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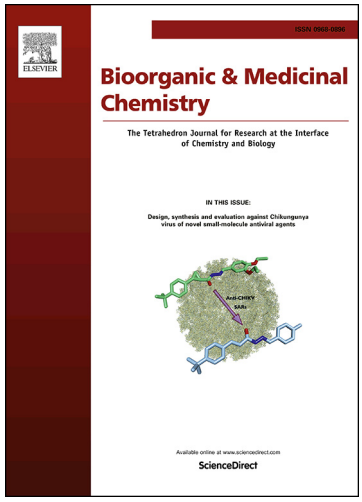

Design, synthesis and biological evaluation of novel 7-azaspiro[3.5]nonane derivatives as GPR119 agonists

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PII: S0968-0896(17)32185-5
DOI: https://doi.org/10.1016/j.bmc.2018.02.032
Reference: BMC 14218

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 9 November 2017
Revised Date: 16 February 2018
Accepted Date: 18 February 2018

Please cite this article as: Matsuda, D., Kawamura, M., Kobashi, Y., Shiozawa, F., Suga, Y., Fusegi, K., Nishimoto, S., Kimura, K., Miyoshi, M., Takayama, N., Kakinuma, H., Ohtake, N., Design, synthesis and biological evaluation of novel 7-azaspiro[3.5]nonane derivatives as GPR119 agonists, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.02.032

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Design, synthesis and biological evaluation of novel 7-azaspiro[3.5]nonane derivatives as GPR119 agonists

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Abstract

The design and synthesis of a novel class of 7-azaspiro[3.5]nonane GPR119 agonists are described. In this series, optimization of the right piperidine *N*-capping group (R^2) and the left aryl group (R^3) led to the identification of compound **54g** as a potent GPR119 agonist. Compound **54g** showed a desirable PK profile in Sprague-Dawley (SD) rats and a favorable glucose lowering effect in diabetic rats.

1. Introduction

Diabetes mellitus is a chronic disease characterized by high blood glucose levels (hyperglycemia). In 2015, it was estimated that the number of people with diabetes worldwide was more than 400 million, and was expected to rise to 642 million by 2040.[1] Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, characterized by hyperglycemia resulting from impaired insulin secretion and insulin resistance. Long-term hyperglycemia can cause serious complications such as blindness, renal failure, diabetic foot disorders, heart attacks and strokes. Although multiple oral antidiabetic agents, such as sulfonylureas, meglitinides, biguanides, thiazolidinediones, α-glucosidase inhibitors and dipeptidyl-peptidase-4 (DPP-4) inhibitors, have been used for the treatment of T2DM, many patients fail to achieve the desired glycemic control.[2,3] Recently, a sodium-dependent glucose co-transporter 2 (SGLT2) inhibitor has been used clinically; this inhibitor exerts a glucose-lowering effect without causing hypoglycemia or weight gain. However, a significant need for the development of new antidiabetic agents with greater safety and efficacy

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continues to exist.

GPR119 is a G-protein coupled receptor (GPCR) that is predominantly expressed in pancreatic β-cells and gastrointestinal L-cells. Oleoyl-lysophosphatidylcholine and oleoylethanolamide (OEA) have been identified as endogenous agonists for the GPR119 receptor.[4,5] The activation of the GPR119 receptor increases the cellular cAMP levels, leading to glucose-dependent insulin secretion from pancreatic β-cells.[6] In addition, the activation of the GPR119 receptor in the gut results in the release of incretins, such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), from enteroendocrine cells.[7] GLP-1 and GIP stimulate insulin secretion from β-cells in a glucose-dependent manner and protect β-cells against apoptosis.[8,9] This glucose-dependent dual mechanism of action suggests that GPR119 agonists can improve glycemic control without inducing hypoglycemia. Furthermore, this mechanism might produce beneficial anti-obesity effects such as suppressed food intake and body weights.[4]

To date, many research groups have investigated small-molecule GPR119 agonists,[10,11] which have led to the development of clinical compounds such as APD668,[12] GSK1292263,[13] and MBX-2982[14] (Figure 1).

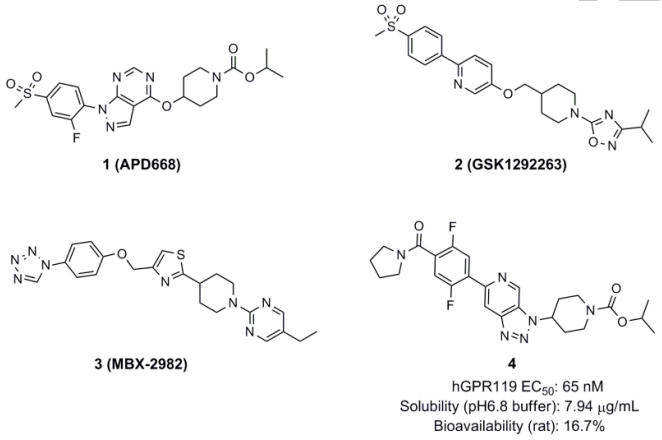

Figure 1. Representative GPR119 receptor agonists

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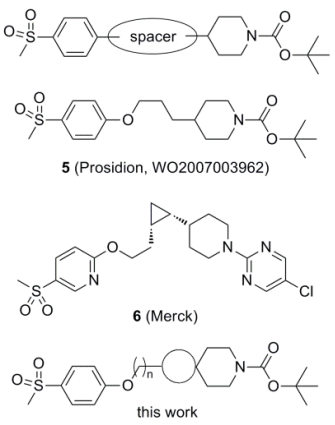

Figure 2. GPR119 agonists with carbon chain spacer

We previously reported a series of 1*H*-pyrazolo[3,4-*c*]pyridine and 3*H*-[1,2,3]triazolo[4,5-*c*]pyridine derivatives (**4**) as a new class of GPR119 agonists[15, 16] (Figure 1). However, these derivatives suffered from a poor aqueous solubility and a low bioavailability because of a strong molecular interaction associated with their molecular planarity. To overcome these issues, we explored an alternative structure class with new central spacers between the left pharmacophore (substituted phenyl group) and the right *N*-capped piperidine with greater three-dimensionality.[17] Using Prosidion's (now Astellas) GPR119 agonists, which have a simple carbon chain as a central spacer (represented by compound **5**; Figure 2), as a starting point, we attempted to introduce conformational rigidity into the carbon chain of **5** to improve its agonist activity. Merck researchers have already identified cyclopropane derivatives, such as compound **6**,[18,19] which are considered the representative of the successful realization of conformational rigidity. Our strategy was to install spiro structures as central spacers (Figure 2) to achieve both conformational rigidity and three-dimensionality.

Here, we describe the synthesis and evaluation of spiro-piperidine derivatives and the discovery of novel 7-azaspiro[3.5]nonane GPR119 agonists, including their structure-activity relationships (SARs) and an *in vivo* efficacy study in diabetic rats.

2. Chemistry

The synthesis of 6-azaspiro[2.5]octane derivatives **13** and **17** is shown in Schemes 1 and 2. Compound **13** was derived from a commercially available starting material, 6-*tert*-butyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate (**7**), which was reduced with diisobutylaluminum hydride (DIBAL); the resulting alcohol **8** was oxidized with Dess-Martin periodinane to yield aldehyde **9**. The Wittig reaction of **9** with $MeOCH_2P^+Ph_3Cl^-$ followed by hydrolysis with trifluoroacetic acid

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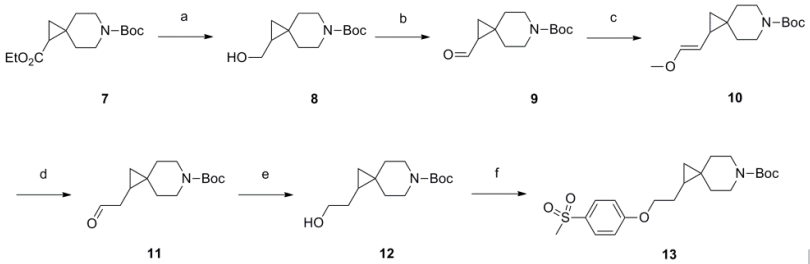
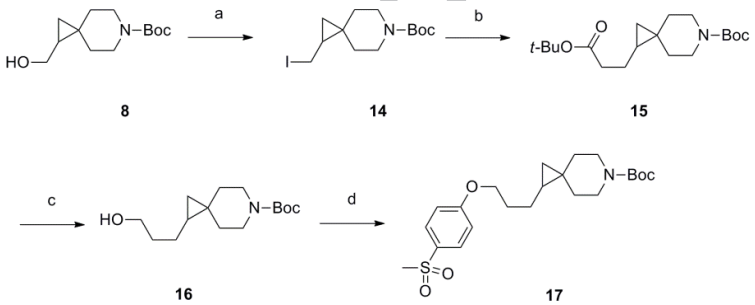
(TFA) in aqueous MeCN yielded **11**. The subsequent reduction of aldehyde **11** with $NaBH_4$ yielded **12**. Finally, the Mitsunobu reaction of **12** with 4-(methanesulfonyl)phenol using *N*,*N*,*N*',*N*'-tetramethylazadicarboxamide (TMAD) and tributylphosphine yielded the 6-azaspiro[2.5]octane derivative **13**.



Scheme 1. (a) DIBAL, THF, 0 ˚C; (b) Dess-Martin periodinane, $CHCl_3$, rt; (c) $MeOCH_2P^+Ph_3Cl^-$, KO-*t*-Bu, toluene, 70 ˚C; (d) TFA, H_2O, CH_3CN, rt; (e) $NaBH_4$, MeOH, rt, (f) 4-(methanesulfonyl)phenol, TMAD, PBu_3, THF, 60 ˚C.

Compound **17** was synthesized from alcohol **8**. The alcohol group was iodinated with I_2 to produce compound **14**. The substitution reaction of **14** with lithium enolate of *tert*-butyl acetate in the presence of *N*,*N*'-dimethylpropyleneurea (DMPU) yielded **15**. The reduction of compound **15** with $LiBH_4$ at 60 ˚C followed by the Mitsunobu reaction under conditions similar to those described for compound **13** provided the desired product **17**.



Scheme 2. (a) I_2, PPh_3, imidazole, $CHCl_3$, rt; (b) *t*-BuOAc, LDA, DMPU, THF, -78 ˚C; (c) $LiBH_4$, THF, 60 ˚C; (d) 4-(methanesulfonyl)phenol, TMAD, PBu_3, THF, 60 ˚C.

The synthesis of 7-azaspiro[3.5]nonane derivative **26** and 3-azaspiro[5.5]undecane derivative **27** is shown in Scheme 3. The Horner-Wadsworth-Emmons reaction [$(EtO)_2POCH_2CO_2Et$, NaH] of commercially available starting materials **18** and **19** provided **20** and **21**, respectively. Hydrogenation of the double bonds of **20** and **21** in the presence of a catalytic amount of $Pd(OH)_2$ and the subsequent reduction of the ester moieties yielded alcohols **24** and **25**. Finally, the Mitsunobu reaction of the alcohols provided the desired products **26** and **27**.

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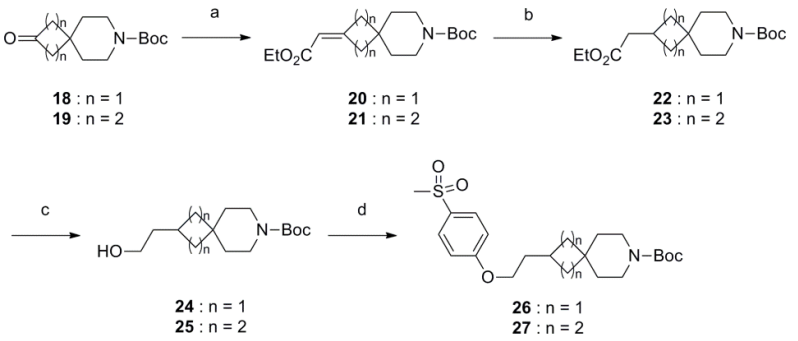
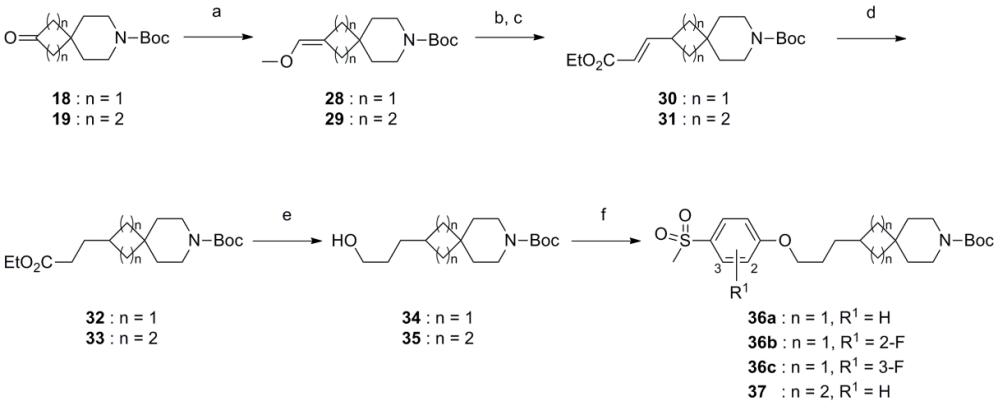


Scheme 3. (a) $(EtO)_2POCH_2CO_2Et$, NaH, DMF, rt; (b) H_2, $Pd(OH)_2/C$, EtOH, rt; (c) $LiBH_4$, THF, reflux; (d) 4-(methanesulfonyl)phenol, TMAD, PBu_3, THF, 60 ˚C.

Scheme 4 shows the synthesis of 7-azaspiro[3.5]nonane derivatives **36a-c** and 3-azaspiro[5.5]undecane derivative **37**. The Wittig reaction ($MeOCH_2P^+Ph_3Cl^-$, KO-*t*-Bu) of **18** and **19** followed by acidic hydrolysis and the Horner-Wadsworth-Emmons reaction of the resulting aldehydes yielded α,β-unsaturated esters **30** and **31**. Catalytic hydrogenation of the double bonds, the subsequent reduction, and the Mitsunobu reaction of the alcohols **34** and **35** with the corresponding phenol provided the desired products **36a-c** and **37**, respectively.



