333   | 3.00         | 2.0±0.2 | 0.22±0.06       | -7.32           | -31.08          | 0.44            | -6.63          | -30.39          |
| 338   | 2.96         | 2.0±0.2 | 0.37±0.13       | -6.82           | -30.58          | 0.74            | -6.12          | -29.88          |
| 340*  | 2.94         | 1.9    | 0.38            | -6.80           |                  | 0.72            | -6.11          |                 |
| 343   | 2.91         | 1.9±0.2 | 0.34±0.06       | -6.93           | -30.69          | 0.64            | -6.28          | -30.04          |
| 348   | 2.87         | 2.0±0.2 | 0.47±0.04       | -6.61           | -30.37          | 0.94            | -5.91          | -29.67          |
| 353   | 2.83         | 2.0±0.2 | 0.60±0.07       | -6.38           | -30.14          | 1.20            | -5.68          | -29.44          |

*340 K data from fitting of equation

Figure S5. Eyring Plot for \( cis \to trans \) and \( trans \to cis \) Isomerization of the Thr-Pro Amide Bond of Ac-Thr-Pro-NHMe (1)

For the analogous plot of \( \ln(k/T) - \ln(k_B/h) \) vs 1000/T, the following equations were derived from linear least squares fitting of the data:

\( cis \to trans \quad y = -4961x - 15.4; \quad R^2 = 0.85 \)
\( trans \to cis \quad y = -4964x - 16.1; \quad R^2 = 0.88 \)

Thus, the activation parameters for the two processes were calculated:

| \( cis \to trans \) | \( trans \to cis \) |
|---------------------|---------------------|
| slope = (-\( \Delta H^\ddagger / R \)) | slope = (-\( \Delta H^\ddagger / R \)) |
| \( \Delta H^\ddagger = 4961 \text{ K} \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) | \( \Delta H^\ddagger = 4964 \text{ K} \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) |
| 9.86 \text{ kcal mol}^{-1} | 9.86 \text{ kcal mol}^{-1} |
| intercept = \( \Delta S^\ddagger / R \) | intercept = \( \Delta S^\ddagger / R \) |
| \( \Delta S^\ddagger = -15.4 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) | \( \Delta S^\ddagger = -16.10 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) |
| = -30.6 \text{ cal K}^{-1} \text{ mol}^{-1} | = -32.6 \text{ cal K}^{-1} \text{ mol}^{-1} |
| \( \Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \) | \( \Delta G^\ddagger = \Delta H^\ddagger - T\Delta S \) |
| \( \Delta G^\ddagger \) at 298K, \( \Delta G^\ddagger = 9.86 \text{ kcal mol}^{-1} \) – (298 x \( -30.6 \times 10^{-3} \)) kcal mol\(^{-1} = 18.98 \text{ kcal mol}^{-1} \) | \( \Delta G^\ddagger \) at 298K, \( \Delta G^\ddagger = 9.86 \text{ kcal mol}^{-1} \) – (298 x \( -32.6 \times 10^{-3} \)) kcal mol\(^{-1} = 19.57 \text{ kcal mol}^{-1} \) |
Table S6: Integration vs. $d_2$ for Ac-Thr-ProNHMe (1) at 60 °C

| $d_2$ (s) | Integration | Integration |
|-----------|-------------|-------------|
|           | trans       | cis         |
| 0.0001    | -0.05       | 1.35        |
| 0.005     | -0.09       | 1.28        |
| 0.01      | -0.12       | 1.27        |
| 0.05      | -0.04       | 1.27        |
| 0.10      | -0.01       | 1.25        |
| 0.15      | 0.14        | 1.25        |
| 0.20      | 0.18        | 1.23        |
| 0.25      | 0.22        | 1.23        |
| 0.40      | 0.35        | 1.19        |
| 0.60      | 0.49        | 1.15        |

Figure S6: Inversion recovery of (A) inverted trans Pro-Hδ multiplet and (B) non-inverted cis Pro-Hδ multiplet of compound 1 at 60 °C

