Synthesis and Binding Properties of a Macrocyclic Peptide Receptor

James Dowden,[a] Peter D. Edwards,[b] Stephen S. Flack,[a] and Jeremy D. Kilburn*[a]

Abstract: Macrocyclic receptor **1** has been synthesised, as a racemate and as a single enantiomer, utilising a Stille coupling for the formation of the biphenyl portion and a macrolactamisation as the final step. The binding properties for the racemic and the homochiral macrocycle with amino acid and dipeptide derivatives, in $CDCl_3$ solution, have been investigated. In the case of racemic **1**, addition of homochiral peptide substrates led to two distinct diastereomeric complexes, and the well separated signals for several protons in the ^1H NMR spectrum could be conveniently followed in titration experiments, allowing determination of both binding constants for the two diastereoisomeric complexes, and indicating that **1** is capable of enantioselective recognition. Titration of homochiral **1** with the same peptide substrates allowed the sense of the enantioselectivity to be determined, and experiments with a greater range of substrates indicated that **1** is particularly effective for the recognition of *N*-Cbz-β-alanyl-L-amino acids, the strongest binding being observed with *N*-Cbz-β-alanyl-L-alanine ($-\Delta G_{ass} = 19.9$ kJ mol^{-1}). Notably the binding of *N*-Cbz-β-alanyl-L-lactic acid was considerably weaker ($-\Delta G_{ass} = 13.1$ kJ mol^{-1}), presumably due to replacement of an NH hydrogen-bond donor in the case of *N*-Cbz-β-alanyl-L-alanine with an oxygen lone-pair in the case of *N*-Cbz-β-alanyl-L-lactic acid. Molecular modelling and 2D NMR studies on the free macrocycle **1** and associated complexes did not provide conclusive evidence for the structure of the host–guest complexes, but did serve to emphasise the flexibility of **1**, which despite this flexibility, shows strong, selective binding of certain peptide guests.

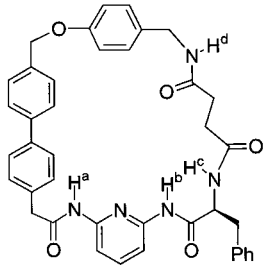
Keywords: host–guest chemistry · macrocycles · peptides · receptors · synthetic methods

Introduction

Over the last thirty years the rapid development of host–guest chemistry has led to the development of synthetic receptors for a whole range of substrates, including many of biological importance, such as nucleotides, carbohydrates and amino acids.[1] The study of such synthetic model systems aids the understanding of fundamental aspects of non-covalent interactions and in many cases inspires the design of new functional molecules and supramolecular systems. The development of synthetic receptors for peptides and amino acid derivatives[2] is of particular interest because the intermolecular interactions involved in complexes between small molecules and peptides are of direct relevance to many biological peptide–protein interactions, and may also lead, for example, to new biosensors, therapeutics and catalysts for peptide hydrolysis. In our own efforts to develop novel receptors for amino acids and peptides we have targeted peptidic guests with a carboxylic acid terminus by preparing a range of macrocyclic structures which feature a specific binding site for the carboxylic acid and additional hydrogen bonding functionality to bind to the backbone of the peptidic guest.[3] Macrocycle **1** is an example of such a receptor, which incorporates a diamidopyridine unit to serve as a carboxylic acid binding site,[4] in a cavity lined on one side with an amide functionality, and on the other with a rigid biaryl fragment, linked by a benzyl ether to a further aromatic ring, to hold open the binding cavity and, in principle, to provide a site for π-stacking interactions or hydrophobic interactions in water-soluble variants of the basic structure.

[a] Dr. J. D. Kilburn, J. Dowden, S. S. Flack
Department of Chemistry
University of Southampton
Southampton SO17 1BJ (UK)
Fax: (+44) 1703-593781
E-mail: jdk1@soton. ac.uk

[b] Dr. P. D. Edwards
Department of Medicinal Chemistry
SmithKline Beecham Pharmaceuticals
New Frontiers Science Park (North)
Third Avenue
Harlow CM19 5AW (UK)



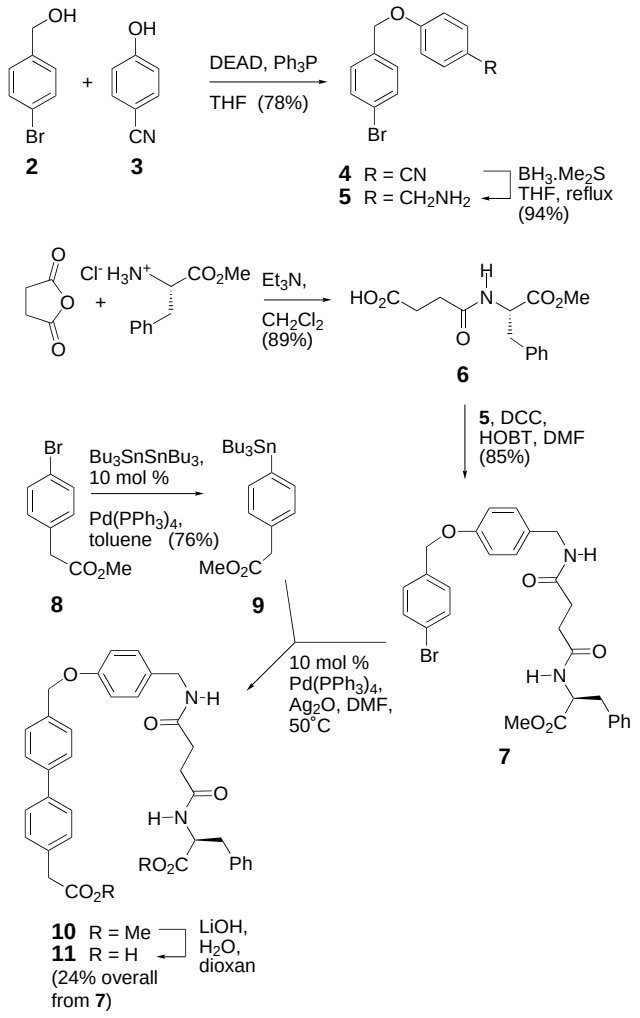
1

  0947-6539/99/0501-0079 $ 17.50+.50/0

We envisioned that variation of the amide-containing side wall of the macrocycle would provide a means of tuning the binding selectivity for specific peptide guests, but initially we wished to prepare a relatively simple example of these structures and establish that it contained appropriate basic features for peptide recognition. Thus we chose to prepare macrocycle **1**, in which the amide side wall is made up of a succinic acid moiety linked to a phenylalanine unit; the latter unit provides a single chiral centre for the structure and thus the possibility of some stereoselective binding properties. In this paper we describe our synthetic efforts to prepare macrocycle **1** in both racemic and chiral forms, and discuss the binding properties of the macrocycle with a range of peptidic guests.[5]

Results and Discussion

Synthesis: Our first attempts to prepare macrocycle **1** involved the synthesis of diacid **11** (Scheme 1) which we hoped to activate and couple in one step to diaminopyridine. This route would be short and allow easy variation of the amide side wall in later variants.



Scheme 1. Synthesis of diacid **11**.

Diacid **11** was assembled by an initial Mitsunobu coupling[6] of commercially available alcohols **2** and **3** (see Scheme 1), and subsequent reduction of nitrile **4** by using borane–dimethyl sulfide complex[7] to give amine **5**. Alternative methods for reduction of the nitrile ($LiAlH_4$, H_2/Raney nickel, $NaBH_4/AlCl_3$) were less successful, since cleavage of the benzyl ether occurs as a competing side reaction. Acid **6** was simply prepared by a base-mediated opening of succinic anhydride with methyl phenylalaninate, and was coupled to amine **5** using dicyclohexyl carbodiimide (DCC) to give aryl bromide **7** in 85% yield. Formation of the biaryl unit then required coupling of aryl bromide **7** with a suitable organometallic reagent derived from bromide **8**. Thus we prepared stannane **9** by treatment of bromide **8** with bis(tributyltin) and a palladium catalyst.[8] The best yields for this reaction (72%) were ultimately achieved by using an excess of bis(tributyltin), $(Ph_3P)_4Pd$ as catalyst, and carrying out the reaction under highly concentrated conditions that minimised unwanted biaryl formation. With the required stannane **9** in hand, the Stille coupling[9] with bromide **7** using $(Ph_3P)_4Pd$ and a stoichiometric amount of silver(I) oxide[10] gave the diester **10** in reasonable yields (50% by NMR analysis of the crude material). Purification of the diester proved to be problematic, and it was instead hydrolysed directly to give the corresponding diacid **11** in 24% overall yield from bromide **7**.

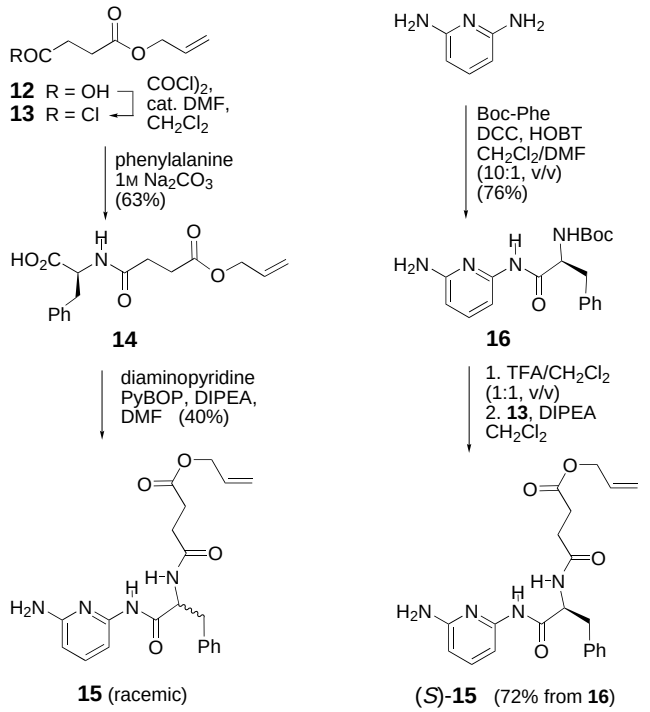
Numerous attempts were made to couple diacid **11** with diaminopyridine. Formation of the bis(acid chloride) was attempted by using oxalyl chloride, thionyl chloride or cyanuric chloride[11] but failed to yield any of the desired product in the subsequent coupling with diaminopyridine. Similarly all efforts with in situ activating agents, such as dicyclohexylcarbodiimide (DCC; with or without auxiliary reagents), carbonyldiimidazole and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[12] were in vain. The use of the bis(acylhydrazide) or bis(activated esters),[13] such as the bis(pentafluorophenol ester), also failed to give the desired macrocycle.

The failure of this approach led us to consider an alternative synthesis which would culminate in the macrolactamisation of amino acid derivative **21** (see Scheme 3), which in turn could be assembled, with appropriate protecting groups, by sequential coupling of a suitable peptidic fragment and a biaryl acid fragment with diaminopyridine.