Scheme 4. (a) $MeOCH_2P^+Ph_3Cl^-$, KO-*t*-Bu, toluene, 70 ˚C; (b) TFA, H_2O, CH_3CN, rt; (c) $(EtO)_2POCH_2CO_2Et$, NaH, DMF, THF, rt; (d) H_2, $Pd(OH)_2/C$, EtOH, rt; (e) for **34**, DIBAL, THF, 0 ˚C; for **35**, $LiBH_4$, toluene, THF, 60 ˚C; (f) ArOH, TMAD, PBu_3, THF, 60 ˚C.

Compound **41** was similarly obtained from **40** (Scheme 5), which was derived *via* the following four reaction steps: 1) Dess-Martin oxidation of **24**, 2) Horner-Wadsworth-Emmons reaction, 3) catalytic hydrogenation, and 4) reduction with $LiBH_4$. The Mitsunobu reaction under conditions similar to those described for **26** in Scheme 3 was then performed, producing **41**.

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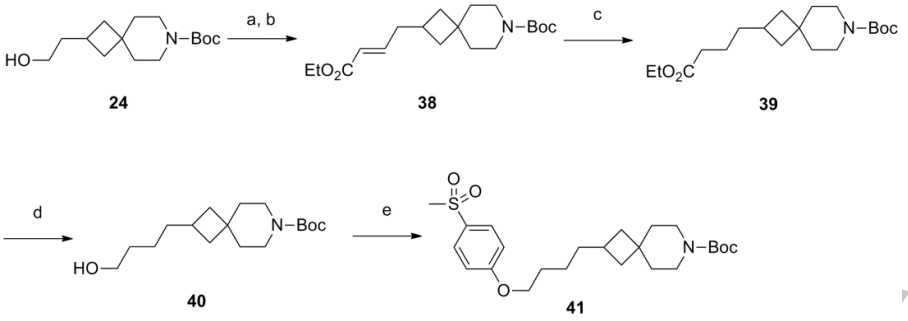
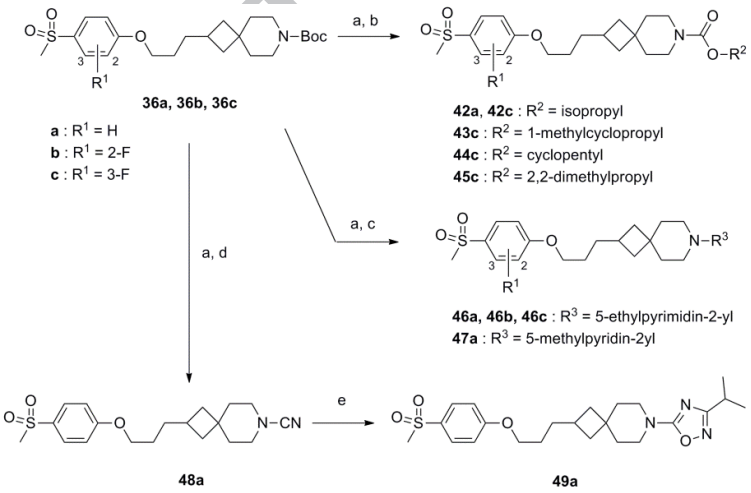


Scheme 5. (a) Dess-Martin periodinane, $CHCl_3$, rt; (b) $(EtO)_2POCH_2CO_2Et$, NaH, DMF, rt; (c) H_2, $Pd(OH)_2/C$, EtOH, rt; (d) $LiBH_4$, THF, 60 ˚C; (d) 4-(methanesulfonyl)phenol, TMAD, PBu_3, THF, 60 ˚C.

Compounds **42a**, **42c**, **43c**, **44c**, **45c**, **46a-c**, **47a** and **49a** were synthesized as shown in Scheme 6. The removal of the *tert*-butoxycarbonyl group (Boc) of **36a-c** followed by treatment with the corresponding carbamating reagents yielded **42a**, **42c**, **43c**, **44c** and **45c**. On the other hand, compounds **46a-c** were prepared from **36a-c** *via* the removal of the Boc group and the subsequent *N*-arylation with 2-chloro-5-ethylpyrimidine. As for **47a**, *N*-arylation was performed using cross-coupling reaction with 2-chloro-5-methylpyridine in the presence of $Pd_2(dba)_3$ and 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) as catalysts. Compound **49a** was prepared *via* cyanide **48a**, which was reacted with *N'*-hydroxy-2-methylpropanimidamide and then dehydrated under acidic conditions to produce **49a**.



Scheme 6. (a) HCl, EtOAc, rt; (b) for **42a**, **42c**, isopropyl chloroformate, Et_3N, $CHCl_3$, rt; for **43c**, 1-methylcyclopropyl 4-nitrophenyl carbonate, Et_3N, $CHCl_3$, rt; for **44c**, cyclopentanol, triphosgene, Et_3N, THF, rt; for **45c**, 2,2-dimethylpropan-1-ol, triphosgene, Et_3N, THF, rt; (c) for **46a-c**,

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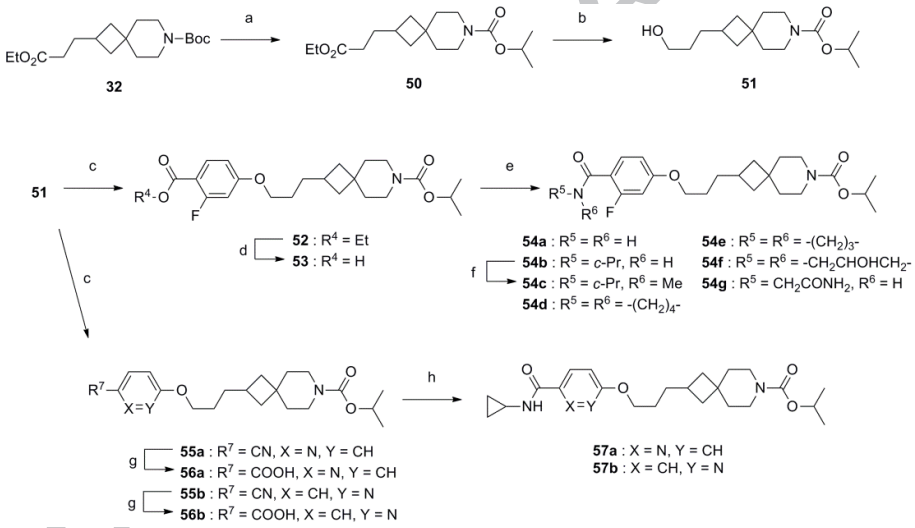
2-chloro-5-ethylpyrimidine, Cs_2CO_3, DMSO, microwave, 180 ˚C; for **47a**, 2-bromo-5-methylpyridine, $Pd_2(dba)_3$, BINAP, NaO-*t*-Bu, 1,2-dimethoxyethane, 60 ˚C; (d) CNBr, $NaHCO_3$, H_2O, $CHCl_3$, rt; (e) (1) *N*'-hydroxy-2-methylpropanimidamide, $ZnCl_2$, Et_2O, rt; (2) conc.HCl, EtOH, reflux.

The synthesis of amide derivatives **54a-g**, **57a** and **57b** is shown in Scheme 7. The common intermediate **51** was derived from **32** using the following reactions: 1) deprotection of the Boc group 2) introduction of an isopropyl carbamate group, 3) and reduction of the ester moiety with DIBAL. The Mitsunobu reaction of **51** with ethyl 2-fluoro-4-hydroxybenzoate followed by basic hydrolysis yielded carboxylic acid **53**. Finally, the condensation of **53** with the corresponding amine under standard conditions yielded the desired amide derivatives **54a**, **54b**, **54d-g**. Compound **54c** was prepared from **54b** through *N*-methylation using NaH and MeI. As for **57a** and **57b**, these compounds were obtained *via* the Mitsunobu reaction of **51** with 5-hydroxypyridine-2-carbonitrile or 6-hydroxypyridine-3-carbonitrile, hydrolysis of the cyano groups of **55a** and **55b**, and the amidation of the resulting carboxylic acid with cyclopropylamine to yield **57a** and **57b**.



Scheme 7. (a) (1) HCl, EtOAc, rt; (2) isopropyl chloroformate, Et_3N, $CHCl_3$, rt; (b) DIBAL, THF, rt; (c) ArOH, TMAD, PBu_3, THF, 60 ˚C; (d) NaOH, H_2O, MeOH, 60 ˚C; (e) R^5R^6NH, EDCI·HCl, HOBt·H_2O, Et_3N, DMF, rt; (f) NaH, MeI, DMF, rt; (g) NaOH, H_2O, EtOH, reflux; (h) cyclopropylamine, EDCI·HCl, HOBt·H_2O, Et_3N, DMF, rt.

3. Results and Discussion

The GPR119 agonist potency of the synthesized compounds was measured using a cAMP assay in the human GPR119 cell line.

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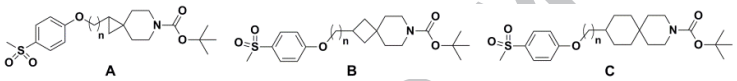
First, we evaluated the potencies of compounds with various types of spacers (including spiro substructures) with the right pharmacophore (4-methylsulfonylphenoxy group) and the left part (*tert*-butoxycarbonylpiperidine) in fixed positions (Table 1). Among the compounds, the 7-azaspiro[3.5]nonane derivative **36a** containing a propylcyclobutyl spacer was the most potent GPR119 agonist. A single carbon contraction (**26**) or elongation (**41**) of the propyl moiety of **36a** led to a 10-fold decrease in the agonist potency. Regarding the cyclobutyl spacer moiety of **36a**, a ring contraction (cyclopropyl: **17**) or ring expansion (cyclohexyl: **37**) resulted in a significant reduction in potency. Furthermore, a single carbon contraction of the propyl spacer moiety of **17** or **37** led to the identification of the potent compound **27** with an EC_{50} value of 55 nM. All the compounds shown in Table 1, except **13** and **17**, exhibited full intrinsic activity. As a result, we identified an alternative chemical class of potent GPR119 agonists exemplified by 7-azaspiro[3.5]nonane derivative **36a** and 3-azaspiro[5.5]undecane derivative **27**. These results indicated that the rigidity of the spiro moiety and the favorable spacer length led to the high agonist activity of **36a** and **27**.

Table 1

SAR of spacers



Cpd.	Spiro	n	hGPR119 EC_{50} (nM)
13	A	2	2388
17	A	3	830
26	B	2	183
36a	B	3	14
41	B	4	154
27	C	2	55
37	C	3	2590

Having obtained a new chemical class, we next focused on optimizing the *N*-capping group of the piperidine of the more potent compound **36a** (Table 2). In addition to the agonist potency, we monitored the solubility of each compound in fasted state simulated intestinal fluid (FaSSIF) as well as the aqueous solubility in pH6.8 phosphate buffer to compare the solubility profiles. Most of the compounds of this series exhibited full intrinsic activity. Although **36a** exhibited a low solubility: <0.13 μg/mL (the lower limit of quantitation) in the phosphate buffer and 3.48 μg/mL in FaSSIF, the three-dimentionallity of the compound was expected to have a positive impact on increasing not only

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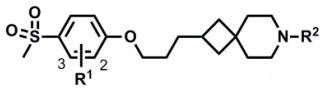
the solubility but also the dissolution rate, leading to improving the oral bioavaialbity.[20] Therefore, we started the optimization study of this series.

The replacement of the acid-labile *tert*-butoxycarbonyl group in **36a** with an isopropyloxycarbonyl group led to the compound **42a**, which was about 5-fold less potent than **36a**. However, the installation of a fluorine atom at the 3-position of the left phenyl ring of **42a** restored the potency (**42c**, EC_{50} = 18 nM). The isopropyl group in **42c** was replaced with 1-methylcyclopropyl (**43c**), cyclopentyl (**44c**) and 2,2-dimethylpropyl (**45c**) to evaluate their effect on the agonist potency and aqueous solubility. The agonist potency of the 1-methylcyclopropyl derivative (**43c**) was comparable to that of **42c**, while the solubility in FaSSIF was nearly half that of **42c**. By contrast, the solubility of the cyclopentyl derivative (**44c**) more than doubled in FaSSIF; however, its agonist potency was not improved despite the higher lipophilicity. The 2,2-dimethylpropyl derivative (**45c**) also showed no improvement in agonist potency. Moreover, replacement of the *tert*-butoxycarbonyl moiety of **36a** with a heteroaromatic group such as pyrimidine, pyridine or oxadiazole as a carbamate isostere, was conducted to enhance the potency.[15] The pyrimidine derivative (**46a**) exhibited a tolerable agonist potency (EC_{50}: 31 nM). However, the installation of a fluorine atom into the phenyl ring of **46a** significantly decreased the potency (**46b** and **46c**). The pyridine analog (**47a**) showed a poor agonist potency, while the potency of the oxadiazole analog (**49a**) was tolerable (EC_{50}: 35 nM). In this series, the carbamate groups appeared to be more favorable than the heteroaryl groups as a piperidine *N*-capping group. Among these carbamate groups, we selected isopropyl carbamate (**42c**) for further SAR study of the left aryl group (R^3) based on its agonist potency, FaSSIF solubility, and lipophilicity (*C*log*P*), as shown in Table 3.

Table 2

SAR of piperidine substituents



Cpd.	R^1	R^2	hGPR119 EC_{50} (nM)	solubility (μg/mL) pH6.8 buffer	solubility (μg/mL) FaSSIF	*C*log*P*[a]
36a	H		14	<0.13	3.48	4.94
36b	2-F		25	NT[b]	NT[b]	4.92
36c	3-F		9	NT[b]	NT[b]	5.12
42a	H		78	0.17	7.09	4.54
42c	3-F		18	<0.16	2.52	4.72

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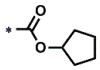
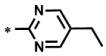
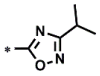
43c	3-F		13	<0.15	1.27	4.76
44c	3-F		23	<0.14	7.65	5.36
45c	3-F		31	NT[b]	NT[b]	5.74
46a	H		31	<0.12	0.64	4.37
46b	2-F		225	NT[b]	NT[b]	4.35
46c	3-F		123	NT[b]	NT[b]	4.55
47a	H		348	NT[b]	NT[b]	4.60
49a	H		35	<0.13	2.51	4.34

[a] The *C*log*P* value was calculated using software from Daylight Chemical Information Systems, Inc.

[b] Not tested.

