Forward- and reverse-synthesis of piperazinopiperidine amide analogs: a general access to structurally diverse 4-piperazinopiperidine-based CCR5 antagonists†

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Piperazinopiperidine amide analogs are among the most promising CCR5 antagonists. As an effective extension of a previously-reported methodology to synthesize such compounds, forward- and reverse-syntheses were successfully developed in which the convergent synthesis of the piperazinopiperidine nucleus, with a building block of 4-substituent-4-aminopiperidine, served as a common key step. The two-way approach affords a comprehensive access to the piperazinopiperidine templated library with variation on the pharmacophore sites. Thus, a SAR study of our synthesized piperazinopiperidine-based CCR5 antagonists was conducted with respect to the structure and configuration of the substituent on the piperazine ring. The *S*-configuration of the benzylic-substituent is vital for the CCR5 binding, and the bulky or aryl substituent on the 2-position in the piperazine ring is detrimental to the activity. By using the forward-synthesis approach, the best compound in the chiral piperazine-based CCR5 antagonist series, Sch-D (Vicriviroc), was conveniently synthesized in an excellent yield.

Introduction

Human immunodeficiency virus (HIV) infection, with its clinical progression to AIDS, has become one of the most fatal diseases in the world. Though highly-active antiretroviral therapy (HAART) has been successful in reducing HIV-1-associated mortality and morbidity, the emergence of multi-drug resistant viral strains and intolerance to available agents provide a compelling need to discover new drugs or targets for effective therapeutic intervention. The C-C chemokine receptor 5 (CCR5) is such an attractive target[1] since it is an essential co-receptor for HIV-1 recognition and entry into $CD4^+$ macrophages and T-cells[2] but not essential for human functions.[3] Many pharmaceutical companies and academic institutions have been enthusiastically investigating novel antagonists against CCR5 as a new class of anti-HIV agents,[4] and indeed several small-molecule CCR5 antagonists (Sch-D,[5] UK-427857,[6] GW-873140,[7] as shown in Fig. 1) are now being evaluated in clinical trials.

The piperazinopiperidine based compounds disclosed by the Schering-Plough Research Institute are among the most promising CCR5 antagonists, as exemplified by Sch-D, which is currently in phase II clinical trials. We are intrigued by the idea of building a piperazinopiperidine templated library to search for new structure CCR5 inhibitors with suitable pharmaceutical properties. Based on our previously-established methodology to construct the piperazinopiperidine scaffold by using a smart building block of 4-substituent-4-aminopiperidine,[8] we developed both forward- and reverse-synthetic routes to piperazinopiperidine amide analogs by sharing the convergent synthesis of the core structure as a key step in common (Scheme 1). Thus, we report herein our new, two-way strategy to synthesize structurally-diverse piperazinopiperidine based CCR5 antagonists, and an SAR with respect to the 1-*N*- and 2-substituent in the piperazine ring is discussed.

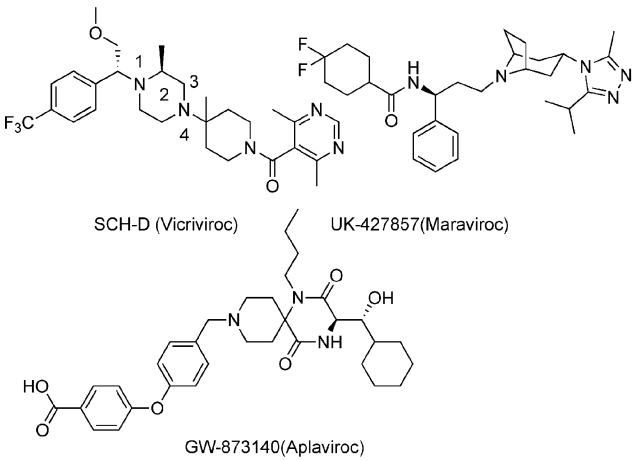

Fig. 1 Representative structures of piperidine-based CCR5 antagonists.

Results and discussion

As depicted in Scheme 1, our originally-developed methodology, starting from the aryl alkyl ketone/aldehyde coupled with an amino acid by reductive amination followed by construction of the piperazinopiperidine nucleus,[9] is referred to as the forward-synthesis approach; accordingly, the synthesis starting with the

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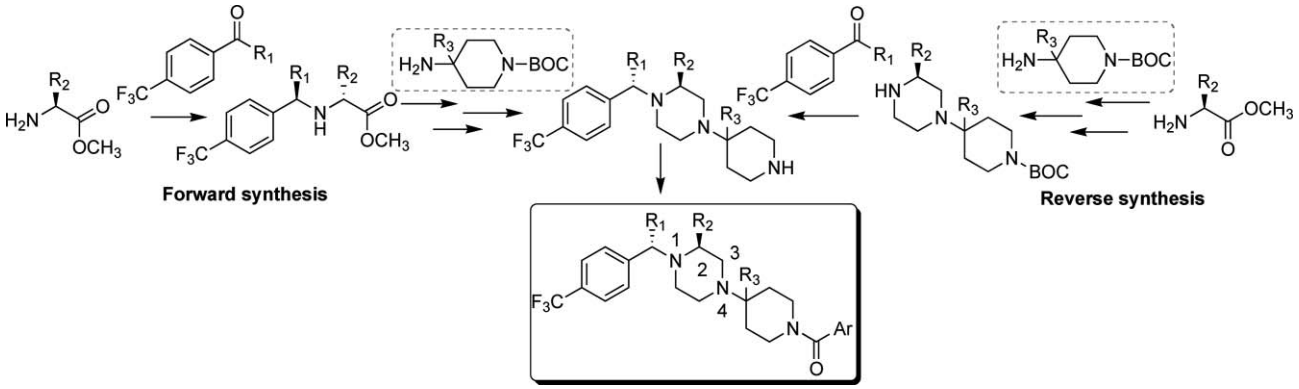


