Bioorganic & Medicinal Chemistry 17 (2009) 5950–5961

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

SAR studies on azasterols as potential anti-trypanosomal and anti-leishmanial agents

Federica Gigante [a,b], Marcel Kaiser [c], Reto Brun [c], Ian H. Gilbert [a,b,*]

[a] Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee DD1 5EH, UK
[b] Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK
[c] Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 30 April 2009
Revised 20 June 2009
Accepted 28 June 2009
Available online 3 July 2009

Keywords:
Trypanosomiasis
Leishmaniasis
Azasterols

ABSTRACT

There is an urgent need for the development of new drugs for the treatment of neglected tropical diseases such as human African trypanosomiasis, Chagas disease and leishmaniasis. Azasterols, have been shown to have activity against the parasites which cause these diseases. In this paper we report synthesis of new azasterols and subsequent analysis of the SAR. The chemistry focused on variations in the ester at the 3β-position of the sterol and the position of the nitrogen in the side chain. The data allowed us to derive preliminary pharmacophore models for the activity of the azasterols against the parasites which cause these diseases.

1. Introduction

Parasitic infections are a major health problem in many countries. Amongst the most neglected diseases are those caused by the kinetoplastids:[1–4] human African trypanosomiasis (Sleeping sickness), endemic in many sub-Saharan African countries, caused by *Trypanosoma brucei* spp.; Chagas' disease, in South and Central America, caused by *Trypanosoma cruzi*; leishmaniasis, in many countries, caused by a number of species and subspecies of *Leishmania*. The treatment of these diseases is mainly based on chemotherapy, but poor efficacy, toxic side effects and cases of resistance are often associated with the drugs currently available. Therefore new effective, safe and affordable drugs are urgently needed.

Azasterols are nitrogen containing sterol compounds, initially developed as antifungal agents,[5,6] with the nitrogen in the sterol side chain (positions 23, 24 or 25) (for a representative example see Fig. 1). These compounds were found to inhibit the enzyme, 24-sterol methyltransferase (24-SMT), responsible for the methenylation of zymosterol.[7–9] This is important for the biosynthesis of 24-alkylated sterols, such as ergosterol, which are the main sterols found in fungi. Ergosterol and other 24-alkylated sterols are the major sterols found in the cell membranes of *T. cruzi*, *Leishmania* spp., and in the vector stage of *T. brucei*. In our group, we have been working on the synthesis and evaluation of azasterols as antiparasitics (Fig. 1).[10–14] We noticed that some of these compounds were active against bloodstream form (bsf) *T. brucei*; however bsf *T. brucei* is reported to scavange cholesterol from the host, rather than biosynthesise ergosterol,[15,16] suggesting alternate modes of action for these compounds. We also discovered that some compounds had multiple modes of action against *Leishmania* spp.[12] Thus compounds with an acetate on the 3β-OH were not inhibitors of 24-SMT; yet they had activity against the parasite, which could not simply be explained by cleavage of the acetate. Furthermore, we demonstrated that whilst some azasterols inhibited the growth of *T. brucei* with an EC_{50} of less than 50 nM, they did not inhibit 24-SMT, suggesting an alternative mode of action.[14]

Interestingly other sterol–amine conjugates have been shown to have antimicrobial activity. A number of sterols with basic side chains have been shown to have antimicrobial activity;[17] this includes a natural product, squalamine, that has been shown to have potent antimicrobial activity.[18–20] We have also prepared some squalamine analogues which have some anti-leishmanial activity.[21]

These azasterols that we have reported[10–14] have interesting in vitro activity against *T. brucei rhodesiense* and *Leishmania donovani*. From the evaluation of these compounds it was possible to construct a preliminary pharmacophore for the activity against *T. brucei rhodesiense*.[10] Important features in these azasterols are (Fig. 2): the acetate at the 3β-OH; a side chain of 3 carbons linked by an amino function to the sterol nucleus; an ester functionality in the side chain. The most potent compounds that we prepared against *T. brucei rhodesiense* was compound **1** (Fig. 1), which had an EC_{50} value of 12 nM.[10,14]

In this paper we report on new series of compounds where modifications were applied to this basic structure, to probe both

* Corresponding author. Tel.: +44 1382 386 240; fax: +44 1382 386 373.
E-mail address: i.h.gilbert@dundee.ac.uk (I.H. Gilbert).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.06.062

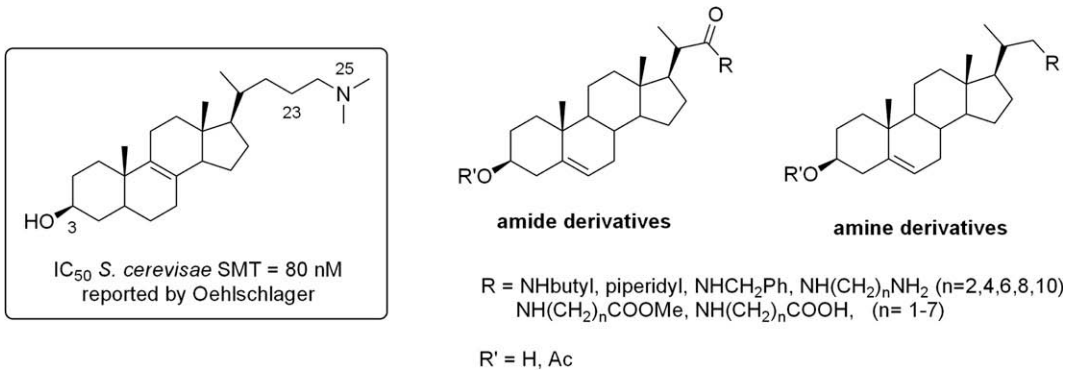


Figure 1. An azasterol reported as an inhibitor of *S. cerevisae* 24-SMT by Oehlschlager et al.[8] and azasterol compounds previously synthesised and reported by our group.

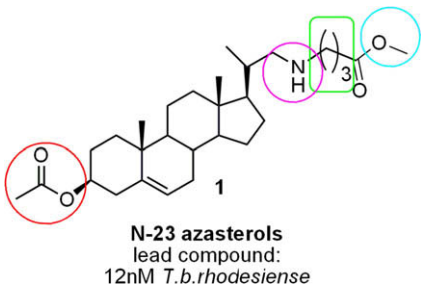


Figure 2. Pharmacophore for activity against *T. brucei rhodesiense*. Data for **1** as reported by Gros et al.:[10] *T. brucei rhodesiense*, EC_{50} 0.012 μM; *T. cruzi*, EC_{50} 24.7 μM; *L. donovani*, EC_{50} 19.2 μM; KB cells, EC_{50} 19.2 μM.

the SAR and to try and improve the physicochemical properties of the compounds. In particular the ester functionality at the 3β-position appears important to this alternate mode of action.[10,12] Loss of the acetate caused a reduction in activity against *T. brucei*.[10] Therefore we decided to prepare a series of compounds with variations to the acetate (series 1, Fig. 3). Similarly the compounds were sensitive to variation in functionality at the side chain;[10] therefore we investigated changing the side chain—in particular the location of the amino moiety in the side chain from the '23-position' to the '25-position' (series 2, Fig. 3). Also previously we had attached the side chain either via an amide linkage in the '23-series',[14,10,11] this was repeated in the '25-series'.

2. Chemistry

2.1. Ester derivatives

The synthetic strategy for the production of the ester analogues is shown in Scheme 1, based on our previously published routes.[10,11,12,14] Starting from the commercially available sterol 3β-acetoxy-23,24-bisnor-5-cholenic acid **2**, the selective reduction of the acid function was carried out with a dimethyl sulfide borane complex (36%). The resulting alcohol **3** was oxidized with PCC in dichloromethane to afford the aldehyde derivative **4** (92%). The acetyl ester at the 3-position was hydrolysed by treatment with potassium carbonate in methanol/water, to afford **5** (90%) as a starting material for the synthesis of various esters. Two different methods were used to produce the ester analogues **6**–**12**. The derivatives **6**–**9** and **12** were obtained by adding various chlorides (propionyl chloride, butyryl chloride, isovalenyl chloride, trimethylacetyl chloride, dimethyl carbamyl chloride, nicotinoyl chloride) to a solution of **5** in pyridine (21–43%). The analogues **10** and **11** were obtained by the coupling of **5** with succinic anhydride or *N*,*N*-dimethylglycine, respectively, in the presence of EDC and DMAP (24–43%). Finally, a one pot reductive amination with sodium cyanoborohydride in methanol was performed to add the side chain and produce the ester analogues **14**–**20** (16–88%). For this purpose, the methyl amino ester **13** was first formed by treating 4-aminobutyric acid with thionyl chloride in methanol.

For compounds **5**–**20**, a doubling of some peaks in the ^{1}H and ^{13}C NMR was observed (see Supplementary data). In particular this affected the peaks corresponding to the D-ring of the sterol and the side chain atoms. Only one compound could be observed by TLC and mass spectrometry. Carrying out the NMR at elevated temperatures gave no significant change in the ^{1}H NMR, probably ruling out the presence of rotamers. The most likely explanation is epimerisation α to the carbonyl at position **20** during the potassium carbonate hydrolysis of the 3β-acetate.

2.2. N-25 amine derivatives

The synthetic route to produce the N-25 amine analogues is outlined in Scheme 2. The selective reduction of the commercially available sterol 3β-acetoxy-5-cholenic acid **21** was carried out with the dimethyl sulfide borane complex (86%). Subsequent oxidation of the resulting alcohol **22** afforded aldehyde **23** (52%), which was reductively aminated with the aminoesters **13** or **26**. Interestingly, when the $n = 3$ aminoester **13** was used, the reaction pro-

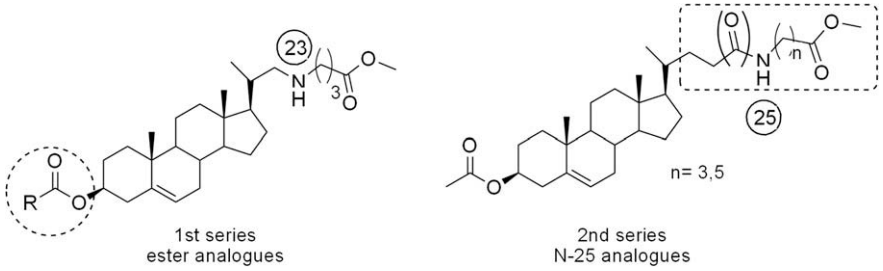
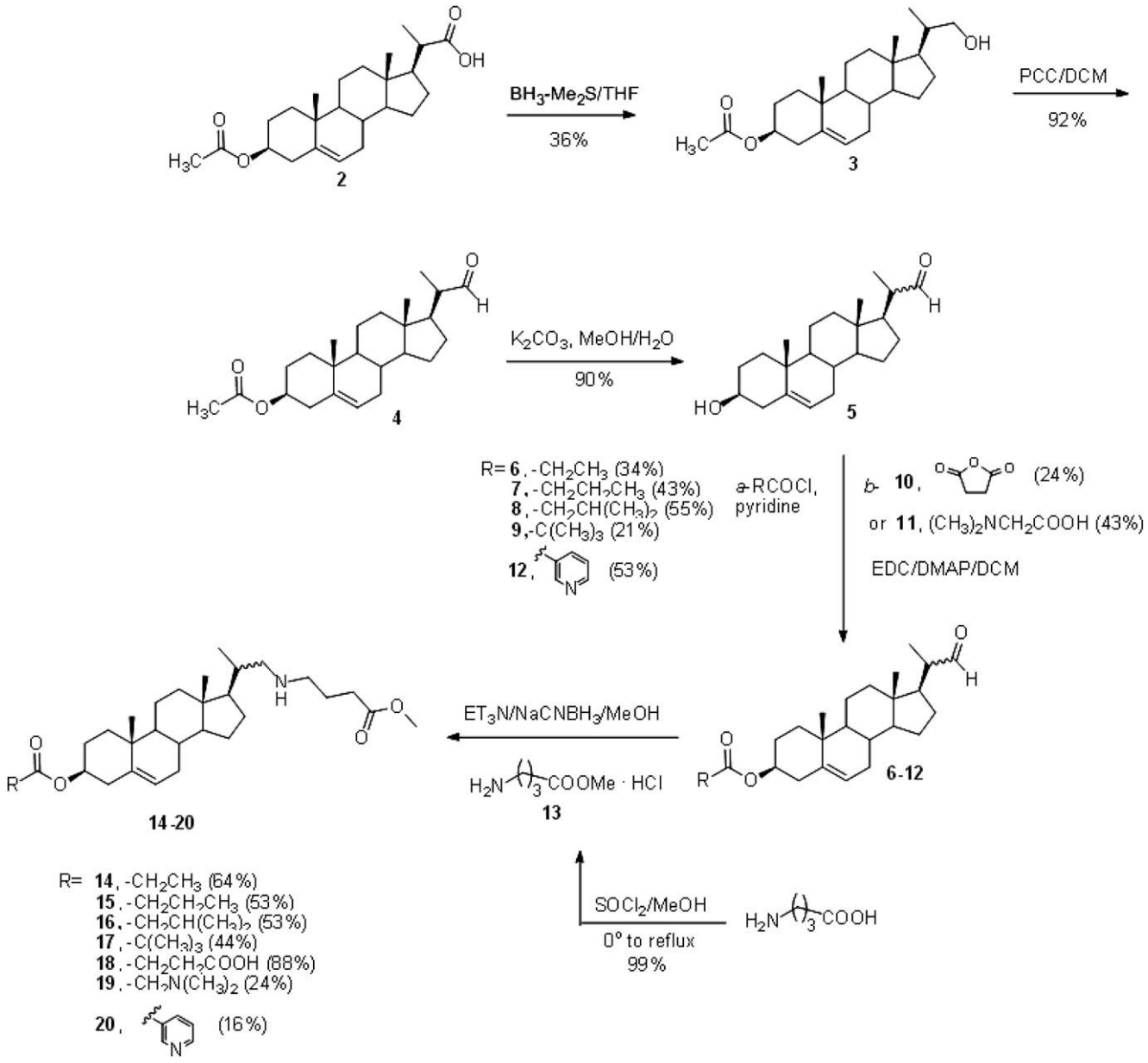


Figure 3. New series of azasterols: esters and N-25 analogues.



