Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 14 December 2009, Revised 12 March 2010, Accepted 17 March 2010 Published online 11 May 2010 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1778

Radiosynthesis of the HIV integrase inhibitor [^{18}F]MK-0518 (Isentress)

Wenping Li,[a]* Wayne Thompson,[b] Thorsten Fisher,[b] John S. Wai,[b] Daria Hazuda,[c] H. Donald Burns,[a] and Terence G. Hamill[a]

The human immunodeficiency virus integrase inhibitor, [^{18}F]MK-0518, was prepared *via* a three-step, one-pot radiosynthesis. [^{18}F]4-Fluorobenzylamine was produced from the fluorination of 4-cyano-*N*,*N*,*N*-trimethylammonium triflate with [^{18}F]fluoride and reduction with borane methylsulfide complex in 50–68% radiochemical yield. The final step, the coupling of [^{18}F]4-fluorobenzylamine with an ester coupling partner, achieved an overall uncorrected radiochemical yield after HPLC purification of ~2%, based on the starting [^{18}F]fluoride. In a typical run, the total synthesis time was about 90 min and gave 0.37–1.74 GBq (10–47 mCi) of [^{18}F]MK-0518. The radiochemical purity of [^{18}F]MK-0518 was > 98% and the specific activity was 243–1275 Ci/mmol (EOS, *n* = 4). A convenient three-step, one-pot radiosynthesis of [^{18}F]MK-0518 *via* [^{18}F]4-fluorobenzylamine has been developed, giving sufficient quantities of [^{18}F]MK-0518 for animal positron emission tomography studies.

Keywords: HIV integrase inhibitor; PET imaging; Isentress; fluorine-18

Introduction

The human immunodeficiency virus (HIV) is a retrovirus that infects cells of the human immune system, destroying or impairing their function. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS). It is estimated that over 33 million people are living with HIV/AIDS worldwide, of which 1.2 million were in the United States at the end of 2008.[1] Although decades of research and the successful development of combination antiretroviral therapies have transferred HIV infection from a fatal to a chronic life-threatening disease, HIV remains one of the most serious health problems in the world.[2–4]

HIV encodes three enzymes: reverse transcriptase (RT), protease (PR), and integrase (IN), each of which play essential roles in viral replication.[5,6] The standard HIV/AIDS treatment models based on nucleosidic and non-nucleosidic RT inhibitors and/or PR inhibitors have been limited due to the multidrug-resistant HIV infection and cross-resistance to agents within a class.[7] Therefore, novel drugs targeting other steps in the HIV-1 life cycle are needed.

HIV-1 IN catalyzes the insertion of the proviral DNA into the host genome (strand transfer) and integration is required for both the stable maintenance of the viral genome and for efficient viral gene expression and replication.[8–10] IN inhibitors are an attractive new class of HIV drugs. Isentress (MK-0518, Raltegravir), shown in Figure 1, is the first IN inhibitor approved by the Food and Drug Administration for use in the treatment of HIV-1 infection in combination with other antiretroviral agents.[11–14]

The goal of this work was to synthesize [^{18}F]MK-0518 to enable the non-invasive study of MK-0518 biodistribution in rhesus monkeys using PET. The planned synthesis of [^{18}F]MK-0518, shown in Scheme 1, would involve using [^{18}F]4-fluorobenzyl amine, a very useful intermediate in F-18 chemistry, which can by synthesized by a variety of routes.[15–20] Generally, it is prepared by the reduction of [^{18}F]4-fluorobenzonitrile. Lithium aluminum hydride ($LiAlH_4$) has been reported as the preferred reducing agent because of its rapid reaction and quantitative yield.[16] However, the use of $LiAlH_4$ is less convenient due to the required anhydrous reaction conditions, the formation of large amounts of aluminum salts, and the troublesome purification. Borane reagents are very effective for the conversion of nitriles to amines and have advantages over $LiAlH_4$.[21] For the work described in this study, borane methylsulfide complex (BH_3-Me_2S, BMS) was selected as the reducing agent, the procedures described in the literature were modified and a one-pot synthesis of [^{18}F]MK-0518 *via* [^{18}F]4-fluorobenzylamine was developed.[16,18]

