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A NEW SYNTHESIS AND NMR-SPECTROSCOPY OF [^{15}N-, 5,4-^{13}C]-AMINOLEVULINIC ACID

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SUMMARY

A new synthesis of [^{15}N-, 5,4-^{13}C]-aminolevulinic acid (ALA) starting from amorphous ^{13}C is described. NMR-spectroscopic data (^{1}H, ^{13}C, ^{15}N) of the product are presented.

KEYWORDS: [^{15}N-, 5,4-^{13}C]-aminolevulinic acid (ALA); ^{13}C-acetic acid

INTRODUCTION

Aminolevulinic acid is an important precursor in porphyrin biosynthesis. Incubation of algae with isotopically labelled δ-aminolevulinic acid (ALA) leads to the production of the correspondingly isotopically labelled porphyrins and bile pigments |1|. Previously described synthesis of ALA was restricted to introduction of one ^{13}C only in various positions |2,3,4,5,6|, whilst the presented new synthesis allows for the introduction of both ^{15}N and up to two ^{13}C in dependence of the chosen starting material. Another merit is the fact that starting from some of the occuring intermediates other labelled compounds of general

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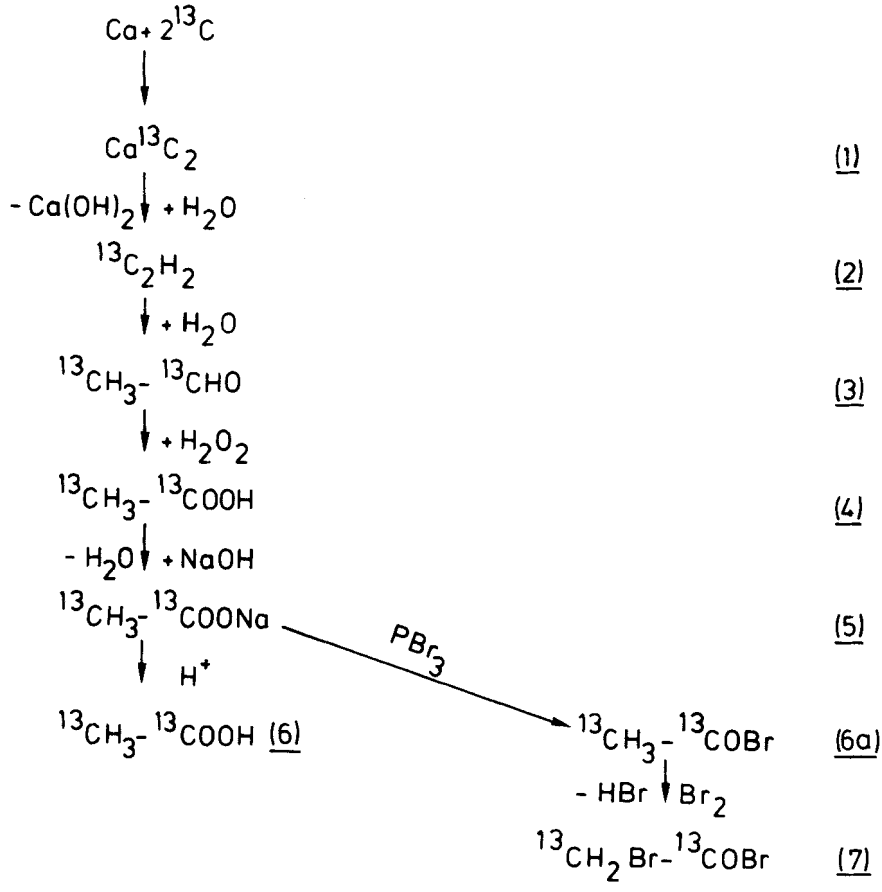
importance can be made (e.g. glycine from (9) by total hydrolysis). The overall reaction scheme is divided into two parts; Scheme 1 describes the synthesis of doubly ^{13}C labelled acetic acid (6) starting from amorphous carbon ^{13}C. In Scheme 2 the production of ALA from acetic acid as starting material is described. Depending on the isotopic labelling of this compound, the various singly or multiply isotopically labelled ALAs can be reached. Scheme 1 represents one way to synthesize $(^{13}C)_2$-isotopically labelled acetic acid (6) from relatively inexpensive amorphous carbon ^{13}C in small quantities but without elaborate equipment.



