Solid-Phase Synthesis of Artificial β-Sheets

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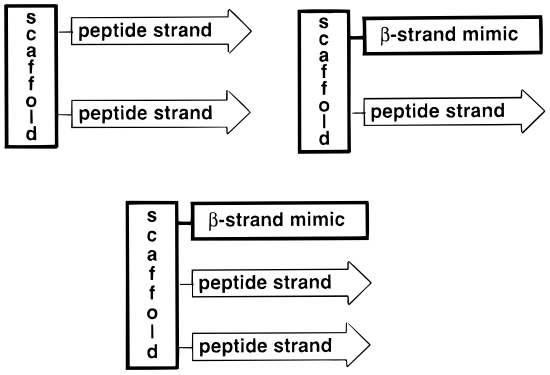
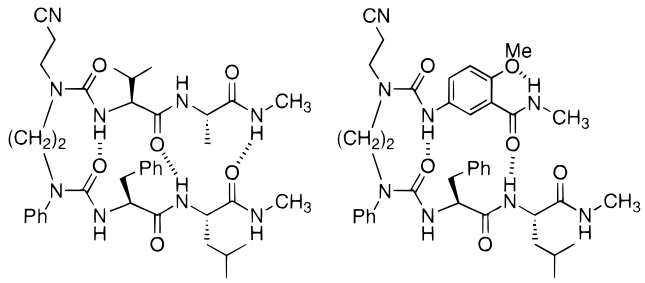
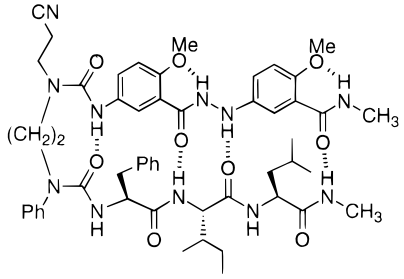
Abstract: This paper describes the solid-phase syntheses of artificial β-sheets **1**−**4**, which mimic the structure and hydrogen-bonding patterns of protein β-sheets. In these compounds, molecular templates induce β-sheet structures in attached peptide strands. The templates consist of di- and triurea derivatives, which hold peptide and peptidomimetic strands in proximity, and β-strand mimics, which hydrogen bond to the peptide strands. The syntheses involve constructing the "lower" peptide strand on Merrifield resin, attaching the di- or triamine portions of the di- or triurea templates, connecting the "upper" peptide and peptidomimetic strands, and cleaving the resulting artificial β-sheets from the resin. The artificial β-sheets were prepared in 8−13 steps from leucine Merrifield in 33−67% overall yield.

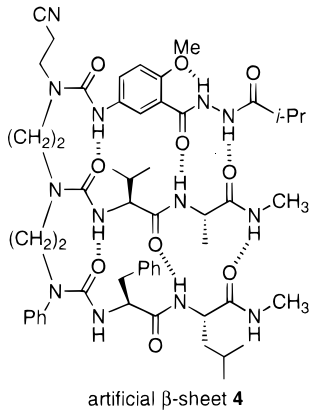
Compounds that mimic the structure and hydrogen-bonding patterns of protein β-sheets are of interest as drug candidates and as model systems with which to study protein structure and stability.[1] We have termed these compounds *artificial β-sheets*, and we are synthesizing and studying artificial β-sheets of increasing size and complexity with the goals of learning about β-sheet structure and developing biologically active peptidomimetic compounds. Our artificial β-sheets consist of an oligourea *molecular scaffold* and attached peptide and peptidomimetic strands. The molecular scaffold holds the strands in proximity, promoting the formation of a hydrogen-bonded β-sheet structure. Some of the structures incorporate a rigid β-strand mimic, which hydrogen bonds to the adjacent peptide strands and further stabilizes the β-sheet structure. The following cartoons illustrate these structures.



We recently reported solution-phase syntheses and structural studies of artificial β-sheets **1**−**3**.[1b,2] In artificial β-sheet **1**, a diurea template links two peptide strands and induces an intramolecularly hydrogen-bonded parallel β-sheet conformation.[2a] Artificial β-sheet **2** mimics an antiparallel β-sheet structure and incorporates both a diurea template and a β-strand mimic.[2b] In artificial β-sheet **3**, the β-strand mimic and peptide strands are elongated.[2c] Artificial β-sheet **4** contains a triurea template, a β-strand mimic, and two peptide strands.[3] In this paper, we report efficient procedures for the solid-phase syntheses of these compounds using Merrifield resin.







