Pergamon

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Tetrahedron Letters 44 (2003) 7467–7470

TETRAHEDRON LETTERS

Calix[8]arene-based glycoconjugates as multivalent carbohydrate-presenting systems

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Received 17 June 2003; accepted 9 August 2003

Abstract—An efficient approach for the introduction of eight mono- or disaccharide sugar moieties (D-glucose, *N*-acetyl-D-glucosamine, D-galactose, L-fucose, D-maltose and D-cellobiose) at the upper rim of calix[8]arene **1**, using thioureido linkers, is reported. The obtained water-soluble, nanosized glycocalix[8]arenes **5b**–**10b** may act as biomimetic carbohydrate systems and as hosts for highly polar organic molecules. Preliminary ^{1}H NMR complexation experiments of octaglycosyl derivative **7b** and **10b** with ionic guests are also reported.

Carbohydrates are key elements in a variety of important biological phenomena involving intermolecular recognition events.[1] In order to better understand molecular details of these processes and to provide biomimetic structures, the development of new glycoconjugated compounds continues to receive great attention. In this context, the creation of multivalent compounds capable of performing a 'glycoside cluster effect'[2] appears to be a promising strategy to provide synthetic high-affinity saccharide ligands or receptors. Thus, sugar moieties have been attached in a multiple fashion to different backbones,[3] including resorc[4]arenes[4] and calix[4]arenes.[5] Introduction of glycoside clusters in these structurally well-defined scaffolds has provided glyco-derivatives able to recognize polar guests and/or specific lectins or potentially promising as molecular delivery systems.[4a,6]

To manipulate multivalent presentation of carbohydrates, larger calix[8]arenes may also constitute an interesting framework.[7] Introduction of sugar groups in this macrocycle would give rise to polyvalent chiral derivatives that not only expose eight carbohydrate groups but, due to the presence of a larger hydrophobic cavity, possess intrinsic potential to act as hosts for higher size polar guests. Noteworthy it is also the possibility to exploit calix[8]arene flexibility for induced fit supramolecular recognition. In this regard, cation templation of calixarene macrocycle has been evidenced by our group on 1,5-bridged calix[8]arenes.[8] In addition, a very interesting mutually host–guest induced fit between conformationally mobile *p*-sulfonatecalix[8]arene and photolabile cholinergic ligands has also been reported.[9]

These considerations prompted us to undertake the scaffolding of saccharide moieties onto the calix[8]arene skeleton. Considering the good capabilities of isothiocyanato sugars to react in chemoselective manner with amino groups[10] and the ability of thiourea-linked sugar-derivatives as neutral hydrogen bonding receptors,[11] we decided to perform glycosylation at the calix[8]arene upper rim using isothiocyanate glycosyl donors. This approach has been successfully used by Santoyo-González et al. to introduce two sugar moieties at the calix[4]arene lower rim[5a] and very recently by Ungaro and co-workers to carry out the partial or exhaustive glycosylation of the calix[4]arene upper rim.[5b]

Octaminocalix[8]arene glycosyl acceptor **1**, was prepared starting from *p*-*tert*-butylcalix[8]arene (**2**)[12] which was exhaustively propylated (*n*-PrI, NaH, DMF) to give **3** (90% yield). This compound was converted by *ipso*-nitration to octanitrocalix[8]arene **4** (40% yield),

