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Synthesis of radioiodine-labeled 2-phenylethyl 1-thio-β-D-galactopyranoside for imaging of *LacZ* gene expression

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Abstract

A potent inhibitor of β-galactosidase (EC 3.2.1.23), 2-phenylethyl 1-thio-β-D-galactopyranoside (PETG), was radioiodinated for noninvasive imaging of *LacZ* gene expression. In order to introduce radioiodine to the phenyl ring of PETG, 2-(4-bromophenyl)ethanethiol was prepared and attached to the C-1 position of β-D-galactose pentaacetate under conditions that resulted in the exclusive formation of the β anomer. The bromo group of PETG was converted to the tributylstannyl group where radioiododemetallation was carried out. Radioiodine-labeled PETG tetraacetate was purified by HPLC, which can be used as a prodrug for biological evaluation or hydrolyzed to 2-(4-[^{123}I/^{125}I]iodophenyl)ethyl 1-thio-β-D-galactopyranoside ([^{123}I/^{125}I]**7**) under basic conditions. The resulting radioiodine-labeled PETG was obtained in overall 62% radiochemical yield (decay-corrected) and with specific activity of 46–74 GBq/μmol.

Keywords: Gene expression; β-Galactosidase; 2-Phenylethyl 1-thio-β-D-galactopyranoside; Radioiodine; SPECT

1. Introduction

With the advance of molecular biology, gene expression has been extensively studied using reporter genes. The most commonly used reporter systems include β-galactosidase, β-glucuronidase, chloramphenicol acetyltransferase, and firefly luciferase.[1] Among these, the *lacZ* gene, which encodes *E. coli* β-galactosidase, is the most attractive reporter gene due to ready availability of the gene, transferring vectors and assaying reagents. Since this reporter gene produces β-galactosidase that hydrolyzes lactose to galactose and glucose, *lacZ* gene expression can be investigated utilizing the β-galactosidase substrates that exhibit either fluorescence or luminescence after they are turned over by the enzyme.[2] However, such methods are limited to in vitro experiments that usually require destruction of cell membranes, and application for in vivo monitoring in living animals is not possible. As a more sensitive and noninvasive approach, nuclear medicine technology has been applied to monitoring of reporter gene expression in living systems using specific radiotracers and positron emission tomography (PET) or single photon emission computed tomography (SPECT).[3] These imaging techniques utilize radiolabeled inhibitors of an enzyme for measurement of the enzyme density as well as the radiolabeled substrates for measurement of the enzyme activity. The most intensively studied system is the herpes simplex virus type 1 thymidine kinase (HSV 1-tk) reporter gene system that uses uracil nucleosides and acycloguanosine derivatives, such as 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl-5-[^{123}I]iodouracil (FIAU) and 8-[^{18}F]fluoro-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (FGCV). Cytosine deaminase and dopamine D_2 receptor reporter gene systems have been also investigated using 5-[^{18}F]fluorocytosine and 3-(2′-[^{18}F]fluoroethyl)spiperone (FESP), respectively. Recently *LacZ* gene expression was evaluated using 2′-[^{18}F]fluorodeoxylactose, a β-galactosidase substrate.[4] Enzymatic synthesis of this radiotracer was troublesome due to its long incubation

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time and low yield, and above all, its biodistribution data suggested the lack of penetration of this radiotracer through the cell membranes.

