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Discovery of 4-[4-({(3*R*)-1-butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride: A highly potent orally available CCR5 selective antagonist

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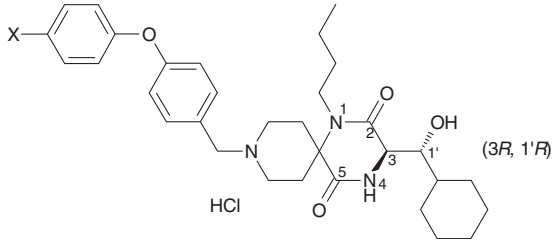
ABSTRACT

Based on the original spirodiketopiperazine design framework, further optimization of an orally available CCR5 antagonist was undertaken. Structural hybridization of the hydroxylated analog **4** derived from one of the oxidative metabolites and the new orally available non-hydroxylated benzoic acid analog **5** resulted in another potent orally available CCR5 antagonist **6a** as a clinical candidate. Full details of a structure–activity relationship (SAR) study and ADME properties are presented.

1. Introduction

HIV-1 infects millions of people worldwide, causing the lethal disease Acquired Immune Deficiency Syndrome (AIDS). To inhibit HIV-1 replication and slow the destruction of the immune system, current therapies utilize a combination of protease and reverse transcriptase inhibitors.[1] While suppression of viral replication through combination therapy delays progression to AIDS, limitations of antiviral therapies for AIDS are exacerbated by complicated regimens, emergence of drug-resistant HIV-1 variants and a number of inherent adverse effects. These issues require new anti-HIV-1 drugs that have a different mode of action from conventional drugs. Agents inhibiting HIV entry into target cells are one of the most promising approaches to treat AIDS.[2] HIV-1 enters target cells via a fusion reaction mediated by the interaction of the viral envelope glycoprotein gp120 and its receptor on the target cell, CD4. This binding induces conformational changes in gp120 that exposes a binding site for a co-receptor, usually either of the chemokine receptors C–C chemokine receptor 5 (CCR5) or C–X–C chemokine receptor 4 (CXCR4) These co-receptors are absolutely imperative for HIV-1 infection and growth. Actually, the endogenous ligands MIP-1α (CCR5) and SDF-1 (CXCR4) have shown

Table 1
Effect of the *p*-substituent X on the activity profiles



Compound	X	Ca assay IC_{50} (nM)	Fusion assay IC_{50} (nM)	% Remaining[a] HLM/RLM	*C* log *P*[b]
4	H	53	7.8	6/18	6.45
7	OMe	91	7.1	NT/NT[c]	6.37
8	OH	68	2.8	3.5/23	5.78
9	$CONH_2$	39	NT[c]	1.5/19	4.96
10	CONHMe	38	7.4	NT/NT[c]	5.17
11	SO_2NH_2	48	3.5	NT/NT[c]	4.61
12	SO_2Me	34	NT[c]	NT/NT[c]	4.81
13	$NHSO_2Me$	26	1.4	1.8/91	5.26
6a	CO_2H	34	5.6	36/39	3.92

[a] The data show the remaining% 15 min after incubating with the 0.5 mg/mL liver microsomes.
[b] Calculated by *C* log *P* 5.1 (Daylight Chemical Information Systems Inc.).
[c] NT: Not tested.

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anti-HIV-1 activity in vitro.[3] In particular, CCR5 is an attractive target because some individuals have a gene encoding a mutant form of CCR5 named CCR5 Δ32 homozygote and these individuals apparently do not have significant health problems and show resistance to HIV-1 infection.[4] Therefore, this previously untargeted receptor, CCR5, has been a promising drug discovery target for many pharmaceutical companies and academic institutions.[5] Maraviroc is the only approved CCR5 antagonist on the market for treatment of HIV-1 infection.[6]

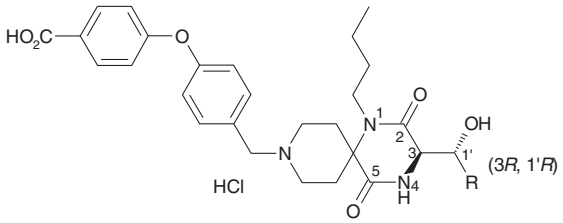
We previously reported the discovery of spirodiketopiperazine-based CCR5 antagonists **1** and **2** using the Ugi multiple component reaction.[7] We also reported the discovery of the hydroxylated analogs **3** and **4** as a biologically active metabolite and a structurally related analog, respectively.[8] Furthermore, we reported the discovery of a non-hydroxylated benzoic acid analog **5** as an orally available CCR5 antagonist.[9] Herein, we present the discovery of another orally available antagonist **6a** as a clinical candidate based on molecular design by structural hybridization of the above-mentioned compounds.

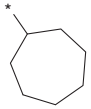
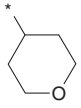
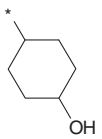
2. Chemistry

Test compounds listed in Tables 1–4 were synthesized as outlined in Schemes 1–5. As shown in Scheme 1, the Ugi coupling reaction of the four components **36a**, an appropriate amine among **37a**–**d**, an appropriate amino acid among **38a–i** and 2-(4-morpholinyl)ethyl isonitrile, followed by deprotection of the *N*-Boc group and cyclization under acidic conditions afforded the 9-*N*-benzyl spirodiketopiperazines represented by the general formula **39a**.[10] Catalytic hydrogenation of **39a** followed by reductive 9-N-alkylation with an appropriate benzaldehyde resulted in **6a**–**d**, **7**–**19**, **20a**, **20b**, **21**, **23** and **25–26**.

Synthesis of **22**, **24** and **27**–**35** is described in Scheme 2. *N*-Phenylmethyl piperidin-4-one **36b** was prepared by the oxidation of the corresponding alcohol **40**, which was prepared by the reductive N-alkylation of commercially available 4-hydroxypiperidine with 4-(4-*N*-methylaminocarbonylphenoxy)benzaldehyde. The Ugi coupling reaction of **36b**, an appropriate amine among **37b**, **37c** and **37e**–**m**, an *N*-Boc protected amino acid **38a** and 2-(4-morpholi-

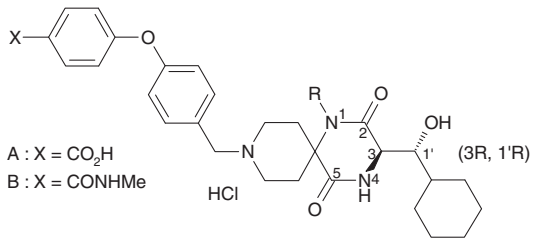
Table 2
Further chemical modification of the cyclohexyl residue of **6a** (3*R*,1′*R*)

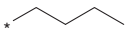
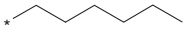
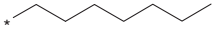
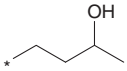
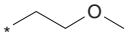
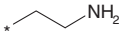
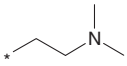
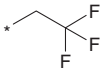
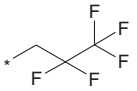


Compound	R	Ca assay IC_{50} (nM)	Fusion assay IC_{50} (nM)	% Remaining[a] HLM/RLM	*C* log *P*[b]
14		48	229	98/100	2.73
15		81	ca. 10,000	89/100	2.25
16		25	66	95/100	2.80
17		33	13	93/100	3.36
18		32	5.2	34/59	4.48
19		120	302	NT[c]	1.52
20a (*cis*) **20b** (*trans*)		140 23	537 129	100/100 100/92	1.84 1.84
6a		34	5.6	36/39	3.92

[a] The data show the remaining % 15 min after incubating with the 0.5 mg/mL liver microsomes.
[b] Calculated by *C* log *P* 5.1 (Daylight Chemical Information Systems Inc.).
[c] NT: Not tested.

Table 3
Effect of the 1-N-substituent on the activity profiles



Compound	X	R	Ca assay IC_{50} (nM)	Fusion assay	*C* log *P*[a]
21	A		50	ca. 10,000	2.34
22	B		59	1862	3.45
23	A		23	295	2.86
24	B		20	178	4.11
25	A		30	112	3.39
26	B		28	21	4.64
6a	A		34	5.6	3.92
10	B		38	7.4	5.17
27	B		68	3.9	5.7
28	B		110	35	6.23
29	B		190	316	6.76
30	B		180	ca. 10,000	3.55
31	B		33	98	3.18
32	B		750	NT[b]	4.05
33	B		640	NT[b]	4.24
34	B		46	1148	5.26
35	B		270	NT[b]	3.86

[a] Calculated by *C* log *P* 5.1 (Daylight Chemical Information Systems Inc.).
[b] NT: Not tested.

Table 4
Activity profiles of all the stereoisomers **6a–d**

Compound	Configuration	Ca assay IC_{50} (nM)	Fusion assay IC_{50} (nM)
6a	(3*R*,1′*R*)	34	5.6
6b	(3*R*,1′*S*)	44	182
6c	(3*S*,1′*R*)	470	380
6d	(3*S*,1′*S*)	51	447

nyl)ethyl isonitrile, followed by deprotection of the *N*-Boc group and cyclization under acidic conditions afforded **22**, **24**, **27**–**31**, **33**–**35** and **41**. Removal of the allyloxycarbonyl residue of **41** under conventional reaction conditions produced **32**.

Optically active β-hydroxy-*N*-Boc-α-amino acids **38a**, **38d**, **38e**, **38h** and **38j** were prepared using Sharpless asymmetric epoxidation.[11] The synthesis procedures for **38a**, **38e**, **38h** and **38j** are described as representative examples in Scheme 3. Doebner condensation of malonic acid with optional aliphatic aldehydes in the presence of piperidine afforded α,β-unsaturated carboxylic acids **42a**, **42e**, **42h** and **42j**, esterification of which under conventional reaction conditions produced the corresponding methyl esters **43a**, **43e**, **43h** and **43j**, respectively. Reduction of the esters with diisobutyl aluminum hydride afforded the corresponding allylic alcohols **44a**, **44e**, **44h** and **44j**, respectively. Asymmetric epoxydation of **44a** using diethyl D-tartrate afforded the optically active epoxy alcohol **45a** with high enantiomeric purity. Following the same reaction conditions as described above, the optically active epoxy alcohols **45e**, **45h** and **45j**, were obtained from **44e**, **44h** and **44j**, respectively. Stepwise oxidation of **45a**, **45e**, **45h** and **45j** with sulfur trioxide–pyridine complex in DMSO and triethylamine, followed by sodium hypochlorite afforded the corresponding epoxy carboxylic acids, regioselective ring opening reaction of which with benzyl amine afforded **46a**, **46e**, **46h** and **46j**, respectively. N-debenzylation of these by catalytic hydrogenation, followed by protection of the resulting amino residue as a *N*-Boc residue, afforded **38a**, **38e**, **38h** and **38j**, respectively. Sharpless asymmetric epoxydation of **44a** using diethyl L-tartrate afforded the corresponding enantiomer **45d**, which was converted to **38d** following the same procedure as described for the preparation of **38a** from **45a**.

The synthesis method for (1*S*,2*R*) β-hydroxy-*N*-Boc-α-amino acid **38c** is outlined in Scheme 4.[12] Cyclocarbamation of **45a** with benzyl isocyanate in the presence of sodium hydride afforded **47c**. Jones oxidation of **47c** afforded the corresponding carboxylic

acids **48c**. Esterification of **48c** afforded **49c**, epimerization followed by alkaline hydrolysis of which with ethanolic potassium hydroxide afforded the corresponding diastereomer **50c**. The ring opening reaction of the cyclic carbamate **50c** under aqueous alkaline condition, followed by N-debenzylation and then N-protection of the resulting amino residue as a *N*-Boc residue, provided **38c**. (1*R*,2*S*) β-hydroxy-*N*-Boc-α-amino acid **38b** was prepared from the corresponding enantiomer **45d** following the same procedure as described for the preparation of **38c** from **45a**.

The other optically active β-hydroxy-*N*-Boc-α-amino acids **38f**, **38g**, **38i**, **38k** and **38l** were prepared by the method, that is, appropriate for conversion of the 2′ site, as described in Scheme 5.[13] Stereoselective aldol condensation of the chiral Shiff base of ethyl glycinate, which was prepared from the chiral hydroxypinanone and ethyl glycinate, with appropriate aliphatic aldehydes afforded optically active aldols **51f**, **51g**, **51i**, **51k** and **51l**, respectively. Acidic hydrolysis of these afforded **52f**, **52g**, **52i**, **52k** and **52l**, respectively. Protection of the resulting primary amino residues as *N*-Boc residues afforded **38f**, **38g**, **38i**, **38k** and **38l**, respectively as their optically active forms.

