Sequence-Selective Recognition

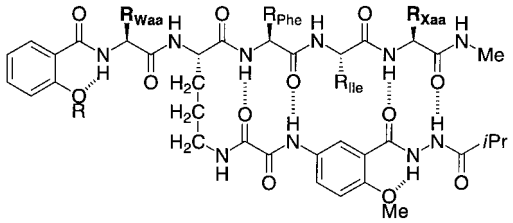
Sequence-Selective Molecular Recognition between β Sheets**

James S. Nowick and De Michael Chung*

Dedicated to Professor Larry Overman on the occasion of his 60th birthday

Interactions between the hydrogen-bonding edges of β sheets occur widely in protein quaternary structure, protein–protein interactions, and protein aggregation and are involved in both healthy biological processes and in diseases ranging from cancer and AIDS to anthrax and Alzheimer's.[1,2] These protein–protein interactions constitute a form of molecular recognition that deserves more attention, both because of its importance in biological processes and because of its fundamental nature. This paper asks whether interactions between β-sheet-forming peptides can be sequence selective and finds that patterning valine and threonine residues across the non-hydrogen-bonded rings formed at the interface between antiparallel β sheets can impart dramatic sequence selectivity.[3]

We had previously reported that peptides such as **1a**, which contain the amino acid Orn(*i*PrCO-Hao), fold into β-



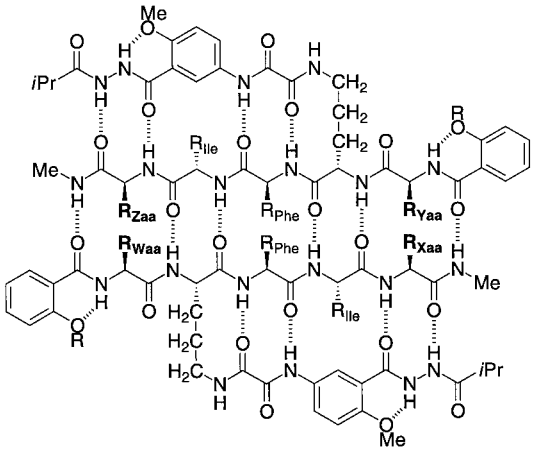
o-MeO-C_6H_4CO-Val-Orn(*i*PrCO-Hao)-Phe-Ile-Leu-NHMe (**1a**)
o-BuO-C_6H_4CO-**Thr**-Orn(*i*PrCO-Hao)-Phe-Ile-**Val**-NHMe (**1b**, **Thr-Val**)
o-BuO-C_6H_4CO-**Val**-Orn(*i*PrCO-Hao)-Phe-Ile-**Thr**-NHMe (**1c**, **Val-Thr**)
o-BuO-C_6H_4CO-**Thr**-Orn(*i*PrCO-Hao)-Phe-Ile-**Thr**-NHMe (**1d**, **Thr-Thr**)
o-BuO-C_6H_4CO-**Val**-Orn(*i*PrCO-Hao)-Phe-Ile-**Val**-NHMe (**1e**, **Val-Val**)

sheetlike structures that dimerize through β-sheet interactions in organic solvents (Scheme 1).[4] In the present study, we chose to replace the valine and leucine residues of **1a** with threonine and valine in all possible orders: Thr–Val (**1b**), Val–Thr (**1c**), Thr–Thr (**1d**), and Val–Val (**1e**). We selected these residues with the expectation that Val would pair preferentially with Val, and that Thr would pair preferentially with Thr, through self-complementary noncovalent interactions

[*] Prof. J. S. Nowick, D. M. Chung
Department of Chemistry
University of California, Irvine
Irvine, CA 92697-2025 (USA)
Fax: (+1) 949-824-9920
E-mail: jsnowick@uci.edu

[**] We thank the NIH for grant support (GM-49076). D.M.C. thanks the Bill and Melinda Gates Foundation and the UCI Institute for Brain Aging and Dementia for a fellowship and training grant support (NIA-5T32AG000096).

