Diazabicyclo Analogues of Maraviroc: Synthesis, Modeling, NMR Studies and Antiviral Activity

L. Legnani,[a] D. Colombo,[b] A. Venuti,[c] C. Pastori,[c] L. Lopalco,[c] L. Toma [a] and S. Villa*[d]

Two diazabicyclo analogues of Maraviroc, in which the azabicycloctane moiety is replaced by a diazabicycloctane or diazabicyclononane, were synthesized and tested, through a viral neutralization assay, on a panel of six pseudoviruses. The diazabicycloctane derivative maintained a significant infectivity reduction power, whereas the diazabicyclononane was less effective. Biological data were rationalized through a computational study that allowed to determine the conformational preferences of the compounds and hypothesize a correlation between the inhibitory activity and the different distribution of the population of the located geometries. A high-field NMR analysis supported the modeling results.

Introduction

The AIDS disease, caused by the Human Immunodeficiency Virus (HIV), is pandemic at global level,[1] and, in the absence of an effective preventive vaccine, all world health organizations consider anti-HIV drugs a priority. A growing research field in order to find new drugs is the identification of entry inhibitors,[2] able to interfere with the events that occur between the anchorage of the virion to CD4 and the fusion of the membranes.

It is known that the HIV-1 entry process in the host cell depends on the interaction of the viral envelope protein gp120 with the receptor/coreceptors on host cell surface. Because CCR5 is the predominant coreceptor for clinical HIV isolates, it has become a very attractive target for anti-HIV therapy. All CCR5 antagonists inhibit HIV-1 entry into target cells by blocking the interaction between gp120 and CCR5 and sharing a common binding site, the pocket formed by the transmembrane domains of CCR5.

Maraviroc **1**, a triazolotropane-based compound synthesized by Pfizer,[3] is the first US Food and Drug Administration-approved drug from the new class of CCR5 antagonists and is sold under the trade name Selzentry (Celsentri outside the United States) as film-coated tablets for oral administration.[4] It shows excellent antiviral potency and pharmacological properties,[5] resulting active against 200 clinically derived HIV-1 envelope-recombinant pseudoviruses, 100 of which derived from viruses resistant to existing drug classes.[6]

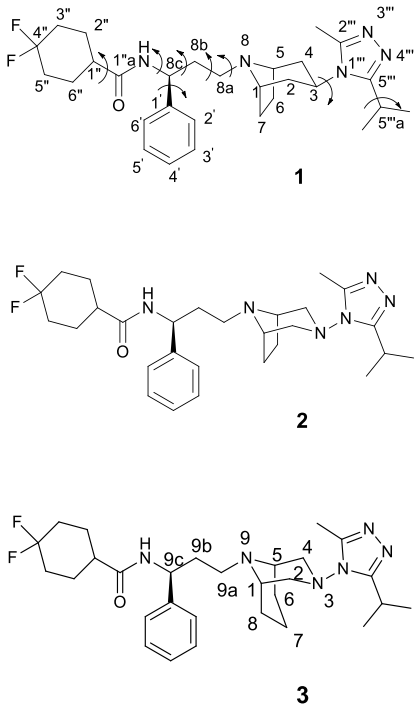


Fig. 1 Structures of Maraviroc (**1**) and its diazabicyclo analogues **2** and **3**

Recently, the crystal structure of human CCR5 bound to **1** has been reported.[7] The nitrogen atom of tropane is protonated and engaged in a salt-bridge interaction with Glu-283, the carboxamide nitrogen gives a hydrogen bond with Tyr-251 and the length of the carbon chain between these two nitrogens is considered critical for the anti-HIV infection activity. Nitrogen atoms N3 and N4 of the triazole group form hydrogen bonds with Tyr-37 and with a water molecule, respectively. A fluorine atom gives two hydrogen bonds with Thr-195 and Thr-259. Phenyl group, triazole, tropane, and cyclohexane groups make hydrophobic interactions.

Starting from **1**, the design of structural analogues represents an important tool to the obtainment of new compounds with possibly higher potency and devoid of the above described side effects. Some of us have a long-lasting experience in the synthesis of pharmacologically active compounds with a diazabicyclo moiety in their structure. So, our interest has been directed to the obtainment of analogues of **1** in which the tropane moiety is replaced by the 3,8-diazabicyclo[3.2.1]octane and 3,9-diazabicyclo[3.3.1]-nonane systems (**2**[8] and **3**, respectively) (Fig.1).

The two compounds were prepared and, together with **1**, submitted to a viral neutralization assay[9] in order to investigate their HIV-1

inhibitory activity. Moreover, a modeling study was carried out on compounds **1–3** to correlate their pharmacological properties with the geometrical and conformational preferences, and the results supported by a detailed NMR analysis.

Result and Discussion

Synthetic procedures for synthesis of Maraviroc analogues

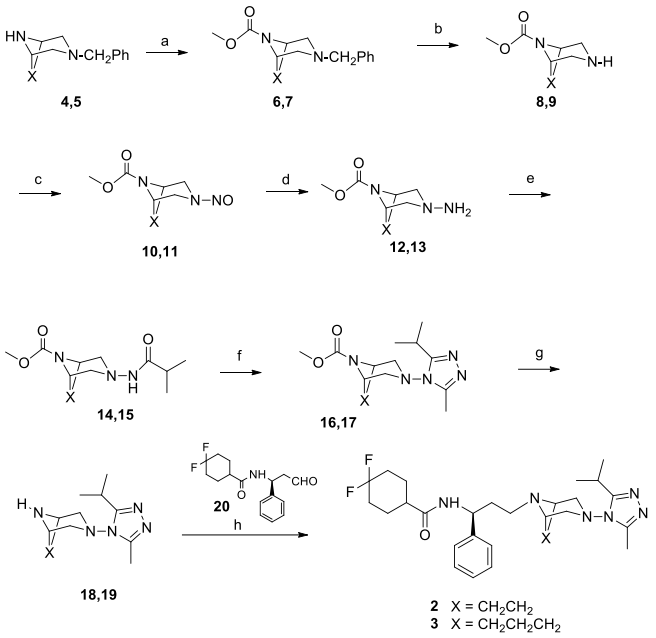
Maraviroc derivatives **2** and **3** were synthesized with a procedure similar to that already used for the reference compound **1**,[10] starting from known diazabicyclo precursors **4** and **5**.[11] After the proper protection of nitrogen at position 8 (9) in diazabicyclo scaffold with methyl chloroformiate (**6, 7**) and debenzylation steps by catalytic hydrogenation using heterogeneous Pd/C catalyst, **4** and **5** were converted into the intermediates **8** and **9**. Their treatment with sodium nitrite in hydrochloric acid afforded the nitroso derivatives **10** and **11** that were immediately reduced by reaction with zinc in acetic acid, to the corresponding amino derivatives **12** and **13**.[12] The amino groups were acylated with isobutyric acid, using a condensing agent (HATU) to obtain the acyl derivatives **14** and **15** that were converted into the triazoles **16** and **17** through a three steps reaction: activation as their corresponding imidoyl chloride by PCl_5, trapping with acetic hydrazide, and finally cyclization to the triazole by acid catalysis.

