SYNTHESIS OF RACEMIC, R- AND S-[1-^{11}C]-β-HYDROXYBUTYRIC ACID

Jan-Olov Thorell[1,2], Sharon Stone-Elander*[1,2], Wilfried A. König[3], Christer Halldin[4] and Lennart Widén[2]

[1]Karolinska Pharmacy, Box 60024, S-104 01 Stockholm, Sweden

[2]Department of Clinical Neurophysiology, Karolinska Hospital and Institute, Stockholm, Sweden

[3]Organic Chemistry Institute, University of Hamburg, Hamburg, Germany

[4]Department of Psychiatry and Psychology, Karolinska Hospital and Institute, Stockholm, Sweden

Summary

Racemic, R- and S-β-hydroxybutyric acid were labelled with ^{11}C in the carboxylic position by a two-step stereospecific synthesis starting with carrier-added [^{11}C]cyanide and R/S, R- or S-propylene oxide. Hydrolysis of the intermediate nitrile with hydrochloric acid gave racemic [1-^{11}C]-β-hydroxybutyric acid and R- or S-[1-^{11}C]-β-hydroxybutyric acid with an enantiomeric excess of 87-97%. The total synthesis time (including HPLC purification) was 45-50 min from end of trapping. The isolated decay-corrected radiochemical yield was 20-30% based on [^{11}C]cyanide. The radiochemical purity of the products was > 99%

Key words: [1-^{11}C]-β-Hydroxybutyric acid, [^{11}C]cyanide, stereoselective synthesis, PET

*Author for correspondence

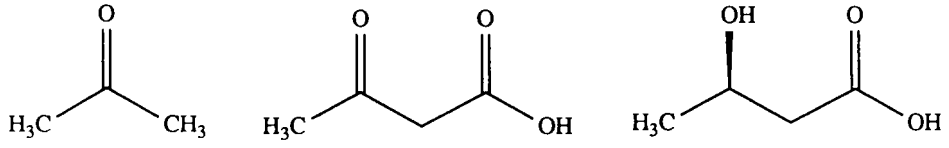
Introduction

D-Glucose is the primary substrate for energy metabolism of the human brain. Under abnormal conditions, however, such as starvation (1,2) the brain may fulfill its nutritional needs at

0362–4803/91/060709–10$05.00

Received 25 February, 1991

least partly with one or more of the ketone bodies: acetone (A), acetoacetic acid (B) and β-hydroxybutyric acid (C) (Scheme 1), which are formed in the liver from the incomplete metabolism of fatty acids. Labelling these compounds with the positron-emitting radionuclide ^{11}C ($t_{1/2}$=20.3 min) and investigation of their *in vivo* transport, distribution and metabolism using positron emission tomography (PET) might allow an estimation of the extent to which they serve as an alternative energy source under different physiological conditions.



A B C

Scheme 1

Both acetone and acetoacetic acid have previously been labelled with ^{11}C (3,4). The *in vivo* distribution of acetoacetic acid has been studied in cats with PET (5,6).

R (=D(-)) and S (=L(+)) β-Hydroxybutyric acid (β-HBA) have not, to our knowledge, previously been labelled with a positron-emitting radionuclide. *In vivo* studies of ^{14}C-labelled β-HBA in the rat (7) have indicated that not only is the metabolism of β-HBA different from that of acetoacetic acid, but also that both the R- and S-forms of β-HBA are metabolically active. The R-form is more favored in oxidative processes and the S-form in lipid syntheses. Production of the radiotracer with a high degree of enantiomeric purity is therefore desirable for the *in vivo* studies of its metabolism.

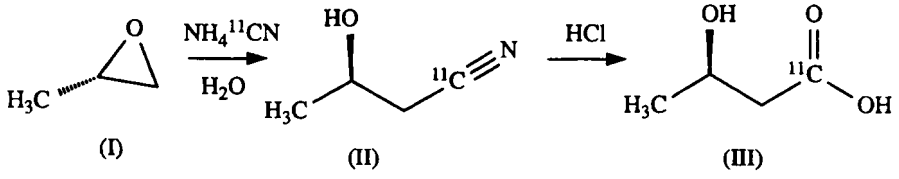
Stereoselective or stereospecific radiolabelling procedures have been accomplished previously in a number of ways, for example:

1. Synthesis of a racemic mixture followed by isolation of the desired isomer either chromatographically or enzymatically. This method suffers from the disadvantage that half of the radionuclide starting material will automatically be lost.

