Synthesis of [1'-^{15}N]-Biotin

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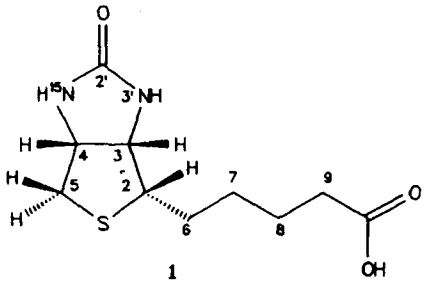
Summary

Racemic [1'-^{15}N]-biotin was synthesized from [^{15}N]-glycine as starting material in 7 steps. The overall yield was 13%.

Key words: Nitrogen-15; [^{15}N]-biotin, [^{15}N]-glycine; C-N, N-H spin coupling constants

Introduction

Biotin (1) is the most important prosthetic group for carbon dioxide transferring enzymes[1]. In our investigation on the mechanisms of CO_2 fixation with biotin dependent enzymes[2] isotopically labelled biotin was required. The synthesis of [2'-^{13}C]-biotin has been reported by us recently[3]. Now we wish to present the synthesis of the nitrogen-15 labelled species. Since our studies required biotin that was specifically labelled at 1' all biological methods to produce biotin with bacteria[4] grown in a medium with ^{15}N ammonium chloride were ruled out. By analogy to our ^{13}C labelling sequence we considered opening the urea ring of biotin and changing the amino group at the 1'-position into a nitrogen-15 labelled amino group. However, no satisfactory method could be found for this transformation[5].



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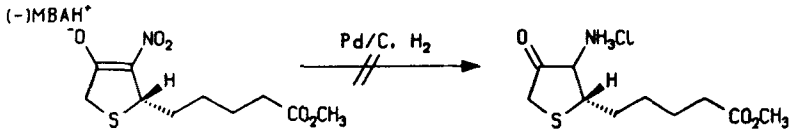
Most of the literature synthesis of biotin are symmetric with respect to the urea moiety, thus a specific labelling of the 1'-nitrogen is difficult[6]. However, 1'N-labelled biotin was already obtained by Vasilevski et al.[7] as reported in a short communication. Thus, we first set out to repeat this sequence. In our hands, however, the hydrogenation of the nitro compound (2) to the amino ketone (3) failed although several attempts were made with different catalysts, various pressures and reaction times; (2) remained either unreacted or was destroyed.



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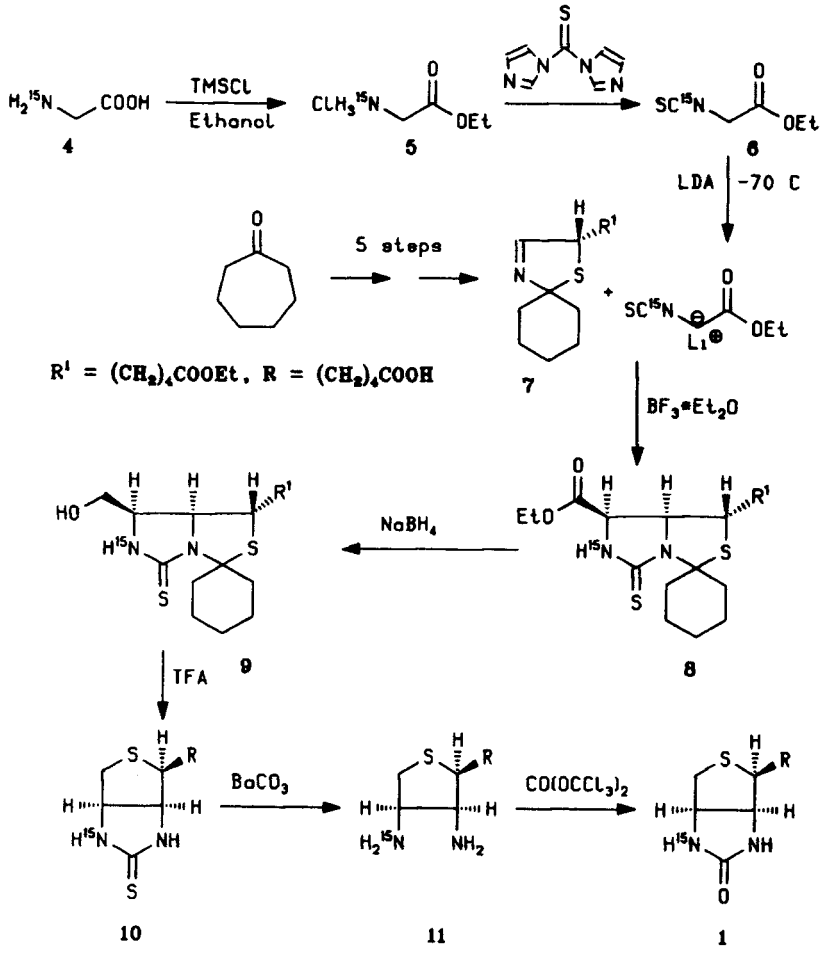
We therefore addressed our attention to another approach communicated by Volkman[8] which seemed, after appropiate modification, suitable for our labelling purpose. Thus, [^{15}N]-glycine (4) was converted in two steps via [1'-^{15}N]-glycine ethyl ester hydrochloride (5) to [^{15}N]-ethyl isothiocyanato acetate (6) using N,N'-thiocarbonyl diimidazole which gave a better yield than using thiophosgene[9]. In the presence of BF_3Et_2O the lithium enolate of (6) was added to the racemic thiazoline (7), which was prepared from cycloheptanone in five steps[10], to give 4 stereoisomers of the diester (8). The required racemate was separated by silica gel (Na_2HPO_4 buffered) chromatography and was then treated with $NaBH_4$ resulting in the racemic alcohol (9). Unlike the original synthesis this racemic mixture was not resolved, but directly hydrolyzed in aqueous trifluoroacetic acid to yield dl-thiobiotin (10). The thiourea/urea transformation in the presence of 2-bromoethanol in N-methylpyrrolidinone, as proposed by the original authors, was replaced by the two step reaction sequence already applied in our synthesis of [2'-^{13}C]-biotin[3] in order to open a convenient way to synthesize doubly labelled [1'-^{15}N, 2'-^{13}C]-biotin. Hydrolysis of (10) was carried out under the same condition as in the case of [2'-^{13}C]-biotin.

