Design and synthesis of screening libraries based on the muurolane natural product scaffold†

Emma C. Barnes,^a Vanida Choomuenwai,^{a,b} Katherine T. Andrews,^{a,b} Ronald J. Quinn,^a and Rohan A. Davis^{a,*}

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The plant-derived natural product 14-hydroxy-6,12-muuroladien-15-oic acid (**1**) was identified as a unique scaffold that could be chemically elaborated to generate novel lead- or drug-like screening libraries. Prior to synthesis a virtual library was generated and prioritised based on drug-like
10 physicochemical parameters such as Log P, Log D_{5.5}, hydrogen bond donors/acceptors, and molecular weight. The natural product scaffold (**1**) was isolated from the endemic Australian plant *Eremophila mitchellii* and then utilised in the parallel solution-phase generation of two series of analogues. The first library consisted of six semi-synthetic natural product amide derivatives, whilst the second contained six carbamate analogues. These libraries have been evaluated for antimalarial activity using a chloroquine-
15 sensitive *Plasmodium falciparum* line (3D7) and the compounds displayed low to moderate activity with IC₅₀ values ranging from 14 to 33 µM.

Introduction

Narrowing down the vast number of molecules found in chemical
20 space to those areas that are biologically relevant, and then to those that have potential to be pursued for drug development is a significant challenge.^{1–5} Chemists have therefore continuously refined compound library generation in order to build smarter libraries.^{6–8} Current synthetic library strategies include the
25 selection of novel scaffolds, particularly those that adhere to lead- and drug-like physicochemical profiles.^{2–6}

Nature remains an untapped source of unique and desirable scaffolds for library production, and subsequent drug discovery.^{2–}

⁸ Indeed, numerous scaffolds that have been identified in natural
30 products (NPs) have led to approved drugs or drug candidates for a range of diseases.^{7–12} Examples include antibacterials (β-lactams, tetracyclins, erythromycins), antivirals (modified nucleosides), anticholesterolemics (lovastatin), and anti-tumour agents (paclitaxel, rapamycins, epothilones).⁹

³⁵ The success of NPs and their semi-synthetic derivatives as therapeutic agents is intrinsically linked to the fact that NPs have been biologically validated, since they have been selected during evolution to bind to biosynthetic enzymes.^{2, 5, 6, 13, 14} It has been hypothesised that this inherent capacity to bind in biological
40 space allows NPs to also recognise human therapeutic targets.^{2, 13,}

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NPs are often cited as being structurally diverse, drug-like, and containing privileged motifs.^{2, 5, 6, 8, 15} Computational studies have shown that NP scaffolds occupy complementary areas of
45 chemical space compared with synthetic compounds, and can be used to increase the chemical complexity and drug-likeness of libraries.^{2, 4–6} Whilst NPs and their semi-synthetic analogues have been widely utilised by the pharmaceutical industry, they have also been used as molecular probes to increase our understanding
50 of biological pathways.^{3, 16}

A major focus of our research is the design and synthesis of drug discovery libraries based on unique NP scaffolds^{17, 18} that would complement, and potentially expedite, current NP drug discovery methods that typically involve the high-throughput
55 screening (HTS) of pre-fractionated or extract libraries.¹⁹ The use of fully characterised NP analogue libraries is advantageous to drug discovery since “hits” resulting from HTS can be quickly evaluated in an identical manner to those from synthetic libraries. Because the chemical structure of the hit compound is already
60 known (unlike extract or pre-fractionated library screening), the potential for lead evaluation can be progressed more rapidly. Furthermore, if the design of a NP library is given due consideration before the synthesis is undertaken, then adherence

^aEskitis Institute, Griffith University, Nathan, QLD 4111, Australia.
E-mail: r.davis@griffith.edu.au; Fax: +61 7 3735 6001; Tel: +61 73735 6043
^bQueensland Institute for Medical Research, Herston, QLD 4006, Australia
† Electronic Supplementary Information (ESI) available: ¹H and ¹³CNMR spectra of **1–14**. See DOI: 10.1039/b000000x/

⁶⁵ to important physicochemical parameters known to be associated

with lead- or drug-like molecules such as Log P, Log D_{5.5}, hydrogen bond donors (HBD)/acceptors (HBA), and molecular weight (MW)^{20, 21} can be addressed that, ultimately, has a positive impact on the progression of any potential hit.

Clearly, if NP analogue libraries are to become more common place in drug discovery, then the supply of suitable NP scaffolds is crucial. While the total synthesis of the required scaffold is a possible solution to the supply issue, the structural complexity and need for multi-step synthesis to make some templates can be prohibitive.^{12, 16, 22} Since we have access to the Eskitis Institute's Nature Bank, which includes 50,055 plant and marine biota samples and 2,738 pure NP compounds, our approach has been to use this valuable resource to acquire relevant NP scaffolds, thus bypassing the *de novo* synthetic strategy for scaffold production. A number of studies have been reported in the literature in which NP scaffolds have been isolated from various biota sources and subsequently used for screening library generation. Examples include the use of the tambjamine, teicoplanin aglycone and yohimbine scaffolds.^{17, 23, 24} Parameters used to initially identify and prioritise potential scaffolds from the NP collection included MW (< 300), quantity available (> 100 mg), Log P (< 5), and the inclusion of chemical handles for modification (*e.g.* -COOH, -OH, -CHO, -NH₂, Ar-Br). NPs possessing stereogenic centres were also given high priority, as stereochemistry confers a unique 3D shape on a molecule and has the potential to improve target binding.^{2, 16, 25, 26} The availability of biota for the re-extraction and re-isolation of each compound also had to be taken into account. Around 200 NPs were initially identified using this approach from the NP library, one of which was the previously reported sesquiterpene 14-hydroxy-6,12-muuroloadien-15-oic acid (**1**).^{27, 28}

The low MW (250), favourable Log P (2.42), multiple stereogenic centres (4) and potential chemical handles (*i.e.* the carboxylic acid²⁹ and allylic hydroxyl group³⁰⁻³²) for synthetic elaboration made **1** a particularly attractive NP scaffold.

Scaffold **1** has previously been obtained from the Australian endemic plant genus *Eremophila*. It was originally isolated from *Eremophila virgata* in 1989,²⁷ and then subsequently from *Eremophila mitchellii*.²⁸ This species is native to inland northern New South Wales and Queensland.³³ Oil extracts from this plant have been found to possess termiticidal, insecticidal, cytotoxic, and antimicrobial activity.³⁴⁻³⁸

Results and discussion

A sample of the air-dried and ground leaves of *E. mitchellii* (27.5 g) was exhaustively extracted with sequential washes of *n*-hexane, CH₂Cl₂, and CH₃OH. The CH₂Cl₂ and CH₃OH extracts were combined and fractionated by diol-bonded silica flash column chromatography using a *n*-hexane/EtOAc gradient. Further purification by diol-bonded silica HPLC (*i*-PrOH/*n*-hexane) yielded 14-hydroxy-6,12-muuroloadien-15-oic acid (**1**, 201.7 mg, 0.734% dry wt). The structure of **1** was assigned after NMR, IR, UV, and MS data analysis and comparison with literature values.^{27, 28}

It was envisaged that both the carboxylic acid and allylic hydroxyl of **1** would be utilised to create library members in order to increase the structural diversity of the analogue series. Initially **1** was modified at the carboxylic acid by generating a small library of amides. The amine partners used for the coupling reactions were chosen following the construction of a virtual library of potential products using a list of commercially available amines (895 in total),³⁹ and the Reactor⁴⁰ and Instant JChem software packages.⁴¹ A number of physicochemical parameters (Table 1) including Lipinski's "Rule of Five" for drug-like molecules (MW < 500, HBD < 5, HBA < 10, Log P < 5)²⁰ and Log D_{5.5} were calculated in an effort to select the most viable molecules for subsequent synthesis.

The distribution coefficient at pH 5.5 (Log $D_{5.5}$) was included since it has been proposed that it is a better descriptor (*c.f.* Log P) of the lipophilic nature of drug-like molecules, particularly NPs,¹⁹ in the small intestine, where the majority of oral drug absorption occurs.²¹ Whilst Log P calculations specifically predict the partitioning of neutral (*i.e.* un-ionized) species between *n*-octanol and water, Log D is a more appropriate measure as it considers the distribution of both ionized and un-ionized species at a given pH. On the basis of the *in silico* data, amine reagents were subsequently selected that would ensure the drug-likeness of the products generated.

