様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２９　年　　６　月　２０　日現在

機関番号：　１２５０１
研究種目：　研究活動スタート支援
研究期間：　2015～2016
課題番号：　１５Ｈ０６０９７

研究課題名（和文）68Ga-Labeled Compounds for PET Myocardial Perfusion Imaging

研究課題名（英文）68Ga-Labeled Compounds for PET Myocardial Perfusion Imaging

研究代表者
マムード　アッシュファック（Mahmood, Ashfaq）

千葉大学・大学院薬学研究院・特任教授

研究者番号：３０７５４７７５

交付決定額（研究期間全体）：（直接経費）　　2,300,000円

研究成果の概要（和文）：PETを用いた心血流画像診断(MPI)診断は心疾患の診断に重要である。本研究では、PET用MPI薬剤として新規68Ga標識薬剤の開発を行った。N4S2あるいはN402骨格を基本骨格とし、種々の置換基を導入した新規6座配位子を合成し、67Ga標識を行った。両骨格を用いた配位子においても標識時間30分で高収率で67Ga錯体を得た。既存の99mTc-MIBIに比較して、低い脂溶性のため、心臓への集積は低値であった。今後、脂溶性の改善による心筋への集積向上に向けたさらなる設計が必要と考えられる。

研究成果の概要（英文）：An important clinical PET application is in myocardial perfusion imaging (MPI) for heart disease. The aim of this research was to synthesize and evaluate new 67/68Ga-complexes for PET MPI.
We studied two separate classes of new hexadentate chelates (N4S2 and N402) that can easily be modified with substituents, provide stable complexation of Ga(III), and result in lipophilic, monocationic [Ga-complexes]+, properties necessary for myocardial uptake. Ligands for both chelate classes were synthesized and their Ga-complexes provided mono-cationic Ga-complexes. Radiolabeling with 67Ga(III) achieved high radiochemical yield in 30 min. In-vivo studies in normal mice display in-vivo stability, rapid distribution and clearance with low myocardial uptake due to lower lipophilicity compared to 99mTc-MIBI. Further modifications for increased lipophilicity and improved myocardial retention are required to attain a effective PET myocardial perfusion agent with high myocardial uptake.

研究分野：　Radiopharmaceutical chemistry

キーワード：　PET　gallium　heart

１．研究開始当初の背景 *Background of the Research:*

Molecular imaging in nuclear medicine has become increasingly important in early diagnosis of disease as well as in better understanding the pathophysiology of diseases. For this, both positron emission tomography (PET) and single-photon emission tomography (SPECT) imaging has contributed in important ways to modern clinical medicine, particularly in oncology, neurology and cardiology. However, the widespread use of PET imaging agents is hampered by the necessity of the close proximity to a cyclotron production facility, particularly in non-oncological settings such as cardiology, where an important clinical application of PET is in the determination of regional myocardial perfusion imaging (MPI), which relies on either a cyclotron for the production of short-lived $^{13}NH_3$ (half-life 9.9 min) or an expensive $^{82}Sr/^{82}Rb$ generator for the radionuclide ^{82}Rb (half-life 76 sec). Recent interest in the metallic radioisotope ^{68}Ga (half-life 68 min), a positron-emitting isotope, has emerged suitable for PET radiopharmaceuticals. It has been further facilitated by the availability of a long life (271 days) commercial $^{68}Ge/^{68}Ga$ generator system and the clinical demonstration in humans of chelated ^{68}Ga-labeled peptides in PET imaging.

The aim of this study was to synthesize small-molecule ^{68}Ga-myocardial perfusion imaging probes that could be used for myocardial PET imaging, independent of an on-site cyclotron facility.

