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Polymer-Supported Organotin Reagent for Prosthetic Group Labeling of Biological Macromolecules with Radioiodine

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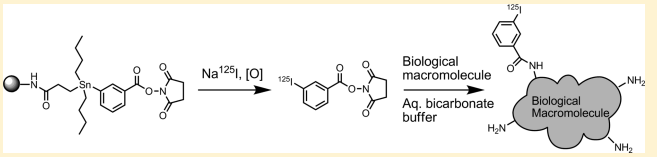
Supporting Information

ABSTRACT: In this study, we investigated the use of polymer-bound precursor for generating a radiolabeled prosthetic group to be used for conjugate labeling of biological macromolecules. For the approach, a trialkyltin chloride in which the tin was bound to a hydrophilic PEG-based resin support via one of the alkyl groups was synthesized. This resin was then used to prepare a resin-bound trialkyltin benzoic acid, which in some cases was further derivatized on-resin by converting it to a succinimidyl ester. Exposure of the resin-bound compounds to electrophilic radioiodine (^{125}I) in either an aqueous or methanol solvent liberated either free radiolabeled [^{125}I]iodobenzoic acid or its succinimidyl ester without co-release of the resin-bound precursors. Radiochemical yield was between 35% and 75%, depending on the solvent system and precursor. As example applications for the released compounds, the amine-reactive *N*-succinimidyl-[^{125}I]iodobenzoate prosthetic group was used for conjugate radiolabeling of a peptide, tomato plant systemin, and two proteins, albumin and IgG antibody. These results demonstrate that resin-bound organotin precursors in which the compound to be labeled is tethered to the support via the tin group to be substituted can be used to produce radioiodine-labeled aromatic prosthetic groups in good specific activity without the need for HPLC purification. This solid-phase approach is potentially adaptable to kit-formulation for performing conjugate radiolabeling of biological macromolecules.



INTRODUCTION

Peptides, proteins, and other biological macromolecules tagged with radioiodine have widespread uses in the biological sciences. The most common method of labeling these molecules with radioiodine is by the direct reaction of electrophilic radioiodine with tyrosine or other phenolic sites on the molecule.[1,2] However, this direct approach has some disadvantages, including the fact that the molecule must be exposed to oxidative conditions in order to produce the electrophilic radioiodine from its sodium salt. This can oxidize methionine and other sensitive residues on the protein or peptide and lead to a reduction or loss its biological activity.[3,4] Additionally, in the case of labeled macromolecules destined for in vivo applications, a further disadvantage is the fact that radioiodine on tyrosine rings is unstable in biological fluids and free iodine slowly accumulates in the thyroid and stomach.[5,6] Due to these limitations, an alternative method of labeling biological macromolecules with radioiodine that has been developed is conjugation of the macromolecule with a small preradioiodinated prosthetic group. Although a variety of prosthetic groups have been developed for this purpose,[7] one of the most commonly used is *N*-succinimidyl iodobenzoate.[8,9] In this molecule, the radioiodine is attached directly onto a nonactivated aromatic ring and is resistant to deiodination in vivo.[9,10] The succinimidyl ester on the molecule allows it to be readily conjugated to lysine amine groups on peptide or proteins under mild aqueous, nonoxidative conditions. Following the conjugation reaction, a gel filtration or ultrafiltration step is typically used to separate the radiolabeled macromolecular conjugate from any unreacted radiolabeled prosthetic group remaining in solution to give the final purified product.

The most effective method for labeling nonactivated aromatic compounds with radioiodine is via radioiododestannylation of the corresponding organotin precursor.[11] This approach has similarly been used to produce radiolabeled *N*-succinimidyl-iodobenzoate.[8,9] The organotin precursors have good stability in storage and are stable to air and moisture, and the radioiododestannylation reaction itself proceeds readily and in high yield.[11] To ensure consumption of the radioiodine in radioiododestannylation reactions, and thus a good overall radiochemical yield, the organotin precursor is typically present in an amount between 1 and 2 orders of magnitude excess over the concentration of radioiodine. However, in the case of prosthetic groups used for conjugate radiolabeling of macromolecules, a consequence of the presence of a large excess of organotin compounds in the reaction mixture is that an HPLC or equivalent purification step is normally needed following the radiolabeling reaction to separate the final radiolabeled product from unreacted organotin precursors. This is necessary in order to avoid unnecessary blockade of amine sites on the biomolecule by the amine-reactive organotin precursors. Complete removal of tin precursors and byproducts is also especially important where the products may

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have potential for use in clinical studies, since organotin is highly toxic to humans. However, HPLC purifying the radiolabled prosthetic group prior to conjugation to biological protein or macromolecules to remove organotin precursors and byproducts introduces extra complexity into the labeling process and creates significant volumes of radioactive waste. Additionally, HPLC has limitations in effectively removing toxic organotin byproducts from solution-phase reactions. Thus, trialkyltin−aromatic precursors are strongly retained by reverse-phase material, and hence, HPLC columns must be washed well between runs, while cleaved trialkyltin salt byproducts are prone to absorption onto exposed silanol groups on the silica resulting in broad peaks that can potentially overlap with product peaks (ref 12 and figures given in the Supporting Information).

