5-*O*-(4-[^{125}I]Iodobenzyl)-L-ascorbic Acid: Electrophilic Radioiodination and Biodistribution in Mice

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As a part of our efforts to develop potential imaging agents for ascorbate bioactivity, 5-*O*-(4-[^{125}I] iodobenzyl)-L-ascorbic acid ([^{125}I]1) was prepared through a two-step sequence which involved radioiododestannylation of a protected tributylstannyl precursor 6, followed by hydrolysis in acidic methanol of the protecting groups in 61% overall radiochemical yield, with a radiochemical purity of over 98% and a specific activity of more than 15.4 GBq/μmol. Tissue distribution of [^{125}I]1 in tumor-bearing mice showed signs of distribution profiles similar to the reported results for 6-deoxy-6-[^{18}F]fluoro-L-ascorbic (6-^{18}FAsA) acid and 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA) but with notable differences in the adrenal glands, in which considerably lower uptake of radioactivity and rapid clearance with time were observed. Pretreatment of mice with a known inhibitor of ascorbate transport, sulfinpyrazone, did not produce any significant change in the adrenal uptake of radioactivity after injection of [^{125}I]1 compared to the control, suggesting that uptake in the adrenal glands is independent of the sodium-dependent vitamin C transporter 2 transport mechanism. Introduction of a bulky substituent at C-5 on AsA, such as an iodobenzyloxy group, may not be suitable for the design of analogs that may still be able to maintain characteristic distribution properties *in vivo* seen with AsA itself.

Key words ascorbic acid analog; radioiodine; iododestannylation; biodistribution; mouse; imaging agent

L-Ascorbic acid (AsA), the reduced form of vitamin C, is highly concentrated in the neurons in the brain in mammalian bodies, which likely indicates its essential roles in neuronal function and protection against oxidative stress.[1,2] Currently, the numerous functions of AsA in the brain have stimulated multidisciplinary interest in this molecule.[3,4] In recent years, two transport mechanisms by which AsA enters the central nervous system (CNS) from plasma have been identified and characterized.[5] One is that AsA enters the cerebrospinal fluid (CSF) directly through the choroid plexus *via* the sodium-dependent vitamin C transporter 2, SVCT-2, the likely major pathway to brain cells. The other route of entry into the CNS involves the uptake of dehydroascorbic acid (DHA), the oxidized form of AsA, on glucose transporters of the GLUT family in the blood-brain barrier endothelium, followed by rapid intracellular reduction of transported DHA to AsA. However, it is argued that this DHA transport is unlikely to play a major role in AsA supply to the brain due to only minimal quantities of DHA in the plasma and competition with glucose for GLUT transport.[5,6] Literature reports have also shown that AsA can enter neurons through SVCT-2,[1,7] and that there is a regional difference in the concentration of AsA within the brain, being potentially reflective of the level of SVCT-2 distribution.[4,8,9]

We are interested in exploring radiotracer probes of AsA analogs for the visualization of biochemical events associated with the functions and transport of AsA in the brain, utilizing a nuclear imaging technique. In previous studies, we reported the synthesis of several AsA analogs, focusing on the introduction of radioisotopes such as ^{18}F and ^{131}I at the C-6 position of the AsA core, and their potential imaging characteristics were also evaluated in rodents. Among them, 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid (6-^{18}FAsA)[10—13] and 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA)[14,15] showed the expected distribution of highest uptake in the adrenal glands in rodents, a neuroendocrine organ that is known to highly express the SVCT-2 transporter,[16] thus demonstrating their suitability as radiotracer analogs of AsA. However, the application of these radiotracers as brain-targeted imaging probes for *in vivo* studies was found to be limited because of their poor delivery from blood circulation into the brain.

In an attempt to improve the brain targeting of a radiolabeled AsA analog, we have become interested in the use of the oxidized form of AsA, in view of the uptake behavior of the pro-drug type of DHA *in vivo*, and have designed 5-*O*-(4-iodobenzyl)-L-ascorbic acid (**1**) as a potential probe molecule. This 5-*O*-substituted AsA analog can be expected to have the hydrated bicyclic hemiketal structure like AsA in aqueous solution (Chart 1), which appears to be a molecular species capable of interacting with the GLUTs.[17,18] Recently, we developed a synthetic route for the preparation of 5-*O*-(4-iodobenzyl)-L-ascorbic acid through a multi-step sequence starting from AsA, such that it would be amenable to preparation of the corresponding labeled analog, and this compound

