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Research Article

Carbon-11 labelling of an *N*-sulfonylamino acid derivative: a potential tracer for MMP-2 and MMP-9 imaging

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Summary

Matrix metalloproteinases (MMPs) are a family of proteinases that play an important role in cancer. Non invasive imaging of MMPs would allow the evaluation of MMP activity in cancer and the assessment of the response of MMP inhibitor based therapies. In this paper, we investigated the synthesis and labelling by methylation with $[^{11}C]CH_3I$ of an *N*-sulfonylamino acid derivative, the (*2R*)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid, a selective and high potent MMP-2 and MMP-9 inhibitor, for cancer imaging with positron emission tomography. Labelling of (*2R*)-3-methyl-2-[[4-[(4-hydroxybenzoyl) amino]benzenesulfonyl] amino] butanoic acid was carried out in a radiochemical yield of 50%–60% within 40 min with a specific activity of 11–26 GBq/μmol (EOS). *In vitro* inhibitory activity studies, biodistribution and *in vivo* serum stability in normal mice are also described.

Key Words: matrix metalloproteinases inhibitors; carbon-11; positron emission tomography

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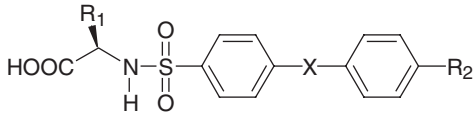
Introduction

Matrix metalloproteinases are a family of zinc-dependent endopeptidases. According to the structure of the enzymes and their substrate specificity, the 20 members described so far are classified into 5 groups. They are all collectively capable of degrading the components of the extracellular matrix to ensure its remodelling and repairing.[1–3] Their activity is tightly controlled by the endogenous co-expression of tissue inhibitors.[4] The implication of MMPs in cancer, and particularly of MMP-2 and MMP-9, also called gelatinases, has been demonstrated by a large number of preclinical and clinical studies.[5–8] The abnormal degradation of the basement membrane due to the over-expression of gelatinases: (i) is necessary for angiogenesis; (ii) promotes tumour growth by increasing the bioavailability of the growth factors released during the degradation and (iii) facilitates the invasion of tumour cells through the connective tissue and blood vessel walls resulting in the establishment of metastases.[9–14] Therefore gelatinases are potential targets for therapeutic intervention in cancer as well as for imaging of malignant tumours.

Phage display,[15] substrate based or structure based design[16,17] and combinatorial chemistry[18–20] have been extensively used to design and develop MMP inhibitors (MMPIs). Today, about half a dozen MMPIs are currently under clinical trials in patients.[21–24] The opportunity to label MMPIs with positron emitters (carbon-11 or fluorine-18) would offer a tool to image MMP activity in cancer and to assess the response to an MMP inhibitor based therapy,[25,26] with positron emission tomography.[27,28] Among the different chemical classes of MMPIs, a family of *N*-sulfonylamino acid derivatives[29–34] has been described as selective and orally active gelatinases inhibitors (general structure, Figure 1). Some members of this MMPI family display high selectivity and nanomolar IC_{50} for MMP-2 and MMP-9. Furthermore, the versatility of the para-substituent of the benzoylaminobenzene side chain (Figure 1, substituent R2) provides a straightforward strategy for isotopic labelling via carbon-11 methylation without the need for protected precursors.

Fei *et al.* [27,28] recently described the synthesis of 6 analogues of CGS 27023A,[29–34] an MMPI from Ciba Geygy/Novartis under clinical trials. CGS 27023A is a broad spectrum MMPI[1–3] belonging to the family of the *N*-sulfonylamino acid derivatives.[29] These compounds were successfully labelled with carbon-11. Furthermore, they demonstrated



R_1 = hydrophobic amino acid side chain
R_2 = SMe, OMe, NO_2, H....
X = tetrazolyl or amide

Figure 1. General structure of the *N*-sulfonylamino acid derivatives

that the analogues exhibited the same inhibitory activity as the parent compound, CGS 27023A, at a concentration of 200 nM. However, no detailed data on the affinity of these compounds as well as *in vivo* stability and biodistribution are available.

