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Removal of Sphingosine 1-Phosphate Receptor-3 ($S1P_3$) Agonism is Essential, But Inadequate to Obtain Immunomodulating 2-Aminopropane-1,3-diol $S1P_1$ Agonists with Reduced Effect on Heart Rate

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A series of 2-substituted 2-aminopropane-1,3-diols having a biphenyl moiety and their phosphate esters were synthesized to obtain sphingosine 1-phosphate receptor-1 ($S1P_1$) receptor agonists with potent immunomodulatory activity accompanied by little or no effect on heart rate. Many of the synthesized compounds sufficiently decreased the number of peripheral blood lymphocytes. Some of the phosphates had potent agonism at $S1P_1$ but no agonism at $S1P_3$, which had been reported to be a receptor responsible for heart rate reduction. Although high $S1P_1/S1P_3$ selectivity was considered to be favorable to reduce the effect on heart rate, almost all the phosphates showed a remarkable heart rate lowering effect in vivo. The results suggest that other factors in addition to $S1P_3$ agonism should be responsible for the heart rate reduction caused by $S1P_1$ agonists. Only 2-amino-2-[2-[2′-fluoro-4′-(4-methylphenylthio)biphenyl-4-yl]ethyl]propane-1,3-diol (**6d**) was identified as a desired $S1P_1$ receptor agonist having both the immunomodulatory activity and an attenuated effect on heart rate by a unique screening flow using in vivo evaluating systems primarily.

Introduction

Sphingosine 1-phosphate (S1P[a]) is a ubiquitous lysophospholipid and influences multiple physiological systems.[1] Genetic deletion of S1P receptor-1 ($S1P_1$) in mice showed that S1P has important roles in vascular maturation and resulted in embryonic lethality.[2,3] Our group previously reported that a unique immunomodulator **1** (FTY720, Chart 1) was discovered by the exploration of chemically modified analogues of a natural compound myriocin using in vivo screens as the main evaluating systems[4,5] and that compound **1** showed the activity by decreasing the number of lymphocytes in peripheral blood and lymph drastically.[6] During the course of research to clarify the mechanism of action of **1**, it was revealed that **1** is converted to its phosphate (**1-P**) by sphingosine kinase 2[7] and **1-P** acts as an agonist for four S1P receptors ($S1P_{1,3,4,5}$) among five known S1P receptors ($S1P_{1-5}$).[8,9] Currently, a large body of evidence shows that $S1P_1$ plays the main role in the lymphocyte-decreasing effect of **1**.[10] Thus the physiological roles of S1P and its receptors have become clear by complementary approaches of genetics and medicinal chemistry.

Compound **1** was shown to be effective in the treatment of multiple sclerosis in phase 2 clinical trials[11] and is currently in phase 3 clinical trials. Additionally, (1*R*,2*S*,3*R*)-2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole (THI), which inhibits S1P lyase in vivo, caused lymphopenia-like **1**[12] and exerted an immunomodulatory effect.[13] These facts suggest that modulators of lymphocyte circulation are highly promising as an immnunomodulator.

On the other hand, the administration of **1** was typically associated with a mild and transient reduction in heart rate as an adverse event in the clinical trials.[14] It was reported that intravenous administration of **1-P** at a dose of 0.005 mg/kg to normal mice evoked a severe reduction of heart rate, whereas in $S1P_3$-deficient mice the administration of **1-P** produced no change in heart rate.[15] This genetic evidence clearly indicates that $S1P_3$ is mainly responsible for the heart rate decreasing effect of **1** in mice. Consequently, $S1P_1$-selective agonists against $S1P_3$ would be expected as a next generation of the S1P receptor modulator with little or no effect on heart rate. On the basis of this presumption, research to find selective $S1P_1$ agonists have been actively performed.[16,17]

Our attempt to obtain a desired $S1P_1$ receptor agonist having both the immunomodulatory activity and a reduced effect on heart rate was initiated by reassessment of **1**-related compounds in our library. Among the phosphates of **1**-related compounds, the phosphate of biphenyl compound **2** was found to be inactive at the $S1P_3$ receptor, although **2** showed much weaker potency in lymphocyte-decreasing activity than **1** (Table 1). We modified the lipophilic side chain of **2** and succeeded in obtaining compound **6d**, which had a reduced heart rate decreasing effect as well as a sufficient immunomodulatory activity (Table 4). Interestingly, it was confirmed in this research that most of the compounds with no $S1P_3$ agonism still reduce heart rate remarkably. This fact strongly suggests that some complicated mechanisms independent of

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[a] Abbreviations: S1P, sphingosine 1-phosphate ; $S1P_1$, sphingosine 1-phosphate receptor-1; THI, (1*R*,2*S*,3*R*)-2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole; DMF, *N*,*N*-dimethylformamide; DME, 1, 2-dimethoxyethane; S-Phos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; X-Phos, 2-dicychlohexylphosphino-2′,4′,6′-triisopropylbiphenyl; Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; $Pd_2(dba)_3 \cdot CHCl_3$, tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct; *m*-CPBA, 3-chloroperoxybenzoic acid; DIPEA, diisopropylethylamine; DMSO, dimethyl sulfoxide; HPLC, high performance liquid chromatography; UPLC, ultra performance liquid chromatography; TMS, tetramethylsilane; TFA, trifluoroacetic acid.

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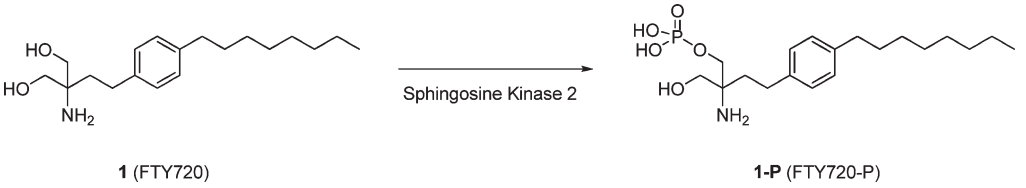
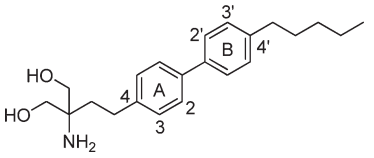
Chart 1. Structures of FTY720 (**1**) and the Active Form, FTY720-P (**1-P**)



1 (FTY720) **1-P** (FTY720-P)

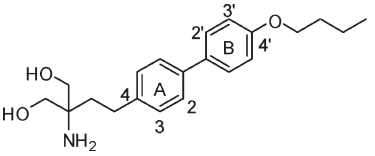
Table 1. Lymphocyte-Decreasing Effect, Agonistic Activity at $S1P_1$ and $S1P_3$, and Heart Rate Decreasing Effect of **1** and Lead Compounds



compd	structure A	structure B	ED_{50}[a] (mg/kg)	phosphate	$S1P_1$ EC_{50}[b,c] (nM)	$S1P_3$ EC_{50}[b] (nM)	ID_{50}[d] (mg/kg)
1			0.02	**1-P**	0.35 (0.14−0.91)	12 (11−13)	0.012
2			0.40	**2-P**	1.0 (0.33−3.5)	> 1000	0.091
3a	3-Cl		0.11	**3a-P**	0.64 (0.20−2.1)	> 1000	NT[e]
4		2′-F	0.10	**4-P**	1.1 (0.40−3.0)	> 1000	NT

[a] Dose (mg/kg) required to decrease the number of lymphocytes by 50% of vehicle-treated control in mice (5 animals for each compound). Alcohol form of compounds was administered intraperitoneally. [b] EC_{50} (nM) of phosphate form was determined by Ca mobilization assays. 95% confidence limits are given in parentheses. [c] Concentration required to show 50% of agonistic activity at S1P (300 nM). [d] Dose (mg/kg) required to decrease heart rate by 50% of vehicle-treated control in rats. Phosphate form of compounds was administered intravenously (3−6 animals for each compound). [e] Not tested.

Table 2. Lymphocyte-Decreasing Effect, Agonistic Activity at $S1P_1$ and $S1P_3$, and Heart Rate Decreasing Effect of Butoxy Derivatives



compd	structure A	structure B	ED_{50}[a] (mg/kg)	phosphate	$S1P_1$ EC_{50}[b,c] (nM)	$S1P_3$ EC_{50}[b] (nM)	ID_{50}[d] (mg/kg)
3b			0.22	**3b-P**	0.81 (0.31−2.4)	> 1000	0.008
3c	3-Cl		0.08	**3c-P**	0.62 (0.22−1.8)	> 1000	0.013
3d		2′-F	0.06	**3d-P**	0.53 (0.18−1.6)	51 (17−150)	0.009
3e		2′-Me	3.4				
3f		2′-F, 5′-F	0.15	**3f-P**	1.2 (0.40−3.3)	> 1000	NT[e]
3g		3′-F	0.21	**3g-P**	1.3 (0.40−4.3)	> 1000	NT
3h		3′-Cl	1.3	**3h-P**	3.1 (0.42−23)	> 1000	NT

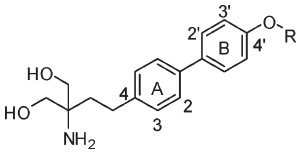
[a] Dose (mg/kg) required to decrease the number of lymphocytes by 50% of vehicle-treated control in mice (5 animals for each compound). Alcohol form of compounds was administered intraperitoneally. [b] EC_{50} (nM) of phosphate form was determined by Ca mobilization assays. 95% confidence limits are given in parentheses. [c] Concentration required to show 50% of agonistic activity at S1P (300 nM). [d] Dose (mg/kg) required to decrease heart rate by 50% of vehicle-treated control in rats. Phosphate form of compounds was administered intravenously (3−6 animals for each compound). [e] Not tested.

$S1P_3$ or species difference would contribute to heart rate reduction caused by **1**. In this paper, we describe the details of the discovery of **6d** and the relationships between $S1P_3$ agonism and heart rate decreasing effect.

Lead Compound and Pharmacological Evaluation. To obtain a lead compound that had selective activity at $S1P_1$, we tried to check our **1**-related compound library. We synthesized the phosphate form of compounds in this library (e.g., **1-P**) and evaluated their $S1P_1$ and $S1P_3$ agonistic activities. The results revealed that **2-P** having a biphenyl moiety in the hydrophobic part had no agonistic activity at $S1P_3$ (Table 1). Though the lymphocyte-decreasing effect of **2** (ED_{50} = 0.40 mg/kg) was weaker than that of **1** (ED_{50} = 0.02 mg/kg), compounds **3a** and **4**, which have substituents on the biphenyl moiety of **2**, were approximately 3-fold more potent than **2** and their phosphates retained the inactivity at $S1P_3$. These results prompted us to select **2** as a lead compound and start our research to obtain $S1P_1$ agonists with little or no effect on heart rate.

The first step of our research procedure was the synthesis and evaluation of the alcohol form of designed compounds. The immunomodulating activity of the alcohol form was estimated by counting the number of lymphocytes in peripheral blood. Since promoting the sequestration of peripheral blood lymphocytes in secondary lymphoid organs dependent on the $S1P_1$ agonism has been considered to cause immunomodulating activity of **1**, the lymphocyte-decreasing effect in peripheral blood can be used as a marker for the

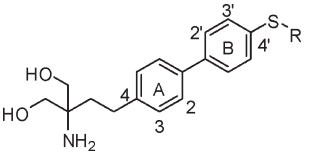
Table 3. Lymphocyte-Decreasing Effect, Agonistic Activity at $S1P_1$ and $S1P_3$, and Heart Rate Decreasing Effect of Benzyloxy and Phenoxy Derivatives



compd	structure A	structure B	structure R	ED_{50}[a] (mg/kg)	phosphate	$S1P_1$ EC_{50}[b,c] (nM)	$S1P_3$ EC_{50}[b] (nM)	ID_{50}[d] (mg/kg)
3i			phenyl	0.25	**3i-P**	21[e]	> 1000	0.14
3j	3-Cl		phenyl	0.16	**3j-P**	4.7 (0.24−92)	> 1000	0.087
3k	3-Cl		benzyl	0.13	**3k-P**	1.6 (0.27−8.9)	13 (3.9−44)	0.017
3l		2′-F	benzyl	0.01	**3l-P**	1.1 (0.25−4.0)	4.1 (2.9−5.6)	0.014
5		2′-F	4-Me-phenyl	0.04	**5-P**	1.8 (0.58−5.9)	83 (35−199)	0.016

[a] Dose (mg/kg) required to decrease the number of lymphocytes by 50% of vehicle-treated control in mice (5 animals for each compound). Alcohol form of compounds was administered intraperitoneally. [b] EC_{50} (nM) of phosphate form was determined by Ca mobilization assays. 95% confidence limits are given in parentheses. [c] Concentration required to show 50% of agonistic activity at S1P (300 nM). [d] Dose (mg/kg) required to decrease heart rate by 50% of vehicle-treated control in rats. Phosphate form of compounds was administered intravenously (3−6 animals for each compound). [e] This value is reported as mean for $n = 2$ determinations.