In the current study, we investigated the use of a solid phase approach to labeling the radioiodinated prosthetic group with the goal of avoiding the need for postlabeling purification steps prior to conjugation to the biological macromolecule. In this solid-phase approach, the organotin precursor to be iodinated is tethered to a resin support via the alkyl tin group to be substituted. Following the iodination, the radioiodinated product is released free into the solvent, whereas unreacted tin precursors and byproducts remain bound to the insoluble resin beads, enabling the latter to be easily separated from the radioiodinated product by a simple filtration or extraction step without HPLC.

Polymer-bound tin compounds have been previously examined for catalytic applications[13,14] and for preparation of cold (unlabeled) iodinated compounds.[15] However, there have only been limited prior studies reported in the literature in which polymer-bound organotin precursors have been used to produce radiolabeled compounds, namely, metataiodobenzylguanidine[16,17] and Congo Red.[18] In analogous approaches, organogermanium[19] and organoborane[20] precursors that were linked to a solid support have been examined for their potential to produce radio-iodinated compounds, but these precursors have reduced reactivity to radio-iodine compared to organotin compounds.[11] With respect to prosthetic groups for conjugate radioiodine labeling of macromolecules, Donovan et al.[21,22] have reported a solution-phase analogue of the solid-phase labeling strategy in which a perfluoroakyl tin group was used as the leaving group. The high affinity of the perfluoroalkyl moiety for fluorous solid-phase extraction media enabled its easy removal from the reaction mix. However, despite these latter variants, as far as we are aware, a prosthetic group for radiolabeling macromolecules in which the organotin precursor is covalently bound to the polymer support has not yet been examined.

EXPERIMENTAL PROCEDURES

General. Organometallic reagents were handled under a nitrogen atmosphere using oven-dried glassware and anhydrous solvents. Elemental analysis on resin samples was conducted by Robertson Microlit Laboratories (Madison NJ). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR. Mass spectrometry data was obtained using a Agilent 6300 ion trap LC/MS. RadioHPLC was performed using a Knauer HPLC system. HPLC chromatographic conditions (A) were as follows: C-18 Microsorb-MV column 50 mm × 4.7 mm, flow rate of 2 mL/min, gradient of 10% MeCN with 0.1% TFA to 60% MeCN with 0.1% TFA over 25 min, UV detection at either 254 or 210 nm, and radioactivity detection using an Ludlum sodium iodide detector placed over the effluent line from the UV detector.

Acrylamide-Functionalized Resin (1). To a suspension of amino NovaPEG resin (4.5 g; loading 0.66 mmol/g; EMD Chemicals Inc., Gibbstown, NJ) in DMF (50 mL) was added acryloyl chloride (1.05 mL, 13 mmol) and TEA (1.85 mL, 13 mmol). The mixture was allowed to react for 2 h at room temperature with gentle shaking. The solvent was then removed and the resin washed twice with DMF (10 mL), once with methanol (10 mL), and then evaporated to dryness. A ninhydrin test performed on samples of the resin was negative following the reaction, indicating complete coupling of the acryloyl chloride to the resin amines.

Dibutyltin Dihydride. Sodium borohydride (2.2 g, 58 mmol) was dissolved in ice−cold water (45 mL) with continuous stirring and nitrogen bubbling. To this mixture was added Bu_2SnCl_2 (3 g, 10 mmol) in ether. Stirring and nitrogen bubbling was continued and the mixture allowed to warm to room temperature over 15 min. The ether layer was then separated, washed with water (2 × 15 mL), dried over $MgSO_4$, and evaporated to yield Bu_2SnH_2 (1.5 g) as a colorless oil. Since alkyltin hydrides have poor stability in storage, the Bu_2SnH_2 was used directly in the next step without further characterization.

Dibutyltin Chloride-Functionalized Resin (2). To an oven-dried Schlenk flask under nitrogen was added 4.35 g of the acrylamide functionalized resin and THF (50 mL). Once the resin was fully swollen, Bu_2SnCl_2 (2 g, 6.6 mmol), Bu_2SnH_2 (1.5 g, 6.4 mmol), and 2,2′-azobisisobutyronitrile (AIBN; 58 mg, 0.35 mmol) were added. The mixture was allowed to react overnight at room temperature with gentle shaking and irradiation from a halogen light source (150 W). During this process, the resin was observed to change appearance from yellow−orange color to more of an opaque off-white color. Following the reaction, the resin was washed with THF, butanol, and methanol and evaporated to dryness to give a yellow−white powder (5.05 g). Elemental analysis (ICP-MS) indicated a tin content of the resin of 5.5% and a chloride content of 2.5%.

