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[^{76}Br]BMK-I-152, a non-peptide analogue for PET imaging of corticotropin-releasing hormone type 1 receptor (CRHR1)

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The study of corticotropin-releasing hormone is of significant interest in mental health. We have developed a radiobromination procedure for the preparation of [^{76}Br]BMK-I-152, a high-affinity corticotropin-releasing hormone type 1 receptor antagonist. The radiobromination procedure resulted in the formation of two radiobrominated products from the same trialkyltin precursor. Utilizing the results of several reaction conditions and the chromatographic and mass spectral data obtained from Waters Acquity and Q-TOF, we determined that both 3-bromo and 4-bromo isomers could be obtained. The authentic sample of the 3-bromo isomer was prepared to confirm the identity of a previously unknown radioactive side product; affinity assays revealed that the 4-bromo isomer had ~70 times higher affinity than that of the 3-bromo compound. By manipulation of reaction conditions, the individual products could be selected. Under no-carrier-added conditions at room temperature in aqueous acetonitrile, the major radioactive product (>80%) was identified as the 3-[^{76}Br]bromo-4-tributylstannyl analogue of BMK-I-152. The 4-[^{76}Br]bromo isomer accounted for less than 1% of the total activity. The 3-[^{76}Br]bromo BMK-I-152 could be obtained by treating this intermediate with trifluoroacetic acid to effect removal of the trialkyltin. If the radiobromination was conducted after first evaporating the water from the aqueous ammonium hydroxide solution of [^{76}Br]bromide, the desired 4-[^{76}Br]bromo isomer was obtained with a 58% radiochemical yield.

Keywords: corticotropin-releasing hormone; radiobromination; bromine-76; CRH; PET

Introduction

Considerable effort has been put into the development of an imaging probe for corticotropin-releasing hormone receptors (CRHR). Corticotropin-releasing hormone (CRH) is an endogenous 41-amino-acid neuropeptide found in the brain and was first isolated from the hypothalamus of sheep.[1] The CRH system plays an important role in regulating the brain functions in response to stress. Abnormal function of CRH receptors has been implicated in anxiety,[2] depression[3] and stress-related syndromes.[4–6] Specifically, CRHR1 densities were reduced in the prefrontal cortex of patients with depression, while CRHR1 densities were increased in the cerebral cortex of Alzheimer's patients.[7,8] A PET ligand for CRHR1 would be able to monitor *in vivo* receptor changes in normal and abnormal states and evaluate the effect of treatment of disorders involving the CRH system. BMK-I-152 [8-(4-bromo-2,6-dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-4-yl]-*N*,*N*-*bis*(2-methoxyethyl) amine is an improved non-peptide high-affinity ligand designed for imaging CRH type 1 receptor over our previously reported ligand MJL 1-109-2 labeled with bromine-76 (Figure 1).[9] Previous attempts to develop a PET or a SPECT ligand based on antalarmin or CP-154,526 yielded ligands with very high lipophilicity and, thus, were unsuitable for imaging the brain.[10,11] [^{76}Br]MJL 1-109-2, a derivative of CP-154,526 with *bis*-methoxyethyl substituent on the 4-amino of the triazolotriazine, was the first high-affinity radioligand that crossed the blood brain barrier due to its lower lipophilicity (C log $p = 3.05$). Recently, a C-11-labeled compound C-11 DMP696 with a chemical structure similar to that of MJL 1-109-2 has also been evaluated for PET imaging.[12] Our newest molecule, BMK-I-152, contains two extra methoxy groups on the phenyl ring further

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reducing the lipophilicity, increasing the brain uptake, and reducing the non-specific binding. In this article, we describe the radiochemical challenges in the radiobromination of BMK-I-152.

