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Novel Deep Cavity Calix[4]pyrroles Derived from Steroidal Ketones*

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Novel steroidal calix[4]pyrroles were prepared in excellent yields from commercially available cholic acid derivatives using an efficient synthetic sequence. Once in hand, it was found that these calix[4]pyrroles exist in the form of four different configurational isomers. Separation of these isomers was achieved readily using normal phase HPLC techniques. Once purified, the steroidal calix[4]pyrroles were screened via -FABMS analyses to judge their utility in effecting the enantioselective recognition of appropriate organic anions. Results that provided support for antipodal *R* > *S* selectivity were obtained in the case of both tartaric acid and mandelic acid. Direct extraction studies were then carried out and these confirmed the pattern of *R* > *S* selectivity observed by -FABMS.

Keywords: Ketones; Isomer; Calix[4]pyrroles; FABMS; Porphyrins; Steroid

INTRODUCTION

One of the current goals of supramolecular chemistry is to create selective receptors tailored for specific substrates. An elegant approach to reaching this goal involves the construction of receptors from building blocks with distinct geometries. Here, inspiration has often come from Nature [3,4] where the ability of certain (bio)molecules to recognize and bind other molecules abets a number of biological processes, including information transduction [5], immune response, and enzyme function [6]. In order to mimic natural receptors with their selectivity for a targeted substrate, stereogenic subunits are often used [7–10]. In this context, one of the more interesting chiral building blocks, other than the widely used 1,1′-binapthyl-2,2′-diol subunit, is the steroid skeleton, namely cholic acid derivatives. These compounds have been used in the past for the construction of many elegant and specific receptors and carriers as well as transmembrane channel mimics and more complex systems capable of undergoing self-assembly in solution and in the solid state [11,12].

In this paper we describe a new way whereby a steroid building block may be employed to construct chiral calix[4]pyrroles, specifically **1**, **2**, and **3** (Fig. 1).

To date, most receptors containing steroid moieties have been prepared from cholic acid derivatives (e.g. **4**) through the use of linking amide or ester bonds. Unfortunately, this approach is generally not conducive to the construction of receptors wherein such rigid subunits are incorporated into a polypyrrolic core. In previous work [2], we described the synthesis of an aldehyde generated from cholic acid and its use in the construction of macrocycles, namely porphyrins and corresponding resorcinol-based systems. Now, we wish to report the preparation of a series of steroid ketones **5a**, **5b**, and **6**. We also show how these building blocks may be used for the construction of novel enantioselective calix[4]pyrrole receptors, namely **1**–**3**, that are characterized by rigid steroid backbones and paired hydrophobic and hydrophilic faces rich in hydroxyl group functionality.

*For preliminary communication see Refs. [1,2].

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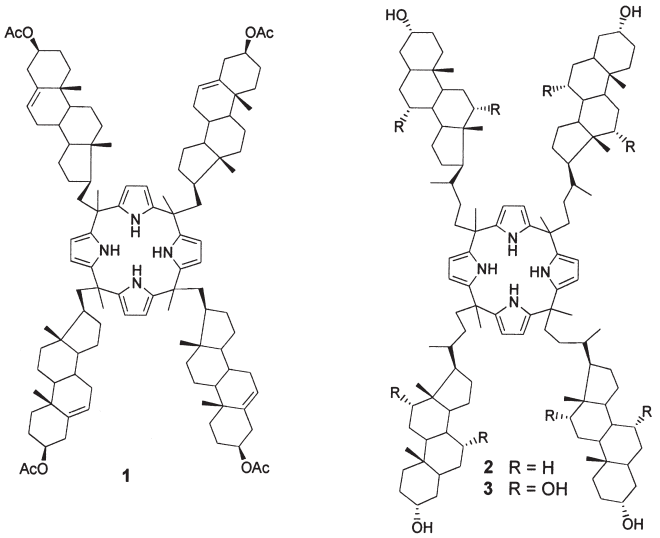


FIGURE 1 Structures of steroid-containing calix[4]pyrrole receptors.

