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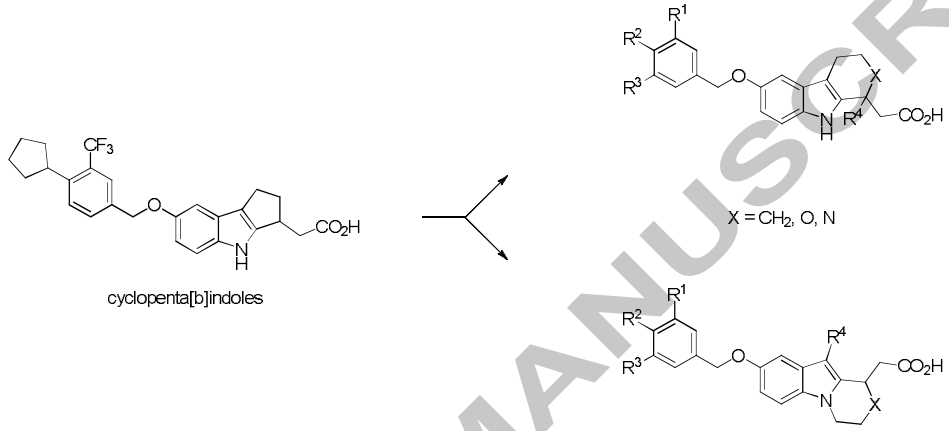

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Design and Synthesis of New Tricyclic Indoles as Potent Modulators of the $S1P_1$ Receptor

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[a] *Arena Pharmaceuticals Inc. 6154 Nancy Ridge Drive, San Diego, CA 92121.*

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ABSTRACT

Modulators of $S1P_1$ have proven utility for the treatment of autoimmune disease and efforts to identify new agents with improved safety and pharmacokinetic parameters are ongoing. Several new $S1P_1$ chemotypes were designed and optimized for potency and oral bioavailability. These new agents are characterized by a "tricyclic fused indole array" and are highly potent agonists of the $S1P_1$ receptor.

Gilenya® (**1**, Figure 1) is a sphingosine 1-phosphate (S1P) receptor modulator indicated for the treatment of relapsing remitting multiple sclerosis (RRMS). *In vivo* **1** is phosphorylated to the *(S)*-monophosphate **2** which is a potent agonist of $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$.[1] Sustained activation of lymphocyte expressed $S1P_1$ results in the internalization and degradation of the receptor.[2] Loss of $S1P_1$ from the cell surface restricts T- and B-cells to the lymphoid tissues and out of the periphery, thus limiting the auto reactive potential of these cell types. This mechanism is believed to be broadly applicable for the treatment of various autoimmune diseases, and has encouraged efforts to identify alternative $S1P_1$ chemotypes.[3]

We recently disclosed the medicinal chemistry effort leading to the discovery of a new series of cyclopenta[b]indoles (*e.g.* **3**, Figure 1) as potent and selective $S1P_1$ agonists.[4] To expand upon this motif, we sought to identify alternatives to the indole-fused cyclopentane ring. We conceived of two generalized structures that are defined by a six atom carbocycle or heterocycle, wherein X = CH_2, O, or NH (**4** and **5**).

Compound **11** was synthesized utilizing a Fischer Indole strategy to prepare the 2,3,4,9-tetrahydro-1H-carbazole ring system (Scheme 1). Intermediate **9** was synthesized in three steps from 4-nitrophenol (**6**) utilizing sodium nitrite and tin chloride to introduce the hydrazine. The Fischer Indole transformation was carried out in acetic acid and was complete within one hour at 70 °C. Saponification of the resultant ester **10** with lithium hydroxide afforded the target molecule **11**.

