JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 99–106.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.802

Research Article

The synthesis of tritium labelled neurokinin-1 receptor ligands

Terence G. Hamill* and H. Donald Burns
Imaging Research Department, Merck Research Laboratories, West Point, PA 19486, USA

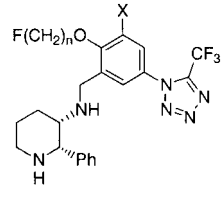
Summary

Radiolabelled neurokinin-1 (NK_1) receptor antagonists **1b** and **2b** were required for *in vitro*/*in vivo* characterization to support the development of **1a** and **2a** as fluorine-18 labelled PET ligands. These tritium labelled compounds were synthesized from aryl iodide precursors giving the final tritiated tracers with specific activities of 28 (**1b**) and 14 (**2b**) Ci/mmol. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: neurokinin-1; substance P; tritium

Introduction

The agonist substance P, acting through the neurokinin-1 (NK_1) receptor, is believed to play an important role in pain, emesis, inflammation and psychiatric disorders such as depression.[1] Work is ongoing to identify NK_1 antagonists as therapeutic agents to treat conditions such as chemotherapy-induced emesis (CIE) and depression.[2] As part of an NK_1 clinical program for



1a, n=1, X=H
1b, n=1, X=T
2a, n=2, X=H
2b, n=2, X=T

Figure 1. Chemical structures of neurokinin-1 compounds

*Correspondence to: T. G. Hamill, Merck Research Laboratories, WP44C-2, West Point, PA 19486, USA.
E-mail: terence_hamill@merck.com

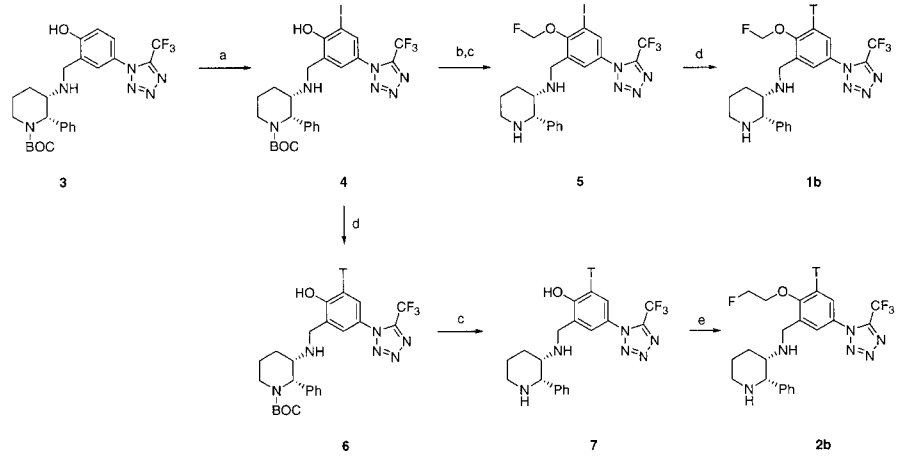
Received 11 June 2003
Revised 29 September 2003
Accepted 3 November 2003

CIE and depression, we were interested in developing tracers for positron emission tomography (PET) studies of the NK_1 receptor to determine receptor occupancy of clinical candidates. To support the development of the fluorine-18 labelled PET ligands,[3] the tritiated compounds were required for *in vivo*/*in vitro* characterization. This paper describes the synthesis of the tritium labelled compounds **1b** and **2b**.

Results and discussion

At the time this work was undertaken, data on [^{18}F]**1** as a potential NK_1 PET ligand was very promising,[3] so in addition to specifically targeting **1b**, a route giving an intermediate that could be used to make other fluorine-containing alkyl ether analogs of **1b** was also desired. For this reason, we also targeted the synthesis of tritium labelled phenol **6** (Sch 1), which could be a common precursor for a variety of tritium labelled, fluorine-containing alkyl aryl ether analogs.

Scheme 1 summarizes the chemistry that was followed to synthesize **1b**, **2b** and their precursors. Phenol **3** (Compound **3** was synthesized at Merck Research Laboratories) was chosen as the starting point. Halogenation of **3** would ultimately allow incorporation of tritium by catalytic reductive dehalogenation. Iodination of **3** was attempted using various conditions (Other conditions used were ICl, NaI with either chloramine-T or NaOCl and iodine/morpholine) with the best results coming when $IPy_2BF_4^4$ in methylene chloride was used. The intermediate iodophenol **4**, itself a precursor to **2b**, was



Key: a) IPy_2BF_4, CH_2Cl_2, RT b) FCH_2I, Cs_2CO_3, DMF, RT c) TFA, CH_2Cl_2, RT d) T_2, 10% Pd/C, Et_3N, DMF e) FCH_2CH_2Br, Cs_2CO_3, DMF, RT.