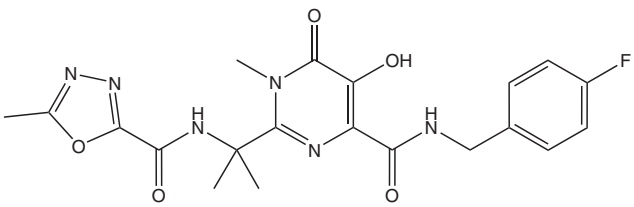


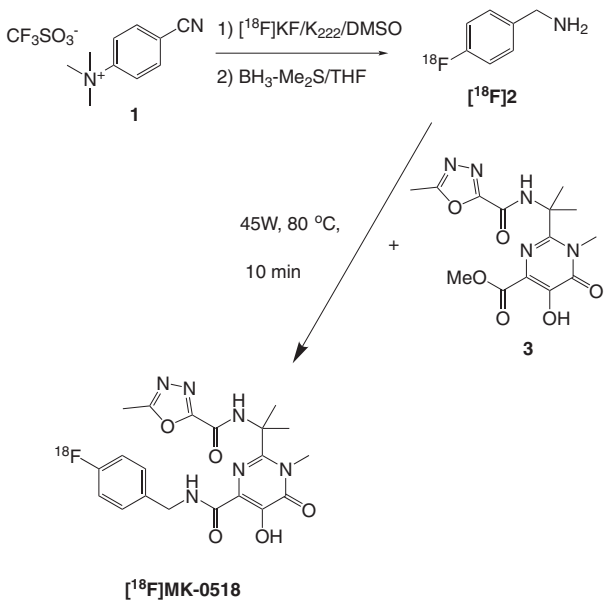
Figure 1. Chemical structure of MK-0518 (Isentress, Raltegravi) (Reference:[12]).

[a]*Imaging Research, Merck Research Laboratories, West Point, PA, USA*

[b]*Medicinal Chemistry, Merck Research Laboratories, West Point, PA, USA*

[c]*Infectious Diseases, Merck Research Laboratories, West Point, PA, USA*

**Correspondence to: Wenping Li, Imaging Research, Merck Research Laboratories, WP 44D-2, West Point, PA 19486, USA.*
E-mail: wenping_li@merck.com



Scheme 1. Radiosynthesis of [^{18}F]MK-0518.

Results and discussions

Radiochemistry

The three-step, one-pot radiosynthesis of [^{18}F]MK-0518 is outlined in Scheme 1. In the first step, [^{18}F]4-fluorobenzonitrile was synthesized *via* a nucleophilic aromatic substitution reaction. 4-Cyano-*N*,*N*,*N*-trimethylammonium triflate was chosen as the starting material, due to trimethylammonium being the preferred leaving group over halo or nitro substituents.[16–20] In previous reports, the reaction of **1** with [^{18}F]KF-K_{222} complex was performed by heating **1** in dimethyl sulfoxide (DMSO) for 10 to 20 min at temperatures ranging from 120 to 180°C with a radiochemical yields of 60–85%.[16,18–20] Kuhnast *et al.* reported the preparation of [^{18}F]4-fluorobenzonitrile by microwave heating at 100 W for 1 min resulting in a similar radiochemical yield.[17] However, no temperature was reported for the substitution.

