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A CONVENIENT PREPARATION OF DERIVATIVES OF 3(S)-AMINO-10(R)-CARBOXY-1,6-DIAZA-CYCLODECA-2,7-DIONE THE DILACTAM OF L-α,γ-DIAMINOBUTYRIC ACID AND D-GLUTAMIC ACID: A β-TURN TEMPLATE

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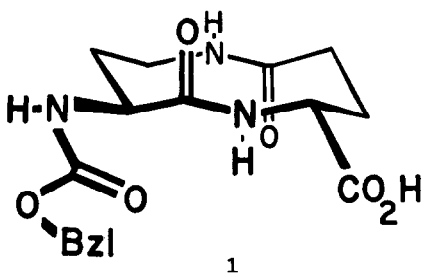
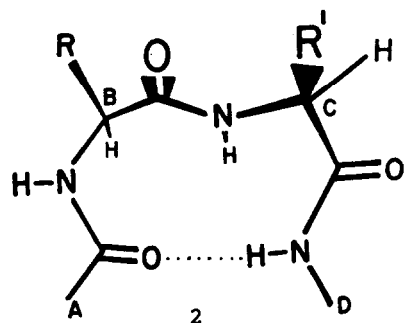
Abstract: The title compound has been prepared as its N-benzyloxycarbonyl urethane in a three-step one-pot reaction in 20-30% yield from the dipeptide derivative Z-Gln-Glu(OH)$_2$. Evidence concerning the configuration and chiral purity of the title compound is given.

As part of a study[1] of rigid analogs of amino acids that enforce local secondary structure on attached linear peptide sequences we wish to report synthesis of the novel macrocyclic dilactam 1, a template that may promote formation of Type IIa β-turns.[2] Since their recognition as major secondary structural elements of proteins, much attention has been directed to type II β-turns, defined by four amino acid residues in a conformation shown in 2.



1



2

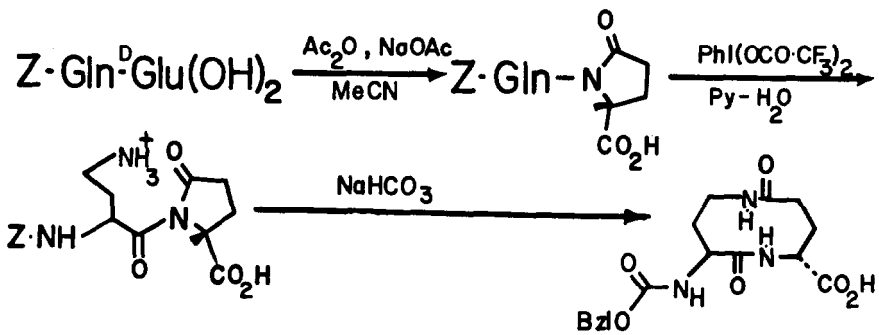
Recent studies imply that the conformations of many peptide hormones when bound to their receptors contain β-turns, and substantial increases in potency and duration of action have been observed for cyclic or bicyclic β-turn-bearing hormone analogs.[3a] Turns have also been implicated in posttranslational protein modification, particularly in phosphorylation and glycosylation of proteins, they often appear to define sites of high antigenicity, and they may provide conformational signals for the first stages of protein folding.[3b]

A normal tetrapeptide sequence has relatively free rotation about the four single bonds of 2, and thus β-turns are stabilized only weakly by the natural amino acids. The strongest turn biases are provided by a conformationally restricted proline residue at site 2 and a mobile glycine residue at site 3. Several groups have studied ring containing analogs of peptides that favor turns by restricting conformations available to linked peptide backbones.[4] We were attracted to 1 as a turn-enhancing template because 1) its constraining atoms lie in the

turn plane without modifying the turn amide function, 2) conformational constraint appears at two of the four single bonds of the turn, and 3) choice of L,D vs D,L chirality should selectively stabilize either type IIa or type IIb β-turns.