(A) Ac-Thr-Pro-NHMe 60 °C

(B) Ac-Thr-Pro-NHMe 60 °C
Table S7: Integration vs. $d_2$ Ac-Thr-ProNHMe (1) at 65 °C

| $d_2$ (s) | Integration | | $d_2$ (s) | Integration |
|-----------|-------------| | 0.8      | 0.25 | 0.82 |
| 0.0001    | -0.53       | 1.05 | 1.0     | 0.36 | 0.81 |
| 0.005     | -0.62       | 1.02 | 1.5     | 0.56 | 0.84 |
| 0.01      | -0.62       | 1.02 | 2.0     | 0.68 | 0.87 |
| 0.05      | -0.55       | 1.00 | 4.0     | 0.92 | 1.00 |
| 0.10      | -0.45       | 1.01 | 6.0     | 1.00 | 1.06 |
| 0.15      | -0.3        | 0.98 | 8.0     | 1.03 | 1.07 |
| 0.20      | -0.24       | 0.92 | 12.0    | 1.08 | 1.08 |
| 0.25      | -0.18       | 0.91 | 14.0    | 1.10 | 1.09 |
| 0.40      | -0.04       | 0.86 | 0.12    | 0.83 |

Figure S7: Inversion recovery of (A) inverted trans Pro-Hδ multiplet and (B) non-inverted cis Pro-Hδ multiplet of compound 1 at 65 °C
Table S8: Integration vs. d₂ Ac-Thr-ProNHMe (1) at 70 °C

| d₂ (s) | Integration | d₂ (s) | Integration |
|--------|-------------|--------|-------------|
|        | trans       | cis    | trans       | cis          |
| 0.0001 | -0.64       | 0.99   | 0.8         | 0.54         |
| 0.005  | -0.48       | 1.09   | 1.0         | 0.66         |
| 0.01   | -0.45       | 1.12   | 1.5         | 0.86         |
| 0.05   | -0.30       | 1.16   | 2.0         | 1.02         |
| 0.10   | -0.24       | 1.12   | 4.0         | 1.25         |
| 0.15   | -0.17       | 1.09   | 6.0         | 1.34         |
| 0.20   | -0.10       | 1.08   | 8.0         | 1.31         |
| 0.25   | -0.02       | 1.07   | 12          | 1.15         |
| 0.40   | 0.17        | 1.02   | 14          | 1.16         |
| 0.60   | 0.38        | 0.98   |             |              |

Figure S8. Inversion recovery of (A) inverted trans Pro-Hδ multiplet and (B) non-inverted cis Pro-Hδ multiplet of compound 1 at 70 °C

(A) [Diagram of Ac-Thr-Pro-NHMe 70 °C with intensity vs. d₂ (sec) for trans]

(B) [Diagram of Ac-Thr-Pro-NHMe 70 °C with intensity vs. d₂ (sec) for cis]
Table S9. Integration vs. d₂ Ac-Thr-ProNHMe (1) at 75 °C

| d₂ (s) | Integration  | Integration  |
|--------|--------------|--------------|
|        | d₂ (s) | trans | cis |
| 0.0001 | -0.51 | 1.26 |
| 0.005  | -0.64 | 1.24 |
| 0.01   | -0.70 | 1.23 |
| 0.05   | -0.61 | 1.19 |
| 0.10   | -0.47 | 1.15 |
| 0.15   | -0.35 | 1.11 |
| 0.20   | -0.25 | 1.08 |
| 0.25   | -0.16 | 1.05 |
| 0.40   | 0.07  | 0.96 |
| 0.60   | 0.29  | 0.89 |

| d₂ (s) | trans | cis |
|--------|-------|-----|
| 0.8    | 0.45  | 0.89|
| 1.0    | 0.57  | 0.91|
| 1.5    | 0.78  | 1.00|
| 2.0    | 0.93  | 1.10|
| 4.0    | 1.29  | 1.36|
| 6.0    | 1.44  | 1.44|
| 8.0    | 1.51  | 1.47|
| 12     | 1.44  | 1.40|
| 14     | 1.38  | 1.34|

Figure S9: Inversion recovery of (A) inverted trans Pro-Hδ multiplet and (B) non-inverted cis Pro-Hδ multiplet of compound 1 at 75 °C

(A)

