Tetrahedron Vol. 33, pp 2299 to 2302 Pergamon Press 1977 Printed in Great Britain

SYNTHESIS OF γ-TETRAZOLE ANALOGUES OF L-GLUTAMIC ACID AND ITS DERIVATIVES

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(*Received in the UK 23 February 1977; accepted for publication 2 March 1977*)

Abstract—The γ-(5-tetrazolyl)-α-amino-L-butyric acid, an analogue of L-glutamic acid, was synthesized from methyl γ-cyano-α-benzyloxycarbonylamino-L-butyrate by transformation of the nitrile group into tetrazole nucleus by using tri-*n*-butyl-tin azide and removal of protecting groups. The synthesis of peptide derivatives containing γ-(5-tetrazolyl)-α-amino-L-butyryl residue is also described.

The free carboxyl group of hormones and other biologically active peptides appearing as a C-terminal or in aspartic and glutamic acid residues plays an important role in interaction with a protein receptor. For example in bradykinin[1] and angiotensin II[2] the presence of C-terminal carboxyl groups is essential for their hormonal activities. Likewise the activity of gastrin is connected with the presence of the free carboxyl group in aspartic acid residue in position 16.[3] Excellent structure-function relationship studies in the gastrin field conducted by Morley[4-7] on the C-terminal tetrapeptide responsible for the hormonal activity, revealed that analogues derived by the exchange of Asp† position results in loss of activity. The only exception displaying gastrin hormonal activity was a peptide containing a β-tetrazole analogue of aspartic acid residue in place of the parent amino acid.[7] It conformed with Herbst's well-known hypothesis according to which the replacement of the carboxyl group for the chemically and physically similar 5-tetrazolyl group in biologically active carboxylic acids may furnish compounds of interesting properties.[8]

The introduction of acidic tetrazolyl group in place of the carboxyl group may have substantial importance in studies of structure activity relationship in the peptide field, as it may enable the role of carboxyl group in its interaction with the protein receptor to be defined. The tetrazole analogues of hormones in which the carboxyl group is bonded through the covalent bond should have different biological activities in comparison to the parent hormones. On the other hand, when the hormone carboxyl group is bonded to the base centre in protein receptor through acid-base interaction, the tetrazole analogues of hormones should have similar biological properties.

Moreover, the tetrazole analogues can be helpful in structural studies of those peptides in which the conformation is stabilized by intermolecular acid-base interaction, as for instance in bradykinin or secretin.

To date, the syntheses of optical active tetrazole analogues of some α-amino acids including α- and β-analogues of aspartic acid, as well as α-analogue of glutamic acid were described.[7-9, 11] This paper presents the synthesis of the γ-tetrazole analogue of L-glutamic acid and its derivatives.

The most frequently applied method of preparing a tetrazole nucleus is a 1,3-dipolar cycloaddition of ammonium[12] or aluminium[13] azides to nitriles. This reaction has, in general, been found to proceed most rapidly with negatively substituted nitriles. The preparation of tetrazole analogues of amino acids from the appriopriate nitriles is a good illustration of the influence of inductive effects. Yields for the reaction of *N*-protected α-aminonitriles are *ca.* 80–95%,[9] and for the β-derivatives despite considerable prolongation of time reaction only 40%.[10] On the other hand in analogous conditions the γ-cyano-α-benzyloxycarbonylamino-butyric acid undergoes practically no transformation to the tetrazole derivative.

Recently Sisido *et al.*[14] described a new method of synthesis of 5-substituted tetrazoles by 1,3-dipolar cycloaddition of trialkyltin azides to nitriles followed by the splitting of the N–Sn bond. The authors stated that trialkyltin azide reacted more readily with a nitrile containing an electron-releasing substituent than with those containing electron-withdrawing groups. Our successful synthesis of γ-(5-tetrazolyl)-α-amino-*L*-butyric acid confirmed their results.

RESULTS

We started with methyl *N*-benzyloxycarbonyl-L-glutamate **1**. The γ-carboxamide group was dehydrated to nitrile by use of benzenesulphonyl chloride in pyridine. The prepared *N*-protected γ-cyano-α-aminobutyrate **2** was refluxed with an equimolar amount of tri-*n*-butyltin azide in tetrahydrofuran. The reaction was controlled spectrophotometrically by observing the decrease of C≡N (2250 cm^{-1}) and N_3 (2075 cm^{-1}) IR bands. After 70 h the reaction was stopped and the tin derivative **3** obtained was transformed to the 5-substituted tetrazole **4** by splitting the N–Sn bond using hydrogen chloride in ether. The carboxyl protecting group was removed by alkaline hydrolysis. The free γ-tetrazole analogue of L-glutamic acid **6** was obtained by removal of the benzyloxycarbonyl protection by the action of hydrobromic acid in acetic acid followed by neutralization of the hydrobromide formed with lithium hydroxide.