Scheme 1. Synthesis of the ester derivatives **14**–**20**.

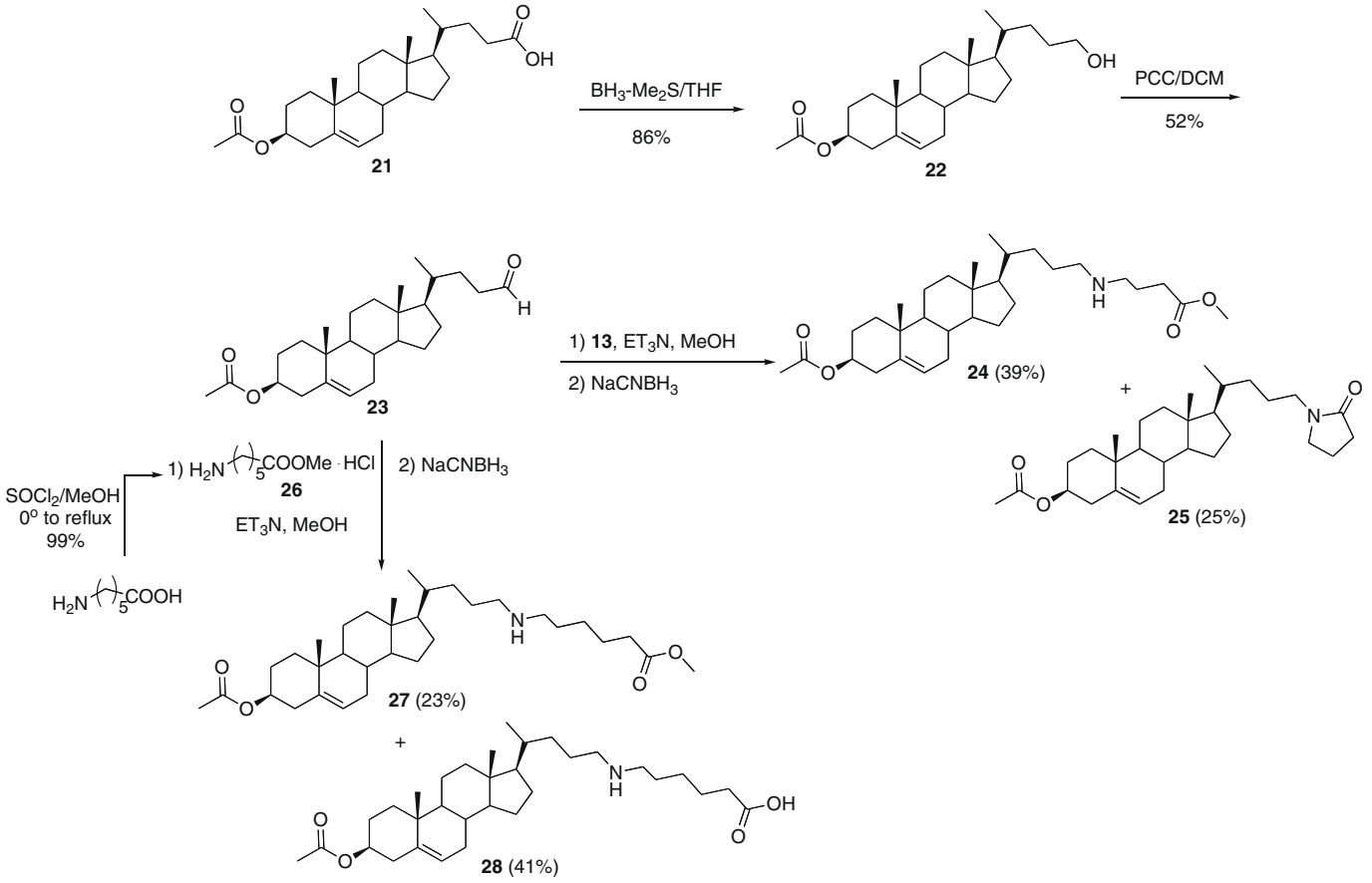
duced the desired product **24** (39%) and the lactam derivative **25** as a side product (25%), which it was possible to separate. When the $n = 5$ aminoester **26** was used, the hydrolysed derivative **28** was recovered (41%), after chromatographic purification, together with the desired product **27** (23%). The formation of side products affected the yields for these reactions.

2.3. N-25 amide derivatives

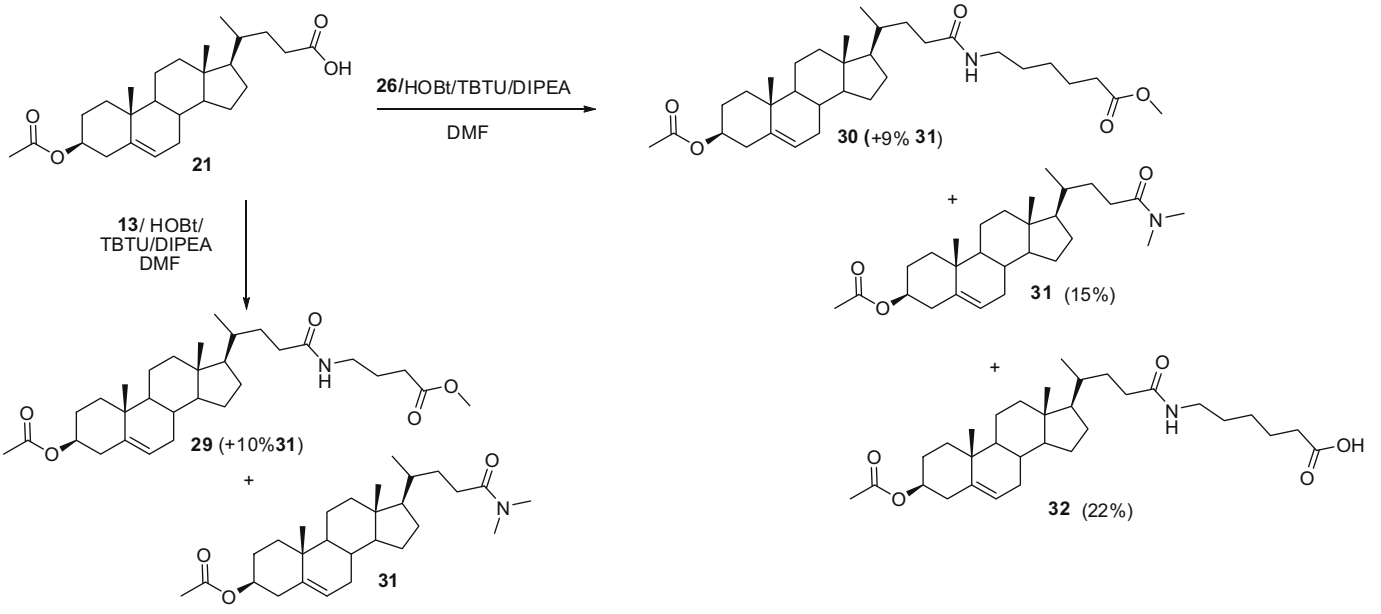
The synthesis of the N-25 amide derivatives is summarized in Scheme 3. The sterol 3β-acetoxy-5-cholenic acid **21** was coupled with the aminoesters **13** or **26**, with either a 3 or 5 carbon chain. The coupling was carried out using the combination of HOBT and TBTU in basic conditions (DIPEA). Although both the desired products were obtained, the dimethylamide derivative **31** was detected as an impurity in both samples, which it was not possible to remove, even after several chromatographic purifications. The formation of **31** might be due to the presence of dimethylamine Me_2NH as a contaminant of the DMF used as solvent for this reaction: this could react with the activated acid starting material and produce **31** as side product (see Supplementary data). Finally, it was possible to isolate **31** pure (15% yield) and calculate that its amount was of 10% in the sample of **29** and of 9% in the sample of **30**. The proportion of compound **31** was calculated from the integration of the CH_3 peaks in the ^1H NMR. The biological analysis of **31** suggested that its presence did not affect the activity of the analogues **29** and **30** (Table 2). A second fraction was collected from the purification of **30**: this was identified as the hydrolysed derivative **32** (22%).

3. Results and discussion

Compounds were screened for activity against the clinically relevant forms of the kinetoplastids: *T. brucei rhodesiense* bloodstream form; *T. cruzi* intracellular amastigotes; *L. donovani* axenic amastigotes. The data for this is shown in Tables 1 and 2.



Scheme 2. Synthesis of N-25 amine derivatives **24**–**25** and **27**–**28**.



Scheme 3. Synthesis of the N-25 amide derivatives **29**–**32**.

Compounds **14–18** were simple analogues of the acetate, with the aim of probing the effect of size at the 3β-position. Increasing the size led to a small loss in activity against *T. brucei* from EC_{50} = 0.8 μM to 2.2 μM. Against the other parasites there was no clear trend, with compounds showing low micromolar activity.

The analogues **18–20** were prepared with water solubilising groups with the aim of reducing the log *D*. In addition, they should probe the SAR of having a polar substituent at the 3β-position. The succinate derivative **18** should be negatively charged at physiological pH, the dimethylglycine derivative **19** should be partially pos-

Table 1
Biological results for ester derivatives **13**–**19**

	EC_{50} (μM) *T. brucei rhodes*	EC_{50} (μM) *T. cruzi*	EC_{50} (μM) *L. don.*	TD_{50} (μM) L6-cells	log *D*	pK_a	% Ionizat. pH 7.4
14, R = CH_2CH_3	0.8	7.6	12.1	21.9	5.1		
15, R = $CH_2CH_2CH_3$	1.6	1.7	6.8	30.7	5.6		
16, R = CH_2 $CH(CH_3)_2$	3.9	10.8	4.9	33.2	6.0		
17, R = $C(CH_3)_3$	2.2	9.7	11.3	11.1	5.8		
18, R = $CH_2CH_2CO_2H$	9.8	13.7	35.7	76.7	4.1	4.4	~99%
19, R = $CH_2N(CH_3)_2$	6.2	11.6	18.5	24	4.4	7.1	~34%
20 R=	0.8	2.51	13.7	9.8	5.2		
Controls	0.01	0.99	~0.5	0.014			

L6-cells are rat skeletal myoblasts and are used as an indication of toxicity to mammalian cells. Log *D* and pK_a values were calculated using the program ACD/Labs 7.00. Controls are: *T. brucei rhodesiense*, melarsoprol; *T. cruzi*, benznidazole; *L. donovani*, miltefosine; L6-cells, podophyllotoxin.

itively charged and the 3-pyridyl **20** contains a polar nitrogen, although in this compound the overall log *D* value is unchanged. The negatively charged and positively charged substituents **18** and **19** were less potent than the uncharged derivatives against all parasites. Interestingly the pyridyl derivative was the most potent against *T. brucei rhodesiense*, although there was a slight increase in potency against L6 cells.

The data for the side chain analogues (series 2) is shown in Table 2. For *T. brucei* the '25-position' compounds showed lower potency than the '23-position' series. The distance between the amine and the ester in the side chain appeared to make relatively little difference to the activity (compare **24** and **27**). Hydrolysis of the methyl ester to the free carboxylic acid caused a drop in activity (**27**/**28** and **30**/**32**) as has been found previously[10] and replacing the amine linkage with an amide linkage caused a drop in activity (**24**/**29** and **27**/**30** and **28**/**32**). With *T. cruzi* the situation is less clear, with a much smaller variation in potency. Again we can see that the free carboxylic acids (**28** and **32**) are less potent. Against *L. donovani* the amide series compounds generally appear more potent than the amine series.

