Triurea Derivatives of Diethylenetriamine as Potential Templates for the Formation of Artificial β-Sheets[1]

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Abstract: This paper describes synthetic and structural studies of triurea derivatives of an *N*,*N*″-disubstituted diethylenetriamine. Diethylenetriamine triureas **1** (PhN($CONHR_1$)CH_2CH_2N($CONHR_2$)CH_2CH_2N($CONHR_3$)CH_2CH_2CN; **2a**, $R_1 = R_2 = R_3 =$ Ph; **2b**, $R_1 = R_2 = R_3 = CH_3$; **2c**, $R_1 =$ (*S*)-CH(CH_2Ph)CO_2CH_3, $R_2 =$ (*S*)-CH(*i*-Pr)CO_2CH_3, $R_3 =$ (*S*)-CH((*S*)-*s*-Bu)CO_2CH_3)) are efficiently prepared in five or six steps from *N*-phenylethylenediamine. Infrared spectroscopy, ^{1}H NMR spectroscopy, and X-ray crystallography indicate that triureas **1** adopt intramolecularly hydrogen-bonded conformations, both in chloroform solution and in the solid state. The three urea groups form a hydrogen-bonded network: The carbonyl group of urea $NCONHR_1$ is hydrogen bonded to the NH group of urea $NCONHR_2$, and the carbonyl group of urea $NCONHR_2$ is hydrogen bonded to the NH group of urea $NCONHR_3$. The three R groups are aligned along the triurea backbone, pointing in roughly the same direction, like three fingers on a hand. Molecular modeling suggests that the triurea backbone will be a suitable template for the creation of artificial β-sheets. When molecular mechanics energy minimization calculations are performed upon a triurea bearing three N-terminally linked peptide strands, a parallel β-sheet is formed.

Although β-sheets are fundamental elements of protein structure, their structure and stability remain poorly understood. One approach to studying protein structure and stability involves the design, synthesis, and study of peptidomimetic model systems.[2] Over the past decade, a number of research groups have developed chemical models for β-sheets in which a synthetic template (turn unit) holds two peptide strands or a peptide strand and a peptide strand mimic in proximity. Beginning in 1986, Feigel and co-workers have employed several aromatic templates to form two-stranded antiparallel and parallel β-sheet structures in cyclopeptides.[3] While earlier studies had focused upon the mimicry and induction of β-turns,[4] Feigel's work marked the beginning of interest in the mimicry of β-sheets. In 1988, Kemp and co-workers reported using an epindolidione β-strand mimic in conjunction with a β-turn unit and a peptide strand to create artificial antiparallel β-sheets.[5] Subsequent reports have described parallel β-sheets that incorporate this template.[6] Since 1991, Kelly and co-workers have described a series of antiparallel β-sheet models in which a dibenzofuran template holds two peptide strands in proximity, while hydrophobic interactions between the template and peptide side chains help stabilize β-sheet formation.[7] Within the past year, a number of other templates capable of bringing two peptide strands together and inducing β-sheet structure have been reported.[8] Complementary to these strategies, which rely upon peptidomimetic surrogates to help stabilize protein structure, are systems that are wholly peptide-based. These systems include "template-assembled synthetic proteins" (TASPs),[9] small naturally occurring β-sheet proteins,[10] and de novo designed proteins.[11]

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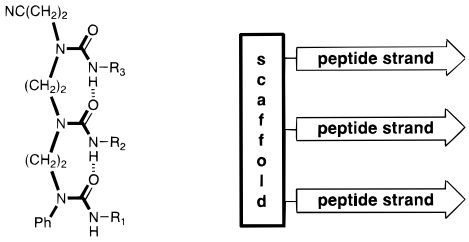
At present, none of the template-based strategies have allowed the development of artificial β-sheets comprising more than two peptide strands and exhibiting patterns of hydrogen bonding characteristic of β-sheet structures. With the goal of creating artificial β-sheets of greater size and complexity than those that have been prepared previously, we are developing a new approach in which an oligourea template holds multiple peptide strands in proximity. Since 1992, we have reported the development of amino acid ester isocyanate and peptide isocyanate building blocks[12] and the creation of oligourea templates, which we have termed "molecular scaffolds".[13] Recently, we have synthesized and studied a small artificial β-sheet comprising a diurea molecular scaffold and two peptide strands[14] and a second small artificial β-sheet comprising a diurea molecular scaffold, a peptide strand, and a β-strand mimic.[15] We now report synthetic and structural studies of a triurea molecular scaffold (**1**) tailored for the creation of three-stranded artificial parallel β-sheets.

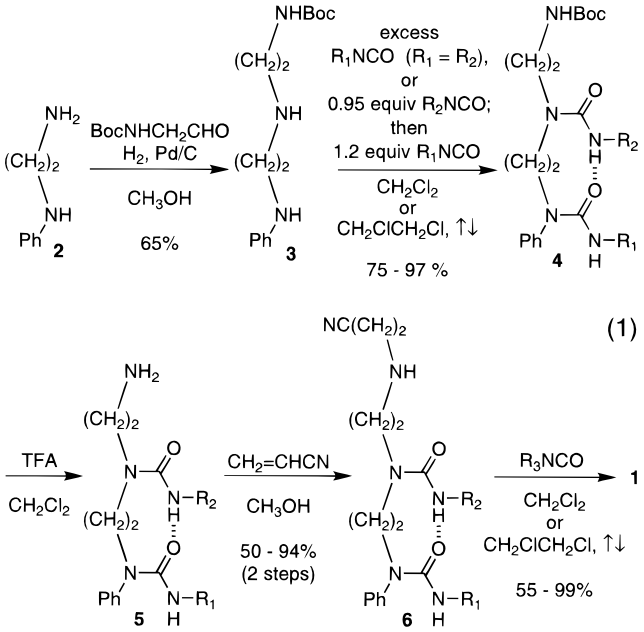


triurea "molecular scaffold" artificial parallel β-sheet

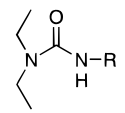
1a $R_1 = R_2 = R_3 = Ph$
1b $R_1 = R_2 = R_3 = CH_3$
1c $R_1 = (S)\text{-}CH(CH_2Ph)CO_2Me$
$R_2 = (S)\text{-}CH(i\text{-}Pr)CO_2Me$
$R_3 = (S)\text{-}CH((S)\text{-}s\text{-}Bu)CO_2Me$