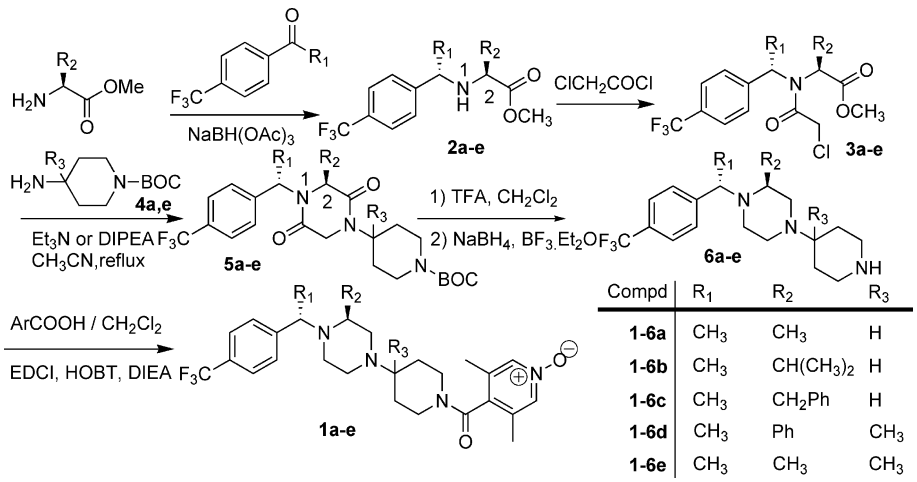
Scheme 1 Two-way strategy of synthesizing piperazinopiperidine amide analogs by employing the 4-substituted-4-aminopiperidine mediated construction of the piperazinopiperidine nucleus as a common key step.

construction of the piperazinopiperidine scaffold followed by coupling with the aryl alkyl ketone/aldehyde *via* a reductive amination is denoted the reverse-synthesis approach. The latter is an alternative approach to provide those chiral piperazine based compounds which are produced in poor stereoselectivity and yield by forward synthesis.

As reported in our previous work,[9] the forward synthesis is flexible for introducing various substituents and the desired chirality into key positions of the pharmacophore, thus compounds **1a**–**e** were conveniently synthesized with variation at the benzylic (1*N*-) position and 2-position of the piperazine ring (Scheme 2). The configuration of the benzylic position was investigated, so the (1*R*,2*S*)-isomers of compounds (1*S*,2*S*)-**1a**–**c** were synthesized as well.

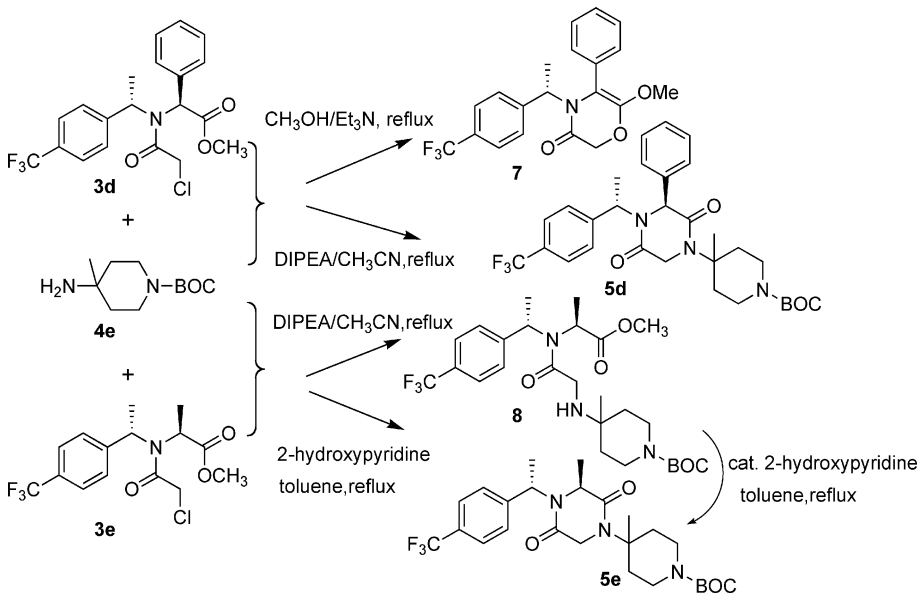
The absolute configuration of the stereogenic centre benzylic-C was assigned on the basis of H^1-NMR data using a well-documented methodology.[10] Basically, the H^1-NMR chemical shifts of substituents at C-2 are highly dependent on their absolute configuration, owing to phenyl shielding brought about by the adjacent chiral 1-phenethyl amine moiety. The phenyl group with *R*- or *S*-configuration exerted a different shielding effect on the C-2 substituents, resulting in an upfield shift of the *cis* proton.[10] In the *anti* (1*S*,2*S*) isomer, the (C-2)–**H** suffered a phenyl shielding by the (*S*)-phenyl moiety, whereas the (C-2)–$\mathbf{CH_3}$ is significantly shielded by the (*R*)-phenyl group in the *syn* (1*R*,2*S*) isomer. Typically, the analysis of ^{1}H-NMR spectra indeed reveals that the (C-2)–CH_3 is more shielded in (1*R*,2*S*)-**5a** (0.82 ppm) than in (1*S*,2*S*)-**5a** (1.47 ppm),[9] while the (C-2)–H is more shielded in (1*S*,2*S*)-**5a** (3.75 ppm)[9] than in (1*R*,2*S*)-**5a** (4.00 ppm). The case is true for other diastereoisomers (**5b**, **5c**) with different 2-substituents. However, the absolute configuration of 1*N*-substituent, assigned as described above, was further confirmed by stereoselectively synthesizing the (1*S*,2*S*)-**2a**, **c** according to the literature method by employing asymmetric reduction with (*S*)-CBS catalyst followed by an S_N2 reaction, as indicated in Scheme 4.[11]

