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**Research Article**

# Design and synthesis of fluorine-18 labeled matrix metalloproteinase inhibitors for cancer imaging

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## Summary

Matrix metalloproteinases (MMPs) are key enzymes involved in cancer invasion and metastasis. New $^{18}$F-labeled MMP inhibitors (**1a–c**) has been designed and synthesized for cancer imaging by positron emmision tomography. The precursors were synthesized in four steps starting from D-form of amino acids. Radiosynthesis of **1a–c** were carried out by simple one-pot synthesis. The resulting radiofluorinated MMP inhibitors were obtained in overall radiochemical yields of 13–43% (EOB, decay corrected) within 60–70 min (including final preparative HPLC separation). Copyright © 2002 John Wiley & Sons, Ltd.

**Key Words:** Pet; matrix metalloproteinase; inhibitor; tumor imaging; fluorine-18

## Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases involved in the extracellular matrix degradation. Under strict control of their proteolytic activity in normal tissue, MMPs play a

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significant physiological role in a variety of tissue remodeling processes. However, the aberrant overexpression of MMPs has been implicated in a number of pathological disease states such as rheumatoid arthritis,[1] periodontal disease,[2] multiple sclerosis,[3] and malignant tumors.[4]

The mammalian MMP family is now known to include at least 20 enzymes and is categorized into several classes based on substrate specificity and domain structure. Among the subfamilies of MMPs, gelatinases (MMP-2 and MMP-9) have become attractive targets for research on cancer and development of anticancer drugs. They have been closely involved in the invasion, metastasis, and angiogenesis that are crucial for the progression of malignant tumors.[5,6] Moreover, increased expressions and activities of gelatinases, especially MMP-2, have been observed in human malignant tumor tissues of various organs including the stomach, prostate, breast, colon, and lung.[5] Thus, clinical MMP measurements are considered to provide important data on the metastatic potential of malignant tumors.[7] For these reasons, we believe that MMP inhibitors labeled with a positron emitter should become a unique type of tracer that might be clinically useful for predicting cancer metastasis through the use of positron emission tomography (PET).

Considerable effort has been devoted to the development of potent MMP inhibitors and numerous inhibitors are reported in the literature.[8] Recent X-ray[9] and NMR[10] studies on the structures of the MMP catalytic domain/inhibitor complexes indicate that the inhibitor interactions with the active-site zinc (II) ion are essential for defining the binding mode and the inhibitory potency. In addition, a number of structure–activity relationship studies show that inhibitor side chains, which may interact with specific enzyme subsites within the active site, play a critical role in determining the inhibitory selectivity as well as the potency.[11] Current focus in the field of MMP inhibitor development is directed towards the synthesis of selective inhibitors. Shionogi[12] and Bayer[13] have reported gelatinase selective inhibitors which contain a carboxylic acid group and a linear side chain (Figure 1). The carboxylic acid group binds effectively to the catalytic zinc ion and the linear side chain may specifically interact with the S′ 1 subsite of the gelatinase.

Based on these structural features of the selective inhibitors, we began our research to develop a novel PET tracer labeled with fluorine-18 for evaluating gelatinase expression and/or activity in malignant tumors. The design and radiosynthesis of the new MMP inhibitors selective for gelatinases are reported in this publication.