Results

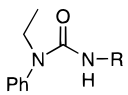
Synthesis of Triureas 1. Triureas **1** were synthesized from *N*-phenylethylenediamine (**2**) as shown in eq 1. Reductive alkylation of *N*-phenylethylenediamine with Boc-glycinal[16] affords diamine **3**. Diamine **3** reacts with isocyanates to generate diureas **4**. Diurea **4a** ($R_1 = R_2 = Ph$) was prepared by treatment of **3** with an excess of phenyl isocyanate; diurea **4b** ($R_1 = R_2 = CH_3$) was prepared by using an excess of methyl isocyanate. The aliphatic amino group of diamine **3** is substantially more basic and nucleophilic than the aromatic amino group, allowing the regioselective synthesis of diureas bearing different substitutents. Thus, diurea **4c** ($R_1 = (S)$-$CH(CH_2Ph)CO_2Me$, $R_2 = (S)$-$CH(i\text{-}Pr)CO_2Me$) was prepared by sequential treatment of diamine **3** with 0.95 equiv of L-valine methyl ester isocyanate and 1.2 equiv of L-phenylalanine methyl ester isocyanate.[12a] ^{1}H NMR analysis of the unpurified reaction mixture indicates that L-valine methyl ester isocyanate reacts with the upper amino group with complete (>98:2) regioselectivity.[17] Diureas **4** are converted to triureas **1** by removal of the *tert*-butyloxycarbonyl protective group to generate amines **5**, conjugate addition of the primary amino group to acrylonitrile to form secondary amines **6**, and reaction of the secondary amino group with the appropriate isocyanate. The triureas were obtained as analytically pure samples by recrystallization or column chromatography. The yields for the various steps shown in eq 1 are not optimized but are generally good. The yields associated with the synthesis of triurea **1c** are probably most representative: **3** → **4c**, 97%; **4c** → **6c**, 72%; and **6c** → **1c**, 99%. The methyl ureas (**b** series) were generated in lower yields because these compounds are water soluble, hygroscopic, and thus more difficult to handle.



Infrared and ^{1}H NMR Spectroscopic Studies of Triureas 1. IR and ^{1}H NMR spectroscopic studies of triureas **1** indicate that these compounds adopt intramolecularly hydrogen-bonded conformations in chloroform solution. To aid in the interpretation of the data, the spectra were compared to those of monoureas **7** and **8**. Monoureas **7** bear two ethyl groups on one nitrogen atom and resemble the "upper" two urea groups of triureas **1**. Monoureas **8** have a phenyl group and an ethyl group on one nitrogen atom and are comparable to the "lower" urea group of triurea **1**. Studies of related diureas have been described in detail in ref 13b, and the reader is directed to this paper for a discussion in greater detail than is presented here.



7a R = Ph
7b R = CH_3



8a R = Ph
8b R = CH_3

The IR spectra of triureas **1a**,**b** and monoureas **7a**,**b** and **8a**,**b** are shown in Figure 1 (10 mM solutions in $CHCl_3$). Triurea **1a** exhibits a non-hydrogen-bonded NH stretching band at 3425 cm^{-1} and a hydrogen-bonded NH stretching band at 3305 cm^{-1}. The band at 3425 cm^{-1} is similar in position, intensity, and shape to that of monourea **8a** (3428 cm^{-1}) and is attributed to the "bottom" NH group of **1a**, which is not hydrogen bonded.

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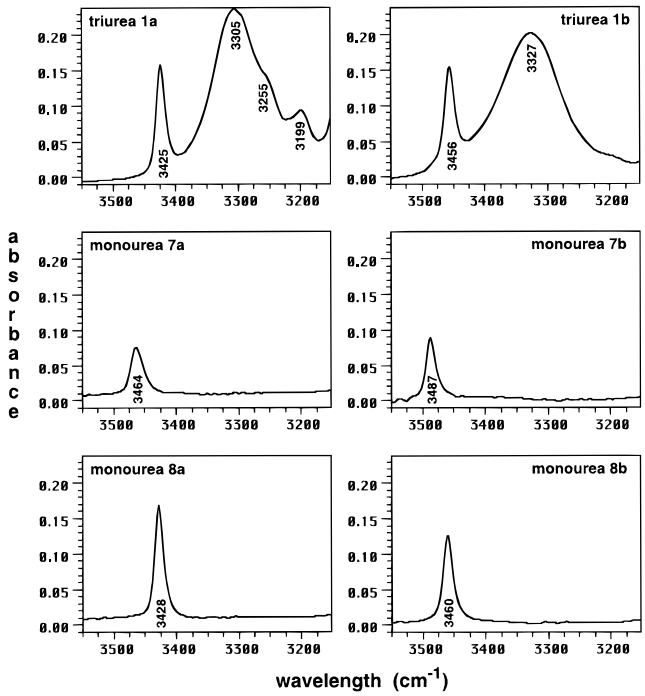

Figure 1. Infrared spectra (3150−3550 cm^{-1}) of ureas **1a**,**b**, **7a**,**b**, and **8a**,**b**. Spectra were recorded at 295 K using a 10 mM solution in $CHCl_3$ (1.0 mm path length) against a $CHCl_3$ reference.

The band at 3305 cm^{-1} arises from the "upper" two NH groups. The position of this band indicates that these groups are hydrogen bonded. The absence of a band at ca. 3464 cm^{-1} (the NH stretching frequency of **7a**) indicates the absence of a detectable population in which one or both of the "upper" two NH groups of **1a** are not hydrogen bonded. Compound **1a** also exhibits bands at 3255 (a shoulder), 3199, and 3142 cm^{-1}. Although the origin of these bands is unclear, similar bands are apparent in every hydrogen-bonded di- and triurea with phenyl substituents that we have studied. (Related examples are described in ref 13.) Apparently these bands are associated with interactions between the hydrogen-bonded NH groups and the adjacent phenyl substituents. Triurea **1b** also exhibits hydrogen-bonded and non-hydrogen-bonded NH stretching bands, at 3327 and 3456 cm^{-1}, respectively. The non-hydrogen-bonded band is similar in position, intensity, and shape to that of monourea **8b** (3460 cm^{-1}). The absence of a band at ca. 3487 cm^{-1} (the NH stretching frequency of **7b**) indicates the absence of a detectable population of a non-hydrogen-bonded conformation of **1b**. The infrared spectrum of triurea **1c** (not shown in Figure 1) also shows hydrogen-bonded and non-hydrogen-bonded bands (3300 and 3425 cm^{-1}, respectively). These studies indicate that triureas **1** are intramolecularly hydrogen bonded in $CHCl_3$ solution.

^1H NMR spectroscopic studies corroborate the IR studies. In 1.0 mM $CDCl_3$ solution, the NH resonances of **1a** appear at 9.21, 9.02, and 6.25 ppm, respectively, at 295 K. Under comparable conditions, the NH resonances of controls **7a** and **8a** appear at 6.24 and 6.08 ppm.[18] Similarly, the NH resonances of **1b** appear at 6.86, 6.77, and 4.24 ppm, while those of **7b** and **8b** appear at 4.32 and 4.06 ppm. These shifts indicate that two of the NH groups of **1** are hydrogen bonded and are consistent with our previous observation that intramolecularly hydrogen-bonded urea NH resonances shift downfield by about 2.5 ppm.[13] Triurea **1c** exhibits a similar pattern of shifting in the ^1H NMR spectrum; the isoleucine and valine NH resonances appear at 6.84 and 6.56 ppm, respectively, while the phenylalanine NH resonance appears at 4.68 ppm. Under the conditions of the IR and ^1H NMR studies, no evidence of intermolecular hydrogen bonding is detected, and the NH groups of triureas **1** exhibit no significant differences in chemical shift (≤0.01 ppm) at varying concentrations (1 and 10 mM).

