Recognition of quaternary ammonium salts with tetrapeptides containing α-aminoisobutyric acid as a conformational constraint

Ryoji Yanagihara,* Masaki Katoh, Masayuki Hanyuu, Toshifumi Miyazawa and Takashi Yamada

Department of Chemistry, Faculty of Science, Konan University, Higashinada, Kobe 658-8501, Japan

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Tetrapeptides Trp-Aib-Gly-Leu-NH-Ar (Aib: α-aminoisobutyric acid, 2-amino-2-methylpropanoic acid, Ar = phenyl or 3,5-dimethylphenyl) were synthesized. The peptides bound quaternary ammonium salts as guests in $CDCl_3$. For every guest, the binding constant *K* of the peptide host which has a 3,5-dimethylphenyl group was larger than that of the host which has a phenyl group. ROESY analysis of the complex revealed that the N^+–CH_3 groups of the guests were close to the aromatic moieties of the host in the complex. The charge in cation guests, the π-basicity of the host, and the turn conformation of the peptides were important factors for the complexation.

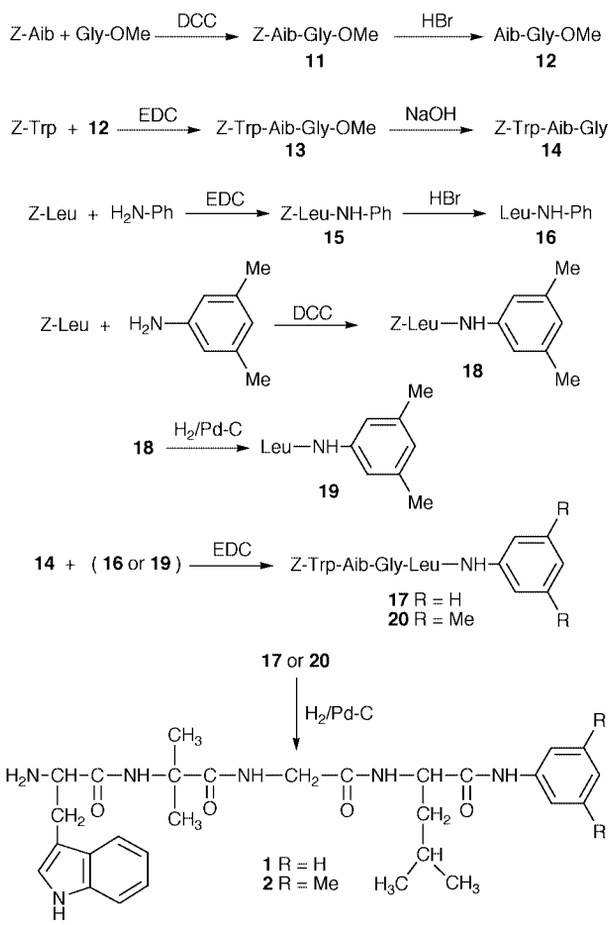
Introduction

α-Aminoisobutyric acid (Aib), one of the α,α-dialkylglycines, is a sterically hindered amino acid that acts as a conformational constraint in peptides.[1] We previously reported that the peptide Dnp-Val-Aib-Gly-Leu-pNA[2] prefers the β-turn structure due to the steric effect of the methyl groups of Aib, the intramolecular hydrogen bonds, and stacking of Dnp and pNA groups.[3] Analyses of NMR and CD spectra and X-ray diffraction revealed that the Dnp and pNA groups are stacked and interact with each other.

As an application of the ability of Aib to control conformation, we focused on the molecular recognition ability of oligopeptides containing Aib. Two aromatic residues at both terminals of an oligopeptide that adopts the β-turn structure will be close to each other, and so should act as a π-basic guest binding site.

The fact that π-basic aromatic residues form a binding site for an ammonium ion has been reported in natural and synthetic receptors. The proteins that bind choline derivatives have aromatic side chains of Trp, Tyr, and Phe at the binding site for the quaternary ammonium group of the choline derivatives.[4] The attractive interaction between aromatic moieties and cations is believed to be effective for the recognition of ammonium salts. Dougherty *et al.* reported, on the complexation of aromatic hosts with methylammonium guests, that cation–π interaction between highly polarized CH_3–N^+ moieties of the guests and electron-rich aromatic moieties of the hosts is the primary binding force.[5] In previous studies, rigid cyclic compounds called cyclophanes that contain four or more aromatic rings were synthesized, and the complexation of these compounds with ammonium guests in aqueous and organic solutions were determined.[5–7] This research showed that multiple aromatic moieties, highly preorganized by covalent bonds, are very efficient for guest binding.

On the other hand, proteins assemble aromatic side chains by means of non-covalent intramolecular forces—hydrogen bonding, ionic and hydrophobic interaction, and steric hindrance. We report here the ammonium-binding ability of Aib-containing tetrapeptides Trp-Aib-Gly-Leu-NH-Ar (the structures and the syntheses of peptides **1** and **2** are shown in Scheme 1) to which Trp and anilide residues were introduced as π-basic moieties. These peptides were designed as models for the protein that consists of an acyclic backbone and that forms a guest binding site. The conformation and complexation of the peptides with ammonium salts were studied.



