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Syntheses of α-dystroglycan derived sialylated glycosyl amino acids carrying a novel mannosyl serine/threonine linkage

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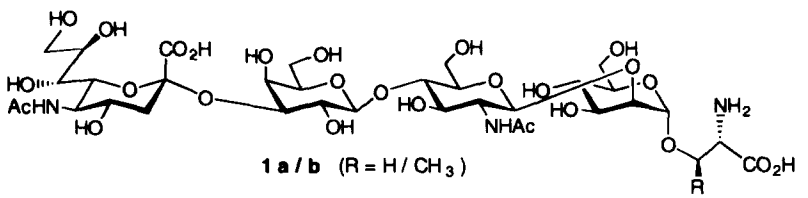
Abstract

An efficient access to the Fmoc protected glycosyl amino acids **20a–d** which correspond to the unique sequence NeuNAc-α-(2,3)-Gal-β-(1,4)-GlcNAc-β-(1,2)-Man-α-Ser/Thr recently found in laminin-binding protein α-dystroglycan, is described. Tetrasaccharide donor **18** was constructed from monosaccharide blocks **2**, **3**, **8** and **9** and coupled with Ser/Thr derivatives **19a–d**. Resultant glycosyl amino acid **20d** was completely deprotected into **1a**.

Keywords: amino acids; amino acid derivatives; glycopeptides; glycoproteins; glycosidation.

α-Dystroglycan (α-DG) is a membrane-associated, extracellular glycoprotein. It is anchored to the cell-membrane via binding to the transmembrane glycoprotein β-dystroglycan (β-DG) to form an α/β-DG-complex which is widely expressed in various types of tissues including skeletal muscle[1a] and schwannoma cells.[1b] The α/β-DG-complex serves as a transmembrane linker between the extracellular matrix and the intracellular skeleton as α-DG binds with a high affinity to extracellular matrix components such as laminin in the striated muscle whereas the intracellular domain of β-DG binds to cytoskeletal proteins such as dystrophin.[1c] Disruption of this transmembrane linkage leads to skeletal muscular dystrophies.[1d]



1a/b (R = H / CH_3)

At present little is known about the biological roles of the carbohydrate moieties of α-DG. Investigations on bovine peripheral nerve α-DG have revealed, that the tetrasaccharide **1a,b**, containing a novel *O*-mannosyl serine/threonine moiety,[1e] is the major constituent of the O-linked carbohydrates and

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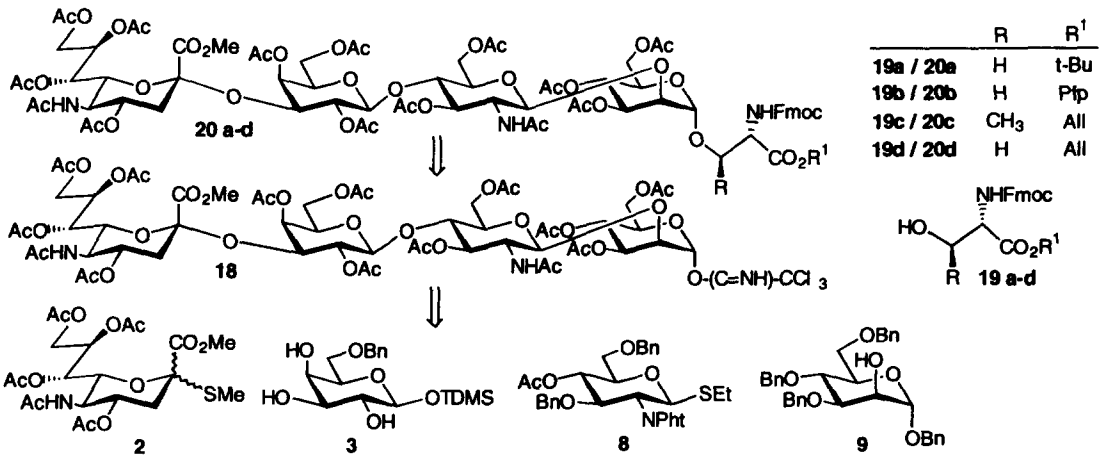
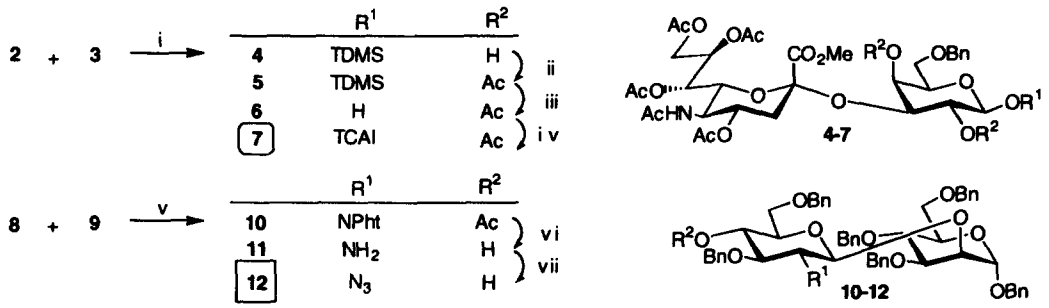
contributes to laminin binding.[1a] To provide molecular probes for elucidating the binding process of bovine peripheral nerve α-DG to laminin, we started the program to synthesize the tetrasaccharidic serine/threonine building blocks **20a–d**. These glycosyl amino acids are designed to be applied to the construction of glycopeptide sequences of α-DG,[1c] possibly to correlate the laminin binding properties with ligand density or sequence specifity in comparison to the native α-DG.

