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Differentially-protected steroidal triamines; scaffolds with potential for medicinal, supramolecular, and combinatorial chemistry†

Vicente del Amo,[a] Laura Siracusa,[a,b] Theodoros Markidis,[a] Beatriz Baragaña,[b] Khadga M. Bhattarai,[a] Marta Galobardes,[a] Gregorio Naredo,[a] M. Nieves Pérez-Payán[b] and Anthony P. Davis*[a]

[a] *School of Chemistry, University of Bristol, Cantock's Close, Bristol, UK BS8 1TS. E-mail: anthony.davis@bristol.ac.uk; Fax: +44 (0) 117 929 8611; Tel: +44 (0) 117 954 6334*

[b] *Trinity College, Dublin 2, Ireland*

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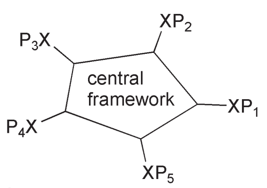
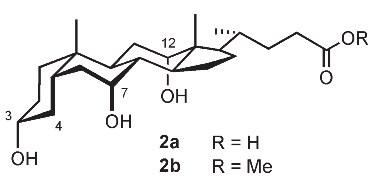
Cholic acid **2a** has been converted into two new orthogonally-protected triamino scaffolds, **13** and **14**. The synthesis proceeds *via* the bis-Boc-NH-substituted azide **10**, for which an improved preparation is described. After removal of the Boc groups, the two axial amines are differentiated through a novel monoprotection employing 1-(2-nitrobenzenesulfonyloxy)-benzotriazole **29**. Regioselectivity of ≥50 : 1 is achieved, presumably reflecting an exceptional sensitivity to steric hindrance. Protection of the remaining amino group as Boc or Alloc gives the scaffolds in ∼40% overall yield from cholic acid. Scaffold **13** has been sequentially deprotected and derivatised with *N*-carbamoyl amino acids, to give a model for tripodal peptide libraries.

Introduction

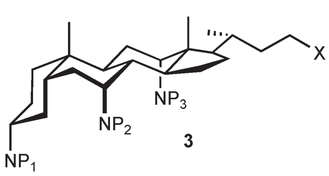
Polyfunctionalised scaffolds **1** are key components for many areas of chemistry. They are needed as core units for combinatorial libraries,[1] dendrimers,[2] template-assembled synthetic proteins (TASPs),[3] glycoclusters,[4] and podand-type synthetic receptors.[1e,f,5] For most of these applications, scaffolds should possess well-defined architectures which confer distinctive shapes on derived compounds or libraries. The steroid nucleus is an attractive starting point for such systems, being readily available, rigid, extended and chiral. Bile acids, such as cholic acid **2a**, have proved especially useful.[1c–e,2b,3b,4c,5c] Firstly, they are inexpensive; cholic acid, at *ca.* €0.4 g^{-1},[6] is the second-cheapest of the commercially-available steroids (after cholesterol). Secondly, they possess high degrees of functionality



1 X = O,N
P = protecting/masking groups



2a R = H
2b R = Me



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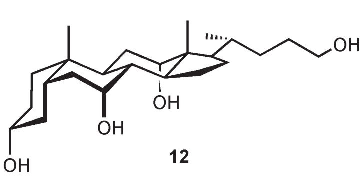
P_1, P_2, P_3 = orthogonal *N*-protection

in well-spaced arrays. Thirdly, they are readily adapted for solid-phase synthesis; the carboxyl-functionalised side-chain provides a natural point of attachment to a resin. Fourthly, they provide co-directed functionality, especially useful if the appended groups are to act cooperatively (*e.g.* in synthetic receptors or catalysts, TASPs, or most biological applications).

In developing the bile acids as scaffolds, two issues must be addressed. Firstly, the "natural" hydroxyl groups are relatively unreactive and difficult to derivatise (especially the axial 7- and 12-OH). The problem can be solved in individual cases, but for rapid, trouble-free elaboration (especially on solid phase), amino groups are preferable. Secondly, differential and orthogonal protection adds greatly to the versatility of any scaffold. The "ideal" bile acid scaffold would therefore be of form **3**. The synthesis of such compounds, however, is not straightforward, especially considering that gram quantities are needed for most applications. The conversion of **2a** to **3** requires efficient stereocontrol at three sterically different centres, and also good regiocontrol. A number of "less ambitious" amino-scaffolds have been described (Fig. 1), including (a) the difunctional **4** and **5** due to Still;[1e,7] (b) ketone **6** due to Kasal *et al.*;[8] (c) undifferentiated triamine **7** due to Savage;[9] (d) alcohol **8**,[10] tris-Boc-protected triamine **9**[11] and bis-Boc-azide **10**,[12] all due to our own group. However, only one fully-differentiated triamino scaffold has been reported. Compound **11** was prepared by Savage and coworkers in ∼15% overall yield from tetraol **12**, in a sequence, consisting of 12 steps.[13] The process is workable on a gram scale but, as all steps require chromatography, is probably not ideal for routine use.



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As mentioned above, we have previously described a partially-differentiated triamino-scaffold, the azide **10**. An intermediate for tris-ammonium "facial amphiphiles"[14] and "cholapod" anion receptors,[5c,15] this compound is available *via* a high-yielding, large-scale process. In principle, deprotection at positions 7 and 12 followed by stepwise reprotection could give a synthesis of **3** in just three further steps. However, this requires a reagent of exceptional selectivity, able to distinguish between two axial

† Electronic supplementary information (ESI) available: Regiochemical assignment of monoprotected bile acid derivatives and estimation of regioselectivities. See http://www.rsc.org/suppdata/ob/b4/b412298d/

DOI: 10.1039/b412298d

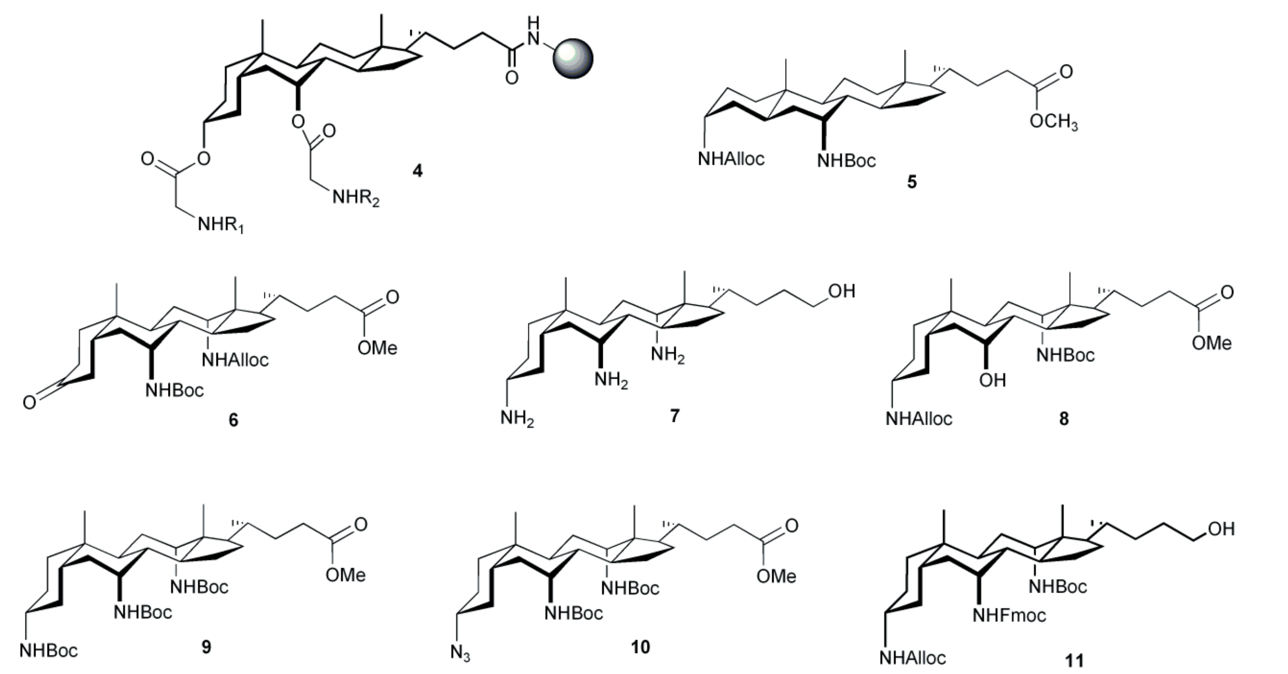
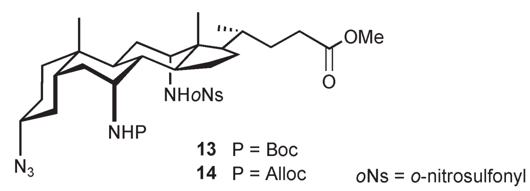


