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Discovery of Phenylpropanoic Acid Derivatives Containing Polar Functionalities as Potent and Orally Bioavailable G Protein-Coupled Receptor 40 Agonists for the Treatment of Type 2 Diabetes

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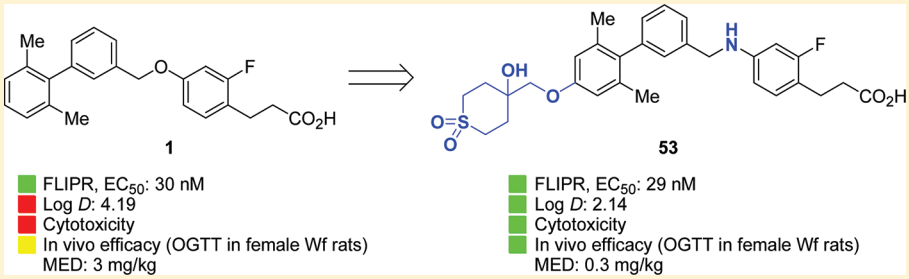

ABSTRACT: As part of a program to identify potent GPR40 agonists with drug-like properties suitable for clinical development, the incorporation of polar substituents was explored with the intention of decreasing the lipophilicity of our recently disclosed phenylpropanoic acid derivative **1**. This incorporation would allow us to mitigate the cytotoxicity issues observed with compound **1** and enable us to move away from the multifunctional free fatty acid-like structure. Substitutions on the 2′,6′-dimethylbiphenyl ring were initially undertaken, which revealed the feasibility of introducing polar functionalities at the biphenyl 4′-position. Further optimization of this position and the linker led to the discovery of several 4′-alkoxybiphenyl derivatives, which showed potent GPR40 agonist activities with the best balance in terms of improved cytotoxicity profiles and favorable pharmacokinetic properties. Among them, 3-{2-fluoro-4-[({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]phenyl}propanoic acid (**35**) exhibited a robust plasma glucose-lowering effect and insulinotropic action during an oral glucose tolerance test in rats with impaired glucose tolerance.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease characterized by defects in insulin secretion from pancreatic β-cells and/or insulin resistance in target tissues of insulin.[1−3] Although the relative importance of diminished insulin secretion and insulin resistance varies among patients with T2DM, many studies indicate that impaired insulin secretion caused by β-cell dysfunction is frequently present in patients.[4] In the clinical management of T2DM, oral insulinotropic agents such as sulfonylureas have been widely prescribed as a mono- or combination therapy.[5,6] However, because sulfonylureas act to stimulate continuous insulin release independently of blood glucose levels, they often elicit hypoglycemia[7,8] and possibly promote the deterioration of islet function, resulting in attenuated efficacy.[9,10] To complement the major drawbacks of sulfonylurea agents currently in use, research exploring novel insulin secretagogues with distinct mechanism of action has attracted a great deal of attention in the pharmaceutical industry and in academia. As a result, novel drugs that potentiate insulin secretion in a glucose-dependent manner, such as dipeptidyl peptidase IV inhibitors and incretin hormone glucagon-like peptide 1 analogues[11] have been recently developed.

Free fatty acids (FFAs) are well-known to amplify glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. Before the discovery of G protein-coupled receptor 40 (GPR40), it was postulated that these effects were mainly mediated by the active metabolites of FFAs.[12−15] GPR40 is highly expressed in pancreatic β-cells and rodent insulin-secreting cell lines and is activated by naturally occurring medium- to long-chain FFAs.[16−18] The mechanism of action of GPR40 remains to be fully elucidated; however, several reports have shown that GPR40 is mainly coupled with the G protein α-subunit of the Gq family (Gαq).[16,17,19] Generally, the activation of Gαq protein-coupled receptors results in increased phospholipase C (PLC) activity and, in turn, inositol 1,4,5-triphosphate (IP_3)-mediated intracellular calcium mobilization and protein kinase C (PKC) activation, which are related to enhanced insulin secretion in pancreatic β-cells.[20,21] In fact, it has been reported that endogenous ligands and small molecule

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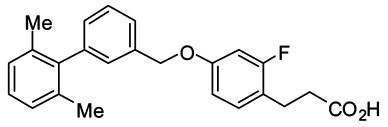
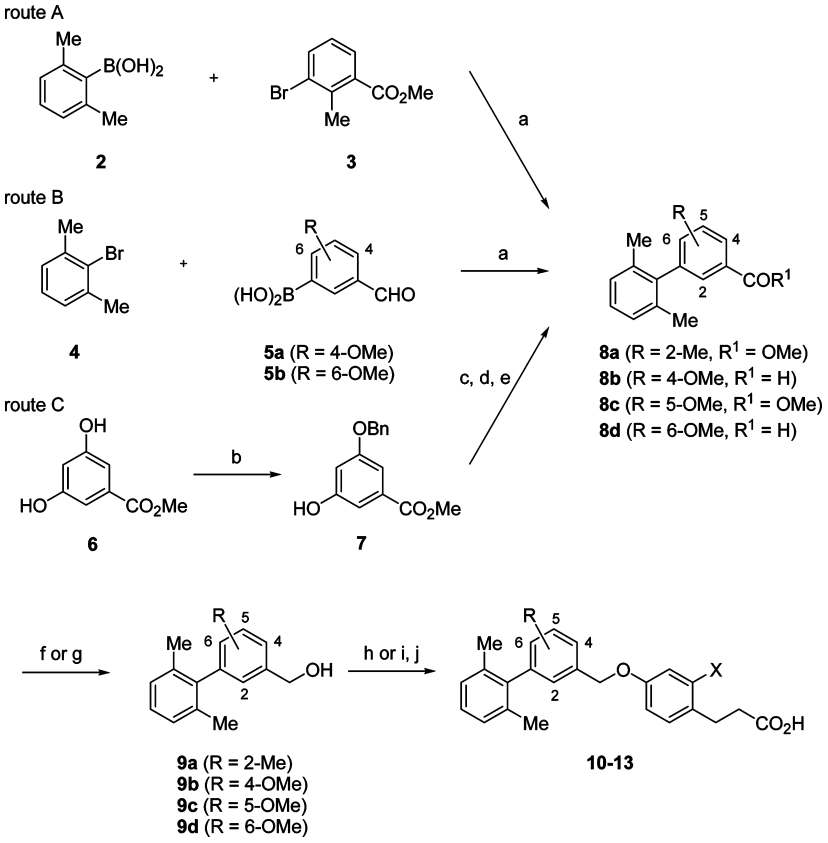


1

binding[a]		h-FLIPR[b]	cytotoxicity		
h-K_i (nM)	r-K_i (nM)	EC_{50} (nM)	ATP[c]	Caspase[d]	Log D[e]
32	54	30 (13–69)	51.8	53.6	4.19

Figure 1. Structure of recently disclosed GPR40 agonist **1** and its profiles. [a]Binding affinities for human and rat GPR40 receptors. All values are average of n = 2 or 3 measurements in the presence of 0.2% BSA. [b]Fluorometric imaging plate reader (FLIPR) functional assay for human GPR40 receptor. The value is an average of n = 3 measurements in the presence of 0.1% BSA. The 95% confidence intervals are shown in parentheses. [c]Intracellular ATP content (%) at 30 μM relative to untreated control. Lowered ATP content relative to untreated control (100%) indicates a decrease in cell viability. [d]Caspase-3/7 activity (%) at 30 μM relative to the response of the reference compound (staurosporine), which was set to 100%. A larger value is interpreted as a higher risk of apoptotic cytotoxicity. [e]The Log D value was determined at pH 7.4 as previously described.[55]

Scheme 1. Synthesis of 2′,6′-Dimethylbiphenyl Derivatives 10–13[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, Cs_2CO_3, toluene, EtOH or MeOH, reflux, 45–87%; (b) benzyl bromide, K_2CO_3, DMF, 70 °C, 46%; (c) (i) $(CF_3SO_2)_2O$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 87%, (ii) **2**, $Pd(PPh_3)_4$, K_3PO_4, DME, 80 °C, 87%; (d) H_2 (balloon pressure), 10% Pd/C, MeOH, THF, rt, 91%; (e) MeI, K_2CO_3, DMF, 50 °C, 93%; (f) LAH, THF, 0 °C or rt, 43–99%; (g) $NaBH_4$, EtOH, 0 °C, 88%–quant; (h) methyl 3-(4-hydroxyphenyl)propanoate, ADDP, *n*-Bu_3P, THF, rt, 71–87%; (i) ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate, ADDP, *n*-Bu_3P, toluene, rt, 87%; (j) KOH or NaOH aq, THF, MeOH or EtOH, rt, 91–98%.

GPR40 agonists increase intracellular calcium concentration via GPR40 and lead to GSIS in pancreatic β-cells.[16–18,22–27] In addition, several reports have shown that synthetic GPR40 agonists stimulate insulin secretion in a glucose-dependent manner and correct impaired glucose tolerance in a single dose in rodents, suggesting that GPR40 agonists would be novel and potential insulinotropic drugs.[26–30]

In our recent publications,[29,30] we described our research efforts that began with a screening hit from our in-house library of compounds selected on the basis of ligand-based drug design, which culminated in the identification of the potent phenylpropanoic acid derivative **1** as a novel GPR40 agonist.[30] Although compound **1** displayed not only favorable agonist activity (EC_{50} = 30 nM) in a fluorometric imaging plate reader (FLIPR)-based functional assay but also significant glucose-lowering action during an oral glucose tolerance test in diabetic rat models, it exhibited cytotoxicity potential in human hepatocellular carcinoma HepG2 cells, as evidenced by both decreased intracellular ATP content and caspase-3/7 induction activity as shown in Figure 1. It is generally recognized that

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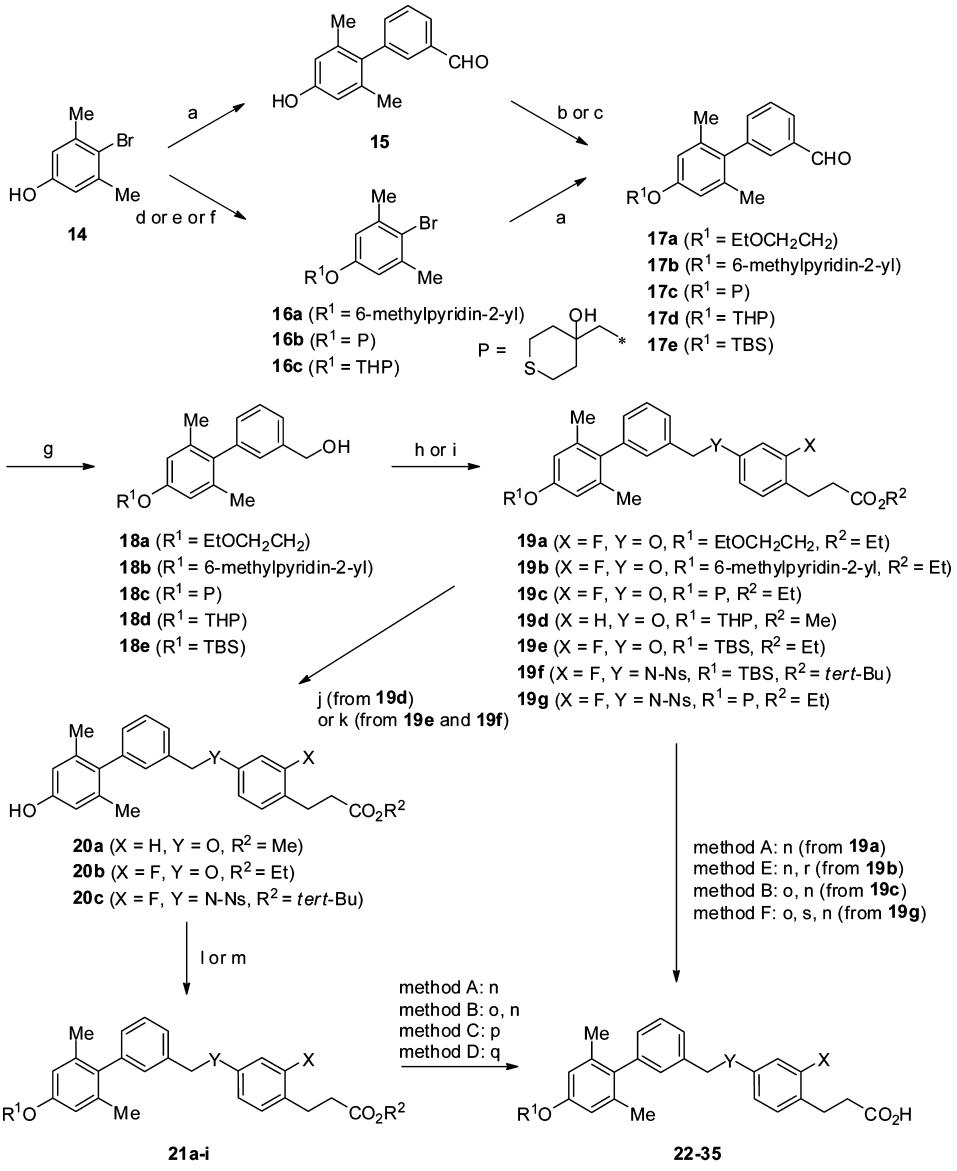
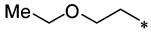
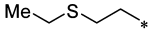
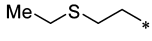
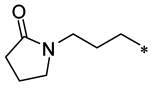
Scheme 2. Synthesis of 4′-Alkoxybiphenyl Derivatives 22−35[a]



compd	R^1	X	Y	R^2	compd	R^1	X	Y	R^2
21a	*c*-PrCH_2	H	O	Me	**21f**		F	O	Et
21b	Bn	H	O	Me	**21g**		F	O	Et
21c		H	O	Me					
21d		F	O	Et	**21h**		F	N-Ns	*tert*-Bu
21e		F	O	Et	**21i**		F	N-Ns	*tert*-Bu

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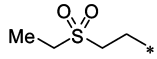
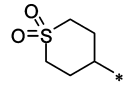
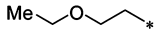
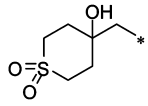
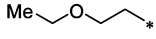
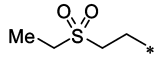
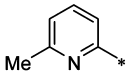
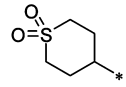
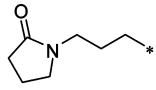
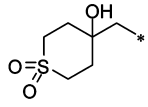
Scheme 2. continued

compd	R^1	X	Y	compd	R^1	X	Y
22	Me	H	O	**30**		F	O
23	*c*-PrCH$_2$	H	O	**31**		F	O
24	Bn	H	O				
25		H	O	**32**		F	O
26		F	O				
27		F	O	**33**		F	NH
28		F	O	**34**		F	NH
29		F	O	**35**		F	NH

[a]Reagents and conditions: (a) (3-formylphenyl)boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH or DME, H_2O, 80 °C, 66−83%; (b) $EtOCH_2CH_2Cl$, K_2CO_3, KI, DMF, 80 °C, 89%; (c) TBSCl, imidazole, rt, 77%; (d) (i) NaOH, MeOH, rt, (ii) Cu, 2-bromo-6-methylpyridine, 185 °C, 74% (2 steps); (e) NaH, 1-oxa-6-thiaspiro[2.5]octane **34**, DMF, 0−80 °C, 44%; (f) 3,4-dihydro-2*H*-pyran, PPTS, CH_2Cl_2, rt, 77%; (g) $NaBH_4$, THF, DME, or MeOH, 0 °C, 83%−quant; (h) ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate or **38a** or **38b**, ADDP, *n*-Bu_3P, THF or toluene, rt, 75−94%; (i) methyl 3-(4-hydroxyphenyl)propanoate or **38b**, DEAD, Ph_3P, THF, rt, 37%; (j) *p*-TsOH·H_2O, MeOH, rt, 88%; (k) TBAF, THF, rt, 79−99%; (l) ROH, DEAD or DIAD, Ph_3P, THF, rt, 51−100%; (m) ROH, ADDP, *n*-Bu_3P, THF, rt, 64−67%; (n) 1 M NaOH aq, THF, MeOH, rt, 49−91%; (o) *m*-CPBA, CH_2Cl_2 or EtOAc, 0 °C to rt, 47−91%; (p) AcOH, concd H_2SO_4, H_2O, 90 °C, 61%; (q) (i) *m*-CPBA, EtOAc, 0 °C to rt, (ii) $HSCH_2CO_2H$, LiOH·H_2O, DMF, rt, (iii) TFA, toluene, rt, (iv) MsOH, EtOAc, rt, 47−60% (4 steps); (r) HCl, EtOAc, rt, 83%; (s) $HSCH_2CO_2H$, LiOH·H_2O, DMF, rt.

reducing the potential for cytotoxicity of drug candidates during the lead optimization process is a prudent strategy to minimize the potential risk for adverse effects in animals and humans.[31−34] Therefore, we sought to minimize the cytotoxicity liability observed for this compound series.

