Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 4

SYNTHESIS AND ^{1}H-NMR OF DEUTERIUM LABELED D,L-HOMOSERINE LACTONE HYDROCHLORIDES

Kondareddiar Ramalingam and Ronald W. Woodard*
Department of Medicinal Chemistry
College of Pharmacy
The University of Michigan
Ann Arbor, Michigan 48109-1065

SUMMARY

The synthesis of six regiospecific deuterated D,L-homoserine lactone hydrochlorides from the appropriately deuterated 2-bromo-1-(tetrahydropyranyloxy) ethanes which were obtained by reduction of the corresponding ethyl bromoacetates are described. The proton nmr of each lactone is recorded and the chemical shift and coupling constants are reported.

Key Words : Deuterium labeling, ^{1}H-nmr, homoserine lactone

INTRODUCTION

L-Homoserine (2-amino-4-hydroxybutanoic acid) is a pivotal amino acid utilized in a variety of pyridoxal-phosphate-dependent enzymatic reactions. (1) These enzyme reactions convert L-homoserine derivatives such as *O*- acetyl-L-homoserine, *O*-phosphoryl-L-homoserine, and (or) *O*- succinyl-L-homoserine into L-methionine (2), L-threonine (3), and L-cystathionine (4), respectively. In order to study the mechanism of these enzymatic transformations, it is necessary to prepare regio- and stereo-specifically deuterated homoserine derivatives and to assign both the chemical shifts and coupling constants of the hydrogens in the ^{1}H-nmr spectra of homoserine. Since the lactone ring form of homoserine is more stable than the open-chain form and the proton chemical shifts in the five-membered ring are more distinct than in the open-chain form (5), we chose the lactone ring system as our synthetic target. The deuterated homoserine lactone-HCl salts shown in Scheme 2 were synthesized and their ^{1}H-nmr spectra were recorded (see Table 1).

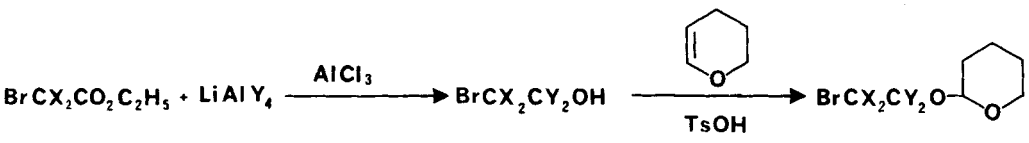
*To whom correspondence should be addressed.

0362-4803/87/040369–08$05.00
© 1987 by John Wiley & Sons, Ltd.

Received June 3, 1986
Revised August 20, 1986

RESULTS AND DISCUSSION

The necessary 2-bromo-1-(2'-tetrahydropyranyloxy)ethanes were obtained by , reduction of the appropriately labeled ethyl bromoacetates with either $LiAlH_4$ or $LiAlD_4$ (6) and $AlCl_3$, followed by reaction with dihydropyran (7) (see Scheme 1).

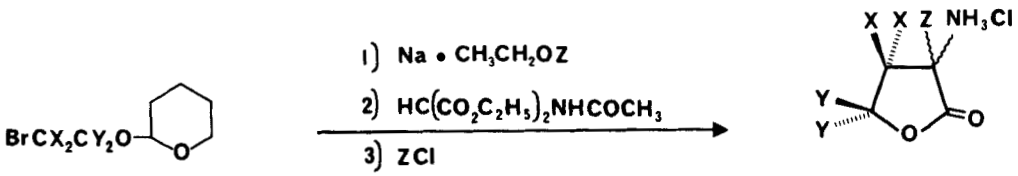


1	2	3	4
a X = H	a Y = H	a X = H Y = H	a X = H Y = H
b X = H	b Y = ^{2}H	b X = H Y = ^{2}H	b X = H Y = ^{2}H
c X = ^{2}H	c Y = H	c X = ^{2}H Y = H	c X = ^{2}H Y = H
d X = ^{2}H	d Y = ^{2}H	d X = ^{2}H Y = ^{2}H	d X = ^{2}H Y = ^{2}H

The next step in the reaction scheme involved a Sn2-type displacement of the bromide from the appropriately deuterated ethane derivative by the *in situ* - generated sodium salt of diethyl acetamidomalonate in either ethanol or ethanol-OD.(8) Hydrolysis and decarboxylation of the condensation product in either 6N HCl or DCl permitted the isolation of the desired deuterated lactone as depicted in Scheme 2.