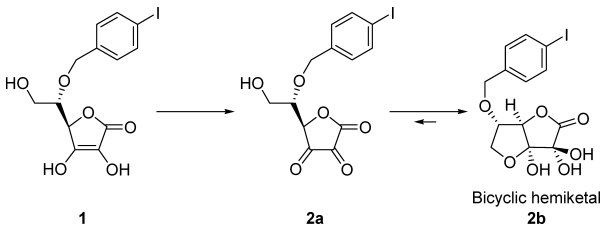

Chart 1. 5-*O*-(4-Iodobenzyl)-L-ascorbic Acid (**1**) and Predicted Conversion to Its Oxidized Form (**2**)

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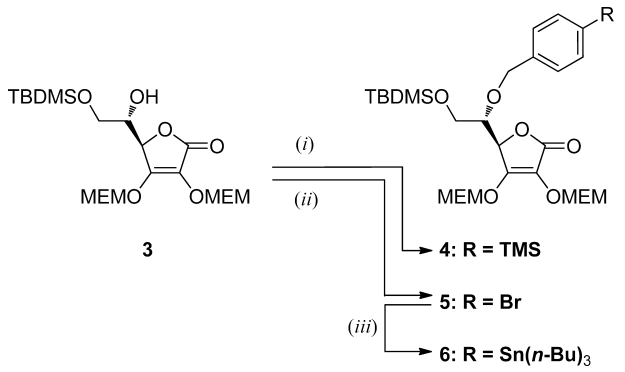
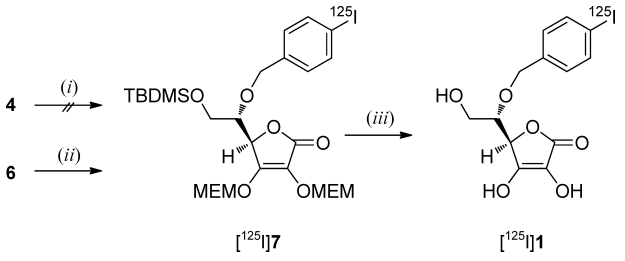


Reagents and conditions: (i) 4-trimethylsilylbenzyl bromide, Ag_2O, $CaSO_4$, benzene, r.t. (ii) 4-bromobenzyl bromide, Ag_2O, $CaSO_4$, r.t. (iii) $Pd(PPh_3)_4$, $Sn_2(n\text{-}Bu)_6$, toluene, reflux.

Chart 2. Synthesis of Labeling Precursors



Reagents and conditions: (i) Na[^{125}I]I, *N*-chlorosuccinimide, AcOH or AcOH–CF_3COOH (ii) Na[^{125}I]I, chloramine-T, AcOH–EtOH, r.t., 2 h (iii) erythorbic acid, 1.0 M HCl–MeOH, 60°C, 30 min.

Chart 3. Radiosynthesis of 5-*O*-(4-[^{125}I]Iodobenzyl)-L-ascorbic Acid ([^{125}I]**1**)

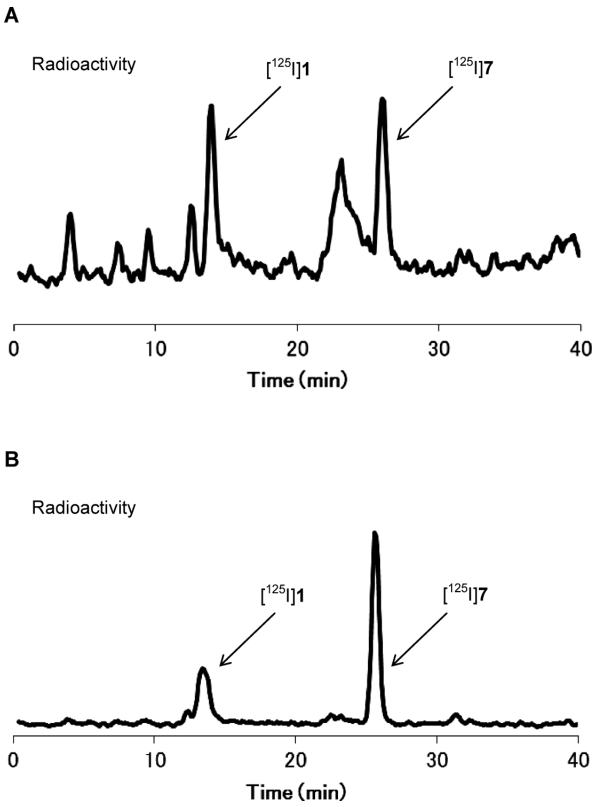


Fig. 1. (A) Analytical HPLC Chromatogram of Deprotection Reaction Mixture of Intermediate [^{125}I]**7** with 1.0 M HCl–MeOH at 50°C for 30 min, Showing Radioactive Profile, and (B) Under the Same Reaction Conditions in the Presence of Erythorbic Acid, Showing Radioactive Profile

HPLC analytical conditions: COSMOSIL 5C18AR-II, 15 mM phosphate buffer (pH=6.0)/MeOH=60/40, flow rate=0.8 mL/min.

was found to have almost the same reducing activity as AsA itself.[19] We report here the radiosynthesis of 5-*O*-(4-[^{125}I] iodobenzyl)-L-ascorbic acid ([^{125}I]**1**) *via* a two-step procedure and its biodistribution in tumor-bearing mice, in order to obtain reference information for the subsequent development of its oxidized form.

