Water-Soluble Cavitands: Synthesis of Methylene-Bridged Resorcin[4]arenes Containing Hydroxyls and Phosphates at Their Feet and Bromomethyls and Thiomethyls at Their Rims

Adam R. Mezo and John C. Sherman*

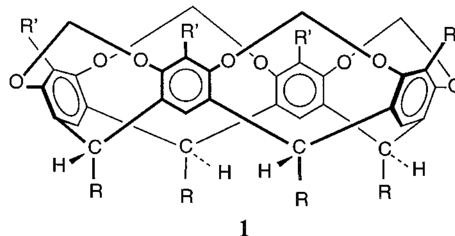
Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada, V6T 1Z1

Received February 18, 1998

The synthesis of rim-functionalized methylene-bridged resorcin[4]arenes ("cavitands") containing hydrophilic propanol or water-soublilizing propylphosphate feet is described. The cavitands possess the synthetically useful benzylthiol (cavitands **6** and **16)** or benzylbromide (cavitands **9** and **11**) functionalities at their rims, which are suitable for further derivatization near the hydrophobic cavity of the cavitand. These water-soluble cavitands represent new building blocks that are ideal for use in aqueous supramolecular chemistry. As an example of their synthetic utility in supramolecular studies, we have reacted phosphate-footed cavitands **11** and **16** with cysteine-containing peptide **17** and chloroacetylated peptide **19**, respectively, to afford the corresponding de novo proteins **18** and **20**.

Introduction

The study of supramolecular assemblies in water is an important stepping stone toward understanding and emulating the noncovalent interactions found in nature.[1] The development of new supramolecular building blocks that are soluble in water would be a great asset to this area. Methylene-bridged resorcin[4]arenes,[2] or cavitands, of the general structure **1** have been used extensively in supramolecular assemblies because of their rigidity, enforced cavities and synthetic availability.[2e,3] They have been used to (1) synthesize carceplexes, where chemical and physical properites of molecules can be altered by encapsulation,[3a,4] (2) bind neutral guest molecules,[5] useful, for example, in the removal of toxins from wastewater,[5f] and (3) form monolayers which have potential as molecular sensors.[6] However, these cavitands have all incorporated a hydrophobic pendent group (R, so-called "feet" in structure **1**) which limits their solubility to organic solvents and hence, their versatility as models for supramolecular study. We recently reported the first



1

water-soluble cavitand,[7] but only by incorporating water-solubilizing groups at the rim positions (R′); this position is vital for the incorporation of functional groups near the cavity. To effectively break into aqueous cavitand chemistry, it is essential to incorporate water-solubilizing functionalities into the pendent group (R, cavitand **1**) while retaining useful functionalities at the rim position (R′) suitable for further derivatization.

In 1989 Cram and co-workers introduced a method to incorporate hydroxyl groups into the pendent groups during the initial condensation step to form butanol-footed, unbridged resorcin[4]arenes.[8] More recently, our group developed a method to selectively bridge the phenolic hydroxyls to afford the first hydroxyl-footed methylene-bridged cavitands.[9] Here, we use this methodology in conjunction with a series of orthogonal protection and functionalization steps to yield rim-functional-

(1) (a) For a review of cyclodextrins, see: Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803−822. (b) Diederich, F.; Dick, K.; Griebel, D. *Chem. Ber.* **1985**, *118*, 3588−3619. (c) Kobayashi, K.; Asakawa, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10307−10313.

(2) For recent examples of unbridged resorcin[4]arenes, see: (a) Lewis, P. T.; Davis, C. J.; Saraiva, M. C.; Treleaven, W. D.; McCarley, T. D.; Strongin, R. M. *J. Org. Chem.* **1997**, *62*, 6110−6111. (b) Botta, B.; Monache, G. D.; Salvatore, P.; Gasparrini, F.; Villani, C.; Botta, M.; Corelli, F.; Tafi, A.; Gacs-Baitz, E.; Santini, A.; Carvalho, C. F.; Misti, D. *J. Org. Chem.* **1997, *62***, 932−938. (c) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6676−6677. For recent reviews, see: (d) Schneider, H. J.; Schneider, U. *J. Inclusion Phenom.* **1994**, *19*, 67−83. (e) Timmerman, P.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron* **1996**, *52*, 2663−2704.

(3) (a) Cram, D. J.; Cram, J. M. *Container Molecules and Their Guests, Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; Royal Society of Chemistry: Oxford, UK, 1994; Vol. 4.; (b) Moran, J. R.; Karbach, S.; Cram, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 5826−5828.

(4) (a) Sherman, J. C. *Tetrahedron* **1995**, *51*, 3395−3422. (b) Sherman, J. C. In *Large Ring Molecules*; Semlyen, J. A., Ed.; J. Wiley & Sons: West Sussex, England, 1996; pp 507−524. (c) Yoon, J.; Cram, D. J. *J. Chem. Soc. Chem. Commun.* **1997**, 497−498.

(5) (a) Tucker, J. A.; Knobler, C. B.; Trueblood, K. N.; Cram, D. J. *J. Am. Chem. Soc.* **1989**, *111*, 3688−3699. (b) Rudkevich, D. M.; Hilmersson, G.; Rebek, J. *J. Am. Chem. Soc.* **1997**, *119*, 9911−9912. (c) Careri, M.; Dalcanale, E.; Mangia, A.; Ruffini, M. *Anal. Comm.* **1997**, *34*, 13−15. (d) Vincetti, M.; Dalcanale, E. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1069−1076. (e) Boerrigter, H.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1997**, *62*, 7148−7155. (f) Dalcanale, E.; Costantini, G.; Soncini, P. *J. Inclusion Phenom.* **1992**, *13*, 87−92.

