RSC Advances

RSC Publishing

PAPER

Synthesis of [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione: an agent for specific radiolabelling of tyrosine

Cite this: *RSC Adv.*, 2013, **3**, 24936

Flagothier Jessica,*[ab] Warnier Corentin,[b] Dammicco Sylvestre,[ab] Lemaire Christian[b] and Luxen André[ab]

Received 26th August 2013
Accepted 10th October 2013

DOI: 10.1039/c3ra44666b

www.rsc.org/advances

We developed a new [^{18}F] prosthetic group, the [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione ([^{18}F]F-PTAD), used for its specific ligation with tyrosine-containing peptides or protein in order to develop a new and versatile radiolabelling technique that could provide a useful tool for new developments in PET imaging.

Introduction

Peptides and proteins are largely used as radiopharmaceuticals for positron emission tomography (PET) studies. The most frequently used radionuclides for PET provide a wide range of physical half-lives that are compatible with the peptide or protein biological half-lives. They can be classified according to their mode of labelling. The peptide is covalently coupled, often *via* a spacer, to a chelator (*e.g.* DOTA, NOTA) which can complex radiometals as gallium-68, copper-64, yttrium-86[1,2] or carry a prosthetic group that can be labelled with iodine-124 or fluorine-18 ions.[3–5] The introduction of fluorine-18 requires harsh conditions (high temperature and strong base) that are not compatible with direct labelling of biomolecules. Therefore, two strategies are usually explored.

The first one is the incorporation of a linker onto the amino acid. The peptide is coupled *via* the linker to the [^{18}F] prosthetic group. For instance, the coupling can be realized *via* Huisgen cycloaddition between [^{18}F]fluoroethylazide[6] or 1-(azidomethyl)-4-[^{18}F]-fluorobenzene[7] and a alkyne or azide-bearing peptide or protein, *via* photoclick to avoid the use of copper,[8] *via* oxime formation between [^{18}F]fluorobenzaldehyde and modified peptides[9] or *via* the tetrazine *trans*-cyclooctene reaction.[10]

The second strategy is the [^{18}F] prosthetic group ligation directly on naturally available function of amino acid side chains of peptides or proteins. *E.g.*: [^{18}F]SFB,[11] [^{18}F]FBEM[12,13] can be linked under mild conditions on –NH_2, –SH group of the peptide.

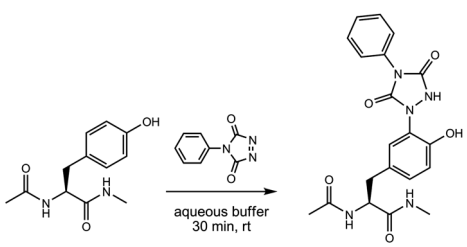
Lysine and cysteine are the most commonly functionalized amino acids: however, the high abundance of lysine makes specific modification difficult and cysteine is most often found in disulfide linked pairs in protein natural environment.

Much recent research developments have focused on the modification of tyrosine and tryptophan. These residues are relatively rare on protein surface, and offer opportunities for controlled single-site modification.

We hereby focus on the coupling between a new prosthetic group and a tyrosine-containing peptide. In literature, these reactions often use transition-metal-mediated processes.[14–17] However, transition metal mediated reactions are to be avoided as much as possible in radiochemistry. The transition metals are indeed often toxic and must be removed efficiently from the sample prior to injection, which complicates and lengthens both the synthesis and quality control of the radiotracer. Francis and co-workers have also explored the labelling of tyrosine residues through a three-component Mannich-type reaction with aldehydes and anilines.[18–20] But these reactions are not transposable in [^{18}F] radiochemistry due to the long synthesis time (20–24 h).

Recently, Ban and co-workers have reported a tyrosine bioconjugation through ene-type reactions[21,22] (Scheme 1).