Table 4. Lymphocyte-Decreasing Effect, Agonistic Activity at $S1P_1$ and $S1P_3$, and Heart Rate Decreasing Effect of Benzylthio and Phenylthio Derivatives



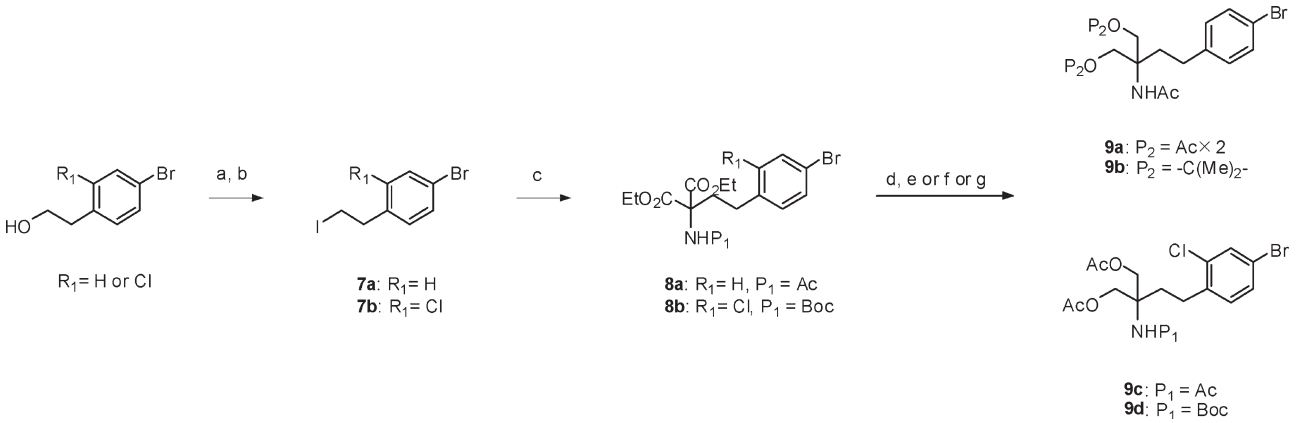
compd	structure A	structure B	structure R	ED_{50}[a] (mg/kg)	phosphate	$S1P_1$ EC_{50}[b,c] (nM)	$S1P_3$ EC_{50}[b] (nM)	ID_{50}[d] (mg/kg)
6a	3-Cl		benzyl	0.08	**6a-P**	2.1 (0.77−5.8)	> 1000	0.018
6b		2′-F	benzyl	0.04	**6b-P**	0.84 (0.24−3.0)	4.9 (3.3−7.4)	0.008
6c		2′-F	phenyl	0.42	**6c-P**	3.6[f]	> 1000	NT[e]
6d		2′-F	4-Me-phenyl	0.05	**6d-P**	3.0 (0.56−16)	> 1000	0.10
6e		2′-F	3-Me-phenyl	0.13	**6e-P**	2.0 (0.29−14)	> 1000	0.063
6f	3-Cl	2′-F	4-Me-phenyl	0.04	**6f-P**	4.2 (0.46−38)	> 1000	0.016
6g		2′-F	4-Et-phenyl	0.04				
6h		2′-F	4-*i*-Pr-phenyl	0.15				
6i		2′-F	4-F-phenyl	0.07				
6j		2′-F	3-F-phenyl	0.16				
6k		2′-F	4-Cl-phenyl	0.18				
6l		2′-F	4-CF_3-phenyl	0.34				
6m		2′-F	4-MeO-phenyl	0.28				
6n		2′-F	3-MeO-phenyl	0.11				

[a] Dose (mg/kg) required to decrease the number of lymphocytes by 50% of vehicle-treated control in mice (five animals for each compound). Alcohol form of compounds was administered intraperitoneally. [b] EC_{50} (nM) of phosphate form was determined by Ca mobilization assays. 95% confidence limits are given in parentheses. [c] Concentration required to show 50% of agonistic activity at S1P (300 nM). [d] Dose (mg/kg) required to decrease heart rate by 50% of vehicle-treated control in rats. Phosphate form of compounds was administered intravenously (3−6 animals for each compound). [e] Not tested. [f] This value is reported as mean for $n = 2$ determinations.

immunomodulating efficacy of the compounds. This method has been adopted as a simple in vivo screening since the early stage of **1**-related research when its mechanism of action was uncertain[5] and has been widely used for the evaluation of immnomodulatory effects of S1P receptor modulators.[17,18] The number of lymphocytes was counted 24 h after intraperitoneal administration to mice, and the results are shown as ED_{50} values. Next, the phosphate esters of alcohols having a sufficient lymphocyte-decreasing effect (ED_{50} < 0.20 mg/kg) were synthesized, and their $S1P_1/S1P_3$ selectivity was evaluated by calcium mobilization assays using transfectants stably expressing the $hS1P_1$ or $hS1P_3$ receptor. The EC_{50} values of **1-P** for $S1P_1$ and $S1P_3$ were 0.35 and 12 nM, respectively. Then, the influence of the phosphates on heart rate was determined by intravenous administration to anesthetized rats. Compound **1-P** lowered heart rate intensively, and the ID_{50} value was 0.012 mg/kg. Pharmacokinetic profiles usually reflect to in vivo results; however, the maximal heart rate reduction in this experiment was observed promptly (in 1 min) after the intravenous administration of **1-P**. Therefore, we assumed that the potency of phosphates on heart rate decreasing effect could be evaluated with little influence of pharmacokinetic difference among the compounds by this method.

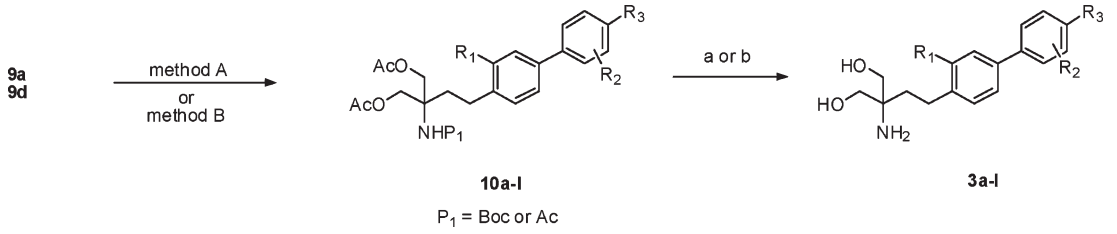
At the beginning of this study, we planned to evaluate mainly the in vitro S1P receptor selectivity of the phosphates because searching for $S1P_1$ selective compounds seemed to be a reasonable approach to obtain compounds with a

Scheme 1. Synthesis of Intermediate Bromides **9**[a]



[a] Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, rt; (b) NaI, 2-butanone, reflux; (c) *N*-protected diethyl aminomalonate, NaH, DMF, 60 °C; (d) $NaBH_4$, $CaCl_2$, EtOH/H_2O, rt; (e) Ac_2O, pyridine, rt; (f) 2,2-dimethoxypropane, *p*-TsOH·H_2O, acetone, rt; (g) (i) 4 M HCl/EtOAc, (ii) Ac_2O, pyridine, rt.

Scheme 2. Synthesis of Biphenyl Compounds **3**[a]



[a] Reagents and conditions: (method A) boronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O, 65 °C; (method B) (i) bis(pinacolate)diboron, $PdCl_2(Cy_3P)_2$, AcOK, dioxane, 100 °C, (ii) phenyl bromide, $Pd(OAc)_2$, S-Phos, K_3PO_4, toluene, 100 °C; (a) 35% HCl, EtOH, reflux; (b) LiOH, THF/MeOH/H_2O, reflux.

reduced effect on heart rate. On the basis of the result in $S1P_3$ knock out mice, we expected that **2-P** had no heart rate deceasing effect. However **2-P** clearly decreased heart rate. Furthermore, the heart rate decreasing effect of **2-P** was not reduced at all despite the loss of $S1P_3$ agonism. Although the ID_{50} value of **2-P** (0.091 mg/kg) was higher than that of **1-P** (0.012 mg/kg), the lymphocyte-decreasing effect of **2** was much weaker than that of **1** (0.40 mg/kg/0.020 mg/kg). This observation made us presume that in vitro agonistic activity at $S1P_3$ does not always correlate well with the in vivo potential of heart rate reduction. Therefore, we decided to adopt an in vivo evaluation of the effect of test compounds on heart rate in addition to in vitro assay.

On the basis of the above preliminary results, the research flow in this study was established as follows: (i) designing compounds and syntheses of the alcohol form, (ii) determination of the lymphocyte-decreasing effect of the alcohols, (iii) syntheses of the phosphate of sufficiently effective compounds (ED_{50} < 0.20 mg/kg) and key compounds, and (iv) evaluations of the heart rate decreasing effect in anesthetized rats and in vitro $S1P_1/S1P_3$ selectivity of the phosphates.

Synthesis. Scheme 1 describes the synthesis of intermediate bromides **9**. After conversion of phenethyl alcohols into iodides **7**, condensation of **7** and *N*-protected diethyl aminomalonate gave diesters **8**. The ester groups of **8** were reduced to hydroxymethyl groups, which were subsequently protected to give the corresponding bromides **9**.

Biphenyl compounds **3a**−**l** were synthesized from bromides **9** through Suzuki coupling, followed by removal of the protecting groups (Scheme 2).

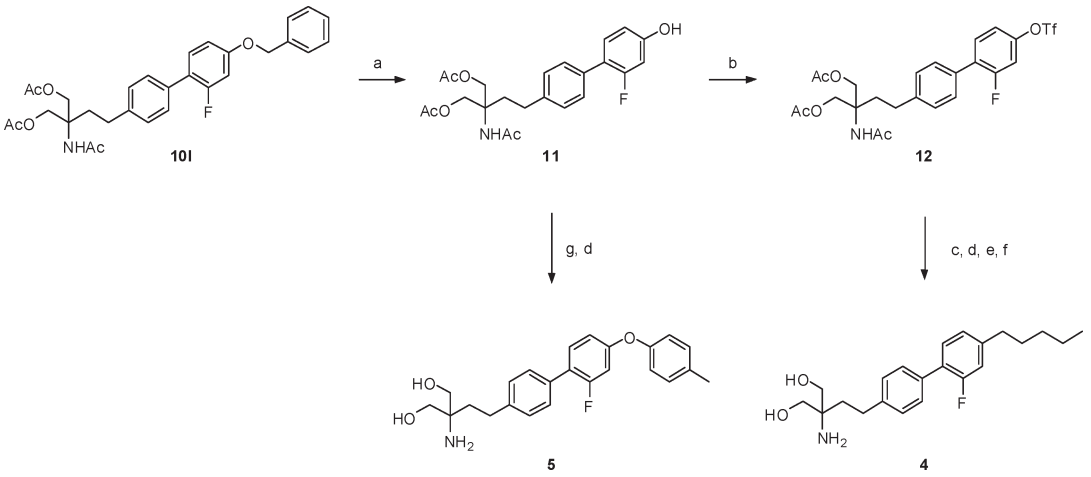
The syntheses of compounds **4** and **5** were carried out as shown in Scheme 3. Hydrogenolysis of biphenyl compound **10l** gave phenol **11**. After activation of the phenolic hydroxy group of **11** by conversion into a triflate, Sonogashira reaction of **12** and 1-pentyne, hydrogenation, and removal of the protecting groups yielded the desired compound **4**. The diaryl ether **5** was obtained by coupling of phenol **11** and *p*-tolylboronic acid using copper acetate, followed by deprotection.

Diaryl sulfides **6a**−**n** were prepared from phenyl bromides **9** through biphenyl bromides **13** (Scheme 4). The bromides **13** transformed from **9** by a similar palladium-mediated coupling to that described in Scheme 2 were converted to the diaryl sulfides **6a**−**n** by a coupling reaction using 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene as the phosphine ligand followed by deprotection.

The phosphate esters of **3**, **4** and **5** (**3-P**, **4-P**, and **5-P**, respectively) were prepared as outlined in Scheme 5. First, the 2-aminopropane-1,3-diols were converted into oxazolines to protect the amino group and one of the hydroxyl groups. The remaining hydroxyl group was phosphorylated by a phosphoramidite with 1*H*-tetrazole, followed by oxidation to give the protected phosphate esters **14**. Desired phosphate esters were obtained by removing all protective groups under acidic conditions.

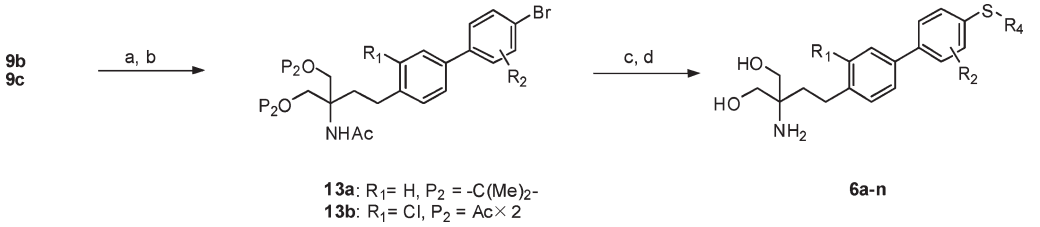
The phosphate esters of compounds **6**, which have a sulfur atom, were synthesized through another route shown in Scheme 6. Bromides **13** were phosphorylated to give intermediates **15** before coupling with thiols because the process of phosphorylation included an oxidative step. Compounds **6-P** were prepared by the palladium coupling reaction of intermediates **15** with thiols, followed by acidic deprotection.

Scheme 3. Synthesis of Compounds **4** and **5**[a]



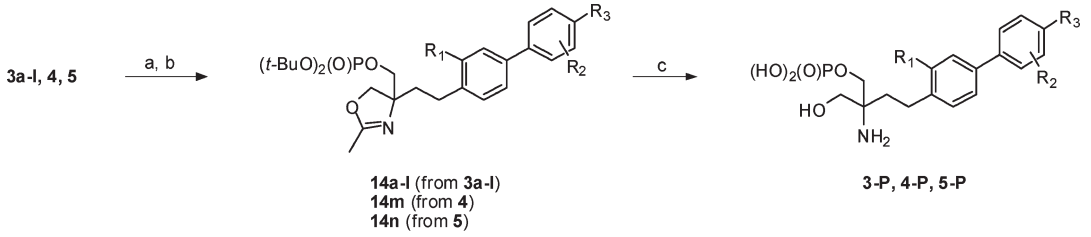
[a] Reagents and conditions: (a) 10% Pd/C, NH_4CO_2H, MeOH, rt; (b) Tf_2O, pyridine, CH_2Cl_2, rt; (c) 1-pentyne, $PdCl_2(CH_3CN)_2$, X-Phos, Cs_2CO_3, CH_3CN, 60 °C; (d) LiOH, $MeOH/H_2O$, reflux; (e) 10% Pd/C, MeOH, rt; (f) 4 M HCl/EtOAc, MeOH/EtOAc, rt; (g) *p*-tolylboronic acid, $Cu(OAc)_2$, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt.

Scheme 4. Synthesis of Diaryl Sulfides **6**[a]



[a] Reagents and conditions: (a) bis(neopentyl glycolate)diboron, $PdCl_2(Cy_3P)_2$, AcOK, dioxane, 100 °C; (b) iodobenzene, $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O, 100 °C; (c) thiol, Xantphos, $Pd_2(dba)_3 \cdot CHCl_3$, DIPEA, dioxane, reflux; (d) 35% HCl, EtOH, 70 °C.

Scheme 5. Phosphorylation of **3**, **4**, and **5**[a]

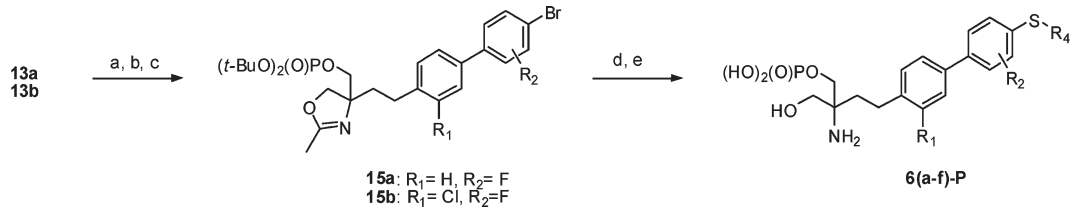


[a] Reagents and conditions: (a) $CH_3C(OEt)_3$, DIPEA, DMF, 120 °C; (b) (i) $(t\text{-BuO})_2PN(i\text{-Pr})_2$, 1*H*-tetrazole, CH_2Cl_2, rt; (ii) *m*-CPBA, rt; (c) 35% HCl, EtOH, 50 °C.

