Synthesis of the Neurotransmitter 4-Aminobutanoic Acid (GABA) from Diethyl Cyanomalonate

Matthew C. Cook, Ross D. Witherell and Robert L. White*

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4J3, Canada

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Abstract: GABA was synthesized by deethoxycarbonylation, ester hydrolysis and nitrile reduction of a highly functionalized intermediate obtained by alkylation of diethyl cyanomalonate with ethyl bromoacetate. By judicious employment of D_2O or $NaBD_4$ in one of the three functional group transformation steps, deuterium was selectively introduced into each of the three possible sites in GABA.

Keywords: GABA, Diethyl cyanomalonate, Neurotransmitter, Deuterium labeling, Deethoxycarbonylation.

INTRODUCTION

4-Aminobutanoic acid (γ-aminobutyric acid, GABA, **1**), the most widespread neurotransmitter in mammals, is responsible for synaptic inhibition in the central nervous system [1], thereby playing an important role in neurological function and disease. Hypo- or hyperactivities of the GABAergic neurotransmitter system lead to epilepsy, schizophrenia and other neurological conditions [2,3], and drugs affecting GABAergic activity are clinically useful for the treatment of neurological illnesses [3,4]. As a result, the detection and quantification of GABA (**1**) continues to be an important focus [5], and deuterium-labeled GABA is particularly sought after as an internal standard for the mass spectral analysis of GABA in human plasma and cerebrospinal fluid [5-7]. Isotopically labeled GABA has been used in drug synthesis (e.g., progabide [8]) and, currently, there is interest in improving drug pharmacokinetics by selective deuterium substitution [9]. Labeled GABA also has been employed as a substrate for NMR molecular dynamics [10], and investigations of the stereochemistry [11] and mechanism [12] of enzyme-catalyzed reactions.

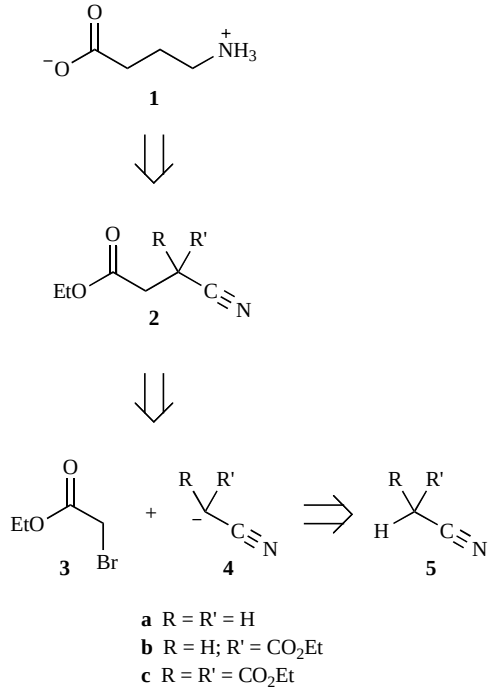
Previously, isotopically labeled versions of GABA have been synthesized *via* multi-step reaction sequences. Typically, [2-$^2H_2$]GABA was obtained from unlabeled GABA after several successive exchanges in DCl/D_2O [7,8,10,12] or $NaOD/D_2O$ [6]. Similarly, successive exchange reactions on succinimide (pyridine/D_2O) [13], followed by imide reduction and lactam hydrolysis in DCl/D_2O, provided GABA deuterated at C-2 and C-3. Alternatively, hydrogenation of a triple bond in a synthetic precursor has been used to place tritium at C-2,3 of GABA [14]. Selective deuteration at C-4 of GABA has been achieved by $LiAlD_4$ reduction of ester [8,12] and imide [13] groups in synthetic precursors. Enzyme-catalyzed reactions, placing one deuterium at C-3 [15] or C-4 [11], have introduced chirality, producing single isomers of monodeuterated GABA.

