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Design, Synthesis, and Biological Evaluation of the Combinatorial Library with a New Spirodiketopiperazine Scaffold. Discovery of Novel Potent and Selective Low-Molecular-Weight CCR5 Antagonists

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Received January 16, 2006

We previously reported the discovery of several spirodiketopiperazine derivatives as potent CCR5 antagonists with anti-HIV activity. Herein, we describe in detail the identification of these lead compounds using a combinatorial chemistry approach. A novel spirodiketopiperazine scaffold was designed on the basis of the concept of the privileged structure of G-protein-coupled receptors (GPCRs). This new framework was obtained in acceptable yield with high purity from the readily prepared isonitrile resin through the Ugi reaction, sequential transformations, and cyclative cleavage. By measuring the inhibitory activity of each compound in the initial library against the intracellular calcium mobilization stimulated by MIP-1α, several compounds were found to show modest but selective CCR5 antagonistic activity. After the rapid evaluation of these hit compounds, several single-digit nanomolar, low-molecular-weight CCR5 antagonists that can potently block the infectivity and replication of laboratory and clinical strains of HIV as well as those of highly drug-resistant HIV variants with minimal cytotoxicity have been identified.

Introduction

Chemokines are a large family of small cytokines that selectively control the adhesion, chemotaxis, and activation of various leukocyte populations and are known to be involved in the initiation and progress of inflammation and allergic diseases.[1] Chemokines, by binding to their receptors on the surface of specific cells, exert a variety of biological activities. Because chemokines are classified into two main groups CC and CXC on the basis of their conserved *N*-terminal cysteine residues, their receptors are also categorized into two groups, CCR and CXCR. Human immunodeficiency virus type 1 (HIV) also binds to chemokine receptors including CCR5 and CXCR4, leading to the infection of its target cells by HIV.[2,3,4,5] Thus, it is thought that interference in the binding of chemokines or HIV to the specific receptor may open a new avenue for the development of novel class of antiinflammatory drugs, anti-allergic drugs, immunosuppressants, and/or antiviral drugs for HIV infection.

In 1991, the first chemokine receptors CXCR1 and CXCR2 were cloned.[6,7] Unlike traditional cytokine and interleukin receptors, these two receptors were found to belong to the superfamily of seven transmembrane (7TM) G-protein-coupled receptors (GPCRs). This information encouraged the pharmaceutical industry to work toward the discovery of chemokine receptor antagonists because drugs that target GPCRs make up greater than 30% of all known marketed medicines.[8] Several pharmaceutical companies and academic institutions have been enthusiastically investigating novel antagonists against chemokine receptors with suitable pharmaceutical properties, and several subtype-selective antagonists are now in clinical trials.[9] Although there are now many approaches to discover GPCR agonists or antagonists, most of the reported leads of chemokine receptor antagonists were identified by high-throughput screening from the company's historical chemical collection.[10] Herein, we describe our approach to identifying subtype-selective antagonists through the synthesis of newly designed combinatorial library targeting chemokine receptors and the rapid evaluation process from hit to lead using iterative focused libraries to identify several single-digit nanomolar, low-molecular-weight, and selective CCR5 antagonists.

Design of the Spirodiketopiperazine Scaffold. During the past decade, many reports have appeared describing combinatorial libraries targeting GPCRs.[11] One promising approach to identify selective small molecule ligands of GPCRs is the use of privileged structures.[12] Among various reported privileged structures, the spiropiperidine structure has been seen in various GPCR ligands, including neurokinin antagonists, growth hormone secretagogues, somatostatin receptor agonists, C5a receptor agonists, melanocortin receptor agonists, and serotonin receptor antagonists.[13]

The 1,4,9-triazaspiro[5.5]undecane-2,5-dione (spirodiketopiperazine) was an attractive scaffold for us because of the following three reasons. (1) Along with spiropiperidine, 2,5-diketopiperazine (DKP) represents an important class of biologically active natural products and synthetic compounds.[14] DKP is a simple heterocyclic scaffold in which diversity can be introduced at up to four positions, and can be prepared from readily available α-amino acids. (2) When we initiated this project, only one compound with this spirodiketopiperazine scaffold, a homodimer of 1,2,5-trimethyl-4-aminopiperidine-4-carboxylic acid, had been known in the chemical database.[15]

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10.1021/jm060051s CCC: $33.50 © 2006 American Chemical Society
Published on Web 06/14/2006

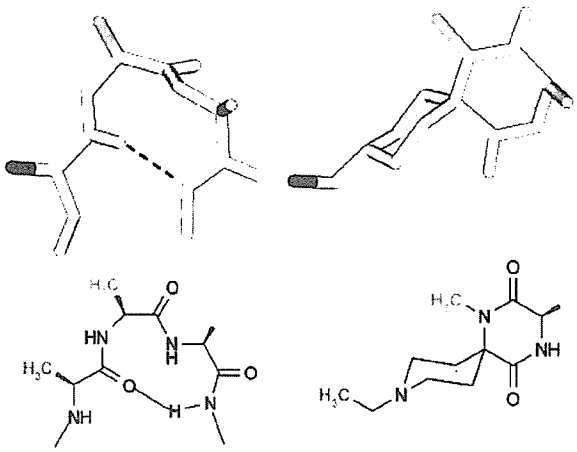


Figure 1. Molecular modeling of type-I β-turn (left) and spirodiketopiperazine (right) showing their structural similarity.

(3) The predicted 3D structure of spirodiketopiperazine suggested that three substituents on this template might have the similar orientation of three side chains on the type I β-turn structure of the protein (Figure 1). These reasons prompted us to develop the synthetic method for spirodiketopiperazine derivatives, produce a library with this scaffold, and investigate their biological action toward several GPCRs, especially chemokine receptors.

Chemistry

To generate a spirodiketopiperazine-based library, we investigated the use of the Ugi multiple component reaction (MCR) on the solid phase followed by cyclative cleavage from the resin.[16] Ugi MCR is one of the most versatile methods to construct diverse dipeptide derivatives by the mixing of an amine, an amino acid derivative, a carbonyl compound, and an isonitrile.[17] This reaction followed by cyclative cleavage from the resin is one of the most reliable approaches to synthesize diketopiperazine derivatives in high purity. An efficient and reliable synthetic route is shown in Scheme 1.

There are several reports regarding the use of this MCR on solid support.[18,19] Two types of isonitrile resins were readily prepared from commercially available resins in two steps. Rink-isonitrile resin (resin A) and methylene-isonitrile resin (resin B) could be prepared from Rink-amide resin and aminomethylated resin, respectively. The conversion of formamide to isonitrile on resin was easily monitored by infrared spectroscopy (IR). As the intensity of the absorption corresponding to the amide decreased, the intensity of the absorption corresponding to the isonitrile increased. The reaction of the isonitrile resin with amine, piperidone, and carboxylic acid could also be monitored by IR measurements. Heating (65 °C) was required to achieve the complete conversion of the reaction. After the Ugi reaction, the Boc group was removed with TMSOTf in the presence of 2,6-lutidine (resin A)[20] or with 25% trifluoroacetic acid in CH_2Cl_2 (resin B). Cyclative cleavage from the support using toluene in the presence of acetic acid at 90 °C yielded the desired spirodiketopiperazine derivatives in high purity.[21] The crude purities and isolated yields of **1a–g** are shown in Table 1.[22]

Table 1. Yield and Purity of Spirodiketopiperazine Derivatives 1a–g on Solid-Phase Synthesis

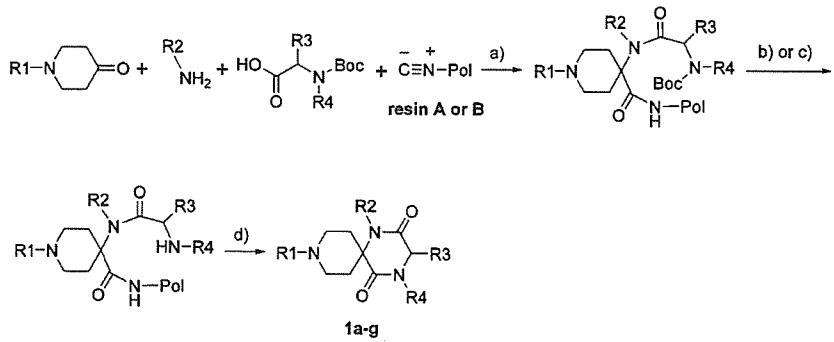
compds	R1	R2	R3	R4	resin[a]	purity (%)[b]	yield (%)[c]
1a	Me	Bn	Bn	H	A	100.0	62
					B	100.0	91
1b	Bn	Bn	Bn	H	A	95.7	67
					B	100.0	88
1c	$Ph(CH_2)_5$	Bn	Bn	H	B	100.0	96
1d	$Ph(CH_2)_5$	Bn	^{i}Bu	H	B	100.0	96
1e	$Ph(CH_2)_5$	Ph	^{i}Bu	H	B	81.3	15
1f	$Ph(CH_2)_5$	^{i}Pr	^{i}Bu	H	B	41.7	30
1g	Bn	Bn	$-CH_2CH_2CH_2-$		A	92.4	68
					B	96.9	99

[a] Resin A is the isonitrile resin prepared from the Rink-amide resin. Resin B is the isonitrile resin prepared from the aminomethylated resin. [b] The crude sample was analyzed by LC-MS/ELSD. Its purity was calculated by the % area of the corresponding peak to the desired product on ELSD. [c] The isolated yield is based upon the loading level of the isonitrile resin. The loading level was determined by elemental analysis.

To expand the diversity at the R1 position, we used both solid- and solution-phase techniques because of the limited number of commercially available 1-substituted piperidone derivatives. In solid-phase synthesis, the use of 1-allyloxycarbonyl-4-piperidone was examined (Scheme 2).[23] 1-Allyloxycarbonyl-4-piperidone was reacted with isonitrile resin in the presence of propylamine and *N*-Boc-leucine. After the Ugi reaction, the allyloxycarbonyl group was removed with $Pd(PPh_3)_4$-Bu_3SnH-AcOH in CH_2Cl_2.[24] The secondary amine was reductively alkylated by the reaction with aldehyde in the presence of sodium triacetoxyborohydride.[25,26] The removal of the Boc group followed by cyclative cleavage gave the desired spiro-compounds (**2a–g**) in high purity (Table 2).

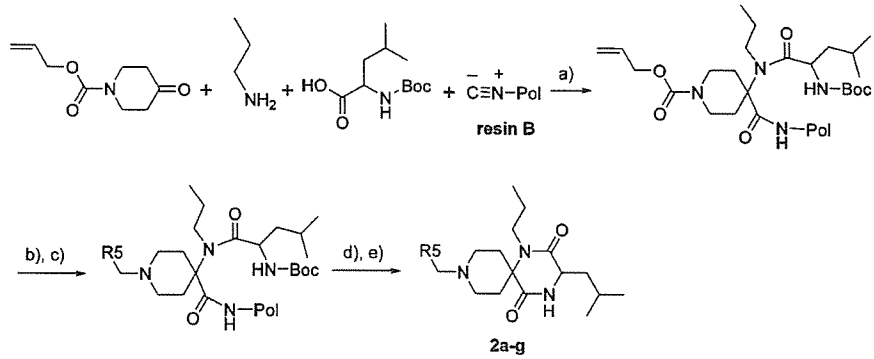
In the solution phase, desired product **4** was obtained by the reductive alkylation of secondary amine **3** with the corresponding aldehyde, followed by catch and release purification utilizing the cation-exchange resin.[27]

Scheme 1. Solid-Phase Synthesis of Spirodiketopiperazine Derivatives[a]



[a] (a) THF-MeOH (1:1), 65 °C, 16 h; (b) TMSOTf, 2,6-lutidine/CH_2Cl_2, rt, 1 h (resin A); (c) TFA-CH_2Cl_2 (1:3), rt, 0.5 h (resin B); (d) AcOH/toluene, 90 °C, 24 h.

Scheme 2. Solid-Phase Synthesis of Spirodiketopiperazine Derivatives **2a–g**[a]



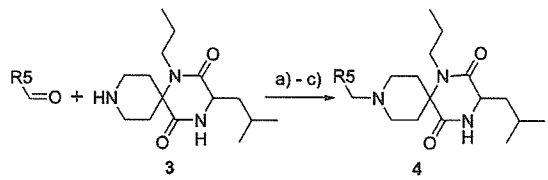
[a] (a) THF–MeOH (1:1), 65 °C, 16 h; (b) $Pd(PPh_3)_4$, Bu_3SnH, $AcOH/CH_2Cl_2$, rt, 4 h; (c) R5-CHO, $NaBH(OAc)_3$, AcOH/DMF, rt, 16 h; (d) TFA–CH_2Cl_2 (1:3), rt, 0.5 h; (e) AcOH/toluene, 90 °C, 24 h.

Table 2. Yield and Purity of Spirodiketopiperazine Derivatives on Solid-Phase Synthesis

compds	R5	purity (%)[a]	yield (%)[b]
2a	Ph	100.0	86
2b	4-Cl-Ph	100.0	97
2c	2,4,6-trimethoxy-Ph	95.9	81
2d	4-methoxy-Ph	98.9	81
2e	$PhCH_2CH_2$	100.0	86
2f	2-furanyl	91.1	72
2g	cyclohexyl	99.0	89

[a] The crude sample was analyzed by LC-MS/ELSD. Its purity was calculated by the % area of the corresponding peak to the desired product on ELSD. [b] The isolated yield is based upon the loading level of the isonitrile resin. Its loading level was determined by elemental analysis.