Results and Discussion

Two organometallic compounds (**4**, **6**) were prepared as precursors for electrophilic radioiodination,[20] as depicted in Chart 2. Trimethylsilylated precursor **4** was synthesized by 4-trimethylsilylbenzylation of the 2,3,6-tri-*O*-modified derivative of AsA (**3**) and tributylstannane **6** was prepared by heating to reflux a mixture of 5-*O*-bromobenzylated compound **5** with an excess of hexabutylditin, and a catalytic amount of $Pd(PPh_3)_4$ in dry toluene for 5 h. The first attempt to prepare [^{125}I]**7** by radioiodo-desilylation of **4** with a mixture of no-carrier-added Na[^{125}I]I and *N*-chlorosuccinimide in AcOH, or additionally containing a small amount of trifluoroacetic acid at various temperatures proved to be inefficient, and the silicon precursor showed significant decomposition, leading to no formation of the desired compound (Chart 3). On the other hand, treatment of tributylstannyl precursor **6** with no-carrier-added Na[^{125}I]I in the presence of chloramine-T in an AcOH–EtOH solution at room temperature for 2 h yielded a radiochemically and chemically pure product of [^{125}I]**7** after HPLC purification, with a radiochemical yield of 77.5±8.3%.

In this study, deprotection of intermediate [^{125}I]**7** was found to be a critical step. During the initial phases of studying the acid hydrolysis reaction to remove all the protecting groups, this step yielded complicated reaction mixtures, giving an unacceptable low yield of the desired product, as illustrated by a typical HPLC profile in Fig. 1A (reaction conditions: 1.0 M HCl–MeOH, 50°C, 30 min). It was assumed that the formation of byproducts, probably resulting from oxidative damage of the desired product, would be induced by exposure to both the acidic medium and elevated temperatures. We considered, therefore, incorporation of anti-oxidant stabilizers into the reaction mixture. Thus, the presence of erythorbic acid (10 μg), a stereoisomer of AsA and a well-known antioxidant food additive,[21] in a reaction mixture was found to be effective in minimizing the formation of byproducts occurring during the reaction, as analyzed by HPLC (Fig. 1B). Based on these preliminary observations, the deprotection reactions of intermediate [^{125}I]**7** in the presence of erythorbic acid were carried out under some different conditions, giving good conversion to [^{125}I]**1** on heating with 1.0 M HCl–MeOH at 60°C for 30 min as shown in Table 1. Thus, the preparation of [^{125}I]**1** from intermediate [^{125}I]**7** and isolation by HPLC (COSMOSIL 5C18AR-II, 15 mM phosphate buffer (pH=6.0)/MeOH=60/40) was repeated. However, considerable decomposition of the final product was observed in the HPLC isolation step, demonstrating an inherent instability of the radiotracer. In an additional attempt to overcome this unwanted outcome and, also, to find a suitable means for retaining high radiochemical purity for some period of storage, we prepared an HPLC solvent system

Table 1. Deprotection Reaction of Intermediate [^{125}I]**7** by Acid Hydrolysis in an H_2O–MeOH Mixture Containing Erythorbic Acid

Entry	Temperature (°C)	Solvent	Time (min)	Yield[a] (%)
1	r.t.	HCl (0.1 M)	30	No reaction
2	50	HCl (0.1 M)	30	22.4
3	60	HCl (0.1 M)	30	68.0
4	60	HCl (1.0 M)	30	78.7

a) Isolated radiochemical yield by HPLC (COSMOSIL 5C18AR-II, 15 mM phosphate buffer (pH=6.0)/MeOH=60/40, flow rate=0.8 mL/min).