２．研究の目的 *Purpose of the study:*

The design strategy in this investigation was to synthesize two separate class of chelates, both of which could provide lipophilic, monocationic $[Ga\text{-complexes}]^+$, properties necessary for myocardial uptake. The strategy adopted was to synthesize two separate core chelate platforms with the ability to easily modify synthetically the structure of the complex so as to change and optimize the molecules physiochemical properties for the development of a PET myocardial imaging agent that has high myocardial uptake and fast clearance from non-target tissue such as blood, liver and lungs. The chelates designed and synthesized in this study are hexa-dentate (a property necessary for stable and complete coordination of $^{67/68}Ga$) and form mono-cationic, lipophilic metal complexes with the Ga-radioisotope. The chelating ligands and their respective non-radioactive Ga-complexes were synthesized and characterized using standard synthetic methods. Radiolabeling with the ^{67}Ga isotope have also been successfully optimized for labeling conditions to achieve high radiochemical yield in 30 min of radiolabeling time. The radiolabeled complexes co-elute with the previously characterized non-radioactive Ga-complexes, and establishing the identity of the ^{67}Ga complexes as monocationic complexes. Additionally, in-vitro physiochemical properties, plasma stability and in-vivo biodistribution studies in normal mice were also conducted to evaluate the in-vivo behavior and potential to localize in myocardial tissue.

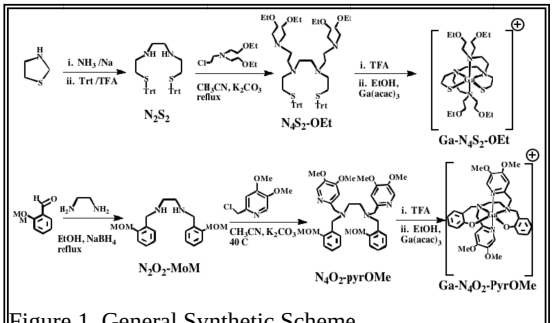

Figure 1. General Synthetic Scheme

３．研究の方法 Materials and Methods :

Tetradentate N_2S_2 ligand: Thiazolidine (9.81 gm; 110 mmol) was dissolved in liquid NH_3 (120mL) at -68℃ and the mixture was stirred vigorously till all solid material was dissolved. Solid Na metal was then added to this solution until dark blue color persisted for at least 15 min at -68 ℃. The reaction was quenched by addition of solid NH_4Cl in portions until discoloration occurred and persisted. The resulting reaction mixture was allowed to come to room temperature and allow slow evaporation of the NH_3 gas to obtain a cloudy-white slurry. Ice cold distilled water (150mL) was added and the solution was neutralized to pH=1 with HCl(con). The clear solution with white precipitates was filtered through a glass filter and the filtrate was washed 3 times with 100mL EtOAc. The aqueous solution was evaporated at 45℃ on rotary evaporator and the white solid precipitates were dried overnight under high vacuum. Yield =9.2g (36mmol, 27.9%)

Tetradentate $N_2S_2(Trt)_2$ Ligand: Solid N_2S_2 crude (3.49g, 13.7mmol) was dissolved in 100mL TFA and triphenylmethanol (3.57g, 13.7mmol) was added to this solution. The yellow solution was stirred at room temperature for 2h. After rotary-evaporation of the TFA from the solution, the resulting greenish yellow solid was dissolved in $CHCl_3$ and washed with 1N NaOH. The product was purified on 2 mm silica preparative plates using 2-5% NH_3-MeOH(7M) in $CHCl_3$. A pale yellow oil was obtained (1.73g) which was characterized by Mass Spec and ^1H NMR.

3-chloropropyl-N-bis(ethoxyethylether)amine: Bis(ethoxyethyl)amine (750mg, 4.65mmol) was mixed with

1-bromo-3-chloropropane (5.0g, 32mmol) and K_2CO_3 (1.93g, 14.0mmol). The mixture was stirred at room temperature overnight and the product was purified via a silica chromatography using $CHCl_3$ as the the eluent. The clear liquid was obtained. Yield = 949mg(4.0mmol, 85.8%), was characterized by Mass Spec and ^{1}H NMR.

