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Research Article

Novel synthesis of [1-^{11}C]γ-vinyl-γ-aminobutyric acid ([1–^{11}C]GVG) for pharmacokinetic studies of addiction treatment

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Summary

γ-Vinyl-γ-aminobutyric acid (GVG, Vigabatrin®), a suicide inhibitor of GABA-transaminase (GABA-T), has been suggested as a new drug for the treatment of substance abuse. In order to better understand its pharmacokinetics and potential side effects, we have developed a radiosynthesis of carbon-11 ($t_{1/2} = 20$ min) labeled GVG for positron emission tomographic (PET) studies. We report here a novel synthetic strategy to prepare the precursor and to efficiently label GVG with C-11. 5-Bromo-3-(carbobenzyloxy)amino-1-pentene was synthesized in five steps from homoserine lactone, including reduction and methylenation. This was used in a one-pot, two-step radiosynthesis. Displacement of bromide with no-carrier-added [^{11}C]cyanide followed by acid hydrolysis afforded [1-^{11}C]GVG with decay corrected radiochemical yields of 27 $\pm$ 9% ($n = 6$, not optimized) with respect to [^{11}C]cyanide in a synthesis time of 45 min. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: GVG; vinyl-GABA; Vigabatrin; GABA-T; C-11 positron emission tomography (PET)

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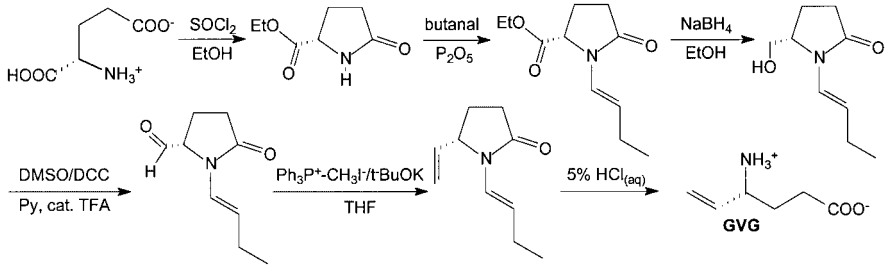
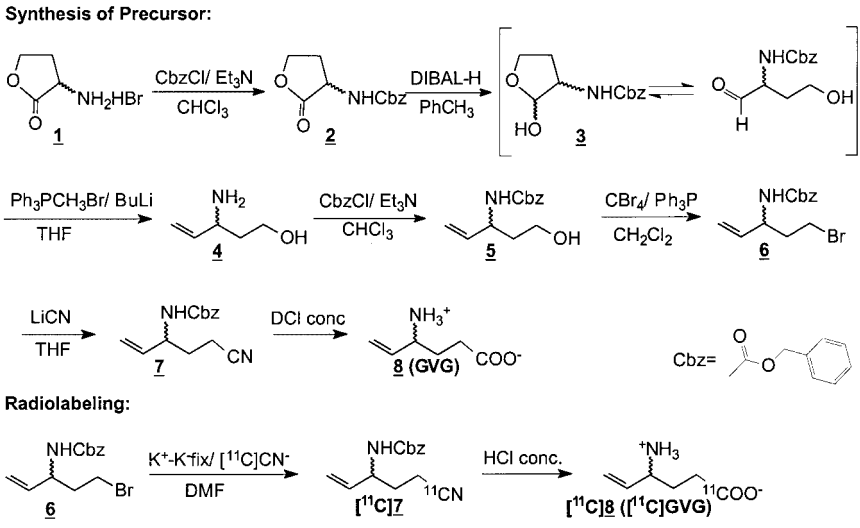
Introduction

γ-Vinyl-γ-aminobutyric acid (γ-vinyl-GABA, GVG, Vigabatrin®) is a suicide inhibitor of GABA transaminase (GABA-T).[1,2] Inhibition of GABA-T by GVG increases the concentration of γ-aminobutyric acid (GABA) in CNS[3] which accounts for the antiepileptic effect of GVG.[4,5] We have shown that GVG inhibits the effects of heroin, cocaine, methamphetamine, nicotine and alcohol both biochemically (attenuation of drug-induced neostriatal synaptic dopamine increase) and behaviorally in rodents and primates. These data suggest that the use of GVG may represent a new strategy for the treatment of substance abuse.[6–9] GVG has been used as an antiepileptic drug in several countries. However, side effects of GVG, such as visual field constriction,[10] place a sense of urgency on better understanding the mechanisms underlying its efficacy in addiction treatment and those associated with vision impairment. Therefore, we developed a radiosynthesis of [^{11}C]GVG ($t_{1/2}$ for C-11 = 20 min) for PET studies in order to measure its pharmacokinetics in the brain and in the body using PET.

