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Synthesis of 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes by intramolecular haloetherification and/or transannular hydroxycyclization of alkenes in [4+3]-cycloadducts

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ABSTRACT

The synthesis of new difunctionalized 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes is described. This type of structure is an interesting synthetic building block for potential bioactive molecules and it was prepared from 8-oxabicyclo[3.2.1]oct-6-en-3-one having a NHBoc function on C-1. This precursor was obtained by a [4+3] cycloaddition reaction of 2-*tert*-butoxycarbonylaminofuran and the oxyallyl cation generated in situ from 2,4-dibromo-3-pentanone. Reduction of the carbonyl group at C-3 was accomplished in high yield and stereoselective manner to afford the corresponding axial alcohol at C-3 as a major product. Further intramolecular haloetherification of this type of alcohols with NBS and I(py)2BF$_4$ led to the corresponding bromo and iodo-derivatives at C-8 of the 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonane framework, in high yield. Epoxidation of 8-oxabicyclo[3.2.1]oct-6-en-3-ol followed by treatment with NaCN, NaN$_3$, and/or NaOH in MeOH afforded 8-hydroxy-2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes in high yield via a transannular hydroxycyclization mediated by a base and through an alkoxide intermediate. The new 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes were tested for biological activity against HIV-1 virus and MT-4 lymphoid cell line, showing a low anti-HIV activity and a high degree of cytotoxicity.

1. Introduction

One of our research interests is the synthesis of new molecular scaffolds with antiviral and/or anticancer activity. In this context we report here the synthesis and biological evaluation of new difunctionalized 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonane entities via two important methodologies: [4+3]-cycloaddition and intramolecular haloetherification and/or transannular hydroxycyclization of C=C bonds (Fig. 1).

The [4+3]-cycloaddition reaction has been widely reviewed and used by several authors[1] and also by us[2] for the preparation of building blocks of natural products, with interesting biological activities, and of non-natural molecules having interesting physical–chemical properties or with a structural added value. Also, enantioselective versions of this type of reaction have been developed.[3]

The intramolecular haloetherification and/or transannular cyclization deserves some comments about its precedents, many of them related with the reactivity and derivatization of [4+3]-cycloadducts (Fig. 2). Aside the 5- and 6-*exo-trig* cyclizations of alkenoxyl radicals that have been extensively used as a synthetic methodology of heterocycles,[4] whose stereoselectivity have been

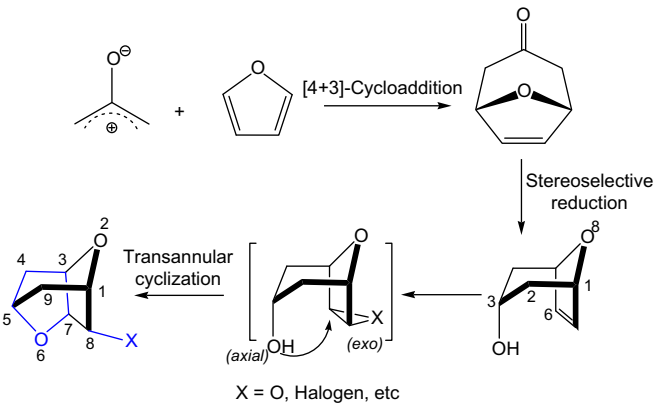

Figure 1. Synthetic methodology for the preparation of 2,6-dioxatricyclo[3.3.1.0^{3,7}]-nonanes.

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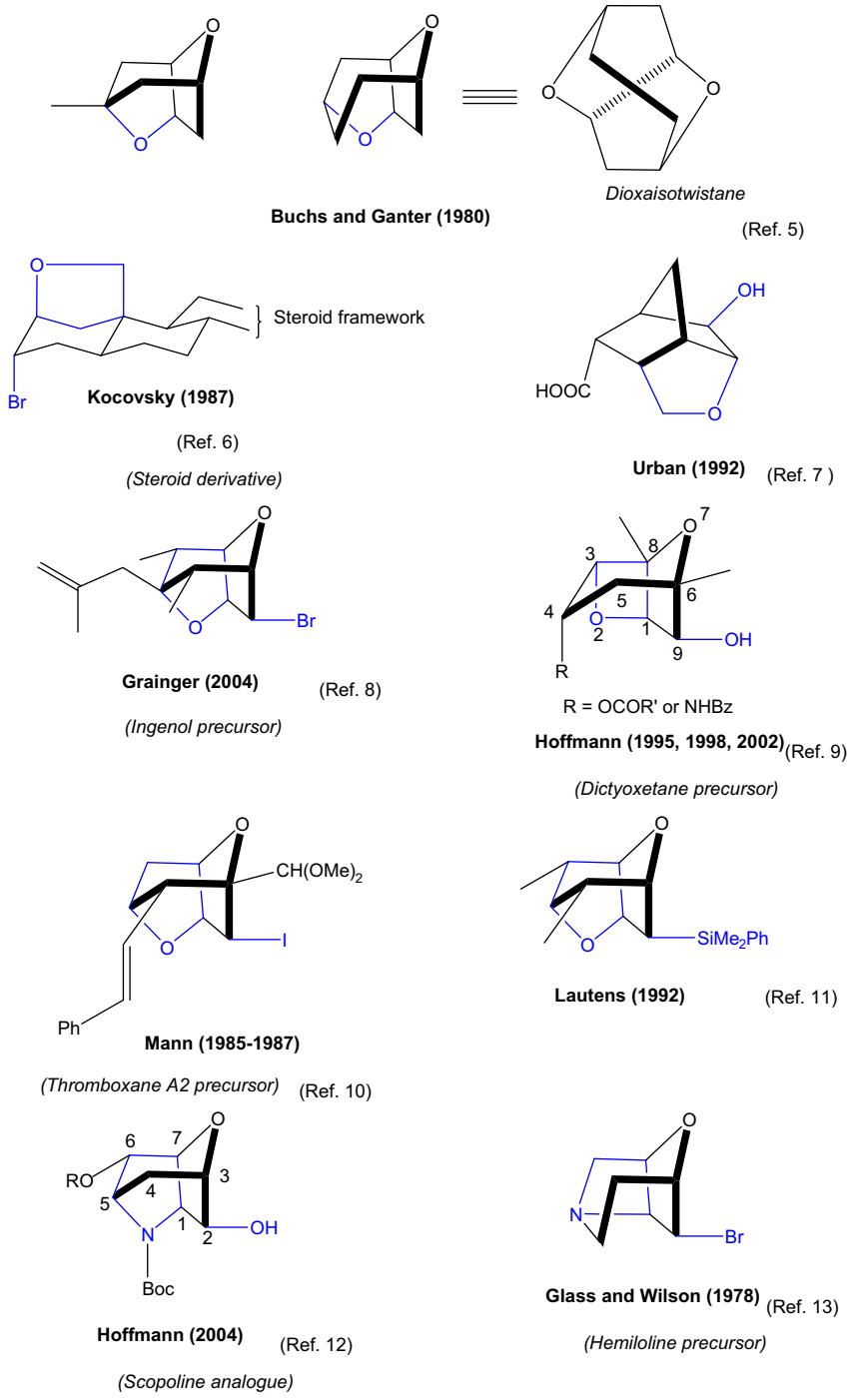


Figure 2. Dioxa- and oxaza-tricycles previously synthesized in the literature by haloetherification and/or transannular hydroxycyclization.

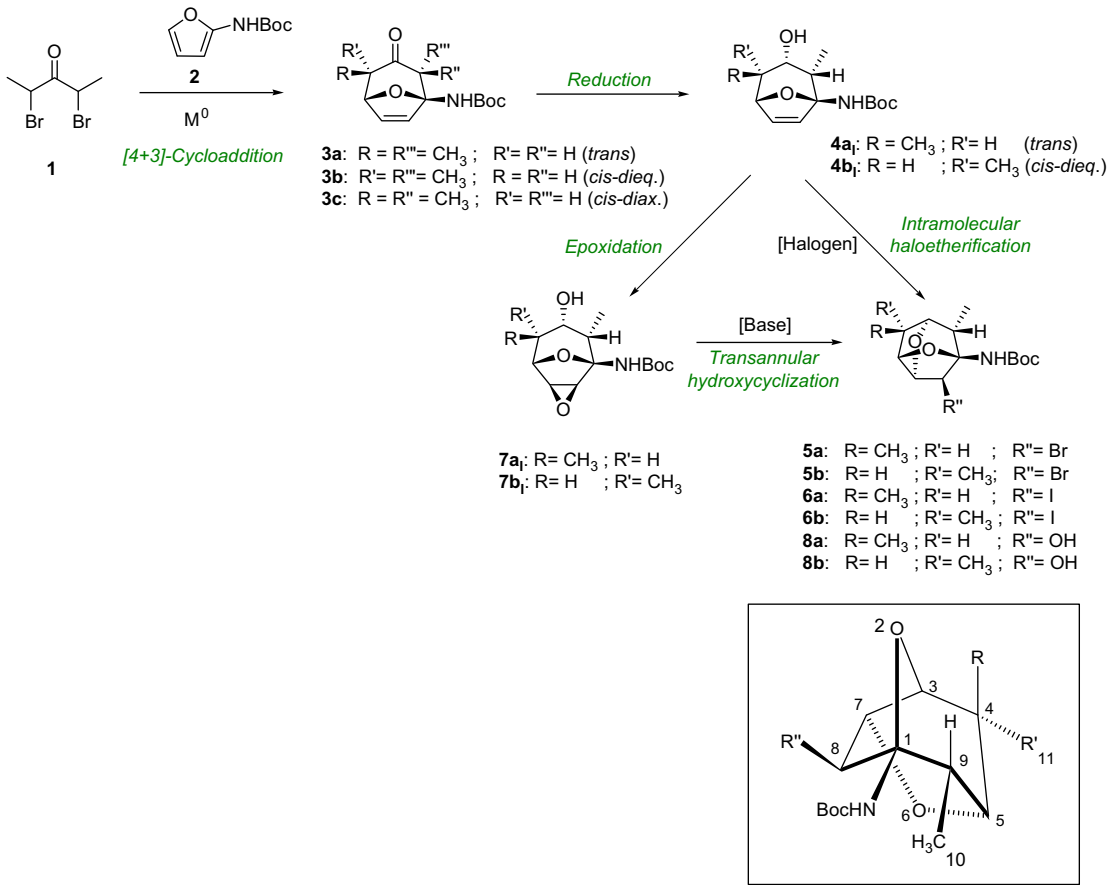
recently studied,[4l] we consider here only the intramolecular transannular cyclizations mediated by ionic intermediates.

This methodology has been used by Buchs and Ganter[5] as early as 1980 for the synthesis of the 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonane core from 3-methyl-8-oxabicyclo[3.2.1]oct-6-en-3-ol and the 2,6-dioxatricyclo[3.3.2.0^{3,7}]decane core from 3-hydroxy-9-oxabicyclo[4.2.1]non-7-en-3-ol by reaction with $Hg(OAc)_2$ followed by $NaBH_4$ in order to prepare the new structure of dioxaisotwistane.

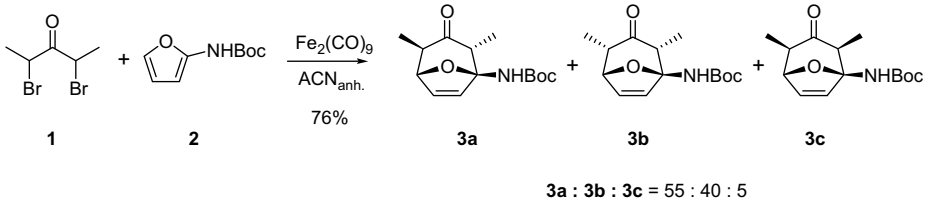
Haloetherification has been applied by Kocovsky et al.[6] as a synthetic tool in a number of transformations in steroids. Thus, the tetrahydrofuran ring formation by a S_Ni attack of an oxygenated function on a halogenonium three-membered cation in *anti* disposition (5(*O*)^n-*exo*-*trig* process), with generation of a bromoether having a *trans* relationship, has been observed in the reaction of some steroidal olefins with hypobromous acid (see Fig. 2).