For the synthesis of the Fmoc-protected glycosyl amino acids **20a–d** (Scheme 1) we decided to introduce the amino acid moiety at the end of the synthesis. This option would offer maximum flexibility in choosing the amino acid protection and facilitates the construction process of the tetrasaccharidic moiety by eliminating any technical problem that may arise in the presence of acid or base sensitive amino acid component. The trichloroacetimidate technology[2] was adopted for the final coupling with serine/threonine derivatives **19a–d**,[3] which calls for **18** as a suitable glycosyl donor. Compound **18** was constructed via the two disaccharides **7** and **12** (Scheme 2) which were prepared in a multigram scale from easily available building blocks **2**,[4d] **3**,[5] **8**[6] and **9**.[7]



Scheme 1. Retrosynthetic disconnection of the glycosyl amino acids **20a–d** into the trichloroacetimidate **18** and the Fmoc-protected amino acids **19a–d**. The tetrasaccharide **18** was formed by the readily available building blocks **2**, **3**, **8** and **9** (All=allyl, Pfp=pentafluorophenyl, TDMS=thexyldimethylsilyl).



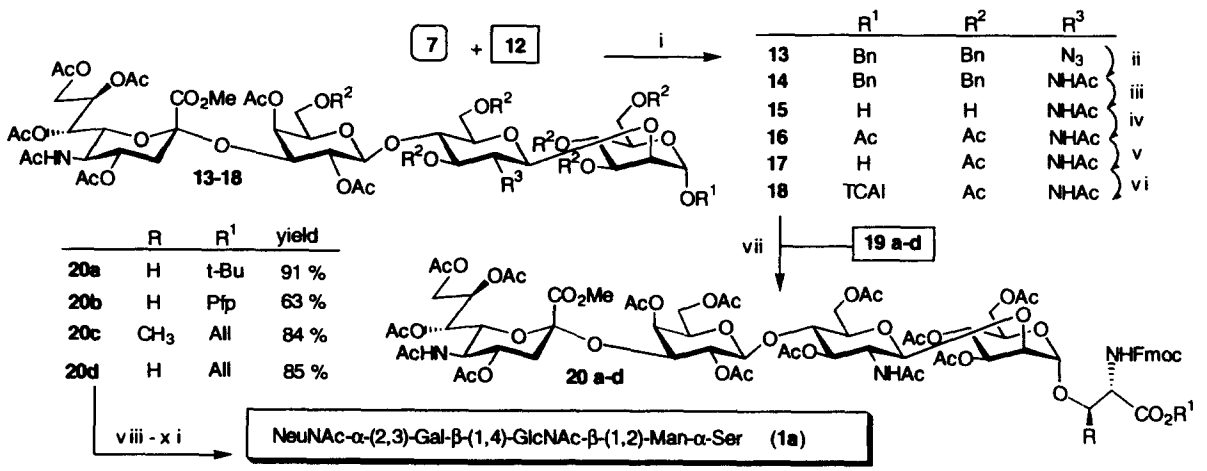
Scheme 2. Formation of the disaccharides **7** and **12**; (i) NIS, TfOH, CH_3CN, MS 3 Å, –40°C; (ii) pyridine, Ac_2O (57%, two steps); (iii) TBAF, HOAc, THF (92%); (iv) Cl_3CCN, DBU, CH_2Cl_2, 0°C (93%); (v) NIS, TfOH, CH_3CN, MS 3 Å, –40°C (95%); (vi) ethylenediamine, *n*-BuOH, 100°C; (vii) TfN_3, DMAP, CH_2Cl_2 (82%, two steps); (TCAI=–(C=NH)–CCl_3)

Synthesis of the α-(2,3)-sialylated disaccharide **7** was accomplished by reaction of thiomethyl-sialoside **2** with the galactose derived triol **3** (Scheme 2). The reaction was performed under standard conditions[4] (NIS, TfOH, CH_3CN[4d]) to afford, after acetylation of **4**, the α-(2,3)-linked product **5** in 57%

yield (Scheme 2, i, ii). Subsequent treatment with tetrabutylammoniumfluoride (TBAF) in the presence of excess acetic acid (Scheme 2, iii) cleaved the thexyldimethylsilyl group. The reaction of the resulting hemiacetal **6** with trichloroacetonitrile and DBU[2] afforded the trichloroacetimidate **7** (Scheme 2, iv) in 93% yield.

