[13] a) B. J. Coe, M. C. Chamberlain, J. P. Essex-Lopresti, S. Gaines, J. C. Jeffery, S. Houbrechts, A. Persoons, *Inorg. Chem.* **1997**, *36*, 3284; b) B. J. Coe, J. P. Essex-Lopresti, J. A. Harris, S. Houbrechts, A. Persoons, *Chem. Commun.* **1997**, 1645; c) B. J. Coe, J. A. Harris, L. J. Harrington, J. C. Jeffery, L. H. Rees, S. Houbrechts, A. Persoons, *Inorg. Chem.* **1998**, *37*, 3391.
[14] a) J. L. Oudar, D. S. Chemla, *J. Chem. Phys.* **1977**, *66*, 2664; b) J. L. Oudar, *J. Chem. Phys.* **1977**, *67*, 446; c) J. Zyss, J. L. Oudar, *Phys. Rev. A* **1982**, *26*, 2016.
[15] E. Hendrickx, C. Dehu, K. Clays, J. L. Brédas, A. Persoons, *ACS Symp. Ser.* **1995**, *601*, 82.
[16] M. Stähelin, D. M. Burland, J. E. Rice, *Chem. Phys. Lett.* **1992**, *191*, 245.
[17] J. N. Woodford, M. A. Pauley, C. H. Wang, *J. Phys. Chem. A* **1997**, *101*, 1989.
[18] J. C. Curtis, B. P. Sullivan, T. J. Meyer, *Inorg. Chem.* **1983**, *22*, 224.
[19] This was confirmed by the use of 100-fold concentrated solutions (ca. 10^{-3} M) which afforded larger HRS signals, allowing the derivation of β_{1064} values of 20, 35, and 62×10^{-30} esu for the oxidized forms of [**1**]Cl_3, [**2**]Cl_3, and [**3**]Cl_3, respectively (referenced to pNA in methanol). Excessive UV absorption by H_2O_2 prevents measurement of the spectra of the Ru^{III} forms, precluding the estimation of β_0 values.

Amphiphilic *p-tert*-Butylcalix[4]arene Scaffolds Containing Exposed Carbohydrate Dendrons**

René Roy* and Jin Mi Kim

Calixarenes are cyclic molecules containing a cavity useful in host–guest chemistry.[1] Their intrinsic amphiphilic architecture also makes them ideal candidates for the study of water–monolayer surface interactions. In this respect, they surpass their cyclodextrin counterparts.[2] In spite of these interesting features—and in addition possible variation of conformational organization, substitution of the upper and lower rims, and shape and size—only limited efforts have been made to construct biologically relevant calixarenes containing carbohydrate moieties.[3, 4] In line with this concept, the synthesis of nondendritic galactose octamers attached to a calix[4]resorcarene scaffold possessing lipophilic side chains has been described. However, as opposed to our work presented herein, the hydrophilic carbohydrate residues were used for polar attachment to a polar quartz surface.[5]

We describe here the first synthesis of dendritic,[6] water-soluble, carbohydrate-containing *p-tert*-butylcalix[4]arenes and their lectin-binding properties. These carbohydrate-containing calix[4]arenes can serve as models to further investigate factors influencing multivalent carbohydrate–protein interactions at the molecular level. The lipophilic *p-tert*-butyl substituents provide the driving force for stable assembly and/or adhesion of a calixarene monolayer to a surface, while the hydrophilic carbohydrate ligands mimic the cell's saccharide-rich surface. This new type of hybrid molecules can serve as coating carbohydrate ligands in competitive solid-phase immunoassays (Figure 1).

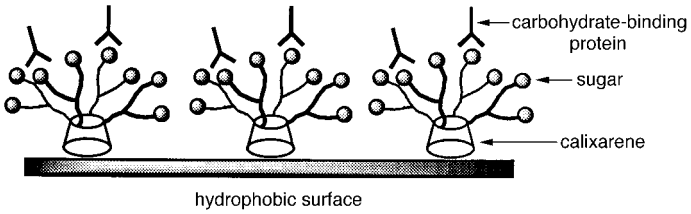


Figure 1. Glycocalix[4]arene hybrids used as coating antigens on a hydrophobic polystyrene surface.

