

Research Article

Synthesis of [N-methyl-¹¹C]-3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidine-7-amine: A potential PET ligand for *in vivo* imaging of CRF₁ receptors

J.S. Dileep Kumar^{1,*}, Vattoly J. Majo¹, Jaya Prabhakaran¹,
Norman R. Simpson², Ronald L. Van Heertum² and
J. John Mann^{1,2}

¹ *Department of Psychiatry and Division of Neuroscience, Columbia University and New York State Psychiatric Institute, 1051 Riverside Drive, Box: 42, New York, NY 10032, USA*

² *Department of Radiology, Columbia University, New York, NY 10032, USA*

Summary

A convenient synthesis of [N-methyl-¹¹C]-3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidine-7-amine (R121920), a highly selective CRF₁ antagonist has been developed as a potential PET ligand. 3-[(6-methylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidine-7-amine (**7**), the precursor for radiolabelling was synthesized through a novel palladium catalyzed Suzuki coupling of aryl bromide **5** with heteroaryl boronate ester **4**. The requisite boronate ester **4** was synthesized in four steps from 2-amino-4-bromopyridine in 50% overall yield. Although the synthesis of cold R121920 proceeded in 93% yield by sodium hexamethyldisilazide (NaHMDS) mediated N-methylation of the desmethylamine **7** at –78°C, the attempted radiosynthesis under various conditions using

*Correspondence to: J. S. Dileep Kumar, Department of Psychiatry and Division of Neuroscience, Columbia University and New York State Psychiatric Institute, 1051 Riverside Drive, Box: 42, New York, NY 10032, USA. E-mail: dk2038@columbia.edu

Contract/grant sponsor: National Institutes of Health; contract/grant number: R21MH066620

conventional bases were not successful. However, the radiolabeling of [^{11}C]R121920 was successfully carried out with [^{11}C]MeOTf in acetone at -20°C in the absence of added basic reagents. The radiotracer was purified by RP-HPLC followed by RP-solid phase extraction. The yield of the reaction was 5% (at EOB) and the specific activity was $>1000\text{ Ci/mmol}$ (at EOB) with a radiochemical purity $>99\%$. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: CRF antagonist; positron emission tomography; R121920; neuropsychiatric diseases

Introduction

Corticotropin releasing factor (CRF or CRH) is a 41 amino acid neurotransmitter isolated and sequenced by Vale and coworkers.^{1,2} The functions of CRF are mainly mediated through two CRF receptor subtypes (CRF₁ and CRF₂) and a CRF binding protein (CRF-BP).³ The CRF₁ receptors are the most abundant CRF receptor subtype found in rodent and primate pituitary and are widely distributed in the brain including cerebral cortex, hippocampus and amygdala. The CRF₂ receptors are primarily distributed in lateral septum, choroid plexus, hypothalamus and sympathetic nuclei.⁴ The CRF binding protein is widely distributed in the central nervous system (CNS) including hippocampus and amygdala and in human, but not in rodent, it is also found in plasma.⁴ Abnormal expression of CRF₁, mostly over-expression, comprises part of the pathogenesis of a diverse range of neuropsychiatric disorders such as anxiety disorders, mood disorders, obsessive-compulsive disorders, posttraumatic stress disorders and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.^{5–10} One of the potential therapeutic strategies to block the effect of CRF elevation is the utilization of selective non-peptidic CRF₁ receptor antagonists.¹¹ To be effective, these molecules must have receptor subtype specificity (a high CRF₁/CRF₂ ratio for affinity), aqueous solubility, good oral bioavailability and rapid permeability across the blood brain barrier (BBB).

