SYNTHESIS OF OPTICALLY ACTIVE DEUTERIUM LABELLED SERINE AND GLYCYLSERINE DERIVATIVES

J. Kovacs*, G. Jham** and K. Y. Hui
St. John's University, Department of Chemistry
Grand Central & Utopia Parkways, Jamaica, N.Y. 11439, U.S.A.

SUMMARY

Optically active α-deuterated serine dipeptide active ester derivatives were prepared to study the racemization mechanism. N-Acetyl-O-benzyl-DL-serine, 1, was converted to 5(4H)-oxazolone 2 and deuterated with CH_3-COOD in α-position. 5(4^2H)-Oxazolone 3 was converted to Z-(α^2H)-L-Ser(Bzl)-OH*** 7, after opening the 5(4^2H)-oxazolone ring with D_2O, removal of the N-acetyl group, treatment with carbobenzoxy chloride and resolution with (+)-α-phenylethylamine. Pentachlorophenyl ester 8 of 7 was converted to Z-Gly-(α^2H)-L-Ser(Bzl)-OPcp, 10, after removal of carbobenzoxy group by catalytic hydrogenation. The deuterium content of the intermediates was determined by mass spectrometry.

Key Words: Derivatives of α^2H-Serine, α^2H-Glycylserine, 5(4^2H)-Oxazolone Serine Active Ester, Optical Resolution

*To whom reprint requests should be addressed.

**Present address: Universidade Federal De Vicosa
Department de Quimica, Vicosa, MG. 36.570, Brasil
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***The following abbreviations are used: benzyloxycarbonyl = Z; tert-butyloxycarbonyl = Boc; benzyl = Bzl; pentachlorophenyl = Pcp; dicyclohexylcarbodiimide = DCC; dicyclohexylurea = DCU, tetrahydrofuran = THF; thin layer chromatography = TLC

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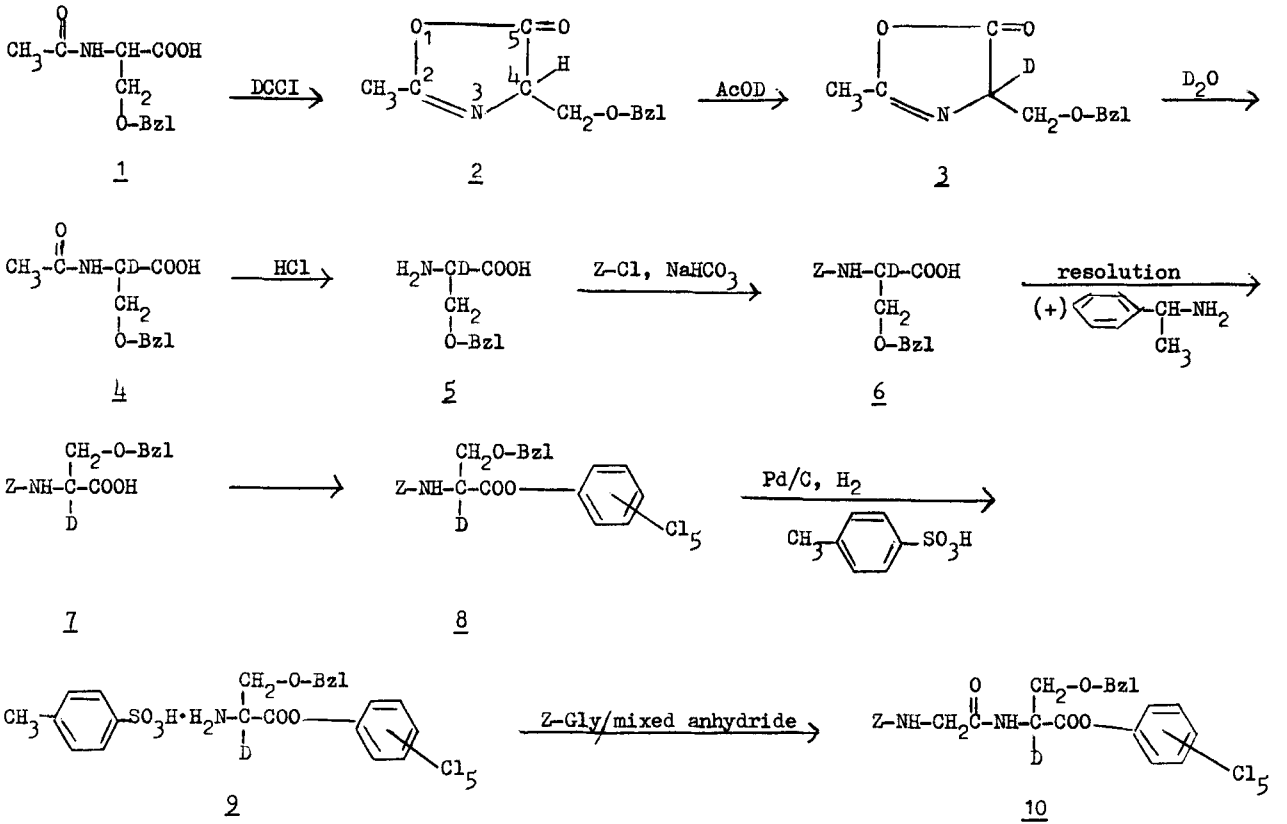
The unusual slow rate of racemization of N-carbobenzoxyglycyl-S-benzyl-L-cysteine active esters and the absence of 5(4H)-oxazolone peak in the IR spectrum during racemization led us to conclude that these active esters probably racemize via the α-hydrogen abstraction mechanism instead of the usual 5(4H)-oxazolone route (1). Detailed investigation of the racemization mechanism of N-carbobenzoxyglycyl-S-benzyl-L-cysteine p-nitrophenyl ester was carried out and the α-hydrogen abstraction mechanism was verified (2,3). Since the Z-Gly-Ser(Bzl)-OPcp also racemizes slower than expected (4) it seemed necessary to investigate the racemization mechanism of the N-carbobenzoxyglycyl-O-benzyl-L-serine active esters. In order to study the mechanism of racemization (5) we needed optically active deuterium labelled serine derivatives. In this communication we describe the synthesis of α-deuterated serine and glycylserine pentachlorophenyl ester derivatives.