Several synthetic routes for the preparation of GVG including radiolabeling with long-lived ^{14}C ($t_{1/2}$ for C-14 = 5730 year) have been published; however, they are not suitable for C-11 radiosynthesis.[11–15] For example, a procedure to prepare the key 2-oxopyrrolidine-5-carboxaldehyde precursor that would in principle allow the incorporation of C-11 by employing the Wittig condensation with [^{11}C]methyltriphenylphosphonium iodide (Scheme 1) was not straightforward.[14–16] We experienced this in our initial trials, and other researchers (authors in References[14,15]) made similar observations. Difficulties encountered during the isolation of the aldehyde precursor, its instability for storage, as well as the subsequent time-consuming Wittig reaction prompted us to pursue another synthetic pathway.



Scheme 1. Previously reported synthesis of GVG via Wittig reaction[14]



Scheme 2. Synthesis of [^{11}C]GVG via bromide via [^{11}C]cyanide displacement

We report here a novel synthetic route to prepare the precursor (from homoserine lactone) and to efficiently label GVG with C-11 (Scheme 2). The entire synthetic procedure should be extendable to the labeling of the pharmacologically active S- form of GVG when S-homoserine lactone is used as the starting material.

Results and discussion

Five-Bromo-3-(carbobenzyloxy)amino-1-pentene (**6**) was synthesized in five steps from homoserine lactone (Scheme 2). Homoserine lactone was chosen as the starting material in anticipation of developing a synthetic procedure for both racemic mixture and individual enantiomers of [^{11}C]GVG. The amino group of homoserine lactone **1** was protected as a benzyl carbamate (Cbz-) **2**. Compound **2** was obtained in low yield when aqueous NaOH was used as a base (34%); however, a significant improvement in yield (97%) was achieved when Et_3N was used as the base in nonaqueous conditions. Reduction of **2** with diisobutylaluminum hydride (DIBAL-H) afforded 2-(carbobenzyloxy)amino-4-butyrolactol (**3**) in 72% yield.[17,18] Efforts were made to optimize reaction conditions by modifying the ratios of DIBAL-H to **2**, reaction temperature and reaction time. ^{1}H NMR of the product (**3**) was

rather complex, most likely due to a mixture of diastereomers arising from lactol–aldehyde equilibrium (although an aldehyde peak was always present, as expected). Compound **3** was found to be stable in solid state and neutral organic solvents. It appeared to be less stable in acidic solution. Wittig methylenation of **3** with DMSO–NaH–Ph_3PCH_3Br[19,20] led to unidentifiable products; however, THF–BuLi–Ph_3PCH_3Br[21] or THF–$NaNH_2$-Ph_3PCH_3Br[22] afforded a crude mixture containing-3-amino-4-penten-1-ol (**4**).

In order to facilitate product isolation, this crude mixture was further treated with benzyl chloroformate to convert **4** to **5** (15% yield, not optimized). We attribute the low yields of the Wittig reaction to the cleavage of the Cbz- protecting group leading to unknown side reactions. Ideally, a protecting group should survive all the chemical manipulations prior to its deprotection, it should bear a chromophore for easy detection and be readily removed at the end of synthesis. We have tested a few other protecting groups, and the choice of benzyl chloroformate to protect amino group, though not ideal, was the best so far.[23,24]

Bromination of **5** with CBr_4/PPh_3 led to the desired precursor 5-bromo-3-(carbobenzyloxy)amino-1-pentene (**6**) in 89% yield. Kinetic studies of the cyanation of **6** showed that the reaction went to near 50% completion in DMF at an equimolar ratio of **6** and LiCN in 10 min at 110°C. The reaction was complete without the formation of noticeable byproduct in THF, even with excess LiCN under extended refluxing. No desired product was found when KCN was used. Solubility of metal cyanides might be a critical factor influencing the cyanation.[25,26] DMSO, MeOH, EtOH and CH_3CN were found to be poor solvents for rapid synthesis. Reaction in DMSO lead to formation of significant quantities of byproducts, while the rest of the solvents gave unacceptable product yields ($<5\%$).