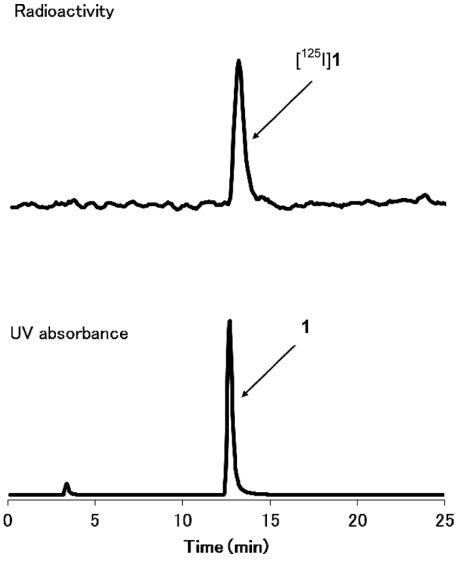


Fig. 2. An HPLC Analysis of 5-*O*-(4-[^{125}I]Iodobenzyl)-L-ascorbic Acid ([^{125}I]**1**), Coinjected with Authentic Sample, after Purification by HPLC

15 mM phosphate buffer (pH=6.0)–MeOH=60 : 40 containing 400 μM DL-homocysteine, flow rate=0.8 mL/min.

of 15 mM phosphate buffer (pH=6.0)–MeOH=60 : 40 containing 400 μM DL-homocysteine, a sulfur–containing amino acid, as an antioxidant stabilizer, based on literature reports of various HPLC analytical techniques for AsA analysis.[21,22] A consequence was that a buffer solution of [^{125}I]**1,** obtained after HPLC isolation using this solvent system, had a reproducible radiochemical purity of above 98% (Fig. 2), and its radiochemical purity was maintained at 92% up to 3 h after storage at 4°C, but dropped to about 70% in 12 h.

Thus, by the two-step procedure described above, the target [^{125}I]**1**, free of erythorbic acid contaminants, was successfully isolated in 61% overall radiochemical yield, ready for intravenous injection into mice with specific activity of over 15 GBq/μmol. The total preparation time, including two HPLC purifications and isolation procedures, was about 4 h.

To explore the *in vivo* tissue distribution characteristics of [^{125}I]**1**, biodistribution studies were performed using C3H/He mice bearing fibrosarcoma, and the results are expressed as % of injected dose (ID)/g of tissue, as shown in Table 2. There was a somewhat slower clearance of the radioactivity from the blood when compared to 6-^{18}FAsA[10—13] and 6-^{131}IAsA[14,15] studied previously. The accumulation of radioactivity in selected organs was highest in the kidneys>liver>lungs>adrenals>heart at 2 min postinjection and then the radioactivity levels gradually decreased with time, similar to those seen for 6-^{18}FAsA and 6-^{131}IAsA. As expected, the brain uptake was limited, ranging from 0.62±0.08 ID%/g at 2 min postinjection to 0.22±09 ID%/g at 60 min, suggesting the inability of [^{125}I]**1** to reach the brain. Moreover, tumor accumulation in the fibrosarcoma was not significant, also similar to those of 6-^{18}FAsA and 6-^{131}IAsA. Recent studies have shown that high-grade tumor tissue has reduced capacity to accumulate AsA relative to normal tissue.[23] On the other hand, contrary to our expectations, the maximum adrenal uptake of radioactivity (12.52±2.52 ID%/g) was seen at 2 min postinjection, which was 3.9-fold lower than that observed for 6-^{131}IAsA in mice at the same time point,[14] and then it rapidly decreased to 2.20±1.44 ID%/g at 30 min after injection with an adrenal-to-liver ratio of only 0.4 for [^{125}I]**1** *versus* 4.3 for 6-^{131}IAsA at 10 min postinjection. Thus, mouse biodistribution studies showed very low accessibility of [^{125}I]**1** to the adrenal glands, different from ^{14}C-AsA,[24] 6-^{18}FAsA and 6-^{131}IAsA, all of which showed the preferential uptake of radioactivity in the adrenal glands. Attempts to identify accumulated radioactivity in the adrenals and blood of mice failed to show optimal conditions for analysis: TLC analysis was considerably complex, being accompanied by the formation of many radioactive species probably produced in the attempted tissue homogenization and/or extraction steps, and chromatographic separation due to the labile nature of compound.

DL-Homocysteine, a sulfur amino acid, is an intermediate metabolite of methionine. An increased homocysteine level in plasma is a risk factor for several chronic pathologies, including cardiovascular diseases, atherosclerosis and chronic renal failure.[25] The amount of DL-homocysteine administered for the distribution studies was estimated to be 0.04 μmol/mouse. In separate experiments, mice (n=3) bearing fibrosarcoma were injected with a buffer solution of [^{125}I]**1** in the absence of DL-homocysteine, although its radiochemical purity was not determined (probably less than 70% based on our experience), and its biodistribution at 10 min postinjection was examined for comparison with that (n=3) using an injectable solution containing DL-homocysteine. The accumulation pattern of radioactivity in the tissues was similar between the two groups (data not shown), seemingly indicating that homocysteine was without significant influence on the mouse distribution profile of [^{125}I]**1**, although under limited experimental conditions.