Results

Artificial β-sheets **1**−**4** were synthesized by constructing the "lower" peptide strand on Merrifield resin, attaching the di- or

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(1) (a) Schneider, J. P.; Kelly, J. W. *Chem. Rev.* **1995**, *95*, 2169. (b) Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, *25*, 401. (c) Service, R. F. *Science* **1996**, *274*, 34.

(2) (a) Nowick, J. S.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1995**, *60*, 7386. (b) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. *J. Am. Chem. Soc.* **1996**, *118*, 2764. (c) Nowick, J. S.; Pairish, M.; Lee, I. Q.; Holmes, D. L.; Ziller, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 5413.

(3) Structural studies of artificial β-sheet **4** will be described in a forthcoming paper. Studies of a closely related artificial β-sheet comprising a triurea template, a β-strand mimic, and two peptide strands are described in ref 1b.

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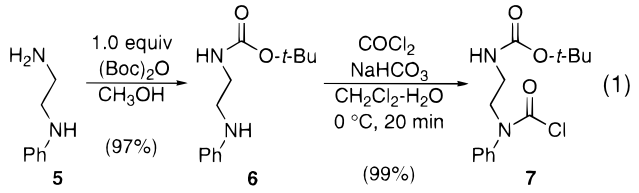
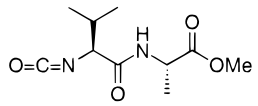
triamine portions of the di- or triurea templates, connecting the "upper" peptide and peptidomimetic strands, and cleaving the resulting artificial β-sheets from the resin. The compounds were prepared in 0.4−0.6 g batches starting with 1.5 g (1.2 mmol) of *tert*-butoxycarbonyl (Boc)-leucine Merrifield resin. The syntheses involve 8−13 steps and proceeded in 33−67% overall yield.

Artificial β-Sheet 1. Artificial β-sheet **1** was prepared by constructing the lower dipeptide on the solid support, adding the diamine portion of the diurea template as carbamoyl chloride **7**, and connecting the upper dipeptide strand as the corresponding peptide isocyanate. Carbamoyl chloride **7** was prepared from *N*-phenylethylenediamine (**5**), as shown in eq 1. Treatment



(1)

of *N*-phenylethylenediamine with 1.0 equiv of di-*tert*-butyl dicarbonate afforded Boc-protected amine **6**. Amine **6** was converted to carbamoyl chloride **7** using modified Schotten−Baumann conditions.[4] These conditions involve addition of a solution of phosgene in toluene to a solution of amine **6** in a stirred, ice-cooled, biphasic mixture of methylene chloride and saturated aqueous sodium bicarbonate solution. This procedure is exceptionally convenient; the carbamoyl chloride is generated in near quantitative yield and good purity and is used without further purification. The top peptide strand was introduced as a peptide isocyanate, valylalanine methyl ester isocyanate.