Scheme 3. Solution-Phase Synthesis of Spirodiketopiperazine Derivatives **4**[a]



[a] (a) $NaBH(OAc)_3$/DMF–1,2-dichloroethane, rt, 36 h; (b) cation-exchange resin (BondElute SCX); (c) elution with 10% Et_3N in MeOH.

Results and Discussion

Hit Identification. According to the synthetic procedure shown in Scheme 1, we synthesized an initial library of 576 compounds employing 8 piperidones, 9 amines, and 8 amino acids (Supporting Information, Figure 1). Each crude member of this library was analyzed for purity using LC-MS/ELSD. In 539 samples, the purity exceeded 80% on ELSD, and the mass spectrum of the major peak was consistent with the expected structure.

The library was tested at 30 μM for inhibition against the calcium mobilization of several subtypes of human chemokine receptor-overexpressed CHO cells stimulated by the corresponding endogenous ligand (hMIP-1α/hCCR5, hMCP-1/hCCR2, hMDC/hCCR4, hSDF-1α/hCXCR4, etc.). At this stage, several compounds showed greater than 50% inhibitory activity in hMIP-1α/hCCR5.[28] Among them, two compounds (R1 = benzyl, R2 = benzyl, R3 = isobutyl; R1 = 6-phenylhexyl, R2 = *n*-propyl, R3 = isobutyl) were selected, resynthesized in an optically pure form, purified, and evaluated as representative hit compounds. Compounds **5–8** were identified as selective micromolar CCR5 antagonists (Table 3). Because no significant difference in antagonistic activities between the enantiomers could be observed, we decided to synthesize the following lead evaluation libraries in racemic form. To evaluate their receptor selectivity, the newly synthesized compounds were also tested by the binding assay as shown in Tables 4 and 5.

From Hit to Lead. Iterative Evaluation of Individual Sites. Among more than 15 subtypes of chemokine receptors, CCR5 is one of the most attractive drug targets to treat HIV infections,[30] asthma,[31] rheumatoid arthritis,[32] and acute/chronic transplant rejections.[33,34] We decided to follow the evaluation process from hit to lead in CCR5 antagonistic activity.

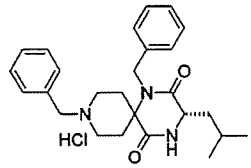
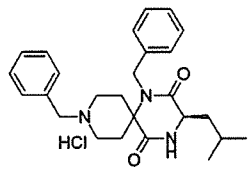
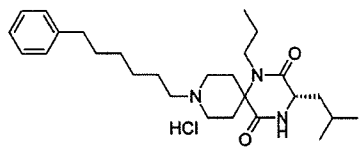
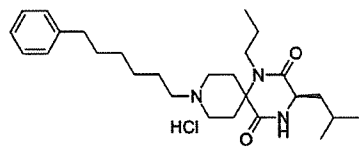
The racemic forms of compounds **7** and **8** (R1 = 6-phenylhexyl, R2 = *n*-propyl, R3 = isobutyl) were used as the lead compounds. Initially, each diversity site (R1, R2, and R3) was individually evaluated. This iterative evaluation approach was reported to be useful for the exploration of a relatively large number of building blocks to select the most suitable fragments at each site.[35]

1. R1 Library. To explore the optimal structures at the R1 site, we synthesized 80 derivatives by using the solution-phase synthesis shown in Scheme 3. The two other sites were fixed (R2 = *n*-propyl; R3 = isobutyl). A list of aldehydes used in the R1 library (Supporting Information, Figure 2) was selected among the commercially available reagents by chemical compatibility, molecular weight, and structural diversity. By considering the substructures of compounds **5–8**, we enriched the 3- or 4-substituted benzaldehyde derivatives in this evaluation set.

Upon screening the R1 library, several compounds with diverse groups (Figure 3) were identified with significantly improved antagonistic activity.[36] These results indicated that the conversion of the 6-phenylhexyl or the benzyl groups in compounds **5–8** to the 4-substituted benzyl group led to a significant improvement in CCR5 antagonistic activity and that the introduction of diverse long substructures at this position might be suitable for the improvement of activity. Along with the preparation of this R1 library, compound **3** was converted to amides, sulfonamides, and ureas by the reaction with the corresponding acyl chlorides, sulfonyl chlorides, and isocyanates, respectively. Some of the acylated products showed modest CCR5 antagonistic activity (data not shown). However, further derivatization from these acylated analogues was not pursued. The activity of representative compound **9** was confirmed through resynthesis and purification (Table 4, compound **9**).

2. R2 Library. In a manner similar to the R1 library, we synthesized 80 derivatives of **7** and **8** using the solid-phase synthesis shown in Scheme 1. The 80 amines were selected

Table 3. Hit Identification[29]

		Ca Assay, IC_{50} (μM)			
		hMIP-1α hCCR5	hMCP-1 hCCR2	hMDC hCCR4	hSDF-1α hCXCR4
5		3.2	> 30.0	> 30.0	> 30.0
6		7.6	> 30.0	> 30.0	> 30.0
7		2.0	> 10.0	> 10.0	> 10.0
8		3.0	> 10.0	> 10.0	> 10.0

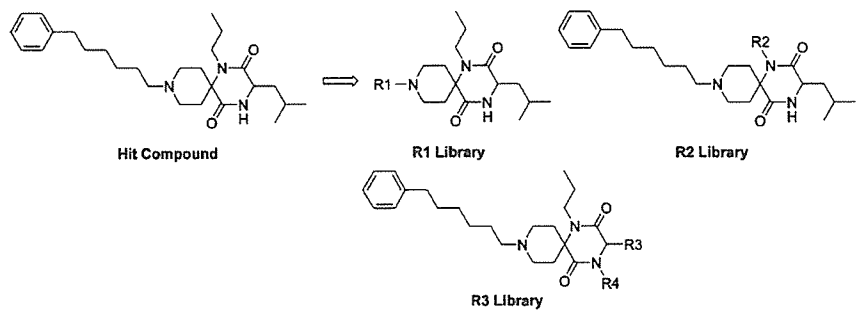


Figure 2. Iterative library for the hit evaluation.

from the chemically compatible and commercially available amines, taking into account the similarity to *n*-propylamine or benzylamine (Supporting Information, Figure 3).

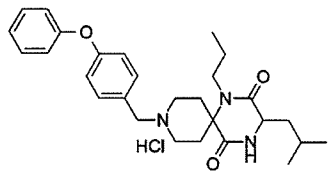
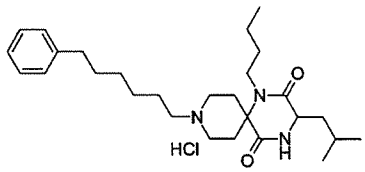
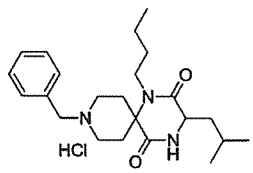
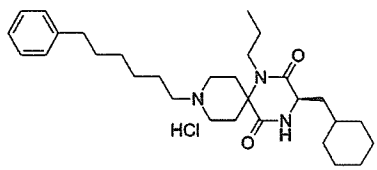
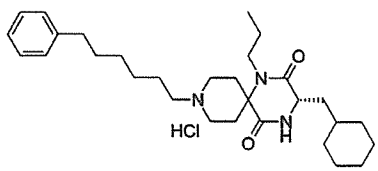
The screening results of the R2 library is shown in Table 4. An *n*-butyl group at the R2 position showed significantly higher potency than the *n*-propyl (**10** vs **7** and **8**) and benzyl groups (**11** vs **5** and **6**). The *n*-butyl group was selected as the optimal substituent at the R2 position.

3. R3 Library. The R3 site was the next site to be optimized. Eighty commercially available Boc-protected amino acid derivatives were employed to construct the R3 Library. The other two sites were fixed (R1 = 6-phenylhexyl; R2 = *n*-propyl). As the R3 library, the optically pure Boc-protected amino acid was used when it was commercially available (Supporting Information, Figure 4). After the evaluation of the antagonistic activity of each compound, compounds **12** and **13** with a cyclohexylmethyl group at R3 showed comparable activity to **7** (Table 4). No significant difference in activity was observed between enantiomers **12**/**13** and **7**/**8**. On the basis of these observations, the following R1R2R3 library was synthesized using racemic amino acid derivatives.

4. R1R2R3 Library. In the final library, all three sites around the spirodiketopiperazine scaffold were simultaneously examined (Figure 4). R1R2R3 library consisted of 14 compounds (R1 × R2 × R3 = 7 × 1 × 2), which were synthesized by the solid-phase synthesis shown in Scheme 3. The more potent hits (compounds **9**−**14**) were selected for resynthesis and purification and were subsequently re-screened against a panel of chemokine receptors (Table 5). Biological evaluation of the purified compounds provided highly selective and potent CCR5 antagonists.

Selectivity. Compound **18**, which showed the most potent affinity for CCR5 among the tested compounds **14** − **19**, was selected as a representative compound to evaluate receptor selectivity. The binding affinity of **18** for a variety of GPCRs was examined by the binding assay using the respective radioisotope-labeled ligand. Compound **18** showed 1000-fold more potent affinity for CCR5 relative to that of the other GPCRs and also showed modest affinity for the muscarinic M3 receptor

Table 4. Hits from the Iterative Libraries

		Ca Assay, IC_{50} (μM)				Binding Assay, IC_{50} (μM)		
		hMIP-1α hCCR5	hMCP-1 hCCR2	hMDC hCCR4	hSDF-1α hCXCR4	hMIP-1α hCCR5	hMCP-1 hCCR2	hSDF-1α hCXCR4
9		0.50	> 30.0	> 30.0	> 30.0	0.017	> 30.0	> 30.0
10		0.27	> 10.0	> 10.0	6.7	0.038	> 10.0	> 10.0
11		0.90	> 30.0	> 30.0	> 30.0	0.56	NT[a]	> 10.0
12		0.27	> 10.0	> 10.0	> 10.0	0.86	NT	> 10.0
13		0.30	> 10.0	> 10.0	> 10.0	0.61	NT	> 10.0

[a] NT: not tested.

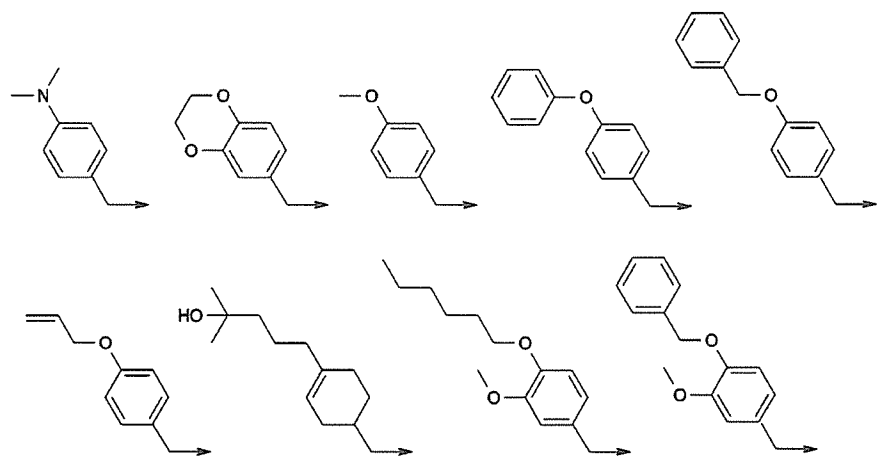


Figure 3. Representative substructures of the R1 hit compounds.

and sigma receptor (for the muscarinic M3 receptor, IC_{50} = 2.07 μM; for the sigma receptor, IC_{50} = 0.183 μM).

Anti-HIV Activity.[37] We next examined in vitro anti-HIV activities of these potent CCR5 antagonists. Compounds **10**, **15**, and **18** potently inhibited not only the replication of laboratory and primary R5 HIV-1 strains but also that of various multidrug-resistant monocyte/macrophage tropic (R5) HIV-1 (see data in ref 37). All examined compounds were inactive against T cell

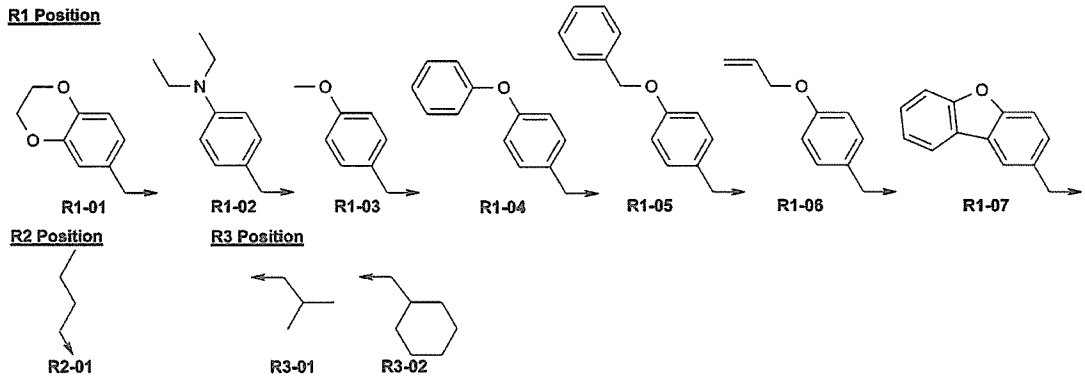
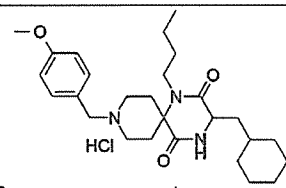
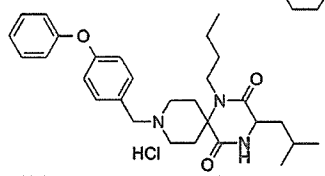
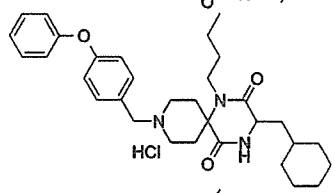
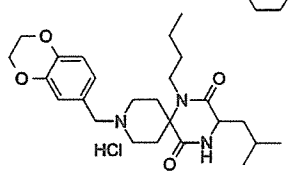
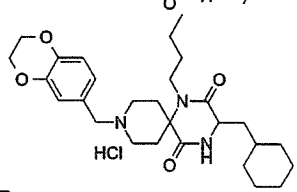
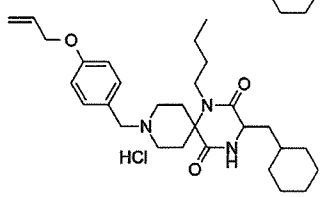


Figure 4. R1R2R3 library components.

