Chiral Vinylphosphonate and Phosphonate Analogues of the Immunosuppressive Agent FTY720

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\[ \text{4-BrC}_6\text{H}_4\text{CHO} \quad \text{HO-} \quad \text{P} \quad \text{HO} \quad \text{C}_8\text{H}_{17-n} \]

(S)-4, (S)-5: \( X = \text{NH}_2, \ Y = \text{CH}_2\text{OH} \) Both have anti-apoptotic activity; (S)-5 is not a S1P\(_1\) agonist

(R)-4, (R)-5: \( X = \text{CH}_2\text{OH}, \ Y = \text{NH}_2 \) Both lack anti-apoptotic activity but are S1P\(_1\) agonists

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capable of recirculation to peripheral inflammatory tissues.3 The cells unresponsive to S1P; therefore, lymphocytes are not receptors leads to their proteasomal degradation and renders analogues, FTY720-(E)-vinylphosphonate (S)-5, but not its R enantiomer, elicited a potent antiapoptotic effect in intestinal epithelial cells, suggesting that it exerts its action via the enantioselective activation of a receptor. (S)-5 failed to activate the sphingosine 1-phosphate type 1 (S1P) receptor.

The first enantioselective synthesis of chiral isosteric phosphonate analogues of FTY720 is described. One of these analogues, FTY720-(E)-vinylphosphonate (S)-5, but not its R enantiomer, elicits a potent antiapoptotic effect in intestinal epithelial cells, suggesting that it exerts its action via the enantioselective activation of a receptor. (S)-5 failed to activate the sphingosine 1-phosphate type 1 (S1P) receptor.

Thus, FTY720 has therapeutic potential and, in fact, is the first S1P receptor modulator that has entered into the stage of a phase-III clinical study.4

Several syntheses of 1 and of phosphates 3 have been accomplished.5 In contrast to phosphates such as 3, phosphonate analogues are resistant to the action of lipid phosphatases and may offer improved cellular stability. A racemic mixture of the nonhydrolyzable phosphate analogue of FTY720 (4) was reported in which the C—O—P bond is replaced with a C=C—P bond;2b rac-4 was found to be a high-affinity agonist of the S1P-type 1 receptor (S1P), with a similar potency as (S)-3.7 We report here the first asymmetric syntheses of the chiral phosphate analogues of FTY720, (R)-4 and (S)-4. Oxazoline intermediate (S)-14 (Scheme 1), prepared by a modification of our previous route,6c was further elaborated to give the corresponding (E)-vinylphosphonate analogue (S)-5.

We have included a preliminary pharmacological characterization of the effects of these analogues on the nontransformed rat intestinal epithelial cell line IEC-6. This study revealed that (S)-5, but not its (R) enantiomer, exerts a potent antiapoptotic effect in a camptothecin (CPT)-induced apoptosis model.8 Unlike phosphate (S)-3, (S)-5 did not activate the S1P receptor of the Endothelial Differentiation Gene (EDG) family of G protein-coupled receptors, making it a novel enantioselective probe activating a cytoprotective mechanism against apoptosis induced by DNA damage.

Wittg reaction of 4-bromobenzaldehyde with the ylde of n-heptiltrifluorophosphonium bromide gave arylalkene 6 as an E2 (1:3) mixture (Scheme 1). Sonogashira coupling between 6 and 4-(phenylmethoxy)-1-butyne delivered enyne 7 as a 1:3 E2 mixture in 92% yield. Alcohol 8 was obtained on reduction of the unsaturated bonds and hydrogenolysis of the O-benzyl group in the presence of Pearlman’s catalyst. After Swern oxidation of 8 provided aldehyde 9, use of a Mannich reagent, Internalization and subsequent polyubiquitination of the S1P receptors leads to their proteosomal degradation and renders the cells unresponsive to S1P; therefore, lymphocytes are not capable of recirculation to peripheral inflammatory tissues.3

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Eschenmoser’s salt,\textsuperscript{9} afforded $R$-methylene aldehyde 10. Reduction of 10 with NaBH\textsubscript{4} in the presence of CeCl\textsubscript{3} (to suppress conjugate reduction) gave allyl alcohol 11.\textsuperscript{10} CeCl\textsubscript{3}, which is a mild Lewis acid, is not required, since CsCl also provided 11 as the only product. Asymmetric Sharpless epoxidation\textsuperscript{11} of 11 with cumene hydroperoxide (CHP) in the presence of L-\((+)-\)DIPT, Ti(OPr\textsubscript{i})\textsubscript{4}, and molecular sieves gave epoxide (\(S\))-12.\textsuperscript{12} The synthesis of (\(S\))-12 was accomplished in 7 steps from $p$-bromobenzaldehyde and in 46\% overall yield. Reaction of alcohol (\(S\))-12 with trichloroacetonitrile in the presence of DBU gave 2,3-epoxy-1-trichloroacetimidate (\(R\))-13. The tetrasubstituted carbon in oxazoline 14 was set up bearing the desired nitrogen substituent by opening of epoxide (\(R\))-13 with catalytic Et\textsubscript{2}AlCl,\textsuperscript{13} affording (\(S\))-14 in 74\% yield for the two steps.