Keywords: glycocalixarenes; calix[8]arenes; thiourea; glycosyl isothiocyanates; molecular recognition.
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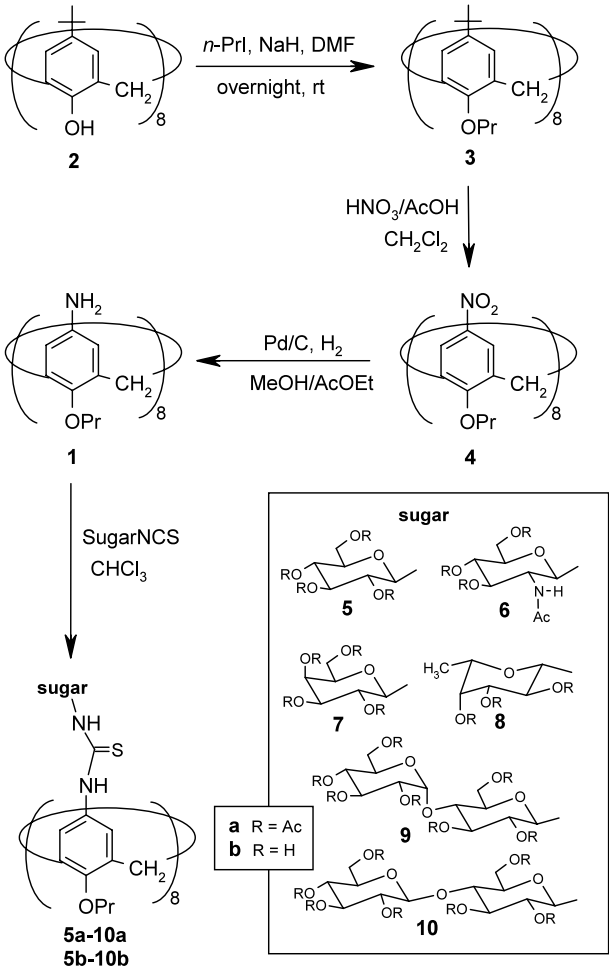
doi:10.1016/j.tetlet.2003.08.039

which in turn was subjected to catalytic hydrogenation to give **1** (90% yield).[13] Glycosylation of **1** was performed by overnight treatment with 8.1 equivalents of peracetylated mono- or disaccharide (D-glucose, *N*-acetyl-D-glucosamine, D-galactose, L-fucose, D-maltose and D-cellobiose) glycosyl isothiocyanates[14] in dry $CHCl_3$ at room temperature. The corresponding thioureido-glycocalix[8]arenes **5a–10a** were obtained in very good yield (70–90%) after a quick purification by column chromatography.



The structure of obtained compounds was confirmed by mass spectrometry and NMR spectroscopy (^1H and ^{13}C).[15] In particular, the presence in the ^1H NMR spectra of a single signal pattern for the eight equivalent sugar units indicated the calix[8]arene upper rim exhaustive functionalization. On the basis of coupling constant values (8.6–9.2 Hz) it was evident that all the eight sugar moieties in derivatives **5a–10a** possess the β-configuration at the anomeric position. The high stereoselectivity was in line with the results of other glycosylation reactions, in which the configuration at C_1 is determined by the glycosyl isothiocyanate used.[10]

Dynamic ^1H NMR spectra ($CDCl_3$, 230–330 K) of compounds **5a–10a** revealed a hindered conformational freedom ascribable to the formation of hydrogen bonds involving the thioureido linkers and to the restricted rotation of the pseudoamide NH–C=S bonds, previously also observed for thioureido-glycocalix[4]arenes[5b] and for other thioureido-sugars.[16] As expected, the conformational mobility was higher in DMSO-d_6 in accordance with the weakening of hydrogen bonds in this H-bonding acceptor solvent.

Peracetylated thioureido-glycocalix[8]arenes **5a–10a** were deprotected by treatment with catalytic amount of sodium methoxide in methanol at room temperature, affording the corresponding hydroxylated derivatives **5b–10b** in quantitative yield. De-*O*-acetylation was confirmed by the absence of *O*-acetyl signals in their NMR spectra. In accordance, they showed a good solubility in pure water, which was lower for fucosyl (8.6×10^{-6} M) and galactosyl (1.8×10^{-5} M) derivatives **8b** and **7b**, and higher for glucosyl (1.4×10^{-4} M) and *N*-acetylglucosamine (5.2×10^{-4} M) calix[8]arenes **5b** and **7b**. A further increase in solubility was observed for maltosyl (2.0×10^{-3} M) and cellobiosyl (2.3×10^{-3} M) disaccharide derivatives **9b** and **10b**. As expected, hydroxylated compounds **5b–10b** showed in DMSO-d_6 a lower conformational mobility than the corresponding peracetates, influenced by the nature of sugar substituent.