To determine the optimum reaction conditions in our hands, this reaction was carried out under various temperatures (110–160°C), input power (75, 100 W), and reaction times (1–5 min). All reactions were carried out in DMSO. Similar radiochemical yields (75–85%) were observed when heated at 120°C or higher temperatures. Lower temperatures were preferred to prevent the loss of volatile [^{18}F]4-fluorobenzonitrile. Heating for 3 min provided the highest radiochemical yields ($\geqslant$85%) when varying the reaction time. Heating at 75 W or 100 W was also tested, and no difference in the radiochemical yield was observed. Therefore, the substitution was carried out in DMSO using microwave heating at 75 W, 120°C for 180 s, providing the desired [^{18}F]4-fluorobenzonitrile. Typically, the fluorination reaction under these conditions resulted in a radiochemical yield of $\geqslant$80% from [^{18}F]fluoride, which was comparable with the methods reported in the literature.[16–20]

The second step in this sequence was the conversion of [^{18}F]4-fluorobenzonitrile to [^{18}F]**2** with BMS. After cooling the crude reaction from step 1 to room temperature, the crude [^{18}F]4-fluorobenzonitrile was treated with an excess of BMS. [^{18}F]**2** was formed with a 50–68% radiochemical purity based on analytical HPLC. It was noticed that this conversion rate was higher in a completely cooled reaction mixture (room temperature) compared with warmer solutions in trial reactions. Reduction with $LiAlH_4$ needs to be performed in harsh conditions, such as refluxing THF at 120–140°C for 2 min, followed by quenching the excess of $LiAlH_4$ and Sep-pak purification.[15–17,19] This procedure is somewhat troublesome and time consuming. The use of borane reagents, such as borane-THF and borane-Me_2S (BMS), have been described to be very effective for the conversion of [^{18}F]fluorobenzonitrile to [^{18}F]**2** under mild conditions.[18] Borane-Me_2S in THF was selected over $LiAlH_4$ as the reducing agent not only to avoid the complicated purification of excess reducing agent but also to prevent the formation of by-products.

Initially, the purification of [^{18}F]**2** with a C18 Light Sep-Pak was attempted. The reduction mixture was quenched with 5 M HCl and basified with 5 M NaOH and water. The basic solution was loaded onto two prewashed C18 Light Sep-Pak cartridges, and the cartridges were washed with 0.01 N NaOH. [^{18}F]**2** was finally eluted from the cartridges in ethanol through a drying column containing a mixture of potassium carbonate and sodium sulfate (60:40). Using this procedure, pure [^{18}F]**2** was obtained as a dry ethanolic solution with an uncorrected radiochemical yield of ~25% based on the initial activity of [^{18}F]F^-.

A significant amount of radioactivity (~30%) remained on the Sep-Pak cartridges and drying column. Increasing the elution volume of ethanol resulted in more radioactivity eluting from the cartridge, suggesting the radioactivity retained on the Sep-Pak cartridge was due to [^{18}F]**2**. This purification sequence required about 25 min but resulted in large volumes of elution solvent making it difficult to perform an automated synthesis of [^{18}F]**2**. To shorten the synthesis time and improve the radiochemical yield, omitting the Sep-Pak purification step was investigated. This led to decreasing the overall synthesis time by nearly 15 min and increasing the available activity of [^{18}F]**2**, ultimately improving the radiochemical yield of [^{18}F]MK-0518.

The final step, coupling [^{18}F]**2** with ester **3** was performed in the same reaction vial. Previous model reactions using unlabeled materials had been carried out to determine the best conditions for the final coupling step. The coupling of methyl ester **3** with unlabeled 4-fluorobenzylamine was explored under different reaction conditions including solvents (ethanol and DMSO), molar ratios of ester:amine (1:1, 1:0.5, 1:0.1), reaction times (30, 60 min), and temperatures (80, 100°C), based on the synthetic route reported by Summa *et al.*[12] The coupling of **3** was achieved with a chemical yield of 80% or greater within 30 min under all conditions tested, and no differences in the chemical yield were observed between different solvents or temperatures. Therefore, the final coupling reaction was carried out at 80°C in either ethanol or DMSO for 30 min. However, under no-carrier added conditions, the coupling of [^{18}F]**2** in DMSO provided a slightly higher yield than in ethanol with a shorter reaction time 10 min. Longer reaction times (30 min) did not improve the yield of [^{18}F]MK-0518. The coupling [^{18}F]**2** with ester **3** at 45 W, 80°C for 10 min in DMSO provided the highest radiochemical yield among the conditions used, providing [^{18}F]MK-0518 in an overall uncorrected radiochemical yield (decay uncorrected) of 1–4%. The trace from the final preparative HPLC purification of [^{18}F]MK-0518 is shown in Figure 2. The desired product eluted at 15.5 min (Panel a) and only the fractions across the narrow peak were collected to avoid the contamination of a UV by-product, eluting at 16.5 min (Panel b). The final [^{18}F]MK-0518 displayed no by-product UV

