Synthesis of I-125 Labeled Photoaffinity Rapamycin Analogs

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Summary

Two no-carrier-added ^{125}I-labeled photoaffinity rapamycin analogs were prepared: 7-demethoxy-7-(4-azido-3-^{125}I-benzyloxy)rapamycin (**2**) and its C_{28}-C_{29} seco analog **3**. The key reactions of the synthesis were substitution of the C_7 methoxyl of rapamycin (**1**) with 4-azido-3-tributylstannylbenzyloxy group, exchange of tributyltin with ^{125}I using Na^{125}I and Chloramine-T, and a $ZnCl_2$ mediated retro-Aldol cleavage of the C_{28}-C_{29} bond of rapamycin.

Key words: ^{125}I-labeled rapamycin photoaffinity labeling analogs, electrophilic destannylation.

Introduction

Rapamycin (**1**), discovered in the 1970s, is a macrolide produced by *Streptomyces hydroscopus* with potent antifungal and immunosuppressant activities.[1,2] It has received renewed interest due to its structural resemblance to FK506 (tacrolimus),[3] a drug recently approved for use in organ transplantation therapy. Although rapamycin and FK506 bind to the same intracellular protein (FKBP12),[4] the immunophilin-ligand complexes interfere with different signaling pathways. FK506-FKBP12 (and cyclosporin A-cyclophilin) bind to and inhibit the Ca^{2+}/calmodulin dependent serine/threonine phosphatase calcineurin (PP2B).[5,6] Recently, several communications have been published on the isolation of proteins which bind to the rapamycin-FKBP12 complex using methods of affinity chromatography.[7,8] These reports have prompted us to describe our

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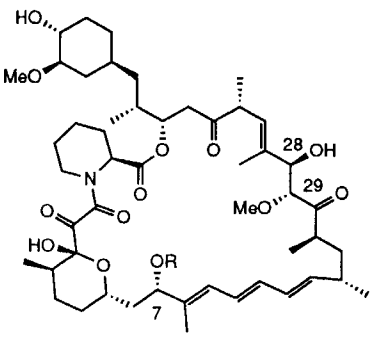

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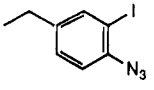
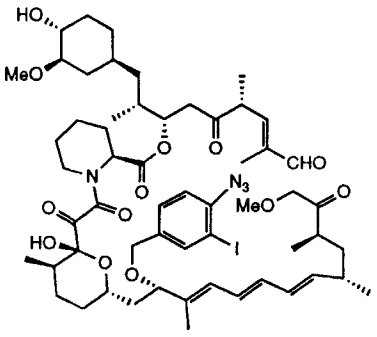
2, R = 



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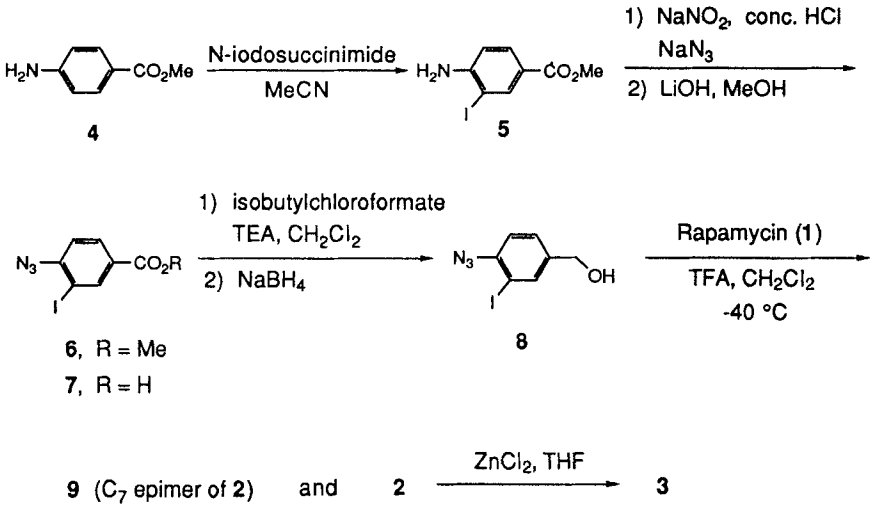
approach to identify the rapamycin downstream target by radiophotoaffinity labeling experiments. In order to sort out the variety of FKBPs from the downstream target, we designed two probes, **2** (7-demethoxy-7-(4-azido-3-iodobenzyloxy)rapamycin), an analog of rapamycin that retained immunosuppressive activity in our splenocyte proliferation assays and **3**, the C_{28}-C_{29} seco derivative of **2**, that retained FKBP12 rotamase inhibition activity, but was devoid of immunosuppressive activity. Thus, **2** would be used to label FKBPs in addition to the downstream target, and **3** would be used to only label FKBPs; thus, by eliminating the common protein bands, we hoped to identify the rapamycin downstream target.