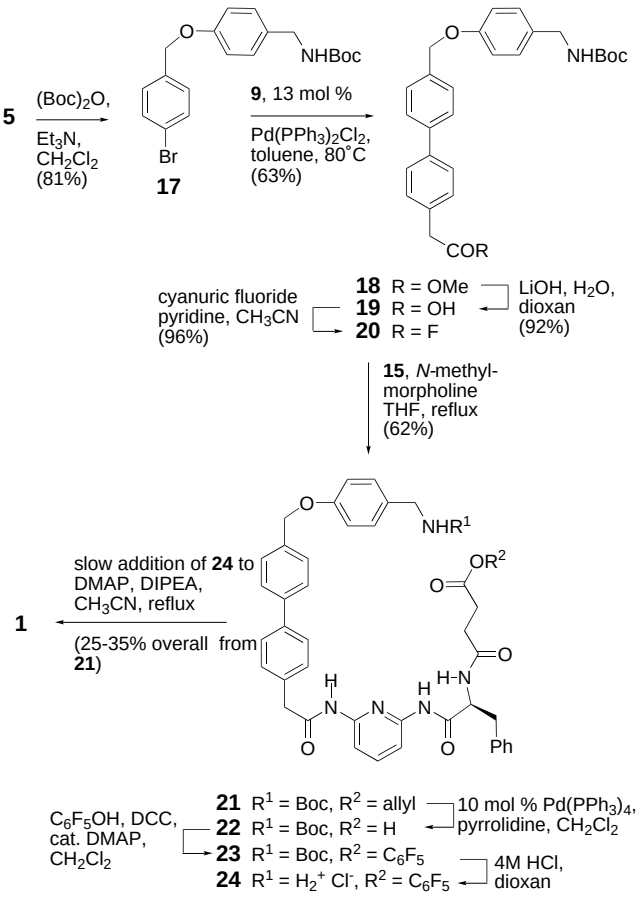
The peptidic fragment was prepared, as the allyl ester **14** (Scheme 2), by reaction of succinic anhydride with allyl alcohol, followed by conversion of monoacid **12**[14] to the acid chloride **13**, which, in turn, was used to acylate phenylalanine. A PyBOP- (PyBOP = benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate[15] or DCC-mediated coupling of acid **14** to 2,6-diaminopyridine gave the desired monoacylated product **15**, but with complete racemisation of the phenylalanine derived chiral centre, presumably via an oxazolone intermediate.[16] In retrospect the formation of **15** as a racemate is not surprising given that **14** is an acylated amino acid (and therefore prone to racemisation on activation) and diaminopyridine is a poor nucleophile.

Racemisation was avoided in the subsequent synthesis of the enantiomerically pure macrocycle by first coupling 2,6-diaminopyridine to *N-tert*-butoxycarbonyl-L-phenylalanine to

0947-6539/99/0501-0080 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 1



Scheme 2. Synthesis of monoacylated product **15**.



Scheme 3. Synthesis of **1**.

give **16**, followed by removal of the amine protecting group, and selective acylation of the more reactive amino functionality with acid chloride **13** (Scheme 2).

Biaryl ester **18** was prepared by Stille coupling of stannane **9** with the protected amino bromide **17** (Scheme 3). Initial attempts at this reaction under $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$ catalysis in *N*-methylpyrollidinone gave poor yields of the desired biaryl ester **18** (30–40%). The use of more polar solvents,[9] copper iodide co-catalysis[17] and softer ligands such as triphenylarsane[18] gave no improvement. Variations of the $Pd_2(dba)_3$ catalytic system developed by Farina and Krishman[19] gave none of the desired product with apparent rapid decomposition of the catalyst. However, when the reaction was performed in a *less* polar solvent such as toluene with $Pd(PPh_3)_2Cl_2$ as catalyst, the yield of the biaryl ester improved to an acceptable and reproducible level of 60–65%. The ester **18** was readily hydrolysed to give the required acid **19** in good yield.

Coupling of the relatively unreactive monoacylated diaminopyridine **15** (as the racemate or as a single enantiomer) with biaryl acid **19** could be achieved in moderate yield (30–40%) by using either DCC or 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in refluxing THF. Better yields however, were achieved by converting acid **19** to the acid fluoride **20**[20] and refluxing this with **15** and *N*-methylmorpholine in THF to give the protected macrocyclisation precursor **21** in 62% yield (Scheme 3). The allyl ester was deprotected to give acid **22** by using $(Ph_3P)_4Pd$ in dioxan, with water or pyrrolidine as the allyl scavenger.[21] Subsequent DCC coupling with pentafluorophenol and removal of the amine protecting group by treatment with 4M HCl in dioxan, followed by trituration with diethyl ether, provided the hydrochloride salt **24**. The crude hydrochloride salt, dissolved in DMF, was added by syringe pump to a refluxing solution of diisopropylethylamine (DIPEA) in acetonitrile to give the desired macrocycle (as a racemate or as a single enantiomer) in 25–35% yield from the allyl ester **21**.

Macrocycle **1** was isolated as an amorphous solid which resisted all our attempts to obtain single crystals for X-ray analysis. It is sparingly soluble in relatively apolar solvents such as chloroform (≈2 mg per mL), and the analysis of **1** by ^1H NMR spectroscopy (in $CDCl_3$) gave a well resolved spectrum which could be fully assigned with the help of 2D NMR experiments. A 2D ROESY[22] spectrum revealed no NOEs between protons on opposite sides of the macrocyclic ring, suggesting that **1** exists in an open conformation in $CDCl_3$ solution, as desired.

Binding studies and molecular modelling

In order to determine the extent to which the receptor was able to bind amino acids and peptides with a free carboxylic acid terminus, as it was designed to do, a series of binding studies with a range of carboxylic acid substrates was carried out. All binding studies with macrocycle **1** were carried out in deuteriochloroform (in which hydrogen bonds can be expected to provide the dominant binding interactions), using a standard NMR titration experiment, monitoring the shift of the NH signals, and analysing the resultant binding curves using the Hostest programme.[23] In each titration experiment significant downfield shifts of NH^b were observed with no

apparent shift of NH[a], consistent with a strong association between the carboxylic acid and the amidopyridine moiety, presumably involving NH[b] and not NH[a]. (Addition of corresponding methyl esters to a solution of **1** led to no significant changes in the NMR spectrum for **1** further confirming the importance of the interaction between the carboxylic acid and the amidopyridine in the observed binding). In each binding experiment a 1:1 binding stoichiometry has been assumed which was generally supported by the good fit of the measured data to the theoretical model, on analysis. (A Job plot[24] was attempted to confirm the 1:1 binding stoichiometry, but the low solubility of **1** meant that acceptable NMR spectra could not be obtained across a suitable concentration range). Dilution experiments were also carried out on both the receptor **1** and on the guest molecules to provide estimates of dimerisation constants for each compound. No appreciable dimerisation or aggregation was detected for **1** and although dimerisation constants were measurable for the dipeptide substrates these were sufficiently small ($0-30\,\text{M}^{-1}$) that inclusion of these numbers did not affect the measured binding constants with receptor **1** within the margin of error for the measurements. As would be anticipated,[25] for the more strongly bound substrates ($-\Delta G_{ass} > 17\,\text{kJ mol}^{-1}$), the titration data showed a poorer fit to the theoretical model and were therefore repeated several times to give an average value. Repeat experiments did, however, give consistent results and the comparison between data obtained for the homochiral macrocycle and that obtained for the racemate was also good (vide infra). Furthermore, titration of receptor **1** with strongly binding substrates in $CDCl_3$ contaminated with $\simeq 2\,\%$ MeOH, gave, as expected, reduced binding energies, but now the data again gave an excellent fit for the assumed 1:1 binding.

Macrocycle **1** was at first obtained as a racemate, but was none-the-less used in initial binding studies (Table 1).

Titration of racemic **1** with phenylacetic acid gave $-\Delta G_{ass} = 11.5\,\text{kJ mol}^{-1}$ which places an upper limit on the strength of the amidopyridine–carboxylic acid interaction.[26] *N*-Cbz-β-alanine ($-\Delta G_{ass} = 12.9\,\text{kJ mol}^{-1}$) showed a modest increase in binding over phenylacetic acid, while *N*-Cbz-glycine ($-\Delta G_{ass} = 14.4\,\text{kJ mol}^{-1}$) showed a more pronounced increase. When the racemic receptor was titrated with homochiral peptide substrates two distinct diastereomeric complexes were immediately evident in the ^{1}H NMR spectrum, and the well separated signals for NH[b] of the two complexes (and to a lesser extent for NH[c] and NH[d]) could be conveniently monitored throughout the titration experiment. Analysis of these data using the Hostest software[23] gave estimates of the two binding energies for the diastereomeric complexes. Thus, titration of racemic **1** with *N*-Cbz-L-alanine gave $-\Delta G_{ass} = 15.8$ and $15.2\,\text{kJ mol}^{-1}$ for the two diastereomeric complexes (which was essentially mirrored, as expected, by titration with *N*-Cbz-D-alanine) and titration with *N*-Cbz-L-phenylalanine gave $-\Delta G_{ass} = 14.2$ and $13.7\,\text{kJ mol}^{-1}$ for the two diastereomeric complexes.

Titration of racemic **1** with the dipeptide *N*-Cbz-L-alanyl-L-alanine showed no significant increase in binding over simple amino acid derivatives for either of the two diastereomeric complexes formed, and *N*-Cbz-β-alanylglycine similarly showed only weak binding ($-\Delta G_{ass} = 15.0\,\text{kJ mol}^{-1}$) (although the measured binding constant of the latter must be treated with some caution as the poor solubility of the substrate in $CDCl_3$ meant that only 49 % saturation was achieved in the titration experiment). However, titration of **1** with *N*-Cbz-β-alanyl-L-alanine gave two significantly different binding energies for the two diastereomeric complexes ($-\Delta G_{ass} = 19.4$ and $16.2\,\text{kJ mol}^{-1}$, $\Delta\Delta G = 3.2\,\text{kJ mol}^{-1}$), representing a binding enantioselectivity of $\approx 80{:}20$, although the sense of the enantioselectivity could not be determined from these experiments).

The ^{1}H NMR of a 1:1 complex between racemic **1** and *N*-Cbz-β-alanyl-L-alanine (7.5 mM in $CDCl_3$) also showed the most significant differences between the diastereomeric complexes so formed (as compared to 1:1 complexes with any other substrate) (Figure 1).

Thus NH[b] shifted downfield (relative to uncomplexed **1**) from $\delta = 7.97$ to $\delta = 8.79$ and 8.28, respectively, for the two

Table 1. Binding constants (K_{ass}) and free energies of complexation[a] ($-\Delta G_{ass}$) for the 1:1 complexes formed between racemic macrocycle **1** and various acid substrates in $CDCl_3$ at 20 °C.

Substrate	K_{ass} [M^{-1}]	$-\Delta G_{ass}$[b] [kJ mol^{-1}]
phenylacetic acid	113	11.5
Cbz-Gly-OH	376	14.4
Cbz-L-Ala-OH	665/505	15.8/15.2
Cbz-D-Ala-OH	608/492	15.6/15.2
Cbz-L-Phe-OH	344/279	14.2/13.7
*t*Boc-L-Ala-OH	335/275	14.2/13.7
Cbz-β-Ala-OH	202	12.9
Cbz-L-Ala-L-Ala-OH	723/405	16.0/14.6
Cbz-β-Ala-L-Ala-OH	2920/781	19.4/16.2
Cbz-β-Ala-Gly-OH	313	15.0

[a] Where two figures are reported these refer to the binding constants or binding energies for the two diastereomeric complexes formed (see text). [b] Errors for binding energies were estimated as 0.5–0.9 kJ mol^{-1} for the substrates listed.

