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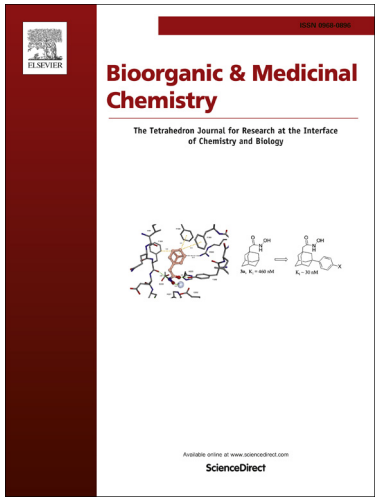

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Graphical Abstract

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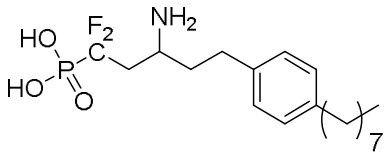

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Synthesis of fluorinated agonist of sphingosine-1-phosphate receptor 1

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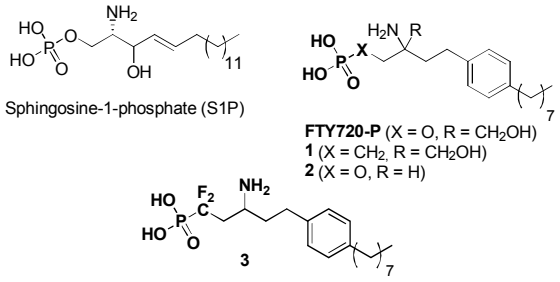
ABSTRACT

The bioactive metabolite Sphingosine-1-phosphate (S1P), a product of sphingosine kinases (SphKs), mediates diverse biological processes such as cell differentiation, proliferation, survival and angiogenesis. A fluorinated analogue of S1P receptor agonist has been synthesized by utilizing a ring opening reaction of oxacycles by a lithiated difluoromethylphosphonate anion as the key reaction. In vitro activity of this S1P analogue is also reported.

1. Introduction

Sphingosine-1-phosphate (S1P) is a phospholipid regulating pleiotropic biological activities including proliferation, survival, inflammation and angiogenesis.[1] The bioactive sphingolipid metabolite, sphingosine-1-phosphate (S1P) is a high affinity ligand for five G-protein coupled receptors (S1P1-5)[2] that functions as a key regulator in many biological processes including blood vessel development, hearth rhythm, blood pressure, and immune regulationS.[3-6]

In the 1990s, Fujita's group isolated the natural compound, myriocin, from the fungus *Isaria sinclairii*, which showed impressive immunosuppressive activity.[7] Various structural modifications of myriocine led to the development of the immunomodulator FTY720 (Fingolimod™). FTY720 is a prodrug requiring metabolization *in vivo* by sphingosine kinase-2 isoform into its bioactive S-isomeric monophosphate ester FTY720-phosphate (FTY720-P),[8] which interacts with four S1P receptors ($S1P_1$, $S1P_{3-5}$); however, the majority of biological effects of FTY720-P are attributed to its interaction with $S1P_1$. The interaction of FTY720-P with $S1P_1$ is thought to induce sequestration of lymphocytes in lymph nodes thereby preventing their movement to the central nervous system.[9] This is turn would limit the autoimmune responses in multiple sclerosis and was actually proposed as an antirejection medication after transplantation. With regards to mechanism of action underlying lymphopenia, it is mostly suggested that FTY720-P causes receptor internalization and proteosomal degradation, leading to a $S1P_1$-null state.[10]



Scheme 1. Structures of S1P and $S1P_1$ receptor agonists

Therefore, the discovery of subtype selective $S1P_1$ modulators are highly desirable as potential therapeutics.[11, 12] The success

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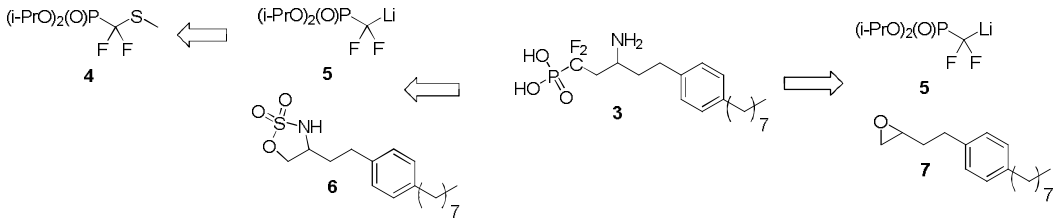


Scheme 2. Two retrosynthetic pathways to prepare α,α-difluorophosphonate derivative 3