Swern oxidation of oxazoline (\(S\))-14 gave oxazoline aldehyde 15 (Scheme 2), which on Horner-Wadsworth-Emmons reaction with tetramethyl methylenediphosphonate afforded ester (\(S\))-16 in 87\% yield and with an \(E/Z\) ratio of \(\sim 10:1\). Simultaneous demethylation and release of the hydroxy and amino groups by treatment with trimethylsilyl bromide (TMSBr) provided (\(S\))-16, but the yield was low. Therefore, the hydroxy and amino groups were first released by treatment with 1 M HCl. After the amine hydrochloride was neutralized (saturated aq Na\textsubscript{2}CO\textsubscript{3}), amino alcohol 17 was converted to (\(S\))-5 with TMSBr followed by 95\% methanol; 84\% yield for the two steps. Reduction of (\(S\))-5 using Pearlman’s catalyst gave (\(S\))-4.

Asymmetric epoxidation of 11 with D-\((-)-\)DIPT gave epoxide (\(R\))-12, which was converted via (\(R\))-14 to (\(R\))-5 in six steps (Scheme 3). Catalytic hydrogenation of (\(R\))-5 afforded (\(R\))-4.


\textsuperscript{10} Luche, J. L. J. Am. Chem. Soc. 1978, 100, 2226–2227.


\textsuperscript{12} Sharpless epoxidation of an analogue of 11 with L-diethyl tartrate gave the corresponding (\(S\))-2,3-epoxy alcohol in 95\% ee: Li, X.; Borhan, B. J. Am. Chem. Soc. 2008, 130, 16126–16127 (Table 1, entry 20).


S1P promotes the survival of many cell types.\textsuperscript{14} Both 2a and (\(S\))-3 protect oligodendrocyte progenitor cells from apoptotic cell death in response to growth factor withdrawal, and (\(S\))-3 was also shown to be cytoprotective in response to pro-apoptotic.


cytokines and microglial activation.15 The ability of 2a, (S)-3, and phosphonate analogues 4 and 5 to protect IEC-6 cells from apoptotic cell death in response to the topoisomerase inhibitor CPT was assessed by DNA fragmentation. Pretreatment with 2a, (S)-3, (R)-4, or (R)-5 did not result in a significant reduction in DNA fragmentation in response to a 4-h treatment with 20 μM CPT. However, we found that the cytotoxic effect was enantioselective, since pretreatment with 1 μM of (S)-4 or (S)-5 showed a significant reduction (21 and 50%, respectively) in DNA fragmentation in response to CPT.

In a preliminary study of the activity of the FTY720-phosphonate analogues on SIP receptors, we performed Ca2+-mobilization assays with HTC4 cells that were stably transfected with SIP1. As shown in Figure 1, the SIP1 transfectants were activated by (S)-3 to 76% of the maximal SIP1-induced activation, and displayed a similar potency as SIP1 (13 ± 2 nM for SIP vs 9 ± 1 nM for (S)-3). (R)-5 and (R)-4 both showed a modest activity against SIP1, with Emax values that ranged from 73 to 93% of the maximal SIP1-induced responses, and EC50 values that were increased by ~2- to 3-fold. (S)-4 activated SIP1 to 36% of the maximal SIP1-induced response, and the EC50 value was increased by ~5-fold to 75 ± 21 nM. Since (S)-5 did not elicit a Ca2+ response from cells transfected with the SIP1 receptor, we conclude that the potent cytotoxic effect of (S)-5 is not mediated by SIP1.

In conclusion, we have described the synthesis of the enantiomers of FTY720 phosphonate analogues 4 and 5. (S)-4 and (S)-5, but not 2a or (S)-3, all at 1 μM, protected IEC-6 cells from apoptosis. The extent of CPT-induced DNA fragmentation was reduced by 50% and 21% in the presence of 1 μM of (S)-5 and (S)-4, respectively. The potent cytotoxic activity of (S)-5 is not mediated by SIP1. Experiments are underway to characterize the cellular effects of these analogues.