In biological phenomena based on multivalent carbohydrate presentation a large surface contact area is usually involved. Due to their large dimension glycocalix[8]arenes **5b–10b** may be considered valid mimics for sugar presentation. In fact, molecular modeling reveals that these compounds originate nanosized structures comparable to calix[4]resorcarene-based glycoclusters reported by Aoyama and co-workers.[6] Figure 1 shows the space-filling CPK model of octacellobiosyl calix[8]arene **10b** having an estimated molecular dimension of 4.5 nm in the 'open' unfolded conformation

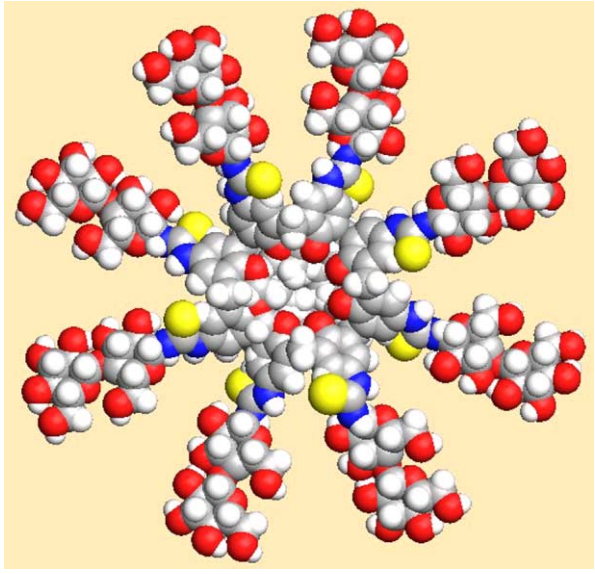


Figure 1. Space-filling CPK computer model of octacellobiosyl calix[8]arene **10b** in the 'open' conformation, built on a calix[8]arene pleated-loop skeleton.

built on the typical calix[8]arene pleated-loop structure.[7] Octamaltosyl derivative **9b** possess similar dimension (4.2 nm), while a smaller dimension is obviously obtained with monosaccharide octaglucosyl derivative **5b** (3.2 nm).

Considering the ability of urea-linked sugar-derivatives to interact with polar molecules, we were induced to test the host–guest properties of glycocalix[8]arenes **5b**–**10b**. Preliminary ^{1}H NMR complexation experiments of octagalactosyl or octacellobiosyl derivatives, **7b** or **10b**, with ionic guests, such as D-glucosamine hydrochloride, histidine hydrochloride, pyromellitic acid tetrasodium salt, and adenosine-5′-triphosphate disodium salt, were undertaken. In all cases, changes in the chemical shift of the guest signals in the protonic spectra in DMSO-d_6 at rt, indicative of interaction phenomena, were observed upon addition of **7b** or **10b**. For example, amino and C_1-OH signals of D-glucosamine hydrochloride undergo an upfield shift up to 1.6 and 0.9 ppm respectively, in the presence of **7b**. A concomitant large broadening of the CH-sugar signals of **7b** was indicative of the involvement of its carbohydrate moieties in the interaction with D-glucosamine hydrochloride.

An association constant of 934±90 M^{-1} and 764±65 M^{-1} of D-glucosamine hydrochloride with **7b** or **10b**, respectively, and a 1:1 complex stoichiometry were determined by non-linear regression analysis of NMR titration data.[17] This indicates an higher affinity for the guest of monosaccharide receptor with respect to disaccharide one. Clearly, increasing the number of sugar-CHOH groups in the host does not automatically imply an increase in affinity, but more subtle interactional and stereochemical aspects must be considered.

In conclusion, we have reported the first example of water soluble glycocalix[8]arenes bearing eight mono- or disaccharide units at the upper rim. These compounds may be used as model systems to address questions related to carbohydrate–carbohydrate or carbohydrate–protein binding events. Their conformational flexibility, at first glance detrimental for molecular recognition, could result in advantageous adaptive supramolecular interactions. The ability of glycocalix[8]arenes to interact with specific molecules of biological relevance and their potential utility as blockers or inhibitors of pathological saccharide-receptor associations are currently under investigation.