In this study, we prepared the (*2R*)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino] benzenesulfonyl]amino] butanoic acid, a member of the *N*-sulfonyl amino acid derivative family and tested its MMP-2 and MMP-9 inhibitory activity in vitro. Starting from the corresponding 4-hydroxybenzoyl analogue, the *N*-sulfonylamino acid derivative was labelled with carbon-11 by methylation with $[^{11}C]CH_3I$. Biodistribution and serum stability studies of the tracer were evaluated *in vivo* in normal mice.

Results and discussion

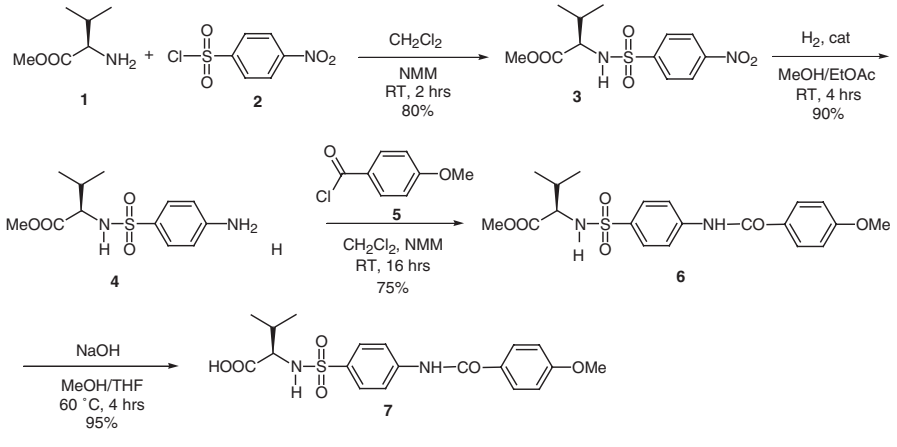
Chemistry

There are at least 3 requirements for a molecule to be an effective MMP inhibitor: (i) the presence of a functional group capable of participating in the chelation of the zinc atom in the active site of the enzyme (hydroxamic, carboxylic, sulfhydryl groups) is required; (ii) at least one functional group providing hydrogen bonds to the enzyme backbone and (iii) one or more side chains fitting with the subsites of the active site.[16,17]

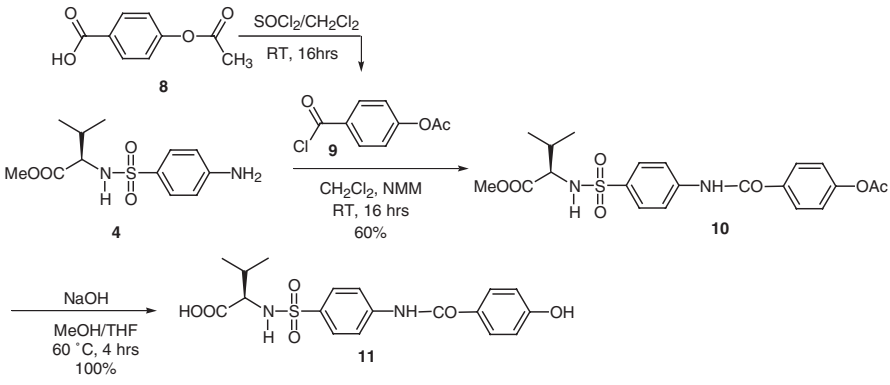
The *N*-sulfonylamino acid derivatives meet most of these criteria. The carboxylic group is able to participate in the chelation of the zinc atom, the presence of the sulfonyl group provides the hydrogen bonds[35,36] and the benzoyl aminobenzene side chain makes hydrophobic contacts with the S'1 subsite of the enzyme.[37–40] Furthermore, analogues of compound **7** have demonstrated selective gelatinases inhibition *in vitro*

