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The First Total Synthesis of the Core Class II Disialylated Hexasaccharide as a Building Block for Glycopeptide Synthesis

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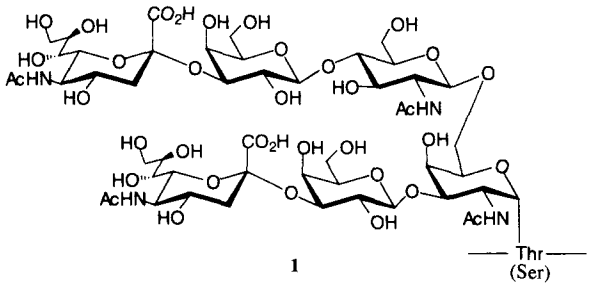
Abstract: The first total synthesis of the protected core class II sialylated glycosyl-Thr hexasaccharide (**3**), utilizing suitably protected building blocks **4**, **5**, **7** and **8** was accomplished.

The title core class II hexasaccharide **1** is the major oligosaccharide of the cell surface glycoprotein, leukosialin,[1] of activated T lymphocytes and is associated with immunological disorders such as leukemia,[2] Wiscott Aldrich Syndrome[3] and AIDS.[4] It is also the major carbohydrate component of the β-subunit of the equine and human chorionic gonadotropin which is responsible for the production of the steroid sex hormones.[5]

In the framework of a project designed to elucidate the nature of the functional importance of the oligosaccharide structures on cell surface glycoproteins, the disialylated core II hexasaccharide **3** was constructed as a building block for glycopeptide synthesis using the Fmoc strategy.



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The protected L-threonine conjugate **3** was synthesized via stereocontrolled glycosylations employing the readily accessible **4**[6], **5**[7], **6**[8], and **8**[9] synthons as shown in Scheme 1. The NIS/TfOH promoted glycosylation of **4** with **5** at -40 °C in acetonitrile led exclusively to the α (2→3) linked disaccharide in 85% yield. Desulfurization followed by lactonization (DBU, THF), oxidative removal of the anomeric

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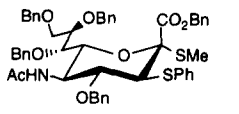
p–methoxyphenyl group and its conversion to the trichloroacetimidate afforded **9**.[10] The TMSOTf promoted glycosylation of the α-trichloroacetimidate **9** with the glucosamine acceptor **8** afforded a mixture of the α and β (1→4) linked trisaccharides **12** (CH_2Cl_2 : hexane 1 : 1, -40 ℃, α : β = 1 : 2, 75%). Treatment of **12** with tetra-n-butylammonium fluoride in the presence of excess acetic acid in tetrahydrofuran gave a mixture of the four diastereomeric hemiacetals **13** quantitatively. The separation of the β and α linked trisaccharides **13** was achieved by preparative thin layer chromatography ($CHCl_3$: t-BuOMe = 9 : 1) at this stage. The β-linked hemiacetal **13** was then converted into its trichloroacetimidate **14** (CCl_3CN, DBU, -10 ℃, α : β = 1 : 1, 96%) which was used as such for the next glycosylation.



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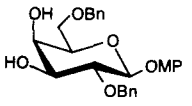

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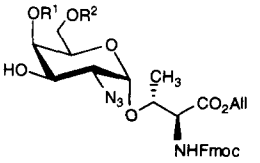
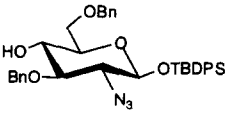


6 : R^1, R^2 = –(Ph)CH–

7 : R^1 = H R^2 = TBDMS



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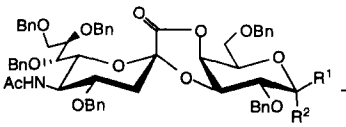
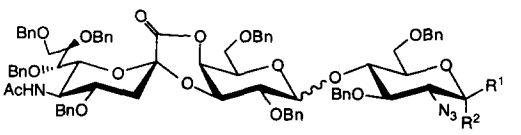
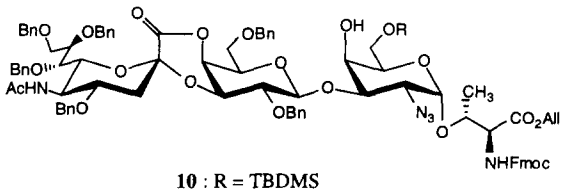


