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The discovery of 3-(N-alkyl)aminopropylphosphonic acids as potent S1P receptor agonists

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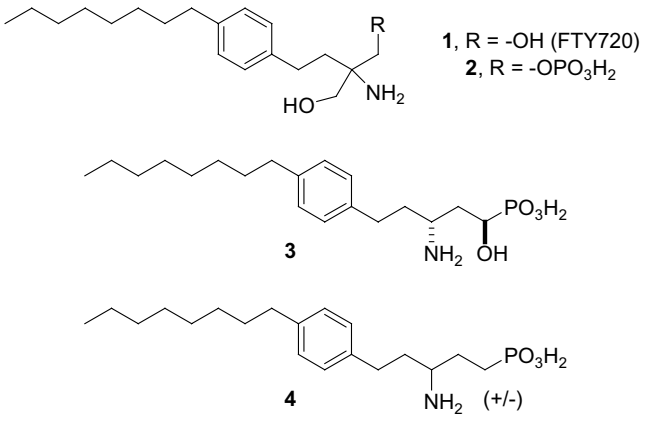
Abstract—3-(N-Alkyl)aminopropylphosphonic acids are potent agonists of four of the five known sphingosine-1-phosphate (S1P) receptor subtypes.

Sphingosine-1-phosphate (S1P) has been demonstrated to be an extracellular signaling factor for a family of related G-protein coupled receptors, which are now named for this ligand.[1] S1P receptors (of which there are five known subtypes, $S1P_{1,2,3,4,5}$) have been implicated to play roles in a variety of biological processes for multiple organ systems.[2] It has been proposed that the immunosuppressive actions of FTY720 (**1**) result from the formation in vivo of an active phosphate ester metabolite (**2**), which is a potent agonist of four of the five known S1P receptors.[3,4]

In order to better understand the pharmacology of FTY720 phosphate (**2**), we sought compounds that would remove the complicating factor of the observed in vivo equilibrium between **1** and **2** and directly target S1P receptors.[5] Nonhydrolyzable phosphonate analogs of **2** were found to be S1P agonists; deletion of the hydroxymethyl group from the quaternary center of these compounds was found to have minimal effect on S1P receptor affinity while an α-hydroxy phosphonate group was demonstrated to be a suitable bioisostere for the phosphate ester. A compound (**3**) identified as part of this work had an S1P receptor profile similar to **2** and was found to replicate the primary pharmacodynamic effect (a dose-dependent lowering of circulating lymphocytes) and the immunosuppressive efficacy of **1** in rodents. While this does not prove that the efficacy of **1** is due the actions of the phosphate ester metabolite, the similarities observed between **2** and **3** suggest that S1P receptor agonists could find utility in immunosuppressive therapy.



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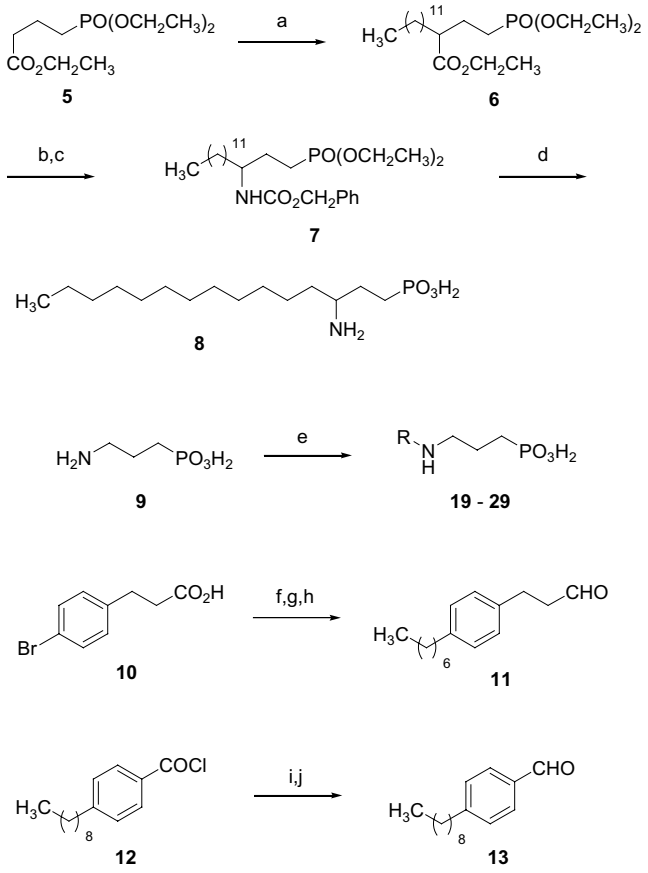
Compound **4** was identified as part of our initial investigations of nonhydrolyzable phosphonate S1P receptor agonists. While this analog was found to have 3–80-fold lower affinity for S1P receptors than either **2** or **3**, it was structurally simpler than either compound, lent itself to a more rapid preparation of analogs and was therefore the basis for some early range finding regarding which structural elements in **4** were required for S1P receptor affinity. These investigations revealed that the lipophilic tail of these compounds could be transposed from the carbon bearing the amino group onto the amino group itself and the resulting 3-(N-alkylamino)propyl-phosphonic acids were ligands for S1P receptors. Some details of the structure–activity relationships for this new class of S1P receptor agonists is the subject of this report.