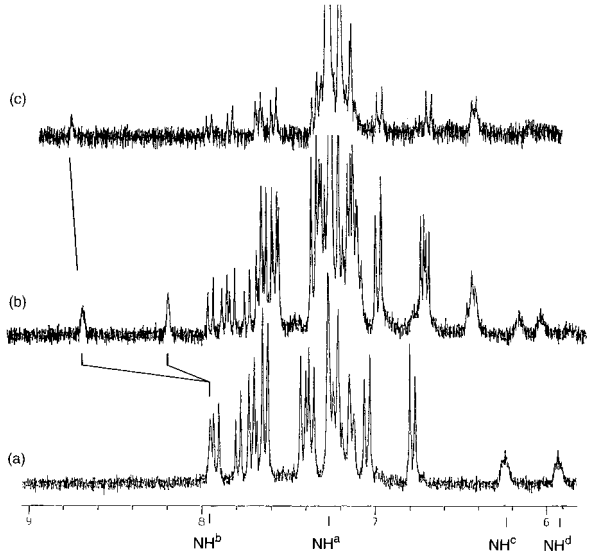


Figure 1. ^{1}H NMR spectra of the aromatic/NH region, in $CDCl_3$ of: a) Macrocycle **1;** b) Racemic macrocycle **1** + *N*-Cbz-β-alanyl-L-alanine; c) homochiral macrocycle **1** + *N*-Cbz-β-alanyl-L-alanine

0947-6539/99/0501-0082 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 1

diastereomeric complexes. NH^c shifted downfield from $\delta = 6.25$ to $\delta = 6.84$ and 6.52, respectively, and NH^d shifted downfield from $\delta = 5.95$ to $\delta = 6.21$ and 6.09, respectively. The position of the signal for NH^a, however, again appeared to be unaffected by the addition of the substrate. Essentially all other signals in the ^{1}H NMR spectrum were separated and could be assigned to the two diastereomeric complexes. (Thus macrocycle **1** may serve as a chiral shift reagent for the ^{1}H NMR spectrum of such peptidic substrates, although perhaps not a very practical one!).

With homochiral receptor **1** in hand we were able to extend the binding studies and determine the sense of the enantioselective binding initially observed with the racemic receptor. Titration experiments were carried out with homochiral **1** and a range of dipeptide substrates (Table 2).

Table 2. Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1:1 complexes formed between homochiral macrocycle **1** and dipeptide substrates in $CDCl_3$ at 20 °C.

Substrate	K_{ass} [M^{-1}]	$-\Delta G_{ass}$ [kJ mol^{-1}]
Cbz-L-Ala-L-Ala-OH	1940 ± 485	18.4 ± 0.7
Cbz-D-Ala-D-Ala-OH	869 ± 124	16.5 ± 0.3
Cbz-β-Ala-L-Ala-OH	3598 ± 900	19.9 ± 0.7
Cbz-β-Ala-D-Ala-OH	925 ± 230	16.6 ± 0.7
Cbz-β-Ala-L-Phe-OH	321 ± 82	16.2 ± 0.5
Cbz-β-Ala-D-Phe-OH	828 ± 143	14.0 ± 0.5
Cbz-β-Ala-L-Val-OH	995 ± 364	16.8 ± 0.6
Cbz-β-Ala-D-Val-OH	186 ± 15	12.7 ± 0.2
Cbz-β-Ala-L-Lac-OH	221 ± 24	13.1 ± 0.3
Cbz-β-Ala-D-Lac-OH	100 ± 10	11.2 ± 0.2

Whereas titration of such guests with the racemic macrocycle had led to the formation of two diastereomeric complexes, clearly distinguishable in the ^{1}H NMR spectra, we now observed formation of a single diastereomeric complex (see Figure 1), allowing independent determination of binding energies for each enantiomer of the various guests, and incidentally confirming that no racemisation had occurred in the modified synthesis of **1**. As before, in each titration experiment significant downfield shifts of NH^b were observed with no apparent shift of NH^a.

Initially, identical dipeptide substrates to those explored with racemic **1** were examined. The results compared well with those obtained for racemic **1** with the best agreement observed for the *N*-Cbz-β-alanylalanine enantiomeric pair, which were bound with very similar levels of enantioselectivity ($ee \approx 60\,\%$) to those observed in the racemic experiment. The overall levels of binding were also in good agreement, *N*-Cbz-β-alanyl-L-alanine bound with a free energy of binding of $-\Delta G_{ass} = 19.9$ kJ mol^{-1}, which compared favourably with the strongest diastereomeric complex in the racemic experiment ($-\Delta G_{ass} = 19.4$ kJ mol^{-1}).

In order to probe the relatively high enantioselective binding of the *N*-Cbz-β-alanylalanine dipeptides the binding of related substrates was studied. Thus with the *N*-Cbz-β-alanylphenylalanine dipeptides as guests, binding energies were somewhat lower ($-\Delta G_{ass} = 16.2$, 14.0 kJ mol^{-1}), with enantioselectivity also being slightly reduced ($\Delta\Delta G = 2.2$ kJ mol^{-1}, $ee \approx 44\,\%$). With the more sterically bulky valine side chain incorporated into the dipeptide, the binding for *N*-Cbz-β-alanyl-L-valine was of a similar order of magnitude ($-\Delta G_{ass} = 16.8$ kJ mol^{-1}) to *N*-Cbz-β-alanyl-L-phenylalanine, but the binding energy for the enantiomer *N*-Cbz-β-alanyl-D-valine was much lower ($-\Delta G_{ass} = 12.7$ kJ mol^{-1}), in fact reduced to a value only slightly larger than that for phenylacetic acid. The enantioselectivity ($\Delta\Delta G = 4.1$ kJ mol^{-1}, $ee \approx 70\,\%$) for the *N*-Cbz-β-alanylvaline dipeptides is quite high and although it does not compare with the high enantioselectivities observed for other recently described peptide receptors,[2b] it is notable for such a flexible receptor that bears only one chiral centre.

From these results it would appear that the presence of a methyl group attached to the chiral centre adjacent to the carboxylic acid receives more stabilisation from complexation than other substituents, reflected by the higher levels of binding for all alanyl containing peptides compared to other substrates, and in particular the strong binding of *N*-Cbz-β-alanyl-L-alanine in comparison to the binding of *N*-Cbz-β-alanylglycine ($\Delta\Delta G = \approx 5$ kJ mol^{-1}). In the ^{1}H NMR spectrum of the 1:1 complex between macrocycle **1** and *N*-Cbz-β-alanyl-L-alanine the alanine methyl signal was shifted upfield from its unbound position at $\delta = 1.44$ to $\delta = 1.28$ in the complex, which could be due to a weak interaction between the methyl group and the biphenyl unit.

We also measured the binding of the ester-linked *N*-Cbz-β-alanyllactic acids as substrates for receptor **1**, effectively replacing a hydrogen bond donor substituent (NH) with a hydrogen bond acceptor substituent (lone pair of electrons on oxygen) in the guest structure. The binding energies for both enantiomers of *N*-Cbz-β-alanyllactic acid ($-\Delta G_{ass} = 13.1$, 11.2 kJ mol^{-1}) are comparable to the binding energy measured for phenyl acetic acid ($-\Delta G_{ass} = 11.5$ kJ mol^{-1}) indicating that these substrates apparently gain little stabilisation other than from the carboxylic acid interaction with the amidopyridine binding site. The substantially lower binding energies for these esters compared to the corresponding *N*-Cbz-β-alanylalanine dipeptides, strongly suggests that a hydrogen bond from the guest NH to the receptor side wall is a key interaction in the binding of the latter.[27] This result is also of interest in view of recent observations that certain bacteria have mutated their cell wall precursor structure from a terminal D-Ala-D-Ala-CO_2H sequence to a D-Ala-D-Lac-CO_2H sequence, thus providing immunity to conventional antibacterials such as the natural D-Ala-D-Ala-CO_2H receptor vancomycin.[28, 29]

The 1:1 complex of *N*-Cbz-β-alanyl-L-alanine with both racemic and homochiral **1**, in chloroform, was studied by 2D ROESY and NOESY NMR spectroscopy in an attempt to identify which intermolecular interactions were contributing to complexation. No useful intermolecular NOEs, however, were observed in any of these experiments. One possible explanation for this is that, due to the flexibility of the macrocycle and guest peptides a large number of conformations are occupied that are suitable for binding and it is therefore, impossible to observe any intermolecular interactions. If this is correct then the level of selectivity observed for some of the substrates is surprisingly good (in general higher selectivity is anticipated with less flexible receptors).[2]

Molecular modelling was also carried out in an attempt to visualise the structure of the complex. Thus the geometry of the free macrocycle **1** and its complexes with dipeptide substrates were examined by using a combination of simulated annealing calculations and molecular dynamics using the MacroModel[30] program. The AMBER*[31] force field was used[32] as implemented in MacroModel V5.0 and the effect of solvent was included through the use of GB/SA chloroform model.[33] Initially, the free macrocycle was energy minimised by using a conjugate gradient (PRCG) and five simulated annealing calculations of 1 ns, involving slow cooling from 600 to $\approx$0 K, were performed, followed by 5×1 ns calculations of molecular dynamics at 300 K to examine the behaviour at room temperature.

The results of the simulation were not conclusive as a large number of conformations were generated in the course of the simulation, reflecting the flexibility of the macrocycle, but it was possible to draw some general conclusions. None of the structures generated showed any intramolecular hydrogen bonds and most of the conformations featured both of the pyridyl-amide NH groups of the diamidopyridine binding site pointing into the macrocyclic cavity. Furthermore, in all of the structures the aromatic units provided an open, box shaped binding cavity, as they were designed to do, and they could be seen to effectively stretch out the amide side wall, holding it apart from the carboxylic acid binding site. The most flexible portion of the macrocycle was the amide side wall which adopted many conformations during the calculation and as a consequence it was not possible to identify a preferred orientation for the benzyl residue of the phenylalanine moiety. The five structures produced at the end of the successive 1 ns molecular dynamics runs at 300 K are shown in Figure 2, and reflect the open binding cavity, the consistent orientation of the amidopyridine unit, and the flexibility of the amide portion.

Dipeptide guests were docked by eye into the binding cavity of the final structures from the molecular dynamic calculations, such that the carboxylic acid carbonyl formed a hydrogen bond with the pyridyl-amide NH^b which was justified by the earlier NMR experiments that showed that only this pyridyl-amide NH group is involved in binding. This single hydrogen-bonding interaction was constrained at a reasonable hydrogen-bonding distance (2.0 Å) throughout all future calculations, thus tethering the guest to the binding site, although this was the only bias placed on the system. The same simulated annealing and molecular dynamics protocol was used as before to examine the conformation of the complex between the macrocycle **1** and dipeptide substrates.

