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S1P receptor mediated activity of FTY720 phosphate mimics

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ABSTRACT

Various carboxylic acids, phosphonic acids, sulfonic acids, tetrazoles as well as sulfonylhydantoins were prepared as phosphate mimics of the chiral aminophosphate **1-P** to act as agonists on the $S1P_1$ receptor. It was found that amino phosphonates and amino carboxylates are potent $S1P_1$ binders. β-Amino acid **11** could be shown to reversibly reduce blood lymphocyte counts in rats after po administration.

FTY720 (fingolimod™) is a novel orally active immunomodulator that has shown efficacy in the treatment of multiple sclerosis.[1] The agent has a unique mode of action, namely modulating lymphocyte trafficking. Specifically, immunoreactive T-cells are trapped in secondary lymphoid organs and cannot exert their physiological role in plasma or tissue.[2] On a molecular level, this reversible reduction of blood lymphocyte count is thought to be mediated by FTY720-P, generated in vivo, acting as an agonist on sphingosine-1-phosphate receptor 1 ($S1P_1$), thereby internalizing the receptor and thus acting as functional antagonist.[3]

The discovery that FTY720 is stereoselectively phosphorylated by sphingosine kinases (SPHKs) in vivo to the active principle (S)-FTY720-phosphate (Fig. 1)[4] explained the activity of the chiral amino alcohol **1** and the inactivity of its enantiomer.[5] Amino alcohol **1** reversibly reduces blood lymphocytes in vivo to a similar extent as FTY720. The activity can be rationalized in vitro by the high turnover of **1** to its phosphate **1-P** by SPHKs as well as potent binding of **1-P** to $S1P_1$. In line with the mode of action, the enantiomer of **1** is not a good substrate for SPHKs, and the enantiomer of **1-P** binds far less potently to $S1P_1$.[6] These findings encouraged us to explore chiral phosphate mimics based on **1-P** that would act without the need for a phosphorylation step in vivo. This study complements the work of other groups,[7] in particular investigations of various phosphate replacements by Merck researchers.[7a]

We chose to investigate carboxylic acids, phosphonic acids, sulfonic acids, but also heterocyles like tetrazoles and sulfonylhydantoins for their potential to mimic the phosphate group of **1-P**.

To allow for maximal overlap of the acidic moieties of these mimics with **1-P**, we initially designed molecules **3**, **4**, and **6** featuring a 2-carbon spacer next to the chiral center (Scheme 1). Our synthetic strategy made use of the availability of aldehyde **2** as a key intermediate[6] as well as Horner–Wadsworth–Emmons (HWE) methodology. Thus, HWE reaction with ethyl (diethoxyphospho-

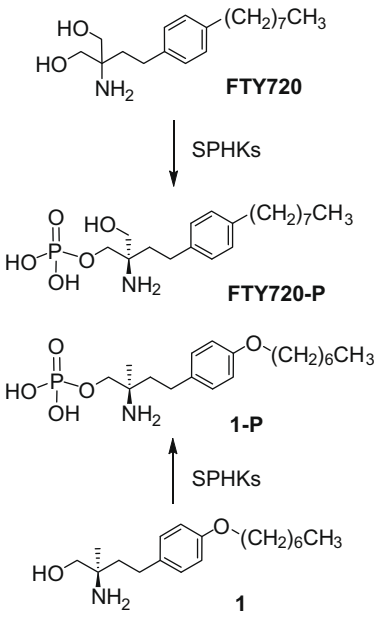

Figure 1. Phosphorylation of FTY720 and amino alcohol **1** in vivo.

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ryl)methanesulfonate, followed by hydrogenation of the double bond and treatment with acid to simultaneously saponify the sulfonic ester and to remove the Boc group led to amino sulfonic acid **3**. Amino phosphonic acid **4** was prepared by reaction of aldehyde **2** with dibenzyl methylenediphosphonate, followed by double bond hydrogenation/debenzylation with H_2/Pd-C and removal of the Boc group. HWE reaction of aldehyde **2** with ethyl 2-(diethoxyphosphoryl)acetate followed by double bond hydrogenation and LiOH mediated ester saponification gave carbamate **5**. Acidic deprotection finally liberated γ-amino acid **6**.