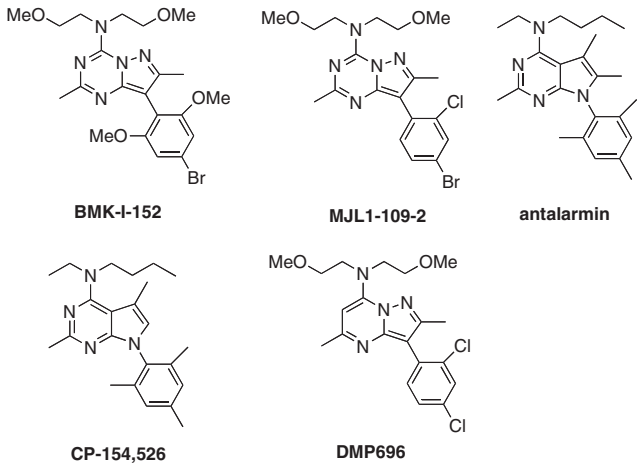


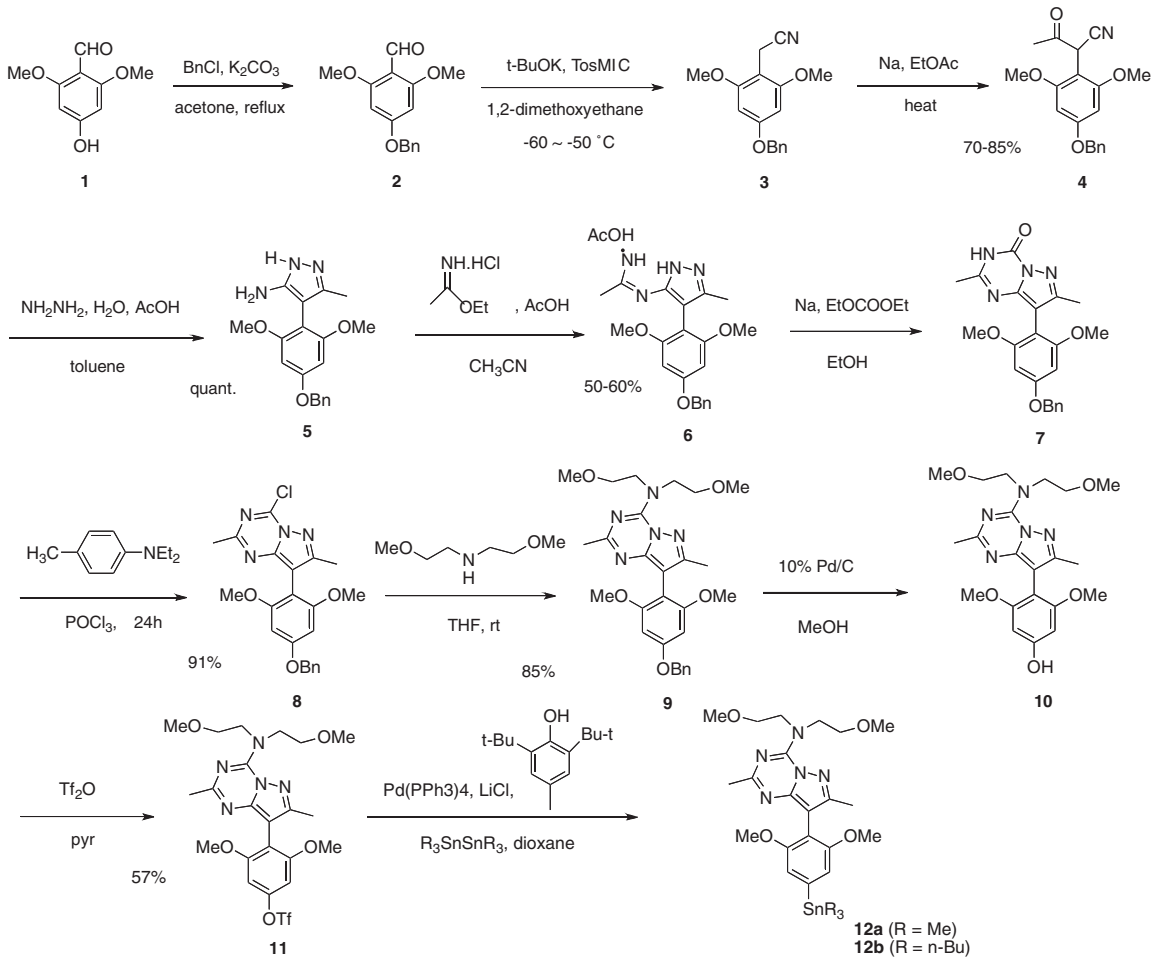
Figure 1. Structures of CRH ligands.

Results and discussion

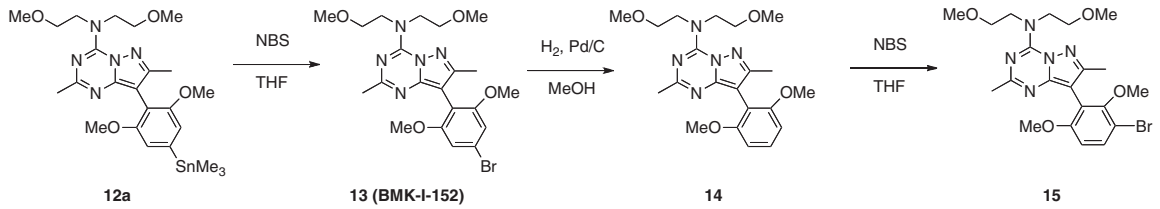
Chemistry

The syntheses of BMK-I-152 (**13**), its 3-bromo analogue (**15**), and its tributylstannyl analogue (**12b**), the precursor for radiolabeling, are outlined in Schemes 1 and 2. A commercially available aldehyde (**1**) was protected with a benzyl group on its phenol moiety and the corresponding benzyl ether **2** was then treated with toluenesulfonylmethyl isocyanide (TosMIC)[13] to provide the arylacetonitrile derivative **3** in 84% yield. Synthesis from the cyanide **3** to compound **9** was carried out basically following the procedure developed by DuPont chemists.[14] Deprotection of the benzyl protecting group (H_2, Pd/C) followed by conversion to triflate yielded compound **11**, which was converted to the trimethyltin precursor **12a** or tributyltin precursor **12b** for the radiobromination.