3. Results and discussion

The compounds listed in Tables 1–4 were evaluated for their inhibitory activities against calcium mobilization of human CCR5 over-expressed CHO cells (hCCR5/CHO), stimulated by MIP-1α (Ca assay),[7a] and for their inhibitory activity against the cell–cell fusion reaction between target cells expressing CD4/CCR5 and effector cells expressing the envelope protein of HIV-1 (fusion assay).[14] To estimate their anti-HIV activities as exactly as possible, the representative compounds were evaluated for their inhibitory activities of virus entry mediated by membrane fusion in the presence of 10% FCS in addition to their receptor antagonist activity.[15]

In our previous paper, we reported the discovery of spirodiketopiperazines **1** and **2** as structurally novel CCR5 antagonists.[7] Both showed potent CCR5 antagonist activity, while their bioavailability was low. After incubation of **1** with human liver microsomes, the corresponding 1′-hydroxylated analog was identified as a biologically active metabolite.[8] Because the proposed structure of the metabolite had two chiral centers, we synthesized all four possible stereoisomers in their optically pure forms from the corresponding β-hydroxylated leucine to evaluate each of their biological activities. Compound **3**, having a (3*R*,1′*R*)-configuration, exhibited approximately 4.5–12-fold more potency than the other stereoisomers in the Ca assay. Based on the unexpected increase in antagonist activity of compound **3**, we used this information to optimize compound **2**, which showed more potent antagonistic activity than compound **1**. As a result, it was found that the (3*R*,1′*R*)-hydroxy compound **4** exhibits strong in vitro activities. Although, these hydroxylated analogs did not improve bioavailability in rodents, they showed more favorable pharmaceutical properties, such as removal of CYP 3A4 inhibition at 30 μM (e.g., **1**: 9.3 μM of IC_{50}) and improved aqueous solubility (pH 6.8) relative to the corresponding non-hydroxylated analog (e.g., **1**: less than 0.2 μg/ml; **3**: 2.7 μg/ml). Further optimization of **4** was continued to identify a clinical candidate that possesses further improved pharmacokinetic (PK) profiles and more potent anti-HIV activity. Optimization by the chemical modification of each of the diversity sites, such as the terminal phenoxy moiety, the cyclohexyl moiety and the 1-*N*-*n*-butyl moiety, was carried out. Results are summarized in Tables 1–4.

These introduction of a hydrophilic substituent into the *p*-position of the terminal phenoxy moiety of the non-hydroxylated analog **4** led us to the discovery of **5** (Fig. 1), which had a significant improvement in oral availability in rodents, as described in our previous paper.[9] This information was applied to further optimize

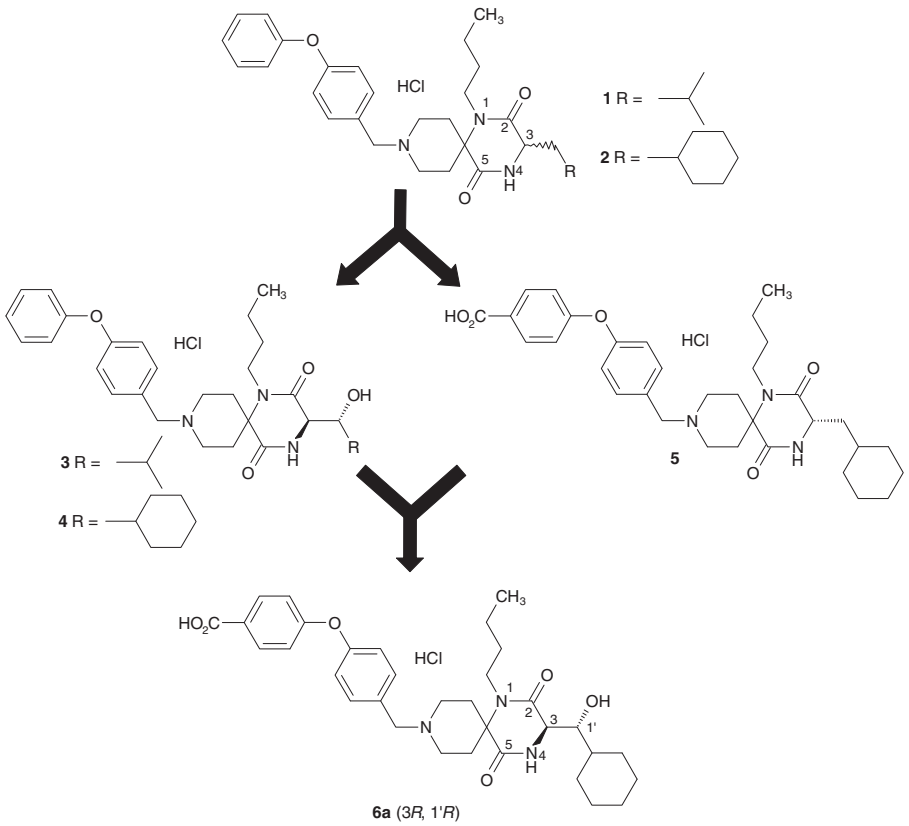
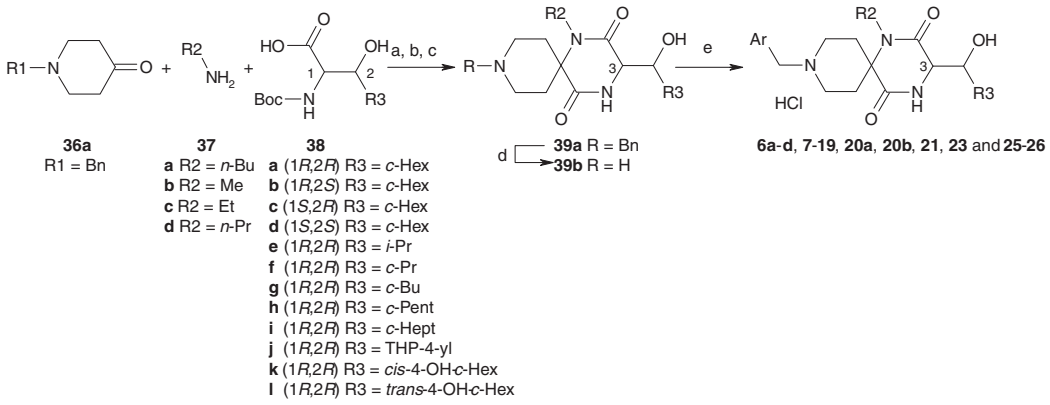
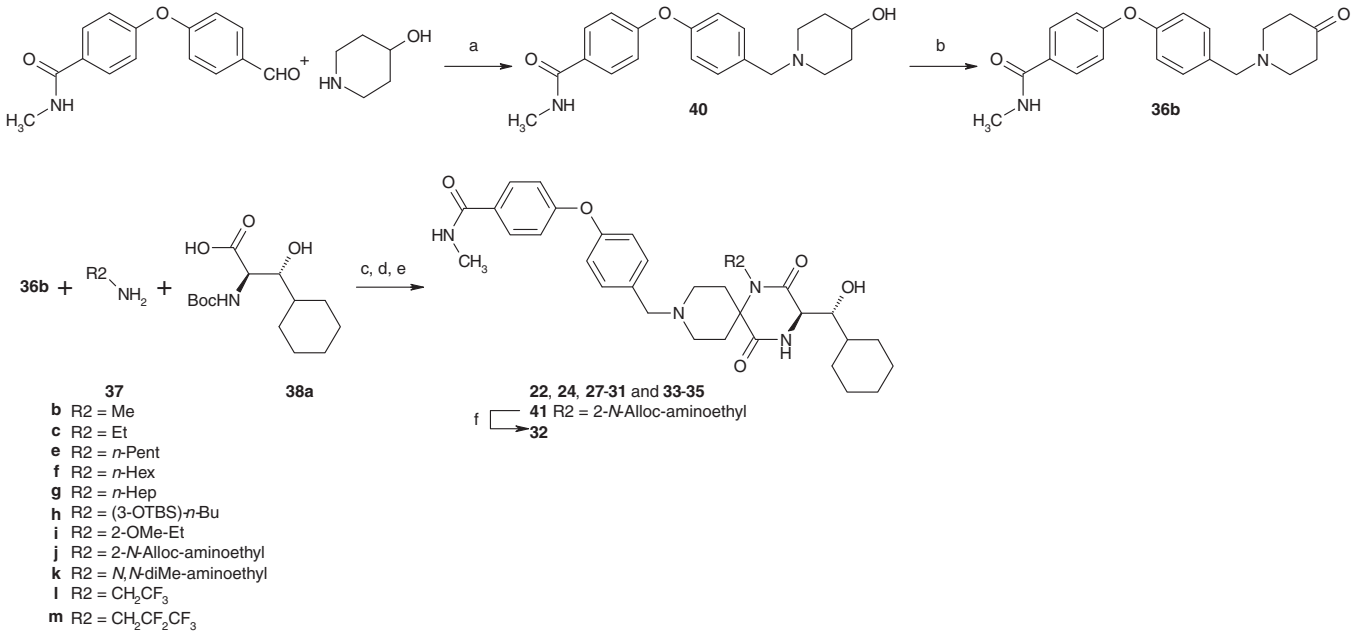


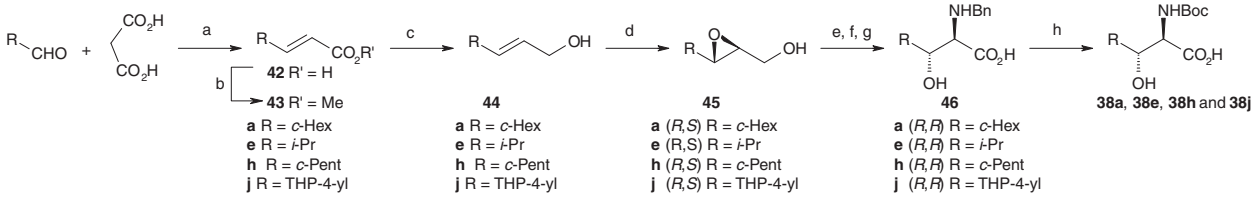
Figure 1. Molecular design of orally available CCR5 antagonists.



Scheme 1. Synthesis of spirodiketopiperazines **6a**–**d**, **7**–**19**, **20a**, **20b**, **21**, **23** and **25**–**26**. Reagents and conditions: (a) 2-morpholinoethyl isocyanide, MeOH, 55 °C; (b) concd HCl, 55 °C; (c) AcOH/toluene, 80 °C; (d) H_2, $Pd(OH)_2$/C, EtOH, 50 °C, then 4 N HCl/AcOEt; (e) Ar-CHO, $NaBH(OAc)_3$, AcOH, DMF and then 4 N HCl/AcOEt.



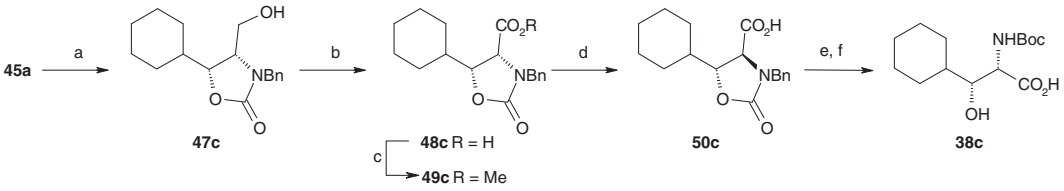
Scheme 2. Synthesis of spirodiketopiperazines **22**, **24** and **27**–**35**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DMF; (b) SO_3·pyr, Et_3N, DMSO (c) 2-morpholinoethyl isocyanide, MeOH, 55 °C; (d) concd HCl, 55 °C; (e) AcOH/toluene, 80 °C; (f) $Pd(PPh_3)_4$, n-Bu_3SnH, AcOH, CH_2Cl_2, then 4 N HCl/AcOEt.



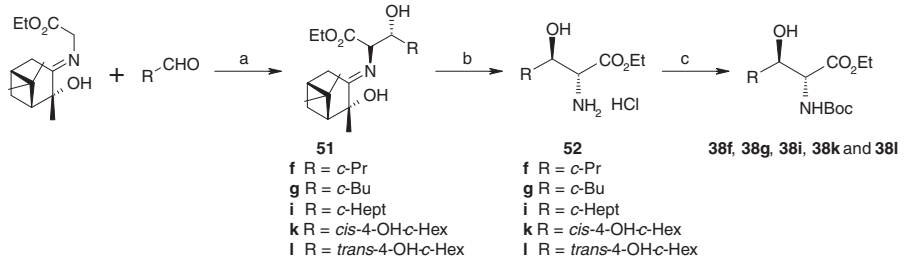
Scheme 3. Synthetic method of optically active β-hydroxy-*N*-Boc-α-amino acids **38a**, **38e**, **38h** and **38j**. Reagents: (a) piperidine, pyridine; (b) concd H_2SO_4, MeOH; (c) DIBAL, THF; (d) (−)-diethyl-D-tartrate, $Ti(Oi\text{-}Pr)_4$, cumene hydroperoxide, MS4A, CH_2Cl_2; (e) SO_3·pyridine, Et_3N, DMSO; (f) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, MeCN, H_2O; (g) $BnNH_2$, 5.0 M NaOH, H_2O; (h) H_2, $Pd(OH)_2$/C, MeOH, then Boc_2O, 1.0 M NaOH.

