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Syntheses of the Phosphodiesterase-4 Inhibitors [^{11}C]Ro 20-1724, *R*-, *R/S*- and *S*-[^{11}C]Rolipram

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SUMMARY

The high affinity and selective cAMP-specific phosphodiesterase-4 inhibitors Ro 20-1724, *R*-, *R/S*- and *S*-rolipram were labeled with ^{11}C by *O*-[^{11}C]methylation of their respective phenolic precursors using [^{11}C]methyl iodide. The desmethyl precursor of Ro 20-1724 was prepared by selective dealkylation with iodotrimethylsilane, whereas, dealkylation of racemic rolipram was not selective and yielded several products. Enantiomeric separation of *R*- and *S*-desmethylrolipram was carried out by chiral semi-preparative high performance liquid chromatography. The final ^{11}C-labeled products were prepared in high radiochemical purity (>99%), yields (45-75%, decay-corrected) and specific activities (18.5-92.5 GBq/μmol), within 30 min from end-of-bombardment.

Key Words: carbon-11, *O*-[^{11}C]methylation, cAMP, PDE4, enzyme, positron emission tomography.

INTRODUCTION

Phosphodiesterases (PDEs) are composed of at least ten families of enzymes that hydrolyze cyclic 3',5'-adenosine monophosphate (cAMP) and/or cyclic 3',5'-guanosine monophosphate (cGMP) to their corresponding inactive 5'-monophosphate (1,2). Therefore, PDEs play an essential regulatory role in signal

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transduction pathways using these second messenger cyclic nucleotides. Among all PDE isozymes, the Ca^{2+}/calmodulin-independent cAMP-specific PDE type-4 (PDE4) enzymes have been the most intensely studied (1). This is largely due to the therapeutic potential of PDE4 inhibitors in the treatment of neuropsychiatric disorders (3), and inflammatory diseases such as asthma (4). PDE4 is one of the main isozymes that selectively terminates the actions of cAMP following the stimulation of many G-protein coupled receptors, including at least β-adrenergic (5), A_2-adenosine (5,6,7) and H_2-histamine receptors (8).

Four distinct PDE4 genes (A-D) with multiple splice variants have recently been cloned and are abundantly expressed in human brain regions. PDE4A, B and D, but not C, are present in the rat brain (1,9,10). Binding studies and molecular cloning techniques have demonstrated that, in addition to their catalytic site, all PDE4 subtypes contain a high-affinity site to which the archetypal PDE4-selective inhibitor rolipram (*R/S*-4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidinone) binds saturably, reversibly and with high affinity (K_d 1-2 nM) (11,12). With the exception of PDE4C (with a slightly different IC_{50} for the high-affinity state), similar binding affinities are obtained for isozymes A, B and D using a series of PDE4 inhibitors (9,10). The nature and function of the high-affinity [^{3}H]rolipram-binding site have yet to be fully elucidated and many hypotheses have been put forward. Recent reports suggested that PDE4 proteins have a single catalytic binding site that can exist in two different conformational binding states, one of low- and the other of high-affinity for rolipram (13,14,15,16).

Previous studies have shown that PDE4 activity and density are regulated by endogenous cAMP levels following short- and long-term regulatory processes, including phosphorylation of PDE4 by protein kinase A, and *de novo* mRNA and protein synthesis: treatments with agents increasing cAMP levels upregulate PDE4; conversely, treatments decreasing cAMP concentrations downregulate PDE4 (6,12,17). Reports suggesting that the cAMP-mediated signaling system is disrupted in several neuropsychiatric disorders, including depression (18), and dementia of the Alzheimer's and vascular types (19,20), have led to current efforts by our group (21,22,23), and more recently others (24,25), to develop new radioligands to image the high-affinity [^{3}H]rolipram-binding site of PDE4 with positron emission