Due to the low quantity of starting material, only ~ 10 mg of **1** was used in each reaction. Three standard coupling reagents were initially investigated to examine which would give optimal yields. These reagents included 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM),²⁹ *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI),¹⁸ and oxalyl chloride [(COCl)₂].⁴²

Reactions using either DMTMM or EDCI afforded low yields (< 35%), believed to be due to a combination of the hygroscopic nature of the NP scaffold and the small reaction scale employed. The first reaction utilising (COCl)₂ and phenethylamine gave a high yield of 77%, however, subsequent reactions employing other amines resulted in yields ranging from 5 to 48%. In all (COCl)₂ coupling reactions minor side products were observed by (+)-LRESIMS and ¹H NMR spectroscopy. These data suggested some of the minor products included the substitution of the primary hydroxyl (14-OH) with a chlorine atom,^{43, 44} and the formation of oxalic acid derivatives at 14-OH.^{45, 46} Due to the low yields and difficulty associated with purifying these potentially unstable minor side-products, full spectroscopic characterisation was not possible. While protection chemistry was considered as an option for reducing the (COCl)₂ side products, this was not pursued due to the number of additional reaction and purification steps required, the scarcity of the NP scaffold, and our desire to generate other analogues based on the murrolane scaffold **1**.

Therefore, we decided to use the allylic hydroxyl group of **1** in reactions with commercially available isocyanates to generate a library of carbamates.³⁰ In an identical manner to the amide

library, a virtual collection of potential carbamates was initially generated using the software Reactor⁴⁰ and Instant JChem⁴¹ coupled with a list of commercially available isocyanates (315 in total).³⁹ Analysis of the *in silico* physicochemical properties (MW, HBD, HBA, Log P, Log $D_{5.5}$) of the virtual library identified those members that were compliant with drug-like properties.^{20, 21}

Literature reports indicated that the carboxylic acid of **1** would react with the isocyanate reagents to form anhydrides or amides,⁴⁷ hence this was protected by methylation using TMS-diazomethane⁴⁸ prior to carbamate library synthesis. In contrast to the amide library production where no protection chemistry was utilised, we decided to use protection chemistry in the carbamate library series since the methylation could be achieved in high yield (> 90%), with no deprotection or purification required. Subsequent reactions of the methyl ester scaffold **8** with various isocyanates resulted in six carbamates with yields ranging from 31 to 76%.

Typically all of the amides and carbamates produced in this study were first purified by fractionation using a silica solid-phase extraction (SPE) cartridge employing either a *n*-hexane/EtOAc or *n*-hexane/*i*-PrOH gradient. Further purification was achieved by semi-preparative diol-bonded silica HPLC (*i*-PrOH/*n*-hexane) or C₁₈-bonded silica HPLC (CH₃OH/H₂O) when required. The structures of all library members (**2-14**) were determined following 1D and 2D NMR (COSY, HSQC, HMBC and ROESY) and (+)-HRESIMS data analysis.

During the large-scale isolation of **1**, mitchellenes A-E (**15-19**) were also obtained; the purification and structural identification of these NPs has been reported elsewhere.²⁸

Compounds **1-19** have recently been added to the open-access small molecule repository at the Queensland Compound Library (QCL)⁴⁹ located at the Eskitis Institute where they are available for drug discovery and chemical biology research. Prior to submitting compounds **1-19** to the QCL, purity studies were performed using analytical C₁₈ HPLC. Analysis of the HPLC data and integration of all UV peaks at 210 nm determined that all compounds had purities > 90%.

To date, compounds **1-19** have been screened for antiplasmodial activity in an *in vitro* growth inhibition assay using a chloroquine sensitive *Plasmodium falciparum* line (3D7). Initially all compounds were screened at 50 μM , and those displaying a percent inhibition of $\geq 50\%$ at this concentration were further evaluated and IC_{50} values determined (Table 1). All compounds were also screened for cytotoxicity towards a normal mammalian cell line [neonatal foreskin fibroblasts (NFF)] in order to determine the selectivity of the compounds towards the malaria parasite.

Scaffold **1** and those library members with simple modifications at the carboxylic acid, such as the amide **3** and the methyl ester **8**, exhibited no activity against *P. falciparum* 3D7. The related natural products **18-19** also demonstrated low

inhibition in this assay.

In contrast, the introduction of larger substituents at the carboxylic acid increased the antimalarial activity, with the aromatic analogues eliciting a low to moderate response against the parasite (IC_{50} 14-19 μM). Of the phenethylamine derivatives (**2, 5-7**) only compound **7** was inactive, suggesting that the polar hydroxyl group on the benzene system was responsible for the reduced activity.

All members of the carbamate library demonstrated low to moderate activity against the *P. falciparum* 3D7 parasites (IC_{50} 16-33 μM). These compounds only show minor variations in their activity, with no particular carbamate substituent eliciting a greater response against the parasite than the others.

Table 1 Physicochemical profiling and biological activity of **1-19**

compound	physicochemical parameters ^a					biological activity	
	MW	Log P	Log D _{5.5}	HBA	HBD	3D7 ^b ($\text{IC}_{50} \pm \text{SD}$)	NFF ^c (% $\pm \text{SD}$)
1	250	2.42	1.70	3	2	> 50	^d
2	353	3.85	3.85	2	2	14 $\pm$ 4	32 $\pm$ 16
3	249	1.61	1.61	2	2	> 50	^d
4	334	0.86	0.86	3	3	> 50	^d
5	387	4.45	4.45	2	2	19 $\pm$ 2	86 $\pm$ 5
6	383	3.69	3.69	3	2	19 $\pm$ 1	16 $\pm$ 5
7	369	3.54	3.54	3	3	> 50	^d
8	264	2.80	2.80	2	1	> 50	^d
9	427	4.77	4.77	5	1	33 $\pm$ 8	^e
10	397	4.85	4.85	2	1	28 $\pm$ 7	44 $\pm$ 10
11	363	4.45	4.45	2	1	20 $\pm$ 3	15 $\pm$ 4
12	389	4.93	4.93	2	1	16 $\pm$ 1	41 $\pm$ 15
13	427	4.69	4.69	3	1	27 $\pm$ 3	45 $\pm$ 9
14	457	4.54	4.59	4	1	18 $\pm$ 3	86 $\pm$ 5
15	248	1.78	1.78	2	1	24 $\pm$ 8	49 $\pm$ 10
16	232	3.09	3.09	1	0	30 $\pm$ 7	53 $\pm$ 15
17	248	1.94	1.94	2	1	20 $\pm$ 7	^e
18	266	2.98	1.14	4	2	> 50	^d
19	252	2.51	1.73	3	2	> 50	^d
chloroquine	319	3.93	-0.76	3	1	0.03 $\pm$ 0.01	^f

^a*In silico* calculations performed using Instant JChem software,⁴¹ MW = molecular weight, Log P = partition coefficient, Log D_{5.5} = distribution coefficient at pH 5.5, HBA = H bond acceptors, HBD = H bond donors; ^b3D7 = *Plasmodium falciparum* (chloroquine sensitive) strain; ^cNFF = neonatal foreskin fibroblast cells, percent inhibition measured at 100 μM ; ^dnot tested; ^enot active; ^fchloroquine IC_{50} = 34 $\pm$ 3 μM .

The tetracyclic NPs **15-17** also showed low to moderate antiplasmodial inhibitory activity (IC_{50} 12-30 μM). While being

structurally similar and possessing almost identical MWs to NPs **18-19**, the rigid structures of **15-17** may allow them to bind more effectively to the target than compounds **18-19**, which possess a greater number of rotatable bonds.²⁵ However, **15-17** do not possess chemical handles that would be useful in the synthesis of derivatives for the exploration of structure activity relationships.

Conclusions

The natural product scaffold 14-hydroxy-6,12-muuroloadien-15-oic acid (**1**) was isolated from the plant *E. mitchellii* and was utilised in the generation of two screening libraries. Initial screening data suggests that scaffold **1** is a valid starting point for the generation of antimalarial compounds, as although the scaffold itself was inactive against *P. falciparum* 3D7, many of the library members generated from this molecule demonstrated improved activity. NP scaffolds such as the one utilised in this study have the potential to be used as tools for investigating biological pathways and developing novel therapeutics.³ While the biological activity demonstrated here against *P. falciparum* is only modest, the library members generated may prove to be useful probes in future screening campaigns against other targets.