(B)
Table S10. Integration vs. d₂ Ac-Thr-ProNHMe (1) at 80 °C

| d₂ (s) | Integration | d₂ (s) | Integration |
|--------|-------------|--------|-------------|
|        | trans       | cis    | trans       | cis    |
| 0.0001 | -0.09       | 1.23   | 0.0001      | 0.78   | 0.92 |
| 0.005  | -0.17       | 1.19   | 0.005       | 0.88   | 0.97 |
| 0.01   | -0.12       | 1.18   | 0.01        | 1.08   | 1.09 |
| 0.05   | -0.06       | 1.09   | 0.05        | 1.19   | 1.18 |
| 0.10   | 0.03        | 1.02   | 0.10        | 1.26   | 1.19 |
| 0.15   | 0.11        | 0.97   | 0.15        | 1.37   | 1.28 |
| 0.20   | 0.19        | 0.94   | 0.20        | 1.41   | 1.31 |
| 0.25   | 0.27        | 0.94   | 0.25        | 1.45   | 1.35 |
| 0.40   | 0.46        | 0.89   | 0.40        | 1.52   | 1.38 |
| 0.60   | 0.64        | 0.88   | 0.60        | 1.52   | 1.38 |

Figure S10: Inversion recovery of (A) inverted trans Pro-Hδ multiplet and (B) non-inverted cis Pro-Hδ multiplet of compound 1 at 80 °C
Table S11. Data for Eyring Analysis for Isomerization About the Thr-Hyp Amide Bond of Ac-Thr-Hyp-NHMe (2)

| T (K) | 1000/T (K\(^{-1}\)) | K | \(k_{tc}\) (s\(^{-1}\)) | \(ln(k_{tc}/T)\) | \(ln(k_{tc}/T) - ln(k_B/h)\) | \(k_{ct}\) (s\(^{-1}\)) | \(ln(k_{ct}/T)\) | \(ln(k_{ct}/T) - ln(k_B/h)\) |
|-------|------------------|---|----------------|----------------|-----------------|----------------|----------------|----------------|
| 333   | 3.00             | 6.7 | 0.14±0.08       | -7.77          | -31.53          | 0.94±0.08       | -5.87          | -29.63         |
| 338   | 2.96             | 6.3 | 0.21±0.10       | -7.38          | -31.14          | 1.32±0.10       | -5.55          | -29.31         |
| 340*  | 2.94             | 6.3 | 0.22±0.10       | -7.36          | -31.12          | 1.33±0.10       | -5.50          | -29.27         |
| 343   | 2.91             | 6.2 | 0.30±0.10       | -7.04          | -30.08          | 1.86±0.10       | -5.22          | -28.98         |
| 348   | 2.87             | 6.2 | 0.49±0.14       | -6.57          | -30.33          | 3.04±0.14       | -4.74          | -28.50         |

*340 K data from fitting of equation

Figure S11. Eyring Plot for cis→trans and trans→cis Isomerization of the Thr-Hyp Amide Bond of Ac-Thr-Hyp-NHMe (2)

For the analogous plot of \(\ln(k/T) - \ln(k_B/h)\), the following equations were derived from linear least squares fitting of the data:

\textit{cis→trans} \quad y = -8537x - 4.04; \quad R^2 = 0.99

\textit{trans→cis} \quad y = -9118x - 4.18; \quad R^2 = 0.99

Thus, the activation parameters for these two processes were calculated:

\textit{cis→trans} \quad \text{slope} = (-\Delta H^\ddagger/R) \quad \Rightarrow \Delta H^\ddagger = 8537 \text{ K} \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} = 16.96 \text{ kcal mol}^{-1}

\Rightarrow \Delta S^\ddagger = -4.04 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} = -8.0 \text{ cal K}^{-1} \text{ mol}^{-1}

\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad \Rightarrow \text{ at 298K, } \Delta G^\ddagger = 16.96 \text{ kcal mol}^{-1} - (298 \times -8.0 \times 10^3) \text{ kcal mol}^{-1} = 19.34 \text{ kcal mol}^{-1}

\textit{trans→cis} \quad \text{slope} = (-\Delta H^\ddagger/R) \quad \Rightarrow \Delta H^\ddagger = 9118 \text{ K} \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} = 18.12 \text{ kcal mol}^{-1}

\Rightarrow \Delta S^\ddagger = -4.18 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} = -8.3 \text{ cal K}^{-1} \text{ mol}^{-1}