In our previous study, a fluorine substituted alkylcarbamoylphenyl group as an R^3 moiety was shown to enhance potency. Therefore, the replacement of the methylsulfonyl group of **42c** with a carbamoyl (**54a**), *N*-cyclopropylcarbamoyl[18] (**54b**), or *N*-cyclopropyl-*N*-methylcarbamoyl group (**54c**) was conducted (Table 3). Among these three options, compound **54b** exhibited an enhanced potency (EC_{50}: 8 nM) with a slightly improved solubility in the phosphate buffer (0.21 μg/mL) as well as an improved FaSSIF solubility (8.58 μg/mL). The 4-(*N*-cyclopropylcarbamoyl)-3-fluorophenyl ring of **54b** was replaced with 4-(*N*-cyclopropylcarbamoyl)-3-pyridyl (**57a**) or the corresponding 2-pyridyl (**57b**) ring, leading to a 10-fold improvement in the phosphate buffer solubility. Unfortunately, the agonist potency of **57a** and **57b** was decreased by 6-fold and 8-fold, respectively. Further replacement of the *N*-cyclopropylcarbamoyl moiety in **54b** with a 1-pyrrolidinylcarbonyl group led to the compound **54d**, which showed an improved solubility (3.85 μg/mL) and an EC_{50} value of 29 nM. Unfortunately, compound **54d** showed a high lipophilicity (*C*log*P*: 5.34), often causing pharmacokinetic issues such as the inhibition of cytochrome P450 (CYP) isozymes. To reduce the lipophilicity, further derivatization by replacing the 1-pyrrolidinylcarbamoyl moiety of **54d** with 1-azetidinylcarbonyl (**54e**), 3-hydoxy-1-azetidinylcarbonyl (**54f**), and *N*-(carbamoylmethyl)carbamoyl (**54g**) groups was conducted. Compound **54g** exhibited reasonable lipophilicity (*C*log*P*: 3.51) and agonist potency (EC_{50}: 48 nM, full intrinsic activity), while its solubility in phosphate buffer (1.77 μg/mL) and FaSSIF (18.4 μg/mL) would require further improvement.

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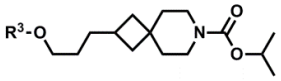
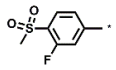
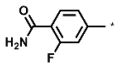
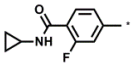
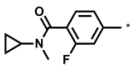
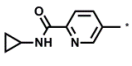
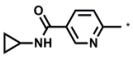
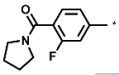
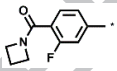
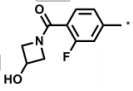
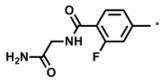
Table 3

SAR of R^3 group



Cpd.	R^3	hGPR119 EC_{50} (nM)	Solubility (μg/mL) pH6.8 buffer	Solubility (μg/mL) FaSSIF	*C*log*P*[a]	CYP inhibition IC_{50} < 10 μM[c]
42c		18	<0.16	2.52	4.72	NT[b]
54a		52	NT[b]	NT[b]	4.38	NT[b]
54b		8	0.21	8.58	5.21	NT[b]
54c		67	NT[b]	NT[b]	5.29	NT[b]
57a		47	2.11	NT[b]	5.14	NT[b]
57b		63	2.24	NT[b]	5.19	NT[b]
54d		29	3.85	92.4	5.34	1A2, 2C9, 2C19
54e		34	3.43	NT[b]	4.78	2C9, 2C19
54f		40	1.89	NT[b]	4.75	2C9, 2C19
54g		48	1.77	18.4	3.51	None

[a] The *C*log*P* value was calculated using software from Daylight Chemical Information Systems, Inc.

[b] Not tested.

[c] The inhibitory effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A were evaluated in human liver microsomes.

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Given the promising profile of compound **54g** (TP0459004), we conducted a pharmacokinetic study in SD rats. As shown in Table 4, compound **54g** displayed excellent oral bioavailability (F: 88.6%) despite its low solubility in the phosphate buffer. The increased three-dimensionality might lead to increase the dissolution rate,[20] resulting in the improved oral bioavailability. Subsequently, the glucose-lowering effect of **54g** was evaluated using an oral glucose tolerance test (oGTT) in Zucker diabetic fatty rats, a model of obesity and impaired glucose tolerance. Compound **54g** was orally administered 30 min before glucose loading, and the plasma glucose concentration was then measured over 2 h. The reductions in glucose excursion (ΔAUC from 0 to 2 h) were 34.0% and 40.2% at 10 and 30 mg/kg, respectively (Figure 3). The glucose-lowering effect was not increased in a dose-dependent manner, probably because of the low solubility or the maximum effect of **54g**. Together, these results suggest that compound **54g** could potentially be useful as a therapeutic agent in patients with T2DM.

Table 4

Pharmacokinetic parameters of **54g** in SD rats

Compound	IV (3 mg/kg)[a]			PO (10 mg/kg)[b]	
	CL (mL/h/kg)	Vdss (mL/kg)	$t_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng · h/mL)	%F
54g	931	1010	0.842	9510	88.6

[a] Dosing vehicle: 20% 2-hydroxypropyl-β-cyclodextrin

[b] Dosing vehicle: 0.5% methylcellulose containing 0.1% Tween80

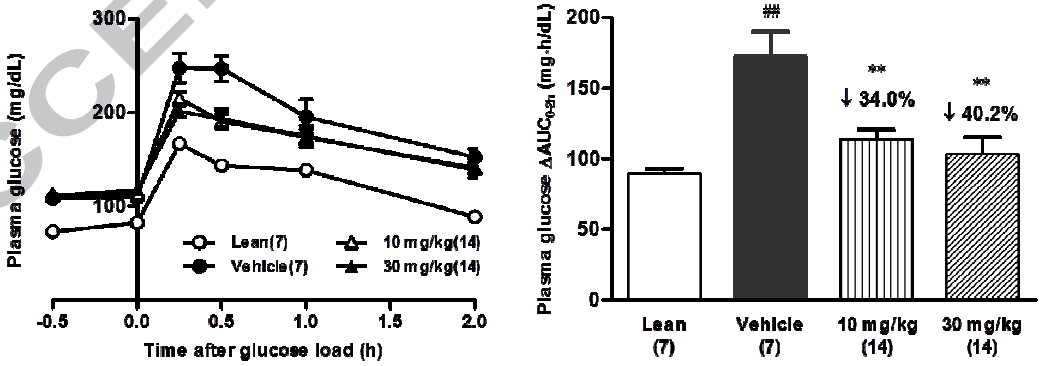

Figure 3. Effects of oral administration of **54g** (10 or 30 mg/kg) on plasma glucose during oGTT in Zucker diabetic fatty rats. Each point represents the mean ± SE. The numbers in parentheses indicate the number of rats per group. $^{\#\#}P < 0.01$ versus Lean, Welch's t-test. $^{**}P < 0.01$ versus vehicle,

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Dunnett's test.

4. Conclusion

In our medicinal chemistry effort toward the identification of an orally active small molecule GPR119 agonist, we designed and synthesized spiro-piperidine derivetives with the aim of increasing solubility and bioavailability by introducing three-dimensionality into the molecule, which resulted in the discovery of a series of novel 7-azaspiro[3.5]nonane derivatives. An optimization study for the right piperidine *N*-capping group (R^1) as well as the left aryl group (R^3) led to the identification of compound **54g**, which possesses good PK properties (including oral bioavailability) in SD rats and exhibits a glucose-lowering effect at 10 mg/kg (po) in Zucker diabetic fatty rats, suggesting its potential for the treatment of T2DM. Further optimization of **54g** aimed at improving its solubility is ongoing, and will be reported in due course.

5. Experimental section

5.1. Human GPR119 agonist activity

GPR119 agonists were evaluated in Flp-In-T-Rex-HEK293 cells overexpressing human GPR119. The cells were treated with tetracycline for 24 h and plated on to 96-well plates at 5000 cells/well in assay buffer (D-MEM, 1 mM 3-isobutyl-1-methylxanthine, 0.01% bovine serum albumin), then incubated with the test compound for 30 min at 37 ˚C. Changes in the cellular cAMP levels were measured using a cAMP HiRange assay kit (Cisbio), according to the manufacturer's protocol. Responses were determined by subtracting the basal cAMP levels from the agonist-stimulated cAMP levels. The EC_{50} values were determined as the concentration of the test compound required to achieve 50% of the maximal response. Data were calculated from the dose-response curves using XLfit software (IDBS).

5.2. Solubility in pH6.8 phosphate buffer

An excess amount of each compound was added to pH6.8 phosphate buffer and shaken on a shaker (model SR-2DS; TAITEC) at 25 °C for 24 h. The suspensions were centrifuged at 3000 and 11000 rpm for 10 min, and the resulting supernatant was diluted with 50% aqueous acetonitrile solution. The concentrations were measured using HPLC. The HPLC analysis was performed using a Shimadzu HPLC system composed of a LC-20AD, SPD-20A and SIL-20AC. The conditions for HPLC were as follows: mobile phase, 0.1% phosphoric acid aqueous solution/acetonitrile; flow rate, 0.8 mL/min; column, reversed- phase (Shimpack XR-ODS, 2.2 μm, 3.0 x 75 mm; Shimadzu) at 40 °C; and detection wavelength, 210 nm.

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5.3. Solubility in fasted state simulated intestinal fluid (FaSSIF)

An excess amount of each compound was added to FaSSIF (pH6.5)[21] and shaken on a shaker (model SR-2s; Yamato Kagaku) at 25 °C for 2 h and then kept at 37 °C for 22 h in a water bath (model LT-10s; Yamato Kagaku). The suspensions were centrifuged at 3000 and 11000 rpm for 10 min, and the supernatant was diluted with 50% aqueous acetonitrile solution or an acetonitrile and methanol mixture (1:1). The concentrations were measured using HPLC. The HPLC analysis was performed using a Shimadzu HPLC system composed of a LC-20AD, SPD-20A and SIL-20AC. The conditions for HPLC were as follows: mobile phase, 0.1% phosphoric acid aqueous solution/acetonitrile; flow rate, 0.8 mL/min; column, reversed- phase (Shimpack XR-ODS, 2.2 μm, 2.0 x 75 mm; Shimadzu) at 40 °C; and detection wavelength, 210 nm.

5.4. Pharmacokinetic evaluation

The pharmacokinetic profile of the test article was investigated in fasted male Sprague-Dawley (SD) rats. After a single intravenous or oral administration of the test article, blood was obtained from the tail vein at each sampling time point and centrifuged to prepare the plasma samples. The quantitative analysis of the target analyte in the plasma samples was performed using liquid chromatography-tandem mass spectrometry. The pharmacokinetic parameters were calculated using a non-compartmental analysis with Phoenix WinNonlin (pharmacokinetic analysis software).

5.5. Oral glucose tolerance test (oGTT) in Zucker diabetic fatty rats

Male Zucker diabetic fatty and lean rats (9 weeks of age) were fasted overnight. Compound **54g** was dissolved in 20 w/v% HP-β-CD and administered orally. After 30 min, glucose solution was orally administered at 2 g/kg body weight. Blood samples were collected from the tail vein, and the plasma glucose levels were determined using the Glucose CII test (Wako Pure Chemical Industries).

5.6. Chemistry

All the solvents and reagents were obtained from commercial suppliers and were used without further purification or were prepared according to published procedures. The melting points were taken with a Yanaco micro-melting point apparatus MP-500D and were uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JOEL JNM-ECA600, Varian Inova300, or Gemini2000, and all the chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using a Shimadzu LCMS 2010 EV spectrometer. High resolution mass spectral data were acquired using a Shimadzu LCMS-IT-TOF equipped with an ESI/APCI dual ion source. Purity was determined by LC-MS (Agilent 1290 Infinity) *via* the following conditions. Column: Waters ACQUITY UPLC CSH C18, 2.1 x 50 mm,

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1.7 µm. Flow: 0.8 ml/min. Gradient: 20 to 99% MeCN/water (0.1% formic acid) linear gradient in 1.2 min, held at 99% for 0.2 min. Detector: UV at 254 nm. All final compounds were obtained with ≥95% purity.

*tert-Butyl 1-(hydroxymethyl)-6-azaspiro[2.5]octane-6-carboxylate (**8**)*

To a solution of 6-*tert*-butyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate (2.0 g, 7.1 mmol) in tetrahydrofuran (THF) (70 mL) was added diisobutylaluminum hydride (DIBAL) (1.0 M in toluene, 20 mL, 20 mmol) under ice cooling. After stirring under ice cooling for 1 h, the reaction was quenched with water (1.4 mL). To the mixture were added 2.5 M NaOH aqueous solution (1.4 mL) and water (4.3 mL), and the mixture was stirred at room temperature overnight. The mixture was filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30-60% EtOAc in hexanes) to afford **8** as a colorless oil (1.8 g, quantitative yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.25 (t, J = 4.7 Hz, 1H), 0.58 (dd, J = 8.6, 4.7 Hz, 1H), 0.92-1.05 (m, 1H), 1.12-1.73 (m, 13H), 3.21-3.37 (m, 2H), 3.49-3.80 (m, 4H); MS ESI/APCI Dual *m/z* 264 $[M+Na]^+$.

*tert-Butyl 1-formyl-6-azaspiro[2.5]octane-6-carboxylate (**9**)*

To a solution of **8** (40 mg, 0.17 mmol) in $CHCl_3$ (1.7 mL) was added Dess-Martin periodinane (91 mg, 0.22 mmol). After stirring at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous Na_2SO_3 and $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20-50% EtOAc in hexanes) to afford **9** as a colorless oil (37 mg, 93% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.13 (dd, J = 7.8, 4.5 Hz, 1H), 1.42-1.51 (m, 12H), 1.64-1.75 (m, 2H), 1.80-1.85 (m, 1H), 3.24-3.31 (m, 1H), 3.41-3.55 (m, 3H), 7.26 (s, 1H); MS ESI/APCI Dual *m/z* 262 $[M+Na]^+$.

*tert-Butyl 1-[2-methoxyethenyl]-6-azaspiro[2.5]octane-6-carboxylate (**10**)*

To a solution of $MeOCH_2P^+Ph_3Cl^-$ (689 mg, 2.01 mmol) in toluene (5.15 mL) was added *t*-BuOK (226 mg, 2.01 mmol) and the mixture was stirred at room temperature for 1 h. To the mixture was added a solution of **9** (370 mg, 1.55 mmol) in toluene (3.09 mL) under ice cooling, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl solution under ice cooling and the resulting mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10-20% EtOAc in hexanes) to afford **10** as a colorless oil (257

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mg, 62% yield, a mixture of cis/trans isomers).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.28-0.36 (m, 1H), 0.59-0.68 (m, 0.5H), 0.71-0.78 (m, 0.5H), 1.15-1.62 (m, 14H), 3.31-3.48 (m, 4H), 3.50 (s, 1.5H), 3.62 (s, 1.5H), 4.03-4.11 (m, 0.5H), 4.58-4.68 (m, 0.5H), 5.95-6.00 (m, 0.5H), 6.31-6.39 (m, 0.5H); MS ESI/APCI Dual *m/z* 290 $[M+Na]^+$.

