SYNTHESIS OF [^{18}F]RO41-0960, A POTENT CATECHOL-O-METHYLTRANSFERASE INHIBITOR, FOR PET STUDIES

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SUMMARY

Ro41-0960 (3,4-dihydroxy-5-nitro-2'-fluorobenzophenone) is a potent, fluorine containing COMT inhibitor. In order to map catechol-O-methyltransferase (COMT) *in vivo* with PET, no-carrier-added [^{18}F]Ro41-0960 was synthesized by the nucleophilic aromatic substitution of [^{18}F]fluoride for 2'-nitro on 3,4-dimethoxy-5,2'-dinitrobenzophenone, followed by hydrolysis with HBr. During the course of this study it was found that [^{18}F]fluoromethane ([^{18}F]CH_3F) was generated as the side product of nucleophilic aromatic substitution reaction. Various precursors with different hydroxyl protecting groups were then investigated for the effects on this side reaction.

Key words: catechol-O-methyltransferase (COMT), Ro41-0960, COMT inhibitor, PET, [^{18}F]fluoromethane.

INTRODUCTION

Catechol-O-methyltransferase (COMT; EC 2.1.1.6) catalyses the O-methylation of a wide range of catechol substrates, such as DOPA, dopamine (DA), norepinephrine (NE) and catecholestrogens, utilising S-adenosylmethionine (SAM) as the methyl donor and requiring Mg^{+2} as a cofactor. Several investigators have reported elevated levels of COMT activity in malignant breast tumors [1, 2] where COMT plays a role in estrogen metabolism. It is also a new molecular target in the development of drugs to treat Parkinson's disease [3]. Since it regulates the concentration of important catecholamine neurotransmitters such as DA and NE, there is speculation that abnormalities in its activity may be associated with neurological and psychiatric disorders. However, due to the lack of suitable *in vivo* inhibitors, the functional significance of COMT in the living body or changes in its activity occurring in diseases has been less studied. With the recent development of selective and potent COMT inhibitors, we now have the opportunity to probe the distribution of COMT *in vivo*.

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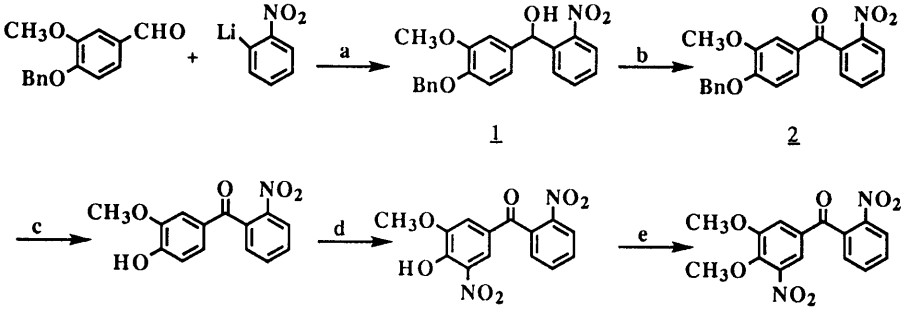
Ro41-0960 (3,4-dihydroxy-5-nitro-2'-fluorobenzophenone) is a potent, fluorine containing COMT inhibitor with *in vitro* IC_{50} values of 16 and 42 nM for rat brain and liver soluble COMT, respectively [3]. It is structurally similar to Ro40-7592 (3,4-dihydroxy-5-nitro-4'-methylbenzophenone, Tolcapone) which is currently undergoing clinical trials in Parkinson's disease [4, 5]. We have recently developed a synthetic route to [^{18}F]Ro41-0960 and have initiated studies in baboon and mouse in order to evaluate it as a radioligand for mapping COMT *in vivo* [6]. We report here the total synthesis of [^{18}F]Ro41-0960, the first positron emitter labeled COMT inhibitor. The implications of testing a series of precursors with different hydroxyl protecting groups are also discussed from a mechanistic point of view.

RESULTS AND DISCUSSION

3,4-Dimethoxy-5,2'-dinitrobenzophenone (5) was first selected as the radiosynthetic precursor to [^{18}F]Ro41-0960. The synthetic route for the preparation of this dimethyl protected catechol substrate is shown in Scheme 1. Coupling reaction between 3-methoxy-4-benzyloxybenzaldehyde and 2-lithionitrobenzene, which was generated from reaction of 2-bromonitrobenzene with phenyllithium, afforded benzhydrol 1. The isolated product was used in the subsequent oxidation step without further purification. Selective deprotection of the benzyl group allowed regiospecific introduction of a nitro group ortho to the free hydroxyl group due to the directive effect of hydroxyl group during the electrophilic nitration. Methylation was achieved by reaction of compound 4 with either sodium hydride followed by dimethylsulfate, or trifluoromethanesulfonate in the presence of diisopropylethylamine.

