Synthesis and binding properties of a macrobicyclic receptor for N-protected peptides with a carboxylic acid terminus

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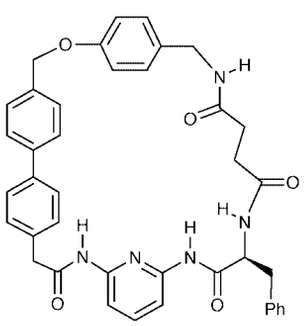
A novel macrobicyclic receptor, **3**, has been synthesised by linking together a diaminopyridine with suitable amino acids, followed by a double intramolecular cyclisation of a suitably activated precursor. Macrobicycle **3** features a diamidopyridine unit, designed to serve as a specific binding site for carboxylic acid functionality, at the base of an open, bowl-shaped cavity. Incorporation of additional amide functionality around the rim of the bowl-shaped structure provides further hydrogen bonding sites to interact with peptidic guests. The binding properties of **3** with N-protected amino acid and peptide derivatives have been investigated by NMR titration experiments, which reveal that **3** is a strong and selective receptor for peptides with a carboxylic acid terminus in $CDCl_3$ solution, the strongest binding being observed with Cbz-β-alanyl-D-alanine ($-\Delta G_{ass} = 22.8$ kJ mol^{-1}). The macrobicycle is reasonably enantioselective (Cbz-β-alanyl-L-alanine, $-\Delta G_{ass} = 19.1$ kJ mol^{-1}) and notably the binding of Cbz-β-alanyl lactic acids is considerably weaker than the binding of the corresponding Cbz-β-alanyl alanines ($\Delta\Delta G_{ass} \sim 8$–9 kJ mol^{-1}). Molecular modelling and 2D NMR studies have been carried out on the free macrobicycle and the 1:1 complex formed with the most strongly bound substrate (Cbz-β-alanyl-D-alanine). These studies provide a consistent picture of the macrobicycle as a flexible receptor, which is able to bind the Cbz-β-alanyl-D-alanine substrate in the macrobicyclic cavity with a series of well defined hydrogen bonds to the alanylalanine amide, and less well defined hydrogen bonds to the benzylcarbamate functionality.

Introduction

The development of synthetic receptors for peptides and amino acid derivatives is of considerable interest because the intermolecular interactions involved in small molecule–peptide complexes are of direct relevance to many biological peptide–protein interactions, and may also lead to new bio-sensors, therapeutics and catalysts for peptide hydrolysis.[1] We have focused on developing receptors specifically for peptides with a free carboxylic acid terminus, by incorporating a carboxylic acid binding site into macrocyclic structures with additional hydrogen bonding functionality to bind to the backbone of peptidic guests. We have described one such macrocyclic structure **1**[2] which utilises a diamidopyridine unit to serve as a carboxylic acid binding site in a cavity lined on one side with amide functionality, and on the other by a rigid biaryl unit. This macrocycle selectively binds to amino acid and peptide derivatives with a carboxylic acid terminus, in chloroform solution. As an alternative approach to recognition of such peptidic substrates we have prepared a range of macrobicycles designed to have a more 3-dimensional architecture, and which feature a specific binding site for carboxylic acid functionality at the base of an open, bowl-shaped cavity. By incorporating additional amide functionality around the rim of such a bowl-shaped structure we intended to provide further hydrogen bonding sites suitably preorganised to interact with guests, such as amino acid derivatives, bound within the macrobicyclic cavity. Thus, for example, we have described macrobicycle **2**[3] in which a carboxylate binding site is provided by a thiourea moiety.



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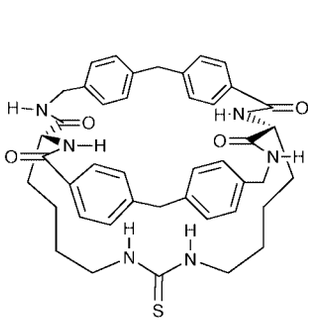

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Macrobicycle **2** binds a range of *N*-acetyl amino acids (as tetrabutylammonium carboxylates), with *N*-Ac-D-amino acid substrates binding through a carboxylate–thiourea interaction on the outside of the cavity, while *N*-Ac-L-amino acid substrates are bound in the cavity of the macrobicyclic receptor, but with the *N*-acetyl unit in a *cis* amide configuration. In order to bind larger substrates than the derivatised amino acids accommodated by **2**, we wished to prepare macrobicycle **3** with a deeper cavity and, in addition, to investigate the use of a diamidopyridine moiety to serve as a carboxylic acid (as opposed to carboxylate) binding site in this type of system. In order to deepen the cavity we chose to link the rim of the macrobicycle, formed from the same biaryl unit used previously in **2**, through glutamic acid and phenylalanine residues, to a diamidopyridine unit at the base of the cavity. We chose to incorporate phenylalanine into the structure to increase the amount of chiral information in the receptor (which might therefore lead to greater enantioselective binding properties) to aid the solubility of the receptor in non-polar solvents such as chloroform, and, assuming that the benzyl residues would prefer to be on the outside of the macrobicycle, this should aid in the preorganisation of the cavity. In this paper we describe the synthesis of macrobicycle **3**, which indeed shows strong and selective binding to amino acid and dipeptide derivatives in chloroform solution.[4]

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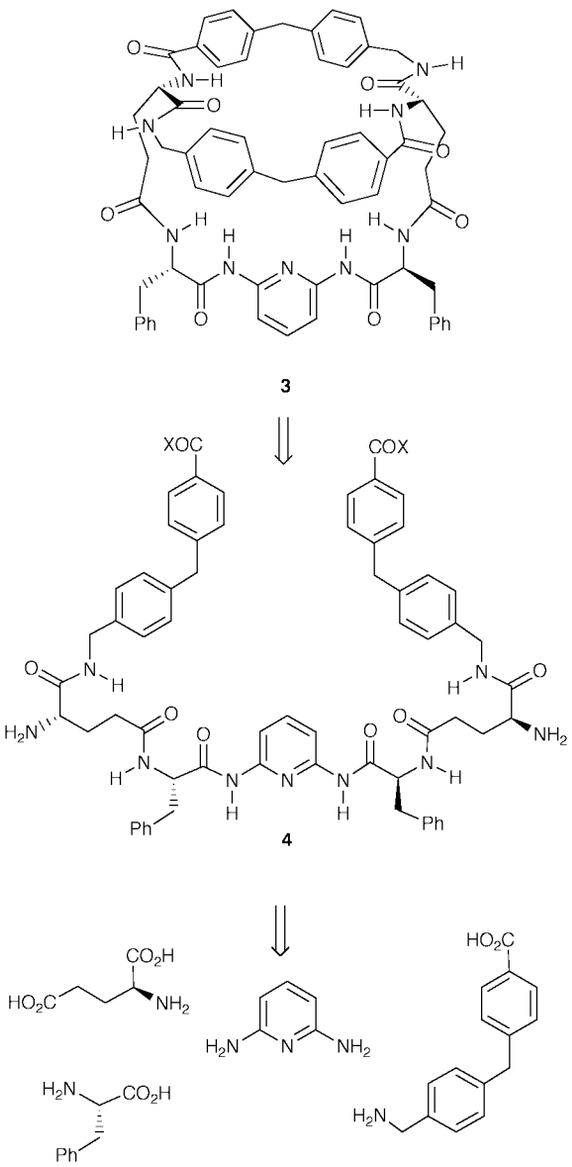
Synthesis

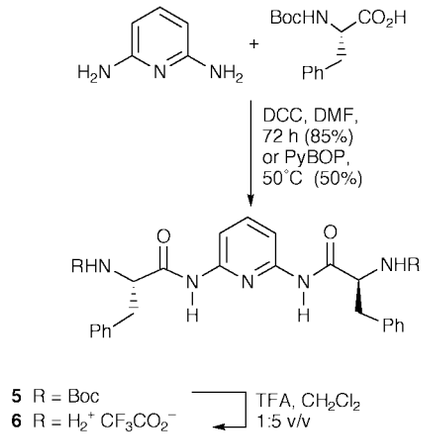
The synthesis of macrobicycle **3** follows the same strategy as for the synthesis of **2** and related macrobicycles,[3,5] with a double intramolecular cyclisation of a suitably activated acyclic precursor **4** as the key step (Scheme 1). Assembly of the cyclisation



Scheme 1

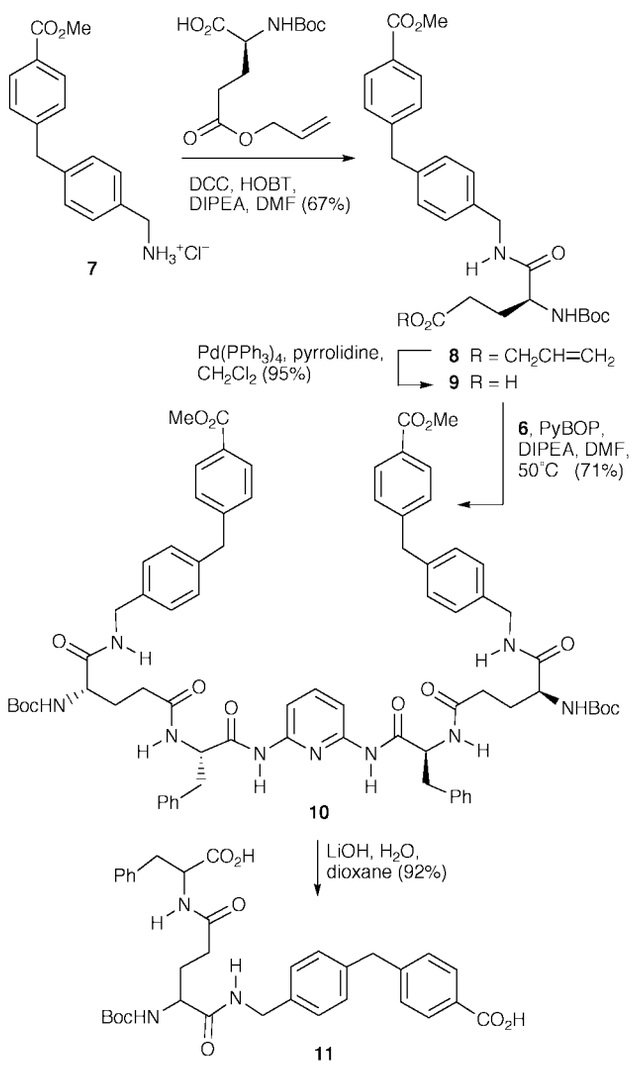
precursor then requires coupling together of the various amino acid building blocks around the carboxylic acid binding site, in this case diaminopyridine.

