Selective inhibition of human immunodeficiency 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 4" 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 Finally, the thymine of the prototype compound has been replaced by N-methylthymine, uracil or SPh.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. 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. Some bicyclic nucleosides have shown to inhibit moderately HIV replication 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. 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) 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 °C, 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 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 °C (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\(^3\) (78%), CAM-293\(^3\) (79%), CAM-285 (72%) and CAM-370 (83%) in high yields (58-83%).

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\(^5\) Figures and chemical reactions were adapted from the original text for clarity and readability.
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, 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.

On the other hand, to determine the importance of the ethoxy moiety, we tested the nucleoside analogues CAM-509, CAM-354, CAM-315 and CAM-321 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 86% yield.

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' (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. 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 1H NMR 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 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

\[ \text{CAM-263, } R = \text{CH}_2\text{CH}_3 \]  
\[ \text{CAM-103-2 : } R = \text{CH}_2\text{CH}_3 \]

Scheme 3 Synthesis of CAM-103-2

15 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 cyanomesyl nucleosides with a benzoyl, instead of tosyl, moiety at the 5' position of the sugar.8 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 sugar8 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
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 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...
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 °C 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 alkynyl 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

<table>
<thead>
<tr>
<th>CAM</th>
<th>MT-4</th>
<th>CEM</th>
<th>CEM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50} (µM)^{[a]}</td>
<td>CC_{50} (µM)^{[b]}</td>
<td></td>
</tr>
<tr>
<td>HIV-1</td>
<td>HIV-2</td>
<td>HIV-1</td>
<td>HIV-2</td>
</tr>
<tr>
<td>286</td>
<td>8.4 ± 3.9</td>
<td>&gt;50</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>300</td>
<td>5.1 ± 0.4</td>
<td>&gt;10</td>
<td>1.4 ± 0.8</td>
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<tr>
<td>361</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
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<tr>
<td>293</td>
<td>≥5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>285</td>
<td>5.2 ± 0.6</td>
<td>&gt;10</td>
<td>8.0 ± 2.8</td>
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<td>370</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
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<tr>
<td>391</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>15.0 ± 5.0</td>
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<td>392</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>&gt;25</td>
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<td>318 (2)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>295</td>
<td>&gt;5</td>
<td>&gt;5</td>
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<tr>
<td>299</td>
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</tr>
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<td>316</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;50</td>
</tr>
<tr>
<td>380</td>
<td>30.4 ± 23.5</td>
<td>&gt;125</td>
<td>30.0 ± 7.1</td>
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<tr>
<td>509</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>&gt;250</td>
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<tr>
<td>536</td>
<td>-</td>
<td>-</td>
<td>&gt;2</td>
</tr>
<tr>
<td>354</td>
<td>31.1 ± 16.5</td>
<td>&gt;50</td>
<td>32.5 ± 3.5</td>
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<tr>
<td>314</td>
<td>≥50</td>
<td>≥50</td>
<td>&gt;50</td>
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<tr>
<td>321</td>
<td>≥125</td>
<td>≥125</td>
<td>≥125</td>
</tr>
<tr>
<td>291 (2)</td>
<td>-</td>
<td>-</td>
<td>&gt;250</td>
</tr>
<tr>
<td>589</td>
<td>-</td>
<td>-</td>
<td>&gt;10</td>
</tr>
<tr>
<td>400</td>
<td>≥250</td>
<td>≥250</td>
<td>≥250</td>
</tr>
<tr>
<td>103 (2)</td>
<td>3.6 ± 2.3</td>
<td>≥50</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>546</td>
<td>≥50</td>
<td>≥50</td>
<td>&gt;50</td>
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<tr>
<td>442</td>
<td>-</td>
<td>-</td>
<td>&gt;10</td>
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</table>
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 alkyl groups leads to an inactive compound (CAM-370).

Compounds CAM-318 and CAM-295, bearing an ethoxy chain functionalized with a hydroxy or phenoxy moiety, were inactive. 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 O-TBDMS resulted in compounds (CAM-509, CAM-536) that lack activity. The presence of other functional groups (CN and CONH2) 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 active than the prototype.

Substitution of the TBDMS group at 2’ position of the ribofuranose by other lipophilic moieties, such as tert-hexylmethylsilyl (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-butylmethylsilyl 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, propoxy, 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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Notes and references


Further reading

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