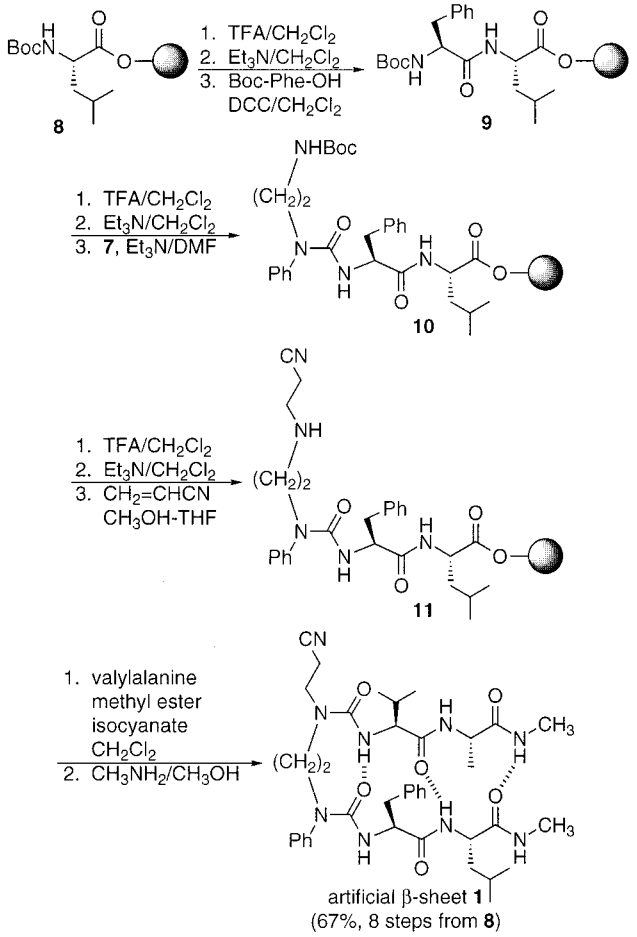


valylalanine methyl ester isocyanate

Peptide isocyanates are readily prepared by treatment of peptide hydrochloride salts with a solution of phosgene in toluene, using similar modified Schotten−Baumann conditions.[4] Peptide isocyanates react cleanly and in high yield with amines to form ureas, making these isocyanates particularly attractive building blocks for the construction of peptide derivatives and peptidomimetic compounds.[4]

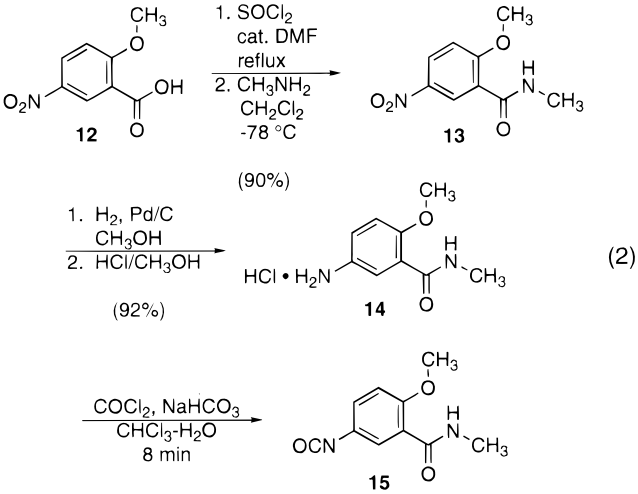
Scheme 1 illustrates the synthesis of artificial β-sheet **1**. Boc-leucine Merrifield resin (**8**) was homologated to the corresponding Boc-phenylalanylleucine dipeptide (**9**) by standard solid-phase peptide synthesis techniques.[5] The Boc protective group was removed by treatment with trifluoroacetic acid (TFA), the free amino group was then liberated by treatment with triethylamine (TEA), and the amino group was coupled with carbamoyl chloride **7** in the presence of TEA. Because carbamoyl chlorides are less reactive than most activated carboxylic acid derivatives, the reaction of carbamoyl chloride **7** with resin-bound peptides requires prolonged (e.g., 20 h) reaction times at ambient temperature or heating (e.g., 50 °C for 2 h). The resulting intermediate (**10**) was treated with TFA to remove the Boc protective group and TEA to liberate the amino group. Michael addition of the amino group to acrylonitrile proceeds smoothly to afford intermediate **11** when a 3:1:1 mixture of tetrahydrofuran (THF), methanol, and acrylonitrile is used as solvent. This solvent swells the resin and gives complete reaction within ca.

Scheme 1



12−15 h. Under these reaction conditions, the Michael addition proceeds to completion cleanly, with little addition of a second equivalent of acrylonitrile.[6] Reaction of amine **11** with valylalanine methyl ester isocyanate, followed by aminolysis with methylamine, affords artificial β-sheet **1**. The aminolysis step cleaves both the alanine methyl ester group and the leucine linkage to the resin, generating both methyl amide groups of **1**. Chromatographic purification afforded artificial β-sheet **1** in 67% overall yield.

Artificial β-Sheet 2. Artificial β-sheet **2** contains a β-strand mimic in place of the valylalanine peptide strand that is present in **1** and was prepared in an analogous fashion. The β-strand mimic was introduced as isocyanate **15**, which was prepared from 5-nitro-2-methoxybenzoic acid[2c,7] (**12**), as shown in eq 2.