Results

First, we searched for a strategy to improve the potency of lymphocyte-decreasing effect exhibited by **2** (Table 2). Replacement of the pentyl group of **2** with butoxy (**3b**) resulted in a slight increase in in vivo activity, and the agonistic activity of the phosphate (**3b-P**) at $S1P_1$ was slightly potentiated. This result suggested that the heteroatom was tolerated as a linkage between the biphenyl and the side chain binding to the 4′ position. In addition, the heteroatom linkage was useful for access to various types of side chains. To improve the potency of **3b**, introduction of chlorine or fluorine into the biphenyl moiety was carried out. The introductions of chlorine into ring A (**3c**) and fluorine into ring B (**3d**) were both useful to improve the in vivo activity of **3b** like the case of **2**. The fluorine on ring B was more effective than the chlorine on ring A to improve in vivo activity; however, **3d-P** showed agonistic activity at $S1P_3$. Replacing the fluorine with methyl (**3e**) resulted in notably reduced activity. In attempts to increase the number of fluorine (**3f**) and to change the substitution position of halogens (**3g**, **3h**), no improvement in activity was observed compared to **3b** although these phosphates had no $S1P_3$ agonism. The phosphates of the compounds having high activity (**3b-P**, **3c-P**, and **3d-P**) were evaluated for the heart rate decreasing effect. Compound **3d-P**, which had agonistic activity at $S1P_3$, reduced heart rate intensively (ID_{50} = 0.009 mg/kg), supporting the hypothesis that the heart rate reduction caused by **1** is associated with $S1P_3$ agonism. On the other hand, ID_{50} values of **3b-P** and **3c-P** were also low (0.008 and 0.013 mg/kg, respectively) in spite of their inactivity at $S1P_3$. This result contradicts the reported observation that **1-P** had no effect on heart rate in $S1P_3$-deficient mice. Although the reason why our results of compound-based approach disagreed with the genetic observation was not clear, we

Scheme 6. Synthesis of Phosphate Esters of **6**[a]



[a] Reagents and conditions: (a) 35% HCl, EtOH, 80 °C; (b) $CH_3C(OEt)_3$, DIPEA, DMF, 120 °C; (c) (i) $(t\text{-BuO})_2PN(i\text{-Pr})_2$, 1*H*-tetrazole, CH_2Cl_2, rt; (ii) *t*-Butyl hydroperoxide, rt; (d) thiol, Xantphos, $Pd_2(dba)_3 \cdot CHCl_3$, DIPEA, dioxane, reflux; (e) 35% HCl, EtOH, 50 °C.

continued our research mainly using in vivo evaluating system rather than in vitro $S1P_1$/ $S1P_3$ selectivity.

The results of replacement of the butoxy group of **3b** with phenoxy or benzyloxy groups were summarized in Table 3. Compound **3i** exhibited comparable lymphocyte-decreasing activity to the butoxy analogue **3b** regardless of weak $S1P_1$ agonistic activity of the phosphate **3i-P**. The heart rate decreasing effect of **3i-P** (ID_{50} = 0.14 mg/kg) was remarkably weaker than that of **3b-P** (0.008 mg/kg), suggesting that the phenyl ring in the side chain would be a favorable substituent in order to reduce the effect on heart rate without losing lymphocyte-decreasing activity. The introduction of chlorine on ring A (**3j** and **3k**) led to a slight improvement in lymphocyte-decreasing activity, and the introduction of fluorine on ring B (**3l**) drastically improved the activity equal to that of **1**. The phosphate of **3l** (**3l-P**) still had $S1P_3$ agonistic activity; however, **5-P** which had a tolyl group instead of the benzyl group showed a weaker agonistic activity at $S1P_3$ than **3l-P**. On the other hand, **5** maintained a comparable lymphocyte-decreasing effect to **3l**. These findings verified that the conversion of the alkoxy group into the phenoxy or benzyloxy group was effective in increasing the lymphocyte-decreasing effect and that the *p*-tolyl group was a substituent to attenuate the $S1P_3$ agonism. The phosphates of compounds having the improved activity (**3k-P**, **3l-P**, and **5-P**) strongly decreased heart rate like **1-P** irrespective of their different potencies of $S1P_3$ agonism. Consequently, no desired compounds having both potent lymphocyte-decreasing activity and little or no effect on heart rate were found in Table 3.

Our next attempt was replacement of the side chain of **3b** with a phenylthio or benzylthio group (Table 4).

Because we established and reported an efficient synthetic method for the preparation of diphenyl sulfide in the research of S1P receptor modulators,[19] the method was applied to the biphenyl compounds in this study. First we replaced the side chain with the benzylthio group because the benzyloxy derivatives **3k** and **3l** showed good activity. Compound **6a**, which has a chlorine atom on ring A, exhibited comparable activity to the phenoxy analogue **3k**. Changing chlorine into fluorine (**6b**) led to better in vivo activity. However, the phosphate of **6b** (**6b-P**) showed miserable results in $S1P_1$/$S1P_3$ selectivity and the heart rate decreasing effect. The potency of **6b-P** on heart rate decreasing (ID_{50} = 0.008 mg/kg) was even higher than that of **1-P** (0.012 mg/kg). On the basis of results of Table 3, we expected that replacement of the benzylthio group with the phenylthio group could be effective to reduce agonistic activity at the $S1P_3$ receptor. Fortunately, our expectation was supported by the result that **6c-P** was not an agonist at $S1P_3$ although the lymphocyte-decreasing effect of **6c** was approximately 10-fold weaker than that of **6b**. Next, we added substituents on the terminal phenyl ring of **6c** to improve the activity. Because the phenylthio group is smaller than the benzylthio group by one methylene, a methyl group was introduced into the terminal phenyl ring to retain the total length of the side chain. Both the para- and meta-substituted derivatives, **6d** and **6e**, respectively, showed improved activity in terms of lymphocyte-decreasing effect. Especially, **6d** was equipotent to **6b**. The results of the phosphates (**6d-P** and **6e-P**) in the heart rate measuring study were interesting. The heart rate decreasing effect of **6d-P** was attenuated. More precisely, the ID_{50} values of **6d-P** and **1-P** were 0.10 and 0.012 mg/kg, respectively whereas their ED_{50} values on lymphopenia (0.05 and 0.02 mg/kg) were closer than their ID_{50} values. On the other hand, **6e-P** produced more intense reduction of heart rate than **6d-P** although **6e-P** had a similar in vitro profile to **6d-P**. Chlorine was introduced into ring A of **6d** to explore more active compounds. The compound **6f** was equipotent to **6d**, but the phosphate **6f-P** showed a strong effect on heart rate like **1-P**. Next, the substituents on the terminal phenyl were examined. Although replacement of the methyl group of **6d** with an ethyl group (**6g**) was tolerated, replacement with a more bulky group (**6h**) yielded less lymphocyte-decreasing activity. Adoption of halogens (**6i**, **6j**, and **6k**) also led to weaker activity. Among the halogens, fluorine (**6i**) was preferable to chlorine (**6k**), and para-substituted derivative (**6i**) was better than meta-substituted derivative (**6j**) like the case of the methyl substituent (**6d** and **6e**). Introduction of a trifluoromethyl group into unsubstituted compound **6c** did not influence activity (**6l**). The activity of *meta*-methoxy compound **6n** was stronger than that of para-substituted compound **6m**. The modification of the terminal phenyl of **6d** resulted in no significant improvement. Consequently, **6d** was the only compound having both the significant lymphocyte-decreasing activity and the reduced effect on heart rate in this study.

The most advantageous compound **6d** was subjected to detailed pharmacological evaluations. Compounds **1** and **6d** had comparable oral lymphocyte-decreasing activity in rats with ED_{50} values of 0.040 mg/kg (0.029−0.056; 95% confidence limit) and 0.084 mg/kg (0.064−0.11; 95% confidence limit), respectively. Next, the effect of orally administered **6d** on heart rate was measured in conscious rats using a telemetry system. Compound **1** significantly reduced the heart rate at doses of 3 mg/kg and higher in this test. On the other hand, **6d** had no clear influence on heart rate at a dose of 10 mg/kg (Figure 1). These data indicated that **6d** have both the sufficient immunomodulatory activity and a low adverse action on heart.

Discussion

The genetic studies using fetal liver chimeric mice with specific deletion of $S1P_1$ from hematopoietic cells and $S1P_3$-deficient mice revealed that $S1P_1$ and $S1P_3$ regulate lymphocyte circulation and reduction of heart rate, respectively.[10,15] It was reported that KRP-203 (not shown), which has the same 2-aminopropane-1,3-diol skeleton as **1**, had a comparable lymphocyte-decreasing effect to **1** and a reduced heart rate decreasing effect.[20] It appears that KRP-203 reduced the

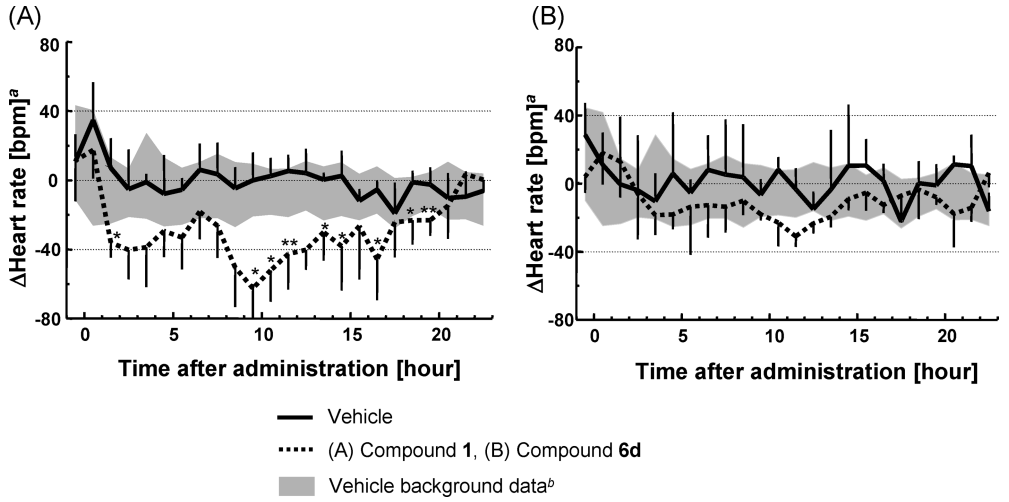


Figure 1. Effects of compounds **1** (A) and **6d** (B) on heart rate in conscious rats (10 mg/kg, po). ΔHeart rate means the change (beat/min) of post-treatment value (day 0) compared to the mean of pretreatment values (from day −3 to day −1) at the corresponding time of each day in the same animals. *: $p < 0.05$, ** : $p < 0.01$, significantly different from the corresponding value in the vehicle group ($n = 3$ or 4, paired t-test). Vehicle background date represent the mean ± SD of each time point data ($n = 38$).

effect on heart rate to almost the same level as compound **6d** according to the report. The phosphate (KRP-203-P) was an agonist at $S1P_1$, but inactive at $S1P_3$. The $S1P_1/S1P_3$ selectivity was presumed to be important for reduction of the effect on heart rate.

In the beginning of our research, we identified **2** as a lead compound that was inactive against $S1P_3$ on this hypothesis by the screening of our **1**-related compound library. Then the pentyl group of **2** was converted into alkoxy, phenoxy, and phenylthio groups to improve the activity. As a result, lots of compounds showed a remarkable lymphocyte-decreasing effect. All the phosphates of the favorably active compounds having $S1P_3$ agonism (e.g., **3k-P**, **3l-P**, **5-P**, and **6b-P**) provoked an intensive decrease of heart rate. This result supported that $S1P_3$ was responsible for the reduction of heart rate caused by **1**. Additionally, the fact that **6d-P** was inactive at $S1P_3$ receptor-like KRP-203-P indicates that elimination of $S1P_3$ receptor agonism should be necessary to reduce the effect on heart rate. However, most of the phosphates having no agonism at $S1P_3$ also significantly influenced heart rate. For instance, the phosphates of both *meta*-methyl (**6e-P**) and chloro-substituted (**6f-P**) derivatives remarkably lowered heart rate, although neither of them showed any agonistic activity at $S1P_3$. This fact contradicted the result of the genetic study that **1** had no effect on heart rate in $S1P_3$-deficient mice and exploded the previously reported assumption that the research of compounds having no agonism at $S1P_3$ would directly lead to find the desirable $S1P_1$ agonist with little or no effect on heart rate. Although the cause of the disagreement between the genetic and our compound-based results is unclear, there are some possibilities: (i) species difference of the heart rate reduction mechanism between the $S1P_3$-deficient mice[15] and the rats used here, (ii) difference of intravital environments between $S1P_3$-deficient mice and wild-type animals exposed to no exogenous $S1P_3$ agonism but to endogenous $S1P_3$ agonism by natural S1P, and (iii) existence of other signaling pathways undetectable by using our calcium mobilization assay.[21] It is likely that other factors in addition to $S1P_3$ participate in the heart rate reduction caused by S1P receptor modulators.

Although it is relatively easy to obtain compounds without $S1P_3$ agonism, it is rather difficult to find compounds with a reduced effect on heart rate among them. The structure of **6d** is similar to that of KRP-203, which has two additional phenyl rings compared to **1** and a sulfur atom in the hydrophobic part. These structural and physicochemical properties may relate to the reduced effect on heart rate of both compounds. Furthermore, the pathogenic mechanism of S1P receptor modulators on the heart rate reduction may be revealed by using compounds having a similar structure and a different potency on heart rate reduction (e.g., **6d** and **6f**).

Because $S1P_3$ agonism showed no consistent relationship to the heart rate decreasing effect at the early stage of this research, the in vivo evaluation in rats was promptly adopted to estimate the potency of the phosphates for heart rate reduction. Thereby we constructed the original screening strategy based on not only in vitro $S1P_1/S1P_3$ selectivity but also simple in vivo systems and found compound **6d** whose effect on heart rate was attenuated. The detailed pharmacological evaluation revealed that oral administration of **6d** in rats provided the excellent lymphocyte-decreasing effect and the attenuated effect on heart rate. To the best of our knowledge, this is the first successful example on finding $S1P_1$ agonists with sufficient immunomodulatory activity and little or no effect on heart rate by a medicinal chemical approach.

Conclusion

2-Substituted 2-aminopropane-1,3-diols having a biphenyl moiety were synthesized to obtain immunomodulating $S1P_1$ receptor agonists with little or no effect on heart rate. The phosphate of **2** was identified as a lead compound with $S1P_1$ selectivity against $S1P_3$ by a screening of **1** analogue library. The structural evolution of **2** led to lots of biphenyl compounds showing considerable lymphocyte-decreasing effect. Several phosphate esters of them were potent $S1P_1$ receptor agonists having high selectivity against $S1P_3$; however, significant effects on heart rate remained in almost all of the phosphates. The results suggested that other factors in addition to $S1P_3$ agonism should be considered to clarify the cause of heart rate reduction by $S1P_1$ receptor agonists. The syntheses of analogues and our original screening strategy using in vivo evaluating systems primarily revealed that **6d** had potent lymphocyte-decreasing effect by intraperitoneal administration to mice and that the effect on heart rate of the phosphate

6d-P was attenuated by intravenous administration to anesthetized rats. It was then confirmed that the oral administration of **6d** to rats brought a similar immnomodulatory efficacy to that of **1** and no influence on heart rate at a dose of 10 mg/kg.