Over the years many research laboratories and pharmaceutical companies have developed a variety of small molecule non-peptide CRF₁ antagonists and some of them have undergone clinical studies (Figure 1).¹² Most of the published preclinical studies using CRF₁ receptor antagonists are based on CP-154526 and antalarmin.¹³ According to these reports CP-154526 binds with high affinity

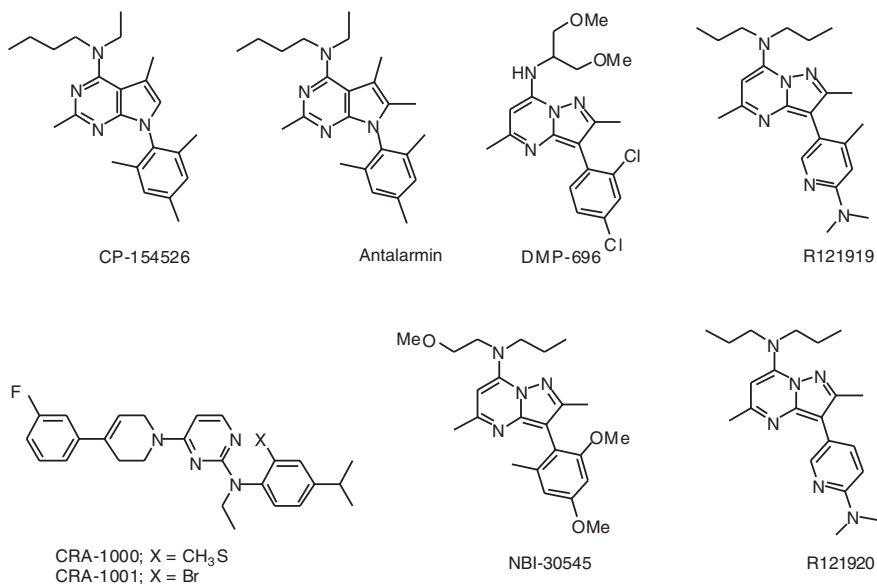


Figure 1. Highly selective CRF₁ receptor antagonists identified recently.

($K_i = 2.7 \text{ nmol/l}$) to CRF₁ receptor and inhibits intracellular signaling through g-protein coupled receptor. The intraperitoneal administration of this compound reduces anxiety-like behavior assessed on an elevated level and shows antidepressant-like effects.^{14,15} Although the radio-synthesis of a number of fluorinated and iodinated analogues of CP-154526 and antalarmin have been developed, the details of the *in vivo* validations of these tracers are not reported.^{16–18}

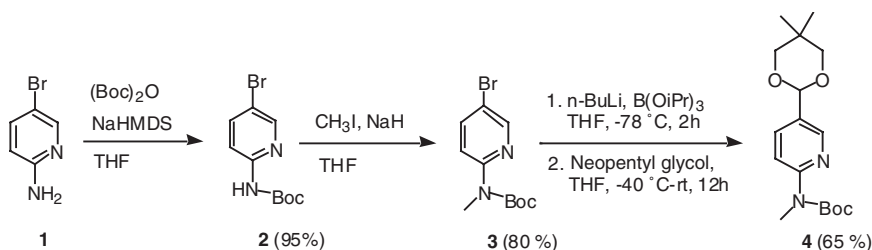
DMP-696, is another potent CRF₁ receptor antagonist ($K_i = 1.6 \text{ nmol/l}$), which reduces stress induced anxiety related behavior without compromising the HPA system in rodent and primates models.¹⁹ Similarly CRA 1000 and CRA 1001 also show potency against CRF-induced conditioned fear.²⁰

NBI 30545 is a highly selective CRF₁ receptor antagonist, which could not get into clinical trial due to poor BBB permeability and the lack of its aqueous solubility.²¹ However, R121919, one of the second-generation CRF₁ receptor antagonists in the NBI series was found to be a high affinity ligand ($K_i = 3 \text{ nmol/l}$) with selectivity greater than 1000 times that of CRF₂ receptor.²² After successful pre-clinical trials against anxiety-like behaviors, R121919 was used in a clinical trial for patients with major depression.²³ However, a parallel toxicological study in normal controls showed significant increases in liver enzymes and