(6) (a) Schierbaum, K. D.; Weiss, T.; Thoden van Velzen, E. U.; Engbersen, J. F. J.; Reinhoudt, D. N.; Göpel, W. *Science* **1994**, *265*, 1413−1415. (b) Thoden van Velzen, E. U.; Engbersen, J. F. J.; de Lange, P. J.; Mahy, J. W. G.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6853−6862.

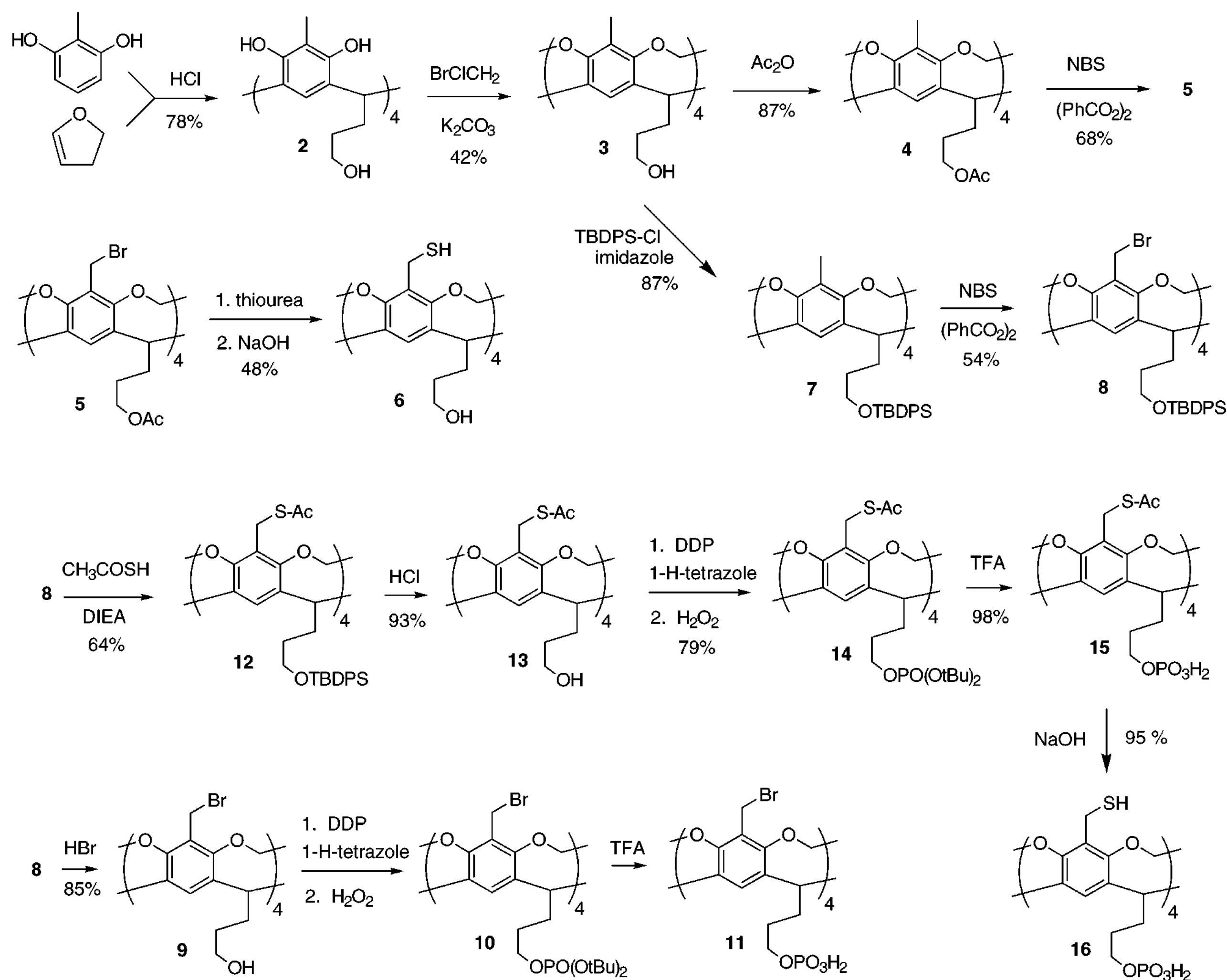
(7) Fraser, J. R.; Borecka, B.; Trotter, J.; Sherman, J. C. *J. Org. Chem.* **1995**, *60*, 1207−1213.

(8) Tunstad, L. M.; Tucker, J. A.; Dalcanale, E.; Weiser, J.; Bryant, J. A.; Sherman, J. C.; Helgeson, R. C.; Knobler, C. B.; Cram, D. J. *J. Org. Chem.* **1989**, *54*, 1305−1312.

(9) Gibb, B.C.; Chapman, R. G.; Sherman, J. C. *J. Org. Chem.* **1996**, *61*, 1505−1509.

S0022-3263(98)00305-3 CCC: $15.00 © 1998 American Chemical Society
Published on Web 09/09/1998

Scheme 1



ized water-soluble cavitands. More specifically, we present a method to introduce benzylbromides or benzylthiols at the rim position (R′), while incorporating either hydroxyl or phosphate groups into the feet of the cavitands. Among the many potential water-solubilizing groups, we chose phosphates because of their synthetic viability and to incorporate as many charges as possible into the feet.

We are also interested in developing templates for the organization of peptide structure. We recently linked four 14-residue peptides to a cavitand containing methyl feet to create a de novo protein based upon a hydrophobic template.[10] In this paper, we illustrate the synthetic utility of phosphate-footed cavitands by reacting suitably activated peptides with both phosphate-footed bromomethyl and phosphate-footed thiomethyl cavitands, thus forming a de novo protein linked to a hydrophilic template.

