Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2355–2363

BIOORGANIC & MEDICINAL CHEMISTRY

Functionalized Oligoanthranilamides: Modular and Conformationally Controlled Scaffolds

Yoshitomo Hamuro[a] and Andrew D. Hamilton[b,*]

[a]*Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA*
[b]*Department of Chemistry, Yale University, New Haven, CT, 06511, USA*

Received 15 March 2001; accepted 27 April 2001

Abstract—This paper describes the use of functionalized oligoanthranilamides as conformationally controlled scaffolds for molecular recognition. Oligomers of anthranilamides are stabilized by the formation of intramolecular six-membered hydrogen bonds in a linear strand conformation. Onto alternate anthranilic acid units, we have attached di- or tripeptide recognition units with the potential to form intramolecular hydrogen bonds to an intercalated peptide strand. Using ^{1}H NMR dilution experiments in $CDCl_3$, we have observed chemical shift changes that are consistent with the formation of an extended hydrogen bonded sheet dimer. We also demonstrate that the bis-alanine functionalized strands are able to form discrete hydrogen bonded complexes with dipeptide substrates and to bind hexanoyl alanylalanine selectively over its benzyl ester. In the presence of excess hydrogen bond donors and acceptors, the oligoanthranilamide strand retained its linear conformation, pointing to the potential of this modular design as a useful and stable scaffold for molecular recognition studies.

Introduction

In recent years the preparation of oligomeric materials that fold into well defined shapes and structures has received considerable attention. The principal goal is to develop a modular design using non-peptidic and variable monomers that can potentially be used as a scaffold for recognition and catalysis.[1–3] One attractive approach is to mimic proteins and exploit intramolecular hydrogen bonding to control the spatial arrangement of the monomeric units.[4–7] We have previously described the use of different oligoanthranilamide derivatives to form well-defined strand, turn, and helical secondary structures in chloroform solution and in the solid state. In particular, we have established that an oligomer of anthranilamides forms a linear strand conformation stabilized by six-membered intramolecular hydrogen bonding between adjacent amides, and the trans preference of the secondary amide bond (Scheme 1).[8] Oligoanthranilamide scaffolds can have several advantages over simple monomeric scaffolds because of their modular design, their iterative synthesis and their ease of modification.[9]

In the present paper, we extend the application of oligoanthranilamides with the preparation of a series of functionalized derivatives that are used to project di- and tripeptide functionality from the linear strand. We further describe preliminary results that show that these parallel positioned peptides can dimerize through intermolecular hydrogen bonds to form a sheet-like aggregate. Intramolecular β-sheet formation is one of the key elements in protein folding and many groups have attempted to template formation of this secondary structure,[10] including Feigel,[11] Kemp,[12] Kelly,[13] Nowick,[14] and Gellman.[15] The design and synthesis of intermolecular β-sheet models are particularly challenging due to the entropically unfavorable process of bringing two or more strands together. Elegant examples in this regard are the stacked cyclic hydrogen bonded peptide sheets of Ghadiri[16] and the interdigitated dibenzofuran peptides of Kelly.[17]

Preparation of Functionalized Strands

Functionalized oligoanthranilamides can be easily synthesized from monomeric 2-nitrobenzoic acid derivatives. For example, the synthesis of scaffold **2** started with reduction of of 2-nitrobenzoic acid *n*-hexyl ester to the amine followed by coupling with the acid chloride of nitrophthalic acid mono methyl ester to give the dimeric

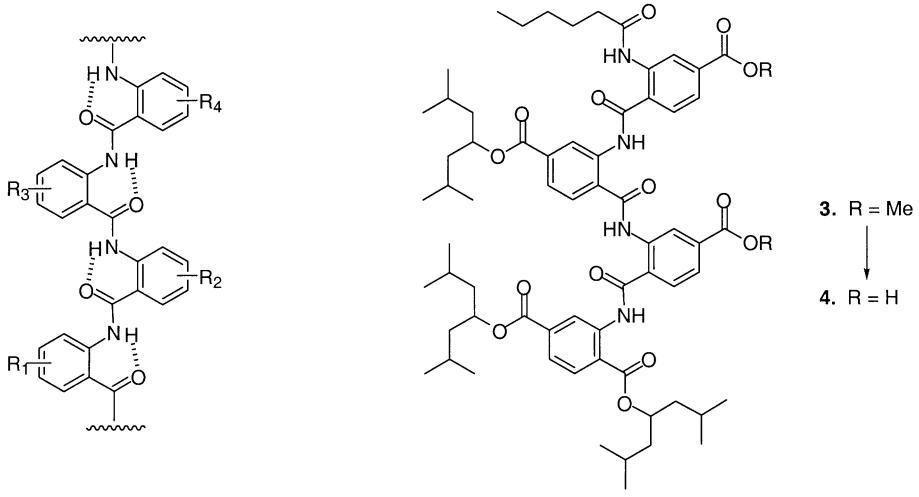
*Corresponding author. Tel.: +1-203-432-5570; fax: +1-203-432-3221; e-mail: andrew.hamilton@yale.edu

