Synthesis, Conformational Studies and Binding Properties of Acyclic Receptors for *N*-Protected Amino Acids and Dipeptides

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Solid-phase syntheses of novel receptors featuring a 2,6-diamidopyridine "head" group and bearing sulfonamidopeptide sidearms are described. NMR conformational studies show that the "two-armed" receptors collapse into an intramolecularly folded structure through formation of a hydrogen-bonding network. In order to accommodate the guests, receptors have to unfold, breaking the intramolecular hydrogen bonds. The absolute binding constants of receptors with *N*-protected amino acids and dipeptides are therefore relatively weak. However, one receptor shows a high selectivity for *N*-Cbz-D-Ala-D-AlaOH over its enantiomer *N*-Cbz-L-Ala-L-AlaOH.

Introduction

Noncovalent interactions play an important role in stabilising the secondary structures of natural biopolymers (peptides, polysaccharides and oligonucleotides) and promoting the binding of natural or synthetic receptors to their targets. Synthetic receptors for biologically relevant substrates such as amino acids and peptides are of increasing importance for possible applications in molecular recognition (enantioselective binding, separation of racemates) and as potential biosensors, therapeutics and catalysts.

In the field of synthetic receptors, several macrocyclic hosts have been prepared and have been shown to bind amino acids or peptide fragments with excellent selectivity.[1−3] During the last decade, a few studies focused on a different class of receptors for peptides; these were called "tweezer receptors" or "two-armed" receptors.[4] In the structure of tweezer receptors, the "linker" (or "head group" or "hinge") is typically a conformationally restricted moiety such as a 1,2-diamine,[5] a steroidal core,[6] a guanidinium,[7a] a thiourea,[7b] or a dibenzofuran.[8] The "linker" covalently binds and directs two functionalised substrate-binding arms. The two sidearms may be macrocyclic oligomers[5] or simple peptides.[6−8] Whereas in many tweezer receptor systems the head group plays only a limited role in the binding of the guest, the group in Southampton has recently developed tweezer receptors that make use of a diamidopyridine moiety as the head group. This can specifically bind to carboxylic acid functionalities, and such tweezers, with peptidic arms, have proved to be selective receptors for peptides with a carboxylic acid terminus.[9] The diamidopyridine moiety also proved to be a highly efficient carboxylic acid binding site (CBS) when incorporated into macrocyclic receptors.[1b,10]

Recently, an interest in synthetic receptors based on peptidosulfonamide sidearms has also emerged.[3,11−13] Over the past five years, the group in Milano has been studying the conformational preferences of unnatural biopolymer scaffolds containing β-sulfonamidopeptides[14] and vinylogous sulfonamidopeptides.[15] Sulfonamidopeptides display an aspect of the covalent framework that is essential for the formation of folded structures by intramolecular hydrogen bonding: The repeating backbone structure contains both hydrogen-bond donors (strong: CONH; very strong: SO_2NH) and hydrogen-bond acceptors (strong: C=O; weak: SO_2N).[14,15] In this paper we report the synthesis and conformational and binding studies of a novel class of "two-armed" synthetic receptors for *N*-protected amino acids and dipeptides, each containing a 2,6-diamidopyridine binding unit bearing pendant pseudo-peptide legs consisting of an amino acid (L-Phe) and chiral β-aminosulfonamides.[16] One of these receptors in particular (**5**, Figure 1) displays a high binding selectivity for the dipeptide *N*-Cbz-D-Ala-D-AlaOH (Cbz = benzyloxycarbonyl) over its enantiomer *N*-Cbz-L-Ala-L-AlaOH.[16]

Results and Discussion

The synthesis of compounds **1**−**3** was accomplished on solid phase, starting from *N*-Fmoc-Gly Wang resin **6** (load-

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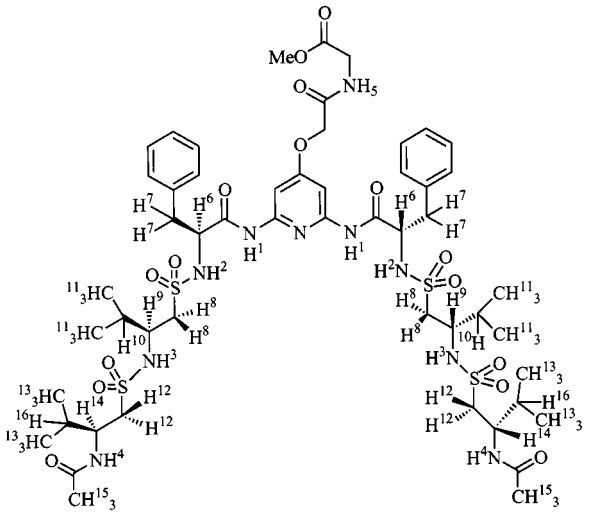

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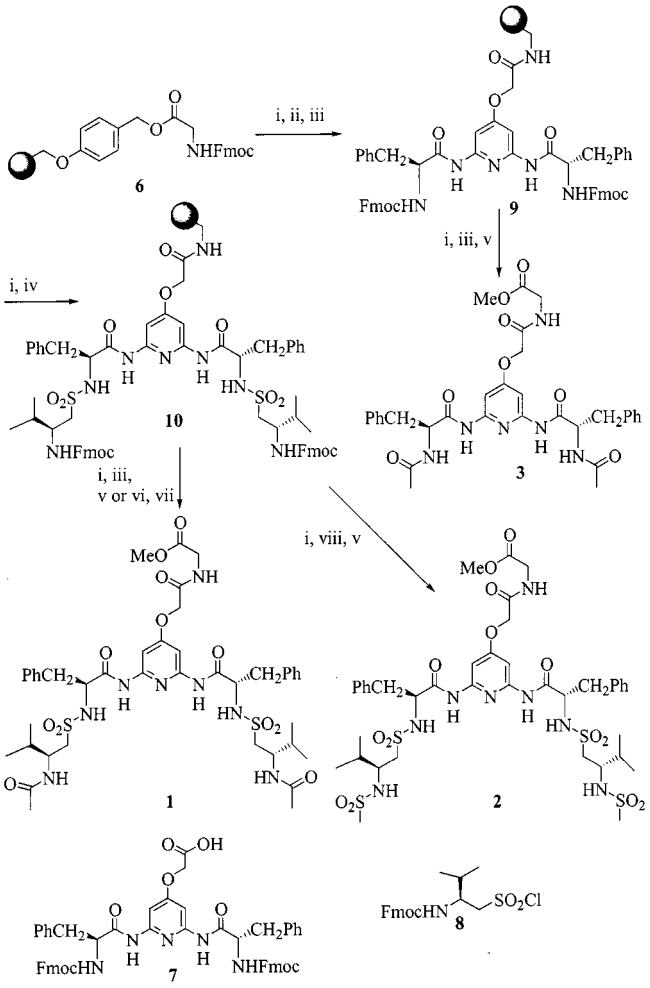
Figure 1. Labelling of hydrogen atoms in receptor **5**

ing 0.88 mmol/g) (Scheme 1) [Fmoc = *N*-(9-fluorenylmethoxycarbonyl)]. After deprotection of the Fmoc-Gly Wang resin, "hinge" **7**[9] was coupled using a 20% excess of resin to give **9**. The unchanged amino groups on the resin were capped with acetylimidazole (AcIm). Deprotection of the Fmoc groups and treatment with the L-valine-derived β-*N*-Fmoc-aminosulfonyl chloride **8**, with DMAP as catalyst and methyl trimethylsilyl dimethylketene acetal (MTDA) as HCl scavenger,[14,17] gave the bis(sulfonamide) **10**. Two cycles were required to ensure completion of the coupling, until no free amino groups could be detected by two different colour tests.[18] Cleavage of the Fmoc protecting groups, followed by acetylation or mesylation, gave bis(sulfonamide) derivatives with two different terminal groups. Release of the resin-bound products was accomplished either by a direct basic methanolysis to provide the methyl ester derivatives **1** and **2**, or alternatively by acidic TFA/H_2O cleavage followed by esterification (two steps, but easier to purify). Compound **3** was synthesized starting from compound **9** by Fmoc deprotection followed by acetylation and cleavage from the resin (basic methanolysis).

