A New Generation of "Cholaphanes": Steroid-Derived Macrocyclic Hosts with Enhanced Solubility and Controlled Flexibility

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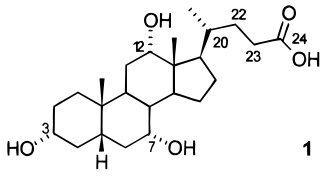
The macrocyclic "cholaphanes" **3a**−**c** were synthesized from the inexpensive steroid cholic acid. Like earlier relatives they feature substantial cavities with inward-directed hydroxyl groups, suitable for binding polar molecules such as carbohydrates in nonpolar media. New features are the externally directed alkyl chains, promoting solubility in organic solvents, and (in the case of **3b/c**) reduced conformational freedom resulting from truncation of the steroidal side-chain. In particular, modeling shows that the smallest macrocycle **3c** possesses very little flexibility, preferring an open conformation which is also revealed in the X-ray crystal structure of its pentahydrate. NMR studies indicated that all three cholaphanes form 1:1 complexes with octyl β-D-glucoside in $CDCl_3$, with K_a = 600−1560 M^{-1}. Cholaphanes **3b/c** proved able to extract methyl β-D-glucoside from aqueous solutions into $CHCl_3$. The transport of methyl β-D-glucoside across a chloroform barrier was also demonstrated for **3c**.

Introduction

Conformational control is a central challenge in the practice of supramolecular chemistry. The programmed association of molecules must be based on specific three-dimensional complementarities, which are hard to achieve in the absence of well-defined conformational preferences. Control in most (synthetic) supramolecular systems derives largely from the use of rigid "construction modules" which restrict the freedom of the overall structure. Thus benzene rings, aromatic heterocycles, alkyne units, and aliphatic six-membered rings have all featured strongly in supramolecular architecture.[1]

The steroid nucleus can make unique contributions to this area owing to its size, chirality, and rigid polycyclic framework.[2] The bile acids, such as cholic acid (**1**), have proved especially useful, because of their availability and useful levels of functionalization.[2b,c] On one hand, the presence of functionality at both ends of these units suggests the construction of macrocyclic structures which may possess a high level of inward-directed polar functionality.[3] On the other, the codirected hydroxyl groups present in most bile acids may be exploited (directly or indirectly) in podand-type receptors,[4] linear dimeric hosts,[5] or "facial amphiphiles".[6]

The typical bile acid structure, as in **1**, may be viewed as two segments, the large rigid tetracyclic nucleus and the smaller, flexible side-chain. Derived architectures often inherit this combination of rigidity and flexibility, which is useful for many applications. However, in the case of synthetic receptors, even moderate levels of flexibility may be undesirable. The restricted preorganization may limit the selectivity attainable[7] and reduce the strength of binding to well-matched substrates. Almost certainly, it will hamper attempts to model the system and to predict and interpret its behavior.



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This problem arose in our early studies on the "cholaphanes" **2**,[2a,8] the first of the above-mentioned macrocyclic systems, and among the first synthetic receptors for carbohydrate derivatives in nonpolar sol-

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.

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(4) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7955. Cheng, Y. A.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813. Hsieh, H.-P.; Muller, J. G.; Burrows, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 12077. Maitra, U.; D'Souza, L. J. *J. Chem. Soc., Chem. Commun.* **1994**, 2793. Davis, A. P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793.

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(7) For recent illustrations of this principle, featuring inverse correlations between flexibility and enantioselectivity, see: (a) Yoon, S. S.; Georgiadis, T. M.; Still, W. C. *Tetrahedron Lett.* **1993**, *34*, 6697. (b) Cuntze, J.; Owens, L.; Alcazar, V.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 367.

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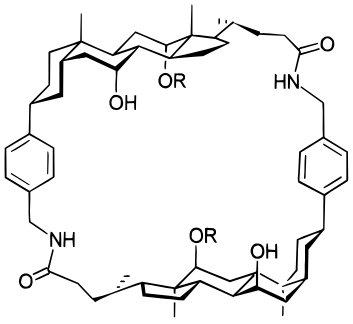
vents.[9] While these hosts showed pleasing affinities and selectivities for alkyl glucosides in chloroform,[8b,c] a detailed interpretation of the binding results proved unrealistic. Molecular modeling showed that the framework of **2** could adopt various conformations, arising from rotations about the series of single bonds connecting the inflexible arylsteroid units.[8b,e] Consideration of all low energy conformers, combined with an adequate range of substrate orientations, was beyond our computational resources.

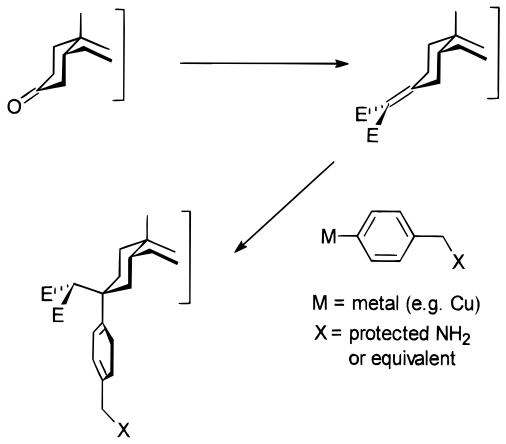


2 (R = H or CH_2Ph)

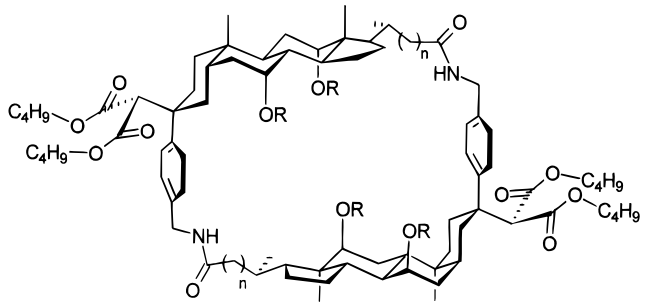
A second problem encountered in our early work was the limited solubility of **2** (R = H), the more effective of the two receptors. Maximum concentrations in $CDCl_3$ (ca. 2 mM) were barely sufficient for the binding measurements. An increase in rigidity appeared likely to exacerbate this difficulty, and we therefore sought a way to separate the two problems, i.e., to tune solubility independently of the central framework.

The answer seemed to lie in Scheme 1, a sequence for introducing both an aryl spacer group and an externally directed 3β solubilizing group, employing a chemoselective organometallic reagent. Preliminary work confirmed that this sequence was practical for E = CN,[10] and that the product could be converted to a cholaphane.[11] We now report the synthetic culmination of this program, the preparation of a range of cholaphanes **3** in which (a) core

Scheme 1



flexibility is controlled through steroidal side-chain adjustment, and (b) solubility in nonpolar solvents is maintained by four externally directed butyl esters. Preliminary binding studies, computer-based molecular modeling and X-ray crystallography suggest that **3c**, in particular, has unusual potential for the selective (and interpretable) recognition of carbohydrates in nonpolar media



3 R = H **a** n = 2
4 R = CHO **b** n = 1
c n = 0

Results and Discussion

Synthesis of 3a and 3b. The syntheses of **3a**, from cholic acid (**1**), and **3b**, from norcholic acid (**5**), followed parallel paths as outlined in Schemes 2 and 3. Although our previous cholaphane syntheses had relied mainly on acetyl protection for the 7,12α-OH groups,[8,11] removal had proved troublesome. Considering the presence of the dibutylmalonyl groups in **3**, we decided to use the more labile formyl groups in this case.[12] The preparation of norcholic acid (**5**), and the formylation of **1** and **5** to give **6a** and **6b**, respectively, were accomplished using high-yielding literature procedures.[13,14] Esterification with diazomethane, selective deformylation of the equatorial C3-oxygens, and oxidation to ketones **8** proceeded smoothly as expected.

The next step, malonylidenation of the 3-keto group, had appeared the major obstacle to success at the start of our program. While the Knoevenagel condensation between malonates and aldehydes is a well-established

(8) (a) Bonar-Law, R. P.; Davis, A. P. *J. Chem. Soc., Chem. Commun.* **1989**, 1050. (b) Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1407. (c) Bhattarai, K. M.; Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *J. Chem. Soc., Chem. Commun.* **1992**, 752. (d) Bonar-Law, R. P.; Davis, A. P. *Tetrahedron* **1993**, *49*, 9829. (e) Bonar-Law, R. P.; Davis, A. P. *Tetrahedron* **1993**, *49*, 9845. (f) Bonar-Law, R. P.; Davis, A. P.; Dorgan, B. J. *Tetrahedron* **1993**, *49*, 9855.

(9) For other studies on carbohydrate recognition, see e.g. refs 3b, 3f, 3h, 7b, and: (a) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397. (b) Kikuchi, Y.; Tanaka, Y.; Sutaro, S.; Kobayashi, K.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10302. (c) Liu, R.; Still, W. C. *Tetrahedron Lett.* **1993**, *34*, 2573. (d) Savage, P. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 10448. (e) Das, G.; Hamilton, A. D. *J. Am. Chem. Soc.* **1994**, *116*, 11139. (f) Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778. (g) Coterón, J. M.; Vicent, C.; Bosso, C.; Penadés, S. *J. Am. Chem. Soc.* **1993**, *115*, 10066. (h) Eliseev, A. V.; Schneider, H.-J. *J. Am. Chem. Soc.* **1994**, *116*, 6081. (i) Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 2998. (j) Morin, G. T.; Hughes, M. P.; Paugam, M.-F.; Smith, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 8895. (k) Deng, G.; James, T. D.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, **116**, 4567. (l) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1911. (m) Mizutani, T.; Murakami, T.; Matsumi, N.; Kurahashi, T.; Ogoshi, H. *J. Chem. Soc., Chem. Commun.* **1995**, 1257. (n) Anderson, S.; Neidlein, U.; Gramlich, V.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1596. (o) Neidlein, U.; Diederich, F. *J. Chem. Soc., Chem. Commun.* **1996**, 1493. (p) Inouye, M.; Miyake, T.; Furusyo, M.; Nakazumi, H. *J. Am. Chem. Soc.* **1995**, *117*, 12416.

(10) Davis, A. P.; Egan, T. J.; Orchard, M. G.; Cunningham, D.; McArdle, P. *Tetrahedron* **1992**, *48*, 8725.

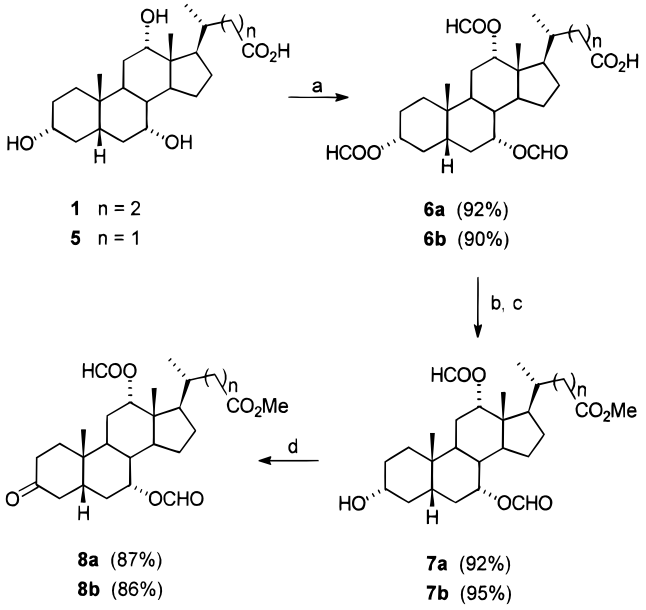
(11) Davis, A. P.; Orchard, M. G. *J. Chem. Soc., Perkin Trans 1* **1993**, 919.

(12) cf. refs 3a−c.

(13) Schteingart, C. D.; Hofmann, A. F. *J. Lipid Res.* **1988**, *29*, 1387.

(14) Tserng, K.-Y.; Klein, P. D. *Steroids* **1977**, *29*, 635.