So, the forward synthesis is efficient for synthesizing chiral piperazine-based compounds in most cases. However, during the synthesis of **1d** and **1e**, the construction of the core structure under the original reaction condition encountered some problems. As shown in Scheme 3, when phenyl-substituted (2-chloroacetyl)aminoacetic acid methyl ester (**3d**) was treated with 4-methyl-4-aminopiperidine (**4e**) in refluxing methanol, an intramolecular cyclization occurred to generate predominantly 6-methoxy-5-phenyl-2*H*-1,4-oxazin-3(4*H*)-one (**7**), in which the

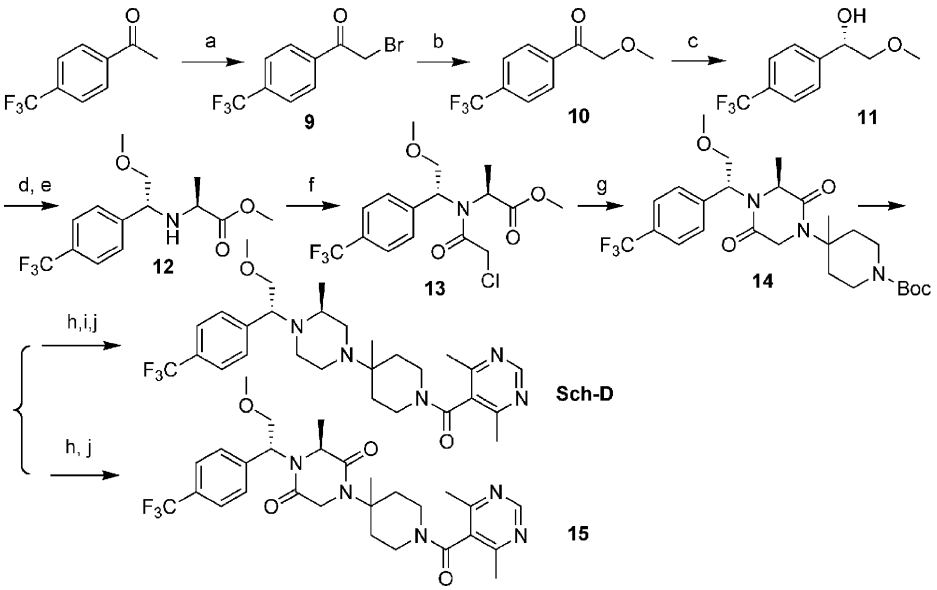


Compd	R_1	R_2	R_3
1-6a	CH_3	CH_3	H
1-6b	CH_3	$CH(CH_3)_2$	H
1-6c	CH_3	CH_2Ph	H
1-6d	CH_3	Ph	CH_3
1-6e	CH_3	CH_3	CH_3

Scheme 2 Forward synthesis.[9]



Scheme 3 Optimized conditions of the 4-substituted-4-aminopiperidine-mediated construction of the central structure.



Scheme 4 Modified forward-synthetic route toward Sch-D. Reagents and conditions: (a) Br_2, CH_2Cl_2, reflux, 88%; (b) Ag_2O, $BF_3{\cdot}Et_2O$, CH_3OH, 0 °C to r.t., 60%; (c) $BH_3{\cdot}Me_2S$, (*S*)-CBS catalyst, $B(OMe)_3$, THF, r.t., 92%; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C, 96%; (e) (*S*)-methyl 2-amino propanoate, CH_3CN, K_2CO_3, reflux, 60%; (f) chloroacetyl chloride, DCE, reflux, 96%; (g) **4e**, 2-hydroxypyridine, toluene, reflux, 54%; (h) TFA, CH_2Cl_2, r.t.; (i) $BH_3{\cdot}Me_2S$, THF, reflux; (j) 4,6-dimethylpyrimidine-5-carboxylic acid, EDCI, HOBt, DIPEA, CH_2Cl_2, r.t., 30% overall yield of three steps for Sch-D, 95% overall yield of two steps for **15**.

4-aminopiperidine served as a base instead of a building block. The resulting conjugate system might be the driving force for the enolization followed by intramolecular nucleophilic substitution. When the solvent (methanol) was changed to acetonitrile, the intermolecular nucleophilic substitution followed by lactamization proceeded smoothly in one step in the presence of triethylamine or diisopropylethylamine under reflux. For the synthesis of 4-methyl-4-aminopiperidine-containing compounds, when the substituent in the 2-position is an alkyl group, the mono-substituted intermediate was isolated and underwent intramolecular lactamization in different reaction conditions (with refluxing toluene and catalytic 2-pyridinol),[12] but the cyclization can be readily accomplished in one step by using directly 2-pyridinol as a base in refluxing toluene.

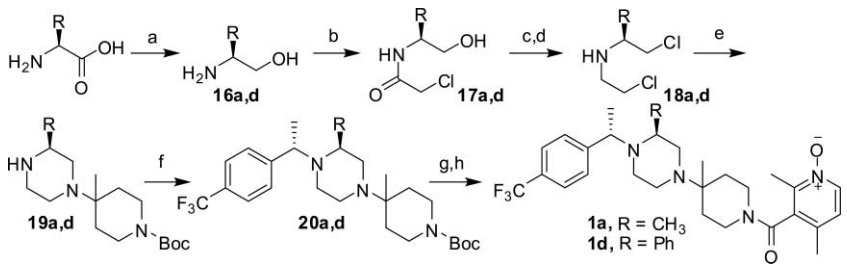
So, with the optimized conditions in hand, we started to synthesize Sch-D *via* our forward-synthesis approach. The starting material, *i.e.* α-methoxyacetophenone (compound **10**) was prepared from α-bromoacetophenone (compound **9**) by the nucleophilic substitution of the methoxy group under the conditions of Ag_2O, methanol and $BF_3{\cdot}Et_2O$.[13] This reaction proceeded well at an ambient temperature, while Schering-Plough's Weinreb amide methodology required a temperature of −78 °C for the addition of aryllithium.[5] However, the reductive amination of

