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Bolaforms with Fourteen Galactose Units: A Proposed Site-Directed Cohesion of Cancer Cells

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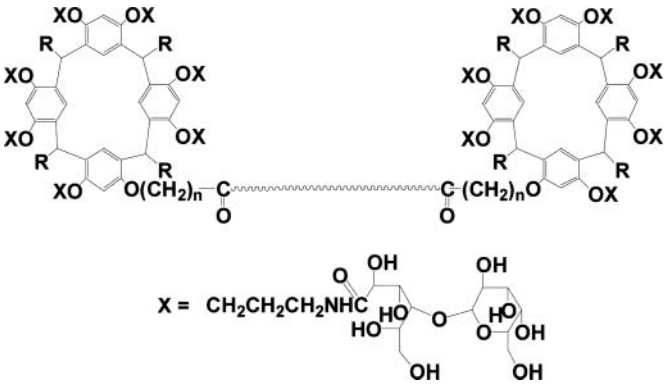
ABSTRACT



The multistep synthesis of a calixarene joined to a second calixarene via a long spacer is described. Since each calixarene bears multiple galactose-based units (known to bind strongly to rat hepatoma cells), there existed the possibility of cross-linking the cancer cells into a network. The compounds did not serve this purpose, a fact potentially correctable by adjusting or rigidifying the spacer. Formation of a "cancer net" around a solid tumor remains a viable approach to retarding growth and/or inhibiting metastasis.

Consider the possibility of an anti-cancer drug that functions by interconnecting a layer of cancer cells. Formation of such a "cancer-net" around a solid tumor might, conceivably, retard growth and/or inhibit metastasis. Since the therapy depends on chemistry occurring at the periphery of the tumor, the common problem of delivering a drug to an entire cell mass, often via an impaired blood supply, is mitigated. To be effective, of course, a cancer-net drug must selectively adhere to pathological cells in preference to healthy cells.

Interconnecting cells into networks is not a new concept. W. Meier,[1] for example, showed that certain cells, surface-modified with a biotin-terminated polymer, are subsequently agglutinated by addition of streptavidin. The polymer thus mimics natural "cell adhesion molecules",[2] i.e., proteins that unite cells via intermembrane ligand−receptor interactions. Kiessling et al.[3] synthesized polymer chains with up to 142 mannose substituents that promote the aggregation of leukemia cells. Polymers can also be used to coat cells so that adhesion is sterically blocked.[4]

The work of Y. Aoyama et al.[5] gave us the impetus to pursue nonpolymeric cancer-net compounds. They found that rat hepatoma cells bind a calixarene having two galactoses on each of its four aromatic rings. Spleen cells lacking specific galactoside receptors do not bind this calixerene. Nor do rat hepatoma cells bind the calixarene substituted with glucose instead of galactose.

Thus, it possible, that molecules with *two* galacto-calixarene macrocycles, covalently joined by a long spacer, might "glycotarget" a group of cancer cells and thereby

(1) Meier, W. *Langmuir* **2000**, *16*, 1457.
(2) Dembo, M.; Bell, G. I. *Curr. Top. Membr. Transp.* **1987**, *29*, 71.
(3) Gestwicki, J. E.; Strong, L. E.; Cairo, C. W.; Boehm, F. J.; Kiessling, L. L. *Chem. Biol.* **2002**, *9*, 163.
(4) Elbert, D. L.; Hubbell, J. A. *Chem. Biol.* **1998**, *5*, 177.
(5) (a) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397. (b) Fujimoto, K.; Miyata, T.; Aoyama, Y. *J. Am. Chem. Soc.* **2000**, *122*, 3558.

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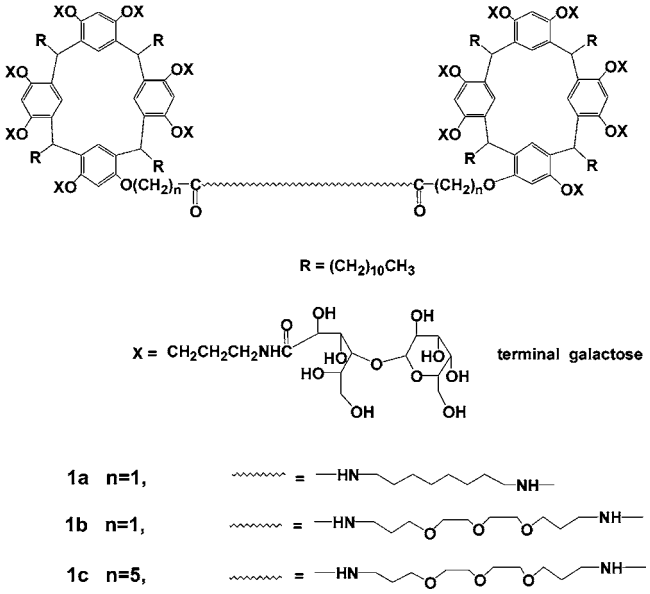
Scheme 1