The structure of this new amino acid was confirmed by IR, NMR, ORD and CD spectra. The ORD and CD spectra (Fig. 1) of the γ-tetrazole analogue of L-glutamic

†The abbreviations follow IUPAC-IUB approved rules (1974) In addition the abbreviation GluT for γ-tetrazole analogue of glutamic acid [γ-(5-tetrazolyl)-α-amino-butyric acid] has been used All amino acids are of the L-configuration

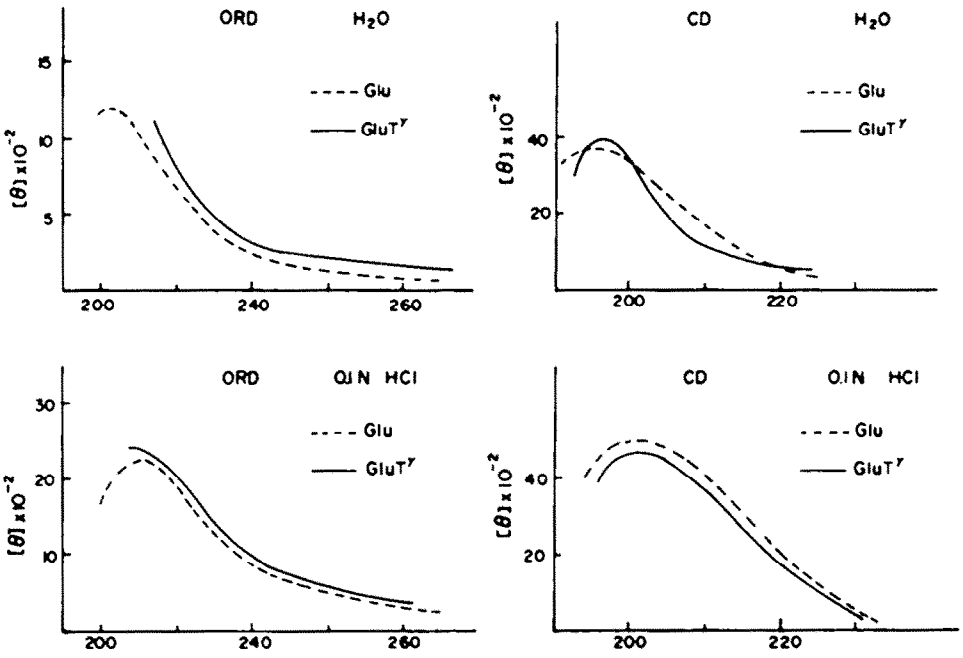
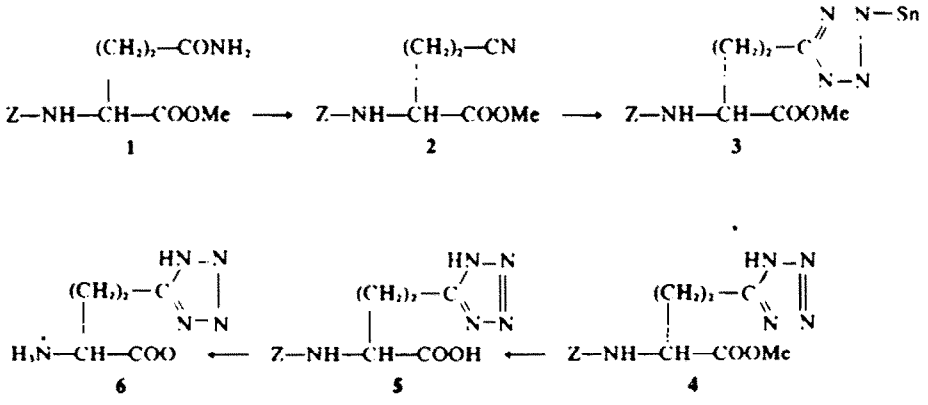


Fig. 1. ORD and CD spectra of L-glutamic acid and its γ-tetrazole analogue.



acid show a good convergence with the parent amino acid spectra. The positive Cotton effect on the CD spectrum due to the optical active $n \rightarrow \pi^*$ of the carboxyl group shows the characteristic dependence on the pH of the solution: at pH 1 $\lambda_{max} = 200$ nm, $\theta = +4750$, while at pH 6 $\lambda_{max} = 197$ nm, $\theta = +3950$.