and anti-tumour activity in mice after oral administration.[29] The synthesis of **7** was conducted according to the preparation of other biphenyl-*N*-sulfonylamino acid derivatives described by Tamura *et al.*[29] Starting from the protected commercially available D-valine, it was possible to synthesize **7** in 4 steps with an overall yield of 52% (Scheme 1). D-valine **1** was first reacted with 4-nitrobenzene sulfonylchloride **2**. The nitro function was then reduced to the corresponding amine **4** by catalytic hydrogenation. After purification, compound **4** was acylated either with 4-methoxybenzoyl chloride **5** to provide the cold reference **7** after hydrolysis of **6** with sodium hydroxide (Scheme 1) or with acetoxy benzoylchloride **9** to provide the labelling precursor (Scheme 2). To demonstrate MMP inhibitory activity, *in vitro* gelatin degradation assay were carried out with **7** as MMP-2 and MMP-9 inhibitor.

The preparation of the labelling precursor (Scheme 2) by activation of the 4-acetoxybenzoic acid **8** with thionyl chloride takes advantage of a simple purification step of **9** by evaporation of non-reacted thionyl chloride, by-products and solvents before reaction with the amine **4**. The double ester **10** was hydrolysed in one step with sodium hydroxide to provide the precursor **11**. Compared to this two step method, trials to produce **10** in one step starting from **4** and **8** using the standard carbodiimide coupling reagents for peptide synthesis DCC (*N,N′*-dicyclohexylcarbodiimide) and WSC (1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide) resulted in low transformation yields (less than



Scheme 1. Synthesis of the cold reference 7, (2R)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid

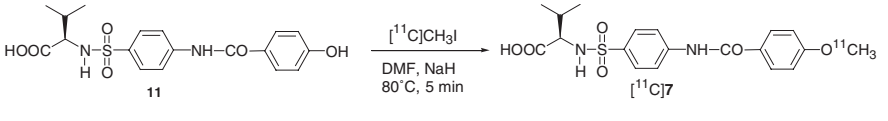


Scheme 2. Synthesis of compound 11, (2R)-3-methyl-2-[[4-[(4-hydroxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid

20%). Alternative methods to prepare the precursor **11**, such as the transformation of the 4-methoxybenzene group of **6** (Scheme 1) into the corresponding 4-hydroxynezene with EtSNa41 or L-selectride,[42] followed by the hydrolysis of the carboxylate, were not investigated but seem to be promising routes .

Radiochemistry

The synthesis of [^{11}C]**7** was performed by carbon-11 methylation of the precursor **11** with [^{11}C]CH_3I in DMF in the presence of NaH for 5 min at 80°C (Scheme 3). The product [^{11}C]**7** was purified by RP-HPLC. The total synthesis time (from end of bombardment) was about 40 min including HPLC purification and formulation. The decay corrected yield for the methylation, determined by HPLC, was 50–60%. Radiochemical purity was $>98\%$ and the specific radioactivity of [^{11}C]**7** was 11–26 GBq/μmol EOS (0.3–0.7 Ci/μmol).



Scheme 3. Synthesis of (2R)-3-methyl-2-[[4-[(4-[^{11}C]methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid

In vitro gelatin degradation assay

The phage display selected cyclic decapeptide (CTTHWGFTLC), containing the amino acids sequence HWGF responsible for its selective MMP-2 and MMP-9 inhibitory activity (**12**),[15] the non selective metalloproteinases inhibitor 1,10 phenanthroline (**13**) and **7** were evaluated for their inhibitory activity towards MMP-2 and MMP-9 in a gelatin degradation assay. The compounds **7**, **12** and **13** were first dissolved in DMF before dilution in the assay buffer. We tested that the presence of DMF had no influence on the degradation of the gelatin by the purified activated MMP-2 and MMP-9. Among these three compounds, **7** displayed the highest inhibitory activity (Figure 2a, b). We determined an IC_{50} for **7** of 110 nM for MMP-2 and 200 nM for MMP-9. Peptide **12** displayed an inhibitory activity of 10 μM for MMP-2 but a more than 10 fold lower inhibitory activity for MMP-9 ($IC_{50} > 100$ μM). Compound **13** showed a typical inhibitory profile for a non specific and low potent metalloproteinases inhibitor.