Our model carbohydrate is the T_N antigen (GalNAcα1 $\rightarrow$O-Ser/Thr) corresponding to one of the immunodominant epitopes found in human adenocarcinomas mucins.[7] This family of carbohydrate-associated tumor markers is usually cryptic in normal cells. We have also recently shown that the O-linked Ser/Thr residues in the analogous T antigen [Gal(β1-3)-GalNAc(α1 $\rightarrow$O-Ser/Thr)] were not essential to generate mouse monoclonal antibodies that recognize cancer tissues.[8] Consequently, the α-linked GalNAc moieties described herein were deprived of the O-Ser/Thr aglycon.

The synthetic strategy for the construction of glycocalix[4]arenes was to attach suitable spacer-substituted α-GalNAc residues to the calix[4]arene core; both convergent and divergent approaches were employed. The key α-D-GalNAc derivative **3** was prepared in four steps from *N*-acetyl-D-galactosamine (**1**) (Scheme 1). The required calix[4]arene core **7** was prepared by transforming commercial *p-tert*-butylcalix[4]arene (**6**) into the known tetraethyl ester[9] followed by hydrolysis and treatment with thionyl chloride (Scheme 2). Direct amidation of **7** with amine **3** and subsequent de-O-acetylation provided tetravalent glycocalix[4]arene **8**.

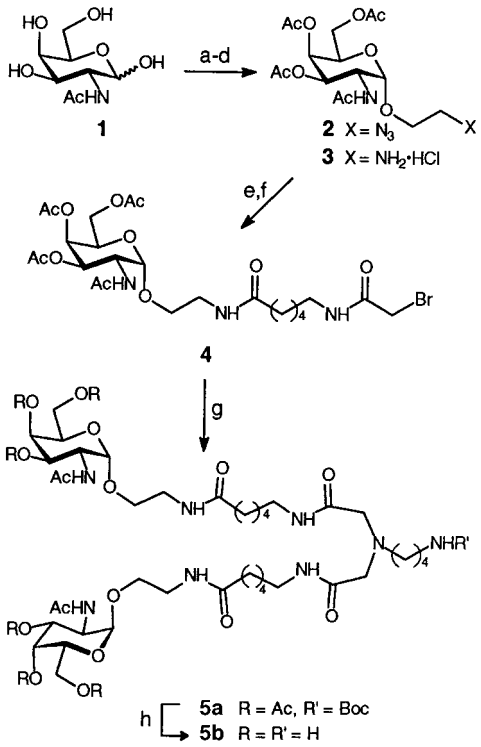
Glycocalix[4]arenes of higher valencies were synthesized by a semiconvergent approach. Divalent α-D-GalNAc precursor **5a** and its deprotected form **5b** were first obtained by treatment of amine **3** with *N*-Boc-6-aminohexanoic acid followed by trifluoroacetolysis and N-bromoacetylation to give **4** in 85% yield (see Scheme 1). Double N-alkylation of mono-*N*-Boc-1,4-diaminobutane with **4** gave dimer **5a** in 73% yield, which was deprotected to provide **5b**. Treatment of acid chloride **7** with mono-*N*-Boc-1,4-diaminobutane afforded **9** in 63% yield. Trifluoroacetolysis of **9** gave **10**, which was N,N-dialkylated with **4** to provide octavalent glycocalix[4]arene **11** in 64% yield after deprotection (Scheme 3). Octameric tetraamine derivative **13** was also obtained by double N-alkylation of **10** using 4-bromoacetamido-1-Boc-butanediamine (51%, Scheme 4). Finally, hexadecameric glycocalix[4]arene **14** was prepared from octameric amine **13** and bromoacetamido-GalNAc derivative **4** after de-O-acetylation.

The ligands **5b**, **8**, **11**, and **14** were purified by size-exclusion chromatography (Sephadex LH20, MeOH). ^{1}H NMR spec-

[*] Prof. R. Roy, J. M. Kim
Department of Chemistry, University of Ottawa
10 Marie Curie, Ottawa, ON K1N 6N5 (Canada)
Fax: (+1) 613-562-5170
E-mail: rroy@science.uottawa.ca

[**] We thank the National Science and Engineering Research Council of Canada (NSERC) for financial support and Dr. P. Thibault, National Research Council of Ottawa (NRC), for MALDI-TOF mass spectrometry experiments.