In the NMR spectrum of γ-(5-tetrazolyl)-α-aminobutyric acid (Fig. 2) signals due to the nonequivalent protons in the metylene group in β-position, as well as in the γ-position were observed. Examination of the Dreiding models indicate that the interaction between the tetrazole nucleus and carboxylate anion is a factor restricting rotation around C_β–C_γ bond. Thus the specific conformation of the side chain is stabilized.

The tetrazole analogue of *N*-protected isoglutamine was obtained by the ammonolysis of ester 4. The reaction was carried out using aqueous and methanol solutions of ammonia. However, in the case of ammonia in methanol the reaction was so slow that after 48 h a high yield of the starting ester was recovered.

The γ-(5-tetrazolyl)-α-aminobutyryl residue was incorporated as N- or C-terminal to two model dipeptides. Coupling of the *p*-nitrophenyl ester of *N*-benzyloxy-

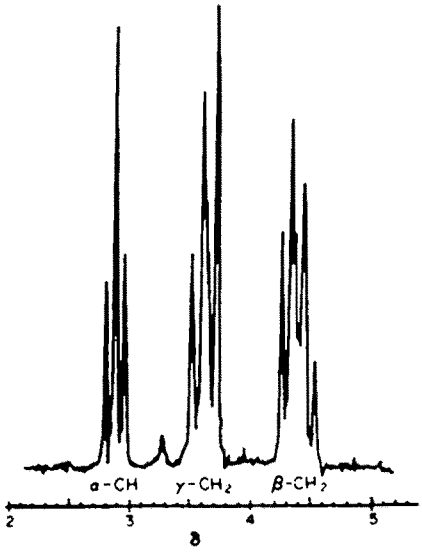
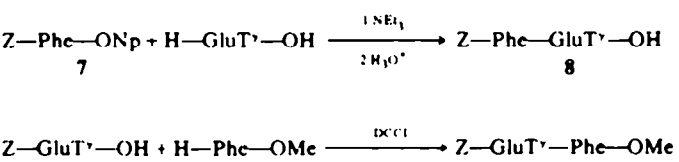


Fig. 2. NMR spectrum of γ-tetrazole analogue of L-glutamic acid.



carbonyl-L-phenylalanine **7** with the triethylammonium salt of γ - (5 - tetrazolyl) - α - aminobutyric acid afforded N-protected dipeptide **8** containing the free carboxyl group with a 73% yield. Protected dipeptide with N-terminal residue of γ-tetrazole analogue of L-glutamic acid was prepared in 90% yield by condensation of methyl phenylalaninate **9** with γ - (5 - tetrazolyl) - α - benzyloxycarbonylamino - L - butyric acid using dicyclohexylcarbodiimide.

The preparation of analogues of biologically active peptides containing tetrazole analogues of amino acids is in progress.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were determined on a Perkin-Elmer Model 257 spectrophotometer. NMR spectra were taken on a Tesla BS 487 spectrometer operating at 80 MHz. ORD and CD curves were determined with a JASCO J-20 spectropolarimeter at 25°.

Methyl N - benzyloxycarbonyl - L - glutaminate. A solution of *N* - benzyloxycarbonyl - L - glutamine (5.6 g–20 mmoles) and sulphuryl chloride (0.08 ml) in dry methanol (40 ml) was left for 3 days at room temp. The methanol was then evaporated, the residue was washed with AcOEt and crystallized twice from methanol. Yield 4.1 g (73%); m.p. 141–141.5°. Lit.[15] m.p. 140–141°.

Methyl γ - cyano - α - benzyloxycarbonylamino - L - butyrate. Benzenesulphonyl chloride (2.3 ml) was added to a suspension of methyl *N* - benzyloxycarbonyl - L - glutaminate (4.19 g–15 mmoles) in dry pyridine (5 ml). The solution was stirred at 50° for 20 min, cooled to room temp. and then acidified with 2 N hydrochloric acid. The mixture was extracted three times with AcOEt. The combined AcOEt layers were washed with 1 N hydrochloric acid, $NaHCO_3$ solution, water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was crystallized from ether–hexane. Yield 3.74 g (90%); m.p. 42–43°; $[\alpha]_D^{20}$ 21° (c = 0.6, MeOH). IR (nujol): 3300 (ν NH), 2330 (ν CN), 1750 (ν CO_{ester}), 1675 (ν $CO_{urethane}$); NMR ($CDCl_3$) δ: 7.10 (5H, s, aromatics), 4.98 (2H, s, benzyl CH_2), 4.30 (H, m, CH), 3.58 (3H, s, OCH_3), 2.45 (4H, m, CH_2CH_2). (*Anal.* Calc. for $C_{14}H_{16}N_2O_4$: C, 60.85; H, 5.84; N, 10.14. Found: C, 60.72; H, 5.91; N, 10.35%).

