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Iodophenyl tagged sphingosine derivatives: Synthesis and preliminary biological evaluation

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ABSTRACT

A facile synthesis of six 4-iodophenyl tagged sphingosine (SP) derivatives bearing alkyl chain lengths from 6 to 13 is described. The key steps for the assembly of these molecules, **5a–f**, are Suzuki–Miyaura cross-coupling and cross-metathesis reactions. The feasibility of radiolabeling was demonstrated by synthesizing two ^{125}I labeled compounds, [**^{125}I**]**5c** and [**^{125}I**]**5e**. In vitro enzyme assays indicated that the molecules, **5c–e**, are potent inhibitors. Thus, they deserve further evaluation as potential radioactive probes for tumor imaging.

Phospholipids play important roles in a diverse range of cellular processes, including cell growth, apoptosis, migration, angiogenesis, and neurogenesis. Ceramide (Cer, **1**), sphingosine (SP, **2**), and sphingosine-1-phosphate (S1P, **3**) are key elements in this lipid family (Fig. 1). They are responsible for regulating various signal transductions in the sphingolipid signaling cascade. The dynamic balance between ceramide and sphingosine levels versus S1P, often described as the 'sphingolipid rheostat', provides an important factor in determining whether cells survive or die. One of the key enzymes balancing these three elements is sphingosine kinase (SphK), whose role is to catalyze the phosphorylation of SP to S1P. It has been demonstrated that one isoform of SphK, SphK1, is an oncogene and often overexpressed in many human solid tumors.[1–3] Therefore, SphK1 upregulation may be a useful biomarker for tumor and a potential target for cancer therapeutics.[4,5] Developing imaging tools that can non-invasively monitor changes of SphK1 in cells may assist in localizing tumors and also provide clues to the stages of tumor proliferation. Often, attaching a fluorescent dye or radioactive nuclide moiety to a biologically active molecule may provide a congener, which can serve as a surrogate for easily detecting biological activity via imaging. Several papers have been published on introducing different fluorescent dyes into Cer, SP, or S1P molecules. The bioactivities of these SP analogs containing a fluorescent dye at the tail-end of the alkyl chain (Fig. 2a) have also been evaluated and the results demonstrated the feasibility of using the substitution approach for developing SP analogs for fluorescence imaging of SphK.[6–10] So far, there are no reports of radionuclide labeled probes suitable for single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging. We have chosen to use the same terminal substitution method for preparing radionuclide labeled SP. These SP analogs, similar to the fluorescent dye-containing derivatives, are neutral and relatively small. Therefore, we expect that they would also fit into the SphK binding pocket. Particularly, we wish to test and delineate the effects of carbon chain length on the binding affinity toward SphK1 and SphK2. For this reason, SP analogs containing alkyl chain lengths from 6 to 13 were prepared and tested. It is important to note that these SP analogs may either serve as substrates for the SphK, or as competing inhibitors blocking the phosphorylation of the parent substrate, SP.

We report herein a facile synthesis of a series of 4-iodophenyl tagged SP derivatives, **5a–f**. Additionally, we also demonstrate the feasibility of radiolabeling these SP analogs by synthesizing two ^{125}I labeled SP derivatives, [**^{125}I**]**5c** and [**^{125}I**]**5e**. Moreover, we further evaluate the biological activity of this series of SP derivatives by SphK enzyme assays. The inhibition constants (IC_{50}) are correlated with the structures and added carbon chain length of these derivatives.

Various methods for the synthesis of sphingolipids were recently reported based upon different carbon–carbon double bond formation approaches as the key synthetic steps. These approaches include the traditional Wittig or Horner–Wadsworth–Emmons olefination,[11,12] stereoselective Birch reduction of alkyne,[9,10]

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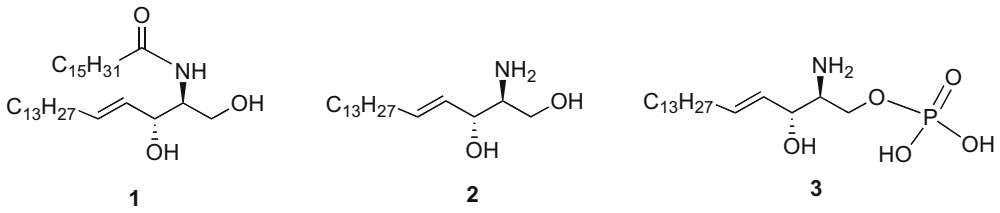


Figure 1. Three key sphingolipids: ceramide (Cer, **1**); sphingosine (SP, **2**); sphingosine-1-phosphate (S1P, **3**).