The lactam derivative **25** and the tertiary amide **31** were generally amongst the least potent. The fact that **31** did not show particular effect against any parasite indicates its presence as a small impurity in the samples of **29** and **30** should not affect their activity.

The derivatives **24**, **25** and **27** showed some cytotoxicity at values comparable with their activity, discouraging further studies on these compounds.

From the data obtained, pharmacophores against the parasites were further refined.

Against *T. brucei rhodesiense* (Fig. 4) the presence of bulkier moieties at the 3β-position resulted in decreased activity, with the exception of the pyridyl group. A side chain of 3 carbons gave higher inhibition than a longer one and the methyl ester derivatives were more potent than the hydrolysed ones.[10] Also the lactam ring

Table 2
Biological results for N-25 amine and amide analogues **24**–**25** and **27**–**32**

	EC_{50} (μM) *T. brucei rhodes.*	EC_{50} (μM) *T. cruzi*	EC_{50} (μM) *L. donov.*	TD_{50} (μM) L6 cells
24, R = $CH_2NH(CH_2)_3COOMe$	1.8	7.4	23.8	5.8
25, R=	38.3	25	13.9	2.5
27, R = $CH_2NH(CH_2)_5COOMe$	2.0	11.1	11.7	7.4
28, R = $CH_2NH(CH_2)_5COOH$	11.2	28.9	11.4	56
29, R = $CONH(CH_2)_3COOMe$	18.4	19.7	3.1	>174
30, R = $CONH(CH_2)_5COOMe$	14.6	23	1.6	>165
31, R = $CONH(CH_3)_2$	21.7	28.2	24.6	>202
32, R = $CONH(CH_2)_5COOH$	56	41.9	2.0	63
Controls	0.007	1.3	0.21	0.017

Controls are: *T. brucei rhodesiense*, melarsoprol; *T. cruzi*, benznidazole; *L. donovani*, miltefosine; L6-cells, podophyllotoxin.

in this position gave poor activity. Finally, the N-23 analogues were in general better inhibitors than the N-25 analogues.

Against *L. donovani* (Fig. 5) the best activity was achieved when the nitrogen was at the 25-position and the side chain was attached through an amide bond.

Against *T. cruzi* (Fig. 6) there was no clear tendency and the new azasterols showed only poor activity. Variations in the side chain and in the nitrogen position did not seem to make a significant difference, however the 3 carbon chain amine derivatives gave better results; also, the methyl ester analogues were more potent than the carboxylic acid. Finally interesting activity was noticed when the acetoxy ester at the 3-position was replaced by a linear 4 carbon chain or by the pyridyl ring.

4. Conclusions

Two new series of azasterols were successfully synthesised as potential anti-parasitics: ester and N-25 derivatives of the lead compound **1**. Some of these analogues showed activity in the low micromolar range against *T. brucei rhodesiense*, in particular **14** and **20** from the ester series, and **24** and **27** from the N-25 amine series. In general, the N-25 amide analogues **29**, **30** and **32** all showed promising activity against *L. donovani*.

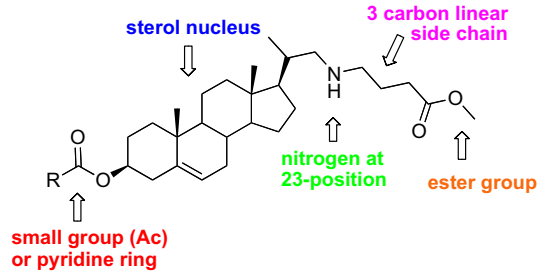


Figure 4. Pharmacophore for activity against *T. brucei rhodesiense*.

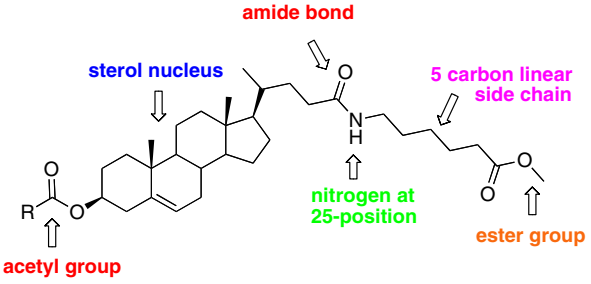


Figure 5. Pharmacophore for activity against *L. donovani*.

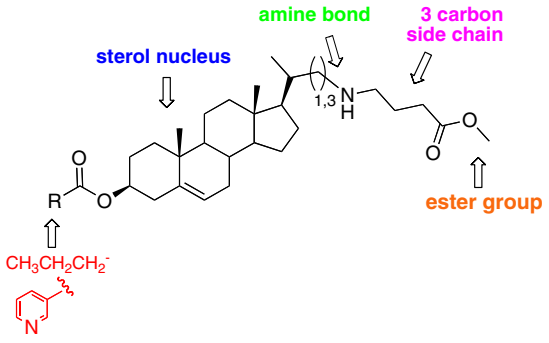


Figure 6. Pharmacophore for activity against *T. cruzi*.

Although the mode of action remains unclear, these new compounds gave useful information about the SAR and allowed further refinement of the pharmacophore against *T. brucei rhodesiense*, *L. donovani* and *T. cruzi* (Figs. 4–6).

Therefore these azasterols represent an important class of compounds and further investigation into the SAR and the mode of action could be useful in the development of new antiparasitics.

5. Experimental

5.1. General experimental details

When applicable, all glassware was oven-dried overnight and all reactions were carried out under Argon atmosphere. Sensitive liquids and solutions were transferred via syringe and were introduced into reaction vessels through rubber septa. All the reactions were carried out using dry solvents unless otherwise stated. All dry solvents, ethanol, methanol, dichloromethane, tetrahydrofuran, dimethylformamide were purchased from Aldrich or Fluka in sure sealed bottles. Analytical TLCs were performed on Silica-Gel 60 F254 plates (Merck). Visualisation of spots was effected by one of the following techniques: (a) UV illumination, (b) immersion of the plate in a 3% solution of ninhydrin in ethanol followed by heating and (c) immersion of the plate in a 48 g L^{-1} solution of phosphomolybdic acid in methanol followed by heating. Column chromatography was carried out on Silica Gel 60 (40–60 μm) purchased from Fluka.

NMR spectra were recorded on a Bruker Avance DPX 300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C or a Bruker Avance DPX 500 MHz spectrometer at 500 MHz for ^{1}H, 125 MHz for ^{13}C. Chemical shifts are reported downfield in parts per million and coupling constants (*J* values) are in hertz. Melting points were determined with a GallenKamp melting point apparatus. Low-resolution mass measurements were performed on Applied Biosystem Mariner API-TOF. LCMS and accurate mass measurements were performed in house with Agilent 1100 HPLC in series with Bruker MicroTof spectrometer or at EPSRC National Mass Spectrometry Service Centre in the Chemistry Department, University of Wales Swansea, Swansea, Wales, UK.

5.2. Biological in vitro assays

The in vitro assays against *T. brucei rhodesiense*, *T. cruzi*, *L. donovani* and L6 cells were performed following the procedure reported by Ganapaty et al.[22]

5.3. 3β-Acetoxy-23,24-bisnorchol-5-en-22-ol (3)

To a solution of 3β-acetoxy-5-cholenic acid **2** (4.505 g, 11.60 mmol) in THF (50 mL), kept below 0 °C, borane–dimethyl sulfide complex 2 M solution in THF (8 mL, 16.0 mmol, 1.4 equiv) was added dropwise and the reaction mixture was stirred overnight at room temperature. It was then diluted with water (2 × 10 mL), and extracted with Et_2O (100 mL). The organic layer was washed with a saturated solution of K_2CO_3 (100 mL, then 75 mL), a saturated solution of NaCl (100 mL) and dried over sodium sulfate. The solvent was removed and the crude purified by column chromatography (hexane/AcOEt 100:0→80:20) to afford **3** as a white solid (1.56 g, 36% yield); R_f = 0.42 (hexane/AcOEt: 80/20); LRMS, *m*/*z* (ES^+ mode): 397 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.61; H, 10.28. Mp = 136–137 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.71 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.06 (3H, d, *J* = 6.6, 21-CH_3), 2.05 (3H, s, 2′-CH_3), 0.90–2.07 (19H, 7 × CH_2, 5 × CH), 2.33 (2H, d, *J* = 7.9, 4-CH_2), 3.38 (1H, dd, J_1 = 7.1, J_2 = 10.5, 22-CH_2), 3.64 (1H, dd,

J_1 = 3.0, J_2 = 10.5, 22′-CH_2), 4.61 (1H, m, 3-CH), 5.39 (1H, m, 6-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.4 (18-CH_3), 17.2 (21-CH_3), 19.7 (19-CH_3), 21.4 (CH_2), 21.9 (2′-CH_3), 24.8 (CH_2), 28.2 (CH_2), 28.2 (CH_2), 32.3 (CH_2), 32.3 (CH), 37.0 (10-C) 37.4 (CH_2), 38.5 (4-CH_2), 39.2 (CH), 40.0 (CH_2), 42.8 (13-C), 50.4 (CH), 52.8 (CH), 56.8 (CH), 68.4 (22-CH_2), 74.4 (3-CH), 123.0 (6-CH), 140.1 (5-C), 171.0 (1′-C=O).

5.4. 3β-Acetoxy-23,24-bisnorchol-5-en-22-al (4)

Compound **3** (0.378 g, 1.01 mmol) and pyridinium chlorochromate (0.417 g, 1.93 mmol, 1.9 equiv) were stirred in DCM (15 mL) overnight at room temperature, in presence of molecular sieves, then poured onto a silica pad, and eluted with 1.25 L of DCM. The filtrate was collected and concentrated under reduced pressure, to afford **4** pure as a white solid (0.347 g, 92% yield); R_f = 0.56 (hexane/AcOEt: 80/20); LRMS, m/z (ES^+ mode): 395 ($[M+Na]^+$, 100%). Anal. Calcd for $C_{24}H_{36}O_3$: C, 77.38; H, 9.74. Found: C, 77.07; H, 9.94. Mp = 105–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 1.18 (3H, d, J = 6.8, 21-CH_3), 2.11 (3H, s, 2′-CH_3), 1.02–1.08 (18H, 7 × CH_2, 4 × CH), 2.46 (3H, m, 4-CH_2 and 20-CH), 4.61 (1H, m, 3-CH), 5.42 (1H, m, 6-CH), 9.60 (1H, d, J = 3.3, 22-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.2 (18-CH_3), 13.5 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.7 (CH_2), 27.0 (CH_2), 27.8 (CH_2), 31.8 (CH_2), 31.9 (CH), 36.6 (10-C), 37.0 (CH_2) 38.1 (4-CH_2), 39.4 (CH_2), 43.0 (13-C), 49.5 (20-CH), 50.0 (CH), 51.0 (CH), 55.9 (CH), 73.9 (3-CH), 122.4 (6-CH), 139.7 (5-C), 170.5 (1′-C=O), 205.1 (22-C=O).

5.5. 23,24-Bisnorchol-5-en-3β-ol-22-al (5)

Compound **4** (0.505 g, 1.35 mmol), and potassium carbonate (0.697 g, 5.04 mmol, 3.7 equiv) were stirred in MeOH/H_2O (36/12 mL) overnight. After evaporating MeOH under reduced pressure, HCl 1.0 M was added to the remaining water solution until a precipitate formed. This was then filtered and purified by column chromatography (hexane/EtOAc 100:0→70:30), to afford **5** as a white solid (0.403 g, 90% yield); R_f = 0.25 (hexane/AcOEt: 80/20); LRMS, m/z (ES^+ mode): 331 ($[M+H]^+$, 100%). Anal. Calcd for $C_{22}H_{34}O_2$: C, 75.77; H, 9.97. Found: C, 75.62; H, 9.95. Mp = 152–153 °C; ^{1}H NMR (300 MHz, MeOD): δ 0.74 and 0.75 (3H, 2s, 18-CH_3), 1.02 and 1.12 (3H, 2d, J_1 = 6.8, J_2 = 6.8, 21-CH_3), 1.09 (3H, s, 19-CH_3), 0.70–2.20 (18H, 7 × CH_2, 4 × CH), 2.25 (3H, m, 4-CH_2 and 20-CH), 3.42 (1H, m, 3-CH), 5.36 (1H, m, 6-CH), 9.52 (1H, 2d, J_1 = 3.2, J_2 = 5.0, 22-CH); ^{13}C NMR (75 MHz, MeOD): δ 12.7 and 13.3 (18-CH_3), 13.9 and 14.0 (21-CH_3), 19.8 (19-CH_3), 21.2 and 21.4 (CH_2), 24.3 and 25.0 (CH_2), 26.9 (CH_2), 27.5 (CH_2), 32.0 and 32.2 (CH_2), 32.3 and 32.3 (CH), 36.9 and 37.0 (10-C), 37.7 (CH_2), 38.8 and 39.9 (CH_2), 42.5 (4-CH_2), 42.7 and 43.4 (13-C), 49.2 (20-CH), 49.9 and 50.5 (CH), 51.4 and 52.4 (CH), 56.4 and 56.6 (CH), 72.1 (3-CH), 121.8 and 121.9 (6-CH), 141.2 and 141.3 (5-C), 205.6 and 206.3 (22-C=O).

