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An Imidazopiperidine Series of CCR5 Antagonists for the Treatment of HIV: The Discovery of *N*-{(1*S*)-1-(3-Fluorophenyl)-3-[(3-*endo*)-3-(5-isobutyryl-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]propyl}acetamide (PF-232798)

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Preventing entry of HIV into human host cells has emerged as an attractive approach to controlling viral replication. Maraviroc **1** is an approved antagonist of the human CCR5 receptor which prevents the entry of HIV. Herein, we report the design and discovery of a series of imidazopiperidine CCR5 antagonists which retain the attractive antiviral profile and window over hERG activity of maraviroc **1**, combined with improved absorption profiles in rat and dog. Furthermore, this series of compounds has been shown to retain activity against a laboratory generated maraviroc-resistant HIV-1 strain, which indicates an alternative resistance profile to that of maraviroc **1**. Compound **41f** (PF-232798) was selected as a clinical candidate from the imidazopiperidine series and is currently in phase II clinical trials.

Introduction

Highly active antiretroviral therapy (HAART[a]) for the treatment of human immunodeficiency virus (HIV) involves a combination of antiretroviral drugs which can deliver sustained reductions in viral load.[1] Initial classes of such drugs were nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors. More recently, a fusion inhibitor (enfuvirtide) and an integrase inhibitor (raltegravir) have been approved. Although these both target new mechanisms, as with the initial drug classes, they rely on interactions with viral proteins. The strategy of preventing viral entry into the host cell by interacting with a human receptor is an attractive alternative which could deliver an agent with a broad spectrum of activity against different viral clades.

Antagonism of the chemokine coreceptor CCR5 has received the most attention within this arena.[2] One of the reasons for this is the excellent genetic evidence for validation of this target provided by CCR5 homozygotes with a 32 base pair deletion in the coding region for the CCR5 gene, which confers almost complete resistance to HIV infection.[3] A number of compounds have been progressed to clinical trials and of these maraviroc **1** became the first CCR5 antagonist to be approved in the USA and Europe for treatment experienced patients in 2007. Compound **1** has more recently been approved for treatment naive patients in the USA.[2,4] The absence of alternative approved agents within this drug class is at least in part explained by the challenge of achieving the required drug-like properties (antiviral activity, absence of hERG activity, acceptable clearance, suitable bioavailability, etc.) in molecules which are typically high molecular weight.

Compound **1** is characterized by excellent antiviral (AV) activity across a range of HIV isolates, a significant window over any hERG (human ether-a-go-go related gene) mediated cardiovascular effects and selectivity over a broad panel of other G-protein coupled receptors.[4,5] Compound **1** is dosed b.i.d. clinically and has moderate bioavailability in man (23%) with food effects on oral exposure.[6,7] During the discovery of **1**, achieving the desired pharmacological profile in an orally biovailable molecule was a significant challenge. In particular, few compounds combined acceptable oral absorption with an appropriate window over hERG channel inhibition. To address this, we initiated a follow-up program with the aim of identifying a new series of CCR5 antagonists with improved oral absorption. Within such a template, we hoped to optimize metabolic stability to increase the chances of identifying an agent suitable for clinical q.d. dosing. Additionally, demonstrating activity against maraviroc-resistant virus was a key factor during the design of new CCR5 antagonists.[8]

Compound Design

As a result of the attractive profile of **1**, we were keen to retain the tropane core from this series. In particular, we had observed excellent antiviral activity with this chemotype and

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[a] Abbreviations: AB, apical to basal; AV, antiviral; BA, basal to apical; CYP, cytochrome P450; DLM, dog liver microsomes; ECL2, extracellular loop 2; HAART, highly active antiretroviral therapy; HEK, human embryonic kidney; hERG, human ether-a-go-go related gene; HLM, human liver microsomes; HIV, human immunodeficiency virus; MVC^{RES}, expanded B-clade maraviroc-resistant HIV-1 isolate CC185; NMP, *N*-methyl-2-pyrrolidone; PBMC, peripheral blood mononucleocyte cells; RLM, rat liver microsomes; SAR, structure−activity relationship.

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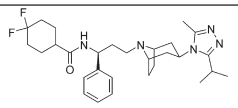
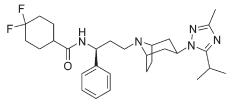
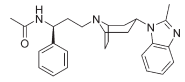
incorporation of the tropane had overcome problems such as CYP inhibition.[5] Optimization of other properties was planned by replacing the *N*- and *C*- substituents within the tropane scaffold.

Table 1. gp160 Fusion Activity, hERG Channel Affinity, and Permeability for Lead Compounds

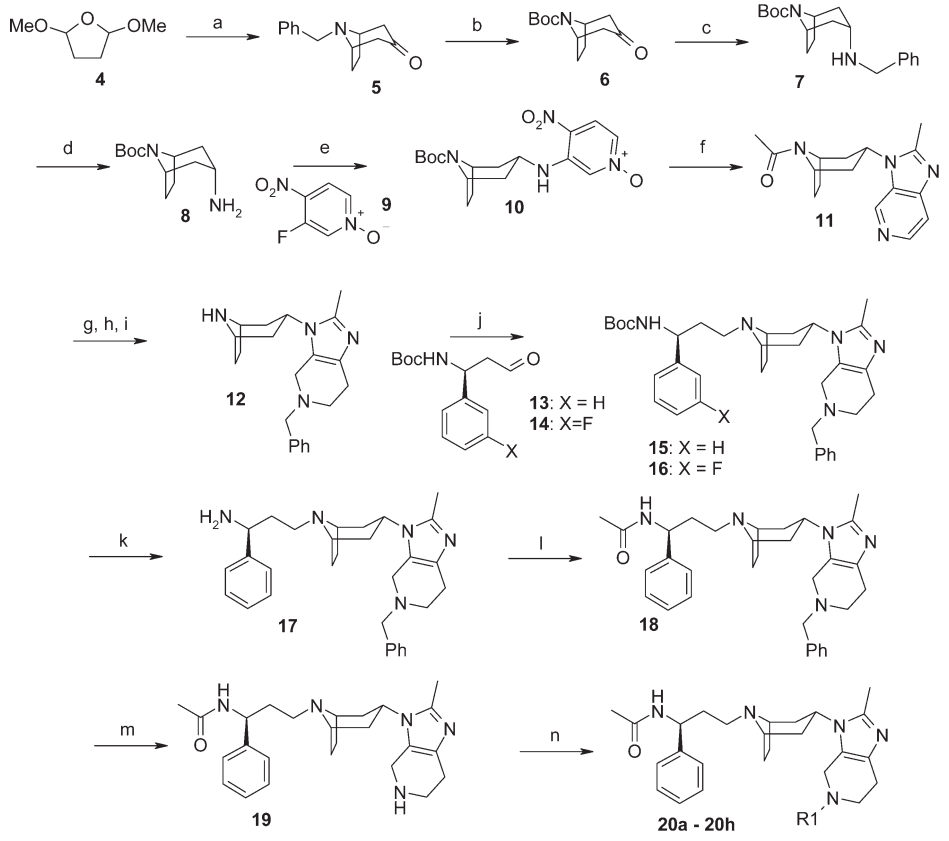
	Compound	gp160 Fusion IC_{50} (nM)[a]	hERG binding[b]	Caco-2 AB/BA[c]
1		0.2	0% @ 300 nM	<1/12
2		1.4	IC_{50} 4230 nM	11/26
3		0.5	IC_{50} 1500 nM	13/35

[a] IC_{50} determinations were the mean of at least two replicates. [b] Inhibition of [^{3}H]-dofetilide binding to hERG stably expressed on HEK-293 cells. [c] $P_{app} \times 10^{-6}$ cms^{-1}.

The incomplete absorption of **1** in man was not surprising as this had also been observed preclinically (approximately 20% absorption in rat and 50% absorption in dog).[9] We were confident that permeability was limiting exposure, which was consistent with in vitro data as shown in Table 1 (caco-2 AB/BA < 1/12). Encouragement that optimization was possible was provided by close structural analogues with isomeric triazoles, which had shown improved caco-2 profiles, e.g. **2** (caco-2 AB/BA 11/26). The enhancement in caco-2 AB flux was hypothesized to result from a reduction in the extent of hydration of the heterocycle due to a change in dipole moment as the aromatic heterocycle is varied.[5,10,11] This was one of the factors which encouraged us to consider replacements for the 1,2,4-triazole in future targets.