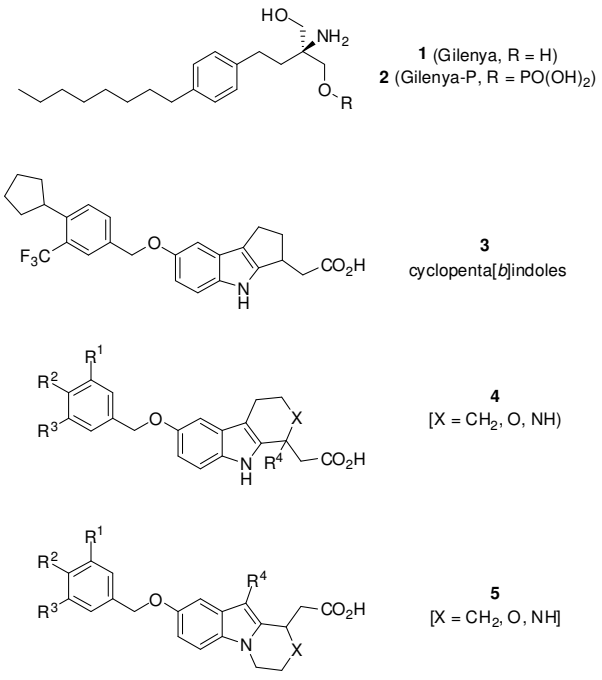
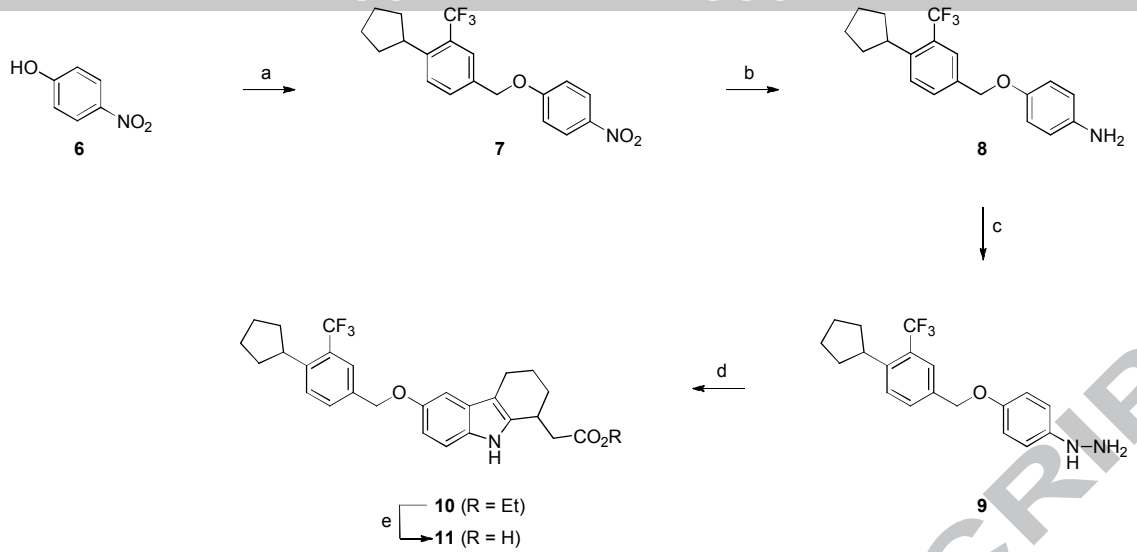
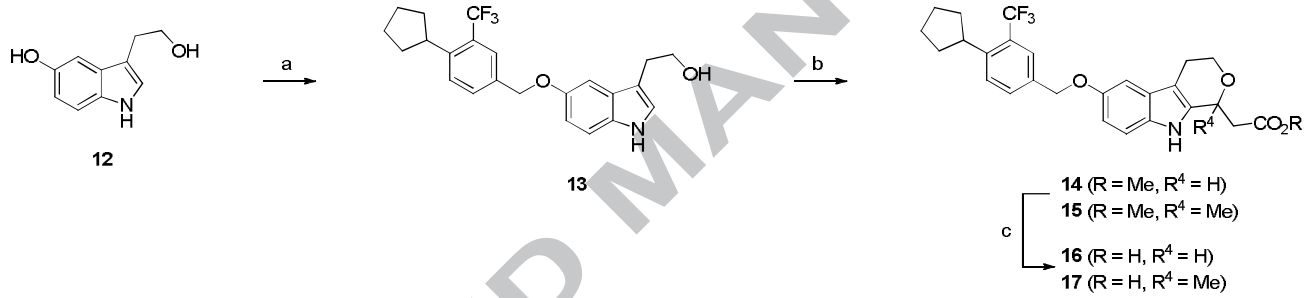