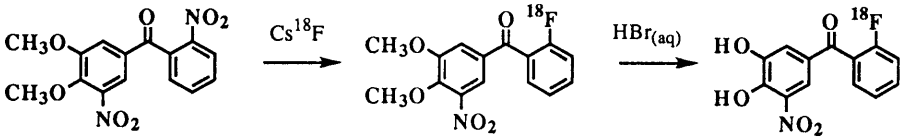
Scheme 1. Synthesis of Precursors for F-18 Labeled COMT Inhibitor Ro41-0960



(a) THF; (b) PCC, CH_2Cl_2; (c) 30%HBr in HOAc; (d) HNO_3/HOAc; (e) NaH, $(CH_3)_2SO_4$

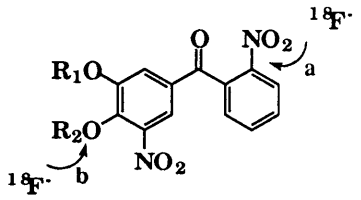
With the precursor (5) in hand, a two-step radiosynthesis, that is nucleophilic aromatic substitution with no-carrier-added [^{18}F]fluoride followed by hydrolysis with HBr, was then carried out (Scheme 2). To our surprise, [^{18}F]fluoromethane ([^{18}F]CH_3F) was identified by radio-GC as a side product during the fluorination reaction, presumably generated by $^{18}F^-$ attack on the methyl moiety ortho to the nitro group on the A ring (Scheme 3). The resulting F-18 labeled by-product (4-hydroxy-3-methyoxy-5-nitro-2'-[^{18}F]fluorobenzophenone) was also identified by radio-TLC and HPLC. This interesting phenomenon has never been reported in any previous cases involving fluorination of catechol substrates with [^{18}F]F^-, for example, syntheses of F-18 labeled 6-fluoro-DOPA, 6-fluoro-DA and 6-fluoro-NE [7, 8, 9, 10] using 3,4-dialkyl protected catechol 6-nitrobenzaldehyde as precursors. The formation of [^{18}F]CH_3F may account for the relatively low total radiochemical yield (5-10% at the end of bombardment) of the final product ([^{18}F]Ro41-0960) after HPLC purification.

Scheme 2. Synthesis of F-18 Labeled COMT Inhibitor (Ro41-0960)



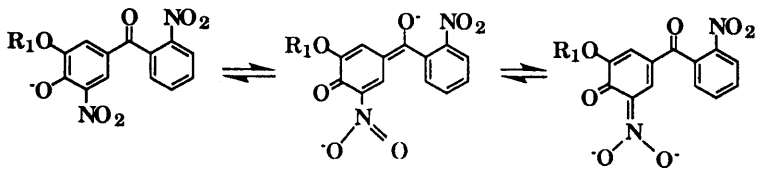
16 [^{18}F]Ro41-0960

Scheme 3. Formation of side product [^{18}F]CH_3F ($R_2 = CH_3$) due to the fact that the corresponding oxygen anion can be stabilized through resonance via the adjacent nitro group as well as the para position ketone carbonyl



a. Formation of desired product

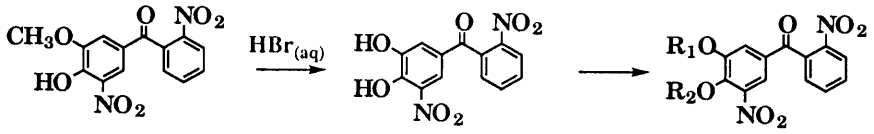
b. Formation of side product--alkyl fluoride



It appears that the methyl protected phenol ortho to the nitro group is extremely susceptible to an attack by $[^{18}F]F^-$ ion, since the corresponding oxygen anion is doubly stabilized through resonance via the adjacent nitro group and the para position ketone carbonyl, resulting in the formation of the side product $[^{18}F]CH_3F$ (Scheme 3). In contrast, for the syntheses of F-18 labeled 6-fluoro-DOPA, 6-fluoro-DA and 6-fluoro-NE where no alkyl fluorides are generated, there is only one electron withdrawing, activating group at the para position to either protected hydroxyl group on the ring. We then investigated structural alterations of the precursor by building up steric hindrance with bulky protecting groups in hopes of slowing down this side reaction, as shown in Scheme 4. We found that the addition of the first protecting group (R_1) always occurred at the phenol which is meta to the nitro group due to the difference in reactivity of the two hydroxyl groups. The introduction of the second protecting group (R_2) required harsh reaction conditions and the yields were low; that is, R_2 is more difficult to introduce and is more easily removed than R_1. This is consistent with the proposed theory that the nucleophilicity of the phenolic anion ortho to the nitro group is minimized as the charge is stabilized through resonance with both ketone carbonyl and nitro group (Scheme 4). It is extremely difficult to introduce two bulky protecting groups on the ring and it is preferred to have benzyl or its analogues as R_1 since they are relatively easier to remove than methyl. The results indicated that the bulky protecting group indeed slowed down the formation of the F-18 labeled alkyl fluoride side product (in the case of compound 7, $[^{18}F]$benzylfluoride was the side product). However, this decrease in the rate of side product formation was accompanied by a decreased yield of desired product. In the case of methylene acetal protected substrate (14), simple fluoride exchange reaction (F-18 for F-19) was also carried out. It appeared that decomposition occurred during the reaction resulting from the fracture of the dioxolane ring. The presence of an electrowithdrawing protective group, such as the trimethylacetoxy moiety in precursor 15, did not give satisfactory yields. The best radiochemical yield obtained by far was with the dimethyl protected precursor (5). This was consistent with our ^{13}C-NMR analyses using a methodology we developed a few years ago employing ^{13}C-NMR as a probe for electron density [8]. A higher ppm value of C-13 chemical shift at the reaction center (the carbon where the nitro group is attached on the B ring) of the dimethyl protected precursor (5) as compared to monobenzyl (8) or dibenzyl protected precursor (9) (Scheme 5), indicated a