While several multi-step sequences and exchange processes are available, the route devised in the present investigation offers greater flexibility, providing a single precursor into which deuterium can be introduced specifically into any combination of three different locations, including C-3, a position less accessible from known routes.

RESULTS AND DISCUSSION

Synthesis of GABA

From a retrosynthetic perspective (Scheme **1**), dissection of the central carbon-carbon bond in GABA (**1**) leads to readily available electrophilic and nucleophilic synthons, such as a monohalogenated acetate ester (e.g., **3**) and the anion of acetonitrile (**4a**) or equivalent (**4b**, **4c**). The ethoxycarbonyl group introduced by using a stabilized anion as the nucleophile can be removed selectively from the intermediates **2b** and **2c** under neutral conditions *via* the Krapcho reaction [16]; subsequent ester hydrolysis and nitrile reduction furnishes GABA.



a R = R' = H
b R = H; R' = CO_2Et
c R = R' = CO_2Et

Scheme 1.

For the route outlined above, carbon isotopes would be introduced most easily from commercially available ^{13}C-labeled ethyl bromoacetate, whereas the use of deuterated reagents during the conversion of the highly functionalized intermediate (**2**) to GABA (**1**) would likely lead to the introduction of hydrogen isotopes at specific locations in GABA. For example, the use of D_2O in the Krapcho deethoxycarbonylation of diethyl ethylmalonate provided ethyl [2-$^2H_2$]butanoate containing 80-90% deuterium [17].

Initially, the carbon-carbon bond forming step was attempted in acetone using the K_2CO_3-mediated reaction of methyl bromoacetate (**6**) with ethyl cyanoacetate (**5b**) (Scheme **2**), but an equimolar mixture of dimethyl 3-cyano-3-ethoxycarbonylpentanedioate (**7**) and unreacted ethyl cyanoacetate (**5b**) was isolated. Based on the initial electrophile, the dialkylation product **7** was obtained in nearly

*Address correspondence to this author at the Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4J3, Canada; Tel: 1-902-494-6403; Fax: 1-902-494-1310; E-mail: robert.white@dal.ca

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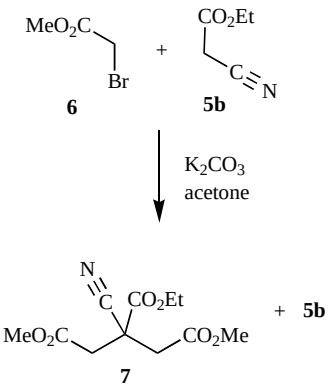
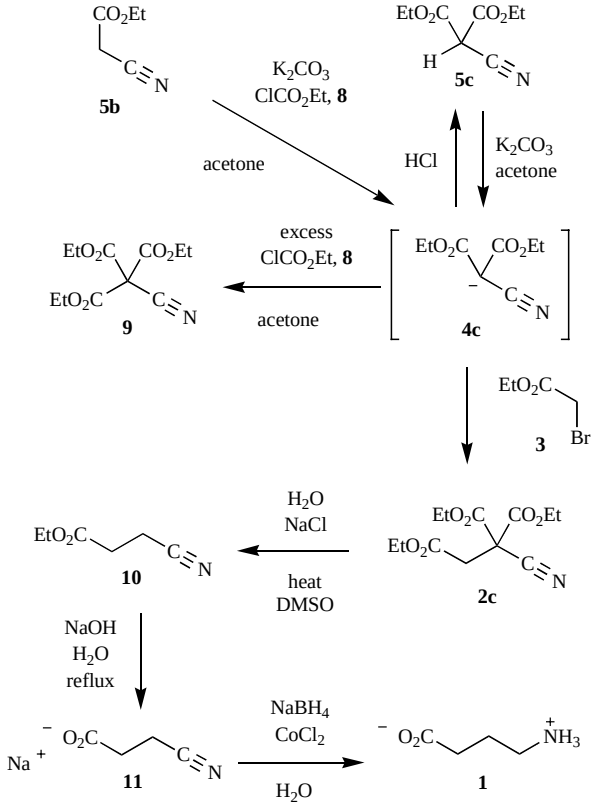
quantitative yield, reflecting the fact that the anion of the monoalkylated product is formed in preference to that of ethyl cyanoacetate and is readily alkylated. Dialkylation of ethyl cyanoacetate has been observed when the anion is generated from sodium hydride [18], but selective monoalkylation also has been reported [19].