Polymer-Bound 3-(Dibutylstannyl)benzoic Acid (4). To an oven-dried Schlenk flask under nitrogen was added the dibutyltin chloride-functionalized resin (1.3 g) and THF (10 mL). Once the resin was fully swollen, the solvent was aspirated off to just above the level of the resin and 3-(ethoxycarbonyl)-phenylzinc (0.5 M in THF; 6 mL) added. The mixture was allowed to react overnight at room temperature with gentle shaking. The resin was then washed with THF (2 × 10 mL), butanol (1 × 10 mL), and water (2 × 10 mL). Following washing, the resin was transferred to a plastic 50 mL tube and 1 M aq. NaOH (20 mL) added. The tube was then incubated with gentle shaking at 55 °C for 4 h. The resin was washed with water (2 × 10 mL) and methanol (1 × 10 mL) and evaporated to dryness.

To test for formation of polymer-bound 3-(dibutylstannyl)-benzoic acid, a sample of the resin (1 mg) was transferred to a glass vial and mixed with methanol (0.1 mL) containing 0.1% acetic acid. To this, *N*-chlorosuccinimide (100 μg, 0.75 μmol in 10 μL methanol) and either NaI (100 μg, 0.67 μmol in 10 μL water) or Na[^{125}I] (6 μCi in 1 μL of 0.01 M NaOH) added. A yellow color from molecular iodine formed in the tube containing NaI. The mixture was allowed to react for 30 min at room temperature with slow rotation of the vial. The solvent containing the released iodobenzoic acid was then separated from the resin and analyzed on HPLC. A primary UV and radioactive peak eluting at 12.8 min (conditions A) was observed. Co-elution of both the UV and radioactive peaks with an authentic

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3-iodobenzoic acid chromatographic standard was used to confirm identity. ESI-MS analysis from incubation of resin (**4**) with excess cold iodine is given in the Supporting Information.

The degree of loading of the resin by the alkyltin benzoic acid was determined by incubating a preweighed sample of the resin overnight in neat TFA to cleave the tin−aromatic bond, followed by quantitation of the released benzoic acid using HPLC. Using this approach, a loading value of 0.32 mmol/g was obtained, representing about half the initial substitution of the resin. Due to the prior incubation step in aqueous sodium hydroxide the remaining unoccupied tin sites on the resin are likely to be predominately in the form of the alkyltin oxide or hydroxide.

Polymer-Bound *N*-Succinimidyl 3-(Dibutylstannyl)benzoate (5). To a suspension of polymer-bound 3-(dibutylstannyl)benzoic acid **4** (5 mg) in DMF (0.2 mL) in a glass vial was added 4-dimethylaminopyridine (DMAP; 4 mg, 33 μmol), *N,N,N′N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU; 10 mg, 33 μmol) and benzoic acid (1 mg, 8 μmol). The purpose of the benzoic acid was to cap any free amines present in the resin, which may have developed during the prior alkaline hydrolysis step. The mixture was allowed to react for 4 h at room temperature with slow rotation of the vial. The resin was then washed twice with DMF and stored in toluene at −20 °C.

To test for formation of polymer-bound *N*-succinimidyl 3-(dibutylstannyl)benzoate, a sample of the resin was reacted with either NaI or Na[^{125}I] and *N*-chlorosuccinimide and the solvent containing the released *N*-succinimidyl 3-iodobenzoate analyzed by HPLC. A primary peak eluting at 15.6 min (conditions A) was observed. Co-elution of both the UV and radioactive peaks with an *N*-succinimidyl 3-iodobenzoate chromatographic standard was used to confirm identity.

Typical Radioiodination Procedure for 3-[^{125}I]Iodobenzoic Acid (6). Between 0.5 and 1 mg of the resin-bound tin precursor was placed in a 1.5 mL glass tube to which was added methanol (1 mL) containing sodium acetate (100 mM) and acetic acid (16 mM). The tube was slowly rotated for 1−2 h to allow the resin to equilibrate with the solvent and wash out any impurities present in the resin. The solvent was then aspirated off and the resin washed briefly with fresh solvent (1 mL). The resin was then resuspended in fresh methanol (100 μL) containing sodium acetate and acetic acid. *N*-Chlorosuccinimide (10 μg, 0.075 μmol in 1 μL of methanol) and Na^{125}I (10−200 μCi, 0.37−7.4 MBq, in 1 μL of 0.01 M NaOH) was added to the tube. The tube was sealed and the tube placed at 45° angle with slow rotation (10 rpm) to ensure efficient mixing of the resin beads with the small amount of solvent in the bottom of the tube. After allowing the mixture to react for 50 min at room temperature, the solvent containing the released radioiodinated compounds was separated from the resin beads and transferred to a new vial. A sample of the mixture was injected into the HPLC for analysis. Co-elution of the radioactive peak with a 3-iodobenzoic acid chromatographic standard was used to confirm identity.

