# Myocardial Imaging Agents: Synthesis, Characterization and Evaluation of [(Z) and (Z,E)-(1-[$^{82}$Br]Bromo-1-penten-5-yl)]triphenylphosphonium Cations

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## SUMMARY

The phosphonium compounds, [(Z) and (Z/E)-(1-bromo-1-penten-5-yl)]triphenylphosphonium iodides (yield, 65-70%) and the corresponding bromine-82-labeled analogues have been prepared as potential myocardial imaging agents. The 2/1 isomer composition of the Z/E mixture has been confirmed by single crystal X-ray analysis. The radiobrominated (Z)-isomer and (Z/E)-mixture both show high heart uptake and high heart/blood ratios in rats. These data suggest that the bromine-75 and bromine-76-labeled agents could be good candidates for myocardial imaging using positron emission computed tomography.

Key Words: Radiobromine, phosphonium, synthesis, structure, heart uptake

## INTRODUCTION

The design, synthesis and molecular structure, and biological activity relationship studies of various radioiodinated organic cations (**1**, M = P, As, N; R = alkyl or aryl) of phosphorus, arsenic, and nitrogen as potential substitutes for thallium-201 for myocardial imaging have recently been

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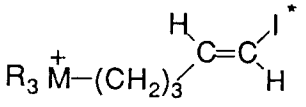
described.[1-3] These agents appear to concentrate in myocytes due to the transmembrane potential gradient[4-7] and show high heart/blood ratios in rats.[1-3] However, our studies with several of these agents in both rats and dogs demonstrated rapid myocardial clearance with excretion through the hepatobiliary route.[1,2]



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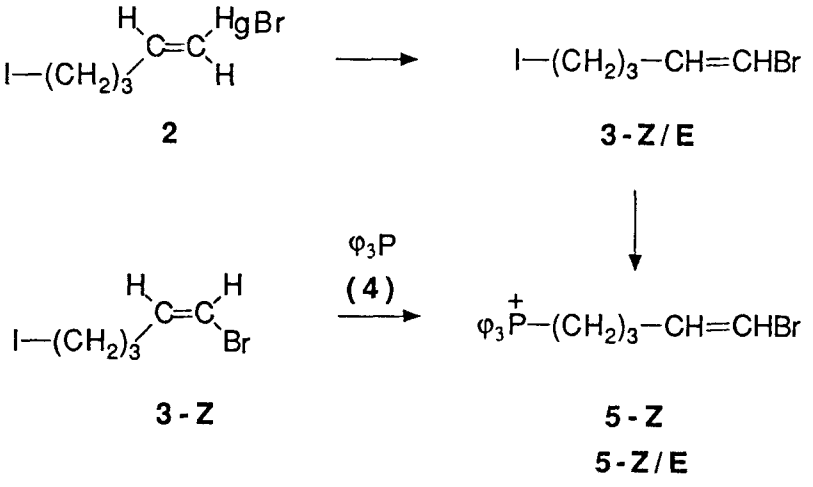
Both the atomic size and electronic properties of bromine are different than iodine and the bromine-carbon bond is more stable than the corresponding iodine-carbon bond. Differences in the *in vivo* uptake, clearance and metabolism of radiobrominated cations in comparison with the analogous radioiodinated cations are thus anticipated. In addition, bromine-75 (half-life = 101 min) and bromine-76 (half-life = 15.9 h) decay by positron emission and are suitable for studies using positron emission tomography (PET).[8] Bromine-82 ($^{82}$Br), although not clinically useful, is commercially available and has a relatively long half-life (35.7 h) both of which make this radioisotope suitable for performing developmental studies as a model for clinically useful bromine radioisotopes. For these reasons, the radiobrominated cations, (1-[$^{82}$Br]bromo-1-penten-5-yl)triphenylphosphonium iodide ([$^{82}$Br-5) as *cis* (Z) and *cis/trans* (Z/E) isomers, were prepared and evaluated in rats.

## RESULTS AND DISCUSSION

### Chemistry

Synthesis. Triphenylphosphine (4) readily reacts with (iodoalkyl) vinyl iodides selectively replacing the primary iodide to form the corresponding (iodovinyl)alkyltriphenylphosphonium iodides.[1,2] Using this strategy, (Z)-1-bromo-5-iodo-1-pentene[9] (**3-Z**) was condensed with **4** to yield [(Z)-1-bromo-1-penten-5-yl]triphenylphosphonium iodide (**5-Z**). Similarly, 1-bromo-5-iodo-1-pentene (3), prepared by bromodemercuration of (E)-(5-iodo-1-penten-1-yl)mercuric bromide[10] (2), was condensed with **4** to provide (Z/E)-1-bromo-1-penten-5-yl)triphenylphosphonium iodide (5-Z/E) (Scheme I).