4, which showed more potent CCR5 antagonist activity than the corresponding non-hydroxylated analog **2**.

Thus, a further optimization process was initiated with introduction of a *p*-substituent into the terminal phenoxy moiety of **4**. Results are summarized in Table 1. Based on the results reported in a previous paper,[9] methoxy, hydroxyl, aminocarbonyl, *N*-methyl aminocarbonyl, aminosulfonyl, methanesulfonyl, methanesulfonylamino and hydroxycarbonyl groups, which were expected to increase the hydrophilicity of the designed molecules relative to **4** based on their *C* log *P* listed values, were introduced as a *p*-substituent to afford **7**–**13** and **6a**, respectively. As expected, all the compounds listed above were found not to exhibit substantial differences in potency in their Ca and fusion assays relative to the *p*-unsubstituted analog **4**. These results showed that relatively polar substituents at this site are acceptable for CCR5. Decreasing hydrophobicity by introducing a polar functional group was thought to favor improvement of some



Scheme 4. Synthetic method of optically active β-hydroxy-*N*-Boc-α-amino acids **38c**. Reagents: (a) benzyl isocyanate, NaH, THF; (b) Jones reagent, acetone; (c) TMS-diazomethane, diethyl ether; (d) KOH, EtOH; (e) 2.0 M KOH; (f) H_2, $Pd(OH)_2$/C then Boc_2O.



Scheme 5. Synthesis of optically active β-hydroxy-*N*-Boc-α-amino acids **38f**, **38g**, **38i**, **38k** and **38l** using hydroxypinanone as the chiral auxiliary. Reagents and conditions: (a) $ClTi(OEt)_3$, Et_3N, CH_2Cl_2; (b) 1.2 M HCl, THF; (c) 1.0 M LiOH, EtOH then Boc_2O.

pharmaceutical properties. Several compounds, **4**, **8**, **9**, **13** and **6a**, were evaluated for their in vitro metabolic stability in rat liver microsomes (RLM) and human liver microsomes (HLM). Compound **6a** exhibited the best in vitro metabolic stability in HLM among those tested, while compound **13** showed the best stability in RLM. Only compound **6a** showed improved in vitro metabolic stability in both of the tested species relative to **4**. On the basis of the results described above, an acidic and hydrophilic *p*-substituent of lower *C* log *P* value, such as carboxylic acid and methansulfonylamino functions, were identified as having a tendency to improve metabolic stability in rat and/or human liver microsomes.

Further optimization of the lipophilic 1′-cyclohexyl moiety of **6a** was also carried out. Results are summarized in Table 2. Replacement of the cyclohexyl moiety of **6a** with isopropyl, cyclopropyl, cyclobutyl, cyclopentyl, cycloheptyl, tetrahydropyran-4-yl, *cis*-4-hydroxycyclohexyl and *trans*-4-hydroxycyclohexyl moieties afforded **14**–**20**, respectively. Isopropyl analog **14** showed an equipotent activity with **6a** in the Ca assay but less potent activity in the fusion assay. Cyclopropyl analog **15** showed 2.4-fold less potent activity in the Ca assay and much less potent activity in the fusion assay. Cyclobutyl analog **16** and cyclopentyl analog **17** showed nearly equipotent activity in the Ca assay and less potent activity in the fusion assay. Cycloheptyl analog **18** showed equipotent activity in both of the assays.

Tetrahydropyan-4-yl analog **19** showed less potent activity in both of the assays because of its presumed increase in hydrophilicity (*C* log *P* = 1.52) relative to **6a**. It was interesting that *trans*-4-hydroxycyclohexyl analog **20b**, which showed nearly equipotent activity with **16**–**18**, exhibited more potent antagonist activity than the corresponding *cis*-isomer **20a**, while both of them did not show much difference in their fusion assays. These compounds were also investigated for their metabolic stability in rat and human liver microsomes. Compounds **14**–**17**, **20a** and **20b**, possessing relatively more hydrophilic *C* log *P* values (1.8–3.4), tended to show much better in vitro metabolic stabilities in both the RLM and HLM than compounds **6a** and **18**, which possessed relatively more hydrophobic substituents on this site. These conflicting results implied the difficulties faced in improving metabolic stability by introducing polar functional group into this residue.

The effect of the 1-*N*-alkyl substituent of the diketopiperazine moiety of **6a** and the corresponding *N*-methyl carboxyamide analogs **10** (Table 1) on the activity profiles was also investigated. Results are summarized in Table 3. Based on the results obtained in our previous paper,[9] straight alkyl chains were predicted to be more favorable than branched ones as a 1-*N*-alkyl substituent. As such, 1-*N*-methyl analog **21**, 1-*N*-ethyl analog **23** and 1-*N*-*n*-propyl analog **25** were synthesized and evaluated as less hydrophilic analogs. Their corresponding *N*-methyl carboxyamide analogs **22**, **24** and **26** were also synthesized and evaluated. Both series of analogs exhibited nearly equipotent antagonist activities relative to **6a**. However, the fusion activity reduced with reduction of the 1-*N*-alkyl chain to *n*-propyl (**25** and **26**), ethyl (**23** and **24**) and methyl (**21** and **22**).

Further chemical modification of the 1-*N*-alkyl residue was carried out with respect to the carboxyamide analogs for synthetic reasons because amide analogs do not need protection and deprotection processes and were predicted to show equipotent in vitro activities to the corresponding carboxylic acid analogs. 1-*N*-*n*-pentyl analog **27**, 1-*N*-*n*-hexyl analog **28** and 1-*N*-*n*-heptyl analog **29** were synthesized and evaluated. Compound **27** showed equipotent activity with **6a** and **10** in the fusion assay, while it showed a slight reduction of the antagonist activity. Compounds **28** and **29** showed less potent activity relative to **10** in both of the assays. As such, 1-*N*-*n*-butyl (C4) and 1-*N*-*n*-pentyl (C5) were identified as the most optimal straight alkyl chains.

Synthesis and evaluation of analogs **30**–**35** possessing a heteroatom-containing 1-*N*-alkyl substituent was also expected to be an interesting chemical modification. 1-*N*-(3-Hydroxybutyl) analog **30** showed less potent activities relative to **10** in both of the assays. 1-*N*-(2-Methoxyethyl) analog **31** retained the potent antagonist activity of **10** but showed much lower potency in the fusion assay. Both the analogs **32** and **33** possessing a nitrogen-containing 1-*N*-alkyl substituent demonstrated more than a 10-fold less potent antagonist activity relative to **10**. These results strongly suggest that introduction of a hydrophilic function into the 1-*N*-alkyl chain is not favorable for the targeted activities. Replacement of the 1-*N*-*n*-butyl moiety with 2,2,2-trifluoroethyl and 2,2,3,3,3-pentafluoropropyl afforded **34** and **35**. 2,2,2-Trifluoroethyl analog **34**

showed a retained antagonist activity but much less potency in the fusion assay. 2,2,3,3,3-Pentafluoropropyl analog **35** showed sevenfold less potent antagonist activity relative to **10**. As such, the fluorine-containing alkyl chains described above were not favored as a 1-*N*-alkyl substituent because of the presumed inherent physical properties of fluorine atoms, which are distinct from hydrogen atoms.

Based on the data described in Tables 1–3, compound **6a** was found to show the most balanced activity profiles and PK profiles in the in vitro evaluations. To investigate their SAR, all possible stereoisomers **6a–d** were synthesized and evaluated by the calcium assay and the fusion assay.[16] Results are summarized in Table 4. Isomers **6a** (3*R*,1′*R*), **6b** (3*R*,1′*S*) and **6d** (3*S*,1′*S*) showed nearly equipotent antagonist activity, while **6c** (3*S*,1′*R*) showed nearly 10-fold less potent antagonist activity relative to the others. Regarding the 1′*R*-isomers **6a** and **6c**, the 3*R*-isomer **6a** showed more potent activity than the corresponding 3*S*-isomer **6c**. Regarding the 1′*S*-isomers, both the 3*R*-isomer **6b** and 3*S*-isomer **6d** exhibited nearly equipotent activity. In the fusion assay, **6a** showed nearly two orders of magnitude more potency (nearly 100-fold) than the other three stereoisomers **6b–d**, as shown in Table 4. Lack of correlation observed between the calcium assay and the fusion assay was considered to be due to the difference in the assay conditions. The calcium assay was conducted using the natural ligand (MIP-1α) and CCR5 overexpressed CHO cell, while the fusion assay was conducted using effector cells expressing the envelope protein of HIV-1 and target cells expressing CD4/CCR5.

As shown in Table 5, compounds **6a**, **10**, **13**, **14** and **26**, which had relatively better AUC in their preliminary tests, such as cassette dosing (data not shown), were again evaluated for their pharmacokinetics after single-dose oral and intravenous administration in rats. For comparison, PK values of the chemical leads **1**, **2** and **5** are also listed. Among the compounds tested, **6a**, **10**, **13**, **14** and **26**, and benzoic acid analog **6a** showed the best oral exposure (AUC = 3422 ng h/mL) and maximum plasma concentration (C_{max} = 2360 ng/mL). The remarkable improvement of the AUC value of **6a** relative to chemical lead **1** after oral dosing was estimated to be due to a marked reduction in clearance (CL) and tissue distribution (V_{ss}) after intravenous dosing. The *N*-methyl caboxyamide analog **10** also showed better AUC and C_{max} relative to **1**, while the sulfonamide analog **13** showed poor PK values. One of the main factors behind the significantly improved AUC of **10** relative to **1** and **13** was again considered to be the significantly improved CL and V_{ss} values. Compound **14**, possessing a carboxylic acid residue at the terminal phenoxy moiety and 1′-isopropyl residue, showed unexpectedly lower AUC and C_{max} values relative to **6a**. Also, compound **26**, possessing an *N*-methyl carboxyamide residue and 1-*N*-*n*-propyl residue, showed relatively poor AUC and C_{max} values compared with **10**. As a result, the carboxylic acid analog **6a** and the corresponding *N*-methyl carboxyamide analog **10** were identified as orally available CCR5 antagonists.

Table 6
Anti-HIV activity of **1**, **5**, **6a**and **14**

Compound	Anti-HIV-1 activity[a]		
	HIV-1Ba-L (R5)		HIV-1MM (R5MDR)
	IC_{50} ± SD(nM)	IC_{90} (nM) ± SD	IC_{50} (nM) ± SD
1	160 ± 40	ND[c]	ND[c]
5	39 ± 38	171 ± 146	ND[c]
6a	0.4 ± 0.3	12 ± 10	0.6 ± 0.2
14	59 ± 39	591 ± 211	ND[d]
TAK-779	28 ± 32	256 ± 169	14 ± 8.0
SCH-351125	4.0 ± 2.0	79 ± 52	3.0 ± 0.5
Zidovudine[b]	7.0 ± 4.0	48 ± 21	250 ± 98
Nelfinavir[c]	12 ± 8.0	105 ± 48	>1000

[a] IC_{50} and IC_{90} values are based on the inhibition of HIV p24 antigen expression in PBMC (mean ± SD). Assays were performed in triplicate.
[b] Zidovudine is a reverse transcriptase inhibitor.
[c] Nerfinavir is a HIV-1 protease inhibitor.
[d] ND: Not determined.

Non-hydroxylated phenoxy analog **1**, non-hydroxylated benzoic acid analog **5**, hydroxylated benzoic acid analogs **6a** and **14**were evaluated for their anti-HIV activity using CCR5-tropic (R5) HIV-1 strains and multidrug-resistant HIV-1 strains. Results are shown in Table 6.[15] For overall estimation of the therapeutic potential of these novel CCR5 antagonists, other CCR5 antagonists TAK-779[17] and SCH-351125,[18] a clinically used reverse transcriptase inhibitor Zidovudine and HIV-1 protease inhibitor Nelfinavir were also evaluated for their in vitro anti-HIV activities. Analogs **6a** showed potent anti-HIV activity, as shown by a significant decrease in virus p24 antigen production relative to **1**, **5** and **14**. These results indicate that the hydroxylated cyclohexylmethyl residue at the 3-position contributed strongly to anti-HIV-1 activity rather than the corresponding non-hydroxylated side chains. Moreover, compound **6a** potently inhibited not only the replication of laboratory and primary HIV-1 R5 strains but also that of various multidrug-resistant monocyte/macrophage tropic (R5) HIV-1 strains.[6b]

The potent anti-HIV-1 activity and good PK profiles of compound **6a** led us to further evaluate it for the inhibition of human MIP-1α binding to CCR5 and chemotaxis toward MIP-1α. According to the data summarized in Table 7, compound **6a** was expected to have a potential as an immunosuppressant in addition to acting as a therapeutic agent towards AIDS. Inactivity of **6a** against hCXCR4/hSDF-1 was considered to support the hypothesis that it shows potent anti-HIV-1 activity selectively through CCR5-mediated antagonist activity. Compound **6a** showed no significant activity against a broad panel of receptors and transporters including the other chemokines.