\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad \Rightarrow \text{ at 298K, } \Delta G^\ddagger = -18.12 \text{ kcal mol}^{-1} - (298 \times -8.3 \times 10^3) \text{ kcal mol}^{-1} = 20.59 \text{ kcal mol}^{-1}
**Table S12.** Integration vs. d$_2$ Ac-Thr-Hyp-NHMe (2) at 60 ºC

| d$_2$ (s) | Integration | | Integration |
|-----------|-------------|---|-------------|---|---|
|           | trans       | cis | d$_2$ (s)   | trans | cis |
| 0.1403    | -0.43       | 0.31 | 2.1781     | 1.67  | 0.34 |
| 0.1977    | -0.28       | 0.29 | 2.5853     | 1.78  | 0.35 |
| 0.2346    | -0.19       | 0.29 | 3.0687     | 1.86  | 0.36 |
| 0.2785    | -0.09       | 0.28 | 3.6424     | 1.92  | 0.37 |
| 0.3924    | 0.14        | 0.28 | 4.3234     | 1.95  | 0.37 |
| 0.4657    | 0.27        | 0.27 | 5.1318     | 1.97  | 0.38 |
| 0.5528    | 0.42        | 0.27 | 7.2301     | 1.97  | 0.37 |
| 0.7788    | 0.72        | 0.27 | 8.5819     | 1.96  | 0.37 |
| 0.9245    | 0.91        | 0.28 | 10.180     | 1.95  | 0.37 |
| 1.0993    | 1.08        | 0.29 | 12.091     | 1.94  | 0.37 |
| 1.5460    | 1.40        | 0.31 | 14.352     | 1.93  | 0.37 |
| 1.8350    | 1.55        | 0.33 |           |       |    |

**Figure S12.** Inversion recovery of (A) inverted trans Thr-H$_{\gamma}$ doublet and (B) non-inverted cis Thr-H$_{\gamma}$ doublet of compound 2 at 60 ºC
**Table S13.** Integration vs. $d_2$ Ac-Thr-Hyp-NHMe (2) at 65 °C

| $d_2$ (s) | trans  | cis  |
|----------|--------|------|
| 0.1403   | -0.23  | 0.26 |
| 0.1977   | -0.02  | 0.26 |
| 0.2346   | 0.09   | 0.26 |
| 0.2785   | 0.17   | 0.25 |
| 0.3924   | 0.36   | 0.25 |
| 0.4657   | 0.47   | 0.25 |
| 0.5528   | 0.59   | 0.25 |
| 0.7788   | 0.84   | 0.26 |
| 0.9245   | 0.98   | 0.27 |
| 1.0993   | 1.12   | 0.28 |
| 1.5460   | 1.39   | 0.31 |
| 1.8350   | 1.51   | 0.33 |

**Figure S13.** Inversion recovery of (A) inverted *trans* Thr-H$_\gamma$ doublet and (B) non-inverted *cis* Thr-H$_\gamma$ doublet of compound 2 at 65 °C

(A)

**Ac-Thr-Hyp-NHMe 65 °C**

(B)

**Ac-Thr-Hyp-NHMe 65 °C**
Table S14. Integration vs. d₂ Ac-Thr-Hyp-NHMe (2) at 70 °C

| d₂ (s) | Integration | d₂ (s) | Integration |
|-------|-------------|-------|-------------|
|       | trans       | cis   | trans       | cis   |
| 0.1403| -0.39       | 0.28  | 2.1781      | 1.96  | 0.39 |
| 0.1977| -0.22       | 0.25  | 2.5853      | 2.01  | 0.4  |
| 0.2346| -0.12       | 0.24  | 3.0687      | 2.12  | 0.42 |
| 0.2785| -0.01       | 0.23  | 3.6424      | 2.2   | 0.43 |
| 0.3924| 0.24        | 0.22  | 4.3234      | 2.26  | 0.43 |
| 0.4657| 0.39        | 0.22  | 5.1318      | 2.29  | 0.44 |
| 0.5528| 0.56        | 0.22  | 7.2301      | 2.29  | 0.44 |
| 0.7788| 0.89        | 0.24  | 8.5819      | 2.28  | 0.43 |
| 0.9245| 1.07        | 0.26  | 10.180      | 2.26  | 0.43 |
| 1.0993| 1.24        | 0.28  | 12.091      | 2.25  | 0.42 |
| 1.5460| 1.59        | 0.32  | 14.352      | 1.96  | 0.39 |
| 1.8350| 1.75        | 0.35  |             |       |     |

