Evaluation of Azasterols as Anti-Parasitics

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In this article, the design and synthesis of some novel azasterols is described, followed by their evaluation against *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*, the causative agents of human African trypanosomiasis, Chagas disease, leishmaniasis, and malaria, respectively. Some of the compounds showed anti-parasitic activity. In particular, a number of compounds appeared to very potently inhibit the growth of the blood stream form *T. b. rhodesiense*, with one compound giving an IC_{50} value of 12 nM. Clear structure activity relationships could be discerned. These compounds represent important leads for further optimization. Azasterols have previously been shown to inhibit sterol biosynthesis in *T. cruzi* and *L. donovani* by the inhibition of the enzyme sterol 24-methyltransferase. However, in this case, none of the compounds showed inhibition of the enzyme. Therefore, these compounds have an unknown mode of action.

Introduction

Parasitic diseases are a major problem throughout the world.[1,2] Leishmaniasis and Chagas disease, human African trypanosomiasis (HAT), and malaria are endemic in many parts of the world, and millions of people are either infected or at risk. Control depends mainly on chemotherapy, which is often associated with toxic side effects and the development of drug resistance. Therefore, there is an urgent need for new drugs to treat these diseases.

Azasterols have potential as anti-parasitics. They are thought to inhibit sterol biosynthesis in the parasites *T. cruzi* and *Leishmania* spp. which give rise to Chagas disease and leishmaniasis, respectively. Sterol metabolism is of particular interest in the parasites that give rise to these diseases because the major sterol present in them is ergosterol and other 24-alkylated sterols, whereas in humans it is cholesterol.[3−5] Azasterols inhibit the enzyme 24-sterol methyltransferase.[6−19] This enzyme catalyzes the alkylation at the 24-position of sterols during the biosynthesis of ergosterol but is not found in the pathway to cholesterol and, hence, is a potential drug target.

The most studied azasterol in the case of Chagas disease and leishmaniasis is 22,26-azasterol (AZA, Figure 1). AZA has been shown to have potent activity in vitro against both *T. cruzi* epimastigotes and intracellular amastigotes[22−26] and in vivo against a rodent model of *T. cruzi*.[27] Also, AZA has been shown to have activity against both the promastigote and intracellular amastigotes forms of various *Leishmania* spp.[28,29] AZA is probably acting by the alteration of the sterol composition leading to cell lysis. Related compounds also appear to show anti-parasitic activity.[30]

Recently, we reported the design, synthesis, and evaluation of some modified azasterols designed to inhibit 24-SMT (Figure 1).[20,21] These compounds contained either a free hydroxyl or an acetate group at position 3 of the sterol and on the sterol side chain, either an alkyl group or an amino derivative. These compounds were active in the micromolar range against *L. donovani* and *T. cruzi*, and interestingly, in the submicromolar range against the blood stream form *T. b. rhodesiense*. The result against the blood stream form of the parasite was unexpected because this particular parasite is reported not to produce its own ergosterol but to salvage cholesterol from the host.[31,32] Hence, inhibitors of 24-SMT would not be expected to be effective against the blood stream form of this parasite.

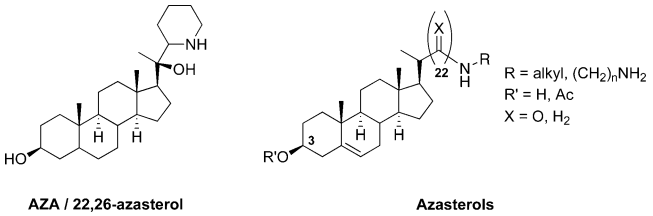

Figure 1. AZA and azasterols previously reported by the authors.[20,21]

We wanted to further investigate the role of the acetate at position 3 (R′, Figure 1) as well as the different functionalities in the side chain (R, Figure 1) to design more potent and selective inhibitors and also to try and understand the mode of action of these compounds. Therefore, we prepared four series of compounds (Figure 2). Series 1 and 2 have a carbonyl group at position 22, whereas series 3 and 4 do not. In series 1 and 3, there is an acetate on the 3β-OH, whereas in series 2 and 4, this position is deprotected.

In this current article, we report the synthesis of the compounds of series 1, 2, 3 and 4. Data are reported for all compounds against the causative organisms of leishmaniasis, Chagas disease, human African trypanosomiasis, and malaria, and structure activity relationships are discussed. We have recently reported the activity and mode of action studies for compounds **3a**−**g** against *T. b. rhodesiense*.[33] The mode of action of these compounds is discussed, but it does not appear to be by the inhibition of 24-SMT.

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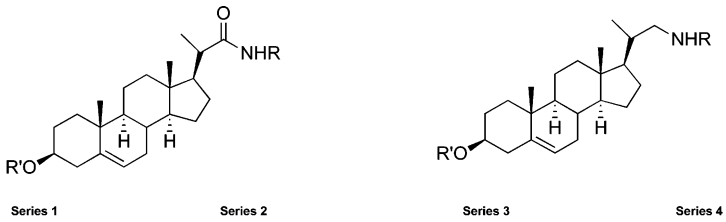


Series 1

1a, R' = CH_3CO, R = CH_2CO_2Me
1b, R' = CH_3CO, R = $(CH_2)_2CO_2Me$
1c, R' = CH_3CO, R = $(CH_2)_3CO_2Me$
1d, R' = CH_3CO, R = $(CH_2)_4CO_2Me$
1e, R' = CH_3CO, R = $(CH_2)_5CO_2Me$
1f, R' = CH_3CO, R = $(CH_2)_6CO_2Me$
1g, R' = CH_3CO, R = $(CH_2)_7CO_2Me$
1h, R' = CH_3CO, R = Boc-Dap-OH
1i, R' = CH_3CO, R = Boc-Dab-OH
1j, R' = CH_3CO, R = Z-Orn-OH
1k, R' = CH_3CO, R = Z-Lys-OBn

Series 2

2a, R' = H, R = CH_2CO_2H
2b, R' = H, R = $(CH_2)_2CO_2H$
2c, R' = H, R = $(CH_2)_3CO_2H$
2d, R' = H, R = $(CH_2)_4CO_2H$
2e, R' = H, R = $(CH_2)_5CO_2H$
2f, R' = H, R = $(CH_2)_6CO_2H$
2g, R' = H, R = $(CH_2)_7CO_2H$
2h, R' = H, R = Boc-Dap-OH
2i, R' = H, R = Boc-Dab-OH
2j, R' = H, R = Z-Orn-OH
2k, R' = H, R = H-Orn-OH
2l, R' = H, R = Z-Lys-OH
2m, R' = H, R = H-Lys-OMe