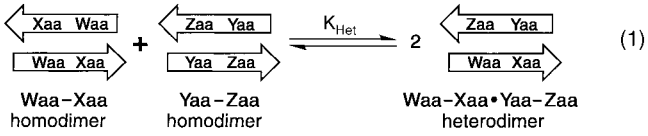
 DOI: 10.1002/anie.200250750



Scheme 1. The β-sheet dimer of peptides **1**.

and with the knowledge that these pairings frequently occur in the non-hydrogen-bonded rings of antiparallel β sheets.[5]

To evaluate the sequence selectivity of molecular recognition between threonine- and valine-containing β-sheet peptides **1b–e**, we measured the propensity of these peptides to form heterodimers. Mixing solutions of (homodimeric) peptides ArCO-**Waa**-Orn(*i*PrCO-Hao)-Phe-Ile-**Xaa**-NHMe (**Waa–Xaa**) and ArCO-**Yaa**-Orn(*i*PrCO-Hao)-Phe-Ile-**Zaa**-NHMe (**Yaa–Zaa**) gives an equilibrium mixture of homodimers [(**Waa–Xaa**)$_2$ and (**Yaa–Zaa**)$_2$] and heterodimer [(**Waa–Xaa**)·(**Yaa–Zaa**)] [Eq. (1)]. A heterodimeric equilibrium constant K_{Het} can be defined as [(**Waa–Xaa**)·(**Yaa–Zaa**)]^2/[(**Waa–Xaa**)$_2$][(**Yaa–Zaa**)$_2$]. If the equilibrium is statistical, $K_{Het} = 4$; if the heterodimer forms preferentially, $K_{Het} > 4$; if the homodimers are preferred, $K_{Het} < 4$. The quantity $K_{Het}/4$ provides a convenient gauge of the dimerization preference, with a value of 1.0 indicating no preference.



(1)

^{1}H NMR spectroscopic studies readily permit the direct measurement of K_{Het}. Chemical exchange between the homo- and heterodimers occurs slowly on the NMR time scale at ambient and subambient temperatures in $CDCl_3$ or in [D_6]DMSO/$CDCl_3$. The hydrazide and anilide NH resonance signals of the Orn(*i*PrCO-Hao) group are well dispersed and allow the various species present to be quantified accurately. Each homodimer presents two doublets associated with the hydrazide NH resonance signals in the $\delta = 11$–12 ppm region of the spectrum and a singlet near $\delta = 10$ ppm associated with the anilide NH resonance signal; the heterodimer presents four doublets and two singlets. Integration of these peaks allows the determination of the ratios of the species; deconvolution of the spectra by fitting the peaks with Lorentzian functions provides even greater accuracy. The spectra shown in Figure 1 and 2 are representative of these studies.

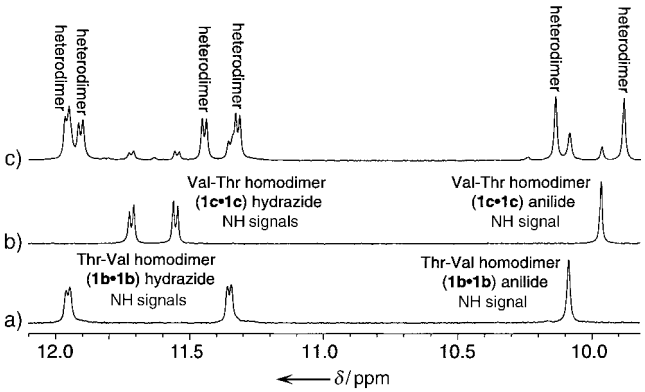


Figure 1. ^{1}H NMR spectra of the hydrazide and anilide NH groups of Thr–Val peptide **1b** (a), Val–Thr peptide **1c** (b), and a mixture of the two peptides (c). The *heterodimer* predominates in the mixture. Spectra were recorded at 500 MHz and 253 K in 10% [D_6]DMSO in $CDCl_3$ at a) 2.0 mM **1b**, b) 2.0 mM **1c** , and c) 2.1 mM **1b** and 1.5 mM **1c**.