Results and Discussion

The preparation of rapamycin photoaffinity labeling agent **2** is depicted in Scheme I.[9] Monoiodination of methyl 4-aminobenzoate (**4**) gave iodoester **5**, which was in turn diazotized and treated with NaN_3 to furnish azido derivative **6**. Hydrolysis of the ester and reduction of the corresponding acid **7** led to alcohol **8**. This multifunctional subunit was attached to rapamycin at the C_7 position under protic acid promoted solvolytic conditions, leading to target compound **2**. Its C_7 epimer **9** was also produced among an array of byproducts in this coupling reaction. The desired isomer **2** was isolated in 15% yield by HPLC purification, which was necessary to effect separation from epimer **9** (isolated in 7% yield). Selective retroaldol cleavage at C_{28} and C_{29} of the macrocycle, mediated by $ZnCl_2$ in THF, afforded seco derivative **3**.[10]

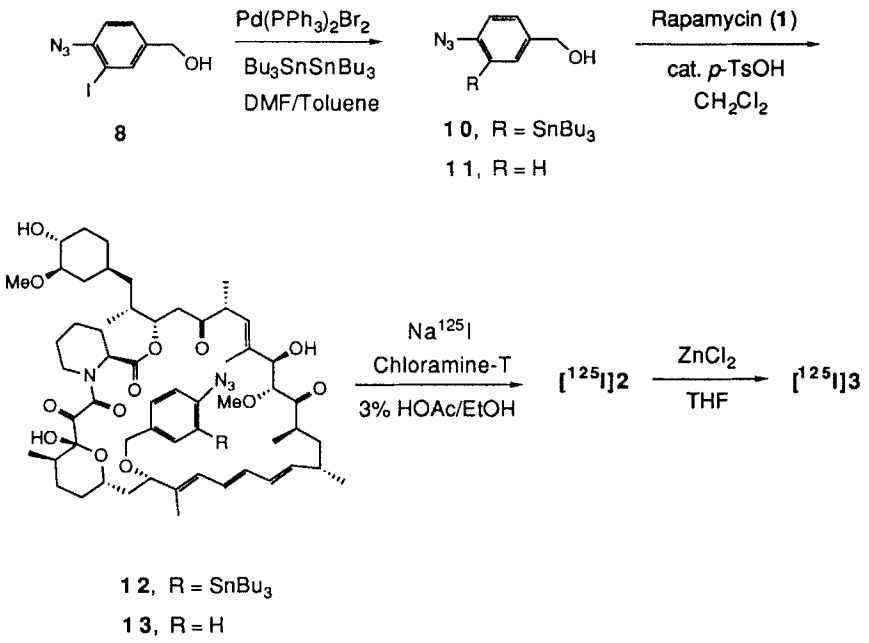
Scheme I



Although the above scheme could lend itself to the preparation of radioiodinated analogs, label would have to be introduced at the very outset of the sequence and carried through a number of steps including the low yielding solvolytic coupling reaction. An ideal alternative would be a one-step procedure to incorporate I-125 nuclide into rapamycin analog **2**. Recent applications of organotin compounds in radiohalogenation drew our attention to the stannylated substrate **12** as a possible substrate for electrophilic tin-halogen exchange to fulfill the above strategy.[11,12] The most direct approach to **12** would be immediate replacement of the iodo group by a stannyl substituent in rapamycin photoaffinity agent **2**. However, the complexity of the multifunctional macrocycle and limited supply of **2** led us instead to adopt a more conservative plan involving first incorporating the tin moiety into alcohol **8**, and then attaching the resulting azido-trialkyltin benzyl alcohol onto rapamycin.

Palladium-mediated coupling of **8** with hexabutyldistannane furnished the tin derivative **10** in 62% isolated yield.[13,14] Initial attempts to attach **10** onto rapamycin employing trifluoroacetic acid (TFA) as the acid promoter met with limited success.[15] When rapamycin was treated with an equivalent amount of TFA at -40 °C to generate a C_7 carbocation with extended conjugation, followed by quenching with excess stannyl alcohol **10**, substantial quantities of protodestannylation product **11** and its corresponding rapamycin adduct **13** were recovered. A better yield of the desired rapamycin tin derivative **12** was obtained by an alternative procedure: rapamycin and mixing of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) together with an excess of tin alcohol **10** in