therefore further clinical development of R121919 was discontinued.¹¹ More recently neuroprotective effect of the CRF₁ receptor antagonist 3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-*N,N*-dipropylpyrazolo[1,5-*a*]pyrimidine-7-amine (R121920) was reported in rat models of permanent focal ischemia.²⁴ There is increasing evidence to support the involvement of CRF in the pathogenesis of ischemic brain damage.²⁵ Up-regulation of CRF mRNA has been reported in the cerebral parenchyma of rats after permanent middle cerebral artery occlusion (MCAO) and traumatic brain injury.²⁶ R121920 is a water-soluble high affinity CRF₁ receptor selective antagonist (K_i = 4 nmol/l for CRF₁; K_i = > 10000 nmol/l for CRF₂ receptor), which rapidly crosses the BBB after intravenous administration with a peak brain concentration approximately 2-fold greater than those in plasma.²⁴ Hence targeting R121920 as an imaging probe might provide the detailed pathophysiology of CRF expression in brain. Herein we report the synthesis of [¹¹C]R121920, a potential PET imaging probe for CRF₁ receptors.

Results and discussion

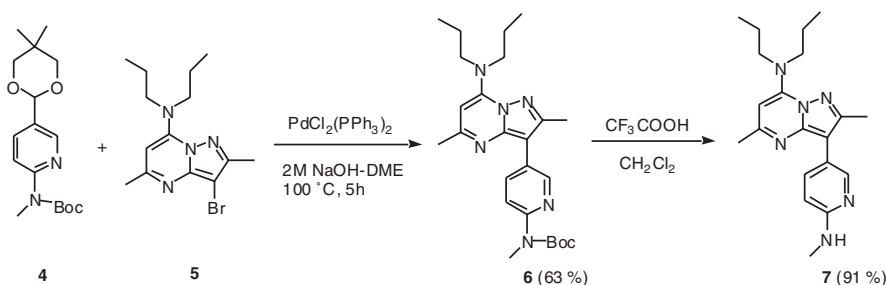
The key step in the synthesis of 3-[(6-methylamino)pyridin-3-yl]-2,5-dimethyl-*N,N*-dipropylpyrazolo[1,5-*a*]pyrimidine-7-amine (**7**), the demethyl precursor for radiosynthesis was envisaged to be a biaryl coupling of aryl bromide **5** with arylboronate ester **4**. To this end, 3-bromo-2,5-dimethyl-7-dipropylaminopyrazolo[1,5-*a*]pyrimidine (**5**) was synthesized by the condensation of 3-iminobutyronitrile and 3-amino-4-bromo-5-methylpyrazole followed by dipropylation with *n*-propyl iodide in presence of NaH in *N,N*-dimethyl formamide.²⁷ [5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)pyridin-2-yl)methylcarbamic acid *tert*-butyl ester (**4**), the requisite counterpart for the biaryl coupling was prepared by the following sequence of reactions as represented in Scheme 1. 2-Amino-4-bromopyridine (**1**) was protected as its *N*-Boc derivative in 95% yield in the presence of NaHMDS. Subsequent methylation of **2** was achieved with methyl iodide in the presence of NaH to afford **3**. Initially, we prepared the requisite Boc-protected 6-methylamino-3-pyridylboronic acid by lithiation of the corresponding bromide **3** using *n*-BuLi at -78°C followed by boronylation with B(O*i*Pr)₃. However, the difficulty in isolation, purification and handling of this boronic acid prompted us to use the corresponding neopentyl boronate ester **4** in the Suzuki coupling, which could be easily prepared