  1433-7851/99/3803-0369 $ 17.50+.50/0

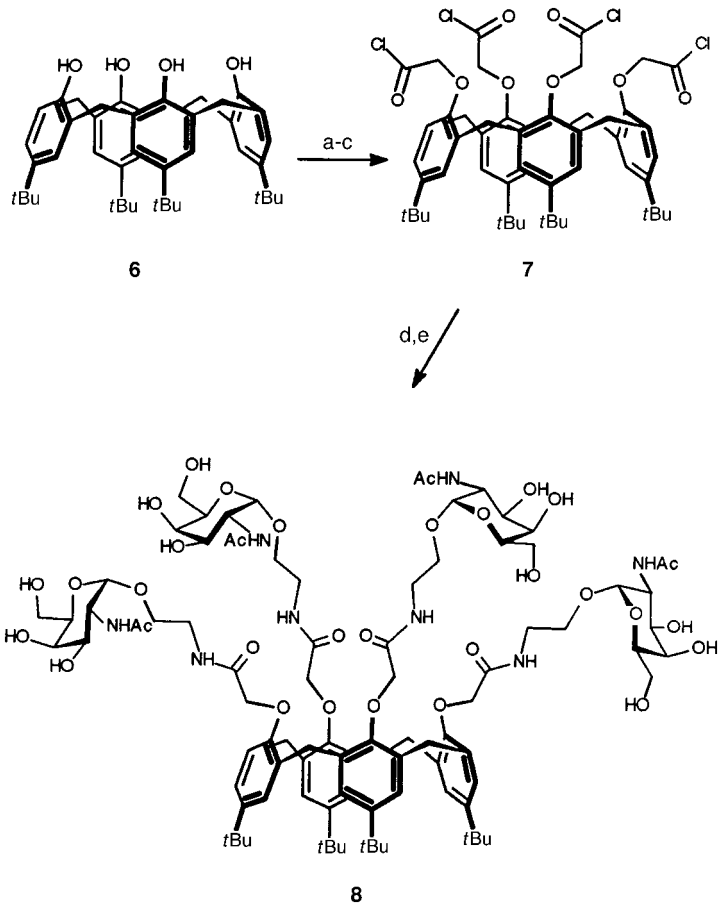


Scheme 1. Synthesis of key monomer **3** and dimer **5b**. a) $HOCH_2CH_2Cl$, $BF_3 \cdot OEt_2$, reflux for 4 h, 25 °C for 48 h; b) NaN_3 (10 equiv), NaI (1 equiv), CH_3CN, reflux, 48 h, 85 % (2 steps); c) Ac_2O, pyridine, 25 °C, 16 h, 80 %; d) 1. H_2-Pd/C, AcOH, MeOH, 16 h; 2. Amberlite IRA-400(Cl) resin, MeOH, 16 h, quantitative; e) $HO_2C(CH_2)_5NHBoc$ (1.2 equiv), DIPEA (2.5 equiv), TBTU (1.2 equiv), CH_2Cl_2, 0 °C, 30 min, 76 %; f) 1. 20 % TFA, CH_2Cl_2, 25 °C, 2 h; 2. $ClCOCH_2Br$ (1.2 equiv), DIPEA (2.5 equiv), CH_2Cl_2, 0 °C, 30 min, 85 %; g) $H_2N(CH_2)_4NHBoc$ (0.9 equiv), DIPEA (1.2 equiv), CH_3CN, reflux, 48 h, 73 %; h) 1. 1M NaOMe, MeOH, pH 9, 25 °C, 3 h; 2. 20 % TFA, CH_2Cl_2, 25 °C, 2 h, 83 % (2 steps). Boc = *tert*-butoxycarbonyl, DIPEA = diisopropylethylamine, TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate, TFA = trifluoroacetic acid.