Scheme 1. Synthetic routes for tritium labelled neurokinin-1 compounds

alkylated using fluoromethyliodide[5] and deprotected to give the desired iodoaryl ether **5**.

When a model hydrogenation reaction of **5** was carried out using 10% Pd/C in DMF with triethylamine at room temperature, the desired product **1a** formed, as determined by HPLC coelution with the authentic standard. Repeating this reaction using tritium gas gave crude **1b** with a radiochemical purity of ~95%. HPLC purification of this material gave **1b** with a radiochemical purity of >99% and a specific activity of 28 Ci/mmol.

In an analogous fashion, gas tritiation of **4** gave crude **6**. This material was first deprotected using trifluoroacetic acid in methylene chloride to give **7**, and was then alkylated using 1-bromo-2-fluoroethane to give **2b**. This sequence was carried out in a one-pot procedure without purifying **7**. However, the specific activity of **7** was determined by purifying an aliquot and its specific activity was determined to be 14.2 Ci/mmol. Purification of crude **2b** by HPLC gave **2b** with a radiochemical purity of >98% and an estimated specific activity of 14 Ci/mmol based on the specific activity of **7**. If this deprotection/alkylation sequence was reversed so that the alkylation was carried out first, followed by the deprotection step, a product resulted which did not coelute with the authentic standard. Treatment of unlabelled **2a** with trifluoroacetic acid showed that this compound is stable to trifluoroacetic acid and that the unknown product did not arise from decomposition of the desired product.

The characterization of fluorine-18 labelled **1** has been reported[3] and the *in vitro* and *in vivo* characterization of **2** labelled with tritium and fluorine-18 and its suitability as an NK_1 receptor ligand and PET radiotracer will be reported elsewhere.

Conclusion

The synthesis of two tritiated neurokinin-1 receptor ligands has been carried out. Each ligand was synthesized from an iodoaryl precursor giving the final products with specific activities of 14 or 28 Ci/mmol. These fluorine-containing compounds also lend themselves to fluorine-18 labelling chemistry and use as neurokinin-1 receptor PET ligands.

Experimental

Materials and methods

^{1}H NMR spectra were recorded using a Varian Infinity-300 spectrometer operating at 300 MHz or a Varian Unityplus operating at 500 MHz. Mass spectral analyses were carried out using a VG 7070E mass spectrometer. Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 100 µl heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson

FC203 Microfraction collector. The acetonitrile used for the HPLC analyses was Fisher Optima grade. The HPLC radiodetector used was a Beckman 171 Radioisotope detector with a Beckman 110B solvent delivery system and Beckman Ready Flow III scintillation cocktail. A Vydac C-18 column, 4.6×250 mm (The Nest Group) was used for analytical and preparative HPLC. Solutions of radioactivity were concentrated using a Jouan vacuum centrifuge. Calibration curves and chemical concentrations used to determine specific activities were determined using a Hewlett Packard Model 8452A UV/Vis Diode Array Spectrophotometer. Sample radioactivities were determined in an LKB Wallac 1410 liquid scintillation counter. The identity of labelled compounds was determined by HPLC coelution with authentic compounds. Reagents were purchased from Aldrich Chemical Co. The IPy_2BF_4 was prepared according to the literature procedure.[4] Compound **3** was synthesized at Merck Research Laboratories.

Nonradioactive syntheses

[2-Fluoromethoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**1a**): A room temperature solution of **3** (100 mg, 0.19 mmol) in DMF (6.6 ml) was treated with Cs_2CO_3 (313 mg, 0.96 mmol) giving a yellow mixture. After stirring for several minutes at room temperature a solution of fluoromethyl iodide (0.09 ml) in DMF (1.2 ml) was added discharging the yellow color. After stirring at room temperature for 30 min, the reaction was diluted with H_2O/brine/aq. saturated NH_4Cl. The aqueous layer was extracted with ethyl acetate and the organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 113 mg of the crude alkylated compound: ^{1}H NMR (δ, $CDC1_3$): 7.54 (3H, m), 7.38–7.22 (6H, m), 5.72 (1H, m), 5.71 (1H, m), 5.48 (1H, m), 3.95 (3H, m), 3.02 (2H, m), 1.87–1.26 (3H, m), 1.39 (9H, s); MS m/z (relative intensity) for $C_{26}H_{30}F_4N_6O_3$: 551 (M + 1, 100%).