A similar process used by Urban et al.[7] is the transannular cyclization that goes through epoxides as intermediates (instead of halogenonium intermediates) via a S_N2 attack of an oxygenated function on an *exo* epoxide ring to afford analogue hydroxytetrahydrofuran systems by an aqueous acid treatment.

Intramolecular haloetherification has been also accomplished by the reaction of 8-oxabicyclo[3.2.1]-oct-2-en-3-ol derivative with bromine in a new synthetic approach to the AB ring system of ingenol developed by Grainger et al.[8] The hydroxyl group has an axial disposition and it is in a close proximity to the highly reactive C6–C7 double bond. This reactivity is due to the high strain of the



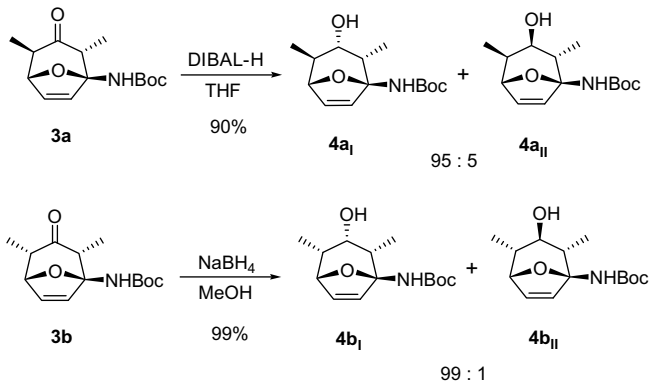
Scheme 1. Pathway for the synthesis of 2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes **5a**,**b**; **6a**,**b** and **8a**,**b**.



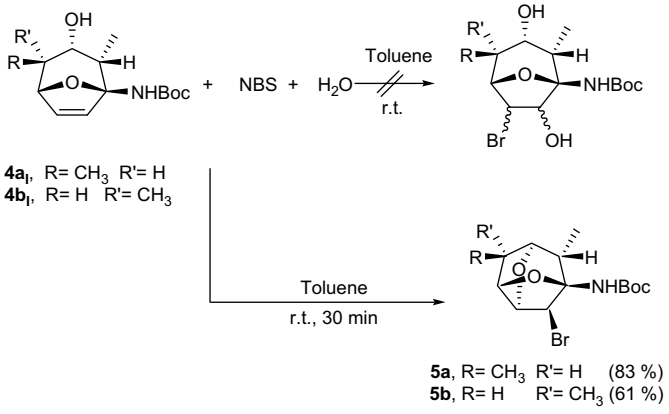
Scheme 2. Synthesis of 8-oxabicyclo[3.2.1]oct-6-en-3-one by a [4+3] cycloaddition reaction.

cyclopentene bridge in the bicyclic system. Also a *trans* relationship of the new oxygen bridge with respect to the *exo* bromine atom is observed. The authors consider that the generation of this stereochemistry is due to the previous attack of bromine on the less hindered *exo*-face of the C=C, followed by intramolecular capture of the *exo* bromonium ion intermediate by the axial alcohol.

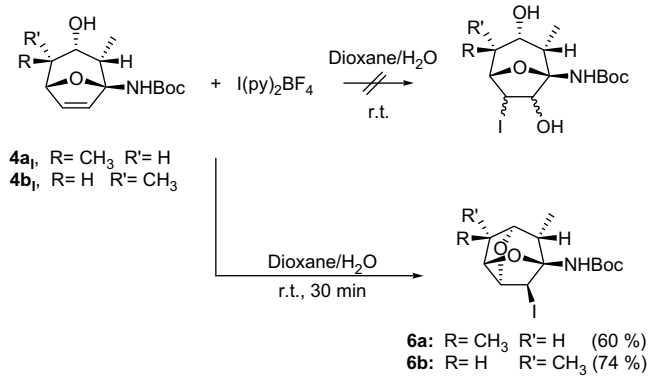
Hoffmann et al. used a similar approach to generate the 2,7-dioxatricyclo[4.2.1.0^{3,8}] core of the antitumor terpenic natural



Scheme 3. Reduction of the ketone group in cycloadducts **3a**,**b**.



Scheme 4. Intramolecular bromoetherification of unsaturated alcohols **4a**$_{\mathbf{I}}$ and **4b**$_{\mathbf{I}}$.

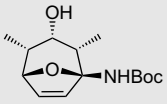
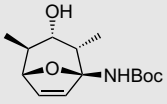


Scheme 5. Iodoetherification of unsaturated alcohols **4a$_I$** and **4b$_I$**. Synthesis of 8-iodo-2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes **6a** and **6b**.

group, usually a halogen atom, which could be displaced by an appropriate nucleophilic nitrogen leading to *Lolium* alkaloids. The formation of the aza bridge could be accomplished by reaction of the unsaturated amine with bromine to generate the intermediate bromoalkaloid, followed by reduction with $LiAlH_4$, which affords the hemiloline alkaloid.

Our contribution to this synthetic methodology deals with the synthesis of 1-*tert*-butoxycarbonylamino-2,6-dioxatricyclo[3.3.1.0^{3,7}]nonanes, which are differently substituted at C-8 with an halide group (bromide **5a**,**b** or iodide **6a**,**b**), in only three steps, or with an hydroxyl group **8a**,**b** in four steps. The preparation of these new compounds has been carried out, starting from 8-oxabicyclo[3.2.1]oct-6-en-3-ones. The tricyclic C-8-functionalized products are very versatile synthons due to the broad range of different group interconversions that it is possible to carry out in order to get a chemical library for biological testing purposes (Scheme 1).

Table 1
Epoxidation reactions of substrates **4a$_I$** and **4b$_I$**

Entry	Substrate	Oxidazing agent (no equiv)	Solvent	t_R (h)	T (°C)	DAS *endo*/*exo*	Conversion (%)	Yield (%)
1	 **4b$_I$**	H_2O_2, NaOH (5)	MeOH anhyd	28	(1) rt, (2) $\Delta_{Ref.}$	—	0	0
2		*m*-CPBA (2)	CH_2Cl_2 anhyd	72	t.a.	57/43	100	55
3		Oxone®/acetone (5)	ACN/H_2O (1:1.5)	17	t.a.	68/32	92	47
4	 **4a$_I$**	*m*-CPBA (2)	CH_2Cl_2 anhyd	72	t.a.	48/52	100	60

product dictyoxetane from 8-oxabicyclo[3.2.1]oct-6-en-3-ones. The generation of the new oxetane ring was carried out by the reaction of the 2-hydroxy-6,7-epoxy derivative with either a Lewis acid ($BF_3 \cdot OEt_3$) or a base (LiH and/or KO^{t}Bu).[9]

The elaboration of the oxetane and oxolane rings of thromboxane A2 analogues has been elegantly carried out by Mann et al. through an iodoetherification process with I_2/aq $NaHCO_3$ to afford the dioxotricyclic iodides in good yield.[10]

Lautens et al. also got the formation of dioxatricyclic compounds by silacupration of the C=C double bond in 8-oxabicyclo[3.2.1]oct-6-en-3-ol with *n*-BuLi followed by $PhMe_2SiCu \cdot LiCN$. Silacupration of the alkoxide, prepared by deprotonation of the alcohol by *n*-BuLi, afforded a net *exo*–*endo* difunctionalization of the olefin. This successful process confirms again that the reactivity arises from the release of strain in the olefin rather than any special features associated with the oxygen bridge.[11]

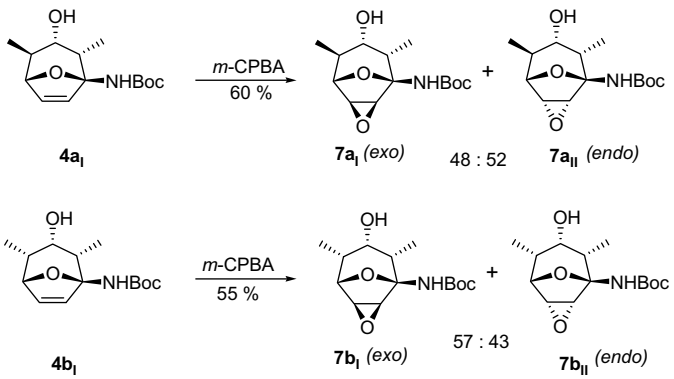
This useful route has been elegantly applied by Hoffmann to synthesize oxazatricyclic noradamantanes such as scopoline and analogues for the study of their anticancer activity. These molecules are closely related to the tropane alkaloids and have a wide variety of biological activities. These authors obtained this oxazatricyclic framework from oxabicyclo[3.2.1]oct-6-en-3-one by transformation of the C6–C7 double bond in an *exo* epoxide and the ketone group in an axial amine (protected as a Boc derivative). This precursor was reacted with ^{t}BuMgCl affording the scopoline scaffold in a regioselective manner.[12]

Analogues of pirrolizidin alkaloids like the *Lolium* alkaloids have been independently synthesized by Wilson and Glass[13] by a transannular addition of a suitable electrophile to an unsaturated amine coming from oxabicyclo[3.2.1]oct-6-en-3-one, leading to an oxazatricyclic skeleton (Fig. 2). This tricyclic intermediate contains a leaving

2. Results and discussion

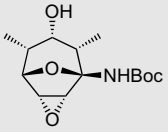
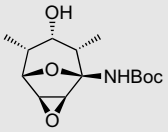
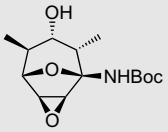
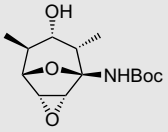
The 1-*tert*-butoxycarbonyl-amino-8-oxabicyclo[3.2.1]oct-6-en-3-ones **3a**–**c** and their precursors (**1** and **2**) were obtained as described by the authors in a previous work:[2r] the bicyclic structures were prepared by a [4+3]-cycloaddition reaction between a furan derivative, functionalized at C-2 by a protected amino group, **2**, and an oxyallyl cation generated, in situ, from 2,4-dibromo-3-pentanone,**1**, in the presence of $Fe_2(CO)_9$ (Scheme 2).

Oxabicyclic ketones **3a**,**b**, were separated by flash column chromatography and their stereochemistry was unequivocally established by NMR correlation studies and confirmed by X-ray diffraction analysis. The reduction of the ketone group at C-6 was approached by using different reducing agents and different



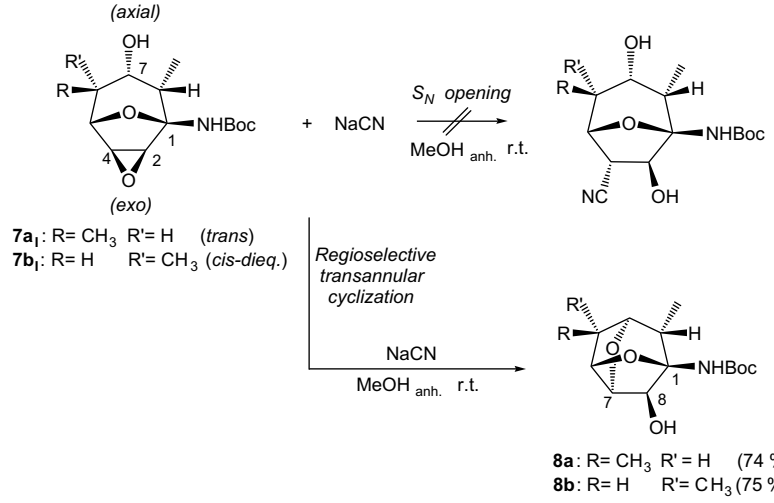
Scheme 6. Synthesis of epoxides **7a$_{I,II}$**, **7b$_{I,II}$** from substrates **4a$_I$** and **4b$_I$**, respectively.