The isolation of **5** as the major product**,** when the reaction was carried out in KCN/DMSO at 90°C for 5 h, suggests that this displacement reaction might be sensitive to the presence of H_2O. 4-(Carbobenzyloxy)-amino-5-hexenenitrile (**7**) was isolated by treating compound **6** with excess LiCN for 2 h in refluxing THF. Compound **7** afforded GVG (**8**) as essentially the only product after being heated in concentrated DCl (deuterated HCl, for the purpose of monitoring reaction via NMR) for 20 min. The alkene of GVG appeared to be unreactive toward concentrated DCl at 100°C. Acrylamide and allyl cyanide underwent partial hydrochlorination under the similar conditions.

Gas phase [^{11}C]cyanide was produced from a [^{11}C]CO_2 based on a procedure described previously; it was then delivered to a hot cell.[27–29] It requires 15 min for the radiosynthesis of [^{11}C]cyanide after EOB. Initially, [^{11}C]cyanide was trapped in an aqueous solution of LiOH (0.05 M, 0.2 ml). Water was removed under reduced pressure as an azeotrope along with CH_3CN. [^{11}C]4-(Carbobenzyloxy)amino-5-hexenenitrile ([^{11}C]**7**) was rapidly generated (10 min) in DMF at 110°C. However, the radiochemical yields were unreliable and depended on whether or not the reaction was carried out under anhydrous conditions. [^{11}C]Cyanide trapping was eventually achieved at −5°C (ethanol–ice bath) with a solid mixture of Kryptofix and K_2CO_3 without using a conventional aqueous trapping procedure.[29] The efficiency for the "dry" trapping procedure was $84 \pm 10\%$ ($n = 6$, the error is a standard deviation). At this temperature, most of the unreacted NH_3 from the system that may interfere with the synthesis would not be trapped.[30] This procedure would be advantageous to any moisture sensitive radiosynthetic steps, as it was the case for our displacement reaction. When a conventional aqueous trapping procedure was used, any trace amount of water left, even after prolonged heating, resulted in either no reaction or extremely low yields for displacement. Furthermore, the use of a column filled with P_2O_5 on silica support (Sicapent®) could reduce the excess amount of ammonia in the gas stream from the cyanide production system. However, directly trapping [^{11}C]cyanide in the reaction solvent (DMF), instead of dry trapping, would result in low radiochemical yields of [^{11}C]**7**, probably due to inhibition of the reaction by ammonia that transferred from the cyanide system and broke through the Sicapent trap.[31]

Hydrolysis of [^{11}C]**7** in a procedure similar to that for the synthesis of GVG afforded [1-^{11}C]GVG ([^{11}C]**8**) quantitatively in 10 min. The resulting mixture was neutralized with aqueous $(NH_4)_2HPO_4$ and injected directly onto an HPLC column. This procedure requires less time and is much more efficient than evaporation of HCl prior to HPLC purification.[28,32] Because of the high solubility of $(NH_4)_2HPO_4$ in water, as much as 0.5 ml of the 12 M HCl can be neutralized by a small volume of $(NH_4)_2HPO_4$ ($\sim$1 ml). The peak resolution on semipreparative HPLC used for the radiotracer purification was not affected. Compared to alternative quenching agents such as $(NH_4)_2CO_3$, the use of $(NH_4)_2HPO_4$ is superior since it does not generate gas bubbles leading to poor HPLC separation. Though the post-column collected fraction of [^{11}C]GVG could be directly injected into baboon (phosphate buffer

solution), the eluent was evaporated in order to diminish the UV-adsorbing volatile impurities in the solution.

This two-step, one-pot radiosynthesis—displacement of bromide with [^{11}C]cyanide followed by acid hydrolysis—afforded [^{11}C]GVG with decay corrected radiochemical yield with respect to [^{11}C]cyanide ($27 \pm 9\%$, $n = 6$, the error is the standard deviation, the synthesis was not optimized) and specific radioactivity > 0.5 Ci/μmol after 45 min. (Due to poor UV adsorption of GVG, more precise measurements could not be achieved. Typical specific radioactivity at the EOB in our previous radiosyntheses was 2.4–3.2 Ci/μmol.[34]) Radiochemical purity was $> 98\%$. This simple and straightforward radiosynthesis provides several advantages over the possible C-11 labeling of GVG via the Wittig reaction (Scheme 1). The starting bromide precursor **6** for our synthesis (Scheme 2) was very stable for storage, as compared to the extremely unstable aldehyde precursor used in the Wittig reaction. In addition to the difficulties encountered during the isolation of the aldehyde, it was claimed that the aldehyde was so unstable that it had to be used immediately after its preparation.[15] All of this precludes the use of the aldehyde as a precursor for our radiosynthesis. Our current one pot radiosynthesis involves simple cyanide displacement and hydrolysis, avoiding the extra labor and care needed for the Wittig reaction. The radiochemical yields were consistently high, more reliable and reproducible than that we have experienced with the Wittig reaction. The procedure is fully applicable to the synthesis of the routine preparations for human PET application.