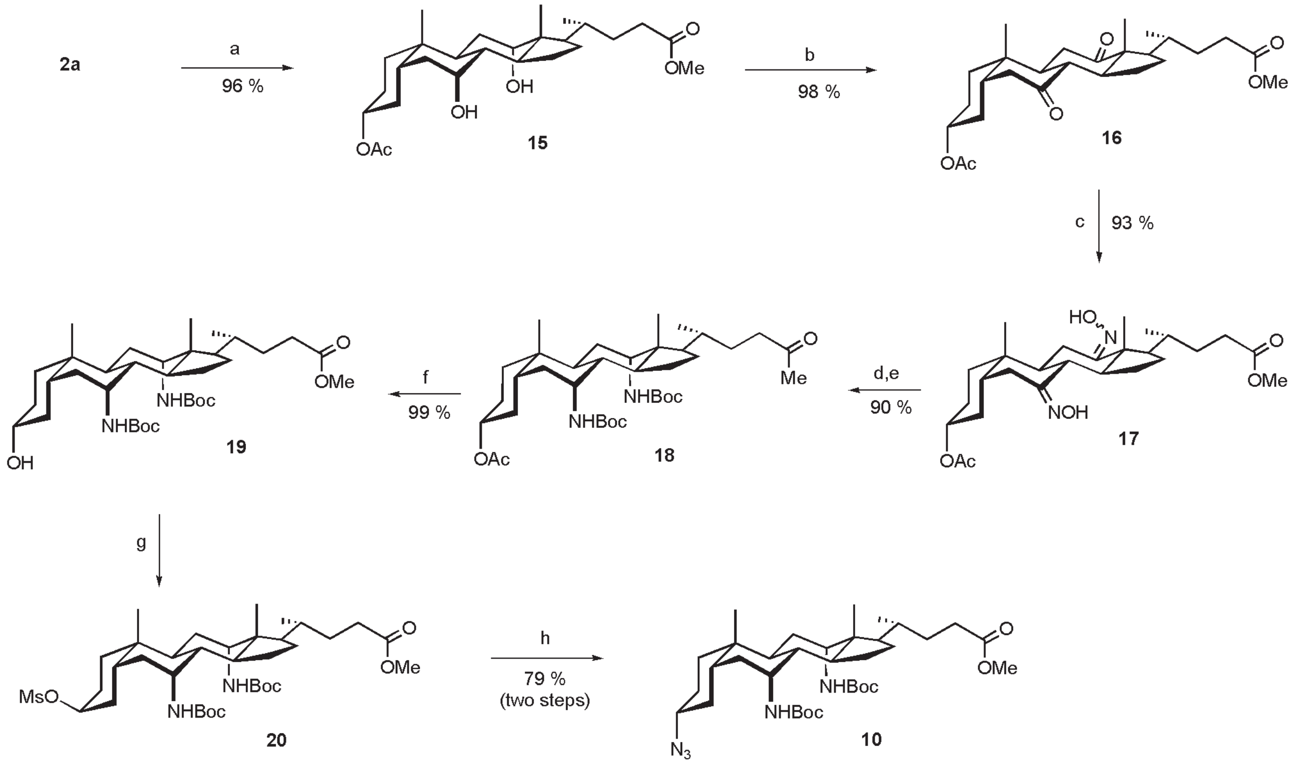
Fig. 1 Amino-scaffolds derived from bile acids, previously reported in the literature.

amino groups. We now report a novel protection method which demonstrates this capability, and enables practical syntheses of two new orthogonally-protected triamino scaffolds, **13** and **14**, in ~40% yield from cholic acid **2a**. We also give details of an updated preparation of **10**, and show that **13** can be sequentially deprotected and derivatised with *N*-carbamoyl amino acids to give a model for tripodal peptide libraries.



Results and discussion

Our current, optimised preparation of **10** is summarised in Scheme 1. Compared to the earlier process[12] this version incorporates a new oxidation method for **15** → **16** which avoids toxic Cr(VI) reagents, a new method for **18** → **19**, and modified procedures for **16** → **17** and **20** → **10**. Only the last two steps require chromatography. The steps up to **19** are performed routinely on a 20 g scale, and the final 2 steps on a 10 g scale. The overall yield is 62% from cholic acid **2a**. Treatment of **10**

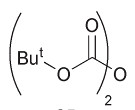


Scheme 1 Optimised synthesis of bis-differentiated scaffold **10**. Reagents and conditions: (a) MeOAc, *p*-TsOH·H_2O (cat.), reflux, 48 h, then $MgSO_4$, reflux, overnight; (b) $Ca(ClO)_2$, KBr (cat.), AcOH/H_2O, 0 °C to rt, overnight; then 2-propanol, rt, 1.5 h; (c) NH_2OH·HCl, NaOAc, MeOH, reflux, overnight; (d) H_2, PtO_2, AcOH, rt, 3 d then Zn, AcOH, rt, 24 h; (e) Boc_2O, $NaHCO_3$, THF/H_2O, rt, 2 d; (f) NaOH, MeOH (dry), 0 °C., 4 h; (g) MsOH, PPh_3, DEAD, DMAP, THF, 0 °C then rt, 48 h; (h) NaN_3, DMF, 47 °C, 3 d.

with TFA in DCM, followed by washing with sat. aq. Na_2CO_3, gave diamine **21** for studies on regioselective protection.

The differentiation of positions 7 and 12 on the bile acid skeleton is a long-standing problem in steroid chemistry. A classical solution is the 3,7-bis-acetylation of methyl cholate with acetic anhydride/pyridine, due to Fieser and Rajagopalan.[16] Although this might imply that the 7-OH is less hindered, detailed studies by Blickenstaff showed that, in the general case, the 12-OH is more accessible; Fieser's 3,7-bis-acetylation relies on a specific acceleration of acetylation at 7 by a free 12-OH.[17] Blickenstaff's results accord with expectation, the steroidal C4 is very close to a 7α substituent and seems likely to lower its reactivity. Other acylation conditions can indeed lead preferentially to 3,12-derivatisation.[18] However, regioselectivities are generally quite modest for both 3,7- and 3,12-bis-acylations. These preparations are only useful because of fortuitious crystallisations.

Initial studies on diamine **21** suggested similar tendencies to the 7,12-diols. Treatment with 1 equivalent of the common protecting agents $(Boc)_2O$ **25**, BOC-ON **26** and AllocCl **27** gave mixtures in which the 12-protected monoamine **22** predominated (Scheme 2 and Table 1, entries 1–4). However, yields were low, and substantial quantities of diprotected compound **24** were also present. Such product mixtures are expected if the 12α-NH_2 continues to react rapidly after monoprotection at 7α-NH_2, so that much of the **23** formed is converted to **24**.‡ Separation of **22** and **23** could not be achieved, so none of these methods could be considered useful.



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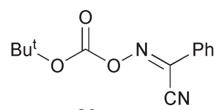

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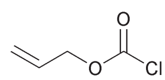

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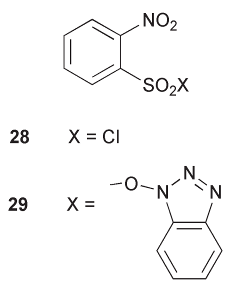

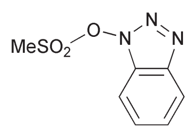

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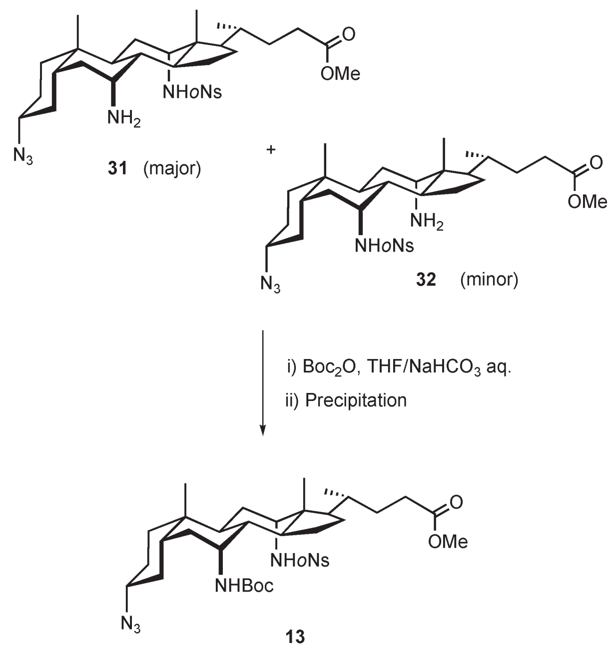
In the hope that a sulfonyl-based electrophile might behave differently to the carbonyl-based **25**–**27**, we investigated *o*-nitrobenzenesulfonyl chloride (*o*NsCl, **28**). The *o*-nitrobenzenesulfonyl group has been used to both activate and protect primary amines.[19] Removal is accomplished by nucleophilic attack with thiolate. Diamine **21** was treated with **28** in the presence of triethylamine or 2,6-lutidine (Table 1, entries 5 and 6). Regioselectivities were still moderate, but the yields of monoprotected products **22** and **23** improved significantly. However, once again, these regioisomers could not be separated.

To increase sensitivity to steric hindrance, we considered changing the leaving group. Methanesulfonyloxy benzotriazole **30** had been used as a selective *N*-mesylating agent, capable of distinguishing between amino nitrogens in differing steric environments.[20] *o*-Nitrobenzenesulfonyl analogue **29** had been employed as a peptide coupling agent,[21] but not for *N*-protection. **29** was prepared from **28** and 1-hydroxybenzotriazole, and applied to the monoprotection of **21**. As shown in Table 1 (entry 7), the yield remained high and the regioselectivity did indeed improve significantly. The mixture of **31** and **32** (≥50 : 1) was treated with $(Boc)_2O$ and the crude product precipitated from Et_2O–hexane to give **13** (Scheme 3). The overall yield was 67% from **10** and 41% from cholic acid **2a**. A 2.6 g batch of **13** has been prepared, and we estimate that up to 7 g should be feasible with standard laboratory equipment.

