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Synthesis and antibacterial activity of some binaphthyl-supported macrocycles containing a cationic amino acid

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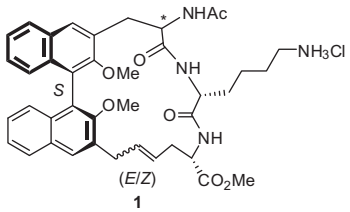
ABSTRACT

As part of a programme investigating antibacterial cyclic macrocycles containing a cationic amino acid with an internal aromatic hydrophobic scaffold, we previously reported a macrocycle anchored at the 3,3′-positions of a 1,1′-binaphthyl unit. This was prepared via key intermediates containing an internal allylglycine and an allyl-substituted binaphthyl unit for a subsequent ring-closing metathesis reaction. This paper presents some structure–activity relationship studies with additional macrocycles based on this lead compound against *Staphylococcus aureus* together with the antibacterial activity of two related acyclic compounds.

1. Introduction

Major health care issues are currently posed by multidrug resistant human pathogenic bacteria.[1,2] The emergence of Gram-positive bacteria, for example, *Staphylococcus aureus* and *Enterococcus faecium*, resistant to the glycopeptide antibiotic, vancomycin, is of particular concern.[3–5] Some recent approaches aimed at overcoming this resistance have involved the development of other novel, high molecular weight vancomycin derivatives such as oritavancin[6] or vancomycin dimers with a rigid actinocin linking group.[7] An alternative approach is to design simpler cationic biaryl-templated amino acid monomacrocycles with some similarity to structural aspects of vancomycin. Such compounds could still potentially act in a similar way to vancomycin but have added flexibility for interacting strongly with the changed peptido-glycan cell wall moiety[5] in vancomycin-resistant bacteria. In this context, the recent report of significant antimicrobial activity in some biaryl-based cyclic β-hairpin cationic peptidomimetics is of interest.[8] We instigated a programme investigating the synthesis of antibacterial agents using some structural elements of vancomycin, but dramatically simplifying the hydrophobic scaffold to core moieties that support a tripeptide unit in a defined orientation. We have previously reported cationic amino acid containing macrocycles anchored by hydrophobic scaffolds based on 3,3′-amino acid linked 1,1′-binaphthyls,[9] carbazoles,[10,11] indoles,[12] benzenes[13] and benzo[*b*] thiophene.[14]

While numerous derivatives showed antibacterial activity, at the time, none matched the activity shown by the originally reported binaphthyl-based macrocycle **1** (Fig. 1).[9] Although **1** was likely to possess singular stereogenicity at C*, its configuration was not known and further, **1** was likely to be a mixture of *E/Z* isomers. Therefore, we embarked on a structure–activity relationship (SAR) study based on **1** in an attempt to improve the antibacterial activity of this new class of cyclic compounds.



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MIC 17 μg/mL against *S. aureus*

Figure 1. The lead cyclic binaphthyl macrocycle antibacterial agent. The MIC reported (17 μg/mL) was an average while the median value was 16 μg/mL.

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2. Results and discussion

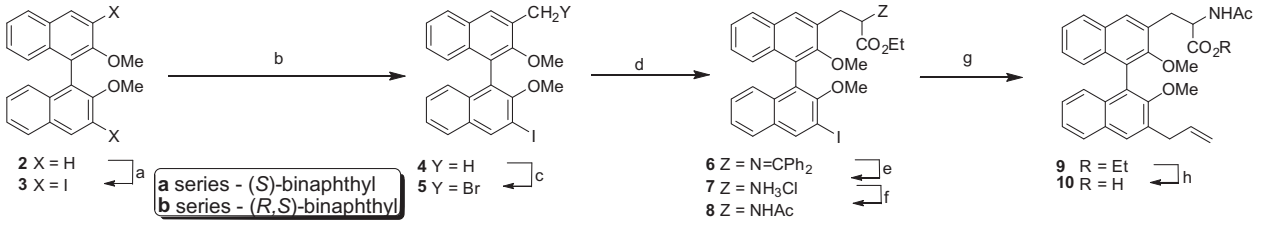
All our target compounds required the synthesis of the binaphthyl scaffold **10** (Scheme 1) which contained olefinic and carboxylic acid moieties to enable incorporation of different, suitably protected dipeptide units and eventual transformation to our cyclic target compounds (Fig. 2).[15]

We did not systematically vary individual features, instead choosing a range of variations including the use of racemic and (*R*)- and (*S*)-binaphthyls, and different basic amino acid side chains. The incorporation of an allylglycine facilitated a ring-closing metathesis (RCM) reaction and the inclusion of basic amino acid side chains, either a lysine or arginine unit, formed part of our design concept. These cationic side chains are thought to undergo an ionic interaction with the D-Ala-D-Ala(Lac) moieties of the growing bacterial cell wall. Most of our derivatives started with the racemic binaphthyl; however, the (*S*)-enantiomer was synthesized in an analogous manner using the same chemistry.[9]

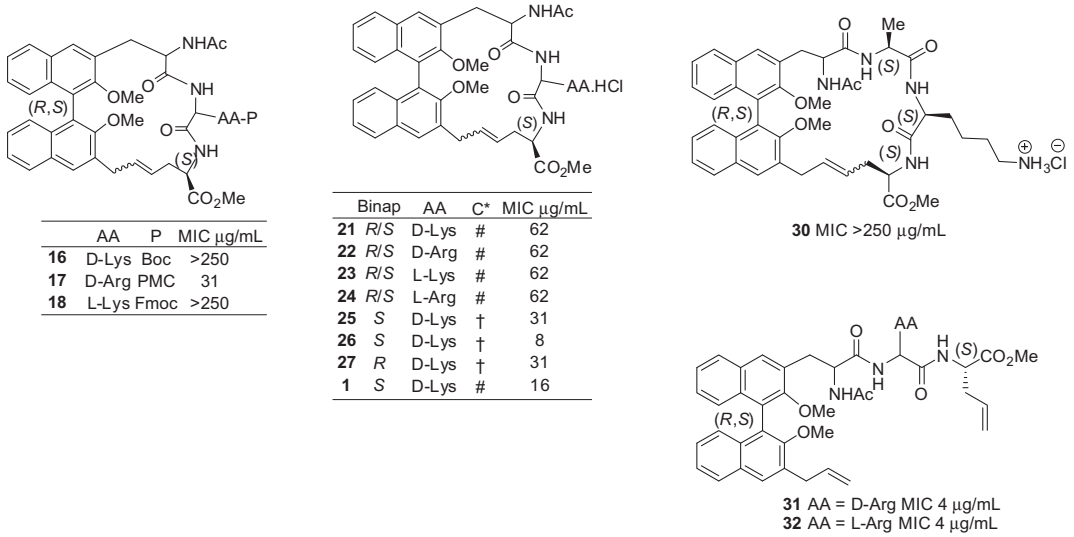
Therefore, the synthesis of (*S*)-**10a**[9] and (*R,S*)-**10b** (Scheme 1) started with either (*S*)-**2a** or the (*R,S*)-**2b**, respectively, following our previously reported procedure for **10a**.[9]

With the scaffolds **10a** and **10b** in hand,[16] we turned our attention to the different dipeptides available. Treatment of **10b** with dipeptides under standard diimide peptide coupling conditions gave the protected acyclic derivatives **11**–**14** (Scheme 2) in satisfactory yields[17]—the corresponding single enantiomer (*S*)-**15** was made starting from **10a**.[9] Subsequent RCM reactions with Grubbs' I ruthenium catalyst in CH_2Cl_2 at reflux produced the macrocycles **16**–**20**,[18] as a mixture of *E/Z* isomers; the assignment of the ratio of the diastereomers could not be determined due to the overlapping broad peaks assigned to the olefinic protons. Deprotection of the amino acid side chains under standard conditions, followed by treatment with HCl in ether gave the binaphthyl-supported products **21**–**24** and **1**[9] as their hydrochlorides.[19]

In a separate experiment, a sample of **16** was subjected to silica gel column chromatography, giving rise to three separate diastereomers (Scheme 3). The chirality of the axis of these isomers was determined by CD spectroscopy, and compared with the independently synthesised macrocycle containing the (*S*)-binaphthyl isomer **20**[9] (from **10a** via **15**). This indicated the presence of two (*S*)-binaphthyl diastereoisomers, **25** and **26**, and one (*R*)-binaphthyl diastereomer **27**. Unfortunately, a second (*R*)-binaphthyl diastereomer produced could not be isolated pure. The two (*S*)-binaphthyl stereoisomers could differ either at the stereogenic C_α carbon β to the naphthyl moiety, or the olefingeometry. Although the assignment of complete configuration was not possible, we had likely refined the (*S*)-binaphthyl isomers down to single stereoisomers. Further evidence suggesting that these two (*S*)-binaphthyl isomers might be diastereomers comes from the biological testing as they show different antibacterial activities; for example, compare **1** (MIC 16 μg/mL) with **26** (MIC 8 μg/mL) (Fig. 2). Further attempts to separate these diastereomers using silica gel HPLC