Coupling of diaminopyridine to activated carboxylic acid derivatives to generate diamidopyridines can be complicated by the low reactivity of the amino groups and, in our case, the normal use of acid chlorides as the coupling partner was precluded if racemisation of the phenylalanine was to be avoided. We found that *tert*-butoxycarbonylphenylalanine could be coupled to diaminopyridine in ~50% yield, using the coupling agent benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBop), and carrying out the reaction at 50 °C (Scheme 2). Alternatively stirring diaminopyridine with an excess of Boc-phenylalanine and dicyclohexylcarbodiimide, as the coupling agent, in DMF for 72 hours gave the desired bis adduct in 85% yield.[6] Treatment of **5** with TFA then gave the bis-TFA salt **6**. Macrobicycle **3** uses the same biarylmethane fragment as the rigidifying unit in the rim of the structure as



Scheme 2

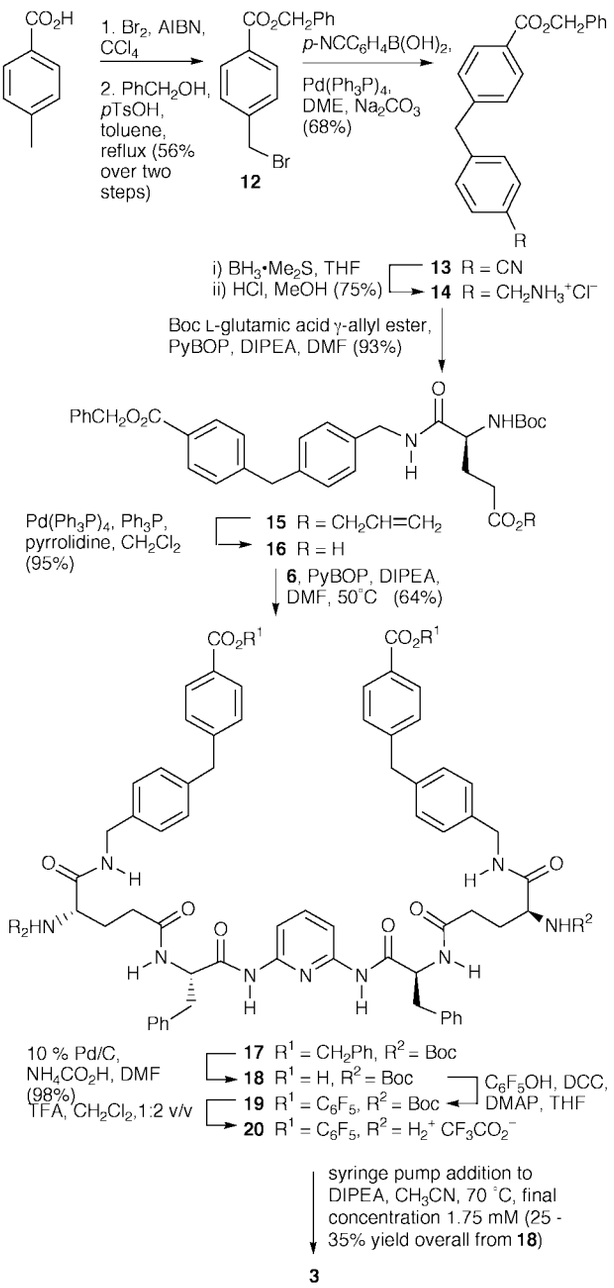
used previously in receptor **2**, and so in our first attempts at the synthesis of **3** the previously described amino methyl ester **7**[3a] was coupled with *tert*-butoxycarbonyl-L-glutamic acid γ-allyl ester in 67% yield, followed by removal of the allyl protecting



Scheme 3

group, using Pd(0) catalysis, to give acid **9** (Scheme 3).[7] Two equivalents of **9** were then coupled to the bis-TFA salt **6** to give the protected macrocyclisation precursor **10**. However, all attempts at the selective cleavage of the methyl esters of **10** were unsuccessful. Thus alkaline hydrolysis led to competitive cleavage of the amide bonds of the amidopyridine unit, giving diacid

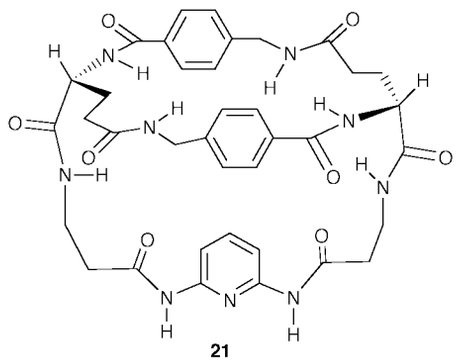
fragment **11** (stereochemistry unproven) in good yield, and treatment of diester **10** with alkyl thiolates,[8] iodide[9] or cyanide anion[10] similarly failed to give the desired diacid. We therefore prepared the biarylmethane unit as the benzyl ester **13** by a Suzuki coupling[11] of benzyl 4-bromomethylbenzoate **12** with 4-cyanophenylboronic acid[3] and reduction of the resulting nitrile to give the amine **14** (Scheme 4). Coupling of **14** with



Scheme 4

tert-butoxycarbonyl-L-glutamic acid γ-allyl ester, followed by removal of the allyl protecting group now gave acid **16**, which in turn was coupled to the bis-TFA salt **6** to give the protected macrocyclisation precursor **17**. Now debenzylation to give the diacid **18** was successfully accomplished using Pd/C with ammonium formate in essentially quantitative yield. Diacid **18** was converted to the bis(pentafluorophenyl ester) **19** and the amine protecting groups were removed with trifluoroacetic acid. Finally, slow addition of the resulting bis(trifluoroacetate salt) **20** to a solution of diisopropylethylamine, in acetonitrile, gave the desired macrobicycle **3** in ~30% overall yield from **18**.

Macrobicycle **3** is soluble in a range of organic solvents, including chloroform, and gave well resolved NMR spectra which could be unambiguously assigned. The ^{1}H NMR spectrum also indicated that macrobicycle **3** was formed as a single diastereoisomer, which ruled out the possibility that racemisation of any of the chiral centres might have occurred during the synthesis. Earlier work on a related, but smaller, macrobicycle **21**[5] had shown that in solution this macrobicycle had



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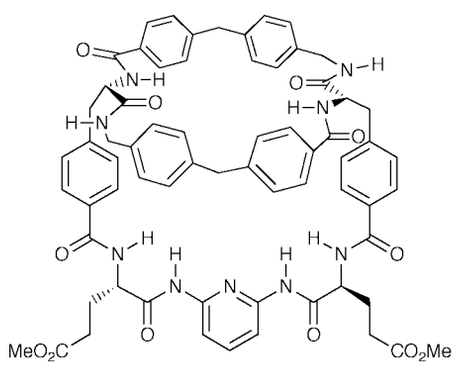

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a preference for a conformation with the amidopyridine unit inverted inside the rim of the macrobicycle so that the amidopyridine (and hence the carboxylic acid binding site) was pointing away from the intended binding cavity, which was clearly unfavourable for binding of amino acids or peptidic guests. For macrobicycle **3**, a 2D ROESY[12] spectrum revealed no NOE's from the pyridine ring protons to the rim of the macrobicycle, suggesting that the pyridine ring is not inverted into the macrobicyclic cavity in this case. This notion was supported by detailed molecular modelling studies on the macrobicycle (*vide infra*). The ^{1}H NMR spectrum of **3** also revealed that the signal for the carboxamide proton NH^5 (for the atom labelling used, see Fig. 2) occurs at 8.16 ppm in $CDCl_3$ solution. In comparison the other amide protons in macrobicycle **3**, NH^3 and NH^7, resonate at 6.37 and 6.47 ppm respectively in $CDCl_3$ solution. Furthermore, for the related macrobicyclic structure **22**, incorporating an identical biaryl rim portion, the carboxamide proton resonates at 6.42 ppm in $CDCl_3$ solution.[13] This strongly suggests that for macrobicycle **3** there is an intramolecular hydrogen bond to NH^5, and this is borne out by the molecular modelling results for **3** (*vide infra*) which showed structures in which a consistent feature was the presence of intramolecular hydrogen bonds, particularly from NH^5 to C=O^4. In the case of macrobicycle **22**, such an intramolecular hydrogen bond is not possible with the rigid aromatic ring, which links the biaryl rim to the diamidopyridine unit.

Binding studies

Binding studies with macrobicycle **3** were carried out with a series of substrates in deuteriochloroform, using a standard NMR titration experiment, by monitoring the shift of the amidopyridine NH signal (H^1) and analysing the resulting binding curves using the Hostest software.[14] Examples of

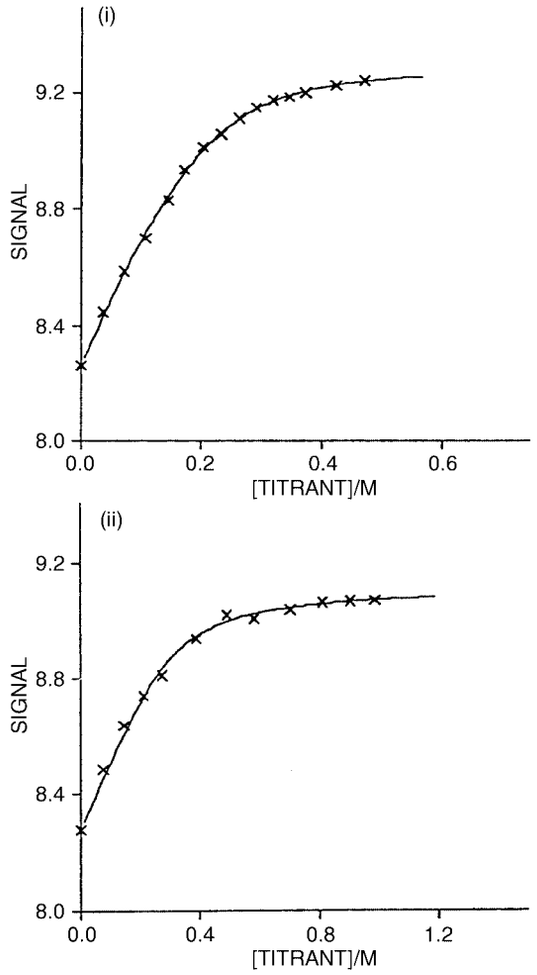

Fig. 1 Binding isotherms showing the shift of NH^1 (ppm) of macrobicycle **3**, in $CDCl_3$ (3.11×10^{-3} M) against guest concentration (M) on titration with (i) Cbz-β-alanyl-D-alanine, and (ii) Cbz-β-alanyl-L-alanine. The ×'s represent the experimental data points and —— the calculated fit.

