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A Photoreactive Analogue of the Immunosuppressant FTY720

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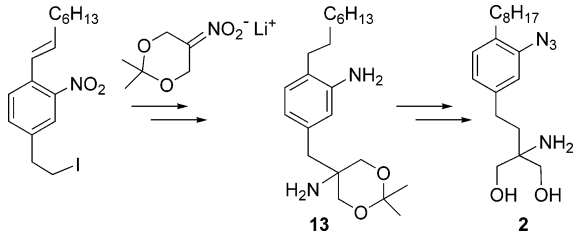

An azido group was incorporated into the immunomodulatory agent FTY720, accomplishing the first synthesis of a photoactivatable analogue of this ligand (**2**) in 9 steps from 2-(4-hydroxyphenyl)ethanol and in 34% overall yield. The key steps are formation of a primary amine at a quaternary center of aniline derivative **13** followed by selective diazotization of the arylamine.

FTY720 (2-amino-[2-(4-octylphenyl)ethyl]-1,3-propanediol, Figure 1, **1**) is a potent immunosuppressive agent that causes lymphocytes to be sequestered in secondary lymphoid organs, without decreasing host immune defense responses to most infections.[1] FTY720 may be approved by the U.S. FDA for treatment of multiple sclerosis, and is in phase III clinical studies to evaluate its effects with respect to kidney graft rejection in humans.[2] In addition, **1** is efficacious against other autoimmune diseases.[1,3] As an analogue of sphingosine, FTY720 is phosphorylated in vivo by sphingosine kinases, converting **1** to a chiral FTY720-phosphate, which is a high affinity agonist of four of the five known sphingosine 1-phosphate (S1P) G protein-coupled receptors in thymocytes and lymphocytes.[4] Internalization of the receptor renders the cells unresponsive to S1P; thus, lymphocytes are not capable of recirculation to peripheral inflammatory tissues and sites of the transplant. Unphosphorylated FTY720 (**1**) also causes internalization of some S1P receptors, but this action is independent of agonism and affects a different spectrum of S1P receptors than the phosphorylated derivative.[5]

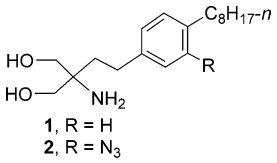

FIGURE 1. Structures of FTY720 (**1**) and its azido analogue **2**.

The potent biological activity of **1** has led to the development of efficient routes to its chemical synthesis. These methods include the Petasis reaction of vinylboronic acid with hydroxyacetone in the presence of an amine,[6] alkylation of diethyl 2-acetamidomalonate with iodo or bromo compounds,[7−9] and bis-formylation of 1-nitro-2-(4-octylphenyl)ethane with aqueous formaldehyde.[10] However, photoreactive analogues of **1** have not yet been synthesized. Aryl azides are highly photoreactive,[11] producing a nitrene intermediate on photolysis. Since the small size of the azido group is desirable for ligand binding to target proteins with minimal perturbation of ligand structure, we synthesized aryl azido analogue **2** (Figure 1) for use in identifying binding sites between this ligand and its receptors.[12]

The synthesis of **2** began with commercially available 2-(4-hydroxyphenyl)ethanol, which was converted to monoacetate **3** in almost quantitative yield in the presence of immobilized $NaHSO_4/SiO_2$.[7] After nitration of **3**[13] and reaction with triflic anhydride afforded triflate **4** in 80% overall yield, we attempted an $Fe(acac)_3$-catalyzed cross-coupling reaction with *n*-octylmagnesium bromide. This step was used with an aryl triflate in the preparation of **1**;[7] however, we found that application of this procedure to nitro compound **4** did not result in cross-coupling in the expected position (C-1). Instead, the *n*-octyl group underwent net substitution (presumably via an anionic nitronate adduct)[14] into the benzene ring at C(5) in the presence

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(12) Compound **2** is phosphorylated in an analogous manner as **1** in primary mouse lymphocytes, demonstrating the utility of **2** as a potential photoaffinity probe.

(13) Yamane, T.; Kondo, H.; Fuse, Y.; Hashizume, T.; Kano, F.; Yamashita, K.; Hosoe, K.; Watanabe, K. Japanese Patent 63045282 A2, 1988; *Chem. Abstr.* **1989**, *110*, 57408.