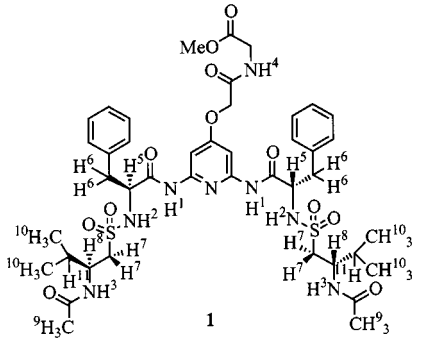
The conformations of compounds **1**−**3** in solution were studied by NMR spectroscopy. In the ^{1}H NMR spectrum of **1** in $CDCl_3$, the peaks were rather broad, indicating a slow conformational equilibrium at room temperature (on the NMR timescale). Assignment of all the proton signals of receptor **1** could, however, be made unambiguously by means of COSY and TOCSY experiments (Figure 2). In CD_3COCD_3 or CD_3SOCD_3, in contrast, the spectra were well resolved, showing single sets of resonances and proving the absence of diastereomeric mixtures (also proved by the ^{13}C NMR spectrum). The N−H protons, H^1 to H^3, displayed a fairly small concentration dependence in $CDCl_3$ (or in CD_2Cl_2), which suggests that compound **1** essentially does not aggregate in the concentration range under consideration (1.25−20 mM, see associated electronic Supporting Information).[19] At a concentration of 8.5 mM in $CDCl_3$,



Scheme 1. Reagents and conditions: (i) piperidine [20% in *N*,*N*-dimethylformamide (DMF)]; (ii) **7**, 1,3-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole hydrate (HOBT), 4-(dimethylamino)pyridine (DMAP), dichloromethane (DCM); (iii) acetylimidazole (Aclm), DCM; (iv) **8**, DMAP, methyl trimethylsilyl dimethylketene acetal (MTDA), DCM, two cycles; (v) Et_3N, MeOH, DMF; (vi) trifluoroacetic acid (TFA), H_2O; (vii) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), DMAP, MeOH, THF; (viii) methanesulfonyl chloride (MsCl), Et_3N, DCM

the signal of the amidopyridine NH^1 protons appeared at unexpectedly low field (δ = 9.41, see Table 1, standard values δ = 8.00−8.50[1b][10a]), whilst the sulfonamide protons NH^2 were also strongly deshielded (δ = 6.32 compared to their standard values δ = 4.50−4.74).[14] The acetamide NH^3 proton signals were located closer to their traditional chemical shifts (δ = 6.22 compared to 6.00−6.20).

The temperature dependence of protons H^1 to H^3 was studied between 298 and 313 K, at a concentration of 1.5 mM in $CDCl_3$. Unfortunately, the dependence at low temperatures could not be studied because of the limited solubility of compound **1** in $CDCl_3$ and in CD_2Cl_2. Calculated values of $\Delta\delta/\Delta T$ for H^1, H^2, H^3 are −9.8, −3.8, and −5.8 ppb/K, respectively, over the range of temperatures studied (see associated electronic Supporting Information).[19] These temperature coefficients indicate various degrees of intramolecular hydrogen bonding and equilibria with non-hydrogen-bonded states.[14,15,20]



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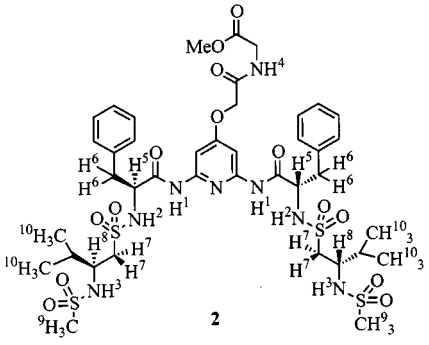

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Figure 2. Labelling of hydrogen atoms in receptors **1** and **2**

Table 1. Chemical shifts of NH protons H^1 to H^3 for compounds **1**−**5** (8.5 mM in $CDCl_3$)

Proton	**1**	**2**	**3**	**4**	**5**
N−H^1	9.41[a]	9.07[a]	8.83[a]	8.87/8.75[a]	9.32[a]
N−H^2	6.32[b]	5.13 (6.34)[b] [c]	6.56[d]	5.98[b]	5.78[b]
N−H^3	6.22[d]	6.34 (5.13)[b] [c]		5.81[d]	6.38[b]
N−H^4					6.22[d]

[a] Reference value for the amidopyridine protons: δ = 8.00−8.50, see refs.[1b,10a] − [b] Reference value for the sulfonamide protons: δ = 4.50−4.74, see ref.[14] − [c] N−H^2 and N−H^3 could not be unequivocally attributed. − [d] Reference value for the carboxyamide protons: δ = 6.00−6.20.

NOESY and ROESY experiments were performed for compound **1** at two different concentrations (5 and 10 mM in $CDCl_3$) and showed the same contact pattern. The CH_3 acetyl hydrogen atoms (H^9) gave cross-peaks with the proton of the valine stereogenic centre (H^8) and with the NH of the terminal acetamide (H^3). While the cross-peak between H^9 and H^3 is typical of a *trans*-amide rotamer, the cross-peak between H^9 and H^8 is indicative of the presence of a *cis*-amide rotamer,[21,22] which is usually present as a small percentage in secondary amides (ca. 3% for *N*-methylacetamide in $CHCl_3$).[23] The acetyl CH_3 hydrogen atoms (H^9) also gave cross-peaks with the aromatic protons of the phenylalanine ring and with the benzylic protons (H^6). Other interesting contacts are: The isopropyl CH_3 protons (H^{10}) gave cross-peaks with the aromatic protons of the phenylalanine ring; the proton at the phenylalanine stereogenic centre (H^5) gave cross-peaks with protons CH_2SO_2 (H^7), and with the isopropyl protons CH_3 (H^{10}) and the isopropyl proton CH (H^{11}). From the NOE data, and the relatively downfield position of the NH signals in the ^1H NMR, we can conclude that "two-armed" receptor **1** collapses into an intramolecularly folded structure through formation of a hydrogen-bonding network involving the amidopyridine moiety, the sulfonamide NH and the terminal acetamide.

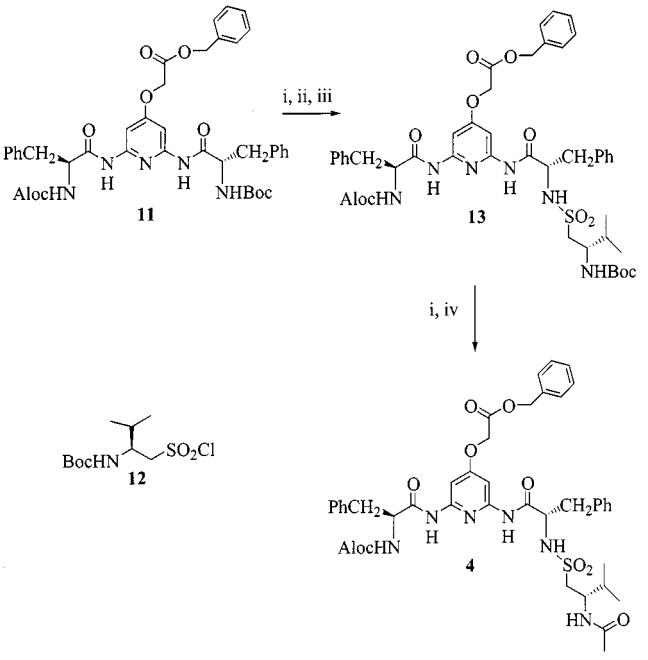
Analogous studies were conducted on the "two-armed" receptor **2** and gave similar results. In summary: (a) the peaks were rather broad in the ^1H NMR spectrum of **2** in $CDCl_3$, indicating a slow conformational equilibrium at room temperature (on the NMR timescale); (b) protons H^1, H^2 and H^3 displayed a fairly small concentration dependence in $CDCl_3$, which suggests that compound **2** essentially does not aggregate in the concentration range considered (1.0−22.5 mM, see associated electronic Supporting Information);[19] (c) the signal of the amidopyridine NH^1 protons appeared at low field (δ = 9.07; see Table 1), whilst the sulfonamide NH^2 and NH^3 proton signals appeared at δ = 6.34 and 5.13, indicating hydrogen bonding; (d) ROESY experiments in $CDCl_3$ (concentration: 5 mM) showed the presence of several cross-peaks indicative of an intramolecularly collapsed structure similar to **1**. In particular, the cross-peaks between mesyl CH_3 (H^9) and isopropyl CH_3 (H^{10}), mesyl CH_3 (H^9) and CH of the valine stereocentre (H^8) were indicative of a *cisoid* conformation of the terminal mesyl group. It was therefore concluded that an intramolecularly folded structure was also highly populated in $CDCl_3$ in this case, and that the presence of a poor hydrogen-bond acceptor such as the methanesulfonamide group[14,15] in the terminal position was compensated for by the very strong hydrogen-bond-donating ability of the methanesulfonamide NH group.[14,15]

In comparison, the spectrum of the control compound **3** (Scheme 1) in $CDCl_3$ was perfectly resolved, and the amidopyridine NH^1 signal was found at δ = 8.83 (for a concentration of 8.5 mM, see Table 1), whereas the acetamide N−H proton was found at δ = 6.56. Protons H^1 and H^2 displayed a fairly small concentration dependence in $CDCl_3$, suggesting that compound **3** essentially does not aggregate in the concentration range under consideration (see associated electronic Supporting Information).[19] ROESY experiments in $CDCl_3$ (concentration: 10 mM) showed cross-peaks consistent with the presence of *trans*-amide rotamers (a minor contact between CH_3 of the terminal acetamide and CH of the phenylalanine stereocentre was also detected, indicating the presence of a small percentage of *cis*-amide rotamer).[22,23]