X-ray Crystallographic Studies of Triureas 1. Triureas **1a**,**b** afforded small crystals suitable for X-ray crystallography. Crystals of **1a** were obtained from a warm mixture of dimethyl sulfoxide and water (ca. 4:1), while crystals of **1b** were produced by slow evaporation of a mixture of methylene chloride and petroleum ether. Triurea **1c** was obtained as a viscous oil, which began to solidify over a period of months but resisted our most valiant efforts to grow crystals for X-ray crystallography.

Figure 2 shows the X-ray crystallographic structures of triureas **1a**,**b**. In these structures the carbonyl group of the "bottom" urea is hydrogen bonded to the NH group of the "middle" urea, and the carbonyl group of the "middle" urea is hydrogen bonded to the NH group of the "top" urea. Triurea **1b** crystallized as a monohydrate, and its structure was refined to a w*R*2 value of 13.4% as a 65:35 ratio of two superimposed conformers differing only in the geometry of the diethylenetriamine backbone. Triurea **1a** was refined to a w*R*2 value of 27.2% as a single conformer. The distances, angles, and thermal parameters involving atoms C2, C3, N2, C4, and C5 of **1a** are not consistent with a well-ordered model. Attempts to refine the structure as a disordered model with two or more components for each of the above atoms proved unsatisfactory.

Discussion and Conclusions

Triureas of the general structure **1** are remarkably easy to synthesize by the route shown in eq 1. In five straightforward and efficient steps, diamine **3** can be converted to a triurea in which three different groups are presented along a well-structured backbone. Because of their structure and ease of preparation, triureas of this sort may be of interest in combinatorial approaches to drug discovery[19] and in the construction

(18) At the suggestion of one of the referees, the temperature dependencies of the ^1H NMR chemical shifts of the NH resonances of **1a**, **7a**, and **8a** were determined. In 1.0 mM $CDCl_3$ solution, over the range of 253−293 K, the following values were observed, **1a** (most downfield NH), −4.5 ppb/K; **1a** (next most downfield NH), −3.8 ppb/K; **1a** (upfield NH), −0.9 ppb/K; **7a**, −1.4 ppb/K; **8a**, −0.8 ppb/K. The greater temperature dependencies of the downfield NH resonances of urea **1a** provide further evidence that these NH groups are hydrogen bonded and that the upfield group is not. (In peptides and related amides, hydrogen-bonded NH groups generally exhibit greater temperature dependencies of chemical shifts than non-hydrogen-bonded NH groups. For examples, see: (a) Ribeiro, A. A.; Goodman, M.; Naider, F. *Int. J. Pept. Protein Res.* **1979**, *14*, 414. (b) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048. (c) Gellman, S. H.; Adams, B. R. *Tetrahedron Lett.* **1989**, *30*, 3381.)

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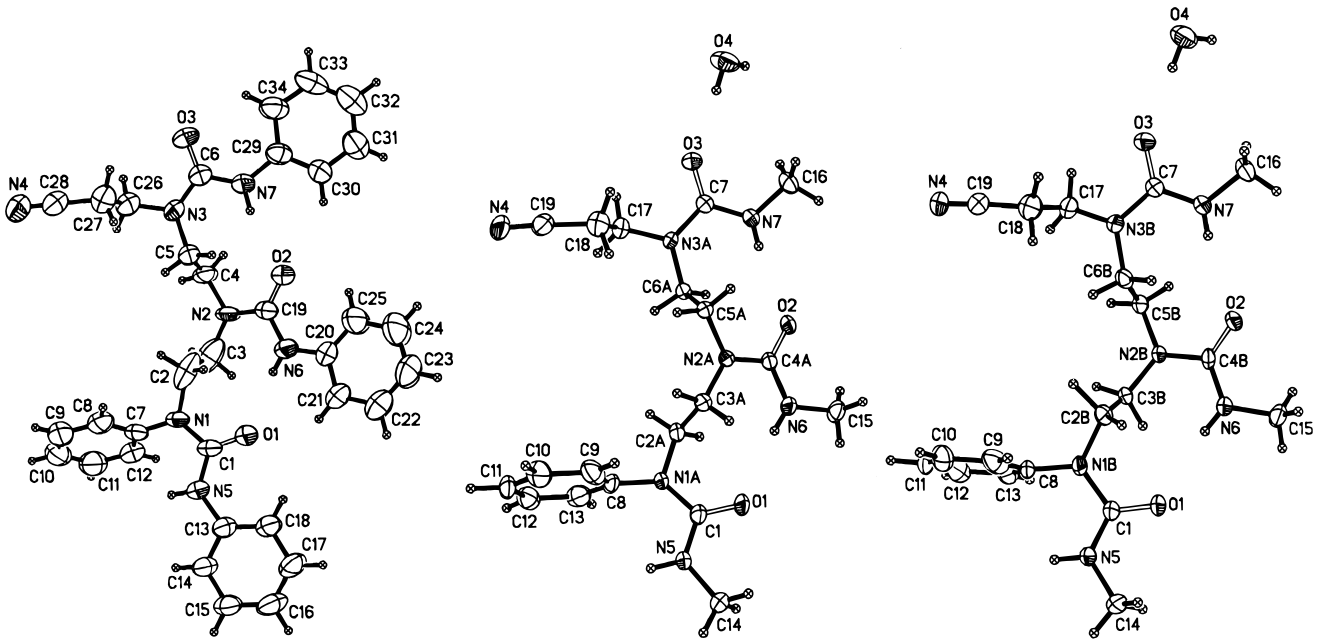

Figure 2. X-ray crystallographic structures of triureas **1a**,**b**. Triurea **1b** crystallized as the monohydrate (water shown). The major (65%) and minor (35%) conformers of **1b** are shown middle and right, above.

of "molecular tweezers"[20] or related structures consisting of three groups appended to a rigid scaffold.[21] Preliminary studies suggest that it may be possible to prepare even larger oligourea homologs (e.g., tetra- and pentaureas) by an iterative route involving the reductive alkylation of amines **5** with Boc-glycinal.