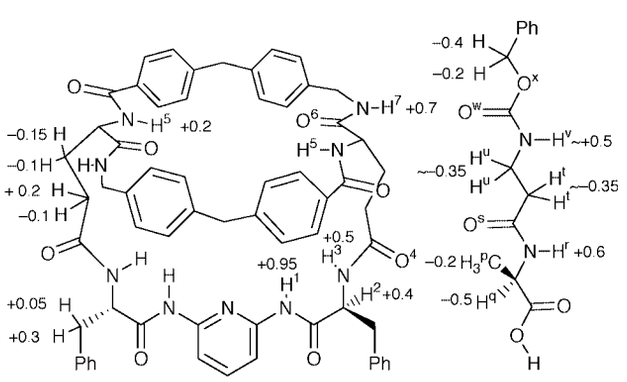

Fig. 2 Atom labelling for macrobicycle **3** and guest Cbz-β-alanyl-D-alanine, and shifts (ppm) for the various protons indicated, on formation of the 1:1 complex between the two, relative to the signals for uncomplexed materials.

titration isotherms for the most strongly bound guest Cbz-β-alanyl-D-alanine and its enantiomer Cbz-β-alanyl-L-alanine are shown in Fig. 1. A number of simple carboxylic acids, α- and β-amino acid derivatives, and dipeptides were studied to allow a thorough investigation of how stereochemistry, relative disposition of functionality and the steric bulk of side chains affected the binding properties of the receptor (Table 1). In each binding experiment a 1:1 binding stoichiometry has been assumed which was supported by the good fit of the measured data to the theoretical model, on analysis. On titration with

Table 1 Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1:1 complexes formed between macrobicycle **3** and various substrates in $CDCl_3$ at 20 °C

Entry	Substrate	K_{ass} [a]/ M^{-1}	$-\Delta G_{ass}$ [b]/ kJ mol^{-1}
1	$PhCH_2CO_2H$	350	14.2
2	$CH_3(CH_2)_4CO_2H$	470	15.0
3	Cbz-Gly-OH	800	16.3
4	Cbz-L-Ala-OH	620	15.6
5	Cbz-D-Ala-OH	1370	17.6
6	Boc-L-Phe-OH	400	14.6
7	Boc-D-Phe-OH	660	15.8
8	Cbz-D-Phe-OH	1370	17.6
9	Ac-D-Phe-OH	2970	19.5
10	Boc-L-Val-OH	180	12.6
11	Boc-D-Val-OH	85	10.7
12	Boc-L-Ser-OH	1360	17.6
13	Boc-D-Ser-OH	450	14.8
14	Cbz-β-Ala-OH	870	16.5
15	Cbz-Gly-L-Phe-OH	280	13.4
16	Cbz-L-Phe-Gly-OH	300	13.6
17	Cbz-Gly-L-Ser-OH	2670	19.2
18	Cbz-Gly-D-Ser-OH	570	15.4
19	Cbz-L-Ala-L-Ala-OH	410	14.6
20	Cbz-D-Ala-D-Ala-OH	1420	17.7
21	Cbz-β-Ala-L-Ala-OH	2220	19.1
22	Cbz-β-Ala-D-Ala-OH	12200	22.8
23	Cbz-β-Ala-L-Lac-OH	110	11.3
24	Cbz-β-Ala-D-Lac-OH	260	13.2

[a] Errors for K_{ass} were estimated as <10%. [b] Errors for $-\Delta G_{ass}$ were estimated as <0.3 kJ mol^{-1}. Errors were estimated from the quality of the fit of the experimental to the calculated data and by carrying out several titration experiments to obtain several estimates of K_{ass} and averaging the values obtained.

each of the substrates the largest shift in the ^1H NMR spectrum was for the amidopyridine NH^1 signal (for the atom labelling used, see Fig. 2), which was consistent with our expectation that the primary binding interaction would be between the carboxylic acid terminus of the guests and the diamidopyridine of the host. Other shifts in the ^1H NMR spectrum of **3** were noted on addition of most substrates and, in particular, we recorded the shift of all the macrobicycle amide NH's in the titration experiments, since some tentative inferences may be drawn concerning hydrogen bonding interactions in the complexes from the magnitude of these shifts.[15] Small shifts in the aromatic signals for the pyridine and phenylalanine rings were also consistently observed, presumably reflecting conformational changes of the macrobicycle on binding, although the shifts of these aromatic protons overlapped with other signals in the course of the titration, or were not generally large enough (<0.1 ppm), to provide additional data for determination of association constants.[16]

Titration of macrobicycle **3** with phenylacetic acid (Table 1, entry 1) gave $-\Delta G_{ass} = 14.2$ kJ mol^{-1} with a limiting downfield shift of the amidopyridine NH^1 signal in the ^1H NMR of 0.45 ppm, but with no significant changes to the rest of the signals for **3**. Similarly, titration with hexanoic acid (entry 2) gave $-\Delta G_{ass} = 15.0$ kJ mol^{-1} with a limiting downfield shift of the amidopyridine NH^1 signal in the ^1H NMR of 0.28 ppm. Again, these results are consistent with the anticipated binding of the carboxylic acid functionality by the amidopyridine unit, and give a good indication of the strength of this interaction (~14–15 kJ mol^{-1}), in this system, against which to compare the binding of the other substrates.[17] Binding of simple amino acid derivatives (entries 3–14) generally showed improved binding over phenylacetic acid and hexanoic acid, with correspondingly larger shifts of the amidopyridine NH^1 signal. In addition, binding of these substrates resulted in downfield shifts of the benzylic amide proton H^7. For alanine and phenylalanine substrates (entries 4–9) there is a small preference for the D-amino

acid, but there is a reversal of this selectivity for the valine and serine substrates (entries 10–13). For the D-phenylalanine substrates we investigated the effect of varying the N-protecting group (*t*Boc, Cbz, Ac). The binding of these substrates increased significantly with decreasing size of the N-protecting group (entries 7–9) which may be attributed to a binding interaction between the N-protecting group functionality and the macrobicycle, which is weakened with the increasing steric demands of the protecting group (although the change from carbamate (*t*Boc, Cbz) to amide (Ac) may also influence the relative binding of these three substrates). In comparison to the *t*Boc phenylalanine substrates, the poor binding of the *t*Boc valine substrates (entries 10, 11) may be attributed to steric demands of the side-chain as well as of the bulky *t*Boc group, while the *t*Boc serine substrates (entries 12, 13) showed substantially better binding, indicating a positive interaction between the hydroxymethyl moiety of the substrate and the receptor. Binding of the *t*Boc serine substrates was accompanied by a substantial downfield shift of the benzylic amide proton H^7 (>0.3 ppm) and *upfield* shift of amide proton H^5 (>0.3 ppm), which was not evident on binding of the other *t*Boc amino acids. One possible explanation for this latter observation is that binding the serine substrates requires a conformational change in the macrobicycle or involves formation of a hydrogen bond to $C{=}O^4$, which in turn breaks the intramolecular hydrogen bond (found in both molecular modelling and NMR studies) to NH^5.

Dipeptide substrates did not generally show substantially better binding compared with the single amino acid derivatives [although strong binding was observed for Cbz-glycinyl-L-serine (entry 17) with large downfield shifts of H^1 (~1 ppm), H^3 (>0.4 ppm) and H^7 (>0.35 ppm), and an upfield shift of H^5 (~0.3 ppm)] until the β-alanylalanine substrates were used (entries 21 and 22). Macrobicycle **3** again showed a preference for the D-substrate (Cbz-β-alanyl-D-alanine, $-\Delta G_a = 22.8$ kJ mol^{-1}, Cbz-β-alanyl-L-alanine, $-\Delta G_a = 19.1$ kJ mol^{-1}), and both the β-alanylalanine substrates were significantly better bound than the corresponding alanine substrates (entries 4, 5) or the alanylalanine substrates (entries 19 and 20). The extent of this increase in binding suggests that strong additional binding interactions had been formed.

We also measured the binding of the ester linked Cbz-β-alanyllactic acids (entries 23 and 24) as substrates for macrobicycle **3**, effectively replacing a hydrogen bond donor substituent (NH) with a hydrogen bond acceptor substituent (O-lone pair) in the guest structure. The binding energies for both enantiomers of Cbz-β-alanyllactic acid (11.3, 13.2 kJ mol^{-1}) are smaller than the binding energy measured for phenylacetic acid (14.2 kJ mol^{-1}) indicating that these substrates apparently gain no stabilisation on binding other than from the carboxylic acid interaction with the amidopyridine binding site. The substantially lower binding energies for these esters compared to the corresponding Cbz-β-alanylalanines strongly suggest that a hydrogen bond from the guest amide NH^r (for the atom labelling, see Fig. 2) to the receptor side wall is a key interaction in the binding of the latter—a result which is strongly supported by the molecular modelling studies (*vide infra*), which suggest that binding of Cbz-β-alanyl-D-alanine involves a hydrogen bond from NH^r to $C{=}O^4$ (although the weaker binding might alternatively result from the replacement of the relatively good hydrogen bond acceptor provided by the alanyl amide carbonyl with the less effective lactate ester carbonyl, and as a consequence of the ester being inherently more flexible than the amide). Coincidentally, a very similar result was observed with receptor **1**,[1d] which also bound Cbz-β-alanylalanine substrates strongly (Cbz-β-alanyl-L-alanine, $-\Delta G_a = 19.9$ kJ mol^{-1}, Cbz-β-alanyl-D-alanine, $-\Delta G_a = 16.6$ kJ mol^{-1}) and Cbz-β-alanyllactic acids much more weakly (Cbz-β-alanyl-L-lactic acid, $-\Delta G_a = 13.1$ kJ mol^{-1}, Cbz-β-alanyl-D-lactic acid, $-\Delta G_a = 11.2$ kJ mol^{-1}), although the selectivity is more pronounced with macrobicycle **3**. This result is of some interest since certain bacteria have mutated their cell wall precursor structure from a terminal Cbz-D-alanyl-D-alanine sequence to a Cbz-D-alanyl-D-lactic acid sequence, thus providing immunity to conventional antibacterials such as the natural D-alanyl-D-alanine receptor, vancomycin.[18]

NMR studies

The binding studies show that macrobicycle **3** is a potent and selective receptor for certain dipeptide guests, as it was designed to be. In order to try to visualise the mode of binding of such guests we undertook further NMR and molecular modelling studies. In particular we wished to ascertain whether the binding of dipeptide guests was indeed within the cavity of the macrobicycle, with the dipeptide backbone threading through the rim formed by the biarylmethane units, or whether the observed binding might simply involve interactions between the guests and the exterior of the macrobicyclic structure. The mode of binding of the most strongly bound guest (Cbz-β-alanyl-D-alanine) was therefore investigated by variable temperature 1D and 2D ^{1}H NMR and molecular modelling.