By analysis of the ^{1}H NMR spectrum, Winkler and Leutert[5] have established the preference of a chair-like conformation with roughly parallel s-trans amide planes for 1,6-diaza-cyclodeca-2,7-dione, the parent ring of 1, and have noted that the barrier for internal amide rotation is ca. 70 kJ/mol. The synthesis of this ring system by conventional amide chemistry has been shown to be difficult by several workers, doubtless owing to the van der Waals strain of generating the hindered sp^3 carbon of a tetrahedral intermediate in a ten-membered ring. Rothe[6a] and Rappoport[6b] have discovered the isomerization of N(γ-aminobutyryl)-pyrrolidones, which generate macrocycles via bicyclic tetrahedral intermediates with transannular N-C bonds. Although these intermediates might be expected to decompose efficiently to two pyrrolidiones, under proper conditions a reasonable yield of the macrocycle is obtained. In our hands 1 was only available by N-acylpyrrolidione routes. Scheme I outlines the most efficient of our procedures for the synthesis of 1; this route provides a one-pot three-step conversion of the dipeptide Z-Gln-Glu(OH)$_2$ to 1 in an overall yield of ca. 20 %, after purification.[7]

Scheme I



Several features of the Scheme are noteworthy. Use of L- Gln as a source in situ for the costly α,γ-L-diaminobutyric acid allows greater versatility in deployment of protective groups and is made possible by the studies of Loudon[8a] and of Waki[8b] on the selectivity of the reagent bis-(trifluoroacetoxy)iodobenzene for inducing Hoffman-like degradation of peptide primary amides to amines under mildly acidic conditions. Satisfactory yields were obtained for the pyrrolidone-forming step only with acetic anhydride in the presence of sodium acetate at temperatures of 60-80°C. A cyclic glutamic acid anhydride is a likely intermediate in the reaction, and these conditions are expected to epimerize highly activated amino acid derivatives. It is thus not surprising that the crude product of the reaction consists of a 4:1 mixture of isomers, shown by the reasoning given below to be the L,D and L,L epimers, and from which the desired L,D product can be isolated in 98-99 % purity by repeated precipitation. A mixture identical in all respects with that prepared above is obtained if the starting Z-L-Gln-D-Glu(OH)$_2$ is replaced by its L,L diastereomer, implying equilibration of the chiral Glu residue during the synthesis. (The 4:1 ratio of

diastereomeric products most probably results from differing efficiencies of cyclization of the acylpyrrolidones; analysis at the pyrrolidione step reveals a 3:1 L:D Glu ratio.)

Since epimerization at the Glu site occurs, the chiral integrity of 1 rests solely on retention of the L-Gln configuration throughout the synthetic sequence. Proof of chiral purity of the α,γ-diaminobutyric acid residue of 1 was unexpectedly difficult. The simplest chiral test for amino acids involves conversion to a dipeptide and separation of its diastereomers by ion exchange, as first reported by Manning and Moore.[9] A purified, homogeneous sample of 1 was subjected to acidic hydrolysis, and the resulting mixture of amino acids was subjected to assay., which unfortunately could only be carried out for the Glu fraction, found to be 92 % D and 8 % L. (Assay of a standard sample of H-L-Gln-OH, certified to be 99.9 % L, gave after hydrolysis and Manning-Moore assay 98 % L and 2 % D, and a reference sample of unhydrolyzed H-L-Glu-OH was found to be >99.9% L.) The simplest explanation of these findings is the operation of a racemization path specific for 1. A likely explanation is an unusually slow hydrolysis of the macrocyclic amides by an addition-elimination mechanism, coupled with intervention of an epimerizing acylpyrrolidone intermediate. Strong evidence is provided by the observation that when the hydrolysis of 1 is conducted in DCl-$CH_3CH_2CO_2D$, followed by H_2O exchange, substantial formation of the species $H3N^+$-CD(CH_2-CH_2-$NH3^+$)-CO_2H and H_3N^+-CD(CH_2-CD_2CO_2H)-CO_2H can be detected by ^2H NMR and by mass spectrometry.