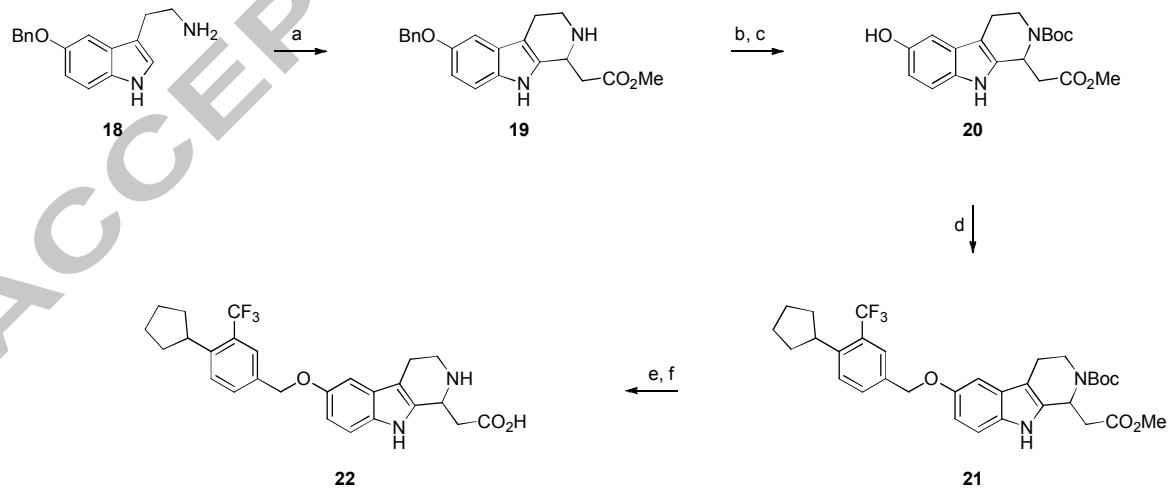
Figure 1. **1** (Gilenya®), **2** (monophosphate), **3** (cyclopenta[b]indoles), **4** and **5**



Scheme 1. (a) 4-(chloromethyl)-1-cyclopentyl-2-(trifluoromethyl)benzene, K_2CO_3, IPA, 80 °C, 72% (b) Zn, 3.0M aqueous NH_4Cl, MeCN, rt, 75% (c) $NaNO_2$, 12M aqueous HCl, $SnCl_2$, water, 0 °C to rt, 95% (d) ethyl 2-(2-oxocyclohexyl)acetate, AcOH, 70 °C, 44% (e) 1.0 M LiOH, dioxane, rt, 66%



Scheme 2. (a) 4-(chloromethyl)-1-cyclopentyl-2-(trifluoromethyl)benzene, Cs_2CO_3, DMF, rt, 94% (b) methyl 3-methoxyacrylate, BF_3 etherate, DCM or methyl 3-oxobutanoate, TsOH, toluene, 80 °C, 56% (c) 1.0 M LiOH, dioxane, rt, 95%



Scheme 3. (a) methyl 3,3-dimethoxypropanoate, TFA, MeCN, H_2O, 80 °C, 79% (b) Pd/C, H_2, MeOH/THF, 95% (c) Boc_2O, THF, rt, 67% (d) 4-(chloromethyl)-1-cyclopentyl-2-(trifluoromethyl)benzene, Cs_2CO_3, DMF, rt (e) 4.0M HCl in dioxane, rt 62% (2 steps) (f) 1.0 M LiOH, dioxane, rt, 74%

Preparation of the 1,3,4,9-tetrahydropyrano[3,4-b]indole analogs (**16**, **17**) was somewhat more difficult depending on the nature of the R^4 substituent (Scheme 2). Both compounds were derived from **13** utilizing either methyl 3-methoxyacrylate or methyl 3-oxobutanoate to introduce the pyran ring. **16** proved the most challenging, with the ring forming step proceeding in very low yield (*i.e.* <5%). Compound **17** on the other hand, was synthesized in moderate yield (*i.e.* 56%) from methyl 3-oxobutanoate.

2,3,4,9-Tetrahydro-1H-pyrido[3,4-b]indole **22** was made by the synthetic route outlined in Scheme 3. The synthesis was relatively facile despite the need for several protecting group manipulations. A Pictet-Spengler reaction was utilized to construct the 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole ring system and proceeded in good yield (79%). Etherification of this product afforded **21** which provided the target piperidinyl carboxylic acid **22** after treatment with acid and base.