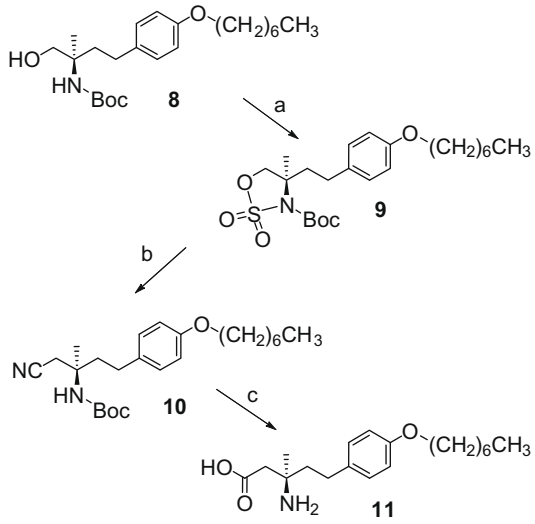
To assess the influence of the spacer length, we prepared carboxylic acid derivatives **7** and **11** (Schemes 1 and 2). Pinnick oxidation of aldehyde **2**, followed by acidic deprotection of the amino group gave α-amino acid **7**. Sulfamidate **9**, generated in two steps from amino alcohol **8** via the corresponding diastereomeric sulfamidites,[8] served as the precursor for the one-carbon chain elongation with sodium cyanide to give nitrile **10**.[9] Simultaneous saponification and Boc removal led to formation of β-amino acid **11**.

To generate the tetrazole, we converted acid **5** to amide **12** (Scheme 3). This amide was then transformed to tetrazole **13** using $TMSN_3$.[10] Basic cleavage of the cyanoethyl group, followed by acidic amine deprotection liberated tetrazole **14**.

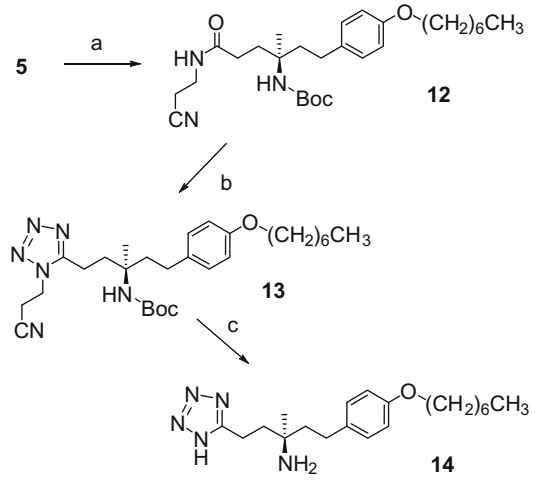
Scheme 4 shows the preparation of sulfonylhydantoin **18**.[11] Reductive amination of aldehyde **2** with glycine methyl ester gave amine **15**. Sulfamoylation to **16** was achieved via in situ generation of H_2NSO_2Cl. Cyclization with sodium methoxide generated carbamate **17** which was deprotected to give sulfonylhydantoin **18**.

All phosphate mimics were tested for S1P isoform affinity in a ligand induced [γ-^{35}S]GTP binding assay with membrane fragments from CHO transfectants stably expressing human S1P receptors.[7g] Although $S1P_3$ has been shown to be responsible for transient heart rate reduction in rodents,[7b] it could recently be demonstrated that $S1P_1$ mediates these heart rate lowering effects in humans.[12] In parallel to the in vitro binding affinity, reduction of in vivo lymphocyte count was assessed 6 h after po application to Lewis rats.[7g]