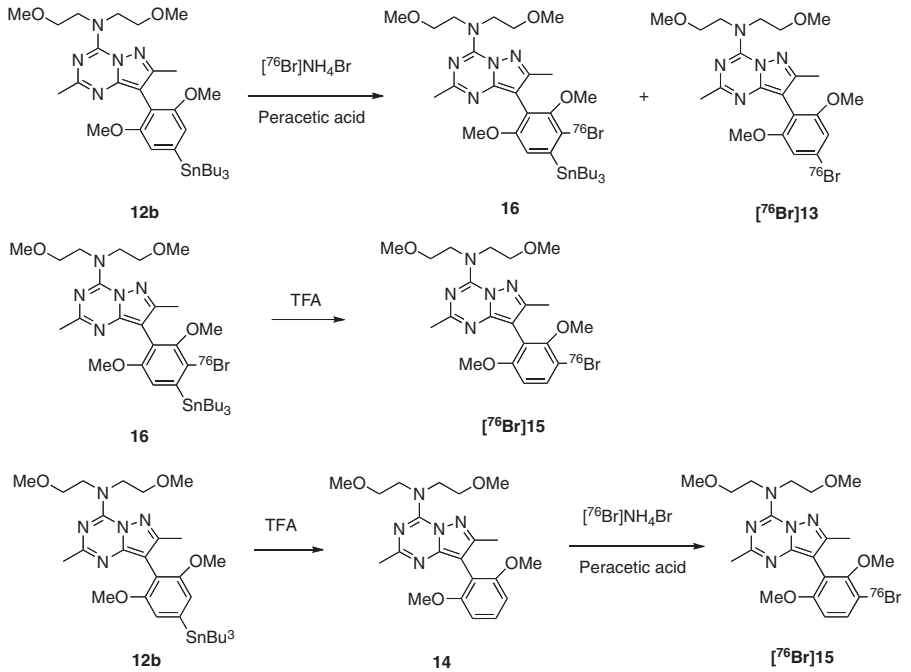
The trimethyltin precursor **12a** was treated with *N*-bromosuccinimide (NBS) in THF to provide the non-radioactive 4-bromo derivative, **13** (BMK-I-152), almost quantitatively. An authentic standard of the 3-bromo compound **15** was prepared by catalytic hydrogenolysis of the 4-bromo substituent using 10% Pd/C, which provided **14** in 92.6% yield. Treatment of **14** with NBS provided the 3-bromo compound **15**.



Scheme 1. Synthesis of precursors for radiolabeling.



Scheme 2. Synthesis of BMK-I-152 and its 3-bromo analogue.



Scheme 3. Radiosynthesis of [^{76}Br]BMK-I-152 and its 3-bromo analogue.

Radiochemistry

Bromine-76 was produced by cyclotron irradiation of high-purity arsenic targets and isolated by chromic acid dissolution and oxidation followed by simple distillation of [^{76}Br]HBr.[15] The radionuclide was trapped in an aqueous solution of NH_4OH. Our initial radiolabeling attempts followed previously published procedures.[9] An aliquot of aqueous solution containing [^{76}Br]NH_4Br was added to a reaction vial. The trimethylstannyl substrate in acetonitrile was added, followed by the addition of a dilute solution of peracetic acid at room temperature (rt) (Scheme 3). Unlike the previous labeling experience with MJL 1-109-2, only a small amount of radioactive BMK-I-152 was observed. Most of the bromine-76 radioactivity was highly retained on the C-18 HPLC column until a 40+min elution with 20% methanol and 80% acetonitrile. In order to analyze this long-retained peak, a carrier-added reaction was performed. However, when NH_4Br was added at equal molar ratio to the tin substrate, only the desired product was obtained with a low radiochemical yield and the long-retained radioactive peak was negligible. Since this long-retained peak had even longer retention time than the tributyltin precursor, we postulated that this radioactive peak still had the tributyltin attached to the molecule (compound **16** in Scheme 3). To test our hypothesis, the long-retained peak from this no-carrier-added synthesis was collected from the HPLC and treated with trifluoroacetic acid to remove the tributyltin and reaction mixture was re-injected onto the HPLC. A single radioactive product was obtained with the same retention time as the BMK-I-152 standard and the long-retained radioactive component had completely disappeared. After analyzing the tin precursor, we concluded that in order to explain this result, the bromine-76 must be attached at a position ortho to the tributyltin group. We also obtained this radioactive product by treating the tributyltin precursor first with TFA in order to remove the tributyltin and then conduct the direct radiobromination.

In the mean time, we also utilized the newly acquired high-resolution Waters Acquity Ultra Performance LC (UPLC) system coupled with highly sensitive Waters Q-TOF MS to analyze the radioactive products. The observed mass of the long-retained chromatographic peak was *m/z* 783/785, which is consistent with that of tributyltin precursor plus bromine. Most interestingly, the mass spectral data for the products obtained from two TFA treatments to remove tributyltin group before and after radio-bromination as described earlier (*m/z* 493) were consistent with the desired BMK-I-152 but the elution time was not co-incident with the authentic standard prepared by chemical means. The subsequent chemical synthesis and characterization of the 3-bromo isomer (**15**)

proved that the radiochemical product formed was indeed the 3-bromo isomer. The Acquity UPLC chromatography system proved capable of separating 3-bromo from 4-bromo isomers by about 0.2 min, a separation not obtained on standard HPLC columns.

Since the 3-bromo isomer (**15**) only differed from the desired product by a single position change, we decided to investigate the biological properties. To our dismay, the affinity of 3-bromo isomer (**15**) is much lower than that of 4-bromo compound (**13**). We evaluated the K_i's of the two non-labeled isomers in monkey frontal cortex via competition with ^{125}I ovine-CRH, a ligand specific for CRHR1. The BMK-I-152 (**13**) had a K_i of 0.35 ± 0.05 nM, whereas the 3-bromo isomer (**15**) had a K_i of 24.4 ± 4.9 nM.