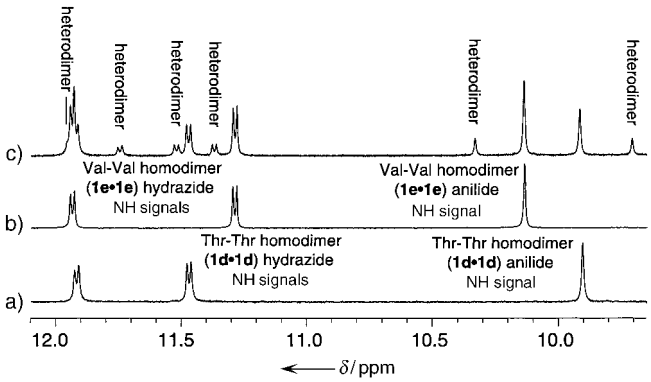


Figure 2. ^{1}H NMR spectra of the hydrazide and anilide NH groups of Thr–Thr peptide **1d** (a), Val–Val peptide **1e** (b), and a mixture of the two peptides (c). The *homodimers* predominate in the mixture. Spectra were recorded at 500 MHz and 253 K in 10% [D_6]DMSO in $CDCl_3$ at a) 2.0 mM **1d**, b) 2.0 mM **1e**, and c) 1.5 mM **1d** and 1.7 mM **1e**.

Analysis of spectra collected in 10% [D_6]DMSO in $CDCl_3$ (Figure 1 and 2) reveals a strong preference for Thr–Val (**1b**) and Val–Thr (**1c**) to form a heterodimer ($K_{Het}/4 = 8.9$) and for Thr–Thr (**1d**) and Val–Val (**1e**) to remain as homodimers ($K_{Het}/4 = 0.083$).[6] In contrast with these results, controls involving all four other possible combinations of peptides **1b–e** show little or no preferences for forming either homo- or heterodimers ($K_{Het}/4 = 0.8$–1.0; Table 1). These results confirm the hypothesis that pairing of like residues is preferred, Thr with Thr, and Val with Val. Calculation of free energies based on these K_{Het} values reveals that a Thr–Thr pair and a Val–Val pair collectively are 0.6 kcal mol^{-1} more stable than two Thr–Val pairs, under the conditions of these experiments.

The [D_6]DMSO cosolvent is essential for sequence selectivity. When pure $CDCl_3$ is used for these studies, no

Table 1: Propensities of peptides **1b–e** to form heterodimers.

	1b&1c	**1d&1e**	**1b&1d**	**1b&1e**	**1c&1d**	**1c&1e**
$K_{Het}/4$	8.9	0.083	0.87	0.80	1.0	1.0