A number of intramolecularly hydrogen-bonded systems that are related to triureas **1** have been reported. We previously described the propylene homolog of **1a** ($PhN(CONHPh)CH_2CH_2CH_2N(CONHPh)CH_2CH_2CH_2N(CONHPh)CH_2CH_2CN$).[13a] IR studies indicated this compound to adopt both hydrogen-bonded and non-hydrogen-bonded conformations in $CHCl_3$ solution. In the main conformer, the "upper" two NH groups are hydrogen bonded; however, there is a significant population that lacks one or both hydrogen bonds. In contrast, the IR spectrum of **1a** shows this compound to be wholly hydrogen bonded. We have also previously described diurea derivatives of 1,2-diaminoethane.[13b] These compounds adopt hydrogen-bonded conformations in which the urea groups form nine-membered, hydrogen-bonded rings that are similar in structure to those seen in triureas **1**. Gellman and co-workers have extensively studied intramolecular hydrogen bonding in di- and triamides and found intramolecularly hydrogen-bonded nine-membered rings to be especially stable in these systems.[22] Araka and co-workers have determined that the hexaphenylurea derivative of pentaethylenehexamine is intramolecularly hydrogen bonded in chloroform solution.[23]

Two structural elements of triureas **1** deserve comment: the phenyl group at the "bottom" of the triurea backbone and the cyanoethyl group at the "top" of the triurea backbone. In *N*-phenyl-*N*-alkyl amides and ureas, there is a strong bias for the phenyl group to be s-trans to the carbonyl group.[24] This bias makes the "lower" carbonyl group point "upward"; intramolecular hydrogen bonding then aligns the "upper" two carbonyl groups. For the "top" carbonyl group to align properly, the "top" backbone urea nitrogen must have two alkyl substituents. The cyanoethyl group provides a convenient "cap" for this nitrogen atom because the reaction of primary amines (e.g., **4**) with acrylonitrile generally proceeds with high yield and selectivity for monoaddition.[25] From the observed selectivity, we estimate that the rate of reaction of primary amines **5** with acrylonitrile is about 10^2 times greater than the rate of addition of the resulting secondary amine adducts (**6**) to acrylonitrile. To avoid overaddition, we generally monitor the reaction by thin-layer chromatography.

The solid-state structures of triureas **1a**,**b** are remarkable. The three urea groups and pendant substituents (R_1, R_2, and R_3 in structure **1**) are approximately coplanar, resembling in geometry a hand with three fingers. The shape of the triureas can best be seen by inspection of space-filling models (e.g., **1a**, shown in Figure 3). The diethylenetriamine backbone curves, arraying the substituents in a slightly divergent fashion. The divergence of the substituents can be quantified by the angles between the vectors of the N−R bonds. The vectors of the "lower" and "middle" bonds ($N-R_1$ and $N-R_2$) of **1a** form an angle of 48°, while those of the "middle" and "upper" bonds ($N-R_2$ and $N-R_3$) form an angle of 52°. In **1b**, the angles are 50° and

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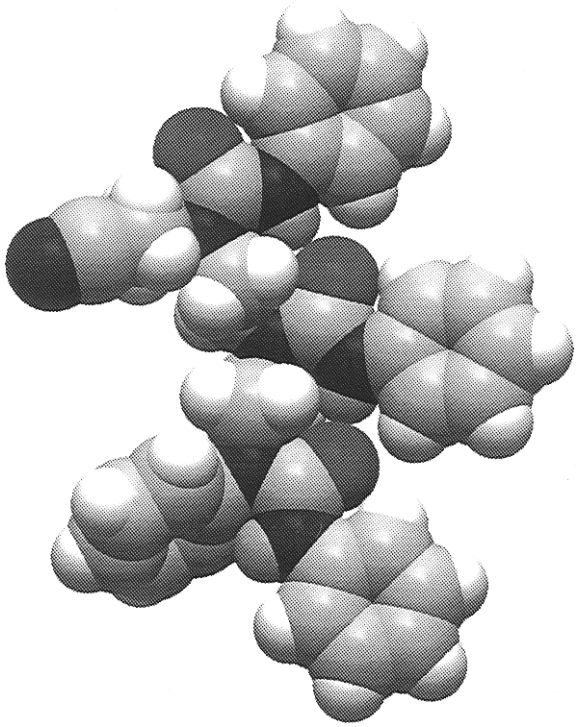
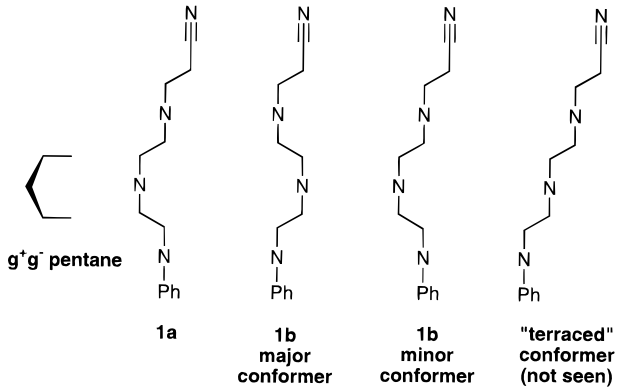


Figure 3. Space-filling representation of the X-ray crystallographic structure of diurea **1a**. Atoms are displayed at 85% of their van der Waals radii.

Chart 1



51°, respectively. Related diureas that we have studied exhibit a similar divergence, with angles ranging from 41° to 62°.[13b]

The crystallographically observed conformations of the diethylenetriamine backbones of triureas **1a**,**b** are also noteworthy. Each 1,2-diaminoethane unit adopts an anti conformation, with N−C−C−N torsion angles ranging from 166° to 170°. The related diureas that we have studied also adopt anti conformations in the solid state, with torsion angles ranging from 159° to 169°.[13b] In each structure, the central CH_2CH_2-NCH_2CH_2 unit of the diethylenetriamine backbone resembles the g^+g^- conformer of pentane.[26] This relationship is easily seen when the triureas are viewed in profile, as shown in Chart 1. Because severe steric interactions disfavor g^+g^- pentane, the observed conformations of the diethylenetriamine backbone might have been thought to be unstable, and the "terraced" conformation shown in Chart 1 might have been anticipated. The distance between the proximal methylene groups in the crystallographically observed conformers (3.6−3.9 Å) is larger than the sum of the van der Waals radii of these groups, however, and the observed conformers do not appear to be disfavored.

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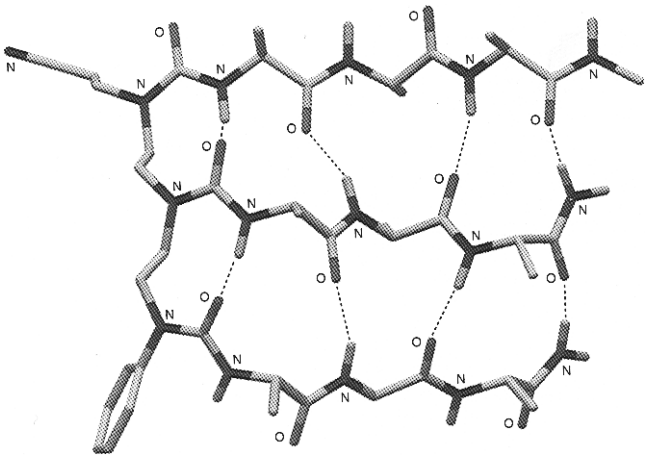

Figure 4. Model of a three-stranded artificial parallel β-sheet consisting of three tripeptide strands attached to the triurea molecular scaffold. The model is in a minimum energy conformation (local minimum) as calculated using MacroModel V5.0 with the AMBER* force field.[27] Hydrogen atoms that are attached to carbon atoms are omitted for clarity.

