Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIX, No. 5

SYNTHESIS OF CARBON-11 LABELLED (R)-CARNITINE

M.Holschbach*, W.Hamkens, W.Roden and L.E.Feinendegen

Institute of Medicine
Research Center Jülich, P.O. Box 1913
D-5170 Jülich, FRG.

KEYWORDS

^{11}C-labelled carnitine, vitamin B_T, synthesis, Positron Emission Tomography (PET).

SUMMARY

A route to ^{11}C-labelled (R)-carnitine (**1**), based on the methylation of the dimethyl derivative (**2**) is described. Furthermore, a five-step synthesis for the enantiomerically pure precursor (**2**) is outlined.

INTRODUCTION

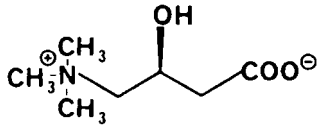
(R)-Carnitine, (R)-3-hydroxy-4-(trimethylammonio)butanoate, (**1**), first isolated from meat extract in 1905[1] and synthesized for the first time by Tomita[2] in 1927 was shown to be a facilitator for the transportation of long-chain fatty acids through the mitochondrial membranes, thus allowing their metabolic oxidation[3-5]. It is a regulator of blood lipid levels and is used as a therapeutic agent to increase cardiac output and improve myocardial function.

---

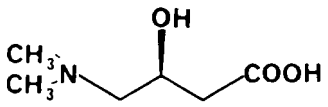
*To whom all correspondence should be addressed

0362–4803/91/050599–08$05.00

Received 27 December, 1990



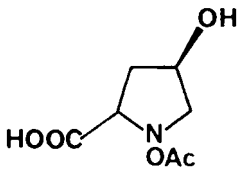
1



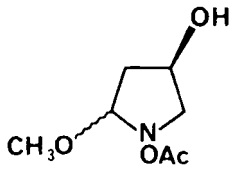
2

SYNTHESIS

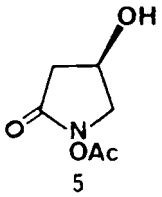
Syntheses of (R)-carnitine either involve a resolution step of the initially formed racemic products[6-16] or start directly from chiral synthons[17-31]. To the best of our knowledge there has been no synthesis for (R)-carnitine starting from (R)-3-hydroxy-4-dimethylaminobutyric acid (**2**); the compound itself has not yet been described in the literature. Based on the elegant work of Seebach et al.[23] our synthesis started with the electrochemical oxidative decarboxylation of (2R,4S)-N-acetyl-4-hydroxyproline (**3**), a readily available chemical, to give (4R)-N-acetyl-2-methoxypyrrolidin-4-ol (**4**). Oxidation of (**4**) with 3-chloroperbenzoic acid (MCPBA) led to the pyrrolidone (**5**), which, after hydrolysis, yielded (R)-4-amino-3-hydroxybutanoic acid (**6**). Methylation of (**6**) with formaldehyde/hydrogen by the method of Bowman and Stroud[32] produced the precursor (**2**) in about 60% overall yield from the acetylproline (**3**). Methylation of (**2**) with $^{11}CH_3I$ under neutral conditions and subsequent treatment with



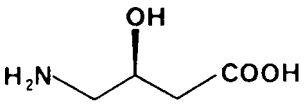
3



4

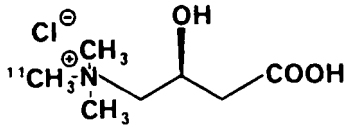


5



6

aqueous HCl gave[^{11}C]-(R)-carnitine hydrochloride (7) in a good radiochemical yield in a purity of 99%.