Scheme I

Structural Studies. Elemental analysis and low resolution $^1$H NMR spectra were consistent with the structural formula of 5. Elemental analyses (C,H,P) were within ±0.4% of the expected values and all proton signals were assignable. The *cis* (Z) and *cis/trans* (Z/E) compositions of 5 were derived from the respective **3-Z**[11] and **3-Z/E**[9] precursors of known structures and from $^1$H NMR which exhibited olefinic protons as unresolved multiplets for 5-Z and 5-Z/E similar to 3-Z and 3-Z/E reported previously.[9,11] The structure of 5-Z/E was unequivocally confirmed by single-crystal x-ray analysis[12]. The structure showed the extended alkyl chain to be disordered between the *cis* (Z) and *trans* (E) conformers with occupancies 2/3 and 1/3, respectively (Figure 1). This static disorder confirmed the presence of a Z/E mixture. Details of the structure and its analysis have been described elsewhere[12].

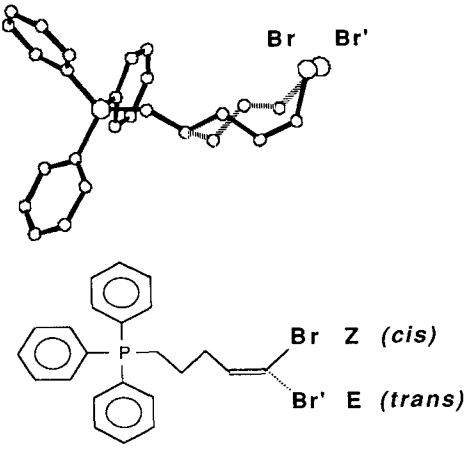



Figure 1. Conformation and structure of Z/E-mixture 5.

**Radiolabeling**

The radiobrominated analogues, [$^{82}$Br]-5-Z and [$^{82}$Br]-5-Z/E, were prepared by coupling radiobrominated 3-Z or 3-Z/E with triphenylphosphine in a manner similar to that described for the corresponding unlabeled standards. The syntheses of radiobrominated 3-Z and 3-Z/E has been reported earlier.[9] [$^{82}$Br]-5-Z (specific activity, 100 mCi/mmol) and [$^{82}$Br]-5-Z/E (specific activity, 128 mCi/mmol) each exhibited one radioactive component on TLC which cochromatographed with the corresponding unlabeled standard.

**Biological Studies**

The [$^{82}$Br]-5-Z and [$^{82}$Br]-5-Z/E radioactive compounds were administered intravenously via a lateral tail vein to female Fischer rats (5/group). The distribution of radioactivity in the heart and other organs was determined at 5 min, 30 min, and 1 h after administration. The biodistribution results are typically expressed as % injected dose/g (% ID/g) and as heart/blood ratios (cpm/g) of the concentration in the heart relative to the blood. For the thyroid gland, results are expressed as % ID (Table 1). For the sake of comparison, the corresponding radioiodinated analogue[2], [(E)-1-[$^{125}$I]-1-penten-5-yl)]triphenylphosphonium iodide ([$^{125}$I]-1-E), was also evaluated in rats and the data provided in Table 1.

Both the *cis* isomer [$^{82}$Br]-5-Z and the *cis/trans* mixture [$^{82}$Br]-5-Z/E, showed high myocardial extraction and high heart/blood ratios. The myocardial concentration of compound 5-Z showed a gradual decrease (5 min, 6.08% ID/g: 1 h, 4.38% ID/g) whereas mixture 5-Z/E showed a gradual increase (5 min, 4.50% ID/g; 1 h 5.75% ID/g). Nevertheless, the myocardial retention values for these radiobrominated agents were higher than the values for the corresponding radioiodinated congeners at all time periods. In addition, 5-Z/E quickly cleared from the liver and showed high heart/blood ratios.

