An Extraction-Based Assay for Neutral Anionophores: The Measurement of High Binding Constants to Steroidal Receptors in a Nonpolar Solvent

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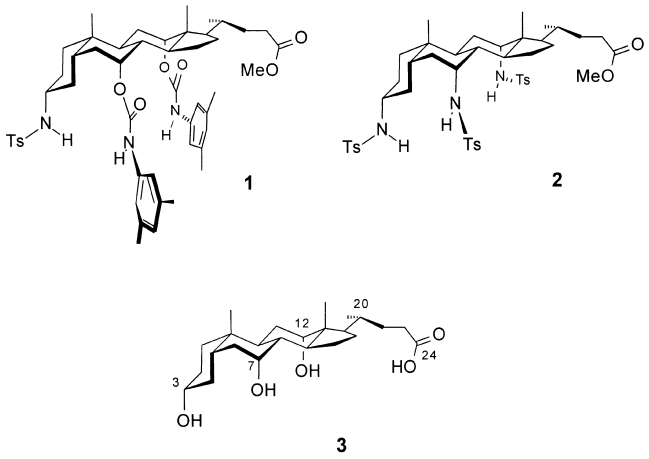
Abstract: The extraction-based protocol for measuring binding constants, developed by Cram and co-workers, has been extended for use with anionic substrates. The method is especially useful for high-affinity receptors, allowing very high binding constants to be measured in nonpolar solvents. Distribution constants K_d between chloroform and water have been obtained for tetraethylammonium chloride and bromide, thus calibrating the method for these two substrates. Application to steroidal podands **5**–**9** has confirmed the ability of electron-withdrawing groups to enhance hydrogen-bond donor capabilities. Binding constants of $\approx 3 \times 10^7\,\text{M}^{-1}$ have been measured for the most powerful receptor **7**. An X-ray crystal structure of **15**, the methyl ester analogue of **7**, reveals a well-defined binding site preorganised for anion recognition.

Keywords: anion recognition · ionophores · molecular recognition · receptors · supramolecular chemistry

Introduction

The study of anion recognition has become an active area of supramolecular chemistry.[1] While much early work focused on positively charged systems, in recent years there has been increasing emphasis on electroneutral anionophores.[2–4] Such molecules are more compatible with organic solvents, have scope for "phase-transfer" applications (e.g. in ion-selective electrodes), and are of special interest if only because they may be viewed as anion-binding counterparts to the classical, cation-binding crown ethers and cryptands.[3] A recent communication from this laboratory described two such anionophores, **1** and **2**, with steroidal organising frameworks derived from cholic acid **3**.[4] Both showed marked affinities for halide anions, binding chloride in $CDCl_3$ with $K_a = 7200$ and $92\,000\,\text{M}^{-1}$, respectively.



The development of improved systems related to **1** and **2** was impeded by problems of measurement. The ^{1}H NMR titrations used in our initial work can only be employed for K_a up to $\approx 10^5\,\text{M}^{-1}$,[5] the level already reached by **2**. The obvious remedy was a change to a more competitive solvent, such as acetonitrile or DMSO. However, comparisons with our earlier results would then be more difficult; moreover the performance of receptors in more polar solvents might not parallel that in nonpolar media of interest, such as biological membranes or the polymer materials used in ion-selective electrodes.

We therefore sought a method which could measure binding constants of, ideally, any magnitude in a nonpolar medium such as chloroform. An option, realised for cation-binding by the group of Cram,[6] relies on the extraction of salts from water into the nonpolar solvent. Though applied

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regularly to cationophores,[7] the method had not been adapted to the study of inorganic anion recognition.[8]

Following Cram, the extraction may be represented by Equations (1) and (2), where H ≡ receptor, X^- ≡ anionic substrate, Y^+ ≡ counterion and K_e ≡ extraction constant.

$$H_{org} + X^-_{aq} + Y^+_{aq} \overset{K_e}{\rightleftharpoons} HX^-Y^+_{org} \quad (1)$$

$$K_e = \frac{[HXY]_{org}}{[H]_{org}[X^-]_{aq}[Y^+]_{aq}} \quad (2)$$

At equilibrium there will also be a concentration of free X^-Y^+ in the organic phase, governed by Equations (3) and (4) (K_d ≡ distribution constant).

$$X^-_{aq} + Y^+_{aq} \overset{K_d}{\rightleftharpoons} X^-Y^+_{org} \quad (3)$$

$$K_d = \frac{[X^-Y^+]_{org}}{[X^-]_{aq}[Y^+]_{aq}} \quad (4)$$

Combining Equations (2) and (4);

$$K_a = \frac{K_e}{K_d} \quad (5)$$

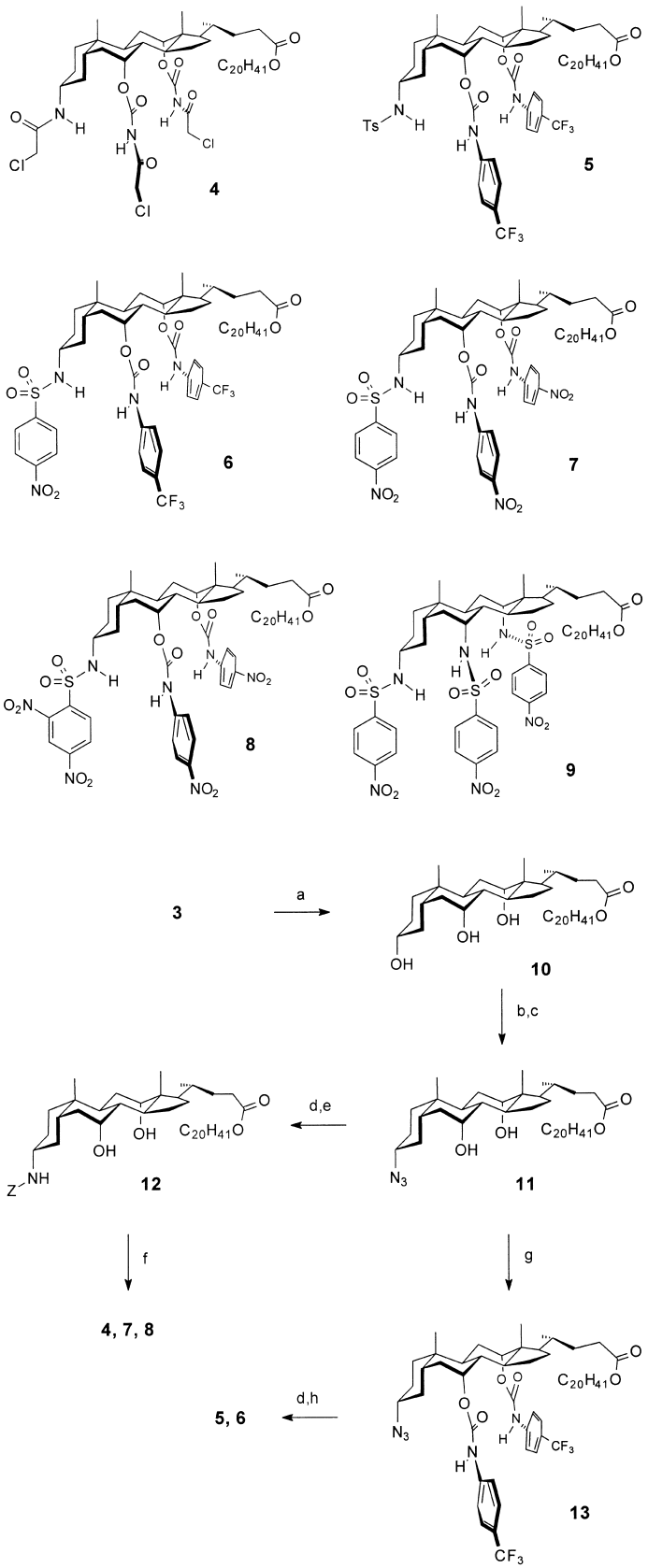
K_a may therefore be determined provided $[HX^-Y^+]_{org}/[H]_{org}$ and K_d can be measured. The method is especially useful for powerful receptors, because quantitative complex formation (receptor saturation) can be avoided by a) using a relatively hydrophilic substrate, and b) adjusting the aqueous concentration of X^-Y^+ used for the extraction experiments. The assumption [implicit in Eq. (4)] that X^- and Y^+ are separate in water but tightly bound in the organic phase does, of course, need to be validated. However, as discussed below, the consistency of our results provides support in the present case.