1D and 2D TOCSY[19] ^{1}H NMR spectra of the 1 : 1 complex (3.0 mM concentration) at 25 °C allowed assignment of most protons for both the host and the guest, and showed that many of the signals are shifted significantly on complexation relative to the free host and free guest (Fig. 2). Thus, for example, three of the NH's of the host (NH^1, NH^3 and NH^7) are shifted significantly downfield (0.5–1 ppm) whereas NH^5 is shifted downfield by only 0.2 ppm, but this latter signal is considerably broadened on complexation. For the guest the alanyl amide NH^r is shifted downfield by 0.6 ppm. The alanyl methyl (CH_3^p) and alanyl α-proton (H^q) of the guest are shifted upfield upon complexation by 0.2 and 0.5 ppm respectively, whilst the phenylalanyl α-proton (H^2) of the host was shifted downfield by 0.4 ppm indicating a significant change in environment for these protons on complexation that is consistent with binding of the substrate within the cavity of the macrobicyclic receptor **3** and is supported by the molecular modelling results (*vide infra*). The signals for the β-alanyl portion of the guest in the 1 : 1 complex could not be assigned with certainty, either by inspection of the 1D ^{1}H NMR or from the 2D TOCSY where no cross peaks attributable to the β-alanyl portion were observed. However a reverse titration experiment (addition of a solution of macrobicycle **3** to a solution of Cbz-β-alanyl-D-alanine) showed an upfield shift of the signals for $CH_2^tCH_2^u$ and a downfield shift for NH^v, which could be extrapolated to give the shifts in the 1 : 1 complex.

At an elevated temperature (40 °C), the ^{1}H NMR spectrum showed little change from that at 25 °C, indicating that the guest is still bound at the higher temperature. 2D ROESY spectra of the 1 : 1 mixture at 25 and 40 °C showed only one assignable intermolecular cross peak between host and guest, that being between the alanyl methyl (CH_3^p) of the guest and the phenylalanyl α-proton (H^2) of the host. Comparison of the intensity of this cross peak with other intramolecular cross peaks allowed an estimate of the upper limit of the distance between these signals as 3.6 Å.

It was evident from these NMR studies that various exchange processes were occurring on the NMR timescale which were leading to poor resolution of, in particular, the signals for the β-alanyl portion of the guest. In order to overcome this, a series of 1D NMR spectra at −60, −40, −20 and 0 °C was obtained of free host, free guest and 1 : 1 complex in an attempt to freeze out any exchange between bound and unbound material. As the temperature was lowered for the mixtures, resolution was improved, but many more peaks appeared indicating that a number of conformations of the complex were being observed. An example of this was clearly seen in the region above 8.5 ppm, where at 40 °C, only one signal was seen for the amido-

pyridyl NH[1] but at −40 °C, at least four separate signals could be observed.

Thus the data from the variable temperature and 2D NMR studies indicated a flexible host–guest system with several low energy conformations and in particular the signals for the β-alanyl portion of the guest were poorly resolved. However, the significant shifts in the various signals for the host and the guest on complexation are consistent with complexation of the guest within the cavity of the receptor utilising several binding interactions. The location of the alanyl methyl of the guest close to the phenylalanyl α-proton of the host also provides a useful constraint for subsequent molecular modelling studies.

Molecular modelling

Molecular modelling was also carried out in an attempt to visualise the structure of the complex. The geometry of the free macrobicycle **3** and its complex with Cbz-β-alanyl-D-alanine was examined using a combination of simulated annealing calculations and molecular dynamics using the MacroModel program.[20] The AMBER*[21] force field was used[22] as implemented in MacroModel V5.0 and the effect of solvent was included through the use of the GB/SA chloroform model.[23] Initially the free macrobicycle was energy-minimised using a conjugate gradient (PRCG) and ten simulated annealing calculations of 1 ns, involving slow cooling from 600 to 0.01 K. Three of the structures from these simulated annealing calculations were selected for further 1 ns molecular dynamics calculations at 300 K to examine the behaviour of **3** at room temperature. The simulations generated a large number of low energy structures, which indicated that the free macrobicycle is a very flexible structure. Thus many conformations were observed, including structures in which the pyridine unit was inverted inside the rim of the macrobicycle (*cf.* structure **21**) or one of the phenylalanine-derived benzyl groups was folded inside the rim, but these structures were significantly higher in energy than the predominant form of the macrobicycle, with an open cavity and the diamidopyridine pointing inwards, suitable for binding substrates as desired. A consistent feature of these structures was the presence of intramolecular hydrogen bonds, particularly from NH^5 to $C{=}O^4$, which is entirely consistent with the observed downfield position for the signal for NH^5 in the ^1H NMR of macrobicycle **3** (*vide supra*).

A structure from the room temperature simulation was chosen and the dipeptide Cbz-β-alanyl-D-alanine was docked within the cavity with the peptide chain threaded through the rim of the macrobicyclic receptor, such that the carboxylic acid proton was constrained at 1.8 Å from the pyridyl nitrogen atom and the distance from the alanyl methyl carbon of the guest to the phenylalanyl α-proton (H^2) of the host was constrained at 2.5 ± 2.5 Å, based on distance constraint determined from the 2D NMR studies on the same complex. Similarly, in a separate calculation, the dipeptide Cbz-β-alanyl-D-alanine was docked with the same constraints on the carboxylic acid proton and on the alanyl methyl carbon, but with the peptide chain on the outside of the cavity of the macrobicyclic receptor. The resulting complexes were both subjected to 10 cycles of the same energy minimisation and simulated annealing protocol (from 600 to 0.01 K) used for the free macrobicycle.

With the peptide docked initially within the rim of the macrobicyclic cavity, the simulated annealing procedure led to a series of related structures with several hydrogen bond interactions between host and guest. The two lowest energy structures were selected for a 5 ns molecular dynamics calculation at 300 K (sampling 100 structures for each) to examine the behaviour of the complex at room temperature. The molecular dynamics calculations for both of these two starting structures generated a series of closely related structures which consistently involved the expected motif of two hydrogen bonds from the carboxylic acid to the amidopyridine unit, as well as a clear

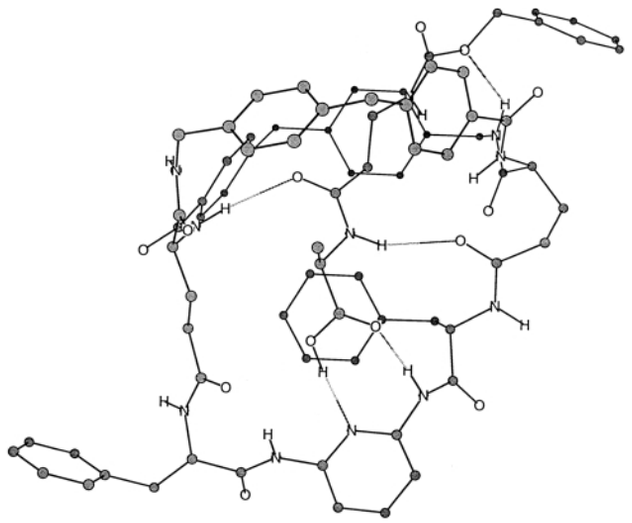

Fig. 3 Typical structure of a 1 : 1 complex between macrobicycle **3** and Cbz-β-alanyl-D-alanine from molecular dynamics calculations, showing hydrogen bond interactions between receptor and substrate.

preference for a hydrogen bond from NH^r of the guest to $C{=}O^4$ of the host—maintained for 81% of the structures sampled during the course of the molecular dynamics—and for a hydrogen bond from $C{=}O^s$ of the guest to $NH^{5'}$ of the host—maintained for 86% of the structures sampled (Fig. 3). The NMR studies revealed that NH^r is shifted 0.6 ppm downfield on complexation, which is entirely consistent with formation of a hydrogen bond to $C{=}O^4$ of the host (with concomitant breaking of the intramolecular hydrogen bond to NH^5). The NMR studies also revealed that NH^5 is only shifted by 0.2 ppm on complexation, but this, again, is consistent with breaking the intramolecular hydrogen bond from NH^5 to $C{=}O^4$ and forming an intermolecular hydrogen bond to $C{=}O^s$. (Conversely, binding with serine substrates was observed in the titration experiments to involve an *upfield* shift of NH^5, which can be explained by formation of a hydrogen bond from the hydroxy side chain of the serine substrate to $C{=}O^4$, which breaks the intramolecular hydrogen bond to H^5.)

The Cbz-β-alanyl portion of the guest, however, adopted many positions relative to the receptor throughout the molecular dynamics calculations, allowing various hydrogen bond interactions from the benzyl carbamate NH^v to $C{=}O^6$ of the receptor, or from the carbamate $C{=}O^w$ or O^x to NH^7 of the receptor. This observation is entirely consistent with the NMR studies, which showed that, in the 1 : 1 complex with **3**, the ^1H signals for the β-alanyl portion of the guest were poorly resolved.

In contrast, structures generated by the simulated annealing protocol, but starting with the peptide guest initially docked on the outside of the cavity, gave a series of structures devoid of any hydrogen bond interactions between host and guest, other than those between the carboxylic acid and the amidopyridine unit. (For one of the calculations the peptide chain was observed to work its way from the outside of the cavity and through the rim to produce a structure closely related to those found in the initial annealing calculations starting with the guest docked *within* the cavity.) The structures generated with the peptide guest on the outside of the cavity were therefore not consistent with the experimentally determined binding selectivities or observed changes to proton signals in the ^1H NMR spectra on complexation.

Thus the modelling and experimental data produce a consistent picture of a flexible receptor, which binds the most strongly bound substrate Cbz-β-alanyl-D-alanine within the cavity of the receptor as hoped, utilising hydrogen bonds to both the NH and C=O of the substrate amide (which are, of course, lost on changing to the substrate Cbz-β-alanyl-D-lactic acid) and

a less well-defined interaction with the benzyl carbamate functionality.

Based on this work it is clear that the basic design of an amidopyridine-derived macrobicycle is an appropriate structure for peptide recognition, but more selective binding properties can be anticipated for structures with greater rigidity—and thus greater levels of preorganisation—as has been borne out by more recent work with macrobicycle **22** which is indeed more selective (particularly for Cbz-L-alanyl-L-alanine) than **3**.[13]

Experimental

General methods

Whenever possible all solvents and reagents were purified according to literature procedures.[24] Thin layer chromatography (TLC) was performed on aluminium-backed sheets (CamLab) coated with either silica gel (SiO_2; 0.25 mm) or neutral alumina, containing fluorescent indicator UV_{254}. Unless otherwise indicated column chromatography was performed on Sorbsil C60, 40–60 mesh silica. All melting points were determined in open capillary tubes using Gallenkamp Electrothermal Melting Point Apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 Fourier Transform Spectrophotometer. ^{1}H NMR spectra at 270 MHz were obtained on a JEOL GX 270, at 300 MHz on a Bruker AC 300, at 360 MHz on a Bruker AM 360 and at 500 MHz on a Varian VXR 500 spectrometer. ^{13}C NMR spectra were recorded at 68 MHz on the JEOL GX 270, at 75 MHz on the Bruker AC 300 and at 90 MHz on the Bruker AM 360. Chemical shifts are reported in ppm on the δ scale, coupling constants in Hz. The multiplicities of the signals were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique. Microanalytical data were obtained from Wellcome Research Laboratories, Beckenham, Kent or The University College of London. Mass spectra were recorded either on a Micromass Platform quadrupole mass analyser with an electrospray ion source, or on a VG Analytical 70-250-SE normal geometry double focusing mass spectrometer at Southampton University. Optical rotations were measured on an AA-100 Polarimeter, and are given in units of 10^{-1} deg cm^2 g^{-1}.