2. Stereoselective incorporation of the radionuclide forming only one of two possible stereoisomers.

3. Stereospecific synthesis in which reaction with a chiral starting material proceeds with retention of configuration at the asymmetric carbon.

The production of ^{11}C-labelled β-HBA, preliminary reported previously (8) and described in detail here, is based on method #3 and is shown in scheme 2.



Scheme 2

[^{11}C]Cyanide (CN^-), a useful labelling precursor, has been used previously to generate a variety of functionalities such as nitriles, amines, carboxylic acids, aldehydes and amides (for a review see 9). In the synthesis reported here, the nucleophilic attack by [^{11}C]CN^- at the least substituted carbon of racemic or chiral propylene oxide (I) proceeded similarly to the reaction of CN^- with epichlorohydrin (10) with ring opening of the epoxide to generate 3-hydroxybutyronitrile (II). The regiospecificity of the ring opening reaction was confirmed by ^{13}C-NMR spectroscopy.

Hydrolysis of the nitrile (II) was accomplished with either concentrated acid or base. The enantiomeric purity of (III) was determined by chiral gas chromatography (11).

Experimental

General

R/S, R- and S-Propylene oxide were obtained from Fluka and trifluoroacetic anhydride (for gas chromatography) from Merck. The solvents used were of analytical grade and commercially available.

Analytical HPLC was performed using an LDC Constametric III pump, a Rheodyne injector (7125 with a 250 µl loop), an Erma ERC 7510 refractrometer (RI) in series with a Beckman model 170 β-flow radiodetector. The column used was a Waters µ-Porasil (300 x 7.8 mm, 10 µm). The mobile phase was CH_2Cl_2:EtOH:HOAc = 800:20:7.5 with a flow rate of 6 ml/min. Peak areas were integrated using a Shimadzu Chromatopac C-R2AX. Semi-preparative HPLC was performed using a Shimadzu LC-6A pump, a Rheodyne injector (7126 with a 1 ml loop), an Aminex HPX 87-H column (300 x 7.8 mm, 9 µm), heated at 65°C, and a GM tube for radioactivity detection. The mobile phase used was 0.01 N H_2SO_4 with a flow rate of 0.75 ml/min.

The NMR spectral analysis was performed on a Varian XL-300. A Carlo Erba Mega 5360 gas chromatograph with split injection and flame ionization detector was employed for enantioselective gas chromatography. The separation of the enantiomers of 3-O-trifluoroacetoxy-butyric acid methyl ester was achieved using a Pyrex glass capillary (30 m, i.d. 0.25 mm) with octakis(3-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin as a chiral stationary phase. Hydrogen at an inlet pressure of 0.6 bar served as a carrier gas; the column temperature was 95°C. Peak areas were integrated with a Merck-Hitachi D-2000 integrator.

[^{11}C]NH_4CN

[^{11}C]Carbon dioxide (CO_2) was produced at the Karolinska Hospital with a Scanditronix MC 16 cyclotron using 17 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. After batch production, the [^{11}C]CO_2 was converted in a two step process (12) to [^{11}C]ammonium cyanide (NH_4CN) and was trapped in water (0.4 ml) with or without carrier NaCN at 0-5°C.

Racemic, R- or S-[1-^{11}C]-β-hydroxybutyric acid

At the end-of-trapping (E.O.T.) of [^{11}C]NH_4CN, R/S, R- or S-propylene oxide (100 µl) was added and the solution was heated at 40°C with stirring for 10 min. HCl(g) was bubbled through the solution for 5 min at room temperature. The solution was then stirred and heated at 150°C for 10 min. The solvent was evaporated, the residue was redissolved in H_2O (1 ml) and injected onto the semi-preparative Aminex HPX 87-H ion exchange column. [^{11}C]-β-HBA, which eluted between 9.5-12 min, was collected directly in an injection vial. Phosphate buffer (6 ml, 12,3 mM) was added and the resulting solution filtered through a Millipore filter (0.22 µm). [^{11}C]-β-HBA thus prepared was sterile and free from pyrogens (Limulus test).