To evaluate the potential cytotoxicity of the compounds, we employed a combination of two assays that focus on different aspects of cell death. The two experiments measured different end points: intracellular ATP content as an indicator of viable cells and caspase-3/7 induction activity as a marker for apoptotic cells. In these assays, precultured HepG2 cells were exposed to test compounds. After 24 h of incubation, the intracellular ATP content and caspase-3/7 induction activity were measured using ATPliteTM-M and Caspase-GloTM 3/7 assay kit, respectively (for more details, refer to the Experimental Section). ATP assays were expressed as a percentage of viable cells compared to untreated controls. Caspase-3/7 induction activity was also expressed as a percentage relative to the response of the reference compound (staurosporine), which was set to 100%.

In the meantime, extensive searches for small molecule GPR40 agonists have been recently pursued in pharmaceutical companies and academia;[35−43] however, the synthetic GPR40 agonists reported in the literature to date have been often plagued by high lipophilicity. This is likely due in part to the fact that endogenous GPR40 ligands are FFAs; the ligand-binding site in the receptor might therefore be hydrophobic in nature. Indeed, compound **1**, designed based on the structural features of FFAs, has a high Log *D* value of 4.19. It is reported that less polar, more lipophilic compounds have an increased likelihood of promiscuous binding to multiple targets, resulting in adverse toxicological outcomes in vivo.[44−46] We postulated that the cytotoxicity identified in compound **1** is attributable to its highly lipophilic character. Therefore, our chemistry efforts were directed toward decreasing the lipophilicity of compound **1**. This strategy would also allow structural differentiation between GPR40 agonists and FFAs that are known to exert pleiotropic physiological effects and cellular toxicity[47−54] and consequently lead to the generation of selective GPR40 agonists with more drug-like physicochemical properties. Herein we describe our research efforts toward identifying positions that tolerate polar functionalities and the subsequent optimization of the 4′-position on the biphenyl ring and the linker that connects the biphenyl ring to the phenylpropanoic acid moiety. These efforts led us to discover several 4′-alkoxylbiphenyl derivatives as potent and bioavailable GPR40 agonists, as represented by 3-{2-fluoro-4-[({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]phenyl}propanoic acid (compound **35**; bold numbers in parentheses indicate specific compounds illustrated in the schemes). In addition, this new series successfully overcame the potential cytotoxicity issues associated with compound **1** and demonstrated the compelling pharmacological profiles that may help demonstrate that GPR40 agonists might constitute an attractive therapeutic approach to the treatment of T2DM.

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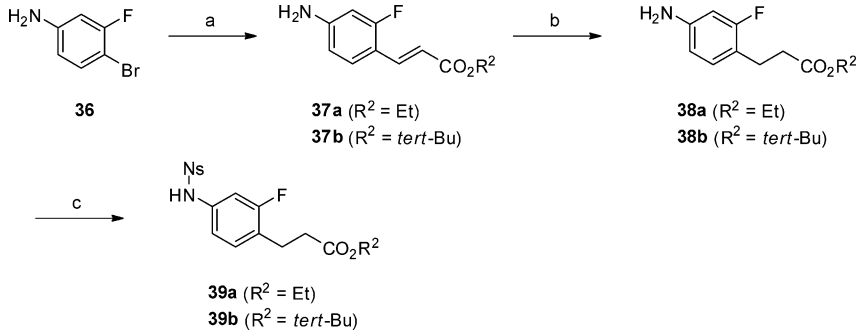
Scheme 3. Synthesis of 4-Aminophenylpropanoic Acid Moieties 38 and 39[a]



36

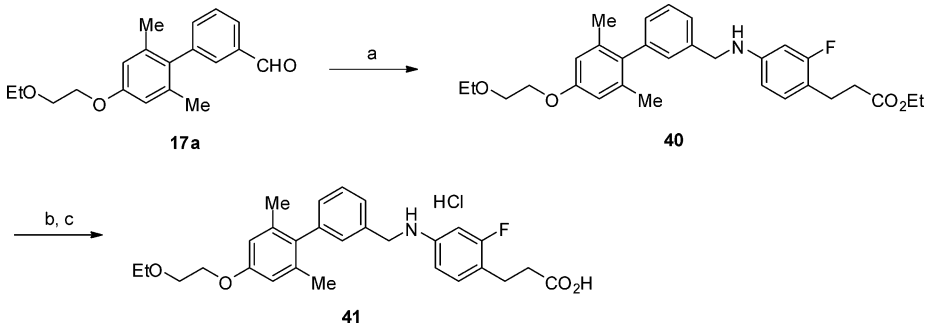
37a (R^2 = Et)
37b (R^2 = *tert*-Bu)

38a (R^2 = Et)
38b (R^2 = *tert*-Bu)

39a (R^2 = Et)
39b (R^2 = *tert*-Bu)

[a]Reagents and conditions: (a) ethyl acrylate or *tert*-butyl acrylate, $Pd(OAc)_2$, $(o\text{-tol})_3P$, *i*-Pr_2NEt, DMF, 110 °C, 80–96%; (b) H_2, 10% Pd/C, EtOH, rt, 79–98%; (c) NsCl, pyridine, rt, 76–77%.

Scheme 4. Synthesis of Compound 41[a]



17a

40

41

[a]Reagents and conditions: (a) (i) **38a**, MS4A, toluene, rt, (ii) H_2, 10% Pd/C, EtOH, rt, 83%; (b) 1 M NaOH aq, THF, EtOH, rt, 99%; (c) HCl, AcOEt, rt, 97%.

CHEMISTRY

2′,6′-Dimethylbiphenyl derivatives **10**–**13** with a substituent on the central phenyl ring were synthesized following the route illustrated in Scheme 1. The synthesis of biphenyl intermediates **8a**–**d** was straightforward, but due to the limitations of commercially available starting materials, three different approaches (route A–C) were required utilizing Suzuki–Miyaura cross-coupling reactions of the appropriate phenyl bromides **3** or **4** or the triflate of phenol **7** with the corresponding boronic acids **2**, **5a**, or **5b** as the key steps. The preparation of the 5-substituted biphenyl intermediate **8c** began with an alkylation of phenol **6** with benzyl bromide (route C). This alkylation provided a mixture of mono- and disubstituted products, which was successfully separated by silica gel chromatography to afford phenol **7**. Conversion of phenol **7** to the corresponding triflate and the subsequent Suzuki–Miyaura cross-coupling reaction with boronic acid **2** proceeded smoothly to yield the desired coupling product. Elaboration into the intermediate **8c** was accomplished in an additional two-step sequence of reactions, involving deprotection of the benzyl group by catalytic hydrogenation followed by methylation of the phenol. Next, the intermediates **8a**–**d** underwent reduction with lithium aluminum hydride (LAH) or sodium borohydride ($NaBH_4$) to give alcohols **9a**–**d**, which were then treated with methyl 3-(4-hydroxyphenyl)propanoate or ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate[30] under Mitsunobu conditions followed by hydrolysis of the ester moieties to deliver the final products **10**–**13**.

A series of 4′-alkoxybiphenyl derivatives **22**–**35** were prepared following the procedures depicted in Scheme 2. Starting from a Suzuki–Miyaura cross-coupling reaction between 4-bromo-3,5-dimethylphenol (**14**) and (3-formylphenyl)boronic acid, phenol **15** was then alkylated with 1-chloro-2-ethoxyethane or protected with a *tert*-butyldimethylsilyl (TBS) group to provide **17a** or **17e**. Alternatively, copper-mediated arylation of phenol **14** with 2-bromo-6-methylpyridine, alkylation of **14** with 1-oxa-6-thiaspiro[2.5]octane, or protection of phenol **14** as the tetrahydropyranyl (THP) ether were carried out prior to palladium-mediated cross-coupling reactions to afford **17b**, **17c**, and **17d**. The resulting aldehydes **17b**–**d** were then converted to **19a**–**d** and **19g** in an analogous fashion to that described in Scheme 1. Subsequently, **19d** and **19e**–**f** were deprotected with tetrabutylammonium fluoride (TBAF), or under acidic conditions, to give phenols **20a** and **20b**–**c**, respectively, which were then coupled with a range of alcohols under Mitsunobu conditions. Finally, the desired compounds **22**–**35** were obtained from **19a**–**c**, **19g**, or **21a**–**i** via the following sets of conditions: methods A and C, simple hydrolysis of the esters to carboxylic acids; method B, oxidation of sulfides to the corresponding sulfones prior to hydrolysis; method D, sequential oxidation to the sulfone, removal of the 2-nitrobenzenesulfonyl (nosyl = Ns) groups, hydrolysis to the carboxylic acid, and acid salt formation; method E, hydrolysis to the carboxylic acid, followed by HCl salt formation; method F, sequential oxidation to the sulfone, removal of the nosyl group, and hydrolysis to the carboxylic acid.

Preparation of 4-aminophenylpropanoic acid esters **38a**–**b** was accomplished by a Mizorogi–Heck reaction of bromobenzene **36** with ethyl acrylate followed by hydrogenation of

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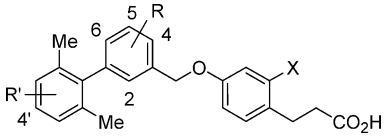
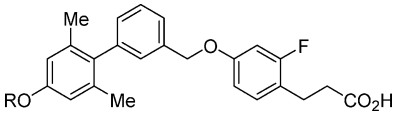
Table 1. In Vitro Profiles of Compounds with Substitutions on 2′,6′-Dimethylbiphenyl Ring

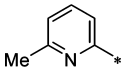
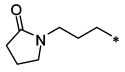
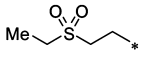
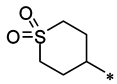
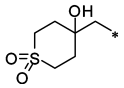


			binding[a]		h-FLIPR[a]	
compd	X	R or R′	h-K_i (nM)	r-K_i (nM)	EC_{50} (nM)	Log D^a
10	H	2-Me	58	30	40 (24–68)	4.22
11	H	4-MeO	520	10000	ND[b]	4.04
12	F	5-MeO	99	120	ND[b]	4.16
13	H	6-MeO	47	87	20 (12–33)	3.38
22	H	4′-MeO	25	6.2	12 (7.7–19)	3.83
23	H	4′-*c*-$PrCH_2O$	16	5.6	15 (9.0–25)	4.61
24	H	4′-BnO	15	6.7	14 (8.5–22)	5.08
25	H	4′-$EtOCH_2CH_2O$	23	10	13 (8.7–19)	3.89
26	F	4′-$EtOCH_2CH_2O$	12	4.5	4.5 (2.6–7.7)	4.14
42	H	H	46	27	22 (10–48)	4.04
1	F	H	32	36	30 (13–69)	4.19

[a]Refers to Figure 1. [b]Not determined.

Table 2. In Vitro Activities and Cytotoxicity Profiles of 4′-Alkoxybiphenyl Derivatives with O Linker



		binding[a]		h-FLIPR[b]	cytotoxicity		
compd	R	h-K_i (nM)	r-K_i (nM)	EC_{50} (nM)	ATP[c]	Caspase[d]	Log D^e
27		20	6.7	23 (16–34)	38.1 (60.3)	42.8 (0.9)	4.10
28[f]		12	6.7	31 (20–50)	47.5 (56.7)	41.1 (–0.4)	4.23
29		21	9.7	32 (21–49)	30.3 (54.1)	20.3 (0.2)	3.51
30		15	4.2	25 (16–38)	32.5 (79.8)	17.7 (–1.1)	3.02
31		16	6.4	25 (18–35)	38.0 (79.8)	28.9 (–0.5)	3.06
32		19	8.2	15 (12–19)	61.5 (88.6)	2.8 (–0.2)	2.78

[a]Refers to Figure 1. [b]Refers to Figure 1. [c]Intracellular ATP content (%) at 30 μM relative to untreated control. Values in parentheses represent intracellular ATP content (%) at 10 μM. [d]Caspase-3/7 activity (%) at 30 μM relative to the response to the reference compound (staurosporine), which was set to 100%. Values in parentheses represent caspase-3/7 activity (%) at 10 μM. [e]Refers to Figure 1. [f]HCl salt.

the olefin moieties in **37a–b**. Nosylation of the anilines **38a** and **38b** afforded **39a** and **39b**, respectively (Scheme 3).

Compound **41**, possessing an amino linker, was readily prepared in three steps from the intermediate **17a** as illustrated in Scheme 4. Aldehyde **17a** was subjected to reductive amination with 4-aminophenylpropanoic acid ethyl ester **38a** to furnish the coupled product **40**, which was saponified and then transformed into its hydrochloride salt **41** for crystallization.

RESULTS AND DISCUSSION

In our search for sites where polar functionalities would be tolerated, we initially investigated the effects of substitutions in the 2′,6′-dimethylbiphenyl ring on potency. Synthesized compounds were first evaluated for in vitro receptor affinities in a competitive binding assay in the presence of 0.2% bovine serum albumin (BSA) to characterize the ligand's ability to displace a synthetic radioligand (3-[4-({2′,6′-dimethyl-6-[(4-^{3}H)phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]-propanoic acid)[29] bound to human and rat GPR40 receptors stably expressed on Chinese hamster ovary (CHO) cells. Compounds showing potency comparable to or better than compounds **1** and **42**[30] in the binding assay were then measured for agonist activity against human GPR40 receptor expressed in CHO cells by a FLIPR assay in the presence of 0.1% BSA. Table 1 summarizes the in vitro profiles of

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compounds bearing a substituent at various positions in the 2′,6′-dimethylbiphenyl ring. Incorporation of a methoxy group into the 4- or 5-position (**11** and **12**, respectively) led to a significant loss in binding affinities against both the human and rat receptors when compared with **42** and **1**, respectively. These potency losses could be attributed to the limited space in the binding pocket of the GPR40 receptor around the 4- and 5-positions of the biphenyl ring. In contrast, although the 6-methoxy derivative **13** displayed somewhat weaker activity against the rat receptor (K_i = 87 nM), the 2-methyl derivative **10** and **13** maintained their potency against human receptors. The observation that potency was preserved with the introduction of a methyl group at the 2-position indicates that this modification has little impact on the overall binding conformation of compound **42**. On the other hand, methoxy substitution at the 4′-position in compound **22** was shown to improve binding affinities for human and rat GPR40 receptors. In light of the enhanced binding affinity and the preserved agonist activity of compound **22**, we next directed our attention to variations of substituents at the 4′-position. More lipophilic and bulkier substituents such as cyclopropylmethoxy or benzyloxy groups (**23** and **24**) were also well tolerated. Notably, the incorporation of a 2-ethoxyethoxy group (**25**), characterized as a moderately polar substituent, restored both the binding affinities and agonist activity in a FLIPR assay. Our previous study demonstrated that the introduction of a fluorine atom at the 2-position of the right-hand phenyl ring remarkably improved pharmacokinetic profiles in terms of maximum plasma concentration (C_{max}) and area under the curve (AUC), with no loss in potency, although it was accompanied by a slight increase in the lipophilicity of the molecule (ΔLog *D* ~0.15).[30] On the basis of these observations, the 2-fluorophenylpropanoic acid derivative **26** with a 2-ethoxyethyl group at the 4′-position of the biphenyl ring was prepared and profiled for its GPR40 activity (Table 1) and rat pharmacokinetics (Table 3). Consistent with previous findings, the oral absorption of the fluoro compound **26** turned out to be better than that of the nonfluoro compound **25**. Surprisingly, in comparison, compound **26** exhibited more potent binding and agonist activity. Thus, preliminary structure−activity relationships of substitutions on the 2′,6′-dimethylbiphenyl ring revealed that polar functionalities could potentially be tolerated at the 4′-position.