Furthermore, the benzyl derivatives **16** and **17** were deprotected by hydrolysis to **18** and **19**. Finally, coupling of aldehyde **20**[13] with diazabicycle derivatives **18** and **19** via reductive amination in presence of sodium triacetoxyborohydride in dichloromethane furnished the final compounds **2** and **3**.

Antiviral activity

A standardized neutralization assay[9] was performed to assess the infectivity reduction of all compounds under examination; in detail, Maraviroc (**1**) and its two analogues **2** and **3** were tested. The infectivity reduction power was challenged on a panel of six pseudoviruses, including one laboratory strain, SF162, four Clade B isolates, QH0692, 6535, PVO, and AC10, and one Clade C primary virus, ZM214. As a specificity negative control, an HIV-unrelated virus, SVA.MLV#922, was also included in the experiment to show that none of the compounds under assay affected its infectivity (data not shown).

Figure 2 reports the results of 90% of infectivity reduction (IC_{90}, μM) with the six pseudoviruses obtained with the three compounds. As expected, Maraviroc (**1**) efficiently neutralized all the viruses tested with a mean IC_{90} of 0.21 μM (ranging 0.08-0.47); compound **2** showed a mean IC_{90} of 0.48 μM (ranging 0.02 to 1.52). Compound **3** had a lower infectivity reduction power with a mean IC_{90} of 1.57 μM (ranging 0.09 to 3.54), exhibiting a statistically significant difference with compound **1** ($p \leq 0.05$). The data showed a progressive decrease of infectivity reduction from **1** to **3**.



Scheme 1. *Reagents and conditions:* (a) $ClCOOCH_3$, TEA, Et_2O, 0°C-r.t., 20 h. (b) H_2,Pd/C 10%, EtOH. (c) aq $NaNO_2$, HCl 2N, 0°C, N_2 atm. (d) Zn powder, AcOH, H_2O, N_2 atm. (e) isobutyric acid, TEA, HATU, DMF, 17 h. (f) 1) PCl_5, CH_2Cl_2, 0°C; 2) $AcNHNH_2$, 2-methyl-2-butanol; 3) AcOH, 2-methyl-2-butanol, 80°C. (g) aq NaOH 10%, MeOH, 80°C; (h) $NaBH(OAc)_3$, CH_2Cl_2, AcOH.

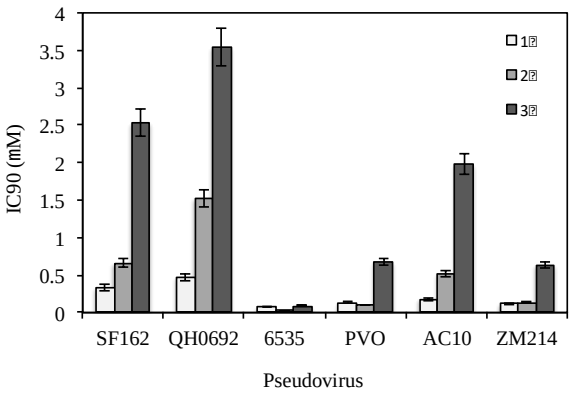


Fig. 2 Infectivity reduction of the positive control Maraviroc (**1**) and its analogues **2** and **3** on a panel of six pseudoviruses. The values are expressed as concentration (μM) leading 90% of infectivity reduction (IC_{90}). Mean plus Standard Deviations of three independent experiments were shown.

Table 1. Relative energies (kcal/mol), equilibrium percentages at 298 K, and significant torsional angles[a] (°) of conformers (P_i > 1%) of compound **1**.

	E_{rel} (kcal/mol)	P_i(%)	τ_1 (°)[a]	τ_2 (°)[b]	τ_3 (°)[c]	τ_4 (°)[d]	τ_5 (°)[e]	τ_6 (°)[e]	τ_7 (°)[g]	τ_8 (°)[h]
1A	0.00	39.3	-4	-164	67	165	-60	-168	-112	41
1B	0.12	32.3	-4	-165	67	165	-60	-168	-125	-41
1C	0.92	8.4	-3	-164	68	168	-60	-167	64	2
1D	1.13	5.8	-2	-162	77	105	-63	-65	63	7
1E	1.38	3.8	-1	-162	77	103	-64	-65	-112	41
1F	1.52	3.0	-2	-162	77	103	-64	-66	-127	-41
1G	1.67	2.3	37	-162	67	174	-61	-76	-123	-41
1H	1.81	1.8	24	-162	67	173	-61	-76	-108	40

[a] τ_1: H-C1"-C1"a-N; τ_2: C1"a-N-C8c-C1'; τ_3: C1'-C8c-C8b-C8a; τ_4: C8c-C8b-C8a-N8; τ_5: N-C8c-C1'- C2'; τ_6: C8b-C8a-N8-C5; τ_7: C4-C3-N1'"-C5'" ; τ_8: N1'"-C5'"-C5'"a-H.

Modeling of Maraviroc (1) and its analogues 2 and 3

Theoretical calculations were performed within the DFT approach at the B3LYP level with the 6-31G(d) basis set.[14] Solvent effects were also considered through single-point calculations on the gas-phase optimized geometries, by using a self-consistent reaction field (SCRF) method, based on the polarizable continuum model (PCM),[15] and water as the solvent. Data provided by EMEA (European Medicines Agency) show that in **1** the nitrogen atom of tropane and those of the triazole ring present different pKa values, precisely 7.9 and 3.3 respectively and, at physiological pH, only the first one is protonated.[16] Moreover, docking studies on **1**, reported in literature, revealed that the positively charged tertiary nitrogen of the tropane moiety could give strong electrostatic interactions in the binding site.[17] Thus, particular attention has been focused on the ionizable nitrogen atoms present in the molecules. All the optimizations have been performed on compounds in their cationic form. Maraviroc (**1**) is a highly flexible molecule with several degrees of conformational freedom related to the orientation around the single bonds exemplified by curved arrows in Figure 1.

In Table 1, the relative energies, the equilibrium percentages, and the geometrical features of the significantly populated (>1%) conformers are reported and the corresponding three-dimensional plots are shown in Figure 3. The two preferred conformations **1A** and **1B** are almost identical, a slightly different orientation of the isopropyl group being the only difference. The left portion of the molecule extends equatorially from the tropane nitrogen atom, with the phenyl ring facing the ethylenic tropane bridge and ends with the carboxyamido function equatorially linked to the difluorocyclohexane ring. The triazole ring is perpendicular to tropane. Conformation **1C** corresponds to **1A** and **1B** with a 180° rotation around the C3-N1''' bond. The two possible orientations described by τ_7, that can be ≈ -120°or 60°, lead, respectively, the isopropyl group on the same (**A**, **B**) or on the opposite side (**C**) of the tropane ethylenic bridge. In **1D-1F** the three-carbon C8a-C8c chain is folded (as highlighted by the τ_4 values), with the carbonyl oxygen pointing towards the H5 at short distance (about 2 Å). Also conformations with the carboxamide moiety bonded in axial position of the difluorocyclohexane ring have been found to be populated (**1G** and **1H**), though, with a small contribution to the overall population (~ 4%).