interconnect them (not unlike the cross-linking of polymer chains). Compound **1** in Scheme 1 sprung from this idea.

Several features of **1** need explanation. (a) The compounds are polyvalent at both termini; multiple simultaneous interactions are known to enhance associative processes in biology.[6] Previously described scaffolds bearing polyvalent glycoconjugates include cyclodextrins,[7] proteins,[8] dendrimers,[9] gold nanoparticles,[10] vesicles,[11,12] self-assembled monolayers,[13] self-aggregates,[14] and cluster compounds.[15] (b) Spacers separating our calixarenes vary in length from 16 atoms (**1a**), to 21 atoms (**1b**), to 29 atoms (**1c**).

The variations were prompted by the fact that we did not know if undesirable "looping" (i.e., intracellular binding competitive with the transcellular connectivity) would be minimized with certain spacer lengths. Both polar and nonpolar spacers were investigated. (c) In addition to 14 galactose units, bis-calixarenes **1** each possess 8 undecyl groups in accordance with Aoyama's cell-binding monomer.[5] It is likely, but not yet established, that the hydrocarbon chains (assumed to be all-cis[16]) contribute hydrophobically to docking at the cancer cells' outer membrane surface.

The nine-step synthesis of **1** (Scheme 2) encountered special problems related to the handling of polyfunctional