Experimental

Chemicals were purchased from Aldrich Chemical Co. Authentic GVG was bought from Aventis Co. Anhydrous THF was prepared by distillation from sodium under nitrogen. Anhydrous DMF was purchased in Sure-seal® bottles. Techniques for preparing and handling moisture sensitive compounds were followed.[33] Macherey-Nagel polygram sil G/UV$_{254}$ plastic-back TLC plates (4×8 cm) were used. NMR spectra were recorded with a Brucker 400 MHz NMR spectrometer. Proton chemical shifts were reported in ppm relative to TMS (δ 0 ppm) or DSS (δ 0.015 ppm). Melting points were determined with a Fisher–Johns melting point apparatus. Mass spectra were recorded with a Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact

ionization at 70 eV. Microanalyses were conducted by Schwarzkopf Microanalytical Laboratory, Inc.

[^{11}C]Cyanide was produced from [^{11}C]CO_2 by an in-house made automatic synthesizer following a standard synthetic route[27–29] and passed through Sicapent® (EM Science, Gibbstown, NJ) before being trapped in the reaction vessel. Purification and analyses of radioactive mixtures were performed with HPLC pump, in-line UV detector (both from Knauer) and radioactivity detector. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. A short wavelength ultraviolet lamp, NaI well counter and automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) were used as UV- and radioactivity detectors for TLC analysis. The radiochemical purity was determined by radio TLC and analytical radio HPLC in the presence of authentic unlabeled compound as a carrier.

Attempt of [6-^{11}C]GVG synthesis (Scheme 1)

Attempt of GVG synthesis via Wittig reaction was performed according to the procedures described in References.[14,15]

*2-(Carbobenzyloxy)amino-4-butyrolactone (**2**, Scheme 2)*

Method A: Solution of Benzyl chloroformate (4.25 ml, 30 mmol) in aqueous NaOH (2 N, 17.5 ml) was slowly (1 h) added to a stirred solution of racemic homoserine lactone [(±)-α-amino-γ-butyrolactone hydrobromide (**1**, 4.55 g, 25 mmol) in aqueous NaOH (2.5 M, 10 ml) cooled in ice-water bath. The mixture was stirred for another 4 h, and the product was extracted with $CHCl_3$ (4 × 60 ml). The extracts were dried ($MgSO_4$), and the solvent was rotary-evaporated yielding a colorless oil that changed to a solid upon addition of diethyl ether (20 ml). The crystals were filtered and dried to give 2.0 g (34%) of **2** as a white solid.

Method B: Benzyl chloroformate (6.34 ml, 44 mmol) followed by triethylamine (6.0 ml, 43 mmol) were added dropwise to a stirred solution of **1** (6.08 g, 33 mmol) and triethylamine (5.5 ml, 39 mmol) in $CHCl_3$ (60 ml) cooled in ice-water bath. The solution was left overnight at room temperature while stirring. The mixture was acidified (12 M HCl) and washed with water (3 × 20 ml). After the aqueous phase was backwashed with diethyl ether (3 × 40 ml), organic fractions were combined, dried ($MgSO_4$) and rotary-evaporated leaving a crystalline

solid. The crystals were washed with 1 : 1 diethyl ether: hexane and vacuum-dried to give 7.65 g (97%) of **2** as white solids. Mp 105–109°C. ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 5 H, C_6H_5-), 5.34 (bs, 1 H, -N*H*-), 5.12 (s, 2 H, -CH_2Ph), 4.43 (m, 2 H, -CH_2O-), 4.25 (m, 1 H, -C*H*(NHZ)-), 2.76 (m. 1 H, -OCH_2CH_2-), 2.33 (m, 1 H, -OCH_2CH_2-). MS m/z 235 (M^+), 91 ($PhCH_2$), 108 ($PhCH_2OH$). Anal. Calcd. for $C_{12}H_{13}NO_4$ (%): C, 61.27; H, 5.57; N, 5.95. Found: C, 61.30; H, 5.92; N, 5.75.