Calix[4]pyrroles, a venerable class of porphyrinogen-like, cyclic tetrapyrroles prepared from the condensation of ketones with pyrrole, have attracted interest of late as novel receptors for anions and for neutral substrates [13]. While generally easy to prepare once the requisite ketones are in hand, one limitation of these agents is that, although the "strength" of binding is easily tunable by, e.g. changing the electronics of the pyrrole moiety [14], the intrinsic substrate selectivities ($F^- \gg Cl^- \cong H_2PO_4^- > Br^-$, $I^- \gg$ neutrals) have remained roughly the same regardless of most changes made. Even though we have recently been able to alter the core size of calixpyrroles, preparing systems containing as many as eight pyrrolic subunits [15], modifying the substrate recognition behavior of calixpyrroles remains a daunting challenge. It was this challenge that provided the impetus for the present work. Specifically, appreciating that cyclic oligo-steroidal layouts based on porphyrins act as selective carbohydrate receptors, we reasoned that calix[4]pyrroles built from **5** or **6** might show modified substrate affinities, perhaps even displaying a degree of enantioselectivity.

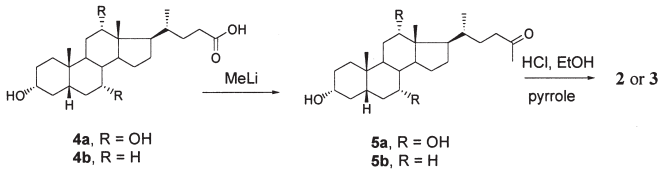
RESULTS AND DISCUSSION

The synthesis of the steroid containing calixpyrroles **2** and **3** departs from the cholic acid derivatives **4a** and **4b**. These commercially available materials were converted without protection into the corresponding ketones **5a** and **5b** by treatment with excess methyllithium. Once in hand, ketones **5a** and **5b** were condensed with pyrrole in a 1:1 mixture of methanol/dichloromethane under conditions of HCl catalysis to give the unresolved tetrasteroidyl calixpyrroles **2** and **3** (Scheme 1).

A related steroid-containing *meso*-tetramethyltetrakis(3β-acetoxy-pregn-5-en-21-yl)-calix[4]pyrrole **1** could also be made from compound **6**. In this case, the key intermediate, **6**, was prepared from methyl 3β-hydroxypregn-5-en-21-oate by treatment with the sodium salt of dimethylsulfoxide followed by acetylation with acetic anhydride and reduction with amalgamated aluminum. Standard condensation then produced **1** (Scheme 2).

In each case, the total yields were very high, being over 90%. However, the products were not pure. Rather, they were obtained as a mixture of up to four different configurational isomers. This large number of isomers made separation by conventional chromatography impractical. Accordingly, we turned to HPLC methods. These structures vary considerably in their degree of compactness, as can be seen by inspection of compounds **2a–d** and **3a–d** in Fig. 2, and this we reasoned would allow for their separation.

Using a Varian 9000 series isocratic pump/detector with UV detection at 254 nm on a Supelco column packed with 5 Å spherical SiO_2 and a mixture of DCM/MeOH as the eluent (for **3** 19:1; for **2** 99:1), separation of all four isomers was achieved in the case of both **2** and **3**. Independent HPLC - +CIMS analysis using 5% methanol in DCM as the eluent for **3** and DCM for **2**, led to the separation and identification of all four configurational isomers as well as two minor by-products. On the basis of +CIMS analysis, these latter were assigned to open chain species arising as the result of either incomplete cyclization or, more likely, degradation on the column. Among the cyclized isomers, the



SCHEME 1 Synthesis of hydroxylated calix[4]pyrroles **2** and **3**.

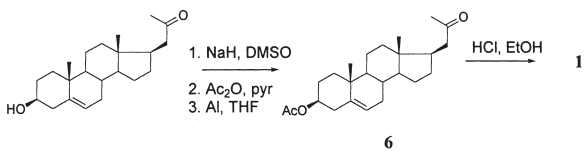
ratios of product formation as calculated by HPLC are summarized in Table I. Using the polarity argument that was demonstrated and recently shown to be true for our deep cavity calixpyrroles [16], we tentatively assigned these peaks, in order of elution, to structures **2a**, **2b**, **2c**, and **2d**, and **3a**, **3b**, **3c**, and **3d**, in the case of both **2** and **3**, respectively. Careful evaluation of the ^{1}H NMR spectra of the individual species in question confirmed this assignment.

