for $C_{13}H_{17}O_5$ 253.1077. Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.58; H, 7.09.

Preparation of Disulfone 8b. A colorless crystalline compound was obtained: mp 114.5–115.0 °C; R_f 0.2 (33% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 7.93–7.85 (m, 4 H), 7.69–7.61 (m, 2 H), 7.55–7.48 (m, 4 H), 5.71–5.67 (m, 1 H), 5.56–5.52 (m, 1 H), 4.72 (d, J = 3 Hz, 1 H), 3.75–3.63 (m, 7 H), 3.43 (d, J = 10 Hz, 1 H), 3.35–3.30 (m, 1 H), 2.46–2.41 (m, 1 H), 2.08–1.99 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 168.8, 139.8, 138.5, 134.5, 134.2, 133.6, 130.4, 129.7, 129.3, 129.0, 128.9, 85.5, 55.9, 52.3, 44.7, 44.0, 32.8; mass spectrum, exact mass m/e 351.0896 ($M^+ - SO_2Ph$, 10%), calcd for $C_{17}H_{19}O_6S$ 351.0993. Anal. Calcd for $C_{23}H_{24}O_8S_2$: C, 56.08; H, 4.91; S, 13.02. Found: C, 55.99; H, 4.92; S, 13.53.

Preparation of Disulfone 10. The general procedure given above also afforded this compound (along with **8b**) as a colorless crystalline compound: mp 180.0–180.5 °C; R_f 0.3 (33% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 7.98–7.84 (m, 4 H), 7.67–7.60 (m, 2 H), 7.56–7.46 (m, 4 H), 5.78–5.75 (m, 1 H), 5.48–5.45 (m, 1 H), 5.40 (d, J = 2 Hz, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 3.52 (m, 1 H), 3.43–3.39 (m, 2 H), 2.92–2.82 (m, 1 H), 2.20–2.09 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 168.9, 139.9, 138.6, 134.5, 134.3, 133.7, 130.4, 129.8, 129.5, 129.1, 128.9, 85.7, 56.0, 52.4, 44.8, 44.1, 33.0; mass spectrum, not informative. Anal. Calcd for $C_{23}H_{24}O_8S_2$: C, 56.08; H, 4.91; S, 13.02. Found: C, 56.06; H, 4.77; S, 12.99.

Preparation of Diketone 9a. A pale yellow oil was obtained by the general procedure describe above: R_f 0.3 (40% ethyl acetate in hexane); IR (film, cm^{-1}) 3404 (br s), 3003 (w), 2954 (m), 2870 (w), 1753 (s), 1733 (vs), 1696 (s), 1433 (m), 1358 (m), 1295 (m), 1270 (m), 1193 (m), 1151 (s), 1023 (w), 951 (w), 876 (w); ^{1}H NMR ($CDCl_3$) δ 5.68–5.62 (m, 1 H), 5.56–5.50 (m, 1 H), 3.73 (s, 6 H), 3.65 (d, J = 11 Hz, 1 H), 3.30 (d, J = 9 Hz, 1 H), 3.01–2.99 (m, 1 H), 2.90–2.86 (m, 1 H), 2.18 (s, 3 H), 2.16 (s, 3 H), 1.74–1.24 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 203.3, 203.0, 168.5, 168.4, 130.1, 129.4, 73.9, 56.0, 52.4, 34.8, 34.6, 30.3, 29.3, 23.9, 23.2; mass spectrum, exact mass m/e 267.1208 (M^+ - COMe, 12%), calcd for $C_{14}H_{19}O_5$ 267.1232. Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.14. Found: C, 61.76; H, 7.08.

Preparation of Disulfone 9b. From the general procedure described above **9b** was obtained as a colorless crystalline solid: mp 163.5–164.0 °C; R_f 0.4 (40% ethyl acetate in hexane); IR (film, cm^{-1}) 1750 (vs), 1731 (s), 1585 (w), 1447 (s), 1435 (s), 1332 (s), 1312 (s), 1151 (s), 1078 (m), 1021 (w), 999 (w), 979 (w), 950 (w), 902 (w); ^{1}H NMR ($CDCl_3$) δ 7.94–7.88 (m, 4 H), 7.69–7.61 (m, 2 H), 7.55–7.49 (m, 4 H), 5.68–5.63 (m, 1 H), 5.51 (d, J = 10 Hz, 1 H), 4.54 (d, J = 2 Hz, 1 H), 3.71 (s, 3 H), 3.70 (s, 3 H), 3.55 (d, J = 11 Hz, 1 H), 3.38–3.32 (m, 1 H), 2.88–2.84 (m, 1 H), 2.21–2.15 (m, 1 H), 1.76–1.67 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 168.8, 168.5, 139.8, 138.6, 134.5, 134.3, 129.7, 129.3, 129.0, 127.6, 86.7, 55.3, 52.4, 36.5, 33.0, 25.5, 23.1; mass spectrum exact mass m/e 506.1057 (M^+, 0.2%). Anal. Calcd for $C_{22}H_{26}O_8S_2$: C, 56.20; H, 5.17; S, 12.66. Found: C, 56.39; H, 5.08; S, 12.38.

Acknowledgment. Drs. J. Saunders and P. Leyton are thanked for help and advice with the NMR experiments, Drs. I. Fleming and B. M. Trost for helpful comments, and Professor Sir Jack Lewis and Girton College for financial support.

Synthesis and Chemical Properties of Tetrazole Peptide Analogues

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Received August 14, 1986

Tetrazole dipeptide analogues in which the amide bond is replaced with the tetrazole ring were synthesized from the corresponding Z or Pht protected dipeptide esters via the imidoyl chloride and imidoyl azide intermediates. Of the various imidoyl chloride/imidoyl azide forming reagents that were investigated for this conversion, the best combination was found to consist of PCl_5/HN_3. The success of this reaction was found to be dependent upon the amino protecting group employed and also upon the amino acid sequence of the starting dipeptide. Racemization of the α-carbon of the N-terminal amino acid residue was found to occur during the formation of the tetrazole dipeptide analogue. A hypothetical mechanism involving the formation of a ketene amine intermediate is proposed to account for this racemization. Although racemization of the α-carbon of the C-terminal amino acid residue did not occur during tetrazole formation, it did take place when the tetrazole dipeptide ester was saponified with base, as well as when the tetrazole dipeptide acid was coupled with an amino acid ester by using diphenylphosphoryl azide as the coupling reagent. Racemization of the C-terminal amino acid residue did not take place when the normal mixed anhydride, DCC–HOBt, and *N,N*-bis[2-oxo-3-oxazolidinyl]-phosphorodiamidic chloride coupling methods were employed.

The use of peptide bond surrogates in the design and synthesis of analogues of biologically active peptides has seen extensive use in recent years.[1] One such peptide bond surrogate is the trans olefinic moiety. This group has been successfully employed in a number of different peptides as a mimic of the trans configuration of the peptide bond.[2] Although the corresponding cis olefinic group would serve as the ideal mimic of the cis amide bond, the ease with which the cis β,γ-unsaturated carbonyl system isomerizes to the more stable trans α,β-unsaturated carbonyl system[2b] has precluded the use of this particular peptide bond surrogate in the design of peptide analogues. To get around this problem, the tetrazole ring system has been proposed by Marshall et al.[3] as an alternate means of mimicking the cis configuration of a peptide bond. The use of this particular peptide bond surrogate requires the synthesis of 1,5-disubstituted tetrazoles in which the 1 and

(1) For a recent review, see: Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1983; Vol. 7, p 267.

(2) (a) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Chem. Commun.* **1980**, 234. (b) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 307. (c) Cox, M. T.; Gormley, J. J.; Hayward, C. F.; Petter, N. N. *J. Chem. Soc., Chem. Commun.* **1980**, 800. (d) Johnson, R. L. *J. Med. Chem.* **1984**, *27*, 1351.

(3) Marshall, G. R.; Humblet, C.; Van Opdenbosch, N.; Zabrocki, J. *Peptides: Synthesis-Structure-Function*; Proc. 7th American Peptide Symposium, Rich, D. H., Gross, E., Eds.; Pierce Chemical Company: Rockford, IL, 1981; p 669.

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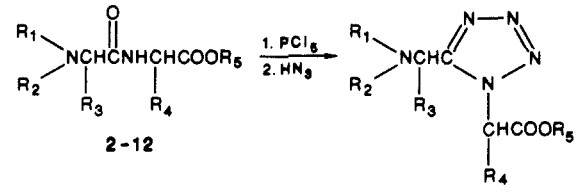
Table I. Synthesis of Tetrazole Dipeptides Using PCl_5 and HN_3

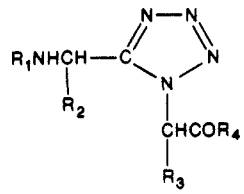


2-12 13-21

dipeptide compound	tetrazole compound	R_1	R_2	R_3	R_4	R_5	yield, %	ratio of diastereoisomers[a] a:b
2	13	Z	$-(CH_2)_3-$		*i*-Bu	CH_3	82	50:50
3	14	Z	$-(CH_2)_3-$		CH_3	CH_3	56	60:40
4	15	Z	H	*i*-Bu	H	CH_3	34	*b*
5	16	Z	H	*i*-Pr	CH_2Ph	CH_3	41	53:47
6		Z	H	H	CH_2Ph	CH_3	0	*b*
7		Z	H	CH_3	*i*-Bu	CH_3	0	*b*
8	17	-Pht-		*i*-Bu	H	CH_3	73	*b*
9	18	-Pht-		*i*-Bu	CH_3	CH_3	71	86:14
10	19	-Pht-		CH_2Ph	CH_3	CH_3	74	63:37
11	20	-Pht-		H	*i*-Bu	CH_2Ph	80	*b*
12	21	-Pht-		H	*sec*-Bu	CH_2Ph	*c*	*b*

[a] Determined by HPLC. The solvent system used and the retention time of the diastereoisomer are given in the Experimental Section. [b] Not applicable. [c] Tetrazole product and starting dipeptide could not be separated from one another.

5 substituents may both be chiral. The general structure of such tetrazoles is illustrated by structure 1.[4] Since the



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chemistry of such tetrazole derivatives has not been described in detail before, we describe in this paper the synthesis and some of the chemical properties of tetrazole analogues of peptides.