Scheme 2



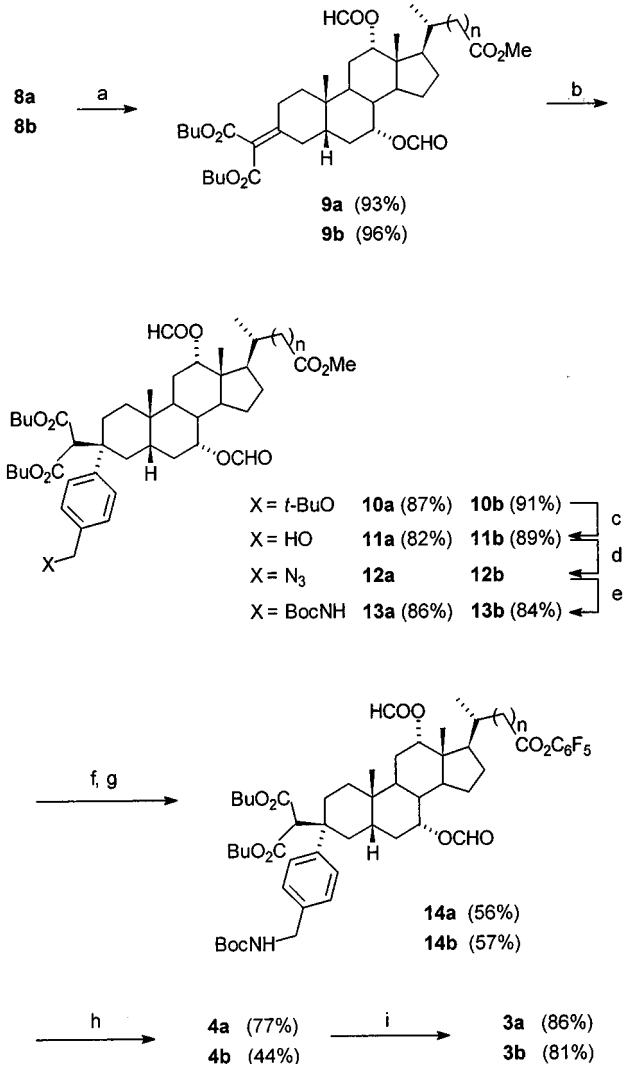
[a] Reaction conditions. (a) HCO_2H, $HClO_4$ (cat.), Ac_2O; (b) CH_2N_2, CH_2Cl_2, MeOH; (c) $NaHCO_3$, MeOH, 0 °C; (d) pyridinium chlorochromate, CH_2Cl_2, SiO_2.

process,[15] the reaction between malonates and ketones does not proceed well using traditional methodology. However, as described elsewhere,[16] the problem was overcome through a "forcing" equivalent of the Knoevenagel reaction, employing 2,2-dibromomalonates and tributylstibine, and driven by oxidation of Sb(III) to Sb(V). Application of this method to ketones **8** gave dibutyl malonylidene derivatives **9** in excellent yields.

Introduction of the spacer group required an organometallic reagent which could add to C3 chemoselectively (in the presence of 5 ester groups) and stereoselectively (equatorial attack) and which bore a substituent capable of conversion to an aminomethyl group. Previous work on related systems[10,11] implied that a "higher-order cuprate" derived from a Grignard reagent would have the necessary chemo- and stereoselectivity. Regarding the substituent, our choice was limited by the dearth of N-protecting groups which are compatible with Grignard and organocuprate reagents.[17] Although an (*N*-pyrrolo)-methyl group had proved viable in similar circumstances,[18] the deprotection method required a hydrolysis step which would probably have been incompatible with the formate esters. We therefore reverted to our earlier procedure,[11] involving a *tert*-butoxymethyl substituent which is subsequently converted to hydroxymethyl, mesylated, treated with azide, reduced to aminomethyl, and Boc-protected. The sequence from **9** through to **13** proceeded well in both series, with overall yields of 61% ($n = 2$) and 68% ($n = 1$).

Conversion of **13** to macrocycles **3** required activation of the side-chain carboxyls, deprotection of the amino

Scheme 3



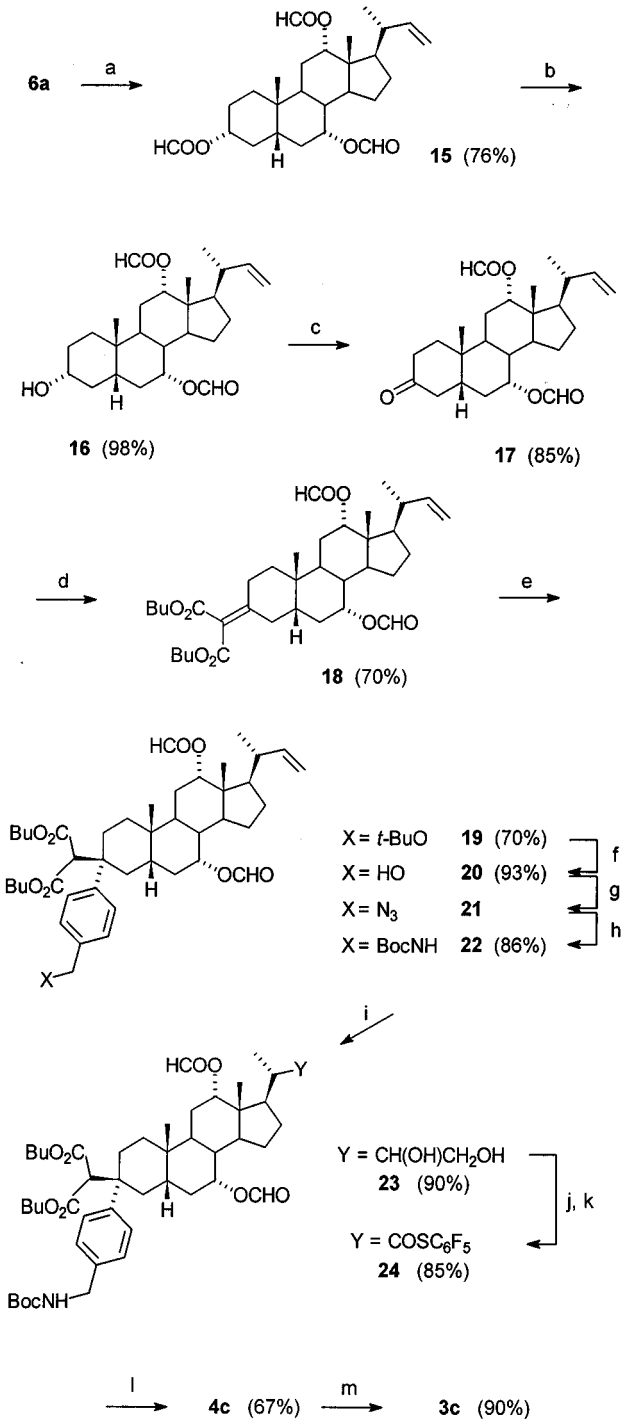
[a] Reaction conditions. (a) $SbBu_3$ (3.5 equiv), dibutyl 2,2-dibromomalonate (1.4 equiv), THF, 60−65 °C; (b) $Ar_2Mg_2Br_2CuCN$, THF, −60 °C [Ar = *p*-$(C_6H_4)CH_2Ot$-Bu]; (c) TFA, CH_2Cl_2, 50 °C, then NH_3 (aq), Et_2O; (d) MsCl, *i*-Pr_2NEt, CH_2Cl_2, −14−0 °C, then $(Me_2N)_2CNH_2^+N_3^-$, 0−30 °C; (e) Ph_3P, THF, H_2O, 60 °C, then $(Boc)_2O$; (f) NaOH, MeOH, THF, then HCO_2H, $HClO_4$ (cat.), Ac_2O, then $(Boc)_2O$, Et_2O, THF, $NaHCO_3$ (aq); (g) C_6F_5OH, DCC, CH_2Cl_2; (h) TFA, CH_2Cl_2, then DMAP, CH_2Cl_2; (i) K_2CO_3, THF, MeOH, H_2O, 60 °C.

groups, cyclodimerization, and deformylation. The first of these steps proved somewhat troublesome, as hydrolysis of the methyl esters could not be accomplished without removal of the formyl groups in positions 7 and 12. The latter could be reinstated, but the acidic conditions necessary removed the N-Boc protection. However, after reprotection of the amino groups and treatment with DCC/pentafluorophenol, the activated esters **14** were obtained in acceptable yields. N-Deprotection and cyclodimerization at high dilution (ca. 1 mM) gave macrocycles **4a** and **4b** in yields of 77% and 44%, respectively, the difference perhaps reflecting an element of strain in **4b**. Finally, deformylation gave the tetrahydroxycholaphanes **3a/b**.

Synthesis of 3c. The sequence leading to the smallest cholaphane, **3c**, is shown in Scheme 4. The synthesis differs from those in Schemes 2 and 3 in that side-chain degradation was accomplished in two widely separated stages. Removal of C(24) through oxidative decarboxy-

(15) Jones, G. *Org. React.* **1967**, *15*, 204.
(16) (a) Davis, A. P.; Bhattarai, K. M. *Tetrahedron* **1995**, *51*, 8033. See also: (b) Liao, Y.; Huang, Y.-Z. *Tetrahedron Lett.* **1990**, *31*, 5897. (c) Chen, C.; Huang, Y.-Z.; Shen, Y.; Liao, Y. *Heteroatom Chem.* **1990**, *1*, 49.
(17) Most common N-protecting groups contain acidic protons or polarized multiple bonds which will quench a Grignard reagent. Obvious exceptions, such as silyl groups, do not eliminate N-basicity, which can interfere with the reactivity of a derived cuprate. For further discussion see ref 11.
(18) Davis, A. P.; Egan, T. J. *Tetrahedron Lett.* **1992**, *33*, 8125.

Scheme 4



[a] Reaction conditions. (a) $Pb(OAc)_4$, $Cu(OAc)_2$ cat., py, C_6H_6; (b) $NaOAc{\cdot}3H_2O$, MeOH; (c) pyridinium chlorochromate, CH_2Cl_2, SiO_2; (d) $SbBu_3$ (3 equiv), dibutyl 2,2-dibromomalonate (1.2 equiv), THF, 60−65 °C; (e) $Ar_2Mg_2Br_2CuCN$, THF, −60 °C [Ar = *p*-(C_6H_4)CH_2O*t*-Bu]; (f) TFA, CH_2Cl_2, 50 °C, then NH_3 (aq), Et_2O; (g) MsCl, *i*-Pr_2NEt, CH_2Cl_2, −14−0 °C, then $(Me_2N)_2CNH_2^+N_3^-$, 0−30 °C; (h) Ph_3P, THF, H_2O, 60 °C, then $(Boc)_2O$; (i) *N*-methylmorpholine *N*-oxide, OsO_4 (cat.), py, Bu_4NOAc; (j) $NaIO_4$, CH_3CN, H_2O, then $NaClO_2$, H_2NSO_3H, CH_3CN, H_2O; (k) C_6F_5SH, DCC, CH_2Cl_2; (l) TFA, CH_2Cl_2, then DMAP, CH_2Cl_2; (m) K_2CO_3, THF, MeOH, H_2O, 60 °C.

lation of triformate **6a** yielded the 22,23-alkene in **15**.[3a,19] As in previous work involving "bis-nor" monomer units,[3a,b] this group was employed as a "masked carboxyl", being carried through the introduction of the spacer group and then revealed via oxidative cleavage (OsO_4-catalyzed bis-

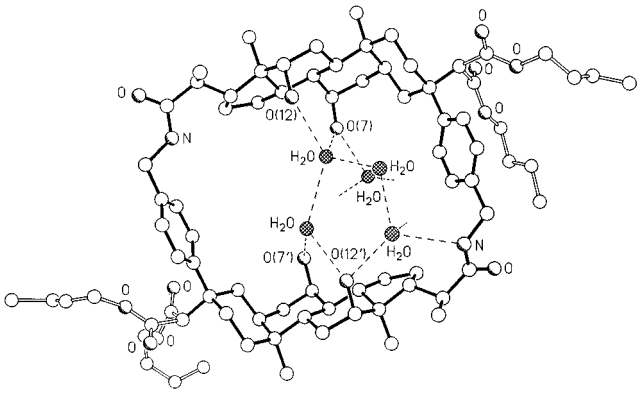


Figure 1. The structure of **3c** in the crystal showing also the entrapped hydrogen bonded solvent water molecules. Nonterminated bonds represent hydrogen bonds to symmetry-related molecules.

hydroxylation,[20] treatment with sodium metaperiodate, and oxidation of the resulting aldehyde with sodium chlorite[21]). In this case the carboxyl group was activated as the (more reactive) pentafluoro*thio*phenyl ester[22] to compensate for the sterically hindered environment of the carbonyl carbon. Cyclodimerization to **4c** (in 67% yield) was followed by deformylation, to give the target cholaphane **3c**.

