Base-Induced Dimerization of Urethane-Protected Amino Acid *N*-Carboxanhydrides

Johann J. Leban*,† and Kimberly L. Colson‡

Divisions of Organic Chemistry and Bioanalytical Sciences, Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709

Received July 13, 1995⊗

tert-Butyloxycarbonyl-protected *N*-carboxanhydrides of amino acids dimerize in the presence of base in aprotic media to form 3,5-dialkyl-2,4-dioxo-1-pyrrolidine analogs. Depending on the nature of the base, different ratios of isomers were obtained. The reaction with lithium bis(trimethylsilyl)-amide lead to one isomer only. After deprotection of the *tert*-butyloxycarbonyl groups and coupling of (benzyloxycarbonyl)valine, a homogeneous product was obtained. Reduction with sodium borohydride again gave a homogeneous product. Nuclear Overhauser enhancement spectroscopy and X-ray crystallography identified the stereochemistry in positions 3 and 5 of the pyrolidine as *Z*. When 1, 8-diazabicyclo[5.4.0]undec-7-ene was used as the base, the condensation led to a 1:3 ratio of isomers. The major isomer was different from the one obtained with lithium bis-(trimethylsilyl)amide. The (benzyloxycarbonyl)valine derivative from this compound was obtained as a 1:1 mixture of isomers, leading to the conclusion that this condensation product was an enantiomeric mixture of the *E* isomers. The pure *Z* isomer from the lithium bis(trimethylsilyl)-amide reaction was converted to a mixture of *Z* and *E* isomers in a ratio of 1:3 when treated with 1,8-diazabizycol[5.4.0]undec-7-ene. The (benzyloxycarbonyl)valine derivative of the *E* isomer from this conversion was again a 1:1 mixture; therefore, the *Z* isomer obtained with lithium bis-(trimethylsilyl)amide was believed to have been an enantiomeric mixture. Several other examples indicated that this reaction occurred also with other *tert*-butyloxycarbonyl-protected *N*-carboxy-anhydrides.

Introduction

N-Carboxy-α-amino acid anhydrides (NCAs), or Leuchs' anhydrides,[1] represent a unique species of amino acid derivatives where both the carboxylate activation and the amino group protection are achieved simultaneously. Their sensitivity to moisture and tendency to polymerization, however, limited broader application. For these reasons, additional protection of the α-amino group was attempted early on.

Tosyl-protected Gly-*N*-carboxanhydride was reported first by Zaoral et al.[2] Nitrophenylsulfonyl-protected Phe-*N*-carboxanhydride was used by Kricheldorf and Halstrøm for peptide synthesis.[3,4] UNCAs (Boc, Cbz, and Fmoc) and their use in peptide synthesis were described only recently.[5] These derivatives represent valuable new tools for peptide chemistry. The application of Boc-Xaa-NCAs for solid-phase synthesis in the presence of triethylamine (TEA) was reported.[6] Fmoc amino acid *N*-carboxanhydrides were reported to esterify to *p*-alkoxybenzyl alcohol resins in the presence of *N*-methylmorpholine.[7]

UNCAs have been utilized to replace isopropyl chloroformate-activated *N*-protected amino acids for various chemical transformations, such as reductions and nucleophilic reactions.[8] UNCAs are stable and can be stored for some time. They can be obtained commercially and are an attractive alternative to other activated amino acid species. Many of these reactions require the presence of base. We found that base can cause a dimerization of two molecules of the amino acid to yield pyrrolidine-2,4-diones. A few selected examples of this reaction and the characterization of the products are presented.