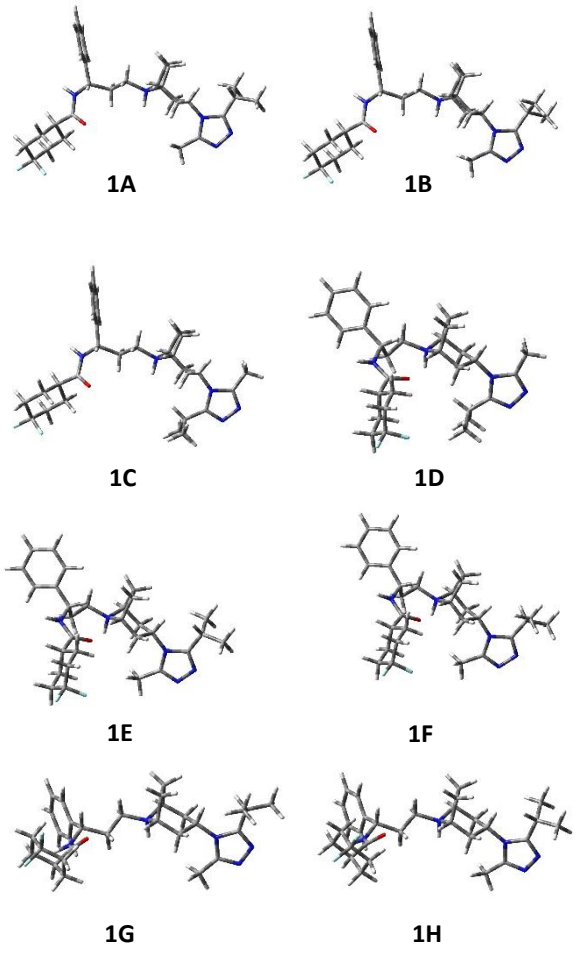


Fig. 3 3D-plots of populated conformers (Pi>1%) of compound **1**.

Table 2. Relative energies (kcal/mol), equilibrium percentages at 298 K, and significant torsional angles[a] (°) of conformations of compounds **2** and **3**.

	E_{rel} (kcal/mol)	P_i(%)	τ_1 (°)[a]	τ_2 (°)[b]	τ_3 (°)[c]	τ_4 (°)[d]	τ_5 (°)[e]	τ_6 (°)[f]	τ_7 (°)[g]	τ_8 (°)[h]
2A	0.37	21.1	7	-163	66	169	-60	-169	-111	29
2B	0.20	28.0	7	-163	67	168	-60	-169	-114	-31
2C	0.00	39.3	-2	-164	68	165	-60	-168	68	11
2D	1.47	3.3	-2	-162	77	103	-63	-66	68	9
2E	1.79	1.9	-3	-162	78	102	-63	-67	-112	31
2F	1.68	2.3	0	-162	78	101	-63	-67	-115	-31
3A	0.88	7.0	7	-163	69	168	-60	-161	-110	37
3B	0.62	11.0	7	-163	65	165	-60	-166	-118	-36
3C	0.00	31.2	-1	-164	66	160	-61	-167	67	-11
3D	0.10	26.1	7	-163	67	175	-60	-71	67	-3
3E	0.73	9.1	8	-163	67	178	-61	-69	-110	36
3F	0.76	8.7	9	-163	69	-179	-60	-67	-118	-36
3I	1.51	2.4	4	-163	66	174	-60	-167	67	8

[a] τ1: H-C1"-C1''a-N; τ2: C1''a-N-C8c-C1' for **2**, C1''a-N-C9c-C1' for **3**; τ3: C1'-C8c-C8b-C8a for **2**, C1'-C9c-C9b-C9a for **3**; τ4: C8c-C8b-C8a-N8 for **2**, C9c-C9b-C9a-N9 for **3**; τ5: N-C8c-C1'- C2' for **2**, τ5: N-C9c-C1'- C2' for **3**; τ6: C8b-C8a-N8-C5 for **2**, C9b-C9a-N9-C5 for **3**; τ7: C4-C3-N1'''-C5'''; τ8: N1'''-C5'''-C5'''a-H.

Then, the same computational approach was applied to compounds **2** and **3**, considering also in these cases the cationic form. We assumed that, at physiological pH, protonation occurs only at N8 (compound **2**) and N9 (compound **3**) in spite of the presence of a second nitrogen atom (N3) on the bicyclic system, as N3 is involved in a N-N bond with triazole and the other triazole nitrogen atoms are expected to be much less basic than N8 (N9) in analogy with the reference compound **1** (see Experimental Section). Also for **2** and **3** tenths of conformations were located, but only some resulted significantly populated (see Table 2 and Figure 4). In the case of **3** the number of located conformers was larger, because of the greater conformational freedom of the bicyclic moiety, that gives rise to "boat" geometries resulting not significantly populated and hence not reported in Table 2.

The same **A**-**C** low energy conformers already found for **1** were located for **2** and **3** but with a reverted relative energy order. In fact, conformers **A** and **B** are significantly populated (**2A** 21.1%, **3A** 7.0%, **2B** 28.0%, **3B** 11.0%), but the global energy minimum is, in both cases, conformer **C**, showing a different orientation of the triazole ring (see τ_7). However, **2A**-**C** and **3A**-**C** represent about 88% and 49% of the overall population, respectively.

Moreover, for **3**, conformer **3D**, showing a different value of τ_6 with respect to **3C**, but the orientation of the isopropyl group on the opposite side of the tropane ethylenic bridge, resulted populated for 26.1%. In the case of **3**, one populated conformer (**3I**) shows the carboxamide moiety bonded in axial position of the difluorocyclohexane ring (2.4%).

In order to better show analogies and differences between the geometries, conformations **A** of compounds **1**-**3** were overlapped through rms fitting of the atoms of the piperidine ring of the byciclic system (Figure 5). From the overlay no significant differences have been observed between the corresponding conformations of the three compounds and, consequently, their different inhibitory activity may be attributable to the observed different distribution of the population of the various conformations.