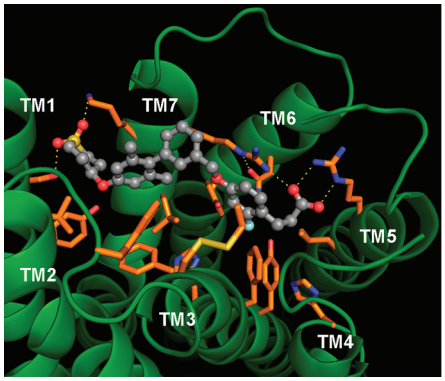
We next focused on installing a range of polar functionalities at the biphenyl 4′-position with the aim of decreasing compound lipophilicity, as measured by Log *D*, to a more druggable range (preferably less than ca. 3)[46] and of improving the cytotoxicity profiles. The resulting in vitro activities, cytotoxicity profiles and Log *D* values of 4′-alkoxybiphenyl derivatives are summarized in Table 2. A wide variety of polar substituents, represented by ether (**27**), heteroaryl (**28**), amide (**29**), and sulfone (**30**, **31**, and **32**) moieties, were tolerated fairly well in terms of potency, and no significant difference in binding affinities between human and rat was observed in this series. Introduction of pyran or pyridine functionality (**27** and **28**, respectively) was found to have little impact on the overall Log *D* value, and these compounds exhibited significant cytotoxicity at 30 μM in both the ATP and caspase assays. In addition, compound **29** maintained comparable activity despite having a lower Log *D* value; however, its cytotoxicity profile remained unacceptable. It should be noted that installation of sulfone substituents, as seen in compounds **30**−**32**, offered the advantage of dramatically reducing the Log *D* value without compromising binding and agonist activities. Moreover, compounds **30** and **31** exhibited a propensity toward improved cytotoxicity profiles based on ATP content, especially at 10 μM, although those at 30 μM were still not improved. These results encouraged us to incorporate an additional polar moiety into the sulfone substituent to further decrease the Log *D* value. Compound **32**, combining the sulfone moiety and a tertiary alcohol group, had a Log *D* value of 2.78 and exhibited considerable improvement in cytotoxicity even at the 30 μM concentration. Thus, the examination of various substituents at the 4′-position uncovered not only the promising attributes of sulfone groups toward lowering the lipophilicity of the molecules but also the possibility that lowering Log *D* to less than 3 could alleviate concerns about cytotoxicity. Pharmacokinetic evaluation of this series in rats also revealed additional advantageous aspects of the sulfone substituents. As can be seen in Table 3, sulfone derivatives (**30**−**32**) exhibited excellent

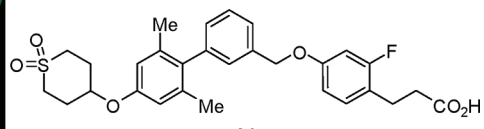
Table 3. Pharmacokinetic Profiles for 4′-Alkoxybiphenyl Derivatives[a]

compd	C_{max} (ng/mL)	$AUC_{0-8\ h}$ (ng·h/mL)	*F* (%)	HLM[b] (μL/min/mg)	RLM[c] (μL/min/mg)
25	9.4	35.4	13.9	0	0
26	32.2	120.4	24.6	8	0
27	67.4	206.9	36.9	0	0
28	25.3	142.7	27.8	0	0
29	53.3	232.3	84.8	7	5
30	184.1	780.7	145.1	6	10
31	221.4	758.8	132.9	3	0
32	185.2	448.8	53.3	ND[d]	ND[d]
33	80.3	207.7	54.5	2	0
34	129.7	433.2	78.1	0	0
35	207.7	689.0	109.9	2	0
41	28.0	77.7	24.1	0	0
42	5.3	2.0	0.9	28	34
1	86.0	249.0	21.5	69	39

[a]Rat cassette dosing at 0.1 mg/kg, iv and 1 mg/kg, po (nonfasted). Average of 3 rats. [b]Metabolic stability in the human hepatic microsome. [c]Metabolic stability in rat hepatic microsome. [d]Not determined.

pharmacokinetic profiles characterized by high C_{max} and AUC compared with the nonsulfone derivatives **25**−**29** and the 4′-unsubstituted biphenyl derivative **1**. Given the fact that most of the compounds were generally quite stable toward oxidative metabolism in both human and rat liver microsomes, which do not allow the evaluation of β-oxidation or conjugation metabolism of the phenylpropanoic acid moieties, the superior pharmacokinetic profiles noted with sulfone derivatives may result from less propensity to β-oxidation and/or conjugation metabolism as a result of the decreased lipophilicity of these molecules. The putative major metabolic pathways from the metabolite studies of compound **42** are believed to be β-oxidation and conjugation metabolism of the acids.[30] Our previous extensive studies of the in vivo efficacy of compounds in an oral glucose tolerance test (OGTT) using diabetic rat models demonstrated that the magnitude of the plasma concentration, coupled with the in vitro activity of tested compounds, were crucial determinants of in vivo efficacy in this series. Accordingly, improved pharmacokinetic parameters with





31

Figure 2. Docking model of **31** inside a GPR40 homology model.

higher C_{max} and AUC, as seen with the sulfone derivatives, were anticipated to translate well into in vivo activity.

Computational modeling served to provide some speculations about the tolerance of different functional groups at the 4′-position of the biphenyl ring for a wide range of substituents. A representative compound **31** was docked into the putative binding pocket of the GPR40 homology model (Figure 2). The results suggested that compound **31** binds in a fashion similar to that of TAK-875, recently reported from our group.[29] In this model, the sulfone substituent of compound **31** extends toward the relatively polar region sandwiched between Ser8 (TM1) and Lys259 (TM7), and each oxygen atom of the sulfone group makes hydrogen bonds with Ser8 and Lys259. The other polar side chains listed in Table 3 also contain hydrogen-bond acceptors that could potentially form hydrogen bonds with Ser8 and/or Lys259. In addition, lipophilic substituents, such as cyclopropylmethoxy and benzyloxy groups, could interact with the hydrophobic residues ($-CH_2-$) of Lys259 and/or Ser8.

Although compound **31** still possessed a risk of cytotoxicity, this compound was considered valuable as an in vitro pharmacological tool for validating the potential therapeutic benefits of GPR40 agonists for the treatment of diabetes. Therefore, we first examined in vitro insulinotropic effects of compound **31** in rat insulinoma, INS-1 cells (clone 833/15) (Figure 3a). The INS-1 cells maintained glucose responsiveness, secreting 9.5-fold the amount of insulin at 11 mM glucose concentration as that at 1 mM. Compound **31** augmented insulin secretion in a dose-dependent manner in the presence of 11 mM glucose and statistically significant increases in insulin secretion were observed at doses of above 0.1 μM of the compound compared to that seen at 11 mM glucose alone. Next, we examined the effect of glucose concentration on the insulinotropic action of **31**. As shown in Figure 3b, compound **31**, at a concentration of 1 μM, augmented insulin secretion in the presence of >6 mM glucose, while it did not enhance insulin secretion in the presence of either 1 or 4 mM glucose. In the same experiment, nateglinide and glibenclamide, well-known as sulfonylurea agents, potentiated insulin secretion even at lower glucose concentrations. Moreover, the amount of secreted insulin in the presence of high glucose levels (11 and 16 mM) was greater with **31** than with nateglinide ($p \leq 0.01$ and $p \leq 0.01$, respectively) or glibenclamide ($p \leq 0.05$ and $p \leq 0.01$, respectively). These results indicate that GPR40 agonists augment insulin secretion through a mechanism strictly dependent on glucose concentration and, furthermore, that they are more potent in enhancing insulin secretion than sulfonylurea agents at high glucose concentrations.

In an effort to rescue the poor cytotoxicity profiles of compounds **30** and **31**, and also to further examine the

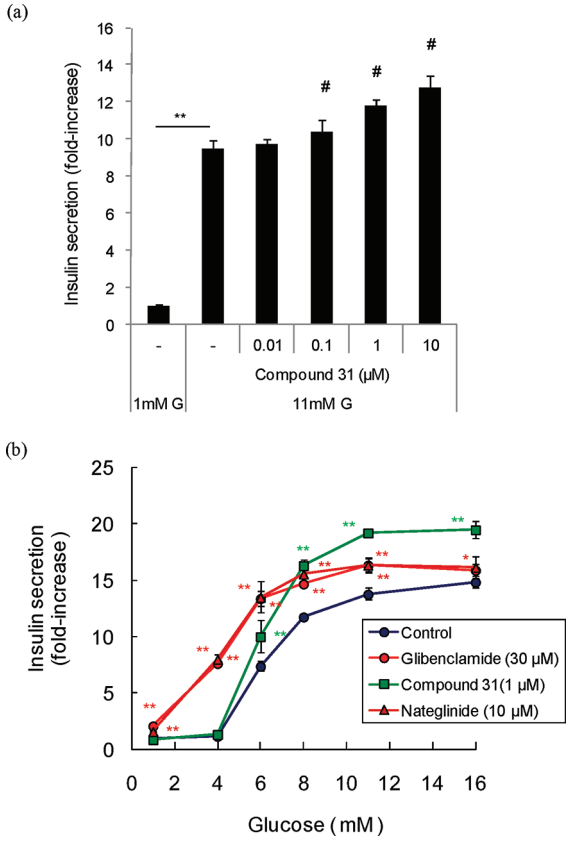


Figure 3. Enhancement of GSIS by compound **31** in INS-1 833/15 cells. (a) INS-1 cells (clone 833/15) were stimulated with 11 mM glucose in the presence or absence of **31** (0.01−10 μM), and the amount of insulin secretion was quantified by enzyme immunoassay (EIA). Data are expressed as the mean fold-increase over control (1 mM glucose stimulation alone) ± SD (n = 3). **$p \leq 0.01$ by Aspin−Welch test. #$p \leq 0.025$ vs 11 mM glucose alone by one-tailed Williams test. (b) INS-1 cells (clone 833/15) were stimulated with various concentrations of glucose (1−16 mM), in the absence or presence of 1 μM of **31**, 10 μM of nateglinide, or 30 μM of glibenclamide, and the amount of insulin secreted was quantified by EIA. Data are expressed as the mean fold-increase over control (1 mM glucose stimulation alone) and represent as the mean values of six wells (each glucose stimulation alone) ± SD (n = 3−6). *$p \leq 0.05$ and **$p \leq 0.01$ vs control (stimulation by glucose alone at each concentration) by Dunnett's test.

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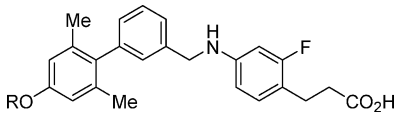
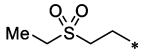
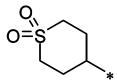
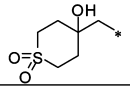
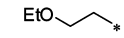
Table 4. In Vitro Activities and Cytotoxicity Profiles of 4′-Alkoxybiphenyl Derivatives with NH Linker



compd	R	binding[a] h-K_i (nM)	binding[a] r-K_i (nM)	h-FLIPR[b] EC_{50} (nM)	cytotoxicity ATP[c]	cytotoxicity Caspase[d]	Log D[e]
33		20	4.5	99 (76–130)	45.7 (79.8)	–0.9 (–1.0)	2.39
34		18	5.1	23 (17–30)	60.7 (78.8)	–0.7 (–0.5)	2.44
35		19	4.5	29 (19–44)	75.9 (88.1)	0.5 (0.8)	2.14
41		18	6.2	26 (17–39)	48.5 (9.9)	1.2 (5.3)	3.49

[a]Refers to Figure 1. [b]Refers to Figure 1. [c]Refers to Table 2. [d]Refers to Table 2. [e]Refers to Figure 1.

relationship of cytotoxicity and compound lipophilicity, the O linker in 4′-alkoxybiphenyl derivatives **30**–**32** was converted to the corresponding NH linker (Table 4). We anticipated that this modification would lead to a significant drop in the Log D value and result in attenuation of cytotoxicity issues. As a result, the binding affinities and agonist activities of compounds **33**–**35** were generally comparable to those of the O linker counterparts **30**–**32**, although compound **33** displayed somewhat weaker agonist activity than compound **30**. Notably, compounds **33**–**35** had significantly lowered Log D values in the range of 2.1–2.5 and exhibited substantially improved cytotoxicity profiles, with the only exception of a somewhat decreased ATP content at the 30 μM dose in the case of compound **33**. These results indicate that the ability to induce cytotoxicity appears to be roughly correlated with the molecule's lipophilicity, and Log D values below 2.8 would be desirable to minimize the potential cytotoxicity risk for this series. It appears certain, however, that the NH linker itself contributed to the mitigation of caspase-3/7 activation in a distinct manner apart from just lowering the lipophilicity. This is because the NH linker derivative **41**, possessing a high Log D value of 3.49, showed no signs of apoptotic cytotoxicity, whereas the O linker derivative **29** with a similar Log D value of 3.51 did display significant apoptotic cytotoxicity. In rat pharmacokinetic studies (Table 3), oral plasma exposure of compounds **33** and **34** turned out to be inferior to that of the corresponding O linker-containing derivatives **30** and **31**. In contrast, compound **35** preserved the excellent oral absorption noted with compound **32**.

On the basis of its excellent in vitro potency, pharmacokinetic profile, and minimal risk of cytotoxicity, compound **35** was selected for further in vivo pharmacological evaluation. Compound **35** was assessed for its ability to improve glucose tolerance in female Wistar fatty rats, a disease model that develops obese and obesity-related features such as impaired glucose tolerance, hyperinsulinemia, and hyperlipidemia.[56] Single oral doses of **35** robustly lowered the blood glucose excursion (Figure 4a) and augmented insulin secretion (Figure 4b) during an oral glucose tolerance test in a dose-proportional manner from 0.1 to 1 mg/kg when the compound was administered 30 min before the oral glucose challenge. It should be noted that compound **35** exhibited a significant

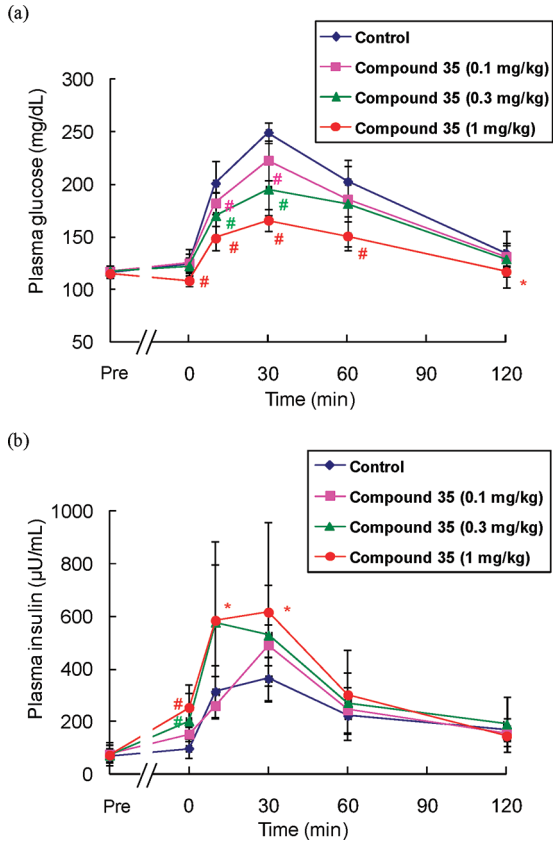


Figure 4. Effects of compound **35** on plasma insulin and glucose levels during an oral glucose tolerance test in female Wistar fatty rats. Overnight-fasted rats were orally given compound **35** (0.1, 0.3, 1 mg/kg) 30 min before glucose load (1 g/kg), and time-dependent changes in (a) plasma glucose and (b) plasma insulin were monitored. #$p \leq 0.025$ vs control by one-tailed Williams' test. *$p \leq 0.025$ vs control by Shirley–Williams' test. Data are means ± SD for six animals.

blood glucose-lowering effect at a much lower dose (0.3 mg/kg) in comparison to compound **1**[30] (3 mg/kg). These results can be at least in part rationalized by the improved oral exposure of **35**. In addition, the risk of hypoglycemia was also assessed in fasting normal Sprague–Dawley (SD) rats by oral administration of a high dose of compound **35** in comparison

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with glibenclamide, a well-known sulfonylurea insulin secretagogue that acts on ATP-sensitive potassium channels in pancreatic β-cells. As shown in parts a and b of Figure 5,

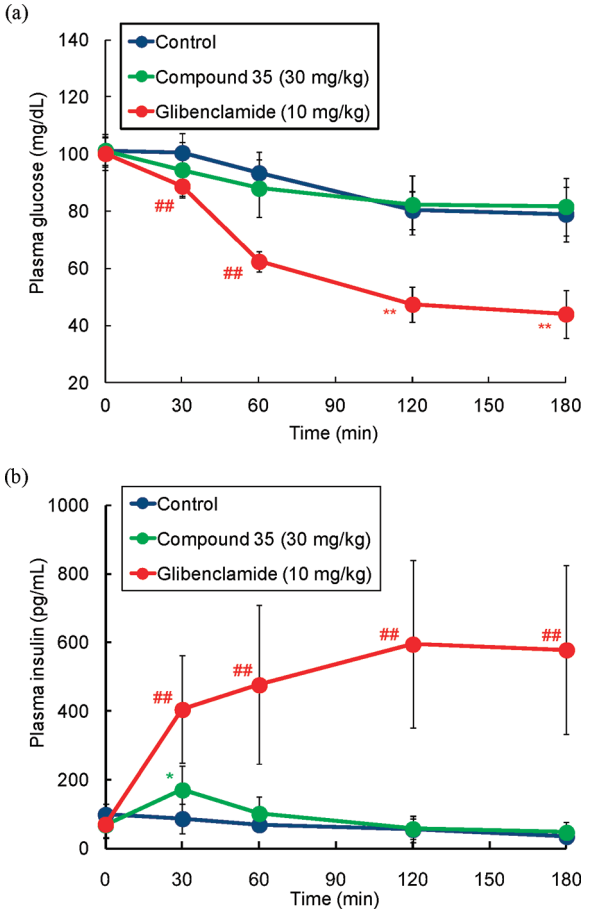

Figure 5. Effects of compound **35** (30 mg/kg, po) and glibenclamide (10 mg/kg, po) on fasting plasma glucose and insulin levels in normal Sprague–Dawley rats. (a) and (b) show time-dependent changes in plasma glucose and plasma insulin level after oral administration of compound **35** or glibenclamide, respectively. *$p \leq 0.05$ and **$p \leq 0.01$ vs control by Student's t test. ##$p \leq 0.01$ vs control by Aspin–Welch test. Data are means ± SD for six animals.

glibenclamide (10 mg/kg) lowered plasma glucose levels below normal fasting levels in SD rats by significantly increasing plasma insulin. In contrast, compound **35**, even at the high dose of 30 mg/kg, did not alter fasting glucose levels in SD rats with normal glucose homeostasis (Figure 5a). In agreement with the absence of hypoglycemic effects, insulin secretion induced by compound **35** was statistically significant only at 30 min after the administration, but the amount was much lower compared to that by caused by administration of glibenclamide (Figure 5b). Thus, our results indicate that compound **35** not only enhances GSIS and effectively improves postprandial hyperglycemia in the diabetic state, but also it may present a low risk of hypoglycemia, an adverse effect common to sulfonylureas.