Scheme 1. Synthesis of boronate ester **4**

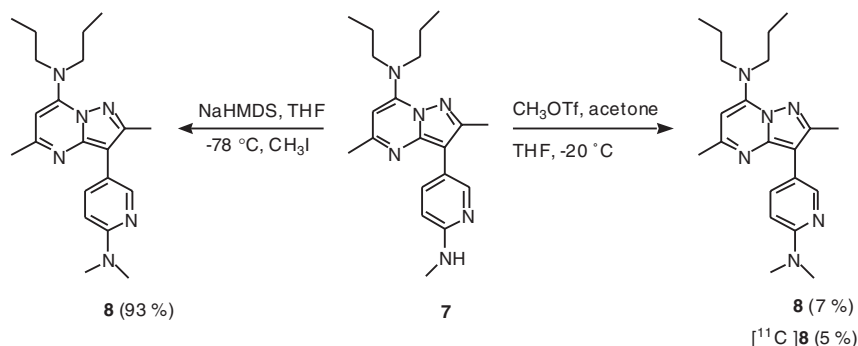
in good yield by the in situ quenching of the boronylation reaction mixture with neopentyl glycol at -40°C .

We then set out to optimize the yield of the Suzuki reaction of heteroaryl bromide **5** with phenylboronic acid under several conditions using various palladium catalysts and bases.²⁸ The ideal condition for the coupling was obtained by using dichlorobis(triphenylphosphine)-palladium (II) ($\text{PdCl}_2(\text{PPh}_3)_2$) as the catalyst in the presence of 3 equivalents of 2 M NaOH in DME- H_2O . Under these conditions, the biaryl coupling of **4** with **5** in the presence of catalytic amount of $\text{PdCl}_2(\text{PPh}_3)_2$ proceeded to give the 3-arylpyrazolo[1,5-a]pyrimidine derivative **6** in 63% yield (Scheme 2). The deprotection of the Boc group was then achieved with 50% trifluoroacetic acid in dichloromethane to yield the desmethyl precursor **7** (Scheme 2).



Scheme 2. Synthesis of the precursor amine **7**

The synthesis of cold R121920 was achieved in 93% yield by sodium hexamethyldisilazide mediated N-methylation of **7** using methyl iodide at -78°C (Scheme 3). Various bases such as NaH, NaOH, K_2CO_3 and KOtBu were ineffective for the desired N-methylation of **7**, and instead, formation of more polar products were observed in these reactions. However, our labeling experiments of **7** with [^{11}C]MeI or [^{11}C]MeOTf in the



Scheme 3. Synthesis of R121920 and [^{11}C]R121920.

presence of NaHMDS did not afford [^{11}C]R121920. This may be due to the interaction of hexamethyldisilazide with the trace amount of [^{11}C]MeI or [^{11}C]MeOTf, which is the limiting reagent under hot experimental conditions. The use of other conventional bases such as NaOH and KO t Bu were also not effective for the [^{11}C]N-methylation reaction due to the predominant formation of polar radiolabelled products. The radiolabeling was finally achieved by the use of [^{11}C]MeOTf in acetone at -20°C without using any base (Scheme 3). The crude product was purified by reverse-phase HPLC to afford [^{11}C]8 in 5% (EOB) yield. Under identical conditions, reaction of 7 with methyl triflate at -20°C in acetone provided 8 in 7% yield. The chemical identity of [^{11}C]8 was confirmed by co-injection with the authentic sample of 8 on analytical reverse-phase HPLC (Figure 2). The specific activity was calculated based on a standard mass curve using HPLC technique. The chemical and radiochemical purity of [^{11}C]8 was found to be $>99\%$ with a specific activity >1000 Ci/mmol (EOB).

Conclusions

A convenient method for the synthesis of [N-methyl- ^{11}C]-3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-*N,N*-dipropylpyrazolo[1,5-*a*]pyrimidine-7-amine ([^{11}C]R121920) and its desmethyl precursor 7 is achieved. The total time required for the synthesis of [^{11}C]R121920 was 30 min from EOB by using [^{11}C]-methyl triflate in acetone at -20°C . The radiochemical purity was $>99\%$ with a specific activity >1000 Ci/mmol. Thus the synthesis of [^{11}C]R121920, a highly selective CRF $_1$ nonpeptide antagonist has been developed as a potential radioligand for the *in vivo* quantification of CRF $_1$ receptors using PET.