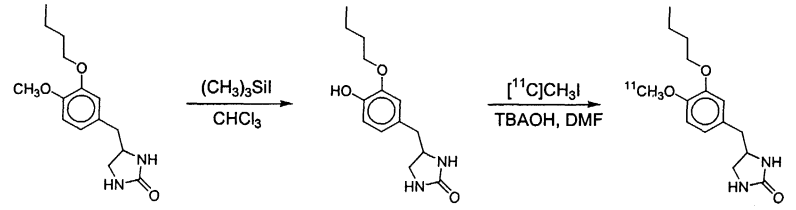
tomography (PET). In this paper, we describe the synthesis of the desmethyl-precursors and ^{11}C-labeling of the widely used high-affinity and selective PDE4 inhibitors Ro 20-1724 (*R/S*-4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone), and *R*-, *R/S*- and *S*-rolipram.

RESULTS AND DISCUSSION

R-Rolipram (IC_{50} = 2-5 nM), *R/S*-rolipram (IC_{50} = 5-7 nM), *S*-rolipram (IC_{50} = 42-95 nM) and Ro 20-1724 (IC_{50} = 39-190 nM) were previously reported to bind selectively to the PDE4 high-affinity site (7,8,10,11,12) and produce behavioral effects (26,27). We present here the synthesis of the first radiotracers developed for PET imaging of PDE4, which is an essential intracellular component of the cAMP-mediated signal transduction pathway used by a variety of receptor systems.

Synthesis and Purification of Precursors

Using 5 equivalents of iodotrimethylsilane (28,29), Ro 20-1724 was dealkylated in high yields and selectivity at the methoxy position (Scheme 1). In contrast, dealkylation with BBr_3 (1.1 or 3.3 equivalents, for 0.5-1 h) or BI_3 (1 equivalent, 5 min) in CH_2Cl_2 (30,31) resulted in the demethylated derivative in poor yields (<40%), with more by-products and a higher proportion of unreacted starting material.

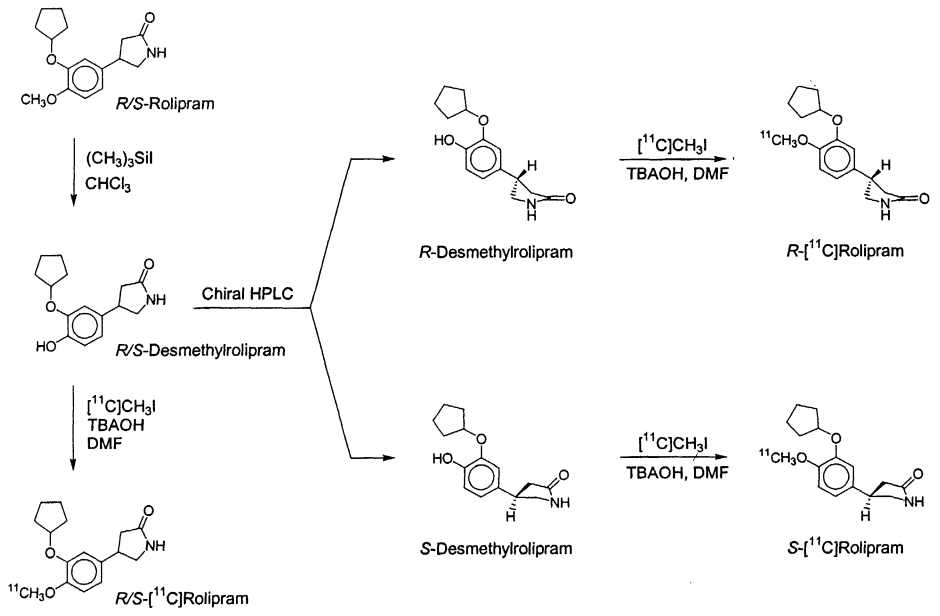


Ro 20-1724 Desmethyl-Ro 20-1724 [^{11}C]Ro 20-1724

Scheme 1. Demethylation and carbon-11 labeling of Ro 20-1724.