Scheme 2.

To avoid similar dialkylation of diethyl malonate ($(EtO_2C)_2CH_2$), a third ethoxycarbonyl group was introduced to limit the reaction to the monoalkylation of a tertiary carbanion ($(EtO_2C)_3C^-$) [20]. Using this strategy and conditions adapted from literature procedures [21,22], the analogous trisubstituted substrate, diethyl cyanomalonate (**5c**), was prepared by acylation of ethyl cyanoacetate (**5b**) (Scheme **3**). Under optimized conditions (dry acetone, vigorous stirring and anhydrous, powdered K_2CO_3), reproducible yields (> 80% after distillation) were obtained.



Scheme 3.

The presence of two components in purified diethyl cyanomalonate (**5c**) was supported by spectral evidence. Two nitrile bands (2262, 2225 cm^{-1}) were apparent in the IR spectrum (neat), while the ^1H and ^{13}C NMR spectra of **5c** in $CDCl_3$ each displayed prominent resonances accompanied by an equal number of lower intensity resonances (ca. 10:1 ratio). After addition of triethylamine to the NMR solution, only one set of ethyl ester resonances (shifted slightly to lower frequency) was observed, suggesting the deprotonation of both components and the formation of anion **4c**, the conjugate base of **5c** (pK_a 1.3 [23]). The ^1H NMR spectrum of **5c** exhibited a very broad signal at δ 13.2, attributed to an enolic hydrogen in the minor component. Enolization of **5c** in acetonitrile was indicated in a study of proton transfer reactions [24] and is precedented by the high enol content documented for 20 structurally analogous cyanomalonamides [25].

Solvent had a large effect on the enol content of diethyl cyanomalonate (**5c**). Only one set of ethyl ester resonances was observed in the ^1H NMR spectra of **5c** recorded in CD_3CN and CD_3OD, and the measured UV extinction coefficient (log ε) was larger in hexane (3.23) than in acetonitrile (2.97). The larger proportion of enol observed for **5c** in nonpolar solvents is consistent with the relationship between enol content and solvent polarity documented for cyanomalonamides [25] and β-diketones [26]. The larger UV extinction coefficients measured for diethyl cyanomalonate (**5c**) in methanol (3.93) and water (4.28) were attributed to ionization in polar protic solvents, forming **4c**, the conjugate base of the strong carbon acid **5c** [23].

When more than two equivalents of ethyl chloroformate (**8**) were included in the acylation reaction mixture (Scheme **3**), a second product was detected by NMR spectroscopy. Repetition of the reaction using 10 equivalents of **8**, led to the isolation of the second product in high yield, and its identification as the cyano triester **9**. Diethyl cyanomalonate (**5c**), formed by the initial acylation, is readily deprotonated under basic conditions. The conjugate base **4c**, thus formed, undergoes acylation, to yield the cyano triester **9**, effectively demonstrating the nucleophilicity of **4c**. As well, several literature reports [22,27] of the alkylation of the diethyl cyanomalonate anion (**4c**) reinforce the inherent nucleophilicity of this stabilized anion.

Using the above K_2CO_3/acetone conditions, the diethyl cyanomalonate anion (**4c**) reacted with ethyl bromoacetate (**3**). Upon addition of a catalytic amount of KI, a higher yield of diethyl 2-cyano-2-ethoxycarbonylbutanedioate (**2c**) was obtained. In acetone, displacement of chloride by iodide is well known [28], suggesting the intermediacy of ethyl iodoacetate, an electrophile with a better leaving group.