*tert-Butyl 1-(2-oxoethyl)-6-azaspiro[2.5]octane-6-carboxylate (**11**)*

To a solution of **10** (200 mg, 0.748 mmol) in CH_3CN (7.48 mL) were added water (1.87 mL) and trifluoroacetic acid (TFA) (0.256 g, 2.24 mmol) and the mixture was stirred at room temperature for 7 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution under ice cooling and organic solvent was removed *in vacuo*. The aqueous residue was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20-50% EtOAc in hexanes) to afford **11** as a colorless oil (110 mg, 58% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.16 (t, J = 5.0 Hz, 1H), 0.65-0.69 (m, 1H), 0.91-0.97 (m, 1H), 1.16-1.22 (m, 1H), 1.25-1.32 (m, 1H), 1.46 (s, 9H), 1.49-1.57 (m, 2H), 2.36-2.43 (m, 1H), 2.47-2.54 (m, 1H), 3.21-3.29 (m, 2H), 3.54-3.70 (m, 2H), 9.82 (br s, 1H); MS ESI/APCI Dual *m/z* 276 $[M+Na]^+$.

*tert-Butyl 1-(2-hydroxyethyl)-6-azaspiro[2.5]octane-6-carboxylate (**12**)*

To a solution of **11** (100 mg, 0.395 mmol) in MeOH (1.97 mL) was added $NaBH_4$ (17.9 mg, 0.474 mmol). After stirring at room temperature for 1 h, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (40-60% EtOAc in hexanes) to afford **12** as a colorless oil (74.0 mg, 73% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.04-0.12 (m, 1H), 0.47-0.56 (m, 1H), 0.57-0.71 (m, 1H), 1.06-1.18 (m, 1H), 1.20-1.66 (m, 13H), 1.70-1.87 (m, 1H), 3.16-3.30 (m, 2H), 3.51-3.79 (m, 4H); MS ESI/APCI Dual *m/z* 278 $[M+Na]^+$.

*tert-Butyl 1-{2-[4-(methanesulfonyl)phenoxy]ethyl}-6-azaspiro[2.5]octane-6-carboxylate (**13**)*

To a solution of **12** (74 mg, 0.29 mmol) in THF (2.9 mL) were added 4-(methanesulfonyl)phenol (75 mg, 0.43 mmol), *N*,*N*,*N*',*N*'-tetramethylazodicarboxamide (TMAD) (75 mg, 0.43 mmol) and tributylphosphine (88 mg, 0.43 mmol). After stirring at 60 ˚C for 2 h, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (30-50% EtOAc in hexanes) to afford **13** as a colorless solid (95 mg, 80% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.12 (t, J = 5.0 Hz, 1H), 0.56 (dd, J = 8.7, 4.5 Hz, 1H), 0.72-0.79

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(m, 1H), 1.09-1.16 (m, 1H), 1.30-1.37 (m, 1H), 1.47 (s, 9H), 1.49-1.56 (m, 1H), 1.59-1.73 (m, 2H), 1.96-2.05 (m, 1H), 3.01-3.05 (m, 3H), 3.19-3.26 (m, 2H), 3.59-3.74 (m, 2H), 4.06-4.13 (m, 2H), 7.00-7.04 (m, 2H), 7.84-7.89 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 17.1, 20.2, 21.6, 28.5, 30.4, 36.8, 43.5 (br), 44.9, 68.7, 79.4, 115.0, 129.6, 132.2, 155.0, 163.2; HRMS ESI/APCI Dual *m/z* calcd for $C_{21}H_{31}NO_5S$ 410.1996 $[M+H]^+$, found 410.1981.

*tert-Butyl 1-(iodomethyl)-6-azaspiro[2.5]octane-6-carboxylate (**14**)*

Under argon atmosphere, to a solution of PPh_3 (936 mg, 3.57 mmol) in $CHCl_3$ (27.0 mL) were added imidazole (423 mg, 6.21 mmol) and I_2 (867 mg, 3.41 mmol) under ice cooling. After the mixture being stirred at 0 ˚C for 20 min, a solution of **8** (749 g, 3.10 mmol) in $CHCl_3$ (9.00 mL) was added to the mixture. The mixture was allowed to warm to room temperature and was stirred for 3 h. The reaction mixture was diluted with $CHCl_3$ and washed with saturated aqueous $Na_2S_2O_3$ solution and then washed with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5-10% EtOAc in hexanes) to afford **14** as a colorless oil (950 mg, 87% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.23 (t, J = 4.8 Hz, 1H), 0.74 (dd, J = 8.4, 4.8 Hz, 1H), 1.12-1.41 (m, 3H), 1.43-1.59 (m, 10H), 1.64-1.79 (m, 1H), 3.07-3.43 (m, 4H), 3.51-3.81 (m, 2H); MS ESI/APCI Dual *m/z* 374 $[M+Na]^+$.

*tert-Butyl 1-(3-tert-butoxy-3-oxopropyl)-6-azaspiro[2.5]octane-6-carboxylate (**15**)*

Under argon atmosphere, to a solution of diisopropylamine (1.5 mL, 11 mmol) in THF (27 mL) was added *n*-BuLi (2.76 M in hexane, 3.9 mL, 11 mmol) under ice cooling. After being stirred at 0 ˚C for 30 min, the mixture was cooled to -78 ˚C and *t*-BuOAc (1.5 mL, 11 mmol) was added to the mixture. After the mixture being stirred at -78 ˚C for 30 min, a solution of **14** (0.95 g, 2.7 mmol) in THF (14 mL) and *N*,*N*'-dimethylpropyleneurea (DMPU) (1.3 mL) were added to the mixture and the mixture was stirred at -78 ˚C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution and the resulting mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4-10% EtOAc in hexanes) to afford **15** (0.77 g, 84% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.01-0.07 (m, 1H), 0.44-0.51 (m, 1H), 0.53-0.67 (m, 1H), 1.05-1.85 (m, 24H), 2.23-2.37 (m, 2H), 3.18-3.30 (m, 2H), 3.49-3.70 (m, 2H); MS ESI/APCI Dual *m/z* 340 $[M+H]^+$, 362 $[M+Na]^+$.

*tert-Butyl 1-(3-hydroxypropyl)-6-azaspiro[2.5]octane-6-carboxylate (**16**)*

To a solution of **15** (770 mg, 2.27 mmol) in THF (23.0 mL) was added $LiBH_4$ (137 mg, 5.67 mmol) and the mixture was stirred at 60 ˚C overnight. The reaction was quenched with saturated aqueous

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NH_4Cl solution and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4-65% EtOAc in hexanes) to afford **16** as a colorless oil (203 mg, 28% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.02-0.04 (m, 1H), 0.43-0.52 (m, 1H), 0.53-0.64 (m, 1H), 1.04-1.17 (m, 1H), 1.18-1.36 (m, 3H), 1.39-1.77 (m, 13H), 3.14-3.31 (m, 2H), 3.48-3.76 (m, 4H); MS ESI/APCI Dual *m/z* 292 $[M+Na]^+$.

*tert-Butyl 1-{3-[4-(methanesulfonyl)phenoxy]propyl}-6-azaspiro[2.5]octane-6-carboxylate (**17**)*

The title compound was synthesized according to the procedure described for compound **13** from **16** (96% yield).

Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.01-0.06 (m, 1H), 0.46-0.55 (m, 1H), 0.56-0.68 (m, 1H), 1.05-1.18 (m, 1H), 1.21-1.73 (m, 14H), 1.86-2.00 (m, 2H), 3.03 (s, 3H), 3.16-3.28 (m, 2H), 3.55-3.71 (m, 2H), 4.06 (t, J = 6.4 Hz, 2H), 6.96-7.04 (m, 2H), 7.83-7.89 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 17.4, 21.7, 23.2, 25.3, 28.6, 29.5, 30.3, 36.9, 43.5 (br), 44.9, 68.2, 79.3, 115.0, 129.6, 132.2, 155.1, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{22}H_{33}NO_5S$ 424.2152 $[M+H]^+$, found 424.2144.

*tert-Butyl 2-(2-ethoxy-2-oxoethylidene)-7-azaspiro[3.5]nonane-7-carboxylate (**20**)*

To a solution of $(EtO)_2POCH_2CO_2Et$ (0.822 mL, 4.11 mmol) in *N*,*N*-dimethylformamide (DMF) (10.0 mL) was added NaH (60% in oil, 164 mg, 4.11 mmol) under ice cooling. After the mixture being stirred for 30 min, a solution of *tert*-butyl 2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (**18**) (655 mg, 2.74 mmol) in DMF (4.00 mL) was added to the mixture and the mixture was allowed to warm to room temperature. After stirring at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (9-15% EtOAc in hexanes) to afford **20** as a colorless oil (800 mg, 94% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.27 (t, J = 7.0 Hz, 3H), 1.46 (s, 9H), 1.52-1.62 (m, 4H), 2.55-2.61 (m, 2H), 2.85-2.92 (m, 2H), 3.25-3.44 (m, 4H), 4.15 (q, J = 7.0 Hz, 2H), 5.67-5.73 (m, 1H); MS ESI/APCI Dual *m/z* 332 $[M+Na]^+$.

*tert-Butyl 2-(2-ethoxy-2-oxoethyl)-7-azaspiro[3.5]nonane-7-carboxylate (**22**)*

To a solution of **20** (797 mg, 2.58 mmol) in EtOH (13.0 mL) was added $Pd(OH)_2$ (20% on carbon, 199 mg), and the mixture was stirred under hydrogen atmosphere at room temperature overnight. The mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced

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pressure to afford **22** as a colorless oil (730 mg, 91% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.25 (t, J = 7.0 Hz, 3H), 1.37-1.68 (m, 15H), 1.98-2.09 (m, 2H), 2.41 (d, J = 7.6 Hz, 2H), 2.57-2.72 (m, 1H), 3.19-3.39 (m, 4H), 4.11 (q, J = 7.0 Hz, 2H); MS ESI/APCI Dual *m/z* 334 $[M+Na]^+$.

*tert-Butyl 2-(2-hydroxyethyl)-7-azaspiro[3.5]nonane-7-carboxylate (**24**)*

To a solution of **22** (732 mg, 2.35 mmol) in THF (12.0 mL) was added $LiBH_4$ (174 mg, 8.88 mmol) and the mixture was stirred under reflux for 5 h. The reaction was quenched with water under ice cooling and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford **24** as a colorlessoil (580 mg, 92% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35-1.49 (m, 13H), 1.51-1.62 (m, 2H), 1.63-1.74 (m, 2H), 1.94-2.03 (m, 2H), 2.25-2.43 (m, 1H), 3.20-3.38 (m, 4H), 3.52-3.64 (m, 2H); MS ESI/APCI Dual *m/z* 292 $[M+Na]^+$.

*tert-Butyl 2-{2-[4-(methanesulfonyl)phenoxy]ethyl}-7-azaspiro[3.5]nonane-7-carboxylate (**26**)*

The title compound was synthesized according to the procedure described for compound **13** from **24** (57% yield).

Colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.40-1.62 (m, 15H), 1.85-2.09 (m, 4H), 2.36-2.52 (m, 1H), 3.03 (s, 3H), 3.22-3.40 (m, 4H), 3.97 (t, J = 6.5 Hz, 2H), 6.95-7.03 (m, 2H), 7.82-7.90 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 25.9, 28.5, 34.5, 36.3, 36.8, 37.9, 39.7, 40.7 (br), 44.9, 66.9, 79.3, 114.9, 129.6, 132.2, 155.0, 163.2; HRMS ESI/APCI Dual *m/z* calcd for $C_{22}H_{33}NO_5S$ 424.2152 $[M+H]^+$, found 424.2144.

*tert-Butyl 9-(2-ethoxy-2-oxoethylidene)-3-azaspiro[5.5]undecane-3-carboxylate (**21**)*

The title compound was synthesized according to the procedure described for compound **20** from *tert*-butyl 9-oxo-3-azaspiro[5.5]undecane-3-carboxylate (**19**) (77% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.27 (t, J = 7.1 Hz, 3H), 1.41-1.59 (m, 17H), 2.17-2.27 (m, 2H), 2.79-2.90 (m, 2H), 3.34-3.45 (m, 4H), 4.14 (q, J = 7.1 Hz, 2H), 5.63 (s, 1H); MS ESI/APCI Dual *m/z* 360 $[M+Na]^+$.

*tert-Butyl 9-(2-ethoxy-2-oxoethyl)-3-azaspiro[5.5]undecane-3-carboxylate (**23**)*

The title compound was synthesized according to the procedure described for compound **22** from **21** (92% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.06-1.74 (m, 25H), 2.20 (d, J = 7.0 Hz, 2H), 3.30-3.41 (m, 4H), 4.12 (q, J = 7.0 Hz, 2H); MS ESI/APCI Dual *m/z* 362 $[M+Na]^+$.

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*tert-Butyl 9-(2-hydroxyethyl)-3-azaspiro[5.5]undecane-3-carboxylate (**25**)*

The title compound was synthesized according to the procedure described for compound **24** from **23** (quantitative yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.77-1.72 (m, 24H), 3.29-3.39 (m, 4H), 3.69 (t, J = 6.7 Hz, 2H); MS ESI/APCI Dual *m/z* 320 $[M+Na]^+$.

*tert-Butyl 9-{2-[4-(methanesulfonyl)phenoxy]ethyl}-3-azaspiro[5.5]undecane-3-carboxylate (**27**)*

The title compound was synthesized according to the procedure described for compound **13** from **25** (64% yield).

Colorless amorphous; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.01-1.36 (m, 6H), 1.40-1.80 (m, 18H), 3.03 (s, 3H), 3.29-3.44 (m, 4H), 4.02-4.11 (m, 2H), 6.98-7.04 (m, 2H), 7.81-7.90 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 27.9, 28.5, 31.0, 31.7 (br), 34.8, 35.6, 36.0, 40.1 (br), 44.9, 66.6, 79.2, 115.0, 129.6, 132.1, 155.1, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{37}NO_5S$ 452.2465 $[M+H]^+$, found 452.2455.