The reaction of *R/S*-rolipram with iodotrimethylsilane (Scheme 2) was not selective and yielded the desmethyl, descyclopentyl and cathecol derivatives in significant amounts. The putative descyclopentyl and catechol derivatives were analyzed by ^{1}H-NMR and showed the absence of the methyl group in the former, and absence of both the methyl and cyclopentyl groups in the latter (data not shown). The highest yield of desmethylrolipram was obtained using 3.3 equivalents of

iodotrimethylsilane. Under these conditions, most of the starting material was dealkylated, which is important due to the difficulty of separating the mono-hydroxy derivatives from rolipram by column chromatography. Reaction of racemic rolipram with BBr_3 (1.1 equivalent, 1 h reaction time) or BI_3 (1 equivalent, 5 min) in CH_2Cl_2 primarily produced the descyclopentyl and catechol derivatives, and a minimal amount of desmethylrolipram, along with a high quantity of unreacted rolipram. Due to its lower reactivity, higher selectivity for sterically hindered methoxy groups was previously reported using iodotrimethylsilane in similar *O*-dealkylation reactions, in comparison to reactions with BBr_3 (29). Contrary to desmethyl-Ro 20-1724, desmethylrolipram could not be purified by recrystallization following the decomposition of the silane-complex. Successive silica gel column chromatography purifications were thus necessary to separate the phenolic derivatives and unreacted racemic rolipram. Enantiomeric separation of *R*- and *S*-desmethylrolipram from the racemic mixture was achieved readily by successive chiral semi-preparative high performance liquid chromatography (HPLC) (Scheme 2), and produced both precursors in pure form (>99% ee), as established by chiral analytical HPLC.



Scheme 2. Dealkylation of *R/S*-rolipram, enantiomeric separation of *R*- and *S*-desmethylrolipram, and radiosynthesis of *R*-, *R/S*- and *S*-[^{11}C]rolipram.

Radiochemistry

[^{11}C]Ro 20-1724, and *R*-, *R/S*- and *S*-[^{11}C]rolipram were synthesized by *O*-[^{11}C]methylation of the corresponding phenolic precursors with [^{11}C]methyl iodide in the presence of TBAOH (Scheme 1 and 2), using a similar approach that has been previously utilized in the radiosynthesis of [^{11}C]tetrabenazine (32). These ^{11}C-labeled PDE4 inhibitors were prepared in high radiochemical yields (45-75%, decay-corrected, based on [^{11}C]CH_3I), purity (>99%) and specific activity (18.5-92.5 GBq/μmol or >500-2,500 mCi/μmol, at end-of-synthesis), in a synthesis time of 30 min (including quality control assays). Attempts to produce *R*- and *S*-[^{11}C]rolipram using less precursor (0.3 mg) and 70% less base were successful and produced the desired radioligands in similar yields, purities and specific activities in comparison to reactions using 1 mg of precursors. As expected, similar results were obtained in the [^{11}C]methylation reactions, reverse-phase semi-preparative and analytical HPLC in the preparation of *R*-, *R/S*- and *S*-[^{11}C]rolipram. In tracer doses, these radiotracers are expected to bind selectively to the high-affinity conformational binding state over the low-affinity state of PDE4. PDE4 is abundantly expressed in the brain (B_{max} > 100 fmol/mg of rat brain tissues) (7), and contributes to 30-70% of total rat brain cAMP-PDE activity (33). Due to their favorable binding characteristics and metabolism, these radioligands have the potential for studying PDE4 with PET. Previous studies have shown that rolipram is metabolized by ether cleavage at the methoxy and cyclopentoxy groups followed by sulphation (34), producing metabolites that do not cross the blood-brain barrier, as analyzed in rat brain homogenates with [^{3}H]rolipram (35), which is important for quantitative measurements in living brains.