Acknowledgements

Financial support from the Italian MIUR (Supramolecular Devices Project) is gratefully acknowledged. We thank Professor M. Piattelli for a critical reading of the manuscript. Thanks are also due to Mr. R. Rapisardi (I.C.T.P.-Sez. di Catania, C.N.R.) for ESI-MS measurements and to Mr. A. Renda (I.C.B.-Sez. di Catania, C.N.R.) for technical assistance.

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13. **Procedure for the preparation of compound 1**: *ipso*-nitration of the octapropoxy-*p*-*tert*-butylcalix[8]arene (**2**) was performed by treatment with HNO_3/CH_3COOH in CH_2Cl_2 at rt, using the procedure reported in: Verboom, W.; Durie, A.; Egberink, R. J. M.; Asfari, Z.; Reinhoudt D. N. *J. Org. Chem.* **1992**, *57*, 1313. The crude reaction mixture was suspended in acetone and the pure octanitrocalix[8]arene derivative (**3**) was precipitated as white solid (40% yield). Compound **3** was dissolved in methanol/ethyl acetate and catalytic amount of Pd/C was added. The mixture was stirred under H_2 (2 bar) at rt for 24 h. The catalyst was removed and the filtrated was evaporated to give the corresponding octamino derivative **4** (90%). All new compounds **1** and **3**–**4** were fully characterized. For brevity we report here only the **^{1}H NMR data** for compound **1**: (400 MHz, $CDCl_3$, 297 K) δ 0.98 (t, $J=7.3$ Hz, 24H), 1.76 (q, $J=6.8$ Hz, 16H), 3.28 (br s, 16H), 3.69 (t, $J=6.5$ Hz, 16H), 3.86 (s, 16H), 6.17 (s, 16H).
14. The commercially unavailable isothiocyanate sugars in β-form were synthesized by the solution method reported in: Camarasa, M. J.; Fernández-Resa, P.; García-López, M. T.; De Las Heras, F. G.; Méndez-Castrillón, P. P.; San Felix, A. *Synthesis* **1999**, 509.
15. Glycocalix[8]arenes **5a–10a** and **5b–10b** gave satisfactory microanalytical and spectral data. For brevity we report here ^{1}H NMR data for **5a–10a**. **Compound 5a**: (400 MHz, DMSO-d_6, 340 K) δ 0.73 (t, $J=7.3$ Hz, 24H), 1.50 (q, $J=7.3$ Hz, 16H), 1.94, 1.97, 1.98 (s, 48H, 24H, 24H), 3.48 (t, $J=6.5$ Hz, 16H), 3.91 (s, 16H), 3.92–3.96 (m overlapped, 8H), 3.99 (dd, $J=2.5$, 12.3 Hz, 8H), 4.15 (dd, $J=4.9$, 12.3 Hz, 8H), 4.92 (dd, $J=9.4$, 9.5 Hz, 16H), 5.29 (dd, $J=9.4$, 9.5 Hz, 8H), 5.85 (dd, $J=9.1$, 9.2 Hz, 8H), 7.16, (s, 16H), 7.78 (d, $J=9.0$ Hz, 8H), 9.46 (s, 8H). **Compound 6a**: (400 MHz, DMSO-d_6, 340 K) δ 0.73 (t, $J=7.3$ Hz, 24H), 1.51 (q, $J=6.8$ Hz, 16H), 1.77, 1.92, 1.97, 1.98 (s, 24H each), 3.49 (br t, 16H), 3.76 (br m, 8H), 3.92 (s, 16H), 3.96–4.07 (overlapped, 16H), 4.16 (dd, $J=4.7$, 12.1 Hz, 8H), 4.84 (dd, $J=9.4$, 9.5 Hz, 8H), 5.12 (dd, $J=9.4$, 9.5 Hz, 8H), 5.62 (br t, $J=8.6$ Hz, 8H), 7.18, (s, 16H), 7.62 (br d, $J=6.8$ Hz, 8H), 7.96 (d, $J=8.3$ Hz, 8H), 9.55 (s, 8H). **Compound 7a**: (400 MHz, DMSO-d_6, 340 K) δ 0.75 (t, $J=7.3$ Hz, 24H), 1.52 (q, $J=7.1$ Hz, 16H), 1.91, 1.95, 1.96, 2.07 (s, 24H each), 3.49 (br t, $J=5.3$ Hz, 16H), 3.91 (s, 16H), 3.98–4.04 (br d, $J=6.2$ Hz, 16H, overlapped), 4.19 (br t, $J=6.2$ Hz, 8H), 5.04 (dd, $J=9.4$, 9.5 Hz, 8H), 5.26 (dd, $J=3.6$, 10.0 Hz, 8H), 5.32 (br d, $J=3.2$ Hz, 8H), 5.84 (br t, $J=9.2$ Hz, 8H), 7.18 (s, 16H), 7.81 (d, $J=8.2$ Hz, 8H), 9.37 (s, 8H). **Compound 8a**: (400 MHz, DMSO-d_6, 340 K) δ 0.74 (t, $J=7.4$ Hz, 24H), 1.05 (d, $J=6.5$ Hz, 24H), 1.52 (q, $J=6.5$ Hz, 16H), 1.91, 1.95, 2.11 (s, 24H each), 3.49 (br t, $J=6.2$ Hz, 16H), 3.92 (s, 16H), 4.03 (br q, $J=6.5$ Hz, 8H), 4.99 (dd, $J=9.5$, 9.6 Hz, 8H), 5.15 (br d, $J=3.2$ Hz, 8H), 5.19 (dd, $J=3.5$, 10.0 Hz, 8H), 5.78 (br t, $J=9.2$ Hz, 8H), 7.16, (s, 16H), 7.74 (d, $J=9.2$ Hz, 8H), 9.35 (s, 8H). **Compound 9a**: (400 MHz, DMSO-d_6, 340 K) δ 0.76 (t, $J=7.1$ Hz, 24H), 1.52 (br q, $J=7.3$ Hz, 16H), 1.92, 1.94, 1.96, 1.97, 2.01, 2.03 (s, 24H, 24H, 24H, 24H, 48H, 24H), 3.49 (br t, 16H), 3.81–3.95 (overlapped, 32H), 4.03 and 4.06 (br d, $J=10.6$ Hz, 16H, overlapped), 4.15 (dd, $J=4.7$, 12.4 Hz, 8H), 4.17–4.21 (overlapped, 8H) 4.35 (br d, $J=12.1$ Hz, 8H), 4.79–4.83 (overlapped, 8H), 4.85 (dd, $J=3.8$, 10.4 Hz, 8H), 4.96 (dd, $J=9.7$, 9.8 Hz, 8H), 5.23 (br t, $J=9.7$ Hz, 8H), 5.28 (d, $J=3.7$ Hz, 8H) 5.25–5.35 (overlapped, 8H), 5.83 (br t, 8H) 7.15, (br s, 16H), 7.71 (br s, 8H), 9.44 (br s, 8H). **Compound 10a**: (400 MHz, DMSO-d_6, 340 K) δ 0.71 (br t, $J=7.1$ Hz, 24H), 1.49 (br q, $J=6.7$ Hz, 16H), 1.91, 1.96, 1.97, 2.01, 2.03 (s, 48H, 48H, 24H, 24H, 24H), 3.43 (br s, 16H), 3.78 (br s, 16H), 3.79–4.12 (overlapped, 40H), 4.20 (dd, $J=4.6$, 12.7 Hz, 8H), 4.29 (br d, $J=11.4$ Hz, 8H), 4.67 (dd, $J=8.3$, 8.9 Hz, 8H), 4.80 (br d, $J=7.6$ Hz, 16H), 4.88 (dd, $J=9.5$, 9.9 Hz, 8H), 5.10–5.27 (overlapped, 8H), 5.21 (dd, $J=9.2$, 9.5 Hz, 8H), 5.73 (dd, $J=8.6$, 8.8 Hz, 8H), 7.14, (s, 16H), 7.77 (d, $J=6.3$ Hz, 8H), 9.49 (s, 8H).
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17. **NMR titration experiments** (400 MHz, 295 K) were performed in DMSO-d_6 by titrating a 1.5×10^{-4} M solution of guest with a solution mixture of host (**7b** or **10b**, both 2.3×10^{-3} M) and guest (1.5×10^{-4} M).