In order to obtain a simplified view of the system, and to study the behaviour of the single legs of receptor **1**, we decided to prepare the differently substituted scaffold **4**, starting from the unsymmetrically protected "hinge" **11** (Scheme 2). As shown in Scheme 2, **11** was deprotected (25% TFA in DCM), and treated with the L-valine-derived β-*N*-Boc-aminosulfonyl chloride **12**, with DMAP as catalyst and methyl trimethylsilyl dimethylketene acetal

(MTDA) as HCl scavenger,[14,17] to give the monosulfonamide **13**. Removal of the Boc group (25% TFA in DCM) and acetylation with acetylimidazole yielded receptor **4**. ^{1}H NMR studies on receptor **4** (8.5 mM in $CDCl_3$) showed a single set of well-resolved signals (see Table 1): The amidopyridine N−H proton signals (two different signals at δ = 8.75 and 8.87) were shifted substantially upfield compared to that of H^1 of receptor **1** (δ = 9.41), and the same effect could be seen for the sulfonamide N−H proton (δ = 5.98 vs. 6.32 for **1**) and the acetamide N−H proton (δ = 5.81 vs. 6.22 for **1**). From these data we concluded that the hydrogen-bonding network in **1** and **2** was strengthened by the cooperative effect of both legs. In the ROESY spectrum (8.3 mM in $CDCl_3$) the acetamide methyl group gave cross-peaks with the isopropyl CH_3, the CH of the valine stereocentre, and the acetamide NH. These contacts are typical of the presence both of a *trans*-amide rotamer and of a *cis*-amide rotamer,[22,23] usually present in small percentages in secondary amides (see the above discussion for **1**).

Binding studies with receptor **1** in $CDCl_3$ were carried out with a series of substrates, by a standard titration experiment, by monitoring the shift of the NH and CH signals on addition of successive aliquots of guest and analysing the resulting binding curves by nonlinear regression analysis.[24] In agreement with the above results, and as a further proof of strong intramolecular self-association, compound **1** proved to be a rather poor receptor for *N*-protected (*N*-Boc and *N*-Cbz) amino acids and dipeptides (see Table 2), even in a noncompetitive solvent such as chloroform. A slightly higher affinity for *N*-Cbz-Ala-OH (either D or L) in preference to *N*-Boc-L-Ala-OH was observed, with binding constants of 207 (*N*-Cbz-L-Ala-OH), 270 (*N*-Cbz-D-Ala-OH) and 119 (*N*-Boc-L-Ala-OH), but little enantioselective binding was observed with the *N*-Cbz-amino acids. Binding of all three amino acid substrates gave rise to significant downfield shifts in the amidopyridine NH^1 signal (≥ 0.5 ppm), but whereas binding of the L enantiomers produced a small upfield shift in the acetamide NH^3 signal (ca. 0.1) and little change to the sulfonamide NH^2 signal, the binding of *N*-Cbz-D-AlaOH produced a downfield shift of the sulfonamide NH^2 signal (ca. 0.4) and little change to the acetamide NH^3 signal. Saturation was reached upon addition of an excess of *N*-protected amino acid as guest (20−30 equiv.). In more polar solvents such as [D_6]acetone, CD_3CN or [D_6]DMSO, the spectra were well resolved, but the chemical shifts of the host signals did not change upon addition of an excess of *N*-protected amino acid.



Scheme 2. Reagents and conditions: (i) TFA (25% in DCM); (ii) K_2CO_3 (10% in H_2O); (iii) **12**, DMAP, MTDA, DCM; (iv) Aclm, DCM

Binding of dipeptide guests gave only slightly higher binding constants, and titration of **1** with, for example, *N*-Cbz-L-Ala-L-Ala-OH gave a binding constant of 245 M^{-1} and resulted in downfield shifts both of the amidopyridine and of the sulfonamide NH signals (NH^1: Δδ = 0.25; NH^2: Δδ = 0.30) and an upfield shift of acetamide NH^3 (Δδ = 0.12). A change in the *N*-protecting group from Cbz to Boc had only a small effect on the binding (or on the shifts of the NH signals), while a change in the *C*-terminal amino

Table 2. Binding constants K_{ass} (M^{-1}) for the 1:1 complexes formed between receptors **1** and **5** and various amino acids and dipeptides derivatives, calculated from the chemical shifts of various proton signals of **1** and **5**, in $CDCl_3$ at 25 °C

Guest	Host **1** K_{ass} [M^{-1}]	(Data[a])	Host **5** K_{ass} [M^{-1}]	(Data[a])
N-Boc-L-Ala-OH	119	(NH^1, NH^3)	–[b]	
N-Cbz-L-Ala-OH	207	(NH^1, NH^3, $CH^7_2SO_2$)	32	(NH^1)
N-Cbz-D-Ala-OH	270	(NH^1, $CH^6H^{6'}$Ph, $CH^6H^{6'}$Ph)	–[b]	
N-Boc-L-Ala-L-Ala-OH	361	(NH^1, NH^2)	–[b]	
N-Cbz-L-Ala-L-Ala-OH	245	(NH^1, NH^2)	107	(NH^1, CH_2)
N-Cbz-D-Ala-D-Ala-OH	242	(NH^2, NH^3)	2404[c]	(NH^1, NH^3, $CH^7H^{7'}$Ph, $CH^7H^{7'}$Ph, $CH^{6,14}$)
N-Boc-L-Ala-L-Phe-OH	297	(NH^1, NH^2)	109	(NH^1)
N-Cbz-L-Ala-L-Phe-OH	292	(NH^1, NH^2, NH^3, $CH^6H^{6'}$Ph)	–[b]	
N-Boc-L-Ala-L-Trp-OH	377	(NH^1, NH^2)	ND[d]	

[a] Data from signals used in the binding calculation. – [b] Experiment not performed. – [c] Error was estimated as < 5%, with data from five different proton signals. – [d] Changes in the spectrum were too small to allow reliable estimation of the binding constant.

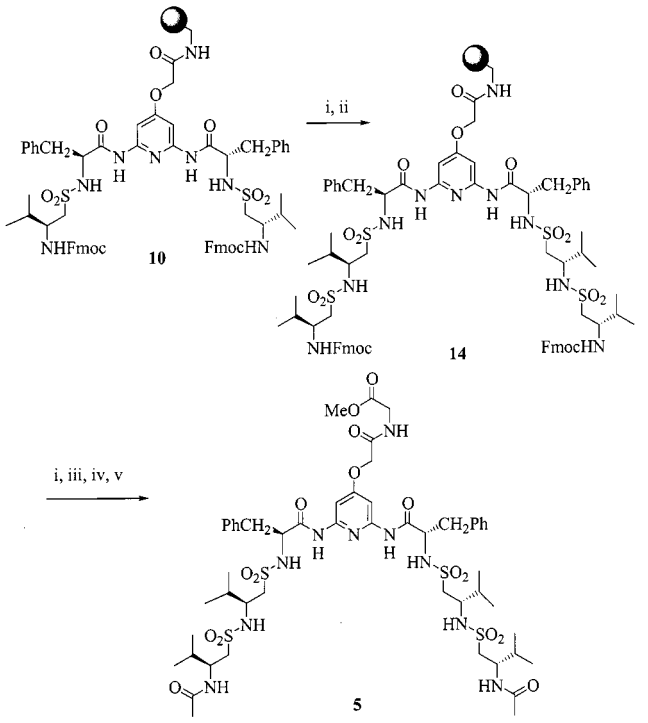
acid from alanine to phenylalanine or tryptophan similarly had only a limited effect. Titration with *N*-Cbz-D-Ala-D-Ala-OH resulted in little change to the amidopyridine NH^1 signal ($\Delta\delta \leq 0.03$), but analysis of the downfield shift of the sulfonamide NH^2 signal ($\Delta\delta = 0.28$) and of the upfield shift of the acetamide NH^3 signal ($\Delta\delta = 0.19$) gave a binding constant of 242 M^{-1}, indicating that **1** also shows little enantioselectivity for dipeptides [K_a(*N*-Cbz-L-Ala-L-AlaOH) = 245 M^{-1}; Table 2]. Binding of amino acids and dipeptides was also accompanied by a small shift of several CH signals in host **1** (≤ 0.12). The 1:1 stoichiometry of the complexes between host **1** and the amino acidic and peptidic guests was established by Scatchard plots,[25] and by the good fit of the experimental data to the 1:1 model.[26]

In the binding experiments with host **1**, we also monitored the chemical shifts of the *N*-protected amino acid and dipeptide guests. The carbamate NH^a signals were shifted downfield on binding of both amino acids ($\Delta\delta = 0.08-0.14$) and dipeptides ($\Delta\delta = 0.10-0.35$).[27] The amide NH^b signals on binding the dipeptides were also shifted downfield with a $\Delta\delta$ value similar to that of the NH^a signals ($\Delta\delta = 0.10-0.35$).[27]

Thus, receptor **1** does appear to bind dipeptides and simple amino acid derivatives, but the absolute binding constants are rather low in comparison to those observed in other diamidopyridine-based receptors, and the shifts of the various NH signals on binding are also rather small in comparison to those seen in other related receptor systems.[1b,10]

In order to study the influence of the length of the legs and possibly to increase the affinity of our "two-armed" receptors for *N*-protected amino acids and dipeptides, we decided to test the new receptor **5**, with longer pseudo-peptide legs (Scheme 3). Compound **5** was synthesized from the bis(sulfonamide) **10** (Scheme 1) by removal of the Fmoc groups, treatment with the L-valine-derived β-*N*-Fmoc-aminosulfonyl chloride **8**, and subsequent deprotection, acetylation and cleavage (Scheme 3).