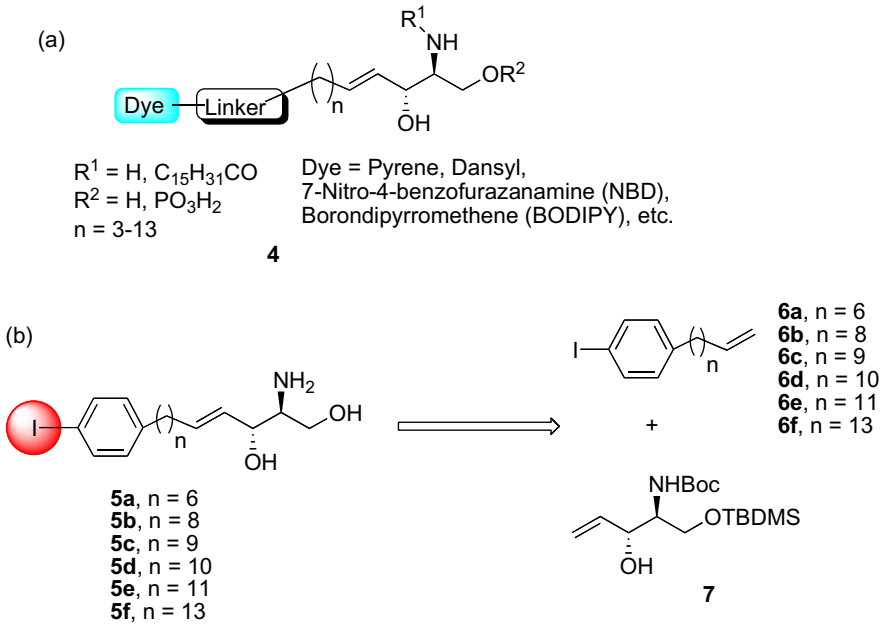


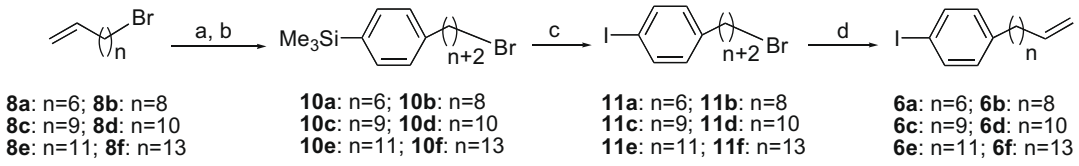
Figure 2. (a) Reported fluorescent dye labeled SP derivatives, **4**. (b) Designed 4-iodophenyl SP tagged derivatives, **5**, and their retrosynthesis analysis.

de-hydrohalogenation,[13] as well as palladium catalyzed thioester-boronic acid cross-coupling method[14] and Grubbs catalyst catalyzed cross-metathesis olefination.[6–8,15–23] We chose the cross-metathesis approach (Fig. 2b) to assemble the E-double bond of the SP skeleton. There are several advantages of using this method: very mild reaction conditions, high functional group tolerance and relative high stereoselectivity toward formation of trans olefins.[24]