Series 3

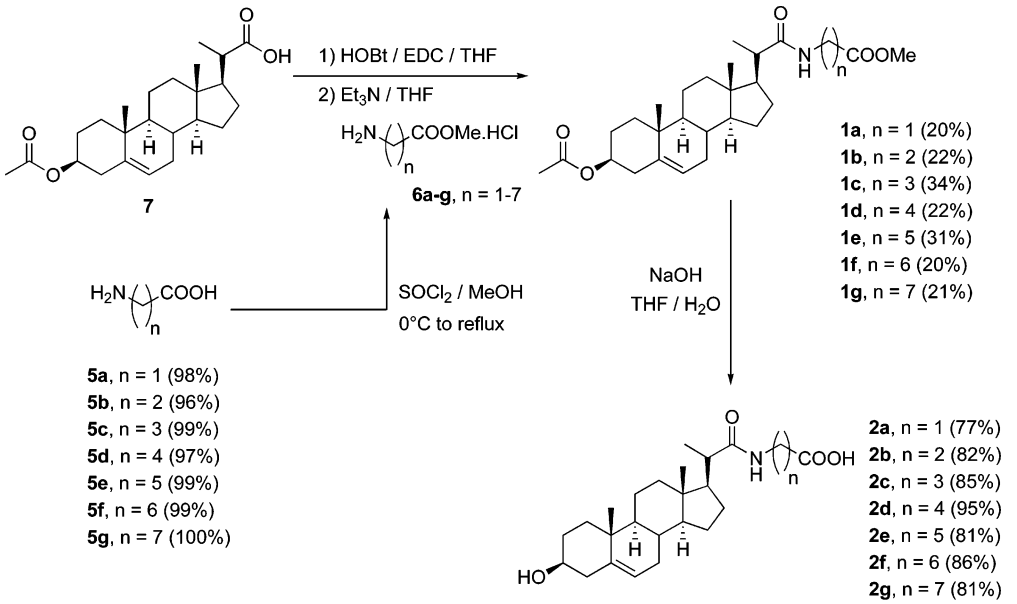
3a, R' = CH_3CO, R = CH_2CO_2Me
3b, R' = CH_3CO, R = $(CH_2)_2CO_2Me$
3c, R' = CH_3CO, R = $(CH_2)_3CO_2Me$
3d, R' = CH_3CO, R = $(CH_2)_4CO_2Me$
3e, R' = CH_3CO, R = $(CH_2)_5CO_2Me$
3f, R' = CH_3CO, R = $(CH_2)_6CO_2Me$
3g, R' = CH_3CO, R = $(CH_2)_7CO_2Me$
3h, R' = CH_3CO, R = $(CH_2)_3CO_2H$
3i, R' = CH_3CO, R = $(CH_2)_5CO_2H$
3j, R' = CH_3CO, R = $(CH_2)_7CO_2H$
3k, R' = CH_3CO, R = Boc-Dap-OH
3l, R' = CH_3CO, R = Boc-Dab-OH
3m, R' = CH_3CO, R = Z-Orn-OH
3n, R' = CH_3CO, R = Z-Lys-OMe
3o, R' = CH_3CO, R= Z-Lys-OBn

Series 4

4a, R' = H, R = CH_2CO_2H
4b, R' = H, R = $(CH_2)_2CO_2H$
4c, R' = H, R = $(CH_2)_3CO_2H$
4d, R' = H, R = $(CH_2)_4CO_2H$
4e, R' = H, R = $(CH_2)_5CO_2H$
4f, R' = H, R = $(CH_2)_6CO_2H$
4g, R' = H, R = $(CH_2)_7CO_2H$
4h, R' = H, R = CH_2CO_2Me
4i, R' = H, R = $(CH_2)_3CO_2Me$
4j, R' = H, R = $(CH_2)_5CO_2Me$
4k,R' = H, R = $(CH_2)_7CO_2Me$
4l, R' = H, R = Boc-Dap-OH
4m, R' = H, R = Boc-Dab-OH
4n, R' = H, R = Z-Orn-OH
4o, R' = H, R=Z-Lys-OH

Figure 2. Compounds prepared and assayed. The precise structures of compounds containing Dap (2,3-diaminopropionic acid), Dab (2,4-diaminobutyric acid), Orn (ornithine), and Lys (lysine) are shown in Schemes 2 and 5.

Scheme 1. Synthesis of Amide Derivatives **1a**−**g** and **2a**−**g**



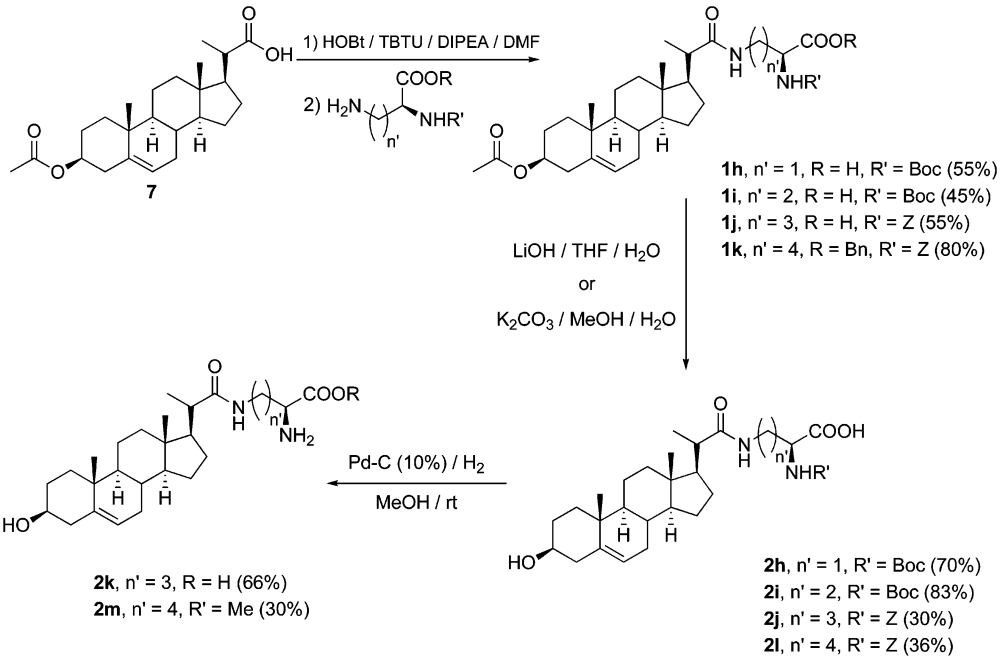
Chemistry. Amide Derivatives. The synthetic strategy for the synthesis of the amide derivatives (series 1 and 2) is outlined in Schemes 1 and 2. The commercially available sterol **7** was coupled to amino acids using various methods. The amino acids (**5a**−**g**) were first protected as methyl esters by treatment with thionyl chloride in methanol. These were then coupled to the sterol **7** via an HOBt activated ester using EDC as a coupling agent in quite low yields (20−34%). The use of DIPC instead of EDC in the first place resulted in greater yields (62−80%), but the samples were contaminated with DIPU as a byproduct which could not be removed by column chromatography or recrystallization. Finally, hydrolysis with sodium hydroxide afforded compounds **2a**−**g** in high yields (77−95%).

A different coupling method was used for the synthesis of compounds **1h**−**k** because the previous method led to no reaction (Scheme 2). The combination of HOBt with TBTU in basic conditions, DIPEA, generated the desired derivatives in reasonable yields (45−80%). Again, hydrolysis with either potassium carbonate or lithium hydroxide afforded compounds **2h**−**j** and **2l**. Finally, the removal of the benzylcarboxy group on the amine function was carried out using Pd−C (10%) and H_2 affording compounds **2k** and **2m** in 30 to 66% yields.

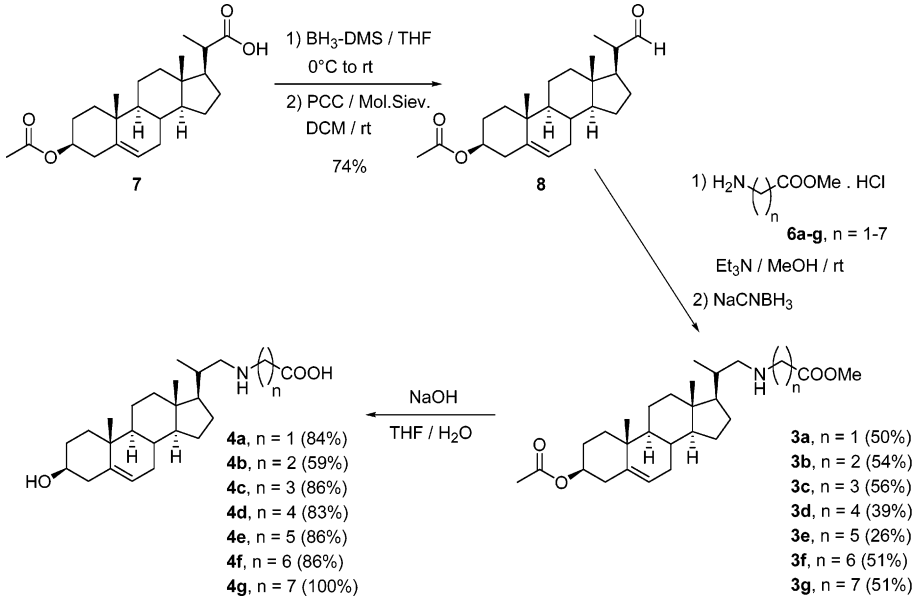
Amine Derivatives. The synthesis of the amine derivatives (series 3 and 4) is summarized in Schemes 3, 4, and 5. The selective reduction of the acid function of starting material **7** was first carried out with a dimethyl sulfide borane complex in a quantitative yield (98%, Scheme 3). The oxidation of the resulting alcohol with PCC in dichloromethane afforded aldehyde **8** (74%). Then, a one pot reductive amination with compounds **6a**−**g** and sodium cyanoborohydride in methanol led to the synthesis of derivatives **3a**−**g** in reasonable yields (26−56%). Hydrolysis with sodium hydroxide finally afforded derivatives **4a**−**g** in good yields (59−100%) in which the acetate group was removed from the 3-position and the ester hydrolyzed on the side chain to give the fully deprotected derivative.