In the ^1H NMR spectrum of **5** in $CDCl_3$, the peaks were rather broad, indicating a slow conformational equilibrium at room temperature, as in the case of compound **1**. Assignment of all the proton signals of receptor **5** could, however, be accomplished by means of COSY and TOCSY experiments (Figure 1). The ^1H NMR spectra at 320 K in $CDCl_3$ and at 300 K in CD_3COCD_3 and in CD_3CN were well resolved and showed single sets of resonances, proving the absence of diastereomeric mixtures (also proved by the ^{13}C NMR spectra). The signals of N−H protons H^1, H^2, H^3 and H^4 displayed a fairly small concentration dependence in $CDCl_3$ (slightly higher for H^3 compared to H^1, H^2 and H^4), suggesting that compound **5** essentially does not aggregate in the concentration range under consideration (0.47−20 mM, see associated electronic Supporting Information).[19] At a concentration of 8.5 mM in $CDCl_3$, the signal of the amidopyridine NH^1 protons appeared at low field ($\delta = 9.32$ compared to $\delta = 8.00-8.50$ for reference values,[1b][10a] see Table 1). The signals of the sulfonamide protons NH^2 and NH^3 appeared at low field as well ($\delta = 5.78$ and 6.38 compared to $\delta = 4.50-4.74$ for reference values[14]), suggesting intramolecular hydrogen bonding. In contrast, the signal of the acetamide protons NH^4 was closer to the conventional value ($\delta = 6.22$ compared to $\delta = 6.00-6.20$ for reference values).



Scheme 3. Reagents and conditions: (i) piperidine (20% in DMF); (ii) **8**, DMAP, MTDA, DCM, two cycles; (iii) Aclm, DCM; (iv) TFA, H_2O; (v) EDC, DMAP, MeOH, THF

The temperature dependence of protons H^1 to H^4 was studied between 300 and 320 K, at a concentration of 1 mM in $CDCl_3$. Calculated values of $\Delta\delta/\Delta T$ for H^1, H^2, H^3, H^4 are − 2.5, − 4.8, − 4.5, and − 4.5 ppb/K, respectively, over the range of temperatures studied (see associated electronic Supporting Information).[19] These temperature coefficients revealed a small temperature dependence in protons H^1 to H^4, indicating various degrees of intramolecular hydrogen bonding.

NOESY experiments were performed at a concentration of 12 mM in $CDCl_3$, and showed some interesting contacts. The CH_3 acetyl hydrogen atoms (H^{15}) gave a cross-peak with the NH of the acetyl group (H^4), which is typical of a *trans*-amide rotamer [a minor contact between CH_3 of the terminal acetamide (H^{15}) and H^{14} at the vicinal stereocentre was also detected, indicating the presence of a small percentage of a *cis*-amide rotamer].[22,23] The CH_3 acetyl hydrogen atoms (H^{15}) also gave distinct cross-peaks with the isopropyl CH_3 hydrogen atoms of two different valine residues (H^{11} and H^{13}). The NH proton of the amidopyridine (H^1) gave cross-peaks with the proton of the valine stereogenic centre (H^9) and with the isopropyl CH proton (H^{10}). The aromatic protons of the phenylalanine ring gave cross-peaks with the proton of the valine stereogenic centre (H^9), with CH_2SO_2 (H^8), with the isopropyl CH_3 (H^{11}), and with the isopropyl CH proton (H^{10}). The benzylic protons (H^7) gave cross-peaks with the isopropyl CH_3 (H^{11}), and

with the isopropyl CH proton (H^{10}). The proton of the phenylalanine stereogenic centre (H^6) also gave cross-peaks with the isopropyl CH_3 (H^{11}) and with the isopropyl CH proton (H^{10}). All these NOE contacts suggested an intramolecularly folded structure for the "two-armed" receptor **5**, produced by formation of a hydrogen-bonding network involving the amidopyridine moiety and the different sulfonamide and acetyl groups of the two legs.

As further evidence of the strong self-association, compound **5** initially proved to be a poorer receptor than compound **1** for *N*-protected amino acids and *N*-protected dipeptides (see Table 2). Compound **5** binds the amino acid *N*-Cbz-L-Ala-OH approximately one order of magnitude less tightly than compound **1** does (K_a = 32 vs. 207 M^{-1}). The NH^1 chemical shift was shifted downfield, but saturation could not be reached even after addition of 20 equiv. of guest.

Receptor **5** associates less strongly than receptor **1** with *N*-Cbz-L-Ala-L-Ala-OH and with *N*-Boc-L-Ala-L-Phe-OH (K_a = 107 and 109 vs. 245 and 297 M^{-1}, see Table 2). The NH^1 chemical shift was shifted downfield (ca. 0.3) but saturation could not be reached even after addition of 15−20 equiv. of dipeptide. The 1:1 stoichiometry of the complexes between host **5** and the amino acidic and peptidic guests was established by Scatchard plots[25] and by the good fit of the experimental data to the 1:1 model.[26]

Titration of **5** with *N*-Cbz-L-Ala-L-Ala-OH gave a binding constant of 107 M^{-1} and a downfield shift of $\delta = 0.3$ for the amidopyridine NH^1, but only small changes to the sulfonamide and acetamide NH protons (NH^2, NH^3, NH^4; $\Delta\delta \leq 0.07$). In contrast, titration of **5** with *N*-Cbz-D-Ala-D-Ala-OH gave a binding constant of 2404 M^{-1}, with a particularly large downfield shift for the sulfonamide NH^3 signal ($\Delta\delta = 0.72$) but an upfield shift for the sulfonamide NH^2 ($\Delta\delta = 0.38$) and even a small *upfield* shift for the amidopyridine NH^1 ($\Delta\delta = 0.05$)! Binding of *N*-Cbz-D-Ala-D-Ala-OH was also accompanied by significant shifts of several CH signals in the host (up to $\delta = 0.4$ vs. ≤ 0.08 in the case of *N*-Cbz-L-Ala-L-Ala-OH). We also monitored the chemical shifts of the *N*-protected amino acid and dipeptide guests. The carbamate NH^a signal of the amino acid substrate was shifted downfield very modestly (ca. 0.03) on binding with **5**.[27] The carbamate NH^a and amide NH^b signals of the dipeptide substrates were more significantly shifted downfield, most notably in the case of *N*-Cbz-D-Ala-D-Ala-OH in which the NH^a signal shifted by $\delta = 0.50$ and the NH^b signal shifted by $\delta = 0.42$ on binding with **5** (in the case of *N*-Cbz-L-Ala-L-Ala-OH, the NH^a signal shifted by $\delta = 0.11$ and the NH^b signal shifted by $\delta = 0.10$ on binding with **5**).[27] Thus, receptor **5** displays remarkably good enantioselectivity for the biologically significant Ala-Ala-OH dipeptide sequence, albeit in a noncompetitive solvent.

Conclusions

In conclusion, we have described the synthesis, the conformational behaviour and the binding properties of a novel class of synthetic "two-armed" receptors containing a 2,6-diamidopyridine binding unit bearing pendant pseudopeptide legs consisting of an amino acid (L-Phe) and various chiral β-aminosulfonamides. These receptors collapse into intramolecularly folded structures in $CDCl_3$ solution, through formation of a network of intramolecular hydrogen bonds. Addition of excesses of *N*-protected amino acids and dipeptides to receptors **1** or **5** involves unfolding of the receptor and breaking of intramolecular hydrogen bonds (with an associated energy cost) to allow interaction with the guest, resulting in rather low binding constants and only small changes of the signals for the hydrogen-bonding NH protons. Only a very qualitative picture of the conformational properties of the hosts and of the structure of their complexes with protected amino acids and dipeptides could be established; this is unfortunately also true for the interesting enantioselectivity shown by host **5** in the recognition of *N*-Cbz-D-Ala-D-Ala-OH.[28] In any event, the significant shifts of several CH and NH signals throughout the spectrum of **5** (in particular the very large downfield shift for the sulfonamide NH^3) suggest that the structure of the complex of *N*-Cbz-D-Ala-D-Ala-OH is very different from that of the enantiomeric guest *N*-Cbz-L-Ala-L-Ala-OH. Such high enantioselectivity (> 20:1, effectively discriminating between methyl groups and hydrogen atoms) has seldom been observed in synthetic receptors,[1−3][10b] and is particularly noteworthy in such a structurally simple acyclic receptor, which appears to lack much, if any, preorganisation for binding. Work to develop a combinatorial approach to the binding of dipeptides is in progress, either by the screening of a library of receptors towards single substrates of interest,[29] or by the screening of a library of dipeptides towards a particular receptor.[30]

Experimental Section

General: Manipulations involving air-sensitive compounds were carried out under argon. Anhydrous solvents were obtained from sodium benzophenone ketyl (THF), or by refluxing over CaH_2 for at least 4 h prior to use. Reagents were used as received, without any further purification, and were generally purchased from Aldrich and Fluka AG. Reactions in solution were monitored by analytical thin layer chromatography (TLC) by using Merck 60 F_{254} silica gel glass plates. The chromatograms were viewed under UV light and by staining with a cerium reagent followed by heating. Flash chromatography was performed with 60 silica gel (230−400 Mesh) purchased from Macherey−Nagel. NMR spectra were recorded with Bruker instruments (AC 200, AC 300 and AVANCE 400). The spectra are reported in ppm relative to tetramethylsilane.