Table 2
Reactions of epoxide breaking and/or transannular hydroxycyclization

Entry	Substrate	Reagent	No equiv	Solvent	t_R (h)	T (°C)	Lewis acid (no equiv)	Conversion (%)	Product	Yield (%)
1		$HCOONH_4$, Pd/C	3	EtOH abs.	48	rt	—	0	—	—
2		NaCN	10	MeOH anhyd	96	(1) rt	—	0	—	—
					168	(2)Δ_{Reflux}				
3		$LiAlH_4$	3	THF anhyd	3	−70	—	100	CM[a]	—
4			3		2	−30				
5	**$7b_{II}$**		3		1	0				
6		$LiAlH_4$	3	THF anhyd	5	0	—	100	CM[a]	—
7			3		10	rt				
8		$NaBH_4$	22	EtOH abs.	12	(1) 0	—	0	—	—
9			22		84	(2) rt				
10		$NaNH_2$	5	THF anhyd	48	rt	—	100	CM[a]	—
11		*t*BuMgCl	1	THF anhyd	2	0	—	100	CM[a]	—
12		NaCN	5	MeOH anhyd	72	rt	—	0	—	—
13			5		17	rt	SiO_2 (5)	0	—	—
14			5		17	rt	$MgBr_2$ (5)	0	—	—
15			5		22	rt	$CeCl_3$ (5)	0	—	—
16			5		2.5	−70	$BF_3.OEt_2$ (1)	0	—	—
17			5		1.5	0				
18			5		19	rt				
19	**$7b_I$**		10	MeOH/H_2O (10:1)	72	(1) rt	$LiClO_4$ (20)	100	**8b**	75
					96	(2)Δ_{Reflux}				
20		NaOH	10	MeOH/H_2O (10:1)	24	rt	—	0	—	—
21		NaN_3	10	MeOH anhyd	18	rt	—	0	—	—
22			10		18	rt	$MgBr_2$ (5)	0	—	—
23			10		5	rt	$CeCl_3$ (5)	0	—	—
24			10		16	Δ_{Reflux}				
25			10		72	(1) rt	$LiClO_4$ (20)	100	**8b**	74
					96	(2)Δ_{Reflux}				
26		NaCN	10	MeOH anhyd	168	rt	—	100	**8a**	75
	$7a_I$									
27		NaCN	10	MeOH anhyd	96	(1)rt	—	0	—	—
					168	(2)$\Delta_{Ref.lux}$				
	$7a_{II}$									

[a] CM: complex mixture.

reaction conditions in order to synthesize the *endo* alcohols with high diastereoselectivity and in high yield. Thus, reduction of **3a** was performed with DIBAL-H in anhydrous THF at −78 °C, obtaining the *endo* alcohol **$4a_I$** in 90% yield and 95:5 diastereomeric ratio. On the other hand, **3b** was reduced with $NaBH_4$ in anhydrous methanol at room temperature obtaining the *endo* alcohol **$4b_I$** in 99% yield and 99:1 diastereomeric ratio (Scheme 3). In both cases the diastereoisomeric alcohols were separated by flash column chromatography. Cycloadduct **3c**, with a *trans* configuration for the methyl groups on C-2 and C-4, due to its stereochemistry and its



Scheme 7. Transannular hydroxyetherification of epoxides **$7a_I$** and **$7b_I$**. Synthesis of the 8-hydroxy-2,6-dioxatricyclo[3.3.1.$0^{3,7}$]nonanes **8a** and **8b**.

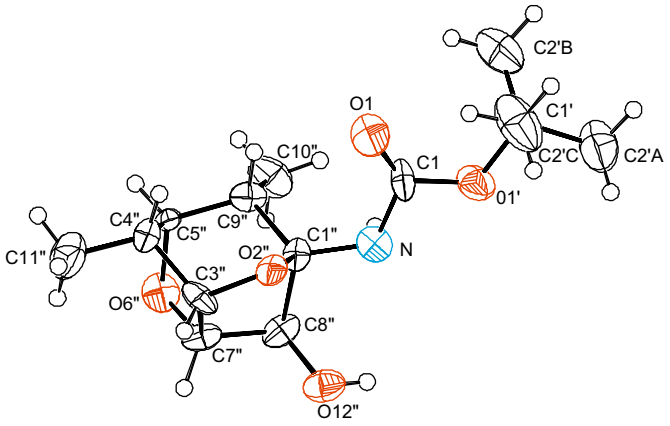


Figure 3. ORTEP representation of **8b** from X-ray diffraction analysis. The numbering of atoms corresponds to the IUPAC nomenclature.

low proportion in the cycloaddition reaction outcome, was not used further for our synthetic purposes.

The tricyclic compounds **5a** and **5b**, substituted at C-8 with a halogen atom, were obtained by the reaction of $\mathbf{4a_I}$ and/or $\mathbf{4b_I}$ with NBS in the presence of water. In this reaction we did not observe the formation of halohydrines but the generation of 8-bromo-2,6-dioxatricyclo[3.3.1.$0^{3,7}$]nonanes **5a** and **5b**, respectively (Scheme 4). The reaction was also performed in absence of water obtaining the same products **5a** and **5b** in 83% and 61% yield (on isolated product), respectively. These last reaction conditions and their results made us to consider that the reaction was taking place by a process of intramolecular cyclization.

The preparation of 8-iodo-2,6-dioxatricyclo[3.3.1.$0^{3,7}$]nonanes **6a** and **6b** was carried out by the reaction of cycloadducts $\mathbf{4a_I}$ and $\mathbf{4b_I}$, respectively, with $I(py)_2BF_4$,[14] in methanol (Scheme 5).

Another strategy to synthesize the dioxatricyclic compounds was the preparation of epoxides of the C6–C7 double bond of precursors $\mathbf{4a_I}$ and $\mathbf{4b_I}$ followed by the oxirane ring opening by S_Ni or transannular hydroxycyclization.

The epoxidation reaction of substrates $\mathbf{4a_I}$ and $\mathbf{4b_I}$ was studied by using different reagents and reaction conditions: hydrogen peroxide and NaOH,[15] *m*-CPBA,[16] and dioxirane generated in situ from Oxone® and acetone.[17] The reaction conditions and results from these epoxidation reactions are quoted in Table 1 and Scheme 6.

The best conditions tested so far were the treatment of $\mathbf{4a_I}$ and $\mathbf{4b_I}$ with *m*-CPBA in anhydrous CH_2Cl_2 at room temperature. Both compounds gave the corresponding pair of diastereomeric epoxides (*endo*/*exo*), in a 1:1 approximated ratio (Scheme 6). Each pair of epoxides were separated and purified by column chromatography and physically and spectroscopically characterized.

The transannular hydroxyetherification of epoxides, to form the dioxatricyclic framework, was approached by the oxirane ring opening under basic conditions with powerful nucleophiles like: NaCN, NaN_3 or even NaOH (see Table 2).[18] We also tried hydrides like $LiAlH_4$ [19] and reducing agents like $HCOONH_4$/Pd–C.[20] In the first trials we avoided the use of Lewis acids due to the sensitivity of the Boc protecting group to acidic conditions. Due to the resistance of these epoxides to break the oxirane ring we started using weak Lewis acids of increasing strength like SiO_2, $LiClO_4$ or $MgCl_2$ and stronger Lewis acids like $CeCl_3$ or $BF_3{\cdot}OEt_2$. In all of these reactions, even under reflux of solvent, we did not observe the formation (from either *endo* or *exo* epoxides) of dihydroxylation products at C-6 and C-7 of cycloadducts, neither the formation of cyanohydrins (from the reaction with NaCN) nor the generation of azides (by the reaction with NaN_3). These expected products from the opening of the epoxide ring by a nucleophilic attack of NaOH, NaCN or NaN_3 (with the nucleophiles alone or in the presence of a Lewis acid as a catalyst) were not observed at all under any of the reaction conditions assayed. It is remarkable that *endo* epoxides $\mathbf{7a_{II}}$ (which are not prone to undergo transannular cyclization through the axial hydroxyl group at C-7) did not react under the aforementioned conditions even after eleven days of reaction time (Table 2, entry 27).

However, when *exo* epoxides $\mathbf{7b_I}$ and $\mathbf{7a_I}$ reacted with the nucleophiles (NaCN or NaN_3) acting as weak bases, but for a very long reaction time (see Table 2, entries 19, 25, and 26), they afforded the dioxatricyclic products **8b**, **8a**, hydroxylated at C-8, as the only products and in high yield (74% and 75%, respectively), as the result of an intramolecular cyclization (Scheme 7). The use of stronger bases like ^{t}BuMgCl,[12] $NaNH_2$ or $LiAlH_4$ converted the starting material in a complex reaction mixture under all the reaction conditions tested. These strong bases seem to affect the Boc protecting group, even at low temperatures, due to the fact that the NHBoc group forms part of a cyclic aminoketal function. The hydrogen of the NHBoc group ($pK_a \approx 11$),[21] is abstracted by the base in first instance with respect to the hydrogen from the OH group ($pK_a \approx 15$). Once that hydrogen is abstracted the cyclic aminoketal opens and follows different reaction pathways generating a complex mixture of products.

The common behavior of epoxides in organic chemistry is that they should react readily in the presence of nucleophiles like CN^- or N_3^-. This process is thermodynamically favored because the opening of the oxirane ring releases its high steric strength and inner energy. However, in our substrates, probably due to steric reasons, the oxirane ring is quite hindered by the methyl groups and the bulky NHBoc group. Only when the OH group at C-7 is axial and the epoxide is *exo* the opening is possible by transannular S_N2. Even in this case the reaction kinetics is quite slow as can be deduced from the experimental results.

It is worth noting that this transannular cyclization is a regiospecific reaction, because no other regioisomer was detected (by GC or NMR). The formation of these particular isomers **8a** and/or **8b** could be explained by the preferential attack of the C-7 hydroxyl

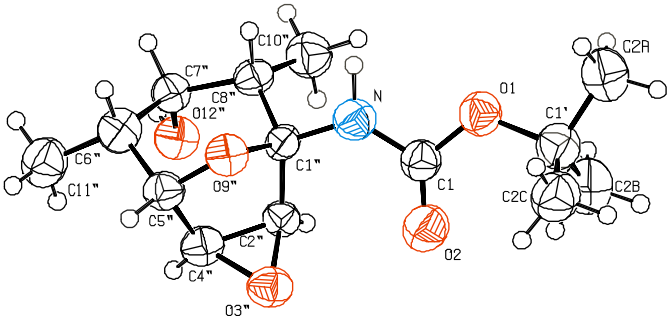


Figure 4. ORTEP representation of $\mathbf{7b_I}$ from X-ray diffraction analysis. The numbering of atoms corresponds to the IUPAC nomenclature.

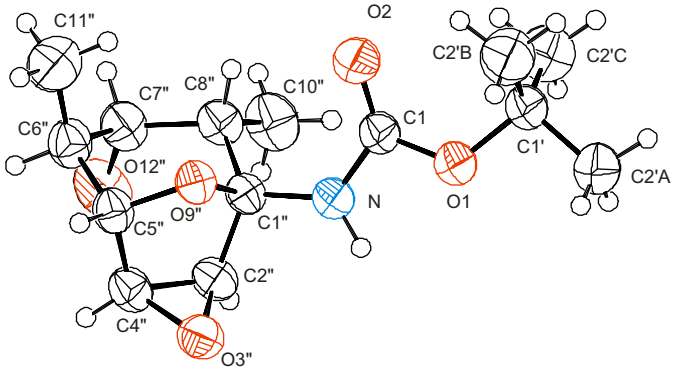


Figure 5. ORTEP representation of $\mathbf{7a_I}$ from X-ray diffraction analysis. The numbering of atoms corresponds to the IUPAC nomenclature.

