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Selective inhibition of human inmunodeficiency virus type 1 (HIV-1) by nucleoside analogues with an unusual tricyclic structure

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Abstract

The nucleoside **CAM-263**, with an unusual tricyclic carbohydrate moiety, specifically inhibits HIV-1 replication while being inactive against HIV-2 or other (retro) viruses. In an attempt to increase the inhibitory efficacy against HIV-1, and to further explore the structural features required for anti-HIV-1 activity, different types of modifications have been carried out on this prototype compound. These include substitution of the the ethoxy group at the C-4" position by alkoxy groups of different length, ramification, conformational freedom or functionalization. In addition, the 4"-ethoxy group has been removed or substituted by other functional groups. Next, we studied the role of the *tert*-butyldimethylsilyl (TBDMS) group at the 2' position by preparing the corresponding 2'-deprotected derivative or by replacing it by other silyl (*tert*-hexyldimethylsilyl) or acetyl moiety. Finally, the thymine of the prototype compound has been replaced by *N*-methylthymine, uracil or SPh. Some of these compounds showed moderate and specific anti-HIV-1 activity.

Introduction

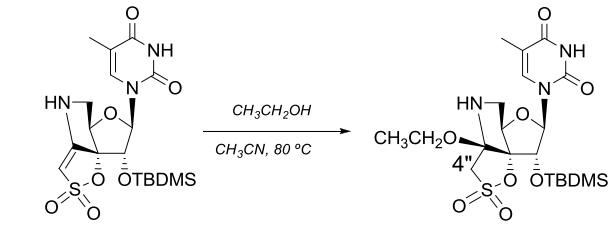
In the last few years the design and synthesis of nucleosides with bicyclic carbohydrate moieties have attracted considerable attention to restrict the conformational flexibility of the nucleoside into determined conformations which was ideal for nucleic acid recognition.[1] On the other hand, a great diversity of bicyclic nucleosides have been synthesized in order to identify conformational preferences of some receptors and enzymes involved in the metabolism or polymerization of nucleosides and to study the interaction of such compounds with their enzymatic targets.[2] Some bicyclic nucleosides have shown to inhibit moderately HIV replication[3] although none of them are specific for HIV-1.

In order to induce further conformational restriction in the bicyclic nucleosides the introduction of additional bridges generating tricyclic nucleosides was also undertaken.[4] However, no biological evaluation against HIV of these compounds have been reported so far.

In an earlier work, we described the efficient transformation (one-step, high yields, and easy purifications) of the nucleoside **CAM-261** (Figure 1)[5] into bi- and tricyclic nucleosides with rather unusual molecular skeletons in a completely regio- and stereoselective fashion. When these polycyclic nucleosides were evaluated in cell culture against different classes of viruses, it

was found that the tricyclic nucleoside **CAM-263** (Figure 1), obtained by reaction of **CAM-261** with ethanol at 80 ºC, specifically inhibits HIV-1 replication while being inactive against HIV-2 or other (retro) viruses. The good selectivity displayed by **CAM-263** prompted us to perform systematic modifications on this prototype with the objective of determining the minimal structural features essential for HIV-1 inhibition. This study form the subject matter of this paper.

As a starting point, we focused our attention on the replacement of the ethoxy group at the C-4" position by alkoxy groups of different length, ramification, conformational freedom or functionalization. In addition, the ethoxy group has been removed or substituted by other functional groups. Next, we studied the role of the *tert*-butyldimethylsilyl (TBDMS) group at the 2' position by