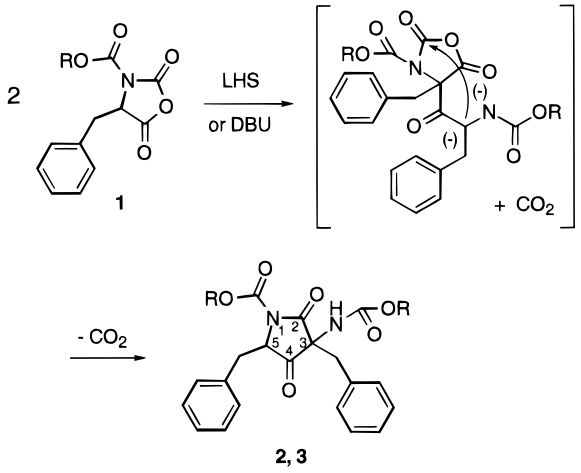
Results and Discussion

The reaction of Boc-Phe-NCA with lithium bis(trimethylsilyl)amide (LHS) in THF at −78 °C yielded, after purification, a single product with the observed mass of a di-Boc-oxopiperazine. The ^{1}H-NMR spectrum, however, was not consistent with this structure. The presence of an NH signal, one α-proton, two distinctly different benzylic signals, and two distinct signals for the Boc groups suggested structure **2** as shown in Scheme 1. HPLC analysis of the crude reaction mixture indicated one main product with the a second minor peak (9%). When the products were separated by HPLC and analyzed by mass spectroscopy, both had the same mass. The major isomer from the LHS reaction was obtained pure after workup and purification in 35% yield. Most of the nonreacted product is hydrolyzed UNCA. We avoided the use of more base and longer reaction times to encrease the yield, because such conditions tended to yield an elevated amount of the second isomer. In contrast to the reaction with LHS, the reaction with 1 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) yielded a 1:3 ratio of isomers. The major isomer **3** crystallized in pure form

* To whom correspondence should be addressed.
† Present address: Hauptplatz 24, 2421-Kittsee, Austria.
‡ Present address: P.O. Box 4392, Chapel Hill, NC 27515.
⊗ Abstract published in *Advance ACS Abstracts,* January 1, 1996.
(1) Leuchs, H.; Manasse, W. *Ber. Dtsch. Chem. Ges.* **1906**, *39*, 857.
(2) Zaoral, M.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2316−2332.
(3) Kricheldorf, H. R. *Angew. Chem.* **1973**, *85*, 86−87.
(4) Halstrøm, J.; Brunfeldt, K.; Kovacs, K. *Acta Chem. Scand., Ser. B* **1979**, *B33*, 685−689.
(5) Fuller, W. D.; Cohen, M. P.; Shabankareh, M.; Blair, R. K.; Goodmann, M.; Naider, F. R. *J. Am. Chem. Soc.* **1990**, *112*, 7414−7416.
(6) Xue, C.-B.; Naider, F. *J. Org. Chem.* **1993**, *58*, 350−355.
(7) Fuller, W. D.; Krotzer, N. J.; Swain, P. A.; Anderson, B. L.; Comer, D.; Goodman, M. *Peptides 1992: Proceedings of the 22nd European Peptide Symposium*; Escom: Leiden, The Netherlands, 1993; pp 231−232.
(8) Fehrentz, J.-A.; Bourdel, E.; Califano, J.-C.; Chaloin, O.; Devin, C.; Garrouste, P.; Lima-Leite, A.-C.; Llinares, M.; Rieunier, F.; Vizavonna, J.; Winternitz, F.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1994**, *35*, 1557−1560.

0022-3263/96/1961-0228$12.00/0

Scheme 1



from methanol and was identical to the minor product of the LHS reaction by HPLC and TLC; it also had the same molecular weight and an ^{1}H-NMR very similar to **2**. The product had no optical rotation, and therefore, we believed that it was a mixture of enantiomers diastereomeric to **2**. We made a (carbobenzyloxy)valine derivative **6**. HPLC analysis indicated the presence of two compounds in a 1:1 ratio, which confirmed that **3** was an enantiomeric mixture. Although we have no structural data yet to prove the *E* stereochemistry for compound **3**, one can assume this stereochemistry safely.

We used compound **2** and applied one-dimensional nuclear Overhauer enhancment spectroscopy (NOE) for the determination of the stereochemstry. Compound **2** did not yield the critical nuclear Overhauser enhancement (NOE) signal between the benzylic methylene groups that would confirm the Z stereochemistry. To identify the stereochemistry by NOE experiments, a (benzyloxycarbonyl)valine analog (**4**) was synthesized. This product was obtained in 82% yield as a single component on HPLC and TLC.

