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Research Article

Design of a radiopharmaceutical for the palliation of painful bone metastases: rhenium-186-labeled bisphosphonate derivative

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Summary

To develop a radiopharmaceutical for the palliation of painful bone metastases based on the concept of bifunctional radiopharmaceuticals, we designed a bisphosphonate derivative attached to a stable ^{186}Re-monoaminemonoamidedithiol (MAMA) chelate (^{186}Re-MAMA-BP) to improve the instability of ^{186}Re-HEDP. The precursor (Tr-MAMA-BP) of ^{186}Re-MAMA-BP was synthesized by coupling the carboxyl group of the Tr-MAMA derivative with the amino group of the bisphosphonate derivative. This ^{186}Re-labeled compound was prepared by a ligand exchange reaction using ^{186}Re-glucoheptonate with a radiochemical yield of 32.0 $\pm$ 4.1%. In the incubation study in buffered solution (pH 7.0), ^{186}Re-MAMA-BP was more stable than ^{186}Re-HEDP. This suggests that ^{186}Re-MAMA-BP is a potential radiopharmaceutical for the palliation of painful bone metastases.

Key Words: bone metastases; palliation; bisphosphonate; rhenium-186; bifunctional

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Introduction

Malignant tumors, especially breast and prostate carcinomas, frequently metastasize to the bone.[1] A prominent symptom caused by these metastases is pain, which has a significant impact on the patients' quality of life. Localized radiation therapy is an effective modality in the treatment of bone pain,[2] however, a common problem in cases of bone metastases is the development of multiple sites of metastases, so internal radiotherapy using specifically localized beta emitters is preferable. Recently, rhemium-186-1-hydroxyethylidene-1,1-diphosphonate (^{186}Re-HEDP), a polynuclear complex, has been proposed for the palliation of pain resulting from the metastatic bone lesions of tumors.[3–5] However, this complex showed slow blood clearance and high gastric uptake of radioactivity upon injection, leading to unnecessary radiation in the patients. The cause of these findings has been reported to be the *in vivo* instability of this ^{186}Re complex leading to the generation of $^{186}ReO_4^-$.[6,7] Thus, development of a more stable ^{186}Re-labeled compound than ^{186}Re-HEDP for the palliation of painful bone metastases has been desired. For this purpose, based on the concept of bifunctional radiopharmaceuticals, we designed a stable ^{186}Re-labeled bisphosphonate derivative (^{186}Re-MAMA-BP) (***1***) by the conjugation of the monoaminemonoamidedithiol (MAMA) moiety to a bisphosphonate analogue (Figure 1) because MAMA derivatives form stable chelates with rhenium.[8,9] This paper describes the synthesis and stability of ^{186}Re-MAMA-BP.

Results and discussion

Tr-MAMA-BP (**8**), the precursor of ^{186}Re-MAMA-BP, was synthesized by coupling the carboxyl group of a Tr-MAMA derivative (**4**) with the amino group of a bisphosphonate derivative (**6**). Namely, bisphosphoric tetraethyl ester (**6**) as a bisphosphonate analogue containing an amino group was coupled with a Tr-MAMA derivative (**4**) in the presence of dicyclohexylcarbodiimide (DCC) in chloroform to yield the desired compound (**7**) in good yield (65%). After the selective hydrolysis of bisphosphoric tetraethyl ester via

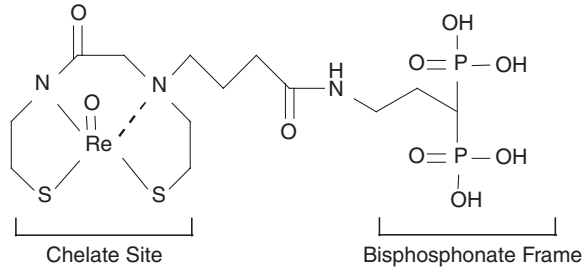


Figure 1. Chemical structure of ^{186}Re-MAMA-BP (1)

trimethylsilylbromide,[10] Tr-MAMA-BP was obtained by reversed phase (RP)-HPLC. Trityl groups of Tr-MAMA-BP were deprotected just before the ligand exchange reaction by treatment with trifluoroacetic acid (TFA) and triethylsilane.