Derivatization to 3-O-trifluoroacetoxy-butyric acid methyl ester

The aqueous fraction containing [^{11}C]-β-HBA was evaporated to dryness and the residue obtained was used for analysis of the enantiomeric purity of the products obtained under different hydrolysis conditions. After waiting until the sample was no longer radioactive, methanolic HCl (1.5 M, 1 ml) was added. The solution was kept at room temperature for 1 h in a screw cap vial (1.5 ml). The excess reagent was removed with a stream of nitrogen gas to dryness. CH_2Cl_2 (200 µl) and trifluoroacetic anhydride (50 µl) were added. After standing at room temperature for 30

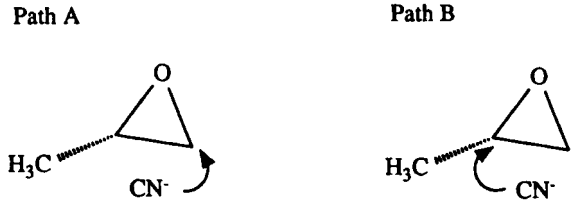
min the reagent was again removed in a nitrogen stream. Finally the samples were dissolved in CH_2Cl_2 (100 µl) and an aliquot was injected into the gas chromatograph.

Results and Discussion

In addition to optimizing the radiochemical yields of two synthesis steps and the purification procedure in the production of [1-^{11}C]-β-HBA , it was also necessary to investigate the regio- and stereospecificity of the reactions.

Formation of II

The normal mode of reaction in a nucleophilic aliphatic substitution involves attack of the nucleophile at the least hindered carbon. In this case CN^- would attack at the methylene carbon of propylene oxide, generating a secondary alcohol (Scheme 3, path A). However, it has been previously proposed in the literature (13,14) that resonance stabilization of the developing carbonium ion may favor the opposite mode of attack by triply bound nucleophiles such as cyanide or acetylene (Scheme 3, path B). A considerable amount of the primary alcohol could therefore be obtained.



Scheme 3

To establish the identity of the main product the reaction was performed with non-radioactive NaCN. The isolated product was examined by Attached Proton Test (APT) ^{13}C-NMR. The shifts observed (Figure 1) [(CD_3Cl), δ: 21.9 (CH_3), 26.7 (CH_2), 63.1 (CH) and 117.6 ppm (CN)] were assigned by comparison with literature values. (15)

In the APT-spectrum, the carbons containing an odd number of protons point down (CH_3 and CH), and those with an even number or no protons point up (CH_2 and CN). The carbon attached to the OH group has a shift of 63.1 ppm and the APT-spectrum shows that this carbon is

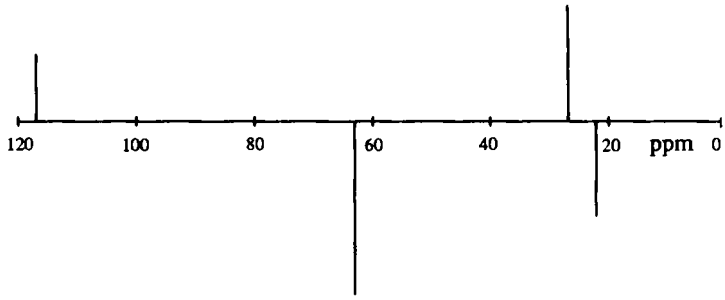


Figure 1. APT-spectrum of 3-hydroxybutyronitrile.

a methine carbon. It was therefore concluded that the major product is the secondary alcohol resulting from attack of $[^{11}C]CN^-$ at the least sterically hindered carbon (Path A, Scheme 3).

The nucleophilic attack of $[^{11}C]CN^-$ on propylene oxide to form (II) was optimized with respect to time, temperature, effect of carrier and amount of starting material. When 100 µl of (I) was reacted with $[^{11}C]CN^-$ for 10 min at 40°C, the conversion of $[^{11}C]CN^-$ to (II) was 60-80 % (Figure 2). The yield of (II) was not appreciably affected by the addition of carrier NaCN.

Hydrolysis of (II) to (III)

The hydrolysis of (II) to (III) (Figure 2) was accomplished by heating an additional 10 min after the addition of NaOH (10 M), H_2SO_4 (18 M), HCl (12 M) or HCl(g) (Table 1). In general, higher yields were obtained with NaOH at lower temperatures (110°C) than for the acids (150°). The amount of the hydrolysis reagent added also affected the radiochemical conversion.

Reagent	Added mmol	Ratio *	Temp °C	Yield %
NaOH	2.5	2:1	110	>90
	1.25	4:1	110	67
	0.625	8:1	110	63
H_2SO_4	7.2	1:1	150	>90
	3.6	2:1	150	78
HCl	**	-	150	75
	14.4	3:1	150	57
	9.6	2:1	150	47

Table 1. Effect of hydrolysis conditions on conversion of (II) to (III).

(*) Ratio between the volume of the aqueous solution of (II) and the added volume of the hydrolysis reagent.

(**) HCl(g) was bubbled through the reaction mixture for 5 min.