The mobile nature of the linking arms used to attach the steroid subunits to the *meso*-positions in **2** and **3** imparts a large degree of flexibility that should allow these systems to adopt geometries suitable for substrate complexation. To the extent this proved true, we reasoned that these steroid functionalized calixpyrroles would be useful as anion and/or neutral substrate receptors. As a first test of the above hypothesis, -FABMS was used to probe the molecular recognition properties of **2** and **3**. After looking at a number of sugars, and amino acids, using -FABMS to screen for their affinity toward receptors **2** and **3**, we finally got a hit when tartaric acid was used as a substrate in the case of **3**. Here, complex formation was evident in the gas phase as inferred from the presence of ion peaks corresponding to the complex anion. Further, a fair degree of enantioselectivity was observed, with the extent of this selectivity being highest for the αααβ isomer. This positive result for receptor **3** stands in contrast to what was observed with **2**. Here, a much lower degree of interaction was seen in the gas phase as can be inferred from an inspection of Figs. 3 and 4. In fact, in contrast to what proved true for **2**, calixpyrrole **3** showed such strong binding that enantioselective recognition is apparent even when this system was tested in the form of a crude mixture of unseparated configurational isomers.

From the above data, as well as independent studies, important insights were gained into the way in which the steroid calixpyrrole receptor **3** interacts with tartaric acid. One key hint came from noting that the extent of interaction with the squaric acid remained relatively constant for both **2** and **3**. However, when a more complex substrate, such as tartaric acid, was employed, a pronounced relative enhancement in binding is seen in the case of **3**, with a corresponding decrease in binding to **2** being observed. Further, in the case of **3**, enhanced enantioselectivity is observed. By contrast, no stereoselectivity is seen for **1**, a system that is also a poor receptor as far as net overall affinity is concerned. This leads us to conclude that the large number of hydroxyl groups present in **3** are required to achieve appreciable selectivity. It also raises the intriguing possibility, at least in our mind, that normal tetrapyrrolic NH-to-anion hydrogen bonding interactions may not be responsible for the binding of tartaric acid and the other complex substrates of this study. To test this latter hypothesis, we studied the binding of R-tartaric acid to **3** in competition with tetrabutylammonium fluoride. Under these conditions, both anions are bound as inferred from -FABMS analysis, a finding which is consistent with tartaric acid binding taking place largely within the core of the steroidal scaffold.

Once the above findings were made, we sought to find a substrate that might provide an alternative spectroscopic handle. After further screening studies, it was found that mandelic acid also gave a positive hit, as judged from -FABMS analysis. Again, the best enantioselectivity was seen in the case of the αααβ isomer and once again for the R stereoisomer, as can be seen in Fig. 5.

To put on a more solid footing the preliminary results we were observing in the gas phase using -FABMS analysis (a technique that is at best only semi-quantitative), direct extraction studies were carried out. As shown in Fig. 6, the R antipodes of both tartaric acid and mandelic acid were extracted



SCHEME 2 Synthesis of control calix[4]pyrrole **1**.