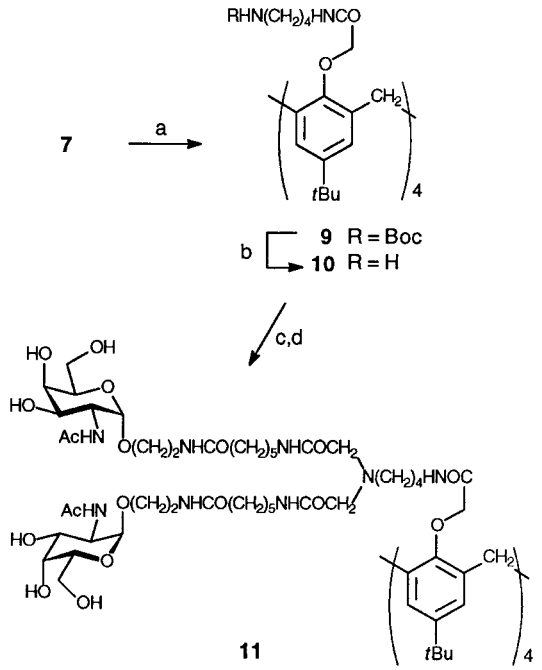
troscopy (D_2O) shows that water-soluble glycocalix[4]arenes **8**, **11**, and **14** exist in the *cone* conformation as judged from the singlets in the aromatic region ($\delta = 6.8$) and the *tert*-butyl signals at $\delta = 1.1$ (Table 1). The ratios for the signals attributed to the anomeric ($\delta = 4.9$) and *tert*-butyl protons ($\delta = 1.1$) were used to confirm complete glycosylation.

These ligands were then evaluated for their relative lectin-binding properties against *Vicia villosa* agglutinin (VVA). This plant lectin has been used previously for binding studies against α-D-GalNAc derivatives.[10] The direct binding abilities and cross-linking behavior of di- (**5b**), tetra- (**8**), octa- (**11**), and hexadecavalent ligands (**14**) toward VVA were initially determined by turbidimetric analysis. Hypervalent glycocalix[4]arene dendrimers demonstrated direct binding to VVA by the rapid formation of insoluble precipitates (Figure 2). Allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (allyl α-D-GalNAc) was used as an inhibitor for the cross-linking of octavalent **11** with VVA, thus demonstrating the sugar specificity of the binding. The interaction between **11** and VVA was so strong that a 250-fold molar excess of allyl α-D-GalNAc was necessary to disrupt the cross-linking interaction.

The efficiency of each glycocalixarene to inhibit the binding of asialoglycophorin, a natural glycoprotein of human eryth-