Attempts to determine the relative stereochemistry of **4** using NOE spectroscopy failed. No NOEs from the protons in positions 5 or 6 were observed to the protons in position 11 (^{1}H NMR data not shown).

To obtain another analog for more structural information we reduced the ketone at position 4 with sodium borohydride ($NaBH_4$). This reduction yielded a product that was homogeneous by TLC and HPLC, and **5** was obtained in 77% yield.

NOE data on **5** provided the information necessary to assign the relative stereochemistry at positions 3 and 5. A NOE observed between the protons on H11 (3.06 ppm) and H6 (2.24 ppm) indicated the close spatial proximity of these two protons. The observation of this NOE could only be accommodated by the relative stereochemistry as shown in Figure 1, which also displayed the key NOEs. The ^{1}H spectral data on **5** are summarized in Table 1. The results obtained identified the product as the 3,5 *Z* isomer.

We also obtained an X-ray structure of **5**. The X-ray structure confirmed the *Z* configuration. The configuration of the chiral carbons (Figure 1) corresponds to 3*R*, 4*S*, and 5*R*.

If **2** was an optically pure product, we assumed that, if it could be converted to the *E* isomer with DBU, it should again yield a single (benzyloxycarbonyl)valine derivative. We treated **2** with DBU, and again a 1:3 mixture of isomers was obtained. The major component

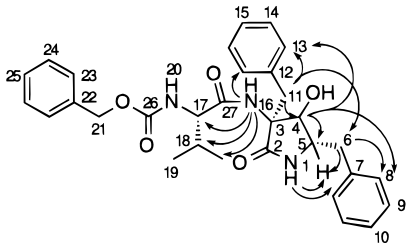


Figure 1. Summary of key NOEs observed in **5**.

Scheme 2

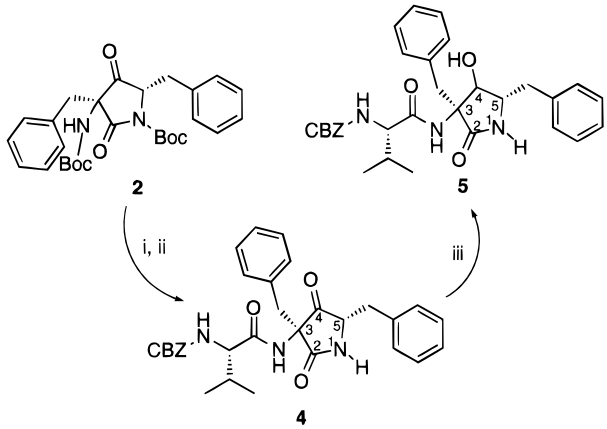


Table 1. ^{1}H NMR Chemical Shift Assignments of 5 and the Observed Nuclear Overhauser Enhancements

assignment	δ	mult	int	obsd NOEs				
1	7.87	s	1	7.11	5.49	3.81		
16	7.63	s	1	7.21	3.85	1.83	0.77	0.75
arom	7.36−7.10	m	16					
4-OH	5.49	s	1	7.87	4.37			
21	5.03	dd	2					
4	4.37	d	1	7.21	7.11	5.49	3.81	
17	3.85	d	1					
5	3.81	m	1					
11	3.06	s	2	7.21	5.49	4.37	2.24	
6	2.80	dd	1	7.11	3.81	2.24		
6′	2.24	dd	1	7.87	7.11	3.81	2.82	3.06[a]
18	1.84	s	1					
19	0.77	d	3					
19′	0.75	d	3					

[a] The intensity of this NOE was very small.

crystallized in pure form from methanol. The (benzyloxycarbonyl)valine derivative was made, and we obtained a product that was a 1:1 mixture of isomers and totally identical to **6**; therefore, **3** was an enantiomeric mixture. Although compounds **4** and **5** were diastereomeric mixtures with the *Z* configuration they did not separate by HPLC and TLC. The conditions used to get crystals for the X-ray determination then produced only one diastereomer. The fact that carbon 3 (Scheme 2) was *R* further substantiated that notion.