lower electron density; therefore a better radiochemical yield was obtained for the nucleophilic aromatic substitution reaction. However, the second radiosynthetic step (the deprotection of the catechol moiety) required more vigorous conditions to cleave both methyl groups of precursor (5) (particularly the methyl group at R_1 position) than the benzyl group of precursor (8).

Scheme 4. Synthesis of Precursors with Various Protected Catechol Moieties



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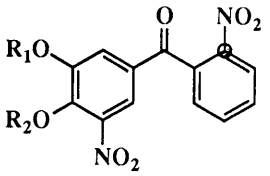
5. $R_1 = CH_3$, $R_2 = CH_3$
7. $R_1 = CH_3$, $R_2 = Bn$
8. $R_1 = Bn$, $R_2 = CH_3$
9. $R_1 = Bn$, $R_2 = Bn$
10. $R_1 = Bn$, R_2 = isopropyl
11. R_1 = MOM, R_2 = MOM
12. R_1 = cyclohexyl , $R_2 = CH_3$
13. R_1 = 2,6-diCl-Bn , $R_2 = CH_3$
14. R_1, $R_2 = CH_2$
15. $R_1 = Bn$, $R_2 = Me_3CCO$

Scheme 5. ^{13}C NMR as a probe for electron density



	Reaction Center (PPM)
5. $R_1 = CH_3$, $R_2 = CH_3$	154.58
8. $R_1 = Bn$, $R_2 = CH_3$	153.24
9. $R_1 = Bn$, $R_2 = Bn$	150.15

All of the nucleophilic aromatic substitution reactions using precursors with various protected catechol moieties as described above were conducted using $Cs^{18}F$, since other forms of [^{18}F]fluoride, such as $N^+Bu_4{}^{18}F^-$ and $K^{18}F$ (in the presence of Kryptofix 222), did not afford the desired products. The use of $Rb^{18}F$ gave similar radiochemical yields as $Cs^{18}F$; however, a longer reaction time was required. Microwave heating, instead of conventional oil bath heating, did not provide any advantages. As shown in Scheme 3, the production of [^{18}F]Ro41-0960 for baboon and mouse studies using the dimethyl protected

precursor (5) was performed using a one-pot, two-step procedure, without isolation or purification of the product from the first step. Complete deprotection was achieved by acid hydrolysis (48% HBr) under refluxing condition (140°C) for 30 min. The use of BBr_3/CH_2Cl_2 for deprotection did not give satisfactory results. The total radiosynthesis time was 100 min with a radiochemical yield of 5-10%, specific activity of 1-2 Ci/μmol (EOB) and radiochemical purity of >98%.

EXPERIMENTAL

All the reagents used for the preparation of precursors were obtained from Aldrich Chemical Co.. [^{18}F]Fluoride ion was made by 17.4 Mev proton irradiation of [^{18}O]H_2O in a silver target. ^{1}H NMR spectra were obtained in $CDCl_3$ on a Bruker 300 MHz NMR spectrometer and are reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded with a Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact ionization at 70 eV. HPLC analyses were carried out with a Perkin-Elmer liquid chromatograph equipped with a radioactivity monitor and detector.

4-benzyloxy-3-methoxy-2'-nitro benzhydrol (1)

A solution of 17 g (0.11 mol) of bromobenzene in tetrahydrofuran (120 mL) was stirred at -78° C under a nitrogen atmosphere while a solution of 1.8M n-butyllithium in hexane (66 ml, 0.106 mol) was introduced. After 30 minutes, the prepared phenyllithium solution was added dropwise within 20 minutes to a solution of of dried 2-bromonitrobenzene (20.2 g, 0.1 mol) in tetrahydrofuran at -120°C under a nitrogen atmosphere. After stirring at -120°C for 30 minutes, a solution of 3-methoxy-4-benzyloxybenzaldehyde (23 g, 0.095 mol) in 120 ml of tetrahydrofuran was added dropwise at -120° and the mixture was then stirred at -120°C for 30 minutes, -78°C for 2 hours, and 0°C for 1 hour. It was then poured into a mixture of ice and 50 mL of 2 N hydrochloric acid and extracted three times with ether (150 mL x 3). The combined ether phases were washed with saturated sodium chloride solution, dried over sodium sulphate and evaporated. The excess of bromobenzene and nitrobenzene was removed under a vacuum and the titled compound which was thus obtained was used without further purification in the subsequent reaction step. ^{1}H NMR ($CDCl_3$): 7.88 (dd, J = 8.1, 1.2 Hz, 1H), 7.70 (dd, J = 7.7, 1.4 Hz, 1H), 7.59 (ddd, J = 7.7, 7.5, 1.2 Hz, 1H), 7.2-7.5 (m, 6H), 6.92 (d, J = 2.0 Hz, 1H), 6.79