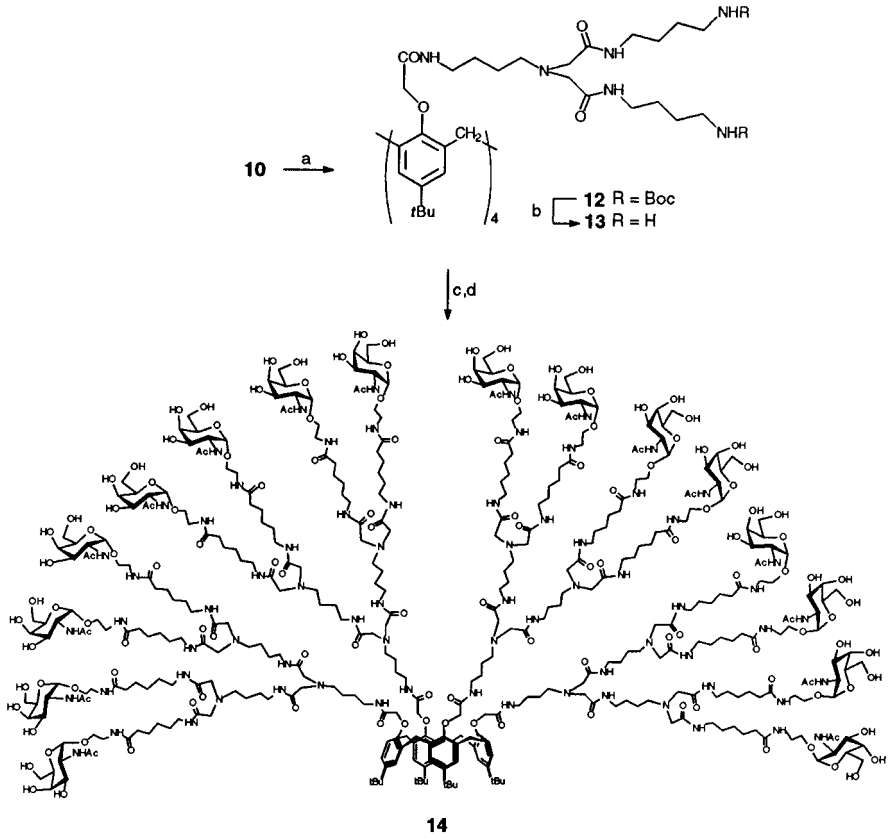


Scheme 2. Synthesis of tetramer **8**. a) $BrCH_2CO_2Et$ (20 equiv), K_2CO_3 (20 equiv), acetone, 4-Å molecular sieves, reflux, 24 h, 84 %; b) 1M KOH, EtOH (1/1.1 v/v), reflux, 9 h, 90 %; c) $SOCl_2$, reflux, 2 h, quantitative; d) **3** (6 equiv), Et_3N (12 equiv), CH_2Cl_2, 0 → 25 °C, 2 h, 74 %; e) NaOMe, MeOH, pH 9, 25 °C, 2 h, 94 %.



Scheme 3. Synthesis of octamer **11**. a) $BocHN(CH_2)_4NH_2$ (6 equiv), DIPEA (12 equiv), CH_2Cl_2, 0 °C, 3 h, 63 %; b) 20 % TFA, CH_2Cl_2, 25 °C, 2 h; c) **4** (10 equiv), DIPEA, CH_3CN, 60 °C, 48 h; d) 1M NaOMe, MeOH, 25 °C, 16 h, 64 % (2 steps).

  1433-7851/99/3803-0370 $ 17.50+.50/0



Scheme 4. Syntheses of hexadecamer **14**. a) $BocHN(CH_2)_4NHCOCH_2Br$ (10 equiv), DIPEA (14 equiv), CH_3CN, 60 °C, 48 h, 51 %; b) 20 % TFA, CH_2Cl_2, 25 °C, 2 h; c) **4** (20 equiv), DIPEA, CH_3CN, 60 °C, 48 h; d) 1 M NaOMe, MeOH, 25 °C, 16 h, 69 % (2 steps).

rocytes, to VVA was measured by enzyme-linked lectin assay (ELLA). Asialoglycophorin (MN) was used as a coating antigen for the solid-phase competitive experiments (MN is a blood group serotype). Horseradish peroxidase labeled VVA (VVA-HRP) was used for the quantitative detection of inhibition by optical density. The results for the inhibition of asialoglycophorin – VVA binding are shown in Figure 3. The best result was obtained from the hexadecavalent conjugate **14** ($IC_{50} = 13.4\,\mu M$), which represents a 12-fold increase in potency over that of the allyl α-D-GalNAc monomer ($IC_{50} = 158.3\,\mu M$).

Our most important finding was the monolayer-forming ability of the GalNAc-containing calix[4]arenes. Tetra- and hexadecavalent glycocalix[4]arenes **8** and **14**, respectively,

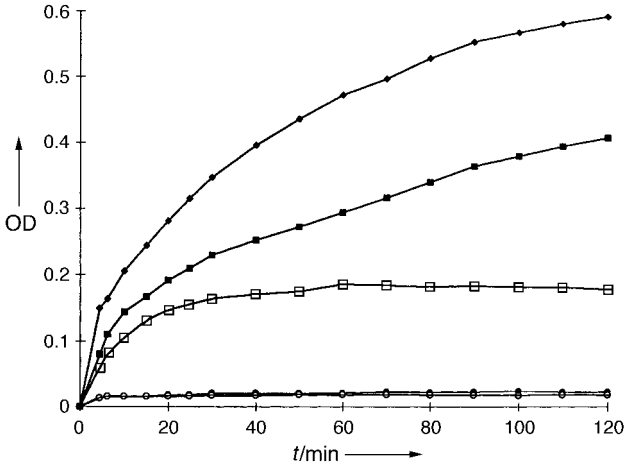


Figure 2. Turbidimetric analysis of the binding of glycocalix[4]arenes with VVA. ●: **5b** (2-mer), ■: **8** (4-mer), ◆: **11** (8-mer), □: **14** (16-mer), ○: **11** with allyl α-D-GalNAc as an inhibitor. OD = optical density measured at 490 nm.