DISCUSSION

The scheme below shows the synthesis of N-carbobenzoxyglycyl-O-benzyl-α-deuterio-L-serine pentachlorophenyl ester. N-Acetyl-O-benzyl-DL-serine, 1, (6) was converted to 5(4H)-oxazolone derivative 2 with dicyclohexylcarbodiimide (7). 5(4H)-Oxazolone derivative was needed to introduce the deuterium in α-position since the α-hydrogen is more acidic in 2 than the α-hydrogen in 1 due to easy enolization of the neighboring carbonyl group (9); the driving force in this reaction is the formation of the aromatic oxazole derivative (9). The deuteration was achieved by treatment of 2 with CH_3-COOD. The 5(4^2H)-oxazolone 3 was treated with deuterium oxide to open the ring without the loss of deuterium. The α-deuterium content of N-acetyl-O-benzyl-α-deuterio-DL-serine, 4, was 98% as determined by mass spectrometry. Compound 4 was then hydrolyzed with HCl to O-benzyl-α-deuterio-DL-serine, 5, which was converted to N-carbobenzoxy-O-benzyl-α-deuterio-DL-serine, 6, with carbobenzoxy chloride the usual way (9). Optically active N-carbobenzoxy-O-

SCHEME FOR SYNTHESIS OF O-BENZYL-α-DEUTERIO-L-SERINE DERIVATIVES



benzyl-α-deuterio-L-serine, 7, was obtained from 6 by resolving it with (+)-α-phenylethylamine. Mass spectrometry showed at least 95% deuterium content; the possible loss of some deuterium during this procedure is due to the basic α-phenylethylamine. N-Carbobenzoxy-O-benzyl-α-deuterio-L-serine pentachlorophenyl ester, 8, was prepared from 7 with DCC and pentachlorophenol. The deuterium content of 8 was found to be 87 $\pm$ 10%; however this value must be higher than 87% since compound 9, which was prepared from 8, has at least 95% deuterium content. The carbobenzoxy group was removed by catalytic hydrogenation in the presence of p-toluenesulphonic acid to obtain O-benzyl-α-deuterio-L-serine pentachlorophenyl ester p-tolueneshulphonate, 9. Active ester salt, 9, was coupled with N-carbobenzoxyglycine using the mixed anhydride procedure (10) to obtain N-carbobenzoxyglycyl-O-benzyl-α-deuterio-L-serine pentachlorophenyl ester, 10, which was needed for racemization rate studies. The deuterium content of 10 was at least 85%. The loss of deuterium might be due to the presence of N-methyl morpholine during the coupling procedure since the strongly electron withdrawing pentachlorophenyl ester residue increases the acidity of the α-deuterium (12). The deuterium content was measured by chemical ionization mass spectrometry by comparing the relative intensities of M^+, $(M+1)^+$ and $(M+2H)^+$ of the labelled and unlabelled compounds. In the case of the pentachlorophenyl esters, calculations were made on the basis of M+1 $-HOPhCl_5$.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. The purity of compounds were checked routinely using Brinkman pre-coated TLC plates, silica gel 60F-254. Optical rotations were determined on a Rudolph spectropolarimeter, Model 200S-340-8006 and on a Cary 60 spectropolarimeter. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Mass spectra were made at the Rockefeller University, N.Y. at Mass Spectrometric Biotechnology Resource.