*2-(Carbobenzyloxy)amino-4-butyrolactol (**3**)*

Compound **2** (2.0 g, 8.5 mmol) was added to a flame dried flask under N_2 atmosphere. After the introduction of toluene (anhydrous, 20 ml) the flask was cooled to −72°C (acetone–dry ice). Diisobutylaluminum hydride (DIBAL-H, 24 ml of 1.0 M solution in CH_2Cl_2) was added over 30 min while the reaction mixture was stirred. After stirring for another 6.5 h at −72°C, the solution was treated with ethyl acetate (50 ml), methanol (1 ml), water (5 ml) and excess of $NaHCO_3$ and stirred for 2 h at room temperature. The residue was filtered and washed with ethyl acetate. The washes were combined and concentrated to give an oil as a crude product. Chromatography on silica gel column using ethyl acetate followed by crystallization in diethyl ether–pentane produced 1.45 g (72%) **3** in a form of white crystals. Mp 80–82°C. ^{1}H NMR ($CDCl_3$) δ 9.58 (s, 1 H, -C*H*O), 7.31 (m, 5 H, -C_6H_5), 5.41 (m, 1 H, -N*H*Z), 5.28 (s, 1 H, -O*H*), 5.10 (m, 3 H, PhCH_2-, HOC*H*-), 4.14-3.81 (m, 3 H, =C*H*NZ, -CH_2OH), 2.27-2.03 (m. 1 H, -CH_2CH_2OH), 1.85-1.72 (m, 1 H, -CH_2CH_2OH). MS *m/z* 219 (M^+-H_2O), 208 (M^+ -$MeCH_2$), 146 (M^+-$PhCH_2$), 108 ($PhCH_2OH$), 91 ($PhCH_2$). Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.94; H, 6.59; N, 5.87.

*3-Amino-4-penten-1-ol (**4**)*

Synthesis of this compound was similar to reaction described in the synthesis of **5** (see next section). After methylenation of **3** (3.43 g, 14.5 mmol) using Wittig reaction, 0.124 g (4%) of **4** was isolated by silica gel flash chromatography using 5–50% methanol in ethyl acetate as eluent. ^{1}H NMR (CD_3OD) δ 5.82 (ddd, J = 5.8, 10.3, 22.7 Hz, 1 H, -C*H*=), 5.24 (d, J = 21.5 Hz, 1 H, CH_2=), 5.20 (d, J = 15.9 Hz, 1 H, CH_2=), 4.21 (m, 2 H, HOCH_2-), 4.03 (m, 1 H, -C*H*$(NH)_2$-), 2.09 (m, 1 H, -CH_2CH_2OH), 1.80 (m, 1 H, -CH_2CH_2OH). MS *m/z* 101 (M^+).

*3-(Carbobenzyloxy)amino-4-penten-1-ol (**5**)*

Methyltriphenylphosphonium bromide (6.79 g, 19 mmol) was added to a flame dried flask under the N_2 atmosphere. After addition of THF (25 ml) the flask was cooled in ice–water bath, and butyllithium (1.6 M in hexanes, 11.8 ml, 18.9 mmol) was added dropwise. The mixture was stirred for 30 min, and the solution of **3** (1.50 g, 6.3 mmol) in THF (8 ml) was added over 30 min. The mixture was stirred at room temperature for another 6.5 h. The flask was cooled in an ice-water bath, and water (0.4 ml) was added followed by the slow addition of the solution of benzyl chloroformate (2.7 ml, 18.9 mmol) and triethylamine (2.6 ml, 18.7 mmol) in THF (10 ml). After 16 h stirring at room temperature, the solid was filtered and washed with THF (2×20 ml). THF fractions were combined, acidified (HCl conc.) and washed with water saturated with NH_4Cl (3×10 ml). Combined aqueous phase was backwashed with diethyl ether (3×30 ml). Ether was added to THF fractions, and the combined organic phase was dried ($MgSO_4$) and rotary-evaporated. Chromatography of the residue on silica gel column eluted with 1:1 ethyl acetate:hexanes yielded 0.23 g (15%) of **5** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5 H, -C_6H_5), 5.83 (m, 1 H, -C*H*=), 5.31-5.08 (m, 4 H, CH_2=, -CH_2Ph), 4.37 (s, 1 H, -C*H*(NHZ)-), 4.70 (m, 2 H, HOCH_2-), 2.25 (bs, 1 H, -N*H*Z), 1.93 (m, 1 H, -CH_2CH$_2$OH), 1.55 (m, 1 H, -CH_2CH$_2$OH). MS *m*/*z* 235 (M^+),190 (M^+ -CH_2CH_2OH), 91 ($PhCH_2$).