MAMA-BP (**9**) contains a MAMA site as a ligand to form a stable complex with ^{186}Re. In addition, MAMA-BP also contains a bisphosphonate site that is capable of chelating with ^{186}Re. Accordingly, to obtain the desired complex, the reaction condition where Re is chelated not with the bisphosphonate site but with the MAMA site is required.

Since it has been reported that MAMA chelates with rhenium in oxidation state (V) to form a stable oxorhenium(V) complex,[8,11] ^{186}Re(V)-glucoheptonate was first prepared by reacting rhenium with glucoheptonate, and then a ligand exchange reaction was performed between ^{186}Re-glucoheptonate and MAMA-BP to produce ^{186}Re-MAMA-BP (**1**).

To be certain that ^{186}Re was selectively bound to the MAMA site in MAMA-BP by the transchelation reaction with ^{186}Re-glucoheptonate, the mixture containing MAMA and HEDP at equal concentrations was reacted with ^{186}Re-glucoheptonate. When this reaction mixture was analyzed by RP-HPLC, almost all radioactivity was eluted in the fraction with the same retention time as that of nonradioactive Re-MAMA on UV detection at 254 nm. ^{186}Re-HEDP was, however, eluted in a shorter retention time than Re-MAMA. These results suggest that ^{186}Re is chelated with the MAMA site when ^{186}Re was bound to MAMA-BP via ^{186}Re-glucoheptonate.

In the labeling reaction of ^{186}Re-MAMA-BP, the same reaction conditions were investigated. Since the labeling reaction of ^{186}Re-MAMA-BP was difficult to proceed at room temperature, we heated the labeling solution in boiling water. After 5, 20 and 60 min of heating, the sample was drawn, and the radioactivity was analyzed by RP-HPLC. The reactions proceeded equally on heating for 5 and 20 min, but the radiochemical yield decreased slightly after 60 min of heating. Therefore, 10 min was used as the reaction time. Furthermore, to test slightly moderate conditions, the labeling solution was reacted at 80°C, and the radiochemical yield of ^{186}Re-MAMA-BP was improved from 21.3 $\pm$ 4.5 to 32.0 $\pm$ 4.1%. After purification by RP-HPLC, ^{186}Re-MAMA-BP showed a radiochemical purity of over 95% (96.4 $\pm$ 1.4%).

In general, the radiochemical yield of ^{186}Re-MAMA is very high (over 95%). However, the radiochemical yield of ^{186}Re-MAMA-BP was not high (32%). The reason for the low radiochemical yield of ^{186}Re-MAMA-BP compared to that of ^{186}Re-MAMA is not clear, but it may be that the steric hindrance occurred with the conjugation of the MAMA moiety to a bisphosphonate analogue.

Figure 2 shows the stability of ^{186}Re-MAMA-BP and ^{186}Re-HEDP in buffered solutions. After 24 h of incubation, 81.8 $\pm$ 1.7% of ^{186}Re-MAMA-

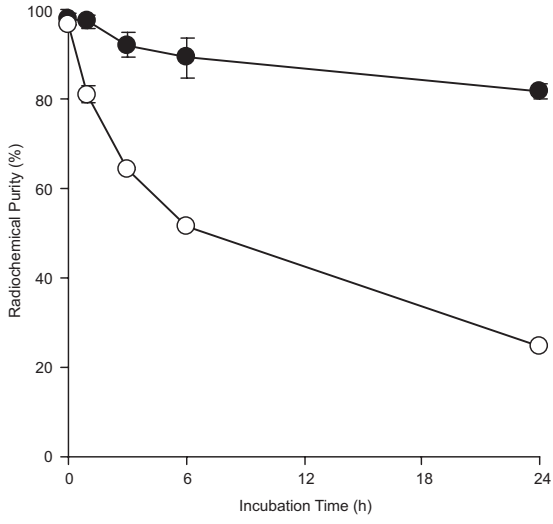


Figure 2. Stability of ^{186}Re-MAMA-BP (closed circles) and ^{186}Re-HEDP (open circles) in buffered-solution

BP remained intact, compared to 24.8 $\pm$ 0.2% in the case of ^{186}Re-HEDP. This indicates that the novel compound ^{186}Re-MAMA-BP, based on the concept of bifunctional radiopharmaceuticals, is more stable than ^{186}Re-HEDP.

