SYNTHESIS OF BIOPTERIN-8a-^{13}C

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SUMMARY

The synthesis of biopterin labeled at the angular 8a position with ^{13}C is described. Cyano-^{13}C-acetic acid was converted in three steps to benzyl 2-aminocyanoacetate. Condensation of the amino cyano ester with the monoxime of 5-deoxyl-L-arabinosone by a modification of the literature method afforded 2-amino-3-benzyloxycarbonyl-5-(L-erythro-1', 2'-dihydroxypropyl) pyrazine-1-oxide-2-^{13}C. This intermediate was condensed with guanidine to give biopterin-8-oxide-8a-^{13}C, which was reduced to afford biopterin-8a-^{13}C.

Key Words: ^{13}C-Biopterin, Carbon-13, Cyano-^{13}C-acetic Acid

INTRODUCTION

The importance of tetrahydrobiopterin as a cofactor in the enzymatic hydroxylation of aromatic amino acids has been established by Kaufman.[1] The precise mechanism of this process and the nature of the intermediate transformation products has not been completely elucidated. Recent investigation[2] has shown that transiently stable intermediates are observed. The use of ^{13}C-NMR with substrates enriched with ^{13}C at the key 4a and 8a angular carbons may provide some insight into the nature of the intermediates in the enzymatic reaction. The synthesis of biopterin-8a-^{13}C, (1) a stable precursor of tetrahydrobioterin-8a-^{13}C, is the subject of this manuscript.

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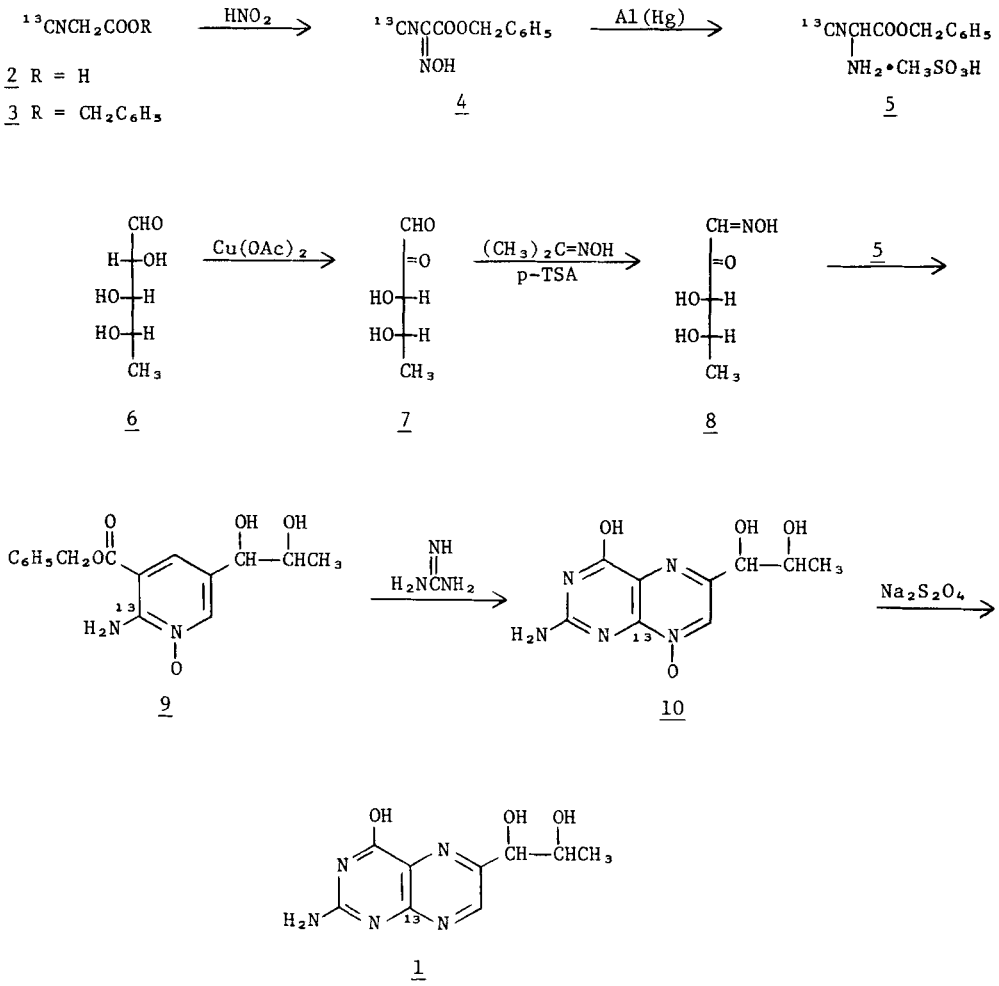
$^{13}CNCH_2COOR$
2 R = H
3 R = $CH_2C_6H_5$
HNO_2
$^{13}CNCCOOCH_2C_6H_5$
NOH
4
Al(Hg)
$^{13}CNCHCOOCH_2C_6H_5$
$NH_2 \cdot CH_3SO_3H$
5
CHO
H—OH
HO—H
HO—H
CH_3
6
$Cu(OAc)_2$
CHO
=O
HO—H
HO—H
CH_3
7
$(CH_3)_2C=NOH$
p-TSA
CH=NOH
=O
HO—H
HO—H
CH_3
8
5
$C_6H_5CH_2OC$
O
OH OH
CH—$CHCH_3$
H_2N
^{13}
N
O
9
NH
H_2NCNH_2
OH
N
N
H_2N
N
^{13}
N
O
OH OH
CH—$CHCH_3$
10
$Na_2S_2O_4$
OH
N
N
H_2N
N
^{13}
N
OH OH
CH—$CHCH_3$
1