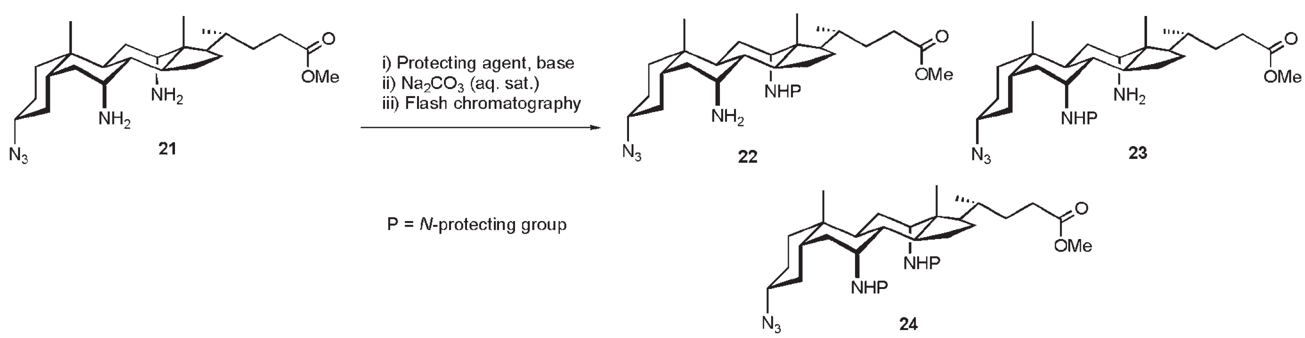


Scheme 3

Steroid **13** conforms well to the ideal of **3**, being furnished with fully orthogonal protection (Boc removable with acid, *o*Ns with thiolate, N_3 by treatment with R_3P/H_2O). None of the groups is sensitive to the basic conditions required to hydrolyse the side-chain methyl ester. To demonstrate its potential the groups were unmasked and derivatised in turn as shown in Scheme 4. The protecting groups were removed in the order 12-*o*Ns, 7-Boc and 3-N_3. The sequence proceeded without incident; the conditions required to remove the *o*Ns, though seemingly aggressive, did not appear to affect any other functional group in **13**.

Finally, the scaffold **13** presents a potential difficulty if one wishes to prepare template-assembled synthetic proteins, or peptide libraries of general form **37**, using Fmoc-based solid

‡ Kinetic modelling suggests that, for these entries, reaction at position 12 might be accelerated by 7-protection. This acceleration does not seem to apply in the case of *o*-nitrobenzenesulfonyl protection (entries 5–7).

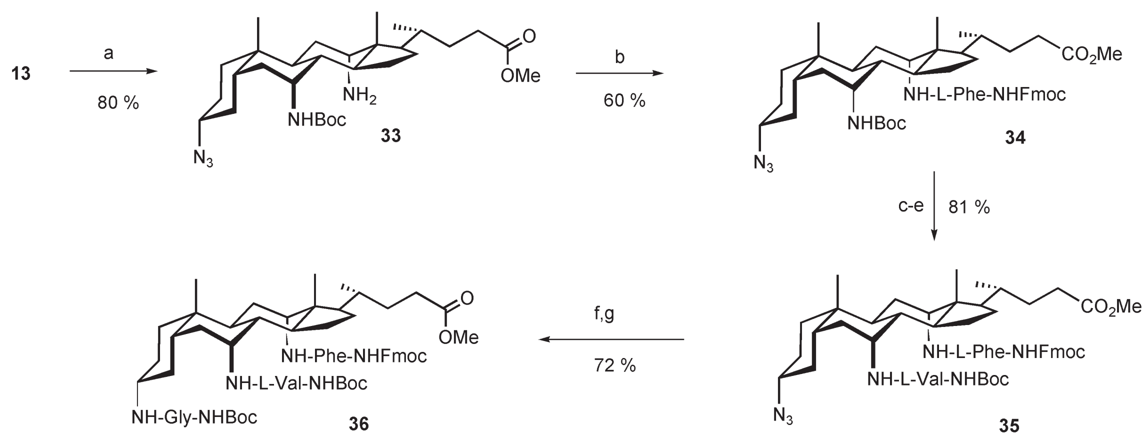


Scheme 2

Table 1 Attempted regioselective protections of diamine **21**[a]

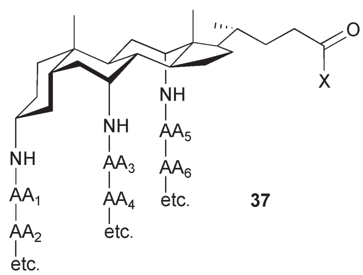
Entry	Reagent[b]	Base[c]	Time/h	Ratio **22**/**23**[d]	Yield **22** + **23** (%)[e]	Yield **24** (%)[e]
1.	Boc_2O **25**	—	48	16 : 1	50	24
2.	Boc_2O **25**	TEA	48	8 : 1	46	25
3.	BOC-ON **26**	—	48	7 : 1	39	27
4.	AllocCl **27**	2,6-lutidine	24	4 : 1	30	29
5.	*o*NsCl **28**	TEA	12	7 : 1	71	13
6.	*o*NsCl **28**	2,6-lutidine	12	6 : 1	72	11
7.	**29**	—	12	≥50 : 1	75	10

[a] All reactions were performed at room temperature with THF as solvent. [b] 1 equivalent. [c] 1.1 equivalents, where relevant. [d] Determined by ^{1}H-NMR integration (C18 methyl group). For further details, including regiochemical assignments, see electronic supplementary information.† [e] After isolation by flash chromatography.



Scheme 4 Reagents and conditions: (a) PhSH, Cs_2CO_3, DMF, 55 °C, overnight; (b) *N*-Fmoc–L-Phe-OH, TBTU, HOBt, DIPEA, DMF/DCM; (c) TFA, DCM, 0 °C, 2 h; (d) sat. Na_2CO_3 aq.; (e) *N*-Boc–L-Val-OH, TBTU, HOBt, DIPEA, DMF/DCM; (f) PMe_3, THF, rt, 5 h then H_2O, rt, overnight; (g) *N*-Boc–Gly-OH, TBTU, HOBt, DIPEA, DMF/DCM.

phase synthesis. The standard *N*-Fmoc-protected amino acid derivatives possess acid-labile protecting groups in their side-chains, so acidic conditions cannot be used to reveal a steroid nitrogen once a peptide chain is in place. The 7-Boc group in **13** must thus be the first to be removed. However, this means that the 12-*o*Ns must be removed later in the sequence, after synthesis of at least one peptide. Although *N*-*o*Ns protection has been advanced as a general method for peptide synthesis,[19b] the removal conditions are vigorous and might not be compatible with all sequences and side-chain protections. On this basis, the 12-*o*Ns should be removed before the others in **13**. The conflict may be resolved by replacing the 7-Boc protection with a group removable by non-acidic conditions, and still orthogonal to *o*Ns and N_3. Alloc fulfils these criteria, deprotection being accomplished with a nucleophile under Pd catalysis. Derivative **14** was therefore prepared by treatment of **31** with reagent **27** (83% yield, 38% overall from cholic acid). Scaffold **14** should complement **13**, being suitable for tris-peptide libraries and other cases where acidic conditions must be avoided.



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Conclusion

In conclusion, we have developed practical syntheses for two new orthogonally-protected triamino scaffolds based on the bile acid framework. The key step in these preparations is a highly regioselective monoprotection of 7α,12α-diamine **21** by *o*-nitro-benzenesulfonyl derivative **29**. The results suggest that reagent **29** is exceptionally sensitive to steric hindrance, a property which should find further use in synthesis. Scaffolds **13** and **14** may be used to construct a wide range of tripodal molecules for use as receptors, catalysts, pharmaceuticals *etc.* They are especially well suited to the preparation of combinatorial libraries using solid phase synthesis.

Experimental

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Methanol was distilled over calcium chloride, magnesium and iodine. DMF was obtained dry from Aldrich. THF and DCM were obtained dry from an Anhydrous Engineering Solvent Purification System (AESPS). Analytical TLC was carried out on DC-Alufolien Kieselgel 60F_{254} 0.2 mm plates (Merck) and compounds were visualised by UV fluorescence, 5% phosphomolybdic acid in ethanol, ninhydrine solution or by charring over a Bunsen burner flame. Flash chromatography of reaction products was carried out using Silica 60A, particle size 35–70 micron (Fischer Scientific). IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. The most intense bands were only quoted. Melting points were obtained using Gallekamp melting point blocks and are quoted as uncorrected values. ^{1}*H*-NMR and ^{13}*C*-NMR spectra were recorded on a Jeol Delta/GX270 or Jeol Delta/GX400 spectrometer, using deuterated solvents and were referenced internally to the residual solvent peak or TMS (δ_H = 0.00 ppm, δ_C = 0.00 ppm) signal. Coupling constants (*J*-Values) are given in Hz. The DEPT 135° technique was used to assign (CH_2) signals. Chemical shifts are reported as follows: value (description of absorption, coupling constant(s) where applicable, number of protons, assignment). NMR spectra assignation was aided by comparison with literature values for similar compounds. In all this experimental section only clear identifiable peaks are assigned.

Methyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate 15.[22]

Cholic acid **2a**, (62.00 g, 151.70 mmol) and *p*-toluenesulfonic acid monohydrate (3.00 g, 15.70 mmol) were suspended in methyl acetate (750 mL), refluxed for 48 h, then allowed to cool to room temperature. $MgSO_4$ (20 g, 166.15 mmol) was added to the resulting yellow solution and the mixture refluxed overnight. $NaHCO_3$ (1.32 g, 15.70 mmol) in H_2O (15 mL) was added dropwise. The mixture was filtered through a plug of silica (*ca.* 35 g) washing thoroughly with EtOAc to obtain a solution which was evaporated under reduced pressure. The resulting white foam was recrystallised from EtOAc–hexane in several crops to give diol **15** (67.98 g, 96%) as a white powder. An analytical sample was recrystallised from DCM–hexane. Mp 150–151 °C (lit.[22] mp 153–155 °C); R_f = 0.46 (Et_2O); IR (solid state): ν_{max} = 3300, 2938, 1723 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.70 (s, 3 H, 18-H_3), 0.91 (s, 3 H, 19-H_3), 0.98 (d, *J* = 6.3 Hz, 3 H, 21-H_3), 2.01 (s, 3 H, CH_3CO_2), 2.18–2.41 (m, 4 H), 3.67 (s, 3 H, CO_2CH_3), 3.85 (broad s, 1 H, 7β-*H*), 3.99 (broad s, 1 H, 12β-*H*), 4.58 (m, 1 H, 3β-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.57 (18-CH_3), 17.37 (21-CH_3), 21.43 (CH_3CO_2), 22.54 (19-CH_3), 23.15 (CH_2), 26.66 (*C*H), 27.41 (CH_2), 28.44 (CH_2), 30.92 (CH_2), 31.08 (CH_2), 34.38 (CH_2), 34.70 (*C*), 34.91 (CH_2), 35.13 (*C*H), 35.25 (CH_2), 39.65 (*C*H), 41.24 (*C*H), 42.09 (*C*H), 46.58 (*C*), 47.26 (*C*H), 51.47 (CO_2CH_3), 68.25 (*C*H), 72.88 (*C*H), 74.30 (*C*H), 170.71 (CH_3CO_2), 174.63 (CO_2CH_3); Anal. found: C, 69.64; H, 9.49%. $C_{27}H_{44}O_6$ requires: C, 69.79; H, 9.49%.

Methyl 3α-acetoxy-7,12-dioxo-5β-cholan-24-oate 16

Calcium hypochlorite (65% by weight, 14.2 g, 64.6 mmol) suspended in H_2O (150 mL) was added dropwise to a solution of diol **15** (20.00 g, 43.04 mmol), acetic acid (260 mL) and a few crystals of potassium bromide at 0 °C. The reaction was stirred overnight at room temperature before the addition of isopropanol (6 mL). After 1.5 h the solution was poured into an ice–water mixture under vigorous stirring. The resulting white solid was filtered and washed thoroughly with water to give diketone **16** (19.4 g, 98%) as a white powder. An analytical sample was recrystallised from AcOH–H_2O. Mp 159–160 °C (lit.[23] mp 163–164 °C); R_f = 0.31 (Toluene–EtOAc 4 : 1); IR (solid state): ν_{max} = 2970, 1738, 1714 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84 (d, *J* = 6.2 Hz, 3 H, 21-H_3), 1.04 (s, 3 H, 18-H_3), 1.31 (s, 3 H, 19-H_3), 2.00 (s, 3 H, CH_3CO_2), 2.21–2.31 (m, 3 H), 2.36–2.44 (m, 1 H), 2.71 (t, *J* = 12.8 Hz, 1 H), 2.80 (t, *J* = 11.7 Hz, 1H), 2.90 (q, *J* = 6.6 Hz, 1 H), 3.67 (s, 3 H, CO_2CH_3), 4.67 (m, 1 H, 3β-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.77 (18-CH_3), 18.60 (21-CH_3), 21.15 (CH_3CO_2), 22.40 (19-CH_3), 25.13 (CH_2), 25.80 (CH_2), 27.61 (CH_2), 30.49 (CH_2), 31.28 (CH_2), 33.14 (CH_2), 33.75 (CH_2), 35.51 (*C*H), 35.78 (*C*), 38.31 (CH_2), 44.95 (*C*H), 45.18 (CH_2), 45.46 (*C*H), 45.64 (*C*H), 48.92 (*C*H), 51.39 (CO_2CH_3), 51.82 (*C*H), 56.84 (*C*), 72.34 (*C*H), 170.41 (CH_3CO_2), 174.46 (CO_2CH_3), 209.07 (*C*=O), 212.34 (*C*=O); Anal. found: C, 70.18; H, 8.72%. $C_{27}H_{40}O_6$ requires C, 70.41; H, 8.75%.

Methyl 3α-acetoxy-7,12-dioximino-5β-cholan-24-oate 17

The diketone **16** (14 g, 30.5 mmol), sodium acetate (12.5 g, 152 mmol) and hydroxylamine hydrochloride (6.32 g, 91 mmol) were suspended in methanol (290 mL). The mixture was heated under reflux overnight. The resulting pale suspension was cooled, to give a precipitate which was collected by filtration. The solid was suspended in water and again collected by filtration. The filtrate from the initial filtration (methanol solvent) was concentrated to a small volume (*ca.* 10 mL). Water (*ca.* 100 mL) was added and the resulting precipitate was collected by filtration. The solids were combined to give dioxime **17** (13.9 g, 93%) as a white powder. An analytical sample was recrystallised from $CHCl_3$–hexane. Mp >261 °C (decomp); R_f = 0.70 (Et_2O); IR (solid state): ν_{max} = 3259, 2940, 1737, 1659 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.92 (d, *J* = 6.0 Hz, 3 H, 21-H_3), 0.93 (s, 3 H, 18-H_3), 1.15 (s, 3 H, 19-H_3), 2.01 (s, 3 H, CH_3CO_2), 2.35–2.41 (m, 1 H), 2.44 (t, *J* = 11.2 Hz, 1 H), 3.10 (dd, *J* = 13.2, 1.4 Hz, 1 H), 3.25 (dd, *J* = 13.2, 4.80 Hz, 1 H), 3.66 (s, 3 H CO_2CH_3), 4.71 (m, 1 H, 3β-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.92 (18-CH_3), 18.88 (21-CH_3), 19.72 (CH_2), 20.93 (CH_3CO_2), 22.23 (19-CH_3), 25.06 (CH_2), 25.84 (CH_2), 27.17 (CH_2), 27.63 (CH_2), 30.45 (CH_2), 31.40 (CH_2), 32.36 (CH_2), 34.07 (CH_2), 35.60 (*C*H), 35.76 (*C*), 41.81 (*C*H), 43.71 (*C*H), 43.73 (*C*H), 45.93 (*C*H), 49.12 (*C*), 51.23 (CO_2CH_3), 52.52 (*C*H), 73.18 (*C*H), 158.78 (*C*), 163.77 (*C*), 170.94 (CH_3CO_2), 175.07 (CO_2CH_3); Anal. found: C, 65.82; H, 8.54; N, 5.76%. $C_{27}H_{42}N_2O_6$ requires C, 66.10; H, 8.63; N, 5.71%.

Methyl 3α-acetoxy-7α,12α-di[*N*-(*t*-butyloxycarbonyl)amino]-5β-cholan-24-oate 18

A mixture of the dioximino compound **17** (10 g, 20.4 mmol) and platinum (IV) oxide hydrate (Adams' catalyst) (1 g, 10% by weight) in glacial acetic acid (52 mL) was stirred under 1 atmosphere of H_2 for 3 d, during which the initial slurry turned into a greenish solution. The reaction mixture was filtered,§ washing with acetic acid, and zinc powder (20 g) was added to the combined filtrates. The mixture was stirred for 24 h before removing the zinc by filtration. The solvents were evaporated under reduced pressure and the residue was dissolved in a mixture of THF (162 mL) and saturated aqueous $NaHCO_3$ (80 mL). Di-*tert*-butyl dicarbonate (11.3 g, 60.8 mmol) was added to the mixture, which was then stirred for 2 d. The layers were separated and the aqueous phase was extracted with ethyl acetate. The organic volumes were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give bis-carbamate **18** (11.9 g, 90%) as a white foam. An analytical sample was recrystallised from DCM–hexane. Mp 217–222 °C; R_f = 0.73 (hexane–EtOAc 1 : 1); IR (solid state): ν_{max} = 3370, 2958, 1738, 1708 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.79 (s, 3 H, 18-H_3), 0.92 (d, *J* = 7.4 Hz, 3 H, 21-H_3), 0.93 (s, 3 H, 19-H_3), 1.43 (s, 18 H, $(CH_3)_3C$), 2.02 (s, 3 H, CH_3CO_2), 2.18–2.50 (m, 2 H), 3.68 (broad s, 1 H, 7β-*H*), 3.71 (s, 3 H, CO_2CH_3), 4.00 (broad s, 1 H, 12β-*H*), 4.57 (m, 1 H, 3β-*H*), 5.20 (broad s, 1 H, 7-CH–N*H*R), 5.40 (broad s, 1 H, 12-CH–N*H*R); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.53 (18-CH_3), 17.50 (21-CH_3), 21.30 (CH_3COO), 22.76 (19-CH_3), 22.91 (CH_2), 26.68 (CH_2), 27.13 (CH_2), 27.81 (CH_2), 28.49 ($(CH_3)_3C$), 28.52 ($(CH_3)_3C$ and *C*H), 30.48 (CH_2), 31.72 (CH_2), 32.23 (CH_2), 34.76 (CH_2 and *C*), 34.92 (CH_2), 35.80 (*C*H), 37.06 (*C*H), 41.40 (*C*H), 44.31 (*C*H), 44.64 (*C*), 47.19 (*C*H), 49.49 (*C*H), 52.27 (CO_2CH_3), 53.23 (*C*H), 74.39 (*C*H), 78.30 ($(CH_3)_3C$), 78.48 ($(CH_3)_3C$), 155.44 (NH*C*O), 155.53 (NH*C*O), 170.08 (CH_3CO_2), 176.65 (CO_2CH_3); Anal. found: C, 67.19; H, 9.47; N, 3.95%. $C_{37}N_{62}N_2O_8$ requires C, 67.04; H, 9.43; N, 4.23%.