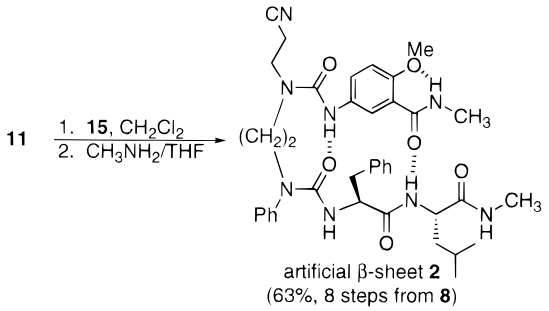


(2)

(4) Nowick, J. S.; Holmes, D. L.; Noronha, G.; Smith, E. M.; Nguyen, T. M.; Huang, S.-L. *J. Org. Chem.* **1996**, *61*, 3929.

(5) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Company: Rockford, IL, 1984.

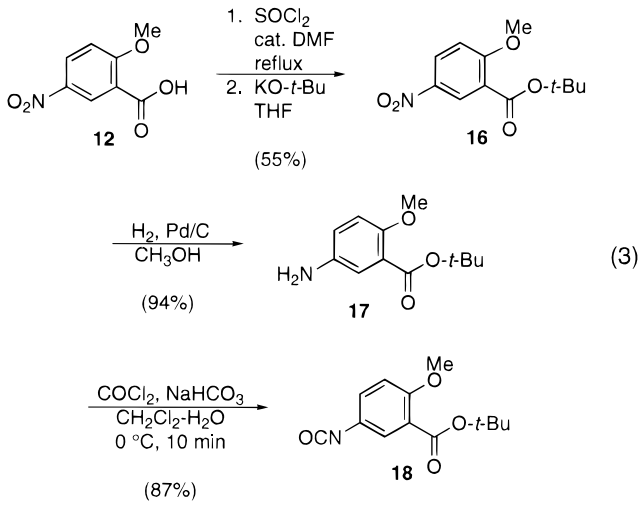
Scheme 2



Carboxylic acid **12** was converted to the corresponding methylamide (**13**) by successive treatment with thionyl chloride and methylamine. Reduction of the nitro group of **13** afforded *N*-methyl-5-amino-2-methoxybenzamide, which darkened rapidly in air and was stored and handled as its amine hydrochloride salt (**14**). Amine hydrochloride **14** was converted to the corresponding isocyanate **15** using modified Schotten−Baumann conditions; chloroform was used as a solvent, instead of methylene chloride, to minimize the formation of emulsions.

Treatment of resin-bound amine **11** with isocyanate **15**, followed by aminolysis with methylamine, generated artificial β-sheet **2** (Scheme 2). Chromatography of the crude aminolysis product afforded pure **2** in 63% yield from Boc-leucine Merrifield resin.

Artificial β-Sheet 3. Artificial β-sheet **3** comprises a peptide strand and a β-strand mimic attached to a diurea molecular scaffold. The peptide strand and β-strand mimic of **3** are longer than those of **2**: the peptide is a tripeptide instead of a dipeptide, and the β-strand mimic is composed of two aromatic rings rather than one. The synthesis of **3** is similar to that of **2**, with the exception that the β-strand mimic is assembled from two halves on the solid support. The first half is introduced as isocyanate **18**, which was prepared as shown in eq 3. Carboxylic acid **12**