Structural and Recognition Properties of 3a−c. As discussed earlier, the design of **3a−c** was influenced by two major considerations. First, we wished to control and, in particular, lower the flexibility of the cholaphane framework, to increase selectivity and binding power (to well-matched substrates). Second, we wished to maintain solubility in nonpolar organic media, irrespective of the core structure. Initial observations implied that we had succeeded in both respects. On one hand, all the tetrahydroxycholaphanes were freely soluble in $CDCl_3$, to the level of at least 25 mg/mL. On the other, computer-based molecular modeling[23] showed that, while **3a** and **3b** could undertake a variety of conformations (including collapsed structures with no substantial cavity), the macrocyclic framework of **3c** had very little freedom. Open toroidal or bowl-shaped structures dominated the lower reaches of the energy spectrum, the only important mode of flexibility being a folding motion with the Ar−CH_2−N bonds acting as hinges.

The structure of **3c**, and its potential as a host for polar molecules, was confirmed by X-ray crystallography (Figure 1). Crystals were grown from a solution in 1:1 CH_2Cl_2−MeOH, which was allowed to evaporate in an atmosphere of methanol. Although there was no deliberate introduction of water, the crystals were found to be heavily hydrated with each molecule of **3c** accompanied by five water molecules. The X-ray analysis reveals the molecule in an open, approximately C_2-symmetric conformation which might be described as a "skewed bucket" by analogy to e.g. cyclodextrins (Figure 2). The transannular separations of the pairs of C(12) and C(7) hydroxyl oxygen atoms are 7.4 and 5.5 Å respectively. These effectively determine the breadth of the cavity while the

(19) Carlson, G. L.; Belobaba, D. T. E.; Hofmann, A. F.; Wedmid, Y. *Steroids* **1977**, *30*, 787.
(20) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(21) Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888.
(22) Davis, A. P.; Walsh, J. J. *Tetrahedron Lett.* **1994**, *35*, 4865.
(23) Details to be published separately.

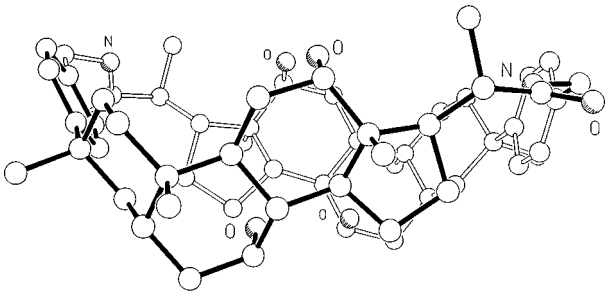


Figure 2. Side view of the macrocyclic portion of **3c** showing the skewed conformation.

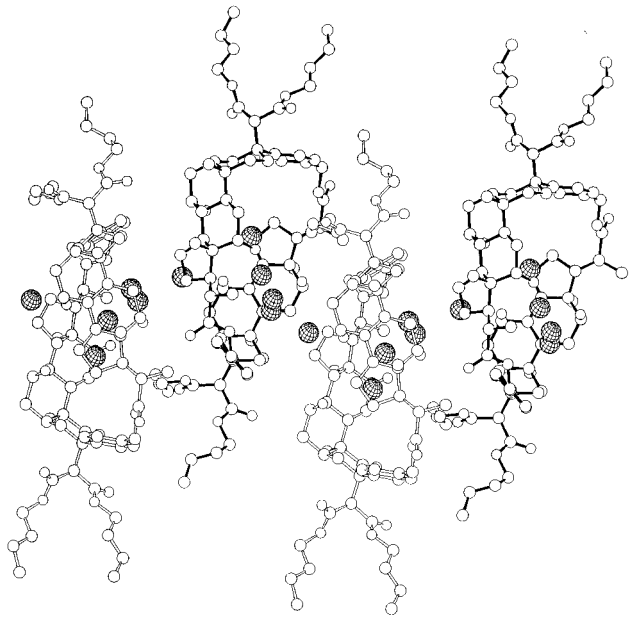
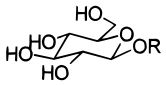


Figure 3. Side view of the packing of molecules of **3c** in the crystal. The water molecules that occupy the helical channel passing through the macrocycles are depicted as large spheres.

surfaces of the aromatic spacer units (ring-centroid···ring-centroid distance 11.5 Å) define its length. The inward-directed hydroxyls and amide N−H groups create a hydrophilic interior within which the five included water molecules are bound via O−H···O and N−H···O hydrogen bonds.[24] It is interesting to note that the included water molecules are concentrated toward one end of the macrocyclic cavity, and also that only one of the amide N−H groups is involved in hydrogen bonding. Inspection of the packing of the molecules in the crystal shows one of the crystallographic screw axes to pass through this region of the macrocycle. The effect of this is to produce partial overlap of the cavities of symmetry related macrocycles creating helical water-containing channels that extend in the *b* direction (Figure 3).

As demonstrated in previous work, the three-dimensional arrangement of polar functionality in cholaphanes is particularly suitable for the recognition of carbohydrate nuclei in nonpolar media.[8b,c] In a preliminary test of their carbohydrate-binding properties, macrocycles **3** were subjected to NMR titration experiments in $CDCl_3$ with octyl β-D-glucoside (**25**) as guest. Addition of **25** to **3** resulted in downfield shifts of the receptor N*H* NMR signals, analyzable in terms of 1:1 binding. In the case of **3a** the motions were consistent with a binding constant (K_a) of 1305 M^{-1}, and a limiting chemical shift change ($\Delta\delta$) of 1.35 ppm. This binding constant is significantly lower than that of 3100 M^{-1} observed previously for **2** (R = H),[8c] perhaps implying that the rigidly constrained aromatic spacer group interferes with the preferred binding geometry of the carbohydrate. In the case of **3b**, a still lower K_a of 600 M^{-1} was measured ($\Delta\delta$ = 0.84 ppm). For the smallest and least flexible macrocycle **3c**, we naturally hoped for a substantial increase in binding constant, reflecting the improved preorganization of the cavity. In fact, the measured value of 1560 M^{-1} ($\Delta\delta$ = 1.07 ppm) was somewhat disappointing. However, we had no reason to assume, *a prioiri*, that **25** would be especially complementary to **3c**, and this rather modest figure may be eclipsed when we survey a wider range of substrates.



25 R = nC_8H_{17}
26 R = Me

The tetrahydroxycholaphanes were also tested for their abilities to extract the hydrophilic methyl β-D-glucoside (**26**) from aqueous solution into an organic medium.[25] Solutions of the macrocycles in chloroform (1 mM) were stirred with aqueous **26** (concentrations from 2.5 M down to 1 M) and the separated organic phases analyzed for carbohydrate using TLC. No extraction was detected in the absence of receptor. The technique could be operated in a semiquantitative fashion by applying the samples in a standard manner using a micropipet and making comparisons with solutions of **26** at known concentrations. When the method was applied to **3c**, extraction was clearly detectable over the full range of glucoside concentrations. With [**26**] (aq) = 1.75 M, the analysis indicated that roughly 0.2 equiv of the glucoside were carried into the organic phase. When the full range of cholaphanes [including **2** (R = H)] were tested under the same conditions, the results were somewhat surprising. The order of effectiveness appeared to be **3c** > **3b** > **2** (R = H) > **3a**. In the case of **3a**, the degree of extraction was below the limit of detection. Although the poor performance of **2** (R = H) and **3a** was unexpected, in view of their effectiveness in the homogeneous binding studies, it may be noted that different substrates (octyl vs methyl glucosides) and conditions (dry vs water-saturated $CHCl_3$) are involved in the two types of experiment.

The extraction ability of **3c** was confirmed by a transport experiment conducted in a U-tube apparatus, employing a gently stirred solution of the cholaphane in chloroform (2 mM), overlaid by aqueous **26** (2 M) on one side and an aqueous receiving phase on the other. The carbohydrate could be detected in the receiving phase after 24 h and increased steadily in concentration over a number of days. Transport was not observed in the absence of the receptor.

In conclusion, we have developed a new method for constructing cholaphanes with heightened solubility in organic solvents and applied it to the synthesis of a series of receptors which vary in size and flexibility. The smallest and least flexible example (**3c**) possesses a well-

(24) There are 15 O−H···O hydrogen bonds which range in distance from 2.73 to 3.05 Å, and one N−H···O hydrogen bond (3.07 Å) from one of the amide nitrogen atoms to one of the included water molecules.

(25) For the extraction of **26** into CCl_4, as a 2:1 host:guest complex, see ref 9b.

defined central famework and a highly preorganized, polar interior. The structural and preliminary recognition studies on this new cholaphane suggest that it will prove useful in investigations of carbohydrate binding and transport in nonpolar organic media.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker MSL300 (^{1}H at 300 MHz, ^{13}C at 75 MHz) or WP80 (^{1}H at 80 MHz) spectrometers, with TMS and $CDCl_3$, respectively, as internal standards. IR spectra were recorded on a Perkin-Elmer 298 spectrometer. Flash chromatography[26] was performed using Kieselgel 60 (400−230 mesh) purchased from Merck. Reactions were monitored by TLC on DC Alufolien Kieselgel 60 F_{254} (0.2 mm) and visualized by charring or developing with iodine as appropriate. $CHCl_3$ and $CDCl_3$ used for extraction and binding experiments were deacidified and partially dried by storage over potassium carbonate. Anhydrous solvents were prepared by following standard procedures.[27] Melting points were uncorrected.

Starting Materials. Cholic acid (**1**) was supplied by Freedom Chemical Diamalt GmbH of Raubling, Germany. Triformate **6a** was prepared from **1**, formic acid, and acetic anhydride, employing a modification of the procedure of Tserng and Klein.[14] 24-Norcholic acid (**5**) was prepared from triformate **6a** via α-nitrosation−fragmentation to the nornitrile, using a method based on that of Schteingart and Hofmann.[13] Formylation of **5**, as above, gave triformate **6b**. Details of these preparations are given as Supporting Information.

Methyl 3α-Hydroxy-7α,12α-bis(formyloxy)-5β-cholan-24-oate (7a). Diazomethane was bubbled into a solution of **6a** (20 g, 41 mmol) dissolved in CH_2Cl_2 (400 mL) and methanol (10 mL) maintained at −5 °C to 0 °C. After the completion of the reaction as judged by TLC (hexane−ethyl acetate, 7:3), evaporation of solvent yielded the corresponding methyl ester. This material (19.5 g, ca. 39 mmol) was dissolved in methanol (300 mL) and cooled to 0 °C. Solid sodium bicarbonate (5 g, 60 mmol) was added in portions with stirring. Deformylation was followed by TLC (CH_2Cl_2−ether, 1:1). After completion of the reaction (1.5 to 2 h) unreacted bicarbonate was neutralized with dilute acetic acid and the solvent evaporated to give a residue. The residue was dissolved in ether, washed with water, dried over Na_2SO_4, and filtered. Evaporation of the filtrate gave crude **7a** as a gum (18 g, ca. 92% overall from **6a**), homogeneous by TLC. A portion of this material was further purified by flash chromatography (ether as eluent) to give **7a** as a white solid: TLC R_f 0.3 (CH_2Cl_2−ether, 1:1); IR (film from $CDCl_3$), 3422, 1718, 1180 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.14 (1H, s), 8.10 (1H, s), 5.26 (1H, br s), 5.05 (1H, s), 3.65 (3H, s), 3.49 (1H, m), 0.92 (3H, s), 0.83 (3H, d, J = 6.2 Hz), 0.75 (3H, s); ^{13}C NMR δ 174.4, 160.67, 160.5, 75.19, 71.31, 70.75, 51.42, 47.05, 44.85, 42.83, 40.85, 38.38, 37.61, 34.76, 34.62, 34.14, 31.34, 30.76, 30.53, 30.20, 28.43, 27.04, 25.38, 22.66, 22.25, 17.34, 11.99. Anal. Calcd for $C_{27}H_{42}O_7 \cdot H_2O$: C, 65.32; H, 8.46. Found: C, 65.34; H, 8.65.