(d, J = 8.3 Hz, 1H), 6.72 (dd, J = 8.3, 2.0 Hz, 1H), 6.34 (s, 1H), 5.10 (s, 2H), 3.828 (s, 3H). MS, m/e (rel. intensity): 365 (M^+, 2), 256 (6), 169 (4), 150 (4), 91 (100), 65 (13).

4-benzyloxy-3-methoxy-2'-nitro benzophenone (2)

A solution of crude 4-benzyloxy-3-methoxy-2'-nitro benzhydrol in 500 ml of methylene chloride was treated within 20 minutes at 20°C with 25 g (0.12 mol) of pyridinium chorochromate and stirred at 20°C for another 2 hours. The reaction mixture was treated with 300 mL of methylene chloride and 200 mL of ether, followed by filteration through a silica gel column (100 g). Upon evaporation, the residue was dissolved in 150 mL of methylene chloride, followed by addition of 400 mL of ether. The solid thus formed was collected by filtration and washed with 25 % methylene chloride in ether to give 20 g (58%) of 2. ^{1}H NMR ($CDCl_3$): 8.20 (dd, J = 8.2, 1.1 Hz, 1H), 7.75 (ddd, J = 7.5, 7.5, 1.1 Hz, 1H), 7.65 (ddd, J = 8.2, 7.5, 1.4 Hz, 1H), 7.62 (d, J = 2.0 Hz, 1H),7.48 (dd, J = 7.5, 1.4 Hz, 1H), 7.3-7.4 (m, 5 H), 6.94 (dd, J = 8.4, 2.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 5.20 (s, 2H), 3.96 (s, 3H). MS, m/e (rel. intensity): 363 (M^+, 4), 231 (3), 149 (4), 141 (4), 119 (7), 117 (8), 91 (100), 71 (12), 57 (17).

4-hydroxy-3-methoxy-2'-nitro benzophenone (3)

To a solution of 16 g (40.7 mmol) of 4-benzyloxy-3-methoxy-2'-nitrobenzophenone in 60 ml methylene chloride, 60 ml of 30% hydrobromic acid in acetic acid was added within 10 minutes at 0°C. After stirring at 20°C for 1 hour, the reaction mixture was poured into 200 mL of ice water, and extracted three times with 70 mL methylene chloride. The combined methylene chloride extracts were washed with water, dried over magnesium sulphate and evaporated. The residual oil was dissolved in 60 mL of chloroform, followed by addition of 300 mL of ether. The yellow crystals thus formed were collected by filtration, washed with ether, and dried to give 9.5 g (85%) of 3. From the mother liquor, 1.5 g (13%) of the title compound was obtained from further recrystallization. ^{1}H NMR ($CDCl_3$): 8.23 (dd, J = 8.2, 1.1 Hz, 1H), 7.75 (ddd, J = 7.5, 7.5, 1.1 Hz, 1H), 7.66 (ddd, J = 8.2, 7.5, 1.5 Hz, 1H), 7.64 (d, J = 1.9 Hz, 1H),7.48 (dd, J = 7.5, 1.5 Hz, 1H), 6.98 (dd, J = 8.3, 1.9 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 3.98 (s, 3H). MS, m/e (rel. intensity): 273 (M^+, 34), 151 (68), 139 (100), 111 (30), 104 (41), 91 (24), 79 (29), 57 (37), 43 (36).

4-hydroxy-3-methoxy-5,2'-dinitrobenzophenone (4)

A solution of 1.3 ml of 85 % nitric acid (18.7 mmol) in acetic acid was added within 15 minutes at 0°C to a solution of 5.0 g (18.3 mmol) of 4-hydroxy-3-methoxy-2'-nitrobenzophenone in 120 mL acetic acid. The reaction mixture was stirred for 1.5 hours at 20°C until the color changed to dark yellow. Water (400 mL) was added, and the mixture was stirred for another 1 hour. The precipitated product was collected on a glass filter, washed with water and then ether, and dried to give 4.5 g (77%) of 4 as dark yellow crystals. ^{1}H NMR ($CDCl_3$): 11.18 (s, 1H), 8.31 (dd, J = 8.2, 1.2 Hz, 1H), 7.88 (d, J = 1.9 Hz, 1H), 7.84 (ddd, J = 7.4, 7.4, 1.2 Hz, 1H), 7.75 (ddd, J = 8.2, 7.4, 1.3 Hz, 1H), 7.70 (d, J = 1.9 Hz, 1H), 7.49 (dd, J = 7.4, 1.3 Hz, 1H), 4.06 (s, 3H). MS, m/e (rel. intensity): 318 (M^+, 60), 196 (64), 150 (89), 135 (69), 134 (100), 104 (96), 91 (29), 79 (50), 77 (33), 76 (45).