Table 1. Selected physical data for representative compounds.

5a: $[\alpha]_D^{20} = +59.1$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): $\delta =$ 1.24 – 1.32 (q, $J = 7.4$ Hz, 4H; $2 \times CH_2$), 1.39 (s, 9H; *t*Bu), 1.47 – 1.53 (m, 8H; $4 \times CH_2$), 1.93, 1.96, 2.00, 2.11 (4 s, 24H; OAc), 2.13 – 2.17 (t, $J =$ 7.4 Hz, 4H; CH_2CONH), 3.02 – 3.33 (m, 14H; CH_2, C*H*H), 3.45 – 3.52 (m, 2H; CH*H*), 3.55 – 3.66 (m, 2H; CH_2), 3.68 – 3.75 (m, 2H; CH*H*), 4.02 – 4.10 (m, 4H; CH_2), 3.70 – 3.76 (m, 2H; C*H*H), 4.02 – 4.10 (m, 4H; H-6), 4.12 – 4.16 (m, 2H; H-5), 4.54 (ddd, $J_{2,3} = 11.4$ Hz, $J_{NH,2} = 9.5$ Hz, 2H; H-2), 4.84 (d, $J_{1,2} = 3.6$ Hz, 2H; H-1), 4.93 – 4.99 (m, 1H; NHBoc), 5.10 (dd, $J_{3,4} =$ 3.3 Hz, 2H; H-3), 5.32 (m, 2H; H-4), 6.90 – 7.05 (br, 4H; NH), 7.50 – 7.65 (br, 2H; NH); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta = 20.7$ (CH_3CO_2), 23.0 ($NHCOCH_3$), 25.0, 26.1, 27.0 ($3 \times CH_2$), 28.4 (*t*Bu), 29.0, 36.2, 38.6, 39.0, 39.7, 42.0 ($6 \times CH_2$), 47.4 (C2), 58.8 (CH_2), 62.0 (C6), 66.7 (C5), 67.3 (C4), 68.1 (CH_2), 68.5 (C3), 98.4 (C1), 156.6 (Ar), 170.4, 170.5, 170.6, 170.7, 173.5 (C=O); positive-ion FAB-MS: *m/z* (%): 1275.6 (10.2) [M^++1].

8: positive-ion FAB-MS: *m/z* (%): 1865.8 (2.4) [M^++1].

11: $[\alpha]_D^{20} = +53.6$ ($c = 0.5$ in DMSO); MALDI-TOF MS calcd for $C_{212}H_{352}N_{32}O_{72}$: 4498: found: 4499 [*M*+1]. **12**: positive-ion FAB-MS calcd for $C_{156}H_{264}N_{24}O_{32}$:2985; found: 2986 (0.2 %) [*M*+1].

12: positive-ion FAB-MS caled for $C_{156}H_{264}N_{24}O_{32}$: 2985; found: 2986 (0.2%) [*M*+1].

14: $[\alpha]_D^{20} = +66.4$ ($c = 1.0$ in MeOH); ^1H NMR (D_2O): $\delta = 1.11$ (br s, 9H, *t*Bu), 1.32 – 1.43, 1.45 – 1.70 (m, 32H; internal CH_2), 2.11 (s, 48H; NAc), 2.25 – 2.37 (m, 32H; CH_2CONH), 2.50 – 2.66 (m, 24H; CH_2N), 3.14 – 3.44 (m, 124H; $CONHCH_2$, NCH_2CO, ArC*H*H, C*H*HO), 3.54 – 3.64 (m, 32H; NC*H*H, CH*H*O), 3.76 – 3.87 (m, 52H; H-6, 4 ArC*H*H, NCH*H*), 3.92 – 4.00 (m, 32H; H-2, H-5), 4.04 (dd, $J_{3,4} = 2.8$ Hz, 16H; H-4), 4.25 (dd, $J_{2,3} =$ 11.0 Hz, 16H; H-3), 4.94 (d, $J_{1,2} = 3.4$ Hz, 16H; H-1), 6.92 (br s, 8H; Ar); ^{13}C NMR (D_2O): $\delta = 21.7$ (NAc), 23.4, 24.7, 25.4, 27.9, 30.8 (*t*Bu), 35.4, 38.5, 49.4 (C2), 54.8, 57.9, 60.7 (C6), 66.0, 67.4 (C3), 68.1 (C4), 70.6 (C5), 96.8 (C1), 128.4 (Ar), 172.4, 173.7, 174.0, 175.9, 176.3 (C=O).

