

Synthesis of I-125 Labeled Photoaffinity Rapamycin Analogs

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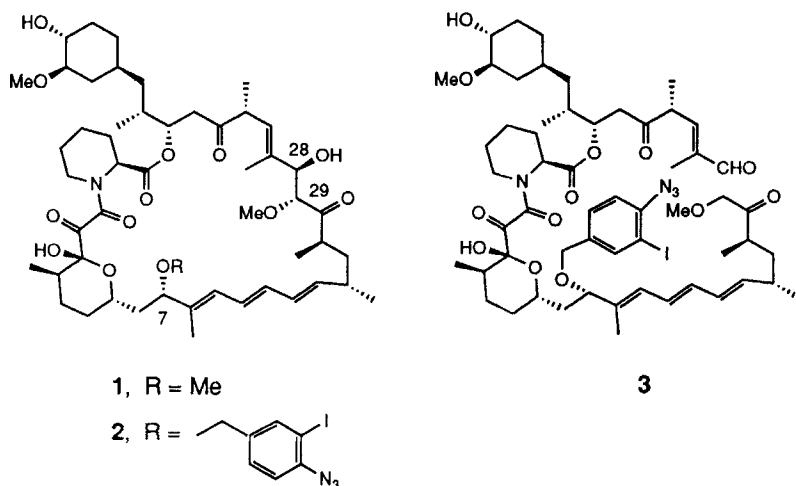
Summary

Two no-carrier-added ¹²⁵I-labeled photoaffinity rapamycin analogs were prepared: 7-demethoxy-7-(4-azido-3-¹²⁵I-benzyloxy)rapamycin (**2**) and its C₂₈-C₂₉ seco analog **3**. The key reactions of the synthesis were substitution of the C₇ methoxyl of rapamycin (**1**) with 4-azido-3-tributylstannylbenzyloxy group, exchange of tributyltin with ¹²⁵I using Na¹²⁵I and Chloramine-T, and a ZnCl₂ mediated retro-Aldol cleavage of the C₂₈-C₂₉ bond of rapamycin.

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

Introduction

Rapamycin (**1**), discovered in the 1970s, is a macrolide produced by *Streptomyces hydropiscus* 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 Ca²⁺/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

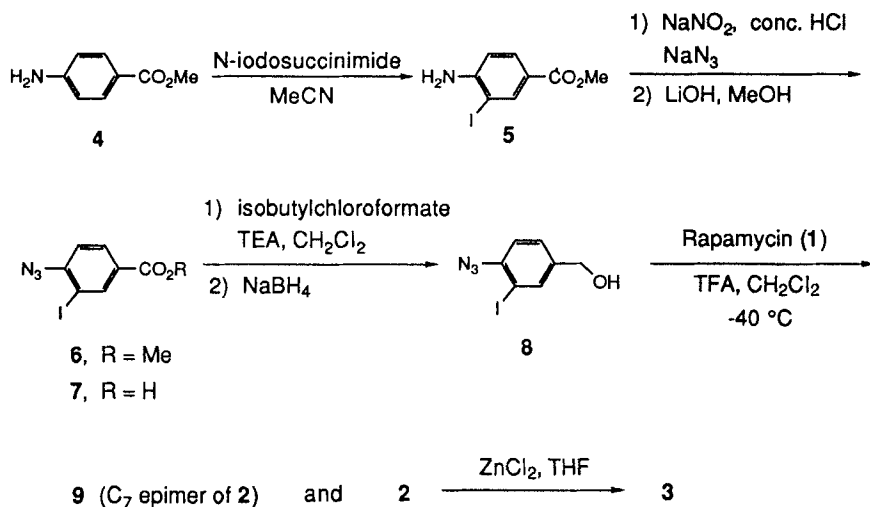


approach to identify the rapamycin downstream target by radio-photoaffinity labeling experiments. In order to sort out the variety of FKBP s from the downstream target, we designed two probes, **2** (7-demethoxy-7-(4-azido-3-iodobenzoyloxy)rapamycin), an analog of rapamycin that retained immunosuppressive activity in our splenocyte proliferation assays and **3**, the C₂₈-C₂₉ seco derivative of **2**, that retained FKBP12 rotamase inhibition activity, but was devoid of immunosuppressive activity. Thus, **2** would be used to label FKBP s in addition to the downstream target, and **3** would be used to only label FKBP s; 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 1.⁹ Monoiodination of methyl 4-aminobenzoate (**4**) gave iodoester **5**, which was in turn diazotized and treated with NaN₃ 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 C₇ position under protic acid promoted solvolytic conditions, leading to target compound **2**. Its C₇ 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 C₂₈ and C₂₉ of the macrocycle, mediated by ZnCl₂ in THF, afforded seco derivative **3**.¹⁰

