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Synthesis of 4(5)-phenylimidazole-based analogues of sphingosine-1-phosphate and FTY720: Discovery of potent $S1P_1$ receptor agonists

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Abstract—The novel immunosuppressant FTY720 has been demonstrated to elicit immunomodulating effects via interaction with the G-protein coupled receptor $S1P_1$. FTY720 induced agonism at the $S1P_3$ receptor, however, has been shown to result in mild bradycardia, a minor side-effect of initial FTY720 therapy. This report describes the synthesis of several potent 4(5)-phenylimidazole-based $S1P_1$ receptor agonists that are accompanied by poor agonist activity at $S1P_3$. For instance, compound **20** displayed an $EC_{50} = 4.7 \pm 1.3$ nM at the $S1P_1$ receptor and $EC_{50} = 780 \pm 1.3$ nM at the $S1P_3$ receptor using a [γ-^{35}S]GTP-binding assay as compared to phospho-FTY720 ($S1P_1$: $EC_{50} = 1.3 \pm 1.3$ nM, $S1P_3$: $EC_{50} = 2.0 \pm 2.4$ nM).

The development of FTY720 for treatment against organ transplant rejection has generated a great deal of interest in the discovery of similar immunosuppressive agents.[1] The active metabolite of FTY720, phospho-FTY720, acts as a potent agonist at four of the five sphingosine-1-phosphate (S1P) receptors, a family of G-protein coupled receptors whose natural ligand is S1P.[2] The recent discovery that phospho-FTY720 elicits immunomodulatory effects via interaction with the $S1P_1$ receptor has made that receptor a target for the synthesis of selective agonists.[3] Agonism at the $S1P_3$ receptor has been shown to induce undesired cardiovascular effects including mild bradycardia, a minor side-effect of initial FTY720 therapy.[4] Therefore, the development of S1P/FTY720 analogues with greater selectivity for $S1P_1$ relative to $S1P_3$ is desired in the investigation for new immunomodulators (see Fig. 1).

In an effort to further our understanding of the SAR of S1P and FTY720 with respect to the S1P receptors and to develop possible therapeutic candidates, we have synthesized a class of analogues incorporating a 4(5)-phenylimidazole functionality in the linker region. This class of S1P receptor agonists essentially constitutes the insertion of a single carbon–carbon bond spacer into the benzimidazole functionality of our previously reported $S1P_4$ selective agonists.[5a] The 4(5)-phenylimidazole class of S1P/FTY720 analogues was found to possess potent agonism at $S1P_1$ accompanied by a relatively low affinity for the $S1P_3$ receptor. In this class of agonists, we explored the effects of stereochemistry at the C2 amino group, methylation at the C2 position, and the use of a variety of head groups.

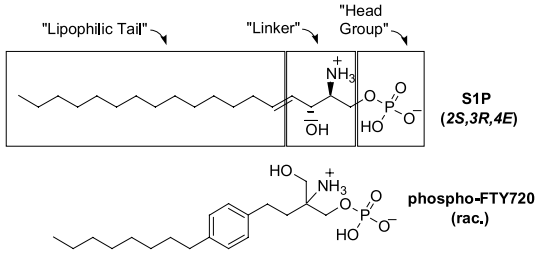

Figure 1. Structures and regions of S1P and phospho-FTY720.