Results and Discussion

Synthesis. Two criteria were born in mind in designing the water-soluble cavitands: (1) the rims of the cavitands must be easily derivatized and (2) their feet must be short and water-soluble. With respect to the first criteria, we chose 2-methylresorcinol to exploit the synthetically versatile aryl methyl group, which can lead to various functional groups on the rim of the cavitand.[11] In accord with the second criteria, 2,3-dihydrofuran was used as the latent aldehyde to introduce short propanol feet and thus aid in reducing the cavitand's overall hydrophobicity. The resulting acid-catalyzed condensation reaction of 2-methylresorcinol and 2,3-dihydrofuran proceeded in 78% yield to form C_{4v}-symmetric dodecol **2** (Scheme 1). Selective bridging[9] of the phenolic hydroxyl groups with CH_2BrCl afforded the rigid methylene-bridged 2-methyl cavitand **3** (42%).

Our first target was hydroxyl-footed benzylthiol **6** accessible via Sorrell's bromination method.[11] Initial attempts to directly brominate hydroxyl-footed cavitand **3** failed due to poor solubility in the desired solvent CCl_4. Acetylation of the pendent hydroxyl groups with acetic anhydride in pyridine afforded cavitand **4** (87%), which was soluble in CCl_4. Radical bromination with NBS and benzoyl peroxide as an initiator afforded acetyl-protected benzyl bromide **5** (68%). Treatment of cavitand **5** with thiourea followed by base hydrolysis produced benzylthiols and regenerated the hydroxyls in one step to form hydroxyl-footed benzylthiol **6** (48%). This route to a benzylthiol cavitand was found to be more efficient than one previously developed by Cram.[12]

(10) Gibb, B. C.; Mezo, A. R.; Sherman, J. C. *Tetrahedron Lett.* **1995**, *36*, 7587−7590.

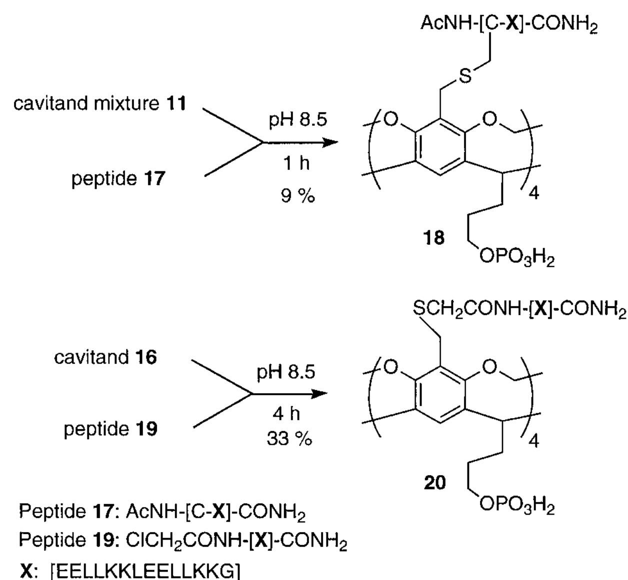
(11) Sorrell, T. N.; Pigge, F. C. *J. Org. Chem.* **1993**, *58*, 784−785.

While planning the syntheses for both phosphate-footed benzyl bromide **11** and phosphate-footed benzylthiol **16**, it was evident that positions R and R′ (structure **1**) on the cavitand should possess orthogonal reactivities. As base-sensitive benzyl bromides were one target for position R′, we chose to use base-sensitive protecting groups in position R′ and acid-sensitive groups in position R. The chosen acid-sensitive protecting group for the hydroxyl feet must be stable to NBS yet be removable without affecting the acetal bridges of the cavitand. Thus, cavitand **3** was treated with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) and imidazole to form TBDPS-protected cavitand **7** (87%). Subsequent selective bromination with NBS and benzoyl peroxide afforded benzyl bromide **8** (54%). Removal of the TBDPS protecting groups with HBr afforded hydroxyl-footed benzyl bromide **9** (85%).

For the synthesis of cavitand **11**, we chose to phosphorylate the hydroxyl feet with di-*tert*-butyl *N,N*-diethylphosphoramidite[13] (DDP) followed by oxidation with H_2O_2. The use of DDP has four main advantages: DDP (1) eliminates the need to generate alkoxides in base-sensitive compounds such as cavitand **9**, (2) provides acid-labile phosphate protecting groups consistent with our protection strategy, (3) generates phosphite esters in high yields, and (4) is quite stable, thus allowing convenient storage. Phosphorylation of cavitand **9** with DDP followed by oxidation with hydrogen peroxide at −78 °C afforded *tert*-butyl-protected phosphate **10**. Subsequent removal of the *tert*-butyl groups with TFA afforded phosphate-footed benzyl bromide **11**. Unfortunately, the phosphorylation did not proceed to completion (most likely a function of the purity of the starting material available only in technical grade),[13] and resulted in residual amounts of the corresponding tris-phosphate impurity, which were evident in the ^{1}H NMR and LSIMS mass spectra. Attempts to further purify the compound before and after removal of the *tert*-butyl groups were unsuccessful. We hoped that subsequent derivatization would facilitate purification. Indeed, in the pursuit of de novo proteins, reaction of impure benzyl bromide **11** with cysteine-containing peptide **17** in pH 8.5 phosphate buffer gave a mixture of products that was separable by reversed phase HPLC to afford de novo protein **18** (Scheme 2).[14]