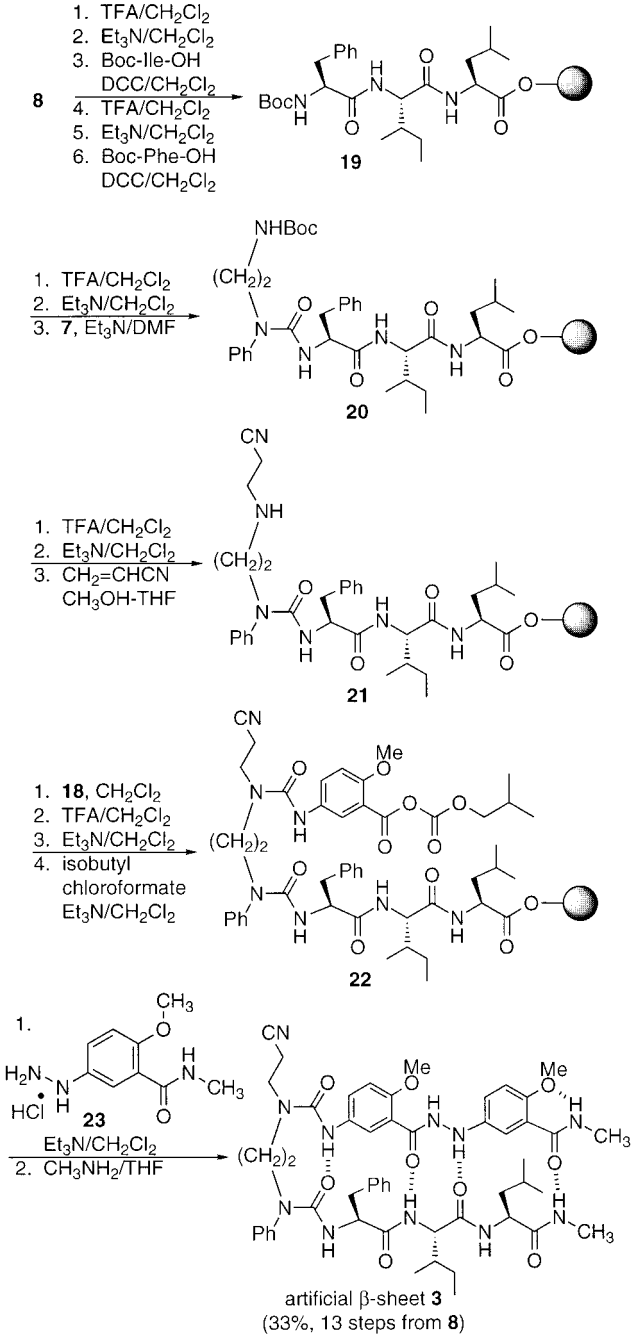


(3)

was converted to *tert*-butyl ester **16** by successive treatment with thionyl chloride and potassium *tert*-butoxide. Reduction of the nitro group generated amine **17**, which was converted to the isocyanate using modified Schotten−Baumann conditions.

The isocyanate was used in the preparation of artificial β-sheet **3**, as shown in Scheme 3. Boc-leucine Merrifield resin (**8**) was