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00161-4

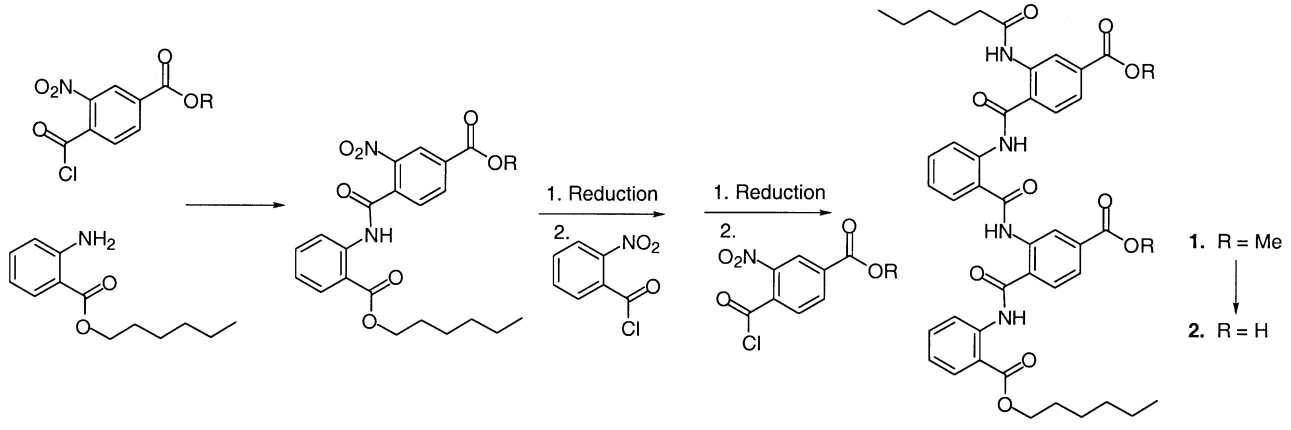
precursor (Scheme 2).[18] The dimer was reduced, coupled with 2-nitrobenzoic acid to give the trimeric derivative and one further reduction and coupling sequence with the acid chloride of nitrophthalic acid mono methyl ester gave the tetrameric compound. The tetramer was reduced and capped with hexanoyl chloride to give protected scaffold **1**. Alkaline hydrolysis using LiOH solution gave unprotected scaffold **2**. Later derivatives of scaffold **2** were found to have poor solubility in organic solvents. A simple modification of the above synthetic route allowed the preparation of more soluble versions, such as scaffolds **3** and **4** (Scheme 1).[18] In an attempt to project peptide chains from the scaffold in a parallel relationship we reacted the free carboxylic acid groups in scaffolds **2** and **4** with bis- and tris-alanine derivatives **5** and **6**, respectively, which in turn were prepared by sequential peptide coupling methods using CDI. From scaffolds **2** and **4** and two oligo-alanine derivatives **5** and **6**, we could prepare four functionalized strands. The molecules resulting from a combination of **2** and **6** had poor solubility properties and no further investigations were carried out. The three other combinations (**7**, **8**, and **9**) were synthesized by acid chloride coupling in good yields (Scheme 3). This simple synthetic strategy should also allow the attachment of oligopeptide sidechains to the monomeric anthanilamide precursors prior to strand formation, raising the potential of attaching different peptides onto the scaffold.

Self-aggregation Properties of the Peptide Functionalized Strands

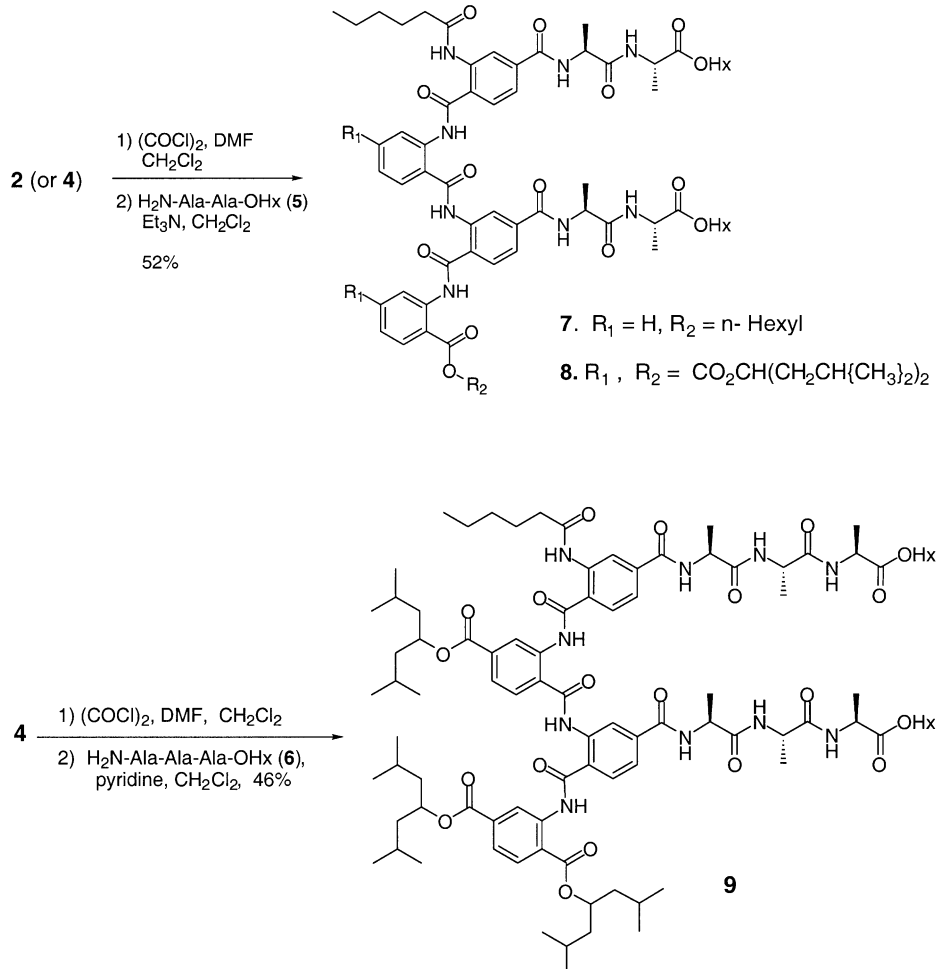
The self-aggregation properties of compound **8** were investigated by following the changes of ^{1}H NMR chemical shifts as a function of concentration in $CDCl_3$ at room temperature. One possibility was that this compound would dimerize as depicted in **10**, by six intermolecular hydrogen bonds in a sheet arrangement. The results of the dilution experiments are shown in Figure 1. Significant downfield shifts of all the alanine NH protons upon increasing concentration are seen, consistent with the formation of intermolecular hydrogen bonds (Fig. 1a). It was not possible to unequivocally distinguish between NH_e and NH_h or NH_f and NH_i in **10**. However, in Figure 1a one of NH_e or NH_h shifts 0.6 ppm and the other 0.4 ppm and one of NH_f and NH_i shifts 0.8 ppm and the other shifts 0.5 ppm, upon increasing concentration from 0.5 to 200 mM. This



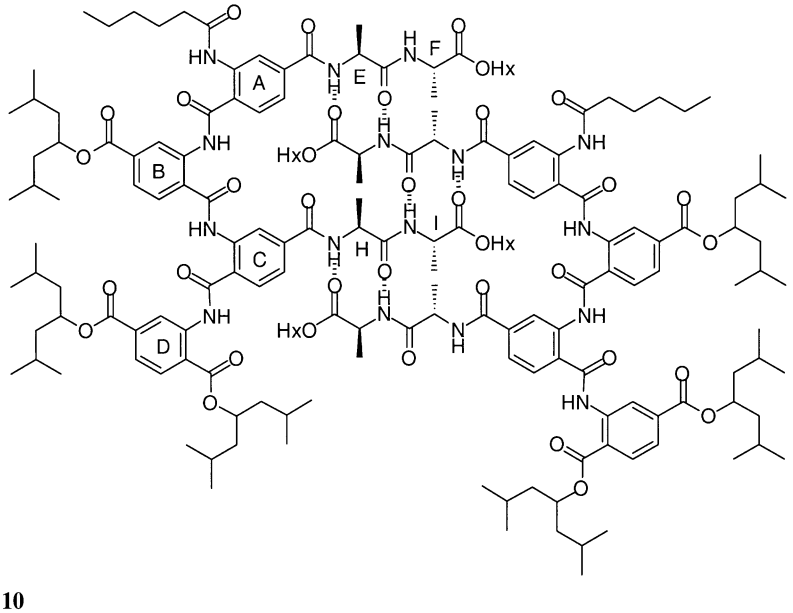
Scheme 1. Modular design of oligoanthranilamide.