An elegant synthesis of biopterin has been reported by Taylor and Jacobi.[3] This method formed the basis for the labeled synthesis which initiated from cyano-^{13}C-acetic acid (2). The cyano acid (2) was esterified with benzyl alcohol as catalyzed by sulfuric acid to give benzyl cyano-^{13}C-acetate (3). Treatment of the benzyl ester with sodium nitrite[4] in acetic acid afforded the crystalline oximino ester (4) in 47% yield. Reduction of the oxime with aluminum amalgam in ether readily gave benzyl 2-amino-cyano-^{13}C-acetate (5) isolated as the methanesulfonic acid salt.

5-Deoxy-L-arabinose (6) was prepared from L-rhamnose according to the literature procedure.[3] Oxidation of 6 with cupric acetate in aqueous ethanol generated 5-deoxy-L-arabinosone (7) which was freed of inorganic salts by passage through Dowex 50 resin. The crude, syrupy product was shown to contain 45% osone by-conversion to imino-5-deoxyl-L-arabino-ascorbic acid and subsequent titrations with iodine. Reaction of 7 with acetoxime in aqueous p-toluenesulfonic acid at pH 3.5 served to prepare the sugar monooxime (8). We were unable to obtain satisfactory or reproducible yields of the oxime by adherence to the literature process.[3] The use of p-toluenesulfonic acid to catalyze this transoximation reaction afforded modest, but reproducible yields of the oxime (8).

Condensation of the crude oxime (8) with the ^{13}C-amino cyano ester (5) in ethanol at ambient temperature for 36 hours afforded the pyrazine-N-oxide (9) in 31% yield from 5. Subsequent ring closure with guanidine gave biopterin-8-oxide-8a-^{13}C (10) in 75% yield. The final reduction of the N-oxide (10) to biopterin-8a-^{13}C (1) was accomplished with sodium dithionite in pH 7 buffer at 100°. Attempts to remove the N-oxide via reduction with trimethyl phosphite were unsuccessful.

Hydrogenation of 10 in 0.1N-HCl over a platinum catalyst readily afforded tetrahydro biopterin, based on ultra violet evidence.

The ^{13}C-NMR spectra for compounds 9, 10, and 1 showed only single signals for ^{13}C-enriched carbon. Biopterin-8a-^{13}C gave a signal at 156.3 ppm, in agreement with the value assigned to the 8a-carbon by Schircks et al.[5] for the natural abundance ^{13}C-NMR spectrum of biopterin.

EXPERIMENTAL

Benzyl Cyano-^{13}C-acetate (3)

A mixture of 3.85 g of cyano-^{13}C-acetic acid,[6] 9 ml of benzyl alcohol and 0.3 ml of concentrated sulfuric acid was heated at 100-105° for 2 hours. After cooling, the mixture was poured into 30 ml of ice water, neutralized with solid sodium bicarbonate and extracted twice with 30-ml portions of ether. The extract was dried over sodium sulfate and evaporated *in vacuo*. The residual liquid was fractionally distilled through a short Vigreux column to afford 7.0 g (82%), bp 110-120°/0.1 mm, lit[4] bp 133-134°/0.5 mm.

Benzyl Oximino-cyano-^{13}C-acetate (4)

A mixture of 7.0 g (0.04 mole) of 3, 12 ml of acetic acid and 4.6 ml of water was chilled to 0-5° in an ice bath. A solution of 3.68 g (0.053 mole) of sodium nitrite in 7.4 ml of water was added dropwise with stirring with maintenance of the temperature below 10°. The mixture was stirred for 3 hours at 0-5°, diluted with 50 ml of ice water and acidified with 5.0 ml of 12 N hydrochloric acid to precipitate a yellow oil. The oil was extracted twice with 50-ml portions of benzene, dried over sodium sulfate and concentrated to a volume of 30 ml. Light petroleum ether was added to induce crystallization. The solution was chilled and the crystals were

collected, washed with petroleum and dried to leave 3.2 g (47%) of 5 mp 112-113° (lit.[4] mp 115°).

