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Optical Resolution of *N*-Carbobenzoxy-α-methoxyglycine

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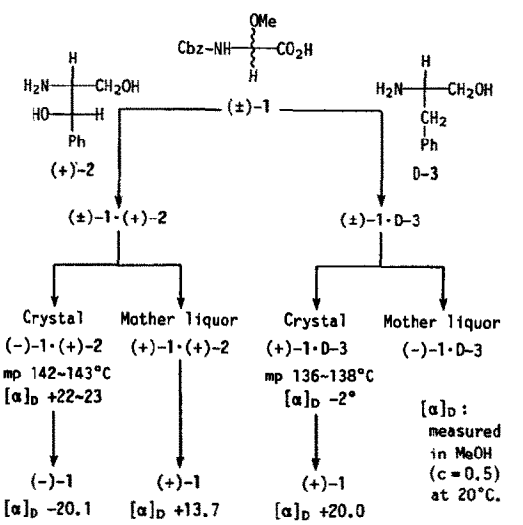
Abstract: *Optical resolution of racemic N-carbobenzoxy-α-methoxyglycine using* DL*-phenylalaninol or (1S,2S)-2-amino-1-phenyl-1,3-propanediol afforded (+)- and (-)-enantiomers, to which* D*- and* L*-configurations, respectively, were assigned based on the* ^{1}*H NMR data and biological activity of dermorphin N-terminal tetrapeptide analogs.*

α-Methoxyglycine, Gly(OMe), is a unique unnatural α-amino acid possessing an electronegative oxygen atom directly attached to the α-carbon atom. Protected Gly(OMe), namely *N*-carbobenzoxy-α-methoxyglycine methyl ester Cbz-Gly(OMe)-OMe, has been synthesized either by dehydrochlorination-MeOH addition of the *N*-chloro derivative of Cbz-Gly-OMe[1] or by *O*-methylation of the α-hydroxyglycine derivative Cbz-Gly(OH)-OH.[2] Although the *N*-deprotected Gly(OMe) residue was too labile to be used as an amino component for peptide bond formation under standard conditions, we have developed a method for synthesizing Gly(OMe)-containing peptides, which involves hydrogenolytic deprotection of the Cbz-Gly(OMe) group in the presence of a mixed anhydride prepared from a carboxyl component and isobutyl chloroformate. Diastereomeric analogs of dermorphin, a potent μ-opioid peptide, namely L-Tyr-D/L-Gly(OMe)-L-Phe-Gly-NH_2, were synthesized using racemic Cbz-Gly(OMe)-OMe as starting material. Chromatographic separation of the protected tetrapeptide enabled preparation of each diastereomer.[1] The optically active form of protected Gly(OMe), however, has not been described and optical resolution of Cbz-Gly(OMe)-OH (**1**) has been studied.

Resolution of the racemic acid (±)-**1** using alkaloids such as (-)-quinine, (-)-brucine, and (-)-norephedrine was unsuccessful. The diastereomeric salt with (+)-(1*S*,2*S*)-2-amino-1-phenyl-1,3-propanediol [(+)-**2**], however, was found to give crystals showing constant melting point (142-143°C) and optical rotation ($[\alpha]_D^{20}$ +22 → +23, c=0.5 in MeOH) on repeated recrystallizations from MeOH-H_2SO_4. Levorotatory free acid (-)-**1**, mp 91-92°C, $[\alpha]_D^{20}$ -20.1 (c=0.5 in MeOH), was obtained from the crystal in 15% yield based on (±)-**1**. From the mother liquor dextrorotatory acid (+)-**1** was obtained, which pos-

Scheme 1. Optical resolution of (±)-**1**.

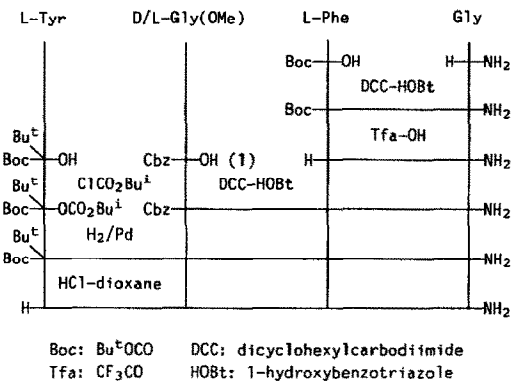


sessed low optical purity (Scheme 1). Unfortunately the enantiomeric amine (−)-**2**, which could be used to prepare the dextrorotatory acid (+)-**1** with high optical purity, was not available commercially. Since (−)-**2** corresponds to a 3-hydroxy analog of L-phenylalaninol [(*S*)-2-amino-3-phenyl-1-propanol] (L-**3**), resolution of (±)-**1** was attempted using L-**3**. Repeated recrystallizations of the diastereomeric salt (±)-**1**·L-**3** from MeOH–Et_2O yielded crystals possessing constant melting point (136–138°C) and rotation ($[\alpha]_D^{20}$ +2, c=0.5 in MeOH). Contrary to expectation, the free acid obtained from the crystallized salt was found to be levorotatory ($[\alpha]_D^{20}$ −20.0, c=0.5 in MeOH). Consequently, by the use of D-phenylalaninol (D-**3**) as the resolving agent the desired dextrorotatory acid (+)-**1** ($[\alpha]_D^{20}$ +20.0, c=0.5 in MeOH) was prepared in 20% yield as shown in Scheme 1. Optical purity of the thus obtained (+)-**1** and (−)-**1** was estimated to be quite high (>99.5%) by HPLC analysis of the synthetic peptides described below.

Analogs of the dermorphin *N*-terminal tetrapeptide amide[3] L-Tyr-D-Ala-L-Phe-Gly-NH_2 containing the optically active Gly(OMe) residue were synthesized by using each enantiomer of **1** according to Scheme 2. The ^1H NMR spectra of the intermediate protected tetrapeptides were superimposable to those of the diastereomeric peptides obtained by column chromatographic separation of Boc-L-Tyr(Bu^t)-DL-Gly(OMe)-L-Phe-Gly-NH_2,[1] and the peptides synthesized from (+)-**1** and (−)-**1** corresponded to the diastereomers with lower and higher R_f values, respectively, on silica gel TLC ($CHCl_3$:MeOH=9:1). Since D- and L-configurations had been assigned to the Gly(OMe) residues in the former and the latter diastereomers, respectively, on the basis of the ^1H NMR chemical shifts of the OMe groups,[1,4] (+)-**1** and (−)-**1** were assumed to possess D- and L-configurations, respectively.

The effect of these dermorphin analogs on rat brain membranes was examined.[5] The inhibitory concentration (IC_{50}) of the D-Gly(OMe)-containing analog on the binding of ^3H-labelled [L-MePhe^3, D-Pro^4]morphiceptin used as a μ-receptor selective opioid ligand was $1x10^{-8}$ mol/L, i.e., 20% as active as the parent dermorphin. The activity of the L-Gly(OMe)-containing diastereomer, on the other hand, was less than 1% that of dermorphin. Since the analog containing an L-Ala in place of the D-Ala residue is known to be much less active,[6] the result also supports the configurational assignment described above.

Scheme 2. Synthesis of dermorphin analogs.



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