*5-Bromo-3-(carbobenzyloxy)amino-1-pentene (**6**)*

A solution of triphenylphosphine (0.885 g, 3.37 mmol) in 10 ml of CH_2Cl_2 was added during 200 min to a stirred solution of **5** (0.159 g, 0.675 mmol) and carbon tetrabromide (1.12 g, 3.37 mmol) in CH_2Cl_2 (20 ml) cooled in ice–water bath, and the mixture was further stirred at 0°C for 1 h. The solvent was rotary-evaporated, and the residue was purified using silica gel column with 30% ethyl acetate–hexane as an eluent. Yield was 0.179 g (89%), with **6** obtained as white crystalline solid. Mp 40–42°C. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5 H, -C_6H_5), 5.76 (m, 1 H, -C*H*=), 5.26-5.12 (m, 4 H, CH_2=, -CH_2Ph), 4.75 (bs, 1 H, -N*H*-), 4.36 (m, 1 H, -C*H*(NHZ)-), 3.41 (m, 2 H, HOCH_2-), 2.10 (s, 2 H, -CH_2CH$_2$OH). ^{1}H NMR (DMSO) δ 7.33 (m, 5 H, -C_6H_5), 5.74 (m, 1 H, -C*H*=), 5.15-5.03 (m, 4 H, -CH_2=, -CH_2Ph), 4.15 (s, 1 H, -C*H*NH-), 3.47 (m, 2 H, HOCH_2-), 1.97 (m, 2 H). MS *m*/*z* 299 (M^+, ^{81}Br), 297

(M^+, ^{79}Br), 91 ($C_7H_7^+$). Anal. Calcd for $C_{13}H_{16}BrNO_2$: C, 52.37; H, 5.41; N, 4.70. Found: C, 52.58; H, 5.73; N, 4.48.

4-(Carbobenzyloxy)amino-5-hexenenitrile (7)

Synthesis: Compound **6** (30 mg, 0.10 mmol) was added to a solution of LiCN (66.6 mg, 2.0 mmol) in THF (5 ml). The resulting mixture was refluxed for 2 h and the solvent was rotary- evaporated. The residue was chromatographically purified on a silica gel column using 30–40% ethyl acetate in hexane as eluent to afford **7** as a white solid (14.4 mg, 70% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 5 H, -C_6H_5), 5.78 (m, 1 H, -C*H*=), 5.36-5.21 (m, 2 H, -CH_2=), 5.12 (s, 2 H, -CH_2Ph), 4.74 (bs, 1 H, -N*H*-), 4.27 (m, 1 H, -C*H*(NHZ)-), 1.96 (m, 2 H, NCCH_2-), 1.29 (m, 1 H, $NCCH_2CH_2$-), 0.95 (m, 1 H, $NCCH_2CH_2$-). MS *m*/*z* 244 (M^+), 190 (M^+-CH_3CH_2CN), 91 ($C_7H_7^+$).

Finding optimal solvent for synthesis of **7**

Compound **6** (2 mg, 7 μmol) and LiCN (0.33 mg, 10 μmol) were dissolved in 0.25 ml of the studied solvent and heated DMF, 105°C; DMSO, 90°C; THF and MeOH, 80°C. The samples (2 μl) were analyzed at 0, 10 and 25 min using HPLC: Phenomenex, Ultremex-10 (C18) column, 10 μm, 250 × 4.6 mm eluted with 10 mM KH_2PO_4 pH 2.6 – 55% CH_3CN at 2 ml/min; t_R (min): **6**, 3.9; **7**, 2.4. The progress of the reaction was estimated based on changes in the areas of the peaks for the starting material (**6**), product (**7**) and byproducts (t_R of the byproducts formed in DMSO are 2.2 and 4.1 min).