Scheme 1: Synthesis of $^{13}CH_3$-$^{13}COOH$ and $^{13}CH_2Br$-$^{13}COBr$ from ^{13}C-carbon amorphous.

$$^{13}CH_3 - {}^{13}COOH \quad (6)$$

$$\downarrow -HBr \; +Br_2$$

$$^{13}CH_2Br - {}^{13}COBr \quad (7)$$

$$\downarrow -HBr \; +C_2H_5OH$$

$$^{13}CH_2Br - {}^{13}COOC_2H_5 \quad (8)$$

$$\downarrow -KBr \; +C_6H_4(CO)_2{}^{15}NK,$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}COOC_2H_5 \quad (9)$$

$$\downarrow -C_2H_5OH \; H^+$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}COOH \quad (10)$$

$$\downarrow -SO_2, -HCl \; +SOCl_2$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}COCl \quad (11)$$

$$\downarrow -HCl \; +CH_2N_2$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}CO - CHN_2 \quad (12)$$

$$\downarrow -N_2 \; +HBr$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}CO - CH_2Br \quad (13)$$

$$\downarrow -NaBr \; +NaCH(COOC_2H_5)_2$$

$$C_6H_4(CO)_2{}^{15}N - {}^{13}CH_2 - {}^{13}CO - CH_2 - CH(COOC_2H_5)_2 \quad (14)$$

$$\downarrow -C_6H_4(COOH)_2 \; H^+ \; -C_2H_5OH \; -CO_2$$

$$^{15}NH_2 - {}^{13}CH_2 - {}^{13}CO - CH_2 - CH_2 - COOH \cdot HCl \quad (15)$$

Scheme 2: Synthesis of [^{15}N-, 5,4-^{13}C]-ALA from (^{13}C)$_2$-acetic acid.

EXPERIMENTAL

Materials and methods: All chemicals have been as pure and dry as possible. NMR-spectra are obtained on a Bruker WP 80 spectrometer, mass spectra on a Varian Mat CH5 spectrometer.

Reaction 1: 3.8 g Calcium (82 mmol) is covered with 1.45 g ^{13}C carbon amorphous (112 mmol) in a ceramic vessel, which is placed in a quartz tube to react under argon (purity 5.0) at 1170°C for 60 minutes.

Reaction 2: The crude product (4.5 g) $Ca^{13}C_2$(1) is very porous and hygroscopic. It is advisable to react it further immediately with H_2O in a flask; the evolving gaseous $^{13}C_2H_2$(2),(1.5l) is captured in a polyethylenic bag.

Reaction 3: Using a tube pump (2) is bubbled through a catalyst containing solution (0.85 g $HgSO_4$, 2 ml H_2SO_4 conc., 25 ml H_2O) at 60°C for 4 h. The evolving $^{13}CH_3$-^{13}CHO(3) is condensed in a NaCl/ice cooled trap whilst the non reacted (2) is fed back into the polyethylenic bag.

Reaction 4 - 5: The oxidation of (3) with 20 ml 30% H_2O_2 leads to $^{13}CH_3$-$^{13}COOH$(4). The pH-value is kept between 8 and 10 by 10 n NaOH. Before drying with $CaCl_2$ the solution is extremely narrowed.

Yield (1 - 5): 2.9 g of $^{13}CH_3$-$^{13}COONa$(5)(34 mmol) = 62% of ^{13}C educt.

Reaction 6 - 8: To achieve $^{13}CH_2Br$-$^{13}COBr$(7) there are two alternatives: Either 3 ml bromine (9.4 g, 59 mmol) are added to 2 g acetic acid(6)(32 mmol)- which can be made from (5) with 85% H_3PO_4- in which 0.3 g phosphorous red (9.6 mmol) is suspended, leads to (7) or via the direct reaction from (5) to (7), where to 10 g of (5)(120 mmol) 50 ml PBr_3 (142 g, 0.5 mol) are added

and stirred for 12 h at 70°C under reflux. 8.75 g $^{13}CH_3$-$^{13}COBr$(6a) (71 mmol) are removed by destillation, immediately added to 3.6ml bromine (11.4 g, 71 mmol) and stirred for 12 h at 45°C. Under ice cooling the produced (7) is added slowly to 10 ml absolute ethanol (7.9 g, 170 mmol). By destillation 10.35 g of $^{13}CH_2Br$-$^{13}COOC_2H_5$(8)(62 mmol) are separated.