3,4-dimethoxy-5,2'-dinitrobenzophenone (5)

To a suspension of 150 mg (8.3 mmol) of sodium hydride in 2 ml of dimethylformamide, 500 mg (1.57 mmol) of 4-hydroxy-3- methoxy-5,2'-dinitrobenzophenone was added slowly at 0°C. After 10 minutes, 4.0 g (32 mmol) of dimethyl sulphate was added. Upon heating to 80° for 20 minutes, the color of the mixture changed from dark yellow to colorless. At 25°C, 1 ml of water was added carefully, followed by stirring for 1 hour. Water (50 ml) was added and the mixture was extracted three times with 50 ml of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel using methylene chloride to elute the product, followed by 5% methol in methylene chloride to recover the starting material. After recrystallization from chloroform/ether, 350 mg (67%) of 5 was obtained as colorless crystals. Some of the starting compound (140 mg, 28%) was recovered from the silica gel column. ^{1}H NMR ($CDCl_3$): 8.29 (dd, J = 8.2, 1.2 Hz, 1H), 7.84 (ddd, J = 7.4, 7.5, 1.2 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.75 (ddd, J = 8.2, 7.5, 1.5 Hz, 1H), 7.49 (dd, J = 7.4, 1.5 Hz, 1H), 7.32 (d, J = 2.0 Hz, 1H), 4.07 (s, 3H), 4.02 (s, 3H). MS, m/e (rel. intensity): 332 (M^+, 64), 210 (33), 198 (100), 150 (35), 137 (45), 134 (86), 104 (90), 77 (23), 76 (56).

3,4-dihydroxy-5,2'-dinitrobenzophenone (6)

A suspension of 1.0 g (3.14 mmol) of 4-hydroxy-3-methoxy-5,2'- dinitrobenzophenone in

l ml of acetic acid and 8 ml of 48 % hydrobromic acid was refluxed for six hours. After addition of 20 ml of water, the reaction mixture was stirred for 20 minutes at 0°C. The yellow solid thus formed was collected by filtration and washed with water and then ether to give 880 mg (70%) of 6. The product was recrystallized from chloroform/ether. ^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.2, 1.2 Hz, 1H), 7.83 (ddd, J = 7.5, 7.5, 1.2 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.74 (ddd, J = 8.2, 7.5, 1.5 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.48 (dd, J = 7.5, 1.5 Hz, 1H). MS, m/e (rel. intensity): 304 (M^+, 32), 182 (22), 135 (26), 134 (54), 104 (100), 79 (55), 77 (46), 76 (72)

4-benzyloxy-3-methoxy-5,2'-dinitrobenzophenone (7)

To a solution of 159 mg (0.5 mmol) of 4-hydroxy-3-methoxy-5,2'-dinitrobenzophenone (4) in 2 ml of dimethyl formamide, 24 mg (l mmol) of sodium hydride was added. After 10 minutes, 171 mg (l mmol) of benzyl bromide was added, and the reaction mixture was stirred for one hour at 80°C. Water (50 mL) was then added, followed by extraction three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel (methylene chloride: hexane = 1:1 as eluent) to yield 70 mg (34%) of 7. ^{1}H NMR ($CDCl_3$): 8.27 (dd, J = 8.2, 1.2 Hz, 1H), 7.6-7.8 (m, 3H), 7.2-7.5 (m, 7H), 5.2 (s, 2H), 4.0 (s, 3H).

3-benzyloxy-4-methoxy-5,2'-dinitrobenzophenone (8)

To a solution of 1.4 g (4.6 mmol) of 3,4-dihydroxy-5,2'-dinitrobenzophenone (6) in 40 ml of dimethylformamide, 240 mg (10 mmol) of sodium hydride was added. After 10 minutes, 940 mg (5.5 mmol) of benzyl bromide was added. The reaction mixture was then stirred for 2 hours at 35°C. At 0°C, 100 ml of water and 5 ml of 2N hydrochloric acid was added. The mixture was then extracted twice with 40 ml of dichloromethane, and the combined dichloromethane extracts were washed with water, dried over sodium sulphate and evaporated. The residual oil was crystallized from ethyl acetate. The yellow crystals was collected by filtration to give 1.2 g (68%) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone. ^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.0, 1.2 Hz, 1H), 7.7-7.9 (m, 3H), 7.3-7.5 (m, 7H), 5.26 (s, 2H). For the subsequent methylation, two methods were used.