GVG (4-amino-5-hexenoic acid, **8**)

A solution of **7** (5 mg) in 0.8 ml of 38% DCl-D_2O was heated for 20 min at 100°C (in 5 mm diameter NMR tube) to give GVG as the only product. No work-up was done. The NMR spectrum of product was identical to that of authentic GVG. ^{1}H NMR (D_2O) δ 5.90 (m, 1 H, *H*C=), 5.54 (s, ^{1}H, CH_2=), 5.50 (d, J = 5.8 Hz, 1 H, CH_2=), 3.96 (m, 1 H, -C*H*(NH_2)-), 2.56 (m, 2 H, -CH_2COOH), 2.17 (m, 1 H, -CH_2CH_2COOH), 2.04 (m, 1 H, -CH_2CH_2COOH).

*[1-^{11}C]GVG ([^{11}C]**8**)*

[^{11}C]Cyanide (150–450 mCi at the EOB) from home-made synthesizer carried by nitrogen was passed through a Sicapent® column (50 × 5 mm column), to reduce the amount of ammonia, and trapped in an ethanol–ice cooled vessel (−5°C) containing dry K_2CO_3 (3.5 mg, 25 μmol) and Kryptofix 222 (4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 12 mg, 32 μmol). [^{11}C]Cyanide which was not trapped in the reaction vessel was quantitatively retained on a column (70 × 5 mm) filled with slime. The radioactivity trapping efficiency was therefore calculated as percentage of the [^{11}C]cyanide retained in the reaction vessel versus the total amount of the [^{11}C]cyanide delivered to the system.

A dry-ice cooled solution of **6** (3 mg, 10 μmol) in 0.2 ml of DMF was added, and the vessel was heated at 125°C for 10 min. After short cooling (cold water), aqueous HCl (0.5 ml, 12 M) was introduced, and the hydrolysis of [1-^{11}C]**7** was done at 125°C for 10 min. The solution was rapidly cooled (cold water) and neutralized by addition of $(NH_4)_2HPO_4$ (400 mg in 0.8 ml of water). [^{11}C]GVG was purified by HPLC on Phenomenex, Lichrosorb RP-18, 10 μm, 250 × 10 mm column with eluent 10 mM KH_2PO_4 pH 2.6 at 2 ml/min; t_R (retention time) 12.3 min. The solvent from the collected fraction was removed by rotary evaporation, and the residue was dried by azeotroping with CH_3CN (2 × 1 ml). The product was redissolved in saline and filtered through a 0.22 μm Millipore filter into a sterile vial. Radiochemical purity was determined by both HPLC and TLC. HPLC was equipped with radio- and UV (235 nm) detectors. HPLC: Phenomenex, Ultremex-10 (C18), 10 μm, 250 × 4.6 mm column with gradient system 0–7 min, 10 mM KH_2PO_4 pH 2.6 at 1 ml/min and 7–15 min, 10 mM KH_2PO_4 pH 2.6 – 60% CH_3CN at 2 ml/min, t_R 5.7 min. TLC analysis was performed using 10 mM KH_2PO_4 pH 2.6 : CH_3CN 1 : 1 as eluent, R_f of GVG was 0.68.

Specific radioactivity of [^{11}C]GVG was estimated by the injection of 0.2 ml of the final radiotracer solution on the HPLC. The same HPLC conditions as for [^{11}C]GVG quality control were used except an isocratic elution was performed with 10 mM KH_2PO_4 pH 2.6 at 1 ml/min. A calibration curve was obtained by the injection of 0.2 ml solutions containing 1–10 mg (7.8–78 nmol) of authentic GVG in saline (0.9% NaCl in water). The EOB-corrected specific radioactivity based on the samples from two radiosyntheses exceeded 0.5 Ci/μmol.

Conclusion

GVG, a suicide inhibitor of GABA transaminase (GABA-T), was efficiently labeled with carbon-11 via a [^{11}C]cyanation of 5-bromo-3-(carbobenzyloxy)amino-1-pentene followed by acid hydrolysis. These synthetic procedures should be applicable to the labeling of the pharmacologically active S form of GVG when the S-enantiomer of homoserine lactone is used. The availability of this radiotracer should allow us to study the pharmacokinetics as well as the potential side effects of the drug. It also sets the stage for future studies in human to better understand its role in addiction treatment.