Typical Radioiodination Procedure for *N*-Succinimidyl 3-[^{125}I]Iodobenzoate (7). Procedure was as described for 3-[^{125}I]iodobenzoic acid except that preincubation and radioiodination were performed in methanol-containing acetic acid (0.1%; 16 mM) only. Following the radioiodination, sodium metabisulfite (100 μg in 10 μL water) and 0.4 mL of either water or aq phosphate buffer (pH 6) was added to the vial and the radioiodinated *N*-succinimidyl 3-[^{125}I]iodobenzoate **7** extracted into toluene (2 × 0.5 mL). After drying over a few crystals of $MgSO_4$, the toluene extract containing the radioiodinated *N*-succinimidyl 3-[^{125}I]iodobenzoate **7** was stored at −20 °C until needed for coupling reactions. The *N*-succinimidyl 3-[^{125}I]iodobenzoate was stable when stored in this manner over several weeks. Co-elution of the radioactive peak with a *N*-succinimidyl 3-iodobenzoate chromatographic standard on HPLC was used to confirm identity.

Conjugation of *N*-Succinimidyl 3-[^{125}I]Iodobenzoate (7) to a Plant Peptide. An aliquot of the *N*-succinimidyl 3-[^{125}I-[iodobenzoate **7** (4 μCi, 148 KBq) in toluene was added to a vial containing a trace (1 μL) of acetic acid to stabilize the activated ester group and evaporated to dryness. To the vial was then added tomato plant systemin peptide (100 μg in 10 μL water) and bicarbonate buffer (10 μL of 100 mM aq solution, pH 8). The mixture was allowed to react for 1 h at room temperature and then analyzed using HPLC. Co-elution of the radioactive peaks (t_R 7.5−9.5 min; HPLC conditions (A) with a systemin−iodobenzamide chromatographic standard was used to confirm identity.

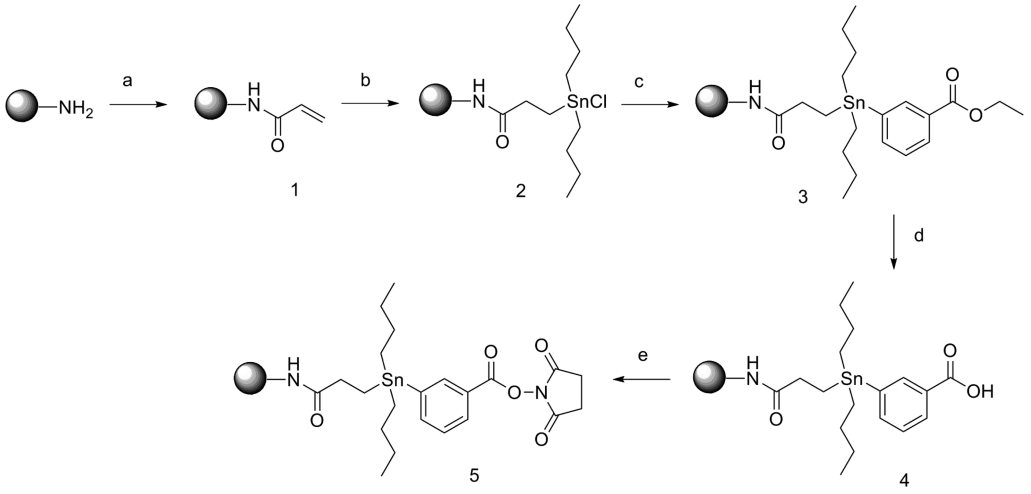
Conjugation of *N*-Succinimidyl 3-[^{125}I]Iodobenzoate (7) to Proteins. An aliquot of the *N*-succinimidyl 3-[^{125}I-[iodobenzoate **7** (5 μCi, 185 KBq) in toluene was evaporated to dryness in the presence of a trace (1 μL) of acetic acid . To the vial was then added bicarbonate buffer (50 μL of 100 mM aq solution, pH 8) and the protein to be labeled (bovine serum albumin or IgG; 200 μg in 10 μL water). The mixture was allowed to react for 2 h at rt. A sample of the mixture was then transferred to ultrafiltration tubes (Centricon, 3500 MW cutoff) together with 2 mL of aq bicarbonate buffer containing bovine serum albumin (100 μg/mL) and benzoic acid (2 mg/mL). The purpose of the latter two additives was to inhibit any nonspecific absorption of either the [^{125}I]-labeled protein or unreacted [^{125}I]iodobenzoic acid to the ultrafiltration filters. The tubes were centrifuged at 6000 × g for 1 h after which an additional 2 mL of water was added to the top of the filter assembly and the tubes recentrifuged. Following the second centrifugation, the retentate (approximately 0.2 mL) and combined filtrate (3.8 mL) were withdawn from the spin columns, transferred to separate tubes, and counted in a gamma counter for ^{125}I.

Chromatographic Standards. Synthesis and characterization of *N*-succinimidyl 3-iodobenzoate and systemin−iodobenzamide conjugate chromatographic standards are given in the Supporting Information.