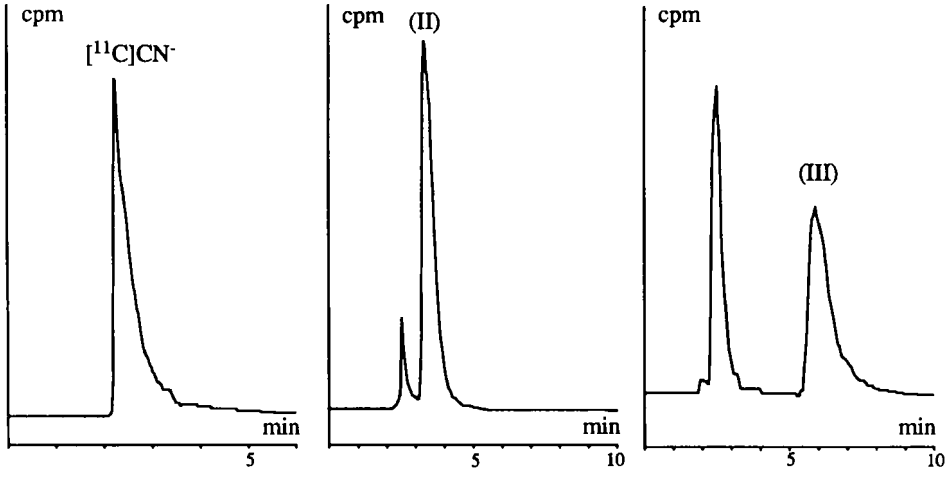


Figure 2. Analytical HPLC chromatograms (radioactivity vs time) of **Left**: trapped [^{11}C]CN$^-$, **Middle**: conversion of [^{11}C]CN$^-$ to (II) and **Right**: hydrolysis of (II) to (III).

Purification

Separation from radioactive by-products was obtained using an Aminex HPX-87 H column (Figure 3). Since an aqueous acidic mobile phase was used, only adjustment of pH was necessary prior to the Millipore filtration. The radiochemical purity of the product thus isolated was >99%.

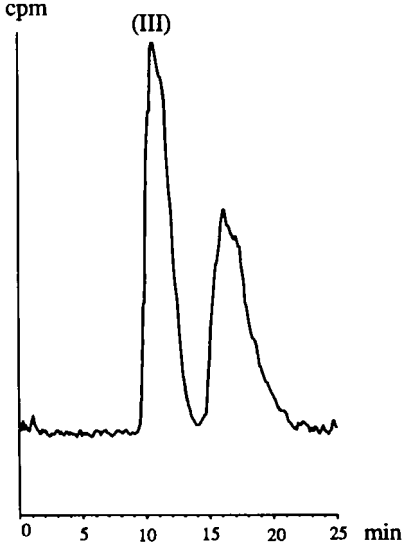


Figure 3. Semi-preparative chromatogram (radioactivity vs time) of [1-^{11}C]-β-HBA.

Determination of enantiomeric purity

β-HBA was synthesized either with or without carrier NaCN under different hydrolysis conditions. The products isolated after decay of the radioactivity were derivatized to 3-O-trifluoroacetoxy-butyric acid methyl esters. The derivatives were analyzed on a octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin glass capillary GC-column. The derivatives of R- and S-β-HBA were resolved and eluted after 7.5 and 11.5 min, respectively (Figure 4).

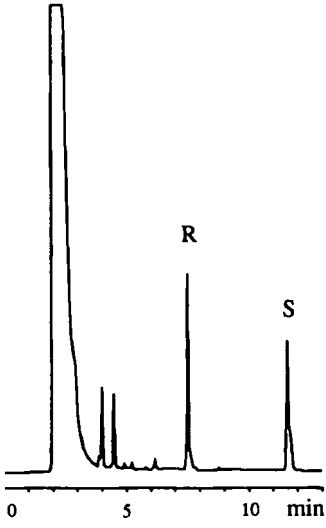


Figure 4. GC-chromatogram of the separation of R-and S-β-hydroxybutyric acid as 3-O-trifluoroacetoxy-butyric acid methyl esters.

Propylene oxide	Carrier NaCN(mg)	Hydrolysis condition	% e.e.
R	10	5 M NaOH	48
S	10	5 M NaOH	37
R	10	9 M H_2SO_4	9
R	5	12 M HCl	87
S	5	12 M HCl	88
R	10	9 M HCl	93 -97
R	5	9 M HCl	97

Table 2. Enantiomeric excesses of (III) obtained under different hydrolysis conditions.