7

MATERIALS AND METHODS

(2R,4S)-N-Acetyl-4-hydroxyproline, MCPBA and BF_3-etherate were purchased from Fluka (Buchs,Switzerland). A sample of (R)-carnitine was obtained from Sigma (Deisenhofen, FRG.). Melting points were determined in glass capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1700, values are in cm^{-1}. ^{1}H-NMR spectra were performed on a Varian EM 390 and a Varian VXRD 300 spectrometer; values are in δ with TMS as internal standard. TLC plates (Silica, Polygram^R Sil G/UV_{254}) were purchased from Macherey Nagel (Düren,FRG.). The solvent systems used were a) diethylether/methanol, 80/20 (v/v) and b) methanol/acetone/HCl, 90/10/4 (v/v). $^{11}CH_3I$ was produced at the CV 28 Compact Cyclotron in Jülich via the $^{14}N(p,\alpha)^{11}C$-reaction, using a nitrogen gas target. The radiochemical purity exceeded 98% as measured by radio gaschromatography. The carbon-11-labelled compound was purified by HPLC on a silica column using water/ethanol, 70/30 (v/v) as eluent.

(4R)-N-Acetyl-2-methoxypyrrolidin-4-ol, (4)

The anodic oxidation of (3) was performed in a simple, undivided electrolysis cell (Pyrex glass vessel, 16 x 7cm, fitted with an internal cooling coil and a magnetic stirring bar. The electrodes consisted of one platinum plate, 4 x 2,5 x 0,2cm, as anode and a coil of platinum wire, 30cm long, as

cathode). A mixture of (**3**), 17,3 g (100 mmol), triethylamine, 2.8 ml, (20 mmol) and methanol, 120 ml, was electrolysed with a current density of 300 mA/cm^2 for 16 hours at 15°C in an atmosphere of nitrogen. Evaporation of the methanol in a rotary evaporator at room temperature yielded a slightly brownish oil (14,4 g), which was chromatographed on silica gel with diethylether/ethanol, 70/30 (v/v) as eluent.
Yield: 13,3 g (83%) of a colourless oil, mixture of enantiomers. ^{1}H-NMR ($CDCl_3$): 2,09+2,14 (2s, 6H, 2 x CH_3CO), 1,85-2,31 (m, 4H, 2 x C-3) 3,26+3,41 (2s, 6H, 2 x OCH_3) 3,25-3,93 (m, 6H, 2 x C-5, 2 x OH) 4,32-4,76 (m, 2H, 2 x C-4) 5,09-5,53 (m, 2H, 2 x C-2) IR (cap): 3354 (OH) 2925 (CH_3, CH_2) 1628 (C=O) 1445 (C-O) $C_7H_{13}NO_3$, 159,17 g x mol^{-1}

(R)-N-Acetyl-4-hydroxy-2-pyrrolidone, (**5**)
At 0°C, to a solution of (**4**), 7,95 g, (50 mmol), in 150 ml CH_2Cl_2 were added MCPBA, 13,2 g, (65 mmol) and BF_3-etherate, 0,3 ml, (2,4 mmol). After stirring overnight at room temperature, 150 ml of pentane were added, the white, cloudy precipitate was filtered by suction and washed with a 1:1 (v/v) mixture of cooled CH_2Cl_2/pentane. Evaporation and chromatography on silica gel yielded the product as an oil.
Yield: 5,3 g (73%), colourless oil. ^{1}H-NMR ($CDCl_3$): 2,46 (s, 3H, CH_3CO) 2,58-2,83 (m, 2H, C-3) 3,05 (s_{broad}, 1H, OH) 3,90 (d, 2H, C-5) 4,36-4,71 (m, 1H, C-4) IR (cap): 3442 (OH) 2936 (CH_2) 1745, 1690 (C=O) 1389, 1312 (C-O) $C_6H_9NO_3$, 143,14 g x mol^{-1}

(R)-4-Amino-3-hydroxybutanoic acid, (**6**)
One g (7 mmol) of (**5**) in 15 ml of 4N HCl was refluxed for 6,5 hours. After cooling the water was evaporated and the residue dried under high vacuum. The crude hydrochloride of (**6**) was treated with an acidic ion exchange resin (Dowex 1x8) and the

free acid recrystallized from water/ethanol.
Yield: 0,82 g (98%) of a white solid. Mp: 212°C (Lit[23]: 213-214°C). ^{1}H-NMR (DMSO-d_6): 2,31 (d, 2H, C-2) 2,57-3,19 (m, 2H, C-4) 3,91-4,28 (m, 1H, C-3) 4,41 (s, 2H, NH_2) 11,12 (s_{broad}, 1H, COOH) $C_4H_9NO_3$, 119,12 g x mol^{-1}

