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# AN IMPROVED PROCEDURE FOR THE SYNTHESIS OF DL-[2-$^{13}$C]-GLUTAMIC ACID

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## SUMMARY

A convenient two-step chemical synthesis of DL-[2-$^{13}$C]-glutamic acid from methyl acrylate and diethyl acetamidomalonate is described. Synthesis of the Michael adduct (III) was effected in quantitative yield by employing strong amino bases as catalysts. The reactivities of three such catalysts, tetramethyl guanidine (TMG), 1,8-diazobicyclo [5,4,0]-undec-7-ene (1,5-5) (DBU) and 1,5-diazobicyclo [4,3,0] non-5-ene (DBN) were compared by $^{13}$C NMR and all are shown to catalyse the reaction quantitatively, however at markedly different rates.

Keywords: DL-[2-$^{13}$C]-Glutamic acid, Michael addition, $^{13}$C NMR

## INTRODUCTION

In addition to being a requisite component of proteins, glutamic acid occupies a central role in both respiration and nitrogen assimilation as well as providing an amine source for amino acid biosynthesis via interconversion to α-ketoglutarate. The conversion of glutamate to α-ketoglutarate can either be catalyzed oxidatively by glutamate

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dehydrogenase or through pyridoxal phosphate mediated transamination catalyzed by aspartate aminotransferase. Due to our interest in the latter enzyme and the mechanistic importance of C2 during the transamination reaction, a convenient synthesis of glutamate $^{13}C$-enriched at position 2 would be of considerable value.

The classical synthetic methods of racemic glutamic acid can be classified into three main groups:

i) synthesis involving a reduction step on either α-oxiiminoglutaric acid, α-diazoglutaric acid, or α-oxoglutaric acid and their esters[1-8].

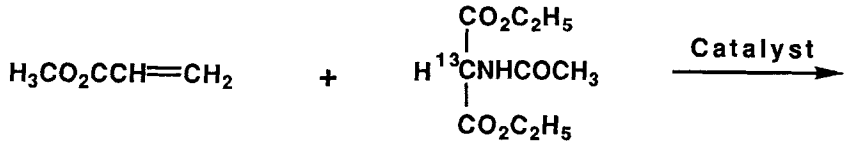
ii) the Strecker reaction performed on β-aldehydopropionic acid[9,10].

iii) condensation of diethyl malonate derivatives with various compounds[11-18].

More modern synthetic methods offer the syntheses of optically pure stereoisomers[19-21], or utilize other types of reactants[22-26]. Of all these methods, few are practical for the efficient introduction of $^{13}C$, especially at C2, into glutamic acid. The best strategy towards such a synthesis involves condensation of malonic acid derivatives with methyl acrylate[14,15]. The Michael type addition is a well known reaction with generally simple experimental procedures[27]. Recently, the use of new types of catalysts has made this process more effective, even for very unreactive systems[28,29]. In this paper we report the application of the strong amino bases; tetramethyl guanidine (TMG), 1,8-diazobicyclo[5,4,0] undec-7-ene (1,5-5) (DBU), and 1,5-diazobicyclo [4,3,0] non-5-ene (DBN) as efficient catalysts for the synthesis of racemic [2-$^{13}C$]-glutamic acid.

## RESULTS AND DISCUSSION

The synthesis of glutamic acid containing the requisite label at C2 proceeds through the Michael addition of methyl acrylate (1) to commercially available diethyl [2-$^{13}C$]-acetamidomalonate (2) (Scheme 1), followed by acid hydrolysis of the resulting intermediate (3). The critical step is the formation of (3), hence the proper choice of



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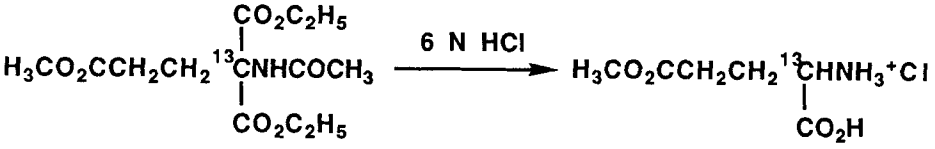