  1433-7851/99/3803-0371 $ 17.50+.50/0

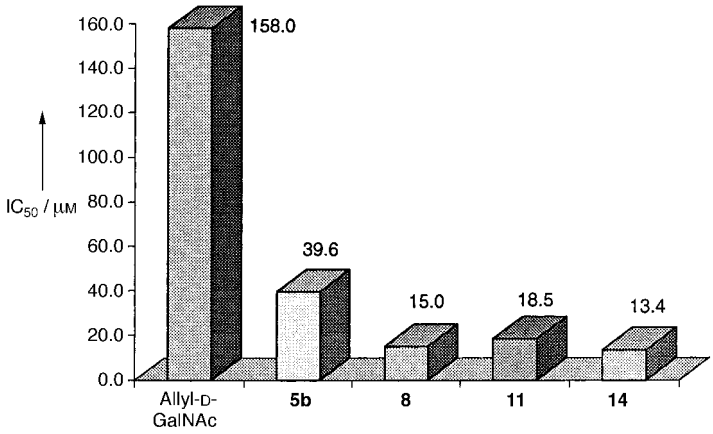


Figure 3. Enzyme-linked lectin assay of the inhibition of the binding of asialoglycophorin to horseradish peroxidase labeled VVA B_4 by allyl D-GalNAc and by ligands **5b**, **8**, **11**, and **14** (VVA B_4 is the isolectin made up of four B subunits).

were incubated at various concentrations (0.1 – 2.5 μg per well) on polystyrene microtiter plates in phosphate-buffered saline, and VVA-HRP was used to detect the direct binding properties. As shown in Figure 4, hexadecavalent glyco-

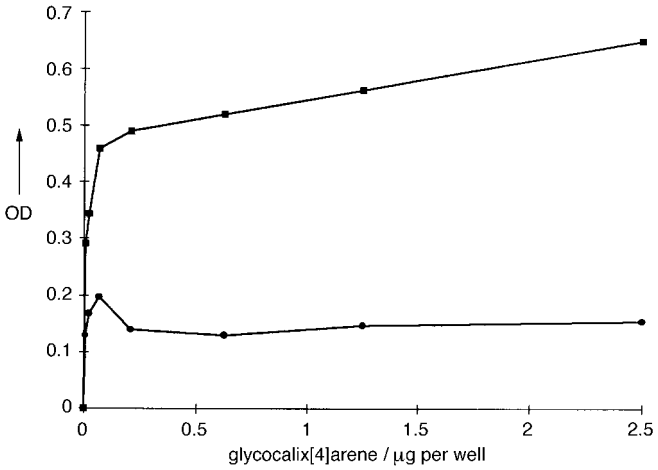


Figure 4. Enzyme-linked lectin assay (VVA-HRP) showing the hydrophobic adsorption of glycocalix[4]arenes onto the surface of a polystyrene microtiter plate. ■: **14** (16-mer), ●: **8** (4-mer). OD = optical density measured at 410 nm.

calix[4]arene **14** was hydrophobically adsorbed onto the microtiter plate surface with as little as 0.2 μg of material per well. Moreover, the interaction could be again inhibited by adding excess allyl α-D-GalNAc monomer (data not shown).

Amphiphilic α-GalNAc-containing *p-tert*-butylcalix[4] arenes with up to 16 carbohydrate units were efficiently synthesized by a novel double N-alkylation strategy. In these novel biomaterials the carbohydrate residues are well exposed to aqueous environments as shown by their lectin-binding properties. Moreover, the materials can be directly adsorbed onto the lipophilic surface of polystyrene microtiter plates and thus should be useful in bioanalytical devices.