Results and Discussion

With respect to our original designs **1** and **2**, the receptors **4**–**9** used for this study incorporated two modifications. Firstly, the methyl ester side chain was replaced by the highly lipophilic eicosyl ester, to restrict loss of receptor during extractions. Secondly, a range of moderately to strongly electron-withdrawing substituents were attached to the pendant NH groups, to increase hydrogen-bond donor potency and therefore affinity.

Receptors **4**–**8** were synthesized from **3** via eicosyl azidocholanoate **11**, as indicated in Scheme 1.[9] Two routes were used, depending on whether the carbamoyl groups in the product contained a reducible substituent. Receptor **9** was prepared from **14**[10] as shown in Scheme 2.

As substrates we chose tetraalkylammonium salts because of a) their widespread use in anion-binding measurements, b) their tunable lipophilicity, and c) ease of analysis by ^{1}H NMR spectroscopy. A preliminary survey indicated that $Et_4N^+Cl^-$ and $Et_4N^+Br^-$ would be suitable for measurements on relatively powerful anionophores.[11] Two methods were explored for the determination of K_d. The first (method A) involved the equilibration of fairly concentrated aqueous salt



Scheme 1. a) Cs_2CO_3, $MeOH/H_2O$ (10:1), evaporate then $C_{20}H_{41}Br$, NaI (cat.), DMF; b) Ph_3P, DEAD, $MeSO_3H$, Et_3N, THF; c) NaN_3, DMPU; d) Zn, AcOH; e) wash with aq. NaOH, then ZCl, CH_2Cl_2 or DCE, Et_3N [Z = $ClCH_2CO$, p-$O_2NC_6H_4SO_2$, o,p-$(O_2N)_2C_6H_4SO_2$]; f) R-NCO, CH_2Cl_2, Me_3SiCl cat. [R = $ClCH_2$, p-$O_2NC_6H_4$]; g) p-$F_3CC_6H_4NCO$, $(CHCl_2)_2$, Me_3SiCl cat., 60 °C; h) Z-Cl, CH_2Cl_2, Et_3N [Z = Ts, p-$O_2NC_6H_4SO_2$].

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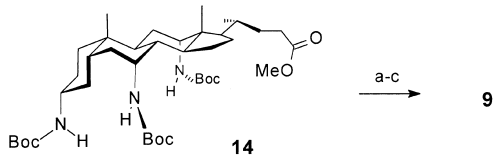


Scheme 2. a) CsOH, MeOH/H_2O (10:1), evaporate then $C_{20}H_{41}Br$, NaI (cat.), DMF; b) TFA, CH_2Cl_2; c) p-$O_2N(C_6H_4)SO_2Cl$, Et_3N, DMAP (cat.), CH_2Cl_2.

solutions with large volumes of chloroform (typically 500 mL), phase separation, evaporation of the organic phase and analysis of the dissolved salt by ^{1}H NMR after addition of an internal standard. The method proved difficult to operate reproducibly, probably due to the large concentration differences between the phases (typically, for $Et_4N^+Cl^-$, aqueous and organic concentrations of 0.5 M and ≈2 μM, respectively). However, values of K_d ($Et_4N^+Cl^-$) $= 1.16 \times 10^{-5}$ M^{-1}, and K_d ($Et_4N^+Br^-$) $= 2.69 \times 10^{-4}$ M^{-1}, were obtained by averaging over five and three experiments, respectively.

This second approach (method B) was less direct. Rearranging Equation (5), gives:

$$K_d = \frac{K_e}{K_a} \qquad (6)$$

In other words, measurement of both K_e and K_a for a receptor-substrate pair may be used to determine K_d for the substrate. K_a must be obtained by an independent method (e.g. NMR titration) under the conditions pertaining to the extraction experiment (water-saturated chloroform). Receptor **4** was employed as our "reference" anionophore. The least powerful of **4**–**9**, it proved amenable to study by ^{1}H NMR titrations; indeed, these measurements could be performed with unusual accuracy, as all three NH signals could be followed independently, analysed by curve fitting, and the results averaged. Binding constants K_a for **4**+$Et_4N^+Cl^-$ and $Et_4N^+Br^-$ in water-saturated $CDCl_3$ were found to be 16 500 M^{-1} (average of three titrations) and 8 400 M^{-1} (average of four titrations), respectively. When solutions of **4** in $CHCl_3$ were equilibrated with aqueous $Et_4N^+Cl^-$ and $Et_4N^+Br^-$, quantities of the salts were extracted into the organic phase as detailed in Table 1. For this rather weak receptor it was necessary to use quite high concentrations of aqueous substrate (0.3–0.5 M), resulting in significant amounts of unbound substrate in the organic phases. The straightforward application of Equation (6) was therefore not possible, as K_e could not be obtained directly from the measured ratio of substrate to receptor in the organic phase (R_m). However, algebraic manipulation yielded a more complex expression for K_d in terms of R_m and K_a (see Experimental Section), which was applied to give the figures in Table 1. Notably, the extractions of $Et_4N^+Br^-$ at three different aqueous concentrations gave closely similar results, while the averaged K_d values for this method are only slightly different from those obtained by method A. Both observations provide support for the model underlying the calculations [Eqs. (1)–(4)]. As method B seems the more reliable, we have adopted this second set of figures [K_d ($Et_4N^+Cl^-$) $= 1.27 \times 10^{-5}$ M^{-1}, and K_d ($Et_4N^+Br^-$) $= 2.18 \times 10^{-4}$ M^{-1}] as standards for K_a determinations by extraction.

