Synthesis and Conformational Preferences of a Potential β-Sheet Nucleator Based on the 9,9-Dimethylxanthene Skeleton

Kurt McWilliams and Jeffery W. Kelly*

Department of Chemistry, Texas A&M University, College Station, Texas, 77843-3255

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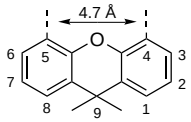
A 4,5-disubstituted-9,9-dimethylxanthene-based amino acid (**10**) has been synthesized for incorporation into peptide sequences which have a propensity to adopt β-sheet structure. Molecular dynamics studies support the FT-IR and NMR results which demonstrate that amides based on this residue utilize the NH and the C═O from the xanthene residue to form an intramolecular hydrogen bond (13-membered ring), unlike the previously studied dibenzofuran-based amino acid residues in which the NH and the C═O of the attached amide groups participate in intramolecular hydrogen bonding (15-membered ring). Interestingly, residue **10** derivatized as a simple amide prefers to adopt a *trans* conformation where the aliphatic side chains are placed on opposite sides of the plane of the 9,9-dimethylxanthene ring system. This is different than the conformational preferences of the dibenzofuran-based amino acids which adopt a *cis* conformation that is preorganized to nucleate β-sheet formation. It will be interesting to see how these conformational differences effect nucleation in aqueous solution.

Introduction

Unnatural amino acids have been widely used as templates to induce protein-like secondary structure in small peptides.[1] For β-sheet nucleators, these templates are generally conformationally restricted molecules, which in addition to reversing the chain direction provide stabilizing contacts with the attached α-amino acid sequence.[2−15] These contacts include hydrophobic and/or electrostatic interactions and have been used to nucleate both parallel and antiparallel β-sheet structures. Recently, this laboratory reported the synthesis of a series of unnatural amino acids and diacids which are capable of nucleating β-sheet structure in peptide sequences of less than 20 α-amino acids.[16−19] These nucleators function by forming a hydrogen-bonded hydrophobic cluster which preorganizes pendant peptide chains for hydrogen bonding and subsequent β-sheet formation. While these templates eliminated most of the entropic penalty associated with chain reversal due to their U shape, the divergent projection of the peptide strands in the dibenzofuran-based peptides and the rotational isomerization in the biphenyl- and bipryidyl-based templates could limit nucleating efficacy. In designing a potentially improved nucleator it was desirable to enhance the characteristics associated with nucleation (hydrophobic cluster formation, chain reversal, and appropriate intramolecular hydrogen bonding) and modify the properties which possibly hinder β-sheet nucleation.

An unnatural amino acid based on 9,9-dimethylxanthene (**1**) was chosen as the target because it offered several potential advantages relative to the amino acids previously prepared by this labortory. Unlike the planar dibenzofuran skeleton, the incorporation of an isopropylidene spacer at the nine position results in a nonplanar xanthene ring system. This leads to the realization of a range of accessible xanthene "puckering angles" which could prove useful in optimizing packing within the desired hydrophobic cluster.[20] In addition to enhancing the hydrophobic cluster through packing interactions, hydrophobicity is also improved as a result of the projection of one of the methyl groups of the isopropylidene spacer into a void existing between the side chains of the flanking α-amino acid residues. Our expectation is that the combination of these two effects should compensate for any increase in the entropic penalty associated with hydrophobic cluster formation as a result of the increased flexibility of the xanthene skeleton. Previous studies involving the rigid aromatic templates



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* To whom correspondence should be addressed: phone 409-845-4242; fax 409-845-9452; e-mail kelly@chemvx.tamu.ed.

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(1) Schneider, J.; Kelly, J. *Chem. Rev.* **1995**, *95*, 2169−2187.
(2) Brandmeier, V.; Feigel, M.; Bremer, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 486−488.
(3) Brandmeier, V.; Feigel, M. *Tetrahedron* **1989**, *45*, 1365−1376.
(4) Brandmeier, V.; Sauer, W. H. B.; Feigel, M. *Helv. Chim. Acta* **1994**, *77*, 70−85.
(5) Gardner, R. R.; Liang, G. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280−3281.
(6) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5081−5082.
(7) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650−4657.
(8) Kemp, D. S. *Trends Biotechnol.* **1990**, *8*, 249−255.
(9) Nowick, J. S.; Powell, N. A.; Martinez, E. J.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763−3765.
(10) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Smith, E. M.; Noronha, G.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 88−99.
(11) Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. J. *J. Am. Chem. Soc.* **1994**, *116*, 9947−9962.
(12) Wagner, G.; Feigel, M. *Tetrahedron* **1993**, *49*, 10831−10842.
(13) Winningham, M. J.; Sogah, D. Y. *J. Am. Chem. Soc.* **1994**, *116*, 11173−11174.
(14) Nesloney, C.; Kelly, J. *Bioorg. Med. Chem.* **1996**. In Press.
(15) Mueller, K.; Obrecht, D.; al., e. In *Perspectives in Medicinal Chemistry*; Testa, B., Ed.; VCH: New York, 1994.
(16) Choo, D.; Schneider, J.; Graciani, N.; Kelly, J. *Macromolecules* **1996**, *29*, 355−366.
(17) Diaz, H.; Kelly, J. *Tetrahedron Lett.* **1991**, *32*, 5725−5728.
(18) Schneider, J.; Kelly, J. *J. Am. Chem. Soc.* **1995**, *117*, 2533−2546.
(19) Tsang, K.; Diaz, H.; Graciani, N.; Kelly, J. *J. Am. Chem. Soc.* **1994**, *116*, 3988−4005.
(20) Schaefer, T.; Sebastian, R. *Can. J. Chem.* **1990**, *68*, 1548.

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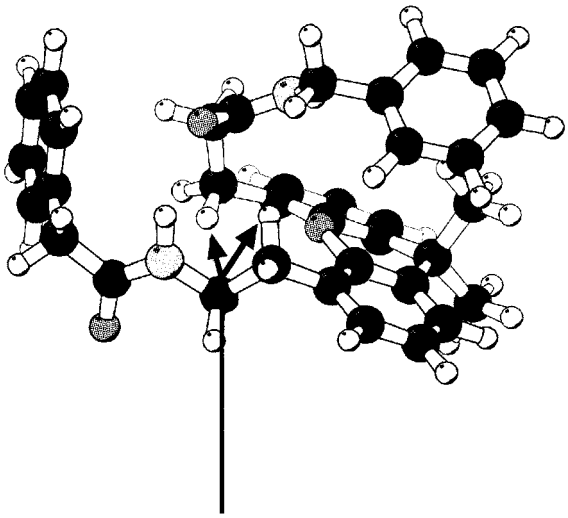


Figure 1. Ball-and-stick rendition of a representative low-energy conformer of benzyl diamide **12**, determined by molecular dynamics/molecular mechanics. Arrows indicate hydrogens used for *trans*/*cis* conformer determination. Note that the ethylene spacers adopt a *trans* conformation.