**Experimental Section**

(25)-2-(2′-(Trichloromethyl)-4′,5′-dihydrooxazol-5-yl)-4-(4′-octyloxyphosphonyl)-butan-1-ol [(S)-(+)-14]. To a solution of 435 mg (1.5 mmol) of epoxy alcohol (S)-12 in 25 mL of CHCl3 at 0 °C were added Cl3CCN (0.17 mL, 1.65 mmol) and DBU (0.023 mL, 0.15 mmol). After being stirred at 0 °C for 1.5 h, the reaction mixture was diluted with EtO (20 mL) and water (20 mL). The organic layer was separated, washed with brine (20 mL), dried (MgSO4), and concentrated. The residue was purified by chromatography (hexane/EtOAc 20:1) to give 565 mg (87%) of (R)-13. Rf 0.58 (EtOAc/hexane 1:3). To an ice-cold solution of 565 mg (1.31 mmol) of (R)-13 in 20 mL of CHCl3 was added Et3AlCl (0.75 mL, 0.75 mmol, a 1.0 M solution in hexane). After the mixture was stirred at 0 °C for 20 min and then at rt for 3 h, the reaction was quenched with saturated aq NaHCO3 solution (20 mL). The organic layer was washed with brine (20 mL), dried (MgSO4), and concentrated. The residue was purified by chromatography (hexane/EtOAc 3:1) to give 480 mg (85%) of (R)-14 as a white solid; mp 56.7–57.5 °C; Rf 0.29 (EtOAc/hexane 1:3); [α]25D +24.9 (c 1.60, CHCl3); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26–1.38 (m, 10H), 1.58 (m, 2H), 1.84 (m, 1H), 2.01 (m, 1H), 2.57 (m, 4H), 3.30 (s, 1H), 3.55 (dd, 1H, J = 11.6, 8.4 Hz), 3.85 (dd, 1H, J = 11.6, 4.8 Hz), 4.45 (d, 1H, J = 8.4 Hz), 4.65 (d, 1H, J = 8.4 Hz), 7.08 (d, 4H), 7.14 (d, 4H), 7.63 (d, 4H), 8.05 (d, 4H), 8.09 (d, 4H). HRMS (MNa+) m/z calc for C21H30Cl3NO2Na 456.1234, found 456.1241. Data for (R)-14: [α]25D −25.0 (c 2.75, CHCl3); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26–1.38 (m, 10H), 1.58 (m, 2H), 1.85 (m, 1H), 2.01 (m, 1H), 2.14 (s, 1H), 2.60 (m, 4H), 3.54 (d, 1H, J = 11.6 Hz), 3.85 (s, 3H), 3.97 (s, 3H), 4.45 (d, 1H, J = 8.4 Hz), 4.63 (d, 1H, J = 8.4 Hz), 7.09 (s, 4H). 1C NMR (CDCl3) δ 14.2, 22.7, 29.0, 29.3, 29.5, 29.7, 31.4, 31.8, 35.6, 37.6, 66.8, 70.6, 86.5, 128.2, 128.9, 140.8, 163.2. HRMS (MNa+) m/z calc for C3H5ClNO2Na 456.1234, found 456.1241. Data for (R)-14: [α]25D −25.0 (c 2.75, CHCl3); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26–1.38 (m, 10H), 1.58 (m, 2H), 1.85 (m, 1H), 2.01 (m, 1H), 2.14 (s, 1H), 2.60 (m, 4H), 3.54 (d, 1H, J = 11.6 Hz), 3.85 (s, 3H), 3.97 (s, 3H), 4.45 (d, 1H, J = 8.4 Hz), 4.63 (d, 1H, J = 8.4 Hz), 7.09 (s, 4H). 1C NMR (CDCl3) δ 14.2, 22.7, 29.0, 29.3, 29.5, 29.7, 31.4, 31.8, 35.6, 37.6, 66.8, 70.6, 86.5, 128.2, 128.9, 140.8, 163.2.