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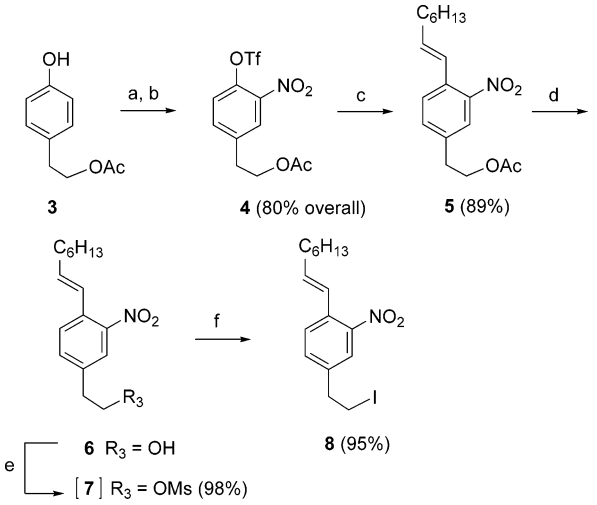
SCHEME 1. Synthesis of Iodide 8[a]



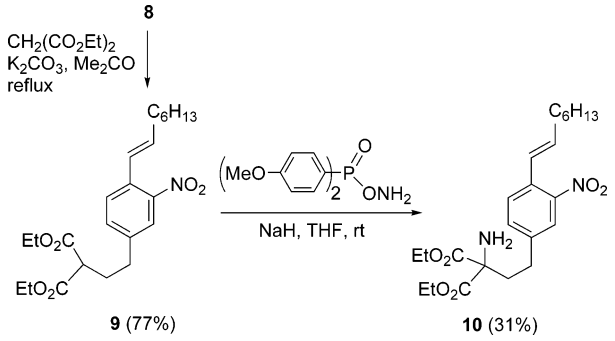
[a] Reagents and conditions: (a) HOAc, HNO_3, 10−15 °C; (b) Tf_2O, Py, CH_2Cl_2, 0 °C−rt; (c) (*E*)-$(HO)_2BCH{=}CHC_6H_{13}$-*n*, Pd(dppf)$_2Cl_2$·CH_2Cl_2, K_2CO_3, THF/H_2O (10:1), reflux, overnight; (d) NaOMe, MeOH, rt; (e) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C; (f) LiI, *n*-Bu_4NI, THF, rt, 1 h.

of Fe(III) with retention of the triflate group, which was hydrolyzed during workup, giving compound **5a**[15] in 50% yield. Fortunately, we found that the Suzuki cross-coupling of triflate **4** with (*E*)-1-octen-1-ylboronic acid in the presence of catalytic Pd(dppf)Cl_2 proceeded as expected to provide **5**. The yield of **5** increased from 53% to 89% when the boronic acid concentration was increased from 0.77 to 1.5 equiv. Hydrolysis of **5** with NaOMe in MeOH, followed by treatment of alcohol **6** with methanesulfonyl chloride and displacement of mesylate **7** with iodide ion afforded **8** in 95% overall yield.

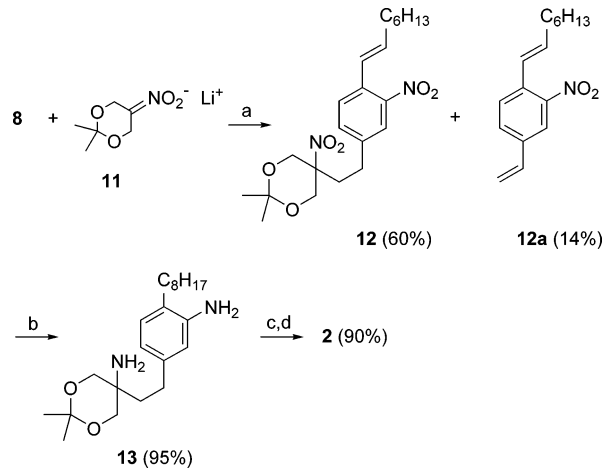
Initially, we tried to establish the quaternary center by means of an electrophilic amination reaction[16] (Scheme 2). After alkylation of **8** with diethyl malonate afforded **9**, the amino group was installed with *O*-(di-*p*-methoxyphenyl)phosphinylhydroxylamine.[17] Unfortunately, this method was inefficient, providing amine **10** in 31% yield; the corresponding tertiary alcohol byproduct was obtained in 15% yield according to NMR analysis, with recovery of 26% of compound **9**.