Sulfinpyrazone is a uricosuric medication used to treat gout and has also been used as a blocker of ascorbate transport in some cell types *in vitro*.[26,27] We previously reported that 6-^{131}IAsA uptake by the adrenal glands of rats was significantly inhibited by pretreatment with sulfinpyrazone (1.5 μg/g per animal) *in vivo*.[15] In this study a similar experiment was carried out to see the response to sulfinpyrazone-treatment for [^{125}I]**1**. As shown in Fig. 3, predosing of mice with sulfinpyrazone (1.5 μg/g per animal) did not produce any change of uptake in the adrenal glands compared to the control group. This is a strong indication that the distribution of [^{125}I]**1** to the adrenal glands is not SVCT-mediated (the SVCT-2 subtype that is the main isoform in adrenal glands), although we do

Table 2. Biodistribution of Radioactivity in C3H/He Mice Bearing Fibrosarcoma at Various Time Points after i.v. Injection of 5-*O*-(4-[^{125}I] Iodobenzyl)-L-ascorbic Acid ([^{125}I]**1**)

Tissue	Tissue concentrations of radioactivity (% injected dose/g tissue)			
	2 min	10 min	30 min	60 min
Blood	15.05±0.41	8.15±1.00	4.30±0.30	3.30±0.72
Adrenals	12.52±2.52	7.49±2.25	2.20±1.44	1.83±0.73
Spleen	4.51±0.43	2.16±0.14	0.81±0.19	0.70±0.15
Pancreas	4.02±0.29	1.89±0.21	0.82±0.24	0.85±0.49
Stomach	3.04±0.13	2.24±0.54	0.82±0.24	0.91±0.38
Stomach content	0.07±0.05	0.07±0.02	0.14±0.09	0.87±1.46
Small intestine	4.13±0.65	4.72±1.13	3.93±1.64	6.82±3.60
Small intestine content	2.18±0.83	8.50±2.56	8.53±3.61	13.93±3.32
Large intestine	3.38±0.53	3.25±2.72	1.59±0.56	2.10±0.68
Large intestine content	0.57±0.26	0.38±0.12	7.53±8.11	2.66±2.89
Kidneys	61.30±20.21	19.69±4.66	9.38±6.29	5.28±4.88
Liver	28.97±3.84	12.70±3.14	3.34±1.55	2.38±0.79
Heart	8.17±0.57	3.76±0.34	1.32±0.36	1.16±0.25
Lungs	15.56±4.31	5.40±0.51	2.33±1.04	2.29±0.49
Muscle	1.99±0.21	1.34±0.24	0.52±0.11	0.53±0.38
Skin	3.07±0.51	1.87±0.34	1.09±0.24	1.13±0.96
Thyroid	5.33±0.74	2.40±0.76	1.00±0.33	2.41±1.12
Brain	0.62±0.08	0.52±0.11	0.34±0.02	0.22±0.09
Bone	2.92±0.51	1.43±0.24	0.54±0.29	0.71±0.38
Ovaries	4.23±0.74	2.32±1.06	1.90±0.41	1.30±0.57
Tumor	1.69±0.26	1.63±0.20	0.91±0.06	0.59±0.13

Results expressed as mean %ID/g tissue±S.D., n=3 per data point.

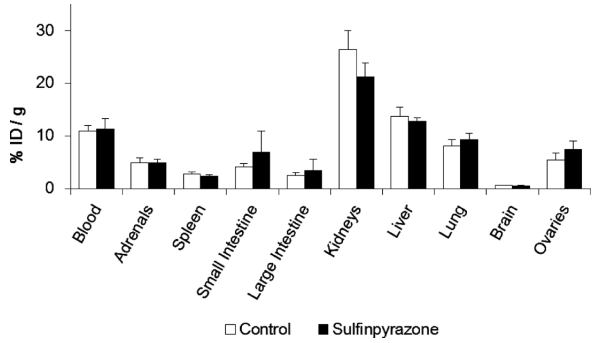


Fig. 3. Radioactivity Levels in Selected Mice Tissues in Control and Sulfinpyrazone Pre-treatment Conditions, 2 min Following i.v. Injection of 5-*O*-(4-[^{125}I]Iodobenzyl)-L-ascorbic Acid ([^{125}I]**1**)

Results expressed as mean %ID/g tissue±S.D., n=3—4 for each group.

not have information on the levels of the SVCT transporters in our tissue sample.