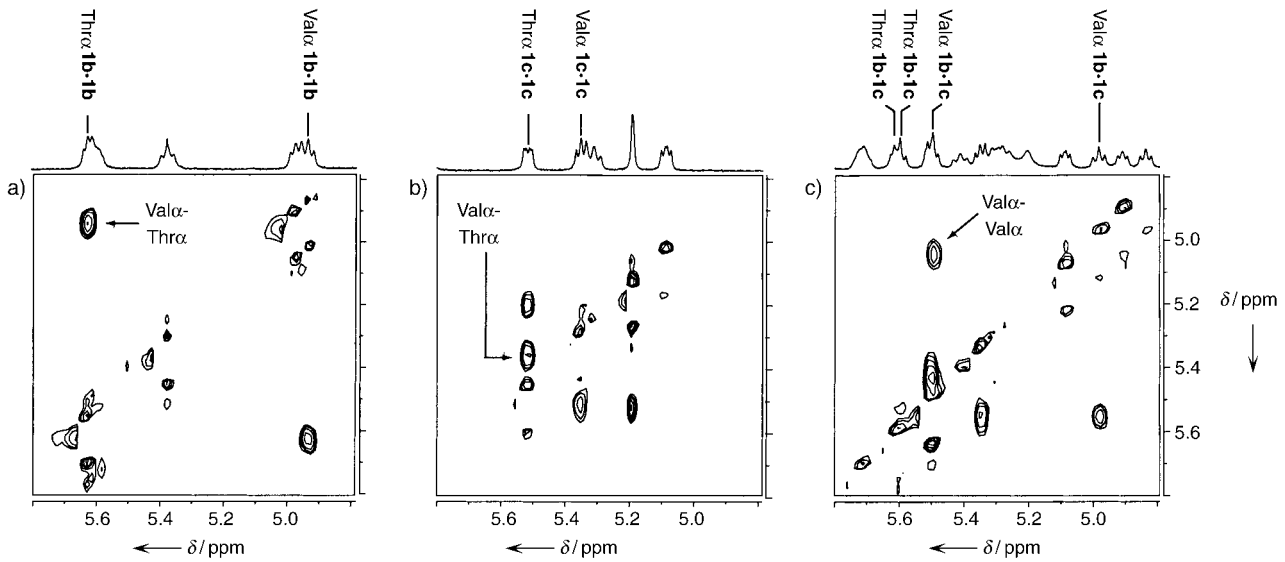


Figure 3. ^{1}H NMR Tr-ROESY spectra of the α-proton region of Thr–Val peptide **1b** (a), Val–Thr peptide **1c** (b), and a mixture of the two peptides (c), illustrating intermolecular NOEs associated with the respective homodimers and the heterodimer. Spectra were recorded at 500 MHz and 253 K with a 250 ms mixing time in 10% [D_6]DMSO in $CDCl_3$ at a) 2.0 mM **1b**, b) 2.0 mM **1c**, and c) 1.4 mM **1b** and 2.6 mM **1c**.

significant selectivity is observed. Selectivity emerges and increases as [D_6]DMSO is added. At 3.9% [D_6]DMSO, for example, $K_{Het}/4 = 2.7$ for Thr–Val (**1b**) and Val–Thr (**1c**) and 0.28 for Thr–Thr (**1d**) and Val–Val (**1e**).[7] The DMSO cosolvent may effect selectivity by promoting interaction between Val side chains (e.g., through solvophobic interactions), destabilizing interactions between Val and Thr side chains, or enhancing hydrogen-bonding interactions between the Thr side chains. In light of the known interactions between DMSO and water dimers, the latter explanation appears to be most likely.[8]

^{1}H NMR transverse-ROESY (Tr-ROESY)[9] studies (Figure 3) corroborate that the peptides form well-defined homo- and heterodimers. Strong NOEs between the Thr and Val α protons in the Tr-ROESY spectra of unmixed **1b** and **1c**, similar to those we reported previously for peptide **1a**,[4] help establish the formation of homodimers. A strong NOE between the two different Val α protons of the new species formed upon mixing **1b** and **1c** confirms the formation of the heterodimer.[10]

The experiments described above establish that sequence-selective molecular recognition of β sheets may be achieved through side chain interactions. The measurement of equilibrium constants by these experiments is appealing, because it provides the same information about side chain interaction energies as a double-mutant cycle experiment, but does so directly in a single measurement.[11,12] The peptide model system developed here is arguably simpler and better defined than other related model systems.[13,14] We envision modifying this system to generate peptides that recognize protein β sheets in a sequence-selective fashion.

Received: December 11, 2002 [Z50750]

Keywords: β sheets · molecular recognition · peptides · proteins · supramolecular chemistry

[1] S. Maitra, J. S. Nowick in *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science* (Eds.: A. Greenberg, C. M. Breneman, J. F. Liebman), Wiley, New York, **2000**, pp. 495–518.

[2] C. Petosa, R. J. Collier, K. R. Klimpel, S. H. Leppla, R. C. Liddington, *Nature* **1997**, *385*, 833–838.

[3] For related observations in proteins associated with genetic diseases, see: a) P. Hammarström, F. Schneider, J. W. Kelly, *Science* **2001**, *293*, 2459–2462; b) E. G. Huizinga, S. Tsuji, R. A. P. Romijn, M. E. Schiphorst, P. G. de Groot, J. J. Sixma, P. Gros, *Science* **2002**, *297*, 1176–1179.

[4] J. S. Nowick, K. S. Lam, T. V. Khasanova, W. E. Kemnitzer, S. Maitra, H. T. Mee, R. Liu, *J. Am. Chem. Soc.* **2002**, *124*, 4972–4973.