On the basis of the promising in vivo pharmacological profile of compound **35**, a 4-week toxicity study was subsequently carried out in normal rats. In this study, no particular adverse effects were observed at oral doses of 15, 50, and 150 mg/kg, indicating the possibility that minimized off-target pharmacology as a result of decreased lipophilicity (Log D = 2.14) and reduced potential cytotoxicity may contribute to the clean toxicological profile of **35**.

CONCLUSION

From the hypothesis that the cytotoxicity observed with compound **1** would be ascribed to its highly lipophilic nature, we envisioned that imparting polarity to compound **1** could lead to the generation of GPR40 agonists with a minimized risk of in vitro cellular toxicity as well as more druggable physicochemical properties. In addition, we anticipated that this strategy would also contribute to moving away from the multifaceted FFA-like structure and result in the identification of the drug candidates that specifically activate the GPR40 receptor. Preliminary investigations of substituents on the 2′,6′-dimethylbiphenyl ring indicated the potential for incorporating polar side chains at the 4′-position. Through the subsequent exploration of polar functionalities at the 4′-position and on the linker, we discovered a series of 4′-alkoxybiphenyl derivatives with a combination of excellent in vitro activities and dramatically improved pharmacokinetic and cytotoxicity profiles. Cytotoxicity issues were successfully overcome by reducing the lipophilicity to a Log D value of less than 2.8. Compound **35** displayed a superior blood glucose-lowering effect in female Wistar fatty rats over compound **1**, and no significant adverse effects were observed in a 4-week toxicity study in rats. Potent and safe GPR40 agonists, such as compound **35**, are expected to provide a novel therapeutic approach to enhance insulin secretion with a low risk of hypoglycemia.

EXPERIMENTAL SECTION

General. Melting points were determined on a Büchi B-545 melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Ultra Shield-300 (300 MHz) instruments. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet, br = broad. Coupling constants (J values) are given in hertz (Hz). The acidic protons of carboxylic acids, alcohols, or anilines were not frequently observed in ^{1}H NMR spectra. Elemental analyses were carried out by Takeda Analytical Laboratories, Ltd., and were within 0.3% of the theoretical values unless otherwise noted. Low-resolution mass spectra (MS) were determined on a Waters liquid chromatography–mass spectrometer system (MS), using a CAPCELL PAK UG-120 ODS (Shiseido Co., Ltd.) column (2.0 mm i.d. × 50 mm) with aqueous CH_3CN (10–95%) containing 0.05% trifluoroacetic acid (TFA), and an HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. All MS experiments were performed using electrospray ionization (ESI) in positive ion mode. Analytical HPLC was performed on a Shimadzu LC-VP instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 2.0 mm × 50 mm column with a 4 min linear gradient from 90/10 to 5/95 and subsequently with a 1.5 min isocratic elution 5/95 A/B, where A = H_2O–0.1%TFA, B = CH_3CN–0.1%TFA, at a flow rate of 0.5 μL/min, with UV detection at 220 and 254 nm, at column temperature of 25 °C, or performed on a Waters Quattro micro API (Agilent HP1100, Gilson215) instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 1.5 mm × 35 mm column, by gradient elution: 0.00 min (A/B = 100/0), 2.00 min (A/B = 0/100), 3.00 (A/B = 0/100), 3.01 (A/B = 100/0), 3.30 (A/B = 100/0) where A = 2% CH_3CN/H_2O with 5 mM NH_4OAc, B = 95% CH_3CN/H_2O with 5 mM NH_4OAc, at a flow rate of 0.5 mL/min, with UV detection at 220 nm, at column temperature of 40 °C. Purity of all tested compounds was determined to be >97% by elemental analyses and/or analytical HPLC using the aforementioned conditions. Reagents and solvents were obtained from commercial sources and used without

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further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was carried out on silica gel columns [(Merck Kieselgel 60, 70−230 mesh or 230−400 mesh, Merck) or (Chromatorex NH-DM 1020, 100−200 mesh)] or on Purif-Pack (SI or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd.). Abbreviations of the solvents are used as follows: EtOAc, ethyl acetate; THF, tetrahydrofuran; EtOH, ethanol; DMF, *N*,*N*-dimethylformamide; Et_2O, diethyl ether; MeOH, methanol; CH_3CN, acetonitrile; DME, 1,2-dimethoxyethane; DMSO, dimethyl sulfoxide.

Methyl 3-(Benzyloxy)-5-hydroxybenzoate (7). To a mixture of methyl 3,5-dihydroxybenzoate (**6**) (8.41 g, 50.0 mmol) and benzyl bromide (3.57 mL, 300 mmol) in DMF (30 mL) was added potassium carbonate (4.15 g, 30.0 mmol). The reaction mixture was stirred at 70 °C for 16 h under nitrogen, followed by solvent removal in vacuo. The residue was partitioned between EtOAc and water. The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 19:1 to 7:3) and then crystallized from hexane/EtOAc to give 7 (3.56 g, 46%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (3H, s), 5.07 (2H, s), 5.18 (1H, s), 6.68 (1H, t, J = 2.3 Hz), 7.14 (1H, dd, J = 2.3, 1.3 Hz), 7.24−7.28 (1H, m), 7.30−7.46 (5H, m). MS m/z 259 $(M + H)^+$.

Methyl 2,2′,6′-Trimethylbiphenyl-3-carboxylate (8a). A mixture of methyl 3-bromo-2-methylbenzoate (**3**) (2.60 g, 11.4 mmol), (2,6-dimethylphenyl)boronic acid (**2**) (2.00 g, 13.3 mmol), tetrakis(triphenylphosphine)palladium(0) (200 mg, 0.170 mmol), and cesium carbonate (5.60 g, 17.2 mmol) in toluene (50 mL) and MeOH (10 mL) was refluxed for 18 h under argon. The insoluble materials were filtered off and washed with EtOAc. The filtrate was concentrated in vacuo to give a crude oil, which was purified by column chromatography (silica gel, hexane/EtOAc, 10:1) to afford **8a** (1.30 g, 45%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93 (6H, s), 2.03 (3H, s), 3.09 (3H, s), 7.07−7.96 (6H, m). MS m/z 255 $(M + H)^+$.

4-Methoxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (8b). The title compound was prepared in 82% yield as a pale-yellow solid from **4** and **5a** using the procedure analogous to that described for the synthesis of **8a**, except that EtOH was employed as a solvent in place of MeOH. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (6H, s), 3.99 (3H, s), 7.03−7.23 (4H, m), 7.35 (1H, dd, J = 2.6, 8.4 Hz), 7.63 (1H, d, J = 2.2 Hz), 10.52 (1H, s). MS m/z 241 $(M + H)^+$.

Methyl 5-Methoxy-2′,6′-dimethylbiphenyl-3-carboxylate (8c). To a solution of 7 (3.10 g, 12.0 mmol) and *N*-ethyldiisopropylamine (2.51 mL, 14.4 mmol) in dichloromethane (30 mL) at 0 °C was added dropwise trifluoromethanesulfonic anhydride (2.22 mL, 13.2 mmol). The reaction mixture was stirred at the same temperature for 1 h and then washed with water, dried over $MgSO_4$, and concentrated in vacuo to give methyl 3-(benzyloxy)-5-{[(trifluoromethyl)sulfonyl]-oxy}benzoate as a brown oil. A mixture of the triflate obtained above, (2,6-dimethylphenyl)boronic acid (2.25 g, 15.0 mmol), tetrakis-(triphenylphosphine)palladium(0) (0.693 g, 0.600 mmol), and potassium triphosphate (3.82 g, 18.0 mmol) in 1,2-dimethoxyethane (40 mL), was stirred at 80 °C for 15 h under argon. After concentration in vacuo, the residue was diluted with EtOAc, washed sequentially with water and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 100:0 to 4:1) to afford methyl 5-(benzyloxy)-2′,6′-dimethylbiphenyl-3-carboxylate (3.60 g, 87%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (6H, s), 3.91 (3H, s), 5.13 (2H, s), 6.97 (1H, dd, J = 2.5, 1.4 Hz), 7.06−7.20 (3H, m), 7.29−7.48 (6H, m), 7.65 (1H, dd, J = 2.5, 1.4 Hz). MS m/z 347 $(M + H)^+$. Methyl 5-(benzyloxy)-2′,6′-dimethylbiphenyl-3-carboxylate was hydrogenated under atmospheric hydrogen with 10% Pd on carbon (containing 50% water, 0.4 g) as a catalyst in MeOH (20 mL) and THF (10 mL) at room temperature for 24 h. The reaction mixture was filtered by Millipore filtration and concentrated in vacuo. The resulting solid was recrystallized from hexane/EtOAc to afford methyl 5-hydroxy-2′,6′-dimethylbiphenyl-3-carboxylate (1.64 g, 91%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (6H, s), 3.91 (3H, s), 5.14 (1H, s), 6.86 (1H, dd, J = 2.5, 1.4 Hz), 7.07−7.21 (3H, m), 7.43 (1H, t, J = 1.4 Hz), 7.51 (1H, dd, J = 2.5, 1.4 Hz). MS m/z 257 $(M + H)^+$. Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.79; H, 6.44. To a mixture of methyl 5-hydroxy-2′,6′-dimethylbiphenyl-3-carboxylate (0.500 g, 1.95 mmol) and iodomethane (0.243 mL, 3.90 mmol) in DMF (4 mL) was added potassium carbonate (0.324 g, 2.34 mmol). The reaction mixture was stirred at 50 °C for 14 h, diluted with water, and extracted with EtOAc. The organic phase was washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 100:0 to 4:1) to afford **8c** (0.490 g, 93%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (6H, s), 3.87 (3H, s), 3.91 (3H, s), 6.91 (1H, dd, J = 2.6, 1.4 Hz), 7.08−7.13 (2H, m), 7.14−7.21 (1H, m), 7.45 (1H, t, J = 1.4 Hz), 7.55 (1H, dd, J = 1.4 Hz). MS m/z 271 $(M + H)^+$.

6-Methoxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (8d). The title compound was prepared in 87% yield as a pale-yellow oil from **4** and **5b** using the procedure analogous to that described for the synthesis of **8a**, except that EtOH was employed as a solvent in place of MeOH. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (6H, s), 3.84 (3H, s), 6.98−7.22 (4H, m), 7.60 (1H, d, J = 2.2 Hz), 7.91 (1H, dd, J = 2.2, 8.8 Hz), 9.92 (1H, s). MS m/z 241 $(M + H)^+$.

(2,2′,6′-Trimethylbiphenyl-3-yl)methanol (9a). To a solution of **8a** (1.30 g, 5.11 mmol) in THF (30 mL) at 0 °C was added portionwise lithium aluminum hydride (220 mg, 5.80 mmol). The reaction mixture was stirred 0 °C for 3 h, and $Na_2SO_4{\cdot}10H_2O$ (2.00 g, 3.83 mmol) was slowly added at the same temperature. After being stirred at room temperature, the mixture was filtered through a pad of Celite using EtOAc and then the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 5:1) to afford **9a** (500 mg, 43%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94 (6H, s), 1.97 (3H, s), 4.69 (2H, d, J = 6.0 Hz), 7.01 (1H, s), 7.06−7.32 (5H, m).

(4-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methanol (9b). To a solution of **8b** (2.90 g, 12.1 mmol) in EtOH (40 mL) at 0 °C was added portionwise sodium borohydride (460 mg, 12.2 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 4 h. After being cooled to 0 °C, the reaction mixture was quenched with citric acid aqueous solution and evaporated under reduced pressure to remove EtOH. The residue was extracted with EtOAc, washed with saturated aqueous NaCl, and then dried over $MgSO_4$. The organic layer was concentrated in vacuo to afford **9b** (3.00 g, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (6H, s), 2.37 (1H, t, J = 6.6 Hz), 3.92 (3H, s), 4.71 (2H, d, J = 6.6 Hz), 6.94 (1H, d, J = 8.8 Hz), 7.02−7.22 (5H, m).

(5-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methanol (9c). To a solution of **8c** (0.485 g, 1.79 mmol) in THF (5 mL) at room temperature was added portionwise lithium aluminum hydride (68.1 mg, 1.79 mmol). The reaction mixture was stirred at room temperature for 15 h under nitrogen and then $Na_2SO_4{\cdot}10H_2O$ (0.577 g, 1.79 mmol) was slowly added at the same temperature. After being stirred for 1 h, the mixture was filtered through a pad of Celite using THF and the filtrate was concentrated in vacuo to afford **9c** (0.431 g, 99%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67 (1H, t, J = 5.7 Hz), 2.05 (6H, s), 3.83 (3H, s), 4.71 (2H, d, J = 5.7 Hz), 6.63 (1H, dd, J = 2.2, 1.4 Hz), 6.72 (1H, s), 6.90−6.94 (1H, m), 7.06−7.19 (3H, m).

(6-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methanol (9d). The title compound was prepared in 88% yield as a colorless oil from **8d** using the procedure analogous to that described for the synthesis of **9b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (6H, s), 3.74 (3H, s), 4.65 (2H, d, J = 5.2 Hz), 6.97 (1H, d, J = 8.4 Hz), 7.03 (1H, d, J = 2.2 Hz), 7.06−7.24 (3H, m), 7.35 (1H, dd, J = 8.4, 2.6 Hz).

General Procedure for the Synthesis of Compound 10−13. To a mixture of **9a−d**, methyl 3-(4-hydroxyphenyl)propanoate or ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate (1.06−1.18 equiv) and tributylphosphine (1.28−2.00 equiv) in THF or toluene was added portionwise 1,1′-(azodicarbonyl)dipiperidine (1.28−2.00 equiv). The

reaction mixture was stirred at room temperature for 18−21 h. After addition of hexane or Et_2O, the insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to afford the coupled ethyl esters. The above ethyl esters were dissolved in a 1:1 mixture of THF and MeOH (or EtOH). To the solution were added potassium hydroxide (2.20 equiv) aqueous solution or 2 M sodium hydroxide aqueous solution (2.61 equiv). The reaction mixture was stirred at room temperature for 7−72 h and then partitioned between EtOAc and 10% citric acid aqueous solution. The phases were separated, and the organic phase was washed with saturated aqueous NaCl, dried over $MgSO_4$ or Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **10**−**13**.

3-{4-[(2,2′,6′-Trimethylbiphenyl-3-yl)methoxy]phenyl}-propanoic Acid (10). Starting from **9a** and methyl 3-(4-hydroxyphenyl)propanoate, the procedures summarized above provided the title compound in 76% yield as a white solid after recrystallization from hexane/Et_2O. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.92 (6H, s), 1.96 (3H, s), 2.63 (2H, t, J = 7.4 Hz), 2.89 (2H, t, J = 7.4 Hz), 5.04 (2H, s), 6.88 (2H, d, J = 8.4 Hz), 7.05−7.34 (8H, m). MS m/z 375 $(M + H)^+$; mp 106−107 °C. HPLC purity: 100%. Anal. Calcd for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found: C, 80.04; H, 7.04.

3-{4-[(4-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-phenyl}propanoic Acid (11). Starting from **9b** and methyl 3-(4-hydroxyphenyl)propanoate, the procedures summarized above provided the title compound in 79% yield as a white solid after recrystallization from hexane/Et_2O. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98 (6H, s), 2.63 (2H, t, J = 7.7 Hz), 2.89 (2H, t, J = 7.7 Hz), 3.90 (3H, s), 5.14 (2H, s), 6.84−6.99 (3H, m), 7.00−7.24 (7H, m). mp 130−131 °C. HPLC purity: 100%. Anal. Calcd for $C_{25}H_{26}O_4$: C, 76.90; H, 6.71. Found: C, 76.77; H, 6.61.

3-{2-Fluoro-4-[(5-methoxy-2′,6′-dimethylbiphenyl-3-yl)-methoxy]phenyl}propanoic Acid (12). Starting from **9c** and ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate, the procedures summarized above provided the title compound in 86% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (6H, s), 2.64 (2H, t, J = 7.6 Hz), 2.91 (2H, t, J = 7.6 Hz), 3.82 (3H, s), 5.05 (2H, s), 6.63−6.71 (3H, m), 6.76 (1H, s), 6.93 (1H, dd, J = 2.3, 1.5 Hz), 7.06−7.19 (4H, m). MS m/z 409 $(M + H)^+$. HPLC purity: 99.5%.

3-{4-[(6-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-phenyl}propanoic Acid (13). Starting from **9d** and methyl 3-(4-hydroxyphenyl)propanoate, the procedures summarized above provided the title compound in 67% yield as a white solid after recrystallization from hexane/Et_2O. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.74 (3H, s), 5.00 (2H, s), 6.85−7.44 (10H, m). mp 103−104 °C. Anal. Calcd for $C_{25}H_{26}O_4 \cdot 0.5H_2O$: C, 75.16; H, 6.81. Found: C, 75.44; H, 6.64.

