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Bicyclic Ligand-Biased Agonists of $S1P_1$: Exploring Side Chain Modifications to Modulate the PK, PD, and Safety Profiles

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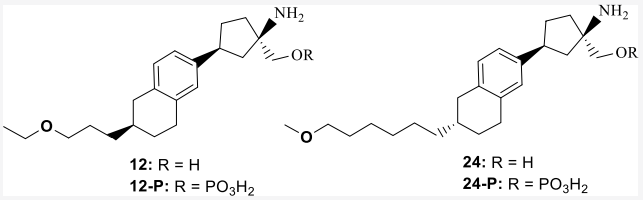
ABSTRACT: Sphingosine-1-phosphate (S1P) binds to a family of sphingosine-1-phosphate G-protein-coupled receptors ($S1P_{1-5}$). The interaction of S1P with these S1P receptors has a fundamental role in many physiological processes in the vascular and immune systems. Agonist-induced functional antagonism of $S1P_1$ has been shown to result in lymphopenia. As a result, agonists of this type hold promise as therapeutics for autoimmune disorders. The previously disclosed differentiated $S1P_1$ modulator BMS-986104 (**1**) exhibited improved preclinical cardiovascular and pulmonary safety profiles as compared to earlier full agonists of $S1P_1$; however, it demonstrated a long pharmacokinetic half-life ($T_{1/2}$ 18 days) in the clinic and limited formation of the desired active phosphate metabolite. Optimization of this series through incorporation of olefins, ethers, thioethers, and glycols into the alkyl side chain afforded an opportunity to reduce the projected human $T_{1/2}$ and improve the formation of the active phosphate metabolite while maintaining efficacy as well as the improved safety profile. These efforts led to the discovery of **12** and **24**, each of which are highly potent, biased agonists of $S1P_1$. These compounds not only exhibited shorter in vivo $T_{1/2}$ in multiple species but are also projected to have significantly shorter $T_{1/2}$ values in humans when compared to our first clinical candidate. In models of arthritis, treatment with **12** and **24** demonstrated robust efficacy.



INTRODUCTION

Sphingosine-1-phosphate (S1P),[1,2] a key regulator of numerous vascular and immune functions, elicits its effects in large part via signaling through five G-protein-coupled receptors ($S1P_{1-5}$).[3] Interaction of S1P with the $S1P_1$ receptor in particular has been shown to regulate trafficking of T and B cells, with agonist-induced functional antagonism of $S1P_1$ blocking lymphocyte egress from the thymus and secondary lymphoid organs.[4−8] Agonist-induced functional antagonism of $S1P_1$ is the consequence of prolonged receptor internalization, followed by degradation rather than recycling back to the surface, resulting in the deletion of the S1P receptor from the cell surface.[6a] Many lymphocyte subsets rely on cell surface $S1P_1$ to detect the gradient of S1P that is maintained from the lymphatic system to the blood in order to migrate into circulation.[6b] In response to this functional antagonism, these cells lose the ability to migrate and are entrapped in the thymus and secondary lymphoid organs resulting in immunosuppresion.[8]

The S1P modulators first gained validation as therapeutics with the approval of fingolimod for the treatment of multiple sclerosis.[9] Fingolimod is a non-selective S1P receptor agonist prodrug which is converted to an active phosphate species via in vivo phosphorylation, akin to the bioconversion of sphingosine to S1P.[10] Action on $S1P_1$ by fingolimod-phosphate has been linked to its beneficial immunomodulatory properties, whereas concurrent agonism of $S1P_3$ was initially associated with several safety issues, such as cardiovascular and pulmonary effects.[11,12] Subsequently, there was a significant amount of research aimed at the identification of $S1P_1$ agonists that were sparing of $S1P_3$ activity.[13] Recently, the $S1P_3$-sparing $S1P_1$ full agonist, siponimod, was approved for the treatment of secondary progressive MS. However, clinical evaluations of this as well as other $S1P_3$-sparing $S1P_1$ full agonists have indicated that agonism of $S1P_1$ contributes to the cardiovascular side effects in humans.[14] Our research efforts shifted to the discovery of third-generation $S1P_1$ receptor modulators that

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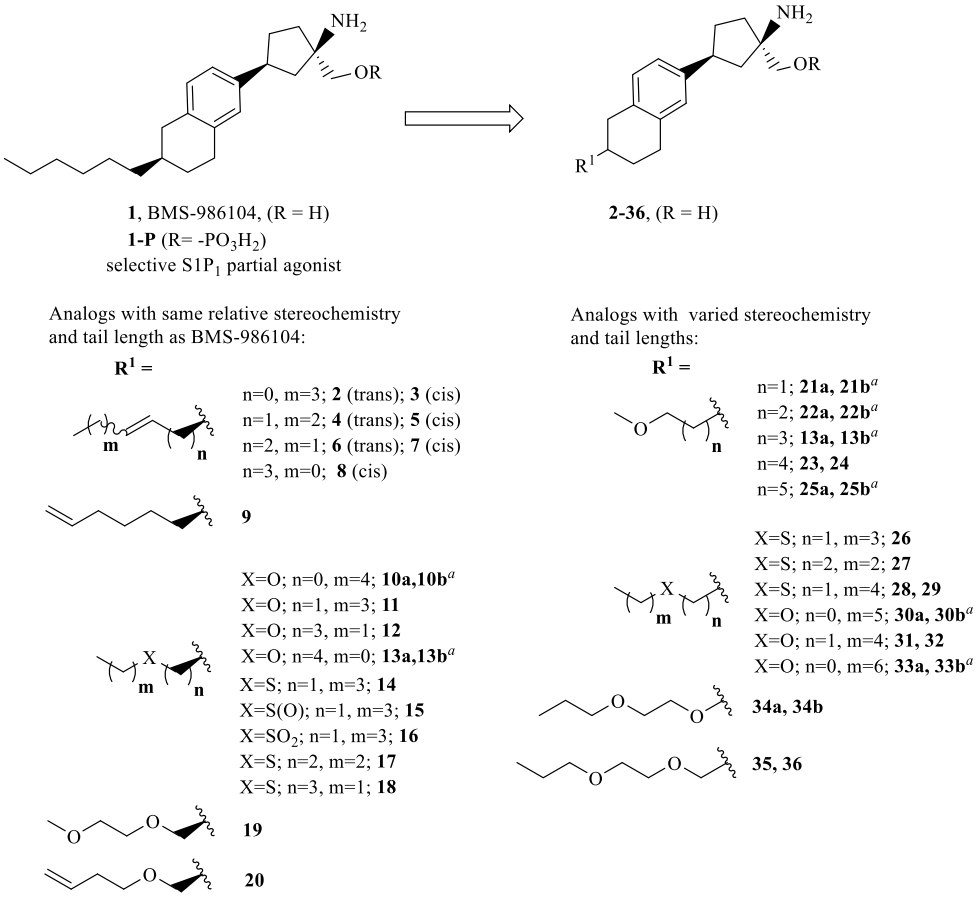

Figure 1. Incorporation of alkene and heteroalkyl side chains. [a] Compounds with an a/b designation were obtained by separation of the diastereoisomers using SFC conditions with a chiral column. The stereochemistry at the side chain connection was not unambiguously determined for these analogues.[18]

demonstrate ligand-biased signaling through $S1P_1$, in addition to maintaining excellent selectivity against $S1P_3$, which was the hallmark of second-generation compounds. As detailed for our earlier compounds,[15a,17] the phosphorylated forms demonstrated comparable potency and efficacy in some GPCR signaling assays, while in others, they showed either reduced Y_{max} values (partial agonism) or reduced potency. This ligand-biased profile is believed to be relevant to the improved profile observed in the pulmonary and cardiovascular safety evaluations. These efforts led to the discovery of the clinical compound BMS-986104 (**1**).[15a] Despite being a partial agonist of $S1P_1$ in several in vitro assays, the bioactive phosphate metabolite of **1** (**1-P**) was shown to fully block S1P-induced T cell migration in vitro and to induce a comparable level of lymphopenia and efficacy as fingolimod in rodent studies. Furthermore, **1** is differentiated from fingolimod in preclinical studies of pulmonary and cardiovascular safety.[15a] However, in clinical studies, the pharmacokinetic half-life of **1** was found to be long (18 days) and the conversion of **1** to **1-P** was lower than expected, leading to a suboptimal level of lymphocyte reduction. Efforts in our labs then shifted to discovering novel compounds with an improved bioconversion to the active phosphate metabolite as well as a reduced human pharmacokinetic $T_{1/2}$. The compounds must also maintain the improved safety profile of **1** and have comparable or better efficacy. Because fingolimod exhibited a superior conversion to the active phosphate metabolite in rat studies when compared to **1** and was subsequently found to also have a higher ratio in humans, the initial triage of compounds involved using a rat blood lymphocyte [blood lymphocyte reduction (BLR)] PK/PD study to determine if our compounds met the desired parameters.[16,17]

As the cyclopentyl amino alcohol has been shown to exhibit the desired $S1P_1$-biased agonist profile,[15,17] this fragment was left unchanged in our new analogues, and herein, we describe modifications of the hexyl side chain of the tetralin moiety (Figure 1). Our initial strategy involved using the same relative stereochemistry and side chain length as **1** and incorporating olefins (**2**–**9**), ethers (**10**–**13**, **20**), thioethers/sulfoxides/sulfones (**14**–**18**), and glycols (**19**) into the side chain in an effort to produce compounds with the desired PK, PD, and safety profiles (see Table 1). We anticipated that adding these potential sites of metabolism in the side chain of our compounds would lead to a reduction of their $T_{1/2}$ values due to a reduction in intrinsic clearance. Next, within the terminal methoxy series, the length of the side chain was modified from the initial hexyl side chain to include side chains with lengths ranging from four to eight atoms (**21**–**25**) and both side chain isomers in this series were studied (see Table 2). Last, a variety of ethers, thioethers, and glycols were prepared with varying lengths and side chain stereochemistry (**26**–**36**).

CHEMISTRY

The synthesis of the alkene compounds **2**–**9** is shown in Scheme 1. Starting from (5*R*,7*S*)-7-((*R*)-6-(hydroxymethyl)-

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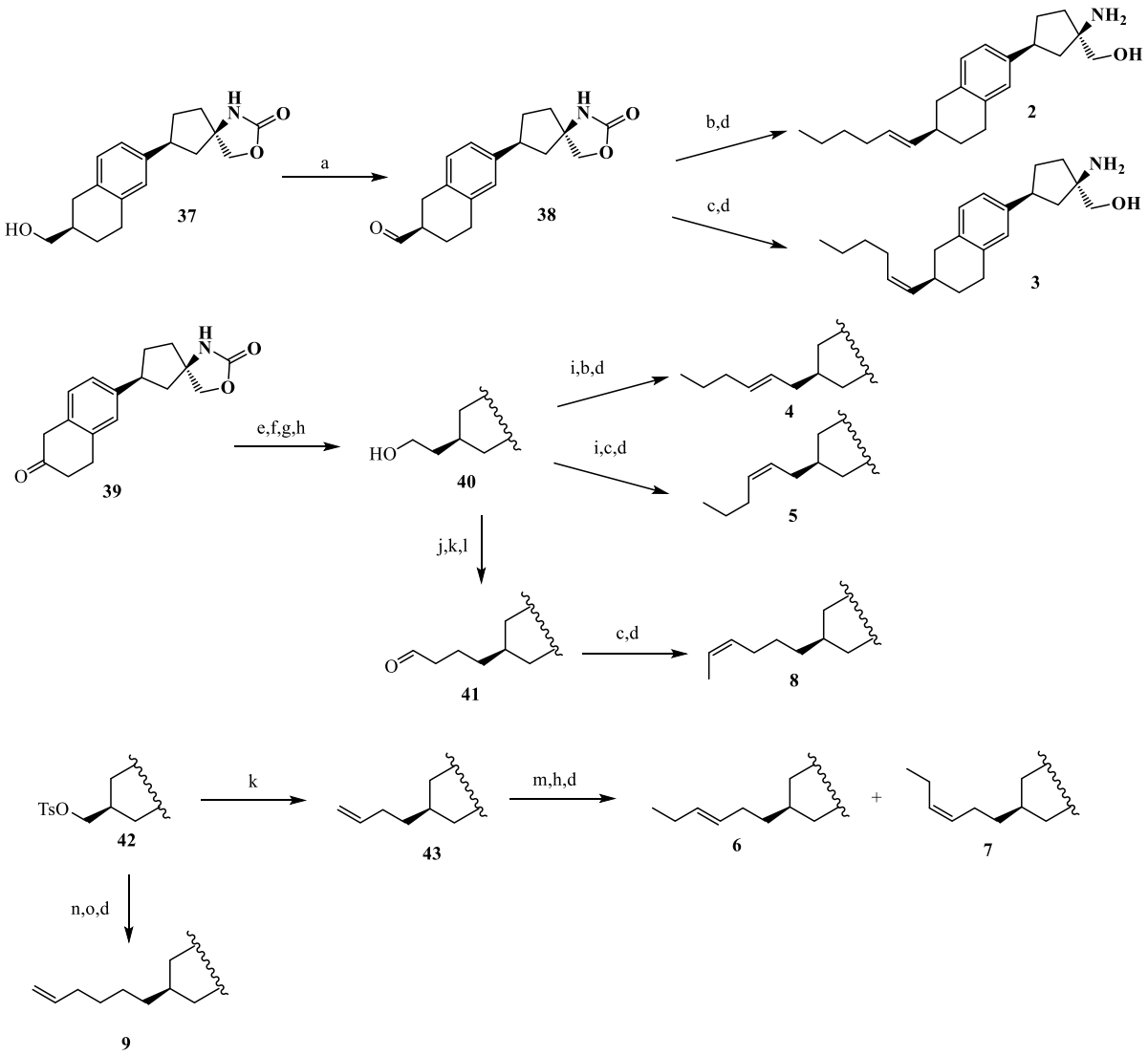
Scheme 1. Synthesis of 6-Alkene-Substituted-((1*R*,3*S*)-1-amino-3-(5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol Compounds[a]



[a]Reagents and conditions: (a) oxalyl chloride, DMSO, TEA, DCM, −78 °C, 84%; (b) Julia−Kocienski olefination: step 1; R−OH, DEAD,1-phenyl-1*H*-tetrazole-5-thiol, Ph_3P, THF, 0 °C, 77%. Step 2; ammonium molybdate tetrahydrate, 30% H_2O_2, 0 °C, 5-(*R*-thio)-1-phenyl-1*H*-tetrazole, EtOH, 0 °C, 95%. Step 3; KHMDS, 5-(R^1-sulfonyl)-1-phenyl-1*H*-tetrazole, THF, 20−30%; (c) Wittig olefination: *R*-(PPh_3)Br, *n*-BuLi, THF, −78 °C, 50−70%; (d) LiOH, water, dioxane or 1 N NaOH, DMSO, MeOH, 100 °C, 50−90%; (e) triethyl phosphonoacetate, THF, 0 °C; (f) $Pd(OH)_2$, MeOH, H_2, 80% (two steps); (g) $LiBH_4$, THF, 80 °C; (h) chiral SFC separation; (i) Dess−Martin periodinane, DCM, 95%; (j) *p*-TsCl, pyridine, 82%; (k) allyl-MgBr, copper(I) bromide, THF, −78 °C, 76−96%; (l) OsO_4, 50% NMO in ^{t}BuOH, THF, $NaIO_4$ in water, 57%; (m) *trans*-3-hexene, second-generation Grubbs catalyst, −78 °C, 74%; (n) LiBr, THF, 50 °C, 100%; (o) pent-4-en-1-yl-MgBr, copper(I) bromide, THF, −78 °C, 77%.

5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]-nonan-2-one (**37**),[17a] a Swern oxidation of the alcohol afforded aldehyde **38**. The hex-1-ene compounds were then synthesized from this aldehyde using two methods. A Julia−Kocienski olefination,[19] followed by base-catalyzed hydrolysis furnished *trans*-alkene isomer **2**, or a Wittig olefination,[20] followed by base-catalyzed hydrolysis provided the *cis*-isomer **3**. The hex-2-ene compounds (**4**, **5**) and the hex-4-ene derivative (**8**) were synthesized starting from tetralone **39**.[17a] The tetralone was reacted with triethyl phosphonoacetate using a Horner−Emmons reaction to yield the homologated ethyl ester, which was then reduced to the alcohol using lithium borohydride. Chiral SFC separation afforded compound **40** after determination of the desired diastereomer.[21] A Dess−Martin oxidation of this alcohol provided the aldehyde, which was then converted to the *trans*- (**4**) and *cis*- (**5**) alkenes using the same methods as described above. Compound **8** was also synthesized from **40** by first converting the alcohol to a tosylate and then reacting this tosylate with allylmagnesium bromide in the presence of copper(I) bromide. The resulting terminal alkene was oxidized using osmium tetroxide to provide aldehyde **41**, which was converted to **8** using the methods described above. To synthesize additional alkene compounds, **37** was first converted to tosylate **42**.[17a] To prepare the two hex-3-ene derivatives, this tosylate was reacted with allylmagnesium bromide, as previously described to form **43**. An olefin metathesis reaction was performed between *trans*-3-hexene and **43** using the second-generation Grubbs catalyst to afford the

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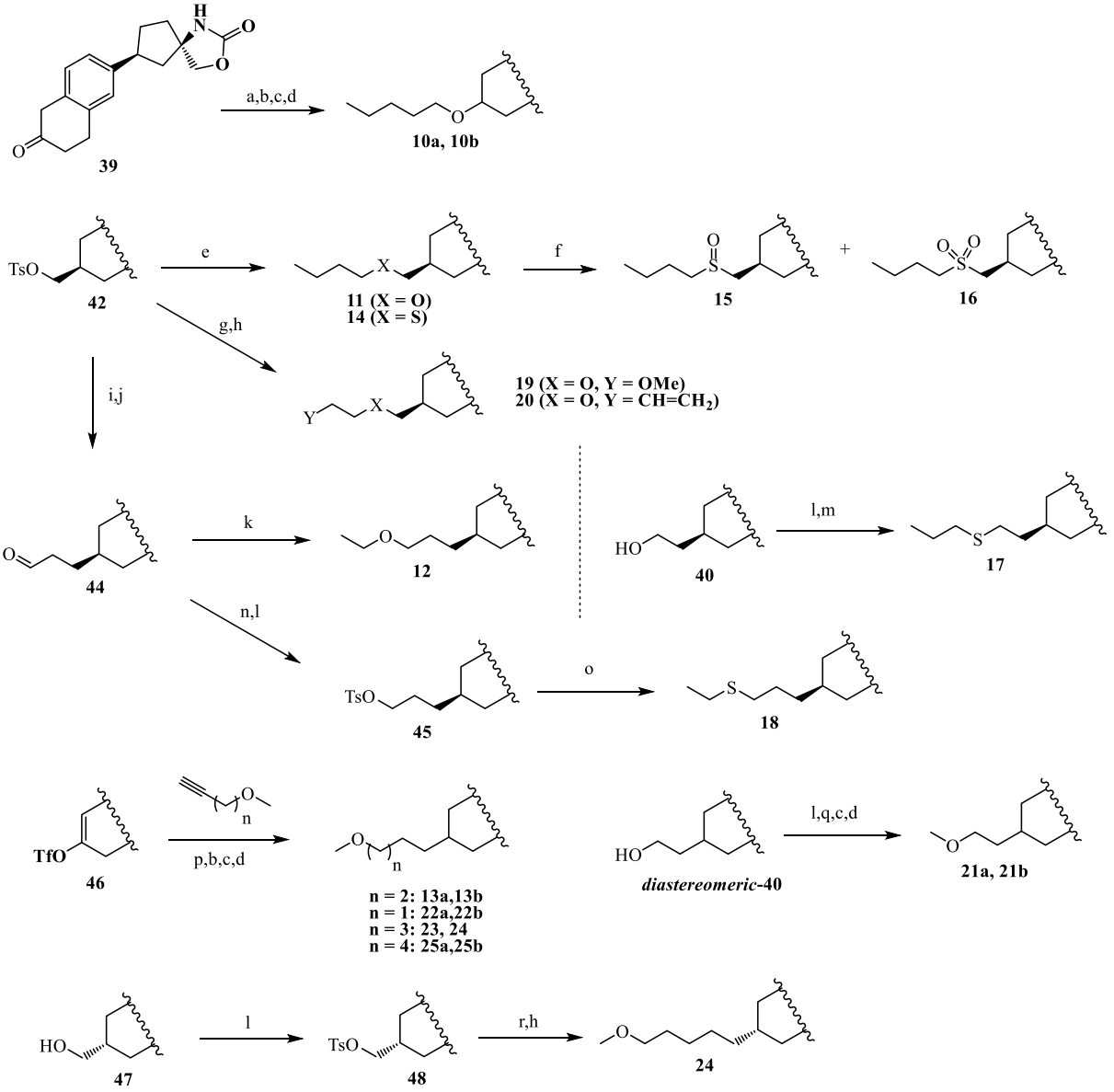
Scheme 2. Synthesis of 6-Ether/Thioether-Substituted-((1*R*,3*S*)-1-amino-3-(5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol Compounds[a]



[a]Reagents and conditions: (a) 1-pentanol, *p*-TsOH, trimethoxymethane, 100 °C, 70%; (b) 10% Pd/C, MeOH, H_2; (c) chiral SFC separation; (d) LiOH, water, dioxane or 1 N NaOH, DMSO, MeOH, 100 °C, 50−72%; (e) *n*-butanol or butyl mercaptan, KO^{t}Bu, THF, 70 °C and then step d, 70−90%; (f) L-10-(−)-camphor sulfonic acid, *m*-CPBA, DCM and then step d, 62−64%; (g) 2-methoxyethanol or but-3-en-1-ol, KO^{t}Bu, DMF/THF, 70 °C and then step d, 70−90%; (h) copper(I) bromide, THF, −78 to 0 °C and then step d, 40−60%; (i) allyl-MgBr, copper(I) bromide-DMS complex, THF, −78 °C, 65%; (j) OsO_4, 50% NMO in ^{t}BuOH, THF, $NaIO_4$ in water, 78%; (k) ethoxytrimethylsilane, triethylsilane, $FeCl_3$, CH_3NO_2, 0 °C, 73% and then step d, 65%; (l) *p*-TsCl, pyridine, 60−82%; (m) propane-1-thiol, 2 N NaOH, dioxane, 90 °C, 98%; (n) $NaBH_4$, EtOH, DCM; (o) ethanethiol, 2 N NaOH, dioxane, 90 °C, 85%; (p) CuI, bis(triphenylphosphine)palladium(II) chloride, TEA, various alkynes, 60 °C, 37−80%; (q) Na°, MeOH, 30%; (r) Mg°, 1,2- dibromoethane, 1-bromo-4-methoxybutane, 60 °C.

cis- and *trans*-hex-3-ene derivatives as a mixture. Chiral SFC separation of these two isomers followed by base-promoted hydrolysis afforded compounds **6** and **7**.[22] Compound **9** was also synthesized from **42** using a copper(I) bromide mediated coupling with pent-4-en-1-ylmagnesium bromide and base hydrolysis.