As shown in Table 2, considerable racemization was observed when NaOH and H_2SO_4 were used at optimal hydrolysis conditions (Table 1), but very little racemization with HCl. This

observation is consistent with the retention of enantiomeric purity reported recently (16) in the hydrolysis of R-α-hydroxynitriles with concentrated HCl. Since β-HBA was produced in high enantiomeric purity, the formation of the nitrile (II) in step 1 in scheme 2 must also proceed with a high retention of chirality under these conditions.

Examination of the enantiomeric purity of the no-carrier added β-HBA was also attempted but the chemical mass obtained was below our detection limit. If use of the no-carrier-added radiotracer is required, a more sensitive analysis must be performed. Since β-HBA is endogenous to the human body there are no toxicological or pharmacological objections for using the carrier added radiotracer for which the enantiomeric purity has been determined. The maximum amount of β-HBA produced with 5 mg NaCN added ranges from 0.02-0.1 μmole. It has been shown (2) that the brain uptake of β-HBA decreases as the total concentration increases, indicating saturation of the blood-brain-barrier transport mechanism. However, these effects were appreciable at concentrations studied between 0.2 to 20.2 mM.

Conclusions

Racemic, R- and S-β-HBA were labelled in the carboxylic position with ^{11}C in a two-step reaction using [^{11}C]CN^- as the radiolabelling precursor. The time for synthesis including HPLC purification was 45-50 min from E.O.T. The radiochemical yield was 20-30 % based on [^{11}C]CN^- and the radiochemical purity was >99%. A high enantiomeric excess (≥87%) for either R- or S-β-HBA was obtained when the chiral nitrile was hydrolyzed with HCl, but not with NaOH or H_2SO_4. Typically with 100 mCi [^{11}C]NH_4CN approximately 5 mCi [1-^{11}C]-β-HBA (end of synthesis) was obtained. The radiotracer produced by this method was sterile and free from pyrogens and either the R- or S-enantiomer is now available for the study of their *in vivo* transport and distribution using PET.

Acknowledgments

The authors would like to thank Mr. G. Printz and Mr. F. Hamnqvist for technical assistance with the radionuclide production and Mr. K-O Schoeps for the NMR-spectra. This work has been supported by grants from Astra Pharmaceutical Company, the Swedish Medical Research Council and the Karolinska Institute.

References

1. Owen O.E., Morgans A.P., Kemp H.G., Sullivan J.M., Herrera M.G. and Cahill Jr G.F.- J. Clin. Invest. 46: 1589 (1967)
2. Gjedde A. and Crone C.- Am. J. Physiol. 229: 1165 (1975)
3. Berger G., Maziere M., Prenant C. and Comar D.- Int. J. Appl. Radiat. Isot. 31: 577 (1980)
4. Straatman M.G., Hortman A.G. and Welch M.J.- J. Lab. Comp. Radiopharm. 10: 175 (1974)
5. Prenen G.H.M., Go K.G., Paans A.M.J., Zuiderveen F., Vaalburg W., Kamman R.L., Moleenaar W.M., Zijlstra S., Elsinga P.H., Seben J.B. and Korf J.- Acta Neurochir. (Wien) 99: 166 (1989)
6. Prenen G.H.M., Go K.G., Zuiderveen F., Paans A.M.J. and Vaalburg W.- Appl. Radiat. Isot. 41: 1209 (1990)
7. Webber R.J. and Edmond J.- J. Biol. Chem. 252: 5222 (1977)
8. Thorell J-O., Stone-Elander S., Halldin C. and Widén L.- Acta Neurol. in press (Abstract)(1991)
9. Fowler J.S. and Wolf A.P.- Positron Emission Tomography and Autoradiography: Principles and Applications for Brain and Heart, Raven Press, New York. 391 (1989)
10. Johnsson F. and Panella B.C.- Org. Syn. Coll. vol 5: 614 (1973)
11. König W.A, Krebber R. and Mischnik P.- J. High Res. Chromatogr. 12: 732 (1989)
12. Christman D.R., Finn R.D., Karlström K.I. and Wolf A.P.- Int. J. Appl. Radioat. Isot. 26: 435 (1975)
13. Parker R.E. and Isaac N.S.- Chem. Rev. 59: 737 (1959)
14. Davies H.S. and Redmon B.C.- U.S. patent 2,390,519 (December 11, 1945)
15. Williams D.H. and Flemming I.- Spectroscopic methods in organic chemistry (Third edition), McGraw-Hill, London (1980)
16. Ziegler T., Hörsch B. and Effenberger F.- Synthesis 7: 575 (1990)