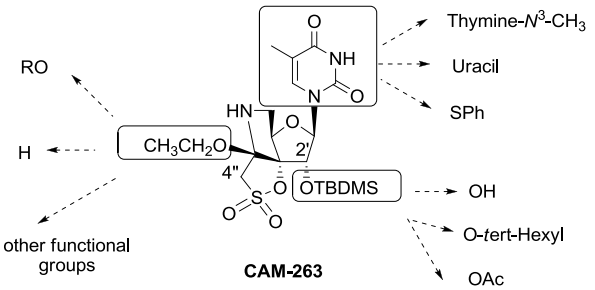


Fig. 1 Modifications carried out on the tricyclic nucleoside **CAM-263**

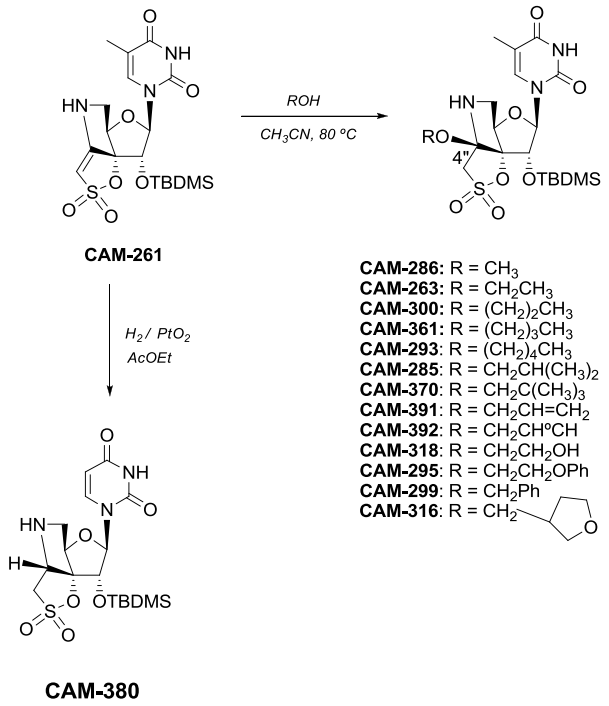
preparing the corresponding 2'-deprotected derivative or by replacing it by other silyl (*tert*-hexyldimethylsilyl) or lipophilic (acetyl) moieties.

Modifications on the nucleobase were also examined. First, the *N-3* methyl analog of **CAM-263** has been prepared in order to determine the importance of H-bond-donating ability of the amide proton at 3-N position of the thymine nucleobase. Finally, the thymine of the prototype compound has been replaced by uracil or by an aromatic moiety such as SPh.

Results and discussion

Chemical Synthesis

We studied first the effect of the substitution of the ethoxy moiety of the prototype compound **CAM-263** by flexible alkoxy moieties of different length and ramification. These compounds were readily prepared in one step by reaction of **CAM-261** with the corresponding alcohols in refluxing acetonitrile at 80 ºC (Scheme 1).[5] Thus, reaction of **CAM-261**, under reflux, with different primary alcohols such as methanol, propanol, butanol, pentanol, isobutyl alcohol and 2,2-dimethyl-1-propanol afforded the corresponding tricyclic derivatives: **CAM-286** (58%), the prototype **CAM-263** (70%), **CAM-300** (60%),[5] **CAM-361**[5] (78%), **CAM-293**[5] (79%), **CAM-285** (72%) and **CAM-370** (83%) in high yields (58-83%).



Scheme 1 Synthesis of **CAM-xxx** to **CAM-xxx**

To study how the conformational restriction of the alkoxy moiety affects in the activity, we prepared compounds **CAM-391** (80%) and **CAM-392** (76%) in which a double or triple bond was present (Scheme 1). On the other hand, the functionalization of the alkoxy moiety was studied by preparing compounds **CAM-318** (74%) and **CAM-295** (67%), with an hydroxy or phenoxy moiety at the terminal end of the alkoxy moiety, or compounds **CAM-299** (80%) or **CAM-316** (67%) with benzyloxy or tetrahydrofuranylmethoxy groups at the C-4" position (Scheme 1). These compounds were prepared following a similar procedure to that described for the synthesis of **CAM-286-xxxx**. Thus, the reaction of **CAM-261** with 2-propen-1-ol, 2-propyn-1-ol, 1,2-ethanediol, 2-phenoxy-1-ethanol, benzyl alcohol and tetrahydro-2-furanmethanol in refluxing acetonitrile afforded the corresponding tricyclic nucleosides **CAM-391-xxxx** in high yields (67-80%).

As it was previously determined for the prototype **CAM-263**,[5] the stereochemistry of the new stereogenic center created on C-4" in this series of compounds was unequivocally determined as *S* on the basis of NOE difference experiments.

Compound **CAM-380** in which the ethoxy moiety at the C-4" position has been removed was also prepared (Scheme 1). The synthesis of this compound was attempted first by hydrogenation of **CAM-261** using 10% palladium on charcoal as catalyst. However, under these conditions, the starting compound **CAM-261** remained unchanged, even upon prolonged reaction times. Instead, when platinum oxide was used as catalyst, compound **CAM-380** was obtained in 30% yield.

In compound **CAM-380**, the signal at δ 4.54 ppm, corresponding to the H-4" proton, correlates with the signal of the H-2' proton of the sugar, at δ 4.90 ppm, indicating that the H-4" proton is on the upper side (β-face) of the furanose ring (Figure 2). This result confirms that the conjugate addition of the hydrogen proceeded with complete stereoselectivity on the β-face of the sugar-fused cyclic enamine **CAM-261**.