5.6. General procedure A: preparation of compounds 6–9 and 12

The aldehyde derivative **5** (1 equiv) was dissolved in pyridine (8.3 mL/mmol) at room temperature, then the chloride (1.2 equiv) was added at 0 °C and the solution was stirred overnight at room temperature. The solution was then diluted with H_2O (60 mL/mmol) and extracted with EtOAc (60 mL/mmol). The EtOAc layer was washed with HCl 1 M (80 mL/mmol) to remove pyridine. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by column chromatography (hexane/EtOAc).

5.6.1. 3β-Propoxy-23,24-bisnorchol-5-en-22-al (6)

Following the general procedure A, starting from **5** (0.330 g, 1.00 mmol) solubilised in pyridine (8.3 mL) propionyl chloride (0.104 mL, 1.20 mmol, 1.2 equiv) was added. The product **6** was collected as a white solid (0.130 g, 34% yield) after extraction and chromatographic purification (hexane/AcOEt 100:0→85:15); R_f = 0.78 (hexane/AcOEt: 80/20); LRMS, m/z (ES^+ mode): 409.3 ($[M+Na]^+$, 100%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 and 0.74 (3H, 2s, 18-CH_3), 1.01 (3H, m, 3′-CH_3), 1.08 and 10.9 (3H, 2s, 19-CH_3), 1.15 and 1.16 (3H, 2d, J_1 = 7.6, J_2 = 7.6, 21-CH_3), 0.70–2.10 (18H, 7 × CH_2, 4 × CH), 2.35 (5H, m, 4-CH_2, 20-CH and 2′-CH_2), 4.63 (1H, m, 3-CH), 5.40 (1H, m, 6-CH), 9.59 (1H, dd, J_1 = 3.3, J_2 = 5.1, 22-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.2 (3′-CH_3), 12.2 and 12.8 (18-CH_3), 13.5 and 13.6 (21-CH_3), 19.3 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 and 24.6 (CH_2), 26.4 (CH_2), 27.0 (CH_2), 27.8 and 27.9 (CH_2), 31.8 and 31.8 (CH_2), 31.9 (CH), 36.6 and 36.6 (10-CH), 37.0 (CH_2), 38.1 and 38.4 (4-CH_2), 39.4 (CH_2), 43.0 (13-C), 48.8 (20-CH), 49.5 (CH), 50.0 and 51.0 (CH), 55.9 and 56.2 (CH), 73.7 (3-CH), 122.3 and 122.4 (6-CH), 139.8 and 139.8 (5-C), 173.9 (1′-C=O), 205.0 and 205.8 (22-C=O).

5.6.2. 3β-Butoxy-23,24-bisnorchol-5-en-22-al (7)

Following the general procedure A, starting from **5** (0.200 g, 0.60 mmol) solubilised in pyridine (5 mL), butyryl chloride (0.075 mL, 0.73 mmol, 1.2 equiv) was added. The product **7** was obtained after extraction and purification by column chromatography (hexane/EtOAc 100:0→90:10) as a white solid (0.105 g, 43% yield); R_f = 0.87 (hexane/AcOEt: 80/20); LRMS, m/z (ES^+ mode): 423 ($[M+Na]^+$, 7%), 823 ($[2M+Na]^+$, 47%). Anal. Calcd for $C_{26}H_{40}O_3 \cdot 0.5$EtOAc: C, 75.63; H, 9.97. Found: C, 75.05; H, 10.06. Mp = 105–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 and 0.73 (3H, 2s, 18-CH_3), 0.96 and 0.98 (3H, m, 4′-CH_3), 1.02 and 1.02 (3H, m, 21-CH_3), 1.16 (3H, 2s, 19-CH_3), 0.70–2.10 (20H, 8 × CH_2, 4 × CH), 2.32 (5H, m, 4-CH_2, 20-CH and 2′-CH_2), 4.53 (1H, m, 3-CH), 5.40 (1H, m, 6-CH), 9.52 (1H, 2d, J_1 = 3.2, J_2 = 5.1, 22-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.2 and 12.8 (18-CH_3), 13.5 and 13.6 (21-CH_3), 13.6 (4′-CH_3), 18.6 (3′-CH_2), 19.3 and 19.3 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 and 24.7 (CH_2), 26.5 and 27.0 (CH_2), 27.8 (CH_2), 31.8 and 31.8 (CH_2), 31.9 (CH), 36.6 (2′-CH_2) 36.6 (10-C), 37.0 (CH_2), 38.1 and 38.4 (4-CH_2), 39.4 (CH_2), 43.0 (13-C), 48.8 (20-CH), 48.8 and 49.5 (CH), 50.0 and 51.0 (CH), 55.9 and 56.2 (CH), 73.6 (3-CH), 122.3 and 122.4 (6-CH), 139.7 and 139.8 (5-C), 173.1 (1′-C=O), 205.0 and 205.8 (22-C=O).

5.6.3. 3β-(3′-Methylbutoxy)-23,24-bisnorchol-5-en-22-al (8)

Following the general procedure A, starting from **5** (0.250 g, 0.76 mmol) solubilised in pyridine (6.5 mL), isovaleryl chloride (0.110 mL, 0.91 mmol, 1.2 equiv) was added. The product **8** was collected after extraction and purification by column chromatography (hexane/EtOAc 100:0→90:10) as a white solid (0.173 g, 55% yield); R_f = 0.92 (hexane/EtOAc: 50/50); LRMS, m/z (ES^+ mode): 437 ($[M+Na]^+$), 851 ($[2M+Na]^+$). Anal. Calcd for $C_{27}H_{42}O_3 \cdot 0.5$EtOAc: C, 75.94; H, 10.11. Found: C, 75.78; H, 10.28. Mp = 105–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 and 0.73 (3H, 2s, 18-CH_3), 0.89 (6H, m, 4′ and 5′-CH_3), 1.05 (6H, m, 19-CH_3 and 21-CH_3), 0.60–2.10 (19H, 7 × CH_2, 5 × CH), 2.08 (2H, m, 2′-CH_2), 2.25 (3H, m, 4-CH_2, 20-CH), 4.52 (1H, m, 3-CH), 5.31 (1H, m, 6-CH), 9.52 (1H, 2d, J_1 = 3.2, J_2 = 5.1, 22-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.2 and 12.8 (18-CH_3), 13.5 and 13.6 (21-CH_3), 19.3 (19-CH_3), 20.7 and 21.0 (CH_2), 22.4 (4′-CH_3 and 5′-CH_3), 23.9 and 24.7 (CH_2), 25.8 (3′-CH), 26.4 and 27.0 (CH_2), 27.8 (CH_2), 31.8 and 31.8 (CH_2), 31.9 (CH), 36.6 and 36.7 (10-C), 37.0 (CH_2), 38.2 and 38.4 (4-CH_2), 39.4 (CH_2), 43.0 (13-C), 43.8 (2′-CH_2), 48.8 (20-CH), 49.5 (CH), 50.0 and 51.0 (CH), 55.9 and 56.2 (CH), 73.5 (3-CH), 122.3 and 122.4 (6-CH), 139.7 and 139.8 (5-C), 172.6 (1′-C=O), 205.0 and 205.8 (22-C=O).

5.6.4. 3β-Trimethylacetoxy-23,24-bisnorchol-5-en-22-al (9)

Following the general procedure A, starting from **5** (0.250 g, 0.76 mmol) solubilised in pyridine (6.3 mL), trimethyl acetyl chlo-

ride (0.111 mL, 0.91 mmol, 1.2 equiv) was added. The product **9** was isolated after extraction and purification by column chromatography (hexane/EtOAc 100:0→90:10) as a white solid (0.066 g, 21% yield); R_f = 0.93 (hexane/EtOAc: 50:50); LRMS, *m*/*z* (ES^+ mode): 437.28 ($[M+Na]^+$, 37%); 851.57 ($[2M+Na]^+$, 46%). Anal. Calcd for $C_{27}H_{42}O_3{\cdot}0.8H_2O$: C, 75.59; H, 10.24. Found: C, 75.63; H, 10.18. Mp = 124–130 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.62 (3H, 2s, 18-CH_3), 1.07 (3H, s, 19-CH_3), 1.08 (3H, d, J = 6.8, 21-CH_3), 1.20 (9H, s, 3′-CH_3, 4′-CH_3 and 5′-CH_3), 0.70–2.10 (18H, 7 × CH_2, 4 × CH), 2.33 (3H, m, 4-CH_2, 20-CH), 4.60 (1H, m, 3-CH), 5.40 (1H, m, 6-CH), 9.57 (1H, 2d, J_1 = 3.1, J_2 = 5.1, 22-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.3 and 12.8 (18-CH_3), 13.5 and 13.6 (21-CH_3), 19.3 and 19.4 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 and 24.7 (CH_2), 26.5 and 27.0 (CH_2), 27.2 (3′-CH_3, 4′-CH_3, 5′-CH_3), 27.6 (CH_2), 31.8 and 31.8 (CH_2), 31.9 (CH), 36.6 and 36.7 (10-C), 37.0 (CH_2), 38.0 and 38.4 (4-CH_2), 38.6 (2′-C) 39.4 (CH_2), 43.0 (13-C), 48.8 (20-CH), 49.5 and 50.0 (CH), 51.9 (CH), 56.0 and 56.2 (CH), 73.5 (3-CH), 122.2 and 122.3 (6-CH), 139.8 and 139.9 (5-C), 178.0 (1′-C═O), 205.1 and 205.8 (22-C═O).

5.6.5. 3β-Nicotinoyloxy-23,24-bisnorchol-5-en-22-al (12)

Following the general procedure A, starting from **5** (0.110 g, 0.33 mmol) solubilised in pyridine (4.5 mL), nicotinoyl chloride hydrochloride (0.074 g, 0.41 mmol, 1.2 equiv) was added. After extraction, product **12** was collected as a white solid (0.077 g, 53% yield) and not further purified due to its poor stability under column chromatography, but was used as crude for next step; R_f = 0.31 (hexane/AcOEt: 80/20); LRMS, *m*/*z* (ES^+ mode): 436.6 ($[M+H]^+$, 100%). Anal. Calcd for $C_{28}H_{37}NO_3{\cdot}0.5HCl$: C, 74.10; H, 8.33; N, 3.09. Found: C, 74.24; H, 8.44; N, 3.19. Mp = 137 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.63 and 0.68 (3H, 2s, 18-CH_3), 0.98 and 1.07 (3H, 2d, J_1 = 6.8, J_2 = 6.8, 21-CH_3), 0.99 and 1.01 (3H, 2s, 19-CH_3), 0.60–2.10 (18H, 7 × CH_2, 4 × CH), 2.30 (1H, m, 20-CH), 2.42 (2H, m, 4-CH_2), 4.83 (1H, m, 3-CH), 5.36 (1H, m, 6-CH), 7.32 (1H, m, 6′-CH), 8.22 and 8.23 (1H, 2dd, J_1 = 1.8, J_2 = 3.9, J_3 = 1.8, J_4 = 3.9, 5′-CH), 8.73 (1H, m, 4′-CH), 9.16 (1H, s, 3′-CH), 9.51 (1H, dd, J_1 = 3.3, J_2 = 5.2, 22-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.9 (18-CH_3), 13.5 and 13.7 (21-CH_3), 19.4 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 and 24.7 (CH_2), 26.5 and 27.1 (CH_2), 27.8 (CH_2), 31.8 (CH_2), 31.9 (CH), 36.7 (10-C), 37.0 (CH_2), 38.1 and 38.4 (4-CH_2), 39.4 (CH_2), 42.1 and 43.0 (13-C), 48.9 (20-CH), 49.5 and 50.0 (CH), 51.0 and 51.9 (CH), 55.9 and 56.2 (CH), 75.1 (3-CH), 122.8 and 122.9 (6-CH), 123.2 (5′-C), 126.6 (2′-C), 135.1 (5-C), 137.1 (6′-C), 151.0 (3′-C), 153.3 (4′-C), 164.1 (1′-C═O), 206.0 (22-C═O).