As previously mentioned, we wanted to probe the importance of the acetate group at the 3-position of the sterol structure and the ester on the side chain. Compounds with an acetate at the 3-position but a free carboxylic acid on the side chain were prepared by reductive amination of aldehyde **8** with amino acids in which the carboxylate was not protected, giving compounds **3h**−**j** in good yields (63−86%) (Scheme 4). Conversely, compounds in which there was an ester in the side chain but no acetate at the 3-position were obtained by hydrolysis of aldehyde

Scheme 2. Synthesis of Amide Derivatives **1h**−**k** and **2h**−**n**



Scheme 3. Synthesis of Amine Derivatives **3a**−**g** and **4a**−**g**



8 followed by a reductive amination with methyl protected amino acids **6**, giving derivatives **4h**−**k** in overall 48 to 71% yields.

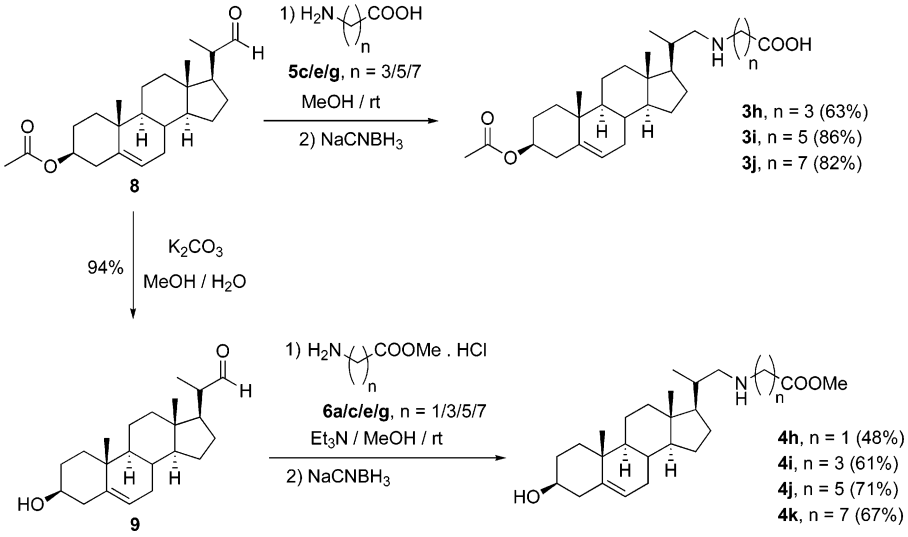
Derivatives **3k**−**o** were also obtained through a reductive amination, with good yields for compounds **3k**−**m** (57−80%) (Scheme 5). However, compound **3o** was obtained in only 23% yield because during the reaction, a transesterification with methanol occurred, leading to the production of compound **3n** in 9% yield, which was enough to allow its purification and the determination of any in vitro activity. Derivatives **4l**−**o** were synthesized by hydrolysis of the former with potassium carbonate in a mixture of methanol/water with good yields (41−70%).

Biological Assays. The compounds were assayed against the recombinant *L. major* and *T. brucei brucei* 24-SMT enzymes (Tables 1, 2, 3, and 4). These two enzymes were overexpressed in *E. coli*, and the enzyme assays were carried out using *E. coli* cell-free extracts. The compounds were also evaluated in vitro against the clinically relevant stage of the parasites, which is the intracellular amastigote for *L. donovani* and *T. cruzi*, and the blood stream form for *T. brucei rhodesiense*, and the two strains of *P. falciparum*, 3D7 and K1, the latter being resistant to chloroquine. Toxicity against macrophages was evaluated as TD_{50}, which is a measure of the cytotoxicity toward mammalian cells (KB cells).

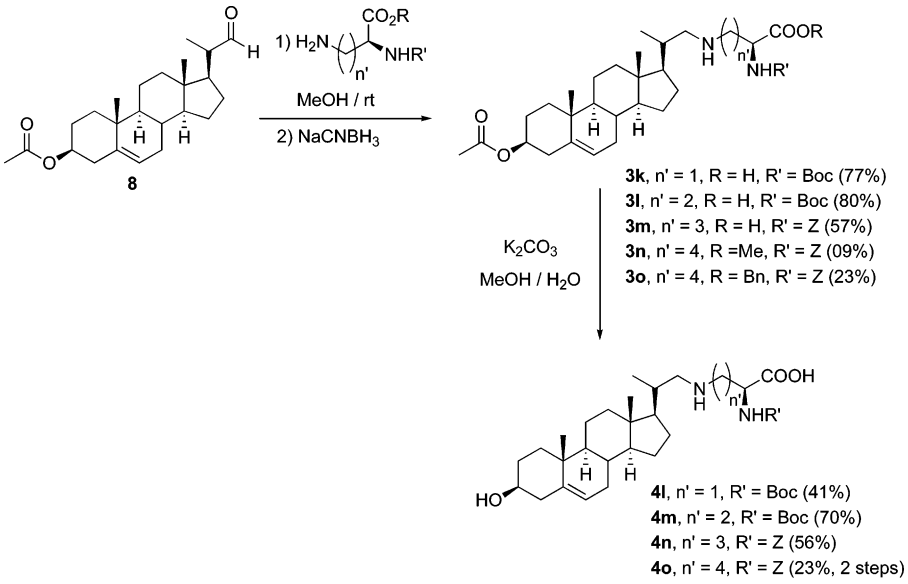
Enzyme Assays. The compounds were assayed against both the recombinant *L. major* and *T. b. brucei* 24-SMT. Of the compounds investigated, none showed significant activity, except for **4c**, which gave an IC_{50} value of 4 μM, and compound **4d**, which is the homologue with an extra carbon in the side chain, with an IC_{50} of 50 μM. This indicates that the compounds in general are not significant inhibitors of the 24-SMT.

Parasite Assays. Similarly, neither series 1 nor 2 showed significant growth inhibition of *L. donovani*. Interestingly, several members of series 3 showed growth inhibition of this parasite, notably compounds **3c** (ED_{50} 3.2 μM) and **3f** (ED_{50} 9.5 μM). Compound **3c** appears to have a more potent inhibition

Scheme 4. Synthesis of Amine Derivatives **3h**−**j** and **4h**−**k**



Scheme 5. Synthesis of Amine Derivatives **3k**−**o** and **4l**−**o**

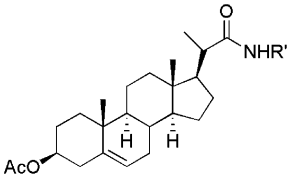


of parasite growth than both AZA and the control. Furthermore, compound **3c** appears to have a greater selectivity for the *L. donovani* than mammalian (K1) cells, compared to that of AZA. There is no clear SAR for this inhibition of growth, which may reflect the complex nature of the intracellular localization of *L. donovani*, which is found in a low pH parasitophorous vacuole within the host macrophage. Thus, the access of compounds to this parasite may be problematic.