Scheme II



8

10, R = $SnBu_3$

11, R = H

12, R = $SnBu_3$

13, R = H

methylene chloride at room temperature. Since *p*-TsOH was consumed in the protodestannylation of **10** and **12**, periodic replenishment of this reagent was needed to drive the coupling reaction. The desired rapamycin stannylated substrate **12** was purified by HPLC in 14% isolated yield. Subsequent electrophilic radioiodine-trialkyltin exchange of **12** turned out to be straightforward. Stability studies showed that rapamycin itself remained intact under slightly acidic conditions (3% acetic acid in ethanol as solvent) in the presence of chloramine-T and NaI.[16] Hence, no effort was made to screen other oxidants. During trial runs of iodine-trialkyltin exchange of substrate **12** under the above conditions (reaction time of 30-40 min), protodestannylation product **13** was detected but found to be separable from **2** by reverse-phase HPLC. Subsequently, several radioiodinations were performed starting from carrier-free $Na^{125}I$ in scales ranging from 1 to 7 mCi (Table I). After HPLC purification, **[^{125}I]2** was obtained in 44-64% radiochemical yields with 96-99% radiochemical purities. The seco derivative was prepared by treament of thoroughly dried **[^{125}I]2** (3.7 mCi) with $ZnCl_2$ in THF at room temperature for 17 h. HPLC purification gave **[^{125}I]3** in 19% radiochemical yield with 96% radiochemical purity.

Table I

Amount of $Na^{125}I$	Yield of $[^{125}I]2$	RCP of $[^{125}I]2$[a]
7.0 mCi	4.2 mCi (60%)	96.2%
5.0 mCi	3.2 mCi (64%)	93.8%
5.0 mCi	2.9 mCi (58%)	80.6%[b]
3.2 mCi	1.5 mCi (47%)	97.3%
1.0 mCi	0.44 mCi (44%)	85.6%[c]
1.0 mCi	0.63 mCi (63%)	99.1%

[a]Radiochemical purities (RCPs) were determined immediately after HPLC purification.

[b]RCP was determined after 72 h storage at -80 °C.

[c]Sample was evaporated to dryness and redissolved in ethanol.

In summary, ^{125}I-labeled rapamycin photoaffinity labeling agents were synthesized by a sequence consisting of replacement of an iodo group with trialkyltin in a benzyl alcohol subunit, attachment of this stannyl subunit onto rapamycin, and radioiodination of the resulting rapamycin tin derivative under electrophilic iodine-trialkyltin exchange conditions.