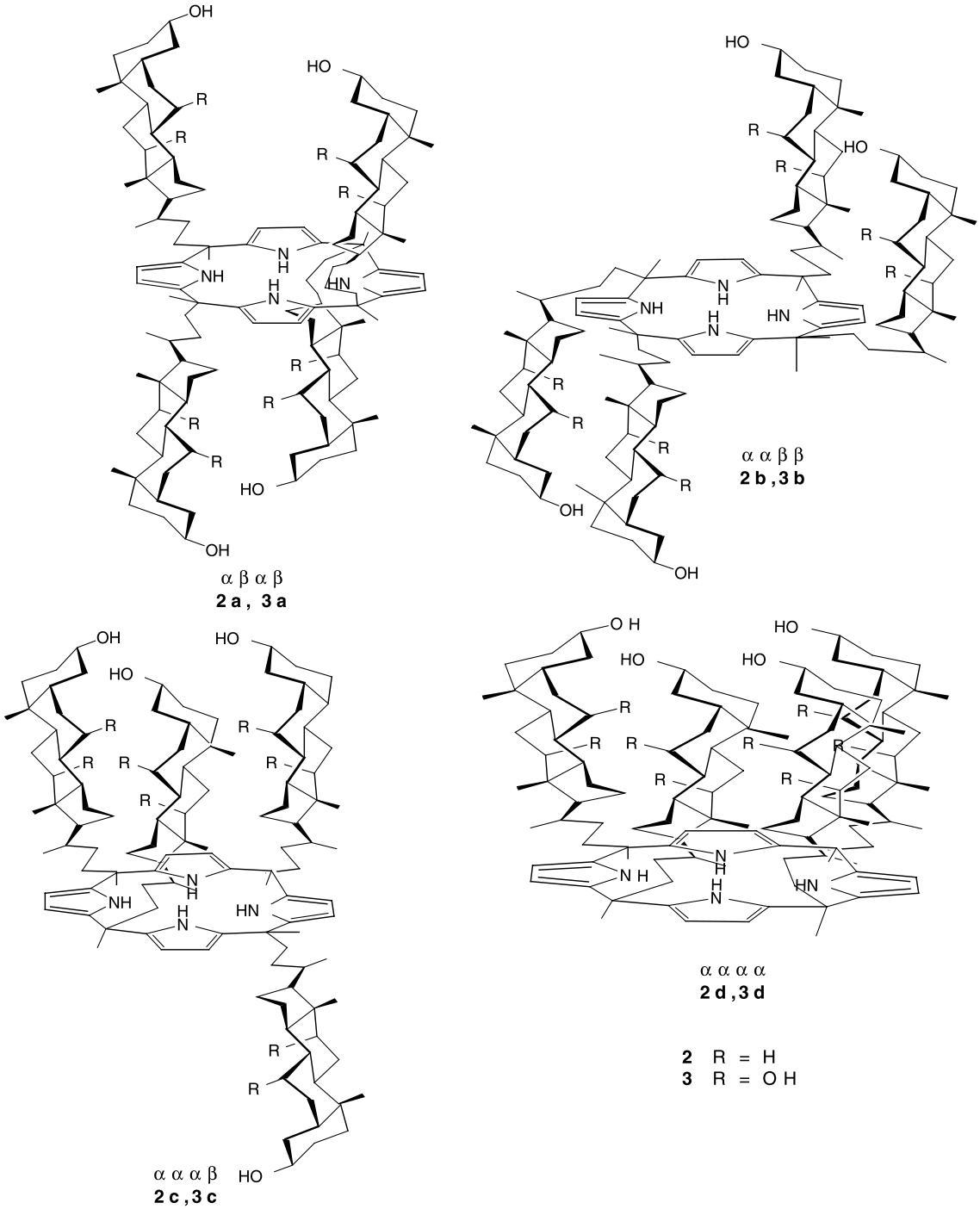


FIGURE 2 Depiction of the four separate configurational isomers obtained during the preparation of receptors **2** and **3**.

TABLE I Product ratio analysis. (A) All product ratios were calculated from HPLC analysis after isolation and determination of structure. (B) Ratios were normalized relative to the peak area of the αβαβ isomer. In the case of both **2** and **3**, it was this isomer that was obtained in the lowest yield

Isomer	αβαβ "a"	ααββ "b"	αααβ "c"	αααα "d"
2	1	2.60	3.86	2.30
3	1	1.56	2.73	1.46

more efficiently when isomer **3c** was used as an extractant. Further, as in the case of the -FABMS analyses, the enantioselectivity matched what would be expected for a mechanism that involved direct interaction between the chiral, steroid-derived walls of the extended cavity calixpyrrole and the anionic form of the carboxylic acid in question. Consistent with this hypothesis were the expected further findings that neither the isomeric mixtures of **2** or **3**,

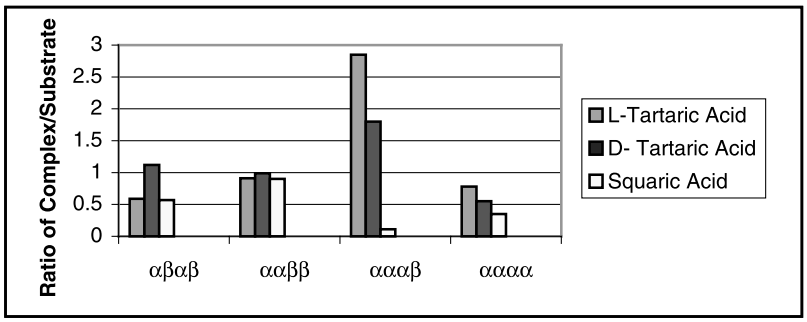


FIGURE 3 -FABMS results for compound **3** with selected anions. For these studies a large excess (>100-fold) of the carboxylic acid in question was added to a MeOH solution of the receptor and subjected to -FABMS analysis. The ratios depicted, are the ratios in percentages with relation to the parent ion peak. It is of note that in all cases the parent ion peak was either **3a**, **3b**, **3c**, **3d**, or that associated with the complex anion formed from these receptors and the deprotonated form of the acid in question. As noted in the text, these results give a semi-quantitative approximation only of the relative concentrations of the species in question.

or even the αααβ isomer of **2**, proved as effective as extractants, as judged by both enantioselectivity and absolute into-dichloromethane mass transfer ability.