Compounds **11**, **16**, **17**, and **22** were all obtained as racemic mixtures, thus requiring separation into their respective enantiomers. This was accomplished chromatographically for **11**, **17**, and **22**, but due to the low yield in preparing **16**, this compound was characterized as the racemic mixture. The stereochemistry of each enantiomer was not determined at this time. Human $S1P_1$ EC_{50} values for these compounds were determined in a homogeneous time resolved fluorescence (HTRF) cyclase assay (Table 1). All of these analogs exhibited good activity against $S1P_1$, with the 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole enantiomers **22a** and **22b** being the most potent in this assay.

In light of the favorable potency of the 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indoles, a brief SAR effort focused on benzyl substitution was performed (Table 2). Various R^1, R^2, and R^3 substituents were explored and were consistent with the SAR observed for the cyclopenta[b]indoles. Larger lipophilic R^2 substituents improved potency, with isobutyl (**32**) and isopropoxy (**33**) affording racemic mixtures of comparable potency to **22a** and **22b**.

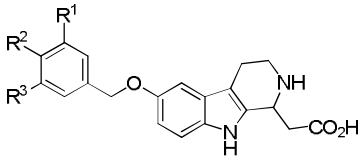
Table 1. Human $S1P_1$ cAMP EC_{50} values

Enantiomer	EC_{50}, nM[a]	E_{max} (%)[b]
11a	2.0	105
11b	1.4	102
rac-**16**	0.41	102
17a	2.2	103
17b	1.2	109
22a	0.03	92
22b	0.06	82

[a] EC_{50} values are then mean of three or more replicates.
[b] % agonism relative to S1P

22a was selected for further profiling and was evaluated against mouse, rat, dog, and monkey $S1P_1$ (Table 3). **22a** was observed to retain good potency, being in the low picomolar range across all preclinical species. Additionally, human $S1P_1$ selectivity was evaluated in the β-arrestin platform, and **22a** was found to possess no agonist activity for $S1P_2$ or $S1P_3$. Discovery of $S1P_3$-sparing agonists have been a major focus in this area due to the association of this receptor with bradycardia,[6] though this effect appears to be species dependent. **22a** is an agonist of $S1P_4$ and $S1P_5$, though it did not produce as large a response as S1P in this assay. $S1P_4$ and $S1P_5$ may play a role in disease amelioration in MS patients,[7,8] but they are not involved in the primary mechanism of lymphocyte trafficking. **22a** was stable in mouse, rat, and human liver microsomes (Table 4), and no significant interaction with any of the major CYP p450 enzymes was observed. Sprague-Dawley rat pharmacokinetics were subsequently assessed and **22a** was observed to have very low oral bioavailability (*i.e.* <2%). This is perhaps due to the zwitterionic properties of this molecule. Intravenous administration revealed a sufficiently long half-life (9.49 h) and

Table 2. Tetrahydro-1H-pyrido[3,4-b]indole Human $S1P_1$ cAMP EC_{50} Values



Compound	R^1	R^2	R^3	EC_{50}, nM[a]	E_{max} (%)[b]
22a	CF_3	cyclopentyl	H	0.03	92
23	CF_3	H	H	170	86
24	CF_3	Cl	H	150	102
25	H	CF_3	H	400	114
26	Cl	OCF_3	H	0.30	110
27	Cl	Cl	H	10,000	-
28	H	H	OCF_3	290	106
29	CN	H	OCF_3	220	97
30	H	SO_2Me	H	10,000	-
31	H	*tert*-Butyl	H	19	96
32	CF_3	isobutyl	H	0.04	78
33	CN	isopropoxy	H	0.04	105

[a] EC_{50} values are the mean of three or more replicates. [b] % agonism relative to S1P (E_{max}).

Table 3. 22a *In vitro* $S1P_1$ Preclinical Species EC_{50} Data and Human $S1P_{1\text{-}5}$ EC_{50} Values

Preclinical Species $S1P_1$ EC_{50} cAMP, nM (E_{max} %)					Human $S1P_{1\text{-}5}$ EC_{50} β-Arrestin, nM (E_{max} %)				
human	mouse	rat	dog	monkey	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
0.03 (92)	0.05 (104)	0.04 (102)	0.04 (83)	0.04 (82)	1.2 (104)	>10,000	>10,000	26 (77)	5.8 (82)

Table 4. 22a Sprague-Dawley Rat Pharmacokinetics and Lymphocyte Lowering (LL)