(3S)-3-(Amino)-3-(hydroxymethyl)-5-(4′-octyloxyphenyl)-pent-(1E)-enyl-phosphonic acid [(S)+(+)-5]. To a solution of 269 mg (0.50 mmol) of (S)-16 in 10 mL of THF at rt was added 3 mL of 1 M HCl. After the reaction mixture was stirred overnight, the solvent was evaporated and the residue was extracted with a mixture of CHCl3 and saturated Na2CO3 aq solution. The organic phase was dried (MgSO4) and concentrated. The residue was purified by chromatography (CHCl3/MeOH/NH4OH 135:25:4) to give 185 mg (90%) of (S)-17 as a pale yellow oil after filtration through a Teflon syringe filter. Rf 0.37 (CHCl3/MeOH/NH4OH 135:25:4); [α]25D +18.8 (c 1.52, CHCl3); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.4 Hz), 1.26–1.29 (m, 10H), 1.58 (m, 2H), 1.48–1.86 (m, 5H), 2.49–2.58 (m, 4H), 3.50 (dd, 1H, J = 21.2, 10.6 Hz), 3.73 (s, 3H), 3.75 (s,
3H), 5.93 (dd, 1H, J = 20.0, 17.6 Hz), 6.85 (dd, 1H, J = 22.8, 17.6 Hz), 7.05–7.10 (m, 4H); 13C NMR (CDCl3) δ 14.1, 22.7, 29.3, 29.36, 29.4, 29.44, 29.5, 31.6, 31.9, 35.5, 39.4, 52.4 (d, J = 6.0 Hz), 59.4 (d, J = 19.1 Hz), 69.2, 115.0 (d, J = 188.1 Hz), 128.1, 128.3, 128.6, 138.6, 138.7, 138.9, 157.9 (d, J = 6.0 Hz); 31P NMR (CDCl3) δ 21.8. Data for (R)-(17): [α] D 25 +17.1 (c 1.51, CHCl3); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.4 Hz), 1.26–1.29 (m, 10H), 1.58 (m, 2H), 1.79–1.82 (m, 5H), 2.53–2.57 (m, 4H), 3.50 (dd, J = 21.2, 10.6 Hz), 3.73 (s, 3H), 3.75 (s, 3H), 5.93 (dd, 1H, J = 20.0, 17.6 Hz), 6.85 (dd, 1H, J = 22.8, 17.6 Hz), 7.05–7.10 (m, 4H); 13C NMR (CDCl3) δ 14.1, 22.7, 29.3, 29.4, 29.42, 29.5, 31.6, 31.9, 35.5, 39.4, 52.4 (d, J = 2.0 Hz), 52.5 (d, J = 2.0 Hz), 59.4 (d, J = 19.1 Hz), 69.1, 115.0 (d, J = 188.1 Hz), 128.1, 128.4, 128.5, 138.7, 140.7, 157.9 (d, J = 6.0 Hz); 31P NMR (CDCl3) δ 21.8. To a solution of (S)-17 in 10 mL of dry CH2Cl2 at rt was added 0.66 mL (5.0 mmol) of TMSBr. After the reaction mixture was stirred for 4 h, the solvent was removed, and the residue was dried and dissolved in 2 mL of 95% MeOH with stirring for 1 h. Removal of the solvent afforded 224 mg (93%) of (S)-5 as a white solid: mp 159.2–161.1 °C; Rf 0.14 (CHCl3/MeOH/H2O/AcOH 65: 25:4:1); [α] D 25 +12.2 (c 1.04, CHCl3/MeOH 9:1); 1H NMR (CDCl3/CD2OD 9:1) δ 0.87 (t, 3H, J = 6.8 Hz), 1.26 (br s, 10H), 1.51 (s, 2H), 1.95–2.10 (m, 2H), 2.47 (t, 2H, J = 7.6 Hz), 2.55–2.68 (m, 2H), 3.83 (br s, 2H), 4.08 (br s, 4H), 6.30 (m, 1H), 6.60 (m, 1H), 6.99 (d, 2H, J = 8.0 Hz), 7.07 (d, 2H, J = 8.0 Hz); 13C NMR (CDCl3/CD2OD 9:1) δ 14.1, 22.9, 29.1, 29.5, 29.6, 31.8, 32.3, 35.8, 36.5, 62.2 (d, J = 20.1 Hz), 64.4, 123.3, 125.2, 128.3, 128.9, 137.6, 141.4, 144.2; 31P NMR (CDCl3/CD2OD 9:1) δ 13.1. HRMS (MNa+) m/z calcld for C20H34NO4PNa 406.2118, found 406.2106.

Data for (R)-5. [α] D 25 −13.0 (c 1.05, CHCl3/MeOH 9:1); 1H NMR (CDCl3/CD2OD 9:1) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26–1.27 (m, 10H), 1.52–1.55 (m, 2H), 2.01–2.11 (m, 2H), 2.52 (t, 2H, J = 7.6 Hz), 2.55–2.68 (m, 2H), 3.26 (br s, 4H), 3.82 (br s, 2H), 6.30 (m, 1H), 6.59 (dd, 1H, J = 23.2, 18.0 Hz), 7.03 (d, 2H, J = 8.0 Hz), 7.08 (d, 2H, J = 8.0 Hz), 8.34 (br s, 1H); 13C NMR (CDCl3/CD2OD 9:1) δ 14.2, 22.9, 29.0, 29.5, 29.6, 29.7, 29.9, 31.8, 32.3, 35.8, 36.5, 62.2 (d, J = 20.1 Hz), 64.4, 123.4, 125.2, 128.3, 128.9, 137.6, 141.4, 144.2; 31P NMR (CDCl3/CD2OD 9:1) δ 13.1.

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Supporting Information Available: Experimental details for the synthesis of compounds 6–8, 10, 11, 12, 16, and 4, and NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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