The structures of triureas **1** in chloroform solution are similar in several ways to the solid-state structures. The ^{1}H NMR and IR spectroscopic studies described in the Results section indicate that the molecules are intramolecularly hydrogen bonded. Analysis of the coupling patterns associated with several of the ^{1}H NMR resonances of the diethylenetriamine backbones of triureas **1** reveals vicinal coupling constants of 5 and 10 Hz. These coupling constants indicate that the N−C−C−N groups of the backbone adopt anti conformations in solution, as in the solid state. (A similar, but more detailed, treatment of the solution-phase conformation of related diureas is described in ref 13b.) From the IR and NMR data, it is not possible to determine whether the "terraced" conformer or crystallographically observed conformers are present, and there is little reason to believe that any one of the backbone conformers shown in Chart 1 should be present exclusively.

Molecular modeling suggests that the triurea backbone will be a suitable template for the creation of artificial β-sheets. Beginning with the crystallographic geometry of the major conformer of **1b**, a model for the "terraced" conformation was prepared, three tripeptide strands (Ala-Ala-Ala-NHMe) were introduced, and an energetic minimum (local minimum) was located using MacroModel V5.0 and the AMBER* force field.[27] The model adopts a hydrogen-bonded parallel β-sheet conformation and is shown in Figure 4. In this model, the N−C−C−N torsion angles are 138°.[28] The diminished torsion angles reduce the angles between the N−R vectors to 22° and the distance between the R substituents on the triurea backbone (the attached peptide α-carbons in Figure 4) to 4.9−5.1 Å.[29] This distance is similar to the separation of peptide strands in parallel

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(28) The calculated torsion angles of 138° (−138°, to be more specific) seem somewhat small, particularly in light of the values that we have observed crystallographically (159−170°) for various di- and triureas. The calculated values may reflect the absence of high-quality parameters for the N−C−C−N torsion angle and the treatment of hydrogen bonding in the AMBER* force field. If the N−C−C−N torsion angles are constrained to a larger value (e.g., −150°), a well-formed parallel β-sheet is still generated upon minimization.

(29) In **1b**, the distance between adjacent methyl groups is 6.5 Å.

β-sheets (ca. 4.9 Å).[30] This model may be viewed as a working hypothesis, which we will evaluate and refine through future experimental studies.

Experimental Section

General. Reaction mixtures were magnetically stirred under a nitrogen atmosphere, unless otherwise noted. Commercial grade reagents and solvents were used without further purification. IR studies were performed using hydrocarbon-stabilized chloroform that was passed through furnace-dried Al_2O_3 prior to use. ^{1}H NMR resonances of aliphatic amino groups that were coincident with the H_2O resonance (δ 1.6) in $CDCl_3$ are not reported in the ^{1}H NMR spectra. Samples of triureas **1** were dried under vacuum (ca. 50 °C, 0.01 mmHg) prior to elemental analysis.

Diamine 3 (PhNHCH$_2$CH$_2$NHCH$_2$CH$_2$NHCO$_2$-*t*-Bu). A 500-mL, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a glass stopper, a gas inlet adapter fitted with a hydrogen balloon, and a magnetic stirring bar was charged with 200 mL of methanol, Boc-glycinal[16] (6.6 g, 41 mmol), *N*-phenylethylenediamine (5.0 mL, 38 mmol), and 6.1 g of 10% Pd/C. The flask was evacuated and filled with nitrogen twice and then evacuated, filled with hydrogen, and maintained under a hydrogen atmosphere for 16 h. The reaction mixture was filtered, concentrated by rotary evaporation, and chromatographed on silica gel (CH_3OH−EtOAc, 1:9) to afford 6.9 g (65%) of diamine **3** as a light brown oil: IR ($CHCl_3$) 3452, 1706 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (dd, J = 8.5, 7.4 Hz, 2 H), 6.71 (app t, J = 7.3 Hz, 1 H), 6.64 (dd, J = 8.6, 0.9 Hz, 2 H), 4.89 (br s, 1 H), 4.07 (br s, 1 H), 3.24−3.19 (m, 4 H), 2.88 (app t, J = 5.8 Hz, 2 H), 2.75 (app t, J = 5.9 Hz, 2 H), 1.45 (s, 9 H); HRMS (FAB) *m/e* for $C_{15}H_{26}N_3O_2$ (M + H)$^+$ calcd 280.2025, found 280.2024.

Diurea 4a (PhN(CONHPh)CH$_2$CH$_2$N(CONHPh)CH$_2$CH$_2$NHCO$_2$-*t*-Bu). A solution of diamine **3** (0.755 g, 2.70 mmol) and phenyl isocyanate (0.88 mL, 8.1 mmol) in 5 mL of 1,2-dichloroethane was heated at reflux for 21 h and then concentrated by rotary evaporation to afford 1.80 g of a dark yellow oil. Column chromatography on silica gel (EtOAc−hexanes, 3:2) afforded 1.108 g (79%) of diurea **4a** as a foamy white solid: IR (KBr) 3423, 3307, 1708 (sh), 1660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (s, 1 H), 7.69 (d, J = 8.2 Hz, 2 H), 7.52 (t, J = 7.5 Hz, 2 H), 7.43 (t, J = 7.2 Hz, 1 H), 7.33−7.24 (m, 8 H), 7.06−6.98 (m, 2 H), 6.29 (s, 1 H), 5.12 (br s, 1 H), 3.87−3.82 (m, 2 H), 3.70−3.65 (m, 2 H), 3.46 (app t, J = 6.2 Hz, 2 H), 3.28 (app q, J = 5.8 Hz, 2 H), 1.40 (s, 9 H); HRMS (FAB) *m/e* for $C_{29}H_{36}N_5O_4$ (M + H)$^+$ calcd 518.2767, found 518.2768.

Amine 5a (PhN(CONHPh)CH$_2$CH$_2$N(CONHPh)CH$_2$CH$_2$NH$_2$). A solution of diurea **4a** (1.108 g, 2.140 mmol) in 11 mL of methylene chloride and 3.3 mL of trifluoroacetic acid was stirred for 4 h and then concentrated by rotary evaporation to afford a dark yellow oil. The oil was dissolved in 100 mL of methylene chloride, and the solution was washed with 100 mL of a mixture of equal volumes of saturated aqueous NaCl solution and saturated aqueous $NaHCO_3$ solution and then with three 50-mL portions of saturated aqueous $NaHCO_3$ solution. The organic layer was concentrated by rotary evaporation to yield 0.881 g (99%) of amine **5a** as a foamy white solid: IR (CH_2Cl_2) 3423, 3307, 1664 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.53 (br s, 1 H), 7.54−7.47 (m, 4 H), 7.43−7.23 (m, 9 H), 7.02 (t, J = 7.2 Hz, 1 H), 6.97 (t, J = 7.4 Hz, 1 H), 6.43 (s, 1 H), 3.92−3.87 (m, 2 H), 3.68−3.63 (m, 2 H), 3.44 (t, J = 5.5 Hz, 2 H), 2.95 (t, J = 5.5 Hz, 2 H); HRMS (FAB) *m/e* for $C_{24}H_{28}N_5O_2$ (M + H)$^+$ calcd 418.2243, found 418.2238.