Figure S14. Inversion recovery of (A) inverted trans Thr-Hγ doublet and (B) non-inverted cis Thr-Hγ doublet of compound 2 at 70 °C
Table S15. Integration vs. $d_2$ Ac-Thr-Hyp-NHMe (2) at 75 °C

| $d_2$ (s) | Integration |   | Integration |
|----------|------------|---|-------------|
|          | trans      | cis|             |
| 0.1403   | -0.39      | 0.17| 2.1781 | 1.79 | 0.26 |
| 0.1977   | -0.22      | 0.14| 2.5853 | 1.92 | 0.28 |
| 0.2346   | -0.12      | 0.13| 3.0687 | 2.03 | 0.29 |
| 0.2785   | -0.01      | 0.13| 3.6424 | 2.11 | 0.3  |
| 0.3924   | 0.22       | 0.12| 4.3234 | 2.16 | 0.31 |
| 0.4657   | 0.36       | 0.12| 5.1318 | 2.19 | 0.31 |
| 0.5528   | 0.51       | 0.12| 7.2301 | 2.2  | 0.31 |
| 0.7788   | 0.81       | 0.15| 8.5819 | 2.19 | 0.31 |
| 0.9245   | 0.98       | 0.17| 10.180 | 2.18 | 0.32 |
| 1.0993   | 1.15       | 0.18| 12.091 | 2.17 | 0.34 |
| 1.5460   | 1.49       | 0.23| 14.352 | 2.17 | 0.33 |
| 1.8350   | 1.65       | 0.25|         |      |     |

Figure S15. Inversion recovery of (A) inverted trans Thr-H$_\gamma$ doublet and (B) non-inverted cis Thr-H$_\gamma$ doublet of compound 2 at 75 °C

(A)

**Ac-Thr-Hyp-NHMe 75 °C**

(B)

**Ac-Thr-Hyp-NHMe 75 °C**
Table S16. Data for Eyring Analysis for rotation about the Thr-Hyp Amide Bond of Ac-[(α-1,4)GlcNAc-Hyp]-NHMe (5)

| T (K) | 1000/T (K⁻¹) | K | kᵣird (s⁻¹) | ln(kᵣird/T) | ln(kᵣird/T) - ln(kᵢr/h) | kᵣird (s⁻¹) | ln(kᵣird/T) - ln(kᵢr/h) |
|-------|---------------|---|--------------|-------------|--------------------------|--------------|--------------------------|
| 333   | 3.00          | 3.0 | 0.15±0.03   | -7.70       | -31.46                   | 0.45         | -6.61                   |
| 338   | 2.96          | 3.0 | 0.18±0.03   | -7.56       | -31.32                   | 0.54         | -6.44                   |
| 340   | 2.94          | 2.6 | 0.34        | -6.90       | -31.00                   | 0.94         | -5.89                   |
| 343   | 2.91          | 2.6 | 0.46±0.08   | -6.61       | -30.37                   | 1.20         | -5.96                   |
| 348   | 2.87          | 2.6 | 0.51±0.11   | -6.53       | -30.29                   | 1.33         | -5.57                   |
| 353   | 2.83          | 2.5 | 0.73±0.16   | -6.19       | -29.95                   | 1.83         | -5.26                   |

*340 K data from fitting of equation

Figure S16. Eyring Plot for cis→trans and trans→cis Isomerization of the Thr-Hyp Amide Bond of Ac-[(α-1,4)GlcNAc-Hyp]-NHMe (5)

For the analogous plot of ln(k/T) – ln(kᵢr/h) vs. 1000/T, the following equations were derived from linear least squares fitting of the data:

**cis→trans**  \( y = -9011x - 3.42; \ R^2 = 0.93 \)

**trans→cis**  \( y = -9522x - 2.92; \ R^2 = 0.91 \)

Thus, the activation parameters for these two processes were calculated:

| cis→trans | trans→cis |
|-----------|-----------|
| slope = -ΔH°/R | slope = -ΔH°/R |
| \( \Rightarrow \Delta H° = -9011 \text{ K x 1.987 cal K}^{-1} \text{ mol}^{-1} \) | \( \Rightarrow \Delta H° = -9522 \text{ K x 1.987 cal K}^{-1} \text{ mol}^{-1} \) |
| \( = 17.90 \text{ kcal mol}^{-1} \) | \( = 18.9 \text{ kcal mol}^{-1} \) |
| intercept = ΔS°/R | intercept = ΔS°/R |
| \( \Rightarrow \Delta S° = -3.42 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) | \( \Rightarrow \Delta S° = -2.92 \times 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) |
| \( = -6.8 \text{ cal K}^{-1} \text{ mol}^{-1} \) | \( = -5.8 \text{ cal K}^{-1} \text{ mol}^{-1} \) |
| \( \Delta G° = \Delta H° - T \Delta S° \) | \( \Delta G° = \Delta H° - T \Delta S° \) |
| \( \Rightarrow \text{at 298K, } \Delta G° = 17.90 \text{ kcal mol}^{-1} - (298 \times -6.8 \times 10^{-3}) \text{ kcal mol}^{-1} = 19.93 \text{ kcal mol}^{-1} \) | \( \Rightarrow \text{at 298K, } \Delta G° = 18.9 \text{ kcal mol}^{-1} - (298 \times -5.8 \times 10^{-3}) \text{ kcal mol}^{-1} = 20.63 \text{ kcal mol}^{-1} \) |
Table S17. Integration vs. d$_2$ Ac-[(α-1,4)GlcNAc-Hyp]-NHMe (5) at 60 °C

| d$_2$ (s) | Integration | Integration |
|-----------|-------------|-------------|
|           | trans       | cis         | d$_2$ (s) | trans | cis |
| 0.000     | -10.00      | 7.65        | 0.800     | 1.150 | 5.49 |
| 0.005     | -10.27      | 6.99        | 1.000     | 3.220 | 5.40 |
| 0.010     | -10.96      | 6.40        | 1.500     | 7.100 | 5.42 |
| 0.050     | -9.617      | 6.86        | 2.000     | 10.25 | 5.66 |
| 0.100     | -8.740      | 6.57        | 4.000     | 16.42 | 6.72 |
| 0.150     | -7.710      | 6.37        | 6.000     | 18.51 | 7.36 |
| 0.200     | -6.740      | 6.45        | 8.000     | 19.37 | 7.59 |
| 0.250     | -5.870      | 6.21        | 12.00     | 19.72 | 7.50 |
| 0.400     | -3.750      | 5.87        | 16.00     | 19.45 | 7.37 |
| 0.600     | -1.210      | 5.66        |

Figure S17. Inversion recovery of (A) inverted trans GlcNAc-H1 doublet and (B) non-inverted cis GlcNAc-H1 doublet of compound 5 at 60 °C

(A)

Ac-[(α-1,4)GlcNAc]Hyp-NHMe 60 °C

(B)

Ac-[(α-1,4)GlcNAc]Hyp-NHMe 60 °C
Table S18. Integration vs. $d_2$ Ac-$[(\alpha-1,4)\text{GlcNAc-Hyp}]$-NHMe (5) at 65 °C

| $d_2$ (s) | Integration | $d_2$ (s) | Integration |
|-----------|-------------|-----------|-------------|
|           | trans      | cis       | trans      | cis       |
| 0.000     | -10.00     | 8.47      | 0.800      | 3.76      | 7.23      |
| 0.005     | -9.12      | 9.17      | 1.000      | 5.81      | 7.16      |
| 0.010     | -8.87      | 9.17      | 1.500      | 9.85      | 7.26      |
| 0.050     | -8.02      | 9.02      | 2.000      | 12.69     | 7.30      |
| 0.100     | -6.96      | 8.68      | 4.000      | 18.82     | 8.77      |
| 0.150     | -5.90      | 8.32      | 6.000      | 21.02     | 9.43      |
| 0.200     | -5.09      | 8.11      | 8.000      | 21.98     | 9.67      |
| 0.250     | -4.06      | 7.86      | 12.00      | 22.40     | 9.89      |
| 0.400     | -1.56      | 7.45      | 16.00      | 22.40     | 9.68      |
| 0.600     | 1.14       | 7.31      |             |           |           |