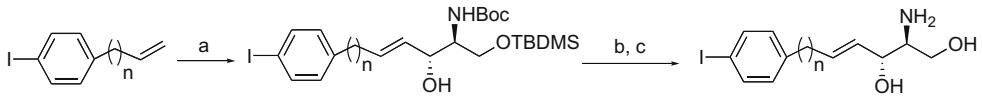
Allyl alcohol, **7**, was synthesized following a well-established method reported by Yamamoto et al.[15] The other intermediate 4-iodophenyl tagged terminal alkenes, **6**, were assembled through a four-step synthetic pathway starting from 1-bromo-ω-alkenes, **8a**–**f** (Scheme 1). Three 1-bromo-ω-alkene starting materials, **8d**–**f**, which are not commercially available, were prepared using either *t*-BuOK promoted de-hydrohalogenation method[25] or Li_2-$CuCl_4$ catalyzed Grignard reagent and alkyl halide coupling strategy (see Supplementary data).[26–28] After hydroboration with 9-borabicyclo[3.3.1]nonane (9-BBN), the formed alkylboranes were subsequently coupled with 1-bromo-4-trimethylsilylbenzene, **9**, through a Suzuki–Miyaura cross-coupling reaction.[29–31] This palladium catalyzed reaction that was carried out in an aqueous NaOH/THF biphasic system afforded 1-(ω-bromoalkyl)-4-trimethylsilyl-benzenes, **10a**–**f**, in 60–80% yields. After iodo-desilylation reactions, these aryltrimethylsilanes, **10a**–**f**, were converted to corresponding aryliodides, **11a**–**f**, in virtually quantitative yields.[31] Finally, a de-hydrobromination reaction with an excess of *t*-BuOK smoothly provided the desired alkenes, **6a**–**f**.[25,32]

With both intermediate alkenes, **6a**–**f**, and allyl alcohol, **7**, in hand, the olefin cross-metathesis reactions were conducted in CH_2Cl_2 with 2 equiv of **6a**–**f** to 1 equiv of **7** under the catalysis of 0.03 equiv of second generation Grubbs catalyst.[15] The desired *E*-alkenes, **12a**–**f**, were isolated in 47–57% yields after 2 h at reflux. Subsequent de-silylation with 1% HCl in 95% EtOH and THF and de-Boc protection with trifluoroacetic acid afforded final products, **5a**–**f**, with yields ranging from 65% to 90% (Scheme 2).[33]

Next, we chose two intermediates, **12c** and **12e**, for radiolabeling test. Palladium catalyzed trans-stannylation in toluene at 75 °C



Scheme 1. Reagents and conditions: (a) 9-BBN, THF, rt, 2 h, 60 °C, 2 h; (b) 1-bromo-4-trimethylsilylbenzene, **9**, $Pd(PPh_3)_4$, 2.0 M NaOH, 75 °C, 18 h; (c) ICl, CH_2Cl_2, 0 °C, 25 min; (d) *t*-BuOK, THF, 0 °C to rt, 2 h.



Scheme 2. Reagents and conditions: (a) alkene **7**, DCM, Grubbs 2nd generation catalyst, 40 °C, 2 h; (b) 1% HCl in 95% EtOH/THF, 0 °C to rt, 2 h; (c) TFA/CH_2Cl_2, 0 °C to rt, 50 min.

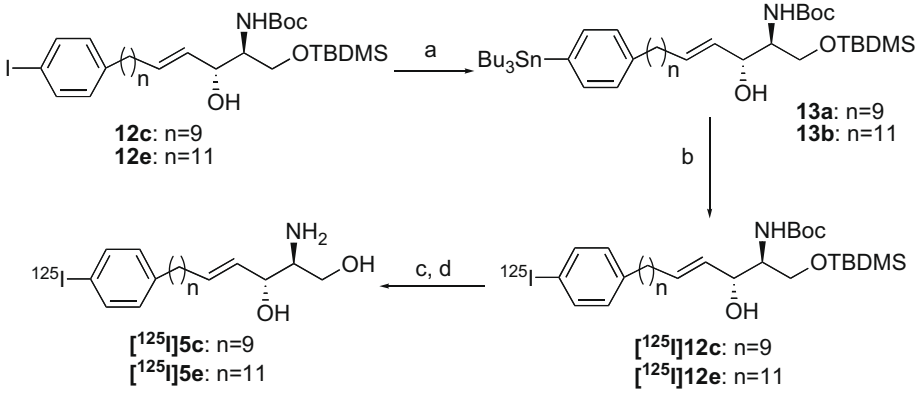
provided tributyltin substituted radiolabeling precursors, **13a** and **13b**, with moderate yields (47% and 49%, respectively). Standard iododestannylation reactions, using no-carrier-added sodium [^{125}I]iodide, H_2O_2, and HCl, were successfully conducted on both precursors.[34,35] Further deprotection with HCl and TFA to remove TBDMS and Boc, two protecting groups, afforded two radioactive products, [**^{125}I**]**5c** and [**^{125}I**]**5e**. Radiochemical yields were 40% and 60%, respectively (Scheme 3). The radiochemical purities were 94% and 98%, respectively. The chemical identities of both ^{125}I -labeled SP analogs were confirmed by co-injection of authentic standards ('cold' **5c** and **5e**) to show identical retention times on HPLC.

