Synthesis of Edatrexate (2-^{13}C-Glutamate)

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Summary

The experimental antitumor drug Edatrexate, labeled with 99% ^{13}C at the 2-position of the glutamate acid group was required for ^{13}C-magnetic resonance spectroscopy studies in biological media. Coupling of 2,4-diamino-4-deoxy-10-ethyl-10-deazapteroic acid with diethyl L-2-^{13}C-glutamate as promoted by BOP reagent afforded Edatrexate (2-^{13}C-glu) diethyl ester in 60% yield following purification by column chromatography. Saponification by aqueous NaOH in 2-methoxyethanol gave the target molecule in 44% yield or 26% overall.

Key Words: Edatrexate, antitumor, ^{13}C-magnetic resonance spectroscopy

Introduction

We have previously reported[1] the synthesis and biological activity of the antifolate drug 10-ethyl-10-deazaaminopterin (Edatrexate, Scheme 1, compound 5). Further clinical studies have demonstrated its efficacy in the treatment of breast and advanced non-small cell lung cancers [2-4]. An important property of Edatrexate was its ability to selectively accumulate in tumor cells *vs* sensitive normal host tissue. Key

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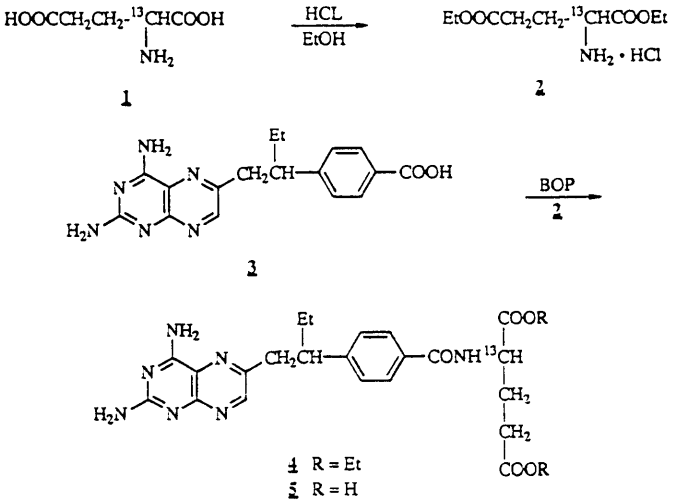
factors in the selective accumulation were enhanced active transport and polyglutamylation in tumor cells. In an attempt to demonstrate the potential of ^{13}C-magnetic resonance spectroscopy *in vivo* we required Edatrexate labeled at the 2-position of the glutamate unit with 99%-^{13}C. Besides the expectation of enhanced signal strength in the ^{13}C-NMR spectrum we hope to demonstrate formation of polyglutamates *via* observation of the chemical shift of the 2-^{13}C signal following conjugation with another glutamate moiety.

Discussion

Edatrexate (2-^{13}C-glu) was prepared from 2,4-diamino-4-deoxy-10-ethyl-10-deazapteroic acid[1] (3) by the method shown in Scheme 1. The procedure consisted of esterification of commercially obtained L-2-^{13}C-glutamic acid (1) with ethanolic HCl followed by coupling of the ester (2) with the pteroic acid (3). The coupling reaction was conducted with benzotriazole oxophosphonate reagent (BOP) rather than the traditional isobutyl chloroformate mediated process in order to conserve the amount of ^{13}C-glutamate expended. The original mixed anhydride procedure requires



Scheme 1

repetitive treatment with 2 to 3 equivalents of glutamate ester compared to only one equivalent for the BOP method. The crude reaction product was purified by flash column chromatography to afford the Edatrexate diethyl ester (4) in about 60% yield. This material was found to be identical to unlabeled compound 4 prepared in the same manner. The labeled diester 4 was shown to be be equivalent to authentic (4) by TLC and ^{1}H-NMR comparisons. Hydrolysis of the labeled diester (4) with NaOH in aqueous 2-methoxyethanol afforded Edatrexate (2-^{13}C-glu) in 44% yield. The ^{1}H-NMR spectrum was equivalent to authentic edatrexate.

Experimental

Analtech silica gel GF plates were used for thin layer chromatography. NMR measurements were conducted on a Varian 300XL spectrometer. Preparative chromatography was performed with Baker flash chromatography silica gel.

Diethyl 2-^{13}C-L-Glutamate Hydrochloride (2)

L-2-^{13}C (99%) Glutamic acid (Cambridge isotopes, 301 mg) was added to 2.5 ml of absolute ethanol saturated with dry HCl at 0-5^{0}C. The mixture was stirred at reflux for 3 hours and evaporated in vacuo. Another 2 ml of ethanol was added followed by a 20-minute reflux period and evaporation. The residue was treated with 10 ml of ether to give a white gum. The ether was decanted and the gum dissolved in 5 ml of $CHCl_3$. The solvent was evaporated and the residue treated twice with 10 ml portions of ether. After drying under high vacuum, the semi-solid product weighed 437 mg (89%).