Diamino carboxylic acid (11) was then treated with 1 equiv. of bis-(trichloromethyl)carbonate, which corresponded to 3 equiv. of phosgene, in the absence of any base so that the rate of phosgene hydrolysis would not be accelerated by alkaline. Later the mixture was neutralized with 1.5 equiv. of Na_2CO_3 and the reaction was allowed to complete.



Performing the ring closure under this modified conditions we could raise the yield from 51% to 71%. The overall yield was therefore 13%. If pure d-biotin is required, dl-biotin can be resolved with l-(+)-arginine[11].

In the ^{1}H NMR spectrum the labelling was observed by the change of the ABM-system of 5-H multiplets to a ABMX-system (see figure 1). The following ^{3}J(N,H) couplings were determined: $^3J(N1',H5)_{endo}$=2.2 Hz, $^3J(N1',H5)_{exo}$=4.6 Hz. The ^{3}J(N1',H3) spin coupling constant could not be resolved. The ^{1}J(N,C) couplings were measured for ^{1}J(N1',C4)=9.5 Hz, ^{1}J(N1',C2')=20.0 Hz. The ^{15}N chemical shift in alkaline D_2O vs external nitromethane was δ=-290.1.

The labelled material will be introduced into various proteins in order to further investigate the mechanism of CO_2 fixation by biotin dependent enzymes[3].

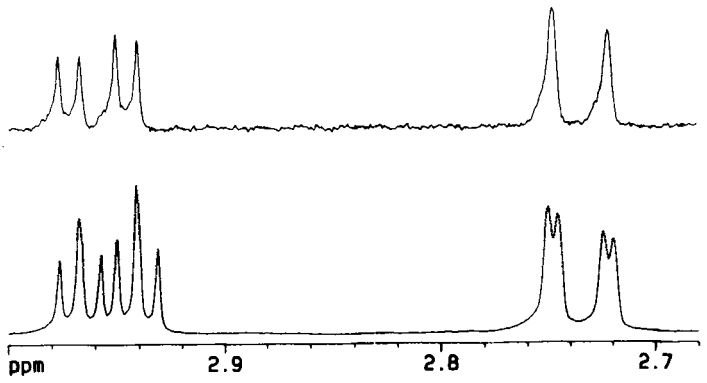


Figure 1. ^{1}H-NMR spectra of H-5_{exo} and H-5_{endo} of unlabelled biotin (1) (upper spectra) and [1'-^{15}N]-biotin (spectra below).

Acknowledgements

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Experimental

Materials

[^{15}N]-glycine was purchased from Aldrich, Steinheim, Germany, bis-(trichloromethyl)carbonate from Fluka, Neu-Ulm, Germany and thiophosgen from Carbolabs, Inc., New Haven, USA. N,N'-thiocarbonyl diimidazole was prepared after the method of Pullukat and Urry[12]. The preparation of thiazoline[10] (7) and glycine ethyl ester hydrochloride[13] (5) is described in the literature. NMR-spectra were recorded on a Bruker AMX-500 (500 MHz) or a Bruker AC-300 (300 MHz).