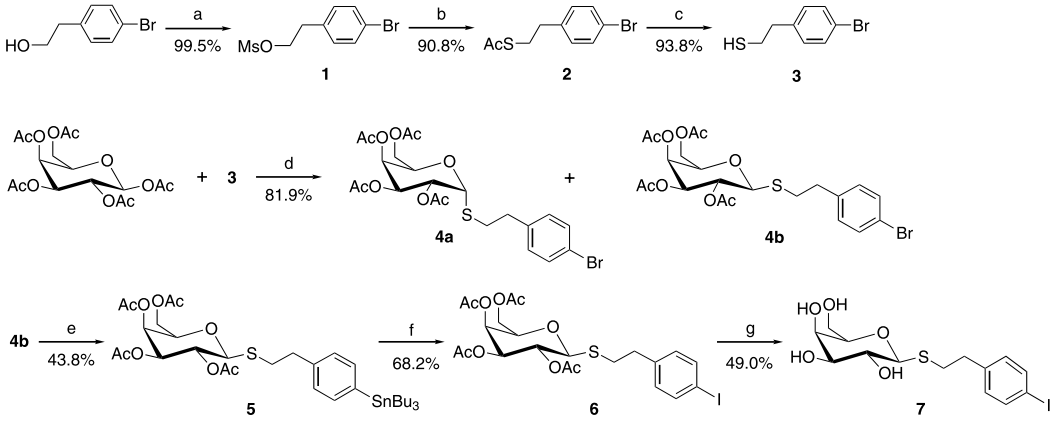
In this study, therefore, PETG, a potent competitive inhibitor of β-galactosidase with K_i ranging from 0.94–2.5 μM,[5–7] was radioiodinated for noninvasive imaging of *LacZ* gene expression.

2. Results and discussion

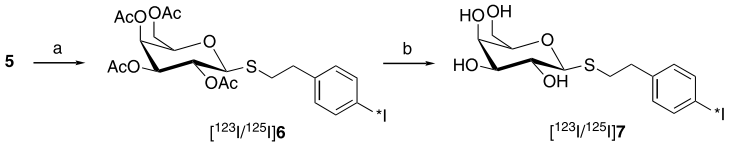
Radioiodine was introduced to the phenylethylthio moiety rather than to the sugar part of PETG, a potent inhibitor of β-galactosidase, in order to minimize perturbation of its binding affinity to the enzyme. In order to carry out radioiododemetallation, a bromo group was introduced at the C-4 position of the phenyl ring, and was further converted to a tributylstannyl group at later step.

2-(4-Bromophenyl)ethanethiol (**3**) was prepared from 4-bromophenethyl alcohol in three steps in high yields. Although the Mitsunobu reaction does not require activation of a hydroxyl group for displacement with a sulfur nucleophile,[8] the desired product **3** can be contaminated with PPh_3 due to their similar polarities. Therefore, the hydroxyl function was activated by conversion to the mesylate **1**, followed by displacement with a suitable sulfur nucleophile. Reduction of the thioester **2** gave the desired thiol **3**.[9,10] The thiol was then introduced into the C-1 position of β-D-galactose pentaacetate, which gave the α and β anomers, in varying proportions depending on the reaction conditions (Scheme 1). The reaction in chloroform at room temperature for 1 h afforded both **4a** and **4b** (α and β anomers in a 33:17 ratio[11]) in a total yield of 73.9%. On the other hand, the β anomer **4b** was solely produced in 81.9% yield when the reaction was carried out in a 3:2 mixture of chloroform and diethyl ether at low temperature for 3 days.[12] Since the PETG has the β configuration, the latter conditions were employed to produce the β anomer (**4b**) stereoselectively. The bromo group on the phenyl ring of **4b** was then substituted with a tributylstannyl group to give **5** that was further converted to an iodo group on **6**. Deprotection of **6** was readily carried out at room temperature under basic conditions to give **7**.

Radioiodination of **5** with Na[^{123}I/^{125}I]I in the presence of Chloramine-T gave [^{123}I/^{125}I]**6** quantitatively (Scheme 2). The reaction produced a polar radioactive compound when an excess amount of Chloramine-T was used. Although the byproduct has not been identified, it may be a compound derived from sulfur oxidation. The tetraacetyl compound [^{123}I/^{125}I]**6** was purified by high performance liquid chromatography (HPLC) (Fig. 1) and could be used as a prodrug for biological studies, since it is expected to cross the cell membranes and then undergo hydrolysis intracellularly to the [^{123}I/^{125}I]**7**. This was the case when 1,3,4,6-tetraacetyl-2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]AFDG) was incubated with the cells, which readily penetrated the cell membranes and was intracellularly converted to [^{18}F]FDG.[13] The identity of [^{123}I/^{125}I]**6** was confirmed by HPLC



Scheme 1. Reagents and conditions: (a) CH_3SO_2Cl, *i*-Pr_2NEt, CH_2Cl_2; (b) Cs_2CO_3, CH_3COSH, DMF; (c) $LiAlH_4$, THF; (d) $BF_3{\cdot}OEt_2$, $CHCl_3$–ether; (e) $Pd(PPh_3)_4$, $(SnBu_3)_2$, toluene; (f) I_2, $CHCl_3$; (g) 1.0 N NaOH–MeOH.