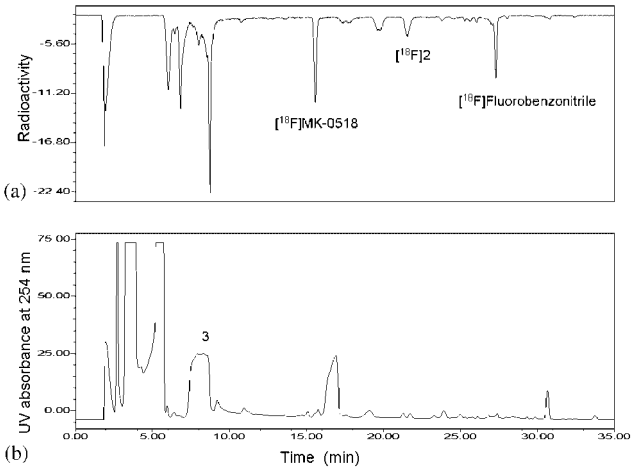


Figure 2. HPLC chromatograms (radioactivity and UV absorbance vs time) of the purification of [^{18}F]MK-0518 with a semi-preparative reversed-phase HPLC column: (a) radioactivity and (b) UV absorbance at 254 nm.

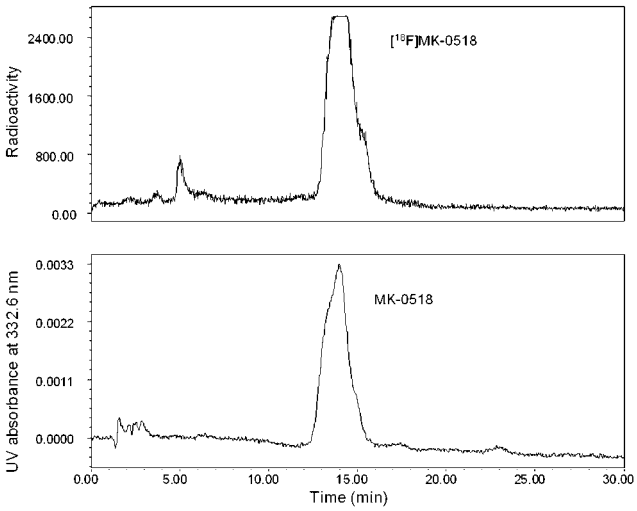


Figure 3. Chromatograms of analytical HPLC of no-carrier added [^{18}F]MK-0518: (a) radioactivity and (b) UV absorbance at 332.6 nm.

peak detected on the analytical HPLC chromatogram (Figure 3). The total synthesis time was about 90 min and gave 0.37–1.74 GBq (10–47 mCi) of pure [^{18}F]MK-0518 with a specific activity of 587 ± 480 Ci/mmol ($n = 4$) and a radiochemical purity > 98%. The identity of [^{18}F]MK-0518 was confirmed by co-elution with the authentic standard using analytical HPLC analysis (Figure 4).