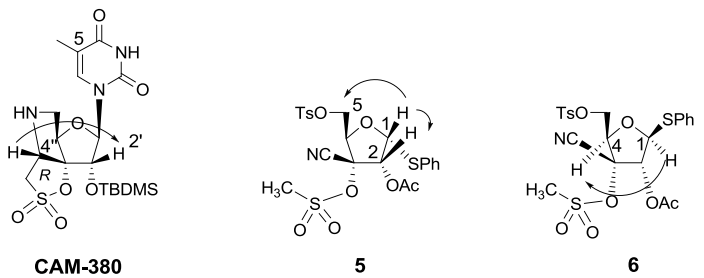


Fig. 2 Observed NOE's for comppunds **CAM-380**, **5** and **6**

On the other hand, to determine the importance of the ethoxy moiety, we tested the nucleoside analogues **CAM-509,**[6] **CAM-354,**[5] **CAM-315**[5] and **CAM-321**[5] with hydroxy, ethylthio, cyano and carboxamide moieties at the C-4" position that have been previously

synthesized in our laboratory (Figure 3). In addition, the 4"-*O*-*tert*-butyldimethylsilyl derivative **CAM-536** was prepared in 73% yield by treatment of the 4"-hydroxy derivative **CAM-509** with *tert*-butyldimethylsilyl chloride in pyridine at room temperature.

Next, modifications at the 2' position was investigated (Scheme 2). Starting from **CAM-263** the 2' deprotected derivative **CAM-291** was obtained in 69% yield by treatment with ammonium fluoride in methanol. Acetylation of **CAM-291** using acetic anhydride/pyridine afforded the 2' acetyl derivative **CAM-400** in 86% yield. Alternatively, reaction of **CAM-291** with *tert*-hexyl dimethylsilyl chloride in acetonitrile at room temperature afforded the corresponding 2'-*O*-silylated nucleoside **CAM-589** in xxxx% yield.

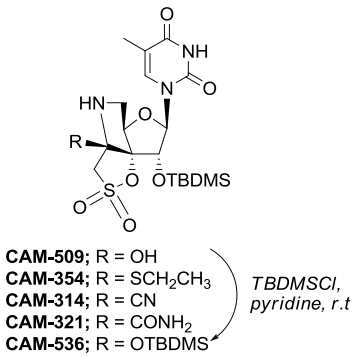
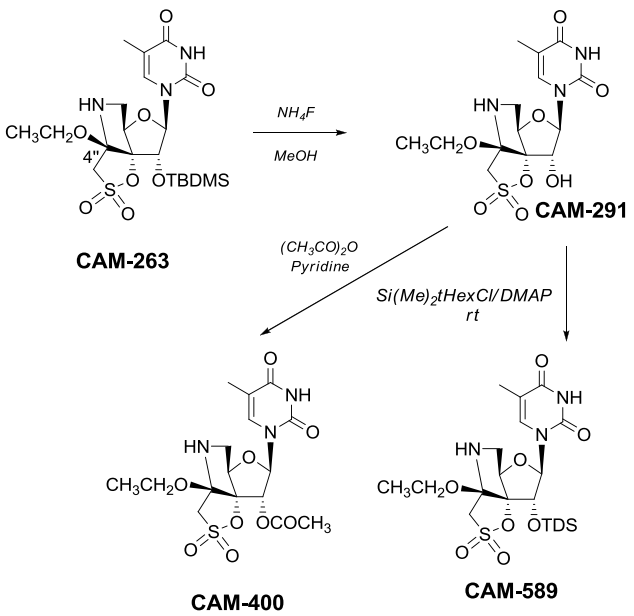


Fig. 3 Structure of compounds **CAM-xxx** to **CAM-xxx**



Scheme 2 Synthesis of **CAM-xxx** to **CAM-xxx**

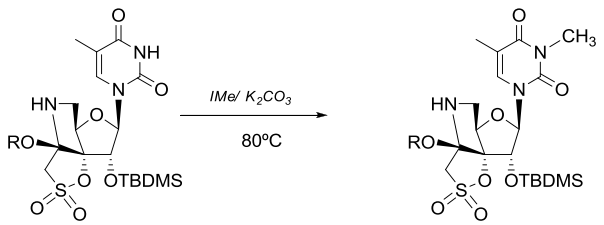
With respect to the modifications on the nucleobase the introduction of a methyl group at the *N*-3 position of the thymine was first carried out (Scheme 3). Thus, compound **CAM-263** was transformed to the corresponding 3-*N*-methyl nucleoside **CAM-103-2** by selective *N*-3-alkylation using methyl iodide in the presence of potassium carbonate.

Secondly, we prepared compounds **CAM-546** and **CAM-442** in which the thymine of the prototype has been replaced by uracil or thiophenyl moieties (Scheme 4). The synthesis of these compounds consisted on a convergent strategy in which the 5-*O*-tosyl-3-cyano mesyl ribofuranose **3**[7] (Scheme 4) has been employed as a common sugar precursor in glycosylation reactions.