In conclusion, ^{186}Re-MAMA-BP showed much greater stability than ^{186}Re-HEDP. Although further investigation of its behavior *in vivo* is required, these results indicate that ^{186}Re-MAMA-BP is a potential radiopharmaceutical for the palliation of painful bone metastases.

Experimental

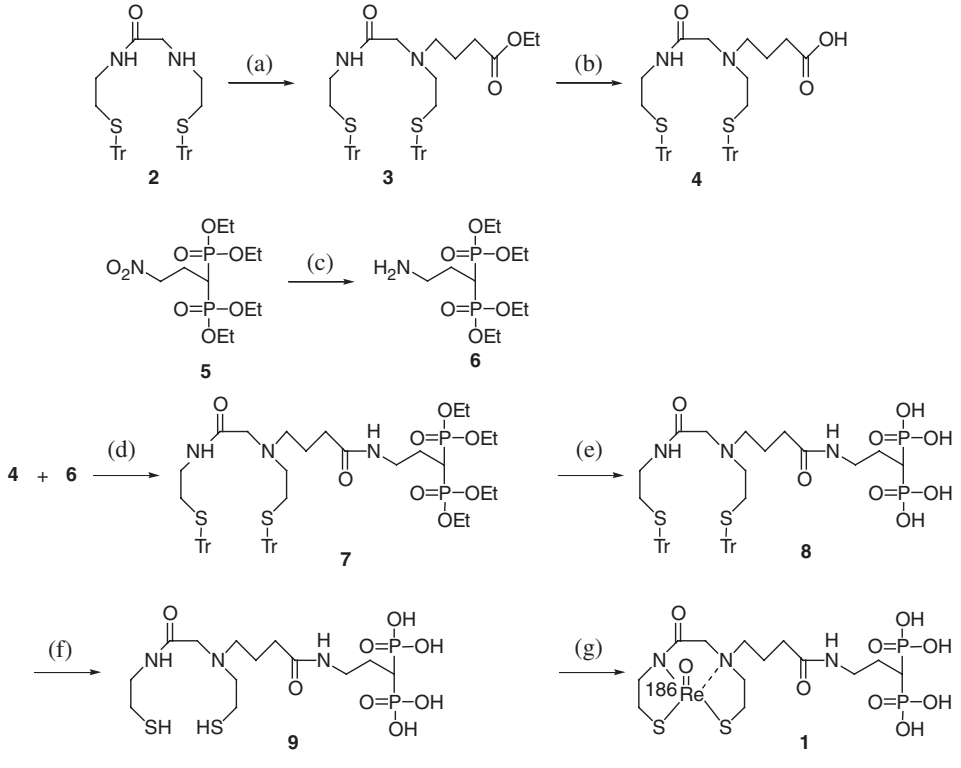
General

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AC-200 spectrometer (JEOL Ltd., Tokyo, Japan), and the chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Fast atom bombardment mass spectra (FAB-MS) were obtained with a JMS-HX/HX 110 A (JEOL Ltd.). ^{186}Re was supplied by the Japan Atomic Energy Research Institute (Tokai-mura, Japan) as perrhenate ($^{186}ReO_4^-$).[12] Cellulose acetate electrophoresis (CAE, Separax-SP; Joko Co. Ltd., Tokyo, Japan) was run with an electrostatic field of 0.8 mA/cm for 20 min in veronal buffer ($I = 0.06$, pH 8.6). TLC analyses were performed with silica plates (Merck Art 5553) with acetone as a developing solvent. Other reagents were of reagent grade and used as received.