In the case of *T. cruzi*, another intracellular parasite, there appears to be more significant activity. For the straight chain derivatives of series 1, compounds of intermediate chain length (**1b**−**e**, chain length 3−6 atoms) gave ED_{50} values in the range of 2−5 μM, which represents interesting leads. As the chain length was increased (**1f**, **1g**) or decreased (**1a**), the activity dropped. Promisingly, these compounds also showed good selectivity compared to that of mammalian cells. None of the branched chain derivatives of series 1 showed significant activity, except perhaps **1h**. The removal of the ester groups on the 3-position and the side chain (series 2) appeared to reduce activity significantly, with only **2g**, one of the more lipophilic derivatives, showing an ED_{50} value below 10 μM. In series 3, for the straight chain ester (**3a**−**g**) or acid (**3h**−**j**) series, none of the compounds showed ED_{50} values below 10 μM, with the exception of the smallest chain derivative **3a**, which had a value of 5.6 μM. Interestingly, this compound was also more active and selective than AZA, although, given the lack of activity of **3a** against 24-SMT, this is not its primary mode of action. Several of the amino acid substituted analogues showed growth inhibition of *T. cruzi*, notably, the carboxylate and amino-protected derivatives of lysine, **3n** and **3o**. For series **4**, none of the fully deprotected derivatives with the free acid and no acetate on the 3β-OH (**4a**−**g**) showed any activity. However, esterifying the carboxylate led to significant activity (**4h**−**k**); although in this case, it may be due to general cell toxicity. These results with series 4 may reflect that the fully deprotected compounds are not sufficiently lipophilic to get into the cells, particularly as these are intracellular parasites.

In the case of *T. brucei rhodesiense*, a number of the compounds showed potent growth inhibition of the parasite. In series 1, the longest ester derivative (**1g**) and the fully protected lysine analogue (**1k**) gave ED_{50} values of less than 1 μM; in the case of **1k**, this was actually 10 nM. A number of other compounds in this series gave ED_{50} values below 5 μM. For series 2 compound **2g**, the longest acid derivative, and **2m**, the methyl ester lysine derivative, gave ED_{50} values of less than 1 μM. In series 3, there was some very potent activity observed,

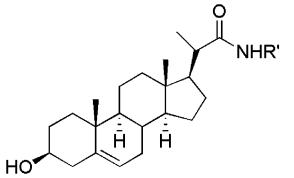
Table 1. Activities of the Amide Derivatives with an Acetate on the 3β-OH (Series 1)[a]



	R′	log D[b]	24-SMT enzyme IC_{50} (μM)		ED_{50} (μM)					TD_{50} (μM)
			L. major	*T. b. brucei*	*L. dono.*	*T. cruzi*	*T. b. rho.*	*P. falc.* (3D7)	*P. falc.* (K1)	toxicity
control		-	-	-	>10	~1	~0.001	~0.003	~0.1	0.001
AZA		2.60	0.028	1.76	8.9	7.4	3.3		-	11.9
1a	R′ = CH_2CO_2Me	5.34	-	-	>65	42.2	24.0	60.0	9.6	>653
1b	R′ = $(CH_2)_2CO_2Me$	5.60	-	-	>63	3.46	6.00	17.44	19.09	597
1c	R′ = $(CH_2)_3CO_2Me$	5.88	-	-	>62	4.70	9.15	16.18	11.59	>615
1d	R′ = $(CH_2)_4CO_2Me$	5.58	-	-	>60	2.43	3.01	0.74	8.43	>598
1e	R′ = $(CH_2)_5CO_2Me$	6.11	-	-	>58	2.54	3.45	21.33	19.78	>582
1f	R′ = $(CH_2)_6CO_2Me$	6.64	-	-	>57	10.82	7.91	14.42	1.26	>566
1g	R′ = $(CH_2)_7CO_2Me$	7.16	-	-	>55	>55	0.607	21.46	2.87	>552
1h	R′ = Boc-Dap-OH	3.38	>100	-	>52	8.70	2.68	-	-	-
1i	R′ = Boc-Dab-OH	3.02	>100	-	>51	26.84	17.48	-	-	-
1j	R′ = Z-Orn-OH	4.16	>100	-	>47	>47	1.70	>47	-	147
1k	R′ = Z-Lys-OBn	9.69	>100	-	>40	>24	0.010	3.4	-	>400

[a] Controls are *T. b. rhodesiense*, pentamidine; *T. cruzi*, benznidazole; *L. donovani*, pentostam; *P. falciparum*, chloroquine; K1 cells, podophyllotoxin. [b] LogD values were calculated with ACD software. Hyphens indicates data not determined. Toxicity was measured using KB cells.

Table 2. Activities of the Amide Derivatives without an Acetate on the 3β-OH (Series 2)



	R′	log D	24-SMT enzyme IC_{50} (μM)		ED_{50} (μM)					TD_{50} (μM)
			L. major	*T. b. brucei*	*L. dono.*	*T. cruzi*	*T. b. rho.*	*P. falc.* (3D7)	*P. falc.* (K1)	toxicity
control		-	-	-	>10	~1	~0.001	~0.003	~0.1	0.001
AZA		2.60	0.028	1.76	8.9	7.4	3.3	-	-	11.9
2a	R′ = CH_2CO_2H	0.73	>100	-	-	>74	>74	>74	-	>743
2b	R′ = $(CH_2)_2CO_2H$	1.33	>100	-	-	>72	>72	>72	-	615
2c	R′ = $(CH_2)_3CO_2H$	1.41	>100	-	29.19	39.8	49.8	45.6	-	616
2d	R′ = $(CH_2)_4CO_2H$	2.17	>100	-	>67	32.85	7.85	>67	-	561
2e	R′ = $(CH_2)_5CO_2H$	2.44	>100	-	>65	11.40	10.44	>65	-	303
2f	R′ = $(CH_2)_6CO_2H$	2.99	>100	-	>63	34.52	2.32	>63	-	252
2g	R′ = $(CH_2)_7CO_2H$	3.53	>100	-	25.84	6.50	0.431	>62	-	377
2h	R′ = Boc-Dap-OH	2.53	-	-	>56	>56	34.3	-	-	109
2i	R′ = Boc-Dab-OH	2.16	-	-	>55	11.2	>55	-	-	1267
2j	R′ = Z-Orn-OH	3.31	>100	-	>50	>50	1.33	-	-	32
2k	R′ = H−Orn-OH	1.71	-	-	>65	>65	2.38	-	-	651
2l	R′ = Z-Lys-OH	3.27	>100	-	>49	>49	1.80	-	-	276
2m	R′ = H-Lys-OMe	3.81	>100	-	25.0	11.20	0.55	-	-	42

with ED_{50} values in the nanomolar range. For the series with an acetate on the 3β-OH and the methyl protected carboxylate on the side chain, the activity of compounds increased as the chain length increased, with maximum activity for compound **3c**, which had an ED_{50} value of 12nM. The activity slowly decreased as the chain length increased. Compounds **3n** and **3o**, the fully protected lysine analogues, also showed growth inhibition with ED_{50} values of less than 1 μM. For series 4, the compounds were less active, with only the longest chain derivative, **4k**, showing an ED_{50} value of less than 1 μM.

Compounds were also evaluated against the protozoan parasite *P. falciparum*. This parasite does not have the biosynthetic machinery for sterol biosynthesis.[34] Both a chloroquine sensitive and a chloroquine resistant line were tested. Some of the compounds showed submicromolar ED_{50} values against this parasite: **1d**, **3n**, **3o**, and **4k**, with the latter 3 showing submicromolar activities against both lines.

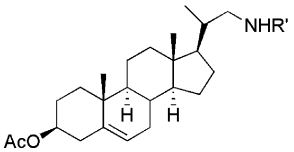
Discussion

None of the azasterols showed significant inhibition of enzyme 24-SMT. This is expected for compounds of series 1 and 3 because it has been shown that 3β-OH is important for the inhibition of 24-SMT, whilst series 1 and 3 have the 3β-OH blocked. Indeed we previously reported a series of azasterols with and without an acetate on the 3β-OH.[20,21] The removal of the acetate caused significant increase in the inhibition of 24-SMT (Table 5).