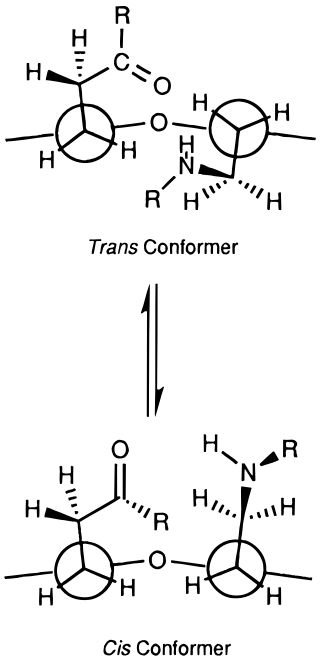
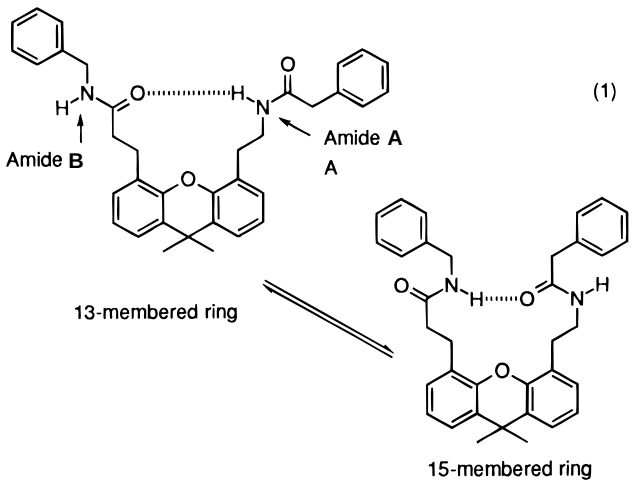


Figure 2. Newman projections of *trans* and *cis* conformations of the propionic acid and aminoethyl substructures within the xanthene-based unnatural amino acid residue.

have demonstrated the need for flexible ethylene linkers between the aromatic ring system and the peptide strands to achieve the desired "perpendicular" conformation associated with the hydrophobic cluster conformation. This perpendicular conformation refers to the preferred perpendicular orientation of the aliphatic carbon−carbon bond relative to the plane of the aromatic ring (Figures 1 and 2).[21,22] In a peptide, these CH_2CH_2 linkers should orient the xanthene ring system perpendicular to the plane of the β-sheet, allowing the side chains of the flanking hydrophobic α-amino acids to interact with the face of the hydrophobic xanthene ring system. The placement of these spacers at the 4 and 5 positions of the 9,9-dimethylxanthene skeleton appears optimal for sheet formation with attachment points 4.7 Å apart, which is close to the strand−strand separation observed in naturally occurring β-sheets (4.85 Å).

One anticipated difference between the previously studied dibenzofuran-based system and this xanthene-based system is in the preferred intramolecularly hydrogen-bonded conformations (*vide infra*). In dibenzofuran-based simple amides, the C═O and the NH of attached amide groups intramolecularly hydrogen bond affording a 15-membered ring exclusively.[19] This arises, in part, from divergent projection of substituents from the fused ring skeleton. For xanthene, substituents radiating from the 4 and 5 positions are parallel with respect to each other creating the possibility of a 13-membered intramolecular hydrogen-bonded ring (eq 1). It will be interesting to discern what effect these anticipated conformational differences have on β-sheet nucleation in aqueous solution.

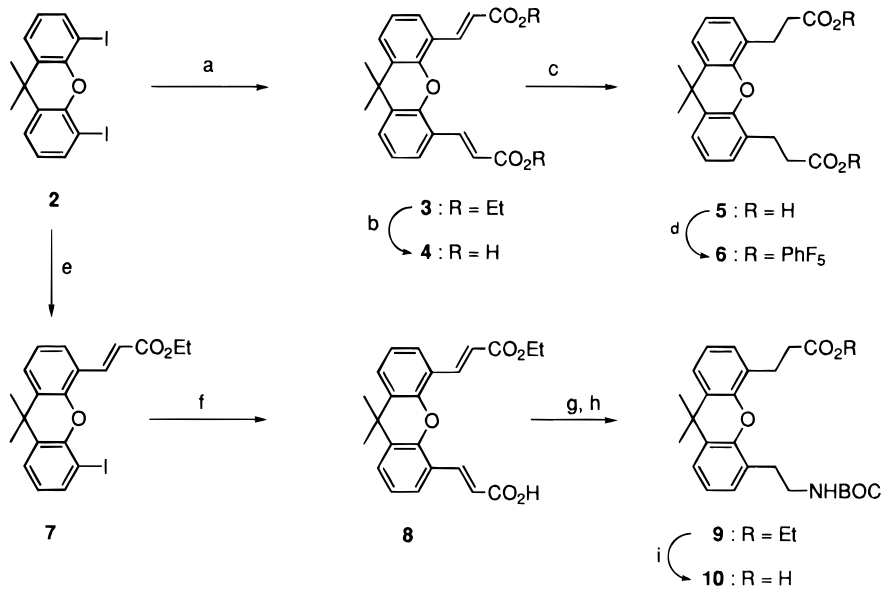


(1)

Owing to the potential of a 4,5-disubstituted-9,9-dimethylxanthene-based amino acid, a convenient route for its regioselective synthesis was therefore sought. The synthesis of xanthene derivatives has previously been accomplished utilizing substituted phenols in the Ullmann condensation to form the xanthone skeleton. In some cases, subsequent electrophilic aromatic substitution was necessary to further functionalize the skeleton. Unfortunately, the Ullmann condensation requires strongly acidic conditions and is sensitive to the substitution pattern of the phenol.[23−28] The commercial availability of both xanthene and xanthone have made the electrophilic aromatic substitution route popular. However, due to the enhanced reactivity of the 2 and 7 positions to classical electrophilic aromatic substitution conditions blocking groups must be used at the 2 and 7 positions to achieve 4,5 electrophilic substitution.[29] Since

(21) Kriz, J.; Jakes, J. *J. Mol. Struct.* **1972**, *12*, 367.
(22) Scharfenberg, P.; Rozsondai, B.; Hargittai, I. *Naturforscher* **1980**, *35a*, 431.
(23) Goldberg, A.; Walker, H. *J. Chem. Soc.* **1953**, 1348−1357.
(24) Kimura, M.; Okabayashi, I. *Chem. Pharm. Bull.* **1987**, *35*, 136−141.
(25) Shi, J.; Zhang, X.; Neckers, D. *J. Org. Chem.* **1992**, *57*, 4418−4421.
(26) Rewcastle, G.; Atwell, G.; Baguley, B.; Calveley, S.; Denny, W. *J. Med. Chem.* **1989**, *32*, 793−799.
(27) Rewcastle, G.; Atwell, G.; Zhuang, L.; Baguley, B.; Denny, W. *J. Med. Chem.* **1991**, *34*, 217−222.
(28) Rewcastle, G.; Atwell, G.; Palmer, B.; Boyd, P.; Baguley, B.; Denny, W. *J. Med. Chem.* **1991**, *34*, 491−496.