In vivo evaluation in rats revealed that *R/S*-[^{11}C]rolipram exhibited higher brain uptake than [^{11}C]Ro 20-1724, and that this binding was selective for PDE4 (23). *R*- & *S*-[^{11}C]Rolipram were synthesized with the goal to use high affinity *R*-[^{11}C]rolipram to study PDE4 with a higher signal in comparison to the racemic form (22), while the less potent *S*-[^{11}C]rolipram has the potential to measure the nonspecific binding of *R*-[^{11}C]rolipram.

EXPERIMENTAL

General

Racemic Ro 20-1724 and rolipram were purchased from Sigma-Research Biochemicals (Oakville, Canada). *R*-Rolipram and *S*-rolipram were a generous gift from Berlex-Canada Inc. (Lachine, Canada). Tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained from Aldrich (Oakville, Canada), and were freshly distilled under nitrogen from $LiAlH_4$ (THF), or under reduced pressure from BaO and stored over 4 Å molecular sieves (DMF). All other chemicals were obtained from commercial sources and used without further purification. All dealkylating reactions were monitored by analytical HPLC using an Alltech Econosil C_{18} column (250 x 4.6 mm, 10 μ) eluted with CH_3CN/ammonium formate (0.1 M) 45/55 at a flow rate of 1 mL/min. Products were detected using ultraviolet (254 nm) and scintillation radioactivity detectors. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity 500 spectrometer at 500 MHz for ^{1}H-NMR or 127.5 MHz for ^{13}C-NMR spectra in deuterated chloroform ($CDCl_3$), using tetramethylsilane as an internal standard. Electron impact (70 eV) low-resolution (MS) and high-resolution mass spectra (HRMS) were obtained on a Micromass 70-250S mass spectrometer. MS data are expressed in *m/z* (intensity relative to base peak = 100).

Chemistry

Desmethyl-Ro 20-1724: Iodotrimethylsilane (385 μL, 2.69 mmol) was slowly added under nitrogen through a silicon septum in a 5 mL reacti-vial to a solution of Ro 20-1724 (150 mg, 0.54 mmol) in anhydrous $CHCl_3$ (1.4 mL) at room temperature. The reaction mixture was stirred for 64 h at room temperature. The solution was then successively treated with methanol (100 μL), and aqueous saturated solutions of sodium bisulfite (300 μL) and sodium bicarbonate (200 μL). The mixture was stirred until it became colorless, then it was diluted with methylene chloride (20 mL), stirred in the presence of Celite (10 min) and filtered. The resulting solution was filtered a second time through Celite. Following evaporation, the resultant product was recrystallized from ethyl acetate to give desmethyl-Ro 20-1724 as a white powder (127 mg, 90%): mp 112-114 °C; ^{1}H-NMR ($CDCl_3$): δ 0.98 (t, J = 7.4 Hz, 3H, CH_3),

1.50 (sext., 2H, J = 7.5 Hz, $C\underline{H}_2CH_3$), 1.78 (quint., 2H, J = 7.1 Hz, $OCH_2C\underline{H}_2$), 2.67 (dd, J = 13.6 and 7.0 Hz, 1H, $ArCH_2$) and 2.76 (dd, J = 13.4 and 6.1 Hz, 1H, $ArCH_2$), 3.19 (dd, J = 8.9 and 6.3 Hz, 1H, CH_2N) and 3.44 (virt. t, J = 8.9 Hz, 1H, CH_2N), 3.96 (dt, J = 15.1 and 6.6 Hz, 1H, CHN), 4.00 (t, J = 6.6 Hz, 1H, CH_2OAr), 6.63 (d, J = 7.8 Hz, 1H, 5-H or 6-H), 6.73 (d, J = 8.1 Hz, 1H, 6-H or 5-H), 6.77 (s, 1H, 2-H); ^{13}C-NMR ($CDCl_3$): δ 14.1 (CH_3), 20.2 ($\underline{C}H_2CH_3$), 32.5 ($\underline{C}H_2CH_2O$), 42.1, 46.7, 55.6 (CHN), 70.0 (CH_2OAr), 115.4 (Ar), 116.4 (Ar), 122.8 (6-C), 130.0 (1-C), 146.7 (ArO), 148.3 (ArO), 166.3 (CO); MS (*m/z*, %): 264 (M^+, 24%), 180 (M^+-$C_3H_4N_2O$, 100%), 124 (M^+-$C_7H_{12}N_2O$, 59%), 85 ($C_3H_5N_2O^+$, 66%); HRMS calculated for $C_{14}H_{20}N_2O_3$ (M^+) 264.1474, found 264.1476.