Table 3
Crystal data refinement for **8b** and **7b$_I$** and **7a$_I$**

Parameters	Compound **8b**	Compound **7b$_I$**	Compound **7a$_I$**
Empirical formula	$C_{14}H_{23}NO_5$	$C_{14}H_{23}NO_5$	$C_{14}H_{23}NO_5$
Formula weight	285.33	285.33	285.33
Temperature	150(2) K	293(2) K	293(2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Orthorhombic	Monoclinic	Monoclinic
Space group	*Pcab* (61)	$P2_1/n$	$P2_1/a$
Unit-cell dimensions	a=10.61300(10) Å α=90°	a=5.937(2) Å α=90°	a=12.409(6) Å α=90°
	b=12.69500(10) Å β=90°	b=21.013(13) Å β=90.15(4)°	b=9.996(4) Å β=110.73(2)°
	c= 21.9340(3) Å γ=90°	c=11.439(6) Å γ=90°	c=13.590(5) Å γ=90°
Volume	2955.21(5) Å^3	1426.8(17)Å^3	1576.6(11)Å^3
Z	8	4	4
Calculated density	1.283 Mg/m^3	1.328 Mg/m^3	1.202 Mg/m^3
Absorption coefficient	0.097 mm^{-1}	0.100 mm^{-1}	0.091 mm^{-1}
F(000)	1232	616	616
Crystal size	0.60×0.50×0.40 mm	0.20×0.10×0.10 mm	0.21×0.14×0.11 mm
Theta range for data collection	3.12–30.03°	2.63–32.58°	2.79–32.30°
Limiting indices	$-14\le h\le 14$, $-17\le k\le 17$, $-29\le l\le 30$	$-8\le h\le 8$, $-31\le k\le 31$, $-17\le l\le 16$	$-15\le h\le 17$, $-13\le k\le 13$, $-18\le l\le 16$
Reflections collected	48189	13,300	15,073
Independent reflections	4309 [R(int)=0.0496]	4428 [R(int)=0.0521]	4251 [R(int)=0.0463]
Completeness to theta	30.03 (99.6%)	25.00 95.2%	25.00 95.8%
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	4309/0/192	4428/10/187	4251/10/180
Goodness of fit on F^2	1.042	0.865	1.105
Final R indices [I>2σ(I)]	R_1=0.0411, wR_2=0.0996	R_1=0.0519, wR_2=0.1154	R_1=0.0521, wR_2=0.1295
R indices (all data)	R_1=0.0514, wR_2=0.1051	R_1=0.1718, wR_2=0.1443	R_1=0.0574, wR_2=0.1330
Largest diff. peak and hole	0.374 and −0.245 e Å^{-3}	0.163 and −0.388 e A^{-3}	0.279 and −0.234 e A^{-3}

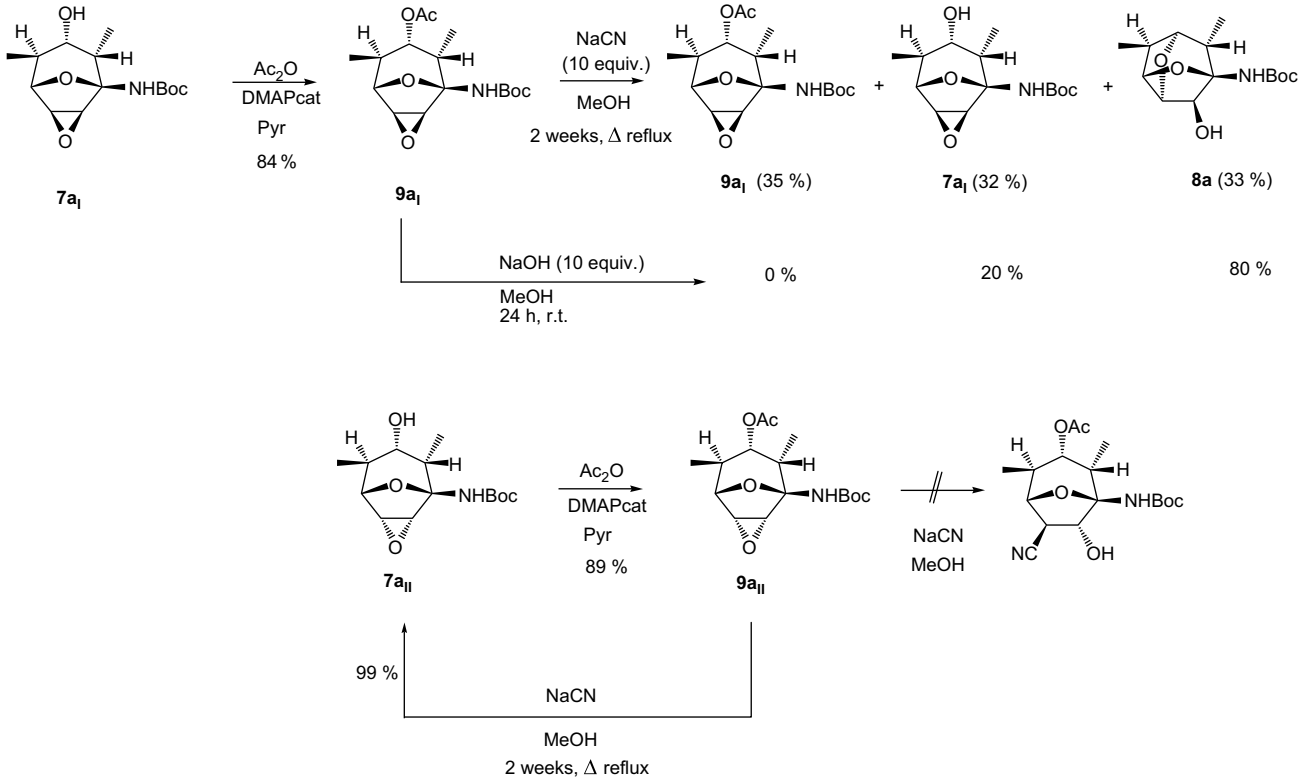
group at C-4 position because C-2 is more hindered by the bulky NHBoc group, which is in a closer proximity to it.

The stereochemistry of both the epoxidic substrates and the dioxatricyclic products has been established by careful ^{1}H and ^{13}C NMR correlation studies and confirmed by X-ray diffraction analysis (see Figs. 3–5 and Tables 3 and 4).

In order to better understand the reactivity and/or stability of the epoxide ring in our substrates and to clarify some aspects of the mechanism of intramolecular cyclization, we protected the hydroxyl group as acetate with two objectives: on one hand, to avoid the intramolecular cyclization in the reaction with NaCN and, on the other hand, to indirectly generate the alkoxide (from the

Table 4
Selected bond lengths (Å) and bond angles (°) for **8b**, **7a$_I$** and **7b$_I$**

	8b		**7b$_I$**	**7a$_I$**
O(2″)–C(3″)	1.446	O(3″)–C(2″)	1.436	1.458
O(2″)–C(1″)	1.451	O(3″)–C(4″)	1.483	1.452
O(12″)–C(8″)	1.411	O(12″)–C(7″)	1.414	1.423
O(6″)–C(7″)	1.434	O(9″)–C(5″)	1.422	1.428
O(6″)–C(5″)	1.452	O(9″)–C(1″)	1.436	1.449
C(1″)–C(8″)	1.535	C(1″)–C(8″)	1.502	1.546
C(1″)–C(9″)	1.553	C(1″)–C(2″)	1.509	1.523
C(8″)–C(7″)	1.527	C(8″)–C(10″)	1.518	1.519
C(7″)–C(3″)	1.568	C(8″)–C(7″)	1.543	1.552
C(5″)–C(4″)	1.529	C(7″)–C(6″)	1.494	1.534
C(5″)–C(9″)	1.544	C(6″)–C(5″)	1.495	1.532
C(4″)–C(11″)	1.522	C(6″)–C(11″)	1.530	1.523
C(4″)–C(3″)	1.528	C(5″)–C(4″)	1.467	1.507
C(9″)–C(10″)	1.527	C(4″)–C(2″)	1.446	1.446
C(3″)–O(2″)–C(1″)	103.80	C(2″)–O(3″)–C(4″)	59.37	59.57
C(7″)–O(6″)–C(5″)	102.93	C(5″)–O(9″)–C(1″)	102.74	104.69
N–C(1″)–C(9″)	115.14	N–C(1″)–O(9″)	106.99	109.18
C(8″)–C(1″)–C(9″)	111.13	C(8″)–C(1″)–C(2″)	110.68	111.59
O(12″)–C(8″)–C(7″)	111.33	C(1″)–C(8″)–C(7″)	112.35	110.40
O(6″)–C(7″)–C(8″)	107.45	C(10″)–C(8″)–C(7″)	110.52	112.07
O(6″)–C(7″)–C(3″)	105.79	O(12″)–C(7″)–C(6″)	112.37	106.94
O(6″)–C(5″)–C(4″)	101.57	C(6″)–C(7″)–C(8″)	111.94	113.36
O(6″)–C(5″)–C(9″)	108.93	C(5″)–C(6″)–C(11″)	112.57	110.47
C(4″)–C(5″)–C(9″)	111.08	O(9″)–C(5″)–C(6″)	109.48	108.96
C(11″)–C(4″)–C(5″)	113.75	C(4″)–C(5″)–C(6″)	111.03	111.52
C(3″)–C(4″)–C(5″)	97.25	C(2″)–C(4″)–C(5″)	107.58	105.79
O(2″)–C(3″)–C(4″)	108.31	C(2″)–C(4″)–O(3″)	58.71	60.42
C(4″)–C(3″)–C(7″)	103.01	C(5″)–C(4″)–O(3″)	113.76	111.46
C(10″)–C(9″)–C(5″)	111.47			
C(5″)–C(9″)–C(1″)	108.58			



Scheme 8. Study of the reactivity of acetates **9a$_I$** and **9a$_{II}$** with NaCN/MeOH.

alcohol group at C-7) by a process of addition–elimination (saponification) on the acetate by using NaOH. This objective was rationalized in the sense that the OH is flanked and hindered by two methyl groups and a NHBoc group, making difficult the access to its hydrogen. The acetate is placed away from the core of the molecule and it is more accessible as observed by computational modeling. For this purpose, epoxides **7a$_I$** and **7a$_{II}$** were treated with Ac_2O and DMAP as catalyst and the corresponding acetyl esters **9a$_I$** and **9a$_{II}$** were obtained in good yield. These acetates were reacted with NaCN in anhydrous MeOH. As with the precursor, the *endo* epoxide **9a$_{II}$** did not react at all, recovering in quantitative yield the compound **7a$_{II}$** as the result of deprotection of the alcohol group, but the oxirane ring was intact. On the other hand, the *exo* epoxide **9a$_I$** partially reacted after two weeks under reflux of solvent and the reaction crude mixture showed the presence of the starting material **9a$_I$** (35%), unprotected alcohol **7a$_I$** (32%) and the corresponding dioxatricycle **8a** (33%) (Scheme 8). These results confirmed a low and peculiar reactivity of the C-2, C-4 epoxide ring.

In a parallel experiment, epoxide **9a$_I$** was treated with NaOH (10 equiv) in MeOH. The saponification of the acetate group was complete after 24 h at rt and we isolated alcohol **7a$_I$** in a 20% and dioxatricycle **8a** in 80% yield. This confirms our hypothesis that the alkoxide intermediate, generated in the addition–elimination process is the key intermediate. Also with this strategy the kinetics of the transannular alkoxycyclization is faster compared to the results observed in entry 20 of Table 2 (even that the substrate is different).

In Scheme 9 it is shown the internal consistency of the results observed in the reactions carried out on [4+3]-cycloadducts and on their corresponding epoxides with nucleophiles acting as weak bases. This scheme shows also a proposal of the reaction mechanisms to explain the haloetherification and the transannular hydroxycyclization processes as well as their regioselectivity. It is worth noting that this transannular cyclization is a highly regioselective reaction, because no other regioisomer was detected (by GC or NMR). As mentioned before, the formation of isomers **8a** and/or **8b** could be explained by the preferential attack of the C-7 hydroxyl group at C-4 position due to C-2 is more hindered by the bulky NHBoc group, which is in closer proximity to it.

