Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 1127-1134 (2000)

Synthesis of tritium labeled (±)-1-[2-(triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid: A possible compound to determine the efficacy of potential GABA transporter substances *in vitro*

Ralf Schirrmacher[1], Wilhelm Hamkens[1], Hartmut Lüddens[2], Frank Rösch[1]

[1]Institute for Nuclear Chemistry, [2]Department of Psychiatry,
Johannes Gutenberg-University Mainz, Germany

address for correspondence:
Prof. Dr. Frank Rösch
[1]Institute for Nuclear Chemistry, Johannes Gutenberg-University
Fritz-Strassmann-Weg 2, D-55128-Mainz, Germany,
tel: +49-6131-392 5302, fax: +49-6131-392 5253,
e-mail: frank.roesch@uni-mainz.de

SUMMARY

(±)-1-[2-(Triphenyl[^{3}H]methoxy)ethyl]-3-piperidinecarboxylic acid ([^{3}H]SNAP-5114) with a specific activity of 40 Ci/mmol was prepared in a two step synthesis starting from ethyl (2-(4-hydroxyphenyl)bis(4-methoxyphenyl)methoxy)-ethyl)piperidine-3-carboxylate and [^{3}H]methyliodide with subsequent hydrolysis of the resulting ester with lithium hydroxide to yield the desired [^{3}H]SNAP-5114.

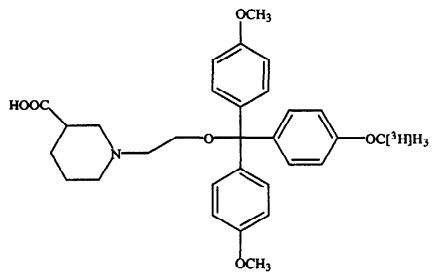
Key Words:
gamma amino butyric acid (GABA), GABA transporter, GAT-3 transporter ligands, nipecotic acid derivatives, tritium

Received 1 February 2000
Revised 29 June 2000
Accepted 8 July 2000

INTRODUCTION

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the mammalian central nervous system. The level of GABA is regulated by rapid uptake of the neurotransmitter via specific, high-affinity transporters located in the presynaptic terminal and/or surrounding glial cells [1]. A group of transporter subtypes has been discovered and characterized [2, 3]. The distribution of subtypes varies greatly, GAT-1 and GAT-2 are ubiquitious whereas GAT-3 is restricted to some well defined areas [4]. Little is known about the role of GABA-transporter subtype GAT-3 in psychiatric or neurological disorders which my be linked to a dysfunction of the GABAergic system, e.g., epilepsy or Parkinson syndrome.

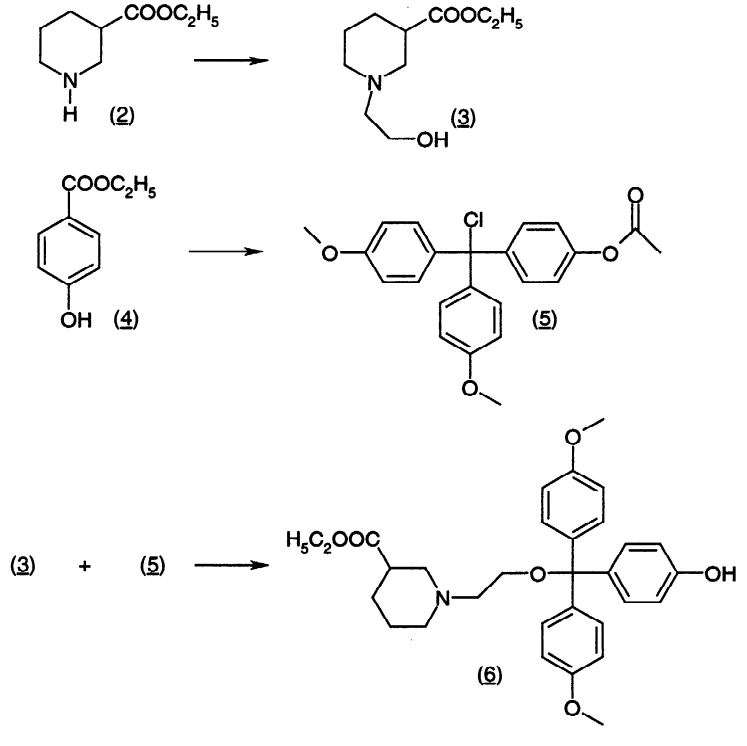
Positron emission tomography (PET) could become a useful tool for evaluating new GAT-3 ligands *in vivo*. To pre-screen possible compounds which are eventual candidates to be tested for their affinity to the GAT-3 subtype, a tritiated compound was needed to perform *in vitro* evaluations. Recently, (±)-1-[2-(triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid (SNAP 5114) was reported by Dhar et al. to show the highest affinity for the GAT-3 subtype out of a series of nipecotic acid derivatives [5]. The racemic compound shows nearly the same affinity to the transporter subtype as GABA itself [10 μM] and was therefore considered to be suitable for labeling with tritium.