There are some literature reports on the substrate specificity of the AsA transporter using biological tissues or cells.[28—32] As most AsA exists as a monovalent anion at physiological pH and SVCT has a high affinity for the ionic form, it has been suggested that the ionic interactions between SVCT and its substrates are the predominant driving forces for the binding of SVCT.[31,32] Studies by Rumsey *et al.*[33] demonstrated that one of the structural requisites of AsA and its analogs for transport in cells is an S-absolute configuration at the C-4 position in a five-membered reduced ring with no substitution on the C-2 and C-3 positions, and that 6-deoxy-6-halogeno-L-ascorbic acid is an effective compound for inhibiting AsA uptake. For the case of [^{125}I]**1**, it is conceivable that the bulky hydrophobic iodobenzyl substituent at the C-5 position of AsA may not be able to interact with the transporter, probably due to its steric hindrance within the site.

Conclusion

We successfully prepared 5-*O*-(4-[^{125}I]iodobenzyl)-L-ascorbic acid ([^{125}I]**1**) with reasonable radiochemical yields, high radiochemical purity and specific activity. Biodistribution studies in mice showed low uptake of radioactivity for [^{125}I]**1** in the adrenal glands, followed by rapid elimination of radioactivity with time. The insensitivity of a blocking effect by pretreatment with sulfinpyrazone is indicative of no or only poor affinity of this radiotracer for the SVCT-2 transporter, although further experiments are needed to confirm this point. Such biological properties of [^{125}I]**1** might be problematic for *in vivo* application of its oxidized form as a prodrug-type radiotracer of AsA, because, unlike AsA itself, this analog may be not able to enter neurons in the brain through SVCT2 from cerebrospinal fluid. Nevertheless, our proposed strategy is now a starting point for the development of radiolabeled AsA analogs targeting the central nervous system, and further research is going to prepare the oxidized form of [^{125}I]**1** to evaluate its biodistribution.

Experimental

Chemical reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. Benzene and toluene were purified by drying over CaH_2 and distillation. ^{1}H-NMR spectra were obtained on a Varian Inova 400 (400 MHz) and were referenced to tetramethylsilane (δ=0 ppm). IR spectra were recorded with a Shimadzu FTIR-8400 spectrometer. Mass spectra were obtained with a JEOL JMS DX-610 (FAB-MS) or an Applied Biosystems Mariner

System 5299 spectrometer (electrospray ionization (ESI)-MS)). Column chromatography was performed on Silica gel 60N (63—210 mesh, Kanto Chemical Co., Inc., Japan), the progress of the reaction was monitored by TLC on Silica gel 60F 254 plates (Merck, Germany), and the spots were visualized with UV light or by spraying with 5% alcoholic molybdophosphoric acid. In the synthetic procedures, the organic extracts were routinely dried over anhydrous Na_2SO_4 and evaporated with a rotary evaporator under reduced pressure. HPLC was done by using a Liquid Chromatograph system (GL-7410/GL-7450, GL Science, Japan) coupled in series with an NaI(Tl) detector (B-FC-3200, Bioscan Inc., Washington, DC, U.S.A.) by monitoring the radioactivity as well as the UV absorption (at 254 nm). All solvents used as mobile phases in HPLC procedures were bubbled with nitrogen gas before use. The radioactivity was quantified with an auto-well gamma counter (ARC-370, Aloka, Japan). Identity of the labeled compound was confirmed from co-injection with authentic samples by HPLC under the same conditions. Specific radioactivity and radiochemical purity were determined by the same HPLC system. No-carrier-added sodium [^{125}I]iodide (0.01 N NaOH solution; >14.8 GBq/mL) was purchased from MP Biomedicals (U.S.A.). Animal experiments were carried out in accordance with our institutional guidelines and were approved by the Animal Care and Use Committee, Kyushu University. Statistical analysis: Quantitative data were expressed as mean±S.D. Means were compared using Student's *t*-test and *p* values <0.05 were considered statistically significant.