Keywords: S1P receptor agonists; FTY720; S1P1; Bradycardia; Phosphothioate; Sphingosine-1-phosphate.
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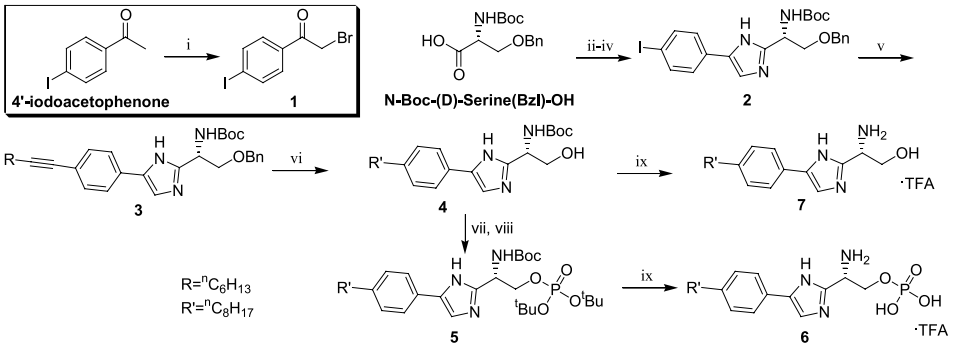
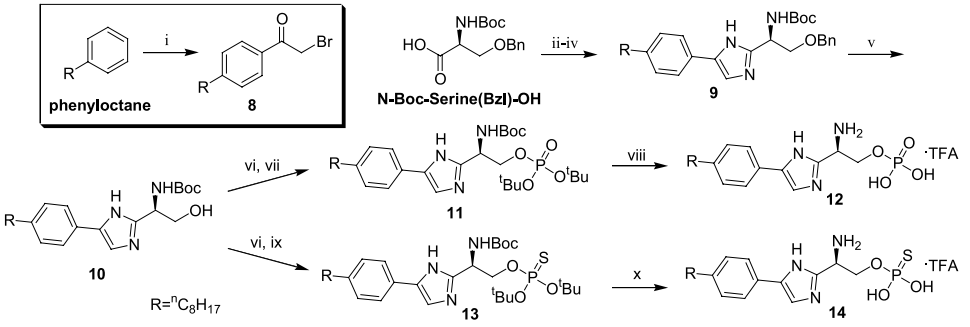
Preparation of the 4(5)-phenylimidazole-based S1P receptor agonists commenced with the synthesis of the (2*S*) 4′(5′)-phenylimidazole compounds **6** and **7** (Scheme 1). After treatment of N-Boc-(D)-serine(Bzl)-OH with cesium carbonate, the cesium salt was allowed to displace the bromine of compound **1**, generated by bromination of 4′-iodoacetophenone, to give an intermediate ketoester that was cyclized to the imidazole **2** on treatment with ammonium acetate and azeotropic removal of water.[6] Compound **2** was then subjected to a Sonogashira coupling to 1-octyne generating the aryl alkyne compound **3**. Compound **3** then underwent chemoselective, simultaneous reduction of the aryl alkyne, as well as removal of the benzyl ether group under Birch reduction conditions to give the free alcohol **4** without affecting the 4′(5′)-phenylimidazole functionality. Alcohol **4** was next phosphorylated with subsequent oxidation by hydrogen peroxide to give the protected phosphate **5**.[7] Global deprotection of compound **5** provided the phosphate **6** as the TFA salt. Alcohol **7** was obtained as a TFA salt from **4** on removal of the N-Boc-protecting group.

To shorten the synthesis of the (2*R*)-4′(5′)-phenylimidazole based phosphate and phosphothioate compounds **12** and **14**, respectively, the alkyl chain was installed prior to imidazole formation (Scheme 2). After treatment with cesium carbonate, the cesium salt of N-Boc-serine(Bzl)-OH was allowed to displace the bromine of **8**, a product of the Friedel–Crafts acylation of phenyl octane with bromoacetyl bromide, resulting in the clean formation of the intermediate ketoester that was cyclized to the imidazole **9** on treatment with ammonium acetate and azeotropic removal of water. Deprotection of the benzyl ether of **9** under Birch reduction conditions yielded alcohol **10**, which was then phosphorylated with subsequent oxidation by hydrogen peroxide to give the protected phosphate **11**. Compound **11** was next globally deprotected, yielding the phosphate **12** as the TFA salt. The phosphothioate **14** was obtained from compound **10** after phosphorylation with subsequent oxidation by elemental sulfur to give compound **13**. Compound **14** was then obtained as the TFA salt on global deprotection of **13** in the presence of benzenethiol as a cation scavenger.[8]

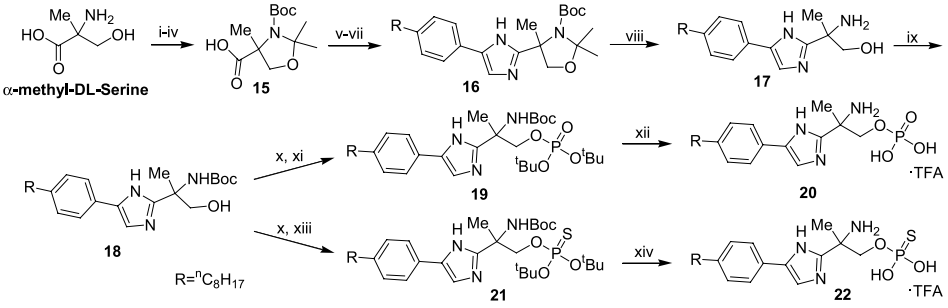
Synthesis of the racemic C2-methylated alcohol, phosphate, and phosphothioate compounds **17**, **20**, and **22**, respectively, began with the Fisher esterification of α-methyl-DL-serine, followed by N-Boc protection, acetonide protection, and finally saponification of the initially formed methyl ester to give carboxylic acid **15** (Scheme 3). Acid **15** was then coupled to **8** (synthesis described in Scheme 1) and the resulting ketoester was