Methyl 3α-hydroxy-7α,12α-di[*N*-(*t*-butyloxycarbonyl)amino]-5β-cholan-24-oate 19

The acetoxy cholanoate **18** (14 g, 21.6 mmol) was dissolved in dry methanol (125 mL), and a solution of sodium hydroxide (1.26 g, 31.5 mmol) in dry methanol (10 mL) was added dropwise. The mixture was stirred at 0 °C for 4 h before evaporating the solvent under reduced pressure. The residue was redissolved in diethyl ether, washed thoroughly with H_2O, dried with $MgSO_4$ and evaporated under reduced pressure to give hydroxy cholanoate **19** (13.3 g, 99%) as a white foam. An analytical sample was recrystallised from DCM–hexane. Mp 245–247 °C; R_f = 0.42 (hexane–EtOAc 1 : 1); IR (solid state): ν_{max} = 3379, 2928, 1706 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, 18-H_3), 0.92 (d, *J* = 7.8 Hz, 3 H, 21-H_3), 0.93 (s, 3 H, 19-H_3), 1.43 (s, 18 H, $(CH_3)_3C$), 2.15–2.25 (m, 1 H), 2.45 (m, 1 H), 3.49 (m, 1 H, 3β-*H*), 3.71 (m, 4 H, 7β-*H* and CO_2CH_3), 3.98 (broad s, 1 H,

§ Provided the catalyst is washed thoroughly with acetic acid, it may be dried, resuspended and used to hydrogenate further batches of **17**.

12β-*H*), 5.21 (broad s, 1 H, 7-CH–N*H*R), 5.35 (broad s, 1 H, 12-CH–N*H*R); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.72 (18-CH_3), 17.37 (21-CH_3), 22.89 (19-CH_3), 23.05 (CH_2), 26.95 (CH_2), 27.41 (CH_2), 28.50 ((CH_3)$_3$C), 28.54 ((CH_3)$_3$C), 29.04 (*C*H), 30.26 (CH_2), 30.75 (CH_2), 31.61 (CH_2), 32.17 (CH_2), 34.65 (*C*), 35.00 (CH_2), 35.24 (*C*H), 35.26 (*C*H), 37.07 (*C*H), 39.14 (CH_2), 41.48 (*C*H), 44.79 (*C*), 44.89 (*C*H), 48.89 (*C*H), 51.97 (CO_2CH_3), 53.29 (*C*H), 71.88 (*C*H), 79.00 ((CH_3)$_3$*C* × 2), 155.45 (NH*C*O), 155.47 (NH*C*O), 175.44 (*C*O_2CH_3); Anal. found: C, 67.51; H, 9.68; N, 4.49%. $C_{35}H_{60}N_2O_7$ requires C, 67.71; H, 9.74; N, 4.51%.

Methyl 3α-azido-7α,12α-di[*N*-(*t*-butyloxycarbonyl)amino]-5β-cholan-24-oate 10, (*via* methyl 3β-methanesulfonyl-7α,12α-di[N-(t-butyloxycarbonyl)amino]-5β-cholan-24-oate 20)

The 3α-hydroxy cholanoate **19** (5.00 g, 8.05 mmol), DMAP (2.58 g, 21.4 mmol) and triphenylphosphine (6.35 g, 24.5 mmol) were dissolved in dry THF (75 mL) under nitrogen and cooled in an ice-bath. Methanesulfonic acid (1.40 mL, 17 mmol) was added to the stirred solution to give a white suspension, and DEAD (4.03 mL, 24 mmol) was added dropwise. With the addition of DEAD the reaction mixture turned into a clear yellow solution then after 3–5 min into a white slurry. After 15 min the mixture was warmed to room temperature and was maintained with vigorous stirring for 48 h. The white solid was removed by filtration, and the mother liquors were evaporated under reduced pressure. The dry residue was purified by flash chromatography (hexane–EtOAc, 3 : 1 to 1 : 1, loading the product mixed with silica) to give the required 3β-methanesulfonate derivative **20** (with small impurities of DEAD and its reduced product; and triphenylphosphine and its oxidised product). The 3β-methanesulfonate derivative **20** and sodium azide (6.9 g, 106 mmol) were stirred in dry DMF (85 mL) under nitrogen at 47 °C for 3 d. The solvent was evaporated under reduced pressure. The residue was redissolved in ethyl acetate, washed with H_2O, dried with $MgSO_4$ and evaporated under reduced pressure. The dry residue was purified by flash chromatography (hexane–EtOAc, 4 : 1, dry loading by evaporation from EtOAc onto silica) to give 3α-azide **10** (4.11 g, 79%) as a white solid. An analytical sample was recrystallised from DCM–hexane. Mp 218–220 °C; R_f = 0.27 (hexane–EtOAc 4 : 1); IR (solid state): ν_{max} = 3384, 2930, 2087, 1702 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, 18-H_3), 0.92 (d, *J* = 5.8 Hz, 3 H, 21-H_3), 0.95 (s, 3 H, 19-H_3), 1.42 (s, 9 H, (CH_3)$_3$C), 1.43 (s, 9 H, (CH_3)$_3$C), 2.20–2.25 (m, 1 H), 2.45–2.55 (m, 1 H), 3.26 (broad s, 1 H, 3β-*H*), 3.65 (broad s, 1 H, 7β-*H*), 3.73 (s, 3 H, CO_2CH_3), 3.99 (broad s, 1 H, 12β-*H*), 5.25 (broad s, 1 H, 7-CH–N*H*R), 5.47 (broad s, 1 H, 12-CH–N*H*R); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.64 (18-CH_3), 17.42 (21-CH_3), 22.96 (CH_2), 23.00 (19-CH_3), 26.91 (CH_2), 27.02 (CH_2), 27.60 (CH_2), 28.51 ((CH_3)$_3$C × 2), 28.78 (*C*H), 30.64 (CH_2), 31.66 (CH_2), 31.93 (CH_2), 34.79 (*C*), 35.24 (CH_2 × 2), 35.54 (*C*H), 37.03 (*C*H), 41.84 (*C*H), 44.52 (*C*H), 44.71 (*C*), 47.22 (*C*H), 49.21 (*C*H), 52.17 (CO_2CH_3), 53.21 (*C*H), 61.75 (*C*H), 78.87 ((CH_3)$_3$*C* × 2), 155.45 ((CH_3)$_3$O*C*ONH × 2), 176.92 (*C*O_2CH_3); Anal. found: C, 64.99; H, 9.23; N, 11.10%. $C_{35}H_{59}N_5O_6$ requires C, 65.09; H, 9.21; N, 10.84%.

Methyl 3α-azido-7α, 12α-diamino-5β-cholan-24-oate 21

To a stirred solution of the biscarbamate **10** (2.02 g, 3.13 mmol) in dry dichloromethane (50 mL) at 0 °C was added trifluoroacetic acid (30 mL). The solution was stirred for 1 h at 0 °C, then allowed to reach room temperature and stirred for a further 2 h. The solvent was evaporated under reduced pressure and the trifluoroacetic acid removed by addition and evaporation of further dichloromethane, to give the bis(trifluoroacetate) salt **21**·2TFA as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (s, 3 H, 18-H_3), 0.99 (d, *J* = 6.3 Hz, 3 H, 21-H_3), 1.01 (s, 3 H, 19-H_3), 2.23–2.31 (m, 2 H), 2.34–2.41 (m, 2 H), 3.27 (m, 1 H, 3β-*H*), 3.60 (broad s, 1 H, 7β-*H*), 3.68 (s, 3 H, COOCH_3), 3.82 (broad s, 1 H, 12β-*H*), 7.73 (broad s, 6 H, NH_3^+).

The bisammonium salt was dissolved in chloroform and washed with three portions of saturated aqueous Na_2CO_3. The organic phase was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield diamine **21** (1.38 g, 99%) as an off-white solid. R_f = 0.32 (ethyl acetate–methanol 9 : 1); IR (solid state): ν_{max} = 3370, 2917, 2090, 1736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.73 (s, 3 H, 18-H_3), 0.93 (s, 3 H, 19-H_3), 0.97 (d, *J* = 6.3 Hz, 3 H, 21-H_3), 2.13–2.49 (m, 2 H), 3.10 (s, 1 H, 7β-*H*), 3.15 (broad s, 1 H, 12β-*H*), 3.17 (m, 1 H, 3β-*H*), 3.66 (s, 3 H, CO_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.22 (18-CH_3), 16.86 (21-CH_3), 22.53 (19-CH_3), 23.21 (CH_2), 25.58 (*C*H), 26.59 (CH_2), 27.27 (CH_2), 28.10 (CH_2), 30.60 (CH_2), 30.73 (CH_2), 34.42 (CH_2), 34.76 (*C*), 34.82 (*C*H), 35.24 (CH_2), 35.87 (CH_2), 39.31 (*C*H), 41.50 (*C*H), 41.98 (*C*H), 45.84 (*C*), 47.16 (*C*H), 47.40 (*C*H), 51.03 (*C*H), 53.59 (CO_2CH_3), 61.16 (*C*H), 174.04 (*C*O_2CH_3); MS (FAB$^+$): *m*/*z* (%): 446 (100) [M + H]$^+$; 403 (40) [M − N_3]$^+$; 468 (10) [M + Na]$^+$.