R/S-Desmethylrolipram: To a solution of *R/S*-rolipram (300 mg, 1.09 mmol) in anhydrous $CHCl_3$ (3.5 mL) at room temperature, iodotrimethylsilane (515 μL, 3.60 mmol) was slowly added under nitrogen through a silicon septum in a 5 mL reacti-vial. The reaction mixture was stirred for 64 h at room temperature, and then quenched with water (200 μL), stirred and transferred into an Erlenmeyer flask using ethyl acetate (75 mL). Aqueous saturated solutions of sodium bisulfite (500 μL) and sodium bicarbonate (200 μL) were added to the stirred mixture, until it became colorless. The resulting solution was washed with brine and extracted (EtOAc) three times. The combined organic extracts were dried over Na_2SO_4, filtered and evaporated. The resulting resin was purified by column chromatography (silica gel, gradient EtOAc/hexanes/triethylamine 39/60/1-49/50/1). The fractions containing *R/S*-desmethylrolipram (as analyzed by analytical HPLC) were combined and further purified by column chromatography as above to give the desired precursor as a resin (71 mg, 25%): ^{1}H-NMR ($CDCl_3$): δ 1.61-1.71 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.75-1.84 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.84-1.98 (m, 4H, 2 x $CH_2C\underline{H}_2CHO$), 2.46 (dd, J = 17.0 and 8.9 Hz, 1H, CH_2CO) and 2.71 (dd, J = 17.0 and 8.9 Hz, 1H, CH_2CO), 3.37 (dd, J = 9.3 and 7.6 Hz, 1H, CH_2N) and 3.75 (virt. t, J = 8.5 Hz, 1H, CH_2N), 3.62 (quint., J = 8.4 Hz, 1H, CHAr), 4.82 (m, 1H, CHOAr), 5.64 (br s, 1H, NH), 6.12 (br s, 1 H, ArOH), 6.72 (m, 2H, 2-H and 5-H or 6-H), 6.87 (d, J = 8.5 Hz, 1H, 6-H or 5-H); ^{13}C-NMR ($CDCl_3$): δ 23.9 ($\underline{C}H_2CH_2CHO$), 32.9 ($\underline{C}H_2CHO$), 38.1, 40.1, 49.8 ($\underline{C}H_2CO$), 80.7 ($\underline{C}HOAr$), 111.3 (Ar), 114.5 (Ar), 119.1 (Ar), 133.8 (1-C), 145.0 (ArO), 145.3 (ArO),

177.6 (CO); MS (*m/z*, %): 261 (M^+, 32%), 193 (M^+-C_5H_8, 100%), 136 (M^+-$C_7H_{11}NO$, 87%); HRMS calculated for $C_{15}H_{19}NO_3$ (M^+) 261.1365, found 261.1353.