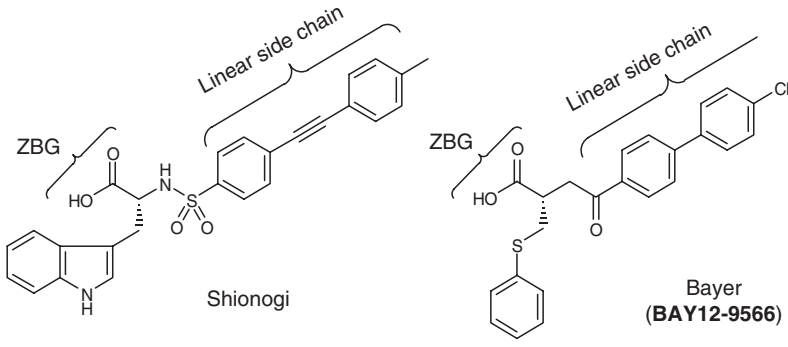



**Figure 1. Selective MMP inhibitors especially for gelatinases. ZBG: zinc binding group**

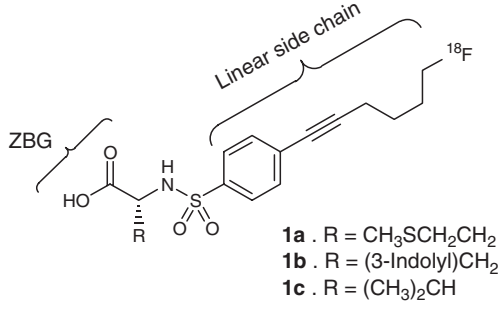



**Figure 2. New radiopharmaceuticals designed for gelatinase inhibition**

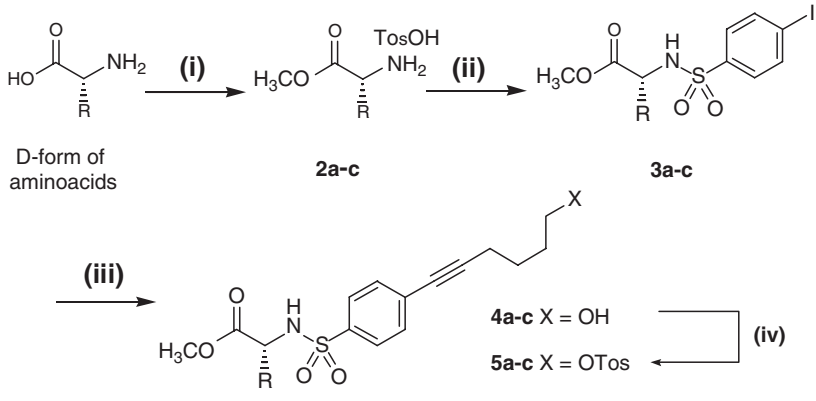
## Result and discussion

As shown in Figure 2, we designed carboxylic acid-based fluorine-18 labeled inhibitors containing the sulfonamide group. This type of inhibitor can be easily constructed from the unnatural D-form of amino acids and aryl sulfonyl chlorides. Moreover, the sulfonamide is suitable sterically for carrying the sulfonyl substituent deeply into the gelatinase S′ 1 subsite. The latter forms a channel-like structure having a straight, narrow, and deep pocket shape.[14] The selectivity and potency of MMP inhibitors increase with linear elongation of the substituent on the sulfonyl group. Substituents were thus designed carrying *para*-substituted aryl alkynes attached to alkyl chains so as to form a long straight structure. The alkyl terminal of the side chain was chosen for the labeling position since this could be easily labeled by simple

nucleophilic displacement of a primary leaving group, such as the tosyl group, with [$^{18}$F]fluoride. On the basis of these design strategies, we approached the development of fluorine-18 labeled gelatinase inhibitors.

The precursors (**5a–c)** were synthesized from the commercially available D-form of methionine, tryptophan, and valine, respectively, as shown in Scheme 1. Protection of the carboxyl group was carried out in methanol under reflux with *p*-toluenesulfonic acid and *p*-toluene-



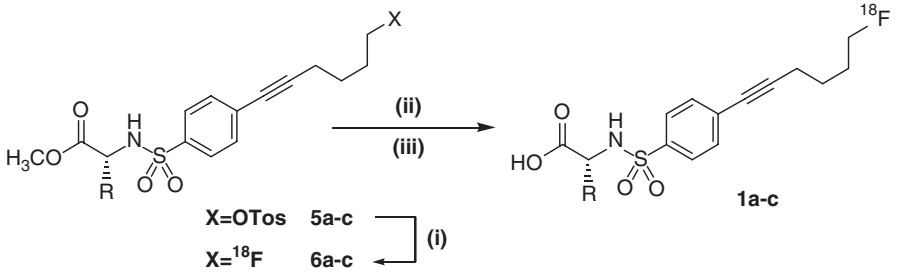

**Scheme 1. Precursor synthesis of the newly designed MMP inhibitors from the D-form of various amino acids. (i) TosCl, TosOH, MeOH, reflux; (ii) 4-Iodobenzenesulfonyl chloride, NMM, $CHCl_3$; (iii) 5-hexyne-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, TEA, DMF; and (iv) TosCl, NMM, $CHCl_3$**