En route to phosphate-footed benzylthiol **16**, it was necessary to introduce a protected thiol from TBDPS-protected benzyl bromide **8**; the *S*-protecting group must be stable to the acidic conditions required to remove the TBDPS group (HCl), the mildly basic conditions required for phosphorylation (1*H*-tetrazole), the oxidatitive conditions to generate the phosphate (H_2O_2, −78 °C), and must be removable in the presence of a phosphate. To this end, the *S*-acetyl protecting group was chosen and introduced by treatment of benzyl bromide **8** with thioacetic acid and diisopropylethylamine to afford *S*-acetyl cavitand **12** (64%). Removal of the TBDPS groups with HCl afforded hydroxyl-footed *S*-acetyl cavitand **13** (93%). Phosphorylation of the hydroxyl groups with DDP followed by oxidation with H_2O_2 yielded *S*-acetyl *tert*-butyl-protected phosphate **14** (79%). Removal of the *tert*-butyl groups with TFA followed by removal of the acetyl groups with NaOH afforded phosphate-footed tetrathiol **16** (93% for 2 steps). Both phosphate-footed cavitands **11** and **16** have eight potential negative charges and are thus soluble in water (pH 8). As an example of the synthetic utility of cavitand **16**, we reacted tetrathiol **16** with chloroacetylated peptide **19** in phosphate buffer (pH 8.5) to afford de novo protein **20** (Scheme 2).[14]

Scheme 2



Conclusion

We have developed synthetic pathways for both hydroxyl-footed and phosphate-footed cavitands that are functionalized at their rims. These cavitands possess either electrophilic benzyl bromides or nucleophilic benzylthiols at their rims which are suitable for further manipulation. These rigid, water-soluble cavitands open the door to a variety of new areas of cavitand chemistry ideal for supramolecular study. A number of studies are currently underway in our labs, including the investigation of water-soluble carceplexes and hemicarceplexes.[15]

Another program of research in our lab entails the use of cavitands as templates for the organization of peptide structure.[10,16] We have shown in this paper that both benzyl bromide **11** and benzylthiol **16**, when reacted with suitably activated peptides, provide four peptides that are linked by highly charged templates. Studies on the structure and stability of these de novo proteins are currently underway. We are also extending this meth-

(12) Cram, D. J.; Karbach, S.; Kim, Y. H.; Baczynshkyj, L.; Marti, K.; Sampson, R. M.; Kalleymeyn, G. W. *J. Am. Chem. Soc.* **1988**, *110*, 2554−2560.

(13) Perich, J. W.; Johns, R. B. *Synth. Commun.* **1988**, 142−144. Attempts to purify DDP were unsuccessful.

(14) The amino acid sequences of peptides **17** and **19** were designed such that each peptide would have a high propensity to form an amphiphilic α-helix and bundle with the other helices attached to the cavitand (ref 10). In addition, peptide **17** included a *N*-terminal cysteine for reaction with benzyl bromide **11** while peptide **19** included a *N*-terminal chloroacetyl group for reaction with benzylthiol **16**. The structures and stabilities of de novo proteins **18** and **20** will be reported elsewhere. Standard one letter code abbreviations have been used for the amino acids: C = cysteine; E = glutamic acid; G = glycine; K = lysine; L = leucine.

(15) The first water-soluble hemicarceplex was recently reported;[4c] however, its aqueous solubility was derived from its inter-bowl linkages. Such a method to attain water solubility greatly limits the variety of potential inter-bowl bridges and thus limits the cavity size. By attaining water solubility via the cavitand's pendent groups, carceplexes and hemicarceplexes are far more versatile candidates for supramolecular study and potential drug delivery systems.

(16) Gibb, B. C.; Mezo, A. R.; Causton, A. S.; Fraser, J. R.; Tsai, F. C. S.; Sherman, J. C. *Tetrahedron* **51**, 8719−8732.

odology to study the aqueous binding of neutral organic molecules by phosphate-footed cavitands. Cram and coworkers have shown that methyl-footed cavitands bind molecules such as CH_2Cl_2 in a 1:1 stoichiometry in organic solvents.[5a] Moreover, we have demonstrated binding of neutral guests in a 2:1 cavitand:guest stoichiometry.[17] Such complexation may indeed be far stronger in aqueous solution.

Experimental Section

General. Chemicals were reagent grade (Aldrich) except for di-*tert*-butyl *N,N*-diethylphosphoramidite (Toronto Research Chemicals) which was technical grade and used without further purification. THF was distilled under N_2 from sodium benzophenone ketyl. DMF and *N,N*-dimethylacetamide (DMA) were dried over 4 Å molecular sieves. LSIMS were run in positive mode (unless otherwise noted) and recorded using thioglycerol (TG), *m*-nitrobenzyl alcohol (NBA), chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), or methanol (MeOH) as the matrixes. MALDI-TOF mass spectra were run using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Melting points are uncorrected. Silica gel (230−400 mesh, BDH) was used for column chromatography, and silica gel glass-backed analytical plates (0.2 mm, Aldrich) were used for TLC with UV detection. Size exclusion chromatography was performed using Sephadex LH-20. All products were dried overnight at room temperature and 0.1 Torr.

2-Methyldodecol (2). 2,3-Dihydrofuran (3.04 mL, 40.3 mmol) was added over 4 h with a syringe pump to a solution of 2-methylresorcinol (5.00 g, 40.3 mmol) and concentrated HCl (7.6 mL) in MeOH (30 mL) and stirred for 16 h under N_2. The reaction mixture was then heated to 50 °C for 5 days. The precipitate was filtered, washed rigorously with H_2O, and dried in vacuo for 24 h. The solid was then suspended in THF, sonicated, and filtered to afford dodecol **2** as an off-white solid (6.1 g, 78%): mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 8.62 (s, 8H), 7.27 (s, 4H), 4.19 (t, J = 7.8 Hz, 4H), 4.10 (br s, 4H), 3.44 (t, J = 6.7 Hz, 8H), 2.24 (m, 8H), 1.94 (s, 12H), 1.35 (m, 8H); MS (TG + DMSO) m/z 776 ((M + H)$^+$, 90), 717 ((M − $(CH_2)_3OH$ + H)$^+$, 100). Anal. Calcd for $C_{44}H_{56}O_{12}\cdot H_2O$: C, 66.48; H, 7.35. Found: C, 66.71; H, 7.28.