Experimental

All reactions were carried out under an inert atmosphere. Reaction solvents were distilled by standard methods prior to usage. Chemicals were purchased from the Aldrich Chemical Company. Rapamycin was supplied by the SB Biologicals Pilot Plant, Brockham Park. Carrier-free $Na^{125}I$ (specific activity: 2175 Ci/mmol) was purchased from New England Nuclear with a concentration of 134 mCi/mL in pH=8-10 aqueous solution. ^1H NMR were recorded on a Bruker AM400 instrument with $CDCl_3$ as solvent. Analytical radio-HPLC profiles were recorded on a Ramona-D radioactivity detector (tritium channel). Radioactive concentrations were determined by scintillation counting using an external quenching curve. Mass spectroscopic data were determined in chemical ionization (CI) mode with specified reagent gases. The tin containing ions are diagnostic by their clusters of ten isotopes.

Methyl 4-Amino-3-iodobenzoate (5)

To a stirred homogeneous solution of methyl 4-aminobenzoate (8.0 g, 53 mmol) in CH_3CN (50 mL) at room temperature was added one drop of concentrated sulfuric acid. A precipitate developed instantly. To the resulting mixture was added N-iodosuccinimide (12.8 g, 57 mmol) in one portion. After stirring at room temperature for 2 h, the suspension was quenched with pH=7 buffer (50 mL). Extraction with EtOAc, drying of the organic phase over anhydrous $MgSO_4$, filtration, concentration *in vacuo*,

and purification by flash column chromatography gave **5** (10.0 g, 67% yield). ^{1}H NMR: 3.85 (3H, s, CO_2**Me**), 6.70 (1H, d, J = 8.4 Hz, 5-**H**), 7.81 (1H, dd, J = 8.4 and 1.7 Hz, 6-**H**), 8.33 (1H, d, J = 1.7 Hz, 2-**H**); MS (CI/CH_4), m/z (%): 278 (100, $(M+H)^+$), 246 (46, $(M+H\text{-}MeOH)^+$), 151 (40, $(M+H\text{-}I)^+$).

Methyl 4-Azido-3-iodobenzoate (**6**)

To a stirred solution of **5** (2.66 g, 9.7 mmol) in MeOAc (25 mL) cooled in an ice bath was added concentrated hydrochloric acid (8 mL) over a period of of 2 min, resulting in a milky suspension. To this suspension was added $NaNO_2$ (2.00 g, 29.0 mmol in 5 mL of H_2O) in one portion. The mixture was stirred vigorously at ice-bath temperature for 45 min, then NaN_3 (2.00 g, 31.0 mmol) was added portionwise over a period of 15 min. After another 15 min period when gas evolution ceased, the mixture was quenched with pH=7 buffer (50 mL). Extraction with EtOAc (180 mL), drying of the organic phase over anhydrous $MgSO_4$, filtration, concentration *in vacuo* gave **6** (2.81 g, 105% yield). ^{1}H NMR: 3.91 (3H, s, CO_2**Me**), 7.17 (1H, d, J = 11.2 Hz, 5-**H**), 8.05 (1H, dd, J = 11.2 and 2.5 Hz, 6-**H**), 8.46 (1H, d, J = 2.4 Hz, 2-**H**); MS (CI/CH_4), m/z (%): 304 (57, $(M+H)^+$), 276 (100, $(M+H\text{-}N_2)^+$), 149 (28, $(M+H\text{-}N_2\text{-}I)^+$).