Methyl 3α-Hydroxy-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (7b). A solution of **6b** (15 g, 31 mmol) dissolved in CH_2Cl_2 (200 mL) and CH_3OH (20 mL) was treated with diazomethane as described above to give the corresponding methyl ester. Deformylation as above with sodium bicarbonate (4.1 g, 49 mmol) in methanol (300 mL), followed by flash chromatography with ether as eluent, gave **7b** (13.84 g, 95%): TLC R_f 0.3 (CH_2Cl_2−ether, 1:1); IR (film from $CDCl_3$) 3422, 2958, 2878, 1714, 1186, 908, 734 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.14 (1H, s), 8.09 (1H, s), 5.25 (1H, br s), 5.05 (1H, br s), 3.65 (3H, s), 3.49 (1H, tt), 0.92 (3H, d, J = 6.0 Hz), 0.88 (3H, s), 0.79 (3H, s); ^{13}C NMR δ 173.55, 160.68, 160.38, 75.06, 71.25, 70.70, 51.29, 47.10, 44.93, 42.86, 40.95, 40.84, 38.38, 37.62, 34.75, 34.12, 33.02, 31.35, 30.19, 28.40, 27.17, 25.35, 22.63, 22.24, 18.6, 12.00. Anal. Calcd for $C_{26}H_{40}O_7$: C, 67.22; H, 8.68. Found: C, 66.69; H, 8.66.

Methyl 3-Oxo-7α,12α-bis(formyloxy)-5β-cholan-24-oate (8a). Silica gel (7 g) was dried overnight at 110 °C and then stirred under nitrogen with pyridinium chlorochromate (5.6 g, 26 mmol) and CH_2Cl_2 (50 mL). A solution of **7a** (6.81 g, 14.2 mmol) in CH_2Cl_2 (50 mL) was added dropwise. Analysis by TLC after 2 h showed the reaction to be complete. The mixture was filtered and the insoluble material washed with ether. Concentration of the filtrate and washings gave a greenish yellow residue which was dissolved in ether, washed with water and brine, dried ($MgSO_4$), and filtered through a short pad of silica. Evaporation of the eluent and crystallization from CH_2Cl_2-hexane to yielded **8a** as white needles (5.93 g, 87%): TLC R_f 0.6 (CH_2Cl_2−ether, 4:1); IR (film from $CDCl_3$) 2965, 2880, 1716, 1180 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.16 (1H, s), 8.10 (1H, s), 5.31 (1H, br s), 5.16 (1H, br s), 3.66 (3H, s), 3.01 (1H, t, J = 15 Hz), 1.04 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 0.79 (3H, s); ^{13}C NMR δ 211.52, 174.33, 160.34, 160.25, 75.0, 70.47, 51.44, 47.10, 44.94, 44.44, 42.76, 42.06, 37.61, 36.43, 36.05, 34.62, 33.33, 30.93, 30.75, 30.51, 29.39, 27.03, 25.77, 22.66, 21.45, 17.36, 12.07. Anal. Calcd for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 67.70; H, 8.47.

Methyl 3-Oxo-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (8b). **7b** (4.95 g, 10.65 mmol) was treated as above with silica gel (5 g) and pyridinium chlorochromate (3.8 g, 18.6 mmol) in CH_2Cl_2 (80 mL), to give **8b** as white needles (4.25 g, 86%): TLC R_f 0.6 (CH_2Cl_2−ether, 4:1); IR (film from $CDCl_3$) 2963, 2878, 1715, 1178, 730 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.16 (1H, s), 8.09 (1H, s), 5.30 (1H, br s), 5.15 (1H, br d), 3.65 (3H, s), 3.01 (1H, t, J = 14 Hz), 1.04 (3H, s), 0.91 (3H, d, J = 6.2 Hz), 0.83 (3H, s); ^{13}C NMR δ 211.48, 173.46, 160.23, 74.87, 70.40, 51.34, 47.09, 45.02, 44.43, 42.79, 42.02, 40.92, 37.60, 36.41, 36.02, 34.31, 33.02, 30.92, 29.37, 27.15, 25.74, 22.65, 21.44, 18.63, 12.08. Anal. Calcd for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.63; H, 8.33.

Methyl 3-[Bis(butoxycarbonyl)methylidene]-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (9b). According to the previously reported method,[16a] tributylstibine (8.82 g, 8 mL, 30.1 mmol) was injected slowly into a stirred solution of ketone **8b** (4.0 g, 8.65 mmol) and dibutyl dibromomalonate (4.5 g, 12.04 mmol) in dry THF (30 mL) under nitrogen at room temperature. The reaction vessel was lowered into an oil bath at 60−65 °C and stirring was maintained for 72 h. After this time interval, TLC analysis (eluent CH_2Cl_2) of the reaction mixture indicated complete disappearance of starting ketone (visualization by 2,4-DNP) and appearance of the faster-moving product. The reaction mixture was cooled and stirred open to the atmosphere for 2 h. The solvent was evaporated and the residue dissolved in a minimum volume of hexane−ether (4:1). This solution was applied to to a column of silica gel column (2.5 × 20 cm) packed using hexane and eluted with hexane (200 mL), hexane−ether (200 mL, 9:1), hexane−ether (200 mL, 4:1) and finally with hexane−ether (7:3) until no further product emerged. Fractions containing the product were evaporated to yield **9b** (5.50 g, 96%): TLC R_f 0.33 (hexane−ether, 1:1); IR (film from $CHCl_3$) 2960, 2878, 1719, 1634, 1204, 1174, 1064 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.14 (1H, s), 8.09 (1H, s), 5.28 (1H, br s), 5.11 (1H, br s), 4.15 (4H, t, J = 5.5 Hz), 3.65 (3H, s), 0.81 (3H, s); ^{13}C NMR δ 173.40, 165.65, 165.42, 160.81, 160.30, 121.49, 74.90, 70.36, 64.67, 64.56, 51.26, 47.04, 44.89, 43.05, 42.77, 40.87, 37.53, 37.16, 34.66, 34.55, 32.97, 31.23, 30.34, 29.06, 27.13, 26.97, 25.56, 22.60, 21.65, 18.92. 18.56, 13.49, 11.99. Anal. Calcd for $C_{37}H_{56}O_{10}$: C, 67.24; H, 8.54. Found: C, 67.29; H, 8.20.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-*tert*-butoxymethyl)phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oate (10a). A solution of *tert*-butyl *p*-bromobenzyl ether (5 g, 20.56 mmol) in dry THF (30 mL) was added in one portion to magnesium turnings (0.7 g, 28.5 mmol) in flame-dried apparatus under argon. The Grignard reaction was initiated by warming and sonication. Sonication continued for 1 h after which the solution was transferred via cannula to a second flame dried reaction vessel. The magnesium-free Grignard reagent was cooled to ca. −10 °C (ice-salt bath), and CuCN (0.895 g, 10 mmol) was added with stirring against a strong

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(27) Perrin, D. D.; Armanego, W. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; 2nd ed.; Pergamon: Oxford, 1980.