1-(2-nitrobenzenesulfonyloxy)-benzotriazole 29.[21]

In a three-necked round bottomed flask equipped with two dropping funnels was prepared a solution of 1-hydroxy benzotriazole (4.05 g, 30.0 mmol), in chloroform (10 mL). The temperature was brought to 0 °C with an ice-bath. To this solution were added at the same time and under vigorous stirring, 2-nitrobenzene sulfonyl chloride **28** (6.65 g, 30 mmol) in chloroform (50 mL), and Na_2CO_3 (4.24 g, 40 mmol) in water (50 mL). Once the additions were finished the reaction mixture was allowed to reach room temperature and stirred overnight. The two layers were separated, the organic phase dried ($MgSO_4$) and evaporated under reduced pressure. The residue was crystallised from chloroform–hexane to obtain the desired compound **29** (6.84 g, 71%) as a crystalline pale yellow solid. IR (solid state) ν_{max} = 3103, 2160, 1979, 1618, 1539, 1398, 1134, 1015 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (ddd, *J* = 8.4, 7.0, 1.1 Hz, 1 H, Ar*H*), 7.64 (ddd, *J* = 7.0, 7.0, 0.9 Hz, 1 H, Ar*H*), 7.73 (dt, *J* = 8.4, 1.0 Hz, 1 H, Ar*H*), 7.80 (ddd, *J* = 8.9, 7.4, 1.5 Hz, 1 H, Ar*H*), 7.95–8.06 (m, 4 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 109.63 (Ar*C*H), 120.81 (Ar*C*H), 125.97 (Ar*C*H), 126.18 (Ar*C*H), 128.38 (Ar*C*), 130.13 (Ar*C*H), 133.16 (Ar*C*H), 133.18 (Ar*C*), 133.44 (Ar*C*H), 137.42 (Ar*C*H), 143.28 (Ar*C*), 150.75 (Ar*C*).

Methyl 3α-azido-7α-amino, 12α-[*N*-(*o*-nitrobenzenesulfonyl)amino]-5β-cholan-24-oate 31

Diamine **21** (2.07 g, 4.65 mmol) was dissolved in dry THF (2.5 mL) under nitrogen and 1-(2-nitrobenzenesulfonyloxy)-benzotriazole **29** (1.48 g, 4.62 mmol) was added at room temperature under vigorous stirring, to give a yellow paste. Stirring was continued overnight after which the solvent was removed. The crude solid was redissolved in DCM, washed with a saturated aqueous Na_2CO_3 solution (3 × 30 mL), the organic phase dried ($MgSO_4$) and the organic solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (DCM–methanol 95 : 5) to yield **31** (2.21 g, 75%) as a yellowish solid. R_f = 0.14 (DCM–methanol 95 : 5); IR (solid state): ν_{max} = 3350, 2941, 2089, 1732, 1360, 1150 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.14 (q, *J* = 12.4 Hz, 1 H), 0.67–0.79 (m, 1 H), 0.81 (s, 3 H, 18-H_3), 0.83 (s, 3 H, 19-H_3), 1.01 (d, *J* = 6.6 Hz, 3 H, 21-H_3), 2.19–2.41 (m, 2 H), 2.92 (m, 1 H, 3β-*H*), 3.10 (broad s, 1 H, 7β-*H*), 3.66 (s, 3 H, CO_2CH_3), 4.12 (d, *J* = 6.8 Hz, 1 H, 12β-*H*), 5.79 (d, *J* = 9.8 Hz, 1 H, N*H*SO_2), 7.68–7.74 (m, 2 H, Ar*H*), 7.85–7.89 (m, 1 H, Ar*H*), 8.09–8.13 (m, 1 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.23 (18-CH_3), 17.41 (21-CH_3), 22.52 (19-CH_3), 23.56 (CH_2), 25.66 (CH_2), 25.90 (*C*H), 26.18 (CH_2), 27.32 (CH_2), 28.32 (*C*H), 30.64 (CH_2), 30.93 (CH_2), 34.56 (CH_2), 35.12 (CH_2), 35.17 (*C*), 35.34 (*C*H), 35.86 (CH_2), 39.08 (*C*H), 41.79 (*C*H), 43.73 (*C*H), 45.52 (*C*), 47.02 (*C*H), 51.23 (CO_2CH_3), 58.09 (*C*H), 60.93 (*C*H), 125.26 (Ar*C*H), 129.71 (Ar*C*H), 132.65 (Ar*C*H), 133.05 (Ar*C*H), 136.51 (Ar*C*), 148.27 (Ar*C*), 174.35 (*C*O_2CH_3); MS (FAB$^+$): *m*/*z* (%): 631 (100)

[M + H]$^+$; 588 (25) [M − N_3]$^+$; 653 (12) [M + Na]$^+$; HRMS (ES$^+$): *m*/*z* calcd for [$C_{31}H_{46}N_6O_6S$ + H]$^+$ 631.3272 found 631.3270. ^{1}H-NMR analysis implied that the level of contamination by regioisomer **32** was below 2%. See electronic supplementary information (ESI).†

Also isolated was methyl 3α-azido-7α,12α-di[*N*-(*o*-nitrobenzenesulfonyl)amino]-5β-cholan-24-oate **24** (P = *o*Ns) (381 mg, 10%). R_f = 0.45 (DCM/methanol 95 : 5); IR (solid state): ν_{max} = 3265, 3097, 2951, 2872, 2090, 1732, 1699, 1540, 1442, 1423, 1353, 1162, 742, 730 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.16 (q, *J* = 12.7 Hz, 1 H), 0.80 (s, 3 H, 18-H_3), 0.86 (s, 3 H, 19-H_3), 0.96 (d, *J* = 6.8 Hz, 3 H, 21-H_3), 2.17–2.25 (m, 1 H), 2.32–2.40 (m, 1 H), 2.87 (m, 1 H, 3β-*H*), 3.67 (s, 3 H, CO_2CH_3), 3.78 (broad s, 1 H, 7β-*H*), 4.09 (d, *J* = 8.8 Hz, 1 H, 12β-*H*), 5.48 (d, *J* = 7.8 Hz, 1 H, 7-CH–N*H*SO_2), 5.65 (d, *J* = 8.8 Hz, 1 H, 12-CH–N*H*SO_2), 7.68–7.75 (m, 4 H, Ar*H*), 7.87–7.90 (m, 2 H, Ar*H*), 8.08–8.11 (m, 2 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.05 (18-CH_3), 17.67 (21-CH_3), 23.22 (19-CH_3), 25.98 (CH_2), 26.24 (CH_2), 27.54 (CH_2), 29.69 (*C*H), 30.99 (*C*H), 31.34 (*C*), 35.22 (*C*H), 35.60 (CH_2), 40.42 (*C*H), 44.30 (*C*H), 46.01 (*C*), 48.05 (*C*H), 51.78 (CO_2CH_3), 57.74 (*C*H), 60.76 (*C*H), 61.09 (*C*H), 121.26 (Ar*C*H), 121.42 (Ar*C*H), 125.93 (Ar*C*H), 126.09 (Ar*C*H), 129.78 (Ar*C*H), 133.03 (Ar*C*H), 133.05 (Ar*C*H), 174.79 (*C*O_2CH_3); MS (CI$^+$): *m*/*z* (%) = 817 (7) [M + H]$^+$, 788 (19) [M − N_2]$^+$.

Methyl 3α-azido-12α-*N*-(*o*-nitrobenzenesulfonyl)–amino-7α-[*N*-(*t*-butyloxycarbonyl)–amino]-5β-cholan-24-oate 13

Amine **31** (2.50 g, 3.96 mmol) was dissolved in THF (34 mL) and an aqueous saturated solution of $NaHCO_3$ (17 mL). To the solution, di-*tert*-butyl dicarbonate (1.73 g, 7.92 mmol) was added and the reaction mixture stirred at room temperature for 4 d. The two layers were separated and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried with $MgSO_4$ and evaporated under reduced pressure. The crude material was precipitated from diethyl ether–petroleum ether to yield **13** (2.6 g, 90%) as a white solid. Mp 148–150 °C; R_f = 0.21 (hexane–EtOAc 3 : 1); IR (solid state): ν_{max} = 3389, 2933, 2089, 1699, 1363, 1160 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.22 (q, *J* = 12.0 Hz, 1 H), 0.82 (s, 3 H, 18-H_3), 0.88 (s, 3 H, 19-H_3), 0.92 (d, *J* = 6.2 Hz, 3 H, 21-H_3), 1.49 (s, 9 H, C(CH_3)$_3$), 2.14–2.22 (m, 1 H), 2.32–2.40 (m, 1 H), 2.96–3.04 (m, 1 H, 3β-*H*), 3.67 (broad s, 4 H, CO_2CH_3 + 7β-*H*), 4.05–4.08 (m, 1 H, 12β-*H*), 4.61 (broad s, 1 H, 7-CH–N*H*Boc), 5.59 (d, *J* = 9.1 Hz, 1 H, 12-CH–N*H*Nos), 7.70–7.77 (m, 2 H, Ar*H*), 7.86–7.88 (m, 1 H, Ar*H*), 8.11–8.13 (m, 1 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.59 (18-CH_3), 17.33 (21-CH_3), 22.81 (19-CH_3), 23.34 (CH_2), 25.92 (CH_2), 26.33 (CH_2), 27.25 (CH_2), 28.22 ((CH_3)$_3$C), 28.42 (*C*H), 30.66 (CH_2), 31.00 (CH_2), 31.57 (*C*), 31.59 (*C*H), 34.89 (CH_2), 35.02 (CH_2), 35.21 (CH_2), 35.33 (*C*H), 37.06 (*C*H), 41.44 (*C*H), 44.50 (*C*H), 45.64 (*C*), 46.82 (*C*H), 47.70 (*C*H), 51.48 (CO_2CH_3), 57.84 (*C*H), 60.55 (*C*H), 79.52 ((CH_3)$_3$*C*), 125.65 (Ar*C*H), 129.82 (Ar*C*H), 132.79 (Ar*C*H), 133.34 (Ar*C*H), 138.59 (Ar*C*), 148.61 (Ar*C*), 155.42 (NH*C*O), 174.46 (*C*O_2CH_3); MS (FAB$^+$): *m*/*z* (%) 753 (100) [M + Na]$^+$; 631 (45) [*M* − Boc]$^+$; Anal. found: C, 59.56; H, 7.77; N, 11.18%. $C_{36}H_{54}N_6O_8S$ requires: C, 59.16; H, 7.45; N, 11.50%.