4-Azido-3-iodobenzyl alcohol (**8**)

To a stirred suspension of **6** (2.30 g, 7.6 mmol) in a mixture of MeOH (15 mL) and H_2O (5 mL) was added $LiOH.H_2O$ (2.50 g, 59.5 mmol) in one portion. The mixture was stirred at room temperature for 3 h and 6 °C for 18 h. The reaction was quenched by acidification with 6N aqueous HCl at ice-bath temperature. Extraction with EtOAc (150 mL), drying of the organic phase over anhydrous $MgSO_4$, filtration, and concentration *in vacuo* gave the corresponding acid as a white solid (2.14 g, 97% yield). To a stirred solution of a portion of the acid **7** (1.11 g, 3.9 mmol) and triethylamine (500 mg) in CH_2Cl_2 (15 mL) at room temperature was added isobutylchloroformate (1.5 mL, 12 mmol) in one portion. Stirring at room temperature was continued for 45 min, followed with addition of a suspension of $NaBH_4$ (1.20 g, 31.8 mmol) in absolute EtOH (8 mL) over a period of 20 minutes. The reaction was quenched with pH=7 buffer (30 mL). Extraction with EtOAc (120 mL), drying of the organic phase over anhydrous $MgSO_4$, filtration, concentration *in vacuo*, and purification by flash column chromatography afforded **8** as a solid (0.74 g, 70% yield). ^{1}H NMR: 4.64 (2H, s, Ar-CH_2OH), 7.12 (1H, d, J = 8.2 Hz, 5-**H**), 7.39 (1H, dd, J = 8.2 and 1.7 Hz, 6-**H**), 7.81 (1H, d, J = 1.7 Hz, 2-**H**); MS (CI/CH_4), m/z (%): 276 (11, $(M+H)^+$), 258 (13, $(M+H\text{-}H_2O)^+$), 248 (13, $(M+H\text{-}N_2)^+$), 230 (59, $(M+H\text{-}H_2O\text{-}N_2)^+$).

7-Demethoxy-7-(4-azido-3-iodobenzyloxy)rapamycin (**2**) and
7-Demethoxy-7-epi-(4-azido-3-iodobenzyloxy)rapamycin (**9**)

Trifluoroacetic acid (20 μL, 0.26 mmol) was added to a solution of rapamycin (20 mg, 0.02 mmol) in CH_2Cl_2 (mL) at -45 °C, and the resulting bright yellow solution was stirred for 5 min. Alcohol **8** (20 mg, 0.07 mmol) in CH_2Cl_2 (0.2 mL) was added and the mixture was stirred for 15 min. The reaction was diluted with pH=7 buffer (5 mL) and extracted with EtOAc (3 x 10 mL), dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Preparative TLC (silica gel plate, 250 micron, mobile phase: MeOH/CH_2Cl_2/EtOAc/petroleum ether (v/v/v/v) 6/54/20/20) separated excess alcohol **8** from rapamycin type adducts. The rapamycin related products were then purified by preparative HPLC (Rainin silica column (S2-125), 10/190/50/50 (v/v/v/v) MeOH/CH_2Cl_2/petroleum ether/EtOAc, 20 mL/min, UV at 254 nm) to afford **2** (3 mg, 15% yield, R_t: 5.7 min). ^1H NMR (4:1 mixture of *trans,cis*-rotamers; data for *trans*-rotamer): 0.67 (q, J = 12 Hz, 41-**H**), 0.92 (3H, d, J = 6.7 Hz), 0.95 (3H, d, J = 6.6 Hz), 0.99 (3H, d, J = 6.6 Hz), 1.05 (3H, d, J = 6.7 Hz), 1.10 (3H, d, J = 6.7 Hz), 1.15 (3H, t, J = 6.9 Hz), 1.66 (3H, s), 1.75 (3H, s), 2.58 (dd, J = 17, 6.3 Hz, 23-**H**), 3.34 (3H, s), 3.41 (3H, s), 3.54 (d, J = 14 Hz, 16-**H**), 3.69 (d, J = 5.9 Hz, 29-**H**), 3.78 (dd, J = 7.8, 7 Hz, 7-**H**), 3.82 (1H, m, 9-**H**), 4.16 (d, J = 5.9 Hz, 28-**H**), 4.38 (2H, AB q, J_{AB} = 15.2 Hz, $\Delta\delta$ = 0.032 ppm), 4.85 (s, 13-O**H**), 5.14 (m, 22-**H**), 5.23 (m, 20-**H**), 5.41 (d, J = 9.7 Hz, 26-**H**), 5.53 (dd, J = 14.9, 9.2 Hz, 1-**H**), 5.92 (d, J = 10.3 Hz, 5-**H**), 6.13 (dd, J = 14.9, 9.2 Hz, 2-**H**), 6.30 (dd, J = 14.8, 9.6 Hz, 3-**H**), 6.38 (dd, J = 14.8, 10.3 Hz, 4-**H**), 7.10 (1H, d, J = 7.8 Hz), 7.31(1H, dd, J = 2.3, 7.8 Hz,), 7.72 (1H, d, J = 2.3 Hz).

