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Synthesis of Symmetrical 4,4'- and 6,6'- Bis(D-Glucose)-based Probes as Tools for the Study of D-Glucose Transport Proteins

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Abstract: Two glucose moieties have been linked from positions -4 or –6 and hydrophobic groups have been placed symmetrically between the hexose moities. To crosslink glucose and obtain 6,6'-probes, advantage has been taken of the unusual reactivity of D-glucurono-γ-lactone with amines. To obtain 4,4'-linked probes, crosslinking via reductive amination has been used. The affinities of these probes for the glucose carrier have been determined and found to be 20 to 200 times that of D-glucose.
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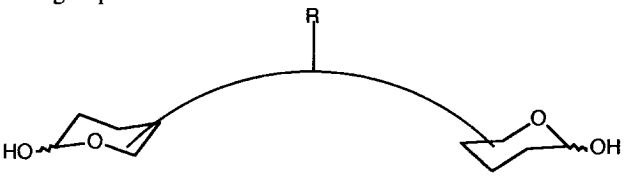
Polyvalent effects in carbohydrate-protein interactions have resulted in a broad and increasing interest in multivalent glycoconjugates, glycopolymers and glycoclusters. [1] Although an impressive number of glycoconjugates has been obtained, [2-4] the assembly of their carbohydrate units has been almost exclusively performed from their anomeric end, with only very few exceptions. [5-8] In the present work, two types of probes in which glucose moieties are linked from their non-reducing end (positions -4 or -6) are described. These can be depicted by the general formula **1** in which the properties and lengths of the spacer with its associated R group can be varied.



1

The glucose transporters (GLUTs) are a family of proteins which facilitate the entry of carbohydrates into the cells by facilitated diffusion;[9] these glucose transporters do not tolerate the introduction of bulky groups at the carbohydrate reducing end [10] and therefore, in the design of probes of type **1**, consideration has to be made of the positions around the carbohydrate ring that could be linked together.

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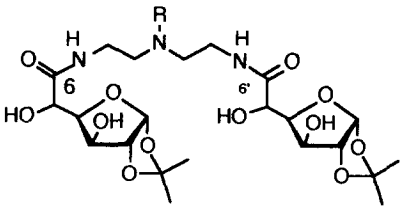
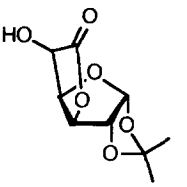
Application of this approach first resulted in the linking of two D-mannose units by an alkylamino spacer , which has yielded useful tools for the study of glucose transport proteins. [11-15] However as D-glucose is the physiologically important substrate, the preparations of glucose-based symmetrical 4,4'- and 6,6'-linked probes are now described.

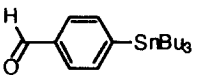
To obtain 6,6'-linked probes, advantage was taken of the peculiar reactivity of **2**, a tricyclic derivative of D-glucurono-γ-lactone, [16,17] in which lactone-ring opening by amines has been shown to occur under particularly mild conditions. [18,19] This led us to consider condensation of two molecules of **2** with a linear α–ω diamino spacer. When reacted (CH_3CN, 2 hrs, RT) with diethylenetriamine in a 1:2 ratio, **2** gave a single product whose structure was assigned as symmetrical **3** (96 %). No alkylation of the secondary amine [20] occurred and this is a key feature of this scheme as the observed chemoselectivity avoids protection of this central amino group.