The preparation of compound **8** is an example of synthetic chemistry used to prepare the (±)-3-(amino)-alkylphosphonic acids described herein (Scheme 1). Treatment of triethyl 4-(phosphono)butyrate (**5**) with potassium bis(trimethylsilyl)amide at −78 °C followed by 1-iodododecane afforded monoalkylated product **6** in good yield. The carboxylate ester of this compound was converted to a protected amine (**7**) with a straightforward sequence of reactions featuring a Curtius rearrangement. Global deprotection of **7** was carried out using iodotrimethylsilane affording target compound **8**. The racemic 3-aminoalkylphosphonic acid analogs **14**–**18** were readily prepared by substituting the appropriate alkyl halide in the first step of this sequence.

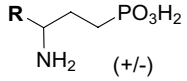


Scheme 1. Reagents and conditions: (a) 1-iodododecane, KHMDS, THF, −78 °C to rt (37%); (b) NaOH, aq MeOH, 50 °C, then CH_3OCOCl, TEA, THF, 0 °C then NaN_3, aq THF; (c) benzyl alcohol, toluene, 85 °C (56%, three steps); (d) iodotrimethylsilane, CH_2Cl_2 (95%); (e) R–CHO, $Bu_4N^+OH^-$, $Na(CN)BH_3$, CH_3OH (20–40%); (f) *i*BuOCOCl, NMM, THF, 0 °C, then $NaBH_4$, aq THF, 0 °C (95%); (g) 1-heptene, 9-BBN, THF, rt, then cat. $(Ph_3P)_4Pd$, KOH, aq THF, reflux (92%); (h) cat. TPAP, NMMO, 4 Å sieves, CH_2Cl_2, (63%); (i) $CH_3NHOCH_3{\cdot}HCl$, toluene/aq NaOH (91%); (j) DIBALH, CH_2Cl_2, −78 °C (75%).

The 3-(N-alkylamino)propylphosphonic analogs **19**–**29** were most conveniently synthesized in 20–40% yield by treating the appropriate aldehyde and equivalent amounts of 3-aminopropylphosphonic acid (**9**) and tetrabutylammonium hydroxide in methanol with sodium cyanoborohydride at 50 °C. Neutralization of the reaction mixtures to give the zwitterionic 3-(N-alkylamino)propyl phosphonic acids occurred during reverse-phase HPLC purification. Some of the aldehydes required for the reductive aminations were commercially available and in other instances they were readily prepared via straightforward functional group manipulations. The preparation of aldehydes **11** and **13** from **10** and **12**, respectively, is representative of the synthetic chemistry that was used to prepare aldehyde intermediates (Scheme 1). The phosphinic and carboxylic acid analogs **30**–**36** were prepared using chemistry analogous to that described above substituting the appropriate amino phosphinic or carboxylic acid for 3-aminopropylphosphonic acid.