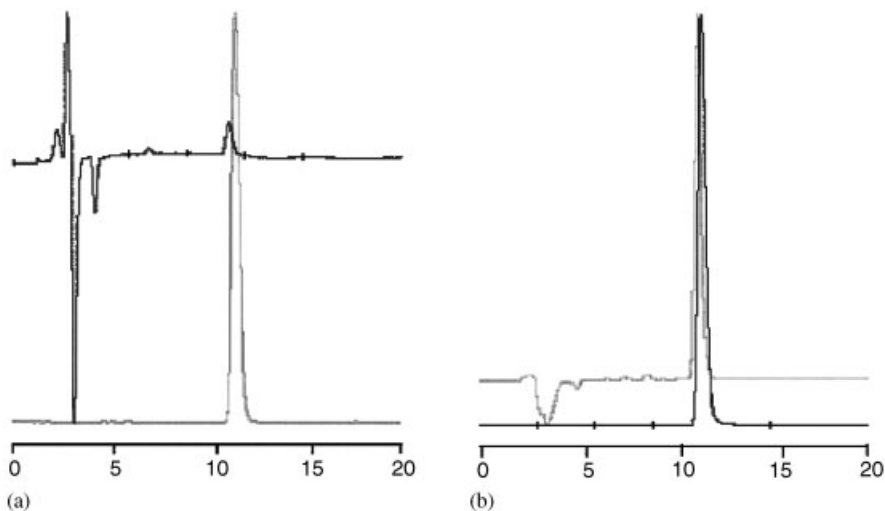


Figure 2. (a) Radiosynthesis of [^{11}C]R121920 and (b) co-injection with R121920.

Experimental

Melting points were determined on a Fisher Melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker PPX 300 and 400 MHz spectrometer. Spectra are recorded in CDCl_3 and chemical shifts are reported in ppm relative to TMS as internal standards. The mass spectra were recorded on JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer in the FAB+ mode. The HPLC analyses were performed using Waters 1525 HPLC system (column: Phenomenex, Prodigy ODS 4.6×250 mm, 5μ). Flash column chromatography was performed on silica gel (Fisher 200–400 mesh) using the solvent system indicated. [^{11}C]-Methyl triflate was synthesized in the radioligand laboratory of Columbia University by transferring [^{11}C]-methyl iodide through a glass column containing AgOTf at 200°C .²⁹ The radiochemical and chemical purities were analyzed by RP-HPLC with PDA and NaI detectors.

[5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)pyridin-2-yl]methylcarbamate tert-butyl ester (4)

A solution of the aryl bromide **3** (630 mg, 2.195 mmol) in THF (10 ml) under argon was treated with *n*-BuLi (1.0 ml, 2.415 mmol, 2.5 M) dropwise at -78°C and allowed to stir for 20 min. Triisopropyl borate

(1.0 ml, 4.349 mmol) was rapidly added to this solution and the reaction mixture was further stirred for 2 h; maintaining the temperature at -78°C . The temperature was then gradually allowed to reach -40°C and finely powdered neopentyl glycol (250 mg, 2.195 mmol) was added. The solution was warmed to r.t. and stirred for further 12 h. The reaction mixture was then quenched with water, the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (3×20 ml). The combined organic layer was concentrated and column chromatographed (70:30 hexane: EtOAc) to give 500 mg (65%) of the boronate ester **4** as a viscous solid. ^1H NMR (400 MHz, CDCl_3): δ 8.70 (d, 1 H, $J=1.8$ Hz, Ar-H), 7.95 (dd, 1 H, $J=1.9$, 8.4 Hz, Ar-H), 7.63 (d, 1 H, $J=8.3$ Hz, Ar-H), 3.76 (s, 4 H, $2 \times \text{OCH}_2$), 3.41 (s, 3 H, NCH_3), 1.51 (s, 9 H, NBoc), 1.02 (s, 6 H, $2 \times \text{CH}_3$); HRMS calculated for $\text{C}_{16}\text{H}_{26}\text{BN}_2\text{O}_4$ (MH^+): 321.1986, found: 321.2010.