R- and S-Desmethylrolipram: Dealkylation of racemic rolipram (200 mg, 0.73 mmol and 3.3 equivalents of iodotrimethylsilane) was carried out two more times as described above, followed by column chromatography purification of *R/S*-desmethylrolipram (as above) obtained from all attempted dealkylating reactions. Enantiomeric separation of the combined fractions containing *R/S*-desmethylrolipram was performed by successive chiral semi-preparative HPLC (Chirex S-Leu & R-NEA column, 250 x 10 mm, Phenomenex, CA, U.S.A., hexane/ethanol 95/5, 10 mL/min, *S*-desmethylrolipram R_t = 15.0 min and k' = 7.1, *R*-desmethylrolipram R_t = 17.5 min and k' = 8.5). The fractions enriched in *R*- or *S*-desmethylrolipram were combined and further purified (hexane/ethanol 97/3, 10 mL/min, *S*-desmethylrolipram R_t = 33.5 min and k' = 16.1, *R*-desmethylrolipram R_t = 35.0 min and k' = 16.9) until pure *R*- or *S*-desmethylrolipram was obtained, both as white powders. The enantiomeric purity of the isolated products was determined by chiral analytical HPLC with a Chirex S-Leu & R-NEA column (250 x 4.6 mm, Phenomenex) eluted with hexane/ethanol 95/5 at a flow rate of 2 mL/min (*S*-desmethylrolipram R_t = 18.5 min and k' = 8.8, and *R*-desmethylrolipram R_t = 20.5 min and k' = 9.9). Identity of *R*-desmethylrolipram purified by chiral semi-preparative HPLC was established by co-injection of *R*-desmethylrolipram, prepared from authentic *R*-rolipram and purified as described above for the racemate, and gave only one peak using the chiral analytical column (at R_t = 20.5 min and k' = 9.9). In contrast, co-injection of *R*-desmethylrolipram (prepared from authentic *R*-rolipram) with *S*-desmethylrolipram gave two peaks in chiral analytical HPLC (R_t = 18.5 min & k' = 8.8, and 20.5 min & k' = 9.9).

R-Desmethylrolipram. mp 124.0-124.5 °C; ^{1}H-NMR ($CDCl_3$): δ 1.60-1.70 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.74-1.83 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.83-1.97 (m, 4H, 2 x $CH_2C\underline{H}_2CHO$), 2.45 (dd, *J* = 16.8 and 9.0 Hz, 1H, CH_2CO) and 2.70 (dd, *J* = 17.0 and 8.9 Hz, 1H, CH_2CO), 3.36 (dd, *J* = 9.5 and 7.6 Hz, 1H, CH_2N) and 3.74 (virt. t, *J* = 8.9 Hz, 1H, CH_2N), 3.60 (quint., *J* = 8.4 Hz, 1H, CHAr), 4.81 (m, 1H, CHOAr), 5.79 (s, 1H, NH), 6.70 (m, 1H, 5-H or 6-H), 6.71 (s, 1H, 2-H), 6.73 (br s, 1 H,

ArOH), 6.86 (m, 1H, 6-H or 5-H); ^{13}C-NMR ($CDCl_3$): δ 23.9 ($\underline{C}H_2CH_2CHO$), 32.9 ($\underline{C}H_2CHO$), 38.2, 40.1, 49.9 ($\underline{C}H_2CO$), 80.7 ($\underline{C}HOAr$), 111.4 (Ar), 114.5 (Ar), 119.1 (Ar), 133.8 (1-C), 145.1 (ArO), 145.4 (ArO), 177.8 (CO); MS (*m/z*, %): 261 (M^+, 8%), 193 (M^+-C_5H_8, 66%), 136 (M^+-$C_7H_{11}NO$, 100%); HRMS calculated for $C_{15}H_{19}NO_3$ (M^+) 261.1365, found 261.1366.