Further elution gave the C_7 epimer **9** (1.5 mg, 7% yield, R_t: 6.5 min). ^1H NMR (3:1 mixture of *trans,cis*-rotamers; data for *trans*-rotamer): 0.65 (q, J = 12 Hz, 41-**H**), 0.86 (3H, d, J = 6.6 Hz), 0.93 (3H, d, J = 6.5 Hz), 0.94 (3H, d, J = 6.5 Hz), 1.01 (3H, d, J = 6.6 Hz), 1.06 (3H, d, J = 6.7 Hz), 1.18 (3H, t, J = 7 Hz), 1.65 (3H, s), 1.75 (3H, s), 2.38 (dd, J = 17.4, 8.5 Hz, 23-**H**), 2.72 (dd, J = 17.4, 2.7 Hz, 23-**H**), 3.33 (3H, s), 3.39 (3H, s), 3.70 (dd, J = 17.4, 2.7 Hz, 7-**H**), 4.01 (1H, d, J = 3.6 Hz, 29-**H**), 4.04-4.10 (m, 9-**H**), 4.26 (s, 28-**H**), 4.39 (2H, AB q, J_{AB} = 8 Hz, $\Delta\delta$ = 0.047 ppm), 4.66 (s, 13-O**H**), 5.20-5.25 (2H, m, 20-**H** and 22-**H**), 5.42 (d, J = 10.3 Hz, 26-**H**), 5.47 (dd, J = 14.4, 9.1 Hz, 1-**H**), 6.05-6.12 (m, 2-**H** and 5-**H**), 6.18 (dd, J = 14.2, 10.4 Hz, 3-**H**), 6.37 (dd, J = 14.2, 11 Hz, 4-**H**), 7.12 (1H, d, J = 7.8 Hz), 7.37 (1H, dd, J = 7.8, 2.3 Hz), 7.74 (1H, d, J = 2.3 Hz).

7-Demethoxy-7-(4-azido-3-iodobenzyloxy)-28(29)-secorapamycin (**3**)

Rapamycin derivative **2** (2 mg) and anhydrous $ZnCl_2$ (2 mg) in tetrahydrofuran (0.4 mL) was stirred under argon at room temperature for 36 h. The mixture was diluted with pH=7 buffer, extracted with EtOAc. Drying of the organic phase over anhydrous $MgSO_4$, filtration,