A room temperature solution of the crude alkylated compound (113 mg, 0.205 mmol) in methylene chloride (5 ml) was treated with trifluoroacetic acid (2.5 ml) and stirred at room temperature for 50 min. The reaction was concentrated *in vacuo* to give a yellow oil and diluted using ethyl acetate/aq. saturated $NaHCO_3$. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 136 mg of crude **1a** as a yellow oil. TLC (5:93:2 methanol:ethyl acetate:triethylamine) shows **1a** at $0.35R_f$. This material was dissolved in chloroform and purified by radial chromatography (5:93:2 methanol:ethyl acetate:triethylamine) to give 41.2 mg of pure **1a** along with 50 mg of **1a** containing small amounts of the corresponding phenol. ^{1}H NMR (δ, $CDCl_3$): 7.29 (2H, dm, $J \sim 7.7$ Hz), 7.21

(1H, dd, $J = 8.6$, 2.7 Hz), 7.16 (2H, t, $J \sim 7.6$ Hz), 7.11 (1H, d, $J = 8.6$ Hz), 7.05 (1H, tt, $J \sim 7.3$, 1.2 Hz), 6.97 (1H, d, $J = 2.7$ Hz), 5.59 (1H, dd, $J = 53.5$, 3.2 Hz), 5.57 (1H, dd, $J = 54.0$, 3.2 Hz), 3.94 (1H, d, $J = 2.6$ Hz), 3.76 (1H, d, $J = 16.1$ Hz), 3.61 (1H, d, $J = 16.1$ Hz), 3.29 (1H, dm, $J = 11.8$ Hz), 2.83 (1H, ddd, $J \sim 12.4$, 12.4, 3.1Hz), 2.78 (1H, q, $J \sim 2.7$ Hz), 2.10 (1H, dm, $J \sim 13.6$ Hz), 1.94 (1H, qt, $J \sim 13.1$, 3.8 Hz), 1.62 (1H, dddd, $J \sim 13.6$, 13.6, 4.3, 3.1 Hz), 1.53 (1H, dm, $J = 13.7$ Hz). A standard NOESY sequence with a 500-ms mixing period showed a strong NOE between the fluoromethylene protons and the ortho aromatic proton at δ 7.11. MS *m/z* (relative intensity) for $C_{21}H_{22}F_4N_6O_1$: 451 (M + 1, 100%).

[2-Hydroxy-3-iodo-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-1-t-butoxycarbonyl-2-phenyl-piperidin-3-yl)-amine (**4**): A room temperature solution of **3** (30 mg, 0.06 mmol) in methylene chloride (3 ml) was treated with IPy_2BF_4 (26 mg, 0.066 mmol). The reaction flask was shielded from light and stirred for 1 h at room temperature, giving a slightly white opaque mixture. The reaction mixture was concentrated *in vacuo* to remove the methylene chloride and the residue was rinsed with ethyl acetate and placed in a separately funnel containing 10% aqueous $Na_2S_2O_3$. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 41 mg of crude **4** as a yellow oil: ^{1}H NMR (δ, $CDCl_3$): 7.75 (1H, d, $J = 2.44$ Hz), 7.5–7.33 (5H, m), 7.08 (1H, d, $J = 2.44$ Hz), 5.42 (1H, d, $J = 6.1$ Hz), 4.22–3.99 (3H, m), 3.21–3.07 (2H, m), 2.0–1.25 (4H, m), 1.37 (9H, s); MS *m/z* (relative intensity) for $C_{25}H_{28}F_3IN_6O_3$: 645 (M + 1, 100%).

[2-Fluoromethoxy-3-iodo-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**5**): A room temperature solution of **4** (41 mg, 0.064 mmol) in DMF (2 ml) was treated with Cs_2CO_3 (98 mg, 0.3 mmol) giving a yellow mixture. After stirring for several minutes a solution of fluoromethyl iodide (0.03 ml) in DMF (0.5 ml) was added giving an off-white opaque mixture. After stirring for 2.5 h at room temperature, the reaction was diluted with brine/saturated aqueous NH_4Cl/H_2O/ethyl acetate and placed in a separatory funnel. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), filtered and concentrated to give 41 mg of a yellow oil. A room temperature solution of this crude material (40 mg, 0.059 mmol) in methylene chloride (2.2 ml) was treated with trifluoroacetic acid (1.1 ml) and stirred at room temperature. After 20 min at room temperature, the reaction was concentrated *in vacuo*, treated with methylene chloride/aqueous saturated $NaHCO_3$ and placed in a separatory funnel. The layers were separated and the aqueous layer was extracted with methylene chloride. The organic layers were combined, dried ($MgSO_4$), filtered and concentrated to give 30 mg of **5** as a yellow oil. The crude **5** was dissolved in chloroform and purified by radial chromatography (3:96:1 methanol:ethyl