α-methoxyacetophenone (**10**) with L-alanine methyl ester failed, so the asymmetric reduction with CBS catalyst followed by an S_N2 displacement route was adapted to afford **12** diastereoselectively in high yield. In the following procedures, the 4-methyl-4-aminopiperidine building block strategy was successfully applied to the synthesis of the piperazinopiperidine scaffold in Sch-D (**14**). Considering that spirodiketopiperazine is another active scaffold of CCR5 antagonists,[14] we kept the diketopiperazine moiety unchanged to build the diketopiperazinopiperidine amide **15** under standard conditions, while the final synthesis of the piperazinopiperidine amide Sch-D was conveniently achieved by using our reported methodology.[9]

In the forward-synthetic approach, the amino acid was used as a chiral pool to introduce and then induce the desired stereochemistry, however, the asymmetric induction effect is dependent on the structure of the side chain of the amino acid, thus the *dr* value [the diastereomeric ratio of (1*S*,2*S*) over (1*R*,2*S*)] of the reductive amination product is low (dr = 1.2–1.5) when the side-chain bears a bulky group. We suppose that a conformationally-constrained amine might improve the stereoselectivity of the reductive amination due to the steric hindrance, so we proposed the reverse-synthesis strategy, in which the piperazinopiperidine core was constructed first, then served as an amine component to react with the phenyl acetone *via* a reductive amination, in order to achieve a better chiral induction effect. On the other hand, this approach could overcome the enolization problem during the cyclization step (as indicated in Scheme 3) when the 2-substituent on the piperazine ring is an aryl or α,β-unsaturated group, which is apt to drive the formation of enolate.

As depicted in Scheme 5, the reverse synthetic approach is featured with the rigid bulky piperazinopiperidine moiety participated in the reductive amination of the acetophenone (**19** → **20**), which might induce a higher ratio of the *anti* isomer over the *syn* isomer with respect to the substituents on the 1*N*- and 2-positions of the piperazine ring. The key intermediate of 3-substituted-1-(4-methylpiperidin-4-yl)piperazine **19** was synthesized in a similar way to that described above, just starting with an amino alcohol **16**. Amidation of **16** with chloroacetyl chloride followed by the reduction of the amide with borane generated by $NaBH_4$ and $BF_3{\cdot}Et_2O$ *in situ*, and further treatment with $SOCl_2$ furnished the dichloride **18**. The following cyclization still employed the nucleophilic substitution of 1-Boc-4-amino-4-methylpiperidine as a common step. The reductive amination of **19** with 4-trifluoromethylacetophenone indeed afforded a higher dr value (dr = 3) even when the 2-substitutent is a bulky phenyl group (**20d**). The reverse-synthesis approach provides an efficient alternative to prepare structurally-diverse piperazinopiperidine amide analogs with modification on the benzylic position and 2-position of the piperazine ring.

The compounds prepared above were evaluated for their inhibitory effects on RANTES-stimulated [^{35}S]-GTPγS binding to CCR5-expressing CHO cell membranes. The results are summarized in Table 1 as IC_{50} values. It is obvious that the configuration of the 1*N*-substituent is vital for the CCR5 inhibitory activity, with the (*S*)-configuration being preferred, which was amply demonstrated by the significant difference of the activity between the diastereoisomers of (1*S*,2*S*)-**1a** and (1*R*,2*S*)-**1a**, (1*S*,2*S*)-**1b** and (1*R*,2*S*)-**1b**, (1*S*,2*S*)-**1c** and (1*R*,2*S*)-**1c**. The structure of the 2-substituent is another key factor for the CCR5 affinity. The bulky substituent (compounds **1b**, **1c**) or aryl substituent (compound **1d**) on the 2-position in the piperazine ring is detrimental to the inhibitory activity against CCR5. This finding is consistent



Scheme 5 Reverse synthesis. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C to reflux, 95%; (b) $ClCOCH_2Cl$, H_2O and CH_2Cl_2, 1 N NaOH solution, 0 °C, 70%; (c) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, reflux; (d) $SOCl_2$, $CHCl_3$, r.t.; (e) **4e**, CH_3CN, DIPEA, reflux, 56% overall yield for three steps; (f) 4-(trifluoromethyl)acetophenone, $NaBH(OAc)_3$, HOAc, THF, r.t., 42%; (g) TFA, CH_2Cl_2, r.t.; (h) 3-carboxy-2,4-dimethylpyridine 1-oxide, EDCI, HOBt, DIPEA, CH_2Cl_2, r.t.

Table 1 CCR5 binding data of the piperazinopiperidine based compounds with modification on the benzylic position and 2-position on the piperazine ring

Compounds	IC_{50}/μM[a]	Compounds	IC_{50}/μM[a]
(1*S*,2*S*)-**1a**[9]	0.03	(1*R*,2*S*)-**1c**	Inactive
(1*R*,2*S*)-**1a**	1.42	**1d**	22% inhibition @10 μM
(1*S*,2*S*)-**1b**[9]	31% inhibition @10 μM	**1e**	0.018
(1*R*,2*S*)-**1b**[9]	Inactive	Sch-D	0.0039
(1*S*,2*S*)-**1c**[9]	20	**15**	Inactive

[a] Inhibition of RANTES-stimulated [^{35}S]-GTPγS binding to CCR5-expressing CHO cell membranes.

with literature-reported results.[5] Unfortunately, the incorporation of a diketopiperazine moiety into the piperazinopiperidine-based CCR5 antagonist resulted in a substantial loss of binding with CCR5 (compound **15**).