N-Acetyl-O-benzyl-α-deuterio-DL-serine, 4.

N-Acetyl-O-benzyl-DL-serine, 1, (6) (4.74 g, 0.02 mol) was added with stirring to 30 ml of sodium dried tetrahydrofuran solution of DCC (4.33 g 0.021 mol). The solution was stirred at room temperature for 1 h. The DCU was removed by filtration and washed with sodium dried tetrahydrofuran; to the filtrate containing the 5(4H)-oxazolone 30 equivalent (36 ml) freshly prepared deuterated acetic acid was added and the solution was left at room temperature for 8 h. to obtain 5(4D)-oxazolone 3.

Deuterated acetic acid was prepared by refluxing an equimolar mixture of D_2O and freshly distilled acetic anhydride for 10 minutes. In another experiment the THF solution, after filtration from DCU, was evaporated and the IR (film) of the oily residue showed the characteristic 5(4H)-oxazolone peak (11) at 5.45 μ.

At the end of 8 h., 55 ml of D_2O (99.8% deuterium) was added to the solution when more DCU precipitated out, and the mixture was allowed to stand at room temperature overnight, then filtered. The filtrate was evaporated in vacuo at 40°C to 10 ml and was acidified with 2N HCl until precipitation was complete. The crystalline N-acetyl-O-benzyl-α-deuterio-DL-serine, 4, was filtered, washed with water and dried under vacuum; yield 3.15g, 75%; mp 133-135°C. It was recrystallized from hot water, mp 137-138°C; literature (6) mp for the unlabelled compound is 139-140°C. The deuterium content was 98% as determined by mass spectrometry.

N-Carbobenzoxy-O-benzyl-α-deuterio-DL-serine, 6.

N-Acetyl-O-benzyl-α-deuterio-DL-serine, 4, (24.89 g, 0.104 mol) was suspended in 186 ml of 1 N HCl and the solution was refluxed for 2 h. The solution was neutralized with ammonia to give 8.45 g of O-benzyl-α-deuterio-DL-serine, 5, which was used without purification for the preparation of 6.

Crude O-benzyl-α-deuterio-DL-serine, 5, (8.45 g, 0.043 mol) was added to a solution of sodium bicarbonate (7.31 g, 0.086 mol) in 225 ml of water and then 225 ml

ether containing carbobenzoxy chloride (0.0665 mol) was added. The reaction mixture was vigorously stirred for 1.5 h. at room temperature. The ether layer was separated and the aqueous layer was extracted with two 100 ml portions of ether then acidified to pH 2 with 1 N HCl. The aqueous layer was extracted with three 35 ml portions of ether and the combined ether layer was extracted with 25 ml of water, dried over anhydrous sodium sulfate and evaporated to dryness. The oily residue was triturated with petroleum ether to give 9.35 g of N-carbobenzoxy-O-benzyl-α-deuterio-DL-serine, yield 65%; mp 98-100°C. The literature value (6,13) for the unlabelled compound is 99-100°C. The deuterium content was calculated to be 92.4% from the relative intensity data presented in Table I.

TABLE I

Compound	Molecular Wt., M	M^+	$(M+H)^+$	$(M+2H)^+$	$\left(\frac{M+2H}{M+H}\right)^+ \times 100$
Z-DL-Ser(Bzl)	329	0.62	100	20.85	20.85
Z-(α^2H)-DL-Ser(Bzl)	330	6.78	100	22.57	22.57

Resolution of N-Carbobenzoxy-O-benzyl-α-deuterio-DL-serine, 7.

N-Carbobenzoxy-O-benzyl-α-deuterio-DL-serine (8.23 g, 0.025 mol) was suspended in 10.7 ml of isopropanol, followed by addition of (+)-1-phenylethylamine (3.34 g, 0.027 mol) to give a clear solution. The solution was allowed to stand at room temperature for 4 h., the solid which precipitated was filtered and washed with 15 ml of isopropanol and two 15 ml portions of dry ether and then dried in vacuum to give 4.16 g salt; yield 74% based on the L isomer, mp 115-122°C. The crude compound was recrystallized from 10 ml isopropanol to give 3.06 g (54%) pure salt of 7, mp 123-125°C, $[\alpha]_{313}^{19.8}$ + 150 (c 1, EtOH). The following physical constants have been reported in the literature (14) for the undeuterated N-carbobenzoxy-O-benzyl-D-serine (-)-1-phenylethylamine salt; mp 124°C; $[\alpha]_{313}^{20}$ - 147.6 (c 1, EtOH).