In light of the *in vitro* affinity data, we required a new synthetic approach to allow the high-yield preparation of the 4-[^{76}Br]bromo isomer (**13**). We first tested the effect of temperature on the labeling yield. By gradually increasing the temperature, the yield of the desired product showed an upward trend with the yield reaching about 5% at 100°C. At higher temperatures (120°C in acetonitrile), the yield of 4-[^{76}Br]bromo compound was increased to 10% with the 3-[^{76}Br]bromo compound still as the major product. We then concluded that lower temperature favored the formation 3-[^{76}Br]bromo isomer, whereas higher temperature favored the formation of 4-[^{76}Br]bromo isomer. With even higher temperatures (150°C, DMF as the solvent and preheating before adding the peracetic acid), the yield increased to 30%; however, the product also contained the 3-[^{76}Br]bromo isomer (**15**), which was not separable from the 4-[^{76}Br]bromo isomer (**13**) using regular reversed-phase HPLC. The 3-[^{76}Br]bromo isomer (**15**) was probably produced at a high temperature from the 3-[^{76}Br]bromo tributyltin compound due to the presence of acetic acid. Although we had a procedure that would give useable amounts of the 4-[^{76}Br]bromo isomer, the reaction was variable as to yield and ratio of the 3-[^{76}Br]bromo to the 4-[^{76}Br]bromo isomer.

We formed the hypothesis that the high-temperature reaction procedure would result in evaporation of most of the water. The reaction was done in an open vial with small volumes of aqueous [^{76}Br]bromide and the precursor was preheated before the addition of the oxidant. Perhaps the presence of water was detrimental to the desired formation of the 4-isomer via bromodestannylation. We changed the procedure to first evaporate the aqueous [^{76}Br]-bromide to dryness, followed by the addition first of the substrate in dry DMF and, second, by oxidizing the reagent with a minimal amount of water. Under these conditions, the 4-[^{76}Br]bromo isomer was obtained in 50% radiochemical yield contaminated with about 1% of the 3-[^{76}Br]bromo isomer. The radiochemical yield for 4-[^{76}Br]bromo isomer was high even when the temperature was reduced to 50°C. The final radiochemical product was purified by HPLC and obtained in $57.9 \pm 9.0\%$ ($n = 8$) radiochemical yield with a radiochemical purity of $>98\%$ and a specific activity of 33.0 ± 7.8 GBq/μmol ($n = 4$).

Experimental

General

Chemicals were purchased from Aldrich® and used without further purification unless otherwise noted. TLC analyses were carried out on Analtech silica gel GHLF 0.25 mm plates with UV and I_2 detection. Column chromatography was carried out with silica gel (ICN SiliTech 32-63, 60 Å). Melting points (m.p.) were determined in open glass capillaries on a Thomas-Hoover m.p. apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Gemini spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard. Mass spectra (MS) and HRMS were recorded on JEOL SX 102a. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, USA.

All UPLC-MS experiments were performed with a Waters Q-TOF Premier (Waters, Miford, MA, USA) coupled with Waters Acquity UPLC system. LC/MS analysis employed an Acquity BEH Shield RP18 column (150×2.1 mm) interfaced to the Waters Q-TOF MS. The elution profile had the following components: initial conditions 95% 25 mM ammonium acetate; 5% CH_3CN; gradient 5–95% acetonitrile over 5 min; isocratic elution at 95% acetonitrile for an additional 10 min.

*4-Benzyloxy-2,6-dimethoxybenzaldehyde (**2**)*

To a solution of 2,6-dimethoxy-4-hydroxybenzaldehyde (10.04 g, 52.35 mmol) in acetone (150 mL) were added K_2CO_3 (21.71 g, 157.1 mmol) and benzyl bromide (8.10 mL, 68.06 mmol). The mixture was heated to reflux for 14 h, at which time TLC indicated no starting material. The mixture was cooled to rt, filtered through a fritted glass filter, and concentrated under reduced pressure. The residue was recrystallized from $CHCl_3$/petroleum ether. The precipitate was filtered and washed with copious amount of petroleum ether to provide 14.02 g (98% yield) of tan solid after drying. ^{1}H NMR ($CDCl_3$) δ 0.36 (1H, s), 7.43 (4H, m), 6.16 (2H, s), 5.13 (2H, s), 3.86 (6H, s); m.p. 118–119°C; MS $(M+1)^+$ 273.2; HRMS calcd. for $C_{16}H_{17}O_4$ 273.1127, obsd. 273.1124.