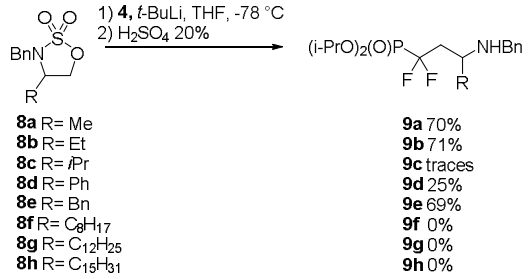
of FTY720 in human clinical investigations has prompted synthetic efforts to provide both pharmacological tools to study S1P signaling and therapeutics.[13] Since FTY720-P is not stable in physiological medium, there remains a tremendous need to develop a nonhydrolysable methylene-phosphonate **1** analogue as a S1P receptor agonist.[14, 15] Previous work has demonstrated that deletion of hydroxymethyl group (compound **2**) have no effect on S1P receptor affinity.[14] Other results demonstrated that enzymatically and hydrolytically stable α,α-difluoromethylphosphonates mimics are better isopolar and isosteric analogues than methylene-phosphonate ones.[16,17] The combination of these reports has generated significant interest in our group to develop a physiologically stable α,α-difluorophosphonate derivative **3** of FTY720-P. Herein, we report the synthesis and biological evaluation of a fluorinated agonist for the sphingosine-1-phosphate receptor through a novel ring opening of oxacycles with α,α-difluoromethylphosphonate anion. Two pathways have been explored to obtain the key γ-amino-α,α-difluoromethylphosphonates moiety : the first one based on ring opening reaction of 1,2 cyclic sulfamidates, and the second one based on an epoxide ring opening (scheme 2).