Scheme 1

Results and discussion

Conformation of peptides

The dependence of NH chemical shifts of the tetrapeptides (**1** and **2**) on temperature and solvents are shown in Tables 1

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Table 1 Temperature dependence of NH chemical shift of **1** and **2**[a]

		$(-d\delta/dT)/10^{-3}$ K^{-1}				
Peptide	Solvent	Indole	Aib	Gly	Leu	NHPh
1	$CDCl_3$	1.2	1.6	1.4	3.0	1.4
	DMSO-d_6	1.8	2.7	2.5	1.0	2.6
2	$CDCl_3$	1.6	1.9	1.8	2.8	1.8
	DMSO-d_6	1.8	2.7	2.6	0.9	2.6

[a] [Peptide]: 1.0 mM, temperature: 298–328 K.

Table 2 Solvent effect on the NH chemical shift of **1** and **2** at 298 K[a]

		δ				
Peptide	Solvent	Indole	Aib	Gly	Leu	NHPh
1	$CDCl_3$	8.15	7.63	6.38	7.78	8.71
	DMSO-d_6	10.82	8.39	8.19	7.81	9.55
2	$CDCl_3$	8.12	7.62	6.45	7.75	8.34
	DMSO-d_6	10.84	8.50	8.17	7.82	9.36

[a] [Peptide]: 1.0 mM.

and 2, respectively. Very little dependence of the chemical shifts of Leu-NH of both **1** and **2** on temperature were observed in DMSO-d_6. The hydrogen bonding of Leu-NH was not to the solvent (intermolecular) but to the carbonyl oxygen in the same molecule (intramolecular). On the other hand, a relatively large dependence of Leu-NH in $CDCl_3$ demonstrated the existence of a hydrogen bond even in a non-polar solvent. Furthermore, the chemical shifts of Leu-NH were approximately equal in both solvents. Thus, the environment of Leu-NH was free from solvents. These results show that Leu-NH participates in the intramolecular hydrogen-bonding, as well as in the case of the previously reported peptide.[3] Therefore, **1** and **2** probably adopt a β-turn structure and the two aromatic rings on both terminals can easily approach to each other.

Complexation of peptides with ammonium salts

Guests studied included phenyltrimethylammonium chloride (**3**), benzyltrimethylammonium chloride (**4**), dodecyltrimethylammonium chloride (**5**), acetylcholine chloride (**6**), *N*-butylpyridinium chloride (**7**) and *N*-methylisoquinolinium iodide (**8**) as quaternary ammonium salts, and *tert*-butylbenzene (**9**) and *N*,*N*-dimethylaniline (**10**) as structurally similar but non-ionic guests. The complexation of the peptide host and ammonium guest in $CDCl_3$ was clarified by monitoring the host-induced upfield shifts of the guest ^{1}H signals in response to the ring current effect of the benzene and indole rings of the host.

The host-induced shifts of the guests exhibited saturation ($\Delta\delta_{sat}$) with an increase in the concentration of the host (see eqns. (1)–(5)). Some titration data are shown in Fig. 1. The

$$\text{host} + \text{guest} \rightleftharpoons \text{complex} \quad (1)$$

$$[\text{host}] = [\text{host}]_t - [\text{complex}] \quad (2)$$

$$[\text{guest}] = [\text{guest}]_t - [\text{complex}] \quad (3)$$

$$K = \frac{[\text{complex}]}{[\text{host}][\text{guest}]} \quad (4)$$

$$\Delta\delta_{obs} = \frac{[\text{complex}]}{[\text{guest}]_t} \times \Delta\delta_{sat} \quad (5)$$

binding constants (K) were obtained by non-linear data fitting to eqn. (6) and are summarized in Table 3. The $\Delta\delta_{sat}$ values

Table 3 Binding constants of (K/M^{-1}) of hosts **1** and **2** with ammonium salts (**3**–**8**) in $CDCl_3$ at 298 K

	K/M^{-1}					
Host	**3**	**4**	**5**	**6**	**7**	**8**
1	340	445	220	255	730	180
2	460	580	280	335	1000	230

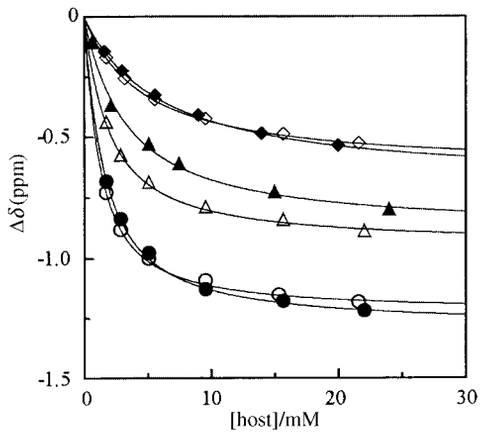

Fig. 1 Complexation-induced shifts (negative values indicate an up-field shift) for guest N^+–CH_3 (▲ and △ for **3**, ◆ and ◇ for **8**) and N^+–CH of pyridine ring (● and ○ for **7**) as a function of [**1**] (▲, ●, and ◆) or [**2**] (△, ○, and ◇) in $CDCl_3$ at 298 K ([guest] = 0.50 mM).