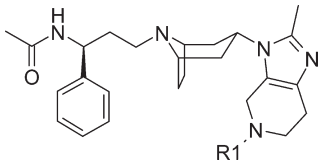
It was found that changing the nature of the amide substituent within the tropane scaffold had a significant effect on hERG channel inhibition. Previous data indicated that the difluoro moiety of **1** was not tolerated by the hERG channel, which is consistent with the findings that addition of polar groups, e.g. amides, sulfonamides, and sulfones, in this part of the molecule can significantly reduce hERG activity.[12,13] Furthermore, docking of examples from this series into a model of the hERG channel suggested that the amide alkyl substituent overlaps with a lipophilic region of the hERG pharmacophore. We reasoned that reducing the size of the

Scheme 1. Synthesis of 3-Substituted 1,4,6,7-Tetrahydro-imidazo[4,5-*c*]pyridine



Reagents and conditions: (a) (1) aq HCl, 0 °C, (2) $PhCH_2NH_2$, $CO(CH_2CO_2H)_2$, NaOAc, H_2O, 50 °C, 47% (2 steps); (b) $(Boc)_2O$, 20% $Pd(OH)_2$ on carbon, H_2, EtOAc, RT, quant; (c) $PhCH_2NH_2$, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, RT, 50%; (d) NH_4HCO_2, 20% $Pd(OH)_2$ on carbon, EtOH, 50 °C, 94%; (e) K_2CO_3, MeCN, reflux, 52%; (f) (1) Fe, AcOH, 130 °C, (2) Ac_2O, 140 °C, 65% (2 steps); (g) H_2, AcOH, 60 °C, 95% yield; (h) PhCHO, $Na(OAc)_3BH$, AcOH, CH_2Cl_2, RT, 47% yield; (i) 6N aq HCl, reflux, 87%; (j) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, RT, 59−74%; (k) 2N HCl, MeOH, 91%; (l) CH_3COCl, CH_2Cl_2, RT, 90%; (m) 20% Pd on carbon, NH_4HCO_2, EtOH, reflux, 93%; (n) for **20a** and **20b**, $(CH_3)_2CO$ or paraformaldehyde, AcOH, $Na(OAc)_3BH$, RT, 22−65%, for **20c** $MeSO_2Cl$, Et_3N, CH_2Cl_2, RT, 68%, for **20d** CH_3COCl, CH_2Cl_2, RT, 94%, for **20e−h** RCO_2Cl or RCOCl, Et_3N, CH_2Cl_2, RT, 67−86%.

Table 2. Profiles of 3-Substituted 1,4,6,7-Tetrahydro-imidazo[4,5-*c*]pyridines



compd	R1	gp160, IC_{50} (nM)[a]	hERG binding (%)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
18	$PhCH_2$	29				
19	H	9				
20a	Me	5	0	< 1/9	< 7	0.4
20b	*i*-Pr	3			46	0.8
20c	$MeSO_2$	2	10		24	0.9
20d	MeCO	0.4	0	< 1/5	< 7	0.6
20e	EtCO	0.4	13	< 1/17	< 7	1.2
20f	*i*-PrCO	0.4	0	< 1/20	42	1.6
20g	$MeCO_2$	0.3	20	3/16	18	1.5
20h	*i*-$PrCO_2$	< 0.1	75	5/28	44	2.7

[a] IC_{50} determinations were the mean of at least two replicates. [b] Inhibition of [^{3}H]-dofetilide binding to hERG stably expressed on HEK-293 cells with a compound concentration of 300 nM. [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

amide to acetyl would minimize these lipophilic interactions and therefore reduce affinity to the channel.

Benzimidazole **3**[14] was identified as an attractive lead with potent activity in the gp160 fusion assay, which we have shown to be a good surrogate for antiviral activity.[15] This was combined with moderate activity in a hERG binding assay consistent with our hypothesis about small amide substituents. The permeability of **3** was also considered to be attractive (Table 1), and molecular weight (MW 416) was relatively low compared with **1** (MW 513). For these reasons, **3** was selected as a suitable template for further work.

The previously reported hERG modeling studies also suggested that the benzimidazole of **3** overlaps with a lipophilic binding area of the ion channel.[12,13] With this in mind, we were keen to investigate replacements which featured a polar group to disrupt lipophilic interactions within the binding site. Furthermore, by reducing the size of the amide substituent of **1** and introducing a larger tropane *C*-substituent, we reasoned that we were introducing the potential for alternative interactions with the CCR5 receptor, thus increasing our chances of seeing a different resistance profile to that of **1**. As a result of these hypotheses, the initial targets designed featured an imidazopiperidine as a replacement for the benzimidazole of **3**.

On the basis of the SAR determined during the discovery of **1** (logD 1.9), we felt we were most likely to achieve an appropriate balance of permeability, potency, and metabolic stability with a logD in the range 1.5−2.5. Final compounds were therefore designed to lie in appropriate physicochemical space to achieve a logD within this range. In addition, the design of compounds with increased molecular weight was avoided.[16]

Synthetic Chemistry

Synthesis of the initial imidazopiperidine template is shown in Scheme 1.[17] The tropane core was constructed by reaction of dimethoxy tetrahydrofuran **4**, benzylamine, and a ketodiacid, which gave **5**. After a change of *N*-protecting group, a primary amine was introduced stereoselectively under reductive amination conditions, followed by debenzylation. Reaction between tropane **8** and pyridine *N*-oxide **9** gave aminopyridine *N*-oxide **10**. Reduction of the nitro group, followed by cyclization, furnished the imidazopyridine **11**, which was accompanied by an exchange of protecting group from Boc to acetyl. Hydrogenation of the pyridine followed by protection of the resulting secondary amine and deacetylation gave imidazopiperidine **12**. Alkylation using homochiral aldehyde **13**[18,19] gave the versatile Boc-protected intermediate **15**. Removal of the Boc, acetylation, and debenzylation formed tropane **19**. From intermediate **19**, a range of functionalities (R1) was introduced to give final compounds **20a**−**h** (see Table 2).

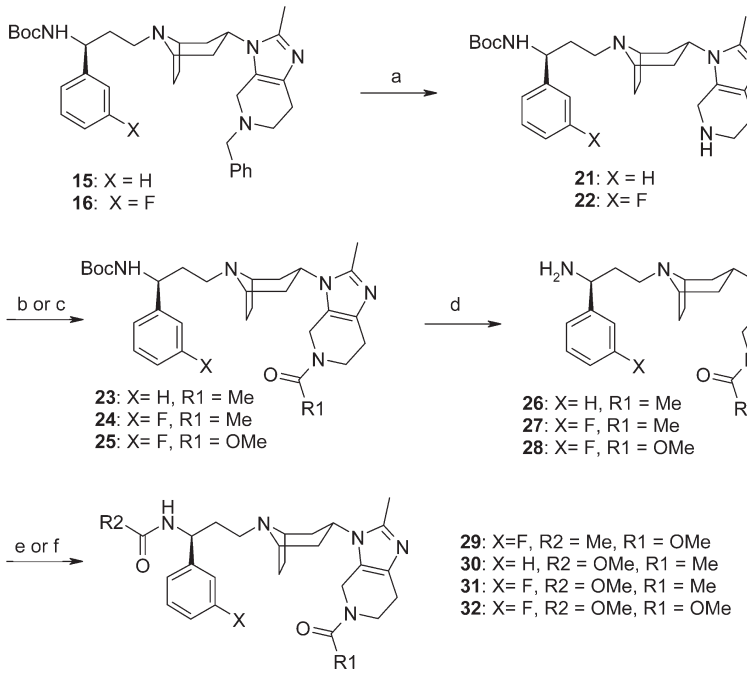
Scheme 2 shows the synthesis of a set of analogues in which the primary amino substituent was derivatized in the final step.[17] Debenzylation of **15**/**16** and subsequent introduction of either an acetyl (**23** and **24**) or a methyl carbamate (**25**), followed by Boc deprotection, gave the primary amine intermediates **26**, **27**, and **28**.