2. Results and Discussion

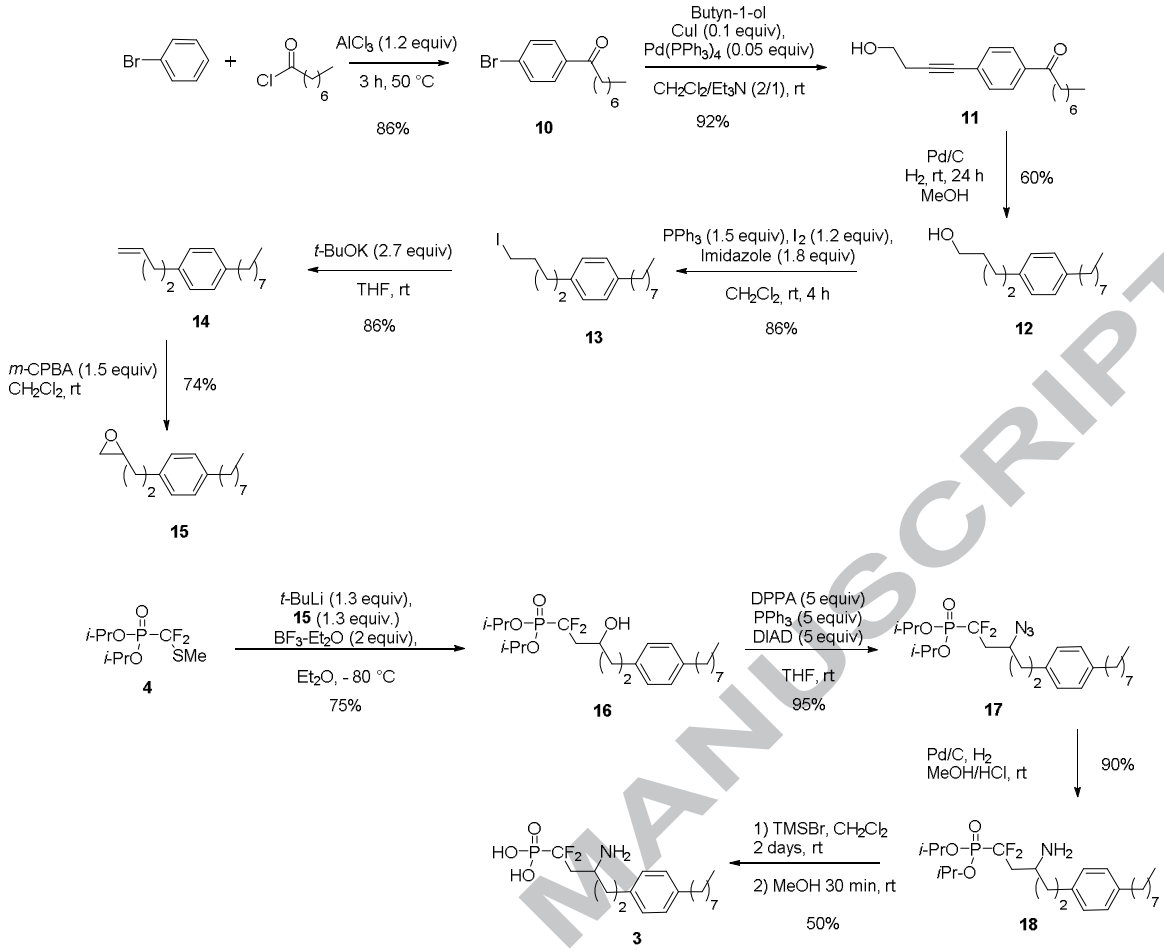
2.1. *Chemistry*

Preparation of the α,α-difluoromethyl phosphonates is commonly conducted through lithiation of commercially available diethyl difluoromethylphosphonate.[18-25] However, the strict use of HCFC and CFC from Montreal's protocol has severely limited access to these starting materials. To circumvent this limitation, our developed an alternative approach utilizing readily available phosphonodifluoromethyl sulfide **4**, prepared from nucleophilic fluoride sources.[26] In contrast to the previously described methods using diethyl α,α-difluoromethylphosphonate, lithiation of **4** in the presence of *t*-BuLi and subsequent capture with a Lewis acid can effectively control the reactivity of the corresponding carbanion species, which in turn, enables rapid access to γ, δ and ε-hydroxyl α,α-difluoromethylphosphonates after addition to strained heterocycles such as epoxides, trimethylene oxide and even THF.[27]

To expand the scope of this ring opening reaction with other heterocycles, we applied this methodology towards the generation of γ-amino-α,α-difluoromethylphosphonates in an effort to synthesize fluorinated analogues of **1**. As shown in scheme 3, sulfamidates **8a-h**, prepared from the corresponding aminoalcohols,[28] were subjected to the in situ derived phosphonodifluoromethyl lithium anion to readily generate various γ-amino-α,α-difluoromethylphosphonates **9a, 9b, 9d** and **9e** after acidic workup. However, the methodology failed in the presence of longer carbon chain sulfamide derivatives **9f-h**. Despite the various tested conditions (Lewis acid addition, higher temperature …), none of sulfamidates **8f-h** did successfully react with the anion **4**.



Scheme 3. Ring opening reaction of 1,2-cyclic sulfamidates 8a-h



Scheme 4. Synthesis of fluorinated S1P agonist 3

In accordance with our desired goal to synthesize a fluorinated analogue of **1**, we altered our original strategy to incorporate an epoxide, instead of a sulfamidate as the electrophile, which after ring opening, the alcohol would be transformed into the necessary amine group. As shown in scheme 4, preparation of the pivotal epoxide **15** commenced with the Friedel Crafts acylation of bromobenzene with octanoyl chloride[29] followed by Sonogashira coupling of bromide **10** to furnish the desired ketone **11**. Simultaneous reduction of the alkyne and ketone moieties with H_2 afforded alcohol **12** which was subsequently transformed into the alkyl iodide **13** with I_2 and PPh_3. After extensive optimization, only the combination of the **13** and tBuOK[30] could the elimination product **14** be obtained in high yields which was then converted to the key epoxide **15** under standard epoxidation conditions with *m*-CPBA.[31]

With the necessary epoxide in hand, we next turned our attention towards the incorporation of the difluorophosphonate moiety. Following our previous protocols and under optimized conditions, lithiation of phosphonate **4** with *t*-BuLi in the presence of $BF_3{\cdot}Et_2O$ generated the necessary nucleophilic equivalents to react with epoxide **15** to rapidly gain access to y-hydroxy-a,a-difluoromethylphosphonate **16** in high yields. To obtain the desired y-aminophosphonate analogue **3**, the alcohol was converted to the necessary amine functionality in a three-step process. Accordingly, azide formation under Mitsunobu conditions with diphenylphosphoryl azide DPPA[32] as the azide source followed by acidic reduction with H_2 afforded amine **18**. Gratifyingly, careful hydrolysis of the phosphonate ester **18** with TMSBr and methanol quench generated the phosphonic acid **3** in 50% yield.

2.2. Biological tests

We compared the proliferative effects of fluorinated S1P agonist **3** (C3) with S1P the active (S)-enantiomer of FTY720-P, which is the in vivo phosphorylated product of FTY720 in representative PC-3 and C4-2B prostate cancer cell lines.[33, 34] As shown in Figure 1, whereas treatment with S1P and FTY720-P resulted in a robust proliferative effect peaking at 100 nM, compound **3** did not exhibit any proliferative effect after 24 h of treatment in both PC-3 and C4-2B cell lines. On the contrary, compound **3** showed an inhibitory effect at concentrations above 1 µM with a marked effect at 10 µM ($IC_{50} \approx 3$ and 20 µM for C4-2B and PC-3, respectively) somehow similar to the inhibition of proliferation observed with high doses of FTY720-P. A similar finding was observed using an MTT-based cell viability (data not shown).

3. Conclusion

Two synthetic pathways were assayed to obtained the targeted γ-amino-α,α-difluoromethylphosphonic acid **3** stable FTY720-P analogue. If the ring opening of cyclic sulfamidates route was

successfully achieved on sulfamidate substituted with short alkyl or aryl chains (25% and 71% yields), this strategy failed on sulfamidates bearing longer alkyl chains. Stable FTY720-P analogue **3** was obtained through the epoxide ring opening route in ten steps in 9% overall yield.