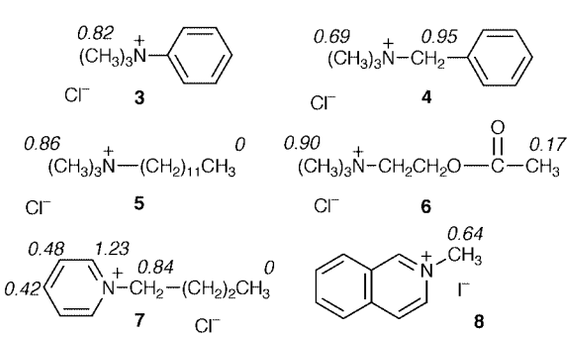

Fig. 2 Guests with the values of $-\Delta\delta_{sat}$ (ppm) of C–H when they were bound to **2** (italic numbers). Values not included in this figure were not precisely determined due to overlap with other C–H signals.

$$\Delta\delta_{obs} = \frac{\Delta\delta_{sat}}{2[\text{host}]_t}\left\{\frac{1}{K} + [\text{host}]_t + [\text{guest}]_t - \sqrt{\left(\frac{1}{K} + [\text{host}]_t + [\text{guest}]_t\right)^2 - 4[\text{host}]_t[\text{guest}]_t}\right\} \quad (6)$$

of the guests **3**–**8** were calculated by extrapolation of the titration curves and shown in Fig. 2. The results show that the nearer the proton was to the charged nitrogen atom, the larger the $|\Delta\delta_{sat}|$ value was. In marked contrast to guests **3**–**8**, the ^{1}H NMR shifts of neutral guests **9** and **10** as references were not affected at all by the host. Thus, guests must be charged for complexation to occur.

The 1:1 host–guest stoichiometry was confirmed by the continuous variation (Job) plots of the concentration of the complex *vs.* mole fraction of the guest (f_{guest}) under conditions where [host] + [guest] was kept constant at 16 mM; the maximum occurs when $f_{guest} = 0.5$. The plots in complexation between **1** and **3** are shown in Fig. 3.

As reference compounds containing two aromatic moieties, Z-Trp-Aib-Gly-OCH_3 (**13**) and Z-Leu-NHC_6H_5 (**15**) were also tested. Markedly less affinity was observed for these compounds with guest **3** than for either **1** or **2**. (K: 40 for **13** and 50

for **15** [M^{-1}]) A turn structure of the hosts in which the two aromatic rings are arranged well seems to be important for guest binding to occur.

ROESY analysis of the complex revealed that the guest was located closely to the aromatic sites of the host. Correlation signals of the 3,5-dimethylphenyl (xylyl) and indolyl groups of **2** to the pyridyl and N^+–CH_2 groups of **7** were observed (shown in Fig. 4). Correlations of the trimethyl groups of guests **3**–**5** to the aromatic C–H groups of the hosts were also observed.[8] Therefore, it is proposed that the quaternary ammonium ion is located on the binding site composed of aromatic moieties at both terminals of the peptide (shown in Fig. 5).

The *K* values of **2** were larger than those of **1** for all of ammonium salts (Table 3). This shows that the π-electron density of the aromatic moiety is an important factor for complexation. The guest binding ability of **2** was greater than those of the reported cyclophanes in complexation with quaternary ammonium salts in organic solvents.[5,7] Therefore, the cyclic structure of a host molecule is not always indispensable for recognition of quaternary ammonium salts. Intramolecular hydrogen bonding and conformational constraints enable acyclic receptors to bind guests.

The peptides reported here are conformational control models for a protein that forms a guest binding site by means of multiple non-covalent intramolecular forces. The ability to form an appropriate conformation due to the steric hindrance of Aib enables the aromatic residues of both terminals to approach each other where the residues effectively act as π-basic units of the receptor. The synthetic hosts that show the cation–π interaction have not yet been applied for medical use. In terms of safety, the peptide host may be more suitable for medical applications than a cyclophan composed of many aromatic moieties. A wide variety of design for peptides that recognize bio-active choline derivatives are expected.

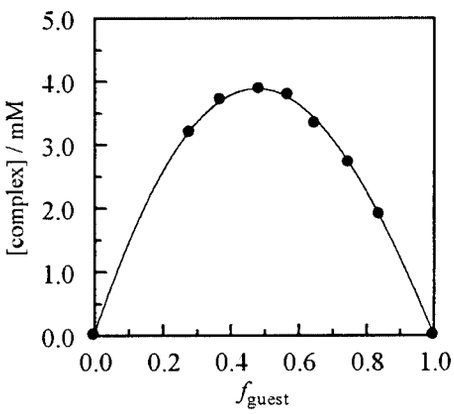

Fig. 3 Job plots of [complex] *vs.* mole fraction of guest **3** (f_{guest}) for the complexation of **1** in $CDCl_3$ at 298 K under condition where [**1**] + [**3**] is maintained at 16 mM.

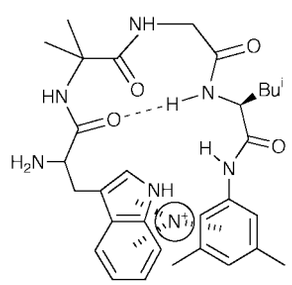

Fig. 5 The proposed structure of complex between an ammonium and **2**.