Experimental Section

Chemistry. Silica gel column chromatography was performed on a Moritex Purif-α2 system using Purif-Pack 30 or 60 μm silica gel columns and the described solvents as eluent under gradient condition. Preparative HPLC was performed on a Shimadzu HPLC system using Capcell Pak C18 UG80 (5 μm 20 mm × 250 mm) under gradient condition (CH_3CN/H_2O + 0.05% TFA). ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were measured in a combination with a Waters Acquity UPLC system (CH_3CN + 0.05% TFA/H_2O + 0.05% TFA) and a Micromass ZQ (ESI) spectrometer. Melting points were obtained on a Büchi 535 melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 II CHN analyzer. All compounds showed >95% purity, according to UPLC or the elemental analyses.

2-(4-Bromophenyl)ethyl Iodide (7a). Methanesulfonyl chloride (11.5 mL, 148 mmol) was added to a solution of 2-(4-bromophenyl)ethyl alcohol (25.0 g, 124 mmol) and triethylamine (22.6 mL, 163 mmol) in CH_2Cl_2 (250 mL) at 0 °C. The suspension was stirred at room temperature for 2 h. The reaction mixture was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to yield a red oil (39.1 g). Sodium iodide (18.6 g, 124 mmol) was added to a solution of the oil in 2-butanone (400 mL), and the suspension was heated under reflux for 4.5 h. The reaction mixture was diluted with EtOAc and washed with water, 10% aqueous sodium thiosulfate, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/EtOAc) gave the title compound (34.2 g, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.13 (2H, t, J = 7.5 Hz), 3.32 (2H, t, J = 7.5 Hz), 7.07 (2H, d, J = 8.4 Hz), 7.44 (2H, d, J = 8.4 Hz).

2-(4-Bromo-2-chlorophenyl)ethyl Iodide (7b). The title compound was synthesized from 2-(4-bromo-2-chlorophenyl)ethyl alcohol using a similar procedure to that described for **7a**. ^{1}H NMR ($CDCl_3$) δ 3.22−3.28 (2H, m), 3.32−3.38 (2H, m), 7.12 (1H, d, J = 8.1 Hz), 7.36 (1H, dd, J = 1.8, 8.4 Hz), 7.53 (1H, d, J = 2.1 Hz).

Diethyl 2-Acetamido-2-[2-(4-bromophenyl)ethyl]malonate (8a). A solution of diethyl acetamidomalonate (28.7 g, 132 mmol) in DMF (55 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 5.72 g, 143 mmol) in DMF (110 mL) at 0 °C. After stirring at room temperature for 45 min, **7a** (34.2 g, 110 mmol) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 1 h and an additional 2 h at 60 °C, the reaction mixture was poured into iced water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/EtOAc) gave the title compound (35.4 g, 78%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, t, J = 7.2 Hz), 2.00 (3H, s), 2.44 (2H, dd, J = 9.0, 10.8 Hz), 3.32 (2H, dd, J = 9.0, 10.8 Hz), 4.16−4.25 (4H, m), 6.76 (1H, brs), 7.02 (2H, d, J = 8.4 Hz), 7.38 (2H, d, J = 8.4 Hz).

Diethyl 2-[2-(4-Bromo-2-chlorophenyl)ethyl]-2-(*tert*-butoxycarbonylamino)malonate (8b). The title compound was synthesized from **7b** and diethyl *tert*-butoxycarbonylaminomalonate using a similar procedure to that described for **8a**. ^{1}H NMR ($CDCl_3$) δ 1.26 (6H, t, J = 7.1 Hz), 1.44 (9H, s), 2.57 (4H, brs), 4.16−4.30 (4H, m), 6.01 (1H, brs), 7.05 (1H, d, J = 8.1 Hz), 7.30 (1H, dd, J = 1.8, 8.4 Hz), 7.49 (1H, d, J = 1.8 Hz).

2-Acetamido-2-[2-(4-bromophenyl)ethyl]propane-1,3-diol Diacetate (9a). $NaBH_4$ (13.4 g, 354 mmol) was added portionwise to a solution of **8a** (35.4 g, 88.4 mmol) and $CaCl_2$ (20.6 g, 185 mmol) in water (55 mL) and EtOH (310 mL). After stirring at room temperature overnight, 1 M aqueous HCl was added to the reaction mixture and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in pyridine (300 mL). Acetic anhydride (25.0 mL, 340 mmol) was added to the solution at 0 °C. After stirring at room temperature overnight, ice and water (300 mL) were added to the reaction mixture. The mixture was extracted with EtOAc, and the organic layer was washed successively with water, 0.5 M aqueous HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was precipitated from hexane/EtOAc (1/1) to give the title compound (11.4 g) as a white solid. The mother liquid was concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/EtOAc) gave the second crop of the title compound (4.8 g). The total yield was 16.2 g (46%). ^{1}H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.10 (6H, s), 2.16−2.22 (2H, m), 2.53−2.59 (2H, m), 4.32 (4H, s), 5.68 (1H, brs), 7.06 (2H, d, J = 8.4 Hz), 7.40 (2H, d, J = 8.4 Hz).

***N*-[5-[2-(4-Bromophenyl)ethyl]-2,2-dimethyl-1,3-dioxane-5-yl]-acetamide (9b).** $NaBH_4$ (21.8 g, 576 mmol) was added portionwise to a solution of **8a** (57.8 g, 144 mmol) and $CaCl_2$ (32.0 g, 288 mmol) in water (100 mL) and EtOH (500 mL). After stirring at room temperature overnight, 1 M aqueous HCl was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in acetone (200 mL). 2,2-Dimethoxypropane (41.9 g, 402 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate was added to the solution. After stirring at room temperature overnight, the mixture was concentrated in vacuo. The residue was washed with saturated $NaHCO_3$ to give the title compound as a white solid (41.5 g, 81%). ^{1}H NMR ($CDCl_3$) δ 1.43 (6H, s), 2.03 (3H, s), 2.01−2.06 (2H, m), 2.46−2.52 (2H, m), 3.67 (2H, d, J = 11.7 Hz), 3.95 (2H, d, J = 12.0 Hz), 5.76 (1H, brs), 7.05 (2H, d, J = 8.4 Hz), 7.38 (2H, d, J = 8.4 Hz).

2-Acetamido-2-[2-(4-bromo-2-chlorophenyl)ethyl]propane-1,3-diol Diacetate (9c). $NaBH_4$ (66.6 g, 1760 mmol) was added portionwise to a solution of **8b** (217 g, 440 mmol) and $CaCl_2$ (97.7 g, 880 mmol) in THF (543 mL), water (1085 mL), and EtOH (2170 mL) at 0 °C. After stirring at room temperature for 14 h, 1 M HCl (1085 mL) was added to the reaction mixture and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in 4 M HCl solution in EtOAc (326 mL). After stirring at 30 °C for 1 h, the mixture was concentrated in vacuo. Aqueous NaOH (0.1 M, 2713 mL) was added to the reaction mixture. The mixture was extracted with EtOAc, and the organic layer was washed with brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in pyridine (271 mL). Acetic anhydride (270 mL) was added to the solution at 0 °C. After stirring at room temperature for 11 h, EtOAc (2710 mL), 1 M NaOH (1084 mL), and 9% $NaHCO_3$ (5420 mL) were added to the reaction mixture. The mixture was extracted with EtOAc, and the organic layer was washed successively with 1 M HCl, 9% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was precipitated from IPE/*n*-heptane (1/1) to give the title compound (135 g, 62%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.00 (3H, s), 2.10 (6H, s), 2.12−2.16 (2H, m), 2.66−2.72 (2H, m), 4.36 (4H, s), 5.75 (1H, brs), 7.10 (1H, d, J = 8.2 Hz), 7.32 (1H, dd, J = 1.9, 7.5 Hz), 7.70 (1H, d, J = 1.9 Hz).

2-[2-(4-Bromo-2-chlorophenyl)ethyl]-2-(*tert*-butoxycarbonylamino)propane-1,3-diol Diacetate (9d). The title compound was synthesized from **8b** using a similar procedure to that described for **9a**. ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 2.03−2.07 (2H, m), 2.09 (6H, s), 2.67−2.73 (2H, m), 4.29 (4H, s), 4.75 (1H, brs), 7.09 (1H, d, J = 8.2 Hz), 7.32 (1H, dd, J = 2.0, 8.4 Hz), 7.50 (1H, d, J = 2.0 Hz).

2-(*tert*-Butoxycarbonylamino)-2-[2-(3-chloro-4′-pentylbiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10a). A mixture of **9d** (493 mg, 1.00 mmol), 4-pentylphenylboronic acid (230 mg, 1.19 mmol), tetrakis(triphenylphosphine)palladium(0) (12 mg, 0.010 mmol), and $NaHCO_3$ (504 mg, 5.99 mmol) in 1,2-dimethoxyethane (6.0 mL) and water (2.0 mL) was heated at 65 °C for 4 h. Water was added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (450 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 6.6 Hz), 1.32−1.35 (4H, m), 1.47 (9H, s), 1.64−1.66 (2H, m), 2.04−2.20 (2H, m), 2.09 (6H, s), 2.64 (2H, t, J = 7.5 Hz), 2.75−2.80 (2H, m), 4.32 (4H, s), 4.79 (1H, brs), 7.23−7.27 (3H, m), 7.39−7.47 (3H, m), 7.56 (1H, d, J = 2.0 Hz).

The compounds **10b**−**l** were synthesized using a similar procedure to that described for **10a**. Synthetic route for each compound is summarized in the Supporting Information.

2-Acetamido-2-[2-(4′-butoxybiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10b). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.2 Hz), 1.48−1.54 (2H, m), 1.75−1.82 (2H, m), 1.96 (3H, s), 2.10 (6H, s), 2.22−2.26 (2H, m), 2.62−2.66 (2H, m), 4.00 (2H, t, J = 6.4 Hz), 4.37 (4H, s), 5.65 (1H, brs), 6.94−6.96 (2H, m), 7.23 (2H, d, J = 8.0 Hz), 7.46−7.50 (4H, m).

2-[2-(4′-Butoxy-3-chlorobiphenyl-4-yl)ethyl]-2-(*tert*-butoxycarbonylamino)propane-1,3-diol Diacetate (10c). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.5 Hz), 1.46 (9H, s), 1.47−1.58 (2H, m), 1.70−1.88 (2H, m), 2.05 (6H, s), 2.04−2.10 (2H, m), 2.73−2.79 (2H, m), 4.00 (2H, t, J = 6.3 Hz), 4.32 (4H, s), 4.78 (1H, brs), 6.95 (2H, d, J = 8.4 Hz), 7.23−7.26 (1H, m), 7.35−7.38 (1H, m), 7.45−7.52 (3H, m).

2-Acetamido-2-[2-(4′-butoxy-2′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10d). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.6 Hz), 1.48−1.53 (2H, m), 1.75−1.82 (2H, m), 1.96 (3H, s), 2.09 (6H, s), 2.23−2.27 (2H, m), 2.63−2.67 (2H, m), 3.98 (2H, t, J = 6.4 Hz), 4.37 (4H, s), 5.64 (1H, brs), 6.67−6.76 (2H, m), 7.24 (2H, d, J = 8.0 Hz), 7.30 (1H, t, J = 8.8 Hz), 7.42−7.44 (2H, m).

2-Acetamido-2-[2-(4′-butoxy-2′-methylbiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10e). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.4 Hz), 1.48−1.54 (2H, m), 1.74−1.80 (2H, m), 1.97 (3H, s), 2.10 (6H, s), 2.24 (3H, s), 2.26−2.29 (2H, m), 2.63−2.68 (2H, m), 3.98 (2H, t, J = 6.4 Hz), 4.37 (4H, s), 5.66 (1H, brs), 6.75−6.81 (2H, m), 7.11 (1H, d, J = 8.4 Hz), 7.21 (4H, brs).

2-Acetamido-2-[2-(4′-butoxy-2′,5′-difluorobiphenyl-4-yl)ethyl]-propane-1,3-diol Diacetate (10f). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.6 Hz), 1.49−1.57 (2H, m), 1.79−1.86 (2H, m), 1.97 (3H, s), 2.10 (6H, s), 2.23−2.27 (2H, m), 2.63−2.67 (2H, m), 4.04 (2H, t, J = 6.4 Hz), 4.36 (4H, s), 5.65 (1H, brs), 6.76 (1H, dd, J = 7.1, 11.7 Hz), 7.15 (1H, dd, J = 7.3, 11.8 Hz), 7.24−7.26 (2H, m), 7.41 (2H, d, J = 8.3 Hz).

2-Acetamido-2-[2-(4′-butoxy-3′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10g). ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J = 7.6 Hz), 1.50−1.55 (2H, m), 1.79−1.86 (2H, m), 1.97 (3H, s), 2.10 (6H, s), 2.22−2.64 (2H, m), 2.62−2.67 (2H, m), 4.07 (2H, t, J = 6.4 Hz), 4.36 (4H, s), 5.67 (1H, brs), 7.00 (1H, t, J = 8.6 Hz), 7.22−7.31 (4H, m), 7.44 (2H, d, J = 8.0 Hz).

2-Acetamido-2-[2-(4′-butoxy-3′-chlorobiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10h). ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, J = 7.6 Hz), 1.52−1.58 (2H, m), 1.82−1.86 (2H, m), 1.97 (3H, s), 2.01 (6H, s), 2.22−2.26 (2H, m), 2.62−2.67 (2H, m), 4.07 (2H, t, J = 6.4 Hz), 4.36 (4H, s), 5.67 (1H, brs), 6.97 (1H, d, J = 8.4 Hz), 7.24 (2H, d, J = 8.0 Hz), 7.38−7.40 (1H, m), 7.44 (2H, d, J = 8.0 Hz), 7.57 (1H, d, J = 2.4 Hz).

2-Acetamido-2-[2-(4′-phenoxybiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10i). ^{1}H NMR ($CDCl_3$) δ 1.97 (3H, s), 2.10 (6H, s), 2.22−2.28 (2H, m), 2.63−2.68 (2H, m), 4.37 (4H, s), 5.68 (1H, brs), 7.04−7.11 (5H, m), 7.23−7.26 (2H, m), 7.32−7.38 (2H, m), 7.47−7.54 (4H, m).