The disaccharide **12**, designed to be coupled with trichloroacetimidate **7**, was prepared from the monosaccharides **8** and **9**. Glycosylation of the mannose derivative **9** with the thioglycoside **8**, promoted by NIS/TfOH,[4a–c] afforded disaccharide **10** in high yield (Scheme 2, v). Compound **10** was subsequently reacted with ethylenediamine in *n*-butanol at 100°C[8] furnishing the 2-amino disaccharide **11** (Scheme 2, vi) which was subjected to the azidotransfer reaction with trifluoromethanesulfonyl azide[9] (Tf-N_3) to give the azido compound **12** (Scheme 2, vii) in 82% yield from **10**.

After preparation of the disaccharides **7** and **12**, the formation of the tetrasaccharide **13** was next examined. The best results were obtained with equimolar amounts of **7** and **12** using TMSOTf (0.2 equiv.) as promoter to give tetrasaccharide **13** in 83% yield (Scheme 3, i). Conversion of compound **13** into the peracetylated trichloroacetimidate **18** was accomplished by a series of standard functional group manipulations (Scheme 3, ii–vi). Thus, the azido group of **13** was reduced by 1,3-propanedithiol[10] to afford the corresponding amine which was treated with acetic anhydride to furnish the *N*-acetylated compound **14** in 95% yield (Scheme 3, ii). Complete removal of the benzyl groups (Scheme 3, iii) afforded heptaol **15** which was acetylated to **16** (Scheme 3, iv) and subsequently treated with hydrazine acetate in DMF at 0°C (Scheme 3, v) to give the hemiacetal **17**. Finally, the reaction of **17** with trichloroacetonitrile/DBU[2] (Scheme 3, vi) afforded the designed trichloroacetimidate **18**. The stereoselective attachment of the Fmoc amino acids **19a–d** to **18** was performed at –20°C in CH_2Cl_2:toluene (1:1) by activation with TMSOTf (0.2 equiv.). These conditions (Scheme 3, vii) exclusively provided the α-linked glycosyl amino acids **20a–d**[11] in good yields, which are designed to be incorporated into Fmoc-based solid phase peptide synthesis.



Scheme 3. Formation of the glycosyl amino acids **20a–d** and deprotection of compound **20d**; (i) TMSOTf, CH_2Cl_2, MS 4 Å, –20°C, (83%); (ii) (a) 1,3-propanedithiol, DIPEA, pyridine:H_2O (7:3); (b) Ac_2O (95%, two steps); (iii) H_2, $Pd(OH)_2$, MeOH, HOAc; (iv) pyridine, Ac_2O, 0°C (95%, two steps); (v) H_2NNH_2×HOAc, DMF, 0°C; (vi) Cl_3CCN, DBU, CH_2Cl_2, 0°C (91%, two steps); (vii) TMSOTf, CH_2Cl_2:toluene (1:1), MS 4 Å, –20°C; (viii) $Pd(Ph_3P)_4$, dimedone, THF (90%); (ix) morpholine, $CHCl_3$; (x) NaOMe/MeOH, pH≈9.0; (xi) 5 mM NaOH/aq. MeOH (90%, three steps); (DIPEA=diisopropylethylamine, TCAI=–(C=NH)–CCl_3)

Completely deblocked tetraosyl serine **1a** was obtained from **20d** as follows (Scheme 3, viii–xi). First, the allyl ester was cleaved by $Pd(Ph_3P)_4$ in the presence of dimedone (Scheme 3, viii). Subsequent

removal of the Fmoc group was effected by morpholine (Scheme 3, ix) followed by complete removal of acetyl groups (NaOMe/MeOH, pH≈9.0, (Scheme 3, x)) and saponification of methyl ester (5 mM NaOH/aq. MeOH, (Scheme 3, xi)). Purification by size exclusion chromatography (Bio-Gel® P-2) furnished the deprotected compound **1a** in 81% overall yield from **20d**. The structure of **1a** was confirmed by NMR and ESI mass spectroscopy.[12]

In summary, we have established a versatile and efficient synthetic route to the α-(2,3)-sialylated tetrasaccharidic glycosyl amino acids **20a–d** containing a novel *O*-mannosyl serine/threonine linkage.