Table 1. Extraction of $Et_4N^+Cl^-$ and $Et_4N^+Br^-$ from aqueous solution into $CHCl_3$ at 303 K by receptor **4**, and derived values for K_d.[a]

Substrate	[substrate]$_{aq}$ [M]	R_m[b]	K_d [M^{-1}][c]
$Et_4N^+Cl^-$	0.5	0.055	1.27×10^{-5}[d]
$Et_4N^+Br^-$	0.5	0.41	2.23×10^{-4}[e]
$Et_4N^+Br^-$	0.4	0.28	2.17×10^{-4}[e]
$Et_4N^+Br^-$	0.3	0.172	2.14×10^{-4}[e]

[a] Solutions of receptor **4** in $CHCl_3$ (5 mL, 0.0006 M) were equilibrated with aqueous substrate solutions at 303 K. The organic phases were separated, evaporated and analysed by ^{1}H NMR. For a detailed procedure, see Experimental Section. [b] Ratio [substrate]/[receptor] in organic phase, as measured by NMR integration. [c] Calculated from R_m and the NMR derived K_a values; see text and Experimental Section. [d] Average of two determinations, with results differing by < 2%. [e] Average $= 2.18 \times 10^{-4}$ M^{-1}.

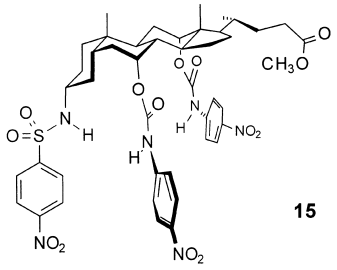
The results from extraction experiments performed with **5**–**9** on $Et_4N^+Cl^-$ and $Et_4N^+Br^-$ are summarised in Table 2. As expected,[12] the electron-withdrawing CF_3 and NO_2 groups do indeed cause significant increases in affinity. Receptor **7** is found to be ≈5000 times more potent than the prototypical sulfonamido-bis-carbamate **1**, despite the change from dry to wet medium.[13] Perhaps surprisingly, affinities peak at **7**. The additional nitro group in **8** may inhibit binding through intramolecular hydrogen bonding, while the nitro groups in tris-sulfonamide **9** seem to have a less dramatic effect than in the bis-carbamate series. The consistency of the three results quoted for **7**+$Et_4N^+Cl^-$, obtained using substantially differing substrate concentrations, serves to further validate the extraction method.

Table 2. Extraction of $Et_4N^+Cl^-$ and $Et_4N^+Br^-$ from aqueous solution into $CHCl_3$ at 303 K by receptors **5**–**9**.[a]

Receptor	$Et_4N^+Cl^-$				$Et_4N^+Br^-$			
	[substrate]$_{aq}$ [M]	K_e [M^{-2}]	K_a [M^{-1}][b]	$-\Delta G^0$ [kJ mol^{-1}]	[substrate]$_{aq}$ [M]	K_e [M^{-2}]	K_a [M^{-1}][c]	$-\Delta G^0$ [kJ mol^{-1}]
5	0.5	1.14	9×10^4	28.7	0.2	23.3	1.07×10^5	29.2
6	0.1	69.4	5.5×10^6	39.1	0.025	1500	6.9×10^6	39.7
7	0.0125	402	3.2×10^7	43.5	0.01	6250	2.9×10^7	43.3
7	0.033	401	3.2×10^7	43.5				
7	0.1	430	3.4×10^7	43.7				
8	0.05	93	7.3×10^6	39.8	0.01	1110	5.1×10^6	38.9
9	0.125	41.2	3.2×10^6	37.8	0.0625	239	1.1×10^6	35.0

[a] Receptor (0.01M) in $CHCl_3$ (1 mL) was vigorously stirred with aqueous substrate (1–8 mL) for 15 min. After separation of the phases the organic layer was passed through hydrophobic filter paper then evaporated. Analysis by ^{1}H NMR gave the ratio of substrate to receptor. After taking account of unbound substrate, K_e was calculated according to ref. [6]. [b] K_e/K_d, assuming $K_d = 1.27 \times 10^{-5}$ M^{-1}. [c] K_e/K_d, assuming $K_d = 2.18 \times 10^{-4}$ M^{-1}.

Finally, NMR and X-ray crystallographic studies supported our hypothesis that these receptors bind halide anions with 1:1 stoichiometry by H-bond donation. A ^{1}H NMR titration of **7** versus $Bu_4N^+Br^-$ yielded the expected downfield motions of the N*H* signals, linear with concentration until equivalence. Although we have not yet obtained crystals of a complex, the sulfonamido-bis-carbamate **15** (the methyl ester analogue of



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7) has been prepared, crystallised, and subjected to X-ray crystallography. As shown in Figure 1, the structure reveals a well-defined binding site occupied by a molecule of acetone solvent.[14] The NH groups converge, despite the presence of just one host–guest hydrogen bond, and may thus be preorganised for anion recognition.

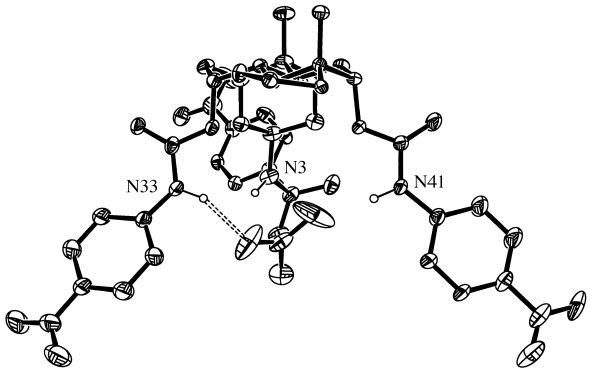

Figure 1. Structure of **15** in the crystal. Hydrogens bound to carbon, the steroidal side-chain (C20–C24; see **3** for numbering) and chloroform solvent are omitted for clarity. The structure is viewed down the long axis of the steroid nucleus, from the C20 end. The proposed anion-binding site is occupied by a molecule of acetone solvent, hydrogen-bonded to N33–H (steroidal C7–OCONH).

In conclusion, we have extended Cram's extraction procedure for measuring association constants, so that it can be applied to the binding of tetraethylammonium chloride and bromide in chloroform. The method is not restricted by an upper limit for K_a, and is therefore suitable for use with high-affinity receptors. Applied to a series of steroidal podand anionophores, it has served to illustrate the dramatic effect that electron-withdrawing groups can have on H-bond donor capabilities, and therefore on receptor potencies. In other work, we have shown that still higher binding constants can be measured for systems containing additional H-bond donor groups.[15] A future priority will be the extension of the method to other anions, so that the selectivities of a range of receptors may be examined under a standard set of conditions.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were run on Bruker DPX-400 or Jeol Eclipse 400 spectrometers using deuterated chloroform as solvent with tetramethylsilane as internal standard. Elemental analyses were carried out in the microanalytical laboratory, Department of Chemistry, University College Dublin. Melting points were recorded on a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 883 spectrophotometer. Thin-layer chromatography was carried out on aluminium-based Kieselgel 60 F_{254} 0.2 mm plates. Spots due to steroidal compounds were visualised by charring over a Bunsen burner. Cholic acid was obtained as a gift from Diamalt GmbH and used without further purification. Solvents were distilled before use and dried using standard techniques. Flash chromatography of reaction products was carried out using Kieselgel 60 (Merck) 400–230 mesh.