flow of argon. Stirring was continued as the temperature was allowed to rise to ca. 0 °C, and the resulting homogeneous solution was then cooled to −60 °C. A solution of **9a**[16a] (5.00 g, 7.4 mmol) in THF (20 mL) was added dropwise, and then the mixture was allowed to attain room temperature. The reaction was quenched with saturated NH_4Cl (aq) and the mixture extracted with ether. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated to give a gummy residue. Flash chromatography eluting with hexane−ether (4:1, followed by 7:3), gave **10a** (5.39 g, 87%): TLC R_f 0.5 (hexane−ether, 1:1); IR (film from $CHCl_3$) 2966, 2876, 1722, 1618, 1467, 1439, 1389, 1364, 1243, 1178, 1063, 1020 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (1H, s), 8.01 (1H, s), 7.34, 7.26 (4H, ABq, J = 8.6 Hz), 5.23 (1H, br s), 5.05 (1H, br s), 4.38 (2H, s), 4.06 (1H, s), 4.03−3.86 (4H, m), 3.65 (3H, s), 1.28, (9H, s), 1.03 (3H, s), 0.89−0.80 (9H, m), 0.73 (3H, s); ^{13}C NMR δ 174.41, 168.14, 160.63 and 160.55, 144.50, 137.58, 127.13, 126.67, 74.92, 73.16, 70.57, 64.73, 63.76, 54.63, 51.41, 47.02, 44.80, 42.64, 42.5, 38.01, 37.89, 37.60, 34.62, 34.22, 32.09, 31.49, 30.76, 30.53, 30.23, 29.10, 28.54, 27.58, 27.05, 25.42, 22.78, 22.69, 18.93, 17.32, 13.54, 11.98. Anal. Calcd for $C_{49}H_{74}O_{11}$: C, 70.13; H, 8.89. Found: C, 70.42; H, 8.83.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-*tert*-butoxymethyl)phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (10b). Organocuprate addition to **9b** (5.00 g, 7.6 mmol) as above yielded **10b** (5.7 g, 91%): TLC R_f 0.38 (hexane−ether, 1:1); IR (film from $CHCl_3$) 2966, 2876, 1722, 1174 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (1H, s), 8.00 (1H, s), 7.35, 7.26 (4H, ABq, J = 8.5 Hz), 5.23 (1H, br s), 5.05 (1H, br d), 4.38 (2H, s), 4.05 (1H, s), 4.03−3.86 (4H, m), 3.64 (3H, s), 1.28 (9H, s), 1.02 (3H, s), 0.89−0.82 (9H, m), 0.77 (3H, s); ^{13}C NMR δ 173.65, 168.17, 168.06, 160.55, 144.53, 137.62, 127.16, 126.71, 74.82, 73.20, 70.55, 66.77, 63.78, 54.6, 51.37, 47.16, 44.92, 42.72, 42.52, 41.03, 38.03, 37.92, 37.64, 34.25, 33.10, 32.11, 31.52, 30.26, 27.61, 27.23, 25.42, 22.79, 22.70, 18.96, 18.91, 18.63, 13.57, 12.04. Anal. Calcd for $C_{48}H_{72}O_{11}$: C, 69.87; H, 8.80. Found: C, 69.76; H, 8.81.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-hydroxymethyl)phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oate (11a). To a solution of **10a** (5.08 g, 6.04 mmol) in dry CH_2Cl_2 (50 mL) under inert atmosphere was added trifluoroacetic acid (5 mL, 65 mmol). The mixture was heated at 50 °C. After 5 h a small aliquot was withdrawn and treated with aqueous alkali.[28] Analysis by TLC confirmed the disappearance of the starting material. The reaction mixture was poured into a biphasic mixture of ether (400 mL) and aqueous ammonia (100 mL, 4 M) under vigorous stirring. After 5 h the hydrolysis was complete as evidenced by TLC. The phases were separated, and the aqueous phase was extracted with ether (2 × 20 mL). The organic phases were combined, washed with brine until neutrality, and dried ($MgSO_4$). Evaporation and flash chromatography with hexane−ether (2:3) as eluent gave **11a** (3.87 g, 82%) as a solid: TLC R_f 0.3 (hexane−ethyl acetate, 3:2); IR (film from $CDCl_3$) 3528, 2962, 2879, 1721, 1174, 1062, 1018; ^1H NMR (300 MHz) δ 8.04 (1H, s), 8.02 (1H, s), 7.40, 7.31 (4H, ABq, J = 8.4 Hz), 5.24 (1H, br s), 5.06 (1H, br s), 4.67 (2H, s), 4.08 (1H, s), 4.04−3.87 (4H, m), 3.64 (3H, s), 1.04 (3H, s), 0.89−0.80 (9H), 0.73 (3H, s); ^{13}C NMR δ 174.43, 168.13, 168.04, 160.61, 145.08, 138.97, 127.41, 126.05, 75.06, 70.65, 64.95, 64.84, 53.77, 51.47, 47.12, 44.88, 42.83, 42.64, 38.09, 37.88, 37.65, 34.67, 34.29, 31.99, 31.55, 30.83, 30.58, 30.26, 29.33, 28.58, 27.09, 25.56, 22.94, 22.72, 18.98, 17.37, 13.58, 12.06. Anal. Calcd for $C_{45}H_{66}O_{11}$: C, 69.02; H, 8.49. Found: C, 68.82; H 8.50.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-hydroxymethyl)phenyl]-7α-12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (11b). 10b (5.76 g, 6.98 mmol) was treated as above with trifluoroacetic acid (5 mL, 65 mmol), followed by aqueous ammonia, to yield **11b** as a white solid (4.8 g, 89%): mp 120−123 °C; TLC R_f 0.25 (hexane−ether, 2:3); IR (film from $CDCl_3$) 3548, 1721, 1177 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (1H, s), 8.03 (1H, s), 7.46, 7.31 (4H, ABq, J = 8.6 Hz), 5.23 (1H, br s), 5.06 (1H, br s), 4.67 (2H, s), 4.08 (1H, s), 4.04−3.87 (4H, m), 3.63 (3H, s), 1.04 (3H, s), 0.89−0.82 (9H, m), 0.78 (3H, s); ^{13}C NMR δ 173.60, 168.12, 168.01, 160.52, 145.05, 138.98, 127.40, 126.04, 74.93, 70.60, 64.93, 64.83, 54.38, 51.36, 47.18, 44.96, 42.87, 42.63, 41.02, 38.08, 37.87, 37.65, 34.28, 33.07, 32.03, 31.54, 30.28, 29.33, 28.58, 27.21, 25.52, 22.92, 22.69, 18.97, 18.93, 18.63, 13.57, 12.06. Anal. Calcd for $C_{44}H_{64}O_{11}$: C, 68.72; H, 8.39. Found: C, 69.06; H, 8.41.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-azidomethyl)phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oate (12a). A solution of **11a** (3.28 g, 4.19 mmol) in CH_2Cl_2 (20 mL) was cooled to −14 °C (ice-salt bath) under nitrogen. Methanesulfonyl chloride (0.42 mL, 5.32 mmol) and then *N*,*N*-diisopropylethylamine (0.95 mL, 5.47 mmol) were added dropwise with stirring. The mixture was allowed to warm to 0 °C. TLC analysis of the reaction mixture after 1 h indicated conversion of the starting material into two faster moving products.[29] *N*,*N*,*N*′,*N*′−Tetramethylguanidinium azide[30] (1.29 g, 8.15 mmol) was added with stirring. The mixture was allowed to reach room temperature then warmed to 30 °C for 30 min. The mixture was diluted with ether (40 mL) and filtered through a short pad of silica, which was then washed with ether. The filtrate and washings were combined, washed with water and saturated brine, dried ($MgSO_4$), and evaporated to yield **12a** (3.8 g) of sufficient purity for further elaboration. A portion of the crude product (0.05 g) was purified by flash chromatography using hexane−ether (4:1) as eluent to yield pure **12a** (0.033 g): TLC R_f 0.57 (hexane−ethyl acetate, 3:2); IR (film from $CDCl_3$) 2963, 2879, 2110, 1722, 1468, 1243, 1176 cm^{-1}; ^1H NMR (300 MHz) δ 8.06 (1H, s), 8.03 (1H, s), 7.42, 7.25 (4H, ABq, J = 8.5 Hz), 5.24 (1H, br s), 5.06 (1H, br s), 4.33 (2H, s), 4.07 (1H, s), 4.03−3.84 (4H, m), 3.64 (3H, s), 1.04 (3H, s), 0.89−0.79 (9H, m), 0.74 (3H, s); ^{13}C NMR δ 174.35, 168.05, 167.95, 160.53, 145.75, 133.56, 127.65, 127.08, 75.03, 70.59, 64.82, 54.40, 51.41, 47.10, 44.86, 42.85, 42.65, 38.07, 37.76, 37.62, 34.65, 34.22, 31.99, 31.50, 30.79, 30.56, 30.21, 29.25, 28.62, 27.06, 25.56, 22.91, 22.67, 18.89, 17.34, 13.49, 12.02. Anal. Calcd for $C_{45}H_{65}N_3O_{10}$: C, 66.89; H, 8.11; N, 5.2. Found: C, 66.83; H, 8.16, N, 4.96.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[*p*-(azidomethyl)phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (12b). 11b (3.57 g, 4.65 mmol) was treated as above with methanesulfonyl chloride (0.47 mL, 6.04 mmol) and *N*,*N*-diisopropylethylamine (1.1 mL, 6.3 mmol), followed by *N*,*N*,*N*′,*N*′-tetramethylguanidinium azide (1.43, g, 9.06 mmol), to yield crude **12b** (3.96 g). Flash chromatography of a portion of this material (0.06 g) yielded pure **12b**: mp 130−133 °C; TLC R_f 0.5 (hexane−ethyl acetate, 3:2); IR (film from $CDCl_3$) 2964, 2880, 2101, 1723, 1178 cm^{-1}; ^1H NMR (300 MHz) δ 8.06 (1H, s), 8.03 (1H, s), 7.43, 7.25 (4H, ABq, J = 8.5 Hz), 5.24 (1H, br t), 5.07 (1H, br d), 4.33 (2H, s), 4.08 (1H, s), 4.07−3.84 (4H, m), 3.63 (3H, s), 1.04 (3H, s), 0.89−0.82 (9H, m), 0.78 (3H, s); ^{13}C NMR δ 173.53, 168.05, 167.94, 160.48, 145.73, 133.56, 127.65, 127.08, 74.90, 70.53, 64.82, 54.40, 51.33, 47.15, 44.94, 42.88, 42.62, 40.98, 38.05, 37.75, 37.61, 34.22, 33.05, 31.96, 31.50, 30.21, 29.25, 28.58, 27.19, 25.52, 22.91, 22.66, 18.92, 18.61, 13.52, 12.03. Anal. Calcd for $C_{44}H_{63}N_3O_{10}$: C, 66.56; H, 7.99; N, 5.29. Found: C, 66.48; H, 8.05; N, 4.83.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oate (13a). To a solution of azide **12a** (3.75 g) in THF (20 mL) were added triphenylphosphine (2.0 g, 7.6 mmol) and water (3 mL). The resulting mixture was heated to 60 °C for 1 h. Di-*tert*-butyl dicarbonate (1.47 g, 6.5 mmol) in THF (5 mL) was added, and the reaction mixture

(28) Cleavage of the *tert*-butyl ether unit by TFA yields a trifluoroacetate which must then be hydrolyzed under basic conditions. The trifluoroacetate is not readily distinguished by TLC from the starting ether, necessitating hydrolysis of reaction samples as well as the bulk product.

(29) The benzyl mesylate (major component) is accompanied by the corresponding benzyl chloride. Both react in the second step to yield azide **12a**. The second step is difficult to monitor by TLC as the benzyl chloride and **12a** have very similar R_f values.

(30) CAUTION: when used in chlorinated solvents, this reagent can lead to explosive side-products. Workers intending to repeat this preparation should seek an alternative to dichloromethane as solvent. See ref 11 and: Li, C.; Shih, T.-L.; Jeong, J. U.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 2645.

was cooled to room temperature, sonicated for 30 min, and then left to stir overnight. The solvent was evaporated and the residue dissolved in ether (150 mL), washed with water and then brine, and dried ($MgSO_4$). Evaporation gave a residue which was dissolved in minimum volume of ether and cooled (ice−salt bath). Insoluble material (Ph_3PO) was removed by filtration. Evaporation of the filtrate gave a residue which was purified by flash chromatography eluting with hexane−ethyl acetate (9:1 and then 2:5) to yield **13a** (3.21 g, 86% from **11a**): TLC R_f 0.32 (hexane−ethyl acetate, 2:1); IR (film from $CDCl_3$) 3411, 2963, 2878, 1722, 1515, 1367, 1246, 1174 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (1H, s), 8.02 (1H, s), 7.36, 7.22 (4H, ABq, J = 8.5 Hz), 5.23 (1H, br s), 5.06 (1H, br s), 4.89 (1H, br t), 4.29 (2H, d, J = 5.8 Hz), 4.07 (1H, s), 4.04−3.86 (4H, m), 3.64 (3H, s), 1.46 (9H, s), 1.04 (3H, s), 0.89−0.79 (9H, m), 0.74 (3H, s); ^{13}C NMR δ 174.29, 168.02, 167.92, 160.45, 155.74, 144.63, 136.90, 127.37, 126.32, 79.23, 74.97, 70.55, 64.72, 54.30, 51.35, 47.03, 44.80, 44.05, 42.76, 42.52, 38.01, 37.76, 37.57, 34.59, 34.20, 31.95, 31.46, 30.73, 30.50, 30.19, 29.25, 28.53, 28.28, 27.01, 25.49, 22.86, 22.63, 18.85, 17.20, 13.48, 11.96. Anal. Calcd for $C_{50}H_{75}NO_{12}$: C, 68.10; H, 8.57, N, 1.58. Found: C, 67.89; H, 8.61; N, 1.32.

Methyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (13b). **12b** (3.9 g) was treated as above with triphenylphosphine (1.9 g, 7.2 mmol) and water (3 mL) in THF (20 mL), followed by di-*tert*-butyl dicarbonate (1.65 g, 7.3 mmol), to yield **13b** (3.41 g, 84% from **11b**): TLC R_f 0.44 (hexane−ethyl acetate, 3:2); IR (film from $CDCl_3$) 3410, 2965, 2879, 1723, 1173, 733 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (1H, s), 8.02 (1H, s), 7.36, 7.22 (4H, ABq, J = 8.5 Hz), 5.23 (1H, br s), 5.06 (1H, br s), 4.94 (1H, br t), 4.29 (2H, d, J = 5.8 Hz), 4.07 (1H, s), 4.04−3.86 (4H, cm), 1.04 (3H, s), 0.89−0.82 (9H, m), 0.78 (3H, s); ^{13}C NMR δ 173.36, 167.94, 167.84, 160.34, 155.68, 144.54, 136.88, 127.29, 126.23, 79.13, 74.76, 70.43, 64.62, 54.25, 51.16, 47.02, 44.82, 43.98, 42.73, 42.45, 40.85, 37.93, 37.70, 37.50, 34.13, 32.93, 31.88, 31.39, 30.10, 29.17, 28.45, 28.20, 27.06, 25.39, 22.76, 22.54, 18.77, 18.48, 13.39, 11.89. Anal. Calcd for $C_{49}H_{73}NO_{12}$: C, 67.80; H, 8.47; N, 1.61. Found: C, 67.70; H, 8.53; N, 1.55.

Pentafluorophenyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oate (14a). Aqueous sodium hydroxide (3 mL, 1.5 M) was added to a stirred solution of **13a** (0.5 g, 0.57 mmol) in THF−methanol (12 mL, 2:1). The mixture was stirred at room temperature for 24 h and then neutralized with $NaHSO_4$ (0.400 g, 3.3 mmol). Evaporation gave a residue which was dissolved in ether, washed thoroughly with water then brine, and dried (Na_2SO_4). Evaporation of the solvent and drying under high vacuum gave crude dihydroxy acid as a white solid. This was dissolved in formic acid (5 mL), a catalytic amount of 70% perchloric acid was added, and the solution was heated at 50 °C (bath temperature) for 24 h. The mixture was cooled to 40 °C. Acetic anhydride (2 drops) was added, and the mixture was stirred for 1 h and then evaporated under high vacuum at ambient temperature. The residue was dissolved in THF−ether (10 mL, 1:1) and neutralized with a slight excess of 1% aqueous sodium bicarbonate. A solution of di-*tert*-butyl dicarbonate (0.140 g, 0.64 mmol) in ether (2 mL) was added with stirring. The mixture was left overnight and then diluted with ether and dried ($MgSO_4$). Evaporation gave a residue which was purified by flash chromatography eluting with $CHCl_3$−methanol (99:1 then 98:2) to give 3β-[bis(butoxycarbonyl)-methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-5β-cholan-24-oic acid (0.361 g): TLC R_f 0.3 (ether). This material was dissolved in CH_2Cl_2 (3 mL) with pentafluorophenol (0.084 g, 0.456 mmol) and cooled to −10 °C under an inert atmosphere. Dicyclohexyl carbodiimide (DCC) (0.094 g, 0.50 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The mixture was left stirring overnight, after which a drop of distilled water was added and stirring continued under an open atmosphere for 1 h. The mixture was cooled to −10 °C, precipitated dicyclohexyl urea was separated by filtration, and the insoluble material washed with ether−hexane (1:1) precooled to −10 °C. Evaporation of the filtrate gave a residue which on flash chromatography eluting with hexane−ether (1:1) gave **14a** (0.327 g, 56%): TLC R_f 0.3 (hexane−ether, 1:1); IR (film from $CDCl_3$) 3414, 2964, 2879, 1791, 1755, 1722, 1519, 1469, 1174; δ_H (300 MHz) δ 8.07 (1H, s), 8.03 (1H, s), 7.37, 7.22 (4H, ABq, J = 8.4 Hz), 5.26 (1H, br s), 5.07 (1H, br s), 4.87 (1H, br t), 4.30 (2H, d, J = 5.9 Hz), 4.08 (1H, s), 4.04−3.86 (4H, m), 1.46 (9H, s), 1.05 (3H, s), 0.89−0.83 (9H, m), 0.77 (3H, s). Anal. Calcd for $C_{55}H_{72}NO_{12}F_5$: C, 63.87; H, 7.02; N, 1.35. Found: C, 63.25; H, 6.96; N, 1.15.