concentration *in vacuo*, and HPLC purification (Rainin silica column (S2-125), 10/190/50/50 (v/v/v/v) MeOH/CH_2Cl_2/petroleum ether/EtOAc, 20 mL/min, UV at 254 nm, R_t: 7.7 min) gave seco derivative **3** (1.3 mg, 65% yield). ^{1}H NMR (2:1 mixture of *trans,cis*-rotamers; data for *trans*-rotamer): 0.71 (q, J = 12 Hz, 41-**H**), 0.88 (3H, d, J = 6.5 Hz), 0.94 (3H, d, J = 6.6 Hz), 0.99 (3H, d, J = 6.7 Hz), 1.05 (3H, d, J = 6.6 Hz), 1.08 (3H, J = 6.6 Hz), 1.76 (3H, s), 1.87 (3H, d, J = 1.7 Hz), 3.42 (3H, s), 3.43 (3H, s), 4.09 (d, J = 10 Hz, 29-**H**), 4.35 (2H, AB q, J_{AB} = 15 Hz, $\Delta\delta$ = 0.03 ppm), 4.67 (s, 13-O**H**), 5.54 (dd, J = 14.5, 9.2 Hz, 1-**H**), 6.02 (d, J = 10.5 Hz, 5-**H**), 6.15 (dd, J = 14.7, 9.2 Hz, 2-**H**), 6.38 (dd, J = 14.7, 9.3 Hz, 3-**H**), 6.47 (dd, J = 14.7, 10 Hz, 4-**H**), 7.18 (1H, dd, J = 7.1, 2.3 Hz), 7.33 (1H, d, J = 6.1 Hz), 7.71 (1H, d, J = 2.3 Hz), 9.47 (s, 28-**H**).

4-Azido-3-tributylstannylbenzyl alcohol (**10**)

A mixture of **8** (300 mg, 1.09 mmol), $Pd(PPh_3)_2Br_2$ (50 mg, 0.06 mmol), bis(tributyltin) (1.0 mL, 2 mmol) in toluene (6 mL), and DMF (2 mL) in a 20 mL vial purged with argon was heated in an oil bath with a temperature of 95-105 °C for 25 min. The mixture was cooled in an ice bath, diluted with saturated aqueous sodium carbonate solution (10 mL) and extracted with EtOAc (60 mL). The organic phase was washed with water (3 x 10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was passed through a short silica gel column (packed in a disposable pipette), and eluted with hexane (12 mL), CH_2Cl_2 (8 mL), and then EtOAc (8 mL). The CH_2Cl_2 and EtOAc fractions were combined and concentrated under reduced pressure. The residue was quickly purified by flash silica gel column chromatography to provide **10** (295 mg, 62% yield). ^{1}H NMR: 0.88 (9H, t, J = 7.3 Hz, CH_3CH_2-), 1.08 (6H, t, J = 8.0 Hz, -CH_2-Sn), 1.29-1.35 (6H, m, CH_3CH_2-), 1.48-1.54 (6H, m, -CH_2-CH_2Sn), 4.66 (2H, s, Ar-CH_2OH), 7.13 (1H, d, J = 8.6 Hz), 7.31-7.41 (2H, br s); MS (CI/CH_4), m/z: 436 $(M+H)^+$.

7-Demethoxy-7-(4-azido-3-tributylstannylbenzyloxy)rapamycin (**12**)

To a mixture of **10** (60 mg, 0.14 mmol) and rapamycin (20 mg, 0.02 mmol) in CH_2Cl_2 (0.4 mL) at room temperature was added a tiny crystal of *p*-TsOH at 15 min intervals. After 4 additions (total reaction time of 1 h), the mixture was passed through a short silica gel column, and eluted with CH_2Cl_2 (8 mL), CH_2Cl_2/EtOAc (6 mL/2 mL), EtOAc (16 mL). The EtOAc fraction was collected. The solvent was removed by evaporation under a stream of nitrogen gas. The residue was purified by normal-phase HPLC (Dynamax silica gel preparative column (8 μm, 2.14 cm I.D. x 25 cm), 7/200/60/40 (v/v/v/v) MeOH/CH_2Cl_2/hexane/EtOAc, 14 mL/min, UV at 254 nm, R_t: 18-20 min). Removal of HPLC mobile phase under nitrogen and drying under vacuum gave **12** (4 mg). The residue was stored in CH_2Cl_2 (2.5