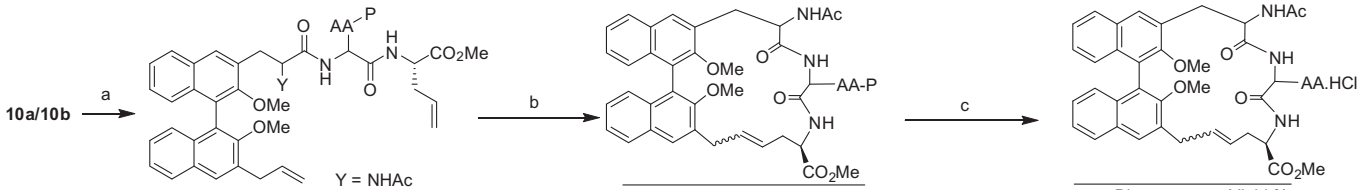
Scheme 1. Reagents and conditions: (a) *n*-BuLi, TMEDA, Et_2O, rt, 3 h, then I_2, −80 °C → rt, overnight, 51% ((*R,S*)-binap), 65% ((*S*)-binap); (b) *n*-BuLi, THF, −80 °C, 1 h, then MeI, −80 °C → rt, 3 h, 51% ((*R,S*)-binap), 48% ((*S*)-binap); (c) NBS, CCl_4, reflux, then irradiation with a mercury lamp, 1 h, 46% ((*R,S*)-binap), 62% ((*S*)-binap); (d) HMPA, DIPA, *n*-BuLi, THF, −10 °C, 10 min, then *N*-(diphenylmethylene)glycinate, then addition of **5**, THF, −78 °C → rt, overnight; (e) 3% HCl, Et_2O, rt, overnight; (f) $MgSO_4$, CH_2Cl_2, 0 °C, then Et_3N, Ac_2O, DMAP, 0 °C → rt, overnight, 75% ((*R,S*)-binap, three steps), 71% ((*S*)-binap, three steps); (g) $PdCl_2$, PPh_3, allyltributyltin, dioxane, reflux, 5 h, 85% ((*R,S*)-binap), 89% ((*S*)-binap); (h) LiOH·H_2O, H_2O, THF, rt, 5 h, 94% ((*R,S*)-binap), 62% ((*S*)-binap[9]).



	AA	P	MIC μg/mL
16	D-Lys	Boc	>250
17	D-Arg	PMC	31
18	L-Lys	Fmoc	>250

	Binap	AA	C*	MIC μg/mL
21	*R/S*	D-Lys	#	62
22	*R/S*	D-Arg	#	62
23	*R/S*	L-Lys	#	62
24	*R/S*	L-Arg	#	62
25	*S*	D-Lys	†	31
26	*S*	D-Lys	†	8
27	*R*	D-Lys	†	31
1	*S*	D-Lys	#	16

Figure 2. In vitro antibacterial activity of compounds against *S. aureus*. Vancomycin was used as the standard (MIC 2.0 μg/mL). Values quoted are the median based on triplicate measurements. AA refers to the amino acid side chain as listed. #Analogues contain a mixture of stereoisomers at C*. †Analogues are presumed to be stereochemically pure at C* but the absolute stereochemistry could not be established.

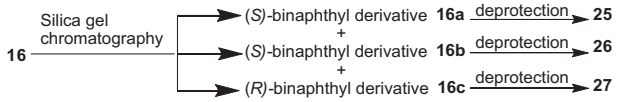


	Binap	AA	P	Yield %
11	*R/S*	D-Lys	Boc	92
12	*R/S*	D-Arg	PMC	66
13	*R/S*	L-Lys	Fmoc	54
14	*R/S*	L-Arg	PMC	68
15	*S*	D-Lys	Boc	49^

	Binap	AA	P	Yield %
16	*R/S*	D-Lys	Boc	74
17	*R/S*	D-Arg	PMC	75
18	*R/S*	L-Lys	Fmoc	72
19	*R/S*	L-Arg	PMC	77
20	*S*	D-Lys	Boc	72^

	Binap	AA	Yield %
21	*R/S*	D-Lys	60#
22	*R/S*	D-Arg	89
23	*R/S*	L-Lys	51
24	*R/S*	L-Arg	58
1	*S*	D-Lys	84^

Scheme 2. Reagents and conditions: (a) $NH_2CH(AA\text{-}P)CO\text{-}L\text{-}allylGly\text{-}OMe$, DCC, DMAP, rt, overnight; (b) benzylidine-bis(tricyclohexylphosphine)-dichlororuthenium (Grubbs' I catalyst), CH_2Cl_2, reflux, 18–20 h; (c) for Boc and Pmc (2,2,5,7,8-pentamethylchromane) protected compounds: TFA, CH_2Cl_2, rt, 25 min, then 1 M HCl–Et_2O; for Fmoc protected compound: 0.02 M piperidine in CH_3CN, 60 °C, 43 h, then 1 M HCl–Et_2O. AA refers to the amino acid side chain as listed. ^Synthesis previously reported, see Ref. 9. #Compound **21** was synthesized from an Fmoc protected D-Lys in a similar sequence to **23**—the yield reflects the outcome of the Fmoc deprotection.



Scheme 3. The separation of diastereomers of cyclic peptide **16** based upon the chirality of the binaphthyl scaffold and their subsequent deprotection.

were not successful, being hampered by the formation of molecular aggregates.

Further, the binaphthyl **10b** was treated with a protected Gly-Ala-Lys-tripeptide ester to give the acyclic structure **28** which was subsequently cyclised using typical Grubbs' RCM conditions, yielding the larger macrocycle **29** (Scheme 4). This was then deprotected giving the hydrochloride **30**, and thus access to the larger macrocycle containing four amino acid units.[20]

The antibacterial activities for the synthesised compounds against *S. aureus* (wild type; ATCC 6538P) are shown in Figure 2. Included are the five analogues **21**, **25**–**27** and **1**, which differ only in their stereochemical elements. The (*S*)-binaphthyl derivative **26** was the most active cyclic derivative synthesised (MIC 8 μg/mL) with the other (*S*)-binaphthyl derivative **1** (MIC 16 μg/mL) and a corresponding (*R*)-binaphthyl derivative **27** (MIC 31 μg/mL) decreasing in activity. Interestingly, the independently synthesised (*S*)-binaphthyl derivative **1**, which was presumably a diastereomeric mixture of **25** and **26**, had an MIC value between that for **25** and **26** (MIC 16 μg/mL). Finally the more complex mixture of diastereomers (**21**),[21] showed weaker activity (MIC 62 μg/mL) again. Although the overall effects observed here may be due to differences in molecular aggregation, an effect originally observed in associated HPLC studies, and/or antagonist effects between diastereomers, it is likely that differences in activity arise from the different configurations of the isomers.

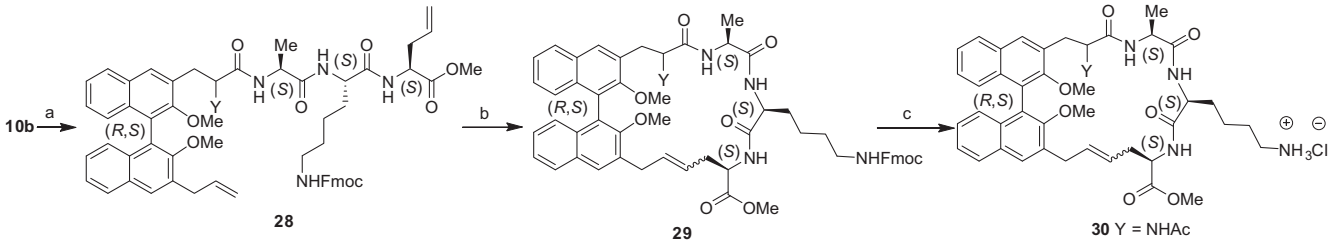
The protected macrocycles **16** and **18** showed no activity whereas the PMC-protected **17** surprisingly revealed moderate activity (MIC 31 μg/mL). The larger macrocycle containing four amino acid residues **30** was inactive (MIC >250 μg/mL). We have also previously reported[19,20] the activity of the macrocycle **1** with the double bond reduced to the corresponding 'alkane' linker, this molecule revealed an MIC value of 16 μg/mL, suggesting that the presence of the double bond in either form (*E* or *Z*) is not relevant.