5.7. 3β-Oxalyloxy-23,24-bisnorchol-5-en-22-al (10)

Compound **5** (0.150 g, 0.45 mmol) was stirred with succinic anhydride (0.082 g, 0.82 mmol, 1.8 equiv) and DMAP (0.093 g, 0.76 mmol, 1.7 equiv) in DCM (7.5 mL), then EDC (0.122 g, 0.64 mmol, 1.4 equiv) was added. The reaction was monitored by TLC and stirred overnight. Subsequently DMAP (0.072 g, 0.59 mmol, 1.3 equiv) and EDC (0.104 g, 0.54 mmol, 1.2 equiv) were added and the mixture was stirred for a further 24 h. The solution was diluted with H_2O (10 mL) and extracted with DCM (10 mL); the organic layer was washed with NaCl (10 mL) and dried over $MgSO_4$. Purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10) afforded **10** as a white sticky solid (0.047 g, 24% yield); R_f = 0.60 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 453.3 ($[M+Na]^+$, 100%); ^1H NMR (500 MHz, $CDCl_3$): δ 0.65 and 0.66 (3H, 2s, 18-CH_3), 0.93 and 0.95 (3H, 2s, 19-CH_3), 0.97 and 1.06 (3H, 2d, J_1 = 6.8, J_2 = 6.9, 21-CH_3), 0.55–2.12 (18H, 7 × CH_2, 4 × CH), 2.25 (3H, m, 4-CH_2, 20-CH), 2.52 (4H, m, 2′-CH_2 and 3′-CH_2), 4.55 (1H, m, 3-CH), 5.30 (1H, m, 6-CH), 9.46 and 9.50 (1H, 2d, J_1 = 3.3, J_2 = 5.1, 22-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 and 12.9 (18-CH_3), 13.5 and 13.6 (21-CH_3), 19.3 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 (CH_2), 26.5 and 27.1 (CH_2), 27.7 (CH_2), 29.3 (2′-CH_2), 29.6 (3′-CH_2), 31.8 (CH_2), 31.8 (CH), 36.6 (10-C), 36.9 (CH_2), 38.0 and 38.1 (4-CH_2), 38.4 (CH_2), 43.0 (13-C), 48.9 (20-CH), 49.5 and 50.0 (CH), 51.9 (CH), 55.9 and 56.1 (CH), 744 (3-CH), 122.5 and 122.5 (6-CH), 139.6 (5-C), 171.7 (1′-C═O), 177.7 (4′-C═O), 205.2 and 206.0 (22-C═O).

5.8. 3β-Dimethylaminoacetoxy-23,24-bisnorchol-5-en-22-al (11)

Compound **5** (0.055 g, 0.17 mmol) was stirred with dimethyl glycine (0.023 g, 0.22 mmol, 1.3 equiv) and DMAP (0.029 g, 0.24 mmol, 1.4 equiv) in DCM (2.5 mL) and EDC (0.043 g, 0.22 mmol, 1.3 equiv) was added. The reaction was monitored by TLC and stirred overnight. Subsequently DMAP (0.026 g, 0.39 mmol, 1.3 equiv) and EDC (0.038 g, 0.20 mmol, 1.2 equiv) were added and the mixture was stirred for a further 24 h. The solution was diluted with H_2O (5 mL) and extracted with DCM (5 mL); the organic layer was then washed with NaCl (5 mL) and dried over $MgSO_4$. Purification by column chromatography (hexane/EtOAc 100:0→80:20), afforded **11** as a white sticky solid (0.030 g, 43% yield); R_f = 0.8 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 416.6 ($[M+H]^+$, 100%); ^1H NMR (500 MHz, $CDCl_3$): δ 0.62 and 0.66 (3H, 2s, 18-CH_3), 0.93 and 0.95 (3H, 2s, 19-CH_3), 0.96 and 1.06 (3H, 2d, J_1 = 6.8, J_2 = 6.8, 21-CH_3), 0.60–2.10 (18H, 7 × CH_2, 4 × CH), 2.25 (3H, m, 4-CH_2, 20-CH), 2.28 (6H, s, 3′-CH_3 and 4′-CH_3) 3.07 (2H, s, 2′-CH_2), 4.62 (1H, m, 3-CH), 5.31 (1H, m, 6-CH), 9.49 (1H, d, J_1 = 3.3, J_2 = 5.1 Hz, 22-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 and 12.9 (18-CH_3), 13.5 and 13.6 (21-CH_3), 19.3 (19-CH_3), 20.7 and 21.0 (CH_2), 23.9 and 24.7 (CH_2), 26.4 and 27.0 (CH_2), 27.7 (CH_2), 29.7 (CH_2), 31.8 and 31.8 (CH), 36.6 and 36.6 (10-C), 36.9 (CH_2), 38.0 and 38.3 (4-CH_2), 39.4 (CH_2), 42.1 and 43.0 (13-C), 44.1 (3′ and 4′-CH_3) 48.9 (20-CH), 49.5 and 49.9 (CH), 51.0 and 51.9 (CH), 55.9 and 56.1 (CH), 59.1 (2′-CH_2), 74.8 (3-CH), 122.7 and 122.8 (6-CH), 139.3 and 139.4 (5-C), 168.5 (1′-C═O), 205.2 and 206.0 (22-C═O).

5.9. Methyl 4-aminobutanoate hydrochloride (13)

Thionyl chloride (3.32 mL, 45.474 mmol, 2.2 equiv) was added dropwise to a stirred solution of 4-aminobutanoic acid (2.235 g, 20.671 mmol) in MeOH (7.8 mL, 194.3 mmol, 9.4 equiv) at 0 °C. The resulting solution was refluxed (60 °C) overnight. The solvent and thionyl chloride in excess were then removed under reduced pressure, to collect **13** as a white solid (3.160 g, 99% yield).

5.10. General procedure B: preparation of compounds 14–20

To a solution of **13** (2.3 equiv) in methanol (22.5 mL/mmol) triethylamine (TEA) (2.3 equiv) was added and the solution stirred for 30 min. Then the aldehyde derivative (**6–12**) was added as a solid and the solution stirred for a further 30 min. Finally $NaCNBH_3$ was added as THF solution 1 M (2.1 equiv) and the reaction stirred overnight at room temperature. The solution was then diluted with H_2O (80 mL/mmol), extracted with $CHCl_3$ (160 mL/mmol), and washed with a saturated solution of NaCl (80 mL/mmol). The organic phase was dried over $MgSO_4$, then concentrated under reduced pressure and the crude purified by column chromatography ($CHCl_3$/MeOH).

5.10.1. 3β-Propoxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (14)

Following the general procedure B, starting from a solution of **13** (0.044 g, 0.28 mmol, 2.3 equiv) and TEA (0.039 mL, 0.28 mmol, 2.3 equiv) in MeOH (2.8 mL), **6** (0.048 g, 0.12 mmol) and $NaCNBH_3$ (0.23 mL of 1 M solution in THF, 0.23 mmol, 1.8 equiv) were added and the reaction stirred at room temperature overnight. Product **14** was isolated after extraction and purification by column chromatog-

raphy ($CHCl_3$/MeOH 100:0→90:10) as a yellow sticky solid (0.039 g, 64% yield); R_f = 0.33 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 488.3 ($[M+H]^+$, 100%). Anal. Calcd for $C_{30}H_{49}NO_4 \cdot 0.3H_2O$: C, 73.07; H, 10.14; N, 2.84. Found: C, 72.79; H, 9.99; N, 2.96. HRMS (ES^+ mode), calculated for $C_{30}H_{50}NO_4$ ($[M+H]^+$): 488.3740; found: 488.3736; mp = 76–78 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.71 (3H, s, 18-CH_3), 0.92 and 0.98 (3H, 2d, J_1 = 6.8, J_2 = 6.8, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.16 (3H, t, J = 7.5, 3′-CH_3), 0.70–2.10 (21H, 8 × CH_2, 5 × CH, 20-CH), 2.31 (4H, m, 4-CH_2, 2′-CH_2), 2.40 (3H, m, 22^a-CH_2 and 26-CH_2), 2.62 (2H, m, 24-CH_2), 2.79 (1H, m, 22^b-CH_2), 3.70 (3H, s, 28-CH_3), 4.62 (1H, m, 3-CH), 5.38 (1H, m, 6-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.2 (3′-CH_3), 11.9 and 12.2 (18-CH_3), 17.6 and 17.7 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 24.1 and 24.3 (CH_2), 25.1 and 25.1 (25-CH_2), 27.7 and 27.8 (CH_2), 27.9 (2′-CH_2), 28.0 (CH_2), 31.8 and 31.9 (CH_2), 32.0 (CH), 35.5 (20-CH), 36.4 (26-CH_2), 36.6 and 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.4 and 39.7 (CH_2), 42.3 and 42.5 (13-C), 49.3 and 49.3 (24-CH_2), 49.9 and 50.0 (CH), 51.6 (28-CH_3), 54.0 and 54.2 (CH), 55.3 (22-CH_2), 56.5 and 56.6 (CH), 73.7 (3-CH), 122.5 (6-CH), 139.7 and 139.7 (5-C), 173.9 and 174.1 (1′-C=O), 175.2 and 175.4 (27-C=O).

5.10.2. 3β-Butoxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (15)

Following the general procedure B, starting from a solution of **13** (0.051 g, 0.33 mmol, 2.4 equiv) and TEA (0.045 mL, 0.33 mmol, 2.4 equiv) in MeOH (2.1 mL), **7** (0.055 g, 0.14 mmol) and $NaCNBH_3$ (0.23 mL of a 1 M solution in THF, 0.23 mmol. 1.8 equiv) were added and the reaction stirred at room temperature overnight. Product **15** was recovered as a sticky yellow solid after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10), 53% yield (0.037 g); R_f = 0.39 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 502.37 ($[M+H]^+$, 100%). Anal. Calcd for $C_{31}H_{51}NO_4 \cdot 0.5H_2O$: C, 72.90; H, 10.26; N, 2.79. Found: C, 72.62; H, 9.97; N, 2.31. HRMS (ES^+ mode), calculated for $C_{31}H_{52}NO_4$ ($[M+H]^+$): 502.3896; found: 502.3893; mp = 85–88 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.62 (3H, s, 18-CH_3), 0.77 and 0.86 (3H, 2d, J_1 = 6.9, J_2 = 6.9, 21-CH_3), 0.94 (3H, t, J = 7.4, 4′-CH_3), 1.04 (3H, s, 19-CH_3), 0.60–1.90 (23H, 9 × CH_2, 5 × CH), 2.22 (2H, m, 2′-CH_2), 2.22 (2H, m, 4-CH_2), 2.31 (2H, m, 26-CH_2), 2.58–2.82 (4H, m, 22-CH_2 and 24-CH_2), 3.69 (3H, s, 28-CH_3), 4.52 (1H, m, 3-CH), 5.29 (1H, m, 6-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.9 and 12.2 (18-CH_3), 13.6 (4′-CH_3), 17.5 and 17.7 (21-CH_3), 18.6 (3′-CH_2), 19.3 (19-CH_3), 21.0 (CH_2), 24.1 and 24.3 (CH_2), 24.7 and 24.8 (25-CH_2), 27.7 (CH_2), 27.8 and 28.0 (CH_2), 31.8 and 31.9 (CH_2), 31.9 (CH), 35.3 (20-CH), 36.2 (26-CH_2), 36.6 (10-C), 36.6 (2′-CH_2), 37.0 (CH_2), 38.3 (4-CH_2), 39.4 and 39.6 (CH_2), 42.3 and 42.5 (13-C), 49.2 and 49.2 (24-CH_2), 50.0 and 50.0 (CH), 51.6 (28-CH_3), 54.1 and 54.2 (CH), 54.5 and 55.2 (22-CH_2), 56.5 and 56.6 (CH), 73.6 (3-CH), 122.5 (6-CH), 139.7 (5-C), 173.1 (1′-C=O), 174.1 (27-C=O).

5.10.3. 3β-(3′-Methylbutoxy)-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (16)

Following the general procedure B, starting from a solution of **13** (0.075 g, 0.49 mmol, 3.2 equiv) and TEA (0.068 mL, 0.49 mmol, 3.2 equiv) in MeOH (6.5 mL), **8** (0.064 g, 0.15 mmol) and $NaCNBH_3$ (0.35 mL of 1 M solution in THF, 0.35 mmol, 2.3 equiv) were added and the reaction stirred overnight at room temperature. Product **16** was collected as a white solid (0.042 g, 53% yield) after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10); R_f = 0.93 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 516 ($[M+H]^+$, 100%). Anal. Calcd for $C_{32}H_{53}O_4N \cdot 0.18HCl$: C, 74.00; H, 10.36; N, 2.70. Found: C, 73.78; H, 10.44; N, 2.56. HRMS (ES^+ mode), calculated for $C_{32}H_{54}NO_4$ ($[M+H]^+$): 516.4053; found: 516.4061; mp = 75–80 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (3H, s, 18-CH_3), 0.89 and 0.96 (3H, 2d, J_1 = 6.9, J_2 = 6.9, 21-CH_3), 0.95 (3H, s, 19-CH_3), 0.97 (6H, s, 4′-CH_3 and 5′-CH_3), 0.60–2.20 (22H, 8×CH_2, 6 × CH), 2.19 (2H, m, 2′-CH_2), 2.33 (2H, m, 4-CH_2), 2.49 (2H, m, 26-CH_2), 2.52–2.73 (3H, m, 22^a-CH_2 and 24-CH_2), 2.78 (1H, m, 22^b-CH_2), 3.69 (3H, s, 28-CH_3), 4.61 (1H, m, 3-CH), 5.39 (1H, m, 6-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.9 and 12.2 (18-CH_3), 17.6 and 17.7 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 22.4 (4′-CH_3 and 5′-CH_3), 24.2 and 24.3 (CH_2), 25.2 and 25.2 (25-CH_2), 25.8 (3′-CH), 27.7 (CH_2), 27.8 and 28.0 (CH_2), 31.9 (CH_2), 32.0 (CH), 35.5 (20-CH), 36.4 (26-CH_2), 36.6 (10-C), 37.0 (CH_2), 38.2 (4-CH_2), 39.4 and 39.7 (CH_2), 42.3 and 42.5 (13-C), 43.8 (2′-CH_2), 49.3 and 49.4 (24-CH_2), 50.0 and 50.0 (CH), 51.6 (28-CH_3), 54.0 and 54.2 (CH), 54.7 and 55.3 (22-CH_2), 56.5 and 56.6 (CH), 73.6 (3-CH), 122.5 (6-CH), 139.7 (5-C), 172.6 (1′-C=O), 174.1 (27-C=O).