Interaction of S1P to its cognate receptors mostly results in internalization and consequent recycling of the receptors back to the membrane, yet the binding of FTY720-P to $S1P_1$ is associated with $S1P_1$ receptor internalization and degradation,[10] making FTY720-P a "functional antagonist".[35] Depending on the cell type, receptor expression, and receptor availability, FTY720-P could modify $S1P_1$ coupling to specific intracellular signals such as Ca^{2+}, cAMP or MEK/ERK 1/2 signalling[36] and therefore altering proliferative signaling in cells. This last characteristic could explain the inhibitory effects of fluorinated FTY720-P analogue **3** on cell proliferation and viability of prostate cancer cells.

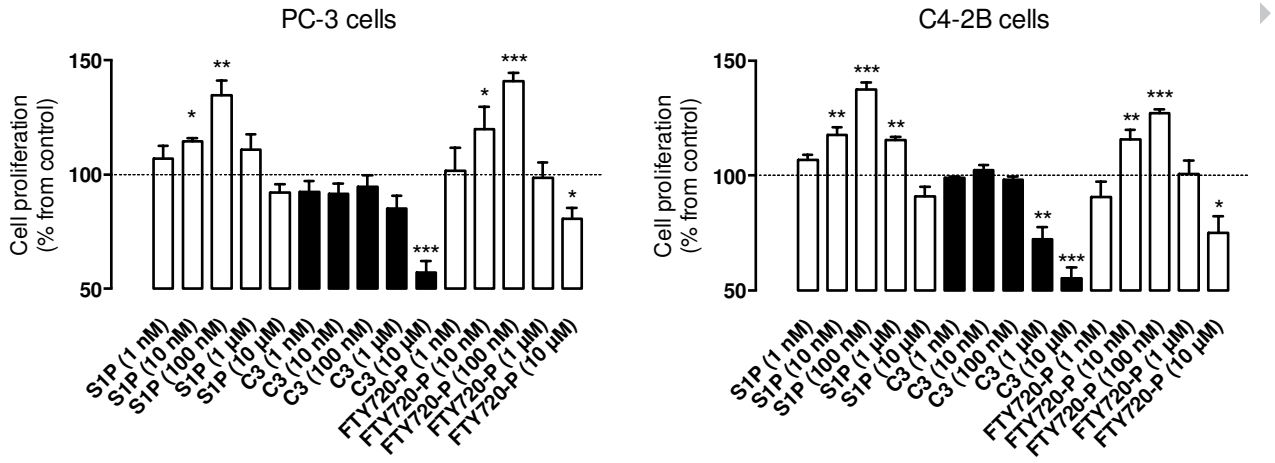


Figure 1. Evaluation of cell proliferation in PC-3 and C4-2B prostate cancer cell lines treated by S1P, FTY720-P and FTY720-P analogue 3 (C3).

4. Experimental

4.1. Chemistry

All solvents were dried following standard procedures (dichloromethane: distillation over CaH_2, THF: distillation over Na/benzophenone).

Column chromatography purifications were performed on Geduran® Si 60 silica gel (40-63 µm) from Merck. TLC were carried out on Merck DC Kieselgel 60 F-254 aluminium sheets. Compounds were visualised by one or more of the following methods: (1) illumination with a short wavelength UV lamp (i.e., λ = 254 nm), (2) spray with a 0.2% (w/v) ninhydrin solution in absolute ethanol, (3) spray with a 3.5% (w/v) phosphomolybdic acid solution in absolute ethanol.

Instruments and methods. ^{1}H, ^{13}C (C13APT or C13CPD experiments), 31P and 19F NMR spectra were recorded either on a Bruker AC 200, on a Bruker DPX 300 or on a Bruker avance III400 spectrometers working at 200, 300 and 400 MHz respectively. Chemical shifts are expressed in parts per million (ppm) from the residual non-deuterated solvent signal $CDCl_3$ (δ_H = 7.26, δ_C = 77.00) or DMSO-d_6 (δ_H = 2.50, δ_C = 39.43). *J* values are in hertz (Hz). High-resolution mass spectra were recorded on a LCT Premier XE benchtop orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer (Waters Micromass) equipped with an ESI source and in the positive mode.