Hexadentate-N,N'propy-bis(Ethoxy ethylether)amine-N_2S_2Trt: $N_2S_2(Trt)_2$ (482mg, 726μmol) was dissolved in anhydrous CH_3CN, 3-Chloropropyl-N-bis(Ethoxyethylether)ami ne (380 mg, 1.6 mmol), potassium carbonate(200 mg, 1.45mmol), potassium iodide(241 mg, 1.45 mmol) were added in sequence. The mixture was refluxed overnight, cooled to room temperatureand evaporated to remove the CH_3CN and purified on 2mm preparative silica plate using a 2.5% of a NH_3-MeOH(7M) in CH_3Cl as a solvent. A yellow-oil was obtained in a yield of 353 mg (330μmol, 45.4%) and was characterized by Mass Spec and ^{1}H NMR.

N,N'propy-bis(Ethoxyethylether)a mine-N_2S_2: $(EtOEt)_2N_4S_2(Trt)_2$ was dissolved in TFA and the solution was stirred at room temperature for 30 min, Triethylsilane was added dropwise till the bright-yellow color becomes pale. White precipitate of triphenylmethane appeared, that were filtered. The filtrate was evaporated to dryness using rotary evaporator. HCl/EtOH was added to the residue and evaporated twice to convert the product to an HCl salt.

Hexadentate-N,N'-(3,4-diMeOPyri dyl)-$N_2S_2(Trt)_2$: $N_2S_2(Trt)_2$ (300 mg, 451μmol) was dissolved in anhydrous CH_3CN (20mL) and 2-Chloromethyl-3,4-dimethoxypridine hydrochloride salt(303mg, 1.35mmol), potassium carbonate(312 mg, 2.26 mmol), potassium iodide(150mg, 902μmol) were added in sequence. The mixture was stirred at 40℃ overnight and purified on 2mm preparative silica plates using 2.5% of NH_3-MeOH(7M) in CH_3Cl as a solvent. A pale yellow oil was obtained yielding 150 mg (155 μmol, 34.4%) of the desired product which was characterized by Mass Spec and ^{1}H NMR.

N,N'-(3,4-diMeOPyridyl)-$N_2S_2(Trt)_2$: N, N'-(3, 4-diMeOPyridyl)-$N_2S_2(Trt)_2$ (122 mg, 126 μmol) was dissolved in TFA (10 mL) and the bright yellow solution was stirred at room temperature for 30 min. Triethylsilane was added dropwise to this solution crude till the yellow color discharged and the solution became pale yellow. The white precipitates of triphenylmethane appeared upon standing and were filtered off, while the filtrate was evaporated to dryness using a rotary evaporator. HCl/EtOH was added to the residue and evaporated twice to convert the product to an HCl salt.

2-methoxymethoxy-4-methoxybenz aldehyde:
2-hydroxy-4-methoxybenzaldehyde (1.0 g, 6.57 mmol) and DIPEA (1.7g, 13.1mmol) were dissolved in CH_2Cl_2 (100mL) and cooled to -35℃. Methoxymethylchloride (530 mg, 6.57 mmol) to then slowly added to the reaction mixture dropwise at -35℃ with stirring. After stirring at -35℃ for 30 min, the temperature was raised to room temperature and stirred overnight. The product was purified using a silica column with 70% $CHCl_3$ /hexane to obtain a pale yellow solid in a yield = 1.101 g (5.61 mmol, 85.4 %). This compound was characterized by Mass Spec and ^{1}H NMR.

Tetradentate MOM protected N_2O_2M:
2-methoxymethoxy-4-methoxybenzaldehyde (2.0 mmoles) was dissolved in anhydrous EtOH (20mL) and ethylenediamine (61.3mg, 1.02mmol) diluted in EtOH (5mL) was slowly added drop-wise while stirring at 80℃. After a 6 hour reflux, the solution was cooled to room temperature and solid $NaBH_4$ in excess was added to the reaction with further stirred at room temperature overnight. A few drops of water were added to quench $NaBH_4$, and the solution was subsequently evaporated to dryness using a rotary evaporator. The product was extracted in CH_3Cl from water and purified on 2mm preparative silica plate using 3 % NH_3MeOH(7M) in CHCl3. A yellow solid was obtained in a yield of 3.68 g (877μmol, 86 %). This compound was characterized by Mass Spec and ^{1}H NMR.