6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-dimethoxyethoxymethyl-5-*O*-(4′-trimethylsilylbenzyl)-L-ascorbic Acid (4) To a solution of 6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-dimethoxyethoxymethyl-L-ascorbic acid **3** (511 mg, 1.10 mmol)[19] in dry benzene (15 mL), in a flask covered with aluminum foil, was added $CaSO_4$ (530 mg), Ag_2O (570 mg, 1.37 mmol) and 4-trimethylsilylbenzyl bromide (332 mg, 1.37 mmol) sequentially. The mixture was stirred for 24 h. Additional aliquots of Ag_2O, $CaSO_4$ and 4-trimethylsilylbenzyl bromide were then added in the same quantities as before and the reaction mixture was stirred for an additional 4 d. The mixture was diluted with EtOAc and filtered through a short pad of Celite®. The combined filtrate was evaporated to dryness. The residue was chromatographed on silica gel (EtOAc : hexane=1 : 3) to give the required product **4** (325 mg, 47.2%) as a viscous oil. ^{1}H-NMR ($CDCl_3$) δ: 0.04 (s, 3H Hz), 0.05 (s, 3H), 0.25 (s, 9H), 0.88 (s, 9H), 3.34 (s, 3H), 3.35 (s, 3H), 3.52—3.49 (q, 4H, *J*=4.3 Hz), 3.77—3.89 (m, 7H), 4.55 (d, 1H, *J*=12.0 Hz), 4.59 (d, 1H, *J*=12.0 Hz), 4.89 (s, 1H), 5.20 (d, 1H, *J*=5.8 Hz), 5.26 (d, 1H, *J*=5.8 Hz), 5.39 (d, 1H, *J*=5.5 Hz), 5.58 (d, 1H, *J*=5.5 Hz), 7.23 (d, 2H, *J*=9.1 Hz), 7.47 (d, 2H, *J*=7.6 Hz); IR (neat) cm^{-1}: 1770, 1687; FAB-MS (*m*/*z*): 629.3 $[M+H]^+$.

6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-dimethoxyethoxymethyl-5-*O*-(4′-bromobenzyl)-L-ascorbic Acid (5) Compound **5** was prepared from **3** using 4-bromobenzyl bromide following a procedure similar to that for **4**. After the reaction mixture was stirred for 3 d, the crude product was chromatographed on silica gel (EtOAc : hexane=1 : 3) to give the required product **5** (37%) as a viscous oil. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 3.35 (s, 6H), 3.51 (q, 4H, *J*=4.9 Hz), 3.76—3.87 (m, 7H), 4.49 (d, 1H, *J*=12.0 Hz), 4.58 (d, 1H, *J*=12.0 Hz), 4.87 (d, 1H, *J*=1.2 Hz), 5.20 (d, 1H, *J*=5.8 Hz), 5.25 (d, 1H, *J*=5.8 Hz), 5.42 (d, 1H, *J*=5.5 Hz), 5.57 (d, 1H, *J*=5.5 Hz), 7.14 (d, 2H, *J*=8.5 Hz), 7.44 (dd, 2H, *J*=1.8, 6.5 Hz); IR (neat) cm^{-1}: 1770, 1681; FAB-MS (*m*/*z*): 635.2 $[M]^+$.

6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-dimethoxyethoxymethyl-5-*O*-(4′-tri-*n*-butylstynylbenzyl)-L-ascorbic Acid (6) A solution of **5** (202 mg, 318 μmol), $Pd(PPh_3)_4$ (24.5 mg, 21.2 μmol) and hexabutylditin (369 μL, 636 μmol) in dry toluene (15 mL) was heated to reflux for 5 h. The reaction mixture was diluted with $CHCl_3$ and filtered through a short pad of Celite®. The combined filtrate was concentrated *in vacuo*. The residue was purified with silica flash chromatography (hexane–EtOAc, 1 : 1 v/v) to give **6** (127 mg, 47%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 0.04 (s, 3H), 0.05 (s, 3H), 0.87—0.94 (m, 18H), 1.04 (t, 6H, *J*=8.1 Hz), 1.28—1.42 (m, 6H), 1.49—1.63 (m, 6H), 3.34 (s, 3H), 3.35 (s, 3H), 3.49—3.53 (m, 4H), 3.76 (s, 3H), 3.78—3.82 (m, 2H), 3.85—3.89 (m, 2H), 4.55 (s, 2H), 4.90 (s, 1H), 5.21 (d,1H, *J*=5.8 Hz), 5.22 (d, 1H, *J*=5.8 Hz), 5.42 (d, 1H, *J*=5.5 Hz), 5.60 (d, 1H, *J*=5.5 Hz), 7.21 (d, 2H, *J*=7.6 Hz), 7.41 (d, 2H, *J*=7.6 Hz) ; IR (neat) cm^{-1}: 2954, 1770; ESI(+)-MS (*m*/*z*) Calcd for $C_{39}H_{71}O_{10}SiSn$ 847.3842 $[M+H]^+$, Found 847.3813 $[M+H]^+$.