Edatrexate (2-^{13}C-Glutamate) Diethyl Ester (4)

To a solution of 612 mg (1.8 mmole) of the 2,4-diamino-4-deoxy-10-deazapteroic acid[1] (3) in 24 ml of dimethyl formamide (DMF) was added 0.49 ml (3.6 mmole) of triethylamine and 798 mg (1.8 mmole) of benzotriazole oxophosphonate (**BOP**) reagent. The mixture was stirred 1.5 hours at room temperature and 437 mg (1.8 mmole) of diethyl L-2-^{13}C-glutamate HCL in 1.5 ml of DMF was added. The mixture

was stirred for 20 hours, the solvent removed *in vacuo* , and the residue thoroughly mixed with 20 ml of water. The aqueous phase was decanted and the thick gum dissolved in 35 ml of $CHCl_3$, and washed with 15 ml of water. The $CHCl_3$ extract was dried over $MgSO_4$ and evaporated to leave 1.29g of a yellow gum. The material was twice shaken with 20-ml portions of ether followed by decantation and drying *in vacuo* to leave 0.88g. The gum was chromatographed on silica gel with elution by $CHCl_3$ - MeOH, 95:5, to afford 0.57 g (60%) of a yellow glass following treatment with 15 ml of ether. The thin layer chromatogram showed a single U.V.-absorbing spot at Rf 0.4, equivalent to the unlabeled diester prepared by the same procedure. The unlabeled diester was shown to be equivalent to authentic material previously synthesized by comparison of ^{1}H-NMR spectra. ^{1}H-NMR ($CDCl_3$), δ ppm,0.78(3H,t,CH_3), 1.25(6H,dt,$OCH_2\underline{CH_3}$), 1.72(2H,m,$\underline{CH_2}CH_3$), 2.10(2H,m,glu-3-CH_2), 2.45(2H,bs,glu-4-CH_2),3.10(4H,bs,C-9H,C-10H),4.18(4H,dq,$O\underline{CH_2}CH_3$),4.80(1H,m,NH), 6.05(1H,bs,NH_2),6.75(1H,bs,NH_2), 7.15(2H,d,3',5'-H), 7.72(2H,d,2',6'-H), 8.35(1H,s,C-7H). The 2- and 4-NH_2 typically integrate to 1H in $CDCl_3$.

Edatrexate (2-^{13}C-Glutamic acid) (5)

The ^{13}C-labeled diester (4) above (0.57g) was dissolved in 2 ml of 2-methoxyethanol followed by addition of 4 ml of 1.25 N NaOH. The mixture was stirred at room temperature for 2.75 hours and the resulting yellow solution was adjusted to pH 7-8 with glacial acetic acid. The solvent was removed *in vacuo* and the residue was dissolved in 20 ml of water followed by treatment with acetic acid to precipitate the product at pH 5-6. After one hour the yellow crystalline precipitate was collected, washed with water, and dried to afford 224 mg (44%) of product. Edatrexate ^{1}H-NMR (NaOD-D_2O), δ ppm, 0.71(3H,t,CH_3), 1.68(2H,m,$\underline{CH_2}CH_3$),1.95 and 2.08(2H,m,glu-3-CH_2), 2.23(2H,m,glu-4-CH_2), 2.99(2H,m,C-9H), 3.14(1H,m,C-10H), 4.23(1H,q,glu-2-CH), 7.11(2H,d,3',5'-H), 7.60(2H,d,2',6'-H), 8.20(1H,s,C-7H). Except for the signal at 4.23 ppm, corresponding to the C-2 glutamate proton, the ^{1}H NMR spectra of ^{13}C-C_2

glutamate labeled edatrexate are identical to the native molecule. Every peak and multiplet structure match precisely. The ^{1}H spectra of the ^{13}C labeled molecule display a residual, < 0.1 proton intensity, ^{13}C-^{1}H glutamate signal at 4.23 ppm, but symmetrically dispaced upfield and downfield by 70 Hz at 4.06 and 4.41 ppm are the $^{13}C_2$-^{1}H glutamate signals. Such splitting is consistent with the heteronuclear ^{13}C-^{1}H J coupling. Comparing the integrated area of the ^{13}C satellites and the $^{13}C_2$-^{1}H glutamate signal reveals that the ^{13}C enrichment is >90%.

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