We wanted to better understand the influence of different types of bases in the condensation reaction and their influence on the stereochemistry of the product. A series of small scale experiments (10 μmol) were run using Boc-Phe-NCA, with various bases and solvents. The base was added at −78 °C, and the reaction was allowed to warm to room temperature. The reaction mixture was

Table 2. Diastereomeric Ratios for 2 and 3 Obtained from Reaction of Boc-Phe-NCA with Various Bases

solvent	base (equiv)	ratio of cis/trans[a]
THF	LHS (0.5)	3.6:1
THF	LHS (1)	3.7:1
DCM	TEA (0.5)	1:1.1
DCM	TEA (1)	1:1
DCM	TEA (5)	1:1.4
DCM	TEA (10)	1:1.4
THF	TEA (1)	no reaction
DCM	DMAP (1)	no reaction
DCM	NMM (1)	no reaction
DCM	DBU (1)	1:2.6
THF	DBU (0.5)	reaction
THF	DBU (1)	1:3
THF	DBU (1)[b]	1:2.7
THF	DBU (1)[c]	1:3

[a] Diastereomeric content measured by HPLC. [b] Compound **2** was incubated with DBU. [c] Compound **3** was incubated with DBU.

directly injected into the HPLC, and the ratio of isomeric products obtained was estimated from the peak areas of **2** and **3**. These HPLC conditions distinguished between the cis/trans isomers but did not indicate the enantiomeric purity of the products. The results are shown in Table 2. Little product was obtained with 0.5 equiv of DBU, while the reaction proceeded more with 1 equiv of DBU. It was important to notice that the reaction did not occur with TEA in THF but did proceed in DCM, indicating that with the proper choice of solvent the condensation can be avoided. Furthermore, no reaction was observed with NMM or 4-(dimethylamino)pyridine (DMAP). This is important because these reagents are common when UNCAs are used as activated amino acid species. The last three experiments in Table 2 again show on an analytical scale the formation of a 1:3 mixture of **2** and **3** with DBU. The interconversion of pure **2** into a 1:3 mixture of **2** and **3** and finally the conversion of pure **3** into a 1:3 mixture of **2** and **3** occurs in the presence of DBU. It is worth noticing that weaker bases DBU and TEA yielded more of the *E* isomers while LHS and LDA (data for LDA not shown) resulted in more *Z* isomer. We believe that the slower reaction with the weaker bases is thermodynamicly controlled. It is also interesting to note that TEA in THF did not give much of the product while it did in DCM. More data will be needed to come to a precise conclusion on the nature of the relationship between solvent, base, and condensation product.

We were interested in whether this reaction is applicable to other Boc-amino acid NCA's and followed the dimerization with a few more examples. The condensation of UNCAs using LHS was not restricted to the Phe analog and worked well with Boc-Ile-NCA and Boc-Thr-(Bzl)-NCA, yielding single isomers in both cases. When the α-side chain was bulky, one isomer was preferred, while a 1:1 mixture of isomers was obtained with Boc-Ala-NCA. No further experiments to determine the stereochemistry of these analogs were performed. It can, however, be expected that an enantiomeric mixture of *Z* isomers is obtained with Boc-Ile-NCA and Boc-Thr(Bzl)-NCA and the enantiomers of both *E* and *Z* in the case of Boc-Ala-NCA. Preliminary data indicated (data not shown) that the condensation was applicable to Cbz-Phe-NCA, but the reaction was less clean than for the Boc-derivative.