General Procedure for Fmoc Removal from Resin-Bound Fmoc Derivatives: *N*-Fmoc-protected resins were treated with a 20% piperidine/DMF solution (7 mL, 3 × 15 min). Between each cycle the solution was drained and the resin was washed with DMF (1×). At the end of the third cycle, the resin was drained, washed with DMF (3×), MeOH (3×) and DCM (4×), and dried in vacuo.

2,6-Bis(*N*-Fmoc-L-Phe-NH)pyridine-4-OCH_2CO-Gly-O-Wang-Merrifield Resin (9): A solid-phase reactor was charged with *N*-Fmoc-Gly-Wang-Merrifield resin **6** (0.260 mmol, 294 mg). Fmoc

removal was accomplished according to the general procedure. Acid **7** (0.217 mmol, 200 mg), HOBt (0.868 mmol, 117 mg), DIC (0.868 mmol, 0.134 mL) and DMAP (0.108 mmol, 13 mg) were then added. DCM (8 mL) was added and the resulting mixture was shaken at room temperature for 20 h. The resin was drained, washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo. Yield: 426 mg, 98%. Acetylimidazole (2.170 mmol, 239 mg) and DCM (8 mL) were then added and the mixture was shaken at room temperature for 2 h. The solution was then filtered and the resin was washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo.

2,6-Bis[(*S*)-*N*-FmocNHCH(*i*Pr)CH$_2$SO$_2$-L-Phe-NH]pyridine-4-OCH$_2$CO-Gly-O-Wang-Merrifield Resin (10): A solid-phase reactor was charged with 2,6-bis(Fmoc-L-Phe-NH)pyridine-4-OCH$_2$CO-gly-O-Wang-Merrifield resin **9** (0.163 mmol, 320 mg), and Fmoc removal was performed according to the general procedure. Sulfonyl chloride **8** (0.652 mmol, 267 mg), DMAP (0.130 mmol, 16 mg) and DCM (8 mL) were then added and the mixture was shaken for 5 min; MTDA (1.300 mmol, 0.264 mL) was added and the mixture was shaken at room temperature overnight. The suspension was then filtered and the resin was washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo. Yield: 334 mg, 92%. The free amino content was checked by two different tests: The TNBS test was negative,[31] while the NF31 test,[32] a more sensitive assay, revealed the presence of approximately 2% free amino content (slightly pink-red beads). A basic washing was performed by addition of a solution of 5% DIPEA in DMF (4 mL). This washing was done twice and the resin was washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo. A second coupling cycle was then performed under the same conditions: **8** (0.163 mmol, 66 mg), DMAP (0.032 mmol, 4 mg), MTDA (0.326 mmol, 66 μL), and DCM (4 mL). Yield: 338 mg, 93%. After washing, both tests were clearly negative (colourless beads).

2,6-Bis[(*S*)-*N*-FmocNHCH(*i*Pr)CH$_2$SO$_2$-(*S*)-NHCH(*i*Pr)CH$_2$SO$_2$-L-Phe-NH]pyridine-4-OCH$_2$CO-Gly-O-Wang-Merrifield Resin (14): A solid-phase reactor was charged with 2,6-bis[(*S*)-*N*-FmocNHCH(*i*Pr)CH$_2$SO$_2$-L-Phe-NH]pyridine-4-OCH$_2$CO-gly-O-Wang-Merrifield resin **10** (0.095 mmol, 169 mg), and Fmoc removal was performed according to the general procedure. Sulfonyl chloride **8** (0.380 mmol, 155 mg), DMAP (0.076 mmol, 9 mg) and DCM (6 mL) were then added and the mixture was shaken for 5 min; MTDA (0.760 mmol, 0.154 mL) was added and the mixture was shaken at room temperature overnight. The suspension was then filtered and the resin was washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo. Yield: 163 mg, 84%. The free amino content was checked by two different tests: The TNBS test was negative,[31] while the NF31 test[32] revealed the presence of free amino content (slightly pink-red beads). A basic washing was performed by addition of a solution of 5% DIPEA in DMF (4 mL). This washing was performed twice and the resin was washed with DMF (3 × 8 mL), MeOH (3 × 8 mL) and DCM (4 × 8 mL), and dried in vacuo. A second coupling cycle was then performed under the same conditions: **8** (0.190 mmol, 78 mg), DMAP (0.032 mmol, 4 mg), MTDA (0.380 mmol, 77 μL) and DCM (4 mL). Yield: 179 mg, 92%. After washing, both tests were clearly negative (colourless beads).

General Procedure for the Acetylation of Resin-Bound Fmoc Derivatives: A solid-phase reactor was charged with Fmoc-protected resins (**9** or **10**) and Fmoc removal was performed according to the general procedure. Acetylimidazole (10 equiv.) and DCM (2 mL per 0.054 mmol) were then added and the mixture was shaken overnight at room temperature. The solution was then filtered and the resin was washed with DMF (3×), MeOH (3×) and DCM (4×) and dried in vacuo.

Mesylation of 2,6-Bis[(*S*)-*N*-FmocNHCH(*i*Pr)CH$_2$SO$_2$-L-Phe-NH]pyridine-4-OCH$_2$CO-Gly-O-Wang-Merrifield Resin (10): A solid-phase reactor was charged with resin **10** (0.054 mmol), and Fmoc removal was performed according to the general procedure. Mesyl chloride (10 equiv.), TEA (12 equiv.) and DCM (4 mL) were added and the mixture was shaken at room temperature overnight. The resin was drained, washed with a 10% solution of TEA in DCM (3×), DMF (3×), MeOH (3×) and DCM (4×), and dried in vacuo.

Cleavage of the 2,6-Diamidopyridine Derivatives from the Resin

General Procedure A (Basic Cleavage):[33] The appropriate resin was treated with an anhydrous TEA/MeOH/DMF solution (4.7 mL, 1.9:1.9:0.9, v/v/v) under argon. The mixture was magnetically stirred at 50° C for 24 h. The suspension was then filtered and the resin was washed with DCM (3×). The eluates were pooled and the solvents were evaporated under reduced pressure to afford a residue that was subsequently purified.

General Procedure B (Acidic Cleavage): The appropriate resin was treated with TFA/H$_2$O solution (95:5 v/v) (5 mL). The mixture was shaken for 4 min. The solution was then drained and the resin was washed with DCM (3×). A second cycle was performed. The eluates were pooled and the solvents were evaporated under reduced pressure to afford a residue that was treated with dry toluene and sonicated for 1 or 2 min. The solvent was evaporated (the treatment was repeated three more times). The resulting white solid was dried in vacuo, to yield the acid derivative in 85−90% overall yield. The acid was mixed with EDC (4 equiv.), DMAP (0.5 equiv.) and dry THF (4.5 mL/0.043 mmol of acid), and the mixture was stirred for 5 min. Dry MeOH (6−9 equiv.) was then added and the reaction mixture was stirred at room temperature overnight. Evaporation of the solvent under reduced pressure afforded a residue, which was subsequently purified.