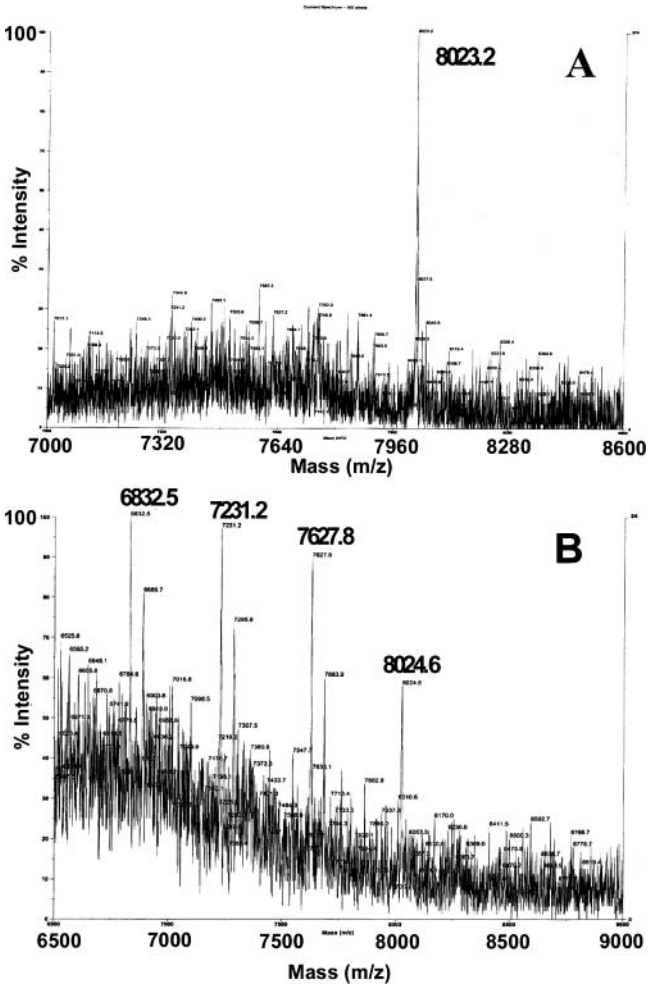


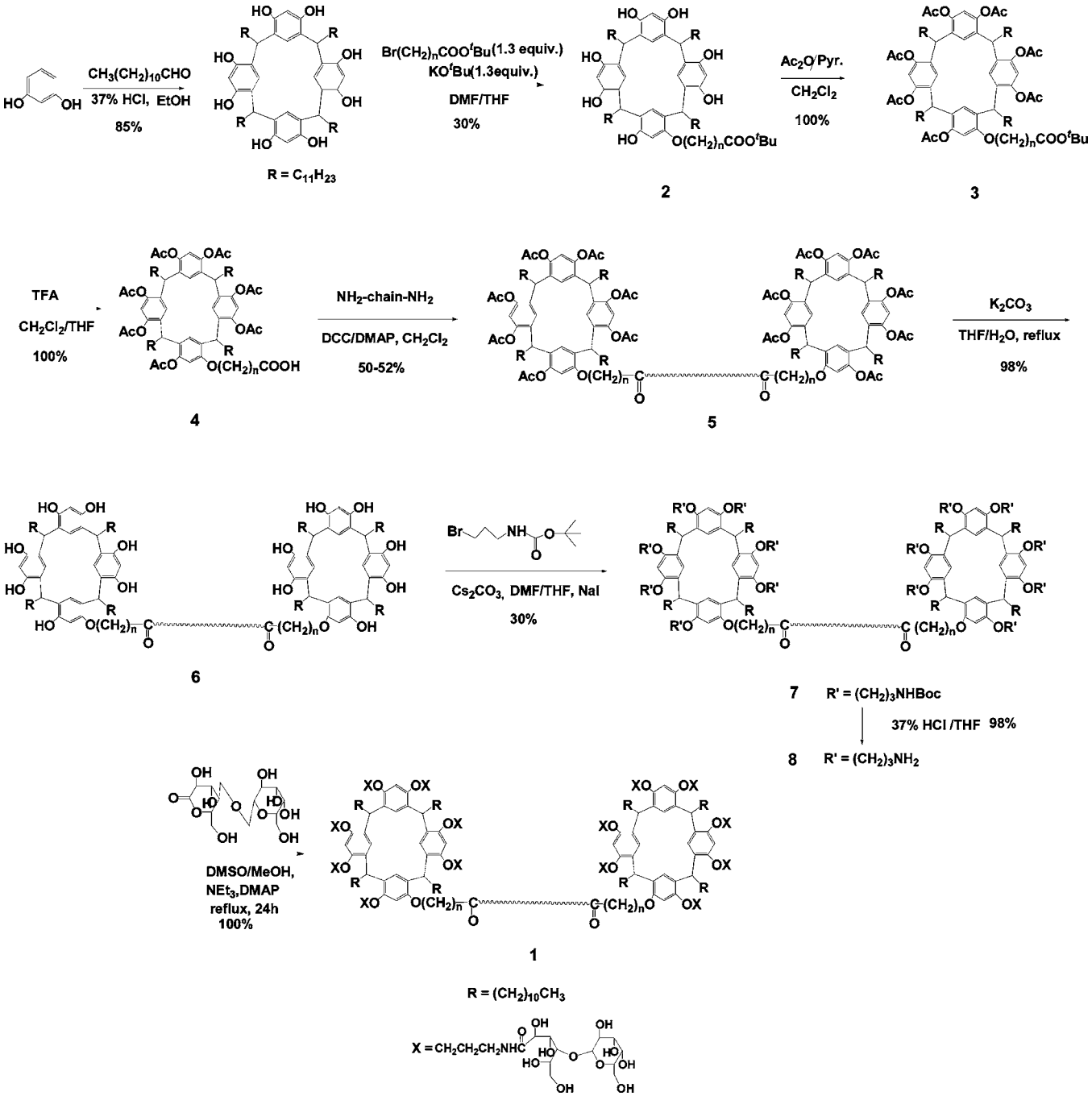
Figure 1. MALDI-MS of **1a** after using (A) Cs_2CO_3 and (B) K_2CO_3 in the **6**-to-**7** transformation.

compounds. First and foremost, we faced the need to monoderivatize one of eight equivalent hydroxyls in calix[4]resorcarene to form **2**. By using 1.3 equiv of bromo-ester, it was possible to obtain **2** in 30% yield along with, roughly, 50% starting calixarene and 20% material with 2−4 alkyl groups. Chromatography with gradient elution (0−20% CH_3CN in $CHCl_3$ on silica) allowed isolation of **2**. Acetylation of the remaining seven hydroxyls with Ac_2O followed by deprotection of the carboxyl (leaving the acetates untouched) gave **4**. Compound **4** allowed a DCC-promoted coupling with a diamine "spacer" in moderate yields. Under our reaction conditions, the diamines preferentially linked the calixarenes (as opposed to deacylating them) to give **5**. Coupling yields were low when the hydroxyls were not acetylated. Removal of the acetate groups in the presence of the amide groups was surprisingly touchy: since neither aqueous HCl nor NaOH performed well, we resorted to aqueous K_2CO_3 to secure **6** in high yield.