Scheme 1[a]

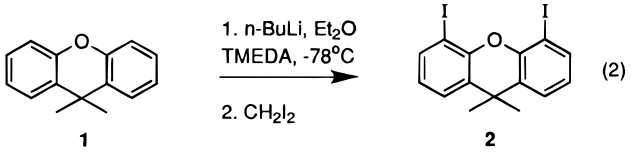


[a] (a) 2 mol % Pd $(OAc)_2$, 4 mol % P $(Ph)_3$, TEA, ethyl acrylate, acetonitrile, 75 °C, (78%); (b) NaOH, ethanol, reflux, (95%); (c) 10% Pd/C, H_2, H_2O, (93%); (d) 2.5 equiv of DIC, 2.5 equiv pentafluorophenol, ethyl acetate/DMF 8:3, (75%); (e) 4 mol % Pd $(OAc)_2$, 2 mol % P $(Ph)_3$, ethyl acrylate, TEA, 75 °C, (25%); (f) 2 mol % Pd $(OAc)_2$, 4 mol % P $(Ph)_3$, TEA, acrylic acid, 75 °C, (67%); (g) 1.1 equiv diphenylphosphoryl azide, TEA, toluene, reflux, 2.0 equiv of *tert*-butyl alcohol, (54%); (h) 10% Pd/C, 35 psi H_2, ethanol, acetic acid, (97%); (i) $LiOH \cdot H_2O$, ethanol, 0 °C, (92%).

a tetrasubstituted product (2, 4, 5, and 7 positions) is inconsistent with the design target, an alternative route was pursued. Metalation studies on xanthone using lithium 1,3-diaminopropane-*N*,*N*,*N*′,*N*′-d_4 as the base and deuterium oxide as the quencher have demonstrated that deprotonation occurs very rapidly at the 4 and 5 positions.[30] Therefore, this anionic strategy was adopted for the regioselective synthesis of 4,5-disubstituted xanthenes and the subsequent synthesis of the unnatural amino acid **10** and the potential parallel β-sheet nucleator, active diester **6** (Scheme 1).

Results and Discussion

Xanthone was converted to 9,9-dimethylxanthene **1** using trimethylaluminum following a previously reported procedure.[29] The preparation of **6** and **10** from **1** employs the common synthetic intermediate 4,5-diiodo-9,9-dimethylxanthene (**2**) (Scheme 1). Diiodide **2** was formed by the treatment of **1** with n-BuLi in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) at −78 °C producing the 4,5-dianion, which was subsequently quenched by rapid addition of diiodomethane (eq 2). Less than 5% of the undesirable alkylation product resulting from nucleophilic displacement of iodine from diiodomethane was observed. However, slow addition of diiodomethane to the dianion resulted in a greater yield of the undesirable nucleophilic addition product.



(2)

A number of other iodine-quenching agents were tested; however, these sources of iodine proved to be problematic with regard to purification and/or product yield.[31] Carbon tetrabromide and tetrachloride quenching agents gave better yields, but the resulting products were for the most part unreactive to the cross-coupling conditions employed in subsequent steps.[32]

The activated diester **6** was prepared by conversion of the common intermediate **2** to the symmetrical diester **3** through a cross-coupling with ethyl acrylate (2.5 equiv) in the presence of palladium acetate (2 mol %), triphenylphosphine (4 mol %), and triethyl amine at 75 °C in acetonitrile.[31,33] Diester **3** was saponified with NaOH in ethanol followed by hydrogenation with 10% Pd/C and H_2 to produce the saturated diacid **5**. Diacid **5** was converted to the activated potential parallel β-sheet nucleator, diester **6**, by treatment with pentafluorophenol in the presence of diisopropylcarbodiimide. Diester **6** could be directly incorporated into peptides by solution fragment condensation with the amino terminus of purified, side chain protected peptides.

Unnatural amino acid **10** was prepared from the common intermediate **2** through sequential palladium-catalyzed cross-coupling reactions to produce the unsymmetrical unsaturated monoacid monoester **8**. The first cross-coupling employed palladium acetate (4 mol %), triphenylphosphine (2 mol %), ethyl acrylate (1.5 equiv), and triethyl amine at 75 °C to produce the monoiodide monoester **7** in a 25% yield.[31,33] The remaining starting material (75%) did not react and was recovered and recycled. A second cross-coupling employing **7** in the

(29) Nowick, J.; Ballester, P.; Ebmeyer, F.; Rebek, J. *J. Am. Chem. Soc.* **1990**, *112*, 8902−8906.

(30) Abrams, S. *J. Labelled Compd. Radiopharm.* **1987**, *29*, 941−948.

(31) Arnold, R.; Kulenovic, S. *J. Org. Chem* **1978**, *43*, 3687−3689.

(32) Quenching the reaction mixture of **1** with carbon tetraiodide produced **2** in a 40% yield; however, purification proved difficult due to iodinated xanthene side products. The aryl halide products resulting from quenching the reaction mixture with either carbon tetrabromide (88% yield) or carbon tetrachloride (74% yield) were easily purified but were unreactive in subsequent palladium cross-coupling reactions.

(33) Plevyak, J.; Dickerson, J.; Heck, R. *J. Org. Chem.* **1979**, *44*, 4078−4080.