*tert-Butyl 2-(methoxymethylidene)-7-azaspiro[3.5]nonane-7-carboxylate (**28**)*

Under argon atmosphere, to a suspension of $MeOCH_2P^+Ph_3Cl^-$ (32.0 g, 93.4 mmol) in toluene (200 mL) was added *t*-BuOK (10.5 g, 93.4 mmol) and the mixture was stirred at room temperature for 20 min. To this mixture was added a solution of *tert*-butyl 2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (**18**) (17.2 g, 71.9 mmol) in toluene (160 mL), and the mixture was stirred at 70 ˚C for 4 h. The reaction was quenched with saturated aqueous NH_4Cl solution under ice cooling and the resulting mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5-20% EtOAc in hexanes) to afford **28** as a pale yellow oil (12.8 g, 67% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.52-1.59 (m, 4H), 2.29-2.47 (m, 4H), 3.26-3.37 (m, 4H), 3.53-3.57 (m, 3H), 5.80-5.87 (m, 1H); MS ESI/APCI Dual *m/z* 290 $[M+Na]^+$.

*tert-Butyl 2-[(1E)-3-ethoxy-3-oxoprop-1-en-1-yl]-7-azaspiro[3.5]nonane-7-carboxylate (**30**)*

To a solution of **28** (12.8 g, 47.9 mmol) in CH_3CN (456 mL) were added water (114 mL) and trifluoroacetic acid (TFA) (4.00 mL) and the mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution under ice cooling and organic solvent was removed *in vacuo*. The resulting aqueous residue was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford *tert*-butyl 2-formyl-7-azaspiro[3.5]nonane-7-carboxylate.

To a solution of $(EtO)_2POCH_2CO_2Et$ (11.0 mL, 57.5 mmol) in DMF (60.0 mL) and THF (60.0 mL)

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was added NaH (60% in oil, 2.30 g, 57.5 mmol) under ice cooling. After the mixture being stirred for 20 min, a solution of *tert*-butyl 2-formyl-7-azaspiro[3.5]nonane-7-carboxylate in DMF (60.0 mL) and THF (60.0 mL) was added to the mixture and the mixture was allowed to warm to room temperature. After stirring at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl solution under ice cooling and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5-20% EtOAc in hexanes) to afford **30** as a colorless oil (13.6 g, 87% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.1 Hz, 3H), 1.45 (s, 9H), 1.53-1.78 (m, 4H), 2.01-2.16 (m, 4H), 2.98-3.14 (m, 1H), 3.23-3.40 (m, 4H), 4.19 (q, J = 7.1 Hz, 2H), 5.74 (dd, J = 15.5, 1.6 Hz, 1H), 7.05 (dd, J = 15.5, 6.7 Hz, 1H); MS ESI/APCI Dual *m/z* 346 $[M+Na]^+$.

*tert-Butyl 2-(3-ethoxy-3-oxopropyl)-7-azaspiro[3.5]nonane-7-carboxylate (**32**)*

To a solution of **30** (13.6 g, 41.9 mmol) in EtOH (420 mL) was added $Pd(OH)_2$ (20% on carbon, 2.72 g), and the mixture was stirred under hydrogen atmosphere at room temperature overnight. The mixture was filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5-30% EtOAc in hexanes) to afford **32** as a yellow solid (13.3 g, 97% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.25 (t, J = 7.1 Hz, 3H), 1.32-1.61 (m, 15H), 1.67-1.78 (m, 2H), 1.88-2.00 (m, 2H), 2.13-2.28 (m, 3H), 3.19-3.38 (m, 4H), 4.12 (q, J = 7.1 Hz, 2H); MS ESI/APCI Dual *m/z* 348 $[M+Na]^+$.

*tert-Butyl 2-(3-hydroxypropyl)-7-azaspiro[3.5]nonane-7-carboxylate (**34**)*

To a solution of **32** (10.4 g, 31.8 mmol) in THF (160 mL) was added DIBAL (1.0 M in toluene, 80.0 mL, 80.0 mmol) under ice cooling and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with saturated aqueous citric acid solution and extracted with EtOAc. The organic layer was washed with saturated aqueous citric acid solution, 1 M HCl aqueous solution and brine, and then dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (40-70% EtOAc in hexanes) to afford **34** as a colorless oil (6.64 g, 74% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31-1.60 (m, 19H), 1.89-2.00 (m, 2H), 2.10-2.31 (m, 1H), 3.19-3.39 (m, 4H), 3.56-3.68 (m, 2H); MS ESI/APCI Dual *m/z* 306 $[M+Na]^+$.

*tert-Butyl 2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**36a**)*

The title compound was synthesized according to the procedure described for compound **13** from **34** (85% yield).

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Colorless solid (mp 154-155 ˚C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34-1.49 (m, 13H), 1.51-1.63 (m, 5H), 1.64-1.80 (m, 2H), 1.90-2.04 (m, 2H), 2.16-2.35 (m, 1H), 3.03 (s, 3H), 3.21-3.40 (m, 4H), 4.00 (t, *J* = 6.5 Hz, 2H), 6.95-7.03 (m, 2H), 7.82-7.91 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 26.9, 28.5, 28.6, 34.0, 36.5, 38.0, 39.7, 41.0 (br), 44.9, 68.5, 79.2, 114.9, 129.6, 132.1, 155.0, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{23}H_{35}NO_5S$ 438.2309 $[M+H]^+$, found 438.2297.

*tert-Butyl 2-{3-[2-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**36b**)*

The title compound was synthesized according to the procedure described for compound **13** from **34** and 2-fluoro-4-(methanesulfonyl)phenol (51% yield).

Colorless solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.24-1.67 (m, 17H), 1.69-1.86 (m, 2H), 1.90-2.07 (m, 2H), 2.14-2.38 (m, 1H), 3.04 (s, 3H), 3.20-3.41 (m, 4H), 4.08 (t, *J* = 6.4 Hz, 2H), 6.98-7.13 (m, 1H), 7.57-7.74 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 26.7, 28.5, 33.9, 34.0, 36.4, 37.9, 39.7, 41.0 (br), 44.7, 69.6, 79.2, 114.0, 115.6 (d, *J* = 21.0 Hz), 124.6 (d, *J* = 5.9 Hz), 132.3, 151.9 (d, *J* = 252.0 Hz), 151.8 (d, *J* = 12.0 Hz), 155.0; HRMS ESI/APCI Dual *m/z* calcd for $C_{23}H_{34}FNO_5S$ 456.2214 $[M+H]^+$, found 456.2199.

*tert-Butyl 2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**36c**)*

The title compound was synthesized according to the procedure described for compound **13** from **34** and 3-fluoro-4-(methanesulfonyl)phenol (86% yield).

Colorless solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.26-1.64 (m, 17H), 1.64-1.84 (m, 2H), 1.90-2.05 (m, 2H), 2.12-2.38 (m, 1H), 3.18 (s, 3H), 3.21-3.40 (m, 4H), 3.99 (t, *J* = 6.2 Hz, 2H), 6.64-6.85 (m, 2H), 7.76-7.94 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 26.8, 28.5, 34.0, 34.0, 36.5, 38.0, 39.7, 40.9 (br), 44.2, 69.0, 79.3, 103.3 (d, *J* = 27.0 Hz), 110.6, 120.2, 131.0, 155.1, 160.8 (d, *J* = 252.0 Hz), 165.2; HRMS ESI/APCI Dual *m/z* calcd for $C_{23}H_{34}FNO_5S$ 456.2214 $[M+H]^+$, found 456.2203.

*tert-Butyl 9-(methoxymethylidene)-3-azaspiro[5.5]undecane-3-carboxylate (**29**)*

The title compound was synthesized according to the procedure described for compound **28** from *tert*-butyl 9-oxo-3-azaspiro[5.5]undecane-3-carboxylate (**19**) (44% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36-1.44 (m, 8H), 1.45 (s, 9H), 1.90-1.99 (m, 2H), 2.12-2.23 (m, 2H), 3.34-3.42 (m, 4H), 3.53 (s, 3H), 5.77 (t, *J* = 1.2 Hz, 1H); MS ESI/APCI Dual *m/z* 318 $[M+Na]^+$.

*tert-Butyl 9-(-3-ethoxy-3-oxoprop-1-en-1-yl)-3-azaspiro[5.5]undecane-3-carboxylate (**31**)*

The title compound was synthesized according to the procedure described for compound **30** from

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29 (82% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.06-1.80 (m, 24H), 2.06-2.23 (m, 1H), 3.28-3.44 (m, 4H), 4.19 (q, J = 7.2 Hz, 2H), 5.74-5.82 (m, 1H), 6.86-6.98 (m, 1H); MS ESI/APCI Dual *m/z* 374 $[M+Na]^+$.

*tert-Butyl 9-(3-ethoxy-3-oxopropyl)-3-azaspiro[5.5]undecane-3-carboxylate (**33**)*

The title compound was synthesized according to the procedure described for compound **32** from **31** (90% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.99-1.82 (m, 27H), 2.23-2.38 (m, 2H), 3.27-3.42 (m, 4H), 4.12 (q, J = 7.2 Hz, 2H); MS ESI/APCI Dual *m/z* 376 $[M+Na]^+$.

*tert-Butyl 9-(3-hydroxypropyl)-3-azaspiro[5.5]undecane-3-carboxylate (**35**)*

To a solution of **33** (470 mg, 1.33 mmol) in THF (7.00 mL) and toluene (7.00 mL) was added $LiBH_4$ (87.0 mg, 3.99 mmol) and the mixture was stirred at 60 ˚C for 16 h. The reaction was quenched with saturated aqueous NH_4Cl solution and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0-20% EtOAc in hexanes) to afford **35** as a colorless oil (370 mg, 89% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.98-1.73 (m, 26H), 3.28-3.40 (m, 4H), 3.57-3.67 (m, 2H); MS ESI/APCI Dual *m/z* 334 $[M+Na]^+$.

*tert-Butyl 9-{3-[4-(methanesulfonyl)phenoxy]propyl}-3-azaspiro[5.5]undecane-3-carboxylate (**37**)*

The title compound was synthesized according to the procedure described for compound **13** from **35** (61%yield).

Colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.02-1.20 (m, 4H), 1.20-1.43 (m, 6H), 1.45 (s, 9H), 1.52-1.63 (m, 3H), 1.64-1.73 (m, 2H), 1.76-1.92 (m, 2H), 3.03 (s, 3H), 3.29-3.43 (m, 4H), 4.01 (t, J = 6.5 Hz, 2H), 6.97-7.03 (m, 2H), 7.82-7.89 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 26.5, 28.0, 28.5, 31.1, 31.6, 33.2, 35.7, 37.7, 39.5 (br), 40.1, 44.9, 68.9, 79.2, 115.0, 129.6, 132.1, 155.1, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{25}H_{39}NO_5S$ 466.2622 $[M+H]^+$, found 466.2612.

*tert-Butyl 2-[(2E)-4-ethoxy-4-oxobut-2-en-1-yl]-7-azaspiro[3.5]nonane-7-carboxylate (**38**)*

To a solution of **24** (526 mg, 1.95 mmol) in $CHCl_3$ (10.0 mL) was added Dess-Martin periodinane (1.10 g, 2.54 mmol) under ice cooling and the mixture was stirred at room temperature for 2 h. The residue was diluted with $CHCl_3$ and washed with saturated aqueous Na_2SO_3 and $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford *tert*-butyl 2-(2-oxoethyl)-7-azaspiro[3.5]nonane-7-carboxylate.

To a solution of $(EtO)_2POCH_2CO_2Et$ (0.552 mL, 2.93 mmol) in DMF (10.0 mL) was added NaH

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(60% in oil, 117 mg, 2.93 mmol) under ice cooling. After the mixture being stirred for 20 min, a solution of *tert*-butyl 2-(2-oxoethyl)-7-azaspiro[3.5]nonane-7-carboxylate in DMF (3.00 mL) was added to the mixture and the mixture was allowed to warm to room temperature. After stirring at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (8-15% EtOAc in hexanes) to afford **38** as a colorless oil (360 mg, 55% yield).
^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.1 Hz, 3H), 1.36-1.49 (m, 13H), 1.50-1.60 (m, 2H), 1.93-2.06 (m, 2H), 2.23-2.46 (m, 3H), 3.21-3.37 (m, 4H), 4.18 (q, J = 7.1 Hz, 2H), 5.73-5.83 (m, 1H), 6.78-6.94 (m, 1H); MS ESI/APCI Dual *m/z* 360 $[M+Na]^+$.

*tert-Butyl 2-(4-ethoxy-4-oxobutyl)-7-azaspiro[3.5]nonane-7-carboxylate (**39**)*

The title compound was synthesized according to the procedure described for compound **32** from **38** (96% yield).
^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.25 (t, J = 7.0 Hz, 3H), 1.30-1.61 (m, 19H), 1.89-2.00 (m, 2H), 2.12-2.31 (m, 3H), 3.19-3.39 (m, 4H), 4.12 (q, J = 7.0 Hz, 2H); MS ESI/APCI Dual *m/z* 362 $[M+Na]^+$.

*tert-Butyl 2-(4-hydroxybutyl)-7-azaspiro[3.5]nonane-7-carboxylate (**40**)*

The title compound was synthesized according to the procedure described for compound **16** from **39** (92% yield).
^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.16-1.61 (m, 21H), 1.87-1.99 (m, 2H), 2.10-2.30 (m, 1H), 3.20-3.38 (m, 4H), 3.58-3.70 (m, 2H); MS ESI/APCI Dual *m/z* 320 $[M+Na]^+$.

*tert-Butyl 2-{4-[4-(methanesulfonyl)phenoxy]butyl}-7-azaspiro[3.5]nonane-7-carboxylate (**41**)*

The title compound was synthesized according to the procedure described for compound **13** from **40** (92% yield).
Colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.28-1.60 (m, 19H), 1.71-1.85 (m, 2H), 1.89-2.01 (m, 2H), 2.16-2.29 (m, 1H), 3.03 (s, 3H), 3.22-3.38 (m, 4H), 4.01 (t, J = 6.5 Hz, 2H), 6.97-7.04 (m, 2H), 7.81-7.90 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 23.7, 28.5, 28.8, 29.0, 34.0, 36.5, 37.6, 38.0, 39.8, 40.9 (br), 44.9, 68.6, 79.2, 115.0, 129.6, 132.1, 155.1, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{37}NO_5S$ 452.2465 $[M+H]^+$, found 452.2456.