Scheme 2. Reagents and conditions: (a) Na[^{125}I/^{123}I]I, Chloramine-T; (b) 0.1 N NaOH–EtOH.

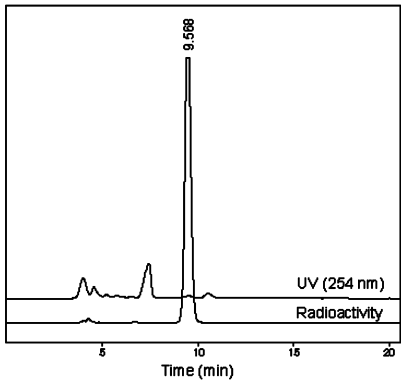


Fig. 1. HPLC profile of 1,3,4,6-tetraacetyl 4-[^{123}I/^{125}I]iodo-PETG. HPLC conditions: 80:19:1 *n*-hexanes–CH_2Cl_2–2-propanol; flow rate, 4.0 mL/min; t_R 9.57 min. The eluant was simultaneously monitored by a UV detector (254 nm) and a NaI(Tl) radioactivity detector.

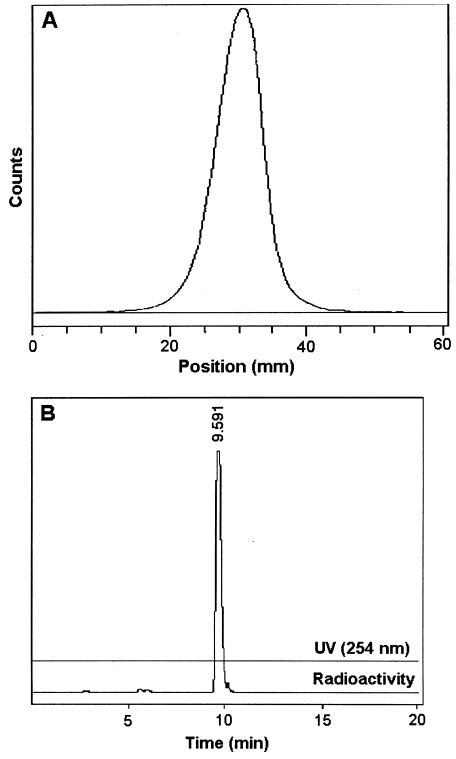


Fig. 2. (A) Radio thin-layer chromatogram of radioiodine-labeled PETG. Developing solvents: 19:1 CH_3CN–H_2O; R_f 0.66; (B) HPLC analysis. HPLC conditions: 7:3 H_2O–CH_3CN; flow rate, 1.0 mL/min; t_R 9.59 min. The eluant was simultaneously monitored by a UV detector (254 nm) and a NaI(Tl) radioactivity detector.

co-injection with unlabeled compound **6**. [^{123}I/^{125}I]**6** was further hydrolyzed to [^{123}I/^{125}I]**7** in 0.1 N NaOH–EtOH at room temperature, and the product was purified by use of a C-18 Sep-Pak® cartridge. The [^{123}I/^{125}I]**7** was identified by co-elution with unlabeled compound **7** on both radio-thin layer chromatography (TLC) and HPLC (Fig. 2(A and B)). Radiochemical yield of the final radiotracer was 62%, with a radiochemical purity $>98.5\%$ and specific activity of 46–74 GBq/μmol.

In conclusion, radioiodine-labeled PETG was synthesized in high yield for noninvasive imaging of *LacZ* gene expression. Its prodrug, [^{123}I/^{125}I]**6**, was also prepared in the course of synthetic route of [^{123}I/^{125}I]**7** to facilitate the penetration through cell membranes.