acetate:triethylamine) to give 11.4 mg (31%) of **5** as an oil: ^{1}H NMR (δ, $CDC1_3$): 7.65 (1H, d, $J = 2.45$ Hz), 7.28 (2H, m), 7.09 (1H, t, $J = 7.57$ Hz), 6.92 (2H, m), 5.59 (1H, dd, $J = 53.96$, 2.44 Hz), 5.55 (1H, dd, $J = 54.3$, 2.4 Hz), 3.87 (1H, s), 3.80 (2H, s), 3.25 (1H, m), 2.79 (1H, m), 2.67 (1H, m), 2.1–1.5 (6H, m); MS *m/z* (relative intensity) for $C_{21}H_{21}F_4IN_6O$: 577 (M + 1, 97%).

[2-Fluoroethoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**2a**): A room temperature solution of 2-hydroxy-5-(5-trifluoromethyl-tetrazol-l-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine, bis HC1 salt (50 mg, 0.102 mmol) in DMF (2 ml) was treated with cesium carbonate (230 mg, 0.714 mmol). After stirring for several minutes, bromofluoroethane (9 µl, 0.122 mmol) was added and the reaction was stirred at room temperature overnight giving a yellow slurry. The reaction was diluted with aqueous saturated NH_4Cl/brine/H_2O and ethyl acetate. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, washed with H_2O, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 44 mg of a yellow oil. Purification by rotary chromatography (5% MeOH/EtOAc to 10% MeOH/EtOAc) gave 33.2 mg (70%) of **2a**: ^{1}H NMR (δ, $CDC1_3$): 7.25 (2H, m), 7.17 (1H, d × d, $J = 8.7$ Hz, 2.8 Hz), 7.14 (2H, m), 7.02 (1H, m), 6.87 (1H, d, $J = 2.8$ Hz), 6.82 (1H, d, $J = 8.7$ Hz), 4.64 (1H, m), 4.63 (1H, m), 4.2–4.0 (2H, m), 3.87 (1H, d, $J = 2.4$ Hz), 3.75 (1H, d, $J = 16$ Hz), 3.58 (1H, d, $J = 16$ Hz), 3.25 (1H, m), 2.80 (1H, m), 2.74 (1H, m), 2.10 (1H, m), 1.89 (1H, m), 1.59 (1H, m), 1.48 (1H, m). A standard NOESY sequence with an 800-ms mixing time showed a correlation between the δ 4.11 methylene on the fluoroethyl sidechain and the phenyl proton at δ 6.82 (ortho to the fluoroethoxy chain). There is also a correlation between the δ 6.87 phenyl proton (ortho to the tetrazole and aminomethyl moieties) and the δ 7.25 proton of the phenyl group. MS *m/z* (relative intensity) for $C_{22}H_{24}F_4N_6O$: 465 (M + l, 100%).

Radioactive syntheses

[2-Fluoromethoxy-3-tritio-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**1b**): A room temperature solution of **5** (8.5 mg, 0.015 mmol) in DMF (2 ml) was treated with triethylamine (0.08 ml) and 10% Pd/C (6.5 mg). The reaction vessel and its contents were degassed by a single evacuation cycle and then exposed to 5 Ci of tritium gas. After 2.8 h at room temperature and atmospheric pressure, the reaction was passed through a short column of celite and rinsed with 3 × 1 ml of DMF and 3 × 1 ml of methanol. The solvents were removed *in vacuo* and labile tritium was washed out by evaporation with methanol (3 × 2 ml). The residue was dissolved in ethanol (20 ml) to give 200 mCi of activity. An aliquot (15 mCi) was concentrated *in vacuo*, dissolved in ethanol (75 µl) for purification by HPLC (C18 Vydac protein and peptide column, 4.6 × 250 mm, 1 ml/min, linear gradient of 10%

MeCN:H_2O (0.1% TFA) to 95% MeCN over 15 min, hold at 95% MeCN for 10 min, 254 nm). Fractions (0.2 ml) were collected beginning at 9 min and the product eluted at 14 min. The center cut fractions were pooled, concentrated *in vacuo* and diluted with 91% ethanol/H_2O (6.6 ml) to give 5 mCi of **1b** with a specific activity of 27.8 Ci/mmol and a radiochemical purify >98%.