[5-(7-Dipropylamino-2,5-dimethyl-1H-pyrazolo[1,5-a]pyrimidin-3-yl)-pyridin-2-yl]methyl carbamic acid tert-butyl ester (6)

A suspension of the aryl bromide **5** (174 mg, 0.535 mmol), aryl boronate ester **4** (150 mg, 0.643 mmol) and $\text{PdCl}_2(\text{PPh}_3)_2$ (37 mg, 10 mol%) in 1,2-dimethoxyethane (2 ml) was thoroughly deaerated and stirred under argon. Deionised water (200 μl) and aqueous NaOH (550 μl , 2 M) were added to the reaction mixture and heated at 100°C for 5 h. The reaction mixture was diluted with EtOAc, dried over MgSO_4 , passed through a short pad of celite, concentrated and column chromatographed (90:10 Hexane: EtOAc) to give the desired product as a pale yellow viscous solid in 150 mg (63%) yield. ^1H NMR (400 MHz, CDCl_3) δ 8.77 (d, 1 H, $J=2.3$ Hz, Ar-H), 8.05 (dd, 1 H, $J=2.4$, 8.6 Hz, Ar-H), 7.65 (d, 1 H, $J=8.5$ Hz, Ar-H), 5.82 (s, 1 H, $^6\text{C-H}$), 3.71 (t, 4 H, $J=7.8$ Hz, $2 \times \text{NCH}_2$), 3.43 (s, 3 H, NCH_3), 2.56 (s, 3 H, Ar- CH_3), 2.46 (s, 3 H, Ar- CH_3), 1.72 (m, 4 H, $2 \times \text{CH}_2$), 1.53 (s, 9 H, NBoc), 0.95 (t, 6 H, $J=7.4$ Hz, $2 \times \text{CH}_3$); HRMS calculated for $\text{C}_{25}\text{H}_{37}\text{N}_6\text{O}_2$ (MH^+): 453.2978, found: 453.2989.

3-[(6-methylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidine-7-amine (7)

A solution of the Boc-amine **6** (68 mg, 0.1504 mmol) in CH_2Cl_2 (1 ml) was treated dropwise with CF_3COOH (1 ml) and was stirred at r.t. for 3 h. The reaction mixture was then diluted with CH_2Cl_2 and neutralized

with saturated NaHCO_3 solution. The aqueous layer was extracted with EtOAc, the combined organic layer was washed with H_2O , brine and concentrated and column chromatographed (4% CH_2Cl_2 in CH_3OH) to yield the product as pale yellow solid in 91% (48 mg) yield. m.p. $136\text{--}137^\circ\text{C}$, ^1H NMR (400 MHz, CDCl_3): δ 8.39 (d, 1 H, $J=9.0$ Hz, Ar-H), 8.14 (s, 1 H, Ar-H), 6.79 (d, 1 H, $J=9.2$ Hz, Ar-H), 5.82 (s, 1 H, $^6\text{C-H}$), 3.71 (t, 4 H, $J=7.3$ Hz, $2 \times \text{NCH}_2$), 3.00 (s, 3 H, N- CH_3), 2.52 (s, 3 H, Ar- CH_3), 2.46 (s, 3 H, Ar- CH_3), 1.71 (m, 4 H, $2 \times \text{CH}_2$), 0.95 (t, 6 H, $J=7.2$ Hz, $2 \times \text{CH}_3$); HRMS calculated for $\text{C}_{20}\text{H}_{29}\text{N}_6$ (MH^+): 353.2431, found: 353.2454.