S-Desmethylrolipram. mp 125.0-126.0 °C; ^{1}H-NMR ($CDCl_3$): δ 1.58-1.68 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.73-1.82 (m, 2H, $C\underline{H}_2CH_2CHO$), 1.82-1.96 (m, 4H, 2 x $CH_2C\underline{H}_2CHO$), 2.45 (dd, J = 17.0 and 8.9 Hz, 1H, CH_2CO) and 2.69 (dd, J = 16.9 and 9.0 Hz, 1H, CH_2CO), 3.36 (virt. t, J = 8.5 Hz, 1H, CH_2N) and 3.73 (virt. t, J = 9.0 Hz, 1H, CH_2N), 3.58 (quint., J = 8.4 Hz, 1H, CHAr), 4.80 (m, 1H, CHOAr), 6.0 (br s, 1H, NH), 6.69 (d, J = 8.3 Hz, 1H, 5-H or 6-H), 6.71 (s, 1H, 2-H), 6.84 (d, J = 8.1 Hz, 1H, 6-H or 5-H), 7.12 (br s, 1H, ArOH); ^{13}C-NMR ($CDCl_3$): δ 23.9 ($\underline{C}H_2CH_2CHO$), 32.8 ($\underline{C}H_2CHO$), 38.4, 40.1, 50.0 ($\underline{C}H_2CO$), 80.6 ($\underline{C}HOAr$), 111.6 (Ar), 114.6 (Ar), 119.1 (Ar), 133.8 (1-C), 145.1 (ArO), 145.4 (ArO), 178.2 (CO); MS (*m/z*, %): 261 (M^+, 7%), 193 (M^+-C_5H_8, 64%), 136 (M^+-$C_7H_{11}NO$, 100%); HRMS calculated for $C_{15}H_{19}NO_3$ (M^+) 261.1365, found 261.1365.

Radiochemistry

[^{11}C]Ro 20-1724: [^{11}C]Methyl iodide, produced from [^{11}C]CO_2 as described previously (36), was swept at 10 mL/min with N_2 and trapped in a 1 mL reacti-vial containing desmethyl-Ro 20-1724 (1 mg) and tetrabutylammonium hydroxide (TBAOH, MeOH 1 M solution, 1.1 equivalent) in DMF (185 μL) at -15 to -40°C. When radioactivity accumulation in the reaction vial was maximal (approx. 3 min), the solution was heated at 85°C for 5 min. After cooling, the mixture was quenched with the HPLC solvent (0.5 mL), and then [^{11}C]Ro 20-1724 was purified by semi-preparative HPLC (Econosil C_{18}, 250 x 10 mm, 10 μ, CH_3CN/ammonium formate (0.1 M) 40/60, 6 mL/min, R_t = 8.5 min). After evaporation of the solvent, the residue was dissolved in saline (10 mL), passed through a 0.22 μm filter into a sterile vial containing aqueous sodium bicarbonate (1 mL, 8.4%). The purity and specific activity of the final formulation of [^{11}C]Ro 20-1724 (pH 6.5-8) was established by analytical HPLC (Econosil C_{18}, 250 x 4.6 mm, 10 μ, CH_3CN/ammonium formate (0.1 M) 40/60, 3 mL/min, R_t = 3.7 min). Identity of the radioactive product as

[^{11}C]Ro 20-1724 was determined by co-elution of authentic standard using analytical HPLC.

R/S-[^{11}C]Rolipram: *R/S*-[^{11}C]Rolipram was prepared from *R/S*-desmethylrolipram and [^{11}C]methyl iodide, purified and analyzed using similar conditions as described above for [^{11}C]Ro 20-1724 (semi-preparative HPLC: 7 mL/min, R_t = 8 min; analytical HPLC: 4 mL/min, R_t = 3 min).

R-[^{11}C]Rolipram: *R*-[^{11}C]Rolipram was synthesized, purified and analyzed using the same procedure as for *R/S*-[^{11}C]rolipram, except that it was prepared from *R*-desmethylrolipram and [^{11}C]methyl iodide.

S-[^{11}C]Rolipram: *S*-[^{11}C]Rolipram was prepared from *S*-desmethylrolipram using the same procedure and HPLC conditions as for *R/S*-[^{11}C]rolipram.

CONCLUSION

The selective PDE4 inhibitors [^{11}C]Ro 20-1724, *R*-, *R/S*- and *S*-[^{11}C]rolipram were synthesized in high radiochemical yields and purity by *O*-[^{11}C]methylation of the corresponding phenolic precursors with [^{11}C]methyl iodide. As the first radioligands developed to image PDE4 with PET, they have the potential to be used for studying *in vivo* this major intracellular component of the cAMP signal transduction system in a variety of receptor systems.

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