5.10.4. 3β-Trimethylacetoxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (17)

Following the general procedure B, starting from a solution of **13** (0.077 g, 0.50 mmol, 3.6 equiv) and TEA (0.070 mL, 0.50 mmol, 3.6 equiv) in MeOH (6.5 mL), **9** (0.059 g, 0.137 mmol) and $NaCNBH_3$ (0.31 mL of 1 M solution in THF, 0.31 mmol, 2.3 equiv) were added and the reaction stirred at room temperature overnight. Product **17** was collected after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10) as a white solid (0.031 g, 44% yield); R_f = 0.96 ($CHCl_3$/MeOH: 80/20); mp = 140–145 °C. Anal. Calcd for $C_{32}H_{53}O_4N \cdot H_2O$: C, 71.59; H, 10.24; N, 2.61. Found: C, 71.51; H, 10.08; N, 2.27. LRMS, *m*/*z* (ES^+ mode): 516 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{32}H_{54}NO_4$ ($[M+H]^+$): 516.4053; found: 516.4054; ^1H NMR (300 MHz, $CDCl_3$): δ 0.62 (3H, s, 18-CH_3), 0.88 and 0.92 (3H, 2d, J_1 = 6.8, J_2 = 6.8, 21-CH_3), 0.97 (3H, s, 19-CH_3), 1.12 (9H, s, 3′-CH_3, 4′-CH_3 and 5′-CH_3), 0.60–1.95 (21H, 8 × CH_2, 5 × CH), 2.22 (2H, m, 4-CH_2), 2.31 (2H, m, 26-CH_2), 2.48–2.63 (3H, m, 22^a-CH_2 and 24-CH_2), 2.72 (1H, m, 22^b-CH_2), 3.61 (3H, s, 28-CH_3), 4.50 (1H, m, 3-CH), 5.31 (1H, m, 6-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.9 and 12.2 (18-CH_3), 17.6 and 17.7 (21-CH_3), 19.4 (19-CH_3), 21.0 (CH_2), 24.1 and 24.3 (CH_2), 25.0 and 25.0 (25-CH_2), 27.2 (3′-CH_3, 4′-CH_3 and 5′-CH_3), 27.7 (CH_2), 28.0 (CH_2), 31.9 (CH_2), 32.0 (CH), 35.4 (20-CH), 36.3 (26-CH_2), 36.6 (10-C), 37.0 (CH_2), 38.0 (4-CH_2), 38.6 (2′-C), 39.4 and 39.7 (CH_2), 42.3 and 42.5 (13-C), 49.2 (24-CH_2), 50.0 and 50.0 (CH), 51.6 (28-CH_3), 54.1 and 54.2 (CH), 54.6 (22-CH_2), 56.5 and 56.6 (CH), 73.5 (3-CH), 122.4 (6-CH), 139.8 (5-C), 174.1 (1′-C=O), 178.0 (27-C=O).

5.10.5. 3β-Oxalyloxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (18)

Following the general procedure B, starting from a solution of **13** (0.044 g, 0.28 mmol, 2.6 equiv) and TEA (0.039 mL, 0.28 mmol, 2.6 equiv) in MeOH (4 mL), **10** (0.047 g, 0.11 mmol) and $NaCNBH_3$ (0.23 mL of 1 M solution in THF, 0.23 mmol, 2.1 equiv) were added and the reaction stirred overnight at room temperature. Product **18** was collected after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→80:20) as a yellow sticky solid (0.051 g, 88% yield); R_f = 0.46 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 532.7 ($[M+H]^+$, 84%); ^1H NMR (500 MHz, $CDCl_3$): δ 0.65 and 0.67 (3H, 2s, 18-CH_3), 0.93 (3H, d, J = 6.6, 21-CH_3), 1.18 (3H, s, 19-CH_3), 0.60–2.20 (21H, 8 × CH_2, 5 × CH), 2.25 (2H, m, 4-CH_2), 2.36 (2H, m, 26-CH_2), 2.51 (5H, m, 2′-CH_2, 3′-CH_2 and 22^a-CH_2), 2.92 (2H, m, 22^b-CH_2 and 24^a-CH_2), 3.17 (1H, m, 24^b-CH_2), 3.60 (3H, s, 28-CH_3), 4.51 (1H, m, 3-CH), 5.28 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.5 and 12.0 (18-CH_3), 16.7 and 17.1 (21-CH_3), 19.3 and 19.4 (19-CH_3), 21.0 and 21.1 (CH_2), 22.6 and 22.7 (CH_2), 25.8 (25-CH_2), 27.5 and 27.7 (CH_2), 27.8 (CH_2), 28.0 (CH_2), 29.7 (2′-CH_2), 31.0 (3′-CH_2), 31.7 and 31.8 (CH_2), 31.9 (CH), 33.7 and 33.8 (20-CH), 36.6 (26-CH_2), 36.6 (10-C), 37.3 (CH_2), 38.4 (4-CH_2), 39.6 and 39.7 (CH_2), 41.0 and 42.1 (13-C), 50.1 (24-CH_2), 51.8 and 51.9 (28-CH_3), 54.5 and 54.6 (CH), 54.7 (22-CH_2), 56.7 and 57.0 (CH), 74.0 (3-CH), 122.4 (6-CH), 139.8 (5-C), 172.5 (1′-C=O), 173.1 (27-C=O), 175.7 (4′-C=O).

5.10.6. 3β-Dimethylaminoacetoxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (19)

Following the general procedure B, starting from a solution of **13** (0.029 g, 0.19 mmol, 2.6 equiv) and TEA (0.026 mL, 0.19 mmol, 2.6 equiv) in MeOH (2.5 mL), **11** (0.030 g, 0.07 mmol) and $NaCNBH_3$ (0.15 mL of 1 M solution in THF, 0.15 mmol, 2.1 equiv) were added and the reaction stirred at room temperature overnight. Product **19** was collected after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10) as a white sticky solid (0.009 g, 24% yield); R_f = 0.57 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 517.7 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{31}H_{53}N_2O_4$ ($[M+H]^+$): 517.4000; found: 517.3977; mp = 287–291 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.63 and 0.64 (3H, 2s, 18-CH_3), 0.88 (3H, d, J = 6.6, 21-CH_3), 0.94 (3H, s, 19-CH_3), 0.70–2.10 (21H, 8 × CH_2, 5 × CH), 2.27, (2H, m, 4-CH_2), 2.28 (6H, s, 3′-CH_3, 4′-CH_3), 2.37 (3H, m, 22^a-CH_2 and 26-CH_2), 2.65 (1H, m, 24^a-CH_2), 2.76 (1H, m, 24^b-CH_2), 2.95 (1H, m, 22^b-CH_2), 3.07 (2H, s, 2′-CH_2), 3.61 (3H, s, 28-CH_3), 4.62 (1H, m, 3-CH), 5.32 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 and 12.3 (18-CH_3), 17.6 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 24.0 (CH_2), 24.3 and 24.3 (25-CH_2), 27.6 (CH_2), 27.8 (CH_2), 29.7 (CH_2), 31.6 and 31.6 (CH_2), 31.8 and 31.8 (CH), 33.8 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.5 (CH_2), 42.3 and 42.6 (13-C), 45.4 (3′-CH_3 and 4′-CH_3), 49.2 (24-CH_2), 49.9 (CH), 51.9 (28-CH_3), 53.8 and 53.8 (22-CH_2), 54.2 (CH), 56.5 (CH), 60.9 (2′-CH_2), 74.2 (3-CH), 122.6 (6-CH), 139.5 (5-C), 170.1 (1′-C═O), 175.3 (27-C═O).

5.10.7. 3β-Nicotinoyloxy-23,24-bisnorchol-5-en-22-yl-(*N*-3-methoxycarbonylpropyl)-amine (20)

Following the general procedure B, starting from a solution of **13** (0.071 g, 0.46 mmol, 2.6 equiv) and TEA (0.064 mL, 0.46 mmol, 2.6 equiv) in MeOH (4.5 mL), **12** (0.077 g, 0.18 mmol) and $NaCNBH_3$ (0.32 mL of 1 M solution in THF, 0.32 mmol, 1.8 equiv) were added and the reaction stirred at room temperature overnight. Product **20** was collected after extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→95:5) as a white solid (0.015 g, 16% yield); R_f = 0.56 ($CHCl_3$/MeOH: 80/20). Anal. Calcd for $C_{33}H_{48}N_2O_4 \cdot 0.7H_2O$: C, 70.49; H, 8.73; N, 4.98. Found: C, 70.13; H, 8.68; N, 4.55. LRMS, *m*/*z* (ES^+ mode): 537.7 ($[M+H]^+$); HRMS (ES^+ mode), calculated for $C_{33}H_{49}N_2O_4$ ($[M+H]^+$): 537.3687; found: 537.3680; ^1H NMR (500 MHz, $CDCl_3$): δ 0.65 and 0.66 (3H, s, 18-CH_3), 0.92 (3H, d, J = 6.5, 21-CH_3), 0.99 (3H, s, 19-CH_3), 0.70–2.00 (21H, 8 × CH_2, 5 × CH), 2.33 (3H, m, 26-CH_2 and 22^a-CH_2), 2.41 (2H, d, J = 8.1, 4-CH_2), 2.52–2.78 (3H, m, 24-CH_2 and 22^b-CH_2), 3.61 (3H, s, 28-CH_3), 4.83 (1H, m, 3-CH), 5.38 (1H, m, 6-CH), 7.32 (1H, dd, J_1 = 4.8, J_2 = 7.9, 5′-CH), 8.23 (1H, m, 6′-CH), 8.73 (1H, d, J = 3.8, 4′-CH), 9.16 (1H, s, 3′-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.0 and 12.3 (18-CH_3), 17.7 and 18.1 (21-CH_3), 19.4 (19-CH_3), 21.0 (CH_2), 24.2 and 24.3 (CH_2), 24.5 (25-CH_2), 27.5 and 27.8 (CH_2), 28.1 (CH_2), 31.8 and 31.9 (CH_2), 31.9 (CH), 35.0 (20-CH), 36.6 (26-CH_2), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.5 and 39.6 (CH_2), 42.3 and 42.7 (13-C), 47.5 and 47.6 (CH), 49.8 and 49.9 (24-CH_2), 49.9 (28-CH_3), 52.0 (22-CH_2), 54.5 and 54.6 (CH), 56.3 and 56.5 (CH), 75.2 (3-CH), 123.0 (6-CH), 123.3 (5′-C), 127.8 (2′-C), 137.1 (6′-C), 139.4 (5-C), 150.9 (3′-C), 153.3 (4′-C), 164.7 (1′-C═O), 175.2 and 175.4 (27-C═O).

5.11. 3β-Acetoxychol-5-en-24-ol (22)

Borane–dimethyl sulfide complex BH_3Me_2S (1.57 mL of a 2 M solution in THF, 3.14 mmol, 1.37 equiv) was added to a solution of 5-cholenic acid-3β-ol-acetate **21** (0.958 g, 2.29 mmol), at 0 °C. After 30 min the ice bath was removed and the solution stirred at room temperature overnight. The reaction mixture was then diluted with H_2O (40 mL) and extracted with Et_2O (40 mL), the organic layer was washed with a saturated solution of K_2CO_3 (2 × 30 mL), NaCl (30 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. After purification by column chromatography (hexane/EtOAc 100:0→70:30) **22** was obtained as a white solid (0.793 g, 86% yield); R_f = 0.17 (hexane/AcOEt: 80/20); LRMS, *m*/*z* (ES^+ mode): 425.49 ($[M+Na]^+$, 30%), 827.98 ($[2M+Na]^+$, 100%). Anal. Calcd for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.64; H, 10.79. Mp = 140 °C; ^1H NMR (500 MHz, MeOD): δ 0.75 (3H, s, 18-CH_3), 0.99 (3H, d, J = 6.6, 21-CH_3), 1.07 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.98–2.12 (23H, 9 × CH_2, 5 × CH), 2.36 (2H, m, 4-CH_2), 3.52 (2H, m, 24-CH_2), 4.52 (1H, m, 3-CH), 5.41 (1H, m, 6-CH); ^{13}C NMR (125 MHz, MeOD): δ 12.3 (18-CH_3), 19.2 (21-CH_3), 19.8 (19-CH_3), 21.3 (2′-CH_3), 22.2 (CH_2), 25.3 (CH_2), 28.8 (CH_2), 29.3 (23-CH_2), 30.3 (22-CH_2), 33.0 (CH_2), 33.2 (CH), 36.8 (CH_2), 37.0 (20-CH) 37.8 (10-C), 38.3 (4-CH_2), 39.2 (CH_2), 41.1 (CH_2), 43.5 (13-C), 51.6 (CH), 57.5 (CH), 58.1 (CH), 63.6 (24-CH_2), 75.5 (3-CH), 123.7 (6-CH), 141.0 (5-C), 172.4 (1′-C═O).