Experimental

General

Optical rotations were recorded on a JASCO P-1020 polarimeter. UV spectra were recorded on a Bruker Tensor 27 spectrophotometer. NMR spectra were recorded at 30 °C on either a Varian 400 MHz, 500 MHz or 600 MHz Unity INOVA spectrometer. The ¹H and ¹³C chemical shifts were referenced to the solvent peaks for DMSO-*d*₆ at δ_H 2.49 and δ_C 39.5, respectively. *J* values are given in Hz. LRESIMS were recorded on a Mariner Time-of-Flight spectrometer equipped with a Gilson 215 eight probe injector. HRESIMS were recorded on a Bruker Apex III 4.7 Tesla fourier transform ion cyclotron resonance mass spectrometer. An Edwards Instrument company Bio-line orbital shaker was used for plant extractions. Fluka silica gel TLC aluminium cards were used for TLC. Phenomenex solid phase extraction (SPE) polypropylene cartridges were used for reaction purifications. For analytical HPLC purity analysis a Waters 600 pump equipped with a Waters 966 PDA detector and Gilson 715 liquid handler (5 mL syringe, 200 µL Rheodyne sample loop) was used. A Waters 600 pump equipped with a Waters 966 PDA detector and a Waters 717 Plus Autosampler connected to a Gilson FC204 fraction collector were used for semi-preparative separations. A Phenomenex C₁₈ Onyx monolithic (4.6 × 100 mm) column was used for analytical HPLC purity analysis. Alltech Davisil diol-bonded silica, 30-40 µm, 60 Å, or Alltech C₁₈ bonded silica, 35-75 µm, 150 Å, or Merck silica gel 60 (0.040-0.063 mm) were used for pre-adsorption work. Either a YMC diol 5 µm 120 Å (20 × 150 mm) column or a ThermoElectron C₁₈ Betasil 5 µm 143 Å (21.2 × 150 mm) column were used for semi-preparative HPLC separations. All solvents used for chromatography, [α]_D, UV, and MS were Lab Scan HPLC grade, and the H₂O was Millipore Milli-Q PF filtered. All synthetic reagents were obtained from Sigma-Aldrich and used without further purification.

Plant material

The leaves of *Eremophila mitchellii* were collected from Currawinya National Park, QLD, Australia, in March, 1996. Collection and identification was undertaken by P. Forster and G. Guymer from the Queensland Herbarium. A voucher specimen (AQ603041) has been deposited at the Queensland Herbarium, Brisbane, Australia.

Extraction and isolation

The air-dried and ground leaves of *E. mitchellii* (27.5 g) were extracted exhaustively with *n*-hexane (250 mL), CH₂Cl₂ (2 × 250 mL), and CH₃OH (2 × 250 mL). All CH₂Cl₂/CH₃OH extractives were combined to yield a dark-brown gum (4.94 g). This crude extract was divided into ~ 500 mg portions and purified by diol-bonded silica flash column (35 × 130 mm) using a 20% stepwise gradient from *n*-hexane to EtOAc followed by CH₃OH. The 20% *n*-hexane/80% EtOAc and EtOAc fractions were combined and pre-adsorbed to diol-bonded silica, packed into stainless steel cartridges (10 × 30 mm), and subjected to semi-preparative HPLC using a YMC diol column. Isocratic conditions of *n*-hexane were held for the first 10 min, followed by a linear gradient to 20% *i*-PrOH/80% *n*-hexane over 40 min, then isocratic conditions of 20% *i*-PrOH/80% *n*-hexane for 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected then analysed by (+)-LRESIMS and ¹H NMR spectroscopy. Scaffold **1** eluted in fractions 32-33 (201.7 mg, 0.734% dry wt).

General procedure for amide formation using DMTMM²⁹

DMTMM was generated following a literature procedure from its precursor 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine.²⁹ Scaffold **1** (8.4 mg, 0.0336 mmol) and phenethylamine (45 µL, 0.3360 mmol) were stirred in dry THF (4 mL) for 15 min at rt under Ar. DMTMM (93 mg, 0.3360 mmol) was added and the solution stirred at rt for 4 h. The solution was poured into H₂O (30 mL) and extracted with CH₂Cl₂ (30 mL). The organic phase was further washed with NaHCO₃ (30 mL), 3.5 % HCl (30 mL), and H₂O (30 mL) before being dried over MgSO₄. The crude product was purified using a SPE cartridge packed with diol-bonded silica (10 × 40 mm) and 10 mL eluent flushes of *n*-hexane, 5% *i*-PrOH/*n*-hexane, 10% *i*-PrOH/*n*-hexane, 20% *i*-PrOH/*n*-hexane, then CH₃OH. The 10% *i*-PrOH/90% *n*-hexane fraction was found to contain impure product following TLC and ¹H NMR spectroscopy analysis. This fraction was pre-adsorbed to diol-bonded silica, packed into a stainless steel cartridge (10 × 30 mm), and subjected to semi-preparative HPLC using a YMC diol column at a flow rate of 9 mL/min and isocratic conditions of *n*-hexane for 10 min, followed by a linear gradient to 15% *i*-PrOH/85% *n*-hexane over 40 min, then isocratic conditions of 15% *i*-PrOH/85% *n*-hexane for 10 min. Sixty fractions (60 × 1 min) were collected. Fraction 46 was found to contain **2** (2.3 mg, 19%). Compound **3** was purified in a similar manner to **2**, except that a linear gradient to 20% *i*-PrOH/80% *n*-hexane over 40 min was utilised in the diol-bonded HPLC step to give **3** in fractions 46-48 (3.7 mg, 27%).

Compound **4** was first purified off a SPE cartridge packed with diol-bonded silica (10 × 40 mm) using 10 mL washes from *n*-hexane to EtOAc in 20% steps followed by CH₃OH. Impure product was found in the CH₃OH fraction, and was further
5 purified by C₁₈ bonded silica semi-preparative HPLC at a flow rate of 9 mL/min and isocratic conditions of 10% CH₃OH/90% H₂O for 10 min, followed by a linear gradient to CH₃OH over 40 min, then isocratic conditions of CH₃OH for 10 min. Sixty fractions (1 min each) were collected and fraction 37 contained **4**
10 (3.2 mg, 31%).

Compound 2

Yield 19%, white gum; $[\alpha]_D^{25} - 85$ (*c* 0.073, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 204 (4.13); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 6.6 Hz, H-11), 1.11 (1H, dddd, *J* = 13.2, 12.6, 12.6, 3.0 Hz, H-2 β), 1.31 (1H, ddd, *J* = 12.6, 12.6, 3.0 Hz, H-3 α), 1.37 (1H, m, H-2 α), 1.38 (1H, m, H-9 β), 1.59 (1H, brdd, *J* = 12.5, 5.4 Hz, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.72 (1H, m, H-1), 1.81 (1H, ddd, *J* = 12.6, 11.4, 2.4 Hz, H-4), 1.95 (1H, m, H-8 α), 2.22 (1H, ddd, *J* = 11.4, 6.0, 4.2 Hz, H-5), 2.40 (1H, brdd, *J* = 18.0, 5.4 Hz, H-8 β), 2.71 (2H, t, *J* = 7.8, 7.8 Hz, H-18), 3.27 (2H, dt, *J* = 7.8, 7.8, 5.4 Hz, H-17), 3.82 (1H, d, *J* = 15.0 Hz, H-14), 3.87 (1H, d, *J* = 15.0 Hz, H-14), 4.79 (1H, brs, 14-OH), 4.90 (1H, s, H-13), 5.07 (1H, s, H-13), 6.45 (1H, brd, *J* = 4.2 Hz, H-6), 7.17 (2H, d, *J* = 7.8 Hz, H-20), 7.18 (1H, dd, *J* = 7.2, 7.2 Hz, H-22), 7.27 (2H, dd, *J* = 7.8, 7.2 Hz, H-21), 7.66 (1H, t, *J* = 5.4, 5.4 Hz, 16-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.0 (C-9), 19.3 (C-11), 25.0 (C-8), 28.9 (C-2), 33.9 (C-3), 34.2 (C-1), 35.1 (C-18), 38.7 (C-10), 40.5 (C-17), 40.8 (C-5), 42.3 (C-4), 63.3 (C-14), 107.3 (C-13), 125.9 (C-22), 128.2 (C-21), 128.5 (C-20), 132.8 (C-7), 135.1 (C-6), 139.6 (C-19), 152.6 (C-12), 167.4 (C-15); (+)-LRESIMS *m/z* (rel. int.) 354 (100) [M + H]⁺; (+)-HRESIMS *m/z* 376.2250 (C₂₃H₃₁NO₂Na [M+Na]⁺ requires 376.2247).