Scheme 2 shows the methods used to synthesize the various ether, thioether, and glycol compounds (**10**−**25**). Compounds **10a** and **10b** were prepared by heating tetralone **39** with pentanol in the presence of trimethoxymethane and *p*-toluenesulfonic acid. The resulting intermediate was then hydrogenated with Pd/C to afford the penultimate compound as a mixture of diastereomers. Chiral SFC separation of the two diastereomers followed by base-catalyzed hydrolysis afforded compounds **10a** and **10b**. Tosylate **42** was used to prepare several of the ether and thioether analogues. Compounds **11** and **14** were synthesized by heating this tosylate with *n*-butanol or *n*-butyl mercaptan in the presence of base, followed by the addition of water and additional base to hydrolyze the carbamate. Compound **14** was then oxidized using *m*-CPBA to form sulfoxide **15** and sulfone **16**. Compound **17** was synthesized in a manner similar to **11** and **14** starting from

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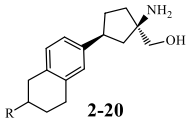
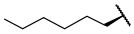
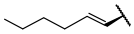
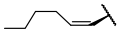
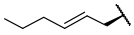
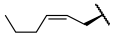
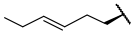
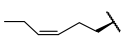
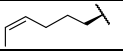
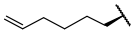
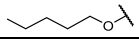
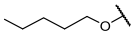
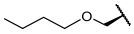
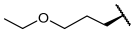
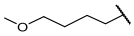
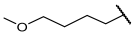
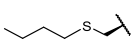
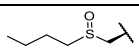
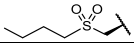
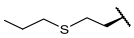
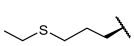
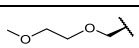
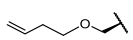
Table 1. PK/PD/Tox Screening of Compounds with the Same Relative Stereochemistry and Side Chain Length.



2-20

Compd	R	4h			24h			Parent *in vivo* $T_{1/2}$ (h) [c]	Phos./parent Ratio (24h)	BAL fold over vehicle (dose, mg/kg)	CM, % change in beat rate (conc, nM) [d]
		Lymph Red.[a] (dose, mg/kg)	Parent [b] (nM)	Phos.[b] (nM)	Lymph Red.[a] (dose, mg/kg)	Parent [b] (nM)	Phos.[b] (nM)				
1		23% (1)	36	11	76%* (1)	51	63	>100	1.2	1.1 (1)	-2±2 (1) -33±4 (10)
2		0% (1)	57	33	72%* (1)	77	200	>75	2.6	nt	nt
3		4% (1)	24	blq	0% (1)	24	44	nd	0.27	nt	nt
4		10% (1)	31	blq	62%* (1)	51	110	62	2.3	nt	-9.6±3.4 (1) -23±1 (10)
5		13% (1)	10	16	71%* (1)	14	120	59	8.6	nt	-11±1.7 (1) -25±3.2 (10)*
6		27% (1)	66	64	74%* (1)	140	810	4	5.8	1.1 (1)	-11±1.2 (1) -33±8.5 (10)
7		31%* (1)	165	44	69%* (1)	180	240	5	1.3	nt	-21±5.2 (1) -38±5.4 (10)
8		0% (0.3)	nt	nt	22% (0.3)	nt	nt	nt	nt	nt	nt
9		9% (1)	30	75	75%* (1)	40	760	47	19	nt	nt
10a		62%* (1)	244	92	78%* (1)	210	180	nt	0.86	0.94 (1)	nt
10b		32%* (1)	215	75	67%* (1)	240	75	nt	0.27	0.94 (1)	-12±5.6 (1) -49±8.5 (10)
11		42%* (1)	66	22	71%* (1)	39	25	nt	0.64	0.95 (1)	nt
12		78%* (2)	172	122	83%* (2)	110	200	26	1.8	1.2 (2)	-14±3.4 (1) -29±0.6 (10)
13a		78%* (2)	300	196	87%* (2)	110	150	nt	1.4	1.7 (10)	nt
13b		65%* (2)	271	78	78%* (2)	78	60	16	0.77	2.1 (10)	-15±7.3 (1) -29±7.5 (10)
14		6% (1)	blq	blq	63%* (1)	73	26	27	0.36	1.1 (1)	-28±7.1 (1) -46±8.3 (10)
15		0% (5)	109	11	75%* (5)	10	23	nt	2.3	1.5 (5)	nt
16		13% (5)	44	blq	16% (5)	blq	blq	nt	nt	0.8 (5)	nt
17		63%* (2)	193	17	73%* (2)	72	12	nt	0.17	1.1 (2)	-43±1.1 (1)* -42±12.3 (10)*
18		65%* (2)	190	nt	74%* (2)	42	nt	14	nt	nt	-42±3.8 (1) -53±9 (10)
19		16% (1)	85	15	13% (1)	blq	blq	nt	nt	1.3 (1)	nt
20		55%* (2)	170	41	74%* (2)	48	43	11	0.9	nt	nt

[a]Compounds tested in Lewis rats (n = 2–4), *p < 0.05 versus vehicle ANOVA w/Dunnett's test. [b]Blood concentration at the time indicated. [c]The in vivo $T_{1/2}$ is estimated from PK profiles from oral dosing observed at four time points: 4, 24, 48, and 72 h (see Table S3). [d]For the cardiomyocyte evaluations, the phosphate of each compound was prepared and assayed. Beat rate reductions are an average of three or more replicates at each concentration unless otherwise noted; *n = 2. Lymph Red. = lymphocyte reduction; Phos. = phosphate; BAL = bronchoalveolar lavage, CM = cardiomyocyte; conc = concentration; blq = below limit of quantitation; nt = not tested; and nd = not able to determine.

alcohol **40**. To move the ether and thioether to the 4-position of the alkyl side chain, **42** was converted to **44** by first reacting with allylmagnesium bromide and copper(I) bromide, followed by an oxidation of the resulting terminal alkene to afford the aldehyde. A reductive etherification of **44** was then employed using ethoxytrimethylsilane in the presence of triethylsilane and iron chloride to form **12** after hydrolysis. The thioether (**18**) was synthesized by converting **44** to tosylate **45** and then

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reacting with ethanethiol. Finally, glycol **19** and ether **20** were prepared from tosylate **42** by heating this tosylate with 2-methoxyethanol or but-3-en-1-ol in the presence of base, followed by a hydrolysis. The initial syntheses of the terminal methoxy analogues involved their preparation as a mixture of diastereomers, which were then separated via chiral HPLC. To synthesize most of the terminal methoxy compounds, a Sonogashira coupling between **46**[17a] and a variety of terminal alkynes was utilized. The resulting coupled products were then hydrogenated, and the diastereomers were separated using chiral SFC conditions. Subsequent hydrolysis afforded **13a**, **13b**, **22a**, **22b**, **23**, **24**, **25a**, and **25b**. Compound **24** was subsequently resynthesized using a chiral intermediate, **47**.[17a] Compound **47** was converted to tosylate **48**, which was then reacted with 4-methoxybutylmagnesium bromide in the presence of copper(I) bromide. A base hydrolysis of the resulting compound afforded **24** as a homochiral product. The final set of terminal methoxy derivatives (**21a** and **21b**) were synthesized by converting **diastereomeric-40** to the corresponding tosylate and reacting this material with sodium methoxide. The coupled product was then separated using chiral SFC conditions and each diastereomer was hydrolyzed to afford **21a** and **21b**. The compounds in Table 3 (**26**−**36**) were prepared using the various methods described above (see Scheme S1). All phosphate metabolites were prepared by reacting the final compounds with pyrophosphoryl chloride and then quenching with water.

RESULTS AND DISCUSSION

Because our compounds require a bioconversion to form the active phosphate metabolites, new analogues were initially assessed directly in vivo using a pharmacodynamic/pharmacokinetic/tox screening approach. A rat BLR PD/PK model determined whether the modifications to the side chain resulted in an acceptable degree of lymphocyte reduction. Based on the lymphocyte reduction of **1** at various doses (see Table S1), we considered compounds tested at 1 mg/kg or greater with at least 70% reduction 24 h post-dose and compounds tested at 0.3 mg/kg or 0.5 mg/kg with at least 45 or 60% reduction 24 h post-dose, respectively, to be acceptable. This model also provided the level of phosphate metabolite formation and an estimate of the pharmacokinetic $T_{1/2}$ from oral dosing. Compound **1** exhibited a pharmacokinetic $T_{1/2}$ over 100 h in rat and was subsequently found to have a long $T_{1/2}$ in a human clinical trial; therefore, we looked for compounds with an estimated $T_{1/2}$ of 30 h or less in our study. In addition to the long $T_{1/2}$, **1** was also found to have poorer than expected phosphate metabolite formation in humans, so we sought compounds with a phosphate-to-parent ratio in the rat PK/PD study based on the 24 h concentration levels better than that of **1** (ratio of 1.2). Maximizing the phosphate-to-parent ratio in rodents was hoped to lead to an increase in the phosphate metabolite formation in humans, which would drive the desired pharmacology while minimizing exposure to the parent and any off-target effects that it might carry. To assess the potential for pulmonary toxicity, the level of protein in bronchoalveolar lavage (BAL) fluid, a marker for vascular leakage and pulmonary edema,[23,24] was measured at the conclusion of the study. Compound **1** exhibited very little BAL protein elevation and we strove to identify compounds that gave similar results. In order to reflect the intrinsic activity of the compounds, the correlation to BAL effects was made based on the apparent in vivo PD ED_{50}. To assess the potential for heart rate effects in the clinic, the phosphate metabolites of our compounds were prepared and evaluated in cultured cardiomyocytes derived from human-inducible pluripotent stem cells. The concentration-dependent responses of these cells to cardioactive drugs are consistent with observed clinical effects.[25] Exposure of these cardiomyocytes to $S1P_1$ agonists has been shown to reduce the rate of beating regardless of their activity on $S1P_3$. Compounds with a biased agonist profile demonstrated a more limited reduction in this cardiomyocyte assay.[15a,b] Analogues with a beat rate change in the cardiomyocyte assay of less than 15% at 1 nM and less than 30% at 10 nM were deemed to be progressible. Many of the compounds tested in the cardiomyocyte assay were also screened in a functional $S1P_1$ GTPγS assay, and, as is shown in Table S2, increased activity on $S1P_1$ is not by itself sufficient to elicit an increase in the cardiomyocyte response.

Table 1 shows the results for compounds which have the same side chain length and relative stereochemistry as **1** since previously published work indicated this to be the optimal stereoisomer based on in vitro evaluation of phosphorylation potential, followed by an in vivo PK/PD/Tox screening.[15a] In our effort to shorten the $T_{1/2}$ and maintain the efficacy and safety profile of **1**, a series of compounds were prepared in which the hexyl side chain was replaced with a variety of hexene derivatives (**1**−**9**). With the exception of **3** and **8**, the ability of the compounds to reduce circulating lymphocytes was similar to **1**. In general, the change to the various hexene side chains did result in compounds with shorter estimated $T_{1/2}$ values. Compounds **3**, **6**, and **7** were significantly shorter with $T_{1/2}$ values of 4 to 5 h. Many of these compounds also provided significant improvement in their ability to form the active phosphate metabolite with the most notable being **5**, **6**, and **9**, which had phosphate-to-parent ratios of 8.6, 5.8, and 19, respectively. Compound **6** was the only compound that met the criteria for lymphocyte reduction, estimated $T_{1/2}$, and phosphate metabolite formation, so the level of BAL protein elevation was measured, and it was found to be negligible, similar to what had been found with **1**. Of the compounds from this series tested in the cardiomyocyte assay, **4** and **5** were the only ones that had a beat rate reduction we deemed progressible. Compound **6** did meet the criteria at the lower concentration; however, it was slightly above the 30% rate reduction at the higher concentration, again similar to **1**. None of the hexene derivatives met all the specified criteria.

An ether linkage was incorporated along the hexyl side chain to afford various derivatives (**10**−**13**). The pentoxy compounds (**10a** and **10b**) which have the ether linkage at the position adjacent to the tetralin moiety were prepared, and while both diastereomers were isolated, the stereochemistry of the side chain was not determined.[18] Both compounds were effective at reducing lymphocyte count at 24 h with 78 and 67% reductions, respectively; however, the phosphate-to-parent ratios were poor for each. While neither isomer increased levels of BAL protein in vivo, the one isomer tested (**10b**) performed poorly in the cardiomyocyte assay at the higher concentration. The butoxymethyl compound **11** was similar to the pentoxy derivatives in that it had desirable results for lymphocyte reduction and BAL protein elevation but also exhibited a low phosphate-to-parent ratio. Next, the ethoxypropyl analogue **12** was analyzed. This compound met the specified criteria for all the assays with an 83% reduction of lymphocytes at 24 h, an estimated $T_{1/2}$ of 26 h, a phosphate-to-parent ratio of 1.8, little to no BAL protein elevation, and an

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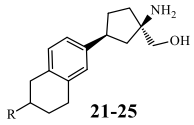
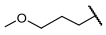
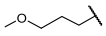
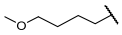
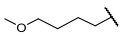
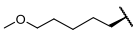
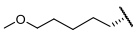
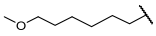
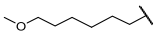
Table 2. PK/PD/Tox Screening of Terminal Methoxy Series



21-25

Compd	R	4h Lymph Red.[a]	4h Parent[b] (nM)	4h Phos.[b] (nM)	24h Lymph Red.[a]	24h Parent[b] (nM)	24h Phos.[b] (nM)	Parent *in vivo* $T_{1/2}$ (h)[c]	Phos./parent Ratio (24h)	BAL fold over vehicle, (dose, mg/kg)	CM, % change in beat rate (conc, nM)[d]
21a		0	nt	nt	12%	nt	nt	nt	nt	nt	nt
21b		0	nt	nt	2%	nt	nt	nt	nt	nt	nt
22a		76%*	141	173	74%*	33	92	13	2.8	3 (15)	nt
22b		62%*	215	70	66%*	86	74	14	0.9	1.6 (2)	-20±2.4 (1) -25±4.3 (10)*
13a		78%*	300	196	87%*	110	150	11	1.4	1.7 (10)	nt
13b		65%*	271	78	78%*	78	60	16	0.77	2.1 (10)	-15±7.3 (1) -29±7.5 (10)*
23		78%*	129	221	81%*	51	420	15	8.2	1.9 (2)	-20±3.9 (1) -40±5.3 (10)*
24		67%*	188	173	82%*	98	250	18	2.6	1.5 (2)	-7±2.8 (1)* -23±0.0 (10)*
25a		70%*	265	234	84%*	69	360	31	3.9	1.7 (2)	-16±2.5 (1) -32±5.5 (10)
25b		45%*	320	41	79%*	295	170	26	2.5	1.2 (2)	-25±6.8 (1)* -32±1 (10)*

[a]Compounds tested in Lewis rats (n = 2–4) at 2 mg/kg, *p < 0.05 versus vehicle ANOVA w/Dunnett's test. [b]Blood concentration at the time indicated. [c]The in vivo $T_{1/2}$ is estimated from oral dosing over 72 h (see Table S3). [d]For the cardiomyocyte evaluations, the phosphate of each compound was prepared and assayed. Beat rate reductions are an average of three or more replicates at each concentration unless otherwise noted; *n = 2. Lymph Red. = lymphocyte reduction; Phos. = phosphate; BAL = bronchoalveolar lavage, CM = cardiomyocyte; conc = concentration; blq = below limit of quantitation; and nt = not tested.

acceptable beat rate change in the cardiomyocyte assay (−14 ± 3.4% at 1 nM and −29 ± 0.6% at 10 nM). Last, in the ether series, the two methoxybutyl analogues (**13a** and **13b**) were assayed. These two diastereomers performed quite well in the BLR assay exhibiting 87 and 78% reduction in the lymphocyte count at 24 h. Compound **13b** also had a desirable $T_{1/2}$ of 16 h and performed well in the cardiomyocyte assay; however, it had a low parent-to-phosphate ratio and a marginal result in BAL protein elevation assay, with a 2-fold elevation of protein relative to vehicle control.

Compounds **14**, **17**, and **18** represent analogues in which a sulfur is incorporated into the hexyl side chain instead of the oxygen linkage. In general, these compounds performed rather poorly in the cardiomyocyte assay and had low phosphate-to-parent ratios. For the butylthiomethyl derivative **14**, the corresponding sulfoxide **15** and sulfone **16** were also prepared. These two compounds had very poor lymphocyte reduction at the 4 h time point and **16** was also poor at the 24 h time point. While the sulfoxide compound (**15**) exhibited an improved phosphate-to-parent ratio (2.3) and modest BAL protein elevation (1.5 fold over vehicle), the compound was not studied further due to the low 24 h phosphate metabolite concentrations at a dose of 5 mg/kg. The glycol variant, **19**, was found to have poor lymphocyte reduction and was not tested in the BAL protein or cardiomyocyte assays. Finally, the compound with an ether linkage combined with a terminal alkene was studied (**20**), and while the compound had acceptable 24 h lymphocyte reduction, the phosphate-to-parent ratio was low and no further studies were warranted.

The compounds in Table 2 represent analogues in the terminal methoxy series in which the side chain length was varied from four to eight atoms. Because the side chain was modified in both composition and length, each stereoisomer at the side chain connection for this series was studied.[18] The methoxyethyl compounds (**21a** and **21b**) represent the shortest chain length investigated, and these compounds showed little to no lymphocyte reduction at either time point. The compounds with one additional atom in the side chain (**22a** and **22b**), however, displayed fairly robust lymphocyte reduction at both the 4 and 24 h time points. Additionally, both compounds had desirable $T_{1/2}$ values (13–14 h). The phosphate-to-parent ratio was significantly better for **22a** in comparison to **22b**. The phosphate metabolite of **22b** was also tested in the cardiomyocyte assay and showed favorable percent beat rate change at the higher concentration (−25 ± 4.3%) but higher than desired at the lower concentration (−20 ± 2.4%). The compounds with a side chain length of six atoms, **13a** and **13b**, were included in this table for comparison purposes as they were discussed in the previous section. The next set of compounds studied had a side chain length of seven atoms (**23** and **24**). Both diastereomers again displayed robust lymphocyte reductions and short $T_{1/2}$ values in comparison to **1**. Furthermore, these compounds both had good to excellent phosphate metabolite formation

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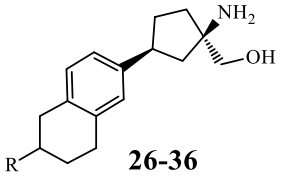
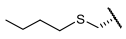
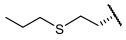
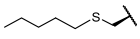
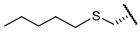
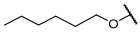
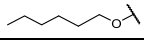
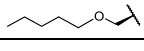
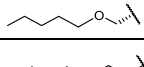
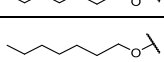
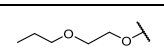
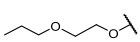
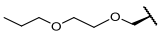
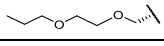
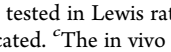
Table 3. PK/PD/Tox Screening of Miscellaneous Compounds



26-36

Compd	R	4h			24h			Parent *in vivo* $T_{1/2}$ (h)[c]	Phos./parent Ratio (24h)	BAL fold over vehicle, (dose, mg/kg)	CM, % change in beat rate (conc, nM)[d]
		Lymph Red.[a] (dose, mg/kg)	Parent[b] (nM)	Phos.[b] (nM)	Lymph Red.[a] (dose, mg/kg)	Parent[b] (nM)	Phos.[b] (nM)				
26		20% (2)	138	blq	44%* (2)	110	25	nt	0.23	1.1 (2)	-14±0.01 (1)* -25±2 (10)*
27		0% (0.5)	43	blq	60%* (0.5)	20	blq	nt	nt	nt	-26±6.3 (1) -61±4.3 (10)
28		0% (0.5)	blq	blq	63%* (0.5)	<31	33	nt	nt	nt	nt
29		0% (0.5)	blq	blq	56%* (0.5)	blq	blq	nt	nt	nt	-29±0.01 (1)* -41±1.7 (10)*
30a		39%* (1)	53	blq	82%* (1)	28	23	nt	0.82	1.1 (1)	-39±5.1 (1)* -40±6.5 (10)
30b		20% (1)	362	20	82%* (1)	417	77	nt	0.18	1.4 (1)	nt
31		27% (1)	27	blq	80%* (1)	28	23	nt	0.5	2 (1)	nt
32		7% (1)	12	blq	32% (1)	18	blq	nt	nt	1.7 (1)	nt
33a		13% (1)	32	blq	80%* (1)	54	84	29	1.6	0.85 (1)	nt
33b		14% (1)	40	blq	80%* (1)	25	42	nt	1.7	1 (1)	nt
34a		72%* (1)	46	64	70%* (1)	blq	36	nt	4.5	1.3 (1)	nt
34b		0% (2)	100	61	40%* (2)	blq	19	nt	2.4	nt	nt
35		72%*(5)	360	240	70%* (5)	28	74	nt	2.6	1.4 (5)	nt
36		67%* (1)	78	33	20% (1)	blq	blq	nt	nt	2.4 (1)	nt

[a]Compounds tested in Lewis rats (n = 2–4) at 0.5, 1, 2, or 5 mg/kg, *p < 0.05 versus vehicle ANOVA w/Dunnett's test. [b]Blood concentration at the time indicated. [c]The in vivo $T_{1/2}$ is estimated from oral dosing over 72 h (see Table S3). [d]For the cardiomyocyte evaluations, the phosphate of each compound was prepared and assayed. Beat rate reductions are an average of three or more replicates at each concentration unless otherwise noted; *n = 2. Lymph Red. = lymphocyte reduction; Phos. = phosphate; BAL = bronchoalveolar lavage, CM = cardiomyocyte; conc = concentration; and nt = not tested.

(ratios of 8.2 and 2.6, respectively), showing a significant improvement over **1** (ratio of 1.2). At a dose of 2 mg/kg, the amount of BAL protein elevation was low to moderate. In the cardiomyocyte assay, the phosphate metabolite of **24** exhibited a favorable result at both concentrations tested (−7 ± 2.8% at 1 nM and −23 ± 0.01% at 10 nM), while **23** performed rather poorly at both concentrations (−20 ± 3.9% at 1 nM and −40 ± 5.3% at 10 nM). Last, the compounds with eight atoms in the side chain were studied (**25a** and **25b**). These compounds both performed well in the lymphocyte assay at 24 h, had good phosphate-to-parent ratios, and exhibited low BAL protein elevation; however, the $T_{1/2}$ values were slightly longer than the other compounds in this series (26–31 h), and the cardiomyocyte results did not meet the specified criteria for either compound. From this series, **24** had the best overall profile. While **23**, the diastereomer with the same relative stereochemistry as **1**, had a superior phosphate-to-parent ratio, **24** still exhibited a 2-fold improvement relative to **1** in this aspect. Additionally, **24** demonstrated more favorable results versus **23** in the pulmonary toxicity assay (1.5- vs 1.9-fold BAL protein increase) as well as in the cardiovascular safety assay (−7 ± 2.8% at 1 nM and −23 ± 0.01% at 10 nM vs −20 ± 3.9% at 1 nM and −40 ± 5.3% at 10 nM).