RESULTS AND DISCUSSION

Synthesis of Polymer-Bound 3-(Dibutylstannyl)benzoic Acid and *N*-Succinimidyl 3-(Dibutylstannyl)benzoate. The synthetic scheme for producing polymer-bound 3-(dibutylstannyl)benzoic acid **4** and its succinimidyl ester derivative **5** is shown in Scheme 1. For the resin support, we employed a PEG-based support (NovaPEG) rather than the more traditional polystyrene based resins (e.g., Merrifield resin) due to the fact the PEG-based resins are able to swell in water and alcoholic solvents, as well as organic solvents. This is in contrast to the polystyrene-based resins which show little to no swelling in water and alcohol solvents due to their strongly hydrophobic backbone. Good swelling in alcohol was considered advantageous for the radioiododestannylation reaction, since this reaction is normally performed in a methanol solvent. Creation of the polymer-bound dibutyltin chloride **2** was performed using an adaptation of the methods described by Newmann et al.[13] and Zhu et al.[15] A terminal alkene group was introduced onto the amino-PEG resin, and this was then stannylated using

Scheme 1. Preparation of Polymer-Bound 3-(Dibutylstannyl)benzoic Acid (4) and Its Succinimidyl Ester (5)[a]



[a] Reagents and conditions: (a) acryloyl chloride, TEA, DMF, room temperature, 2 h; (b) Bu_2SnCl_2, Bu_2SnH_2, AIBN, THF, light, room temperature, overnight; (c) 3-(ethoxycarbonyl)phenylzinc, THF, room temperature, overnight; (d) aq NaOH, 55 °C, 4 h; (e) TSTU, DMAP, benzoic acid, DMF, room temperature, 4 h.

Bu_2SnHCl together with AIBN and light irradiation. The Bu_2SnHCl was prepared in situ from a 1:1 mixture of Bu_2SnH_2 and Bu_2SnCl_2.

Although characterization of organotin bound to polystyrene-based resins has been previously performed using gel-phase ^{13}C NMR, we had limited success using this approach with the PEG-based resin used as the polymer support in the current study. This was due the fact that large background peaks from the PEG resin made it difficult to distinguish peaks coming from the terminal functionalization of the resin. Similar problems were encountered when trying to analyze the resin using IR. We thus relied on elemental analysis of tin and chloride to determine the degree of stannylation of the resin. Following tethering of aromatic compounds to the resin, the progress of reactions was monitored by cleaving off the compounds from resin samples using molecular iodine and assaying the released products with HPLC.

Once the polymer-bound chloride was synthesized, it was used to generate a resin-bound dibutyltin ethyl benzoate **3** via transmetalation of a commercially available zinc precursor. In order to cleave the ethyl ester and generate a free carboxylic acid on the molecule, necessary for producing a reactive functional group for subsequent conjugation to amine-containing biomolecules, the resin was incubated in aqueous sodium hydroxide to give **4**. HPLC analysis on samples of the reaction products that had been cleaved off the resin using iodine indicated $a > 95\%$ hydrolysis of the ester. Interestingly, in parallel experiments in which we used Merrifield resin rather than NovaPEG as the polymer support, treatment of the polymer-bound dibutyltin ethyl benzoate with aqueous sodium hydroxide did not produce cleavage of the ester, even with overnight incubation in the hydroxide. PEGA resin, which consists of a polystyrene backbone on which PEG is grafted, was also found to be unsatisfactory as the polymer support since incubation in sodium hydroxide caused it to become brittle and fragile. The NovaPEG resin by contrast appeared unaffected. Following the hydrolysis step, the resin was washed, dried, and stored in a darkened desiccator at room temperature until needed for radioiodinations or for conversion to an activated ester. The resin showed good stability under these conditions when stored over a period of several months.

To enable the released radiolabeled benzoic acid to couple to proteins, we chose a succinimidyl ester as the activating group. This activating group has a combination of reasonable water solubility when at tracer levels and good reactivity to amine groups. Although the succinimidyl ester group could in principle be added once the radiolabeled benzoic acid had been released from the resin in solution, we chose instead to convert the benzoic acid to an activated ester directly on the resin, since this would simplify radiolabeling of biological macromolecules using the solid-phase approach and would facilitate enabling the resin to be developed in a kit form for protein radiolabeling. However, a drawback of this preactivation approach is that resin is likely to be less stable in storage when in the activated state, and also care has to be taken for the succinimidyl ester not to be prematurely hydrolyzed during incubation of the resin in radioiodine. For the activation of the resin-bound alkyltin benzoic acid, we initially used hydoxysuccinimide in the presence of DIC and base. However, only a partial conversion to the succinimidyl ester **5** was obtained using this approach, despite several variations in reaction conditions. Subsequently, we used the uronium-based activating agent, *N,N,N′N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) to produce the succinimidyl ester **5**. This gave a more satisfactory conversion. Following conversion of the resin to the succinimidyl ester, the final polymer-bound *N*-succinimidyl 3-(dibutylstannyl)benzoate **5** was stored in toluene at −20 °C until needed for radioiodinations.