The α-phenylethylamine salt of N-carbobenzoxy-O-benzyl-deuterio-L-serine was suspended in 25 ml of water and 2 N NaOH was added until the solid dissolved. The solution was extracted with two 30 ml portions of ether and the aqueous layer was acidified to pH 2 with 1 N HCl. On cooling crystalline 7 was precipitated which was filtered and washed with 10 ml water to give 2.19 g of N-carbobenzoxy-O-benzyl-α-deuterio-L-serine, mp 95-97°C; $[\alpha]_D^{23}$ + 13 (c 1, EtOH); $[\alpha]_{313}^{23}$ + 80 (c 1, EtOH). The following physical constants have been reported in the literature (14) for the resolved undeuterated N-carbobenzoxy-O-benzyl-D-serine; mp 98°C; $[\alpha]_D^{20}$ - 16.8 (c 1, EtOH); $[\alpha]_{313}^{20}$ - 89.6 (c 1, EtOH). The extent of deuteration was calculated to be at least 95%.

N-Carbobenzoxy-O-benzyl-α-deuterio-L-serine Pentachlorophenyl Ester, 8.

N-Carbobenzoxy-O-benzyl-α-deuterio-L-serine (0.33 g, 1 mmol) and pentachlorophenol (0.293 g, 1.1 mmol) were dissolved in 5 ml of ethyl acetate. To this solution 0.227 g (1.1 mmol) DCC was added. The DCU was filtered, the filtrate left overnight at 0°C and filtered again to remove additional DCU. The solvent was concentrated in vacuo at 40°C, and on the addition of petroleum ether the crude ester precipitated out in crystalline form, filtered and washed 3 times with 4 ml portions of ether-petroleum ether (1:3); yield 0.46 g, 80%, mp 124-124.5°C and the mixed melting point with the unlabelled 8 gave no depression; this compound shows the characteristic active ester peak (11) at 5.62 μ. The extent of deuteration was calculated to be 87%.

N-Carbobenzoxy-O-Benzyl-L-Serine Pentachlorophenyl Ester, 8.

This unlabelled ester was synthesized as described for the labelled compound. The crude compound was recrystallized from absolute methanol, yield 62%; mp 123-125°C; $[\alpha]_{400}^{22}$ + 9.58 (c 3.6, THF); IR (KBr) showed the characteristic active ester peak at 5.62 μ.

Anal. Calcd. for $C_{24}H_{18}Cl_5NO_5$: C, 49.99; H, 3.14; N, 2.45. Found: C, 49.77; H, 3.38; N, 2.28.

O-Benzyl-L-Serine Pentachlorophenyl Ester Trifluoroacetate.

Boc-L-Ser(Bzl)-OPcp (10) (3.3 g; 6.07 mmol) was dissolved in 18 ml of anhydrous trifluroacetic acid and after 15 minutes 40 ml of anhydrous ether was added and the solution was refrigerated for 1 h. The crystalline trifluoroacetate was filtered and washed with two 50 ml portions of dry ether and dried in a desiccator over sodium hydroxide and P_2O_5; yield 3.1 g, 90%; mp 139-141°C. Another portion of the trifluoroacetate salt was converted to the HBr salt as described below for characterization.

O-Benzyl-L-Serine Pentachlorophenyl Ester Hydrobromide.

N-tert-Butyloxycarbonyl-O-benzyl-L-serine pentachlorophenyl ester, 10, (1 g, 1.84 mmol) was dissolved in 5 ml of trifluoroacetic acid. After 10 minutes 4 ml of HBr/AcOH (40%) was added to the clear solution. Crystalline HBr salt was formed immediately; the solution was diluted with ether and the solid filtered, washed with ether and dried; yield 0.80 g (96%); mp 180-182°C. The crude HBr salt was recrystallized from ethanol-ether, mp 182.5-183.5°C; $[\alpha]_D^{23}$ + 15.45 (c 1.1, DMF); IR (KBr) showed the active ester peak at 5.59 μ.

Anal. Calcd. for $C_{16}H_{13}BrCl_5NO_3$: C, 36.66; H, 2.50; N, 2.67. Found: C, 36.63; H, 2.56; N, 2.84.