The synthesis of an isomeric imidazopiperidine is shown in Scheme 3 using a similar set of transformations to those shown in Scheme 1.[17,20] A related series of imidazopiperidine CCR5 antagonists has recently been reported[21] in addition to the disclosure of alternative series of tropanes.[22−24]

Results and Discussion

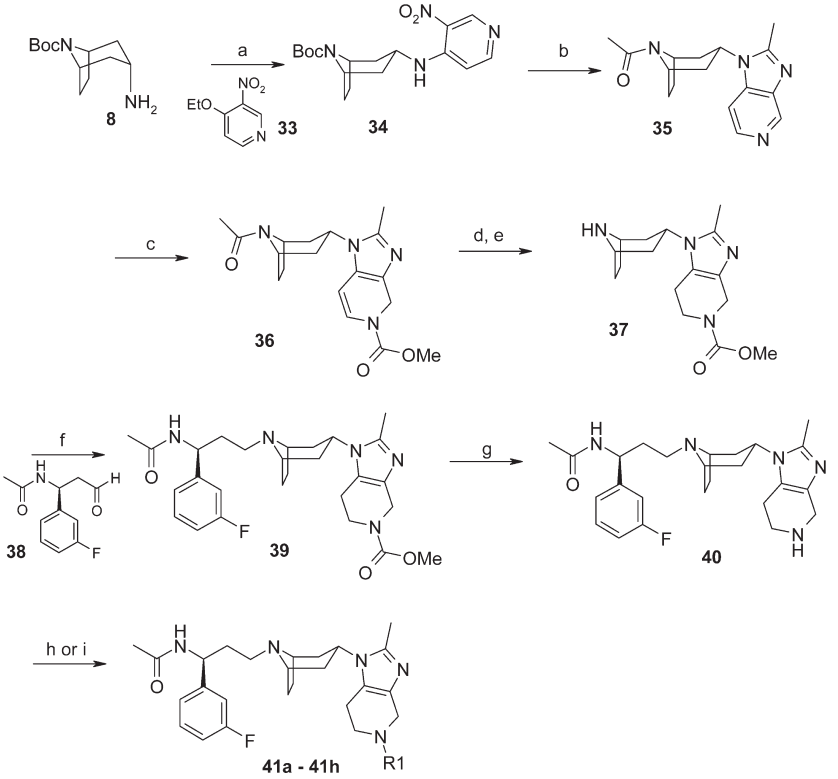
The first indication of activity from the imidazopiperidine scaffold was provided by benzyl intermediate **18**, for which the IC_{50} in the gp160 fusion assay was an encouraging 29 nM (Table 2). Trimming back the benzyl group to methyl gave **20a**, which showed a 6-fold potency improvement. Furthermore, hERG binding activity was not detected and the in vitro intrinsic clearance in human liver microsomes (HLM) was low (< 7 μL/min/mg). However, caco-2 flux was poor (AB/BA < 1/9), which was perhaps not surprising based on the dibasic nature and the resulting polarity (logD 0.4). Despite the caco-2 data, **20a** provided encouragement that balancing the required in vitro properties in this series may well be possible. Although the potency of the more lipophilic isopropyl analogue **20b** was similar (IC_{50} 3 nM), metabolic stability was reduced, which discouraged us from introducing further alkyl substituents. Sulfonamide **20c** showed a similar overall profile. Introduction of an acetyl substituent (**20d**) showed exquisite potency in the gp160 assay (IC_{50} 0.4 nM), similar for the first time to that of **1** (IC_{50} 0.2 nM). This level of potency is

Scheme 2. Synthesis of 3-Substituted 1,4,6,7-Tetrahydro-imidazo[4,5-*c*]pyridine



Reagents and conditions: (a) NH_4HCO_2, Pd/C (10% w/w) or 20% $Pd(OH)_2$ on carbon, EtOH, 60 °C, 86%-quant; (b) for **23** and **24**, CH_3COCl, CH_2Cl_2, RT, 90−99%; (c) for **25**, $ClCO_2CH_3$, CH_2Cl_2, RT, 93%; (d) HCl(g), MeOH, CH_2Cl_2, 0 °C−RT, 66−92%; (e) for **29**, CH_3COCl, Hunig's base, CH_2Cl_2, RT, 86%; (f) for **30**−**32**, CH_3CO_2Cl, Hunig's base, CH_2Cl_2, RT, 78−87%.

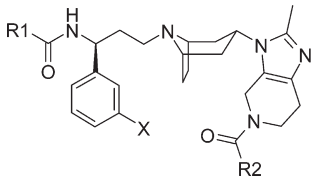
Scheme 3. Synthesis of 1-Substituted 1,4,6,7-tetrahydro-imidazo[4,5-*c*]pyridine



Reagents and conditions: (a) Hunig's base, NMP, 120 °C, 33%; (b) (1) Fe, AcOH, 60 °C, (2) Ac_2O, 120 °C, 91%; (c) (1) $ClCO_2CH_3$, EtOH, −70 °C, (2) $NaBH_4$, −70 °C to RT, 74%; (d) 10% Pd on carbon, H_2, EtOH, 50 °C, 91%; (e) 2 M aq H_2SO_4, reflux, 80%; (f) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, RT, 67%; (g) NaOH, H_2O, propan-2-ol, reflux, 73%; (h) for **41a** and **41b**, (i) 1. 4,6-dichloropyrimidine or 2,3-dichloropyrazine, Et_3N, *n*-BuOH, 100 °C, 74−80%, (2) H_2, Pd/C, EtOH, RT, 57%; for **41d**, **41e**, **41g**, and **41h**, R1COCl, Hunig's base, CH_2Cl_2, RT 55−97%, for **41f**, $(CH_3)_2CHCOCl$, Et_3N, THF, RT, 90%.

particularly impressive when considering the polarity of **20d** (logD 0.4; 1.5 units lower than **1**), which is consistent with significantly improved lipophilic efficiency compared to that of **1**. It was hoped that the increased polarity of **20d** would

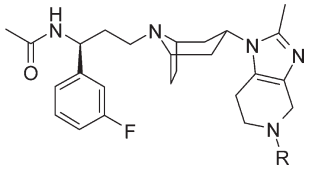
Table 3. Profiles of 3-Substituted 1,4,6,7-Tetrahydro-imidazo[4,5-c]pyridines



compd	R1	R2	X	gp160 IC_{50} (nM)[a]	hERG binding (%)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
29	Me	OMe	F	0.1	0	2/23	19	1.9
30	OMe	Me	H	0.6	57	4/27	42	1.4
31	OMe	Me	F	0.2	28	6/23	24	1.7
32	OMe	OMe	F	0.2	69	17/17	92	2.6

[a] IC_{50} determinations were the mean of at least two replicates. [b] Inhibition of [^{3}H]-dofetilide binding to hERG stably expressed on HEK-293 cells with a compound concentration of 300 nM. [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

Table 4. Profiles of 1-Substituted 1,4,6,7-Tetrahydro-imidazo[4,5-*c*]pyridines



compd	R	gp160, IC_{50} (nM)[a]	hERG binding IC_{50} (μM)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
41a	pyrimidin-4-yl	< 0.1	0.01	4/15	35	2.6
41b	pyrazin-2-yl	< 0.1	1		183	3.0
39	CO_2Me	< 0.1	2	3/11	< 8	1.9
41d	COMe	< 0.1	5	< 1/6	< 8	1.4
41e	COEt	< 0.1	5	2/13	11	1.7
41f	CO^{i}Pr	< 0.1	12	2/8	13	2.0
41g	COPr	< 0.1	6	1/26	25	2.2
41h	CO^{t}Bu	< 0.1	5	1/24	35	2.8

[a] IC_{50} determinations were the mean of at least two replicates. [b] Fluorescence polarization assay using a fluorescently labeled analogue of dofetilide.[26] [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

result in the attractive potency being combined with favorable HLM stability, which was indeed found to be the case (Cl_{int} < 7 μL/min/mg). There were no signs of hERG inhibition, but once again we were unable to achieve caco-2 AB flux values above the limit of quantification, which was also the case for the more lipophilic amides **20e** and **20f** (caco-2 AB < 1 for all three examples).

Alternative changes to acetamide **20d** were considered that would result in increased lipophilicity to target improved permeability. A methyl carbamate was proposed as a less polar expression of the corresponding acetamide. Carbamate **20g** retained excellent activity in the gp160 fusion assay and good metabolic stability. Significantly, **20g** exhibited the potential for improved oral absorption (caco-2 AB 3) compared with **1** (caco-2 AB < 1). The increase in AB flux between equilipophilic **20f** and **20g** can be rationalized by the weaker H-bond acceptor capacity of the carbamate, which would reduce the dehydration penalty on entering a membrane. A positive effect on permeability/transporter activity of this change has recently been reported elsewhere.[25] Unfortunately, there were signs of hERG inhibition, which only increased with the more lipophilic carbamate **20h**, although low but measurable caco-2 AB flux was once again observed for **20h**.

A thorough analysis of the hERG binding data within the tropane series revealed a trend that affinity was generally reduced by incorporation of a *meta*-fluoro substituent onto the phenyl ring (data not shown), which had little effect on activity in the gp160 fusion assay. This trend continued, as inhibition in the hERG binding assay appeared to be reduced in compound **29** compared with *des*-fluoro analogue **20g** (0% and 20% at 300 nM, respectively) with the other in vitro parameters being similar (Table 3). Swapping the substituent of the primary amine from acetyl to methyl carbamate was investigated with **30** and **31**, which were found to have higher hERG inhibition than the original compounds (inhibition at 300 nM: **30**/**20g** 57%/20% and **31**/**29** 28%/0%). These data indicated that once again hERG inhibition was reduced with the *meta*-fluoro in **31**. A significantly improved caco-2 profile was achieved in the bis-carbamate **32** (caco-2 AB/BA 17/17), although this also resulted in unacceptably high hERG activity (69% at 300 nM) and reduced metabolic stability (Cl_{int} 92 μL/min/mg), all of which were presumably driven by the extra lipophilicity (logD 2.6).