The reaction mechanism of the condensation can be explained by the increased acidity of the α-proton in the UNCAs compared to the protected amino acid. After deprotonation with LHS, another UNCA molecule acylated the first at the α-carbanion, and then the acylated UNCA species, in turn, acylated the amino group of the incoming molecule (see Scheme 1). Since the anion was planar an enantiomeric mixture was formed. The *Z* isomeric enantiomers were formed in a kinetic controlled reaction. Contrary to the LHS reaction, the reaction with 1 equiv of DBU led to a racemic mixture of *E* enantiomers in a thermodynamicly controlled reaction due to the slow deprotonation. This proposed mechanism is corroborated by the fact that both the *Z* and *E* condensation products equilibrate to the same ratio of *Z* and *E* isomers when incubated with DBU.

It would be interesting to learn if such a intermolecular condensation could be accomplished with two different amino acids. One would expect a clean product only if the acidity of the α-proton of the respective UNCAs were sufficiently different or if steric effects favor the condensation of different species of UNCA. Preliminary data indicate that such condensations indeed could be achieved under special circumstances. Such work will be the scope of future reports.

Conclusion

Pyrrolidine-2,4-diones are novel amino acid derived heterocycles obtained by base-induced condensation of UNCAs and may be useful as a chiral auxillary or intermediate in medicinal chemistry. The reaction can be controlled to obtain the enantiomeric mixtures of *Z* and *E* isomers, depending on what base is used. The correct choice of base and solvent can eliminate this reaction when UNCAs are used during peptide synthesis.

Experimental Section

Urethane-protected amino acids were obtained from SNPE, Inc., of Princeton, NJ. Other chemicals were supplied by the Aldrich Chemical Co., Milwaukee, WI.

Structure Determination of 5 by Proton NMR. ^{1}H NMR data on **5** were acquired on a Varian Unity-400 equipped with a Nalorac 5 mm inverse detection triple resonance gradient probe at 26 °C. ^{1}H NOE spectra were acquired for the protons resonating at 7.87, 7.63, 5.49, 4.37, 3.06, 2.80, and 2.24 ppm using a sweep width of 5000 Hz, an acquisition time of 0.499 s, and a relaxation delay of 6 s. ^{1}H NMR data on **4** were acquired on a Varian VXR-300 equipped with a Nalorac 5 mm quad nucleus probe at 26 °C. ^{1}H NOE spectra of **4** were acquired for the protons resonating at 9.04, 8.44, 5.01, 3.11, 2.78, 1.97, and 1.03 ppm using a spectral width of 4400 Hz, an acquisition time of 0.502 s, and a relaxation delay of 8 s. Selective irradiation to accomplish the dipolar exchange was employed throughout the relaxation delay. All NOE experiments were processed using the difference method. The ^{1}H chemical shift assignments were made with coupling data obtained from a COSY experiment. The COSY experiments were acquired using a sweep width of 5000 Hz, a relaxation delay of 1 s, and an acquisition time of 0.205 s. The COSY experiments were acquired using 512 points in F1 and 2048 points in F2 and processed, using a sinebell weighting, to give a 2K by 2K matrix.

Structure determination of 5 by X-ray crystallography: $C_{31}H_{35}N_3O_5$; $^1/_2H_2O$, $M_r = 538.64$; monoclinic, $P21$; $a =$ 1 7.100(4) Å, $b = 5.180(2)$ Å, $c = 23.292(2)$ Å, $V = 5972(1)$ Å^3, $Z = 8$, $D_c = 1.198$ g/cm^3; graphite-monochromated Cu Kα radiation $\lambda = 1.541\ 78$ Å; reflections: total 9580, unique 9233 ($R_{int} = 0.149$), $F(000) = 2296.00$; structure solution by direct methods[9] and structure refinement using full-matrix least-squares methods.[10]

HPLC Analysis. Analysis of diastereomer content was determined by HPLC using a Waters Novapak C_{18} radialpak column. The sample was eluted with 0.1% TFA/H_2O (A) and 0.1% TFA/acetonitrile (B) (20−90% B over 44 min).