Scheme 2. Synthesis of scaffold **2** from monomers.



Scheme 3. Synthesis of functionalized strands **7**, **8** and **9** from scaffolds **2** and **4**.



10

observation is consistent with the predicted dimerization, shown in **10** where two NH_e protons and two NH_i protons are used for hydrogen bonding but only one NH_f and one NH_h proton of each pair hydrogen bond upon dimerization. The dimerization constant (K_{dim}) derived from curve fitting analysis of the four NH protons was $10 \pm 2\,M^{-1}$.[19] The phenyl protons on the anthranilamide scaffold also showed two distinct concentration dependencies (Fig. 1b). The phenyl 3-protons on rings A and ring C moved upfield ($\Delta\delta = -0.09$ and

−0.15 ppm, respectively) while those on rings B and D did not shift. These upfield shifts are presumably due to the inductive effect of forming hydrogen bonds to the A and C ring carboxamides on dimerization. Figure 2 shows the chemical shift changes of alanine α-CH protons as a function of concentration. Protons CH_e and CH_h moved downfield by 0.2 ppm over the course of the dilution, while protons CH_f and CH_i shifted slightly upfield. Neither pair separated into distinct resonances. No significant changes occurred in the anthranilamide NH protons indicating that intramolecular hydrogen bonding within the scaffold was sustained through the experiment.

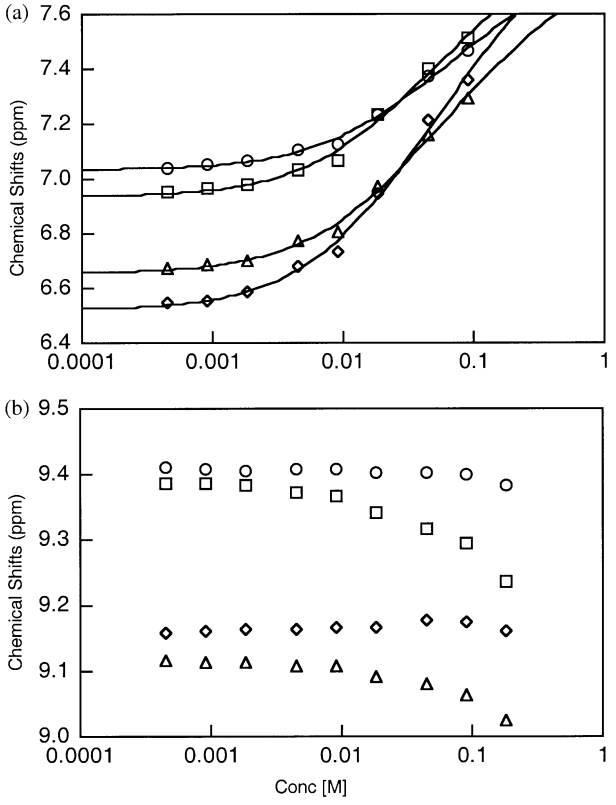


Figure 1. Dilution experiment of **8** at room temperature. (a) Chemical shifts of alanine NH protons. ○, NH_h (or NH_e), □, NH_e (or NH_h), ◇, NH_f (or NH_i), △, NH_i (or NH_e). (b) Chemical shifts of the phenyl 3-protons. ○, ring B; □, ring C; ◇, ring D; △, ring A.

In these room temperature experiments, the low K_{dim} values and the lack of saturation at high concentration and thus of sigmoidal shapes in the dilution curves suggested that a full population of the aggregated state had not been achieved. In order to investigate the oligomerization further, a low temperature ^1H NMR dilution experiment of compound **8** was performed in $CDCl_3$ at −20 °C. At high concentrations, severe peak broadening was observed, presumably due to higher aggregation. This broadening prevented the measurement of the chemical shifts of the alanine NH resonances, however the alanine C–H resonances could be followed. Figure 2 shows the chemical shifts of alanine α-protons at room temperature and −20 °C. At −20 °C, the dilution curves

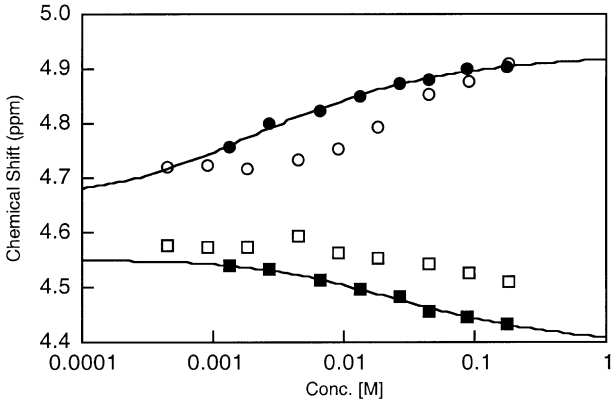
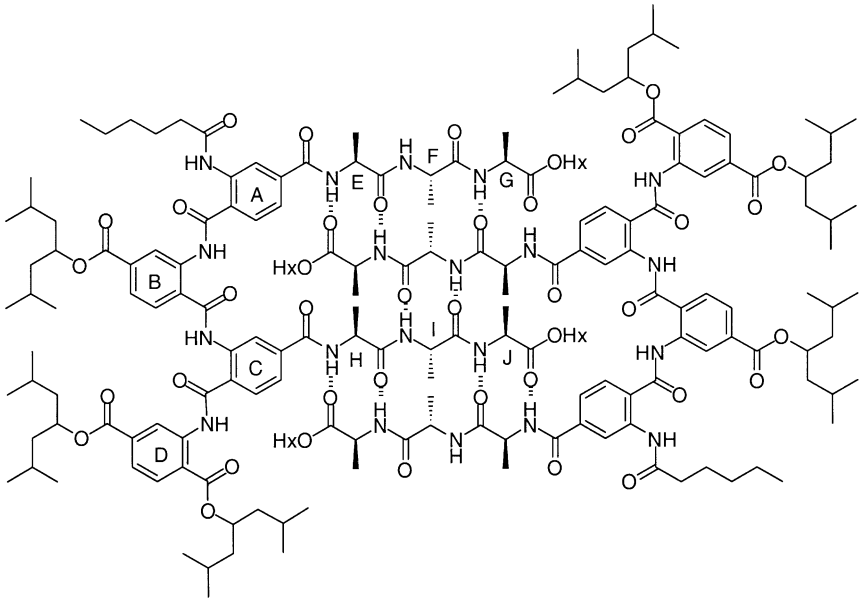
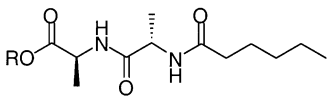


Figure 2. The comparison of two dilution experiments of **8**, at room temperature (open) and at −20 °C (closed). Chemical shifts of alanine α-protons. ○, CH_e and CH_h; □, CH_f and CH_i.