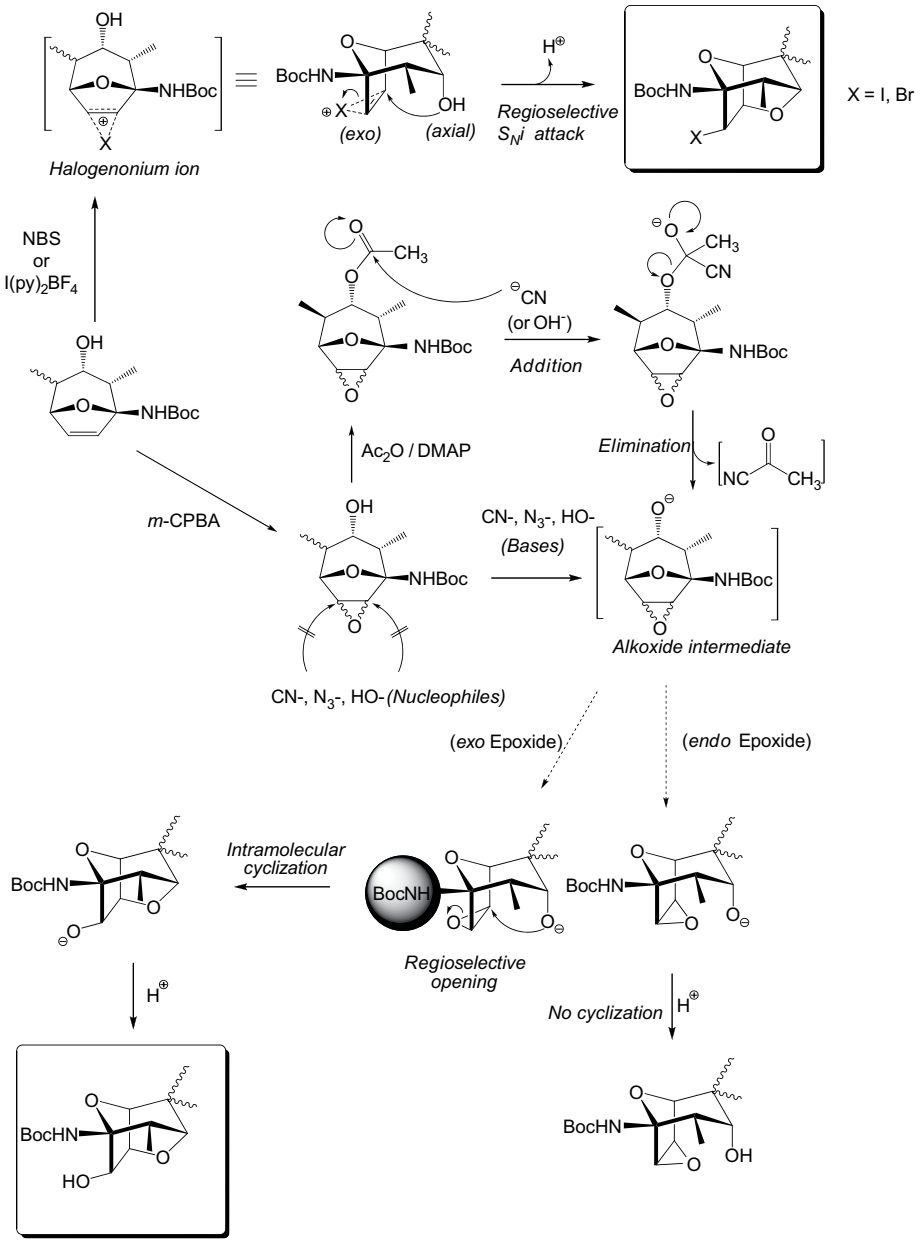
3. Biological tests

All compounds described in this work were synthesized with the aim of forming a selected library of compounds to be evaluated as anti-HIV-1 agents, acting as inhibitors of the catalytic centre of α-glucosidases, enzymes of great importance in the anchoring and union of virions on the human cells membrane.

The evaluation of the anti-HIV activity was carried out on lymphoid MT-4 cells infected by the NL4-3 strain of HIV-1 virus (see Experimental section for details of the operating procedure). The antiviral activity was measured by quantification of the host cells viability after addition of the development reagent MTT.[22]

As internal standards four clinical drugs were used: AZT,[23] an inhibitor of the reverse transcriptase of HIV virus; AMD-3100,[24] an inhibitor of the entry of virions on the human cells; Ritonavir,[25] inhibitor of the HIV-1 protease and finally Nevirapine[26] that is a non-nucleoside reverse transcriptase inhibitor.

In Table 5 the results from the biological tests are quoted, showing that dioxatricycles are only slightly active against HIV-1 virus at EC_{50} values far away from those of the clinical drugs used as standards or references. With this small library only rough structure–activity relationships (SAR) could be drawn: dioxatricycles **5a**,**b**; **6a**,**b** and **8a**,**b**, even they are low active, they are much more active than their epoxide precursors **7a$_{I,II}$** and **7b$_{I,II}$**. On the other hand, the acetylated epoxides **9a$_{I,II}$** are as active as the dioxatricycles. Finally, within the family of dioxatricycles, iodo-derivatives **6a**,**b** are more active than bromo-derivatives **5a**,**b** and these more active than hydroxy-derivatives **8a**,**b**, which could be associated with the size of the substituent. No apparent implications of



Scheme 9. Proposal of a reaction mechanism to explain the reactivity of the epoxide group in the substrates and the formation of dioxatricycles.

hydrogen bonds seem to be involved in the interaction of these molecules with the active site as shows the low apparent activity of **8a**,**b** or even of the epoxides with the free hydroxyl group in **7a$_{I,II}$** and **7b$_{I,II}$**. On the other hand, a high degree of cytotoxicity (CC_{50}) is observed, with values at the same level of the effective dose, so the adverse effects of these drugs could be important and no therapeutic margin is expected at the present level.

4. Conclusion

In summary, we have established a methodology to obtain 8-functionalized 1-*tert*-butoxycarbonylamino-2,6-dioxatricyclo [3.3.1.0^{3,7}]nonanes, in three or four steps with good yield and a high degree of regio- and stereoselectivity. By this method it is possible to prepare a wide variety of derivatives (bromo, iodo, hydroxy, etc.) under mild conditions to preserve the protecting group of the amino group. A reaction mechanism has been proposed to explain the formation of products and the special reactivity of the oxirane ring of substrates. This mechanism is consistent with the precedents found in the literature.

All compounds synthesized have been isolated purified and physically and spectroscopically characterized. The stereochemistry has been unequivocally assigned by a careful correlation of ^{1}H and ^{13}C NMR data and confirmed by X-ray diffraction analysis.

The compounds have been tested for anti-HIV-1 activity, following a standard protocol, and observing a low degree of antiretroviral activity and a high cytotoxicity, compared to standard drugs used in clinics.

5. Experimental section

5.1. General methods

Unless otherwise noted, all reactions were conducted under an atmosphere of dry nitrogen or argon in oven-dried glassware. Raw materials were obtained from commercial suppliers and used as

Table 5
Anti-HIV-1 activity of dioxatricyclic compounds and precursors

Compound	EC_{50} (μM)[a]	CC_{50} (μM)[b]	Structural features
5a	>72	>72	Bromo-dioxatricycle
5b	>72	>72	Bromo-dioxatricycle
6a	>63	>63	Iodo-dioxatricycle
6b	>63	>63	Iodo-dioxatricycle
8a	>88	>88	Hydroxy-dioxatricycle
8b	>88	>88	Hydroxy-dioxatricycle
$7a_I$	>440	>440	Epoxy-alcohol
$7a_{II}$	>440	>440	Epoxy-alcohol
$7b_I$	>440	>440	Epoxy-alcohol
$7b_{II}$	>440	>440	Epoxy-alcohol
$9a_I$	>76	>76	Acetylated epoxide
$9a_{II}$	>76	>76	Acetylated epoxide
AZT	0.0022	>3.75	Standard
Nevirapine	0.0721	>2.77	Standard
AMD-3100	0.0020	>9.94	Standard
Ritonavir	0.0140	>1.39	Standard

[a] EC_{50}: Effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with the MTT method in MT-4 lymphoid cells.
[b] CC_{50}: Cytotoxic concentration 50 or needed concentration to induce 50% death of non-infected MT-4 cells.

received. All solvents were purified using standard techniques before use: ether, tetrahydrofuran, hexane, and pentane were distilled under nitrogen from sodium/benzophenone. Acetonitrile was distilled under nitrogen from CaH_2. Infrared spectra were recorded on an FTIR NICOLET 510 spectrophotometer as thin films over NaCl plates. NMR spectra were obtained in $CDCl_3$ on spectrometers at 400 MHz (MERCURY-400) and/or 500 MHz (UNITY-500) for ^1H NMR, and at 100 MHz for ^{13}C NMR. For ^1H NMR tetramethylsilane was used as internal standard. ^{13}C NMR spectra were referenced to the 77.0 ppm resonance of chloroform. When necessary, assignments were established by DEPT, ^1H–^1H COSY, and HMBC or gHMQC ^{13}C–^1H correlation experiments. Mass spectra were measured on a HEWLETT-PACKARD 5890 mass spectrometer using the chemical ionization technique and ammonia as ionizing gas. GC analyses were performed on HP-8790 gas chromatograph equipped with a HEWLETT-PACKARD-crosslinked MePhe-Silicone capillary column (L=25 m, Φ=0.2 mm, δ=2.5 μm) using helium as gas carrier and a FID detector (T=250 °C, P_{H2}=4.2 psi, P_{air}=2.1 psi). The elemental analyses were obtained in a FISONS elemental analyzer, Model Na-1500. The samples were previously pyrolized at 1000 °C under oxygen atmosphere and the content of carbon, hydrogen and nitrogen was determined by evaluation of the combustion gases by gas chromatography using a FID detector.

5.2. X-ray diffraction analysis protocol

Suitable crystals (0.1×0.1×0.2 mm) from compounds **8b**, **$7a_I$**, and **$7b_I$** were selected and mounted on a MAR345 apparatus with image plate detector, or on a Nonius Kappa CCD equipped with a low temperature device, using graphite monochromated Mo Kα radiation (λ=0.71070 Å). Unit-cell parameters were determined from automatic centering of reflections and refined by least-squares method. Intensities were collected with graphite monochromatized Mo Kα radiation. Lorentz-polarization and absorption corrections were made. The structures were solved by direct methods and refined by full-matrix least-squares method using SHELXS-97/2 [27] or SIR-97 [28] computer programs, on the basis of the non-equivalent reflections by symmetry (very negative intensities were not assumed). The function minimized was: $\Sigma w[(F_o)^2-(F_c)^2]^2$, where $w=[\sigma^2(I)+(0.0745P)^2+0.4463\ P)^{-1}]$, and $P=[(F_o)^2+2\ (F_c)^2]/3$; f, f′ and f″ were taken from the International Tables of X-ray Crystallography.[29] All the H atoms were computed and refined, using a riding model, with isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the atom, which are linked. The final R(on F) factors and goodness of fit are shown on Table 3. Number of refined parameters was 127. Max. shift/esd=0.00. Mean shift/esd=0.00. Refinement of F^2 was done against all reflections. The weighted R-factor wR and goodness of fit S are based on F^2, conventional R-factors R are based on F, with F set to zero for negative F^2. The threshold expression of $F^2>2\sigma(F^2)$ is used only for calculating R-factors(gt) and is not relevant to the choice of reflections for refinement. R-factors based on F^2 are statistically about twice as large as those based on F, and R- factors based on all data will be even larger. All esds (except the esd in the dihedral angle between two ls planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving ls planes. The molecular illustrations (Figs. 3–5) were made by using the ORTEP-3 program.[30]

The main X-ray data are quoted in Table 3. Also a selection of most significant bond lengths and bond angles for compounds **8b**, **$7a_I$** and **$7b_I$** are included in Table 4. On the other hand, hydrogen coordinates as well as anisotropic thermal parameters are included as Supplementary data. Crystallographic data for the structures **8b**, **$7a_I$** and **$7b_I$** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 731404, 720950 and 720951, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

5.3. Bicyclic ketone reduction

The 1-*tert*-butoxycarbonyl-amino-8-oxabicyclo[3.2.1]oct-6-en-3-ones **3a–c** and their precursors (**1** and **2**) were obtained as described by the authors in a previous work:[2r] the bicyclic structures were prepared by a [4+3]-cycloaddition reaction between a furan derivative, functionalized at C-2 by a protected amino group, **2**, and an oxyallyl cation generated, in situ, from 2,4-dibromo-3-pentanone,**1**, in the presence of a reducing metal. With this particular substrate several reducing metals and metallic pairs were used, however, $Fe_2(CO)_9$ showed to be the most efficient reagent for the cycloaddition reaction, affording, with a 76% yield, the diastereoisomeric mixture of cycloadducts (**3a**/**3b**/**3c**)=55/40/5. Oxabicyclic ketones **3a**,**b**, were separated and purified by flash column chromatography and their stereochemistry was unequivocally established by NMR correlation studies and confirmed by X-ray diffraction analysis.