sulfonyl chloride.[15] The obtained methyl esters of the amino acids (**2a–c**) were coupled with 4-iodobenzenesulfonyl chlorides in the presence of *N*-methylmoropholine (NMM). Utilizing the reaction conditions developed by Sonogashira,[16] the obtained iodophenylsulfonamides (**3a–c**) were coupled with 5-hexyn-1-ol in the presence of palladium(II), CuI(I), and triethylamine (TEA) to yield the desired alkynyl phenylsulfonamides (**4a–c**) in good yield. Conversion of the hydroxyl group to the tosylate by treating with *p*-toluenesulfonyl chloride and NMM completed the precursor **5a–c** synthesis.

The non-radioactive fluorinated inhibitors were synthesized from the corresponding precursors by fluorination with tetrabutylammonium fluoride in acetonitrile under reflux followed by removing the methyl ester protective groups with aqueous sodium hydroxide in methanol.

The gelatinase inhibitors labeled with fluorine-18 (**1a–c**) were easily prepared via a one-pot synthesis outlined in Scheme 2. The reactions




**Scheme 2. Radiosynthesis of 1a–c by a one-pot procedure: (i) [$^{18}$F]KF, $K_2CO_3$, Kryptofix 2.2.2, $CH_3CN$, reflux; (ii) aqueous NaOH; and (iii) aqueous HCl**

**Table 1. Radio-TLC analysis of the radiosynthesis of 1a–c from 5a–c**

| Precursor | **R**[a] | [$^{18}$F]Fluorination (%)[b] | Deprotection (%)[b] |
|---|---|---|---|
| **5a** | $CH_2CH_2SCH_3$ | **61** (6 min) | **99** (6 min) |
| **5b** | $CH_2$(3-indolyl) | **29** (12 min) | **95** (10 min) |
| **5c** | $CH(CH_3)_2$ | **78** (12 min) | **57** (15 min) |

[a] Side chains of **5a–c** indicated in Scheme 2.
[b] Decay corrected yields.

were monitored by radio-thin-layer chromatography (radio-TLC) to optimize the reaction time. The one-pot synthesis, radiofluorination and deprotection, was done in the same vial with heating at 110°C using an oil bath.

The initial fluorination of the precursor was performed by nucleophilic displacement with [$^{18}$F]fluoride in the presence of potassium carbonate[17] and Kryptofix 2.2.2. After fluorination, basic hydrolysis of the methyl ester group of **6a–c** was carried out by adding 2 N NaOH to the reaction solution. The results of radiofluorination and deprotection profiled by radio-TLC are summarized in Table 1.

The radiofluorination of **5b** did not proceed smoothly compared to **5a** or **5c** and the yield was not improved by prolonged reaction time. The reason for this could not be clarified in this study, but might be caused by involvement of the indolyl side chain. On the other hand, the deprotection of **6a** and **6b** proceeded easily and was almost complete within a short time (6 and 12 min, respectively), while **6c** was not deprotected completely even after 15 min.The difficulty with the methyl ester hydrolysis of **6c** might be due to steric hindrance of the isopropyl group at the carbonyl α-position.

The basic hydrolysis of the methyl ester was quenched by acidifying the solution with aqueous HCl. The resulting acidic solution was diluted with $H_2O$ followed by solid phase extraction using a Sep-Pak C18 cartridge. The final products (**1a–c**) were isolated from the extract by semi-preparative reverse phase HPLC. The products (**1a–c**) had retention times identical to those of the corresponding authentic non-radioactive standards

From the results of the TLC analysis the optimum radiofluorination time was set to 12 min (**5a–c**) and the deprotection time to 6 min (for **6a**) or 12 min (for **6b** and **6c**). Under these conditions the total radiosynthesis times were about 60–70 min including the preparative HPLC separation. The average of the overall radiosynthesis yields of **1a**–**c** were 43, 13 and 33%, respectively ($n = 5$–7, decay corrected).