In this reaction, the cyclic diazodicarboxamide PTAD, an electrophilic compound, reacts selectively on the *o*-position of the phenol side chain of tyrosine in mild aqueous conditions (aqueous buffer). They showed that histidine, serine and cysteine were not modified by PTAD and determined that tryptophan and lysine did not interfere with the modification of tyrosine. That indicates that this reagent exhibits a high degree



Scheme 1 Tyrosine bioconjugation through ene-type reaction developed by Ban and co-workers.

[a]*Département de chimie, Université de Liège, 3 allée du 6 aout, B-4000 Liège, Belgium. E-mail: jflagothier@ulg.ac.be; Fax: +32 4 3662946; Tel: +32 4 3662334*

[b]*Centre de Recherches du Cyclotron, Université de Liège, 8 allée du 6 aout, B-4000 Liège, Belgium. E-mail: aluxen@ulg.ac.be; Fax: +32 4 3662946; Tel: +32 4 3663687*

of chemoselectivity. They also demonstrated that the 1,2,4-triazolidine-3,5-dione linkage is hydrolytically and thermally stable, a great advantage for future *in vivo* injections.

Due to these positive properties, this technique was chosen to link a [^{18}F] prosthetic group to the tyrosine included in a peptide. We herein present the synthesis of [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione [^{18}F]F-PTAD and the preliminary results for the coupling with tyrosine.

Results and discussion

We initially focused on the direct introduction of fluorine-18 on the cyclic diazodicarboxamide **1**. Compound **1** can be easily prepared in three steps with 4-nitrobenzylisocyanate as starting material[21,23,24] but cannot be isolated due to its high instability.[25] The second approach to obtain compound **4** was the labelling of 4-(4-nitrophenyl)-1,2,4-triazolidine-3,5-dione **2** followed by the triazolidine ring oxidation step (Scheme 2).

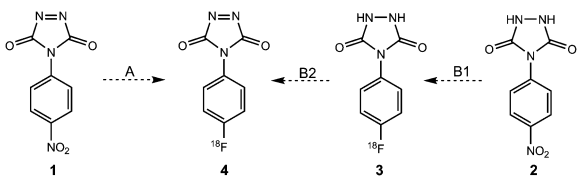
Unfortunately, all the attempts to obtain this compound [^{18}F]**3** (Kryptofix/K_2CO_3, organic bases method[26] or "C14" method[27]) at temperatures between 90 °C and 180 °C were unsuccessful. The labelling conditions are too harsh and only ^{18}F fluoride and decomposition products of 4-(4-nitrophenyl)-1,2,4-triazolidine-3,5-dione precursor were recovered.

A second possibility was the addition of an aliphatic chain containing a tosylate leaving group on the PTAD compound (Scheme 3). Because of the poor results obtained during this aliphatic precursor synthesis including high instability of the triazolidine ring (data not shown), the strategy was changed and the fluorine-18 was introduced at the beginning of the synthesis.

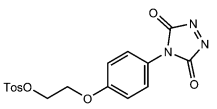
Precursor **5** was synthesized according to the literature using 4-nitroaniline as starting material[28] and involved two reaction steps: dimethylation of 4-nitroaniline with iodomethane and trimethylammonium triflate formation with methyltrifluoromethanesulfonate. Precursor **5** was obtained with a global yield of 28%.

During the radiosynthesis of compound [^{18}F]**10** (Scheme 4), the identification of [^{18}F] compounds **6–10** was based on radio-TLC and radio-HPLC analyses showing the same eluting factor or retention time as the corresponding cold references compounds.

Compound **5** was labelled in DMSO at 100 °C in 10 minutes with standard KF[^{18}F]-K_{222}/potassium carbonate conditions to afford [^{18}F]**6** with 91 ± 4% of radiochemical yield (decay corrected). [^{18}F]**6** was diluted in water and washed on a C18 Sep-Pak cartridge to eliminate the remaining precursor and other



Scheme 2 (A) Radiolabelling not tested due to the high instability of compound **1**. (B) Radiolabelling followed by the triazolidine ring oxidation.