Ligand competition studies between [^{33}P]-S1P and **2**, **3** and all new compounds were carried out for each of the

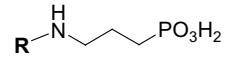
Table 1. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]

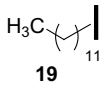
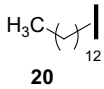
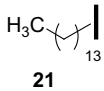
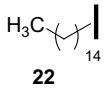
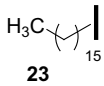
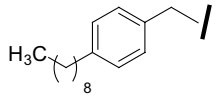
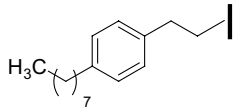
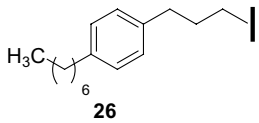
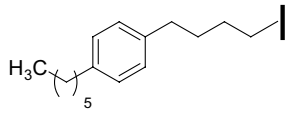
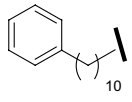


R (Compd)	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
2	0.3	1100	6.3	15	0.8
3	0.10	>10,000	18	16	4.5
4	8.4	>10,000	280	240	100
H_3C(11) **8**	150	>10,000	40	2500	86
H_3C(12) **14**	25	>10,000	10	140	86
H_3C(13) **15**	19	1200	14	76	830
H_3C(14) **16**	85	>10,000	47	1400	450
H_3C(17) **17**	1600	>10,000	130	>10,000	1500
Ph(10) **18**	15	2100	2	94	110

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 3 for assay protocol.

Table 2. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]



R (Compd)	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
 **19**	30	>10,000	13	650	19
 **20**	2.5	150	2.8	200	38
 **21**	2.3	580	3.6	140	13
 **22**	6.6	740	2.7	230	11
 **23**	7.0	740	15	800	12
 **24**	0.6	>10,000	21	26	5.5
 **25**	6.3	>10,000	4.3	24	45
 **26**	5.3	2200	14	840	42
 **27**	360	2000	1000	>10,000	590
 **28**	1.1	530	0.91	140	59

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 3 for assay protocol.

five human S1P receptors stably expressed in Chinese Hamster Ovary (CHO) cell membranes.[3] S1P receptor agonism by the test compounds was also determined by measurement of ligand-induced [^{35}S]-5′-O-3-thiotriphosphate (GTPγS) binding; all of the compounds tested were found to be agonists of S1P receptors (data not shown).[6] The (±)-3-(amino)alkylphosphonic acid compounds in Table 1 are analogs of **4** containing simple *n*-alkyl side chains. The length of the alkyl chain does not contribute to selectivity for any single S1P receptor and all of the compounds in this series have minimal $S1P_2$ affinity. It is apparent that a side chain length of 13 or 14 carbon atoms (compounds **14** and **15**) leads to optimal affinity for the other four S1P receptors, although none of these is greater than that of **4** for $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$. It is noteworthy that a correlation between *n*-alkyl chain length in analogs of **1** and in vivo immunosuppressive activity has been reported;[7] the S1P receptor data for the compounds in Table 1 suggests that may be due in part to the monophosphate esters of those 2-alkyl-2-amino-1,3-propanediols demonstrating similar trends in S1P receptor affinity.

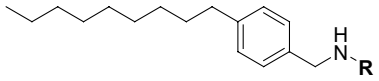
Transposition of the *n*-alkyl side chains of the analogs in Table 1 from the carbon bearing the primary amino group onto the amino group itself afforded the

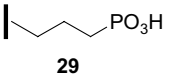
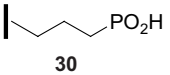
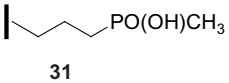
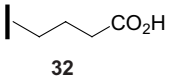
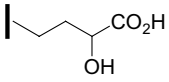
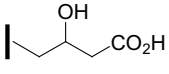
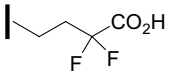
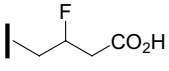
3-(N-alkylamino)propyl phosphonic acid analogs in Table 2. The relationship between *n*-alkyl chain length and S1P receptor affinity for **19**–**23** appears similar to that observed for the corresponding analogs in Table 1 with a length of 13 or 14 carbon atoms being optimal. These same analogs (**19**–**23**) had increases in receptor affinity of 5–10-fold for both $S1P_1$ and $S1P_2$ with more modest increases generally seen for $S1P_3$, $S1P_4$, and $S1P_5$ as compared to the corresponding compounds in Table 1. Insertion of a phenyl ring in the alkyl chain could bring about further enhancements in potency. Compounds **24**–**28** all maintain the same approximate alkyl chain length of compounds **21** or **22**; benzyl amine analog **24** has a subnanomolar $S1P_1$ IC_{50} and 10-fold or greater enhanced affinity for $S1P_3$, $S1P_4$, and $S1P_5$ as compared to **4**. Phenyldecyl analog **28** was found to have high affinity for both $S1P_1$ and $S1P_3$.