For the Krapcho deethoxycarbonylation reaction [16] of **2c**, reaction mixtures in DMSO-d_6 were monitored using ^1H NMR spectroscopy to optimize reaction times and temperatures. Deethoxycarbonylation was complete after 16 h at 140 °C or after only 0.5 h at 180 °C, yielding ethyl 3-cyanopropanoate (**10**) and ethanol. Products requiring no further purification were isolated in good yield (87% at 140 °C and 71% at 180 °C) from the DMSO reaction mixtures, simply by addition of water and extraction into ether. If necessary, residual DMSO was removed by back extraction into water.

Ester hydrolysis was accomplished by heating ethyl 3-cyanopropanoate (**10**) in aqueous NaOH at reflux for 1.5 h. Nitrile hydrolysis was minimized by using only 10% excess base, and nitrile reduction [29] was achieved by carefully adding $NaBH_4$ and $CoCl_2$ directly to the aqueous reaction mixture after ester hydrolysis. Following cation-exchange chromatography, GABA (**1**) was obtained as a colorless powder in an overall yield of 32% from ethyl cyanoacetate (**5b**).

Deuterium Labeling

In principle, Krapcho deethoxycarbonylation [17] of **2c** in the presence of D_2O would form ethyl [3,3-$^2H_2$]-3-cyanopropanoate

upon loss of the geminal ester groups from the quaternary carbon. Under conditions (140 °C, 16 h) similar to those employed in the synthetic sequence, deethoxycarbonylation of **2c** yielded deuterated ethyl 3-cyanopropanoate; the reduced intensity of the AA'BB' protons (δ 2.72-2.62) relative to the ethyl CH_2 signal (δ 4.20) indicated about 80% deuteration at C-2 and C-3. In the ^{13}C NMR spectrum of the isolated product, the signal at δ 13.0 (C-3, α to the nitrile) was absent, and the intensity of the signal at δ 30.0 (C-2, α to the ester) was reduced significantly because of the relaxation, NOE and multiplicity effects of deuterium [30]. These observations are consistent with complete and partial (about 60%) deuteration at C-3 and C-2, respectively.

The introduction of deuterium during Krapcho deethoxycarbonylation of **2c** was evaluated in a series of experiments monitored by ^1H NMR spectroscopy. Deuterium incorporation at 140 °C was not influenced by the use of DMSO-d_6 and/or a longer reaction time (24 h). At a higher temperature (180 °C) in DMSO-d_6, similar levels of deuterium incorporation were observed at 0.5 and 2 h; at 17 h 90% deuteration was obtained, but the isolated yield of deuterated product was only 43%.

The lack of substantially higher deuterium incorporation at longer reaction times (i.e., > 16 h at 140 °C and > 0.5 h at 180 °C) suggests that exchange does not occur readily after deethoxycarbonylation is complete. Also, no exchange was detected when **10**, the product of deethoxycarbonylation, was subjected to Krapcho reaction conditions (D_2O/DMSO-d_6, 140 °C, 18 h). Together these results strongly suggest that exchange leading to deuterium incorporation at C-2 of **10** occurs prior to deethoxycarbonylation. On the other hand, the corresponding protons in the starting cyano triester **2c** and the monodeethoxycarbonylated intermediate **2b** are more acidic because of the additional electron-withdrawing inductive effect provided by the β-ester substituents on C-3. Thus, at the temperatures investigated, exchange and deethoxycarbonylation occur as competing reactions. The high level of deuterium incorporation suggests that exchange in **2c** is more rapid than deethoxycarbonylation.

When C-2,3-deuterated ethyl 3-cyanopropanoate was subjected to ester hydrolysis in base and subsequent nitrile reduction, the resulting GABA contained deuterium (4% d_3, 60% d_2, 30% d_1 and 6% d_0 by ESI-MS) located predominantly at C-2 (^1H NMR). The significant exchange adjacent to the cyano group in deuterated 3-cyanopropanoate most likely occurred during base-promoted ester hydrolysis, a result noted previously under basic conditions [31].