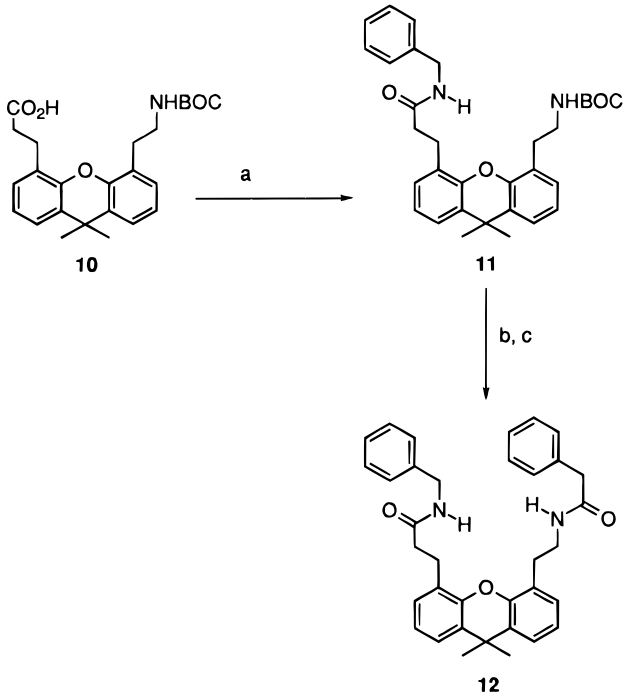


Figure 3. Conversion of compound **10** to compound **12** through **11**: (a) 1.5 equiv of BOP, 1.5 equiv of benzylamine, 3.0 equiv of DIEA, CH_2Cl_2; (b) 25% TFA/CH_2Cl_2, 50 min; (c) 1.5 equiv of BOP, 2.0 equiv phenylacetic acid, 3.5 equiv of DIEA, CH_2Cl_2, (25%).

presence of palladium acetate (2 mol %), triphenylphosphine (4 mol %), and acrylic acid gave the monoacid monoester **8** in a 67% yield.[31,33] The initial strategy called for hydrogenation of the monoacid monoester and subsequent Curtius rearrangement of the propionic acid side chain. This strategy yielded a myriad of products via cyclic intramolecular intermediates, presumably due to the proximity of the propionic acid derived side chains to one another throughout the course of the reaction.[34−37] Therefore, the unsymmetrical unsaturated monoacid monoester **8** was treated with diphenylphosphoryl azide and triethylamine in refluxing toluene to produce the unsaturated monoester monoisocyanate intermediate, which was trapped with anhydrous *tert*-butyl alcohol to afford the unsaturated monoester monocarbamate **9** in a 67% yield.[38−40] The purified material was hydrogenated using 10% Pd/C and H_2 in ethanol/acetic acid (4:1) followed by hydrolysis with LiOH in ethanol at 25 °C to give the amino acid **10** directly, in a 40% overall yield from **7**.

Diamide **12** was prepared using the route shown in Figure 3, to evaluate the preferred hydrogen-bonding pattern in amides derived from **10**. The Boc-protected xanthene-based amino acid **10** was treated with benzylamine in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) to afford monobenzamide monocarbamate **11**. The BOC protecting group was then removed using 25% TFA in dichloromethane at room temperature for 50 min. The free amine was treated with phenylacetic acid in the presence of BOP to afford diamide **12**.

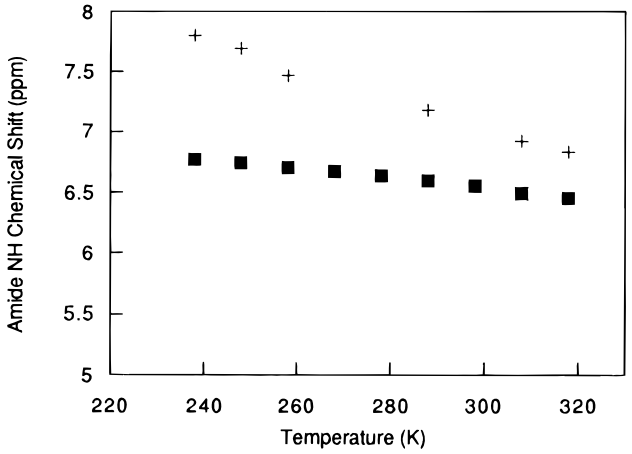


Figure 4. Temperature dependency of amide proton chemical shift for diamide **12** in 1.5 mM $CDCl_3$ solution. Data points at 268, 278, and 298 K for amide proton A are not represented due to overlap with the aromatic region of xanthene: (+) NH of **12**, amide A; (■) NH of **12**, amide B.

Previous hydrogen-bonding studies on acyclic amides, in noncompetitive solvents such as CH_2Cl_2, have shown that clear differences exist between the temperature dependence of hydrogen-bonded vs non-hydrogen-bonded amide proton chemical shifts.[41−43] A temperature coefficient less than −10 ppb/K is indicative of a hydrogen-bonded amide proton, while a dependence greater than −5 ppb/K is characteristic of a non-hydrogen-bonded amide proton. Generally, hydrogen-bonded amides are downfield of a free amide NH. FT-IR spectroscopy has also been used to detect the presence of hydrogen-bonded amide hydrogens due to an altered force constant which shifts the NH stretching band from 3400 to 3500 cm^{-1} for non-hydrogen-bonded amide hydrogens to a broadened 3200−3400 cm^{-1} band for hydrogen-bonded amide NHs.

Variable temperature NMR experiments verified the presence of both a hydrogen-bonded and non-hydrogen-bonded amide pair in amide **12** (Figure 4). Corroboration was provided by the IR spectra of **12** in chloroform which showed a sharp band at 3443 cm^{-1}, indicative of a non-hydrogen-bonded amide hydrogen, and a broad band at 3327 cm^{-1}, corresponding to a hydrogen-bonded amide hydrogen (see supporting information, concentration independent from 0.5 to 6 mM). In order to assign the amide proton involved in an intramolecular hydrogen bond, a DQCOSY experiment was carried out at 258 K to unambiguously assign both protons. It was determined that the amide signal at 7.46 ppm (258 K) corresponded to amide A and that the signal at 6.7 ppm (258 K) was that of amide B, (eq 1). This result demonstrates that a 13-membered hydrogen-bonded ring is present in $CDCl_3$ solution (eq 1) due to the fact that amide A exhibited a temperature dependence of −12 ppb/K, characteristic of hydrogen bonding, while amide B showed a temperature dependence of only −4 ppb/K.[44] The curvature in the temperature dependent amide plots

(34) Chin, J.; Breslow, R. *Tetrahedron Lett.* **1982**, *23*, 4221−4224.
(35) Kluger, R.; Hunt, J. *J. Am. Chem. Soc.* **1989**, *111*, 5921−5925.
(36) Sauers, C.; Gould, C.; Ioannou, E. *J. Am. Chem. Soc.* **1972**, *94*, 8156−8163.
(37) Ishikawa, K.; Endo, T. *J. Am. Chem. Soc.* **1988**, *110*, 2016−2017.
(38) Hocking, M. *Can. J. Chem.* **1968**, *46*, 2275−2282.
(39) Pfister, J.; Wymann, W. *Synthesis* **1983**, 38−40.
(40) Shioiri, T.; Ninomiya, S.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205.
(41) Stevens, E.; Sugawara, N.; Bonora, G.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048−7050.
(42) Liang, G.; Rito, C.; Gellman, S. *J. Am. Chem. Soc.* **1992**, *114*, 4440−4442.
(43) Gellman, S.; Adams, B.; Dado, G. *J. Am. Chem. Soc.* **1990**, *112*, 460−461.