Scheme 1. Reagents and conditions: (i) Br_2, 1:1 Et_2O/dioxane, rt, 12 h, 65%; (ii) Cs_2CO_3, EtOH, rt, 1 h; (iii) **1**, DMF, rt, 1 h, 81% (two steps); (iv) NH_4OAc, xylenes, 110 °C, 6 h, 46%; (v) 1-octyne, $Pd(dba)_2$, Ph_3P, CuI, DIEA, THF, rt, 12 h, 91%; (vi) Na °, NH_3, −78 °C, 5 min, 68%; (vii) tetrazole, di-*tert*-butyl diisopropylphosphoramidite, 1:1 CH_2Cl_2/THF, rt, 12 h; (viii) H_2O_2, rt, 4 h, 73% (two steps); (ix) 1:1 TFA/CH_2Cl_2, rt, 4 h, quant.



Scheme 2. Reagents and conditions: (i) $AlCl_3$, $BrC(O)CH_2Br$, 1,2-DCE, 0 °C → rt, 2 h, 57%; (ii) Cs_2CO_3, EtOH, rt, 1 h; (iii) **8**, DMF, rt, 1 h; (iv) NH_4OAc, xylenes, 110 °C, 6 h, 76% (three steps); (v) Na °, NH_3, −78 °C, 5 min, 42%; (vi) tetrazole, di-*tert*-butyl diisopropylphosphoramidite, 1:1 CH_2Cl_2/THF, rt, 12 h; (vii) H_2O_2, rt, 4 h, 62% (two steps); (viii) 1:1 TFA/CH_2Cl_2, rt, 4 h, 93%; (ix) S_8, rt, 3 h, 39% (two steps); (x) PhSH, TMSBr, 1:1 TFA/CH_2Cl_2, rt, 4 h, quant.

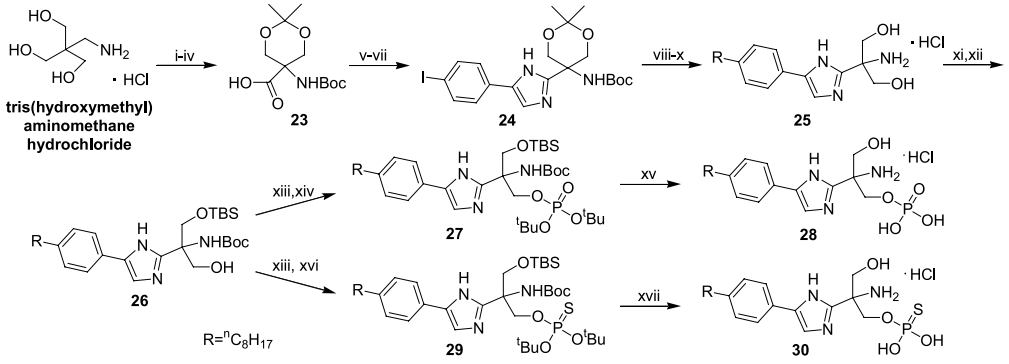


Scheme 3. Reagents and conditions: (i) MeOH, $SOCl_2$, 0 °C → rt, 12 h; (ii) $(Boc)_2O$, $NaHCO_3$, 1:1 H_2O/THF, rt, 12 h, 33% (two steps); (iii) 2,2-DMP, $BF_3 \cdot OEt_2$, rt, 12 h, 85%; (iv) 2 M NaOH, MeOH, rt, 12 h, 85%; (v) Cs_2CO_3, EtOH, rt, 1 h; (vi) **8**, DMF, rt, 1 h; (vii) NH_4OAc, xylenes, 110 °C, 6 h, 20% (three steps); (viii) *p*-TsOH, MeOH, 70 °C, 3 h, 60%; (ix) $(Boc)_2O$, Na_2CO_3, 1:1 H_2O/THF, rt, 12 h, 57%; (x) tetrazole, di- *tert*-butyl diisopropylphosphoramidite, CH_2Cl_2/THF, rt, 12 h; (xi) H_2O_2, rt, 3 h, 46% (two steps); (xii) 1:1 TFA/CH_2Cl_2, rt, 4 h, 91%; (xiii) S_8, rt, 3 h, 46% (two steps); (xiv) PhSH, TMSBr, 1:1 TFA/CH_2Cl_2, rt, 4 h, 94%.

cyclized to the imidazole compound **16**. Alcohol **17** was obtained as the free base after global deprotection of compound **16** under acidic conditions with a basic work-up. To produce the phosphate and phosphothioate compounds **20** and **22**, respectively, **17** was regioselectively protected as the primary N-Boc to give the protected alcohol **18**. Compound **18** was then phosphorylated and subsequently oxidized by hydrogen peroxide to give the protected phosphate **19**. Global deprotection of **19** yielded the racemic C2-methylated phosphate **20** as the TFA salt. Oxidation of the phosphite obtained from the phosphorylation of **18** with elemental sulfur supplied compound **21**. Global deprotection of **21** in the presence of a cation scavenger furnished the racemic C2-methylated phosphothioate **22** as the TFA salt.