Received: June 24, 1998
Revised version: October 13, 1998 [Z12049IE]
German version: *Angew. Chem.* **1999**, *111*, 380 – 384

Keywords: calixarenes · dendrimers · glycosides · lectins

[1] For recent reviews, see: a) A. Ikida, S. Shinkai, *Chem. Rev.* **1997**, *97*, 1713 – 1734; b) M. Takeshita, S. Shinkai, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1088 – 1097; c) C. D. Gutsche, *Aldrichimica Acta* **1995**, *28*, 3 – 9; c) V. Böhmer, *Angew. Chem.* **1995**, *107*, 785 – 818; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713 – 745.
[2] a) J. Szejtli, *J. Inclusion Phenom.* **1992**, *14*, 25 – 36; b) G. Wenz, *Angew. Chem.* **1994**, *106*, 851 – 870; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 803 – 822; c) S. Li, W. C. Purdy, *Chem. Rev.* **1992**, *92*, 1457 – 1470.
[3] S. J. Meunier, R. Roy, *Tetrahedron Lett.* **1996**, *37*, 5469 – 5472.
[4] a) A. Marra, A. Dondoni, F. Sansone, *J. Org. Chem.* **1996**, *61*, 5155 – 5158; b) A. Dondoni, M. Kleban, A. Marra, *Tetrahedron Lett.* **1997**, *38*, 7801 – 7804; c) A. Dondoni, A. Marra, M.-C. Scherrmann, A. Casnati, F. Sansone, R. Ungaro, *Chem. Eur. J.* **1997**, *3*, 1774 – 1782.
[5] a) T. Fujimoto, C. Shimizu, O. Hayashida, Y. Aoyama, *J. Am. Chem. Soc.* **1997**, *119*, 6676 – 6677; b) O. Hayashida, C. Shimizu, T. Fujimoto, Y. Aoyama, *Chem. Lett.* **1998**, 13 – 14.
[6] a) R. Roy, *Curr. Opin. Struct. Biol.* **1996**, *6*, 692 – 702; b) R. Roy, *Polym. News* **1996**, *21*, 226 – 232; c) R. Roy, *Top. Curr. Chem.* **1997**, *187*, 241 – 274; d) N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1997**, *3*, 1193 – 1199.
[7] a) T. Toyokuni, A. Singhal, *Chem. Soc. Rev.* **1995**, *24*, 231 – 308; b) S. Hakomori, *Curr. Opin. Immunol.* **1991**, *3*, 646 – 653.
[8] K. Rittenhouse-Diakun, Z. Xia, D. Pickhardt, M.-G. Baek, R. Roy, *Hybridoma* **1998**, *17*, 165 – 173.
[9] K. Iwamoto, S. Shinkai, *J. Org. Chem.* **1992**, *57*, 7066 – 7073.
[10] S. Sakai, T. Sasaki, *J. Am. Chem. Soc.* **1994**, *116*, 1587 – 1588.

Organically Templated Mixed-Valent Ti^{III}/Ti^{IV} Phosphate with an Octahedral – Tetrahedral Open Framework**

Sambandan Ekambaram and Slavi C. Sevov*

The zeolitic aluminum silicates and the more recently discovered microporous aluminum phosphates have attracted tremendous interest due to their application as catalysts, ion-exchangers, and molecular sieves in many technologically important processes. Nevertheless, due to the presence of main group elements only, these materials have no potential for redox reactions and redox catalysis. It is highly desirable, therefore, to build microporous compounds containing d-block metals as an integral part of the framework and in close proximity to the voids. Titanium has been of particular interest as a potential substitute for silicon owing to the available oxidation state of four and appropriate size. Substitutions of the tetrahedral silicon have been achieved at the doping level,[1] and despite the small amounts of titanium, the resulting materials have shown highly improved

[*] Prof. S. C. Sevov, S. Ekambaram
Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, IN 46556 (USA)
Fax: (+1) 219-631-6652
E-mail: ssevov@nd.edu

[**] We thank the National Science Foundation (DMR-9701550 and DMR-9703732) and the Bayer Corporation (Postdoctoral Fellowship) for the financial support.

  1433-7851/99/3803-0372 $ 17.50+.50/0