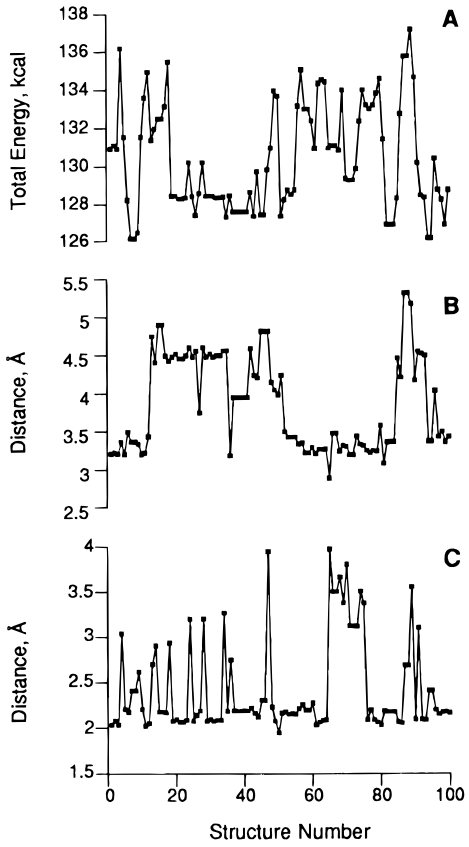


Figure 5. Molecular dynamics results for benzyl diamide **12**. (A) Total energy in kilocalories for each minimized structure. (B) Distance between indicated interior hydrogens (Figure 1) of the α-carbons of the ethylene spacer. Distances less than 4.0 Å are for *trans* arrangement of the side chains, while those greater than 4.0 Å are characteristic of *cis* arrangement (Figure 2). (C) Hydrogen bond distance of the 13-membered intramolecular ring measured from the nitrogen of amide A to the carbonyl oxygen of amide B.

at low temperature indicates some intermolecular association; however, this is not a problem at ambient temperature since the FT-IR peaks are concentration independent from 0.5 to 6.5 mM.

In an effort to understand the hydrogen-bonding results, molecular modeling studies on alkyl amide **12** and related amides (where the benzyl group is replaced by an ethyl or methyl group) were performed. A molecular dynamics study was carried out for 100 ps at 700 K starting from the extended *trans* conformation. Conformers were sampled every picosecond and minimized to a low-energy conformation, resulting in a trajectory of 100 possible conformations. Figure 1 is a ball-and-stick representation of the lowest energy conformer of **12**, as determined from these studies.

The ethylene spacers adopt a "perpendicular conformation", not the *cis* conformer observed in dibenzofuran systems, but rather a *trans* conformation, as shown in Figure 2.

Approximately 50% of the conformers of **12** sampled were in this *trans* conformation (see Figures 1 and 5, and all of the low-energy conformers had *trans*, 13-membered intramolecularly hydrogen-bonded rings. As the size of the amide substituent decreased from benzyl to methyl, the population of *trans* conformers sampled increased. This result seems to indicate that as the steric bulk of the amide substituents is decreased, the molecule is capable of adopting a more extended conformation, which favors the *trans* arrangement of the ethylene spacers (Figure 2).

It is important to point out that for the 4-(2-aminoethyl)-6-dibenzofuranpropionic acid derived amides, the 15-membered ring hydrogen-bonded *cis* conformation was favored. This conformation is thought to be important for β-sheet nucleation since it allows intramolecular hydrogen bonding between the flanking α-amino acids, analogous to the hydrogen bond pair that forms between the *i* and *i*+3 residues in a naturally occurring β-turn. In addition, this conformation facilitates hydrophobic cluster formation involving the dibenzofuran skeleton and the hydrophobic side chains of the flanking α-amino acids. It will be interesting to see what effect, if any, this altered hydrogen bond preference has on the β-sheet nucleating ability of **10** in aqueous solution. It is possible that the 15-membered ring *cis* conformation would be favored in peptides composed of **10** dissolved in aqueous buffers as a result of the hydrophobic effect, which is not operational in hydrocarbon solvents. In any case, residue **10** will prove useful in developing a further understanding of the requirements for β-sheet nucleation.

Conclusion

The potentially useful parallel β-sheet nucleator **6** and antiparallel β-sheet nucleator **10**, based on 9,9-dimethylxanthene, were regioselectively synthesized for future incorporation into peptides composed of less than 20 α-amino acid residues. Amides based on residue **10** prefer to adopt a 13-membered ring hydrogen-bonded *trans* conformation, unlike the 15-membered ring hydrogen-bonded *cis* conformation adopted by the dibenzofuran-based nucleators. Peptides composed of **10** *will* serve as useful probes to evaluate the mechanistic requirement of an effective β-sheet nucleator and to determine whether hydrophobic cluster formation could change the preferred conformation of **10** in the context of a water soluble peptide.

Experimental Section

General Methods and Material for Synthesis of 6, 10, and 12. The xanthone used in these studies was purchased from Aldrich. The *n*-BuLi used for the metalation reactions was purchased from Aldrich and titrated with 9-phenylanthracene and isopropyl alcohol prior to use. Diethyl ether was distilled from sodium/benzophenone ketyl. Toluene was distilled from calcium hydride. Acetonitrile was distilled spectral grade purchased from EM Science. *N*,*N*,*N*′,*N*′- tetramethylethylenediamine (TMEDA), diisopropylethylamine (DIEA), and triethylamine (TEA) were purchased from Aldrich and refluxed over ninhydrin, distilled, and distilled again from KOH. *tert*-Butyl alcohol was dried with magnesium sulfate and fractionally distilled onto activated 4 Å molecular sieves. Triphenylphosphine was purchased from Aldrich and recrystallized from hexanes. All other reagents were used without further purification. Thin layer chromatography was performed using aluminum sheets precoated with a 200 μm coating of 60 F_{254} silica gel as purchased from Alltech. Flash chromatography was performed according to Still.[45] Melting points were determined using a Fisher melting point apparatus and are uncorrected. Routine NMR spectra were

(44) Variable temperature NMR studies were conducted on **12** at 6.5, 1, and 0.5 mM concentrations and indicated no concentration dependence. There was downfield deviation observed for amide B at 6.5 mM, below 243 K. We ascribe this deviation to the beginning of intermolecular aggregation.