For the synthesis of **3** our previously described 5-*O*-tosyl derivative **1** (Scheme 5) has been used.[5] Thus, hydrolysis of the 1,2-*O*-isopropylidene moiety of **1**, with aqueous trifluoroacetic acid, followed by reaction with acetic anhydride/pyridine afforded a 1:1.5 mixture (deduced from ^{1}HNMR spectrum) of the two anomeric forms (α and β) of the diacetate derivative **3** in 90% yield. Condensation of **3** with silylated uracil under modified Vorbrüggen[8] conditions afforded the 3'-cyano mesyl nucleoside **4** in 58% yield. The coupling

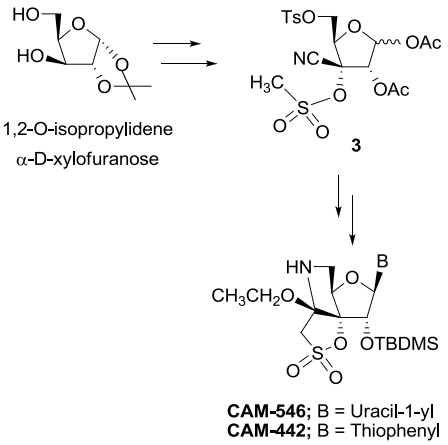
constant value $J_{1',2'}$ = 7 Hz observed for this compound is in reasonably good agreement with the data for other cyanomesyl nucleosides described in our group.[9]

On the other hand, reaction of **3** with thiophenol in the presence of boron trifluoride etherate afforded the β aryl thioglycoside **6** in 62% yield together with the α aryl thioglycoside **5** as a minor compound (1%). In these sugar derivatives the similar values of the coupling constant $J_{1,2}$ observed between the α ($J_{1,2}$ = 5.9 Hz) and β anomers ($J_{1,2}$ = 5.8 Hz) precluded its use to establish the anomeric configuration. Therefore, their anomeric configuration was unequivocally determined by a NOE experiment (Figure 2). Thus, for the major



CAM-263, R = CH_2CH_3 **CAM-103-2** : R = CH_2CH_3

Scheme 3 Synthesis of **CAM-103-2**

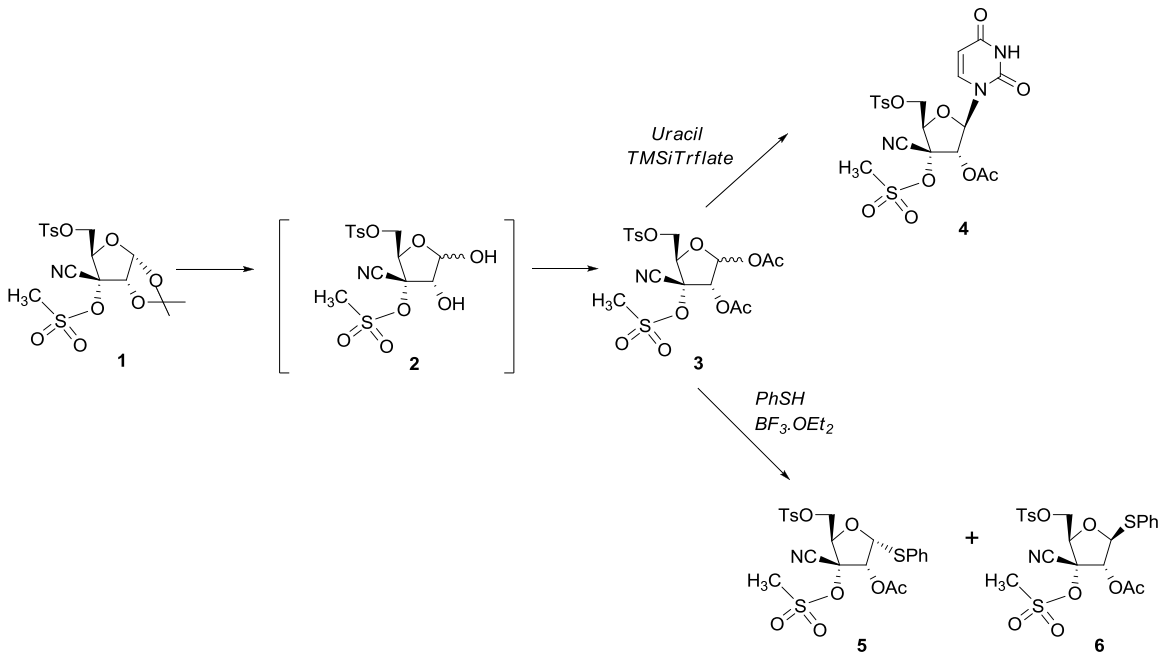


Scheme 4 Key glycosylation approach for the synthesis of **CAM-546** and **CAM-442**