General Procedure for Base-Induced Dimerization of UNCAs. A solution of 10 mmol of urethane-protected amino acid−NCA in 25 mL of dry THF was cooled to −78 °C under N_2. A solution of 1 M LHS in THF (5 mL) was added slowly via syringe. The reaction mixture was allowed to warm to room temperature (ca. 2 h). It was then quenched with 20 mL of 1 N HCl and extracted with EtOAc. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo* to yield a thick oil. The product was purified by flash column chromatography (hexane/EtOAc 2:1, unless otherwise described). Optical rotations were measured in EtOAc (1 g/100 mL).

***tert*-Butyl 3,5-(*Z*)-Dibenzyl-3-[(*tert*-butoxycarbonyl)-amino]-2,4-dioxo-1-pyrrolidinecarboxylate (2) (Derived from Boc-Phe-NCA).** The product was purified by hexane/EtOAc (3:1) to obtain 1.73 g (35%). ^{1}H NMR (d_6-DMSO) δ: 8.4 (s, 1H), 7.4−6.9 (m, 10H), 3.2 (m, 1H), 3.1−2.8 (m, 4H), 1.5 (s, 9H), 1.4 (s, 9H). Mass spectra: *m/e* 517.2 $(M + Na)^+$. Anal. Calcd for $C_{28}H_{34}N_2O_6$: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.23; H, 7.12; N, 5.60. HPLC: t_R = 34.71 min.

***tert*-Butyl 3,5-(*E*)-Dibenzyl-3-[(*tert*-butoxycarbonyl)-amino]-2,4-dioxo-1-pyrrolidinecarboxylate (3) (Derived from Boc-Phe-NCA).** DBU was substituted for LHS. Crude product was recrystallized from MeOH to yield 0.63 g (26%). This is the second peak by HPLC. ^{1}H NMR (d_6-DMSO) δ: 8.5 (s, 1H), 7.4−7.0 (m, 10H), 3.3 (m, 1H), 3.2−2.9 (m, 4H), 1.45 (s, 9H), 1.4 (s, 9H). Mass spectra: *m/e* 295.0 $(MH - 2Boc)^+$. Anal. Calcd for $C_{28}H_{34}N_2O_6$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.98; H, 6.91; N, 5.74. HPLC: t_R = 35.45 min.

***N*-α-(Benzyloxycarbonyl)-L-valine *N*-(3,5-(*Z*)-Dibenzyl-2,4-dioxo-3-pyrrolidinyl)amide (4).** A mixture containing **2** (1.0 g, 2 mmol) in a solution of 10 mL of 1 M HCl/EtOAc was stirred for 1 h, concentrated, and dried. This residue, Cbz-Val-OH (0.51 g, 2 mmol), EDC (0.39 g, 2 mmol), HOBt (0.27 g, 2 mmol) and NMM (0.22 mL, 2 mmol) in DMF were stirred for 16 h at 25 °C. The reaction was quenched with 5% citric acid and extracted with EtOAc. The organic phase was washed with 5% $NaHCO_3$, 5% citric acid, and water, dried with $MgSO_4$, and concentrated. The residue was purified over silica gel (hexane/EtOAc, 1.5:1) to yield 0.43 g of a white solid (82%). ^{1}H NMR (d_6-DMSO) δ: 9.0 (s, 1H), 8.4 (s, 1H), 7.4−7.1 (m, 13H), 6.8 (m, 2H), 5.0 (m, 2H), 4.0−3.9 (m, 2H), 3.2−2.7 (m, 4H), 2.0−1.8 (m, 2H), 1.0 (m, 1H), 0.8 (m, 6H). Mass spectra: *m/e* 528.4 $(MH)^+$. Optical rotation: $[\alpha]^{20}_{589} = -131.0°$.

***N*-α-(Benzyloxycarbonyl)-L-valine *N*-(3,5-(*Z*)-Dibenzyl-4-hydroxy-2-oxo-3-pyrrolidinyl)amide (5).** Compound **4** (0.9 g, 1.7 mmol) was dissolved in 50 mL of MeOH. An excess of $NaBH_4$ was added and the reaction stirred 20 min. The reaction was concentrated, water was added, and a white solid was filtered. Recrystallization from 30 mL of MeOH yielded 0.3 g of white solid (77%). ^{1}H NMR (see Figure 1). Mass spectra: *m/e* 530.4 $(MH)^+$. Anal. Calcd for $C_{31}H_{35}N_3O_5$(0.65 mol H_2O): C, 68.80; H, 6.71; N, 7.77. Found: C, 68.84; H, 6.68; N, 7.82. $[\alpha]^{20}_{589} = -113.9°$.

