PERKIN 1

Steroidal guanidines as enantioselective receptors for *N*-acyl α-amino acids. Part 1. 3α-Guanylated carbamates derived from cholic acid

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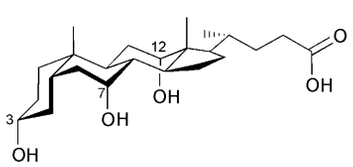
Receptors **5–11**, bearing guanidinium, carbamate and (in some cases) other functional groups, were synthesized from cholic acid **1**. These cations were shown to extract chiral carboxylate anions from aqueous buffer into chloroform with significant enantioselectivities. The most successful receptors bore two carbamate substituents and achieved, in the best cases, ratios (L:D) of 7–10:1 for a series of five *N*-acetyl α-amino acids.

Introduction

The design of enantioselective receptors is one of the central challenges of supramolecular chemistry.[1] Interest derives from the importance of enantioselective recognition in biology, and the practical need for enantiomerically pure compounds within the pharmaceutical industry. Amino acid derivatives[2] are relevant substrates on both counts. They are chiral units of fundamental biological importance, and are critical to pharmaceutical research (especially since the advent of combinatorial chemistry).

For practical purposes, the enantioselective *extraction* of amino acid derivatives is especially interesting,[3] as it raises the possibility of separation methods which are catalytic in receptor.[4] As part of our programme on receptors based on cholic acid **1**,[5] we were interested to use the chirality of the steroidal



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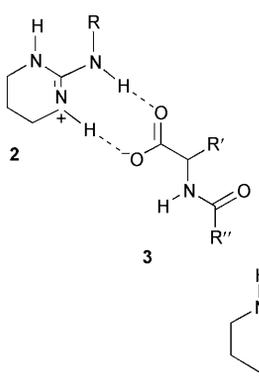
framework to achieve enantioselective phase transfer. We now give details of a family of receptors with useful selectivities in the case of *N*-acyl α-amino acids.[6]

Results and discussion

Receptor design

Phase transfer from aqueous into organic solvents is most easily achieved for ionic but fairly hydrophobic substrates. Pairing with a sufficiently lipophilic counterion virtually guarantees that extraction will take place. For selectivity, it is also useful if the ion pair is structured by specific, directional forces such as hydrogen bonding. We therefore chose the carboxylates **3** as substrates, and the guanidinium moiety **2** as the primary extraction/binding unit.[7] Although the related five-membered ring system was better known,[8] we preferred the six-membered **2** because of its greater stability and lipophilicity, and also because it should hold a substrate slightly closer to the remainder of the receptor (represented by R). By placing **2** on the lipophilic steroidal skeleton of **1**, we could ensure that the complexes would favour organic media, and by exploiting the functionality of **1** we could create highly asymmetric binding sites. For example, with the guanidinium at steroidal C-3, selective manipulations at C-7 and C-12 could give a range of molecules **4** in which groups A and B possess varying, and contrasting, recognition abilities.



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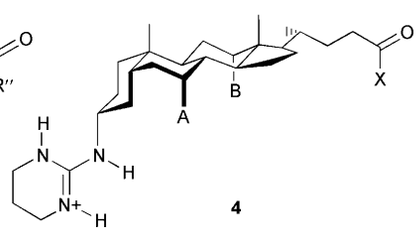

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At this early stage of our programme, we sought to demonstrate the concept and develop our methodology through simple variants of **4**, with synthetic considerations dominating the choice of A and B. Carbamates could be accessed by derivatisations with isocyanates, and had previously been shown to contribute to anion recognition.[5a] On this basis, guanidinium **5** was chosen as the initial target. As our studies progressed, we also prepared guanidinium **6**, for which it was hoped that the more acidic carbamoyl NH groups would increase affinities and selectivities (see below), and the series of "asymmetrically-substituted" receptors **7–11** (Fig. 1).

Receptor synthesis

The synthesis of receptor **5** is summarised in Scheme 1. The first step, conversion of **12** to **13**, employs a Mitsunobu reaction with methanesulfonic acid as nucleophile, followed by back

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Table 1 ^{1}H NMR chemical shifts (δ, $CDCl_3$) for **11**·*N*-Ac-DL-valinates, and controls

	Receptor carbamate N*H*	Receptor guanidinium N*H*	Substrate *H*NCHCO$_2^-$	Substrate HNC*H*CO$_2^-$	Substrate CH(C*H*$_3$)$_2$
11·Cl^-	8.82, 9.05	8.71, 6.4 (2 H, br)			
TBA·*N*-Ac-valinate[a]			6.70	4.00	0.81, 0.83
11·*N*-Ac-L-valinate[b]	9.64, 10.01	9.82, 11.33, 4.8	5.87	4.34	0.88, 0.25
11·*N*-Ac-D-valinate[c]			6.06	4.21	

[a] TBA ≡ tetrabutylammonium. [b] 100% Extraction of carboxylate with respect to receptor. [c] 55% Extraction of carboxylate with respect to receptor.

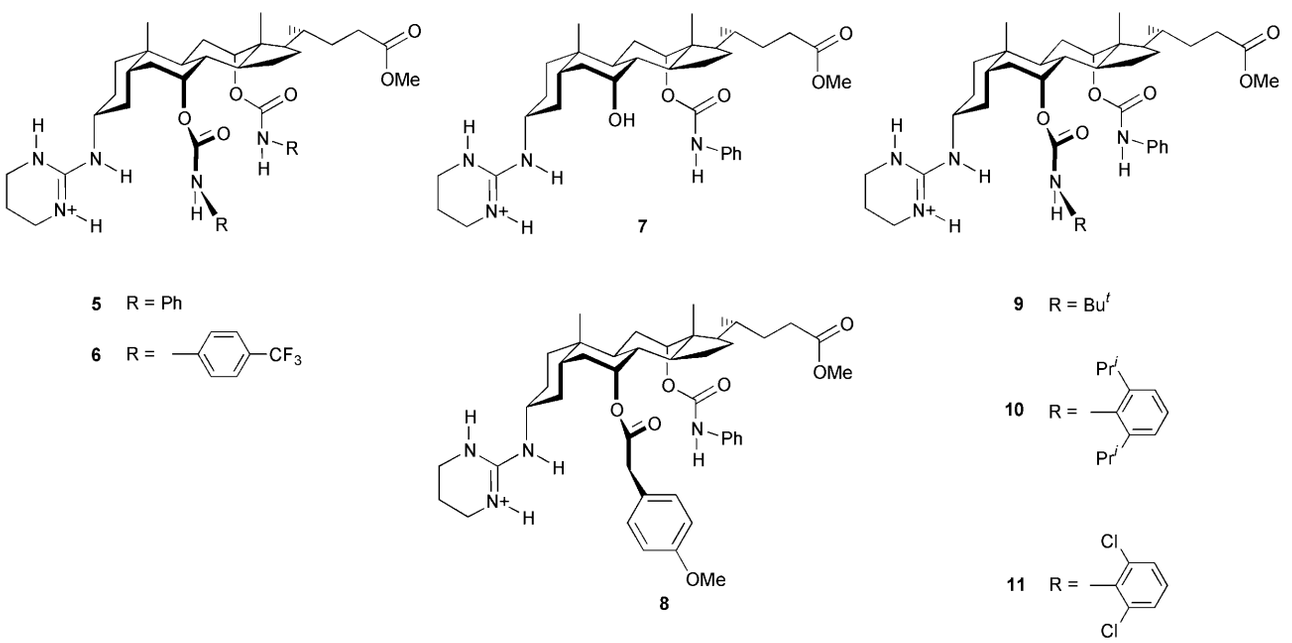
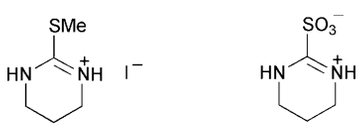


Fig. 1 Steroidal guanidinium receptors studied in this work.

displacement with azide. The reaction is selective for the less-hindered, equatorial 3α-OH. Our initial version of this method, reported in a preliminary communication,[9] employed just DEAD, Ph_3P and $MeSO_3H$ as reagents. However, subsequent experience has shown that this combination gives variable yields. Following the lead of Anderson *et al.*,[10] we have found that basic additives improve the reproducibility and scope[11] of the method. Of several bases tested, the most generally effective appears to be DMAP. Herein we report a new procedure employing this reagent, giving **13** from **12** reliably in >60% yield.

After reduction of the azido group in **13**, the guanidinium unit was introduced stepwise[12] *via* thiourea **14**. Although the yield for this sequence was disappointing (*ca.* 10% overall from **13**), we were unable to find a more workable procedure. Reagents **16**[13] and **17**,[14] though capable in principle of effecting



16 **17**

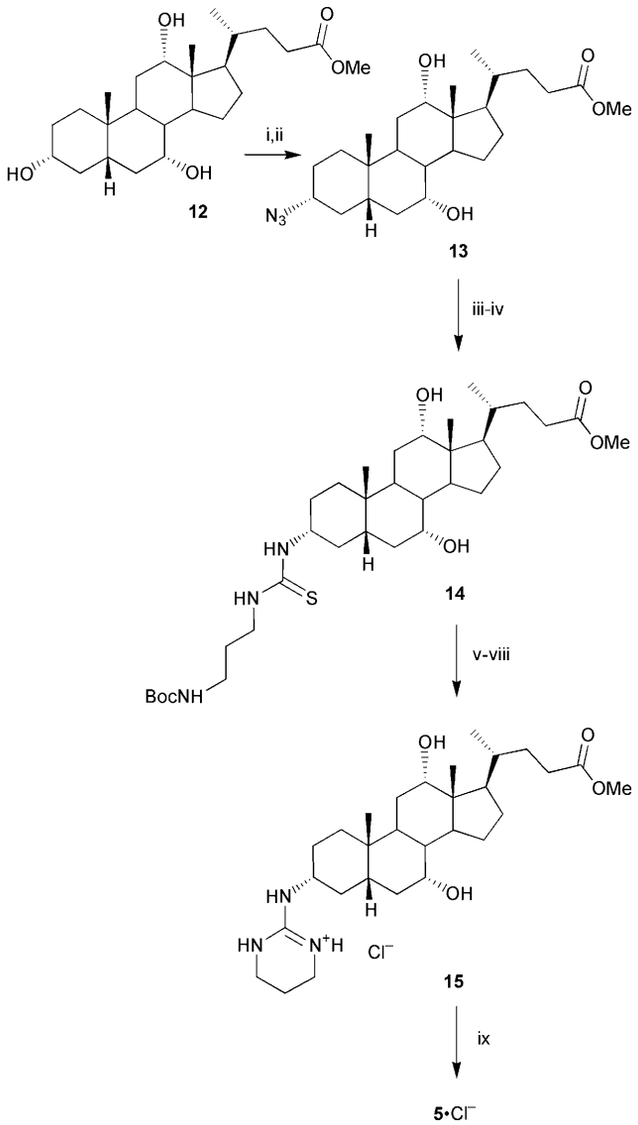
the guanylation in a single step, were found to react very slowly with cholanyl 3α-NH_2 groups. In the final step, guanidinium salt **15** was treated with phenyl isocyanate under acid catalysis to give **5**·Cl^- in 83% yield.

Receptor **6** was prepared using similar methodology, although in this case the carbamates were inserted before guanylation (Scheme 2).

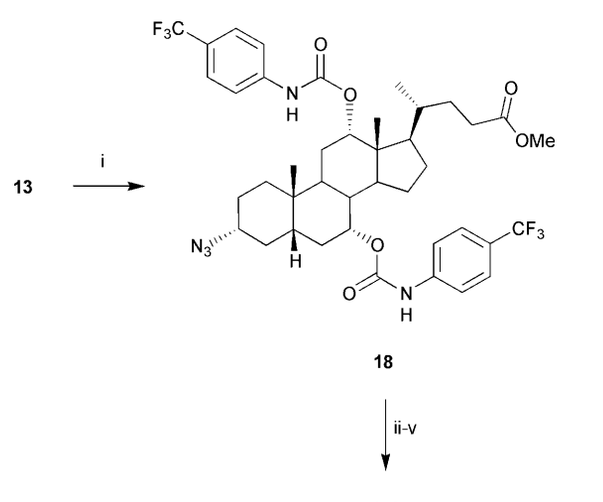
For the "asymmetrically substituted" receptors **7–11**, a longer sequence was necessary (Scheme 3). Acetate **19**, available *via* the classical two-step method of Fieser and Rajagopalan,[15] was converted to **20** by a conventional Mitsunobu reaction (63% yield), derivatised at C-12 with phenyl isocyanate (53% yield), then deacylated to diol **22** (65% yield). Selective mesylation, azide displacement, reduction and Boc-protection gave **23** (57% yield), then *N*-deprotection followed by guanylation (as above) gave **7**·Cl^- in 11% yield. Finally, receptors **8–11** were prepared by direct derivatisation of the C-7 hydroxy group. Acylation of **7**·Cl^- with 4-methoxyphenylacetyl chloride and Pr^i_2NEt gave ester **8**·Cl^- in 14% yield, while treatment with the corresponding isocyanates, with HCl as catalyst, gave carbamates **9**, **10** and **11** in yields of 63, 14 and 33% respectively.