4′-Hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (15). A flask was charged with **14** (10.3 g, 51.0 mmol), (3-formylphenyl)-boronic acid (7.67 g, 51.2 mmol), 1 M sodium carbonate aqueous solution (150 mL), EtOH (50 mL), and toluene (150 mL). The system was placed under argon by evacuation and filling with argon five times. After tetrakis(triphenylphosphine)palladium(0) (2.95 g, 2.55 mmol) was added, the system was again placed under argon: the above pump−flush cycle was repeated three times. The reaction mixture was stirred at 80 °C for 24 h under argon. After cooling to room temperature, the reaction mixture was passed through a pad of Celite. The filtrate was washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 9:1 to 3:2) to afford **15** (9.53 g, 83%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (6H, s), 4.69 (1H, s), 6.62 (2H, s), 7.42 (1H, dt, J = 7.7, 1.4 Hz), 7.59 (1H, t, J = 7.6 Hz), 7.66 (1H, t, J = 1.7 Hz), 7.86 (1H, dt, J = 7.6, 1.5 Hz), 10.05 (1H, s). MS m/z 227 $(M + H)^+$.

2-(4-Bromo-3,5-dimethylphenoxy)-6-methylpyridine (16a). To a solution of sodium hydroxide (0.230 g, 5.81 mmol) in MeOH (50 mL) was added **14** (1.17 g, 5.81 mmol). After being stirred at room temperature for 10 min, the reaction mixture was concentrated to dryness in vacuo to afford sodium salt of **14** (1.30 g). The resultant sodium salt was subsequently combined with 2-bromo-6-methlypyridine (1.00 g, 5.81 mmol) and copper powder (11 mg, 0.17 mmol), and the resulting reaction mixture was stirred at 185 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed sequentially with water and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 100:0 to 5:1) to afford **16a** (1.25 g, 74%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (6H, s), 2.46 (3H, s), 6.57 (1H, d, J = 8.3 Hz), 6.83−6.92 (3H, m), 7.54 (1H, t, J = 8.3 Hz). MS m/z 292 $(M + H)^+$.

4-[(4-Bromo-3,5-dimethylphenoxy)methyl]tetrahydro-2*H*-thiopyran-4-ol (16b). To a solution of **14** (6.07 g, 30.2 mmol) in DMF (60 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 1.21 g, 30.2 mmol) in a single portion. After being stirred for 1 h at room temperature, the reaction mixture was cooled to 0 °C and a solution of 1-oxa-6-thiaspiro[2.5]octane (2.62 g, 20.1 mmol) in DMF (20 mL) was slowly added. The reaction mixture was allowed to warm to 80 °C and stirred overnight at the same temperature. After concentration in vacuo to remove DMF, the residue was partitioned between EtOAc and NaCl aqueous solution. The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed sequentially with 2 M NaOH aqueous solution and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 5:1) to afford **16b** (2.92 g, 44%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73−1.88 (2H, m), 2.01−2.14 (3H, m), 2.34−2.52 (8H, m), 3.01−3.16 (2H, m), 3.73 (2H, s), 6.65 (2H, s).

2-(4-Bromo-3,5-dimethylphenoxy)tetrahydro-2*H*-pyran (16c). To a mixture of **14** (10.5 g, 52.2 mmol) and 3,4-dihydro-2*H*-pyran (8.83 g, 105 mmol) in dichloromethane (160 mL) was added pyridinium *p*-toluenesulfonate (2.64 g, 10.5 mmol). The reaction mixture was stirred at room temperature for 80 h and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 4:1) to afford **16c** (11.5 g, 77%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56−1.75 (3H, m), 1.80−2.07 (3H, m), 2.37 (6H, s), 3.55−3.64 (1H, m), 3.83−3.93 (1H, m), 5.37 (1H, t, J = 3.1 Hz), 6.80 (2H, s).

4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-carbaldehyde (17a). To a mixture of **15** (8.52 g, 37.7 mmol) and 1-chloro-2-ethoxyethane (6.15 g, 56.6 mmol) in DMF were added potassium carbonate (6.25 g, 45.2 mmol) and potassium iodide (1.25 g, 7.54 mmol). The reaction mixture was stirred at 80 °C for 18 h and then poured into water. The mixture was extracted with EtOAc, washed with saturated aqueous NaCl, and then dried over $MgSO_4$. The organic layer was concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 3:1) to afford **17a** (10.0 g, 89%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 7.0 Hz), 1.99 (6H, s), 3.62 (2H, q, J = 7.0 Hz), 3.81 (2H, t, J = 4.9 Hz), 4.15 (2H, t, J = 4.9 Hz), 6.71 (2H, s), 7.42 (1H, dt, J = 7.5, 1.5 Hz), 7.58 (1H, t, J = 7.5 Hz), 7.66 (1H, t, J = 1.5 Hz), 7.86 (1H, dt, J = 7.5, 1.5 Hz), 10.05 (1H, s). MS m/z 299 $(M + H)^+$.

2′,6′-Dimethyl-4′-[(6-methylpyridin-2-yl)oxy]biphenyl-3-carbaldehyde (17b). The title compound was prepared in 94% yield as a colorless oil from **16a** using the procedure analogous to that described for the synthesis of **15**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (6H, s), 2.50 (3H, s), 6.64 (1H, d, J = 8.1 Hz), 6.85−6.93 (3H, m), 7.46 (1H, dt, J = 7.6, 1.4 Hz), 7.53−7.66 (2H, m), 7.71 (1H, t, J = 1.4 Hz), 7.88 (1H, dt, J = 7.6, 1.4 Hz), 10.07 (1H, s). MS m/z 318 $(M + H)^+$.

4′-[(4-Hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-carbaldehyde (17c). The title compound was prepared in 66% yield as a white solid from **16b** using the procedure analogous to that described for the synthesis of **15**, except that 1,2-dimethoxyethane was employed as a solvent in place of EtOH. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70 (1H, t, J = 5.8 Hz), 1.76−1.90 (2H, m), 2.01 (6H, s), 2.05−2.16 (2H, m), 2.20 (1H, s), 2.40−2.53 (2H, m), 3.03−3.18 (2H, m), 3.80 (2H, s), 4.73 (2H, d, J = 5.8 Hz), 6.67 (2H, s), 7.02−7.09 (1H, m), 7.12 (1H, s), 7.31−7.37 (1H, m), 7.41 (1H, t, J = 7.4 Hz).

2′,6′-Dimethyl-4′-(tetrahydro-2*H*-pyran-2-yloxy)biphenyl-3-carbaldehyde (17d). The title compound was prepared in 83% yield as a yellow oil from **16c** using the procedure analogous to that described for the synthesis of **15**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57−1.78 (3H, m), 1.82−1.93 (2H, m), 1.99 (6H, s), 2.04 (1H, m), 3.65 (1H, m), 3.97 (1H, m), 5.47 (1H, t, J = 3.0 Hz), 6.84 (2H, s), 7.42 (1H, m), 7.58 (1H, t, J = 7.5 Hz), 7.67 (1H, s), 7.86 (1H, m), 10.05 (1H, s).

4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-carbaldehyde (17e). To a mixture of **15** (9.00 g, 39.8 mmol) and imidazole (2.98 g, 43.8 mmol) in DMF (100 mL) was added *tert*-butyldimethylsilyl chloride (6.60 g, 43.8 mmol). After being stirred for 4 h, the reaction mixture was diluted with EtOAc and washed sequentially with water and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 4:1) to afford **17e** (10.5 g, 77%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.25 (6H, s), 1.02 (9H, s), 1.97 (6H, s), 6.62 (2H, s), 7.44 (1H, dt, J = 1.5, 7.5 Hz), 7.59 (1H, t, J = 7.5 Hz), 7.68 (1H, t, J = 1.5 Hz), 7.86 (1H, dt, J = 1.5, 7.5 Hz), 10.06 (1H, s).

[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methanol (18a). To a solution of **17a** (2.39 g, 9.70 mmol) in THF (20 mL) and 1,2-dimethoxyethane (20 mL) at 0 °C was added sodium borohydride (0.227 g, 6.00 mmol). The reaction mixture was stirred at the same temperature for 3 h and quenched with NH_4Cl aqueous solution. The mixture was extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:1) to afford **18a** (3.55 g, 98%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.66 (1H, t, J = 5.9 Hz), 2.00 (6H, s), 3.62 (2H, q, J = 7.1 Hz), 3.80 (2H, t, J = 5.1 Hz), 4.14 (2H, t, J = 5.1 Hz), 4.73 (2H, d, J = 5.9 Hz), 6.69 (2H, s), 7.06 (1H, d, J = 7.3 Hz), 7.12 (1H, s), 7.33 (1H, d, J = 7.3 Hz), 7.40 (1H, t, J = 7.3 Hz). MS m/z 301 $(M + H)^+$.

{2′,6′-Dimethyl-4′-[(6-methylpyridin-2-yl)oxy]biphenyl-3-yl}methanol (18b). The title compound was prepared in 98% yield as a colorless oil from **17b** using the procedure analogous to that described for the synthesis of **18a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98−2.03 (6H, m), 2.47−2.53 (3H, m), 4.75 (2H, d, J = 5.1 Hz), 6.62 (1H, d, J = 8.3 Hz), 6.83−6.92 (3H, m), 7.07−7.13 (1H, m), 7.14−7.19 (1H, m), 7.33−7.39 (1H, m), 7.43 (1H, t, J = 7.5 Hz), 7.56 (1H, t, J = 7.7 Hz). MS m/z 320 $(M + H)^+$.

4-({[3′-(Hydroxymethyl)-2,6-dimethylbiphenyl-4-yl]oxy}methyl)tetrahydro-2*H*-thiopyran-4-ol (18c). The title compound was prepared in quantitative yield as a white solid from **17c** using the procedure analogous to that described for the synthesis of **18a**, except that MeOH was employed as a solvent in place of 1,2-dimethoxyethane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70 (1H, t, J = 5.8 Hz), 1.76−1.90 (2H, m), 2.01 (6H, s), 2.05−2.16 (2H, m), 2.20 (1H, s), 2.40−2.53 (2H, m), 3.03−3.18 (2H, m), 3.80 (2H, s), 4.73 (2H, d, J = 5.8 Hz), 6.67 (2H, s), 7.02−7.09 (1H, m), 7.12 (1H, s), 7.31−7.37 (1H, m), 7.41 (1H, t, J = 7.4 Hz).

[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-pyran-2-yloxy)biphenyl-3-yl]methanol (18d). The title compound was prepared in quantitative yield as a colorless oil from **17d** using the procedure analogous to that described for the synthesis of **18a**, except that MeOH was employed as a solvent in place of 1,2-dimethoxyethane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−1.79 (4H, m), 1.80−1.93 (2H, m), 2.00 (6H, s), 2.03 (1H, m), 3.64 (1H, m), 3.97 (1H, m), 4.73 (2H, d, J = 5.7 Hz), 5.45 (1H, t, J = 3.0 Hz), 6.81 (2H, s), 7.07 (1H, d, J = 7.5 Hz), 7.13 (1H, s), 7.33 (1H, d, J = 7.5 Hz), 7.40 (1H, t, J = 7.8 Hz).

(4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-yl)methanol (18e). The title compound was prepared in quantitative yield as a white solid from **17e** using the procedure analogous to that described for the synthesis of **18a**, except that MeOH was employed as a solvent in place of 1,2-dimethoxyethane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.23 (6H, s), 1.00 (9H, s), 1.67 (1H, t, J = 5.4 Hz), 1.96 (6H, s), 4.73 (2H, d, J = 5.4 Hz), 6.58 (2H, s), 7.07 (1H, d, J = 7.2 Hz), 7.13 (1H, s), 7.30−7.36 (1H, m), 7.39 (1H, t, J = 7.5 Hz).

Ethyl 3-(4-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2-fluorophenyl)propanoate (19a). To a mixture of **18a** (0.330 g, 1.10 mmol), ethyl 3-(2-fluoro-4-hydroxyphenyl)-propanoate (0.233 g, 1.10 mmol), and tributylphosphine (0.448 mL, 1.80 mmol) in toluene (20 mL) at 0 °C was added portionwise 1,1′-(azodicarbonyl)dipiperidine (0.454 g, 1.80 mmol). The reaction mixture was stirred at room temperature for 16 h. After addition of hexane, the insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 19:1 to 3:1) to afford **19a** (0.505 g, 93%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.28 (6H, m), 1.98 (6H, s), 2.57 (2H, t, J = 7.7 Hz), 2.89 (2H, t, J = 7.7 Hz), 3.62 (2H, q, J = 7.0 Hz), 3.80 (2H, t, J = 7.0 Hz), 4.08−4.15 (4H, m), 5.06 (2H, s), 6.63−6.70 (4H, m), 7.05−7.11 (2H, m), 7.16 (1H, s), 7.34−7.35 (1H, m), 7.42 (1H, t, J = 7.4 Hz). MS m/z 495 $(M + H)^+$.

Ethyl 3-[4-({2′,6′-Dimethyl-4′-[(6-methylpyridin-2-yl)oxy]-biphenyl-3-yl}methoxy)-2-fluorophenyl]propanoate (19b). The title compound was prepared in 94% yield as a colorless oil from **18b** using the procedure analogous to that described for the synthesis of **19a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.99 (6H, m), 2.50 (3H, s), 2.58 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 4.12 (2H, q, J = 7.2 Hz), 5.08 (2H, s), 6.60−6.73 (3H, m), 6.84−6.92 (3H, m), 7.05−7.16 (2H, m), 7.21 (1H, s), 7.36−7.49 (2H, m), 7.56 (1H, t, J = 7.8 Hz). MS m/z 514 $(M + H)^+$.

Ethyl 3-[2-Fluoro-4-({4′-[(4-hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-phenyl]propanoate (19c). The title compound was prepared in 89% yield as a colorless oil from **18c** (0.900 g, 2.51 mmol) using the procedure analogous to that described for the synthesis of **19a**, except that THF was employed as a solvent in place of toluene. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.75−1.90 (2H, m), 1.99 (6H, s), 2.05−2.16 (2H, m), 2.19 (1H, s), 2.39−2.52 (2H, m), 2.57 (2H, t, J = 7.6 Hz), 2.89 (2H, t, J = 7.6 Hz), 3.03−3.19 (2H, m), 3.79 (2H, s), 4.12 (2H, q, J = 7.2 Hz), 5.06 (2H, s), 6.60−6.73 (4H, m), 7.01−7.19 (3H, m), 7.33−7.48 (2H, m).

Methyl 3-(4-{[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-pyran-2-yloxy)biphenyl-3-yl]methoxy}phenyl)propanoate (19d). To a mixture of **18d** (5.15 g, 16.5 mmol), methyl 3-(4-hydroxyphenyl)-propanoate (3.28 g, 18.2 mmol), and triphenylphosphine (5.63 g, 21.5 mmol) in THF (100 mL) was added dropwise diethyl azodicarboxylate (40 wt % in toluene, 9.70 mL, 24.8 mmol). The reaction mixture was stirred at room temperature for 2 days. After concentration in vacuo, the residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 3:1) to afford **19d** (2.92 g, 37%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57−1.78 (3H, s), 1.82−1.90 (2H, m), 1.98 (6H, s), 2.02 (1H, m), 2.59 (2H, t, J = 7.8 Hz), 2.89 (2H, t, J = 7.8 Hz), 3.62 (1H, m), 3.66 (3H, s), 3.97 (1H, m), 5.08 (2H, s), 5.45 (1H, t, J = 3.0 Hz), 6.81 (2H, s), 6.89 (2H, d, J = 8.4 Hz), 7.05−7.14 (3H, m), 7.18 (1H, s), 7.34−7.47 (2H, m).

Ethyl 3-{4-[(4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2-fluorophenyl}propanoate (19e). The title compound was prepared in 89% yield as a white solid from **18e** using the procedure analogous to that described for the synthesis of **19a**, except that THF was employed as a solvent in place of toluene. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.23 (6H, s), 1.00 (9H, s), 1.23 (3H, t, J = 7.2 Hz), 1.95 (6H, s), 2.57 (2H, t, J = 7.6 Hz), 2.90 (2H, t, J = 7.6 Hz), 4.12 (2H, q, J = 7.2 Hz), 5.06 (2H, s), 6.58 (2H, s), 6.62−6.73 (2H, m), 7.04−7.21 (3H, m), 7.32−7.48 (2H, m).

***tert*-Butyl 3-(4-{[(4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-yl)methyl][(2-nitrophenyl)sulfonyl]amino}-2-fluorophenyl)propanoate (19f).** The title compound was prepared in 92% yield as a yellow oil from **18e** and *tert*-butyl 3-(4-amino-2-fluorophenyl)propanoate (**38b**) using the procedure analogous to that described for the synthesis of **19d**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.20−0.24 (6H, m), 0.96−1.02 (9H, m), 1.36−1.41 (9H, m), 1.81 (6H, s), 2.45 (2H, t, J = 7.7 Hz), 2.82 (2H, t, J = 7.7 Hz), 4.92 (2H, s), 6.54 (2H, s), 6.71−6.80 (2H, m), 6.90−7.07 (3H, m), 7.19−7.33 (2H, m), 7.46−7.54 (1H, m), 7.56−7.61 (1H, m), 7.63−7.72 (2H, m).