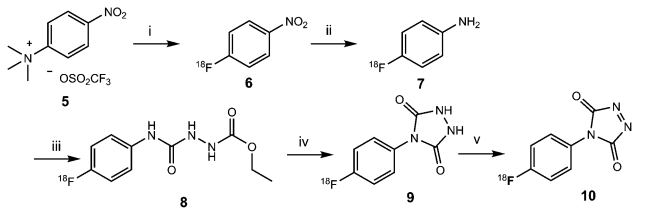
Scheme 3 Aliphatic precursor derived from PTAD compound.

impurities. This is why precursor **5** was preferred to the 1,4-dinitrobenzene which is hardly separated from 4-fluoronitrobenzene. Reduction of the nitro group with sodium borohydride in the presence of palladium on activated carbon in methanol gave compound [^{18}F]**7** with a radiochemical yield (DC) of 80 ± 6%.[28]

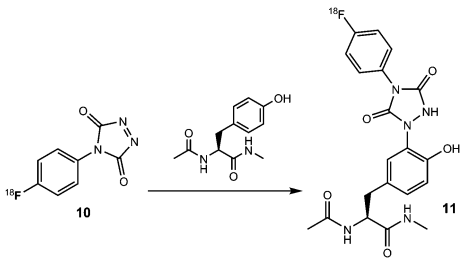
This reduction method is well suited because Pd/C can be easily filtered through a glass fibre filter (Millex) before the trapping onto a *t*C18 environmental Sep-Pak® cartridge. Since the next step requires a dry reaction medium, the cartridge is dried under a stream of nitrogen. [^{18}F]**7** is then eluted with dry dichloromethane and coupled with ethyl carbazate in the presence of triphosgene and triethylamine.[29,30] This method was preferred to the one proposed by Mallakpour *et al.*,[31] which is more longer, inapplicable to the fluorine-18 chemistry, and affords lower yield. Directly after the evaporation of dichloromethane, cyclisation of semicarbazide [^{18}F]**8** was realized as described by Cookson *et al.*[24] in basic aqueous medium and [^{18}F]**9** was obtained in acetonitrile after its purification on a tC18$^+$ short Sep-Pak® cartridge. The radiochemical yield (DC) for the coupling and cyclisation steps is 65 ± 5%. The last step is the triazolidine ring oxidation with *N*-bromosuccinimide and pyridine in acetonitrile. The global radiochemical yield for the synthesis of [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione is 50% (DC).

Because of the high instability of the F-PTAD compound, the crude solution of [^{18}F]**10** was directly used, without purification, in tyrosine ligation reaction. We chose *N*-acyl tyrosine methylamide as a model for the preliminary studies of the ligation of [^{18}F]F-PTAD with a tyrosine, as described by Ban and coworkers[21] (Scheme 5).

[^{18}F]**11** was obtained after the coupling between [^{18}F]**10** and *N*-acyl tyrorine methylamide in PBS/CH_3CN (1/1, v/v) solution with 65 ± 5% yield. When the [^{18}F]F-PTAD was added continuously over a period of 2 minutes, instead of a single addition, the reaction yield increased significantly from 40% to 70%. In some cases, when the tyrosine of the target compound or protein are



Scheme 4 Preparation of [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione and coupling with *N*-acyl tyrosine methylamide. (i) KF[^{18}F]-K_{222}/K_2CO_3, DMSO, 10 min, 100 °C, 94%; (ii) $NaBH_4$, Pd/C, MeOH, 5 min, rt, 88%; (iii) triphosgene, rt, NEt_3, 5 min, 0 °C, ethylcarbazate, 10 min, 80%; (iv) 4 M KOH, 5 min, rt, 85%; (v) NBS, pyridine, CH_3CN.



Scheme 5 Ligation of [^{18}F]F-PTAD and *N*-acyl tyrosine methylamide: pH = 7 phosphate buffer (PBS), CH_3CN, 5 min, rt, 65%.

less accessible, the isocyanate decomposition products of PTAD compounds may be formed and urea formation at lysines is likely.[22,32] This problem is solved by using Tris buffer. In our case, the use of Tris buffer in the [^{18}F]F-PTAD–*N*-acyl tyrosine methylamide ligation instead of PBS provides the desired tyrosine ligation product with the same yield. The crude reaction mixture was diluted with water and injected onto a semipreparative HPLC to obtain pure [^{18}F]**11**. The global radiochemical yield (synthesis of [^{18}F]F-PTAD, ligation with *N*-acyl tyrosine methylamide and semipreparative HPLC purification) is 20% (DC) and the synthesis lasts 2 h 15 min.