1-Methyl 9-allyl (7*S*)-7-*tert*-butoxycarbonylamino-6-oxo-5-aza-1(1),3(1,4)-dibenzenanonaphane-1,9-dicarboxylate 8

Dicyclohexylcarbodiimide (467 mg, 2.27 mmol) was added to a stirred solution of amine salt **7** (668 mg, 2.27 mmol), *N*-*tert*-butoxycarbonylglutamic acid γ-allyl ester (650 mg, 2.27 mmol), HOBT (311 mg, 2.27 mmol), and diisopropylethylamine (DIPEA) (397 μL, 2.28 mmol) in DMF (20 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for a further 24 hours. The resulting suspension was filtered and the filtrate was concentrated under reduced pressure to give a crude orange solid which was dissolved in EtOAc (10 mL) and washed with 1 M HCl solution (2 × 10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an orange solid which was purified by column chromatography [petroleum ether (bp 40–60 °C)–EtOAc (3 : 2 v/v)] to give **8** as a white powder (797 mg, 67%); R_f [petroleum ether (bp 40–60 °C)–EtOAc (3 : 2 v/v)] 0.47; ν_{max}($CHCl_3$)/cm^{-1} 3429, 3327, 3018, 1716, 1672, 1512; δ_H(300 MHz, $(CD_3)_2SO$) 8.30 (1H, t, *J* 6, N*H*CH_2), 7.85 (2H, d, *J* 8, ArH), 7.35 (2H, d, *J* 8, ArH), 7.18 (4H, s, ArH), 6.95 (1H, d, *J* 8, N*H*CH), 5.90 (1H, m, C*H*=CH_2), 5.28 (1H, d, *J* 16, CH=CH_AH$_B$), 5.18 (1H, d, *J* 9, CH=CH$_A$$H_B$), 4.50 (2H, d, *J* 7, C$H_2$CH=$CH_2$), 4.21 (2H, d, *J* 6, ArCH_2NH), 3.95 (2H, s, ArCH_2Ar), 3.98 (1H, m, NHC*H*), 3.81 (3H, s, OCH_3), 2.36 (2H, t, *J* 7, CH_2COO), 1.90 (1H, m, CH_AH$_B$$CH_2$CO), 1.76 (1H, m, CH$_A$$H_B$$CH_2$CO), 1.38 (9H, s, C($CH_3$)); δ_C(75.5 MHz, $(CD_3)_2SO$) 172.0 (C), 171.6 (C), 166.2 (C), 155.4 (C), 147.2 (C), 138.9 (C), 137.4 (C), 132.8 (CH), 129.4 (CH), 129.0 (CH), 128.7 (CH), 127.5 (C), 127.4 (CH), 117.6 (CH_2), 78.2 (C), 64.4 (CH_2), 53.8 (CH), 52.1 (CH_3), 41.8 (CH_2), 40.6 (CH_2), 30.2 (CH_2), 28.2 (CH_3), 27.2 (CH_2); LRMS (FAB) *m*/*z* 525 (M$^+$ + H, 21%), 469 (12), 425 (32), 407 (20), 154 (100) (Found C, 66.74; H, 7.18; N, 5.67. $C_{29}H_{36}N_2O_7$ requires C, 66.40; H, 6.92; N, 5.34%).

(7*S*)-1^4-Methoxycarbonyl-7-*tert*-butoxycarbonylamino-6-oxo-5-aza-1(1),3(1,4)-dibenzenanonaphane-9-carboxylic acid 9

Tetrakis(triphenylphosphine)palladium(0) (77 mg, 0.067 mmol) was added to a stirred solution of **8** (710 mg, 1.35 mmol), triphenylphosphine (43 mg, 0.17 mmol) and pyrrolidine (0.284 mL, 3.37 mmol) in degassed CH_2Cl_2 (7 mL). The solution was stirred for 30 minutes and the reaction was shielded from light. 1 M HCl (10 mL) was added and the aqueous layer extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with 1 M HCl (10 mL) and brine (10 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give an off-white solid. The product was purified by column chromatography [CH_2Cl_2–MeOH (9 : 1 v/v)] to yield the acid **9** as a white solid (622 mg, 95%); R_f [CH_2Cl_2–MeOH (9 : 1 v/v)] 0.20; ν_{max}($CHCl_3$)/cm^{-1} 3700–3350, 3016, 2930, 1714, 1666; δ_H(270 MHz, $(CD_3)_2SO$) 8.33 (1H, br s, N*H*CH_2), 7.90 (2H, d, *J* 8, ArH), 7.33 (2H, d, *J* 8, ArH), 7.19 (4H, s, ArH), 7.01 (1H, d, *J* 6, N*H*CH), 4.24 (2H, d, *J* 6, ArCH_2NH), 4.00 (2H, s, ArCH_2Ar), 3.93 (1H, m, NHC*H*), 3.82 (3H, s, OCH_3), 2.12 (2H, m, CH_2COO), 1.80 (1H, m, CH_AH$_B$$CH_2$CO), 1.72 (1H, m, CH$_A$$H_B$$CH_2$CO), 1.39 (9H, s, C($CH_3$)$_3$); δ_C(75.5 MHz, $(CD_3)_2SO$) 174.8 (C), 172.0 (C), 166.2 (C), 155.4 (C), 147.0 (C), 138.8 (C), 137.3 (C), 129.4 (CH), 128.9 (CH), 128.7 (CH), 127.6 (C), 127.4 (CH), 78.1 (C), 54.7 (CH), 51.9 (CH_3), 41.9 (CH_2), 40.8 (CH_2), 31.4 (CH_2), 28.1 (CH_3), 27.7 (CH_2); LRMS (FAB) *m*/*z* 485 (M$^+$ + H, 14%), 423 (24), 385 (38), 335 (73), 239 (100).

(1*S*,7*S*)-1,7-Dibenzyl-1,7-bis(*tert*-butyloxycarbonylamino)-3,5-diaza-4(2,6)-pyridinaheptaphane-2,6-dione 5

Dicyclohexylcarbodiimide (4.8 g, 23 mmol) was added to a solution of 2,6-diaminopyridine (0.52 g, 4.8 mmol) and *N*-*tert*-butyloxycarbonylphenylalanine (6.2 g, 24 mmol) in DMF (30 mL) and the mixture was stirred for 72 hours. The resulting yellow suspension was filtered and the filtrate concentrated to half of the original volume. EtOAc (75 mL) was added and the solution was washed with 1 M HCl (3 × 50 mL), 1 M NaOH (3 × 50 mL), brine (2 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give a foam, which was purified by flash column chromatography [petroleum ether (bp 40–60 °C)–EtOAc (7 : 3 v/v)] to yield the diamidopyridine **5** as a foam (2.42 g, 85%), mp 88–90 °C; $[a]_D$ +32.2 (c = 1.0 in MeOH); ν_{max}(KBr)/cm^{-1} 2980, 1685, 1585, 1510, 1448; δ_H(300 MHz, $(CD_3)_2SO$) 10.22 (2 H, s, pyrNH), 7.77 (3 H, br s, pyrH), 7.39–7.16 (12 H, m, PhH, NHBoc), 4.48 (2 H, m, CHCO), 3.03 (2 H, dd, *J* 13 and 5, CH_AH$_B$Ph), 2.82 (2 H, dd, *J* 13 and 11, CH$_A$$H_B$Ph), 1.31 (18 H, s, CH_3); δ_C(75.5 MHz, $(CD_3)_2SO$) 171.8 (C), 155.6 (C), 150.2 (C), 140.2 (CH), 138.0 (C), 129.4 (CH), 128.1 (CH), 126.4 (CH), 109.3 (CH), 78.3 (C), 56.5 (CH), 37.1 (CH_2), 28.2 (CH_3); LRMS (ES) *m*/*z* 626.2 (M$^+$ + Na, 13%), 604.2 (M$^+$ + H, 25) (Found C, 65.27; H, 6.92; N 11.28. $C_{33}H_{41}N_5O_6$ requires C, 65.65; H, 6.85; N, 11.60%).

Bis(trifluoroacetate) salt 6

Trifluoroacetic acid (2.0 mL) was added to a solution of **5** (0.57 g, 0.95 mmol) in CH_2Cl_2 (10 mL) and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the resulting yellow–brown oil was triturated with ether (10 mL) and filtered to give the ammonium salt **6** as a pale yellow powder (0.485 g, 85%), mp 140–143 °C; ν_{max}(KBr)/cm^{-1} 3032, 2935, 1669, 1587, 1540, 1499; δ_H(300 MHz, $CDCl_3$/$(CD_3)_2SO$, 95 : 5 v/v) 10.56 (s, 2 H, pyrNH), 8.45 (s, 6 H, NH_3), 7.74 (d, *J* 7.4, 2 H, pyrH), 7.60 (t, *J* 7.4, 1 H, pyrH), 7.23–7.08 (m, 10 H,

PhH), 4.41 (br s, 2 H, NCHCO), 3.19 (dd, *J* 14.0 and 7.0, 2 H, C$H_A$$H_B$Ph), 3.08 (dd, *J* 14.0 and 7.0, 2 H, CH$_A$$H_B$Ph).