***N*-α-(Benzyloxycarbonyl)-L-valine *N*-(3,5-(*E*)-Dibenzyl-2,4-dioxo-3-pyrrolidinyl)amide (6).** Compound **3** (2 g, 4.04 mmol) was dissolved in 10 mL of 1 M HCl in EtOAc and stirred for 1 h. The solvent was removed in vacuo, and the remaining solid was crystallized with ether yielding 1.5 g (91%) of white solid that was used without further purification. ^{1}H NMR (d_6-DMSO) δ: 2.7−2.8 (m, lH), 3.1−3.4 (m, 3H), 3.7−3.75 (m, lH), 7.2−7.4 (m, 10H), 9.0−9.2 (m, 3H). Mass spectra: *m/e* 295.0 $(MH)^+$.

A DMF mixture consisting of the deprotected **3** (1.23 g, 3 mmol), CBZ-Val-OH (753 mg, 3 mmol), HOBt (406 mg, 3 mmol), NMM (300 μL, 3 mmol), and EDC (575 mg, 3 mmol) was stirred at rt for 12 h. Volatiles were removed, and the residue was dissolved in EtOAc. This was then washed with 5% citric acid and 5% $NaHCO_3$, dried ($MgSO_4$), and concentrated. This material was purified over silica gel (5% MeOH/CH_2C1_2) to obtain 1.11 g (70%) of **6**. ^{1}H NMR (d_6-DMSO) δ: 0.85−0.95 (m, 6H), 1.9−2.1 (m, lH), 2.85−3.0 (m, 2H), 3.1−3.4 (m, 2H), 4.0−4.2 (m, 2H), 5.0−5.2 (m, 2H), 7.1−7.5 (m, l5H), 7.95 (s, lH), 8.4 (m, lH), 9.0 (s, lH). Mass spectra: *m/e* 550.2 $(M + Na)^+$. Anal. Calcd from $C_{33}H_{31}N_{30}$s: C, 70.57; H, 6.30; N, 7.96. Found: C, 68.84; H, 6.23; N, 8.32. HPLC: two isomers (50:50) were observed [t_R = 23.12 min (A) and 24.80 min (B)].

Conversion of 2 to 3 and Synthesis of 6. A soluton of 1.6 g of 2 (3.3 mmol) in dry THF was cooled to −78 °C under N_2, DBU (500 mL, 3.3 mmol) was added, and the reaction mixture was stirred for 2 h. The solvent was removed in vacuo and the residue partitioned between EtOAc and 5% citric acid. The organic phase was washed with 5% $NaHCO_3$, dried with $MgSO_4$, and concentrated to yield a white solid. Recrystallization from MeOH yielded 0.6 g (36%) of **3**. ^{1}HNMR (d_6-DMSO) δ: 1.35 (s, 9H), 1.45 (s, 9H), 2.9−3.2 (m, 4H), 3.3 (m, 1H), 6.9−7.3 (m, 10H), 8.45 (s, 1H). Mass spectra: *m/e* 517.4 $(M + Na)^+$. Anal. Calcd from $C_{28}H_{34}N_2O_6$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.90; H, 6.88; N, 5.74. HPLC (**3**, t_R = 35.49 min).