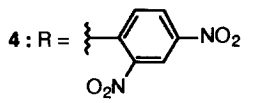
3 : R = H



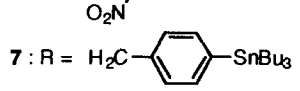
2



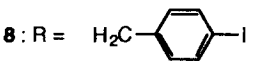
6



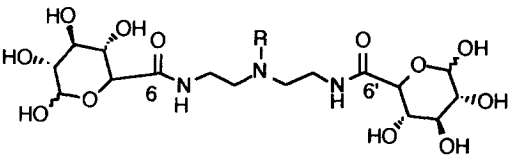
4 : R =

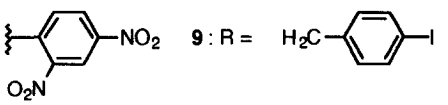


7 : R =



8 : R =





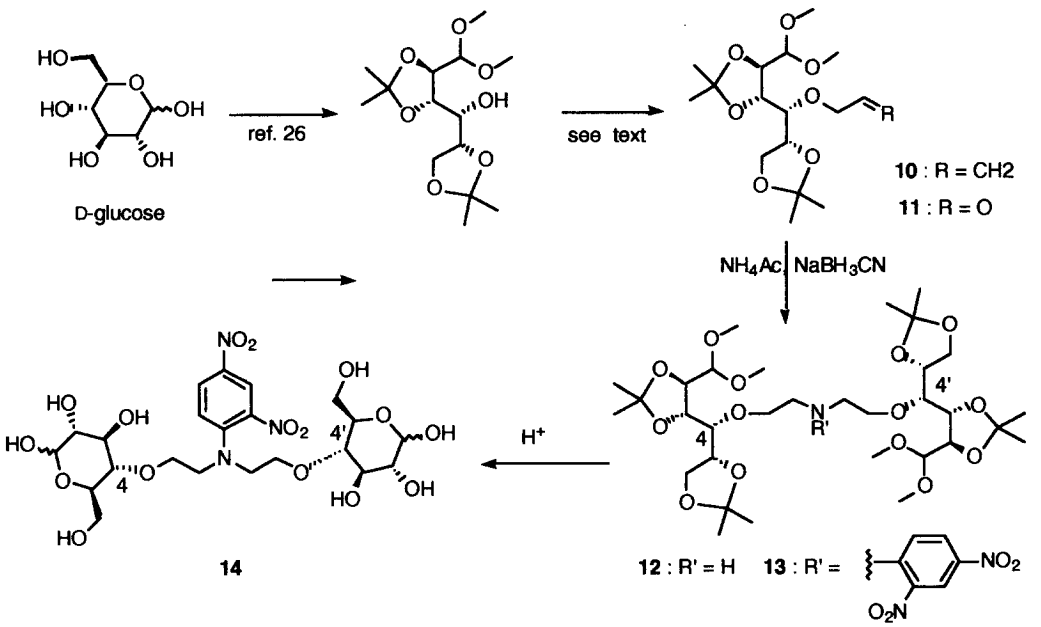
5 : R =

9 : R =

With readily-available synthon **3** in hand, its potential to produce functionalized probes was first explored by introduction of an affinity marker. Thus probe **5** [21] could be obtained by arylation of **3** with 2,4-dinitrofluorobenzene, in the presence of pyridine, to give **4** (68 %), which was followed by acidic hydrolysis (88 %) of the acetal groups. A reductive amination was also performed ($NaBH_3CN$, pH 3.4) after reaction of **3** with stannylated aldehyde **6** [22,23] to give **7**; treatment of the latter with iodine chloride afforded **8** (50 % from **3**) and acidic cleavage of the two acetals finally yielded probe **9**.[24] The introduction of an iodine to obtain the reporter group of **9** is related to its potential use in Single Photon Emitted Computed Tomography (SPECT) medical imaging of glucose transporters. [25]

For the preparation of a 4,4'-linked bis(glucose) probe, the use of reductive amination to crosslink two glucose-derived ethanal derivatives was explored as an alternative to use of a 2-propylamine spacer. [11] Treatment of the known 2,3-5,6-bis-O-isopropylidene-*aldehydo*-D- glucose dimethylacetal [26] with NaH and allyl bromide gave **10** (88 %) . Oxidation with $OsO_4/NaIO_4$ treatment then afforded aldehyde **11**(75%). When **11** was treated with ammonium acetate in a 1: 20 molar ratio, sodium cyanoborohydride

reduction gave the desired secondary amine product **12** (21%), the tertiary amine (35%) being the main product. [27] By reacting **12** with 1-fluoro-2,4-dinitrobenzene in the presence of solid potassium carbonate, **13** was rapidly produced (80%), which was followed by acidic hydrolysis of the six acetal groups (1M HCl - 90 min , 100°C) to give **14** (62 %). [28]



With probes **5**, **9** and **14** in hand, their affinities for a glucose transporter was evaluated [29] and the Ki values were found to be 314 µM, 360 µM and 39 µM respectively . These affinities are higher than that of D-glucose (Ki = 8 mM), the parent compound.[30] The higher affinity for the 4,4-linked probe **14** (200 times > D-glucose and 8 times > **5**) suggests that linking of the nitrophenyl moiety to the C4 hydroxyl position is optimal for the GLUT4 transporter ; [31] however, introduction of bulky groups into both the C6 and C4 positions is tolerated and therefore either may be suitable for the preparation of ligands for SPECT.