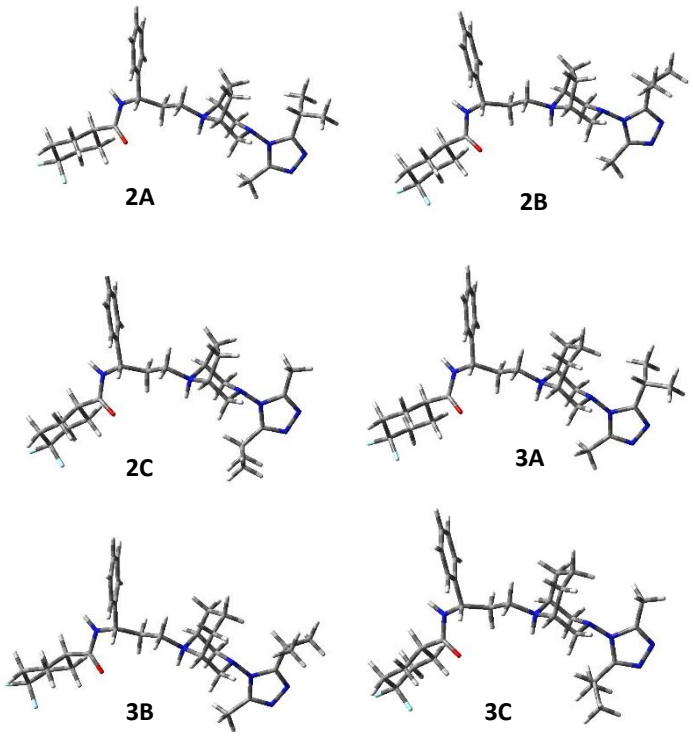
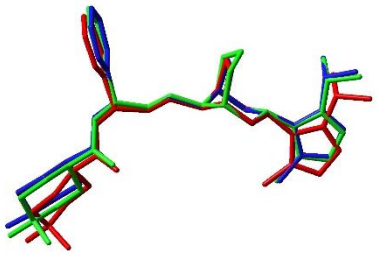


Fig. 4 3D-plots of conformers **A-C** of compounds **2** and **3**.

Considering that the preferred conformers of **1** are **A** and **B** (71.6%), it is possible to hypothesize that the presence of the isopropyl group on the same side of the bridge of the bicyclic system could address its antiviral activity. The global minimum of **2** and **3** is conformation **C** with the isopropyl oriented in the opposite side respect to the bridge. Nevertheless conformers **A** and **B** were significantly populated both for **2** and **3** with percentages of 49.1 and 18.0% respectively. This result might give reason of the observed biological activity of the two analogues and allow to explain the better values of derivative **2** with respect to **3**.



1-3 A

Fig. 5 Overlay of conformers **A** of compounds **1** (red), **2** (blue) and **3** (green).

NMR spectroscopy

^{1}H NMR chemical shift of compounds **1**-**3** was obtained through high field 1D and 2D spectroscopy and the data are showed in Table 3. Starting from the characteristic H-3 (**1**), H-8c (**2**), H-9c (**3**), and H-1", H-5"'a, H-1, H-5 (**1**-**3**), easily assigned by HSQC and COSY, the other resonances were assigned. Some signals, specifically methyl, isopropyl methyls and H-5'''a of compounds **2** and **3**, appeared as couples of signals (Table 3), which gave coalescence when recorded at increasing temperature (see supplementary), suggesting the presence of slowly interconverting conformations as indicated also by their positive cross peaks in the NOESY experiments due to chemical exchange (see supplementary).

In order to support the eterocycle rings assignments, the chemical shifts of the piperidine or piperazine protons of **1**-**3** were calculated and reported in Table 3 compared to the experimental data.

NOESY experiments allowed the assignment of the axial and equatorial protons of the bicyclic systems (Table 3). Specifically equatorial H-2/H-4 of compound **1** were given at 1.81 ppm due to their NOESY cross peaks with H-3 (4.41 ppm); consequently the axial

(4.41 ppm) with C*H*$_3$ at 2.51 ppm accounted for the **1C** and **1D** conformers with the isopropyl placed at the opposite side of the molecule. **1C** and **1D** were also supported by a cross peak between H-5"'a (3.26 ppm) and H-2ax/H-4ax (2.29 ppm). Finally a cross peak of H-1/H-5 (3.50/3.53 ppm) with H-8c (5.03 ppm) and with H-8b (2.03 ppm) accounted for **1D**-**F**.

H-2/H-4 were assigned at 2.29 ppm. Similar correlations were observed between equatorial H-2/H-4 at 3.32 and 3.35 ppm and H-6/H-7 at 2.34 of compound **2** and between equatorial H-2/H-4 at 3.29 and equatorial H-6/H-8 at 1.95 ppm of compound **3**.

Table 3. ^{1}H NMR chemical shift (δ) of compounds **1**-**3**. B3LYP/6-31G(d) GIAO calculated (calcd) δ of the piperidine or piperazine protons based on the geometries optimized at the same level are also showed in comparison with the experimental values.

^{1}H position	δ(ppm)					
	(**1**) [a]		(**2**) [a]		(**3**) [b]	
	Exp	calcd[(c)]	Exp	calcd[(c)]	Exp	calcd[(c)]
1 (5)	3.50	4.11	4.30	3.78	3.59	4.09
2-ax (4-ax)	2.29	2.47	4.11	3.61	3.99	3.95
2-eq (4-eq)	1.81	2.18	3.32	3.24	3.29	3.50
3	4.41	4.28	-	-	-	-
4-ax (2-ax)	2.29	2.26	4.11	3.45	3.95	3.82
4-eq (2-eq)	1.81	2.11	3.35	3.18	3.29	3.51
5 (1)	3.53	3.84	4.21	3.71	3.55	3.26
6-ax	2.10	2.18	2.34	2.54	2.20	2.08
6-eq	2.10	2.08	2.34	1.98	1.95	2.10
7-ax	2.10	2.23	2.34	2.57	2.58	2.74
7-eq	2.10	2.20	2.34	2.10	1.79	2.22
8-ax	-	-	-	-	2.20	2.30
8-eq	-	-	-	-	1.95	2.19
8a (9a)	2.54	-	3.11	-	3.11	-
8a (9a)	2.54	-	3.19	-	3.26	-
8b (9b)	2.03	-	2.35	-	2.24	-
8b (9b)	2.03	-	2.42	-	2.24	-
8c (9c)	5.03	-	4.99	-	4.99	-
1"	2.35	-	2.45	-	2.40	-
2",3",5",6"	1.68-2.18	-	1.65-2.15	-	1.64-2.13	-
2'-6'	7.23-7.34	-	7.27-7.45	-	7.27-7.43	-
2C*H*$_3$(*i*Pr)	1.35	-	1.36	-	1.32	-
2C*H*$_3$(*i*Pr)	-	-	1.40	-	1.36	-
C*H*$_3$	2.51	-	2.70	-	2.54	-
C*H*$_3$	-	-	2.47	-	2.40	-
5"'a	3.26	-	3.26	-	3.15	-
5"'a	-	-	3.63	-	3.29	-

[(a)] CD_3OD, 298 K

[(b)] CD_3OD:D_2O 70:30, 320 K

[(c)] Mean values weighted on population percentages.

NOESY data also confirmed the presence of the expected conformers **A**-**F** of **1**-**3** (Table 1 and 2). Hence, for compound **1**, H-3 (4.41 ppm) cross peaks with H-5"'a (3.26 ppm) and 2CH_3*i*Pr (1.35 ppm) and a cross peak between axial H-2/H-4 (2.29 ppm) and CH_3 (2.51 ppm) accounted for the triazole isopropyl facing the ethylenic bridge of conformers **1A**, **1B**, **1E** and **1F** whereas a cross peak of H-3

Analogously, a cross peak between H-5"'a (3.26 ppm) and H-6/H-7 (2.34 ppm) and between axial H-2/H-4 (4.11 ppm) and CH_3 (2.70 ppm) accounted for **2A**, **2B**, **2E** and **2F** conformers whereas a cross peak of H-5"'a (3.63 ppm) with axial H-2/H-4 (4.11 ppm) and of CH_3 (2.47 ppm) with equatorial H-2/H-4 (3.35 ppm) accounted for the **2C** and **2D**. The H-1/H-5 (4.30/4.21 ppm) - H-8b (2.40 ppm) contact supported **2D**-**F** conformations.