Pentafluorophenyl 3β-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,-12α-bis(formyloxy)-24-nor-5β-cholan-23-oate (14b). Methyl ester **13b** (0.4 g, 0.46 mmol) was converted as above to the corresponding carboxylic acid (0.26 g). This was treated as above with pentafluorophenol (0.062 g, 0.33 mmol) and DCC (0.07 g, 0.33 mmol) to give **14b** (0.262 g, 57%): TLC R_f 0.25 (hexane−ether, 1:1); IR (film from $CDCl_3$) 3414, 2964, 2880, 2455, 1789, 1757, 1722, 1520, 1176 cm^{-1}; ^1H NMR (300 MHz) δ 8.07 (1H, s), 8.03 (1H, s), 7.37, 7.22 (4H, ABq, J = 8.3 Hz), 5.26 (1H, br s), 5.08 (1H, br s), 4.83 (1H, br t), 4.30 (2H, d), 4.07 (1H, s), 4.04−3.86 (4H, m), 1.05 (3H, s), 1.00 (3H, d, J = 6.4 Hz), 0.90−0.83 (6H, m), 0.81 (3H, s); ^{13}C NMR δ 168.69, 168.07, 167.96, 160.47, 155.85, 144.66, 136.90, 127.40, 126.35, 79.40, 74.73, 70.50, 64.78, 54.33, 46.78, 44.98, 44.07, 42.90, 42.55, 40.10, 38.03, 37.80, 37.62, 34.23, 33.29, 31.97, 31.48, 30.23, 29.27, 28.57, 28.29, 27.27, 25.52, 22.86, 22.64, 18.92, 18.32, 13.50, 11.97. Anal. Calcd for $C_{54}H_{70}NO_{12}F_5$: C, 63.57; H, 6.92; N, 1.35. Found: C, 62.97; H, 6.84; N, 1.30.

Tetrakis(formyloxy) Cholaphane 4a. Trifluoroacetic acid (1 mL) was added to a well-stirred solution of **14a** (0.327 g, 0.31 mmol) in dry CH_2Cl_2 (2 mL) cooled to 0 °C under argon. The mixture was allowed to warm gradually to ambient temperature. After 4 h, removal of organic volatiles under high vacuum gave a solid residue. CCl_4 (5 mL) was added, the mixture stirred, and solvent removed under high vacuum. The above procedure was repeated twice to afford the corresponding ammonium salt as a white solid. This material was dissolved in dry CH_2Cl_2 (50 mL) and added dropwise to a well-stirred solution of DMAP (0.58 g) in dry CH_2Cl_2 (300 mL) at ambient temperature under an atmosphere of argon. The reaction mixture was stirred for 3 days, after which evaporation and flash chromatography, eluting with $CHCl_3$−ethyl acetate (7:3), gave macrocycle **4a** (0.194 g, 77%): TLC R_f 0.36 ($CHCl_3$−ethyl acetate, 7:3); IR (film from $CDCl_3$) 3405, 2961, 2877, 1724, 1662, 1519, 1467, 1178 cm^{-1}; ^1H NMR (300 MHz) δ 8.01 (2H, s), 7.99 (2H, s), 7.37, 7.23 (8H, ABq, J = 8.5 Hz), 5.60 (2H, t, J = 5.3 Hz), 5.18 (2H, br s), 5.04 (2H, br s), 4.40, 4.26 (4H, dq, ABX, J_{AB} = 13.7 Hz, $J_{A/BX}$ = 5.5 and 5.0 Hz), 4.05 (2H, s), 4.01−3.83 (8H, m, 4 × OCH_2), 1.03 (6H, s), 0.87−0.80 (18H, m), 0.74 (6H, s); ^{13}C NMR δ 172.82, 168.01, 167.93, 160.39, 145.17, 136.27, 127.77, 127.64, 75.45, 70.79, 64.85, 54.31, 45.94, 44.77, 43.55, 42.97, 42.56, 38.04, 37.67, 37.59, 34.29, 34.26, 31.97, 31.93, 31.57, 31.38, 30.25, 29.25, 28.49, 27.26, 25.67, 22.93, 22.67, 18.94, 17.46, 13.56, 11.95. Anal. Calcd for $C_{88}H_{126}N_2O_{18}\cdot 4H_2O$:[31] C, 67.24; H, 8.59; N, 1.78. Found: C, 67.41; H, 8.16; N, 1.59; FABMS *m*/*z* 1521, 100% $[M + Na]^+$.

Tetrakis(formyloxy) Cholaphane 4b. **14b** was deprotected as above with trifluoroacetic acid to yield the corresponding ammonium salt. This material was treated as above with DMAP (0.472 g) in dry CH_2Cl_2 (250 mL) to yield **4b** (0.082 g, 44%): TLC R_f 0.4 ($CHCl_3$−ethyl acetate, 7:3); IR (film from $CDCl_3$) 3410, 2961, 2877, 1754, 1748, 1727, 1718, 1680, 1667, 1514, 1467 cm^{-1}; ^1H NMR (300 MHz) δ 8.12 (2H, s), 8.04 (2H, s), 7.33, 7.13 (8H, ABq, J = 8.3 Hz), 5.36 (2H, d, J = 7.4 Hz), 5.11 (2H, br s), 5.01 (2H, br s), 4.80 (2H, ABX, J = 8.0, 12.9 Hz), 4.02−3.82 (12H, br m), 1.00 (6H, s), 0.88−0.78 (24H, m); ^{13}C NMR δ 171.18, 167.95, 167.84, 161.43, 160.45, 145.98, 135.84, 128.04, 76.50, 71.02, 64.93, 64.83, 55.70, 49.17, 45.25, 44.21, 43.86, 43.74, 42.62, 42.56, 38.13, 38.01, 37.59, 33.84, 32.76, 31.53, 30.37, 29.66, 26.72, 26.19, 22.38, 22.32, 18.98,

(31) In line with previous experience (refs 8d, 11) analytical samples of cholaphanes **3** and **4** appeared to be solvated. In all cases the data was consistent with integral numbers of water molecules, possibly acquired from atmospheric exposure after drying.

17.57, 13.52, 12.19, 12.24. Anal. Calcd for $C_{86}H_{122}N_2O_{18}\cdot 2H_2O$:[31] C, 68.50; H, 8.42; N, 1.86. Found: C, 68.67; H, 8.29; N, 1.71. FABMS *m/z* 1494, 100% $[M + Na]^+$.

Tetrahydroxy Cholaphane 3a. To a stirred solution of **4a** (0.045 g, 0.028 mmol) in THF (4 mL) was added a 3% solution of K_2CO_3 in methanol−water (3 mL, 4:1). The temperature was raised to 60 °C for 24 h, after which analysis by TLC showed total consumption of the starting material. Evaporation gave a residue to which CH_2Cl_2 and a small volume of 1% aqueous sodium hydrogen sulfate were added. The organic phase was washed with water and saturated brine till neutrality and then dried (Na_2SO_4). Evaporation gave a residue which was subjected to flash chromatography eluting with $CHCl_3$−ethyl acetate−methanol (44:5:1 and then 40:5:5) to give **3a** (0.034 g, 86%): TLC R_f 0.25 ($CHCl_3$−methanol, 9:1); IR 3340, 3332, 3325, 2963, 2875, 2336, 1755, 1722, 1649, 1535, 1463, 1415, 754 cm^{-1}; ^1H NMR (300 MHz) δ 7.38, 7.13 (8H, ABq, J = 8.3 Hz), 5.73 (2H, br t), 4.39, 4.21 (4H, ABX, J_{AB} = 13.9, $J_{A/BX}$ = 5.4 and 4.6 Hz), 4.08 (2H, s), 4.02−3.80 (8H, m, 4 x OCH_2), 1.04 (6H, s), 0.93 (6H, d, J = 6.0 Hz), 0.88−0.81 (12H, m), 0.67 (6H, s); ^{13}C NMR δ 173.19, 168.35, 168.24, 145.48, 135.84, 127.76, 127.45, 72.76, 68.08, 64.76, 54.92, 46.43, 46.36, 43.65, 42.84, 41.80, 39.60, 38.67, 38.33, 35.13, 34.68, 34.54, 32.87, 32.49, 31.45, 30.30, 29.30, 28.35, 27.45, 26.89, 23.03, 22.94, 18.99, 17.40, 13.59, 12.47. Anal. Calcd for $C_{84}H_{126}N_2O_{14}\cdot 2H_2O$:[31] C, 70.85; H, 9.20; N, 1.96. Found: C, 70.96; H, 9.27; N, 1.58. FABMS *m/z* 1409, 100% $[M + Na]^+$.

Tetrahydroxy Cholaphane 3b. 4b was treated as above with K_2CO_3 in methanol−water. Flash chromatography of the crude product, eluting with $CHCl_3$-methanol (98:2, then 95:5) gave **3b** (0.03 g, 81%): IR 3415, 2966, 2877, 1755, 1724, 1662 cm^{-1}; ^1H NMR (300 MHz) δ 7.35, 7.12 (8H, ABq, J = 8.4 Hz), 5.37 (2H, br s), 4.5 (2H, brq), 4.14−3.81 (16H, m), 0.97−0.94 (12H, d), 0.84 (12H, q, J = 7.1 Hz), 0.72 (6H, s); ^{13}C NMR δ 171.95, 168.12, 146.12, 135.56, 128.05, 72.77, 67.95, 65.82, 64.76, 54.94, 48.99, 46.54, 44.96, 43.88, 42.39, 39.34, 38.92, 38.37, 34.59, 34.34, 33.35, 32.66, 30.33, 28.47, 27.14, 22.72, 18.99, 18.05, 15.25, 13.6, 12.52. Anal. Calcd for $C_{82}H_{122}N_2O_{14}\cdot 2H_2O$:[31] C, 70.56; H, 9.10; N, 2.01. Found: C, 70.68; H, 9.05; N, 1.60. FABMS *m/z* 1381, 100% $[M + Na]^+$.