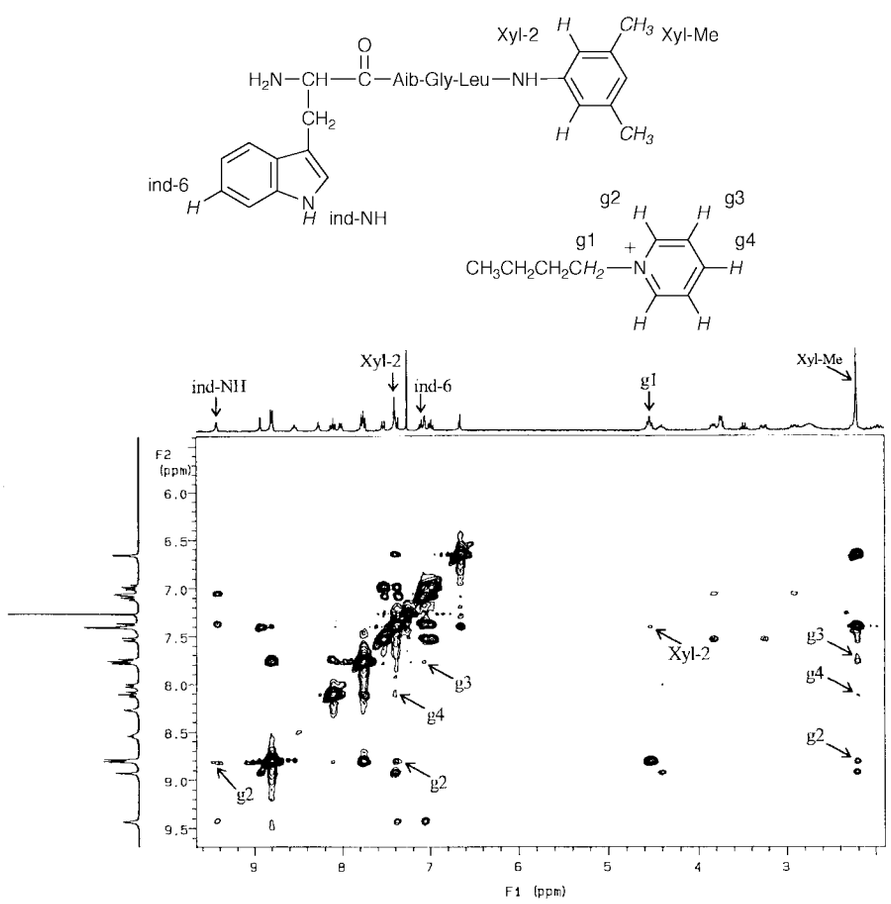

Fig. 4 ROESY spectrum of the complex of **2** (28 mM) and **7** (28 mM) in $CDCl_3$ at 298 K.

Experimental

General

The ^{1}H NMR spectra were recorded on a Varian Unity 300 MHz instrument at 299.94 MHz. Tetramethylsilane was used as the internal standard. Assignments of signals for the peptides and the complexes were made by COSY and ROESY correlations. Specific rotations were measured on a JASCO DIP 1000 digital polarimeter. MALDI-TOF mass spectra were recorded on a PerSeptive Biosystems Voyager DE PRO Biospectrometry Workstation, where α-cyano-4-hydroxycinnamic acid as a matrix reagent.

Peptide synthesis

The peptides used as hosts were prepared by the usual Z strategy in the liquid phase (Scheme 1). Z-Trp,[9,10] Z-Leu,[9,11] Z-Aib,[12] and Gly-OMe·HCl[13] were prepared according to the method described in the literature. The coupling reactions were performed according to the carbodiimide–HOBT method. Aniline, 3,5-dimethylaniline, and all guest samples (**3**–**10**) were obtained commercially.

Z-Aib-Gly-OMe 11.[14] To a solution of Z-Aib (47.4 g, 0.200 mol), Gly-OMe·HCl (23.7 g, 0.189 mol), triethylamine (19.4 g, 0.192 mol), and HOBT (27.0 g, 0.200 mol) in dichloromethane (250 cm^3), DCC (41.3 g, 0.200 mol) was slowly added at 0 °C. The solution was stirred for 3 days at room temperature. The precipitated urea was then filtered off and the filtrate was dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (150 cm^3). The solution washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3), and saturated NaCl (50 cm^3), and then dried over anhydrous sodium sulfate. The solid obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane to give **11** (41.9 g, 72%), mp 63–64 °C; δ_H ($CDCl_3$) 1.55 (6H, s, Aib-CH_3), 3.75 (3H, s, O-CH_3), 4.02 (2H, br s, Gly-CH_2), 5.10 (2H, s, Z-CH_2), 5.26 (1H, s, Aib-NH), 6.80 (1H, br s, Gly-NH), 7.33–7.37 (5H, m, Ph); *m*/*z* (MALDI-TOFMS) [Found: 331.13. ($C_{15}H_{20}N_2O_5$ + Na)$^+$ requires 331.13].

Aib-Gly-OMe·HBr 12.[14] To a solution of 30% hydrogen bromide in acetic acid (14 cm^3), **11** (4.35 g, 14.1 mmol) was added and allowed to stand for 2.5 h at 35 °C. White crystals precipitated with the addition of ether (100 cm^3) were collected, and then washed with ether. Recrystallization from MeOH–ether gave **12** (3.50 g, 97%), mp 194–195 °C; δ_H (DMSO-d_6) 1.47 (6H, s, Aib-CH_3), 3.63 (3H, s, O-CH_3), 3.90 (2H, d, Gly-CH_2), 8.18 (3H, br s, Aib-NH_3^+), 8.77 (1H, t, Gly-NH); *m*/*z* (MALDI-TOFMS) [Found: 175.11. ($C_7H_{14}N_2O_3$ + H)$^+$ requires 175.11].