Now an even greater diversity of structures was produced for the conformation of the complexes, in the course of the simulation. Both the macrocycle and the dipeptide guests were very flexible and did not show any clear preference for a single binding geometry. However, complexation of the carboxylic acid to the diamidopyridine unit through two hydrogen bonds in an eight-membered ring arrangement was commonly observed, and this generally preferred orientation for carboxylic acid binding placed the rest of the dipeptide guest in a position close to the amide side wall of the macrocycle. Further hydrogen bonding interactions between the backbone of the guest and the amide side wall of the receptor frequently featured in the structures generated by the molecular modelling, but no specific hydrogen bonds, other than the constrained one, could be consistently identified. The structure for the complex of macrocycle **1** with *N*-Cbz-β-alanyl-L-valine shown in Figure 3 is an illustrative example.

From the modelling it was not possible to provide a rationalisation for the measured enantioselective binding properties of macrocycle **1**. Similarly it was not possible to

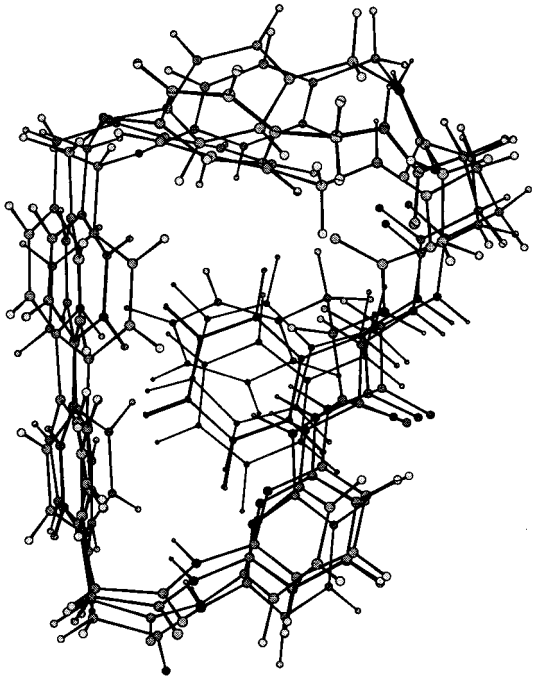


Figure 2. Structures of macrocycle **1** generated at the end of each of five successive molecular dynamics calculations (1 ns at 300 K).

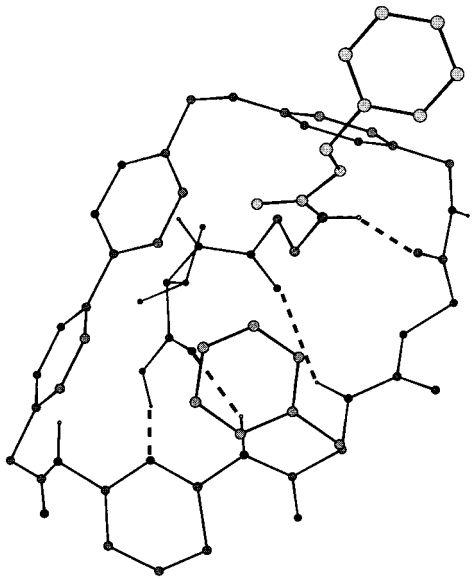


Figure 3. Complex between macrocycle **1** and Cbz-β-alanyl-L-valine illustrating possible H-bonding interactions that may be formed (all H atoms, except those in NH groups, have been omitted for clarity).

0947-6539/99/0501-0084 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 1

elucidate how the selectivity for alanyl amides over lactate esters may arise. The results do, however, support the hypothesis that the macrocycle is very flexible, and indicate that in addition to a strong interaction between the carboxylic acid and the diamidopyridine unit, additional hydrogen bonding interactions between the backbone of the guest and the amide side wall of the receptor are perfectly feasible, consistent with the observed stronger binding of *N*-Cbz-*β*-alanyl-L-amino acid guests compared to simple amino acid derivatives. The modelling of such a flexible system would undoubtedly benefit from structural experimental data, since it would allow known interactions to be confidently and accurately constrained, but such data was not forthcoming from 2D NMR experiments (vide supra).

Conclusion

In conclusion, we have been able to show that macrocycle **1** is an effective receptor for dipeptides with a carboxylic acid terminus, and shows some selectivity, particularly for *N*-Cbz-*β*-alanyl-L-amino acids, and thus the basic receptor design was shown to be appropriate for peptide recognition. Although the observed binding enantioselectivity is not as great as has been observed in some other peptide binding systems it is surprisingly good given the flexibility of the receptor and the minimal chirality which it incorporates. Evidence from the binding studies suggests that the binding of *N*-Cbz-*β*-alanyl-L-amino acid substrates involves interaction of the carboxylic acid of the peptide guest with the amidopyridine unit (with a strong hydrogen bond to NH[b]) and involves a hydrogen-bond interaction with the amide NH group of the substrate. Variation of the peptide side wall of the receptor, using the synthetic route we have developed, should be straightforward, allowing the preparation of less flexible variants, with increased binding functionality, and potentially providing structures which can be solubilised in water.

Experimental Section

General methods: Whenever possible all solvents and reagents were purified according to literature procedures.[34] 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 and at 360 MHz on a Bruker AM 360 spectrometer. ^{13}C NMR spectra were recorded at 68 MHz on the JEOL GX 270, and at 75 MHz on the Bruker AC 300 and at 90 MHz on the Bruker AM 360. Microanalytical data were obtained from SmithKline Beecham Pharmaceuticals, Brockham Park, Surrey, UK. 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.

11-Bromo-4-cyano-3-oxa-1(1,4),4(1,4)-dibenzatetraphane (**4**): Diethyl diazodicarboxylate (11.10 g, 64.0 mmol) was added dropwise over a period of one hour to a stirred solution of 4-bromobenzyl alcohol (**2**) (10.00 g, 53.3 mmol), 4-cyanophenol (**3**) (6.32 g, 53.3 mmol) and triphenylphosphane (16.79 g, 64.0 mmol) in dry, freshly distilled THF (80 mL) at 0 °C under nitrogen. The reaction mixture was gradually allowed to warm to room temperature and stirred overnight. The reaction was concentrated in vacuo to give an orange gum and trituration with MeOH gave a white powder which was recrystallised from MeOH to give nitrile **4** (11.68 g, 78 %), m.p. 130–131 °C (MeOH); IR ($CHCl_3$): $\tilde{\nu}$ = 2225, 1605, 1574, 1507, 1488, 1465, 1406, 1377, 1298, 1254 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.60 (d, J = 8.0 Hz, 2H, ArH), 7.55 (d, J = 8.0 Hz, 2H, ArH), 7.3 (d, J = 8.0 Hz, 2H, ArH), 6.95 (d, J = 8.0 Hz, ArH), 5.10 (s, 2H, ArCH_2O); ^{13}C NMR (68 MHz, $CDCl_3$): δ = 161.9, 134.9, 134.2, 132.1, 129.3, 122.5, 119.3, 115.8, 104.6, 69.7; MS (EI): *m*/*z* (%): 289 (7), 287 (7), 171 (97), 169 (100), 90 (40); elemental analysis calcd for $C_{14}H_{10}ONBr$: C 58.36, H 3.5, N 4.86; found: C 58.09, H 3.38, N 4.96.

11-Bromo-5-amino-3-oxa-1(1,4), 4(1,4)-dibenzapentaphane (**5**): Borane–dimethyl sulfide reagent (22 mL of a 2 M solution in THF, 44.0 mmol) was added to a refluxing solution of nitrile **4** (10.02 g, 34.7 mmol) in dry THF (200 mL) under nitrogen. The reaction was refluxed for two days, allowed to cool, and 1 M HCl (105 mL) was added. The resulting mixture was refluxed for a further two hours, cooled to room temperature and sodium hydroxide pellets (8.42 g, 209 mmol) were added. The mixture was stirred at room temperature for thirty minutes. The organics were extracted into Et_2O (3 × 100 mL) and the combined organic layer washed with H_2O (3 × 300 mL) and brine (2 × 100 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Recrystallisation from EtOH yielded amine **5** as a white solid (9.44 g, 94 %), m.p. 155–157 °C (EtOH/H_2O); IR (Nujol): $\tilde{\nu}$ = 2918, 2856, 1605, 1595, 1503, 1457, 1401, 1375, 1303, 1237, 1171 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.51 (d, J = 8.5 Hz, 2H, ArH), 7.31 (d, J = 8.5 Hz, 2H, ArH), 7.23 (d, J = 8.5 Hz, 2H, ArH), 6.92 (d, J = 8.5 Hz, 2H, ArH), 5.01 (s, 2H, ArCH_2O), 3.81 (s, 2H, ArCH_2NH$_2$), 1.66 (bs, 2H, NH$_2$); ^{13}C NMR (68 MHz, $CDCl_3$): δ = 157.5, 136.3, 136.1, 131.8, 129.2, 128.5, 122.0, 115.0, 69.4, 46.0; MS (EI): *m*/*z* (%): 293 (30), 291 (30), 171 (100), 169 (100), 90 (25); elemental analysis calcd for $C_{14}H_{14}ONBr$: C 57.55, H 4.83, N 4.79; found: C 57.35, H 4.92, N 4.83.

***N*-Succinyl-L-phenylalanine methyl ester** (**6**): Succinic anhydride (1.0 g, 10 mmol), phenyl alanine methyl ester hydrochloride (2.16 g, 10 mmol) and triethylamine (2.02 g, 2.8 mL, 20 mmol) were suspended in dry CH_2Cl_2 (50 mL) and the mixture was stirrred at room temperature for 12 h. 1 M HCl was added (50 mL) until the aqueous layer was at pH 1. The layers were separated and the aqueous layer further extracted with CH_2Cl_2 (50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give a clear oil which was crystallised overnight in a freezer from EtOAc/toluene to give acid **6** as a white amorphous solid (2.48 g, 89 %); m.p. 70–72 °C; IR ($CHCl_3$): $\tilde{\nu}$ = 3425, 2926, 1736, 1676, 1514, 1438 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.08–7.31 (m, 5H, ArH), 6.36 (d, J = 8 Hz, 1H, N*H*CO), 4.88 (q, J = 7 Hz, 1H, C*H*NH), 3.72 (s, 3H, COOMe), 3.15 (dd, J = 6 Hz, 14 Hz, 1H, PhCH_AH$_B$), 3.07 (dd, J = 6 Hz, 14 Hz, 1H, PhCH$_A$$H_B$), 2.66 (t, J = 6 Hz, 2H, CH_2COOH), 2.50 (t, J = 6 Hz, 2H, CH_2CONH); ^{13}C NMR (68 MHz, $CDCl_3$): δ = 177.1, 172.2, 171.6, 135.8, 129.4, 128.7, 127.3 (1) 53.5, 52.6, 37.9, 30.6, 29.4 (2); MS (CI, NH_3): *m*/*z* (%): 297 ([*M*+NH$_4$]$^+$, 30), 280 ([*M*+H]$^+$, 100), 180 (45).

