Supplementary Information

# Chemoenzymatic Synthesis of $GM_3$ and $GM_2$ Gangliosides Containing a Truncated Ceramide Functionalized for Glycoconjugation and Solid Phase Applications

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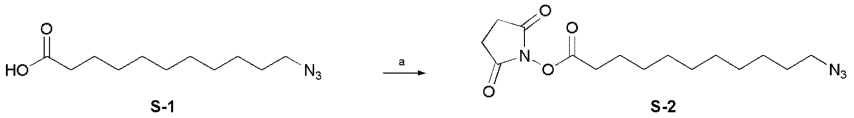
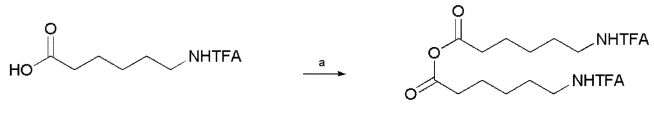


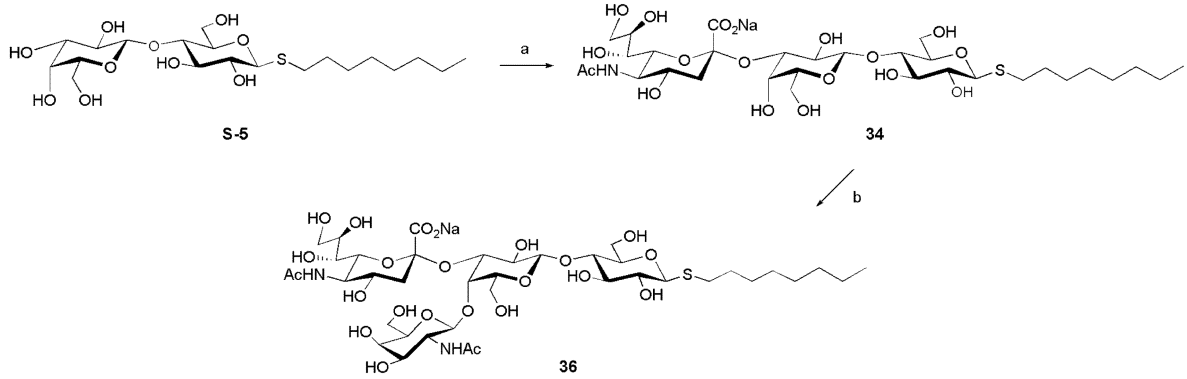

**Scheme S-1.** *Reagents and conditions*: (a) 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide, *N*-hydroxysuccinimide, dichloromethane, 43%.

***N*-(11-Azidoundecanoyloxy)succinimide (S-2).** 11-Azidoundecanoic acid[1] (156 mg; 0.687 mmol), 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide (395 mg; 2.1 mmol; 3.0 eq), and *N*-hydroxysuccinimide (237 mg; 2.1 mmol; 3.0 eq) were dissolved in dry dichloromethane (15 mL). The reaction mixture was stirred overnignt until the reaction was completed. The solvent was then evaporated and purification by silica gel colum chromatography afforded **S-2** as a white solid (97 mg; 0.30 mmol; 43%): $^1$H NMR (500 MHz, $CD_3OD$): $\delta_H$ 3.25 (t, 2H, $J$ = 6.9 Hz, $\underline{CH_2}N_3$), 2.81 (s, 4H, $H_{Suc}$), 2.60 (t, 2H, $J$ = 7.2 Hz, $\underline{CH_2}C(O)$), 1.71 (quintet, 2H, $J$ = 7.4 Hz, $\underline{CH_2}CH_2N_3$), 1.57 (quintet, 2H, $J$ = 7.1 Hz, $\underline{CH_2}CH_2C(O)$), 1.43-1.32 (m, 12H, alkane $\underline{CH_2}$); $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta_C$ 171.9 (2C), 170.3, 52.5, 31.6, 30.5, 30.4, 30.2, 30.1, 29.9, 29.7, 27.8, 26.5 (2 C), 25.7. ESI HRMS calc'd for $C_{15}H_{24}N_4O_4Na$: 347.16898. Found: 347.16876.




**Scheme S-1.** *Reagents and conditions*: (a) 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide, dichloromethane, 55%.