Synthesis of the 2-amino-1,3-propanediol based compounds **25**, **28**, and **30** was initiated with acetonide protection of tris(hydroxymethyl)aminomethane hydrochloride, followed by N-Boc protection, Swern oxidation, and finally sodium chlorite oxidation to give the carboxylic acid **23** (Scheme 4). Compound **23** was subsequently coupled to **1** (synthesis described in Scheme 1) and cyclized to imidazole **24**. Compound **24** was then subjected to a Sonogashira coupling to 1-octyne generating the aryl alkyne compound, which was then hydrogenated to reduce the newly formed triple bond. Global deprotection provided the 2-amino-1,3- propanediol **25** as the HCl salt. To provide the mono-phosphate and mono-phosphothioate compounds **28** and **30**, respectively, **25** was regioselectively protected as the primary N-Boc and the resulting diol was subjected to conditions for mono-TBS protection to give the racemic alcohol **26**. Compound **26** was next phosphorylated and then oxidized by hydrogen peroxide to provide compound **27**. Concurrent deprotection of the TBS, phosphate ester, and N-Boc groups of **27** supplied the mono-phosphate **28** as the HCl salt. To obtain the mono-phosphothioate **30**, alcohol **26** was first phosphorylated and then oxidized with elemental sulfur to give compound **29**. Concurrent deprotection of the TBS, phosphothioate ester, and N-Boc groups of **29** with a cation scavenger present supplied the mono-phosphothioate **30** as the HCl salt.

Analysis of the phosphate compounds **6** and **12** demonstrated them to be very potent agonists at $S1P_1$ and good agonists at $S1P_5$ with good efficacy at each receptor (Table 1).[9] The (2*R*)-compound **12** displayed slightly



Scheme 4. Reagents and conditions: (i) PTSA (cat.), 2,2-DMP, DMF, rt, 12 h; (ii) $(Boc)_2O$, $NaHCO_3$, 1:1 THF/H_2O, rt, 12 h, 69% (two steps); (iii) $(COCl)_2$, DMSO, TEA, DCM, −78 °C rt, 2 h, 74%; (iv) $NaClO_2$, $NaH_2PO_4 \cdot H_2O$, ^{t}BuOH/H_2O, 2-methyl-2-butene, rt, 1 h, 95%; (v) Cs_2CO_3, EtOH, rt, 1 h; (vi) **1**, DMF, rt, 1 h, 86% (two steps); (vii) NH_4OAc, xylenes, 110 °C, 6 h, 50%; (viii) 1-octyne, $Pd(dba)_2$, Ph_3P, CuI, DIEA, THF, rt, 12 h, 92%; (ix) H_2, 10% Pd/C, EtOH, rt, 12 h, quant.; (x) 3 N HCl, THF, rt, 4 h, 87%; (xi) $(Boc)_2O$, $NaHCO_3$, 1:1 THF/H_2O, 50 °C, 12 h, 68%; (xii) TBSCl, imid., DMAP (cat.), CH_2Cl_2, rt, 1 h, 52%; (xiii) tetrazole, di-*tert*-butyl diethylphosphoramidite, CH_2Cl_2/THF, 50 °C, 1 h; (xiv) H_2O_2, rt, 3 h, 48% (two steps); (xv) 3N HCl, THF, rt, 4 h, 38%; (xvi) S_8, rt, 3 h, 24% (two steps); (xvii) PhSH, TMSBr, 3 N HCl, THF, rt, 4 h, 50%.