Conclusions

In this study, we successfully developed a two-way strategy to synthesize piperazinopiperidine amide analogs as CCR5 antagonists with variation on the configuration and structure of the 1*N*- and 2-substituent on the piperazine ring. The forward-synthesis and the reverse-synthesis employ our previously-established methodology to construct the piperazinopiperidine nucleus with a building block of 4-substituted 4-aminopiperidine as a common key step. The significant advantage of the reverse-synthesis approach can provide an improved asymmetric induction effect of the reductive amination. By using the modified forward-synthesis approach, the best compound in the chiral piperazine-based CCR5 antagonist series, Sch-D (Vicriviroc) was conveniently synthesized in good yield. The currently-developed two-way strategy affords a comprehensive access to the structurally-diverse piperazinopiperidine amide analogs with variation on the pharmacophore sites. A preliminary SAR study of our synthesized piperazinopiperidine-based CCR5 antagonists was investigated with respect to the structure and configuration of the substituent on the piperazine ring. The *S*-configuration of the benzylic-substituent is critical for the effective CCR5 binding, and the bulky or aryl substituent on the 2-position in the piperazine ring caused a substantial loss of the activity.

Experimental

All reactions were performed under a nitrogen atmosphere with flame-dried glassware. Solvents were distilled and dried according to standard procedures. ^{1}H-NMR spectra were recorded on a Varian 300-MHz or 400-MHz spectrometer. ^{13}C-NMR spectra were recorded on a Varian Mercury VX 400-MHz spectrometer. Melting points (uncorrected) were determined on a Buchi-510 capillary apparatus. Specific rotations (uncorrected) were determined in a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a DTGS spectrometer in KBr pellets. Low and high resolution mass spectra were determined on a Finnigan MAT-95 mass spectrometer. TLC was performed on 0.25 mm HSGF 254 silica gel plates. The key products were characterized by NMR, MS and high resolution mass spectra. The synthetic procedures and physicochemical data of compounds **3d**, (1*R*,2*S*)-**3a**, (1*R*,2*S*)-**3c**, **7**, **8**, **13**–**15**, **16a**–**18a** and **20a** are reported in the ESI.†

(*S*)-Methyl-2-phenyl-2-((*S*)-1-(4-(trifluoromethyl)phenyl)-ethylamino)acetate (2d)

The mixture solution of L-phenylglycine methyl ester hydrochloride (1.285 g, 6.41 mmol) and Et_3N (1.117 mL, 6.41 mmol) in dry 1,2-dichloroethane (15 mL) was stirred at room temperature for 0.5 h, the 4′-(trifluoromethyl)acetophenone (1.0 g, 5.34 mmol) was added, treated with sodium triacetoxyborohydride (2.266 g, 10.69 mmol) and HOAc (0.61 mL, 10.69 mmol). The mixture was stirred at room temperature for 22 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with Et_2O. The organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4. The solvent was removed under vacuum. The residue was purified by chromatography using petroleum ether–ether = 10 : 1 to give compound **2d** as colorless oil (0.975 g, 54% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (d, 2H, J = 8.0 Hz), 7.36–7.31 (m, 5H), 7.24–7.17 (m, 2H), 4.12 (s, 1H), 3.59 (s, 3H), 3.58 (m, 1H), 2.42 (br-s, 1H), 1.32 (d, 3H, J = 6.7 Hz).

tert-Butyl-4-((*S*)-2,5-dioxo-3-phenyl-4-((*S*)-1-(4-(trifluoromethyl)-phenyl)ethyl)piperazin-1-yl)-4-methylpiperidine-1-carboxylate [(1*S*,2*S*)-5d]

The compound **3d** (939 mg, 2.276 mmol), *tert*-butyl-4-amino-4-methylpiperidine-1-carboxyate **4e** (0.5 g, 2.3 mmol) and Et_3N (0.35 mL) in MeCN (10 mL) was refluxed overnight and the solvent was removed under reduced pressure. The residue was purified by chromatography using petroleum ether–EtOAc = 1 : 1 to give compound (1*S*,2*S*)-**5d** as white solid (712 mg, 56% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59–7.20 (m, 7H), 6.92–6.90 (d, 2H, J = 8.1 Hz), 5.17–4.97 (m, 2H), 4.65–4.40 (m, 1H), 4.15–3.81 (m, 1H), 3.82–3.73 (m, 4H), 3.14–3.09 (m, 1H), 2.16–1.90 (m, 2H), 1.87 (s, 2H), 1.45 (s, 9H), 1.42–1.22 (m, 2H), 1.26 (s, 3H). EI-MS (m/z): 559 [M]$^+$. IR (KBr): 3446, 2976, 1745, 1673, 1423, 1327, 1168, 1070, 1016, 850 cm^{-1}. $[\alpha]_D^{20}$ = +16.8 (c = 2.5, $CHCl_3$).

tert-Butyl-4-((*S*)-3-methyl-2,5-dioxo-4-((*R*)-1-(4-(trifluoromethyl)-phenyl)ethyl)piperazin-1-yl)piperidine-1-carboxylate [(1*R*,2*S*)-5a]

The procedure is similar to the preparation of **5d** to give (1*R*,2*S*)-**5a** as a white solid (62% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (d, 2H, J = 8.2 Hz), 7.52 (d, 2H, J = 8.2 Hz), 5.87 (q, 1H, J = 7.1 Hz), 4.46 (m, 1H), 4.22 (brs, 2H), 4.00 (q, 1H, J = 7.1 Hz), 3.86 (q_{AB}, 2H, J = 16.8 Hz), 2.77 (m, 2H), 1.60 (d, 3H, J = 7.2 Hz), 1.61–1.45 (m, 4H), 1.46 (s, 9H), 0.82 (d, 3H, J = 7.1Hz). EI-MS (m/z): 483 [M]$^+$. IR (KBr): 3431, 2982, 1676, 1659, 1443, 1327, 1142, 1072 cm^{-1}. $[\alpha]_D^{20}$ = +119 (c = 1.45, $CHCl_3$).

tert-Butyl-4-((*S*)-3-benzyl-2,5-dioxo-4-((*R*)-1-(4-(trifluoromethyl)-phenyl)ethyl)piperazin-1-yl)piperidine-1-carboxylate [(1*R*,2*S*)-5c]