Ethyl 3-(2-Fluoro-4-{({4′-[(4-hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)[(2-nitrophenyl)sulfonyl]amino}phenyl)propanoate (19g). The title

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compound was prepared in 92% yield as a pale-yellow amorphous solid from **18c** and ethyl 3-(4-amino-2-fluorophenyl)propanoate (**38a**) using the procedure analogous to that described for the synthesis of **19d**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.22 (6H, s), 1.00 (9H, s), 1.21 (3H, t, J = 7.2 Hz), 1.81 (6H, s), 2.53 (2H, t, J = 7.8 Hz), 2.87 (2H, t, J = 7.8 Hz), 4.10 (2H, q, J = 7.2 Hz), 4.92 (2H, s), 6.54 (2H, s), 6.71−6.81 (2H, m), 6.90 (1H, s), 6.96−7.08 (2H, m), 7.22−7.36 (2H, m), 7.52 (1H, m), 7.60 (1H, m), 7.63−7.73 (2H, m).

Methyl 3-{4-[(4′-Hydroxy-2′,6′-dimethylbiphenyl-3-yl)-methoxy]phenyl}propanoate (20a). A mixture of **19d** (2.92 g, 6.15 mmol) and *p*-toluenesulfonic acid monohydrate (0.120 g, 0.620 mmol) in MeOH (60 mL) was stirred at room temperature for 2 h and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 1:2) to afford **20a** (2.12 g, 88%) as a red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (6H, s), 2.59 (2H, t, J = 7.8 Hz), 2.89 (2H, t, J = 7.8 Hz), 3.66 (3H, s), 4.63 (1H, s), 5.08 (2H, s), 6.59 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.05−7.13 (3H, m), 7.17 (1H, s), 7.35−7.45 (2H, m).

Ethyl 3-{2-Fluoro-4-[(4′-hydroxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]phenyl}propanoate (20b). To a solution of **19e** (11.6 g, 20.8 mmol) in THF (110 mL) at 0 °C was added dropwise tetrabutylammonium fluoride (1 M solution in THF, 22.9 mmol, 22.9 mmol). The reaction mixture was stirred at room temperature overnight, diluted with saturated aqueous NaCl, and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 9:1 to 2:1) to afford **20b** (8.70 g, 99%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.96 (6H, s), 2.58 (2H, t, J = 7.7 Hz), 2.90 (2H, t, J = 7.7 Hz), 4.12 (2H, q, J = 7.2 Hz), 4.78 (1H, s), 5.06 (2H, s), 6.59 (2H, s), 6.61−6.72 (2H, m), 7.00−7.20 (3H, m), 7.31−7.47 (2H, m). MS m/z 423 $(M + H)^+$.

***tert*-Butyl 3-(2-Fluoro-4-{[(4′-hydroxy-2′,6′-dimethylbiphenyl-3-yl)methyl][(2-nitrophenyl)sulfonyl]amino}phenyl)-propanoate (20c).** The title compound was prepared in 79% yield as a yellow oil from **19f** using the procedure analogous to that described for the synthesis of **20b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, t, J = 7.2 Hz), 1.82 (6H, s), 2.53 (2H, t, J = 7.8 Hz), 2.87 (2H, t, J = 7.8 Hz), 4.10 (2H, q, J = 7.2 Hz), 4.58 (1H, s), 4.93 (2H, s), 6.55 (2H, s), 6.71−6.81 (2H, m), 6.88 (1H, s), 6.96−7.09 (2H, m), 7.23−7.37 (2H, m), 7.52 (1H, m), 7.60 (1H, m), 7.64−7.73 (2H, m).

Methyl 3-(4-{[4′-(Cyclopropylmethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}phenyl)propanoate (21a). To a mixture of **20a** (0.200 g, 0.512 mmol), cyclopropymethanol (73.6 mg, 1.02 mmol), and triphenylphosphine (0.175 g, 0.666 mmol) in THF (4 mL) was added diethyl azodicarboxylate (40 wt % solution in toluene, 0.302 mL, 0.768 mmol). The reaction mixture was stirred for 12 h and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 9:1 to 2:1) to afford **21a** (0.157 g, 69%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31−0.39 (2H, m), 0.60−0.69 (2H, m), 1.27 (1H, m), 1.98 (6H, s), 2.59 (2H, t, J = 7.8 Hz), 2.89 (2H, t, J = 7.8 Hz), 3.66 (3H, s), 3.81 (2H, d, J = 6.9 Hz), 5.08 (2H, s), 6.66 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.05−7.13 (3H, m), 7.18 (1H, s), 7.35−7.45 (2H, m).

Methyl 3-(4-{[4′-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]-methoxy}phenyl)propanoate (21b). The title compound was prepared in 63% yield as a colorless oil from **20a** and benzyl alcohol using the procedure analogous to that described for the synthesis of **21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (6H, s), 2.59 (2H, t, J = 7.8 Hz), 2.89 (2H, t, J = 7.8 Hz), 3.66 (3H, s), 5.07 (2H, s), 5.08 (2H, s), 6.75 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.05−7.13 (3H, m), 7.18 (1H, s), 7.30−7.49 (7H, m).

Methyl 3-(4-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}phenyl)propanoate (21c). The title compound was prepared in 51% yield as a colorless oil from **20a** and 2-ethoxymethanol using the procedure analogous to that described for the synthesis of **21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, J = 7.0 Hz), 1.98 (6H, s), 2.59 (2H, t, J = 7.7 Hz), 2.89 (2H, t, J = 7.7 Hz), 3.56−3.69 (5H, m), 3.80 (2H, t, J = 5.0 Hz), 4.14 (2H, t, J = 5.0 Hz), 5.08 (2H, s), 6.68 (2H, s), 6.85−6.93 (2H, m), 7.03−7.20 (4H, m), 7.33−7.46 (2H, m).

Ethyl 3-[4-({4′-[2-(Ethylsulfanyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2-fluorophenyl]propanoate (21d). To a mixture of **20b** (1.67 g, 2.75 mmol), 2-(ethylsulfanyl)ethanol (0.325 mL, 3.03 mmol), and tributylphosphine (1.03 mL, 4.13 mmol) in THF (33 mL) was added dropwise 1,1′-(azodicarbonyl)dipiperidine (1.04 g, 4.13 mmol). After the reaction mixture was stirred at room temperature for 16 h, 2-(ethylsulfanyl)ethanol (0.325 mL, 3.03 mmol), tributylphosphine (1.03 mL, 4.13 mmol), and 1,1′-(azodicarbonyl)dipiperidine (1.04 g, 4.13 mmol) were sequentially added to the mixture, which was stirred for another 16 h. The insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **21d** (1.22 g, 64%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.31 (3H, t, J = 7.2 Hz), 1.98 (6H, s), 2.59 (2H, t, J = 7.8 Hz), 2.67 (2H, q, J = 7.2 Hz), 2.85−2.97 (4H, m), 3.66 (3H, s), 4.15 (2H, t, J = 6.9 Hz), 5.08 (2H, s), 6.66 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.04−7.14 (3H, m), 7.17 (1H, s), 7.35−7.47 (2H, m).

Ethyl 3-[4-({2′,6′-Dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)-propoxy]biphenyl-3-yl}methoxy)-2-fluorophenyl]propanoate (21e). To a mixture of **20b** (0.400 g, 0.947 mmol), 1-(3-hydroxypropyl)pyrrolidin-2-one (0.134 mL, 1.04 mmol), and tributylphosphine (0.354 mL, 1.42 mmol) in THF (5 mL) was added dropwise 1,1′-(azodicarbonyl)dipiperidine (0.358 g, 1.42 mmol). After the reaction mixture was stirred at room temperature for 16 h, the insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **21e** (0.350 g, 67%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.96−2.10 (4H, m), 1.98 (6H, s), 2.40 (2H, t, J = 7.8 Hz), 2.57 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.38−3.53 (4H, m), 4.00 (2H, t, J = 6.0 Hz), 4.12 (2H, q, J = 7.2 Hz), 5.06 (2H, s), 6.62−6.71 (4H, m), 7.05−7.13 (2H, m), 7.16 (1H, s), 7.34−7.46 (2H, m). MS m/z 548 $(M + H)^+$.

Ethyl 3-(4-{[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-pyran-4-yloxy)biphenyl-3-yl]methoxy}-2-fluorophenyl)propanoate (21f). The title compound was prepared in 92% yield as a colorless oil from **20b** and tetrahydro-2*H*-pyran-4-ol using the procedure analogous to that described for the synthesis of **21d**, except that diethyl azodicarboxylate and triphenylphosphine were employed in place of 1,1′-(azodicarbonyl)dipiperidine and tributylphosphine, respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.74−1.89 (2H, m), 1.93−2.10 (8H, m), 2.57 (2H, t, J = 7.6 Hz), 2.89 (2H, t, J = 7.6 Hz), 3.55−3.66 (2H, m), 3.95−4.06 (2H, m), 4.12 (2H, q, J = 7.2 Hz), 4.44−4.55 (1H, m), 5.06 (2H, s), 6.61−6.71 (4H, m), 7.04−7.13 (2H, m), 7.15−7.19 (1H, m), 7.34−7.46 (2H, m).

Ethyl 3-(4-{[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-thiopyran-4-yloxy)biphenyl-3-yl]methoxy}-2-fluorophenyl)propanoate (21g). The title compound was prepared in 91% yield as a yellow oil from **20b** and tetrahydro-2*H*-thiopyran-4-ol using the procedure analogous to that described for the synthesis of **21d**, except that diethyl azodicarboxylate and triphenylphosphine were employed in place of 1,1′-(azodicarbonyl)dipiperidine and tributylphosphine, respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.98 (6H, s), 1.99−2.11 (2H, m), 2.16−2.27 (2H, m), 2.53−2.64 (4H, m), 2.85−3.01 (4H, m), 4.07−4.17 (2H, m), 4.32−4.42 (1H, m), 5.06 (2H, s), 6.62−6.71 (4H, m), 7.05−7.12 (2H, m), 7.17 (1H, s), 7.34−7.46 (2H, m).

***tert*-Butyl 3-(4-{({4′-[2-(Ethylsulfanyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)[(2-nitrophenyl)sulfonyl]amino}-2-fluorophenyl)propanoate (21h).** The title compound was prepared in 86% yield as a yellow oil from **20c** and 2-(ethylsulfanyl)ethanol using the procedure analogous to that described for the synthesis of **21d**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, t, J = 7.5 Hz), 1.39 (9H, s), 1.85 (6H, s), 2.45 (2H, t, J = 7.8 Hz), 2.67 (2H, q, J = 7.5 Hz), 2.82 (2H, t, J = 7.8 Hz), 2.91 (2H, t, J = 6.9 Hz), 4.14 (2H, t, J = 6.9 Hz), 4.93 (2H, s), 6.62 (2H, s), 6.70−6.81 (2H, m), 6.91 (1H, s), 6.96−7.07 (2H, m), 7.24 (1H, d, J = 7.8 Hz), 7.31 (1H, t, J = 7.5 Hz), 7.46−7.54 (1H, m), 7.58 (1H, d, J = 7.8 Hz), 7.64−7.72 (2H, m). MS m/z 723 $(M + H)^+$.

***tert*-Butyl 3-[4-({[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-thiopyran-4-yloxy)biphenyl-3-yl]methyl}[(2-nitrophenyl)sulfonyl]-**

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amino)-2-fluorophenyl]propanoate (21i). The title compound was prepared in quantitative yield as a pale-yellow oil from **20c** and 2-(ethylsulfanyl)ethanol using the procedure analogous to that described for the synthesis of **21d**, except that diethyl azodicarboxylate and triphenylphosphine were employed in place of 1,1′-(azodicarbonyl)-dipiperidine and tributylphosphine, respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (9H, s), 1.85 (6H, s), 1.97−2.11 (2H, m), 2.14−2.28 (2H, m), 2.45 (2H, t, J = 7.7 Hz), 2.52−2.66 (2H, m), 2.82 (2H, t, J = 7.7 Hz), 2.89−3.02 (2H, m), 4.30−4.41 (1H, m), 4.93 (2H, s), 6.61 (2H, s), 6.76 (2H, t, J = 8.3 Hz), 6.88−7.08 (3H, m), 7.18−7.35 (2H, m), 7.46−7.62 (2H, m), 7.63−7.73 (2H, m). MS m/z 735 $(M + H)^+$.

3-{4-[(4′-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-phenyl}propanoic Acid (22). To a mixture of **20a** (0.200 g, 0.512 mmol), MeOH (0.0410 mL, 1.02 mmol), and triphenylphosphine (0.175 g, 0.666 mmol) in THF (4 mL) at 0 °C was added dropwise diethyl azodicarboxylate (40 wt % solution in toluene, 0.302 mL, 0.768 mmol). The reaction mixture was stirred for 12 h and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 3:1) to afford methyl 3-{4-[(4′-methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-phenyl}propanoate (0.134 g, 65%) as a yellow oil. To a solution of the above ester (0.134 g, 0.331 mmol) in THF (4 mL) and MeOH (2 mL) at room temperature was added 1 M NaOH aqueous solution (0.662 mL). The reaction mixture was stirred at the same temperature for 1 h, and then 1 M HCl aqueous solution was added until the solution reached pH ∼ 3. The mixture was extracted with EtOAc, washed sequentially with water and saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:2) to afford **22** (0.115 g, 89%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.81 (3H, s), 5.08 (2H, s), 6.66 (2H, s), 6.87−6.93 (2H, m), 7.06−7.15 (3H, m), 7.18 (1H, br), 7.35−7.45 (2H, m). mp 148−149 °C. HPLC purity: 99.4%. Anal. Calcd for $C_{25}H_{26}O_4$: C, 76.90; H, 6.71. Found: C, 76.98; H, 6.76.

3-(4-{[4′-(Cyclopropylmethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}phenyl)propanoic Acid (23). To a solution of **21a** (0.157 g, 0.353 mmol) in THF (5 mL) and MeOH (2.5 mL) at room temperature was added 1 M NaOH aqueous solution (0.706 mL). The reaction mixture was stirred at the same temperature for 4 h, and then 1 M HCl aqueous solution was added until the solution reached pH ∼ 3. The mixture was extracted with EtOAc, washed sequentially with water and saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:2) to afford **23** (0.115 g, 76%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30−0.40 (2H, m), 0.60−0.70 (2H, m), 1.20−1.36 (1H, m), 1.98 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.81 (2H, d, J = 6.9 Hz), 5.08 (2H, s), 6.66 (2H, s), 6.90 (2H, d, J = 8.4 Hz), 7.05−7.19 (4H, m), 7.35−7.45 (2H, m). HPLC purity: 99.8%; mp 128−129 °C. Anal. Calcd for $C_{28}H_{30}O_4$: C, 78.11; H, 7.02. Found: C, 78.21; H, 7.03.

3-(4-{[4′-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-phenyl)propanoic Acid (24). The title compound was prepared in 91% yield as a white solid from **21b** using the procedure analogous to that described for the synthesis of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 5.07 (2H, s), 5.08 (2H, s), 6.75 (2H, s), 6.90 (2H, d, J = 8.4 Hz), 7.05−7.15 (3H, m), 7.18 (1H, s), 7.29−7.48 (7H, m). HPLC purity: 98.5%; mp 122−123 °C. Anal. Calcd for $C_{31}H_{30}O_4$: C, 79.80; H, 6.48. Found: C, 79.84; H, 6.50.

3-(4-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]-methoxy}phenyl)propanoic Acid (25). The title compound was prepared in 75% yield as a white solid from **21c** using the procedure analogous to that described for the synthesis of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, J = 6.9 Hz), 1.98 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.62 (2H, q, J = 6.9 Hz), 3.77−3.84 (2H, m), 4.10−4.17 (2H, m), 5.08 (2H, s), 6.68 (2H, s), 6.90 (2H, d, J = 8.7 Hz), 7.05−7.20 (4H, m), 7.34−7.45 (2H, m). MS m/z 449 $(M + H)^+$. HPLC purity: 100%. Anal. Calcd for $C_{28}H_{32}O_5$: C, 74.97; H, 7.19. Found: C, 74.82; H, 7.17.

3-(4-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]-methoxy}-2-fluorophenyl)propanoic Acid (26). The title compound was prepared in 77% yield as a white solid from **19a** using the procedure analogous to that described for the synthesis of **23**, except that 10% citric acid aqueous solution was employed in place of 1 M HCl aqueous solution. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, J = 7.0 Hz), 1.98 (6H, s), 2.64 (2H, t, J = 7.6 Hz), 2.91 (2H, t, J = 7.6 Hz), 3.62 (2H, q, J = 7.0 Hz), 3.80 (2H, t, J = 5.0 Hz), 4.14 (2H, t, J = 5.0 Hz), 5.06 (2H, s), 6.63−6.70 (4H, m), 7.06−7.13 (2H, m), 7.16 (1H, s), 7.33−-7.38 (1H, m), 7.42 (1H, t, J = 7.4 Hz). MS m/z 467 $(M + H)^+$. HPLC purity: 99.8%.