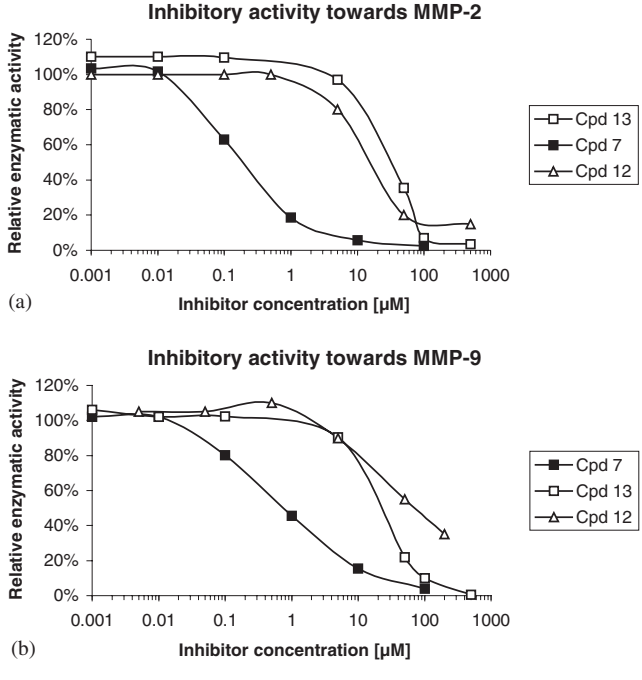


Figure 2. (a) and (b) Inhibitory activity of compounds 7, 12 and 13 towards MMP-2 and MMP-9. The results are the mean of 2 to 3 independent experiments

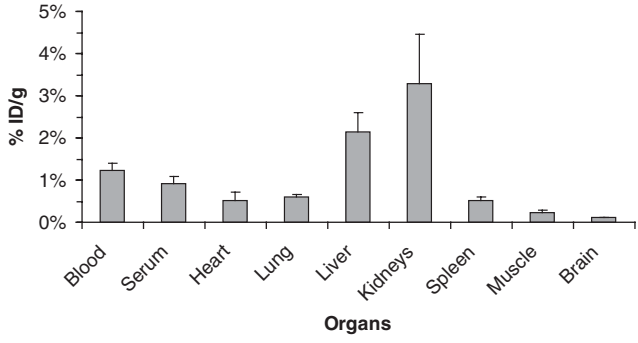


Figure 3. Biodistribution in normal mice ($n = 3$) of [^{11}C]7. Radioactivity concentration was expressed as a percentage of injected dose per gram of tissue (% ID/g) and reported as the mean ± standard deviation (SD)

Biodistribution and Serum stability.

In the biodistribution study, 30 min post injection (Figure 3), there was only a moderate uptake of [^{11}C]**7** in the liver and kidneys (2.15 ± 0.45%ID/g and 3.29 ± 1.1%ID/g, respectively). The corresponding concentration of radioactivity in the plasma was 0.93 ± 0.17%ID/g. In the heart, lungs, spleen, muscles and brain, the uptake of radioactivity was lower than 1%ID/g.

For metabolite analyses, blood was collected and centrifuged. To evaluate the partition of the [^{11}C]**7** between blood cells and serum, a blood pellet and aliquots of serum were counted showing that 75% ($\pm$ 5%) of the radioactivity was bound to the blood cells. The serum was deproteinized by acetonitrile precipitation. Less than 10% of the radioactivity precipitates with the proteins. No metabolites were detectable by HPLC analyses of the extractable fraction of the radioactivity. All the radioactivity was recovered in one peak co-eluting with the cold reference **7**. The compound was found to be rapidly excreted and stable towards degradation up to 30 minutes post injection and thus seems to be suitable for PET imaging of gelatinases activity. The evaluation of the [^{11}C]**7** derivate in Lewis lung tumour bearing mice is in progress.