LM Stability ($t_{1/2}$)[a]	Sprague-Dawley Rat Pharmacokinetics (1.0 mg/kg iv/po)						SD Rat LL IC_{50}[b]
	Cl (L/h/kg)	V_{ss} (L/kg)	C_{max} (μg/mL)	AUC_{last} (hr*μg/mL)[c]	$t_{1/2}$ (h)	%F	
>60 min (m,r,h)	0.643	6.71	1.96	1.32	9.49	<2	13.0 ng/mL

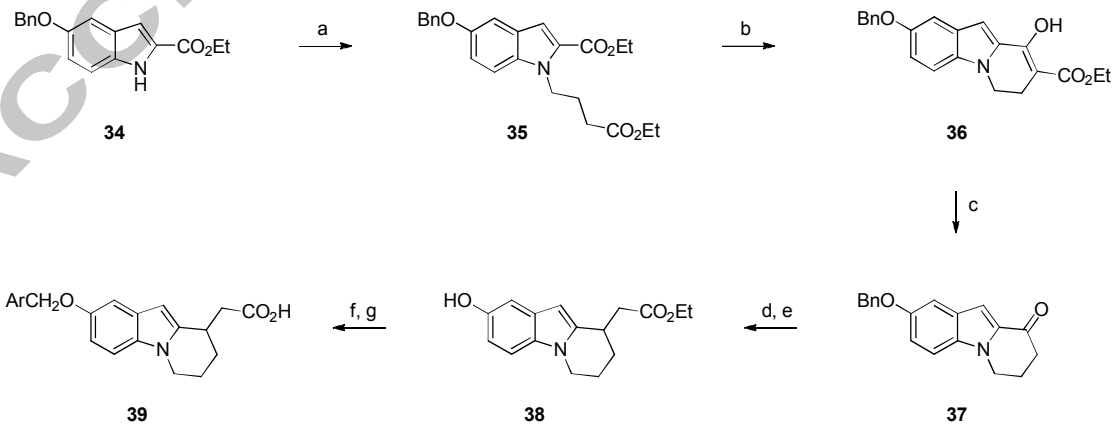
[a.] microsomal stability [b.] plasma lymphocyte lowering (LL) IC_{50} value, compound delivered by intravenous infusion [c.] area under the curve out to the last measurement taken

prompted a lymphocyte lowering pharmacokinetic / pharmacodynamic (PK/PD) analysis. A reduction in circulating lymphocytes (lymphocyte lowering) is the primary mechanism by which $S1P_1$ agonists exert their immunomodulating effect. An indirect PK/PD model was created to estimate the blood concentration of the drug needed to suppress lymphocyte levels by 50% (IC_{50}). **22a** was determined to have an *in vivo* lymphocyte lowering IC_{50} value of 13.0 ng/mL.