Z-Trp-Aib-Gly-OMe 13. To a solution of Z-Trp (4.06 g, 12.0 mmol), **12** (2.55 g, 10.0 mmol), triethylamine (1.01 g, 10.0 mmol), and HOBT (1.62 g, 12.0 mmol) in dichloromethane (15 cm^3) and DMF (2 cm^3), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) (2.30 g, 12.0 mmol) was slowly added at 0 °C. The solution was stirred for 24 h at room temperature, and then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (100 ml), and the solution was washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (2 × 50 cm^3), and saturated NaCl (30 cm^3) and dried over anhydrous sodium sulfate. The solid obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane to give **13** (4.01 g, 81%), mp 102–104 °C; $[\alpha]_D^{25}$ +17.4° (*c* 0.20 in MeOH); δ_H ($CDCl_3$) 1.32 (6H, s, Aib-CH_3), 3.25 (2H, ddd, Trp-β-CH_2), 3.73 (3H, s, O-CH_3), 3.91 (2H, d, Gly-CH_2), 4.42 (1H, q, Trp-αCH), 5.11 (2H, s, Z-CH_2), 5.44 (1H, br s, Trp-NH), 5.89 (1H, s, Aib-NH), 6.97 (1H, br s, Gly-NH), 7.09–7.68 (10H, m, Ar), 8.17 (1H, br s, indolyl-NH); *m*/*z* (MALDI-TOFMS) [Found: 517.20. ($C_{26}H_{30}N_4O_6$ + Na)$^+$ requires 517.21].

Z-Trp-Aib-Gly 14. To a solution of **13** (3.00 g, 6.07 mmol) in MeOH (60 cm^3), 1 M sodium hydroxide (8 cm^3) was added. The solution was stirred for 5 h at room temperature and then dried under reduced pressure. The residue was dissolved in water and washed with ether (2 × 50 cm^3). The aqueous layer was acidified with 10% citric acid and extracted with ethyl acetate (3 × 50 cm^3). The organic layer was dried over anhydrous sodium sulfate. The powder obtained by evaporation of the solution was recrystallized from dichloromethane–hexane to give **14** (2.83 g, 97%), mp 106–110 °C; $[\alpha]_D^{25}$ +12.7° (*c* 0.20 in MeOH); δ_H ($CDCl_3$) 1.20 (6H, s, Aib-CH_3), 3.18 (2H, m, Trp-β-CH_2), 3.75 (2H, d, Gly-CH_2), 4.46 (1H, q, Trp-αCH), 5.03 (2H, dd, Z-CH_2), 5.90 (1H, br s, Trp-NH), 6.27 (1H, s, Aib-NH), 6.91 (1H, br s, Gly-NH), 7.04–7.59 (10H, m, Ar), 8.76 (1H, br s, indole-NH); *m*/*z* (MALDI-TOFMS) [Found: 503.22. ($C_{25}H_{28}N_4O_6$ + Na)$^+$ requires 503.19].

Z-Leu-NHPh 15. To a solution of Z-Leu (9.68 g, 36.5 mmol), aniline (3.73 g , 40.0 mmol), and HOBT (5.41 g, 40.0 mmol) in dichloromethane (120 cm^3), EDC·HCl (7.67 g, 40.0 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature. The solution was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (100 cm^3). The solution was washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3), and saturated NaCl (50 cm^3) and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane to give **15** (10.9 g, 87%), mp 141–142 °C; $[\alpha]_D^{25}$ −25.6° (*c* 0.20 in MeOH); δ_H ($CDCl_3$) 0.96 (6H, dd, Leu-δ-CH_3), 1.55–1.79 (3H, m, Leu-β-CH_2 and Leu-γ-CH), 4.31 (1H, br s, Leu-α-CH), 5.11 (2H, dd, Z-CH_2), 5.26 (1H, d, Leu-NH), 7.10 (1H, t, Ph(anilide)-4H), 7.25 (2H, t, Ph(anilide)-3H), 7.31 (5H, m, Ph(Z)), 7.48 (2H, d, Ph(anilide)-2H), 8.16 (1H, s, anilide-NH); *m*/*z* (MALDI-TOFMS) [Found: 363.18. ($C_{20}H_{24}N_2O_3$ + Na)$^+$ requires 363.17].

Leu-NHPh 16.[15] Z-Leu-NHPh (5.10 g, 15.0 mmol) was added to a solution of 30% hydrogen bromide in acetic acid (20 cm^3) and allowed to stand for 2.5 h at 35 °C. Precipitation occurred with the addition of ether (100 cm^3). It was filtered and then washed with ether. White crystals of **16**·HBr were obtained (4.05 g, 94%), mp 99–102 C°; $[\alpha]_D^{25}$ +34.2° (*c* 0.20 in MeOH); δ_H ($CDCl_3$) 0.84 (6H, dd, Leu-δ-CH_3), 1.67–1.85 (3H, m, Leu-β-CH_2 and Leu-γ-CH), 4.68 (1H, br s, Leu-α-CH), 7.06 (1H, t, Ph-4H), 7.21 (2H, t, Ph-3H), 7.57 (2H, d, Ph-2H), 7.97 (3H, br s, Leu-NH_3^+), 9.85 (1H, s, anilide-NH); *m*/*z* (MALDI-TOFMS) [Found: 207.14. ($C_{12}H_{18}N_2O$ + H)$^+$ requires 207.15]. The obtained **16**·HBr was dissolved in 1 M NaOH and extracted with ethyl acetate. The free base **16** was quantitatively obtained by evaporation of the solution under reduced pressure and used without further purification.