Method A. To a solution of 400 mg (1.0 mmol) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone in 5 ml of dimethylformamide, 300 mg (13 mmol) of sodium hydride and 1.6 g (13 mmol) of dimethyl sulphate were added, whereupon the mixture was heated to reflux for 20 minutes. At 0°C, 1 ml of water was carefully added, and the solution was then stirred another 1 hour at 25°C. The reaction mixture was treated with 50 ml of water and extracted three times with 50 ml of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel (methylene chloride: hexane = 1:1 as eluent). After recrystallization from methylene chloride/ether, 240 mg (58%) of 8 was obtained. ^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.2, 1.2 Hz, 1H), 7.7-7.9 (m, 3H), 7.3-7.5 (m, 7H), 5.22 (s, 2H), 4.09 (s, 3H).

Method B. To a solution of 95 mg (0.24 mmol) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone in 4 ml dichloromethane, 93 mg (125 μL, 0.72 mmol) of diisopropylethylamine was added. After a few minutes, a solution of 118 mg (90 μL, 0.79 mmol) of methyl trifluoromethanesulfonate in 1 mL methylene chloride was added dropwise at 0°C. After 15 minutes, the solution was poured into ice water and extracted twice with 50 mL of ether. The combined ether extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel. After recrystallization from ether 90 mg (92%) of 8 was obtained.

3,4-dibenzyloxy-5,2'-dinitrobenzophenone (9)

To a solution of 425 mg (1.4 mmol) of 3,4-dihydroxy-5,2'-dinitrobenzophenone in 2 ml of dimethylformamide, 72 mg (3 mmol) of sodium hydride and 513 mg (3 mmol) of benzyl bromide was added. After 20 minutes, 300 mg (12.5 mmol) of sodium hydride and 2 g (11.7 mmol) of benzyl bromide were added, and the resulting mixture was refluxed for one hour, then was stirred for 12 hours at 25°C. The reaction mixture was then treated with 50 mL of water and extracted three times with 50 mL of ethyl acetate. The combined ethyl acetate extracts was washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 41 mg (8%) of 9 along with 415 mg (75%) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone. ^{1}H NMR ($CDCl_3$): 8.46 (d, 1H), 7.83 (d, 1H), 7.3-7.5 (m, 14H), 5.35 (s, 2H), 5.23 (s, 2H).

3-benzyloxy-4-isopropyl-5,2'-dinitrobenzophenone (10)

To a solution of 63 mg (1.05 mmol) of isopropyl alcohol and 79 mg (l mmol) of pyridine in 7 mL of methylene chloride, a solution of 282 mg (l mmol) of trifluoromethanesulfonic anhydride in 3 mL of methylene chloride was added slowly at 0°C. After 15 minutes, an insoluble salt was removed. The solution was then added to a mixture of 60 mg (0.152 mmol) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone and 129 mg (l m mol) of diisopropylethylamine in methylene chloride at 0°C. After 15 minutes, the reaction mixture was quenched with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 42 mg (63%) of 10. ^{1}H NMR ($CDCl_3$): 8.27 (m, 1H),7.7-7.8 (m, 3H), 7.5-7.3 (m, 7H), 5.195 (s, 2H), 4.88 (m, 1H), 1.30 (s, 6H).

3,4-di(methoxymethyl)-5,2'-dinitrobenzophenone (11)

To a solution of 3,4-dihydroxy-5,2'-dinitrobenzophenone (6, prepared from 500 mg (1.57 mmol) of 4) in 10 ml of tetrahydrofuran, 800 mg (8.24 mmol) of diisopropylethylamine was added. A solution of 500 mg (6.21 mmol) chloromethylmethylether in 10 mL of methylene chloride was then added slowly at 0°C during a period of 10 mnutes. After 30 minutes, the reaction mixture was quenched with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel (hexane: methylene chloride: methanol = 50:48:2 to 0:98:2) to yield 300 mg (49%) of 3,4-di(methoxymethyl)-5,2'-dinitrobenzophenone (^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.2, 1.2 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H), 7.83 (ddd, J = 7.5, 7.4, 1.2 Hz, 1H), 7.74 (ddd, J = 8.2, 7.5, 1.4 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.48 (dd, J = 7.5, 1.4 Hz, 1H), 5.32 (s, 2H), 5.30 (s, 2H), 3.54 (s, 3H), 3.51 (s, 3H)) and 225 mg (41%) of 4-hydroxy-3-methoxymethyl)-5,2'-dinitro benzophenone ((^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.2, 1.2 Hz, 1H), 7.83 (ddd, J = 7.5, 7.4, 1.2 Hz, 1H), 7.74 (ddd, J = 8.2, 7.5, 1.4 Hz, 1H), 7.67 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.48 (dd, J = 7.5, 1.4 Hz, 1H), 5.21 (s, 2H), 3.69 (s, 3H)).

3-cyclohexyloxy-4-methoxy-5,2'-dinitrobenzophenone (12)

A solution of 400 mg (1.31 mmol) of 3,4-dihydroxy-5,2'-dinitrobenzophenone, 1 g

(12.17 mmol) cyclohexene and 1.5 g (10.8 mmol) boron trifluoride-ether complex was refluxed for 12 hours. The reaction mixture was treated with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 120 mg (23.7%) of 4-hydroxy-3-cyclohexyloxy-5,2'-dinitrobenzophenone.