Compound 3

Yield 27%, white gum; $[\alpha]_D^{25} - 113$ (*c* 0.107, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 202 (3.84), 219 (3.71); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 7.2 Hz, H-11), 1.11 (1H, dddd, *J* = 12.6, 12.6, 12.6, 3.0 Hz, H-2 β), 1.31 (1H, ddd, *J* = 13.2, 12.6, 2.4 Hz, H-3 α), 1.35 (1H, m, H-9 β), 1.37 (1H, m, H-2 α), 1.55 (1H, brdd, *J* = 12.6, 5.4 Hz, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.73 (1H, m, H-1), 1.80 (1H, ddd, *J* = 12.0, 11.4, 3.0 Hz, H-4), 1.96 (1H, m, H-8 α), 2.21 (1H, ddd, *J* = 11.4, 6.0, 4.2 Hz, H-5), 2.38 (1H, brdd, *J* = 18.0, 5.4 Hz, H-8 β), 3.81 (1H, d, *J* = 15.0 Hz, H-14), 3.86 (1H, d, *J* = 15.0 Hz, H-14), 4.75 (1H, brs, 14-OH), 4.90 (1H, s, H-13), 5.05 (1H, s, H-13), 6.49 (1H, brd, *J* = 4.2 Hz, H-6), 7.07 (2H, s, 16-NH₂); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.1 (C-9), 19.3 (C-11), 25.1 (C-8), 29.0 (C-2), 33.8 (C-3), 34.2 (C-1), 38.7 (C-10), 41.0 (C-5), 42.3 (C-4), 63.3 (C-14), 107.4 (C-13), 132.5 (C-7), 135.9 (C-6), 152.6 (C-12), 169.3 (C-15); (+)-LRESIMS *m/z* (rel. int.) 250 (100) [M + H]⁺; (+)-HRESIMS *m/z* 272.1620 (C₁₅H₂₃NO₂Na [M+Na]⁺ requires 272.1621).

Compound 4

Yield 31%, orange gum; $[\alpha]_D^{26} - 53$ (*c* 0.04, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 202 (4.00), 221 (3.82); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 7.2 Hz, H-11), 1.11 (1H, dddd, *J* = 13.2, 12.6, 12.6, 3.6 Hz, H-2 β), 1.29 (1H, ddd, *J* = 12.6, 12.6, 3.0 Hz, H-3 α), 1.34 (1H, m, H-9 β), 1.36 (1H, m, H-2 α), 1.56 (1H, m, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.72 (1H, m, H-1), 1.78 (3H, s, H-21), 1.80 (1H, dd, *J* = 12.6, 4.2 Hz, H-4), 1.98 (1H, m, H-8 α), 2.21 (1H, ddd, *J* = 11.4, 6.0, 5.4 Hz, H-5), 2.38 (1H, brdd, *J* = 18.6, 4.2 Hz, H-8 β), 3.08 (2H, m, H-17), 3.13 (2H, m, H-18), 3.81 (1H, d, *J* = 15.0 Hz, H-14), 3.86 (1H, d, *J* = 15.0 Hz, H-14), 4.83 (1H, brs, 14-OH), 4.90 (1H, s, H-13), 5.06 (1H, s, H-13), 6.47 (1H, brd, *J* = 4.8 Hz, H-6), 7.64 (1H, brd, *J* = 12.0 Hz, 16-NH), 7.92 (1H, brd, *J* = 13.2 Hz, 19-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.0 (C-9), 19.3 (C-11), 22.5 (C-21), 25.0 (C-8), 28.9 (C-2), 33.8 (C-3), 34.2 (C-1), 38.3 (C-17), 38.7 (C-10), 39.0 (C-18), 40.9 (C-5), 42.2 (C-4), 63.3 (C-14), 107.3 (C-13), 132.5 (C-7), 135.6 (C-6), 152.6 (C-12), 167.5 (C-15), 169.5 (C-20); (+)-LRESIMS *m/z* (rel. int.) 357 (100) [M + Na]⁺, 335 (100) [M + H]⁺; (+)-HRESIMS *m/z* 335.2318 (C₁₉H₃₁N₂O₃ [M+H]⁺ requires 335.2329).

General procedure for amide formation using EDCI¹⁸

Scaffold **1** (9.8 mg, 0.0392 mmol), EDCI (11.3 mg, 0.0588 mmol), and DMAP (0.5 mg, 0.00392 mmol) were stirred in CH₃CN (3 mL) for 1 h at rt under Ar. Phenethylamine (50 μ L, 0.3920 mmol) was added and the mixture stirred for 16 h. A further 50 μ L of the amine was added and the reaction stirred for a further 2 h. The solution was poured into CH₂Cl₂ (20 mL),
80 before being extracted with H₂O (20 mL) then 2 N HCl (20 mL). The crude product was purified as above for the DMTMM coupling reaction to give **2** (1.6 mg, 12%). Compound **4** was prepared in a similar manner (3.2 mg, 22%).

General procedure for amide formation using (COCl)₂⁴²

Scaffold **1** (10.7 mg, 0.0428 mmol) was dissolved in dry CH₂Cl₂ (2 mL) under Ar. (COCl)₂ (11 μ L, 0.1270 mmol) was initially added, then anhydrous DMF (10 μ L, 0.1293 mmol) was added dropwise. Gas evolution was observed on addition of DMF. In a
95 second vial, phenethylamine (28 μ L, 0.2233 mmol) and anhydrous pyridine (100 μ L) were stirred in dry CH₂Cl₂ (2 mL) under Ar. This vial was cooled to 0 °C and the acid chloride generated added dropwise. The solution was stirred for 20 min before being poured into CH₂Cl₂ (10 mL) and extracted with 2 N HCl (30 mL). The crude product was purified as above for the DMTMM coupling reaction to give **2** (11.6 mg, 77%). Compound **4** was prepared in a similar manner (0.9 mg, 5%). Compound **5** was purified off a silica SPE cartridge (10 × 40 mm) by using a 10% stepwise gradient from *n*-hexane to EtOAc followed by CH₃OH (10 mL elutions). Pure product was found in fractions 5-6 (8.6 mg, 48%). Compound **6** was first purified by silica SPE cartridge (10 × 40 mm) using a 10% stepwise gradient from *n*-hexane to EtOAc followed by CH₃OH (10 mL elutions). Fractions 5-7 were then subjected to semi-preparative HPLC
100 using a YMC diol column at a flow rate of 9 mL/min and isocratic conditions of *n*-hexane for 10 min, followed by a linear gradient to 20% *i*-PrOH/80% *n*-hexane over 40 min, then isocratic conditions of 20% *i*-PrOH/80% *n*-hexane for 10 min.

Sixty fractions (60 × 1 min) were collected. Fraction 41 was found to contain **6** (2.5 mg, 5%). Compound **7** was purified in the same manner as **6**, and eluted in fractions 53-54 (1.7 mg, 8%) after diol-bonded silica HPLC.

Compound 5

Yield 48%, white gum; $[\alpha]_D^{25} - 55$ (*c* 0.07, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 202 (4.23), 221 (4.21); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 7.2 Hz, H-11), 1.11 (1H, dddd, *J* = 13.2, 13.2, 12.6, 2.4 Hz, H-2 β), 1.31 (1H, m, H-3 α), 1.34 (1H, m, H-9 β), 1.36 (1H, m, H-2 α), 1.56 (1H, brdd, *J* = 12.6, 6.0 Hz, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.72 (1H, m, H-1), 1.80 (1H, ddd, *J* = 12.6, 11.4, 3.0 Hz, H-4), 1.93 (1H, m, H-8 α), 2.21 (1H, ddd, *J* = 11.4, 6.0, 4.2 Hz, H-5), 2.40 (1H, brdd, *J* = 17.4, 4.8 Hz, H-8 β), 2.70 (2H, t, *J* = 7.2, 7.2 Hz, H-18), 3.24 (2H, m, H-17), 3.81 (1H, d, *J* = 15.0 Hz, H-14), 3.87 (1H, d, *J* = 15.0 Hz, H-14), 4.80 (1H, brs, 14-OH), 4.89 (1H, s, H-13), 5.07 (1H, s, H-13), 6.45 (1H, brd, *J* = 4.2 Hz, H-6), 7.18 (2H, d, *J* = 7.8 Hz, H-20), 7.32 (2H, d, *J* = 7.8 Hz, H-21), 7.66 (1H, t, *J* = 5.4, 4.8 Hz, 16-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.0 (C-9), 19.3 (C-11), 25.0 (C-8), 29.0 (C-2), 33.9 (C-3), 34.2 (C-1), 34.3 (C-18), 38.7 (C-10), 40.2 (C-17), 40.9 (C-5), 42.3 (C-4), 63.3 (C-14), 107.3 (C-13), 128.1 (C-21), 130.5 (C-20), 130.6 (C-22), 132.8 (C-7), 135.1 (C-6), 138.6 (C-19), 152.6 (C-12), 167.5 (C-15); (+)-LRESIMS *m/z* (rel. int.) 388 (100) [M + H]⁺; (+)-HRESIMS *m/z* 410.1865 (C₂₃H₃₀NO₃Na [M+Na]⁺ requires 410.1857).