The biological results are summarized in Table 1 with FTY720-P and **1-P** as reference compounds. Sulfonic acid **3**, tetrazole **14** as well as sulfonylhydantoin **18** only exhibited potency above 100 nM on $S1P_1$. Phosphonate **4** showed 20 nM potency on $S1P_1$, but weak selectivity over $S1P_3$. For the carboxylic acid derivatives, the S1P agonist profile was strongly dependent on the length of the spacer between the chiral center and the acidic moiety. Whereas γ-amino acid **6** lacked significant $S1P_1$ potency, the corresponding β-amino acid **11** as well as α-amino acid **7** were considerably more potent. Interestingly, **7** also showed pronounced selectivity over $S1P_3$. Comparing these results to those of an earlier Merck study[7a] shows the influence of the quaternary stereogenic center at the carbon attached to the amino group on $S1P_1$ potency. The racemic

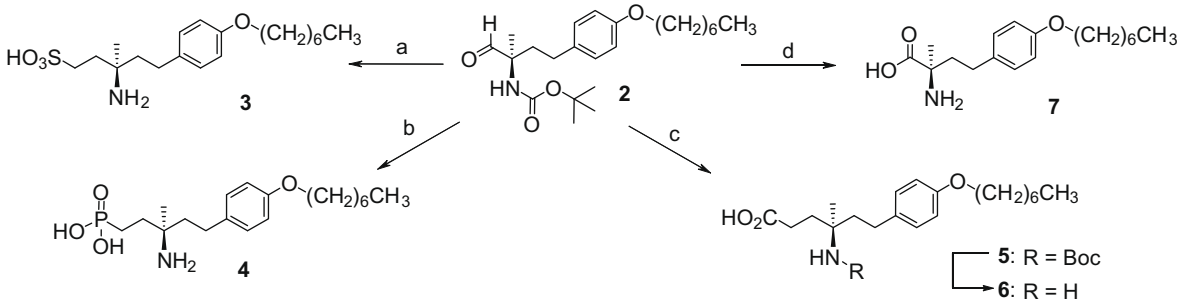


Scheme 2. Reagents and conditions: (a) (i) $SOCl_2$, pyridine, CH_3CN, −40 °C→−10 °C, 86%; (ii) $RuCl_3$ cat., $NaIO_4$, CH_3CN, H_2O, rt, 60%; (b) NaCN, DMF, rt, 92%; (c) HCl, dioxane, microwave, 160 °C, 45%.

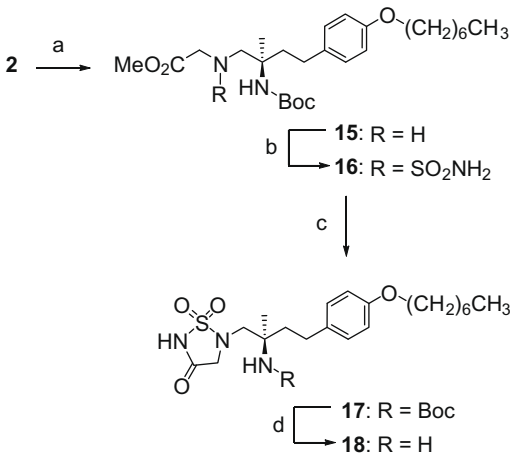


Scheme 3. Reagents and conditions: (a) $NC(CH_2)_2NH_2$, EDC, HOAt, DMF, rt, 72%; (b) Ph_3P, DIAD, $TMSN_3$, CH_3CN, rt, 57%; (c) (i) DBU, CH_2Cl_2, rt, 90%; (ii) HCl, MeOH, rt, 77%.