Table 1. Distribution of Radioactivity[a] in Tissues of Female Fischer Rats after Intravenous Administration[c] of Radiolabeled Cations

| Min after injection and agent | Mean % injected dose/g (range) | | | | | | Mean Heart/ Blood |
|---|---|---|---|---|---|---|---|
| | Heart | Blood | Liver | Lungs | Kidneys | Thyroid[d] | |
| **5 min** | | | | | | | |
| [$^{125}$I]-1[b] | 4.40 (3.80-5.74) | 0.29 (0.27-0.33) | 1.48 (1.04-2.56) | 1.72 (1.41-2.17) | 1.48 (1.04-2.56) | 0.36 (0.27-0.48) | 15 |
| [$^{82}$Br]-5-Z | 6.08 (4.82-6.85) | 0.28 (0.27-0.29) | 0.77 (0.59-0.87) | 2.50 (1.64-3.91) | 11.79 (10.42-14.16) | 0.42 (0.33-0.49) | 22 |
| [$^{82}$Br]-5-Z/E | 4.50 (3.53-5.82) | 0.21 (0.14-0.27) | 0.94 (0.57-1.36) | 1.59 (1.29-1.92) | 8.50 (0.39-12.86) | 0.34 (0.27-0.40) | 22 |
| **30 min** | | | | | | | |
| [$^{125}$I]-1[b] | 3.99 (3.17-4.70) | 0.12 (0.10-0.13) | 0.62 (0.55-0.71) | 1.28 (0.91-1.74) | 9.36 (8.28-10.72) | 0.31 (0.25-0.37) | 34 |
| [$^{82}$Br]-5-Z | 5.23 (3.79-6.56) | 0.20 (0.19-0.20) | 0.25 (0.21-0.29) | 1.69 (1.45-1.96) | 8.07 (7.06-8.94) | 0.35 (0.28-0.39) | 26 |
| [$^{82}$Br]-5-Z/E | 5.26 (4.69-5.59) | 0.14 (0.13-0.15) | 0.30 (0.25-0.34) | 1.59 (1.41-1.75) | 8.66 (7.07-10.16) | 0.35 (0.30-0.40) | 38 |
| **60 min** | | | | | | | |
| [$^{125}$I]-1[b] | 4.19 (3.51-5.44) | 0.14 (0.12-0.14) | 0.49 (0.44-0.54) | 1.18 (1.07-1.26) | 6.80 (5.67-8.41) | 0.30 (0.25-0.36) | 32 |
| [$^{82}$Br]-5-Z | 4.38 (3.65-5.37) | 0.20 (0.19-0.22) | 0.18 (0.15-0.22) | 1.46 (1.22-1.80) | 5.57 (5.13-6.50) | 0.32 (0.29-0.36) | 22 |
| [$^{82}$Br]-5-Z/E | 5.48 (4.28-7.55) | 0.14 (0.12-0.16) | 0.17 (0.15-0.18) | 1.33 (1.12-1.59) | 5.76 (4.96-6.79) | 0.28 (0.23-0.38) | 39 |

[a]The percent dose/g data are the mean and range for five rats.

[b]See ref. 2.

[c]Radioactivity dose in $\mu$Ci (of compounds) administered in animals: 4.20 (1), 4.0 (5-Z), 1.0 (5-Z/E).

[d]This data is % ID/thyroid.

## CONCLUSION

These studies have demonstrated the usefulness of a simple method for the preparation of radiobrominated *cis/trans* mixtures of vinyl bromide substituted triphenylphosphonium compounds. The **Z/E** mixture of a model $^{82}$Br-labeled phosphonium compound appears to behave biologically very similar to the corresponding *trans*-vinyl iodide analogue, suggesting that replacement of bromide for iodide does not significantly change the myocardial uptake. We have earlier reported the synthesis of **3-E** (unlabeled)[9] via $Br_2$ treatment of the alane analogue of **2**. However, when this alane was treated with ammonium bromide, the model for ammonium [$^{82}$Br]bromide, with or without an oxidizing agent, pure **3-E** was not formed. Our similar attempts to prepare [$^{82}$Br]-**3-E** as a precursor for [$^{82}$Br]-**5-E** were also futile. Recent reports indicate interest[13] in radiobrominated radiopharmaceuticals. The two-step technique described in this report, involving the initial synthesis of radiobrominated substrate and then coupling, may be applicable only for the preparation of $^{82}$Br compounds to be used as models for $^{75}$Br and $^{76}$Br compounds. However, the direct radiobromination of mercurated substrates could provide compounds labeled with $^{75}$Br or $^{76}$Br for positron emission tomographic (PET) studies.

