Pergamon

Tetrahedron Letters, Vol. 37, No. 31, pp. 5469-5472, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01167-7

Polysialosides Scaffolded on *p-Tert*-Butylcalix[4]arene

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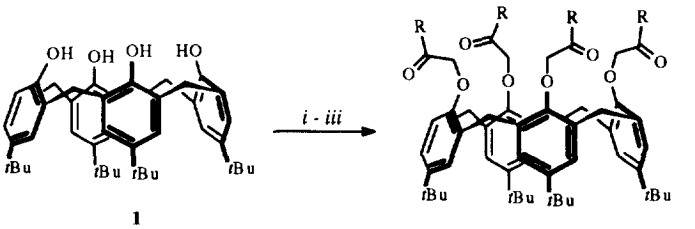
Abstract: *p-Tert*-butylcalix[4]arene (**1**) was transformed into known tetraethyl ester **2** to provide cone-shaped calix[4]arene used for the scaffolding of tetravalent α-sialoside. Ester **2** was transformed into acid chloride **4** which after treatment with mono-Boc-1,4-butanediamine, trifluoroacetolysis and N-chloroacetylation afforded tetraamine **7**. Conjugation of α-thiosialoside **9** by nucleophilic substitution and deprotection furnished water-soluble tetravalent α-thiosialoside **12** which bound strongly to the plant lectin wheat germ agglutinin.

Cell surface sialic acid residues are critically involved in numerous biological functions.[1] In particular, sialylated oligosaccharides constitute low affinity receptors for the adhesion of viruses and bacteria such as influenza, rotaviruses and *E. coli*.[2] In order to study factors influencing carbohydrate-protein interactions and to provide high affinity ligands for potential anti-adhesion therapy, divalent clusters,[3] neoglycoproteins,[4] polymers,[5] and dendrimers[6] containing α-sialosides have been prepared.[7] Although very promising, each of these conjugates has its own drawback spanning from low affinity, immunogenicity,[4b] heterogeneity and synthetic complexity. As an extension of these findings, we became interested by chemically well defined carbohydrate clusters of high affinity with the potential for cell targeting and drug delivery. To this end, we describe herein a novel prototype structure which has the intrinsic abilities for both carbohydrate scaffolding and drug inclusion complex formation.

Calix[n]arenes have been previously used as scaffolding elements for carbohydrate attachment.[8] However, previous syntheses afforded structures deprived of suitable spacer arm and of low water solubility. Since calix[n]arenes can be prepared with different ring sizes and conformations,[9] they represent an interesting family of precursors for the synthesis of biologically active carbohydrate clusters. Their built-in architecture allows desired carbohydrate orientation to be readily manipulated.

p-Tert-butylcalix[4]arene **1** was transformed into the known tetraethyl ester **2** following slight modifications of the published procedure ($BrCH_2CO_2Et$, K_2CO_3, acetone, reflux, 85%).[10] Chromatographic purification followed by exhaustive aqueous washings were necessary to remove excess ethyl bromoacetate and cations encapsulated in the cavity formed by the four ionophoric OCH_2CO_2Et groups.[11] Compound **2**, having a fixed cone-shaped conformation allowing carbohydrate orientation in only one direction, was hydrolyzed (1 M, KOH, H_2O, EtOH, reflux, 94%) to form tetraacid **3**[12] in 94% yield (Scheme 1).[13] The acid **3** was transformed into acid chloride **4** ($SOCl_2$, reflux, 2 h, 100%) which, after treatment with excess (5 eq.) mono-N-Boc-1,4-butanediamine (Et_3N, CH_2Cl_2, 0 °C→25 °C, 16 h), afforded key precursor **5** in 62% yield.