*5.3.1. Reduction of the ketone group of **3a**. Synthesis of **$4a_I$** and **$4a_{II}$***

To a solution of ketone **3a** (304 mg, 1.14 mmol) in anhydrous THF (3 mL), a solution 1 M in hexane of DIBAL-H (3.42 mmol) was added at −78 °C. After 75 min at −78 °C, a saturated aqueous solution of NH_4Cl (12 mL) was added and the crude mixture was extracted with diethyl ether (3×10 mL) and the organic layers were combined, dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of chloroform and ethyl acetate of increasing polarity, isolating pure diastereoisomeric compounds **$4a_I$** (0.262 g) and **$4a_{II}$** (0.014 g). Yield=90%. Diastereoselectivity: **$4a_I$** (*endo*)/**$4a_{II}$** (*exo*)=95/5.

*5.3.1.1. tert-Butyl rac-N-{(1S,2S,3S,4S,5R)-3-hydroxy-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-1-yl}-carbamate (**$4a_I$**).* Colorless oil. IR (film) ν_{max} 3330 (OH, st), 2973, 2932, 1717 (C=O, st), 1526 (N–H, δ), 1366, 1250 (^{t}Bu), 1161 (C–O–C, st), 995 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ=1.04 (3H, d, $J_{9,2}$=7.4 Hz, H-9″), 1.21 (3H, d, $J_{10,4}$=7.6 Hz,

H-10″), 1.44 (9H, s, H-2′), 1.85 (1H, q, $J_{4,10}$=7.6 Hz, H-4″), 2.03 (1H, d, J=10.6 Hz, OH), 2.34 (1H, dq, $J_{2,9}$=7.4 Hz, $J_{2,3}$=5.6 Hz, H-2″), 3.55 (1H, br s, H-3″), 4.65 (1H, d, $J_{5,6}$=1.6 Hz, H-5″), 5.10 (1H, s, NH), 6.40 (1H, dd, $J_{6,5}$=1.6 Hz, $J_{6,7}$=5.6 Hz, H-6″), 6.50 (1H, d, $J_{7,6}$=5.6 Hz, H-7″) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ=12.4 (C-10″), 17.6 (C-9″), 28.5 (C-2′), 39.6 (C-4″), 39.9 (C-2″), 75.7 (C-3″), 82.9 (C-5″), 94.9 (C-1″), 135.0 (C-7″), 135.1 (C-6″), 154.2 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 270 (43, M+H), 214 (89, M+2-^{t}Bu), 196 (96, M−O^{t}Bu), 170 (100, M+2−COO^{t}Bu), 152 (83, M−NH_2Boc). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.44; H, 8.60; N, 5.15.

*5.3.1.2. tert-Butyl rac-N-{(1S,2S,3R,4S,5R)-3-hydroxy-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-1-yl}-carbamate (**4a$_{II}$**).* Colorless oil. IR (film) ν_{max} 3342 (N–H, st), 3336 (OH, st), 2975, 2932, 1717 (C=O, st), 1526 (N–H, δ), 1324, 1248 (^{t}Bu), 1161 (C–O–C, st), 1094, 1053 (C–O–C, st as), 1014, 982 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.03 (3H, d, $J_{9,2}$=6.7 Hz, H-9″), 1.14 (3H, d, $J_{10,4}$=6.9 Hz, H-10″), 1.44 (9H, s, H-2′), 1.48 (1H, d, J=7.0 Hz, OH), 1.75 (1H, dq, $J_{2,9}$=6.7 Hz, $J_{2,3}$=8.0 Hz, H-2″), 1.87 (1H, ddq, $J_{4,5}$=1.5 Hz, $J_{4,3}$=6.9 Hz, $J_{4,10}$=6.9 Hz, H-4″), 3.50 (1H, dd, $J_{3,4}$=6.9 Hz, $J_{3,2}$=8.0 Hz, H-3″), 4.67 (1H, br s, H-5″), 5.08 (1H, s, NH), 6.15 (1H, dd, $J_{6,5}$=1.7 Hz, $J_{6,7}$=5.9 Hz, H-6″), 6.22 (1H, d, $J_{7,6}$=5.9 Hz, H-7″) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ=11.8 (C-10″), 13.5 (C-9″), 28.5 (C-2′), 34.6 (C-4″), 41.2 (C-2″), 73.5 (C-3″), 82.9 (C-5″), 94.9 (C-1″), 131.0 (C-7″), 131.3 (C-6″), 153.9 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 270 (28, M+H), 214 (51, M+2−^{t}Bu), 170 (100, M+2−COO^{t}Bu), 152 (63, M−NH_2Boc). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.41; H, 8.62; N, 5.23.

*5.3.2. Reduction of the ketone group of **3b**. Synthesis of **4b$_I$** and **4b$_{II}$***

To a suspension of $NaBH_4$ (364 mg, 9.62 mmol) in MeOH (1.5 mL), at 0 °C, a solution of **3b** (427 mg 1.60 mmol) in MeOH (2 mL) was added. Afterward, the reaction was stirred at rt for 1.5 h. Then, a saturated aqueous solution of NH_4Cl (4 mL) was added at 0 °C and the reaction mixture extracted with diethyl ether (3×10 mL). The organic layers were combined together, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of chloroform and ethyl acetate of increasing polarity, isolating pure diastereoisomeric compounds **4b$_I$** (422 mg) and **4b$_{II}$** (4.1 mg). Yield=99%. Diastereoselectivity: **4b$_I$**(*endo*)/**4b$_{II}$** (*exo*)=99/1.

*5.3.2.1. tert-Butyl rac-N-{(1S,2S,3S,4R,5R)-3-hydroxy-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-1-yl}-carbamate (**4b$_I$**).* White solid. Mp 137–138 °C (CCl_4). IR (film) ν_{max} 3334 (OH, st), 2973, 2930, 1723 (C=O, st), 1624, 1520 (N–H, δ), 1456, 1364, 1250 (^{t}Bu), 1165 (C–O–C, st), 1084, 1016 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.98 (3H, d, $J_{10,4}$=7.6 Hz, H-10″), 1.08 (3H, d, $J_{9,2}$=7.6 Hz, H-9″), 1.44 (9H, s, H-2′), 1.62 (1H, br s, OH), 2.25–2.35 (2H, m, H-2″ y H-4″), 3.73–3.79 (1H, m, H-3″), 4.62 (1H, ddd, $J_{5,6}$=1.6 Hz, $J_{5,3}$=1.6 Hz, $J_{5,4}$=3.2 Hz, H-5″), 5.18 (1H, s, NH), 6.47 (1H, dd, $J_{6,5}$=1.6 Hz, $J_{6,7}$=6.2 Hz, H-6″), 6.50 (1H, d, $J_{7,6}$=6.2 Hz, H-7″) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ=12.8 (C-10″), 12.9 (C-9″), 28.5 (C-2′), 38.7 (C-2″), 41.9 (C-4″), 73.1 (C-3″), 82.8 (C-5″), 94.7 (C-1″), 134.7 (C-6″), 136.5 (C-7″), 154.2 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 214 (40, M+2−^{t}Bu), 196 (53, M−O^{t}Bu), 170 (100, M+2−COO^{t}Bu), 152 (63, M−NH_2Boc), 110 (93). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.40; H, 8.63;N, 5.17.

*5.3.2.2. tert-Butyl rac-N-{(1R,2S,3R,4R,5R)-3-hydroxy-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-1-yl}-carbamate (**4b$_{II}$**).* White solid. Mp 58–59 °C (CCl_4). IR (film) ν_{max} 3334 (OH, st), 2973, 2930, 1713 (C=O, st), 1526 (N–H, δ), 1456, 1393, 1366, 1250 (^{t}Bu), 1163 (C–O–C, st), 1024, 993, 955 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.95 (3H, d, $J_{10,4}$=7.0 Hz, H-10″), 1.04 (3H, d, $J_{9,2}$=7.0 Hz, H-9″), 1.44 (9H, s, H-2′), 1.71–1.79 (3H, m, H-2″,H-4″ y OH), 2.85 (1H, dd, $J_{3,2}$=8.6 Hz, $J_{3,4}$=8.6 Hz, H-3″), 4.63 (1H, d, $J_{5,6}$=1.8 Hz, $J_{5,4}$=3.6, H-5″), 5.14 (1H, s, NH), 6.19 (1H, dd, $J_{6,5}$=1.8 Hz, $J_{6,7}$=6.4 Hz, H-6″), 6.24 (1H, d, $J_{7,6}$=6.4 Hz, H-7″) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ=13.8 (C-9″), 14.3 (C-10″), 28.5 (C-2′), 40.8 (C-4″), 44.4 (C-2″), 78.5 (C-3″), 81.9 (C-5″), 95.3 (C-1″), 130.0 (C-7″), 132.0 (C-6″), 154.0 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 214 (34, M+2−^{t}Bu), 196 (90, M−O^{t}Bu), 170 (100, M+2−COO^{t}Bu), 152 (55, M−NH_2Boc). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.41; H, 8.59; N, 5.24.

5.4. Epoxidation of 4a$_I$ and 4b$_I$. Synthesis of 7a$_I$, 7a$_{II}$, 7b$_I$ and 7b$_{II}$

In a round bottomed flask fitted with a magnetic stirrer, compound **4a$_I$** (158 mg, 0.587 mmol) was placed and a solution of *m*-CPBA (203 mg, 1.174 mmol) in CH_2Cl_2 (3.5 mL) at rt was added at once. After 3 days, CH_2Cl_2 (10 mL) was added and the solution was extracted with a 10% aqueous solution of Na_2CO_3 (3×5 mL), the organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of hexane and ethyl acetate of increasing polarity, isolating pure diastereoisomeric compounds **7a$_{II}$** (0.104 g) and **7a$_I$** (0.096 g). Yield=60%. Diastereoselectivity: **7a$_I$**(*exo*)/**7a$_{II}$**(*endo*)=48/52.

In an independent experiment, but following the same operating procedure, compound **4b$_I$** was epoxidated to afford products **7b$_I$** and **7b$_{II}$**. Yield=55%. Diastereoselectivity: **7b$_I$**(*exo*)/**7b$_{II}$**(*endo*)=57/43.

*5.4.1. tert-Butyl rac-N-{(1R,2S,4S,5S,6S,7S,8S)-7-hydroxy-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-1-yl}-carbamate (**7a$_I$**)*

Colorless oil. IR (film) ν_{max} 3415 (N–H, st), 2975, 2934, 1730 (C=O, st), 1505, 1456, 1369, 1246 (^{t}Bu), 1165 (C–O–C, st), 1080 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.07 (3H, d, $J_{10,8}$=7.6 Hz, H-10″), 1.17 (3H, d, $J_{11,6}$=7.2 Hz, H-11″), 1.45 (9H, s, H-2′), 1.82 (1H, q, $J_{11,6}$=7.2 Hz, H-6″), 2.93–2.99 (1H, m, H-8″), 3.59 (1H, d, J=3.6 Hz, H-4″), 3.68 (1H, d, J=3.6 Hz, H-7″), 3.71 (1H, d, J=3.6 Hz H-2″), 4.11 (1H, s, H-5″), 5.27 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.8 (C-11″), 14.3 (C-10″), 29.1 (C-2′), 35.4 (C-8″), 42.8 (C-6″), 74.0 (C-7″), 81.1 (C-4″), 83.2 (C-5″), 83.6 (C-2″) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 286 (1, M+1), 230 (15), 212 (22), 186 (100, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.94; H, 8.10; N, 4.89.