Radioiodination of Polymer-Bound Precursors. Initial tests to optimize reaction conditions for the release of [^{125}I]-labeled compounds following incubation of the resin with electrophilic radioiodine were conducted using the polymer-bound alkyltin benzoic acid resin **4** (Scheme 2a). Figure 1 shows an HPLC analysis of the solvent after incubating polymer-bound alkyltin benzoic acid resin **4** with radioiodine in a methanol solvent containing acetic acid and sodium acetate. Most of the free radioiodine, which elutes close to the void volume, has disappeared, and a large peak from 3-[^{125}I]iodobenzoic acid released into the solution is apparent. The absence of significant peaks in the UV trace suggested a good chemical purity for

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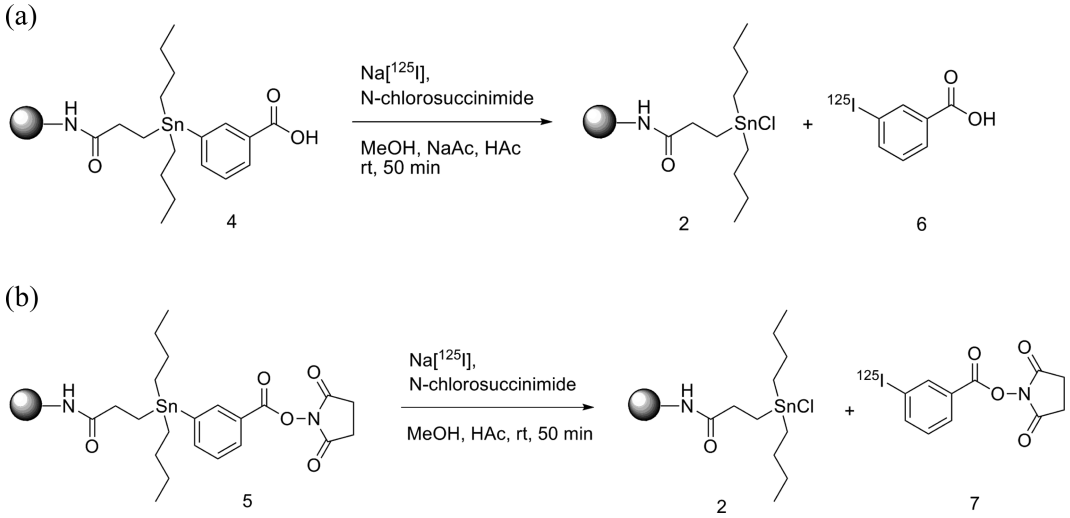
Scheme 2. Radioiodination of Polymer-Bound Organotin Precursors and Products Formed (a) Polymer-Bound 3-(Dibutylstannyl)benzoic Acid (4), (b) Polymer-Bound *N*-Succinimidyl 3-(Dibutylstannyl)benzoate (5)



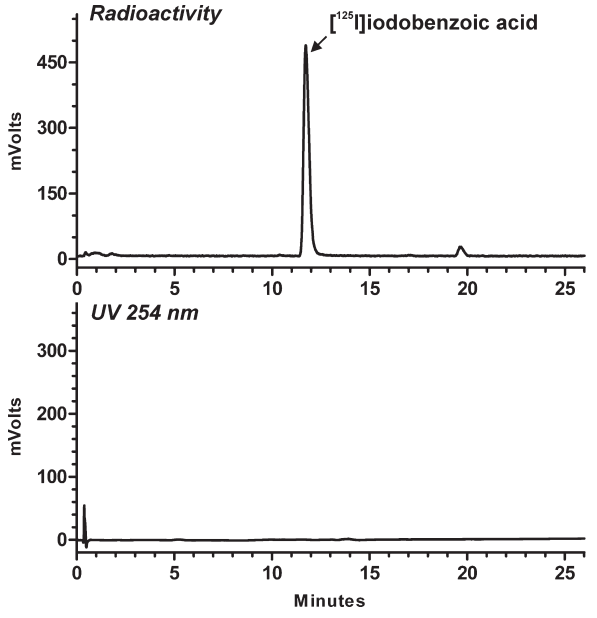


Figure 1. HPLC analysis of the reaction medium following incubation of polymer-bound 3-(dibutylstannyl)benzoic acid (**4**) with Na[^{125}I] and *N*-chlorosuccinimide. HPLC conditions A.

the released radioactive compound. Radiochemical yield for 3-[^{125}I]iodobenzoic acid using a methanol with acetic acid solvent typically ranged from 50% to 65% over different runs, with the remaining radioactivity found to be mostly absorbed to the resin.