The structure–biological activity relationships of these newly synthesized SP analogs were evaluated by using well-established combined human SphK1 and SphK2 enzyme assays (see Supplementary data for experimental details).[5] Such preliminary tests help to evaluate how the addition of a 4-iodophenyl group at the end of alkyl chain and the change of alkyl chain length affect their enzyme activity with respect to the natural substrate, SP. The competition phosphorylation between the new ligands, **5a**–**f**, SP and a known SphK enzyme inhibitor, *N*,*N*-dimethylsphingosine (DMSP) toward a radiolabeled ligand provided the inhibition constants, IC_{50} values (Table 1). The IC_{50} values for sphingosine in inhibiting SphK enzyme assays were 3.86 and 1.56 μM for SphK1 and SphK2, respectively. The enzyme assays also showed that the IC_{50} of DMSP for SphK1 was 2.16 μM; while the IC_{50} for SphK2 was >80 μM. The data for sphingosine and its inhibitor, DMSP, demonstrated that the enzyme assays provided inhibition values similarly to the previously reported results, except for the DMSP showed less activity for SphK2.[36] We speculate that this difference may be caused by the attachment of 4-[^{125}I]phenoxy group in that radiolabeled ligand we used for the competition phosphorylation. The IC_{50} values of **5a**–**f** to the SphK1 are in the same order of those determined for SP and DMSP suggesting that they are close analogs. It is worthy to mention that the addition of a 4-iodophenyl group does not affect the binding affinity to this enzyme. Among these new derivatives, **5b**–**d**, the carbon chain length ranged from 8 to 10 and displayed high affinities (low IC_{50} values) toward SphK1 (<10 μM); while the IC_{50} values toward SphK2 displayed more variability with two of the derivatives, **5c** and **5f**, and showed IC_{50} >15 μM. Apparently, compound **5b** (n = 8) displayed the highest binding affinity to SphK1 with IC_{50} of 0.5 μM.

Table 1
Inhibition constants (IC_{50}) of SP and its analogs to SphK1 and SphK2

Test compounds	Alkyl chain length (n)	IC_{50} (μM)	
		SphK1	SphK2
Sphingosine (SP)	13	3.86 ± 0.001	1.56 ± 0.001
N,*N*-Dimethylsphingosine	13	2.17 ± 0.001	>80
5a	6	3.96 ± 0.001	7.23 ± 0.001
5b	8	0.50 ± 0.001	5.73 ± 0.001
5c	9	2.78 ± 0.001	16.6 ± 0.001
5d	10	2.19 ± 0.001	4.38 ± 0.001
5e	11	2.25 ± 0.001	13.3 ± 0.001
5f	13	3.98 ± 0.001	29.2 ± 0.001

In summary, we report a facile synthetic route to synthesize a series of SP analogs, 4-iodophenyl tagged SP derivatives relying on a cross-metathesis reaction as the stitching strategy. Meanwhile, we also report, to the best of our knowledge, the first synthesis of radionuclide, ^{125}I labeled sphingosine derivatives. In principle, the flexibility of this synthetic route will allow further exploration of labeling with various other radioisotopes, such as ^{18}F, ^{76}Br, or ^{11}C, to the SP derivatives with minimum perturbation of their biological functions. In addition, the preliminary biological evaluation of **5a**–**f** using a competition of phosphorylation enzyme assay demonstrated that **5b**–**d** bound to SphK1 with high affinity, while the binding affinity to SphK2 in general was lower. On the basis of these initial results, these newly synthesized 4-iodophenyl tagged SP derivatives deserve further investigations as tools for studying sphingosine biology in tumor proliferation and as potential in vivo tumor imaging agents.