[^{15}N]-ethyl isothiocyanato acetate (6)

5.50 g (30.86 mmol, 1.2 eq.) N,N'-thiocarbonyl diimidazole was suspended in 400 ml of $CHCl_3$ under ice cooling. Within 1 h, 3.61 g (25.68 mmol) of (6) was added in portions. After 15 min 3.6 ml (25.68 mmol, 1.0 eq.) of triethylamine was slowly added dropwise to the suspension which was stirred further for 30 min in an ice bath. Removing the ice bath the reaction mixture was heated to 40°C and stirred overnight at this temperature. The solvent was distilled and 19 g of an oily residue was obtained. Purification by silica gel chromatography (CH_2Cl_2) gave 3.06 g (20.96 mmol, 82% yield) of a yellow oil, Rf=0.656.

^{1}H-NMR ($CDCl_3$, int. TMS): δ=1.30 (t, 3H, ester-CH_3), 4.20 (d, 2H, H-4), 4.27 (q, 2H, ester-CH_2). ^{13}C-NMR ($CDCl_3$, int. TMS): δ=14.1 (ester-CH_3), 46.4 (C-4), 62.6 (ester-CH_2), 138.5 (d, C-2'), 166.1 (C-5).

[1'-^{15}N]-diester (8)

LDA was prepared at -78°C (from 4.0 ml (28.25 mmol, 1.3 eq.) of diisopropyl amine, 25.7 ml (28.25 mmol, 1.3 eq.) of 1.2 M n-butyl lithium solution) in 115 ml THF. Then 3.06 g (20.96 mmol) of (8) dissolved in 20 ml THF was slowly added dropwise to the LDA to give the lithium salt, which was added during 2 h to a solution of 10.00 g (35.28 mmol, 1.7 eq.) thiazoline (7) in 50 ml THF which had prior been treated with 4.8 ml (38.51 mmol, 1.8 eq.) BF_3Et_2O and then stirred for 30 min at -78°C. After 1.5 h the reaction mixture was allowed to warm up to -40°C following by adding an aqueous solution of 7.69 g (143.81 mmol, 6.9 eq.) ammonium chloride in 88 ml water. The aqueous layer was extracted three times with 100 ml diethyl ether. The organic layer was dried over sodium sulfate and the solvent was evaporated. The two diastereomeric racemates were separated by silica gel chromatography (CH_2Cl_2:Et_2O=20:1). The buffered silica gel was prepared by shaking it with an aqueous solution of $Na_2HPO_4 2H_2O$ (per 100 g silica gel 3.52 g phosphate in 30 ml water) and was allowed to stand for 2 h. The requested diastereomers, the more polar isomers (Rf=0.225), were obtained as an oil which was recrystalized in hexane to give 3.57 g (8.32 mmol, 40% yield) of light brown powder.

^{1}H-NMR ($CDCl_3$, int. TMS): δ=1.26-1.32 (2t, 6H,2 ester-CH_3), 1.24-2.04 (m, 14H, H-7,8, 5 cy-CH_2), 2.30 (t, 2H, H-9), 3.09, 3.18, 3.44 (3m, 3H, H-2,6), 4.13 (q, 2H, ester-CH_2), 4.22-4.32 (m, 3H, H-3, ester-CH_2), 4.65 (m, 1H, H-4), 6.10 (d, 1H, H-1'). ^{13}C-NMR ($CDCl_3$, int. TMS): δ=14.3 (ester-CH_3), 24.4, 24.6, 24.8, 25.1 (C-7,3 cy-CH_2), 28.2 (C-6), 31.3 (C-8), 34.0 (C-9), 35.8, 36.7 (2 cy-CH_2), 44.4 (C-2), 58.2 (d, C-4), 60.3, 62.2 (2 ester-CH_2), 75.1 (C-3), 75.8 (cy-C), 168.6 (C-5), 173.2 (C-10), 179.1 (d, C-2').

Alcohol (9)

3.57 g (8.32 mmol) (8) was dissolved in 50 ml of methanol and 50 ml of THF and was cooled in an ice bath. 0.36 (9.56 mmol, 1.2 eq.) $NaBH_4$ was added at once and the reaction mixture was stirred for 17 h. The solvent was evaporated and the precipitate was dissolved in diethyl ether and water. The aqueous layer was extracted three times with 100 ml diethyl ether. After drying over sodium sulfate diethyl ether was removed and 2.75 g (7.09 mmol, 85% yield) of (9) was obtained.