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References

1. Jung MJ, Lippert BW, Metcalf BW, Bohlen P, Schechter PJ. *J Neurochem* 1977; **29**: 797–802.
2. Lippert B, Metcalf BW, Jung MJ, Casara P. *Eur J Biochem* 1977; **74**: 441–445.
3. Weber OM, Verhagen A, Duc CO, Meier D, Leenders KL, Boesiger P. *Magn Reson Imaging* 1999; **17**: 417–425.
4. Sabers A, Gram L. *Pharm Toxicol* 1992; **70**: 237–243.
5. Grant SM, Heel RC. *Drugs* 1991; **41**: 889–926.
6. Dewey SL, Morgan AE, Ashby CRJ, Horan B, Kushner SA, Logan J, Volkow ND, Fowler JS, Gardner EL, Brodie JD. *Synapse* 1998; **30**: 119–129.
7. Dewey SL, Brodie JD, Gerasimov M, Horan B, Gardner EL, Ashby CRJ. *Synapse* 1999; **31**: 76–86.
8. Gerasimov MR, Ashby CRJ, Gardner EL, Mills MJ, Brodie JD, Dewey SL. *Synapse* 1999; **34**: 11–19.
9. Gerasimov MR, Schiffer WK, Brodie JD, Lennon IC, Taylor SJ, Dewey SL. *Eur J Pharmacol* 2000; **395**: 129–135.

10. Eke T, Talbot JF, Lawden MC. *BMJ* 1997; **314**: 180–181.
11. Schuster AJ, Wagner ER. *J Labelled Cpd Radiopharm* 1993; **33**: 213–217.
12. Gill HS. *J Labelled Cpd Radiopharm* 1995; **36**: 425–430.
13. Wei ZY, Knaus EE. *Tetrahedron* 1994; **50**: 5569–5578.
14. Kwon TW, Keusenkothen PF, Smith MB. *J Org Chem* 1992; **57**: 6169–6173.
15. Wei Z-Y, Knaus EE. *J Org Chem* 1993; **58**: 1586–1588.
16. Kihlberg T, Gullberg P, Langstron B. *J Labelled Cpd Radiopharm* 1990; **28**: 1115–1119.
17. Barrett AGM, Broughton HB. *J Org Chem* 1986; **51**: 495–503.
18. Yoon NM, Gyoung YS. *J Org Chem* 1985; **50**: 2443–2450.
19. Greenwald R, Chaykovsky M, Corey E. *J Org Chem* 1963; **28**: 1128–1129.
20. Corey EJ, Weinshenker NM, Schaaf TK, Huber W. *J Am Chem Soc* 1969; **91**: 5675–5677.
21. Reitz AB, Nortey SO, Maryanoff BE. *J Org Chem* 1987; **52**: 4191–4202.
22. Pike RM, Mayo DW, Butcher DJ, Hinkle RJ. *J Chem Ed* 1986; **63**: 917–918.
23. Greene TW, Wuts PGM. *Protective Groups in Organic Synthesis*, Wiley: New York, 1991.
24. Two syntheses of fully protected homoserine lactones were made at the different stages of this research. *N*-(2,5,5-Tetramethyl-2,5-disila-1-azacyclopentyl)-homoserine lactone was found to be moisture sensitive and decomposed during purification, while 2-[1,3-Dimethyl-1,3-diaza-2-oxo-5-azacyclohexanyl)-4-butyrolactone was synthesized in rather low yield.
25. Grashey R. *Comprehensive Organic Synthesis*, Vol 6. Pergamon Press: New York, 1991.
26. Ding Y-S, Fowler JS, Wolf AP. *J Labelled Cpd Radiopharm* 1993; **33**: 645–654.
27. Christman DR, Finn RD, Karlstrom KI, P. WA. *Int J Appl Radiat Isot* 1975; **26**: 435–442.
28. Iwata R, Ido T, Takahashi T, Nakanishi H, Iida S. *Appl Radiat Isot* 1986; **38**: 97–102.
29. Ding Y-S, Antoni G, Fowler JS, Wolf AP, Langstrom B. *J Labelled Cpd Radiopharm* 1989; **27**: 1079–1090.
30. Ponchant M, Hinnen F, Demphel S, Crouzel C. *Appl Radiat Isot* 1997; **48**: 755–762.
31. Westerberg G, Langstrom B. *Appl Radiat Isot* 1996; **48**: 459–461.
32. Ponchant M, Galea H, Bottlaender M, Coulon C, Fuseau C, Ottaviani M, Crouzel C. *J Labelled Cpd Radiopharm* 2000; **43**: 1311–1320.
33. Brown HC. *Organic syntheses via boranes*, Wiley: NY, 1975.
34. Studenov AR, Wegner AM, Ding YS. *J Labelled Cpd Radiopharm* 2001; **44**: 1–12.