5.12. 3β-Acetoxychol-5-en-24-al (23)

Pyridinium chlorochromate (0.646 g, 3.00 mmol, 2.1 equiv) and **22** (0.580 g, 1.44 mmol) were stirred in DCM (20 mL) overnight at room temperature, in presence of molecular sieves, and then poured onto a silica pad, and eluted with 1.5 L of DCM. After collecting and concentrating the filtrate under reduced pressure, **23** was recovered pure as a white solid (0.300 g, 52% yield); R_f = 0.85 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 418.34 ($[M+NH_4]^+$, 42%). Anal. Calcd for $C_{26}H_{40}O_3 \cdot 0.2H_2O$: C, 77.26; H, 10.07. Found: C, 77.18; H, 10.13. ^1H NMR (500 MHz, $CDCl_3$): δ 0.67 (3H, s, 18-CH_3), 0.93 (3H, d, J = 6.5, 21-CH_3), 1.01 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.86–2.03 (21H, 8 × CH_2, 5 × CH), 2.32 (2H, d, J = 7.5, 4-CH_2) 2.42 (2H, m, 23-CH_2), 4.60 (1H, m, 3-CH), 5.37 (1H, m, 6-CH), 9.78 (1H, m, 24-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.4 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 27.8 (CH_2), 28.0 (22-CH_2), 28.2 (CH_2), 29.7 (CH_2), 31.9 (8-CH), 35.4 (20-CH), 36.6 (10-C), 37.0 (CH_2) 38.1 (4-CH_2), 39.7 (CH_2), 41.0 (23-CH_2), 42.4 (13-C), 50.0 (CH), 55.8 (CH), 56.6 (CH), 74.0 (3-CH), 122.5 (6-CH), 139.7 (5-C), 171.6 (1′-C═O), 203.9 (24-C═O).

5.13. Methyl 6-aminohexanoate hydrochloride (26)

Starting from thionyl chloride (4 mL, 54.80 mmol, 2.6 equiv) and 6-aminohexanoic acid (2.764 g, 21.07 mmol) in MeOH (8 mL, 197.50 mmol, 9.4 equiv), **26** was isolated as a white solid (99% yield).

5.14. General procedure C: preparation of compounds 24–25 and 27–28

Triethylamine (TEA) (2.1 equiv) was added to a solution of methyl ester amine $H_2N(CH_2)_nCOOMe \cdot HCl$ (2.1 equiv) in MeOH (24 mL/mmol) and the solution stirred for 30 min. Then **23** was added as a solid and the solution stirred for further 30 min. Finally $NaCNBH_3$ was added as THF solution 1 M (1.8 equiv) and the reaction stirred overnight at room temperature. The solution was then diluted with H_2O (80 mL/mmol), extracted with $CHCl_3$ (120 mL/mmol) and washed with saturated NaCl solution (80 mL/mmol). The organic layer was dried over $MgSO_4$, then concentrated under reduced pressure and the crude was purified by column chromatography ($CHCl_3$/MeOH).

5.14.1. 3β-Acetoxychol-5-en-24-yl-(*N*-3-methoxycarbonylpropyl)-amine (24)

Following the general procedure C, starting from a solution of **13** (0.080 g, 0.52 mmol, 2.1 equiv) and TEA (0.073 mL, 0.52 mmol, 2.1 equiv) in MeOH (6 mL), **23** (0.100 g, 0.25 mmol) and $NaCNBH_3$ (0.45 mL of 1 M solution in THF, 0.45 mmol, 1.8 equiv) were added and the reaction stirred overnight at room temperature. After

extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→95:5), **24** was collected pure as a white solid (0.049 g, 39% yield); R_f = 0.29 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 502.62 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{31}H_{52}NO_4$ ($[M+H]^+$): 502.3891; found: 502.3887; mp = 219 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.69 (3H, s, 18-CH_3), 0.96 (3H, d, *J* = 6.6, 21-CH_3), 1.04 (3H, s, 19-CH_3), 2.06 (3H, s, 2′-CH_3), 0.92–2.14 (25H, 10 × CH_2, 5 × CH), 2.34 (2H, m, 4-CH_2), 2.52 (2H, t, *J* = 7.0, 28-CH_2), 2.85 (2H, m, 24-CH_2), 2.96 (2H, t, *J* = 3.0, 26-CH_2), 3.70 (3H, s, 30-CH_3), 4.62 (1H, m, 3-CH), 5.40 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.6 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 27.8 (CH_2), 28.3 (CH_2), 31.4 (27-CH_2), 31.8 (CH_2), 31.9 (28-CH_2), 33.1 (CH), 35.6 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.7 (CH_2), 42.4 (13-C), 47.4 (23-CH_2), 48.6 (22-CH_2), 48.7 (26-CH_2), 48.8 (24-CH_2), 50.0 (CH), 52.0 (30-CH_3), 56.0 (CH), 56.5 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.7 (5-C), 170.6 (1′-C=O), 173.4 (29-C=O).

The side product of this reaction, **25**, was also isolated pure as a white solid (0.031 g, 25% yield).

5.14.2. *N*-(3β-Acetoxychol-5-en-24-yl)-2-oxopyrrolidine (25)

R_f = 0.78 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 492.58 ($[M+Na]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{30}H_{48}NO_3$ ($[M+H]^+$): 470.3629; found: 470.3614; mp = 144–145 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.60 (3H, s, 18-CH_3), 0.85 (3H, d, *J* = 6.6, 21-CH_3), 0.94 (3H, s, 19-CH_3), 1.96 (3H, s, 2′-CH_3), 0.78–2.00 (25H, 10 × CH_2, 5 × CH), 2.25 (2H, d, *J* = 7.2, 4-CH_2), 2.31 (2H, t, *J* = 8.1, 28-CH_2), 3.16 (2H, t, *J* = 7.3, 24-CH_2), 3.31 (2H, t, *J* = 7.0, 26-CH_2), 4.53 (1H, m, 3-CH), 5.32 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.0 (27-CH_2), 18.7 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 23.8 (23-CH_2), 24.3 (CH_2), 27.8 (CH_2), 28.2 (CH_2), 31.2 (22-CH_2), 31.8 (CH_2), 31.9 (CH), 33.0 (28-CH_2), 35.5 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.7 (CH_2), 42.3 (13-C), 42.9 (26-CH_2), 47.1 (24-CH_2), 50.0 (CH), 55.9 (CH), 56.7 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.7 (5-C), 170.6 (1′-C=O), 174.8 (29-C=O).

5.14.3. β-Acetoxychol-5-en-24-yl-(*N*-5-methoxycarbonylpent-1-yl)-amine (27)

Following the general procedure C, starting from a solution of **26** (0.095 g, 0.52 mmol, 2.1 equiv) and TEA (0.073 mL, 0.524 mmol, 2.1 equiv) in MeOH (6 mL), **23** (0.100 g, 0.25 mmol) and $NaCNBH_3$ (0.45 mL of 1 M solution in THF, 0.45 mmol, 1.8 equiv) were added and the reaction stirred overnight at room temperature. After extraction and purification by column chromatography ($CHCl_3$/MeOH 100:0→92:8), **27** was collected pure as a white solid (0.031 g, 23% yield); R_f = 0.56 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 530.68 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{33}H_{56}NO_4$ ($[M+H]^+$): 530.4204; found: 530.4209; mp = 238–240 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.66 (3H, s, 18-CH_3), 0.93 (3H, d, *J* = 6.5, 21-CH_3), 1.01 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.83–2.10 (29H, 12 × CH_2, 5 × CH), 2.32 (4H, t, *J* = 7.4, 4-CH_2 and 30-CH_2), 2.81 (4H, m, 24-CH_2 and 26-CH_2), 3.66 (3H, s, 32-CH_3), 4.59 (1H, m, 3-CH), 5.40 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.6 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 24.3 (28-CH_2), 26.4 (29-CH_2), 27.8 (CH_2), 28.3 (CH_2), 30.4 (23-CH_2), 31.8 (CH_2), 32.9 (CH), 33.1 (30-CH_2), 33.8 (22-CH_2 and 27-CH_2), 35.6 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.7 (CH_2), 39.8 (24-CH_2 and 26-CH_2), 42.4 (13-C), 50.0 (CH), 51.6 (32-CH_3), 56.0 (CH), 56.6 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.7 (5-C), 170.6 (1′-C=O), 175.2 (31-C=O).

The side product of this reaction, **28**, was also isolated pure as a white solid (0.054 g, 41% yield).

5.14.4. *N*-6-(3β-Acetoxychol-5-en-24-yl)-aminohexanoic acid (28)

R_f = 0.16 ($CHCl_3$/MeOH: 90/10); LRMS, *m*/*z* (ES^+ mode): 516.41 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{32}H_{54}NO_4$ ($[M+H]^+$): 516.4047; found: 516.4033; mp = 258–262 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.66 (3H, s, 18-CH_3), 0.93 (3H, d, *J* = 6.5, 21-CH_3), 0.93 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.85–2.10 (29H, 12 × CH_2, 5 × CH), 2.32 (4H, m, 4-CH_2 and 30-CH_2), 2.92 (4H, m, 24-CH_2 and 26-CH_2), 4.60 (1H, m, 3-CH), 5.37 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.6 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 22.7 (28-CH_2), 24.3 (CH_2), 25.3 (29-CH_2), 27.8 (CH_2), 28.3 (CH_2), 31.8 (CH_2), 33.0 (CH), 33.8 (22-CH_2), 33.9 (27-CH_2), 35.5 (20-CH), 36.6 (10-C), 37.0 (CH_2), 37.1 (30-CH_2), 38.1 (4-CH_2), 39.7 (CH_2), 42.4 (13-C), 47.1 (23-CH_2), 47.8 (26-CH_2), 48.4 (24-CH_2), 50.0 (CH), 56.0 (CH), 56.6 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.7 (5-C), 170.6 (1′-C=O).

5.15. General procedure D: preparation of compounds 29–32

DIPEA (3.4 equiv) was added to a solution of **21** (1 equiv), HOBt (1.6 equiv) and TBTU (1.6 equiv) in DMF (28 mL/mmol) and the reaction stirred and monitored by TLC. When the formation of the activated ester with HOBt was complete, the methyl ester amine $H_2N(CH_2)_nCOOMe{\cdot}HCl$ was added (3.2 equiv) and the reaction stirred overnight at room temperature. The mixture was then diluted with H_2O (50 mL/mmol) and extracted with $CHCl_3$ (50 mL/mmol); the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure and the crude purified by column chromatography (hexane/EtOAc).

5.15.1. 3β-Acetoxychol-5-en-24-oyl-(*N*-3-methoxycarbonylpropyl)-amide (29)

Following the general procedure D, starting from a solution of **21** (0.150 g, 0.36 mmol, 1 equiv), HOBt (0.779 g, 0.58 mmol, 1.6 equiv) and TBTU (0.185 g, 0.58 mmol, 1.6 equiv) in DMF (10 mL), DIPEA (0.21 mL, 1.22 mmol, 3.4 equiv) was added and the reaction stirred at room temperature. After 1 h **13** was added (0.177 g, 1.15 mmol, 3.2 equiv) and the reaction stirred overnight at room temperature. After extraction, purification of the crude was attempted 3 times by column chromatography ($CHCl_3$/MeOH 100:0→95:5; hexane/EtOAc 100:0→75:25), but it was not possible to separate **29** from the side product **31**, still detected in the sample. The amount of **31** in the sample was 10%, as calculated by the ^1H NMR; R_f = 0.70 ($CHCl_3$/MeOH: 80/20); LCMS, *m*/*z* (ES^+ mode): t_R 4.6 min, 516.37 ($[M+H]^+$, M = 29); t_R 5.0 min, 444.34 ($[M+H]^+$, M = 31); HRMS (ES^+ mode), calculated for $C_{31}H_{50}NO_5$ ($[M+H]^+$): 516.3684; found: 516.3696; ^1H NMR (500 MHz, $CDCl_3$): δ 0.69 (3H, s, 18-CH_3), 0.95 (3H, d, *J* = 6.5, 21-CH_3), 1.03 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.92–2.14 (23H, 9 × CH_2, 5 × CH), 2.06 (1H, m, 23^a-CH_2), 2.22 (1H, m, 23^b-CH_2), 2.31 (2H, d, *J* = 7.0, 4-CH_2), 2.36 (2H, t, *J* = 7.2, 28-CH_2), 3.29 (2H, m, 26-CH_2), 3.67 (3H, s, 30-CH_3), 4.60 (1H, m, 3-CH), 5.40 (1H, m, 6-CH), 5.67 (1H, m, 25-NH); signals for **31**: δ 2.96 (3H, s, 26-CH_3), 3.03 (3H, s, 27-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.6 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 24.7 (27-CH_2), 27.8 (CH_2), 28.2 (CH_2), 31.5 (28-CH_2), 31.8 (22-CH_2), 31.8 (CH_2), 31.9 (CH), 33.6 (23-CH_2), 35.5 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 38.9 (26-CH_2), 39.7 (CH_2), 42.4 (13-C), 50.0 (CH), 51.8 (30-CH_3), 55.8 (CH), 56.6 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.6 (5-C), 170.6 (1′-C=O), 173.7 (24-C=O), 174.0 (29-C=O); signals for **31**: δ 37.4 (26-CH_3 and 27-CH_3).