Methyl 3α-azido-7α-[*N*-(*t*-butyloxycarbonyl)–amino]-12α-amino-5β-cholan-24-oate 33

Thiophenol (3.65 mL, 35.5 mmol) was added to a suspension of the scaffold **13** (2.6 g, 3.56 mmol) and caesium carbonate (6.9 g, 21.2 mmol) in dry DMF (32 mL) under nitrogen at room temperature. The reaction was heated at 55 °C overnight before removing the DMF under reduced pressure. The mixture obtained was dissolved in ethyl acetate, washed with saturated aqueous $NaHCO_3$, then brine, dried with $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexane–EtOAc 1 : 1 to remove all the by-products and then EtOAc–TEA 9 : 1) gave 12α-amino cholanoate **33** (1.57 g, 80%) as a yellowish solid. R_f = 0.33 (hexane/EtOAc 1 : 1); IR (solid state): ν_{max} = 3394, 2934, 2090, 1699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.73 (s, 3 H, 18-H_3), 0.94 (s, 3 H, 19-H_3), 0.97 (d, *J* = 6.4 Hz, 3 H, 21-H_3), 1.45 (s, 9 H, (CH_3)$_3$C), 2.15–2.41 (m, 2 H), 3.14–3.21 (m, 1 H, 3β-*H*), 3.23 (broad s, 1 H, 12β-*H*), 3.67 (m, 4 H, CO_2CH_3 + 7β-*H*), 4.95 (broad s, 1 H, 7-CH–N*H*Boc); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.59 (18-CH_3), 17.17 (21-CH_3), 22.91 (19-CH_3), 22.95 (CH_2), 27.10 (CH_2), 27.48 (CH_2), 28.15 (*C*H), 28.49 ((CH_3)$_3$C), 28.84 (CH_2), 30.91 (CH_2), 31.15 (CH_2), 31.42 (CH_2), 34.66 (*C*), 35.11 (*C*H), 35.20 (CH_2), 35.31 (CH_2), 36.39 (*C*H), 37.38 (*C*H), 41.94 (*C*H), 42.29 (*C*H), 46.12 (*C*), 47.88 (*C*H), 51.45 (CO_2CH_3), 53.66 (*C*H), 61.27 (*C*H), 79.05 ((CH_3)$_3$*C*), 155.50 (NH*C*O), 174.43 (*C*O_2CH_3); MS (FAB$^+$) *m*/*z* (%): 547 (100) [M + 1]$^+$; 569 (15), [M + Na]$^+$.

Compound 34

In a dry round bottomed flask *N*-Fmoc–L-phenylalanine (355 mg, 0.92 mmol), TBTU (295 mg, 0.92 mmol), HOBt (124 mg, 0.92 mmol) and DIPEA (160 µL, 0.92 mmol) were dissolved in dry DMF (0.5 mL) and the solution was vigorously sonicated for 5 min. To this activated solution was then added amine **33** (201 mg, 0.37 mmol) dissolved in dry DCM (0.2 mL). The reaction mixture was stirred for 24 h at room temperature and under nitrogen atmosphere before the solvent was removed under reduced pressure, the crude was washed with brine, extracted with DCM, and dried over $MgSO_4$. Flash chromatography (diethyl ether–TEA 99 : 1) gave **34** (197 mg, 60%) as a white solid. R_f = 0.75 (diethyl ether–TEA 99 : 1) IR (solid state): ν_{max} = 3380, 2950, 2091, 1700, 1513, 1450, 1365, 1247, 1165, 1053 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.62 (d, *J* = 6.5 Hz, 3 H, 21-H_3), 0.69 (s, 3 H, 18-H_3), 0.90 (s, 3 H, 19-H_3), 1.44 (s, 9 H, C(CH_3)$_3$), 2.01–2.16 (m, 1 H), 2.21–2.36 (m, 1 H), 3.06–3.21 (m, 3 H, PheCH_2 + 3β-*H*), 3.65 (m, 4 H, CO_2CH_3 + 7β-*H*), 3.90–4.58 (m, 5 H, FmocCH_2 + 2 H + 12β-*H*), 5.12 (broad s, 1 H, N*H*), 5.34 (broad s, 1 H, N*H*), 6.98–7.74 (m, 13 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.3 (18-CH_3), 17.1 (21-CH_3), 23.1 (19-CH_3), 23.2 (CH_2), 24.5 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 28.6(C(CH_3)$_3$), 28.8 (*C*H), 30.8 (CH_2), 31.1 (CH_2), 34.8(*C*), 34.9 (*C*H), 33.6 (CH_2), 35.7 (CH_2), 35.8 (CH_2), 37.2 (CH_2), 39.2 (*C*H), 41.9 (*C*H), 44.2 (*C*H), 44.3 (*C*H), 44.8 (*C*), 45.6 (*C*H), 48.6 (*C*H), 51.5 (CO_2CH_3), 52.1 (CH), 55.0 (*C*H), 61.8 (*C*H), 66.6(CH_2), 77.5 (*C*(CH_3)$_3$), 119.5 (Ar*C*H), 126.8 (Ar*C*H), 127.4 (Ar*C*H), 128.0 (Ar*C*H), 128.9 (Ar*C*H), 137.3 (Ar*C*), 141.2 (Ar*C*), 143.8 (Ar*C*), 155.4 (NH*C*O), 157.0 (NH*C*O), 173.4 (*C*), 174.5 (*C*O_2CH_3); MS (FAB$^+$): *m*/*z* (%): 815 (100) [M − Boc]$^+$, 937 (75) [M + Na]$^+$, 915 (48) [M + 1]$^+$; HRMS (FAB$^+$): *m*/*z* calcd. for [$C_{54}H_{70}N_6O_7$ + Na]$^+$: 937.5204, found 937.5201.

Compound 35

Compound **34** (200 mg, 0.22 mmol) was dissolved in DCM (50 mL). TFA (5 mL) was added at 0 °C and the reaction was stirred for 2 h. The solvent was evaporated under reduced pressure and the resulting pale yellow foam was dissolved again in DCM (15 mL), washed with saturated aqueous Na_2CO_3 (2 × 10 mL), and dried over $MgSO_4$. Evaporation gave the 7α-amine (170 mg, 95%) as a pale yellow solid. R_f = 0.39 (ethyl acetate–TEA 9.9 : 0.1); IR (solid state): ν_{max} = 2917, 2090, 1734, 1448, 1251, 1167 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.66 (s, 3 H, 18-H_3), 0.85–2.30 (m, 28 H), 3.00–3.21 (m, 4 H, PheCH_2 + 7β-*H* + 3β-*H*), 3.63 (s, 3 H, CO_2CH_3), 3.90–4.58 (m, 5 H, FmocCH_2 + 2 H + 12β-*H*), 5.30 (broad s, 1 H, N*H*), 6.98–7.74 (m, 13 H, Ar*H*).