Table 5. Hits from the R1R2R3 Library

		Ca Assay, IC_{50} (μM)			Binding Assay, IC_{50} (μM)		
		hMIP-1α hCCR5	hMCP-1 hCCR2	hSDF-1α hCXCR4	hMIP-1α hCCR5	hMCP-1 hCCR2	hSDF-1α hCXCR4
14		0.12	> 30.0	> 30.0	0.0045	> 30.0	> 30.0
15		0.12	> 10.0	> 10.0	0.083	> 10.0	> 10.0
16		0.17	> 10.0	> 10.0	0.0061	> 10.0	> 10.0
17		0.17	> 10.0	> 10.0	0.020	> 10.0	> 10.0
18		0.020	> 10.0	> 10.0	0.0025	> 10.0	> 10.0
19		0.050	> 10.0	> 10.0	0.011	> 10.0	> 10.0

tropic (X4) HIV-1. These results support the hypothesis that spirodiketopiperazines such as **18** show potent anti-HIV activity through their antagonistic effects on CCR5.

Conclusions

Through the design and synthesis of a novel spirodiketopiperazine scaffold, several potent and selective CCR5 antagonists were identified. Further optimization of these potential anti-HIV agents will be reported soon.

Experimental Section

General. All reactions were carried out under a positive pressure of argon. 4-(1-(9-Fluorenylmethyloxycarbonylamino)-1-(2,4-dimethoxyphenyl)methyl)phenyloxy-polystyrene (1−2% divinylbenzene) (*N*-

Fmoc-Rink resin) and aminomethylpolystyrene (1−2% divinylbenzene) (aminomethylated resin) were purchased from Watanabe Chemicals. Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian VXR300 spectrometer with tetramethylsilane as an internal standard. Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectra were obtained on Perseptive Elete. Infrared spectra (IR) were measured on a Perkin-Elmer FT-IR 1760X spectrometer. Column chromatography was carried out on silica gel (Merck silica gel 60 (0.063−0.200 mm) or Fuji Silysia FL60D). Thin-layer chromatography was performed on silica gel (Merck TLC, silica gel 60 F_{254}). The solvents and reagents were purchased and used for the reaction without further purification. The following abbreviations for solvents and reagents are used: tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), acetic acid (AcOH). For compounds where analysis was not obtained, HPLC-MS/UV/ELSD analysis was used, and purity was determined to be >95%.

Rink-Isonitrile Resin (Resin A). The *N*-Fmoc-Rink resin (0.61 mmol/g, 4.0 g, 2.42 mmol) was successively washed with DMF (40 mL × 2) and 20% piperidine in DMF (40 mL × 1). The resin was suspended in 20% piperidine in DMF (40 mL), and the mixture was agitated for 30 min at room temperature. The mixture was filtered. The resin was washed with DMF (40 mL × 7) and suspended in DMF (15 mL). To it was added ethyl formate (25 mL). The mixture was stirred under reflux for 6 h. After cooling to room temperature, the resin was collected by filtration and washed with DMF (50 mL × 2), CH_2Cl_2 (50 mL × 4), MeOH (50 mL × 4), and CH_2Cl_2 (50 mL × 4). Drying under reduced pressure gave the *N*-formylated Rink resin (3.00 g, 0.71 mmol/g calculated by nitrogen content of elementary analysis: N%, 1.00) as a white resin. IR (KBr): 1692 (CONH) cm^{-1}. The *N*-formylated Rink resin (0.71 mmol/g, 3.00 g, 2.13 mmol) was washed with CH_2Cl_2 (30 mL × 2) and suspended in CH_2Cl_2 (30 mL). To the mixture were successively added triethylamine (1.70 mL, 12.2 mmol), CCl_4 (1.18 mL, 12.2 mmol), and triphenylphosphine (3.20 g, 12.2 mmol). The suspension was stirred under reflux for 1 h. After cooling to room temperature, the mixture was filtered. The resin was successively washed with CH_2Cl_2 (30 mL × 4), MeOH (30 mL × 2), and CH_2Cl_2 (30 mL × 4). Drying under reduced pressure gave the Rink-isonitrile resin (2.56 g, 0.84 mmol/g) as a pale yellow resin. IR (KBr): 2137 cm^{-1}.

Methylene-Isonitrile Resin (Resin B). The aminomethyl resin (0.52 mmol/g, 10.0 g, 5.2 mmol) was washed with DMF (40 mL × 2) and suspended in a mixture of DMF (40 mL) and ethyl formate (60 mL). The suspension was stirred under reflux for 6 h. After cooling to room temperature, the resin was filtered and washed with DMF (50 mL × 2), CH_2Cl_2 (50 mL × 4), MeOH (50 mL × 4), and CH_2Cl_2 (50 mL × 4). The resin was dried under reduced pressure to give the *N*-formylated aminomethyl resin (10.0 g, 0.44 mmol/g calculated by nitrogen content of elementary analysis) as a white resin. IR (KBr): 1682 (CONH) cm^{-1}. The *N*-formylated aminomethyl resin (0.44 mmol/g, 10.0 g, 4.4 mmol) was washed with CH_2Cl_2 (100 mL × 2) and then suspended in CH_2Cl_2 (100 mL). To it were successively added triethylamine (3.62 mL, 26.0 mmol), CCl_4 (2.51 mL, 26.0 mmol) and triphenylphosphine (6.82 g, 26.0 mmol). The suspension was stirred under reflux for 1 h. After cooling to room temperature, the mixture was filtered. The resin was successively washed with CH_2Cl_2 (100 mL × 4), MeOH (100 mL × 2), and CH_2Cl_2 (100 mL × 4). Drying under reduced pressure gave the methylene-isonitrile resin (9.73 g, 0.45 mmol/g) as a pale yellow resin. IR (KBr): 2146 cm^{-1}.

Typical Procedure for the Preparation of Spirodiketopiperazine Derivative from Rink-Isonitrile Resin. 1,3-Dibenzyl-9-methyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1a). The Rink-isonitrile resin (0.84 mmol/g, 350 mg, 0.294 mmol) was washed with THF−MeOH (1:1, 4 mL × 2). To a mixture of the above resin in THF−MeOH (1:1, 4 mL) were successively added *N*-methyl-4-piperidone (166 mg, 1.47 mmol), benzylamine (158 mg, 1.47 mmol), and *N*-Boc-phenylalanine (390 mg, 1.47 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the mixture was filtered. The resin was successively washed with THF−MeOH (1:1) (4 mL × 3) and CH_2Cl_2 (4 mL × 3). To a mixture of the resin in a 1.5 M solution of 2,6-lutidine in CH_2Cl_2 (2 mL) was added a 1.0 M solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was allowed to warm up to room temperature and then stirred for 30 min. The resin was collected by filtration. To a mixture of the resin in a 1.5 M solution of 2,6-lutidine in CH_2Cl_2 (2 mL) was added a 1.0 M solution of TMSOTf in CH_2Cl_2 (2 mL) at 0 °C. The mixture was allowed to warm up to room temperature and then stirred for 30 min. The resin was collected by filtration and successively washed with CH_2Cl_2 (4 mL × 3), toluene (4 mL × 3), and 1.25 M acetic acid in toluene (4 mL). The suspension of the resin in 1.25 M acetic acid in toluene (4 mL) was shaken for 24 h at 90 °C. After cooling to room temperature, the mixture was filtered, and the resin was washed with $CHCl_3$−MeOH (1:1) (4 mL × 2). The filtrate and washings were combined and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel with a gradient of AcOEt−MeOH from 1:0 to 10:1 to give the title compound (68.8 mg, 62% yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (m, 8 H), 7.03 (m, 2 H), 4.84 (m, 1 H), 4.54 (t, J = 4.5 Hz, 1 H), 4.53 (m, 1 H), 3.38 (m, 1 H), 3.16 (m, 1 H), 3.03 (dd, J = 13.8, 4.5 Hz, 1 H), 2.90 (m, 2 H), 2.70 (m, 1 H), 2.45 (s, 3 H), 2.06 (m, 1 H), 1.92−1.71 (m, 3 H); IR (KBr): 3448, 2928, 2855, 1682, 1497, 1455, 1430, 1361, 1261, 1206, 1137, 840, 802, 724, 703 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 755 $(2M + H)^+$, 378 $(M + H)^+$. HRMS (MALDI-TOF, Pos., Internal Standard PEG) calcd for $C_{23}H_{27}N_3O_2$·H: 378.2182. Found: 378.2192.

1,3,9-Tribenzyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1b). Using a procedure identical to that described for the preparation of **1a**, the title compound was obtained from 1-benzyl-4-piperidone, benzylamine, *N*-Boc-phenylalanine, and Rink-isonitrile resin in 67% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36−7.15 (m, 15 H), 5.95 (m, 1 H), 4.89 (d, J = 16.2 Hz, 1 H), 4.74 (d, J = 16.2 Hz, 1 H), 4.37 (m, 1 H), 3.77 (m, 1 H), 3.42 (dd, J = 13.8, 3.9 Hz, 1 H), 3.12−2.88 (m, 5 H), 2.24 (m, 3 H), 1.83 (m, 1 H), 1.24 (m, 1 H); IR (KBr): 3445, 3063, 3032, 1677, 1570, 1490, 1455, 1417, 1350, 1280, 1258, 1169, 1032, 735, 702, 639 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 907 $(2M + H)^+$, 454 $(M + H)^+$. HRMS (MALDI-TOF, Pos., Internal Standard PEG) calcd for $C_{29}H_{31}N_3O_2$·H: 454.2495. Found: 454.2495.

1,9-Dibenzyl-3,4-propylene-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1g). Using a procedure identical to that described for the preparation of **1a**, the title compound was obtained from 1-benzyl-4-piperidone, benzylamine, *N*-Boc-proline, and Rink-isonitrile resin in 68% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33−7.16 (m, 10H), 5.07 (m, 1H), 4.46 (m, 2H), 3.65 (m, 4H), 3.22 (m, 1H), 2.90 (m, 2H), 2.45 (m, 1H), 2.30−1.78 (m, 8H); IR (KBr): 3482, 1655, 1496, 1455, 1416, 1363, 1260, 1169, 1034, 981, 763, 740, 701, 641 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 404 $(M + H)^+$. Anal. Calcd for $C_{25}H_{29}N_3O_2$·1.1H_2O: C, 70.93; H, 7.43; N, 9.93. Found: C, 70.82; H, 7.37; N, 9.65.

Typical Procedure for the Preparation of Spirodiketopiperazine Derivative from Methylene-Isonitrile Resin. 1,3-Dibenzyl-9-(5-phenylpentyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1c). The methylene-isonitrile resin (0.45 mmol/g, 500 mg, 0.225 mmol) was washed with THF−MeOH (1:1) (4 mL × 2). To a suspension of the resin in THF−MeOH (1:1) (4 mL) were successively added *N*-(5-phenylpentyl)-4-piperidone (206 mg, 1.125 mmol), benzylamine (213 mg, 1.125 mmol), and *N*-Boc-phenylalanine (299 mg, 1.125 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the mixture was filtered. The collected resin was washed with THF−MeOH (1:1) (4 mL × 3), and CH_2Cl_2 (4 mL × 3). The resin was then added to 25% TFA in CH_2Cl_2 (4 mL) at 0 °C. The mixture was allowed to warm up to room temperature and then stirred for 30 min. After filtration, the resin was washed with CH_2Cl_2 (4 mL × 3), toluene (4 mL × 3), and 1.25 M acetic acid in toluene (4 mL). The suspension of the resin

in 1.25 M acetic acid in toluene was agitated for 24 h at 90 °C. After cooling to room temperature, the mixture was filtered. The resin was washed with $CHCl_3$−MeOH (1:1) (4 mL × 2). The filtrate and washings were concentrated under reduced pressure. The residue was purified by column chromatography over silica gel with a gradient of AcOEt−MeOH from 1:0 to 10:1 to give the title compound (103 mg, 96% yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (m, 15H), 5.95 (m, 1H), 4.80 (m, 2H), 4.43 (m, 1H), 3.29 (m, 5H), 2.82 (m, 2H), 2.58 (m, 3H), 2.41 (m, 1 H), 1.64 (m, 6H), 1.34 (m, 3H), 0.86 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.6, 166.6, 142.0, 137.7, 135.3, 130.2, 129.4, 128.9, 128.6, 128.0, 127.3 126.6, 126.1, 58.7, 57.1, 55.8, 49.2, 45.3, 40.4, 35.7, 30.9, 30.2, 30.1, 26.4, 23.9; IR (KBr): 3448, 3239, 3062, 3029, 2938, 2860, 1679, 1496, 1454, 1415, 1361, 1200, 1130, 830, 799, 732, 720, 701 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 510 $(M + H)^+$. HRMS (MALDI-TOF, Pos., Internal Standard PEG) calcd for $C_{33}H_{39}N_3O_2$·H: 510.3120. Found 510.3103.