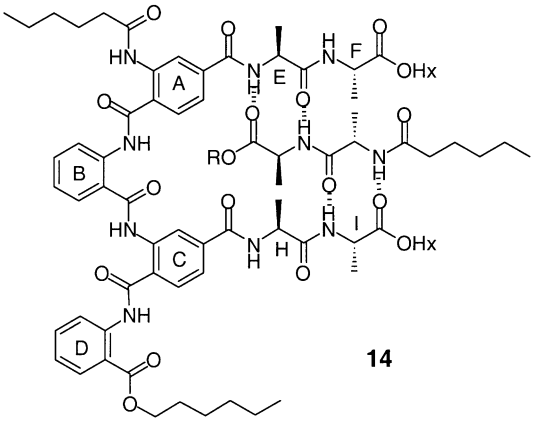


11



12. R = H

13. R = Bn



14

shift to the left indicating that transition from monomer to oligomer occurs at lower concentrations and that the aggregated state has reached saturation. The dimerization constants at −20 °C were calculated from the CH_e resonances to be $330 \pm 220\ M^{-1}$.[19] Scheme 4 shows two possible dimeric structures which could account for the observations above. These two aggregates are interconvertible since the intercalation dimer transforms into the stacked dimer by breaking the hydrogen bonds between A2 and B1. At this point it is impossible to distinguish between the two forms, however, it is likely that the stacked dimer would more readily form higher aggregates.

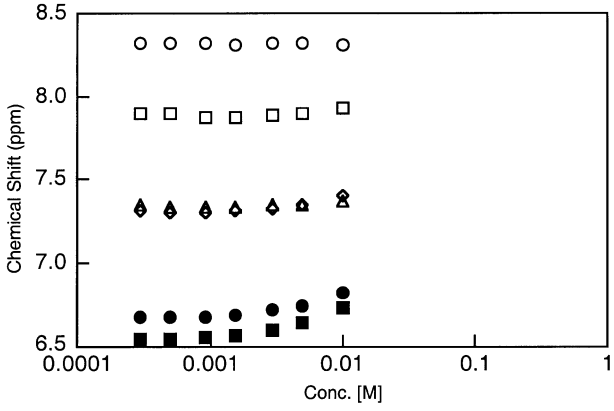
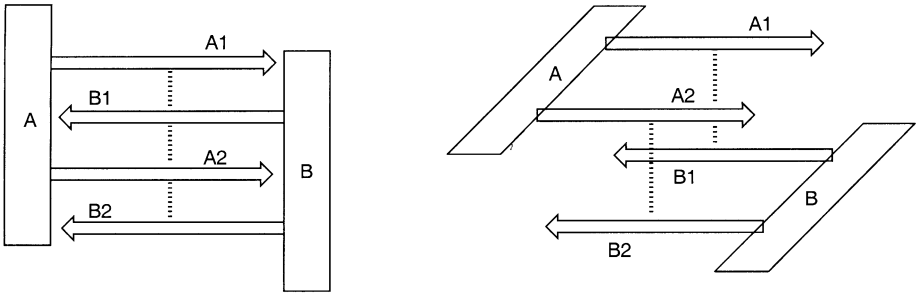


Figure 3. Dilution experiment of **9** at room temperature. Chemical shifts of alanine NH's. ○, NH_j (or NH_g); □, NH_g (or NH_j); ◇, NH_e (or NH_h); △, NH_h (or NH_e); ●, NH_i (or NH_f); ■, NH_f (or NH_i).

We anticipated that the incorporation of an additional amino acid into the side chains of **9** would lead to the more extensive hydrogen bonding interaction in the dimer (shown in **11**) involving 10 hydrogen bonds instead of six in **10**. A ^1H NMR dilution experiment with **9** in $CDCl_3$ at room temperature showed very little change in the chemical shifts of the alanine NH resonances over a 10^{-4}–10^{-2} M concentration range (Fig. 3). Significantly the NH_j or NH_g resonances were already downfield shifted (8.3 and 7.9 ppm, respectively) at the lowest concentration (10^{-3} M) compared with those of the terminal residues in **8** (6.5 and 6.7 ppm at 10^{-3} M). These downfield shifts in **9** showed no change with concentration and are most likely due to intramolecular hydrogen bonding between two tris-alanine strands. However, aggregation of a less regular nature can occur with **9**. Concentrations of **9** above 10^{-2} M were able to effectively gel $CHCl_3$ at room temperature.[20]

Binding Properties of the Functionalized Strands

The rigid separation of the side chains provided by the anthranilamide strand prompted us to investigate the ability of **7** to act as a host for small molecule guests. In particular, the position of the amide bonds in the flanking bis-alanine chains suggested that dipeptide carboxylate or ester derivatives, such as **12** and **13**, might be recognized through formation of a hydrogen bonded β-sheet, as in **14**. We had earlier discussed the use of peptide strands within a rigid framework as recognition units for peptide carboxylates, in direct analogy to the antibiotic vancomycin.[21] This macrobicyclic



Scheme 4. Schematic representation of possible dimers, intercalated and stacked.