Compound **6a** was also evaluated for its species differences and found to show selective inhibitory activity to humans over the hamster, guinea pig, rat and rabbit, as shown in Table 8.

Table 5
Pharmacokinetic evaluations of **1**, **6a**, **10**, **13**, **14** and **26** in rats

Compound	10 mg/kg, po				3 mg/kg, iv			
	C_{max} (ng/mL)	$T_{1/2}$ (min)	AUC (ng·h/mL)	BA (%)	AUC (ng h/mL)	$T_{1/2}$ (min)	CL (mL/min/kg)	V_{ss} (mL/kg)
1	33.3[a]	75.7	96.7[a]	1.3	372	13	137	2349
2	5.6[a]	103	24.8[a]	1.9	400	19.9	113	2542
5	2400	48.4	10,532	34	3091	11.1	16	145
6a (3*R*,1′*R*)	2360	120	3422	23	4402	21.6	11.3	132
10	333[a]	73.2	1296[a]	10	3782	27.6	12.8	492
13	50[a]	205	242[a]	5	1545	137	25	3826
14	137	270	150	5.9	2307	13.2	21.2	317
26	133[a]	120	513[a]	11.2	1373	43.2	35.9	1725

[a] PK values after oral dosing are normalized to 10 mg/kg.

Table 7
Activity profiles of **6a**

Compound	hCCR5/hMIP-1α IC_{50} (nM)			hCXCR4/hSDF-1 IC_{50} (nM)
	Ca assay	Binding	Chemotaxis	Ca assay
6a	34	5.8	5.8	>30,000

Table 8
Species difference of **6a**

Compound	CCR5/hRANTES Ca assay IC_{50} (nM)				
	Human	Hamster	Guinea pig	Rat	Rabbit
6a	46	>30,000	>30,000	>30,000	>30,000

Table 9
Pharmacokinetics data of **6a** after po administration

	Rat	Dog	Monkey
po dose (mg/kg)	10	3	10
C_{max} (ng/mL)	2360	135	136
$T_{1/2}$ (min)	120	270	1464
AUC (ng·h/mL)	3422	189	777
iv dose (mg/kg)	1	1	1
V_{ss} (mL/kg)	295	1586	45,045
CL (ml/min/mL)	10	33	15
BA (%)	23	12	4.6

PK profiles of compound **6a** in the three species rat, dog and monkey were evaluated. Results are summarized in Table 9. Bioavailability (BA) of **6a** in rat, dog and monkey was 23%, 12% and 4.6%, respectively. It is well known that their blood concentration has to be maintained higher than the IC_{90} level for anti-HIV drugs to show systemic efficacy. Compound **6a**, having highly potent and long-acting anti-HIV activity, was found to maintain a higher blood concentration than its IC_{90} values for 6 h after its oral administration in all of the species tested and is thus expected to have therapeutic potential in clinical use. Furthermore, **6a** did not show any significant safety problem in AMES test, 14-day rat safety study and dog cardiac study up to the dosage of 100 mg/kg, po.

4. Conclusions

In summary, further optimization of the structurally novel spirodiketopiperazine-based CCR5 antagonist **2** resulted in the discovery of an orally available drug candidate **6a**, that is, expected to have therapeutic potential for the treatment of AIDS. Rational molecular design was performed, based on the information reported by our group.[7–9] As briefly described in Figure 1, the optimization process starting from the chemical lead **2** essentially consisted of three steps: introduction of 1′*R*-OH, which resulted in **4**; introduction of *p*-CO_2H into the terminal phenoxy moiety, which resulted in **5**; and then introduction of both the functions 1′*R*-OH and *p*-CO_2H, which resulted in **6a**. As described in Tables 1–4, *p*-CO_2H, 1′-cyclohexyl, 1*N*-*n*-butyl and stereochemistry of 1′-OH were confirmed to be optimal at each of their sites from the viewpoint of biological and PK profiles. Various in vitro evaluations for its anti-HIV activity and PK profiles, as summarized in Tables 6–9, strongly suggest that **6a** has promise as a clinically effective drug candidate for the treatment of AIDS.

5. Experimental

5.1. Chemistry

5.1.1. General methods

Analytical samples were homogeneous as confirmed by thin layer chromatography (TLC), and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian Gemini-200 or a MERCURY-300 spectrometer with tetramethylsilane as an internal standard. The chemical shift values δ are reported in ppm and coupling constants (J) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionization (EI) mass spectra were obtained with a JEOL JMS-700 spectrometer. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were obtained on Perseptive Voyager Elete. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by Hitachi M-1200H spectrometer. IR spectra were measured on a JASCO FTIR-430 spectrometer. Elemental analyzes were performed with a Perkin–Elmer PE2400 series II CHNS/O Analyzer and were only indicated as the elements within ±0.4% of the theoretical values unless otherwise noted. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm), Fuji Silysia BW235 or Fuji Silysia FL60D]. TLC was performed on silica gel (Merck TLC, silica gel 60 F_{254}).

5.1.2. Typical procedures for the solution phase Ugi four-component condensation and reductive alkylation of 9-*N*-substituent

5.1.2.1. (3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (4). To a stirred solution of 1-benzyl-4-piperidone (75 g, 261 mmol), *n*-butylamine (258 ml, 261 mmol) and (2*R*,3*R*)-2-(*t*-butoxycarbonylamino)-3-cyclohexyl-3-hydroxypropanoic acid (49.4 g, 261 mmol) in methanol (1.0 L) was added 2-morpholinoethyl isocyanide (36 mL, 261 mmol). After being stirred at 55 °C overnight, the reaction mixture was treated with concentrated hydrochloric acid (261 L) with cooling. After being stirred at 55 °C for another 2 h, the reaction mixture was evaporated, treated with water, sodium bicarbonate and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated to give deprotected Ugi product as a yellow oil, a solution of which dissolved in acetic acid/toluene (1.25 M/1.1 L) was stirred at 80 °C for 1 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed twice with a small amount of water. The organic layer was washed with aqueous sodium hydrogen carbonate, brine, dried over sodium sulfate and evaporated to afford *N*-benzylpiperidinodiketopiperazine as an oil (103 g). Debenzylation of the resulting oily product was carried out by the catalytic hydrogenation at an atmospheric pressure in ethanol (1.0 L) in the presence of 20% $Pd(OH)_2$/C (20 g) for 3 h at 50 °C. Catalyst was removed by filtration through a pad of Celite. The filtrate was treated with 4 N hydrogen chloride/ethyl acetate (130 mL) and evaporated. The resulting powder was washed with *t*-butyl methyl ether to afford the title compound as a white powder (83 g, 82% yield in four steps). To a stirred solution of the resulting residue (100 mg, 0.258 mmol), 4-phenoxybenzaldehyde (61 mg, 0.309 mmol) in 1% acetic acid/*N*,*N*-dimethylformamide (2 mL) was added sodium triacetoxyborohydride (71 mg, 0.335 mmol). After being stirred overnight, the reaction mixture was evaporated. The resulting residue was purified by column chromatography on silica gel and treated with 4 N hydrogen chloride/ethyl acetate (2 mL) to give the title compound in 46% yield. $[\alpha]_D^{29}$ +2.51° (*c* 0.97, MeOH) TLC R_f 0.46 (AcOEt/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.7 Hz, 2H), 7.42–7.37 (m, 2H), 7.18 (m, 1H), 7.07–7.01 (m, 4H), 4.31 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 3.97 (m, 1H), 3.71 (m, 1H), 3.60–3.05 (m, 5H), 2.55–1.90 (m, 6H), 1.90–1.60 (m, 5H), 1.60–1.10 (m, 6H), 1.10–0.90 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3365, 3190, 2924, 2852, 2362, 1660, 1590, 1509, 1489, 1469, 1423, 1243, 1172, 1115 cm^{-1}; MS (APCI, Pos 20v) *m/z* 534 $(M+H)^+$, 422; HRMS Calcd 534.3332, Obsd 534.3332.

Compounds **7**–**13**, **6a**–**d**, **14**–**19**, **20a**, **20b**, **21**, **23** and **25**–**26** were synthesized according to the same procedures as described above for the preparation of **4** using the corresponding *N*-alkylamine for *n*-butyl amine, *N*-Boc-amino acid for (2*R*,3*R*)-2-(*t*-butoxycarbonylamino)-3-cyclohexyl-3-hydroxypropanoic acid and benzaldehyde for 4-phenoxybenzaldehyde.

5.1.2.2. (3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-9-[4-(4-methoxyphenoxy)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (7). TLC R_f 0.50 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.49 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.7 Hz, 2H), 7.02–6.92 (m, 4H), 4.30 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 3.97 (m, 1H), 3.79 (s, 3H), 3.72 (m, 1H), 3.58–3.38 (m, 3H), 3.30–3.13 (m, 2H), 2.55–2.40 (m, 2H), 2.32 (m, 1H), 2.16–1.86 (m, 3H), 1.81–1.60 (m, 5H), 1.50–1.10 (m, 6H), 1.03–0.80 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); MS (APCI, Pos 20v) *m/z* 564 $(M+H)^+$; HRMS Calcd 564.3437, Obsd 564.3441.

5.1.2.3. (3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-9-[4-(4-hydroxyphenoxy)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (8). TLC R_f 0.35 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 9.0 Hz, 2H), 4.30 (s, 2H), 4.16 (d, J = 1.8 Hz, 1H), 3.96 (m, 1H), 3.72 (m, 1H), 3.58–3.36 (m, 3H), 3.30–3.06 (m, 2H), 2.52–1.86 (m, 6H), 1.82–1.58 (m, 5H), 1.50–1.10 (m, 6H), 1.04–0.90 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); MS (APCI, Pos 20v) *m/z* 550 $(M+H)^+$; HRMS Calcd 550.3281, Obsd 550.3277.

5.1.2.4. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzamide hydrochloride (9). TLC R_f 0.25 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.90 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 4.02 (m, 1H), 3.76 (m, 1H), 3.56–3.42 (m, 3H), 3.33–2.99 (m, 2H), 2.54–1.88 (m, 6H), 1.81–1.60 (m, 5H), 1.48–1.12 (m, 6H), 1.04–0.81 (m, 5H); MS (APCI, Pos 20v) *m/z* 577 $(M+H)^+$, 465; HRMS Calcd 577.3390, Obsd 577.3391.

5.1.2.5. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (10). TLC R_f 0.45 (AcOEt/MeOH, 5/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.0 Hz, 1H), 4.00 (m, 1H), 3.76 (m, 1H), 3.56–3.45 (m, 3H), 3.30–3.16 (m, 2H), 2.91 (s, 3H), 2.51–2.28 (m, 3H), 2.16–1.92 (m, 3H), 1.76–1.69 (m, 5H), 1.39–1.15 (m, 6H), 1.00–0.86 (m, 5H); MS (APCI, Pos 20v) *m/z* 591 $(M+H)^+$; HRMS Calcd 591.3546, Obsd 591.3536.

5.1.2.6. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzenesulfonamide hydrochloride (11). TLC R_f 0.28 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.89 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 4.01 (m, 1H), 3.75 (m, 1H), 3.58–3.42 (m, 3H), 3.32–3.14 (m, 2H), 2.55–2.40 (m, 2H), 2.32 (m, 1H), 2.13 (m, 1H), 2.07–1.89 (m, 2H), 1.82–1.60 (m, 5H), 1.50–1.12 (m, 6H), 1.06–0.80 (m, 5H); MS (APCI, Pos 20v) *m/z* 613 $(M+H)^+$; HRMS Calcd 613.3060, Obsd 613.3065.

5.1.2.7. (3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-9-{4-[4-(methylsulfonyl)phenoxy]benzyl}-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (12). TLC R_f 0.46 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.95 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.25–7.16 (m, 4H), 4.38 (s, 2H), 4.15 (d, J = 2.4 Hz, 1H), 4.02 (m, 1H), 3.76 (m, 1H), 3.60–3.44 (m, 3H), 3.30–3.10 (m, 2H), 3.11 (s, 3H), 2.54–2.26 (m, 3H), 2.18–1.88 (m, 3H), 1.82–1.62 (m, 5H), 1.50–1.10 (m, 6H), 1.02–0.82 (m, 5H); MS (APCI, Pos 20v) *m/z* 612 $(M+H)^+$; HRMS Calcd 612.3107, Obsd 612.3104.