4.1.1. 4-Bromo-octanoylbenzene **10**

Octanoyl chloride (0.17 mL, 1.0 mmol, 1.0 equiv) was added dropwise to a mixture of bromobenzene (0.21 mL, 2.0 mmol, 2.0 equiv) and aluminium chloride (160 mg, 1.2 mmol, 1.2 equiv). The reaction mixture was then stirred at 50 °C for 3 h, poured into ice water, and extracted with CH_2Cl_2. The organic phase was washed with aq solution HCl (1 N) and brine, dried over $MgSO_4$ and concentrated under reduced pressure The resulting residue was purified by flash chromatography (pentane/EtOAc, 100/0, 90/10, 80/20) to afford **10** as a yellow solid (243 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.25-1.32 (m, 8H), 1.32-1.35 (m, 2H), 1.57-1.71 (m, 2H), 2.89 (t, $^3J_{HH}$ = 7.4 Hz, 2H), 7.57 (d, $^3J_{HH}$ = 7.4 Hz, 2H), 7.80 (d, $^3J_{HH}$ = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 24.4, 29.3, 29.4, 31.8, 38.7, 128.1, 129.7, 132.0, 135.9, 198.8; HMRS (ESI) m/z calcd for $C_{14}H_{19}BrO$ 282.0619 [M+H$^+$], found 282.0620.

4.1.2. 1-(4-(4-hydroxybut-1-ynyl)phenyl)octan-1-one **11**

A solution of butyn-1-ol (50 mg, 0.71 mmol, 1.0 equiv) in a mixture CH_2Cl_2/triethylamine (v/v: 2/1) were added to a solution of bromobenzene **10** (200 mg, 0.71 mmol, 1.0 equiv) in CH_2Cl_2 (2 mL) at room temperature. The reaction mixture was degassed by softly bubbling argon during 30 min and then CuI (6 mg, 0.07 mmol, 0.1 equiv) and $Pd(PPh_3)_4$ (40 mg, 0.35 µmol, 0.05 equiv) were successively added. The reaction mixture was further degassed by argon bubbling and stirred overnight at room temperature under an argon atmosphere. After filtration on celite followed by removal of the solvent under reduced pressure, the residue was purified by flash chromatography (pentane/EtOAc, 50/50) to give **11** as a white solid (178 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.28-1.34 (m, 8H), 1.69-1.74 (m, 2H), 2.72 (t, $^3J_{HH}$ = 6.4 Hz, 2H), 2.93 (t, $^3J_{HH}$ = 9.6 Hz, 2H), 3.84 (t, $^3J_{HH}$ = 8.4 Hz, 2H), 7.48 (d, $^3J_{HH}$ = 8.8 Hz, 2H), 7.89 (d, $^3J_{HH}$ = 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2 (s), 22.7, 22.8, 24.4 (s), 29.3, 29.4, 31.8, 38.7, 61.3, 83.4, 98.7, 128.1, 129.7, 131.9, 135.9, 198.8.

4.1.3. 3-(4-octylphenyl)propan-1-ol **12**

Pd/C (30 mg, 10 wt. %) was added to a solution of **11** (200 mg, 0.73 mmol, 1.0 equiv) in MeOH (2 mL). The reaction mixture was degassed by using argon bubbling during 30 min

followed by hydrogen and stirred overnight at room temperature under hydrogen atmosphere (1 bar). After filtration on celite followed by concentration under reduced pressure, the residue was purified by flash chromatography (pentane/EtOAc, 80/20) to afford **12** as a yellow solid (115 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.27-1.30 (m, 10H), 1.61-1.72 (m, 6H), 2.54-2.64 (m, 4H), 3.66 (t, $^3J_{HH}$ = 8.4 Hz, 2H), 7.10 (s, Ar, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.8, 25.4, 27.6, 29.2, 29.4, 29.5, 31.6, 32.3, 35.2, 35.2, 62.8, 125.9, 128.2, 128.3, 128.4; HMRS (ESI) m/z calcd for $C_{18}H_{30}O$ 262.2297 [M+H$^+$], found 262.2299.

4.1.4. 1-(4-iodobutyl)-4-octylbenzene **13**

Iodine (223, 0.88 mmol, 1.2 equiv) and imidazole (90 mg, 1.32 mmol, 1.8 equiv) were successively added to a stirred solution of triphenylphosphine (300 mg, 1.1 mmol, 1.5 equiv) in CH_2Cl_2 (5 mL) at room temperature. The resulting solution was stirred for 10 min before addition of a solution of the alcohol **12** (191 mg, 1.0 mmol, 1.0 equiv) in CH_2Cl_2. The reaction was stirred 4 h at room temperature, and then was quenched with addition of solution of $Na_2S_2O_3$, extracted with dichloromethane, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (pentane) to give **13** as a colourless oil (346 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.24-1.27 (m, 10H), 1.67-1.79 (m, 4H), 1.81-1.86 (m, 2H), 2.52-2.60 (m, 4H), 3.18 (t, $^3J_{HH}$ = 9.2 Hz, 2H), 7.07 (s, Ar, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 6.8, 14.1, 22.8, 25.4, 27.6, 29.2, 29.4, 29.5, 31.6, 32.3, 35.2, 35.2, 128.2, 128.4, 138.9, 140.5; HMRS (ESI) m/z calcd for $C_{18}H_{29}I$ 372.1314 [M+H$^+$], found 372.1307.