The NOESY spectrum of compound **3** showed correlations similar to those found for **1** and **2**. Thus the **3A**, **3B**, **3E** and **3F** family was confirmed by cross a peak between axial H-2/H-4 (3.99/3.95 ppm) and CH_3 (2.54 ppm), and by a contact between H-5'''a (3.15 ppm) and axial H-7 (2.58 ppm). H-5'''a (3.15 ppm) and axial H-2/H-4 (3.99/3.95 ppm) correlation accounted for **3C** and **3D** and a cross peak between H-1/H-5 (3.59/3.55 ppm) and H-9c (4.99 ppm) was related to **3D**-**F** conformers.

Actually, the observed contacts correspond to distances of less than 3 Å as measured on the computed most populated conformations of compounds **1**-**3** (Fig. 3 and 4).

Conclusions

Two structural analogues of Maraviroc (**1**), in which the azabicyclooctane moiety is replaced by a diazabicyclooctane or diazabicyclononane, were synthesized and their infectivity reduction power determined through a viral neutralization assay on a panel of six pseudoviruses. Diazabicyclooctane **2** demonstrated a biological activity similar to Maraviroc, whereas diazabicyclononane **3** showed a lower infectivity reduction. The modeling study revealed that Maraviroc and its derivatives are highly flexible molecules with several degrees of conformational freedom and high-field 1D and 2D NMR experiments confirmed this hypothesis showing the existence in solution of the calculated conformers as evidenced by specific NOESY contacts. From a conformational point of view, no significant differences were observed between the corresponding conformations of the three compounds and, consequently, their different inhibitory activity could be attributable only to a different distribution of the population of conformations. In particular, attention was focused on the isopropyl group. The reference compound **1** orients it on the same side of the ethylene bridge of the bicyclic system, whereas **2** and **3** show a preference for the opposite orientation. This behavior may explain the antiviral activity of the two analogues with respect to Maraviroc, that remains significant for compound **2** and more reduced for **3**.

Experimental

Chemistry

Reagents were purchased from commercial suppliers and used as received. Commercial plates on aluminum backed Silica Gel 60 plates (0.2 mm, Merck) were used for analytical TLC to follow the course of the reaction. Silica gel 60 (Merck 40–63 μm) was used for flash chromatography to purify intermediates and final compounds. The purity of final compounds was determined by HPLC analysis and was ≥ 95%. Melting points were determined in open capillary tubes with a Büchi Melting Point 510. ^{1}H NMR spectra were acquired at ambient temperature with a 300 MHz Oxford-Varian instrument. Chemical shifts are expressed in ppm from tetramethylsilane resonance in the indicated solvent (TMS: δ = 0.0 ppm). Derivatives **4** and **5** were synthesized according to literature methods.[11] The structures of all compounds are consistent with their analytical and spectroscopic data.

Synthesis of Maraviroc (**1**): Maraviroc (**1**) was prepared according to literature procedure.[10] ^{1}H-NMR: see Table 3. ^{13}C-NMR (CD_3OD): δ = 10.4 (*C*H_3), 20.1 (2 *C*H_3 *i*Pr), 24.7 (C5'''), 24.8 (C6 and C7), 25.0 (C6''or C2'', d, $J_{C\text{-}F}$ = 9.5 Hz), 25.2 (C2''or C6'', d, $J_{C\text{-}F}$ = 9.5 Hz), 31.8-31.9 (C3'' and C5'', t, $J_{C\text{-}F}$ = 25 Hz), 33.7 (C8b), 34.6 (C2 and C4), 41.7 (C1''), 47.2 (C3), 47.9 (C8a), 50.7 (C8c), 58.4 (C1 or C5), 59.2 (C5 or C1), 121.9 (C4'', t, $J_{C\text{-}F}$ = 240 Hz), 125.7 (C2' and C6'), 126.4 (C4'), 127.7 (C3' and C5'), 141.7 (C1'), 150.6 (C5''' or C2'''), 159.4 (C2''' or C5''') and 174.9 (C1''a). Experimental: pKa 1 = 7.919; pKa 2 = 3.443 (see supplementary).

General procedure A to synthesize methyl 3-benzyl-3,8-diazabicyclo[3.2.1]octane-8-carboxylate compound (6) and methyl 3-benzyl-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (7): to a cooled solution of the appropriate diazabyciclo derivatives (**4,5**) (8.75 mmol) and triethyl amine (1.83 mL, 13 mmol) in diethyl ether (50 mL) methyl chloroformate (1.01 mL, 13 mmol) was slowly added and the mixture stirred overnight at room temperature. After completion of reaction, the mixture was washed with 5% $NaHCO_3$ solution (20 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the final products.

Methyl-3-benzyl-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (6): general procedure A was followed for the synthesis of compound **6**, which was obtained as a pale yellow oil after purification by flash column chromatography (cyclohexane/ethyl acetate, 80:20); 88% yield. TLC (cyclohexane/ethyl acetate, 80:20): Rf = 0.45. ^{1}H-NMR (300 MHz, acetone-d_6) δ (ppm): 1.70-1.95 (m, 4H, $2H_6$,$2H_7$); 2.15-2.30 (m, 2H, $2H_2$(H_4)); 2.55-2.65 (m, 2H, $2H_4$(H_2)); 3.45 (s,2H, CH_2Ph); 3.60 (s, 3H, OCH_3); 4.10-4.20 (m, 2H, H_1,H_5); 7.15-7.40 (m, 5H, Ph).

Methyl-3-benzyl-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (7): general procedure A was followed for the synthesis of compound **7**, which was obtained as an orange oil after purification by flash column chromatography (cyclohexane/ethyl acetate, 90:10); 69% yield. TLC (petroleum ether/ethyl acetate, 90:10): Rf = 0.3. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.50-1.90 (m, 5H, $2H_6$, H_7, $2H_8$); 2.25-2.40 (m, 2H, H_7, H_2 (H_4)); 2.75-2.95 (m, 3H, H_2 (H_4), $2H_4$ (H_2)); 3.40 (s, 2H, CH_2Ph); 3.70 (s, 3H, OCH_3); 4.05-4.25 (m, 2H, H_1, H_5); 7.20-7.40 (m, 5H, Ph).

General procedure B to synthesize methyl-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (8) and methyl-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (9): the appropriate diazabicyclo derivatives (**6**,**7**) was dissolved in ethanol (10 mL), to the solution 10% Pd-C (10% in weight) was added, and the mixture was hydrogenated at room temperature and external pressure. After the uptake of hydrogen ceased, the catalyst was filtered off, the solvent evaporated to give final compounds.