Scheme 3

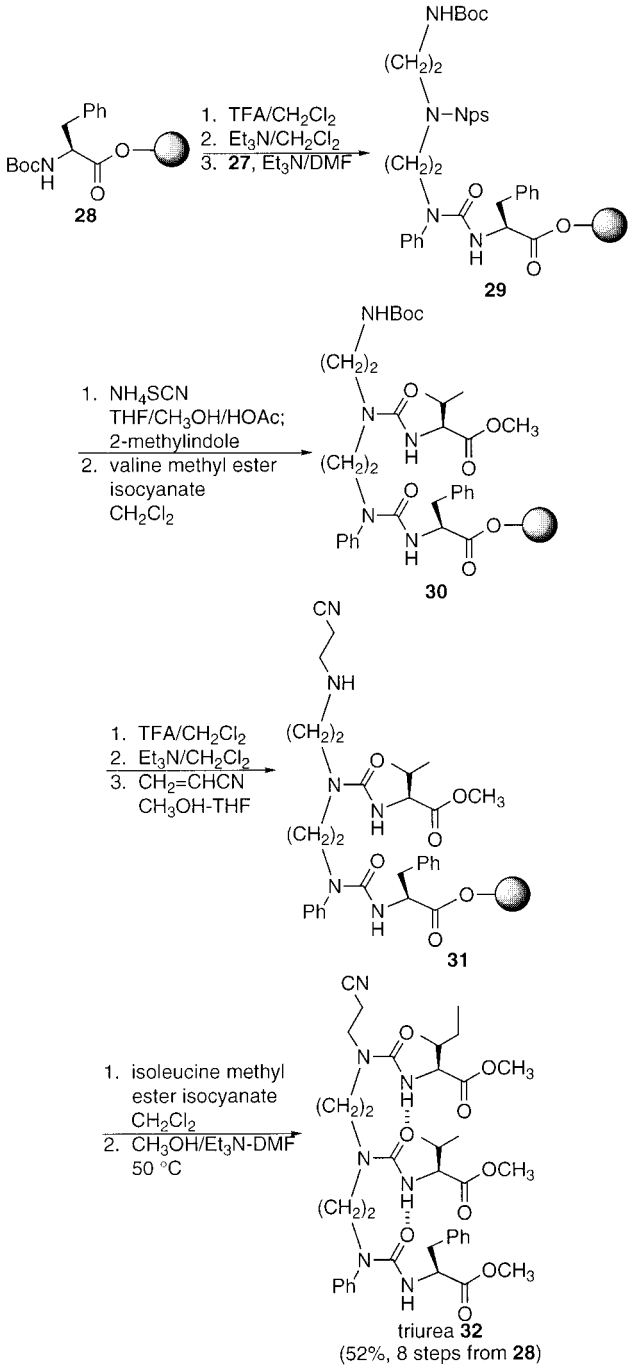


converted to the Boc-phenylalanylisoleucylleucine tripeptide **19** by standard solid-phase techniques. The Boc group was removed by treatment with TFA, the free amino group was then liberated by treatment with TEA, and the amino group was coupled with carbamoyl chloride **7** to form intermediate **20**. This intermediate was converted to cyanoethylamine **21** by removal of the Boc group with TFA, liberation of the free amino group with TEA, and Michael addition to acrylonitrile. The first half of the β-strand mimic was then introduced by reaction of the amino group of **21** with isocyanate **18**. Removal of the *tert*-butyl ester protective group with TFA and treatment with isobutyl chloroformate afforded mixed anhydride **22**. The second half of the β-strand mimic was introduced by coupling mixed anhydride **22** with hydrazine **23**.[2c] Artificial β-sheet **3** was liberated from the resin by aminolysis with methylamine. Column chromatography, followed by preparative reverse-phase HPLC, afforded pure **3** in 33% overall yield.

(6) Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066.

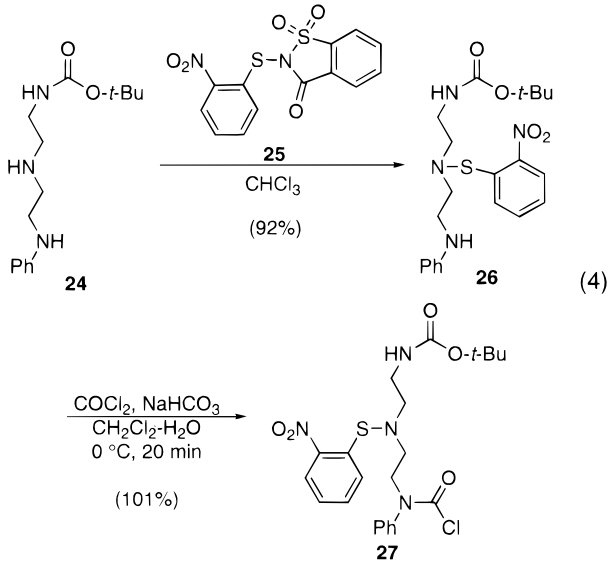
(7) de Paulis, T.; Janowsky, A.; Kessler, R. M.; Clanton, J. A.; Smith, H. E. *J. Med. Chem.* **1988**, *31*, 2027.

Scheme 4



Triurea 32. In the solid-phase syntheses of artificial β-sheets **1**−**3**, the diurea molecular scaffold is introduced as a derivative of ethylenediamine (**7**), in which one of the amino groups is protected as a Boc carbamate and the other is activated as a carbamoyl chloride. Because artificial β-sheet **4** is based upon a triurea molecular scaffold, a means of constructing the triurea molecular scaffold was required. We initially investigated a variety of S_N2 alkylation and reductive alkylation strategies for constructing the requisite diethylenetriamine portion of the molecular scaffold on the solid support. These alkylation reactions proved insufficiently clean to allow an efficient solid-phase synthesis.

For this reason, we ultimately settled upon introduction of the triurea molecular scaffold as a derivative of diethylenetriamine (**27**), in which two of the amino groups are protected with Boc and *o*-nitrophenylsufenyl[8] (Nps) groups. Derivative **27** was prepared from diamine **24**,[6] as shown in eq 4. Diamine



24 was converted to Nps-protected monoamine **26** by treatment with *N*-(2-nitrophenylsufenyl)saccharin.[9] The reaction proceeds at the "middle" nitrogen with high yield and selectivity, because this nitrogen is aliphatic and is thus much more basic and nucleophilic than the "lower" nitrogen, which is aromatic. Monoamine **26** was then converted to carbamoyl chloride **27** using the modified Schotten−Baumann conditions. This compound is not stable and is used immediately after preparation, without further purification.