Figure S18. Inversion recovery of (A) inverted trans GlcNAc-H1 doublet and (B) non-inverted cis GlcNAc-H1 doublet of compound 5 at 65 °C
**Table S19.** Integration vs. d$_2$ Ac-[(α-1,4)GlcNAc-Hyp]-NHMe (5) at 70 °C

| d$_2$ (s) | Integration | Integration |
|----------|-------------|-------------|
| 0.00     | trans: -10.00 | cis: 8.47   |
| 0.10     | trans: -10.84 | cis: 6.73   |
| 0.20     | trans: -8.73  | cis: 5.69   |
| 0.25     | trans: -6.03  | cis: 4.37   |
| 0.40     | trans: -3.75  | cis: 3.85   |
| 0.60     | trans: -1.05  | cis: 3.44   |
| 0.80     | trans: 1.25   | cis: 3.29   |
| 1.00     | trans: 3.33   | cis: 3.25   |

| d$_2$ (s) | Integration | Integration |
|----------|-------------|-------------|
| 1.50     | trans: 6.87 | cis: 3.43   |
| 2.00     | trans: 9.41 | cis: 3.85   |
| 3.00     | trans: 13.19| cis: 4.95   |
| 4.00     | trans: 15.59| cis: 5.87   |
| 5.00     | trans: 17.19| cis: 6.56   |
| 6.00     | trans: 18.04| cis: 6.79   |
| 7.00     | trans: 18.34| cis: 6.77   |
| 8.00     | trans: 18.85| cis: 6.89   |

**Figure S19.** Inversion recovery of (A) inverted *trans* GlcNAc-H1 doublet and (B) non-inverted *cis* GlcNAc-H1 doublet of compound 5 at 70 °C
Table S20. Integration vs. \( d_2 \) Ac-[(\( \alpha \)-1,4)GlcNAc-Hyp]-NHMe (5) at 75 °C

| \( d_2 \) (s) | Integration \( trans \) | Integration \( cis \) |
|----------------|-------------------------|-------------------------|
| 0.00           | -10.00                  | 8.47                    |
| 0.10           | -9.83                   | 9.01                    |
| 0.20           | -7.21                   | 7.65                    |
| 0.25           | -3.83                   | 6.11                    |
| 0.40           | -1.02                   | 5.32                    |
| 0.60           | 2.03                    | 4.72                    |
| 0.80           | 4.37                    | 4.63                    |
| 1.00           | 6.46                    | 4.81                    |

| \( d_2 \) (s) | Integration \( trans \) | Integration \( cis \) |
|----------------|-------------------------|-------------------------|
| 1.50           | 9.87                    | 5.35                    |
| 2.00           | 12.44                   | 5.84                    |
| 3.00           | 16.09                   | 7.14                    |
| 4.00           | 18.71                   | 7.83                    |
| 5.00           | 21.43                   | 9.51                    |
| 6.00           | 22.70                   | 9.95                    |
| 7.00           | 22.35                   | 9.24                    |
| 8.00           | 22.96                   | 9.46                    |

Figure S20. Inversion recovery of (A) inverted \( trans \) GlcNAc-H1 doublet and (B) non-inverted \( cis \) GlcNAc-H1 doublet of compound 5 at 75 °C

(A) **Ac-[(\( \alpha \)-1,4)GlcNAc]Hyp-NHMe 75 °C**

(B) **Ac-[(\( \alpha \)-1,4)GlcNAc]Hyp-NHMe 75 °C**
Table S21. Integration vs. d₂ Ac-[(α-1,4)GlcNAc-Hyp]-NHMe (5) at 80 °C

| d₂ (s) | Integration | | d₂ (s) | Integration |
|-------|-------------|---|-------|-------------|
|       | trans       | cis |       | trans       | cis |
| 0.00  | -10.00      | 9.76 | 1.50  | 12.53       | 4.74 |
| 0.10  | -10.73      | 8.95 | 2.00  | 15.67       | 5.81 |
| 0.20  | -9.64       | 9.80 | 3.00  | 23.41       | 8.70 |
| 0.25  | -7.61       | 8.66 | 4.00  | 27.14       | 10.02|
| 0.40  | -5.99       | 7.21 | 5.00  | 28.48       | 10.35|
| 0.60  | -4.16       | 6.62 | 6.00  | 30.03       | 11.04|
| 0.80  | -2.31       | 6.18 | 7.00  | 30.74       | 11.60|
| 1.00  | -0.87       | 5.67 | 8.00  | 12.53       | 4.74 |

Figure S21. Inversion recovery of (A) inverted trans GlcNAc-H1 doublet and (B) non-inverted cis GlcNAc-H1 doublet of compound 5 at 80 °C

(A)

(B)
**Figure S22.** Selected region of TOCSY Spectrum for Compound 1 in 90% H$_2$O, 10% D$_2$O, pH 7.51, at 500 MHz and 300 K with DSS as internal reference. Water suppression by excitation sculpting.