Results and Discussion

Tetrazole Peptide Synthesis. The synthetic approach that we used for the synthesis of tetrazole dipeptide analogues was based on the standard method of synthesizing tetrazoles whereby an amide bond is converted to the corresponding tetrazole via the imidoyl chloride and imidoyl azide intermediates.[5] The dipeptides Z-Pro-Leu-OCH_3 (2) and Z-Leu-Gly-OCH_3 (4) served as the model peptide substrates in these studies. Initially both of these dipeptides were treated with a variety of reagents known to convert amides into their corresponding imidoyl chlorides.[6] Among these reagents were PCl_5, PCl_3, $POCl_3$, $SOCl_2$, and oxalyl chloride. After treatment of either Z-Pro-Leu-OCH_3 or Z-Leu-Gly-OCH_3 with one of the above imidoyl chloride forming reagents the reaction mixtures were treated with HN_3 in benzene.[7] Only in the case where the imidoyl chloride was generated with 1.1 equiv of PCl_5 in benzene were tetrazole products isolated. The tetrazole derivative of Z-Pro-Leu-OCH_3, Z-*ambo*-Proψ-[CN_4]Leu-OCH_3 (13), was isolated in a 82% yield while the tetrazole analogue of Z-Leu-Gly-OCH_3, Z-DL-Leuψ-[CN_4]Gly-OCH_3 (15), was isolated in a 34% yield.

The formation of the tetrazole ring was verified through ^{13}C NMR by the disappearance of the amide carbonyl carbon resonance and the appearance of a new signal for the tetrazole C-5 carbon between 150 and 160 ppm.[8] It was also observed in the ^{13}C NMR spectrum of the tetrazole products that the signal for the C-terminal α-carbon was shifted downfield on the order of 6–9 ppm compared with that in the parent dipeptide ester, while the signal for the N-terminal α-carbon was shifted upfield 8–12 ppm. Such shifts probably are due to changes in the electron density that occur in the atoms adjacent to the two α-carbon atoms when the amide is converted to a tetrazole ring. Because the lone-pair electrons of the tetrazole N-1 nitrogen are delocalized throughout the tetrazole ring, this nitrogen atom is relatively more electron deficient when compared with the nitrogen atom of the amide bond. The C-5 carbon atom of the tetrazole, on the other hand, is more electron rich than the carbonyl carbon of an amide bond because of the mesomeric effect of the tetrazole ring. In the ^1H NMR, the chemical shifts of the protons on the N- and C-terminal α-carbon atoms of the tetrazole derivatives were found to be downfield from the corresponding signals in the starting dipeptides.

In order to determine the scope and limitations of the reaction sequence that converts dipeptides to their corresponding tetrazoles a variety of dipeptides containing either the benzyloxycarbonyl or phthalyl amino protecting

(4) The ψ[ ] nomenclature for peptide backbone modifications proposed by A. F. Spatola in ref 1 is used in this paper to describe the tetrazole peptide analogues. The ψ symbol indicates the absence of the amide bond between the specified amino acid residues while the structure that replaces the amide group is shown within the brackets. We propose the term CN_4 be used to designate the tetrazole peptide bond surrogate. Thus, the tetrazole peptide analogue of the dipeptide Xxx-Yyy would be designated as Xxxψ[CN_4]Yyy. The use of the *ambo* and ξ prefixes in the naming of some of the tetrazole peptide analogues is based on the recommendations made in 1983 by the IUPAC-IUB Joint Commission on Biochemical Nomenclature and published in the *Biochem. J.* **1984**, *219*, 345. The prefix *ambo* is used in those cases where a mixture of diastereoisomers exist. Thus, Z-*ambo*-Proψ[CN_4]-L-Leu-OCH_3 would signify a mixture of the two diastereoisomers Z-L-Proψ[CN_4]-L-Leu-OCH_3 and Z-D-Proψ[CN_4]-L-Leu-OCH_3. The prefix ξ is used to indicate that the configuration of a chiral center is unknown. Thus, in the case of Pht-ξ-Leuψ[CN_4]-L-Ala-OH, a single diastereoisomer of a tetrazole peptide is being referred to in which the chirality of the leucyl residue is unknown.

(5) Smith, P. A. S. In *Molecular Rearrangement*; de Mayo, P., Ed.; Wiley-Interscience: New York, 1963; p 457.

(6) Ulrich, H. *The Chemistry of Imidoyl Halides*; Plenum Press: New York, 1968; p 55.

(7) Von Braun, J. *Ann.* **1931**, *490*, 125.

(8) Butler, R. N.; McEvoy, T. M. *Proc. R. Irish. Acad., Sec. B* **1977**, *77(B)*, 359.

group were examined. As can be seen from the results that are summarized in Table I, the yields of the tetrazoles were dependent upon the amino protecting group and the amino acid sequence. When the benzyloxycarbonyl (Z) protecting group was used, the yield of the tetrazoles was found to be dependent upon the nature of the N-terminal amino acid. When proline was the N-terminal amino acid the amount of tetrazole formed was very good. On the other hand, when either leucine or valine was the N-terminal amino acid, dipeptides **4** and **5**, the amount of tetrazole formed was only in the range of 30–40%, while in the case where either alanine or glycine was the N-terminal amino acid residue, dipeptides **6** and **7**, no tetrazole was formed at all.

The reason for these differences in reactivity is not known at present, but the fact that little starting dipeptide is recovered in the case of **4–7** would indicate that there are competing side reactions taking place. Shorter reaction times with respect to the HN_3 treatment did not result in an increase in tetrazole products in the case of **4–7**. Furthermore, when Z-Gly-Phe-OCH_3 (**6**) was treated with PCl_5 and the resulting imidoyl chloride then treated with water instead of HN_3 only 35% of the starting dipeptide was recovered unchanged. Thus, it would appear that whatever side reactions are taking place during the synthesis of the tetrazole peptides from dipeptides **4–7**, they are occurring primarily during the PCl_5 reaction. The products of the side reaction(s), although unknown, are quite polar since they are extracted into the aqueous layer upon workup of the reaction. In addition, since ^1H NMR of the residue obtained after evaporation of the aqueous extract shows the absence of the benzyloxycarbonyl moiety, it appears that one of the side reactions taking place is the loss of this functionality. It is unlikely, however, that the loss of this moiety in the reactions with dipeptides **4–7** is due to a simple acid cleavage type of mechanism as is generally seen in classical peptide chemistry, because the benzyloxycarbonyl groups of Z-Gly-OCH_3 and Z-Pro-Leu-OCH_3 are stable under the reaction conditions employed. Atempts at minimizing these side reactions by carrying out the imidoyl chloride step at 0 °C in toluene were unsuccessful.

In contrast to the results obtained with the benzyloxycarbonyl protecting group, the yields of the tetrazoles were always high regardless of the nature of the N-terminal amino acid residue when the phthalyl (Pht) group was the amino protecting group. It thus seems that the use of an amino protecting group that leaves no free NH is necessary in order for tetrazole formation to proceed in a good yield under the PCl_5/HN_3 reaction conditions.

Racemization of the α-Carbon of the N-Terminal Amino Acid Residue. The results obtained in this study indicated that the α-carbon of the N-terminal amino acid residue of the dipeptide ester undergoes racemization during the conversion of the dipeptide to the tetrazole peptide. Thus, the tetrazole analogue of the dipeptide Z-Pro-Leu-OCH_3 was obtained as a pair of diastereoisomers, Z-*ambo*-Proψ[CN_4]-L-Leu-OCH_3 (**13a** and **13b**) while the tetrazole analogue of the dipeptide Z-Leu-Gly-OCH_3 was obtained as a racemic mixture, Z-DL-Leuψ[CN_4]Gly-OCH_3 (**15**). The contention that it was the α-carbon of the N-terminal amino acid residue and not the α-carbon of the C-terminal amino acid of the dipeptide that was racemizing during the formation of the tetrazole was strongly supported by the following two experiments. In one case, the dipeptide Pht-Gly-Leu-OBzl (**11**) was converted to its corresponding tetrazole derivative Pht-Glyψ[CN_4]-L-Leu-OBzl (**20**) by using PCl_5 and HN_3. The benzyl ester was removed from **20** by hydrogenolysis to give the free acid Pht-Glyψ[CN_4]-L-Leu-OH (**22**), which was coupled to alanine methyl ester by using the mixed anhydride method[9] to give the tetrazole tripeptide Pht-Glyψ[CN_4]-L-Leu-L-Ala-OCH_3 (**23**). The product obtained was shown to contain only one component by HPLC and ^{13}C NMR.

In a second experiment Pht-Gly-Ile-OBzl (**12**) was converted to its corresponding tetrazole derivative Pht-Glyψ[CN_4]-L-Ile-OBzl (**21**) with PCl_5 and HN_3. ^{13}C NMR showed the presence of only one tetrazole product in the reaction mixture, thereby indicating that the α-carbon atom of the isoleucyl residue had not racemized during the formation of the tetrazole.

As part of an effort directed toward understanding the mechanism of the racemization of the dipeptide's N-terminal α-carbon during the tetrazole conversion process several experiments were performed. First, Z-Pro-Leu-OCH_3 (**2**) was treated with PCl_5 to give an imidoyl chloride. Instead of treating this material with HN_3 as was normally done to obtain the tetrazole, the reaction was quenched with water. The starting dipeptide was recovered unchanged with no apparent racemization of the prolyl residue. Secondly, one of the pure diastereoisomers of Z-*ambo*-Proψ[CN_4]-L-Leu-OCH_3, **13a**, was treated with PCl_5 followed by the addition of HN_3. The tetrazole was recovered unchanged and only the starting diastereoisomer could be detected by HPLC and ^{13}C NMR, thus indicating that no racemization of the tetrazole took place under the PCl_5/HN_3 reaction conditions. The results of these two experiments indicated that the racemization of the N-terminal α-carbon atom occurred after the imidoyl chloride stage but before the formation of the tetrazole.

In a final experiment, a dipeptide analogue, L-(2-(benzyloxy)-4-methylpentanoyl)-L-Ala-OCH_3 (**25**), in which the protected amino moiety had been replaced with the benzyloxy functionality and which was prepared by coupling L-2-benzyloxy-4-methylpentanoic acid (**24**) with alanine methyl ester, was treated sequentially with PCl_5 and HN_3. Although only a small amount of the desired tetrazole was formed, with ^{13}C NMR we were able to show that the tetrazole product obtained, compound **26**, existed as a pair of diastereoisomers. This observation suggests that the Z or Pht amino protecting groups need not be involved in the racemization process as is typically the case when amino acid derivatives racemize.

On the basis of the evidence above, a hypothetical mechanism that could account for the racemization occurring during the tetrazole formation reaction is proposed in Scheme I. It is proposed that the imidoyl chloride **27** reacts with HN_3 to give an imidoyl chloride (**28**). This intermediate could cyclize to form a tetrazole or it could lose a molecule of HN_3 either through a 1,4 syn elimination process or through formation of a nitrilium ion (**29**). The former process would give rise to ketene imine **30** directly, while the nitrilium ion could tautomerize to give **30**. The addition of HN_3 to the ketene imine would result in the formation of an imidoyl azide (**31**) in which the N-terminal α-carbon would have either the *R* or *S* configuration. Cyclization of **31** would provide in this case tetrazoles in which the N-terminal carbon would be racemic.