To investigate the effect of altering the vector of the polar group attached to the bicycle, the 1-substituted 1,4,6,7-tetrahydro-imidazo[4,5-c]pyridine was also considered (Table 4). Early signs that the nature of the polar substituent could significantly affect the interactions with the hERG channel were provided by heterocycle **41a**, which showed very potent inhibition (IC_{50} 0.01 μM). We were very interested to see that a 100-fold reduction in hERG binding was observed with the isomeric compound **41b**. These data encouraged us that the nature of the polar group could have significant effects on

Table 5. Antiviral Activity of Lead Compounds

compd	gp160 IC_{50} (nM)[a]	AV IC_{90} (nM)[b]
1	0.2	3.2 ($n = 4$)
29	0.1	1.6 ($n = 6$)
39	< 0.1	0.8 ($n = 8$)
41f	< 0.1	2.0 ($n = 5$)

[a] IC_{50} determinations were the mean of at least two replicates. [b] Antiviral activity against HIV BaL in PBMCs.

hERG activity. Carbamate **39**, the isomeric analogue of **29**, possessed a very attractive profile. Excellent potency (IC_{50} < 0.1 nM) was combined with what appeared to be an acceptable window over hERG activity, low but measurable flux in caco-2 cells (AB/BA 3/11) and excellent metabolic stability (Cl_{int} < 7 μL/min/mg). Encouraged by the difference between the hERG data for **41a** and **41b**, we were keen to investigate alternative substituents to attempt to further reduce hERG binding activity. The more polar bisacetamide **41d** showed decreased hERG binding but also reduced flux in the caco-2 assay consistent with previous SAR. Significantly, hERG binding did not increase as the amide substituent became increasingly more lipophilic (**41e**−**h**). A clear disconnect between the gp160 and hERG binding SARs was identified in compound **41f**, which demonstrated particularly weak hERG binding affinity (IC_{50} 12 μM). These data are consistent with the introduction of the steric bulk in **41f** introducing a clash with the hERG channel. Furthermore, the elevated logD of **41f** compared to acetamide **41d** (2.0 and 1.4, respectively) contributed to improved flux in caco-2 cells (AB/BA 2/8), with sustained metabolic stability (Cl_{int} 13 μL/min/mg).

Compounds **29**, **39**, and **41f** were selected for further screening as these offered the best balance of in vitro properties. It is worth noting the similarity in logDs (1.9, 1.9, 1.9, and 2.0 for **1**, **29**, **39**, and **41f**, respectively), which highlights the value of having defined a target range prior to compound design/synthesis.

The data from evaluation in an antiviral assay using mitogen-stimulated peripheral blood mononucleocyte cells (PBMC) from pooled donors and HIV Ba-L are shown in Table 5. The three new imidazopiperidine compounds possessed enhanced antiviral activity compared to that of **1** with up to a 4-fold improvement for **39**.

We were keen to assess if the improved caco-2 profiles of **29**, **39**, and **41f** translated to improved in vivo oral absorption. As the oral absorption of **1** in rat was estimated to be 20% (see Table 6), we were very pleased to find that a similar assessment for **29** and **39** was consistent with complete absorption. Unfortunately, the low bioavailability of **41f** prevented an estimation of absorption in rat; however, exposure in hepatic portal vein cannulated rats was found to be similar to **39**, which gave us confidence that **41f** was also well absorbed. On the basis of oral bioavailabilities of 44%, 50%, and 31%, respectively, in dog, oral absorption was estimated to be complete in each case, compared with 70% for **1**. The data from both rat and dog gave us confidence that we had identified compounds with the potential for complete oral absorption in man.

Table 7 shows in vitro data for the stability of the lead imidazopiperidines in human, rat, and dog liver microsomes. Compounds **39** and **41f** showed identical profiles to **1** in rat and dog, with only compound **29** having intrinsic clearances above the limit of detection. Despite the attractive metabolic stability, in vivo rat pharmacokinetic studies revealed high total/unbound clearances in all cases as had been found for **1**.

Table 6. Pharmacokinetic Properties of Lead Compounds in Rat and Dog iv/po Studies[a]

	1[b]	**29**[c]	**39**[d]	**41f**[c]
rat PPB (%)[e]	46	71	68	62
rat Cl (mL/min/kg)	74	146	91	201
rat Cl_u (mL/min/kg)[f]	137	518	253	526
rat $t_{1/2}$ (h)	2.3	1.7	2.3	1.4
rat F (%)	6	8	20	< 5
estimated rat absorption (%)	20	complete	complete	
dog PPB (%)[e]	60	62	73	47
dog Cl (mL/min/kg)	21	36	36	36
dog Cl_u (mL/min/kg)[f]	53	93	150	63
dog V_d (L/kg)	4.3	11.8	9.9	10.5
dog $t_{1/2}$ (h)	2.3	3.8	3.3	3.4
dog F (%)	42	44	50	31
estimated dog absorption (%)	70	complete	complete	complete

[a] Assuming rat liver blood flow is 100 mL/min/kg and dog is 50 mL/min/kg. [b] Rat iv 1 mg/kg, po 10 mg/kg; dog iv 0.5 mg/kg, po 1 mg/kg. [c] Rat iv 1 mg/kg, po 2 mg/kg; dog iv 0.5 mg/kg, po 0.5 mg/kg. [d] Rat iv 2 mg/kg, po 2 mg/kg; dog iv 0.5 mg/kg, po 0.5 mg/kg. [e] % of drug bound to plasma proteins; blood:plasma partitioning is 1:1 for all compounds in rat and dog. [f] Cl_u is unbound clearance.

Table 7. Human, Rat and Dog Intrinsic Clearance Values from in Vitro Liver Microsome Assays

	1	**29**	**39**	**41f**
HLM Cl_{int}[a,b]	49		17	7
RLM Cl_{int}[a]	< 6	15	< 6	< 6
DLM Cl_{int}[a]	< 9	32	< 9	< 9

[a] Units: μL/min/mg protein. [b] Assay was performed using alternative batch of mircosomes to those used previously.

Dog pharmacokinetic studies also showed similar clearance data for the three lead imidazopiperidines compared to **1**. Because of the varying role of transporters in clearance of **1** across species,[27] we were cautious when making predictions of human clearance for **29**, **39**, and **41f**. On the basis of the translation between the rat, dog, and human clearance data for **1**,[27] in addition to the structural/physicochemical similarities with the imidazopiperidines, we felt confident that the lead imidazopiperidines would display acceptable clearance in man. When in vitro HLM stability data was generated in the same assay for the lead compounds (Table 7), reduced intrinsic clearances were apparent for **39** and **41f** compared with **1**, e.g. 49 and 7 μL/min/mg protein for **1** and **41f**, respectively. These data indicated the possibility of reduced clearance in man should the in vitro assay be predictive for in vivo clearance. Reduced human clearance was considered beneficial to both reducing dose size and also extending half-life to increase chances of suitability for q.d. dosing.

Identifying compounds with the potential to address Maraviroc-resistant virus was another key driver for the development of second generation CCR5 antagonists. With this in mind, activity of the new imidazopiperidines was assessed against expanded B-clade Maraviroc-resistant HIV-1 isolate CC185 (MVC^{RES}).[8] In addition, representatives of alternative chemotypes were tested (Aplaviroc **42**[28] and Vicriviroc **43**[29]) along with examples of previously reported tropanes (**44**−**47**) as shown in Chart 1.

The compounds were tested in parallel against MVC^{RES} HIV-1 isolate CC185 and its passaged controlled parental isolate, i.e. Maraviroc-sensitive "start" isolate CC185. Each compound was tested at the predetermined IC_{90} against the lab adapted isolate HIV BaL (as in Table 5) in addition to 10- and 100-fold above this concentration. The IC_{90} value

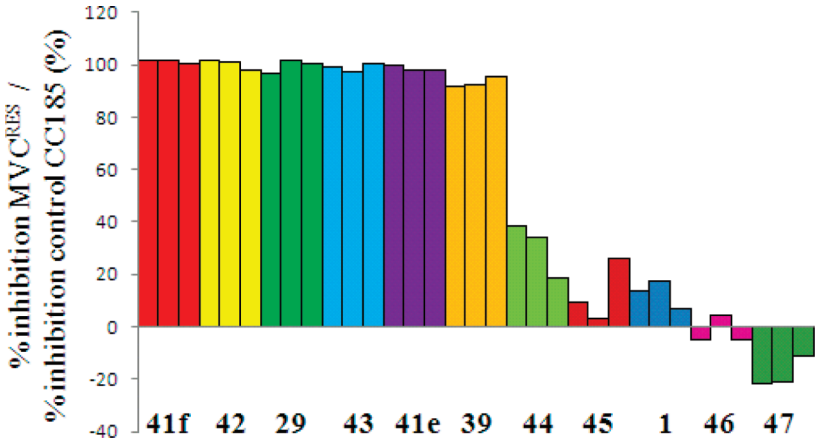


Figure 1. Relative activity of various CCR5 antagonists against MVC^{RES} HIV-1 (isolate CC185) versus passaged controlled parental virus in PBL culture. For each compound the first column indicates a concentration of compound equivalent to the IC_{90} against HIV BaL, the second $10 \times IC_{90}$, and the third $100 \times IC_{90}$.