1-Benzyl-3-(2-methylpropyl)-9-(5-phenylpentyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1d). Using a procedure identical to that described for the preparation of **1c**, the title compound was obtained from 1-(5-phenylpentyl)-4-piperidone, benzylamine, *N*-Boc-leucine, and methylene-isonitrile resin in 96% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (m, 10 H), 6.18 (m, 1 H), 4.83 (m, 2 H), 4.12 (m, 1 H), 3.54 (m, 1 H), 3.43 (m, 3 H), 2.94 (m, 2 H), 2.72 (m, 2 H), 2.60 (t, J = 7.5 Hz, 2 H), 2.37 (m, 1 H), 2.05 (m, 1 H), 1.88−1.60 (m, 7 H), 1.39 (m, 2 H), 1.01 (m, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.6, 167.8, 141.7, 137.7, 128.7, 128.3, 127.1, 126.3, 125.9, 58.4, 56.9, 52.6, 49.3, 49.1, 45.2, 43.4, 35.4, 30.6, 30.3, 29.7, 26.1, 24.4, 23.7, 23.3, 21.0; IR (KBr): 3433, 3233, 2956, 2869, 1678, 1496, 1455, 1414, 1361, 1329, 1200, 1131, 830, 799, 720, 700 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 951 $(2M + H)^+$, 476 $(M + H)^+$. HRMS (MALDI-TOF, Pos., Internal Standard PEG) calcd for $C_{30}H_{41}N_3O_2$·H: 476.3277. Found 476.3288.

3-(2-Methylpropyl)-1-phenyl-9-(5-phenylpentyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione (1e). Using a procedure identical to that described for the preparation of **1c**, the title compound was obtained from 1-(5-phenylpentyl)-4-piperidone, aniline, *N*-Boc-leucine, and methylene-isonitrile resin in 15% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−6.98 (m, 10 H), 6.22 (m, 1 H), 4.15 (m, 1 H), 3.63 (m, 1 H), 3.47 (m, 2 H), 3.26 (m, 1 H), 2.86 (m, 2 H), 2.57 (m, 3 H), 2.24 (m, 2 H), 2.06 (m, 2 H), 1.65 (m, 6 H), 1.30 (m, 2 H), 0.97 (m, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.1, 168.2, 141.7, 135.7, 130.0, 129.6, 129.3, 128.3, 125.9, 118.3, 58.7, 56.8, 52.9, 49.2, 48.7, 41.8, 35.4, 31.4, 30.7, 30.6, 26.0, 24.5, 23.5, 23.1, 21.3; IR (KBr): 3422, 3247, 2956, 2868, 1678, 1493, 1545, 1347, 1200, 1131, 831, 799, 752, 720, 700 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 923 $(2M + H)^+$, 462 $(M + H)^+$. HRMS (MALDI-TOF, Pos., Internal Standard PEG) calcd for $C_{29}H_{39}N_3O_2$·H: *m/z* 462.3120. Found: 462.3087.

Typical Procedure for the Preparation of Spirodiketopiperazine Derivative from 1-Alloc-4-piperidone and Methylene-Isonitrile Resin. 9-(4-Chlorophenylmethyl)-3-(2-methylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2b). The resin (0.45 mmol/g, 500 mg, 0.225 mmol) was washed with THF−MeOH (1:1) (4 mL × 2). To the suspension of the resin in THF−MeOH (1:1) (4 mL) were added *N*-allyloxycarbonyl-4-piperidone (206 mg, 1.13 mmol), 1-propylamine (66.5 mg, 1.125 mmol), and *N*-Boc-leucine (213 mg, 1.125 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the mixture was filtered. The resin was successively washed with THF−MeOH (1:1) (4 mL × 3) and CH_2Cl_2 (4 mL × 3). To the suspension of the resin in CH_2Cl_2 (4 mL) were successively added acetic acid (135 mg, 2.25 mmol), tetrakis(triphenylphosphine)palladium (0) (52.0 mg, 0.045 mmol), and tributyltinhydride (327 mg, 1.125 mmol). The mixture was shaken for 4 h at room temperature. After filtration, the resin was washed with CH_2Cl_2 (4 mL × 4) and DMF (4 mL × 4). To the suspension of the resin in 1% acetic acid in DMF (4 mL) were successively added 4-chlorobenzaldehyde (158 mg, 1.125 mmol) and sodium triacetoxyborohydride (238 mg, 1.125 mmol). The mixture was shaken for 16 h at room temperature and filtered. The resin was successively washed with MeOH (4 mL × 2), DMF (4 mL × 4), and CH_2Cl_2 (4 mL × 4). The resin was suspended in 25% TFA in CH_2Cl_2 (4 mL) at 0 °C. The mixture was allowed to warm up to room temperature and then stirred for 30 min. After filtration, the resin was rinsed with CH_2Cl_2 (4 mL × 3), toluene (4 mL × 3), and 1.25 M acetic acid in toluene (4 mL). The resin was suspended with 1.25 M acetic acid in toluene (4 mL). The suspension was shaken for 24 h at 90 °C. After cooling to room temperature, the mixture was filtered. The resin was washed with $CHCl_3$−MeOH (1:1) (4 mL × 2). The filtrate and washings were concentrated under reduced pressure. The residue was purified by column chromatography over silica gel with a gradient of AcOEt−MeOH from 1:0 to 10:1 to give the title compound (89 mg, 97% yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (m, 4H), 6.59 (m, 1H), 3.96 (m, 1H), 3.50 (s, 2H), 3.33 (m, 2H), 2.85 (m, 2H), 2.57 (m, 1H), 1.75 (m, 10H), 0.93 (m, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.1, 168.3, 137.1, 132.7, 130.3, 128.4, 61.9, 60.1, 52.5, 50.2, 49.8, 44.3, 42.7, 32.9, 24.3, 23.4, 23.1, 21.2, 11.4; IR (KBr): 3437, 3205, 3081, 2953, 2869, 1682, 1659, 1490, 1469, 1415, 1364, 1329, 1087, 1068, 1016, 844 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 406 $(M + H)^+$. Anal. Calcd for $C_{22}H_{32}N_3O_2Cl$: C, 65.09; H, 7.95; N, 10.35. Found: C, 65.23; H, 8.17; N, 10.20.

9-Benzyl-3-(2-methylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]-undeca-2,5-dione (2a). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and benzaldehyde in 86% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (m, 5 H), 6.80 (m, 1 H), 3.96 (m, 1 H), 3.57 (s, 2 H), 3.39 (m, 2 H), 2.88 (m, 2 H), 2.62 (m, 1 H), 1.88 (m, 7 H), 1.57 (m, 3 H), 0.96 (m, 9 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.2, 168.3, 129.1, 128.3, 127.2, 62.6, 60.0, 52.5, 50.2, 49.8, 44.3, 42.7, 32.8, 32.6, 24.2, 23.4, 23.1, 21.2, 11.3; IR (KBr): 3437, 3202, 3063, 2959, 2870, 1677, 1658, 1470, 1418, 1366, 1329, 1266, 1199, 1158, 1068, 742, 698 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 372 $(M + H)^+$. Anal. Calcd for $C_{22}H_{33}N_3O_2$·0.67H_2O; C, 68.90; H, 9.02; N, 10.96. Found: C, 68.99; H, 8.87; N, 10.88.

3-(2-Methylpropyl)-1-propyl-9-((2,4,6-trimethoxyphenyl)-methyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2c). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 2,4,6-trimethoxybenzaldehyde in 81% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (m, 1H), 6.12 (s, 2H), 3.94 (m, 1H), 3.81 (s, 3H), 3.79 (s, 6H), 3.67 (s, 2H), 3.36 (m, 2H), 2.96 (m, 2H), 2.66 (m, 1H), 1.88 (m, 7H), 1.55 (m, 3H), 0.94 (m, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.2, 168.5, 160.1, 159.7, 90.6, 60.4, 55.7, 55.3, 52.5, 49.7, 49.2, 48.5, 44.2, 42.6, 32.4, 24.3, 23.3, 23.2, 21.1, 11.3; IR (KBr): 3440, 2955, 1671, 1608, 1468, 1417, 1227, 1205, 1151, 1132, 1064, 952 cm^{-1}. MS (ESI, Pos. 20 V) 462 $(M + H)^+$, 181. Anal. Calcd for $C_{25}H_{39}N_3O_5$·0.33H_2O: C, 64.22; H, 8.55; N, 8.99. Found: C, 64.34; H, 8.65; N, 8.84.

3-(2-Methylpropyl)-9-(4-methoxyphenymethyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2d). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 4-methoxybenzaldehyde in 81% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.2 Hz, 2H), 6.55 (m, 1H), 3.96 (m, 1H), 3.80 (s, 3H), 3.48 (s, 2H), 3.36 (m, 2H), 2.79 (m, 2H), 2.56 (m, 1H), 1.88 (m, 7H), 1.56 (m, 3H), 0.95 (m, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.2, 168.4, 158.7, 130.3, 113.6, 62.0, 60.2, 55.2, 52.5, 50.2, 49.7, 44.2, 42.7, 32.8, 24.3, 23.4, 23.1, 21.2, 11.3; IR (KBr): 3435, 3205, 2955, 1667, 1612, 1513, 1468, 1399, 1366, 1244 cm^{-1}. MS (ESI, Pos. 20 V) *m/z* 402 $(M + H)^+$. Anal. Calcd for $C_{23}H_{35}N_3O_3$: C, 68.80; H, 8.79; N, 10.46. Found: C, 68.69; H, 9.09; N, 10.46.

3-(2-Methylpropyl)-9-(3-phenylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2e). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 3-phenylpropanal in

86% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (m, 2H), 7.17 (m, 3H), 6.60 (m, 1H), 3.96 (m, 1H), 3.39 (m, 2H), 2.84 (m, 3H), 2.64 (m, 3H), 2.42 (m, 2H), 2.09 (m, 2H), 1.85 (m, 6H), 1.57 (m, 3H), 0.93 (m, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.1, 168.3, 141.9, 128.3, 125.8, 60.1, 57.7, 52.5, 50.3, 49.9, 44.2, 42.7, 33.8, 32.7, 28.6, 24.2, 23.3, 23.2, 21.2, 11.3; IR (KBr): 3433, 3220, 3065, 2956, 2868, 1677, 1661, 1469, 1417, 1370, 1328, 1262, 1200, 1158, 1124, 1071, 742, 701 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 400 $(M + H)^+$. Anal. Calcd for $C_{24}H_{37}N_3O_2$: C, 72.14; H, 9.33; N, 10.52. Found: C, 71.82; H, 9.42; N, 10.44.

9-(2-Furanylmethyl)-3-(2-methylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2f). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 2-furfural in 72% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (m, 1H), 6.42 (m, 1H), 6.31 (m, 1H), 6.19 (m, 1H), 3.94 (m, 1H), 3.57 (s, 2H), 3.37 (m, 2H), 2.87 (m, 3H), 2.62 (m, 1H), 2.15 (m, 2H), 1.92 (m, 2H), 1.74 (m, 2H), 1.54 (m, 3H), 0.93 (m, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 174.2, 170.9, 168.3, 162.0, 142.3, 110.1, 108.9, 60.0, 54.7, 52.5, 50.1, 49.8, 44.2, 42.7, 32.8, 24.3, 23.3, 23.1, 21.2, 11.3; IR (KBr): 3448, 3207, 3113, 2958, 2871, 2816, 1680, 1655, 1470, 1403, 1364, 1340, 1303, 1150, 1075, 1012, 923, 745, 602 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 362 $(M + H)^+$. Anal. Calcd for $C_{20}H_{31}N_3O_3$: C, 66.45; H, 8.64; N, 11.62. Found: C, 66.36; H, 8.86; N, 11.50.

9-Cyclohexylmethyl-3-(2-methylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (2g). Using a procedure identical to that described for the preparation of **2b**, the title compound was obtained from 1-allyloxycarbonyl-4-piperidone, 1-propylamine, *N*-Boc-leucine, methylene-isonitrile resin, and cyclohexylaldehyde in 89% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (m, 1H), 3.96 (m, 1H), 3.39 (m, 2H), 2.79 (m, 3H), 2.51 (m, 1H), 2.17 (m, 3H), 1.93 (m, 4H), 1.79 (m, 7H), 1.58 (m, 4H), 1.21 (m, 3H), 0.95 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.3, 168.5, 65.2, 60.3, 52.5, 50.7, 50.3, 44.2, 42.6, 35.3, 32.8, 32.0, 26.8, 26.1, 24.2, 23.4, 23.2, 21.2, 11.3; IR (KBr): 3436, 3203, 3076, 2924, 2871, 2851, 1679, 1468, 1414, 1369, 1329, 1152, 1130, 1066 cm^{-1}. MS (ESI, Pos. 20 V) *m*/*z* 378 $(M + H)^+$. Anal. Calcd for $C_{22}H_{39}N_3O_2$: C, 69.98; H, 10.41; N, 11.13. Found: C, 69.83; H, 10.71; N, 11.07.