In an attempt to circumvent the problem of racemization, we treated Z-Pro-Leu-OCH_3 with PCl_5 to form the corresponding imidoyl chloride and then reacted this material with a variety of different azide reagents in order to convert it to the imidoyl azide and subsequently to the desired tetrazole product. The products obtained were

(9) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

Scheme I

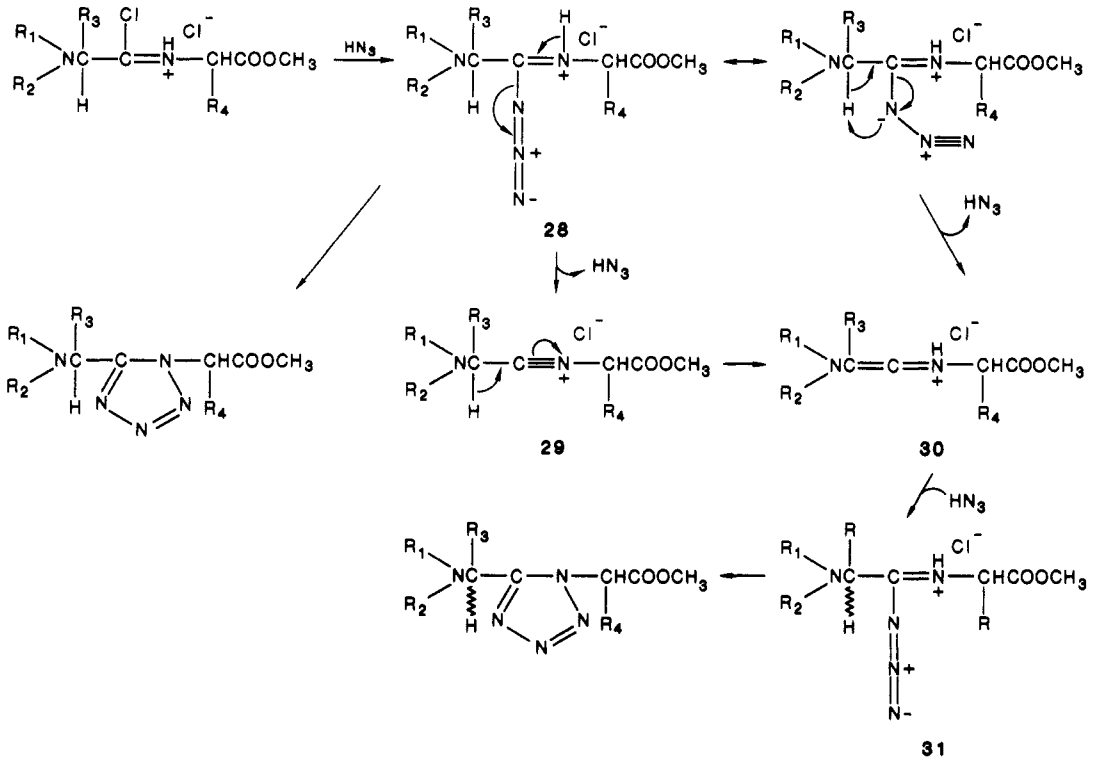

Table II. Synthesis of the Z-Proψ[CN_4]-L-Leu-OCH_3 Isomers 13a and 13b from 2 Using PCl_5 and Different Azide Reagents

azide reagents	ratio of diastereoisomers 13a:13b	unreacted 2, %	isolated yield (13a + 13b), %
HN_3	1:1	12	82
NaN_3–NH_4Cl[a]	1:3	28	45
$(n\text{-Bu})_3SnN_3$	4:1	42	*b*
Me_3SiN_3	2:1	47	21

[a] Reaction run at 90 °C for 4 h. [b] Yield could not be determined since the tetrazole products could not be separated from $(n\text{-Bu})_3SnN_3$.

analyzed by HPLC and the results are summarized in Table II. The azide reagent HN_3 was found to give the best yield of the desired tetrazole peptides. In addition, this reagent gave an equal ratio of the two tetrazole diastereoisomers **13a** and **13b**. Other reagents, such as NaN_3/NH_4Cl,[10] trimethylsilyl azide (Me_3SiN_3),[11] and tri-*n*-butyltin azide ($(n\text{-Bu})_3SnN_3$)[12] gave low overall yields of tetrazole dipeptides, as well as unequal amounts of the two diastereoisomers **13a** and **13b**. The reagent, NaN_3/NH_4Cl, generated a diastereoisomeric mixture with a ratio of **13a:13b** equal to 1:3. Since the reaction employing this reagent was heated at 90 °C, this ratio most likely represents a thermodynamic equilibrium. In contrast, the latter two reagents produced predominantly the **13a** tetrazole diastereoisomer. When the reaction employing Me_3SiN_3 was heated at 80 °C, the ratio of diastereoisomers obtained became 1:1. These results suggest that the mechanism of tetrazole formation with the reagents Me_3SiN_3 and $(n\text{-Bu})_3SnN_3$ is different from that with HN_3. In possibly a more direct route to the imidoyl azide, the amide was converted to the lithium imine enolate by using either *n*-BuLi or LiH and then reacted with diphenylphosphoryl azide[13] (DPPA). Intramolecular azide transfer to give an imidoyl azide did not take place, however.

Racemization of the α-Carbon of the Tetrazole Peptide C-Terminal Residue. The tetrazole peptide analogues were found to be stable to 4 N HCl in dioxane. However, when each of the pure diastereoisomers of Z-*ambo*-Proψ[CN_4]-L-Leu-OCH_3, **13a** and **13b**, was saponified with 1 equiv of 1 N NaOH at room temperature, a pair of diastereoisomeric acids was obtained in each case. Interestingly, the ratio of the two acids obtained from either **13a** or **13b** was about 2:5 in each case, clearly indicating that a thermodynamic equilibrium was achieved during the saponification process. When the two diastereoisomeric acids obtained from **13a** were treated with diazomethane, a mixture consisting of two esters was obtained. These were readily separated by flash chromatography. The higher R_f ester was found to have the same R_f value, ^1H and ^{13}C NMR spectra, and specific rotation as **13a**. The lower R_f ester, on the other hand, had the same R_f value and ^1H and ^{13}C spectra as **13b**, but its specific rotation while of the same magnitude as that of **13b** had the opposite sign. Thus it was clear that the saponification and reesterification process carried out on **13a** had yielded a mixture composed of **13a** and the enantiomer of **13b**, compound **13c**. Carrying out the same saponification and reesterification process with **13b**, likewise, yielded a pair of esters. The lower R_f ester was found to be **13b**, while the higher R_f ester was found to be the enantiomer of **13a**, compound **13d**. When the saponification and reesterification process was carried out on a mixture of **13a** and **13b**, the two sets of esters obtained, while having the same R_f values and ^1H and ^{13}C spectra as **13a** and **13b**, were, nevertheless, optically inactive.

The above experiments can be analyzed in the following manner in order to determine whether it is the N-terminal α-carbon or C-terminal α-carbon that racemizes during the

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saponification reaction. Since the proline α-carbon of **2** was racemized during the synthesis of tetrazole **13**, the configurations of the two diastereoisomers of **13** should be *RS* and *SS*. Now if the N-terminal α-carbon of **13** racemized during the saponification reaction (case A), then acids and the corresponding esters having the configurations of *RS* and *SS* would be obtained no matter whether **13a** or **13b**, or a mixture of **13a** and **13b** was carried through the saponification and reesterification process. On the other hand, if the racemization occurred at the C-terminal α-carbon of **13** (case B), then the corresponding pairs of acids and their respective esters that would be obtained when **13a** and **13b** were carried through the saponification an reesterification reactions would have either the *RS* and *RR* or the *SS* and *SR* configurations. If a mixture of **13a** and **13b** was saponified and then reesterified, two sets of esters should be obtained. Each set would contain enantiomers with the configurations of either *RR* and *SS* or *RS* and *SR*. The results of the experiments described above for **13a** and **13b** clearly fit case B and thus support the proposition that it is the C-terminal α-carbon of the tetrazole dipeptide that is racemized during the ester hydrolysis. The use of milder conditions such as 1 equiv of 1 N LiOH at 0 °C[14] or nucleophilic cleavage of the ester with LiBr in refluxing DMF[15] to hydrolyze the tetrazole esters also led to racemization of the C-terminal α-carbon.

As mentioned above, when Pht-Glyψ[CN_4]-L-Leu-OH (**22**) was coupled to Ala-OCH_3 by using the mixed anhydride method, only one diastereoisomer of the tripeptide was obtained. Interestingly, when the same coupling reaction was carried out by using diphenylphosphoryl azide, a mixture of two diastereoisomers was obtained due to the racemization of the α-carbon of the C-terminal residue of **22**. This observation was confirmed in another coupling study where one of the diastereoisomers of Pht-*ambo*-Leuψ[CN_4]-L-Ala-OH (**32**) was coupled with Gly-OCH_3. It was found that the coupling of **32** with Gly-OCH_3 by using diphenylphosphoryl azide gave a mixture of the tetrazole tripeptides Pht-ξLeuψ[CN_4]-D-Ala-Gly-OCH_3 (**33a**) and Pht-ξLeuψ[CN_4]-L-Ala-Gly-OCH_3 (**33b**). In contrast, when either the normal mixed anhydride, DCC–HOBt, or *N,N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride coupling methods were employed, only the tetrazole tripeptide **33b** was obtained. Interestingly, when 2 equiv of Et_3N were used in the generation of the mixed anhydride for the coupling of **32** with Gly-OCH_3, a mixture of **33a** and **33b** resulted, thus indicating that racemization took place under these conditions.

It is clear from the above saponification and coupling studies that the proton α to the carboxyl group in a tetrazole dipeptide analogue is quite labile. This is in sharp contrast to the situation seen in a normal dipeptide where the C-terminal α C-H is normally not racemized under the saponification and coupling reactions employed in this study.

Conclusion. In summary, we have shown in this study that the synthesis of tetrazole analogues of dipeptides can readily be carried out by using PCl_5 and HN_3, but that the success of this reaction is dependent upon the amino protecting group employed as well as the amino acid sequence. Moreover, this study has shown that this reaction sequence leads to racemization of the α-carbon atom of the N-terminal residue and that the α-carbon of the C-terminal residue of the tetrazole peptide is readily racemized in the presence of base.

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Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Specific rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D line). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^1H NMR spectra were recorded on either a JEOL FX-90 MHz or a Nicolet Zeta 300-MHz spectrometer. Splitting multiplicity is given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, (br) broad. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si) in $CDCl_3$ or Me_2SO-d_6. ^{13}C NMR was performed on the JEOL FX-90 MHz instrument at 25 MHz. When either $CDCl_3$ or Me_2SO-d_6 were used as the solvent, they also served as the internal standard at δ 77.0 and 39.5 ppm, respectively. Column chromatography was performed with Silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF uniplates. HPLC analysis was performed on a Waters HPLC system using a 8SI 10μ Radial-PAK cartridge at a flow rate of 1.5 mL/min. Z and Pht protected peptides were detected at 260 and 290 nm, respectively, by using an ISCO 1840 UV absorbance monitor. The solvent systems used in both TLC and HPLC consisted of the following: (A) EtOAc/*n*-hexane (1:1), (B) EtOAc/*n*-hexane (1:2), and (C) EtOAc/*n*-hexane (1:3), (D) *n*-propanol/NH_4OH (5:1).