*Propan-2-yl 2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**42a**)*

To a suspension of **36a** (2.7 g, 6.1 mmol) in EtOAc (31 mL) was added 4 M HCl in EtOAc (31 mL). After stirring at room temperature overnight, the mixture was concentrated *in vacuo*. To the resulting

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residue were added 1 M NaOH aqueous solution and $CHCl_3$. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford 2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (2.0 g).

To a solution of the above crude material (60 mg, 0.18 mmol) in $CHCl_3$ (2.0 mL) were added Et_3N (0.050 mL, 0.36 mmol) and isopropyl chloroformate (0.031 mL, 0.27 mmol) under ice cooling. After stirring at room temperature for 6 h, the reaction mixture was quenched with water and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (25-40% EtOAc in hexanes) and solidified with EtOAc and hexanes to afford **42a** as a colorless solid (47 mg, 62% yield).

Mp 130 ˚C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.20-1.25 (m, 6H), 1.37-1.43 (m, 2H), 1.43-1.50 (m, 2H), 1.54-1.61 (m, 4H), 1.69-1.78 (m, 2H), 1.95-2.01 (m, 2H), 2.20-2.32 (m, 1H), 3.03 (s, 3H), 3.25-3.44 (m, 4H), 4.00 (t, J = 6.6 Hz, 2H), 4.86-4.94 (m, 1H), 6.97-7.02 (m, 2H), 7.81-7.87 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 26.9, 28.6, 34.0, 36.4, 37.9, 39.7, 40.6, 40.9, 44.9, 68.3, 68.4, 114.9, 129.6, 132.1, 155.4, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{22}H_{33}NO_5S$ 424.2152 $[M+H]^+$, found 424.2147.

Propan-2-yl

*2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**42c**)*

The title compound was synthesized according to the procedure described for compound **42a** from **36c** (47% yield).

Colorless solid (mp 105-106 ˚C); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.35-1.62 (m, 8H), 1.69-1.77 (m, 2H), 1.95-2.02 (m, 2H), 2.21-2.30 (m, 1H), 3.18 (s, 3H), 3.25-3.42 (m, 4H), 3.99 (t, J = 6.4 Hz, 2H), 4.86-4.94 (m, 1H), 6.68-6.74 (m, 1H), 6.76-6.80 (m, 1H), 7.82-7.88 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 26.7, 28.5, 33.9, 34.0, 36.3, 37.9, 39.6, 40.6, 40.8, 44.2, 68.3, 69.0, 103.3 (d, J = 24.0 Hz), 110.6, 120.1 (d, J = 15.0 Hz), 131.0, 155.4, 160.8 (d, J = 255.3 Hz), 165.2 (d, J = 12.0 Hz); HRMS ESI/APCI Dual *m/z* calcd for $C_{22}H_{32}FNO_5S$ 442.2058 $[M+H]^+$, found 442.2051.

1-Methylcyclopropyl

*2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**43c**)*

The title compound was synthesized according to the procedure described for compound **42a** from **36c** and 1-methylcyclopropyl 4-nitrophenyl carbonate (31% yield).

Colorless solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.59-0.64 (m, 2H), 0.83-0.88 (m, 2H), 1.35-1.60 (m, 11H), 1.70-1.76 (m, 2H), 1.95-2.01 (m, 2H), 2.21-2.30 (m, 1H), 3.19 (s, 3H), 3.21-3.45 (m, 4H), 3.99 (t, J = 6.4 Hz, 2H), 6.68-6.75 (m, 1H), 6.77-6.81 (m, 1H), 7.81-7.89 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 13.0, 21.8, 26.7, 28.5, 33.9, 36.3, 37.9, 39.6, 40.8 (br s), 44.2, 56.3, 69.0, 103.3

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(d, *J* = 24.0 Hz), 110.6, 120.1 (d, *J* = 15.0 Hz), 131.0, 155.3, 160.8 (d, *J* = 255.3 Hz), 165.2 (d, *J* = 12.0 Hz); HRMS ESI/APCI Dual *m/z* calcd for $C_{23}H_{32}FNO_5S$ 454.2058 $[M+H]^+$, found 454.2051.

Cyclopentyl
*2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**44c**)*

To a suspension of **36c** (0.42 g, 0.92 mmol) in EtOAc (9.0 mL) was added 4 M HCl in EtOAc (9.0 mL). After stirring at room temperature overnight, the mixture was concentrated *in vacuo* to afford 2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane hydrochloride. To a solution of cyclopentanol (16 mg, 0.18 mmol) in THF (0.90 mL) were added triphosgene (19 mg, 0.064 mmol) and Et_3N (0.051 mL, 0.37 mmol) under ice cooling. After the mixture being stirred for 30 min, 2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane hydrochloride (36 mg, 0.092 mmol) was added to the mixture. The mixture was allowed to warm to room temperature and was stirred overnight. The mixture was then quenched with water and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20-35% EtOAc in hexanes) to afford **44c** as a colorless solid (24 mg, 55% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.35-1.48 (m, 4H), 1.49-1.63 (m, 6H), 1.66-1.77 (m, 6H), 1.79-1.90 (m, 2H), 1.94-2.01 (m, 2H), 2.20-2.31 (m, 1H), 3.18 (s, 3H), 3.24-3.44 (m, 4H), 3.98 (t, *J* = 6.4 Hz, 2H), 5.06-5.12 (m, 1H), 6.68-6.74 (m, 1H), 6.76-6.81 (m, 1H), 7.81-7.88 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 23.7, 26.8, 28.5, 32.9, 34.0, 34.0, 36.4, 38.0, 39.7, 40.7, 40.9, 44.2, 69.0, 77.7, 103.3 (d, *J* = 27.0 Hz), 110.6, 120.2 (d, *J* = 18.0 Hz), 131.0, 155.6, 160.8 (d, *J* = 252.3 Hz), 165.2 (d, *J* = 9.0 Hz); HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{34}FNO_5S$ 468.2214 $[M+H]^+$, found 468.2209.

2,2-Dimethylpropyl
*2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**45c**)*

The title compound was synthesized according to the procedure described for compound **44c** from **36c** and 2,2-dimethylpropan-1-ol (51% yield).

Colorless solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.94 (s, 9H), 1.36- 1.63 (m, 8H), 1.69-1.77 (m, 2H), 1.95-2.03 (m, 2H), 2.20-2.31 (m, 1H), 3.18 (s, 3H), 3.31-3.37 (m, 2H), 3.39-3.45 (m, 2H), 3.76 (s, 2H), 3.99 (t, *J* = 6.6 Hz, 2H), 6.69-6.74 (m, 1H), 6.76-6.81 (m, 1H), 7.82-7.88 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 26.6, 26.8, 28.5, 31.6, 34.0, 34.0, 36.3 (br), 38.0, 39.7 (br), 40.8, 41.0, 44.2, 69.0, 74.7, 103.3 (d, *J* = 24.0 Hz), 110.6, 120.2 (d, *J* = 15.0 Hz), 131.0, 155.9, 160.8 (d, *J* = 255.0 Hz), 165.2 (d, *J* = 9.0 Hz); HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{36}FNO_5S$ 470.2371 $[M+H]^+$, found 470.2362.

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*7-(5-Ethylpyrimidin-2-yl)-2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (**46a**)*

To a suspension of **36a** (0.19 g, 0.43 mmol) in EtOAc (4.0 mL) was added 4 M HCl in EtOAc (4.0 mL). After stirring at room temperature for 5 h, the mixture was concentrated *in vacuo*. To a solution of the residue in DMSO (4.0 mL) were added 2-chloro-5-ethylpyrimidine (0.10 mL, 0.87 mmol) and Cs_2CO_3 (0.71 g, 2.2 mmol), and the mixture was stirred at 180 ˚C under microwave irradiation for 20 min. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (30-50% EtOAc in hexanes) to afford **46a** as a pale yellow solid (145 mg, 75% yield).

Mp 165-166 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.17 (t, J = 7.6 Hz, 3H), 1.37-1.82 (m, 10H), 1.97-2.09 (m, 2H), 2.20-2.36 (m, 1H), 2.44 (q, J = 7.5 Hz, 2H), 3.03 (s, 3H), 3.60-3.77 (m, 4H), 4.01 (t, J = 6.5 Hz, 2H), 6.96-7.04 (m, 2H), 7.82-7.89 (m, 2H), 8.15 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 15.7, 22.7, 26.9, 28.6, 34.1, 34.4, 36.4, 38.1, 39.7, 41.0, 41.2, 44.9, 68.5, 115.0, 124.0, 129.6, 132.1, 157.1, 161.0, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{33}N_3O_3S$ 444.2315 $[M+H]^+$, found 444.2307.

*7-(5-ethylpyrimidin-2-yl)-2-{3-[2-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (**46b**)*

The title compound was synthesized according to the procedure described for compound **46a** from **36b** (33% yield).

Colorless solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.11-1.23 (m, 3H), 1.35-1.91 (m, 10H), 1.96-2.10 (m, 2H), 2.20-2.37 (m, 1H), 2.38-2.52 (m, 2H), 3.04 (s, 3H), 3.57-3.80 (m, 4H), 4.09 (t, J = 6.4 Hz, 2H), 6.99-7.13 (m, 1H), 7.56-7.75 (m, 2H), 8.15 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 15.7, 22.7, 26.8, 28.6, 34.0, 34.4, 36.4, 38.1, 39.7, 41.0, 41.2, 44.8, 69.6, 114.1, 115.6 (d, J = 21.0 Hz), 124.0, 124.7, 132.3 (d, J = 5.7 Hz), 151.9 (d, J = 253.0 Hz), 152.0, 157.1, 161.0; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{32}FN_3O_3S$ 462.2221 $[M+H]^+$, found 462.2212.

*7-(5-Ethylpyrimidin-2-yl)-2-{3-[3-fluoro-4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (**46c**)*

The title compound was synthesized according to the procedure described for compound **46a** from **36c** (26% yield).

Colorless solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.11-1.23 (m, 3H), 1.34-1.84 (m, 10H), 1.94-2.11 (m, 2H), 2.19-2.38 (m, 1H), 2.38-2.52 (m, 2H), 3.18 (s, 3H), 3.58-3.80 (m, 4H), 4.00 (t, J = 6.4 Hz, 2H), 6.63-6.84 (m, 2H), 7.78-7.90 (m, 1H), 8.15 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 15.7, 22.7, 26.8, 28.6, 34.0, 34.4, 36.4, 38.1, 39.7, 41.0, 41.2, 44.2, 69.0, 103.3 (d, J = 24.0 Hz), 110.6, 120.1 (d, J = 15.0 Hz), 124.0, 131.0, 157.1, 160.8 (d, J = 255.0 Hz), 161.0, 165.3 (d, J = 12.0

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Hz); HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{32}FN_3O_3S$ 462.2221 $[M+H]^+$, found 462.2213.

*2-{3-[4-(Methanesulfonyl)phenoxy]propyl}-7-(5-methylpyridin-2-yl)-7-azaspiro[3.5]nonane (**47a**)*

To a suspension of **36a** (2.7 g, 6.1 mmol) in EtOAc (31 mL) was added 4 M HCl in EtOAc (31 mL). After stirring at room temperature overnight, the mixture was concentrated *in vacuo*. To the resulting residue were added 1 M NaOH aqueous solution and $CHCl_3$. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford 2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (2.0 g).

A suspension of the above crude material (0.10 g, 0.30 mmol), 2-bromo-5-methylpyridine (61 mg, 0.36 mmol), $Pd_2(dba)_3$ (14 mg, 0.015 mmol) 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (18 mg, 0.030 mmol) and *t*-BuONa (85 mg, 0.89 mmol) in 1,2-dimethoxyethane (3.0 mL) was stirred at 60 ˚C for 9 h. The insoluble material was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10-40% EtOAc in hexanes) and solidified with EtOAc and hexanes to afford **47a** as a colorless solid (26 mg, 20% yield).
^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38-1.47 (m, 2H), 1.50-1.64 (m, 4H), 1.65-1.81 (m, 4H), 1.96-2.06 (m, 2H), 2.18 (s, 3H), 2.22-2.35 (m, 1H), 3.03 (s, 3H), 3.32-3.39 (m, 2H), 3.41-3.48 (m, 2H), 4.01 (t, J = 6.37 Hz, 2H), 6.57-6.62 (m, 1H), 6.97-7.03 (m, 2H), 7.25-7.31 (m, 1H), 7.81-7.89 (m, 2H), 7.96-8.04 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 17.3, 26.9, 28.6, 34.1, 34.2, 36.2, 38.2, 39.4, 42.8, 43.1, 44.9, 68.5, 107.3, 115.0, 121.6, 129.6, 132.1, 138.3, 147.7, 158.4, 163.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{32}N_2O_3S$ 429.2206 $[M+H]^+$, found 429.2206.

*2-{3-[4-(Methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carbonitrile (**48a**)*

To a solution of 2-{3-[4-(methanesulfonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane (0.10 g, 0.30 mmol) in $CHCl_3$ (3.0 mL) were added water (1.0 mL) and $NaHCO_3$ (50 mg, 0.59 mmol), then CNBr (36 mg, 0.33 mmol) was added under ice cooling. After stirring overnight at room temperature, the reaction was quenched with water and extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (40-70% EtOAc in hexanes) to afford **48a** as a colorless solid (111 mg, quantitative yield).
^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.38-1.44 (m, 2H), 1.55-1.62 (m, 4H), 1.68-1.76 (m, 4H), 1.96-2.02 (m, 2H), 2.21-2.31 (m, 1H), 3.03 (s, 3H), 3.06-3.11 (m, 2H), 3.14-3.19 (m, 2H), 4.00 (t, J = 6.4 Hz, 2H), 6.96-7.02 (m, 2H), 7.83-7.88 (m, 2H).

*2-{3-[4-(Methanesulfonyl)phenoxy]propyl}-7-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]-7-azaspiro[3.5]nonane (**49a**)*

To a solution of **48a** (0.11 g, 0.32 mmol) in THF (1.0 mL) were added

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N'-hydroxy-2-methylpropanimidamide (39 mg, 0.38 mmol) and 1 M $ZnCl_2$ diethyl ether solution (0.38 mL, 0.38 mmol). After stirring at room temperature for 30 min, the precipitate was collected by filtration. The resulting solid was dissolved in EtOH (2.0 mL) and concentrated HCl solution (2.0 mL). After stirring under reflux for 3 h, the mixture was quenched with saturated $NaHCO_3$ aqueous solution under ice cooling and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (35-50% EtOAc in hexanes) and solidified with EtOAc and hexanes to afford **49a** as a colorless solid (53 mg, 37% yield).