2-Methyl Cavitand 3. Dodecol **2** (20.0 g, 25.8 mmol) was dissolved in degassed DMA (100 mL) and added via a syringe pump over 48 h under N_2 at room temperature to a solution of DMA (700 mL), bromochloromethane (7.50 mL, 113 mmol), and potassium carbonate (46.3 g, 335 mmol). After an additional 24 h at room temperature, CH_2BrCl (7.50 mL, 113 mmol) was added, and the reaction mixture was heated to 45 °C. An additional aliquot of CH_2BrCl (7.50 mL, 113 mmol) was added the next day, and the reaction mixture was heated to 65 °C. After another 24 h at 65 °C, the reaction mixture was evaporated in vacuo followed by careful neutralization of the carbonate salts with 2 M HCl. The crude precipitate was filtered, washed with water until the filtrate was neutral, dissolved in THF, and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The solid was purified by column chromatography ($CHCl_3$:MeOH, 9:1) to afford 2-methyl cavitand **3** as an off-white solid (8.84 g, 42%): mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 7.43 (s, 4H), 5.86 (d, J = 7.4 Hz, 4H), 4.59 (t, J = 6.1 Hz, 4H), 4.43 (t, J = 5.1 Hz, 4H), 4.19 (d, J = 7.4 Hz, 4H), 3.48 (m, 8H), 2.35 (m, 8H), 1.88 (s, 12H), 1.42 (m, 8H); MS (TG) m/z 825 ((M + H)$^+$, 100). Anal. Calcd for $C_{48}H_{56}O_{12}\cdot H_2O$: C, 68.39; H, 6.93. Found: C, 68.07; H, 6.82.

Acetylated 2-Methyl Cavitand 4. 2-Methyl cavitand **3** (0.72 g, 0.87 mmol) was dissolved in pyridine (8 mL) and acetic anhydride (8 mL) and stirred overnight under N_2. The solvent was removed in vacuo, and the crude product purified by column chromatography ($CHCl_3$:MeOH, 96:4) to afford acetylated 2-methyl cavitand **4** as a white solid (0.76 g, 87%): mp 131 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 4H), 5.86 (d, J = 6.9 Hz, 4H), 4.82 (t, J = 9.1 Hz, 4H), 4.23 (d, J = 6.9 Hz, 4H), 4.15 (t, J = 6.5 Hz, 8H), 2.26 (m, 8H), 2.03 (s, 12H), 1.95 (s, 12H), 1.69 (m, 8H); MS (TG) m/z 993 ((M + H)+, 100). Anal. Calcd for $C_{56}H_{64}O_{16}$: C, 67.73; H, 6.50. Found: C, 67.65; H, 6.30.

Acetylated Benzyl Bromide 5. A solution of 2-methyl acetylated cavitand **4** (0.74 g, 0.75 mmol), NBS (0.58 g, 3.3 mmol), and benzoyl peroxide (10 mg) in CCl_4 (50 mL) was refluxed for 16 h. The reaction mixture was then cooled to room temperature and filtered, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (EtOAc:hexanes, 1:1) to afford benzyl bromide **5** as a white solid (0.66 g, 68%): mp 136 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 7.09 (s, 4H), 6.01 (d, J = 6.7 Hz, 4H), 4.82 (t, J = 8.1 Hz), 4.55 (d, J = 6.7, 4H), 4.39 (s, 8H), 4.15 (t, J = 6.5 Hz, 8H), 2.28 (m, 8H), 2.05 (s, 12H), 1.67 (m, 8H); MS (TG) m/z 1229 ((M − Br + H)$^+$, 100), 1309 ((M + H)$^+$, 80). Anal. Calcd for $C_{56}H_{60}Br_4O_{16}$: C, 51.40; H, 4.62. Found: C, 51.60; H, 4.42.

Hydroxyl-Footed Benzylthiol 6. Thiourea (5.1 mg, 0.072 mmol) was added to a solution of bromocavitand **5** (20 mg, 0.015 mmol) in degassed DMF (2 mL), and the mixture was stirred for 2 h. The reaction mixture was poured onto degassed 2 M NaOH (2 mL) and stirred for 1 h. The reaction mixture was evaporated in vacuo, dissolved in water, and acidified with 5% acetic acid. The precipitate was filtered, thoroughly washed with water, dissolved in $CHCl_3$:MeOH (9:1), dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by column chromatography (9:1, $CHCl_3$:MeOH) to afford tetrathiol **6** as a white solid (7.0 mg, 48%): mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 4H), 5.95 (d, J = 7.5 Hz, 4H), 4.59 (t, J = 7.9 Hz, 4H), 4.43 (m, 8H), 3.47 (m, 16H), 2.71 (t, J = 7.8 Hz, 4H), 2.38 (m, 8H), 1.42 (m, 8H); MS (LSIMS$^-$, NBA) m/z 917 ((M − SH − H)$^-$, 100), 951 ((M − H)$^-$, 50). Anal. Calcd for $C_{48}H_{56}O_{12}S_4\cdot H_2O$: C, 59.36; H, 6.02. Found: C, 59.43; H, 5.73.