Amine 6a (PhN(CONHPh)CH$_2$CH$_2$N(CONHPh)CH$_2$CH$_2$NHCH$_2$CH$_2$CN). A solution of amine **5a** (0.201 g, 0.481 mmol) and acrylonitrile (0.0475 mL, 0.722 mmol) in 10 mL of methanol was stirred for 28 h and then concentrated by rotary evaporation to afford 0.215 g (95%) of amine **6a** as a foamy white solid: IR (CH_2Cl_2) 3421, 3307, 2252, 1662 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1 H), 7.59 (d, J = 7.8 Hz, 2 H), 7.52 (app t, J = 7.4 Hz, 2 H), 7.43 (app t, J = 7.3 Hz, 1 H), 7.37−7.23 (m, 8 H), 7.06−6.97 (m, 2 H), 6.29 (s, 1 H), 3.92−3.87 (m, 2 H), 3.70−3.66 (m, 2 H), 3.47 (t, J = 5.9 Hz, 2 H), 2.95 (t, J = 6.6 Hz, 2 H), 2.87 (t, J = 5.9 Hz, 2 H), 2.48 (t, J = 6.5 Hz, 2 H); HRMS (FAB) *m/e* for $C_{27}H_{31}N_6O_2$ (M + H)$^+$ calcd 471.2508, found 471.2510.

Triurea 1a (PhN(CONHPh)CH$_2$CH$_2$N(CONHPh)CH$_2$CH$_2$N(CONHPh)CH$_2$CH$_2$CN). A solution of amine **6a** (0.166 g, 0.353 mmol) and phenyl isocyanate (0.043 mL, 0.39 mmol) in 3 mL of 1,2-dichloroethane was heated at reflux for 16 h and then concentrated by rotary evaporation to afford 0.219 g of a foamy tan solid. Column chromatography on silica gel (EtOAc−hexanes, 3:1), followed by recrystallization from methanol, yielded 0.123 g (59%) of triurea **1a** as white crystals: mp 177.5−178.8 °C; IR (CH_2Cl_2) 3421, 3305, 2251, 1659 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1 H), 9.01 (s, 1 H), 7.74 (app t, J = 8.7 Hz, 4 H), 7.53 (t, J = 7.7 Hz, 2 H), 7.47 (t, J = 7.2 Hz, 1 H), 7.31 (app t, J = 7.5 Hz, 10 H), 7.09−7.02 (m, 3 H), 6.25 (s, 1 H), 3.85−3.83 (m, 2 H), 3.75−3.73 (m, 2 H), 3.59 (t, J = 6.2 Hz, 2 H), 3.58−3.47 (m, 4 H), 2.69 (t, J = 6.1 Hz, 2 H); HRMS (FAB) *m/e* for $C_{34}H_{36}N_7O_3$ (M + H)$^+$ calcd 590.2879, found 590.2874. Anal. Calcd for $C_{34}H_{35}N_7O_3$: C, 69.25; H, 5.98; N, 16.63. Found: C, 69.35; H, 6.18; N, 16.44.

Diurea 4b (PhN(CONHCH$_3$)CH$_2$CH$_2$N(CONHCH$_3$)CH$_2$CH$_2$NHCO$_2$-*t*-Bu). A solution of diamine **3** (0.307 g, 1.10 mmol) and methyl isocyanate (0.38 mL, 6.6 mmol) in 3 mL of methylene chloride was stirred for 11 h and then concentrated by rotary evaporation to afford 0.406 g of a foamy deliquescent tan solid. Column chromatography on silica gel (CH_3OH−EtOAc, gradient from 5:95 to 10:90) afforded 0.325 g (75%) of diurea **4b** as a deliquescent foamy white solid: IR (CH_2Cl_2) 3454, 3336, 1705, 1645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (app t, J = 7.6 Hz, 2 H), 7.37 (app t, J = 7.3 Hz, 1 H), 7.18 (d, J = 7.1 Hz, 2 H), 6.60 (br s, 1 H), 5.22 (br s, 1 H), 4.26 (app q, J = 4.4 Hz, 1 H), 3.67−3.62 (m, 2 H), 3.46−3.40 (m, 2 H), 3.34 (t, J = 6.1 Hz, 2 H), 3.17 (q, J = 5.6 Hz, 2 H), 2.87 (d, J = 4.4 Hz, 3 H), 2.74 (d, J = 4.7 Hz, 3 H), 1.40 (s, 9 H); HRMS (FAB) *m/e* for $C_{19}H_{32}N_5O_4$ (M + H)$^+$ calcd 394.2454, found 394.2445.

Amine 5b (PhN(CONHCH$_3$)CH$_2$CH$_2$N(CONHCH$_3$)CH$_2$CH$_2$NH$_2$). A solution of diurea **4b** (0.309 g, 0.785 mmol) in 12 mL of methylene chloride and 1.2 mL of trifluoroacetic acid was stirred for 3 h and then concentrated by rotary evaporation to afford a yellow oil. The oil was dissolved in 50 mL of methylene chloride, and the solution was washed with 150 mL of a saturated aqueous Na_2CO_3 solution. The aqueous phase was extracted with three 50-mL portions of methylene chloride, concentrated to 90 mL by rotary evaporation, and extracted with three 100-mL portions of methylene chloride. The combined organic layers were concentrated to afford 0.201 g (87%) of amine **5b** as a yellow oily solid: IR (CH_2Cl_2) 3454, 3338, 1643 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (app t, J = 7.4 Hz, 2 H), 7.34 (app t, J = 6.8 Hz, 1 H), 7.20 (d, J = 7.2 Hz, 2 H), 6.63 (app q, J = 4.0 Hz, 1 H), 4.39 (app q, J = 4.3 Hz, 1 H), 3.70−3.65 (m, 2 H), 3.47−3.42 (m, 2 H), 3.29 (t, J = 6.2 Hz, 2 H), 2.84 (d, J = 4.4 Hz, 3 H), 2.79−2.73 (m, 5 H); HRMS (FAB) *m/e* for $C_{14}H_{24}N_5O_2$ (M + H)$^+$ calcd 294.1930, found 294.1923.