3α,7α,12α-Tris(formyloxy)-24-nor-5β-chol-22(23)-ene (15). The procedure was closely based on that of Carlson et al.[19] A mixture of cholic acid triformate (**6a**) (10.96 g, 22.2 mmol), cupric acetate monohydrate (0.5 g, 2.5 mmol), and pyridine (1.2 mL, 14.8 mmol) in benzene (600 mL) under nitrogen was subjected to azeotropic removal of water for 3 h. During this period three 20 mL portions of benzene and water were collected in a Dean−Stark apparatus. The mixture was cooled to about 40 °C, and lead tetraacetate (40 g, 86 mmol) was added in one portion. The reaction vessel was then lowered into an oil bath maintained at 90 °C, and the mixture was vigorously stirred. Progress of the reaction was monitored by TLC eluting with hexane−ethyl acetate (7:3). After 4 h, the mixture was cooled and filtered through a pad of Celite. The insoluble lead salts remaining on the filter were thoroughly washed with benzene. The combined filtrates were washed successively with HCl (aq) and water, dried, and evaporated. The residue was dissolved in ether and filtered through a pad of silica, which was then washed with further ether. Filtrate and washings were combined, concentrated, and recrystallized from hexane to afford **15** (7.32 g, 76%): mp 185−188 °C (lit.[19] mp 186−188 °C); IR 1720, 1710, 1605 cm^{-1}; ^1H NMR (300 MHz) δ 8.17 (1H, s), 8.09 (1H, s), 8.02 (1H, s), 5.61 (1H, m), 5.26 (1H, br t), 5.06 (1H, br d), 4.87 (2H, m), 4.73 (1H, m), 0.95 (3H, s), 0.94 (3H, d, J = 6.5 Hz), 0.78 (3H, s); ^{13}C NMR δ, 160.42, 144.07, 112.2, 75.01, 73.61, 70.57, 46.76, 44.86, 42.89, 40.71, 40.37, 37.61, 34.43, 34.35, 34.20, 31.23, 28.55, 27.17, 26.47, 25.48, 22.67, 22.23, 19.45, 12.26.

3-Oxo-7α,12α-bis(formyloxy)-24-nor-5β-chol-22(23)-ene (17). Sodium acetate trihydrate (0.050 g, 0.37 mmol) was added to a stirred solution of **15** (0.100 g, 0.22 mmol) in methanol (24 mL). Analysis by TLC, using ethyl acetate as eluent, showed complete disappearance of starting material after 96 h. The solvent was evaporated, and the residue was dissolved in ether, washed with water and then brine, and dried. Evaporation and drying under high vacuum afforded alcohol **16** as a white solid (0.089 g, 97.8%): ^1H NMR (80 MHz) δ 8.13 (1H, s), 8.08 (1H, s), 5.8−5.4 (1H, m), 5.19 (1H, br t), 5.16−4.71 (3H, m, 1H), 3.4 (1H, m), 0.88 (3H, s), 0.74 (3H, s). A portion of this material (0.040 g, 0.096 mmol) was oxidized with pyridinium chlorochromate (0.034 g, 0.16 mmol), as described above for **7a**, to yield **17** (0.035 g, 85% overall): IR (film from $CDCl_3$) 3025, 2963, 2878, 1716, 1642, 1220, 1179 cm^{-1}; ^1H NMR (300 MHz) δ 8.17 (1H, s), 8.09 (1H, s), 5.62 (1H, m), 5.30 (1H, br t), 5.15 (1H, br d), 4.88 (2H, m), 3.01 (1H, t, J = 14 Hz), 1.04 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 0.81 (3H, s); ^{13}C NMR δ 211.56, 160.29, 160.41, 144.06, 112.4, 74.99, 70.58, 46.87, 45.01, 44.54, 42.89, 42.16, 40.45, 37.73, 36.50, 36.15, 34.44, 31.04, 29.62, 27.22, 25.91, 22.76, 21.54, 19.57, 12.41. Anal. Calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.80; H, 8.79.

3β-[Bis(butoxycarbonyl)methyl]-3α-[(*p*-(*tert*-butoxymethyl)phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-cholan-22(23)-ene (19). Prepared by treating **18**[16a] (3.98 g, 6.47 mmol) with the organocuprate derived from *tert*-butyl *p*-bromobenzyl ether (4.23 g, 16.5 mmol), magnesium (0.56 g, 23 mmol), and CuCN (0.77 g, 8.59 mmol), as described above for **10a**. Flash chromatography of the crude product eluting with hexane−ether (9:1, 200 mL; 17:3, 200 mL; 4:1, remaining fractions) yielded **19** (3.51 g, 70%):[32] TLC R_f 0.3 (hexane−ether, 7:3); IR (film from $CDCl_3$) 2967, 2877, 1758, 1724, 1639, 1467, 1382, 1364, 1239, 1188, 1066, 1021, 959, 733 cm^{-1}; ^1H NMR (300 MHz) δ 8.07 (1H, s), 8.05 (1H, s), 7.35, 7.26 (4H, ABq, J = 8.5 Hz), 5.59 (1H, m), 5.23 (1H, br s), 5.05 (1H, br s), 4.85 (2H, m), 4.39 (2H, s), 4.05 (1H, s), 3.95 (4H, m), 1.03 (3H, s), 0.86 (3H, d, J = 6.9 Hz), 0.75 (3H, s); ^{13}C NMR δ 168.16, 168.07, 160.65, 160.55, 144.53, 144.20, 137.65, 127.16, 126.68, 112.17, 74.82, 73.19, 70.62, 64.76, 63.79, 54.70, 46.73, 45.82, 42.70, 42.50, 40.40, 38.11, 37.96, 37.68, 34.29, 32.17, 31.34, 30.28, 29.17, 28.72, 27.62, 27.23, 25.51, 22.82, 22.74, 19.46, 18.97, 13.56, 12.26. Anal. Calcd for $C_{47}H_{70}O_9$: C, 72.46; H, 9.06. Found: C, 72.72; H, 8.88.

3β-[Bis(butoxycarbonyl)methyl]-3α-[*p*-(hydroxymethyl)phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-chol-22(23)-ene (20). Prepared from **19** (8.27 g, 10.60 mmol), as described above for **11a**. Flash chromatography of the crude product eluting with hexane−ethyl acetate (3:2) yielded **20** (7.11 g, 92.7%): TLC R_f 0.3 (hexane−ether, 3:7); IR (film from $CHCl_3$) 3546 (br), 2961, 2876, 1753, 1720, 1176; δ_H (300 MHz) δ 8.06 (1H, s), 8.01 (1H, s), 7.40, 7.31 (4H, ABq, J = 8.6 Hz), 5.59 (1H, m), 5.24 (1H, br t), 5.06 (1H, br s), 4.85 (2H, m), 4.67 (2H, s), 4.09 (1H, s), 3.96 (4H, m), 1.05 (3H, s), 0.86 (3H, d, J = 6.4 Hz), 0.87 (6H, 2 t, J = 6.7 Hz), 0.76 (3H, s); ^{13}C NMR δ, 168.14, 168.03, 160.63, 160.55, 145.08, 144.16, 138.97, 127.42, 126.05, 112.23, 74.93, 70.66, 64.93, 64.83, 54.38, 46.75, 44.83, 42.86, 42.64, 40.46, 38.10, 37.87, 37.64, 34.31, 32.04, 31.54, 30.28, 29.33, 28.69, 27.24, 25.59, 22.94, 22.71, 19.46, 18.98, 13.58, 12.29. Anal. Calcd for $C_{43}H_{62}O_9$: C, 71.43; H, 8.64. Found: C, 71.52, H, 8.71.

3β-[Bis(butoxycarbonyl)methyl]-3α-[*p*-(azidomethyl)phenyl]-7α,12α-bis(formyloxy)-24-nor-5β-chol-22(23)-ene (21). Prepared from **20** (0.51 g, 0.705 mmol), methanesulfonyl chloride (0.09 g, 61 μL, 0.77 mmol), and *N*,*N*-diisopropylethylamine (0.14 mL, 0.80 mmol), followed by *N*,*N*,*N*′,*N*′-tetramethylguanidinium azide (0.22 g, 1.39 mmol), as described above for **12a**. The crude azide obtained (0.535 g) was of sufficient purity for further elaboration. A portion (0.05 g) was purified by flash chromatography, eluting with hexane−ether (4:1) to yield **21** (0.04 g): TLC R_f 0.2 (hexane−ether, 4:1); IR (film from $CDCl_3$) 2963, 2879, 2100, 1756, 1722, 1639, 1516, 1468, 1176 cm^{-1}; ^1H NMR (300 MHz) δ 8.07 (1H, s), 8.03 (1H, s), 7.43, 7.23 (4H, ABq, J = 8.2 Hz), 5.64−5.52 (1H, m), 5.24 (1H, br s), 5.06 (1H, br s), 4.91−4.79 (2H, m), 4.33 (2H, s), 4.08 (1H, s), 3.99−3.86 (4H, m), 1.05 (3H, s), 0.92−0.82 (9H, m), 0.76 (3H, s); ^{13}C NMR δ 168.04, 167.94, 160.52, 145.7, 144.11, 133.54, 127.64, 127.07, 112.18, 74.87, 70.58,

(32) A minor slower-running impurity proved to be 3β-[bis(butoxycarbonyl)methyl]-3α-[*p*-(*tert*-butoxymethyl)phenyl]-7α-formyloxy-12α-hydroxy-24-nor-5β-cholan-22(23)-ene, presumably arising from deformylation of **19**. In large-scale work this was reformylated and carried through to **20**.

64.82, 54.34, 46.69, 44.78, 42.60, 40.42, 38.04, 37.72, 37.58, 34.23, 31.95, 31.48, 30.19, 29.23, 28.67, 27.20, 25.57, 22.92, 22.66, 19.41, 18.91, 13.60, 12.25. Anal. Calcd for $C_{43}H_{61}NO_8$: C, 68.92; H, 8.25; N, 5.16. Found: C, 69.05; H, 8.22; N, 5.62.

3*β*-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-24-nor-5*β*-cholan-22(23)-ene (22). Triphenylphosphine (2.75 g, 10.5 mmol) was added to a solution of **21** (6.48 g, ca. 8.66 mmol) in THF−water mixture (6:1, 70 mL). The mixture was heated with stirring at 60 °C. After the completion of the reduction, as indicated by TLC, the mixture was cooled to ambient temperature. Di-*tert*-butyl dicarbonate (2.46 g, 13.06 mmol) and triethylamine (2.4 mL, 17.3 mmol) were added, and the mixture stirred overnight. The solvent was evaporated and the residue dissolved in ether (150 mL), washed with water and then brine, and dried ($MgSO_4$). Evaporation gave a residue which was dissolved in minimum volume of ether and cooled (ice−salt bath). Insoluble material (Ph_3PO) was removed by filtration. Evaporation of the filtrate gave a residue which was purified by flash chromatography, eluting with hexane−ethyl acetate (9:1 and then 4:1), to yield **22** (6.10 g, 86% from **20**): TLC R_f 0.35 (hexane−ethyl acetate, 4:1); IR (film from $CDCl_3$) 3410, 2965, 2879, 1721, 1517, 1468, 1417, 1245, 1176 cm^{-1}; ^1H NMR (300 MHz) δ 8.07 (1H, s), 8.02 (1H, s), 7.37, 7.22 (4H, ABq, J = 8.4 Hz), 5.65−5.28 (1H, m), 5.23 (1H, br s), 5.05 (1H, br s), 4.91−4.79 (3H, m), 4.30 (2H, d, J = 5.8 Hz), 4.08 (1H, s), 4.04−3.86 (4H, m), 1.46 (9H, s), 1.04 (3H, s), 0.97−0.83 (9H, m), 0.76 (3H, s); ^{13}C NMR δ 168.12, 168.02, 160.64, 155.81, 144.71, 144.15, 136.95, 127.45, 126.41, 112.22, 79.37, 74.92, 70.65, 64.82, 54.31, 46.73, 44.81, 44.12, 42.84, 42.57, 40.46, 38.09, 37.81, 37.62, 34.30, 32.01, 31.52, 30.25, 29.31, 28.66, 28.35, 27.24, 26.84, 25.60, 22.95, 22.70, 19.45, 18.96, 13.57, 12.27. Anal. Calcd for $C_{48}H_{71}O_{10}N$: C, 70.13; H, 8.70; N, 1.70. Found: C, 69.96; H, 8.78; N, 1.38.