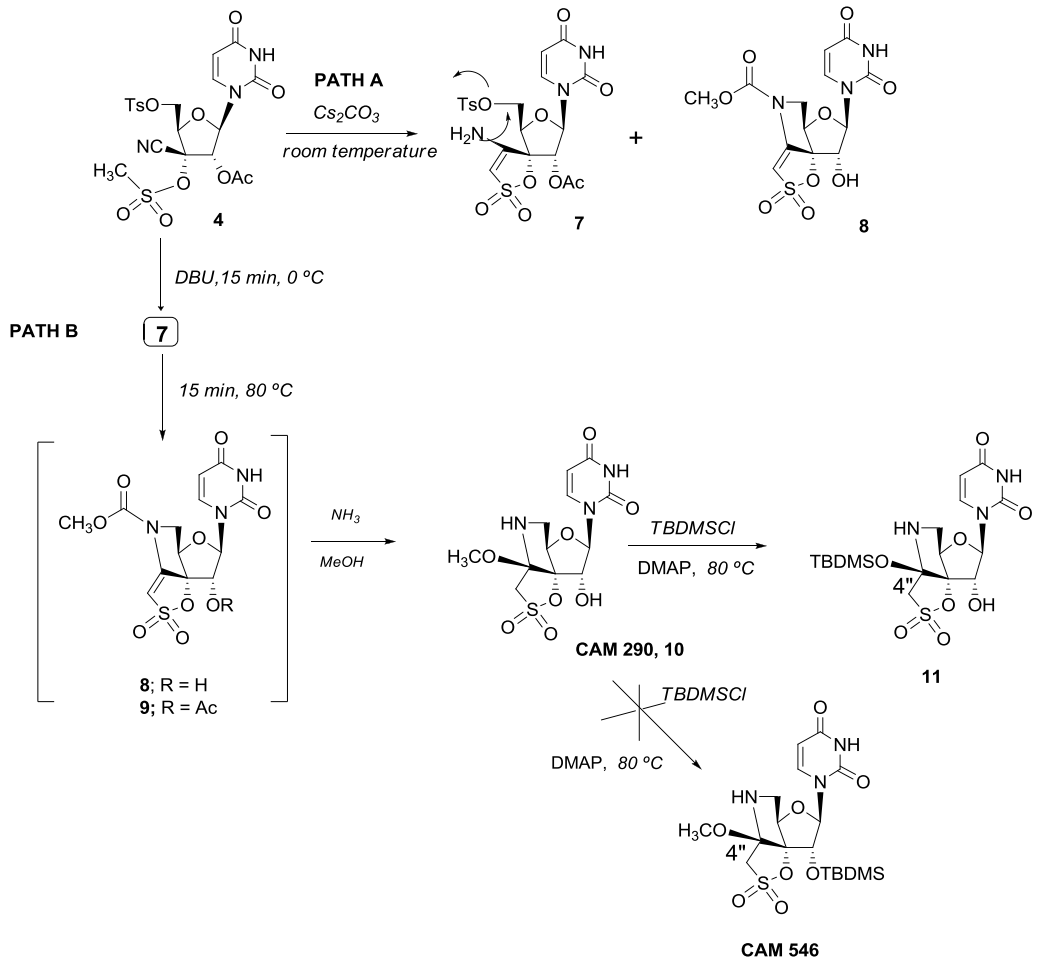
isomer **6,** irradiation of H-1 caused enhancement of the signals for H-4 indicating that both protons, H-1 and H-4, are at the lower face (α face) of the furanose ring, and therefore the anomeric configuration of this compound is β. For the minor isomer **5**, irradiation of H-1 causes enhancement of the signals H-2 and H-5 indicating that all of these protons are at the upper face (β face) of the furanose ring, and therefore the anomeric configuration of this compound is α.

Once the 5'-*O*-tosyl cyano mesyl nucleoside **4** was obtained, we attempted its transformation into the spiro nucleoside **7** (Scheme 6, Path A) following a protocol similar to that previously established for other cyano mesyl nucleosides with a benzoyl, instead of tosyl, moiety at the 5' position of the sugar.[9] Thus, treatment. of **4** under non-nucleophilic basic conditions (potassium carbonate/acetonitrile) at room temperature afforded the expected spiro derivative **7** in 30% yield, together with an unexpected compound (**8**) that was formed in 20% yield. Formation of **8** could be explained through the formation of **7** followed by the *"in situ"* intramolecular attack of the 4"-amino group to the tosyl leaving group at the 5' position of the sugar[5] and acetyl group migration from the 2' position to the NH of the pyrroline ring. Compound **8** could be considered as an attractive synthetic intermediate for the synthesis of the desired **CAM-546** and for this reason we decided to drive the formation of **8** to completion, avoiding the isolation of **7**.