(45) Still, W. C.; Kahn, M.; Maitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

recorded on a Varian XL-200E, unless otherwise specified. Variable temperature studies and DQCOSY spectra were recorded on a Varian 500 Unity Plus spectrometer. FT-IR data were collected on a Galaxy 6021 spectrometer using a CaF_2 solution cell having a 3 mm path length. Analytical HPLC was carried out using a Perkin Elmer series 410 Bio pump equipped with a variable wavelength UV detector and integrator. HPLC was reverse phase employing C_{18} column packing unless otherwise specified. Solvent A was 95% distilled water, 5 % spectral grade acetonitrile, and 0.2% triflouroacetic acid. Solvent B was 95 % spectral grade acetonitrile, 5% distilled water, and 0.2% triflouroacetic acid. Mass determinations were carried out by the Texas A&M University Mass Spectrometry Applications Laboratory using a VG-70S double-focusing high-resolution mass spectrometer. Molecular dynamics runs of 100 ps were carried out at 700 K without constraints using the CVFF force field in discover (Biosym Technologies). Conformations were sampled at 1 ps intervals. Each structure was then minimized using conjugate gradients until the first derivative reached a minimum value of 0.001 kcal mol^{-1} $Å^{-1}$. No constraints were used in the dynamics run or the minimization. The bulk dielectric used in the trajectories was 1, to mimic the solvent properties of CH_2Cl_2 in which the experimental studies were carried out. Modeling graphics were created using MolScript.[46]

Synthesis of 4,5-Diiodo-9,9-dimethylxanthene (2). An oven-dried 250 mL round bottom flask cooled under argon was charged with 20.0 g (95 mmol) of 9,9-dimethylxanthene (**1**) and 125 mL of freshly distilled diethyl ether. The flask was flushed with argon, and the mixture was stirred for 10 min at room temperature. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine [TMEDA, 36 mL (236 mmol)] was added via syringe, and the mixture was cooled to −78 °C using a dry ice/acetone bath. After 20 min of stirring at −78 °C, 140 mL (280 mmol) of *n*-BuLi (2.0 M in hexanes) was added via syringe at a rate of 4 mL/min. The resulting dark red solution was slowly warmed to room temperature and stirred for 24 h. To an oven-dried 500 mL round bottom flask cooled under argon were added 150 mL of freshly distilled diethyl ether and 20 mL (248 mmol) of diiodomethane. The diiodomethane/ether solution was cooled to 0 °C using an ice bath and stirred for 20 min. The solution of dimethylxanthene dianion prepared above was cooled to −78 °C and transferred to the diiodomethane/ether (0 °C) solution via cannula. Upon complete addition of the dimethylxanthene dianion, the cream-colored suspension was warmed to room temperature and stirred for 12 h. Distilled water (200 mL) was added, and the mixture was stirred until two *clear* layers were observed. The layers were filtered through a Whatman (no.1) filter paper and separated, with the water layer being extracted with two 100 mL portions of diethyl ether. The ether was removed from the combined ethereal layers under vacuum to give a viscous brown oil. The oil was dissolved in acetone, filtered to remove inorganic salts, and evaporated to dryness by rotary evaporator to give 30 g (68% crude yield) of a dark brown oil. The oil was used without further purification. The reaction was monitored by TLC (95:5 petroleum ether/ethyl acetate): diiodoxanthene ($R_f = 0.56$) and monoiodoxanthene ($R_f = 0.64$). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 (s, 6H), 6.85 (t, $J = 7.8$ Hz, 2H), 7.36 (dd, $J = 1.4$, 7.8 Hz, 2H), 7.71 (dd, $J = 1.4$, 7.8 Hz, 2H). ^{13}C $CDCl_3$, 50 MHz) δ 31.99, 84.48, 125.21, 125.95, 131.45, 137.73, 150.51 $C_{15}H_{12}OI_2$ MS (EI) calcd $[M^+] = 461.8978$ found $[M^+] = 461.8956$.

Synthesis of Diethyl 9,9-Dimethylxanthene-4,5-dipropenoate (3). An oven-dried 250 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 4.0 g (8.6 mmol) of **2**. The flask was held under high vacuum for 2 h, followed by addition of 0.100 g (5 mol %) of palladium acetate and 0.46 g (20 mol %) of triphenylphosphine. The flask was capped with a rubber septum and purged with argon for 10 min. Distilled anhydrous DMF (75 mL) was added via syringe, followed by 10 mL of ethyl acrylate (92 mmol) and 2.5 mL of distilled triethylamine. The septum was replaced with an oven-dried condenser cooled under argon, and the flask was heated to 75 °C using a thermostat-regulated oil bath. The reaction was held at 75 °C for 6 h or until precipitated palladium could be seen. The flask was cooled to room temperature, and the DMF, ethyl acrylate, and triethylamine were removed under high vacuum. The resulting dark oil was partitioned between diethyl ether and water and filtered using a 45 μm nylon membrane. The layers were separated, with the water layer being extracted twice with 50 mL portions of diethyl ether. The ether layers were combined, and the ether was removed under vacuum to give a dark thick oil, further purified by flash chromatography (90:10 hexanes/ethyl acetate). The purification resulted in 2.34 g (67% yield) of **3**: ^{1}H ($CDCl_3$, 200 MHz) δ 1.35 (t, $J = 7.2$ Hz, 6H), 4.36 (q, $J = 7.2$ Hz, 4H), 6.50 (d, J, $= 16.0$ Hz, 2H), 7.12 (t, $J = 7.8$ Hz, 2H), 7.48 (m, 4H), 8.32 (d, $J = 16.0$ Hz, 1H). ^{13}C ($CDCl_3$, 50 MHz) δ 14.56, 31.56, 35.25, 60.04, 84.91, 119.36, 122.88, 123.58, 125.23, 125.89, 127.74, 131.38, 137.41, 138.94, 167.04 $C_{25}H_{26}O_5$ MS (FAB-NBA) calcd $[M + H]^+ = 407.1859$ found $[M + H]^+ = 407.1846$.