Conclusion

We synthesized a new [^{18}F] prosthetic group, the [^{18}F]4-(4-fluorophenyl)-1,2,4-triazole-3,5-dione ([^{18}F]F-PTAD) with good yield and proved that this [^{18}F]F-PTAD can be efficiently coupled with a tyrosine, the *N*-acyl tyrosine methylamide. This radiosynthesis was designed to be implemented on an automatic synthesizer. Its automation is in progress in our laboratory on a Fastlab® synthesizer from GE Healthcare.

Experimental procedure

General procedure

Reagents and solvents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. NMR spectra were recorded on Bruker Avance DRX-400 instruments (^{1}H at 400 MHz and ^{13}C at 100 MHz), ^{1}H and ^{13}C spectra were referenced to TMS using the ^{13}C or residual proton signals of the deuterated solvents as internal standards. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. All the values are expressed in part per million (ppm). TLC analyses were performed on Macherey-Nagel Polygram SIL G/UV254 plates using UV light as visualizing agent. A Bioscan TLC scanner model AR2000 was used for analysis of the ^{18}F labelled compounds. HPLC analyses were run on a Waters system (616 pump, a manual Rheodyne injector, 996 PDA detector and NaI(Tl) scintillation detector from Eberline) controlled by Empower® software. Analytical HPLC Analyses were performed on an Xbridge® column C18 (4.6 × 150 mm; 5 μm) with a flow rate of 1 mL min^{-1}. During the radiochemical synthesis, analytical HPLC analyses were performed using the following conditions (grad1): CH_3CN/H_2O, linear 5 min from 5/95 to 15/85, linear 15 min from 15/85 to 50/50 and the washout linear 10 min from 50/50 to 90/10. Semipreparative HPLC analyses were performed on an Xbridge® column C18 (10 × 250 mm; 5 μm) using the following conditions (grad 2): CH_3CN/H_2O, linear 30 min from 5/95 to 25/75 and linear 5 min from 25/75 to 50/50 with a flow rate of 5 mL min^{-1}.

Chemistry

***p*-Fluoro-4-phenyl-1-carbethoxysemicarbazide.** This compound was synthesized according to the literature.[23]

4-(4-Fluorophenyl)-1,2,4-triazolidine-3,5-dione. This compound was synthesized according to the literature.[24]

4-(4-Fluorophenyl)-3*H*-1,2,4-triazole-3,5(4*H*)-dione. This compound was synthesized according to the literature.[21] The red solution should be used for bioconjugation without isolation.

(*S*)-2-Acetamido-3-(4-hydroxy-3-(4-(4-fluorophenyl)-3,5-dioxo-1,2,4-triazolidin-1-yl)phenyl)-*N*-methylpropanamide. This compound was synthesized according to the literature.[21]

RMN ^{1}H(400 MHz, DMSO-d^6): 1.77 (s, 3H), 2.53 (d, 3H, J = 4 Hz), 2.61–2.66 (dd, 1H, J = 8 Hz, J = 12 Hz), 2.83–2.87 (dd, 1H, J = 4 Hz, J = 12 Hz), 4.28–4.34 (m, 1H, J = 4 Hz, J = 4 Hz, J = 8 Hz), 6.69 (d, 1H, J = 8 Hz), 6.84 (d, 1H, J = 8 Hz), 7.26 (*t*, 2H, J = 8 Hz), 7.58 (dd, 2H, J = 4 Hz, J = 8 Hz), 7.70 (s, 1H), 7.98 (d, 1H(NH), J = 4 Hz), 8.16 (d,1H(NH), J = 4 Hz).