*(4-Benzyloxy-2,6-dimethoxyphenyl)acetonitrile (**3**)*

A solution of TosMIC (Aldrich®, 11.80 g, 60.4 mmol) in 50 mL 1,2-dimethoxyethane was added dropwise to a stirred suspension of *tert*-BuOK (13.70 g, 114 mmol) in 50 mL of 1,2-dimethoxyethane in a three-necked flask while maintaining the reaction temperature at −40°C to −30°C. A solution of 4-benzyloxy-2,6-dimethoxybenzaldehyde (15.0 g, 55.1 mmol) in 1,2-dimethoxyethane (150 mL) was added to the reaction mixture dropwise over a period of about 30 min while keeping the reaction temperature at −60 to −50°C. Stirring was continued for 2.5 h at −60 to −50°C. At this point, TLC (hexane–EtOAc 3:1, v/v) of the reaction mixture showed no trace of the starting material. Methanol (120 mL) was added at once and the mixture was heated to reflux for 15 min. The mixture was cooled to rt, concentrated under reduced pressure, and partitioned between 0.5 M citric acid (100 mL) and dichloromethane (50 mL) and the aqueous layer was extracted with more dichloromethane (50 mL × 2). Combined organic layer was washed with saturated aqueous $NaHCO_3$ solution (50 mL), the organic layer dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ and filtered through a silica gel bed (ca 10 cm) and briefly eluted with hexane–EtOAc 3:1 (v/v). The residue was recrystallized from EtOAc–hexane to give 13.02 g (84% yield) of pale yellow plate crystals. ^{1}H NMR ($CDCl_3$) δ 7.42 (5H, m), 6.21 (2H, s), 5.06 (2H, s), 3.82 (6H, s), 3.60 (2H, s); m.p. 96–97°C; MS $(M+1)^+$ 283; HRMS calcd. for $C_{17}H_{18}O_3N$ 283.1208, obsd. 283.1203; elemental analysis calcd. for $C_{17}H_{17}O_3N$ C 72.07, H 6.05, N 4.94, found C 71.92, H 6.03, N 4.98.

*2-(4-Benzyloxy-2,6-dimethoxyphenyl)-3-oxobutyronitrile (**4**)*

To a solution of (4-benzyloxy-2,6-dimethoxyphenyl)acetonitrile (10.77 g, 38.01 mmol) in ethyl acetate (45 mL) were added

sodium pellets portionwise at rt. Upon completion of adding the sodium, the reaction mixture was heated to reflux. After ca 50 min, a turbid white precipitate started to appear. The reaction mixture was allowed to stir at the reflux temperature for another hour. After cooling to rt, more ethyl acetate (90 mL) was added to the white suspension to facilitate stirring, and the resultant mixture was stirred at rt overnight. The mixture was filtered and the white solid collected was washed with copious amounts of ethyl ether (500 mL). The solid was collected and suspended in water (100 mL) and the solution was acidified to pH = ca 5 using 0.1 N aqueous HCl solution. It was extracted with ethyl acetate (50 mL × 3) and combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. After pumping at high vacuum, the oily residue converted to a white solid (9.08 g, 73% yield). ^{1}H NMR ($CDCl_3$) δ 7.41 (5H, m), 6.24 (2H, s), 5.08 (1H, s), 5.07 (2H, s), 3.82 (6H, s), 2.16 (3H, s); m.p. 109–110°C; MS $(M+1)^+$ 326.2; HRMS calcd. for $C_{19}H_{20}O_4N$ 326.1393, obsd. 326.1390; elemental analysis calcd. for $C_{19}H_{19}O_4N$ C 70.14, H 5.89, N 4.31, found C 70.00, H 5.93, N 4.43.

*4 - (4 - Benzyloxy-2,6 - dimethoxyphenyl)-5-methyl-2H-pyrazol-3 - yl - amine (**5**)*

A mixture of 2-(4-benzyloxy-2,6-dimethoxyphenyl)-3-oxobutyronitrile (7.29 g, 22.4 mmol), hydrazine monohydrate (1.63 mL, 33.6 mmol), and acetic acid (2.14 mL, 37.4 mmol) in toluene (60 mL) was heated to reflux while removing water using a Dean-Stark trap for 5 h. The reaction mixture was cooled to rt and concentrated using rotary evaporation. To the residue was added 6 N aqueous HCl solution and the resulting suspension was extracted with ether (30 mL × 2). The aqueous layer was cooled to 0°C and basified with concentrated ammonium hydroxide solution to pH ca 11. The resulting mixture was extracted with ethyl acetate (100 mL × 3) and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to give a pale yellow foam (7.20 g, 95% yield). ^{1}H NMR ($CDCl_3$) δ 7.41 (5H, m), 6.30 (2H, s), 5.09 (2H, s), 3.75 (6H, s), 2.07 (3H, s); MS $(M+1)^+$ 340.2; HRMS calcd. for $C_{19}H_{22}O_3N_3$ 340.1662, obsd. 340.1655.

*N-[4-(4-benzyloxy-2,6-dimethoxyphenyl)-5-methyl-2H-pyrazol-3-yl]acetamidine; acetic acid salt (**6**)*

Glacial acetic acid (1.21 mL, 21.2 mmol) was added to a stirred mixture of 4-(4-benzyloxy-2,6-dimethoxyphenyl)-5-methyl-2H-pyrazol-3-ylamine (7.15 g, 21.7 mmol) and ethyl acetimidate hydrochloric acid salt (3.93 g, 31.6 mmol) in acetonitrile (90 mL) and the mixture was stirred for 18 h at rt. A white precipitate was formed. Ethyl ether (70 mL) was added and the solid was filtered, washed with ethyl ether (40 mL), and dried under vacuum to give a white solid (7.33 g, 77% yield), which was used directly for the next step. ^{1}H NMR ($CDCl_3$) δ 9.66 (1H, br s), 9.00 (1H, br s), 8.59 (1H, br s), 7.43 (5H, m), 6.42 (2H, s), 5.17 (2H, s), 3.69 (6H, s), 2.30 (3H, s), 2.11 (3H, s), 2.00 (3H, s); m.p. 214–215°C.