![TOCSY Spectrum Diagram](image)

**Figure S23.** Variation in amide chemical shifts for compound 1 with temperature in 90% H$_2$O, 10% D$_2$O, pH 7.51, at 500 MHz with DSS as internal reference. The NHMe is represented by ■ (-5.9 ppb K$^{-1}$) and the AcNHThr by ▲ (-7.3 ppb K$^{-1}$).

![Amidechemical_shifts_Diagram](image)
Figure S24. Variation in amide chemical shifts for compound 1 with temperature in 90% H₂O, 10% D₂O, pH 7.51, at 500 MHz with DSS as internal reference.
**Figure S25.** Selected Region of TOCSY Spectrum for Compound 2 in 90% H₂O, 10% D₂O, pH 7.13, at 500 MHz and 300 K with DSS as internal reference. Water suppression by excitation sculpting.

**Figure S26.** Variation in Amide Chemical Shifts for Compound 2 with temperature in 90% H₂O, 10% D₂O, pH 7.13, at 500 MHz with DSS as internal reference. The NHMe is represented by ■ (-7.7 ppb K⁻¹) and the AcNHThr by ▲ (-7.8 ppb K⁻¹).
Figure S27. Variation in amide chemical shifts for compound 1 with temperature in 90% H$_2$O, 10% D$_2$O, pH 7.13, at 500 MHz with DSS as internal reference.
**Figure S28.** Selected Region of TOCSY Spectrum for Compound 4 in 90% H₂O, 10% D₂O, pH 6.35, at 500 MHz and 301 K with DSS as internal reference. Water suppression by excitation sculpting.

**Figure S29.** Variation in Amide Chemical Shifts for Compound 4 with Temperature in 90% H₂O, 10% D₂O with DSS as internal reference. The GlcNHAc is represented by ● (-7.1 ppb K⁻¹), the NHMe is represented by ■ (-6.3 ppb K⁻¹) and the AcNHThr by ▲ (-8.2 ppb K⁻¹).
Figure S30. Variation in amide chemical shifts for compound 1 with temperature in 90% H₂O, 10% D₂O, pH 6.35, at 500 MHz with DSS as internal reference.
**Figure S31.** Selected Region of TOCSY Spectrum for Compound 5 in 90% H$_2$O, 10% D$_2$O at 500 MHz, pH 7.53, and 301 K with DSS as internal reference. Water suppression by excitation sculpting.

**Figure 32.** Variation in Amide Chemical Shifts for Compound 5 with Temperature in 90% H$_2$O, 10% D$_2$O at 500 MHz with DSS as internal reference. The GlcNHAc of the trans rotamer is represented by ● (-9.0 ppb K$^{-1}$) and the NHMe is represented by ■ (-6.9 ppb K$^{-1}$).
Figure S33. Variation in amide chemical shifts for compound 5 with temperature in 90% H₂O, 10% D₂O, pH 7.53, at 500 MHz with DSS as internal reference.
Figure S34. NOESY spectrum for compound 4 in 90% H₂O, 10% D₂O, pH 6.35, at 500 MHz and 301 K with DSS as internal reference. Water suppression by excitation sculpting.
**Figure S35.** GOESY experiments for compound 4 in D$_2$O at 500 MHz and 301 K with DSS as internal reference. Mixing time 300 ms.
**Figure S36.** NOESY spectrum for compound 5 in 90% H$_2$O, 10% D$_2$O, pH 7.53, at 500 MHz and 301 K with DSS as internal reference. Water suppression by excitation sculpting.
Figure S37. GOESY experiments for compound 5 in D$_2$O at 500 MHz and 301 K with DSS as internal reference. Mixing time 300 ms.