As part of the SAR studies we also assessed the activity of an acyclic analogue. This analogue, **31** (Fig. 2), which was readily accessed from the synthetic intermediate **12** by acid catalysed cleavage of the PMC group, displayed excellent activity against *S. aureus* (MIC 4 μg/mL). The activity of **31** was close to that of vancomycin (MIC 2 μg/mL in this test), as was that of the L-Arg congener **32**, obtained from deprotection of the intermediate **14**. While these were surprising results at the time, subsequent studies by us revealed[22,23] that related acyclic peptides anchored with a binaphthyl unit at the 2′ position possess significant activity against a range of Gram-positive bacteria.

In conclusion, we have assessed the antibacterial activity against *S. aureus* of 12 different macrocyclic derivatives, including **1**, incorporating a cationic amino acid moiety and a hydrophobic binaphthyl scaffold. While some examples showed notable activity, the D-Lys-analogue **26**, the diastereomer of the original lead compound **1**, showed an increased activity. Importantly, this work also revealed a new acyclic lead compound, **31**, with strong antibacterial activity. The general principles defined by this latter molecular structure as an antibacterial agent have been subsequently explored by us[22,23] and other related work will be reported in future papers.

3. Experimental

General methods were as described previously.[23] All NMR spectra were determined in $CDCl_3$ solution at 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR).



Scheme 4. Reagents and conditions: (a) $NH_2\text{-}L\text{-}Ala\text{-}L\text{-}(N_\omega\text{-}Fmoc)Lys\text{-}L\text{-}allylGly\text{-}OMe$, DCC, DMAP, rt, overnight, 39%; (b) benzylidine-bis(tricyclohexylphosphine)dichlororuthenium, CH_2Cl_2, reflux, 15 h, quantitative; (c) 0.02 M piperidine in CH_3CN, DMF, 70 °C, 12 h, then 1 M HCl–Et_2O, 19%.

3.1. Synthesis of the hydrophobic scaffold 10b

3.1.1. (*R*,*S*)-2,2′-Dimethoxy-1,1′-binaphthyl 2b

This was synthesized as previously reported.[20]

3.1.2. (*S*)-2,2′-Dimethoxy-1,1′-binaphthyl 2a[9]

This was prepared in the same manner as **2b** above (95%); $[\alpha]_D^{23}$ −53.1 (*c* 1.2, $CDCl_3$), CD ($CHCl_3$) 235 nm + 100 mdeg (*c* 0.08 mg/10 mL).

3.1.3. (*R*,*S*)-3,3′-Diiodo-2,2′-dimethoxy-1,1′-binaphthyl 3b

This was synthesized as previously reported.[20]

3.1.4. (*S*)-3,3′-Diiodo-2,2′-dimethoxy-1,1′-binaphthyl 3a[9]

This was prepared in the same manner as **3b** above (65%); $[\alpha]_D^{23}$ +29.8 (*c* 1.2, $CDCl_3$), CD ($CHCl_3$) 245 nm + 100 mdeg (*c* 0.09 mg/10 mL).[9]

3.1.5. (*R*,*S*)-2,2′-Dimethoxy-3-iodo-3′-methyl-1,1′-binaphthyl 4b

To a −80 °C solution of **3b** (2.016 g, 3.6 mmol) in dry THF (80 mL, 0.044 M) was added *n*-butyllithium (2.8 mL, 3.9 mmol) dropwise. The reaction mixture turned yellow and was stirred for 1 h before dry methyl iodide (0.35 mL, 5.6 mmol) was added. The reaction mixture was allowed to gradually warm to rt and stirred for a further 3 h before saturated aqueous ammonium chloride solution (four drops) was added. The reaction mixture was evaporated to dryness, the residue taken up in diethyl ether and washed with water. The organic layer was dried ($MgSO_4$), and the filtrate evaporated to dryness to give a pale yellow solid. Purification of the crude material by flash column chromatography (4% ethyl acetate/hexane) yielded **4b** (0.678 g, 42%). TLC (10% ethyl acetate/hexane), R_f of 0.54; ^{1}H NMR δ 2.55, s, 3H; 3.36, s, 6H; 7.06, d, *J* = 8.0 Hz, 1H; 7.12, d, *J* = 8.0 Hz, 1H; 7.16–7.42, m, 2H; 7.80, t, *J* = 8.0 Hz, 2H; 7.81, s, 3H; 8.52, s, 1H. ^{13}C NMR δ 17.2, 60.2, 60.9, 92.6, 124.0, 124.7, 125.4 (×2), 125.5, 126.0, 126.7, 126.8, 127.2, 130.1, 130.6, 131.5, 132.1, 132.7, 134.1, 139.3, 154.4, 155.4. *m/z* (CI, +ve) 455 ($M+H^+$, 62), 329 ($[455–I]^{+\cdot}$, 100), 313 (57) and 185 (62). $C_{23}H_{20}O_2I$ + H^+ requires *m/z* 455.0508, Found 455.0505.

3.1.6. (*S*)-3-Iodo-2,2′-dimethoxy-3′-methyl-1,1′-binaphthyl 4a

This was prepared in the same manner as **4b** above (28%); $[\alpha]_D^{23}$ +39.5 (*c* 1.4, $CHCl_3$), CD ($CHCl_3$) 237 nm + 29 mdeg (*c* 0.11 mg/10 mL).

3.1.7. (*R*,*S*)-3-Bromomethyl-3′-iodo-2,2′-dimethoxy-1,1′-binaphthyl 5b

To a solution of **4b** (1.737 g, 3.8 mmol) in carbon tetrachloride (100 mL) was added *N*-bromosuccinimide (1.316 g, 7.4 mmol). The mixture was heated to reflux followed by irradiation with a 500 W mercury lamp for 1.5 h. The cooled reaction mixture was filtered (to remove succinimide), and the filtrate evaporated to dryness to give a red solid. The residue was purified by flash column chromatography (4% ethyl acetate/hexane) to yield a colourless crystalline solid (1.130 g) that was a mixture from which **5b** (0.942 g, 46%) was isolated by column chromatography using the same conditions. ^{1}H NMR δ 3.36, s, 3H; 3.40, s, 3H; 4.72 d, *J* = 9.9 Hz, 1H; 4.91, d, *J* = 9.9 Hz, 1H; 7.08, d, *J* = 8.7 Hz, 1H; 7.18, d, *J* = 8.7 Hz, 1H; 7.23–7.44, m, 4H; 7.80, d, *J* = 8.4 Hz, 1H; 7.88, d, *J* = 8.4 Hz, 1H; 8.07, s, 1H; 8.54, s, 1H. ^{13}C NMR ($CDCl_3$) δ 29.3; 61.0; 61.4; 92.5; 124.4; 125.1; 125.3; 125.6; 125.9; 126.8; 127.0; 127.1; 128.0; 130.2; 131.4; 132.1; 132.2; 133.9; 134.1; 139.7; 154.38; 154.44; *m/z* (CI, +ve) 535 (94) & 533 (M+H, 92), 455 (74), 453 (100), 409 (14), 407 (27), 405 (18), 329 (24) and 313 (14).

3.1.8. (*S*)-3-Bromomethyl-3′-iodo-2,2′-dimethoxy-1,1′-binaphthyl 5a

This was prepared in the same manner as **5b** above (62%).

3.1.9. Ethyl (*R*,*S*)-3-[3-(3′-iodo-2,2′-dimethoxy-1,1′-binaphthyl)]-2-[(diphenylmethylene)amino]propanoate 6b

To a −10 °C solution of HMPA (0.7 mL, 4.02 mmol) and diisopropylamine (0.24 mL, 1.71 mmol) in dry THF (40 mL) was added *n*-butyllithium (1.32 M in hexanes, 1.4 mL, 1.85 mmol). The pale yellow solution was stirred for 10 min then cooled to −78 °C. To this solution was added a solution of ethyl *N*-(diphenylmethylene)glycinate (0.450 g, 1.68 mmol) in dry THF (20 mL) using a cannula and precooling the addition solution by running the drops down the side of the receiving flask. The resulting mixture was stirred for 30 min then a solution of the binaphthyl derivative **5b** (0.895 g, 1.68 mmol) in dry THF (40 mL) was added using a cannula. The reaction mixture was allowed to slowly warm to rt and stirred overnight. The yellow solution was quenched with aqueous ammonium chloride solution (1 mL). The reaction mixture was evaporated to dryness to give a yellow oil that contained **6b** which was used without further purification. TLC (10% ethyl acetate/hexane), R_f 0.15; ^{1}H NMR δ 1.21, t, *J* = 5.0 Hz, 3H; 3.00, s, 3H; 3.20, s, 3H; 3.40, dd, *J* = 7.0, 10.0 Hz, 1H; 3.71, dd, *J* = 3.0, 10.0 Hz, 1H; 4.11–4.22, m, 2H; 4.50, q, *J* = 3.0 Hz, 1H; 6.82, d, *J* = 5.0 Hz, 1H; 7.78–6.98, m, 19H; 8.49, s, 1H; *m/z* (CI, +ve) 720 ($M+H^+$, 52%), 674 (9), 672 (8), 648 (17), 608 (8), 556 (27) and 268.