Preparation of the Benzyloxycarbonyl and Phthaloyl Protected Dipeptides 2–12. The benzyloxycarbonyl and phthaloyl protected dipeptides **2–12** were prepared by using the mixed anhydride procedure of Anderson et al.[9] Since the benzyloxycarbonyl protected dipeptides have all been described previously, only the previously unreported ^{13}C NMR spectra are reported here.

Z-Pro-Leu-OCH_3 (2): mp 78–80 °C; $[\alpha]_D$ −42.3° (*c* 1.07, DMF) [lit.[16] mp 74–75 °C; $[\alpha]_D$ − 42.5° (*c* 1.0 DMF)]; ^{13}C NMR ($CDCl_3$) δ 21.59, 22.30 (q, Leu δC), 23.76 (t, Pro γC), 24.60 (d, Leu γC), 28.96 (t, Pro βC), 41.10 (t, Leu βC), 46.78 (t, Pro δC), 50.63 (d, Leu αC), 51.53 (q, OCH_3), 60.14 (d, Pro αC), 66.83 (t, CH_2Ph), 127.40, 127.98 136.37 (Ph), 155.14 (Z C=O), 171.24, 172.52 (Pro and Leu C=O).

Z-Pro-Ala-OCH_3 (3): mp 79–80 °C; $[\alpha]_D$ −74.6° (*c* 1.30, EtOH) [lit.[17] mp 79–80 °C; $[\alpha]_D$ −74.2° (*c* 0.84, EtOH)]; ^{13}C NMR ($CDCl_3$) δ 17.80 (q, Ala βC), 23.93 (t, Pro γC), 29.26 (t, Pro βC), 46.97 (t, Pro δC), 47.92 (d, Ala αC), 51.89 (q, OCH_3), 60.34 (d, Pro αC), 67.03 (t, CH_2Ph), 127.58 127.73, 128.21, 136.51 (Ph), 155.27 (Z C=O), 171.31, 172.78 (Pro and Ala C=O).

Z-Leu-Gly-OCH_3 (4): mp 89–91 °C; $[\alpha]_D$ −25.0° (*c* 1.0, EtOH) [lit.[18] mp 93–94 °C; $[\alpha]_D$ −26.6° (*c* 5, EtOH)]; ^{13}C NMR ($CDCl_3$) δ 21.70, 22.59 (q, Leu δC), 24.54 (d, Leu γC), 40.93 (t, Leu βC), 41.31 (t, Gly αC), 51.74 (q, OCH_3), 53.64 (d, Leu αC), 66.72 (t, CH_2Ph), 127.68 128.11, 136.29 (Ph), 156.12 (Z C=O), 169.88 (Gly C=O), 172.76 (Leu C=O).

Z-Val-Phe-OCH_3 (5): mp 141–143 °C; $[\alpha]_D$ +16.1° (*c* 1.14, dioxane) [lit.[19] mp 138–139 °C; $[\alpha]_D$ +14° (dioxane)]; ^{13}C NMR ($CDCl_3$) δ 17.69, 18.95 (q, Val γC), 31.00 (d, Val βC), 38.02 (t, Phe βC), 51.95 (q, OCH_3), 53.19 (d, Phe αC), 60.49 (d, Val αC), 66.90 (t, CH_2Ph), 126.97, 127.82, 127.95 128.38, 128.44, 129.14, 135.83, 136.48 (Z and Phe Ph), 156.25 (Z C=O), 170.87, 171.61 (Val and Phe C=O).

Pht-Leu-Gly-OCH_3 (8): oil; $[\alpha]^{24}_D$ −10.4° (*c* 1.38, MeOH); TLC R_f(A) 0.55; ^1H NMR ($CDCl_3$) δ 0.94 (d, *J* = 5.9 Hz, 6 H, Leu δCH_3), 1.20–1.68 (m, 1 H, Leu γCH), 1.68–2.08 (m, 1 H, Leu βCH), 2.20–2.60 (m, 1 H, Leu βCH), 3.73 (s, 3 H, OCH_3), 4.05 (d, *J* = 5.1 Hz, 2 H, Gly αCH_2), 4.96 (dd, *J* = 5.1, 11.0 Hz, 1 H, Leu αCH), 6.78 (br t, 1 H, NH), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 21.15, 22.71 (q, Leu δC), 25.23 (d, Leu γC), 37.45 (t, Leu βC), 41.33 (t, Gly αC), 51.95 (q, OCH_3), 53.10 (d, Leu αC), 123.36, 131.70, 134.08 (Pht), 167.87 (Pht C=O), 169.36, 169.80 (Leu and Gly C=O). Anal. Calcd for $C_{17}H_{20}N_2O_5$: C, 61.43; H, 6.07; N, 8.43. Found: C, 61.40; H, 6.15; N, 8.45.

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Pht-Leu-Ala-OCH$_3$ (9): mp 83–87 °C; $[\alpha]_D^{24}$ −17.7° (*c* 1.36, MeOH); TLC R_f(A) 0.71; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, *J* = 6.2 Hz, 6 H, Leu δCH$_3$), 1.40 (d, *J* = 7.3, 3 H, Ala βCH$_3$), 1.60–2.04 (m, 2 H, Leu γCH and βCH), 2.16–2.60 (m, 1 H, Leu βCH), 3.72 (s, 3 H, OCH$_3$), 4.40–4.80 (m, 1 H, Ala αCH), 4.93 (dd, *J* = 4.9, 11.1 Hz, 1 H, Leu αCH), 6.76 (br d, *J* = 7.2 Hz, 1 H, NH), 7.68–8.00 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 17.45 (q, Ala βC), 20.83, 22.54 (q, Leu δC), 24.90 (d, Leu γC), 37.11 (t, Leu βC), 47.95 (d, Ala αC), 51.71 (q, OCH$_3$), 52.61 (d, Leu αC), 122.96, 131.44, 133.83 (Pht), 167.53 (Pht C=O), 168.42 (Ala C=O), 172.59 (Leu C=O). Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.08. Found: C, 62.29; H, 6.32; N, 7.95.

Pht-Phe-Ala-OCH$_3$ (10): mp 150–151 °C; $[\alpha]^{24}_D$ −146.5° (*c* 1.42, MeOH); TLC R_f(A) 0.68; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, *J* = 7.0 Hz, 3 H, Ala βCH$_3$), 3.55 (m, 2 H, Phe βCH$_2$), 3.72 (s, 3 H, OCH$_3$), 4.40–4.80 (m, 1 H, Ala αCH), 5.13 (dd, *J* = 7.0, 9.7 Hz, 1 H, Phe αCH), 6.64 (br d, *J* = 5.4 Hz, 1 H, NH), 7.16 (s, 5 H, Phe), 7.50–7.80 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 18.09 (q, Ala βC), 34.91 (t, Phe βC), 48.48 (d, Ala αC), 52.19 (q, OCH$_3$), 55.79 (d, Phe αC), 123.37, 131.66, 134.05 (Pht), 126.84, 128.52, 128.78, 136.75 (Phe Ph), 167.72, 167.88 (Pht and Ala C=O), 172.92 (Phe C=O). Anal. Calcd for $C_{21}H_{20}N_2O_5$: C, 66.30; H, 6.54; N, 7.37. Found: C, 66.16; H, 6.56; N, 7.32.

Pht-Gly-Leu-OBzl (11): mp 99–100 °C; $[\alpha]_D$ − 2.9° (*c* 1.31, $CHCl_3$); TLC R_f(A) 0.75; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, *J* = 5.7 Hz, 6 H, Leu δCH$_3$), 1.30–1.80 (m, 3 H, Leu γCH and βCH$_2$), 4.39 (s, 2 H, Gly αCH$_2$), 4.52–4.80 (m, 1 H, Leu αCH), 5.15 (s, 2 H, CH$_2$Ph), 6.30 (br d, 1 H, NH), 7.83 (s, 5 H, Ph), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 21.72, 22.31 (q, Leu δC), 24.53 (d, Leu γC), 40.33 (t, Leu βC), 41.17 (t, Gly αC), 51.08 (d, Leu αC), 66.65 (t, *C*H$_2$Ph), 123.08, 131.82, 133.75 (Pht), 127.68, 127.98, 128.19, 135.18 (Ph), 165.87, 167.25, (Pht and Gly C=O), 172.10 (Leu C=O). Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.44; H, 5.78; N, 6.82.

Pht-Gly-Ile-OBzl (12): mp 128–130 °C; $[\alpha]^{24}_D$ −36.0° (*c* 1.11, MeOH); TLC R_f(B) 0.37; ^{1}H NMR ($CDCl_3$) δ 0.78–0.91 (m, 6 H, Ile βCH$_3$ and δCH$_3$), 1.00–1.52 (m, 2 H, Ile γCH$_2$), 1.68–2.10 (m, 1 H, Ile βCH), 4.30 (d, *J* = 16.0 Hz, 1 H, Gly αCH), 4.49 (d, *J* = 16.0 Hz, 1 H, Gly αCH), 4.67 (dd, *J* = 4.5, 8.4 Hz, 1 H, Ile αCH), 5.09 (d, *J* = 12.1 Hz, 1 H, CH$_2$Ph), 5.24 (d, *J* = 12.1 Hz, 1 H, CH$_2$Ph), 6.37 (br d, 1 H, NH), 7.34 (s, 5 H, Ph), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 11.30 (q, Ile δC), 15.31 (q, Ile βC), 25.14 (t, Ile γC), 38.10 (d, Ile βC), 40.79 (t, Gly αC), 56.78 (d, Ile αC), 66.98 (t, *C*H$_2$Ph), 123.41, 132.15, 133.99 (Pht), 128.18, 128.29, 128.47, 135.31 (Ph), 165.87, 167.49 (Gly and Pht C=O), 171.33 (Ile C=O). Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.89; H, 5.83; N, 6.84.

General Procedure for the Preparation of the Tetrazole Dipeptides 13–21. The tetrazole dipeptides were synthesized by using a modification of the standard literature method for synthesizing tetrazoles.[20] Protected dipeptide ester (1 mmol) and PCl_5 (1.1 mmol) were mixed together in 4 mL of dry benzene. The mixture was stirred at room temperature for 1.5 h. Usually the reaction mixture became clear in 15 min; however, longer reaction times were needed for those peptides that were only partially soluble in benzene (**10** and **12**). The solution was stripped of solvent under reduced pressure and the residue treated with a solution of HN_3 in benzene (3 mL).[7] The reaction mixture was stirred at room temperature overnight. For compound **12** the resulting solution was refluxed for 2 h. Removal of solvent under reduced pressure gave a residue that was partitioned between EtOAc and H_2O. The organic layer was washed with 1 N $NaHCO_3$, H_2O, and saturated NaCl solution and then dried over $MgSO_4$. Filtration and concentration in vacuo gave a crude product, which was then purified through flash chromatography or recrystallization.