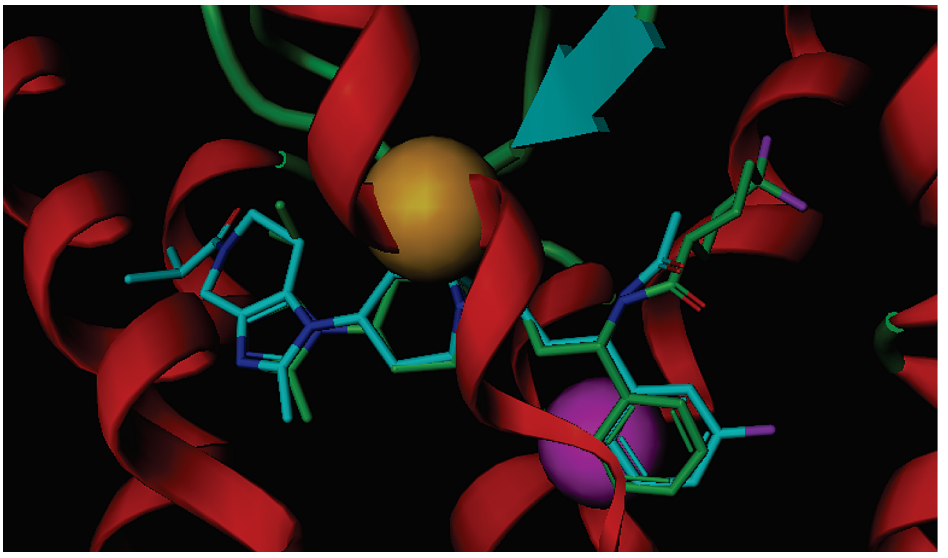
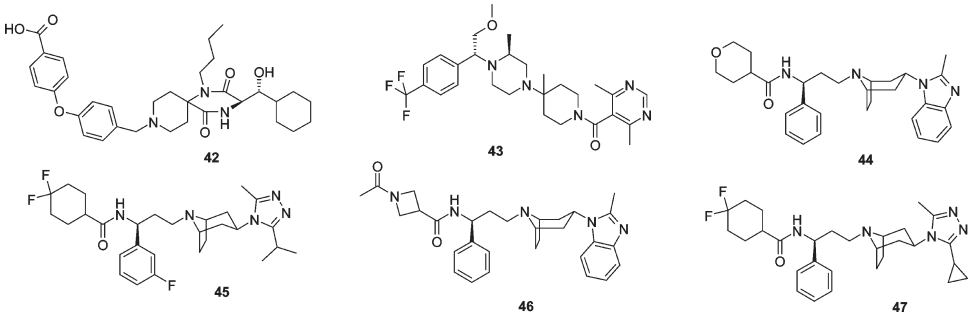


Figure 2. Docking of **1** (green) and **41f** (cyan) into a model of CCR5. E283 is indicated in orange, and Y108 is shown in magenta.

Chart 1. Comparator Compounds Tested against Maraviroc-Resistant Virus



against HIV-BaL was deemed an appropriate benchmark for primary isolates based upon comparative activities for **1** against lab-adapted versus primary isolates[15] and that the same IC_{90} values were observed for **39** and **41f** against CC185 and HIV-BaL in full dose−response studies (data not shown). The inhibition against MVC^{RES} virus at the three concentrations for each compound is shown in Figure 1 as a percentage of the inhibition against the passaged controlled parental CC185 virus.

The MVC^{RES} virus was found to be sensitive to the alternative chemotypes **42** and **43**, which demonstrated the potential for nonidentical resistance profiles for CCR5 antagonists. Tropanes **45** and **47** were found to be effectively inactive against the MVC^{RES} virus, which is consistent with their structural similarity with **1**. Replacing the exocyclic triazole substituted tropane with an endocyclic benzimidazole substituted tropane was found to have little effect, as benzimidazoles **44** and **46** also showed weak inhibition of MVC^{RES} virus. We were pleased to find that all four imidazopiperidines tested (**29**, **39**, **41e** and **41f**) demonstrated very similar inhibition of both MVC^{RES} virus and the parental virus, as was found to be the case for the alternative

chemotypes **42** and **43**. These data provided evidence that resistance profiles distinct from that of **1** can be achieved within the tropane chemotype.

A CCR5 homology model has previously been reported based on the bovine rhodopsin crystal structure.[30] A binding site for the tropane series was proposed which was consistent with site directed mutagenesis studies that showed a loss of functional binding of multiple examples to E238A and Y108A mutants.[31] The proposed binding mode is allosteric with respect to the gp120-CD4 complex binding site to CCR5. An ionic interaction between the tropane nitrogen of **1** and the E283 carboxylate moiety in addition to a hydrophobic interaction between the phenyl group and the Y108 phenol moiety were proposed (see Figure 2).[32] Like **1**, imidazopiperidine **41f** demonstrated a loss of functional binding to the E238A and Y108A mutants, which is consistent with these compounds occupying similar binding sites.[31]

The computer-assisted overlay shown in Figure 2 indicates the differential occupation of the transmembrane region of CCR5. As all of the tropanes which demonstrate activity against the MVC^{RES} virus possess a substituted imidazopiperidine in place of the triazole within **1**, it is possible that additional interactions made by this substituent affect the resistance profile. Modeling suggests that this group points toward the hinge region for extracellular loop 2 (ECL2), hence it may well control the presentation of the receptor to the virus.

Conclusion

A series of imidazopiperidines has been identified which in the case of **41f** combine complete oral absorption in rat and dog and improved in vitro metabolic stability compared to **1**. Antiviral activity of **41f** against HIV BaL in PBMCs is also enhanced compared to that of **1**. Furthermore, antiviral activity of **41f** is retained against an HIV isolate which has been shown to be resistant to **1**, consistent with a distinct resistance profile. Because of a favorable profile in preclinical safety studies, **41f** was progressed to clinical studies.[33] Phase II clinical trials of **41f** (PF-232798)[20] are currently ongoing, and further data will be reported in due course.

Experimental Procedures

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Flash column chromatography was carried out using 40−63 μm silica gel. NMR spectra were carried out on a Varian Mercury 400 or Varian Inova 500 in the solvents specified. HPLC purity determinations were carried out using a Phenomenex phenyl hexyl 150 mm × 4.6 mm column with 5 μm particle size; gradient: 98−2% A over 18 min, 2 min hold, 1 mL/min flow rate (A, 10 mM ammonium acetate in H_2O; B, 10 mM ammonium acetate in MeOH), temperature 50 °C. All test compounds were confirmed to be ≥95% pure by either combustion analysis or HPLC. Combustion analyses were conducted by Exeter Analytical UK, Ltd., Uxbridge, Middlesex, UK. Compounds **42**,[28] **43**,[29] **44**,[14] **45**,[34] **46**,[14] and **47**[34] were prepared using methods previously reported. The FluorAce β-galactosidase kit for the gp-160 fusion assay was purchased from Bio-Rad (Hemel Hempstead, UK). Black, clear bottom, 384-well tissue culture−treated plates were obtained from Greiner (Stonehouse, UK). Cell culture media, with the exception of serum, was obtained from Gibco (Paisley, UK). Fetal calf serum was obtained from PAA (Yeovil, UK). All other reagents for the gp160 fusion assay were purchased from Sigma (Poole, UK).

8-Benzyl-8-azabicyclo[3.2.1]octan-3-one (5). A solution of 2,5-dimethoxytetrahydrofuran **4** (50 g, 378 mmol) in hydrochloric acid (0.025 N, 160 mL) was cooled to 0 °C for 16 h. Benzylamine hydrochloride (65 g, 453 mmol), ketomalonic acid (55 g, 377 mmol), and an aqueous solution of sodium acetate (300 mL, 0.69 M) were added and the reaction was stirred at room temperature for 1 h. The mixture was heated to 50 °C for further 90 min, then cooled in an ice bath and basified to pH 12 with 2N aqueous sodium hydroxide solution. The layers were separated, and the aqueous phase extracted with ethyl acetate (3 × 300 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residual brown oil was distilled under reduced pressure (126 °C, 3 mmHg) to afford the title compound as an off-white solid (37.8 g, 47%). LRMS m/z $[M + H]^+$ 216.