TBDPS-Protected 2-Methyl Cavitand 7. TBDPS-Cl (6.06 mL, 23.3 mmol) was added to a solution of cavitand **3** (2.40 g, 2.91 mmol) and imidazole (3.16 g, 46.6 mmol) in DMF (20 mL) and stirred overnight under N_2 at room temperature. The DMF was then evaporated in vacuo, $CHCl_3$ (20 mL) was added, and the organic layer was washed with water (3 × 10 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by column chromatography (9:1, hexanes: EtOAc) to afford cavitand **7** as a white foam (4.62 g, 87%): ^{1}H NMR ($CDCl_3$) δ 7.60 (m, 16H), 7.28 (m, 24H), 6.91 (s, 4H), 5.85 (d, J = 6.9 Hz, 4H), 4.77 (t, J = 8.2 Hz, 4H), 4.23 (d, J = 6.9 Hz, 4H), 3.66 (t, J = 6.4 Hz, 8H), 2.22 (m, 8H), 1.95 (s, 12H), 1.59 (m, 8H), 0.99 (s, 36H); MS (LSIMS$^-$, NBA + TG + $CHCl_3$) m/z 1824 ((M − H)$^-$, 40), 1931 ((M·thioglycerol − H)$^-$, 100). Anal. Calcd for $C_{112}H_{128}O_{12}Si_4$: C, 75.64; H, 7.25. Found: C, 75.49; H, 7.12.

TBDPS-Protected Benzyl Bromide 8. NBS (2.93 g, 16.5 mmol) and benzoyl peroxide (0.10 g, 0.41 mmol) were added to a solution of cavitand **7** (6.83 g, 3.74 mmol) in CCl_4. The solution was refluxed for 18 h under N_2 at which point the reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated in vacuo, and the crude residue was purified by column chromatography (hexanes:EtOAc, 95:5; followed by 92:8) to afford benzyl bromide **8** as a white foam (2.41 g, 54%): ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 16H), 7.30 (m, 24H), 7.06 (s, 4H), 6.00 (d, J = 6.2 Hz, 4H), 4.78 (t, J = 7.9 Hz, 4H), 4.54 (d, J = 6.2 Hz, 4H), 4.39 (s, 8H), 3.65 (t, J = 6.3 Hz, 8H), 2.22 (m, 8H), 1.56 (m, 8H), 0.99 (s, 36H); MS (LSIMS$^-$, NBA + $CHCl_3$) m/z 2093 ((M − H)$^-$, 100). Anal. Calcd for $C_{112}H_{124}Br_4O_{12}Si_4$: C, 64.24; H, 5.97. Found: C, 64.64; H, 5.96.

Hydroxyl-Footed Benzyl Bromide 9. HBr (48%, 420 μL, 2.49 mmol) was added to a solution of tetrabromocavitand **8** (80 mg, 0.038 mmol) in THF:MeOH (4:1, 4 mL), and the reaction was stirred for 4 h. The reaction mixture was then evaporated in vacuo and purified by column chromatography (9:1, $CHCl_3$:MeOH) to afford hydroxyl-footed cavitand **9** as a white solid (37 mg, 85%): mp > 250 °C; ^{1}H NMR (acetone-d_6)

(17) Chapman, B. C.; Sherman, J. C. *J. Am. Chem. Soc.* **1995**, *117*, 9081−9082.
(18) The ^{1}H NMR signals from the tris-phosphate appeared as broad signals under those of the tetraphosphate.
(19) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787−3788.

δ 8.02 (s, 4H), 6.03 (d, $J = 7.5$ Hz, 4H), 4.73 (t, $J = 8.2$ Hz, 4H), 4.55 (s, 8H), 4.49 (d, $J = 7.5$ Hz, 4H), 3.96 (t, $J = 5.9$ Hz, 4H), 3.64 (dt, $J = 5.9$ Hz, $J = 5.9$ Hz, 8H), 2.70 (m, 8H), 1.51 (m, 8H); MS (LSIMS$^-$, TG + $CHCl_3$) m/z 1140 ((M)$^-$, 60), 1219 ((M·Br)$^-$, 100). Anal. Calcd for $C_{48}H_{52}Br_4O_{12}·H_2O$: C, 49.91; H, 4.72. Found: C, 50.17; H, 4.62.

***tert*-Butyl Phosphorylated Benzyl Bromide 10.** 1*H*-Tetrazole (46 mg, 0.66 mmol) was added to a THF (10 mL) solution of hydroxyl-footed benzyl bromide cavitand **9** (25 mg, 0.022 mmol) and di-*tert*-butyl diethylphosphoramidite (61 μL, 0.22 mmol), and the mixture was stirred for 15 min at room temperature. The reaction mixture was then cooled to −78 °C, H_2O_2 (30 μL, 0.26 mmol) was added, and the reaction mixture was allowed to warm to room temperature over 30 min. The reaction mixture was poured onto H_2O, extracted three times with $CHCl_3$, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by size exclusion chromatography (EtOAc:MeOH:H_2O, 40:10:4) followed by column chromatography ($CHCl_3$:MeOH; 96:4) to afford a white solid, which was a mixture of tetraphosphate **10** and ca. 15% of the corresponding tris-phosphate derivative; tetraphosphate **10** was not purified further.[18] For **10**: ^{1}H NMR ($CDCl_3$) δ 7.15 (bs, 4H), 5.99 (d, $J = 6.5$ Hz, 4H), 4.82 (t, $J = 7.5$ Hz, 4H), 4.54 (d, $J = 6.5$ Hz, 4H), 4.38 (s, 8H), 4.00 (m, 8H), 2.35 (bm, 8H), 1.67 (bm, 8H), 1.45 (s, 72H); ^{31}P NMR ($CDCl_3$) δ −9.97 (s, 4P); MS (NBA) m/z 1461 ((M − 8(C(CH_3)$_3$) + 9H)$^+$, 100), 1798 (M − 2(C(CH_3)$_3$) + 9H)$^+$, 20), 1855 (M − C(CH_3)$_3$ + 9H)$^+$; 10); HRMS (NBA) calcd for (M − 8(C(CH_3)$_3$) + 9H)$^+$ 1460.8910, found 1460.8924.