Z-*ambo*-Proψ[CN$_4$]-L-Leu-OCH$_3$ (13). The two diastereoisomers obtained were separated by flash chromatography using solvent system B.

13a: $[\alpha]_D$ −8.2° (*c* 1.02, MeOH); TLC R_f(B) 0.47; HPLC t_R(B) 7.9 min; a pair of rotamers were shown in the ^{1}H NMR with a ratio of 10:3, ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74–0.87 and 0.96, 0.94 (m and two dd, *J* = 5.8, 5.9 Hz, 6 H, Leu δCH$_3$), 1.23–1.45 (m, 1 H, Leu γCH), 2.00–2.60 (m, 6 H, Pro βCH$_2$, γCH$_2$ and Leu βCH$_2$), 3.68 and 3.55–3.80 (s over m, 5 H, OCH$_3$ and Pro δCH$_2$), 5.00–5.25 (m, 3 H, CH$_2$Ph and Pro αCH), 5.67 (dd, *J* = 4.2, 11.1 Hz, 1 H, Leu αCH), 7.32 (br s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.86, 22.11 (q, Leu δC), 23.79 (t, Pro γC) 24.54 (d, Leu γC), 31.42 (t, Pro βC), 39.50 (t, Leu βC), 46.43 (t, Pro δC), 51.42 (d, Pro αC), 52.56 (q, OCH$_3$), 58.95 (d, Leu αC), 67.02 (t, *C*H$_2$Ph), 127.59, 128.08, 136.10 (Ph), 154.63 (CN$_4$), 156.41 (Z C=O), 168.40 (Leu C=O). Anal. Calcd for $C_{20}H_{27}N_5O_4$: C, 59.83; H, 6.78; N, 17.45. Found: C, 60.03; H, 6.91; N, 17.55.

13b: $[\alpha]_D$ −90.4° (*c* 1.38, MeOH); TLC R_f(B) 0.37; HPLC t_R(B) 10.2 min; a pair of rotamers were shown in the ^{1}H NMR with a ratio of 10:3, ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73 and 0.97 (d and d, *J* = 6.4 and 6.7 Hz, 3 H, Leu δCH$_3$), 0.76 and 1.00 (d and d, *J* = 6.4 and 6.6 Hz, 3 H, Leu δCH$_3$), 1.12–1.26 and 1.60–1.75 (m, 1 H, Leu γCH), 1.90–2.15 (m, 2 H, Pro γCH$_2$), 2.18–2.35 (m, 3 H, Pro βCH$_2$ and Leu βCH), 2.54–2.65 (m, 1 H, Leu βCH), 3.75 and 3.54–3.90 (s over m, 5 H, OCH$_3$ and Pro δCH$_2$), 4.85–5.14 (m, 3 H, Pro αCH and CH$_2$Ph), 5.71 (dd, *J* = 4.9, 9.9 Hz, 1 H, Leu αCH), 7.32 (br s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 21.40, 22.27 (q, Leu δC), 24.06 (t, Pro γC), 24.65 (d, Leu γC), 31.42 (t, Pro βC), 38.85 (t, Leu βC), 46.43 (t, Pro δC), 50.71 (d, Pro αC), 52.83 (q, OCH$_3$), 58.52 (d, Leu αC), 67.08 (t, *C*H$_2$Ph), 127.65, 128.14, 136.21 (Ph), 154.58 (CN$_4$), 156.91 (Z C=O), 168.66 (Leu C=O). Anal. Calcd for $C_{20}H_{27}N_5O_4$: C, 59.83; H, 6.78; N, 17.45. Found: C, 59.65; H, 6.96; N, 17.20.

Z-*ambo*-Proψ[CN$_4$]-L-Ala-OCH$_3$ (14). An oil consisting of two diastereoisomers was obtained. They were separated from one another by flash chromatography using solvent system A. Anal. Calcd for $C_{17}H_{21}N_5O_4$: C, 56.81; H, 5.89; N, 19.48. Found: C, 56.47; H, 5.88; N, 19.47.

14a: TLC R_f(A) 0.34; HPLC t_R(A) 7.13 min; ^{1}H NMR ($CDCl_3$) δ 1.94 and 1.60–2.80 (d over m, *J* = 7.3 Hz, 7 H, Ala βCH$_3$ and Pro β, γCH$_2$), 3.64 and 3.24–3.76 (s over m, 5 H, OCH$_3$ and Pro δCH$_2$), 4.68–5.04 (m, 3 H, CH$_2$Ph and Pro αCH), 5.80 (q, *J* = 7.3 Hz, 1 H, Ala αCH), 7.22 (s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 15.96 (q, Ala βC), 24.42 (t, Pro γC), 31.46 (t, Pro βC), 46.62 (t, Pro δC), 50.33 (d, Pro αC), 53.01 (q, OCH$_3$), 55.55 (d, Ala αC), 67.30 (t, *C*H$_2$Ph), 128.07, 127.71, 128.44, 136.10 (Ph), 154.67 (CN$_4$), 156.60 (Z C=O), 168.88 (Ala C=O).

14b: TLC R_f(A) 0.30; HPLC t_R(A) 9.00 min; ^{1}H NMR ($CDCl_3$) δ 1.85 and 1.50–2.4 (d over m, *J* = 7.7 Hz, 7H, Ala βCH$_3$ and Pro β, γCH$_2$), 3.60 and 3.20–3.68 (s over m, 5 H, OCH$_3$ and Pro δCH$_2$), 4.98 and 4.84–5.20 (s over m, 3 H, CH$_2$Ph and Proαcontinued CH), 5.24–5.80 (m, 1 H, Ala αCH), 7.23 (br s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 17.20 (q, Ala βC), 23.98 (t, Pro γC), 31.80 (t, Pro βC), 46.75 (t, Pro δC), 51.70 (d, Pro αC), 52.91 (q, OCH$_3$), 56.16 (d, Ala αC), 67.35 (t, *C*H$_2$Ph), 127.83, 128.10, 128.46, 136.22 (Ph), 154.84 (CN$_4$), 156.11 (Z C=O), 168.64 (Ala C=O).

Z-DL-Leuψ[CN$_4$]Gly-OCH$_3$ (15): mp 65–68 °C; TLC R_f(B) 0.25; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (d, *J* = 5.6 Hz, 3 H, Leu δCH$_3$), 0.95 (d, *J* = 6.1 Hz, 3 H, Leu δCH$_3$), 1.65–1.80 (m, 1 H, Leu βCH), 1.86–2.20 (m, 2 H, Leu βCH and γCH), 3.78 (s, 3 H, OCH$_3$), 4.92–5.05 (m, 1 H, Leu αCH), 5.00 (d, *J* = 12.1 Hz, 1 H, CH$_2$Ph), 5.09 (d, *J* = 12.1 Hz, 1 H, CH$_2$Ph), 5.35 (d, *J* = 17.7 Hz, 1 H, Gly αCH$_2$), 5.52 (d, *J* = 17.7 Hz, 1 H, Gly αCH$_2$), 7.25–7.41 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 21.21, 22.27 (q, Leu δC), 24.18 (d, Leu γC), 41.61 (t, Leu βC), 43.26 (d, Leu αC), 47.68 (t, Gly αC), 52.64 (q, OCH$_3$), 66.89 (t, *C*H$_2$Ph), 127.51, 127.98, 135.75 (Ph), 155.93 (CN$_4$) 156.55 (Z C=O), 165.87 (Gly C=O). Anal. Calcd for $C_{17}H_{23}N_5O_4$: C, 56.50; H, 6.41; N, 19.38. Found: C, 56.53; H, 6.47; N, 19.38.

Z-*ambo*-Valψ[CN$_4$]-L-Phe-OCH$_3$ (16). The two diastereoisomers were separated by flash chromatography using solvent system B and were obtained as oils. Anal. Calcd for $C_{23}H_{27}N_5O_4$: C, 63.14; H, 6.22; N, 16.00. Found: C, 63.33; H, 6.28; N, 15.82.

16a: TLC R_f(B) 0.64; HPLC t_R(B) 8.16 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (d, *J* = 6.6 Hz, 3 H, Val γCH$_3$), 0.83 (d, *J* = 6.7 Hz, 3 H, Val γCH$_3$), 1.94–2.09 (m, 1 H, Val βCH), 3.60 and 3.60–3.80 (s over m, 5 H, OCH$_3$ and Phe βCH$_2$), 4.26 (t, *J* = 9.3 Hz, 1 H, Val αCH), 5.03 (s, 2 H, CH$_2$Ph), 5.39 (br d, *J* = 7.7 Hz, 1 H, NH), 5.64 (dd, *J* = 4.0, 11.6 Hz, Phe αCH), 7.02–7.08 and 7.12–7.20 (m, 5 H, Phe), 7.24–7.35 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 17.88, 18.79 (q, Val γC), 31.26 (d, Val βC), 37.17 (t, Phe βC), 50.41 (d, Val αC), 52.97 (q, OCH$_3$), 61.83 (d, Phe αC), 67.21 (t, *C*H$_2$Ph), 127.36, 127.79, 128.16, 128.44, 128.83, 128.92, 135.47,

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136.01 (Z and Phe Ph), 155.99, 156.03 (CN_4 and Z C=O), 167.15 (Phe C=O).

16b: TLC R_f(B) 0.59; HPLC t_R(B) 9.20 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (d, J = 6.7, 3 H, Val γCH_3), 1.01 (d, J = 6.6 Hz, 3 H, Val γCH), 2.28–2.42 (m, 1 H, Val βCH), 3.75 and 3.62–3.82 (s over m, 5 H, OCH_3 and Phe βCH_2), 4.57 (t, J = 9.2 Hz, 1 H, Val αCH), 4.96 (d, J = 12.2 Hz, 1 H, CH_2Ph), 5.09 (d, J = 12.2 Hz, 1 H, CH_2Ph), 5.64 (dd, J = 5.8, 9.8 Hz, 1 H, Phe αCH), 7.34 (br s, 5 H, Phe), 7.26–7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 18.36, 19.10 (q, Val γC), 32.21 (d, Val βC), 36.93 (t, Phe βC), 50.80 (d, Val αC), 53.01 (q, OCH_3), 61.23 (d, Phe αC), 67.29 (t, CH_2Ph), 127.17, 127.90, 128.23, 128.51, 128.57, 129.05, 135.10, 136.03 (Z and Phe Ph), 155.83, 155.92 (CN_4 and Z C=O), 167.32 (Phe C=O).