RMN ^{13}C (100 MHz, DMSO-d^6): 22.7, 25.5, 37.3, 54.6, 115.1 (d, J = 23 Hz), 117.2, 121.6, 125.1, 127.5 (d, J = 9 Hz), 128.2, 130.6, 149.8, 153.0, 159.7 (d, J = 236 Hz), 162.2, 166.2, 169.1, 171.7.

***N*,*N*-Dimethyl-4-nitroaniline.** This compound was synthesized according to the literature using 4-nitroaniline as starting material.[28] The compound was purified by recrystallization and an orange solid was obtained with a yield of 70%.

RMN ^{1}H(400 MHz, $CDCl_3$): 3.11 (s, 6H), 6.60 (d, 2H, J = 8 Hz), 8.11 (d, 2H, J = 8 Hz).

RMN ^{13}C (100 MHz, $CDCl_3$): 40.3, 110.2, 126.1, 136.9, 154.9.

***N*,*N*,*N*-Trimethyl-4-nitrobenzenammonium triflate.** This compound was synthesized according to the literature using *N*,*N*-dimethyl-4-nitroaniline as starting material.[28] The product was obtained as a pale yellow solid with a yield of 47%.

RMN ^{1}H(400 MHz, $CO(CD_3)_2$): 4.03 (s, 9H), 8.52 (m, 4H).

RMN ^{13}C (100 MHz, $CO(CD_3)_2$): 57.1, 121.3 (q, J = 320 Hz), 122.8, 125.4, 149.1, 151.9.

Radiochemistry

No-carrier-added [^{18}F] fluoride was obtained by proton bombardment of an [^{18}O]-enriched water target *via* the ^{18}O(p,n)^{18}F reaction. The activity was trapping by passing the target water through a Sep-Pak light QMA cartridges (Waters). The fluoride ions on the cartridge were eluted by 700 μL of 50/50 CH_3CN/H_2O solution of K_2CO_3 (8 mg) and Kryptofix 222 (26 mg) into a heated conical glass vial (120 °C). This eluate was brought to dryness by azeotropic distillation after three additions of acetonitrile (150 μL) under a stream of nitrogen gas to give the no-carrier-added K[^{18}F]-K_{222} complex. A solution of 10 mg of *N*,*N*,*N*-trimethyl-4-nitrobenzenammonium triflate in 1 mL of

DMSO was added to the dried residue and the mixture was heated at 100 °C during 8 minutes. Labelling efficiency was checked by radio-TLC (silica gel, ethyl acetate/hexane 1/1 v/v), R_f values: [^{18}F]fluoride = 0, [^{18}F]4-fluoronitrobenzene = 0.8. HPLC analysis (grad1): t_R = 22 min. TLC radiochemical conversion = 91 ± 4% (n > 10).

The reaction medium was poured into water (10 mL) and trapped onto a previously activated with acetonitrile (5 mL) and washed with water (5 mL) C18 environmental Sep-Pak® cartridge (Waters). The [^{18}F] 4-fluoronitrobenzene was rinsed with 10 mL of water in order to eliminate the starting precursor and was eluted with 2 mL of methanol in a vial containing 3 mg of Pd/C and 25 mg of $NaBH_4$. The mixture was stirred 5 minutes at room temperature. The reduction efficiency was checked by radio-TLC (silica gel, ethyl acetate/hexane 1/1 v/v), R_f values: [^{18}F]4-fluoroaniline = 0.4. HPLC analysis (grad1): t_R = 14 min. Radiochemical yield (decay corrected) = 80 ± 6% (n > 10). The methanolic solution was poured into water (10 mL) and passed through a glass fiber filter (Millex) and trapped onto a *t*C18 environmental Sep-Pak® cartridge (Waters) previously activated with acetonitrile (5 mL) and washed with water (5 mL). The cartridge was flushed by a stream of nitrogen during 5 minutes and then eluted with 2.5 mL of dry dichloromethane in a vial containing 10 mg of triphosgene. The vial was cooled at 0 °C and 20 μL of dry triethylamine was added. The mixture was stirred for 5 min before the addition of 40 mg of ethylcarbazate. The solution was stirred 10 minutes at 0 °C to give [^{18}F]*p*-fluoro-4-phenyl-1-carbethoxysemicarbazide. The reaction efficiency was checked by radio-TLC (silica gel, ethyl acetate/hexane 1/1 v/v), R_f values: [^{18}F]*p*-fluoro-4-phenyl-1-carbethoxysemicarbazide = 0.1. HPLC analysis (grad1): t_R = 15 min. HPLC radiochemical conversion: 80 ± 10% (n = 9). Dichloromethane was evaporated and 0.5 mL of 4 M KOH were added, the mixture was stirred for 5 minutes and finally quenched by 0.5 mL of HCl 6 M. The cyclisation reaction efficiency was checked by radio-TLC (silica gel, ethyl acetate/hexane 1/1 v/v), R_f values: [^{18}F]4-(4-fluorophenyl)-1,2,4-triazolidine-3,5-dione = 0.02. HPLC analysis (grad1): t_R = 4 min. Radiochemical yield DC for coupling step and cyclisation step = 65 ± 5% (n = 7).