Scheme 3. Reagents and conditions: (a) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, toluene, rt, 75 °C, 6 h; (b) H_2O_2, Na^{125}I, HCl, EtOH, 0 °C to rt, 10 min; (c) 2 M HCl/EtOH, 0 °C to rt, 40 min; (d) TFA/CH_2Cl_2, 0 °C to rt, 40 min.

Acknowledgment

The authors thank Ms. Catherine Hou for editorial assistance.

Supplementary data

General experimental procedures, details of the preparation, biological evaluation, and analytical data for compounds **8d–f**, **6a–f**, **10a–f**, **11a–f**, **12a–f**, **13c**, and **13e**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.035.

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32. Representative experimental procedures for synthesizing intermediates **6a–f**: *(4-(11-Bromoundecyl)phenyl)trimethylsilane* (**10c**). To an ice bath cooled stirred solution of **8c** (2.45 g, 10.5 mmol) was added a solution of 9-BBN in THF (0.5 M, 24 mL, 12 mmol) under N_2. After the addition, the ice bath was removed, the mixture was stirred for 2 h at room temperature and then was heated to 60 °C for additional 2 h. The reaction solution was cooled to room temperature, 1-bromo-4-trimethylsilylbenzene (2.29 g, 10 mmol), $Pd(Ph_3)_4$ (0.578 g, 0.5 mmol) and an aqueous solution of NaOH (2.0 M, 10 mL) were added. This mixture was purged with N_2 for 20 min and heated to 75 °C for 18 h. A standard workup with $CHCl_3$ followed flash chromatography (FC) with hexanes afforded **10c** as a clear oil (2.94 g, 77%). ^{1}H NMR ($CDCl_3$) δ 7.45 (d, 2H, J = 7.9 Hz), 7.18 (d, 2H, J = 7.8 Hz), 3.42 (t, 2H, J = 6.8 Hz), 2.60 (t, 2H, J = 7.7 Hz), 1.87 (p, 2H, J = 7.0 Hz), 1.63 (t, 2H, J = 7.2 Hz), 1.29 (br s, 14H), 0.28 (s, 9H). *1-(11-Bromoundecyl)-4-iodobenzene* (**11c**). To an ice bath cooled stirred solution of **10c** (0.383 g, 1.0 mmol) in 2 mL CH_2Cl_2 under N_2 was added a solution of ICl in CH_2Cl_2 (1.0 M, 1.2 mL). After stirring at 0 °C for 25 min, the reaction was quenched by 10 mL 10% $NaHSO_3$ solution. Standard workup with CH_2Cl_2 provided a quantitative amount of product and it was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.59 (dt, 2H, J_1 = 8.3 Hz, J_2 = 2.1 Hz), 6.93 (d, 2H, J = 8.3 Hz), 3.42 (t, 2H, J = 6.8 Hz), 2.55 (t, 2H, J = 7.6 Hz), 1.86 (p, 2H, J = 7.0 Hz), 1.58 (t, 2H, J = 6.8 Hz), 1.28 (br s, 14H). *1-Iodo-4-(undec-10-enyl)benzene* (**6c**). To an ice bath cooled stirred solution of **11c** (0.437 g, 1.0 mmol) in 5 mL THF under N_2 was added a solution of *t*-BuOK in THF (1.0 M, 3.0 mL). The reaction was stirred at room temperature for 4 h and then quenched with H_2O. After standard workup with $CHCl_3$, FC on silica gel using hexanes as solvent gives **6c** as a colorless oil (0.356 g, 64.5%). ^{1}H NMR ($CDCl_3$) δ 7.59 (dt, 2H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.93 (d, 2H, J = 8.3 Hz), 5.93–5.72 (m, 1H), 5.06–4.91 (m, 2H), 2.55 (t, 2H, J = 7.6 Hz), 2.05 (quartet, 2H, J = 6.8 Hz), 1.58 (br s, 2H), 1.28 (br s, 12H).