Compound **3** from this reaction was dissolved in 10 mL of 1 M HCl in EtOAC and stirred for 1 h. The solution was concentrated in vacuo and yielded 0.24 g of white solid. Mass spectrum: *m/e* 295.3 $(MH)^+$. A DMF solution containing 0.407 mg, 1 mmol) of this product (0.25 g, 1 mmol), CBZ-Val-OH, (0.25 g, 1 mmol) EDC, HOBt (0.176 g, 1.3 mmol), and NMM (0.22 mL, 2 μmol) was stirred for 18 h at rt. The DMF was removed in vacuo, and the residue was partitioned between EtOAc and 5% NaHCO3. The organic phase was washed with 5% citric acid, dried with $MgSO_4$, and concentrated to yield 0.48 g of white solid. Purification by silica gel chromatography (5% MeOH/CH_2Cl_2) yielded 0.21 of a material that was identical to **6**. Mass spectra: *m/e* 528.5 $(MH)^+$. HPLC: indicated the presence of two isomers (50:50). (t_R = 23.17 and 24.81 min). Both isomers were isolated by HPLC on a small scale and analyzed by mass spectroscopy. Both peaks gave a *m/e* of 528.3 $(MH)^+$.

***tert*-Butyl 3[(*tert*-butoxycarbonyl)amino]-3,5-dimethyl-2,4-dioxo-1-pyrrolidinecarboxylate (7) (Derived from Boc-Ala-NCA).** The yield was 1.14 g (23%). ^{1}H NMR (d_6-DMSO) δ: 8.25 (s, 1H), 8.15 (s, 1H), 4.65 (m, 1H), 4.35 (m, 1H), 1.5 (s, 18H), 1.45 (m, 6H), 1.35 (m, 18H), 1.3−1.25 (m, 6H). Mass spectra: *m/e* 143 $(MH - 2Boc)^+$. Anal. Calcd for $C_{16}H_{26}N_2O_6$: C, 56.11; H, 7.66; N, 8.11. Found: C, 55.90; H, 7.67; N, 8.06.

***tert*-Butyl 3,5-Bis((*R*)-1-Methylpropyl)-3-[(*tert*-butoxycarbonyl)amino]-2,4-dioxo-1-pyrrolidinecarboxylate (8) (Derived from Boc-Ile-NCA).** The yield was 1.06 g (50%). The product was purified by hexane/EtOAc (3:1). ^{1}H NMR (d_6-DMSO) δ 7.9 (s, 1H), 4.2 (m, 1H), 2.0 (m, 1H), 1.8 (m, 1H), 1.5 (s, 9H), 1.3 (s, 9H), 1.1−0.7 (m, 16H). Mass spectra: *m/e* 449 $(M + Na)^+$. Anal. Calcd for $C_{22}H_{38}N_2O_6$: C, 61.93; H, 8.98; N, 6.57. Found: C, 61.86; H, 8.93; N, 6.52. $[\alpha]^{20}_{589} = -6.35°$.

***tert*-Butyl 3,5-Bis[(*R*)-1-(benzyloxy)ethyl]-3-[(*tert*-butoxycarbonyl)amino]-2,4-dioxo-1-pyrrolidinecarboxylate (9) (Derived from Boc-Thr(Bzl)-NCA).** The yield was 1.4 g (24%). ^{1}H NMR (d_6-DMSO) δ: 8.05 (s, 1H), 7.4−7.05 (m, 10H), 4.6−4.5 (m, 2H), 4.3 (m, 2H), 4.1 (m, 1H), 4.0-3.8 (m, 2H), 1.35 (s, 18H), 1.3−1.2 (m, 6H). Mass spectra: *m/e* 605 $(M + Na)^+$. Anal. Calcd for $C_{32}H_{42}N_2O_8$: C, 65.95; H, 7.27; N, 4.81. Found: C, 65.22; H, 7.29; N, 4.76. $[\alpha]^{20}_{589} = -20.6°$.

Acknowledgment. We thank Tony Landavazo for helping us to prepare this manuscript, Brad Cozart and Brian Herring for technical assistance, and Andrew Spaltenstein for helpful discussions. We thank the scientists from MolecularStructures for solving the X-ray structure of **5**.

JO951260F

(9) Miller, R.; Gallo, S. M.; Khalak, H. G.; Weeks, C. M. *J. Appl. Crystallogr.* **1994**, *27*, 613−621.

(10) The author has deposited atomic coordinates for **5** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.