Experimental

Materials and methods

General

BMS (2 M in THF), 4-nitrobenzonitrile, 4-fluorbenzylamine, and all solvents were purchased from Aldrich Chemical Co. and were used without further purification. Compounds **1**, **3** and MK-0518 were provided by Merck Research Laboratories. [^{18}F]F^- was obtained from Siemens Biomarker Solutions (North Wales, PA) on an anion exchange resin transported to the radiochemistry laboratory. Radiochemical procedures were performed using a

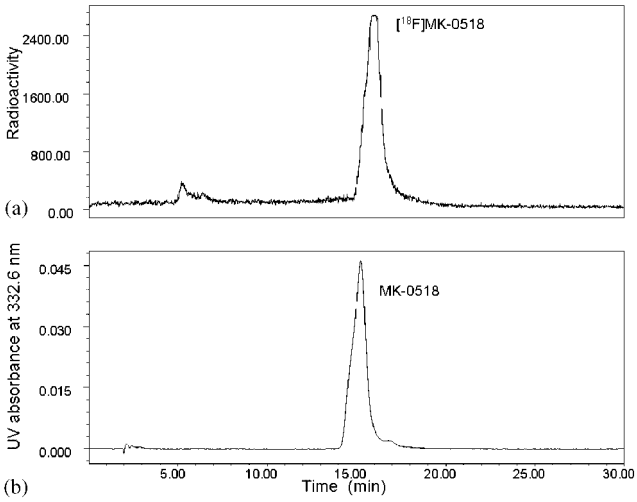


Figure 4. Chromatograms of analytical HPLC of [^{18}F]MK-0518 co-injected with authentic standard: (a) radioactivity and (b) UV absorbance at 332.6 nm. The small shoulder peak at ~17 min of RT was attributed from the authentic standard, and was not observed in HPLC analysis of no-carrier added [^{18}F]MK-0518.

Gilson (Worthington, OH) 233XL liquid handler and microwave reactions were carried out in a Resonance Instruments Model 521A Microwave Cavity with integrated temperature control. A Capintec CRC-35R dose calibrator was used for radioactivity measurements. Waters C-18 Light Sep-pak cartridges were pre-activated with ethanol (5 ml), followed by sequential washing with water (10 ml) and 0.01 N NaOH (10 ml) before use.

The [^{18}F]MK-0518 was purified on a semi-preparative reverse-phase HPLC system equipped with a Waters (Milford, MA) 600 pump and 600 Controller using a Waters XBridge C18 column (5 μm, 10 × 150 mm). The mobile phase consisted of acetonitrile (CH_3CN) containing 0.1% TFA (A) and phosphate buffer (10 mM Na_2HPO_4, pH 7.4) (B) and a gradient method was used. A linear gradient of 10% A to 30%A over 20 min and 30% A to 60% A over 5 min, holding at 60% A for 10 min with a run time of 35 min at 4 ml/min was used. The preparative run was monitored at 254 nm with an Amersham Bioscience (Piscataway, NJ) UV-M II detector and a Bioscan (Missisauga, Ont., Canada) FlowCount radioactivity detector. [^{18}F]MK-0518 eluted at 15 min and its radiochemical purity and identity was determined with analytical HPLC by coinjection with the authentic standard. Analytical reverse-phase HPLC was accomplished on a Waters (Milford, MA) 600E chromatography system with a Waters 991 photodiode array detector and a Bioscan (Missisauga, Ontario, Canada) FlowCount radioactivity detector. All samples were analyzed on a Waters X-Terra C18 column (5 μm, 4.6 × 150 mm). The mobile phase was CH_3CN (0.1% TFA) (A) and phosphate buffer (10 mM Na_2HPO_4, pH 7.4) (B). Gradient elution consisted of a linear gradient in 20 min from 10/90 to 30/70 and another linear gradient followed in 5 min from 30/70 to 60/40 at 1 ml/min. A 5-min washing-out at 60/40 was followed to the end of run (30 min run time). Both [^{18}F]4-fluorobenzonitrile ($R_t = 27$ min) and [^{18}F]**2** ($R_t = 22$ min) were confirmed with commercial standards with UV absorbance at 254 nm, respectively. [^{18}F]MK-0518 co-eluted with the authentic standard at 15.3 min at 332.6 nm of UV maximum absorption wavelength. Specific activities for [^{18}F]MK-0518 were determined by using analytical HPLC using the same system described above against a calibration curve prepared with the cold standard.