In synthesizing their monomeric calixarene, Aoyama et al.[5] octa-alkylated the parent calixarene with bromo-aceto-

(6) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755.
(7) Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem.* **2001**, *12*, 655.
(8) Lemieux, G. A.; Bertozzi, C. R. *Chem. Biol.* **2001**, *8*, 265.
(9) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088.
(10) Hernáiz, M. J.; de la Fuente, J. M.; Barrientos, A. G.; Penadés, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 1554.
(11) Ma, Z.; Li, J.; Liu, M.; Cao, J.; Zou, Z.; Tu, J.; Jiang, L. *J. Am. Chem. Soc.* **1998**, *120*, 12678.
(12) Chenevier, P.; Grandjean, C.; Loing, E.; Malingue, F.; Angyalosi, G.; Gras-Masse, H.; Roux, D.; Melnyk, O.; Bourel-Bonnet, L. *Chem. Comm.* **2002**, *20*, 2446.
(13) Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11782.
(14) Akiyoshi, K.; Deguchi, S.; Moriguchi, N.; Yamaguchi, S.; Sunamoto, J. *Macromolecules* **1993**, *26*, 3062.
(15) Grandjean, C.; Angyalosi, G.; Loing, E.; Adriaenssens, E.; Melnyk, O.; Pancre, V.; Auriault, C.; Gras-Masse, H. *Chem. BioChem.* **2001**, *2*, 747.
(16) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6676.

Scheme 2



nitrile followed by reduction to an octa-amine. The octa-amine was subsequently reacted with a sugar lactone. This strategy was unsuccessful with our compounds due to the difficulty of reducing the fourteen nitriles in the presence of the two spacer amides. Hence, we shifted to a nonreductive pathway: intermediate **6** was treated with $Br(CH_2)_3NHBoc$, a "14-site" alkylation that proceeded to completion only in the presence of Cs_2CO_3. Removal of the Boc groups from **7** with HCl produced polyamine **8** that could now be reacted with the sugar lactone to give **1**. Figure 1A, the spectrum with the correct $[MW + Na]^+$ of 8024, was critical in affirming the proposed structure of **1**. When K_2CO_3 was used instead of Cs_2CO_3 in the **6**-to-**7** step, the spectrum (Figure 1B) displayed an abundance of lower homologues.

Our cell experiments used epithelial-like 4-dimethyl-amino azobenzene-transformed rat liver cells (Riken RLC-16, Japan) cultured in Williams' Medium E containing 1% L-glutamine, 1% penicillin/streptomycin, and 10% v/v fetal bovine serum. The surface-adhered cells were washed three times with Dulbecco's phosphate-buffered saline prior to being trypsinized via a quick rinse with 0.25% trypsin/EDTA followed by a 30 min incubation at 37 °C also with trypsin/EDTA. Fetal bovine serum in medium or buffer was then added to retard the trypsin. Repeated pipeting broke the

suspension into single cells. Samples (1.0 mL) placed in wells of a 24-well polystyrene microtiter plate contained light to heavy cell concentrations (50−1000 K per well) and one of our compounds (1.5×10^{-4} to 5×10^{-2} mM). Exposure of the cells to **1**, with and without gentle shaking, or with and without the presence of Ca^{2+}, was allowed to proceed for 5−30 min at room temperature. Subsequent inspection of the cell suspensions by light microscope and light scattering gave no convincing evidence for aggregation. Since the work of Aoyama et al.[5] with simple analogues of **1** affirmed the presence of binding to the identical cell line, lack of cell flocculation may have more to do with spacer length than with binding equilibria. Spacer lengths must, of course, allow intercellular cohesion to compete with intracellular looping. Rigid spacers[17] would have, in retrospect, favored the former. Despite our apparent setback, the concept of a cancer-net drug, in addition to the design and synthesis of polyglycosylated bolaforms that address the concept (if not actually manifesting it), clears the way for future work in the area.

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Supporting Information Available: Synthetic procedures for the compounds in Scheme 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Schwab, P. F. H.; Levin, M. D.; Michl, J. *Chem. Rev.* **1999**, *99*, 1863.