Synthesis of ^{186}Re-MAMA-BP (Scheme 1)

N-[2-(Tritylthio)ethyl]-2-[2-(tritylthio)ethylamino]acetamide (Tr-MAMA) (***2***). Compound **2** was synthesized according to the published procedure.[13] ^{1}H NMR ($CDCl_3$): δ 7.42–7.17 (overlapped m,30H), 3.06 (td,2H), 3.02 (s,2H), 2.45(t,2H), 2.38–2.31 (overlapped m,4H). FAB-MS calcd for $C_{44}H_{42}N_2OS_2$ $(M+H)^+$: m/z 679. Found: 679.

N-[2-(Tritylthio)ethylaminocarbonylmethyl]-N-[2-(tritylthio)ethyl]-4-amino-butyric Acid (***4***). Compound **2** (3.30 g, 4.86 mmol) was dissolved in 50 ml of *N*,*N*-dimethylformamide (DMF), and diisopropylethylamine (689 mg, 5.34 mmol) and ethyl 4-bromo-*n*-butyrate (1.04 g, 5.34 mmol) were added to the solution. After the reaction mixture had been stirred at 90°C for 3 h, the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed with brine. The organic layer was dried over anhydrous $CaSO_4$, and the solvent was removed *in vacuo*. The oily residue was purified by flash chromatography on silica gel using ethyl acetate-hexane (1:1)



Scheme 1. Synthesis of ^{186}Re-MAMA-BP
Reagents: (a) ethyl 4-bromo-*n*-butyrate, (*i*-Pr)$_2$NEt; (b) NaOH; (c) Pd-C; (d) DCC; (e) $(CH_3)_3SiBr$; (f) TFA, triethylsilane; (g) ^{186}Re-glucoheptonate

as the eluent to provide compound **3** (2.03 g, 52.9%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.41–7.15 (overlapped m,30H), 4.06 (q,2H), 3.01 (td,2H), 2.83 (s,2H), 2.38–2.12 (overlapped m,10H), 1.68–1.54 (overlapped m,2H), 1.20 (t,3H). FAB-MS calcd for $C_{50}H_{52}N_2O_3S_2$ $[M + H]^+$: *m/z* 793. Found: 793.

After hydrolysis of the ester bond, compound **4** (1.92 g, 98.0%) was obtained as brown crystals. ^{1}H NMR ($CDCl_3$): δ 7.40–7.14 (overlapped m,30H), 2.99 (td,2H), 2.85 (s,2H), 2.38–2.23 (overlapped m,10H), 1.65–1.60 (overlapped m,2H). FAB-MS calcd for $C_{48}H_{48}N_2O_3S_2$ $(M + H)^+$: *m/z* 765. Found: 765.

Tetraethyl-3-nitropropylidene-1,1-bisphosphonate (***5***). Compound **5** was synthesized according to the published procedure.[14,15] ^{1}H NMR ($CDCl_3$): δ 4.71 (t,2H), 4.27–4.14 (overlapped m,8H), 2.68–2.40 (overlapped m,3H), 1.36 (t,12H). FAB-MS calcd for $C_{10}H_{22}O_6P_2$ $(M + H)^+$: *m/z* 362. Found: 362.

Tetraethyl-3-aminopropylidene-1,1-bisphosphonate (***6***). Compound **5** (100 mg, 0.28 mmol) was dissolved in a mixture of 1.13 ml of methanol and 0.03 ml of water, and 70.0 mg of the catalyst (10% Pd on activated carbon) was added to the solution. The mixture was hydrogenated at room temperature for 6 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to provide crude compound **6** as a pale yellow oil. Compound **6** was used in the next reaction without further purification.