A comparison of the effect of several different solvent conditions on radiochemical yield and radiochemical purity of the released 3-[^{125}I]iodobenzoic acid is shown in Table 1. Both methanol-only and fully aqueous solvent systems were examined. Acid versus basic conditions were adjusted using acetic acid and sodium acetate for the methanol solvent and sodium phosphate for the aqueous solvent. Interestingly, a similar radiochemical yield was obtained using an aqueous solvent as

Table 1. Radiochemical Yields and Purities for Production of 3-[^{125}I]Iodobenzoic Acid (6) from Incubation of the Polymer-Bound Precursor with Na[^{125}I] and *N*-Chlorosuccinimide in Different Solvent Systems

solvent	radiochemical yield (%)	radiochemical purity (%)
MeOH, 16 mM HAc	51.8	91.9
MeOH, 16 mM HAc, 100 mM NaAc[a]	59.2	95.0
MeOH, 100 mM NaAc	74.8	93.4
Water, 100 mM $NaPO_4$, pH 6.2	68.3	93.7
Water, 100 mM $NaPO_4$, pH 7.0	66.8	94.5
Water, 100 mM $NaPO_4$, pH 8.0	65.5	80.5

[a] HPLC shown in Figure 1.

with the methanol solvent, despite the fact that tributyltin compounds are normally insoluble in the former. This was presumably a consequence of conjugation of the alkyl tin to the hydrophilic PEG support. For the methanol-based solvents, best yields (75%) were obtained with a relatively basic rather than acidic solvent. In the case of the aqueous phosphate-buffered solvent, similar radiochemical yields were obtained at the three different pH values tested. However, at the most alkaline pH tested, a free radioiodine peak eluting close to the void volume in the HPLC chromatogram was observed, which hence reduced the overall radiochemical purity of the released 3-[^{125}I]iodobenzoic acid.

In the case of the succinimidyl ester-activated resin **5**, a mildly acidic reaction medium was chosen as the radioiodination solvent, despite a lower total yield of radioiodinated products compared with a more basic solvent (Scheme 2b). This was to avoid premature loss of the activated ester group under basic conditions. However, because of the potential for cleavage of the tin−aromatic bond under strongly acidic conditions, the level of acetic acid in the solvent was minimized. For the iodination reaction, we thus chose 0.1% (16 mM) acetic acid in methanol as providing a suitable compromise between providing sufficient acid to neutralize sodium hydroxide in which the Na[^{125}I] is

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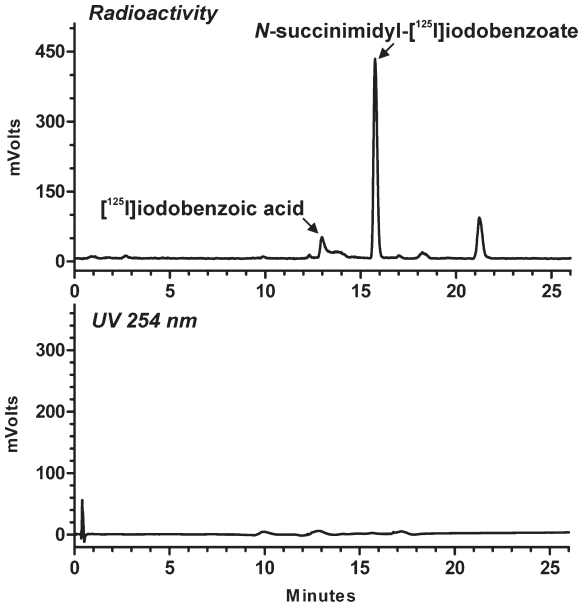

Figure 2. HPLC analysis of the reaction medium following incubation of polymer-bound *N*-succinimidyl 3-(dibutylstannyl)benzoate (**5**) with Na[^{125}I] and *N*-chlorosuccinimide. HPLC conditions A.

shipped and thus preserving the succinimidyl ester group, but at the same time minimizing the potential for concomitant acidolysis of the tin−aromatic bond. HPLC analysis of the reaction solution following incubation of the resin in radioiodine and with *N*-chlorosuccinimide oxidant is shown in Figure 2. In the radioactivity trace, a large peak from released *N*-succinimidyl 3-[^{125}I]iodobenzoate **7** was apparent. Smaller peaks from unesterified 3-[^{125}I]iodobenzoic acid and a late-running peak from a radiochemical impurity (at 22 min) were also present. The identity of the late-running impurity was not established, but it was observed to remain unchanged following hydrolysis of the released *N*-succinimidyl 3-[^{125}I]iodobenzoate with bicarbonate buffer. Some trace levels of chemical impurities were observed on the UV trace in different runs but were at or close to the detection limits of the UV and were not further characterized. Median radiochemical yield for *N*-succinimidyl 3-[^{125}I]iodobenzoate over several runs was 35% (range 28−36%) at low levels of radioiodine (5−10 μCi, 0.19−0.37 MBq), but was slightly higher at 44% for a run performed with a higher level (1 mCi, 37 MBq) of radioiodine (Supporting Information). A slightly lower radiochemical yield of the succinimidyl ester compared to the free acid can be explained by the electron withdrawing effect of the activated ester reducing the susceptibility of the aromatic tin to electrophilic attack, combined with the fact that not all of the free acid sites on the resin were completely converted to the succinimidyl ester by the TSTU activating agent. Following the radioiodination reaction, the released *N*-succinimidyl 3-[^{125}I]-iodobenzoate was extracted into toluene and was stable in this formulation when stored in the freezer.