3.1.10. Ethyl (*S*)-3-[3-(3′-iodo-2,2′-dimethoxy-1,1′-binaphthyl)]-2-[(diphenylmethylene)amino]propanoate 6a

This was prepared in the same manner as **6b** above and was used without isolation.

3.1.11. Ethyl (*R*,*S*)-2-amino-3-[3-(2,2′-dimethoxy-3′-iodo-1,1′-binaphthyl)]propanoate hydrochloride salt 7b

To a solution of the alkylated product **6b** (1.208 g, 1.68 mmol) in diethyl ether (30 mL) was added HCl (15 mL, 3% aqueous, 4.6 mmol) and the mixture was stirred at rt overnight giving a yellow oil underneath the aqueous layer and a yellow organic layer. The mixture was evaporated to dryness, the sticky yellow residue was taken up in ethanol and evaporated to dryness. This was repeated twice. The final residue was dried under high vacuum then freeze dried. The crude product was used without further purification. ^{13}C NMR δ 13.8, 13.9, 33.0, 33.6, 53.2, 53.7, 61.0, 61.1, 61.2, 62.3, 92.3, 117.4, 124.1, 124.2, 125.1, 125.3, 125.5, 125.6, 125.8, 126.0, 126.2, 126.5, 126.70, 126.75, 126.9, 127.05, 127.65, 127.85, 128.2, 130.0, 130.4, 131.3, 132.0, 132.1, 132.4, 132.8, 132.9, 133.3, 134.8, 133.9, 134.1, 139.7 (×2), 152.5, 154.4, 154.7, 154.9, 168.6, 169.0. $C_{27}H_2NO_4I_2$ + H^+ (ammonium ion) requires *m/z* 556.0985, Found 556.0991.

3.1.12. Ethyl (*S*)-2-amino-3-[3-(3′-iodo-2,2′-dimethoxy-1,1′-binaphthyl)]propanoate hydrochloride salt 7a

This was prepared in the same manner as **7b** above and was used directly in the next step.

3.1.13. Ethyl (R_a,S_a)-2(*R*,*S*)-acetamino-3-[3-(2,2′-dimethoxy-3′-iodo-1,1′-binaphthyl)]propanoate 8b

The residue obtained after acid hydrolysis of **6b** containing the binaphthyl salt **7b** (0.993 g, 1.68 mmol) was dissolved in CH_2Cl_2 (dry, distiled, 100 mL). The solution was stirred with $MgSO_4$ (anhydrous) briefly then cooled in an ice/salt bath. Triethylamine (0.70 mL, 5.02 mmol) was added, followed by acetic anhydride (0.4 mL, 4.24 mmol) and DMAP, after stirring for 5 min. The reaction mixture was allowed to warm to rt and was stirred overnight. To the reaction mixture was added a solution of HCl (50 mL, aqueous 3%) and CH_2Cl_2 (50 mL). The aqueous layer was removed and

extracted with CH_2Cl_2. The combined organic layers were washed with a solution of 3% aqueous hydrochloric acid (1×), a solution of 1:1 aqueous saturated LiCl (2×) then water (×1). The solution was dried ($MgSO_4$) and the filtrate evaporated to dryness to give a yellow liquid. The crude product was purified by (short) column chromatography (10% ethyl acetate/hexane → ethyl acetate) to yield **8b** as a pale yellow solid (0.75 g, 75% from **5b**) (average 91% yield per step). TLC (25% ethyl acetate/hexane), R_f of 0.06; ^{1}H NMR δ 1.25, t, J = 7.0 Hz, 3H; 1.98, s, 3H; 3.26, s, 3H; 3.28, s, 3H; 3.30–3.42, m, 2H; 4.23, m, 2H; 4.73, q, J = 7.0 Hz, 1H; 6.79, d, J = 7.0 Hz, 1H; 7.05–7.82, m 7H; 7.86, s, 1H; 8.54, s, 1H; ^{13}C NMR[24] δ 14.2, 22.7, 22.9, 32.8, 33.0, 34.9, 54.2, 54.8, 60.3, 60.5, 60.6, 60.7, 60.8, 61.1, 61.2, 116.0, 116.1, 123.7, 124.1, 124.5, 124.6, 124.7, 124.9, 125.3, 125.5, 125.6, 126.0, 126.1, 127.4, 127.5, 129.2, 129.3, 129.4, 129.7, 130.2, 130.3, 130.40, 130.45, 130.5, 132.8, 133.0, 133.3, 133.4, 133.6, 136.7, 154.7, 154.7, 155.1, 155.3, 170.0, 170.1, 171.2, 171.7; m/z 598 (M+H$^+$, 35%), 552 (13), 550 (12), 472 (100), 328 (20), 85 (79).

3.1.14. Ethyl (S_a)-2(*R*,*S*)-acetamino-3-[3-(2,2′-dimethoxy-3′-iodo-1,1′-binaphthyl)]propanoate 8a

This was prepared in the same manner as **8b** above (71% over three steps).

3.1.15. Ethyl (R_a,S_a)-2(*R*,*S*)-acetylamino-3-[3-(3′-allyl-2,2′-dimethoxy-1,1′-binaphthyl)]propanoate 9b

To a solution of the binaphthyl derivative **8b** (0.740 g, 1.24 mmol) in 1,4-dioxane (dry, 40 mL) was added palladium chloride (0.025 g, 0.14 mmol) and triphenylphosphine (0.136 g, 0.52 mmol). The solution was deoxygenated with argon for 10 min then allyltributyltin (0.39 mL, 1.26 mmol) was added. The resulting mixture was heated at reflux for 5 h. After cooling the solution was filtered through celite and evaporated to dryness. The residue was purified by (short) column chromatography, to remove stannanes, then flash column chromatography (50% ethyl acetate/hexane) to give **9b** as a colourless oil (0.54 g, 85%). ^{1}H NMR δ 0.92, t, J = 7.2 Hz, 3H; 1.26, t, J = 7.2 Hz, 3H; 1.98, s, 3H; 2.04, s, 3H; 3.17, s, 3H; 3.24, s, 3H; 3.25–3.33, m, 2H; 3.58–3.72, br m, 2H; 4.12, q, J = 7.3 Hz, 2H; 4.21, q, J = 7.1 Hz, 2H; 4.71, q, J = 7.2 Hz, 1H; 4.84, q, J = 7.0 Hz, 1H; 5.13–5.19, m, 2H; 6.08–6.22, m, 1H; 6.78, d, J = 7.2 Hz, 1H; 6.91, d, J = 7.2 Hz, 1H; 7.14–7.26, m, 4H; 7.35–7.46, m, 2H; 7.81–7.85, m, 4H; ^{13}C NMR[24] δ 14.2; 22.7; 22.9; 32.8; 33.0; 34.9; 54.2; 54.8; 60.3; 60.5; 60.5; 60.7; 60.8; 61.1; 61.2; 116.0; 116.0; 123.7; 124.1; 124.5; 124.6; 124.6; 124.9; 125.3; 125.5; 125.6; 126.0; 126.1; 127.4; 127.4; 129.2; 129.3; 129.3; 129.7; 130.2; 130.3; 130.4; 130.4; 130.5; 132.8; 133.0; 133.3; 133.3; 133.6; 136.7; 154.7; 154.7; 155.1; 155.3; 170.0; 170.1; 171.2; 171.7. m/z (CI, +ve) 512 (100%). $C_{32}H_{33}NO_5$ + H$^+$ requires m/z 512.2437. Found 512.2452.

3.1.16. Ethyl (S_a)-2(*R*,*S*)-acetylamino-3-[3-(3′-allyl-2,2′-dimethoxy-1,1′-binaphthyl)]propanoate 9a

This was prepared in the same manner as **9b** above (75% over the three steps from **6b**).