The procedure is similar to the preparation for **5d** to give (1*R*,2*S*)-**5c** as a white foam (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 4H), 7.25 (m, 3H), 6.85 (d, 2H, J = 7.2 Hz), 5.85 (q, 1H, J = 7.2 Hz), 4.40–4.32 (m, 2H), 4.13 (m, 2H), 3.28 (d, 1H, J = 16.8 Hz), 2.88 (dd, 1H, J = 10.5, 3.6 Hz), 2.72 (m, 2H), 2.16 (d, 1H, J = 13.6 Hz), 2.15 (d, 1H, J = 16.4 Hz), 1.69 (d, 3H, J = 7.2 Hz), 1.43 (s, 9H), 1.40–1.04 (m, 4H). EI-MS (m/z): 559 [M]$^+$. HR-EI-MS calcd for $C_{30}H_{36}F_3N_3O_4$ 559.2658, found 559.2671. IR (KBr): 3481, 2978, 2935, 1693, 1659, 1454, 1425, 1367, 1327, 1167, 1124, 1072, 1016, 851, 702 cm^{-1}. $[\alpha]_D^{20}$ = +113 (c = 1.35, EtOAc).

2,4-Dimethyl-3-(4-methyl-4-((*S*)-3-phenyl-4-((*S*)-1-(4-(trifluoro-methyl)phenyl)ethyl)piperazin-1-yl)piperidine-1-carbonyl)pyridine 1-oxide [(1*S*,2*S*)-1d]

The mixture solution of compound **5d** (0.28 g, 0.5 mmol) and trifluoroacetic acid (5 mL) in methylene chloride (2.5 mL) was stirred at room temperature for 2 h. After removing the solvent and trifluoroacetic acid under reduced pressure, 2 N NaOH was added and extractive work up with EtOAc. The organic layer

was washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was removed under vacuum to give the crude diketopiperazine, which was dissolved in dimethoxyethane (5 mL). To the solution were added sodium borohydride (0.189 g, 5.0 mmol) and boron trifluoride etherate (0.38 mL, 3.0 mmol). The mixture was stirred under reflux for 3 h and then cooled to 0 °C. Methanol (6 mL) and concentrated hydrogen chloride (3.6 mL) were added, respectively. The reaction mixture was stirred for 15 minutes at room temperature, then refluxed for 45 minutes. The mixture was concentrated, and basified with 6 N sodium hydroxide, then extracted with EtOAc. Usual work-up was applied to the combined organic layers to give the compound **6d** as a glassy solid. The crude piperidine **6d** was dissolved in methylene chloride (2 mL) and treated with 2,4-dimethylnicotinic acid-*N*-oxide (0.1 g, 0.6 mmol), EDCI (0.144 g, 0.75 mmol), HOBT (0.101 g, 0.75 mmol) and 0.175 mL DIPEA. The mixture was stirred at room temperature overnight and the solvent was removed under reduced pressure. The residue was purified by chromatography using CH_2Cl_2–CH_3OH (30 : 1) to give the compound **1d** as white foam (62 mg, 21.3% overall yield of three steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.57–8.54 (d, 1H, J = 8.7 Hz), 8.33–8.30 (d, 1H, J = 6.9 Hz), 8.07–8.04 (d, 1H, J = 8.7 Hz), 7.94–7.92 (d, 1H, J = 8.1 Hz), 7.71–7.68 (d, 2H, J = 7.8 Hz), 7.31–7.12 (m, 5H), 3.62–3.58 (m, 2H), 3.51–3.36 (m, 3H), 3.20–2.95 (m, 4H), 2.41–2.26 (m, 4H), 2.26 (s, 3H), 2.04–1.87 (m, 4H), 1.41–1.25 (m, 2H), 1.24–1.22 (d, 3H, J = 7.2 Hz), 1.20 (s, 3H). ESI-MS (m/z): 581.3 [M+1]$^+$. $[\alpha]_D^{20}$ = +12.05 (c = 0.95, $CHCl_3$).

2,4-Dimethyl-3-(4-((*S*)-3-methyl-4-((*R*)-1-(4-(trifluoromethyl)-phenyl)ethyl)piperazin-1-yl)piperidine-1-carbonyl)pyridine 1-oxide [(1*R*,2*S*)-1a]

The procedure is similar to the preparation of **1d** to give (1*R*,2*S*)-**1a** as a white foam (70% overall yield of three steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (d, 1H, J = 6.9 Hz), 7.56 (d, 2H, J = 8.1 Hz), 7.37 (d, 2H, J = 8.1 Hz), 6.99 (d, 1H, J = 6.9 Hz), 4.74 (br-t, 1H), 4.10 (m, 1H), 3.46 (br-d, 1H), 2.98–2.85 (m, 3H), 2.67–2.59 (m, 3H), 2.43 (d, 3H, J = 16.2 Hz), 2.44–2.11 (m, 4H), 2.22 (d, 3H, J = 17.1 Hz), 2.01–1.95 (m, 1H), 1.78–1.61 (m, 2H), 1.42 (d, 3H, J = 6.6 Hz), 1.32–1.26 (m, 1H), 1.10 (d, 3H, J = 6.0 Hz). EI-MS (m/z): 504 [M]$^+$. HR-EI-MS calcd for $C_{27}H_{35}O_2F_3N_4$ 504.2712, found 504.2679. $[\alpha]_D^{20}$ = +14.1 (c = 1.18, $CHCl_3$).