After purification of **1a–c** their radiochemical purities were evaluated by analytical reverse phase HPLC. The purities of **1b** and **1c** stored in preparative HPLC mobile phase remained 99% or more for at least 2 h. On analysis of **1a**, however, a slight impurity was seen 10 min after purification by preparative HPLC (Figure 3A). The impurity seemed to result from the decomposition of **1a** because the radioactivity of the impurity relative to **1a** increased gradually with time (Figure 3B).The chemical instability of **1a** would make it unsuitable as a PET tracer even though it was synthesized in the best radiochemical yield among the three types of tracer.

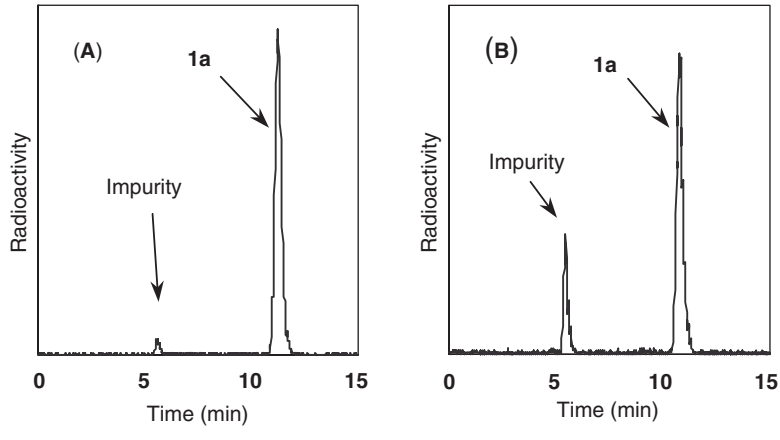



**Figure 3. Analytical HPLC profiles of 1a 10 min (A) and 180 min (B) after preparative HPLC purification. Radiochemical purities of 1a after 10 and 180 min were 95 and 74%, respectively**

## Experimental section

### *General*

All commercially available materials were of reagent grade and used without further purification. Reactions were monitored by TLC using Merck silica gel 60 $F_{254}$ precoated aluminium sheets. Column chromatography was performed using Silica Gel 60 (spherical, 40–50 μm) purchased from Kanto Chemical Co., Inc. $^1$H NMR spectra were recorded on a JEOL GX-400 (400 MHz), reporting the chemical shifts ($\delta$) in parts per million (ppm). Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 or -DX500. Radio-TLCs were analyzed with a BAS 5000 system (Fuji film, Japan).

### *Precursor synthesis*

D-*Valine methyl ester (**2c**)*. To a solution of D-valine (2.34 g, 20.0 mmol) and *p*-toluenesulfonic acid monohydrate (4.18 g, 22.0 mmol) in methanol (40 ml) was added *p*-toluenesulfonyl chloride (4.58 g, 24.0 mmol) and thereafter reflux for 20 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure. The residue was crystallized from methanol and ethyl ether to give 5.69 g (18.7 mmol, 93%) of **2c** as a colorless crystal.

*(2R)-2-[(4-iodophenyl)sulfonylamino]-3-methylbutyric acid methyl ester (**3c**)*. To a solution of **2c** (4.90 g, 16.1 mmol) and *N*-methylmorpholine (10 ml) in $CHCl_3$ (50 ml) was added 4-idobenzenesulfonyl chloride (5.37 g, 17.7 mmol). After stirring overnight at room temperature, $CHCl_3$ was evaporated under vacuum and the residue was dissolved in ethyl acetate followed by washing with $H_2O$ and brine. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The oil residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (5:2) to give 5.52 g (13.9 mmol, 86%) of **3c** as a colorless crystal. $^1$H NMR ($CDCl_3$/TMS): $\delta$ 0.88 (3 H, d, $J = 6.8$ Hz), 0.95 (3 H, d, $J = 6.8$ Hz), 2.00–2.09 (1 H, m), 3.49 (3 H, s), 3.74 (1 H, dd, $J = 10.4$, 5.2 Hz), 5.28 (1 H, d, $J = 10.4$ Hz), 7.55 (2 H, d, $J = 6.0$ Hz), 7.85 (2 H, d, $J = 6.0$ Hz). MS (EI): 398 ($[M + H]^+$).