Compound 6

Yield 5%, white gum; $[\alpha]_D^{25} - 78$ (*c* 0.153, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 203 (4.62), 225 (4.60), 278 (3.54), 284 (3.48); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 7.2 Hz, H-11), 1.11 (1H, dddd, *J* = 13.2, 12.6, 12.6, 3.0 Hz, H-2 β), 1.31 (1H, ddd, *J* = 13.2, 12.0, 2.4 Hz, H-3 α), 1.35 (1H, m, H-9 β), 1.37 (1H, m, H-2 α), 1.56 (1H, brdd, *J* = 12.6, 6.0 Hz, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.72 (1H, m, H-1), 1.81 (1H, ddd, *J* = 12.0, 11.4, 2.4 Hz, H-4), 1.95 (1H, m, H-8 α), 2.22 (1H, ddd, *J* = 11.4, 5.4, 4.8 Hz, H-5), 2.40 (1H, brdd, *J* = 17.4, 4.8 Hz, H-8 β), 2.71 (2H, t, *J* = 7.8, 7.2 Hz, H-18), 3.22 (2H, m, H-17), 3.70 (3H, s, H-23), 3.82 (1H, d, *J* = 14.4 Hz, H-14), 3.87 (1H, d, *J* = 14.4 Hz, H-14), 4.79 (1H, brt, *J* = 4.2, 4.2 Hz, 14-OH), 4.90 (1H, s, H-13), 5.07 (1H, s, H-13), 6.45 (1H, brd, *J* = 4.8 Hz, H-6), 6.84 (2H, d, *J* = 7.8 Hz, H-21), 7.07 (2H, d, *J* = 7.8 Hz, H-20), 7.62 (1H, t, *J* = 5.4, 4.8 Hz, 16-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.1 (C-9), 19.3 (C-11), 25.0 (C-8), 28.9 (C-2), 33.9 (C-3), 34.0 (C-1), 34.2 (C-18), 38.7 (C-10), 40.7 (C-17), 40.8 (C-5), 42.3 (C-4), 54.9 (C-23), 63.3 (C-14), 107.3 (C-13), 113.7 (C-21), 129.5 (C-20), 131.4 (C-19), 132.8 (C-7), 135.1 (C-6), 152.6 (C-12), 157.6 (C-22), 167.4 (C-15); (+)-LRESIMS *m/z* (rel. int.) 384 (100) [M + H]⁺; (+)-HRESIMS *m/z* 406.2339 (C₂₄H₃₃NO₃Na [M+Na]⁺ requires 406.2353).

Compound 7

Yield 8%, white gum; $[\alpha]_D^{25} - 48$ (*c* 0.087, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 203 (4.06), 224 (4.00), 278 (3.02), 287 (2.88); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 6.6 Hz, H-11), 1.11 (1H, dddd, *J* = 13.2, 13.2, 12.6, 2.4 Hz, H-2 β), 1.30 (1H, m, H-3 α), 1.35 (1H, m, H-9 β), 1.37 (1H, m, H-2 α), 1.56 (1H, m, H-9 α), 1.61 (1H, m, H-3 β), 1.62 (1H, m, H-10), 1.72 (1H, m, H-1), 1.81 (1H, ddd, *J* = 12.6, 11.4, 1.8 Hz, H-4), 1.95 (1H, m, H-8 α), 2.21 (1H, ddd, *J* = 11.4, 6.0, 4.8 Hz, H-5), 2.40

(1H, brdd, *J* = 17.4, 4.8 Hz, H-8 β), 2.58 (2H, t, *J* = 7.8, 7.2 Hz, H-18), 3.20 (2H, m, H-17), 3.82 (1H, d, *J* = 15.0 Hz, H-14), 3.87 (1H, d, *J* = 15.0 Hz, H-14), 4.90 (1H, s, H-13), 5.07 (1H, s, H-13), 6.43 (1H, brd, *J* = 4.8 Hz, H-6), 6.65 (2H, d, *J* = 7.8 Hz, H-21), 6.94 (2H, d, *J* = 7.8 Hz, H-20), 7.60 (1H, t, *J* = 5.4, 4.8 Hz, 16-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 16.1 (C-9), 19.3 (C-11), 25.0 (C-8), 28.9 (C-2), 33.9 (C-3), 34.2 (C-1), 34.3 (C-18), 38.7 (C-10), 40.1 (C-17), 40.8 (C-5), 42.3 (C-4), 63.3 (C-14), 107.3 (C-13), 115.0 (C-21), 129.3 (C-20), 129.5 (C-19), 132.8 (C-7), 135.1 (C-6), 152.6 (C-12), 155.6 (C-22), 167.4 (C-15); (+)-LRESIMS *m/z* (rel. int.) 370 (100) [M + H]⁺; (+)-HRESIMS *m/z* 392.2199 (C₂₃H₃₁NO₃Na [M+Na]⁺ requires 392.2196).

Methylation of **1** using TMS-diazomethane⁴⁸

Scaffold **1** (12.8 mg, 0.0512 mmol) was dissolved in CH₃OH:CH₂Cl₂ (1:1, 1 mL) before TMS-diazomethane (2.0 M in diethyl ether, 77 μ L, 0.1536 mmol) was added dropwise. The reaction was stirred for 20 min at rt then quenched with AcOH (0.5 mL). The solvent was removed under reduced pressure to give **8** (13.7 mg, 99%).

Compound 8

White gum; $[\alpha]_D^{25} - 134$ (*c* 0.087, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ nm (log ϵ) 202 (3.87), 222 (3.93); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.89 (3H, d, *J* = 6.5 Hz, H-11), 1.11 (1H, dddd, *J* = 12.5, 12.5, 12.0, 2.0 Hz, H-2 β), 1.32 (1H, ddd, *J* = 13.0, 11.5, 2.5 Hz, H-3 α), 1.35 (1H, m, H-2 α), 1.37 (1H, m, H-9 β), 1.56 (1H, m, H-9 α), 1.62 (1H, m, H-3 β), 1.63 (1H, m, H-10), 1.71 (1H, m, H-1), 1.83 (1H, ddd, *J* = 11.5, 11.5, 2.5 Hz, H-4), 2.03 (1H, m, H-8 α), 2.32 (1H, m, H-5), 2.37 (1H, dd, *J* = 18.5, 6.0 Hz, H-8 β), 3.60 (3H, s, H-16), 3.79 (1H, d, *J* = 15.0 Hz, H-14), 3.86 (1H, d, *J* = 15.0 Hz, H-14), 4.90 (1H, s, H-13), 5.04 (1H, s, H-13), 6.83 (1H, brd, *J* = 5.5 Hz, H-6); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 15.9 (C-9), 19.2 (C-11), 25.0 (C-8), 28.9 (C-2), 33.7 (C-3), 34.0 (C-1), 38.4 (C-10), 40.9 (C-5), 42.2 (C-4), 51.3 (C-16), 63.0 (C-14), 107.6 (C-13), 128.9 (C-7), 142.8 (C-6), 152.2 (C-12), 167.1 (C-15); (+)-LRESIMS *m/z* (rel. int.) 287 (100) [M + Na]⁺; (+)-HRESIMS *m/z* 287.1606 (C₁₆H₂₄O₃Na [M+Na]⁺ requires 287.1618).

General procedure for carbamate formation³⁰

Scaffold **8** (8 mg, 0.0299 mmol) was dissolved in dry CH₂Cl₂, and 3,4-(methylenedioxy)-phenyl isocyanate (97.5 mg, 0.5980 mmol) was added followed by Et₃N (cat. 10 μ L). The solution was stirred at rt under Ar for 16 h then was quenched with CH₃OH. The solution was dried under N₂ and the crude loaded onto a SPE silica column (10 × 40 mm) that was washed with *n*-hexane, 10% EtOAc/90% *n*-hexane, 20% EtOAc/80% *n*-hexane, 50% EtOAc/50% *n*-hexane, 80% EtOAc/20% *n*-hexane, EtOAc, then CH₃OH in 10 mL elutions. Fractions containing impure product by ¹H NMR spectroscopy were further purified by semi-preparative diol HPLC. These fractions were injected onto a YMC diol column at a flow rate of 9 mL/min and isocratic conditions of *n*-hexane for 10 min, followed by a linear gradient to 20% *i*-PrOH/80% *n*-hexane over 40 min, then isocratic conditions of 20% *i*-PrOH/80% *n*-hexane for 10 min. Sixty fractions (60 × 1 min) were collected. Fractions 28-29 were found to contain **9** (6.9 mg, 49%). Compound **10** eluted in the 20%

EtOAc/80% *n*-hexane fraction when purified off a SPE silica column in the same manner as compound **9** (8.2 mg, 64%). Compound **11** was purified in an identical way to compound **9**, and eluted in fractions 19-20 off the diol-bonded silica HPLC column (9.7 mg, 76%). Compound **12** was first purified using a silica packed SPE column (10 × 40 mm) and elutions of *n*-hexane, 10% EtOAc/90% *n*-hexane, 20% EtOAc/80% *n*-hexane, then CH₃OH. Impure fractions were further purified by C₁₈ bonded silica semi-preparative HPLC at a flow rate of 9 mL/min (120 × 0.5 min fractions) and isocratic conditions of 10% CH₃OH/90% H₂O for 10 min, followed by a linear gradient to CH₃OH over 40 min, then isocratic conditions of CH₃OH for 10 min. Fractions 95-97 contained **12** (4.5 mg, 32%). Impure compound **13** was pre-adsorbed to silica and loaded onto a silica flash column (15 × 40 mm) that was washed with 20% EtOAc/80% *n*-hexane and collected in 1 mL fractions. Compound **13** eluted in fractions 20-26 (2.9 mg, 31%). Compound **14** was purified in the same manner as **13**, except that 25% EtOAc/75% *n*-hexane was utilised. Compound **14** eluted into fractions 20-26 (8 mg, 69%).