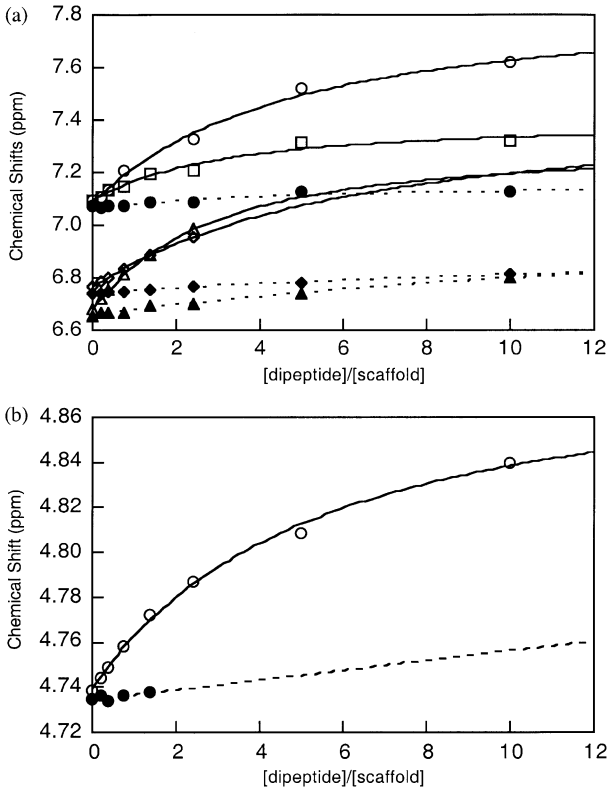


Figure 4. Chemical shifts of **7** upon the titration of **7** with **12** and **13** (The concentration of **7** was kept constant at 10^{-3} M). (a) NH protons of **7**. ○, NH_e; □, NH_h; ◇, NH_f; △, NH_i; with **12**. ●, NH_e and NH_h; ■, NH_f; ▲;, NH_i; with **13** (NH_e and NH_h were inseparable in this titration). (b) ○, CH_e and CH_h of **7** with **12**; ●, CH_e and CH_h of **7** with **13**.

heptapeptide derivative binds to acyl-D-alanine-D-alanine carboxylate derivatives through several hydrogen bonds that mimic one strand of a β-sheet.[22] Our initial studies with the functionalized anthranilamides involved acyl-L-alanine-L-alanine carboxylic acid and ester derivatives **12** and **13**.[23]

The complexation properties of compound **7** were measured by ^{1}H NMR titration experiments in $CDCl_3$ at room temperature. We had earlier determined that the dimerization constant for **7** was $55 \pm 10\,M^{-1}$ indicating that at 10^{-3} M (the concentration of **7** in the titration experiments) self-aggregation should be only minor.[19] On titration of **7** with bis-alanine carboxylic acid **12**, significant shifts in several of the NH and CH protons were observed (Fig. 4a). Analysis of the titration curve using non-linear regression analysis gave a K_a value of $350 \pm 120\,M^{-1}$.[24] The NH resonances from amides E, and I showed the largest downfield shifts (0.35–0.5 ppm) consistant with their direct participation in a hydrogen bond to carbonyl groups of the dipeptide substrate (seen in **14**). Amide NHs from residues F and H shifted downfield by a lesser amount (~0.2 ppm) as would be expected from an involvement of the residue CO in hydrogen bonding to acidic protons in the substrate. In the case of the amide NH from residue H this may involve the carboxylic acid proton. One complication in this analysis is the presence of additional amide NH and CO groups on the exterior of the sheet in **14** capable of forming further hydrogen bonds to dipeptide substrates in higher order (2:1 or 3:1) complexes. This may account for the observation that even at 10 equivalents of dipeptide, clear saturation in the shifts of the NH_e and $CH_{e,h}$ resonances has not been reached. That a discrete interaction is taking place between **7** and **12** is seen in the behavior of benzyl ester **13**. In contrast to **12**, when **13** is titrated into a $CDCl_3$ solution of **7** no significant shifts in the amide NH or α-CH resonances of the functionalized strand are seen, even at high concentrations of the dipeptide ester (Fig. 4b). The absence of a strong interaction between **7** and **13** is likely due to the bulkiness of the benzyl group which, if the four hydrogen bonds shown in **14** were formed, would encounter unfavorable steric interactions with the oligoanthranilamide strand. During both titrations, the anthranilamide proton resonances moved little indicating that the conformation of the scaffolds remained intact even in the presence of excess dipeptide substrate.

In summary, we have described the synthesis of a new class of functionalized oligomers in which parallel di- and tripeptide strands are displayed from one face of an oligoanthranilamide scaffold. The self-aggregation properties of the bis-alanine functionalized strands were analyzed by ^{1}H NMR dilution experiments which showed chemical shift changes that were consistent with the formation of an extended hydrogen bonded sheet dimer. In contrast, the tris-alanine derivatives did not self-associate suggesting that intramolecular hydrogen bonding blocked the intermolecular binding sites. The bis-alanine strands were also able to form discrete hydrogen bonded complexes with dipeptide substrates. ^{1}H NMR experiments were consistent with the formation of an intermolecular sheet structure that was available to hexanoylalanylalanine carboxylic acid but not its benzyl ester. During both dilution and titration experiments, the anthranilamide CH or NH protons barely shifted suggesting that the intramolecularly hydrogen bonded conformation of the scaffolds was retained. These results demonstrate both the stability and usefulness of linear oligoanthralinamides as conformationally well-controlled scaffolds.

Experimental

General methods

CH_2Cl_2 was obtained from Fisher and distilled from P_2O_5. Et_2O and THF were obtained from Fisher and distilled from sodium benzophenone ketyl. All other reagents, unless otherwise noted, were obtained from the Aldrich Chemical Company and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300 MHz). NMR chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were determined at the Department of Chemistry, University of Pittsburgh, USA. EI and FAB mass spectra (MS) were obtained using a Varian MAT CH-5 or VG 7070 mass spectrometer under the direction of Dr. Kasi V. Somayajula. Melting points were determined using an Electrothermal capillary

melting point apparatus and are uncorrected. Elemental analysis was carried out by Atlantic Microlab, Inc., Norcross, GA, USA. Analytical thin layer chromatography (TLC) was conducted using PolyGram 0.25 mm silica gel precoated plates with fluorescent indicator UV_{254}. Silica gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM; EM Science) was used for flash chromatography.

***N-tert*-Butoxycarbonyl-L-alanyl-L-alanine benzyl ester.** A solution of *N-tert*-butoxycarbonyl-L-alanine (3.78 g, 20 mmol) and CDI (3.41 g, 21 mmol) in dry CH_2Cl_2 (40 mL) was stirred at room temperature for 25 min. L-Alanine benzyl ester hydrochloride (4.31 g, 20 mmol) and Et_3N (2.02 g, 20 mmol) were added to the solution and the reaction mixture was stirred at room temperature overnight. CH_2Cl_2 (250 mL) was added to the mixture which was then washed with 1 N HCl (200 mL), aqueous $NaHCO_3$ (200 mL) and brine (200 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give the desired product as a white wax (6.43 g, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 5H), 6.69 (broad d, $J=6.0$ Hz, 1H), 5.17 (m, 2H), 5.06 (s, 1H), 4.61 (m, 1H), 4.18 (broad m, 1H), 1.44 (m, 15H).