Hexadentate-N,N'propy-bis(Ethoxy ethylether)amine-MOM-N_2O_2M:
MOM-N_2O_2M (368.7 mg, 877 μmol) was dissolved in dry CH_3CN and 3-Chloropropyl-N-bis(Ethoxyethylether)ami ne (500 mg, 2.1 mmol), potassium carbonate (242 mg, 1.75mmol), potassium iodide(291 mg, 1.75 mmol) were added in sequence. The mixture was refluxed overnight and purified on 2mm preparative silica plates using 5% of an NH3•MeOH(7M) solution in CH_3Cl as the solvent. The desired product was obtained as a yellow oil was obtained in a yield of 352 mg (428μmol, 48.8%) and the compound was characterized by Mass Spec and ^{1}H NMR.

N-N'propy-bis(Ethoxyethylether)a mine-MOM-N_2O_2M: N,N'propy-bis(Ethoxy ethylether)amine-MOM-N_2O_2M (403 mg, 490 μmol) was dissolved in 2M HCl/EtOH(6.5 mL) and stirred at room temperature for 2 hours. The HCl/EtOH was then evaporated using rotary evaporator and the product was extracted into $CHCl_3$ from 5% $NaHCO_3$. The $CHCl_3$ was evaporated to yield the desired product that was characterized by Mass Spec and ^{1}H NMR.

Hexadentate-N,N'-3,4-diMeOPyrid yl-MOM-N_2O_2M: MOM-N_2O_2M (540 mg,

1.284 mmol) was dissolved in anhydrous CH_3CN (50mL), 2-Chloromethyl-3,4-dimethoxypridine hydrochloride salt (863.2 mg, 3.852 mmol), potassium carbonate(887.3 mg, 6.42 mmol), potassium iodide(426.4 mg, 2.568 mmol) were added in sequence. The mixture was stirred at 40 ℃ for 24 hours and subsequently purified on 2mm preparative silica plate using 10% of NH3 • MeOH(7M) in CH_3Cl as a solvent. A yellow oil was obtained in a yield of 717.5 mg (993 μmol, 77.3%) and was characterized as the desired compound by Mass Spec and ^{1}H NMR.

N,N'-3,4-diMeOPyridyl-MOM-N_2O_2M: N,N'-3,4-diMeOPyridyl-MOM-N_2O_2M (500 mg, 691.7 μmol) was dissolved in 2M HCl/EtOH (8 mL) and stirred at room temperature for 5 hours. Then HCl/EtOH was then evaporated using rotary evaporator and the product was extracted into $CHCl_3$ from 5% $NaHCO_3$ solution. The and $CHCl_3$ was then concentrated to obtain the desired compound that was characterized by Mass Spec and ^{1}H NMR.

2-methoxymethoxybenzaldehyde: 2-hydroxybenzaldehyde (4.0 g, 32.8 mmol) and DIPEA (8.478 g, 65.6 mmol) were dissolved in CH_2Cl_2 and cooled to -35℃. Methoxymethylchloride (3.961 mg, 49.2 mmol) was then added to the reaction mixture dropwise at -35℃. After stirring at -35 ℃ for 3 hours, the temperature was raised to room temperature and stirred overnight. The product was purified on a silica column using $CHCl_3$ as the eluent. A pale yellow solid was obtained in a yield of 5.325 g (32.1 mmol, 97.9 %) and was characterized by ^{1}H NMR

Tetradentate-MOM-N_2O_2: 2-methoxymethoxybenzaldehyde(4.69 g, 28.3 mmol) was dissolved in dried EtOH (100mL) and ethylenediamine(775.3mg, 12.9 mmol) diluted with EtOH was added drop-wise while stirring at 80℃. After 2 hours reflux, the solution was cooled and $NaBH_4$ was added in portions. The reaction was stirred at room temperature overnight. A few drops of water were added to quench $NaBH_4$ and the ethanol was evaporated using rotary evaporator. The product was extracted in CH_3Cl from saturated a $NaHCO_3$ aqueous solution and purified on 2mm preparative silica plate using a 3 % NH_3-MeOH(7M) in CHCl3. A yellow solid was obtained in a yield of 3.2 g (8.88 mmol, 68.9 %) and was characterized by Mass Spec and ^{1}H NMR.