Merck β- and γ-amino acids **19** and **20** (Fig. 2) were found to be poor $S1P_1$ binders assessed by inhibition of [^{33}P]-$S1P_1$ binding



Scheme 1. Reagents and conditions: (a) (i) $EtO_3SCH_2P(O)(OEt)_2$, *n*-BuLi, THF, −78 °C→rt, 74%; (ii) Pd-C, H_2, EtOAc, rt, 61%; (iii) HCl, Et_2O, rt, 53%; (b) (i) $(BnO)_2P(O)CH_2P(O)(OBn)_2$, *n*-BuLi, −78 °C→rt, 53%; (ii) Pd-C, H_2, EtOAc, rt; (iii) HCl, MeOH, rt, 56% over two steps; (c) (i) $EtO_2CCH_2P(O)(OEt)_2$, *n*-BuLi, THF, −78 °C→rt, 80%; (ii) Pd-C, H_2, EtOAc, rt, 89%; (iii) LiOH, MeOH, THF, rt, 99%; (iv) TFA, CH_2Cl_2, rt, 99%; (d) (i) $NaClO_2$, 2,3-dimethyl-2-butene, KH_2PO_4, *t*-BuOH, rt, 96%; (ii) TFA, CH_2Cl_2, rt, 50%.



Scheme 4. Reagents and conditions: (a) $H_2NCH_2CO_2Me{\cdot}HCl$, $NaCNBH_4$, pH 5 buffer, rt, 29%; (b) $ClSO_2NCO$, HCOOH, rt, then add **15**, Et_3N, CH_2Cl_2, rt, 93%; (c) NaOMe, MeOH, rt, 35%; (d) HCl, Et_2O, rt, 90%.

Table 1
[γ-^{35}S]GTP binding assay results of phosphate mimics

Compound	$S1P_1$ EC_{50}[a] (nM)	$S1P_3$ EC_{50}[a] (nM)	$S1P_4$ EC_{50}[a] (nM)	$S1P_5$ EC_{50}[a] (nM)
FTY720-P	0.14	2.5	0.70	0.46
1-P	0.85	5.4	5.7	2.2
3	110	nd	nd	nd
4	20	151	19	19
6	240	3100	800	nd
7	44	>22,000	140	280
11	19	300	120	200
14	960	6400	670	3600
18	460	2400	1700	1000

[a] All compounds shown are full agonists.

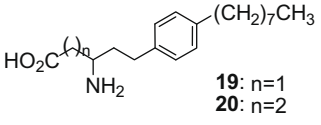


Figure 2. Achiral Merck amino carboxylates **19** and **20**.

(IC_{50} = 1900 nM for **19**, 710 nM for **20**, and 0.16 nM for FTY720-P as reference). In contrast, various racemic phosphonates of the Merck series were shown to retain high affinity for $S1P_1$.

The compounds in Table 1 were also tested for $S1P_2$ activity, but all of them showed an IC_{50} >22,000 nM.

Comparing the $S1P_1$ potency of the phosphate mimics to amino phosphate **1-P**, it is not too unsurprising that none of them displayed pronounced activity in reducing blood lymphocyte counts in vivo. For compound throughput reasons, we assessed this by measuring the ED_{50} at a single time point (6 h). The ED_{50} for β-amino acid **11** was determined to be 3.3 mg/kg, all other derivatives did not show significant reduction of lymphocyte count after a po dose of 3 mg/kg.[13] It should be noted that it is quite likely that there are significant differences in the PK parameters of these derivatives.[14] However, these remained undetermined in this single time point PD experiment.

In summary, we have shown that the phosphate group of **1-P** can be mimicked by various functional groups in a potency range on $S1P_1$ between 20 nM and 1 μM. β-Amino acid **11** also showed efficacy on peripheral lymphocyte count in rats after 6 h, illustrating the potential of amino carboxylates as oral S1P modulators.