Compound 9

Yield 49%, light yellow gum; $[\alpha]_D^{27} - 152$ (*c* 0.067, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ (log ϵ) 207 (3.60), 248 (3.03), 297 (2.58) nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 6.5 Hz, H-11), 1.15 (1H, dddd, *J* = 12.5, 12.0, 12.0, 2.5 Hz, H-2 β), 1.35 (1H, m, H-3 α), 1.38 (1H, m, H-9 β), 1.40 (1H, m, H-2 α), 1.58 (1H, brdd, *J* = 12.0, 6.0 Hz, H-9 α), 1.67 (1H, m, H-10), 1.68 (1H, m, H-3 β), 1.74 (1H, m, H-1), 1.99 (1H, ddd, *J* = 12.0, 11.0, 3.0 Hz, H-4), 2.04 (1H, m, H-8 α), 2.35 (1H, m, H-5), 2.38 (1H, brdd, *J* = 18.5, 5.0 Hz, H-8 β), 3.59 (3H, s, H-16), 4.49 (1H, d, *J* = 14.5 Hz, H-14), 4.58 (1H, d, *J* = 14.5 Hz, H-14), 5.06 (1H, s, H-13), 5.11 (1H, s, H-13), 5.95 (2H, s, H-22'), 6.82 (1H, s, H-20'), 6.83 (1H, brd, *J* = 7.0 Hz, H-24'), 6.84 (1H, brd, *J* = 5.0 Hz, H-6), 7.10 (1H, brs, H-25'); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 15.9 (C-9), 19.2 (C-11), 25.0 (C-8), 28.7 (C-2), 33.2 (C-3), 33.9 (C-1), 38.3 (C-10), 40.5 (C-5), 42.9 (C-4), 51.3 (C-16), 65.0 (C-14), 100.7 (C-25'), 100.8 (C-22'), 108.0 (C-24'), 111.0 (C-13), 111.1 (C-20'), 129.2 (C-7), 133.4 (C-19'), 142.3 (C-6), 142.4 (C-23'), 147.0 (C-12), 147.1 (C-21'), 153.1 (C-17'), 167.0 (C-15); (+)-LRESIMS *m/z* (rel. int.) 450 (100) [M + Na]⁺, 438 (100) [M + H]⁺; (+)-HRESIMS *m/z* 450.1880 (C₂₄H₂₉NO₆Na [M+Na]⁺ requires 450.1887).

Compound 10

Yield 64%, opaque gum; $[\alpha]_D^{24} - 94$ (*c* 0.08, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ (log ϵ) 203 (4.31), 210 (4.30), 217 (4.22), 229 (4.04) nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.90 (3H, d, *J* = 6.6 Hz, H-11), 1.11 (1H, dddd, *J* = 12.6, 12.6, 12.6, 2.4 Hz, H-2 β), 1.34 (1H, m, H-3 α), 1.37 (1H, m, H-2 α), 1.38 (1H, m, H-9 β), 1.58 (1H, brdd, *J* = 12.6, 5.4 Hz, H-9 α), 1.64 (1H, m, H-3 β), 1.65 (1H, m, H-10), 1.71 (1H, m, H-1), 1.95 (1H, ddd, *J* = 12.0, 11.4, 3.0 Hz, H-4), 2.04 (1H, m, H-8 α), 2.35 (1H, dd, *J* = 11.4, 5.4 Hz, H-5), 2.38 (1H, brdd, *J* = 18.0, 5.4 Hz, H-8 β), 3.60 (3H, s, H-16), 4.17 (2H, d, *J* = 6.0, H-19'), 4.42 (1H, d, *J* = 13.8 Hz, H-14), 4.50 (1H, d, *J* = 13.8 Hz, H-14), 5.00 (1H, s, H-13), 5.05 (1H, s, H-13), 6.84 (1H, brd, *J* = 5.4 Hz, H-6), 7.22 (1H, dd, *J* = 7.2, 7.2

Hz, H-23'), 7.23 (2H, d, *J* = 7.8 Hz, H-21'), 7.30 (2H, dd, *J* = 7.2, 7.8 Hz, H-22'), 7.78 (1H, t, *J* = 6.0, 6.0 Hz, 18'-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 15.8 (C-9), 19.2 (C-11), 25.0 (C-8), 28.7 (C-2), 33.2 (C-3), 34.0 (C-1), 38.3 (C-10), 40.4 (C-5), 42.9 (C-4), 43.7 (C-19'), 51.3 (C-16), 64.9 (C-14), 110.9 (C-13), 126.7 (C-23'), 126.9 (C-21'), 128.2 (C-22'), 129.1 (C-7), 139.7 (C-20'), 142.5 (C-6), 147.3 (C-12), 156.1 (C-17'), 167.1 (C-15); (+)-LRESIMS *m/z* (rel. int.) 420 (100) [M + Na]⁺, 398 (100) [M + H]⁺; (+)-HRESIMS *m/z* 398.2308 (C₂₄H₃₂NO₄ [M+H]⁺ requires 398.2326).

Compound 11

Yield 76%, light yellow gum; $[\alpha]_D^{29} - 52$ (*c* 0.073, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ (log ϵ) 202 (4.41), 221 (4.43) nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.85 (3H, t, *J* = 7.5, 7.5 Hz, H-22'), 0.91 (3H, d, *J* = 7.0 Hz, H-11), 1.12 (1H, dddd, *J* = 12.5, 12.5, 12.5, 2.5 Hz, H-2 β), 1.25 (2H, m, H-21'), 1.34 (1H, m, H-3 α), 1.36 (2H, m, H-20'), 1.37 (1H, m, H-9 β), 1.38 (1H, m, H-2 α), 1.59 (1H, m, H-9 α), 1.64 (1H, m, H-3 β), 1.65 (1H, m, H-10), 1.73 (1H, m, H-1), 1.94 (1H, brdd, *J* = 11.5, 11.5 Hz, H-4), 2.04 (1H, m, H-8 α), 2.34 (1H, m, H-5), 2.38 (1H, brdd, *J* = 18.5, 5.0 Hz, H-8 β), 2.96 (2H, m, H-19'), 3.61 (3H, s, H-16), 4.37 (1H, d, *J* = 14.5 Hz, H-14), 4.45 (1H, d, *J* = 14.5 Hz, H-14), 4.99 (1H, s, H-13), 5.03 (1H, s, H-13), 6.83 (1H, brd, *J* = 3.5 Hz, H-6), 7.12 (1H, t, *J* = 5.5, 4.5 Hz, 18'-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 13.6 (C-22'), 15.9 (C-9), 19.2 (C-11), 19.3 (C-21'), 25.0 (C-8), 28.7 (C-2), 31.4 (C-20'), 33.2 (C-3), 34.0 (C-1), 38.3 (C-10), 39.0 (C-19'), 40.4 (C-5), 42.9 (C-4), 51.3 (C-16), 64.5 (C-14), 110.6 (C-13), 129.1 (C-7), 142.5 (C-6), 147.4 (C-12), 155.8 (C-17'), 167.0 (C-15); (+)-LRESIMS *m/z* (rel. int.) 386 (100) [M + Na]⁺, 364 (100) [M + H]⁺; (+)-HRESIMS *m/z* 364.2498 (C₂₁H₃₄NO₄ [M+H]⁺ requires 364.2482).