9 : R^1, R^2 = H, OC(=NH)CCl_3



12 : R^1 = OTBDPS R^2 = H

13 : R^1, R^2 = OH, H



10 : R = TBDMS

11 : R = H

MP = 4-methoxyphenyl

TBDMS = t-butyldimethylsilyl

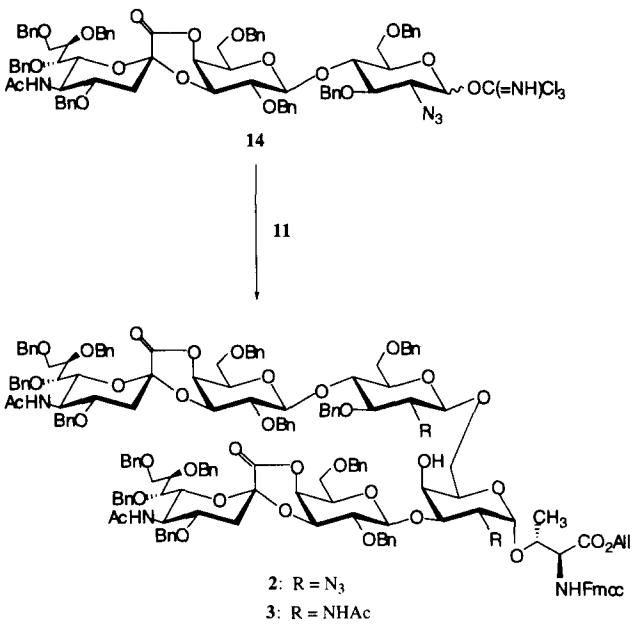
All = allyl

Fmoc = 9-fluorenylmethoxycarbonyl

TBDPS = t-butyldiphenylsilyl

Scheme 1

The galactosamine derivative **6** was debenzylidenated (aq. CF_3CO_2H–CH_2Cl_2, 0°C, 97%) and silylated selectively at the 6 position (TBDMSCl, imidazole, DMF, 90%) to afford the acceptor **7**. Glycosylation of **9** with the galactosamine acceptor **7** promoted with BF_3•OEt_2 (0.8 equiv.) in toluene–CH_2Cl_2 (2 : 1) at –15 ~ -10 ℃ afforded the β (1→3)-linked trisaccharide **10** in 54% yield. Compound **10** was desilylated (aq. CF_3CO_2H-CH_2Cl_2, 0 ℃, 90%) to afford **11** and then glycosylated with **14** (TMSOTf, CH_2Cl_2, -40 °C, β : α = 3 : 1, 80%) to yield the hexasaccharide **2**.



Scheme 2

The hexasaccharide **2** upon treatment with freshly distilled AcSH in pyridine yielded the title compound **3**.[11] Separation of the two diastereomers could be easily achieved by column chromatography at this stage. The structural assignments were made from ^{13}C NMR measurements and their comparison with those of the inner disaccharide, N-(9-fluorenylmethoxycarbonyl)-*O*-[2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester.

In conclusion a facile total synthesis of the core class II disialylated hexasaccharide **3** in a fully protected form has been achieved for the first time.