Methyl (1S)-1-benzyl-12-bromo-3,6-dioxo-10-oxa-2,7-diaza-9(1,4),12(1,4)-dibenzenadodecaphane-1-carboxylate (**7**): Dicyclohexyl carbodiimide (1.78 g, 8.63 mmol) was added to a stirred solution of amine **5** (2.09 g, 7.16 mmol), acid **6** (2 g, 7.16 mmol) and 1-hydroxybenzotriazole monohydrate (0.97 g, 7.16 mmol) in dry distilled DMF (65 mL) at 0 °C, under N_2, and stirred at 0 °C for 30 min then at room temperature for six hours. The solvent was removed by distillation at reduced pressure and the crude solid was purified by flash column chromatography (CH_2Cl_2/MeOH, 99:1 v/v) to give ester **7** as a white amorphous solid (3.36g, 85 %); m.p. 175–177 °C; IR ($CHCl_3$): $\tilde{\nu}$ = 3281, 2924, 1740, 1633, 1538, 1516, 1456, 1427, 1375, 1254, 1178 cm^{-1}; ^{1}H NMR (270 MHz, $(CD_3)_2SO$): δ = 8.49 (d, J = 8 Hz, 1H, MeOOCCHN*H*), 8.37 (t, J = 6 Hz, 1H, ArCHN*H*CO), 7.69 (d, J = 8 Hz, 2H, ArH), 7.51 (d, J = 8 Hz, 2H, ArH), 7.25–7.40 (m, 7H, ArH), 7.04 (d, J = 8 Hz, 2H, ArH), 5.18 (s, 2H, ArCH_2O), 4.56 (q, J = 7 Hz, 1H, C*H*COOMe), 4.28 (d, J = 6 Hz, 2H, ArCH_2NH), 3.70 (s, 3H, COOMe), 3.13 (dd, J = 6 Hz,14 Hz, 1H, CHCH_AH$_B$Ph), 3.05 (dd, J = 6 Hz,14 Hz, 1H, CHCH$_A$$H_B$Ph), 2.41–2.46 (m, 4H, OCCH$_2$CH$_2$CO); ^{13}C NMR (68 MHz, $(CD_3)_2SO$): δ = 172.0, 171.4, 170.9, 156.8, 137.1, 136.6, 131.8, 131.2, 129.6, 128.9, 128.4, 128.1, 126.4, 120.7, 114.5, 68.2, 53.5, 51.7, 41.4, 36.7, 30.4, 30.3; MS (FAB): *m*/*z* (%): 553 (45), 262 (37), 225 (60), 169 (65); elemental

analysis calcd for $C_{28}H_{29}BrN_2O_5$: C 60.77, H 5.28, N 5.06; found: C 60.93, H 4.97, N 5.21.

Methyl 4-tri-*n*-butylstannylphenylacetate (**9**): Tetrakis(triphenylphosphane)palladium(0) (250 mg, 2.2 mmol) was added to a thoroughly degassed solution of methyl 4-bromophenylacetate **7** (5.00 g, 22.0 mmol) and bis(tributylditin) (22.0 mL, 25.0 g, 44 mmol) in toluene (5 mL) under nitrogen. The reaction mixture was heated to reflux and stirred for five hours. The mixture was cooled to room temperature and concentrated under reduced pressure. Dry flash silica chromatography, using petroleum ether as eluent, removed unwanted bis(tributylditin) and tributyltin bromide and the product was then obtained by elution with petroleum ether/EtOAc (99.5:0.5, v/v) to give stannane **9** as a clear, colourless oil (7.05 g, 76 %), IR (neat): $\tilde{\nu}$ = 2957, 2925, 1736, 1458, 1440, 1371, 1340, 1249, 1154; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48 (dd, J = 7.7 Hz, $J_{H,Sn}$ 37.9 Hz, 2H, ArH), 7.13 (d, J = 7.7 Hz, 2H, ArH), 3.73 (s, 3H, COOMe), 3.65 (s, 2H, CH_2COO), 1.67 – 1.61 (m, 6H, Sn(CH_2)$_2$CH_2CH3), 1.51 – 1.36 (m, 6H, SnCH$_2$CH_2CH$_2$CH$_3$), 1.11 (t, J = 8.1 Hz, 6H, SnCH_2(CH$_2$)$_2$CH$_3$), 0.95 (t, J = 7.4 Hz, 9H, Sn(CH$_2$)$_3$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1, 140.4, 136.7 ($J_{C,Sn}$ = 32 Hz), 133.6, 128.8 ($J_{C,Sn}$ = 40 Hz), 52.0, 41.2, 29.1 ($J_{C,Sn}$ = 20 Hz), 27.4 ($J_{C,Sn117/119}$ = 55/57 Hz), 13.7, 9.6 ($J_{C,Sn117/119}$ = 326/340 Hz); MS (CI, NH_3): m/z (%): 458 ([M+NH$_4$]$^+$, 55), 441 ([M+H]$^+$, 12), 400 (25), 308 (100), 168 (57), 35 (85).

(14S)-14-Benzyl-9,12-dioxo-5-oxa-8,13-diaza-2(1,4),3(1,4),6(1,4)-tribenzenatetradecaphane-1,14-dicarboxylic acid (**11**): Tetrakis(triphenylphosphane)palladium (20 mg) and silver(I) oxide (83 mg, 0.36 mmol) were added to a thoroughly degassed solution of bromide **7** (200 mg, 0.36 mmol) in dry DMF (7 mL) and the mixture was heated with stirring to 50 °C under nitrogen. A degassed solution of stannane **9** (192 mg, 0.44 mmol, 1.2 equiv) in dry DMF (2 mL) was added and the mixture stirred for 12 hours at 50 °C. The mixture was allowed to cool and was filtered through celite. The filtrate was diluted with EtOAc (100 mL) and washed with H_2O (100 × 2 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated, to give a crude solid which was purified by flash column chromatography (petroleum ether/EtOAc 90:10 v/v, then MeOH/CH_2Cl_2/Et_2O 1:50:50 v/v) to give the intermediate diester **10** (152 mg) contaminated with the starting bromide **7** in 4:1 ratio (yield of **10** ≈ 54 % by ^{1}H NMR spectroscopy), which was subjected to hydrolysis without further purification.

Diester **10** (contaminated with bromide **7**) (152 mg) was dissolved in 1,4-dioxane (20 mL) with gentle warming. Lithium hydroxide monohydrate (70 mg, 1.6 mmol) in H_2O (5 mL) was added and the mixture stirred at room temperature overnight. The solution was adjusted to pH 1 (1 M HCl) and the solvents were removed in vacuo to give a crude solid, which was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 95:5 v/v) to give the diacid **11** as a yellowish solid (52 mg, 24 % from **7**); m.p. 185 – 187 °C; ^{1}H NMR (270 MHz, (CD$_3$)$_2$SO): δ = 8.39 (t, J = 6 Hz, 1H, ArCH$_2$N*H*CO), 8.33 (d, J = 8 Hz, 1H, MeOOCCHN*H*), 7.79 (d, J = 8 Hz, 2H, ArH), 7.74 (d, J = 8 Hz, 2H, ArH), 7.63 (d, J = 8 Hz, 2H, ArH), 7.47 (d, J = 8 Hz, 2H, ArH) 7.27 – 7.42 (m, 7H, ArH), 7.08 (d, J = 8 Hz, 2H, ArH), 5.24 (s, 2H, ArCH_2O), 4.53 (m, 1H, C*H*COOMe), 4.30 (d, J = 6 Hz, 2H, ArCH_2NH), 3.74 (s, 2H, ArCH_2COOH), 3.19 (dd, J = 5 Hz, 14 Hz, 1H, CHCH_AH$_B$Ph), 2.97 (dd, J = 9 Hz, 14 Hz, 1H, CHCH$_A$$H_B$Ph), 2.41 – 2.46 (m, 4H, OCCH$_2$CH$_2$CO); ^{13}C NMR (68 MHz, (CD$_3$)$_2$SO): δ = 173.1, 172.6, 171.3, 171.0, 157.1, 139.3, 138.1, 137.6, 136.2, 134.3, 131.7, 129.9, 129.0, 128.4, 128.1, 126.5, 126.4, 126.3, 114.5, 68.7, 53.5, 41.4, 40.2, 36.7, 30.6, 30.5; IR ($CHCl_3$): $\tilde{\nu}$ = 1698, 1634, 1231, 1004 cm^{-1}; MS (FAB): m/z (%): 595 (5), 225 (20), 169 (52), 85 (100); elemental analysis calcd for $C_{35}H_{34}N_2O_7$: C 70.69, H 5.76, N 4.71; found C 70.39, H 5.61, N 4.63.

4-Hydrogen-1-prop-2-enyl-butane dicarboxylate (**12**): A mixture of succinic anhydride (5 g, 50 mmol) and allyl alcohol (3 mL, 50 mmol) were refluxed for 3 h. The mixture was then distilled at reduced pressure to afford monoester **12** as a colorless liquid (5 g, 72 %), bp 128 °C (4 mm of Hg) [ref.[14] 148 °C (8 mm of Hg)]; IR (CH_2Cl_2): $\tilde{\nu}$ = 2930, 1737, 1717, 1649, 1420, 1170 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.90 (br. s, 1H, COOH), 5.85 (ddt, J = 17.3 Hz, 10.3 Hz, 5.5 Hz, 1H, CH_2=C*H*), 5.26 (ddt, J = 17.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{trans}), 5.18 (ddt, J = 10.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{cis}), 4.55 (dt, J = 5.5 Hz, 1.5 Hz, 2H, CH_2O), 2.62 (m, 4H, CH_2CH$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 178.5, 172.1, 132.0, 118.4, 65.5, 29.1, 28.9.

Acid chloride (**13**): Oxalyl chloride (4.2 mL, 47.5 mmol) was added to a stirred solution of monoester **12** (5 g, 31.7 mmol) in CH_2Cl_2 (20 mL). Two drops of DMF were added and the mixture stirred for two hours under nitrogen. The solvent was removed under reduced pressure to yield acid chloride **13** as a pale yellow oil (6.0 g), which was used without further purification; IR (CH_2Cl_2): $\tilde{\nu}$ = 1794, 1737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.85 (ddt, J = 17.3 Hz, 10.3 Hz, 5.5 Hz, 1H, CH_2=C*H*), 5.26 (ddt, J = 17.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{trans}), 5.18 (ddt, J = 10.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{cis}), 4.55 (dt, J = 5.5 Hz, 1.5 Hz, 2H, CH_2O), 3.22 (t, J = 6.6 Hz, 2H, CH_2CH$_2$), 2.71 (t, J = 6.6 Hz, 2H, CH$_2$CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 173.2, 170.0, 131.8, 118.9, 66.0, 41.9, 29.4.