Extraction studies

As expected, receptors **5–11** proved capable of extracting a range of *N*-acyl α-amino acids from aqueous phosphate buffer (pH = 7.4) into chloroform. NMR analysis of the extracts revealed substantial interactions between substrates and receptors. The effects are typified by the combination of receptor **11** with the enantiomers of *N*-acetyl valinate, illustrated in Table 1. Comparing the figures for **11**·Cl^- and **11**·*N*-Ac-L-valinate, it is apparent that all receptor N*H* signals bar one move downfield when the chloride ion is replaced by the carboxylate. This is consistent with a complex in which the substrate is bound through H-bond donation from both carbamates and two of the three guanidinium protons. Similar effects could be perceived for **11**·*N*-Ac-D-valinate, although in this case extensive broadening precluded detailed analysis. Comparing the complexes with the tetrabutylammonium salt of *N*-Ac-valinate, it is seen that the substrate N*H* signal moves *upfield* on complex formation. This suggests that it is less strongly hydrogen-bonded in the complex, possibly implying weakening of a strong intramolecular hydrogen bond to a carboxylate oxygen. The substrate α-C*H* resonance moves downfield on complex



Scheme 1 *Reagents and conditions*: i, DEAD, Ph_3P, DMAP, $MeSO_3H$, THF; ii, NaN_3, DMPU, 48 h; iii, Zn dust, AcOH, RT, 24 h; iv, $SCN(CH_2)_3NHBoc$, Pr^i_2NEt, CH_2Cl_2, RT, 72 h; v, MeI, MeOH, reflux; vi, TFA, CH_2Cl_2, RT; vii, Pr^i_2NEt, MeOH, RT, 24 h; viii, NaOH aq. then HCl aq.; ix, PhNCO, conc. HCl aq. (cat.), CH_2ClCH_2Cl, reflux, 72 h.



Scheme 2 *Reagents and conditions*: i, *p*-$F_3CC_6H_4NCO$, TMSCl (cat.), CH_2Cl_2, RT; ii, Zn dust, AcOH, RT, 1.5 h; iii, $SCN(CH_2)_3NHBoc$, Pr^i_2NEt, MeOH, RT, 72 h; iv, MeI, MeOH, reflux, then TFA, CH_2Cl_2, RT, then Pr^i_2NEt, MeOH; v, NaOH aq. then HCl aq.

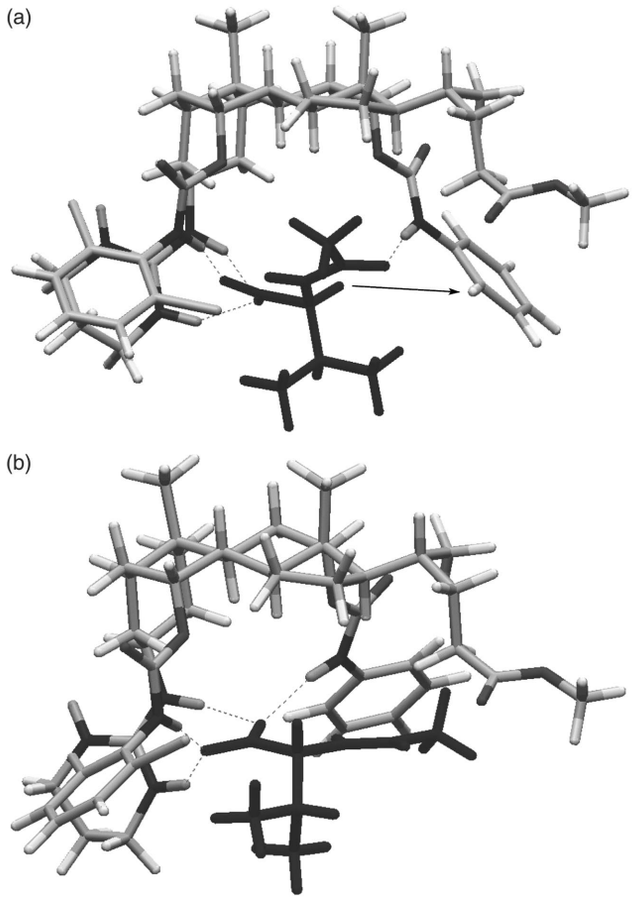


Fig. 2 Structures of (a) **11**·*N*-Ac-L-valinate and (b) **11**·*N*-Ac-D-valinate derived from computer-based molecular modelling. The substrate anions are coloured black, intermolecular hydrogen bonds are shown as broken lines. The arrow in (a) represents the observed intermolecular NOE.

formation and, specific to this case, a substantial shielding effect is observed for one of the valinate methyl groups. Finally, comparing the two diastereomeric complexes, there are significant differences between substrate N*H* and α-C*H* signals.

Further structural information was sought from NOE studies. One intermolecular effect was discernible for **11**·*N*-Ac-L-valinate, an enhancement of 1.07% in the *ortho* protons of **11**-NHPh on irradiation of the α-CH in *N*-Ac-L-valinate (Fig. 2a).

Extended Monte Carlo Molecular Mechanics (MCMM) searches were also undertaken on the above complexes. Although the calculations were not influenced by the NMR data (see Experimental section), they yielded structures which were consistent with the spectroscopic information. The results are shown in Fig. 2. In both cases, the carbamate and two guanidinium NH groups in the receptor were found to act as H-bond donors, while the substrate NH makes an intramolecular H-bond with a carboxylate oxygen. The major difference between the two structures is that the 12-carbamoyl NH of **11** is bound to the *acetyl* oxygen of the L-valinate, but a *carboxylate* oxygen of the D-valinate. Although the protons linked by the intermolecular NOE are ~3.8 Å from each other (Fig. 2a), they can be brought to within 2.2 Å by rotation about the N–Ph bond. The valyl methyls in Fig. 2a are close to the aromatic rings of the receptor, and are in quite different environments; hence it is not surprising that one is shifted upfield in the NMR spectrum of the complex. The calculations predict a slightly lower energy for the L complex, in keeping with the enantioselectivities discussed below.

The separation of signals for L- and D-substrates was also observed when extractions were performed on racemic mixtures of the *N*-acyl α-amino acids. This allowed the measurement of

Table 2 Extraction of *N*-acyl α-amino acids by "symmetrical" biscarbamate receptors **5** and **6**

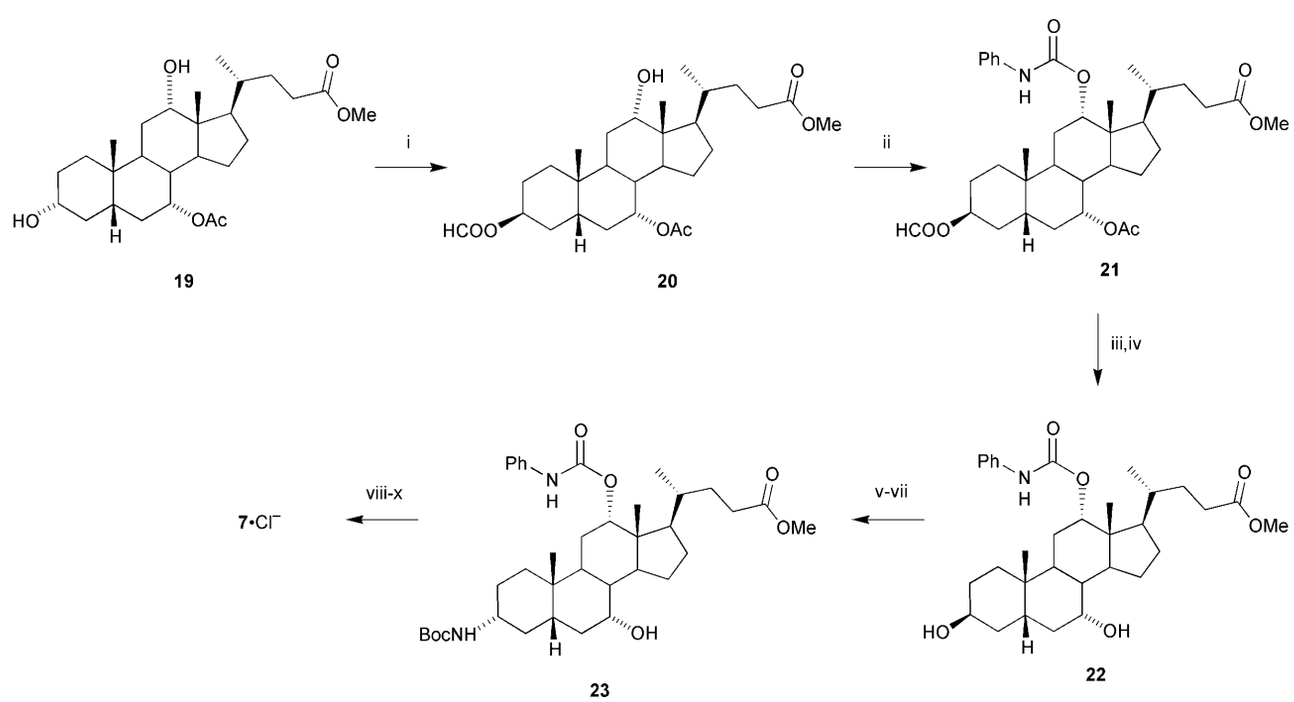
Receptor	Substrate	Extraction efficiency (%)[a]	Enantioselectivity (L:D)[b]
5	*N*-Ac-DL-alanine	52	7:1
5	*N*-Ac-DL-phenylalanine	87	7:1
5	*N*-Ac-DL-valine	71	7:1
5	*N*-Ac-DL-tryptophan	83	7:1
5	*N*-Ac-DL-asparagine	~0	—
5	*N*-*t*-Boc-DL-valine	98	1:1
5	*N*-*t*-Boc-DL-serine	92	3:1
5	*N*-*t*-Boc-DL-histidine	66	3.5:1
6	*N*-Ac-DL-alanine	41	10:1
6	*N*-Ac-DL-valine	63	9:1
6	*N*-Ac-DL-phenylalanine	90	9:1
6	*N*-Ac-DL-tryptophan	83	9:1
6	*N*-Ac-DL-methionine	74	7:1

[a] Concentration of substrate as percentage of receptor concentration, determined by NMR integration. [b] Determined by NMR integration of signals for complexed substrates in extracts. Signals were assigned through comparison with diastereomerically pure complexes.

Table 3 Extraction of *N*-acyl α-amino acids by hydroxy- and acyloxycarbamate receptors **7** and **8**

Receptor	Substrate	Extraction efficiency (%)[a]	Enantioselectivity (L:D)[b]
7	*N*-Ac-DL-alanine	~0	—
7	*N*-Ac-DL-valine	35	2:1
7	*N*-Ac-DL-tryptophan	72	1:1
7	*N*-*t*-Boc-DL-histidine	35	1:1
8	*N*-Ac-DL-valine	76	1:1
8	*N*-*t*-Boc-DL-histidine	72	2:1

[a] Concentration of substrate as percentage of receptor concentration, determined by NMR integration. [b] Determined by NMR integration of signals for complexed substrates in extracts. Signals were assigned through comparison with diastereomerically pure complexes.



Scheme 3 *Reagents and conditions*: i, DEAD, Ph_3P, HCO_2H, THF; ii, PhNCO, TMSCl (cat.), CH_2Cl_2; iii, $NaHCO_3$, MeOH; iv, NaOMe, MeOH, then H_2SO_4 (cat.), MeOH; v, MsCl, Et_3N, CH_2Cl_2; vi, NaN_3, DMPU; vii, Zn, AcOH, then $(Boc)_2O$, THF, $NaHCO_3$ aq.; viii, TFA, CH_2Cl_2, then $SCN(CH_2)_3NHBoc$, Pr^i_2NEt, MeOH; ix, MeI, MeOH, reflux, then TFA, CH_2Cl_2, RT, then Pr^i_2NEt, MeOH; x, NaOH aq. then HCl aq.

enantioselectivities through straightforward NMR integrations. Results from such experiments are summarised in Tables 2–5. All extractions were based on the typical procedure given in the Experimental section, unless otherwise indicated in Table footnotes. Control experiments established that no significant extraction of substrates took place in the absence of receptor.

Initial experiments involving bis(phenylcarbamate) **5** showed that good enantioselectivities are indeed possible with this system. Several *N*-acetyl α-amino acids were extracted with remarkably consistent L:D ratios of 7:1 (Table 2). Extraction efficiencies varied according to the lipophilicities of the substrates, as expected. The polar asparagine derivative was not extracted to a measurable extent. In the case of valine, replacing acetyl with Boc raised extraction levels but extinguished selectivity. However, the more polar *N*-Boc derivatives of serine and histidine were extracted with appreciable L:D ratios of ~3:1.

Enhanced receptor power may also lead to improved selectivity; as binding free energies increase, differences between them

Table 4 Extraction of *N*-acyl α-amino acids by "unsymmetrical" biscarbamate receptors **9–11**

Receptor	Substrate	Extraction efficiency (%)[a]	Enantioselectivity (L:D)[b]
9	*N*-Ac-DL-alanine	45	3:1
9	*N*-Ac-DL-valine	69	4:1
9	*N*-Ac-DL-tryptophan	89	4:1
9	*N*-*t*-Boc-DL-valine	88	3:2
9	*N*-*t*-Boc-DL-histidine	61	3:1
10	*N*-Ac-DL-phenylalanine	53	3.5:1
11	*N*-Ac-DL-alanine	76	6:1
11	*N*-Ac-DL-phenylalanine	93	9:1
11	*N*-Ac-DL-valine	89	9:1
11	*N*-Ac-DL-tryptophan	92	6:1
11	*N*-Ac-DL-methionine	93	9:1
11	*N*-Ac-DL-proline	74	4:1
11	*N*-Ac-DL-*tert*-leucine	82	2.5:1
11	*N*-*t*-Boc-DL-valine	76	1:1
11	*N*-*t*-Boc-DL-histidine	46	4:1

[a] Concentration of substrate as percentage of receptor concentration, determined by NMR integration. [b] Determined by NMR integration of signals for complexed substrates in extracts. Signals were assigned through comparison with diastereomerically pure complexes.