3-(4-((*S*)-3-Benzyl-4-((*R*)-1-(4-(trifluoromethyl)phenyl)ethyl)-piperazin-1-yl)piperidine-1-carbonyl)-2,4-dimethylpyridine 1-oxide [(1*R*,2*S*)-1c]

The procedure is similar to the preparation of **1d** to give (1*R*,2*S*)-**1c** as a white foam (55% overall yield of three steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.16 (d, 1H, J = 6.3 Hz), 7.62 (d, 2H, J = 8.1 Hz), 7.50 (d, 2H, J = 8.1 Hz), 7.14 (m, 3H), 7.00 (t, 1H, J = 6.3 Hz), 6.76 (m, 2H), 4.78 (m, 1H), 4.02 (q, 1H, J = 6.3 Hz), 3.37 (m, 1H), 2.97–2.76 (m, 7H), 2.64 (m, 1H), 2.52 (m, 1H), 2.44 (d, 3H, J = 12.6 Hz), 2.30 (m, 1H), 2.23 (d, 3H, J = 12.1 Hz), 2.10 (m, 1H), 1.98 (m, 1H), 1.71–1.65 (m, 2H), 1.38 (d, 3H, J = 6.3 Hz), 1.40–1.36 (m, 2H). ESI-MS (m/z): 581.2 [M+1]$^+$. HR-ESI-MS calcd for $C_{33}H_{39}N_4O_2F_3$ +H 581.3098, found 581.3116. $[\alpha]_D^{20}$ = +13.8 (c = 1.04, $CHCl_3$).

2-Bromo-1-(4-(trifluoromethyl)phenyl)ethanone (9)[14]

The solution of 4-trifluoromethylacetophenone (3.7 g, 20 mmol) in 60 mL of methylene chloride was heated to reflux. Within 1 h, a solution of bromine (1.05 mL, 20 mmol) in 20 mL of methylene chloride was added dropwise to the boiling solution under vigorous stirring. During the reaction, HBr gas was removed by a positive nitrogen pressure. The organic reaction mixture was stirred overnight at room temperature, then evaporated. The residue was recrystallized from PE to give white crystals (4.7 g, 88% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.11 (d, 2H, J = 8.1 Hz), 7.77 (d, 2H, J = 7.8 Hz), 4.47 (s, 2H). mp 53–54 °C (lit.[15] mp 53–54 °C).

2-Methoxy-1-(4-(trifluoromethyl)phenyl)ethanone (10)

Ag_2O (2.85 g, 12.3 mmol) was added to the solution of 20 mL of $BF_3{\cdot}Et_2O$ and 10 mL of CH_3OH at 0 °C. After the silver oxide was dissolved completely, the bromoketone **9** (1.64 g, 6.1 mmol) in 10 mL of CH_3OH was added to the reaction solution at room temperature, and the stirring continued for 22 h. Then, the insoluble material was filtered off and washed with methanol and diethyl ether. The organic layer was washed with water and extracted with ether. The combined organic extracts were dried over anhydrous Na_2SO_4. Silica gel column chromatography using ethyl ester–PE = 1 : 6 afforded the pure product (863 mg, 60% yield) as a white crystal. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.04 (d, 2H, J = 7.8 Hz), 7.72 (d, 2H, J = 8.1 Hz), 4.70 (s, 2H), 3.50 (s, 3H). mp 48–50 °C.

(*S*)-2-Methoxy-1-(4-(trifluoromethyl)phenyl)ethanol (11)

To the solution of (*S*)-2-(diphenylhydroxymethyl)-pyrrolidine (101 mg, 40 mmol) in dry THF (15 mL) was added trimethyl borate (50 mg, 0.48 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 2 h. After a 2 M borane–dimethyl sulfide complex in Et_2O (2 mL, 4 mmol) was added, the solution of compound **10** (820 mg, 3.75 mmol) in dry THF (15 mL) was added dropwise over 1 h with a syringe pump. The mixture was stirred at room temperature for another 1 h, until compound **10** disappeared on TLC monitoring. The resulting mixture was quenched with methanol in an ice bath and concentrated under reduced pressure. The residue was purified on a silica gel column, using petroleum ether and ethyl acetate (6 : 1, v/v) as an eluent, to give yellowish oil **11** (761 mg, 92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, 2H, J = 7.8 Hz), 7.49 (d, 2H, J = 8.1 Hz), 4.93 (d,1H, J = 5.1 Hz, 3.3 Hz), 3.55 (q, 1H, J = 3.0 Hz, 6.9 Hz, 3.3 Hz), 3.42 (s, 3H), 3.41 (q, 1H, J = 3.3 Hz), 2.94 (br-s, 1H).

(*S*)-Methyl 2-((*R*)-2-methoxy-1-(4-(trifluoromethyl)phenyl)-ethylamino)propanoate (12)

MsCl (0.22 mL, 2.8 mmol) and Et_3N (0.7 mL, 5.1 mmol) were added to the solution of compound **11** (512 mg, 2.3 mmol) in 25 mL of CH_2Cl_2 in an ice bath. It was stirred at the same temperature for 0.5 h. After the evaporation of the solvent, the residue was diluted with Et_2O, washed with brine and dried over anhydrous Na_2SO_4. The removal of the solvent gave the mesylate (662 mg, 96% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, 2H, J = 9.0 Hz), 7.53 (d, 2H, J = 8.1 Hz), 5.74 (d,1H, J = 3.6 Hz, 4.8 Hz, 3.3 Hz), 3.76 (q,

1H, $J = 7.8$ Hz, 3.0 Hz, 8.7 Hz), 3.62 (q, 1H, $J = 3.3$ Hz, 7.5 Hz, 3.9 Hz), 3.42 (s, 3H), 3.01 (s, 3H).

The mixture of the mesylate (1062 mg, 3.56 mmol), alanine methyl ester (741 mg, 7.12 mmol) and dry K_2CO_3 (738 mg, 5.34 mmol) in 20 mL of CH_3CN was refluxed for 4 days. The reaction was monitored by TLC until all the mesylate was consumed. After the solvent was removed *in vacuo*, the residue was diluted with EtOAc, washed with brine and dried over anhydrous Na_2SO_4. Concentration *in vacuo* left a yellowish oil, which was chromatographed (PE–EtOAc = 6 : 1) to give the desired (*R*,*S*) diastereomer **12** (654 mg, 60% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58 (d, 2H, $J = 8.7$ Hz), 7.48 (d, 2H, $J = 8.1$ Hz), 3.96 (t, 1H, $J = 6.6$ Hz), 3.71 (s, 3H), 3.39 (s, 3H), 3.36 (q, 1H, $J = 3.6$ Hz), 3.10 (q, 1H, $J = 7.5$ Hz, 6.9 Hz, 6.9 Hz), 2.59 (br-s, 1H). $[a]_D^{24} = -111.3$ ($c = 3.8$, $CHCl_3$).