To a solution of 100 mg (0.26 mmol) of 4-hydroxy-3-cyclohexyloxy-5,2'-dinitrobenzophenone in 5 ml of dimethylformamide, 62 mg (2.8 mmol) of sodium hydride and 315 mg (2.5 mmol) of dimethyl sulphate was added. The mixture was heated to reflux for 110 minutes. At 0°C, 1 mL of water was carefully added, the solution was then stirred for another 1 hour at 25°C. The reaction mixture was treated with 30 mL of water and extracted three times with 20 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 8 mg (7.7%) of 12. ^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.2, 1.2 Hz, 1H), 7.6-8.0 (m, 4H), 7.48 (dd, J = 7.5, 1.4 Hz, 1H), 4.08 (s, 3H), 3.92 (m, 1H), 0.8-2.2 (m, 10 H).

3-(2,6-dichlorobenzyloxy)-4-methoxy-5,2'-dinitrobenzophenone (13)

The same procedure as for 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone (see synthesis of compound 8) was used. From 250 mg (0.82 mmol) of 3,4-dihydroxy-5,2'-dinitrobenzophenone, 50 mg (2.1 mmol) of sodium hydride and 311 mg (1.3 mmol) of 2,6-dichlorobenzylbromide, 250 mg (65.8 %) of 3-(2,6-dichlorobenzyloxy)-4-hydroxy-5,2'-dinitrobenzophenone was obtained.

For the subsequent methylation, the same procedure as for 3-benzyloxy-4-methoxy-5,2'-dinitrobenzophenone (8) was used. From 250 mg (0.64 mmol) of 3-(2,6-dichlorobenzyloxy)-4-hydroxy-5,2'-dinitrobenzophenone, 120 mg (5 mmol) of sodium hydride and 500 mg (4.76 mmol) of dimethylsulphate, 80 mg (31%) of 13 was obtained. ^{1}H NMR ($CDCl_3$): 8.30 (dd, J = 8.2, 1.1 Hz, 1H), 7.99 (d, J = 1.9 Hz, 1H), 7.85 (ddd, J = 7.5, 7.4, 1.1 Hz, 1H), 7.75 (ddd, J = 8.2, 7.5, 1.4 Hz, 1H), 7.50 (dd, J = 7.4, 1.4 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.25-7.45 (m, 3H).

3,4-methylenedioxy-5,2'-dinitrobenzophenone (14)

Upon stirring, 150 mg (1.6 mmol) of potassium fluoride dihydrate was added to a solution

of 100 mg of 3,4-dihydroxy-5,2'-dinitrobenzophenone (0.33 mmol) in 5 mL of dimethylformamide. To the resulting mixture, 570 mg of methylene bromide (3.3 mmol) was then added. The reddish solution was refluxed for 2 hours. Another 150 mg (1.6 mmol) of potassium fluoride dihydrate and 570 mg of methylene bromide was added. After 10 hours, the reaction mixture was treated with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 40 mg (38%) of 14. ^{1}H NMR ($CDCl_3$): 8.30 (dd, J = 8.2, 1.1 Hz, 1H), 7.84 (ddd, J = 7.5, 7.4, 1.1 Hz, 1H), 7.76 (ddd, J = 8.2, 7.4, 1.5 Hz, 1H), 7.76 (d, J = 1.6 Hz, 1H), 7.67 (d, J = 1.6 Hz, 1H), 7.49 (dd, J = 7.5, 1.5 Hz, 1H), 6.35 (s, 2H).

3,4-methylenedioxy-5-nitro-2'-fluorobenzophenone (fluoro analogue of 14)

Upon stirring, 97 mg (1.0 mmol) of potassium fluoride dihydrate was added to a solution of 26 mg (0.1 mmol) of 3,4-dihydroxy-5,2'-dinitrobenzophenone in 4 mL of dimethylformamide. To the mixture, 300 mg of methylene bromide (1.7 mmol) was then added. After refluxing for 12 hours, the reaction mixture was quenched with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 25.5 mg (88%) of the titled compound. ^{1}H NMR ($CDCl_3$): 8.05 (dd, 1H), 7.63 (dd, 1H), 7.4-7.6 (m, 2H), 7.32 (ddd, 1H), 7.21 (ddd, 1H), 6.35 (s, 2H).

3-benzyloxy-4-(trimethylacetoxy)-5,2'-dinitrobenzophenone (15)

To a solution of 80 mg (0.2 mmol) of 3-benzyloxy-4-hydroxy-5,2'-dinitrobenzophenone in 5 ml pyridine, 120 mg (1.0 mmol) of trimethylacetyl chloride was added slowly at 0^{o}C. The reaction mixture was stirred at room temperature for 2 hours, quenched with 50 mL of water and extracted three times with 30 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 88 mg (92%) of 15. ^{1}H NMR ($CDCl_3$): 8.28 (dd, J = 8.0, 1.0 Hz, 1H), 7.89 (d, J = 1.7 Hz, 1H), 7.84 (ddd, J = 7.6, 7.4, 1.0 Hz, 1H), 7.76 (ddd, J = 8.0, 7.6, 1.4 Hz, 1H), 7.68 (d, J = 1.7 Hz, 1H), 7.48 (dd, J = 7.4, 1.4 Hz, 1H), 7.38 (br., 5H), 5.16 (s, 2H), 1.28 (m, 9H).