L-Alanine hexyl ester. L-Alanine (13.4 g, 150 mmol) was suspended in 1-hexanol (100 mL) and benzene (80 mL). HCl gas was bubbled into the mixture which was then refluxed for 2 h. The mixture was cooled to room temperature and washed with aqueous $NaHCO_3$ (2×100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and the drying reagent was filtered off. The filtrate was evaporated in vacuo and purified by column chromatography (SiO_2, AcOEt) to give the titled product as a pale yellow oil (12.2 g, 47%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.11 (m, 2H), 3.54 (q, $J=5.4$ Hz, 1H), 1.64 (m, 2H), 1.54 (broad, 2H), 1.32 (m, 9H), 0.89 (t, 6.7. 3H).

***N-tert*-Butoxycarbonyl-L-alanyl-L-alanine hexyl ester.** A solution of *N-tert*-butoxycarbonyl-L-alanine (3.78 g, 20 mmol) and CDI (3.41 g, 21 mmol) in dry CH_2Cl_2 (40 mL) was stirred at room temperature for 10 min. A solution of L-alanine hexyl ester (3.47 g, 20 mmol) and Et_3N (2.02 g, 20 mmol) in dry CH_2Cl_2 (20 mL) was added to the CDI solution and the reaction mixture was stirred at room temperature overnight. CH_2Cl_2 (100 mL) was added to the mixture which was then washed with 1N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give the desired product as a clear oil (6.65 g, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (broad, 1H), 5.11 (broad, 1H), 4.53 (m, 1H), 4.14 (m, 3H), 1.63 (m, 2H), 1.40 (m, 15H), 0.89 (t, 6.6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 172.4, 155.4, 79.9, 65.5, 49.8, 48.0, 31.3, 28.4, 28.3, 25.4, 22.5, 18.5, 18.2, 13.9; HR-MS *m/e* calcd for $C_{17}H_{32}N_2O_5$ 344.2314, found 344.2311.

L-Alanyl-L-alanine hexyl ester hydrochloride (HCl·5). To a solution of *N-tert*-butoxycarbonyl-L-alanyl-L-alanine hexyl ester (3.45 g, 10 mmol) in dry CH_2Cl_2 (20 mL) in an ice bath was added TFA (10 mL) and the mixture was stirred for 1 h in the ice bath. The reaction mixture was evaporated in vacuo to afford the TFA salt of the desired compound. The salt was treated with CH_2Cl_2 (120 mL) and washed with aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give the free amine of the desired compound. The free amine was dissolved in hexane and HCl gas was bubbled through. The solution was evaporated in vacuo to give the titled compound as a clear oil (2.54 g, 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (broad d, $J=5.4$ Hz, 1H), 8.18 (broad s, 2H), 4.48 (m, 2H), 4.09 (m, 2H), 1.62 (m, 5H), 1.46 (m, 3H), 1.29 (m, 6H), 0.88 (t, $J=6.6$ Hz, 3H).

***N-tert*-Butoxycarbonyl-L-alanyl-L-alanyl-L-alanine hexyl ester.** A solution of *N-tert*-butoxycarbonyl-L-alanine (1.14 g, 6.0 mmol) and CDI (1.07 g, 6.6 mmol) in dry CH_2Cl_2 (20 mL) was stirred at room temperature for 15 min. A solution of HCl·51 (1.40 g, 5.0 mmol) and Et_3N (0.61 g, 6.0 mmol) in dry CH_2Cl_2 (40 mL) was added to the CDI solution and the reaction mixture was stirred at room temperature overnight. CH_2Cl_2 (100 mL) was added to the mixture which was then washed with 1 N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give the desired product as a white solid (1.95 g, 94%): mp 148–150°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (broad, 2H), 5.31 (broad, 1H), 4.51 (m, 2H), 4.14 (m, 3H), 1.63 (m, 2H), 1.38 (m, 24H), 0.89 (t, $J=5.9$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 172.7, 171.9, 155.4, 79.8, 65.5, 50.0, 48.7, 48.1, 31.3, 28.4, 28.3, 25.4, 22.5, 18.8, 18.1, 13.9; HR-MS *m/e* calcd for $C_{16}H_{28}N_3O_5$ ($M^+-C_4H_9O$) 342.2034, found 342.2029.

L-Alanyl-L-alanyl-L-alanine hexyl ester (6). To a solution of *N-tert*-butoxycarbonyl-L-alanyl-L-alanyl-L-alanine hexyl ester (1.25 g, 3.0 mmol) in dry CH_2Cl_2 (10 mL) in an ice bath was added TFA (5 mL) and the mixture was stirred for 1 h in the ice bath. The reaction mixture was evaporated in vacuo to afford the TFA salt of the desired compound. The salt was treated with CH_2Cl_2 (100 mL) and washed with aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The evaporated residue was solidified from hexane to give the titled compound as a white powder (496 mg, 47%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (broad, 1H), 6.72 (broad, 1H), 4.49 (m, 2H), 4.14 (m, 2H), 3.57 (broad, 1H), 1.81 (broad, 2H), 1.64 (m, 2H), 1.39 (m, 15H), 0.89 (t, $J=6.7$ Hz, 3H).

Hx-Ant(Ala-Ala-OHx)-Ant-Ant(Ala-Ala-OHx)-Ant-OHx (7). A solution of **2**[18] (75 mg, 0.10 mmol) and oxalyl chloride (75 mg, 0.60 mmol) in dry THF (20 mL) was stirred at room temperature. DMF (0.025 mL) was added through a silica gel filter and the mixture was stirred at room temperature for 25 min. The reaction mixture was evaporated in vacuo to obtain the diacid chloride (102 mg). To a solution of HCl·5 (max. 82%, 273 mg, max. 0.80 mmol) and Et_3N (506 mg, 5.0 mmol) in dry THF (5 mL) was added a solution of the diacid chloride in dry THF (5 mL) and the mixture was stirred