Mp 124-125 ˚C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.28 (d, J = 7.0 Hz, 6H), 1.41-1.48 (m, 2H), 1.54-1.63 (m, 4H), 1.68-1.78 (m, 4H), 2.00-2.06 (m, 2H), 2.23-2.36 (m, 1H), 2.83-2.92 (m, 1H), 3.03 (s, 3H), 3.44-3.48 (m, 2H), 3.51-3.57 (m, 2H), 4.01 (t, J = 6.4 Hz, 2H), 6.97-7.02 (m, 2H), 7.83-7.88 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 20.5, 26.9, 27.1, 28.6, 33.5, 34.0, 35.7, 37.8, 38.9, 43.0, 43.2, 44.9, 68.4, 114.9, 129.6, 132.2, 163.3, 171.0, 175.8; HRMS ESI/APCI Dual *m/z* calcd for $C_{23}H_{33}N_3O_4S$ 448.2265 $[M+H]^+$, found 448.2249.

*Propan-2-yl 2-(3-ethoxy-3-oxopropyl)-7-azaspiro[3.5]nonane-7-carboxylate (**50**)*

To a suspension of **32** (5.7 g, 18 mmol) in EtOAc (88 mL) was added 4 M HCl in EtOAc (88 mL). After stirring at room temperature for 1.5 h, the mixture was concentrated *in vacuo*. The resulting residue was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc and with $CHCl_3$. The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. To an ice cooled solution of the residue in $CHCl_3$ (90 mL) were added Et_3N (4.9 mL, 35 mmol) and a solution of isopropyl chloroformate (2.4 mL, 21 mmol) in $CHCl_3$ (24 mL), and the mixture was stirred at room temperature for 4 h. The reaction was quenched with water and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (15-25% EtOAc in hexanes) to afford **50** as a colorless solid (5.5 g, quantitative yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.15-1.30 (m, 9H), 1.31-1.61 (m, 8H), 1.66-1.78 (m, 2H), 1.89-2.01 (m, 2H), 2.16-2.28 (m, 3H), 3.23-3.41 (m, 4H), 4.12 (q, J = 7.1 Hz, 2H), 4.82-4.96 (m, 1H); MS ESI/APCI Dual *m/z* 312 $[M+H]^+$, 334 $[M+Na]^+$.

*Propan-2-yl 2-(3-hydroxypropyl)-7-azaspiro[3.5]nonane-7-carboxylate (**51**)*

The title compound was synthesized according to the procedure described for compound **34** from **50** (91%yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.4 Hz, 6H), 1.31-1.62 (m, 10H), 1.88-2.02 (m, 2H), 2.12-2.31 (m, 1H), 3.15-3.43 (m, 4H), 3.55-3.68 (m, 2H), 4.80-4.97 (m, 1H); MS ESI/APCI Dual *m/z* 270 $[M+H]^+$, 292 $[M+Na]^+$.

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Propan-2-yl

*2-{3-[3-fluoro-4-(methoxycarbonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**52**)*

The title compound was synthesized according to the procedure described for compound **13** from **51** and methyl 2-fluoro-4-hydroxybenzoate (quantitative yield).

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.31-1.51 (m, 4H), 1.51-1.63 (m, 4H), 1.63-1.81 (m, 2H), 1.90-2.04 (m, 2H), 2.12-2.36 (m, 1H), 3.23-3.44 (m, 4H), 3.89 (s, 3H), 3.96 (t, J = 6.2 Hz, 2H), 4.79-5.00 (m, 1H), 6.55-6.75 (m, 2H), 7.88 (t, J = 8.6 Hz, 1H); MS ESI/APCI Dual m/z 422 $[M+H]^+$, 444 $[M+Na]^+$.

*2-Fluoro-4-[3-(7-{[(propan-2-yl)oxy]carbonyl}-7-azaspiro[3.5]nonan-2-yl)propoxy]benzoic acid (**53**)*

To a solution of **52** (3.10 g, 7.35 mmol) in MeOH (36.8 mL) was added 2 M NaOH aqueous solution (36.8 mL). After the mixture being stirring at 60 ˚C for 3 h, the organic solvent was removed *in vacuo*. The resulting aqueous residue was washed with diethylether, acidified with 1 M HCl aqueous solution and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford **53** as a colorless solid (2.96 g, 99% yield).

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.31-1.51 (m, 4H), 1.52-1.64 (m, 4H), 1.64-1.84 (m, 2H), 1.90-2.07 (m, 2H), 2.11-2.42 (m, 1H), 3.24-3.44 (m, 4H), 3.92-4.05 (m, 2H), 4.82-4.99 (m, 1H), 6.58-6.78 (m, 2H), 7.96 (t, J = 8.8 Hz, 1H); MS ESI/APCI Dual m/z 408 $[M+H]^+$, 406 $[M-H]^-$.

*Propan-2-yl 2-[3-(4-carbamoyl-3-fluorophenoxy)propyl]-7-azaspiro[3.5]nonane-7-carboxylate (**54a**)*

To a solution of **53** (100 mg, 0.245 mmol) in DMF (2.45 mL) were added 28% NH_3 aqueous solution (22.4 mg, 0.368 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (70.5 mg, 0.368 mmol), 1,2,3-benzotriazol-1-ol monohydrate (HOBt·H_2O) (56.4 mg, 0.368 mmol) and Et_3N (37.2 mg, 0.368 mmol). After the mixture being stirring at room temperature for 15 h, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50% EtOAc in hexanes) and solidified with $CHCl_3$ and hexanes to afford **54a** as a colorless solid (89 mg, 89% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.36-1.42 (m, 2H), 1.43-1.50 (m, 2H), 1.52-1.61 (m, 4H), 1.67-1.76 (m, 2H), 1.93-2.02 (m, 2H), 2.21-2.31 (m, 1H), 3.25-3.33 (m, 2H), 3.34-3.44 (m, 2H), 3.97 (t, J = 6.6 Hz, 2H), 4.86-4.94 (m, 1H), 5.74 (br. s., 1H), 6.52-6.66 (m, 2H),

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6.77 (dd, *J* = 8.7, 2.5 Hz, 1H), 8.06 (t, *J* = 9.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 26.8, 28.6, 34.0, 36.4, 38.0, 39.7, 40.6, 40.9, 68.3, 68.6, 102.0 (d, *J* = 27.0 Hz), 111.2, 112.3 (d, *J* = 12.0 Hz), 133.5, 155.4, 162.1 (d, *J* = 249.3 Hz), 163.6 (d, *J* = 12.0 Hz), 164.8; HRMS ESI/APCI Dual *m/z* calcd for $C_{22}H_{31}FN_2O_4$ 407.2341 $[M+H]^+$, found 407.2340.

*Propan-2-yl 2-{3-[4-(cyclopropylcarbamoyl)-3-fluorophenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**54b**)*

The title compound was synthesized according to the procedure described for compound **54a** from **53** and cyclopropanamine (68% yield).

Colorless solid (mp 94 °C); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.58-0.63 (m, 2H), 0.83-0.89 (m, 2H), 1.23 (d, *J* = 6.2 Hz, 6H), 1.36-1.42 (m, 2H), 1.42-1.49 (m, 2H), 1.52-1.60 (m, 4H), 1.67-1.74 (m, 2H), 1.95-2.00 (m, 2H), 2.21-2.28 (m, 1H), 2.89-2.95 (m, 1H), 3.27-3.32 (m, 2H), 3.35-3.41 (m, 2H), 3.95 (t, *J* = 6.4 Hz, 2H), 4.87-4.93 (m, 1H), 6.57 (dd, *J* = 14.0, 2.5 Hz, 1H), 6.68-6.74 (m, 1H), 6.75 (dd, *J* = 9.1, 2.5 Hz, 1H), 8.05 (t, *J* = 9.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 6.9, 22.3, 23.1, 26.9, 28.6, 34.0, 34.0, 36.4, 38.0, 39.7, 40.6, 40.9, 68.3, 68.5, 101.9 (d, *J* = 27.0 Hz), 111.1, 113.1 (d, *J* = 12.0 Hz), 133.2, 155.4, 161.7 (d, *J* = 246.3 Hz), 163.0 (d, *J* = 12.0 Hz), 164.6; HRMS ESI/APCI Dual *m/z* calcd for $C_{25}H_{35}FN_2O_4$ 447.2654 $[M+H]^+$, found 447.2650.

*Propan-2-yl 2-(3-{4-[cyclopropyl(methyl)carbamoyl]-3-fluorophenoxy}propyl)-7-azaspiro[3.5]nonane-7-carboxylate (**54c**)*

To a solution of **54b** (80 mg, 0.18 mmol) in DMF (1.8 mL) was added NaH (60% in oil, 11 mg, 0.27 mmol) under ice cooling. After the mixture being stirred at room temperature for 30 min, MeI (51 mg, 0.36 mmol) was added to the mixture. After stirring at room temperature for 2 h, the reaction was quenched with water and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (33-50% EtOAc in hexanes) and solidified with EtOAc and hexanes to afford **54c** as a colorless solid (24 mg, 29% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.39-0.47 (m, 2H), 0.49-0.59 (m, 2H), 1.23 (d, *J* = 6.2 Hz, 6H), 1.35-1.42 (m, 2H), 1.43-1.49 (m, 2H), 1.53-1.61 (m, 4H), 1.67-1.74 (m, 2H), 1.94-2.01 (m, 2H), 2.21-2.30 (m, 1H), 2.78-2.85 (m, 1H), 3.10 (br. s., 3H), 3.27-3.33 (m, 2H), 3.35-3.42 (m, 2H), 3.93 (t, *J* = 6.4 Hz, 2H), 4.86-4.94 (m, 1H), 6.57 (dd, *J* = 11.8, 2.2 Hz, 1H), 6.70 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.27-7.33 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 8.6, 22.3, 28.6, 31.8, 34.0, 34.1, 34.8, 38.0, 39.7, 40.6, 40.9, 68.3, 68.4, 76.9, 101.8 (d, *J* = 27.0 Hz), 110.7, 118.5, 130.0, 155.4, 159.4 (d, *J* = 249.2 Hz), 161.4 (d, *J* = 12.0 Hz), 168.7; HRMS ESI/APCI Dual *m/z* calcd for $C_{26}H_{37}FN_2O_4$

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461.2810 $[M+H]^+$, found 461.2797.

Propan-2-yl

*2-{3-[3-fluoro-4-(pyrrolidine-1-carbonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**54d**)*

The title compound was synthesized according to the procedure described for compound **54a** from **53** and pyrrolidine (62% yield).

Colorless solid (mp 60-61 ˚C); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.36-1.42 (m, 2H), 1.42-1.49 (m, 2H), 1.52-1.62 (m, 4H), 1.66-1.73 (m, 2H), 1.84-1.91 (m, 2H), 1.92-2.01 (m, 4H), 2.20-2.30 (m, 1H), 3.27-3.32 (m, 2H), 3.34 (t, J = 6.6 Hz, 2H), 3.36-3.41 (m, 2H), 3.63 (t, J = 7.0 Hz, 2H), 3.92 (t, J = 6.4 Hz, 2H), 4.86-4.93 (m, 1H), 6.58 (dd, J = 11.8, 2.4 Hz, 1H), 6.70 (dd, J = 8.7, 2.4 Hz, 1H), 7.34 (t, J = 8.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 24.6, 26.0, 26.9, 28.6, 34.0, 34.1, 36.4, 38.0, 39.7, 40.6, 40.9, 46.0, 47.9, 68.3, 68.4, 102.1 (d, J = 24.0 Hz), 110.9, 117.9 (d, J = 18.0 Hz), 130.0 (d, J = 6.0 Hz), 155.4, 159.4 (d, J = 246.2 Hz), 161.5 (d, J = 12.0 Hz), 165.3; HRMS ESI/APCI Dual *m/z* calcd for $C_{26}H_{37}FN_2O_4$ 461.2810 $[M+H]^+$, found 461.2793.

Propan-2-yl

*2-{3-[4-(azetidine-1-carbonyl)-3-fluorophenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**54e**)*

The title compound was synthesized according to the procedure described for compound **54a** from **53** and azetidine (75% yield).

Colorless solid (mp 68-69 ˚C); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.35-1.42 (m, 2H), 1.42-1.49 (m, 2H), 1.51-1.59 (m, 4H), 1.67-1.73 (m, 2H), 1.93-2.01 (m, 2H), 2.20-2.35 (m, 3H), 3.27-3.34 (m, 2H), 3.35-3.42 (m, 2H), 3.93 (t, J = 6.6 Hz, 2H), 4.11 (t, J = 7.6 Hz, 2H), 4.19 (t, J = 7.6 Hz, 2H), 4.86-4.94 (m, 1H), 6.57 (dd, J = 12.0, 2.5 Hz, 1H), 6.71 (dd, J = 8.7, 2.5 Hz, 1H), 7.48 (t, J = 8.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 15.7, 22.3, 26.9, 28.6, 34.0, 34.1, 36.4, 38.0, 39.7, 40.6, 40.9, 48.6, 51.1, 68.3, 68.4, 102.0 (d, J = 27.0 Hz), 110.9, 114.2 (d, J = 18.0 Hz), 131.2 (d, J = 6.0 Hz), 155.4, 160.2 (d, J = 249.3 Hz), 162.3 (d, J = 9.0 Hz), 166.7; HRMS ESI/APCI Dual *m/z* calcd for $C_{25}H_{35}FN_2O_4$ 447.2654 $[M+H]^+$, found 447.2645.

Propan-2-yl

*2-{3-[3-fluoro-4-(3-hydroxyazetidine-1-carbonyl)phenoxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**54f**)*

The title compound was synthesized according to the procedure described for compound **54a** from **53** and azetidin-3-ol (77% yield).

Colorless solid (mp 106-107 ˚C); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.35-1.42 (m, 2H), 1.42-1.49 (m, 2H), 1.51-1.60 (m, 4H), 1.65-1.75 (m, 2H), 1.93-2.01 (m, 2H),

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2.20-2.30 (m, 1H), 2.64-2.88 (m, 1H), 3.23-3.33 (m, 2H), 3.34-3.43 (m, 2H), 3.93 (t, *J* = 6.4 Hz, 2H), 3.98-4.05 (m, 2H), 4.23-4.32 (m, 1H), 4.37-4.47 (m, 1H), 4.65-4.73 (m, 1H), 4.85-4.93 (m, 1H), 6.57 (dd, *J* = 12.4, 2.1 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.49 (t, *J* = 8.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 26.8, 28.5, 33.9, 34.0, 36.3, 37.9, 39.6, 40.6, 40.8, 58.3, 60.8, 61.6, 68.4, 68.4, 102.0 (d, *J* = 26.9 Hz), 111.0, 113.9 (d, *J* = 15.0 Hz), 131.2, 155.4, 160.2 (d, *J* = 252.0 Hz), 162.4 (d, *J* = 12.0 Hz), 166.7; HRMS ESI/APCI Dual *m/z* calcd for $C_{25}H_{35}FN_2O_5$ 463.2603 $[M+H]^+$, found 463.2595.