(R)-3-Hydroxy-4-dimethylaminobutyric acid, (**2**)
To 1 g (8,3 mmol) of (**6**) in 50 ml water is added 0,5 g (16,6 mmol) of an aqueous formaldehyde solution (37%) and 1 g of 10% palladised charcoal. With vigorous stirring hydrogen gas is bubbled through the mixture for 12 hours at 20°C and for an additional hour at 40°C. After purging the reaction vessel with nitrogen, the mixture was heated to reflux and filtered while hot. Any methylated product remaining adsorbed on the catalyst was extracted with hot water. Evaporation of the water leaves a residue which is taken up in boiling ethanol, filtered to remove insoluble material and the ethanolic solution evaporated to dryness. Treatment of the crude product with water followed by evaporation to remove paraformaldehyde leaves pure (**2**).
Yield: 1,1 g = 90%, slightly yellow oil. ^{1}H-NMR (DMSO-d_6): 2,37 (d, 2H, C-2) 2,44 (d, 2H, C-4) 3,18 (s, 6H, $N(CH_3)_2$) 3,97 (m, 1H, C-3) 5,24 (s_{broad}, 1H, OH) $C_6H_{13}NO_3$, 147,17 g x mol^{-1}. Calc.: C 48.96% H 8,90% O 32,61% Found: C 49,02% H 8,96% O 32,64%.

[^{11}C]-(R)-Carnitine hydrochloride, (**7**)
In a 5 ml reaction vessel are placed 1 mg (6,8 μmol) (**2**) and 1 ml dry ethanol. Under dry-ice cooling gaseous $^{11}CH_3I$ is bubbled into the solution and after completion of the addition (1 min.), the reaction flask is heated to 100°C for ten minutes. The temperature is then lowered to 50°C and the reaction mixture is evaporated to dryness (2 min.). One ml

of dry 1,36N ethanolic HCl (13,6 μmol) is added and the resulting mixture again evaporated (3 min.). The residue is taken up in water/ethanol, 70/30 (v/v) and subjected to HPLC (5 min., 10 ml/min., detection at 206+254 nm.). The fraction containing the product (7) is collected, evaporated and taken up in isotonic NaCl. After filtration through a sterile filter unit (MILLEX GV, 0,22 μm, Millipore), the solution is ready for injection. Overall synthesis time is 21 minutes.

Radiochemical yield: 39% Specific activity: $2,66 \times 10^5$ Ci/mol

On analysis (Mp., NMR, IR) the product proved in all respects identical with an authentic specimen of (R)-carnitine hydrochloride. Mp.: 136-138°C. ^{1}H-NMR (DMSO-d_6): 1,16-1,76 (m, 2H, C-2) 3,19-3,46 (m, 2H, C-4) 3,42 (s, 9H, $N(CH_3)_3$) 4,24 (m, 1H, C-3) IR (KBr): 3430 (OH, NR_4^+) 3020,2980 (CH_3, CH_2) 2870 (NR_4^+) 1682 (C=O).

RESULTS AND DISCUSSION

(R)-Carnitine plays an important role in human metabolism and transport of long-chain fatty acids. Because (S)-carnitine is a competitive inhibitor of (R)-carnitine transferases and can deplete the (R)-carnitine level of cardiac tissue, (R)-carnitine has been recommended for replacement therapy. Carnitine deficiency primarily results from two different reasons: first, an insufficient synthesis of carnitin in the liver and, second, a defect in the transport mechanisms of carnitine from the blood to the heart and skeletal muscle.

Thus, for kinetic studies with Positron Emission Tomography (PET), a procedure for the synthesis of carbon-11-labelled (R)-carnitine was developed. In an overall synthesis time of 21 minutes enantiomerically pure (99%) (R)-carnitine was prepared in a radiochemical yield of 39%.