5.1.2.8. N-{4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclohexyl (hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]phenyl}methanesulfonamide hydrochloride (13). TLC R_f 0.41 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.53 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 4.33 (s, 2H), 4.15 (d, J = 1.8 Hz, 1H), 3.98 (m, 1H), 3.73 (m, 1H), 3.58–3.40 (m, 3H), 3.32–3.03 (m, 2H), 2.95 (s, 3H), 2.52–2.24 (m, 3H), 2.17–1.88 (m, 3H), 1.80–1.62 (m, 5H), 1.48–1.08 (m, 6H), 1.03–0.82 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); MS (APCI, Pos 20v) *m/z* 627 $(M+H)^+$; HRMS Calcd 627.3216, Obsd 627.3212.

5.1.2.9. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (6a). $[\alpha]_D^{26}$ +2.3° (*c* 1.03, MeOH); TLC R_f 0.58 (AcOEt/AcOH/H_2O, 10/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 9.0 Hz, 2H), 7.19 (d, J = 9.0 Hz, 2H), 7.08 (d, J = 9.0 Hz, 2H), 4.38 (s, 2H), 4.17 (d, J = 2.1 Hz, 1H), 4.02 (m, 1H), 3.78 (m, 1H), 3.60–3.40 (m, 3H), 3.30–3.10 (m, 2H), 2.56–1.86 (m, 6H), 1.82–1.60 (m, 5H), 1.52–1.16 (m, 6H), 1.06–0.82 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H); IR (KBr) 3365, 3226, 2926, 2850, 2507, 1710, 1682, 1635, 1599, 1503, 1467, 1427, 1378, 1312, 1237, 1163, 1113, 1098 cm^{-1}; MS (FAB, Pos, glycerin + m-NBA) 578 $(M+H)^+$, 227; Elemental Analysis Calcd for $C_{33}H_{43}N_3O_6$·HCl: C, 64.54; H, 7.22; N, 6.84. Found: C, 64.23; H, 7.26; N, 6.90.

5.1.2.10. 4-[4-({(3*R*)-1-Butyl-3-[(*S*)-cyclohexyl(hydrox y)methy l]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (6b). $[\alpha]_D^{26}$ +27.7° (*c* 0.46, MeOH); TLC R_f 0.38 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.37 (s, 2H), 4.11–4.04 (m, 2H), 3.74–3.45 (m, 5H), 3.22 (m, 1H), 2.54–1.98 (m, 5H), 1.76–1.66 (m, 5H), 1.47–1.18 (m, 7H), 1.01–0.91 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3404, 2927, 1671, 1599, 1503, 1423, 1244, 1162 cm^{-1}; MS (APCI, Pos 20v) *m/z* 578 $(M+H)^+$; HRMS Calcd 578.3230, Obsd 578.3232.

5.1.2.11. 4-[4-({(3*S*)-1-Butyl-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoicacidhydrochloride (6c). $[\alpha]_D^{26}$ −23.9° (*c* 0.44, MeOH); TLC R_f 0.38 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.37 (s, 2H), 4.11–4.04 (m, 2H), 3.74–3.45 (m, 5H), 3.22 (m, 1H), 2.54–1.98 (m, 5H), 1.76–1.66 (m, 5H), 1.47–1.18 (m, 7H), 1.01–0.91 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 34.1.4, 2927, 1672, 1599, 1503, 1423, 1244, 1162 cm^{-1}; MS (APCI, Pos 20v) *m/z* 578 $(M+H)^+$; HRMS Calcd 578.323, Obsd 578.3239.

5.1.2.12. 4-[4-({(3*S*)-1-Butyl-3-[(*S*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (6d). $[\alpha]_D^{24}$ −2.78° (*c* 0.67, MeOH); TLC R_f 0.56 (CH_2Cl_2/MeOH, 5/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.4 (s, 1H), 8.05 (m, 1H), 7.97 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 7.09 (d,

J = 8.7 Hz, 2H), 5.28 (d, J = 6.9 Hz, 1H) 4.35 (s, 2H), 3.97 (m, 1H), 3.88–3.12 (m, 7H), 2.64–2.20 (m, 3H), 2.06–1.42 (m, 8H), 1.40–1.00 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H), 0.80 (m, 2H); MS (MALDI, Pos) m/z 578 $(M+H)^+$; HRMS Calcd 578.3230, Obsd 578.3228.

5.1.2.13. 4-[4-({(3*R*)-1-Butyl-3-[(1*R*)-1-hydroxy-2-methylpropyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (14). $[\alpha]_D^{26}$ +10.08° (c 1.07, MeOH) TLC R_f 0.32 (AcOEt/MeOH, 2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.37 (s, 2H), 4.15 (d, J = 2.4 Hz, 1H), 4.02 (m, 1H), 3.76 (m, 1H), 3.60–3.44 (m, 3H), 3.24–3.08 (m, 2H), 2.56–1.92 (m, 5H), 1.70 (m, 1H), 1.50–1.26 (m, 3H), 1.08–0.90 (m, 9H); IR (KBr) 2962, 1675, 1599, 1504, 1422, 1245, 1162 cm^{-1}; MS (APCI, Pos 20v) m/z 538 $(M+H)^+$; HRMS Calcd 538.2917, Obsd 538.2925.

5.1.2.14. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclopropyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (15). TLC R_f 0.29 (AcOEt/MeOH, 2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H), 7.17 (d, J = 9.0 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.37 (s, 2H), 4.11 (d, J = 2.5 Hz, 1H), 3.92 (m, 1H), 3.80 (m, 1H), 3.54–3.34 (m, 4H), 3.14 (dd, J = 9.0, 2.5 Hz, 1H), 2.61–2.43 (m, 2H), 2.35 (m, 1H), 2.15 (m, 1H), 1.70–1.49 (m, 2H), 1.49–1.33 (m, 2H), 1.09 (m, 1H), 0.96 (t, J = 7.5 Hz, 3H), 0.60–0.43 (m, 2H), 0.33 (m, 1H), 0.24 (m, 1H); IR (KBr) 3219, 2958, 2873, 2582, 1710, 1672, 1598, 1502, 1422, 1385, 1243, 1161, 1113, 1098, 1049 cm^{-1}; MS (APCI, Neg 20v) m/z 534 $(M-H)^-$; HRMS Calcd 536.2761, Obsd 536.2757.

5.1.2.15. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclobutyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (16). TLC R_f 0.68 (BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.5 Hz, 2H), 4.32 (s, 2H), 3.90 (m, 1H), 3.90 (d, J = 2.0 Hz, 1H), 3.73 (m, 1H), 3.68 (dd, J = 9.0, 2.0 Hz, 1H), 3.53–3.40 (m, 3H), 3.26 (m, 1H), 2.67 (m, 1H), 2.51 (m, 1H), 2.43–2.37 (m, 2H), 2.15–1.29 (m, 11H), 0.94 (t, J = 7.0 Hz, 3H); MS (APCI, Neg 20v) m/z 548 $(M-H)^-$; HRMS Calcd 550.2917, Obsd 550.2924.

5.1.2.16. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cyclopentyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (17). TLC R_f 0.45 ($CHCl_3$/MeOH, 5/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 4.38 (s, 2H), 4.02 (m, 1H), 4.01 (d, J = 1.8 Hz, 1H), 3.78 (m, 1H), 3.62–3.08 (m, 5H), 2.60–2.06 (m, 5H), 2.00–1.08 (m, 12H), 0.96 (t, J = 6.9 Hz, 3H); IR (KBr) 3424, 2957, 2871, 2586, 1665, 1599, 1503, 1421, 1371, 1311, 1244, 1162, 1098, 1049 cm^{-1}; MS (FAB, Pos, glycerin + m-NBA) m/z 564 $(M+H)^+$; HRMS Calcd 564.3074, Obsd 564.3074.

5.1.2.17. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-cycloheptyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (18). TLC R_f 0.28 (CH_2Cl_2/MeOH, 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, J = 9.0 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.37 (s, 2H), 4.15 (d, J = 2.4 Hz, 1H), 4.02 (m, 1H), 3.76 (m, 1H), 3.58–3.44 (m, 3H), 3.30–3.07 (m, 2H), 2.51–1.17 (m, 21H), 0.96 (t, J = 7.0 Hz, 3H); IR (KBr) 2930, 1672, 1599, 1502, 1420, 1244, 1161, 1115 cm^{-1}; MS (APCI, Neg 20v) m/z 590 $(M-H)^-$; HRMS Calcd 592.3387, Obsd 592.3383.

5.1.2.18. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-hydroxy(tetrahydro-2*H*-pyran-4-yl)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (19). TLC R_f 0.30 ($CHCl_3$/MeOH, 4/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (d, J = 9.0 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.37 (s, 2H), 4.12 (d, J = 2.0 Hz, 1H), 4.08–3.93 (m, 3H), 3.75 (m, 1H), 3.57–3.34 (m, 5H), 3.30–3.15 (m, 2H), 2.52–1.69 (m, 8H), 1.50–1.18 (m, 5H), 0.96 (t, J = 7.2 Hz, 3H); MS (APCI, Pos 20v) m/z 580 $(M+H)^+$; HRMS Calcd 580.3023, Obsd 580.3016.

5.1.2.19. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-hydroxy(4-hydroxycyclohexyl)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (20a). TLC R_f 0.17 (AcOEt/MeOH, 2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 4.32 (s, 2H), 4.17 (d, J = 1.8 Hz, 1H), 4.00–3.91 (m, 2H), 3.70 (m, 1H), 3.52–3.37 (m, 3H), 3.30–3.17 (m, 2H), 2.50–2.33 (m, 3H), 2.10 (m, 1H), 1.80–1.18 (m, 12H), 0.94 (t, J = 7.0 Hz, 3H); IR (KBr) 2934, 1672, 1598, 1502, 1422, 1243, 1161, 1112 cm^{-1}; MS (APCI, Pos 20v) m/z 594 $(M+H)^+$; HRMS Calcd 594.3179, Obsd 594.3176.

5.1.2.20. 4-[4-({(3*R*)-1-Butyl-3-[(*R*)-hydroxy(4-hydroxycyclohexyl)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (20b). TLC R_f 0.27 (CH_2Cl_2/MeOH, 3/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, J = 8.9 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 4.36 (s, 2H), 4.14 (d, J = 2.1 Hz, 1H), 3.97 (m, 1H), 3.71 (m, 1H), 3.57–3.41 (m, 4H), 3.30–3.24 (m, 2H), 2.58–2.43 (m, 3H), 2.12–1.68 (m, 6H), 1.40–0.93 (m, 8H), 0.95 (t, J = 7.0 Hz, 3H); IR (KBr) 2934, 1676, 1599, 1502, 1423, 1243, 1161, 1098, 1048 cm^{-1}; MS (APCI, Neg 20v) m/z 592 $(M-H)^-$; HRMS Calcd 594.3179, Obsd 594.3185.

5.1.2.21. 4-[4-({(3*R*)-3-[Cyclohexyl(hydroxy)methyl]-1-methyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (21). TLC R_f 0.55 (CH_2Cl_2/MeOH, 4/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.38 (s, 2H), 4.17 (d, J = 1.8 Hz, 1H), 3.99 (m, 1H), 3.79 (m, 1H), 3.50–3.46 (m, 2H), 3.26 (dd, J = 9.6, 1.8 Hz, 1H), 2.95 (s, 3H), 2.60–2.36 (m, 3H), 2.12–1.94 (m, 3H), 1.80–1.64 (m, 4H), 1.38–1.18 (m, 3H), 1.00–0.88 (m, 2H); IR (KBr) 3385, 2926, 2852, 1684, 1598, 1503, 1427, 1244, 1161 cm^{-1}; MS (APCI, Pos 20v) m/z 536 $(M+H)^+$; HRMS Calcd 536.2761, Obsd 536.2762.

5.1.2.22. 4-[4-({(3*R*)-3-[Cyclohexyl(hydroxy)methyl]-1-ethyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (23). TLC R_f 0.66 (CH_2Cl_2/MeOH, 4/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 9.0 Hz, 2H), 7.17 (d, J = 9.0 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.37 (s, 2H), 4.15 (d, J = 2.0 Hz, 1H), 4.00 (m, 1H), 3.76 (m, 1H), 3.63 (m, 1H), 3.49–3.45 (m, 2H), 3.33–3.26 (m, 2H), 2.50–2.38 (m, 3H), 2.17–1.93 (m, 3H), 1.80–1.65 (m, 4H), 1.34–1.17 (m, 6H), 1.00–0.87 (m, 2H); IR (KBr) 3049, 2924, 1703, 1677, 1649, 1599, 1502, 1422, 1248, 1167, 1113 cm^{-1}; MS (APCI, Pos 20v) m/z 550 $(M+H)^+$; HRMS Calcd 550.2917, Obsd 550.2922.