General Procedure for the Preparation of the Initial, R2, and R3 Libraries. Rink-isonitrile resin (1.6 g, 0.61 mmol/g resin) was suspended in a mixture of $CHCl_3$-MeOH (3:1). This suspension was equally divided into 80 test tubes with a screwed cap. One test tube contained 20 mg (0.122 mmol) of the resin. The solvent was removed by evaporation under reduced pressure with centrifugation. To this test tube were successively added 0.122 mL of a 1.0 M solution of the corresponding amine in THF−MeOH (1:1) (0.122 mmol), 0.122 mL of a 1.0 M solution of the corresponding *N*-Boc-amino acid in THF−MeOH (1:1) (0.122 mmol), and 0.122 mL of a 1.0 M solution of the corresponding 1-substituted 4-piperidone in THF−MeOH (1:1) (0.122 mmol) at room temperature. The mixture was warmed at 65 °C for 18 h. After cooling to room temperature, the mixture was filtered, and the resin was washed with THF (2 mL × 2), MeOH (2 mL × 2), and CH_2Cl_2 (2 mL × 3). The resin was suspended in 0.2 mL of CH_2Cl_2. To this suspension were successively added a 1.5 M solution of 2,6-lutidine in CH_2Cl_2 (0.2 mL) and a 1.0 M solution of TMSOTf in CH_2Cl_2 (0.2 mL) at room temperature. The mixture was agitated for 30 min at room temperature and then filtered. The resin was washed with CH_2Cl_2 (2 mL × 2), MeOH (2 mL × 2), and CH_2Cl_2 (2 mL × 3). After the resin was dried under reduced pressure, it was transferred into a test tube with a screwed cap. The resin was suspended in a 1.25 M solution of AcOH in toluene (0.2 mL). The mixture was warmed at 90 °C for 16 h. After cooling to room temperature, the resin was collected by filtration and washed with MeOH (1 mL × 2). The filtrate and washings were concentrated under reduced pressure with centrifugation. The residue was dissolved in MeOH (0.488 mL). A small amount of this solution (0.050 mL) was saved for LC-MS analysis. The remaining solution was transferred into a 96-well plate. The solvent was removed by evaporation under reduced pressure with centrifugation. The residues were dissolved in DMSO (0.438 mL) (10 mM solution for in vitro biological assay). The overall yield was estimated to be 40% yield from Rink-isonitrile resin.

(3*S*)-1,9-Dibenzyl-3-(2-methylpropyl)-1,4,9-triazaspiro[5.5]-undeca-2,5-dione Hydrochloride (5). To an agitated suspension of Rink-isonitrile resin (1.0 g, 0.56 mmol) in THF−MeOH (10 mL, 1:1) were successively added benzylamine (0.183 mL, 1.68 mmol), *N*-Boc-L-leucine (419 mg, 1.68 mmol), and 1-benzyl-4-piperidone (0.30 mL, 1.68 mmol) at room temperature. The mixture was stirred at 65 °C for 16 h. After cooling to room temperature, the mixture was filtered and washed with THF (6 mL × 3), MeOH (6 mL × 3), and CH_2Cl_2 (6 mL × 3). The resin was suspended in a 1.5 M solution of 2,6-lutidine in CH_2Cl_2 (4 mL) and a 1.0 M solution of TMSOTf in CH_2Cl_2 (4 mL) at room temperature. The mixture was agitated for 30 min at room temperature and then filtered. The resin was suspended in a 1.5 M solution of 2,6-lutidine in CH_2Cl_2 (4 mL) and a 1.0 M solution of TMSOTf in CH_2Cl_2 (4 mL) at room temperature. The mixture was agitated for 30 min at room temperature and then filtered. The resin was washed with CH_2Cl_2 (6 mL × 4), MeOH (6 mL × 4), and CH_2Cl_2 (6 mL × 5). The resin was suspended in a 1.2 M solution of AcOH in toluene (6 mL). The mixture was warmed at 90 °C for 20 h. After cooling to room temperature, the resin was collected by filtration and washed with MeOH (6 mL × 2). The filtrate and washings were loaded on cation-exchanged resin (BondElute SCX, Varian, 1.0 g), which was washed with MeOH (6 mL × 2), H_2O (6 mL), and MeOH (6 mL) prior to use. The resin was washed with MeOH (4 mL × 2). Elution with 10% Et_3N in MeOH (5 mL × 2) was concentrated under reduced pressure to give an oily residue. This residue was purified by flash chromatography over silica gel (40 g) with $CHCl_3$-*i*-PrOH (20:1) to give a colorless oil, which was dissolved in MeOH (10 mL). Then, 1 N HCl (0.60 mL) was added to it. Concentration under reduced pressure gave a white powder, which was triturated with Et_2O to yield **5** (72 mg, 28% yield, 95% ee determined by HPLC) as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.48 (m, 5 H), 7.23 (m, 5 H), 5.06−4.82 (m, 2 H), 4.31 (s, 2 H), 4.17 (dd, J = 8.0, 4.6 Hz, 1 H), 3.72 (m, 2 H), 3.40 (m, 2 H), 2.52 (m, 2 H), 2.08 (m, 2 H), 2.00−1.60 (m, 3 H), 0.98 (t, J = 6.0 Hz, 6 H); IR (KBr): 3435, 3231, 3063, 2956, 2550, 1673, 1497, 1456, 1413, 1364, 1327, 1155, 1131, 968, 750, 700 cm^{-1}. MS (APCI, Pos., 40 V) *m*/*z* 420 $(M + H)^+$. Anal. Calcd for $C_{26}H_{33}N_3O_2$·HCl·H_2O: C, 65.88; H, 7.65; N, 8.86. Found: C, 65.99; H, 7.77; N, 8.83. HPLC (Chiralcel OJ-R 4.6 × 150 mm. 0.5 M $NaClO_4$/CH_3CN = 65/35. 0.8 mL/min. UV 210 nm. Temp 40 °C) retention time, 11.44 min.

(3*R*)-1,9-Dibenzyl-3-(2-methylpropyl)-1,4,9-triazaspiro[5.5]-undeca-2,5-dione Hydrochloride (6). Using a procedure identical to that described for the preparation of **5**, the title compound (62 mg, 94% ee determined by HPLC) was obtained from Rink-isonitrile resin, benzylamine, *N*-Boc-D-leucine, and 1-benzyl-4-piperidone in 24% yield as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.48 (m, 5 H), 7.23 (m, 5 H), 4.82 (m, 2 H), 4.31 (s, 2 H), 4.17 (dd, J = 8.0, 4.6 Hz, 1 H), 3.72 (m, 2 H), 3.40 (m, 2 H), 2.52 (m, 2 H), 2.08 (m, 2 H), 2.00−1.60 (m, 3 H), 0.98 (t, J = 6.0 Hz, 6 H); IR (KBr): 3434, 3215, 3063, 2956, 2660, 2532, 2419, 2361, 1674, 1496, 1456, 1413, 1364, 1327, 1154, 1131, 1073, 1032, 1005, 968, 929, 750, 733, 700 cm^{-1}. MS (APCI, Pos., 40 V) 420 $(M + H)^+$. Anal. Calcd for $C_{26}H_{33}N_3O_2$·HCl·H_2O: C, 65.88; H, 7.65; N, 8.86. Found: C, 66.18; H, 7.76; N, 8.80. HPLC (Chiralcel OJ-R 4.6 × 150 mm. 0.5 M $NaClO_4$/CH_3CN = 65/35. 0.8 mL/min. UV 210 nm. Temp 40 °C) retention time, 9.46 min.

(3*S*)-3-(2-Methylpropyl)-9-(6-phenylhexyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (7). Using a procedure identical to that described for the preparation of **5**, the title compound (69 mg, 91% ee. determined by HPLC) was obtained from Rink-isonitrile resin, 1-propylamine, *N*-Boc-L-leucine, and 1-(6-phenylhexyl)-4-piperidone in 26% yield as a white powder. $[\alpha]^{26}_D$ −24.6° (c 0.680, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ 7.18 (m, 5 H), 4.02 (dd, J = 7.6, 4.8 Hz, 1 H), 3.70 (m, 2 H), 3.56

(m, 2 H), 3.39 (m, 2 H), 3.11 (m, 2 H), 2.63 (dd, J = 7.8, 7.2 Hz, 2 H), 2.48 (m, 2 H), 2.17 (m, 2 H), 1.95−1.50 (m, 9 H), 1.42 (m, 4 H), 1.00−0.89 (m, 9 H); IR (KBr): 3435, 3205, 3082, 3026, 2935, 2870, 2493, 2361, 1674, 1454, 1417, 1370, 1331, 1155, 1070, 1004, 961, 750, 700 cm^{-1}. MS (FAB, Pos., glycerin + m-NBA) 442 (M + H) $^+$, 232, 171, 79. Anal. Calcd for $C_{27}H_{43}N_3O_2$·HCl: C, 67.83; H, 9.28; N, 8.79. Found: C, 67.56; H, 9.50; N, 8.71. HPLC (Chiralcel OD-R 4.6 × 250 mm. 0.5 M $NaClO_4/CH_3CN$ = 55/45. 0.5 mL/min. UV 210 nm. Temp 40 °C) retention time, 23.32 min.

(3*R*)-3-(2-Methylpropyl)-9-(6-phenylhexyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (8). Using a procedure identical to that described for the preparation of **5**, the title compound (63 mg, 89% ee determined by HPLC) was obtained from Rink-isonitrile resin, 1-propylamine, *N*-Boc-D-leucine, and 1-(6-phenylhexyl)-4-piperidone in 23% yield as a white powder. $[\alpha]^{26}_D$ +23.0° (c 0.453, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ 7.18 (m, 5 H), 4.02 (dd, J = 7.6, 4.6 Hz, 1 H), 3.70 (m, 2 H), 3.56 (m, 2 H), 3.39 (m, 2 H), 3.11 (m, 2 H), 2.63 (dd, J = 7.8, 7.2 Hz, 2 H), 2.48 (m, 2 H), 2.17 (m, 2 H), 1.95−1.50 (m, 9 H), 1.42 (m, 4 H), 1.00−0.89 (m, 9 H); IR (KBr): 3441, 3204, 3082, 3026, 2935, 2870, 2660, 2499, 2413, 2361, 1674, 1455, 1417, 1370, 1330, 1267, 1205, 1154, 1070, 1003, 960, 928, 899, 750, 700 cm^{-1}. MS (FAB, Pos., glycerin + *m*-NBA) 442 (M + H) $^+$, 294, 232, 202, 171, 79. Anal. Calcd for $C_{27}H_{43}N_3O_2$·HCl: C, 67.83; H, 9.28; N, 8.79. Found: C, 67.52; H, 9.51; N, 8.70. HPLC (Chiralcel OD-R 4.6 × 250 mm. 0.5 M $NaClO_4/CH_3CN$ = 55/45. 0.5 mL/min. UV 210 nm. Temp 40 °C) retention time, 25.17 min.

3-(2-Methylpropyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione (3). To a stirred solution of 1-allyloxycarbonyl-4-piperidone (1.83 g, 10.0 mmol) in MeOH (20 mL) were successively added 1-propylamine (1.64 mL, 20.0 mmol), *N*-Boc-leucine (4.99 g, 20.0 mmol), and benzylisonitrile (1.22 mL, 10.0 mmol) under argon at room temperature. The mixture was left for 3 days at room temperature and then diluted with AcOEt (150 mL). The mixture was washed with H_2O, 1 N HCl, H_2O, sat. $NaHCO_3$, and sat. NaCl. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give a yellow oil, which was purified by flash chromatography over silica gel (Fujisilicia FL-60D, 200 g) with AcOEt−hexane (1:2). The obtained oil was triturated with hexane and gave the Ugi product (3.84 g, 67% yield) as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.40−7.10 (m, 5 H), 5.96 (m, 1 H), 5.30 (m, 1 H), 5.21 (m, 1 H), 4.58 (m, 2 H), 4.45 (d, J = 15.0 Hz, 1 H), 4.42 (m, 1 H), 4.19 (d, J = 15.0 Hz, 1 H), 3.96 (m, 2 H), 3.50 (m, 2 H), 3.25 (m, 2 H), 2.62 (m, 1 H), 2.25 (m, 1 H), 2.00−1.50 (m, 6 H), 1.41 (s, 9 H), 1.23 (m, 1 H), 1.00−0.80 (m, 9 H). The above powder was dissolved in 4 N HCl in 1,4-dioxane (20 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C. Concentration under reduced pressure gave a solid residue, which was dissolved in MeOH (10 mL). The mixture was stirred for 1 h at room temperature, and concentrated under reduced pressure to give an oily residue. The residue was suspended in 1,2-dichloroethane (20 mL). *N,N*-Diisopropylethylamine (4.0 mL) was added to it. The mixture was refluxed for 16 h. After cooling to room temperature, the mixture was diluted with AcOEt, and washed with 1 N HCl, sat. $NaHCO_3$, and sat. NaCl. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give a yellow amorphous substance, which was purified by column chromatography over silica gel with AcOEt−hexane (2:3) to yield the cyclic compound (651 mg, 27%) as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 6.10−5.85 (m, 1 H), 5.31 (m, 1 H), 5.22 (m, 1 H), 4.59 (m, 2 H), 4.16−3.92 (m, 3 H), 3.70−3.40 (m, 4 H), 2.10−1.40 (m, 8 H), 1.30 (m, 1 H), 1.10−0.80 (m, 9 H); MS (APCI, Pos., 40 V) m/z 316 (M + H)$^+$, 308 (M − OAllyl)$^+$. To a stirred solution of the above powder in CH_2Cl_2 (4.0 mL) under argon were successively added AcOH (0.24 mL, 4.2 mmol), tributyltin hydride (0.57 mL, 2.1 mmol), and tetrakis-(triphenylphosphine)-palladium (0) (40 mg, 0.035 mmol) at room temperature. The mixture was stirred for 20 min at room temperature and then diluted with MeOH (20 mL). The mixture was loaded on cation-exchange resin (Bondesil SCX, 5.0 g, 3.0 mmol), which was washed with MeOH (10 mL), H_2O (10 mL), and MeOH (10 mL) prior to use. The resin was washed with MeOH (10 mL × 3). Elution with 10% Et_3N in MeOH (10 mL × 3) was concentrated under reduced pressure to give a red oil. Purification by column chromatography over silica gel with $CHCl_3$−MeOH−Et_3N (38:2:1) gave a solid, which was triturated with Et_2O to yield the title compound (189 mg, 38% yield) as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 3.99 (dd, J = 7.8, 4.4 Hz, 1 H), 3.50−3.20 (m, 4 H), 3.05−2.85 (m, 2 H), 2.10−1.75 (m, 5 H), 1.75−1.40 (m, 4 H), 1.00−0.85 (m, 9 H); MS (APCI, Pos., 40 V) m/z, 282 (M + H)$^+$, 159.