Hydrolysis of 1 and measurement of the optical rotation of the resulting mixture of α,γ-diaminobutyric acid and H-Glu-OH was carried out with quantitation by amino acid analysis using an internal standard. The observed rotation at 633 nm was -0.019°, in approximate agreement with -0.025°calculated for a mixture containing the observed amounts of α,γ-diaminobutyric acid and H-Glu-OH, of which 2x0.08 % of the latter is assumed to be racemic. Since the rotations of the two amino acids are of opposite sign, and the observed rotation is small, this measurement has a large error and can be used to confirm the configurations at the two chiral centers of 1 but not to establish their enantiomeric purities. The latter point is established by an independent synthesis of the OtBu ester of 1 by the reactions of Scheme I. Thus the product from Z-L-Gln-D-Glu(OH)-OtBu after TFA treatment yielded a substance identical with 1 prepared by the earlier route, while a similarly treated Z-L-Gln-L-Glu-OtBu yielded a single species identical with the minor stereoisomer formed by the earlier route. HPLC experiments established the baseline separability of these two species and allowed the estimate that considerably less than 2% of the latter could be present in the sample of 1. Since the application of Scheme I to the t-butyl esters thus does not result in epimerization at the glutamate chiral center, independent epimerization at the Gln (or α,γ-diaminobutyrate) center would necessarily generate diastereomers. Since none were detected, the t-butyl ester route must preserve chiral integrity at both centers, and since the properties of the samples of 1 prepared from the dipeptide acid and ester are identical, the former product is also free of large amounts of its enantiomer.

We have carried out explorations of the removal of the Z group of 1 and incorporation of the resulting dipeptide analog into larger peptides. In general the manipulation of 1 and its tetrapeptide derivatives is complicated by poor solubility, tendency toward gel formation, and lack of crystallinity, suggesting that the presence of the two macrocyclic secondary

amide functions perpendicular to the plane of the turn facilitate intermolecular association. (The ^{1}H NMR spectra of **1** and its peptide derivatives support the structural analogy with the parent 1,6-diaza-cyclodeca-2,7-dione studied by Winckler and Leutert.[5]) Results of these and related studies will be reported subsequently.

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7. Z-L-Gln-D-Glu(OH)-OH , 10.4 g (20 mM) prepared by DCC/HOBt coupling of Z-L-Gln-OH and H-D-Glu(OtBu)$_2$, followed by TFA treatment, was suspended in 80 mL MeCN containing 3.3 g NaOAc and 5.6 mL Ac_2O and stirred in a 115°C bath for 10 min after start of reflux. The oil obtained after evaporation was dissolved in 14 mL 1:1 MeCN-H_2O and treated with 8.6 g (20 mM) of Ph-I(OTFA)$_2$. After 24 h at 25°C, the MeCN was removed, 350 mL H_2O was added, the pH was brought to pH 8.5, and after 4 h the solution was extracted with EtOAc, then acidified to pH 1-2. A crude yield of 2.8 g macrocycle Na salt was obtained, purified by repeated solution in 10 mL H_2O and precipitation with 300 mL MeCN. Yield as Na salt·3H_2O: 1.75 g, 20 %, mp 230-247°C dec. MS (FAB) M+1 386. $[\alpha]_{589}$ +35°(c=1.0 5% $NaHCO_3$). HPLC: 15:85 MeCN-1% TFA; 1 mL/min, Vydac 218TP54 column, 10.58 min (L,D), 13.45 min (L,L). Anal. Calcd for $C_{17}H_{21}N_3O_6$: C,56.19; H, 5.83; N, 11.56. Found: C, 55.39; H, 5.84; N, 11.25. High Res. MS: Calcd: 363.14301. found: 363.14272 ± 0.0007
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