Compound 12

Yield 32%, yellow gum; $[\alpha]_D^{27} - 101$ (*c* 0.120, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ (log ϵ) 197 (3.13), 220 (3.41) nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.91 (3H, d, *J* = 7.0 Hz, H-11), 1.04 (2H, m, H-22'), 1.10 (1H, m, H-2 β), 1.11 (2H, m, H-20'), 1.18 (2H, m, H-21'), 1.33 (1H, m, H-3 α), 1.36 (1H, m, H-2 α), 1.38 (1H, m, H-9 β), 1.52 (2H, brd, *J* = 12.0 Hz, H-22'), 1.58 (1H, brdd, *J* = 12.5, 5.5 Hz, H-9 α), 1.63 (1H, m, H-3 β), 1.64 (2H, m, H-21'), 1.66 (1H, m, H-10), 1.71 (2H, m, H-20'), 1.72 (1H, m, H-1), 1.94 (1H, brdd, *J* = 11.5, 10.5 Hz, H-4), 2.04 (1H, m, H-8 α), 2.34 (1H, m, H-5), 2.38 (1H, brdd, *J* = 18.0, 5.0 Hz, H-8 β), 3.22 (1H, m, H-19'), 3.59 (3H, s, H-16), 4.36 (1H, d, *J* = 14.0 Hz, H-14), 4.44 (1H, d, *J* = 14.0 Hz, H-14), 4.98 (1H, s, H-13), 5.02 (1H, s, H-13), 6.83 (1H, brs, H-6), 7.05 (1H, d, *J* = 8.0 Hz, 18'-NH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 15.9 (C-9), 19.2 (C-11), 24.5 (C-21'), 25.0 (C-8), 25.1 (C-22'), 28.7 (C-2), 32.6 (C-20'), 33.2 (C-3), 34.0 (C-1), 38.3 (C-10), 40.4 (C-5), 42.9 (C-4), 49.4 (C-19'), 51.3 (C-16), 64.4 (C-14), 110.5 (C-13), 129.1 (C-7), 142.5 (C-6), 147.4 (C-12), 155.0 (C-17'), 167.0 (C-15); (+)-LRESIMS *m/z* (rel. int.) 413 (100) [M + Na]⁺, 390 (100) [M + H]⁺; (+)-HRESIMS *m/z* 390.2634 (C₂₃H₃₆NO₄ [M+H]⁺ requires 390.2639).

Compound 13

Yield 31%, opaque gum; $[\alpha]_D^{27} - 67$ (c 0.093, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 202 (4.07), 225 (4.00), 276 (2.88), 282 (2.82) nm; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 0.91 (3H, d, $J = 7.0$ Hz, H-11), 1.11 (1H, brddd, $J = 15.0, 14.0, 13.0$ Hz, H-2 β), 1.33 (1H, m, H-3 α), 1.36 (1H, m, H-2 α), 1.38 (1H, m, H-9 β), 1.58 (1H, m, H-9 α), 1.63 (1H, m, H-3 β), 1.65 (1H, m, H-10), 1.71 (1H, m, H-1), 1.95 (1H, brdd, $J = 12.0, 10.5$ Hz, H-4), 2.04 (1H, m, H-8 α), 2.34 (1H, m, H-5), 2.38 (1H, brdd, $J = 18.5, 5.0$ Hz, H-8 β), 3.61 (3H, s, H-16), 3.71 (3H, s, H-24'), 4.10 (2H, d, $J = 6.0, 19.9$ Hz, H-19'), 4.41 (1H, d, $J = 14.0$ Hz, H-14), 4.49 (1H, d, $J = 14.0$ Hz, H-14), 4.99 (1H, s, H-13), 5.04 (1H, s, H-13), 6.83 (1H, brs, H-6), 7.16 (2H, d, $J = 8.5$ Hz, H-21'), 6.85 (2H, d, $J = 8.5$ Hz, H-22'), 7.66 (1H, t, $J = 6.0, 6.0$ Hz, 18'-NH); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 15.8 (C-9), 19.2 (C-11), 25.0 (C-8), 28.7 (C-2), 33.2 (C-3), 33.9 (C-1), 38.3 (C-10), 40.4 (C-5), 42.9 (C-4), 43.2 (C-19'), 51.3 (C-16), 55.0 (C-24'), 64.8 (C-14), 110.8 (C-13), 113.6 (C-22'), 128.3 (C-21'), 129.1 (C-7), 131.7 (C-20'), 142.4 (C-6), 147.2 (C-12), 156.0 (C-17'), 158.1 (C-23'), 167.0 (C-15); (+)-LRESIMS m/z (rel. int.) 450 (100) $[\text{M} + \text{Na}]^+$, 428 (100) $[\text{M} + \text{H}]^+$; (+)-HRESIMS m/z 428.2448 ($\text{C}_{25}\text{H}_{34}\text{NO}_5$ $[\text{M} + \text{H}]^+$ requires 428.2432).

Compound 14

Yield 69%, opaque gum; $[\alpha]_D^{26} - 79$ (c 0.08, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 202 (4.16), 226 (3.65), 278 (2.88) nm; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 0.90 (3H, d, $J = 7.0$ Hz, H-11), 1.10 (1H, brddd, $J = 15.0, 13.5, 13.0$ Hz, H-2 β), 1.32 (1H, m, H-3 α), 1.36 (1H, m, H-2 α), 1.37 (1H, m, H-9 β), 1.58 (1H, brdd, $J = 13.0, 5.0$ Hz, H-9 α), 1.64 (1H, m, H-3 β), 1.65 (1H, m, H-10), 1.70 (1H, m, H-1), 1.95 (1H, brddd, $J = 12.0, 11.5, 2.5$ Hz, H-4), 2.04 (1H, m, H-8 α), 2.33 (1H, m, H-5), 2.38 (1H, brdd, $J = 17.5, 4.5$ Hz, H-8 β), 3.60 (3H, s, H-16), 3.71 (6H, s, H-26', H-27'), 4.10 (2H, d, $J = 6.0, 19.9$ Hz, H-19'), 4.41 (1H, d, $J = 14.5$ Hz, H-14), 4.49 (1H, d, $J = 14.5$ Hz, H-14), 4.99 (1H, s, H-13), 5.04 (1H, s, H-13), 6.83 (1H, brd, $J = 4.5$ Hz, H-6), 6.75 (1H, brd, $J = 8.0$ Hz, H-21'), 6.85 (1H, s, H-25'), 6.86 (1H, brd, $J = 8.0$ Hz, H-22'), 7.67 (1H, t, $J = 6.0, 6.0$ Hz, 18'-NH); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 15.8 (C-9), 19.2 (C-11), 25.0 (C-8), 28.7 (C-2), 33.2 (C-3), 33.9 (C-1), 38.3 (C-10), 40.4 (C-5), 42.9 (C-4), 43.5 (C-19'), 51.3 (C-16), 55.3 (C-27'), 55.5 (C-26'), 64.8 (C-14), 110.7 (C-13), 111.1 (C-25'), 111.7 (C-22'), 119.0 (C-21'), 129.1 (C-7), 132.2 (C-20'), 142.5 (C-6), 147.3 (C-23'), 147.7 (C-24'), 148.6 (C-12), 156.0 (C-17'), 167.0 (C-15); (+)-LRESIMS m/z (rel. int.) 480 (100) $[\text{M} + \text{Na}]^+$, 458 (100) $[\text{M} + \text{H}]^+$; (+)-HRESIMS m/z 458.2551 ($\text{C}_{26}\text{H}_{36}\text{NO}_6$ $[\text{M} + \text{H}]^+$ requires 458.2537).

Compound Purity analysis

Compounds **1-19** were prepared at concentrations of 0.1 mg/100 μL in DMSO and injected (100 μL) onto a Phenomenex C₁₈ Onyx monolithic column. HPLC fractionation conditions consisted of a linear gradient (curve #6) from 90% H₂O (0.1% TFA)/10% CH₃OH (0.1% TFA) to 50% H₂O (0.1% TFA)/50% CH₃OH (0.1% TFA) in 3 min at a flowrate of 4 mL/min, a convex gradient (curve #5) to CH₃OH (0.1% TFA) in 3.50 min at a flowrate of 3 mL/min, held at CH₃OH (0.1% TFA) for 0.50 min

at a flowrate of 3 mL/min, held at CH₃OH (0.1% TFA) for a further 1.0 min at a flowrate of 4 mL/min, then a linear gradient (curve #6) back to 90% H₂O (0.1% TFA)/10% CH₃OH (0.1% TFA) in 1 min at a flowrate of 4 mL/min, then held at 90% H₂O (0.1% TFA)/10% CH₃OH (0.1% TFA) for 2 min at a flowrate of 4 mL/min. Total run time for each analytical injection was 11 min. Compound purity was determined by extracting each chromatogram at 210 nm and integrating all UV peaks. The percent purity and retention time for **1-19** were as follows: **1** (90%, 6.22 min), **2** (95%, 6.52 min), **3** (90%, 5.89 min), **4** (100%, 5.88 min), **5** (93%, 6.72 min), **6** (96%, 6.51 min), **7** (97%, 6.17 min), **8** (90%, 6.53 min), **9** (100%, 6.92 min), **10** (95%, 6.90 min), **11** (96%, 6.89 min), **12** (97%, 7.03 min), **13** (99%, 6.85 min), **14** (98%, 6.73 min), **15** (90%, 4.46 min), **16** (92%, 6.29 min), **17** (93%, 5.96 min), **18** (97%, 6.19 min) and **19** (90%, 6.16 min).