Because of the differing efficacy and toxicity profiles observed in the diastereomers of the terminal methoxy series, where both the stereochemistry and chain length were modified, the diastereomer of some compounds from Table 1 as well as other sets of diastereomers with longer side chain length were studied (Table 3). The first four compounds are thioether analogues. Compounds **26** and **27** represent the diastereomers of **14** and **17**, respectively. For **26**, much like the difference in the two diastereomers **23** and **24** from the terminal methoxy series, the cardiomyocyte results were significantly improved versus **14** (−14 ± 0.01% at 1 nM and −25 ± 1% at 10 nM vs −28 ± 7.1 at 1 nM and −46 ± 8.3 at 10 nM); however, **26** had modest 24 h lymphocyte reduction

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Table 4. In Vitro Pharmacology[a]

assay	FTY720-P	1-P	12-P	24-P
hS1P$_1$ binding (IC_{50}, nM), Y_{max}	0.008 ± 0.0014 101%	0.010 ± 0.004 100%	0.04 ± 0.017 98%	0.016 ± 0.004 98%
hS1P$_1$ GTPγS (EC_{50}, nM), Y_{max}	0.70 ± 0.28 96%	0.90 ± 0.36 81%	2.1 ± 0.53 83%	2.1 ± 0.92 77%
hS1P$_1$ internalization (EC_{50}, nM), Y_{max}	0.070 ± 0.02 102%	0.11 ± 0.02 68%	0.4[c] 53%	4.9[c] 77%
hS1P$_1$ ERK-P (EC_{50}, nM), Y_{max}	0.02 ± 0.006 100%	8.2 ± 3.6 101%	5.2 ± 2.4 99%	2.7 ± 3.8 100%
hS1P$_3$ GTPγS(EC_{50}, nM)	3.6 ± 2.8	>1000	>1000	>1000
hS1P$_4$ GTPγS (EC_{50}, nM)	1.6 ± 0.43	10.5[d]	10 ± 8.8	5.6 ± 2.9
hS1P$_5$ GTPγS (EC_{50}, nM)	0.67 ± 0.24	10.7[d]	4.9 ± 2.6	3.6 ± 4.7
human lymphocyte chemotaxis (IC_{50}, nM)	1.2[d]	2.5[d]	4.1[d]	0.7[d]

[a] $n \geq 3$ unless otherwise noted. [b] Antagonist mode. [c] $n = 1$. [d] $n = 2$; hS1P = human sphingosine 1-phosphate; Y_{max} = Normalized maximal response.

Table 5. Pharmacokinetic Parameters for Compounds 12 and 24

		12			
species	dose (mg/kg)[a]	IV CL (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	F % (PO)
rat	IV:1 PO:1	6.0 ± 0.3	12 ± 1.1	29 ± 5.5	45%
cynomolgus monkey	IV:1 PO:1	7.4 ± 0.7	8.4 ± 0.5	21 ± 1.9	76%
projected human		2.1	10	79	
		24			
species[a]	dose (mg/kg)[b]	IV CL (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	F % (PO)
rat	IV:2	10 ± 2.7	13 ± 3.8	19 ± 0.9	
cynomolgus monkey	IV:1 PO:1	9.7 ± 3.8	9.9 ± 3.1	17 ± 0.7	69%
dog	PO:1			81 ± 13	
projected human				<30	

[a] $n = 3$ on males; Rat: Sprague-Dawley unless otherwise noted. [b] 18.4% hydroxypropyl-β-cyclodextrin in 13.8 mM citric acid as vehicle. F = bioavailability, Cl = clearance, and V_{ss} = volume of distribution at steady state.

and rather poor phosphate metabolite formation, similar to **14**. This improvement in the cardiomyocyte assay was not observed for **27** as it had equally poor results as **17**. The last two compounds in the thioether series have a chain length of seven atoms (**28** and **29**). The 24 h lymphocyte reductions for these compounds were 63 and 56%, respectively, despite both compounds having rather low phosphate metabolite concentrations. Of this pair, only compound **29** was tested in the cardiomyocyte assay and it performed poorly (−29 ± 0.01 at 1 nM and −41 ± 1.7 at 10 nM).

The next set of compounds **30**−**36** are ether analogues in which the ether position and chain length have been varied. The first four compounds in this series have seven atoms in the side chain and the ether in the first or second position relative to the tetralin core. With the exception of **32**, these compounds showed good lymphocyte reductions, but relatively poor formation of the phosphate metabolite (phosphate/parent <1.0). Additionally, the one compound of this group that was assayed for cardiovascular safety (**30a**) did not meet the desired criteria. The last two compounds in this mono-ether series possess side chains of eight atoms (**33a** and **33b**). These diastereomers both showed impressive 24 h lymphocyte reduction (both 80%) and reasonable phosphate-to-parent ratios (1.6 and 1.7); furthermore, each compound elicited little to no BAL protein elevation. The estimated $T_{1/2}$ for **33a** (29 h) was longer than desired. The final four compounds in this table are sets of glycol side chains containing seven or eight atoms. For these compounds, only one of the diastereomers from each set had reasonable 24 h lymphocyte reduction (**34a** and **35**; both 70%). These compounds also showed significant phosphate metabolite formation and modest BAL protein elevation; however, neither progressed to cardiovascular safety evaluation or estimated $T_{1/2}$ determination.

Based on the assessments for reduction of circulating lymphocytes, projected in vivo $T_{1/2}$, potential for phosphorylation, as well as pulmonary and cardiovascular safety, **12** and **24** were chosen for further evaluation. Previous work showed our third-generation partial S1P$_1$ agonists to have a pulmonary and cardiovascular safety profile superior to that of fingolimod.[15a,b,17] These clinical compounds were found to be partial agonists in two of the in vitro assays. Thus, the phosphate metabolites of compounds **12** (**12-P**) and **24** (**24-P**) were characterized in these in vitro assays and compared to the phosphate metabolite of our first clinical candidate (**1-P**) to ascertain if they had a similar partial agonist in vitro profile (Table 4). The compounds all have equivalent potency in the S1P$_1$ binding assay; however, as we found with **1-P**, **12-P** and **24-P** are differentiated from fingolimod phosphate (**FTY720-P**) in the S1P$_1$ GTPγS and S1P$_1$ internalization assays where they exhibit a partial agonist profile. Our improved preclinical pulmonary safety (lower BAL protein elevation) is postulated to be associated with the partial agonist profile of the compounds, as studies have indicated that maintaining some degree of S1P/S1P$_1$ signaling is critical for controlling vascular tone.[23,25,26] Additionally, **12-P** and **24-P** are differentiated from FTY720-P in the ERK-P assay. While **12-P** and **24-P** are both full agonists in this assay like **FTY20-P**, they exhibit 130- to 260-fold weaker potency than **FTY720-P**. The bradycardia effect in some patients receiving fingolimod is likely a function of their full agonist activity of S1P$_1$ in atrial myocytes prior to the subsequent functional antagonism (receptor down-regulation) in those cells.[27] Additionally, the S1P-dependent activation of G protein-coupled inwardly rectifying potassium (GIRK) channels on atrial myocytes has also been described as a contributing factor. The weaker potency in ERK phosphorylation for the third-generation agonists may contribute to the reduced activity in the cardiomyocyte beating rate assay.

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Compounds **12-P** and **24-P** both maintain excellent selectivity over $S1P_3$. The compounds were also tested for activity against $S1P_4$ and $S1P_5$ where they both exhibited full agonism with IC_{50} values of 10 nM ($S1P_4$) and 4.9 nM ($S1P_5$) for **12-P** and 5.6 nM ($S1P_4$) and 3.6 nM ($S1P_5$) for **24-P**. Finally, **12-P** and **24-P** were tested in a human chemotaxis assay, a functional primary cell assay that correlates well with in vivo lymphopenia. Much like **1-P**, these compounds both completely block the S1P-induced T-cell migration with an IC_{50} of 4.1 and 0.7 nM, respectively.

The in vivo pharmacokinetic parameters for **12** and **24** were investigated in rats, monkeys, and dogs, following intravenous (i.v.) and oral (p.o.) administration (Table 5). For **12**, the results demonstrated favorable drug characteristics with acceptable bioavailability in both rats (F = 45%) and monkeys (F = 76%) using 1 mg/kg oral administration. Compound **12** has a large volume of distribution, with a steady-state volume of distribution (V_{ss}) of 12 ± 1.1 L/kg in rats and 8.4 ± 0.5 L/kg in dogs. The terminal $T_{1/2}$ of **12** after IV dosing was 29 ± 5.5 h in rats and 21 h in monkeys, and these were significantly shorter than that for **1** (131 to 210 h). Low clearance from blood was observed in all the species tested. Using the estimated clearance that was generated from human liver microsomal data and adjusted with IVIVC ratio, the projected human $T_{1/2}$ of **12** is 79 h with a volume of distribution of 10 L/kg and clearance of 2.1 mL/min/kg.[28] For **24**, the results again demonstrated favorable drug characteristics with acceptable bioavailability in monkeys (F = 69%) following oral administration. Compound **24** also had a large volume of distribution, with V_{ss} of 13 ± 3.8 L/kg in rats and 9.9 ± 3.1 L/kg in dogs. The terminal $T_{1/2}$ of **24** was shorter than that of **12** (19 ± 0.9 in rats and 17 ± 0.7 in monkeys) and again low clearance from blood was observed. The projected human $T_{1/2}$ of **24** is less than 30 h.[28]

The phosphate-to-parent blood AUC ratios in rats, dogs, and monkeys after oral dosing for fingolimod, **1**, **12**, and **24** are shown in Table 6. Because the phosphate metabolite is the active species, a compound with greater efficiency of bioconversion to the active phosphate species could afford a lower overall dose, reducing the risk of off-target effects in addition to being more easily formulated. Based on the available data of fingolimod, it appears that although the extent of phosphorylation in humans was reduced in comparison to non-clinical species, an improved phosphorylation in non-clinical species translated to improved phosphorylation in humans. In contrast to the phosphate-to-parent ratios from the PK/PD studies in Lewis rats (see Tables 1 and 2), the ratios for **12** (0.9) and **24** (0.3) in Sprague-Dawley rats were significantly lower than **1** (2.5). These lower ratios in Sprague-Dawley rats were not observed in our previous clinical candidates, both of which had consistent results between these strains of rats.[17] In monkeys, however, **12** was improved over **1** (0.73 vs 0.3). A similar improvement in the monkey phosphate-to-parent ratio (~3-fold) was noted for our second clinical candidate, and this ultimately led to better phosphorylation in humans.[17] The phosphate-to-parent ratio of **24** was found to be lower than **1** in both monkeys (<0.1) and dogs (0.31).

Table 6. Phosphate/Parent Ratios for Fingolimod, 1, 12, and 24[a]

species	Fingolimod	1	12	24
Sprague-Dawley rat	5.6	2.5	0.9	0.3
cynomolgus monkey	5.9	0.3	0.73	<0.1
beagle dog	3.7	2.4	nt	0.31
human	0.42	~0.1	nt	nt

[a]Phosphate-to-parent ratios calculated using the AUC ratio from PO dosing, nt = not tested.

Next, we evaluated **12** and **24** in an in vivo efficacy model. Figure 2 shows the effect of treatment with compounds **12** or **24** in a rat adjuvant-induced arthritis model (rat AA) in Lewis rats. In rats dosed with vehicle only, there was an increase in paw volume beginning at day 11, and this swelling reached a near-maximum level by day 15 to 19 (1.5−3.0 mL increase). Rats given **12** at once daily doses of 0.1, 0.5, and 2.5 mg/kg exhibited a dose-dependent reduction in paw edema. The rats dosed at 0.5 mg/kg exhibited slight paw edema beginning at day 18 and at day 22 had roughly 75% reduction in swelling as compared to the vehicle group. In the groups treated with 2.5 mg/kg of **12**, there was no appreciable paw volume increase observed throughout the duration of the study. From this study, **12** was determined to have an ED_{50} of 0.3 mg/kg in the rat AA model. Rats given **24** at once daily doses of 0.1, 0.3, and 1.0 mg/kg also exhibited a dose-dependent reduction in paw edema. The rats dosed at 0.3 mg/kg exhibited paw edema beginning at day 13 and at day 20 had roughly 50% reduction in swelling as compared to the vehicle group. In the groups treated with 1.0 mg/kg of **24**, there was no appreciable paw volume increase observed throughout the duration of the study. From this study, **24** also had an ED_{50} of 0.3 mg/kg in the rat AA model.

CONCLUSIONS

In summary, after an extensive exploration of alkenes, ethers, thioethers, and glycols as replacements for the hexyl side chain of our previous clinical candidate (**1**), our efforts led to the discovery of **12** and **24**, each of which are highly potent, biased agonists of $S1P_1$. Furthermore, in comparison to our first clinical candidate, both compounds not only exhibited shorter in vivo $T_{1/2}$ in multiple species but are also projected to have significantly shorter $T_{1/2}$ values in humans. These compounds both efficiently reduced blood lymphocyte counts in rats, had acceptable liability profiles, and showed excellent pharmacokinetic properties across multiple species. Studies in rodent models of arthritis demonstrated robust efficacy with **12** and **24** treatment. In preclinical evaluations, both compounds demonstrated good projected safety profiles of undesired cardiovascular and pulmonary effects. Despite the positive attributes of **24**, poor formation of the desired phosphate metabolite was observed in all species tested, leading to deprioritization of this compound. Compound **12**, however, exhibited an improved phosphate ratio in cynomolgus monkeys in comparison to **1**. This improvement in the phosphate metabolite formation in monkeys, along with a reduced projected human $T_{1/2}$, a robust efficacy in a model of arthritis, and an improved safety profile led to further evaluation of **12**.

EXPERIMENTAL SECTION

Chemical Methods. All commercially available chemicals and solvents were used without further purification. Reactions were performed under an atmosphere of nitrogen. All flash column chromatography was performed on EM Science silica gel 60 (particle size of 40−60 μm). All new compounds gave satisfactory ^1H NMR,

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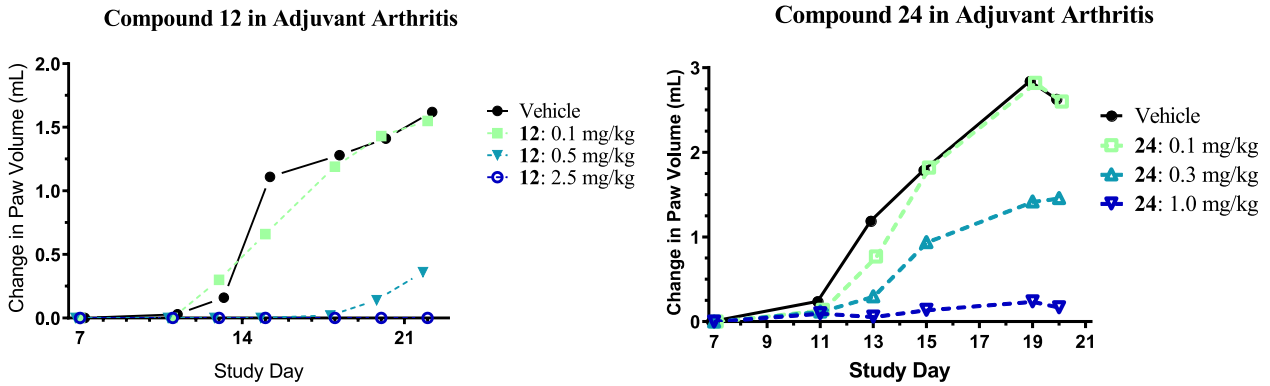

Figure 2. Efficacy of **12** (0.1, 0.5, and 2.5 mg/kg) and **24** (0.1, 0.3, and 1.0 mg/kg) vs vehicle in an adjuvant arthritis model (AA) in Lewis rats. n = 8 in each group.

LC/MS, and mass spectrometry results. ^{1}H NMR spectra were obtained on a Bruker 400 MHz or a JEOL 500 MHz NMR spectrometer using the residual signal of deuterated NMR solvent as internal reference. Electrospray ionization mass spectra were obtained on a Waters ZQ single quadrupole mass spectrometer. High-resolution mass spectral analysis was performed on an LTQ-FT mass spectrometer interfaced to a Waters Acquity ultraperformance liquid chromatography.

HPLC analyses were performed using the following conditions. All final compounds had an HPLC purity of ≥95% unless otherwise stated.

Method A (analytical): Waters Acquity UPLC, BEH C18 2.1 × 50 mm, 1.7 μm particles; mobile phase A: 98:2 water/ACN 0.05%TFA; mobile phase B: 2:98 water/ACN 0.05%TFA; column temp 50 °C; gradient 2−98%B over 1 min then 0.5 min hold at 100% B. Flow 0.8 mL/min; detection: UV 200 nm.

Method B (analytical): Waters Acquity UPLC BEH C18 2.1 × 50 mm, 1.7 μm particles; mobile phase A: 90:10 water/ACN 0.1%TFA; mobile phase B: 10:90 water/ACN 0.1%TFA; temperature: 50 °C; gradient: 0−100% B over 2 min, then a 0.5 min hold at 100% B; flow: 1.0 mL/min; detection: UV at 220 nm.

Method C (analytical): Waters Sunfire C18 2.1 × 50 mm 5 μ, 1.7 μm particles; mobile phase A: 90:10 water/ACN 0.1% TFA; mobile phase B: 10:90 water/ACN 0.1% TFA; temperature: 40 °C; gradient: 0−100% B over 4 min, then a 0.5 min hold at 100% B; flow: 1.0 mL/min; detection: UV at 220 nm.

Method D (analytical purity long run): Shimadzu HPLC, Sunfire C18 (3.0 × 150 mm, 3.5 μm particles; mobile phase A: 5:95 acetonitrile/water with 0.05% TFA; mobile phase B: 95:5 acetonitrile/water with 0.05 TFA; temperature: 25 °C; gradient: 0−100% B over 12 min, then a 3 min hold at 100% B; flow: 1.0 mL/min; detection: UV at 254 nm.

Method E (analytical): Column Waters Acquity BEH C18 2.1 × 50 mm 1.7 μm; linear gradient of 0−100% solvent B over 3 min, then 0.75 min hold at 100% B; flow rate: 1.11 mL/min; solvent A: 5:95 acetonitrile/water with 10 mM ammonium acetate; solvent B: 95:5 acetonitrile/water with 10 mM ammonium acetate; temperature = 50 °C; products detected at 220 wavelength w/positive ionization mode.

*((1R,3S)-1-Amino-3-((R)-6-((E)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**2**).*[29] DEAD (730 μL, 4.6 mmol) was added dropwise to a solution of pentan-1-ol (300 mg, 3.40 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (740 mg, 4.2 mmol), and Ph_3P (1.09 g, 4.2 mmol) in THF (20 mL) at 0 °C. The mixture was stirred at a temperature range from 0 °C to rt for 16 h. The mixture was diluted with EtOAc (30 mL) and washed with brine (2 × 20 mL) and water (20 mL), then dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified on a silica gel cartridge using an EtOAc/Hex gradient (0−50% EtOAc over 12 column volumes) to afford 5-(pentylthio)-1-phenyl-1H-tetrazole (650 mg, 77% yield). LC/MS M^{+1} = 249.2. HPLC t_r = 3.36 (method C).

Ammonium molybdate tetrahydrate (680 mg, 0.550 mmol) was added to 30% H_2O_2 (407 mL, 40 mmol) at 0 °C and the resultant solution was added dropwise to a solution of 5-(pentylthio)-1-phenyl-1*H*-tetrazole (650 mg, 2.6 mmol) in EtOH (20 mL) at 0 °C. The mixture was allowed to warm to rt and stirred at rt for 16 h. Next, 30 mL of brine was added and the mixture was extracted with EtOAc (80 mL), washed with brine, dried (Na_2SO_4), and concentrated in vacuo to afford 5-(pentylsulfonyl)-1-phenyl-1*H*-tetrazole (700 mg, 95% yield). LC/MS M^{+1} = 328. HPLC t_r = 3.36 (method C).

KHMDS (420 μL, 0.21 mmol) was added dropwise to a solution of 5-(pentylsulfonyl)-1-phenyl-1*H*-tetrazole (26 mg, 0.092 mmol) and (*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalene-2-carbaldehyde (**38**, 25 mg, 0.084 mmol) in THF (3 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h. Water (1 mL) was then added and the mixture was warmed to rt. The reaction mixture was diluted with EtOAc (80 mL), washed with brine, dried (Na_2SO_4), and concentrated in vacuo to afford (5*R*,7*S*)-7-((*R*)-6-((*E*)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (5 mg, 17% yield). ^{1}H NMR (400 MHz, MeOD): δ 7.02−6.90 (m, 3H), 5.55−5.46 (m, 1H), 4.44−4.25 (m, 2H), 3.14−2.94 (m, 1H), 2.88−2.67 (m, 3H), 2.53 (dd, *J* = 16.3, 10.1 Hz, 1H), 2.42−2.32 (m, 1H), 2.29 (dd, *J* = 13.1, 7.2 Hz, 1H), 2.19−2.01 (m, 4H), 1.97−1.87 (m, 3H), 1.84−1.71 (m, 2H), 1.62−1.46 (m, 1H), 1.43−1.32 (m, 4H), 1.01−0.86 (m, 3H); LC/MS M^{+1} = 354.

*((1R,3S)-1-Amino-3-((R)-6-((Z)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**3**).*[29] To a solution of pentyltriphenylphosphonium, bromide salt (182 mg, 0.44 mmol) in THF (3 mL) at −78 °C was added *n*-butyllithium (168 μL, 0.421 mmol) dropwise. The reaction was allowed to warm to 0 °C and maintained at 0 °C for 1 h and then cooled back to −78 °C. A solution of (*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalene-2-carbaldehyde (**38**, 60 mg, 0.200 mmol) in THF (2 mL) was then added and the reaction was allowed to warm at rt and stirred for 1.5 h. The reaction was quenched with saturated NH_4Cl (5 mL) and extracted with EtOAc (50 mL). The organic extract was washed with saturated NH_4Cl (3 × 20 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified on a silica gel cartridge using an EtOAc/Hex gradient (0−45% EtOAc over 25 min) to afford (5*R*,7*S*)-7-((*R*)-6-((*Z*)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (40 mg, 57% yield) as a white solid. ^{1}H NMR (400 MHz, *MeOD*): δ 6.98 (s, 3H), 5.52−5.26 (m, 2H), 4.37 (s, 1H), 4.31 (s, 1H), 3.03 (tt, *J* = 11.0, 7.3 Hz, 1H), 2.83 (dd, *J* = 7.9, 4.6 Hz, 2H), 2.72 (s, 2H), 2.58−2.38 (m, 1H), 2.29 (dd, *J* = 12.9, 7.2 Hz, 1H), 2.19−2.05 (m, 4H), 2.01−1.70 (m, 4H), 1.61−1.49 (m, 1H), 1.39 (s, 4H), 1.08−0.72 (m, 3H); LC/MS M^{+1} = 354.3; HPLC t_r = 1.7 (method B).