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5. (a) Compound **3** was prepared from 2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-D-galactopyranosyl[5b] acetate in three steps: (i) N_2H_4×HOAc, DMF, 0°C (85%); (ii) TDMSCl, imidazole, DMF, 0°C–rt (91%); (iii) NaOMe/MeOH, −10°C (93%); for the use of the TDMS group for anomeric protection, see (c). (b) Dekany, G.; Wright, K.; Toth, I. *J. Carbohydr. Chem.* **1997**, *16*, 983–999. (c) Hummel, G.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*, 1173–1176.
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11. Selected ^{1}H and ^{13}C NMR data ($CDCl_3$): **20a** ^{1}H NMR (500 MHz): δ 5.83 (d, 1H, *J*=8.1 Hz, NH^{Ser}), 4.74 (d, 1H, *J*<1.0 Hz, H-1α^{M}), 4.67 (d, 1H, *J*=8.2 Hz, H-1β^{Gal}), 4.57 (d, 1H, *J*=8.1 Hz, H-1β^{GN}); ^{13}C NMR (125 MHz): δ 100.90 (C-1^{Gal}), 99.66 (C-1^{GN}), 98.27 ($J_{C,H}$=169.9 Hz, C-1^{M}), 96.74 (C-2^{SA}); **20b** ^{1}H NMR (500 MHz): δ 6.07 (d, 1H, *J*=8.8 Hz, NH^{Ser}), 4.83 (d, 1H, *J*<1.0 Hz, H-1α^{M}), 4.66 (d, 1H, *J*=8.3 Hz, H-1β^{Gal}), 4.62 (d, 1H, *J*=7.8 Hz, H-1β^{GN}); ^{13}C NMR (125 MHz):

δ 100.86 (C-1^{Gal}), 99.58 (C-1^{GN}), 98.92 ($J_{C,H}$=168.7 Hz, C-1^{M}), 96.75 (C-2^{SA}); **20c** ^{1}H NMR (400 MHz): δ 5.92 (d, 1H, J=8.3 Hz, NH^{Thr}), 4.70 (d, 1H, J<1.0 Hz, H-1α^{M}), 4.67 (d, 1H, J=8.3 Hz, H-1β^{Gal}), 4.56 (d, 1H, J=7.8 Hz, H-1β^{GN}); ^{13}C NMR (100 MHz): δ 100.85 (C-1^{Gal}), 99.85 (C-1^{GN}), 98.79 ($J_{C,H}$=171.7 Hz, C-1^{M}), 96.70 (C-2^{SA}); **20d** ^{1}H NMR (500 MHz): δ 5.92 (d, 1H, J=8.3 Hz, NH^{Ser}), 4.70 (d, 1H, J<1.0 Hz, H-1α^{M}), 4.67 (d, 1H, J=8.3 Hz, H-1β^{Gal}), 4.56 (d, 1H, J=7.8 Hz, H-1β^{GN}); ^{13}C NMR (125 MHz): δ 100.86 (C-1^{Gal}), 99.77 (C-1^{GN}), 98.39 ($J_{C,H}$=170.4 Hz, C-1^{M}), 96.75 (C-2^{SA}).

12. Selected data for **1a**: ^{1}H NMR (500 MHz, D_2O, ^{t}BuOH as internal standard): δ 4.86 (d, 1H, J=1.4 Hz, H-1α^{M}), 4.56 (d, 1H, J=7.6 Hz, H-1β^{GN}), 4.53 (d, 1H, J=7.9 Hz, H-1β^{Gal}), 2.74 (dd, 1H, J_{vic}=4.6 Hz, J_{gem}=12.4 Hz, H-$3_{eq}{}^{SA}$), 2.03, 2.02 (2s, 6H, NAc), 1.79 (dd, 1H, J_{vic}=12.2 Hz, H-$3_{ax}{}^{SA}$); ^{13}C NMR (125 MHz, D_2O, ^{t}BuOH as internal standard): δ 175.70, 175.40, 174.55, 172.53 (C=O), 103.30 (C-1^{Gal}), 100.50 (C-2^{SA}), 100.20 (C-1^{GN}), 98.29 (C-1^{M}), 40.33 (C-3^{SA}), 23.01, 22.73 (CH_3); ESI MS (negative mode): m/z=922.2 $(M-H)^-$; calcd for $C_{34}H_{56}N_3O_{26}$ (M–H): 922.3; $[\alpha]_D{}^{22}$=–3.0 (0.46, H_2O). **1a** was recently synthesized chemoenzymatically by Matsuo, I.; Isomura, M.; Ajisaka, K. *Tetrahedron Lett.* **1999**, *40*, 5047–5050.