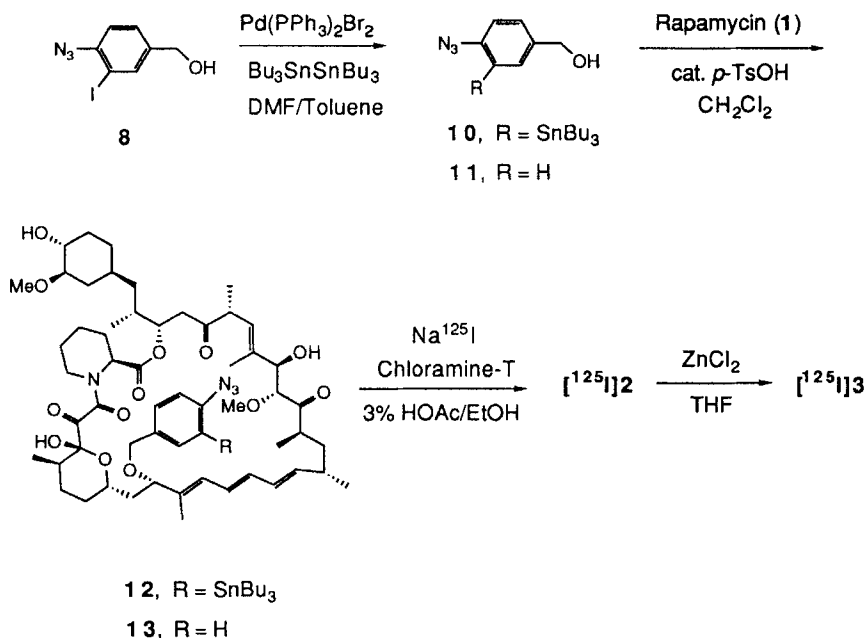
Scheme I



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 C₇ 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



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 Na¹²⁵I in scales ranging from 1 to 7 mCi (Table I). After HPLC purification, [¹²⁵I]**2** was obtained in 44–64% radiochemical yields with 96–99% radiochemical purities. The seco derivative was prepared by treatment of thoroughly dried [¹²⁵I]**2** (3.7 mCi) with ZnCl₂ in THF at room temperature for 17 h. HPLC purification gave [¹²⁵I]**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%

^aRadiochemical purities (RCPs) were determined immediately after HPLC purification.

^bRCP was determined after 72 h storage at -80 °C.

^cSample was evaporated to dryness and redissolved in ethanol.

In summary, ¹²⁵I-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 Na¹²⁵I (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 CDCl₃ 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 CH₃CN (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 MgSO₄, filtration, concentration *in vacuo*,

and purification by flash column chromatography gave **5** (10.0 g, 67% yield). ^1H NMR: 3.85 (3H, s, CO_2Me), 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, $(\text{M}+\text{H})^+$), 246 (46, $(\text{M}+\text{H}-\text{MeOH})^+$), 151 (40, $(\text{M}+\text{H}-\text{I})^+$).

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 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). ^1H NMR: 3.91 (3H, s, CO_2Me), 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, $(\text{M}+\text{H})^+$), 276 (100, $(\text{M}+\text{H}-\text{N}_2)^+$), 149 (28, $(\text{M}+\text{H}-\text{N}_2-\text{I})^+$).

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 $\text{LiOH}\cdot\text{H}_2\text{O}$ (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). ^1H NMR: 4.64 (2H, s, $\text{Ar}-\text{CH}_2\text{OH}$), 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, $(\text{M}+\text{H})^+$), 258 (13, $(\text{M}+\text{H}-\text{H}_2\text{O})^+$), 248 (13, $(\text{M}+\text{H}-\text{N}_2)^+$), 230 (59, $(\text{M}+\text{H}-\text{H}_2\text{O}-\text{N}_2)^+$).