[^{3}H]SNAP 5114 (1)

RESULTS AND DISCUSSIONS

Our aim was to accomplish an efficient labeling process avoiding the decrease of specific activity often associated with multistep synthetic routes. Thus, we synthesized a precursor (6) containing a phenolic hydroxy function which could be labeled with commercially available [^{3}H]methyliodide in two steps. We carried out the synthesis of (6) as shown in scheme 1.

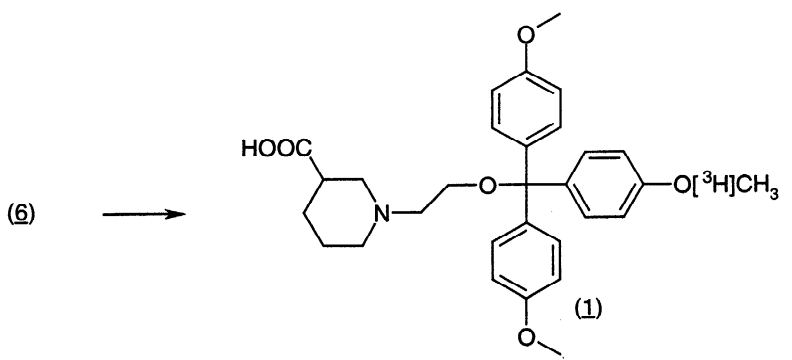


Scheme 1: Synthesis of the tritiation precursor (6)

a) 2-bromoethanol, K_2CO_3, dioxane b) 4-methoxy phenylmagnesium bromide, THF c) acetylchloride d) THF, triethylamine, DMAP e) $CH_3O^-Na^+/CH_3OH$ pH 8.5

Treatment of ethyl piperidine-3-carboxylate (2) with 2-bromo ethanol in dioxane with addition of K_2CO_3 afforded the addition product (3) in 83% yield. After coupling with (5), which was synthesized from 4-methoxy phenylmagnesiumbromide and ethyl 4-hydroxybenzoate (4) in THF, the final product (6) could be obtained after deprotection of the phenolic hydroxy group with sodium methanolate in methanol at pH 8.5 in an overall yield of 27% [6]. The synthesis of the unlabelled SNAP-5114 was performed according to established literature procedures [5].

Tritiation of (6) with [^{3}H]methyliodide (6 mCi, specific activity 60-85 Ci/mmol) was performed in acetonitrile with addition of NaOH (1N) followed by semi preparative HPLC separation and subsequent ester hydrolysis of the pure tritiated ester to yield the product (1) in a purity of >99%. The specific activity of tritium labeled [^{3}H]SNAP-5114 (1) was determined by comparing the UV absorption of the labeled compound with the UV absorption of a known concentration of the unlabelled compound.



Scheme 2: Tritium labeling of 6.

f) acetonitrile, NaOH (5N), [^{3}H]CH_3I in acetonitrile + 0.25% toluene g) ethanol, LiOH (1N), NaH_2PO_4 (5%)

EXPERIMENTAL

Unlabelled reagents were purchased from Merck Darmstadt and Fluka and were used without further purification. Solvents were HPLC grade. Carrier free [^{3}H]methyl iodide was purchased from Amersham (10 mCi/ml acetonitrile + 0.25% toluene, specific activity 60-85 Ci/mmol). HPLC was performed with Sycam S1100 HPLC system and a Sycam S3200 UV detector. NMR spectra were recorded using a Bruker 200-MHz-FT-NMR spectrometer AC 200. Chemical shifts are quoted in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. IR spectra were recorded using a Perkin Elmer FT-IR spectrometer 1760X with KBr discs. MS spectra were obtained on a Finnigan MAT90 spectrometer.