Radiochemistry

The [^{18}F]fluoride was obtained on an anion exchange resin from Siemens Biomarker Solutions, transported to the radiochemistry laboratory, and was eluted with a mixture (0.5 ml) of 80% MeCN: 20% oxalate aqueous solution (a mixture of 0.05 ml of 200 mg $K_2C_2O_4$/3 mg K_2CO_3/5 ml H_2O, 0.25 ml H_2O, and 1.2 ml MeCN) and azeotropically dried under an argon stream adapted from a reported procedure.[22] Briefly, the eluted [^{18}F]F^- was transferred into a 1 ml v-vial containing Kryptofix 222 (0.2 ml, 36 mg/ml in MeCN). The vial was vented with an 18G1 syringe needle and the mixture was dried under an argon stream using microwave heating at 40 W, 80°C for 3 min. Additional aliquots of acetonitrile (3 × 0.5 ml) were added and heated to dryness.

*4-[^{18}F]fluorobenzylamine ([^{18}F]**2**).* The synthesis of [^{18}F]4-fluorobenzylamine ([^{18}F]**2**) was carried out with some modification as previously described.[17,18]

Fluorination: A precursor solution of **1** (5 mg) in anhydrous DMSO (0.2 ml) was added to the dry cryptate of [^{18}F]KF in a 1-ml vial, the vent needle was removed, and the reaction mixture was heated at 75 W, 120°C for 180 s in the microwave reactor system. An aliquot (10 μl) was removed and analyzed by using analytical HPLC as described above to determine the radiochemical yield.

Reduction: The reaction mixture was allowed to cool to room temperature. BMS (400 μl, 2 M in THF) was added, and the mixture was stirred first at room temperature for 5 min with an argon flow. An aliquot (10 μl) was removed and analyzed to determine the radiochemical purity by using the same analytical HPLC method as described above. The THF was then removed under argon flow with microwave heating (35 W, 65°C for 5 min and then at 40 W, 80°C for 5 min).

Synthesis of [^{18}F]MK-0518. Methyl ester **3** (~5 mg) in DMSO (300 μl) was added to the crude reaction mixture. The mixture was heated in the microwave (45 W, 80°C) for 10 min. After cooling for 1 min, the mixture was diluted with water (800 μl) and loaded onto an Xbridge C-18 semi-preparative HPLC column. The peak corresponding to [^{18}F]MK-0518 eluting at 15 min was collected, and most of the solvent was evaporated, and transferred into a sterile vial for animal studies. One typical synthesis for animal PET study provided 47 mCi of [^{18}F]MK-0518 with a specific activity of 1275 Ci/mmol at the end of synthesis and a radiochemical purity > 98% in a radiochemical yield 4%.

Conclusion

[^{18}F]MK-0518 has been successfully synthesized *via* the three-step one-pot procedure described in this study, giving sufficient quantities of [^{18}F]MK-0518 for *in vivo* PET studies. The three-step, one-pot radiosynthesis included nucleophilic fluorination, nitrile reduction, and coupling in the same reaction vial and is simple and convenient to use as no purifications of intermediates are required. The total synthesis time was about 90 min and gave 10–47 mCi (0.37–1.74 GBq) of [^{18}F]MK-0518 with a specific activity ranging from 243 to 1275 Ci/mmol ($n = 4$) and radiochemical purity > 98%. The results of the animal PET studies will be the subject of another study and published elsewhere.