Eicosyl cholate (10): Solid cesium carbonate was added to a solution of cholic acid (20.20 g, 49.4 mmol) in methanol/water (10:1, 200 mL) until the pH was approximately 9 (determined using indicator paper). The solvent was removed in vacuo and re-evaporated with toluene (2×50 mL) and CH_2Cl_2 (2×50 mL). 1-Bromoeicosane (23.92 g, 66.2 mmol, 1.4 equiv) and sodium iodide (0.5 g) were added and the mixture was stirred in dry DMF (300 mL) at 47 °C under argon for one week. The mixture was dissolved in chloroform (250 mL) and washed with water (3×200 mL), dried ($MgSO_4$) and the solvent removed in vacuo. The crude product was re-evaporated with toluene (2×25 mL) and CH_2Cl_2 (2×25 mL) and purified by flash chromatography (hexane/ethyl acetate 1:1), then methanol/CH_2Cl_2 1:4) affording eicosyl cholate **10** as a slightly off-white solid (30.33 g, 44.0 mmol, 89%). $R_f=0.35$ (10% methanol/CH_2Cl_2); m.p. 75–77 °C; IR (film): $\tilde{\nu}_{max}=1738$ cm^{-1} (C=O); ^{1}H NMR (400.13 MHz): $\delta=0.70$ (s, 3H; 18-CH_3), 0.88 (t, $J=7.0$ Hz, 3H; eicosyl CH_3), 0.90 (s, 3H; 19-CH_3), 0.99 (d, $J=6.5$ Hz, 3H; 21-CH_3), 1.26 (m, ≈36H; eicosyl $C_{18}H_{36}$), 3.46 (br m, 1H; 3β-H), 3.85 (s, 1H; 7β-H), 3.98 (s, 1H; 12β-H), 4.05 (t, $J=6.5$ Hz, 2H; eicosyl OCH_2); ^{13}C NMR (100.61 MHz): $\delta=12.47$ (C-18), 14.07 (eicosyl CH_3), 17.31 (C-21), 22.45 (C-19), 22.65 (CH_2), 23.21(CH_2), 25.94 (CH_2), 26.42 (CH), 27.47(CH_2), 28.21 (CH_2), 28.67 (CH_2), 29.26 (CH_2), 29.33 (CH_2), 29.52 (CH_2), 29.58 (CH_2), 29.68 (CH_2), 30.44 (CH_2), 30.96 (CH_2), 31.36 (CH_2), 31.90 (CH_2), 34.69 (CH_2), 34.75, 35.22 (CH), 35.32 (CH_2), 39.57 (2C; CH_2, CH), 41.52 (CH), 41.69 (CH), 46.46, 47.09 (CH), 64.42 (eicosyl OCH_2), 68.44 (CH), 71.90 (CH), 73.05 (CH), 174.39 (C-24); HRMS (FAB): calcd for $C_{44}H_{80}O_5Na$: 711.5903 $[M+Na]^+$, found: 711.5924; elemental analysis calcd (%) for $C_{44}H_{80}O_5$ (689.10): C 76.69, H 11.70; found C 76.21, H 11.64.

Eicosyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate (11): Eicosyl cholate **10** (7.1 g, 10.3 mmol), triphenylphosphine (8 g, 30.5 mmol) methanesulfonic acid (1.4 mL, 21.8 mmol) and triethylamine (0.57 mL, 4.12 mmol) were dissolved in dry THF (50 mL). Diethyl azodicarboxylate (5.3 mL, 33.7 mmol) was added dropwise over 15 min while stirring at 40 °C under an atmosphere of argon. The mixture was evaporated and partially purified by flash chromatography on silica gel with ethyl acetate/chloroform (10:1) as the eluant yielding eicosyl 3β-methanesulfonyloxy-7α,12α-dihydroxy-5β-cholan-24-oate as a partially pure, low-melting solid (5.1 g, 65%). $R_f=0.60$ (ethyl acetate/hexane 1:1); ^{1}H NMR (400.13 MHz): $\delta=0.68$ (s, 3H; 18-CH_3), 0.87 (s, 3H; 19-CH_3), 0.96 (d, $J=6.0$ Hz, 3H; 21-CH_3), 1.25 (m, 36H; eicosyl CH_2), 2.63 (t, $J=15$ Hz, 1H; 4α-H), 3.85 (m, 1H; 7β-H), 3.97 (m, 1H; 12β-H), 4.04 (t, $J=6.5$ Hz, 2H; OCH_2), 4.96 (m, 1H; 3β-H). The foregoing material was dissolved in *N*,*N*′-dimethyl-*N*,*N*′-propylene urea (DMPU) (28 mL) and sodium azide (5 g, 77 mmol) was added. The mixture was allowed to stir at 40 °C for 16 h then poured into cold water (100 mL) and extracted with chloroform (60 mL). The extract was dried over $MgSO_4$ and evaporated. Column chromatography on silica gel with chloroform/hexane/ethyl acetate (1:1:1) as eluant yielded the azide **11** as an amorphous solid (4.18 g, 90%, 59% from **10**). $R_f=0.71$ (ethyl acetate/hexane 1:1); ^{1}H NMR (400.13 MHz): $\delta=0.69$ (s, 3H; 18-CH_3), 0.86 (t, $J=7.0$ Hz, 3H; eicosyl CH_3), 0.89 (s, 3H; 19-CH_3), 0.98 (d, $J=6.5$ Hz, 3H; 21-CH_3), 1.26 (m, 36H; eicosyl CH_2), 3.14 (m, 1H; 3β-H), 3.87 (m, 1H; 7β-H), 4.01 (m, 1H; 12β-H), 4.06 (t, $J=6.5$ Hz, 2H; OCH_2); ^{13}C NMR (100.13 MHz): $\delta=$ 11.90, 13.60, 16.80, 21.98, 22.20, 22.80, 25.48, 25.92, 26.40, 27.14, 27.58, 28.19, 28.80, 28.87, 29.22, 30.36, 30.76, 31.44, 34.32, 34.87, 34.94, 35.08, 38.83, 41.33, 41.49, 46.08, 46.72, 60.84, 63.96, 67.85, 72.73, 174.06 (C24); elemental analysis calcd (%) for $C_{44}H_{79}N_3O_4$ (714.12): C 74.01, H 11.15, N 5.88; found C 74.09, H 10.91, N 5.75.