General Procedure for the Preparation of the R1 Library. Secondary amine **3** (40 mg, 0.143 mmol) was dissolved in 1,2-dichloroethane (3.20 mL) and equally divided into 80 wells of a 96-well plate (0.40 mL). Each well contained 0.00178 mmol of the starting material. Then, 0.017 mL of a 0.5 M DMF solution of the corresponding aldehyde (0.00533 mmol) was added to it. To this mixture was added a 0.33 M DMF solution of sodium triacetoxyborohydride (0.0211 mL, 0.00697 mmol) at room temperature. The mixture was agitated for 36 h at room temperature, acidified by the addition of 5% acetic acid in MeOH (0.050 mL), and then loaded on cation-exchange resin (Waters OASIS MCX Extraction Plate (30 mg) 60 μm (LP), 1.02 meq/g, 0.0306 mmol/well), which was washed with MeOH (0.40 mL) prior to use. The resin was washed with MeOH (0.30 mL × 2). Elution with 10% Et_3N in MeOH (0.30 mL × 2) was collected in a 96-well plate (1.0 mL). A small amount of this elution (0.60 mL) was saved for LC-MS analysis. The remaining solution was concentrated under reduced pressure. The residue was dissolved in 0.064 mL of DMSO to a concentration of 10 mM (estimated yield: 50%), which was used for the in vitro biological assay without further purification.

3-(2-Methylpropyl)-9-(4-phenoxyphenylmethyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (9). To a solution of **3** (20.0 mg, 0.071 mmol) in DMF−AcOH (0.80−0.080 mL) were successively added 4-phenoxybenzaldehyde (0.037 mL, 0.213 mmol) and sodium triacetoxyborohydride (45 mg, 0.213 mmol) at room temperature. The mixture was stirred for 16 h at room temperature and then diluted with MeOH (1.0 mL). The mixture was loaded on cation-exchange resin (Bondesil SCX, 1.0 g, 0.6 mmol), which was washed with MeOH (2.0 mL), H_2O (2.0 mL), and MeOH (2.0 mL) prior to use. The resin was washed with MeOH (2.0 mL × 2). Elution with 10% Et_3N in MeOH (2.0 mL × 3) was concentrated under reduced pressure to give a colorless oil. Purification by column chromatography over silica gel with $CHCl_3$−MeOH (10:1) yielded a colorless oil, which was dissolved in MeOH (10 mL). The solution was acidified by 1 N HCl (0.10 mL) and then concentrated under reduced pressure. Trituration with Et_2O gave the title compound (31 mg, 87% yield) as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.55 (m, 2 H), 7.40 (m, 2 H), 7.18 (m, 1 H), 7.05 (m, 4 H), 4.33 (s, 2 H), 4.01 (dd, J = 7.6, 4.8 Hz, 1 H), 3.79 (m, 2 H), 3.60−3.30 (m, 4 H), 2.46 (m, 2 H), 2.17 (m, 2 H), 1.95−1.40 (m, 5 H), 0.94 (m, 9 H); IR (KBr): 3439, 3220, 3066, 2959, 2872, 2663, 2561, 1672, 1590, 1509, 1489, 1418, 1370, 1330, 1241, 1200, 1172, 1072, 931, 873, 787, 694 cm^{-1}. MS (APCI, Pos. 40 V) m/z 464 (M + H)$^+$. Anal. Calcd for $C_{28}H_{37}N_3O_3$·HCl·H_2O: C, 64.59; H, 7.74; N, 8.07. Found: C, 64.38; H, 7.67; N, 8.07.

1-Butyl-3-(2-methylpropyl)-9-(6-phenylhexyl)-1,4,9-triazaspiro-[5.5]undeca-2,5-dione Hydrochloride (10). Using a procedure identical to that described for the preparation of **1c**, the title compound (34 mg) was obtained from 1-(6-phenylhexyl)-4-piperidone, 1-butylamine, *N*-Boc-leucine, and methylene-isonitrile resin in 35% yield as a white powder. Mp 127−133 °C; ^{1}H NMR (200 MHz, CD_3OD): δ 7.30−7.06 (m, 5 H), 4.02 (dd, J = 7.8, 4.8 Hz, 1 H), 3.70 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.11 (m, 2 H), 2.63 (t, J = 7.8 Hz, 2 H), 2.46 (m, 2 H), 2.18 (m, 2 H), 1.95−1.50 (m, 9 H), 1.50−1.25 (m, 6 H), 0.97 (m, 9 H); IR (KBr): 3447, 3199, 2934, 2869, 2663, 2502, 2440, 1673, 1455, 1418, 1372, 1329, 1152, 1086, 1003, 962, 750, 700 cm^{-1}. MS (MALDI-TOF, Pos.) m/z 456 (M + H)$^+$. Anal. Calcd for

$C_{28}H_{45}N_3O_2$·HCl·0.4H_2O: C, 67.35; H, 9.45; N, 8.41. Found: C, 67.67; H, 9.39; N, 8.42.

9-Benzyl-1-butyl-3-(2-methylpropyl)-1,4,9-triazaspiro[5.5]-undeca-2,5-dione Hydrochloride (11). Using a procedure identical to that described for the preparation of **1c**, the title compound (217 mg) was obtained from 1-benzyl-4-piperidone, 1-butylamine, *N*-Boc-leucine, and methylene-isonitrile resin in 59% yield as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.64−7.44 (m, 5 H), 4.36 (s, 2 H), 4.01 (dd, J = 7.8, 4.8 Hz, 1 H), 3.77 (m, 2 H), 3.55−3.35 (m, 4 H), 2.60−2.30 (m, 2 H), 2.17 (m, 2 H), 1.95−1.75 (m, 1 H), 1.75−1.60 (m, 2 H), 1.60−1.45 (m, 2 H), 1.45−1.20 (m, 2 H), 1.10−0.80 (m, 9 H); IR (KBr): 3435, 3230, 2957, 2871, 2505, 2454, 1680, 1647, 1459, 1413, 1370, 1326, 1147, 955, 746, 698 cm^{-1}. MS (MALDI-TOF, Pos.) m/z 386 $(M + H)^+$, 91. Anal. Calcd for $C_{23}H_{35}N_3O_2$·HCl: C, 65.46; H, 8.60; N, 9.96. Found: C, 65.09; H, 8.63; N, 9.88.

(3*R*)-3-Cyclohexylmethyl-9-(6-phenylhexyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (12). Using a procedure identical to that described for the preparation of **1c**, the title compound (100 mg) was obtained from 1-(6-phenylhexyl)-4-piperidone, 1-propylamine, *N*-Boc-D-cyclohexylalanine, and methylene-isonitrile resin in 74% yield as a white powder. Mp 105−109 °C; $[\alpha]^{25}_D$ +19.5° (c 0.126, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 7.29−7.09 (m, 5 H), 4.05 (dd, J = 7.59, 4.48 Hz, 1 H), 3.85−3.63 (m, 2 H), 3.61−3.46 (m, 2 H), 3.42−3.33 (m, 2 H), 3.19−3.05 (m, 2 H), 2.67−2.59 (m, 2 H), 2.50−2.30 (m, 2 H), 2.29 − 2.08 (m, 2 H), 1.85−1.09 (m, 21 H), 1.08−0.84 (m, 5 H); IR (KCl): 3410, 2926, 2853, 2514, 2439, 1670, 1450, 1417, 1371, 748, 700 cm^{-1}. Exact mass spectrum (FAB, Pos.) m/z calcd for $C_{30}H_{48}N_3O_2$: 482.3747; found: 482.3752.

(3*S*)-3-Cyclohexylmethyl-9-(6-phenylhexyl)-1-propyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (13). Using a procedure identical to that described for the preparation of **1c**, the title compound (100 mg) was obtained from 1-(6-phenylhexyl)-4-piperidone, 1-propylamine, *N*-Boc-L-cyclohexylalanine, and methylene-isonitrile resin in 82% yield as a white powder. $[\alpha]^{25}_D$ −18.7° (c 1.092, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 7.29−7.09 (m, 5 H), 4.05 (dd, J = 7.59, 4.48 Hz, 1 H), 3.85−3.63 (m, 2 H), 3.61−3.46 (m, 2 H), 3.42−3.33 (m, 2 H), 3.19 − 3.05 (m, 2 H), 2.67−2.59 (m, 2 H), 2.50−2.30 (m, 2 H), 2.29−2.08 (m, 2 H), 1.85−1.09 (m, 21 H), 1.08−0.84 (m, 5 H); IR (KCl): 3410, 2926, 2853, 2514, 2439, 1670, 1450, 1417, 1371, 748, 700 cm^{-1}. Exact mass spectrum (FAB, Pos.) m/z calcd. for $C_{30}H_{48}N_3O_2$: 482.3747; found: 482.3752.

General Procedure for the Preparation of the R1R2R3 Library. The methylene-isonitrile resin (1.00 g, 0.94 mmol) was suspended in mixed solvent THF−MeOH (1:1, 6 mL). To it were successively added 1-butylamine (0.464 mL, 4.70 mmol), *N*-Boc-amino acid (4.70 mmol), and 1-allyloxycarbonyl-4-piperidone (0.861 g, 4.70 mmol). The mixture was agitated for 16 h at 65 °C under argon. After cooling to room temperature, the mixture was drained. The resin was washed with THF (6 mL × 2), MeOH (6 mL × 2), and CH_2Cl_2 (6 mL × 3). An aliquot of this resin was dried under reduced pressure to measure its IR spectra. IR (KBr): 1714, 1669 cm-1. The resin was suspended in CH_2Cl_2 (6 mL). To it were successively added acetic acid (0.323 mL, 5.64 mmol), tributyltin hydride (0.759 mL, 2.82 mmol), and tetrakis(triphenylphosphine)palladium (0) (0.109 g, 0.094 mmol) at room temperature. The mixture was agitated for 6 h at room temperature. The mixture was drained. The resin was washed with CH_2Cl_2 (6 mL × 4), MeOH (6 mL × 2), and CH_2Cl_2 (6 mL × 3), and dried under reduced pressure to give a yellow resin. The loading ratio was determined by a picrate assay to be 0.66 mmol/g resin (81% conversion). Then, 100 mg of this resin (0.066 mmol) was washed with DMF (2 mL × 2) and then suspended in DMF (0.2 mL). A 1.0 M solution of the corresponding aldehyde in DMF (0.33 mL, 0.33 mmol) was added. The mixture was agitated for 5 min at room temperature. Then a 0.33 M solution of sodium triacetoxyborohydride in DMF (1.0 mL, 0.33 mmol) and acetic acid (0.10 mL) were added to it. The mixture was agitated for 24 h at room temperature. The mixture was drained, and the resin was washed with DMF (1 mL × 4), CH_2Cl_2 (1 mL × 3), MeOH (1 mL × 2), and CH_2Cl_2 (1 mL × 3). To it was added 50% TFA in CH_2Cl_2 (2 mL). The mixture was agitated for 5 min at room temperature and then drained. The resin was suspended in 50% TFA in CH_2Cl_2 (2 mL). The mixture was agitated for 20 min at room temperature and then drained. The resin was washed with CH_2Cl_2 (2 mL × 4), MeOH (2 mL × 2), toluene (2 mL × 4), and 1.25 M AcOH in toluene (2 mL). The resin was suspended in 1.25 M AcOH in toluene (2.0 mL). The mixture was agitated for 16 h at 90 °C. After cooling to room temperature, the mixture was filtered, and the resin was washed with MeOH (2.0 mL × 2). The filtrate and washings were combined (6.0 mL). Then, 0.10 mL of this solution was saved for LC-MS analysis (0.00050 mmol). The remaining solution was concentrated under reduced pressure. The residue was weighted and dissolved in DMSO to a concentration of 10 mM for the in vitro biological assay.

1-Butyl-3-cyclohexylmethyl-9-(4-methoxyphenylmethyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (14). Using a procedure identical to that described for the preparation of **2b**, the title compound (17 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-cyclohexylalanine, methylene-isonitrile resin, and 4-methoxybenzaldehyde in 59% yield as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.47 (d, J = 8.8 Hz, 2 H), 7.03 (d, J = 8.8 Hz, 2 H), 4.29 (s, 2 H), 4.04 (dd, J = 7.6, 4.8 Hz, 1 H), 3.83 (s, 3 H), 3.74 (m, 2 H), 3.55−3.35 (m, 4 H), 2.41 (m, 2 H), 2.15 (m, 2 H), 1.85−1.55 (m, 7 H), 1.55−1.42 (m, 3 H), 1.42−1.30 (m, 3 H), 1.30−1.10 (m, 2 H), 1.08−0.80 (m, 5 H); IR (KBr): 3436, 3221, 2926, 2851, 2666, 2560, 2362, 1672, 1613, 1585, 1517, 1448, 1419, 1373, 1305, 1255, 1182, 1031, 929, 826, 795, 587 cm^{-1}. MS(FAB., Pos., Glycerin + *m*-NBA) m/z 456 $(M + H)^+$, 121. Anal. Calcd for $C_{27}H_{41}N_3O_3$·HCl·1.5H_2O: C, 62.47; H, 8.74; N, 8.09. Found: C, 62.59; H, 8.35; N, 7.90.