(7*S*,12*S*,18*S*,23*S*)-Dimethyl 12,18-dibenzyl-7,23-bis(*tert*-butoxycarbonylamino)-6,10,13,17,20,24-hexaoxo-5,11,14,16,19,25-hexaaza-1,29(1),3,27(1,4)-tetrabenzena-15(2,6)-pyridinanonacosaphane-1^4,29^4-dicarboxylate 10

DIPEA (0.07 mL, 0.4 mmol) was added to a solution of acid **9** (100 mg, 0.207 mmol), bis-TFA salt **6** (44 mg, 0.068 mmol) and PyBOP (108 mg, 0.207 mmol) in DMF (1 mL) and the mixture was stirred at 50 °C for 18 hours. The reaction mixture was diluted with EtOAc (10 mL) and washed with 2 M HCl solution (8 × 5 mL), sat. $NaHCO_3$ (5 mL), brine (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography [CH_2Cl_2–MeOH (97 : 3 v/v)] to give diester **10** as a pale brown powder (66 mg, 71%); R_f [CH_2Cl_2–MeOH (95 : 5 v/v)] 0.63; $[a]_D$ +6.2 (*c* = 1.0 in MeOH); ν_{max}($CHCl_3$)/cm^{-1} 3427, 1715, 1663, 1530; δ_H(270 MHz, $(CD_3)_2$SO) 10.34 (br s, 2 H, pyrNH), 8.29 (br s, 4 H, CH_2NH and CH_2CONH), 7.91 (d, *J* 8, 4 H, ArH), 7.78 (s, 3 H, pyrH), 7.42–7.14 (m, 22 H, ArH), 6.94 (d, *J* 6, 2 H, BocN*H*), 4.84 (m, 2 H, $PhCH_2CH$), 4.26 (d, *J* 6, 4 H, $ArCH_2NH$), 4.02 (s, 4 H, $ArCH_2Ar$), 3.91 (m, 2 H, $CHCH_2CH_2$), 3.84 (s, 6 H, CO_2CH_3), 3.10 (m, 2 H, PhC$H_A$$H_B$), 2.85 (m, 2 H, PhCH$_A$$H_B$), 2.24–2.04 (m, 4 H, CH_2CH_2CO), 1.82–1.62 (m, 4 H, CH_2CH_2CO), 1.39 (s, 18 H, $C(CH_3)$); δ_C(67.9 MHz, $(CD_3)_2$SO) 171.8 (C), 171.7 (C), 171.1 (C), 166.0 (C), 155.2 (C), 149.9 (C), 147.0 (C), 140.1 (CH), 138.7 (C), 137.5 (C), 137.1 (C), 129.2 (CH), 129.1 (CH), 128.8 (CH), 128.5 (CH), 127.9 (CH), 127.1 (CH), 126.2 (CH), 109.2 (CH), 78.0 (C), 54.5 (CH), 54.0 (CH), 51.9 (CH_3), 41.6 (CH_2), 40.4 (CH_2), 37.0 (CH_2), 31.6 (CH_2), 28.0 (CH_3), 27.7 (CH_2); LRMS (FAB) *m*/*z* 1336 (M^+ + H, 4%), 335 (63), 239 (91), 120 (100) (Found C, 67.39; H, 6.46; N, 9.38. $C_{75}H_{85}N_9O_{14}$ requires C, 67.40; H, 6.41; N, 9.43%).

Attempted hydrolysis of bis-methyl ester 10

1 M LiOH (1 mL) was added to a solution of diester **10** (28 mg, 0.02 mmol) in 1,4-dioxane (1 mL) and the mixture stirred at room temperature for 22 hours. The reaction mixture was concentrated under reduced pressure to give a white solid, which was partitioned between EtOAc (5 mL) and water (5 mL). The aqueous layer was acidified to pH 1 with 1 M HCl and extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give diacid **11** as a white foam (23 mg, 92%); R_f [CH_2Cl_2–MeOH (9 : 1 v/v)] 0.36; ν_{max}($CHCl_3$)/cm^{-1} 3500–3200, 1711, 1642; δ_H(270 MHz, $(CD_3)_2$SO) 8.21 (t, *J* 6, 1 H, N*H*CH_2), 8.15 (d, *J* 8, 1 H, N*H*$COCH_2$), 7.85 (d, *J* 8, 2 H, ArH), 7.33 (d, *J* 8, 2 H, ArH), 7.28–7.10 (m, 9 H, ArH), 6.90 (d, *J* 8, 1 H, CH_2CHNH), 4.40 (m, 1 H, $CHCH_2Ph$), 4.22 (d, *J* 6, 2 H, $ArCH_2NH$), 3.99 (s, 2 H, $ArCH_2Ar$), 3.87 (m, 1 H, CH_2CH_2CH), 3.15 (dd, *J* 5 and 11, 1 H, PhC$H_A$$H_B$), 2.83 (dd, *J* 9 and 11, 1 H, PhCH$_A$CH_B), 2.10 (m, 2 H, CH_2CH_2CO), 1.75 (m, 1 H, C$H_A$$H_B$$CH_2CO$), 1.65 (m, 1 H, CH$_A$$H_B$$CH_2CO$), 1.36 (s, 9 H, CH_3); δ_C(67.9 MHz, $(CD_3)_2$SO) 173.0 (C), 171.9 (C), 171.6 (C), 171.5 (C), 167.1 (C), 155.2 (C), 146.5 (C), 138.8 (C), 137.5 (C), 137.1 (C), 129.4 (CH), 128.9 (CH), 128.7 (CH), 128.5 (CH), 128.0 (CH), 127.1 (CH), 126.2 (CH), 77.9 (C), 54.0 (CH), 53.2 (CH), 41.6 (CH_2), 40.4 (CH_2), 36.6 (CH_2), 31.7 (CH_2), 28.0 (CH_3), 27.7 (CH_2); LRMS (FAB): *m*/*z* 618 (M^+ + H, 47%), 518 (58), 335 (100), 225 (85).

Benzyl α-bromo-*p*-toluate 12

A solution of α-bromo-*p*-toluic acid (21.5 g, 0.10 mol), benzyl alcohol (14.7 mL, 0.13 mol), *p*-TsOH·H_2O (1.9 g, 10 mmol) in toluene (250 mL) was heated to reflux and water removed in a Dean–Stark trap. After 18 hours, the mixture was allowed to cool and most of the toluene was removed under reduced pressure. The residue was dissolved in EtOAc (180 mL) and washed with saturated $NaHCO_3$ (2 × 80 mL), brine (80 mL) and dried (Na_2SO_4). Solvent was removed under reduced pressure to give a colourless oil which solidified upon standing. The product was triturated with cold hexane and filtered to give ester **12** as a white solid (23.9 g, 80%); mp (hexane–EtOAc): 63–65 °C; ν_{max}($CHCl_3$)/cm^{-1} 3033, 2955, 1712, 1608, 1575, 1493, 1447; δ_H(300 MHz, $CDCl_3$) 8.07 (dt, *J* 8 and 1, 2 H, ArH), 7.49–7.36 (m, 7 H, ArH), 5.38 (s, 2 H, CH_2Ph), 4.51 (s, 2 H, $ArCH_2Br$); δ_C(75.5 MHz, $CDCl_3$) 166.0 (C), 142.9 (C), 136.1 (C), 130.4 (CH), 130.2 (C), 129.2 (CH), 128.1 (CH), 128.5 (CH), 128.3 (CH), 67.0 (CH_2), 32.3 (CH_2); LRMS (CI) *m*/*z* 322 (M^+ + NH_4, 100%), 305 (M^+ + H, 6), 244 (52) (Found C, 58.92; H, 4.31. $C_{15}H_{13}BrO_2$ requires C, 59.04; H, 4.29%).

Benzyl 4-(4′-cyanophenyl)methylbenzoate 13

Bromo ester **12** (8.4 g, 28 mmol), 4-cyanophenylboronic acid (5.3 g, 36 mmol) and tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.018 mmol) were stirred in degassed dimethoxyethane (100 mL) for 5 minutes to give a clear yellow solution. Degassed 2 M Na_2CO_3 (25 mL, 50 mmol) was added and the reaction was refluxed for 4 hours. After cooling, water (100 mL) was added and the mixture stirred for 5 minutes. The organic layer was run off and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). The organics were combined, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give an oil, which was purified by flash column chromatography [petroleum ether (bp 40–60 °C)–EtOAc (5 : 95 v/v)], to give the cyano ester **13** as white crystals (6.10 g, 68%), mp 69–70 °C; ν_{max}(KBr)/cm^{-1} 3031, 2957, 2224, 1717, 1604, 1494; δ_H(300 MHz, $CDCl_3$) 8.04 (dt, *J* 8 and 2, 2 H, ArH), 7.60 (dt, *J* 8 and 2, 2 H, ArH), 7.48–7.35 (m, 7 H, ArH), 7.28 (d, *J* 8, 2 H, ArH), 7.25 (d, *J* 8, 2 H, ArH), 5.37 (s, 2 H, CH_2Ph), 4.10 (s, 2 H, $ArCH_2Ar$); δ_C(75.5 MHz, $CDCl_3$) 166.3 (C), 145.8 (C), 144.9 (C), 136.2 (C), 132.6 (CH), 130.4 (CH), 129.8 (CH), 129.2 (CH), 128.9 (C), 128.8 (CH), 128.4 (CH), 128.3 (CH), 119.0 (C), 110.6 (C), 66.9 (CH_2), 42.1 (CH_2) (Found C, 80.58; H, 5.11; N, 4.16. $C_{22}H_{17}NO_2$ requires C, 80.71; H, 5.23; N, 4.28%).

Benzyl 4-(4′-aminomethylphenyl)methylbenzoate hydrochloride salt 14

Borane–dimethyl sulfide complex (3.6 mL of a 2 M solution in THF, 7.2 mmol) was added over 5 min to a stirred solution of benzyl 4-(4′cyanophenyl)methylbenzoate **13** (2.00 g, 6.12 mmol) in THF (60 mL) at reflux. The resulting mixture was stirred at reflux for 5 hours to give a clear solution. The reaction was cooled to room temperature and quenched by adding HCl (9.2 mL of a 0.8 M solution in MeOH). The resulting white suspension was heated at reflux for 15 minutes and concentrated under reduced pressure to give a white solid, which was triturated with dry MeOH (15 mL). The MeOH was removed and the resulting white solid suspended in boiling MeOH (10 mL) for 5 minutes before being isolated by filtration to give the amine hydrochloride salt **14** as a white solid (1.61 g, 72%); mp 220–222 °C; ν_{max}(KBr)/cm^{-1} 3031, 2580, 2360, 1715, 1609, 1591, 1482; δ_H(300 MHz, $(CD_3)_2$SO) 8.48 (s, 3 H, NH_3), 7.93 (d, *J* 8, 2 H, ArH), 7.47–7.34 (m, 9 H, ArH), 7.28 (d, *J* 8, 2 H, ArH), 5.34 (s, 2 H, CH_2Ph), 4.04 (s, 2 H,CH_2N), 3.95 (s, 2 H, $ArCH_2Ar$); δ_C(75.5 MHz, $(CD_3)_2$SO) 165.6 (C), 147.1 (C), 140.9 (C), 136.3 (C), 132.1 (C), 129.6 (CH), 129.3 (CH), 129.2 (CH), 129.0 (CH), 128.6 (CH), 128.2 (CH), 128.0 (CH), 127.6 (C), 66.1 (CH_2), 41.9 (CH_2), 40.7 (CH_2); LRMS (ES^+) *m*/*z* 332.4 (M^+ + H) (Found C, 72.13; H, 5.99; N, 3.64. $C_{22}H_{22}ClNO_2$ requires C, 71.83; H, 6.03; N, 3.81%).