2,6-Bis[(*S*)-*N*-AcNHCH(*i*Pr)CH$_2$SO$_2$-L-Phe-NH]pyridine-4-OCH$_2$-CO-Gly-OMe (1): By general procedure A. The crude residue was purified by flash column chromatography on silica gel, with DCM/acetone (5%) and DCM/MeOH (1−5%) as eluents. The yellow solid obtained was precipitated from DCM/diethyl ether to give a white solid in 30% overall yield. By general procedure B. The crude residue was purified by flash column chromatography on silica gel, with DCM/acetone (5%) and DCM/MeOH (2−6%) as eluents. The white solid obtained was precipitated from DCM/diethyl ether to give a white solid in 35% overall yield. **1:** ^{1}H NMR (200 MHz, 5 mM in [D$_6$]DMSO, 307 K): δ = 10.27 (s, 2 H, Py-NHCO), 8.74 (t, *J* = 5.0 Hz, 1 H, NH-Gly), 7.85 (br. s, 2 H, NHSO$_2$), 7.70 (d, *J* = 10.0 Hz, 2 H, NHAc), 7.62 (s, 2 H, CH-Py), 7.50−7.32 (m, 10 H, aromatic), 4.73 (s, 2 H, O-CH$_2$-Gly), 4.50−4.48 (m, 2 H, CH-Phe), 4.12−4.08 (m, 2 H, CH-*i*Pr), 4.02 (d, *J* = 5.0 Hz, 2 H, CH$_2$-Gly), 3.74 (s, 3 H, OMe), 3.30−2.65 (m, 4 H, CH$_2$Ph), 2.60−2.30 (m, 4 H, CH$_2$SO$_2$), 1.88 (s, 6 H, CH$_3$CO), 1.70−1.68 (m, 2 H, CHMe$_2$), 0.77 (d, 12 H, *J* = 7.0 Hz, CH$_3$). ^{1}H NMR (400 MHz, 3.8 mM in CDCl$_3$, 300 K): δ = 9.30 (br. s, 2 H, Py-NHCO), 7.58 (br. s, 1 H, NH-Gly), 7.37−7.28 (m, 12 H, aromatic), 6.27 (br. s, 2 H, NHSO$_2$), 6.05 (br. s, 2 H, NHAc), 4.50 (br. s, 4 H, O−CH$_2$-Gly and CH-Phe), 4.17−4.12 (m, 4 H, CH-*i*Pr and CH$_2$-Gly), 3.77 (s, 3 H, OMe), 3.37−3.35 (m, 2 H, CH$_2$Ph), 3.03−2.92 (m, 4 H, CH$_2$SO$_2$), 2.82−2.79 (m, 2 H, CH$_2$Ph), 1.97 (s, 6 H, CH$_3$CO), 1.76−1.74 (m, 2 H, CHMe$_2$), 0.80 (d, *J* = 7.0 Hz, 6 H, CH$_3$), 0.79 (d, *J* = 7.0 Hz, 6 H, CH$_3$). ^{13}C NMR (50.3 MHz, 5 mM, [D$_6$] acet-

one, 300 K): δ =171.4, 170.5, 167.8, 167.4 (CO), 151.8, 138.0 (C, arom.), 130.5, 129.1, 127.6 (CH, arom.), 96.5 (CH, Py), 67.3 (CH_2, OCH_2CO), 59.9 (CH, CH-Phe), 55.3 (CH_2, CH_2SO_2), 52.0 (CH_3, OCH_3), 50.4 (CH, CH-*i*Pr), 40.9 (CH_2, CH_2-Gly), 39.0 (CH_2, CH_2Ph), 32.5 (CH, $CHMe_2$), 22.9 (CH_3, CH_3CO), 19.1 (CH_3), 17.5 (CH_3). MS (FAB): *m/z* = 931 $[M + H]^+$; 953 $[M + Na]^+$. HRMS (FAB) calcd. for $C_{42}H_{59}N_8O_{12}S_2$ $[M + H]^+$: 931.36884, found *m/z*: 931.36869.

2,6-Bis[(*S*)-*N*-MsNHCH(*i*Pr)CH_2SO_2-L-Phe-NH]pyridine-4-OCH_2CO-Gly-OMe (2): This compound was prepared by general procedure A. The crude residue was purified by flash column chromatography on silica gel, with DCM/acetone (5%) and DCM/MeOH (3%) as eluents. The yellow solid obtained was precipitated from DCM/diethyl ether to give a white solid in 30% overall yield. **2:** ^1H NMR (200 MHz, 5 mM in $[D_6]$DMSO, 300 K): δ = 10.77 (s, 2 H, Py-NHCO), 8.78−8.76 (m, 1 H, NH-Gly), 8.13−8.11 (m, 2 H, $NHSO_2$), 7.61 (s, 2 H, CH-Py), 7.50−7.32 (m, 12 H, aromatic, $NHSO_2$), 4.73 (s, 2 H, O−CH_2-Gly), 4.40−4.60 (m, 2 H, CH-Phe), 4.02 (d, *J* = 5.0 Hz, 2 H, CH_2-Gly), 3.73 (s, 3 H, OMe), 3.63−3.59 (m, 2 H, CH-*i*Pr), 3.30−2.65 (m, 4 H, CH_2Ph), 2.98 (s, 6 H, CH_3SO_2), 2.60−2.30 (m, 4 H, CH_2SO_2), 1.88−1.74 (m, 2 H, $CHMe_2$), 0.76 (d, *J* = 7.0 Hz, 6 H, CH_3), 0.75 (d, *J* = 7.0 Hz, 6 H, CH_3). ^1H NMR (400 MHz, 5 mM in $CDCl_3$, 300 K): δ = 9.07 (br. s, 2 H, Py-NHCO), 7.65 (br. s, 1 H, NH-Gly), 7.38−7.28 (m, 12 H, aromatic), 6.34 (br. s, 2 H, $NHSO_2$), 5.13 (br. s, 2 H, $NHSO_2$), 4.54 (s, 2 H, O−CH_2-Gly), 4.41 (br. s, 2 H, CH-Phe), 4.20 (br. s, 2 H, CH_2-Gly), 3.79 (s, 3 H, OMe), 3.70−3.60 (m, 2 H, CH-*i*Pr), 3.47−3.16 (m, 2 H, CH_2Ph), 3.14−2.91 (m, 8 H, CH_2Ph and CH_3SO_2), 2.80−2.55 (m, 2 H, CH_2SO_2), 2.50−2.33 (m, 2 H, CH_2SO_2), 2.02−1.90 (m, 2 H, $CHMe_2$), 0.86 (br. s, 12 H, CH_3). ^{13}C NMR (50.3 MHz, 10 mM, $CDCl_3$, 300 K): δ = 170.2, 167.6, 166.1 (CO), 150.2, 136.4 (C, arom.), 129.7, 128.0, 127.4 (CH, arom.), 96.3 (CH, Py), 66.7 (CH_2, OCH_2CO), 59.8 (CH, CH-Phe), 55.0 (CH_3, OCH_3), 53.5 (CH_2, CH_2SO_2), 52.3 (CH, CH-*i*Pr), 41.8 (CH_3, CH_3SO_2), 40.7 (CH_2, CH_2-Gly), 38.6 (CH_2, CH_2Ph), 32.5 (CH, $CHMe_2$), 17.9 (CH_3), 17.6 (CH_3). MS (FAB): *m/z* = 1003 $[M + H]^+$; 1025 $[M + Na]^+$. HRMS (FAB) calcd. for $C_{40}H_{59}N_8O_{14}S_4$ $[M + H]^+$: 1003.30281, found *m/z*: 1003.30330.

2,6-Bis(*N*-Ac-L-Phe-NH)pyridine-4-OCH_2CO-Gly-OMe (3): This compound was prepared by general procedure A. The yellow solid obtained was precipitated twice from DCM/diethyl ether to give a white solid in 30% overall yield. **3:** ^1H NMR (200 MHz, 8.5 in $[D_6]$acetone, 300 K): δ = 9.39 (s, 2 H, Py-NHCO), 8.07 (t, *J* = 6.0 Hz, 1 H, NH-Gly), 7.76 (d, *J* = 8.0 Hz, 2 H, NHAc), 7.42 (s, 2 H, CH-Py), 7.35−7.10 (m, 10 H, aromatic), 4.96−4.85 (m, 2 H, CH-Phe), 4.53 (s, 2 H, O−CH_2-Gly), 4.01 (d, *J* = 6.0 Hz, 2 H, CH_2-Gly), 3.63 (s, 3 H, OMe), 3.29−3.19 (m, 2 H, CH_2Ph), 3.02−2.90 (m, 2 H, CH_2Ph), 1.90 (s, 6 H, CH_3CO). ^1H NMR (400 MHz, 8.5 in $CDCl_3$, 300 K): δ = 8.83 (s, 2 H, Py-NHCO), 7.35 (br. s, 1 H, NH-Gly), 7.33−7.23 (m, 12 H, aromatic), 6.56 (d, *J* = 8.0 Hz, 2 H, NHAc), 4.96−4.91 (m, 2 H, CH-Phe), 4.49 (s, 2 H, O−CH_2-Gly), 4.17−4.11 (m, 2 H, CH_2-Gly), 3.78 (s, 3 H, OMe), 3.33−3.27 (m, 2 H, CH_2Ph), 3.14−3.09 (m, 2 H, CH_2Ph), 2.03 (s, 6 H, CH_3CO). ^{13}C NMR (75.4 MHz, 8.5 mM, $CDCl_3$, 300 K): δ = 171.4, 170.2, 167.6, 166.1 (CO), 150.6, 136.4 (C, arom.), 129.2, 128.6, 127.0 (CH, arom.), 96.3 (CH, Py), 66.8 (CH_2, OCH_2CO), 55.3 (CH, CH-Phe), 52.3 (CH_3, OCH_3), 40.7 (CH_2, CH_2-Gly), 37.1 (CH_2, CH_2Ph), 23.2 (CH_3, CH_3CO). MS (FAB): *m/z* = 633 $[M + H]^+$; 655 $[M + Na]^+$. HRMS (FAB) calcd. for $C_{32}H_{37}N_6O_8$ $[M + H]^+$: 633.26674, found *m/z*: 633.26722.