N-Carbobenzoxyglycyl-O-Benzyl-L-Serine Pentachlorophenyl Ester.

N-Carbobenzoxyglycine and O-benzyl-L-serine pentachlorophenyl ester trifluoroacetate were coupled in tetrahydrofuran in the usual manner by the mixed anhydride procedure (10). The crude dipeptide ester was obtained in 75% yield and recrystallized from tetrahydrofuran-pentane and then from tetrahydrofuran petroleum ether; yield 35%; mp 149.5-151.5°C, $[\alpha]_D^{23}$ -4.57 (c 3.17, THF); IR (KBr) showed the active ester peak at 5.58 μ, amide I and amide II peaks at 6.02 and 6.5 μ respectively. TLC in $CHCl_3$: MeOH (9:1) showed a major spot for the active ester and a very faint spot corresponding to the pentachlorophenol.

Anal. Calcd. for $C_{26}H_{21}Cl_5N_2O_6$: C, 49.2; H, 3.33; N, 4.41. Found: C, 49.36; H, 3.51; N, 4.24.

O-Benzyl-α-Deuterio-L-Serine Pentachlorophenyl Ester p-Toluenesulphonate, 9.

Anhydrous p-toluenesulphonic acid, prepared from the hydrate (2.07 mmol) by drying over P_2O_5 in vacuum to constant weight was dissolved in 30 ml dry methanol, followed by addition of 0.36 g Pd/C (10%). The palladium on charcoal was prehydrogenated for 15 minutes, then finely powdered N-carbobenzoxy-O-benzyl-α-deuterio-L-serine pentachlorophenyl ester (1.2 g, 2.07 mmol) was added and the mixture was hydrogenated for 20 minutes. The solution was warmed and the solid was removed by filtration of the warm solution. The residue was washed with 10 ml of methanol, the filtrate was evaporated to dryness in vacuo at 35°C and diluted with 10 ml of dry ether. The crystalline material was washed with 5 ml of THF and dried; mp 181-182°C when the temperature raised 2°C/min., however, if the temperature was raised fast to 175°C first, then the mp was 211°C. The mother liquor was evaporated to dryness and the solid residue was washed with 10 ml of dry THF and was recrystallized from absolute methanol; total yield 80%; IR (KBr) showed the active ester peak at 5.65 μ.

O-Benzyl-L-Serine Pentachlorophenyl Ester p-Toluenesulphonate.

This compound was synthesized as the labelled ester above, yield 60%; mp 180-182.5°C; IR (KBr) shows identical active ester peak with the unlabelled compound.

Anal. Calcd. for $C_{23}H_{20}Cl_5NO_6S$: C, 44.81; H, 3.27; N, 2.27. Found: C, 44.76; H, 3.3; N, 2.28.

N-Carbobenzoxyglycyl-O-Benzyl-α-Deuterio-L-Serine Pentachlorophenyl Ester, 10.

N-Carbobenzoxyglycine (0.078 g, 0.373 mmol) was dissolved in 3 ml of dry tetrahydrofuran and isobutylchloroformate (0.049 ml, 0.373 mmol) was added followed by the addition of N-methyl morpholine (0.0416 ml, 0.373 mmol). The reaction mixture was stirred at -5°C. After 7 minutes a suspension of O-benzyl-α-deuterio-L-serine pentachlorophenyl ester p-toluenesulphonate, 9, (0.23 g, 0.373 mmol) in 2 ml of dry

tetrahydrofuran was added. A solution of 3 ml of dry tetrahydrofuran containing 0.0416 ml (0.373 mmol) of N-methyl morpholine was added to the above mixture over a period of 2 h. with stirring, the temperature being maintained between -10 and 0°C. The solid was filtered and washed with 10 ml of tetrahydrofuran and the solution was evaporated to dryness in vacuo at 35°C. The solid residue was dissolved in 50 ml of ethyl acetate and washed with 2 portions of H_2O. The organic layer was dried over anhydrous sodium sulfate, filtered, evaporated in vacuo. The solid residue was dissolved in 10 ml ethyl acetate, filtered, and evaporated in vacuum. The residue was dissolved in 4.5 ml of tetrahydrofuran and the product was precipitated with 4.5 ml of petroleum ether. The crystalline 10 was filtered, washed with tetrahydrofuran:petroleum ether (1:1) and dried; yield 86%; mp 153-154°C; the mixed melting point with the unlabelled 10 gave no depression; IR (KBr) showed the active ester peak at 5.62 μ, and the amide I and amide II peaks at 6.15 μ and 6.58 respectively; $[\alpha]_D^{22}$ -4 (c 1, THF).

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