Table 5 Extraction of α-amino acids and Naproxen by **11**

Receptor	Substrate	Extraction efficiency (%)[a]	Enantioselectivity (L:D)[b]
11	DL-valine[c]	~0	—
11	DL-phenylalanine[c]	35	2:1
11	DL-tryptophan[c]	20	2:1
11	*RS*-Naproxen[c] **24**	98	1.5:1 (S:R)

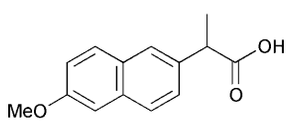
[a] Concentration of substrate as percentage of receptor concentration, determined by NMR integration. [b] Determined by NMR integration of signals for complexed substrates in extracts. Signals were assigned through comparison with diastereomerically pure complexes, or by re-equilibration of extracts with non-racemic substrates. [c] Substrates for extractions dissolved in aqueous borate buffer (pH = 9).

may also increase. Receptor **6** was designed to explore this possibility. Parallel work on receptors for inorganic anions had shown that the *p*-trifluoromethylphenyl substituents can increase potencies for halide ions by factors of >20.[16] In the event, receptor **6** did not show greater extraction ability than **5**, perhaps because its affinity for chloride was also increased. However, it did prove to be marginally more enantioselective, extracting several *N*-acetyl amino acids with selectivities of 9–10:1 (Table 2).

Receptors **7** and **8** possess more obviously asymmetric binding sites, and might therefore be expected to show greater enantioselectivities. Unfortunately, neither performed especially well with *N*-acyl amino acid derivatives, as illustrated in Table 3. One point of interest is that the two molecules showed complementary recognition properties, in the sense that **7** was modestly selective for *N*-Ac-DL-valine but unselective for *N*-*tert*-Boc-DL-histidine, while **8** was unselective for the former but somewhat selective for the latter.

Receptors **9–11** retain the dicarbamoylguanidinium binding site of **5** and **6**, but with increased asymmetry due to differing carbamoyl substituents. The hindered substituents in **9** and **10** yielded no dividend, but the dichlorophenyl group in **11** did give some useful and interesting effects (Table 4). Firstly, **11** showed a small but significant increase in extraction ability for *N*-acetyl amino acids (most obviously for *N*-Ac-DL-alanine; *cf.* 76% for **11** *vs.* 52% for **5**). Secondly, enantioselectivities were slightly higher than those for **5** in some cases.

Receptor **11** was also used to investigate the extraction of other classes of carboxylate. As shown in Table 5, positive results were obtained for underivatised phenylalanine and tryptophan, though not for the less lipophilic valine. For these experiments a more basic aqueous medium was used, to raise the concentration of amino acid carboxylate (presumably the form extracted). The basic medium was also used for Naproxen **24**, to reduce the background of unmediated extraction. Selectivities for these substrates were modest but significant.



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Conclusion

This research has shown that steroidal guanidinium cations derived from **1** can extract chiral carboxylates from aqueous phases with quite encouraging enantioselectivities. These receptors are just the first of many which could, in principle, be prepared from **1** and its relatives. By varying the nature and positions of the three recognition units, we can hope to develop systems with improved selectivities and altered scope. The enantioselective membrane transport of carboxylates is also a realistic proposition, and will be investigated in future work.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 or Bruker MSL-300 spectrometers and were referenced internally to the residual $CHCl_3$ (δ_H 7.26) and $CDCl_3$ (δ_C 77.0 central peak) or TMS (δ_H 0.00, δ_C 0.00) signals respectively. Coupling constants (*J* values) are given in Hz. Carbon spectra were assigned with the aid of DEPT 135° and DEPT 90° techniques. Two-dimensional correlated NMR spectroscopy was employed to verify the proton and carbon spectral assignments of important synthetic intermediates and final receptors. Melting points were determined using open capillary tubes in Griffin or Gallenkamp melting point blocks and are uncorrected. IR spectra were recorded using a Perkin-Elmer 883 or FT-IR Paragon 1000 spectrometers. Sample films were supported on sodium chloride plates. Analytical TLC was carried out on DC-Alufolien Kieselgel 60F_{254} 0.2 mm plates, and compounds

were visualised by UV fluorescence, ninhydrin spray and by charring over a Bunsen burner flame. Elemental analyses were carried out in the microanalytical laboratory, Department of Chemistry, University College Dublin. Mass spectra were recorded at the Universities of Bath or Birmingham. Cholic acid was obtained as a gift from Freedom Chemical Diamalt GmbH and used without further purification. Flash Chromatography of reaction products was carried out using Kieselgel 60 (Merck) 400–230 mesh. All reactions were performed at RT unless otherwise stated. When necessary, THF was distilled from $LiAlH_4$ then from sodium–benzophenone ketyl, both CH_2Cl_2 and CH_2ClCH_2Cl were distilled from calcium hydride, and MeOH was distilled from Mg turnings, immediately before use. Except where specified, all reagents were purchased from commercial sources and were used without further purification.

Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate 13

Methanesulfonic acid (2.26 mL, 34.8 mmol) was added to a solution of methyl cholate **12** (7.00 g, 16.6 mmol), triphenylphosphine (13.11 g, 50 mmol) and DMAP (4.25 g, 34.8 mmol) in dry THF (50 mL) under argon. Diethyl azodicarboxylate (7.9 mL, 50 mmol) was added dropwise over a 15 min period, after which the solution became cloudy and viscous. The mixture was stirred for 48 h. The solvent was evaporated under reduced pressure and the resulting residue partially purified by flash chromatography (200 g SiO_2) eluting with EtOAc–hexane–CH_2Cl_2 (1:4:0.5), yielding impure 3β-methanesulfonate as an off-white solid (7.2 g), R_f 0.57 (EtOAc–CH_2Cl_2–hexane, 7:0.03:1); δ_H(400 MHz; $CDCl_3$) 0.70 (3 H, s, 18-CH_3), 0.94 (3 H, s, 19-CH_3), 0.98 (3 H, d, *J* 6.6, 21-CH_3), 2.09–2.17 (1 H, m), 2.20–2.30 (1 H, m), 2.33–2.41 (1 H, m), 2.65 (1 H, m), 2.98 (3 H, s, OSO_2CH_3), 3.67 (3 H, s, CO_2CH_3), 3.86 (1 H, m, 7β-H), 3.99 (1 H, m, 12β-H), 4.97 (1 H, m, 3α-H). The crude methanesulfonate was dissolved in DMPU (50 mL) and sodium azide (5 g, 0.077 mol) was added. The mixture was stirred under dry conditions (calcium chloride tube) for 48 h and then poured into water (200 mL). The product was extracted into diethyl ether (100 mL) and washed with water (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. Purification by flash chromatography (150 g SiO_2) eluting with EtOAc–hexane (1:2) afforded *azide* **13** as a white solid (4.57 g, 62% from **12**). An analytical sample was obtained by recrystallisation from CH_2Cl_2–hexane, mp 109–110 °C (Found: C, 66.78; H, 9.09; N, 9.09. $C_{25}H_{41}N_3O_4$ requires C, 67.08; H, 9.23; N, 9.39%); R_f 0.73 (EtOAc–hexane, 2:3), 0.43 (EtOAc–hexane, 1:3); ν_{max}(Nujol)/cm^{-1} 3244, 2107, 1748 and 1172; δ_H(400 MHz; $CDCl_3$) 0.70 (3 H, s, 18-CH_3), 0.91 (3 H, s, 19-CH_3), 0.98 (3 H, d, *J* 6.0, 21-CH_3), 3.15 (1 H, br m, 3β-H), 3.67 (3 H, s, CO_2CH_3), 3.86 (1 H, m, 7β-*H*), 3.99 (1 H, m, 12β-H); δ_C(75.5 MHz; $CDCl_3$) 12.33 (C-18), 17.20 (C-21), 22.41 (C-19), 23.14 (CH_2), 26.31 (CH), 26.75 (CH_2), 27.46 (CH_2), 27.98 (CH_2), 30.71 (CH_2), 30.96 (CH_2), 34.60 (CH_2), 34.70 (C-10), 35.24 (CH_2), 35.30 (CH), 35.40 (CH_2), 39.19 (CH), 41.70 (CH), 41.77 (CH), 46.44 (C-13), 47.12 (CH), 51.42 (CO_2CH_3), 61.21 (C-3), 68.23 (C-7), 73.05 (C-12), 174.83 (C-24); *m*/*z* (FAB) 470.3 ([M + Na]$^+$, 96%), 427.3 (9), 420.3 (47), 402.3 (24), 384.3 (100), and 369.3 (39).

Methyl 3α-{[3-(*tert*-butoxycarbonylamino)propylamino]thioxomethylamino}-7α,12α-dihydroxy-5β-cholan-24-oate 14

Acetic acid (4.2 mL) was added to a mixture of zinc dust (826 mg, 12.64 mmol) and azido diol **13** (413 mg, 0.92 mmol). The reaction mixture was protected from the atmosphere by a calcium chloride tube and stirred vigorously for 24 h. The zinc residues were removed by filtration and washed with acetic acid (2 × 2 mL) and toluene (2 × 2 mL). The filtrate was evaporated under reduced pressure. Residual acetic acid was removed by repetitive addition, and evaporation under reduced pressure, of distilled toluene (2 × 1 mL) to give the corresponding alkylammonium acetate as a clear pale yellow oil [one major component by TLC, R_f 0.22 (CH_2Cl_2–MeOH, 5:1)]. 3-(*tert*-Butoxycarbonylamino)propyl isothiocyanate[17] (500 mg, 2.31 mmol) was added to a stirred solution of the alkylammonium acetate and *N*,*N*-diisopropylethylamine (482 μL, 2.77 mmol) in dry CH_2Cl_2 (10 mL) under an atmosphere of argon. The resulting pale yellow solution was stirred for 72 h. The solution was evaporated under reduced pressure, and the resulting oil purified by flash chromatography eluting with hexane–EtOAc (1:2), affording pure *thiourea* **14** (189 mg, 32%) as a white foam, mp 115–122 °C (Found: C, 62.52; H, 9.19; N, 6.38. $C_{34}H_{59}N_3O_6S{\cdot}H_2O$ requires C, 62.25; H, 9.38; N, 6.41%) (HRMS: Found: MH$^+$ 638.4212. $C_{34}H_{60}N_3O_6S$ requires *m*/*z*, 638.4203); R_f 0.61 (EtOAc); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3354, 2934, 2869, 1689, 1540, 1437, 1366, 1253, 1171, 1037, and 737; δ_H(300 MHz; $CDCl_3$) 0.68 (3 H, s, 18-CH_3), 0.90 (3 H, s, 19-CH_3), 0.98 (3 H, d, *J* 6.2, 21-CH_3), 1.42 [9 H, s, C(CH_3)$_3$], 3.17 (2 H, dt, *J* 5.7 and 6.3, CH_2NHCO), 3.56 (3 H, br m, 3β-H and CH_2NHCS), 3.66 (3 H, s, CO_2CH_3), 3.84 (1 H, m, 7β-H), 3.99 (1 H, m, 12β-H); 4.95 (1 H, br t, NHCO), 6.15 (1 H, d, *J* 7.2, 3α-NH), 6.96 (1 H, br s, NHCS); δ_C(75.5 MHz; $CDCl_3$) 12.50 (C-18), 17.32 (C-21), 22.58 (C-19), 23.13 (CH_2), 26.67, 27.43 (CH_2), 28.21 (CH_2), 28.34 [C(CH_3)$_3$], 29.77 (CH_2), 30.87 (CH_2), 31.06 (CH_2), 34.53 (C-10), 34.63 (CH_2), 35.19, 35.57 (CH_2), 36.19 (CH_2), 37.11 (CH_2), 39.41, 40.79 (CH_2), 41.65, 41.96, 46.38 (C-13), 47.06, 51.51 (CO_2CH_3), 53.87 (C-3), 68.17 (C-7), 72.96 (C-12), 79.58 [*C*(CH_3)$_3$], 156.97 (NHCO), 174.78 (C-24); *m*/*z* (FAB) 638.3 (M$^+$ + 1, 100%), 606.4 (22), 582.3 (21), 530.4 (23), 504.3 (7), 420.2 (7), 338.3 (7), 219.1 (18), 178.0 (28), 161.1 (12), 147.1 (22), 131.1 (17), 117.0 (40), 97.1 (40), and 75.1 (18). The above material was combined with less pure fractions for use in the following step.