2-(*tert*-Butoxycarbonylamino)-2-[2-(3-chloro-4′-phenoxybiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10j). ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.10 (6H, s), 2.08−2.15 (2H, m), 2.75−2.81 (2H, m), 4.32 (4H, s), 4.78 (1H, brs), 7.04−7.13 (4H, m), 7.25−7.28 (2H, m), 7.34−7.39 (3H, m), 7.49−7.54 (3H, m).

2-[2-(4′-Benzyloxy-3-chlorobiphenyl-4-yl)ethyl]-2-(*tert*-butoxycarbonylamino)propane-1,3-diol Diacetate (10k). ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.10 (6H, s), 2.04−2.14 (2H, m), 2.74−2.80 (2H, m), 4.32 (4H, s), 4.77 (1H, brs), 5.11 (2H, s), 7.04 (2H, d, J = 8.4 Hz), 7.23−7.26 (1H, m), 7.35−7.52 (9H, m).

2-Acetamido-2-[2-(4′-benzyloxy-2′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Diacetate (10l). ^{1}H NMR ($CDCl_3$) δ 1.98 (3H, s), 2.10 (6H, s), 2.19−2.28 (2H, m), 2.62−2.68 (2H, m), 4.37 (4H, s), 5.09 (2H, s), 5.68 (1H, brs), 6.76−6.84 (2H, m), 7.23−7.44 (10H, m).

2-Amino-2-[2-(3-chloro-4′-pentylbiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3a). A solution of **10a** (450 mg, 0.803 mmol) in EtOH (9.0 mL) was added to 35% HCl (3.0 mL), and the mixture was refluxed for 3 h. After the organic solvent was distilled off in vacuo, the yielded solid was collected to afford the title compound (320 mg, 97%) as white crystals; mp 185−187 °C; MS (ESI$^+$) m/z 376 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, J = 6.6 Hz), 1.19−1.41 (4H, m), 1.49−1.66 (2H, m), 1.73−1.89 (2H, m), 2.52−2.63 (2H, m), 2.68−2.82 (2H, m), 3.57 (4H, d, J = 4.5 Hz), 5.40−5.41 (2H, m), 7.28 (2H, d, J = 8.1 Hz), 7.42 (1H, d, J = 8.1 Hz), 7.58−7.60 (3H, m), 7.68 (1H, d, J = 1.8 Hz), 7.84 (3H, brs). Anal. ($C_{22}H_{30}ClNO_2\cdot HCl$) C, H, N.

2-Amino-2-[2-(4′-butoxybiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3b). A solution of **10b** (570 mg, 1.21 mmol) in MeOH (5.0 mL) and THF (4 mL) was added to a solution of lithium hydroxide monohydrate (255 mg, 6.07 mmol) in water (5.0 mL), and the mixture was refluxed for 4 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in 4 M HCl solution in EtOAc (6.0 mL) and MeOH (6.0 mL), and the solvent was distilled off in vacuo. The yielded white crystalline solid was collected and washed with EtOAc to give the title compound (384 mg, 83%); mp 201−203 °C; MS (ESI$^+$) m/z 344 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J = 7.2 Hz), 1.40−1.50 (2H, m), 1.68−1.75 (2H, m), 1.80−1.85 (2H, m), 2.61−2.66 (2H, m), 3.54 (4H, d, J = 5.2 Hz), 4.00 (2H, t, J = 6.4 Hz), 5.39 (2H, t, J = 5.2 Hz), 6.98−7.01 (2H, m), 7.26 (2H, d, J = 8.4 Hz), 7.53−7.57 (4H, m), 7.87 (3H, brs). Anal. ($C_{21}H_{29}NO_3\cdot HCl$) C, H, N.

The compounds **3c**−**l** were synthesized using a similar procedure to that described for **3a** or **3b**. The synthetic route for each compound is summarized in the Supporting Information. Some compounds were isolated as free base before converting into hydrochloride salt.

2-Amino-2-[2-(4′-butoxy-3-chlorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3c). mp 188−190 °C; MS (ESI$^+$) m/z 378 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J = 7.2 Hz), 1.41−1.49 (2H, m), 1.69−1.84 (4H, m), 2.73−2.76 (2H, m), 3.57 (4H, d, J = 4.8 Hz), 4.01 (2H, t, J = 6.3 Hz), 5.44 (2H, t, J = 4.8 Hz), 7.01 (2H, d, J = 8.7 Hz), 7.40 (1H, d, J = 8.1 Hz), 7.55−7.70 (4H, m), 7.87 (3H, brs). Anal. ($C_{21}H_{28}ClNO_3\cdot HCl\cdot 0.25H_2O$) C, H, N.

2-Amino-2-[2-(4′-butoxy-2′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3d). mp 163−165 °C; MS (ESI$^+$) m/z 362 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J = 7.6 Hz), 1.42−1.47 (2H, m), 1.68−1.75 (2H, m), 1.81−1.85 (2H, m), 2.63−2.67 (2H, m), 3.55 (4H, d, J = 4.8 Hz), 4.02 (2H, t, J = 6.8 Hz), 5.39 (2H, t, J = 4.8 Hz), 6.85−6.92 (2H, m), 7.29 (2H, d, J = 8.4 Hz), 7.38−7.44 (3H, m), 7.84 (3H, brs). Anal. ($C_{21}H_{28}FNO_3\cdot HCl$) C, H, N.

2-Amino-2-[2-(4′-butoxy-2′-methylbiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3e). mp 136−138 °C; MS (ESI$^+$) m/z 358 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J = 7.6 Hz), 1.40−1.49 (2H, m), 1.67−1.74 (2H, m), 1.82−1.87 (2H, m), 2.20 (3H, s), 2.63−2.67 (2H, m), 3.55 (4H, d, J = 5.2 Hz), 3.98 (2H, t, J = 6.4 Hz), 5.38 (2H, t, J = 5.2 Hz), 6.80 (1H, dd,

J = 2.4, 8.4 Hz), 6.85 (1H, d, J = 2.4 Hz), 7.08 (1H, d, J = 8.4 Hz), 7.21−7.26 (4H, m), 7.85 (3H, brs). Anal. ($C_{22}H_{31}NO_3 \cdot HCl \cdot H_2O$) C, H, N.

2-Amino-2-[2-(4′-butoxy-2′,5′-difluorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3f). mp 182−184 °C; MS (ESI^+) m/z 380 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.95 (3H, t, J = 7.2 Hz), 1.42−1.48 (2H, m), 1.70−1.78 (2H, m), 1.80−1.85 (2H, m), 2.50−2.67 (2H, m), 3.54 (4H, d, J = 5.1 Hz), 4.10 (2H, t, J = 6.6 Hz), 5.39 (2H, t, J = 4.9 Hz), 7.21 (1H, dd, J = 7.4, 12.3 Hz), 7.30 (2H, d, J = 8.3 Hz), 7.40 (1H, dd, J = 7.0, 12.2 Hz), 7.46 (2H, d, J = 6.7 Hz), 7.85 (3H, brs). Anal. ($C_{21}H_{27}F_2NO_3 \cdot HCl$) C, H, N.

2-Amino-2-[2-(4′-butoxy-3′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3g). mp 164−166 °C; MS (ESI^+) m/z 362 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.95 (3H, t, J = 7.6 Hz), 1.43−1.48 (2H, m), 1.70−1.77 (2H, m), 1.80−1.84 (2H, m), 2.61−2.66 (2H, m), 3.54 (4H, d, J = 4.8 Hz), 4.08 (2H, t, J = 6.4 Hz), 5.38 (2H, t, J = 4.8 Hz), 7.22 (1H, t, J = 8.8 Hz), 7.27 (2H, d, J = 8.0 Hz), 7.40−7.43 (1H, m), 7.50−7.53 (1H, m), 7.58 (2H, d, J = 8.0 Hz), 7.81 (3H, brs). Anal. ($C_{21}H_{28}FNO_3 \cdot HCl \cdot 0.25H_2O$) C, H, N.

2-Amino-2-[2-(4′-butoxy-3′-chlorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3h). mp 154−156 °C; MS (ESI^+) m/z 378 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.95 (3H, t, J = 7.6 Hz), 1.45−1.51 (2H, m), 1.71−1.78 (2H, m), 1.80−1.85 (2H, m), 2.62−2.66 (2H, m), 3.54 (4H, d, J = 5.2 Hz), 4.10 (2H, t, J = 6.4 Hz), 5.39 (2H, t, J = 5.2 Hz), 7.21 (1H, d, J = 8.8 Hz), 7.28 (2H, d, J = 8.4 Hz), 7.56−7.59 (3H, m), 7.69 (1H, d, J = 2.4 Hz), 7.85 (3H, brs). Anal. ($C_{21}H_{28}ClNO_3 \cdot HCl \cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-(4′-phenoxybiphenyl-4-yl)ethyl]propane-1,3-diol (3i). mp 149−150 °C; MS (ESI^+) m/z 364 [M + H]. ^{1}H NMR ($CDCl_3$) δ 1.73−1.78 (2H, m), 2.67−2.73 (2H, m), 3.53 (2H, d, J = 10.8 Hz), 3.63 (2H, d, J = 10.8 Hz), 7.04−7.12 (4H, m), 7.25−7.27 (4H, m), 7.32−7.38 (1H, m), 7.47−7.54 (4H, m). Anal. ($C_{23}H_{25}NO_3 \cdot 0.3H_2O$) C, H, N.

2-Amino-2-[2-(3-chloro-4′-phenoxybiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3j). mp 211−212 °C; MS (ESI^+) m/z 398 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.80−1.86 (2H, m), 2.75−2.81 (2H, m), 3.58 (4H, d, J = 4.8 Hz), 5.41 (2H, t, J = 4.8 Hz), 7.08 (4H, d, J = 8.4 Hz), 7.18 (1H, t, J = 7.5 Hz), 7.40−7.45 (3H, m), 7.59−7.62 (1H, m), 7.69−7.72 (3H, m), 7.88 (3H, brs). Anal. ($C_{23}H_{24}ClNO_3 \cdot HCl$) C, H, N.

2-Amino-2-[2-(4′-benzyloxy-3-chlorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (3k). mp 186−187 °C; MS (ESI^+) m/z 412 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.79−1.85 (2H, m), 2.73−2.79 (2H, m), 3.57 (4H, d, J = 4.8 Hz), 5.16 (2H, s), 5.40 (2H, t, J = 5.1 Hz), 7.10 (2H, d, J = 8.7 Hz), 7.38−7.48 (6H, m), 7.55−7.66 (4H, m), 7.85 (3H, brs). Anal. ($C_{24}H_{26}ClNO_3 \cdot HCl$) C, H, N.

2-Amino-2-[2-(4′-benzyloxy-2′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol (3l). mp 166−167 °C; MS (ESI^+) m/z 396 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.30 (2H, s), 1.47−1.55 (2H, m), 2.59−2.65 (2H, m), 3.23−3.26 (4H, m), 4.48 (2H, brs), 5.17 (2H, s), 6.93−7.03 (2H, m), 7.26 (1H, d, J = 8.4 Hz), 7.36 (2H, d, J = 8.4 Hz), 7.39−7.49 (7H, m). Anal. ($C_{24}H_{26}FNO_3 \cdot 1.25H_2O$) C, H, N.

2-Acetamido-2-[2-(2′-fluoro-4′-hydroxybiphenyl-4-yl)ethyl]-propane-1,3-diol Diacetate (11). A mixture of **10l** (0.86 g, 1.6 mmol) and ammonium formate (517 mg, 8.19 mmol) in MeOH (30 mL) was stirred under hydrogen atmosphere in the presence of 10% Pd/C (0.30 g) for 6 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo, diluted with water, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (0.56 g, 87%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.04 (3H, s), 2.11 (6H, s), 2.29−2.34 (2H, m), 2.61−2.67 (2H, m), 4.38 (4H, s), 6.06 (1H, brs), 6.64−6.71 (2H, m), 7.10−7.27 (3H, m), 7.38 (2H, dd, J = 1.2, 8.1 Hz), 8.42 (1H, brs).

2-Acetamido-2-[2-[2′-fluoro-4′-(trifluoromethanesulfonyloxy)-biphenyl-4-yl]ethyl]propane-1,3-diol Diacetate (12). A solution of trifluoromethanesulfonic anhydride (0.30 mL, 1.7 mmol) in CH_2Cl_2 (1.0 mL) was added dropwise to a solution of **11** (507 mg, 1.17 mmol) and pyridine (0.58 mL, 11 mmol) in CH_2Cl_2 (3.0 mL) at 0 °C. After stirring at room temperature for 2.5 h, the reaction was quenched with saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (460 mg, 70%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.98 (3H, s), 2.01 (6H, s), 2.25−2.29 (2H, m), 2.65−2.69 (2H, m), 4.36 (4H, s), 5.68 (1H, brs), 7.11−7.18 (2H, m), 7.29 (2H, d, J = 8.0 Hz), 7.43 (2H, dd, J = 1.3, 8.3 Hz), 7.49 (1H, t, J = 8.2 Hz).

2-Amino-2-[2-(2′-fluoro-4′-pentylbiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (4). To a mixture of **12** (150 mg, 0.266 mmol), bis(acetonitrile)dichloropalladium(II) (2.8 mg, 0.010 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-Phos, 15.2 mg, 0.0318 mmol), and cesium carbonate (225 mg, 0.690 mmol) in CH_3CN (1.0 mL) was added 1-pentyne (0.13 mL, 1.3 mmol). The mixture was stirred at 60 °C for 5 h, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in MeOH (2.0 mL) and added to a solution of lithium hydroxide monohydrate (56 mg, 1.3 mmol) in water (2.0 mL). The mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel chromatography ($CHCl_3$/MeOH) gave 2-acetamido-2-[2-[2′-fluoro-4′-(1-pentynyl)biphenyl-4-yl]ethyl]propane-1,3-diol diacetate (67 mg, 70%) as a pale-yellow solid. A solution of the solid in MeOH (10 mL) was stirred under hydrogen atmosphere in the presence of 10% Pd/C (20 mg) for 4 h at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo. To a solution of the residue in EtOAc (2.0 mL) and MeOH (2.0 mL) was added 4 M HCl solution in EtOAc (1.0 mL). After the solution was concentrated in vacuo, the residue was collected by filtration to give the title compound (44 mg, 87%) as a white solid. mp 154−156 °C; MS (ESI^+) m/z 360 [M + H]. ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 6.8 Hz), 1.27−1.35 (4H, m), 1.56−1.64 (2H, m), 1.81−1.85 (2H, m), 2.51−2.67 (4H, m), 3.54 (4H, d, J = 5.0 Hz), 5.41 (2H, t, J = 4.9 Hz), 7.11−7.15 (2H, m), 7.30 (2H, d, J = 8.0 Hz), 7.40 (1H, t, J = 8.5 Hz), 7.46 (2H, d, J = 6.7 Hz), 7.84 (3H, brs). Anal. ($C_{22}H_{30}FNO_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(4-methylphenoxy)biphenyl-4-yl]-ethyl]propane-1,3-diol (5). A mixture of **11** (278 mg, 0.644 mmol), 4-methylphenylboronic acid (175 mg, 1.28 mmol), Cu-$(OAc)_2$ (117 mg, 0.644 mmol), pyridine (255 mg, 2.84 mmol), and powdered molecular sieves 4 Å (500 mg) in CH_2Cl_2 (5.0 mL) was stirred at room temperature for 9 h. The reaction mixture was poured into 2% aqueous solution of citric acid and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the protected title compound (260 mg, 77%) as a white solid. To a solution of the solid in MeOH (10 mL) was added 1 M aqueous LiOH (10 mL), and the mixture was refluxed for 2 h. The reaction mixture was concentrated in vacuo, and the resultant solid was collected by filtration. Purification of the solid by preparative HPLC gave the title compound (59 mg, 21%) as a white solid. mp 154−156 °C; MS (ESI^+) m/z 396 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.72−1.78 (2H, m), 2.36 (3H, s), 2.67−2.72 (2H, m), 3.54 (2H, d, J = 10.5 Hz), 3.63 (2H, d, J = 10.8 Hz), 6.72−6.83 (2H, m), 6.98 (2H, d, J = 8.7 Hz), 7.18 (2H, d, J = 8.4 Hz), 7.25−7.28 (2H, m), 7.34 (1H, t, J = 8.6 Hz), 7.44 (2H, dd, J = 1.5, 8.1 Hz). Anal. ($C_{24}H_{26}FNO_3$) C, H, N.