With this observation, the ester hydrolysis of **10** was carried out in D_2O; subsequent nitrile reduction yielded GABA with a high level of deuterium (97%). The ^1H NMR spectrum exhibited two broad singlets at δ 2.96 and 2.25, consistent with deuterium at C-3. While the ESI mass spectrum confirmed the high deuterium content, it also revealed about 20% incorporation of a third deuterium, presumably by exchange of protons adjacent to the ester carbonyl group prior to hydrolysis.

To place deuterium at C-4 in GABA, the third possible position, $NaBD_4$ was used in the nitrile reduction step; product containing about 70% deuterium at C-4 was isolated. The low incorporation of deuterium is consistent with partial exchange of deuterium during the initial reaction of borodeuteride with $CoCl_2$ and water [29], and preferential transfer of hydride to the nitrile. The latter is indicated by the kinetic isotope effect of 3.3 observed for the reduction of benzonitrile [29].

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were acquired on a Bruker/Tecmag AC-250 MHz NMR spectrometer operating at 250.13 and 62.9 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS, δ = 0) or residual solvent signal (D_2O, δ 4.79). All coupling constants (*J*) are reported in Hz; splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Ultraviolet (UV) and infrared (IR) spectra were recorded on Hewlett-Packard 8452A UV-Vis and Bomem Michelson spectrometers, respectively. Electron ionization mass spectra (EI-MS) and accurate mass determinations (HRMS) were obtained on a CEC-110B sector instrument, and electrospray ionization mass spectra (ESI-MS) were collected on a Finnigan LCQ Duo ion trap using flow injection [32].

Dimethyl 3-Cyano-3-ethoxycarbonylpentanedioate (7)

A mixture of ethyl cyanoacetate (**5b**, 0.74 g, 6.5 mmol), methyl bromoacetate (**6**, 1.0 g, 6.5 mmol) and anhydrous K_2CO_3 (1.8 g, 13 mmol) in acetone (12 mL) was stirred in the dark at -18 °C for 19 h. Ether (100 mL) was added, and the solution was dried (anhydrous $MgSO_4$). Rotary evaporation afforded a colorless oil (1.2 g), composed of equimolar amounts of starting material (**5b**) and dialkylated product (**7**).

5b: ^1H NMR ($CDCl_3$) δ: 4.27 (2H, q, *J* = 7.1), 3.50 (2H, s), 1.33 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ: 163.0, 113.3, 62.9, 24.7, 13.8.

7: (98% from **6**). ^1H NMR ($CDCl_3$) δ: 4.33 (2H, q, *J* = 7.1), 3.75 (6H, s), 3.10 and 3.07 (4H, AB, *J* = 16.9), 1.35 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ: 168.7, 166.9, 117.4, 63.6, 52.4, 42.2, 39.3, 13.9.

Diethyl Cyanomalonate (5c)