3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo [1,5-a]pyrimidine-7-amine (8)

A stirred solution of the amine **7** (30 mg, 0.085 mmol) in THF (1.0 ml) was treated dropwise at -78°C with NaHMDS (1.0 M in THF, 0.128 mmol, 128 μl). A solution of methyl iodide (10% v/v in THF, 159 μl , 0.256 mmol) was added to the reaction mixture and stirred further for 20 min, maintaining the temperature at -78°C . The reaction was quenched by the addition of water. The reaction mixture was diluted with CH_2Cl_2 , dried over MgSO_4 , concentrated and column chromatographed (4% methanol in CH_2Cl_2) to give the product as a pale yellow solid in 93% (28 mg) yield. m.p. 102°C , ^1H NMR (300 MHz, CDCl_3): δ 8.48 (d, 1 H, $J=2.3$ Hz, Ar-H), 7.91 (dd, 1 H, $J=2.3, 8.8$ Hz, Ar-H), 6.65 (d, 1 H, $J=8.8$ Hz, Ar-H), 5.79 (s, 1 H, $^6\text{C-H}$), 3.70 (t, 4 H, $J=7.7$ Hz, $2 \times \text{NCH}_2$), 3.12 (s, 6 H, N(CH_3) $_2$), 2.54 (s, 3 H, Ar- CH_3), 2.45 (s, 3 H, Ar- CH_3), 1.70 (m, 4 H, $2 \times \text{CH}_2$), 0.94 (t, 6 H, $J=7.4$ Hz, $2 \times \text{CH}_3$); HRMS calculated for $\text{C}_{21}\text{H}_{31}\text{N}_6$ (MH^+): 367.2610, found: 367.2599.

*Radiosynthesis of [N-methyl- ^{11}C]-3-[(6-dimethylamino)pyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo [1,5-a]pyrimidine-7-amine ([^{11}C]**8**)*

The desmethylamine **7** (1.5 mg) was dissolved in 400 μl of anhydrous acetone in a capped 5 ml V-vial and was cooled to -20°C . [^{11}C]-Methyl triflate was transported by a stream of argon (20–30 ml/min) into the vial for 5 min at -20°C . At the end of the trapping, the reaction mixture was directly injected into a semi preparative RP-HPLC (Phenomenex C18, 10×250 mm, 10 μ) and eluted with a solvent system (flow rate: 5 ml/min) comprising of acetonitrile: water: triethylamine (85: 15: 0.3).

The precursor **7** appeared at 5–6 min during the HPLC analyses. The product fraction with a retention time of 9–10 min based on γ -detector was collected, diluted with 100 ml of deionized water, and passed through a classic C-18 Sep-Pak cartridge. The Sep-Pak was further washed with deionized water (2×50 ml) to ensure the complete removal of triethylamine. Reconstruction of the product in 1 ml of absolute ethanol afforded [^{11}C]**8** (5% yield based on EOB). A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex C18) to determine the specific activity and radiochemical purity. Then the final solution of the [^{11}C]**8** in 10% ethanol-saline was analyzed to confirm the chemical and radiochemical purities.

Acknowledgements

This work was supported by a research grant from the National Institutes of Health (R21MH066620). The authors also thank Ms Julie Arcement for her assistance in the radiolabeling studies.