Conclusion

(*2R*)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid (**7**) and the corresponding labelled hydroxy precursor

(*2R*)-3-methyl-2-[[4-[(4-hydroxybenzoyl)amino] benzenesulfonyl]amino] butanoic (**11)** have been synthesized. [^{11}C]**7** was prepared by methylation with [^{11}C]CH_3I in high yields. The time required for labelling and purification of the tracer is compatible with the half life of carbon-11. In the gelatine degradation assay (*2R*)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid (**7)** exhibited a strong inhibitory effectiveness for gelatinases. Furthermore early *in vivo* studies demonstrated favourable pharmacokinetics for MMP-2 and MMP-9 activity monitoring and stability towards enzymatic degradation within the first 30 min after injection. Further in vivo evaluations in tumour bearing mice models are currently in progress.

Experimental

General

Chemicals and enzymes were purchased from Aldrich, Sigma, Fluka (Neu-Ulm, Germany) or Chemicon (Hofheim, Germany) and were used without further purification.

Analytical methods

Thin Layer Chromatography (TLC) was run on precoated plates of silica gel $60F_{54}$ (Merck, Darmstadt, Germany). The compounds were localized at 254 nm using a UV lamp. Flash chromatography was conducted on silica 63–200 μm (Merck, Darmstadt, Germany) under compressed air. Semi-preparative HPLC was performed using a S1020 pump (Sykam, Gilching, Germany) equipped with a detector to monitor radioactivity. Analytical HPLC was performed using a S1121 Solvent Delivery System (Sykam, Gilching, Germany), a UV detector and an EG&G detector to monitor radioactivity. Metabolite analyses were performed on an HPLC system composed of a S1121 Solvent Delivery System (Sykam, Gilching, Germany), a UV detector and a Flow Scintillation Analyzer 500TR Series (Packard, Germany) equipped with a PET cell for radioactivity monitoring. For HPLC, the following columns were used : for semi-preparative HPLC : Multospher 100 RP18 5 μm 250 × 10 mm (CS Chromatographie Service GmBH, Germany), for analytical HPLC : Phenomemex C18 150 × 4.60 mm (CS Chromatographie Service GmBH, Germany), for metabolite analyses: Nucleosil

100C18 5 μm 150 × 10 mm (CS Chromatographie Service GmBH, Germany). Mass spectra were recorded on the LC-MS system LCQ from Finnigan (Bremen, Germany) using the Hewlett Packard series 1100 HPLC system. NMR spectra were recorded on an Bruker AMX 250 MHz instrument using the hydrogenated residue of the deuterated solvents as internal standards for ^{1}H NMR. The chemical shifts were reported in ppm.

Chemistry

Synthesis of (2R)-3-methyl-2-[(4-nitrobenzenesulfonyl)amino] butanoic acid methyl ester (**3**). To a solution of D-valine methyl ester (**1**) (2 g, 12 mmol) in CH_2Cl_2 (120 ml) were added 2.6 ml (24 mmol) of *N*-methylmorpholine (NMM) and 3.18 g (14.3 mmol) of 4-nitrobenzensulfonyl chloride (**2**) at 0°C. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was twice washed with 100 ml of 1 N HCl solution, brine, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel using 80:20 hexane-ethyl acetate to give 3.2 g (80%) of **3** as a yellow solid. TLC Rf: 0.36 (7/3/1, hexane/EtOAc/Et_3N, v/v/v). ^{1}H NMR (250 MHz, $CDCl_3$): δ: 8.32 (d, $J = 8.7$ Hz, 2 H), 8.01 (d, $J = 8.7$ Hz, 2 H), 5.33 (d, $J = 10.0$ Hz, 1 H), 3.81 (dd, $J = 4.9$ Hz, 1 H), 3.48 (s, 3 H), 2.08 (m, 1 H), 0.94 (d, $J = 6.8$ Hz, 3 H), 0.84 (d, $J = 6.8$ Hz, 3 H).