***N*-[5-[2-(4′-Bromo-2′-fluorobiphenyl-4-yl)ethyl]-2,2-dimethyl-1,3-dioxane-5-yl]acetamide (13a).** A mixture of **9b** (7.13 g, 20.0 mmol), bis(neopentyl glycolato)diboron (4.97 g, 22.0 mmol), potassium acetate (5.89 g, 60.0 mmol), and dichlorobis(tricyclohexylphosphine)palladium(II) (738 mg, 1.00 mmol) in 1,4-dioxane (80 mL) was heated at 100 °C. After 6 h, the mixture was

allowed to cool to room temperature and poured into water. The mixture was extracted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was precipitated from water to give a brown solid. To a mixture of the solid, 1-bromo-3-fluoro-4-iodobenzene (5.95 g, 19.7 mmol) and $NaHCO_3$ (9.48 g, 112 mmol) in 1,2-dimethoxyethane (120 mL) and water (40 mL) was added tetrakis(triphenylphosphine)palladium(0) (434 mg, 0.375 mmol). After stirring at 100 °C for 8 h, 1-bromo-3-fluoro-4-iodobenzene (2.83 g, 9.4 mmol) and tetrakis(triphenylphosphine)palladium(0) (217 mg, 0.188 mmol) was added to the mixture. The mixture was stirred at 100 °C for another 8 h and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (5.28 g, 58%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.35 (6H, s), 1.87 (3H, s), 1.98−2.04 (2H, m), 2.47−2.53 (2H, m), 3.71 (2H, d, J = 11.8 Hz), 3.97 (2H, d, J = 11.6 Hz), 7.27 (2H, d, J = 8.1 Hz), 7.44−7.52 (4H, m), 7.61−7.66 (2H, m).

2-Acetamido-2-[2-(4′-bromo-3-chloro-2′-fluorobiphenyl-4-yl)-ethyl]propane-1,3-diol Diacetate (13b). The title compound was synthesized from **9c** using a similar procedure to that described for **13a**. ^{1}H NMR ($CDCl_3$) δ 2.01 (3H, s), 2.11 (6H, s), 2.18−2.24 (2H, m), 2.75−2.81 (2H, m), 4.39 (4H, s), 5.74 (1H, brs), 7.24−7.38 (5H, m), 7.50 (1H, s).

2-Amino-2-[2-(4′-benzylthio-2′-fluorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (6b). A mixture of **13a** (450 mg, 0.999 mmol), benzyl mercaptan (137 mg, 1.13 mmol), tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct (25.9 mg, 0.0250 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos, 29.8 mg, 0.0515 mmol), and *N*,*N*-diisopropylethylamine (258 mg, 1.99 mmol) in 1,4-dioxane (5.0 mL) was refluxed for 7 h. After being poured into water, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the protected title compound. The compound was dissolved in MeOH (6.0 mL), and 35% HCl (3.0 mL) was added to the solution. The mixture was stirred at 70 °C for 3.5 h, concentrated in vacuo, and diluted with diisopropyl ether. The yielded solid was collected by filtration to give the title compound (362 mg, 94%) as a pale-yellow solid. mp 218−219 °C; MS (ESI$^+$) m/z 412 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.80−1.85 (2H, m), 2.63−2.68 (2H, m), 3.54 (4H, d, J = 4.5 Hz), 4.34 (2H, s), 5.40 (2H, t, J = 4.9 Hz), 7.22−7.35 (7H, m), 7.41−7.47 (5H, m), 7.83 (3H, brs). Anal. ($C_{24}H_{26}FNO_2S\cdot HCl$) C, H, N.

The compounds **6a**,**c**−**n** were synthesized using a similar procedure to that described for **6b**. Synthetic route for each compound is summarized in the Supporting Information. Some compounds were isolated as free base before converting into hydrochloride salt.

2-Amino-2-[2-(4′-benzylthio-3-chlorobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (6a). mp 167−168 °C; MS (ESI$^+$) m/z 428 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.78−1.84 (2H, m), 2.74−2.79 (2H, m), 3.57 (4H, d, J = 4.8 Hz), 4.30 (2H, s), 5.42 (2H, t, J = 4.8 Hz), 7.24−7.43 (8H, m), 7.60−7.70 (4H, m), 7.87 (3H, brs). Anal. ($C_{24}H_{26}ClNO_2S\cdot HCl\cdot 0.25H_2O$) C, H, N.

2-Amino-2-[2-(2′-fluoro-4′-phenylthiobiphenyl-4-yl)ethyl]propane-1,3-diol Hydrochloride (6c). mp 154−155 °C; MS (ESI$^+$) m/z 398 [M + H]. ^{1}H NMR ($CDCl_3$) δ 1.79−1.85 (2H, m), 2.63−2.69 (2H, m), 3.54 (4H, d, J = 4.5 Hz), 5.41 (2H, t, J = 5.0 Hz), 7.13−7.17 (2H, m), 7.32 (2H, d, J = 8.4 Hz), 7.44−7.53 (8H, m), 7.83 (3H, brs). Anal. ($C_{23}H_{24}FNO_2S\cdot HCl$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(4-methylphenylthio)biphenyl-4-yl]-ethyl]propane-1,3-diol (6d). The title compound was obtained by purification using preparative HPLC. mp 135−136 °C; MS (ESI$^+$) m/z 412 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.30 (2H, brs), 1.49−1.54 (2H, m), 2.35 (3H, s), 2.60−2.65 (2H, m), 3.19−3.29 (4H, m), 4.47 (2H, brs), 7.04 (2H, d, J = 9.9 Hz), 7.29 (4H, m), 7.39−7.48 (5H, m). Anal. ($C_{24}H_{26}FNO_2S$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(3-methylphenylthio)biphenyl-4-yl]-ethyl]propane-1,3-diol Hydrochloride (6e). mp 148−150 °C; MS (ESI$^+$) m/z 412 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.79−1.85 (2H, m), 2.35 (3H, s), 2.63−2.68 (2H, m), 3.53−3.54 (4H, m), 5.39 (2H, t, J = 4.7 Hz), 7.10−7.14 (2H, m), 7.23−7.49 (9H, m), 7.81 (3H, brs). Anal. ($C_{24}H_{26}FNO_2S\cdot HCl\cdot 0.25H_2O$) C, H, N.

2-Amino-2-[2-[3-chloro-2′-fluoro-4′-(4-methylphenylthio)biphenyl-4-yl]ethyl]propane-1,3-diol (6f). mp 98−100 °C; MS (ESI$^+$) m/z 446 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.29 (2H, brs), 1.47−1.53 (2H, m), 2.35 (3H, s), 2.72−2.77 (2H, m), 3.21−3.30 (4H, m), 4.49 (2H, t, J = 4.7 Hz), 7.01−7.06 (2H, m), 7.30 (2H, d, J = 8.0 Hz), 7.38−7.52 (6H, m). Anal. ($C_{24}H_{25}ClFNO_2S\cdot 0.25H_2O$) C, H, N.

2-Amino-2-[2-[4′-(4-ethylphenylthio)-2′-fluorobiphenyl-4-yl]-ethyl]propane-1,3-diol (6g). mp 115−117 °C; MS (ESI$^+$) m/z 426 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, J = 7.5 Hz), 1.29 (2H, brs), 1.49−1.54 (2H, m), 2.60−2.68 (4H, m), 3.21−3.29 (4H, m), 4.46 (2H, t, J = 5.1 Hz), 7.05−7.08 (2H, m), 7.09−7.34 (4H, m), 7.40−7.49 (5H, m). Anal. ($C_{25}H_{28}FNO_2S\cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(4-isopropylphenylthio)biphenyl-4-yl]ethyl]propane-1,3-diol Hydrochloride (6h). mp 245−246 °C; MS (ESI$^+$) m/z 440 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.20 (6H, d, J = 6.9 Hz), 1.77−1.83 (2H, m), 2.61−2.66 (2H, m), 2.91 (1H, sept, J = 6.9 Hz), 3.52 (4H, d, J = 4.6 Hz), 5.39 (2H, t, J = 4.6 Hz), 7.05−7.09 (2H, m), 7.28−7.35 (4H, m), 7.41−7.46 (5H, m), 7.83 (3H, brs). Anal. ($C_{26}H_{30}FNO_2S\cdot HCl$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(4-fluorophenylthio)biphenyl-4-yl]-ethyl]propane-1,3-diol (6i). mp 137−139 °C; MS (ESI$^+$) m/z 416 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.29 (2H, brs), 1.49−1.55 (2H, m), 2.60−2.66 (2H, m), 3.19−3.29 (4H, m), 4.46 (2H, t, J = 5.2 Hz), 7.07−7.26 (2H, m), 7.29−7.60 (9H, m). Anal. ($C_{23}H_{23}F_2NO_2S$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(3-fluorophenylthio)biphenyl-4-yl]-ethyl]propane-1,3-diol (6j). mp 137−139 °C; MS (ESI$^+$) m/z 416 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.30 (2H, brs), 1.50−1.56 (2H, m), 2.61−2.67 (2H, m), 3.19−3.30 (4H, m), 4.47 (2H, t, J = 5.4 Hz), 7.23−7.33 (7H, m), 7.44−7.55 (4H, m). Anal. ($C_{23}H_{23}F_2NO_2S\cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-[4′-(4-chlorophenylthio)-2′-fluorobiphenyl-4-yl]-ethyl]propane-1,3-diol (6k). mp 135−136 °C; MS (ESI$^+$) m/z 432 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.31 (2H, brs), 1.50−1.55 (2H, m), 2.61−2.66 (2H, m), 3.20−3.28 (4H, m), 4.46 (2H, brs), 7.16−7.30 (4H, m), 7.43−7.55 (7H, m). Anal. ($C_{23}H_{23}ClFNO_2S\cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-[4-(trifluoromethyl)phenylthio]biphenyl-4-yl]ethyl]propane-1,3-diol (6l). mp 128−130 °C; MS (ESI$^+$) m/z 466 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.32 (2H, brs), 1.51−1.56 (2H, m), 2.62−2.68 (2H, m), 3.22−3.29 (4H, m), 4.48 (2H, t, J = 5.2 Hz), 7.30−7.38 (3H, m), 7.44−7.52 (5H, m), 7.61 (1H, t, J = 8.2 Hz), 7.74 (2H, d, J = 8.4 Hz). Anal. ($C_{24}H_{23}F_4NO_2S\cdot 0.5H_2O$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(4-methoxyphenylthio)biphenyl-4-yl]ethyl]propane-1,3-diol Hydrochloride (6m). mp 246−247 °C; MS (ESI$^+$) m/z 428 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.75−1.81 (2H, m), 2.59−2.65 (2H, m), 3.50 (4H, d, J = 4.3 Hz), 3.80 (3H, s), 5.36 (2H, brs), 6.92−6.96 (2H, m), 7.05 (2H, d, J = 6.6 Hz), 7.28 (2H, d, J = 8.2 Hz), 7.41−7.43 (3H, m), 7.50 (2H, d, J = 8.8 Hz), 7.69 (3H, brs). Anal. ($C_{24}H_{26}FNO_3S\cdot HCl\cdot 0.75H_2O$) C, H, N.

2-Amino-2-[2-[2′-fluoro-4′-(3-methoxyphenylthio)biphenyl-4-yl]ethyl]propane-1,3-diol (6n). mp 108−110 °C; MS (ESI$^+$) m/z 428 [M + H]. ^{1}H NMR (DMSO-d_6) δ 1.55−1.61 (2H, m), 2.61−2.66 (2H, m), 3.26−3.33 (4H, m), 3.76 (3H, s), 4.64 (2H, brs), 6.92−7.08 (3H, m), 7.15−7.58 (8H, m). Anal. ($C_{24}H_{26}FNO_3S\cdot 1.5H_2O$) C, N. H: calcd, 6.43; found, 6.00.

4-(Di-*tert*-butylphosphoryloxymethyl)-4-[2-(3-chloro-4′-pentylbiphenyl-4-yl)ethyl]-2-methyl-2-oxazoline (14a). A solution of **3a** (180 mg, 0.436 mmol), *N*,*N*-diisopropylethylamine (0.16 mL, 0.89 mmol), and triethyl orthoacetate (0.095 mL, 0.52 mmol) in DMF (2.0 mL) was heated at 120 °C for 5 h. The reaction mixture

was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue in CH_2Cl_2 (4.4 mL) were added 1*H*-tetrazole (61 mg, 0.87 mmol) and di-*tert*-butyl *N*,*N*-diisopropylphosphoramidite (0.28 mL, 0.88 mmol) at 0 °C. After stirring at room temperature for 2 h, *m*-CPBA (65%, 201 mg, 0.75 mmol) was added to the mixture at 0 °C. The mixture was stirred at room temperature for 1 h, poured into saturated $NaHCO_3$, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (159 mg, 62%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 6.6 Hz), 1.23−1.45 (4H, m), 1.48 (18H, s), 1.56−1.72 (2H, m), 1.78−1.98 (2H, m), 2.03 (3H, s), 2.63 (2H, t, J = 7.5 Hz), 2.66−2.88 (2H, m), 3.85−4.02 (2H, m), 4.12 (1H, d, J = 9.0 Hz), 4.38 (1H, d, J = 8.7 Hz), 7.23−7.28 (3H, m), 7.38−7.47 (3H, m), 7.55 (1H, d, J = 1.8 Hz).