*5.4.2. tert-Butyl rac-N-{(1R,2R,4R,5S,6S,7S,8S)-7-hydroxy-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-1-yl}-carbamate (**7a$_{II}$**)*

Colorless oil. IR (film) ν_{max} 3417 (N–H, st), 2975, 2930, 1734 (C=O, st), 1503, 1458, 1371, 1248 (^{t}Bu), 1163 (C–O–C, st), 1082 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.01 (3H, d, $J_{10,8}$=7.2 Hz, H-10″), 1.28 (3H, d, $J_{11,6}$=7.0 Hz, H-11″), 1.45 (9H, s, H-2′), 1.88–1.92 (1H, m, H-6″), 2.97 (1H, q, $J_{10,8}$=7.2 Hz, H-8″), 3.83 (1H, d, J=4.4 Hz, H-7″), 4.01 (1H, s, H-7″), 4.46–4.50 (1H, m, H-4″), 4.50 (1H, d, J=4.0 Hz, H-2″), 5.51 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.8 (C-11″), 14.3 (C-10″), 29.1 (C-2′), 35.4 (C-8″), 42.8 (C-6″), 74.0 (C-7″), 81.1 (C-4″), 83.2 (C-5″), 83.6 (C-2″) ppm. MS (CI, NH_3, 70 eV, 150 °C) m/z (%) 286 (1, M+1), 230 (15), 212 (17), 186 (100, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.90; H, 8.15; N, 4.97.

*5.4.3. tert-Butyl rac-N-{(1R,2R,4R,5S,6S,7S,8S)-7-hydroxy-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-1-yl}-carbamate (**7b$_I$**)*

Colorless oil. IR (film) ν_{max} 3498 (N–H, st), 2975, 1719 (C=O, st), 1524, 1456, 1369, 1248 (^{t}Bu), 1167 (C–O–C, st), 1028 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.03 (3H, d, $J_{11,6}$=7.2 Hz, H-11″), 1.10 (3H, d, $J_{10,8}$=7.2 Hz, H-10″), 1.45 (9H, s, H-2′), 2.22–2.29 (1H, m,

H-6″), 2.90–3.06 (1H, m, H-8″), 3.62 (1H, d, J_4=3.2 Hz, H-4″), 3.67 (1H, d, J_2=3.2 Hz, H-2″), 3.85 (1H, d, J_7=3.2 Hz, H-7″), 4.15 (1H, d, J_5=3.2 Hz, H-5″), 5.31 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=11.6 (C-11″), 11.8 (C-10″), 28.4 (C-2′), 38.1 (C-6″), 40.0 (C-8″), 53.6 (C-4″), 54.5 (C-2″), 72.0 (C-7″), 77.8 (C-5″) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z*, (%) 286 (33, M+1), 247 (45), 229 (45, ^{t}Bu), 186 (100, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.92; H, 8.14; N, 4.89.

*5.4.4. tert-Butyl rac-N-{(1R,2R,4R,5S,6R,7S,8S)-7-hydroxy-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-1-yl}-carbamate (**7b$_{II}$**)*

Colorless oil. IR (film) ν_{max} 3415 (N–H, st), 2975, 2934, 1730 (C=O, st), 1505, 1456, 1369, 1246 (^{t}Bu), 1165 (C–O–C, st), 1080 (C–O–C, asym. st) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.85 (3H, d, $J_{11,6}$=7.2 Hz, H-11″), 1.04 (3H, d, $J_{10,8}$=7.2 Hz, H-10″), 1.44 (9H, s, H-2′), 1.60 (1H, br s, OH), 2.35 (1H, q, $J_{11,6}$=7.2 Hz, H-6″), 2.72 (1H, q, $J_{10,8}$=7.2 Hz, H-8″), 3.79 (1H, s, H-7″), 4.06 (1H, s, H-5″), 4.42 (1H, d, *J*=1.6 Hz, H-4″), 4.44 (1H, d, *J*=3.6 Hz, H-2″), 5.41 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=13.3 (C-11″), 13.7 (C-10″), 28.2 (C-2′), 40.5 (C-8″), 44.4 (C-6″), 74.1 (C-7″), 81.5 (C-2″), 83.1 (C-4″), 83.9 (C-5″) ppm. M. (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 287 (14, M+2), 286 (100, M+H), 247 (46), 230 (29), 186 (92, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.95; H, 8.11; N, 4.96.

5.5. Alcohol acetylation of 7a$_I$ and 7a$_{II}$. Synthesis of 9a$_I$ and 9a$_{II}$

In a round bottomed flask fitted with a magnetic stirring bar, compound **7a$_{II}$** (28 mg, 0.098 mmol) and a catalytic amount of DMAP were placed. Anhydrous pyridine (2 mL) and then Ac_2O (46 mL, 0.49 mmol) were added and the reaction mixture was stirred at rt for 19 h. The reaction crude was concentrated to dryness and the resulting oil was purified by flash column chromatography by using mixtures of hexane and ethyl acetate of increasing polarity, obtaining pure compound **9a$_{II}$** (28,5 mg, 89% yield).

In an independent experiment, but following the same operating procedure, alcohol **7a$_I$** was acetylated to afford product **9a$_I$** with 84% yield.

*5.5.1. rac-(1R,2S,4S,5S,6S,8S)-1-[(tert-Butoxycarbonyl)amino]-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-7-yl acetate (**9a$_I$**)*

Colorless oil. IR (film) ν_{max} 3341 (N–H, st), 2977, 1732 (C=O, st), 1522, 1458, 1369, 1240 (^{t}Bu), 1165 (C–O–C, st), 1086 (C–O–C, st as), 1022 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.97 (3H, d, $J_{10,8}$=7.2 Hz, H-10‴), 1.23 (3H, d, $J_{11,6}$=7.0 Hz, H-11‴), 1.45 (9H, s, H-2″), 1.77 (1H, q, $J_{11,6}$=7.2 Hz, H-6‴), 2.05 (3H s, H-2), 3.04–3.14 (1H, m, H-8‴), 3.51 (1H, d, $J_{2,4}$=4.4 Hz, H-4″), 3.77 (1H, d, $J_{2,4}$=3.0 Hz, H-2″), 4.10 (1H, s, H-5‴), 4.82 (1H, d, $J_{7,8}$=7.2 Hz, H-7‴), 5.30 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=11.1 (C-10‴), 16.3 (C-11‴), 21.4 (C-2), 28.4 (C-2″), 35.4 (C-8‴), 36.4 (C-6‴), 53.5 (C-2‴), 54.5 (C-4‴), 75.9 (C-7‴), 77.8 (C-5‴), 89.6 (C-1‴), 154.1(C-1′), 170.1 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 328 (38, M+1), 289 (100), 272 (69, M+1−^{t}Bu), 228 (100, M−HCOO^{t}Bu), 123 (75). Anal. Calcd for $C_{16}H_{25}NO_6$: C, 58.70; H, 7.70; N, 4.28. Found: C, 58.68; H, 7.73; N, 4.26.

*5.5.2. rac-(1R,2R,4R,5S,6S,8S)-1-[(tert-butoxycarbonyl)amino]-6,8-dimethyl-3,9-dioxatricyclo[3.3.1.0^{2,4}]non-7-yl acetate (**9a$_{II}$**)*

Colorless oil. IR (film) ν_{max} 3372 (N–H, st), 2977, 1734 (C=O, st), 1620, 1506, 1456, 1371, 1244 (^{t}Bu), 1165 (C–O–C, st), 1124, 1065 (C–O–C, st as), 1009 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.05 (3H, d, $J_{10,8}$=6.8 Hz, H-10‴), 1.30 (3H, d, $J_{11,6}$=6.8 Hz, H-11‴), 1.45 (9H, s, H-2″), 1.89–1.93 (1H, m, H-6‴), 2.15 (3H s, H-2), 3.27 (br d, H-8‴), 3.87 (1H, d, $J_{5,4}$=5.0 Hz, H-5‴), 4.46–4.48 (1H, m, H-4‴), 4.57 (1H, dd, *J*=$J_{2,4}$=4.0 Hz, H-4″), 5.02 (1H, s, H-7‴), 5.40 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.2 (C-11‴), 13.7 (C-10‴), 21.3 (C-2), 28.4 (C-2″), 34.5 (C-8‴), 42.3 (C-6‴), 75.7 (C-7‴), 81.0 (C-4‴), 81.4 (C-2‴), 82.9 (C-5‴), 93.0 (C-1‴), 153.8 (C-1′), 171.1 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 328 (38, M+1), 289 (88), 272 (59, M+1−^{t}Bu), 228 (100, M−HCOO^{t}Bu), 123 (42). Anal. Calcd for $C_{16}H_{25}NO_6$: C, 58.70; H, 7.70; N, 4.28. Found: C, 58.72; H, 7.89; N, 4.30.

5.6. Synthesis of brominated dioxatricyclic compounds 5a and 5b. General procedure

In a round bottomed flask fitted with a magnetic stirring bar, compound **4a$_I$** (16.7 mg, 0.062 mmol) and NBS (1.3 mg, 0.0075 mmol) were placed. Then, toluene (1 mL) was added and the mixture was stirred at rt for 30 min. The reaction was quenched by adding a saturated solution of $NaHCO_3$ (2 mL). The reaction crude was extracted with ethyl acetate (10 mL×3) and the organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of chloroform and ethyl acetate of increasing polarity, to isolate pure compound **5a** (17.9 mg, 83% yield).

Under similar reaction conditions, **4b$_I$** gave the corresponding brominated tricyclic compound **5b** in a 61% yield.

*5.6.1. tert-Butyl rac-N-{(1R,3S,4S,5S,7R,8S,9S)-8-bromo-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**5a**)*

Colorless oil. IR (film) ν_{max} 3407 (N–H, st), 2977, 1738 (C=O, st), 1501, 1368, 1242 (^{t}Bu), 1167 (C–O–C, st as), 1076, 1041 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.04 (3H, d, $J_{10,9}$=7.2 Hz, H-10″), 1.31 (3H, d, $J_{11,9}$=6.8 Hz, H-11″), 1.45 (9H, s, H-2′), 1.90–1.98 (1H, m, H-4″), 3.44–3.49 (1H, m, H-9″), 3.91 (1H, d, *J*=4.4 Hz, H-5″), 4.42 (1H, s, H-8″), 4.56–4.58 (1H, m, H-3″), 4.75 (1H, d, $J_{8,7}$=3.6 Hz, H-7″), 5.35 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.4 (C-11″), 13.9 (C-10″), 28.4 (C-2′), 35.9 (C-9″), 42.3 (C-4″), 56.4 (C-8″), 80.7 (C-3″), 83.4 (C-5″), 85.1 (C-7″), 92.5 (C-1″), 153.6 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 350 (30, M+3), 348 (32, M+1), 311 (86), 309 (93), 294 (32, M+4−^{t}Bu), 292 (34, M+2−^{t}Bu), 250 (95), 248 (100−M+1−COO^{t}Bu). Anal. Calcd for $C_{14}H_{22}BrNO_4$: C, 48.29; H, 6.37; N, 4.02. Found: C, 48.30; H, 6.36; N, 4.04.

*5.6.2. tert-Butyl rac-N-{(1R,3S,4R,5S,7R,8S,9S)-8-bromo-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**5b**)*

Colorless oil. IR (film) ν_{max} 3415 (N–H, st), 2971, 2929, 1726 (C=O, st), 1503, 1458, 1369, 1246 (^{t}Bu), 1105, 1047 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.85 (3H, d, $J_{11,4}$=7.2 Hz, H-11″), 1.04 (3H, d, $J_{10,9}$=6.8 Hz, H-10″), 1.44 (9H, s, H-2′), 2.45 (1H, q, $J_{11,4}$=7.2 Hz, H-4″), 3.23–3.29 (1H, m, H-9″), 3.89 (1H, s, H-5″), 4.46 (1H, s, H-8″), 4.52–4.54 (1H, m, H-3″), 4.70 (1H, d, $J_{8,7}$=4.0 Hz, H-7″), 5.32 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=13.5 (C-11″), 14.1 (C-10″), 28.4 (C-2′), 41.6 (C-9″), 44.5 (C-4″), 57.0 (C-8″), 83.4 (C-3″), 83.7 (C-7″), 84.9 (C-5″), 92.3 (C-1″), 153.6 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 350 (43, M+3), 348 (45, M+1), 311 (91), 309 (99), 294 (35, M+4−^{t}Bu), 292 (37, M+2−^{t}Bu), 250 (93), 248 (100, M+1−COO^{t}Bu). Anal. Calcd for $C_{14}H_{22}BrNO_4$: C, 48.29; H, 6.37; N, 4.02. Found: C, 48.27; H, 6.39; N, 4.01.