*8-(4-Benzyloxy-2,6-dimethoxyphenyl)-2,7-dimethyl-3H-pyrazolo[1,5-a][1,3,5]triazin-4-one (**7**)*

Sodium (5.06 g, 0.22 mol) was added portionwise to absolute ethanol (200 mL) in a three-necked r.b. flask equipped with a condenser. The solution was cooled to rt and *N*-[4-(4-benzyloxy-2,6 - dimethoxyphenyl) - 5 -methyl - 2H - pyrazol-3-yl]acetamidine; acetic acid salt (**6**) (7.33 g, 16.7 mmol) was added followed by diethyl carbonate (21.3 mL, 0.176 mol). The mixture was heated to reflux for 6 h. After cooling to rt, the white precipitate was filtered and washed with fresh ethanol (30 mL). The solid was dissolved in 0.1 N aqueous HCl solution and extracted with ethyl acetate (50 mL × 3). Combined organic layers were washed with saturated aqueous sodium bicarbonate solution, dried over $MgSO_4$, and filtered. Concentration under reduced pressure followed by recrystallization from ethyl acetate provided 6.23 g (15.3 mmol, 92% yield) of a white solid. ^{1}H NMR ($CDCl_3$) δ 10.86 (1H, br s), 7.42 (5H, m), 6.31 (2H, s), 5.10 (2H, s), 3.72 (6H, s), 2.46 (3H, 2), 2.56 (3H, s); MS (M+1) 407.2; HRMS calcd. for $C_{22}H_{23}O_4N_4$ 407.1719, obsd. 407.1726.

*8-(4-Benzyloxy-2,6-dimethoxyphenyl)-4-chloro-2,7-dimethylpyrazolo-[1,5-a][1,3,5]triazine (**8**)*

A mixture of 8-(4-benzyloxy-2,6-dimethoxyphenyl)-2,7-dimethyl-3H-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (**7**) (4.50 g, 11.1 mmol) and *N*,*N*-diethyl-*p*-toluidine (4.33 mL, 24.4 mmol) in $POCl_3$ (50 mL) was heated to reflux for 24 h. The mixture was cooled to rt and $POCl_3$ was removed *in vacuo*. The residue was partitioned between ice-water (30 mL) and ethyl acetate (50 mL) and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to a minimal volume. The residue was quickly passed through a silica gel bed (ca 10 cm) and eluted with hexane–EtOAc (1:4, v/v). The concentration of the filtrate provided 3.66 g (78% yield) of a brownish gum, which was used for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 7.42 (5H, m), 6.33 (2H, s), 5.12 (2H, s), 3.71 (6H, s), 2.61 (3H, s), 2.36 (3H, s).

*[8 - (4-Benzyloxy-2,6-dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine (**9**)*

To 8-(4-benzyloxy-2,6-dimethoxyphenyl)-4-chloro-2,7-dimethylpyrazolo-[1,5-*a*][1,3,5]triazine (**8**) (3.65 g, 8.59 mmol) in dry THF (30 mL) was added *bis*(2-methoxyethyl)amine (1.90 mL, 12.9 mmol) and the mixture was stirred for 1 h. The solvent was removed under reduced pressure and the residue was purified on a silica gel chromatographic column (elution with hexane–EtOAc 4:1, v/v) to give 2.89 g (64% yield) of a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.43 (5H, m), 6.31 (2H, s), 5.10 (2H, s), 4.30 (4H, m), 3.76 (4H, t, J = 5.4 Hz), 3.72 (6H, s), 3.38 (6H, s), 2.37 (3H, s), 2.19 (3H, s); m.p. 124–125°C; elemental analysis calcd. for $C_{28}H_{35}N_5O_5$ C 64.47, H 6.76, N 13.43, found C 64.54, H 6.70, N 13.42; MS $(M+1)^+$ 522; HRMS calcd. for $C_{28}H_{36}O_5N_5$ 522.2716, obsd. 522.2715.

*4 - {-[Bis(2-methoxyethyl)amino]-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]-triazin-8-yl} -3,5-dimethoxyphenol (**10**)*

To a solution/suspension of [8-(4-benzyloxy-2,6-dimethoxyphenyl) - 2,7-dimethylpyrazolo[1,5 - *a*][1,3,5]triazin - 4 - yl]*bis*(2 - methoxyethyl)amine (**9**) (2.80 g, 5.37 mmol) in methanol (75 mL) was added 10% Pd/C (de Gussa type, Aldrich) and the mixture was stirred under hydrogen atmosphere for 1 h at rt. After 1 h, when TLC indicated no starting material, the mixture was diluted with ethyl acetate (200 mL) and filtered through a bed of Celite® (4 cm). The filtrate was concentrated under reduced pressure to give a white solid (2.28 g, 98% yield). ^{1}H NMR ($CDCl_3$) δ 5.87 (2H, s), 4.37 (4H, m), 3.79 (4H, t, J = 5.4 Hz), 3.60 (6H, s), 3.40 (6H, s), 2.50 (3H, s), 2.17 (3H, s); m.p. 183–184°C; elemental analysis calcd. for $C_{21}H_{29}O_5N_5.0.5H_2O$ C 57.26, H 6.86, N 15.89, found C 57.56, H 6.61,

N 15.97; MS $(M+1)^+$ 432.2; HRMS calcd. for $C_{21}H_{30}O_5N_5$ 432.2247, obsd. 432.2232.