## EXPERIMENTAL

The melting points (mp) were determined in capillary tubes with a Büchi SP apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed using 250-$\mu$m-thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). Spots on the TLC plates were visualized under short-wave UV light or by exposure to iodine vapor. The $^1$H NMR spectra were obtained at 60 MHz with a Varian 360 A instrument. Samples (30-40 mg) were dissolved in the solvents indicated, and the resonances are reported downfield ($\delta$) from the internal tetramethylsilane standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated by only the symbols, the analytical results for the elements were within ± 0.4% of the theoretical value.

### Materials

All chemicals and solvents were analytical grade and were used without further purification.

The ammonium [$^{82}$Br]bromide was purchased from New England Nuclear, Inc. (North Billerica, MA). The specific activity of $NH_4{}^{82}Br$ was adjusted with carrier $Br_2$ in benzene to a desired specific activity (mCi/mmol) prior to use.

**Animal Tissue Distribution Studies**

The distribution of radioactivity was determined in tissues of 8-12-week-old female Fischer 344 rats (125-170 g) after intravenous administration of $^{82}$Br-labeled **5-Z** and **5-Z/E**. The animals were allowed food and water *ad libitum* prior to and during the course of the experiment. The radiolabeled compounds were dissolved in dimethyl sulfoxide ($Me_2SO$) and diluted with saline to a final concentration of 5% $Me_2SO$. The solution was filtered through a 0.22 $\mu$m Millipore filter and injected via a lateral tail vein into the ether-anesthetized animals. After the times indicated, the animals were sacrificed by cervical fracture, and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. The organs were weighed and counted in a NaI autogamma counter (Packard Instruments). Samples of the injected radioactive solutions were also assayed as standards to calculate the percent injected dose (ID) per gram of tissue values and ID per thyroid gland.

Synthesis of [(Z)-1-bromo-1-penten-5-yl]triphenylphosphonium iodide (**5-Z**) and (Z/E)-1-bromo-1-penten)triphenylphosphonium iodide (**5-Z/E**).

A solution of (Z or Z/E)-1-bromo-5-iodo-1-pentene (308 mg, 1.1 mmol) and triphenylphosphine (510 mg, 2 mmol) in acetone (5 mL) was refluxed for 16 h. The phosphonium product separated from the reaction solution as a crystalline precipitate. The precipitate was collected by filtration, washed with acetone and recrystallized with chloroform and acetone to yield 350-375 mg (65-70%) of the desired phosphonium compound:

**5-Z**: mp 169-170°C, 1H NMR ($CDCl_3$) $\delta$ 7.60-8.20 (m, 15 H, triphenyl), 6.2-6.6 (m, 2H, HC=CHBr), 3.35-4.0 (2H, P-$CH_2$), 1.7 and 2.5 (m and m, 2H and 2H, $CH_2$-$CH_2$). Anal. ($C_{23}H_{23}BrIP$) C, H, P.

**5-Z/E**: mp 145-146°C, 1H NMR ($CDCl_3$) $\delta$ 6.1-6.5 (m, 2H, HC=CHBr) and other protons appeared at positions identical to those for **5-Z**. Anal. ($C_{23}H_{23}BrIP$) C, H, P.

Synthesis of Radiolabeled Compounds. [$^{82}$Br][(Z or Z/E)-1-bromo-1-penten-5-yl] triphenylphosphonium iodide ([$^{82}$Br]-5-Z or [$^{82}$Br]-5-Z/E).

The radiobrominated compound [$^{82}$Br]-3-Z (643 $\mu$Ci, specific activity 100 mCi/mmol) or [$^{82}$Br]-3-Z/E (293 $\mu$Ci, specific activity 128 mCi/mmol) was prepared using a procedure described earlier[9] and refluxed with triphenylphosphine (10 mg) in acetone (1.0 mL) for 3 h. The solvent was evaporated under argon, and the product was purified by silica gel column chromatography. Elution with chloroform removed the unreacted starting material, and further elution with 30% acetone in chloroform (v/v) provided the desired radiobrominated phosphonium compound.

**[$^{82}$Br]-5-Z:** Radiochemical yield 108 $\mu$Ci (17%).

**[$^{82}$Br]-5-Z/E:** Radiochemical yield 55 $\mu$Ci (19%). The radiobrominated compounds were characterized by cochromatography (TLC) (30% acetone in chloroform) with the corresponding unlabeled standards. The low radiochemical yields were due to a relatively short reaction time (3 h) leaving approximately 80% starting material unreacted.

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