Pht-DL-Leuψ[CN_4]Gly-OCH_3 (17): mp 109–110.5 °C; TLC R_f(B) 0.25; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, J = 6.2 Hz, 6 H, Leu δCH_3), 1.28–1.76 (m, 1 H, Leu γCH), 2.08–2.48 (m, 1 H, Leu βCH), 2.68–3.08 (m, 1 H, Leu βCH), 3.54 (s, 3 H, OCH_3), 5.20 (s, 2 H, Gly αCH_2), 5.62 (dd, J = 5.6, 10.2 Hz, 1 H, Leu αCH), 7.72–8.00 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 21.15, 22.28 (q, Leu δC), 24.55 (d, Leu δC), 37.90 (t, Leu βC), 41.78 (d, Leu αC), 47.74 (t, Gly αC), 52.60 (q, OCH_3), 123.27, 130.90, 134.39 (Pht), 153.70 (CN_4), 165.36, 166.76 (Pht and Gly C=O). Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 56.87; H, 5.31; N, 19.40.

Pht-*ambo*-Leuψ[CN_4]-L-Ala-OCH_3 (18). Diastereoisomer **18b** crystallized out of the mixture with EtOAc–Et_2O and isomer **18a** was isolated by flash chromatography using solvent system A. Anal. Calcd for $C_{18}H_{21}N_5O_4$: C, 58.21; H, 5.70; N, 18.86. Found: C, 58.12; H, 5.81; N, 18.75.

18a: obtained as an oil; TLC R_f(A) 0.71; HPLC t_R(C) 15.45 min; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, J = 6.4 Hz, 6 H, Leu δCH_3), 1.32–1.76 (m, 1 H, Leu γCH), 1.89 (d, J = 7.3 Hz, 3 H, Ala βCH_3), 1.96–2.46 (m, 1 H, Leu βCH), 2.60–2.94 (m, 1 H, Leu βCH), 3.77 (s, 3 H, OCH_3), 5.32 (q, J = 7.3 Hz, 1 H, Ala αCH), 5.61 (dd, J = 5.9, 9.5 Hz, 1 H, Leu αCH), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 16.46 (q, Ala βC), 21.61, 22.47 (q, Leu δC), 25.05 (d, Leu γC), 38.70 (t, Leu βC), 42.68 (d, Leu αC), 53.22 (q, OCH_3), 55.74 (d, Ala αC), 123.75, 131.36, 134.64 (Pht), 153.79 (CN_4), 167.22, 168.15 (Pht and Ala C=O).

18b: mp 182–183.5 °C; $[\alpha]^{24}_D$ –142.2° (c 1.09, DMF); TLC R_f(A) 0.65; HPLC t_R(C) 16.05 min; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.4 Hz, 6 H, Leu δCH_3), 1.48–1.72 (m, 1 H, Leu γCH), 1.97 (d, J = 7.3 Hz, 3 H, Ala βCH_3), 2.00–2.42 (m, 1 H, Leu βCH), 2.68–2.94 (m, 1 H, Leu βCH), 3.77 (s, 3 H, OCH_3), 5.25 (q, J = 7.3 Hz, 1 H, Ala αCH), 5.63 (dd, J = 5.5, 10.3 Hz, 1 H, Leu αCH), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 16.84 (q, Ala βC), 21.36, 22.56 (q, Leu δC), 24.69 (d, Leu γC), 38.10 (t, Leu βC), 41.87 (d, Leu αC), 52.62 (q, OCH_3), 55.36 (d, Ala αC), 123.43, 131.09, 134.48 (Pht), 153.17 (CN_4), 166.79, 167.85 (Pht and Ala C=O).

Pht-*ambo*-Pheψ[CN_4]-L-Ala-OCH_3 (19). Diastereoisomer **19a** was obtained from the mixture through crystallization with MeOH. Anal. Calcd for $C_{21}H_{19}N_5O_4$: C, 62.21; H, 4.72; N, 17.28. Found: C, 62.11; H, 4.80; N, 17.17.

19a: mp 225–226 °C; $[\alpha]^{24}_D$ –262.5° (c 0.90, DMF); ^{1}H NMR (Me_2SO-d_6) δ 1.88 (d, J = 7.3 Hz, 3 H, Ala βCH_3), 2.95 (s, 3 H, OCH_3), 3.62 (dd, J = 5.4, 14.0 Hz, 1 H, Phe βCH), 3.97 (dd, J = 10.6, 14.0 Hz, 1 H, Phe βCH), 5.29 (q, J = 7.3 Hz, 1 H, Ala αCH), 6.17 (dd, J = 5.4, 10.6 Hz, 1 H, Phe αCH), 7.20 (s, 5 H, Phe), 7.82 (s, 4 H, Pht Ar); ^{13}C NMR (Me_2SO-d_6) δ 16.23 (q, Ala βC), 34.41 (t, Phe βC), 44.00 (d, Phe αC), 52.18, (q, OCH_3), 54.89 (d, Ala αC), 123.01, 129.00, 134.71 (Pht), 126.59, 128.00 128.68, 135.88 (Phe), 153.02 (CN_4), 166.21, 168.19 (Pht and Ala C=O).

19b: obtained as an oil; TLC R_f(A) 0.71; ^{1}H NMR (Me_2SO-d_6) δ 1.86 (d, J = 7.3, 3 H, Ala βCH), 3.69 (s, 3 H, OCH_3), 3.85 (dd, J = 6.5, 14.2 Hz, 1 H, Phe βCH), 4.13 (dd, J = 9.5, 14.2 Hz, 1 H, Phe βCH), 5.22 (q, J = 7.3 Hz, 1 H, Ala αCH), 5.76 (dd, J = 6.5, 9.5 Hz, 1 H, Phe αCH), 7.19 (s, 5 H, Phe), 7.56–7.88 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 16.03 (q, Ala βC), 35.50 (t, Phe βC), 45.31 (d, Phe αC), 52.89 (q, OCH_3), 55.52 (d, Ala αC), 123.24, 130.63, 134.34 (Pht), 126.81, 128.25, 128.74, 135.51 (Phe), 152.95 (CN_4), 166.71, 167.69 (Pht and Ala C=O).

Pht-Glyψ[CN_4]-L-Leu-OBzl (20): oil; $[\alpha]^{24}_D$ – 33.0° (c 1.12, MeOH); TLC R_f(B) 0.53; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, J = 6.2 Hz, 6 H, Leu δCH_3), 1.20–1.68 (m, 1 H, Leu γCH), 2.00–2.70 (m, 2 H, Leu βCH_2), 4.97 (d, J = 16.0 Hz, 1 H, Gly αCH), 5.08 (s, 2 H, CH_2Ph), 5.17 (d, J = 16.0 Hz, 1 H, Gly αCH), 5.58 (dd, J = 5.2, 10.2 Hz, 1 H, Leu αCH), 7.00–7.4 0 (m, 5 H, Bzl), 7.60–7.92 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 20.77, 21.93 (q, Leu δC), 24.32 (d, Leu γC), 30.33 (t, Gly αC), 38.57 (t, Leu βC), 58.91 (d, Leu αC), 67.60 (t, CH_2Ph), 123.05, 131.15, 133.99 (Pht), 127.46, 128.03, 128.11 (Bzl), 151.14 (CN_4), 166.36, 167.20 (Leu and Ala C=O). Anal. Calcd for $C_{23}H_{23}N_5O_4$: C, 63.73; H, 5.35; N, 16.16. Found: C, 63.69; H, 5.36; N, 16.12.

Pht-Glyψ[CN_4]-L-Ile-OBzl (21): ^{13}C NMR ($CDCl_3$) δ 10.59 (q, Ile δC), 15.36 (q, Ile βC), 25.62 (t, Ile, γC), 31.04 (t, Gly αC), 36.52 (d, Ile βC), 65.84 (d, Ile αC), 68.05 (t, CH_2Ph), 123.48, 131.89, 134.32 (Pht), 151.39 (CN_4), 166.84, 166.97 (Pht and Gly C=O).

Pht-Glyψ[CN_4]-L-Leu-OH (22). To a solution of **20** (710 mg, 1.64 mmol) in a 3:1 mixture of t-BuOH–MeOH (7 mL) was added 10% Pd/C (80 mg) under argon. Hydrogenolysis of the solution was carried out on a Parr apparatus at 25 psi for 2 h. The catalyst was removed by filtration and the filtrate was evaporated to give an oil: TLC R_f(D) 0.42; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, J = 6.2 Hz, 6 H, Leu δCH_3), 1.08–1.68 (m, 1 H, Leu γCH), 1.92–2.64 (m, 2 H, Leu βCH_2), 4.98 (d, J = 5.1 Hz, 1 H, Gly αCH), 5.19 (d, J = 5.1 Hz, 1 H, Gly αCH), 5.53 (dd, J = 4.9, 10.4 Hz, Leu αCH), 7.52–7.84 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 20.77, 22.39 (q, Leu δC), 24.48 (d, Leu γC), 30.52 (t, Gly αC), 38.46 (t, Leu βC), 59.39 (d, Leu αC), 123.54, 131.20, 134.42 (Pht), 151.57 (CN_4), 166.98 (Pht C=O), 169.67 (COOH). An analytical sample was obtained by treating **22** with dicyclohexylamine (1 equiv) to give 611 mg (71%) of the dicyclohexylammonium salt after crystallization from acetone–ether: mp 194–196 °C; $[\alpha]^{24}_D$ –13.5° (c 2.67, MeOH). Anal. Calcd for $C_{28}H_{40}N_6O_4$: C, 64.10; H, 7.69; M, 16.02. Found: C, 64.11; H, 7.52; N, 16.09.

Pht-Glyψ[CN_4]-L-Leu-L-Ala-OCH_3 (23). The free acid **22** (98 mg) was coupled to Ala-OCH_3·HCl by using the mixed anhydride method.[9] The crude tripeptide obtained was purified by flash chromatography (silica gel, EtOAc–hexane = 1:1) to give 90 mg (74%) of **23** as an oil: $[\alpha]^{24}_D$ –19.5° (c 1.10, MeOH); TLC R_f(A) 0.45; HPLC t_R(A) 11.65 min; ^{1}H NMR ($CDCl_3$) δ 0.97 (d, J = 5.7 Hz, 6 H, Leu δCH_3), 1.39 (d, J = 7.3 Hz, 3 H, Ala βCH_3), 1.23–1.74 (m, 1 H, Leu γCH), 1.94–2.62 (m, 2 H, Leu βCH_2), 3.71 (s, 3 H, OCH_3), 4.50 (m, 1 H, Ala αCH), 5.11 (d, J = 16.5 Hz, 1 H, Gly αCH_2), 5.34 (d, J = 16.5 Hz, 1 H, Gly αCH_2), 5.53 (dd, J = 6.2, 9.3 Hz, 1 H, Leu αCH), 7.03 (br d, J = 7.5 Hz, 1 H, NH), 7.68–7.96 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 17.35 (q, Ala βC), 21.39, 22.26 (q, Leu δC), 24.78 (d, Leu γC), 31.20 (t, Gly αC), 40.03 (t, Leu βC), 48.61 (d, Ala αC), 52.22 (q, OCH_3), 61.13 (d, Leu αC), 123.56, 131.85, 134.26 (Pht), 151.49 (CN_4), 166.60 (Pht C=O), 166.88 (Ala C=O), 172.16 (Leu C=O). Anal. Calcd for $C_{20}H_{24}N_6O_5$: C, 56.06; H, 5.65; N, 19.62. Found: C, 55.87; H, 5.86; N, 19.46.