mL). ^{1}H NMR ($CDCl_3$): (major *trans*-rotamer) 0.651 (1H, q, J = 12 Hz), 0.880 (H, t, J = 7.2 Hz), 0.926 (3H, d, J = 7.4 Hz), 0.933 (1H, d, J = 6.3 Hz), 0.985 (3H, d, J = 6.5 Hz), 1.049 (3H, d, J = 6.1 Hz), 1.077 (3H, d, J = 4.8 Hz), 1.256-1.379 (m), 1.470-1.562 (m),1.721 (3H, s), 1.734 (3H, s), 2.545 (1H, dd, J = 17, 6.5 Hz), 2.722 (1H, dd, J = 17, 6.5 Hz), 2.890-2.950 (1H, m), 3.326 (3H, s), 3.396 (3H, s), 3.450-3.553 (2H, m), 3.709 (1H, d, J = 6 Hz), 3.818-3.855 (1H, m), 4.386 (2H, q, J = 12 Hz), 5.140-5.200 (2H, m), 5.390 (1H, d, J = 10 Hz), 5.504 (1H, dd, J = 15, 9.3 Hz), 5.944 (1H, d, J = 11 Hz), 6.164 (1H, d, J = 10 Hz), 6.315 (1H, d, J = 10 Hz), 6.385 (1H, d, J = 11 Hz), 7.086 (1H, d, J = 7.8 Hz); MS (CI/NH_3), m/z: 1288 $(M-N_2)^+$. Tin derivative **12** underwent partial decomposition to protodestannylation product **13** during storage in $CDCl_3$. A mixture containing 20% of **13** was observed in the recovered NMR sample by UV-HPLC.

7-Demethoxy-7-(4-azido-3-^{125}I-benzyloxy)rapamycin ([^{125}I]**2**)

Many radioiodination runs were performed. A typical procedure involved evaporating a 50 µL of the above stock solution of **12** (0.8 µg, 0.055 µmol) in a 0.5 mL conical-bottomed vial to dryness under nitrogen. To the residue was added 50 µL of a 3/100 (v/v) HOAc/EtOH solution, followed by 15 µL of a stock solution of chloramine-T in water (0.0038 µmol). Carrier-free Na^{125}I (1.0 mCi) was transferred from its shipping vial via a 25 µL micro gas-tight syringe into the reaction vial. The former was rinsed with 2 x 25 µL of the 3/100 (v/v) HOAc/EtOH solution. The rinses were added to the reaction vial, then the mixture was allowed to stand at room temperature with occasional shaking. After 30 min, the reaction mixture was purified by HPLC (Zorbax phenyl reverse-phase column (5 µm, 4.6 mm I.D. x 25 cm), 88:12 (v/v) MeOH/$H_2$0, 1 mL/min, simultaneous UV at 270 nm and gamma radioactivity detection, R_t: 18.4 min). The collected HPLC eluate gave 0.63 mCi of [^{125}I]**2** with radiochemical purity of 99%.

7-Demethoxy-7-(4-azido-3-^{125}I-benzyloxy)-28(29)-secorapamycin ([^{125}I]**3**)

The HPLC solvent (2 mL) of a 3.7 mCi portion of [^{125}I]**2** prepared as described above was removed by evaporation under nitrogen at room temperature (30 min). The residue was dried under vacuum for 1 h, and then redissolved in tetrahydrofuran (0.5 mL), followed by addition of anhydrous $ZnCl_2$ (10 mg). The mixture was stirred at room temperature under darkness for 17 h. Tetrahydrofuran was removed by evaporation under nitrogen. The residue was taken up in pH=7 buffer (0.6 mL) and MeOH (0.6 mL), and purified by HPLC (Zorbax Phenyl reverse-phase column (5µm, 4.6 mm I.D. x 25 cm), 88/12 (v/v) MeOH/H_2O, 1 mL/min, detected by UV at 270 nm and a gamma radioactivity detector, R_t: 20.5 min). The collected HPLC eluate gave 0.69 mCi of [^{125}I]**3** with radiochemical purity in excess of 96%.

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