Spiro Compounds

Preparation of 4'-Spirocyclobutyl Nucleoside Analogues as Novel and Versatile Adenosine Scaffolds

Jan Willem Thuring,[d] and Guido Verniest[a, d]

Abstract: Despite the large variety of modified nucleosides that have been reported, the preparation of constrained 4'-spirocyclic adenosine analogues has received very little attention. We discovered that the [2+2]-cycloaddition of dichloroketene to readily available 4'-exo-methylenefuranose sugars efficiently results in the diastereoselective formation of novel 4'-spirocyclobutanones. The reaction mechanism was investigated via density functional theory (DFT) and found to proceed either via an on-synchronous or stepwise reaction sequence, controlled by the stereochemistry at the 3'-position of the sugar substrate. The obtained dichlorocyclobutanones were converted into nucleoside analogues, providing access to a novel class of chiral 4'-spirocyclobutyl adenosine mimetics in eight steps from commercially available sugars. Assessment of the biological activity of designed 4'-spirocyclic adenosine analogues identified potent inhibitors for protein methyltransferase target PRMT5.

In recent years, mimetics of S-adenosyl-L-methionine (SAM, 1) have found applications in drug discovery as protein methyltransferase (PMT) inhibitors for oncology targets,[1] as exemplified by PRMT5 inhibitor LLY-283 (2)[2] and the clinical candidate pinometostat (3) for DOT1L[3] (Figure 1). These selected examples indicate that the nucleoside moiety of SAM (1) is generally well conserved, whereas diverse modifications at the 5’-position of the adenosine analogues are tolerated. Consequently, this encourages an exploration of the chemical space for the discovery of selective SAM (1) analogues for methyltransferase targets of interest.

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Figure 1. Examples of SAM analogues as methyltransferase inhibitors and undescribed spirocyclic scaffold 5.

An interesting example of a constrained SAM (1) analogue and potent (35 nM) methyltransferase inhibitor for PRMT5 is the natural product dehydrosinefungin (4),[4] for which no total synthesis has been described. The impact of the 4'-exocyclic double bond in 4 as a conformational constraint is emphasized by the observation that the saturated analogue of 4 (sinefungin) is nearly 250-fold less potent on the same target, and displays a poor selectivity against a panel of methyltransferases.[5]
In this respect, the incorporation of a spirocyclobutyl ring can be considered as a mimic of the exocyclic alkene in dehydro-sinefungin (4), with the potential to identify novel, biologically active structurally constrained SAM (1) analogues. Indeed, conformational restriction via cyclization is a frequently used approach in drug discovery[6] and has proven to be a valuable strategy towards nucleoside analogues with an enhanced affinity and specificity for the nucleoside binding site in proteins.[7]

Remarkably, despite the example of 5’s modified nucleosides, the preparation of 4'-spirocyclobutyl adenosine scaffolds of type 5 has not been reported. Focussing on 4'-spirocarbocyclic nucleosides, only few examples describe 4'-spirocyclopentyl[8] and -propyl[9] nucleoside analogues, which are typically prepared via lengthy synthetic routes or display limited opportunity for diversification of the spirocyclic scaffold structures. The development of a strategy that enables an efficient synthesis towards unknown adenosine core scaffold 5 is thus of high interest to obtain innovative platform building blocks that can be used in medicinal chemistry for the design of constrained analogues of SAM (1).

In previous reports, Redlich et al. described the synthesis of 4'-spirocyclic furanoses of type 7 using a [2+2]-cycloaddition of dichloroketene on exo-ethylidene furanose substrates 6a and 6b, affording dichlorocyclobutanones 7a and 7b, respectively (Scheme 1).[10] Although this is an interesting transformation, such cycloaddition on the corresponding ribose derivative 8 would generate far more potential as novel building block for nucleoside analogues, however, subjecting exo-ethylidene riboside 8 to identical cycloaddition conditions resulted exclusively in the formation of side product 9. The further derivatization of cyclobutanones 7a and 7b was not investigated.

