Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 8

FOLATE ANALOGUES: 27. SYNTHESES OF ^{14}CARBON-LABELED 10-DEAZAAMINOPTERIN AND 10-ETHYL-10-DEAZAAMINOPTERIN

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SUMMARY

10-Deazaaminopterin (1) and 10-Ethyl-10-deazaaminopterin (2) labeled uniformly with ^{14}C at the glutamate moiety were prepared by the following procedure. Uniformly labeled L-glutamic acid was converted to its dimethyl ester (3). 4-Amino-4-deoxy-10-deazapteroic acid (4) and 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid (5) were converted to their respective mixed anhydrides 6 and 7 with isobutylchloroformate. Reaction of 6 and 7 with labeled dimethyl-L-glutamate gave dimethyl-10-deazaaminopterin (8) and dimethyl 10-ethyl-10-deazaaminopterin (9) respectively, which on mild alkaline hydrolysis gave the target compounds 1 and 2 in ∿22-25% yield, based on the amount of radiolabeled glutamic acid used.

Key Words: 10-Deazaaminopterin, 10-Ethyl-10-deazaaminopterin, ^{14}C, Glutamic acid.

INTRODUCTION

Inhibitors of the enzyme dihydrofolate reductase, are potential anti- cancer drugs because of their ability to block cell division by interferring with folate metabolism (1). The well known anticancer drug methotrexate is one of the most potent inhibitors of dihydrofolate reductase. 10-Deazaaminopterin (1) and 10-ethyl-10-deazaaminopterin (2) are close structural analogues of methotrexate (2,3) which showed more desirable chemotherapeutic indices in several tumor models (4). These two compounds are presently undergoing advanced clinical trials (5), and are under commercial development in Europe. A preliminary report of the use of tritium labeled 1 and 2 for pharmacological studies has appeared recently (6). However, they were made by direct exchange of 1 and 2 with tritium

0362-4803/87/080895-07$05.00

Received January 31, 1986
Revised December 5, 1986

at unspecified positions of the molecule and no details of their preparations were given. In order to facilitate our investigations regarding the metabolism of 1 and 2 to their poly-γ-glutamates (7), these drugs with the radiolabel at well defined positions of the molecule were required. In addition, demand for specifically labeled 1 and 2 for various biochemical and pharmacologic studies is on the increase. In this manuscript we detail a convenient procedure for the synthesis of labeled 10-deazaaminopterin and 10-ethyl-10-deazaaminopterin, with uniform ^{14}carbon labels at the glutamate moieties of the molecules.

CHEMISTRY

Both 4-amino-4-deoxy-10-deazapteroic acid (4) and 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid (5) were prepared according to the procedure of Nair (3). Uniformly labeled L-glutamic acid with a specific activity of 250 milliCurie/millimole was purchased from ICN. The labeled glutamic acid was diluted with crystalline unlabeled L-glutamic acid to a specific activity of 20 milliCurie/millimole, and the diluted product was dried at 25°C under vacuum over P_2O_5 for 24 h. The 4-amino-4-deoxy folate analogues are known to undergo base catalyzed deamination at the 4 position to the corresponding folate analogues under hydrolytic conditions (8). Therefore, a carboxyl protective group which could be removed without affecting deamination of the final product was required. The trimethylsilyl derivative of L-glutamic acid (8) was selected as a suitable choice. Treatment of radiolabeled L-glutamic acid with hexamethyl disilazane (9) at elevated temperatures gave the corresponding tri-trimethylsilyl derivative 10. We have previously reported that the carboxyl group of a 4-amino-4-deoxypteroic acid analogue can be selectively activated as the mixed anhydride with isobutylchloroformate for peptide coupling under a selected set of conditions. Using this procedure (Vide infra) both acids 4 and 5 were converted to their respective mixed anhydrides 6 and 7, and coupled with tri-trimethylsilyl-L-glutamate (10) by standard procedures. Hydrolysis of the resulting coupled products 11 and 12 with aqueous Na_2CO_3, and purification of the hydrolysates by repeated ion exchange chromatography gave the desired labeled target compounds 1

and 2, albeit in very low yields (∿5%).

The unacceptable low yields of the final products obtained by the previous method prompted us to consider the use of the methoxycarbonyl protective groups for the glutamate moiety. Labeled L-glutamic acid was converted to the dimethyl ester hydrochloride (3) and after neutralization with 4-methylmorpholine, it was coupled with the mixed anhydrides 6 or 7. The coupled products were hydrolysed with a mixture of 0.05 N NaOH in CH_3CN, and purified by ion exchange chromatography over DEAE-cellulose (Scheme-I). The yields of the final products 1 and 2 ranged from 22 to 25% which were considered acceptable. The deamination products formed under these hydrolytic conditions, amounted to less than three percent.