*(2R)-2-[[4-(6-hydroxyhex-1-ynyl)phenyl]sulfonylamino]-3-methyl-butyric acid methyl ester (4c)*. To a solution of **3c** (2.05 g, 5.16 mmol) and triethylamine (3.0 ml) in DMF (9.0 ml) was added copper(I) iodide (42.9 mg, 0.225 mmol), dichlorobis(triphenylphosphine)palladium(II) (80.5 mg, 0.114 mmol), and 5-hexyn-1-ol (700 µl, 6.34 mmol) at room temperature. The mixture was stirred under an argon atmosphere at 65°C overnight. After being cooled to room temperature, the reaction solution was diluted with 1 N HCl and extracted with ethyl ether. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The oil residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (7:17) to give 1.62 g (4.41 mmol, 85%) of **4c** as a colorless solid. $^1$H NMR ($CDCl_3$/TMS): $\delta$ 0.870 (3 H, d, $J = 6.8$ Hz), 0.950 (3 H, d, $J = 6.8$ Hz), 1.68-1.77 (4 H, m), 1.99-2.07 (1 H, m), 2.48 (2 H, t, $J = 6.4$ Hz), 3.46 (3 H, s), 3.70-3.75 (3 H, m), 5.18 (1 H, d, $J = 10.4$ Hz), 7.47 (2 H, d, $J = 8.4$ Hz), 7.73 (2 H, d, $J = 8.8$ Hz). MS (EI): 367 ($M^+$).

*(2R)-3-methyl-2-[[4-[6-(toluene-4-sulfonyloxy)hex-1-ynyl]phenyl] sulfonylamino]butyric acid methyl ester (***5c***)*. To a solution of **4c** (1.62 g, 4.41 mmol) and *N*-methylmorpholine (6.0 ml) in $CHCl_3$ (10 ml) was added *p*-toluenesulfonyl chloride (1.29 g, 6.76 mmol). After stirring overnight at room temperature, $CHCl_3$ was evaporated under vacuum. Two normal HCl was added to the residue and the organic extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The oil residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (5:4) to give 1.73 g (3.31 mmol, 75%) of **5c** as colorless heavy oil. $^1$H NMR ($CDCl_3$/TMS): $\delta$ 0.873 (3 H, d, $J = 6.8$ Hz) 0.952 (3 H, d, $J = 6.8$ Hz), 1.63-1.71 (2 H, m), 1.80–1.88 (2 H, m), 1.99–2.07 (1 H, m), 2.43 (2 H, t, $J = 6.8$ Hz), 2.45 (3 H, s), 3.47 (3 H, s), 3.74 (1 H, dd, $J = 10.0$, 5.2 Hz), 5.07 (1 H, d, $J = 10.0$ Hz), 7.35 (2 H, d, $J = 8.0$ Hz), 7.59 (2 H, d, $J = 8.8$ Hz), 7.73 (2 H, d, $J = 8.8$ Hz). 7.80 (2 H, d, $J = 8.4$ Hz). MS (EI): 521 ($M^+$).

Precursors **5a** and **5b** were prepared from D-methionine and D-tryptophan, respectively, using the same procedures as for the synthesis of **5c**.