5.15.2. 3β-A-cetoxychol-5-en-24-oyl-(*N*-5-methoxycarbonylpent-1-yl)-amide (30)

Following the general procedure D, starting from a solution of **21** (0.174 g, 0.42 mmol), HOBt (0.098 g, 0.72 mmol, 1.7 equiv) and TBTU (0.203 g, 0.63 mmol, 1.5 equiv) in DMF (10 mL), DIPEA (0.21 mL, 1.22 mmol, 2.9 equiv) was added and the reaction stirred at room temperature. After 1 h, **26** was added (0.209 g, 1.15 mmol, 3.2 equiv) and the reaction stirred overnight at room temperature.

After extraction, purification of the crude was attempted 3 times by column chromatography ($CHCl_3$/MeOH 100:0→95:5; hexane/EtOAc 100:0→80:20), but it was not possible to separate **30** from the side product **31**, still detected in the sample. The amount of **31** in the sample was 9%, as calculated by the ^{1}H NMR; R_f = 0.67 ($CHCl_3$/MeOH: 80/20); LCMS, *m*/*z* (ES^+ mode): t_R 4.9 min, 544.48 ($[M+H]^+$, M = 30); t_R 5.0 min, 444.34 ($[M+H]^+$, M = 31); HRMS (ES^+ mode), calculated for $C_{33}H_{54}NO_5$ ($[M+H]^+$): 544.3997; found: 544.3984; mp = 103–106 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.66 (3H, s, 18-CH_3), 0.92 (3H, d, *J* = 6.5, 21-CH_3), 1.00 (3H, s, 19-CH_3), 2.03 (3H, s, 2′-CH_3), 0.78–2.08 (27H, 11 × CH_2, 5 × CH), 2.05 (1H, m, 23^a-CH_2), 2.22 (1H, m, 23^b-CH_2), 2.31 (4H, m, 4-CH_2 and 30-CH_2), 3.24 (2H, m, 26-CH_2), 3.66 (3H, s, 32-CH_3), 4.59 (1H, m, 3-CH), 5.37 (1H, m, 6-CH), 5.50 (1H, m, 25-NH); signals for **31**: δ 2.93 (3H, s, 26-CH_3), 3.00 (3H, s, 27-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.5 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 24.5 (28-CH_2), 26.4 (29-CH_2), 27.8 (CH_2), 28.2 (CH_2), 31.2 (22-CH_2), 31.8 (CH_2), 31.9 (CH), 33.7 (23-CH_2 and 30-CH_2), 33.9 (27-CH_2), 35.5 (20-CH), 36.6 (10-C), 37.0 (CH_2), 38.1 (4-CH_2), 39.2 (26-CH_2), 39.7 (CH_2), 42.4 (13-C), 50.0 (CH), 51.6 (32-CH_3), 55.8 (CH), 56.7 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.6 (5-C), 170.6 (1′-C=O), 173.6 (24-C=O), 174.1 (29-C=O); signals for **31**: δ 37.4 (26-CH_3 and 27-CH_3).

The side product of this reaction, **31**, was isolated pure after column chromatography (hexane/EtOAc 100:0→85:15) as a white sticky solid (0.028 g, 15% yield).

5.15.3. 3β-Acetoxychol-5-en-24-oyl-(*N*,*N*-dimethyl)-amide (31)

R_f = 0.67 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 444.35 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{28}H_{46}NO_3$ ($[M+H]^+$): 444.3472; found: 444.3468; mp = 178–180 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.67 (3H, s, 18-CH_3), 0.93 (3H, d, *J* = 6.5, 21-CH_3), 1.00 (3H, s, 19-CH_3), 2.02 (3H, s, 2′-CH_3), 0.78–2.02 (21H, 8×CH_2, 5 × CH), 2.19 (1H, m, 23^a-CH_2), 2.33 (3H, m, 4-CH_2 and 23^b-CH_2), 2.92 (3H, s, 26-CH_3), 3.00 (3H, s, 27-CH_3), 4.59 (1H, m, 3-CH), 5.36 (1H, m, 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9 (18-CH_3), 18.5 (21-CH_3), 19.3 (19-CH_3), 21.0 (CH_2), 21.5 (2′-CH_3), 24.3 (CH_2), 27.8 (CH_2), 28.2 (CH_2), 31.3 (22-CH_2), 31.8 (CH_2), 31.9 (CH), 35.4 (23-CH_2), 35.6 (20-CH), 36.6 (10-C), 37.0 (CH_2), 37.4 (26-CH_3 and 27-CH_3), 38.1 (4-CH_2), 39.7 (CH_2), 42.4 (13-C), 50.0 (CH), 55.9 (CH), 56.6 (CH), 74.0 (3-CH), 122.6 (6-CH), 139.6 (5-C), 170.6 (1′-C=O), 173.7 (24-C=O).

Another side product of this reaction, **32**, was isolated pure after column chromatography ($CHCl_3$/MeOH 100:0→85:15) as a white sticky solid (0.049 g, 22% yield).

5.15.4. *N*-(3β-Acetoxychol-5-en-24-oyl)-6-aminohexanoic acid (32)

R_f = 0.79 ($CHCl_3$/MeOH: 80/20); LRMS, *m*/*z* (ES^+ mode): 530.38 ($[M+H]^+$, 100%); HRMS (ES^+ mode), calculated for $C_{32}H_{52}NO_5$ ($[M+H]^+$): 530.3840; found: 530.3849; ^{1}H NMR (500 MHz, MeOD): δ 0.75 (3H, s, 18-CH_3), 1.00 (3H, d, *J* = 6.5, 21-CH_3), 1.05 (3H, s, 19-CH_3), 2.00 (3H, s, 2′-CH_3), 0.85–2.06 (27H, 11 × CH_2, 5 × CH), 2.10 (1H, m, 23^a-CH_2), 2.22 (1H, m, 23^b-CH_2), 2.30 (4H, m, 4-CH_2 and 30-CH_2), 3.16 (2H, m, 26-CH_2), 4.52 (1H, m, 3-CH), 5.39 (1H, m, 6-CH), 7.98 (1H, m, 25-NH); ^{13}C NMR (125 MHz, MeOD): δ 12.3 (18-CH_3), 18.9 (21-CH_3), 19.7 (19-CH_3), 21.3 (CH_2), 22.1 (2′-CH_3), 25.3 (CH_2), 25.8 (28-CH_2), 27.6 (29-CH_2), 28.8 (CH_2), 29.2 (CH_2), 30.2 (22-CH_2), 33.0(CH_2), 33.2 (CH), 33.4 (23-CH_2), 34.2 (27-CH_2), 35.0 (20-CH), 36.8 (10-C), 37.8 (20-CH_2), 38.2 (4-CH_2), 39.1 (26-CH_2), 41.1 (CH_2), 40.3 (30-CH_2), 43.6 (13-C), 51.6 (CH), 57.2 (CH), 58.1 (CH), 75.5 (3-CH), 123.6 (6-CH), 141.04 (5-C), 172.4 (1′-C=O), 176.7 (24-C=O), 177.6 (29-C=O).

Acknowledgements

We would like to acknowledge the College of Life Sciences, University of Dundee and the Welsh School of Pharmacy for funding (F.G.) and also the UNDP/ World Bank/WHO Programme for Research and Training in Tropical Diseases (T.D.R.) for financial support. The EPSRC National Mass Spectrometry Centre in Swansea and Mrs. Gina Mackay, University of Dundee, acknowledged for accurate mass spectrometry.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.062.

References and notes

1. http://www.who.int/tdr.
2. Barrett, M. P.; Gilbert, I. H. *Curr. Top. Med. Chem.* **2002**, *2*, 471.
3. James, D. M.; Gilles, H. M. *Human Antiparasitic Drugs: Pharmacology and Usage*; Wiley: Chichester, 1985.
4. Barrett, M. P.; Burchmore, R. J. S.; Stich, A.; Lazzari, J. O.; Frasch, A. C.; Cazzulo, J. J.; Krishna, S. *Lancet* **2003**, *362*, 1469.
5. Oehlschlager, A. C.; Czyzewska, E. K. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*; Chapman & Hall: New York, 1992; pp 437–475.
6. Ator, M. A.; Schmidt, S. J.; Adams, J. L.; Dolle, R. E. *Biochemistry* **1989**, *28*, 9633.
7. Ator, M. A.; Schmidt, S. J.; Adams, J. L.; Dolle, R. E.; Kruse, L. I.; Frey, C. L.; Barone, J. M. *J. Med. Chem.* **1992**, *35*, 100.
8. Oehlschlager, A. C.; Angus, R. H.; Pierce, A. M.; Pierce, H. D.; Srinivasan, R. *Biochemistry* **1984**, *23*, 3582.
9. Rahier, A.; Genot, J. C.; Schuber, F.; Benveniste, P.; Narula, A. S. *J. Biol. Chem.* **1984**, *259*, 5215.
10. Gros, L.; Lorente, S. O.; Jimenez, C. J.; Yardley, V.; Rattray, L.; Wharton, H.; Little, S.; Croft, S. L.; Ruiz-Perez, L. M.; Gonzalez-Pacanowska, D.; Gilbert, I. H. *J. Med. Chem.* **2006**, *49*, 6094.
11. Lorente, S. O.; Jimenez, C. J.; Gros, L.; Yardley, V.; de Luca-Fradley, K.; Croft, S. L.; Urbina, J. A.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *Bioorg. Med. Chem.* **2005**, *13*, 5435.
12. Lorente, S. O.; Rodrigues, J. C. F.; Jimenez, C. J.; Joyce-Menekse, M.; Rodrigues, C.; Croft, S. L.; Yardley, V.; de Luca-Fradley, K.; Ruiz-Perez, L. M.; Urbina, J.; de Souza, W.; Pacanowska, D. G.; Gilbert, I. H. *Antimicrob. Agents Chemother.* **2004**, *48*, 2937.
13. Magaraci, F.; Jimenez, C. J.; Rodrigues, C.; Rodrigues, J. C. F.; Braga, M. V.; Yardley, V.; de Luca-Fradley, K.; Croft, S. L.; de Souza, W.; Ruiz-Perez, L. M.; Urbina, J.; Pacanowska, D. G.; Gilbert, I. H. *J. Med. Chem.* **2003**, *46*, 4714.
14. Gros, L.; Castillo-Acosta, V. M.; Jimenez, C. J.; Sealey-Cardona, M.; Vargas, S.; Estevez, A. M.; Yardley, V.; Rattray, L.; Croft, S. L.; Ruiz-Perez, L. M.; Urbina, J. A.; Gilbert, I. H.; Gonzalez-Pacanowska, D. *Antimicrob. Agents Chemother.* **2006**, *50*, 2595.
15. Coppens, I.; Courtoy, P. J. *Annu. Rev. Microbiol.* **2000**, *54*, 129.
16. Coppens, I.; Courtoy, P. J. *Mol. Biochem. Parasitol.* **1995**, *73*, 179.
17. Bellini, A. M.; Mencini, E.; Quaglio, M. P.; Guarneri, M. *Arch. Pharm.* **1990**, *323*, 201.
18. Kikuchi, K.; Bernard, E. M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 1433.
19. Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L.; Bernard, E. M.; Kikuchi, K.; Armstrong, D. *J. Am. Chem. Soc.* **1995**, *117*, 6138.
20. Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N.; McCrimmon, D.; Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1354.
21. Khabnadideh, S.; Tan, C. L.; Croft, S. L.; Kendrick, H.; Yardley, V.; Gilbert, I. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1237.
22. Ganapaty, S.; Thomas, P. S.; Karagianis, G.; Waterman, P. G.; Brun, R. *Phytochemistry* **2006**, *67*, 1950.