L-2-(Benzyloxy)-4-methylpentanoic Acid (24). Methyl L-2-hydroxy-4-methylpentanoate (5 g, 34.2 mmol) and benzyl bromide (8.7 g, 51.3 mmol) in Et_2O were treated with portions of silver oxide (8 g, 34.5 mmol) by using a modification of the procedure of Mislow et al.[21] After the addition of an initial portion of silver oxide the mixture was heated to initiate refluxing. From then on refluxing was maintained by the addition of Ag_2O. After all of the Ag_2O had been added the mixture was refluxed for 2 h. The silver oxide was removed by filtration and the solvent removed in vacuo. The residue was distilled under vacuum to give 6.87 g (85%) of methyl L-2-(benzyloxy)-4-methylpentanoate as a colorless oil: bp 105–110 °C (0.5 mmHg); $[\alpha]^{25}_D$ –67.9° (neat); ^{1}H NMR ($CDCl_3$) δ 0.83 (d, J = 6.6 Hz, 3 H, δCH_3), 0.91 (d, J = 6.2 Hz, 3 H, δCH_3), 1.94–2.00 (m, 3 H, γCH and βCH_2), 3.73 (s, 3 H, OCH_3), 3.99 (dd, J = 4.4, 8.8 Hz, 1 H, αCH), 4.37 (d, J = 11.5 Hz, 1 H, CH_2Ph), 4.70 (d, J = 11.5 Hz, 1 H, CH_2Ph), 7.34 (s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 21.57, 22.87 (q, δC), 24.39 (d, γC), 41.81 (t, βC), 51.30 (q, OCH_3), 72.19 (t, CH_2Ph), 76.83 (d, αC), 127.58, 127.84, 128.14, 137.63 (Ph), 173.30 (C=O). Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.15; H, 8.53. Found: C, 71.30; H, 8.29.

This material (6.50 g, 27.5 mmol) was dissolved in dioxane (25 mL) and 10% NaOH (12.5 mL, 31.25 mmol) was added. The solution was stirred at room temperature for 1 h and then washed with diethyl ether. The aqueous layer was acidified with 10% HCl and extracted twice with EtOAc. The combined EtOAc extracts were dried over $MgSO_4$ and stripped of solvent in vacuo to give 4.30 g (66%) of **24** as a colorless oil: ^{1}H NMR ($CDCl_3$)

(21) (a) Mislow, K. *J. Am. Chem. Soc.* **1951**, *73*, 4043. (b) Mislow, K.; O'Brien, R. E.; Schaefer, H. *J. Am. Chem. Soc.* **1962**, *84*, 1940.

δ 0.84 (d, J = 6.2 Hz, 3 H, δCH_3), 0.93 (d, J = 6.2 Hz, 3 H, δCH_3), 1.24–2.08 (m, 3 H, βCH_2 and γCH), 4.01 (dd, J = 4.4, 8.8 Hz, 1 H, αCH), 4.41 (d, J = 11.4 Hz, 1 H, CH_2Ph), 4.75 (d, J = 11.4 Hz, 1 H, CH_2Ph), 7.34 (s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 21.61, 22.91 (q, δC), 24.52 (d, γC), 41.72 (t, βC), 72.54 (t, $PhCH_2$), 76.57 (d, αC), 127.88, 128.05, 128.31, 137.29 (Ph), 178.07 (C=O). The acid was further characterized as its dicyclohexylammonium salt: mp 97–98 °C; $[\alpha]^{24}_D$ –51° (c 1.0, MeOH). Anal. Calcd for $C_{25}H_{40}NO_3$: C, 74.63; H, 9.95. Found: C, 74.54; H, 10.14.

Methyl (L-2-(Benzyloxy)-4-methylpentanoyl)-L-alaninate (25). Compound **24** (2.10 g, 9.45 mmol) was dissolved in dry DMF (5 mL) and cooled in a dry ice bath to –20 °C. To this solution was added oxalyl chloride (0.91 mL, 10.39 mmol). The resulting solution was stirred at 0 °C for 20 min and then treated with a solution of Et_3N (4.35 mL, 31.18 mmol) and Ala-OCH_3·HCl (1.45 g, 10.39 mmol) in dry DMF (7 mL). The mixture was stirred at 0 °C for 12 h and then at room temperature overnight. The solvent was evaporated in vacuo and the residue was partitioned between EtOAc (100 mL) and 1 N HCl (25 mL). The organic layer was washed with 1 N $NaHCO_3$, H_2O, and saturated NaCl solution and dried ($MgSO_4$). The solution was stripped of solvent and the residue obtained was purified by flash chromatography (EtOAc/hexane = 1:3) to give 2.04 g (70%) of the product as an oil: TLC R_f(C) 0.43; $[\alpha]^{24}_D$ –92.5° (c 4.33, MeOH); ^1H NMR ($CDCl_3$) δ 0.85 (d, J = 6.4 Hz, 3 H, δCH_3), 0.92 (d, J = 6.4 Hz, 3 H, δCH_3), 1.40 and 1.25–2.04 (d over m, J = 7.3 Hz, 6 H, Ala βCH_3 and βCH_2, γCH), 3.76 and 3.70–3.96 (s over m, 4 H, OCH_3 and Ala αCH), 4.44 (d, J = 11.1 Hz, 1 H, CH_2Ph), 4.70 (d, J = 11.1 Hz, 1 H, CH_2Ph), 4.46–4.84 (m, 1 H, αCH), 7.08 (br d, J = 7.92 Hz, 1 H, NH), 7.37 (br s, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 17.46 (q, Ala βC), 21.09, 22.66 (q, δC), 23.96 (d, γC), 41.82 (t, βC), 46.67 (d, Ala αC), 51.54 (q, OCH_3), 72.12 (t, CH_2Ph), 78.30 (d, αCH), 127.38, 127.68, 127.82, 136.73 (Ph), 172.35, 172.46 (C=O and Ala C=O). Anal. Calcd for $C_{17}H_{25}NO_4$: C, 66.42; H, 8.20; N, 4.56. Found: C, 66.36; H, 8.09; N, 4.58.

Methyl 2(*S*)-Methyl-2-[5-[1-(*RS*)-(benzyloxy)-3-methylbutyl]tetrazol-1-yl]acetate (26). This material was synthesized from **25** by using the same general procedure described above. The crude product was isolated in a 6% yield as a mixture of two diastereoisomers after purification by flash chromatography (silica gel, EtOAc/hexane = 1:3): ^{13}C NMR ($CDCl_3$) δ 17.13 (Ala βC), 21.81, 22.00, 22.37, 22.61 (δC), 24.52, 24.67 (γC), 42.96, 43.33 (βC), 52.75, 52.86 (Ala αC), 55.98 (OCH_3), 70.90, 71.63, 71.78, 72.08 (αCH and CH_2Ph), 127.59, 127.86, 127.99, 128.18, 128.38, 128.51, 136.51, 135.72 (Ph), 154.53, 154.84 (CN_4), 168.45, 168.55 (Ala C=O).

Saponification of 13a and 13b. To a solution of **13b** (1.76 g, 4.39 mmol) in MeOH (10 mL) was added 1 N NaOH (4.4 mL, 4.4 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was removed in vacuo and the residue obtained was partitioned between H_2O (60 mL) and EtOAc (15 mL). The aqueous layer was washed with EtOAc (15 mL × 2), acidified with 10% HCl, and then extracted with EtOAc (80 mL × 3). The combined EtOAc extracts were washed with saturated NaCl solution and dried over $MgSO_4$. The solvent was evaported to give 1.62 g (95%) of a diastereoisomeric mixture of acids: ^{13}C NMR ($CDCl_3$) δ 21.07, 21.37, 22.37, (Leu δCH_3), 23.87 (Pro γCH_2), 24.71 (Leu γCH), 31.60, 31.80 (Pro βCH_2), 38.88, 39.51 (Leu βCH_2), 46.68, 46.86 (Pro γCH_2), 51.04, 51.78 (Pro αCH), 58.78, 59.34 (Leu αCH), 67.51, 67.66 (CH_2Ph) 127.60, 127.66, 127.75, 128.01, 128.99, 128.36, 135.83, 135.96 (Ph), 154.92, 156.78, 157.18 (Z C=O and CN_4), 169.94, 170.05 (COOH). Anal. Calcd for $C_{19}H_{25}N_5O_4$: C, 58.90; H, 6.50; N, 18.08. Found: C, 58.81; H, 6.60; N, 17.93.

A portion (200 mg) of the diastereoisomeric mixture of acids was dissolved in 5 mL of MeOH. A slight excess of CH_2N_2 in Et_2O was added to this solution. The solution was stirred at room temperature for 30 min and then stripped of solvent. The two esters were separated by flash chromatography using solvent system B and were found to have the same R_f(B), t_R(B), and ^{13}C NMR spectra as **13a** and **13b**. The higher R_f ester had a specific rotation of $[\alpha]_D$ +6.3° (c 1.20, MeOH) and was thus identified as the enantiomer of **13a, 13d**. The lower R_f ester possessed a specific rotation, $[\alpha]_D$ –90.2° (c 2.05, MeOH), identical with that of **13b**. The ratio of **13d** and **13b**, as shown by HPLC, was found to be equal to 2:5.

Diastereoisomer **13a** was saponified by using the same procedure as that described above for **13b** to obtain a mixture of diastereomeric acids in 92%. A portion of this mixture of acids was converted back into the methyl esters by using diazomethane. The mixture of two esters that was obtained were separated by flash chromatography and as in the case of **13b** were found to have the same R_f(B), t_R(B), and ^{13}C NMR spectra as **13a** and **13b**. The higher R_f ester possessed a specific rotation, $[\alpha]_D$ –8.0° (c 1.71, MeOH), identical with that of **13a**. The lower R_f ester had a specific rotation of $[\alpha]_D$ +89.5° (c 2.91, MeOH) and was thus identified as the enantiomer of **13b, 13c**. The ratio of **13a** and **13c**, as shown by HPLC, was found to be equal to 2:5.