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Eicosyl 3α-(chloroacetylamido)-7α,12α-bis(chloroacetylaminocarbonyloxy)-5β-cholan-24-oate (4): Zinc dust (2.6 g, 40 mmol) was added with vigorous stirring to the azido-diol **11** (2.55 g, 3.57 mmol) in glacial acetic acid (30 mL). After 8 h the mixture was filtered, the reaction solvent was removed by azeotropic distillation with toluene under reduced pressure, and the residue was redissolved in $CHCl_3$. The solution was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated. The white solid was redissolved in dry CH_2Cl_2 (30 mL) with chloroacetyl chloride (0.31 mL, 3.8 mmol) and triethylamine (0.505 g, 5 mmol). The mixture was allowed to stir for 2 h under an atmosphere of argon at room temperature. The solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as eluant yielded **12** (Z = $ClCH_2O$) as an amorphous solid (2.47 g, 92 %). $R_f = 0.28$ (ethyl acetate/hexane 1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 1740 cm^{-1} (C=O); ^1H NMR (400.13 MHz): $\delta = 0.68$ (s, 3H; 18-CH_3), 0.88 (t, J = 7.0 Hz, 3H; eicosyl CH_3), 0.90 (s, 3H; 19-CH_3), 0.96 (d, $J = 6.5$ Hz, 3H; 21-CH_3), 1.28 (m, 36H; eicosyl CH_2), 2.82 (br s, 2H; 2 × OH), 3.65 (m, 1H; 3β-H), 3.84 (m, 1H; 7β-H), 4.00 (m, 1H; 12β-H), 4.02 (t, $J = 6.5$ Hz, 2H; OCH_2), 4.03 (s, 2H; CH_2Cl), 6.48 (d, $J = 6.0$ Hz, 1H; 3α-NH); ^{13}C NMR (100.13 MHz): δ = 12.42, 13.98, 17.23, 22.44, 22.57, 23.10, 25.85, 26.52, 27.45, 28.29, 28.57, 29.16, 29.23, 29.44, 29.48, 29.58, 30.78, 31.24, 31.80, 34.44, 34.51, 35.20, 35.58, 35.96, 39.31, 41.67, 41.90, 42.60, 46.48, 47.21, 49.96, 64.39, 68.26, 73.07, 164.96, 174.32 (C24). To the foregoing material (1.0 g, 1.4 mmol) in dry 1,2-dichloroethane (DCE) (20 mL) was added chloroacetyl isocyanate (0.24 mL, 2.8 mmol) and chlorotrimethylsilane (0.183 mL, 1.44 mmol), under an atmosphere of argon. The solution was allowed to stir at 40 °C for 24 h. The solvent was removed and the product was purified by flash chromatography using hexane/ethyl acetate/chloroform (3:1:1) as eluant affording the bis-carbamate **4** as an amorphous solid (1.09 g, 82 %). R_f = 0.28 (ethyl acetate/hexane 1:3); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 1740 cm^{-1} (C=O); ^1H NMR (400.13 MHz): $\delta = 0.76$ (s, 3H; 18-CH_3), 0.88 (m, 6H; 21-CH_3, OCH_3), 0.97 (s, 3H; 19-CH_3), 1.26 (m, 36H; eicosyl CH_2), 3.65 (m, 1H; 3β-H), 3.40 (s, 2H; 3α-CH_2Cl), 4.04 (t, $J = 6.5$ Hz, 2H; OCH_2), 4.62 (s, 2H; CH_2Cl), 4.70 (s, 2H; CH_2Cl), 4.96 (m, 1H; 7β-H), 5.16 (m, 1H; 12β-H), 6.46 (d, $J = 6.0$ Hz, 1H; 3α-NH), 8.60 (s, 1H; carbamate NH), 9.27 (s, 1H; carbamate NH); ^{13}C NMR (100.13 MHz): δ = 11.37, 13.57, 17.20, 21.98, 21.15, 22.65, 24.51, 25.41, 26.65, 27.26, 28.14, 28.73, 28.82, 29.00, 29.16, 30.14, 30.56, 30.87, 31.39, 34.05, 35.10, 35.17, 37.78, 40.69, 41.67, 42.35, 44.03, 44.65, 46.87, 49.53, 52.99 (C3), 63.95, 74.00 (C7), 77.92 (C12), 150.46 (NHCO), 150.62 (NHCO), 164.53, 167.40, 168.24, 169.54, 173.45 (C24); FAB-MS: *m/z* (%): 1046 (17) $[M+2Na-H]^+$, 1024 (26) $[M+Na]^+$, 949 (50), 729 (100); HRMS (FAB): calcd for $C_{52}H_{85}Cl_3N_3O_9Na_2$ $[M+2Na-H]^+$: 1046.5147, found: 1046.5083.

