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Supporting Information

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Thioglycoligases: Mutant Glycosidases for Thioglycoside Synthesis

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General:

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-300 or Avance-400 Spectrometers. Chemical shifts are reported in δ units (ppm) using residual ^{1}H and ^{13}C signals of the deuterated solvents as reference: δ_H ($CDCl_3$) 7.26, δ_H (CD_3OD) 3.31, δ_C ($CDCl_3$) 77.0, δ_C (CD_3OD) 49.0. Electrospray mass spectra were recorded on a PE Sciex API 300 LC/MS/MS instrument by direct injection of the compounds in a 1:1 CH_3CN/H_2O solution. Melting points were determined with a Mel-Temp II apparatus and are not corrected. Silica gel 60 (230-400 mesh) from SiliCycle was used for column chromatography. The petroleum ether used for column chromatography had a boiling point range from 35-60°C. Amberlite IR-120PLUS from Aldrich was transformed into the H^+-form before use. All reagents and solvents were purchased from Aldrich, Fluka, Sigma or Fisher Scientific. Solvents were dried over CaH_2 (CH_2Cl_2, pyridine, toluene, acetonitrile), over Mg (methanol) or over molecular sieves 4Å (DMF). All reactions were carried out under a dry nitrogen atmosphere.

Chemical Synthesis of deoxythio sugar acceptors:

p-Nitrophenyl 2,3,6-tri-O-benzoyl-β-D-galactopyranoside:

Benzoyl chloride (1.50 ml, 1.82 g, 12.9 mmol) was added dropwise to a solution of *p*-nitrophenyl β-D-galactopyranoside (1.00 g, 3.32 mmol) in DMF (15 ml) and pyridine (15 ml) at -20°C. After stirring for 5 h at -5°C another 0.30 ml of benzoyl chloride (0.36 g, 2.59 mmol) was added dropwise, and the solution was stirred for 2 h at -5°C. Water (10 ml) was added and the mixture was concentrated by evaporation in vacuo. The residue was

dissolved in CH_2Cl_2, and the organic phase was washed sequentially with saturated aqueous $NaHCO_3$, 1 M HCl and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (toluene → 4:1 toluene/EtOAc) followed by crystallization from hot toluene yielded *p*-nitrophenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (850 mg, 1.39 mmol, 42%); mp 180-181°C; ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.05 (m, 2 H, Ar), 8.0 - 7.3 (m, 15 H, 3xBz), 7.06 (m, 2 H, Ar), 6.10 (dd, 1 H, $J_{2,3}$ 10.3 Hz, $J_{2,1}$ 7.9 Hz, H-2), 5.48 (dd, 1 H, $J_{3,2}$ 10.3 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.40 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.76 (dd, 1 H, $J_{6,6}$ 11.7 Hz, $J_{6,5}$ 5.1 Hz, H-6), 4.68 (dd, 1 H, $J_{6,6}$ 11.7 Hz, $J_{6,5}$ 7.7 Hz, H-6), 4.47 (m, 1 H, H-4), 4.31 (m, 1 H, H-5), 2.66 (d, 1 H, $J_{OH,4}$ 4.5 Hz, OH); ^{13}C-NMR (75 MHz): δ_C ($CDCl_3$): 166.4, 165.8, 165.3, 161.3, 143.0, 133.7, 133.5, 129.9, 129.7, 129.7, 129.3, 129.0, 128.6, 128.6, 128.5, 128.5, 125.6, 116.8, 98.8, 73.9, 73.2, 69.0, 67.1, 63.0; ESI-MS: *m/z* = 636.5 $[M + Na]^+$ (expected for $C_{33}H_{27}NO_{11}Na^+$: *m/z* = 636.2).