3,4-dimethoxy-5-nitro-2'-fluorobenzophenone (16)

To a suspension of NaH (7.2 mg, 0.3 mmole) in dimethylformamide (0.5 mL), an authentic sample of Ro41-0960 (26 mg, 0.094 mmole; obtained from Hoffmann-La Roche, Nutley, NJ) was added at 0°C. The reaction mixture was stirred for 10 min, followed by addition of methyl iodide (43 mg, 0.3 mmole). The resulting solution was stirred for another 20 minutes at 25°C. Upon usual work up and purification on silica gel (methylene chloride: methanol = 1:1 as eluent), 4-hydroxy-3-methoxy-5-nitro-2'-fluoro benzophenone (25 mg, 0.085 mmol) was obtained.

To a solution of 4-hydroxy-3-methoxy-5-nitro-2'-fluoro benzophenone (25 mg, 0.085 mmol) in 0.5 ml dimethylformamide, sodium hydride (25 mg, 0.98 mmol) and dimethyl sulphate (3.8 g, 30 mmol) was added, whereupon the mixture was heated to reflux for 30 minutes. At 0°C, 1 mL of water and 15 ml of 2N sod'ium hydroxide aqueous solution was added carefully, and the resulting solution was stirred for another 20 minutes at 25°C. The reaction mixture was treated with 10 mL of water and extracted three times with 20 mL of ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over sodium sulphate and evaporated. The residual oil was chromatographed on silica gel to yield 19 mg (73%) of the tited compound. ^{1}H NMR ($CDCl_3$): 7.72 (d, J = 1.9 Hz, 1H), 7.64 (t, J = 1.9 Hz, 1H), 7.63-7.53 (m, 2H), 7.31 (ddd, J = 7.6, 7.4, 1.0 Hz, 1H), 7.20 (ddd, not well resolved, 1H), 4.08 (s, 3H), 3.99 (s, 3H).

Gas chromatographic analysis of [^{18}F]fluoromethane ([^{18}F]CH_3F)

[^{18}F]fluoromethane was identified through coinjection of an authentic sample with the radioactive labeled sample, and matching the elution times using radio gas chromatography. Chromatographic separations were carried out on a Hewlett Packard 7890 A Series II gas chromatograph that was equipped with a 16 ft x 1/8 in o.d. stainless steel column packed with 50-80 mesh Porapak Q (Analabs, Inc.) connected in series to a 8 ft. x 1/8 in. o.d. stainless steel column packed with 50-80 mesh Porapak R (Analabs, Inc.). The column temperature was programmed from 25°C to 150°C at a 12°C/min rate during the course of analysis. Helium gas was used as the carrier at a flow rate of 50 mL/min. The column outlet was connected in series to a thermal conductivity detector (maintained at 175°C) for mass measurement, and a Geiger-type radiation monitor (Elscint, Inc. Model GRM-1) for measurement of the radioactive component. The analog outputs from both detectors were

connected to a chromatography acquisition station (Scientific Systems, Inc.) and processed using Vision IV chromatography software on a PC computer.

Synthesis of [^{18}F]Ro41-0960

To an azeotropically dried residue of no-carrier-added [^{18}F]CsF was added a solution of 3,4-dimethoxy-5,2'-dinitrobenzophenone (5, 12 mg) in DMSO (0.3 mL). The mixture was heated at 150°C for 10 min in a 10 cc silicone-coated tube (Vacutainer). The vapor generated from the reaction was flushed with a stream of N_2 and trapped on charcoal in an ion chamber (Capintec) to monitor the formation of the side product [^{18}F]CH_3F. The reaction mixture was cooled to room temperature, treated with 48% HBr(aq) (1.5 mL) and then heated at 140°C for 30 min. The crude final product which was evaporated to 1/3 of the original volume was taken up in 1.0 mL of HPLC solvent and injected onto a semipreparative HPLC column for purification. Conditions: Phenomenex Spherex C18, 5 μ, 25 x 1. cm column; mobile phase CH_3CN: 0.05 M H_3PO_4 = 35:65, 4.0 mL/min. [^{18}F]Ro41-0960 eluted at 20 min. After removal of the solvent by rotary evaporation, the product was formulated in saline containing 3-5% of ethanol and filtered (Gelman Sciences Acrodisc, sterile 0.22 μm millipore, 13 mL) into a sterile vial. The total radiosynthesis time was 100 min with a radiochemical yield of 5-10%, specific activity of 1-2 Ci/μmol (EOB) and radiochemical purity of >98%.

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