1-Benzyl 9-allyl (7*S*)-7-*tert*-butoxycarbonylamino-6-oxo-5-aza-1(1),3(1,4)-dibenzenanonaphane-1^4,9-dicarboxylate 15

DIPEA (6.3 mL, 36 mmol) was added to a suspension of amine hydrochloride salt **14** (3.4 g, 9.2 mmol), *N*-*tert*-butoxycarbonyl-

L-glutamic acid γ-allyl ester (3.07 g, 10.6 mmol) and PyBOP (5.9 g, 11 mmol) in DMF (200 mL) and the resulting solution was stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure to give an orange oil which was dissolved in EtOAc (50 mL), washed with 1 M HCl (10 mL), water (5 × 10 mL), brine (10 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure to give a solid which was purified by column chromatography [petroleum ether (bp 40–60 °C)–EtOAc (3 : 2 v/v)] to afford the allyl ester **15** as a white solid (5.10 g, 93%); mp 56–58 °C (EtOAc–hexane); $[a]_D$ +3.6 (c = 1.0 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3030, 2979, 2940, 1720, 1682, 1655, 1609, 1521; δ_H(300 MHz, $CDCl_3$) 8.01 (d, J 8, 2 H, ArH), 7.47–7.12 (m, 12 H, ArH and N*H*CH_2), 6.53 (br s, 1 H, N*H*Boc), 5.91 (ddt, J 17, 10 and 6, 1 H, C*H*=CH_2), 5.36 (s, 2 H, CH_2Ph), 5.32 (dq, J 17 and 1, 1 H, CH=CH_AH$_B$), 5.24 (dq, J 10 and 1, 1 H, CH=CH$_A$$H_B$), 4.58 (dt, J 6 and 1, 2 H, CH_2CH=CH_2), 4.42 (d, J 5, 2 H, NCH_2), 4.17 (m, 1 H, NCHCO), 4.02 (s, 2 H, ArCH_2Ar), 2.55 (dt, J 17 and 7, 1 H, CH_AH$_B$$CO_2$), 2.43 (dt, J 17 and 7, 1 H, CH$_A$$H_B$$CO_2$), 2.17 (m, 1 H, C$H_AH_B$$CH_2CO_2$), 1.96 (m, 1 H, CH$_A$$H_B$$CH_2CO_2$), 1.41 (s, 9 H, CH_3); δ_C(75.5 MHz, $CDCl_3$) 176.2 (C), 172.1 (C), 166.5 (C), 156.4 (C), 146.6 (C), 139.3 (C), 136.1 (C), 136.0 (C), 131.9 (CH), 130.0 (CH), 129.1 (CH), 128.9 (CH), 128.6 (CH), 128.2 (CH), 128.1 (CH), 128.0 (C), 127.7 (CH), 118.5 (CH_2), 80.7 (C), 66.6 (CH_2), 65.4 (CH_2), 53.3 (CH), 43.1 (CH_2), 41.5 (CH_2), 30.0 (CH_2), 28.3 (CH_3), 27.8 (CH_2); LRMS (ES$^+$) m/z 623.6 (M^+ + Na), 601.6 (M^+ + H) (Found C, 69.96; H, 6.72; N, 4.66. $C_{35}H_{40}N_2O_7$ requires C, 69.98; H, 6.71; N, 4.66%).

(7*S*)-1^4-Phenylmethoxycarbonyl-7-*tert*-butoxycarbonylamino-6-oxo-5-aza-1(1),3(1,4)-dibenzenanonaphane-9-carboxylic acid 16

Tetrakis(triphenylphosphine)palladium(0) (0.52 g, 0.45 mmol) was added to a solution of allyl ester **15** (5.0 g, 8.3 mmol), pyrrolidine (1.6 mL, 19 mmol) and triphenylphosphine (0.22 g, 0.85 mmol) in degassed CH_2Cl_2 (100 mL) and the resulting yellow solution was stirred at room temperature for 22 hours. 1 M HCl (50 mL) was added and the CH_2Cl_2 layer was separated. The aqueous phase was extracted with EtOAc (30 mL) and the organics were combined, washed with 1 M HCl (70 mL), brine (50 mL), H_2O (50 mL), dried (Na_2SO_4) and filtered. The solution was concentrated under reduced pressure to give an off-white solid. Recrystallisation from EtOAc–petroleum ether (bp 40–60 °C) gave the title compound as a white solid (4.42 g, 95%), mp 103–106 °C [EtOAc–petroleum ether (bp 40–60 °C)]; $[a]_D$ +2.0 (c = 1.0 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3323, 2978, 2929, 1717, 1653, 1609, 1575; δ_H(300 MHz, $(CD_3)_2SO$) 12.11 (s, 1 H, CO_2H), 8.28 (t, J 6, 1 H, CH_2N*H*), 7.91 (d, J 8, 2 H, ArH), 7.47–7.34 (m, 7 H, ArH), 7.16 (s, 4 H, ArH), 6.94 (d, J 8, 1 H, $NHCO_2$), 5.33 (s, 2 H, CH_2Ph), 4.23 (d, J 6, 2 H, CH_2N), 4.00 (s, 2 H, ArCH_2Ar), 3.93 (m, 1 H, NHC*H*CO), 2.23 (t, J 8, 2 H, CH_2CO_2), 1.87 (m, 1 H, CH_AH$_B$$CH_2CO_2$), 1.72 (m, 1 H, CH$_A$$H_B$$CH_2CO_2$), 1.36 (s, 9 H, CH_3); δ_C(75.5 MHz, $(CD_3)_2SO$) 173.9 (C), 171.7 (C), 165.4 (C), 155.3 (C), 147.3 (C), 138.8 (C), 137.3 (C), 136.2 (C), 129.4 (CH), 129.0 (CH), 128.6 (CH), 128.5 (CH), 128.0 (CH), 127.8 (CH), 127.3 (C), 127.2 (CH), 78.0 (C), 65.9 (CH_2), 53.8 (CH), 41.7 (CH_2), 40.5 (CH_2), 30.2 (CH_2), 28.1 (CH_3), 27.1 (CH_2); LRMS (ES$^+$) m/z 583.2 (M^+ + Na), 561.7 (M^+ + H) (Found C, 68.51; H, 6.39; N, 4.92. $C_{32}H_{36}N_2O_2$ requires C, 68.56; H, 6.47; N, 5.00%).

Dibenzyl (7*S*,12*S*,18*S*,23*S*)-12,18-dibenzyl-7,23-bis(*tert*-butoxycarbonylamino)-6,10,13,17,20,24-hexaoxo-5,11,14,16,19,25-hexaaza-1,29(1),3,27(1,4)-tetrabenzena-15(2,6)-pyridinanonacosaphane-1^4,29^4-dicarboxylate 17

DIPEA (0.30 mL, 1.7 mmol) was added to a solution of acid **16** (464 mg, 0.859 mmol), bis-TFA salt **6** (180 mg, 0.286 mmol) and PyBOP (449 mg, 0.859 mmol) in DMF (5 mL) and the mixture was stirred under nitrogen at 50 °C for 18 hours. Solvent was removed under reduced pressure and the resulting red–brown oil was taken up in EtOAc (20 mL) and washed with 2 M HCl (8 × 10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL), and dried (Na_2SO_4). The crude product was filtered, concentrated under reduced pressure and purified by column chromatography [CH_2Cl_2–MeOH (97 : 3 v/v)] to give a brown powder which was recrystallised from EtOAc–petroleum ether (bp 40–60 °C) to give diester **17** as a pale brown solid (264 mg, 64%); mp 156–158 °C [EtOAc–petroleum ether (bp 40–60 °C)]; $[a]_D$ +46.7 (c = 1.0 in DMF); ν_{max}(KBr)/cm^{-1} 3305, 2974, 2927, 1715, 1684, 1653, 1608, 1585; δ_H(300 MHz, $(CD_3)_2SO$) 10.35 (br s, 2 H, pyrNH), 8.27–8.22 (m, 4 H, N*H*COCH_2 and N*H*CH_2), 7.91 (d, J 8, 4 H, ArH), 7.76 (s, 3 H, pyrH), 7.46–7.13 (m, 32 H, ArH), 6.90 (d, J 8, 2 H, NHBoc), 5.33 (s, 4 H, OCH_2Ph), 4.82 (m, 2 H, PhCH_2C*H*), 4.23 (d, J 5, 4 H, NHCH_2Ar), 3.99 (s, 4 H, ArCH_2Ar), 3.87 (m, 2 H, C*H*$(CH_2)_2$), 3.06 (dd, J 13 and 3, 2 H, PhCH_AH$_B$), 2.83 (dd, J 13 and 11, 2 H, PhCH$_A$$H_B$), 2.12 (m, 4 H, CH_2CH_2CO), 1.72 (m, 4 H, C$H_2$$CH_2$CO), 1.36 (s, 18 H, CH_3); δ_C(75.5 MHz, $(CD_3)_2SO$) 171.8 (C), 171.6 (C), 171.1 (C), 165.3 (C), 155.1 (C), 149.9 (C), 147.2 (C), 140.0 (CH), 138.7 (C), 137.5 (C), 137.1 (C), 136.1 (C), 129.3 (CH), 129.1 (CH), 128.9 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.7 (CH), 127.2 (C), 127.1 (CH), 126.2 (CH), 109.2 (CH), 77.9 (C), 65.8 (CH_2), 54.5 (CH), 54.0 (CH), 41.6 (CH_2), 40.4 (CH_2), 37.0 (CH_2), 31.6 (CH_2), 28.0 (CH_3), 27.7 (CH_2); LRMS (FAB) m/z 1488 (M^+ + H, 5%), 335 (8), 315 (12), 91 (100) (Found C, 69.11; H, 6.25; N, 8.23. $C_{87}H_{93}N_9O_{14}$·H_2O requires C, 69.35; H, 6.36; N, 8.37%).

(7*S*,12*S*,18*S*,23*S*)-12,18-Dibenzyl-7,23-bis(*tert*-butoxycarbonylamino)-6,10,13,17,20,24-hexaoxo-5,11,14,16,19,25-hexaaza-1,29(1),3,27(1,4)-tetrabenzena-15(2,6)-pyridinanonacosaphane-1^4,29^4-dicarboxylic acid 18

A solution of diester **17** (260 mg, 0.175 mmol) with NH_4CO_2H (313 mg, 5 mmol) and 10% Pd/C (50 mg) in DMF (4 mL) was stirred for 3 hours. The reaction was filtered through Celite and concentrated under reduced pressure to give the diacid **18** as a white solid (225 mg, 98%); mp 176–178 °C (EtOAc–hexane); $[a]_D$ +11.7 (c = 1.0 in DMF); ν_{max}(KBr)/cm^{-1} 3295, 2975, 2928, 1711, 1653, 1608, 1584, 1513; δ_H(300 MHz, $CDCl_3$) 10.35 (s, 2 H, pyrNH), 8.27–8.22 (m, 4 H, CH_2N*H* and N*H*COCH_2), 7.86 (d, 4 H, J 8, ArH), 7.76 (s, 3 H, pyrH), 7.34–7.14 (m, 22 H, ArH), 6.91 (d, J 8, 2 H, NHBoc), 4.82 (m, 2 H, C*H*CH_2Ph), 4.23 (d, J 6, 4 H, CH_2N), 3.97 (s, 4 H, ArCH_2Ar), 3.87 (m, 2 H, C*H*NHBoc), 3.06 (dd, J 13 and 4, 2 H, PhCH_AH$_B$), 2.83 (dd, J 13 and 11, 2 H, PhCH$_A$$H_B$), 2.23–1.99 (m, 4 H, CH_2CO), 1.82–1.58 (m, 4 H, C$H_2$$CH_2$CO), 1.36 (s, 18 H, CH_3); δ_C(67.9 MHz, $(CD_3)_2SO$) 172.3 (C), 172.1 (C), 171.4 (C), 167.5 (C), 155.6 (C), 150.3 (C), 146.9 (C), 140.4 (C), 139.2 (C), 137.8 (C), 137.4 (C), 129.8 (CH), 129.5 (CH), 129.3 (CH), 129.0 (CH), 128.9 (CH), 128.3 (CH), 127.5 (CH), 126.6 (CH), 109.6 (CH), 78.4 (C), 54.9 (CH), 54.4 (CH), 42.0 (CH_2), 40.8 (CH_2), 37.4 (CH_2), 32.0 (CH_2), 28.4 (CH_3), 28.1 (CH_2); LRMS (FAB) m/z 1309 (M^+ + H, 19%), 335 (61), 238 (100), 225 (76) (Found C, 66.36; H, 6.26; N, 9.38. $C_{73}H_{81}N_9O_{14}$·H_2O requires C, 66.10; H, 6.31; N, 9.50%).