"Dried" refers to drying the organic layer over anhydrous $MgSO_4$. All compounds were found to be homogeneous by TLC analysis.

Ethyl (2-hydroxyethyl)piperidine-3-carboxylate (3)

To a solution of ethyl piperidine-3-carboxylate (2.48 ml, 16 mmol) in 1,4-dioxane (20 ml) were subsequently added 2-bromo ethanol (1.13 g, 16 mmol), potassium carbonate (6.63 g, 48 mmol) and a small amount of sodium iodide. The mixture was refluxed overnight, concentrated under reduced pressure, extracted with ethyl acetate (30 ml), filtered and dried. The organic layer was evaporated under reduced pressure. The product (3) was obtained as a yellow oil after separation using column chromatography (2:1, ethylacetate/hexane) (2.7 g, 13.3 mmol, 83%) according to a literature procedure [5].

^{1}H-NMR (200 MHz, $CDCl_3$): (3H, m, 1.2 ppm), (8H, m, 1.5-2.6 ppm), (2H, t, 2.6 ppm), (1H, m, 2.7-2.76 ppm), (1H, m, 2.85-2.9 ppm), (2H, t, 3.6 ppm), (2H, q, 4.1 ppm)

(4-Hydroxyphenyl)bis(4-methoxyphenyl)methanol

A Grignard reagent was prepared from p-bromo anisidine (46.18 g, 0.24 mol) and magnesium (6 g, 0.24 mol) in THF (60 ml). The resulting solution was slowly added to a solution of ethyl 4-hydroxybenzoate (10 g, 0.08 mol) in THF (20 ml) and kept at 4°C overnight. Saturated ammonia solution was added to hydrolyze the resulting salt.

The solution was extracted with ether, dried and the solvent was evaporated under reduced pressure. The crude product was recrystallized from acetic acid/water to yield the crystalline product (19.8 g, 0.058 mol, 90%).

1H-NMR (200 MHz, $CDCl_3$): (6H, s, 3.8), (12H, m, 6.5-7.5)

IR: ν(Ph-OH): 3150-3700, ν(Ph-O-CH_3): 2830, ν(C_t-OH): 1000-1200

4-Chloro bis(4-methoxyphenyl)methyl)phenyl acetate (5)

(4-Hydroxyphenyl)bis(4-methoxyphenyl)methanol was added to an excess of acetyl chloride and refluxed for two hours. The remaining acetyl chloride was removed under reduced pressure and the red solid product was obtained quantitatively without further purification.

1H-NMR (200 MHz, $CDCl_3$): (3H, s, 2.1), (6H, s, 3.8), (12H, m, 6.7-7.7)

IR: ν(CO): 1740, ν(CO): 1200, ν(Ph-O-CH_3): 2830, ν(C-Cl): 570-650

4-((2-(3-(Ethoxycarbonyl)piperidyl)ethoxy)bis(4-methoxyphenyl)methyl)phenyl acetate

To a solution of ethyl (2-hydroxyethyl)piperidine-3-carboxylate (1.63 g, 8.1 mmol) in dry THF (10 ml) were subsequently added triethylamine (2 ml, 18.6 mmol), 2-dimethylaminopyridine (DMAP) (98 mg) and 4-chloro bis(4-methoxyphenyl)methyl)-phenyl acetate (3.7 g, 10.5 mmol) and stirred over night at room temperature. The solution was passed into a mixture of dichloromethane (50 ml) and water (10 ml). The organic layer was dried, filtered, evaporated and separated with flash chromatography (ethylacetate/n-hexane/triethylamine: 25/75/0.1%) to yield 4-((2-(3-(ethoxycarbonyl) piperidyl)ethoxy)bis(4-methoxyphenyl)methyl)phenyl acetate as a yellow oil (1.9 g, 40%).