Hydrolysis and Hydrogenation To Afford Diacid 5. An oven-dried 100 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 1.0 g (2.5 mmol) of **3**, 1.0 g (25 mmol) of NaOH, and 50 mL of absolute ethanol. The flask was equipped with an oven-dried condenser, and the mixture was refluxed for 12 h. The flask was cooled to room temperature, and the diacid product was collected by filtration through a Whatman (no. 1) filter paper. The solid material was transferred to a 500 mL Parr hydrogenation vessel containing 0.100 g of 10% Pd/C and 200 mL of distilled water. The vessel was degassed by attaching an aspirator to the exit valve of the hydrogenation apparatus, evacuating the vessel, and pressurizing with H_2. These actions were performed three times, to insure that a H_2 atmosphere was present. The vessel was then pressurized to 55 psi and agitated for 12 h. The palladium was removed by filtration using a 45 μm nylon filter. The product was collected by neutralization with 12 M HCl and filtration through a 45 μm nylon membrane. The material was then redissolved into 100 mL of boiling ethanol, which caused aggregation of any remaining Pd/C, and was subsequently removed by filtration through a 45 μm nylon membrane. The ethanol was removed under aspirator vacuum to give 0.83 g (94% yield) of a white powder **5**. ^{1}H (acetone, 200 MHz) δ 1.76 (s, 6H), 2.88 (t, $J = 7.8$ Hz, 4H), 3.29 (t, $J = 7.8$ Hz, 4H), 7.19 (t, $J = 7.6$ Hz, 2H), 7.34 (d, $J = 7.4$ Hz, 2H), 7.56 (d, $J = 7.8$ Hz, 2H). ^{13}C (acetone, 50 MHz) δ 41.53, 47.72, 49.74, 50.07, 138.92, 140.41, 144.03, 144.1, 146.00, 164.15, 189.25. $C_{21}H_{22}NO_5$ MS (FAB-thioglycerol/1% TFA) calcd $[M + Na]^+ = 354.1467$ found $[M + Na]^+ = 354.1492$.

Pentafluorophenol Active Ester 6. An oven-dried 25 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 50 mg (0.1 mmol) of **5**. The flask was held under high vacuum for 2 h, followed by addition of 50 mg (0.25 mmol) of pentafluorophenol, 0.05 mL of diisopropylcarbodiimide, and 10 mL of an ethyl acetate/DMF solution (70/30 v/v). The flask was capped with a rubber septum, purged with argon for 10 min, and then stirred under argon for 12 h. The precipitated DIC urea was removed by filtration through a 45 μm nylon membrane. The ethyl acetate/DMF solution was removed under aspirator vacuum, and the resulting solid was purified by flash chromatography (85:15 hexanes:ethyl acetate) to give 74 mg of **6** (76% yield). ^{1}H ($CDCl_3$, 200 MHz) δ 1.63 (s, 6H), 3.08 (m, 4H), 3.26 (m, 4H), 7.05 (t, $J = 7.4$ Hz, 2H), 7.13 (dd, $J = 2.1$, 7.8 Hz, 2H), 7.33 (dd, $J = 2.1$, 7.6 Hz, 2H). ^{13}C ($CDCl_3$, 50 MHz) δ 25.69, 32.39, 33.49, 34.22, 123.11, 124.98, 126.34, 127.85, 130.15, 147.96, 168.78. $C_{33}H_{20}F_{10}O_5$ MS (FAB-NBA/MeOH) calcd $[M + F]^+ = 705.1135$; found $[M + F]^+ = 705.1136$, $[M]^+ = 686.1152$.

Synthesis of Ethyl 4-Iodo-9,9-dimethylxanthene-5-propenoate (7). An oven-dried 250 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 10 g (216 mmol) of **2**. The flask was held under high vacuum for 2 h, followed by addition of 0.100 g (4 mol %) of palladium acetate and 0.05 g (2 mol %) of triphenylphosphine. The flask was capped with a rubber septum and purged with argon for 10 min. Distilled spectral grade acetonitrile (50 mL) was added via syringe, followed by 2.5 mL of ethyl acrylate (240

(46) Kraulis, J. *J. Appl. Crystallogr.* **1991**, *24*, 946−950.

mmol) and 7.6 mL of distilled triethylamine. The septum was replaced with an oven-dried condenser cooled under argon, and the flask was heated to 75 °C using a thermostat-regulated oil bath. The reaction was held at 75 °C for 6 h or until precipitated palladium could be seen. The flask was cooled to room temperature, and the acetonitrile was removed under aspirator vacuum. The resulting dark oil was partitioned between diethyl ether and water and filtered using a 45 μm nylon membrane. The layers were separated, with the water layer being extracted twice with 50 mL portions of diethyl ether. The ether layers were combined, and the ether was removed under vacuum to give a dark thick oil which was further purified by flash chromatography (95:5 hexanes/ethyl acetate). the purification resulted in 2.23 g (24% yield) of **7** and 7.5 g of **2**. ^{1}H ($CDCl_3$, 200 MHz) δ 1.36 (t, J = 7.2 Hz, 3H), 1.59 (s, 6H), 4.30 (q, J = 7.2 Hz, 2H), 6.56 (d, J = 16.2 Hz, 1H), 6.85 (t, J = 7.8 Hz, 1H), 7.11 (t, J = 7.2 Hz, 1H), 7.39 (m, 2H), 7.50 (m, 1H), 7.70 (m, 1H), 8.62 (d, J = 16.2 Hz, 1H). ^{13}C ($CDCl_3$, 50 MHz) δ 15.84, 32.96, 36.29, 61.78, 86.20, 120.74, 124.15, 124.96, 126.37, 126.50, 127.27, 129.03, 132.50, 132.70, 150.43, 150.77, 168.38. $C_{20}H_{20}O_3I$ MS (FAB-NBA/CH_2Cl_2) calcd [M + H] = 435.0454 found [M + H] = 435.0466.