However, interestingly, compounds of series 2 and 4 showed no significant inhibition of 24-SMT. This is in contrast to compounds that we previously reported,[20,21] in which there is either an alkyl or alkylamino substitutent on the side chain of the azasterol (see, for example, compounds **10b**, **10d**, and **10f** in Table 5). The ester or carboxylic acid functional groups in the azasterols reported in this present paper clearly do not undergo favorable interactions with the *L. major* 24-SMT. The lack of activity of compounds reported in this article against 24-SMT indicates that any growth inhibition shown by these compounds was not due to the inhibition of this enzyme. Therefore, there must be some other target within the cell.[33]

The compounds appeared to show strong growth inhibition of *T. b. rhodesiense*. The most active compounds were the series **3b**−**g**, which gave nanomolar inhibition of parasite growth. These compounds had an acetate on the 3β-OH and a ester functionality on the sterol side chain. The optimum inhibitor

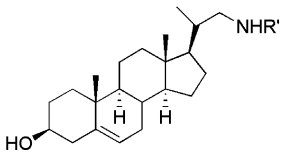
Table 3. Activities of the Amine Derivatives with an Acetate on the 3β-OH (Series 3)[a]



	R′	log D	24-SMT enzyme IC_{50} (μM)		ED_{50} (μM)					TD_{50} (μM)
			L. major	*T. b. brucei*	*L. dono.*	*T. cruzi*	*T. b. rho.*	*P. falc.* (3D7)	*P. falc.* (K1)	toxicity
control		-	-	-	>10	~1	~0.001	~0.003	~0.1	0.001
AZA		2.60	0.028	1.76	8.9	7.4	3.3	-	-	11.9
3a	$R' = CH_2CO_2Me$	6.34	>100	-	-	5.61	7.85	>67.32	-	94.47
3b	$R' = (CH_2)_2CO_2Me$	5.28	>100	-	22.84	28.93	0.302	>65.27	-	17.40
3c	$R' = (CH_2)_3CO_2Me$	4.54	>100	>100	3.19	24.70	0.012	15.43	-	19.21
3d	$R' = (CH_2)_4CO_2Me$	4.54	>100	-	>62	11.73	0.032	8.65	-	26.12
3e	$R' = (CH_2)_5CO_2Me$	4.69	>100	>100	21.92	20.53	0.033	7.37	-	15.55
3f	$R' = (CH_2)_6CO_2Me$	5.19	>100	>100	9.50	53.51	0.025	3.49	-	11.25
3g	$R' = (CH_2)_7CO_2Me$	5.71	>100	>100	14.78	28.69	0.098	2.64	-	10.00
3h	$R' = (CH_2)_3CO_2H$	4.04	>100	-	>65	12.62	54.61	>65	-	505
3i	$R' = (CH_2)_5CO_2H$	4.57	>100	-	>62	>62	55.16	>62	-	433
3j	$R' = (CH_2)_7CO_2H$	5.63	>100	-	-	21.00	9.11	>58	-	59
3k	R′ = Boc-Dap-OH	5.52	>100	-	>54	4.64	>54	-	-	-
3l	R′ = Boc-Dab-OH	5.42	>100	-	>53	13.22	>52	-	-	-
3m	R′ = Z-Orn-OH	6.11	>100	-	>48	18.46	17.36	-	-	-
3n	R′ = Z-Lys-OMe	6.15	-	-	-	<1.71	0.753	0.799	0.215	5.35
3o	R′ = Z-Lys-OBzl	7.53	-	-	-	1.71	0.619	0.839	0.069	29.79

[a] Growth inhibition data for compounds **3a**−**g** against *T. b. rhodesiense* have been reported previously.[33]

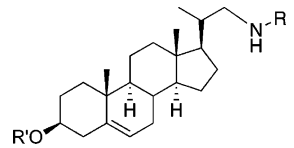
Table 4. Activities of the Amide Derivatives without an Acetate on the 3β-OH (Series 4)



	R′	log D	24-SMT enzyme IC_{50} (μM)		ED_{50} (μM)					TD_{50} (μM)
			L. major	*T. b. brucei*	*L. dono.*	*T. cruzi*	*T. b. rho.*	*P. falc.* (3D7)	*P. falc.* (K1)	toxicity
control		-	-	-	>10	~1	~0.001	~0.003	~0.1	0.001
AZA		2.60	0.028	1.76	8.9	7.4	3.3	-	-	11.9
4a	$R' = CH_2CO_2H$	2.80	>100	-	>77	>77	>77	>77	-	>770
4b	$R' = (CH_2)_2CO_2H$[a]	2.97	-	-	-	-	-	-	-	-
4c	$R' = (CH_2)_3CO_2H$	3.18	4	-	>72	47.29	14.41	56.5	-	451
4d	$R' = (CH_2)_4CO_2H$	3.48	48.9	-	>70	66.19	30.07	>70	-	>695
4e	$R' = (CH_2)_5CO_2H$	3.71	>100	-	54.52	56.34	>67	>67	-	>67313
4f	$R' = (CH_2)_6CO_2H$	4.24	>100	-	>65	44.92	47.60	>65	-	72.00
4g	$R' = (CH_2)_7CO_2H$	4.77	>100	-	26.39	38.38	10.6	>63	-	>633
4h	$R' = CH_2CO_2Me$	5.48	-	-	-	<2.75	4.91	40.56	22.25	45.04
4i	$R' = (CH_2)_3CO_2Me$	3.69	-	-	-	<2.57	5.05	7.53	1.95	1.55
4j	$R' = (CH_2)_5CO_2Me$	3.83	-	-	-	<2.41	2.15	1.31	0.50	6.09
4k	$R' = (CH_2)_7CO_2Me$	4.85	-	-	-	2.64	0.308	0.349	0.062	6.17
4l	R′ = Boc-Dap-OH	4.67	-	-	-	>58	>58	5.76	-	86.1
4m	R′ = Boc-Dab-OH	4.57	-	-	-	<2.08	>56	36.56	38.12	>563
4n	R′ = Z-Orn-OH	5.25	-	-	-	>52	>52	51	-	417.4
4o	R′ = Z-Lys-OH	5.18	-	-	-	9.52	>50	16.66	6.29	>504

[a] No data is provided for **4b** because it was too insoluble.

Table 5. Previously Reported Examples of Inhibitors of 24-SMT[21]



	R′	R	inhibition of *L. major* 24-SMT IC_{50} (μM)
10a	Ac	butyl	>100
10b	H	butyl	0.97
10c	Ac	piperidyl	>100
10d	H	piperidyl	6.4
10e	Ac	$(CH_2)_8NH_2$	35
10f	H	$(CH_2)_8NH_2$	2

was **3c**, which was a butyrate derivative. Shortening or lengthening the chain led to a loss in activity. The removal of the acetate on the 3β-OH (**4h**−**k**) led to about a 100-fold drop in activity. Similarly, retaining the acetate on the 3β-OH but converting the ester group in the side chain to a carboxylic acid (**3h**−**j**) led to a 1000-fold drop in activity. The removal of the acetate group on the 3β-OH and the conversion of the ester to a carboxylic acid (**4a**−**g**) also led to a 1000-fold loss in activity. In series 3 and 4, the side chain was linked to the sterol via an amine bond. However, in series 1 and 2, the side chain was linked via an amide bond. In this case, compounds **1a**−**g** (with an acetate in the 3β-OH position and a ester group in the side chain) and compounds **2a**−**g** (with no acetate in the 3β-OH and a carboxylic acid in the side chain) showed a 100-fold reduced activity compared to that of **3a**−**g**. The small exception to this is **2g**, which showed an ED_{50} value of 0.4 μM. The greater activity of this may be due to the lipophilicity of this compound, improving cellular uptake (see below). This data allows us to establish at least some of the features for a pharmacophore for activity against *T. b. rhodesiense* (Figure 3). The following appear to be essential: an acetate at the 3β-OH; the sterol side chain linked by an amine group; the side chain having a length of about 4−7 carbon atoms; and an ester functionality at the side chain, rather than a carboxylic acid.