**6-Trifluoroacetamidohexanoic anhydride (S-4)**. 6-Trifluoroacetamidohexanoic acid[2] (200 mg; 0.880 mmol) and 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide (88.0 mg; 0.459 mmol; 0.522 eq) were dissolved in dichloromethane (10 mL). The reaction mixture was stirred for 6 hr at room temperature. The organic phase was washed with $H_2O$, dried with anhydrous $Na_2SO_4$, and evaporated to yield **S-4** (100 mg; 0.487 mmol; 55%): $^1$H NMR (500 MHz, $D_2O$) : $\delta_H$ 3.31-3.26 (m, 4H, $\underline{CH_2}$NHTFA), 2.50 (t, 4H, $J$ = 7.3 Hz, $\underline{CH_2}C(O)$), 1.70-1.55 (m, 8H, $\underline{CH_2}CH_2$NHTFA, $\underline{CH_2}CH_2C(O)$), 1.42-1.36 (m, 4H, $CH_2$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta_C$ 171.0, 159.0 (q, $J_{C,F}$ = 37 Hz), 117.6 (q, $J_{C,F}$ = 286 Hz), 40.5, 35.7, 29.4, 27.0, 24.8. ESI HRMS calc'd for $C_{16}H_{20}N_2O_4F_3$: 459.13251. Found: 459.13221.




**Scheme 1.** *Reagents and conditions*: (a) α-(2,3)-Neu5Ac transferase, 87%; (b) β-(1,4)-GalNAc transferase, UDP-GlcNAc 4-epimerase, UDP-GlcNAc, 88%.

**Octyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-*O*-(β-D-galacto-pyranosyl)-(1→4)-*O*-1-thio-β-D-glucopyranoside (34)**: The acceptor **S-5**[3] (28.9 mg; 61.4 μmol; 12.3 mM in reaction), crude CMP-Neu5Ac (~89.3 μmol; ~17.9 mM in reaction; ~1.2 eq), $MnCl_2$ (100 μL of 0.5 M solution; 10 mM in reaction), $MgCl_2$ (100 μL of 0.5 M solution; 10 mM in reaction), dithiothreitol (37.5 μL of 0.1 M solution; 2.5 mM in reaction), 0.05 M HEPES buffer, pH 7.5 containing 10% (v/v) glycerol (2.25 mL), α-(2,3)-sialyltransferase (2.0 mL in 0.05 M HEPES buffer, pH 7.5 containing 10% (v/v) glycerol; ~1.25 U), and alkaline phosphatase (Roche Bioscience) (12.5 μL; ~12.5 U) were combined in a 45 mL Falcon tube. The mixture was tumbled gently at room temperature for 24 hr after which time TLC ($CHCl_3$-MeOH-$H_2O$-AcOH; 10:7:1:2) indicated almost complete disappearance of the starting material **6**. The reaction mixture was centrifuged at 14,000 rpm for 30 min and the supernatant applied to a Sep-Pak (tC18; 10g). Gradient elution (MeOH-$H_2O$) afforded **34**. Further purification by HPLC (Beckman $C_{18}$-silica semi-preparative column) using a gradient of water and methanol yielded **34** (40.6 mg; 53.3 μmol; 87%): $[\alpha]_D$ –28.9° (*c* 0.51, $H_2O$). $^1$H NMR (500 MHz, $D_2O$): $\delta_H$ 4.52 (d, 2H, $J_{1,2}$ = 9.1 Hz, H-$1_{Gal}$, H-$1_{Glc}$), 4.10 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.0 Hz, H-$3_{Gal}$), 3.96 (m, 2H, H-6$a_{Glc}$, H-$4_{Gal}$), 3.91-3.79 (m, 7H, H-$8_{Neu}$, H-9$a_{Neu}$, H-$5_{Gal}$, H-9$b_{Neu}$, H-$6_{Neu}$, H-$7_{Neu}$, H-$2_{Gal}$), 3.75-3.56 (m, 6H, H-$4_{Neu}$, H-6$a_{Gal}$, H-6$b_{Gal}$, H-$5_{Neu}$, H-$3_{Glc}$, H-$4_{Glc}$), 3.37-3.33 (m, 1H, H-$2_{Glc}$), 2.78-2.70 (m, 2H, $\underline{CH_2}$S, H-3$e_{Neu}$), 2.03 (s, 3H, C(O)$\underline{CH_3}$), 1.79 (dd, 1H, $J_{3a,3e}$ = 12.1 Hz, $J_{3a,4}$ = 12.1 Hz, H-3$a_{Neu}$), 1.63 (quintet, 2H, *J* = 7.4 Hz, $\underline{CH_2}CH_2$S), 1.40-1.37 (m, 2H, $\underline{CH_2}CH_2CH_2$S), 1.32-1.26 (m, 8H, alkane $\underline{CH_2}$), 0.86 (t, 3H, *J* = 6.9 Hz, $CH_3$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta_C$ 175.9, 174.7, 103.5, 100.7, 86.1, 79.5, 79.0, 76.7, 76.4, 76.1, 73.8, 72.9, 72.6, 70.2, 69.2, 69.0, 68.4, 63.5, 61.9, 61.1, 52.6, 40.5, 32.0, 30.8, 30.2, 29.1, 29.0, 28.8, 22.9, 14.3. ESI HRMS calc'd for $C_{31}H_{54}NO_{18}S$: 760.30671. Found:760.30648.

**Octyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-[2-acetamido-2-deoxy-*O*-β-D-galactopyranosyl-(1→4)]-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-1-thio-β-D-glucopyranoside (36)** : The acceptor **34** (9.75 mg, 12.8 μmol) and UDP-*N*-acetylglucosamine disodium salt (11.7 mg, 18.0 μmol, 1.4 eq) were combined in a Falcon tube. UDP-*N*-acetylglucosamine 4-epimerase (1.0 mL, ~1.0 U) in 0.2 M NaCl, 1.0 mM EDTA, 20 mM HEPES, 5 mM β-mercaptoethanol, pH 7.0 and *N*-acetylgalactosaminyl transferase (2.9 mL, ~1.0 U) were added to the mixture followed by 48 μL of 0.5 M $MgCl_2$ (6.0 mM in reaction) and alkaline phosphatase (10 μL, ~10 U). The reaction was gently tumbled for 16 hr until the reaction was completed as observed by silica gel TLC (EtOAc-MeOH-$H_2O$-AcOH; 6:3:3:2). The mixture was centrifuged for 20 min at 14,000 rpm, the supernatant was collected and applied to a Sep-Pak (tC18; 5g). Elution with a gradient of water-methanol afforded **36**. Further purification by HPLC (Beckman $C_{18}$-silica semi-preparative column) using a gradient of water and methanol yielded **36** (10.8 mg, 11.3 μmol, 88%): $[\alpha]_D$ –5.71° (*c* 0.30, $H_2O$). $^1$H NMR (500 MHz, $D_2O$): $\delta_H$ 4.74 (m, 1H, H-$1_{GalNAc}$), 4.53-4.51 (m, 2H, H-$1_{Gal}$, H-$1_{Glc}$), 4.14-4.10 (m, 2H, H-$3_{Gal}$, H-$4_{Gal}$), 3.96-3.57 (m, 20H, H-6$a_{Glc}$, H-$4_{GalNAc}$, H-$2_{GalNAc}$, H-9$a_{Neu}$, H-$5_{Neu}$, H-6$b_{Glc}$, H-$4_{Neu}$, H-$5_{Gal}$, H-6$a_{Gal}$, H-6$b_{Gal}$, H-$5_{GalNAc}$, H-6$a_{GalNAc}$, H-6$b_{GalNAc}$, H-$8_{Neu}$, H-$3_{GalNAc}$, H-$3_{Glc}$, H-9$b_{Neu}$, H-$4_{Glc}$, H-$7_{Neu}$, H-$5_{Glc}$), 3.47 (dd, 1H, $J_{6,5}$ = 10.0 Hz, $J_{6,7}$ = 1.2 Hz H-$6_{Neu}$), 3.37-3.32 (m, 2H, H-$2_{Gal}$, H-$2_{Glc}$), 2.80-2.64 (m, 3H, $\underline{CH_2}$S, H-3$e_{Neu}$), 2.03, 2.01 (2 × s, 2 × 3H, 2 × C(O)$\underline{CH_3}$), 1.92 (dd, 1H, $J_{3a,3e}$ = 11.6 Hz, $J_{3a,4}$ = 11.6 Hz, H-3$a_{Neu}$), 1.64 (quintet, 2H, *J* = 7.3 Hz, $\underline{CH_2}CH_2$S), 1.40-1.37 (m, 2H, $\underline{CH_2}CH_2CH_2$S), 1.29-1.27 (m, 8H, alkane $\underline{CH_2}$), 0.86 (t, 3H, *J* = 6.7 Hz, $CH_3$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta_C$ 175.9, 175.7, 174.9, 103.6, 103.5, 102.5, 86.1, 80.0 (2C), 78.0, 76.7, 75.6, 75.2, 74.9, 4.0, 73.2, 72.9, 72.1, 70.9, 69.6, 69.0, 68.7, 62.1, 61.4, 61.2, 52.5, 51.8, 37.8, 32.0, 30.8, 30.2, 29.2, 29.0, 28.8, 23.5, 22.9, 14.3. ESI HRMS calc'd for $C_{39}H_{68}N_2O_{23}S$: 963.38608. Found: 963.38653.

## References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2. C. M. McKeen, L. J. Brown, J. T. G. Nicol, J. M. Mellor, and T. Brown, *Org. Biomol. Chem.*, 2003, **1**, 2267.
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