3*β*-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-24-nor-5*β*-cholane-22(*R*,*S*)-23-diol (23). To a well-stirred heterogeneous mixture of pyridine (0.1 mL, 1.2 mmol), *N*-methylmorpholine *N*-oxide (1.51 g, 12.5 mmol), aqueous Bu_4NOAc (1.4 M, 2.5 mL, 3.5 mmol), and potassium acetate (0.1 g, 1 mmol) in acetone-distilled water mixture (20 mL, 10:1, v/v) was added a 2% solution of regenerated OsO_4 ($NaIO_4$) in dioxane water mixture (1:1, 7 mL, ca. 0.5 mmol). After 5 min, a solution of **22** (3.6 g, 4.37 mmol) in acetone−water (20 mL, 10:1) was added. Stirring was maintained for 5 h at room temperature and then overnight at 40 °C. Analysis by TLC showed complete consumption of **22**. Sodium metabisulfite (2.7 g, 14.2 mmol) was added after cooling, and the mixture was stirred for 1 h. The mixture was diluted with ether (50 mL) and dried (Na_2SO_4) and filtered. Concentration of the filtrate and washings gave crude **23** (4.1 g, ca. 90%) of sufficient purity for further elaboration. A portion of the crude diol was purified by flash chromatography eluting with hexane−ethyl acetate (2:3): TLC R_f 0.17 (ether); IR (film from $CDCl_3$) 3410, 2963, 2877, 1754, 1721, 1513, 1466, 1414, 1384, 1367, 1245, 1173, 1063, 755 cm^{-1}; ^1H NMR (300 MHz) δ 8.04 (1H, s), 8.02 (1H, s), 7.35, 7.20 (4H, ABq, J = 8.3 Hz), 5.19 (1H, br s), 5.06 (1H, br s), 4.93 (1H, br t), 4.28 (2H, d, J = 5.6 Hz), 4.06 (1H, s), 4.03−3.86 (4H, m), 3.69 (1H, br d), 3.56−3.36 (2H, br m), 1.04 (3H, s), 0.89−0.80 (9H, m), 0.75 (3H, s); ^{13}C NMR δ 168.04, 167.94, 160.44, 155.81, 144.61, 136.84, 127.33, 126.33, 79.32, 75.02, 73.48, 70.56, 64.74, 62.19, 54.34, 45.13, 44.19, 44.09, 42.56, 42.42, 39.31, 37.98, 37.80, 37.64, 34.19, 31.95, 31.46, 30.19, 29.26, 28.57, 28.28, 26.55, 25.46, 22.82, 18.87, 13.46, 12.01, 11.72. Anal. Calcd for $C_{48}H_{73}NO_{12}$: C, 67.34; H, 8.59; N, 1.63. Found: C, 67.05; H, 8.62; N, 1.38.

Pentafluorophenyl 3*β*-[Bis(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,-12α-bis(formyloxy)-23,24-bisnor-5*β*-cholane-22-thiolate (24). Sodium metaperiodate (0.3 g, ca. 1.4 mmol) was added to a stirred solution of **23** (1.01 g, 1.18 mmol) in acetonitrile−water (20 mL, 3:1). After complete oxidation of the diol (analysis by TLC), the mixture was filtered. Evaporation of filtrate and washings gave a residue which was dissolved in ether, washed with sodium bisulfite, water, and brine, and dried (Na_2SO_4). Evaporation yielded 3*β*-[bis(butoxycarbonyl)-methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-23,24-bisnor-5*β*-cholan-22-al: (TLC R_f 0.80, ether). The crude aldehyde was dissolved in acetonitrile−water (3:1, 40 mL) and cooled to −20 °C. Sulfamic acid (0.250 g, 2.57 mmol) was added with stirring, followed, after a delay of 5 min, by dropwise addition of a solution of sodium chlorite (1.33 mmol) in water (10 mL). Stirring was continued at 0 °C until analysis by TLC showed consumption of the aldehyde. Sodium metabisulfite (2 g, 10.5 mmol) was added in one portion and the mixture stirred for 30 min, diluted with ether, and filtered. Evaporation of filtrate and washings gave a residue which was dissolved in ether (100 mL), washed with water and brine, and dried (Na_2SO_4). Evaporation yielded 3*β*-[bis-(butoxycarbonyl)methyl]-3α-[[[*p*-(*tert*-butoxycarbonyl)amino]methyl]phenyl]-7α,12α-bis(formyloxy)-23,24-bisnor-5*β*-cholan-22-oic acid: (TLC R_f 0.36, ether). A solution of this material and pentafluorothiophenol (0.36 g, 0.24 mL, 1.74 mmol) in CH_2Cl_2 (10 mL) was cooled to −10 °C under an inert atmosphere and stirred for 10 min. DCC (0.326 g, 1.6 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The mixture was left stirring overnight, after which a drop of water was added and the mixture stirred for 1 h and then cooled to −10 °C. Precipitated dicyclohexylurea was separated by filtration and washed with ether-hexane mixture (1:1) cooled to −10 °C. Evaporation of the filtrate and washings gave a residue which on flash chromatography, eluting with hexane−ether (1:1), gave **24** (1.123 g, 85%): TLC R_f 0.4 (hexane−ether, 1:1); IR (film from $CDCl_3$) 3419, 2968, 2939, 2880, 1757, 1724, 1689, 1516, 1495, 1472, 1176, 983, 962 cm^{-1}; ^1H NMR (300 MHz) δ 8.09 (1H, s), 8.02 (1H, s), 7.37, 7.22 (4H, ABq, J = 8.3 Hz), 5.20 (1H, br s), 5.07 (1H, br s), 4.87 (1H, br t), 4.29 (2H, d, J = 5.8 Hz), 4.07 (1H, s), 4.04−3.88 (4H, m), 2.68 (1H, m), 1.19 (3H, d, J = 7.1 Hz), 1.06 (3H, s), 0.88−0.81 (9H, m); ^{13}C NMR δ 194.86, 168.09, 168.01, 160.44, 155.82, 144.65, 137.03, 134.65, 127.44, 126.43, 79.42, 74.25, 70.41, 64.84, 54.42, 51.33, 45.35, 44.62, 44.16, 42.59, 42.38, 38.05, 37.84, 37.69, 34.32, 32.14, 32.07, 31.52, 30.29, 29.31, 28.77, 28.73, 28.36, 26.10, 25.63, 24.65, 22.90, 18.97, 16.92, 13.60, 12.35, 12.30.

Tetrakis(formyloxy) Cholaphane (4c). Prepared by treating **24** (1.123 g, 1.10 mmol) with TFA (2 mL) and then DMAP (3.07 g) in dry CH_2Cl_2 (1.2 l), as described above for **4a**. The cyclodimerization was carried out over a period of 6 days. Flash chromatography of the crude product, eluting with $CHCl_3$-ethyl acetate (3:2) and drying under high vacuum at 50−55 °C for 4 h, gave **4c** (0.550 g, 67%): TLC R_f 0.3 ($CHCl_3$−ethyl acetate, 3:2); IR (film from $CDCl_3$) 3404, 3300, 2962, 2876, 1753, 1722, 1659, 1517, 1466, 1414, 1384, 1179, 755 cm^{-1}; ^1H NMR (300 MHz) δ 8.06 (2H, s), 7.88 (2H, s), 7.42, 7.23 (8H, ABq, J = 8.3 Hz), 5.62 (2H, d, J = 5.6 Hz), 5.15 (2H, br s), 5.02 (2H, br d), 4.68 (2H, dq, J = 7.1, 13.8 Hz), 4.07 (2H, s), 4.03−3.82 (10H, m), 2.45 (2H, br d), 1.04 (6H, d, J = 6.1 Hz), 1.03 (6H, s), 0.88−0.80 (12H, m), 0.78 (6H, s); ^{13}C NMR δ 175.29, 167.84, 160.28, 159.96, 145.20, 136.08, 127.86, 127.76, 75.01, 71.02, 64.79, 54.03, 45.17, 44.98, 44.82, 43.76, 43.39, 42.41, 38.09, 37.73, 37.52, 34.11, 31.89, 31.34, 30.23, 29.47, 29.21, 26.42, 25.99, 23.08, 22.67, 18.84, 17.16, 13.48, 12.18. Anal. Calcd for $C_{84}H_{118}N_2O_{18}\cdot 3H_2O$:[31] C, 67.36; H, 8.34; N, 1.87. Found: C, 67.13; H, 8.03; N, 1.76. FABMS *m*/*z* 1465, 100% $[M + Na]^+$.

Tetrahydroxy Cholaphane (3c). Prepared by treatment of **4c** (0.087 g, 0.056 mmol) with K_2CO_3 in methanol−water, as described above for **3a**. Flash chromatography of the crude product, eluting with CH_2Cl_2−methanol (99:1, 98:2, and then 95:5) gave **3b** (0.072 g, 90%): TLC R_f 0.25 ($CHCl_3$−methanol, 9:1); IR (film from $CDCl_3$) 3615, 3400, 2966, 2880, 1756, 1724, 1652, 1517, 1468, 1438, 1383, 1144, 1096 cm^{-1}; ^1H NMR (300 MHz) δ 7.42, 7.16 (8H, ABq, J = 8.3 Hz), 5.71 (2H, br d), 4.75 (2H, dq, J = 7.1, 13.8 Hz), 4.11 (2H, s), 4.01−3.84 (14H, m), 1.17 (6H, d, J = 6.9 Hz), 1.00 (6H, s), 0.84 (12H, m), 0.69 (6H, s); ^{13}C NMR δ 176.14, 168.39, 168.29, 145.64, 135.67, 127.65, 127.54, 72.62, 67.98, 64.75, 54.60, 46.61, 45.29, 44.79, 43.57, 42.92, 41.73, 39.47, 38.49, 38.35, 34.67, 34.34, 32.35, 30.23, 29.59, 28.63, 26.81, 26.68, 23.04, 18.94, 18.91, 16.98, 13.57, 12.56. Anal. Calcd for $C_{80}H_{118}N_2O_{14}\cdot 2H_2O$:[31] C, 70.25; H, 8.99;

N, 2.05. Found: C, 70.31; H, 8.99; N, 1.93. FABMS *m/z* 1354, 100% $[M + Na]^+$.

NMR Titration Studies. Solutions of cholaphanes **3a**−**c** (1.2−1.8 mM) in $CDCl_3$ were placed in NMR tubes. Solutions in $CDCl_3$ of octyl β-D-glucopyranoside (**25**) (70−90 mM) were added in portions. The resulting downfield shifts of the N*H* NMR signals were analyzed using an iterative nonlinear least-squares curve-fitting program, with weighting of data points according to the error analysis of Deranleau,[33] to give the binding constants and limiting chemical shifts quoted in the text. Binding curves are provided as Supporting Information.

Extraction Experiments. Aqueous solutions of methyl-β-D-glucoside (**26**) (3 mL, 1−2.5 M) and $CHCl_3$ solutions of cholaphanes **3a**−**c** and **2** (R = H) (3 mL, 1 mM) were placed in sealed flasks and stirred for 24 h in the dark. The two phases were separated by removing the bulk of the aqueous layer with a pipet and then filtering the organic layer through hydrophobic filter paper. The resulting clear organic phase was analyzed by TLC, spotting with a 20 μL pipet onto glass-backed plates, and eluting with ethyl acetate−methanol−water (8:1:1). Visualization with resorcinol/H_2SO_4 (2-propanol as solvent) and heating with a heat gun gave dark spots for the cholaphanes (R_f 0.8−0.95) and for **26** (R_f 0.05−0.1). For quantitation, extracts were evaporated, redissolved in methanol−$CHCl_3$ (4:1), and compared against standard solutions of **26** spotted from the same solvent mixture.

Transport Experiment. A $CHCl_3$ solution of cholaphane **6c** (6 mL, 2.5 mM) was placed in a "U-tube" transport apparatus with an extra port allowing access to the organic phase. The "receiving arm" was charged with distilled water (2 mL) and a solution of **26** in distilled water (2 mL, 2M) was added gently to the "departing arm". The organic phase was stirred gently (ca. 60−80 rpm). After 24 h, all three phases were analyzed by TLC, eluting with ethyl acetate−methanol (4:1) and visualizing with 2,4-DNP. The analyses were repeated after 48, 72, and 168 h. **26** was detected at approximately static levels in the organic phase, and in small but increasing concentrations in the receiving phase. A similar experiment in the absence of **3c** showed no detectable amount of glucoside in the "receiving arm" or organic layer.

(33) Deranleau, D. A. *J. Am. Chem. Soc.* **1969**, *91*, 4044. Connors, K. A. *Binding Constants*; Wiley: New York, 1987; p 66.

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Supporting Information Available: Synthetic procedures for **5**, **6a**, and **6b**; binding curves from NMR titrations (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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