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α,12α-dihydroxy-5β-cholan-24-oate chloride 15

Methyl iodide (84 μL, 1.35 mmol) was added to a stirred solution of crude thiourea **14** (purity *ca.* 70% as judged by ^{1}H NMR; 250 mg, 0.39 mmol) in dry MeOH (2.5 mL) under an atmosphere of argon. The solution was refluxed for 1.5 h while stirring, after which time analysis by TLC (EtOAc) revealed that no starting material remained. The solution was allowed to cool to room temperature, and then evaporated under reduced pressure. Under an atmosphere of argon, the resulting residue was dissolved in dry CH_2Cl_2 (2.5 mL), and 99% trifluoroacetic acid (0.83 mL, 10.77 mmol) was added while stirring. The resulting yellow suspension was stirred for 1 h, after which time analysis by TLC ($CHCl_3$–MeOH, 3:1) revealed that the amine deprotection had reached completion. The solution was evaporated under reduced pressure, and under an atmosphere of argon the resulting residue was dissolved in dry MeOH (6 mL). Over a period of 20 min this solution was added dropwise *via* a syringe to *N*,*N*-diisopropylethylamine (2.3 mL, 13.20 mmol) under an atmosphere of argon with vigorous stirring. The resulting faint pink solution was stirred for 24 h during which time thiol was evolved. At the end of the 24 h period analysis by TLC ($CHCl_3$–MeOH, 3:1) revealed one major component. The solution was evaporated under reduced pressure and the residue dissolved in CH_2Cl_2 (50 mL), and then washed with aq. HCl (2 M, 2 × 50 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with water (50 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue (135 mg) was purified by flash chromatography eluting with CH_2Cl_2–MeOH (20:1 graduating to 5:1) to afford the guanidinium salt (96 mg) which was pure by TLC. To ensure chloride was the counter anion, the salt was taken up in $CHCl_3$–MeOH (60 mL; 25:1) and washed carefully with aq. NaOH (2 M, 2 × 50 mL) followed by triply distilled water (50 mL). The organic layer was then washed with aq. HCl (2 M, 2 × 50 mL)

followed by triply distilled water (2 × 50 mL). The combined acidic aqueous layers were then extracted with CH_2Cl_2–MeOH (50 mL; 25:1); allowing each washing to settle for 30 min before isolating the organic layer. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue was further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **15** (55 mg, 26% from thiourea) as a white powder, mp 214–217 °C (Found: C, 62.22; H, 9.25; N, 7.26. $C_{29}H_{50}N_3O_4Cl{\cdot}H_2O$ requires C, 62.44; H, 9.40; N, 7.54%) (HRMS: Found: M^+, 504.3804. $C_{29}H_{50}N_3O_4$ requires *m*/*z*, 504.3801); R_f 0.35 (CH_2Cl_2–MeOH, 5:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3424, 2940, 1637, 1438, 1376, and 1199; δ_H(300 MHz; $CDCl_3$) 0.64 (3 H, s, 18-CH_3), 0.85 (3 H, s, 19-CH_3), 0.92 (3 H, d, *J* 5.9, 21-CH_3), 3.33 (4 H, br m, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.51 (1 H, br m, 3β-H), 3.65 (3 H, s, CO_2CH_3), 3.77 (1 H, m, 7β-H), 3.91 (1 H, m, 12β-H), 6.79 (1 H, d, *J* 7.9, 3α-NH), 7.83 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring); δ_C(75.5 MHz; $CDCl_3$) 12.47 (C-18), 17.29 (C-21), 20.26 (C-5 of the pyrimidinum ring), 22.21 (C-19), 23.16 (CH_2), 26.67, 27.18 (CH_2), 27.53 (CH_2), 27.93 (CH_2), 30.90 (CH_2), 31.06 (CH_2), 34.28 (CH_2), 34.53 (C-10), 35.24 (CH_2), 35.33, 36.02 (CH_2), 38.48 (C-4,6 of the pyrimidinium ring), 39.38, 41.34, 41.82, 46.50 (C-13), 46.89, 50.98 (C-3), 51.54 (CO_2CH_3), 68.18 (C-7), 73.59 (C-12), 151.89 (C-2 of the pyrimidinium ring), 174.75 (C-24); *m*/*z* (FAB) 504 (M^+, 100%), 486 (3), 154 (4), and 100 (18).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α,12α-bis(phenylaminocarbonyloxy)-5β-cholan-24-oate chloride 5·Cl$^-$

Aq. HCl (11.65 M, 5 μl) was added to a stirred solution of guanidinium chloride **15** (250 mg, 0.46 mmol) and phenyl isocyanate (150 μL, 1.38 mmol) in dry CH_2ClCH_2Cl (3.2 mL) under an atmosphere of argon. The resulting pale yellow solution was refluxed for 72 h, after which period the solution was allowed to cool to room temperature, and phenyl isocyanate (100 μL, 0.92 mmol) and aq. HCl (11.65 M, 10 μl) added portion wise with the solution being brought back to reflux after each addition. The solution was allowed to cool to room temperature, and then stirred in air for 2–3 h. The resulting creamy white suspension was evaporated under reduced pressure. The resulting residue was taken up in a minimum amount of CH_2Cl_2 and any undissolved material removed by filtration. The filtrate was purified by flash chromatography eluting with CH_2Cl_2–MeOH (50:1) to afford the guanidinium salt (336 mg) which was pure by TLC. To ensure chloride was the counter anion, the salt was taken up in CH_2Cl_2 (40 mL) and washed carefully with aq. NaOH (1 M, 2 × 20 mL) followed by triply distilled water (20 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 20 mL) followed by triply distilled water (2 × 20 mL). The organic layer was dried by decanting and evaporated under reduced pressure. Residual CH_2Cl_2 and MeOH from the flash chromatography were removed by repetitive addition and evaporation under reduced pressure of purified $CHCl_3$ (2 × 10 mL). The resulting residue was then further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **5**·Cl$^-$ (300 mg, 83%) as a white solid, mp 186–193 °C (Found: C, 63.39; H, 7.43; N, 8.81. $C_{43}H_{60}N_5O_6Cl{\cdot}2(H_2O)$ requires C, 63.43; H, 7.93; N, 8.61%) (HRMS: Found: M^+, 742.4539. $C_{43}H_{60}N_5O_6$ requires *m*/*z*, 742.4544); R_f 0.40 (CH_2Cl_2–MeOH, 10:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3240, 2948, 1723, 1646, 1598, 1535, 1501, 1443, 1379, 1314, 1223, 1080, 1051, 1028, 754, and 694; δ_H(300 MHz; $CDCl_3$) 0.73 (3 H, s, 18-CH_3), 0.80 (3 H, s, 19-CH_3), 0.92 (3 H, d, *J* 6.2, 21-CH_3), 2.58 (1 H, br m), 3.04 (1 H, br m, 3β-H), 3.16 (4 H, br m, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.60 (3 H, s, CO_2CH_3), 4.82 (1 H, m, 7β-H), 5.11 (1 H, m, 12β-H), 6.65 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 6.96 (1 H, t, *J* 7.4, ArH *para*), 6.97 (1 H, t, *J* 7.2, ArH *para*), 7.24 (4 H, t, *J* 7.5, ArH *meta*), 7.73 (2 H, d, *J* 7.7, ArH *ortho*), 7.75 (2 H, d, *J* 7.9, ArH *ortho*), 8.17 (1 H, br s, 3α-NH), 8.84 (1 H, s, NHCO), 9.27 (1 H, br s, NHCO); δ_C(75.5 MHz; $CDCl_3$) 12.36 (C-18), 17.62 (C-21), 20.05 (C-5 of the pyrimidinium ring), 21.79 (C-19), 22.81 (CH_2), 25.29 (CH_2), 26.67 (CH_2), 27.15 (CH_2), 28.00 (CH), 30.57 (CH_2), 30.89 (CH_2), 33.41 (C-10), 34.07 (CH_2), 34.71 (CH, and CH_2), 37.92 (CH), 38.52 (C-4,6 pyrimidinium ring), 40.25 (CH), 42.74 (CH), 44.87 (C-13), 46.87 (CH), 50.58 (C-3), 51.40 (CO_2CH_3), 70.61 (C-7), 75.17 (C-12), 118.23 (ArCH *ortho*), 119.12 (ArCH *ortho*), 122.39 (ArCH *para*), 122.54 (ArCH *para*), 128.48 (ArCH *meta*), 128.65 (ArCH *meta*), 139.23 (ArC), 139.38 (ArC), 152.99 (C), 153.47 (C), 153.83 (C), 174.70 (C-24); *m*/*z* (FAB) 742 (M^+, 100%), 621 (15), 605 (13), 484 (5), 468 (19), 154 (15), and 100 (53).

Methyl 3α-azido-7α,12α-bis[*p*-(trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate 18

α,α,α-Trifluoro-*p*-tolyl isocyanate (1.50 mL, 10.4 mmol) was added dropwise to a stirred solution of trimethylsilyl chloride (150 μL, 1.2 mmol) and azide **13** (2.14 g, 4.78 mmol) in dry CH_2Cl_2 (26 mL) under an atmosphere of argon. The solution was stirred for 72 h. Further portions of α,α,α-trifluoro-*p*-tolyl isocyanate (700 μL, 5.2 mmol; added dropwise) and trimethylsilyl chloride (70 μL, 270 μmol) were added over this period. After the 3 d period TLC analysis (hexane–EtOAc, 2:1) showed that all the starting material had reacted to give one steroidal product. Water (140 μl) was added to the stirred pale yellow solution. The solution was allowed to stir in air for 1–2 hours, and then evaporated under reduced pressure. The residue was taken up in a minimum amount of EtOAc, purified by flash chromatography eluting with hexane–EtOAc (2:1) and evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 and evaporated repeatedly to remove residual hexane and EtOAc, thus affording *7α*,12*α*-bis[p-(trifluoromethyl)phenylcarbamate] **18** (3.28 g, 82%) as a white solid, mp 124–131 °C (Found: C, 57.85; H, 5.68; N, 8.10. $C_{41}H_{49}N_5O_6F_6{\cdot}0.5(CH_2Cl_2)$ requires C, 57.67; H, 5.83; N, 8.10%); † R_f 0.89 (hexane–EtOAc, 3:2); δ_H(400 MHz; $CDCl_3$) 0.79 (3 H, s, 18-CH_3), 0.88 (3 H, d, *J* 6.4 Hz, 21-CH_3), 0.97 (3 H, s, 19-CH_3), 3.19 (1 H, br m, 3β-H), 3.61 (3 H, s, CO_2CH_3), 4.99 (1 H, m, 7β-H), 5.14 (1 H, m, 12β-H), 6.86 [1 H, s, 7α-O(CO)NH exchanges with D_2O], 6.97 [1 H, br s, 12α-O(CO)NH exchanges with D_2O], 7.58 (8 H, s, Ar-H); δ_C(100.6 MHz; $CDCl_3$) 12.48 (C-18), 17.73 (C-21), 22.66 (C-19), 23.02 (CH_2), 25.97 (CH_2), 26.97 (CH_2), 27.28 (CH_2), 29.26, 30.86 (CH_2), 31.07 (CH_2), 31.57 (CH_2), 34.48 (C-10), 34.65 (CH_2), 34.80, 35.17 (CH_2), 38.00, 41.22, 43.86, 45.57 (C-13), 47.60, 51.62 (CO_2CH_3), 61.23 (C-3), 72.53 (C-7), 76.84 (C-12), 118.33 (ArCH *ortho*), 118.37 (ArCH *ortho*), 126.47 (ArC *para*), 126.51 (ArC *para*), 126.55 (ArCH *meta*), 126.59 (ArCH *meta*), 141.22 (ArC), 152.58 (NHCO), 152.83 (NHCO), 174.71 (C-24), 185.20 (CF_3).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α,12α-bis[*p*-(trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate chloride 6·Cl$^-$

Azide **18** (3.28 g) was dissolved in glacial AcOH (197 mL). Zinc dust (6.56 g, 99.7 mmol) was added to the solution and the resultant mixture stirred for 1.5 h. The zinc salts and remaining zinc were removed by filtration and the filtrate evaporated under reduced pressure to afford the alkylammonium acetate (3.41 g, 3.99 mmol) as a white solid, which was one major component by TLC (R_f 0.24, hexane–EtOAc, 2:1). Under an atmosphere of argon 3-(*tert*-butoxycarbonylamino)propyl isothiocyanate (1.82 g, 8.46 mmol) was added to a stirred solution of the alkylammonium acetate (3.41 g) and *N*,*N*-diisopropylethylamine (1.76 mL, 10.1 mmol) in dry MeOH (26 mL). The

† Residual CH_2Cl_2, *ca.* 0.5 equivalents, was detected in the analytical sample by ^{1}H NMR.

resulting pale yellow solution was stirred for 72 h. The solution was evaporated under reduced pressure, and the resulting oil partially purified by flash chromatography eluting with hexane–EtOAc (2:1), affording the corresponding thiourea (2.22 g, *ca.* 85% pure due to close-running impurities); R_f 0.46 (hexane–EtOAc, 2:1). Methyl iodide (6.87 mL, 110 mmol) was added to a stirred solution of the crude thiourea (2.0 g) in dry MeOH (17 mL) under an atmosphere of argon. The solution was refluxed for 1.5 h while stirring, after which time analysis by TLC (EtOAc) revealed no starting material remained. The solution was allowed to cool to room temperature, and then evaporated under reduced pressure. Under an atmosphere of argon, the resulting residue was dissolved in dry CH_2Cl_2 (17 mL) and trifluoroacetic acid (6.87 mL, 89.1 mmol) added while stirring. The resulting yellow suspension was stirred for 1 h, after which time analysis by TLC (EtOAc) revealed that the amine deprotection had reached completion. The solution was evaporated under reduced pressure and, under an atmosphere of argon, the resulting residue dissolved in dry MeOH (9.5 mL). Over a period of 20 min this solution was added dropwise *via* a syringe to *N*,*N*-diisopropylethylamine (9.4 mL, 54.46 mmol) under an atmosphere of argon with vigorous stirring. The resulting solution was stirred for 24 h during which time methyl thiol was evolved. At the end of the 24 h period analysis by TLC (EtOAc) revealed one major component. The solution was evaporated under reduced pressure and the residue dissolved in CH_2Cl_2 (50 mL), and then washed with aq. HCl (2 M, 2 × 50 mL). The combined aqueous layers were carefully extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with water (50 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue (1.21 g) was purified by flash chromatography eluting with EtOAc to afford the guanidinium salt (521 mg) which was pure by TLC. To ensure chloride was the counter anion, the salt was taken up in $CHCl_3$–MeOH (100 mL, 25:1) and washed carefully with aq. NaOH (2 M, 2 × 60 mL) followed by triply distilled water (60 mL). The organic layer was then washed with aq. HCl (2 M, 2 × 60 mL) followed by triply distilled water (2 × 60 mL). The combined acidic aqueous layers were then extracted with CH_2Cl_2–MeOH (60 mL; 25:1); allowing each washing to settle for 30 min before isolating the organic layer. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue was further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **6**·Cl^- (411 mg, 11% from **18**), mp 203–209 °C (Found: C, 56.49; H, 6.13; N, 7.22. $C_{45}H_{58}N_5O_6F_6Cl{\cdot}3(H_2O)$ requires C, 56.87; H, 6.57; N, 7.37%) (HRMS: Found: M^+, 878.4298. $C_{45}H_{58}N_5O_6F_6$ requires *m*/*z*, 878.4291); R_f 0.81 (EtOAc); δ_H(400 MHz; $CDCl_3$) 0.77 (3 H, s, 18-CH_3), 0.89 (3 H, s, 19-CH_3), 0.95 (3 H, d, *J* 6.5 Hz, 21-CH_3), 3.05 (1 H, br m, 3β-H), 3.29 (4 H, br s, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.61 (3 H, s, CO_2CH_3), 4.90 (1 H, m, 12β-H), 5.20 (1 H, m, 7β-H), 6.15 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 7.51 (4 H, d, *J* 7.9 Hz, ArH *meta*), 7.97 (4 H, m, ArH *ortho*), 8.58 (1 H, br s, 3α-NH), 8.89 (1 H, br s, NHCO), 9.52 (1 H, br s, NHCO); δ_C(100.6 MHz; $CDCl_3$) 12.42 (C-18), 17.75 (C-21), 20.10 (C-5 of the pyrimidinium ring), 21.89 (C-19), 22.89 (CH_2), 25.40 (CH_2), 26.71 (CH_2), 27.19 (CH_2), 28.28 (CH), 30.70 (CH_2), 30.99 (CH_2), 33.63 (C-10), 34.10 (CH_2), 34.82, 34.99, 38.06 (CH), 38.61 (C-4,6 pyrimidinium ring), 40.72 (CH), 42.88 (CH), 45.06 (C-13), 47.07 (CH), 50.58 (C-3), 51.39 (CO_2CH_3), 71.03 (C-7), 75.64 (C-12), 117.95 (ArCH *ortho*), 118.67 (ArCH *ortho*), 123.13 (ArC *para*), 123.95 (ArC *para*), 125.68 (ArCH *meta*), 125.70 (ArCH *meta*), 125.82, 125.86, 125.90, 142.60, 142.74, 153.10 (C), 153.37 (C), 174.61 (C-24); δ_F(376 MHz; $CDCl_3$) −62.29, −62.31.