3-(4-{[2′,6′-Dimethyl-4′-(tetrahydro-2*H*-pyran-4-yloxy)-biphenyl-3-yl]methoxy}-2-fluorophenyl)propanoic Acid (27). The title compound was prepared in 49% yield as a white solid from **21g** using the procedure analogous to that described for the synthesis of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74−1.91 (2H, m), 1.98 (6H, s), 1.98−2.11 (2H, m), 2.64 (2H, t, J = 7.8 Hz), 2.91 (2H, t, J = 7.8 Hz), 3.54−3.66 (2H, m), 3.95−4.06 (2H, m), 4.50 (1H, m), 5.06 (2H, s), 6.62−6.72 (4H, m), 7.05−7.15 (2H, m), 7.17 (1H, s), 7.33−7.47 (2H, m). MS m/z 479 $(M + H)^+$; mp 120−121 °C. HPLC purity: 98.9%. Anal. Calcd for $C_{29}H_{31}FO_5$: C, 72.78; H, 6.53. Found: C, 72.62; H, 6.59.

3-[4-({2′,6′-Dimethyl-4′-[(6-methylpyridin-2-yl)oxy]-biphenyl-3-yl}methoxy)-2-fluorophenyl]propanoic Acid Hydrochloride (28). To a mixture of **18b** (0.920 g, 2.88 mmol), ethyl 3-(2-fluoro-4-hydroxyphenyl)propanoate (0.672 g, 3.16 mmol), and tributylphosphine (1.08 mL, 4.32 mmol) in THF (20 mL) was added portionwise 1,1′-(azodicarbonyl)dipiperidine (1.09 g, 4.32 mmol). The reaction mixture was stirred at room temperature for 70 h. After removal of the solvent in vacuo, Et_2O was added to the residue. The insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 10:1 to 3:1) to afford ethyl 3-[4-({2′,6′-dimethyl-4′-[(6-methylpyridin-2-yl)oxy]biphenyl-3-yl}methoxy)-2-fluorophenyl]propanoate (1.39 g, 94%) as a colorless oil. MS m/z 514 $(M + H)^+$. To a solution of the above compound (1.39 g, 2.71 mmol) in THF (20 mL) and MeOH (10 mL) at room temperature was added 1 M NaOH aqueous solution (5.42 mL, 5.42 mmol). The reaction mixture was stirred at the same temperature for 2 h and then neutralized by addition of 1 M HCl aqueous solution. The mixture was extracted with EtOAc, washed sequentially with water and saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 2:1 to 1:2) to afford the free base of **28** (0.820 g, 62%) as a colorless oil. MS m/z 486 $(M + H)^+$. The obtained oil (0.820 g, 1.69 mmol) was dissolved in EtOAc (8 mL), and then 4 M HCl solution in EtOAc (1.27 mL, 5.07 mmol) was added. After removal of the solvent in vacuo, the residue was crystallized from EtOAc/Et_2O to afford **28** (0.730 g, 48% from **18b**) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.94 (6H, s), 2.38 (3H, s), 2.41−2.56 (2H, m), 2.68−2.84 (2H, m), 5.16 (2H, s), 6.68−6.94 (5H, m), 7.03 (1H, d, J = 7.2 Hz), 7.10−7.30 (3H, m), 7.38−7.56 (2H, m), 7.70−7.85 (1H, m). MS m/z 486 $(M + H)^+$ (as a free base). HPLC purity: 99.4%. Anal. Calcd for $C_{30}H_{29}ClFNO_4$: C, 69.03; H, 5.60; N, 2.68. Found: C, 68.86; H, 5.71; N, 2.66.

3-[4-({2′,6′-Dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)propoxy]-biphenyl-3-yl}methoxy)-2-fluorophenyl]propanoic Acid (29). The title compound was prepared in 88% yield as a white solid from **21e** using the procedure analogous to that described for the synthesis of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90−2.12 (10H, m), 2.41 (2H, t, J = 8.1 Hz), 2.63 (2H, t, J = 7.4 Hz), 2.90 (2H, t, J = 7.4 Hz), 3.41−3.54 (4H, m), 3.99 (2H, t, J = 6.2 Hz), 5.08 (2H, s), 6.59−6.73 (4H, m), 7.04−7.17 (3H, m), 7.31−7.47 (2H, m). MS m/z 520 $(M + H)^+$; mp 138−139 °C. HPLC purity: 100%. Anal. Calcd for $C_{31}H_{34}FNO_5$: C, 71.66; H, 6.60; N, 2.70. Found: C, 71.50; H, 6.49; N, 2.65.

3-[4-({4′-[2-(Ethylsulfonyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2-fluorophenyl]propanoic Acid (30). To a solution of **21d** (0.200 g, 0.392 mmol) in dichloromethane (4 mL) at 0 °C was added portionwise *m*-chloroperbenzoic acid (70%, 0.212 g, 0.862

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mmol). The reaction mixture was stirred at the same temperature for 2 h. The mixture was washed sequentially with 1 M NaOH aqueous solution and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 10:1 to 1:1) to afford ethyl 3-[4-({4′-[2-(ethylsulfonyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2-fluorophenyl]propanoate (0.190 g, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.47 (3H, t, J = 7.5 Hz), 1.99 (6H, s), 2.57 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.19 (2H, q, J = 7.5 Hz), 3.42 (2H, t, J = 5.4 Hz), 4.12 (2H, q, J = 7.2 Hz), 4.44 (2H, t, J = 5.4 Hz), 5.07 (2H, s), 6.62−6.71 (4H, m), 7.05−7.13 (2H, m), 7.15 (1H, s), 7.35−7.48 (2H, m). MS m/z 543 $(M + H)^+$. To a solution of the above ester (0.190 g, 0.350 mmol) in AcOH (4.5 mL) and water (4 mL) was added concd H_2SO_4 (0.65 mL). The reaction mixture was stirred at 90 °C for 4 h. After cooled to room temperature, the mixture was diluted with EtOAc and washed sequentially with water and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 0:100) to afford **30** (0.124 g, 61%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (3H, t, J = 7.5 Hz), 1.99 (6H, s), 2.64 (2H, t, J = 7.8 Hz), 2.91 (2H, t, J = 7.8 Hz), 3.19 (2H, q, J = 7.5 Hz), 3.42 (2H, t, J = 5.4 Hz), 4.44 (2H, t, J = 5.4 Hz), 5.07 (2H, s), 6.63−6.72 (4H, m), 7.05−7.17 (3H, m), 7.35−7.47 (2H, m); mp 123−124 °C. HPLC purity: 99.8%. Anal. Calcd for $C_{28}H_{31}FO_6S$: C, 65.35; H, 6.07. Found: C, 65.14; H, 5.97.

3-[4-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2-fluorophenyl]-propanoic Acid (31). To a solution of **21g** (0.53 g, 1.0 mmol) in EtOAc (10 mL) at 0 °C was added portionwise *m*-chloroperbenzoic acid (70%, 0.55 g, 2.2 mmol). The reaction mixture was stirred at the same temperature for 2 h and diluted with EtOAc. The mixture was washed sequentially with 1 M NaOH aqueous solution and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:1) to afford ethyl 3-[4-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2-fluorophenyl]propanate (0.25 g, 49%) as a pale-yellow oil. MS m/z 555 $(M + H)^+$. Subsequently, the title compound was prepared in 68% yield as a white solid from the above ester using the procedure analogous to that described for the synthesis of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98 (6H, s), 2.29−2.57 (4H, m), 2.65 (2H, t, J = 7.8 Hz), 2.85−3.01 (4H, m), 3.38−3.53 (2H, m), 4.67 (1H, m), 5.07 (2H, s), 6.61−6.73 (4H, m), 7.04−7.18 (3H, m), 7.34−7.49 (2H, m). MS m/z 527 $(M + H)^+$; mp 148−149 °C. HPLC purity: 100%. Anal. Calcd for $C_{29}H_{31}FO_6S$: C, 66.14; H, 5.93. Found: C, 65.92; H, 6.02.

3-[2-Fluoro-4-({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-phenyl]propanoic Acid (32). The title compound was prepared in 56% yield as a white solid from **19c** using the procedure analogous to that described for the synthesis of **31**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (6H, s), 2.17−2.38 (4H, m), 2.65 (2H, t, J = 7.5 Hz), 2.85−3.04 (4H, m), 3.41−3.60 (2H, m), 3.88 (2H, s), 5.08 (2H, s), 6.61−6.77 (4H, m), 7.03−7.21 (3H, m), 7.35−7.49 (2H, m). MS m/z 557 $(M + H)^+$; mp 198−199 °C. HPLC purity: 99.3%. Anal. Calcd for $C_{30}H_{33}FO_7S$: C, 64.73; H, 5.98; S, 5.76; F, 3.41. Found: C, 64.57; H, 5.94; S, 5.83; F, 3.48.

3-{4-[({4′-[2-(Ethylsulfonyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]-2-fluorophenyl}propanoic Acid Methanesulfonate (33). The title compound was prepared in 60% yield as a white solid from **21h** using the procedure analogous to that described for the synthesis of **34**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (3H, t, J = 7.5 Hz), 1.88 (6H, s), 2.34 (3H, s), 2.39 (2H, t, J = 7.5 Hz), 2.65 (2H, t, J = 7.5 Hz), 3.18 (2H, q, J = 7.5 Hz), 3.58 (2H, t, J = 5.4 Hz), 4.28−4.37 (4H, m), 6.28−6.42 (2H, m), 6.72 (2H, s), 6.90−7.00 (2H, m), 7.05 (1H, s), 7.31 (1H, d, J = 7.5 Hz), 7.39 (1H, t, J = 7.5 Hz). MS m/z 514 $(M + H)^+$ (as a free base). HPLC purity: 99.0%. Anal. Calcd for $C_{28}H_{32}FNO_5S{\cdot}CH_3SO_3H{\cdot}0.4H_2O$: C, 56.46; H, 6.01; N, 2.27. Found: C, 56.39; H, 5.95; N, 2.12.

3-{4-[({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]-2-fluorophenyl}-propanoic Acid Methanesulfonate (34). To a solution of **21i** (3.50 g, 4.76 mmol) in EtOAc (20 mL) at 0 °C was added portionwise *m*-chloroperbenzoic acid (70%, 1.41 g, 5.72 mmol). The reaction mixture was stirred at room temperature for 16 h and poured into 1 M NaOH aqueous solution. The mixture was extracted with EtOAc, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 9:1 to 1:1) to afford *tert*-butyl 3-(4-{({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)[(2-nitrophenyl)-sulfonyl]amino}-2-fluorophenyl)propanoate (2.49 g, 68%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (9H, s), 1.87 (6H, s), 2.28−2.58 (6H, m), 2.83 (2H, t, J = 7.6 Hz), 2.88−3.02 (2H, m), 3.36−3.53 (2H, m), 4.61−4.70 (1H, m), 4.94 (2H, s), 6.65 (2H, s), 6.70−6.84 (2H, m), 6.93−7.10 (3H, m), 7.20 (1H, d, J = 7.7 Hz), 7.31 (1H, t, J = 7.8 Hz), 7.46−7.54 (1H, m), 7.54−7.62 (1H, m), 7.63−7.74 (2H, m). To a solution of the above compound (2.49 g, 3.25 mmol) in DMF (16 mL) were added 2-sulfanylacetic acid (0.678 mL, 9.75 mmol) and lithium hydroxide monohydrate (0.818 g, 19.5 mmol). The reaction mixture was stirred at room temperature for 16 h and then concentrated in vacuo. The residue was partitioned between EtOAc and NaCl aqueous solution. The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 9:1 to 1:1) to afford *tert*-butyl 3-{4-[({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]-2-fluorophenyl}propanoate (1.55 g, 82%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (9H, s), 1.97 (6H, s), 2.28−2.61 (6H, m), 2.73−2.86 (2H, m), 2.88−3.03 (2H, m), 3.35−3.54 (2H, m), 4.04−4.22 (1H, m), 4.33 (2H, s), 4.60−4.73 (1H, m), 6.21−6.41 (2H, m), 6.66 (2H, s), 6.88−7.16 (3H, m), 7.28−7.46 (2H, m). To a solution of the above compound (1.55 g, 2.66 mmol) in toluene (15 mL) was added trifluoroacetic acid (15 mL). The reaction mixture was stirred at room temperature for 2 h and then concentrated in vacuo. The residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and the pH of the aqueous solution was adjusted to ca. 6−7 with 1 M HCl aqueous solution. The phases were separated, and the aqueous solution was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 9:1 to 1:1) to afford 3-{4-[({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)-amino]-2-fluorophenyl}propanoic acid (1.30 g, 2.47 mmol) as a white amorphous solid. This compound (1.30 g, 2.47 mmol) was then dissolved in EtOAc (5 mL) and Et_2O (5 mL), and to this solution was added methanesulfonic acid (0.160 mL, 2.47 mmol). The resulting solid was collected by filtration, rinsed with Et_2O/EtOAc (2:1), and dried to afford **34** (1.39 g, 47% from **21i**) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.87 (6H, s), 2.10−2.30 (4H, m), 2.34−2.46 (5H, m), 2.67 (2H, t, J = 7.5 Hz), 3.06−3.28 (4H, m), 4.35 (2H, s), 4.63−4.77 (1H, m), 6.35−6.53 (2H, m), 6.78 (2H, s), 6.92−7.10 (3H, m), 7.28−7.44 (2H, m). MS m/z 526 $(M + H)^+$ (as a free base). HPLC purity: 99.9%. Anal. Calcd for $C_{29}H_{32}FNO_5S{\cdot}CH_3SO_3H{\cdot}0.5H_2O$: C, 57.13; H, 5.91; N, 2.22. Found: C, 57.08; H, 5.85; N, 2.15.

3-{2-Fluoro-4-[({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)-amino]phenyl}propanoic Acid (35). To a solution of **19g** (1.71 g, 2.32 mmol) in dichloromethane (20 mL) at 0 °C was added portionwise *m*-chloroperbenzoic acid (70%, 1.26 g, 5.11 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated in vacuo. The residue was partitioned between EtOAc and 1 M NaOH aqueous solution. The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 9:1 to 1:4) to afford ethyl 3-(2-fluoro-4-{({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-di-

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methylbiphenyl-3-yl}methyl)[(2-nitrophenyl)sulfonyl]amino}phenyl)-propanoate (1.28 g, 72%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, t, J = 7.3 Hz), 1.80−1.94 (6H, m), 2.15−2.38 (4H, m), 2.45−2.64 (3H, m), 2.79−3.05 (4H, m), 3.40−3.61 (2H, m), 3.87 (2H, s), 4.10 (2H, q, J = 7.1 Hz), 4.93 (2H, s), 6.63 (2H, s), 6.71−6.86 (2H, m), 6.87−7.12 (3H, m), 7.19−7.41 (2H, m), 7.46−7.79 (4H, m). To a solution of the above compound (1.28 g, 1.67 mmol) in DMF (9 mL) were added sulfanylacetic acid (0.348 mL, 5.01 mmol) and lithium hydroxide monohydrate (0.420 g, 10.0 mmol). The reaction mixture was stirred at room temperature for 24 h and then concentrated in vacuo. The residue was partitioned between EtOAc and NaCl aqueous solution. The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 17:3 to 1:1) to afford ethyl 3-{2-fluoro-4-[({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methyl)amino]phenyl}propanoate (0.820 g, 84%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.3 Hz), 1.98 (6H, s), 2.14−2.35 (4H, m), 2.44−2.61 (3H, m), 2.75−3.02 (4H, m), 3.37−3.59 (2H, m), 3.87 (2H, s), 4.11 (2H, q, J = 7.2 Hz), 4.33 (2H, s), 6.21−6.39 (2H, m), 6.66 (2H, s), 6.88−7.12 (3H, m), 7.27−7.44 (2H, m). To a solution of the above compound (0.820 g, 1.40 mmol) in THF (3 mL) and MeOH (10 mL) was added 1 M NaOH aqueous solution (4.20 mL, 4.20 mmol). After being stirred at room temperature for 24 h, 10% citric acid aqueous solution was added. The mixture was evaporated under reduced pressure to remove MeOH, extracted with EtOAc, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 17:3 to 1:2). The residue was recrystallized from EtOAc/hexane to afford **35** (0.618 g, 48% from **19g**) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.89−2.07 (6H, m), 2.15−2.36 (4H, m), 2.60 (2H, t, J = 7.6 Hz), 2.84 (2H, t, J = 7.6 Hz), 2.89−3.02 (2H, m), 3.38−3.59 (2H, m), 3.87 (2H, s), 4.34 (2H, s), 6.24−6.40 (2H, m), 6.65 (2H, s), 6.91−7.11 (3H, m), 7.28−7.45 (2H, m). MS m/z 556 $(M + H)^+$; mp 180−181 °C. HPLC purity: 97.8%. Anal. Calcd for $C_{30}H_{34}FNO_6S$: C, 64.85; H, 6.17; N, 2.52. Found: C, 64.55; H, 6.33; N, 2.41.

Ethyl (2*E*)-3-(4-Amino-2-fluorophenyl)prop-2-enoate (37a). To a mixture of **36** (13.3 g, 70.0 mmol), ethyl acrylate (9.48 mL, 87.5 mmol), and tris(2-methylphenyl)phosphine (8.52 g, 28.0 mmol) in *N*,*N*-diisopropylethylamine (50 mL) and DMF (50 mL) was added palladium acetate(II) (0.786 g, 3.50 mmol). The reaction mixture was stirred for 5 h at 110 °C under argon. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was diluted with water and EtOAc, and the insoluble materials were removed by Celite filtration. The organic phase of the filtrate was washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 2:3) to afford **37a** (14.0 g, 96%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 7.2 Hz), 4.03 (2H, s), 4.24 (2H, q, J = 7.2 Hz), 6.28−6.47 (3H, m), 7.32 (1H, t, J = 8.4 Hz), 7.71 (1H, d, J = 16.0 Hz). MS m/z 210 $(M + H)^+$.