To evaluate carbamoyl chloride **27** as building block for the triurea molecular scaffold, we used it to prepare triurea **32**, a compound that we had previously prepared by solution-phase techniques.[6] Triurea **32** was prepared on Merrifield resin, as shown in Scheme 4. Boc-phenylalanine Merrifield resin (**28**) was deprotected by treatment with TFA and TEA and was coupled with carbamoyl chloride **27** to give monourea **29**. Removal of the Nps protective group by treatment with ammonium thiocyanate and 2-methylindole,[10] followed by treatment with valine methyl ester isocyanate,[4,11] generated diurea **30**. The Boc protective group was then removed by treatment with TFA, and the free amine was liberated by treatment with TEA and subjected to acrylonitrile to form cyanoethylamine **31**. The third urea group was introduced by treatment with isoleucine methyl ester isocyanate,[4,11] and the triurea was liberated from the resin by transesterification with methanol and triethylamine in dimethylformamide (DMF).[12] Column chromatography, followed by preparative reverse-phase HPLC, afforded pure triurea **32** in 52% overall yield.

Artificial β-Sheet 4. Artificial β-sheet **4** was prepared on Merrifield resin using carbamoyl chloride **27** to form the triurea molecular scaffold and isocyanate **35** to introduce the β-strand mimic. Isocyanate **35** was prepared from 5-nitro-2-methoxybenzoic acid (**12**), as shown in eq 5. Acid **12** was converted to the corresponding acid chloride by treatment with thionyl chloride and then coupled with isobutyric hydrazide to form intermediate **33**. Reduction of the nitro group to form amine **34**, followed by treatment with phosgene using modified Schotten-Baumann conditions, afforded the isocyanate.

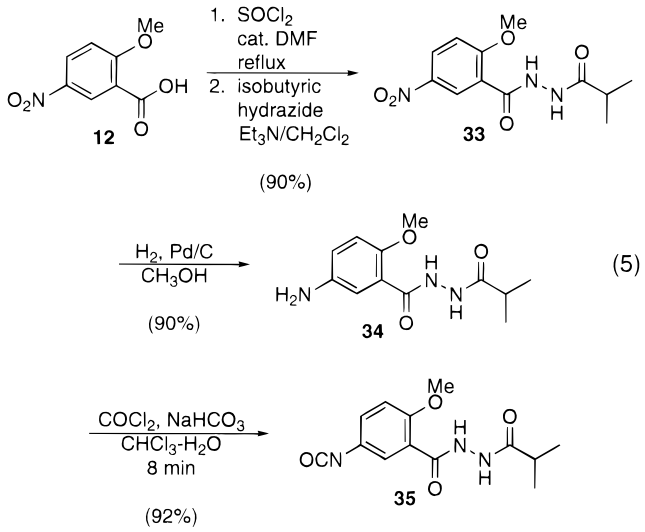
(8) Zervas, L.; Borovas, D.; Gazis, E. *J. Am. Chem. Soc.* **1963**, *85*, 3660.

(9) Romani, S.; Bovermann, G.; Moroder, L.; Wünsch, E. *Synthesis* **1985**, 512.

(10) (a) Wünsch, E.; Spangenberg, R. *Chem. Ber.* **1972**, *105*, 740. (b) Lüscher, I. F.; Schneider, C. H. *Helv. Chim. Acta* **1983**, *66*, 602.

(11) Nowick, J. S.; Powell, N. A.; Nguyen, T. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 7364.

(12) Reference 5, p 91.



Artificial β-sheet **4** was prepared as shown in Scheme 5. The Boc-phenylalanylleucine dipeptide resin (**9**, from Scheme 1) was deprotected and coupled with carbamoyl chloride **27** to afford monourea **36**. The Nps group was removed,[10] and the liberated amino group was coupled with valylalanine methyl ester isocyanate[4] to form diurea **37**. Removal of the Boc protective group and Michael addition of the primary amino group to acrylonitrile afforded cyanoethylamine **38**. Reaction with isocyanate **35**, followed by aminolysis of with methylamine, generated artificial β-sheet **4**. Column chromatography, followed by preparative reverse-phase HPLC, afforded pure artificial β-sheet **4** in 40% overall yield.

Discussion and Conclusions

The solid-phase syntheses of artificial β-sheets **1**−**4** and triurea **32** are efficient, allowing the assembly of each of these complex molecules in a few days to a week. These syntheses encompass a variety of solid-phase synthetic reactions, including peptide coupling, the reaction of carbamoyl chlorides and amines to form ureas, the reaction of isocyanates and amines to form ureas, Michael addition, the removal of three different types of protective groups, and aminolysis and transesterification reactions. Although the syntheses require 8−13 steps, they can be performed rapidly because excess reagents are used to drive the reactions to completion, byproducts and excess reagents are washed away, and purification of resin-bound intermediates is not required. The yields are good, averaging 91−95% per step.