Bis(pentafluorophenyl) (7*S*,12*S*,18*S*,23*S*)-12,18-dibenzyl-7,23-bis(*tert*-butyloxycarbonylamino)-6,10,13,17,20,24-hexaoxo-5,11,14,16,19,25-hexaaza-1,29(1),3,27(1,4)-tetrabenzena-15(2,6)-pyridinanonacosaphane-1^4,29^4-dicarboxylate 19

Dicyclohexylcarbodiimide (113 mg, 0.548 mmol) was added to a solution of diacid **18** (230 mg, 0.176 mmol), pentafluorophenol (100 mg, 0.543 mmol) and DMAP (14 mg, 115 mmol) in THF (5 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 16 hours. The reaction was filtered and the filtrate diluted with CH_2Cl_2 (10 mL). The organics were washed with 0.5 M HCl (10 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude title

compound as a white solid (285 mg) which was used directly in the next step without further purification.

Bis(trifluoroacetate) salt 20

Trifluoroacetic acid (3 mL) was added to a suspension of crude activated ester **19** (285 mg, ~0.18 mmol) in CH_2Cl_2 (6 mL) and the resulting solution was stirred at room temperature for 2 hours. Solvent and most of the trifluoroacetic acid was removed under reduced pressure to give a brown gum, which upon trituration with diethyl ether (3 × 10 mL) yielded a white solid, which was dried under reduced pressure to give the crude bis-TFA salt **20** (315 mg) which was used directly in the next step without further purification; δ_H(of crude **20**) (270 MHz, $(CD_3)_2SO$) 10.47 (s, 2 H, pyrNH), 8.90 (t, *J* 3, 2 H, N*H*CH_2), 8.46 (d, *J* 6, 2 H, N*H*$COCH_2$), 8.23 (br s, 6 H, NH_3^+), 8.13 (d, *J* 8, 4 H, ArH), 7.78 (s, 3 H, pyrH), 7.55 (d, *J* 8, 4 H, ArH), 7.42–7.18 (m, 22 H, ArH), 4.87 (m, 2 H, PhCH_2C*H*), 4.41 (dd, *J* 3 and 13, 2 H, NHC$H_A$$H_B$Ar), 4.32 (dd, *J* 5 and 13, 2 H, NHC$H_A$$H_B$Ar), 4.12 (s, 2 H, ArC$H_2$Ar), 3.78 (m, 2 H, C*H*$CH_2$), 3.11 (dd, *J* 3 and 13, 2 H, PhC$H_A$$H_B$), 2.87 (dd, *J* 11 and 13, 2 H, PhC$H_A$$H_B$), 2.22 (m, 4 H, C$H_2$CONH), 2.00 (m, 4 H, C$H_2$$CH_2$CONH).

(1*S*,6*S*,12*S*,17*S*)-6,12-Dibenzyl-5,8,10,13,18,24,26,32-octaza-20,22,28,30(1,4)-tetrabenzena-9(2,6)-pyridinabicyclo[15.8.8]-tritriacontaphan-4,7,11,14,19,25,27,33-octone 3

A solution of crude bis-TFA salt **20** (315 mg) in DMF (30 mL) was added dropwise over 9 hours to a solution of DIPEA (0.60 mL) in MeCN (150 mL) at 70 °C. The mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc (100 mL) and the resulting solution washed with 0.5 M HCl (2 × 20 mL), water (2 × 20 mL), brine (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by chromatography [CH_2Cl_2 to CH_2Cl_2–MeOH (97 : 3 v/v)] gave a solid which was recrystallised from $CHCl_3$ to give macrobicycle **3** as a white solid (63 mg, 33% from diacid **18**); mp 213–14 °C ($CHCl_3$); $[\alpha]_D$ −5.3 (*c* = 1.0 in DMF); ν_{max}($CHCl_3$)/cm^{-1} 3323, 1684, 1652, 1635, 1604, 1544; δ_H(500 MHz, $CDCl_3$) 8.16 (br s, 2 H, N*H*COAr), 8.08 (br s, 2 H, pyrNH), 7.82 (d, *J* 5, 2 H, pyrH), 7.73 (t, *J* 5, 1 H, pyrH), 7.60 (d, *J* 7, 4 H, ArH), 7.19 (m, 4 H, ArH), 7.16 (m, 2 H, ArH), 7.08 (d, *J* 7, 4 H, ArH), 6.99 (d, *J* 7, 4 H, ArH), 6.88 (d, *J* 7, 4 H, ArH), 6.80 (d, *J* 7, 4 H, ArH), 6.48 (d, *J* 8, 2 H, N*H*CH_2), 6.37 (br s, 2 H, N*H*$CHCH_2$Ph), 4.85 (dd, *J* 9 and 15, 2 H, C$H_A$$H_B$-NH), 4.73 (m, 2 H, NHC*H*$(CH_2)_2$), 4.35 (m, 2 H, C*H*CH_2Ph), 3.83 (dd, *J* 3.0 and 15, 2 H, $CH_A$$H_B$NH), 3.77 (d, *J* 14, 2 H, ArC$H_A$$H_B$Ar), 3.63 (d, *J* 14, 2 H, ArC$H_A$$H_B$Ar), 2.98 (dd, *J* 5 and 13, 2 H, PhC$H_A$$H_B$CH), 2.74 (dd, *J* 7 and 13, 2 H, PhC$H_A$$H_B$CH), 2.45 (m, 2 H, C$H_A$$H_B$$CH_2$CO), 2.42 (m, 4 H, CH_2CH_2CO), 2.20 (m, 2 H, $CH_A$$H_B$$CH_2$CO); δ_C(90.6 MHz, $CDCl_3$) 174.9 (C), 171.1 (C), 169.0 (C), 167.6 (C), 149.3 (C), 145.6 (C), 141.2 (CH), 139.7 (C), 136.1 (C), 135.8 (C), 130.7 (C), 129.4 (CH), 129.3 (CH), 129.2 (CH), 129.1 (CH), 128.5 (CH), 128.0 (CH), 127.8 (CH), 110.1 (CH), 56.6 (CH), 53.7 (CH), 43.3 (CH_2), 41.7 (CH_2), 37.4 (CH_2), 31.9 (CH_2), 25.3 (CH_2); MS (MALDI-TOF): *m*/*z* 1072.9 (MH^+, 20%), 247.6 (67), 207.3 (92) [Found (FAB-MS) MH^+ = 1072.4880. $C_{63}H_{62}N_9O_8$ requires *M*H, 1072.4887] (Found C, 69.07; H, 5.62; N, 11.32. $C_{63}H_{61}N_9O_8$·H_2O requires C, 69.41; H, 5.82; N, 11.56%).

Measurement of binding constants

All titration experiments were conducted on a JEOL GX 270 or Bruker AM300 NMR machine at 293 K. In order to minimise effects of acid impurities in the solvent on the position of the NH signal, all deuteriochloroform used was passed through a column of alumina prior to use and collected over molecular sieves (4 Å, Aldrich). For the host, macrobicycle **3**, 600 μL of a solution of 10 mg in 3 mL deuteriochloroform (3.11 mM) was placed in an NMR tube along with a molecular sieve (4 Å, Aldrich). Guest stock solutions in deuteriochloroform were made to concentrations such that 10 μL of that solution contained typically 0.25 equivalents of guest with respect to the host. The ^{1}H NMR of **3** was recorded for the starting solution and after addition of each aliquot of guest, and the data was analysed using the Hostest software.[14] A dilution experiment was also carried out in order to verify that there were no effects on the shift of the signals due to the dilution of the host throughout the titration experiment.

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15 As has been pointed out by a referee, the observed shifts in the signals for the NH protons in many of the titration experiments are quite small in comparison to the often large shifts reported for

amide–amide hydrogen bonding in chloroform solution, in many other host–guest systems. In the present case this may be explained by several factors, including i) the breaking of intramolecular hydrogen bonds in **3** on complexation (as is particularly suggested for NH^5 by its downfield starting position in the NMR and by the molecular modelling), ii) the general flexibility of the host–guest system and consequent loose binding fit, as revealed by the NMR and molecular modelling studies and iii) the macrobicyclic host **3** possesses C_2 symmetry, so that while a hydrogen bond is only formed with one of two symmetrically related protons, the NMR reports the time-averaged signal for the hydrogen-bonded and non-hydrogen bonded partner.

16 In the case of the titration experiment with what turned out to be the most tightly bound substrate, Cbz-β-alanyl-D-alanine, we did monitor the shift of the phenylalanyl α-proton ($\Delta\delta = 0.4$) and the aromatic rim proton *ortho* to the carboxamide ($\Delta\delta = -0.12$). Analysis of the resulting data gave association constants in agreement with those determined from monitoring the amidopyridine NH, but the quality of the fit for the data from the phenylalanyl α-proton and aromatic rim proton was not as good, due to loss of resolution and the presence of overlapping signals from both host and guest protons as the titration progressed.

17 Comparison of binding constants of structurally different carboxylic acids should be treated with some caution since the strength of the interaction with the amidopyridine unit will be influenced by the acidity of the carboxylic acid moiety (see *e.g.*, M. H. Abraham, *Chem. Soc. Rev.*, 1993, **22**, 73; see also S. Shan, S. Loh and D. Herschlag, *Science*, 1996, **272**, 97). Thus, for example, the difference in binding strength of hexanoic and phenylacetic acid may be partly due to the difference in their acidities. However, the differences in acidities, in chloroform solution, of closely related amino acid substrates are likely to be small and are unlikely to lead to differences in the interaction with the amidopyridine unit which exceed the error in the experimentally determined binding constants and clearly for enantiomers of the same substrate the acidities will be the same.

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