Ethyl cyanoacetate (**5b**, 5.70 mL, 54 mmol) was added to a stirred mixture of K_2CO_3 (24 g, 190 mmol, pre-treated by heating at 130 °C for 16 h) and acetone (70 mL, dried over K_2CO_3 for 16 h). After 5 min, ethyl chloroformate (**8**, 10.2 mL, 108 mmol) was added and the reaction mixture was heated at reflux for 6 h. The reaction was allowed to cool to room temperature, water (100 mL) was added and the acetone was removed *in vacuo*. Additional water (50 mL) was added and the basic solution was extracted with dichloromethane (3 x 20 mL). The aqueous layer was acidified to pH < 0 with concentrated HCl (30 mL) and extracted with diethyl ether (4 x 25 mL). The ether layers were combined, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to yield a crude orange oil (8.86 g). The crude products from three replicate reactions (8.72 g, 7.74 g and 8.86 g) were combined (25.32 g) and 22.5 g of the product was vacuum distilled (b.p. 115-116 °C) yielding **5c** as a clear, colorless oil (20.5 g, 81%). IR (neat) cm^{-1}: 2262, 2225, 1751; UV (solvent λ_{max} (log ε)): acetonitrile 248 nm (2.97), dioxane 252 nm (2.42), hexane 256 nm (3.23), methanol 246 nm (3.93), water 246 nm (4.28); Major tautomer: ^1H NMR ($CDCl_3$) δ: 4.59 (s), 4.34 (q, *J* = 7.3), 1.34 (t, *J* = 7.3); ^{13}C NMR ($CDCl_3$) δ: 160.6, 111.5, 63.8, 44.5, 13.5. Minor tautomer: ^1H NMR ($CDCl_3$) δ: 13.2 (br s), 4.42 (q, *J* = 7.3), 1.40 (t, *J* = 7.3); ^{13}C NMR ($CDCl_3$) δ: 176.0, 113.6, 64.2, 60.3, 13.9. ^1H NMR (CD_3CN) δ: 4.81 (0.7H, s), 4.30 (4H, q, *J* = 7), 1.29 (6H, t, *J* = 7); ^1H NMR (CD_3OD) δ: 4.73 (0.4H, s), 4.31 (4H, q, *J* = 7.3), 1.31 (6H, t, *J* = 7.3); ESI-MS (negative mode) *m*/*z* 184(100%) $[M - H]^-$, 66(12): CID (29%) of *m*/*z* 184: *m*/*z* 156(10), 66(100); HRMS (70 eV) calculated for $C_8H_{11}NO_4$ = 185.0688 amu, found = 185.0696 ± 0.0008 amu.

Diethyl 2-Cyano-2-ethoxycarbonylpropanedioate (9)

Anhydrous K_2CO_3 (4.84 g, 35 mmol, pre-treated by heating at 130 °C for 16 h) was added to acetone (15 mL, dried over K_2CO_3 for 16 h) and the mixture was stirred for 15 min. Ethyl cyanoacetate (**5b**, 1.06 mL, 10 mmol) was added, followed after 5 min by ethyl chloroformate (**8**, 9.56 mL, 100 mmol). The reaction mixture was refluxed with stirring for 6 h, allowed to cool, water (100 mL) was

added and solution was acidified with concentrated HCl (10 mL) to a pH < 0. The product was extracted with diethyl ether (3 x 25 mL). The ether layers were combined and, dried over anhydrous $MgSO_4$; the solvent was removed *in vacuo*. A portion (1.51 g) of the crude product (2.51 g, 98%) was dissolved in ether (50 mL) and washed with saturated sodium bicarbonate (3 x 25 mL). The ether layer was dried over anhydrous $MgSO_4$ and, upon evaporation *in vacuo*, **9** was obtained as colorless oil (1.06 g, 70%). IR (neat) cm^{-1}: 2267, 1778, 1753; ^1H NMR ($CDCl_3$) δ: 4.40 (2H, q, *J* = 7.1), 1.37 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ: 159.6, 111.3, 64.9, 61.7, 13.8; EI-MS (70 eV): 257(10%) [$M^{+\bullet}$], 185(9), 156(10), 139(14), 128(18), 43(15), 28(100); HRMS (70 eV) calculated for $C_{11}H_{15}NO_6$ = 257.0899 amu, found = 257.0881 ± 0.0008 amu.

Diethyl 2-Cyano-2-ethoxycarbonylbutanedioate (2c)