Methyl-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (8): general procedure B was followed for the synthesis of compound **8**, which was obtained as a yellow oil without further purification (99% yield). TLC (dicholromethane/methanol, 85:15): Rf = 0.36. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.65-1.85 (m, 4H, $2H_6$,$2H_7$); 2.50-2.60 (m, 2H, $2H_2$ (H_4)); 2.75-2.95 (m, 2H, $2H_4$ (H_2)); 3.55 (s, 3H, OCH_3); 3.95-4.10 (m, 2H, H_1, H_5).

Methyl-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (9): general procedure B was followed for the synthesis of compound **9**, which was obtained as a yellow oil without further purification (91% yield). TLC (dichloromethane/methanol, 85:15): Rf = 0.35. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.50-2.80 (m, 6H, $2H_6$, H_7,$2H_8$, NH); 2.40-2.55 (m, 1H, H_7); 2.90-3.10 (m, 4H, H_2, H_4); 3.65 (s, 3H, OCH_3); 3.90-4.15 (m, 2H, H_1, H_5).

General procedure C to synthesize methyl-3-nitroso-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (10) and methyl 3-nitroso-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (11): to a stirred and cooled solution of **8** or **9**. (4.86 mmol) in 2 M hydrochloric acid (25 mL) was added, under nitrogen, dropwise a solution of sodium nitrite (20 mmol) in 5 mL of water. The mixture was stirred for 4 h at room temperature, then cooled, made alkaline with 50% sodium hydroxide, and extracted with ether. The extract was dried over Na_2SO_4, the solvent evaporated, and the residue purified by flash chromatography to give the desired products **10** or **11**).

Methyl-3-nitroso-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (10): general procedure C was followed for the synthesis of compound **10**, which was obtained as a yellow oil after purification by flash column chromatography (petroleum ether/ethyl acetate, 60:40); 75% yield. TLC (petroleum ether/ethyl acetate, 60:40): Rf = 0.2. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.36-2.21 (m, 4H, $2H_6$, $2H_7$); 2.76-2.83 (m, 1H, H_2 (H_4)); 3.75 (s, 3H, OCH_3); 3.97-4.04 (m, 1H, H_2 (H_4)); 4.34-4.40 (m, 1H, H_5); 4.51-4.60 (m, 2H, H_1, H_4 (H_2)); 4.76-4.84 (m, 1H, H_4 (H_2)).

Methyl-3-nitroso-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (11): general procedure C was followed for the synthesis of compound **11**, which was obtained as a yellow oil after purification by flash column chromatography (petroleum ether/ethyl acetate, 50:50); 51% yield. TLC (petroleum ether/ethyl acetate, 60:40): Rf = 0.33. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.44-2.00 (m, 6H, $2H_6$, $2H_7$, $2H_8$); 2.74-2.89 (m, 1H, H_2 (H_4)); 3.78 (s, 3H, OCH_3); 3.96-4.09 (m, 1H, H_2 (H_4)); 4.32-4.66 (m, 2H, H_1, H_5); 4.74-5.00 (m, 2H, $2H_4$ (H_2)).

General procedure D to synthesize methyl-3-amino-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (12) and methyl 3-amino-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (13): to a stirred suspension of zinc dust (1.81 mmol) in 1.9 mL of 1:l acetic acid-water was added under nitrogen, maintaining the temperature between 10 and 20 °C, a solution of **10** or **11** (0.4 mmol) in 1.3 mL of acetic acid. The mixture was stirred for 15 min at room temperature and then heated to 80 °C for 15 min. After 5 min of heating, an additional portion of zinc dust (1.21 mmol) was added. The hot solution was then filtered and the zinc and inorganic salts were washed with three 1.5 mL portions of hot 1 N hydrochloric acid. The combined filtrate and washings were basified with 50% aqueous sodium hydroxide solution and extracted with dichloromethane (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated. The compounds **12**,**13** were not purified or further characterized due to their instability, and immediately used for the next reactions. Compound **12** was a yellow oil: TLC (dichloromethane/methanol, 95:5): Rf = 0.2. Compound **13** was a yellow oil: TLC (dichloromethane/methanol, 93:7): Rf = 0.3.

General procedure E to synthesize methyl-3-isobutyramido-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (14) and methyl 3-isobutyramido-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (15): to a solution of the appropriate amino derivatives (**12**,**13**) (2.43 mmol) in dimethylformamide (1 mL), triethylamine (4.01 mmol), isobutyric acid (3.95 mmol) and HATU (2.39 mmol) were added. The resultant mixture was stirred for 17 h at room temperature. Finally, water (3 mL) and ethyl acetate (3 mL) were added and the phases separated. The organic layer was extracted with 6 M HCl (3 × 5 mL) and then NaOH pellet was added until pH 9-10 to the aqueous acidic phase, cooled at 0°C. The basic layers were extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the final products.

Methyl-3-isobutyramido-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (14): general procedure E was followed for the synthesis of compound **14**, which was obtained as a yellow oil after purification by flash column chromatography (dichlorometane/methanol, 95:5); 60% yield from **10**. TLC (dichlorometane/methanol, 95:5): Rf = 0.28. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.11-1.18 (m, 6H, $2CH_3$); 1.96-2.17 (m, 4H, $2H_6$, $2H_7$); 2.60-2.68 (m, 1H, H_2 (H_4)); 2.87-2.95 (m, 3H, H_2 (H_4), $2H_4$ (H_2)); 3.00-3.15 (m, 1H, C*H*(CH_3)$_2$); 3.71 (s, 3H, OCH_3); 4.20-4.38 (m, 2H, H_1, H_5).

Methyl 3-isobutyramido-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (15): General procedure E was followed for the synthesis of compound **15** which was obtained as a yellow oil after purification by flash column chromatography (dichlorometane/methanol, 95:5); 60% yield from **11**. TLC (dichlorometane/methanol, 85:15): Rf = 0.23. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.07-1.17 (m, 6H, $2CH_3$); 1.48-1.94 (m, 5H, $2H_6$, H_7, $2H_8$); 2.40-2.60 (m, 1H, H_7); 2.60-2.75 (m, 2H, $2H_2$ (H_4)); 2.86-3.11 (m, 3H, C*H*(CH_3)$_2$, $2H_4$ (H_2)); 3.68 (s, 3H, OCH_3); 4.11-4.31 (m, 2H, H_1, H_5).

General procedure F to synthesize methyl 3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (16) and methyl-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (17): a mixture of dichloromethane (4.8 mL) and PCl_5 (2.7 mmol) was cooled to -5 °C. A solution of butyramido derivatives **16** or **17** (2.08 mmol) in dichloromethane (1.6 mL) was slowly added keeping the temperature below 10 °C. The solution was warmed to ambient temperature and held at this temperature for 2.5 h, then cooled back to -5 °C. A solution of acetic hydrazide (3.33 mmol) in 2-methyl-2-butanol was prepared by dissolving the acetic hydrazide in acetonitrile (1.8 mL) and 2-methyl-2-butanol (9.8 mL), and then concentrating it to approximately half volume by distillation under reduced pressure. The acetic hydrazide solution was added to the reaction mixture keeping the temperature below 10 °C. The resultant solution was warmed to ambient temperature and stirred for 19 h. The mixture was cooled to -5 °C and 2 M NaOH (3 mL) was added, keeping the temperature below 10 °C and the layers were separated. The organic layer was concentrated under reduced pressure. The residue was treated with 2-methyl-2-butanol (1.5 mL) and acetic acid (0.2 mL) and warmed at 80°C for 2.5 h. The solution was cooled to 0°C and 2 M NaOH was added until pH 12. The phases were

separated and the aqueous layer was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the final products.