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catalyst for the Michael addition will be crucial in determining the overall yield of glutamic acid. For example, sodium hydride, a commonly used agent for carbanion generation[16,17], produced intermediate (3) in only 60% yield. Alternatively, the applicability of the strong amino bases, TMG, DBU and DBN were investigated. While each of the catalysts produced intermediate (3) quantitatively, their relative rate enhancements of the reaction varied markedly. As shown in Fig. 1, DBN was the most effective among the three catalysts, the reaction between (2) and a three-fold excess of methyl acrylate in the presence of 10 mM DBN was complete after 5 hours at room temperature, whereas DBU required almost sixteen hours reaction time. TMG was the slowest catalyst of the three; after 15 h the yield of ester (3) was ca 50 % and the reaction required 72 hours for completion. On the other hand, the advantage of using TMG is that it does not cause polymerization of the olefin. For this reason, TMG was used for the preparative synthesis of glutamic acid. Accordingly, diethyl [2-$^{13}$C] acetamidomalonate (2) was reacted with 1.5 equivalents of methyl acrylate in the presence of 1.5 mM TMG for 72 hours at room temperature. The desired ester (3) was recovered in quantitative yield after crystallization, and upon hydrolysis in 6M HCl afforded the hydrochloride salt of DL-[2-$^{13}$C]-glutamic acid (4) in 95% yield. Although not essential for our experi-

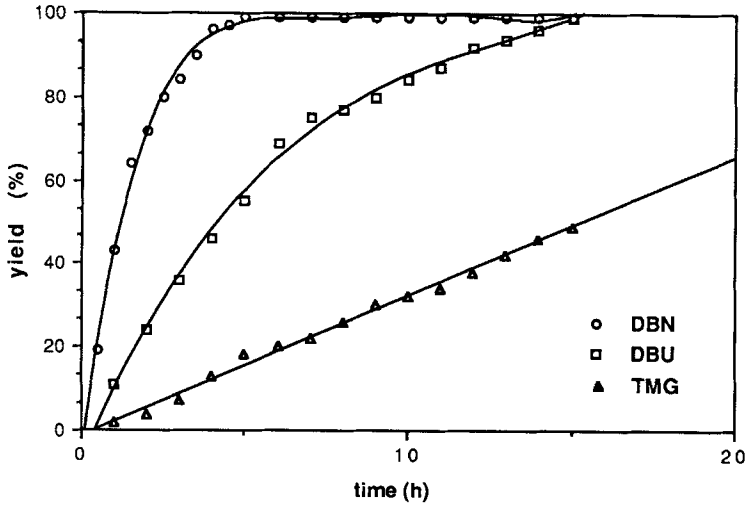


Figure 1. Rates of formation of intermediate (3) catalyzed by either DBN, DBU, or TMG.

ments, the resolution of DL-glutamic acid can be readily effected using the standard protocol of stereo-selective hydrolysis of the N-acetyl derivative by hog renal acylase I[30].

## EXPERIMENTAL

Melting points (mp) were determined on a Buchi 510 apparatus and are uncorrected. Proton magnetic resonance spectra ($^1H$ NMR) were recorded at 500.13 MHz on a Bruker AM 500 spectrometer. Chemical shifts ($\delta_H$) are reported in ppm relative to internal dioxane ($\delta$=3.53) for $D_2O$ solutions or residual $CHCl_3$ ($\delta$=7.24). Carbon magnetic resonance spectra ($^{13}C$ NMR) were recorded at 125.76 MHz on a Bruker AM 500 using the WALTZ 16 sequence for proton decoupling. Carbon chemical shifts are quoted in ppm and are referenced to either internal dioxane ($\delta$=66.5) or $CDCl_3$ ($\delta$=77.0).