33. Representative experimental procedures for synthesizing **5a–f**: *tert-Butyl (2S,3R,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxy-14-(4-iodophenyl)tetradec-4-en-2-ylcarbamate* (**12c**). To a solution of **6c** (0.142 g, 0.4 mmol) in 3 mL CH_2Cl_2 was added allylic alcohol **7** (0.066 g, 0.2 mmol) and Grubbs catalyst 2nd generation (0.005 g, 0.006 mmol) at room temperature. After stirring at 40 °C for 2 h, the solvent was evaporated under vacuum and the residue was purified by FC on silica gel (EtOAc/hexanes, from 10/90 to 15/85) to provide a white waxy solid (0.072 g, 54%). ^{1}H NMR ($CDCl_3$) δ 7.51 (dt, 2H, J_1 = 8.3 Hz, J_2 = 2.1 Hz), 6.85 (d, 2H, J = 8.3 Hz), 5.68 (dt, 1H, J_1 = 15.4 Hz, J_2 = 6.8 Hz), 5.43 (dd, 1H, J_1 = 15.4 Hz, J_2 = 5.8 Hz), 5.15 (d, 1H, J = 8.3 Hz), 4.12 (quartet, 1H, J = 5.9 Hz), 3.86 (dd, 1H, J_1 = 10.3 Hz, J_2 = 3.0 Hz), 3.67 (dd, 1H, J_1 = 10.3 Hz, J_2 = 3.1 Hz), 3.49 (br s, 1H), 3.22 (d, 1H, J = 7.4 Hz), 2.47 (t, 2H, J = 7.6 Hz), 1.98 (quartet, 2H, J = 6.6 Hz), 1.48 (br s, 2H), 1.38 (s, 9H), 1.20 (br s, 12H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 155.9, 142.6, 137.4, 133.2, 130.7, 129.7, 90.7, 79.6, 74.7, 63.6, 54.8, 35.6, 32.4, 31.4, 29.65, 29.62, 29.3, 28.6, 26.0, 18.3, −5.42, −5.44. HRMS calcd for $C_{31}H_{54}INaNO_4Si$ (M+Na)$^+$: 682.2765, found: 682.2755. *(2S,3R,E)-2-Amino-14-(4-iodophenyl)tetradec-4-ene-1,3-diol* (**5c**). To a solution of **12c** (0.032 g, 0.048 mmol) in 2 mL THF was added 2 mL HCl (1% concd HCl in 95% EtOH) at 0 °C. The reaction mixture was then stirred at room temperature for 3 h. The solvent was removed under vacuum and the residue was re-dissolved in 2 mL CH_2Cl_2. After cooling down to 0 °C, 1 mL trifluoroacetic acid (TFA) was added dropwise. The reaction mixture was further stirred at room temperature for another 1 h. It was concentrated under vacuum and the residue was submitted to FC on silica gel (CH_2Cl_2/MeOH/NH_4OH = 200/25/2.5) to give **5c** as a white solid (0.019 g, 88%). ^{1}H NMR (CD_3OD) δ 7.58 (dt, 2H, J_1 = 8.3 Hz, J_2 = 2.0 Hz), 6.96 (d, 2H, J = 8.3 Hz), 5.74 (dt, 1H, J_1 = 15.4 Hz, J_2 = 6.6 Hz), 5.48 (dd, 1H, J_1 = 15.4 Hz, J_2 = 6.7 Hz), 4.00 (br s, 1H), 3.72 (br s, 1H), 3.52 (br s, 1H), 2.80 (br s, 1H), 2.55 (t, 2H, J = 7.5 Hz), 2.06 (quartet, 2H, J = 6.3 Hz), 1.58 (br s, 2H), 1.30 (br s, 12H). ^{13}C NMR (CD_3OD) δ 144.0, 138.6, 135.5, 131.8, 130.7, 91.2, 75.0, 63.9, 57.9, 36.5, 33.5, 32.6, 30.74, 30.66, 30.46, 30.42, 30.35. HRMS calcd for $C_{20}H_{33}INO_2$ (M+H)$^+$: 446.1556; found: 446.1541. $[\alpha]_D^{25} = -2.6$ (*c* 0.83, $CHCl_3$).