3.1.17. (R_a,S_a)-2(*R*,*S*)-Acetylamino-3-[3-(3′-allyl-2,2′-dimethoxy-1,1′-binaphthyl)]propanoic acid 10b

To a solution of the binaphthyl derivative **9b** (0.522 g, 1.02 mmol) in THF (22 mL) in an ice/water bath was added a solution of LiOH (0.196 g, 4.67 mmol) in water (9 mL). The mixture was allowed to gradually warm to rt and was stirred for 5 h. To the reaction mixture was added diethyl ether, the aqueous layer was washed with diethyl ether and the combined diethyl ether layers extracted with water (2×). The combined aqueous layer was acidified (3% aqueous HCl), extracted with diethyl ether (3×) and dried ($MgSO_4$). The filtrate was evaporated to dryness to give **10b** as a white solid (0.462 g, 94%). TLC (ethyl acetate) R_f of 0.05; ^{1}H NMR δ 2.07, s, 3H; 3.07, s, 3H; 3.20, s, 3H; 3.28–3.77, m, 4H; 4.55, 2 × t, J = 5.0 Hz, 1H; 5.11–5.19, m, 2H; 6.07–6.20, m, 1H; 7.16–7.56, m, 6H; 7.74, br s, 1H; 7.84, d, J = 8.0 Hz, 1H; 7.88, s, 4H. ^{13}C NMR[24] δ 15.2; 22.5; 22.7; 25.5; 32.2; 34.9; 55.3; 60.4; 60.6; 60.7; 60.8; 60.9; 65.8; 67.8; 116.0; 116.1; 123.8; 124.3; 124.55; 124.59; 124.7; 124.9; 125.3; 125.5; 125.67; 125.75; 126.0; 126.2; 127.4; 127.6; 129.27; 129.34; 129.7; 130.3; 130.47; 130.50; 130.58; 132.85; 133.0; 133.27; 133.31; 133.6; 136.7; 154.6; 155.0; 155.3; 171.5; 172.0; 173.5; 173.7. m/z (CI, +ve) 484 (M+H$^+$, 100%), 466 (13) and 444 (19).

3.1.18. (S_a)-2(*R*,*S*)-Acetylamino-3-[3-(3′-allyl-2,2′-dimethoxy-1,1′-binaphthyl)]propanoic acid 10a

This was prepared in the same manner as **10b** (75%).

3.2. Synthesis of acyclic peptides 11–15[24]

3.2.1. Methyl (2*S*,5*R*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(R_a,S_a)-binaphthalen-3-yl)]-5-[4-(*tert*-butoxycarbonylamino)-butyl]-8(*R*,*S*)-acetamido-3,6-diaza-4,7-dioxononanoate 11

The binaphthyl derivative **10b** (0.258 g, 0.53 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and a solution of the protected dipeptide (NH$_2$-D-(N_ω-Boc)Lys-L-allylGly-OMe[9] (0.22 g, 0.61 mmol) in CH_2Cl_2 (3 mL) was added. To the resulting solution was added a crystal of 4-dimethylaminopyridine and then the solution was cooled in an ice/water bath. To the chilled solution was added 1,3-dicyclohexylcarbodiimide (0.111 g, 0.54 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. To the reaction mixture was added CH_2Cl_2 (10 mL) which was then filtered through celite. The filtrate was evaporated to give **11** as a pale yellow crystalline solid (0.404 g, 92%). TLC (4% isopropanol/CH_2Cl_2) R_f of 0.50; ^{1}H NMR 1.17–1.93, m, 6H; rotamers 1.41/1.43, s, 9H; rotamers 2.01/2.02 s, 3H; 2.42–2.70, m, 2H; 2.93–3.07, m 2H; rotamers 3.10/3.14, 3H; 3.23–3.74, m, 4H; rotamers 3.23/3.25, s, 3H; rotamers 3.71/3.74, s, 3H; 4.43–4.70, m, 3H; 5.04–5.24, m, 4H; 5.60–5.83, m, 1H; 6.08–6.23, m, 1H; 7.00–7.60, m, 6H; 7.79–7.95, m, 4H; m/z (ES, +ve) 845 (M+Na$^+$, 2%), 823 (M+H$^+$, 2), 723 (823–Boc, 0.6), 415 (8), 393 (9), 145 (79), 106 (83) and 104 (100).

3.2.2. Methyl (2*S*,5*S*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(R_a,S_a)-binaphthalen-3-yl)]-8(*R*,*S*)-acetamido-3,6-diaza-4,7-dioxo-5{3-[3-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-guanidine]propyl}nonanoate 12

The binaphthyl derivative **10b** (0.188 g, 0.39 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and N_ω-PMC-D-arginine-L-allylglycine methyl ester in CH_2Cl_2 (3 mL) was added. To the resulting solution was added a crystal of 4-dimethylaminopyridine and then the solution was cooled in an ice/water bath. To the chilled solution was added 1,3-dicyclohexylcarbodiimide (0.096 g, 0.46 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. To the reaction mixture was added CH_2Cl_2 (10 mL) which was then filtered through celite. The filtrate was evaporated to give **12** as a colourless crystalline solid (0.259 g, 66%). ^{1}H NMR 1.29, s, 6H; 1.40–2.06, m, 6H; 2.09, s, 3H; 2.44, s, 3H; 2.48–2.69, m, 4H; 3.54, s, 3H; 2.60, s, 3H; 3.04–3.43, m, 4H; rotamers 3.07/3.10, s, 3H; rotamers 3.23/3.30, s, 3H; 3.57–3.70, m, 2H; rotamers 3.69/3.71, s, 3H; 4.54–4.65, m, 3H; 5.03–5.18, m, 4H; 5.72–5.81, m, 1H; 6.07–6.21, m, 1H; 6.33–6.44, m, 3H; 7.18–7.55, m, 6H; 7.64–7.95, m, 4H. m/z (ES, +ve) 1017 (M+H$^+$, 15), 920 (4), 316 (37) and 288 (100). $C_{56}H_{69}N_6O_{10}S$ + H$^+$ requires m/z 1017.4796. Found 1017.4796.

3.2.3. Methyl (2*S*,5*R*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(R_a,S_a)-binaphthalen-3-yl)]-8(*R*,*S*)-acetamido-3,6-diaza-5-{4-[(9*H*-fluoren-9-yl)methoxycarbonylamino]butyl}-4,7-dioxononanoate 13

The binaphthyl derivative **10b** (0.447 g, 0.92 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and a solution of the dipeptide (0.450 g, 0.94 mmol) in CH_2Cl_2 (2 mL) was added. To the resulting solution was added a crystal of 4-dimethylaminopyridine and then the solution was cooled in an ice/water bath. To the chilled solution was added 1,3-dicyclohexylcarbodiimide (0.195 g, 0.94 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. To the reaction mixture was added CH_2Cl_2 (10 mL) which was then filtered through celite. The filtrate was evaporated to dryness and the residue purified by flash column chromatography (4% MeOH/CH_2Cl_2) to give **13** as an off-white crystalline solid (0.474 g, 54%). ^{1}H NMR 1.05–1.71, m, 6H; 1.86–2.02, m, 3H; 2.45–2.60, m, 2H; 2.99–3.73, m, 15H; 4.14–4.42, m, 3H; 4.45–4.70, m, 3H; 5.04–5.19, m, 4H; 5.60–5.78, m, 1H; 6.05–6.20, m, 1H; 6.82–6.49, m, 10H; 7.56–7.58, m, 2H; 7.74–7.88, m, 6H; *m/z* (ES, +ve) 945 (M+H$^+$, 36%) and 225 (100). $C_{57}H_{60}N_4O_9$ + H$^+$ requires *m/z* 945.4389. Found 945.4439.

3.2.4. Methyl (2*S*,5*R*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(R_a,S_a)-binaphthalen-3-yl)]-8(*R*,*S*)-acetamido-3,6-diaza-4,7-dioxo-5-{3-[3-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)guanidine]propyl}nonanoate 14

The binaphthyl derivative **10b** (0.127 g, 0.26 mmol) and N_ω-PMC-L-arginine-L-allylglycine methyl ester (freshly deprotected from the terminal Fmoc derivative) (0.150 g, 0.27 mmol) were dissolved in CH_2Cl_2 (dry, 1.5 mL). To the resulting solution was added 4-dimethylaminopyridine (crystal) and then the solution was cooled in an ice/water bath. To the chilled solution was added 1,3-dicyclohexylcarbodiimide (0.053 g, 0.25 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. To the reaction mixture was added CH_2Cl_2 (10 mL) which was then filtered through celite. The filtrate was evaporated to dryness and the residue purified by flash column chromatography (4% MeOH/CH_2Cl_2) to give **14** as a colourless solid (0.176 g, 68%). ^{1}H NMR δ 1.29, s, 6H; 1.54–2.04, m, 6H; 1.91, s, 3H; 2.08, s, 3H; 2.47–2.68, m, 10H; 3.10, s, 3H; 3.12–3.50, m, 4H; 3.19, s, 3H; 3.59–3.68, m, 2H; 3.71, s, 3H; 4.42–4.70, m, 3H; 5.07–5.19, m, 4H; 5.65–5.83, m, 1H; 6.05–6.23, m, 1H; 6.18–6.49, m, 3H; 7.10–8.00, m, 10H; *m/z* (ES, +ve) 1040 (M+Na$^+$, 16), 1018 (M+H$^+$, 64), 1017 (M$^{+\cdot}$, 100), 920 (13), 624 (14) and 225 (24). $C_{56}H_{69}N_6O_{10}S$ + H$^+$ requires *m/z* 1017.4796. Found 1017.4799.