3. Experimental

3.1. Materials and methods

^{1}H NMR spectra were performed on a JNM-LA 300 spectrometer (JEOL Ltd) at 300 MHz, and chemical shifts (δ) are reported in ppm downfield from Me_4Si as an internal reference. Both EI and FAB mass spectra were obtained on a JMS-700 Mstation (JEOL Ltd) instrument in the positive-ion mode. Ethyl acetate or MeOH was used as the solvent, and 3-nitrobenzyl alcohol satd with NaI served as matrix for the FAB mass spectra. TLC was performed on E. Merck F_{254} silica plates, and the sugar compounds were visualized by UV illumination or staining with 1:9 H_2SO_4–EtOH. Radio-TLC was performed on a Bioscan radio-TLC scanner. HPLC was carried out on a Thermo Separation Products System with a semipreparative column (Alltech Econosil silica gel, 10 μm, 10×250 mm). Analysis of the final radiotracer was performed on reversed-phase HPLC using an analytical column (YMC C18, 5 μm, 4.6×250 mm). The eluant was simultaneously monitored by a UV detector (254 nm) and a NaI(Tl) radioactivity detector. Radioactivity was measured in a dose calibrator.

Reaction solvents were distilled prior to use. All other chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and used without further purification. Na[^{123}I]I was purchased from Korea Cancer Center Hospital (Seoul, Korea) and Na[^{125}I]I from NEN Life Science Products, Inc. (Boston, MA, USA). Flash column chromatography was performed using E. Merck silica gel 60 (230–400 mesh).

3.2. 2-(4-Bromophenyl)ethyl methanesulfonate (1)

To a soln of 4-bromophenethyl alcohol (0.300 g, 1.49 mmol) in CH_2Cl_2 (7.0 mL) was added *N*,*N*′-diisopropylethylamine (0.31 mL, 1.79 mmol) at room temperature (rt) for 30 min. The solution was cooled to -20 °C and stirred at the same temperature for 2 h after methanesulfonyl chloride (0.14 mL, 1.79 mmol) was added dropwise. At the end of the reaction, the solution was poured into a mixture of 5 mL of water and 2 mL of 1 N HCl. The organic layer was separated, and the aq layer was extracted with CH_2Cl_2 (10 mL × 2). The

combined organic extracts were washed with 20 mL of satd $NaHCO_3$ and dried over anhyd $MgSO_4$. Flash column chromatography (3:1 n-C_6H_{14}–EtOAc) afforded 2-(4-bromophenyl)ethyl methanesulfonate (**1**) in 99.5% yield (0.414 g) as a white solid: ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J 8.3 Hz, 2 H), 7.12 (d, J 8.3 Hz, 2 H), 4.39 (t, J 6.7 Hz, 2 H), 3.01 (t, J 6.7 Hz, 2 H), 2.89 (s, 3 H). EIMS: m/z 280 (M^+, ^{81}Br), 278 (M^+, ^{79}Br). HRMS: Calcd for $C_9H_{11}{}^{79}BrO_3S$: 277.9612. Found: 277.9600.

3.3. *S*-[2-(4-Bromophenyl)ethyl]thioacetate (2)

Methanesulfonate **1** (0.700 g, 2.51 mmol) was dissolved in anhyd DMF (25 mL) under N_2, and then Cs_2CO_3 (2.45 g, 7.53 mmol) was added. After stirring the suspension for 30 min at rt, thiolacetic acid (0.54 mL, 7.53 mmol) was added into the suspension. The mixture was then stirred for another 30 min, poured into water, and extracted with EtOAc (15 mL × 3). The combined organic extracts were washed with 20 mL of water and dried over anhyd Na_2SO_4. Flash column chromatography (20:1 n-C_6H_{14}–EtOAc) afforded the *S*-[2-(4-bromophenyl)ethyl]thioacetate (**2**) in 90.8% yield (0.590 g) as a pale-yellow solid with a foul odor: ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J 8.4 Hz, 2 H), 7.09 (d, J 8.4 Hz, 2 H), 3.08 (m, 2 H), 2.82 (m, 2 H), 2.32 (s, 3 H). EIMS: m/z 260 (M^+, ^{81}Br), 258 (M^+, ^{79}Br). HRMS: Calcd for $C_{10}H_{11}{}^{79}BrOS$: 257.9714. Found: 257.9698.