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

Trifluoroacetic acid (20 μ L, 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_f : 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-OH), 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_f : 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-OH), 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/CH₂Cl₂/petroleum ether/EtOAc, 20 mL/min, UV at 254 nm, R_t: 7.7 min) gave seco derivative **3** (1.3 mg, 65% yield). ¹H 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, Δδ = 0.03 ppm), 4.67 (s, 13-OH), 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(PPh₃)₂Br₂ (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 MgSO₄, 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), CH₂Cl₂ (8 mL), and then EtOAc (8 mL). The CH₂Cl₂ 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). ¹H NMR: 0.88 (9H, t, J = 7.3 Hz, CH₃CH₂-), 1.08 (6H, t, J = 8.0 Hz, -CH₂-Sn), 1.29-1.35 (6H, m, CH₃CH₂-), 1.48-1.54 (6H, m, -CH₂-CH₂Sn), 4.66 (2H, s, Ar-CH₂OH), 7.13 (1H, d, J = 8.6 Hz), 7.31-7.41 (2H, br s); MS (CI/CH₄), 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 CH₂Cl₂ (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 CH₂Cl₂ (8 mL), CH₂Cl₂/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/CH₂Cl₂/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 CH₂Cl₂ (2.5

mL). ^1H 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 ($\text{M}-\text{N}_2$) $^+$. 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]**12**)

Many radiiodination 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_2O , 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]**12** with radiochemical purity of 99%.

7-Demethoxy-7-(4-azido-3- ^{125}I -benzyloxy)-28(29)-secorapamycin ([^{125}I]**13**)

The HPLC solvent (2 mL) of a 3.7 mCi portion of [^{125}I]**12** 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 (5 μm , 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]**13** with radiochemical purity in excess of 96%.

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Notes and References

1. Venzina, C., Kudelski, A. and Sehgal, S.N.- *J Antibiot (Tokyo)* **28**: 721 (1975).
2. Martell, R. R., Klincius, J. and Galet, S.- *Can. J. Physiol. Pharmacol.* **55**: 48 (1977).
3. Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M. and Aoki, H., Imanaka, H.- *J Antibiotics (Tokyo)* **40**: 1249 (1987).
4. (a) Siekierka, J.J., Hung, S.H., Poe, M., Lin, C.S. and Sigal, N.H.- *Nature* **341**: 755 (1989). (b) Harding, M.W., Galat, A., Uehling, D.E. and Schreiber, S.L.- *Nature* **341**: 758 (1989).
5. Friedman, J. and Weissman, I.- *Cell* **66**: 799 (1991).
6. Liu, J., Farmer, J.D., Lane, W.S., Friedman, J., Weissman, I. and Schreiber, S.L.- *Cell* **66**: 807 (1991).
7. Brown, E.J., Albers, M.W., Shin, T.B., Ichikawa, K., Keith, C.T., Lane, W.S. and Schreiber, S.L.- *Nature* **369**: 756 (1994).
8. Sabatini, D.M., Erdjument-Bromage, H., Lui, M., Tempst, P. and Snyder, S.H.- *Cell* **78**: 35 (1994).
9. Luengo, J.I., Yamashita, D.S., Dunnington, D., Konialian-Beck, A., Rozamus, L.W., Yen, H.-K., Bossard, M.J., Levy, M.A., Hand, A., Newman-Tarr, T., Badger, A., Faucette, L., Johnson, R.K., D'Alessio, K., Porter, T., Shu, A.Y.L., Heys, J.R., Choi, J., Kongsærre, P., Clardy, J. and Holt, D.A.- *Chemistry and Biology* **2**: 471 (1995).
10. Luengo, J.I., Konialian, A. L. and Holt, D.A.- *Tetrahedron Lett.* **34**: 991 (1993).

11. Adam, M.J., Ruth, T.J., Pate, B. and Hall, L.D.- *J. Chem. Soc., Chem. Commun.*: 625 (1982).
12. Seevers, R.H. and Counsell, R.E.- *Chem Rev.* **82**: 575 (1982).
13. Azizian, H., Eaborn, C. and Pidcock, A.- *J. Organomet. Chem.* **215**: 49 (1981).
14. Stille, J.K.- *Angew. Chem. Int. Ed. Engl.* **25**: 508 (1986).
15. Luengo, J.I., Konialian-Beck, A., Rozamus, L.W. and Holt, D.A.- *J. Org. Chem.* **59**: 6512 (1994).
16. Hunter, W.M. and Greenwood, F.C.- *Nature* **194**: 495 (1962).