An alternative route to an intermediate bearing a primary amine at a quaternary carbon involves the use of the lithium salt of 2-nitropropane-1,3-diol acetonide (**11**),[18] followed by reduction (Scheme 3). This salt, which was prepared in situ by deformylation of tris(hydroxymethyl)nitromethane acetonide

SCHEME 2. Electrophilic Amination of 9



SCHEME 3. Completion of the Synthesis of Photoprobe 2[a]



[a] Reagents and conditions: (a) DMF, rt, 20 h; (b) H_2, 10% Pd/C, EtOH, 36 °C, 6 h; (c) 2 M HCl, $NaNO_2$, H_2O, 0 °C; (d) (i) NaN_3, H_2O, 0 °C, (ii) $NaHCO_3$.

with lithium hydroxide,[19] reacted with iodide **8** in DMF to form dinitro compound **12** in 60% yield. The elimination byproduct, styryl derivative **12a**, was formed in 14% yield. Dinitro compound **12** was reduced to diamine **13** under hydrogen atmosphere with Pd/C as the catalyst, with concomitant reduction of the double bond. Chemoselective installation of an azido group in the aromatic ring was accomplished by diazotization of the aryl amino group in **13** and treatment of the resulting intermediate diazonium chloride with sodium azide to give **2** in 90% overall yield.[20]

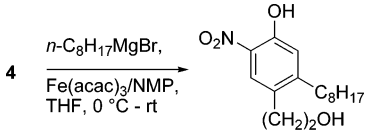
In summary, the first photoreactive analogue of **1**, a new immunosuppressant that traps T cells in lymph nodes, was synthesized in 9 steps and 34% overall yield. The key steps were the generation of a quaternary center via alkylation of iodide **8** with the lithium salt of 2-nitropropane-1,3-diol acetonide (**11**) and the selective diazotization of diamine **13**.

Experimental Section

3-Nitro-4-((*E*)-oct-1-enyl)phenethyl Methanesulfonate (7) and 4-(2-Iodoethyl)-2-nitro-1-((*E*)-oct-1-enyl)benzene (8). To a solution of alcohol **6** (280 mg, 1.0 mmol) in 10 mL of CH_2Cl_2 and

(14) For the reaction of Grignard reagents with nitroarenes, followed by reaction with an oxidant (DDQ, $KMnO_4$, or $Pb(OAc)_4$) see: (a) Bartoli, G.; Bosco, M.; Dalpozzo, R. *Tetrahedron Lett.* **1985**, *26*, 115−118. (b) Bartoli, G. *Acc. Chem. Res.* **1984**, *17*, 109−115.

(15) The structure of compound **5a** was assigned on the basis of mass spectral and NMR data (see the Supporting Information).



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0.28 mL (2.0 mmol) of Et_3N was added methanesulfonyl chloride (0.21 mL, 1.5 mmol) at 0 °C. The reaction mixture was stirred for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), dried (Na_2SO_4), and evaporated to yield crude mesylate **7** (391 mg, ∼109%), which was used without further purification (R_f 0.38, EtOAc/hexane 1:2). To a solution of **7** (391 mg, 1.0 mmol) in 10 mL of THF were added LiI (406 mg, 3.0 mmol) and *n*-Bu_4NI (110 mg, 0.30 mmol). The reaction was completed within 1 h of stirring at room temperature. After evaporation of the volatile components, the residue was purified by flash chromatography (EtOAc/hexane 1:10) to give 370 mg (95%) of iodide **8** as a pale yellow solid: R_f 0.51 (EtOAc/hexane 1:10); mp 34−35 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.31−1.37 (m, 6H), 1.48 (m, 2H), 2.25 (dt, 2H, J = 6.8, 6.8 Hz), 3.22 (t, 2H, J = 7.2 Hz), 3.36 (t, 2H, J = 7.2 Hz), 6.24 (dt, 1H, J = 15.6, 6.8 Hz), 6.80 (d, 1H, J = 15.6 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.54 (d, 1H, J = 8.0 Hz), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 4.2, 14.1, 22.6, 28.8, 28.9, 31.7, 33.2, 38.9, 124.0, 124.6, 128.6, 132.0, 132.8, 136.9, 140.1, 147.6; LR-MS (APPI, MH^+) *m*/*z* calcd for $C_{16}H_{23}NIO_2$ 388.1, found 388.2; $(2M + Na)^+$ calcd *m*/*z* 797.1, found 797.3.