Z-Trp-Aib-Gly-Leu-NHPh 17. To a solution of **14** (2.80 g, 5.83 mmol), **16** (1.17 g, 5.65 mmol), and HOBT (788 mg, 5.83 mmol) in dichloromethane (80 cm^3) and DMF (3 cm^3), EDC·HCl (1.11 g, 5.83 mmol) was slowly added at 0 °C. The solution was stirred for 3 days at room temperature and then dried under reduced pressure. The obtained residue was dissolved in ethyl acetate (150 ml). The solution was washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3), and saturated NaCl (50 cm^3) and then dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was purified by chromatography on silica gel with ethyl acetate to give **17** (3.06 g, 81%), mp 132–134 °C; $[\alpha]_D^{25}$ −34.1° (*c* 1.00 in chloroform); δ_H (DMSO-d_6) 0.87 (6H, dd, Leu-δ-CH_3), 1.19 (6H, s, Aib-CH_3), 1.60 (1H, m, Leu-γ-CH), 1.88 (t, 2H, Leu-β-CH_2), 3.04 (2H, ddd, Trp β-CH_2), 3.61 (2H, d, Gly-CH_2), 4.46 (2H, m, Trp-α-CH and Leu-α-CH), 4.95 (2H, dd, Z-CH_2), 6.96 (1H, t, indolyl-5-H), 7.04 (2H, dt, Ph(anilide)-4-H and indolyl-6-H), 7.13 (1H, d, indolyl-2-H), 7.21–7.27 (7H, m, Ph(Z) and

Ph(anilide)-3-H), 7.32 (1H, d, indolyl 7-H), 7.39 (1H, d, Trp-NH), 7.61 (1H, d, indolyl 4-H), 7.68 (d, 2H, phenyl 2-H), 7.80 (1H, d, Leu-NH), 8.08 (1H, t, Gly-NH), 8.56 (1H, s, Aib-NH), 9.58 (1H, s, anilide-NH), 10.81 (1H, br s, indolyl-NH); *m/z* (MALDI-TOFMS) [Found: 691.33. $(C_{37}H_{44}N_6O_6 + Na)^+$ requires 691.32].

Trp-Aib-Gly-Leu-NHPh 1. Hydrogen was bubbled through a solution of **17** (3.00 g, 4.48 mmol) in MeOH (15 cm^3) in the presence of 5% palladium on activated carbon (0.10 g) for 5 h at room temperature. The catalyst was filtered off and the filtrate was dried under reduced pressure. The crude product was purified by chromatography on silica gel with 15% MeOH–chloroform to give **1** (1.90 mmol, 79%), mp 131–133 °C; $[\alpha]_D^{25}$ +4.7° (*c* 0.55 in MeOH); δ_{CH} ($CDCl_3$) 0.98 (6H, dd, Leu δ-CH_3), 1.43 and 1.45 (6H, s, Aib β-CH_3), 1.77 (1H, m, Leu γ-CH), 1.91 (2H, t, Leu β-CH_2), 3.05 (2H, ddd, Trp β-CH_2), 3.60 (2H, ddd, Gly α-CH_2), 3.69 (1H, m, Trp α-CH), 4.51 (1H, q, Leu α-CH), 6.97 (1H, d, indolyl 2-H), 7.05 (1H, t, phenyl 4-H), 7.16 (1H, t, indolyl 5-H), 7.21 (1H, t, indolyl 6-H), 7.27 (2H, t, phenyl 3-H), 7.33 (1H, d, indolyl 7-H), 7.53 (1H, d, indolyl 4-H), 7.79 (2H, d, phenyl 2-H); δ_C ($CDCl_3$) 21.3, 23.5, 24.8, 25.2, 25.7, 30.3, 44.1, 53.4, 55.5, 56.4, 110.7, 111.6, 118.6, 119.8, 120.2, 122.4, 123.5, 124.1, 127.3, 128.9, 136.3, 138.5, 170.1, 171.6, 175.7, 175.8; *m/z* (MALDI-TOFMS) [Found: 535.28. $(C_{29}H_{38}N_6O_4 + H)^+$ requires 535.30]. (Found: C, 63.63; H, 7.17; N, 15.06. $C_{29}H_{38}N_6O_4 \cdot 0.75H_2O$ requires C, 63.54; H, 7.26; N, 15.33%).