Synthesis of (2R)-3-methyl-2-[(4-aminobenzenesulfonyl)amino] butanoic acid methyl ester (**4**). A solution of **3** (2.7 g, 8.5 mmol) in 30 ml of MeOH and 30 ml of EtOAc was hydrogenated using 200 mg of 10% Pd/C catalyst for 4 h. The solution was filtered on Celite and the filtrate was concentrated under reduced pressure. The residue was crystallized from 1/1 (v/v) CH_2Cl_2-hexane to give 2.23 g (91%) of **4** as colourless crystals. TLC: Rf: 0.0 (7/3/1, hexane/EtOAc/Et_3N, v/v/v). ^{1}H NMR (250 MHz, MeOD): δ: 7.48 (d, $J = 8.8$ Hz, 2 H), 6.66 (d, $J = 8.8$ Hz, 2 H), 3.52 (d, $J = 6.5$ Hz, 1 H), 3.42 (s, 3 H), 1.90 (m, 1 H), 0.90 (d, $J = 0.9$ Hz, 3 H), 0.88 (d, $J = 0.9$ Hz, 3 H). MS ($C_{12}H_{18}N_2O_4S$) calculated 286, measured: m/z: 286.8 $[M + H]^+$.

Synthesis of (2R)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid methyl ester (**6**). To a solution of **4** (400 mg, 1.4 mmol) in CH_2Cl_2 (20 ml), 380 μl (2.8 mmol) of *N*-methylmorpholine and 586 mg of 4-methoxybenzoylchloride (**5**)

(2.1 mmol) were added at room temperature and stirred overnight. The mixture was poured into water and extracted with EtOAc. The organic solution was twice washed with 100 ml of 1 N HCl solution, brine, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel using 70:30 hexane-ethyl acetate to give 441 mg (75%) of **6** as a white solid. TLC: Rf: 0.45 (6/4, hexane/EtOAc, v/v). ^{1}H NMR (250 MHz, DMSO-d_6): δ: 10.37 (s, 1 H), 7.96 (d, $J = 8.7$ Hz, 2 H), 7.92 (d, $J = 8.7$ Hz, 2 H), 7.82 (d, $J = 9.7$ Hz, 1 H), 7.70 (d, $J = 8.1$ Hz, 2 H), 7.09 (d, $J = 8.2$ Hz, 2 H), 3.83 (s, 3 H), 3.52 (dd, $J = 7.8$, 8.9 Hz, 1 H), 3.37 (s, 3 H), 1.87 (m, 1 H), 0.80 (d, $J = 6.8$ Hz, 3 H), 0.77 (d, $J = 6.7$ Hz, 3 H). MS ($C_{20}H_{24}N_2O_6S$) calculated 420, measured: m/z: 421.1 $[M + H]^+$, 863.0 $[2M + Na]^+$.

Synthesis of (2R)-3-methyl-2-[[4-[(4-methoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid (**7**). To a solution of **6** (150 mg, 0.3 mmol) in 4 ml of MeOH and 4 ml of THF, 2.5 ml of 1 M NaOH solution were added at room temperature. The mixture was stirred for 4 h at 60°C. After being cooled to room temperature, the solution was acidified with 1 N HCl solution and extracted with EtOAc. The organic solution was washed with brine, dried over $MgSO_4$ and evaporated. The residue was crystallized from EtOAc-hexane to give 120 mg (>95%) of **7** as colourless crystals. ^{1}H NMR (250 MHz, DMSO-d_6): δ: 12.56 (br s, 1 H), 10.37 (s, 1 H), 7.96 (d, $J = 8.8$ Hz, 2 H), 7.92 (d, $J = 8.7$ Hz, 2 H), 7.82 (d, $J = 9.7$ Hz, 1 H), 7.72 (d, $J = 8.7$ Hz, 2 H), 7.06 (d, $J = 8.8$ Hz, 2 H), 3.83 (s, 3 H), 3.47 (dd, $J = 7.2$ Hz, 6.1 Hz, 1 H), 1.94 (m, 1 H), 0.81 (d, $J = 6.9$ Hz, 3 H), 0.77 (d, $J = 6.9$ Hz, 3 H). MS ($C_{19}H_{22}N_2O_6S$) calculated: 406, measured : m/z: 406.9 $[M + H]^+$, 812.8 $[2M + H]^+$.