The compounds **14b**−**n** were synthesized using a similar procedure to that described for **14a**. Synthetic route for each compound is summarized in the Supporting Information.

4-[2-(4′-Butoxybiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14b). ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t, J = 7.2 Hz), 1.49 (18H, s), 1.50−1.56 (2H, m), 1.75−2.04 (4H, m), 2.02 (3H, s), 2.65−2.70 (2H, m), 3.88−3.91 (1H, m), 3.95−4.02 (4H, m), 4.35 (1H, d, J = 8.7 Hz), 6.95 (2H, d, J = 8.7 Hz), 7.23 (2H, d, J = 8.2 Hz), 7.46 (2H, d, J = 8.1 Hz), 7.48 (2H, d, J = 8.7 Hz).

4-[2-(4′-Butoxy-3-chlorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14c). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.2 Hz), 1.36−1.58 (2H, m), 1.50 (18H, s), 1.68−1.97 (4H, m), 2.03 (3H, s), 2.65−2.90 (2H, m), 3.86−4.02 (2H, m), 4.00 (2H, t, J = 6.6 Hz), 4.12 (1H, d, J = 8.7 Hz), 4.38 (1H, d, J = 8.7 Hz), 6.95 (2H, d, J = 8.7 Hz), 7.25 (1H, d, J = 6.9 Hz), 7.36 (1H, dd, J = 1.5, 7.8 Hz), 7.45−7.52 (3H, m).

4-[2-(4′-Butoxy-2′-fluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14d). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.4 Hz), 1.48 (18H, d, J = 3.2 Hz), 1.51−1.55 (2H, m), 1.75−1.82 (2H, m), 1.85−1.89 (1H, m), 1.97−2.02 (1H, m), 2.02 (3H, s), 2.66−2.71 (2H, m), 3.88−3.92 (1H, m), 3.96−4.03 (4H, m), 4.36 (1H, d, J = 8.8 Hz), 6.67−6.71 (1H, m), 6.73−6.76 (1H, m), 7.24 (2H, d, J = 8.0 Hz), 7.31 (1H, t, J = 8.8 Hz), 7.41−7.43 (2H, m).

4-[2-(4′-Butoxy-2′,5′-difluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14f). ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.4 Hz), 1.48 (18H, d, J = 3.3 Hz), 1.51−1.55 (2H, m), 1.79−1.88 (3H, m), 1.96−2.02 (1H, m), 2.01 (3H, s), 2.67−2.71 (2H, m), 3.89 (1H, dd, J = 4.6, 9.8 Hz), 3.98 (1H, dd, J = 5.2, 10.0 Hz), 4.02−4.13 (3H, m), 4.35 (1H, d, J = 8.9 Hz), 6.75 (1H, dd, J = 7.1, 11.7 Hz), 7.14 (1H, dd, J = 7.3, 11.7 Hz), 7.23−7.26 (2H, m), 7.41 (2H, d, J = 7.8 Hz).

4-[2-(4′-Butoxy-3′-fluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14g). ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, J = 7.2 Hz), 1.48 (18H, d, J = 3.2 Hz), 1.51−1.57 (2H, m), 1.79−1.88 (3H, m), 1.96−2.03 (1H, m), 2.02 (3H, s), 2.66−2.70 (2H, m), 3.89 (1H, dd, J = 4.8, 10.0 Hz), 3.98 (1H, dd, J = 5.2, 10.0 Hz), 4.01 (1H, d, J = 8.8 Hz), 4.07 (2H, t, J = 6.4 Hz), 4.35 (1H, d, J = 8.8 Hz), 7.00 (1H, t, J = 8.4 Hz), 7.22−7.32 (4H, m), 7.41−7.43 (2H, d, J = 8.4 Hz).

4-[2-(4′-Butoxy-3′-chlorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14h). ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, J = 7.2 Hz), 1.48 (18H, d, J = 3.6 Hz), 1.48−1.56 (2H, m), 1.81−1.88 (3H, m), 1.95−2.04 (1H, m), 2.03 (3H, s), 2.66−2.70 (2H, m), 3.89 (1H, dd, J = 4.8, 10.0 Hz), 3.97 (1H, dd, J = 5.2, 10.0 Hz), 4.01 (1H, d, J = 8.8 Hz), 4.07 (2H, t, J = 6.4 Hz), 4.35 (1H, d, J = 8.8 Hz), 6.96 (1H, d, J = 8.8 Hz), 7.23 (2H, d, J = 8.0 Hz), 7.39 (1H, dd, J = 2.4, 8.4 Hz), 7.43 (2H, d, J = 8.0 Hz), 7.58 (1H, d, J = 2.0 Hz).

4-(Di-*tert*-butylphosphoryloxymethyl)-2-methyl-4-[2-(4′-phenoxybiphenyl-4-yl)ethyl]-2-oxazoline (14i). ^{1}H NMR (DMSO-d_6) δ 1.40 (18H, d, J = 2.2 Hz), 1.72−1.86 (2H, m), 1.92 (3H, s), 2.50−2.56 (1H, m), 2.60−2.68 (1H, m), 3.77−3.84 (2H, m), 4.07 (1H, d, J = 8.8 Hz), 4.14 (1H, d, J = 8.8 Hz), 7.05−7.08 (4H, m), 7.16 (1H, t, J = 7.2 Hz), 7.29 (2H, d, J = 8.2 Hz), 7.39−7.44 (2H, m), 7.55 (2H, d, J = 8.1 Hz), 7.64−7.68 (2H, m).

4-(Di-*tert*-butylphosphoryloxymethyl)-4-[2-(3-chloro-4′-phenoxybiphenyl-4-yl)ethyl]-2-methyl-2-oxazoline (14j). ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.80−2.02 (2H, m), 2.04 (3H, s), 2.68−2.92 (2H, m), 3.86−4.02 (2H, m), 4.13 (1H, d, J = 8.6 Hz), 4.39 (1H, d, J = 8.8 Hz), 7.04−7.13 (5H, m), 7.28 (1H, d, J = 8.0 Hz), 7.33−7.39 (3H, m), 7.49−7.54 (3H, m).

4-[2-(4′-Benzyloxy-3-chlorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14k). ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.78−2.00 (2H, m), 2.03 (3H, s), 2.64−2.90 (2H, m), 3.86−4.00 (2H, m), 4.12 (1H, d, J = 8.9 Hz), 4.38 (1H, d, J = 8.9 Hz), 5.11 (2H, s), 7.04 (2H, d, J = 8.5 Hz), 7.25−7.52 (10H, m).

4-[2-(4′-Benzyloxy-2′-fluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (14l). ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.76−1.94 (2H, m), 2.02 (3H, s), 2.61−2.72 (2H, m), 3.87−4.03 (3H, m), 4.33−4.37 (1H, m), 5.09 (2H, s), 6.76−6.84 (2H, m), 7.23−7.46 (10H, m).

4-(Di-*tert*-butylphosphoryloxymethyl)-4-[2-(2′-fluoro-4′-pentylbiphenyl-4-yl)ethyl]-2-methyl-2-oxazoline (14m). ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 6.9 Hz), 1.33−1.38 (4H, m), 1.48 (18H, s), 1.61−1.68 (2H, m), 1.82−1.89 (1H, m), 1.97−2.05 (1H, m), 2.02 (3H, s), 2.62 (2H, t, J = 7.9 Hz), 2.69 (2H, t, J = 8.6 Hz), 3.89 (1H, dd, J = 4.7, 10.0 Hz), 3.97 (1H, dd, J = 4.9, 10.1 Hz), 4.01 (1H, d, J = 8.8 Hz), 4.36 (1H, d, J = 8.8 Hz), 6.94−7.01 (2H, m), 7.24−7.26 (2H, m), 7.31 (1H, t, J = 8.0 Hz), 7.45 (2H, dd, J = 1.3, 8.3 Hz).

4-(Di-*tert*-butylphosphoryloxymethyl)-4-[2-[2′-fluoro-4′-(4-methylphenoxy)biphenyl-4-yl]ethyl]-2-methyl-2-oxazoline (14n). ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.80−2.10 (2H, m), 2.02 (3H, s), 2.36 (3H, s), 2.69 (2H, t, J = 8.6 Hz), 3.90 (1H, dd, J = 4.5, 9.9 Hz), 3.98 (1H, dd, J = 4.8, 9.9 Hz), 4.02 (1H, d, J = 8.4 Hz), 4.36 (1H, d, J = 8.7 Hz), 6.72−6.82 (2H, m), 6.96−6.99 (2H, m), 7.17−7.44 (7H, m).

2-Amino-4-(3-chloro-4′-pentylbiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3a-P). A solution of **14a** (159 mg, 0.268 mmol) in EtOH (2.5 mL) and 35% HCl (0.50 mL) was heated at 50 °C for 3 h. After addition of water (20 mL), the yielded solid was collected and washed with water to afford the title compound (79 mg, 64%) as a white solid. mp 229−238 °C (dec); MS (ESI^+) m/z 456 [M + H]. ^{1}H NMR (CD_3OD) δ 0.93 (3H, t, J = 6.6 Hz), 1.29−1.48 (4H, m), 1.60−1.74 (2H, m), 1.95−2.08 (2H, m), 2.66 (2H, t, J = 7.5 Hz), 2.84−2.94 (2H, m), 3.72−3.85 (2H, m), 3.98−4.14 (2H, m), 7.27 (2H, d, J = 8.1 Hz), 7.43 (1H, d, J = 8.1 Hz), 7.50−7.53 (3H, m), 7.62 (1H, d, J = 1.5 Hz). Anal. ($C_{22}H_{31}ClNO_5P$) C, H, N.

The compounds **3(b**−**l)-P**, **4-P**, and **5-P** were synthesized using a similar procedure to that described for **3a-P**. Synthetic route for each compound is summarized in the Supporting Information.

2-Amino-4-(4′-butoxybiphenyl-4-yl)-2-(phosphoryloxymethyl)-butanol (3b-P). mp 222−225 °C (dec); MS (ESI^+) m/z 424 [M + H]. ^{1}H NMR (CD_3OD) δ 1.00 (3H, t, J = 7.2 Hz), 1.50−1.55 (2H, m), 1.75−1.79 (2H, m), 1.97−2.02 (2H, m), 2.69−2.75 (2H, m), 3.72 (2H, brs), 4.00 (4H, t, J = 6.1 Hz), 6.96 (2H, d, J = 8.4 Hz), 7.29 (2H, d, J = 7.6 Hz), 7.48−7.51 (4H, m). Anal. ($C_{21}H_{30}NO_6P$) C, H, N.

2-Amino-4-(4′-butoxy-3-chlorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3c-P). mp 219 °C (dec); MS (ESI^+) m/z 458 [M + H]. ^{1}H NMR (CD_3OD) δ 1.02 (3H, t, J = 7.3 Hz), 1.46−1.60 (2H, m), 1.74−1.85 (2H, m), 1.99−2.05 (2H, m), 2.85−2.90 (2H, m), 3.72−3.85 (2H, m), 3.98−4.14 (4H, m), 7.00 (2H, d, J = 8.6 Hz), 7.40 (1H, d, J = 7.9 Hz), 7.46−7.54 (3H, m), 7.59 (1H, d, J = 1.6 Hz). Anal. ($C_{21}H_{29}ClNO_6P$) C, H, N.

2-Amino-4-(4′-butoxy-2′-fluorobiphenyl-4-yl)-2-(phosphoryloxymethy)butanol (3d-P). mp 220−223 °C (dec); MS (ESI^+) m/z 442 [M + H]. ^{1}H NMR (CD_3OD) δ 1.00 (3H, t, J = 7.2 Hz),

1.49−1.55 (2H, m), 1.74−1.81 (2H, m), 1.99−2.04 (2H, m), 2.70−2.76 (2H, m), 3.69−3.76 (2H, m), 3.97−4.06 (4H, m), 6.72−6.75 (1H, m), 6.78−6.81 (1H, m), 7.29−7.36 (3H, m), 7.41 (2H, d, J = 8.4 Hz). Anal. ($C_{21}H_{29}FNO_6P$) C, H, N.

2-Amino-4-(4′-butoxy-2′,5′-difluorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3f-P). mp 228−231 °C (dec); MS (ESI$^+$) m/z 460 [M + H]. ^{1}H NMR (CD_3OD) δ 1.00 (3H, t, J = 7.5 Hz), 1.49−1.58 (2H, m), 1.77−1.84 (2H, m), 1.99−2.04 (2H, m), 2.70−2.77 (2H, m), 3.72 (2H, d, J = 2.2 Hz), 3.98−4.04 (2H, m), 4.08 (2H, t, J = 6.4 Hz), 6.96 (1H, dd, J = 7.2, 12.0 Hz), 7.19 (1H, dd, J = 7.6, 11.8 Hz), 7.32 (2H, d, J = 8.2 Hz), 7.43 (2H, d, J = 8.3 Hz). Anal. ($C_{21}H_{28}F_2NO_6P\cdot 0.25H_2O$) C, H, N.

2-Amino-4-(4′-butoxy-3′-fluorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3g-P). mp 224−227 °C (dec); MS (ESI$^+$) m/z 442 [M + H]. ^{1}H NMR (CD_3OD) δ 1.00 (3H, t, J = 7.2 Hz), 1.49−1.58 (2H, m), 1.76−1.83 (2H, m), 1.99−2.04 (2H, m), 2.69−2.76 (2H, m), 3.69−3.76 (2H, m), 3.97−4.04 (2H, m), 4.08 (2H, t, J = 6.4 Hz), 7.10−7.14 (1H, m), 7.30−7.35 (4H, m), 7.50 (2H, d, J = 8.0 Hz). Anal. ($C_{21}H_{29}FNO_6P\cdot 0.1H_2O$) C, H, N.

2-Amino-4-(4′-butoxy-3′-chlorobiphenyl-4-yl)-2-(phosphoryloxymethylbutanol (3h-P). mp 221−224 °C (dec); MS (ESI$^+$) m/z 458 [M + H]. ^{1}H NMR (CD_3OD) δ 1.01 (3H, t, J = 7.5 Hz), 1.54−1.59 (2H, m), 1.78−1.83 (2H, m), 1.98−2.02 (2H, m), 2.69−2.73 (2H, m), 3.72 (2H, d, J = 2.3 Hz), 3.99−4.04 (2H, m), 4.09 (2H, t, J = 6.3 Hz), 7.10 (1H, d, J = 8.6 Hz), 7.31 (2H, d, J = 8.0 Hz), 7.46−7.50 (3H, m), 7.59 (1H, d, J = 2.2 Hz). Anal. ($C_{21}H_{29}ClNO_6P$) C, H, N.