The overall yield of the deuterated lactone in these three steps is only 45-52%. However, only the lactonization step is poor, mainly due to the fact that in acidic aqueous ethanolic solutions homoserine lactone is in equilibrium with homoserine. Mudd (9) has demonstrated that when D,L-homoserine is dissolved in 6N HCl for 1 hr at room temperature, an equilibrium mixture of lactone (60%) and homoserine (40%) is obtained. Armstrong (10) has also reported low yields (41-44%) in the preparation of D- and L- homoserine lactone-HBr from D- and L-*O*- phenyl homoserine *via* treatment with 48% HBr.

Since the enzymes to be studied utilize only the L-homoserine derivative, it is not necessary to resolve the enantiomers. We have, however, resolved several of the target lactones by dissolving them in buffer solution (pH = 8.5) and allowing them to stand for 1-2 hr, then treating the resulting mixture with D-amino acid oxidase and catalase using standard methods.(11) After separation of the amino acid from the keto



4		5
a X = H Y = H	Z = ^{2}H	a X = H Y = H Z = ^{2}H
b X = H Y = ^{2}H	Z = ^{2}H	b X = H Y = ^{2}H Z = ^{2}H
c X = ^{2}H Y = H	Z = ^{2}H	c X = ^{2}H Y = H Z = ^{2}H
d X = ^{2}H Y = ^{2}H	Z = H	d X = ^{2}H Y = ^{2}H Z = H
b X = H Y = ^{2}H	Z = H	e X = H Y = ^{2}H Z = H
c X = ^{2}H Y = H	Z = H	f X = ^{2}H Y = H Z = H

acid, one obtains optically pure homoserine which may be lactonized or derivatized directly.

The ^{1}H-nmr spectra were measured at 270 MHz without deuterium decoupling and the results are summarized in Table 1 along with the Dreiding models of two of the more stable conformations of the five-membered lactone ring system.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. All chemicals were obtained from Aldrich Chemical Co. and were used without further purification. Percent deuterium incorporations were determined from ^{1}H-nmr spectral data. NMR spectra were obtained on an IBM WP-270 MHz NMR spectrometer. Anhydrous diethyl ether (Mallinckrodt) was used without further purification.

2-Bromo[1,1-$^2H_2$]ethanol 1b.

Compound **1b** was prepared by a modification of the previously published procedure(6). A suspension of lithium aluminum deuteride (98.9 atom % D) (1.25 g; 0.03 mol) in dry diethyl ether (150 mL) was stirred at -78°C for 30 min. To a cooled, stirred suspension, anhydrous aluminium chloride (4.00 g; 0.03 mol) was added in small portions from a solid addition device in a manner that allowed the temperature of the stirred suspension to be maintained at -75°C. The mixture was then stirred for an additional 30 min at -78°C. To this suspension, ethyl bromoacetate (5.0 g; 0.03 mol) in

50 mL of dry diethyl ether was added dropwise over a 30 min period from a constant pressure addition funnel. The resultant mixture was then stirred at -78°C for an additional 2 hr and then allowed to warm slowly to room temperature. Excess deuteride was destroyed by the addition of ethyl acetate (5 mL) followed by water (5 mL). The ether solution was decanted and the aluminum salts were washed with ether (3 x 75 mL). The combined diethyl ether extracts were dried (Na_2SO_4), concentrated and distilled to give 2.2 g (58%) of 2-bromo[1,1-$^2H_2$]ethanol **1b**: bp 56-57°C/20 mm Lit (8) 65-68°C/33 mm ^{1}H-nmr ($CDCl_3$) **d** 2.52 (s, 1H, O*H*), 3.64 (s, 2H, -C*H*$_2$Br).

2-Bromo[2,2-$^2H_2$]ethanol 1c.

Using the method described above, 8.45 g (0.05 mol) of ethyl bromo-[2,2-$^2H_2$]acetate(6) and lithium aluminium hydride (2.01 g; 0.05 mol)-anhydrous aluminium chloride (6.6 g; 0.05 mol) were reacted to give 3.40 g of **1c**: bp 55-57°C/20 mm ^{1}H-nmr ($CDCl_3$) **d** 2.56 (s, 1H, O*H*), 3.85 (s, 2H, -C*H*$_2$OH).

2-Bromo[1,1,2,2-$^2H_4$]ethanol 1d.

Compound **1d** was prepared by the same procedure described for the preparation of 2-bromo[1,1-$^2H_2$]ethanol **1b**. Ethyl 2-bromo[2,2-$^2H_2$]acetate (8.45 g; 0.05 mol) and lithium aluminum deuteride (2.01 g; 0.05 mol) and anhydrous aluminum chloride (6.6 g; 0.05 mol) were reacted to give 3.35 g (52%) of 2-bromo[1,1,2,2-$^2H_4$]ethanol **1d**; bp 56-57°C/20 mm.

2-Bromo-1-(2'-tetrahydropyranyloxy)ethane 4a.