2-[(*S*)-*N*-AcNHCH(*i*Pr)CH_2SO_2-L-Phe-NH]-6-(*N*-Alloc-L-Phe-NH)pyridine-4-OCH_2CO-Gly-OBn (4): The asymmetric scaffold **11** (33 mg, 0.044 mmol) was dissolved in a solution of 25% (v/v) TFA in dry DCM (1.5 mL). The solution was stirred at room temperature for 1.5 h, diluted with DCM (100 mL), and washed with a 10% aqueous solution of K_2CO_3 and brine. The organic layer was dried with Na_2SO_4 and filtered, and the solvents were evaporated. The sulfonyl chloride **12** (25 mg, 0.088 mmol), DMAP (8 mg, 0.066 mmol), dry DCM (2 mL) and MTDA (36 μL) were then added to the free amine (28 mg, 0.044 mmol). The mixture was stirred at room temperature for 2 h 30. DCM was removed and the residue was dissolved in EtOAc (75 mL). The organic layer was washed with a 10% aqueous solution of citric acid (3 × 10 mL) and brine (10 mL), dried with Na_2SO_4 and filtered. The solvents were evaporated to give the *N*-Boc-protected compound **13** (39 mg, 99% yield). Compound **13** (39 mg, 0.043 mmol) was dissolved in a solution of 25% (v/v) TFA in DCM (1.0 mL). The solution was stirred at room temperature for 1 h and the DCM was evaporated. Dry toluene (2 mL) was added to the residue. The mixture was sonicated and the toluene was evaporated (3 cycles). The residue was dissolved in DCM (2 mL) and Aclm was added (34 mg, 0.305 mmol). The mixture was stirred at room temperature overnight. DCM was evaporated and the residue was dissolved in EtOAc (75 mL). The organic layer was washed with a 10% aqueous solution of citric acid (3 × 10 mL), and then with a 10% aqueous solution of $NaHCO_3$ (3 × 10 mL) and brine (10 mL), dried with Na_2SO_4 and filtered. The solvents were evaporated to give 33 mg (90%) of compound **4**, which could not be further purified. **4:** ^1H NMR (400 MHz, 8.5 mM in $CDCl_3$, 300 K): δ = 8.87 (s, 1 H, Py-NHCO), 8.75 (s, 1 H, Py-NHCO), 7.41−7.23 (m, 17 H, aromatic), 5.98 (br. s, 1 H, NH-SO_2), 5.87−5.79 (m, 2 H, CH=C and NH-Ac), 5.74 (br. s, 1 H, NH-Alloc), 5.28 (s, 2 H, O−CH_2-Ph), 5.23−5.14 (m, 2 H, CH_2=C), 4.67 (s, 2 H, O−CH_2-CO), 4.57−4.55 (m, 1 H, CH-Phe), 4.53−4.51 (m, 2 H, CH_2-Alloc), 4.47 (br. s, 1 H, CH-Phe), 4.24−4.20 (m, 1 H, CH-*i*Pr), 3.34−3.24 (m, 2 H, CH_2Ph), 3.08−2.82 (m, 4 H, CH_2Ph and CH_2SO_2), 1.95 (s, 3 H, CH_3CO), 1.72−1.70 (m, 1 H, $CHMe_2$), 0.82 (d, *J* = 7.0 Hz, 6 H, CH_3). MS (FAB): *m/z* = 843 $[M + H]^+$; 865 $[M + Na]^+$. HRMS (FAB) calcd. for $C_{43}H_{51}N_6O_{10}S$ $[M + H]^+$: 843.33819, found *m/z*: 843.33657.

2,6-Bis[(*S*)-*N*-AcNHCH(*i*Pr)CH_2SO_2-(*S*)-NHCH(*i*Pr)CH_2SO_2-L-Phe-NH]pyridine-4-OCH_2CO-Gly-OMe (5): By general procedure B. The crude residue was purified by flash column chromatography on silica gel, with DCM/EtOH (5%)/EtOAc (5%) as eluents. The white solid obtained was precipitated from DCM/diethyl ether to give a white solid in 20% overall yield. **5**: ^1H NMR (200 MHz, 20 mM in CD_3CN, 300 K): δ = 9.18 (s, 2 H, Py-NH^1CO), 7.58−7.48 (3 H, NH-Gly and CH-Py), 7.33−7.21 (m, 10 H, aromatic), 6.56 (d, *J* = 6.0 Hz, 2 H, NH^3-SO_2), 6.38 (d, *J* = 10.0 Hz, 2 H, NH^4-CO), 5.71 (d, *J* = 10.0 Hz, 2 H, NH^2-SO_2), 4.58 (s, 2 H, O−CH_2-Gly), 4.38−4.29 (m, 4 H, CH^9-*i*Pr and CH^{14}-*i*Pr), 3.96 (d, *J* = 6.0 Hz, 2 H, CH_2-Gly), 3.71−3.65 (m, 5 H, OMe and CH^6-Phe), 3.26−2.63 (m, 12 H, $CH_2^{12}SO_2$ and $CH_2^8SO_2$ and CH_2^7Ph), 1.88 (s, 6 H, $CH_3^{15}CO$), 1.82−1.73 (m, 4 H, $CH^{10}Me_2$ and $CH^{16}Me_2$), 0.87−0.70 (m, 24 H, CH_3^{13} and CH_3^{11}). ^1H NMR (400 MHz, 2 mM in $CDCl_3$, 300 K): δ = 9.27 (s, 2 H, Py-NH^1CO), 7.43 (br. s, 1 H, NH-Gly), 7.37−7.28 (m, 12 H, aromatic), 6.24 (br. s, 2 H, NH^3-SO_2), 6.17 (br. s, 2 H, NH^4-CO), 5.72 (br. s, 2 H, NH^2-SO_2), 4.57 (s, 2 H, O−CH_2-Gly), 4.46 (br. s, 4 H, CH^9-*i*Pr and CH^{14}-*i*Pr), 4.17 (s, 2 H, CH_2-Gly), 3.79 (br. s, 5 H, OMe and CH^6-Phe), 3.40−3.37 (m, 4 H, $CH_2^{12}SO_2$ and $CH_2^8SO_2$), 3.20−3.12 (m, 2 H, $CH_2^{12}SO_2$), 3.03−2.97 (m, 2 H, $CH_2^8SO_2$), 2.77 (br. s, 2 H, CH_2^7Ph), 2.56 (br. s, 2 H, CH_2^7Ph), 2.04 (br. s, 8 H, $CH_3^{15}CO$ and $CH^{16}Me_2$), 1.88 (br. s, 2 H, $CH^{10}Me_2$), 0.89 (br. s, 12 H, CH_3^{13}), 0.77 (br. s, 12 H, CH_3^{11}). ^{13}C NMR (100.6 MHz, 20 mM in $CDCl_3$, 300 K): δ = 172.3, 170.9, 168.1, 166.7 (CO), 151.0, 137.1 (C,

arom.), 130.2, 129.2, 127.8 (CH, arom.), 97.1 (CH, Py), 67.2 (CH_2, OCH_2CO), 60.2 (CH, CH-Phe), 56.1 (CH_2, CH_2SO_2), 55.1 (CH_3, OCH_3), 52.8 (CH, CH-*i*Pr), 50.8 (CH, CH-*i*Pr), 41.2 (CH_2, CH_2, CH_2-Ph), 39.2 (CH_2-Gly), 33.7 (CH, $CHMe_2$), 32.1 (CH, $CHMe_2$), 23.8 (CH_3, CH_3CO), 19.6 (CH_3), 18.2 (CH_3). MS (FAB): *m*/*z* = 1230 $[M + H]^+$; 1252 $[M + Na]^+$. HRMS (FAB) calcd. for $C_{52}H_{81}N_{10}O_{16}S_4$ $[M + H]^+$: 1229.47094, found *m*/*z*: 1229.47036.

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[1] For a recent review on peptide receptors, see: [1a]M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479−2494. See also: [1b] P. D. Henley, C. P. Waymark, I. Gillies, J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1* **2000**, 1021−1031, and references therein.

[2] W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155−163.

[3] C. Gennari, H. P. Nestler, U. Piarulli, B. Salom, *Liebigs Ann./Recueil* **1997**, 637−647.

[4] For original work on tweezer receptors, see: [4a] C.-W. Chen, H. W. Whitlock Jr., *J. Am. Chem. Soc.* **1978**, *100*, 4921−4922. [4b] S. C. Zimmerman, W. Wu, Z. Zeng, *J. Am. Chem. Soc.* **1991**, *113*, 196−201.