We have also synthesized all of these compounds by solution-phase techniques.[2,6,13] The solution-phase syntheses involve forming the same bonds as the solid-phase syntheses, albeit in a slightly more convergent fashion. The overall yields of the two types of syntheses are comparable; the solution-phase syntheses give slightly lower average yields per step, but require fewer steps in the longest linear sequence. The main advantage of the solid-phase technique is convenience. Because intermediates need not be isolated, purified, and characterized, it is possible to carry out reactions more rapidly. For this reason, solid-phase synthesis is now our preferred method for the preparation of artificial β-sheets. We have recently applied these methods to the multiple parallel solid-phase synthesis of a library of 16 artificial β-sheets, which we are using to study the β-sheet-forming propensities of different amino acids. The solid-phase methods have facilitated this endeavor, and we will publish this study in a forthcoming paper.

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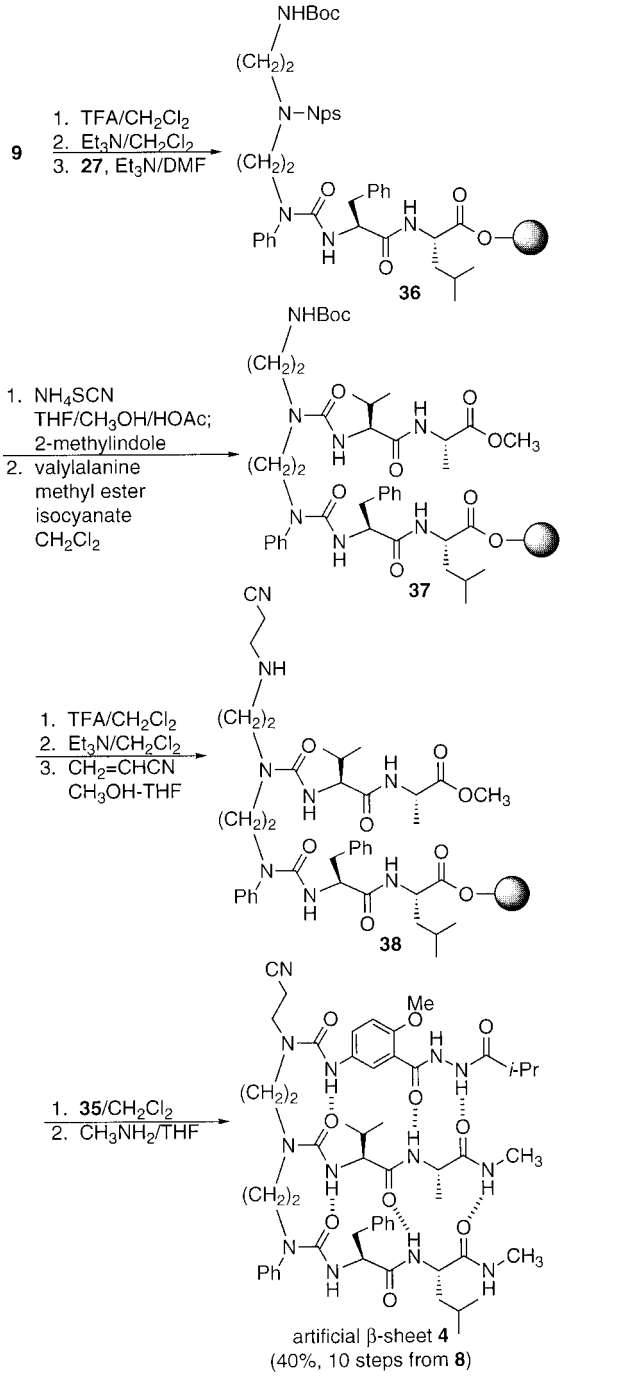
Scheme 5



Supporting Information Available: Experimental procedures; ^{1}H NMR spectra and HPLC traces of artificial β-sheets **1**−**4** (24 pages). See any current masthead page for ordering and Internet access instructions.

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(13) Nowick, J.; Smith, E. M. Unpublished results.