3.2.5. Methyl (2*S*,5*R*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(R_a,S_a)-binaphthalen-3-yl)]-5-[4-(*tert*-butoxycarbonylamino)-butyl]-8(*R*,*S*)-acetamido-3,6-diaza-4,7-dioxononanoate 15[9]

This was prepared in the same manner as described for **11** above using **10a** (0.149 g, 0.31 mmol), the dipeptide (NH_2-D-(N_ω-Boc)Lys-L-allylGly-OMe (0.120 g, 0.33 mmol freshly *N*-Boc deprotected) DMAP (1 crystal) and CH_2Cl_2 (5 mL) to produce **15** (0.123 g, 49%) and was used directly in the following cyclisation reaction; ^{1}H NMR 1.18–1.53, m, 5H; 1.41, s, 9H; 1.54–1.75, m, 1H; 2.01, s, 3H; 2.37–2.69, m, 2H; 2.88–3.16, 2H; 3.09, s, 3H; 3.17–3.42, m, 2H; 3.58–3.80, m, 2H; 3.29, s, 3H; 3.72, s, 3H; 4.43–4.70, m, 3H; 4.75–4.94, m, 1H; 5.01–5.25, m, 4H; 5.59–5.85, m, 1H; 6.04–6.23, m, 1H; 6.98–7.45, m, 6H; 7.54, d, *J* = 7.2 Hz, 1H; 7.75–7.99, m, 4H.

3.3. Synthesis of protected macrocycles 16–20

3.3.1. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-6-[4-(*tert*-butoxycarbonylamino)-butyl]-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene 16

The binaphthyl derivative **11** (0.205 g, 0.25 mmol) was dissolved in CH_2Cl_2 (50 mL). The solution was deoxygenated with argon gas for 10 min before the addition of benzylidenebis(tricyclohexylphosphine)dichloro ruthenium (0.022 g, 0.027 mmol). The reaction mixture was heated at reflux for 18 h, cooled and the evaporated to dryness. The residue was subjected to silica gel column chromatography and elution with MeOH (2.5%) in CH_2Cl_2 gave **16** (0.152 g, 77%) as a pale brown solid. ^{1}H NMR 1.04–2.08, m, 9H; rotamers 1.38/1.39, s, 9H; 2.40–2.75, m, 2H; 2.90–3.79, m, 15H; 4.28–5.20, m, 3H; 5.62–6.30, m, 2H; 6.80–7.60, m, 6H; 7.70–8.00, m, 4H. $C_{45}H_{54}N_4O_9$ + H$^+$ requires 795.4004. Found 795.3969. The resulting residue was subjected to flash column chromatography (4% MeOH in CH_2Cl_2 then 10% MeOH in CH_2Cl_2) to give diastereomers of **16** in three fractions (isomers).

Compound **16a.** Pale yellow glass like solid (0.038 g). *m/z* (ES, +ve) 817 (M+Na$^+$, 4%), 795 (M+H$^+$, 16), 593 (48) and 297 (100);
Compound **16b.** Light brown solid (0.041 g). *m/z* (ES, +ve) 795 (M+H$^+$, 54%), 593 (35), 297 (100), 145 (44), 104 (33) and 86 (64);
Compound **16c.** Very pale yellow glass like solid (0.073 g). *m/z* (ES, +ve) 795 (M+H$^+$, 15%), 147 (32), 145 (66), 106 (16), 104 (52) and 86 (100).

3.3.2. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-9-methoxycarbonyl-6-{3-[3-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)guanidine]propyl}-4,7-dioxocyclododecaphane-11-ene 17

Prepared from the protected acyclic compound **12** (0.20 g, 0.2 mmol) dissolved in CH_2Cl_2 (50 mL). The solution was deoxygenated with argon gas for 10 min before the addition of benzylidenebis(tricyclohexylphosphine)dichloro ruthenium (0.020 g, 0.024 mmol). The reaction mixture was heated at reflux for 18 h, cooled and then evaporated to dryness. The residue was subjected to silica gel column chromatography and elution with MeOH (2.5%) in CH_2Cl_2 gave **17** as a pale brown solid (0.147 g, 74%). ^{1}H NMR δ 1.28, s, 6H; 1.49–2.17, m, 9H; 2.23, s, 3H; 2.52–2.65, m, 10H; 3.01–3.46, m, 4H; 3.13, s, 3H; 3.30, s, 3H; 3.60–3.73, m, 2H; 3.69, s, 3H; 4.37–4.75, m, 2H; 5.00–5.46, m, 1H; 6.10–6.54, m, 5H; 6.89–7.44, m, 6H; 7.61–8.05, m, 4H; 8.25, s, 1H; *m/z* (ES, +ve) 989 (M+H$^+$, 17), 495 (15), 371 (27), 316 (75), 297 (88), 288 (85), 217 (73) and 199 (100).

3.3.3. (6*S*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-6-(4-{[(9*H*-fluoren-9-yl)methoxy]-carbonylamino}butyl)-9-methoxycarbonyl-4,7-dioxocyclododecaphane-11-ene 18

The acyclic compound **13** (0.470 g, 0.50 mmol) was dissolved in CH_2Cl_2 (120 mL). The solution was deoxygenated with argon gas for 10 min before the addition of benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (0.022 g, 0.027 mmol). The reaction mixture was heated at reflux for 18 h. The cooled reaction mixture was evaporated to dryness the resulting residue purified by flash column chromatography (4% methanol/ CH_2Cl_2) to give **18** as an off-white crystalline solid (0.329 g, 72%). ^{1}H NMR 1.05–1.71, m, 6H; 1.99, s, 3H; 2.47–2.60, m, 2H; 2.99–3.16, m, 2H; 3.21, s, 3H; 3.28, s, 3H; 3.58–3.67, m, 2H; 3.69, s, 3H. *m/z* (ES, +ve) 917 (M+H$^+$, 5), 593 (9), 522 (3) and 297 (100%). $C_{55}H_{56}N_4O_9$ + H$^+$ requires 917. $C_{55}H_{56}N_4O_9$ + H$^+$ requires *m/z* 917.4110. Found 917.4126.

3.3.4. (6*S*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-9-methoxycarbonyl-6-{3-[3-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)guanidine]propyl}-4,7-dioxocyclododecaphane-11-ene 19

The acyclic compound **14** (0.176 g, 0.17 mmol) was dissolved in CH_2Cl_2 (50 mL). The solution was deoxygenated with argon for 10 min before the addition of benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (0.015 g, 0.017 mmol). The reaction

mixture was heated at reflux for 20 h. The cooled reaction mixture was evaporated to dryness the resulting residue purified by flash column chromatography (4% methanol/ CH_2Cl_2) to give **19** as a light brown glass (0.132 g, 77%). ^{1}H NMR 1.27, s, 6H; 1.75–2.17, m, 9H; 2.43–2.57, m, 3H; 2.89–3.79, m, 10H; 4.36–4.80, m, 2H; 5.00–5.60, m, 1H; 6.14–6.50, m, 5H; 6.99–7.40, m, 6H; 7.70–7.96, m, 4H; *m/z* (ES, +ve) 1011 (M+Na+) and 989 (M+H+). $C_{54}H_{64}N_6O_{10}S$ + H^+ requires *m/z* 989.4533. Found 989.4483.

3.3.5. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-6-[4-(*tert*-butoxycarbonylamino)-butyl]-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene 20[9]

This was prepared from the (*S*)-acyclic compound **15** (0.120 g, 0.14 mmol) and benzylidenebis(tricyclohexylphosphine)-dichlororuthenium (0.011 g, 0.013 mmol) using the method as set out for **16**. After column chromatography the protected macrocyclic (*S*)-**20** was isolated as a colourless glass (0.083 g, 72%). ^{1}H NMR δ 1.17–1.98, m, 6H; 1.42, s, 9H; 2.17, s, 3H; 2.36–2.77, m, 2H; 2.94–3.17, m, 2H; 3.15, s, 3H; 3.18–3.55, m, 4H; 3.32, s, 3H; 3.52, s, 3H; 4.26–4.39, m, 2H; 4.40–4.65, m, 1H; 4.80–4.90, m, 1H; 5.00–5.20, m, 2H; 6.16–6.23, m, 1H; 6.38, d, *J* = 7.2 Hz, 1H; 6.53, d, *J* = 6.0 Hz, 1H; 6.63, d, *J* = 6.9 Hz, 1H; 6.92, d, *J* = 8.4 Hz, 1H; 7.00, d, *J* = 7.8 Hz, 1H; 7.10–7.19, m, 2H; 7.31–7.38, m, 2H; 7.69, s, 1H; 7.75, s, 1H; 7.81, d, *J* = 8.1 Hz, 2H; *m/z* (ES, +ve) 817 (M+Na^+, 4%), 795 (M+H^+, 13), 593 (20), 297 (100). $C_{45}H_{54}N_4O_9$ + H^+ requires *m/z* 795.3969. Found 795.3974.