5.1.2.23. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-2,5-dioxo-1-propyl-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]benzoic acid hydrochloride (25). TLC R_f 0.21 ($CHCl_3$/MeOH/AcOH, 20/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (d, J = 9.0 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.37 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 4.01 (m, 1H), 3.75 (m, 1H), 3.55–3.38 (m, 3H), 3.30–3.09 (m, 2H), 2.55–2.26 (m, 3H),

2.18–1.88 (m, 3H), 1.83–1.60 (m, 5H), 1.57–1.10 (m, 4H), 1.04–0.80 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H); MS (APCI, Pos 20v) m/z 564 $(M+H)^+$; HRMS Calcd 564.3074, Obsd 564.3073.

5.1.2.24. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-2,5-dioxo-1-propyl-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (26). TLC R_f 0.38 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 9.0 Hz, 2H), 7.60 (d, J = 9.0 Hz, 2H), 7.15 (d, J = 9.0 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.1 Hz, 1H), 3.99 (m, 1H), 3.75 (m, 1H), 3.54–3.39 (m, 3H), 3.30–3.10 (m, 2H), 2.91 (s, 3H), 2.56–2.27 (m, 3H), 2.18–1.88 (m, 3H), 1.83–1.60 (m, 5H), 1.46 (m, 1H), 1.37–1.11 (m, 3H), 1.04–0.80 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H); MS (APCI, Pos 20v) m/z 577 $(M+H)^+$; HRMS Calcd 577.339, Obsd 577.3392.

5.1.3. 4-[4-[(4-Hydroxy-1-piperidinyl)methyl]phenoxy]benzamide (40)

To a stirred suspension of 4-(4-*N*-methylaminocarbonyl phenoxy)benzaldehyde (9.23 g, 36.2 mmol) in 1% acetic acid/*N*,*N*-dimethylformamide (30 ml) was added sodium triacetoxyborohydride (11.5 g, 54.3 mmol). After being stirred overnight, the reaction mixture was poured into water and treated with 5.0 M sodium hydroxide (pH 9–10). The aqueous layer was extracted with chloroform and the organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated to yield the title compound (9.93 g, 81%) as a white powder. TLC R_f 0.10 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.10–6.90 (m, 4H), 6.11 (m, 1H), 3.72 (m, 1H), 3.49 (s, 2H), 3.00 (d, J = 5.1 Hz, 2H), 2.82–2.65 (m, 2H), 2.22–2.06 (m, 2H), 1.96–1.80 (m, 2H), 1.78–1.50 (m, 2H); MS (APCI, Pos) 341 $(M+H)^+$.

5.1.4. 4-[4-[(4-Oxo-1-piperidinyl)methyl]phenoxy]benzamide (36b)

To a stirred solution of **40** (12.2 g, 35.8 mmol) in dichloromethane (100 ml), triethylamine (27 ml) and dimethylsulfoxide (36 mL) was added sulfur trioxide–pyridine complex (11.4 g, 71.6 mmol). After being stirred at room temperature overnight, the reaction mixture was quenched with ice water (500 mL), treated with 5.0 M sodium hydroxide solution (30 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated. The resulting residue was triturated with *t*-butyl methyl ether and dried to yield the title compound (11.0 g, 91%) as a pale yellow solid. TLC R_f 0.45 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.04–6.98 (m, 4H), 6.06 (m, 1H), 3.62 (m, 1H), 3.62 (s, 2H), 3.01 (d, J = 4.8 Hz, 3H), 2.77 (t, J = 6.3 Hz, 4H), 2.47 (t, J = 6.3 Hz, 4H); MS (APCI, Pos) 339 $(M+H)^+$.

5.1.4.1. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-ethyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (24). To a stirred solution of 4-[4-[(4-oxo-1-piperidinyl)methyl]phenoxy]benzamide (100 mg, 0.30 mmol), 2.0 M ethylamine/methanol (0.18 mL, 0.36 mmol) and (2*R*,3*R*)-2-(*t*-butoxycarbonylamino)-3-cyclohexyl-3-hydroxypropanoic acid (103 mg, 0.36 mmol) in methanol (2 mL) was added 2-morpholinoethyl isocyanide (50 μL, 0.36 mmol). After being stirred at 55 °C overnight, the reaction mixture was treated with concentrated hydrochloric acid (0.5 mL) under cooling. The reaction mixture was stirred at 55 °C for another 2 h, evaporated, treated with aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated to give deprotected Ugi product as a yellow oil, a solution of which in acetic acid/toluene (1.25 M/2 mL) was stirred at 80 °C for 1 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed twice with a small amount of water. The organic layer was washed with aqueous sodium hydrogen carbonate, brine, dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel column chromatography, treated with 4.0 N hydrogen chloride/ethyl acetate and evaporated to afford the title compound (130 mg, 40%) as a pale yellow powder. TLC R_f 0.37 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.0 Hz, 1H), 3.99 (m, 1H), 3.78–3.59 (m, 2H), 3.48–3.44 (m, 2H), 3.36 (m, 1H), 3.28 (m, 1H), 2.91 (s, 3H), 2.54–2.36 (m, 3H), 2.15–1.93 (m, 3H), 1.80–1.65 (m, 4H), 1.34–1.15 (m, 6H), 1.00–0.86 (m, 2H); IR (KBr) 3395, 2926, 2851, 1638, 1498, 1421, 1308, 1245, 1174, 1046 cm^{-1}; MS (APCI, Pos 20v) m/z 563 $(M+H)^+$; HRMS Calcd 563.3233, Obsd 563.3231.

Compounds **22** and **27**–**35** were synthesized according to the same procedures as described above for the preparation of **24** using the corresponding amines for ethylamine.

5.1.4.2. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-methyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (22). TLC R_f 0.32 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.37 (s, 2H), 4.17 (d, J = 2.0 Hz, 1H), 3.98 (m, 1H), 3.78 (m, 1H), 3.49–3.46 (m, 2H), 3.25 (dd, J = 9.6, 2.0 Hz, 1H), 2.95 (s, 3H), 2.91 (s, 3H), 2.57–2.35 (m, 3H), 2.12–1.95 (m, 3H), 1.80–1.65 (m, 4H), 1.34–1.15 (m, 3H), 1.00–0.87 (m, 2H); IR (KBr) 3323, 2925, 1642, 1500, 1248, 1175 cm^{-1}; MS (APCI, Pos 20v) m/z 549 $(M+H)^+$; HRMS Calcd 549.3077, Obsd 549.3075.

5.1.4.3. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-2,5-dioxo-1-pentyl-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (27). TLC R_f 0.52 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 2.4 Hz, 1H), 4.00 (m, 1H), 3.75 (m, 1H), 3.59–3.41 (m, 3H), 3.30–3.12 (m, 2H), 2.91 (s, 3H), 2.56–2.24 (m, 3H), 2.18–1.88 (m, 3H), 1.84–1.60 (m, 5H), 1.54–1.06 (m, 8H), 1.04–0.80 (m, 2H), 0.92 (t, J = 6.6 Hz, 3H); IR (KBr) 3393, 2929, 1643, 1498, 1245 cm^{-1}; MS (APCI, Pos 20v) m/z 605 $(M+H)^+$; HRMS Calcd 605.3703, Obsd 605.3705.

5.1.4.4. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-hexyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (28). TLC R_f 0.53 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.15 (d, J = 1.8 Hz, 1H), 3.99 (m, 1H), 3.75 (m, 1H), 3.59–3.42 (m, 3H), 3.33–3.10 (m, 2H), 2.91 (s, 3H), 2.55–2.24 (m, 3H), 2.18–1.87 (m, 3H), 1.83–1.62 (m, 5H), 1.50–1.10 (m, 10H), 1.05–0.80 (m, 5H); IR (KBr) 3368, 2928, 1644, 1498, 1420, 1245 cm^{-1}; MS (APCI, Pos 20 v) m/z 619 $(M+H)^+$; HRMS Calcd 619.3859, Obsd 619.3868.

5.1.4.5. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-heptyl-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide hydrochloride (29). TLC R_f 0.53 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.16 (d, J = 2.1 Hz, 1H), 3.99 (m, 1H), 3.75 (m, 1H), 3.59–3.42 (m, 3H), 3.33–3.05 (m, 2H), 2.91 (s, 3H), 2.60–2.26 (m, 3H), 2.18–1.88 (m, 3H), 1.82–1.60 (m, 5H), 1.52–1.08 (m, 12H), 1.05–0.80 (m, 5H); IR (KBr) 3380, 2927, 1643,

1498, 1420, 1245 cm^{-1}; MS (APCI, Pos 20v) *m/z* 633 $(M+H)^+$; HRMS Calcd 633.4016, Obsd 633.4021.

5.1.4.6. 4-(4-{[(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-(3-hydroxybutyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)-*N*-methylbenzamide hydrochloride (30). TLC R_f 0.24 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.16 (d, J = 3.6, 2.0 Hz, 1H), 4.02 (m, 1H), 3.88–3.74 (m, 2H), 3.60 (m, 1H), 3.50–3.43 (m, 2H), 3.38 (m, 1H), 3.27 (dd, J = 9.6, 2.0 Hz, 1H), 2.91 (s, 3H), 2.59–2.33 (m, 3H), 2.15–1.93 (m, 4H), 1.80–1.65 (m, 5H), 1.35–1.15 (m, 6H), 1.01–0.87 (m, 2H); IR (KBr) 3387, 2922, 2852, 1642, 1499, 1482. 1427, 1306, 1245, 1176 cm^{-1}; MS (APCI, Pos 20v) *m/z* 607 $(M+H)^+$; HRMS Calcd 607.3496, Obsd 607.3494.

5.1.4.7. 4-(4-{[(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-(2-methoxyethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)-*N*-methylbenzamidehydrochloride (31). TLC R_f 0.35 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.36 (s, 2H), 4.18 (d, J = 2.1 Hz, 1H), 3.98 (m, 1H), 3.86–3.18 (m, 8H), 3.31 (s, 3H), 2.91 (s, 3H), 2.60–1.58 (m, 10H), 1.42–0.80 (m, 5H); IR (KBr) 3324, 2927, 2852, 2564, 1652, 1499, 1418, 1306, 1245, 1176, 1113, 1048 cm^{-1}; MS (APCI, Pos 20v) *m/z* 593 $(M+H)^+$; HRMS Calcd 593.3339, Obsd 593.3346.

5.1.4.8. Allyl 2-[(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-9-(4-{4-[(methylamino)carbonyl]phenoxy}benzyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-1-yl]ethylcarbamatehydrochloride (41). TLC R_f 0.44 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 5.91 (m, 1H), 5.28 (m, 1H), 5.15 (m, 1H), 4.52 (d, J = 5.1 Hz, 2H), 4.36 (s, 2H), 4.17 (d, J = 1.8 Hz, 1H), 3.99 (m, 1H), 3.82–3.53 (m, 2H), 3.52–3.17 (m, 6H), 2.91 (s, 3H), 2.70–2.25 (m, 3H), 2.18–1.87 (m, 3H), 1.85–1.60 (m, 4H), 1.41–1.10 (m, 3H), 1.04–0.80 (m, 2H); IR (KBr) 3433, 1698, 1637, 1255 cm^{-1}; MS (APCI, Pos 20v) *m/z* 662 $(M+H)^+$, 256.

5.1.4.9. 4-[4-({(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-1-[2-(dimethylamino)ethyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methyl)phenoxy]-*N*-methylbenzamide dihydrochloride (33). TLC R_f 0.08 ($CHCl_3$/MeOH, 5/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 9.0 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.36 (s, 2H), 4.23 (d, J = 1.5 Hz, 1H), 4.01–3.90 (m, 3H), 3.72 (m, 1H), 3.50–3.44 (m, 2H), 3.33–3.24 (m, 3H), 3.00 (s, 3H), 2.96 (s, 3H), 2.91 (s, 3H), 2.59–2.43 (m, 3H), 2.17–1.94 (m, 3H), 1.80–1.65 (m, 4H), 1.35–1.15 (m, 3H), 1.03–0.82 (m, 2H); IR (KBr) 3413, 2927, 2852, 2701, 1669, 1498, 1420, 1327, 1244, 1174, 1120, 1044 cm^{-1}; MS (APCI, Pos 20v) *m/z* 606 $(M+H)^+$; HRMS Calcd 606.3655, Obsd 606.3654.