[1-Phosphono-3-[4-[[2-(tritylthio)ethyl]-[[2-(tritylthio)ethylcarbamoyl]methyl] amino]-butyrylamino]propyl]phosphonic Acid Tetraethyl Ester (***7***). Compound **4** (206 mg, 0.27 mmol) and crude compound **6** (90.0 mg, 0.27 mmol) were dissolved in chloroform. DCC (56.0 mg, 0.27 mmol) in 2 ml of chloroform was added dropwise to the reaction mixture while the reaction temperature was maintained below 0°C. After 30 min of stirring below 0°C, the reaction solution was stirred at room temperature for 5 h. After filtration, the filtrate was evaporated *in vacuo*, and the residue was purified by flash chromatography on silica gel using chloroform-methanol (10:1) as the eluent to provide compound 7 (190 mg, 65.3%) as yellow crystals. ^{1}H NMR ($CDCl_3$): δ 7.40–7.15 (overlapped m,30H), 4.23–4.11 (overlapped m,8H), 3.38 (td,2H), 3.01 (td,2H), 2.83 (s,2H), 2.38–2.02 (overlapped m,13H), 1.67–1.60 (overlapped m,2H), 1.33 (t,12H). FAB-MS calcd for $C_{59}H_{73}N_3O_8P_2S_2$ $(M + H)^+$: *m/z* 1078. Found: 1078.

N^2-[4-[(3,3-Diphosphonopropyl)amino]-4-oxobutyl]-N^1,N^2-bis[2-(tritylthio)ethyl] glycin-amide (Tr-MAMA-BP) (***8***). Hydrolysis of the phosphonic esters of compound **7** was carried out via trimethylsilylbromide. Compound **7** (100 mg, 92.8 μmol) was dissolved in 2 ml of chloroform, and trimethylsilylbromide (116 mg, 0.76 mmol) was added to the solution. After 3 h of stirring at 50°C, the reaction solution was stirred at room temperature for 36 h. After the

solvent was removed *in vacuo*, the residue was recovered in 2 ml of methanol and stirred at room temperature for 60 min. After the solvent was removed *in vacuo*, the residue was purified by RP-HPLC performed with a Cosmosil $5C_{18}$-AR-300 column (10×150 mm; Nacalai Tesque, Kyoto, Japan) at a flow rate of 4.7 ml/min with a gradient mobile phase from 95% A (0.1% aqueous TFA) and 5% B (acetonitrile with 0.1% TFA) to100% B in 30 min. A fraction containing compound **8** was collected, and the solvent was removed by lyophilization to provide compound **8** (35.0 mg, 39.1%) as white crystals. ^{1}H NMR ($CDCl_3$): δ 7.14–7.37 (overlapped m,30H), 3.36 (m,2H), 3.08 (m,2H), 2.92 (s,2H), 2.84–2.60 (overlapped m,5H), 2.33–2.22 (overlapped m,6H), 2.01 (m,2H), 1.65 (m,2H). FAB-MS calcd for $C_{51}H_{57}N_3O_8P_2S_2$ $(M+H)^+$: m/z 966. Found: 966.

[N-[2-[[3-(3,3-Diphosphonopropylcarbamoyl)propyl](2thioethyl)amino]acetyl]-2-aminoethanethiolate] Oxorhenium(V) (^{186}Re-MAMA-BP) (***1***). ^{186}Re-MAMA-BP (***1***) was formed by transchelation form ^{186}Re-glucoheptonate. Namely, $^{186}ReO_4^-$ solution was added to stock freeze-dried powder of $SnCl_2 \cdot 2H_2O$ and glucoheptonate (made from 1 g of glucoheptonate and 30 mg of $SnCl_2 \cdot 2H_2O$ in 20 ml of aqueous solution pH 8.6) to produce a final freeze-dried powder concentration of about 125 mg/ml. After heating at 80°C for 10 min and standing at room temperature for 50 min, the formation of ^{186}Re-glucoheptonate with a radiochemical yield of over 90% (93.3 ± 2.1%) was ascertained by TLC (Rf = 0). Trityl groups of Tr-MAMA-BP (**8**) (0.3 mg) were deprotected by treatment with TFA and triethylsilane just before radiolabeling. After removal of the solvent under a stream of N_2, 0.2 ml of 0.2 M acetate buffer (pH 3.0) was added to the residue. Then, 0.2 ml of ^{186}Re-glucoheptonate solution was added to this solution, and the reaction mixture was vigorously stirred and allowed to react at 80°C for 10 min. After the reaction mixture was allowed to cool to room temperature, ^{186}Re-MAMA-BP was purified by RP-HPLC performed with a Cosmosil $5C_{18}$-AR-300 column (4.6×150 mm) at a flow rate of 1 ml/min with a mixture of 0.2 M phosphate buffer (pH 6.0) and ethanol (80:20) containing 10 mM tetrabutylammoniumhydroxide.