[5] [5a] H. Wennemers, S. S. Yoon, W. C. Still, *J. Org. Chem.* **1995**, *60*, 1108−1109. [5b] Y. Shao, W. C. Still, *J. Org. Chem.* **1996**, *61*, 6086−6087. [5c] Z. Pan, W. C. Still, *Tetrahedron Lett.* **1996**, *37*, 8699−8702. [5d] M. D. Weingarten, K. Sekanina, W. C. Still, *J. Am. Chem. Soc.* **1998**, *120*, 9112−9113. [5e] M. Torneiro, W. C. Still, *Tetrahedron* **1997**, *53*, 8739−8750.

[6] [6a] R. Boyce, G. Li, H. P. Nestler, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 7955−7956. [6b] Y. Cheng; T. Suenaga, W. C Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813−1814.

[7] [7a] M. Davies, M. Bonnat, F. Guillier, J. D. Kilburn, M. Bradley, *J. Org. Chem.* **1998**, *63*, 8696−8703. [7b] G. M. Kyne, M. E. Light, M. B. Hursthouse, J. de Mendoza, J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1* **2001**, 1258−1263.

[8] S. R. LaBrenz, *J. Am. Chem. Soc.* **1995**, *117*, 1655−1656.

[9] [9a] T. Fessmann, J. D. Kilburn, *Angew. Chem. Int. Ed.* **1999**, *38*, 1993−1996. [9b] T. Braxmeier, M. Demarcus, T. Fessmann, S. McAteer, J. D. Kilburn, *Chem. Eur. J.* **2001**, *7*, 1889−1898.

[10] [10a] J. Dowden, P. D. Edwards, S. S. Flack, J. D. Kilburn, *Chem. Eur. J.* **1999**, *5*, 79−89. [10b] P. D. Henley, J. D. Kilburn, *Chem. Commun.* **1999**, 1335−1336.

[11] C. Gennari, H. P. Nestler, B. Salom, W. C. Still, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1765−1768.

[12] D. W. P. M. Löwik, S. J. E. Mulders, Y. Cheng, Y. Shao, R. M. J. Liskamp, *Tetrahedron Lett.* **1996**, *37*, 8253−8256.

[13] D. W. P. M. Löwik, M. D. Weingarten, M. Broeckema, A. J. Brouwer, W. C. Still, R. M. J. Liskamp, *Angew. Chem. Int. Ed.* **1998**, *37*, 1846−1850.

[14] C. Gennari, M. Gude, D. Potenza, U. Piarulli, *Chem. Eur. J.* **1998**, *4*, 1924−1931.

[15] B. Salom, D. Potenza, C. Gennari, C. Longari, E. Fioravanzo, O. Carugo, N. Sardone, *Chem. Eur. J.* **1996**, *2*, 644−655.

[16] For a preliminary communication on part of this work, see: E. Botana, S. Ongeri, R. Arienzo, M. Demarcus, J. G. Frey, U. Piarulli, D. Potenza, C. Gennari, J. D. Kilburn, *Chem. Commun.* **2001**, 1358−1359.

[17] M. Gude, U. Piarulli, D. Potenza, B. Salom, C. Gennari, *Tetrahedron Lett.* **1996**, *37*, 8589−8592.

[18] Test 1 (TNBS test): W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260−264. Test 2 (NF31 test): A. Madder, N. Farcy, N. G. C. Hosten, H. De Muynk, P. J. De Clercq, J. Barry, A. P. Davis, *Eur. J. Org. Chem.* **1999**, 2787−2791. See more details in the Exp. Sect. and in refs.[31,32]

[19] Electronic Supporting Information available (see footnote on the first page of this article): details on concentration dependence experiments, details of temperature dependence experiments, NMR binding studies and chemical shift data.

[20] L. Belvisi, C. Gennari, A. Mielgo, D. Potenza, C. Scolastico, *Eur. J. Org. Chem.* **1999**, *2*, 389−400.

[21] G. J. Pernia, J. D. Kilburn, J. W. Essex, R. J. Mortishire-Smith, M. Rowley, *J. Am. Chem. Soc.* **1996**, *118*, 10220−10227.

[22] H. J. Dyson, P. E. Wright in *2-Dimensional NMR spectroscopy* (Eds.: W. R. Croasmun, R. M. K. Carlson), VCH-Verlagsgesellschaft, Weinheim, **1994**, p. 672. We checked the possibility that the observed contact between the hydrogen atom at the amino acid stereogenic centre and the acetyl hydrogen atoms might be an artefact due to scalar coupling-transfer through the NH signal; this spin diffusion interference is usually caused by the frequency of the transmitter. However, ROESY spectra recorded with a different transmitter frequency (original transmitter frequency: 5 ppm; modified transmitter frequencies: 6 ppm and 7 ppm) gave the same results. Thus, the observed contacts are real, and account for a certain percentage of *cis*-amide.

[23] R. R. Gardner, S. L. Mc Kay, S. H. Gellman, *Org. Lett.* **2000**, *2*, 2335−2338, and references therein. No separate set of signals due to the *cis* rotamer could be detected in the proton spectra of **1**, and it was therefore concluded that its contribution does not exceed the standard percentage ($\leq$ 5%).

[24] Data were analysed by using a modified version of the algorithm described by C. A. Hunter and used in his software *NMRTit HG* (see: A. P. Bisson, C. A. Hunter, J. C. Morales, K. Young, *Chem. Eur. J.* **1998**, *4*, 845−851) to allow simultaneous evaluation of data from several signals in the NMR of the host. The normalised sum of squares for all the experimental data, $\chi^2 = (\text{obsd.} - \text{calcd.})^2/\sigma^2$, was minimised by floating the host and host−guest chemical shifts for each proton considered and the common binding constant by using the solver function in a Microsoft Excel™ spreadsheet.

[25] R. E. Lenkinski, J. Reuben, *J. Am. Chem. Soc.* **1976**, *98*, 3089−3094.

[26] For a full set of all the binding data and curve-fitting, see ref.[10] and associated electronic Supporting Information; see ref.[19]

[27] H^a and H^b were named as follows: RO−CO−NH^a−CH(Me)COOH; RO−CO−NH^a−CH(Me)CO−NH^b−CH(R^1)COOH.

[28] ROESY spectra were recorded for: (a) host **5** alone (5 mM in $CDCl_3$); (b) host **5** (5 mM in $CDCl_3$) with Cbz-D-Ala-D-Ala-OH (10 mM in $CDCl_3$) − i.e., guest/host ratio of 2:1 (ca. 80% saturation); (c) host **5** (2 mM in $CDCl_3$) with Cbz-D-Ala-D-Ala-OH (10 mM in $CDCl_3$) − i.e., guest/host ratio of 5:1 (ca. 92% saturation). In spectrum (a) we detected the NOE contacts, which we have already described in the text. In spectrum (b) we essentially detected no major changes. The experiment therefore gave no useful information regarding the binding. In spectrum (c) very few signals could be observed and the signal-to-noise ratio was too high.

[29] For examples of the screening of a library of receptors with a chosen substrate, see refs.[2,5d,6a,6b,9b]; see also: [29a] H. P. Nestler, *Mol. Diversity* **1996**, *2*, 35−40. [29b] R. Xu, G. Greiveldinger, L. E. Marenus, A. Coopper, J. A. Ellman, *J. Am. Chem. Soc.* **1999**, *121*, 4898−4899. [29c] H. Hioki, T. Yamada, C. Fujioka, M. Kodama, *Tetrahedron Lett.* **1999**, *40*, 6821−6825.

[30] For examples of the screening of a library of substrates towards a particular receptor, see: H. Wennemers, M. Conza, M. Nold, P. Krattiger, *Chem. Eur. J.* **2001**, *7*, 3342−3347, and refs.[5a,7,11−13]

[31] TNBS test: A few resin beads were sampled and washed several times with ethanol. The sample was placed in a vial and 1−2 drops (25−50 μL) of a 10% of DIPEA solution in DMF and 1−2 drops of 1% 2,4,6-trinitrobenzenesulfonic acid (TNBS) in DMF were added. The sample was inspected under a microscope and colour changes were noted. The TNBS test is considered to be positive (presence of free amino groups) if the resin beads turn orange or red within a minute and negative if the beads remain colourless. For further details see ref.[18]

[32] NF31 test: A few beads of resin were suspended in 100 μL of a 2 mM solution of NF31 {4-nitrophenyl-5-[*N*-ethyl-*N*-[4-(nitrophenyl)azo]phenyl]amino-3-oxapentanoate}. After heating at 70 °C for 10 min, the beads were filtered and washed with DMF (3×), MeOH (3×) and DCM (3×). The beads were inspected under a microscope. Beads containing free amino functions appear as red spheres while completely coupled beads (no free amino groups) remain colourless. For further details see ref.[18]

[33] D. L. Holmes, E. M. Smith, J. S. Nowick, *J. Am. Chem. Soc.* **1997**, *119*, 7655−7669.

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