Synthesis of 4-((Ethoxycarbonyl)ethenyl)-9,9-dimethylxanthene-5-propenoic Acid (8). An oven-dried 100 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 3.13 g (72 mmol) of **7**. The flask was held under high vacuum for 2 h, followed by addition of 0.032 g (2 mol %) of palladium acetate and 0.075 g (4 mol %) of triphenylphosphine. The flask was capped with a rubber septum and purged with argon for 10 min. Distilled spectral grade acetonitrile (50 mL) was added via syringe, followed by 0.54 mL of acrylic acid (7.9 mmol) and 2.5 mL of triethylamine. The septum was replaced with an oven-dried condensor cooled under argon. The flask was heated to 75 °C using a thermostat-regulated oil bath and held for 6 h or until precipitated palladium could be seen. The flask was cooled to room temperature, and the acetonitrile was removed under aspirator vacuum. The resulting dark thick oil was purified by flash chromatography (70:29:1 hexanes/ethyl acetate/acetic acid) to give 1.73 g (63% yield) of a light brown oil. ^{1}H ($CDCl_3$, 200 MHz) δ 1.34 (t, J = 7.1 Hz, 3H), 1.62 (s, 6H), 4.34 (q, J = 7.1 Hz, 2H), 6.51 (d, J = 16.2 Hz, 1H), 6.56 (d, J = 16.0 Hz, 1H), 7.12 (m, 2H), 7.47 (m, 4H), 8.30 (d, J = 16.2 Hz, 1H), 8.38 (d, J = 16.0 Hz, 1H), 9.25 (broad s, 1H) ^{13}C ($CDCl_3$, 50 MHz) δ 14.38, 31.77, 34.32, 60.75, 119.10, 120.36, 122.56, 122.99, 123.52, 125.70, 126.14, 127.70, 128.21, 130.84, 131.01, 138.09, 140.72, 148.38, 148.70, 166.93, 171.57. $C_{23}H_{22}O_5$ MS (FAB-NBA/TFA/$CHCl_3$) calcd $[M + H]^+$ = 379.1546; found $[M + H]^+$ = 379.1523.

Curtius Rearrangement and Subsequent Hydrogenation of 8 To Afford 9. An oven-dried 100 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 1.25 g (3.3 mmol) of **8**. The flask was held under vacuum for 48 h, backfilled with argon, capped with a rubber septum, and purged with argon for 30 min. Freshly distilled toluene (50 mL) was added via syringe, followed by 0.80 mL of diphenylphosphoryl azide (3.7 mmol, 1.1 equiv) and 1.2 mL of distilled triethylamine (2.6 equiv). The septum was replaced with an oven-dried condenser cooled under argon, and the flask was heated to reflux. After 90 min, the flask was cooled to room temperature and 0.24 mL (1 equiv) of dried *tert*-butyl alcohol was added through the condenser. The flask was again heated to reflux. After 8 h, another equivalent of dried *tert*-butyl alcohol was added and the flask was heated to 45 °C for an additional 8 h. The flask was then cooled to room temperature, and the toluene was removed under aspirator vacuum to give a thick pale green oil which was purified by flash chromatography (85:15 hexanes/ethyl acetate) to give 0.81 g (54% yield) of the unsaturated amino acid precursor. The majority (0.71 g) of this material was dissolved in 30 mL of ethanol and 3 mL of glacial acetic acid and transferred to a 75 mL Parr hydrogenation vessel containing 0.400 g of 10% Pd/C. The vessel was degassed by attaching an aspirator to the outlet valve of the hydrogenation apparatus, evacuating the vessel, and pressurizing with H_2. These actions were performed three times to insure that a H_2 atmosphere was present. The vessel was then pressurized to 35 psi and agitated for 12 h. The palladium was removed by filtration through a 45 μm nylon membrane. The product was isolated by removing the ethanol and acetic acid under high vacuum to give 0.69 (52% yield) of **9** and approximately 10% of the *tert*-butyl ester ethyl carbamate product from exchange of the *tert*-butyl and ethyl protecting groups. This product was difficult to remove by flash chromatography; however, failure of the *tert*-butyl ester to be hydrolyzed in the subsequent step provided a convenient means to separate the two compounds. For **9**: ^{1}H ($CDCl_3$, 200 MHz) δ 1.26 (t, J = 7.0 Hz, 3H), 1.43 (s, 9H), 1.60 (s, 6H), 2.67 (t, J = 8.0 Hz, 2H), 2.97 (t, J = 7.0 Hz, 2H), 3.10 (t, J = 8.0 Hz, 2H), 3.45 (m, 2H), 4.19 (q, J = 7.0 Hz, 2H), 7.04 (m, 4H), 7.30 (d, J = 7.6 Hz, 2H). ^{13}C ($CDCl_3$, 50 MHz) δ 14.21, 26.45, 28.36, 31.29, 34.15, 34.83, 40.69, 60.57, 78.86, 122.84, 124.41, 124.45, 126.12, 127.64, 127.82, 128.59, 129.99, 130.0, 148.23, 156.02, 173.36. $C_{27}H_{35}NO_2$ MS (FAB-NBA/NaI/acetone) calcd $[M + H]^+$ = 454.2593; found $[M + H]^+$ = 454.2598.

Hydrolysis od 9 To Afford Unnatural Xanthene Amino Acid 10. An oven-dried 100 mL round bottom flask cooled in a desiccator over calcium sulfate (Drierite) was charged with 0.18 g (0.4 mmol) of **9** and 50 mL of absolute ethanol. This mixture was cooled to 0 °C under argon using an ice bath and stirred for 10 min. $LiOH{\cdot}H_2O$ (0.100 g, 2.3 mmol) was added, and the mixture was slowly warmed to room temperature. The saponification reaction was monitored by HPLC at 254 nm, and upon completion, the reaction was quenched with 10 mL of 1 M citric acid. The ethanol was removed under vacuum, resulting in the precipitation of the product, which was collected by extraction with dichloromethane. The methylene chloride was removed under aspirator vacuum, and the material was held under high vacuum overnight to give 0.155 g (92%) of **10**. ^{1}H ($CDCl_3$, 200 MHz) δ 1.40 (s, 9H), 1.61 (s, 6H), 2.71 (t, J = 7.8 Hz, 2H), 2.95 (t, J = 7.6 Hz, 2H), 3.12 (t, J = 7.8 Hz, 2H), 3.40 (m, 2H), 5.06 (bs, <1H), 7.05 (m, 4H), 7.29 (d, J = 7.8 Hz, 2H). $C_{25}H_{31}NO_5$ MS (FAB-NBA/NaI/polyethylene glycol 400 and 600) calcd [M + Na] = 448.2099; found [M + Na] = 448.2103.

BOP Coupling To Afford Diamide 12. Following a previously reported procedure, diamide **12** was prepared in a 25% yield) of **10**:[19] ^{1}H ($CDCl_3$, 500 MHz) δ 1.6 (s, 6H), 2.6 (m, 2H), 2.9 (m, 2H), 3.1 (m, 2H), 3.4 (m, 4H), 4.4 (d, J = 6.0 Hz, 2H), 6.4 (t, J = 8.2 Hz, 1H), 7.0 (m, 4H), 7.2 (m, 13H) $C_{35}H_{36}N_2O_4$ MS (FAB-NBA/CH_2Cl) calcd $[M + H]^+$ = 533.2804 found $[M + H]^+$ = 533.2833.

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Supporting Information Available: IR data, concentration dependent VT-NMR for **12**, and NMR spectra for all compounds (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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