Amine 6b (PhN(CONHCH$_3$)CH$_2$CH$_2$N(CONHCH$_3$)CH$_2$CH$_2$NHCH$_2$CH$_2$CN). A solution of amine **5b** (0.192 g, 0.655 mmol) and acrylonitrile (0.065 mL, 0.99 mmol) in 10 mL of methanol was stirred for 26 h and then concentrated by rotary evaporation to afford 0.210 g of a yellow oil. Column chromatography on silica gel (*i*-PrOH−$CHCl_3$, 1:1) yielded 0.133 g (58%) of amine **6b** as a viscous yellow oil: IR (CH_2Cl_2) 3454, 3338, 2250, 1645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (app t, J = 7.4 Hz, 2 H), 7.36 (app t, J = 8.0 Hz, 1 H), 7.21 (d, J = 7.2 Hz, 2 H), 6.56 (app q, J = 4.3 Hz, 1 H), 4.33 (app q, J = 4.6 Hz, 1 H), 3.70−3.65 (m, 2 H), 3.49−3.42 (m, 2 H), 3.34 (t, J = 6.1 Hz, 2 H), 2.90 (t, J = 6.6 Hz, 2 H), 2.84 (d, J = 4.4 Hz, 3 H), 2.77−2.73 (m, 5 H), 2.46 (t, J = 6.6 Hz, 2 H); 1.89 (br s, 1 H); HRMS (FAB) *m/e* for $C_{17}H_{27}N_6O_2$ (M + H)$^+$ calcd 347.2195, found 347.2197.

Triurea 1b (PhN(CONHCH$_3$)CH$_2$CH$_2$N(CONHCH$_3$)CH$_2$CH$_2$N(CONHCH$_3$)CH$_2$CH$_2$CN). A solution of amine **6b** (0.133 g, 0.382 mmol) and methyl isocyanate (0.068 mL, 1.15 mmol) in 2 mL of methylene chloride was stirred for 4.5 h and then concentrated by rotary evaporation to afford 0.173 g of a hygroscopic foamy white solid. Repeated recrystallization from CH_2Cl_2−petroleum ether, followed by column chromatography on silica gel (0.7% MeOH in CH_2Cl_2), afforded 0.085 g (55%) of triurea **1b** as a hygroscopic foamy white solid: IR (CH_2Cl_2) 3452, 3323, 2249, 1645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (app t, J = 7.4 Hz, 2 H), 7.38 (t, J = 7.3 Hz, 1 H), 7.18 (d, J = 7.1 Hz, 2 H), 6.86 (app q, J = 3.7 Hz, 1 H), 6.78 (app q, J = 4.3

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Hz, 1 H), 4.29 (app q, J = 4.5 Hz, 1 H), 3.63−3.58 (m, 2 H), 3.49 (t, J = 6.2 Hz, 2 H), 3.45−3.40 (m, 2 H), 3.29 (app s, 4 H), 2.89 (d, J = 4.4 Hz, 3 H), 2.83 (d, J = 4.4 Hz, 3 H), 2.75 (d, J = 4.7 Hz, 3 H), 2.59 (t, J = 6.2 Hz, 2 H); HRMS (FAB) *m/e* for $C_{19}H_{30}N_7O_3$ (M + H)$^+$ calcd 404.2410, found 404.2409. Anal. Calcd for $C_{19}H_{29}N_7O_3$: C, 56.56; H, 7.24; N, 24.30. Found: C, 56.73; H, 7.37; N, 24.04.

Diurea 4c (PhN(CONH-(*S*)-CH(CH_2Ph)CO_2CH_3)CH_2CH_2N-(CONH-(*S*)-CH(*i*-Pr)CO_2CH_3)$CH_2CH_2NHCO_2$-*t*-Bu). A solution of diamine **3** (0.180 g, 0.645 mmol) and L-valine methyl ester isocyanate[12a] (0.096 g, 0.61 mmol) in 15 mL of methylene chloride was stirred for 13 h and then concentrated by rotary evaporation to afford a light brown oil. Column chromatography on silica gel (EtOAc−hexanes, 2:1) afforded 0.259 g (97%) of $PhNHCH_2CH_2N$(CONH-(*S*)-CH(*i*-Pr)CO_2-CH_3)$CH_2CH_2NHCO_2$-*t*-Bu as a colorless oil: IR ($CHCl_3$) 3462, 3365, 1734, 1703, 1643 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (t, J = 7.7 Hz, 2 H), 6.70 (t, J = 7.3 Hz, 1 H), 6.63 (d, J = 8.1 Hz, 2 H), 5.95 (d, J = 7.5 Hz, 1 H), 5.16 (br s, 1 H), 4.39 (br s, 1 H), 4.30 (dd, J = 8.4, 5.6 Hz, 1 H), 3.72 (s, 3 H), 3.62 (dt, J = 14.8 Hz, 6.1 Hz, 1 H), 3.48−3.40 (m, 2 H), 3.35−3.20 (m, 5 H), 2.11−2.04 (m, 1 H), 1.42 (s, 9 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.85 (d, J = 6.6 Hz, 3 H); HRMS (FAB) *m/e* for $C_{22}H_{37}N_4O_5$ (M + H)$^+$ calcd 437.2764, found 437.2750.

A solution of $PhNHCH_2CH_2N$(CONH-(*S*)-CH(*i*-Pr)CO_2CH_3)CH_2-CH_2NHCO_2-*t*-Bu (0.178 g, 0.407 mmol) and L-phenylalanine methyl ester isocyanate[12a] (0.098 g, 0.48 mmol) in 5 mL of 1,2-dichloroethane was heated at reflux for 45 h and then concentrated by rotary evaporation to give a brown oil. Column chromatography on silica gel (EtOAc−CH_2Cl_2, 9:1) afforded 0.262 g (100%) of diurea **4c** as a colorless oil: IR ($CHCl_3$) 3458 (sh), 3427, 3325, 1740, 1703, 1649 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (app t, J = 7.5 Hz, 2 H), 7.29 (app t, J = 7.4 Hz, 1 H), 7.22−7.19 (m, 3 H), 7.07 (app d, J = 7.7 Hz, 2 H), 6.98−6.94 (m, 2 H), 6.59 (d, J = 5.5 Hz, 1 H), 5.19 (br s, 1 H), 4.72−4.66 (m, 2 H), 4.26 (dd, J = 8.0, 6.2 Hz, 1 H), 3.77 (ddd, J = 13.4, 10.5, 5.2 Hz, 1 H), 3.71 (s, 3 H), 3.68 (s, 3 H), 3.65−3.56 (m, 1 H), 3.55−3.46 (m, 1 H), 3.45−3.32 (m, 2 H), 3.29−3.12 (m, 3 H), 3.06 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 5.1 Hz, 1 H), 2.91 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 6.3 Hz, 1 H), 2.22 (octet, J = 6.7 Hz, 1 H), 1.39 (s, 9 H), 1.03 (d, J = 6.8 Hz, 3 H), 1.00 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 172.2, 158.2, 156.0, 141.1, 135.7, 129.8, 128.7, 128.1, 127.5, 127.3, 126.6, 78.6, 59.6, 54.0, 51.7, 51.3, 48.5, 46.7, 45.8, 39.7, 37.6, 29.9, 28.0, 18.9, 18.3; HRMS (FAB) *m/e* for $C_{33}H_{48}N_5O_8$ (M + H)$^+$ calcd 642.3503, found 642.3505.