Eicosyl 3α-(*p*-nitrobenzenesulfonylamido)-7α,12α-(*p*-nitrophenylaminocarbonyloxy)-5β-cholan-24-oate (7): Zinc dust (2.6 g, 40 mmol) was added with vigorous stirring to the azido-diol **11** (2.55 g, 3.57 mmol) in glacial acetic acid (30 mL). After 8 h the mixture was filtered, the reaction solvent was removed by azeotropic distillation with toluene under reduced pressure, and the residue was redissolved in $CHCl_3$. The solution was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated. The white solid was redissolved in dry CH_2Cl_2 (30 mL) with *p*-nitrobenzenesulfonyl chloride (0.84 g, 3.8 mmol) and triethylamine (0.505 g, 5 mmol), and allowed to stir for 2 h under an atmosphere of argon at 0 °C. The solution was allowed to warm to room temperature, washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated. Column chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as eluant yielded **12** (Z = *p*-$O_2NC_6H_4SO_2$) as an amorphous solid (2.47 g, 92 %). R_f = 0.28 (ethyl acetate/hexane/chloroform 1:1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$) = 1730 (C=O), 1380 (SO_2N), 1530 cm^{-1} (NO_2); ^1H NMR (400.13 MHz): $\delta = 0.67$ (s, 3H; 18-CH_3), 0.88 (m, 6H; 19-CH_3, eicosyl CH_3), 0.97 (d, $J = 6.0$ Hz, 3H; 21-CH_3), 1.28 (m, 36H; eicosyl CH_2), 2.74 (br s, 2H; 2 × OH), 3.00 (m, 1H; 3β-H), 3.84 (m, 1H; 7β-H), 4.00 (m, 1H; 12β-H), 4.05 (t, $J = 6.5$ Hz, 2H; OCH_2), 6.10 (d, $J = 6.0$ Hz, 1H; 3α-NH), 8.09 (d, $J = 9.0$ Hz, 2H; aryl H *ortho* to NO_2), 8.33 (d, $J = 9.0$ Hz, 2H; aryl H *meta* to NO_2); ^{13}C NMR (100.13 MHz): δ = 11.42, 13.05, 16.31, 21.43, 21.64, 22.11, 24.91, 25.50, 26.43, 27.09, 27.63, 27.81, 28.23, 28.30, 28.50, 28.55, 28.65, 28.72, 29.88, 30.28, 30.88, 33.40, 33.43, 34.14, 34.77, 36.44, 38.34, 70.87, 40.98, 45.40, 46.14, 53.36, 63.52, 67.32, 71.95, 123.25, 127.13, 146.63, 148.83, 173.42 (C24); HRMS (FAB): calcd for $C_{50}H_{84}N_2O_8S$: 872.5948 $[M]^+$, found: 872.5924. To the foregoing material (0.61 g, 0.66 mmol) in dry DCE (15 mL) was added *p*-nitrophenyl isocyanate (0.43 g, 2.65 mmol) and chlorotrimethylsilane (0.09 mL, 0.71 mmol). The solution was allowed to stir at 60 °C under an atmosphere of argon for 3 d. The reaction mixture was cooled to 0 °C and filtered to remove solid impurities. The filtrate was evaporated and the product purified by flash chromatography using hexane/ethyl acetate/chloroform (1:1:1) as eluant affording the bis-carbamate **7** as an amorphous solid (0.59 g, 75 %). R_f = 0.22 (ethyl acetate/hexane 1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$) = 1730 (C=O), 1380 (SO_2N), 1525 cm^{-1} (NO_2); ^1H NMR (400.13 MHz): $\delta = 0.56$ (m, 1H; 4α-H), 0.81 (s, 3H; 18-CH_3), 0.87 (m, 6H; 19-CH_3, OCH_3), 0.90 (d, $J = 6.0$ Hz, 3H; 21-CH_3), 1.27 (m, 36H; eicosyl CH_2), 2.93 (m, 1H; 3β-H), 3.98 (t, $J = 6.5$ Hz, 2H; OCH_2), 5.03 (m, 1H; 7β-H), 5.17 (m, 1H; 12β-H), 5.43 (br s, 1H; 3α-NH), 7.45 (d, $J = 9.0$ Hz, 2H; carbamato aryl H *ortho* to NO_2), 7.61 (d, $J = 9.0$ Hz, 2H; carbamato aryl H *meta* to NO_2), 7.81 (br s, 1H; carbamate NH), 7.97 (m, 5H; aryl H, carbamate NH), 8.00 (d, $J = 9.0$ Hz, 2H; aryl H), 8.14 (d, $J = 9.0$ Hz, 2H; sulfonamido aryl H *ortho* to NO_2); ^{13}C NMR (100.13 MHz): δ = 11.66, 13.61, 13.71, 17.11, 22.01, 22.19, 25.41, 26.88, 28.11, 28.73, 28.87, 29.01, 29.10, 29.20, 30.25, 30.80, 30.91, 31.43, 33.78, 34.23, 37.55, 41.10, 42.44, 45.00, 47.10, 54.10, 64.03, 72.18, 112.88, 117.00, 124.05, 124.39, 125.90, 127.36, 141.35, 141.62, 144.78, 146.10, 149.52, 151.77, 152.17, 173.70; HRMS (FAB): calcd for $C_{64}H_{92}N_6O_{14}S$: 1200.6392 $[M]^+$, found: 1200.6392.

Eicosyl 3α-(2,4-dinitrobenzenesulfonylamido)-7α,12α-(*p*-nitrophenylamino-carbonyloxy)-5β-cholan-24-oate (8): Zinc dust (0.53 g) was added while vigorously stirring to the azido-diol **11** (0.5 g, 0.72 mmol) in glacial acetic acid (10 mL). After 12 h the mixture was filtered, the reaction solvent was removed by forming an azeotrope with toluene and the solid was redissolved in $CHCl_3$. The liquor was washed with aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was redissolved in dry CH_2Cl_2 (60 mL) at 0 °C with 2,4-dinitrobenzenesulfonyl chloride (0.175 g, 0.75 mmol), and triethylamine (0.2 mL, 1.44 mmol) and the reaction was allowed to stir for 2 h under an atmosphere of nitrogen. The solution was then allowed to warm to room temperature. The solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate (2:1) as eluant yielded **12** (Z = o,p-$(O_2N)_2C_6H_3SO_2$) (0.39 g, 59 %). R_f = 0.28 (ethyl acetate/hexane 1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 1740 (C=O), 1380 cm^{-1} (SO_2N); ^1H NMR (400.13 MHz): $\delta = 0.70$ (s, 3H; 18-CH_3), 0.90 (m, 6H; 19-CH_3, eicosyl CH_3), 0.98 (s, 3H; 21-CH_3), 1.28 (m, 36H; eicosyl CH_2), 3.24 (m, 1H; 3β-H), 4.01 (m, 1H; 12β-H), 4.07 (t, $J = 6.5$ Hz, 2H; OCH_2), 5.47 (d, $J = 6.0$ Hz, 1H; 3α-NH), 8.38 (d, $J = 9.0$ Hz, 1H; aryl H), 8.54 (d, $J = 9.0$ Hz, 1H; aryl H), 8.68 (s, 1H; aryl H); ^{13}C NMR (100.13 MHz): δ = 12.05, 13.62, 16.85, 22.10, 22.20, 25.48, 28.21, 28.80, 28.87, 29.07, 29.12, 29.18, 29.22, 30.38, 31.45, 34.00 34.71, 38.99, 46.10, 46.88, 55.08, 64.05, 67.69, 72.49, 76.22, 76.54, 76.86, 120.22, 126.66, 131.66, 173.92 (C24). To the foregoing material (0.61 g, 0.66 mmol) in dry DCE (10 mL) was added *p*-nitrophenyl isocyanate (0.43 g, 2.65 mmol) and chlorotrimethylsilane (0.06 mL, 0.47 mmol). The mixture was allowed to stir at room temperature under an atmosphere of argon for 3 d then cooled to 0 °C and filtered to remove solid impurities. The filtrate was evaporated and the product was purified by flash chromatography using hexane/ethyl acetate/chloroform (1:1:1) as eluant, affording the bis-carbamate **8** as an amorphous solid (0.51 g, 72 %). R_f = 0.20 (ethyl acetate/hexane/chloroform 1:1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$) = 1725 (C=O), 1380 (SO_2N), 1530 cm^{-1} (NO_2); ^1H NMR (400.13 MHz): δ = 0.83 (s, 3H; 18-CH_3), 0.89 (m, 6H; 19-CH_3, eicosyl CH_3), 0.91 (d, J = 6.0 Hz, 3H; 21-CH_3), 1.26 (m, 36H; eicosyl CH_2), 3.32 (m, 1H; 3β-H), 3.97 (t, $J = 6.5$ Hz, 2H; OCH_2), 5.08 (m, 2H; 7β-H, 12β-H), 5.43 (br s, 1H; 3α-NH), 7.50 – 8.34 (m, 14H; aryl H, carbamate NH, carbamate NH); ^{13}C NMR (100.13 MHz): δ = 11.48, 13.60, 17.09, 22.06, 22.19, 25.40, 28.11, 28.74, 28.86, 29.01, 29.09, 29.20, 30.26, 30.81, 31.42, 34.21, 45.05, 63.97, 110.76, 117.03, 124.31, 130.30, 141.27, 145.02, 148.95, 151.84, 173.68, 177.14; HRMS (FAB): calcd for $C_{64}H_{91}N_7O_{16}S$: 1245.6243 $[M]^+$, found: 1245.6243.