3.4. Synthesis of deprotected macrocycles 22–27

3.4.1. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-6-(4-aminobutyl)-5,8-diaza-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene hydrochloride 25

To a solution of the macrocycle **16a** (0.038 g, 0.05 mmol) in CH_2Cl_2 (2 mL) was added trifluoroacetic acid (2 mL) and the mixture stirred at rt for 25 min. It was then evaporated to dryness, and the residue taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (3 mL) and a solution of 1.0 M hydrogen chloride in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The product **25** was crystallized from the residue with diethyl ether and CH_2Cl_2. The product was isolated using centrifugation to yield afforded **25** as a pale yellow solid (0.021 g, 60%) (*S*-isomer). ^{1}H NMR δ 0.86–2.58, m, 11H; 2.80–3.80, m, 13H; 4.25–4.95, m, 3H; 5.00–5.40, m, 2H; 6.00–6.30, m, 1H; 6.95–7.60, m, 6H; 7.65–8.00, m, 4H; *m/z* (ES, +ve) 697 (32%) and 696 (100) and 695 (M+H^+, 96); $C_{40}H_{46}N_4O_7$ + H^+ requires *m/z* 695.3445. Found 695.3435.

3.4.1.1. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-6-(4-aminobutyl)-5,8-diaza-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene hydrochloride 26.

Similarly **16b** (0.041 g, 0.05 mmol) afforded **26** as a pale yellow solid (0.023 g, 60%) (*S*-isomer). *m/z* (ES, +ve) 696 (9%), 695 (M+H^+, 20); 111 (12) and 60 (100); $C_{40}H_{46}N_4O_7$ + H^+ requires *m/z* 695.3445. Found 695.3427.

3.4.1.2. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-6-(4-aminobutyl)-5,8-diaza-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene hydrochloride 27.

Similarly **16c** (0.073 g, 0.09 mmol) **27** as a pale yellow crystalline solid (0.055 g, 82%) (*R*-isomer). *m/z* (ES, +ve) 696.5 (41%) and 695.8 (53) and 695.4 (M+H^+, 73), 111 (48) and 60 (100); $C_{40}H_{46}N_4O_7$ + H^+ requires *m/z* 695.3445. Found 695.3400.

3.4.2. (6*R*,9*S*)-1(3,3′)-2,2′-dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-6-(3-guanidinopropyl)-9-methoxycarbonyl-4,7-dioxocyclododecaphane-11-ene hydrochloride 22

This was prepared from a solution of the protected macrocyclic **17** (0.099 g, 0.10 mmol) in CH_2Cl_2 (2 mL) and trifluoroacetic acid (2 mL). The mixture was stirred at rt for 25 min and then evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (3 mL) and a solution of 1.0 M hydrogen chloride in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The deprotected macrocyclic **22** was isolated (0.068 g, 89%) as an off-white solid. ^{1}H NMR δ 0.83–2.22, m, 9H; 2.49–2.54, m, 2H; 3.04–4.14, m, 13H; 4.34–4.65, m, 3H; 5.15–5.55, m, 2H; 5.75–5.97, m, 1H; 7.09–7.96, m, 10H; *m/z* (ES, +ve) 723 (M+H^+, 15), 316 (28), 288 (78), 217 (100), 199 (79), 111 (41); $C_{40}H_{46}N_6O_7$ + H^+ requires *m/z* 723.3520. Found 723.3533.

3.4.3. (6*S*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-6-(4-aminobutyl)-5,8-diaza-9-methoxycarbonyl-4,7-dioxocyclododecaphane-11-ene hydrochloride 23

To the macrocyclic **18** (0.123 g, 0.13 mmol) was added dry CH_3CN (9 mL). The mixture was warmed to 60 °C in a sealed system and a solution of piperidine in CH_3CN (0.02 M, 0.54 mL) was added. The reaction mixture was heated at 60 °C for 43 h. The cooled mixture was filtered, the filtrate evaporated to dryness to give a white solid. The solid was purified by flash column chromatography (10% MeOH/CH_2Cl_2, followed by 10% MeOH/CH_2Cl_2containing 2% triethylamine). The isolated product was dissolved in CH_2Cl_2 (5 mL) and 1.0 M HCl in diethyl ether (0.5 mL) was added. The solution was stirred for 10 min then evaporated to dryness and dried under high vacuum to give **23** as a pale yellow crystalline solid (0.048, 51%). ^{1}H NMR δ 0.86–2.55, m, 11H; 3.00–3.71, m, 13H; 4.40–4.92, m, 3H; 5.02–5.60, m, 2H; 6.02–6.29, m, 1H; 7.08–7.61, m, 6H; 7.62–8.20, m, 4H; *m/z* (ES, +ve) 695 (M+H^+, 100%) and 498 (25); $C_{40}H_{46}N_4O_7$ + H^+ requires *m/z* 695.3445. Found 695.3419.

3.4.4. (6*S*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(R_a,S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-5,8-diaza-6-(3-guanidinopropyl)-9-methoxycarbonyl-4,7-dioxocyclododecaphane-11-ene hydrochloride 24

The macrocyclic **19** (0.047 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL) then trifluoroacetic acid (2 mL) was added. The mixture was stirred at rt for 25 min. The mixture was evaporated to dryness, the residue taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (3 mL) and a solution of 1.0 M HCl in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The product **24** was crystallized from the residue with diethyl ether and CH_2Cl_2. The product was isolated using centrifugation to yield **24** as an off-white solid (0.021 g, 58%). ^{1}H NMR δ 0.83–2.22, m, 9H; 2.49–2.54, m, 2H; 3.04–4.14, m, 13H; 4.34–4.65, m, 3H; 5.15–5.55, m, 2H; 5.75–5.97, m, 1H; 7.09–7.96, m, 10H; *m/z* (ES, +ve) 724 (M+H^+, 100%); $C_{40}H_{46}N_6O_7$ + H^+ requires *m/z* 723.3506. Found 723.3488.

3.4.5. (6*R*,9*S*)-1(3,3′)-2,2′-Dimethoxy-(S_a)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-6-(4-aminobutyl)-5,8-diaza-9-methoxycarbonyl-4,7-dioxocyclotridecaphane-11-ene hydrochloride 1[9]

The macrocyclic **20** (0.041 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL) then trifluoroacetic acid (2 mL) was added. The

mixture was stirred at rt for 25 min. The mixture was evaporated to dryness, the residue taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (3 mL) and a solution of 1.0 M HCl in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The product **1** was crystallized from the residue with diethyl ether and CH_2Cl_2. The product was isolated using centrifugation to yield **1** (0.032 g, 84%) as an off-white solid. *m/z* (ESI, +ve) 696 (85%), 695 (M+H$^+$, 100); $C_{40}H_{46}N_4O_7$+H$^+$ requires *m/z* 695.3445 Found *m/z* 695.3455.

3.5. Synthesis of the deprotected acyclic peptides *31*–32

3.5.1. Methyl (2*S*,5*R*)-2-allyl-9-[2-(3′-allyl-2,2′-dimethoxy-1,1′-(*R*$_a$,*S*$_a$)-binaphthalen-3-yl)]-8(*R*,*S*)-acetamido-3,6-diaza-5-(3-guanidinopropyl)-4,7-dioxononanoate 31

The acyclic compound **12** (0.056 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL) then trifluoroacetic acid (3 mL) was added. The mixture was stirred at 0 °C for 45 min, and then warmed to rt followed by stirring for 15 min. The mixture was then diluted with CH_2Cl_2 (15 mL) and then evaporated to dryness. The residue taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (3 mL) and a solution of 1.0 M HCl in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated to dryness again. This was repeated twice more. The product **31** was crystallized from the residue with diethyl ether and CH_2Cl_2 to afford **31** that was isolated as a pale yellow solid (0.034 g, 79%). ^{1}H NMR: 1.25–1.45, m, 1H; 1.50–2.00, m, 6H; 2.44–2.70, m, 2H; 2.89–3.02, m, 1H; 3.22–3.45, m, 9H; 3.68–3.72, m, 2H; 4.52–4.59, m, 2H; 4.62–4.80, m, 1H; 5.11–5.20, m, 4H; 5.71–5.88, m, 1H; 6.12–6.24, m, 1H; 7.06–7.12, m, 2H; 7.22–7.25, m, 2H; 7.39–7.44, m, 2H; 7.89–7.94, m, 4H. *m/z* (ESI, +ve) 751 (M−Cl+H$^+$, 55%); $C_{42}H_{50}N_6O_7$ + H$^+$ requires *m/z* 751.3819 Found 751.3826.