[2-Hydroxy-3-tritio-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-1-t-butoxycarbonyl-2-phenyl-piperidin-3-yl)-amine (**6**): A room temperature solution of **4** (9 mg, 0.015 mmol) in DMF (1.8 ml) was treated with triethylamine (0.035 ml) and transferred to a 2 ml reaction containing 10% Pd/C (8 mg). The reaction vessel and its contents were degassed by a single evacuation cycle and then exposed to 5 Ci of tritium gas. The reaction was terminated after a total of 1 h at room temperature and atmospheric pressure. The catalyst was removed through a short column of celite and the celite was rinsed with DMF (3 × 1 ml) and methanol (3 × 1 ml). The solvents were removed *in vacuo* and any labile tritium was washed out by evaporation with methanol (3 × 2 ml). The residue was dissolved in ethanol to give 334 mCi (~13 mCi/ml) of **6**.

[2-Hydroxy-3-tritio-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**7**): An aliquot containing of crude **6** (~6.5 mCi) was concentrated to dryness and treated with 1:1 CH_2Cl_2:TFA (0.1 ml) and stirred at room temperature for 30 min. The reaction was partially concentrated *in vacuo*, diluted with ethanol (0.08 ml) and purified by HPLC [C18 Vydac, 4.6 × 250 nm, 15 min linear gradient @ 1 ml/min, 10% acetonitrile:H_2O (0.1% TFA) to 95% acetonitrile, retention time ~12.5 min]. The center cut fractions were pooled, concentrated *in vacuo* and diluted with ethanol (3 ml) to give 2.8 mCi of **7** with a specific activity of 14.2 Ci/mmol and radiochemical purity >98%.

[2-Fluoroethoxy-3-tririo-5-(5-trifluoromethyl-tetrazal-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (**2b**): An aliquot of crude **6** (~13 mCi) was concentrated to dryness and treated with methylene chloride (0.1 ml) and TFA (0.1 ml) and stirred at room temperature for 15 min. The reaction mixture was concentrated to dryness and treated with DMF (0.05 ml) and several milligrams of Cs_2CO_3. A 10 µl aliquot of a solution of 1-bromo-2-fluoroethane (3 µl) in DMF (0.5 ml) was added to the reaction solution. After stirring overnight at room temperature an additional 10 µl of the 1-bromo-2-fluoroethane/DMF solution was added and the reaction was stirred for two additional hours. The reaction was quenched with H_2O (20 µl) and purified by HPLC [C18 Vydac, 4.6 × 250 mm, 15 min linear gradient @ 1 ml/min, 10% acetonitrile:H_2O (0.1% TFA) to 95% acetonitrile, retention time ~13.5 min]. The center cut fraction (earlier fractions were contaminated with the unalkylated phenol which elutes at ~12.5 min under these conditions) was concentrated and diluted with ethanol (1 ml) to give 0.97 mCi of **2b**. The specific activity was estimated to be 14 Ci/mmol based on the specific activity of **7**. The radiochemical purity was >98%.

Acknowledgements

The authors wish to thank Chemsyn Science Laboratories for carrying out the gas tritiation reactions described here, Marc Berridge (3D Imaging) for providing the fluoromethyl iodide, Gerard Kieczykowski (Merck Research Laboratories) for providing **3** and 2-hydroxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine, bis HC1 salt (compound **7**, unlabelled) used to synthesize **2a** and to determine the specific activity of **7** and S. Pitzenberger and J. Murphy (Merck Research Laboratories) for carrying out the NOESY ^{1}H NMR analysis of **1a** and **2a**.

References

1. Saria A. *Eur J Pharmacol* 1999; **375**: 51–60.
2. Rupniak NMJ, Kramer MS. *Trends Pharmacol Sci* 1999; **20**: 485–490.
3. Burns HD, Goldberg M, Sciberras D, Hietala J, Solin O, Eskola O, Laakso A, Gronroos T, Forsback S, Bergman J, Haaparanta M, Bergstrom M, Ogren M, Langstrom B, Brooks D, Luthra S, Turjanski N, Hamill TG, Ryan C, Eng W-s, Sanabria-Bohorquez SM, Coimbra A, Gibson R, Petty K, Reines S, Hargreaves RJ. *NeuroImage* 2002; **16**: S5.
4. Barluenga J, Gonzalez JM, Garcia-Martin MA, Campos PJ, Asensio G. *J Org Chem* 1993; **58**: 2058–2060.
5. Zheng L, Berridge MS. *Appl Radiat Isotop* 2000; **52**: 55–61.