Radiochemistry. 2,3-*O*-Dimethoxyethoxymethyl)-5-*O*-4-[^{125}I]iodobenzyl-6-*O*-*tert*-butyldimethylsilyl-L-ascorbic Acid ([^{125}I]7) No-carrier-added Na^{125}I (3.7—18.5 MBq), chloramine-T (10 μg), and acetic acid (3.3 μL) were dissolved sequentially in a solution of **6** (100 μg) in EtOH (50 μL). The mixture was allowed to stand for 2 h at room temperature and the entire reaction mixture was injected into an HPLC column (Nacalai Tesque COSMOSIL 5C18 AR-II, 4.6×250 mm, H_2O/MeOH=13/87, flow rate 0.8 mL/min). The product fraction corresponding to **7** was collected after a retention time of 13 min. The isolated radiochemical yield was 77.5±8.3% with radiochemical purity of above 98%, as determined by HPLC.

5-*O*-(4′-[^{125}I]Iodobenzyl)-L-ascorbic Acid ([^{125}I]1) A solution of intermediate [^{125}I]**7** (3.7—11.1 MBq) in an HPLC eluate (a mixture of H_2O and MeOH) obtained by electrophilic radioiodination was added to a reaction vial and the solvent was evaporated at room temperature. To find out the appropriate reaction conditions for the deprotection step, to this residue erythorbic acid (10 μg) and methanol containing 0.1 M–HCl or 1.0 M HCl (100 μL) were added, and the vial was sealed. The mixture was then allowed to proceed at room temperature, or heated at 50°C or 60°C for 30 min, as shown in Table 1. In preparative runs, the deprotection reaction of intermediate [^{125}I]**7** was carried out by heating with 1.0 M HCl at 60°C for 30 min in the presence of erythorbic acid. This acidic solution was then neutralized with saturated aqueous $NaHCO_3$ and the resulting entire mixture was injected into an HPLC column (Nacalai Tesque COSMOSIL 5C18 AR-II, 4.6×250 mm, 15 mM phosphate buffer containing 400 μM DL-homocysteine (pH= 6.0)/MeOH=60/40, flow rate 0.8 mL/min). The radioactive fraction containing the required product (retention time: 13 min) was collected in a flask and the MeOH from the eluent was evaporated at 30°C. By the two-step procedure described above, the target [^{125}I]**1**, free of erythorbic acid contaminants, was isolated in 61% overall radiochemical yield. Total preparation time starting from the radoiodination step was 4 h. The specific activity of the obtained [^{125}I]**1** was above 15.4 GBq/μmol, as determined by direct measurement of the HPLC eluate using a UV detector. The HPLC-collected fraction of [^{125}I]**1** was allowed to stand at 4°C and at several inter-

vals from zero to 6 h, and the samples were withdrawn and analyzed for stability by HPLC using the above conditions. At 12 h, two radioactive species eluted before intact [^{125}I]**1**, appeared with a radioactive ratio of about 30%, without any indication of the formation of the free ^{125}I-iodide ion.

Biodistribution NFSa-fibrosarcoma was inoculated subcutaneously (s.c.) into the right hind leg muscles of female C3H/He mice (5 weeks old, 15—18 g). These mice bearing tumors which developed with a diameter of about 1 cm at 9—14 d after inoculation were used for the *in vivo* biodistribution studies. The tumor-bearing mice were intravenously injected through the tail vein with a solution of [^{125}I]**1** (37 kBq, 100 μL) in an HPLC eluent containing DL-homocysteine (0.04 μmol/mouse). The mice were sacrificed by ether anesthesia at a predetermined time after injection. A sample (0.2—0.5 mL) of blood was collected at the time of euthanasia. Samples of the tissues and tumors were excised immediately and weighed. The tissue radioactivity was counted by a gamma counter (Aloka, ARC-370). The tissue radioactivity levels were calculated as a percentage of the injected dose per gram of tissue (%ID/g of tissue). In the other group of mice, a blocking experiment to determine the SVCT-specific uptake of the radiotracer was carried out. Each mouse received of sulfinpyrazone (1.5 μg/g per animal, 50 μL, dissolved in 2% ethanol–saline containing 5% Tween 80) *via* intravenous injection 3 min prior to the radiotracer injection. The control group received only a solution of 2% ethanol–saline containing 5% Tween 80. These mice were sacrificed 10 min after tracer injection (37 kBq, 80 μL), and tissue samples of the sulfinpyrazone-pretreated and control mice were assayed for radioactivity as described above.

Acknowledgments Support for this study from the Asahi Glass Foundation and the program for the Creation of Innovation Centers for Advanced Interdisciplinary Research Areas from Special Coordination Funds for Promoting Science and Technology (SCF) commissioned by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan is gratefully acknowledged. We also thank Prof. Takashi Ohshima of Graduate School of Pharmaceutical Sciences, Kyushu University for valuable discussion.

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