EXPERIMENTAL PROCEDURES

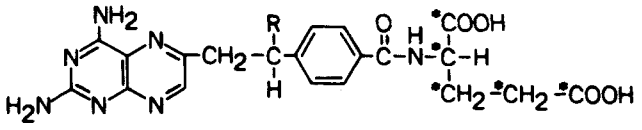
All reagents used were the highest quality available from Aldrich Chemical Company. Isobutylchloroformate and 4-methylmorpholine were redistilled prior to use. Dimethyl formamide (DMF) was dried over type 3-A molecular sieve. DEAE-cellulose in the chloride form was used for ion exchange chromatography. A linear NaCl gradient from 0 to 0.5 M at pH 7.0 in 0.005 M phosphate buffer was used to elute the products from the DEAE column. All radioactive measurements were made on a model LKB 1219 Rackbeta liquid scintillation counter using Fisher brand scintiverse E as the counting medium. Optical density was measured on a Bausch & Lomb spectronic model 2000 spectrophotometer.

^{14}C LABELED-TRI-TRIMETHYLSILYL-L-GLUTAMATE (10)

In a 50 mL round-bottomed flask 14.7 mg (0.1 mmol) of L-glutamic acid (specific activity 20 mCi/mmol) was stirred under reflux with 2.5 mL of hexamethyldisilazane and 1.0 mg (in 0.5 mL benzene) of concentrated H_2SO_4 for 1 h. The clear reaction mixture thus obtained was cooled to ∿ 25°C, and the excess reagent was removed under vacuum. The glassy residue of 10 was placed in a vacuum desicator, and dried for 2 h at 25°C, dissolved in 1 mL of dry DMF, and was used immediately for coupling with the mixed anhydrides 6 or 7.

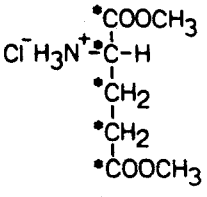
^{14}C-LABELED DIMETHYL GLUTAMATE HYDROCHLORIDE (3)

As in the previous experiment, 0.1 mmol (14.7 mg) of uniformly labeled L-



10-Deaza aminopterin; R=-H (1)

10-Ethyl-10-Deaza aminopterin; R=$-CH_2-CH_3$ (2)



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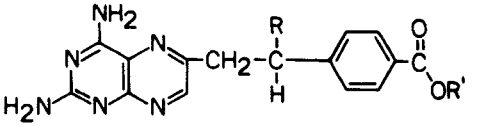
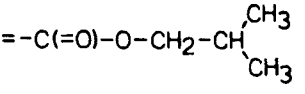


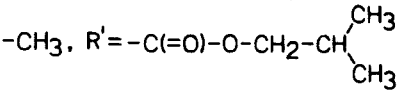
4 ; R=-H, R'=-H

5; R=$-CH_2-CH_3$, R'=-H

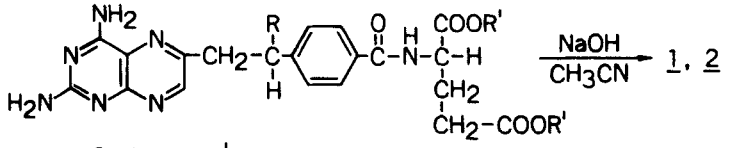
$^{*}14C$



6; R=-H, R'=$-C(=O)-O-CH_2-CH(CH_3)_2$

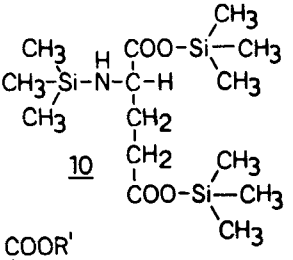


7; R=$-CH_2-CH_3$, R'=$-C(=O)-O-CH_2-CH(CH_3)_2$

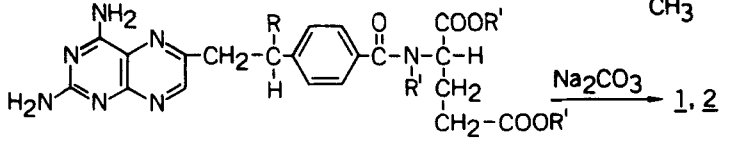


8; R=-H, R'=$-CH_3$

9; R=$-CH_2-CH_3$, R'=$-CH_3$



10



11; R=-H, R'=$-Si(CH_3)_3$

12; R=$-CH_2-CH_3$, R'=$-Si(CH_3)_3$

glutamic acid with a specific activity of 20 mCi/mmole was stirred under reflux with 3.0 mL of a saturated solution of gaseous HCl in absolute methanol for 18 h under strictly anhydrous conditions. The reaction mixture was cooled

to 30°C, 20 mL of dry benzene was added, and the solvent mixture was removed by evaporation under reduced pressure. The crystalline residue of 3 thus obtained was dried in vacuum over P_2O_5 for 24 h, and dissolvced in 1.0 mL of DMF containing 0.0113 mL (0.1 mmol) of 4-methylmorpholine. This neutralized solution was immediately used for the next step.