at room temperature for 40 min. CH_2Cl_2 (50 mL) was added to the mixture which was then washed with 1 N HCl (50 mL), aqueous $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, evaporated in vacuo and solidified from $CHCl_3$ (16 mL) and AcOEt (80 mL) to obtain the desired product as a pale yellow solid (62 mg, 52%): m.p. 255–256°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.46 (s, 1H), 12.39 (s, 1H), 12.35 (s, 1H), 11.19 (s, 1H), 9.14 (s, 1H), 9.05 (d, J = 1.2 Hz, 1H), 8.79 (d, J = 8.4 Hz, 1H), 8.69 (d, J = 8.1 Hz, 1H), 8.11 (dd, J = 8.6, 1.1 Hz, 1H), 7.94 (m, 3H), 7.66 (d, J = 7.5 Hz, 2H), 7.59 (m, 2H), 7.28 (m, 3H), 6.93 (m, 2H), 4.83 (t, J = 7.2 Hz, 1H), 4.75 (t, J = 6.9 Hz, 1H), 4.58 (m, 2H), 4.35 (t, J = 6.6 Hz, 2H), 4.14 (m, 4H), 2.41 (t, J = 7.5 Hz, 2H), 1.82–1.25 (m, 46H), 0.8 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 172.7, 172.3, 171.7, 171.6, 168.6, 167.7, 167.0, 166.9, 166.4, 165.8, 140.7, 140.4, 140.0, 139.9, 138.2, 137.6, 134.7, 133.3, 131.1, 127.9, 127.7, 127.5, 124.1, 123.7, 123.4, 122.7, 122.6, 122.2, 121.7, 120.63, 120.56, 119.5, 116.1, 66.1, 65.7, 65.6, 49.4, 49.3, 48.4, 48.3, 38.4, 31.4, 29.7, 28.5, 25.7, 25.5, 25.1, 22.5, 22.4, 18.7, 18.24, 18.19, 17.8, 14.0; LR-MS (FAB, MNBA) m/e calcd for $C_{66}H_{88}N_6O_{14}Na$ ($M + Na^+$) 1239.6, found 1240.

Hx-Ant(Ala-Ala-OHx)-Ant(ODmh)-Ant(Ala-Ala-OHx)-Ant(ODmh)-ODmh (8). A solution of **4**[18] (58 mg, 0.050 mmol) and oxalyl chloride (135 mg, 0.12 mmol) in dry THF (5 mL) was stirred at room temperature. DMF (0.025 mL) was added through a silica gel filter and the mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated in vacuo to obtain the diacid chloride. A solution of HCl·**5** (112 mg, 0.40 mmol) and Et_3N (101 mg, 1.0 mmol) in dry CH_2Cl_2 (10 mL) was added to the diacid chloride and the mixture was stirred at room temperature for 2 h. CH_2Cl_2 (50 mL) was added to the mixture and which was then washed with 1N HCl (50 mL), saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to obtain the crude product. The crude product was purified by column chromatography (silica gel, 1:1 = AcOEt/hexane) to give the desired product as a yellow foam (154 mg, 80%): m.p. 147-148°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.51 (s, 1H), 12.45 (s, 1H), 12.22 (s, 1H), 11.25 (s, 1H), 9.41 (s, 1H), 9.36 (s, 1H), 9.15 (s, 1H), 9.08 (s, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.00 (m, 5H), 7.82 (m, 4H), 6.83 (d, J = 7.5 Hz, 1H), 6.75 (d, J = 7.5 Hz, 1H), 5.38 (m, 3H), 4.74 (m, 2H), 4.57 (m, 2H), 4.13 (m, 4H), 2.44 (m, 2H), 1.78–1.25 (m, 52H), 0.95–0.80 (m, 45H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 172.7, 172.3, 172.0, 167.7, 167.0, 166.9, 166.8, 166.4, 165.7, 165.1, 165.0, 140.8, 140.5, 140.0, 139.9, 138.5, 138.0, 136.1, 134.9, 131.0, 127.9, 127.8, 127.7, 124.8, 124.3, 123.5, 123.3, 123.2, 122.9, 122.4, 122.1, 121.8, 121.1, 119.9, 119.5, 74.0, 73.2, 72.9, 65.6, 49.5, 48.4, 44.0, 38.4, 31.4, 28.5, 25.5, 24.8, 23.2, 22.5, 18.0, 14.0; HR-MS (FAB, 3-NBA) m/e calcd for $C_{89}H_{130}N_6O_{16}Na$ ($M + Na^+$) 1621.9401, found 1621.9406.

Hx-Ant(Ala-Ala-Ala-OHx)-Ant(ODmh)-Ant(Ala-Ala-Ala-OHx)-Ant(ODmh)-ODmh (9). A solution of **4** (115 mg, 0.10 mmol) and oxalyl chloride (64 mg, 0.50 mmol) in dry THF (5 mL) was stirred at room temperature. DMF (0.025 mL) was added through a silica gel filter and the mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated in vacuo to obtain the diacid chloride. To a solution of **6** (88 mg, 0.25 mmol) and pyridine (79 mg, 1.0 mmol) in dry CH_2Cl_2 (2.5 mL) was added a solution of the diacid chloride in dry CH_2Cl_2 (2.5 mL) and the mixture was stirred at room temperature for 4 h. CH_2Cl_2 (30 mL) was added to the mixture and which was then washed with 1N HCl (30 mL), aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to obtain the crude product (161 mg). The crude product was purified by a column chromatography (SiO_2, 1/1 = THF/hexane) to give the desired product as a yellow foam (45 mg, 50%): mp 225–226°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.97 (s, 1H), 12.94 (s, 1H), 12.50 (s, 1H), 11.85 (s, 1H), 9.62 (s, 1H), 9.61 (s, 1H), 9.45 (s, 1H), 9.22 (s, 1H), 8.33 (m, 3H), 8.17 (m, 2H), 8.10 (d, J = 8.4 Hz, 1H), 7.98 (m, 2H), 7.87 (m, 2H), 7.33 (m, 2H), 6.69 (d, J = 7.2 Hz, 1H), 6.56 (d, J = 7.8 Hz, 1H), 5.40 (m, 3H), 5.26 (m, 1H), 5.08 (m, 1H), 4.79 (m, 1H), 4.64 (m, 1H), 4.50 (m, 2H), 4.17 (m, 4H), 2.5 (t, J = 7.5 Hz, 2H), 1.83–1.25 (m, 58H), 1.00–0.89 (m, 45H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 172.9, 172.8, 172.6, 171.5, 171.2, 167.7, 167.4, 167.0, 166.8, 165.2, 164.5, 141.1, 140.8, 140.7, 140.3, 138.4, 137.8, 136.1, 135.2, 131.1, 128.2, 127.6, 124.6, 124.4, 123.5, 123.0, 122.5, 121.9, 119.6, 119.1, 74.1, 73.2, 72.9, 66.2, 65.7, 49.2, 49.0, 48.9, 48.7, 48.2, 47.8, 44.1, 44.0, 43.8, 38.6, 31.4, 28.5, 25.2, 24.9, 24.8, 23.3, 23.1, 22.6, 20.1, 19.9, 19.4, 18.7, 18.4, 17.8, 14.0; HR-MS (FAB, 3-NBA) m/e calcd for $C_{95}H_{140}N_{10}O_{20}Na$ ($M + Na^+$) 1764.0143, found 1764.0074.