[5] a) S. Lifson, C. Sander, *J. Mol. Biol.* **1980**, *139*, 627–639; b) M. A. Wouters, P. M. G. Curmi, *Proteins Struct. Funct. Genet.* **1995**, *22*, 119–131; c) E. G. Hutchinson, R. B. Sessions, J. M. Thornton, D. N. Woolfson, *Protein Sci.* **1998**, *7*, 2287–2300; d) Y. Mandel-Gutfreund, S. M. Zaremba, L. M. Gregoret, *J. Mol. Biol.* **2001**, *305*, 1145–1159.

[6] Studies were performed at 253 K. Although the resonance signals are resolved in the 500 MHz ^{1}H NMR spectrum at 298 K, they are sharper at lower temperatures. Measurement of the relative concentrations of the homo- and heterodimers at varying ratios of peptides verifies that K_{Het} is invariant and supports its use to gauge the preferences of amino acid pairings.

[7] Further addition of [D_6]DMSO beyond 10% results in additional increases in selectivity, however shimming of the NMR instrument becomes difficult at higher [D_6]DMSO concentrations. In pure [D_6]DMSO, dimers do not form.

[8] B. Kirchner, M. Reiher, *J. Am. Chem. Soc.* **2002**, *124*, 6206–6215, and references therein.

[9] a) T.-L. Hwang, A. J. Shaka, *J. Am. Chem. Soc.* **1992**, *114*, 3157–3159; b) T.-L. Hwang, A. J. Shaka, *J. Magn. Reson. Ser. B* **1993**, *102*, 155–165.

[10] NOEs between the Thr α-protons of the heterodimer could not be observed because the resonance signals of these protons are nearly coincident.
[11] a) C. K. Smith, L. Regan, *Science* **1995**, *270*, 980 – 982; b) C. A. Blasie, J. M. Berg, *Biochemistry* **1997**, *36*, 6218 – 6222.
[12] A. Horovitz, *Folding Des.* **1996**, *1*, R121-R126.
[13] M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479 – 2494.
[14] For some leading examples of related work involving peptide recognition, see: a) S. R. LaBrenz, J. W. Kelly, *J. Am. Chem. Soc.* **1995**, *117*, 1655 – 1656; b) Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813 – 1814; c) X. Cha, K. Ariga, T. Kunitake, *J. Am. Chem. Soc.* **1996**, *118*, 9545 – 9551; d) R. Zutshi, J. Franciskovich, M. Shultz, B. Schweitzer, P. Bishop, M. Wilson, J. Chmielewski, *J. Am. Chem. Soc.* **1997**, *119*, 4841 – 4845; e) C. N. Kirsten, T. H. Schrader, *J. Am. Chem. Soc.* **1997**, *119*, 12061 – 12068; f) T. D. Clark, J. M. Buriak, K. Kobayashi, M. P. Isler, D. E. McRee, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, *120*, 8949 – 8962; g) M. A. Hossain, H.-J. Schneider, *J. Am. Chem. Soc.* **1998**, *120*, 11208 – 11209; h) B. Baumeister, S. Matile, *Chem. Eur. J.* **2000**, *6*, 1739 – 1749; i) Y. Takahashi, A. Ueno, H. Mihara, *ChemBioChem* **2001**, *2*, 75 – 79; j) G. Xu, W. Wang, J. T. Groves, M. H. Hecht, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3652 – 3657; k) A. Cox, M. M. Arroyo, K. H. Mayo, *Biochem. J.* **2001**, *357*, 739 – 747; l) Y. Hamuro, A. D. Hamilton, *Bioorg. Med. Chem.* **2001**, *9*, 2355 – 2363; m) H. Wennemers, M. Conza, M. Nold, P. Krattiger, *Chem. Eur. J.* **2001**, *7*, 3342 – 3347; n) S. T. Phillips, M. Rezac, U. Abel, M. Kossenjans, P. A. Bartlett, *J. Am. Chem. Soc.* **2002**, *124*, 58 – 66; o) H. Zeng, X. Yang, R. A. Flowers, B. Gong, *J. Am. Chem. Soc.* **2002**, *124*, 2903 – 2910; p) K. B. Jensen, T. M. Braxmeier, M. Demarcus, J. G. Frey, J. D. Kilburn, *Chem. Eur. J.* **2002**, *8*, 1300 – 1309.