Biological Experiments

P. falciparum growth inhibition assay

As previously described, *P. falciparum* growth inhibition assays were carried out using an isotopic microtest.⁵⁰ Briefly, ring-stage infected erythrocytes (0.5% parasitemia and 2.5% hematocrit) were seeded into triplicate wells of 96 well tissue culture plates containing serial dilutions of control (chloroquine) or test compounds and incubated under standard *P. falciparum* culture conditions. After 48 h, 0.5 μCi [³H]-hypoxanthine was added to each well after which the plates were cultured for a further 24 h. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and [³H] incorporation determined using a 1450 MicroBeta liquid scintillation counter. Percentage inhibition of growth compared to matched DMSO controls (0.5%) was determined and IC₅₀ values were calculated using linear interpolation of inhibition curves.⁵¹ Chloroquine (Sigma Aldrich, C6628) was used as a positive control. The mean IC₅₀ ($\pm$ SD) was calculated over three independent experiments, each carried out in triplicate, and is shown in Table 1.

Cytotoxicity assays⁵²

Neonatal foreskin fibroblast (NFF) cells were cultured at 37 °C and 5% CO₂ in RPMI 1640 media (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS (CSL Biosciences, Parkville, Victoria, Australia) and 1% streptomycin (Life Technologies, Inc., Rockville, MD; complete medium). Cells were maintained in log phase growth before being seeded (3000/well) into 96 well tissue culture plates (Corning, USA). Treatment was undertaken after 24 h of growth. Compounds were dissolved in DMSO and diluted in complete medium; the DMSO concentration in the medium did not exceed 1%. An equivalent dose of DMSO was used to treat the control cells. The cells were washed with PBS and fixed in methylated spirits after three days of treatment. Total protein was determined using sulforhodamine B as described previously.⁵² Compounds were tested in triplicate in three independent experiments.

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Notes and references

1. J. L. Medina-Franco, K. Martinez-Mayorga, M. A. Giulianotti, R. A. Houghten and C. Pinilla, *Curr. Comput-Aid. Drug*, 2008, **4**, 322.
2. K. Grabowski, K.-L. Baringhaus and G. Schneider, *Nat. Prod. Rep.*, 2008, **25**, 892.
3. V. Hegde, M. Campitelli, R. J. Quinn and D. Camp, *Org. Biomol. Chem.*, 2011, **9**, 4570.
4. J. Hert, J. J. Irwin, C. Laggner, M. J. Keiser and B. K. Shoichet, *Nat. Chem. Biol.*, 2009, **5**, 479.
5. J. Rosen, J. Gottfries, S. Muresan, A. Backlund and T. I. Oprea, *J. Med. Chem.*, 2009, **52**, 1953.
6. P. Ertl, S. Roggo and A. Schuffenhauer, *J. Chem. Inf. Model.*, 2008, **48**, 68.
7. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2007, **70**, 461.
8. D. J. Newman, *J. Med. Chem.*, 2008, **51**, 2589.
9. D. J. Newman and G. M. Cragg, *Future Med. Chem.*, 2009, **1**, 1415.
10. G. M. Cragg, P. G. Grothaus and D. J. Newman, *Chem. Rev.*, 2009, **109**, 3012.
11. G. M. Cragg and D. J. Newman, *Phytochem. Rev.*, 2009, **8**, 313.
12. A. L. Harvey, *Drug Discov. Today*, 2008, **13**, 894.
13. E. Kellenberger, A. Hofmann and R. J. Quinn, *Nat. Prod. Rep.*, 2011, **28**, 1483.
14. B. M. McArdle and R. J. Quinn, *ChemBioChem*, 2007, **8**, 788.
15. K. Kumar and H. Waldmann, *Angew. Chem. Int. Ed.*, 2009, **48**, 3224.
16. J. P. Nandy, M. Prakesch, S. Khadem, P. T. Reddy, U. Sharma and P. Arya, *Chem. Rev.*, 2009, **109**, 1999.
17. R. A. Davis, A. R. Carroll and R. J. Quinn, *Aust. J. Chem.*, 2001, **54**, 355.
18. R. A. Davis, G. K. Pierens and P. G. Parsons, *Magn. Reson. Chem.*, 2007, **45**, 442.
19. D. Camp, R. A. Davis, M. Campitelli, J. Ebdon and R. J. Quinn, *J. Nat. Prod.*, 2011, accepted 28 Nov 2011.
20. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3.
21. S. K. Bhal, K. Kassam, I. G. Peirson and G. M. Pearl, *Mol. Pharm.*, 2007, **4**, 556.
22. J. W.-H. Li and J. C. Vederas, *Science*, 2009, **325**, 161.
23. P. Seneci, C. Sizemore, K. Islam and P. Kocis, *Tetrahedron Lett.*, 1996, **37**, 6319.
24. A. Atuegbu, D. Maclean, C. Nguyen, E. M. Gordon and J. W. Jacobs, *Bioorg. Med. Chem.*, 1996, **4**, 1097.
25. M. Feher and J. M. Schmidt, *J. Chem. Inf. Comput. Sci.*, 2003, **43**, 218.
26. T. M. Gierasch, Z. Shi and G. L. Verdine, *Org. Lett.*, 2003, **5**, 621.
27. E. L. Ghisalberti, P. R. Jefferies, B. W. Skelton, A. H. White and R. S. F. Williams, *Tetrahedron*, 1989, **45**, 6297.
28. E. C. Barnes, A. R. Carroll and R. A. Davis, *J. Nat. Prod.*, 2011, **74**, 1888.
29. M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki and S. Tani, *Tetrahedron*, 1999, **55**, 13159.
30. S. Van Hoof, C. J. Lacey, R. C. Rohrich, J. Wiesner, H. Jomaa and S. Van Calenbergh, *J. Org. Chem.*, 2008, **73**, 1365.
31. Y. Tamaru and M. Kimura, *Pure Appl. Chem.*, 2008, **80**, 979.
32. A. J. Chalk and S. A. Magennis, *J. Org. Chem.*, 1976, **41**, 1206.
33. R. J. Chinnock, *Eremophila and Allied Genera*, Rosenberg Publishing, Dural, NSW, 2007.
34. D. N. Leach, R. N. Spooner-Hart and G. F. Eaton, World Patent WO2004/021784, 2004.
35. G. O. Maupin, J. Karchesy, N. A. Panella and M. C. Dolan, World Patent WO2002/050053, 2002.
36. R. W. Kerr, 1951, Bulletin No. 261. CSIRO: Melbourne.
37. K. D. Beattie, P. G. Waterman, P. I. Forster, D. R. Thompson and D. N. Leach, *Phytochemistry*, 2011, **72**, 400.
38. J. M. Wilkinson and H. M. A. Cavanagh, *Phytother. Res.*, 2005, **19**, 643.
39. Sigma-Aldrich, Castle Hill, NSW; www.sigmaaldrich.com/chemistry/chemistry.
40. Reactor, ChemAxon, version 5.3.1, 2011; www.chemaxon.com/products/reactor.
41. Instant JChem, ChemAxon, version 5.5, 2011; www.chemaxon.com/products/instant-jchem.
42. T. Itoh, T. Taguchi, M. R. Kimberley, K. I. Booker-Milburn, G. R. Stephenson, Y. Ebizuka and K. Ichinose, *Biochemistry*, 2007, **46**, 8181.
43. M. Nakamura, M. Toganoh, H. Ohara and E. Nakamura, *Org. Lett.*, 1999, **1**, 7.
44. D. S. Matteson, R. Soundararajan, O. C. Ho and W. Gatzweiler, *Organometallics*, 1996, **15**, 152.
45. Y. Zhao, C. Mao, Y. Li, P. Zhang, Z. Huang, F. Bi, R. Huang and Q. Wang, *J. Agric. Food. Chem.*, 2008, **56**, 7326.
46. L. Nahar, S. D. Sarker, P. K. Li and A. B. Turner, *Chem. Nat. Cmpd.*, 2004, **40**, 50.
47. I. S. Blagbrough, N. E. Mackenzie, C. Ortiz and A. I. Scott, *Tetrahedron Lett.*, 1986, **27**, 1251.
48. J. Garfinkle, F. Scott Kimball, J. D. Trzupek, S. Takizawa, H. Shimamura, M. Tomishima and D. L. Boger, *J. Am. Chem. Soc.*, 2009, **131**, 16036.
49. A. Hofmann, C. Wang, A. Osman and D. Camp, *Struct. Chem.*, 2010, **21**, 1117.
50. K. T. Andrews, A. Walduck, M. J. Kelso, D. P. Fairlie, A. Saul and P. G. Parsons, *Int. J. Parasitol.*, 2000, **30**, 761.
51. W. Huber and J. C. Koella, *Acta Trop.*, 1993, **55**, 257.
52. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.