3.5.2. Methyl (2*S*,5*S*)-2-allyl-9-[2-(3′-allyl-2,2′dimethoxy-1,1′-(*R*$_a$,*S*$_a$)-binaphthalen-3-yl)]-8(*R*,*S*)-acetamido-3,6-diaza-5-(3-guanidinopropyl)-4,7-dioxononanoate 32

The acyclic compound **14** (0.054 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL) then trifluoroacetic acid (3 mL) was added. The mixture was stirred at 0 °C for 2 h, and then warmed to rt followed by stirring for 15 min. The mixture was then diluted with CH_2Cl_2 (15 mL) and then evaporated to dryness. The residue taken up in $CH_2Cl_2$and evaporated to dryness again. This was repeated twice more. The residue was taken up in CH_2Cl_2 (1 mL) and a solution of 1.0 M HCl in diethyl ether (1 mL) was added. The resulting mixture was stirred at rt for 10 min before being evaporated to dryness. The residue was taken up in CH_2Cl_2 (15 mL) and evaporated to dryness again. This was repeated twice more. The product **32** was crystallized from the residue with diethyl ether and CH_2Cl_2. The deprotected non-cyclic compound **32** that was isolated as a pale yellow solid (0.031 g, 74%). ^{1}H NMR: 1.28–1.40, m, 1H; 1.55–2.00, m, 6H; 2.47–2.67, m, 2H; 2.90–3.00, m, 1H; 3.15–3.49, m, 9H; 3.64–3.72, m, 2H; 4.39–4.64, m, 2H; 4.65–4.75, m, 1H; 4.91–5.20, m, 4H; 5.76–5.82, m, 1H; 6.15–6.23, m, 1H; 7.05–7.12, m, 2H; 7.21–7.27, m, 2H; 7.39–7.44, m, 2H; 7.88–7.94, m, 4H. *m/z* (ESI, +ve) 751 (M−Cl+H$^+$, 100%); $C_{42}H_{50}N_6O_7$ + H$^+$ requires *m/z* 751.3819 Found 751.3817.

3.6. Synthesis of the (5*S*,9*S*,12*S*)-1(3,3′)-2,2′-dimethoxy-(*R*$_a$,*S*$_a$)-1,1′-binaphthylena-3(*R*,*S*)-acetamido-9-(4-aminobutyl)-8,11-diaza-12-methoxycarbonyl-5-methyl-4,7,10-trioxocyclohexadecaphane-14-ene 30

A solution of **10b** (0.25 g, 0.52 mmol), the tripeptide NH_2-L-Ala-L-(N_ω-Foc)Lys-L-allylGly-OMe (0.286 g, 0.52 mmol), DMAP (1 crystal), DCC 90.109 g, (0.53 mmol) in CH_2Cl_2 (3 mL) at 0 °C was then allowed to come to rt and then stirred overnight. The reaction was filtered, concentrated by rotary evaporation and the residue then subjected to column chromatography and elution with 5% isopropanol in CH_2Cl_2 gave the peptide **28** (0.206 g, 39%) as a white solid. ^{1}H NMR δ 1.34, d, *J* = 7.2 Hz, 3H; 1.38–1.51, m, 3H; 1.71–1.93, m, 3H; 1.98, s, 3H; 3.13–3.27, m, 5H; 3.32, s, 3H; 3.60–3.69, m, 2H; 3.73, s, 3H; 3.98–4.07, m, 1H; 4.15–4.22, m, 1H; 4.28–4.53, m, 4H; 4.60–4.67, m, 1H; 5.11–5.17, m, 4H; 5.41–5.61, m, 1H, 5.69–5.78, m, 1H; 6.07–6.19, m, 1H; 6.91, br s, 1H, NH; 7.10–7.13, m, 1H; 7.18–7.30, m, 5H; 7.35–7.41, m, 4H; 7.55–7.60, m, 3H; 7.73–7.75, m, 2H; 7.80–7.86, m, 4H. *m/z* (ES, +ve) 1016 (M+H$^+$, 100%).

Compound **28** (0.068 g, 0.07 mmol) and benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (0.004 g, 0.005 mmol) in deoxygenated CH_2Cl_2 (15 mL) were then heated at reflux for 15 h. The solvent was then evaporated and the residue subjected to flash column chromatography and elution with 10% MeOH in CH_2Cl_2 to give the cyclic protected tetrapeptide **29** (0.069 g). ^{1}H NMR 1.19–2.05, m, 6H; 2.04–2.24, m, 2H; 3.06–3.73, m, 15H; 4.11–4.64, m, 3H; 5.69–5.78, m, 1H; 6.00–6.22, m, 1H; 7.13–7.84, m, 10H. This product was immediately added to dry CH_3CN (4 mL) and DMF (1 mL) with piperidine (0.32 mL, 0.006 mmol) and the mixture heated at 70 °C for 12 h. The reaction mixture was then cooled and centrifuged at 2100 rpm for 5 min and the solid separated. The solid was then added to HCl in ether (1.0 M, 0.3 mL) and MeOH (2 mL), filtered and the filtrate concentrated and dried to yield the cyclic tetrapeptide **30** (10 mg, 19%) as a pale yellow solid. The ^{1}H NMR spectra of **30** was exceptionally broad (see Ref. 18), probably exacerbated by slow conformational tumbling in solution, and was not able to be deconvoluted into meaningful data. *m/z* (ES, +ve) 766.5 (100%), 767 (79) and 767.5 (58); $C_{43}H_{51}N_5O_8$ + H$^+$ requires *m/z* 766.3816. Found 766.3768.

3.7. Determination of minimum inhibitory concentration (MIC)

Compound preparation: Compounds were accurately weighed out (between approx. 1–2 mg) and dissolved in 10% methanol/water (v/v) to a final stock concentration of 10 mg/mL. Compounds were then diluted 1/10 in water to a test concentration of 1000 μg/mL and used immediately.

3.7.1. Starter culture

The day prior to MIC determinations, an overnight (14 h) culture of *Staphylococcus aureus* in Luria Broth (LB) was grown at 37 °C. The culture was then diluted 1 in 1,000 prior to use in the assay. This dilution provided a clear suspension of cells sufficient for overnight growth and to enable the clear visual determination of inhibition of growth.

In a 96 well round bottom plate, 50 μL LB/well was added. Compounds were assayed in triplicate as 8 dilutions in each assay and to the upper well of each of the three columns 50 μL of compound at 1,000 μg/mL was added. The compounds were then diluted 1 in 2 from the top row to the bottom row giving the eight dilutions. After the dilutions, 50 μL of the diluted overnight culture was added to each well.

Each assay also contained a media only control, bacteria but no compound, and bacteria with vancomycin in triplicate. Compounds

were generally tested in the two fold dilution series 250 μg/mL down to 1.95 μg/mL. Vancomycin was tested in the two fold dilution series with a starting concentration of 7.8 μg/mL. The highest concentration of solvent in the assay was 0.25% v/v and assays demonstrated the solvent to have no adverse effect on the overnight growth of the cultures.

Assays were set up in a blinded fashion such that the structures of the compounds were unknown to the operator. MICs were determined by eye, where the compound concentration at the lower dilution to the well of obvious growth was recorded as the MIC. Results of the triplicates were recorded and the median values (rounded to whole numbers) stated in this manuscript.

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15. All new compounds reported in this paper were fully characterised using ^{1}H and ^{13}C NMR, MS, and HRMS unless otherwise stated. All compounds were judged to be of >95% purity based on ^{1}H NMR analysis. The macrocyclic free amines were also judged to be homogenous by TLC analysis and the subsequent salts were recrystallised.
16. We also attempted the stereoselective alkylation of the (*S*)-bromide **5** using the Seebach chiral imidazolidinone auxiliary, see Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem., Int. Ed.* **1996**, *35*, 2708. In our hands, it gave modest yields of the desired alkylated product (~50%) with subsequent hydrolysis being slow and not producing the required amino acid intermediate, but instead an unwanted amide.
17. As a result of the presumed epimeric mixture of the *N*-acetyl protected glycine residue, and the rotamers present, ^{13}C NMR was not productive in the assignment of structure.
18. For all macrocycles synthesised here, peaks in the ^{1}H NMR spectra were notably broad but were consistent with the assigned structures. Hence, a greater emphasis was placed on MS and in particular, HRMS, for elucidation of structure.
19. The synthesis of compounds **15**, **20** and **1**, were described previously, see Ref. 9.
20. For examples of typical procedures used, see Ref. 9 and Bremner, J. B.; Pyne, S. G.; Keller, P. A.; Coghlan, D. R.; Garas, A.; Witchard, H. M.; Boyle, T. P.; Coates, J. A. WO 03/002545 A1, Patent, Peptoid Compounds, AU2002/00850 2003.
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24. The presence of diastereomers and rotamers accounts for the excess peaks appearing in the NMR spectra.