p-Nitrophenyl 4-S-acetyl-2,3,6-tri-O-benzoyl-4-deoxy-4-thio-β-D-glucopyranoside:
Trifluoromethanesulfonic anhydride (0.44 ml, 0.75 g, 2.7 mmol) was added dropwise to a solution of *p*-nitrophenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (813 mg, 1.33 mmol) in 20 ml CH_2Cl_2 and 1.2 ml pyridine at 0°C. After 1 h at 0°C, CH_2Cl_2 (50 ml) was added, and the organic layer was washed with saturated aqueous $NaHCO_3$, 1M HCl and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give 990 mg of a yellowish solid (100%). Potassium thioacetate (460 mg, 4.0 mmol) and HMPA (10 ml) were added, and the suspension was stirred at RT for 1 h. A mixture of EtOAc/Et_2O (1:1, 50 ml) was added, and the organic layer was washed twice with water, with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (19:1 → 3:1 PE/EtOAc) and crystallization from hot EtOAc yielded *p*-nitrophenyl 4-*S*-acetyl-2,3,6-tri-*O*-benzoyl-4-deoxy-4-thio-β-D-glucopyranoside as a white powder (520 mg, 59%); mp 249°C (degradation); ^{1}H-NMR (300 MHz): δ_H ($CDCl_3$): 8.06 (m, 2 H, Ar), 8.02 - 7.31 (m, 15 H, 3xBz), 7.02 (m, 2 H, Ar), 5.84 (dd, 1 H, $J_{3,4}$ 10.8 Hz, $J_{3,2}$ 9.3 Hz, H-3), 5.73 (dd, 1 H, $J_{2,3}$ 9.3 Hz, $J_{2,1}$ 7.6 Hz, H-2), 5.43 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.79 (dd, 1 H, $J_{6,6}$ 12.0 Hz, $J_{6,5}$ 2.2 Hz, H-6), 4.54 (dd, 1 H, $J_{6,6}$ 12.0 Hz, $J_{6,5}$ 7.2 Hz, H-6), 4.37 (m, 1 H, H-5), 4.10 (t, 1 H,

$J_{4,5}$=$J_{4,3}$ 10.8 Hz, H-4), 2.28 (s, 3 H, Ac); ^{13}C-NMR (75 MHz): δ_C ($CDCl_3$): 192.7, 165.9, 165.6, 165.0, 161.1, 143.1, 138.8, 133.6, 133.6, 129.9, 129.8, 129.7, 129.4, 128.8, 128.6, 128.5, 128.4, 125.6, 116.8, 98.3, 73.7, 72.5, 71.2, 63.7, 44.3, 30.8; ESI-MS: *m/z* = 694.0 $[M + Na]^+$ (expected for $C_{35}H_{29}NO_{11}SNa^+$: *m/z* = 694.1).

p-Nitrophenyl 4-deoxy-4-thio-β-D-glucopyranoside (**1**):

A solution of *p*-nitrophenyl 4-*S*-acetyl-2,3,6-tri-*O*-benzoyl-4-deoxy-4-thio-β-D-glucopyranoside (230 mg, 0.34 mmol) in 10 ml MeOH containing catalytic amounts of MeONa was stirred for 3 h at RT. The mixture was neutralized with Amberlite IR-120PLUS (H^+-form). After filtration, DTT (280 mg, 1.8 mmol) in 2 ml of degassed water was added, and N_2 was bubbled through the solution for 5 min. After stirring under N_2 overnight the mixture was concentrated in vacuo. Column chromatography (3:2 toluene/EtOAc → EtOAc) afforded **1** as a white powder (80 mg, 0.25 mmol, 74%); ^{1}H-NMR (400 MHz): δ_H (d_4-MeOH): 8.22 (m, 2 H, Ar), 7.23 (m, 2 H, Ar), 5.10 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.94 (dd, 1 H, $J_{6,6}$ 12.3 Hz, $J_{6,5}$ 1.9 Hz, H-6), 3.84 (dd, 1 H, $J_{6,6}$ 12.3 Hz, $J_{6,5}$ 4.8 Hz, H-6), 3.62 (m, 1 H, H-5), 3.49 (dd, 1 H, $J_{2,3}$ 9 Hz, $J_{2,1}$ 7.6, H-2), 3.41 (dd, 1 H, $J_{3,4}$ 10.2 Hz, $J_{3,2}$ 9.0 Hz, H-3), 2.84 (t, 1 H, $J_{4,5}$=$J_{4,3}$ 10.2 Hz, H-4); ^{13}C-NMR (75 MHz): δ_C (d_4-MeOH): 163.9, 143.9, 126.6, 117.7, 101.5, 79.8, 78.8, 75.7, 62.9, 43.0; ESI-MS: *m/z* = 340.0 $[M + Na]^+$ (expected for $C_{12}H_{15}NO_7SNa^+$: *m/z* = 340.1).