To a solution of the above amine (150 mg, 0.18 mmol) in dry DCM (0.2 mL) was added a pre-activated mixture (sonicated for 5 min) of *N*-Boc–L-valine (80 mg, 0.37 mmol), TBTU (115 mg, 0.36 mmol), HOBt (48 mg, 0.36 mmol) and DIPEA (62 µl, 0.36 mmol) in dry DMF (0.4 mL). The reaction mixture was stirred for 24 h at room temperature and under nitrogen atmosphere before the solvent was removed under reduced pressure. The residue was dissolved in DCM, washed with brine and dried over $MgSO_4$. Flash chromatography (diethyl ether–TEA 99 : 1) gave **35** (156 mg, 85%) as a white solid. R_f = 0.64 (ethyl acetate–TEA 99 : 1); IR (solid state): ν_{max} = 3276, 2928, 2089, 1660, 1533, 1449, 1366, 1297, 1249, 1168, 1043 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.60 (d, *J* = 6.5 Hz, 3 H, 21-H_3), 0.74 (s, 3 H, 18-H_3), 0.89(s, 3 H, 19-H_3), 0.91–2.01 (m, 26 H), 1.46 (s, 9 H, C(CH_3)$_3$), 2.02–2.14 (m, 1 H), 2.20–2.58 (m, 2 H), 3.00–3.21 (m, 3 H, PheCH_2 + 3β-*H*), 3.64 (m, 4 H, CO_2CH_3 + 7β-*H*), 4.01–4.62 (m, 6 H, FmocCH_2 + 2 H + 12β-*H* + C*H* Val), 5.12 (broad s, 1 H, N*H*), 5.57 (broad s, 1 H, N*H*), 6.98–7.74 (m, 14 H, Ar*H* + N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2 (18-CH_3), 17.0 (21-CH_3), 17.5 (CH_3 Val), 19.7 (CH_3 Val), 22.7 (19-CH_3), 23.3 (CH_2), 24.5 (CH_2), 28.2 (CH_2), 28.3 (C(CH_3)$_3$), 28.5 (CH_2), 28.8 (*C*H), 30.4 (CH_2), 31.3(*C*H), 31.9 (CH_2), 34.7(*C*), 34.9 (*C*H), 35.7 (CH_2), 35.8 (CH_2), 37.2 (CH_2), 39.0 (*C*H), 42.0 (*C*H), 44.2 (*C*H), 44.3 (*C*H), 44.8 (*C*), 46.2 (*C*H), 47.0(*C*H), 48.6 (*C*H), 51.5 (CO_2CH_3), 52.1 (*C*H), 55.0 (*C*H), 58.4(*C*), 61.1 (*C*H), 67.5(CH_2), 80.1(*C*H), 119.5 (Ar*C*H), 126.8 (Ar*C*H), 127.4(Ar*C*H), 128.0 (Ar*C*H), 128.9 (Ar*C*H), 136.6 (Ar*C*), 141.3 (Ar*C*), 143.7(Ar*C*), 156.4 (NH*C*O), 157.0 (NH*C*O), 173.4 (*C*), 170.8 (*C*), 174.5 (CO_2CH_3). MS (FAB$^+$): *m*/*z* (%) 915, (100) [M − Boc]$^+$, 1015 (80) [M + H]$^+$, 1037 (64) [M + Na]$^+$; HRMS (FAB$^+$): *m*/*z* calcd. for [$C_{59}H_{79}N_7O_8$ + Na]$^+$: 1036.5889, found 1036.5883.

Compound 36

Azide **35** (150 mg, 0.15 mmol) was dissolved in dry THF (3 mL). Trimethylphosphine (1.0 M in THF, 180 mL, 0.18 mmol) was added to the solution of the steroid and the mixture was stirred at room temperature under nitrogen for 5 h before water (53 µL, 2.9 mmol) was added and the reaction was stirred overnight. The solvents were removed by evaporation, addition of toluene and re-evaporation to obtain the 3α-amine as a solid. The formation of the product was monitored by TLC (R_f = 0.05, ethyl acetate–TEA 99 : 1) and then confirmed by MS (FAB$^+$): *m*/*z* (%) 989 (62) [*M* + H]$^+$, 1011 (30) [*M* + Na]$^+$.

The above amine was dissolved in dry DCM (0.2 mL). A mixture of *N*-Boc–glycine (53 mg, 0.30 mmol), TBTU (96 mg, 0.30 mmol), HOBt (40 mg, 0.30 mmol) and DIPEA (52 µL, 0.3 mmol), previously activated (sonicated for 5 min) in 0.4 mL of dry DMF, was added to the solution of the steroid and the resulting mixture was stirred for 24 h under nitrogen. After removal of the solvents, the crude product was solved in DCM, washed with brine, and dried over $MgSO_4$. Flash chromatography (diethyl ether–TEA 99 : 1) gave **36** (124 mg, 72% over two steps) as a white solid. R_f = 0.72 (ethyl acetate–TEA 99 : 1); IR (solid state): ν_{max} = 3789, 3696, 3661, 3298, 2958, 1659, 1534, 1450, 1250, 1367, 1250, 1169 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.60–2.58 (m, 38 H), 1.46 (s, 9 H, C(CH_3)$_3$),1.48 (s, 9 H, C(CH_3)$_3$), 3.01–3.29 (m, 2 H, PheCH_2), 3.64 (m, 4 H, CO_2CH_3 + 7β-*H*), 4.01–4.62 (m, 9 H, FmocCH_2 + 2 H + 12β-*H* + 3β-*H* + C*H* Val + CH_2 Gly), 5.13 (broad s, 1 H, N*H*), 5.47 (broad s, 1 H, N*H*), 5.57 (broad s, 1 H, N*H*), 6.98–7.74 (m, 14 H, Ar*H* + N*H*), 7.90 (broad s, 1 H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.1 (18-CH_3), 17.2 (21-CH_3), 17.4 (CH_3), 19.7 (CH_3), 22.5 (19-CH_3), 23.3 (CH_2), 24.5 (CH_2), 28.1 (CH_2), 28.3 (C(CH_3)$_3$ × 2), 28.6 (CH_2), 28.8 (*C*H), 30.2 (CH_2), 31.3 (*C*H), 32.0 (CH_2), 34.7 (*C*), 34.9 (*C*H), 35.6 (CH_2), 35.8 (CH_2), 37.2 (CH_2), 39.0 (*C*H), 42.0 (*C*H), 42.7 (CH_2), 44.2 (*C*H), 44.3 (*C*H), 44.8 (*C*), 46.2 (*C*H), 47.0 (*C*H), 48.6 (*C*H), 51.5 (CO_2CH_3), 52.0 (*C*H), 55.2 (*C*H), 58.4 (*C*), 61.1 (*C*H), 67.5 (CH_2), 80.1 (*C*H), 81.0 (*C*), 119.5 (Ar*C*H), 126.8 (Ar*C*H), 127.4(Ar*C*H), 128.0 (Ar*C*H), 128.9 (Ar*C*H), 136.6 (Ar*C*), 141.3 (Ar*C*), 143.7 (Ar*C*), 156.1 (NH*C*O), 156.4 (NH*C*O), 157.0 (NH*C*O), 173.4 (*C*), 170.8 (*C*), 174.5 (CO_2CH_3), 174.8 (*C*); MS (FAB$^+$): *m*/*z* (%): 1145 (60) [M + H]$^+$, 1045 (50) [M − Boc], 1167 (32) [M + Na]$^+$; HRMS (FAB$^+$): *m*/*z* calcd. for [$C_{66}H_{92}N_6O_{11}$ + H]$^+$ 1145.6902 found 1145.6920.

Methyl 3α-azido-12α-*N*-(*o*-nitrobenzenesulfonyl)–amino-7α-[*N*-(allyloxycarbonyl)–amino]-5β-cholan-24-oate 14

Amine **31** (438 mg, 0.69 mmol) was dissolved in THF (7 mL) and an aqueous saturated solution of $NaHCO_3$ (3 mL) was added. Allyl chloroformate (136 mg, 120 µL, 1.13 mmol) was added and the reaction mixture was stirred for 4 d at room temperature. The solvent was removed under reduced pressure, the residue was redissolved in DCM and washed with water and the organic phases were combined and dried with $MgSO_4$. Flash chromatography (DCM to DCM–EtOAc 1 : 1) afforded **14** (409 mg, 83%) as a white solid. R_f = 0.85. (EtOAc–MeOH–TEA 9 : 1 : 0.09); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.23 (q, *J* = 11.9 Hz, 1 H), 0.82 (s, 3 H, 18-H_3), 0.88 (s, 3 H, 19-H_3), 0.91 (d, *J* = 6.6 Hz, 3 H, 21-H_3), 1.03–1.15 (m, 2 H), 2.16–2.27 (m, 1 H); 2.31–2.39 (m, 1H), 2.99–3.07 (m, 1 H, 3β-*H*), 3.66 (s, 3 H, CO_2CH_3), 3.77 (broad s, 1 H, 7β-*H*), 4.06 (d, *J* = 9.0 Hz, 1 H, 12β-*H*), 4.59 (m, 2 H, CO_2CH_2CH=CH_2), 4.86 (d, *J* = 8.7 Hz,1 H, N*H*Alloc), 5.24 (dd, *J* = 10.6, 0.9 Hz, 1 H, C*H* alkene), 5.34 (d, *J* = 17.6 Hz, 1 H, C*H* alkene), 5.57 (d, *J* = 8.6 Hz, 1 H, N*H*SO_2), 5.92–6.01 (m, 1 H, CO_2CH_2C*H*=CH_2), 7.69–7.76 (m, 2 H, Ar*H*), 7.82–7.85 (m, 1 H, Ar*H*), 8.09–8.12 (m, 1 H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.6 (18-CH_3), 17.3 (19-CH_3), 22.8 (21-CH_3), 23.3 (CH_2), 25.9 (CH_2), 26.2 (CH_2), 27.2 (CH_2), 28.2 (*C*H), 30.6 (CH_2), 30.9 (CH_2), 31.8 (CH_2), 34.8 (*C*), 35.0 (CH_2), 35.3 (CH_2), 35.4 (*C*H). 36.9 (*C*H), 41.4 (*C*H), 44.4 (*C*H), 45.6 (*C*H), 47.6 (7-*C*H), 51.5 (CO_2CH_3), 57.8 (*C*H), 60.7 (3-*C*H), 65.5 (OCH_2CH=CH_2), 117.4 (OCH_2CH=CH_2), 125.4 (OCH_2*C*H=CH_2); 129.9 (Ar*C*H); 132.8 (Ar*C*), 132.9(Ar*C*H); 133.4 (Ar*C*H); 134.4 (Ar*C*); 136.3 (Ar*C*), 148.5 (NH*C*OO), 174.6 (CO_2CH_3); MS (ES$^-$): *m*/*z* (%) 713.5, (100) [M − H]$^-$; HRMS (FAB$^+$): *m*/*z* calcd. for [$C_{35}H_{50}N_6O_8S$ + NH_4]$^+$ 732.3749 found 732.3747.

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