4(S) 4-*tert*-butoxycarbonyl-4-benzyl-3-oxo-11,4-diamino-12,2-diaza-1(1,3)-benzatetraphane (**16**): Dicyclohexyl carbodiimide (12.90 g, 56.5 mmol) was added to a stirred solution of 2,6-diaminopyridine (6.17 g, 56.5 mmol), HOBt (5.09 g, 37.7 mmol) and N-*tert*-butoxycarbonylphenylalanine (10.00 g, 37.7 mmol), in CH_2Cl_2/DMF (100 mL, 10:1 v/v) . After stirring overnight the reaction mixture was filtered through celite and concentrated under reduced pressure. The resulting gum was diluted with EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL) and the organic phase dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a green gum, which was purified by flash column chromatography (CH_2Cl_2/MeOH, CH_2Cl_2/MeOH, 99.5:0.5 to 98:2, v/v) followed by recrystallization from aqueous EtOH to give **16** as a white crystalline solid (10.21 g, 76 %), m.p. 198 – 200 °C (EtOH); $[\alpha]_D$ = +3.9 (c = 1.0 in acetone); IR (CH_2Cl_2): $\tilde{\nu}$ = 3467, 3347, 3257, 1695, 1685, 1671, 1617, 1555, 1463, 1388, 1365, 1159 cm^{-1}; ^{1}H NMR (300 MHz, (CD$_3$)$_2$SO): δ = 9.00 (s, 1H, pyrN*H*), 7.50 – 7.10 (m, 7H, *p*-pyrH, PhH, CHN*H*), 6.26 (d, J = 7.3 Hz, 1H, *m*-pyrH), 6.25 (d, J = 7.1 Hz, 1H, *m*-pyrH), 5.30 (bs, 2H, NH_2), 4.56 (br. m, 1H, CHCO), 3.26 (dd, J = 13.8 Hz, 4.8 Hz, 1H, PhCH_AH$_B$), 2.95 (dd, J = 13.8 Hz, 9.6 Hz, 1H, PhCH$_A$$H_B$), 1.30 (9H, s, (CH$_3$)$_3$); ^{13}C NMR (68 MHz, (CD$_3$)$_2$SO): δ = 171.3, 158.6, 155.5, 150.2, 139.0, 138.2, 129.4, 128.9, 126.3, 103.6, 100.9, 78.2, 56.5, 37.2, 28.2; MS (CI): m/z (%):357 (70), 229 (100), 246 (85); elemental analysis calcd for $C_{19}H_{24}N_4O_3$; C 64.03, H 6.79, N 15.72; found: C 63.81, H 6.65, N 15.33.

4(S) Prop-2-enyl-4-benzyl-3,6-dioxo-11-amino-12,2,5-diaza-1(1,3)-benzanonaphane-9-carboxylate (**15**): Amine **16** (2.05 g, 5.64 mmol) was dissolved with TFA/CH_2Cl_2 (25 mL, 1:1 v/v) and stirred for 30 minutes. The reaction mixture was concentrated under reduced pressure and excess TFA was removed by azeotropic distillation with toluene. The resulting gum was triturated with Et_2O to give the amine as the trifluoroacetate salt as a white solid (2.50 g) which was used without further purification, ^{1}H NMR (300 MHz, CD_3OD): δ = 7.86 (dd, J = 8.8 Hz, 7.7 Hz, 1H, *p*-pyrH), 7.38 (m, 5H, ArH), 6.82 (d, J = 7.7 Hz, 1H, *m*-pyrH), 6.68 (d, J = 8.8 Hz, 1H, *m*-pyrH), 4.41 (dd, J = 8.4 Hz, 6.4 Hz, 1H, C*H*NH), 3.39 (dd, J = 14.0 Hz, 6.4 Hz, 1H, PhCH_AH$_B$), 3.20 (dd, J = 14 Hz, 8.4 Hz, 1H, PhCH$_A$$H_B$); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 170.3, 155.4, 146.0, 142.0, 134.9, 130.5, 130.1, 129.0, 108.2, 101.4, 56.4, 38.3.

Acid chloride **13** (1.00 g, 5.57 mmol) in THF (5 mL) was added dropwise to a stirred solution of the trifluoroacetate salt described above (1.44 g, 5.64 mmol) and a large excess of DIPEA (0.14 mL, 29 mmol) in dry freshly distilled THF (5 mL). The reaction was stirred for two hours before being concentrated under reduced pressure and dissolved in EtOAc (100 mL), washed with saturated aqueous sodium bicarbonate (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow oil. Purification by flash column chromatography (CH_2Cl_2/MeOH, 99.5:0.5 to 98:2, v/v) gave (S)-**15** as a colourless foam (1.58 g, 72 %), $[\alpha]_D$ = −24.3 (c, 1.0 in CH_2Cl_2); IR (KBr): $\tilde{\nu}$ = 3407, 1729, 1662, 1458, 1380, 1298, 1247, 1162 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.46 (bs, 1H, pyrN*H*CO), 7.33 (d, J = 7.6 Hz, 1H, *m*-pyrH), 7.29 (dd, J = 7.8 Hz, 7.6 Hz, 1H, *p*-pyr*H*), 7.25 – 7.15 (m, 7H, Ph*H*, CHN*H*), 6.36 (d, J = 7.8 Hz, 1H, *m*-pyr*H*), 6.10 (d, J = 8.1 Hz, 1H, CHN*H*), 5.87 (ddt, J = 17.3 Hz, 10.3 Hz, 5.9 Hz, 1H, CH_2=C*H*), 5.33 (ddt, J = 17.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{trans}), 5.27 (ddt, J = 10.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH$_2$CH=CH_{cis}), 4.92 (dd, J = 6.6 Hz, 6.3 Hz, 1H, C*H*NH), 4.62 (dt, J = 5.9 Hz, 1.5 Hz, 2H, CH_2=CHCH$_2$), 4.32 (br, 2H, NH_2), 3.25 (dd, J = 14.0 Hz, 6.3 Hz, 1H, PhCH_AH$_B$), 3.14 (dd, J = 14.0 Hz, 6.6 Hz, 1H, PhCH$_A$$H_B$), 2.80 – 2.50 (m, 4H, CH$_2$CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.8, 171.9, 170.0, 157.3, 149.4, 140.2, 136.3, 132.1, 129.5, 128.8, 127.2, 118.7, 104.8, 103.6, 65.7, 55.2, 38.3, 31.0, 29.5; LRMS (ES+): m/z (%): 793 ([2MH]$^+$, 10 %), 397 ([MH]$^+$, 100). HRMS (ES+): m/z (%): calcd for $C_{21}H_{25}N_4O_4$ [M+H]: 397.1876, found 397.1894.

Synthesis of racemic prop-2-enyl-4-benzyl-3,6-dioxo-11-amino-12,2,5-diaza-1(1,3)-benzanonaphane-9-carboxylate (**15**): Acid chloride **13** was added

0947-6539/99/0501-0086 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 1

dropwise to L-phenylalanine (7.84 g, 47.5 mmol) in 1M aqueous sodium carbonate solution (100 mL) and the mixture stirred overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL), and the aqueous phase acidified with 1M HCl. The resulting oily suspension was extracted with CH_2Cl_2 (100 mL), the organic phase dried (Na_2SO_4) and the solvent removed at reduced pressure to give crude **14** as a yellow oil (9 g) which was used directly without further purification. Dicyclohexyl carbodiimide (7.84 g, 38 mmol) was added to 2,6-diaminopyridine (3.44 g, 31.7 mmol), 4-dimethylaminopyridine (3.84 g, 31.7 mmol) and the crude **14** (9 g) in CH_2Cl_2 (100 mL). After stirring overnight the mixture was filtered, silica gel (20 g) added and concentrated to dryness. Purification by flash column chromatography (CH_2Cl_2/MeOH, 99.5:0.5 to 98:2, v/v)) gave racemic **15** as a yellow foam (9 g, 72% overall), identical to (*S*)-**15** except $[\alpha]_D = 0.0$ ($c =$ 1.0 in CH_2Cl_2);

1-Bromo-8-*tert*-butoxycarbonylamino-7-oxo-3-oxa-6-aza-1(1,4), 4(1,4)-dibenzaoctaphane (**17**): Di-*tert*-butyldicarbonate (4.51 g, 20.6 mmol) was added to a stirred suspension of amine **5** (5.00 g, 17.3 mmol) and triethylamine (3.6 mL, 25.7 mmol) in CH_2Cl_2 (50 mL). After one hour the reaction mixture was poured into aqueous 1M citric acid solution (50 mL). The organic phase was washed with brine (3×50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a crude pale yellow solid. Precipitation from EtOAc/hexane gave the protected amine **17** as a white solid (5.22 g, 81%), m.p. 98–100 °C (EtOAc/hexane); IR (CH_2Cl_2): $\nu = 3445$, 1698, 1613, 1510, 1241, 1167 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.50$ (d, $J = 8.5$ Hz, 2H, ArH), 7.39 (d, $J = 8.5$ Hz, 2H, ArH), 7.21 (d, $J =$ 8.5 Hz, 2H, ArH), 6.91 (d, $J = 8.5$ Hz, 2H, ArH), 4.99 (s, 2H, CH_2O), 4.90 (br. s, 1H, NH), 4.24 (d, 5.9 Hz, 2H, CH_2NH), 1.47 (s, 9H, $(CH_3)_3$); ^{13}C NMR (68 MHz, $CDCl_3$): $\delta = 157.6$, 156.0, 136.1, 131.8, 131.7, 129.1, 129.0, 121.9, 115.0, 79.5, 69.3, 44.2, 28.5; IR ($CHCl_3$): $\tilde{\nu} = 3448$, 3312, 2981, 2925, 2873, 1682, 1610, 1585, 1531, 1510, 1434, 1392, 1366 cm^{-1}; MS (CI, NH_3): *m*/*z* (%): 411 ($[M+NH_4]^+$, 30), 409 ($[M+NH_4]^+$, 30), 355 (100), 353 (100), 277 (30), 275 (37); elemental analysis calcd for $C_{19}H_{22}O_3NBr$: C 58.17, H 5.65, N 3.57, found: C 57.86, H 5.57, N 3.55.

Methyl-1-*tert*-butoxycarbonylamino-3-oxa-2(1,4), 5(1,4), 6(1,4)-tribenzaheptaphane-7-carboxylate (**18**): Bis(triphenylphosphane) palladium(II) dichloride (94 mg, 0.13 mmol) was added to a thoroughly degassed solution of bromide **17** (390 mg, 1.00 mmol) and stannane **9** (450 mg, 1.00 mmol) in toluene (5 mL) under argon. The reaction was refluxed for 12 h, cooled to room temperature, diluted with EtOAc (50 mL), washed with brine (3x50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the crude product as a brown solid. Purification by flash column chromatogaphy (petroleum ether (b.p. 40–60 °C) to petroleum ether (b.p. 40–60 °C)/EtOAc (7:3, v/v)) followed by recrystallisation from EtOAc/hexane gave ester **18** as a white solid (0.29 g, 63%), m.p. 116–118 °C (EtOAc/hexane); IR (CH_2Cl_2): $\tilde{\nu} = 3450$, 2979, 2870, 1732, 1707, 1509, 1456, 1437, 1367, 1240, 1168 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.61 (d, $J = 8.1$ Hz, 2H, ArH), 7.56 (d, $J = 8.1$ Hz, 2H, ArH), 7.49 (d, $J =$ 8.1 Hz, 2H, ArH), 7.37 (d, $J = 8.1$ Hz, 2H, ArH), 7.22 (d, $J = 8.5$ Hz, 2H, ArH), 6.96 (d, $J = 8.5$ Hz, 2H, ArH), 5.07 (s, 2H, CH_2O), 5.00 (1H, br. s, NH), 4.26 (d, $J = 5.6$ Hz, 2H, CH_2NH), 3.73 (s, 2H, CH_2COOMe), 3.69 (s, 3H, COOMe), 1.39 (s, 9H, $(Me)_3$); ^{13}C NMR (68 MHz, $CDCl_3$): $\delta = 172.2$, 158.2, 156.0, 140.7, 139.8, 136.1, 133.3, 131.5, 129.9, 129.0, 128.1, 127.5, 127.4, 115.1, 79.5, 69.9, 52.3, 44.3, 41.0, 28.6; MS (ES^+): *m*/*z* (%): 945 ($[2M\mathrm{Na}]^+$, 12), 940 ($[2M\mathrm{NH_4}]^+$, 5), 500 ($[M\mathrm{K}]^+$, 15), 484 ($[M\mathrm{Na}]^+$, 100), 479 ($[M\mathrm{NH_4}]^+$, 15).