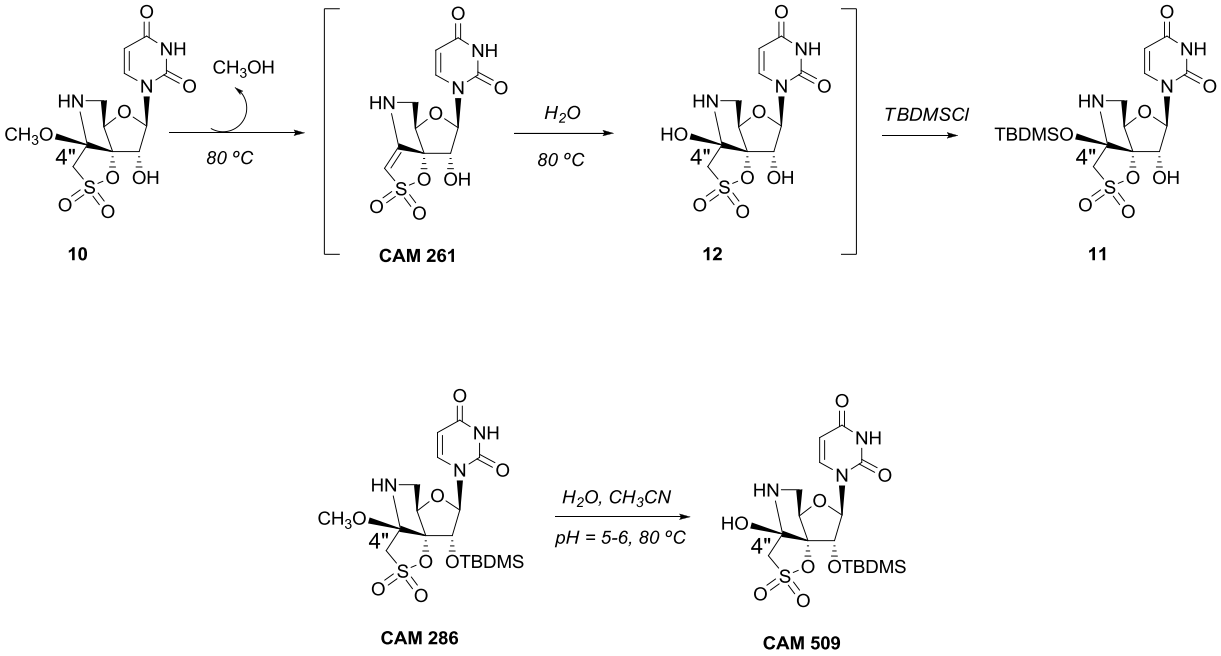
With this aim, the conversion of the starting 5-*O*-tosyl nucleoside **4** to the desired nucleoside **8** was studied as a function of reaction time, temperature and base. Among the set of reactions assayed, the best results were obtained when DBU was used as a base, 0 ºC, and 10 min reaction time. Under these conditions, the clean formation of **7** was detected by TLC analysis (Scheme 6, Path B). On increasing the temperature to 80 ºC for 15 extra minutes the reaction goes to completion and compound **7** was consumed. At this time a mixture of compounds was detected by TLC from which the desired nucleoside **8** together with its 2'-*O*-acetyl derivative **9** was identified by NMR. Treatment of the mixture with methanol ammonia gave the methoxy derivative **10** in 65% yield**.** Noteworthy, the reaction of **10** with *tert*-butyldimethylsilyl chloride and DMAP at 80ºC did not afford the expected uracil nucleoside **CAM-546**, instead the 4"-*O*-silylated derivative **11** was obtained in xxx% yield.