p-Nitrophenyl 2,3-di-O-benzoyl-α-L-arabinopyranoside:

Benzoyl chloride (1.0 ml, 1.25 g, 8.7 mmol) was added dropwise to a solution of *p*-nitrophenyl α-L-arabinopyranoside (1.00 g, 3.8 mmol) in DMF (30 ml) and pyridine (10 ml) at -20°C. The mixture was allowed to warm to RT overnight while stirring and worked up as *p*-nitrophenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside. Column chromatography (5:1 → 2:1 PE/EtOAc) and crystallization from EtOAc/heptane yielded *p*-nitrophenyl 2,3-di-*O*-benzoyl-α-L-arabinopyranoside as a white powder (520 mg, 1.11 mmol, 29%); mp 150-151°C; ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.18 (m, 2 H, Ar), 8.13-7.40 (m, 10 H, 2xBz), 7.08 (m, 2 H, Ar), 5.80 (dd, 1 H, $J_{2,3}$ 6.4 Hz, $J_{2,1}$ 4.2 Hz, H-2), 5.55 (dd, 1 H, $J_{3,2}$ 6.4

Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.52 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 4.46 (m, 1 H, H-4), 4.17 (dd, 1 H, $J_{5,5}$ 11.9 Hz, $J_{5,4}$ 7.0 Hz, H-5), 3.90 (dd, 1 H, $J_{5,5}$ 11.9 Hz, $J_{5,4}$ 3.4 Hz, H-5), 2.40 (d, 1 H, $J_{OH,4}$ 5.3 Hz, OH); ^{13}C-NMR (100 MHz): δ_C ($CDCl_3$): 165.9, 165.0, 161.2, 142.9, 133.8, 133.7, 129.9, 129.8, 128.8, 128.6, 128.6, 125.8, 116.5, 96.8, 71.4, 69.0, 65.1, 62.9; ESI-MS: *m/z* = 502.0 $[M + Na]^+$ (expected for $C_{25}H_{21}NO_9Na^+$: *m/z* = 502.1).

p-Nitrophenyl 4-S-acetyl-2,3-di-O-benzoyl-4-deoxy-4-thio-β-D-xylopyranoside:

The partially protected glycoside *p*-nitrophenyl 2,3-di-*O*-benzoyl-α-L-arabinopyranoside (340 mg, 0.71mmol) was treated as described for the preparation of *p*-nitrophenyl 4-*S*-acetyl-2,3,6-tri-*O*-benzoyl-4-deoxy-4-thio-β-D-glucopyranoside. Column chromatography (6:1 → 2:1 PE/EtOAc) gave *p*-nitrophenyl 4-*S*-acetyl-2,3-di-*O*-benzoyl-4-deoxy-4-thio-β-D-xylopyranoside as a white foam (215 mg, 0.4 mmol, 56%); ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.19 (m, 2 H, Ar), 8.1-7.35 (m, 10 H, 2xBz), 7.09 (m, 2 H, Ar), 5.60 (m, 1 H, H-3), 5.57 (m, 1 H, H-2), 5.50 (d, 1 H, $J_{1,2}$ 4.9 Hz, H-1), 4.42 (dd, 1 H, $J_{5,5}$ 12.2 Hz, $J_{5,4}$ 4.2 Hz, H-5), 4.07 (m, 1 H, H-4), 3.75 (dd, 1 H, $J_{5,5}$ 12.2 Hz, $J_{5,4}$ 7.5 Hz, H-5), 2.35 (s, 3 H, Ac); ^{13}C-NMR (100 MHz): δ_C ($CDCl_3$): 193.4, 165.2, 165.0, 161.1, 143.0, 133.6, 129.9, 128.9, 128.8, 128.5, 125.8, 116.6, 97.7, 70.2, 69.8, 63.4, 41.3, 30.7; ESI-MS: *m/z* = 560.0 $[M + Na]^+$ (expected for $C_{27}H_{23}NO_9SNa^+$: *m/z* = 560.1).