In anticipation of the probable poor oral adsorption of compounds **19**–**28**, a series of zwitterionic compounds bearing a 4-(nonyl)benzyl side chain was prepared (Table 3). Phosphinic and carboxylic acids were found to be poor replacements for the phosphonate based on the significant drop in S1P receptor affinities observed for **30**–**32**. Substitution of the γ-amino butyric acid analog **32** with either hydroxy groups (compounds **33** and **34**) or fluorine atoms (compounds **35** and **36**) had little effect on S1P receptor affinities.

The ability of S1P receptor agonists to lower the number of circulating lymphocytes in the mouse after iv administration can be used as a marker of their immunosuppressive efficacy.[5] Many of the 3-(N-alkylamino)propyl phosphonic acids here were evaluated for that ability, but intravenous (iv) dose–titration measurements in Balb/c mice were often complicated by an acute toxicity, apparently cardiovascular in nature that was characterized by ataxia, labored breathing, ruffling, or reduced activity in mild instances and unconsciousness, seizures, paralysis, or even death in more severe cases. For example, 3 mpk iv doses of compounds **20**, **22**–**25**, and **27** were all found to be severely toxic. Reduced doses of tetradecyl analog **21** or 4-(nonyl)benzyl analog **29** were also acutely toxic, but administration of these compounds via the peritoneal cavity was found to enhance tolerability presumably by blunting maximum compound concentration. A 0.25 mpk ip dose of **29** gave the maximal lymphocyte lowering response at 3 h after

Table 3. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]



R (Compd)	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
 **29**	0.2	750	2.7	40	0.7
 **30**	12	>10,000	1000	900	140
 **31**	2	>10,000	170	590	60
 **32**	7.7	>10,000	1200	>10,000	200
 **33**	22	>10,000	370	1200	99
 **34**	14	>10,000	170	1400	66
 **35**	44	>10,000	390	4000	200
 **36**	41	>1000	350	600	57

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 3 for assay protocol.

compound challenge. Aside from **2** and **3**,[8] none of the other compounds in Tables 1–3 were able to induce the maximal lymphopenic response at this dose level.

In conclusion, 3-(N-alkylamino)propyl phosphonic acids have been discovered to be a novel class of S1P receptor agonists. The fact that these new S1P agonists and those from other structural series (e.g., **2** and **3**) all can induce a lowering of circulating lymphocytes indicates that ligands for S1P receptors indeed have the potential to be novel immunomodulators.[9] The acute toxicity seen in mice with S1P agonists may be an extreme manifestation of the bradycardia that has been reported in the clinic with **1**.[10] The varied distributions of S1P receptor subtypes[11] suggests that the separation of efficacy and acute toxicity may be possible with receptor-selective ligands; reports of the exploitation of these structurally simple 3-(N-alkylamino)propyl phosphonic acids to this end will follow shortly.

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6. All test compounds were found to be agonists of human $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors as evidenced by their ability to induce levels of GTPγS binding comparable to S1P. The magnitudes of the calculated EC_{50} values from these assays were generally +/−5-fold of the IC_{50} values.
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8. Signs of mild toxicity were observed in mice for 15–30 min after doses of 0.5 mpk iv of **2** or 0.25 mpk iv of **3**. Escalating the dose of either compound resulted in more severe toxicity, but not lethality. Metabolic factors or altered binding to plasma proteins of **2** and **3** as compared to the 3-(N-alkylamino)propyl phosphonic acid analogs may account for the observed differences.
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