The title compound was prepared by a modification of the method reported by Witiak *et. al.* (7) To a stirred solution of dihydropyran (2.02 g; 0.024 mol) and a catalytic amount of *p*- toluenesulfonic acid (TsOH) (15 mg) at 0°C was added 2-bromoethanol (3.0 g; 0.02 mol). The reaction mixture was stirred at room temperature for 2 hr. Distillation of the reaction mixture in the presence of solid $NaHCO_3$ afforded 3.55 g (85%) of a colorless liquid: bp 63-64°C/0.5 mm (Lit. (7) bp 67-68°C/0.7 mm).

2-Bromo-[1,1-$^2H_2$]-1-(2'-tetrahydropyranyloxy)ethane 4b.

Dihydropyran (1.09 g; 0.013 mol) was added to [1,1-$^2H_2$]-2-bromoethanol (1.5

g, 0.012 mol) containing a catalytic amount of TsOH. Workup as de-scribed above gave a colorless liquid: bp 64-66°C/0.5 mm. ^{1}H-nmr ($CDCl_3$) **d** 1.3-1.9 (m, 6 H, 3', 4', 5'-C*H*$_2$), 3.3-3.85 (m, 4 H, 6'-C*H*$_2$ and Br-C*H*$_2$-C^{2}H$_2$-), 4.4 (b.s., 1 H, 2'-*H*).

2-Bromo-[2,2-$^2H_2$]-1-(2'-tetrahydropyranyloxy)ethane 4c.

The tetrahydropyranylether from 2-bromo-[2,2-$^2H_2$]ethanol was prepared by the procedure described above. The oil was isolated in 80% yield after vacuum distillation: bp 60-61°C/0.2 mm. ^{1}H-nmr ($CDCl_3$) **d** 1.3-1.9 (m, 6 H, 3', 4', 5'-C*H*$_2$), 3.2-3.85 (m, 4 H, 6'-C*H*$_2$ and Br-C^{2}H$_2$-C*H*$_2$-), 4.28 (b.s., 1 H, 2'-*H*).

2-Bromo-[1,1,2,2-$^2H_4$]-1-(2'-tetrahydropyranyloxy)ethane 4d.

The ether **4d** was prepared by the same method as the pyranylether **4a**. The product was isolated by vacuum distillation as a clear oil: bp 63-64°C/0.5 mm in a yield of 78%. ^{1}H-nmr ($CDCl_3$) **d** 1.3-1.9 (m, 6 H, 3', 4', 5'-C*H*$_2$), 2.9-3.65 (m, 2 H, 6'-C*H*$_2$), 4.2 (b.s., 1 H, 2'-*H*).

D,L-[2-^{2}H]Homoserine lactone hydrochloride 5a.

To a solution of 0.12 g (5 mg atom) of sodium in C_2H_2OD (5 mL) was added, with stirring, diethyl acetamidomalonate (1.08 g; 5 mmol) and the mixture was heated under reflux for 0.5 hr under a nitrogen atomosphere. After the suspension of diethyl sodium acetamidomalonate had cooled, 2-bromo-1-(2'-tetrahydropyranyloxy) ethane (1.2 g; 5.2 mmol) was added and the mixture was stirred at room temperature for 3 hr and heated at reflux for 12 hr. After cooling to 25°C, 15 mL of DCl (20% wt/wt) was added and the solution heated at reflux for 24 hr. The majority of the DCl and C_2H_5OD was removed under water aspiration pressure. Water (5 mL) was added to the dark waxy residue and the aqueous portion of the solution was decanted and concentrated to 2 mL. Absolute ethanol (25 mL) was added to this solution and the resulting mixture was evaporated under reduced pressure. This process was repeated twice and the resulting residue dried *in vacuo* at 100°C. The semi-crystalline solid was dissolved in 25 mL of boiling absolute ethanol and filtered to remove any undissolved sodium bromide. The volume of the filtrate was reduced to 7 mL and left standing at 4°C overnight. The white crystalline product that separated was filtered and dried *in vacuo*

to yield 52% of the title compound (0.364 g) with a mp 203-205°C (Lit. (12) mp 198-199.5°C for the nondeuterated analog). Compound **5a** showed an incorporation of 99.0 atom %of deuterium.

D,L-[2,4,4-$^2H_3$]Homoserine lactone hydrochloride 5b.

Compound **5b** was obtained from 2-Bromo-[1,1-$^2H_2$]-1-(2'-tetrahydropyranyl-oxy)ethane **4b** (1.2 g; 5.7 mmol) and diethyl acetamidomalonate (1.09 g; 5 mmol) by the same procedure described above. Recrystallization from absolute ethanol gave white crystals, mp 203-205°C, (0.34 g; 49%). The atom percent of deuterium in **5b** estimated from the integrated nmr spectrum was 98%.