Pht-ξLeuψ[CN_4]Ala-OH (32). Pht-*ambo*-Leuψ[CN_4]Ala-OBzl was obtained from Pht-Leu-Ala-OBzl by using the same method described above. One of the diastereoisomers was obtained in a 44% yield from EtOAc: mp 156–157 °C; $[\alpha]^{24}_D$ –114° (c 1.12, DMF); TLC R_f(B) 0.64; ^1H NMR ($CDCl_3$) δ 0.97 (d, J = 5.7 Hz, 6 H, Leu δCH_3), 1.40–1.70 (m, 1 H, Leu γCH), 1.97 (d, J = 7.5 Hz, 3 H, Ala βCH_3), 1.92–2.16 (m, 1 H, Leu βCH), 2.64–3.00 (m, 1 H, Leu βCH), 4.62 (s, 2 H, CH_2Ph), 5.35 (q, J = 7.5 Hz, 1 H, Ala αCH), 5.63 (dd, J = 5.5, 10.3 Hz, Leu αCH), 6.80–7.00, 7.08–7.28 (m, 5 H, Ph), 7.73 (br s, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 17.06 (Ala βC), 21.53, 22.65 (Leu δC), 24.91 (Leu γC), 38.30 (Leu βC), 42.07 (Leu αC), 55.76 (Ala αC), 67.81 (CH_2Ph), 123.59, 131.26, 134.38 (Pht), 127.62, 128.49, 134.21 (Ph), 153.28 (CN_4), 166.97, 167.49 (Ala and Pht C=O). Anal. Calcd for $C_{24}H_{25}N_5O_4$: C, 64.41; H, 5.63; N, 15.65. Found: C, 64.68; H, 5.94; N, 15.76.

The benzyl ester (120 mg, 0.268 mmol) was dissolved in DMF and this solution added to a flask containing 10% Pd/C (20 mg). The mixture was hydrogenolized at 40 psi by using a Parr Shaker for 1 h. The catalyst was removed by filtration and the filtrate was stripped of solvent. The residue was crystallized from EtOAc–petroleum ether to give 59 mg (62%) of **32**: mp 208 °C dec; $[\alpha]^{24}_D$ –159° (c 1.07, MeOH); ^1H NMR ($CDCl_3$) δ 0.98 (d, J = 6.15 Hz, 6 H, Leu δCH_3), 1.32–1.76 (m, 1 H, Leu γCH), 1.90 (d, J = 7.5 Hz, Ala βCH_3), 1.92–2.40 (m, 1 H, Leu βCH), 2.68–3.08 (m, 1 H, Leu βCH), 5.18 (q, J = 7.5 Hz, 1 H, Ala αCH), 5.51 (dd, J = 5.1, 10.3 Hz, 1 H, Leu αCH), 7.23 (br s, 1 H, COOH), 7.32–7.72 (m, 4 H, Pht); ^{13}C NMR (Me_2SO-d_6) δ 16.53 (Ala βC), 21.21, 22.69 (Leu δC), 24.16 (Leu γC), 37.51 (Leu βC), 41.28 (Leu αC), 51.19 (Ala αC), 123.23, 130.56, 134.63 (Pht), 153.44 (CN_4), 166.75, 169.52 (Ala and Pht C=O). Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.59. Found: C, 57.06; H, 5.63; N, 19.38.

Pht-ξLeuψ[CN_4]-L-Ala-Gly-OCH_3 (33b). Pht-ξLeuψ[CN_4]-Ala-OH (**32**, 300 mg, 0.81 mmol) was coupled to Gly-OCH_3·HCl by using the mixed anhydride method described by Anderson et al.[9] The crude product obtained was purified by flash chromatography (EtOAc–hexane = 2:1, R_f 0.45). The product **33b** was obtained as an oil (238 mg, 69%): $[\alpha]^{24}_D$ –134° (c 1.36, MeOH); ^1H NMR ($CDCl_3$) δ 0.98 (d, J = 6.2 Hz, 3 H, Leu δCH_3), 1.01 (d, J = 6.6 Hz, 3 H, Leu δCH_3), 1.40–1.84 (m, 1 H, Leu γCH), 1.99 (d, J = 7.0 Hz, 1 H, Ala βCH_3), 2.04–2.48 (m, 1 H, Leu βCH), 2.52–2.92 (m, 1 H, Leu βCH), 3.45 (d, J = 5.7 Hz, 2 H, Gly αCH_2), 3.65 (s, 3 H, OCH_3), 5.13 (q, J = 7.0 Hz, 1 H, Ala αCH), 5.64 (dd, J = 5.3, 10.1 Hz, Leu αCH), 6.12 (br t, NH), 7.60–7.88 (Pht); ^{13}C NMR ($CDCl_3$) δ 17.89 (Ala βC), 21.40, 22.65 (Leu δC), 24.65 (Leu γC), 38.39 (Leu βC), 40.77 (Gly αC), 42.11 (Leu αC), 51.95 (OCH_3), 57.28 (Ala αC), 123.50, 131.13, 134.34 (Pht), 153.67 (CN_4), 166.93, 167.53, 168.92 (Pht, Ala and Gly C=O). Anal. Calcd for $C_{20}H_{24}N_6O_5 \cdot 0.5H_2O$: C, 54.90; H, 5.64; N, 19.21. Found: C, 54.93; H, 5.76; N, 18.97.

Compound **33b** was also obtained through use of the DCC–HOBt[22] and *N,N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride[23] coupling methods. The yields obtained were 86% and 37%, respectively.

Pht-ξLeuψ[CN_4]-D-Ala-Gly-OCH_3 (33a). Compound **32** was coupled to Gly-OCH_3·HCl by using diphenylphosphoryl azide.[24] Gly-OCH_3·HCl (112 mg, 0.89 mmol) and compound **32** (300 mg, 0.81 mmol) were mixed in dry DMF (5 mL). The mixture was cooled to 0 °C in an ice bath. To this solution were added DPPA

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(245 mg, 0.89 mmol) and Et_3N (247 mL, 1.78 mmol) sequentially. The mixture was stirred at 0 °C for 10 h and then warmed up to room temperature. After the solution was stirred for an additional 6 h, the mixture was stripped of solvent under reduced pressure and worked up as described for **33b**. The product was obtained as a pair of diastereoisomers, **33a** and **33b** in an 83% yield. The ratio of **33a** and **33b** was about 7:13 as determined by measuring either the methyl ester or Gly αCH_2 resonances of the two compounds in ^1H NMR. Isomer **33a** was separated from **33b** by using flash chromatography (EtOAc/hexane = 2:1, R_f = 0.51): ^1H NMR ($CDCl_3$) δ 0.96 (d, J = 6.2 Hz, 6 H, Leu δCH_3), 1.40–1.76 (m, 1 H, Leu γCH), 1.95 (d, J = 7.5 Hz, 3 H, Ala βCH_3), 2.00–2.32 (m, 1 H, Leu βCH), 2.58–2.96 (m, 1 H, Leu βCH), 3.74 (s, 3 H, OCH_3), 4.09 (d, J = 5.72 Hz, Gly αCH_2), 5.38 (q, J = 7.5 Hz, 1 H, Ala αCH), 5.69 (dd, J = 5.7, 10.1 Hz, 1 H, Leu αCH), 7.23 (br t, J = 5.7 Hz, 1 H, NH), 7.56–7.88 (m, 4 H, Pht); ^{13}C NMR ($CDCl_3$) δ 17.54 (Ala βC), 21.35, 22.52 (Leu δCH_3), 24.95 (Leu γC), 38.52 (Leu βC), 41.42 (Gly αC), 42.72 (Leu αC), 52.21 (OCH_3), 57.45 (Ala αC), 123.67, 131.26, 134.55 (Pht), 153.93 (CN_4), 167.28, 167.62, 169.31 (C=O).

Synthesis of 13a and 13b from 2 Using PCl_5 and Different Azide Reagents. When PCl_5/Me_3SiN_3 or $PCl_5/(n\text{-}Bu)_3SnN_3$ were the reagents used for synthesis of the tetrazoles **13a** and **13b** the general procedure described above was applied except HN_3 was replaced with Me_3SiN_3 or $(n\text{-}Bu)_3SnN_3$. When the reagents $PCl_5/NaN_3/NH_4Cl$ were used, the reaction was carried out in DMF at 90 °C for 4 h and worked up as described in the general procedure.

Acknowledgment. This work was supported by a grant (NS 20036) from the National Institutes of Health and by a Research Career Development Award (HL 00932) to R.L.J. We also thank Josefina Quirante for synthesizing L-2-benzyloxy-4-methylpentanoic acid and Drs. Thomas Hoye and Thomas Livinghouse for helpful discussions.

The Influence of Ion Pairing on the Electroreductive Cleavage of Substituted 9,10-Anthraquinones in DMF Solution

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Received November 18, 1986

A variety of substituted 9,10-anthraquinones with acetate and trifluoroacetate leaving groups at the 2-methyl position were synthesized from 2-methyl-9,10-anthraquinones containing 0–2 methoxy substituents. Cyclic voltammograms of the acetates in DMF containing $LiClO_4$ as supporting electrolyte exhibited two reduction waves, the first resulting from the formation of Li^+ ion pairs of their radical anions and the second from Li^+ ion pairs of their dianions. Constant potential reduction of the acetates to their dianions followed by air oxidation gave high yields (78–88%) of their reductive cleavage products, the 2-methyl-9,10-anthraquinones. In contrast, reduction of the acetates to their radical anions led to high yields of their alcohols (the 2-(hydroxymethyl)-9,10-anthraquinones) as a result of saponification. Reduction of the trifluoroacetates in DMF/$LiClO_4$ produced comparable yields of their corresponding reductive cleavage products and alcohols via ion pairs of their radical anions or dianions.

Reductive cleavage has been used to deprotect 9,10-anthraquinone esters of amino acids,[1a] peptides,[1a] carboxylic acids,[1b] and primary amines.[1b] Bioreductive cleavage of the antitumor anthracyclines, which possess a substituted 9,10-anthraquinone, has been proposed as a possible mechanism whereby these drugs function as antineoplastic agents.[2,3] There is uncertainty, however, regarding the precise mechanism of this in vivo reaction of anthracyclines.[3b,c] Koch and co-workers[2b] have provided evidence that suggests that a hydroquinone intermediate is the actual species that undergoes cleavage whereas other workers[2c] favor a semiquinone. A third intermediate, which has not been seriously considered in the literature, is a radical anion. This could be an oversight since hydrophobic environments exist in the cell wherein this intermediate could be relatively long-lived.

It was our long-range goal to prepare a variety of substituted anthraquinones with good leaving groups and examine substituent effects upon the cleavage reactions of their hydroquinones in aqueous electrolytes and their radical anions or dianions in nonaqueous electrolytes by using electrochemical techniques. Redox potentials of these compounds, which would serve as models for the anthracyclines, could be useful in the design and synthesis of new anthracyclines that have low cardiotoxicity.[4] In this paper we report the synthesis of anthraquinones **1–4** and their electrochemistry in DMF electrolytes.

Results and Discussion

Synthesis of Anthraquinones 1–4. The synthetic route to anthraquinones **1–4** is outlined in Scheme I with **2**. Bromination of **2a** with *N*-bromosuccinimide gave **2b** in 75% yield. Compound **2b** was converted to **2c** with AgOAc (91%), **2d** with AgO_2CCF_3 (92%), and **2e** with

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