A mixture of diethyl cyanomalonate (**5c**, 7.15 g, 38.6 mmol) and anhydrous K_2CO_3 (2.69 g, 19.5 mmol) in acetone (50 mL) was stirred at reflux for 20 min. Ethyl bromoacetate (**3**, 6.46 g, 38.6 mmol) and KI (0.32 g, 1.9 mmol) were added, and the stirred mixture was heated at reflux for 14 h. The reaction mixture was mixed with chloroform (300 mL); the mixture was dried (anhydrous $CaCl_2$), filtered, and solvent was removed by rotary evaporation. The residue was dissolved in ether (130 mL) and the solution was washed with aqueous $NaHSO_3$ (5 x 100 mL). The ether layer was dried (anhydrous $CaCl_2$), decolorized over activated carbon, and rotary evaporated to yield **2c** as a pale yellow oil (9.56 g, 91%). IR (neat) cm^{-1}: 2256, 1746; ^1H NMR ($CDCl_3$) δ: 4.35 (4H, q, *J* = 7.3), 4.22 (2H, q, *J* = 7.3), 3.27 (2H, s), 1.35 (6H, t, *J* = 7.3), 1.29 (3H, t, *J* = 7.3); ^{13}C NMR ($CDCl_3$) δ: 167.9, 162.8, 114.6, 64.3, 61.8, 51.7, 38.5, 13.8; EI-MS (70 eV) 271(1%) [$M^{+\bullet}$], 197(2), 169(2), 225(3), 152(10), 124(12), 97(23), 43(18), 28(100); HRMS (70 eV): calculated for $C_{12}H_{17}NO_6$ = 271.1056 amu, found = 271.1043 ± 0.0008 amu.

Ethyl 3-Cyanopropanoate (10)

Duplicate reaction mixtures, each consisting of a solution of diethyl 2-cyano-2-ethoxycarbonylbutanedioate (**2c**, 1.36 g, 5.0 mmol), water (2.70 mL, 150 mmol) and sodium chloride (0.073 g, 1.2 mmol) in DMSO (5 mL), were heated at 140 °C for 18 h. Upon cooling to room temperature, the reaction mixtures were combined; water was added (100 mL), and the product was extracted into ether (4 x 50 mL). The combined ether layers were dried over $MgSO_4$ and the solvent was removed *in vacuo*, giving **10** as an oil (1.10 g, 87%). ^1H NMR ($CDCl_3$) δ: 4.20 (2H, q, *J* = 7.1), 2.72-2.62 (4H, m), 1.29 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ: 170.1, 118.6, 61.4, 30.0, 14.1, 13.0; EI-MS (70 eV) 128(2%) [M + H]$^+$ [33], 127(5) [$M^{+\bullet}$], 100(99), 82(91), 56(39), 55(100).

GABA (1)

Ethyl 3-cyanopropanoate (**10**, 0.258 g, 2.0 mmol) and NaOH (1.27 g, 3.2 mmol) were heated at reflux in water (15 mL) for 1.5 h. Upon cooling to room temperature, $CoCl_2{\cdot}6H_2O$ (0.966 g, 4.1 mmol) was added to the reaction mixture, followed by the addition of solid $NaBH_4$ (0.768 g, 20 mmol) in small portions. After stirring at room temperature for 4 h, water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion-exchange column (30 x 2 cm, H^+ form). The column was eluted with 0.5 M aqueous ammonia and 200-mL fractions were collected. Fractions 3–8 were concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried, giving GABA as a white powder (0.10 g, 50%). ^1H NMR (D_2O) δ: 3.00 (2H, t, *J* = 7.6), 2.29 (2H, t, *J* = 7.3), 1.89 (2H, quintet, *J* = 7.3); ^{13}C NMR (D_2O) δ: 181.8, 39.5, 34.6, 24.1; ESI-MS (positive mode) *m/z* 105(5), 104(100%) [M + H]$^+$, 87(13), 86(13): CID (17%) of *m/z* 104: *m/z* 87(100), 86(56).

Deuterated GABA

In trial experiments to assess deuterium incorporation during Krapcho deethoxycarbonylation, diethyl 2-cyano-2-ethoxycarbonylbutanedioate (**2c**, 0.75 mmol), NaCl (0.19 mmol) and D_2O (22.5 mmol) in DMSO (or DMSO-d_6) were heated in a capped tube. Product was isolated by extraction into ether (4 x 20 mL) after addition of water (15 mL). Deuterium incorporation was calculated from the ^1H NMR spectra of isolated products.