Scheme 5 Synthesis of **3-6**



Scheme 6 Synthesis of compounds **7-11**

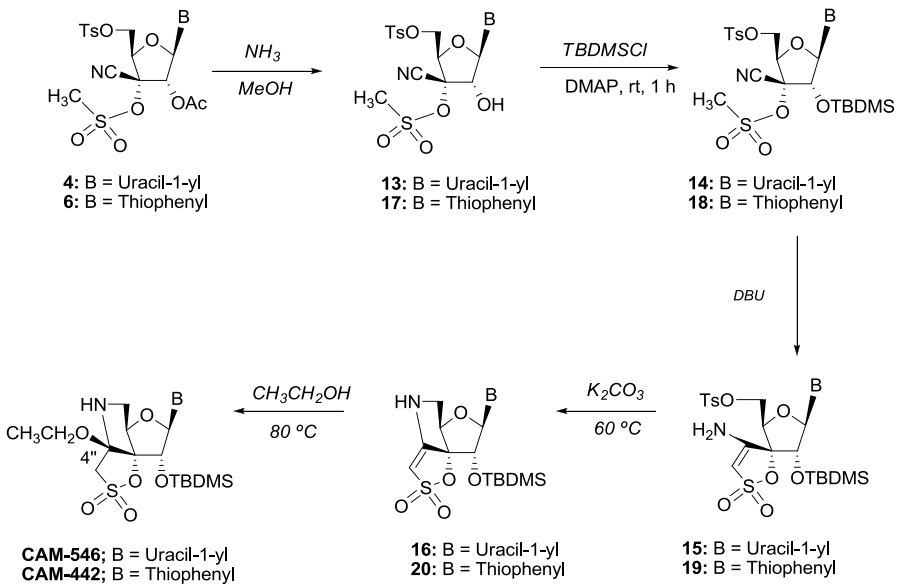


Scheme 7 Proposed mechanism for the synthesis of compound **11**

A plausible explanation for the formation of **11** is illustrated in Scheme 7. We propose that, by heating, nucleoside **10** might experiment the elimination of methanol to give the cyclic enamine **CAM-261**. This transformation is consistent with literature data[10] that indicates that the Michael-type addition products (in our case, compound **10**) can often be reverted to the starting material by heating. Next, the addition of water to the conjugated double bond of **CAM-261** (position 4") might afford the 4"-hydroxy nucleoside **12** that was readily silylated.

To confirm the reversibility of our Michael-type reaction, the 4"-methoxy derivative **CAM-286** was reacted with a mixture of acetonitrile/water at 80ºC under weak acid media (pH adjusted at 5-6 with acetic acid). Under these conditions the 4"-hydroxy derivative **CAM-509** was obtained confirming our hypothesis (Scheme 7).

Finally, synthesis of **CAM-546** and **CAM-442** was undertaken as shown in Scheme 8. Thus, 2' deprotection of nucleoside **4** with saturated methanolic ammonia gave the deprotected nucleoside **13** which was not purified. Reaction of **13** with *tert*-butyldimethylsilyl chloride at 80 ºC for 1 hour afforded the 2'-*O*-silylated nucleoside **14** (68%). It should be mentioned that the substitution of the 5'-tosyl group by chloride was observed upon prolonged reaction times. The 5'-chloro derivative obtained



Scheme 8 Synthesis of **CAM 546** and **CAM 442**

behaves exactly as the corresponding 5'-*O*-tosyl derivative **14** and for this reason when the substitution takes place the resulting mixture was used directly without purification. Nucleoside **14** was treated with DBU to give the spiro derivative **15** (68%). Reaction of **15** with potassium carbonate at 80 ºC for 5 h afforded the cyclic enamine sulfonate **16** in 70% yield that was treated with ethanol to afford **CAM-546** in 61% yield.

A similar synthetic sequence was followed with the thiophenyl derivative **6** to afford the 2'-*O*-silylated derivative **18** that was transformed into **19** (77%), **20** (73%) and **CAM 442** (79%) as described above (Scheme 8).

Biological evaluation

Table 1 summarizes the results of the biological evaluation of the test compounds expressed as EC_{50} values or compound concentrations required to inhibit virus-induced cytopathicity in CEM and MT-4 cell cultures by 50%. The antiviral data on the prototype compound (**CAM-263**) is also reported as reference.

Whereas several compounds inhibited HIV-1 replication in the lower micromolar concentration range, none of the compounds proved active against HIV-2 at subtoxic concentrations (Table 1). Therefore, the active compounds should be considered as specific inhibitors of HIV-1 replication. Their anti-HIV activity in CEM cells were similar than in MT-4 cells.

Nucleosides **CAM-286** and **CAM-300** with one more or one less carbon atom in the alkoxy chain than the prototype showed an antiviral activity comparable to it. However, compounds with longer alkoxy chains (**CAM-361** or **CAM-293**) were devoid of antiviral activity. The propyloxy derivative **CAM-300** was the most inhibitory to HIV-1 replication in CEM cells of this series.

The rigidity of the alkoxy moiety seems to influence the antiviral activity. Thus, compounds **CAM-391** and **CAM-392** with a less flexible alkenyl or alkynyl alkoxy chain were inactives.