4.1.5. 1-(but-3-enyl)-4-octylbenzene **14**

A solution of **13** (372 mg, 1.0 mmol, 1.0 equiv) in dry THF (2 mL) was added dropwise to a solution of *t*-BuOK (111 mg, 2.7 mmol, 2.7 equiv) in dry THF (8 mL) under argon. Precipitate was observed even before the addition was complete and TLC control indicated the reaction was completed after 30 min. The reaction mixture was quenched using an aqueous saturated solution of $NaHCO_3$. The organic layer was separated and concentrated under reduced pressure and the resulting residue was purified by flash chromatography (pentane) to furnish **14** as a colourless oil (212 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, $^3J_{HH}$ = 7.5 Hz, 3H), 1.24-1.27 (m, 10H), 1.47-15.4 (m, 2H), 2.25-2.32 (m, 2H), 2.49 (t, $^3J_{HH}$ = 10.4 Hz, 2H), 2.59 (t, $^3J_{HH}$ = 9.6 Hz, 2H), 4.91 (dd, $^2J_{HHgem}$ = 2.0 Hz, $^3J_{HHcis}$ = 9.2 Hz, 1H), 4.91 (dd, $^2J_{HHgem}$ = 2.0 Hz, $^3J_{HHtrans}$ = 21.6 Hz, 1H), 5.80 (m, 1H), 7.07 (s, Ar, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.6, 31.6, 31.9, 35.0, 35.2, 35.2, 35.2, 114.8, 128.3, 128.4, 138.3, 139.0, 140.5; HMRS (ESI) m/z calcd for $C_{18}H_{28}$ 244.2191 [M+H$^+$], found 244.2201.

4.1.6. 2-(4-octylphenethyl)oxirane **15**

A solution of *m*-CPBA (332 mg, 1.5 mmol, 1.5 equiv) in dry CH_2Cl_2 (2 mL) was added dropwise to a solution of alkene **14** (244 mg, 1.0 mmol, 1.0 equiv) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred overnight at room temperature and then quenched using an aqueous saturated solution of $NaHCO_3$. The organic layer was separated and concentrated under reduced pressure. Purification by flash chromatography (pentane/EtOAc, 100/0 to 95/5) to give **15** as a colourless oil (192 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, $^3J_{HH}$ = 7.5 Hz, 3H), 1.26-1.30 (m, 10H), 1.81-1.83 (m, 2H), 1.86-1.89 (m, 2H), 2.48-2.50 (m, 3H), 2.70-2.78 (m, 3H), 2.95-2.97 (m, 1H), 7.07 (s, Ar, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.6, 31.6, 31.9, 35.0, 35.2, 35.2, 35.2, 47.3, 51.9, 128.2, 128.4, 138.4, 140.6; HMRS (ESI) m/z calcd for $C_{18}H_{28}O$ 260.2140 [M+H$^+$], found 260.2124.

4.1.7. (3-hydroxy-1,1-difluoro-5-phenyloctyl)-phosphonic acid diisopropyl ester **16**

A solution of diisopropyl methylsulfanyl difluoromethylphosphonate **4** (262 mg, 1.0 mmol, 1.0 equiv) in dry Et_2O (1 mL) was added dropwise to a cold solution of *t*-butylithium (1.3 M, 1 mL, 1.3 mmol, 1.3 equiv) in dry Et_2O (5 mL) at - 80 °C. After 10 min of stirring, a solution of **15** (348 mg, 1.3 mmol, 1.3 equiv) in dry Et_2O (1 mL) was added slowly at - 80 °C, followed by $BF_3.OEt_2$ (0.26 mL, 2.0 mmol, 2.0 equiv) at the same temperature. After 15 min, the reaction mixture was quenched at - 80 °C by addition of an aqueous saturated NH_4Cl and the mixture was slowly warmed up to room temperature. The aqueous layer was extracted with Et_2O (twice) and combined organic layers were washed with aqueous solution of $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated under reduced pressure and crude product was purified by flash column chromatography (pentane/EtOAc, 90/10) to give **16** as a colourless oil (357 mg, 75%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.89 (t, $^3J_{HH}$ = 7.5 Hz, 3H), 1.03-1.33 (m, 24H), 1.48-1.52 (m, 2H), 1.97-2.02 (m, 4H), 2.46-2.48 (m, 2H), 2.50-2.53 (m, 2H), 3.75-3.76 (m, 1H), 4.04 (s, OH, 1H), 4.77-4.81 (m, 2H) 7.07 (s, Ar, 5H); ^{19}F NMR: (377 MHz, $CDCl_3$) δ - 111.05 (dddd, $^3J_{FH}$ = 19.4 Hz, $^3J_{FH}$ = 24.3 Hz, $^2J_{FP}$ = 111.3 Hz, $^2J_{FF}$ = 263.1 Hz, 1F), – 105.80 (dddd, $^3J_{FH}$= 19.4 Hz, , $^3J_{FH}$ = 24.3 Hz, $^2J_{FP}$ = 108.3 Hz, $^2J_{FF}$ = 296.1 Hz, 1F); ^{31}P NMR: (162 MHz, $CDCl_3$) δ 6.12 (dd, $^2J_{PF}$ = 111.3 Hz, $^2J_{PF}$ = 108.3, 1P); ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 23.6, 23.7, 23.8, 24.0 (d, $^3J_{CP}$ = 4.8 Hz), 24.1 (d, $^3J_{CP}$ = 3.3 Hz), 29.2, 29.5, 31.4, 31.5, 35.7, 39.2, 51.2, 74.1 (d, $^2J_{CP}$ = 7.0 Hz), CF_2 not detected, 128.3, 128.4, 138.9, 140.4; HMRS (ESI) m/z calcd for $C_{25}H_{43}F_2O_4P$ 476.2867 [M+H$^+$], found 476.2925.