Acknowledgements

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References and notes

1. Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; O'Connor, P.; Polman, C. H.; Haas, T.; Korn, A. A.; Karlsson, G.; Radue, E. W. *N. Engl. J. Med.* **2006**, *355*, 1124.
2. Brinkmann, V. *Pharmacol. Ther.* **2007**, *115*, 84.
3. (a) Matloubian, M.; Charles, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Maria, L.; Proia, R. L.; Cyster, J. G. *Nature* **2004**, *427*, 355; (b) Schwab, S. R.; Pereira, J. P.; Matloubian, M.; Xu, Y.; Huang, Y.; Cyster, J. G. *Science* **2005**, *309*, 1735.
4. For recent reviews see: (a) Cooke, N. G.; Zécri, F. *Annu. Rep. Med. Chem.* **2007**, *42*, 245; (b) Marsolais, D.; Rosen, H. *Nat. Rev. Drug Disc.* **2009**, *8*, 297.
5. Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. *J. Biol. Chem.* **2002**, *277*, 21453.
6. Högenauer, K.; Billich, A.; Pally, C.; Streiff, M.; Wagner, T.; Welzenbach, K.; Nussbaumer, P. *Chem. Med. Chem.* **2008**, *3*, 1027.
7. Earlier studies describing phosphonates and carboxylates: (a) Hale, J. J.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.; Chrebet, G.; Bergstrom, J.; Card, D.; Koo, G. C.; Koprak, S. L.; Jackson, J. J.; Rosen, H.; Mandala, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3351; (b) Hale, J. J.; Doherty, G.; Toth, L.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.; Chrebet, G.; Bergstrom, J.; Card, D.; Forrest, M.; Sun, S.; West, S.; Xie, H.; Nomura, N.; Rosen, H.; Mandala, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3501; (c) Hale, J. J.; Lynch, C. L.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.; Parent, S. A.; Chrebet, G.; Bergstrom, J.; Card, D.; Ferrer, M.; Hodder, P.; Strulovici, B.; Rosen, H.; Mandala, S. *J. Med. Chem.* **2004**, *47*, 6662; (d) Colandrea, V. J.; Legiec, I. E.; Huo, P.; Yan, L.; Hale, J. J.; Mills, S. G.; Bergstrom, J.; Card, D.; Chebret, G.; Hajdu, R.; Keohane, C. A.; Milligan, J. A.; Rosenbach, M. J.; Shei, G.; Mandala, S. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2905; (e) Foss, F. W.; Snyder, A. H.; Davis, M. D.; Rouse, M.; Okusa, M. D.; Lynch, K. R.; Macdonald, T. L. *Bioorg. Med. Chem.* **2007**, *15*, 663; (f) Lu, X.; Sun, C.; Valentine, W. J.; Shuyu, E.; Liu, J.; Tigyi, G.; Bittman, G. *J. Org. Chem.* **2009**, *74*, 3192; (g) Pan, S.; Mi, Y.; Pally, C.; Beerli, C.; Chen, A.; Guerini, D.; Hinterding, K.; Nuesslein-Hildesheim, B.; Tuntland, T.; Lefebvre, S.; Liu, Y.; Gao, W.; Chu, A.; Brinkmann, V.; Bruns, C.; Streiff, M.; Cannet, C.; Cooke, N.; Gray, N. *Chem. Biol.* **2006**, *13*, 1227.
8. Meléndez, R. E.; Lubell, W. D. *Tetrahedron* **2003**, *59*, 2581.
9. Elongation with standard CN displacement of the mesylate failed because of competing cyclic carbamate formation: Curran, T. P.; Pollastri, M. P.; Abelleira, S. M.; Messier, R. J.; McCollum, T. A.; Rowe, C. G. *Tetrahedron Lett.* **1994**, *35*, 5409.
10. Duncia, J. V.; Pierce, M. E.; Santella, J. B., III *J. Org. Chem.* **1991**, *56*, 2395.
11. Campbell, A. D.; Birch, A. M. *Synlett* **2005**, 834. and references cited therein.
12. Gergely, P.; Wallstroem, E.; Nuesslein-Hildesheim, B.; Bruns, C.; Zécri, F.; Cooke, N.; Traebert, M.; Tuntland, T.; Rosenberg, M.; Saltzman, M. *Mult. Scler.* **2009**, *15*, S125.
13. ED_{50} of FTY720 in that assay is 0.09 mg/kg.
14. For assay throughput and project requirement reasons, compounds were routinely tested using po administration. Only amino acid **7** was also dosed ip to give an ED_{50} of ca. 10 mg/kg.