Phosphate-Footed Benzyl Bromide 11. TFA (0.32 mL, 4.2 mmol) was added to a CH_2Cl_2 (4 mL) solution of impure tetraphosphate **10** (25 mg of the mixture described above, ca. 0.01 mmol), and the mixture was stirred for 10 min. The reaction mixture was evaporated in vacuo to afford a white solid, which was a mixture of tetraphosphate-footed benzyl bromide **11** and ca. 15% of the corresponding tris-phosphate derivative (19 mg); tetraphosphate **11** was not purified further.[18] For **11**: ^{1}H NMR ($CDCl_3$ + 20% acetone-d_6) δ 7.36 (bs, 4H), 5.95 (d, $J = 7.3$ Hz, 4H), 4.69 (bt, $J = 7.6$ Hz, 4H), 4.43 (m, 12H), 4.15 (bm, 8H), 2.42 (bm, 8H), 1.59 (bm, 8H); ^{31}P NMR ($CDCl_3$ + 20% acetone-d_6) δ −0.53 (s, 4P); MS (LSIMS$^-$, NBA + $CHCl_3$ + MeOH) m/z 1379 ((M − PO_3H_2 − H)$^-$, 60), 1459 ((M − H)$^-$, 100); HRMS (LSIMS$^-$, NBA + $CHCl_3$ + MeOH) calcd for (M − H)$^-$ 1458.8754, found 1458.8765.

***S*-Acetyl TBDPS Cavitand 12.** Thioacetic acid (219 μL, 3.08 mmol) was added to a solution of benzyl bromide **8** (1.50 g, 0.701 mmol) and diisopropylethylamine (535 μL, 3.08 mmol) in DMF (20 mL). The reaction mixture was stirred for 16 h, evaporated in vacuo, and purified by column chromatography (hexanes:EtOAc, 3:1) to afford cavitand **12** as a white foam (930 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 16H), 7.30 (m, 24H), 6.96 (s, 4H), 5.88 (d, $J = 7.2$ Hz, 4H), 4.73 (t, $J = 8.1$ Hz, 4H), 4.26 (d, $J = 7.2$ Hz, 4H), 4.01 (s, 8H), 3.64 (t, $J = 6.3$ Hz, 8H), 2.31 (s, 12H), 2.19 (m, 8H), 1.55 (m, 8H), 0.99 (s, 36H); MS (MALDI$^+$, DHB) m/z 2097 (M·Na$^+$)$^+$, 100), 2113 ((M·K$^+$)$^+$, 90). Anal. Calcd for $C_{120}H_{136}O_{16}S_4Si_4$: C, 69.46; H, 6.61. Found: C, 69.60; H, 6.51.

***S*-Acetyl Hydroxyl-Footed Cavitand 13.** Concentrated HCl (150 μL, 1.82 mmol) was added to a solution of *S*-Ac-TBDPS cavitand **12** (100 mg, 0.0472 mmol) in THF:MeOH (4:1, 5 mL). The reaction mixture was stirred for 4 h, neutralized with saturated $NaHCO_3$, and evaporated in vacuo. The crude product was dissolved in $CHCl_3$:MeOH (9:1, 10 mL), dried over $MgSO_4$, evaporated in vacuo, and purified by column chromatography (9:1, $CHCl_3$:MeOH) to afford cavitand **13** as a white solid (49 mg, 93%): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 7.58 (s, 4H), 5.79 (d, $J = 7.7$ Hz, 4H), 4.55 (t, $J = 8.1$ Hz, 4H), 4.44 (br s, 4H), 4.27 (d, $J = 7.7$ Hz, 4H), 3.94 (s, 8H), 3.47 (t, $J = 6.5$ Hz, 8H), 2.37 (m, 8H), 2.29 (s, 12H), 1.40 (m, 8H); MS (MALDI$^+$, DHB) m/z 1144 ((M + Na$^+$)$^+$, 90), 1160 ((M + K)$^+$, 100). Anal. Calcd for $C_{120}H_{136}O_{16}S_4·H_2O$: C, 59.03; H, 5.84. Found: C, 59.42; H, 5.72.

***tert*-Butyl Phosphorylated *S*-Acetyl Cavitand 14.** 1*H*-Tetrazole (0.19 g, 2.7 mmol) was added to a THF solution of hydroxyl-footed *S*-Ac-cavitand **13** (0.10 g, 0.089 mmol) and di-*tert*-butyl diethylphosphoramidite (0.25 mL, 0.89 mmol), and the resulting mixture stirred for 10 min under N_2. The reaction mixture was then cooled to −78 °C, at which point H_2O_2 (0.12 mL, 1.1 mmol) was added and the reaction mixture was allowed to warm to room temperature over 30 min. The reaction mixture was then poured onto H_2O and extracted three times with $CHCl_3$. The combined $CHCl_3$ extracts were then washed with 10% $NaHSO_3$ and H_2O, dried over $MgSO_4$, and evaporated. The residue was purified by size exclusion chromatography (EtOAc:MeOH:H_2O; 40:10:4) followed by column chromatography ($CHCl_3$:MeOH; 96:4) to afford *tert*-butyl-protected *S*-Ac phosphate **14** as a white solid (0.13 g, 79%): mp 100 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 4H), 5.82 (d, $J = 7.3$ Hz, 4H), 4.76 (t, $J = 8.1$ Hz, 4H), 4.25 (d, $J = 7.3$ Hz, 4H), 4.00 (m, 16H), 2.29 (br m, 20H), 1.65 (m, 8H), 1.44 (s, 72H); ^{31}P NMR ($CDCl_3$) δ −9.96 (s, 4P); MS (NBA + $CHCl_3$) m/z 1441 (M − (C(CH_3)$_3$)$_8$ + 9H)$^+$, 100). Anal. Calcd for $C_{88}H_{132}O_{28}P_4S_4$: C, 55.92; H, 7.04. Found: C, 56.10; H, 7.00.