In conclusion, we have developed a high yield synthesis of non-racemic, steroid-based carboxylic acid receptors that are predicated on the use of calix[4]pyrroles as a semi-rigid scaffold. The best of these receptors, the polyhydroxylated αααβ configurational isomer, **3c**, shows evidence for enantioselective binding in the case of tartaric acid and mandelic acid. This finding is rationalized in terms of multiple substrate–receptor hydrogen bonding interactions that involve not only specific anion–pyrrole NH contacts, but also less well-defined steroid–substrate interactions. The importance of these latter ancillary contacts is underscored by the fact that receptors **2** and, especially, **1** proved far less effective than **3**, as judged by either mass spectrometric screening or more direct extraction methods. It is also highlighted by the fact that receptors **3a**–**d**, which contained the requisite hydroxyl donor functionality but presumably a less than optimal configurational geometry, displayed the same antipodal selectivity (R > S) as **3c**, albeit at a diminished level. This latter finding is important from a practical perspective since it leads to the suggestion that for certain applications (e.g. bulk extractions) it may not be necessary to effect separation of the individual configurational isomers. This, in turn, augers well for the eventual "real-world" use of systems such as those described herein.

EXPERIMENTAL

Steroids **4a** and **4b** were purchased from Sigma-Aldrich and Steraloids. Other solvents and reagents were purchased from Fluka. Melting points were determined on a Boëtius (Germany) micro melting point apparatus (Kofler block) and are uncorrected. Optical rotations were measured on an Opton (Germany) polarimeter in chloroform at 25°C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were taken and complexation properties measured on a Varian CARY400 SCAN UV–VIS spectrophotometer. Infrared spectra were recorded on a Perkin–Elmer PE 580 spectrometer (wavenumbers in cm^{-1}). Proton NMR spectra were taken on a Varian UNITY-500 (499.8 MHz for ^{1}H) FT NMR spectrometer at 23°C in $CDCl_3$ with tetramethylsilane being used as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (J) and widths of multiplets (W) are given in Hz (unless stated otherwise). Mass spectra were recorded on a VG

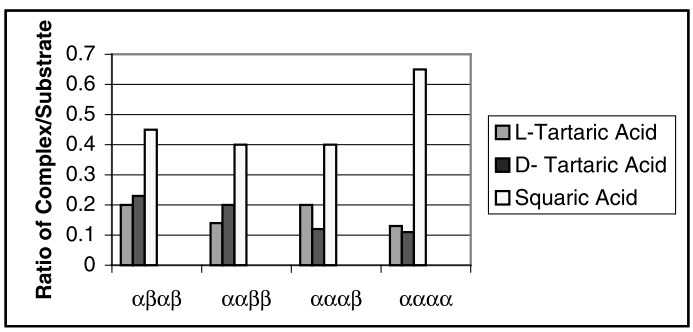


FIGURE 4 -FABMS results for compound **2** with selected anions. These studies were carried out as detailed in the caption to Fig. 3. Note, however, that there is a large difference in scale for this figure as compared to Fig. 3.

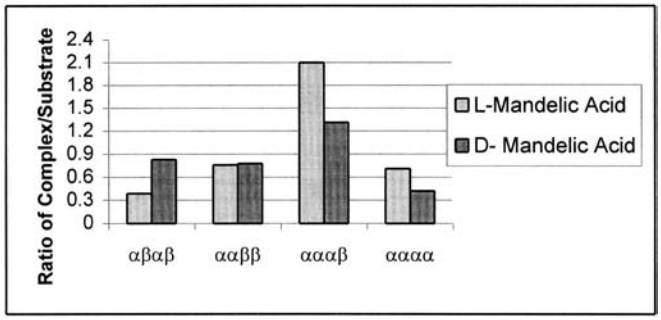


FIGURE 5 -FABMS results for compound **3** with selected anions. These studies were carried out as detailed in the caption to Fig. 3.

Analytical ZAB-EQ spectrometer. Microanalyses were performed on a Perkin–Elmer 2400 Series II CHNS/O Elemental Analyzer. Thin-layer chromatography was performed on silica gel G (ICN Biomedicals) plates, with detection effected by spraying with concentrated sulfuric acid followed by heating. Preparative TLCs were performed on 200 × 200 mm plates made up of the above silica gel (thickness 0.4 mm). For preparative and flash column chromatography, silica gel 32–63, 60 Å (ICN Biomedicals) or Silpearl (Kavalier) were used. The petroleum ether used was a fraction boiling at 40–62°C. Prior to evaporation on a rotary vacuum evaporator (bath temperature <50°C) solutions involving organic solvents were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate.