Methyl 7α-acetoxy-3α,12α-dihydroxy-5β-cholan-24-oate 19

This procedure is modified from that of Fieser and Rajagopalan.[15] Freshly distilled acetyl chloride (100 μL, 1.41 mmol) was added dropwise *via* a syringe to a stirred mixture of methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oate[15] (500 mg, 0.99 mmol) in dry MeOH (4 mL) at 0 °C protected from the atmosphere by a calcium chloride tube. The mixture was stirred for 10 min, then allowed to reach room temperature and stirring continued for 2.5 h, after which a clear solution resulted. After an additional 1.5 h (over which time the progress of the deprotection was carefully monitored by TLC) a saturated aqueous solution of sodium bicarbonate (2 mL) was added slowly with stirring. The solution was carefully evaporated under reduced pressure (maintaining the temperature below 40 °C) and the residue taken up in CH_2Cl_2–water (30 mL; 1:1). The organic layer was isolated, washed with water (20 mL) and dried by decanting. The organic layer was evaporated under reduced pressure and the residue further dried *in vacuo* at 60 °C to afford the 7α-acetate **19** (440 mg, 96%) as a white solid which was over 90% pure as judged by ^1H NMR; R_f 0.20 (hexane–EtOAc, 1:3); δ_H(300 MHz; $CDCl_3$) 0.67 (3 H, s, 18-CH_3), 0.91 (3 H, s, 19-CH_3), 0.97 (3 H, d, *J* 6.2, 21-CH_3), 2.06 (3 H, s, $OCOCH_3$), 3.49 (1 H, br m, 3β-H), 3.66 (3 H, s, CO_2CH_3), 3.99 (1 H, m, 12β-H), 4.88 (1 H, m, 7β-H).

Methyl 3β-formyloxy-7α-acetoxy-12α-hydroxy-5β-cholan-24-oate 20

Diethyl azodicarboxylate (37 mL, 235 mmol) was added dropwise (10–15 min) *via* a syringe to a stirred solution of crude 7α-acetate **19** (36.5 g, 79 mmol), triphenylphosphine (61.8 g, 236 mmol) and 95–97% formic acid (5.92 mL, 157 mmol) in dry THF (360 mL) under an atmosphere of argon. The resulting pale yellow solution was stirred for 2.5 h; after which time analysis by TLC (hexane–EtOAc, 1:2) showed no starting material remained. The solution was evaporated under reduced pressure, and the resulting pale yellow oil was split into two equal portions. Each portion was taken up in a minimum amount of CH_2Cl_2 and partially purified by flash chromatography eluting with hexane–EtOAc (3:1) to afford crude product (44 g; *ca.* 70% pure as judged by TLC). This was recrystallised from MeOH–water (seeding with pure crystals of **20** after a hot filtration) to afford pure *3β*-formate **20** (24.2 g, 63% from crude **19**) as white glassy crystals, mp 188–191 °C (Found: C, 68.04; H, 8.90. $C_{28}H_{44}O_7$ requires C, 68.25; H, 9.01%); R_f 0.51 (hexane–EtOAc, 1:2); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3552, 3433, 2939, 2865, 1717, 1439, 1379, 1365, 1314, 1251, 1224, 1179, 1153, 1069, 1024, 979, and 864; δ_H(300 MHz; $CDCl_3$) 0.68 (3 H, s, 18-CH_3), 0.95 (3 H, s, 19-CH_3), 0.96 (3 H, d, *J* 6.9, 21-CH_3), 2.03 (3 H, s, $OCOCH_3$), 3.65 (3 H, s, CO_2CH_3), 3.99 (1 H, m, 12β-H), 4.89 (1 H, m, 7β-H), 5.16 (1 H, m, 3α-H), 8.04 (1 H, s, OCOH); δ_C(75.5 MHz; $CDCl_3$) 12.50 (C-18), 17.34 (C-21), 21.59 (C-19), 22.92 ($OCOCH_3$), 24.84 (CH_2), 27.22 (CH_2), 27.56, 28.77 (CH_2), 30.23 (CH_2), 30.79 (CH_2), 30.95 (CH_2), 32.72 (CH_2), 34.55 (C-10), 34.91, 36.32, 38.02, 42.09, 46.57 (C-13), 47.18, 51.49 (CO_2CH_3), 70.48 (C-3), 71.00 (C-7), 72.71 (C-12), 160.73 (OCOH), 170.43 ($OCOCH_3$), 174.55 (C-24).

Methyl 3β-formyloxy-7α-acetoxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate 21. Trimethylsilyl chloride (5.2 μL, 0.041 mmol) was added to a stirred solution of **20** (200 mg, 0.41 mmol) and phenyl isocyanate (66 μL, 0.61 mmol) in dry CH_2Cl_2 (0.6 mL) protected from the atmosphere by a calcium chloride tube. The resulting solution was stirred for 24 h, by which time the solvent had evaporated. The residue was taken up in dry CH_2Cl_2 (0.6 mL), and analysis by TLC revealed one major steroidal product was present. The solution was stirred in air for 1–2 h and then evaporated under reduced pressure. The residue was taken up in a minimum amount of CH_2Cl_2 and any undissolved material removed by filtration. The filtrate was purified by flash chromatography eluting with hexane–EtOAc (4:1) to afford the *carbamate* **21** (131 mg, 53%) as a white foam

(Found: C, 68.53; H, 8.03; N, 2.39. $C_{35}H_{49}NO_8$ requires C, 68.70; H, 8.08; N, 2.29%); R_f 0.40 (hexane–EtOAc, 1:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3347, 2949, 2873, 1720, 1600, 1537, 1501, 1443, 1379, 1313, 1250, 1224, 1199, 1154, 1082, 1048, 1027, 755, 736, and 694; δ_H(300 MHz; $CDCl_3$) 0.76 (3 H, s, 18-CH_3), 0.88 (3 H, d, *J* 6.4, 21-CH_3), 0.97 (3 H, s, 19-CH_3), 2.09 (3 H, s, $OCOCH_3$), 3.63 (3 H, s, CO_2CH_3), 4.93 (1 H, m, 7β-H), 5.11–5.13 (2 H, br m, 12β-H and 3α-H), 6.66 (1 H, br s, NHCO), 7.09 (1 H, t, *J* 7.2, ArH *para*), 7.34 (2 H, t, *J* 5.3, ArH *meta*), 7.42 (2 H, br d, *J* 7.7, ArH *ortho*), 8.03 (1 H, s, OCOH); δ_C(75.5 MHz; $CDCl_3$) 12.32 (C-18), 17.54 (C-21), 21.67 (C-19), 22.81 (CH_2), 22.90 ($OCOCH_3$), 24.88 (CH_2), 26.09 (CH_2), 27.15 (CH_2), 28.42 (CH), 30.11 (CH_2), 30.75 (CH_2), 30.93 (CH_2), 32.71 (CH_2), 34.55 (C-10), 34.68 (CH), 36.25 (CH), 37.69 (CH), 43.62 (CH), 45.38 (C-13), 47.42 (CH), 51.49 (CO_2CH_3), 70.22 (C-3), 70.97 (C-7), 76.81 (C-12), 118.97 (ArCH *ortho*), 123.66 (ArCH *para*), 129.08 (ArCH *meta*), 137.80 (ArC), 153.21 (NHCO), 160.67 (OCOH), 170.23 ($OCOCH_3$), 174.56 (C-24). Repetition of the procedure starting with 23.5 g of **20** yielded 25.8 g of **21**, which was used in the following step.

Methyl 3β-hydroxy-7α-acetoxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate. Sodium bicarbonate (15.7 g, 187 mmol) was added to a stirred solution of crude 3β-formate **21** (25 g, 41 mmol) in MeOH (300 mL). The reaction flask was stoppered and the resulting suspension was stirred for 24 h. The white suspension was evaporated under reduced pressure (maintaining the temperature below 45 °C). The resulting residue was taken up in EtOAc (500 mL), and washed with water (2 × 350 mL). The combined aqueous layers were extracted with EtOAc (200 mL). The combined organic extracts were dried by decanting and evaporated under reduced pressure. The resulting white residue (24.4 g) was dissolved in a minimum amount of CH_2Cl_2. Analysis by TLC (hexane–EtOAc, 1:1) revealed the presence of one steroidal component and one higher running UV active impurity. The CH_2Cl_2 solution of crude product was then purified by flash chromatography initially eluting with hexane–EtOAc (3:1), then with EtOAc to afford the *title compound* (22 g, 92% from crude **21**) as a white solid, mp 168–172 °C (Found: C, 69.66; H, 8.36; N, 2.34. $C_{34}H_{49}NO_7$ requires C, 69.94; H, 8.47; N, 2.40%); R_f 0.14 (hexane–EtOAc, 1:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3343, 2951, 2874, 1728, 1601, 1538, 1502, 1443, 1379, 1313, 1226, 1175, 1084, 1028, 754, 736, and 695; δ_H(300 MHz; $CDCl_3$) 0.75 (3 H, s, 18-CH_3), 0.87 (3 H, d, *J* 6.4, 21-CH_3), 0.94 (3 H, s, 19-CH_3), 2.08 (3 H, s, $OCOCH_3$), 3.62 (3 H, s, CO_2CH_3), 4.02 (1 H, m, 3α-H), 4.91 (1 H, m, 7β-H), 5.09 (1 H, m, 12β-H), 6.72 (1 H, s, NHCO), 7.07 (1 H, t, *J* 7.3, ArH *para*), 7.31 (2 H, t, *J* 7.9, ArH *meta*), 7.41 (2 H, br s, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.27 (C-18), 17.51 (C-21), 21.65 (C-19), 22.80 (CH_2), 22.92 ($OCOCH_3$), 26.04 (CH_2), 27.15 (CH_2), 27.69 (CH_2), 28.20, 29.40 (CH_2), 30.72 (CH_2), 30.91 (CH_2), 34.66, 34.77 (C-10), 35.47, 35.66 (CH_2), 37.67, 43.61, 45.36 (C-13), 47.38, 51.45 (CO_2CH_3), 66.51 (C-3), 71.21 (C-7), 76.67 (C-12), 118.87 (ArCH *ortho*), 123.51 (ArCH *para*), 129.02 (ArCH *meta*), 137.90 (ArC), 153.12 (NHCO), 170.32 ($OCOCH_3$), 174.56 (C-24).