Amine 5c (PhN(CONH-(*S*)-CH(CH_2Ph)CO_2CH_3)CH_2CH_2N-(CONH-(*S*)-CH(*i*-Pr)CO_2CH_3)$CH_2CH_2NH_2$). A solution of diurea **4c** (0.271 g, 0.422 mmol), 1.3 mL of trifluoroacetic acid, and 0.02 mL of water in 8 mL of methylene chloride was stirred for 45 min. Saturated aqueous $NaHCO_3$ solution was then added gradually, with stirring, until the bubbling ceased (ca. 10 mL). The phases were separated, the aqueous phase was extracted with two 5-mL portions of methylene chloride, and the combined organic layers were washed with 20 mL of saturated aqueous NaCl solution, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield 0.242 g (106%) of amine **5c** as a colorless oil: IR ($CHCl_3$) 3425, 3296, 1740, 1649 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (t, J = 7.5 Hz, 2 H), 7.28 (t, J = 7.1 Hz, 1 H), 7.20−7.19 (m, 3 H), 7.10 (d, J = 7.8 Hz, 2 H), 7.03−6.94 (m, 3 H), 4.77 (d, J = 7.8 Hz, 1 H), 4.68 (app q, J = 6.6 Hz, 1 H), 4.23 (dd, J = 7.8, 5.8 Hz, 1 H), 3.81−3.73 (m, 1 H), 3.70−3.63 (m, 1 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 3.56−3.48 (m, 1 H), 3.41−3.32 (m, 2 H), 3.31−3.22 (m, 1 H), 3.05 (dd, ABX pattern, J_{AB} = 13.7 Hz, J_{AX} = 5.6 Hz, 1 H), 2.92 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 6.9 Hz, 1 H), 2.84 (br s, 2 H), 2.27 (br s, 2 H), 2.20−2.13 (m, 1 H), 0.96 (d, J = 6.8 Hz, 6 H).

Amine 6c (PhN(CONH-(*S*)-CH(CH_2Ph)CO_2CH_3)CH_2CH_2N-(CONH-(*S*)-CH(*i*-Pr)CO_2CH_3)$CH_2CH_2NHCH_2CH_2CN$). A solution of 0.242 g of amine **5c** (from above) and acrylonitrile (0.042 mL, 0.64 mmol) in 8 mL of methanol was stirred for 65 h and then concentrated by rotary evaporation to afford 0.214 g of a yellow oil. Column chromatography on silica gel (CH_3OH−EtOAc, 1:9) yielded 0.180 g (72% from **4c**) of amine **6c** as a colorless oil: IR ($CHCl_3$) 3425, 3305, 2251, 1740, 1651 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (t, J = 7.5 Hz, 2 H), 7.29 (t, J = 6.8 Hz, 1 H), 7.21−7.19 (m, 3 H), 7.11 (d, J = 7.6 Hz, 2 H), 6.96−6.95 (m, 2 H), 6.72 (d, J = 8.1 Hz, 1 H), 4.73 (d, J = 7.8 Hz, 1 H), 4.67 (app q, J = 6.7 Hz, 1 H), 4.25 (dd, J = 8.3, 5.7 Hz, 1 H), 3.78−3.70 (m, 2 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 3.54−3.48 (m, 1 H), 3.44−3.36 (m, 2 H), 3.33−3.28 (m, 1 H), 3.05 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 5.5 Hz, 1 H), 2.94−2.88 (m, 3 H), 2.80−2.75 (m, 2 H), 2.51−2.48 (m, 2 H), 2.19−2.12 (m, 1 H), 1.79 (br s, 1 H), 0.96 (d, J = 6.7 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 173.5, 159.0, 156.5, 141.5, 136.0, 130.0, 128.9, 128.3, 127.7, 126.8, 119.0, 59.3, 54.2, 52.0, 51.6, 48.8, 48.3, 46.2, 45.2, 37.9, 30.3, 19.2, 18.3.

Triurea 1c (PhN(CONH-(*S*)-CH(CH_2Ph)CO_2CH_3)CH_2CH_2N-(CONH-(*S*)-CH(*i*-Pr)CO_2CH_3)CH_2CH_2N(CONH-(*S*)-CH((*S*)-*s*-Bu)-CO_2CH_3)CH_2CH_2CN). A solution of amine **6c** (0.180 g, 0.303 mmol) and L-isoleucine methyl ester isocyanate[12a] (0.054 g, 0.31 mmol) in 10 mL of methylene chloride was stirred for 20 h and then concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel (CH_3OH−EtOAc, 1:9) to yield 0.231 g (99%) of triurea **1c** as a colorless oil: IR ($CHCl_3$) 3425, 3305, 2249, 1740, 1643 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (t, J = 7.5 Hz, 2 H), 7.33 (t, J = 7.3 Hz, 1 H), 7.22−7.20 (m, 3 H), 7.08 (d, J = 7.4 Hz, 2 H), 6.97−6.95 (m, 2 H), 6.86 (br s, 1 H), 6.58 (d, J = 7.7 Hz, 1 H), 4.72−4.66 (m, 2 H), 4.24 (dd, J = 7.6, 6.0 Hz, 1 H), 4.20 (app t, J = 6.6 Hz, 1 H), 3.75 (ddd, J = 13.1, 10.5, 5.0 Hz, 1 H), 3.70 (s, 3 H), 3.684 (s, 3 H), 3.680 (s, 3 H), 3.60−3.47 (m, 3 H), 3.43−3.32 (m, 5 H), 3.25−3.20 (m, 1 H), 3.07 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 5.2 Hz, 1 H), 2.91 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 6.4 Hz, 1 H), 2.65 (dt, J = 16.8 Hz, J = 6.9 Hz, 1 H), 2.51 (dt, J = 16.9, 5.9 Hz, 1 H), 2.23−2.17 (m, 1 H), 1.95−1.90 (m, 1 H), 1.55−1.50 (m, 1 H), 1.32−1.26 (m, 1 H), 1.00 (d, J = 6.9 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H), 0.922 (d, J = 6.8 Hz, 3 H), 0.920 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 173.5, 172.4, 158.3, 157.6, 156.4, 141.4, 136.0, 130.3, 129.0, 128.4, 128.0, 127.6, 126.9, 118.8, 59.7, 58.9, 54.3, 52.1, 51.6, 49.3, 47.9, 47.0, 46.8, 45.3, 38.1, 36.8, 30.4, 25.7, 19.2, 18.4, 17.6, 15.6, 11.4; HRMS (FAB) *m/e* for $C_{39}H_{56}N_7O_9$ (M + H)$^+$ calcd 766.4139, found 766.4141. Anal. Calcd for $C_{39}H_{55}N_7O_9$: C, 61.16; H, 7.24; N, 12.80. Found: C, 60.97; H, 7.43; N, 12.62.

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Supporting Information Available: Experimental details of the X-ray crystallographic structure determination, tables of distances, angles, fractional coordinates, and thermal parameters, and thermal ellipsoid plots for triureas **1a**, **b** (32 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS, can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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