Yield (5 - 8): 52% of (5).

Reaction 9: 10.35 g (62 mmol) of (8) are dissolved in 125 ml dimethyl formamide (DMF). After addition of 13.2 g (71 mmol) of ^{15}N-K-Phthalimide, the suspension was stirred under argon for 4 h at 45°C und then 16 h at room temperature. DMF is removed by vacuum destillation. The viscous residue is dissolved in CH_2Cl_2 and water(3:1). After drying the organic phase by $MgSO_4$, the solvent is removed. Purification by recrystallization from methanol, if necessary.

Yield: 13.0 g of $C_8H_4O_2$ ^{15}N-$^{13}CH_2$-$^{13}COOC_2H_5$(9) (55 mmol) = 89%, m.p. 98°C.

^{1}H-NMR(80MHz)($CDCl_3$):(ppm) 7.79(m, 4H, C_6H_4);4.42(ddd, 2H, -$^{13}CH_2$-, $^1J(H,C)$=142.1Hz, $^2J(H,C)$=6.1Hz, $^2J(H,N)$=1.0Hz); 4.20(qu, 2H, -O-CH_2-); 1.21(tr, 3H, -CH_3).

^{13}C-NMR(20MHz)($CDCl_3$):(ppm) 167.4 (dtr, -^{13}COO-, $^1J(C,C)$= 62.5Hz, $^2J(C,H)$=6.1Hz); 38.9 (trdd, -$^{13}CH_2$-, $^1J(C,H)$= 142.0Hz, $^1J(C,C)$=62.5Hz, $^1J(C,N)$=12.3 Hz).

^{15}N-NMR(8MHz)($CDCl_3$):(ppm) -225.5 (d, $^1J(N,C)$=12.0Hz).

Reaction 10: 11 g of (9)(46.6 mmol) are suspended in 350 ml 1n HCl and refluxed for 2 h. The hydrolysis product $C_8H_4O_2$ ^{15}N-$^{13}CH_2$-^{13}COOH(10) is precipitated by cooling slowly to -5°C, filtered after 5 h and severely dried with P_2O_5.

Yield: 8,65 g of (10) (41.6 mmol) = 89%.

Reaction 11: 8.6 g of (10)(41.5 mmol), 15 ml $SOCl_2$(24.8g, 208mmol) and 4 drops of DMF are refluxed for 2 h. Surplus $SOCl_2$ is evaporated.

Yield: 10 g of $C_8H_4O_2$ ^{15}N-$^{13}CH_2$-^{13}COOCl(11) (41.5 mmol) = 100% .

Reaction 12: 10 g of (11)(41.5 mmol) are dissolved in 100 ml absolute diethylether and added slowly to a freshly prepared solution of 80 mmol CH_2N_2 in 200 ml diethylether under ice cooling (temperature not over +5°C). After termination of the reaction (2 h) the very voluminous product $C_8H_4O_2{}^{15}N\text{-}{}^{13}CH_2\text{-}{}^{13}CO\text{-}CHN_2$(12) was stirred for 1 h at room temperature.

Reaction 13: This suspension is bubbled with HBr until development of N_2 stops. Then it is refluxed to expel surplus HBr and kept at -20°C for 2 h before the precipitate is filtered, dried, washed with water and severely redried with P_2O_5.

Yield (11 -13): 7.95 g of $C_8H_4O_2{}^{15}N\text{-}{}^{13}CH_2\text{-}{}^{13}CO\text{-}CH_2Br$(13) (30.4 mmol) = 72.5% .