To a solution of (5*R*,7*S*)-7-((*R*)-6-((*Z*)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (45 mg, 0.127 mmol) in dioxane (2 mL) was added water (0.5 mL)

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and lithium hydroxide hydrate (53 mg, 1.3 mmol). The mixture was stirred at 100 °C for 16 h under N_2. After cooling, the mixture was filtered and washed with MeOH, the combined solvents were evaporated, and the residue was purified by HPLC: column Phenomenex Luna C18 5μ 21.2 × 100 mm. Solvent A: 10% MeOH−90% H_2O−0.1% TFA; solvent B: 90% MeOH−10% H_2O−0.1% TFA. Gradient time = 15 min. Start B = 0%, final B 100%. Stop time 20 min. The collected fraction was basified with saturated $NaHCO_3$, concentrated under vacuo and the aqueous layer was extracted with DCM (3 × 20 mL), which was dried (Na_2SO_4) and concentrated under vacuo. The residue was freeze-dried to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((*Z*)-hex-1-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (32 mg, 69% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.12−6.89 (m, 3H), 5.49−5.27 (m, 2H), 3.63−3.47 (m, 2H), 3.07 (ddd, *J* = 10.9, 7.3, 4.1 Hz, 1H), 2.83 (dd, *J* = 8.0, 4.7 Hz, 2H), 2.79−2.64 (m, 2H), 2.57−2.39 (m, 1H), 2.40−2.24 (m, 1H), 2.21−2.00 (m, 3H), 1.98−1.77 (m, 4H), 1.70−1.46 (m, 2H), 1.45−1.32 (m, 4H), 1.00−0.84 (m, 3H); LC/MS M^{+1} = 328.3; HPLC t_r = 8.75 (method D).

*((1R,3S)-1-Amino-3-((S)-6-((E)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**4**).*[29] To a mixture of (5*R*,7*S*)-7-((*R*)-6-(2-hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**40**, 80 mg, 0.254 mmol) in DCM (5 mL) was added Dess−Martin periodinane (140 mg, 0.330 mmol). The reaction mixture was stirred 1 h, diluted with DCM, and washed with 1 N NaOH. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The compound was used without further purification.

1 M KHMDS in THF (0.36 mL, 0.36 mmol) was added dropwise to a solution of 5-(butylsulfonyl)-1-phenyl-1*H*-tetrazole (57 mg, 0.22 mmol) and 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)acetaldehyde (45 mg, 0.144 mmol) in THF (3 mL) at −78 °C and the resultant solution was stirred at the same temperature for 2 h. Water (1 mL) was added and the mixture was warmed to rt, diluted with water (10 mL), and extracted with EtOAc (30 mL). The organic layer was washed with saturated NH_4Cl (2 × 15 mL), brine (20 mL), dried ($MgSO_4$), and concentrated under vacuo to afford the desired product, which was purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 30−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to provide (5*R*,7*S*)-7-((*S*)-6-((*E*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (11 mg, 0.031 mmol, 22% yield).

To a mixture of (5*R*,7*S*)-7-((*S*)-6-((*E*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (11 mg, 0.031 mmol) in MeOH (1 mL) and DMSO (1 mL) was added 1 N NaOH (1 mL). The reaction mixture was heated at 95 °C for 4 h, then cooled, and acidified with TFA. The crude product was filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to provide ((1*R*,3*S*)-1-amino-3-((*S*)-6-((*E*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (13 mg, 0.028 mmol, 90% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 6.99 (s, 3H), 5.53−5.46 (m, 2H), 3.71−3.55 (m, 2H), 3.19−3.03 (m, 1H), 2.89−2.73 (m, 3H), 2.48−2.33 (m, 2H), 2.19−1.87 (m, 9H), 1.81−1.68 (m, 2H), 1.51−1.31 (m, 3H), 0.94 (t, *J* = 7.4 Hz, 3H); LC/MS M^{+1} = 328; HPLC t_r = 8.42 (method D).

*((1R,3S)-1-Amino-3-((S)-6-((Z)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**5**).*[29] To a mixture of butyltriphenylphosphonium, bromide salt (172 mg, 0.43 mmol) in THF (1 mL) was added LiHMDS (0.43 mL, 0.43 mmol). The reaction mixture was stirred for 30 min, then 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)acetaldehyde (45 mg, 0.144 mmol) in THF (1 mL) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was quenched with water, then diluted with EtOAc, and washed with saturated NaCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (12 g) using an 20% MeOH/DCM/DCM gradient (0−25% 20% MeOH/DCM over 17 CV) to afford (5*R*,7*S*)-7-((*S*)-6-((*Z*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (14 mg, 0.040 mmol, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07−7.01 (m, 1H), 6.99−6.91 (m, 2H), 5.58 (br s, 1H), 5.54−5.42 (m, 2H), 4.40−4.23 (m, 2H), 3.11−2.95 (m, 1H), 2.91−2.73 (m, 3H), 2.43 (br dd, *J* = 16.4, 10.7 Hz, 1H), 2.32 (dd, *J* = 13.2, 7.3 Hz, 1H), 2.22−2.09 (m, 4H), 2.09−2.01 (m, 2H), 2.01−1.91 (m, 3H), 1.91−1.71 (m, 2H), 1.46−1.38 (m, 3H), 0.94 (t, *J* = 7.3 Hz, 3H); LC/MS M^{+1} = 354.2; HPLC t_r = 1.23 (method A).

To a mixture of (5*R*,7*S*)-7-((*S*)-6-((*Z*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (14 mg, 0.040 mmol) in MeOH (1 mL) and DMSO (1 mL) was added 1 N NaOH (1 mL). The reaction mixture was heated at 95 °C for 4 h, then cooled, and acidified with TFA. The crude product was filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-((*Z*)-hex-2-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (11 mg, 0.022 mmol, 57% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 6.99 (s, 3H), 5.49 (t, *J* = 4.7 Hz, 2H), 3.72−3.56 (m, 2H), 3.20−3.03 (m, 1H), 2.90−2.71 (m, 3H), 2.49−2.36 (m, 2H), 2.17−2.10 (m, 3H), 2.10−2.02 (m, 2H), 2.02−1.88 (m, 4H), 1.83−1.66 (m, 2H), 1.51−1.33 (m, 3H), 0.94 (t, *J* = 7.4 Hz, 3H); LC/MS M^{+1} = 328; HPLC t_r = 8.28 (method D). Purity = 90%.

*((1R,3S)-1-Amino-3-((R)-6-((E)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**6**) and ((1R,3S)-1-Amino-3-((R)-6-((Z)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (**7**).*[29] Nitrogen was bubbled through a mixture of *trans*-3-hexene (5.8 mL, 47 mmol), (5*R*,7*S*)-7-((*R*)-6-(but-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro-[4.4]nonan-2-one (**43**, 1.5 g, 4.6 mmol), and DCM (50 mL) for 3 min at −78 °C before Grubbs second-generation catalyst (0.25 g, 0.294 mmol) was added. The bubbling was continued for 2 min and then the mixture was stirred under N_2 at 40 °C for 3.5 h. The mixture was concentrated and purified by flash chromatography (24 g silica gel column, gradient elution from 0 to 40% of EtOAc in DCM) to afford (5*R*,7*S*)-7-((*R*)-6-(hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (1.2 g, 3.4 mmol, 74% yield) as a solid. SFC separation (20% MeOH in CO_2, ADH column; 40 °C; 140 bar BPR) gave PK1: (5*R*,7*S*)-7-((*R*)-6-((*E*)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (0.8 g, 2.263 mmol, 49.1% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.08−7.01 (m, 1H), 7.00−6.91 (m, 2H), 6.36 (s, 1H), 5.62−5.34 (m, 2H), 4.37−4.25 (m, 2H), 3.09−2.96 (m, 1H), 2.91−2.76 (m, 3H), 2.40 (dd, *J* = 16.4, 10.5 Hz, 1H), 2.31 (dd, *J* = 13.3, 7.2 Hz, 1H), 2.21−2.08 (m, 4H), 2.08−1.91 (m, 5H), 1.90−1.79 (m, 1H), 1.79−1.69 (m, 1H), 1.53−1.32 (m, 3H), 1.00 (t, *J* = 7.5 Hz, 3H); HPLC t_r = 4.11 min (condition C); LC/MS M^{+1} = 354); and PK2: (5*R*,7*S*)-7-((*R*)-6-((*Z*)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (0.1 g, 0.283 mmol, 6.14% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.04 (d, *J* = 7.8 Hz, 1H), 6.98−6.91 (m, 2H), 5.60 (br s, 1H), 5.44−5.30 (m, 2H), 4.39−4.23 (m, 2H), 3.11−2.96 (m, 1H), 2.91−2.77 (m, 3H), 2.41 (dd, *J* = 16.2, 10.7 Hz, 1H), 2.32 (dd, *J* = 13.3, 7.2 Hz, 1H), 2.22−2.04 (m, 6H), 2.01−1.89 (m, 3H), 1.89−1.80 (m, 1H), 1.79−1.70 (m, 1H), 1.50−1.37 (m, 3H), 1.00 (t, *J* = 7.5 Hz, 3H); HPLC t_r = 4.08 min (condition C); LC/MS M^{+1} = 354). See the Supporting Information for additional NMR analysis.

Each isomer was dissolved independently in dioxane (0.5 mL) and treated with NaOH (0.93 g, 23 mmol) in water (7 mL) at 90 °C under N_2 for 1.5 days. Each mixture was independently extracted with EtOAc (4 × 4 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The resulting residue was purified using reverse-phase HPLC [Phenomenex Luna 5μ 30 × 100 mm (Axia); gradient over 6 min from 40 to 100% of solvent B and holding @ 100% of solvent B for 6 min; solvent A: 10% MeOH: 90% H_2O: 0.1%

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TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA]. The appropriate fractions were concentrated, neutralized with 2 N aqueous NaOH, and extracted with EtOAc. The organic fraction was then concentrated to afford each isomer:

Obtained ((1*R*,3*S*)-1-amino-3-((*R*)-6-((*E*)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**6**, 11 mg, 52% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05−6.93 (m, 3H), 5.56−5.36 (m, 2H), 3.53−3.40 (m, 2H), 3.09−2.96 (m, 1H), 2.88−2.74 (m, 3H), 2.38 (dd, J = 16.4, 10.5 Hz, 1H), 2.28 (dd, J = 13.3, 8.0 Hz, 1H), 2.15−1.85 (m, 8H), 1.83−1.62 (m, 2H), 1.52 (dd, J = 13.2, 11.0 Hz, 1H), 1.46−1.32 (m, 3H), 0.97 (t, J = 7.5 Hz, 3H); HPLC t_r = 3.50 min (method C); LC/MS M^{+1} = 328 (see the Supporting Information for NMR confirmation of the E isomer).

Obtained ((1*R*,3*S*)-1-amino-3-((*R*)-6-((*Z*)-hex-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**7**, 5 mg, 48% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (s, 2H), 7.00 (s, 1H), 5.44−5.33 (m, 2H), 3.58−3.43 (m, 2H), 3.13−2.98 (m, 1H), 2.92−2.77 (m, 3H), 2.41 (br dd, J = 16.2, 10.7 Hz, 1H), 2.30 (dd, J = 13.4, 7.7 Hz, 1H), 2.23−2.03 (m, 6H), 1.85−1.63 (m, 4H), 1.56 (dd, J = 13.0, 11.4 Hz, 1H), 1.50−1.34 (m, 3H), 1.00 (t, J = 7.6 Hz, 3H); LC/MS M^{+1} = 328; HPLC t_r = 3.50 min (method C). Purity 90% (see the Supporting Information for NMR confirmation of *Z* isomer).

*((1R,3S)-1-Amino-3-((R)-6-((Z)-hex-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**8**).*[29] To a stirred mixture of ethyltriphenylphosphonium bromide (650 mg, 1.76 mmol) and anhydrous THF (5 mL) was added a 1 M THF solution of KOtBu (1.8 mL, 1.8 mmol) dropwise at 0 °C. The mixture was stirred at the same temperature for 1 h before a solution of 4-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)butanal (**41**, 120 mg, 0.35 mmol) in anhydrous THF (5 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 40 min. Saturated aqueous NH_4Cl solution (2 mL) was added dropwise to quench the reaction. Water (1 mL) and hexanes (8 mL) were added. The aqueous layer was separated and extracted with EtOAc (3 × 2 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography purification using ISCO (12 g of silica gel column, gradient elution from 15 to 80% of EtOAc in hexanes) afforded (5*R*,7*S*)-7-((*R*)-6-(hex-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (115 mg, 0.33 mmol, 89% yield) as a white solid. This product was further purified using SFC conditions (ChiralPak AD-H 25 × 5 cm ID, 5 μm; Flow rate: 150.0 mL/min; mobile phase: 70/30 CO_2/MeOH) to afford (5*R*,7*S*)-7-((*R*)-6-((*Z*)-hex-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (86 mg, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11−7.01 (m, 1H), 7.00−6.90 (m, 2H), 5.78 (br s, 1H), 5.57−5.36 (m, 2H), 4.43−4.23 (m, 2H), 3.13−2.96 (m, 1H), 2.93−2.73 (m, 3H), 2.49−2.25 (m, 2H), 2.21−2.04 (m, 4H), 2.03−1.91 (m, 3H), 1.89−1.80 (m, 1H), 1.64 (br d, J = 5.9 Hz, 3H), 1.54−1.43 (m, 3H), 1.44−1.33 (m, 3H); LC/MS M^{+1} = 354.4; HPLC t_r = 4.17 min (method C).

A mixture of (5*R*,7*S*)-7-((*R*)-6-((*Z*)-hex-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (40 mg, 0.11 mmol), 2 N aqueous NaOH (1.1 mL, 2.3 mmol), and dioxane (0.5 mL) was stirred under N_2 in a sealed vial at 100 °C for 4 h. The mixture was cooled and extracted with EtOAc (4 × 1 mL), and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 7 min from 35 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The appropriate fractions were concentrated, neutralized with 2 N aqueous NaOH and extracted with EtOAc. The EtOAc layer was concentrated to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((*Z*)-hex-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (35 mg, 0.104 mmol, 92% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02 (s, 2H), 6.99 (s, 1H), 5.57−5.37 (m, 2H), 3.58−3.40 (m, 2H), 3.04 (tt, J = 10.7, 7.5 Hz, 1H), 2.93−2.71 (m, 3H), 2.39 (br dd, J = 16.4, 10.7 Hz, 1H), 2.29 (dd, J = 13.4, 7.9 Hz, 1H), 2.09 (br d, J = 6.5 Hz, 3H), 1.97−1.89 (m, 4H), 1.81−1.67 (m, 4H), 1.63 (dd, J = 6.1, 0.8 Hz, 3H), 1.55−1.44 (m, 3H), 1.42−1.35 (m, 3H); LC/MS M^{+1} = 328.4; HPLC t_r = 9.89 min (method D).

*((1R,3S)-1-Amino-3-((R)-6-(hex-5-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**9**).*[29] To a mixture of ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 150 mg, 0.33 mmol) in dry THF (2 mL) was added lithium bromide (143 mg, 1.646 mmol). The resulting mixture was stirred at 50 °C for 16 h and quenched with water (3 mL). After cooling, the mixture was taken up in EtOAc (20 mL), washed with saturated $NaHCO_3$ (10 mL), dried (Na_2SO_4), and concentrated under vacuo to afford (5*R*,7*S*)-7-((*R*)-6-(bromomethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (120 mg, 0.330 mmol, 100% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.01 (s, 2H), 7.00−6.99 (m, 1H), 4.42−4.27 (m, 2H), 3.51 (d, J = 6.2 Hz, 2H), 3.10−3.00 (m, 1H), 3.00−2.90 (m, 1H), 2.87−2.79 (m, 2H), 2.54 (dd, J = 16.2, 10.0 Hz, 1H), 2.29 (dd, J = 13.0, 7.0 Hz, 1H), 2.18−2.02 (m, 5H), 2.01−1.87 (m, 2H), 1.87−1.74 (m, 1H).

A stirred suspension of copper(I) bromide (31.5 mg, 0.220 mmol) and (5*R*,7*S*)-7-((*R*)-6-(bromomethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (40 mg, 0.110 mmol) in THF (5 mL) was cooled with a dry ice bath in acetone and treated with pent-4-en-1-ylmagnesium bromide (2.2 mL, 1.1 mmol). The resulting mixture was warmed slowly to rt and stirred for 3 h. The mixture was quenched with water (3 mL) at 0 °C, and the mixture was taken up in EtOAc (20 mL). The layers were separated and the organic layer was washed with saturated $NaHCO_3$ (10 mL), dried (Na_2SO_4), and concentrated under vacuo to afford (5*R*,7*S*)-7-((*R*)-6-(hex-5-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (30 mg, 0.085 mmol, 77% yield). HPLC t_r = 1.64 min (method B); LC/MS M^{+1} = 354.

(5*R*,7*S*)-7-((*R*)-6-(hex-5-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (30 mg, 0.085 mmol) was dissolved in dioxane (1.5 mL) and water (0.5 mL) and lithium hydroxide hydrate (36 mg, 0.85 mmol) were added. The reaction mixture was stirred under N_2 at 100 °C for 16 h. The mixture was concentrated and purified by preparative HPLC: Column Phenomenex Luna C18 5μ 21.2 × 100 mm. Solvent A: 10% MeOH−90% H_2O−0.1% TFA; solvent B: 90% MeOH−10% H_2O−0.1% TFA. Gradient time = 15 min. Start B = 0%, final B 100%. Stop time 20 min. The collected fraction was basified with saturated $NaHCO_3$, concentrated under vacuo, and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under vacuo to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-(hex-5-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (20 mg, 0.055 mmol, 65% yield) as a white solid. ^{1}H NMR (400 MHz, *MeOD*): δ 6.97 (d, J = 1.5 Hz, 3H), 5.83 (ddt, J = 17.1, 10.3, 6.8 Hz, 1H), 5.00 (dq, J = 17.2, 1.8 Hz, 1H), 4.93 (ddd, J = 10.2, 2.2, 1.2 Hz, 1H), 3.63−3.48 (m, 2H), 3.12−2.95 (m, 1H), 2.89−2.72 (m, 3H), 2.42−2.23 (m, 2H), 2.16−1.99 (m, 3H), 1.98−1.75 (m, 4H), 1.75−1.53 (m, 2H), 1.47−1.25 (m, 9H); HPLC t_r = 8.33 min (method D); LC/MS M^{+1} = 328.3.

*((1R,3S)-1-Amino-3-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**10a, 10b**).*[29] A mixture of 1-pentanol (10 mL, 92 mmol), *p*-toluenesulfonic acid monohydrate (8 mg, 0.042 mmol), and trimethoxymethane (0.61 mL, 5.6 mmol) was stirred at 100 °C for 2 h with a slow N_2 stream to remove the methanol byproduct. The residual liquid was mixed with (5*R*,7*S*)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]-nonan-2-one (**39**, 400 mg, 1.4 mmol) and stirred at 100 °C under N_2 for 2.5 h. Next, 10% Pd−C (400 mg) was added at rt, followed by EtOAc (5 mL). The mixture was vigorously stirred under a hydrogen balloon for 4 h. The mixture was filtered through a membrane filter and the filtrate was concentrated. Flash chromatography purification (12 g silica gel column, gradient elution from 0 to 100% EtOAc in hexanes) afforded (5*R*,7*S*)-7-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (350 mg, 0.98 mmol, 70% yield) as a sticky solid. LC/MS M^{+1} = 358. Chiral separation (Lux-Amy-2 (3 × 25 cm), 25% MeOH, 120 mL/min, 220 nm, 45 °C)

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of the solid afforded two isomers. Each isomer was hydrolyzed in the following fashion.

Isomer 1: A mixture of (5*R*,7*S*)-7-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (110 mg, 0.31 mmol), lithium hydroxide monohydrate (155 mg, 3.7 mmol), dioxane (1 mL), and water (1 mL) was stirred at 90 °C under N_2 for 15 h. The mixture was cooled and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the resulting reside was purified using reverse-phase HPLC (Phenomenex Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). Fractions with product were pooled and basified with K_2CO_3, then extracted with EtOAc, dried (Na_2SO_4), and concentrated to afford ((1*R*,3*S*)-1-amino-3-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl) methanol (**10a**, 60 mg, 0.17 mmol, 50% yield) as a white solid. ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.03−6.95 (m, 3H), 3.73−3.63 (m, 1H), 3.57−3.42 (m, 4H), 3.09−2.97 (m, 2H), 2.95−2.85 (m, 1H), 2.82−2.68 (m, 2H), 2.27 (dd, *J* = 13.3, 7.8 Hz, 1H), 2.13−2.01 (m, 2H), 1.97−1.46 (m, 7H), 1.37−1.29 (m, 4H), 0.94−0.87 (m, 3H); HPLC t_r = 7.19 min (method D); LC/MS M^{+1} = 332.3.

Isomer 2: A solution of (5*R*,7*S*)-7-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (68 mg, 0.19 mmol) and lithium hydroxide monohydrate (96 mg, 2.3 mmol) in dioxane (1 mL) and water (1 mL) was stirred at 90 °C under N_2 for 15 h. The mixture was cooled and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford ((1*R*,3*S*)-1-amino-3-(6-(pentyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (47 mg, 0.14 mmol, 72% yield) as a yellowish solid. ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.03−6.95 (m, 3H), 3.73−3.64 (m, 1H), 3.57−3.49 (m, 2H), 3.48−3.40 (m, 2H), 3.09−2.96 (m, 2H), 2.95−2.86 (m, 1H), 2.81−2.69 (m, 2H), 2.25 (dd, *J* = 13.2, 7.9 Hz, 1H), 2.13−2.00 (m, 2H), 1.95−1.83 (m, 1H), 1.82−1.55 (m, 5H), 1.48 (dd, *J* = 13.2, 11.0 Hz, 1H), 1.37−1.29 (m, 4H), 0.93−0.87 (m, 3H); HPLC t_r = 7.27 min (method D); LC/MS M^{+1} = 332.3.

*((1R,3S)-1-Amino-3-((R)-(6-(butoxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**11**).*[29] To a mixture of *n*-butanol (500 μL, 5.5 mmol) and a 1 N potassium *tert*-butoxide in THF (33 μL, 0.33 mmol) was added ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 15 mg, 0.033 mmol). The mixture was stirred at 70 °C for 6 h and then quenched with a saturated aqueous NH_4Cl solution (1 mL) and water (1 mL). The reaction mixture was extracted with EtOAc (3 × 2 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford a solid. The solid was mixed with water (0.5 mL), lithium hydroxide monohydrate (27.6 mg, 0.659 mmol), and dioxane (1 mL). The resulting mixture was stirred at 100 °C under N_2 for 7 h. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were concentrated and then purified using reverse-phase HPLC (Waters Xbridge C18 19 × 100 mm; gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). Fractions with a desired product were basified with K_2CO_3 and then extracted with EtOAc to afford ((1*R*,3*S*)-1-amino-3-((*R*)-(6-(butoxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (9.8 mg, 0.029 mmol, 88% yield) as a white solid. ^{1}H NMR (500 MHz, *CDCl*$_3$): δ 7.08−6.97 (m, 3H), 3.47 (br t, *J* = 6.7 Hz, 4H), 3.40 (dd, *J* = 8.9, 6.7 Hz, 2H), 3.14−2.98 (m, 1H), 2.92−2.71 (m, 3H), 2.47 (br dd, *J* = 16.4, 10.5 Hz, 1H), 2.29 (br dd, *J* = 13.3, 7.8 Hz, 1H), 2.20 (br s, 2H), 2.07 (s, 3H), 1.99−1.85 (m, 1H), 1.82−1.66 (m, 2H), 1.65−1.56 (m, 2H), 1.56−1.50 (m, 1H), 1.48−1.37 (m, 3H), 0.96 (t, *J* = 7.5 Hz, 3H); HPLC t_r = 7.19 min (method D); LC/MS M^{+1} = 332.1.