Table 1. EC_{50} (nM) and E_{max} values for S1P and synthetic analogues at S1P receptors determined by a [γ-^{35}S]GTP binding assay[ab]

Compound	$S1P_1$		$S1P_2$		$S1P_3$		$S1P_4$		$S1P_5$	
	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}
S1P	4.5 ± 1.1	1.00	8.3 ± 1.2	1.00	8.7 ± 1.1	1.00	270 ± 1.2	1.00	9.2 ± 1.1	1.00
Phospho-FTY720	1.3 ± 1.3	1.00	naa	0.00	2 ± 2.4	0.50	41 ± 1.2	0.78	40 ± 1.2	0.56
6	7 ± 1.2	0.75	naa	0.00	1700 ± 3.2	0.56	400 ± 1.1	1.00	37 ± 1.2	0.73
7	2700 ± 1.2	0.98	naa	0.00	2700 ± 1.3	0.38	7400 ± 1.2	0.90	860 ± 1.3	0.83
12	4 ± 1.3	0.69	naa	0.00	330 ± 1.4	0.42	150 ± 1.2	0.79	12 ± 1.3	0.69
14	6.4 ± 1.3	0.67	naa	0.00	690 ± 1.2	0.45	190 ± 1.2	0.82	29 ± 1.7	0.82
17	naa	0.00	naa	0.00	naa	0.00	naa	0.00	naa	0.00
20	4.7 ± 1.3	0.88	naa	0.00	780 ± 1.3	0.53	91 ± 1.4	0.94	26 ± 1.1	0.72
22	9.8 ± 1.2	0.87	naa	0.00	1600 ± 1.3	0.44	170 ± 1.4	0.68	51 ± 1.2	0.60
25	5100 ± 1.2	0.66	naa	0.00	naa	0.00	660 ± 2.1	0.79	6700 ± 2.4	0.48
28	7.9 ± 1.1	0.91	18 ± 6.3	0.95	630 ± 3.7	0.21	160 ± 1.2	0.87	17 ± 1.2	0.66
30	150 ± 1.1	0.66	naa	0.00	790 ± 1.3	0.68	89 ± 1.5	0.72	16 ± 2.0	0.56

[a] Values are means of three experiments (naa = no agonist activity).
[b] E_{max} = maximal efficacy of analogue/maximal efficacy of S1P at the indicated receptor.

more potent agonism at $S1P_1$ and $S1P_5$ than its enantiomer **6**. As was expected, alcohol **7** was a poor agonist at all of the S1P receptors, approximately 2- to 3-fold less potent than the phosphate counterpart **6**. The phosphothioate **14** retained relative potency and efficacy at $S1P_1$, as compared to the phosphate counterpart **12**, while displaying slightly decreased potency and a mild increase in efficacy at $S1P_5$.

Examination of the C2-methylated phosphate compound **20** demonstrated retention of agonism as compared to the non-methylated counterparts **6** and **12**. As with the phosphate counterparts, compound **20** displayed potent agonism at $S1P_1$ and good potency at $S1P_5$. The 2-methylated phosphothioate compound **22** retained the agonism of the phosphate counterpart **20** with only slight decreases in potency and efficacy at $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$. The 2-methylated alcohol compound **17** showed a total lack of agonism at each of the S1P receptors.

Incorporation of a 2-amino-1,3-propanediol head group gave several interesting results. As expected, the diol compound **25** was a poor agonist at all of the S1P receptors. The mono-phosphate compound **28**, however, demonstrated very high levels of potency and efficacy as an agonist at $S1P_1$ and $S1P_2$, to our knowledge the first synthetic compound to demonstrate potent agonism at $S1P_2$. Compound **28** showed only moderate selectivity however, as it was also a relatively potent agonist at $S1P_4$, and $S1P_5$. Compared to **28**, the mono-phosphothioate **30** lost potency and efficacy as an agonist at $S1P_1$ and all agonist activity at $S1P_2$. With regard to $S1P_3$, $S1P_4$ and $S1P_5$, compound **30** retained the properties of compound **28**, acting as a poor agonist at $S1P_3$, while displaying rather high potency and moderate efficacy at $S1P_4$ and $S1P_5$.

To summarize, we have synthesized a series of S1P/FTY720 analogues that incorporate a 4(5)-phenylimidazole ring system in the 'linker' region of the pharmacophore. This structural modification has resulted in the generation of highly potent $S1P_1$ agonists. We have determined a slight preference in potency for 2*R*-configuration, as well as a necessity for the phosphate or phosphothioate head group to obtain significant potency. We have also demonstrated the retention of agonism on methylation at the C2 position and that incorporation of a mono-phosphate 2-amino-1,3-propanediol head group results in retention of agonist activity at $S1P_1$ as compared to compounds **6** and **12**. The mono-phosphothioate compound **30**, however, significantly lost agonist activity at $S1P_1$. Our findings have helped to develop the SAR of S1P/FTY720 analogues with regard to selectivity between $S1P_1$ and $S1P_3$ agonism, and will serve as the basis for future SAR and in vivo studies.

Acknowledgments

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