Synthesis of 4-acetoxybenzoylchloride (**9**). To a solution of 1 g (5.5 mmol) of 4-acetoxybenzoic acid (**8**) in CH_2Cl_2 (20 ml), 10 ml of thionyl chloride were added. After the solution was stirred overnight, the solvents were removed under vacuum to give a colourless oil (**9**) that was used for the next step without further purification.

Synthesis of (2R)-3-methyl-2-[[4-[(4-acetoxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid methyl ester (**10**). To a solution of **9** (300 mg, 1.5 mmol) in 30 ml of CH_2Cl_2, 400 mg of **4** (1.4 mmol) and 380 µl (2.8 mmol) of *N*-methylmorpholine were added at room temperature and the solution stirred overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was twice

washed with 100 ml of 1 N HCl solution, brine, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel using 70:30 hexane-EtOAc to give 376 mg (60%) of **10** as a white solid. TLC : Rf : 0.40 (6/4 hexane/EtOAc v/v). ^{1}H NMR (250 MHz, DMSO-d_6 + MeOD) : δ: 10.26 (s, 1 H), 7.94 (d, J = 8.6 Hz, 2 H), 7.86 (d, J = 8.8 Hz, 2 H), 7.68 (d, J = 8.8 Hz, 2 H), 7.21 (d, J = 8.6 Hz, 2 H), 3.52 (d, J = 6.7 Hz, 1 H), 3.32 (s, 3 H), 2.22 (s, 3 H), 1.86 (m, 1 H), 0.80 (d, J = 2.8 Hz, 3 H), 0.77 (d, J = 2.8 Hz, 3 H). MS ($C_{21}H_{24}N_2O_7S$) calculated: 448, measured: m/z: 449.1 $[M+H]^+$, 918.9 $[2M+Na]^+$.

Synthesis of (2R)-3-methyl-2-[[4-[(4-hydroxybenzoyl)amino]benzenesulfonyl]amino] butanoic acid (**11**). To a solution of **10** (200 mg, 0.45 mmol) in 4 ml of MeOH and 4 ml of THF, 2.5 ml of 1M NaOH solution were added at room temperature. The mixture was stirred for 4 h at 60°C. After being cooled to room temperature, the solution was acidified with 1 N HCl solution and extracted with EtOAc. The organic solution was washed with brine, dried over $MgSO_4$ and evaporated. The residue was crystallized from EtOAc-hexane to give 172 mg (99%) of **7** as colourless crystals. ^{1}H NMR (250 MHz,DMSO-d_6) δ: 12.53 (br s, 1 H), 10.22 (s, 1 H), 10.15 (br s, 1 H), 7.91 (d, J = 8.8 Hz, 2 H), 7.84 (d, J = 8.7 Hz, 2 H), 7.70 (d, J = 8.8 Hz, 2 H), 6.86 (d, J = 8.7 Hz, 2 H), 3.48 (d, J = 6.6 Hz, 1 H), 1.91 (m, 1 H), 0.81 (d, J = 7.0 Hz, 3 H), 0.78 (d, J = 7.0 Hz, 3 H). MS ($C_{18}H_{20}N_2O_6S$) calculated: 392, measured: m/z: 392.9 $[M+H]^+$, 823.0 $[2M+K]^+$.

Radiochemistry

No carrier added $[^{11}C]CO_2$ was produced on an RDS 112 cyclotron (Siemens, Germany) via the $^{14}N(p,\alpha)^{11}C$ reaction using nitrogen with 1% oxygen. $[^{11}C]CO_2$ was converted into $[^{11}C]CH_3I$ using the PET Trace MeI Microlab remote system (GE Medical System, Germany).