***tert*-Butyl (2*E*)-3-(4-Amino-2-fluorophenyl)prop-2-enoate (37b).** The title compound was prepared in 80% yield as a yellow solid from **36** and *tert*-butyl acrylate using the procedure analogous to that described for the synthesis of **37a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (9H, s), 3.99 (2H, s), 6.26 (1H, d, J = 16.2 Hz), 6.31−6.45 (2H, m), 7.30 (1H, t, J = 8.4 Hz), 7.62 (1H, d, J = 16.2 Hz).

Ethyl 3-(4-Amino-2-fluorophenyl)propanoate (38a). Compound **37a** (12.4 g, 59.3 mmol) was hydrogenated under atmospheric hydrogen with 10% Pd on carbon (containing 50% water, 4.0 g) as a catalyst in EtOH (120 mL) at room temperature for 12 h. The reaction mixture was filtered by Millipore filtration and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:1) to afford **38a** (9.89 g, 79%) as a pale-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 2.55 (2H, t, J = 7.7 Hz), 2.85 (2H, t, J = 7.7 Hz), 3.66 (2H, s), 4.12 (2H, q, J = 7.2 Hz), 6.32−6.42 (2H, m), 6.96 (1H, t, J = 8.4 Hz). MS m/z 212 $(M + H)^+$.

***tert*-Butyl 3-(4-Amino-2-fluorophenyl)propanoate (38b).** The title compound was prepared in 98% yield as a pale-yellow solid from **37b** using the procedure analogous to that described for the synthesis of **38a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (9H, s), 2.47 (2H, t, J = 7.8 Hz), 2.81 (2H, t, J = 7.8 Hz), 3.65 (2H, s), 6.32−6.40 (2H, m), 6.95 (1H, t, J = 8.4 Hz). MS m/z 240 $(M + H)^+$.

Ethyl 3-(2-Fluoro-4-{[(2-nitrophenyl)sulfonyl]amino}-phenyl)propanoate (39a). To a solution of **38a** in pyridine (70 mL) was added portionwise 2-nitrobenzenesulfonyl chloride (11.4 g, 51.5 mmol). After being stirred at room temperature for 70 h, the reaction mixture was concentrated in vacuo. To the residue were added water and EtOAc, and the resultant mixture was stirred at 80 °C for 15 min and passed through a pad of Celite. The organic phase of the filtrate was washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:1) to afford **39a** (14.2 g, 76%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, t, J = 7.2 Hz), 2.56 (2H, t, J = 7.6 Hz), 2.89 (2H, t, J = 7.6 Hz), 4.10 (2H, q, J = 7.2 Hz), 6.86 (1H, dd, J = 8.3, 1.9 Hz), 6.99 (1H, dd, J = 10.7, 2.3 Hz), 7.10 (1H, t, J = 8.3 Hz), 7.24 (1H, s), 7.62 (1H, td, J = 7.7, 1.4 Hz), 7.72 (1H, td, J = 7.7, 1.4 Hz), 7.87 (1H, dd, J = 3.4, 1.4 Hz), 7.89 (1H, dd, J = 3.4, 1.4 Hz). MS m/z 397 $(M + H)^+$.

***tert*-Butyl 3-(2-Fluoro-4-{[(2-nitrophenyl)sulfonyl]amino}-phenyl)propanoate (39b).** The title compound was prepared in 77% yield as a beige solid from **38b** using the procedure analogous to that described for the synthesis of **39a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (9H, s), 2.48 (2H, t, J = 7.8 Hz), 2.85 (2H, t, J = 7.8 Hz), 6.86 (1H, dd, J = 2.1, 8.1 Hz), 6.98 (1H, dd, J = 2.1, 10.8 Hz), 7.10 (1H, t, J = 8.1 Hz), 7.61 (1H, m), 7.71 (1H, m), 7.87 (2H, dd, J = 1.5, 7.8 Hz).

Ethyl 3-[4-({[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methyl}amino)-2-fluorophenyl]propanoate (40). To a mixture of **17a** (1.48 g, 7.00 mmol) and **38a** (2.09 g, 7.00 mmol) in toluene (15 mL) were added molecular sieves (0.4 nm, beads, 3.5 g). After being stirred at room temperature for 30 h, the reaction mixture was passed through a pad of Celite and the filtrate was concentrated in vacuo to afford the crude imine. The above imine was hydrogenated under atmospheric hydrogen with 10% Pd on carbon (containing 50% water, 0.5 g) as a catalyst in EtOH (20 mL) at room temperature for 12 h. The reaction mixture was filtered by Millipore filtration and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 19:1 to 7:3) to afford **40** (2.88 g, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19−1.28 (6H, m), 1.97 (6H, s), 2.54 (2H, t, J = 7.7 Hz), 2.84 (2H, t, J = 7.7 Hz), 3.61 (2H, q, J = 7.0 Hz), 3.80 (2H, t, J = 5.0 Hz), 4.07−4.16 (5H, m), 4.33 (2H, s), 6.25−6.36 (2H, m), 6.68 (2H, s), 6.95 (1H, t, J = 8.5 Hz), 7.04 (1H, dt, J = 7.3, 1.4 Hz), 7.10 (1H, s), 7.27−7.32 (1H, m), 7.38 (1H, t, J = 7.5 Hz). MS m/z 494 $(M + H)^+$.

3-[4-({[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]-methyl}amino)-2-fluorophenyl]propanoic Acid Hydrochloride (41). To a solution of **40** (2.88 g, 5.83 mmol) in EtOH (90 mL) and THF (90 mL) was added 2 M NaOH aqueous solution (30 mL). The reaction mixture was stirred at room temperature for 16 h, and then 10% citric acid aqueous solution was added until the solution reached pH ∼ 5. The mixture was extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, hexane/EtOAc, 4:1 to 1:4) to give a free base of **41** (2.70 g, 5.80 mmol) as a pale-yellow oil. To a solution of the above carboxylic acid (2.66 g, 5.72 mmol) in EtOAc (15 mL) was added 4 M HCl solution in EtOAc (5 mL). The resulting precipitate was triturated with $EtOAc/Et_2O$, collected by filtration, rinsed with $EtOAc/Et_2O$, and dried to afford **41** (2.78 g, 96%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (3H, t, J = 7.0 Hz), 1.83 (6H, s), 2.65 (2H, t, J = 6.5 Hz), 2.83 (2H, t, J = 6.5 Hz), 3.61 (2H, q, J = 7.0 Hz), 3.78 (2H, t, J = 4.8 Hz), 4.11 (2H, t, J = 4.8 Hz), 4.48 (2H, s), 6.63 (2H, s), 6.82 (1H, d, J = 9.8 Hz), 6.89 (1H, s), 6.94−7.01 (1H, m), 7.02−7.12 (2H, m), 7.41 (1H, t, J = 7.6 Hz), 7.49−7.55 (1H, m). mp 122 °C. Anal. Calcd

for $C_{28}H_{32}FNO_4$·HCl: C, 66.99; H, 6.63; N, 2.79. Found: C, 66.81; H, 6.68; N, 2.72.

Preparation of CHO Membrane for GPR40 Receptor Binding Assay. Cell lines stably expressing human GPR40 and rat GPR40 were used for the experiments. Each cell was cultured in Minimum Essential Medium Alpha (MEM-Alpha, Invitrogen) supplemented with 10% dialyzed fetal bovine serum (dialyzed FBS, Thermo Trace Ltd.), 100 unit/mL penicillin, and 100 unit/mL streptomycin in 5% CO_2/95% air atmosphere at 37 °C. Cells were harvested at confluence in Dulbecco's Phosphate-Buffered-Saline (D-PBS, Invitrogen) containing 1 mM EDTA and centrifuged. Cells were homogenized in ice-cold membrane preparation buffer (50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF (Wako), 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 100 μg/mL Phosphoramidon, (Peptide Institute, Inc.), and centrifuged (700*g*, 10 min, 4 °C). The supernatant was filtered through 40 μm cell strainer (BD Falcon) and ultracentrifuged (100000*g*, 1 h, 4 °C) with Optima L-100 XP ultracentrifuge (Beckman Coulter). The precipitation was suspended in the same buffer, and the protein concentration was determined with the BCA Protein assay reagent (Pierce) following membrane solubilization with 0.1% SDS and 0.1 M NaOH aqueous solution. The membrane suspension was stored at −80 °C until receptor binding assay.

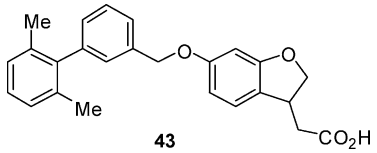
GPR40 Receptor Binding Assay. The frozen cell membranes were resuspended in ice-cold assay buffer (25 mmol/L Tris-HCl (pH 7.5), 5 mmol/L EDTA, 0.5 mmol/L PMSF, 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 0.05% CHAPS (Wako), 0.2% fatty-acid-free BSA (Sigma)), and used for receptor binding assay. To determine the K_d values of 3-[4-({2′,6′-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl] propanoic acid (Amersham Biosciences) for human and rat GPR40, binding assays were performed in the presence of various concentrations of the labeled ligand. After incubation at room temperature for 90 min, the membranes were harvested GF/C filter plates (MILLIPORE) and washed with ice-cold 50 mmol/L Tris-HCl (pH 7.5) using FilterMate Harvester (PerkinElmer). The membrane-associated radioactivities were counted using a TopCount liquid scintillation counter (PerkinElmer). Nonspecific binding was defined as binding in the presence of 10 μmol/L of the unlabeled ligand. To determine the binding affinities of test compounds to human and rat GPR40, binding assays were performed in the presence of both various concentrations of test compounds and 2 or 6 nmol/L of the labeled ligand. The 50% inhibitory concentrations (IC_{50} values) of test compounds for the labeled ligand were calculated using nonlinear regression analysis in GraphPad Prism 3.0 (GraphPad Software). K_i values were converted as $K_i = IC_{50}/\{1 + (\text{the concentration of the labeled ligand})/K_d\}$.

Ca Influx Activity of CHO Cells Expressing Human GPR40 (FLIPR Assay). CHO dhfr cells stably expressing human GPR40 (accession no. NM_005303) were plated and incubated overnight in 5% CO_2 at 37 °C. Then cells were incubated in loading buffer (recording medium containing 2.5 μg/mL fluorescent calcium indicator Fluo 4 a.m. (Molecular Devices), 2.5 mmol/L probenecid (Dojindo), and 0.1% fatty acid-free BSA (Sigma)) for 60 min at 37 °C. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the cells and increases of the intracellular Ca^{2+} concentration after addition were monitored by FLIPR Tetra system (Molecular Devices) for 90 s. The agonistic activities of test compounds and γ-linolenic acid on human GPR40 were expressed as $[(A - B)/(C - B)] \times 100$ (increase of the intracellular Ca^{2+} concentration (A) in test compounds-treated cells, (B) in vehicle-treated cells, and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} value and 95% confidence interval of each compound was obtained with Prism 5.0 software (GraphPad).

Cytotoxicity Test. HepG2 cells were cultured at 37 °C, 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 50 μg/mL streptomycin. Cells were seeded at 2×10^4 cells/well in 96-well white plate (Costar) and cultured with test compounds in DMEM supplemented with 0.5% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 IU/mL penicillin, and 50 μg/mL streptomycin for 1 day. The intracellular ATP content was measured by using ATPliteTM-M (PerkinElmer) according to the manufacture's instruction. ATP content was calculated (n = 3) as follows. ATP content (% of control) = (RLU of compound/RLU of 1% DMSO) × 100. Caspase-3/7 activity was measured by using Caspase-GloTM 3/7 assay kit (Promega) according to the manufacture's instruction. Caspase-3/7 activity was calculated (n = 3) as follows. Caspase-3/7 activity (%) = (RLU of compound − RLU of 1% DMSO)/(RLU of 30 μM Staurosporine − RLU of 1% DMSO) × 100.

Pharmacokinetic Analysis in Rat Cassette Dosing. Test compounds were administered as a cassette dosing to nonfasted rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with CH_3CN containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by a high performance liquid chromatography−tandem mass spectrometry.

Homology Modeling and Ligand Docking. A homology model of GPR40 was constructed using the crystal structure of bovine rhodopsin (PDB code 1GZM),[57] which was obtained from the RCSB Protein Data Bank as a structural template. An alignment of the amino acid sequences between GPR40 and rhodopsin was created using ClustalX (version 2.0.11)[58] and manually revised. Procedures of homology modeling were performed in MOE (version 2008.10).[59] The CL2 loop on the extra cellular domain was excluded except Cys170 forming disulfide bond due to the difficulty of estimation. In the previous step, compound **43** was docked into the obtained receptor model using the program GOLD (version 4.1).[60] Then, the resultant docking models with receptor models, replacing compound **43** with **31**, were subjected to energy minimization with MOE after connecting each residual substituent. In the energy minimization



43

process, the MMFF94s force field was used and the dielectric constant was set to 2r, where r is the distance between two interacting atoms.

In Vitro Insulin Secretion Assay. Rat insulinoma INS-1 cell clone, INS-1 833/15 cells were kindly donated by Duke University Medical Center. INS-1 833/15 cells were maintained in RPMI 1640 (Invitorogen) containing 10% heat-inactivated fetal calf serum (Thermo Trace), 10 mM Hepes, 0.05 mM β-mercaptoethanol, penicillin/streptomycin, and 1 mM sodium pyruvate at 37 °C in a humidified 5% CO_2/95% air. The cells were seeded into 96-well poly-L-lysin coated plates (5×10^4 cells/well) and were subsequently cultured for 2 days at 37 °C in a humidified 5% CO_2/95% air environment. After the culture, media was removed and cells were preincubated with Krebs−Ringer bicarbonate Hepes (KRBH) buffer containing (116 mM NaCl, 4.7 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 2.52 mM $CaCl_2 \cdot 2H_2O$, 24 mM HEPES, 0.2% FFA-free BSA) containing 1 mM glucose. After the preincubation, the buffer solution was discarded and KRBH solution containing various concentration of glucose as indicated in Figure 3, with or without compound **31** dissolved in dimethylsulfoxide, was added. After 2 h incubation, the supernatant in each well was collected. Insulin concentration of each supernatant shown in Figure 3a was measured by EIA kit (Morinaga, Japan), according to the manufacturer's instruction. Insulin concentration shown in Figure 3b was measured by homemade competitive EIA using human insulin specific antibody and biotinated-human insulin.

Oral Glucose Tolerance Test in Female Wistar Rats. Female Wistar fatty rats were obtained from Takeda RABICS (Osaka, Japan). Rats were fed regular chow CE-2 (CLEA, Japan) and tap water ad libitum and were housed in cages in a room with controlled temperature (23 ± 1 °C), humidity (55 ± 5%), and lighting (lights

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on from 07:30 to 19:30). The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company (Osaka, Japan). Thirteen-week-old female Wistar fatty rats were fasted overnight, and blood samples were collected from tail vein. Plasma glucose (PG), triglyceride (TG), and nonesterified free fatty acids (NEFA) levels of each sample were measured using the automatic analyzer Hitachi 7080 (Hitachi, Japan). Rats were divided into several groups based on body weight, PG, TG, and NEFA (n = 6). Compound **31** (0.1−1 mg/kg) or 0.5% methylcellulose (vehicle) was orally administered 30 min before oral glucose load (1 g/kg). Blood samples were collected from tail vein 0 (just before glucose load), 10, 30, 60, and 120 min after the glucose load. After collecting the plasma, PG levels were measured using Hitachi 7080. Plasma insulin levels were measured using rat insulin RIA kit (Linco). Statistical differences versus control were analyzed by one-tailed Williams' test or Shirley−Williams' test.

Effects on Normal Fasting Plasma Glucose and Insulin Levels in Normal Rats. Eight-week-old male SD rats were fasted overnight and divided into 3 groups based on body weight (n = 6). Compound **35** (30 mg/kg), glibenclamide (10 mg/kg), or vehicle (0.5% methylcellulose) was orally administered, and blood was collected from tail vein before 0.5, 1, 2, and 3 h after the administrations. Plasma glucose and levels were measured as described above. Plasma insulin levels were measured by rat insulin ELISA kit (Morinaga, Japan). Statistical differences versus control were analyzed by Student's t test or Aspin−Welch test.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

GPCR, G protein-coupled receptor; GPR40, G protein-coupled receptor 40; T2DM, type 2 diabetes mellitus; FFA, free fatty acids; GSIS, glucose-stimulated insulin secretion; PLC, phospholipase C; IP_3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C; FLIPR, fluorometric imaging plate reader; TBAF, tetrabutylammonium fluoride; CHO, Chinese hamster ovary; EIA, enzyme immunoassay; KRBH, Krebs−Ringer bicarbonate Hepes; PG, plasma glucose; TG, triglyceride; NEFA, nonesterified free fatty acids

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