(4,6-Dimethylpyrimidin-5-yl)(4-((*S*)-4-((*R*)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methylpiperidin-1-yl)methanone (Sch-D)

To the solution of compound **14** (136 mg, 0.25 mmol) in methylene chloride (5 mL) was added trifluoroacetic acid (0.4 mL). The mixture was stirred at room temperature for 1.0 h, and was evaporated under reduced pressure. The resulting residue was dissolved in 5 mL of THF, then borane–dimethylsulfide complex (1.25 mL, 2 M in Et_2O) was added dropwise. The mixture was stirred at reflux under nitrogen overnight, then it was quenched with 2 mL of methanol in an ice bath. The solvent was evaporated *in vacuo*. The residue was used for the next reaction without further purification. The crude piperazine was dissolved in DCM (3 mL) and treated with 4,6-dimethylpyrimidine-5-carboxylic acid (43 mg, 0.28 mmol), EDCI (76 mg, 0.38 mmol), HOBt (53 mg, 0.38 mmol) and DIPEA (0.1 mL). The mixture was stirred at room temperature for 24 h. Then the mixture was diluted with EtOAc, usual work-up was applied. The residue was purified by chromatography (10–50% EtOAc-CH_2Cl_2, then 5% CH_3OH–CH_2Cl_2) to give the title compound as white foam (31 mg, 30% yield for 3 steps). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.94 (s, 1H), 7.58–7.51 (dd, 4H, $J = 9.0$ Hz, 2.1 Hz), 4.24–4.20 (br-d, 1H, $J = 13.2$ Hz), 4.03 (br-s, 1H), 3.80–3.69 (m, 2H), 3.49–3.39 (m, 2H), 3.34 (s, 3H), 3.10 (br-s, 1H), 3.00–2.95 (m, 1H), 2.69–2.62 (m, 1H), 2.48 (s, 3H), 2.45 (s, 3H), 2.44 (br-s, 1H), 2.37–2.23 (m, 3H), 2.00–1.80 (m, 2H), 1.46–1.36 (m, 1H), 1.30–1.23 (m, 2H), 1.19–1.17 (d, 3H, $J = 6.3$ Hz), 0.93 (s, 3H). ESI-MS (*m*/*z*): 534.2 $[M+H]^+$, 535.3 $[M+2H]^+$. $[a]_D^{22} = +6.6$ ($c = 0.5$, free base in MeOH) [Lit.[5] $[a]_D^{25} = +13.3$ (2.44 mg cc^{-1} hydrochloride salt in MeOH)].

(*S*)-2-Amino-2-phenylethanol (16d)

Lithium aluminium hydride (2.75 g, 72.3 mmol) was suspended in 100 mL of THF at 0 °C. L-Phenylglycine (5.0 g, 33 mmol) was added slowly in small portions. The reaction mixture was heated to reflux overnight and then cooled to room temperature. Saturated K_2CO_3 solution was added slowly. Filtration and evaporation of the solvent gave yellow solid. The residue was recrystallized from hexane and EtOAc (3 : 1) to give (*S*)-phenylglycinol as yellow solid (4.4 g, 97% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39–7.23 (m, 5H), 4.04 (dd, 1H, $J = 4.4$ Hz, 8.0 Hz), 3.70 (dd, 1H, $J = 4.4$ Hz, 10.8 Hz), 3.54 (dd, 1H, $J = 8.0$ Hz, 10.8 Hz), 2.60 (br-s, 3H).

(*S*)-2-Chloro-*N*-(2-hydroxy-1-phenylethyl)acetamide (17d)

A vigorously-stirred solution of **16d** (4.0 g, 29.2 mmol) in 20 mL of water at 0 °C was treated successively and slowly with a solution of chloroacetyl chloride (2.78 mL, 35 mmol) in 20 mL of CH_2Cl_2, then was added 4.5 mL of 1 N NaOH solution. When the addition was complete, it was stirred at r.t. for 1 h. After the solvent was removed *in vacuo*, the residue was diluted with EtOAc, washed with brine and dried over anhydrous Na_2SO_4. Concentration *in vacuo* left a yellow solid, which was subjected to the recrystallization (PE–EtOAc) to give **17d** as white solid (4.0 g, 64.3% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36–7.27 (m, 6H), 5.06–5.04 (m, 1H), 4.05 (dd, 2H, $J = 15.2$, 8.8 Hz), 3.90–3.82 (m, 2H), 2.74 (br-s, 1H). $[a]_D^{20} = +31.8$ (c 0.9, $CHCl_3$).

(*S*)-2-Chloro-*N*-(2-chloro-1-phenylethyl)acetamide (18d)

The procedure is similar to the preparation for **18a** to give **18d** (450 mg, 31% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40–7.29 (m, 5H), 4.25 (dd, 1H, $J = 4.0$ Hz, 9.2 Hz), 3.72 (dd, 1H, $J = 4.0$ Hz, 10.8 Hz), 3.68–3.56 (m, 3H), 2.96 (br-s, 2H). EIMS: 217 (M^+).

tert-Butyl 4-methyl-4-((*S*)-3-phenyl-4-((*S*)-1-(4-(trifluoromethyl)-phenyl)ethyl)piperazin-1-yl)piperidine-1-carboxylate (20d)

The procedure is similar to the preparation for **20a** to give **20d** as white foam (42.5% yield), and the other isomer (14.1% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68–7.20(m, 9H), 4.78 (brs, 1H), 3.95–3.48 (m, 2H), 3.45–3.40 (m, 2H), 3.04–2.8 (m,2H), 2.50–2.32 (m, 5H), 1.92–1.84 (m, 2H), 1.46 (s, 9H), 1.50–1.32 (m, 2H), 1.15 (d, 3H, $J = 6.4$ Hz), 0.9 (s, 3H).

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