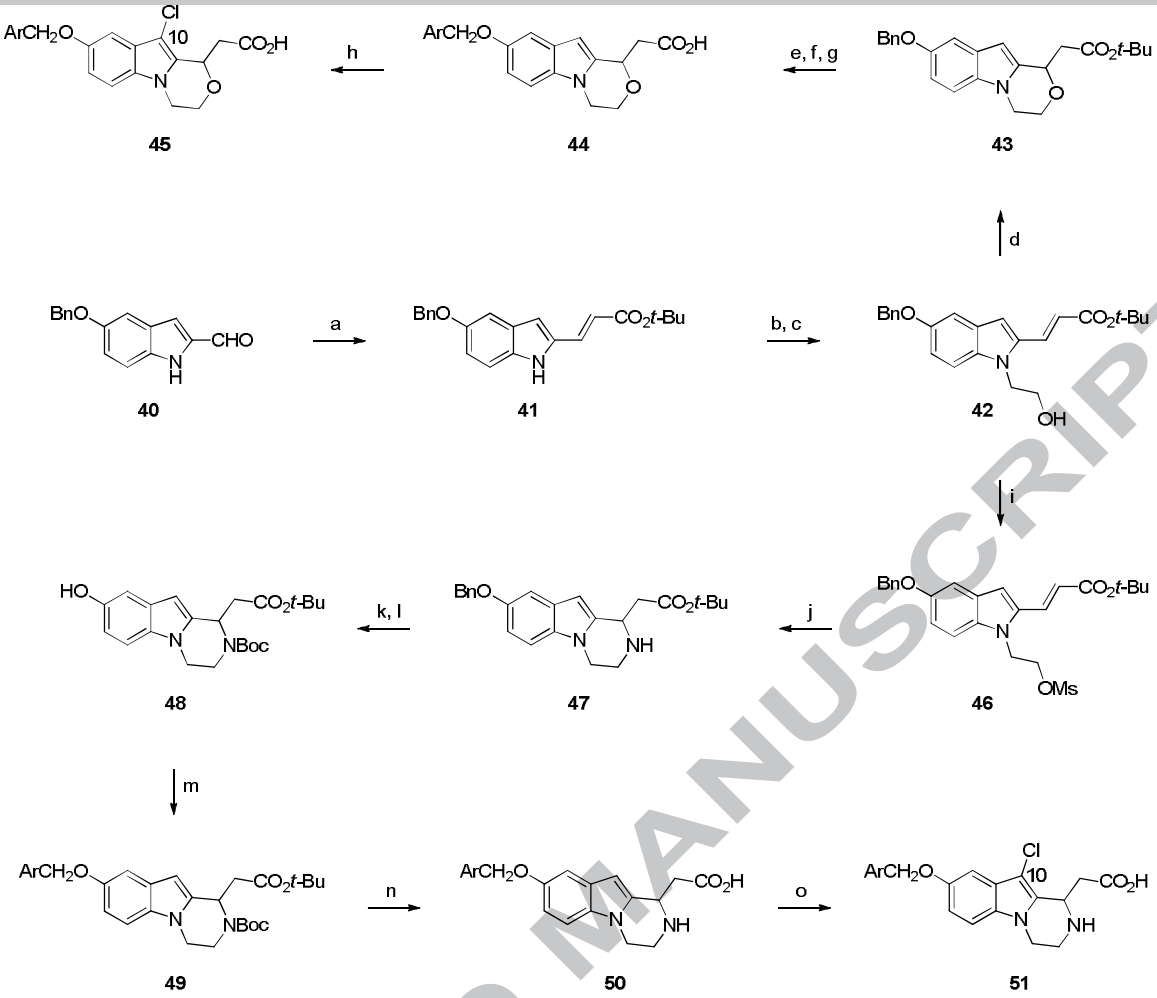
Several tricyclic motifs wherein the indole ring fusion is present on the opposite side were also prepared (e.g. **39**, **45**, **51**) Preparation of indoles **39**, **45** and **51** was carried out utilizing the chemistry shown in Schemes 4 and 5. The carbocyclic indole **39** was synthesized in a similar method employed previously.[5] Alkylation of commercial **34** with ethyl 4-bromobutyrate followed by Dieckmann Condensation afforded **36**. Heating this intermediate in acetic acid and water provided **37** in good yield. Horner-Emmons olefination followed by hydrogenation provided phenol **38**. Etherification and subsequent saponification of the ester under basic conditions afforded the final product **39**. Morpholine **45** and piperizine **51** were constructed from common intermediate **42**, which was synthesized in three steps from commercially available **40.** Intramolecular cyclization to generate the morpholine ring was carried out in moderate yield upon treatment of **42** with CsF in THF. Hydrogenation, alkylation, and deprotection gave the fully elaborated compound **44**. Lastly, chlorine could be optionally introduced in the 10-position by treatment with NCS (*e.g.* **45**).

Piperiazine **47** was prepared from mesylate **46** *via* ammonia promoted intramolecular cyclization. Debenzylation, alkylation, and cleavage of the Boc and *tert*-butyl ester under acidic conditions gave **50**. Chlorination to afford **51** was possible *via* treatment with *N*-chlorosuccinimide. The analogous N-methyl adducts **67** and **68** (Table 5) were prepared in a similar matter utilizing methyl amine instead of ammonia.

The chemistry described in Schemes 4 and 5 was utilized to synthesize a variety of analogs and human $S1P_1$ EC_{50} values were obtained (Table 5). Disubstitution was exclusively examined and larger R^2 groups provided compounds of improved potency. Of the compounds prepared, morpholines **62**-**64** were the most potent in the cyclase assay, and appear to benefit from the chlorine in the 10-position. Racemate **63** was resolved into its respective enantiomers using chiral chromatography, and both enantiomers were very potent in the $S1P_1$ agonist assay (*i.e.* **63a** IC_{50} = 0.23 nM, E_{max} = 93%; and **63b** IC_{50} = 0.01 nM, E_{max} = 98%). **63b** was also evaluated in β-arrestin and had an EC_{50} value of 0.83 nM (E_{max} = 112%) in this platform. Most importantly, this enantiomer was observed to be orally bioavailable in rat when dosed as a suspension in 0.5% methylcellulose in sterile water