***tert*-Butyl 3-Oxo-8-azabicyclo[3.2.1]octan-8-carboxylate (6).** A mixture of ketone **5** (15.0 g, 69.7 mmol), di-*tert*-butyl dicarbonate (18.2 g, 83.4 mmol), and 20% w/w palladium hydroxide on carbon (3.0 g) in ethyl acetate (165 mL) was stirred for 4 h at room temperature under an atmosphere of hydrogen (269 kPa). The mixture was filtered through Arbocel and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel (100:0 to 50:50 hexane: ether) to afford the title compound as a colorless oil which crystallized on standing (16.2 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58−4.35 (2H, m), 2.82−2.48 (2H, m), 2.34−2.26 (2H, m), 2.11−2.00 (2H, m), 1.68−1.60 (2H, m), 1.48 (9H, s).

***tert*-Butyl 3-(Benzylamino)-*endo*-8-azabicyclo[3.2.1]octane-8-carboxylate (7).** A solution of ketone **6** (10.0 g, 44.4 mmol), benzylamine (4.85 mL, 49.7 mmol), and sodium triacetoxyborohydride (14.11 g, 66.6 mmol) was stirred for 16 h at room temperature in a mixture of glacial acetic acid and dichloromethane (1:9 v/v, 290 mL). The solvents were evaporated under reduced pressure and the residue dissolved in ethyl acetate (200 mL) and washed with saturated aqueous sodium carbonate solution (50 mL) and water (50 mL). The organic solution was dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (98:2:0.25 dichloromethane: methanol:concentrated aqueous ammonia) to afford the title compound as a white solid (7.00 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.26 (4H, m), 7.26−7.20 (1H, m), 4.23−4.03 (2H, m), 3.74 (2H, s), 3.03−2.95 (1H, m), 2.19−1.85 (5H, m), 1.61−1.52 (2H, m), 1.48−1.42 (11H, m).

***tert*-Butyl 3-*endo*-Amino-8-azabicyclo[3.2.1]octane-8-carboxylate (8).** A mixture of benzylamine **7** (7.00 g, 22.1 mmol), ammonium formate (7.00 g, 111 mmol), and 20% w/w palladium hydroxide on carbon (0.70 g) in ethanol (200 mL) was heated to 50 °C until gas evolution ceased. The cooled mixture was filtered through Arbocel and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (98:2:0.25 to 95:5:0.5 dichloromethane:methanol:concentrated aqueous ammonia) to afford the title compound as a colorless oil (4.70 g, 94%). LRMS m/z $[M + H]^+$ 227.

***tert*-Butyl 3-*endo*-[(3-Nitro-4-pyridinyl)amino]-8-azabicyclo[3.2.1]-octane-8-carboxylate (34).** *tert*-Butyl 3-amino-*endo*-8-azabicyclo-[3.2.1]octane-8-carboxylate **8** (3.0 g, 13.2 mmol), 4-ethoxy-3-nitropyridine hydrochloride **33** (2.7 g, 13.2 mmol), and *N*-ethyl-*N*, *N*-diisopropylamine (1.89 g, 14.6 mmol) were dissolved in 1-methyl-2-pyrrolidinone (5 mL) and heated at 120 °C for 18 h. The cooled reaction mixture was diluted with ethyl acetate (150 mL) and washed with water (3 × 50 mL), saturated aqueous sodium hydrogen carbonate solution (50 mL), and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure. The residue was triturated with diethyl ether and filtered to afford the title compound as a yellow solid (1.5 g, 33%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.25 (1H, s), 9.00−8.75 (1H, m), 8.35 (1H, m), 6.60 (1H, m), 4.50−4.15 (2H, m), 3.98 (1H, m), 2.50−1.40 (17H, m). LRMS m/z $[M + H]^+$ 349

1-*endo*-(8-Acetyl-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-imidazo[4,5-*c*]pyridine (35). Aminopyridine **34** (4.40 g, 12.6 mmol) and iron powder (2.11 g, 37.8 mmol) were dissolved in glacial acetic acid (50 mL), and the mixture was heated to 60 °C for 2 h.

Acetic anhydride (8 mL) was then added and the mixture heated to 140 °C for 18 h. The cooled reaction mixture was filtered through a pad of Arbocel, and solvent was removed under reduced pressure. The residue was partitioned between dichloromethane (200 mL) and water (200 mL) and the mixture adjusted to pH 9 with 2N aqueous sodium hydroxide solution. The mixture was filtered through a pad of Arbocel and the organic phase separated. The aqueous layer was extracted with dichloromethane (100 mL) and the combined organic extracts dried ($MgSO_4$) and filtered. Solvent was evaporated under reduced pressure and the residue triturated with ethyl acetate, filtered, and dried to give the title compound as a white solid (3.27 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (1H, s), 8.35 (1H, m), 7.22 (1H, m), 4.90 (1H, m), 4.35 (1H, m), 4.25−4.10 (1H, m), 2.70−2.45 (5H, m), 2.35−1.80 (9H, m). LRMS m/z $[M + H]^+$ 285.

Methyl 1-(8-Acetyl-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1,4-dihydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate (36). A solution of imidazopyridine **35** (10.0 g, 35.2 mmol) in ethanol (95 mL) and water (5 mL) was degassed twice by application of a vacuum and then flushing with nitrogen gas prior to cooling to −70 °C. To the cooled, stirred solution was added methyl chloroformate (3.3 mL, 42.2 mmol) dropwise over 10 min. After a further 45 min, sodium borohydride (4.0 g, 105.7 mmol) was added portionwise. The reaction mixture was allowed to warm to room temperature over 1 h. Crushed ice was carefully added, and stirring was continued for 10 min. The supernatant was decanted from the white precipitate, and the ethanol was removed under reduced pressure. The resulting aqueous mixture was recombined with the white precipitate and was diluted with 2 M aqueous HCl (100 mL) to give a solution which was then extracted with ethyl acetate (100 mL). The organic layer was extracted with 2 M aq HCl (50 mL). The two acidic layers were then combined and basified to pH 9 by careful addition of solid potassium hydroxide. The aqueous mixture was extracted with dichloromethane (100 mL and then 2 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The residue was triturated with ethyl acetate, filtered, and dried to give a solid (7.0 g). The mother liquors were concentrated and purified by silica column chromatography (99:1 to 95:5 dichloromethane: methanol) to give a second batch of the title compound as a solid (2.0 g, combined yield 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 and 6.65 (1H, 2 × m), 5.48 (1H, m), 4.90 (2H, s), 4.84 (1H, m), 4.35−4.25 (1H, m), 4.00−3.80 (4H, m), 3.80 (3H, br s), 2.40 (3H, br s), 2.70−2.35 (2H, m), 2.15 (3H, s), 2.30−2.10 (2H, m), 2.00−1.70 (4H, m). LRMS (APCI) m/z $[M + H]^+$ 345

Methyl 1-[(3-*endo*)-8-Azabicyclo[3.2.1]oct-3-yl]-2-methyl-1,4,6,7-tetrahydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate (37) . Part 1: Methyl 1-(8-Acetyl-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1,4,6,7-tetrahydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate (**50**). To a solution of olefin **36** (6.75 g, 19.6 mmol) in ethanol (60 mL) at room temperature was added 10% palladium on carbon (500 mg), and the mixture was stirred at 50 °C under an atmosphere of hydrogen gas (60 psi) for 5 h. After cooling to room temperature, the catalyst was removed by filtration and the filtrate was evaporated. The residue was dissolved in dichloromethane, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in ethyl acetate (10 mL), and the flask was scratched to induce crystallization. The suspension was diluted with diethyl ether, the precipitate was collected by filtration, washed with diethyl ether, and dried to give the desired product as a white solid (6.2 g, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (1H, m), 4.50 (2H, br, s), 4.30 (1H, m), 4.00−3.87 (1H, m), 3.85−3.70 (m, 3H), 3.70 (3H, s) 2.80−2.40 (7H, m), 2.30−2.05 (2H, m), 2.15 (3H, s), 1.90−1.70 (4H, m). LRMS (APCI) m/z $[M + H]^+$ 347. Part 2: A mixture of the acetamide **50** (10.58 g, 30.0 mmol) in 2 M aqueous sulfuric acid (150 mL) was heated at 100 °C for 18 h. After cooling to room temperature, the mixture was basified to pH 11−12 using solid sodium hydroxide and extracted three times with dichloromethane. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give a yellow/brown gum. The crude product was purified by silica column chromatography (99:1:0.1 to 90:10:1 dichloromethane: methanol:concentrated aqueous ammonia) to give the desired product as a solid (7.4 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50−4.35 (3H, m), 3.82−3.65 (7H, br s), 2.80−2.10 (5H, m), 2.40 (3H, s), 1.95−1.55 (6H, m). LRMS (APCI) m/z $[M + H]^+$ 305.