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11. Selected ^{1}H–NMR, ^{13}C–NMR data and $[\alpha]_D$ are presented below. Compound **6**: $[\alpha]_D$ +111.7° (c 0.38), ^{1}H-NMR (270 MHz): 7.77 (d, 2H, J = 7.6, Ar-Fmoc), 7.63 (d, 2H, J = 7.2, Ar-Fmoc), 5.05 (d, 1H, J = 3.6 Hz, H-1), 3.60 (dd, 1H, J = 3.6, 10.9 Hz, H-2), 3.79 (s, 1H, 1H, H-5), 5.95 (m, 1H, C<u>H</u>=CH$_2$), 5.73 (d, 1H, 8.8, NH), 5.59 [s, 1H, PhC<u>H</u>(O)$_2$], 5.37 (dd, 1H, J = 1.3, 17.1 Hz, =CH$_2$), 5.28 (dd, 1H, J = 1.3, 10.2 Hz, =CH2), 4.70 (d, 2H, J = 5.9 Hz, CO$_2$C<u>H</u>$_2$), 1.32 (d, 3H, J = 6.3 Hz, CH$_3$-Thr). Compound **9**: ^{1}H-NMR (270 MHz): 8.52 (s, 1H, α-C=N<u>H</u>), 6.38 (d, 1H, J = 3.4 Hz, H-1a), 5.33 (d, 1H, J = 3.4 Hz, H-4a), 4.74 (d, 1H, J = 8.9 Hz, N<u>H</u> b), 2.26 (dd, 1H, J = 4.4, 13.1 Hz, H-3b equat.), 1.71 (br, H-3b axial), 1.65 (s, 3H, NHCOC<u>H</u>$_3$); ^{13}CNMR (68 MHz) : 93.7 (C-1a), 95.3 (C-2b), 72.3 (C-4a), 37.8 (C-3b), 23.5 (<u>C</u>H$_3$C=O); Compound **12:** ^{1}H-NMR (400 MHz): 5.15 (d, 1H, J = 4.39 Hz, H-4b), 4.25-4.40 (brd, H-1b), 4.25-4.40 (brd, H-1a), 2.28 (dd, 1H, J = 5.12, 13.41 Hz, 3c equat.), 1.75 (s, 3H, NHCOC<u>H</u>$_3$) , 1.1 (s, 9H, ^{t}Bu); ^{13}C-NMR (100 MHz) : 101.7 (C-1b, β-Gal), 96.7 (C-1a, β-GlcN$_3$), 95.3 (C-1c, NeuAc), 23.7 (<u>C</u>H$_3$C=O), 37.7 (C-2c, NeuAc). FAB MS (M+Na)$^+$ 1622.3; Compound **10**: $[\alpha]_D$ +18.7° (c 2.1), ^{1}H-NMR (400 MHz): 7.75 (d, 2H, J = 7.3 Hz, Ar), 7.61 (d, 2H, J = 7.3 Hz, Ar) 5.92 (m, 1H, C<u>H</u>=CH$_2$), 5.68 (d, 1H, J = 9.8 Hz, NH), 5.35 (d, 1H, J = 16.8 Hz, =CH$_2$), 5.25 (d, 1H, J = 10.6 Hz, =CH$_2$), 5.20 (d, 1H, J = 3.6 Hz, H-4b), 5.03 (d, 1H, J = 3.66 Hz, H-1a), 4.42 (brm, H-1b), 2.19 (dd, 1H, J = 4.6, 14.1, H-3c equat.), 1.69 (s, 3H, NHCOCH$_3$), 1.35 (d, 3H, J = 6.5 Hz, CH$_3$-Thr), 0.86 (s, 9H, ^{t}Bu).^{13}C-NMR (100 MHz) : 102.89 (C-1b, J = 158.5 Hz, β-Gal), 100.0 (C-1a, J = 171.4 Hz, α-GalN$_3$), 95.4 (C-2c, NeuAc), 37.5 (C-3c NeuAc), 23.6 (NHCO<u>C</u>H$_3$), 19.0 (<u>C</u>H$_3$-Thr). FAB MS (M+Na)$^+$ 1681.3. ; Compound **3**: $[\alpha]_D$ +27.5° (c 0.4), ^{1}H-NMR, (600 MHz): 7.74 (d, 2H, J = 7.3 Hz, 2H, Ar-Fmoc), 7.61 (br, 2H, Ar-Fmoc) 5.85 (m, 1H, C<u>H</u>=CH$_2$), 5.32 (d, 2H, J = 17.1 Hz, CH=C<u>H</u>$_2$), 5.27 (d, 2H, J = 10.3 Hz, CH=C<u>H</u>$_2$), 5.14 (d, J = 3.4 Hz, H-4 β-Gal), 4.81 (br, 2H, H-1 β-GlcNAc, H-1 α-GalNAc), 1.84 (s, 3H, NHCOC<u>H</u>$_3$), 1.82 (s, 3H, NHCOC<u>H</u>$_3$), 1.75 ((s, 3H, NHCOC<u>H</u>$_3$), 1.70 (s, 3H, NHCOC<u>H</u>$_3$), 1.28 (3H, CH$_3$-Thr). ^{13}CNMR (150 MHz) : 95.3 (C-2 NeuAc), 99.8 (C-1 β-GlcNAc), 100.6 (C-1 α-GalNAc), 102.0 (C-1 β-Gal), 103.4 (C-1, β-Gal') ; FAB MS (M + I)$^+$ 2920.5, (M+Na)$^+$ 2942.9.