Hexadentate-bis-Pyridylmethyl-MOM-N_2O_2: MOM-N_2O_2 (500 mg, 1.39 mmol) was dissolved in anhydrous CH_3CN and 2-Chloromethylpridine hydrochloride (569 mg, 3.5 mmol), potassium carbonate(958 mg, 6.9 mmol), potassium iodide(690 mg, 4.2 mmol) were added in sequence. The mixture was stirred at 40℃ overnight and purified on 2mm preparative silica plates using a 4% NH_3-MeOH in CH_3Cl as a solvent. A yellow oil was obtained in a yield of 533 mg (982 μmol, 70.7%) and was characterized by Mass Spec and ^{1}H NMR.

N,N'-Pyridylmethyl-MOM-N_2O_2: bis-Pyridylmethyl-MOM-N_2O_2 (100mg, 185 μmol) was dissolved in 2M HCl/EtOH (2 mL) and stirred at room temperature overnight. A precipitate occurred overnight. The HCl/EtOH was evaporated using rotary evaporator and the product was extracted into $CHCl_3$ from 5% $NaHCO_3$ and $CHCl_3$ was concentrated to give the desired product as a solid which was characterized by Mass Spec and ^{1}H NMR.

$[Ga(diMeOPyrN_4S_2)]^+$: The deprotected of N, N'-(3, 4-diMeOPyridyl)-N_2S_2 (78 mg, 162 μmol) was dissolved in EtOH(10 mL) and solid Ga(acetylacetonate)$_3$ (60 mg, 162 μmol) was added to the solution. The reaction mixture was stirred at room temperature. The completion of the reaction was comfirmed by MS. The product was purified on the silica TLC with 40% NH_4Cl saturated MeOH in $CHCl_3$ and was characterized by Mass Spec displaying the natural gallium isotopic pattern of the desired gallium complex.

$[Ga(diMeOPyrN_4O_2M)]^+$: The deprotected of N,N'-3,4-diMeOPyridyl-N_2O_2M ligand (46 mg, 73 μmol) was suspended in EtOH(10 mL) and solid Ga(acetylacetonate)$_3$ (27 mg, 73 μmol) was added to the solution. The reaction mixture was stirred at room temperature for 4 hours. The product was purified on the HPLC. [Column: 4.6×150mm C8-MS Waters gradient; A: 10mM NH4Cl pH=6.0 buffer B: MeOH B= 20%(0-5min)20-100%(5-25min)100%(25-30 min)] and characterized Mass Spec displaying the natural gallium isotopic pattern of the desired gallium complex.

$[Ga(PyrN_4O_2)]^+$: The deprotected *N,N'-Pyridylmethyl-N_2O_2 ligand* (53.5mg, 118μmol) was suspended in EtOH (15 mL) and solid Ga(acetylacetonate)$_3$ (43.3 mg, 118 μmol) was added to the solution. The reaction mixture was stirred at room temperature overnight. The product was purified on the HPLC. [Column: 4.6×150mm C8-MS Waters gradient; A: 10mM NH4Cl pH=6.0 buffer B: MeOH B= 20%(0-5min)20-100%(5-25min)100%(25-30 min)] to obtain the desired gallium complex that was characterized by Mass Spec displaying the natural gallium isotopic pattern of the desired gallium complex.

４．研究成果 Research Results: We successfully synthesized and characterized hexadentate chelate ligands, based on two separate N_4S_2 and N_4O_2 metal chelating hexadentate scaffolds. After characterized these organic chelating systems with NMR

and Mass spectroscopy, we also synthesized and characterized non-radioactive Ga-complexes, these non-radioactive complexes displayed the required molecular mass and pattern for desired metal complexes. Using the long-lived ^{67}Ga radioisotope, radiolabeling was also successfully optimized for radiolabeling conditions to achieve high radiochemical yield (>90%) in 30 min of radiolabeling time. The radiolabeled complexes co-elute with the previously characterized non-radioactive Ga-complexes, and established the identity of the ^{67}Ga-radiolabeled complexes.