Eicosyl 3α-azido-7α,12α-bis-*p*-(trifluoromethyl)phenylaminocarbonyloxy-5β-cholan-24-oate (13): (α,α,α-4-Trifluoromethyl)phenyl isocyanate (0.10 mL, 0.70 mmol, 2.5 equiv) and chlorotrimethylsilane (0.01 mL, 0.079 mmol) were added under argon t a solution of the azido diol **11** (200 mg, 0.28 mmol) in dry DCE (3 mL). The solution was stirred at 40 °C for 16 h. The solvent was removed in vacuo and the product was purified by flash chromatography (CH_2Cl_2/hexane 9:1) affording the azido dicarbamate **13** as a slightly off-white solid (246 mg, 0.23 mmol, 80 %). R_f = 0.6

(CH_2Cl_2/hexane 9:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 2150 (azide), 1740 cm^{-1} (C=O); ^1H NMR (400.13 MHz): $\delta = 0.75$ (s, 3H; 18-CH_3), 0.90 (t, $J =$ 7.0 Hz, 3H; eicosyl CH_3), 0.94 (d, $J = 6.0$ Hz, 3H; 21-CH_3), 0.97 (s, 3H; 19-CH_3), 1.25 (m, 36H; eicosyl CH_2), 3.18 (m, 1H; 3β-H), 4.01 (t, $J =$ 6.5 Hz, 2H; OCH_2), 4.97 (m, 1H; 7β-H), 5.13 (m, 1H; 12β-H), 6.94 (br s, 1H; carbamate NH), 7.06 (br s, 1H; carbamate NH), 7.67 (s, 8H; aryl H); ^{13}C NMR (100.13 MHz): $\delta = 12.33$, 14.10, 17.60, 22.53, 22.68, 22.87, 25.84, 25.90, 26.83, 27.14, 28.61, 29.13, 29.22, 29.35, 29.49, 29.56, 29.68, 30.74, 31,19, 31.46, 31.91, 34.38, 34.50, 34.65, 35.04, 37.86, 41.08, 43.73, 45.44, 47.47, 61.20, 64.58, 72.38, 118.18, 118.20, 126.37, 141.07, 152.43, 152.68, 174.22; HRMS (FAB): calcd for $C_{60}H_{87}N_5O_6F_6Na$: 1110.6458 $[M+Na]^+$, found: 1110.6472.

Eicosyl 3α-(*p*-toluenesulfonylamino)-7α,12α-bis(*p*-trifluoromethyl)phenylaminocarbonyloxy-5β-cholan-24-oate (5): Zinc dust (1.07 g, 16 mmol) was added with vigorous stirring to the bis-carbamoyl steroid **13** (0.89 g, 0.87 mmol) in glacial acetic acid (16 mL). After 8 h the mixture was filtered, the reaction solvent was removed by azeotropic distillation with toluene under reduced pressure, and the residue was redissolved in $CHCl_3$. The solution was washed with saturated aqueous sodium hydrogencarbonate and water, dried over $MgSO_4$ and re-evaporated. The white solid was redissolved in dry CH_2Cl_2 (20 mL) with *p*-toluenesulfonyl chloride (0.167 g, 0.87 mmol) and triethylamine (0.24 mL, 1.74 mmol), and the reaction mixture was allowed to stir for 2 h under an atmosphere of argon. The solution was washed with aqueous HCl (2 M) and water, dried, and evaporated. Flash chromatography with hexane/ethyl acetate/chloroform (1:1:1) as eluant yielded the tosylamido steroid **5** as an amorphous solid (0.485 g, 67%). $R_f = 0.44$ (ethyl acetate/hexane 1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 3600–3200 (NH), 1720 (C=O), 1380 cm^{-1} (SO_2N); ^1H NMR (400.13 MHz): $\delta = -0.13$ (m, 1H), 0.69 (s, 3H; 18-CH_3), 0.76 (s, 3H; 19-CH_3), 0.89 (d, 3H; $J = 4.2$ Hz, 21-CH_3), 0.90 (t, $J = 7.0$ Hz, 3H; eicosyl CH_3), 1.28 (m, 36H; eicosyl CH_2), 2.45 (s, 3H; tosylamido CH_3), 2.62 (m, 1H; 3β-H), 4.02 (t, $J = 6.5$ Hz, 2H; OCH_2), 4.87 (m, 1H; 7β-H), 5.21 (m, 1H; 12β-H), 5.89 (d, 1H; $J = 6$ Hz, 3α-NH), 7.19 (d, $J = 9.0$ Hz, 2H; tosylamido aryl H *meta* to SO_2), 7.30 (d, $J = 8.0$ Hz, 2H; aryl H *ortho* to CF_3), 7.36 (d, $J = 8.0$ Hz, 2H; aryl H *meta* to CF_3), 7.51 (d, $J = 8.0$ Hz, 2H; aryl H *ortho* to CF_3), 7.69 (d, $J = 8.0$ Hz, 2H; aryl H *meta* to CF_3), 7.70 (s, 1H; carbamate NH), 7.80 (d, $J = 9.0$ Hz, 2H; tosylamido aryl H *ortho* to SO_2), 7.87 (s, 1H; carbamate NH); ^{13}C NMR (100.13 MHz): $\delta = 12.41$ (C18), 14.07, 17.63 (C21), 21.42 (tosylamido CH_3), 21.82 (C19), 22.66, 22.81, 25.88, 26.04, 27.24, 28.61, 29.21, 29.33, 29.47, 29.54, 29.67, 30.77, 31.11, 31.26, 31.90, 33.36, 34.70, 35.70, 37.68, 40.98, 43.37, 45.23, 47.49, 53.01 (C3), 64.47, 71.71 (C7), 75.80 (C12), 117.6, 118.21, 118.29, 125.86, 126.02, 126.5, 130.25, 137.18, 141.9, 144.80, 152.60 (NHCO), 152.79 (NHCO), 174.20, (C24); HRMS (FAB): calcd for $C_{67}H_{95}N_3O_8F_6S_1$: 1215.6744 $[M]^+$, found: 1215.6744.