^{1}H-NMR(80MHz)($CDCl_3$):(ppm) 7.81(m, 4H, C_6H_4-); 4.77(ddd, 2H, $-{}^{13}CH_2-$, $^1J(H,C)$=140.9Hz, $^2J(H,C)$=4.4Hz, $^2J(H,N)$=1.2Hz); 4.00(d, 2H, $-CH_2Br$, $^2J(H,C)$=5.3Hz).

^{13}C-NMR(20MHz)($CDCl_3$):(ppm) 194.8(d, $-{}^{13}CO-$, $^1J(C,C)$=42.5Hz); 44.4(dd, $-{}^{13}CH_2-$, $^1J(C,C)$=42.5Hz, $^1J(C,N)$=12.2Hz).

MS(70eV):m/e(%): 286(1.1) M^+(Br81); 284(1.1) M^+(Br79); 205(1.2) M^+-Br; 162(100) $M^+-{}^{13}COCH_2Br$; 148(2.0) $C_8H_4O_2{}^{15}NH^+$; 133(1.8) $C_8H_4O_2H^+$.

Reaction 14: 7.9 g of (13)(30.4 mmol) are dissolved in 300 ml dried acetone and slowly added to a solution of 0.76 g Na (33 mmol) and 5.28 g malonic acid ethyl ester (33 mmol) in 50 ml absolute ethanol. This solution is stirred for 24 h at 70°C under reflux, the solvent is removed and the very viscous substance is worked up in CH_2Cl_2 and water. The precipitated $C_8H_4O_2{}^{15}N\text{-}{}^{13}CH_2\text{-}{}^{13}CO\text{-}CH_2\text{-}CH(COOC_2H_5)_2$(14) is used for further reaction without purification.

Reaction 15: (14) is dissolved slowly in a solution of 150 ml HCl 35% and 35 ml methanol, stirred at room temperature for 2 h. Then the temperature is raised to 100°-110°C for 2 - 3 h, 70 ml water are added and it is stirred for 8 h at 80° - 90°C

to complete the hydrolysis. From the filtrate the solvent is removed by destillation; the residue is dried by KOH. Besides $^{15}NH_2$-$^{13}CH_2$-^{13}CO-CH_2-CH_2-COOH·HCl(15) other hydrolysis products with similar physico-chemical properities are produced. Separation of these products is therefore difficult by classical methods. HPLC seems to be the proper technique, if isolation of pure (15) is necessary. For the purpose indicated above, condensation of ALA to porphobilinogen by using the enzyme aminolevulinat dehydratase(ALAD), execution of the purification procedure is not advisable. Because the enzyme works with high selectivity and is not inhibited by the impurities, inevitable losses connected with a separation of a pure (15) can be avoided. Yield: 10 g of mixture of hydrolysis product corresponding to 2.5 - 3.5 g of pure (15) (as judged by NMR).

RESULTS

The overall reaction yield from ^{13}C carbon amorphous to ALA is over 9%; under favourable conditions up to 13% can be achieved. NMR-data of important intermediates can be found in |7|. The chemical shifts and coupling constants of the title compound, which are summarized below, are within experimental uncertainty equal to those observed in ^{15}N-, 5-^{13}C-ALA and ^{15}N-ALA |7|.

^{1}H-NMR (D_2O, Ref. TSP-Na-Salt): δ= 4.12 ppm (dd), -$^{13}CH_2$-,
$^1J(^1H,^{13}C)$ = 144.1 Hz, $^2J(^1H,^{13}C)$ = 4.4 Hz;
δ = 2,79 ppm (multiplett), -CH_2-CH_2-.

^{13}C-NMR (D_2O, Ref. TSP-Na-Salt): δ = 207,4 ppm (d), -^{13}CO-,
$^1J(^{13}C,^{13}C)$ = 39,0 Hz; δ = 50,0 ppm (dd), -$^{13}CH_2$-,
$^1J(^{13}C,^{13}C)$ = 39,0 Hz, $^1J(^{13}C,^{15}N)$ = 7,8 Hz.

^{15}N-NMR (H_2O, Ref. $^{15}NH_4{}^{15}NO_3$, sat. in H_2O, extern): δ = -350,8ppm,
$^1J(^{15}N,^{13}C)$ = 7,6 Hz.

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