^{1}H-NMR (200 MHz, $CDCl_3$): (3H, t, 1.24 ppm), (5H, m, 1.45-2.0 ppm), (3H, s, 2.1 ppm), (1H, t, 2.2 ppm), (1H, m, 2.5-2.55 ppm), (2H, t, 3.2 ppm), (6H, s, 3.7 ppm), (2H, q, 4.0 ppm), (12H, m, 7.3-7.9 ppm)

MS (FD): m/z (% rel. Int.) 562.4 (100, $[M+1]^+$)

Ethyl (2-(4-hydroxyphenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (6)

4-((2-(3-(Ethoxycarbonyl)piperidyl)ethoxy)bis(4-methoxyphenyl)methyl)phenyl acetate (0.9 g, 1.6 mmol) was dissolved in methanol, and a sodium methanolate/methanol solution was added until a pH of 8.5 was adjusted (wet pH-paper). The solution was stirred for 1 h, water was added in small amounts and the resulting precipitate was collected, washed with water and dried under vacuum to yield the product as a yellow solid (0.75 g, 1.35 mmol, 90%).

^{1}H-NMR (200 MHz, $CDCl_3$): (3H, t, 1.24 ppm), (5H, m, 1.45-2.0 ppm), (1H, t, 2.2 ppm), (1H, m, 2.5-2.55 ppm), (2H, t, 3.2 ppm), (6H, s, 3.7 ppm), (2H, q, 4.0 ppm), (12 H, m, 7.3-7.9 ppm)

IR: ν(CO): 1740, ν(CO): 1200, ν(Ph-OH): 3150-3700, ν(Ph-O-CH_3): 2830

MS (FD): m/z (% rel. Int.) 505.4 (100.0, $[M]^+$), 320.1 (5.8, $[(M)-(C_{11}H_{17}NO_3)]^+$

(±)-Ethyl (2-(tris(4-[^{3}H]methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate

Ethyl (2-(4-hydroxyphenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (6 mg, 0.0115 mmol) was dissolved in 0.5 ml acetonitrile and 2.5 µl of an aqueous solution of NaOH (5N) were added. 6 mCi (600 µl) of a commercially available solution of [^{3}H]methyliodide in acetonitrile (0.25% toluene) were added and stirred at 60°C for 3 h. The reaction mixture was purified via HPLC (LiChrosorb RP-8, 250x10, 4 ml/min, methanol/water 70/30 + 0.1% triethylamine). The desired product (1) (Rt: 25 min) was separated from the precursor (6) as evaluated in systematic experiments carried out before using non-tritiated methyliodide. The obtained solution was diluted with five-fold excess of water and fixed on a solid phase column (LiChrolut EN, Merck). The solid phase column was dried in a nitrogen stream and the product was eluted with ethanol (1 ml).

Total activity: 5 mCi, specific activity: 40 Ci/mmol, TLC: (TLC-foil from Merck, 5x10 cm, 60 F_{254}) ethylacetate/n-hexane 1:1, R_f: 0.45.

(±)-1-[2-(Triphenyl[^{3}H]methoxy)ethyl]-3-piperidine-carboxylic acid ([^{3}H]SNAP 5114) (1)

To a solution of (±)-ethyl (2-(tris(4-[^{3}H]methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate in 0.5 ml ethanol and 200 µl LiOH (1N) were added and stirred at room temperature for 18 h.

The pH was adjusted to pH 5.5 using NaH_2PO_4 (5%), the solution was extracted with dichloromethane (1 ml) and dried. No release of tritium compounds was observed. As evaluated in systematic experiments carried out before using non-tritiated methyl-iodide, the ester cleavage was quantitative. Therefore, the specific activity was not determined, but was expected to be the same as for the (±)-ethyl (2-(tris(4-[^{3}H]methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate.

TLC: (TLC-foil from Merck, 5x10 cm, 60 F_{254}) methanol/ethylacetate 3:2, R_f: 0.42.

REFERENCES

[1] Mager S., Naeve J. et al. *Neuron.* **10**: 177 (1993)

[2] Mabjeesh N.J. and Kanner B.I.. *Biochemistry.* **28**: 7694 (1989)

[3] Kanner B.I. and Bendahan A.. *Proc. Natl. Acad. Sci. USA.* **87**: 2550 (1990)

[4] Borden L.A., Smith K.E., Gustafson E.L. et al. *J. Neurochem.* **64**: 977 (1995)

[5] Dhar T.G.M., Borden L.A. et al. *J. Med. Chem.* **37**: 2334 (1994)

[6] Taunton-Rigby A., Kim Y.-Ho et al. *J. Org. Chem.* **37**: 956 (1972)