p-Nitrophenyl 4-deoxy-4-thio-β-D-xylopyranoside (**2**):

p-Nitrophenyl 4-*S*-acetyl-2,3-di-*O*-benzoyl-4-deoxy-4-thio-β-D-xylopyranoside (190 mg, 0.35 mmol) was deprotected as described for compound **1**. Column chromatography (1:1 → 1:3 PE/EtOAc) gave **2** as a white powder (70 mg, 0.24 mmol, 69%); ^{1}H-NMR (400 MHz): δ_H (d_4-MeOH): 8.20 (m, 2 H, Ar), 7.18 (m, 2 H, Ar), 5.03 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.98 (dd, 1 H, $J_{5,5}$ 11.8 Hz, $J_{5,4}$ 5.0 Hz, H-5), 3.52 (t, 1 H, $J_{5,5}$=$J_{5,4}$ 11.8 Hz, H-5), 3.44 (dd, 1 H, $J_{2,3}$ 8.9 Hz, $J_{2,1}$ 7.6 Hz, H-2), 3.32 (m, 1 H, H-3), 2.84 (m, 1 H, H-4); ^{13}C-NMR (100 MHz): δ_C (d_4-MeOH): 163.7, 143.9, 126.6, 117.6, 102.3, 78.6, 75.9, 69.2, 42.0; ESI-MS: *m/z* = 310.0 $[M + Na]^+$ (expected for $C_{11}H_{13}NO_6SNa^+$: *m/z* = 310.0).

Enzymatic synthesis of thiooligosaccharides:

The deoxythio sugars **1** or **2** (20 mM), the DNP-donors 2,4-dinitrophenyl *β*-D-glucopyranoside (DNP-Glc) or 2,5-dinitrophenyl *β*-D-mannopyranoside (DNP-Man) (30 mM) and the mutant enzymes Abg E171A or Man2A E429A (~1 mg ml^{-1}) were incubated for ~3 h at RT in phosphate buffer (80 mM). DNP-Glc or DNP-Man was added to a total concentration of 45 mM, and the solution was incubated at RT for ~1 h. After lyophilization standard per-*O*-acetylation with pyridine/Ac_2O and subsequent workup was performed. The final purification by column chromatography (9:1 → 1:1 toluene/EtOAc) yielded products **3**-**6**.

p-Nitrophenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-S-2,3,6-tri-O-acetyl-4-deoxy-4-thio-β-D-glucopyranoside (**3**):