Preparation of 3α,7α,12α-trihydroxy-26,27-dinor-5β-cholestan-24-one (5a)

Cholic acid (2 g, 4.9 mmol) was dissolved in dry THF (80 ml) under argon at 0°C (ice-bath) and methyl lithium (16 ml, 1.6 M solution in ether; 25 mmol) was added. The reaction mixture was stirred for 8 h at room temperature, and then quenched with TMSCl (30 ml). At this juncture, the volatiles were evaporated off using a rotary evaporator and the residue was dissolved in ethyl acetate (80 ml) and washed with aqueous HCl (5% solution, 80 ml) and water (3 × 80 ml). The organic layer was dried over Na_2SO_4 and concentrated to dryness *in vacuo*. The resulting residue was dissolved in dichloromethane and purified by column chromatography over silica gel using a mixture of dichloromethane–methanol (96:4) as the eluent. This yielded 1.6 g (80.4%) of **2b** of m.p. 92–94°C. IR spectrum (chloroform): 3613, 3428 (OH), 1710 (C═O), 1378 (C-CH_3), 1369 (CO-CH_3), 1076, 1041, 979 (C–OH). ^{1}H NMR spectrum: 0.7 s, 0.91 s, 0.98 d (3 × 3H steroidal methyls), 2.16 s (3H, $COCH_3$), 3.47 m (1H, $C_{(3)}$–H), 3.87 m (1H, $C_{(7)}$–H), 3.98 m (1H, $C_{(12)}$–H). For $C_{25}H_{42}O_4$ (406.60) calculated 73.85% C; 10.41% H; 15.74% O, found: 73.79% C; 10.47% H.

Preparation of 3α-hydroxy-26,27-dinor-5β-cholestan-24-one (5b)

Lithocholic acid (1.85 g, 4.9 mmol) was treated as above to yield 1.2 g (65.2%) of **2b**. M.p. 148–151°C. IR spectrum (chloroform): 3609 (OH), 1710 (C = O), 1376 (C-CH_3), 1365 (CO-CH_3), 1030 (C–OH). ^{1}H NMR spectrum: 0.64 s, 0.90 d, 0.92 s (3 × 3H, steroidal methyls), 2.14 s (3H, CO–CH_3), 3.62 m (1H, $C_{(3)}$–H). For $C_{25}H_{42}O_2$ (37,460) calculated 80.16% C; 11.30% H; 8.54% O, found 79.86% C; 11.50% H.

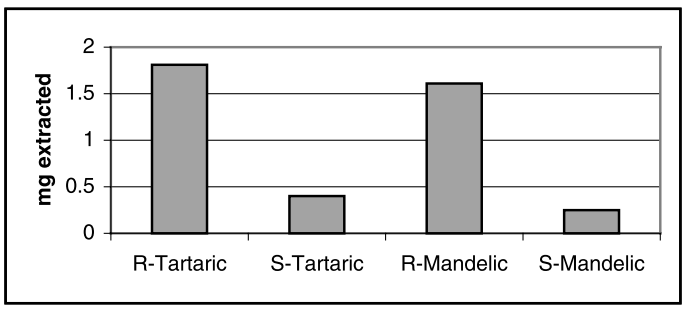


FIGURE 6 Receptor-mediated extraction of non-racemic carboxylic acids from water (pH = 7) into dichloromethane. The data is from an average of two extractions and depicts the amount of anion extracted by **3c** into dichloromethane after vigorous shaking for a total of 5 min, followed by letting the bi-phase system stand for 1 h. For this experiment, 1 ml each of both dichloromethane and water was used from an analytically prepared stock of either calixpyrrole **3c** (0.26 mmol) or α-hydroxy acid (6.60 mmol).