1-Butyl-3-(2-methylpropyl)-9-(4-phenoxyphenylmethyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (15). Using a procedure identical to that described for the preparation of **2b**, the title compound (16 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 4-phenoxybenzaldehyde in 48% yield as a white powder. Mp 158−164 °C; ^{1}H NMR (200 MHz, CD_3OD): δ 7.54 (d, J = 8.8 Hz, 2 H), 7.40 (m, 2 H), 7.18 (m, 1 H), 7.11−7.00 (m, 4 H), 4.33 (s, 2 H), 4.01 (dd, J = 7.6, 4.8 Hz, 1 H), 3.80 (m, 2 H), 3.60−3.35 (m, 4 H), 2.43 (m, 2 H), 2.18 (m, 2 H), 1.80 (m, 1 H), 1.70 (m, 1 H), 1.54 (m, 2 H), 1.37 (m, 3 H), 1.00−0.90 (m, 9 H); IR (KBr): 3440, 3221, 3066, 2957, 2871, 2559, 1673, 1590, 1509, 1489, 1419, 1371, 1329, 1242, 1172, 873, 693 cm^{-1}. MS (FAB, Pos., Glycerin + *m*-NBA) 478 $(M + H)^+$, 183. Anal. Calcd for $C_{29}H_{39}N_3O_3$·HCl·H_2O: C, 65.46; H, 7.96; N, 7.90. Found: C, 65.67; H, 7.89; N, 7.83.

1-Butyl-3-cyclohexylmethyl-9-(4-phenoxyphenylmethyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (16). Using a procedure identical to that described for the preparation of **2b**, the title compound (23 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-cyclohexylalanine, methylene-isonitrile resin, and 4-phenoxybenzaldehyde in 70% yield as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.74−7.56 (m, 1 H), 7.53 (d, J = 8.8 Hz, 2 H), 7.40 (m, 2 H), 7.18 (m, 1 H), 7.10−7.00 (m, 3 H), 4.33 (s, 2 H), 4.04 (dd, J = 7.4, 4.8 Hz, 1 H), 3.80 (m, 2 H), 3.60−3.35 (m, 4 H), 2.43 (m, 2 H), 2.17 (m, 2 H), 1.90−1.60 (m, 7 H), 1.60−1.45 (m, 2 H), 1.45−1.30 (m, 2 H), 1.30−1.15 (m, 4 H), 1.10−0.80 (m, 5 H); IR (KBr): 3434, 3210, 3064, 2926, 2851, 2664, 2558, 1672, 1590, 1509, 1489, 1418, 1373, 1241, 1173, 1118, 1072, 1048, 929, 873, 723, 694, 542 cm^{-1}. MS (FAB., Pos., Glycerin + *m*-NBA) m/z 518 $(M + H)^+$, 334, 279, 183. Anal. Calcd for $C_{32}H_{43}N_3O_3$·HCl·0.5H_2O: C, 68.25; H, 8.05; N, 7.46. Found: C, 68.23; H, 7.88; N, 6.77.

9-(1,4-Benzodioxane-6-yl)-1-butyl-3-(2-methylpropyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (17). Using a procedure identical to that described for the preparation of **2b**, the title compound (147 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-leucine, methylene-isonitrile resin, and 6-formyl-1,4-benzodioxane in 31% yield as a white

powder. Mp 194−198 °C; ^{1}H NMR (200 MHz, CD_3OD): δ 7.08 (d, J = 2.2 Hz, 1 H), 7.01 (dd, J = 8.2, 2.2 Hz, 1 H), 6.93 (d, J = 8.2 Hz, 1 H), 4.27 (s, 4 H), 4.23 (s, 2 H), 4.01 (dd, J = 7.8, 4.8 Hz, 1 H), 3.72 (m, 2 H), 3.55−3.35 (m, 4 H), 2.43 (m, 2 H), 2.16 (m, 2 H), 1.80 (m, 1 H), 1.67 (m, 2 H), 1.55 (m, 2 H), 1.37 (m, 2 H), 1.00−0.90 (m, 9 H); IR (KBr): 3436, 2957, 2565, 1672, 1591, 1512, 1465, 1419, 1294, 1262, 1152, 1067, 888 cm^{-1}. MS (MALDI-TOF, Pos.) m/z 444 (M + H)$^+$, 149. Anal. Calcd for $C_{25}H_{37}N_3O_4$·HCl: C, 62.55; H, 7.98; N, 8.75. Found: C, 62.40; H, 8.20; N, 8.66.

9-(1,4-Benzodioxane-6-yl)-1-butyl-3-cyclohexyl-1,4,9-triazaspiro-[5.5]undeca-2,5-dione Hydrochloride (18). Using a procedure identical to that described for the preparation of **2b**, the title compound (698 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-cyclohexylalanine, methylene-isonitrile resin, and 6-formyl-1,4-benzodioxane in 57% yield as a white powder. Mp 163−169 °C; ^{1}H NMR (200 MHz, CD_3OD): δ 7.08 (d, J = 2.2 Hz, 1 H), 6.99 (dd, J = 8.0, 2.2 Hz, 1 H), 6.92 (d, J = 8.0 Hz, 1 H), 4.27 (s, 4 H), 4.23 (s, 2 H), 4.04 (dd, J = 7.6, 4.8 Hz, 1 H), 3.74 (m, 2 H), 3.60−3.35 (m, 4 H), 2.43 (m, 2 H), 2.15 (m, 2 H), 1.90−1.60 (m, 7 H), 1.60−1.45 (m, 2 H), 1.45−1.30 (m, 2 H), 1.30−1.10 (m, 4 H), 1.10−0.80 (m, 5 H); IR (KBr): 3436, 2926, 2852, 2511, 1675, 1645, 1591, 1511, 1418, 1374, 1294, 1261, 1068, 1050, 930, 888 cm^{-1}. MS (MALDI-TOF, Pos.) m/z 484 (M + H)$^+$, 149. Anal. Calcd for $C_{28}H_{41}N_3O_4$·HCl·0.3H_2O: C, 64.00; H, 8.17; N, 8.00. Found: C, 64.00; H, 7.94; N, 7.90.

9-(4-Allyloxyphenylmethyl)-1-butyl-3-cyclohexyl-1,4,9-triazaspiro[5.5]undeca-2,5-dione Hydrochloride (19). Using a procedure identical to that described for the preparation of **2b**, the title compound (10 mg) was obtained from 1-allyloxycarbonyl-4-piperidone, 1-butylamine, *N*-Boc-cyclohexylalanine, methylene-isonitrile resin, and 4-allyloxybenzaldehyde in 34% yield as a white powder. ^{1}H NMR (200 MHz, CD_3OD): δ 7.46 (d, J = 8.4 Hz, 2 H), 7.04 (d, J = 8.4 Hz, 2 H), 6.06 (m, 1 H), 5.41 (m, 1 H), 5.28 (m, 2 H), 4.59 (m, 2 H), 4.28 (s, 2 H), 4.04 (dd, J = 7.2, 4.8 Hz, 1 H), 3.77 (m, 2 H), 3.55−3.35 (m, 4 H), 2.39 (m, 2 H), 2.16 (m, 2 H), 1.90−1.60 (m, 7 H), 1.60−1.45 (m, 2 H), 1.45−1.30 (m, 2 H), 1.30−1.10 (m, 3 H), 1.10−0.80 (m, 5 H); IR (KBr): 3436, 3202, 2925, 2853, 2661, 2514, 2362, 1669, 1611, 1583, 1515, 1450, 1417, 1372, 1306, 1252, 1183, 998, 838, 796 cm^{-1}. MS (MALDI-TOF, Pos.) m/z 482 (M + H)$^+$, 147. Anal. Calcd for $C_{29}H_{43}N_3O_3$·HCl·0.4H_2O: C, 66.30; H, 8.60; N, 8.00. Found: C, 66.45; H, 8.40; N, 7.61.

Acknowledgment. We thank Mr. Y. Ushio, Mr. T. Inohara, and Mrs. S. Fujita for their technical assistance and valuable comments. We also thank Dr. I. C. Choong for the critical reading of this manuscript.

Supporting Information Available: Epimerization study in Scheme 1 and structural lists of building blocks in R1, R2, and R3 libraries. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) In the synthesis of compound **1e**, 1,9-bis(5-phenylpentyl)-3-(2-methylpropyl)-1,4,9-triazaspiro[5.5]undeca-2,5-dione was obtained in 45% yield. This result indicated that 1-(5-phenypentyl)-4-piperidone might decompose during the reaction to produce 5-phenylpentylamine. For the decomposition of 4-piperidone, see the following: (a) Shimano, M.; Meyers, A. I. Asymmetric Michael-type additions of lithium amides to aromatic systems leading to novel β-amino acids.

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JM060051S

Structure-Based Design of Novel HIV-1 Protease Inhibitors To Combat Drug Resistance

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Received May 11, 2006

Structure-based design and synthesis of novel HIV protease inhibitors are described. The inhibitors are designed specifically to interact with the backbone of HIV protease active site to combat drug resistance. Inhibitor **3** has exhibited exceedingly potent enzyme inhibitory and antiviral potency. Furthermore, this inhibitor maintains impressive potency against a wide spectrum of HIV including a variety of multi-PI-resistant clinical strains. The inhibitors incorporated a stereochemically defined 5-hexahydrocyclopenta[*b*]-furanyl urethane as the P2-ligand into the (*R*)-(hydroxyethylamino)sulfonamide isostere. Optically active (3a*S*,5*R*,6a*R*)-5-hydroxy-hexahydrocyclopenta[*b*]furan was prepared by an enzymatic asymmetrization of meso-diacetate with acetyl cholinesterase, radical cyclization, and Lewis acid-catalyzed anomeric reduction as the key steps. A protein−ligand X-ray crystal structure of inhibitor **3**-bound HIV-1 protease (1.35 Å resolution) revealed extensive interactions in the HIV protease active site including strong hydrogen bonding interactions with the backbone. This design strategy may lead to novel inhibitors that can combat drug resistance.

Introduction

The AIDS epidemic is one of the most challenging problems in medicine in the 21st century.[1] Among many strategies to combat this disease, highly active antiretroviral therapy (HAART) with HIV protease inhibitors (PI) in combination with reverse transcriptase inhibitors continues to be the first line treatment for control of HIV infection.[2] This treatment regimen has definitely improved quality of life, enhanced HIV management, and halted the progression of the disease. Despite these impressive successes, there are serious limitations including major toxicity and complexity of these treatment regimens. Perhaps the most serious problem is that a growing number of patients are developing multi-drug-resistant strains of HIV, and there is ample evidence that these strains can be transmitted.[3,4] Thus far, no effective treatment options exist for these patients. In this context, our research emphasis has been to design nonpeptidyl inhibitors and optimize their potency against mutant strains resistant to currently approved PIs.

We recently designed and developed a number of protease inhibitors with remarkable antiviral potency and drug-resistance profiles.[5,6] As shown in Figure 1, inhibitor **1** (now known as TMC-114, or Darunavir) has shown unprecedented picomolar enzyme inhibitory activity and antiviral potency, favorable drug resistance profiles against multi-drug-resistant HIV and encouraging pharmacokinetic properties.[7,8] This inhibitor has recently been approved by the United States Food and Drug Administration for treatment of drug-resistant HIV.[9] A high-resolution X-ray crystal structure of **1**-bound HIV protease has now revealed critical molecular insight into interactions responsible for the observed resistance profiles.[10] Structural analysis revealed that close contact of inhibitor **1** with the main chains of the protease active site amino acids (Asp-29 and Asp-30) is critical to its potency and wide-spectrum activity against multi-PI-resistant HIV-1 variants. It appears that both P2- and P2′-ligands of inhibitor **1** are involved in extensive hydrogen bonding with the protein backbone. Interestingly, examination of X-ray structures of **1**-bound protein−ligand complexes of wild-type HIV protease and mutant HIV proteases also revealed only a small distortion in the backbone conformations.[10] This is also evident in the X-ray structures of a number of other HIV

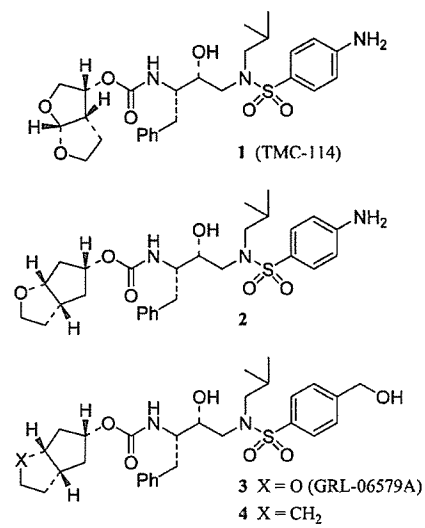

Figure 1. Structure of inhibitors **1**−**4**.