3.4. 2-(4-Bromophenyl)ethanethiol (3)

A soln of **2** (0.500 g, 1.93 mmol) in THF (20 mL) was added dropwise to a suspension of $LiAlH_4$ (0.293 g, 7.72 mmol) in THF (10 mL) at 0 °C under N_2. The mixture was stirred at rt for 30 min, and the excess $LiAlH_4$ was decomposed by the addition of cold water (10 mL), followed by 1 N HCl (20 mL). The reaction mixture was extracted with EtOAc (15 mL × 3), and the combined organic extracts were washed with 20 mL of water and dried over anhyd Na_2SO_4. Flash column chromatography (20:1 n-C_6H_{14}–EtOAc) gave the 2-(4-bromophenyl)ethanethiol (**3**) in 93.8% yield (0.393 g) as a pale-yellow liquid with a foul odor: ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J 8.3 Hz, 2 H), 7.07 (d, J 8.3 Hz, 2 H), 2.87 (m, 2 H), 2.77 (m, 2 H), 1.36 (t, J 7.8 Hz, 1 H). EIMS: m/z 218 (M^+, ^{81}Br), 216 (M^+, ^{79}Br). HRMS: Calcd for $C_8H_9{}^{79}BrS$: 215.9608. Found: 215.9595.

3.5. 2-(4-Bromophenyl)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α,β-D-galactopyranoside (4a and 4b)

3.5.1. Method A (mixture of α and β anomers). To β-D-galactose pentaacetate (0.062 g, 0.16 mmol) in $CHCl_3$ (3 mL) were added thiol **3** (0.030 g, 0.14 mmol), followed by $BF_3{\cdot}OEt_2$ (0.20 mL, 1.6 mmol). Reaction was allowed to proceed at rt in dark room for 1 h. Excess $BF_3{\cdot}OEt_2$ was decomposed by the addition of cold water (10 mL), and the mixture was then extracted with EtOAc (5 mL × 3). The combined organic extracts were dried over anhyd $MgSO_4$. Flash column chromatography (3:1 n-C_6H_{14}–EtOAc) afforded the α anomer (**4a**) in 48.8% yield (0.037 g) and the β anomer (**4b**) in 25.1% yield (0.019 g).

3.5.2. Method B (only β anomer). To β-D-galactose pentaacetate (0.398 g, 1.02 mmol) dissolved in $CHCl_3$ (6 mL) was added Et_2O (4 mL). The mixture was cooled to 0 °C, and to it was added a soln of **3** (0.200 g, 0.92 mmol) in $CHCl_3$ (3 mL), followed by $BF_3{\cdot}OEt_2$ (1.3 mL). The reaction was allowed to proceed at −10 to 0 °C for 3 days. Excess $BF_3{\cdot}OEt_2$ was decomposed by the addition of cold water (20 mL), and the mixture was extracted with EtOAc (15 mL × 3). The combined organic extracts were dried over anhyd $MgSO_4$. Flash column chromatography (3:1 n-C_6H_{14}–EtOAc) afforded the only β anomer (**4b**) in 81.9% yield (0.413 g) as a colorless sticky syrup.

3.5.2.1. Spectral data for 4a. ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J 8.3 Hz, 2 H), 7.06 (d, J 8.3 Hz, 2 H), 5.76 (d, J 5.1 Hz, 1 H, H-1), 5.44 (m, 1 H), 5.27 (dd, J 10.7, 5.4 Hz, 1 H), 5.19 (dd, J 10.7, 3.2 Hz, 1 H), 4.54 (t, J 6.4 Hz, 1 H), 4.10 (m, 2 H), 2.78 (m, 4 H, –SCH_2CH_2–), 2.15, 2.06, 1.99, 1.98 (4s, 12 H, –$COCH_3$). FABMS: m/z 571 ($M^+ + Na$, ^{81}Br), 569 ($M^+ + Na$, ^{79}Br). HRMS: Calcd for $C_{22}H_{27}{}^{79}BrO_9SNa$: 569.0457. Found: 569.0447.