25 mg (64%); mp 161.5-162°C (hot toluene); ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.19 (m, 2 H, Ar), 7.09 (m, 2 H, Ar), 5.29 - 5.16 (m, 4 H, H-1, H-2, H-3', H-3), 5.06 (t, 1 H, $J_{4',3'}$=$J_{4',5'}$ 9.8 Hz, H-4'), 4.94 (t, 1 H, $J_{2',1'}$=$J_{2',3'}$ 9.6 Hz, H-2'), 4.77 (d, 1 H, $J_{1',2'}$ 10 Hz, H-1'), 4.64 (dd, 1 H, $J_{6,6}$ 12.1 Hz, $J_{6,5}$ 1.7 Hz, H-6), 4.38 (dd, 1 H, $J_{6,6}$ 12.1 Hz, $J_{6,5}$ 5.5 Hz, H-6), 4.31 (dd, 1 H, $J_{6',6'}$ 12.4 Hz, $J_{6',5'}$ 2.2 Hz, H-6'), 4.13 (dd, 1 H, $J_{6',6'}$ 12.4 Hz, $J_{6',5'}$ 4.8 Hz, H-6'), 4.06 (m, 1 H, H-5), 3.75 (m, 1 H, H-5'), 3.05 (t, 1 H, $J_{4,5}$=$J_{4,3}$ 10.6 Hz, H-4), 2.10, 2.09, 2.07, 2.06, 2.04, 2.02, 2.00 (7xs, 21 H, 7xAc); ^{13}C-NMR (100 MHz): δ_C ($CDCl_3$): 170.4, 170.1, 170.0, 170.0, 169.3, 169.3, 169.2, 161.3, 143.2, 125.7, 116.7, 98.0, 81.7, 75.9, 74.6, 73.6, 72.4, 70.3, 69.9, 68.1, 63.3, 61.9, 45.8, 20.7-20.4 (7x); ESI-MS: *m/z* = 796.0 $[M + Na]^+$ (expected for $C_{32}H_{39}NO_{19}SNa^+$: *m/z* = 796.2).

p-Nitrophenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-S-2,3-di-O-acetyl-4-deoxy-4-thio-β-D-xylopyranoside (**4**):

29 mg (79%); mp 122-123°C (EtOAc/heptane); ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.20 (m, 2 H, Ar), 7.07 (m, 1 H, Ar), 5.21 - 5.11 (m, 4 H, H-1, H-2, H-3, H-3'), 5.07 (t, 1 H, $J_{4',3'}$=$J_{4',5'}$ 9.9 Hz, H-4'), 5.00 (dd, 1 H, $J_{2',1'}$ 10 Hz, $J_{2',3'}$ 9.3 Hz, H-2'), 4.63 (d, 1 H, $J_{1',2'}$ 10

Hz, H-1'), 4.29 (dd, 1 H, $J_{5,5}$ 12.4 Hz, $J_{5,4}$ 4.5 Hz, H-5), 4.19 (d, 2 H, $J_{6',5'}$ 3.5 Hz, 2xH-6'), 3.74 (dt, 1 H, $J_{5',4'}$ 9.9 Hz and $J_{5',6'}$ 3.5 Hz, H-5'), 3.65 (dd, 1 H, $J_{5,5}$ 12.4 Hz, $J_{5,4}$ 9.6 Hz, H-5), 3.23 (m, 1 H, H-4), 2.10, 2.06, 2.05, 2.03, 2.02, 2.00 (6xs, 18 H, 6xAc); ^{13}C-NMR (100 MHz): δ_C ($CDCl_3$): 170.4, 170.0, 169.9, 169.2, 169.2, 169.0, 161.1, 143.0, 125.7, 116.5, 98.1, 81.9, 76.0 (x2), 73.6, 71.3, 69.9, 67.9, 65.6, 61.8, 42.7, 20.5 (6x); ESI-MS: *m/z* = 724.5 $[M + Na]^+$ (expected for $C_{29}H_{35}NO_{17}SNa^+$: *m/z* = 724.2).

p-Nitrophenyl (2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-S-2,3,6-tri-O-acetyl-4-deoxy-4-thio-β-D-glucopyranoside (**5**):