5.7. Synthesis of iodinated dioxatricyclic compounds 6a and 6b. General procedure

In a 5 mL heart-shaped flask fitted with a magnetic stirring bar, compound **4a$_I$** (24 mg, 0.089 mmol) and $I(py)_2BF_4$ (40 mg, 0.107 mmol) were placed. Afterward, 1,4-dioxane/methanol (10:1) (1 mL) was added and the mixture was stirred at rt for 30 min. The

reaction was quenched by adding a 5% aqueous solution of $Na_2S_2O_3$ (2 mL). The reaction crude was extracted with diethyl ether (10 mL×3) and the organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of chloroform and ethyl acetate of increasing polarity, to isolate pure compound **6a** (21 mg, 60% yield).

Under similar reaction conditions, **4b$_I$** afforded the corresponding brominated tricyclic compound **6b** in a 74% yield.

*5.7.1. tert-Butyl rac-N-{(1R,3S,4S,5S,7R,8S,9S)-8-iodo-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**6a**)*

Colorless oil. IR (film) ν_{max} 3392 (N–H, st), 2975, 1736 (C=O, st), 1495, 1368, 1240 (^{t}Bu), 1165(C–O–C, st), 1074, 1041 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.04 (3H, d, $J_{10,9}$=7.6 Hz, H-10″), 1.30 (3H, d, $J_{11,4}$=6.8 Hz, H-11″), 1.46 (9H, s, H-2′), 1.91–1.98 (1H, m, H-4″), 3.37–3.43 (1H, m, H-9″), 3.97 (1H, d, J=7.6 Hz, H-5″), 4.54 (1H, s, H-8″), 4.58–4.60 (1H, m, H-3″), 4.88 (1H, d, $J_{8,7}$=3.6 Hz, H-7″), 5.17(1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.4 (C-11″), 14.1 (C-10″), 28.5 (C-2′), 35.9 (C-9″), 38.5 (C-8″), 42.3 (C-4″), 80.6 (C-3″), 83.5 (C-5″), 86.8 (C-7″), 92.3 (C-1″), 153.5 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 396 (1, M+1), 340 (60, M+1−^{t}Bu), 296 (32, M−COO^{t}Bu), 168 (25, M−COO^{t}Bu-I). Anal. Calcd for $C_{14}H_{22}INO_4$: C, 42.54; H, 5.61; N, 3.54. Found: C, 42.51; H, 5.59; N, 3.58.

*5.7.2. tert-Butyl rac-N-{(1R,3S,4S,5S,7R,8S,9S)-8-iodo-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**6b**)*

Colorless oil. IR (film) ν_{max} 3388 (N–H, st), 2975, 1736 (C=O, st), 1499, 1458, 1368, 1240 (^{t}Bu), 1165(C–O–C, st), 1047 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.85 (3H, d, $J_{11,4}$=6.8 Hz, H-11″), 1.07 (3H, d, $J_{10,9}$=6.8 Hz, H-10″), 1.45 (9H, s, H-2′), 2.45 (1H, q, $J_{11,4}$=6.8 Hz, H-4″), 3.16–3.22 (1H, m, H-9″), 3.94 (1H, s, H-5″), 4.54–4.56 (1H, m, H-3″), 4.60 (1H, s, H-8″), 4.83 (1H, d, $J_{8,7}$=4.0 Hz, H-7″), 5.14 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=14.1 (C-11″), 14.8 (C-10″), 28.9 (C-2′), 39.5 (C-8″), 42.2 (C-9″), 44.9 (C-4″), 83.7 (C-3″), 85.4 (C-5″), 85.9 (C-7″), 92.4 (C-1″), 153.9 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 396 (1, M+1), 340 (80, M+1−^{t}Bu), 296 (43, M−COO^{t}Bu), 212 (52), 168 (16, M−COO^{t}Bu-I). Anal. Calcd for $C_{14}H_{22}INO_4$: C, 42.54; H, 5.61; N, 3.54. Found: C, 42.56; H, 5.63; N, 3.51.

5.8. Synthesis of hydroxylated dioxatricyclic compounds 8a and 8b

In a 5 mL round bottomed flask fitted with a magnetic stirring bar, compound **7b$_I$** (13.7 mg, 0.048 mmol), dissolved in MeOH–H_2O (10:1)(3 mL), was placed. NaCN (23.7 mg, 0.484 mmol) and $LiClO_4$ (102 mg, 0.96 mmol) were added and the reaction mixture was stirred at rt for 72 h and afterward under reflux for additional 96 h (control by TLC). Methanol and water were removed under vacuum and the resulting residue was lixiviated with diethyl ether (10 mL×4) and the organic layers were combined, dried over anhyd $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was submitted to a flash column chromatography on silica gel, eluting with mixtures of hexane and ethyl acetate of increasing polarity, isolating pure compound **8b** (10.3 mg, 75% yield). Under similar reaction conditions but using NaN_3 as a weak base and anhyd MeOH as a solvent, **8b** was also obtained with a 74% yield.

When **7a$_I$** was reacted with NaCN (with the same molar ratio) in absence of $LiClO_4$ and in anhydrous methanol for 7 days at rt, hydroxylated tricyclic compound **8a** was obtained in a 75% yield.

*5.8.1. tert-Butyl rac-N-{(1R,3S,4S,5S,7R,8S,9S)-8-hydroxy-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**8a**)*

Colorless oil. IR (film) ν_{max} 3417 (N–H, st), 2977, 1736 (C=O, st), 1651, 1503, 1369, 1248 (^{t}Bu), 1163, 1080, 1047 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.01 (3H, d, $J_{10,9}$=7.6 Hz, H-10″), 1.28 (3H, d, $J_{11,4}$=7.2 Hz, H-11″), 1.45 (9H, s, H-2′), 1.86–1.94 (1H, m, H-4″), 2.96 (1H, q, $J_{10,9}$=7.6 Hz, H-9″), 3.82 (1H, d, $J_{5,4}$=4.4 Hz, H-5″), 4.00 (1H, d, $J_{8,7}$=3.6 Hz, H-8″), 4.46 (1H, br d, H-3″), 4.49 (1H, d, $J_{8,7}$=3.6 Hz, H-7″), 5.46 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=10.2 (C-11″), 13.6 (C-10″), 28.5 (C-2′), 34.8 (C-9″), 42.2 (C-4″), 73.4 (C-8″), 80.5 (C-3″), 82.6 (C-5″), 83.0 (C-7″), 93.9 (C-1″), 110.6 (C-1′), 154.4 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 286 (32, M+H), 247 (17), 230 (21), 186 (36, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.95; H, 8.10; N, 4.89.

*5.8.2. tert-Butyl rac-N-{(1R,3S,4R,5S,7R,8S,9S)-8-hydroxy-4,9-dimethyl-2,6-dioxatricyclo[3.3.1.0^{3,7}]non-1-yl}-carbamate (**8b**)*

Colorless oil. IR (film) ν_{max} 3415 (N–H, st), 2971, 2929, 1726 (C=O, st), 1503, 1458, 1369, 1246 (^{t}Bu), 1105, 1047 (C–O–C, st as) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.85 (3H, d, $J_{11,4}$=7.2 Hz, H-11″), 1.04 (3H, d, $J_{10,9}$=7.2 Hz, H-10″), 1.44 (9H, s, H-2′), 2.35 (1H, q, $J_{11,4}$=7.2 Hz, H-4″), 2.73 (1H, q, $J_{10,9}$=7.2 Hz, H-9″), 3.80 (1H, s, H-5″), 4.06 (1H, s, H-8″), 4.41–4.43 (1H, m, H-3″), 4.45 (1H, d, $J_{8,7}$=4.0 Hz, H-7″), 5.45 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=13.9 (C-11″), 14.4 (C-10″), 29.0 (C-2′), 41.2 (C-9″), 45.1 (C-4″), 74.7 (C-8″), 82.1 (C-7″), 83.7 (C-3″), 84.6 (C-5″), 94.2 (C-1″), 155.0 (C-1) ppm. MS (CI, NH_3, 70 eV, 150 °C) *m*/*z* (%) 285 (13, M), 247 (35), 230 (26), 186 (20, M−HCOO^{t}Bu). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.90; H, 8.14; N, 4.92.

5.9. Procedure for the anti-HIV-1 in vitro biological testing

The procedure used to test active agents against the human immunodeficiency virus HIV-1 has been designed to detect drugs that act at any of the steps of the reproductive cycle of the virus. The assay detects the death of host human lymphoid MT-4 cells induced by HIV. Tiny amounts of virus are added to the cells culture and two complete reproductive cycles of the virus are needed to kill the cells. The active principles or drugs that interact with virions or with genetic materials from the virus, will interfere the viral activity and may protect the cells from cytolysis.

The testing system is automatized and allows the evaluation of a great number of drug candidates at a time. However, the compounds that result chemically altered or that are rapidly metabolized, under the culture conditions, may not show antiviral activity in these assays.

In all tests a positive reference, standard or control is introduced. In our particular case four positive controls were used simultaneously and under the same culture conditions.

The evaluation of the anti-HIV activity was carried out on lymphoid MT-4 cells infected by the NL4-3 strain of HIV-1 virus, with an infection multiplicity of 0.002, incubating along 5 days at 37 °C under a 5% CO_2 atmosphere and in the presence of the active principle at concentrations lower than 25 and/or 125 μg/mL. The antiviral activity was measured by quantification of the host cells viability, after addition of the development reagent MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazole) bromide] that is metabolized in the mitochondria of alive cells generating the intensively colored formazan, which is measured by a spectrophotometer.[22]

As references or internal standards four clinical drugs were used: AZT,[23] an inhibitor of the reverse transcriptase of HIV virus; AMD-3100,[24] an inhibitor of the entry of virions on the human cells; Ritonavir[25] or RTV, inhibitor of the HIV-1 protease and finally Nevirapine,[26] also marketed under the trade name Viramune that is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used to treat HIV-1 infection and AIDS.

The protocol followed for the anti-HIV assays is as follows:

(1) The drug candidate (normally 1–2 mg) is dissolved in DMSO to prepare a stock solution. Aliquots of this stock solution are diluted 1:100 with a cellular culture medium. From this new solution new dilutions are carried out by a factor $^1/_2$ $\log_{10}$.
(2) The MT-4 lymphoid cells are added and after a short period of time HIV-1 is added, resulting finally a culture medium, containing the drug, cells and virus, with a 1:200 dilution. As a *control of cytotoxicity* of the drug a parallel test with MT-4 cells in absence of virus (non-infected cells) is also carried out. On the other hand, as *blanks or basic controls* MT-4 cells infected and non-infected, but in absence of drugs, are also used in order to establish a reference of cell viability.
(3) The cultures are incubated at 37 °C under a 5% CO_2 atmosphere for 5 days.
(4) The tetrazolium salt, MTT, is added to the 96-wells plates and the cultures are incubated until the viable cells metabolize MTT and develop color by formation of formazan.
(5) The individual wells are analyzed by a colorimeter to measure the formation of formazan and also they are visualized by a microscope to detect the presence of viable cells and to confirm the possible protecting activity of the evaluated drug.
(6) In the same 96-wells plate, the infected cells treated with the drug are compared to the non-infected cells also treated with the drug and with other controls (infected and non-infected cells not treated with the drug, wells that contain only drug but not cells, etc.).
(7) The experimental data are processed to calculate the following two parameters:

EC_{50}: Effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with the MTT method in MT-4 lymphoid cells.

CC_{50}: Cytotoxic concentration 50 or needed concentration to induce 50% death of non-infected MT-4 cells.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.04.076.

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