3.5.2.2. Spectral data for 4b. ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J 8.3 Hz, 2 H), 7.09 (d, J 8.3 Hz, 2 H), 5.44 (d, J 3.2 Hz, 1 H), 5.25 (t, J 9.9 Hz, 1 H), 5.04 (dd, J 9.9, 3.2 Hz, 1 H), 4.47 (d, J 9.8 Hz, 1 H, H-1), 4.14 (m, 2 H), 3.92 (m, 1 H), 2.99 (m, 2 H), 2.88 (m, 2 H), 2.15, 2.05, 2.03, 1.99 (4s, 12 H, –$COCH_3$). FABMS: m/z 571 ($M^+ + Na$, ^{81}Br), 569 ($M^+ + Na$, ^{79}Br). HRMS: Calcd for $C_{22}H_{27}{}^{79}BrO_9SNa$: 569.0457. Found: 569.0461.

3.6. 2-(4-Tributylstannylphenyl)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (5)

To a stirred soln of **4b** (0.122 g, 0.22 mmol) in $C_6H_5CH_3$ (4 mL) were added sequentially tetrakis(triphenylphosphine)palladium(0) (0.025 g, 0.022 mmol) and hexabutylditin (0.56 mL, 1.1 mmol). The reaction mixture was stirred at 120 °C for 17 h under N_2 and then filtered through a Celite column. The resulting solution was concentrated in vacuo and purified by flash column chromatography (3:1 n-C_6H_{14}–EtOAc) to give the tributylstannyl compound **5** in 43.8% yield (0.073 g) as a colorless sticky syrup: ^{1}H NMR ($CDCl_3$): δ 7.39 (d, J 4.8 Hz, 2 H), 7.17 (d, J 4.8

Hz, 2 H), 5.44 (m, 1 H), 5.26 (t, *J* 6.0 Hz, 1 H), 5.04 (dd, *J* 2.1, 6.0 Hz, 1 H), 4.48 (d, *J* 6.0 Hz, 1 H, H-1), 4.14 (m, 2 H), 3.92 (m, 1 H), 2.91 (m, 4 H), 2.16, 2.06, 2.03, 1.99 (4s, 12 H, –$COCH_3$), 1.54 (m, 6 H), 1.33 (m, 6 H), 1.04 (m, 6 H), 0.90 (m, 9 H). FABMS: *m*/*z* 781 ($M^+ + Na$). HRMS: Calcd for $C_{34}H_{54}NaO_9SSn$: 781.2408. Found: 781.2370.

3.7. 2-(4-Iodophenyl)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (6)

To a soln of **5** (0.027 g, 0.036 mmol) in $CHCl_3$ (3 mL) was added dropwise a soln of iodine in $CHCl_3$ (0.1 M) until iodine color persisted. The solution was stirred for 15 h at rt. Excess iodine was quenched by a soln of KF in MeOH (1 M, 0.1 mL), and the mixture was stirred with 5% aq sodium bisulfite soln (0.3 mL) for 5 min. The solution was then extracted with EtOAc (5 mL × 3), and the combined organic extracts were then dried over anhyd $MgSO_4$. Flash column chromatography (2:1 *n*-C_6H_{14}–EtOAc) afforded the iodo compound **6** in 68.2% yield (0.015 g) as a white solid: ^1H NMR ($CDCl_3$): δ 7.62 (d, *J* 8.3 Hz, 2 H), 6.96 (d, *J* 8.3 Hz, 2 H), 5.44 (m, 1 H), 5.25 (t, *J* 9.8 Hz, 1 H), 5.04 (dd, *J* 3.4, 9.9 Hz, 1 H), 4.46 (d, *J* 9.8 Hz, 1 H, H-1), 4.14 (m, 2 H), 3.92 (m, 1 H), 2.92 (m, 4 H), 2.15, 2.05, 2.03, 1.99 (4s, 12 H, –$COCH_3$). FABMS: *m*/*z* 617 ($M^+ + Na$). HRMS: Calcd for $C_{22}H_{27}INaO_9S$: 617.0318. Found: 617.0297.