Conjugation of *N*-Succinimidyl 3-[^{125}I]Iodobenzoate to Biomolecules. To test the ability of *N*-succinimidyl 3-[^{125}I]-iodobenzoate to couple an amine-containing biological macromolecules, its conjugation to the proteins bovine serum albumin (BSA) and goat antimouse IgG (Table 2), to the peptide, tomato plant systemin (Figure 3), and to an aminoglycoside compound (Supporting Information) was examined. Conjugation to BSA and IgG

Table 2. Labeling Yields, as Indicated by the Percentage of Radioactivity Retained after Ultrafiltration of the Reaction Mixture, from Incubation of *N*-Succinimidyl 3-[^{125}I]Iodobenzoate (7) or Unactivated 3-[^{125}I]Iodobenzoic Acid (6) with Bovine Serum Albumin or IgG Protein

radiolabeling group	protein	radioactivity retained (%)
N-succinimidyl 3-[^{125}I]iodobenzoate	Bovine serum albumin	67.6
N-succinimidyl 3-[^{125}I]iodobenzoate	Goat IgG	67.3
3-[^{125}I]iodobenzoic acid (control)	Bovine serum albumin	2.5

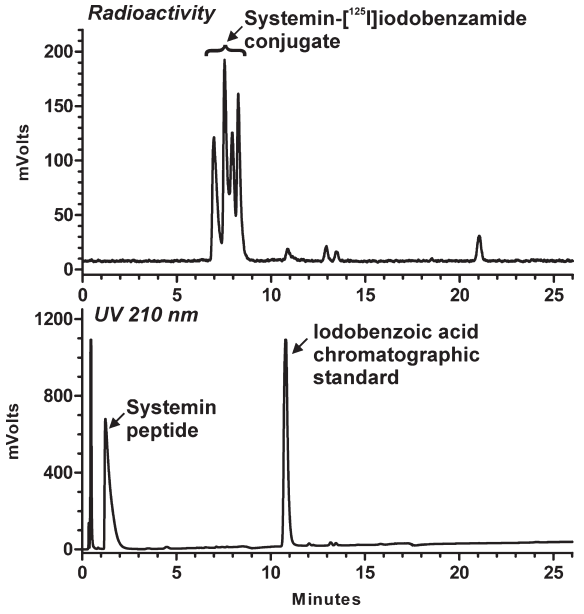

Figure 3. HPLC analysis of the reaction medium following incubation of *N*-succinimidyl 3-[^{125}I]iodobenzoate (**7**) with tomato plant systemin peptide. Unlabeled iodobenzoic acid (10 μg) included as a chromatographic standard.

was performed in an aqueous bicarbonate buffer followed by ultrafiltration through a MW 3500 cutoff membrane to separate the radiolabeled protein from unbound [^{125}I]]iodobenzoate. Using this approach, yields of 67% relative to the added *N*-succinimidyl 3-[^{125}I]iodobenzoate radioactivity were obtained for both proteins. Unactivated 3-[^{125}I]iodobenzoic acid was not significantly retained by the filters. For conjugation to tomato plant systemin, yields of 69% and 92% for the combined peaks and relative to the added *N*-succinimidyl 3-[^{125}I]iodobenzoate radioactivity were obtained in two separate runs, as determined by HPLC analysis of the peptide conjugate. Several individual [^{125}I] peaks could be resolved in the HPLC chromatogram. This probably reflects labeling of different amine residues on the peptide, since this water-soluble peptide contains 3 lysine amine groups and one terminal amino group,[23] although the possibility of partial conjugation to co-eluting truncated impurities that may have been present in the original peptide sample also exists.

■ CONCLUSIONS

In summary, we have examined an approach for conjugate prosthetic group labeling of protein and peptide labeling in which the organotin precursor for the radioiodinated prosthetic group

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is tethered to a solid support via the tin leaving group. Exposing the polymer-bound organotin precursor to electrophilic radioiodine releases the radiolabeled prosthetic group into solution, with the unreacted organotin precursor and organotin byproducts remaining bound to the resin. This approach thus simplifies the process of radiolabeling of proteins and peptides via the conjugate radiolabeled group approach by reducing the need for postiodination purification of the prosthetic group prior to conjugating it to biological macromolecules. By avoiding the need for HPLC, the quantity of radioactive waste generated in the labeling process is also greatly reduced. This solid-phase approach may have additional applications for development of a simple kit formulation for radiochemical labeling of protein and peptide molecules or for development of automated radiolabeling processes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental procedures and analytical characterization of organotin and peptide chromatographic standards. ESI-MS analysis for an example of a solution-phase iododestannylation reaction and from cold (unlabled) iodination of resin (**4**). HPLC radiochromatograms from high-activity radioiodination of resin (**5**) and from conjugation of (**7**) to an aminoglycoside compound. This material is available free of charge via the Internet at http://pubs.acs.org.

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