1-*tert*-butoxycarbonylamino-3-oxa-2(1,4), 5(1,4), 6(1,4)-tribenzaheptaphane-7-carboxylic acid (**19**): Aqueous lithium hydroxide (1.3 mL of a 1M solution, 1.3 mmol) was added to ester **18** (0.52 g, 1.1 mmol) in 1,4 dioxane (4 mL) and the reaction mixture was stirred for two hours. The mixture was concentrated under reduced pressure and diluted with EtOAc (50 mL). 1M HCl (25 mL) was added and the mixture shaken until all of the solid dissolved. The organic phase was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Recrystallisation from acetone/hexane gave carboxylic acid **19** as a white solid (0.45 g, 92%), m.p. >230 °C (acetone/hexane); IR (KBr): $\tilde{\nu} = 3303$, 2726, 1719, 1686, 1517, 1248, 1161, 1109, 1055 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$): $\delta = 12.35$ (br, 1H, COOH), 7.68 (d, $J = 8.1$ Hz, 2H, ArH), 7.63 (d, $J = 8.1$ Hz, 2H, ArH), 7.53 (d, $J = 8.1$ Hz, 2H, ArH), 7.36 (d, $J = 8.1$ Hz, 2H, ArH), 7.31 (br, 1H, NH), 7.17 (d, $J = 8.5$ Hz, 2H, ArH), 6.98 (d, $J = 8.5$ Hz, 2H, ArH), 5.14 (s, 2H, CH_2O), 4.06 (d, $J = 4.5$ Hz, 2H, CH_2NH), 3.63 (s, 2H, CH_2COOH), 1.48 (s, 9H, $(Me)_3$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): $\delta = 172.6$, 157.1, 156.0, 139.3, 138.1, 136.2, 134.3, 132.3, 129.9, 128.2, 128.1, 126.6, 126.5, 114.4, 77.6, 68.7, 42.7, 40.2, 28.2; MS (ES^+): *m*/*z* (%): 465 ($[\mathrm{MNH_4}]^+$, 100); elemental analysis calcd for $C_{27}H_{29}NO_5$: C 72.48, H 6.48, N 3.13; found C 72.08, H 6.50, N 3.19.

1-*tert*-butoxycarbonylamino-3-oxa-2(1,4), 5(1,4), 6(1,4)-tribenzaheptaphane-7-carboxylic acid fluoride (**20**): Cyanuric fluoride (59 mg, 0.44 mmol) was added to a solution of carboxylic acid **19** (100 mg, 0.22 mmol) and pyridine (0.018 mL, 0.22 mmol) in dry acetonitrile (2 mL) and stirred overnight at room temperature. H_2O was added and the precipitated cyanuric acid removed by filtration through a celite pad. CH_2Cl_2 (10 mL) and H_2O (10 mL) were added and the organic layer separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give acid fluoride **20** as a pale yellow solid (96 mg, 96%), m.p. >230 °C; IR (CH_2Cl_2): $\tilde{\nu} = 3054$, 2986, 2305, 1842, 1710, 1509, 1421 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.60$ (d, $J = 8.5$ Hz, 4H, ArH), 7.54 (d, $J = 8.5$ Hz, 2H, ArH), 7.37 (d, $J = 8.5$ Hz, 2H, ArH), 6.96 (d, $J = 8.5$ Hz, 2H, ArH), 5.11 (s, 2H, CH_2O), 4.82 (br, 1H, NH), 4.26 (d, $J = 5.1$ Hz, 2H, CH_2NH), 3.87 (s, 2H, CH_2COF), 1.47 (s, 9H, $(Me)_3$).

13(S) Prop-2-enyl 1-*tert*-butoxycarbonylamino-13-benzyl-8,12,15-trioxo-3-oxa-9,102,11,14-tetraaza-2(1,4), 5(1,4), 6(1,4), 10(1,3)-tetrabenzenaheptadecaphane-17-carboxylate (**21**): *N*-Methylmorpholine (0.027 mL, 0.24 mmol) was added to a solution of acid fluoride **20** (0.05 g, 0.11 mmol) and amine **15** (40 mg, 0.11 mmol) in THF (2 mL). The mixture was heated at reflux under nitrogen for two hours, cooled, diluted with EtOAc (50 mL) washed with saturated sodium bicarbonate (2×50 mL) and brine ($2 \times$ 50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an orange oil. Flash column chromatography on basic alumina Brockman grade II (Et_2O/MeOH (99.5:0.5 to 97:3, v/v)) followed by recrystallisation from EtOAc/Et_2O/hexane gave **21** as a pale yellow solid (56 mg, 62%), m.p. 58–60 °C (EtOAc/Et_2O/hexane); $[\alpha]_D = +7.1$ ($c = 1.0$ in MeOH); IR (CH_2Cl_2): $\nu = 1846$, 1492, 1410, 1086, 1014, 842, 804; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.11$ (s, 1H, pyrN*H*CO), 8.48 (s, 1H, pyrN*H*CO), 7.86 (d, $J = 8.1$ Hz, 1H, *m*-pyrH), 7.73 (d, $J = 7.7$ Hz, 1H, *m*-pyrH), 7.58 (d, $J = 8.1$ Hz, 2H, ArH), 7.56 (d, $J = 8.1$ Hz, 2H, ArH), 7.52 (1H, obscured, *p*-pyr*H*), 7.48 (d, $J = 8.1$ Hz, 2H, ArH), 7.39 (d, $J = 8.1$ Hz, 2H, ArH), 7.25-7.15 (m, 8H, Ph*H*, ArH, ArCH_2N*H*), 6.95 (d, $J = 8.5$ Hz, 2H, ArH), 6.65 (d, $J = 8.1$ Hz, 1H, COCHN*H*), 5.85 (ddt, $J =$ 17.3 Hz,10.3 Hz, 5.5 Hz, 1H, CH_2CH=CH_2), 5.26 (ddt, 17.3 Hz, 3.3 Hz, 1.5 Hz, 1H, CH_2CH=CH_{trans}), 5.20 (ddt, $J = 10.3$ Hz, 3.3 Hz, 1.5 Hz, 1H, CH_2CH=CH_{cis}), 5.08 (s, 2H, CH_2O), 4.90 (bm, 1H, COC*H*NH), 4.50 (dt, $J = 5.9$ Hz, 1.5 Hz, 2H, CH_2CH=CH_2), 4.25 (d, $J = 5.2$ Hz, 2H, CH_2NH), 3.75 (s, 2H, ArCH_2CONH), 3.18 (dd, $J = 13.6$ Hz, 6.6 Hz, 1H, PhC$H_A$$H_B$), 3.10 (dd, $J = 13.6$ Hz, 7.0 Hz, 1H, PhC$H_A$$H_B$), 2.68 (t, $J = 7.0$ Hz, 2H, CH_2CH$_2$), 2.49 (t, $J = 7.0$ Hz, 2H, CH_2CH_2), 1.46 (s, 9H, $(Me)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 172.9$, 172.1, 170.4, 169.9, 158.2, 156.1, 149.9, 149.3, 140.5, 140.4, 140.0, 136. 3, 136.2, 133.8, 131.9, 131.6, 130.1, 129.5, 129.0, 128.7, 128.2, 127.7, 127.4, 127.2, 118.8, 115.1, 110.1, 109.9, 79.7, 69.9, 65.8, 55.2, 44.3, 44.2, 39.5, 30.8, 29.5, 28.8; LRMS (ES^+): *m*/*z* (%): 826 (100); HRMS (ES^+): *m*/*z* calcd for $C_{48}H_{52}N_5O_8$ (M+H): 826.3816; found *m*/*z* 826.3821; elemental analysis calcd for $C_{48}H_{51}N_5O_8$; C 69.80, H 6.22, N 8.48; found: C 69.45, H 6.28, N 8.41.

12(S) 12-Benzyl-2-oxa-8, 92, 10, 13, 18-pentaaza-1(1,4), 4(1,4), 5(1,4), 9(1,3)-tetrabenzenacycloicosaphane-7,11,14,17-tetraone (**1**): Tetrakis(triphenylphosphane)palladium(0) (17 mg, 10 mol%, 0.01 mmol) was added to **21** (0.12 g, 0.15 mmol) and pyrrolidine (0.12 mL, 1.5 mmol) in CH_2Cl_2 (5 mL) at room temperature. The reaction was stirred under argon for half an hour, before being diluted with $CHCl_3$ (25 mL) and washed with 1M HCl (2×25 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated under reduced pressure and dried under high vacuum to give the crude acid **22** as a pale yellow foam (0.12 g 0.15 mmol) which was dissolved in CH_2Cl_2 (5 mL) with DMAP (2 mg, 0.01 mmol) and pentafluorophenol (80 mg, 0.44 mmol). Dicyclohexyl carbodiimide (90 mg, 0.44 mmol) in CH_2Cl_2 (2 mL) was added and the mixture stirred for an hour. The mixture was concentrated under reduced pressure to give the crude pentafluorophenyl ester **22** as an orange gum (0.33 g), which was dissolved in dioxane (2 mL) and HCl (5 mL of a 4M solution in dioxane) was added. The reaction mixture was stirred for half an hour, concentrated under reduced pressure and the resulting gum was triturated with Et_2O to give the crude hydrochloride salt **22** as a pale yellow solid (0.25 g), which was dried for half an hour under high vacuum.