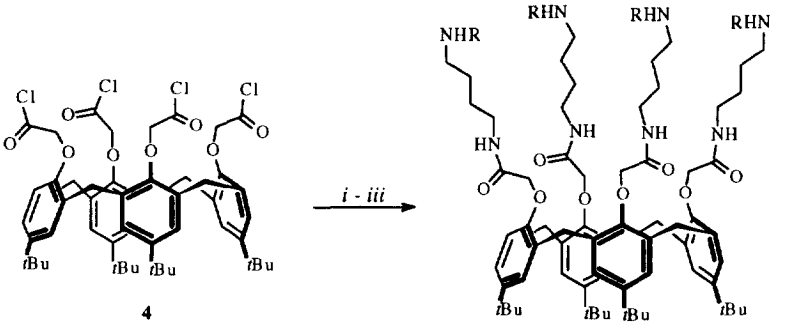


1

2 R = OEt
3 R = OH
4 R = Cl

Scheme 1. *i*) $BrCH_2CO_2Et$, K_2CO_3, acetone, N_2, reflux, 20 h, 85%; *ii*) 1M KOH, EtOH (1:1.1, v/v), reflux, 3.5 h, 94%; *iii*) $SOCl_2$, reflux, 2 h, quant.

Boc-protecting groups of intermediate **5** (mp 198.5-200 °C) were removed (20% TFA in CH_2Cl_2, 25°C, 6h) to provide tetraamine **6** quantitatively. Complete amine deprotection was monitored by ^{1}H-NMR spectroscopy (500 MHz, DMSO-d_6) which revealed the absence of any remaining *t*-butyl signal at 1.40 ppm. Tetraamine calix[4]arene **6** was then treated with chloroacetic anhydride (Et_3N, CH_2Cl_2, 25 °C, 16 h) to give electrophilic tetra-N-chloroacetylated calix[4]arene **7** (63%) as key synthon (Scheme 2). Previous studies from this laboratory have established that N-chloroacetyl groups constitute efficient precursors for multivalent attachment of thiolated sugars.[6]



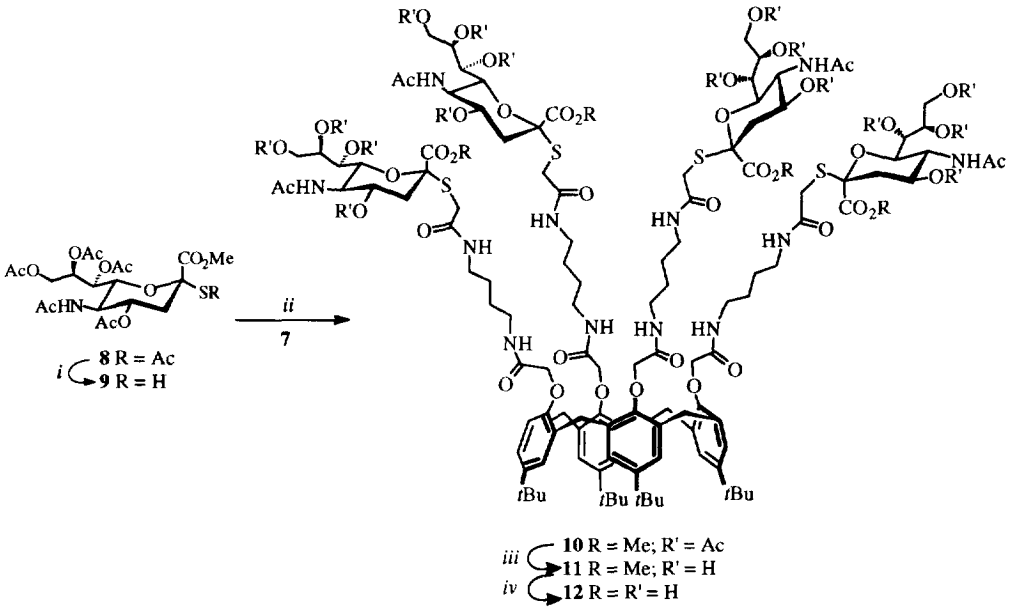
4

5 R = Boc
6 R = H
7 R = C(O)CH_2Cl

Scheme 2. *i*) $H_2N(CH_2)_4NHBoc$, Et_3N, CH_2Cl_2, 0 °C, 30 min, 25°C, 16 h, 62%; *ii*) 20% TFA in CH_2Cl_2, 25°C, 6h, quantitative; *iii*) $(ClCH_2CO)_2O$, Et_3N, CH_2Cl_2, 25 °C, 16 h, 63%.