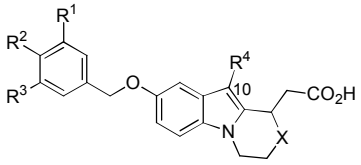


Scheme 4. (a) ethyl 4-bromobutyrate, tetrabutylammonium iodide, NaH, DMF, rt, 97% (b) KOt-Bu, THF, rt, 96% (c) acetic acid / water, 220 °C, 80% (d) ethyl 2-(diethoxyphosphoryl)acetate, NaH, DMF, 65 °C, 45% (e) $Pd(OH)_2/C$, NH_4CO_2H, MeOH, THF, 60 °C, 86% (f) $ArCH_2Cl$, Cs_2CO_3, DMF, 75 °C, 62-84% (g) 1.0 M LiOH, dioxane, rt, 91%



Scheme 5. (a) *tert*-butyl 2-(triphenylphosphoranylidene)acetate, toluene, 70 °C, 63% (b) (2-bromoethoxy)(tert-butyl)dimethylsilane, NaH, DMF, 0-80 °C, 65% (c) TBAF, THF, rt, 93% (d) CsF, THF 100 °C, 33% (e) $Pd(OH)_2/C$, NH_4CO_2H, MeOH, 60 °C, 79% (f) $ArCH_2Cl$, K_2CO_3, DMF, rt to 60 °C, 18-74% (g) TFA, L-cysteine, rt, 13-47% (h) NCS, DCM, 0 °C to rt, 80-100% (i) MsCl, DMAP, DCM, rt, 97% (j) 7.0 M NH_3 in MeOH, dioxane, 100 °C, 89% (k) Boc_2O, THF, rt, 82% (l) $Pd(OH)_2/C$, NH_4CO_2H, MeOH, THF, 60 °C, 94% (m) $ArCH_2Cl$, K_2CO_3, DMF, rt to 60 °C, 66-81% (n) 4N HCl in dioxane, rt, 92% (o) NCS, DCM, 0 °C to rt, 80-100%

Table 5. Human $S1P_1$ cAMP EC_{50} Values



Compound	R^1	R^2	R^3	R^4	X	EC_{50}, nM[a]	E_{max} (%)[b]
52	CF_3	H	CF_3	H	CH_2	22	103
53	CN	H	OCF_3	H	CH_2	25	99
54	CF_3	cyclopentyl	H	H	CH_2	2.3	102
55	CF_3	isopropoxy	H	H	CH_2	3.3	105
56	OEt	OEt	H	H	CH_2	170	105
57	CN	H	OCF_3	H	O	6.8	98
58	CF_3	cyclopentyl	H	H	O	0.14	99
59	CF_3	OCH_2F	H	H	O	11	101
60	CN	isopropoxy	H	H	O	1.7	104
61	Cl	$OCH(CH_2F)_2$	H	H	O	0.48	94
62	CF_3	isopropoxy	H	Cl	O	0.04	94
63	CN	isopropoxy	H	Cl	O	0.04	104
64	CF_3	cyclopentyl	H	Cl	O	0.04	96
65	CF_3	isobutyl	H	H	NH	2.1	101
66	CF_3	isopropoxy	H	H	NH	0.67	99
67	CN	H	OCF_3	Cl	NMe	1.7	101
68	CF_3	cyclopentyl	H	Cl	NMe	0.15	95

[a] EC_{50} values are the mean of three or more replicates. [b] % agonism relative to S1P (E_{max}).

(*%F* = 39). At a 1.0 mg/kg oral dose, this molecule achieved C_{max} and AUC_{last} levels of 0.319 μg/mL and 0.973 hr·μg/mL, respectively. In addition, this molecule has an acceptable half-life ($t_{1/2}$) of 3.4h in rat.

In summary, we have described the synthesis of several new $S1P_1$ agonist series. One of these new $S1P_1$ chemotypes, the 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indoles (*e.g.* **22a**), was found to be highly potent; however, development of this series was limited by poor systemic exposure following oral dosing. Further exploration revealed a morpholinyl chemical series, from which several molecules were identified as low picomolar agonists of $S1P_1$. Moreover, a selected molecule from within this series was shown to have appreciable systemic exposure in rat after oral delivery as a suspension in 0.5% methylcellulose. Further evaluation of this new scaffold is ongoing and results from these studies will be reported in due course.

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