Acknowledgement: We would like to thank the staff of the Jülich Compact Cyclotron for performing the irradiations.

REFERENCES

1. W.Gulewitsch, R.Krimberg, Hoppe-Seyler's Z. Physiol. Chem. 45: 326 (1905).
2. M.Tomita, Y.Sendju, Hoppe-Seyler's Z. Physiol. Chem. 169: 263 (1927).
3. S.Friedman, in "The Vitamins",2nd edition, Academic Press, New York, 5, 329 (1972).
4. I.B.Fritz, E.Kaplan, K.T.N.Yu, Am. J. Physiol. 202: 117 (1962).
5. J.Bremer, Physiol. Rev. 63: 1420 (1983).
6. H.Löster, D.M.Müller, Wiss. Z. Karl-Marx-Univ. Leipzig Math.-Naturwiss. R. 34: 212 (1985).
7. P.de Witt, E.Diamanti, to Sigma Tau, US Patent 4.254.053. (1981).
8. E.Strack, I.Lorenz, Hoppe-Seyler's Z. Physiol. Chem. 318: 129 (1960).
9. G.Kato, E.A.Hosein, Can. J. Chem. 47: 1177 (1969).
10. D.M.Müller, E.Strack, I.Lorenz, J. Prakt. Chem. 317: 689 (1975).
11. Y.Hamari, to Kogyo Co., JP Patent 84.231.048 (1984).
12. V.Ianella, to Isom, EP Patent Appl. 141.408 (1984).
13. H.Kikuchi, to Nisshin Flour Milling Chem., DE Patent 3.536.093 (1986).
14. C.J.Sih, to Wisconsin Alumni Res. Found., PCT Int. Patent Appl. WO 04.900 (1985).
15. F.Aragozzini, M.Manzoni, V.Cavazzoni, Biotechnol. Lett. 8: 95 (1986).
16. E.P.Dropsy, A.M.Klibanov, Biotechnol. Bioeng. 26: 911 (1984).

17. K.Bock, I.Lundt, C.Pederson, Acta Chem. Scand., Ser. B 37: 341 (1983).

18. M.Fiorini, C.Valentini, to Anic, EP Patent Appl. 60.595 (1982).

19. B.Zhou, A.S.Gopalan, F.v.Middlesworth, W.R.Shieh, C.H.Sih, J. Am. Chem. Soc. 105: 5925 (1983).

20. D.Seebach, F.Giovannini, B.Lamatsch, Helv. Chim. Acta 68: 958 (1985).

21. E.Santaniello, R.Casati, F.Milani, J. Chem. Res. Synop. 1984, 132.

22. C.H.Wong, D.G.Drueckhammer, N.M.Sweers, J. Am. Chem. Soc. 107: 4028 (1985).

23. P.Renaud, D.Seebach, Synthesis 1986, 424.

24. R.Pellegata, I.Dosi, M.Villa, G.Lesma, G.Palmisano, Tetrahedron 41: 5607 (1985).

25. T.Kanamaru, S.Iiuna, S.Susumu, to Tokeda, JP Patent 85.214.890 (1985).

26. H.Kulla, P.Lehky, to Lonza, EP Patent Appl. 195.944 (1985).

27. S.V.Pande, Methods Enzymol. 123: 297 (1986).

28. M.Kawamura, S.Akutsu, to Seitetsu, JP Patents 86.234.788 and 86.234.794 (1986).

29. H.Seim, H.Löster, E.Strack, H.P.Kleber, 3rd Eur. Congr. Biotechnol., Verlag Chemie, Weinheim, 1, 481 (1984).

30. K.Yokozeki, K.Kubota, to Ajinomoto, EP Patent Appl. 122.794 (1984).

31. R.Voeffray, J.C.Perlberger, L.Tenud, Helv. Chim. Acta 70: 2058 (1987).

32. R.E.Bowman, H.H.Stroud, Soc. 1950, 1342.