*((1R,3S)-1-Amino-3-((S)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**12**).*[29] To a stirred solution of 3-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propanal (**44**, 440 mg, 1.34 mmol), ethoxytrimethylsilane (1 mL, 6.7 mmol), and triethylsilane (1.07 mL, 6.7 mmol) in nitromethane (10 mL) at 0 °C was added ferric chloride (22 mg, 0.134 mmol). The mixture was stirred at 0 °C for 15 min and then at rt for 12 h. The reaction mixture was diluted with EtOAc and washed with saturated NaCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated and then purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford (5*R*,7*S*)-7-((*S*)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]-nonan-2-one (350 mg, 0.98 mmol, 73% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 6.97 (s, 2H), 6.96 (s, 1H), 4.42−4.26 (m, 2H), 3.58−3.46 (m, 4H), 3.02 (tt, *J* = 11.1, 7.2 Hz, 1H), 2.90−2.72 (m, 3H), 2.37 (dd, *J* = 16.3, 10.3 Hz, 1H), 2.28 (dd, *J* = 13.0, 6.8 Hz, 1H), 2.19−2.03 (m, 2H), 2.01−1.88 (m, 3H), 1.86−1.76 (m, 1H), 1.75−1.64 (m, 3H), 1.52−1.33 (m, 3H), 1.20 (t, *J* = 7.0 Hz, 3H); HPLC t_r = 13.6 min (method D); LC/MS M^{+1} = 358.1.

To a solution of (5*R*,7*S*)-7-((*S*)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (400 mg, 1.12 mmol) in dioxane (40 mL) was added 1 N NaOH (40 mL). The reaction mixture was heated at 100 °C overnight and then cooled, poured into water, and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (250 mg, 0.73 mmol, 65% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.03−6.92 (m, 3H), 3.57−3.42 (m, 6H), 3.01 (tt, *J* = 11.1, 7.2 Hz, 1H), 2.89−2.75 (m, 3H), 2.37 (dd, *J* = 16.2, 10.5 Hz, 1H), 2.20 (dd, *J* = 13.2, 7.0 Hz, 1H), 2.05−1.85 (m, 3H), 1.83−1.75 (m, 1H), 1.75−1.64 (m, 4H), 1.60−1.50 (m, 1H), 1.47−1.33 (m, 3H), 1.20 (t, *J* = 7.0 Hz, 3H); HPLC t_r = 6.63 min (method D); LC/MS M^{+1} = 358.1.

*((1R,3S)-1-Amino-3-((S)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methyl Dihydrogen phosphate (**12-P**).*[29] To a mixture of ((1*R*,3*S*)-1-amino-3-((*S*)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (40 mg, 0.121 mmol) in acetonitrile (4 mL) was added pyrophosphoryl chloride (0.1 mL, 0.723 mmol). After stirring at rt for 1 h, the reaction mixture was quenched with water (0.5) mL) and then purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(3-ethoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methyl dihydrogen phosphate (20 mg, 0.46 mmol, 37% yield). HPLC conditions: Phenomenex Luna 5 μm C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20%−100% gradient over 15 min; and 30 mL/min. LC/MS M^{+1} = 412.4; HPLC t_r = 1.07 (method A).

*((1R,3S)-1-Amino-3-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**13a, 13b**).*[29] To a mixture of 6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-3,4-dihydronaphthalen-2-yl trifluoromethanesulfonate (**46**, 400 mg, 0.96 mmol), copper(I) iodide (18 mg, 0.096 mmol), and bis-(triphenylphosphine)palladium(II) chloride (67 mg, 0.096 mmol) in TEA (3 mL) was added 4-methoxybut-1-yne (161 mg, 1.92 mmol). The reaction mixture was heated at 60 °C for 1 h, diluted with EtOAc, and washed with 1 M HCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (24 g) using an EtOAc/Hex gradient (0−100% EtOAc over 13 CV) to afford (5*R*,7*S*)-7-(6-(4-methoxybut-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (126 mg, 38% yield). ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.06−6.92 (m, 3H), 6.73−6.67 (m, 1H), 6.38−6.32 (m, 1H), 4.38−4.24 (m, 2H), 3.58 (t, *J* = 6.9 Hz, 2H), 3.07−2.93 (m, 1H), 2.81 (t, *J* = 8.1 Hz, 2H), 2.68 (t, *J* = 6.9 Hz, 2H), 2.47−2.36 (m, 2H), 2.31 (dd, *J* = 13.3, 7.2 Hz, 1H), 2.22−2.08 (m, 3H), 2.03−1.91 (m, 2H), 1.92−1.76 (m, 2H); HPLC t_r = 0.94 min (method A); LC/MS M^{+1} = 352.2.

(5*R*,7*S*)-7-(6-(4-Methoxybut-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (126 mg) was dissolved in MeOH (10 mL) and then Pearlman's catalyst (40 mg, 0.29 mmol) was added. The reaction mixture was stirred under a blanket of hydrogen (balloon pressure) for 1 h. The catalyst was then filtered off and the mixture was concentrated in vacuo, and the resulting residue was purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18

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column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford (5*R*,7*S*)-7-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (130 mg, 100% yield). HPLC t_r = 1.04 min (M); LC/MS M^{+1} = 358.3. This product was then separated into diastereomers using SFC chiral separation: chiral AS-H 25 × 3 cm ID, 5 mm; flow rate: 85.0 mL/min; mobile phase: 75/25 CO_2/MeOH, and the two fractions were concentrated and dried. Isomer 1: obtained 32 mg and isomer 2: obtained 33 mg.

To a mixture of (5*R*,7*S*)-7-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer 2, 33 mg, 0.097 mmol) in MeOH (1 mL) and DMSO (0.5 mL) was added 1 N NaOH (0.5 mL). The reaction mixture was heated at 95 °C for 4 h, then cooled, and acidified with TFA. The crude product was filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**13a**, 33 mg, 0.073 mmol, 74% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.18−6.72 (m, 3H), 3.73−3.56 (m, 2H), 3.44 (t, J = 6.4 Hz, 2H), 3.35 (s, 3H), 3.21−3.03 (m, 1H), 2.92−2.75 (m, 3H), 2.49−2.30 (m, 2H), 2.20−2.07 (m, 1H), 2.05−1.87 (m, 4H), 1.73 (t, J = 12.7 Hz, 2H), 1.66−1.56 (m, 2H), 1.55−1.45 (m, 2H), 1.45−1.27 (m, 3H); HPLC t_r = 7.57 min (method D); LC/MS M^{+1} = 332.

To a mixture of (5*R*,7*S*)-7-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer 1, 32 mg, 0.090 mmol) in MeOH (1 mL) and DMSO (0.5 mL) was added 1 N NaOH (0.5 mL). The reaction mixture was heated at 95 °C for 4 h, then cooled, and acidified with TFA. The crude product was filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(4-methoxybutyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**13b**, 29 mg, 0.064 mmol, 71% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.17−6.78 (m, 3H), 3.72−3.55 (m, 2H), 3.44 (t, J = 6.5 Hz, 2H), 3.35 (s, 3H), 3.21−3.02 (m, 1H), 2.95−2.70 (m, 3H), 2.52−2.29 (m, 2H), 2.18−2.06 (m, 1H), 2.02−1.86 (m, 4H), 1.70 (d, J = 12.5 Hz, 2H), 1.66−1.56 (m, 2H), 1.55−1.29 (m, 5H); HPLC t_r = 7.64 min (method D); LC/MS M^{+1} = 332.

*((1R,3S)-1-Amino-3-((R)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**14**).*[29] To a stirred mixture of butyl mercaptan (0.7 mL, 1.6 mmol), anhydrous THF (6 mL) and ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 240 mg, 0.53 mmol) was added a 1 N THF solution of potassium *tert*-butoxide (1.6 mL, 1.6 mmol) dropwise. The resulting mixture was stirred at rt for 1.5 h and then at 50 °C for 1 h before 2 N aqueous NaOH (5.3 mL, 10.6 mmol) was added. The mixture was concentrated to remove THF and dioxane (3 mL) was added. The resulting mixture was stirred at 90 °C overnight, cooled to rt, and extracted with EtOAc (5 mL, 2 × 2 mL). The combined EtOAc extracts were dried (Na_2SO_4), concentrated, and the resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). Fractions with product were pooled, basified to pH = ∼12 with aqueous 1 N NaOH, and then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (153 mg, 0.42 mmol, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11−6.98 (m, 3H), 3.95 (br d, J = 5.5 Hz, 1H), 3.56−3.42 (m, 2H), 3.14−2.95 (m, 2H), 2.90−2.80 (m, 2H), 2.69−2.46 (m, 5H), 2.30 (dd, J = 13.5, 7.6 Hz, 1H), 2.17−2.05 (m, 2H), 2.02−1.87 (m, 2H), 1.83−1.76 (m, 4H), 1.67−1.57 (m, 3H), 1.56−1.38 (m, 4H); HPLC t_r = 8.91 min (method D); LC/MS M^{+1} = 348.1.

*((1R,3S)-1-Amino-3-((6R)-6-((butylsulfinyl)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**15**).*[29] To a stirred clear solution of ((1*R*,3*S*)-1-amino-3-((*R*)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (10 mg, 0.029 mmol) in DMSO (0.061 mL, 0.86 mmol) was added L-10-(−)-camphor sulfonic acid (33 mg, 0.14 mmol) in DCM (0.5 mL) and methanol (0.2 mL) at −78 °C, followed by 77% *m*-CPBA (6.5 mg, 0.030 mmol). The temperature was raised to 0 °C over 30 min. The mixture was stirred at 0 °C for 30 min and rt for 30 min. The mixture was concentrated and the residue was purified using reverse-phase HPLC (Waters Xbridge C18 19 × 100 mm; gradient over 10 min from 10 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). Fractions with product were pooled, basified with aqueous 1 N NaOH to pH = ∼12, and then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford ((1*R*,3*S*)-1-amino-3-((6*R*)-6-((butylsulfinyl)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (7 mg, 0.018 mmol, 64% yield) as a solid. ^{1}H NMR (400 MHz, *MeOD*): δ 7.16−6.88 (m, 3H), 3.56−3.35 (m, 2H), 3.16−2.94 (m, 2H), 2.93−2.73 (m, 6H), 2.60 (br dd, J = 16.2, 10.0 Hz, 1H), 2.44−2.26 (m, 1H), 2.25−2.09 (m, 2H), 2.09−1.82 (m, 3H), 1.80−1.64 (m, 4H), 1.61−1.45 (m, 3H), 0.99 (t, J = 7.3 Hz, 3H); HPLC t_r = 6.36 min (method D); LC/MS M^{+1} = 364.1.

*((1R,3S)-1-Amino-3-((R)-6-((butylsulfonyl)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**16**).*[29] To a stirring solution of ((1*R*,3*S*)-1-amino-3-((*R*)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (10 mg, 0.029 mmol) and L-10-(−)-camphor sulfonic acid (33 mg, 0.14 mmol) in DCM (0.5 mL) and methanol (0.2 mL) at −78 °C was added 77% mCPBA (13 mg, 0.058 mmol). The temperature was raised to 0 °C over 30 min. The mixture was stirred at 0 °C for 30 min and at rt for 30 min. The mixture was concentrated and the residue was purified using reverse-phase HPLC (Waters Xbridge C18 19 × 100 mm; gradient over 10 min from 10 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). Fractions with product were pooled and basified to pH = ∼12 with 1 N NaOH and then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((butylsulfonyl)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (7 mg, 0.018 mmol, 62% yield) as a solid. ^{1}H NMR (400 MHz, *MeOD*): δ 7.09−6.92 (m, 3H), 3.55−3.44 (m, 2H), 3.21−3.11 (m, 4H), 3.11−2.99 (m, 2H), 2.90−2.83 (m, 2H), 2.74−2.60 (m, 1H), 2.59−2.43 (m, 1H), 2.31−2.13 (m, 2H), 2.07−1.88 (m, 2H), 1.87−1.76 (m, 3H), 1.76−1.63 (m, 2H), 1.60−1.46 (m, 3H), 1.01 (t, J = 7.4 Hz, 3H); HPLC t_r = 7.00 min (method D); LC/MS M^{+1} = 380.3.

*((1R,3S)-1-Amino-3-((R)-6-(2-(propylthio)ethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**17**).*[29] (5*R*,7*S*)-7-((*R*)-6-(2-Hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**40**, 180 mg, 0.57 mmol) was dissolved in dry pyridine (1 mL) and *p*-toluenesulfonyl chloride (326 mg, 1.7 mmol) was added in one portion. The resulting mixture was stirred at rt for 2 h and the solvent was removed in vacuo. The residue was dissolved in DCM and loaded onto the column. Flash chromatography purification (4 g of silica gel column, 0−100% EtOAc in DCM) afforded 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (220 mg, 0.47 mmol, 82% yield) as a solid. HPLC t_r = 1.04 min (method A); LC/MS M^{+1} = 470.4.

To a stirred mixture of propane-1-thiol (0.017 mL, 0.19 mmol), 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (30 mg, 0.064 mmol), and dioxane (0.5 mL) was added 2 N aqueous NaOH (0.64 mL, 1.3 mmol), and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated and then purified using reverse-phase HPLC [Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100%

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of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA]. Fractions with product were pooled and basified to pH = ~12 with 1 N NaOH and then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-(2-(propylthio)ethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (23 mg, 0.063 mmol, 98% yield) as a white solid. ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.02 (s, 2H), 6.99 (s, 1H), 3.63−3.36 (m, 2H), 3.15−2.98 (m, 1H), 2.93−2.76 (m, 3H), 2.69−2.60 (m, 2H), 2.57−2.48 (m, 2H), 2.42 (dd, *J* = 16.3, 10.6 Hz, 1H), 2.29 (br dd, *J* = 12.8, 7.6 Hz, 1H), 2.14−2.02 (m, 1H), 2.00−1.84 (m, 3H), 1.66 (br s, 8H), 1.43 (br s, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); HPLC t_r = 9.34 min (method D); LC/MS M^{+1} = 348.4.

*((1R,3S)-1-Amino-3-((S)-6-(3-(ethylthio)propyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**18**).*[29] To a stirred mixture of ethanethiol (0.031 mL, 0.434 mmol), 3-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propyl 4-methylbenzenesulfonate (**45**, 70 mg, 0.145 mmol), and dioxane (1 mL) was added 2 N aqueous NaOH (0.217 mL, 0.434 mmol) at 0 °C. The resulting mixture was stirred at 90 °C overnight. The mixture was cooled to rt, extracted with EtOAc (4 × 1 mL), and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The resulting residue was purified using reverse-phase HPLC [Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA]. Fractions with product were pooled and basified to pH = ~12 with 1 N NaOH and then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(3-(ethylthio)propyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (45 mg, 0.123 mmol, 85% yield) as a white solid. ^{1}H NMR (400 MHz, *MeOD*): δ 7.06−6.90 (m, 3H), 3.57−3.44 (m, 2H), 3.02 (tt, *J* = 11.2, 7.2 Hz, 1H), 2.87−2.74 (m, 3H), 2.63−2.51 (m, 4H), 2.37 (dd, *J* = 16.2, 10.5 Hz, 1H), 2.24 (br dd, *J* = 12.8, 7.3 Hz, 1H), 2.09−1.78 (m, 4H), 1.78−1.65 (m, 4H), 1.57 (br t, *J* = 12.4 Hz, 1H), 1.49 (dd, *J* = 9.4, 7.0 Hz, 2H), 1.44−1.33 (m, 1H), 1.26 (t, *J* = 7.4 Hz, 3H); HPLC t_r = 9.14 min (method D); LC/MS M^{+1} = 348.3.

*((1R,3S)-1-Amino-3-((R)-6-((2-methoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**19**).*[29] To a stirring mixture of 2-methoxyethanol (0.043 mL, 0.549 mmol) and a 1 N THF solution of potassium *tert*-butoxide (0.44 mL, 0.44 mmol) was added ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 70 °C for 1.5 h and at rt for 3 days. The mixture was concentrated in vacuo. The residue was mixed with 2 N aqueous sodium hydroxide (0.5 mL, 1.000 mmol) and dioxane (0.5 mL) and then heated at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 × 150 mm, 5 μm particles; guard column: Waters XBridge C18, 19 × 10 mm, 5 μm particles; mobile phase A: 5:95 acetonitrile/water with 10 mM ammonium acetate; mobile phase B: 95:5 acetonitrile/water with 10 mM ammonium acetate; gradient: 0−100% B over 15 min, then a 5 min hold at 100% B; flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((2-methoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (13.3 mg, 71% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.98 (s, 2H), 6.96 (s, 1H), 3.56−3.44 (m, 6H), 3.30 (br s, 2H), 3.26 (s, 3H), 2.99−2.88 (m, 1H), 2.81−2.67 (m, 3H), 2.36 (dd, *J* = 16.3, 10.4 Hz, 1H), 2.12 (dd, *J* = 12.9, 7.9 Hz, 1H), 1.99−1.83 (m, 3H), 1.86−1.73 (m, 1H), 1.71−1.63 (m, 1H), 1.62−1.53 (m, 1H), 1.42 (dd, *J* = 12.9, 11.4 Hz, 1H), 1.33 (dtd, *J* = 12.3, 10.7, 6.9 Hz, 1H); LC/MS M^{+1} = 333.6; HPLC t_r = 1.19 (method E).

*((1R,3S)-1-Amino-3-((R)-6-((but-3-en-1-yloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**20**).*[29] To a mixture of but-3-en-1-ol (32 mg, 0.44 mmol) in dry DMF (2 mL) at 0 °C was added 1 N THF solution of potassium *tert*-butoxide (440 μL, 0.44 mmol). After stirring at rt for 30 min, ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 40 mg, 0.088 mmol) in DMF (2 mL) was added and the reaction mixture was stirred at rt for 2 h. The reaction mixture was quenched with water (5 mL) at 0 °C, then taken up in EtOAc (30 mL), and washed with saturated $NaHCO_3$ (3 × 20 mL). The organic layer was dried (Na_2SO_4) and concentrated under vacuo. The resulting residue was dissolved in dioxane (1.5 mL) and water (0.5 mL), and then lithium hydroxide hydrate (37 mg, 0.88 mmol) was added. The reaction mixture was stirred at 100 °C for 16 h. The mixture was concentrated and purified with preparative HPLC. HPLC conditions: Phenomenex Luna C18 5μ 21.2 × 100 mm. Solvent A: 10% MeOH−90% H_2O−0.1% TFA; solvent B: 90% MeOH−10% H_2O−0.1% TFA. Gradient time = 15 min. Start B = 0%, final B 100%. Stop time 20 min. The collected fraction was basified with saturated $NaHCO_3$, concentrated under vacuo, and the aqueous layer was extracted with DCM (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((but-3-en-1-yloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)-methanol (15 mg, 47% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.05−6.93 (m, 3H), 5.88 (ddt, *J* = 17.2, 10.3, 6.8 Hz, 1H), 5.11 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.04 (ddt, *J* = 10.3, 2.1, 1.2 Hz, 1H), 3.58−3.44 (m, 4H), 3.43 (d, *J* = 6.4 Hz, 2H), 3.03 (tt, *J* = 11.1, 7.2 Hz, 1H), 2.88−2.73 (m, 3H), 2.44 (dd, *J* = 16.5, 10.3 Hz, 1H), 2.36 (qt, *J* = 6.7, 1.3 Hz, 2H), 2.23 (br dd, *J* = 13.1, 7.4 Hz, 1H), 2.11−1.97 (m, 3H), 1.97−1.85 (m, 1H), 1.85−1.68 (m, 2H), 1.56 (br t, *J* = 12.3 Hz, 1H), 1.44 (dtd, *J* = 12.8, 10.7, 6.7 Hz, 1H); LC/MS M^{+1} = 330.3; HPLC t_r = 6.62 (method D).

*((1R,3S)-1-Amino-3-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**21a, 21b**).*[29] Sodium metal (120 mg, 5.3 mmol) was added to MeOH (2 mL). The reaction mixture was stirred until the metal was dissolved and then 2-(6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (**diastereomeric-40**, 125 mg, 0.27 mmol) was added and the reaction was stirred overnight. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was dried with $MgSO_4$, filtered, and concentrated and then purified using HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford (5*R*,7*S*)-7-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (30 mg, 0.09 mmol, 30% yield). This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: Chiral OD-H 25 × 3 cm ID, 5 μm; flow rate: 85.0 mL/min; mobile phase: 65/35 CO_2/MeOH.

Peak 2: isomer a: recovered (5*R*,7*S*)-7-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (13 mg, 0.039 mmol, 15% yield).

Peak 1: isomer b: recovered (5*R*,7*S*)-7-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (12 mg, 0.036 mmol, 14% yield). Each isomer was taken individually into the next step.

To a mixture of (5*R*,7*S*)-7-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 12 mg, 0.036 mmol) in DMSO (0.5 mL) and MeOH (1 mL) was added 1 N NaOH. The reaction mixture was heated at 95 °C for 2 h, and then it was cooled and acidified with TFA. Purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**21a**, 10 mg, 0.023 mmol, 62% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.03−6.97 (m, 3H), 3.70−3.58 (m, 2H), 3.55 (t, *J* = 6.6 Hz, 2H), 3.37 (s, 3H), 3.18−3.04 (m, 1H), 2.90−2.75 (m, 3H), 2.49−2.33 (m, 2H), 2.20−2.05 (m, 1H), 2.03−1.79 (m, 5H), 1.73 (t, *J* = 12.8 Hz, 1H), 1.65 (qd, *J* = 6.7, 2.8 Hz, 2H), 1.49−1.35 (m, 1H); LC/MS M^{+1} = 304; HPLC t_r = 5.57 (method D).

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To a mixture of (5*R*,7*S*)-7-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer b, 13 mg, 0.039 mmol) in DMSO (0.5 mL) and MeOH (1 mL) was added 1 N NaOH. The reaction mixture was heated at 95 °C, then cooled, and acidified with TFA, and the reaction mixture was purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20%−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(2-methoxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)-methanol, TFA (**21b**, 10 mg, 0.022 mmol, 56.5% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.02−6.97 (m, 3H), 3.71−3.58 (m, 2H), 3.55 (t, J = 6.6 Hz, 2H), 3.37 (s, 3H), 3.21−3.01 (m, 1H), 2.96−2.73 (m, 3H), 2.53−2.32 (m, 2H), 2.23−2.05 (m, 1H), 2.03−1.78 (m, 5H), 1.78−1.56 (m, 3H), 1.42 (dtd, J = 12.8, 10.4, 6.6 Hz, 1H)); LC/MS M^{+1} = 304; HPLC t_r = 5.58 (method D).

*((1R,3S)-1-Amino-3-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**22a, 22b**).*[29] To a mixture of 6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-3,4-dihydronaphthalen-2-yl trifluoromethanesulfonate (**46**, 100 mg, 0.240 mmol), copper(I) iodide (4.6 mg, 0.024 mmol), and bis-(triphenylphosphine)palladium(II) chloride (17 mg, 0.024 mmol) in TEA (3 mL) was added 3-methoxyprop-1-yne (0.10 mL, 1.2 mmol). The reaction mixture was heated at 60 °C for 1 h, cooled to rt, diluted with EtOAc, and washed with 1 M HCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (12 g) using an EtOAc/Hex gradient (0−100% EtOAc over 20 CV) to afford (5*R*,7*S*)-7-(6-(3-methoxyprop-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (30 mg, 0.089 mmol, 37% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.13−7.04 (m, 2H), 7.04−6.94 (m, 1H), 6.75 (s, 1H), 4.41−4.27 (m, 3H), 3.41 (s, 3H), 3.15−2.94 (m, 1H), 2.82 (t, J = 8.1 Hz, 2H), 2.41 (td, J = 8.1, 1.3 Hz, 2H), 2.31 (dd, J = 13.1, 7.2 Hz, 1H), 2.18−2.04 (m, 3H), 2.02−1.89 (m, 2H), 1.88−1.74 (m, 1H).