5 mg (35%); ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.20 (m, 2 H, Ar), 7.09 (m, 2 H, Ar), 5.36 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, $J_{2',1'}$ 1 Hz, H-2'), 5.32 - 5.17 (m, 4 H, H-1, H-2, H-3, H-4'), 5.09 (dd, 1 H, $J_{3',4'}$ 10.1 Hz, $J_{3',2'}$ 3.5 Hz, H-3'), 4.98 (d, 1 H, $J_{1',2'}$ 1 Hz, H-1'), 4.61 (dd, 1 H, $J_{6,6}$ 12.2 Hz, $J_{6,5}$ 2.1 Hz, H-6), 4.46 (dd, 1 H, $J_{6,6}$ 12.2 Hz, $J_{6,5}$ 5.4 Hz, H-6), 4.33 (dd, 1 H, $J_{6',6'}$ 12.4 Hz, $J_{6',5'}$ 2.4 Hz, H-6'), 4.15 (dd, 1 H, $J_{6',6'}$ 12.4 Hz, $J_{6',5'}$ 5.5 Hz, H-6'), 4.08 (m, 1 H, H-5), 3.75 (m, 1 H, H-5'), 3.07 (t, 1 H, $J_{4,5}$=$J_{4,3}$ 10.7 Hz, H-4), 2.20, 2.12, 2.10, 2.07, 2.06, 2.06, 1.98 (7xs, 21 H, 7xAc); ^{13}C-NMR (75 MHz): δ_C ($CDCl_3$): 170.5, 170.3, 170.3, 170.0, 169.8, 169.6, 169.2, 161.2, 143.2, 125.7, 116.6, 98.1, 79.3, 74.3, 72.1, 71.5, 69.9, 69.7, 65.7, 45.1, 20.6 (7x); ESI-MS: *m/z* = 796.0 $[M + Na]^+$ (expected for $C_{32}H_{39}NO_{19}SNa^+$: *m/z* = 796.2).

p-Nitrophenyl (2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-S-2,3-di-O-acetyl-4-deoxy-4-thio-β-D-xylopyranoside (**6**):

25 mg (82%); ^{1}H-NMR (400 MHz): δ_H ($CDCl_3$): 8.20 (m, 2 H, Ar), 7.07 (m, 2 H, Ar), 5.45 (d, 1 H, $J_{2',3'}$ 3.5 Hz, H-2'), 5.26-5.12 (m, 4 H, H-1, H-2, H-3, H-4'), 5.07 (dd, 1 H, $J_{3',4'}$ 10.1 Hz, $J_{3',2'}$ 3.5 Hz, H-3'), 4.87 (s, 1 H, H-1'), 4.28-4.15 (m, 3 H, H-5, H-6', H-6'), 3.75 (m, 1 H, H-5'), 3.66 (dd, 1 H, $J_{5,5}$ 12.4 Hz, $J_{5,4}$ 10.0 Hz, H-5), 3.28 (m, 1 H, H-4), 2.20, 2.10, 2.08, 2.06, 2.04, 2.03, 1.97 (6xs, 18 H, 6xAc); ^{13}C-NMR (75 MHz): δ_C ($CDCl_3$): 170.4, 170.2, 170.0, 169.9, 169.6, 169.3, 161.1, 143.1, 125.8, 116.5, 98.3, 79.8, 76.8, 71.7, 71.5, 70.9,

69.8, 65.5 (2x), 62.6, 43.0, 20.5 (6x); ESI-MS: *m/z* = 724.5 $(M + Na]^+$ (expected for $C_{29}H_{35}NO_{17}SNa^+$: *m/z* = 724.2).

Competition study for evaluation of relative rates:

pNP-Xyl (5.5 mM), **2** (5.5 mM), DNP-Man (3.75 mM) and Man2A E429A (~1 mg ml^{-1}) were incubated in phosphate buffer (50 mM, pH 6.8) for 3 h at RT. After removal of an aliquot DNP-Man was added to a final concentration of 22 mM. After 5 h at RT another aliquot was taken. The aliquots were diluted 1:3 with acetonitrile and centrifuged before applying to the HPLC.

HPLC analysis was performed using a Waters 600E multisolvent delivery system with acetonitrile (A)/ water (B) as mobile phase (linear gradient: 80% A→ 60% A in 15 min, flow: 1 ml min^{-1}), a Waters 2486 Dual λ Absorbance Detector (detection at 280 nm), a TOSO HAAS Amide 80 column (4.6 x 250 mm) and Millenium 3.20 software.