Methyl 1-*endo*-{8-[(3*S*)-3-(Acetamido)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-5-carboxylate (39). Sodium triacetoxyborohydride (11.0 g, 51.9 mmol) was added portionwise to a solution of *N*-[(1*S*)-1-(3-fluorophenyl)-3-oxopropyl]acetamide **38**[17] (11.32 g, 54.1 mmol) and secondary amine **37** (13.73 g, 45.1 mmol) in dichloromethane (150 mL) and the mixture stirred for 1 h at room temperature. The solution was then washed with water (100 mL), brine (50 mL), dried ($MgSO_4$), and filtered. Solvent was evaporated under reduced pressure and the resulting white solid was dissolved in ethyl acetate (100 mL). Slight cooling induced crystallization, which was allowed to complete at room temperature overnight. The resulting white solid was isolated by filtration and recrystalized from acetone (4 mL/g) to give the title compound as a white crystalline solid (15 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.25 (1H, m), 7.10−6.90 (3H, m), 6.80−6.50 (1H, m), 5.15 (1H, m), 4.65−4.38 (3H, m), 3.80−3.60 (5H, m), 3.50−3.30 (2H, m), 2.80−1.90 (16H, m), 1.70−1.40 (4H, m). LRMS (ES) m/z $[M + H^+]$ 498. Found C, 62.80; H, 7.48; N, 13.55. $C_{27}H_{37}FN_5O_3\cdot(H_2O)$ requires C, 62.89; H, 7.43; N, 13.58%

***N*-{(1*S*)-3-[3-*endo*-(2-Methyl-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-(3-fluorophenyl)-propyl}acetamide (40).** To a stirred solution of carbamate **39** (13.27 g, 26.7 mmol) in propan-2-ol (80 mL) was added 2 M aqueous sodium hydroxide solution (120 mL), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the mixture was extracted with ethyl acetate (2 × 200 mL). The combined organic components were washed with brine (150 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product mixture was purified by silica column chromatography (90:10:1 then 80:20:1 dichloromethane:methanol:concentrated aqueous ammonia) to give the title compound as a white foam (8.54 g, 73%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.38−7.29 (1H, m), 7.18−7.12 (1H, m), 7.10−7.04 (1H, m), 7.00−6.91 (1H, m), 5.20−5.12 (1H, m), 4.63−4.52 (1H, m), 3.70 (2H, br s), 3.41−3.33 (2H, m) 3.08−3.02 (2H, m), 2.78−2.72 (2H, m), 2.52−2.41 (2H, m), 2.39 (3H, s), 2.30−2.25 (2H, m), 2.19−2.08 (2H, m), 1.98 (3H, s), 1.95−1.85 (2H, m), 1.70−1.60 (4H, m). LRMS (APCI) m/z $[M + H]^+$ 440. Found C, 66.19; H, 7.99; N, 15.44. $C_{25}H_{34}FN_5O\cdot 0.8(H_2O)$ requires C, 66.14; H, 7.90; N, 15.43%.

***N*-{(1*S*)-3-[3-*endo*-(5-Isobutyryl-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-(3-fluorophenyl)propyl}acetamide (41f).** To a stirred solution of secondary amine **40** (19.9 g, 45.3 mmol) in tetrahydrofuran (500 mL) was added triethylamine (7.0 mL, 50.0 mmol), followed by dropwise addition of isobutyryl chloride (5.3 mL, 50 mmol). After 1 h, a second portion of isobutyryl chloride was added dropwise (0.5 mL, 5.0 mmol). After 0.5 h, the reaction mixture was concentrated to approximately 300 mL and ethylacetate (200 mL) was added. The reaction mixture was washed with 10% aqueous potassium carbonate solution (200 mL; w/v). The aqueous phase was separated and extracted with ethylacetate (100 mL). The organic components were combined, washed with brine (100 mL), dried ($MgSO_4$), filtered, and concentrated until a mobile oil was obtained which had just started to form a foam. The residue was dissolved in ethyl acetate (100 mL) and heated to 90 °C. Water (0.5 mL) was added to the hot solution, and the mixture was allowed to slowly cool to room temperature. The precipitate was collected by filtration, washed with ethyl acetate (50 mL), and dried under reduced pressure to give the title compound as a white solid (20.9 g, 90%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.35−7.29 (1H, m), 7.14−7.12 (1H, m), 7.07−7.05 (1H, m), 6.97−6.92 (1H, m), 5.17−5.13 (1H, m), 4.63−4.52 (1H, m), 4.44−4.43 (2H, m), 3.89−3.78 (2H, m), 3.40−3.31 (2H, m), 3.06−2.90 (1H, m), 2.84−2.77 and 2.75−2.69 (2H, 2 × m), 2.51−2.39 (2H, m), 2.36 and 2.35 (3H,

2 × s), 2.30−2.20 (2H, m), 2.13−2.03 (2H, m), 1.95 (3H, s), 1.90−1.84 (2H, m), 1.65−1.55 (4H, m), 1.11−1.08 and 1.06−1.04 (6H, 2 × m). Rotamers apparent in spectrum. LRMS *m/z* [MH^+] 510. Found C, 66.94; H, 7.92; N, 13.47. $C_{29}H_{40}FN_5O_2 \cdot 0.5(H_2O)$ requires C, 67.16; H, 7.97; N, 13.50%. $[\alpha]_D^{25}$ −23.4° (1.64 mg/mL in MeOH).

Gp160 Fusion Assay.[35] CHO-Tat10 and HeLa-P4 cells were grown in 225 cm^2 flasks or roller bottles to confluence. Cells were washed twice with PBS and harvested using Trypsin EDTA. Cells were pooled into two separate 1 L sterile spinner flasks, one for each cell line. A cell count was performed, and the cell suspension was diluted in medium (DMEM with 2 mM L-glutamine and 2% FCS for HeLa-P4 or RPMI1640 with 2 mM L-glutamine and 2% FCS for CHO-Tat10) to give a suspension at 6.6×10^5 cells per mL. The spinner flasks were connected to a 5% CO_2 gas supply to maintain pH and stirred at 30 rpm at room temperature during dispensing. Then 15 μL of CHO-Tat10 and 15 μL of HeLa-P4 were added sequentially to a sterile tissue culture−treated 384-well plate containing 10 μL compound/control solution (360 wells of test compound and 8 wells each of a maximum [no inhibitor], minimum [saturating concentration of fusion inhibitor], and standard [an IC_{50} concentration of a fusion inhibitor] controls). Plates were lidded and incubated at 37 °C and 5% CO_2 in a humidified (Cytomat) incubator. After 20 h, 20 μL of reaction buffer (13.4% glycerol, 1.3% Triton-X100, 0.34 M Tris [pH7.8], 200 μM 4-methylumbelliferyl-galactoside (MUG), 0.028% β-mercapto-ethanol, 33% Bio-Rad 1× reaction buffer) was added to each well. Plates were incubated at 25 °C for a further 2 h, during which time cell lysis occurred, and then 10 μL of Bio-Rad 10 × stop buffer was added to each well. The fluorescent signal was detected, after a further 45-min incubation, using a Tecan Spectrafluor Plus (Tecan) (λex = 360 nm, λem = 460 nm; bottom reading; 3 flashes; gain = 50−60).