To a mixture of (5*R*,7*S*)-7-(6-(3-methoxyprop-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (30 mg, 0.089 mmol) in MeOH (5 mL) was added Pearlman's catalyst (6.2 mg, 0.044 mmol). The reaction mixture was evacuated and backfilled with hydrogen and then hydrogenated under balloon pressure for 2 h. The reaction mixture was then evacuated and backfilled with N_2 and the catalyst was filtered away. The residue was purified by HPLC to afford (5*R*,7*S*)-7-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (25 mg). HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: Chiral AD-H 25 × 3 cm ID, 5 mm; flow rate: 85.0 mL/min; mobile phase: 60/40 CO_2/MeOH.

Peak 2: isomer a; recovered (5*R*,7*S*)-7-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (10 mg, 0.029 mmol, 33% yield).

Peak 1: isomer b; recovered (5*R*,7*S*)-7-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (13 mg, 0.038 mmol, 43% yield). Each isomer was taken individually into the next step.

To a mixture of (5*R*,7*S*)-7-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 10 mg, 0.038 mmol) in MeOH (1 mL) and DMSO (0.5 mL) was added 1 N NaOH (0.5 mL). The reaction mixture was heated at 95 °C for 2 h, cooled to rt, acidified with TFA, and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20%−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**22a**, 9 mg, 52% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.02−6.96 (m, 3H), 3.71−3.57 (m, 2H), 3.44 (t, J = 6.5 Hz, 2H), 3.35 (s, 3H), 3.22−3.03 (m, 1H), 2.93−2.72 (m, 3H), 2.49−2.30 (m, 2H), 2.18−2.07 (m, 1H), 2.03−1.86 (m, 4H), 1.81−1.64 (m, 4H), 1.51−1.31 (m, 3H)); LC/MS M^{+1} = 318; HPLC t_r = 6.09 (method D).

To a mixture of (5*R*,7*S*)-7-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer b, 13 mg, 0.038 mmol) in MeOH (1 mL) and DMSO (0.5 mL) was added 1 N NaOH (0.5 mL). The reaction mixture was heated at 95 °C for 2 h, cooled to rt, acidified with TFA, and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(3-methoxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**22b**, 9 mg, 52% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.05−6.92 (m, 3H), 3.73−3.55 (m, 2H), 3.44 (t, J = 6.5 Hz, 2H), 3.35 (s, 3H), 3.12 (d, J = 9.2 Hz, 1H), 2.94−2.73 (m, 3H), 2.52−2.28 (m, 2H), 2.19−2.04 (m, 1H), 2.05−1.87 (m, 4H), 1.80−1.62 (m, 4H), 1.53−1.29 (m, 3H)); LC/MS M^{+1} = 318; HPLC t_r = 6.13 (method D).

*((1R,3S)-1-Amino-3-((R)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**23**).*[29] To a mixture of 6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-3,4-dihydronaphthalen-2-yl trifluoromethanesulfonate (**46**, 150 mg, 0.36 mmol), copper(I) iodide (6.8 mg, 0.036 mmol), and bis(triphenylphosphine)-palladium(II) chloride (25 mg, 0.036 mmol) in TEA (3 mL) was added 5-methoxypent-1-yne (176 mg, 1.8 mmol). The reaction mixture was heated at 60 °C for 1 h, cooled to rt, diluted with EtOAc, and washed with 1 M HCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (24 g) using an EtOAc/Hex gradient (0−100% EtOAc over 13 CV) to afford (5*R*,7*S*)-7-(6-(5-methoxypent-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (105 mg, 0.29 mmol, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04−6.95 (m, 3H), 6.69 (s, 1H), 5.62 (s, 1H), 4.38−4.27 (m, 2H), 3.52 (t, J = 6.2 Hz, 2H), 3.40−3.37 (m, 3H), 3.11−2.98 (m, 1H), 2.83 (t, J = 8.1 Hz, 2H), 2.50 (t, J = 7.2 Hz, 2H), 2.46−2.39 (m, 2H), 2.33 (dd, J = 13.2, 7.3 Hz, 1H), 2.23−2.10 (m, 2H), 2.03−1.91 (m, 2H), 1.90−1.79 (m, 3H).

To a mixture of (5*R*,7*S*)-7-(6-(5-methoxypent-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (105 mg, 0.29 mmol) in MeOH (10 mL) was added Pearlman's catalyst (20 mg, 0.14 mmol) The reaction mixture was evacuated and backfilled with hydrogen and then hydrogenated under balloon pressure for 2 h. The reaction mixture was then evacuated and backfilled with N_2 and the catalyst was filtered away. The residue was purified by HPLC to afford (5*R*,7*S*)-7-(6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one. This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: chiral AD-H 25 × 3 cm ID, 5 mm; flow rate: 85.0 mL/min; mobile phase: 70/30 CO_2/MeOH.

Isomer a: peak 2: recovered (5*R*,7*S*)-7-(6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (38 mg, 0.102 mmol, 36% yield).

Isomer b: peak 1: recovered (5*R*,7*S*)-7-(6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (40 mg, 0.108 mmol, 38% yield).

To a mixture of (5*R*,7*S*)-7-(-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 38 mg, 0.11 mmol) in DMSO (0.5 mL) and MeOH (1 mL) was added 1 N NaOH (0.5 mL). The reaction mixture was heated at 95 °C for 2 h, cooled to rt, acidified with TFA, and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (26 mg, 0.055 mmol, 54% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.02−6.96 (m, 3H), 3.71−3.56 (m, 2H), 3.42 (t, J = 6.6 Hz, 2H), 3.34 (s, 3H), 3.18−3.02 (m, 1H), 2.90−2.71 (m, 3H), 2.49−2.29 (m, 2H), 2.12 (d, J = 2.9 Hz, 1H), 2.02−1.87 (m, 4H), 1.79−1.66 (m,

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2H), 1.66−1.55 (m, 2H), 1.52−1.32 (m, 7H); LC/MS M^{+1} = 346; HPLC t_r = 8.16 (method D).

*((1R,3S)-1-Amino-3-((S)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**24**).*[29] To a stirred mixture of magnesium (0.53 g, 22 mmol) and anhydrous THF (30 mL) was added a few drops of 1,2-dibromoethane at rt. The mixture was stirred for 15 min before a solution of 1-bromo-4-methoxybutane (2.9 mL, 22 mmol) in anhydrous THF (10 mL) was added dropwise to keep the reaction mixture warm and not boiling. After the addition, the mixture was stirred at 60 °C for 3 h and cooled to rt. The solution was separated and added to a stirred mixture of copper(I) bromide (0.63 g, 4.4 mmol), ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**48**, 1 g, 2.2 mmol), and THF (10 mL) at −78 °C. The mixture was stirred at −78 °C for 20 min before the temperature was slowly raised to 0 °C. The mixture was stirred at 0 °C for 3 h and then at rt for 15 h. Saturated aqueous NH_4Cl solution (20 mL) was added dropwise to quench the reaction. Water (10 mL) and hexanes (20 mL) were added to the reaction. The aqueous layer was separated and extracted with EtOAc (2 × 15 mL). The combined organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography purification (40 g silica gel column, gradient elution from 15 to 100% of EtOAc in hexanes) afforded (5*R*,7*S*)-7-((*S*)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one as a white solid (680 mg, 1.83 mmol, 83% yield). LC/MS M^{+1} = 372.3; HPLC t_r = 1.12 (method A).

To a mixture of (5*R*,7*S*)-7-((*S*)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (680 mg, 1.83 mmol) in MeOH (20 mL) and DMSO (5 mL) was added 1 N NaOH (10 mL). The reaction mixture was heated at 95 °C for 12 h, cooled to rt, diluted with EtOAc, and washed with sat NaCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The resulting white solid was suspended in EtOAc and enough MeOH was added to dissolve using a heat gun to warm solution. HCl gas was bubbled through for 10 s, then ether was added, and the solution was allowed to stand in a freezer for 1 h. The solid was collected and dried to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol as the HCl salt (450 mg, 1.3 mmol, 70% yield. ^{1}H NMR (400 MHz, *MeOD*): δ 7.04−6.90 (m, 3H), 3.55−3.38 (m, 4H), 3.34 (s, 3H), 3.00 (tt, *J* = 11.1, 7.3 Hz, 1H), 2.88−2.70 (m, 3H), 2.35 (dd, *J* = 16.2, 10.5 Hz, 1H), 2.20 (dd, *J* = 12.8, 7.3 Hz, 1H), 2.08−1.85 (m, 3H), 1.83−1.49 (m, 6H), 1.49−1.26 (m, 7H); LC/MS M^{+1} = 346.6; HPLC t_r = 8.19 (method D).

*((1R,3S)-1-Amino-3-((S)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methyl Dihydrogen Phosphate (**24-P**).*[29] To a mixture of ((1*R*,3*S*)-1-amino-3-((*S*)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (8.4 mg, 0.024 mmol) in acetonitrile (1 mL) was added pyrophosphoryl chloride (0.135 mL, 0.972 mmol). The reaction was stirred for 1 h and then quenched with water (0.5 mL). The reaction mixture was purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(5-methoxypentyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methyl dihydrogen phosphate (4 mg, 8.93 μmol, 37% yield). HPLC conditions: Phenomenex Luna 5 μm C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20%−100% gradient over 15 min; and 30 mL/min. ^{1}H NMR (400 MHz, *MeOD*): δ 7.06−6.97 (m, 3H), 4.04−3.90 (m, 2H), 3.45 (t, *J* = 6.7 Hz, 2H), 3.35 (s, 3H), 3.22−3.09 (m, 1H), 2.88−2.72 (m, 3H), 2.51 (dd, *J* = 13.5, 6.5 Hz, 1H), 2.34 (dd, *J* = 16.7, 9.9 Hz, 1H), 2.19−2.09 (m, 1H), 2.08−2.01 (m, 2H), 2.01−1.89 (m, 2H), 1.79 (t, *J* = 12.5 Hz, 1H), 1.66 (br dd, *J* = 3.6, 1.7 Hz, 1H), 1.61 (dt, *J* = 14.1, 6.8 Hz, 2H), 1.49−1.32 (m); LC/MS M^{+1} = 426.4; HPLC t_r = 0.82 (method A).

*((1R,3S)-1-Amino-3-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**25a**, **25b**).*[29] To a mixture of 6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-3,4-dihydronaphthalen-2-yl trifluoromethanesulfonate (**46**, 100 mg, 0.240 mmol), copper(I) iodide (4.5 mg, 0.024 mmol), and bis(triphenylphosphine)palladium(II) chloride (17 mg, 0.024 mmol) in TEA (3 mL) was added 6-methoxyhex-1-yne (81 mg, 0.72 mmol). The reaction mixture was heated at 60 °C for 1 h, cooled to rt, diluted with EtOAc, and washed with 1 M HCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (24 g) using an EtOAc/Hex gradient (0−100% EtOAc over 13 CV). Isolated fractions with product were concentrated and dried in vacuo to afford (5*R*,7*S*)-7-(6-(6-methoxyhex-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (35 mg, 0.092 mmol, 39% yield). ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.08−6.91 (m, 3H), 6.68 (s, 1H), 5.87 (s, 1H), 4.48−4.20 (m, 2H), 3.44 (t, *J* = 6.3 Hz, 2H), 3.37 (s, 3H), 3.03 (tt, *J* = 10.5, 7.2 Hz, 1H), 2.82 (t, *J* = 8.1 Hz, 2H), 2.47−2.38 (m, 4H), 2.32 (dd, *J* = 13.4, 7.3 Hz, 1H), 2.21−2.08 (m, 2H), 2.03−1.91 (m, 2H), 1.77−1.60 (m, 5H).

To a mixture of (5*R*,7*S*)-7-(6-(6-methoxyhex-1-yn-1-yl)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (34 mg, 0.090 mmol) in MeOH (5 mL) was added Pearlman's catalyst (6.29 mg, 0.045 mmol). The reaction mixture was evacuated and backfilled with hydrogen and then hydrogenated under balloon pressure for 2 h. The reaction mixture was then evacuated and backfilled with N_2 and the catalyst was filtered away. The residue was purified by HPLC to afford (5*R*,7*S*)-7-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one. This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: chiral AD-H 25 × 3 cm ID, 5 mm; flow rate: 85.0 mL/min; mobile phase: 60/40 CO_2/MeOH.

Peak 2: isomer a; recovered (5*R*,7*S*)-7-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (9 mg, 0.023 mmol, 26% yield).

Peak 1: isomer b; recovered (5*R*,7*S*)-7-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (8 mg, 0.021 mmol, 23% yield. Each isomer was taken individually into the next step.

To a mixture of (5*R*,7*S*)-7-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 9 mg, 0.023 mmol) in DMSO (0.5 mL) and MeOH (0.5 mL) was added 1 N NaOH. The reaction mixture was heated at 95 °C for 2 h, cooled to rt, acidified with TFA, and purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (**25a**, 8 mg, 0.017 mmol, 72% yield). HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. ^{1}H NMR (400 MHz, *MeOD*): δ 7.31−6.80 (m, 3H), 3.73−3.57 (m, 2H), 3.42 (t, *J* = 6.5 Hz, 2H), 3.34 (s, 3H), 3.19−3.02 (m, 1H), 2.93−2.71 (m, 3H), 2.50−2.28 (m, 2H), 2.21−2.05 (m, 1H), 2.03−1.86 (m, 4H), 1.82−1.64 (m, 2H), 1.59 (quin, *J* = 6.8 Hz, 2H), 1.50−1.27 (m, 9H); LC/MS M^{+1} = 360; HPLC t_r = 7.59 (method D).

To a mixture of (5*R*,7*S*)-7-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (8 mg, 0.021 mmol) in DMSO (0.5 mL) and MeOH (0.5 mL) was added 1 N NaOH. The reaction mixture was heated at 95 °C for 2 h, cooled to rt, acidified with TFA, and purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-(6-(6-methoxyhexyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol, TFA (**25b**, 7 mg, 0.015 mmol, 71% yield). HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight. ^{1}H NMR (400 MHz, *MeOD*): δ 7.03−6.95 (m, 3H), 3.72−3.56 (m, 2H), 3.42 (t, *J* = 6.5 Hz, 2H), 3.34 (s, 3H), 3.21−3.00 (m, 1H), 2.92−2.70 (m, 3H), 2.51−2.27 (m, 2H), 2.22−2.04 (m, 1H), 2.03−1.86 (m, 4H), 1.81−1.64 (m, 2H), 1.59 (quin, *J* = 6.8 Hz, 2H), 1.50−1.24 (m, 9H); LC/MS M^{+1} = 360; HPLC t_r = 7.58 (method D).

*((1R,3S)-1-Amino-3-((S)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**26**).*[29] To a stirred mixture of butyl mercaptan (0.029 mL, 0.27 mmol) in a 1 N THF solution of potassium *tert*-butoxide (0.27 mL, 0.27 mmol) was added ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**48**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 70 °C overnight and

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concentrated in vacuo. The residue was mixed with water (0.5 mL), lithium hydroxide monohydrate (46 mg, 1.1 mmol), and dioxane (0.5 mL) and then heated at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were dried (Na_2SO_4), concentrated, and purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-((butylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol, TFA (21 mg, 0.044 mmol, 78% yield). HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (br s, 2H), 7.07−6.90 (m, 3H), 5.63 (t, J = 5.0 Hz, 1H), 3.54−3.42 (m, 2H), 3.39 (s, 1H), 3.35−3.29 (m, 1H), 3.07−2.94 (m, 1H), 2.88 (br dd, J = 16.3, 4.5 Hz, 1H), 2.78−2.69 (m, 2H), 2.57−2.53 (m, 2H), 2.41 (dd, J = 16.3, 10.4 Hz, 1H), 2.25 (dd, J = 13.1, 7.2 Hz, 1H), 2.03−1.91 (m, 2H), 1.91−1.72 (m, 4H), 1.61 (t, J = 12.6 Hz, 1H), 1.57−1.44 (m, 2H), 1.43−1.31 (m, 3H), 0.88 (t, J = 7.4 Hz, 3H); LC/MS M^{+1} = 348.1; HPLC t_r = 1.53 (method E).

*((1R,3S)-1-Amino-3-((S)-6-(2-(propylthio)ethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**27**).*[29] (5*R*,7*S*)-7-((*S*)-6-(2-Hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**40b**, 45 mg, 0.14 mmol) was dissolved in dry pyridine (0.5 mL) and *p*-toluenesulfonyl chloride (82 mg, 0.43 mmol) was added in one portion. The resulting mixture was stirred at rt for 2 h and the solvent was removed in vacuo. The residue was dissolved in DCM and loaded onto the column. Flash chromatography purification using ISCO (4 g of silica gel column, 0−100% EtOAc in DCM) afforded 2-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro-[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (57 mg, 0.121 mmol, 85% yield) as a solid. LC/MS M^{+1} = 470.4; HPLC t_r = 1.04 (method A).

To a stirred mixture of propane-1-thiol (0.017 mL, 0.19 mmol), 2-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (30 mg, 0.064 mmol), and dioxane (1 mL) was added 2 N NaOH (0.096 mL, 0.19 mmol) at 0 °C. The resulting mixture was stirred at 60 °C for 6 h. 2 N aqueous NaOH (0.64 mL, 1.28 mmol) was added and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The desired fractions were basified with 2 N NaOH and extracted with EtOAc. The organic layer was dried and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-(2-(propylthio)ethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (21 mg, 0.057 mmol, 90% yield) as a white solid. ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.02 (s, 2H), 6.99 (s, 1H), 3.55−3.41 (m, 2H), 3.16−2.98 (m, 1H), 2.91−2.76 (m, 3H), 2.69−2.60 (m, 2H), 2.57−2.50 (m, 2H), 2.42 (dd, J = 16.2, 10.6 Hz, 1H), 2.29 (br dd, J = 13.3, 7.8 Hz, 1H), 2.17−2.04 (m, 1H), 2.02−1.80 (m, 4H), 1.71−1.65 (m, 5H), 1.47−1.35 (m, 2H), 1.02 (t, J = 7.3 Hz, 3H); LC/MS M^{+1} = 348.4; HPLC t_r = 9.25 (method D).

*((1R,3S)-1-Amino-3-((R)-6-((pentylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**28**).*[29] To a stirred mixture of pentane-1-thiol (0.020 mL, 0.165 mmol), ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 25 mg, 0.055 mmol), and dioxane (0.4 mL) was added 2 N aqueous NaOH (0.082 mL, 0.165 mmol) at 0 °C. The resulting mixture was stirred at 70 °C for 4 h, cooled to rt, and 2 N NaOH (0.549 mL, 1.098 mmol) was added, and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The desired fractions were basified with 2 N NaOH and extracted with EtOAc. The organic layer was dried and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((pentylthio)-methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (19 mg, 0.050 mmol, 92% yield) as a white solid. ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.02 (d, J = 1.8 Hz, 2H), 6.98 (s, 1H), 3.54−3.40 (m, 2H), 3.11−2.92 (m, 2H), 2.88−2.77 (m, 2H), 2.61−2.43 (m, 5H), 2.28 (dd, J = 13.6, 7.9 Hz, 1H), 2.13−2.02 (m, 2H), 2.00−1.83 (m, 2H), 1.81−1.69 (m, 3H), 1.55−1.44 (m, 3H), 1.43−1.27 (m, 4H), 0.95−0.86 (m, 3H); LC/MS M^{+1} = 362.1; HPLC t_r = 9.66 (method D).

*((1R,3S)-1-Amino-3-((S)-6-((pentylthio)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**29**).*[29] To a stirred mixture of pentane-1-thiol (0.020 mL, 0.165 mmol), ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**48**, 25 mg, 0.055 mmol), and dioxane (0.4 mL) was added 2 N NaOH (0.082 mL, 0.165 mmol) at 0 °C. The resulting mixture was stirred at 70 °C for 4 h, cooled to rt, and 2 N aqueous NaOH (0.549 mL, 1.098 mmol) was added and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL) and the combined EtOAc extracts were dried (Na_2SO_4) and concentrated. The resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The desired fractions were basified with 2 N NaOH and extracted with EtOAc. The organic layer was dried and concentrated to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-((pentylthio)-methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (16 mg, 0.042 mmol, 77% yield) as a white solid. ^{1}H NMR (400 MHz, *MeOD*): δ 7.09−6.84 (m, 3H), 3.56−3.38 (m, 2H), 3.08−2.97 (m, 1H), 2.92 (br dd, J = 16.3, 4.8 Hz, 1H), 2.86−2.71 (m, 2H), 2.59−2.50 (m, 4H), 2.45 (br dd, J = 16.1, 10.1 Hz, 1H), 2.28−2.17 (m, 1H), 2.12−1.96 (m, 2H), 1.95−1.83 (m, 2H), 1.84−1.66 (m, 2H), 1.66−1.50 (m, 3H), 1.50−1.29 (m, 5H), 0.92 (t, J = 7.0 Hz, 3H); LC/MS M^{+1} = 362.2; HPLC t_r = 9.78 (method D).

*((1R,3S)-1-Amino-3-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**30a, 30b**).*[29] A mixture of *n*-hexanol (5 mL, 40.1 mmol), *p*-toluenesulfonic acid monohydrate (4 mg, 0.021 mmol), and trimethoxymethane (0.31 mL, 2.8 mmol) was stirred at 100 °C for 2 h with a slow N_2 stream flowing over the mixture to remove methanol. The residual liquid was mixed with (5*R*,7*S*)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**39**, 200 mg, 0.701 mmol) and stirred at 100 °C for 2 h. The mixture was cooled and then 10% Pd−C (100 mg, 0.094 mmol) was added under N_2, followed by EtOAc (3 mL). The mixture was hydrogenated under a balloon of H_2 for 12 h. The mixture was filtered through a membrane filter and the filtrate was concentrated. Flash chromatography purification (12 g of silica gel column, 5->100% EtOAc in hexanes) afforded (5*R*,7*S*)-7-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (220 mg, 0.59 mmol, 84% yield) as a sticky solid. This product was then separated into individual isomers using the following conditions: Berger SFC MGIII; column: Lux Amylose-2 3 × 250 cm, 5μ; flow rate: 180.0 mL/min; mobile phase: CO_2/(MEOH + 0.2%DEA) = 80/20.

Peak 1: isomer a; recovered (5*R*,7*S*)-7-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (50 mg, 0.029 mmol, 23% yield).

Peak 2: isomer b; recovered (5*R*,7*S*)-7-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (47 mg, 0.038 mmol, 21% yield). Each isomer was taken individually into the next step.