*Propan-2-yl 2-(3-{4-[(2-amino-2-oxoethyl)carbamoyl]-3-fluorophenoxy}propyl)-7-azaspiro[3.5]nonane-7-carboxylate (**54g**)*

The title compound was synthesized according to the procedure described for compound **54a** from **53** and glycinamide (58% yield).

Colorless solid (mp 141-142 ˚C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (d, *J* = 6.2 Hz, 6H), 1.33-1.49 (m, 4H), 1.50-1.63 (m, 4H), 1.65-1.79 (m, 2H), 1.92-2.03 (m, 2H), 2.18-2.33 (m, 1H), 3.24-3.43 (m, 4H), 3.97 (t, *J* = 6.3 Hz, 2H), 4.14-4.19 (m, 2H), 4.84-4.95 (m, 1H), 5.42 (br. s., 1H), 6.06 (br. s., 1H), 6.59-6.66 (m, 1H), 6.75-6.80 (m, 1H), 7.99-8.07 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 22.3, 26.8, 28.6, 34.0, 36.4, 38.0, 39.7, 40.6, 40.9, 43.5, 68.3, 68.6, 102.0 (d, *J* = 27.1 Hz), 111.3, 112.4 (d, *J* = 12.0 Hz), 133.0, 155.4, 162.0 (d, *J* = 246.3 Hz), 163.5 (d, *J* = 12.0 Hz), 163.8, 171.1; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{34}FN_3O_5$ 464.2555 $[M+H]^+$, found 464.2543.

*Propan-2-yl 2-{3-[(6-cyanopyridin-3-yl)oxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**55a**)*

The title compound was synthesized according to the procedure described for compound **13** from **51** and 5-hydroxypyridine-2-carbonitrile (62% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (d, *J* = 6.2 Hz, 6H), 1.33-1.64 (m, 8H), 1.67-1.82 (m, 2H), 1.93-2.05 (m, 2H), 2.17-2.35 (m, 1H), 3.24-3.44 (m, 4H), 4.04 (t, *J* = 6.4 Hz, 2H), 4.83-4.97 (m, 1H), 7.17-7.23 (m, 1H), 7.59-7.66 (m, 1H), 8.32-8.37 (m, 1H); MS ESI/APCI Dual *m/z* 372 $[M+H]^+$, 394 $[M+Na]^+$.

*5-[3-(7-{[(Propan-2-yl)oxy]carbonyl}-7-azaspiro[3.5]nonan-2-yl)propoxy]pyridine-2-carboxylic acid (**56a**)*

To a solution of **55a** (200 mg, 0.538 mmol) in EtOH (6.00 mL) was added 2 M NaOH aqueous solution (6.00 mL), and the mixture was stirred at 100 ˚C for 13 h. The reaction mixture was cooled to room temperature, then the reaction was quenched with 2 M HCl aqueous solution (8.00 mL) and the resulting mixture was extracted with $CHCl_3$. The organic layer was concentrated *in vacuo* to

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afford **56a** as a colorless solid (169 mg, 79% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.2 Hz, 6H), 1.34-1.66 (m, 8H), 1.67-1.84 (m, 2H), 1.93-2.06 (m, 2H), 2.19-2.38 (m, 1H), 3.26-3.42 (m, 4H), 4.06 (t, J = 6.5 Hz, 2H), 4.84-4.96 (m, 1H), 7.30-7.36 (m, 1H), 8.14-8.19 (m, 1H), 8.23-8.25 (m, 1H).

*Propan-2-yl 2-(3-{[6-(cyclopropylcarbamoyl)pyridin-3-yl]oxy}propyl)-7-azaspiro[3.5]nonane-7-carboxylate (**57a**)*

The title compound was synthesized according to the procedure described for compound **54a** from **56a** and cyclopropanamine (83% yield).

Colorless solid (mp 74-75 ˚C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.60-0.68 (m, 2H), 0.81-0.91 (m, 2H), 1.23 (d, J = 6.2 Hz, 6H), 1.33-1.50 (m, 4H), 1.51-1.80 (m, 6H), 1.92-2.04 (m, 2H), 2.16-2.36 (m, 1H), 2.85-2.98 (m, 1H), 3.21-3.45 (m, 4H), 4.01 (t, J = 6.4 Hz, 2H), 4.82-4.97 (m, 1H), 7.22-7.26 (m, 1H), 7.86 (br. s, 1H), 8.09-8.17 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 6.6, 22.3, 22.5, 26.9, 28.6, 34.0, 36.4, 38.0, 39.7, 40.6, 40.9, 68.3, 68.5, 120.7, 123.2, 136.7, 142.5, 155.4, 157.4, 165.7; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{35}N_3O_4$ 430.2700 $[M+H]^+$, found 430.2691.

*Propan-2-yl 2-{3-[(5-cyanopyridin-2-yl)oxy]propyl}-7-azaspiro[3.5]nonane-7-carboxylate (**55b**)*

The title compound was synthesized according to the procedure described for compound **13** from **51** and 6-hydroxypyridine-3-carbonitrile (54% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (d, J = 6.4 Hz, 6H), 1.33-1.61 (m, 8H), 1.63-1.76 (m, 2H), 1.91-2.02 (m, 2H), 2.18-2.33 (m, 1H), 3.25-3.43 (m, 4H), 4.32 (t, J = 6.7 Hz, 2H), 4.84-4.95 (m, 1H), 6.76-6.82 (m, 1H), 7.73-7.79 (m, 1H), 8.45-8.48 (m, 1H); MS ESI/APCI Dual *m/z* 372 $[M+H]^+$, 394 $[M+Na]^+$.

*6-[3-(7-{[(Propan-2-yl)oxy]carbonyl}-7-azaspiro[3.5]nonan-2-yl)propoxy]pyridine-3-carboxylic acid (**56b**)*

The title compound was synthesized according to the procedure described for compound **56a** from **55b** (95% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.17-1.27 (m, 6H), 1.32-1.61 (m, 8H), 1.64-1.79 (m, 2H), 1.88-2.05 (m, 2H), 2.16-2.37 (m, 1H), 3.24-3.45 (m, 4H), 4.35 (t, J = 6.6 Hz, 2H), 4.85-4.96 (m, 1H), 6.71-6.80 (m, 1H), 8.13-8.24 (m, 1H), 8.86-8.92 (m, 1H).

*Propan-2-yl 2-(3-{[5-(cyclopropylcarbamoyl)pyridin-2-yl]oxy}propyl)-7-azaspiro[3.5]nonane-7-carboxylate (**57b**)*

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The title compound was synthesized according to the procedure described for compound **54a** from **56b** and cyclopropanamine (83% yield).

Colorless solid (mp 76-77 ˚C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.58-0.66 (m, 2H), 0.80-0.91 (m, 2H), 1.17-1.26 (m, 6H), 1.32-1.47 (m, 4H), 1.48-1.60 (m, 4H), 1.62-1.76 (m, 2H), 1.90-2.03 (m, 2H), 2.16-2.35 (m, 1H), 2.81-2.94 (m, 1H), 3.21-3.44 (m, 4H), 4.24-4.34 (m, 2H), 4.82-4.97 (m, 1H), 6.41-6.62 (m, 1H), 6.67-6.77 (m, 1H), 7.93-8.01 (m, 1H), 8.50-8.56 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 6.8, 22.3, 23.1, 26.8, 28.6, 34.0, 34.1, 36.4, 38.0, 39.7, 40.7, 40.9, 66.6, 68.3, 110.9, 123.4, 137.7, 146.3, 155.4, 165.9, 167.1; HRMS ESI/APCI Dual *m/z* calcd for $C_{24}H_{35}N_3O_4$ 430.2700 $[M+H]^+$, found 430.2694.

Acknowledgements

The authors thank Daisuke Yamamoto for contributing to the oGTT study, Naoto Ohsaki for collecting the solubility data, and Satoshi Wakabayashi for collecting the ADME data.

References

1. International Diabetes Federation, *IDF diabetes atlas seventh edition 2015,* http://www.diabetesatlas.org/.
2. Koro, C. E.; Bowlin, S. J.; Bourgeois, N.; Fedder, D. O. *Diabetes Care* **2004**, *27*, 17.
3. Stein, S. A.; Lamos, E. M.; Davis, S. N. *Expert Opin. Drug Saf.* **2013**, *12*, 153.
4. Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C. T.; Gardner, L. S.; Griffin, G.; Jackson, H. C.; Procter, M. J.; Rasamison, C. M.; Tang-Christensen, M.; Widdowson, P. S.; Williams, G. M.; Reynet, C. *Cell Metabolism* **2006**, *3*, 167.
5. Soga, T.; Ohishi, T.; Matsui, T.; Saito, T.; Matsumoto, M.; Takasaki, J.; Matsumoto, S.; Kamohara, M.; Hiyama, H.; Yoshida, S.; Momose, K.; Ueda, Y.; Matsushime, H.; Kobori, M.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 744.
6. Chu, Z. L.; Jones, R. M.; He, H.; Carroll, C.; Gutierrez, V.; Lucman, A.; Moloney,M.; Gao, H.; Mondala, H.; Bagnol, D.; Unett, D.; Liang, Y.; Demarest, K.; Semple,G.; Behan, D. P.; Leonard, *J. Endocrinology* **2007**, *148*, 2601
7. Chu, Z.-L.; Carroll, C.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Pedraza, M.;Mondala, H.; Gao, H.; Bagnol, D.; Chen, R.; Jones, R. M.; Behan, D. P.; Leonard, J. *Endocrinology* **2008**, *149*, 2038.
8. Farilla, L.; Bulotta, A.; Hirshberg, B.; Li Calzi, S.; Khoury, N.; Noushmehr, H.; Bertoloto, C.; Di Mario, U.; Harlan, D. M.; Perfetti, R. *Endocrinology* **2003**, *144*, 5149.
9. Trümper, A.; Trümper, K.; Trusheim, A.; Arnold, R.; Göke, B.; Horsch, D. *Molecular Endocrinology* **2001**, *15*, 1559.
10. Jones, R. M.; Leonard, J. N.; Buzard, D. J.; Lehmann, J. *Expert Opin. Ther. Pat.* **2009**, *19*, 1339.

ACCEPTED MANUSCRIPT

11. Ritter, K.; Buning, C.; Halland, N.; Pöverlein, C.; Schwink, L. *J. Med. Chem.* **2016**, *59*, 3579.
12. Semple, G.; Ren, A.; Fioravanti, B.; Pereira, G.; Calderon, I.; Choi, K.; Xiong, Y.; Sage, C. R.; Morgan, M.; Xing, C.; Chu, Z.; Leonard, J. N.; Grottick, A. J.; Al-Shamma, H.; Jones, R. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3134.
13. Polli, J. W.; Hussey, E.; Bush, M.; Generaux, G.; Smith, G.; Collins, D.; McMullen, S.; Turner, N.; Nunez, D. J. *Xenobiotica* **2013**, *43*, 498.
14. http://clinicaltrials.gov/ct2/show/NCT01035879.
15. Matsuda, D.; Kobashi, Y.; Mikami, A.; Kawamura M.; Shiozawa, F.; Kawabe, K.; Hamada, M.; Oda, K.; Nishimoto, S.; Kimura, K.; Miyoshi, M.; Takayama, N.; Kakinuma, H.; Ohtake, N. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3441.
16. Matsuda, D.; Kobashi, Y.; Mikami, A.; Kawamura M.; Shiozawa, F.; Kawabe, K.; Hamada, M.; Nishimoto, S.; Kimura, K.; Miyoshi, M.; Takayama, N.; Kakinuma, H.; Ohtake, N. *Bioorg. Med. Chem.* **2017**, *25*, 4339.
17. Lovering, F.; Bikker, J.; Humblet, C. *J. Med. Chem.* **2009**, *52*, 6752.
18. Szewczyk, J.W.; Acton, J.; Adams, D. A.; Chicchi, G.; Freeman, S.; Howard, A. D.; Huang, Y.; Li, C.; Meinke, P. T.; Mosely, R.; Murphy, E. Samuel, R. Santini, C.; Yang, M.; Zhang, Y.; Zhao, K.; Wood, H. B. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2665.
19. Liu, P.; Hu, Z.; DuBois, B. G.; Moyes, C. R.; Hunter, D. N.; Zhu, C.; Kar, N. F.; Zhu, Y.; Garfunkle, J.; Kang, L.; Chicchi, G.; Ehrhardt, A.; Woods, A.; Seo, T.; Woods, M.; Heek, M.; Dingley, K. H.; Pang, J.; Salituro, G. M.; Powell, J.; Terebetski, J. L.; Hornak, V.; Campeau, L.; Lamberson, J.; Ujjainwalla, F.; Miller, M.; Stamford, A.; Wood, H. B.; Kowalski, T.; Nargund, R. P.; Edmondson, S. D. *ACS Med. Chem. Lett.* **2015**, *6*, 936.
20. Press, N. J.; Taylor, R. J.; Fullerton, J. D.; Tranter, P.; McCarthy, C.; Keller, T. H; Arnold, N.; Beer, D.; Brown, L.; Cheung, R.; Christie, J.; Denholm, A.; Haberthuer, S.; Hatto, J. D. I.; Keenan, M; Mercer, M. K.; Oakman, H.; Sahri, H.; Tuffnell, A. R.; Tweed, M.; Trifilieff, A. *J. Med. Chem.* **2015**, *58*, 6747.
21. Galia, E.; Nicolaides, E.; Horter, D.; Lobenberg, R.; Reppas, C.; Dressman, J. B. *Pharm. Res.* **1998**, *15*, 698.

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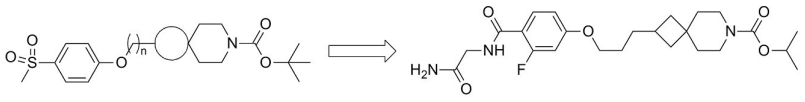

54g

hGPR119 EC_{50}: 48 nM

Glucose lowering effect in diabetic rats

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