Hexanoyl-L-alanyl-L-alanine benzyl ester (13). A solution of hexanoyl-L-alanine (5.62 g, 30 mmol) and CDI (5.11 g, 32 mmol) in dry CH_2Cl_2 (50 ml) was stirred at room temperature for 30 min. A solution of L-alanine benzyl ester hydrochloride (6.80 g, 32 mmol) and Et_3N (3.19 g, 32 mmol) in dry CH_2Cl_2 (150 mL) was added to the previous solution and the reaction mixture was stirred at room temperature overnight. CH_2Cl_2 (100 mL) was added to the mixture which was then washed with 1 N HCl (150 mL), saturated $NaHCO_3$ (150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography (silica gel, 1:1 = AcOEt/CH_2Cl_2) and then recrystallized from AcOEt/hexane to give the desired product as a white cotton (4.06 g, 39%). Mp 130–131°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 5H), 6.56 (d, J = 7.2 Hz, 1H), 6.01 (d, J = 7.5 Hz, 1H), 5.18 (m, 2H), 4.59 (m, 1H), 4.49 (m, 1H), 2.19 (t, J = 6.2 Hz, 2H), 1.60 (m, 2H), 1.42 (d, J = 7.2 Hz, 3H), 1.36 (d, J = 7.0 Hz, 3H), 1.30 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 172.5, 172.4, 135.4, 128.6, 128.4, 128.1, 67.0, 48.5, 48.2, 36.4, 31.4, 25.3, 22.4, 18.8, 17.7, 13.9; HS-MS m/e calcd for $C_{19}H_{28}N_2O_4$ 348.2049, found 348.2059.

Hexanoyl-L-alanyl-L-alanine (12). A solution of **13** (3.49 g, 10 mmol) and 10% Pd/C (350 mg) in dry THF (50 mL) was prepared in a 250-mL round-bottomed

flask provided with a magnetic stirrer. H_2 was introduced after removal of air by an aspirator and the solution was stirred vigorously for 2 h at room temperature. The catalyst was removed by filtration through Celite. The filtrate was evaporated in vacuo to give the crude product. The crude product was recrystallized from AcOEt/hexane to give the desired product as a white powder (1.96 g, 76%): mp 147–148 °; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 7.1 Hz, 1H), 6.53 (d, J = 7.6 Hz, 1H), 4.64 (m, 2H), 4.54 (m, 2H), 2.22 (t, J = 7.5 Hz, 2H), 1.62 (m, 2H), 1.45 (d, J = 7.1 Hz, 3H), 1.37 (d, J = 7.0 Hz, 3H), 1.30 (m, 4H), 0.89 (t, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.0, 172.3, 172.0, 47.6, 47.4, 35.1, 30.9, 24.9, 21.9, 18.2, 17.2, 13.9; MS m/e calcd for $C_{12}H_{22}N_2O_4$ 259.1658, found 259.1665.

Dilution experiment with 8

A representative dilution experiment was as follows: compound **8** (128 mg, 0.08 mmol) was dissolved in 0.4 mL of $CDCl_3$ to give a solution of **8** at 200 mM and the ^{1}H NMR of the solution was measured at room temperature. Sequential dilution of the solution with $CDCl_3$ gave solutions of **8** at 100, 50, 20, 10, 5, 2, 1, 0.5 mM and the ^{1}H NMR of each solution was measured. The same procedure was repeated and ^{1}H NMR spectra were measured at −20 °C. Dimerization constants were calculated by Saunders and Hyne's model[19] using KaleidaGraph.

Titration Experiment of 7 with 12

A representative dilution experiment was as follows: compound **7** (12 mg, 0.01 mmol) was dissolved in 10 mL of $CDCl_3$ to give a solution of **7** at 10 mM (solution A). Compound **12** (13 mg, 0.05 mmol) was dissolved in 5 mL of solution A (solution B, 10 mM of **7** and 100 mM of **12**). After various ratios of solution A and solution B were mixed (including 100% solution A and 100% solution B), ^{1}H NMR spectra of the resulting solutions were measured at room temperature. The resulting binding isotherm was fit to a 1:1 binding model using KaleidaGraph.

Acknowledgements

The authors thank the National Science Foundation for financial support of this work. We also thank the Kureha Chemical Industry for a fellowship to Y.H.

References and Notes

1. Tanatani, A.; Mio, M. J.; Moore, J. S. *J. Am. Chem. Soc.* **2001**, *123*, 1792 and references therein.
2. Louis, A.; Lehn, J.-M.; Homo, J.-C.; Schmutz, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 233.
3. Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173.
4. For a discussion of the role of intramolecular hydrogen bonding in a family of simple diamides, see: Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228.
5. Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574.
6. Seebach, D.; Abele, S.; Siffereln, T.; Hänggi, M.; Gruner, S.; Seiler, P. *Helv. Chem. Acta.* **1998**, *81*, 2218.
7. Nowick, J. S.; Muna, A.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Amer. Chem. Soc.* **1995**, *117*, 89.
8. Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1996**, *118*, 7529. Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1997**, *119*, 10587.
9. Liskamp, R. M. *J. Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 633.
10. (a) For recent reviews, see: Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, 401. (b) Schneider, J. P.; Kelly, J. W. *Chem. Rev.* **1995**, *95*, 2169.
11. Brandmeier, V.; Sauer, W. H. B.; Feigel, M. *Helv. Chim. Acta* **1994**, *77*, 70.
12. Kemp, D. S.; Li, Z. Q. *Tetrahedron Lett.* **1995**, *36*, 4179.
13. Schneider, J. P.; Kelly, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 2533.
14. Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Amer. Chem. Soc.* **1996**, *118*, 1066.
15. Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280.
16. Kobayashi, K.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 95.
17. LaBrenz, S. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 1655.
18. For the syntheses of monomers and scaffolds **1**–**4**, see ref 8.
19. Determined using the method of Saunders and Hyne. Saunders, M.; Hyne, J. B. *J. Chem. Phys.* **1958**, *20*, 1319.
20. For other examples of peptide based gelators see; Estroff, L.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447 and references therein.
21. Pant, N.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 2002.
22. Williams, D. H. *J. Am. Chem. Soc.* **1981**, *1037*, 6580.
23. For other approches to dipeptide carboxylate recognition through sheet hydrogen bonding see; Weiner, W. S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 681. Dowden, J.; Edwards, P. D.; Kilburn, J. D. *Tetrahedron Lett.* **1997**, *38*, 1095. Pan, Z.; Still, W. C. *Tetrahedron Lett.* **1996**, *37*, 8699. Pieters, R. J.; Diederich, F. *J. Chem. Soc., Chem. Commun.* **1996**, 2255.
24. Association constant analysis was carried out using KaleidaGraph ver 2.1.