Z-Leu-NH-C_6H_3(3,5-Me_2) 18. To a solution of Z-Leu (5.31 g, 20.0 mmol) 3,5-dimethylaniline (2.67 g, 22.0 mmol), and HOBT (2.70 g, 20.0 mmol) in dichloromethane (80 cm^3) and DMF (10 cm^3), DCC (4.13 g, 20.0 mmol) was slowly added at 0 °C. The solution was stirred for 24 h at room temperature. The precipitated urea was removed by filtration and the filtrate was dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (100 cm^3) and the solution was washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3), and saturated NaCl (50 cm^3), then dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate to give **18** (5.75 g, 78%), mp 153–154 °C; $[\alpha]_D^{25}$ −23.2° (*c* 1.00 in MeOH); δ_H ($CDCl_3$) 0.94 (6H, dd, Leu-δ-CH_3), 1.55–1.77 (3H, m, Leu-β-CH_2 and Leu-γ-CH), 2.26 (6H, s, xylyl-CH_3), 4.29 (1H, br s, Leu-α-CH), 5.11 (2H, s, Z-CH_2), 5.30 (1H, d, Leu-NH), 6.74 (1H, s, xylyl-4H), 7.12 (2H, s, xylyl-2H), 7.33 (5H, br s, Ph), 8.10 (1H, s, xylidide-NH); *m/z* (MALDI-TOFMS) [Found: 391.20. $(C_{22}H_{28}N_2O_3 + Na)^+$ requires 391.20].

Leu-NH-C_6H_3(3,5-Me_2) 19. Hydrogen was bubbled through a solution of **18** (6.67 g, 18.1 mmol) in DMF (50 cm^3) in the presence of 5% palladium on activated carbon (0.10 g) for 3 h at room temperature. The catalyst was filtered off and the filtrate was dried under reduced pressure. Recrystallizaton of the crude product from ethyl acetate gave **19** (3.94 g, 93%), mp 61–63 °C; $[\alpha]_D^{25}$ −12.1° (*c* 1.00 in MeOH); δ_H ($CDCl_3$) 0.98 (6H, dd, Leu-δ-CH_3), 1.41 (1H, m, Leu-γ-CH), 1.60 (2H, br s, Leu-NH_2), 1.79 (2H, m, Leu-β-CH_2), 2.30 (6H, s, xylyl-CH_3), 3.49 (1H, dd, Leu-α-CH), 6.74 (1H, s, xylyl-4H), 7.24 (2H, s, xylyl-2H), 9.39 (1H, s, xylidide-NH); *m/z* (MALDI-TOFMS) [Found: 235.17. $(C_{14}H_{22}N_2O + H)^+$ requires 235.18].

Z-Trp-Aib-Gly-Leu-NH-C_6H_3(3,5-Me_2) 20. To a solution of **14** (7.69 g, 16.0 mmol), **19** (3.51 g, 15.0 mmol), and HOBT (2.16 g, 16.0 mmol) in dichloromethane (35 cm^3) and DMF (6 cm^3), EDC·HCl (3.07 g, 16.0 mmol) was slowly added at 0 °C. The solution was stirred for 2 days at room temperature and then dried under reduced pressure. The resulting residue was dissolved into ethyl acetate (100 cm^3) and washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3), and saturated NaCl (50 cm^3), then dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was purified by chromatography on silica gel with ethyl acetate to give **20** (8.92 g, 80%), mp 102–104 °C; $[\alpha]_D^{25}$ −38.5° (*c* 1.00 in chloroform); δ_H ($CDCl_3$) 0.99 (6H, dd, Leu-δ-CH_3), 1.29 and 1.32 (6H, s, Aib-CH_3), 1.76 (1H, m, Leu-γ-CH), 1.92 (t, 2H, Leu-β-CH_2), 2.26 (6H, s, xylyl-CH_3), 3.21 (2H, ddd, Trp β-CH_2), 3.73 (2H, br s, Gly-CH_2), 4.26 (1H, q, Trp-α-CH), 4.57 (1H, q, Leu-α-CH), 4.98 (2H, dd, Z-CH_2), 5.44 (1H, d, Trp-NH), 6.29 (1H, s, Aib-NH), 6.71 (1H, s, xylyl-4H), 6.85 (1H, br s, Gly-NH), 7.05 (1H, d, indolyl-2-H), 7.14 (1H, t, indolyl-5-H), 7.21–7.37 (8H, m, Ph and indolyl-(5-7)-H), 7.39 (2H, s, xylyl-2H), 7.50 (1H, d, Leu-NH), 7.58 (1H, d, indolyl 4-H), 8.29 (1H, s, xylydide-NH), 8.56 (1H, br s, indolyl-NH); *m/z* (MALDI-TOFMS) [Found: 719.36. $(C_{39}H_{48}N_6O_6 + Na)^+$ requires 719.35].