References

1. Owens MJ, Nemeroff CB. *Pharmacol Rev* 1991; **43**(4): 425–473.
2. Chalmers DT, Lovernberg TW, Grigoriadis DE, Behan DP, De Souza EB. *Trends Pharmacol Sci* 1996; **17**(4): 166–172.
3. Gilligan PJ, Robertson DW, Zaczek R. *J Med Chem* 2000; **43**(9): 1641–1660.
4. De Souza EB. *Psychoneuroendocrinology* 1995; **20**(8): 789–819.
5. O'Brien D, Skelton KH, Owens, MJ, Numeroff CB. *Hum Psychopharmacol Clin Exp* 2001; **16**:81–87.
6. Skutella T, Probst JC, Renner U, Holsboer F, Behl C. *Neuroscience* 1998; **85**(3):795–805.
7. Mitchell AJ. *Neurosci Biobehav Rev* 1998; **22**(5): 635–651.
8. Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A. *Behav Brain Res* 1999; **100**(1–2): 207–215.
9. Behan DP, Grigoriadis DE, Lovenberg T, Chalmers D, Heinrichs S, Liaw C, De Souza EB. *Mol Psychiatry* 1996; **1**(4): 265–277.
10. De Souza EB, Whitehouse PJ, Kuhar MJ, Price DL, Vale WW. *Nature* 1986; **319**(6054): 593–595.
11. Holsboer F. *CNS Spectrums* 2001; **6**(7): 590–594.
12. McCarthy JA, Heinrichs SC, Grigoriadis DE. *Annu Rep Med Chem* 1999; **34**: 11–20.

13. Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD, Winston EN, Chen YL, Heym J. *Proc Natl Acad Sci USA* 1996; **93**(19): 10477–10482.
14. Hikichi T, Akiyoshi J, Yamamoto Y, Tsutsumi T, Isogawa K, Nagayama H. *Pharmacopsychiatry* 2000; **33**(5): 189–193.
15. Arborelius L, Skelton KH, Thrivikraman KV, Plotsky PM, Schulz DW, Owens MJ. *J Pharm Exp Therapeutics* 2000; **294**(2): 588–597.
16. Martarello L, Kilts CD, Ely T, Owens MJ, Nemeroff CB, Camp M, Goodman MM. *Nucl Med Biol* 2001; **28**(2): 187–195.
17. Tian X, Hsin L-W, Webster EL, Contoreggi C, Chrousos GP, Gold PW, Habib K, Ayala A, Eckelman WC, Jacobson AE, Rice KC. *Bioorg Med Chem Lett* 2001; **11**(3): 331–333.
18. Hsin L-W, Webser, EL, Chrousos GP, Gold PW, Eckelman WC, Contoreggi C, Rice KC. *J Label Compd Radiopharm* 2000; **43**(9): 899–908.
19. Hodge CN, Aldrich PE, Zelda R, Wasserman ZR, Fernandez CH, Nemeth GA, Arvanitis A, Cheeseman RS, Chorvat RJ, Ciganek E, Christos TE, Gilligan PJ, Krenitsky P, Cholfield E, Strucely P. *J Med Chem* 1999; **42**: 819–832.
20. Chaki S, Okuyama S, Nakazato A, Nakazato A, Kumagai T, Okubo T, Ikeda Y, Oshida Y, Hamajima Y, Tomisawa K. *Eur J Pharmacol* 1999; **71**: 205–211.
21. Wilcoxon K, Chen C, Huang C, Haddach M, Xie M, Wing L, Grigoriadas D, De Souza EB, McCarthy JR. Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21–25, 1999, MEDI-002.
22. Heinrichs SC, De Souza EB, Schulteis G, Lapsansky JL, Grigoriadis DE. *Neuropsychopharmacology* 2002; **27**(2): 194–202.
23. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F. *J Psy Res* 2000; **34**(3): 171–181.
24. Mackay KB, Bozigian H, Grigoriadis DE, Loddick SA, Verge G, Foster AC. *J Cereb Blood Flow Metab* 2001; **21**(10): 1208–1214.
25. Strijbos PJ, Relton JK, Rothwell, NJ. *Brain Res* 1994; **656**(2): 405–408.
26. Beaumont A, Marmarou C, Marmarou A. *Neurol Res* 2000; **22**(7): 665–673.
27. Takamizawa A, Hamashima Y. *Jpn Tokkyo Koho* 1966; JP 41015583.
28. Majo VJ, Prabhakaran J, Mann JJ, Kumar JSD. *Adv Synth Catal* 2003; **345**: 620–624.
29. Jewett DM. *Nucl Med Biol* 2001; **28**(6): 733–734.