Materials; [2-$^{13}C$] (99%) Diethyl Acetamidomalonate was purchased from Cambridge Isotope Laboratories; TMG, DBU, DBN and methyl acrylate were purchased from Fluka Chemical Co. and used without purification.

*a)Effect of TMG, DBU and DBN on the rate of Michael addition of methyl acrylate (1) to diethyl acetamidomalonate (2).*

The relative activities of TMG, DBU and DBN were compared by monitoring the conversion of the $^{13}$C-labeled methine carbon ( $\delta$ 52.4) of diethyl acetamidomalonate (2) to the quaternary carbon ($\delta$ 64.5) of ester (3) by $^{13}$C NMR. The experiments were carried out as follows:
To a 5 mm diameter NMR tube containing 7.24 mg (33.3 mmol) of diethyl [2-$^{13}$C]-acetamidomalonate and 8.2 mg (8.58 ml, 100 mmol) of methyl acrylate in 1 ml dry deuterochloroform 10 mmol of catalyst was added. In the three independent experiments 1.15 mg (1.25 ml) of TMG, 1.52 mg (1.49 ml) of DBU, or 1.24 mg (1.19 ml) of DBN were added. The progress of the reactions was monitored for 15h, those with DBU and TMG being recorded every hour, and that with DBN every 30 min. The results of these experiments are summarized in Figure 1.

*b)Preparation of DL-[2-$^{13}$C]-Glutamic Acid.*

N-Acetyl-2-carbethoxy-[2-$^{13}$C]-glutamic acid 1-ethyl 3-methyl ester(3). To a stirred solution of 108.6 mg (0.5 mmol) of diethyl [2-$^{13}$C ] acetamidomalonate in 10 ml of dry dichloromethane at ambient temperature were added TMG (5.76 mg, 6.29 ml, 0.015 mmol) and methyl acrylate (64.6 mg, 67.6 ml, 0.75 mmol). The mixture was stirred for 72 h, then solvent and excess methyl acrylate were removed at reduced pressure. The resulting yellow oil crystallized upon standing at 4°C overnight, and the product was recrystalized from diethyl ether-hexane. Yield 100%, mp= 64-65°C (lit mp = 65-67°C[15]).$^{1}$H NMR ($CDCl_3$): $\delta$ 1.21 (t, 6H, $CH_3$), 1.99 (s, 3H, $CH_3$), 2.18 (t, 2H, $CH_2$), 2.62 (t, 2H, $CH_2$), 3.60 (s, 3H, $CH_3$), 4.20 (q, 4H, $CH_2$), 6.74 (broad s, 1H, NH). $^{13}$C NMR ($CDCl_3$): $\delta$ 13.67 ($CH_3$), 22.91 ($CH_3$), 27.75 ($CH_2$), 28.79 ($CH_2$), 51.70 ($CH_3$), 62.68 ($CH_2$), 64.56 (C), 167.73 (CO), 169.20 (CO), 172.72 (CO).

DL-[2-$^{13}$C]-Glutamic Acid Hydrochloride (4). N-Acetyl-2-Carbethoxy glutamate 1-ethyl 3-methyl ester (3) (151 mg, 0.5 mmol) was heated

under reflux for five hours in 3 ml of 6N HCl. The solution was evaporated in vacuo and the residue recrystalized from alcohol-diether ether. Yield 87 mg (95%). $^{1}H$ NMR($D_2O$): δ 1.98 (m, 2H, $CH_2$), 2.39 (m, 2H, $CH_2$), 3.65 (t, 1H, CH); $^{13}C$ NMR(D2O): δ 25.42 ($CH_2$), 29.94 ($CH_2$), 53.75 (CH), 173.74 (CO), 176.99 (CO).

DL-[2-$^{13}$C]-Glutamic Acid (5). Glutamic acid hydrochloride (4) (87 mg, 0.475 mmol) was dissolved in hot ethanol and one equivalent of pyridine was added, causing an immediate separation of the free amino acid. Yield ca 70 mg (100%) mp. 195-196°C (lit. mp. = 198°C).

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