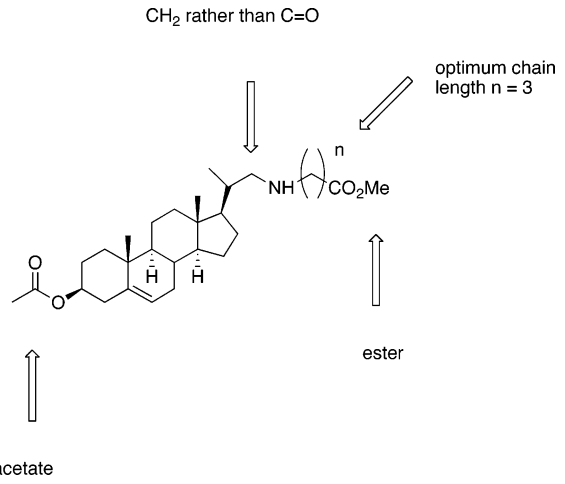


Figure 3. Pharmacophore for growth inhibition of *T. b. rhodesiense*.

The removal of either the acetate or the ester functionality appears to reduce activity. The effect of removing the acetate or ester will be to decrease the lipophilicity of compounds and to increase their hydrophilicity; however, a comparison of the calculated logD values indicates that the deprotected compounds are only about a log factor more hydrophilic (compare **3c**, logD = 4.54; **4c**, logD = 3.18). Therefore, one or both ester functionalities may be important for the pharmacological activity of the compounds and not just for facilitating cellular uptake by increasing lipophilicity.

Previous work by us has indicated the importance of the acetate substituent on the 3β-OH for anti-parasitic activity.[21] To summarize this previous work, compound **10a** (Table 5) has an acetate on the 3β-OH and showed more potent inhibition of the growth of *L. amazonensis* promastigotes than compound **10b** in which the 3β-OH group is deprotected. We hypothesised that compound **10a** was being taken up as the acetate and then the acetate cleaved in the cell to give the 3β-OH compound **10b**, which then inhibited 24-SMT. An indirect way to measure whether a compound is inhibiting 24-SMT in the cell is to study the sterol composition of cells. If a compound inhibits 24-SMT, then there would be a reduction in the levels of 24-alkylated sterols found in the cell membrane. By studying the sterol composition of *L. amazonensis* promastigotes treated with compounds **10a** and **10b**, it was possible to demonstrate that compound **10b** produced a greater reduction in the levels of 24-alkylated sterols than compound **10a**.[20,21] This is consistent with more potent inhibition of 24-SMT by **10b**, but contrasts with the anti-parasitic activity. Thus, the anti-leishmanial activity of compound **10a** is not just due to the uptake of the compound as the acetate and then the cleavage of the acetate in the cell to give compound **10b**, which then subsequently inhibits 24-SMT. Although this data was obtained with the related organism *L. amazonesis*, it is a precedent for a growth inhibition by azasterols with an acetate on the 3β-OH.

We also prepared some amino acid substituents at the side chain. These provide a variety of different functionalities. There is no clear structure activity relationship for the growth inhibition of *T. brucei*. For series 1, lipophilic compound **1k** is the most potent one, which contrasts to series 2, where hydrophilic **2m** is the most potent compound.

The mode of action of the compounds reported in this article against *T. b. rhodesiense* is not known. However, given the potent activity of some of them (especially **3b**−**g**) and their selectivity compared to that of mammalian cells, it is probable that there is a specific mode of action of these compounds against *T. brucei*. This may be a specific interaction with an alternative protein target, or they could conceivably be acting through the perturbations of membrane structure and function.

The lower activity of compounds against *T. cruzi* and *L. donovani* may reflect the fact that these are intracellular parasites, meaning that the access of compounds is restricted. There are several compounds that show some promise against *L. donovani* (notably **3c**), which is more active and selective than AZA, although it is clearly not acting by the inhibition of 24-SMT. In the case of *T. cruzi*, compounds **1b**−**e**, **4h**−**k**, and **4m** gave interesting inhibition, with ED_{50} values close to that of the control drug benznidazole. However, their mechanism of action is not clear. These compounds are worthy of further study, with the aim of improving their activity and selectivity.

Although several compounds showed submicromolar inhibition of the growth of *P. falciparum*, none of them approached the levels of activity of the control drug chloroquine and are not interesting for further optimization. The possible exceptions to this are compounds **3n** and **3o**, which showed significant activity against both chloroquine resistant and sensitive *P. falciparum*.

Conclusion

Successful synthetic strategies were devised for the synthesis of these substituted azasterols. None of the compounds discussed here showed significant inhibition of 24-SMT, although many of the compounds inhibited the growth of the parasites; in particular, **3a**−**g** potently inhibited the growth of *T. b. rhodesiense*. The fact that these compounds do not give inhibition of 24-SMT suggests other modes of action for the azasterols reported in this article, despite various other azasterols previously being shown to inhibit 24-SMT. If the azasterols described in this article have an alternate mode of action, it is conceivable that other azasterols such as AZA have modes of action in addition to the inhibition of 24-SMT.

Further investigation into the mode of action of these compounds would be very interesting. These compounds have potential for further optimization, particularly against *T. brucei* but also against *T. cruzi*.

Experimental Section

Enzyme Activity Assays. Experimental procedures for assay of recombinant *L. major* 24-SMT have been reported previously.[21, 30]

In assays of the inhibition of 24-SMT, soluble protein extracts from *E. coli* BL21(DE3)/pET28a-TbSMT cells were used. *Trypanosoma b. brucei* recombinant 24-SMT is produced as a His-tagged fusion protein and is overexpressed when induced with 1 mM IPTG during 4 h. The cells were disrupted by sonication in a buffer containing 50 mM Tris-HCl at pH 7.4, 2 mM $MgCl_2$, 4 mM CHAPS, 0.5% (v/v) Tween 80, and protease inhibitors. The sonicate was centrifuged at 12 000 rpm for 30 min at 4 °C to obtain the soluble fraction, which contained the active form of the enzyme. A standard 24-SMT activity assay contained 1.7 mg/mL of bacterial soluble extract in buffer containing 50 mM Tris-HCl at pH 7.4, 2 mM $MgCl_2$, 4 mM CHAPS, 0.5% (v/v) Tween 80, 100 μM desmosterol, and 200 μM ^{14}C-*S*-adenosyl-L-methionine, (6×10^5 dpm or 1×10^6 dpm per reaction). Desmosterol was dissolved in chloroform, which was evaporated before adding the rest of components. The inhibitor was resuspended first in a minimal volume of its corresponding solvent and later added to the reaction mixture as an aqueous solution. The reaction was started with the enzyme. Incubations were performed at 30 °C for 45 min, and terminated with 0.5 mL of 10% KOH dissolved in 80% (v/v) methanol. To quantify the efficiency of the extraction, ^{3}H-cholesterol (3 mg, 30000 dpm per reaction) was added as an internal standard. The methylated sterol product was extracted three times

with 1 mL of hexane and the resulting organic layer washed once with the Tris-HCl buffer to remove the ^{14}C-*S*-adenosyl-L-methionine that was not incorporated. Then, 1 mL of the organic layer was added to 10 mL of hydrofluor, and the radioactivity was measured in a scintillation counter. IC_{50} values were obtained from hyperbolic plots of percentage of inhibition versus concentration of inhibitor.

Assays in Vitro. ***L. donovani.***[20] Peritoneal exudate macrophages were harvested from CD1 mice, 24 h after starch induction. After washing, the macrophages were dispensed into Lab-tek 16-well tissue culture slides and maintained in RPMI1640 + 10% heat-inactivated foetal calf serum (HIFCS) at 37 °C in a 5% CO_2/air mixture for 24 h. *Leishmania donovani* (MHOM/ET/67/L82) amastigotes were harvested from an infected Golden hamster spleen and were used to infect the macrophages at a ration of 5 parasites:1 macrophage. Infected cells were left for a further 24 h and then exposed to the drug[35] for a total of 5 days, with the overlay being replaced on day 3.[36] The top concentration for the test compounds was 30 μg/mL, and all concentrations were carried out in quadruplicate. On day 5, the overlay was removed, and the slides were fixed (100% methanol) and stained (10% Giemsa, 10 min) before being evaluated microscopically. ED_{50} (ED_{90}) values were calculated using Ms*xl*fit. The ED_{50} value for the positive control drug, Pentostam, is usually 3−8 $\mu g Sb^V$/mL.