Ethyl [2,2,3,3-$^2H_4$]-3-Cyanopropanoate

Diethyl 2-cyano-2-ethoxycarbonylbutanedioate (**2c**, 0.54 g, 2.0 mmol), D_2O (1.08 mL, 60 mmol) and sodium chloride (0.029 g, 0.50 mmol) were heated in DMSO-d_6 (2 mL) at 180 °C for 14 h. The reaction mixture was allowed to cool to room temperature, saturated brine was added (50 mL) and the aqueous layer was extracted with ether (2 x 25 mL). The ether layers were combined and washed with brine (25 mL), which was back extracted with ether (25 mL). The ether layers were combined, treated with charcoal for 1 h and filtered through Celite. After drying over $MgSO_4$, the solvent was removed *in vacuo* giving ethyl [2,2,3,3-$^2H_4$]-3-cyanopropanoate as an oil (0.18 g, 62%). ^1H NMR ($CDCl_3$) δ: 4.20 (2H, q, *J* = 7.1), 2.67 (0.58H, m), 1.29 (3H, t, *J* = 7.0); ^{13}C NMR ($CDCl_3$) δ: 170.1, 118.7, 61.5, 14.2.

[2,2-$^2H_2$]GABA

Ethyl [2,2,3,3-$^2H_4$]-3-cyanopropanoate (**3-53**, 0.18 g, 1.4 mmol) and NaOH (0.120 g, 3.01 mmol) in water (15 mL) were heated at reflux for 1.5 h. Reduction with $CoCl_2{\cdot}6H_2O$ (0.654 g, 2.75 mmol) and $NaBH_4$ (0.52 g, 14 mmol) followed by ion-exchange chromatography gave [2,2-$^2H_2$]GABA as a white powder (0.07 g, 48%). ^1H NMR (D_2O) δ: 3.00 (2H, t, *J* = 7.3), 2.28 (0.4H, m), 1.88 (2H, br t, *J* = 7); ESI-MS [M + H]$^+$: *m/z* 106(49%) 105 (24), 104(4).

[3,3-$^2H_2$]GABA

As described above, ethyl 3-cyanopropanoate (**10**, 0.635 g, 5.0 mmol) was hydrolyzed in D_2O (15 mL) containing NaOH (0.228 g, 5.7 mmol) and reduced using $CoCl_2{\cdot}6H_2O$ (2.38 g, 10.0 mmol) and $NaBH_4$ (1.89 g, 50 mmol). Ion-exchange chromatography yielded [3,3-$^2H_2$]GABA as a white powder (0.24 g, 46%). ^1H NMR (D_2O) δ: 2.96 (2H, br s), 2.25 (2H, br s); ESI-MS [M + H]$^+$: *m/z* 108(4%), 107(28), 106(100), 105(8), 104(3).

[4,4-$^2H_2$]GABA

As described above, ethyl 3-cyanopropanoate (**10**, 0.255 g, 2.0 mmol) was hydrolyzed in aqueous NaOH (0.106 g, 2.6 mmol; 15 mL H_2O) and reduced using $CoCl_2{\cdot}6H_2O$ (0.952 g, 4.0 mmol) and $NaBD_4$ (0.837 g, 20 mmol). Ion-exchange chromatography yielded [4,4-$^2H_2$]GABA as a white powder (0.18 g, 87%). ^1H NMR (D_2O) δ: 2.95 (0.5H, m), 2.27 (2H, t, *J* = 7.0), 1.85 (2H, m); ESI-MS [M + H]$^+$: *m/z* 107(8%), 106(100), 105(85), 104(21).

CONCLUSIONS

GABA (**1**) was synthesized in five steps, consisting of the introduction of a blocking group, carbon-carbon bond formation, and three functional group modifications. The utility of the reaction sequence was demonstrated by the preparation of deuterated GABA. The isotopic label was introduced predominantly at C-2, C-

3 or C-4 by using an appropriate deuterated reagent in one of the three functional group transformation steps.

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