^{1}H-NMR ($CDCl_3$, int. TMS): δ=1.26 (t, 3H, ester-CH_3), 1.20-1.17 (m, 14H, H-7,8, cy-CH_2), 2.31 (t, 2H, H-9), 2.70, 3.31, 3.63-3.87 (m, 6H, H-2,5,6, OH), 4.13 (q, 2H, ester-CH_2), 4.17-4.20 (m, 2H, H-3,4), 6.87 (d, 1H, H-1'). ^{13}C-NMR ($CDCl_3$, int. TMS): δ=14.3 (ester-CH_3), 24.3, 24.5, 24.8, 25.7 (C-7, cy-CH_2), 28.1, 30.5 (C-6,8), 34.0 (C-9), 36.6, 36.8, 37.8 (C-2, cy-CH_2), 60.4 (ester-CH_2), 63.2 (C-4), 66.8 (C-5), 75.3 (C-3), 75.3 (cy-C), 173.3 (C-10), 177.5 (C-2').

[1'-^{15}N]-dl-thiobiotin (10)

2.75 g (7.09 mmol) of (9) dissolved in 160 ml of an aqueous trifluoroacetic acid (H_2O:TFA=1:3), was stirred at 110°C for 5.5 h. Then the solvent was evaporated in vacuo to give a brown precipitate which was recrystalized from water. 1.34 g (5.11 mmol, 72% yield) of dl-thiobiotin was obtained.

1H-NMR (D_2O, pH 14, int. dioxan δ=3.71): δ=1.25-1.86 (m, 6H, H-6,7,8), 2.22 (t, 2H, H-9), 2.89 (dd, 1H, H-5$_{endo}$), 3.06 (ddd, 1H, H-5$_{exo}$), 3.43 (m, 1H, H-2), 4.35 (m, 1H, H-3), 4.63 (dd, 1H, H-4). ^{13}C-NMR (D_2O, pH 14, int. dioxan δ=67.6):δ=24.7 (C-8), 26.7 (C-6), 27.3 (C-7), 36.3 (C-9), 38.8 (C-5), 55.0 (C-2), 64.4 (d, C-4), 65.8 (C-3), 179.1 (d, C-2'), 182.8 (C-10).

[1'-^{15}N]-3,4-diaminotetrahydro-2-thiophenvalerianic acid (11)

A mixture of 1.67 g (6.38 mmol) (10) and 20 g barium hydroxide in 100 ml water was placed in a glass autoclave and heated to 160°C for 7 d. The resulting mixture was bubbled with carbon dioxide until neutralization. After filtration the solution was concentrated in vacuo to give 1.30 g (5.93 mmol, 93% yield) of (11).

^{1}H-NMR (D_2O, int. dioxan δ=3.71):δ=1.19-1.82 (m, 6H, H-6,7,8), 2.14 (t, 2H, H-9), 2.67 (ddd, 1H, H-5_{endo}), 3.03 (dd, 1H, H-5_{exo}), 3.43 (t, 1H, H-3), 3.54-3.67 (m, 2H, H-2,4). ^{13}C-NMR (D_2O, int. dioxan δ=67.6):δ=27.4, 29.4, 31.69 (C-6,7,8), 32.5 (C-5), 39.1 (C-9), 51.5 (C-2), 58.3 (d, C-4), 58.9 (C-3), 185.5 (C-10).

[1'-^{15}N]-dl-biotin (1)

To a solution of 1.30 g (5.93 mmol) (11) in 50 ml water cooled in an ice water bath was added 1.76 g (5.93 mmol, 3 eq. phosgene) bis-(trichloromethyl)carbonate. After 3 h, 0.94 g (8.90 mmol, 3 eq. base) sodium carbonate was added and the mixture was stirred further for 1 h. Then it was allowed to stir for 20 h at room temperature. Filtration of the solution followed by concentration to 10 ml gave 1.05 g (4.31 mmol, 73% yield) [1'-^{15}N]-dl-biotin.

^{1}H-NMR (D_2O, pH 14, int. dioxan δ=3.71): δ=1.29-1.75 (m, 6H, H-6,7,8), 2.14 (t, 2H, H-9), 2.73 (dd, 1H, H-5_{endo}), 2.96 (ddd, 1H, H-5_{exo}), 3.30 (m, 1H, H-2), 4.39 (dd, 1H, H-3), 4.56 (dd, 1H, H-4).^{13}C-NMR (D_2O, pH 14, int. dioxan δ=67.6): δ=26.6, 28.7, 29.3 (C-6,7,8), 38.4 (C-9), 40.8 (C-5), 56.4 (C-2), 61.3 (d, C-4), 63.0 (C-3), 166.4 (d, C-2'), 184.6 (C-10).

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