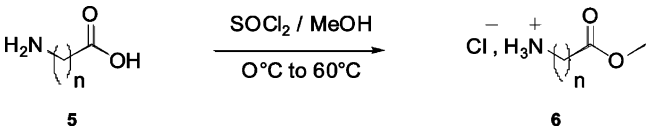
T. cruzi.[20] Murine (CD1) peritoneal macrophages were harvested 24 h after starch induction, and 100 μL was dispensed into 96-well plates at a concentration of 4×10^5/mL. After 24 h, the cells were infected with *Trypanosoma cruzi* Tulahuan LAC-Z trypomastigotes. Twenty-four hours later, the infected cells were exposed to the drug for 3 days. Then, 50 μL of 500 μM CPRG/1% nonidet P-40 was added to each well. The plates were read after 2−5 h at λ570.[37] The ED_{50} (ED_{90}) values were calculated using Ms*xl*fit. L6 fibroblasts are also used as host cells.

T. brucei.[33] *Trypanosoma brucei rhodesiense* STIB900 bloodstream form (bsf) trypomastigotes were maintained in an HMI-18 medium[38] with 15% heat-inactivated fetal calf serum (HIFCS) (Harlan-SeraLab, U.K.) at 37 °C and 5% CO_2/air mixture. Trypomastigotes were washed and resuspended in fresh medium at a concentration of 2×10^5/mL. Then, 100 μL was added to the drug dilutions. The top concentration for the test compounds was 30 μg/mL. The ED_{50} value for pentamidine is usually between 1.0 and 0.1 ng/mL. The plates were incubated for 72 h at 37 °C and 5% CO_2. At 72 h, the plates were assessed microscopically before Alamar Blue was added.[39] Plates were read after 5−6 h on a Gemini Fluorescent plate reader (Softmax Pro. 3.1.1, Molecular Devices, U.K.) at EX/EM 530/585 nm with a filter cutoff at 550 nm. ED_{50} values were calculated with Ms*xl*fit (IDBS, U.K.).

P. falciparum. *Plasmodium falciparum* 3D7 cultures were mantained in an RPMI 1640 medium (Sigma, U.K.) 37 °C and 5% CO_2 in 5% hematocrit. Synchronized ring stage cultures were prepared at 1% parasitemia, and 50 μL was added per well, the top test drug final concentration being 30 μg/mL. After 24 h of incubation at 37 °C and 5% CO_2, 5 μL of (^{3}H)-hypoxanthine was added (0.2 μCi/well),[40,41] and the plates were shaken for 1 min and then incubated for 48 h. The plates were rapidly freeze/thawed, harvested, and dried. (^{3}H)-hypoxanthine uptake was measured using a microbeta counter (Wallac 1450). The ED_{50} values were calculated as before.

Cytotoxicity against Mammalian Cells.[33] The plates were seeded with 100 μL of KB cells[42] at 4×10^4/mL and RPMI 1640 + 10% HIFCS and incubated at 37 °C and 5% CO_2 for 24 h. The overlay was removed and replaced by test drugs in fresh medium at 300, 30, 3, and 0.3 μg/mL. The positive control drug was Podophyllotoxin (Sigma, U.K.). The dilutions were carried out in triplicate. The plates were incubated for a further 72 h, at 37 °C and 5% CO_2. The wells were microscopically assessed for cell growth. The overlay was removed and the wells washed three times with PBS (pH 7.0). Then, 100 μL of PBS + 10 μL of Alamar Blue were added per well and plates incubated for 2−4 h (37 °C and 5% CO_2) before reading at EX/EM 530/585 nm (cutoff 550 nm) in a Gemini plate reader. The ED_{50} (ED_{90}) values were calculated compared to those of blanks and untreated controls.

Scheme 6. Esterification Procedure



5 **6**

Chemical Experimental Details. The numbering schemes for NMR and general information for the chemistry procedures as well as the experimental procedures for all compounds are given in the Supporting Information.

General Experimental Details. When applicable, all glassware was oven-dried overnight, and all reactions were carried out under a nitrogen atmosphere. Sensitive liquids and solutions were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa.

All dry solvents, ethanol, methanol, dichloromethane, tetrahydrofuran, and dimethylformamide were purchased from Aldrich or Fluka in Sure Seal bottles.

Analytical TLC was performed on silica gel 60 F254 plates, purchased from Merck. The visualization of spots was effected by one of the following techniques: (a) UV illumination, (b) the immersion of the plate in a 3% solution of ninhydrin in ethanol followed by heating, and (c) the immersion of the plate in a 48 g L^{-1} solution of phosphomolibdic acid in methanol followed by heating. Column chromatography was carried out on Sorbsil C60A (40−60 micron) silica gel purchased from Merck.

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 DPX 500 MHz spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The chemical shifts are reported downfield in parts per million using $CDCl_3$ as an internal reference ($\delta = 7.26$ for ^{1}H and $\delta = 77.16$ for ^{13}C) unless otherwise stated, and coupling constants (J values) are in hertz.

IR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer. Compounds were ground with KBr and spectra recorded in reflectance mode.

Melting points were determined with a Gallencamp melting point apparatus and are uncorrected.

Mass spectra and exact mass measurements were performed on a Waters ZQ4000 and a Finnigan MAT 95XP, respectively, at EPSRC National Mass Spectrometry Service Centre in the Chemistry Department, University of Wales Swansea, Swansea, Wales, U.K.

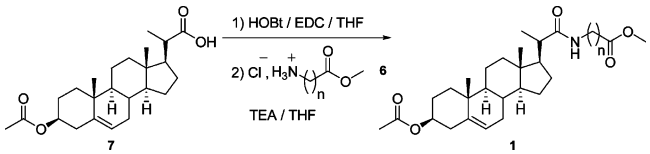
The general procedures and data for key compounds are presented here.

General Procedure A (Scheme 6)**.** Thionyl chloride (3 equiv) was added dropwise to a stirred solution of amino acid **5** (1 equiv) and methanol (10 equiv) at 0 °C. The resulting solution was then heated to reflux in methanol (0.4 mL/mmol) overnight. The solvent and thionyl chloride in excess were then removed under reduced pressure.

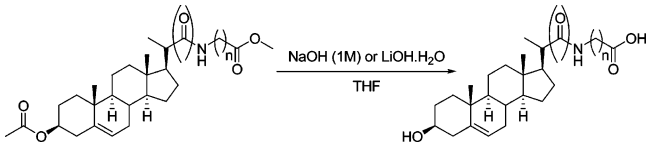
General Procedure B (Scheme 7)**.** HOBt (1.2 equiv) and 3β-acetoxy-5-cholenic acid **7** (85% pure, 1.0 equiv) were dissolved in dry THF (5 mL/mmol). Then, EDC (1.1 equiv) was added, and the solution was stirred for 30 min. This solution was transferred to a second flask, where a solution of methyl *n*-amino ester **6** (1.2 equiv) and triethylamine (1.6 equiv) in THF (4 mL/mmol) had been prepared. The reaction was allowed to stir overnight at room temperature. The reaction was diluted with water (20 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with water (10 mL), a saturated solution of K_2CO_3 (2×10 mL), HCl 1 M (2×10 mL), and finally water again (2×10 mL). The solution was dried with magnesium sulfate and the remaining solvents removed under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/AcOEt (80/20) as the eluant.

General Procedure C (Scheme 8)**.** A sodium hydroxide solution (1 M, 10 equiv) or lithium hydroxide (2 equiv) was added to the starting material (1.0 equiv) dissolved in THF (25 mL/mmol) at room temperature. The mixture was stirred for various lengths of

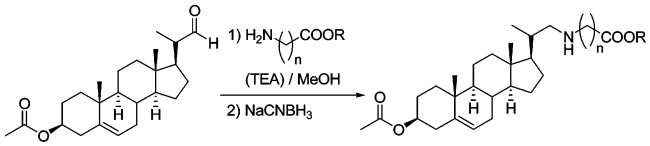
Scheme 7. Coupling Procedure



Scheme 8. Hydrolysis Procedure



Scheme 9. Reductive Amination Procedure



time. Then, THF was removed under reduced pressure. The resulting mixture was diluted with water (50 mL/mmol), and a hydrochloric acid solution (1 M) was added until the pH was neutral. The product was filtered, washed with AcOEt, and dried under vacuum.