Methyl 3β,7α-dihydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate 22. The above hydroxyacetate (2.50 g, 4.28 mmol) was added to methanolic sodium methoxide (2 M, 10 mL) under an atmosphere of argon with stirring. The resulting white suspension was stirred vigorously at 45 °C for 38 h. Over the course of this period the suspension became a clear solution after 15 min, and then a pale yellow suspension within 24 h. The suspension was allowed to cool to room temperature; TLC analysis (hexane–EtOAc, 1:2) revealed no starting material remained. The suspension was then taken up in CH_2Cl_2 (80 mL) and washed successively with aq. HCl (2 M, 50 mL) and water (2 × 50 mL). The organic layer was isolated, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting residue was further dried by evaporation *in vacuo* for 15 min. TLC analysis of the residue revealed that side chain ester cleavage had occurred (up to 40%; as judged by TLC). Re-esterification was carried out; the residue (2.15 g) was protected from the atmosphere by a calcium chloride tube and dry MeOH (16 mL) added. The mixture was then stirred until fully dissolved, and then concentrated sulfuric acid (152 μL) added slowly. The resulting pale yellow solution was stirred for 6 h, after which time TLC analysis (hexane–EtOAc, 1:2) revealed the presence of only one major steroidal component. The solution was evaporated under reduced pressure (maintaining the temperature below 45 °C). The resulting residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aqueous sodium bicarbonate (2 × 40 mL) followed by water (2 × 40 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give the *diol* **22** (1.64 g, 71%) as a flaky glassy material (Found: C, 70.73; H, 8.65; N, 2.53. $C_{32}H_{47}NO_6$ requires C, 70.94; H, 8.75; N, 2.59%); R_f 0.29 (hexane–EtOAc, 1:2); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3484, 2923, 2872, 1719, 1602, 1542, 1501, 1444, 1379, 1313, 1227, 1195, 1176, 1083, 1051, 1029, 754, 736, and 693; δ_H(300 MHz; $CDCl_3$) 0.77 (3 H, s, 18-CH_3), 0.90 (3 H, d, *J* 6.4, 21-CH_3), 0.93 (3 H, s, 19-CH_3), 3.63 (3 H, s, CO_2CH_3), 3.91 (1 H, m, 7β-H), 4.01 (1 H, m, 3α-H), 5.11 (1 H, m, 12β-H), 6.94 (1 H, s, NHCO), 7.05 (1 H, t, *J* 7.0, ArH *para*), 7.31 (2 H, t, *J* 7.4, ArH *meta*), 7.42 (2 H, br d, *J* 8.3, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.26 (C-18), 17.58 (C-21), 22.93 (C-19), 23.02 (CH_2), 25.92 (CH_2), 26.91, 27.32 (CH_2), 27.71 (CH_2), 29.54 (CH_2), 30.80 (CH_2), 30.99 (CH_2), 34.52 (CH_2), 34.80, 35.12 (C-10), 35.86, 36.43 (CH_2), 39.08, 43.55, 45.28 (C-13), 47.48, 51.47 (CO_2CH_3), 66.73 (C-3), 68.52 (C-7), 76.68 (C-12), 118.57 (ArCH *ortho*), 123.27 (ArCH *para*), 129.02 (ArCH *meta*), 138.13 (ArC), 153.06 (NHCO), 174.64 (C-24).

Methyl 3α-azido-7α-hydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate. Methanesulfonyl chloride (18.6 μL, 0.24 mmol) was added dropwise *via* a syringe to a stirred pale yellow solution of 3β-hydroxy steroid **22** (100 mg, 0.19 mmol) and Et_3N (51.6 μL, 0.37 mmol) in dry CH_2Cl_2 (0.6 mL) at 0 °C under an atmosphere of argon. The resulting off-white suspension was stirred at this temperature for 50 min, after which period TLC analysis (hexane–EtOAc, 1:2) showed that all starting material **22** had been consumed. With vigorous stirring saturated aqueous sodium bicarbonate (0.7 mL) was added slowly, the biphasic mixture was allowed to reach room temperature and then taken up in CH_2Cl_2 (2 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (2 × 2 mL) and water (2 × 3 mL), dried ($MgSO_4$) and evaporated under reduced pressure to afford methyl 3β-methylsulfonyloxy-7α-hydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate (102 mg, 89%) as an off-white foam which was stored under argon in the fridge (*ca.* 4 °C; decomposes within two weeks); R_f 0.50 (hexane–EtOAc, 1:2); δ_H(300 MHz; $CDCl_3$) 0.77 (3 H, s, 18-CH_3), 0.89 (3 H, d, *J* 6.2, 21-CH_3), 0.95 (3 H, s, 19-CH_3), 2.60 (1 H, br t, *J* 15), 2.96 (3 H, s, OSO_2CH_3), 3.63 (3 H, s, CO_2CH_3), 3.91 (1 H, m, 7β-H), 4.92 (1 H, m, 3α-H), 5.11 (1 H, m, 12β-H), 6.84 (1 H, br s, NHCO), 7.06 (1 H, t, *J* 7.2 ArH *para*), 7.31 (2 H, t, *J* 7.7, ArH *meta*), 7.41 (2 H, br d, *J* 7.6, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.28 (C-18), 17.57 (C-21), 22.72 (C-19), 22.96 (CH_2), 25.96 (CH_2), 27.18, 27.29 (CH_2), 29.68 (CH_2), 30.78 (CH_2), 30.99 (CH_2), 34.00 (CH_2), 34.60 (CH_2), 34.78, 36.17, 38.50, 39.05, 43.53, 45.28 (C-13), 47.50, 51.48 (CO_2CH_3), 68.26 (C-7), 76.43 (C-12), 80.31 (C-3), 118.54 (ArCH *ortho*), 123.37 (ArCH *para*), 129.07 (ArCH *meta*), 137.98 (ArC), 174.60 (C-24). This procedure was repeated on a larger scale with crude **22** (17.6 g) to give the above methanesulfonate (18.3 g, 91% from crude **22**) which was almost pure as judged by TLC. A portion of the crude methanesulfonate (11 g, 18 mmol) was taken up in DMPU (54 mL) and sodium azide

(11 g, 169 mmol) was added. The pale yellow reaction mixture was protected from the atmosphere by a calcium chloride tube and stirred at 50 °C for 24 h, after which time TLC analysis (hexane–EtOAc, 1:1) of a mini work-up revealed that all starting material had been consumed to give one major steroidal product and one minor steroidal product. The resulting creamy off-white mixture was cooled to room temperature and then taken up in EtOAc–water (500 mL; 1:1). The organic layer was isolated and washed with water (300 mL). The combined aqueous layers were extracted with EtOAc (200 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting pale yellow oil was purified by flash chromatography eluting with hexane–EtOAc (3:1) to afford the *title azide* (8.5 g, 85% from crude methanesulfonate, 76% from **22**) as a clear oil which solidified *in vacuo* to give a white solid, mp 152–160 °C (Found: C, 67.50; H, 8.20; N, 9.45. $C_{32}H_{46}N_4O_5$ requires C, 67.80; H, 8.19; N, 9.89%) (HRMS: Found: M^+, 566.3449. $C_{32}H_{46}N_4O_5$ requires *m*/*z*, 566.3468); R_f 0.72 (hexane–EtOAc, 1:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3520, 3410, 3331, 2948, 2870, 2092, 1726, 1601, 1530, 1501, 1443, 1380, 1313, 1225, 1194, 1082, 1050, 1028, 754, 736, and 693; δ_H(300 MHz; $CDCl_3$) 0.77 (3 H, s, 18-CH_3), 0.90 (3 H, d, *J* 5.6, 21-CH_3), 0.91 (3 H, s, 19-CH_3), 3.11 (1 H, br m, 3β-H), 3.63 (3H, s, CO_2CH_3), 3.91 (1 H, m, 7β-H), 5.13 (1 H, m, 12β-H), 6.86 (1 H, s, NHCO), 7.06 (1 H, t, *J* 7.3, ArH *para*), 7.32 (2 H, t, *J* 7.5, ArH *meta*), 7.43 (2 H, br d, *J* 7.9, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.32 (C-18), 17.59 (C-21), 22.57 (C-19), 22.97 (CH_2), 25.71 (CH_2), 26.62 (CH_2), 27.29 (CH_2), 27.66, 30.78 (CH_2), 30.98 (CH_2), 34.64 (C-10), 34.67 (CH_2), 34.78, 35.28 (CH_2), 35.31 (CH_2), 39.14, 41.63, 43.48, 45.24 (C-13), 47.47, 51.47 (CO_2CH_3), 61.16 (C-3), 68.07 (C-7), 76.04 (C-12), 118.64 (ArCH *ortho*), 123.37 (ArCH *para*), 129.02 (ArCH *meta*), 137.97 (ArC), 153.04 (NHCO), 174.60 (C-24); *m*/*z* (FAB) 566.3 (M^+, 93%), 540.3 (6), 524.3 (12), 447.3 (3), 430.2 (8), 412.2 (86), 402.3 (34), 384.2 (100), 369.2 (84), and 355.2 (5).

Methyl 3α-(*N*-*tert*-butoxycarbonylamino)-7α-hydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate 23. Acetic acid (90 mL) was added to a mixture of zinc dust (22.3 g, 341 mmol) and the above azide (7.44 g, 13.13 mmol). The reaction mixture was protected from the atmosphere by a calcium chloride tube and stirred vigorously for 24 h, after which period TLC analysis (CH_2Cl_2–MeOH, 5:1) of a mini work up revealed one major steroidal component was present. The zinc residues were removed by filtration and washed with acetic acid (2 × 30 mL) and toluene (2 × 30 mL). The filtrate was evaporated under reduced pressure. Residual acetic acid was removed by repetitive addition and evaporation under reduced pressure of distilled toluene (2 × 30 mL) followed by further evaporation *in vacuo* to give the alkylammonium acetate as an off-white foam; R_f 0.46 (CH_2Cl_2–MeOH, 5:1). Di-*tert*-butyl dicarbonate (3.44 g, 15.76 mmol) was added to a stirred biphasic mixture of the ammonium acetate in a saturated aqueous solution of sodium bicarbonate (45 mL) and distilled THF (90 mL). The mixture was stirred for 48 h, after which time TLC analysis (CH_2Cl_2–MeOH, 10:1) revealed that the great majority of the starting material had been consumed to give one main steroidal product. Further portions of di-*tert*-butyl dicarbonate (340 mg, 1.56 mmol) and aqueous solution of sodium bicarbonate (10 mL) were added and the mixture stirred for an additional 45 min; after which time TLC analysis revealed no starting material remained. The mixture was carefully evaporated under reduced pressure (maintaining the temperature below 40 °C). The resulting residue was taken up in EtOAc–water (300 mL; 1:1). The organic layer was isolated and washed with a further portion of water (150 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue was purified by flash chromatography eluting with hexane–EtOAc (3:1) to afford the *protected amine* **23** (6.18 g, 74%) as a white foam (Found: C, 68.26; H, 8.70; N, 4.22. $C_{37}H_{56}N_2O_7 \cdot 0.5(H_2O)$ requires C, 68.37; H, 8.85; N, 4.31%); R_f 0.47 (CH_2Cl_2–MeOH, 10:1), 0.30 (hexane–EtOAc, 2:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3412, 2950, 2870, 1716, 1602, 1522, 1502, 1444, 1380, 1366, 1313, 1227, 1194, 1172, 1082, 1051, 1027, 997, 754, 736, and 693; δ_H(300 MHz; $CDCl_3$) 0.76 (3 H, s, 18-CH_3), 0.89 (3 H, s, 19-CH_3), 0.90 (3 H, d, *J* 5.9, 21-CH_3), 1.40 [9 H, s, $C(CH_3)_3$], 3.22 (1 H, br m, 3β-H), 3.63 (3 H, s, CO_2CH_3), 3.89 (1 H, m, 7β-H), 4.31 (1 H, br s, 3α-NH), 5.12 (1 H, m, 12β-H), 6.87 (1 H, br s, NHCO), 7.07 (1 H, t, *J* 7.4, ArH *para*), 7.32 (2 H, t, *J* 7.9, ArH *meta*), 7.44 (2 H, br d, *J* 8.0, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.25 (C-18), 17.59 (C-21), 22.65 (C-19), 23.00 (CH_2), 25.68 (CH_2), 27.32 (CH_2), 27.61 (CH), 28.17 (CH_2), 28.41 [$C(CH_3)_3$], 30.79 (CH_2), 31.00 (CH_2), 34.57 (C-10), 34.81 (CH and CH_2), 35.71 (CH_2), 37.12 (CH_2), 39.11 (CH), 41.77 (CH), 43.52 (CH), 45.23 (C-13), 47.51 (CH), 50.73 (C-3), 51.47 (CO_2CH_3), 68.31 (C-7), 76.21 (C-12), 78.91 [$C(CH_3)_3$], 118.31 (ArCH *ortho*), 123.27 (ArCH *para*), 129.08 (ArCH *meta*), 138.11 (ArC), 155.20 (NHCO), 174.62 (C-24).