Synthesis of (2R)-3-methyl-2-[[4-[(4-[^{11}C]methoxybenzoyl]amino]-benzenesulfonyl] amino] butanoic acid [^{11}C]7. Precursor **11** (0.5 mg) was dissolved in 300 µl of dry DMF with NaH (0.5 mg) in a 1.1 ml Screw Neck Micro-Vial (Alltech, Germany). $[^{11}C]CH_3I$ was allowed to bubble in to a vial containing the precursor for 2–3 min. The mixture was heated for 5 min at 80°C. The purification was performed using the semi-preparative system (see *Analytical methods*) eluted with a water/MeCN/TFA mixture (60/40/0.1 v/v/v) at a flow rate of 6 ml/min

(t_R: 13–13.5 min). The product fraction was collected and concentrated under vacuum. Radiochemical purity and specific radioactivity were then determined using the analytical HPLC system eluted with a water/MeCN/TFA mixture (60/40/0.1 v/v/v) at a flow rate of 1 ml/min (t_R: 4.9 min).

Enzyme inhibition assays

Gelatine labelling. Gelatine was dissolved in saline to a concentration of 1 mg/ml. 125 µl of this solution was transferred to an Eppendorf cap coated with 150 µg iodogen. 10 µl of n.c.a. [^{125}I]NaI (60 MBq) was added to the solution. After 30 min reaction, the solution was removed and eluted through a NAP-10 column (Amersham Pharmacia Biotech, Freiburg, Germany) to separate non-reacted sodium iodide, according to the manufacturer instructions. The radiochemical purity was controlled by TLC on precaoted plates of silica gel $60F_{254}$ (Merck, Darmstadt, Germany) eluted with a mixture of acetone, water, butanol and ammonia (6.5:0.5:2:1) (v/v/v/v). The labelled gelatine was detected with a radioTLC analyser Trace Master 20 (Berthold, Germany). Rf gelatine: 0.0; Rf [^{125}I]NaI: 1. The radiochemical purity was $>98\%$.

Inhibition assay. Compound **7** (1 mg, 2.46 µmol in 5 µl DMF), HWGF-peptide (**12**) (1 mg, 0.74 µmol in 10 µl DMF) and 1,10 phenanthroline (**13**) (9.9 mg, 50 µmol in 25 µl DMF) were dissolved and diluted in the assay buffer (50 mM Tris-HCL, pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 2 mM NaN_3) to the appropriate concentrations (0.001–200 µM final concentration). For each assay, 100 ng of MMP-2 or MMP-9 were activated for 1 h at 37°C with APMA (p-aminophenylmercuric acetate) (5 mM). Compounds **7**, **12** and **13**, at the desired concentrations, were added to the enzyme solution and incubated for 1 h at 37°C. Finally, 1µg of labelled gelatine was added to each assay and incubated for 1 h at 37°C. The degradation of labelled gelatine was determined by counting the radioactivity in the supernatant after precipitation of the undegraded gelatine with 20% trichloroacetic acid. The 1,10 phenanthroline (**13**) and CTTHWGFTLC (**12**) peptide served as control MMP inhibitors.

Biodistribution and in vivo serum stability

All procedures involving animals were in conformity with the German law for protection of animals that are in compliance with European laws and policies.

Female Balb/c mice, aged 5–7 weeks, were injected in to the tail vein with 22–26 GBq (600–700 μCi) of [^{11}C]**7**. Animals were sacrificed 30 minutes after injection. Tissues of interest were immediately dissected, weighed and counted for radioactivity with a γ-counter. Radioactivity concentration was expressed as a percentage of injected dose per gram of tissue (% ID/g) and reported as the mean $\pm$ standard deviation (S.D.).

Serum stability of [^{11}C]**7** was studied by analytical radioHPLC. After sacrifice, blood was immediately collected and centrifuged. Serum was deproteinised by protein precipitation with acetonitrile. The samples were then centrifuged for 15 min at 12 000 *g*. Aliquots of blood, serum and supernatant after acetonitrile precipitation were counted for radioactivity with a γ-counter to evaluate the partition of the tracer. The supernatants were concentrated and injected onto an HPLC column (see *Analytical methods*) eluted with a water/MeCN/TFA mixture (60/40/0,1 v/v/v).

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