Lipophilicity measurements of the ^{67}Ga-labeled complexes were determined to be log $D_{7.4}$ of -1.44 for the $[Ga(diMeOPyrN_4S_2)]^+$ complex, while $[Ga(PyrN_4O_2)]^+$ displayed a $logD_{7.4}$ of -1.14 and the $[Ga(diMeOPyrN_4O_2M)]^+$ displayed a relatively higher $logD_{7.4}$ of -0.223. Despite the complexes being lipophilic and cation, their lipophilicity was significantly lower then that observed for the clinical MPI SPECT agent ^{99m}Tc-sestaMIBI ($logD_{7.4}$=0.43; literature 0.7).

In-vivo biodistribution studies conducted with these ^{67}Ga-labeled complexes displayed rapid clearance and excreation. The heart/blood ratios over a 2-hour period did

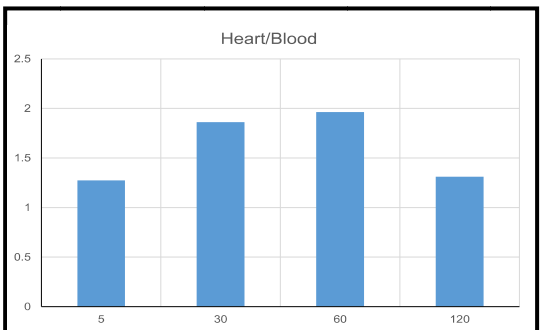

Figure 3. In-vivo heart/blood ratios over time for the $[^{67}Ga(diMeOPyrN_4S_2)]^+$ complex.

not show high uptake and retention in the

Figure 4. In-vivo heart/blood ratios over time for the $[^{67}Ga(diMeOPyrN_4O_2M)]^+$complex.

myocardial tissue, as required for a optimized $^{67/68}$Ga-complex for PET MPI

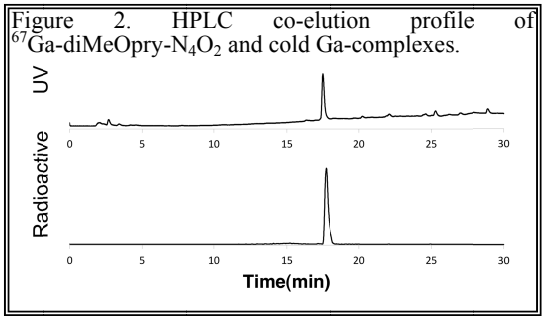

Figure 2. HPLC co-elution profile of ^{67}Ga-diMeOpry-N_4O_2 and cold Ga-complexes.

imaging.

These results suggest that while the core chelate scaffolds provide an adequate platform for the synthesis and radiolabeling of $^{67/68}$Ga complexes, additional modifications maybe necessary to further optimize the complexes for higher lipophilicity and enhanced clearance.

5. 主な発表論文等

〔学会発表〕Conference Presentations:

1. **佐藤優美、Mahmood A、鈴木博元、上原知也、荒野泰.** Diaminodithiol **骨格を基礎とした新規** ^{67}Ga **錯体の設計、作製および評価. 日本薬学会第** 137 **年会. 仙台国際国際センター（宮城県仙台市）** (2017.3.26)

2. Mahmood A, Uehera T, Peng Y, Akgun Z, Limpa-Amara N, Jones AG. Optimizing technetium and rhenium small-molecules for enhanced biological behavior. PACIFCHEM 2015. Honolulu, Hawaii, USA Dec 16th 2015

6. 研究組織

(1)研究代表者

マムード　アッシュファック(Mahmood, Ashfaq)

千葉大学・大学院薬学研究院・特任教授

研究者番号：30754775