Methyl 3α-{[3-(*tert*-butoxycarbonylamino)propylamino]-thioxomethylamino}-7α-hydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate. 99% Trifluoroacetic acid (18.0 mL, 234 mmol) was added slowly *via* a syringe to a stirred solution of protected amine **23** (4.50 g, 7.02 mmol) in dry CH_2Cl_2 (45 mL) under an atmosphere of argon. The resulting pale yellow solution was stirred for 2 h, after which time analysis by TLC (CH_2Cl_2–MeOH, 10:1) revealed that amine deprotection had reached completion to give one major steroidal product (R_f 0.22). The solution was evaporated under reduced pressure (maintaining the temperature below 40 °C). Residual trifluoroacetic acid was removed by repetitive addition and evaporation under reduced pressure of distilled CH_2Cl_2 (2 × 20 mL) followed by evaporation *in vacuo* to give the alkylammonium trifluoroacetate. 3-(*tert*-Butoxycarbonylamino)propyl isothiocyanate (2.97 g, 13.73 mmol) was added to a stirred solution of the alkylammonium salt and *N*,*N*-diisopropylethylamine (3.60 mL, 20.67 mmol) in dry MeOH (45 mL) under an atmosphere of argon. The resulting pale yellow solution was gently refluxed for 16 h. The solution was allowed to cool to room temperature; TLC analysis (CH_2Cl_2–MeOH, 10:1) revealed the reaction had reached *ca.* 75 % completion. A further volume of *N*,*N*-diisopropylethylamine (1.20 mL, 6.89 mmol) was added followed by a further portion of the isothiocyanate (1.50 g, 6.94 mmol). The solution was again gently refluxed under argon for 3 h then allowed to cool to room temperature; TLC analysis (CH_2Cl_2–MeOH, 10:1) revealed that the reaction had reached completion to give one steroidal product. The solution was evaporated under reduced pressure (maintaining the temperature below 40 °C) and the resulting residue purified by flash chromatography sequentially eluting with CH_2Cl_2 followed by CH_2Cl_2–MeOH (100:1) and finally with CH_2Cl_2–MeOH (25:1) to afford the *thiourea* (4.63 g, 87% from **23**) as an off-white foam, mp 124–131 °C (Found: C, 64.04; H, 8.36; N, 7.30; S, 4.47. $C_{41}H_{64}N_4O_7S \cdot 0.5(H_2O)$ requires C, 64.28; H, 8.56; N, 7.32; S, 4.18%); R_f 0.53 (hexane–EtOAc, 1:2); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3312, 2946, 2869, 1720, 1691, 1601, 1534, 1443, 1366, 1313, 1226, 1194, 1172, 1082, 1051, 1027, 753, 736, and 694; δ_H(300 MHz; $CDCl_3$) 0.76 (3 H, s, 18-CH_3), 0.89 (3 H, s, 19-CH_3), 0.90 (3 H, d, *J* 7.0, 21-CH_3), 1.41 [9 H, s, $C(CH_3)_3$], 3.16 (2 H, br dt, *J* 5.4 and 6.0, CH_2NHCO), 3.56 (3 H, br m, 3β-H and CH_2NHCS), 3.66 (3 H, s, CO_2CH_3), 3.88 (1 H, m, 7β-H), 4.84 (1 H, br t, *J* 6.0, CH_2NHCO), 5.13 (1 H, m, 12β-H), 5.98 (1 H, br s, 3α-NH, exchanges with D_2O), 6.90 to 7.40 (2 H, br s, CH_2NHCS and PhNHCO, exchanges with D_2O), 7.04 (1 H, br t, *J* 7.0, ArH *para*), 7.31 (2 H, br t, *J* 7.3, ArH *meta*), 7.45 (2 H, br d, *J* 7.7, ArH *ortho*); δ_C(75.5 MHz; $CDCl_3$) 12.32 (C-18), 17.63 (C-21), 22.62 (C-19), 22.99 (CH_2), 25.82 (CH_2), 27.32 (CH_2), 27.39 (CH_2), 27.68, 28.28 [$C(CH_3)_3$], 29.67 (CH_2), 30.77 (CH_2), 31.00 (CH_2), 34.57 (CH_2), 34.81, 35.34 (CH_2), 36.21 (CH_2), 36.94 (CH_2), 39.18, 40.72 (CH_2), 41.55, 43.46, 45.23

(C-13), 47.47, 51.47 (CO_2CH_3), 53.68 (C-3), 68.18 (C-7), 76.31 (C-12), 79.78 [$C(CH_3)_3$], 118.76 (ArCH *ortho*), 123.24 (ArCH *para*), 129.02 (ArCH *meta*), 138.20 (ArC), 153.17 (C), 157.47 (C), 174.65 (C-24).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α-hydroxy-12α-phenylaminocarbonyloxy-5β-cholan-24-oate chloride 7·Cl⁻. Methyl iodide (14.3 mL, 230 mmol) was added *via* a syringe to a stirred solution of the above thiourea (4.33 g, 5.72 mmol) in dry MeOH (43 mL) under an atmosphere of argon. The solution was refluxed for 105 min while stirring. The solution was allowed to cool to room temperature; analysis by TLC (hexane–EtOAc, 1:2) revealed that all the steroidal starting material had been consumed to give one steroidal product (R_f 0.08). The solution was evaporated under reduced pressure. Under an atmosphere of argon, the resulting residue was dissolved in dry CH_2Cl_2 (43 mL) and 99% trifluoroacetic acid (15.2 mL, 197 mmol) added slowly *via* a syringe while stirring. The resulting dark yellow solution was stirred for 65 min, after which time analysis by TLC (CH_2Cl_2–MeOH, 10:1) revealed one major steroidal product (R_f 0.13) was present. The solution was evaporated under reduced pressure at *ca.* 45 °C. Any residual trifluoroacetic acid was removed by further evaporation of the resulting residue *in vacuo* for 1 h with sporadic heating (35 °C). Under an atmosphere of argon the resulting residue was dissolved in dry MeOH (100 mL). Over a period of 20 min this solution was added dropwise *via* a syringe to a vigorously stirred solution of *N*,*N*-diisopropylethylamine (19.5 mL, 112 mmol) in dry MeOH (19.5 mL). The resulting solution was stirred under an atmosphere of argon for 18 h during which time thiol was evolved. The solution was evaporated under reduced pressure at *ca.* 45 °C and the residue dissolved in CH_2Cl_2 (150 mL); analysis by TLC (CH_2Cl_2–MeOH, 10:1) revealed one major steroidal component. The CH_2Cl_2 phase was washed with aq. HCl (0.5 M, 2 × 100 mL) and then dried by decanting; analysis by TLC (CH_2Cl_2–MeOH, 5:1) revealed one major steroidal component. The organic phase was evaporated under reduced pressure and the resulting residue (4.79 g) was partially purified by flash chromatography eluting with CH_2Cl_2–MeOH (20:1 graduating to 10:1). Thus affording partially purified guanidinium salt (3.38 g, 90% from starting thiourea). A portion (1.04 g) was further purified by flash chromatography eluting with EtOAc–MeOH (10:1 graduating to 20:3). To ensure chloride was the counter anion, the resulting purified salt (505 mg) was taken up in CH_2Cl_2 (50 mL) and washed with aq. NaOH (1 M, 2 × 25 mL) followed by triply distilled water (25 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 25 mL) followed by triply distilled water (2 × 25 mL). The organic phase was then dried by decanting and evaporated under reduced pressure. The resulting residue was then further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **7**·Cl⁻ (473 mg, 13% from starting thiourea) as a white solid, mp 192–194 °C (Found: C, 62.39; H, 8.22; N, 7.89; Cl, 4.98. $C_{36}H_{55}N_4O_5Cl{\cdot}2(H_2O)$ requires C, 62.21; H, 8.56; N, 8.07; Cl, 5.04%) (HRMS: Found: M^+, 623.4161. $C_{36}H_{55}N_4O_5$ requires *m*/*z*, 623.4173); R_f 0.09 (EtOAc–MeOH, 10:1), 0.46 (CH_2Cl_2–MeOH, 5:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3406, 2949, 1725, 1638, 1601, 1542, 1501, 1444, 1376, 1313, 1225, 1194, 1082, 1053, 1027, 754, 734, and 693; δ_H(400 MHz; $CDCl_3$) 0.78 (3 H, s, 18-CH_3), 0.90 (3 H, s, 19-CH_3), 0.99 (3 H, d, *J* 6.5, 21-CH_3), 2.58 (1 H, br q, *J* 12.4), 2.86 (2 H, br s, water), 3.09 (1 H, br s, 7α-OH), 3.30 (4 H, br s, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.49 (1 H, br m, 3β-H), 3.68 (3 H, s, CO_2CH_3), 3.86 (1 H, m, 7β-H), 5.14 (1 H, m, 12β-H), 7.01 (1 H, t, *J* 7.4, ArH *para*), 7.19 (1 H, d, *J* 8.3, 3α-NH), 7.27 (2 H, t, *J* 7.9, ArH *meta*), 7.63 (2 H, d, *J* 7.9, ArH *ortho*), 7.70 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 8.71 (1 H, s, NHCO); δ_C(100.6 MHz; $CDCl_3$) 12.29 (C-18), 17.65 (C-21), 20.12 (C-5 of the pyrimidinium ring), 22.26 (C-19), 23.04 (CH_2), 25.39 (CH_2), 27.19 (CH), 27.25 (CH_2), 27.34 (CH_2), 30.78 (CH_2), 31.04 (CH_2), 34.25 (C-10), 34.42 (CH_2), 34.89 (CH), 34.95 (CH_2), 35.72 (CH_2), 38.42 (C-4,6 pyrimidinium ring), 39.08 (CH), 41.29 (CH), 42.94 (CH), 45.03 (C-13), 47.28 (CH), 51.12 (C-3), 51.39 (CO_2CH_3), 68.43 (C-7), 75.35 (C-12), 119.04 (ArCH *ortho*), 122.56 (ArCH *para*), 128.55 (ArCH *meta*), 139.09 (ArC), 152.23 (C), 153.82 (C), 174.60 (C-24); *m*/*z* (FAB) 623 (M^+, 100%), 502 (4), 486 (12), 154 (12), 100 (21). The partially purified material was dissolved in CH_2Cl_2 (100 mL), washed with aq. HCl (0.5 M, 50 mL), separated from solvent by evaporation, and dried before using in the following step.

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α-[(4-methoxyphenyl)acetoxy]-12α-phenylaminocarbonyloxy-5β-cholan-24-oate chloride 8·Cl⁻. *N*,*N*-Diisopropylethylamine (265 μL, 1.52 mmol) was added to a stirred solution of crude guanidinium chloride **7**·Cl⁻ (500 mg, 0.76 mmol) in dry $ClCH_2CH_2Cl$ (5 mL) under an atmosphere of argon. 4-Methoxyphenylacetyl chloride (350 μL, 2.29 mmol) was added dropwise *via* a syringe, to give a pale yellow solution which was refluxed for 24 h while stirring. The resulting dark orange solution was allowed to cool to room temperature; TLC analysis (CH_2Cl_2–MeOH, 5:1) revealed almost complete conversion of the 7α-hydroxy steroid to one major steroidal product. The solution was evaporated under reduced pressure. Flash chromatography was twice carried out on the resulting residue; the first column was eluted with CH_2Cl_2–MeOH (30:1 graduating to 10:1). The second column was eluted with EtOAc graduating to EtOAc–MeOH (10:1). To ensure chloride was the counter anion, the resulting purified salt (93 mg) was taken up in CH_2Cl_2 (10 mL) and washed carefully with aq. NaOH (1 M, 2 × 5 mL) followed by triply distilled water (5 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 5 mL) followed by triply distilled water (2 × 5 mL). The organic phase was then dried by decanting and evaporated under reduced pressure. The resulting residue was then further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **8**·Cl⁻ (84.5 mg, 14% from crude **7**·Cl⁻) as a white film (HRMS: Found: M^+, 771.4691. $C_{45}H_{63}N_4O_7$ requires *m*/*z*, 771.4697); R_f 0.13 (EtOAc–MeOH, 10:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3406, 2951, 1724, 1647, 1513, 1442, 1377, 1314, 1225, 1198, 1178, 1080, 1046, 1028, 734, 694, and 668; δ_H(400 MHz; $CDCl_3$) 0.68 (3 H, s, 18-CH_3), 0.85 (3 H, s, 19-CH_3), 0.89 (3 H, d, *J* 6.0, 21-CH_3), 3.23 (4 H, br s, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.28 (1 H, br m, 3β-H), 3.61 (1 H, d, *J* 14.0, PhCH*H*), 3.68 (3 H, s, CO_2CH_3), 3.80 (3 H, s, PhOCH_3), 3.93 (1 H, d, *J* 14.6, PhCH*H*), 4.77 (1 H, m, 7β-H), 5.01 (1 H, m, 12β-H), 6.94 (2 H, d, *J* 8.6, ArH at position 7), 7.07 (1 H, t, *J* 7.3, ArH *para* at position 12), 7.34 (2 H, t, *J* 8.0, ArH *meta* at position 12), 7.50 (2 H, d, *J* 8.5, ArH at position 7), 7.57 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 7.73 (2 H, d, *J* 8.5, ArH *ortho* at position 12), 7.91 (1 H, s, NHCO), 8.12 (1 H, br s, 3α-NH); δ_C(100.6 MHz; $CDCl_3$) 12.39 (C-18), 17.54 (C-21), 20.09 (C-5 of the pyrimidinium ring), 22.43 (C-19), 22.59 (CH_2), 26.00 (CH_2), 26.89 (CH_2), 27.82 (CH_2), 28.92 (CH), 30.81 (CH_2), 31.00 (CH_2), 31.27 (CH_2), 33.86 (C-10), 34.76 (CH), 34.88 (CH_2), 35.41 (CH_2), 37.74 (CH), 38.35 (C-4,6 pyrimidinium ring), 41.21 (CH), 41.40 (PhCH_2), 42.98 (CH), 45.12 (C-13), 47.29 (CH), 51.19 (C-3), 51.38 (CO_2CH_3), 55.18 (PhOCH_3), 70.64 (C-7), 75.64 (C-12), 113.70 (ArCH at position 7), 119.06 (ArCH *ortho* at position 12), 122.94 (ArCH *para* at position 12), 127.48 (ArC at position 7), 128.77 (ArCH *meta* at position 12), 130.84 (ArCH at position 7), 138.82 (ArC at position 12), 152.59 (C), 153.56 (C), 158.01 (ArC at position 7), 171.27 (PhCH$_2$*C*=O), 174.41 (C-24); *m*/*z* (FAB) 771.6 (M^+, 100%), 468.4 (5), 100.1 (15).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α-(*tert*-butylaminocarbonyloxy)-12α-phenylaminocarbonyloxy-5β-cholan-24-oate chloride 9·Cl⁻. Aq. HCl (11.65 M, 7 μL) was added to a stirred solution of crude guanidinium chloride **7**·Cl⁻