A mixture of (5*R*,7*S*)-7-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 50 mg, 0.136 mmol), lithium hydroxide monohydrate (51 mg, 1.22 mmol), dioxane (1 mL), and water (1 mL) was stirred at 90 °C for 15 h. The mixture was cooled and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The desired fractions were basified with K_2CO_3 and extracted with EtOAc. The organic layer was then dried (Na_2SO_4), filtered, and concentrated to afford ((1*R*,3*S*)-1-amino-3-(6-(hexyloxy)-5,6,7,8-tetrahydronaph-

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thalen-2-yl)cyclopentyl)methanol (**30a**, 47 mg, 0.122 mmol, 90% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.34−6.76 (m, 3H), 3.83−3.68 (m, 1H), 3.61−3.51 (m, 2H), 3.51−3.40 (m, 2H), 3.09−2.95 (m, 2H), 2.94−2.83 (m, 1H), 2.80−2.65 (m, 2H), 2.22 (dd, J = 13.1, 7.6 Hz, 1H), 2.04 (br d, J = 2.6 Hz, 1H), 2.00−1.95 (m, 1H), 1.94−1.63 (m, 4H), 1.63−1.49 (m, 3H), 1.45−1.28 (m, 6H), 0.97−0.82 (m, 3H); LC/MS M^{+1} = 346.3; HPLC t_r = 8.86 (method D).

A mixture of (5*R*,7*S*)-7-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer b, 47 mg, 0.13 mmol), lithium hydroxide monohydrate (51 mg, 1.2 mmol), dioxane (1 mL), and water (1 mL) was stirred at 90 °C for 15 h. The mixture was cooled and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified using reverse-phase HPLC (Phen Luna 5μ 30 × 100 mm (Axia); gradient over 8 min from 30 to 100% of solvent B; solvent A: 10% MeOH: 90% H_2O: 0.1% TFA; solvent B: 90% MeOH, 10% H_2O, 0.1% TFA). The desired fractions were basified with K_2CO_3 and extracted with EtOAc. The organic layer was then dried (Na_2SO_4), filtered, and concentrated to afford ((1*R*,3*S*)-1-amino-3-(6-(hexyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**30b**, 28 mg, 0.122 mmol, 56% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.08−6.95 (m, 3H), 3.83−3.73 (m, 1H), 3.63−3.55 (m, 2H), 3.54−3.42 (m, 2H), 3.09−2.97 (m, 2H), 2.96−2.86 (m, 1H), 2.83−2.65 (m, 2H), 2.22 (dd, J = 13.2, 7.5 Hz, 1H), 2.14−1.89 (m, 3H), 1.88−1.66 (m, 3H), 1.65−1.50 (m, 3H), 1.46−1.26 (m, 6H), 0.97−0.88 (m, 3H); LC/MS M^{+1} = 346.3; HPLC t_r = 8.85 (method D).

*((1R,3S)-1-Amino-3-((R)-6-((pentyloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**31**).*[29] To a stirred mixture of 1-pentanol (0.119 mL, 1.098 mmol) and a 1 N THF solution of potassium *tert*-butoxide (0.549 mL, 0.549 mmol) was added ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 60 °C for 18 h and concentrated in vacuo. The residue was mixed with water (0.5 mL), lithium hydroxide monohydrate (18 μL, 0.66 mmol), and dioxane (1 mL). The resulting mixture was stirred at 100 °C for 7 h, cooled to rt, and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated and the resulting residue was purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((pentyloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (5.5 mg, 0.016 mmol, 29% yield). ^{1}H NMR (500 MHz, *MeOD*): δ 7.05−6.99 (m, 2H), 6.98 (s, 1H), 3.65−3.53 (m, 2H), 3.47 (t, J = 6.7 Hz, 2H), 3.40 (d, J = 6.9 Hz, 2H), 3.10−2.98 (m, 1H), 2.91−2.73 (m, 3H), 2.44 (dd, J = 16.3, 10.4 Hz, 1H), 2.36 (dd, J = 13.1, 6.7 Hz, 1H), 2.08 (br s, 3H), 1.94−1.79 (m, 3H), 1.69 (t, J = 12.6 Hz, 1H), 1.65−1.54 (m, 2H), 1.46−1.38 (m, 1H), 1.37−1.30 (m, 4H), 0.98−0.84 (m, 3H); LC/MS M^{+1} = 346.2; HPLC t_r = 1.87 (method E).

*((1R,3S)-1-Amino-3-((S)-6-((pentyloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**32**).*[29] To a stirred mixture of 1-pentanol (0.12 mL, 1.1 mmol) and a 1 N THF solution of potassium *tert*-butoxide (0.55 mL, 0.55 mmol) was added ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**48**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 60 °C for 18 h, concentrated in vacuo, and the resulting residue was mixed with water (0.5 mL), lithium hydroxide monohydrate (18 μL, 0.66 mmol), and dioxane (1 mL). The resulting mixture was stirred at 100 °C for 7 h, cooled to rt, and extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated and the resulting residue was purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*S*)-6-((pentyloxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (10.6 mg, 0.031 mmol, 56% yield). ^{1}H NMR (500 MHz, *MeOD*): δ 7.04−6.99 (m, 2H), 6.99−6.95 (m, 1H), 3.62−3.51 (m, 2H), 3.47 (t, J = 6.7 Hz, 2H), 3.40 (d, J = 6.4 Hz, 2H), 3.11−2.97 (m, 1H), 2.92−2.76 (m, 3H), 2.44 (dd, J = 16.1, 10.7 Hz, 1H), 2.34 (dd, J = 13.4, 6.4 Hz, 1H), 2.15−1.96 (m, 3H), 1.96−1.91 (m, 2H), 1.91−1.78 (m, 2H), 1.71−1.55 (m, 3H), 1.50−1.39 (m, 1H), 1.38−1.33 (m, 3H), 0.97−0.87 (m, 3H); LC/MS M^{+1} = 346.2; HPLC t_r = 1.86 (method E).

*((1R,3S)-1-Amino-3-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**33a**, **33b**).*[29] To a mixture of (5*R*,7*S*)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**39**, 100 mg, 0.350 mmol) and 1-heptanol (500 μL, 3.54 mmol) in toluene (2 mL) was added *p*-toluenesulfonic acid monohydrate (5 mg, 0.026 mmol). Oven dried 3A molecular sieves were added and the mixture was heated at reflux overnight. The reaction mixture was cooled to rt, diluted with EtOAc, and washed with saturated NaCl. The organic layer was dried $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified on a silica gel cartridge (40 g) using an EtOAc/Hex gradient (0−100% EtOAc over 20 min) to afford (5*R*,7*S*)-7-(6-(heptyloxy)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (55 mg, 0.14 mmol, 41% yield). LC/MS M^{+1} = 384.4; HPLC t_r = 1.26 (method A).

To a mixture of (5*R*,7*S*)-7-(6-(heptyloxy)-7,8-dihydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (53 mg, 0.138 mmol) in MeOH (10 mL) was added Pearlman's catalyst (19 mg, 0.14 mmol). The reaction mixture was hydrogenated under a balloon of H_2 for 2 h. The catalyst was filtered away and the mixture was concentrated in vacuo. This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: chiral As−H 25 × 3 cm ID, 5μ; flow rate: 85.0 mL/min; mobile phase: 70/30 CO_2/MeOH.

Peak 2: isomer a; recovered (5*R*,7*S*)-7-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (9 mg, 17% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09−7.03 (m, 1H), 7.01−6.92 (m, 2H), 5.29 (br s, 1H), 4.40−4.26 (m, 2H), 3.78−3.67 (m, 1H), 3.62−3.48 (m, 2H), 3.15−2.99 (m, 2H), 2.99−2.86 (m, 1H), 2.85−2.68 (m, 2H), 2.33 (dd, J = 13.2, 7.3 Hz, 1H), 2.24−2.04 (m, 3H), 1.96 (dd, J = 13.1, 10.9 Hz, 2H), 1.89−1.74 (m, 2H), 1.61 (quin, J = 6.9 Hz, 4H), 1.35−1.29 (m, 6H), 0.93−0.87 (m, 3H).

Peak 3: isomer b; recovered (5*R*,7*S*)-7-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (9 mg, 17% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09−7.03 (m, 1H), 7.01−6.92 (m, 2H), 5.25 (s, 1H), 4.40−4.25 (m, 2H), 3.80−3.66 (m, 1H), 3.59−3.50 (m, 2H), 3.15−2.99 (m, 2H), 2.98−2.88 (m, 1H), 2.84−2.71 (m, 2H), 2.33 (dd, J = 13.2, 7.3 Hz, 1H), 2.21−2.03 (m, 3H), 2.03−1.91 (m, 2H), 1.90−1.73 (m, 2H), 1.60 (q, J = 7.0 Hz, 4H), 1.36−1.29 (m, 6H), 0.95−0.87 (m, 3H).

To a mixture of (5*R*,7*S*)-7-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 9 mg, 0.023 mmol) in dioxane (4 mL) was added 1 N NaOH. The reaction mixture was heated at 100 °C overnight, cooled to rt, acidified with TFA, and concentrated in vacuo. The residue was then filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20%−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol, TFA (5 mg, 10 μmol, 45% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.03 (s, 2H), 7.00 (s, 1H), 3.83−3.73 (m, 1H), 3.70−3.61 (m, 2H), 3.61−3.50 (m, 2H), 3.18−3.08 (m, 1H), 3.04 (dd, J = 16.4, 4.7 Hz, 1H), 2.97−2.84 (m, 1H), 2.81−2.66 (m, 2H), 2.42 (ddd, J = 13.4, 7.1, 1.1 Hz, 1H), 2.19−2.01 (m, 2H), 2.00−1.89 (m, 3H), 1.88−1.77 (m, 1H), 1.73 (t, J = 12.8 Hz, 1H), 1.59 (quin, J = 6.9 Hz, 2H), 1.44−1.23 (m, 8H), 0.97−0.87 (m, 3H); LC/MS M^{+1} = 360.2; HPLC t_r = 8.34 (method D).

To a mixture of (5*R*,7*S*)-7-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer b, 8 mg, 0.021 mmol) in dioxane (4 mL) was added 1 N NaOH. The reaction mixture was heated at 100 °C overnight, cooled to rt, acidified with TFA, and concentrated in vacuo. The residue was filtered and purified by HPLC. HPLC conditions: Phenomenex Luna 5μ C18 column (30 × 100 mm); MeCN (0.1% TFA)/water (0.1% TFA); 20−100% gradient over 15 min; and 30 mL/min. Isolated fractions with the correct mass were freeze-dried overnight to afford ((1*R*,3*S*)-1-amino-3-(6-(heptyloxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)-methanol (5 mg, 0.014 mmol, 60% yield). ^{1}H NMR (400 MHz,

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MeOD): δ 7.07−6.98 (m, 3H), 3.83−3.73 (m, 1H), 3.71−3.51 (m, 4H), 3.18−3.08 (m, 1H), 3.04 (dd, *J* = 16.6, 5.0 Hz, 1H), 2.96−2.85 (m, 1H), 2.82−2.68 (m, 2H), 2.42 (ddd, *J* = 13.3, 7.1, 1.2 Hz, 1H), 2.17−2.01 (m, 2H), 2.00−1.89 (m, 3H), 1.88−1.77 (m, 1H), 1.73 (t, *J* = 12.8 Hz, 1H), 1.59 (quin, *J* = 6.9 Hz, 2H), 1.45−1.22 (m, 8H), 0.96−0.86 (m, 3H); LC/MS M^{+1} = 360.2; HPLC t_r = 8.34 (method D).

*((1R,3S)-1-Amino-3-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**34a, 34b**).*[29] To a solution of 2-butoxyethanol (920 μL, 7.01 mmol) was added trimethyl orthoformate (310 μL, 2.8 mmol) and the mixture was stirred at 100 °C for 2 h with the cap open. The reaction mixture was added to (5*R*,7*S*)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**39**, 200 mg, 0.701 mmol) in THF (2 mL), followed by 2-butoxyethanol (920 μl, 7.0 mmol), *p*-toluenesulfonic acid monohydrate (10.00 mg, 0.053 mmol), and molecular sieves. The reaction mixture was stirred at 110 °C for 16 h. After cooling, 10% Pd−C (200 mg) and EtOAc (5 mL) were added. The reaction mixture was stirred under H_2 for 16 h and then poured onto ice, diluted with DCM, and washed with 1 N HCl. The organic layer was collected, dried over Na_2SO_4, and concentrated to afford (5*R*,7*S*)-7-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (140 mg, 0.36 mmol, 52% yield), LC/MS M^{+1} = 388.3; HPLC t_r = 1.05 (method A). This product was then separated into individual isomers using the following conditions: Berger SFC MGII; column: Lux-Cellulose-4 25 × 3 cm, 5 μm; flow rate: 120 mL/min; mobile phase: 65/35 CO_2/MeOH.

Peak 2: isomer a; (5*R*,7*S*)-7-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (60 mg).

Peak 1: isomer b; (5*R*,7*S*)-7-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (60 mg).

Each isomer was taken individually into the next step.

To a solution of (5*R*,7*S*)-7-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer a, 60 mg, 0.155 mmol) in dioxane (3 mL) and water (1 mL) was added lithium hydroxide (37 mg, 1.55 mmol) and the reaction mixture was stirred at 100 °C for 16 h. The reaction mixture was diluted with water and extracted with EtOAc (2x). The organic layer was collected, dried over Na_2SO_4, and concentrated to afford ((1*R*,3*S*)-1-amino-3-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)-methanol (40 mg, 0.105 mmol, 67.9% yield). ^{1}H NMR (400 MHz, *MeOD*): δ 7.06−6.95 (m, 3H), 3.88−3.79 (m, 1H), 3.77−3.66 (m, 2H), 3.64−3.56 (m, 2H), 3.56−3.45 (m, 4H), 3.10−2.98 (m, 2H), 2.98−2.87 (m, 1H), 2.83−2.69 (m, 2H), 2.26 (dd, *J* = 13.2, 7.5 Hz, 1H), 2.13−1.99 (m, 2H), 1.99−1.89 (m, 1H), 1.89−1.70 (m, 3H), 1.65−1.51 (m, 3H), 1.47−1.33 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H); LC/MS M^{+1} = 362.2; HPLC t_r = 7.46 (method D).

To a solution of (5*R*,7*S*)-7-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (isomer b, 60 mg, 0.155 mmol) in dioxane (3 mL) and water (1 mL) was added lithium hydroxide (37 mg, 1.55 mmol), and the reaction mixture was stirred at 100 °C for 16 h. The reaction mixture was diluted with water and extracted with EtOAc (2×). The organic layer was collected, dried over Na_2SO_4, and concentrated to afford ((1*R*,3*S*)-1-amino-3-(6-(2-butoxyethoxy)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol, TFA. ^{1}H NMR (400 MHz, *MeOD*): δ 7.04−6.96 (m, 3H), 3.89−3.79 (m, 1H), 3.77−3.67 (m, 2H), 3.63−3.57 (m, 2H), 3.55−3.42 (m, 4H), 3.11−2.98 (m, 2H), 2.97−2.86 (m, 1H), 2.81−2.69 (m, 2H), 2.26−2.16 (m, 1H), 2.13−2.03 (m, 1H), 2.03−1.95 (m, 1H), 1.95−1.88 (m, 1H), 1.88−1.74 (m, 2H), 1.73−1.64 (m, 1H), 1.60−1.50 (m, 3H), 1.46−1.33 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H); LC/MS M^{+1} = 362.2; HPLC t_r = 7.47 (method D).

*((1R,3S)-1-Amino-3-((R)-6-((2-propoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**35**).*[29] To a stirred mixture of 2-propoxyethanol (0.063 mL, 0.55 mmol) and 1 N THF solution of potassium *tert*-butoxide (0.44 mL, 0.44 mmol) was added ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate[17a] (**42**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 70 °C for 1.5 h and at rt for 3 days before being concentrated in vacuo. The residue was mixed with 2 N aqueous sodium hydroxide (0.5 mL, 1.000 mmol) and dioxane (0.5 mL), and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((2-propoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-cyclopentyl)methanol (9 mg, 44% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.03−6.92 (m, 3H), 3.36−3.28 (m, 11H), 2.97−2.88 (m, 1H), 2.81−2.68 (m, 3H), 2.36 (br dd, *J* = 15.6, 11.1 Hz, 1H), 2.11 (br dd, *J* = 12.6, 8.2 Hz, 1H), 1.97−1.76 (m, 6H), 1.70−1.55 (m, 2H), 1.55−1.46 (m, 2H), 1.46−1.39 (m, 1H), 1.33 (dt, *J* = 10.3, 5.0 Hz, 1H), 0.93−0.77 (m, 3H); LC/MS M^{+1} = 362.3; HPLC t_r = 1.48 (method E).

*((1R,3S)-1-Amino-3-((S)-6-((2-propoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (**36**).*[29] To a stirred mixture of 2-propoxyethanol (0.063 mL, 0.55 mmol) and 1 N THF solution of potassium *tert*-butoxide (0.55 mL, 0.55 mmol) was added ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate[17a] (**48**, 25 mg, 0.055 mmol). The resulting mixture was stirred at 70 °C for 1.5 h. The mixture was concentrated in vacuo. The residue was mixed with 2 N aqueous sodium hydroxide (0.5 mL, 1 mmol) and dioxane (0.5 mL), and the resulting mixture was stirred at 90 °C overnight. The mixture was extracted with EtOAc (4 × 1 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by HPLC to afford ((1*R*,3*S*)-1-amino-3-((*R*)-6-((2-propoxyethoxy)methyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (9 mg, 44% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.02−6.94 (m, 3H), 5.52 (br s, 1H), 3.51 (br dd, *J* = 6.9, 2.5 Hz, 4H), 3.06−2.94 (m, 1H), 2.80−2.70 (m, 7H), 2.64 (br s, 3H), 2.41−2.32 (m, 2H), 2.23 (dd, *J* = 13.1, 6.7 Hz, 1H), 2.03−1.71 (m, 6H), 1.58 (br t, *J* = 12.9 Hz, 1H), 1.51 (sxt, *J* = 7.1 Hz, 2H), 1.40−1.28 (m, 1H), 0.87 (t, *J* = 7.4 Hz, 3H); LC/MS M^{+1} = 362.3; HPLC t_r = 1.46 (method E).

*(R)-6-((5R,7S)-2-Oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalene-2-carbaldehyde (**38**).* A solution of oxalyl chloride (260 μL, 3 mmol) in DCM (5 mL) was stirred for 30 min at rt then cooled to −78 °C. DMSO (420 μL, 6 mmol) was added dropwise and stirred for 1 h at that temperature. Next, a solution of (5*R*,7*S*)-7-((*R*)-6-(hydroxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**37**, 600 mg, 2 mmol) in DCM (3 mL)/DMSO(1 mL) was added dropwise. The mixture was stirred for 30 min at the same temperature. TEA (1.1 mL, 8 mmol) was added dropwise and the mixture was stirred for 15 min, then warmed to rt, and stirred for another 15 min. The reaction mixture was quenched with water (1 mL) at 0 °C, diluted with EtOAc (50 mL), washed with saturated NH_4Cl (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude material was purified on a silica gel cartridge using an EtOAc/Hex gradient (0−100% EtOAc over 12 column volumes) to afford (*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro-[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalene-2-carbaldehyde (500 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.80 (d, *J* = 0.9 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 7.04−6.96 (m, 1H), 6.94 (s, 1H), 5.48 (br s, 1H), 4.38−4.24 (m, 2H), 3.10−2.94 (m, 3H), 2.92−2.81 (m, 2H), 2.77−2.62 (m, 1H), 2.32 (dd, *J* = 13.2, 7.3 Hz, 1H), 2.27−2.19 (m, 1H), 2.17−2.05 (m, 2H), 2.04−1.91 (m, 2H), 1.88−1.72 (m, 2H); LC/MS M^{+1} = 300.1; HPLC t_r = 0.81 (method A).

*(5R,7S)-7-((R)-6-(2-Hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**40**).* To a mixture of triethyl phosphonoacetate (1.68 mL, 8.4 mmol) in THF (3 mL) at 0 °C was added sodium hydride (0.34 g, 8.4 mmol) portionwise. The reaction mixture was stirred for 30 min and then (5*R*,7*S*)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one[17a] (**39**, 1 g, 3.5 mmol) was added. The reaction was allowed to warm to rt and stirred for 3 h. The reaction mixture was diluted with EtOAc and washed with saturated NaCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (40 g) using an EtOAc/Hex gradient (0−100% EtOAc over 12 CV). Isolated fractions with the desired

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product were concentrated in vacuo. This product was dissolved in MeOH (10 mL) and Pearlman's catalyst (0.098 g, 0.70 mmol) was added. The mixture was hydrogenated under a balloon of hydrogen for 12 h. The catalyst was filtered and the mixture was concentrated in vacuo to afford ethyl 2-(6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]-nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)acetate (1 g, 2.8 mmol, 80% yield). LC/MS M^{+1} = 358.4; HPLC t_r = 0.96 (method A).

To a mixture of ethyl 2-(6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]-nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)acetate (650 mg, 1.82 mmol) in THF (10 mL) was added lithium borohydride in THF (5.5 mL, 5.5 mmol). The reaction mixture was heated at 80 °C overnight, and then quenched with water and 1 N HCl. The reaction mixture was extracted with EtOAc (2×), dried with $MgSO_4$, filtered, and concentrated. The crude material was purified on a silica gel cartridge (40 g) using an MeOH/DCM gradient (0−10% MeOH over 15 CV) to afford (5*R*,7*S*)-7-(-6-(2-hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**diastereomeric-40**), which was then separated into the individual isomers under SFC conditions: Column: chiral OD-H 25 × 3 cm ID, 5 mm; flow rate: 85.0 mL/min, mobile phase: 65/35 CO_2/MeOH.

Peak 1: recovered (5*R*,7*S*)-7-((*R*)-6-(2-hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**40**, 180 mg, 0.57 mmol, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06−7.00 (m, 1H), 7.00−6.91 (m, 2H), 5.91 (s, 1H), 4.38−4.25 (m, 2H), 3.81 (t, J = 6.8 Hz, 2H), 3.12−2.95 (m, 1H), 2.93−2.75 (m, 3H), 2.45 (dd, J = 16.2, 10.5 Hz, 1H), 2.31 (dd, J = 13.3, 7.2 Hz, 1H), 2.21−2.07 (m, 2H), 2.05−1.77 (m, 5H), 1.67 (qd, J = 6.7, 2.0 Hz, 2H), 1.53−1.35 (m, 2H).

Peak 2: recovered (5*R*,7*S*)-7-((*S*)-6-(2-hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (181 mg, 0.57 mmol, 32% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07−7.01 (m, 1H), 6.99−6.91 (m, 2H), 5.90 (br s, 1H), 4.39−4.24 (m, 2H), 3.87−3.76 (m, 2H), 3.10−2.95 (m, 1H), 2.91−2.76 (m, 3H), 2.45 (dd, J = 16.2, 10.5 Hz, 1H), 2.31 (dd, J = 13.2, 7.3 Hz, 1H), 2.21−2.06 (m, 2H), 1.99−1.81 (m, 4H), 1.78−1.61 (m, 3H), 1.54−1.36 (m, 2H).

Stereochemistry was assigned by converting the peak 2 material to a compound with known stereochemistry (see the Supporting Information for chiral HPLC traces).