*(2R)-4-methylsulfanyl-2-[[4-[6-(toluene-4-sulfonyloxy)-hex-1-ynyl]-phenyl]sulfonylamino]butyric acid methyl ester (**5a**)*. $^1$H NMR ($CDCl_3$/TMS): $\delta$ 1.62–1.71 (2 H, m), 1.79–1.87 (2 H, m), 1.87–1.95 (1 H, m),

1.98–2.07 (1 H, m), 2.05 (3 H, s), 2.43 (2 H, t, $J = 7.2$ Hz), 2.44 (3 H, s), 2.47–2.61 (2 H, m), 3.54 (3 H, s), 4.05–4.12 (3 H, m), 5.53 (1 H, d, $J = 9.2$ Hz), 7.35 (2 H, d, $J = 8.0$ Hz), 7.46 (2 H, d, $J = 8.8$ Hz), 7.74-7.82 (4 H, m). MS (EI): 494 ($[M-CH_3CO^{\bullet}+H]^+$), 554 ($[M+H]^+$).

*(2R)-3-(1H-indol-3-yl)-2-[[4-[6-(toluene-4-sulfonyloxy)-hex-1-ynyl]-phenyl]sulfonylamino]propionic acid methyl ester (***5b***).* $^1$H NMR ($CDCl_3$/TMS): $\delta$ 1.63–1.71 (2H, m), 1.80-1.89 (2H, m), 2.43 (2H, t, $J = 6.8$ Hz), 2.44 (3H, s), 3.21 (1H, dd, $J = 14.8$, 5.6 Hz), 3.46 (1H, dd, $J = 14.8$, 6.0 Hz), 3.47 (3H, s), 4.10 (2H, t, $J = 6.4$ Hz), 4.23-4.29 (1H, m), 5.14 (1H, d, $J = 8.8$ Hz), 6.99 (1H, s), 7.08 (1H, t, $J = 7.2$ Hz), 7.17 (1H, t, $J = 7.2$ Hz), 7.31–7.46 (6H, m), 7.58 (2H, d, $J = 8.4$ Hz), 7.79 (2H, d, $J = 8.4$ Hz), 8.10 (1H, brs). MS (EI): 608 ($M^+$).

*Synthesis of non-radiolabeled standard*

Non-radiolabeled standards of **1a–c** were synthesized by following the general procedures. To a solution of the corresponding precursor in acetonitrile was added 2–4 equivalents of tetrabutylammonium fluoride (1 M/THF, Aldrich) and the solution was stirred under reflux. After checking the disappearance of the precursor by TLC, acetonitrile was evaporated off under vacuum. To the residue was added $H_2O$ and the organic extracted with ethyl acetate followed by drying over $MgSO_4$. The fluorinated compound was purified by column chromatography on silica gel. It was then dissolved in methanol, after which 2 N NaOH was added to the solution at room temperature. The mixture was heated at 60°C for several hours, monitoring the hydrolysis of the methyl ester by TLC. After completion of the reaction, the reaction solution was acidified with aqueous HCl followed by evaporation under vacuum. To the residue was added $H_2O$ and the organic extracted with ethyl acetate. The extract was dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate with acetic acid (1%). The analytical data for the non-labeled standard are given below.

*(2R)-2-[[4-(6-fluorohex-1-ynyl)phenyl]sulfonylamino]-4-methyl-sulfanylbutyric acid (non-radiolabeled standard of* **1a***).* $^1$H NMR (DMSO-*d*6/TMS): $\delta$ 1.72–1.99 (5H, m), 2.03–2.14 (1H, m), 2.05 (3H, s), 2.47–2.63 (4H, m), 4.12–4.18 (1H, m), 4.51 (2H, dt, $J = 47.2$, 5.8 Hz),

5.44 (1H, d, $J = 8.8$ Hz), 6.50–7.20 (1H, br), 7.49 (2H, d, J = 8.8 Hz), 7.77 (2H, d, $J = 8.4$ Hz).