21-Oxo-21-homopregn-5-en-3β-yl Acetate (6)

A mixture of sodium hydride (3.6 g, 150 mmol) and dimethyl sulfoxide (108 ml) was stirred at 66°C for 2 h under argon. After cooling, the solution was diluted with tetrahydrofuran (100 ml) and methyl 3-hydroxypregn-5-en-21-oate [17] (5.0 g, 14.4 mmol) in tetrahydrofuran (100 ml) was added. The mixture was stirred for 3 h at room temperature, poured into water (700 ml), neutralized with solid ammonium chloride and the product was extracted with ethyl acetate (2 × 200 ml). The organic extracts were washed with water (6 × 200 ml), dried over Na_2SO_4 and concentrated to dryness *in vacuo*. The residue (5.2 g) was practically the pure sulfoxide. (TLC in benzene–acetone 6:4) ^{1}H NMR ($CDCl_3$, 100 MHz): 0.62 s 3H (3 × H-18); 1.01 s, 3H (3 × H-19); 2.68 s, 3H ($SOCH_3$); 3.78 s, 2H ($COCH_2SO$); 3.54 mult, 1H, $W = 32$ (H-3); 5.37 bd, 1H, $J = 4.5$ (H-6).

The crude sulfoxide (5.2 g, ca. 13 mmol) was dissolved in pyridine (72 ml), and acetic anhydride (22 ml) was added. After standing at room temperature overnight, the reaction mixture was poured onto ice (400 g) and the product was extracted with ethyl acetate (2 × 200 ml). The organic extracts were washed with dilute hydrochloric acid (5%, 3 × 200 ml), water (200 ml), aqueous saturated potassium hydrogen carbonate (2 × 200 ml), and water. After drying over Na_2SO_4 the solvents were evaporated off to give a residue (5.6 g) that was practically the pure acetate (TLC in benzene–acetone 6:4). ^{1}H NMR ($CDCl_3$, 100 MHz): 0.62 s 3H (3 × H-18); 1.01 s, 3H (3 × H-19); 2.03 s, 3H (CH_3COO); 2.68 s, 3H ($SOCH_3$); 3.78 s, 2H ($COCH_2SO$); 4.60 mult, 1H, $W = 32$ (H-3); 5.38 bd, 1H, $J = 4.5$ (H-6).

The acetate obtained above (5.6 g, ca 13 mmol) was dissolved in tetrahydrofuran (210 ml) and water (25 ml). Amalgamated aluminum [18] (3.2 g) was then added over a 30 min period to the boiling stirred solution. The resulting mixture was heated at reflux with stirring for an additional 3 h. After cooling, it was poured onto a column of silica gel (50 g), layered with celite (20 g), and subject to elution with ethyl acetate. The resulting crude product (4.5 g) was purified by column chromatography over silica gel (310 g) using light petroleum–benzene–ether (50:50:1) as the eluent. This gave 3.1 g of ketone **6** (58% from the starting ester). M.p. 161–163°C (methanol), $[\alpha]_D$ −59 (c 0.4 $CHCl_3$). Reported [19] m.p. 156–158°C (ethyl acetate–methanol), $[\alpha]_D$ −50 ($CHCl_3$). For **6**: IR ($CHCl_3$): 1724, 1256. ^{1}H NMR ($CDCl_3$, 100-MHz): 0.60 s 3H (3 × H-18); 1.02 s, 3H (3 × H-19); 2.03 s, 3H (CH_3COO); 2.14 s, 3H ($COCH_3$); 4.60 mult, 1H, $W = 32$ (H-3); 5.39 bd, 1H, $J = 4.5$ (H-6).

meso-Tetramethyl-tetrakis(3β-acetoxy-pregn-5-en-21-yl)-calix[4]pyrrole (1)

21-Oxo-21-homopregn-5-en-3β-yl acetate (**6**; 100 mg, 0.27 mmol) was dissolved in a mixture of dichloromethane–ethanol (1:1; 2 ml) and pyrrole was added (17 mg) together with two drops of concentrated aqueous hydrochloric acid. The mixture was then allowed to stir at room temperature for 72 h. At this point, the reaction mixture was evaporated to dryness and the residue dissolved in dichloromethane (4 ml) and purified by column chromatography over silica gel using dichloromethane–methanol 9:1 as the mobile phase. This yielded 46 mg (40.6%) of the calix[4]pyrrole **1**. M.p. 234–237°C, $[\alpha]_D^{25}$ +66.8 (c 0.5, dichloromethane). IR spectrum (chloroform) 3437, 3111, 1573, 976, 957 (NH, pyrrole), 1725 (C═O), 1669 (C═C), 1367 (methyl, acetate), 1199 (C–O, acetate). ^{1}H NMR ($CDCl_3$, 100 MHz): 0.60 and 1.01 2 s, 2x 12H (angular methyls), 2.03 s 2.15 s 2x 6H (CH_3COO–), 4.6, 3.5 2 m 4H (C_3–H), 5.39 m (4H C_6–H), 5.3–5.8 m 8H (pyrrole C–H), 6.7–7.1 range of multiplets 4H (NH pyrrole). Mass spectrum FAB (m/z): 1690, 1675, 1659, 1642, 1628, 1615, 1583, 1313, 1289, 1001, 959, 556. For $C_{112}H_{156}N_4O_8$ (1686.5) calculated 79.77% C, 9.32% H, 3.32% N; found 79.26% C, 9.12% H, 2.95% N.