Synthesis of [N-[2-[(2-Mercaptoethyl)amino]acetyl]-2-aminoethanethiolate] rhenium(V) Oxide (Re-MAMA)

A modified procedure reported previously was employed with slight modification.[16] Compound **2** (100 mg, 0.15 mmol) was suspended in 2 ml of ethanol. $SnCl_2 \cdot 2H_2O$ (31 mg, 0.14 mmol) in 0.5 ml of 0.1 N HCl and $KReO_4$ (40 mg, 0.14 mmol) in 0.5 ml of 0.1 N HCl were added to this stirred suspension, then this reaction mixture was refluxed at 120°C for 12 h. After the solvent was removed *in vacuo*, the residue was purified by RP-HPLC performed with a Cosmosil $5C_{18}$-AR-300 column (10×150 mm) at a flow rate

of 4.7 ml/min with a gradient mobile phase from 95% A (0.1% aqueous TFA) and 5% B (acetonitrile with 0.1% TFA) to 100% B in 30 min. A fraction containing Re-MAMA was collected, and the solvent was removed by lyophilization to provide Re-MAMA (5.0 mg, 9.1%) as reddish violet crystals.

^{1}H NMR ($CDCl_3$): δ 7.98 (m,1H), 4.03 (m,2H) 3.54 (m,2H), 3.20 (m,2H), 3.05 (m,2H), 2.86 (m,2H). FABMS calcd for $C_6H_{11}N_2O_2$ ^{187}ReS2 $(M-H)^-$: m/z 393. Found: 393, $C_6H_{11}N_2O_2$ ^{185}ReS2 $(M-H)^-$: m/z 391. Found: 391.

Preparation of ^{186}Re-HEDP

^{186}Re-HEDP was prepared according to the published procedure,[7] and used after confirmation of its radiochemical purity by TLC (Rf = 0) and CAE (3.5 cm anode from the origin).

Analysis of reaction of ^{186}Re-glucoheptonate with the mixture of MAMA and HEDP

A mixture containing MAMA and HEDP at equal concentrations (15 mM) was reacted with same volume of ^{186}Re-glucoheptonate solution at room temperature for 60 min. This reaction mixture was analyzed by RP-HPLC, performed with a gradient mobile phase from 95% A (0.1% aqueous TFA) and 5% B (acetonitrile with 0.1% TFA) to 100% B in 30 min at a flow rate of 1 ml/min.

In vitro stability

To evaluate the stability of ^{186}Re complexes in buffered-solution, ^{186}Re-labeled compounds were diluted 5-fold with 0.1 M phosphate buffered (pH 7.0) saturated with 95% O_2/5% CO_2, and the solutions were incubated at 37°C for 24 h. After 1, 3, 6, and 24 h of incubation, the samples were drawn, and the radioactivity was analyzed by RP-HPLC or TLC.

Conclusion

Based on the concept of bifunctional radiopharmaceuticals, we have developed a novel rhenium-186-labeled bisphosphonate (^{186}Re-MAMA-BP) for the palliation of painful bone metastases. Precursor (**8**) was prepared by the conjugation of MAMA derivative protected thiol groups with a bisphosphonate derivative. The rhenium-186-labeled compound was synthesized from the precursor by simple reactions, followed by purification by RP-HPLC in high radiochemical purity. ^{186}Re-MAMA-BP showed the greater stability than ^{186}Re-HEDP when incubated in the phosphate buffer (pH 7.0). These results suggest that the newly synthesized ^{186}Re-MAMA-BP could become a potential agent for the palliation of painful bone metastases. The biodistribution of ^{186}Re-MAMA-BP will be reported elsewhere in the near future.

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