3.8. 2-(4-Iodophenyl)ethyl 1-thio-β-D-galactopyranoside (7)

To a soln of **6** (0.015 g, 0.025 mmol) in MeOH (2 mL) was added 1.0 N aq NaOH (1.0 mL) at 0 °C. After stirring the reaction mixture for 30 min at rt, the solution was poured into water (5 mL) and extracted with EtOAc (10 mL × 3). The combined organic extracts were dried over anhyd $MgSO_4$. Flash column chromatography (10:1 CH_2Cl_2–MeOH) afforded the 2-(4-iodophenyl)ethyl 1-thio-β-D-galactopyranoside (**7**) in 49% yield (0.0052 g) as a white solid: ^1H NMR ($CDCl_3$): δ 7.61 (d, *J* 8.3 Hz, 2 H), 6.98 (d, *J* 8.3 Hz, 2 H), 4.31 (d, *J* 9.3 Hz, 1 H, H-1), 3.97 (m, 1 H), 3.79 (m, 2 H), 3.61 (t, *J* 9.3 Hz, 1 H), 3.51 (m, 2 H), 2.93 (m, 4 H). FABMS: *m*/*z* 449 ($M^+ + Na$). HRMS: Calcd for $C_{14}H_{19}INaO_5S$: 448.9896. Found: 448.9884.

3.9. 2-(4-[^{123}I/^{125}I]Iodophenyl)ethyl 1-thio-β-D-galactopyranoside ([^{123}I/^{125}I]7)

An appropriate dose of Na[^{123}I]I (259 MBq) or Na[^{125}I]I (74 MBq) in 0.01 N NaOH was neutralized with 0.05 N H_3PO_4 and then added to an ethanolic soln (200 μL) of the tributylstannyl precursor **5** (1.1 mg, 1.45 μmol). A freshly prepared soln of Chloramine-T (0.5 mg, 2.19 μmol) in water (50 μL) was added to the reaction mixture that was then stirred at rt. The iodination was terminated after 20 min by an addition of saline (2 mL), and the radioactive compounds were extracted with EtOAc (1.5 mL × 3). The organic layer was passed through a Pasteur pipet containing Na_2SO_4, and the solvent was removed under a gentle stream of N_2. The residue was redissolved in 1 mL of HPLC solvents and purified by HPLC using 80:19:1 *n*-C_6H_{14}–CH_2Cl_2–2-propanol at a flow rate of 4.0 mL/min. The product [^{123}I/^{125}I]**6** was eluted at 9.57 min. The solvents were removed, and a portion was taken out for biological studies. The other portion was redissolved in EtOH (200 μL) and diluted with 0.1 N aq NaOH (100 μL). The reaction mixture was stirred for 10 min, and excess NaOH was neutralized by the addition of 0.1 N aq HCl (80 μL). The solution was then diluted with 10 mL of water and passed through a C-18 Sep-Pak® cartridge. The cartridge was washed with water (10 mL), followed by EtOH (1.5 mL) that eluted the product. The solvent was removed and the residue was redissolved in saline. [^{123}I/^{125}I]**7** (144.3 or 41 MBq) was obtained in overall 62% radiochemical yield (decay-corrected) and a radiochemical purity > 98.5%. An aliquot was analyzed by reversed-phase HPLC using 7:3 water–MeCN at a flow rate of 1.0 mL/min, and specific activity of [^{123}I/^{125}I]**7** was determined by using a standard curve obtained from **7** with different concentrations injected on HPLC versus UV absorbance at 254 nm. Another portion of [^{123}I/^{125}I]**7** was co-injected with unlabeled compound **7** on HPLC to confirm its identity.

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