5-(3-Nitro-4-((*E*)-oct-1-enyl)phenethyl)-2,2-dimethyl-5-nitro-1,3-dioxane (12). To a solution of the lithium salt of 2-nitropropane-1,3-diol acetonide (**11**, prepared by the reaction of 494 mg (2.58 mmol) of 2-hydroxymethyl-2-nitropropane-1,3-diol monoacetonide with 111 mg (2.58 mmol) of $LiOH·H_2O$, using a Dean−Stark trap to remove water) in 4 mL of DMF was added 200 mg (0.52 mmol) of **8**. After the reaction mixture was stirred at room temperature for 20 h, H_2O was added (10 mL), and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (EtOAc/hexane 1:2) to give 76 mg (60%) of **12** as an off-white solid: R_f 0.60 (EtOAc/hexane 1:2); mp 80−82 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.31−1.50 (m, 8H), 1.41 (s, 3H), 1.45 (s, 3H), 2.14 (m, 2H), 2.25 (m, 2H), 2.60 (m, 2H), 3.97 (d, 2H, J = 12.8 Hz), 4.52 (d, 2H, J = 12.8 Hz), 6.22 (dt, 1H, J = 15.6, 6.8 Hz), 6.79 (d, 1H, J = 15.6 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.51 (d, 1H, J = 18.0 Hz), 7.66 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 21.1, 22.6, 25.4, 28.4, 28.8, 29.9, 31.7, 33.2, 35.2, 63.9, 85.8, 99.3, 123.8, 124.4, 128.8, 131.9, 132.8, 137.0, 139.1, 147.6; HR-MS (ESI, MNa^+) *m*/*z* calcd for $C_{22}H_{32}N_2O_6Na$ 443.2153, found 443.2146.

5-(3-Amino-4-octylphenethyl)-2,2-dimethyl-1,3-dioxan-5-amine (13). A suspension of **12** (70 mg, 0.17 mmol) and 10% Pd/C (36 mg) in 20 mL of EtOH was stirred under 1 atm of H_2 at 36 °C for 6 h. The mixture was filtered through a short pad of Celite, and the pad was washed with EtOH (20 mL). The filtrate was concentrated to give 58 mg (95%) of **13** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.27−1.44 (m, 8H), 1.41 (s, 3H), 1.46 (s, 3H), 1.59 (m, 2H), 1.66 (m, 2H), 2.42 (dt, 2H, J = 6.8, 6.8 Hz), 2.57 (m, 2H), 3.49 (br s, 4H), 3.56 (d, 2H, J = 11.6 Hz), 3.78 (d, 2H, J = 11.6 Hz), 6.53−6.55 (m, 2H), 6.92 (d, 1H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 19.5, 22.5, 27.4, 28.2, 28.7, 29.2, 29.4, 29.6, 30.8, 31.8, 36.7, 49.7, 49.4, 69.0, 98.3, 115.3, 118.4, 124.5, 129.3, 140.2, 144.0; HR-MS (ESI, MH^+) *m*/*z* calcd for $C_{22}H_{39}N_2O_2$ 385.3006, found 363.3016.

2-(3-Azido-4-octylphenethyl)-2-aminopropane-1,3-diol (2). To a solution of **13** (22 mg, 0.060 mmol) in 0.10 mL of 2 M HCl and 0.05 mL of H_2O was added $NaNO_2$ (4.3 mg, 0.060 mmol) portionwise at 0 °C. After the suspension was stirred for 15 min, NaN_3 (3.9 mg, 0.060 mmol) was added in one portion. The mixture was stirred at 0 °C for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 4:1) to give 20 mg (90%) of **2** as a pale yellow solid: R_f 0.20 (EtOAc/hexane 4:1); mp 63−65 °C; ^{1}H NMR ($CDCl_3$/CD_3OD 10:1) δ 0.82 (t, 3H, J = 6.8 Hz), 1.21−1.24 (m, 10H), 1.47 (m, 2H), 1.68 (m, 2H), 2.44 (t, 2H, J = 7.6 Hz), 2.57 (m, 2H), 3.47 (d, 2H, J = 10.8 Hz), 3.56 (d, 2H, J = 10.8 Hz), 6.83 (d, 1H, J = 7.6 Hz), 6.90 (s, 1H), 7.00 (d, 1H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$/CD_3OD 10:1) δ 13.9, 22.5, 28.8, 29.1, 29.31, 29.32, 30.3, 30.7, 31.8, 35.9, 56.7, 65.3, 117.7, 124.5, 130.3, 131.9, 137.6, 140.8; HR-MS (ESI, MH^+) *m*/*z* calcd for $C_{19}H_{33}N_4O_2$ 349.2598, found 349.2598.

Supporting Information Available: We thank Dr. Markis Gräler for conducting the cell-based phosphorylation experiments. Financial support from NIH Grant HL083187 is gratefully acknowledged.

Supporting Information Available: Experimental procedures for compounds **3**−**6**, **9**, and **10** and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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