*(2R)-2-[[4-(6-fluorohex-1-ynyl)phenyl]sulfonylamino]-3-(1H-indol-3-yl)propionic acid (non-radiolabeled standard of* **1b***).* $^1$H NMR ($CDCl_3$/TMS): $\delta$ 1.78–1.80 (2H, m), 1.80–1.94 (2H, m), 2.49 (2H, t, $J = 7.0$ Hz), 3.18 (1H, dd, $J = 14.4$, 6.6 Hz), 3.28 (1H, dd, $J = 14.4$, 5.2 Hz), 4.22–4.24 (1H, m), 4.51 (2H, dt, $J = 47.2$, 5.8 Hz), 5.39 (1H, d, $J = 8.4$ Hz), 6.94 (1H, s), 7.06 (1H, t, $J = 7.6$ Hz), 7.17 (1H, t, $J = 7.6$ Hz) 7.25–7.48 (4H, m), 7.52 (2H, d, $J = 8.4$ Hz), 7.56–7.90 (1H, br), 8.10 (1H, brs). HRSM (EI): calculated for $C_{23}H_{23}FN_2O_4S$ 442.1357 ($M^+$), found 442.1337.

*(2R)-2-[[4-(6-fluorohex-1-ynyl)phenyl]sulfonylamino]-3-methylbutyric acid (non-radiolabeled standard of* **1c***).* $^1$H NMR ($CDCl_3$/TMS): $\delta$ 0.87 (3H, d, $J = 6.7$ Hz), 0.98 (3H, d, $J = 6.7$ Hz), 1.71–1.96 (4H, m), 2.51 (2H, t, $J = 6.7$ Hz), 3.79–3.84 (1H, m), 4.52 (2H, dt, $J = 47.2$, 5.8 Hz), 5.15 (1H, d, $J = 9.4$ Hz), 7.47 (2H, d, $J = 8.1$ Hz), 9.75 (2H, d, $J = 8.3$ Hz). HRSM (EI): calculated for $C_{17}H_{22}FNO_4S$ 355.1248 ($M^+$), found 355.1224.

*Radiosynthesis (general procedures)*

[$^{18}$F]Fluoride was produced by the $^{18}$O(p, n)$^{18}$F nuclear reaction on a [$^{18}$O]water target using an HM12 cyclotron (Sumitomo Heavy Industries, Japan) and the fluoride was converted to potassium [$^{18}$F]fluoride with potassium carbonate. To the [$^{18}$F]fluoride solution including potassium carbonate (8-10 mg) in a vial (5 ml volume) was added Kryptofix 2.2.2 (16 mg). Azeotropic removal of water with acetonitrile ($3 \times 3$ ml) was carried out by heating at 110°C under a stream of nitrogen. To the activated Kryptofix 2.2.2/potassium [$^{18}$F]fluoride was added the precursor (10 μmol) dissolved in acetonitrile (1 ml). The mixture was heated at 110°C for 12 min before adding 2 N NaOH (300 μL). After 6–12 min, 2 N HCl (450 μl) and $H_2O$ (10 ml) were added to the reaction solution. The resulting acidic aqueous solution was passed through a Sep-Pak C18 column followed by eluting with acetonitrile (10 ml). The acetonitrile solution was evaporated in vacuo to dryness. The residue was purified by semi-preparative reversed phase HPLC using a YMC-Pack ODS-A column ($300 \times 10$ mm, s-5 μm) eluted with acetonitrile/methanol/water/acetic acid (200:400:400:10 for **1a** and

**1b**, and 200:400:380:10 for **1c**) at a flow rate of 5.0 ml/min. The retention times of **1a**, **1b**, and **1c** were 19.3, 24.7, and 16.3 min, respectively. Analytical HPLC was performed using a LiChrosorb® RP-8 ($250 \times 4$ mm, s-7 μm, Cica-MERCK) column eluted with the same mobile phase of semi-preparative HPLC.

## Conclusion

We have designed and synthesized a new type of PET tracer for cancer imaging, carboxylic acid-based MMP inhibitors labeled with fluorine-18. The precursors were easily prepared from the D-form of various amino acids in four steps. Fluorine-18 labeled compounds were synthesized from the precursors by a simple one-pot preparation and purified by HPLC of the three types of labeled compounds. **1a** was prepared in the best total radiochemical yield, but turned out to be unstable chemically and thus unsuitable for use to biological studies. **1b** and **1c** are undergoing biological evaluation as prospective candidates for novel and potent PET tracers for cancer imaging. This evaluation includes *in vitro* enzymatic inhibition and biodistribution studies using several tumor models. The results of these studies will be reported elsewhere in the near future.

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