Methyl-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (16): general procedure F was followed for the synthesis of compound **16**, which was obtained as an orange oil after purification by flash column chromatography (petroleum ether/ethyl acetate, 4:6); 48% yield. TLC (dichlorometane/methanol, 9:1): Rf = 0.38. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.34-1.40 (m, 6H, $2CH_3$); 2.00-2.05 (m, 4H, $2H_6$, $2H_7$); 2.35-2.51 (m, 3H, CH_3); 2.88-2.95 (m, 2H, $2H_2$ (H_4)); 3.03-3.18 (m, 1H, C*H*(CH_3)$_2$); 3.44-3.56 (m, 2H, $2H_4$ (H_2)); 3.76 (s, 3H, OCH_3); 4.38-4.48 (m, 2H, H_1, H_5).

Methyl3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,9-diazabicyclo[3.3.1]nonane-9-carboxylate (17): general procedure F was followed for the synthesis of compound **17**, which was obtained as a white solid after purification by flash column chromatography (dichlorometane/methanol, 95:5); 48% yield. M.p. 176-176.8°C. TLC (dichlorometane/methanol, 9:1): Rf = 0.26. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.36-1.40 (m, 6H, $2CH_3$); 1.66-2.05 (m, 5H, $2H_6$, H_7, $2H_8$); 2.40-2.59 (m, 3H, CH_3); 2.59-2.73 (m, 1H, H_7); 3.05-3.19 (m, 3H, C*H*(CH_3)$_2$, $2H_2$(H_4)); 3.61-3.73 (m, 2H, $2H_4$ (H_2)); 3.80 (s, 3H, OCH_3); 4.31-4.53 (m, 2H, H_1, H_5).

General procedure G to synthesize 3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,8-diazabicyclo[3.2.1]octane (18) and 3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,9-diazabicyclo[3.3.1]nonane (19): to a solution of the appropriate triazole derivatives (**16** or **17**, 0.17 mmol) in methanol (2.9 mL), 10% NaOH was added (2.9 mL). The solution was warmed at 80°C for 5 h. The methanol was removed under reduced pressure and dichloromethane (10 mL) was added. The phases were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the final products.

3-(3-Isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,8-diazabicyclo[3.2.1]octane (18): general procedure G was followed for the synthesis of compound **18**, which was obtained as a pale yellow oil and was utilized without further purification (57% yield). TLC (dichlorometane/methanol, 7:3): Rf = 0.18. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.32-1.36 (m, 6H, $2CH_3$); 1.80-1.86 (m, 2H, $2H_6$ (H_7)); 1.96-2.14 (m, 2H, $2H_7$ (H_6)); 2.32-2.54 (m, 3H, CH_3); 2.84-2.90 (m, 2H, $2H_2$ (H_4)); 3.01-3.18 (m, 1H, C*H*(CH_3)$_2$); 3.44-3.50 (m, 2H, $2H_4$ (H_2)); 3.58-3.64 (m, 2H, H_1, H_5).

3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,9-diazabicyclo[3.3.1]nonane (19): general procedure G was followed for the synthesis of compound **19**, which was obtained as a yellow foam and was utilized without further purification (98% yield). TLC (dichlorometane/methanol, 7:3): Rf = 0.11. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.28-1.37 (m, 6H, $2CH_3$); 1.63-2.00 (m, 5H, $2H_6$, H_7, $2H_8$); 2.34-2.51 (m, 3H, CH_3); 2.51-2.68 (m, 1H, H_7); 2.97-3.14 (m, 2H, C*H*(CH_3)$_2$, H_2 (H_4)); 3.18-3.28 (m, 1H, H_2 (H_4)); 3.55-3.71 (m, 2H, $2H_4$ (H_2)); 4.20-4.44 (m, 2H, H_1, H_5).

Synthesis of (S)-4,4-difluoro-N-(3-oxo-1-phenylpropyl) cyclohexanecarboxamide (20): compound **20** was synthesized following the procedure reported in literature[12a] and its analytical data were comparable to those reported. White solid. M.p. 119°C TLC (petroleum ether/ethyl acetate, 4:6): Rf = 0.52. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.60-2.24 (m, 8H); 2.92-3.12 (m, 2H); 5.44-4.55 (m , 1H); 6.20-6.26 (d, br, 1H); 7.20-7.40 (m, 5H); 9.75 (s, 1H). M/z 296.07 $[M + H]^+$.

Synthesis of 4,4-difluoro-N-((1S)-3-(3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)-1-phenylpropyl)cyclohexanecarboxamide (2): to a suspension of **18** (22.8 mg, 0.097 mmol) in dichloromethane (0.4 mL) under a nitrogen atmosphere, a solution of **20** (31 mg, 0.106 mmol) in toluene (0.5 mL) and acetic acid (0.01 mL) were added. The solution was stirred for 30 min at room temperature then was cooled at 0°C and sodium triacetoxyborohydride (25 mg, 0.116 mmol) was added. The resultant mixture warmed to room temperature was stirred for further 4 h. Finally, the reaction was quenched with water (3 mL) and the pH adjusted to 11-12 by addition of 2 M sodium hydroxide solution. The phases were separated and the organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a yellow oil that was purified by flash column chromatography (dichlorometane/methanol, 9:1); 37.7% yield. TLC (dichlorometane/methanol, 9:1): Rf = 0.24. ^{1}H-NMR: see Table 3. ^{13}C-NMR (CD_3OD): δ = 8.0 (*C*H_3), 10.6 (*C*H_3), 19.3 (2 *C*H_3 *i*Pr), 20.2 (2 *C*H_3 *i*Pr), 23.1 (C6 and C7), 24.0 (C5'''a), 24.7 (C5'''a), 25.0-25.1 (C2'' and C6'', d, $J_{C\text{-}F}$ = 9.5 Hz), 30.0 (C8b), 31.8 (C3'' and C5'', t, $J_{C\text{-}F}$ = 25 Hz), 41.4 (C1''), 48.6-48.7 (C8a), 50.1 (C8c), 54.7-54.9 (C2 and C4), 61.3-61.5 (C1 or C5), 61.9 (C5 or C1), 121.9 (C4'', t, $J_{C\text{-}F}$ = 240 Hz), 125.8 (C2' and C6'), 127.0 (C4'), 128.0 (C3' and C5'), 140.1 (C1'), 151.2 (C5''' or C2'''), 159.9 (C2''' or C5''') and 175.7 (C1''a); $[\alpha]_D^{25}$ = -20.05 (c = 6.3 × 10^{-3} M, $CHCl_3$). ESI-HRMS m/z: $[M + H]^+$ measured: 515.33088; calculated: 515.33044; $[M + Na]^+$ measured: 537,31234; calculated: 537,31239. Experimental: pK_a 1 = 7.268; pK_a 2 = 2.958 (see supplementary).