4.1.8. (3-azido-1,1-difluoro-5-phenyloctyl)-phosphonic acid diisopropyl ester **17**

Triphenylphosphine (1.31 g, 5.0 mmol, 5.0 equiv) was added to a solution of alcohol **16** (476 mg, 1.0 mmol, 1.0 equiv) in THF (3 mL) at 0 °C. The mixture was stirred for a minute. Diisopropyl azodicarboxylate (0.95 mL, 5.0 mmol, 5.0 equiv) was then added dropwise, and a white precipitate appeared. Diphenyl phosphoryl azide (DPPA) (1.04 mL, 5.0 mmol, 5.0 equiv) was added dropwise at 0 °C, and the reaction mixture was allowed to warm to room temperature and stirred for an additional 24 h. After concentration, the residue was directly purified by silica gel chromatography (pentane/EtOAc, 100/0, 98/2, 95/5, 90/10) to give **17** as a colorless oil (482 mg, 95%).^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.86 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.09-1.33 (m, 24H), 1.54-1.59 (m, 2H), 1.83-1.86 (m, 2H), 2.13-2.25 (m, 2H), 2.52-2.57 (m, 2H), 2.67-2.90 (m, 2H), 3.77-3.81 (m, 1H), 4.80-4.86 (m, 2H) 7.07 (s, Ar, 5H); ^{19}F NMR: (377 MHz, $CDCl_3$) δ –112.33 (td, $^3J_{FH}$ = 20.4 Hz, $^2J_{FP}$ = 106.1 Hz, 2F), ^{31}P NMR: (162 MHz, $CDCl_3$) δ 4.55 (t, $^2J_{PF}$ = 106.1 Hz, 1P); ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 23.6, 23.7, 23.8, 24.0 (d, $^3J_{CP}$ = 4.8 Hz), 24.1 (d, $^3J_{CP}$ = 3.3 Hz), 29.2, 29.5, 31.4, 31.5, 35.7, 39.2, 51.2, 74.1 (d, $^2J_{CP}$ = 7.0 Hz), CF_2 not detected, 128.3, 128.4, 138.9, 140.4; HMRS (ESI) m/z calcd for $C_{25}H_{42}F_2N_3O_3P$ 501.2932 [M+H$^+$], found 501.3009.

4.1.9. (3-amino-1,1-difluoro-5-phenyloctyl)-phosphonic acid diisopropyl ester **18**

To a solution of azide **17** (508 mg, 1.0 mmol, 1.0 equiv) in MeOH (2 mL) was added Pd/C (51 mg, 10 wt. %) and aq solution HCl 1 M (1 mL). The reaction mixture was degassed by using argon bubbling during 30 min followed by hydrogen and stirred 48 h at room temperature under hydrogen atmosphere (1 bar). After filtration on celite followed by removal of the solvent under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2/EtOAc, 90/10, 80/20, 70/30, 50/50) to

afford **18** as a yellow solid (427 mg, 90%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.86 (t, $^{3}J_{HH}$ = 7.4 Hz, 3H), 1.26-1.38 (m, 24H), 1.68-1.78 (m, 2H), 2.02-2.31 (m, 2H), 2.36-2.60 (m, 4H), 3.35-3.64 (m, 1H), 4.80-4.86 (m, 2H) 7.07 (s, Ar, 5H); ^{19}F NMR: (377 MHz, $CDCl_3$) δ δ –111.85 (dddd, $^{3}J_{FH}$ = 14.8 Hz, $^{3}J_{FH}$ = 28.1 Hz, $^{2}J_{FP}$ = 107.6 Hz, $^{2}J_{FF}$ = 296.6 Hz, 1F), –109.61 (dddd, $^{3}J_{FH}$= 14.8 Hz, , $^{3}J_{FH}$ = 28.1 Hz, $^{2}J_{FP}$ = 108.4 Hz, $^{2}J_{FF}$ = 296.6 Hz, 1F); ^{31}P NMR: (162 MHz, $CDCl_3$) δ 5.12 (dd, $^{2}J_{PF}$ = 107.6 Hz, $^{2}J_{PF}$ = 108.4, 1P); ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 23.6, 23.7, 23.8, 24.0 (d, $^{3}J_{CP}$ = 4.8 Hz), 24.1 (d, $^{3}J_{CP}$ = 3.3 Hz), 29.2, 29.5, 31.4, 31.5, 35.7, 39.2, 51.2, 74.1 (d, $^{2}J_{CP}$ = 7.0 Hz), CF_2 not detected, 128.3, 128.4, 138.9, 140.4; HMRS (ESI) m/z calcd for $C_{25}H_{42}F_2NO_3P$ 475.3027 [M+H$^+$], found 475.3102.