Two routes to bis(D-glucose) probes in which the carbohydrates have been linked by a secondary amine bridge between their positions -4 or -6 have been described. The flexibility of these approaches should be of help in further enhancing probe affinities for glucose transporters [32] but could also be useful in the preparation of bola-amphiphiles[4] or other functionalised glycolipids.

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21. **5** : Oil $[\alpha]_D^{21}$ = +20 (c = 0.3 - H_2O) at 15 min → +13 (24 h). ^{1}H-NMR (300 MHz - D_2O) : δ 8.8 (broad s, 1 H, H-Ar), 8.3 (d, J = 12 Hz, 1 H, H-Ar), 7.4 (d, J = 12 Hz, 1 H, H-Ar), 5.1 (d, J_{1-2} = 3 Hz, 1 H, H-1α), 4.5 (d, J_{1-2} = 8 Hz, 1 H, H-1β), 4.0 - 3.3 (m, 16 H, H-2, -3, -4, -5 α and β, NC$\underline{H}_2$C$\underline{H}_2$NCO). ^{13}C-NMR (62.5 MHz - D_2O) : δ 174.3, 173.5 (C-6 α and β), 152.4, 140.8 (Cquat-Ar), 131.2, 127.2, 124.1 (CH-Ar), 98.7 (C-1 β); 94.9 (C-1 α), 77.9, 77.6, 76.5, 74.9, 74.4, 74.2, 73.8, 73.4 (C-2, C-3, C-4, C-5 α and β), 53.1 ($\underline{C}H_2$N(Ar)$\underline{C}H_2$), 39.7 (CONH$\underline{C}H_2$).
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24: **9** : Oil $[\alpha]_D^{21}$ = + 4 (c = 0.45 - H_2O) at 5 min (unchanged at 24 h). ^{1}H-NMR (300 MHz - D_2O) : δ 7.8 and 7.2 (AA'XX' system, J_{app}= 8.5 Hz, 2*2 H, H-Ar), 5.4 (d, J_{1-2} = 2.5 Hz, 1 H, H-1α), 4.3 (d, J_{1-2} = 10 Hz, 1 H, H-1β), 4.0 - 3.3 (m, 14 H, H-2, -3, -4, -5 α and β, CONHC$\underline{H}_2$ and CH_2Ar), 2.75 (M, 4 H, NC$\underline{H}_2$$CH_2$NCO). ^{13}C-NMR (75 MHz - D_2O) : δ 172.9; 171.9 (C-6 α and β), 138.8, 133.0 (CH-Ar), 128.2 (C-ipso Ar), 96.8 (C-I), 96.1(C-1 β), 92.4 (C-1 α), 75.3, 74.7, 73.7, 72.4, 71.6, 71.4, 71.0, 70.7 (C-2, C-3, C-4, C-5 α and β), 57.7 (CH_2-Ar), 53.9 ($\underline{C}H_2$N(CH_2Ar)$\underline{C}H_2$), 34.8 (CONH$\underline{C}H_2$).
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27: Surprisingly, although a 1:20 ratio is used, very little primary amine is produced under those conditions.
28: **14** : Oil $[\alpha]_D^{21}$ = + 10 (c = 9, CH_3OH) at 10 min → + 12 (24 h). ^{1}H-NMR (300 MHz - D_2O) : δ 8.8 (broad s, 1 H, H-Ar), 8.4 (d, *J* = 9 Hz, 1 H, H-Ar), 7.5 (d, *J* = 9 Hz, 1 H, H-Ar), 5.25 (d, J_{1-2} = 2.5 Hz, 1 H, H-1α), 4.6 (d, J_{1-2} = 8 Hz, 1 H, H-1β), 4.2-3.2 (M, all other H's). ^{13}C-NMR (75 MHz - D_2O) : δ 152.3, 140.4, 139.8 (Cquat-Ar), 130.8, 128.6, 123.3 (CH-Ar), 98.3(C-1 β), 94.4 (C-1 α), 80.4, 78.4, 77.5, 76.8, (C-2, C-3, C-4, C-5 β), 75.3, 74.1, 72.9(C-2, C-3, C-4, C-5 α), 71.7 (O$\underline{C}H_2$$CH_2$N), 63.0 (C-1 β), 62.9 (C-1 α), 54.6 (CH_2N-Ar) .
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