Synthesis of 4,4-difluoro-N-((1S)-3-(3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-3,9-diazabicyclo[3.3.1]nonan-9-yl)-1-phenylpropyl)cyclohexanecarboxamide (3): to a suspension of **19** (47 mg, 0.187 mmol) in dichloromethane (0.7 mL) under a nitrogen atmosphere, a solution of **20** (60 mg, 0.204 mmol) in toluene (1 mL) and acetic acid (0.02 mL) were added. The solution was stirred for 3h at room temperature then was cooled at 0°C and sodium triacetoxyborohydride (48 mg, 0.224 mmol) was added. The resultant mixture warmed to room temperature and stirred for further 4 h. Finally, the reaction was quenched with water (4 mL) and the pH adjusted to 11-12 by addition of 2 M sodium hydroxide solution. The phases were separated and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a white foam that was purified by flash column chromatography (dichlorometane/methanol, 97:3); 21.3% yield. TLC (dichlorometane/methanol, 9:1): Rf = 0.45. ^{1}H-NMR: see Table 3. ^{13}C-NMR (CD_3OD:D_2O, 70:30): δ = 9.1 (*C*H_3), 11.4 (*C*H_3), 17.0 (C7), 19.1 (2 *C*H_3 *i*Pr), 20.3 (2 *C*H_3 *i*Pr), 23.2 (C6 and C7), 24.2 (C5'''a), 24.8-24.9 (C2'' and C6'', d, $J_{C\text{-}F}$ = 9.5 Hz), 30.0 (C9b), 31.5 (C3'' and C5'', t, $J_{C\text{-}F}$ =

25 Hz), 41.3 (C1''), 47.50 (C9a), 50.5 (C9c), 52.5 (C2 and C4), 52.8 (C1 or C5), 53.4 (C5 or C1), 122.3 (C4'', t, $J_{C\text{-}F}$ = 240 Hz), 125.7 (C2' and C6'), 127.0 (C4'), 128.0 (C3' and C5'), 140.0 (C1'), 150.1 (C5''' or C2'''), 159.7 (C2''' or C5''') and 175.6 (C1''a); $[\alpha]_D^{25}$ = -23.34 (c = 6.3 × 10^{-3} M, $CHCl_3$). ESI-HRMS m/z: $[M + H]^+$ measured: 529,34671; calculated: 529,34609; $[M + Na]^+$ measured: 551,32845; calculated: 551,32804. Experimental: pK_a 1 = 7.155; pK_a 2 = 2.923 (see supplementary).

Viral neutralization assay

Viral Infectivity Reduction Assay: Infectivity reduction was measured as a reduction in Luc reporter gene expression after a single round of virus infection in TZM-bl cells with env-pseudotyped viruses.[18] Virus panel of HIV-related pseudoviruses included one laboratory strain SF162 (Clade B), four from primary infected Subtype B subjects such as QH0692, 6535, PVO, and AC10 isolates (all strains were CCR5-tropic), and one primary infected Subtype C virus, ZM214 (CCR5-tropic). In order to demonstrate the specificity of HIV neutralization, an HIV-unrelated virus (VSV-G virus, strain SVA.MLV#922) was also included. Briefly, 200 TCID50 of pseudoviruses in 50 µL culture media was incubated with 100 µL of serially diluted compounds (from 10 to 0.001 µM) by using D-MEM with 10% foetal bovine serum in a 96-well plate for 1 h at 37 °C. Compound **1**, Maraviroc, was used as positive control. A 100 µL solution of TZM-bl cells (104 cells/well) containing 15 µg/mL DEAE dextran was added; the cultures were then incubated at 37 °C in 5% CO_2/95% air for 48 h. Infection was monitored by evaluating the luciferase activity. Infectivity reduction was calculated as IC_{90}, the compound concentration at which relative luminescence units (RLU) were reduced by 90% relative to virus control wells (wells with no inhibitor) after subtraction of background RLU in the cell control wells (wells without virus infection).

Computational Methods

All the calculations were carried out using the GAUSSIAN09 program package.[19] Optimizations were performed at the B3LYP/6-31G(d) level,[14] considering all degrees of conformational freedom. Vibrational frequencies were computed at the same level of theory to verify that the optimized structures were minima. The solvent effects were considered by single-point calculations, at the same level as above, on the gas-phase optimized geometries, using a self-consistent reaction field (SCRF) method, based on the polarizable continuum model (PCM).[15] The population percentages were calculated through the Boltzmann equation.

NMR Spectroscopy

NMR spectra of compounds **1-3** were recorded at 298 or 320K with a Bruker AVANCE-500 spectrometer operating at 500.13 MHz for ^1H or at 125.76 MHz for ^{13}C NMR spectra using a 5 mm z-PFG (pulsed field gradient) broadband reverse probe. Chemical shifts are reported on the δ (ppm) scale and are relative to residual CH_3OD at 3.30 ppm (central line) for ^1H NMR spectra, and relative to CD_3OD at 47.0 ppm (central line) for ^{13}C NMR spectra. The data were collected and processed by XWIN-NMR software (Bruker) running on a PC with Microsoft Windows xp. Compounds (about 3-5 mg) were dissolved in the proper solvent (0.6 mL) which was CD_3OD for **1** and **2** and CD_3OD/D_2O 70/30 for compound **3** which gave very broad shaped signals in methanol only (see supplementary). The solutions were put in a 5mm NMR tube and the spectra recorded at 298 K (compounds **1** and **2**) or 320 K (compound **3**). The signal assignment was given by a combination of 1D and 2D (COSY and HSQC) experiments, using standard Bruker pulse programs. The ^1H-^1H and ^{13}C-^1H bond correlations were confirmed by COSY and HSQC experiment using Z-PFGs. The pulse widths were 7.50 µs (90°) for ^1H and 14.75 µs (90°) for ^{13}C. Typically 32 K data points were collected for one-dimensional spectra. Spectral width was 11.45 ppm (5733 Hz) for ^1H NMR (digital resolution: 0.17 Hz per point). 2D experiments parameters were as follows. For ^1H-^1H correlations: relaxation delay 2.0 s, data matrix 1 K × 1 K (512 experiments to 1 K zero filling in F_1, 1 K in F_2), 2 or 24 transients in each experiment for COSY and NOESY respectively, spectral width 8.00 ppm (3996 Hz). The NOESY spectra were generated with a mixing time of 1.0 s and acquired in the TPPI mode. There were not significant differences in the results obtained at different mixing times (0.5 – 1.5 s). For ^{13}C-^1H correlations (HSQC): relaxation delay 2.5 s, data matrix 1K × 1K (512 experiments to 1 K zero filling in F_1, 1K in F_2), 6 transients in each experiment, spectral width 8.0 ppm (3996 Hz) in the proton domain and 150.0 ppm (18864 Hz) in the carbon domain. A sinebell weighting was applied to each dimension. All 2D spectra were processed with the Bruker software package.

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