For our first "glyco-calix[4]arene" prototype, we chose α-thiolated sialoside as carbohydrate precursor since it proved useful in glycodendrimer syntheses.[6] Thus, chemoselective de-S-acetylation (NaOMe, MeOH, 15 min, -40 °C, 95%) and low temperature quenching (H^+ resin) of the known 1-thio-α-sialosyl acetate **8**[6] afforded thiol intermediate **9** which was then coupled to tetra-N-chloroacetylated calix[4]arene **7** (Et_3N, CH_3CN, 16 h, 25 °C) to give sialylated calix[4]arene **10** in 65% yield (Scheme 3). The extent of α-thio-sialoside incorporation was readily determined by ^{1}H-NMR spectroscopy. The total disappearance of the N-chloroacetyl signal at 4.01 ppm ($CDCl_3$) along with the integration of the well separated H-4 (4.86 ppm) and

H-3e (2.72 ppm) sialosyl signals relative to the *tert*-butyl signal at 1.11 ppm showed complete incorporation of the four residues. Removal of O-acetates from peracetylated ester **10** (cat. NaOMe, MeOH) gave **11** quantitatively which upon treatment with 1 M aqueous NaOH in EtOH (1:5, v/v, 16 h, 25 °C) afforded fully deprotected α-sialylated-calix[4]arene **12** in quantitative yield.



Scheme 3. *i*) 0.95 equiv. MeONa, MeOH, -40 °C, 15 min, then H^+ resin, 95%; *ii*) Et_3N, CH_3CN, N_2, 25 °C, 16h, 65%; *iii*) cat. MeONa, MeOH, r.t., quant.; *iv*) 1M NaOH, EtOH (1:5, v/v), 25 °C, 16h, quant.

In spite of its amphiphatic structure, acid **12** was fairly water soluble (~1.1 mM, 3 mg/mL). Moreover, after titration (pH 7.0) with 0.5 M NaOH (sodium form) the solubility increased to 4.8 mM (13 mg/mL). The solubility of both compounds was thus adequate for protein binding studies. Using microtiter plate turbidimetric assays (490 nm), the sodium form of **12** (0.5 mg/mL, PBS, 50 µL, pH 7.4) exhibited strong crosslinking ability with tetrameric wheat germ agglutinin (WGA, 2 mg/mL, PBS, 50 µL), a plant lectin known to bind sialosides. Formation of the crosslinked lattice (80 min) could be inhibited by monomeric phenylthio α-sialoside (20 mg/mL PBS, 10 µL). Methyl ester **11**, also water soluble, formed stable complex with WGA at the same concentration, demonstrating the absence of simple electrostatic binding interactions. In conclusion, a water-soluble tetravalent α-sialoside was efficiently scaffolded on the upper rim of calix[4]arene. The availability of higher homologous calix[n]arenes, coupled with the large number of potential modifications at the lower rim, offer interesting new prototypes for the construction of even more complex glycoconjugates of biological relevance.

Acknowledgments: We thank the Natural Science and Engineering Research Council of Canada (NSERC) for financial support. S.J. Meunier is also thankful to NSERC for a postgraduate scholarship.