Table 1 Inhibitory effects of test compounds on HIV-1 and HIV-2 replication in MT-4 and CEM cell culture and recombinant HIV-1 RT

CAM	EC_{50} (μM)[a]					CC_{50} (μM)[b]
	MT-4		CEM			CEM
	HIV-1	HIV-2	HIV-1	HIV-2	HIV1/138K[c]	
286	8.4 ± 3.9	>50	5.5 ± 0.7	>50	>500	111 ± 14.8
300	5.1 ± 0.4	>10	1.4 ± 0.8	>10	>500	28.5 ± 2.0
361	>10	>10	>10	>10		34.8 ± 13.7
293	≥5	>5	>5	>5	>500	10.1 ± 0.1
285	5.2 ± 0.6	>10	8.0 ± 2.8	>10	>500	21.1 ± 3.6
370	>10	>10	>10	>10		21.5 ± 1.6
391	>10	>10	15.0 ± 5.0	>50		57.8 ± 9.3
392	>25	>25	>25	>25		66.2 ± 4.9
318 (2)	-	-	>50	>50		97.7 ± 4.7
295	>5	>5	>5	>5	>500	10.4 ± 0.7
299	>10	>10	≥10	>10	>500	20.4 ± 1.1
316	>10	>10	>50	>50		72.0 ± 1.7
380	30.4 ± 23.5	>125	30.0 ± 7.1	>125		>125
509	>250	>250	>250	>250		>250
536	-	-	>2	>2		7.04 ± 1.8
354	31.1 ± 16.5	>50	32.5 ± 3.5	>50		80.6 ± 8.6
314	>50	>50	>50	>50		103.0 ± 7.9
321	>125	>125	>125	>125		≥125
291 (2)	_	_	>250	>250		≥250
589	_	_	>10	>10		27.0 ± 3.6
400	>250	>250	>250	>250		>250
103 (2)	3.6 ± 2.3	≥50	3.0 ± 1.4	≥50		32.0 ± 2.0
546	>50	>50	>50	>50		111.0 ± 1.4
442	-	-	>10	>50		131.0 ± 12.7

263	13.8 ± 5.4	>10	7.7 ± 4.0	>50	>500	25.0 ± 1.2

[a] 50% effective concentration, or the compound concentration required to inhibit HIV-induced cytopathicity by 50%.
[b] 50% cytostatic concentration, or the compound concentration required to inhibit cell proliferation (CEM) or to reduce cell viability (MT-4) by 50%.
[c] 50% inhibitory concentration, or the compound concentration required to inhibit recombinant HIV-1 RT activity by 50%

The isobutyloxy derivative **CAM-285** showed an antiviral activity comparable to that of the unbranched derivatives. However, an increase in the number of the branch chain alkyl groups leads to an inactive compound (**CAM-370)**.

Compounds **CAM-318-2** and **CAM-295**, bearing an ethoxy chain functionalized with a hydroxy or phenoxy moiety, were inactives. Compounds **CAM-299** and **CAM-316** with a methoxy chain functionalized with phenyl or furanyl moieties were also devoid of antiviral activity. This observation suggests that the functionalization of the alkoxy chain is not well tolerated.

Replacement of the ethoxy moiety in the prototype by OH or OTBDMS resulted in compounds (**CAM-509**, **CAM-536**) that lack activity. The presence of other functional groups (CN and $CONH_2$) also gave inactive compounds (**CAM-314** and **CAM-321**). In contrast, nucleoside **CAM-380** lacking the ethoxy moiety or **CAM-354** with an ethyl chain containing a sulfur atom at C-4" were endowed with anti-HIV-1 activity, although are approximately 2-fold less actives than the prototype.

Substitution of the TBDMS group at 2' position of the ribofuranose by other lipophilic moieties, such as *tert*-hexyldimethylsilyl (**CAM-589**) or acetyl (**CAM-400**), gave inactive compounds. The 2'-deprotected derivative (**CAM-291-2**) was also inactive.

The 3-*N*-methylthymine derivative **CAM-103-2** was 4-fold more active than its unsubstituted counterpart (**CAM-263**). Substitution of the thymine moiety by uracil or SPh gave inactive compounds (**CAM-546** and **CAM-442**) respectively.

Conclusions

In summary, in this paper we report on novel derivatives of the prototype tricyclic nucleoside **CAM-263** by modifying the ethoxy moiety at the C-4" position, the nucleobase and the substituent at the 2' position. Several members of this class of compounds show specific anti-HIV-1 activity comparable and even slightly superior to those of the prototype tricyclic nucleoside. Our structure-activity relationship studies demonstrate that both, the presence of thymine and a *tert*-butyldimethylsilyl group at the 2'-position of the sugar are important structural components since deletion of either of them is detrimental to the anti-HIV-1 activity. Modifications at the alkoxy moiety at C-4" position were less stringent to keep anti-HIV-1 activity. Thus, the ethoxy moiety can be replaced by methoxy, propyloxy, hydrogen and ethylthio moieties. Introduction of a methyl group at the position *N*-3 of the thymine ring enhanced the antiviral activity by 4-fold. The tricyclic nucleosides here described represent a novel type of selective anti HIV-1 inhibitors.

Acknowledgements

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Further reading

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