(350 mg, 0.53 mmol) and *tert*-butyl isocyanate (300 μL, 2.63 mmol) in dry CH_2ClCH_2Cl (3.5 mL) under an atmosphere of argon. The stirred solution was gently refluxed for 6 d. The solution was allowed to cool to room temperature, and *tert*-butyl isocyanate (1.95 mL, 17.08 mmol) and chlorotrimethylsilane (20 μL, 0.16 mmol) were added portion wise with the solution being brought back to reflux after each addition. The mixture was allowed cool to room temperature, and then stirred in air for 2–3 h. Analysis by TLC (EtOAc–MeOH, 10:1) revealed the reaction had reached *ca.* 80% completion. The solution was evaporated under reduced pressure and the residue purified by flash chromatography eluting with EtOAc to afford the guanidinium salt (259 mg). To ensure chloride was the counter anion, the salt was taken up in CH_2Cl_2 (40 mL) and washed with aq. NaOH (1 M, 2 × 20 mL) followed by triply distilled water (20 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 20 mL) followed by triply distilled water (2 × 20 mL). The organic layer was dried by decanting and evaporated under reduced pressure. The resulting residue was then further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **9**·Cl^- (253 mg, 63% from crude **7**·Cl^-) as a white solid, mp 277–280 °C (Found: C, 63.48; H, 8.31; N, 8.97; Cl, 5.01. $C_{41}H_{64}N_5O_6Cl·H_2O$ requires C, 63.45; H, 8.58; N, 9.03; Cl, 4.51%) (HRMS: Found: M^+, 722.4850. $C_{41}H_{64}N_5O_6$ requires *m*/*z*, 722.4857); R_f 0.25 (EtOAc); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3446, 2950, 1718, 1648, 1534, 1442, 1379, 1314, 1273, 1224, 1083, 1049, 1028, 734, and 694; δ_H(400 MHz; $CDCl_3$) 0.74 (3 H, s, 18-CH_3), 0.86 (3 H, s, 19-CH_3), 0.94 (3 H, d, *J* 6.5, 21-CH_3), 1.37 [9 H, s, $C(CH_3)_3$], 2.66 (1 H, br m, CH*H*), 3.13 (1 H, br m, 3β-H), 3.24 (4 H, br m, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.63 (3 H, s, CO_2CH_3), 4.70 (1 H, m, 7β-H), 5.13 (1 H, m, 12β-H), 6.58 (1 H, s, NHCO), 6.74 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 6.97 (1 H, t, *J* 7.5, ArH *para*), 7.23 (2 H, t, *J* 7.8, ArH *meta*), 7.76 (2 H, d, *J* 7.5, ArH *ortho*), 8.65 (1 H, d, *J* 9.0, 3α-NH), 8.77 (1 H, s, NHCO); δ_C(100.6 MHz; $CDCl_3$) 12.46 (C-18), 17.71 (C-21), 20.26 (C-5 of the pyrimidinium ring), 22.07 (C-19), 22.90 (CH_2), 25.49 (CH_2), 26.70 (CH_2), 27.30 (CH_2), 28.36 (CH), 29.13 [$C(CH_3)_3$], 30.78 (CH_2), 31.12 (CH_2), 33.65 (C-10), 34.18 (CH_2), 34.91 (CH), 35.04 (CH_2), 38.10 (CH), 38.60 (C-4,6 pyrimidinium ring), 40.89 (CH), 43.09 (CH), 45.02 (C-13), 47.03 (CH), 50.23 [$C(CH_3)_3$], 50.79 (C-3), 51.40 (CO_2CH_3), 69.38 (C-7), 75.32 (C-12), 119.30 (ArCH *ortho*), 122.45 (ArCH *para*), 128.46 (ArCH *meta*), 139.40 (ArC), 153.18 (C), 153.83 (C), 155.13 (C), 174.71 (C-24); *m*/*z* (FAB) 722.5 (M^+, 100%), 706.5 (3), 621.5 (3), 601.5 (5), 585.5 (7), and 468.4 (11).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-ylamino)-7α-(2,6-diisopropylphenylaminocarbonyloxy)-12α-phenylamino-carbonyloxy-5β-cholan-24-oate chloride 10·Cl^-. Aq. HCl (11.65 M, 2 μL) was added to a stirred solution of crude guanidinium chloride **7**·Cl^- (100 mg, 152 μmol) and 98% 2,6-diisopropylphenyl isocyanate (50 μL, 234 μmol) in dry CH_2ClCH_2Cl (1.0 mL) under an atmosphere of argon. The stirred solution was refluxed for 6 d. The solution was allowed to cool to room temperature, and further aliquots of 2,6-diisopropylphenyl isocyanate (150 μL, 702 μmol) and aq. HCl (11.65 M, 6 μL) were added portion wise with the solution being brought back to reflux after each addition. The resulting yellow solution was allowed cool to room temperature, and then stirred in air for 2–3 h. Analysis by TLC (CH_2Cl_2–MeOH, 10:1) revealed the reaction had reached *ca.* 70% completion. The solution was evaporated under reduced pressure. Flash chromatography was twice carried out on the resulting residue; the first column was eluted with CH_2Cl_2–MeOH (30:1). The second column was eluted with EtOAc to afford the guanidinium salt (21 mg) which was pure by TLC. To ensure chloride was the counter anion, the salt was taken up in CH_2Cl_2 (4 mL) and washed with aq. NaOH (1 M, 2 × 2 mL) followed by triply distilled water (2 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 2 mL) followed by triply distilled water (2 × 2 mL). The organic layer was dried by decanting and evaporated under reduced pressure. The resulting residue was then further dried *in vacuo* at 60 °C to afford the pure *guanidinium chloride* **10**·Cl^- (18 mg, 14% from crude **7**·Cl^-) as a clear film (HRMS: Found: M^+, 826.5455. $C_{49}H_{72}N_5O_6$ requires *m*/*z*, 826.5483); R_f 0.32 (EtOAc), 0.31 (CH_2Cl_2–MeOH, 10:1); δ_H(400 MHz; $CDCl_3$) 0.79 (3 H, s, 18-CH_3), 0.91 (3 H, s, 19-CH_3), 0.99 (3 H, d, *J* 6.5, 21-CH_3), 1.18 [12 H, d, *J* 6.6, $CH(CH_3)_2$], 2.80 (2 H, br m, CH_2), 3.23 [3 H, br m, 3β-H and $CH(CH_3)_2$], 3.65 (3 H, s, CO_2CH_3), 4.77 (1 H, m, 7β-H), 5.21 (1 H, m, 12β-H), 6.79 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 6.93 (1 H, t, *J* 7.5, ArH *para* at position 12), 7.11 (2 H, d, *J* 7.5, ArH *meta* at position 7), 7.21 (3 H, m, 2H ArH *meta* at position 12 and 1H ArCH *para* at position 7), 7.73 (2 H, d, *J* 8.0, ArH *ortho* at position 12), 8.09 (1 H, br s, 3α-NH), 8.91 (2 H, br s, NHCO); δ_C(100.6 MHz; $CDCl_3$) 11.99 (C-18), 17.24 (C-21), 19.79 (C-5 of the pyrimidinium ring), 21.77 (C-19), 22.62 [$CH(CH_3)_2$], 22.74 (CH_2), 22.92 [$CH(CH_3)_2$], 23.10 [$CH(CH_3)_2$], 23.63 [$CH(CH_3)_2$], 25.07 (CH_2), 25.81 (CH_2), 26.95 (CH_2), 27.77 (CH), 28.21 [$CH(CH_3)_2$], 28.41 [$CH(CH_3)_2$], 30.30 (CH_2), 30.82 (CH_2), 32.03 (CH_2), 33.26 (C-10), 34.15 (CH_2), 34.51 (CH_2), 34.69 (CH), 37.69 (CH), 40.52 (CH), 42.64 (CH), 44.61 (C-13), 46.78 (CH), 49.72 (C-3), 51.04 (CO_2CH_3), 69.89 (C-7), 74.35 (C-12), 118.59 (ArCH *ortho* at position 12), 121.81 (ArCH *para* at position 12), 122.49 (ArCH *meta* at position 7), 122.88 (ArCH *meta* at position 7), 127.43 (ArCH), 127.99 (ArCH), 131.66 (ArC), 139.04 (ArC at position 12), 146.55 (ArC), 147.18 (ArC), 152.34 (C), 153.24 (C), 155.44 (C), 174.30 (C-24); *m*/*z* (FAB) 827 (M^+, 100%), 706 (5), 690 (5), 622 (5), 606 (6), and 469 (15).

Methyl 3α-(1,4,5,6-tetrahydropyrimidin-3-ium-2-yl)amino-7α-(2,6-dichlorophenylaminocarbonyloxy)-12α-phenylamino-carbonyloxy-5β-cholan-24-oate chloride 11·Cl^-. Aq. HCl (11.65 M, 6 μL) was added to a stirred mixture of crude guanidinium chloride **7**·Cl^- (350 mg, 0.53 mmol) and 98% 2,6-dichlorophenyl isocyanate (500 mg, 2.66 mmol) in dry CH_2ClCH_2Cl (4 mL) in a flask equipped with a reflux condenser under an atmosphere of argon. The resulting pale yellow suspension was stirred at 50 °C for 2 d. The mixture was allowed to cool to room temperature, and then stirred in air for 2–3 h. Analysis by TLC (EtOAc–MeOH, 10:1) revealed that the reaction had reached *ca.* 80% completion. The mixture was filtered and the filtered powder washed with CH_2Cl_2 (2 × 10 mL). The resulting yellow filtrate was evaporated under reduced pressure and the residue (700 mg) purified by flash chromatography, eluting with EtOAc, followed by successive recrystallisations from hexane–$CHCl_3$ to afford the guanidinium salt (165 mg). To ensure chloride was the counter anion, the salt was taken up in CH_2Cl_2 (20 mL) and washed with aq. NaOH (1 M, 2 × 10 mL) followed by triply distilled water (10 mL). The organic layer was then washed with aq. HCl (0.5 M, 2 × 10 mL) followed by triply distilled water (2 × 10 mL). The organic layer was dried by decanting and evaporated under reduced pressure. The resulting residue was then further dried *in vacuo* at 50 °C to afford the pure *guanidinium chloride* **11**·Cl^- (147 mg, 33% from crude **7**·Cl^-) as a white foam, mp 302–304 °C (HRMS: Found: M^+, 810.3781. $C_{43}H_{58}N_5O_6Cl_2$ requires *m*/*z*, 810.3764); R_f 0.21 (EtOAc), 0.46 (EtOAc–MeOH, 10:1); ν_{max}(film from CH_2Cl_2)/cm^{-1} 3425, 2948, 1723, 1644, 1601, 1531, 1442, 1379, 1314, 1228, 1198, 1081, 1049, 1028, 783, and 695; δ_H(400 MHz; $CDCl_3$) 0.78 (3 H, s, 18-CH_3), 0.92 (3 H, s, 19-CH_3), 0.98 (3 H, d, *J* 6.5, 21-CH_3), 1.63 (6 H, s, water), 2.46 (1 H, br m), 2.94 (1 H, br m, CH*H*), 3.06 (1 H, br m, 3β-H), 3.25 (4 H, br m, 2 × CH_2 at positions 4,6 of the pyrimidinium ring), 3.64 (3 H, s, CO_2CH_3), 4.88 (1 H, m, 7β-H), 5.20 (1 H, m, 12β-H), 6.42 (2 H, br s, 2 × NH at positions 1,3 of the pyrimidinium ring), 6.96 (1 H, t, *J* 7.3, ArH *para* at position 12), 7.10 (1 H, t, *J* 8.0, ArH *para* at position 7),

7.24 (2 H, t, *J* 8.0, ArH *meta* at position 12), 7.32 (2 H, d, *J* 8.0, ArH *meta* at position 7), 7.80 (2 H, d, *J* 8.0, ArH *ortho* at position 12), 8.71 (1 H, br s, 3α-NH), 8.82 (1 H, br s, NHCO), 9.05 (1 H, br s, NHCO); δ_C(100.6 MHz; $CDCl_3$) 12.38 (C-18), 17.72 (C-21), 20.16 (C-5 of the pyrimidinium ring), 22.12 (C-19), 22.91 (CH_2), 25.53 (CH_2), 26.47 (CH_2), 27.39 (CH_2), 28.31 (CH), 30.80 (CH_2), 31.07 (CH_2), 31.15 (CH_2), 33.66 (C-10), 34.56 (CH_2), 34.98 (CH), 35.04 (CH_2), 38.08 (CH), 38.46 (C-4,6 pyrimidinium ring), 40.82 (CH), 43.06 (CH), 45.07 (C-13), 47.23 (CH), 50.68 (C-3), 51.42 (CO_2CH_3), 71.59 (C-7), 75.09 (C-12), 119.12 (ArCH *ortho* at position 12), 122.48 (ArCH *para* at position 12), 128.17 (ArCH), 128.27 (ArCH), 128.50 (ArCH), 132.82 (ArCCl), 134.79 (ArC at position 7), 139.34 (ArC at position 12), 152.88 (C), 153.71 (C), 153.90 (C), 174.65 (C-24); *m*/*z* (FAB) 810.4 ($[M - H]^+$, 100%), 689.4 (4), 673.3 (5), 605.4 (10), and 468.4 (28).

Typical procedure for extraction experiments. A solution of receptor **5**·Cl^- in distilled, acid free $CHCl_3$ (1 mL, 6.4 mM) was added to an aqueous solution of *N*-Ac-DL-valine [5 mL, 7.7 mM, made up using a pH 7.4 (0.1 M) aqueous phosphate buffer (KH_2PO_4/K_2HPO_4)]. The resulting biphasic solution was stirred vigorously in a closed glass tube for 2 h. After cessation of stirring, the solution was left to settle for 10 min. The organic layer was isolated and dried by passing it through hydrophobic filter paper (Aldrich catalogue no. *z*13346–9). The filtrate was evaporated under reduced pressure (maintaining the temperature below 35 °C), the residue dissolved in 0.6 mL $CDCl_3$ and subjected to ^{1}H NMR analysis (> 256 scans).

Monte Carlo Molecular Mechanics (MCMM) calculations on 11·*N*-Ac-L-valine and 11·*N*-Ac-D-valine. Computations were performed with MacroModel V5.5,[18] employing the Amber* force field, $CHCl_3$ GB/SA solvation, and 5000 steps of MCMM. 6 and 3 separate searches were conducted for the L and D substrates respectively, all from widely differing starting geometries. All searches yielded structures essentially similar to those in Fig. 2.

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