5.1.4.10. 4-(4-{[(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-2,5-dioxo-1-(2,2,2-trifluoroethyl)-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)-*N*-methylbenzamide hydrochloride (34). TLC R_f 0.51 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.43 (m, 1H), 4.36 (s, 2H), 4.30 (d, J = 2.1 Hz, 1H), 4.18–3.92 (m, 2H), 3.74 (m, 1H), 3.56–3.42 (m, 2H), 3.35 (dd, J = 9.6, 2.1 Hz, 1H), 2.91 (s, 3H), 2.58 (m, 1H), 2.43–2.11 (m, 3H), 2.02 (m, 1H), 1.89 (m, 1H), 1.84–1.60 (m, 4H), 1.40–1.06 (m, 3H), 1.05–0.80 (m, 2H); IR (KBr) 3383, 2930, 1678, 1498, 1246, 1155, cm^{-1}; MS (APCI, Pos 20v) *m/z* 617 $(M+H)^+$, 505, 256; HRMS Calcd 617.2951, Obsd 617.2957.

5.1.4.11. 4-(4-{[(3*R*)-3-[(*R*)-Cyclohexyl(hydroxy)methyl]-2,5-dioxo-1-(2,2,3,3,3-pentafluoropropyl)-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)-*N*-methylbenzamide hydrochloride (35). TLC R_f 0.69 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.49 (m, 1H), 4.36 (s, 2H), 4.31 (d, J = 1.8 Hz, 1H), 4.10–4.00 (m, 2H), 3.72 (m, 1H), 3.52–3.48 (m, 2H), 3.35 (m, 1H), 2.91 (s, 3H), 2.63 (m, 1H), 2.44–2.20 (m, 3H), 2.03–1.60 (m, 6H), 1.34–1.14 (m, 3H), 1.01–0.85 (m, 2H); IR (KBr) 3371, 2933, 1680, 1600, 1498, 1407, 1246, 1206, 1102, 1032 cm^{-1}; MS (APCI, Pos 20v) *m/z* 667 $(M+H)^+$; HRMS Calcd 667.2919, Obsd 667.2916.

5.1.5. 4-[4-({(3*R*)-1-(2-Aminoethyl)-3-[(*R*)-cyclohexyl(hydroxy)methyl]-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl}methy l)phenoxy]-*N*-methylbenzamide dihydrochloride (32)

To a stirred suspension of **41** (534 mg, 0.81 mmol) in dichloromethane (8.0 mL) was added acetic acid (0.11 mL, 1.94 mmol), tri-*n*-butyltin hydride (0.26 mL, 0.97 mmol) and tetrakis(triphenylphosphine)palladium(0) (47 mg, 0.04 mmol). After being stirred for 3 h at ambient temperature, the reaction mixture was quenched with saturated sodium hydrogen carbonate, extracted with chloroform/methanol, dried over anhydrous sodium sulfate and evaporated to yield the title compound (178 mg, 38%). TLC R_f 0.33 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.12 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 4.35 (s, 2H), 4.23 (d, J = 2.1 Hz, 1H), 4.04–3.65 (m, 4H), 3.51–3.40 (m, 2H), 3.26 (dd, J = 9.9, 2.1 Hz, 1H), 3.15–3.06 (m, 2H), 2.91 (s, 3H), 2.65–2.44 (m, 3H), 2.16 (m, 1H), 2.08–1.91 (m, 2H), 1.83–1.62 (m, 4H), 1.40–1.12 (m, 3H), 1.05–0.80 (m, 2H); IR (KBr) 3416, 2929, 1670, 1497, 1246, cm^{-1}; MS (APCI, Pos 20v) *m/z* 578 $(M+H)^+$, 466, 256.

5.1.6. Typical procedures for optically active β-hydroxy-*N*-Boc-α-amino acid using Sharpless asymmetric epoxidation

5.1.6.1. 3-Cyclohexyl-2-propenoic acid (42a). To a stirred solution of cyclohexanecarboxyaldehyde (400 mL, 3.30 mmol) in pyridine (750 mL) were added malonic acid (290 g, 2.78 mol) and piperidine (7.5 mL, 75 mmol) at room temperature. After being stirred at ambient temperature for 1 h, the reaction mixture was heated at 80 °C for 5 h, cooled to room temperature, poured into ice-cooled 3.0 M aqueous hydrochloric acid (1.2 L) and extracted with ethyl acetate. The organic layer was extracted with 2.0 M sodium hydroxide. The aqueous layer was washed with ethyl acetate, acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and evaporated to yield the title compound (442 g, 100%) as a pale yellow solid. TLC R_f 0.78 ($CHCl_3$/MeOH/AcOH, 20/2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.03 (dd, J = 15.6, 6.6 Hz, 1H), 5.77 (dd, J = 15.6, 1.6 Hz, 1H), 2.30–2.04 (m, 1H), 1.87–1.59 (m, 5H), 1.46–1.02 (m, 5H).

5.1.6.2. Methyl 3-cyclohexyl-2-propenoate (43a). To a stirred solution of cyclohexyl-2-propenoic acid **42a** (442 g, 3.30 mol) in MeOH (1.5 L) was slowly added concentrated sulfuric acid (15 ml) at room temperature. After being stirred under reflux condition for 14 h, the solvent was removed by evaporation. The resulting residue was slowly added to ice water (500 mL) and neutralized with sodium hydrogen carbonate (50 g). The aqueous mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and concentrated in vacuo to yield the title compound (449 g, 96%). TLC R_f 0.83 ($CHCl_3$/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.92 (dd, J = 15.6, 6.6 Hz, 1H), 5.77 (dd, J = 15.6, 1.5 Hz, 1H), 3.72 (s, 3H), 2.15 (m, 1H), 1.80–1.61 (m, 4H), 1.19–1.03 (m, 6H).

5.1.6.3. 3-Cyclohexyl-2-propen-1-ol (44a). To a stirred solution of methyl-3-cyclohexyl-2-propenoate **43a** (80.0 g, 476 mmol) in dry tetrahydrofuran (1.0 L) was slowly added diisobutyl aluminum hydride/toluene (1.0 M, 1.0 L, 1.0 mol) at −70 °C to −60 °C over 2 h under argon atmosphere. The reaction mixture was warmed up to −10 °C–0 °C, quenched with water (5 mL) and then aqueous saturated sodium sulfate (~400 mL). The resulting precipitates were removed by filtration. The filtrate was evaporated, and the resulting residue was purified by distillation (bp 125–128 °C/26 mmHg) to give the title compound (53 g, 88%) as a colorless oil. TLC R_f 0.67 (hexane/AcOEt, 2/1); H NMR (300 MHz, $CDCl_3$) δ 5.70–5.53 (m, 2H), 4.08 (d, J = 4.8 Hz, 2H), 1.96 (m, 1H), 1.80–1.58 (m, 4H), 1.37–1.00 (m, 6H).

5.1.6.4. [(2*R*,3*R*)-3-Cyclohexyloxiran-2-yl]methanol(45a). To a stirred suspension of dried MS4A (75 g) in dichloromethane (1.3 L) was added titanium(IV) isopropoxide (42 ml, 142 mmol) at room temperature under argon atmosphere, a solution of diethyl D-**tartrate** (35.3 g, 171 mmol) in dichloromethane at −30 °C to −20 °C and stirring was continued for 30 min. To the mixture were added a solution of **44a** (100 g, 714 mmol) in dichloromethane (100 mL) at −30 °C to −20 °C and a solution of *t*-butyl hydroxy peroxide in octane (178 mL, 1.07 mol) at −30 °C to −20 °C and the mixture was stirred for 3 h. After the excess peroxide was quenched with the addition of dimethyl sulfide (157 mL, 2.1 mol) at −30 °C to −20 °C and stirring was continued for 1.5 h the reaction mixture was further treated with 2.0 L of water and (±)-tartaric acid (300 g, 2.0 mol) and stirring was continued overnight. The reaction mixture was extracted with ethyl acetate. The organic layer was evaporated. The resulting residue was dissolved in *t*-butyl methyl ether (1.0 L) and the excess diethyl D-tartrate was hydrolyzed by stirring with 1.0 M sodium hydroxide water solution for 1 h. The reaction mixture was diluted with water (800 mL) and extracted with *t*-butyl methyl ether. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/AcOEt, 10/1–1/1) to yield the title compound (100 g, 90%). $[\alpha]_D^{26}$ +32.7° (*c* 1.67, $CHCl_3$); TLC R_f 0.43 (hexane/AcOEt, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (m, 1H), 3.62 (m, 1H), 2.98 (m, 1H), 2.76 (dd, J = 6.9, 2.4 Hz, 1H), 1.90–1.50 (m, 6H), 1.36–1.01 (m, 5H).

5.1.6.5. (2*R*,3*R*)-2-Benzylamino-3-cyclohexyl-3-hydroxypropanoic acid (46a). To a stirred solution of [(2*R*,3*R*)-3-cyclohexyloxiran-2-yl]methanol **45a** (184 g, 1.18 mol) in dimethyl sulfoxide (828 ml) were added triethylamine (644 mL, 4.62 mol) and sulfur trioxide–pyridine complex (368 g, 2.32 mol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was quenched with ice water (3.4 L) and extracted with *t*-butyl methyl ether. The organic layer was washed with ice-cooled 0.5 M aqueous hydrochloric acid and brine, dried over anhydrous magnesium sulfate and evaporated. To the resulting aldehyde in acetonitrile (3.6 L) were added water (1.8 L), 2-methyl-2-butene (550 ml, 5.2 mol) and mono sodium hydrogen phosphate (184 g, 1.53 mol) at room temperature. To the reaction mixture was slowly added sodium hypochlorite (80%, 440 g, 3.9 mol) keeping the temperature at 20 °C–25 °C. After being stirred at room temperature for 30 min, the reaction mixture was diluted with *t*-butyl methyl ether and treated with 1.0 M sodium hydroxide (1.7 L) at room temperature. The precipitates were removed by filtration. The organic layer of the filtrate was extracted with 1.0 M sodium hydroxide. The aqueous layer was acidified with 6.0 M hydrochloric acid (pH 3.0), extracted with *t*-butyl methyl ether, washed with brine, dried over anhydrous magnesium sulfate and evaporated to yield [(2*R*,3*R*)-3-cyclohexyloxiran-2-yl]carboxylic acid. To a stirred solution of [(2*R*,3*R*)-3-cyclohexyloxiran-2-yl]carboxylic acid in water (198 mL) were slowly added benzylamine (269 mL, 2.47 mol) followed by 5.0 M sodium hydroxide (148 mL, 740 mmol) at 0 °C. After being stirred under reflux for 2.5 h, the reaction mixture was treated with 5.0 M sodium hydroxide (16 mL, 82 mmol) at room temperature. The aqueous layer was washed with *t*-butyl methyl ether, and acidified with 2.0 M hydrochloric acid (pH 4–5). The precipitates were collected by filtration and washed with ice water, ice-cooled acetone, *t*-butyl methyl ether and dried to yield **46a** (227 g, 35% in three steps) as a white powder. TLC R_f 0.71 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.48–7.22 (m, 5H), 3.87 (d, J = 13.2 Hz, 1H), 3.69 (d, J = 13.2 Hz, 1H), 3.05 (d, J = 6.0 Hz, 1H), 1.72–1.50 (m, 6H), 1.24–0.80 (m, 5H); MS (FAB, Pos) 278 $(M+H)^+$

5.1.6.6. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-cyclohexyl-3-hydroxypropanoic acid (38a). To a suspension of $Pd(OH)_2$/C (30 g, 20% wt, wet) in methanol (3.0 L) were added (2*R*,3*R*)-2-benzylamino-3-cyclohexyl-3-hydroxypropanoic acid **46a** (150 g, 542 mmol) and 1.0 M sodium hydroxide (540 mL) at 0 °C. After being stirred under hydrogen atmosphere for 8 h, the reaction mixture was treated with 1.0 M sodium hydroxide (80 mL) and then di-*t*-butyl dicarbonate (160 mL, 504 mmol) under argon atmosphere. After being stirred overnight, palladium was removed by filtration through a pad of Celite and washed with methanol and water. Methanol in the filtrate was removed by evaporation and the resulting aqueous solution was washed with *t*-butyl methyl ether. The aqueous layer was acidified with 5% potassium bisulfate (3.0 L) to pH 3.0, extracted with ethyl acetate, dried over anhydrous magnesium sulfate and evaporated to yield **38a** (151.2 g, 97%). $[\alpha]_D^{25}$ = −16.1° (*c* 0.98, MeOH); TLC R_f 0.54 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 4.28 (d, J = 6.0 Hz, 1H), 3.47 (t, J = 6.0 Hz, 1H), 1.95–0.95 (m, 11H), 1.44 (s, 9H).

Compounds **38d**, **38e**, **38h** and **38j** were prepared according the same procedures as described for the preparation of **38a**.