^{14}C-LABELED 10-DEAZAAMINOPTERIN (1) AND 10-ETHYL-10-DEAZAAMINOPTERIN (2).

To 0.05 mmol of pteroic acid 4 placed in a 5 mL oven dried graduated cylinder was added 1.0 mL of DMF and 0.008 mL (0.0625 mmol) of 4-methylmorpholine. The mixture was gently heated till all the solid went in solution. The cylinder was placed in an ice bath for 15 minutes, and 0.0065 mL (0.05 mmol) of freshly distilled isobutylchloroformate was introduced under stirring. The reaction mixture containing 6 was removed from the ice bath, placed at ambient temperature for 30 minutes, and mixed with the solution of 3. After 18 h at 25°C the reaction mixture was placed in a water bath maintained at 80°C for 15 minutes, and evaporated under reduced pressure. The reaction product 8 thus obtained by coupling 6 with 3 was stirred with a mixture of 7.5 mL of 0.05 N NaOH and 2.5 mL of CH_3CN for 8 h and evaporated under reduced pressure to 5.0 mL at 25°C. The solution was diluted to 20 mL, the pH adjusted to 7.5 with .1 N HCl and applied on a DEAE cellulose column (28x2.5 cm). The column was eluted with a linear NaCl gradient from 0 to 0.5 M in 0.005 M phosphate buffer at pH 7.0. The mixing chamber and the reservoir contained one litre each of the respective solutions. Small fractions were collected, and each fraction was monitored for optical density and radioactivity. All fractions corresponding to the product 1 was pooled, diluted ten times its volume with distilled water, and reapplied on a fresh DEAE-cellulose column. After washing the column with 50 mL distilled water, it was eluted with a solution of 15% NH_4OH. All the radioactivity which was applied on the column was eluted in the first 100 mL of the solution. The NH_4OH solution containing the product as the ammonium salt was evaporated to dryness, and the residue dissolved in 10 mL of distilled water. The purity and authenticity of the sample were checked by co-chromatography of a small amount of the radioactive product with authentic non-radioactive 10-deazaaminopterin on a

DEAE-cellulose column as described above. Each fraction eluted from the column was monitored for optical density and radioactivity. There was a perfect correlation between optical density of the standard and radioactivity as shown in Fig. 1. Compound 1 thus obtained had a radiochemical yield of 25.5% (510 uCi) and exhibited a specific activity of 19.56 uCi/umole.

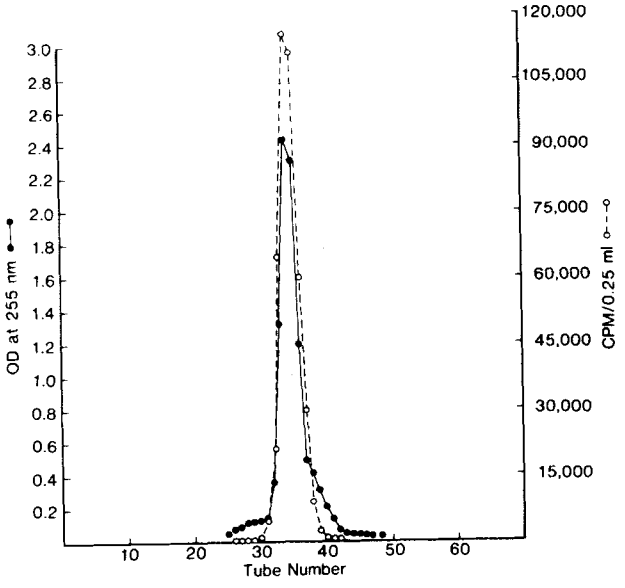


Fig. 1. Co-chromatography of ^{14}C-10-deazaaminopterin with authentic 10-deazaaminopterin on a DEAE-cellulose column.

The above experiment was repeated starting from pteroic acid analogue 5. The mixed anhydride 7 was prepared from 0.05 mmol of 5 and coupled with 0.1 mmol of radiolabeled dimethyl-L-glutamate (3) with a specific activity of 20 mCi/mmol. The reaction product thus obtained was worked up, hydrolysed and purified as described above to obtain compound 2. Radiochemical yield, 22% (440 uCi); specific activity, 19.35 uCi/umole.

These experiments were repeated by substituting the trimethylsilyl derivative 10 for the dimethyl ester 3. The mixed anhydride 6 or 7 was coupled with 3 as described above. After evaporation of DMF, the product was stirred with 50 mL of 0.1 N Na_2CO_3 for 18 h, diluted to 200 mL and the pH of the solution was adjusted to 7.5 with 0.1 N HCl. The crude product of each reaction was

purified by ion exchange chromatography on a DEAE-cellulose column. The radiochemical yield of either 1 or 2 obtained by this method was less than 5% which was found to be unsatisfactory.

Acknowledgement

This work was supported by grants CA 27101 and CA 32687 awarded by the National Cancer Institute, DHHS.

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