General Procedure D (Scheme 9)**.** Amino acid **5** (1.2 equiv) or triethylamine (1.4 equiv) with methyl amino ester **6** (1.2 equiv) in a methanol solution (12 mL/mmol) was stirred 30 min. Then, 3β-acetoxy-23,24-bisnor-chol-5-en-22-al (**8**) (1 equiv) was added as a solid and the solution stirred for a further 30 min. Finally, triacetoxy sodium borohydride or sodium cyanoborohydride as a solid or in a THF solution (1.2 equiv) was added and the mixture stirred at room temperature overnight. The solution was diluted with water (10 mL) and extracted with chloroform (20 mL). The organic layer was washed with a saturated solution of sodium chloride (10 mL), dried, and the solvent removed under reduced pressure. The crude product was purified by column chromatography ($CHCl_3$/MeOH 90:10).

3β-Acetoxy-23,24-bisnor-5-en-22-(methyl pentanoate) Amide (1d). Following general procedure B and starting from HOBt (275 mg, 2.04 mmol, 1.2 equiv), 3β-acetoxy-5-cholenic acid (**7**) (800 mg, 1.75 mmol, 1.0 equiv) and EDC (380 mg, 1.98 mmol, 1.1 equiv) in THF (9 mL), and methyl 5-aminopentanoate (**6d**) (398 mg, 2.38 mmol, 1.4 equiv) and triethylamine (0.35 mL, 2.52 mmol, 1.4 eq) in THF (9 mL), compound **1d** was isolated as a white solid (194 mg, 22%).

3β-Acetoxy-23,24-bisnor-5-en-22-(6-(2-*Z*-amino)benzyl hexanoate) Amide (1k). HOBt (382 mg, 2.83 mmol, 1.1 equiv), TBTU (909 mg, 2.83 mmol, 1.1 equiv), and 3β-acetoxy-5-cholenic acid (**7**) (1 g, 2.57 mmol, 1.0 equiv) were dissolved in dry DMF (30 mL). Then, DIPEA (1.34 mL, 7.72 mmol, 3.0 equiv) was added, and the solution was stirred for 1 h. *Z*-Lys-OBzl (2.040 g, 3.86 mmol, 1.5 equiv) in suspension in DMF (30 mL) was then added, and the resulting solution stirred overnight. The reaction was diluted with water, extracted with chloroform (2 $\times$ l5 mL), dried with magnesium sulfate, and the remaining solvents removed under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution of hexane/AcOEt (80:20) first and $CHCl_3$/MeOH (90:10) second. Compound **1j** was isolated as a white solid (1.585 g, 80%).

3β-ol-23,24-bisnor-5-en-22-(octanoic acid) Amide (2g). Following general procedure C for 48 h starting from 3β-acetoxy-23,-24-bisnor-5-en-22-(methyl octanoate) amide (**1g**) (114 mg, 0.21 mmol, 1.0 equiv) and sodium hydroxide (1.9 mL, C = 1 M, 1.9 mmol, 9.1 equiv) in THF (5.6 mL), compound **2g** was isolated as a white solid (83 mg, 81%).

3β-Acetoxy-23,24-bisnor-chol-5-en-22-(methyl butanoate) Amine (3c). Following general procedure D and starting from triethylamine (73 μL, 0.52 mmol, 2.2 equiv), methyl 4-aminobutanoate (**6c**) (88 mg, 0.57 mmol, 2.4 equiv), 3β-acetoxy-23,24-bisnor-chol-5-en-22-al (**8**) (90 mg, 0.24 mmol, 1.0 equiv), and sodium cyanoborohydride (0.55 mL, 0.55 mmol, 2.3 equiv) in methanol (3.5 mL), compound **3c** was isolated as a white solid (64 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.74 (s, 3H, H^{18}), 1.05 (d, 3H, $H,^{21}$ J = 6.8), 1.07 (s, 3H, H^{19}), 2.09 (s, 3H, $H^{2'}$), 0.90 to 2.10 (m, 21H, H^1, H^2, $H,^7$ $H,^8$ $H,^9$ $H,^{11}$ $H,^{12}$ $H,^{14}$ $H,^{15}$ $H,^{16}$ $H,^{17}$ $H,^{20}$ H^{25}), 2.40 (m, 5H, $H,^4$ H^{26} and H^{22}), 2.71 (m, 3H, H^{24} and $H^{22'}$), 3.73 (s, 3H, H^{28}), 4.65 (m, 1H, H^3), 5.42 (m, 1H, H^6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.3 (C^{18}), 18.2 (C^{21}), 19.7 (C^{19}), 21.9 ($C^{2'}$), 25.4 (C^{25}), 34.3 (C^{26}), 36.7 (C^{20}), 37.0 (C^{10}), 38.5 (C^4), 42.9 (C^{13}), 49.7 (C^{24}), 52.0 (C^{28}), 55.6 (C^{22}), 21.4, 24.7, 28.2, 28.4, 32.3, 32.3, 37.4, 40.0, 50.4, 54.6, 56.9 (C^1, C^2, $C,^7$ $C,^8$ $C,^9$ $C,^{11}$ $C,^{12}$ $C,^{14}$ $C,^{15}$ $C,^{16}$ C^{17}), 74.4 (C^3), 123.0 (C^6), 140.1 (C^5), 171.0 ($C^{1'}$), 174.5 (C^{27}); Mp = 94−95 °C; R_f = 0.30 ($CHCl_3$/MeOH: 90/10); Anal. ($C_{29}H_{47}NO_4$, 0.4H_2O) C, H, N. LRMS, *m*/*z* (ES^+): 474.2 ($[M+H]^+$, 100%); HRMS (ES^+), Calculated for $C_{29}H_{48}NO_4$ ($[M+H]^+$): 474.3583; Found: 474.3579.

3β-Acetoxy-23,24-bisnor-chol-5-en-22-(methyl heptanoate) Amine (3f). Following general procedure D and starting from triethylamine (100 μL, 0.72 mmol, 2.4 equiv), methyl 7-aminoheptanoate (**6f**) (126 mg, 0.64 mmol, 2.1 equiv), 3β-acetoxy-23,24-bisnor-chol-5-en-22-al (**8**) (110 mg, 0.30 mmol, 1.0 equiv), and sodium cyanoborohydride (0.35 mL, 0.35 mmol, 1.2 equiv) in methanol (3 mL), compound **3f** was isolated as a white solid (77 mg, 51%).

3β-ol-23,24-bisnor-5-en-22-(octanoic acid) Amine (4g). Following general procedure C for 48 h starting from 3β-acetoxy-23,24-bisnor-chol-5-en-22-(methyl octanoate) amine (**3g**) (38 mg, 0.07 mmol, 1.0 equiv) and sodium hydroxide (1.0 mL, C = 1 M, 1.0 mmol, 13.9 equiv) in THF (2 mL), compound **4g** was isolated as a white solid (34 mg, 100%).

3β-ol-23,24-bisnor-chol-5-en-22-(methyl octanoate) Amine (4k). Following general procedure D and starting from triethylamine (140 μL, 1.01 mmol, 2.4 equiv), methyl 8-aminooctanoate (**6g**) (154 mg, 0.73 mmol, 1.7 equiv), 3β-ol-23,24-bisnor-chol-5-en-22-al (**9**) (138 mg, 0.42 mmol, 1.0 equiv), and sodium cyanoborohydride (0.6 mL, 0.60 mmol, 1.4 equiv) in methanol (9 mL), compound **4k** was obtained in its pure form (136 mg, 67%).

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Supporting Information Available: Experimental procedures and analytical data for compounds (either known compounds or intermediates) not included in the Experimental Section as well as the Table of elemental analysis data obtained for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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