Eicosyl 3α-(*p*-nitrobenzenesulfonylamino)-7α,12α-bis(*p*-trifluoromethyl)-phenylaminocarbonyloxy-5β-cholan-24-oate (6): Zinc dust (1.0 g, 15 mmol) was added with vigorous stirring to the bis-carbamoyl steroid **13** (0.74 g, 0.73 mmol) in glacial acetic acid (16 mL). After 8 h the mixture was filtered, the reaction solvent was removed by azeotropic distillation with toluene under reduced pressure, and the residue was redissolved in $CHCl_3$. The solution was washed with saturated aqueous sodium hydrogen carbonate and water, then dried over $MgSO_4$ and re-evaporated. The white solid was redissolved in dry CH_2Cl_2 (25 mL) with nitrobenzenesulfonyl chloride (0.193 g, 0.87 mmol) and triethylamine (0.24 mL, 1.74 mmol), and the reaction was allowed to stir for 2 h under an atmosphere of argon. The solution was washed with aqueous HCl (2 M) and water, dried (Na_2SO_4), and the solvent was removed. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as eluant yielded the nosylamido steroid **6** as an amorphous solid (0.55 g, 65%). $R_f = 0.30$ (ethyl acetate/hexane 1:1); IR: $\tilde{\nu}_{max}$ (film from $CDCl_3$): 3600–3200 (NH), 1740 (C=O), 1540 (NO_2), 1380 cm^{-1} (SO_2N); ^1H NMR (400.13 MHz): $\delta = 0.10$ (m, 1H; 4α-H), 0.73 (s, 3H; 18-CH_3), 0.77 (s, 3H; 19-CH_3), 0.88 (d, $J = 6.0$ Hz, 3H; 21-CH_3), 0.90 (t, $J = 7.0$ Hz, 3H; eicosyl CH_3), 1.27 (m, 36H; eicosyl CH_2), 2.71 (m, 1H; 3β-H), 4.01 (t, $J = 6.5$ Hz, 2H; OCH_2), 4.91 (m, 1H; 7β-H), 5.18 (m, 1H; 12β-H), 5.89 (d, $J = 6.0$ Hz, 1H; 3α-NH), 7.34 (s, 1H; carbamate NH), 7.42 (m, 9H; carbamato aryl H, carbamate NH), 8.04 (d, 2H; $J = 8.8$ Hz, nosylamido aryl H), 8.28 (d, 2H; $J = 8.8$ Hz, nosylamido aryl H); ^{13}C NMR (100.13 MHz): $\delta = 13.60$ (C18), 17.16 (C21), 21.47, 22.20, 22.38, 25.42, 28.19, 28.75, 28.87, 29.02, 29.21, 30.30, 30.72, 31.44, 33.09, 34.19, 37.27, 42.91, 44.81, 46.98, 53.63 (OCH_2), 64.08 (C3), 71.57 (C7), 75.88 (C12), 117.41, 117.61, 124.84, 125.69, 127.24, 141.10, 144.13, 145.33, 150.06, 151.91 (NHCO), 152.17 (NHCO), 173.71 (C24); FAB-MS: m/z (%): 1269.5 (100) $[M+Na]^+$, 1064 (15), 837 (30), 635 (20), 455 (10); HRMS (FAB): calcd for $C_{66}H_{92}N_4O_{10}F_6S_1Na$: 1269.6336 $[M+Na]^+$, found: 1269.6366.

Extraction of tetraethylammonium chloride/bromide from aqueous solution into chloroform by receptor 4: Solutions of receptor **4** in water-saturated chloroform (0.6 mM, 5 mL), and tetraethylammonium halide in water (0.3–0.5 M, 1 mL), were sealed in a sample tube with a small magnetic stirring bar. The tube was placed in a water bath held at 30 °C, and the contents were stirred vigorously for 15 minutes. The two phases were allowed to separate. The majority of the aqueous phase was then removed with a pipette and the remaining mixture was filtered through Whatman 1PS hydrophobic filter paper to remove any excess undissolved aqueous phase. Solvent was then evaporated from the filtrate under reduced pressure. The resulting glass-like solid was redissolved in deuterated chloroform and an NMR spectrum was recorded. Integration of the tetraethylammonium versus receptor protons gave the ratio of substrate/receptor present in the organic phase (R_m). The distribution constant K_d for the tetraethylammonium halide was obtained as follows. In their analysis of the extraction protocol,[6] Cram et al. define the quantity R as $[HX^-Y^+]_{org}/[H_i]_{org}$, where $[H_i]_{org}$ is the initial concentration of the receptor in the organic phase. As R_m contains contributions from both bound and unbound substrate, R_m and R are related by Equation (7).

$$R = R_m - \frac{K_d\,[X^-Y^+]^2_{aq}}{[H_i]_{org}} \tag{7}$$

It is shown by Cram et al. that:

$$K_e = \frac{R}{(1-R)\,[X^-Y^+]^2_{aq}} \tag{8}$$

Combining Equations (8) and (5), and rearranging:

$$R = K_d K_a (1-R)\,[X^-Y^+]^2_{aq} \tag{9}$$

where R and K_a are independent measurements made on the same receptor. Substituting Equation (7) into (9):

$$R_m - \frac{K_d[X^-Y^+]^2_{aq}}{[H_i]_{org}} = K_d K_a\,(1 - R_m + \frac{K_d[X^-Y^+]^2_{aq}}{[H_i]_{org}})[X^-Y^+]^2_{aq} \tag{10}$$

Rearranging Equation (10) gives (11), a quadratic equation which can be solved to give K_d.

$$(K_a[X^-Y^+]^4_{aq})K_d^2 + (K_a[X^-Y^+]^2_{aq}[H_i]_{org} - K_a[X^-Y^+]^2_{aq}[H_i]_{org}R_m + [X^-Y^+]^2_{aq})\,K_d - R_m[H_i]_{org} = 0 \tag{11}$$

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[14] $0.10 \times 0.35 \times 0.40$ mm crystal, orthorhombic, obtained from acetone/chloroform as a solvate containing $1 \times$ acetone and $0.5 \times$ chloroform per molecule of **14**. Space group $P22_12_1$ (no. 18), $a = 13.8288(5)$, $b = 14.6236(4)$, $c = 25.6859(9)$ Å, $V = 5194.4(3)$ Å^3, $\rho_{calcd} = 1.346\,Mg\,m^{-3}$, $2\theta_{max} = 27.98^\circ$, $\lambda(Mo_{K\alpha}) = 0.71073$ Å, $T = 173.0 \pm 0.1$ K. 29113 Reflections collected, 12366 of which are independent; 6330 [$I > 2\sigma(I)$] reflections were used for refinement. Reflections were corrected for Lorentz polarisation effects, absorption correction was not applied [$\mu(Mo_{K\alpha}) = 0.212\,mm^{-1}$, max. transmission 97.91% and min. transmission 92.01%]. The structure was solved by direct methods [SHELXS-97, G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467] and refined on F^2 by full-matrix least-squares techniques [SHELXL-97, G. M. Sheldrick, SHELXL-97, A Program for Crystal Structure Refinement, University of Göttingen (Germany), **1997**]. 662 parameters, hydrogen atoms were calculated to their idealised positions and refined as riding atoms (temperature factor 1.2 or 1.5 times the carbon temperature factor). $R = 0.0917$ and $wR = 0.2085$ for data $I > 2\sigma(I)$ and $R = 0.1867$ and $wR = 0.2557$ for all data. The final difference map displayed no electron density higher than $0.90\,e\,Å^{-3}$. CCDC-136298 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; (fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

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