meso-Tetramethyl-tetrakis(3α-hydroxy-26,27-dinor-5β-cholestan-24-yl)-calix[4]pyrrole (2)

The preparation of **2** was carried out as above, using 3α-hydroxy-26,27-dinor-5β-cholestan-24-one (**5b**; 100 mg, 0.267 mmol) as the starting material and chloroform–ethanol (1:1, 2 ml) as the solvent. The reaction was run at room temperature for 72 h. The yield was 104 mg (0.061 mmol, 91%) of calixpyrrole **2** m.p. 164–168°C, $[\alpha]_D^{25}$ +93.1 (dichloromethane). IR spectrum (chloroform) 3609, 1036 (OH), 3439 (NH), 3109, 1576, 1030 (pyrrole), 1718 (CO), 1736 (CH_3). ^{1}H NMR spectrum: 0.4 s 0.91 2 s 2x 12 H (angular methyls), 3.6 m 4H (C_3–H), 5.88 m 8H (C–H, pyrrole). Mass spectrum FAB (m/z): 1695 (M + 1, 40%), 1628 (80%), 1395, 1362.5 (60%), 1204 (100%), 1031, 889, 848, HR 1695.43 $C_{116}H_{182}N_4O_4$. For $C_{116}H_{180}N_4O_4$ (1694.7); calculated 82.21% C, 10.71% H, 3.31% N; found 82.53% C, 11.01% H, 3.14% N.

meso-Tetramethyl-tetrakis(3α,7α,12α-trihydroxy-26,27-dinor-5β-cholestan-24-yl)-calix[4]pyrrole (3)

This compound was prepared as above using 3α,7α,12α-trihydroxy-26,27-dinor-5β-cholestan-24-one (**5a**; 100 mg, 0.246 mmol) as the starting material and dichloromethane–methanol (1:1, 2 ml) as the solvent. The reaction was run at room temperature over 57 h. The yield of **3** was 107 mg (0.059 mmol; 98%). M.p. 227–230°C, $[\alpha]_D^{25}$ +119 (c 0.5, methanol). IR spectrum (KBr) cm^{-1}: 3434, 3270 (OH, NH), 3109

(pyrrole, C–H), 1580 (pyrrole, ring), 1376 (CH_3), 1044, 1028, 1009, 981 (C–OH). ^{1}H NMR spectrum: 0.52 s 0.89, 2 s 2x 12H (angular methyls), 3.2 s 4H 3.6 s 4H, 3.76 s 4H, 4.0 m 8H, 4.3 s 4H, 5.65 br mult 8H (= CH– pyrrole), 6.4–7.35 range of signals 4H (NH). Mass spectrum (*m*/*z*, FAB): 1823 (M + 2, 15%), 1565 (7%), 1536 (18%), 1458.7 (100%), HR MS 1823 $C_{116}H_{182}N_4O_{12}$. For $C_{116}H_{180}N_4O_{12}$ (1822) calculated 76.44% C, 9.95% H, 3.07% N; found 76.14% C, 9.36% H, 2.22% N.

Binding studies

Qualitative -FABMS studies of binding were carried out by mixing a large excess of one or more potential substrates with the calix[4]pyrrole receptor in question and subjecting the resulting mixture, made up as a MeOH solution, to -FABMS analysis. The ratios depicted, are the ratios in percentages with relation to the parent ion peak. It is of note that, in all cases, the parent ion peak was either **3a**, **3b**, **3c**, **3d**, or that associated with the complex anion formed from these receptors and the deprotonated form of the acid in question. These results obtained in this way are semi-quantitative and give a rough approximation only of the relative concentrations of the species in question.

Receptor-mediated extraction studies were carried out by measuring the amount of various non-racemic carboxylate anions extracted from water (pH = 7) into dichloromethane by receptor **3c** after 5 min of vigorous shaking followed by an hour of letting the bi-phase system stand. The data from two separate experiments was averaged to give the values reported herein.

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