The reaction medium was then poured into water (10 mL), trapped onto a tC18 plus short Sep-Pak® cartridge (Waters) previously activated with acetonitrile (5 mL) and washed with water (5 mL) and eluted with 1 mL of acetonitrile. 100 μL of pyridine solution (0.3 M in CH_3CN) was added to the previous solution followed by the addition of 100 μL of *N*-bromosuccinimide solution (0.3 M in CH_3CN). This mixture was directly added to 1 mL of *N*-acyl tyrosine methylamide solution (1 mg in 0.9 mL of PBS 100 mM pH = 7 (or Tris buffer 100 mM pH = 7)) during a period of 2 minutes. The solution was stirred at room temperature during 5 min. The coupling efficiency was checked by radio-TLC (silica gel, ethyl acetate/hexane 1/1 v/v), R_f values: [^{18}F]final compound = 0.20, and by analytical HPLC (grad 1): t_R = 9 min. Radiochemical purity of [^{18}F]11 was 65 ± 5% (n = 5). The radiolabelled compound was purified by semipreparative HPLC (grad 2): t_R = 12 min. The purified sample was analyzed by analytical HPLC to confirm his chemical and radiochemical purity. The total decay corrected radiochemical yield was 25%.

Acknowledgements

The authors wish to acknowledge the financial support from the Biowin Project of the Walloon Region (KeyMarker) and GE Healthcare. We thank the CyclotronTeam at Cyclotron Research Centre at Liege for producing fluorine-18.