*Trifluoromethanesulfonic acid 4-{4-[bis(2-methoxyethyl)amino]-2,7- dimethylpyrazolo[1,5-a][1,3,5]triazin-8-yl} -3,5-dimethoxyphenyl ester (**11**)*

To a solution of 4- {4-[*bis*(2-methoxyethyl)amino]-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-8-yl} -3,5-dimethoxyphenol (**10**) (300 mg, 0.695 mmol) in dry pyridine (1.0 mL) was added trifluoromethanesulfonic anhydride (129 mL, 0.765 mmol) at 0°C. The mixture was stirred at 0°C for 30 min and then at rt overnight. The mixture was partitioned between water (3 mL) and ethyl acetate (3 mL). The aqueous layer was extracted with ethyl acetate (3 mL × 2). Combined ethyl acetate layers were washed with water (5 mL), 1 N aqueous HCl solution (3 mL), water, and brine, dried (over anhydrous $MgSO_4$), and the concentrated crude product was purified on a silica gel chromatographic column (elution with hexane–EtOAc 4:1, v/v) to provide 224 mg (0.397 mmol, 57% yield) of a white foam. ^{1}H NMR ($CDCl_3$) δ 6.54 (2H, s), 4.31 (4H, m), 3.76 (4H, t, J = 5.6 Hz), 3.76 (6H, s), 3.39 (6H, s), 2.38 (3H, s), 2.17 (3H, s); MS $(M+1)^+$ 564.2; HRMS (M^+) calcd. for $C_{22}H_{28}F_3N_5O_7S$ 564.1740, obsd. 564.1730.

*{8 - [2,6 -Dimethoxy-4-(trimethylstannyl)-phenyl]-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-yl} -bis(2-methoxyethyl)amine (**12a**)*

To a solution of trifluoromethanesulfonic acid 4- {4-[*bis*(2-methoxyethyl)amino]-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-8-yl}-3,5-dimethoxyphenyl ester (**11**) (216 mg, 0.383 mmol) in dioxane (2.5 mL) were added lithium chloride (48 mg, 1.13 mmol), tetrakis(triphenylphosphine)palladium (12 mg, 0.0104 mmol), one crystal of 2,6-di-*tert*-butyl-4-methylphenol, and hexamethylditin (180 mg, 0.549 mmol) under argon. The mixture was heated to 110°C for 3 h and cooled to rt. It was diluted with ethyl acetate (3 mL) and washed with 10% aqueous ammonium hydroxide solution (5 mL) and the organic layer was filtered through a bed (1 cm) of Celite. The filtrate was washed with water (5 mL) and brine (5 mL), dried (anhydrous $MgSO_4$), and the concentrated crude product was purified on silica gel column chromatography (elution with 4:1 hexane–EtOAc, v/v) to provide 194 mg (0.335 mmol, 88% yield) of a white solid. ^{1}H NMR ($CDCl_3$) δ 6.75 (2H, s), 4.32 (4H, m), 3.76 (4H, t, J = 6.0 Hz), 3.76 (6H, s), 3.39 (6H, s), 2.36 (3H, s), 2.21 (3H, s), 0.32 (9H, s); m.p. 153–155°C; MS $(M+1)^+$ 578, 580; elemental analysis calcd. for $C_{24}H_{37}N_5O_4Sn$ C 49.85, H 6.45, N 12.11, obsd. C 50.06, H 6.53, N 11.70.

*{8 - [2,6-Dimethoxy-4-(tributylstannyl)-phenyl]-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-yl} -bis(2-methoxyethyl)amine (**12b**)*

To a solution of trifluoromethanesulfonic acid 4-{4-[*bis*(2-methoxyethyl)amino]-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-8-yl}-3,5-dimethoxyphenyl ester (438 mg, 0.777 mmol) in dioxane (4.5 mL) were added lithium chloride (99 mg, 2.33 mmol), tetrakis(triphenylphosphine)palladium (27 mg, 0.0233 mmol), one crystal of 2,6-di-*tert*-butyl-4-methylphenol, and hexabutylditin (506 μL, 1.01 mmol) under argon. The mixture was heated to 110°C for 22 h and then cooled to rt. It was diluted with ethyl acetate (20 mL) and washed with 10% aqueous ammonium hydroxide solution (10 mL) and the organic layer was filtered through a bed (2 cm) of Celite. The filtrate was washed with water (20 mL) and brine (20 mL), dried (over anhydrous $MgSO_4$), and the concentrated crude product was purified on silica gel Chromatotron® (4 mm plate, elution with 5, 7, and 10% EtOAc in hexane, v/v), which provided 284 mg (0.335 mmol, 52% yield) of a white solid. ^{1}H NMR ($CDCl_3$) δ6.70 s), 4.27 (4H, m), 3.75 (4H, t, J = 6.0 Hz), 3.75 (6H, s), 3.37 (6H, s), 2.35 (3H, s), 2.19 (3H, s), 1.58 (6H, m), 1.35 (6H, m), 1.06 (6H, m), 0.90 (9H, t, J = 7.3 Hz); elemental analysis calcd. for $C_{33}H_{55}N_5O_4Sn$ C 56.26, H 7.87, N 9.94, found C 56.44, H 7.89, N 9.69.