D,L-[2,3,3-$^2H_3$]Homoserine lactone hydrochloride 5c.

By following the above procedure, **5c**, mp 202-204°C (absolute ethanol) was obtained in 49% yield from (1.2 g; 5.7 mmol) of **4c**. The isotopic purity of the compound **5c** was determined by nmr to be 98.5%.

D,L-[3,3,4,4-$^2H_4$]Homoserine lactone hydrochloride 5d.

The title compound was prepared in a procedure analogous to that reported for **5a** except that the non-deuterated ethanol was used as the solvent and the hydroylsis, decarboxylation, and deprotection were accomplished with non-deuterated hydrochloric acid to yield 0.33 g (47%) of a white solid with a mp 203-205°C. The atom percent of deuterium in **5d** obtained from the integrated nmr spectrum was 98.5%.

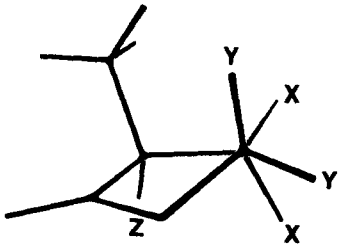
D,L-[4,4-$^2H_2$]Homoserine lactone hydrochloride 5e.

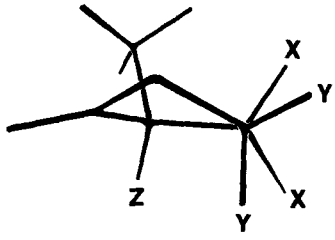
Lactone **5e** was prepared in the same manner as **5d** from **4b** in 46% yield with a mp 202-204°C. The isotopic purity of the compound **5e** was determined by nmr to be 98.7%.

D,L-[3,3-$^2H_2$]Homoserine lactone hydrochloride 5f.

In an experiment similiar to that for **5e**, 0.6 g of **4c** was converted into 0.17 g (51%) of **5f**, mp 201-203°C. Compound **5f** showed an incorporation of 98.5 atom % of deuterium.

Table 1. ^{1}H-nmr Data On Homoserine Lactone Hydrochloride.





Compd	H_2 (Z)	H_3 (X)	H_3 (X)	H_4 (Y)	H_4 (Y)
5a	----	2.56 (q*)	2.29 (q*)	4.47 (q*) J=8.8Hz	4.28 (m)
5b	----	2.54 (d) J=12.3Hz	2.27 (d) J=12.2Hz	----	----
5c	----	----	----	4.47 (d) J=8.8Hz	4.28 (d) J=8.9Hz
5d	4.33 (s)	----	----	----	----
5e	4.33 (dd) J=11.3Hz J=8.9Hz	2.62 (dd) J=12.2Hz J=8.9Hz	2.38 (t*) J=11.8Hz	----	----
5f	4.33 (s)	----	----	4.45 (d) J=8.8Hz	4.28 (d) J=8.9Hz

* apparent

ACKNOWLEDGMENT

This work was supported by U. S. Public Health Service Grant GM 30097. We are grateful to the U. S. P. H. S. and the College of Pharmacy for their contribution to the purchase of the IBM 270 MHz NMR.

REFERENCES

1. Walsh, C. - In "Enzymatic Reaction Mechanisms", W. H. Freeman and Co., N. Y., 1979, pp. 823-827.
2. Kerr, D. and Flavin, M. - *Biochem. Biophy. Res. Comm.* **31**: 124 (1968).
3. Flavin, M. and Kono, T. - *J. Biol. Chem.* **235**: 1109-1111 (1960).
4. Chang, M. N. T. and Walsh, C. T. - *J. Am. Chem. Soc.* **103**: 4921-4917 (1981).

5. Chang, M. N. T. and Walsh, C. T. - *J. Am. Chem. Soc.* **102**: 7370-7372 (1980).
6. Hogg, J. L. and Schowen, R. L. - *J. Pharm. Sci.* **63**: 1620-1623 (1974).
7. Witiak, D. T., Poochikian, G. K., Feller, D. R., Kenfield, N. A. and Neusman, H. A. I. - *J. Med. Chem.* **18**: 992-1000 (1975).
8. Ramalingam, K. and Woodard, R. W. - *J. Labelled Compounds* **XXI**, 563-568 (1984).
9. Mudd, S. H. - *J. Biol. Chem.* **237**: 87-92 (1959).
10. Armstrong, M. D. - *J. Am. Chem. Soc.* **71**: 3399-3402 (1949).
11. Greenstein, J. P., Birnbaum, S. M. and Otey, C. M. - *J. Biol. Chem.* **204**: 307-321 (1953).
12. Fillman, J. and Albertson, N. - *J. Am. Chem. Soc.* **70**: 171-74 (1948).