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13. Selected data: ^{1}H-NMR data of mixture of free and cation encapsulated (parentheses) calix[4]arene tetraethyl ester **2** ($CDCl_3$, δ ppm): 1.05 (1.12) (s, 36H, *t*-Bu), 1.26 (1.39) (t, 12H, J=7.1 Hz, $CH_2\underline{CH_3}$), 3.17 (3.37) (d, 4H, J=13.0, CH_b), 4.18 (4.35) (q, 8H, J=7.1, $\underline{CH_2}CH_3$), 4.78 (4.44) (s, 8H, OCH_2), 4.84 (4.21) (d, 4H, J=13.0, CH_a), 6.75 (7.09) (s, 8H, ArH). Compound **3**: mp 272-274°C (dec.). Compound **5**: mp 198.5-200°C, ^{1}H-NMR ($CDCl_3$, δ ppm): 1.06 (s, 36H, *t*-Bu), 1.40 (s, 36H, O*t*-Bu), 1.44-1.59 (m, 16H, 2 internal CH_2), 3.06-3.10 (m, 8H, $\underline{CH_2}NHBOC$), 3.21 (d, 4H, J=13.1 Hz, CH_b), 3.34-3.38 (m, 8H, $NH\underline{CH_2}$), 4.38-4.47 (d, 4H, CH_a), 4.47 (s, 8H, OCH_2), 4.97-5.07 (m, 4H, NH), 6.76 (s, 8H, ArH), 7.90-7.98 (m, 4H, NH); ^{13}C-NMR ($CDCl_3$, δ ppm): 27.0, 27.6 (2 internal CH_2), 28.5 (O-*t*-Bu), 31.3 (*t*-Bu), 31.4 ($Ar\underline{CH_2}$), 33.9 ($\underline{C}Me_3$), 39.0, 40.4 (2 $NH\underline{CH_2}$), 74.5 (OCH_2), 79.1 ($O\underline{C}Me_3$), 125.9, 132.7, 145.9, 152.8 (Ar), 156.2, 169.6 (C=O); FAB-MS for $C_{88}H_{136}N_8O_{16}$: 1562.9 (M+H, 2.3%). Compound **10**: $[\alpha]_D$ +10.3° (c 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.11 (s, 36H, *t*-Bu), 1.55-1.75 (m, 16H, 2 internal CH_2), 1.85 (s, 12H, NAc), 1.90 (dd, 4H, H-3a), 1.99, 2.02, 2.12, 2.17 (4s, 48H, 4OAc), 2.72 (dd, 4H, H-3e), 3.20-3.31 (m, 12H, CH_2N and SCH_b), 3.35-3.53 (m, 20H, CH_2N, SCH_a, CH_b and OCH_b), 3.75 (s, 12H, OMe), 3.75-3.88 (m, 8H, H-6 and OCH_a), 3.99-4.10 (m, 12H, CH_a, H-5 and H-9b), 4.25 (dd, 4H, $J_{9a,9b}$=11.6, H-9a), 4.64-4.75 (m, 4H, NH), 4.86 (ddd, 4H, H-4), 5.28 (dd, 4H, $J_{7,8}$=8.8, H-7), 5.35-5.46 (m, 4H, H-8), 5.71-5.80 (m, 4H, NH), 7.03-7.14 (m, 4H, NH), 7.09 (s, 8H, Ar); ^{13}C-NMR ($CDCl_3$, δ ppm): 20.8, 21.6 (OAc), 23.1 (NAc), 26.2, 27.0 (2 internal CH_2), 30.3 ($Ar\underline{CH_2}$), 31.2 (*t*-Bu), 32.4 (SCH_2), 34.2 ($\underline{C}Me_3$), 37.5 (C-3), 39.4 (2 $NH\underline{CH_2}$), 49.1 (C-5), 53.4 (OMe), 62.4 (C-9), 67.1 (C-7), 68.0 (C-8), 69.5 (C-4), 74.0 (C-6), 77.0 (OCH_2), 82.0 (C-2), 126.2, 134.3, 149.0, 149.6 (Ar), 168.7, 169.3, 170.0, 170.4, 170.6, 170.8, 171.4 (C=O).

(*Received in USA* 3 *April* 1996; *revised* 7 *June* 1996; *accepted* 9 *June* 1996)