*[8-(4-Bromo-2,6-dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine (**13**)*

To a solution of {8-[2,6-dimethoxy-4-(trimethylstannyl)-phenyl]-2,7 - dimethylpyrazolo[1,5 - *a*][1,3,5]triazin - 4 -yl} - *bis*(2 - methoxyethyl)amine (**12a**) (27.7 mg, 0.0467 mmol) in dry THF (0.3 mL) was added NBS (9.1 mg, 0.0514 mmol) and the mixture was stirred for 1 h at rt. The solvent was removed *in vacuo* and the residue was purified on a silica gel chromatographic column (eluted with hexane–EtOAc 5:1, v/v) to provide 21.0 mg (0.0425 mmol, 91% yield) of a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.79 (2H, s), 4.30 (4H, m), 3.76 (4H, t, J = 5.7 Hz), 3.74 (6H, s), 3.38 (3H, s), 2.37 (3H, s), 2.17 (3H, s); m.p. 112–113°C; elemental analysis calcd. for $C_{21}H_{28}BrNO_4 \cdot 0.5$ acetone C 51.63, H 5.97, N 13.37, obsd. C 51.95, H 6.01, N 13.52; MS $(M+1)^+$ 494; HRMS calcd. for $C_{21}H_{30}BrN_5O_4$ 494.1403, obsd. 494.1403.

*[8-(2,6-Dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine (**14**)*

To compound **13** (107 mg, 0.216 mmol) in methanol (5.0 mL) was added 10 wt% Pd/C (40 mg, Aldrich) and the flask was filled with hydrogen gas. The mixture was stirred under atmospheric hydrogen (balloon) for 30 min. The mixture was filtered through a Celite pad (~2 cm) and the filtrate concentrated under reduced pressure. The residue was purified on a silica gel column (eluted with hexane–EtOAc 5:1–2:1 and 10% methanol/CH_2Cl_2) to give 83.1 mg (0.200 mmol, 92.6% yield) of compound **14** as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.28 (1H, t, J = 8.7 Hz), 6.65 (2H, d, J = 8.7 Hz), 4.30 (4H, br), 3.76 (4H, t, J = 5. Hz), 3.76 (6H, s), 3.39 (6H. s), 2.37 (3H, s), 2.20 (3H, s); HRMS (TOF MS ES+) calcd. for $C_{21}H_{30}N_5O_4$ 416.2298, obsd. 416.2280.

*[8-(3-Bromo-2,6-dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine (**15**)*

To a magnetically stirred solution of [8-(2,6-dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5 - *a*][1,3,5]triazin - 4 -yl]*bis*(2-methoxyethyl)amine (**14**) (50.0 mg, 0.120 mmol) in THF (2.0 mL) was added NBS (29 mg, 0.163 mmol) and the mixture was stirred for 15 min at rt. The solvent was evaporated under reduced pressure and the mixture was purified on a silica gel chromatographic column (eluted with hexane–EtOAc 7:1–4:1) to provide compound **15** (56.0 mg, 95% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (1H, d, J = 8.7 Hz), 6.66 (1H, d, J = 8.7 Hz), 4.32 (4H, br s), 3.77 (4H, t, J = 5.7 Hz), 3.73 (3H, s), 3.54 (3H, s), 3.39 (6H, s), 2.38 (3H, s), 2.19 (3H, s); HRMS (TOF MS ES+) calcd. for $C_{21}H_{29}N_5O_4Br$ 494.1403, obsd. 494.1405.

Radiolabeling

[8-(4-[^{76}Br]bromo-2,6-dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine

An aliquot of the aqueous solution of **[^{76}Br]** ammonium bromide (10–20 μL, 20.0–40.0 MBq) was added to a 1 mL

reaction vial and the solvent was evaporated with argon flow. Tributyltin substrate **12b** (200 μg) in 50 μL of acetonitrile was added to the vial containing the **[^{76}Br]** radioactivity and was followed by the addition of 2 μL of 37% peracetic acid in 10 μL of acetonitrile. The vial was sealed and placed on an 80°C heating block and heated for 30 min. At the end of the reaction, the reaction mixture was loaded onto a Phenomenex C-18 (2) column (250 × 4.6 mm) and eluted with 100 mM ammonium acetate/acetonitrile (40/60) at a flow rate of 1.2 mL/min. The radioactivity peak containing the desired product (t_R = 15 min) was collected and analyzed with another HPLC system for specific activity and radiochemical purity determination. Chemical purity and *m/z* identification utilized UPLC-MS.

[8-(3-[^{76}Br]bromo-2,6-dimethoxyphenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]bis(2-methoxyethyl)amine

Tributyltin substrate **12b** (200 μg) in 50 μL of acetonitrile was treated with 5 μL of trifluoroacetic acid and heated at 100°C for 5 min. Br-76 radioactivity (20 μL) was added to the mixture and was followed by the addition of 2 μL of 37% peracetic acid in 10 μL of acetonitrile. The reaction mixture was heated at 80°C for 20 min and then purified with HPLC using the same conditions as described above. A separate HPLC system was used for specific activity and radiochemical purity determination. Chemical purity and *m/z* identification utilized UPLC-MS.

Conclusion

We demonstrated that by controlling the reaction conditions, either pure 3 (**15**) or 4-[^{76}Br]BMK-152 (**13**) can be prepared from the same tributyltin precursor. Bromination ortho to the trialkyltin group predominates in an aqueous reaction mixture. Removal of water from the radioactivity before adding the substrate and oxidizing agent is critical to obtain the desired bromodestannylation reaction yielding the 4-bromo isomer. The mechanism of reaction remains unclear. The 4-bromo isomer exhibits higher *in vitro* affinity than the 3-bromo isomer. We observe that the 4-bromo isomer has potential as a suitable PET ligand for imaging CRHR1 *in vivo*.

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