***S*-Acetyl Phosphate-Footed Cavitand 15.** TFA (0.40 mL, 5.2 mmol) was added to a solution of cavitand **14** (46 mg, 0.024 mmol) in DCM, and the reaction mixture was stirred for 10 min. The reaction mixture was evaporated in vacuo to afford cavitand **15** as a white solid (34 mg, 98%): mp 100 °C (dec); ^{1}H NMR (methanol-d_4) δ 7.35 (s, 4H), 5.82 (d, $J = 7.3$ Hz, 4H), 4.75 (t, $J = 8.0$ Hz, 4H), 4.32 (d, $J = 7.3$ Hz, 4H), 4.10 (m, 16H), 2.44 (m, 8H), 2.05 (br s, 12H), 1.60 (m, 8H); ^{31}P NMR (methanol−d_4) δ 0.00 (s, 4P); MS (LSIMS$^-$, NBA + MeOH) m/z 1439 ((M − H)$^-$, 100). Anal. Calcd for $C_{56}H_{68}O_{28}P_4S_4·3H_2O$: C, 44.98; H, 4.99. Found: C, 45.06; H, 4.72.

Phosphate-Footed Benzylthiol 16. A 0.190 M degassed solution of NaOH (1.39 mL, 0.264 mmol, 20 equiv) was added to a degassed suspension of *S*-acetylated cavitand **15** (19 mg, 0.0132 mmol) in MeOH and H_2O (1:1, 7 mL). The reaction mixture was stirred for 30 min under N_2 at which point prewashed Amberlite ion-exchange resin (H$^+$) was added via a sidearm until the solution was no longer basic. The mixture was stirred for 2 min and then cannulated to another flask so as to leave the resin behind. The solution was evaporated in vacuo to afford tetrathiol **16** as a white solid (16 mg, 95%): mp 100 °C (dec.); ^{1}H NMR (methanol-d_4) δ 7.32 (s, 4H), 5.92 (d, $J = 7.3$ Hz, 4H), 4.77 (t, $J = 8.3$ Hz, 4H), 4.60 (d, $J = 7.3$ Hz, 4H), 4.03 (dt, $J = 5.8$ Hz, $J = 5.8$ Hz, 8H), 3.61 (s, 8H), 2.45 (m, 8H), 1.64 (m, 8H); ^{31}P NMR (methanol-d_4) δ 3.24 (s, 4P); MS (LSIMS$^-$, TG + MeOH) m/z 1271 (M − H)$^-$, 100). Anal. Calcd for $C_{48}H_{60}O_{24}P_4S_4·4H_2O$: C, 42.86; H, 5.10. Found: C, 42.53; H, 4.73.

Peptide Synthesis. Peptides were synthesized on an Applied Biosystems 431A peptide synthesizer using standard Fmoc/*t*-Bu chemistry, HOBt (1-hydroxybenzotriazole)/HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) activation of the amino acids, and Rink's amide resin[19] to afford C-terminal amides. The last cycle in the synthesis of peptide **17** entailed acetylation of the N-terminus with acetic anhydride in NMP. Peptide **17** was then cleaved from the resin by a 2 h treatment of a mixture of TFA (95%), H_2O (2.5%), and 1,2-ethanedithiol (2.5%). For peptide **19**, the last cycle included chloroacetylation of the N-terminus by treatment with chloroacetyl chloride and DIEA as reported previously.[10] Peptide **19** was cleaved from the resin by a 2 h treatment with 95% TFA. Each crude peptide was then concentrated, precipitated with ice-cold ether, and filtered off. Both peptides **17** and **19** were purified by reversed phase HPLC using linear gradients of aqueous acetonitrile containing 0.1% TFA on a Phenomenex Selectosil C_{18} column (250 × 22.5 mm) at a flow rate of 10 mL/min. The peptides were lyophilized and characterized by analytical HPLC and MS (LSIMS$^+$, TG). For peptide **17**: m/z 1745 ((M + H)$^+$, 100). For peptide **19**: m/z 1814 ((M + H)$^+$, 100).

De Novo Protein 18. Peptide **17** (20.4 mg, 11.2 μmol) and impure cavitand **11** (3.7 mg, ca. 2.3 μmol) were stirred in degassed 150 mM sodium phosphate pH 8.5 buffer (4 mL) for 1 h at room temperature under N_2. The crude product was purified twice by reversed phase HPLC (conditions as above). The major peak eluted at ca. 45% acetonitrile, separable from the corresponding tris-phosphate derivative, and was lyoph-

ilized to afford a white solid (1.9 mg, 9%). Purity was assessed by the observation of one peak by analytical HPLC, and its identity was confirmed by electrospray MS to give a mass of 8394.6 ± 1.2 Da [calcd 8393.7 Da (average isotope composition)].

De Novo Protein 20. Peptide **19** (20.0 mg, 11.5 μmol) and cavitand **16** (3.6 mg, 2.9 μmol) were stirred in a degassed 150 mM sodium phosphate pH 8.5 buffer (4 mL) for 4 h at room temperature under N_2. The crude product was purified twice by reversed phase HPLC (conditions as above). The major peak eluted at ca. 45% acetonitrile and was lyophilized to afford a white solid (7.8 mg, 33%). Purity was assessed by the observation of one peak by analytical HPLC, and its identity was confirmed by electrospray MS to give a mass of 8110.6 ± 1.2 Da [calcd 8109.4 Da (average isotope composition)].

Acknowledgment. We thank NSERC (Canada) for financial support in the form of a grant to J.C.S. and a post-graduate fellowship to A.R.M. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the ACS, for partial support of this research. Warm thanks to Professor Bruce C. Gibb for critical reading of this manuscript.

JO980305K