5.1.6.7. (2*S*,3*S*)-2-(*t*-Butoxycarbonylamino)-3-cyclohexyl-3-h y droxypropanoic acid (38d). TLC R_f 0.85 ($CHCl_3$/MeOH/AcOH, 20/2/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.78 (d, J = 8.7 Hz, 1H), 3.02–3.97 (m, 1H), 3.39–3.32 (m, 1H), 1.80–1.40 (m, 6H), 1.36 (s, 9H), 1.30–0.90 (m, 5H).

5.1.6.8. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-cyclopentyl-3-hydroxypropanoic acid (38h). $[\alpha]_D^{23.5}$ = −24.2° (CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 4.20 (d, J = 3.9 Hz, 1H), 3.54 (dd, J = 8.4, 3.9 Hz, 1H), 2.16 (m, 1H), 1.90–1.76 (m, 2H), 1.71–1.51 (m, 4H), 1.44 (s, 9H), 1.40–1.26 (m, 2H).

5.1.6.9. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-(4-tetrahydropyranyl)-3-hydroxypropanoic (38j). $[\alpha]_D^{26}$ = −18.1° (MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 4.26 (d, J = 5.1 Hz, 1H), 4.00–3.91 (m, 2H), 3.50 (dd, J = 7.2, 5.1 Hz, 1H), 3.45–3.32 (m, 2H), 1.94 –1.63 (m, 3H), 1.54–1.28 (m, 11H).

5.1.7. General methods for the preparation of optically active *syn*-β-hydroxy-*N*-Boc-α-amino acid

5.1.7.1. (4*S*,5*S*)-3-Benzyl-5-cyclohexyl-4-(hydroxymethyl)oxazolidine-2-one (47c). To a stirred suspension of sodium hydride (4.56 g, 114 mmol) in tetrahydrofuran (250 mL) was added dropwise a solution of epoxy alcohol **45d** (8.1 g, 51.8 mmol) in tetrahydrofuran (17 mL) for 15 min at 0 °C. After being stirred for 10 min, the reaction mixture was treated with benzyl isocyanate (9.6 mL, 77.8 mmol) for 20 min. After being stirred for 20 min at 0 °C, the reaction mixture was refluxed for 1.5 h, cooled with ice bath, quenched with saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The resulting residue

was purified by silica gel column chromatography to yield the title compound (4.42 g, 28%) as a white powder. TLC R_f 0.33 (hexane/AcOEt = 1/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.42–7.27 (m, 5H), 4.86 (d, J = 15.0 Hz, 1H), 4.26 (d, J = 15.0 Hz, 1H), 4.08 (dd, J = 10.2, 6.9 Hz, 1H), 3.88–3.68 (m, 2H), 3.50 (dt, J = 6.9, 3.3 Hz, 1H), 2.10 (m, 1H), 1.90–1.49 (m, 5H), 1.38–0.85 (m, 5H).

5.1.7.2. (4*S*,5*S*)-3-Benzyl-5-cyclohexyl-2-oxo-oxazolidine-4-carboxylic acid (48c). To a stirred suspension of (4*S*,5*S*)-3-benzyl-5-cyclohexyl-4-(hydroxymethyl)oxazolidin-2-one (4.22 g, 14.6 mmol) in acetone (200 mL) was added dropwise Jones reagent (2.5 M, 12 mL, 29.2 mmol) at 0 °C. After being stirred for 3 h at ambient temperature, the reaction mixture was quenched with *i*-propanol. The resulting precipitates were removed by filtration and washed with chloroform, and the filtrate was evaporated. The resulting residue was diluted with chloroform, washed with brine and extracted with chloroform. The organic layer was washed with small amount of brine, dried over anhydrous sodium sulfate, and evaporated to yield the title compound quantitatively. TLC R_f 0.48 ($CHCl_3$/MeOH = 10/1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.40–7.24 (m, 5H), 4.80 (d, J = 15.0 Hz, 1H), 4.31 (dd, J = 9.0, 7.5 Hz, 1H), 4.09 (d, J = 7.5 Hz, 1H), 3.95 (d, J = 15.0 Hz, 1H), 1.98 (m, 1H), 1.86–1.52 (m, 5H), 1.36–0.90 (m, 5H).

5.1.7.3. Methyl(4*S*,5*S*)-3-benzyl-5-cyclohexyl-2-oxo-oxazolidine-4-carboxylate (49c). To a stirred suspension of **48c** (4.70 g, 14.6 mmol) in diethyl ether (250 mL) was added 2.0 M trimethylsilyl diazomethane in *n*-hexane (11 mL, 22 mmol). After being stirred for 2 h, the reaction mixture was treated with 2.0 M trimethylsilyl diazomethane in *n*-hexane (4 mL, 8 mmol), stirred overnight, quenched with acetic acid and evaporated. The resulting residue was purified by silica gel column chromatography to yield the title compound (2.87 g, 62%). TLC R_f 0.41 (hexane/AcOEt = 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.21 (m, 5H), 4.86 (d, J = 15.0 Hz, 1H), 4.18 (dd, J = 9.6, 7.5 Hz, 1H), 4.06 (d, J = 7.5 Hz, 1H), 3.93 (d, J = 15.0 Hz, 1H), 3.73 (s, 3H), 2.02 (m, 1H), 1.82–1.54 (m, 4H), 1.46 (m, 1H), 1.31–0.85 (m, 5H).

5.1.7.4. (4*R*,5*S*)-3-Benzyl-5-cyclohexyl-2-oxo-oxazolidine-4-carboxylic acid (50c). To a stirred suspension of **49c** (2.87 g, 9.04 mmol) in dry ethanol (30 mL) was added potassium hydroxide (2.54 g, 45.2 mmol). The reaction mixture was refluxed for 45 min. After cooling to the room temperature, the reaction mixture was evaporated and the resulting residue was acidified with 1.0 M hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated to yield the title compound (2.70 g, 98%). TLC R_f 0.60 ($CHCl_3$/MeOH/AcOH = 20/2;1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.40–7.23 (m, 5H), 4.83 (d, J = 15.0 Hz, 1H), 4.30 (dd, J = 5.4, 4.8 Hz, 1H), 4.15 (d, J = 15.0 Hz, 1H), 3.82 (d, J = 4.8 Hz, 1H), 1.76–1.43 (m, 6H), 1.30–0.85 (m, 5H).

5.1.7.5. (2*R*,3*S*)-2-(*t*-Butoxycarbonylamino)-3-cyclohexyl-3-hydroxy-propanoic acid (38c). A mixture of compound **50c** (2.70 g, 8.90 mmol) and 2.0 M aqueous potassium hydroxide (89 mL, 178 mmol) was refluxed under stirring for 7 h. The reaction mixture was neutralized by 6.0 M hydrochloric acid at 0 °C and stirred for 30 min. The resulting precipitates were collected by filtration and washed with cold water, cold acetone and then diethyl ether. To a stirred suspension of the resulting solid in methanol (89 mL) was added 1.0 M sodium hydroxide (8.9 mL) and 20% $Pd(OH)_2$/C (400 mg). After being stirred for 2.5 h under hydrogen atmosphere, the reaction mixture was treated with di-*t*-butyl dicarbonate (3.1 mL, 13.4 mmol) at argon atmosphere. After being stirred overnight, catalyst was removed by filtration through a pad of Celite and washed with methanol and water. The organic solvent was removed by evaporation and the resulting residue was washed with hexane/ethyl acetate (4/1). The aqueous layer was acidified with 5% potassium bisulfate (pH 3.0) and extracted with ethyl acetate, dried over anhydrous magnesium sulfate and evaporated to yield the title compound (2.15 g, 84%). TLC R_f 0.52 ($CHCl_3$/MeOH/AcOH = 20/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 4.32 (d, J = 2.4 Hz, 1H), 3.71 (dd, J = 9.3, 2.4 Hz, 1H), 2.03 (m, 1H), 1.84–1.60 (m, 4H), 1.54–1.15 (m, 4H), 1.45 (s, 9H), 1.08–0.88 (m, 2H).

5.1.8. Typical procedures for *trans*-β-hydroxy-*N*-Boc-amino acid using hydroxypinanone as a chiral auxiliary

5.1.8.1. Iminoester of hydroxypinanone (51f). To a stirred solution of the iminoglycinate which was prepared by the dehydrative condensation of ethyl glycinate and hydroxyl pinanone, in dichloromethane (6 mL) were added stepwise a solution of titanium chloride triethoxide in dichloromethane (2.0 M, 2.0 mL, 4.0 mmol), cyclopropanecarboxyaldehyde (300 mg, 4.28 mmol) and triethylamine (1.1 mL, 7.89 mmol) at 0 °C. After being stirred for 5 h at 0 °C, the reaction mixture was poured into cold brine. The aqueous layer was extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and evaporated. The resulting residue was purified by silica gel column chromatography to yield the title compound (1.3 g, quant) as a white solid. TLC R_f 0.40 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.63 (d, J = 6.5 Hz, 1H), 4.47 (s, 1H),

5.1.8.2. (2*R*,3*R*)-2-Amino-3-cyclohexyl-3-hydroxypropanoic acid (52f). To a stirred solution of iminoester **51f** (616 mg, 1.90 mmol) in tetrahydrofuran (6.5 mL) was added 1.2 M aqueous hydrochloric acid (13 mL, 15.6 mmol). After being stirred overnight, the reaction mixture was evaporated and the resulting residue was purified by silica gel column chromatography to yield the title compound **52f** (373 mg, 94%) as a colorless viscous oil. TLC R_f 0.52 ($CHCl_3$/MeOH, 5/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (br s, 3H), 5.62 (br s, 1H), 4.18 (q, J = 7.0 Hz, 2H), 3.95 (d, J = 3.5 Hz, 1H), 3.19(m, 1H), 1.22 (t, J = 7.0 Hz, 3H), 1.04 (m, 1H), 0.45–0.38 (m, 2H), 0.28 (m, 1H), 0.18 (m, 1H).

5.1.8.3. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-cyclopropyl-3-hydroxypropanoic acid (38f). To a stirred solution of **52f** (449 mg, 2.25 mmol) in ethanol (20 mL) was added 1.0 M lithium hydroxide (5.5 mL, 5.5 mmol). After being stirred for 2 h, the reaction mixture was treated with di-*t*-butyl dicarbonate (0.8 mL, 3.48 mmol) and stirring was continued for 2 h. The reaction mixture was quenched with water. The aqueous layer was washed with *t*-butylmethylether, acidified with 5% potassium hydrogen sulfate to pH 3.0–4.0 and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and evaporated to yield the title compound **38f** (553 mg) quantitatively. TLC R_f 0.73 ($CHCl_3$/MeOH/AcOH, 10/2/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.36 (br s, 1H), 6.72 (d, J = 9.0 Hz, 1H), 4.91 (br s, 1H), 4.00 (dd, J = 9.0, 5.0 Hz, 1H), 3.07 (m, 1H), 1.37 (s, 9H), 1.01 (m, 1H), 0.35–0.30 (m, 2H), 0.23–0.18 (m, 2H).

Compounds **38g**, **38i**, **38k** and **38l** were prepared according to the same procedures as described above for the preparation of **38f**.

5.1.8.4. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-cyclobutyl-3-hydroxy-propanoic acid (38g). TLC R_f 0.53 ($CHCl_3$/MeOH/AcOH, 10/1/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.33 (br, 1H), 6.68 (d, J = 9.0 Hz, 1H), 3.80 (dd, J = 9.0, 5.5 Hz, 1H), 3.56 (dd, J = 8.0, 5.5 Hz, 1H), 1.89–1.62 (m, 7H), 1.36 (s, 9H).

5.1.8.5. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-cycloheptyl-3-hydroxy-propanoic acid (38i). TLC R_f 0.85 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.49 (m, 1H), 4.42 (m, 1H), 3.56 (m, 1H), 1.88–1.22 (m, 21H).

5.1.8.6. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-*syn*-hydroxy-3-(4 -hydroxycyclohexyl)propanoic acid (38k). TLC R_f 0.62 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 4.32 (d, J = 4.5 Hz, 1H), 3.91 (m, 1H), 3.55 (m, 1H), 1.97–1.43 (m, 9H), 1.44 (s, 9H).

5.1.8.7. (2*R*,3*R*)-2-(*t*-Butoxycarbonylamino)-3-*anti*-hydroxy-3-(4-hydroxycyclohexyl)propanoic acid (38l). TLC R_f 0.64 (*n*-BuOH/AcOH/H_2O, 4/2/1); ^{1}H NMR (300 MHz, CD_3OD) δ 4.25 (d, J = 5.4 Hz, 1H), 3.50–3.42 (m, 2H), 1.98–1.92 (m, 4H), 1.84–1.77 (m, 1H), 1.60–1.50 (m, 2H), 1.44 (s, 9H), 1.28–1.12 (m, 2H).

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