Benzyl 2-Amino-cyano-^{13}C-acetate Methanesulfonate (5)

Heavy duty aluminum foil (0.65 g, 0.3 $cm^{(2)}$ pieces) was treated for 2 minutes with 15.5 ml of 2% mercuric chloride. The solution was decanted and the resulting amalgam was washed twice with water, ethanol tetrahydrofuran and finally covered with 16 ml of tetrahydrofuran. A solution of 3.1 g of the oximino ester (5) in 19 ml of ether was added to the amalgam and the mixture was stirred while 0.93 ml of water was added at a rate to maintain reflux. The mixture was cooled and filtered through a layer of Celite. The cake was washed with 35 ml portions of tetrahydrofuran and ether, followed by treatment of the total filtrate with 1.86 g of methanesulfonic acid. The solution was diluted with 150 ml of ether and the resulting crystalline precipitate was collected and dried to afford 2.4 g (55%) of 6, mp 159-162°; lit.[3] mp 157-160°).

2-Amino-3-benzyloxycarbonyl-5-(L-erythro-1', 2'-dihydroxypropyl) pyrazine-1-oxide-2-^{13}C (9)

A solution of 1.2 g (0.0079 mole) of 5-deoxy-L-arabinose (6) in 6 ml of water was diluted with 75 ml of ethanol. The solution was heated to boiling and 12 g of powdered cupric acetate was added. The mixture was stirred at reflex for exactly 9 minutes and immediately cooled. After filtration of salts the cake was washed with 100 ml of methanol and the total filtrate passed through a 2.5 x 20 cm column of Dowex 50W x 4 resin. Following elution by another 200 ml of methanol the total eluate was evaporated *in vacuo* to leave a pale yellow syrup, containing 45% of the osone (7) as shown by titration with 0.1 N-iodine.[3]

The residual syrup was dissolved in 3 ml of water, the pH adjusted to 3.5 with 5% ammonimum hydroxide and 15 mg of p-toluenesulfonic acid was added. Acetoxime (1.0 g, 0.0137 mole) was added and the solution was heated at 50° for 12 hours. After dilution with 15 ml of water the solution was extracted with three 15-ml portions of ether to remove excess acetoxime. The aqueous portion was freeze-dried to leave a yellow syrup identified as the oxime (8) by the amino malononitrile method of Taylor and Jacobi.[3]

The crude keto aldoxime (8) and 0.09 g (0.0031 mole) of 5 were added to 10 ml of absolute ethanol and the solution was kept at room temperature for ether and chilled 20 hours. The precipitate was collected, washed with ether and water and dried to leave 302 mg of 9; mp 164-165° (lit.[3] 165-166°); UV (EtOH) 229 nm (ε 12,600), 252 (18,600), 380 (8,200); ^{13}C-NMR (d_6-DMSO) one enriched peak, 148.1 ppm.

Biopterin-8-oxide-8a-^{13}C (10)

To a solution of 65 mg (0.20 mmole) of 9 in 0.9 ml of dimethyl formamide was added 43.3 mg (0.45 mmole) of guanidine hydrochloride and 63 mg (1.1 mmole) of sodium methoxide. The mixture was stirred at 70-75° for 16 hours, cooled and diluted with 1.2 ml of water. The pH was adjusted to 3 and the solution kept 20 hours. The yellow precipitate was collected by centrifugation and washed successively with water, ethanol and ether to afford 38 mg (75%); UV (0.1 N NaOH) 262 nm (ε 15,500), 388 (4,100); ^{13}C-NMR (d_6-DMSO) one enriched peak, 151.1 ppm. Paper chromatography showed a single uv absorbing spot at R_f 0.20 in BuOH/HOAc/H_2O, 4/1/1.

Biopterin-8a-^{13}C (1)

A suspension of 15 mg of the N-oxide in 3 ml of Tris buffer (pH 7) was stirred at reflux until solution was obtained. Sodium dithionite (11.0 mg) was added and heating continued for 30 minutes. After cooling the yellow precipitate was collected by centrifugation and washed with water. The material was recrystallized from water to afford 8.2 mg (57%) of biopterin - 8a-^{13}C; uv (0.1 N NaOH) 254 nm (ε 24,500), 363 (7,800); ^{13}C-NMR (3 N NaOD) single enriched signal 156.3 ppm. Paper chromatography showed a single uv absorbing spot at R_f 0.35 (identical to natural biopterin) in Bu/HOAc/H_2O, 4/1/1.

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