References

[1] *2008 Report on the Global AIDS Epidemic, UNAIDS.* Geneva, Switzerland **2008**.
[2] F. J. Palella, K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, G. A. Satten, D. J. Aschman, S. D. Holmberg, *N. Engl. J. Med.* **1998**, *338*, 853–860.
[3] E. Wood, R. S. Hogg, B. Yip, P. R. Harrigan, M. V. O'Shaughnessy, J. S. Montaner, *AIDS* **2003**, *17*, 711–720.
[4] G. Chene, J. A. Sterne, M. May, D. Costagliola, B. Ledergerber, A. N. Phillips, F. Dabis, J. Lundgren, A. D'Arminio Monforte, F. de Wolf, R. Hogg, P. Reiss, A. Justice, C. Leport, S. Staszewski, J. Gill, G. Fatkenheuer, M. E. Egger; The Antiretroviral Therapy Cohort Collaboration, *Lancet* **2003**, *362*, 679–686.
[5] E. Asante-Appiah, A. M. Skalka, *Antiviral Res.* **1997**, *36*, 139–156.
[6] P. hindmarsh, J. Leis, *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 836–843.
[7] J. Cohen, *Science* **2002**, *296*, 2320–2324.
[8] Y. Pommier, A. A. Johnson, C. Marchand, *Nat Rev. Drug Discov.* **2005**, *4*, 236–248.
[9] D. J. Hazuda, P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J. A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau, M. D. Miller, *Science* **2002**, *287*, 646–650.
[10] R. L. LaFemina, C. L. Schneider, H. L. Robbins, P. L. Callahan, K. Legrow, E. Roth, W. A. Schleif, E. A. Emini, *J. Virol.* **1992**, *66*, 7414–7419.
[11] M. Rowley, *Progr. Med. Chem.* **2008**, *46*, 1–28.
[12] V. Summa, A. Petrocchi, F. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. G. Paz, D. J. Hazuda, P. Jones, O. Kinzel, R. Laufer, E. Monteagudo, E. Muraglia, E. Nizi, F. Orvieto, P. Pace, G. Pescatore, R. Scarpelli, K. Stillmock, M. V. Witmer, M. Rowley, *J. Med. Chem.* **2008**, *51*, 5843–5855.
[13] Y. Wang, N. Serradell, J. Bolos, E. Rosa, *Drugs Fut.* **2007**, *32*, 118–122.
[14] T. H. Evering, M. Markowitz, *Drugs Today* **2007**, *43*, 865–877.
[15] P. K. Garg, S. Garg, M. R. Zalutsky, *Bioconjugate Chem.* **1991**, *2*, 44–49.
[16] F. Dolle, F. Hinnen, F. Vaufrey, B. Tavitian, C. Crouzel, *J. Label. Comp. Radiopharm.* **1997**, *39*, 319–330.
[17] B. Kuhnast, F. Hinnen, R. Boisgard, B. Tayitian, F. Dolle, M. R. Kilbourn, G. L. Watkins, S. A. Toorongian, *J. Label Comp. Radiopharm.* **2003**, *46*, 1093–1103.
[18] T. Haradahira, Y. Hasegawa, K. Furuta, M. Suzuki, Y. Watanabe, K. Suauki, *Appl. Radiat. Isot.* **1998**, *49*, 1551–1556.
[19] I. Koslowsky, S. Shahhosseini, J. Wilson, J. Mercer, *J. Label Comp. Radiopharm.* **2008**, *51*, 352–356.
[20] M. S. Haka, M. R. Kilbourn, G. L. Watkins, S. A. Toorongian, *J. Label Comp. Radiopharm.* **1989**, *27*, 823–833.
[21] H. C. Brown, Y. M. Choi, S. Narasimhan, *Synthesis* **1981**, 605–606.
[22] T. G. Hamill, S. Krause, R. Christine, C. Bonnefous, S. Govek, T. J. Seiders, N. D. P. Cosford, J. Roppe, T. Kamenecka, S. Patel, R. E. Gibson, S. Sanabria, K. Riffel, W. Eng, C. King, X. Yang, M. D. Green, S. S. O'Malley, R. Hargreaves, H. D. Burns, *Synapse* **2005**, 205–216.