Methyl γ - (5 - tetrazolyl) - α - benzyloxycarbonylamino - L - butyrate. A solution of methyl γ - cyano - α - benzyloxycarbonylamino - L - butyrate (2.76 g–10 mmoles) and tri-*n*-butyltin azide[16] (3.08 ml–10 mmoles) in dry THF (10 ml) was refluxed for 70 h. Then the cooled solution was treated with hydrogen chloride in ether and left for 1 h. The crystalline product was collected and washed several times with ether. Yield 1.94 g (61%); m.p. 135–138°. Recrystallization from aqueous methanol raised m.p. 145–146° (1.76 g–55%); $[\alpha]_D^{20}$ −14.5° (c = 1, MeOH). IR (Nujol): 3290 (ν NH), 1730 (ν CO_{ester}), 1675 (ν $CO_{urethane}$), 1050 and 1020 cm^{-1} (tetrazole ring). NMR (d_6-acetone) δ: 2.25 (2H, m, β-CH_2), 3.06 (2H, t, γ-CH_2), 3.46 (3H, s, OCH_3), 4.25 (H, m, CH), 5.00 (2H, s, benzyl CH_2), 7.25 (5H, s, aromatics). (*Anal.* Calc. for $C_{14}H_{17}N_3O_4$: C, 52.67; H, 5.37; N, 21.94. Found: C, 52.57; H, 5.43; N, 22.32%).

γ - (5 - Tetrazolyl) - α - benzyloxycarbonylamino - L - butyric acid. Methyl γ - (5 - tetrazolyl) - α - benzyloxycarbonylamino - L - butyrate (1.28 g–4 mmoles) was dissolved in 1N NaOH (8.8 ml). The solution was left for 1 h at room temp. and then acidified with 1N hydrochloric acid. The crystalline product was collected and washed with water. Yield 1.12 g (92%); m.p. 76–82°. Recrystallization from water gave 0.9 g (74%) a material melting at 83–84°; $[\alpha]_D^{20}$ 6.0° (c = 1, MeOH). IR (Nujol): 3270 (ν HN), 1705 (ν CO_{acid}), 1680 (ν $CO_{urethane}$), 1069 and 1014 cm^{-1} (tetrazole ring). NMR (d_6-acetone) δ: 2.29 (2H, m, β-CH_2), 3.06 (2H, t, γ-CH_2), 4.37 (H, m, CH), 5.02 (2H, s, benzyl CH_2), 7.24 (5H, s, aromatics). (*Anal.* Calc. for $C_{13}H_{15}N_5O_4 \cdot H_2O$: C, 48.30; H, 5.30; N, 21.67. Found: C, 48.55; H, 5.37; N, 21.79%).

γ - (5 - Tetrazolyl) - α - benzyloxycarbonylamino - L - butyramide. Methyl γ - (5 - tetrazolyl) - α - benzyloxycarbonylamino - L - butyrate (0.64 g–2 mmoles) was dissolved in conc. aqueous ammonia (3 ml) and the solution was left at room temp. for 4 days. The solvent was removed under reduced pressure, the residue dissolved in water and the amide was separated by acidification with 2 N hydrochloric acid. The product was collected and washed several times with water. Yield 0.48 g (79%); m.p. 190–193°. The material recrystallized from methanol melted at 193–194° (0.36 g–60%); $[\alpha]_D^{20}$ + 5.0° (c = 0.8, MeOH). IR (Nujol): 3370 and 3280 (ν NH), 1680 (broad, ν $CO_{urethane, amide}$), 1090 and 1054 cm^{-1} (tetrazole ring). NMR (d_6-DMSO) δ: 2.28 (2H, m, β-CH_2), 3.13 (2H, t, γ-CH_2), 4.25 (H, m, CH), 5.20 (2H, s, benzyl CH_2), 7.13–7.75 (8H, m, aromatics, NH). (*Anal.* Calc. for $C_{13}H_{16}N_6O_3$: C, 51.31; H, 5.30; N, 27.62. Found: C, 51.36; H, 5.03; N, 27.98%).