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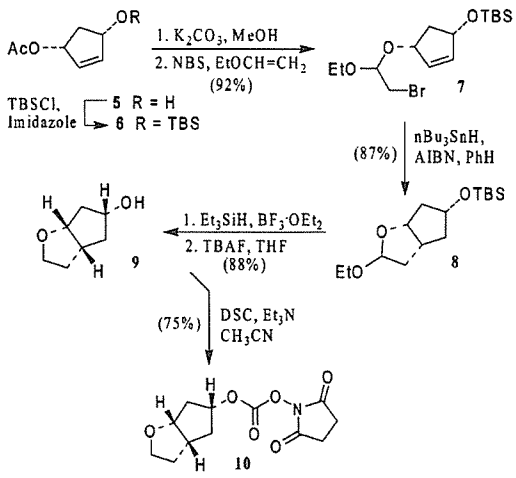
10.1021/jm060561m CCC: $33.50 © 2006 American Chemical Society
Published on Web 08/02/2006

Scheme 1. Synthesis of Hexahydrocyclopenta[*b*]furanyl Carbonate



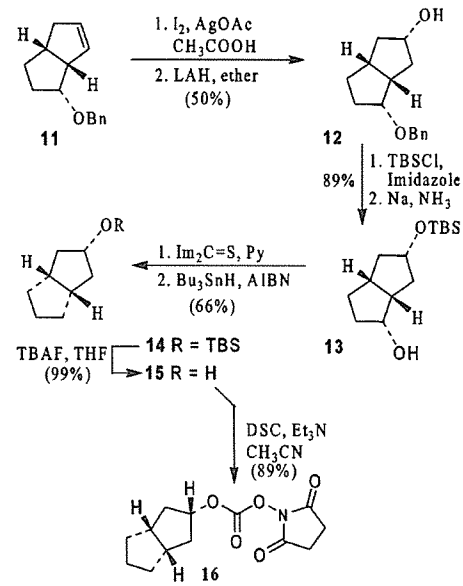
protease-inhibitor and related complexes.[11,12] Our working hypothesis to combat drug-resistance is to design inhibitors that make maximum interactions in the active site of HIV protease, particularly extensive hydrogen bonding interactions with the protein backbone in both S2- and S2′-sites. Conceivably, inhibitors exhibiting maximum hydrogen bonding interactions with the backbone of the wild-type enzyme may also retain potency against the mutant strains.

Upon the basis of this presumption, we have now designed and evaluated protease inhibitors (Figure 1) incorporating a novel ligand that can extensively interact with the backbone residues. The inhibitors incorporate a stereochemically defined bicyclic hexahydrocyclo-pentanofuran as the P2-ligand where the cyclic ether oxygen is positioned to hydrogen bond with the backbone Asp-29 NH. The bicyclic ligand is also expected to fill in the S2-subsite effectively. Furthermore, a 4-hydroxy-methylphenylsulfonamide is introduced as a P2′-ligand so that the hydroxyl group will be optimally positioned to hydrogen bond with the backbone residues in the S2′-subsite. Herein we report structure-based design, synthesis, preliminary biological evaluation, and X-ray crystal structure of inhibitor **3**-bound HIV-1 protease. The inhibitor has shown remarkable enzyme inhibitory and antiviral potency. Preliminary drug resistance profiles also indicated that the inhibitor maintains impressive potency against multi-PI-resistant clinical HIV-1 variants isolated from patients with drug-resistant HIV.

Chemistry

For synthesis of target inhibitors **2** and **3**, we devised an efficient synthetic route to (3a*S*,5*R*,6a*R*)-5-hydroxy-hexahydrocyclopenta[*b*]furan in optically active form. As shown in Scheme 1, enzymatic asymmetrization of meso-diacetate with acetyl cholinesterase provided the monoacetate **5** in multigram scale (85% yield).[13] Formation of Mosher ester of **5** revealed that enantiomeric purity of **5** was 95% ee.[14] Protection of hydroxyl group with TBSCl in the presence of imidazole in THF afforded TBS-ether **6**[15] in 98% yield. Hydrolysis of **6** with potassium carbonate in methanol gave the alcohol. Treatment of the resulting alcohol with ethyl vinyl ether and NBS in CH_2Cl_2 furnished bromo acetal **7**. Radical cyclization[16] of **7** with n-Bu_3-SnH in the presence of AIBN in benzene under reflux furnished bicyclic acetal **8** in excellent yield. Reduction of **8** with Et_3SiH in the presence of $BF_3.OEt_2$ followed by removal of TBS group with TBAF in THF afforded optically active hexahydrocyclo-

Scheme 2. Synthesis of P2-ligand and Active Carbonate



pentafuran-5-ol (**9**) in 88% yield. Treatment of **9** with *N,N*′-disuccinimidyl carbonate in the presence of Et_3N afforded mixed carbonate **10**.[17]

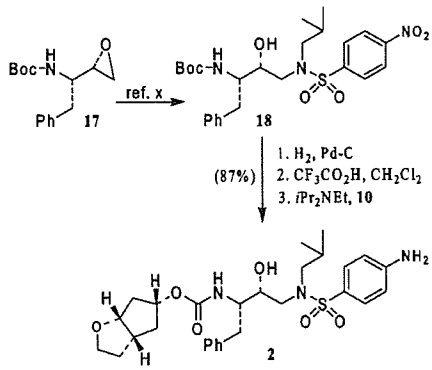
For the synthesis of inhibitor **4**, the corresponding P2-ligand, *endo-cis*-bicyclo[3.3.0]octan-3-ol was synthesized according to Scheme 2. Benzyl ether **11** was synthesized according to the literature procedure.[18] Exposure of **11** to iodine in the presence of silver acetate in acetic acid afforded the corresponding iodoacetate derivative.[19] Reduction of the resulting iodoacetate with LAH furnished hydroxy benzyl ether **12**[20] in 50% yield in two steps. The hydroxyl group in **12** was protected as TBS-ether by reaction with TBSCl and imidazole.[21] Subsequent removal of the benzyl ether with sodium in liquid ammonia[22] furnished hydroxyl TBS ether **13** in 89% yield. Removal of hydroxyl group in **13** was effected by using Barton–McCombie deoxygenation[23] reaction. Thus, alcohol **13** was reacted with *N,N*′-thiocarbonyldiimidazole in a mixture of (2:1) toluene and pyridine at 55 °C for 12 h. The resulting thiocarbonylimidazoyl derivative was treated with *n*Bu_3SnH in toluene at reflux to provide TBS ether **14** in 66% yield in two steps. Deprotection of TBS with TBAF in THF[21] furnished *endo-cis*-bicyclo[3.3.0]-octan-3-ol (**15**).[24] Treatment of alcohol **15** with *N,N*′-disuccin-imidyl carbonate in the presence of Et_3N in acetonitrile afforded succinimidyl carbonate **16** in excellent yield.[17]

The synthesis of inhibitor **2** with hexahydrocyclopenta[*b*]-furanyl urethane as the P2-ligand and 4-aminosulfonamide as the P2′-ligand is shown in Scheme 3. Nitrosulfonamide derivative **18** was prepared from commercially available epoxide **17** as described previously.[25] Catalytic hydrogenation of **18** over 10% Pd/C in ethyl acetate for 11 h afforded the corresponding aminosulfonamide derivative.

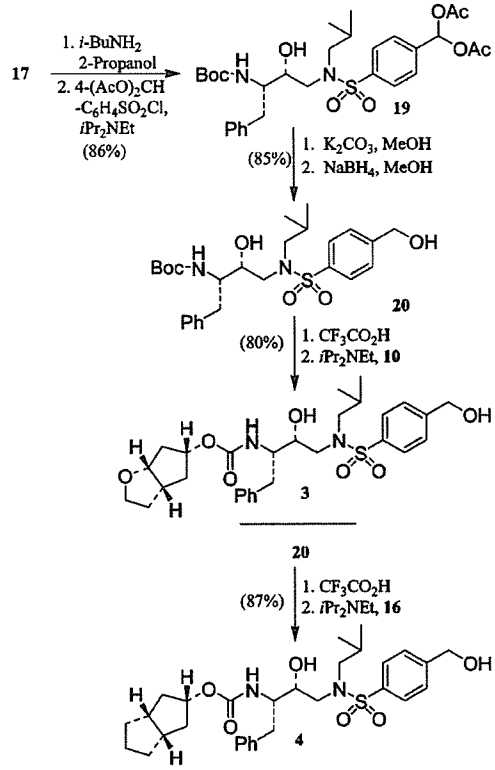
Subsequent removal of Boc-group was effected by exposure of the resulting aminosulfonamide to trifluoroacetic acid in CH_2-Cl_2 to furnish the corresponding diamine. Selective alkoxycarbonylation of the aliphatic amine with succinimidyl carbonate **10** provided inhibitor **2** in 87% yield in the three-step sequence.

Synthesis of inhibitors **3** and **4** was carried out as shown in Scheme 4. For the preparation of hydroxyethylamine sulfonamide inhibitor scaffold, commercially available epoxide **17** was reacted with isobutylamine in 2-propanol. Treatment of the resulting amino alcohol with *p*-(diacetoxymethyl)phenylsulfonyl

Scheme 3. Synthesis of Inhibitor **2**



Scheme 4. Synthesis of Inhibitors **3** and **4**



chloride[26] in the presence of diisopropylethylamine furnished sulfonamide derivative **19**. It was converted to hydroxymethylsulfonamide derivative **20** by deprotection of the acetates with K_2CO_3 and subsequent reduction of the resulting aldehyde with $NaBH_4$ in methanol. Sulfonamide derivative **20** was converted to inhibitor **3** by removal of Boc group by exposure to trifluoroacetic acid and reaction of the resulting amine with succinimidyl carbonate **10** and diisopropylethylamine in CH_2Cl_2. Inhibitor **2** was obtained in 80% yield. Similarly, reaction of the resulting amine with succinimidyl carbonate **16** afforded inhibitor **4** in 87% yield.

Results and Discussion

Inhibitors with hexahydrocyclopenta[*b*]furanyl urethane as the P2-ligand have shown impressive in vitro potency. Inhibitors **2**–**4** were evaluated in enzyme inhibitory and antiviral assays and the results are shown in Table 1. For enzyme inhibitory assay, we utilized the protocol described by Toth and Marshall[27]

Table 1. Enzyme Inhibitory and Antiviral Activity of Inhibitors

inhibitor[a]	K_i (nM)	IC_{50} (nM)
2	0.14 ±0.02	8
3	0.0045 ± 0.001	1.8
4	5.3 ± 0.3	>1000

[a]Inhibitor **1** has exhibited a K_i value of 14 pM and antiviral IC_{50} of 3 nM.

Table 2. Antiviral Data (IC_{50}) of **3** in PBMC and MT-2 Cells (nM)

virus	SQV	RTV	INV	NFV	APV	**3**
$HIV\text{-}1_{LAI}$[a]	14	43	32	14	34	1.8
$HIV\text{-}1_{BA\text{-}L}$[a]	18	36	24	7	29	2.0
$HIV\text{-}1_{LAI}$[b]	24	34	26	10	24	1.8
$HIV\text{-}2_{EHO}$[b]	1.9	290	13	20	440	21

[a] PBMC. [b] MT-2 cells; SQV (saquinavir), RTV (ritonavir), INV (indinavir), NFV (nelfinavir), APV (amprenavir); data represent the mean value of three determinations.

and the K_i-values denote the mean values of at least four determinations. As can be seen, inhibitor **2** which incorporates a stereochemically defined hexahydrocyclopenta[*b*]furanyl urethane as the P2-ligand and 4-aminosulfonamide as the P2′-ligand has shown enzymatic K_i value of 0.14 nM. As described previously, the X-ray crystal structure of protein–ligand complex of **1** and HIV-1 protease revealed that structural changes on the P2′-aryl ring could lead to improved interaction with the Asp-29 and Asp 30 NH.[10] In an effort to make the compound interact with backbone residues in the S2′-pocket more effectively, we incorporated a hydroxymethylsulfonamide derivative as the P2′-ligand in inhibitor **3**. This inhibitor exhibited a very impressive K_i value of 4.5 pM. To examine the importance of the ring oxygen of (*3aS,5R,6aS*)-5-hydroxy-hexahydro-cyclopenta[*b*]furanyl ligand in inhibitor **3**, the corresponding inhibitor **4** with an *endo*-3-bicyclo[3.3.0]octanyl urethane as the P2-ligand was evaluated. As shown, inhibitor **4** has shown an enzymatic K_i value of 5.3 nM, a >1100-fold reduction in potency compared to **3**. This marked difference in enzyme inhibitory potency is also reflected in their antiviral potency. Inhibitor **3** has shown an antiviral IC_{50} value of 1.8 nM in MT-2 human T-lymphoid cells exposed to $HIV\text{-}1_{LAI}$. Consistent with its enzyme inhibitory potency, inhibitor **2** has also shown good antiviral activity. In comparison, compound **4** has exhibited antiviral IC_{50} value of >1 μM, a drastic >500-fold reduction with respect to inhibitor **3**.

We have compared antiviral potency of inhibitor **3** against various FDA approved protease inhibitors. It has maintained a remarkable potency in MT-2 cells exposed to $HIV\text{-}1_{LAI}$ compared to other FDA approved protease inhibitors. Antiviral activity against three different HIV isolates was determined in PHA-PBMC and MT-2 cells. The results are shown in Table 2. This inhibitor exerted far more potent activity against two HIV-1 isolates ($HIV\text{-}1_{LAI}$ and $HIV\text{-}1_{Ba\text{-}L}$) in both MT-2 cells and PHA-PBMC than all currently available approved protease inhibitors examined. In addition, it was as potent against $HIV\text{-}2_{EHO}$ as indinavir and nelfinavir with an IC_{50} value of 21 nM. In vitro cytotoxicity of inhibitor **3** was minimal and its concentration that reduced the viability of target cells by 50% (CC_{50}) was greater than 100 μM.

We also examined inhibitor **3** for its antiviral activity against a panel of multi-drug-resistant HIV-1 variants as shown in Table 3. An HIV-1 clinical strain $HIV\text{-}1_{ET}$, isolated from a drug-naïve patient with HIV-1 infection, was sensitive to all protease inhibitors examined, among which inhibitor **3** was most potent with the lowest IC_{50} value of 3 nM. In contrast, each of six drug-resistant clinical strains containing 10–12 protease inhibi-