Trp-Aib-Gly-Leu-NH-C_6H_3(3,5-Me_2) 2. Hydrogen was bubbled through a solution of **20** (4.53 g, 6.50 mmol) in MeOH (25 cm^3) in the presence of 5% palladium on activated carbon (0.10 g) for 2 h at room temperature. The catalyst was filtered off and the filtrate was dried under reduced pressure. The crude product was purified by chromatography on silica gel with 15% MeOH–chloroform to give **2** (2.60 g, 71%), mp 128–131 °C; $[\alpha]_D^{25}$ −10.1° (*c* 0.20 in MeOH); δ_{CH} ($CDCl_3$) 0.97 (6H, dd, Leu δ-CH_3), 1.43 and 1.45 (6H, s, Aib β-CH_3), 1.75 (1H, m, Leu γ-CH), 1.90 (2H, t, Leu β-CH_2), 2.27 (6H, s, xylyl CH_3), 3.07 (2H, ddd, Trp β-CH_2), 3.65 (2H, ddd, Gly α-CH_2), 3.73 (1H, m, Trp α-CH), 4.55 (1H, q, Leu α-CH), 6.72 (1H, s, xylyl 4-H), 6.99 (1H, d, indolyl 2-H), 7.07 (1H, t, indolyl 5-H), 7.18 (1H, t, indolyl 6-H), 7.42 (2H, s, xylyl 2-H), 7.30 (1H, d, indolyl 7-H), 7.54 (1H, d, indolyl 4-H); δ_C ($CDCl_3$) 21.5, 21.6, 23.3, 25.0, 25.1, 25.2, 29.9, 40.0, 43.9, 53.4, 55.4, 56.5, 110.1, 111.6, 118.1, 118.5, 119.8, 122.4, 123.7, 126.1, 127.2, 136.3, 138.1, 138.5, 170.4, 171.6, 175.3, 175.4; *m/z* (MALDI-TOFMS) [Found: 563.31. $(C_{31}H_{43}N_6O_4 + H)^+$ requires 563.33] (Found: C, 64.12; H, 7.55; N, 14.22. $C_{31}H_{42}N_6O_4 \cdot H_2O$ requires C, 64.12; H, 7.64; N, 14.47%).

Binding assay

The ^1H NMR spectra were taken for a series of solutions containing a guest (**3**–**8**, 0.50 mM) and varying amounts (0 to 25 mM) of a host (**1** or **2**) in $CDCl_3$ at 298 K. The binding constant (*K*) was obtained by non-linear fitting of titration data for N^+–CH_3 or N^+–CH to eqn. (6), where the correlation coefficient $|r|$ was greater than 0.99 in every case. At least three runs were carried out for each guest. The accuracy in *K* is within ±5% in every case. The concentration of a complex in solution, as required for the Job plots (Fig. 3), was evaluated from $\Delta\delta_{obs}$ for the guest according to eqn. (5).

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Notes and references

1 C. Toniolo and E. Benedetti, *ISI Atlas of Science: Biochemistry*, 1988, 225; I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747; C. Toniolo and E. Benedetti, *Macromolecules*, 1991, **24**, 4004; V. Pavone, E. Benedetti, B. D. Blasio, C. Pedone, A. Santini, A. Bavaso, C. Toniolo and M. Crisma, *J. Mol. Biol.*, 1990, **214**, 633; C. Aleman, J. A. Subirana and J. J. Perez, *Biopolymers*, 1992, **32**, 621; K. Okuyama and S. Ohuchi, *Biopolymers*, 1996, **40**, 85.

2 Dnp: 2,4-dinitrophenyl, pNA: *p*-nitroanilide.

3 T. Yamada, M. Nakao, T. Miyazawa, S. Kuwata, M. Sugiura, Y. In and T. Ishida, *Pept. Chem.*, 1991, 71; T. Yamada, M. Nakao, T. Miyazawa, S. Kuwata, M. Sugiura, Y. In and T. Ishida, *Biopolymers*, 1993, **33**, 813.

4 J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker and I. Silman, *Science*, 1991, **253**, 872; J. Novotny, R. Bruccoleri and F. A. Saul, *Biochemistry*, 1989, **28**, 4735; M. Harel, I. Schalk, L. Ehret-Sabatier, F. Bouet, M. Goeldner, C. Hirth, P. H. Axelsen, I. Silman and J. L. Sussman, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 9031.
5 D. A. Dougherty, *Science*, 1996, **271**, 163; D. A. Stauffer and D. A. Dougherty, *Tetrahedron Lett.*, 1988, **29**, 6039; J. C. Ma and D. A. Dougherty, *Chem. Rev.*, 1997, **97**, 1303.
6 K. Kobayashi, Y. Asakawa and Y. Aoyama, *Supramol. Chem.*, 1993, **2**, 133; H.-J. Schneider and U. Schneider, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1994, 67.
7 G. D. Isai and B. Masci, *Tetrahedron Lett.*, 1993, **41**, 6635; A. Cattani, A. D. Cort and L. Mandolini, *J. Org. Chem.*, 1995, **60**, 8313; A. Arduini, W. M. McGregor, D. Paganuzzi, A. Pochini, F. Ugozzoli and R. Ungaro, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 839.
8 The solubilities of guests **6** and **8** in $CDCl_3$ are too low to observe ROESY correlations.
9 E. Klieger, E. Schröder and H. Gibian, *Justus Liebigs Ann. Chem.*, 1961, **640**, 157.
10 E. L. Smith, *J. Biol. Chem.*, 1948, **175**, 39.
11 G. Losse and E. Demuth, *Chem. Ber.*, 1961, **94**, 1762.
12 G. Valle, F. Formaggio, M. Crisma, G. M. Bonora and C. Toniolo, *J. Chem. Soc.*, *Perkin Trans. 2*, 1986, 1371.
13 M. Brenner and W. Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.
14 T. Yamada, S. Nonomura, H. Fujiwara, T. Miayazawa and S. Kuwata, *J. Chromatogr.*, 1990, **515**, 475.
15 H. Tsuzuki, T. Oka and K. Morihara, *J. Biochem.*, 1980, **88**, 669; R. E. Beattie, D. T. Elmore, C. H. Williams and D. J. S. Guthrie, *Biochem. J.*, 1987, **245**, 285.

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