2-Amino-4-(4′-phenoxybiphenyl-4-yl)-2-(phosphoryloxymethyl)-butanol (3i-P). mp 207−209 °C (dec); MS (ESI$^+$) m/z 444 [M + H]. ^{1}H NMR (CD_3OD) δ 1.98−2.04 (2H, m), 2.70−2.76 (2H, m), 3.73 (2H, d, J = 1.1 Hz), 3.98−4.07 (2H, m), 7.01−7.05 (4H, m), 7.12 (1H, t, J = 7.7 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.36 (2H, t, J = 8.3 Hz), 7.53 (2H, d, J = 8.0 Hz), 7.58 (2H, d, J = 8.6 Hz). Anal. ($C_{23}H_{26}NO_6P\cdot 0.25H_2O$) C, H, N.

2-Amino-4-(3-chloro-4′-phenoxybiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3j-P). mp 218 °C (dec); MS (ESI$^+$) m/z 478 [M + H]. ^{1}H NMR (CD_3OD) δ 2.00−2.05 (2H, m), 2.86−2.92 (2H, m), 3.72−3.85 (2H, m), 3.98−4.14 (2H, m), 7.03−7.08 (5H, m), 7.36−7.51 (3H, m), 7.60−7.64 (4H, m). Anal. ($C_{23}H_{25}ClNO_6P$) C, H, N.

2-Amino-4-(4′-benzyloxy-3-chlorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3k-P). mp 241 °C (dec); MS (ESI$^+$) m/z 492 [M + H]. ^{1}H NMR (CD_3OD) δ 2.00−2.04 (2H, m), 2.85−2.90 (2H, m), 3.72−3.85 (2H, m), 3.98−4.14 (2H, m), 5.15 (2H, s), 7.09 (2H, d, J = 8.4 Hz), 7.32−7.60 (10H, m). Anal. ($C_{24}H_{27}ClNO_6P\cdot H_2O$) C, H, N.

2-Amino-4-(4′-benzyloxy-2′-fluorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (3l-P). mp 241−243 °C; MS (ESI$^+$) m/z 476 [M + H]. ^{1}H NMR (CD_3OD) δ 2.02−2.05 (2H, m), 2.72−2.77 (2H, m), 3.71−3.73 (2H, m), 4.07−4.09 (2H, m), 5.12 (2H, s), 6.82−6.90 (2H, m), 7.29−7.46 (10H, m). Anal. ($C_{24}H_{27}FNO_6P\cdot H_2O$) C, H, N.

2-Amino-4-(2′-fluoro-4′-pentylbiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (4-P). mp 225−228 °C (dec); MS (ESI$^+$) m/z 440 [M + H]. ^{1}H NMR (CD_3OD) δ 0.92 (3H, t, J = 6.8 Hz), 1.33−1.37 (4H, m), 1.61−1.67 (2H, m), 1.98−2.04 (2H, m), 2.62−2.66 (2H, m), 2.70−2.76 (2H, m), 3.72 (2H, d, J = 2.4 Hz), 3.97−4.04 (2H, m), 6.98 (1H, d, J = 11.1 Hz), 7.05 (1H, d, J = 8.0 Hz), 7.31−7.36 (3H, m), 7.45 (2H, d, J = 7.9 Hz). Anal. ($C_{22}H_{31}FNO_5P$) C, H, N.

2-Amino-4-[2′-fluoro-4′-(4-methylphenoxy)biphenyl-4-yl]-2-(phosphoryloxymethyl)butanol (5-P). mp 218−220 °C; MS (ESI$^+$) m/z 476 [M + H]. ^{1}H NMR (CD_3OD) δ 1.96−2.08 (2H, m), 2.35 (3H, s), 2.64−2.80 (2H, m), 3.72 (2H, s), 3.95−4.08 (2H, m), 6.71−6.83 (2H, m), 6.96 (2H, d, J = 8.4 Hz), 7.22 (2H, d, J = 8.4 Hz), 7.31−7.45 (5H, m). Anal. ($C_{24}H_{27}FNO_6P$) C, H, N.

4-[2-(4′-Bromo-2′-fluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (15a). To a solution of **13a** (2.50 g, 5.55 mmol) in EtOH (50 mL) was added 35% HCl (25 mL). After stirring at 80 °C for 4 h, the reaction was concentrated in vacuo and neutralized with 1 M aqueous NaOH. The yielded precipitate was collected by filtration to give the deprotected compound (1.36 g, 86%). A solution of the compound, *N*,*N*-diisopropylethylamine (0.57 g, 4.4 mmol), and triethyl orthoacetate (0.72 g, 4.4 mmol) in DMF (5.0 mL) was heated at 120 °C for 3 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. To the solution of the residue in CH_2Cl_2 (37 mL) was added 1*H*-tetrazole (389 mg, 5.55 mmol) and di-*tert*-butyl *N*,*N*-diisopropylphosphoramidite (1.54 g, 5.55 mmol) at 0 °C. After stirring at room temperature for 2 h, a solution of *tert*-butyl hydroperoxide in decane (5−6 M, 2.22 mL) was added to the solution at 0 °C. The mixture was stirred for 10 min, poured into saturated $NaHCO_3$, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the title compound (0.98 g, 30%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.87−2.02 (2H, m), 2.04 (3H, s), 2.70 (2H, t, J = 8.6 Hz), 3.86−4.01 (3H, m), 4.36 (1H, d, J = 8.9 Hz), 7.26−7.44 (7H, m).

4-[2-(4′-Bromo-3-chloro-2′-fluorobiphenyl-4-yl)ethyl]-4-(di-*tert*-butylphosphoryloxymethyl)-2-methyl-2-oxazoline (15b). The title compound was synthesized from **13b** using a similar procedure to that described for **15a**. ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 1.85−1.95 (2H, m), 2.03 (3H, s), 2.73−2.85 (2H, m), 3.92 (1H, dd, J = 4.8, 9.9 Hz), 3.99 (1H, dd, J = 4.8, 9.9 Hz), 4.11 (1H, d, J = 9.0 Hz), 4.38 (1H, d, J = 8.7 Hz), 7.24−7.37 (5H, m), 7.49 (1H, s).

2-Amino-4-(4′-benzylthio-2′-fluorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (6b-P). A mixture of **15a** (280 mg, 0.479 mmol), benzyl mercaptan (66 mg, 0.53 mmol), tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct (12.4 mg, 0.0119 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos, 14.3 mg, 0.0247 mmol), and *N*,*N*-diisopropylethylamine (124 mg, 0.959 mmol) in 1,4-dioxane (2.0 mL) was refluxed for 5 h. After being poured into water, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/EtOAc) gave the protected title compound. To a solution of the compound in EtOH (5.0 mL) was added 35% HCl (1.0 mL). The mixture was stirred at 50 °C for 4 h and diluted with water. The yielded solid was collected by filtration to give the title compound (74 mg, 31%) as a white solid. mMp 167−170 °C; MS (ESI$^+$) m/z 492 [M + H]. ^{1}H NMR (CD_3OD) δ 1.99−2.06 (2H, m), 2.70−2.80 (2H, m), 3.73 (2H, s), 4.09 (2H, d, J = 5.1 Hz), 4.22 (2H, s), 7.10−7.37 (10H, m), 7.43−7.46 (2H, m). Anal. ($C_{24}H_{27}FNO_5PS\cdot 1.5H_2O$) C, H, N.

The compounds **6(a,c−f)-P** were synthesized using a similar procedure to that described for **6b-P**. Synthetic route for each compound is summarized in the Supporting Information.

2-Amino-4-(4′-benzylthio-3-chlorobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (6a-P). mp 236−238 °C; MS (ESI$^+$) m/z 508 [M + H]. ^{1}H NMR (CD_3OD) δ 1.98−2.02 (2H, m), 2.84−2.88 (2H, m), 3.72−3.80 (2H, m), 4.00−4.09 (2H, m), 4.19 (2H, s), 7.19−7.29 (3H, m), 7.33 (2H, d, J = 7.4 Hz), 7.38 (2H, d, J = 8.2 Hz), 7.42 (1H, d, J = 7.9 Hz), 7.48−7.52 (3H, m), 7.61 (1H, d, J = 1.5 Hz). Anal. ($C_{24}H_{27}ClNO_5PS\cdot 1.25H_2O$) C, H, N.

2-Amino-4-(2′-fluoro-4′-phenylthiobiphenyl-4-yl)-2-(phosphoryloxymethyl)butanol (6c-P). mp 208−212 °C; MS (ESI$^+$) m/z 478 [M + H]. ^{1}H NMR (CD_3OD) δ 1.94−2.05 (2H, m), 2.68−2.80 (2H, m), 3.73 (2H, s), 3.93−4.08 (2H, m), 7.03−7.06 (1H, m), 7.13 (1H, d, J = 8.4 Hz), 7.34−7.49 (10H, m). Anal. ($C_{23}H_{25}FNO_5PS\cdot 0.25H_2O$) C, H, N.

2-Amino-4-[2′-fluoro-4′-(4-methylphenylthio)biphenyl-4-yl]-2-(phosphoryloxymethyl)butanol (6d-P). mp 236−238 °C; MS (ESI$^+$) m/z 492 [M + H]. ^{1}H NMR (CD_3OD) δ 1.98−2.08 (2H, m), 2.38 (3H, s), 2.68−2.82 (2H, m), 3.72 (2H, s), 3.94−4.10 (2H, m), 6.91 (1H, d, J = 11.4 Hz), 7.05 (1H, d, J = 8.1 Hz), 7.24−7.45 (9H, m). Anal. ($C_{24}H_{27}FNO_5PS$) C, H, N.

2-Amino-4-[2′-fluoro-4′-(3-methylphenylthio)biphenyl-4-yl]-2-(phosphoryloxymethyl)butanol (6e-P). mp 212−213 °C; MS (ESI$^+$) m/z 492 [M + H]. ^{1}H NMR (CD_3OD) δ 1.97−2.05 (2H, m), 2.34 (3H, s), 2.68−2.82 (2H, m), 3.72 (2H, s), 3.92−4.10 (2H, m), 6.97 (1H, d, J = 11.4 Hz), 7.09 (1H, d, J = 9.9 Hz), 7.18−7.47 (9H, m). Anal. ($C_{24}H_{27}FNO_5PS \cdot 0.75H_2O$) C, H, N.

2-Amino-4-[3-chloro-2′-fluoro-4′-(4-methylphenylthio)biphenyl-4-yl]-2-(phosphoryloxymethyl)butanol (6f-P). mp 214−216 °C; MS (ESI$^+$) m/z 526 [M + H]. ^{1}H NMR (CD_3OD) δ 1.93−2.02 (2H, m), 2.39 (3H, s), 2.85−2.91 (2H, m), 3.68−3.82 (2H, m), 3.95−4.10 (2H, m), 6.91 (1H, d, J = 11.1 Hz), 7.04 (1H, d, J = 8.4 Hz), 7.27 (2H, d, J = 7.8 Hz), 7.35−7.42 (5H, m), 7.53 (1H, s). Anal. ($C_{24}H_{26}ClFNO_5PS$) C, H, N.

Intracellular Ca^{2+} Mobilization Assay. Chem-1 or CHO cells stably expressing human S1P receptors ($hS1P)_1$ or $hS1P_3$ respectively were loaded with 5 μM Fura-2 AM (Dojindo, Kumamoto, Japan) for 90 min at 37 °C. After two washes, the cells were suspended at a concentration of 3×10^5 cells/mL in HBSS containing 0.1% BSA (fatty acid free) and 1.25 mM probenecid (pH 7.4). The cells (3×10^4 cells/well) in HBSS containing 0.1% BSA (fatty acid free) and 1.25 mM probenecid (pH 7.4) were plated in black wall 96-well plates, and then the changes in fluorescence were monitored at 37 °C at excitation wavelengths of 340 and 380 nm and an emission wavelength of 540 nm using a functional drug screening system (FDSS3000, Hamamatsu Photonics, Shizuoka, Japan). Ten seconds after the start of monitoring, the serial dilutions of S1P or test compounds were added and the maximal change in Ca^{2+} concentration after stimulator addition was quantitated.

Mouse Lymphocyte Reduction Assay. Male BALB/c mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Test compounds were intraperitoneally administered. Twenty-four hours later, a peripheral blood sample was collected from the posterior vena cava by using a heparinized syringe and hemolyzed and fixed by using TQ-Prep (Beckman Coulter, CA). The number of CD3$^+$ pan-T cells and CD45R$^+$ pan-B cells was determined by two-color flow cytometry (Cytomics FC 500, Beckman Coulter, CA) using fluorescein isothiocyanate (FITC)-conjugated hamster antimouse CD3e (CD3ε chain) monoclonal antibody (Mab) (Clone 145−2C11, BD Bioscience, CA) and R-phycoerythrin (R-PE)-conjugated rat antimouse CD45R (B220) Mab (Clone RA3−6B2, BD Bioscience, CA).

Rat Lymphocyte Reduction Assay. Male F344 rats were purchased from Charles Liver Japan Inc. (Yokohama, Japan). Test compounds were orally administered. Twenty-four hours later, a peripheral blood sample was collected from the posterior vena cava by using a heparinized syringe and hemolyzed and fixed by using TQ-Prep (Beckman Coulter, CA). The number of CD3$^+$ pan-T cells and CD45RA$^+$ pan-B cells was determined by two-color flow cytometry (Cytomics FC 500, Beckman Coulter, CA) using FITC-conjugated mouse antirat CD3 Mab (Clone 1F4, BD Bioscience, CA) and R-phycoerythrin (R-PE)-conjugated mouse antirat CD45RA Mab (Clone OX-33, BD Bioscience, CA).

Measurement of Heart Rate in Anesthetized Rats. Male Sprague−Dawley rats purchased from Charles Liver Japan Inc. were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The lead II electrocardiogram (ECG) was recorded, and heart rate was counted by the detection of R wave in ECG. Test compounds were intravenously injected for approximately 30 s. The heart rate was measured before administration, and 1, 2, 3, 4, 5, 10, and 15 min after the intravenous administration of test compound. The maximal heart rate was showed at one minute point. This test was carried out at doses ranging from 0.03 mg/kg to 3 mg/kg.

Measurement of Heart Rate Using Telemetry System. Rats (CD (SD) IGS) purchased from Charles Liver Japan Inc. were used at 10−12 weeks of age. A transmitter (TL11M2-C50-PXT, Data Science International) was implanted in the lateral abdominal subcutaneous tissue. The pressure sensor catheter of transmitter was inserted into the abdominal aorta. Test compounds were homogeneously suspended with 0.5% HPMC (METOLOSE 60SH-50, Shin-Etsu Chemical Co., Ltd.) and administered orally. Heart rate was monitored by data acquisition system in the telemetry system (Dataquest, A.R.T., Science International). Frequency of data sampling was for 15 s in every 5 min.

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Supporting Information Available: Tables giving the synthetic routes of alcohol compounds and phosphate compounds, preparation details of **6a** and **6a-P**, and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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