Notes and references

1 B. M. Zeglis and J. S. Lewis, *Dalton Trans.*, 2011, 6168–6195.
2 M. D. Bartholomä, *Inorg. Chim. Acta*, 2012, **389**, 36–51.
3 M. Fani and H. Maecke, *Eur. J. Nucl. Med. Mol. Imaging*, 2012, **39**, 11–30.
4 L. Koehler, K. Gagnon, S. McQuarrie and F. Wuest, *Molecules*, 2010, **15**, 2686–2718.
5 L. Cai, S. Lu and V. W. Pike, *Eur. J. Org. Chem.*, 2008, 2853–2873.
6 M. Glaser and E. Arstad, *Bioconjugate Chem.*, 2007, **18**, 989–993.
7 D. Thonon, C. Kech, J. Paris, C. Lemaire and A. Luxen, *Bioconjugate Chem.*, 2009, **20**, 817–823.
8 D. Thonon, E. Goukens, G. Kaisin, J. Paris, J. Flagothier and A. Luxen, *Tetrahedron*, 2011, **67**, 5572–5576.
9 X.-G. Li, M. Haaparanta and O. Solin, *J. Fluorine Chem.*, 2012, **143**, 49–56.
10 E. J. Keliher, T. Reiner, G. M. Thurber, R. Upadhyay and R. Weissleder, *ChemistryOpen*, 2012, **1**, 177–183.
11 G. Vaidyanathan and M. R. Zalutsky, *Nat. Protoc.*, 2006, **1**, 1655–1661.
12 W. Cai, X. Zhang, Y. Wu and X. Chen, *J. Nucl. Med.*, 2006, **47**, 1172–1180.
13 J. Paris, D. Thonon, E. Goukens, G. Kaisin, S. Lacroix and A. Luxen, *J. Labelled Compd. Radiopharm.*, 2011, **54**, S435.
14 K. L. Seim, A. C. Obermeyer and M. B. Francis, *J. Am. Chem. Soc.*, 2011, **133**, 16970–16976.
15 T. Kodadek, I. Duroux-Richard and J.-C. Bonnafous, *Trends Pharmacol. Sci.*, 2005, **26**, 210–217.
16 S. Meunier, E. Strable and M. G. Finn, *Chem. Biol.*, 2004, **11**, 319–326.
17 S. D. Tilley and M. B. Francis, *J. Am. Chem. Soc.*, 2006, **128**, 1080–1081.
18 N. S. Joshi, L. R. Whitaker and M. B. Francis, *J. Am. Chem. Soc.*, 2004, **126**, 15942–15943.
19 J. M. McFarland, N. S. Joshi and M. B. Francis, *J. Am. Chem. Soc.*, 2008, **130**, 7639–7644.
20 D. W. Romanini and M. B. Francis, *Bioconjugate Chem.*, 2007, **19**, 153–157.
21 H. Ban, J. Gavrilyuk and C. F. Barbas, *J. Am. Chem. Soc.*, 2010, **132**, 1523–1525.
22 H. Ban, M. Nagano, J. Gavrilyuk, W. Hakamata, T. Inokuma and C. F. Barbas, *Bioconjugate Chem.*, 2013, **24**, 520–532.
23 S. Alakurtti, T. Heiska, A. Kiriazis, N. Sacerdoti-Sierra, C. L. Jaffe and J. Yli-Kauhaluoma, *Bioorg. Med. Chem.*, 2010, **18**, 1573–1582.
24 R. C. Cookson, S. S. Gupte, I. D. R. Stevens and C. T. Watts, *Org. Synth.*, 1971, **51**, 121–127.

25 M. A. Zolfigol, M. Bagherzadeh, S. Mallakpour, G. Chehardoli, A. Ghorbani-Choghamarani, N. Koukabi, M. Dehghanian and M. Doroudgar, *J. Mol. Catal. A: Chem.*, 2007, **270**, 219–224.
26 C. F. Lemaire, J. J. Aerts, S. Voccia, L. C. Libert, F. Mercier, D. Goblet, A. R. Plenevaux and A. J. Luxen, *Angew. Chem., Int. Ed.*, 2010, **49**, 3161–3164.
27 J. Aerts, S. Voccia, C. Lemaire, F. Giacomelli, D. Goblet, D. Thonon, A. Plenevaux, G. Warnock and A. Luxen, *Tetrahedron Lett.*, 2010, **51**, 64–66.
28 Y. Seimbille, M. E. Phelps, J. Czernin and D. H. S. Silverman, *J. Labelled Compd. Radiopharm.*, 2005, **48**, 829–843.
29 A. M. Venkatesan, Z. Chen, O. D. Santos, C. Dehnhardt, E. D. Santos, S. Ayral-Kaloustian, R. Mallon, I. Hollander, L. Feldberg, J. Lucas, K. Yu, I. Chaudhary and T. S. Mansour, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5869–5873.
30 S. Gobec, A. Kovac, A. Brajic, S. Pecar, J. Bostock, I. Chopra, R. Lenarsic, S. Bombek, M. Kocevar and S. Polanc, EP 1 889 831 A2, 2008.
31 S. Mallakpour and Z. Rafiee, *Synth. Commun.*, 2007, **37**, 1927–1934.
32 R. Adamo, M. Allan, F. Berti, E. Danieli and Q.-Y. Hu, WO2012US45549, 2011.