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35. Representative experimental procedures for synthesizing [^{125}I]**5c** and **5e**: *tert-Butyl (2S,3R,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxy-14-(4-(tributylstannyl)-phenyl) tetradec-4-en-2-ylcarbamate* (**13a**). A mixture of **12c** (0.033 g, 0.05 mmol), bis(tributyltin) ($(Bu_3Sn)_2$, 0.145 g, 0.25 mmol), and palladium tetrakistriphenylphosphine ($Pd(PPh_3)_4$, 0.006 g, 10 mol %) in toluene (3 mL) was heated at 75 °C for 6 h. The reaction solution was cooled to room temperature and concentrated under vacuum. The residue was submitted to FC on silca gel (EtOAc/hexanes, 15/85) to provide **13a** as a light yellow waxy solid (0.019 g, 47%). ^{1}H NMR ($CDCl_3$) δ 7.37 (d, 2H, J_1 = 7.8 Hz), 7.15 (d, 2H, J = 7.7 Hz), 5.77 (dt, 1H, J_1 = 15.4 Hz, J_2 = 6.8 Hz), 5.51 (dd, 1H, J_1 = 15.4 Hz, J_2 = 5.7 Hz), 5.24 (d, 1H, J = 7.3 Hz), 4.19 (br s, 1H), 3.95 (dd, 1H, J_1 = 10.3 Hz, J_2 = 3.0 Hz), 3.76 (dd, 1H, J_1 = 10.5 Hz, J_2 = 2.9 Hz), 3.56 (br s, 1H), 3.31 (d, 1H, J = 7.9 Hz), 2.58 (t, 2H, J = 7.7 Hz), 2.06 (quartet, 2H, J = 6.6 Hz), 1.72–1.20 (m, 35H), 1.08–0.86 (m, 24H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 156.0, 142.8, 138.4, 136.9, 136.6, 136.3, 133.3, 129.7, 128.8, 128.4, 128.0, 79.7, 74.8, 63.7, 54.8, 36.2, 32.5, 31.7, 29.8, 29.4, 29.3, 29.1, 28.6, 28.2, 27.6, 27.0, 26.0, 18.4, 13.9, 13.1, 13.0, 9.8, 6.5, 6.4, −5.4. HRMS calcd for $C_{43}H_{82}NO_4SiSn$ (M+H)$^+$: 824.5035, found: 824.5035. *Radioiodination*. Tributyltin precursor, **13a**, (200 μg) was dissolved in 100 μL EtOH and cooled with an ice bath. To the solution were added successively 100 μL 1 M HCl, 10 μL [^{125}I]NaI (535 μCi, non-carrier-added, purchased from Perkin–Elmer) and 50 μL H_2O_2 (3%). The ice bath was removed and the reaction was maintained at room temperature for 10 min. Satd $NaHSO_3$ (100 μL) was added to terminate the reaction. The reaction mixture was neutralized with 1.5 mL satd $NaHCO_3$. The whole mixture was passed through an activated C-4 mini-column and washed with 3 mL 20% EtOH. Labeled product was eluted with 1 mL EtOH (65%). Ethanol was removed under a flow of Ar. The residue was dissolved in 200 μL EtOH and 200 μL THF, cooled to 0 °C and treated with the addition of 200 μL HCl (2.0 M). After 10 min at 0 °C and 30 min at room temperature, the solvent was removed under Ar flow. The residue was re-dissolved in 500 μL CH_2Cl_2 and cooled in an ice bath. To this solution was added 200 μL trifluoroacetic acid (TFA). After 5 min at 0 °C and 50 min at room temperature, the reaction mixture was dried under Ar flow. The residue was dissolved in 100 μL EtOH and purified by HPLC (Phenomenex Si-column, CH_2Cl_2/MeOH/0.1% NH_4OH 100/10/1 with a flow rate of 0.7 mL/min). The collected solution was blown to dryness and the final product was re-dissolved in EtOH for biological studies. Radiochemical purity (RCP) of product was determined by HPLC (Phenomenex Si-column, CH_2Cl_2/MeOH/0.1%NH_4OH 100/10/1 with a flow rate of 1 mL/min) and its identity was identified by co-injection with 'cold' **5c**. The overall yield was 40% and RCP was 94%.
36. Paugh, S. W.; Paugh, B. S.; Rahmani, M.; Kapitonov, D.; Almenara, J. A.; Kordula, T.; Milstien, S.; Adams, J. K.; Zipkin, R. E.; Grant, S.; Spiegel, S. *Blood* **2008**, *112*, 1382.