γ - (5 - Tetrazolyl) - α - amino - L - butyrate. γ - (5 - Tetrazolyl) - α - benzyloxycarbonylamino - L - butyric acid (0.327 g–1.07 mmole) was treated with hydrobromic acid in acetic acid (1 ml). After 40 min dry ether was added; the separated hygroscopic hydrobromide was washed several times with dry ethyl ether by decantation and dried in a dessicator over KOH and P_2O_5. The hydrobromide was dissolved in methanol and the solution was neutralized by the addition of 2 N $LiOH_{aq}$ and left overnight in a refrigerator. The crystalline product was collected and washed with ether. Yield 0.18 g (98%); m.p. 231° (dec.). The material recrystallized from water–acetone melted with dec. at 233° (0.12 g–66%); $[\alpha]_D^{20}$ + 11° (c = 0.6, H_2O). IR (Nujol): 3300–2400 (ν NH_3^+), 1650 (δ$_{as}$ NH_3^+), 1582 (ν $CO_{carboxylate}$), 1511 (δ$_{sym}$ NH_3^+), 1082 and 1044 cm^{-1} (tetrazole ring). NMR (D_2O) δ: 2.61 (2H, m, β-CH_2), 3.36 (2H, m, γ-CH_2), 4.10 (H, t, CH). (*Anal.* Calc. for $C_5H_9N_5O_2 \cdot H_2O$: C, 31.74; H, 5.86; N, 37.02. Found: C, 31.82; H, 5.98; N, 38.33%).

Benzyloxycarbonyl - L - phenylalanyl - γ - (5 - tetrazolyl) - α - amino - L - butyric acid. The suspension of *p*-nitrophenyl ester of *N* - benzyloxycarbonyl - L - phenylalanine (0.462 g–1.1 mmole), γ-(5-tetrazolyl)-α-amino-L-butyric acid (0.171 g–1 mmole), NEt_3 (0.28 ml–2 mmoles) in DMF (2 ml) and water (0.3 ml) was stirred for 20 h. Then aqueous $NaHCO_3$ was added and the solution was extracted three times with AcOEt. The aqueous layer was acidified with 2 N hydrochloric acid to pH 2 and then extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was crystallized from acetone–ether. Yield 0.33 g (73%), m.p. 148–150°. IR (Nujol): 3265 (ν NH), 1712 (ν $CO_{carboxyl}$), 1682 (ν $CO_{urethane}$), 1645 (ν $CO_{peptide}$), 1070 and 1045 cm^{-1} (tetrazole ring). (*Anal.* Calc. for $C_{22}H_{24}N_6O_5$: C, 58.39; H, 5.35; N, 18.58. Found: C, 58.53; H, 5.69; N, 19.12%).

γ - (5 - Tetrazolyl) - α - benzyloxycarbonylamino - L - butyryl - L - phenylalanine methyl ester. The dicyclohexylcarbodiimide (0.206 g–1 mmole) was added to the solution of γ - (5 - tetrazolyl) - α - benzyloxycarbonylamino - L - butyric acid (0.305 g–1 mmole) and methyl ester of L-phenylalanine (0.22 g–1.2 mmole) in DMF (4 ml). The mixture was stirred overnight, diluted with AcOEt (20 ml), and the separated dicyclohexylurea was filtered off and washed with AcOEt. The combined filtrates were washed with 1N hydrochloric acid, water and dried over $MgSO_4$. The residue obtained after solvent evaporation was

crystallized from AcOEt. Yield 0.42 g (90%), m.p. 164–166°; $[\alpha]_D^{20} - 17°$ (c = 1, acetone). IR (Nujol): 3330 (ν NH), 1738 ($\nu_s O_{ester}$), 1692 ($\nu CO_{urethane}$), 1660 ($\nu CO_{peptide}$), 1080 and 1064 cm^{-1} (tetrazole ring). NMR (d_6-acetone) δ: 2.23 (2H, m, β-CH_2), 2.99 (4H, m, benzyl CH_2, CH_2-tetrazole), 3.55 (3H, s, OCH_3), 3.80–4.78 (5H, m, NH, CH), 4.98 (2H, urethane CH_2), 7.10 (5H, s, aromatics). (*Anal.* Calc. for $C_{21}H_{26}N_6O_5$: C, 59.21; H, 5.62; N, 18.02. Found: C, 59.28; H, 5.90; N, 17.91%).

Acknowledgements—The authors gratefully acknowledge financial support for this work by a grant from the Polish Academy of Sciences MR-I.12.1.6.1/4.

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