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Supporting Information Available: Additional experimental procedures and analytical data for intermediates and final compounds. Further pharmacokinetic data from rat and dog po studies are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Temesgen, Z; Warnke, D; Kasten, M J. Current status of antiretroviral therapy. *Expert Opin. Pharmacother.* **2006**, *7*, 1541–1554.
(2) Dorr, P.; Perros, M. CCR5 inhibitors in HIV-1 therapy. *Expert Opin. Drug Discovery* **2008**, *3*, 1345–1361.
(3) Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapouméroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **1996**, *382*, 722–725.
(4) Meanwell, N. A.; Kadow, J. F. Drug evaluation: Maraviroc, a chemokine CCR5 receptor antagonist for the treatment of HIV infection and AIDS. *Curr. Opin. Invest. Drugs* **2007**, *8*, 669–681.
(5) Wood, A.; Armour, D. The discovery of the CCR5 receptor antagonist, UK-427,857, a new agent for the treatment of HIV infection and AIDS. *Prog. Med. Chem.* **2005**, *43*, 239–271.
(6) Abel, S.; Russell, D.; Whitlock, L. A.; Ridgway, C. E.; Nedderman, A. N. R.; Walker, D. K. Assessment of the absorption, metabolism and absolute bioavailability of maraviroc in healthy male subjects. *Br. J. Clin. Pharmacol.* **2008**, *65*, 60–67.
(7) Chan, P. L. S.; Weatherley, B.; McFadyen, L. A population pharmacokinetic meta-analysis of maraviroc in healthy volunteers and asymptomatic HIV-infected subjects. *Br. J. Clin. Pharmacol.* **2008**, *65*, 76–85.
(8) Westby, M.; Smith-Burchnell, C.; Mori, J.; Lewis, M.; Mosley, M.; Stockdale, M.; Dorr, P.; Ciaramella, G.; Perros, M. Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *J. Virol.* **2007**, *81*, 2359–2371.
(9) Walker, D. K.; Abel, S.; Comby, P.; Muirhead, G. J.; Nedderman, A. N. R.; Smith, D. A. Species differences in the disposition of the CCR5 antagonist, UK-427,857, a new potential treatment for HIV. *Drug Metab. Dispos.* **2005**, *33*, 587–595.
(10) Barber, C. G.; Blakemore, D. C.; Chiva, J.-Y.; Eastwood, R. L.; Middleton, D. S.; Paradowski, K. A. 1-Amido-1-phenyl-3-piperidinylbutanes—CCR5 antagonists for the treatment of HIV. Part 1. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1075–1079.
(11) Barber, C. G.; Blakemore, D. C.; Chiva, J.-Y.; Eastwood, R. L.; Middleton, D. S.; Paradowski, K. A. 1-Amido-1-phenyl-3-piperidinylbutanes—CCR5 antagonists for the treatment of HIV: Part 2. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1499–1503.
(12) Price, D. A.; Armour, D.; de Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. Overcoming hERG affinity in the discovery of maraviroc; a CCR5 antagonist for the treatment of HIV. *Curr. Top. Med. Chem.* **2008**, *8*, 1140–1151.
(13) Price, D. A.; Armour, D.; de Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. Overcoming hERG affinity in the discovery of the CCR5 antagonist maraviroc. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4633–4637.
(14) Armour, D. R.; Price, D. A.; Stammen, B. L. C.; Wood, A.; Perros, M.; Edwards, M. P. Preparation of acylaminophenylpropylbenzimidazolylazabicycloalkanes and related compounds as CCR5 receptor modulators. Patent WO 00/38680, 2000.
(15) Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721–4732.
(16) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nature Rev. Drug Discovery* **2007**, *6*, 881–890.
(17) Basford, P. A.; Stephenson, P. T.; Taylor, S. C. J.; Wood, A. Preparation of tropane derivatives as CCR5 modulators. Patent WO 03/084954, 2003.
(18) Price, D. A.; Gayton, S.; Selby, M. D.; Ahman, J.; Haycock-Lewandowski, S.; Stammen, B. L.; Warren, A. Initial synthesis of UK-427,857 (maraviroc). *Tetrahedron Lett.* **2005**, *46*, 5005–5007.
(19) Armour, D. R.; Price, D. A.; Stammen, B. L. C.; Wood, A.; Perros, M.; Edwards, M. P. Preparation of azolylpiperidines as CCR5 receptor modulators. Patent WO 00/039125, 2000.
(20) Stupple, P. A. Preparation of imidazopyridine substituted tropane derivatives with CCR5 receptor antagonist activity for the treatment of HIV and inflammation. Patent WO 2005/033107, 2005.
(21) Ernst, J.; Dahl, R.; Lum, C.; Sebo, L.; Urban, J.; Miller, S. G.; Lundström, J. Anti-HIV-1 entry optimization of novel imidazopiperidine-tropane CCR5 antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1498–1501.
(22) Duan, M.; Aquino, C.; Dorsey, G. F.; Ferris, R.; Kazmierski, W. M. 4,4-Disubstituted cyclohexylamine based CCR5 chemokine receptor antagonists as anti-HIV-1 agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4988–4992.
(23) Duan, M.; Aquino, C.; Ferris, R.; Kazmierski, W. M.; Kenakin, T.; Koble, C.; Wheelan, P.; Watson, C.; Youngman, M. [2-(4-Phenyl-4-piperidinyl)ethyl]amine based CCR5 antagonists: derivatizations at the N-terminal of the piperidine ring. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1610–1613.
(24) Kazmierski, W. M.; Aquino, C.; Chauder, B. A.; Deanda, F.; Ferris, R.; Jones-Hertzog, D. K.; Kenakin, T.; Koble, C. S.; Watson, C.; Wheelan, P.; Yang, H.; Youngman, M. Discovery of Bioavailable 4,4-Disubstituted Piperidines as Potent Ligands of the Chemokine Receptor 5 and Inhibitors of the Human Immunodeficiency Virus-1. *J. Med. Chem.* **2008**, *51*, 6538–6546.
(25) Fish, P. V.; Barta, N. S.; Gray, D. L. F.; Ryckmans, T.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. Derivatives of (3*S*)-*N*-(biphenyl-2-ylmethyl)pyrrolidin-3-amine as selective noradrenaline reuptake inhibitors: reducing P-gp mediated efflux by modulation of H-bond acceptor capacity. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4355–4359.
(26) Singleton, D. H.; Boyd, H.; Steidl-Nichols, J. V.; Deacon, M.; de Groot, M. J.; Price, D.; Nettleton, D. O.; Wallace, N. K.; Troutman, M. D.; Williams, C.; Boyd, J. G. Fluorescently Labeled Analogues of Dofetilide as High-Affinity Fluorescence Polarization Ligands for the Human Ether-a-go-go-Related Gene (hERG) Channel. *J. Med. Chem.* **2007**, *50*, 2931–2941.

(27) Walker, D. K.; Abel, S.; Comby, P.; Muirhead, G. J.; Nedderman, A. N. R.; Smith, D. A. Species differences in the disposition of the CCR5 antagonist, UK-427,857, a new potential treatment for HIV. *Drug Metab. Dispos.* **2005**, *33*, 587–595.
(28) Habashita, H.; Hamano, S.; Shibayama, S.; Takaoka, Y. Preparation of triazaspiro[5.5]undecane derivatives as active ingredients in remedies for inflammatory diseases. Patent WO 02/074770, 2002.
(29) Baroudy, B. M.; Clader, J. W.; Josien, H. B.; McCombie, S. W.; McKittrick, B. A.; Miller, M. W.; Neustadt, B. R.; Palani, A.; Smith, E. M.; Steensma, R.; Tagat, J. R.; Vice, S. F.; Laughlin, M. A.; Gilbert, E.; Labroli, M. A. Preparation of piperazine derivatives useful as CCR5 antagonists. Patent WO 00/66558, 2000.
(30) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **2000**, *289*, 739–745.
(31) Mansfield, R.; Able, S.; Griffin, P.; Irvine, B.; James, I.; Macartney, M.; Miller, K.; Mills, J.; Napier, C.; Navratilova, I.; Perros, M.; Rickett, G.; Root, H.; van der Ryst, E.; Westby, M.; Dorr, P. CCR5 pharmacology methodologies and associated applications. *Methods Enzymol.* **2009**, *460*, 17–55.
(32) Kondru, R.; Zhang, J.; Ji, C.; Mirzadegan, T.; Rotstein, D.; Sankuratri, S.; Dioszegi, M. Molecular interactions of CCR5 with major classes of small-molecule anti-HIV CCR5 antagonists. *Mol. Pharmacol.* **2008**, *73*, 789–800.
(33) Dorr, P.; Westby, M.; McFadyen, L.; Mori, J.; Davis, J.; Perruccio, F.; Jones, R.; Stupple, P.; Middleton, D.; Perros, M. PF-232798, a Second Generation Oral CCR5 Antagonist. 15th Conference on Retroviruses and Opportunistic Infections, Boston, MA, 3−6 February, 2008.
(34) Prepared using procedures described in: Perros, M.; Price, D. A.; Stammen, B. L. C.; Wood, A. Preparation of therapeutic tropane derivatives. Patent WO 01/90106, 2001.
(35) Bradley, J.; Gill, J.; Bertelli, F.; Letafat, S.; Corbau, R.; Hayter, P.; Harrison, P.; Tee, A.; Keighley, W.; Perros, M.; Ciaramella, G.; Sewing, A.; Williams, C. Development and automation of a 384-well cell fusion assay to identify inhibitors of CCR5/CD4-mediated HIV virus entry. *J. Biomol. Screening* **2004**, *9*, 516–524.