*4-((R)-6-((5R,7S)-2-Oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)butanal (**41**).* To a mixture of (5*R*,7*S*)-7-((*R*)-6-(2-hydroxyethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (56 mg, 0.18 mmol) in pyridine (3 mL) was added *p*-toluenesulfonyl chloride (135 mg, 0.71 mmol), and the reaction mixture was stirred for 2 h. The analysis indicated that the reaction was incomplete, so more *p*-toluenesulfonyl chloride (135 mg, 0.71 mmol) was added, and the mixture was stirred for 1 more hour. The reaction mixture was concentrated. The crude material was purified on a silica gel cartridge (40 g) using an EtOAc/Hex gradient (0−100% EtOAc over 20 CV) to afford 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (68 mg, 0.145 mmol, 82% yield). LC/MS M^{+1} = 470.4; HPLC t_r = 1.04 (method A).

To a mixture of 2-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]-nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (65 mg, 0.138 mmol) and copper(I) bromide (40 mg, 0.28 mmol) in THF (3 mL) at −78 °C was added allylmagnesium bromide (2.8 mL, 2.8 mmol). The reaction was slowly allowed to warm to rt and stirred overnight. The reaction mixture was diluted with EtOAc and washed with saturated NaCl. The organic layer was dried with $MgSO_4$, filtered, and concentrated to afford (5*R*,7*S*)-7-((*R*)-6-(pent-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (45 mg, 0.133 mmol, 96% yield). LC/MS M^{+1} = 340.4; HPLC t_r = 1.18 (method A).

To a clear solution of (5*R*,7*S*)-7-((*R*)-6-(pent-4-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (47 mg, 0.138 mmol) in THF (1.5 mL) were sequentially added 50% NMO (0.065 mL, 0.28 mmol) and osmium tetroxide in ^{t}BuOH (0.052 mL, 4.2 μmol) at rt. The solution was vigorously stirred at rt overnight, and then sodium periodate (118 mg, 0.55 mmol) in water (1 mL) was added and the mixture was stirred vigorously at rt for 30 min. The mixture was extracted with EtOAc (3 × 2 mL). The combined EtOAc extracts were dried (Na_2SO_4), concentrated, and purified using flash chromatography to afford 4-((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)butanal (27 mg, 0.079 mmol, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.82 (t, J = 1.7 Hz, 1H), 7.07−7.00 (m, 1H), 6.99−6.89 (m, 2H), 5.55 (s, 1H), 4.39−4.23 (m, 2H), 3.08−2.98 (m, 1H), 2.90−2.74 (m, 3H), 2.49 (td, J = 7.3, 1.7 Hz, 2H), 2.46−2.29 (m, 2H), 2.22−2.07 (m, 2H), 2.01−1.90 (m, 3H), 1.89−1.69 (m, 4H), 1.49−1.35 (m, 3H); LC/MS M^{+1} = 342.3; HPLC t_r = 0.92 (method A).

*(5R,7S)-7-((R)-6-(Hydroxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**42**).* (5*R*,7*S*)-7-((*R*)-6-(hydroxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro-[4.4]nonan-2-one[17a] (**37**, 690 mg, 2.289 mmol) was dissolved in pyridine (5 mL) and *p*-toluenesulfonyl chloride (1.3 g, 6.9 mmol) was added in one portion. The resulting mixture was stirred at rt for 4 h. The solvent was removed in vacuo, and the crude material was purified on a silica gel cartridge using an EtOAc/Hex gradient (20−100% EtOAc over 12 column volumes) to afford ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 860 mg, 1.9 mmol, 82% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89−7.79 (m, 2H), 7.40 (s, 2H), 6.99 (br s, 3H), 6.45−6.32 (m, 1H), 4.42−4.23 (m, 2H), 4.07−3.96 (m, 2H), 3.08−2.93 (m, 1H), 2.90−2.70 (m, 3H), 2.51−2.48 (m, 3H), 2.46−2.38 (m, 1H), 2.36−2.25 (m, 1H), 2.21−2.07 (m, 3H), 2.03−1.89 (m, 3H), 1.87−1.74 (m, 1H), 1.52−1.37 (m, 1H); MS $(M + H)^+$ at m/z 456; HPLC t_r = 2.01 min (method A).

*(5R,7S)-7-((R)-6-(But-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**43**).* ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)-methyl 4-methylbenzenesulfonate (**42**, 5 g, 11 mmol) was dissolved in THF (80 mL) and copper(I) bromide (3.15 g, 22 mmol) was added. The mixture was stirred at rt for 30 min and then cooled to −78 °C. Allylmagnesium bromide (55 mL, 55 mmol) was added dropwise over a period of 30 min. The reaction mixture was gradually warmed to rt and stirred overnight. The reaction was quenched by adding aqueous NH_4Cl and then extracted with EtOAc. The organic layers were combined and concentrated. The residue was purified on a silica gel cartridge using an MeOH/DCM gradient to afford (5*R*,7*S*)-7-((*R*)-6-(but-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro-[4.4]nonan-2-one (2.7 g, 8.3 mmol, 76% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07−7.02 (m, 1H), 6.98−6.91 (m, 2H), 5.87 (ddt, J = 17.0, 10.3, 6.6 Hz, 1H), 5.12 (br s, 1H), 5.09−5.03 (m, 1H), 4.98 (ddt, J = 10.2, 2.1, 1.2 Hz, 1H), 4.40−4.26 (m, 2H), 3.14−2.98 (m, 1H), 2.92−2.74 (m, 3H), 2.52−2.28 (m, 2H), 2.25−2.07 (m, 4H), 2.02−1.90 (m, 3H), 1.89−1.68 (m, 2H), 1.54−1.34 (m, 3H); LC/MS M^{+1} = 326.0; HPLC t_r = 1.25 (method A).

*3-((S)-6-((5R,7S)-2-Oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propanal (**44**).* To a solution of ((*R*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**42**, 1.2 g, 2.6 mmol) and copper(I) bromide-dimethyl sulfide complex (1.63 g, 7.90 mmol) in ether (50 mL) was added 1 M allylmagnesium bromide in ether (40 mL, 40 mmol). The reaction mixture was stirred at rt for 16 h. The reaction was diluted with saturated NH_4Cl and water and then extracted with EtOAc. The organic layer was collected, dried over Na_2SO_4, and concentrated in vacuo. The crude material was purified on a silica gel cartridge (40 g) using an EtOAc/Hex gradient (0−100% EtOAc over 13 CV) to afford (5*R*,7*S*)-7-((*R*)-6-(but-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro-[4.4]nonan-2-one (560 mg, 1.71 mmol, 65% yield), LC/MS M^{+1} = 326.

To a clear solution of (5*R*,7*S*)-7-((*R*)-6-(but-3-en-1-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (560 mg, 1.72 mmol) in THF (30 mL) was sequentially added 50% NMO (403 mg, 3.44 mmol) and osmium tetroxide in ^{t}BuOH (0.65 mL, 0.052 mmol). The solution was vigorously stirred overnight.

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Then, sodium periodate (1.47 g, 6.9 mmol) in water (15 mL) was added and the mixture was stirred vigorously at rt under N_2 for 30 min. The mixture was extracted with EtOAc (3 × 2 mL). The combined EtOAc extracts were dried (Na_2SO_4), concentrated, and purified using silica gel chromatography purification (20−100% of EtOAc in hexanes) to afford 3-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propanal (440 mg, 1.34 mmol, 78% yield). ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 9.84 (t, *J* = 1.7 Hz, 1H), 7.07−7.01 (m, 1H), 6.99−6.95 (m, 1H), 6.94 (s, 1H), 5.30 (br s, 1H), 4.39−4.25 (m, 2H), 3.12−2.96 (m, 1H), 2.91−2.78 (m, 3H), 2.57 (td, *J* = 7.4, 1.8 Hz, 2H), 2.43 (br dd, *J* = 16.3, 9.5 Hz, 1H), 2.33 (dd, *J* = 13.4, 7.3 Hz, 1H), 2.21−2.09 (m, 2H), 2.03−1.92 (m, 3H), 1.89−1.79 (m, 1H), 1.79−1.68 (m, 3H), 1.51−1.38 (m, 1H); LC/MS M^{+1} = 328.2; HPLC t_r = 0.91 (method A).

*3-((S)-6-((5R,7S)-2-Oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propyl 4-Methylbenzenesulfonate (**45**).* To a stirred solution of 3-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propanal (42 mg, 0.128 mmol) in 100% ethanol (2 mL) and DCM (0.5 mL) was added $NaBH_4$ (5 mg, 0.13 mmol). The mixture was stirred at rt for 1 h. The mixture was concentrated. The residue was quenched with saturated aqueous NH_4Cl solution (1 mL) and water (1 mL) and then extracted with EtOAc (4 mL, 2 × 1 mL). The combined organic solutions were dried over sodium sulfate and concentrated under reduced pressure to afford (5*R*,7*S*)-7-((*S*)-6-(3-hydroxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (42 mg, 0.127 mmol, 99% yield) as a white solid. LC/MS M^{+1} = 330.3; HPLC t_r = 0.87 (method A).

(5*R*,7*S*)-7-((*S*)-6-(3-hydroxypropyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (42 mg, 0.13 mmol) was dissolved in dry pyridine (1 mL) and *p*-toluenesulfonyl chloride (73 mg, 0.38 mmol) was added in one portion. The resulting mixture was reacted at rt for 4 h and the solvent was removed in vacuo. The residue was dissolved in DCM and purified using flash chromatography (4 g of silica gel column, 25−100% EtOAc in hexanes) to afford 3-((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)propyl 4-methylbenzenesulfonate (40 mg, 0.083 mmol, 65% yield) as a white solid. LC/MS M^{+1} = 484.4; HPLC t_r = 1.08 (method A).

*(5R,7S)-7-((S)-6-(Hydroxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**48**).* (5*R*,7*S*)-7-((*R*)-6-(hydroxymethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro-[4.4]nonan-2-one[17a] (**47**, 690 mg, 2.289 mmol) was dissolved in pyridine (5 mL) and *p*-toluenesulfonyl chloride (1.3 g, 6.9 mmol) was added in one portion. The resulting mixture was stirred at rt for 4 h. The solvent was removed in vacuo, and the crude material was purified on a silica gel cartridge using an EtOAc/Hex gradient (20−100% EtOAc over 12 column volumes) to afford ((*S*)-6-((5*R*,7*S*)-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)-1,2,3,4-tetrahydronaphthalen-2-yl)methyl 4-methylbenzenesulfonate (**48**, 800 mg, 95% yield). ^{1}H NMR (400 MHz, *CDCl*$_3$): δ 7.84−7.79 (m, 2H), 7.39−7.33 (m, 2H), 7.00−6.90 (m, 3H), 6.48−6.41 (m, 1H), 4.34−4.24 (m, 2H), 4.06−3.94 (m, 2H), 3.04−2.91 (m, 1H), 2.87−2.72 (m, 3H), 2.46 (s, 3H), 2.44−2.35 (m, 1H), 2.33−2.21 (m, 1H), 2.20−2.03 (m, 3H), 2.00−1.88 (m, 3H), 1.84−1.78 (m, 1H), 1.48−1.37 (m, 1H); MS $(M + H)^+$ at *m*/*z* 456; HPLC t_r = 2.05 min (method A).

Biological Methods. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication no. 85-23, revised 2011). Phosphate metabolites were dissolved in 0.3 M NaOH as a 3 mM stock prior to being assayed.

SIP$_1$ Binding Assay. Membranes were prepared from CHO cells expressing human $S1P_1$. Cell pellets (l × 109 cells/pellet) were suspended in buffer containing 20 mM HEPES (4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.5, 50 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA) and protease inhibitor cocktail (Roche), and disrupted on ice using the Polytron homogenizer. The homogenate was centrifuged at 20,000 rpm (48,000*g*) and the supernatant was discarded. The membrane pellets were resuspended in buffer containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 2 mM EDTA, and stored in aliquots at −80 °C after protein concentration determination. Membranes (2 μg/well) and a 0.03 nM final concentration of ^{33}P−S1P ligand (1 mCi/mL, PerkinElmer or American Radiolabeled Chemicals) diluted in assay buffer [50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% fatty acid free bovine serum albumin (BSA), and 1 mM NaF] were added to the compound plates [384 Falcon v-bottom plate (0.5 μL/well in a 11 point, 3-fold dilution)]. Binding was performed for 45 min at room temperature (rt), terminated by collecting the membranes onto 384-well Millipore FB filter plates, and radioactivity was measured by TOPCOUNT. The competition data of the test compounds over a range of concentrations was plotted as percentage inhibition of radioligand-specific binding.

Receptor [^{35}S] GTPγS Binding Assays ($S1P_1$ GTPγS/$S1P_3$ GTPγS). Compounds were loaded in a 384 Falcon v-bottom plate (0.5 μL/well in a 11 point, 3- fold dilution). Membranes prepared from $S1P_1$/CHO cells or EDG3-Ga15-bla HEK293T cells (EDG3 equivalent $S1P_3$) were added to the compound plate (40 μL/well, final protein 3 μg/well) with MULTIDROP. [^{35}S]GTP (1250 Ci/mmol, Perkin-3-Elmer) was diluted in assay buffer: 20 mM HEPES, pH7.5, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM ethylene glycol tetraacetic acid, 1 mM dithiothreitol, 10 μM GDP, 0.1% fatty acid free BSA, and 10 μg/mL saponin to 0.4 nM. The [^{35}S]GTP solution (40 μL) was added to the compound plate with a final concentration of 0.2 nM. The reaction was kept at rt for 45 min. At the end of incubation, all the mixtures in the compound plate were transferred to Millipore 384-well FB filter plates via the VELOCITY l Vprep liquid handler. The filter plate was washed with water four times by using the manifold Embla plate washer and dried at 60 °C for 45 min. MicroScint 20 scintillation fluid (30 μL) was added to each well for counting on the Packard TOPCOUNT. EC_{50} is defined as the agonist concentration that corresponds to 50% of the Y_{max} (maximal response) obtained for each individual compound tested.

hS1P$_1$ ERK Phosphorylation. $hS1P_1$/CHO cells were plated in BD Amine 384-well plates the day before the assay. On the day of the assay, the growth medium was removed and replaced with serum-free medium (Ham's F-12; Invitrogen) and incubated for 2 h. Test compounds prediluted in HBSS/20 mM HEPES (Gibco, NY, USA) were transferred to the cell plates and incubated for 7 min at 37 °C. Cells were lysed in a lysis buffer (PerkinElmer, MA, USA), and phospho-ERK was measured using the SureFire pERK kit (PerkinElmer, MA, USA) per manufacturer's instructions. Data were plotted as percentage activation of the test compound relative to the efficacy of 10 μM S1P. The EC_{50} was defined as the concentration of test compound, which produces 50% of the maximal response and was quantified using the four-parameter logistic equation to fit the data.

S1P$_1$ Internalization Assay. For quantification of $S1P_1$ expression using a high content system, GFP was fused to $hS1P_1$ at its C-terminus and a stable CHO cell line expressing $hS1P_1$/GFP was established. Cells were suspended in assay medium (F12 medium with 5% charcoal−dextran-treated FBS, 20 mM HEPES, and 1× Pen/Strep) at 7.5 × 104 cells/mL and plated into 384-well plates (in 20 μL, 1,500 cells/well final) with a Multidrop liquid handler (Thermo Scientific, Waltham, MA). Cell plates were incubated at 37 °C for 48 h. Titrated S1P and test compounds were dispensed into the cell plates (concentration range from 100 to 0.005 μM) and the plates were further incubated at 37 °C for 50 min. Cells were fixed by adding 10 μL of fixation buffer (7.4% v/v formaldehyde containing 15 μM Draq5 in DPBS) directly onto the assay medium and incubated for 15 min at rt, followed by two washes with DPBS. This was followed by the addition of 50 μL DPBS to the cell layer and the plates were sealed with a transparent plate seal (PerkinElmer #6005185). Imaging was carried out using the Evotec Opera High Content Confocal System (PerkinElmer, Boston, MA) equipped with three diode lasers, Peltier-cooled CCD camera detector, and a Nipkow spinning disk. Cell images were quantified using a membrane fluorescent scoring

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algorithm. Data was analyzed using a customized HTS data analysis software package. EC_{50} values were determined using XL-Fit software program.

Chemotaxis Assay. Human T cells were isolated from peripheral blood mononuclear cells by rosetting with sheep red blood cells for 1 h at 4 °C. The sheep red blood cells were removed by lysis using ACK lysing buffer (Gibco). Human T cells from two donors were cultured for 72 h in RPMI-1640 containing 0.5% fatty acid free BSA (Calbiochem) and then labeled with the fluorescent dye Calcein-AM (10 μg/mL; Molecular Probes) in chemotaxis buffer (phenol red-free RPMI-1640, containing 0.5% fatty-acid free BSA) at rt for 45 min. The labeled cells were then washed and resuspended in chemotaxis buffer at 1.2×10^7/mL. Chemotaxis was measured using a chemotaxis plate with 5 μm pores (Neuroprobe). Labeled cells (3×10^5 in 25 μL of chemotaxis buffer) were preincubated with test compound (final assay concentrations ranged from 0.01 to 50 nM) for 10 min at rt before loaded to each of the upper wells of a plate. The bottom wells of the plate were loaded with S1P at concentration that is known to afford 90% maximal chemotaxis without the presence of test compounds. The membrane was placed over the lower wells and the plate was covered and incubated at 37 °C for 120 min. Following incubation, the upper wells were washed twice with PBS to remove unmigrated cells and fluorescence in the lower wells was read at 485 nm/530 nm excitation/emission on a Cytofluor 4000 (Perceptive Biosystems). The inhibition of chemotaxis achieved by titrated concentrations of compounds was calculated as a percentage of the compound-free S1P control signal after plate background and buffer background signal subtraction. The IC_{50} is defined as the concentration of compound required to reach 50% inhibition of chemotaxis and was calculated by the XLFit software program.

BLR Assay. Lewis rats were dosed orally with vehicle alone (polyethylene glycol 300) or with test compounds. Compounds were dosed as a solution or suspension in the vehicle and adjusted to reflect the free amount of test article in the event that salt forms are utilized. Blood was drawn at different time points and blood lymphocyte counts were determined on an ADVIA 120 Hematology Analyzer (Siemens Healthcare Diagnostics). The results were measured as a reduction in the percentage of circulating lymphocytes as compared to the vehicle-treated group at the time of measurement. The results represent the average results of all animals within each treatment group (n = 2−4).

In Vivo Phosphate Metabolite Formation. Lewis rats were dosed orally with compounds. Blood was drawn at 24 h and spotted onto Dried Blood Spot (DBS) Cards. The DBS Cards were stored at ambient temperature in sealed plastic bags with desiccant added. When ready for analysis, a 6 mm punch (equivalent to 12.5 μL of wet blood) was taken at n = 1 and placed in a shallow 96-well filter plate. Next, 105 μL of a mixture of 75% acetonitrile and 25% water containing internal standard was added and gently vortexed for 30 min and then centrifuged. The supernatant was separated from the protein pellet and 5 μL was injected. The parent (alcohol) and active phosphate metabolite compounds were quantitatively analyzed, using a DBS calibration curve, by LC/MS/MS on a Triple Quadrupole Instrument. The area ratios of the phosphorylated compound to the parent (alcohol) compound were determined. A larger value for the ratio of the phosphorylated compound to the parent (alcohol) compound indicated greater phosphate metabolite formation from the parent (alcohol) compound. Reference material (parent alcohol and phosphate metabolite) was analyzed to optimize the LC−MS/MS assay and enable data reporting in concentrations. DBS standard curves containing both parent alcohol and phosphate metabolites were prepared and analyzed in the same manner as the study samples and analyzed by the optimized LC−MS/MS to quantify the amount of phosphate metabolite formed. The results represent the average results of all animals within each treatment group (n = 2−4).

Pulmonary Toxicity Assay. The analysis of protein levels in bronchoalveolar lavage (BAL) fluid obtained from an animal were used to gauge pulmonary side effects. Post-study rats were euthanized with intraperitoneal barbiturate overdose. The animals were placed in a supine position, a skin incision was made, and blunt dissection followed to expose the trachea. The trachea was incised and a catheter was inserted 4−6 mm into the trachea. Phosphate-buffered saline (PBS; 1 mL/mouse) was infused into the lungs and then aspirated. The concentration of the protein in the recovered BAL fluid was determined on an Advia 1800 Chemistry Analyzer (Siemens Healthcare Diagnostics). The results represent the average results of all animals within each treatment group (n = 2−4).

Multi-electrode Array Electrophysiology Studies in Human-Inducible Pluripotent Stem Cell-Derived Cardiomyocytes. Human-inducible pluripotent stem cell-derived cardiomyocytes (hiPSC CMs) were purchased from Cellular Dynamics International (Madison, WI). qPCR analysis showed similar RNA expression levels of $S1P_1$, $S1P_2$, and $S1P_3$ in hiPSC CMs as in adult human heart tissue (results not shown). hiPSC CMs were cultured with 7% CO_2 on 0.1% gelatin treated six-well culture plates for 7 days, then trypsinized, and diluted with cardiac fibroblasts (10%). Suspensions of hiPSC CMs and fibroblasts were then co-cultured on laminin-coated 9-well multi-electrode array (MEA) plates (256−9 well MEA300/30iRITO-mq; Multichannel Systems; Atlanta, GA). After 7 days of culture on MEA plates, cells formed a spontaneously beating monolayer over recording electrodes imbedded in each well. Spontaneous extracellular field potentials (FPs) were recorded from 28 electrodes/well at a sampling frequency of 10 kHz using an USB-MEA256-System and MC Rack acquisition software (Multi Channel Systems). Following a 20−60 min equilibration period in a humidified environment at 37 °C with constant 5% CO_2 and 95% O_2 supply, compounds were added to each well in 300 μL of maintenance medium with final DMSO or NaOH vehicle concentration less than 0.1% or 30 μM. Dilute NaOH (30 μM) was used as control in these studies and had no significant effect on the beating rate of hiPSC CMs. Effects of test agents on FPs were evaluated for at least 2 h. Data were analyzed with MC_DataTool and custom software written in MatLab (Mathworks; Natick, MA).

Rat Adjuvant Arthritis (AA) Studies. AA was induced in male Lewis rats (~200 g) by subcutaneous injection of 100 μL of complete Freund's adjuvant (Sigma-Aldrich, MO) at the base of the tail. Volumes of both hind paws were measured with a water-based plethysmometer (Ugo Basile, Italy). Compounds were dissolved in PEG300 and administered daily by oral gavage (5 mL/kg) from the time of adjuvant injection. Paw volumes were periodically measured during the course of the study, and change in paw volumes was calculated relative to predisease baseline measurements.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01109.

Lymphocyte reduction for **1** at 4 and 24 h at various doses; synthesis of **26**−**36**; $S1P_1$ GTPγS activity of phosphates for select compounds; exposures at 4, 24, 48, and 72 h for $T_{1/2}$ estimation; structure analysis of **6** and **7**; analyses of **12** and **24**; chiral HPLC analysis of **40b**; and method for predicting human $T_{1/2}$ and clearance (PDF)

Molecular formula strings (CSV)

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Notes

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■ ABBREVIATIONS USED

S1P, sphingosine-1-phosphate; $S1P_{1-5}$, sphingosine-1-phosphate receptors 1−5; RRMS, relapsing-remitting multiple sclerosis; BLR, blood lymphocyte reduction; BAL, bronchoalveolar lavage

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