4.1.10. (3-amino-1,1-difluoro-5-phenyloctyl)-pentylphosphonic acid **3**

TMSBr (0.6 mL, 4.2 mmol, 5.0 equiv.) was added to a solution of γ-amino-α,α-difluoromethylphosphonate **18** (400 mg, 0.8 mmol, 1.0 equiv) in CH_2Cl_2 (1 mL). The reaction mixture was stirred 48 h at room temperature under argon atmosphere. Then, solvent and volatile products are removed under reduced pressure. Methanol (1 mL) was added and the reaction mixture was stirred for 30 min at room temperature. The resulting mixture was evaporated under reduced pressure and diluted in Et_2O. Slow addition of water issued in the precipitation of phosphonic acid **3**, which was isolated as a white solid (150 mg, 50%). ^{1}H NMR: (300 MHz, DMSO+DCl) δ 0.75 (t, $^{3}J_{HH}$ = 6.9 Hz, 3H), 0.96-1.03 (m, 12H), 1.14-1.16 (m, 2H), 1.88-1.91 (m, 2H), 2.29-2.58 (m, 5H), 7.04 (dd, $^{3}J_{HH}$ = 8.1 Hz, $^{3}J_{HH}$ = 8.1 Hz, Ar, 4H), ^{19}F NMR: (282 MHz, DMSO+DCl) δ –112.13 (dddd, $^{3}J_{FH}$ = 14.1 Hz, $^{3}J_{FH}$ = 39.4 Hz, $^{2}J_{FP}$ = 95.8 Hz, $^{2}J_{FF}$ = 304.6 Hz, 1F), –109.21 (dddd, $^{3}J_{FH}$ = 14.8 Hz, $^{3}J_{FH}$ = 36.6 Hz, $^{2}J_{FP}$ = 95.8 Hz, $^{2}J_{FF}$ = 304.6 Hz, 1F); ^{31}P NMR: (121 MHz, $CDCl_3$) δ 5.12 (dd, $^{2}J_{PF}$ = 95.8 Hz, $^{2}J_{PF}$ = 95.8, 1P); ^{13}C NMR: (DMSO+DCl, 75 MHz) δ 13.8, 21.9, 28.4, 28.6, 29.9, 30.8 (d, $^{3}J_{CP}$ = 4.8 Hz), 31.1 (d, $^{3}J_{CP}$ = 3.3 Hz), 34.6, 78.8 (t,$^{1}J_{CF}$ = 33.0 Hz), 79.1, CF_2 not detected, 128.0, 128.2, 137.7, 139.9; HMRS (ESI) m/z calcd for $C_{19}H_{32}F_2NO_3P$ 391.2088 [M+H$^+$], found 391.2006.

4.2. Biological assays

4.2.1 Cell proliferation and cell viability assays

C4-2B and PC-3 metastatic prostate cancer cells were plated in 6-well plates ($2x10^5$ for C4-2B and 10_5 cells/well for PC-3, respectively), then cultured for 24h. Cells were treated with S1P at 2.5 µM or with compound 3, at different concentrations. In order to determine cell proliferation, ^{3}H-thymidine was added to the culture medium (1 µCi/mL) 6 h before the end of the experiment. Cells were washed once with ice-cold PBS and 3 times with 10% trichloroacetic acid to cause the precipitation of DNA and proteins. The precipitate was solubilized in 0.3 N NaOH/1% SDS, and radioactivity was measured.[37] Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl-)2,5-diphenyltetrazolium (MTT) bromide assay.[38]

4.2.1 Statistical analysis

The statistical significance of differences between the means was evaluated using the unpaired Student's t test. Statistical tests were two-sided, and the level of significance was set at $P < 0.05$. The IC50 values were calculated from dose–response curves obtained by nonlinear regression analysis. Calculations were done using Prism 6 (GraphPad Software, San Diego, CA).

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References and notes

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Supplementary Material

General procedure for the preparation and characterizations of compounds **8a-h.** General procedure for the ring opening reactions of sulfamidates and characterizations of compounds **9a-b** and **9d-e**.