The solid **22** was dissolved in dry, freshly distilled DMF (10 mL) and added by syringe pump (2 mL h^{-1}) to a refluxing solution of DIPEA (0.075 mL, 0.44 mmol) in dry, freshly distilled MeCN (100 mL). After the addition was complete the reaction mixture was refluxed for a further 12 h after which it was concentrated under reduced pressure, diluted with EtOAc (100 mL) and washed with saturated aqueous sodium bicarbonate solution (2 × 100 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography (on silica pre-washed with Et_2O/ammonia saturated MeOH (99:1, v/v)) and gradient elution (Et_2O/ammonia saturated MeOH (99:1 to 97:3, v/v)) gave macrocycle **1** as a white flaky solid (31 mg, 33 %). Further purification was achieved by dissolving the product in acetone or $CHCl_3$ and precipitating with hexane. $[\alpha]_D = -16.3$ ($c = 0.3$ in $CHCl_3$/MeOH, 1:1 v/v); IR (CH_2Cl_2): $\tilde{\nu} = 3392$, 2919, 1635, 1584, 1509, 1449, 1289, 1241 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$): $\delta = 7.97$ (s, 1H, N*H*COCHCH$_2$Ph), 7.91 (d, 1H, $J =$ 8 Hz, *m*-pyrH), 7.78 (d, 1H, $J = 8$ Hz, *m*-pyrH), 7.71 (d, 2H, $J = 8$ Hz, ArH), 7.65 (t, 1H, $J = 8$ Hz, *p*-pyrH), 7.63 (d, 2H, $J = 8$ Hz, ArH), 7.42 (d, 2H, $J =$ 8 Hz, ArH), 7.37 (d, 2H, $J = 8$ Hz, ArH), 7.28 (s, 1H, N*H*COCH$_2$Ar), 7.26 – 7.12 (m, 5H, Ph), 7.05 (d, 2H, $J = 9$ Hz, ArH), 6.78 (d, 2H, $J = 9$ Hz, ArH), 6.25 (d, 1H, $J = 8$ Hz, PhCH$_2$CHN*H*), 5.95 (t, 1H, $J = 5$ Hz, CH$_2$N*H*), 5.30 (s, 2H, CH$_2$O), 4.62 (m, 1H, C*H*CH$_2$Ph), 4.41 (dd, 1H, $J = 6$ Hz, 14 Hz, C*H*$_A$H$_B$NH), 4.06 (dd, 1H, $J = 5$ Hz, 14 Hz, CH$_A$*H*$_B$NH), 3.80 (s, 2H, NHCOC*H*$_2$Ar), 3.19 (dd, 1H, $J = 7$ Hz, 14 Hz, C*H*$_A$H$_B$Ph), 2.96 (dd, 1H, $J = 8$ Hz, 12 Hz, CH$_A$*H*$_B$Ph), 2.52 – 2.13 (4H, m, CH$_2$CH$_2$); ^{13}C NMR (75.5 MHz, 5 % CD_3OD in $CDCl_3$): $\delta = 173.2$, 172.8, 169.5, 169.1, 156.9, 149.3, 148.9, 140.8, 139.8, 138.9, 137.0, 136.2, 133.1, 130.5, 130.4, 129.4, 129.3, 128.9, 128.1, 128.0, 127.3, 127.1, 116.6, 110.0, 109.8, 69.3, 55.8, 45.0, 43.5, 37.5, 31.3, 31.2; LRMS (ES$^+$) 668 (100); HRMS (FAB) calcd for $C_{40}H_{37}N_5O_5$: 668.2874; found 668.2863.

General procedure for NMR titration experiments

Macrocycle **1** was first dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$, and brine, then dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. All deuterochloroform used was passed through alumina and collected over activated 4Å molecular sieves. A standard solution of **1** was prepared immediately prior to use by dissolving a known amount (typically ≈4 mgs) in $CDCl_3$ (2 mL). A known aliquot of this solution (either 500 or 600 μL) was transferred to a dry NMR tube. A standard solution of the guest solution at 10 – 20 times the concentration of the host solution was similarly prepared. After recording the spectra of the free host solution, aliqouts of the guest solution were added and the NMR spectra recorded after each addition. The chemical shift of the most downfield pyr-N*H*CO relative to residual chloroform ($\delta = 7.27$) was recorded. The signals corresponding to the other NH protons were observed to move during the titration but were either too broad or became obscured as they merged with the aromatic signals and were consequently not monitored throughout the titration. The volume of the added aliquots of guest solution varied from addition to addition and from experiment to experiment so as to obtain the best range of data points for analysis. The experimental data so obtained was analysed by curve fitting to obtain the association constant (K_a) using HOSTEST 5.0.[23] The quality of the curve fit was assessed by stochastic analysis of the curve, also using HOSTEST 5.0, and is indicated by the associated errors (see Table 2).

Acknowledgements

We thank the EPSRC and SmithKline Beecham for a CASE award (J. D.), Dr Jonathan Essex for assistance with the molecular modelling studies and Peter Henley for assistance in preparing this manuscript.

[1] For reviews see a) J. J. B. Perry, J. D. Kilburn, *Contemp. Org. Synth*. **1997**, *4*, 61; b) *Tetrahedron Symposium-In-Print on Molecular Recognition*, (Ed.: A. D. Hamilton) *Tetrahedron* **1995**, *51*, 343; c) T. H. Webb, C. S. Wilcox, *Chem. Soc. Rev.* **1993**, *22*, 383.

[2] For recent examples of synthetic receptors for amino acid and peptide derivatives, see for example: a) R. J. Pieters, J. Cuntze, M. Bonnet, F. Diederich, *J. Chem. Soc., Perkin Trans. 2* **1997**, 1891; b) W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155; c) D. W. P. M. Löwik, S. J. E. Mulders, Y. Cheng, Y. Shao, R. M. J. Liskamp, *Tetrahedron Lett.* **1996**, *37*, 8253; d) M. Asakawa, C. L. Brown, D. Pasini, J. F. Stoddart, P. G. Wyatt, *J. Org. Chem.* **1996**, *61*, 7234; e) C. Gennari, H. P. Nestler, B. Salom, W. C. Still, *Angew. Chem.* **1995**, *107*, 1894; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1765; f) Y. Kuroda, Y. Kato, T. Higashioji, J. Hasegawa, S. Kawanami, M. Takahashi, N. Shiraishi, K. Tanabe, H. Ogoshi, *J. Am. Chem. Soc.* **1995**, *117*, 10950; g) S. R. LaBrenz, J. W. Kelly, *J. Am. Chem. Soc.* **1995**, *117*, 1655; h) H. J. Schneider, *Angew. Chem.* **1993**, *105*, 890; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 848. See also references [3, 5].

[3] a) G. J. Pernia, J. D. Kilburn, J. W. Essex, R. J. Mortishire-Smith, M. Rowley, *J. Am. Chem. Soc.* **1996**, *118*, 10220; b) G. J. Pernia, J. D. Kilburn, M. Rowley, *J. Chem. Soc., Chem. Commun.* **1995**, 305; c) C. P. Waymark, J. D. Kilburn, I. Gillies, *Tetrahedron Lett*. **1995**, *36*, 3051; d) H. K. Patel, J. D. Kilburn, G. J. Langley, P. D. Edwards, T. Mitchell, R. Southgate, *Tetrahedron Lett.* **1994**, *35*, 481.

[4] a) F. Garcia-Tellado, S. Goswami, S. K. Chang, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1990**, *112*, 7393; b) F. Garcia-Tellado, J. Albert, A. D. Hamilton, *J.Chem. Soc. Chem. Commun.* **1991**, 1761.

[5] For preliminary communications of parts of this work, see: a) J. Dowden, P. D. Edwards, J. D. Kilburn, *Tetrahedron Lett*. **1997**, *38*, 1095; b) S. S. Flack, J. D. Kilburn, *Tetrahedron Lett*. **1995**, *36*, 3409.

[6] O. Mitsunobu, J. Kimura, K. Iizumu, N. Yanagida, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 510.

[7] a) H. C. Brown, Y. M. Choi, S. Narasimhan, *Synthesis* **1981**, 605; b) H. C. Brown, Y. M. Choi, S. Narasimhan, *J. Org. Chem.* **1982**, *47*, 3153.

[8] H. Azizian, C. Eaborn, A. Pidcock, *J. Organomet. Chem.* **1981**, *215*, 49.

[9] J. K. Stille, *Angew. Chem.* **1986**, *98*, 504; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508.

[10] J. Malm, P. Björk, S. Gronowitz and A. B. Hörnfeldt, *Tetrahedron Lett.* **1992**, *33*, 2199.

[11] K. Venkataraman, D. R. Wagle, *Tetrahedron Lett.* **1979**, *20*, 3037.

[12] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Lett.* **1989**, *30*, 1927.

[13] M. Bodanszky, A. Bodanszky, *The Practice of Peptide Synthesis*, Springer, Berlin, **1984**.

[14] C. D. Hurd, R. Roach, C. W. Huffman, *J. Am. Chem. Soc.* **1956**, *78*, 104.

[15] J. Coste, D. Le-Nguyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205.

[16] J. Jones, *The Chemical Synthesis of Peptides*, Clarendon Press, Oxford, **1991**.

[17] V. Farina, S. Kapadia, B. Krishnan, C. Wang, L. S. Liebeskind, *J. Org. Chem.* **1994**, *59*, 5905.

[18] V. Farina, B. Krishnan, D. R. Marshall, G. P. Roth, *J. Org. Chem.* **1993**, *58*, 5434.

[19] V. Farina, B. Krishnan, *J. Am. Chem. Soc.* **1991**, *113*, 9585.

[20] a) L. A. Carpino, D. Sadat-Aalaee, H. G. Chao, R. H. DeSelms, *J. Am. Chem. Soc.* **1990**, *112*, 9651; b) J. N. Bertho, A. Loffet, C. Pinel, F. Reuther, G. Sennyey, *Tetrahedron Lett.* **1991**, *32*, 1303.

[21] R. Deziel, *Tetrahedron Lett.* **1987**, *28*, 4371.

[22] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *63*, 207.

[23] Hostest 5 software: C. S. Wilcox, N. M. Glagovich, University of Pittsburgh, USA, C. S. Wilcox and the University of Pittsburgh, **1993**. See also: a) C. S. Wilcox *Design,* Synthesis, and Evaluation of an Efficacious Functional Group Dyad. Methods and Limitations in the Use of NMR for Measuring Host – Guest Interactions, in *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, (Ed.: H. J. Schneider, H. Dürr) VCH, Weinheim, **1991**; b) C. S. Wilcox, J. C. Adrian, Jr, T. H. Webb, F. J. Zawacki, *J. Am. Chem. Soc.*, **1992**, *114*, 10189.

[24] a) K. A. Connors, *Binding Constants, The Measurement of Molecular Complex Stability*, Wiley, **1987**, 24 – 28; b) A. Job, *Annales de Chimie* **1928**, *9*, 113.

[25] G. Weber, *Molecular Biophysics*; (Ed.: B. Pullman, M. Weissbluth) Academic Press, New York, **1965**, 369 – 397.

[26] 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, although differences in the series of substrates presented here seem unlikely to be larger than the experimental errors in the measured binidng constants.

[27] The substantially stonger binding of *N*-Cbz-β-alanyl-L-alanine compared with *N*-Cbz-β-alanyl-L-lactic acid 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.
[28] C. T. Walsh, S. L. Fisher, I. S. Park, M. Prahalad, Z. Wu, *Chemistry and Biology* **1996**, *3*, 21.
[29] R. J. Dancer, A. C. Try, G. J. Sharman, D. H. Williams, *J. Chem. Soc. Chem. Commun.*, **1996**, 1445.
[30] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440.
[31] D. Q. McDonald, W. C. Still, *Tetrahedron Lett.* **1992**, *33*, 7742.
[32] a) W. D. Cornell, P. Ceiplak, C. I. Baoyly, I. R. Gould, K. M. Merz, Jr., D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179; b) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Chio, G. Alagona, S. Profeta, Jr., P. Weiner, *J. Am. Chem. Soc.* **1984**, *106*, 765; c) S. J. Weiner, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **1986**, *7*, 230.
[33] W. C. Still, A. Tempczyk, R. C. Hawley, *J. Am. Chem. Soc.* **1990**, *112*, 6127.
[34] D. D. Perrin, W. L. F. Armarego, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 3rd ed, **1989**.

Received: April 28, 1998 [F 1123]