To obtain 4'-spirocyclic adenosine analogues of type 5, different types of protected 4'-exo-methylene substrates were prepared. In contrast to the failed cycloaddition reaction on ribose analogue 8, we unexpectedly observed that when substrate 12a was initially subjected to standard [2+2]-cycloaddition conditions with dichloroketene,[10] a clean and diastereoselective transformation towards dichlorocyclobutane 13a was obtained (Scheme 2). Encouraged by this, 4'-exo-methylene substrates 12b and 12c were evaluated using identical conditions, which also resulted in a diastereoselective reaction to afford cycloadducts 13b and 13c, respectively. In all cases, the facial selectivity of the [2+2]-cycloaddition is directed by the 3'-substituent, resulting in optically pure dichlorocyclobutanones 13a–13c. Accordingly, the synthesis of stable dichlorocyclobutanones 13a and 13c was successfully reproduced on multigram scale.

Scheme 1. Previous work and current study[11]

Scheme 2. [2+2]-Cycloadditions on 4'-exo-methylene furanoses 12a–12c (ribose) and 14a–14b (xylose), affording cycloadducts 13a–13c and 15a–15b, respectively; [a] after reaction work-up, [b] recrystallized.
To substantiate the observed stereoselectivity in the [2+2]-
cycloaddition on substrates 12a–12c, the energy profiles
of the approach from both the α and β faces were calculated
using DFT. These results were found to corroborate the experi-
mental observations, that is, the observed approach of dichlo-
roketene from the β face towards 13a–13c was found to pro-
cede via a non-synchronous concerted reaction mechanism,
which corresponds to a lower energy transition state com-
pared to an approach from the α face. Indeed, calculations for
the addition of the ketene at the α face showed that a second
energy barrier (16c, Figure 2) needs to be overcome through a
two-step mechanism, as displayed for cycloadduct 13c.

Interestingly, when comparing our findings to the reported
synthesis of 7a and 7b starting from 4′-exo-ethylidene 6a and
6d, the opposite facial selectivity is observed. Moreover, DFT
calculations of the reaction mechanism towards cycloadd-
duct 7b are supportive for the observed addition at the α face
of 6b. To substantiate these observations, we verified if the
stereoselectivity of the cycloaddition can be controlled via
steric interactions at the 3′-position of the furanose ring using
4′-exo-methylene substrates 14a–14b. This hypothesis could
be verified as the structures of cycloadducts 15a–15b, ob-
tained as single isomers, were elucidated via single-crystal X-
ray diffraction (sc-XRD)\(^\text{[13]}\) and found to be opposite compared
to 13a–13c (Scheme 2). The stereochemistry at the 3′-position
of the furanose ring is thus directing the approach of dichloroke-
tene to either the α or the β face, enabling stereoselective access
to both facial isomers by selecting the appropriate substrate.

Having multigram quantities of 4′-spirocyclic furanoses 13a
and 13c in hand, a reductive dechlorination using zinc and
acetic acid afforded ketones 17a and 17b, respectively
(Scheme 3). At this stage, the stable reaction products 17a and
17b could be efficiently purified via silica gel chromatography
and were isolated in good yields. Consecutively, the reduction
of ketone 17a showed a high selectively (94%) for cis-isomer
18a when LIAIH\(_4\), THF, –78 °C (18a), NaBH\(_4\), MeOH, –78 °C (19a).

Next, we investigated the ring puckering of 5a and 5b be-
cause the substitution of the furanose ring is known to have
an important impact on the adopted conformation and hence, affects the biological properties in nucleosides and corresponding oligonucleotides.\[15\] At first, sc-XRD clearly demonstrated that 4'-spirocyclobutanol 5a adopts a South (2-endol) conformation in the crystal lattice. Additionally, NMR spectroscopy was used to relate the H-H scalar couplings (J\textsubscript{1}, J\textsubscript{2}) to the relative percentage of North/South ring puckers,\[16\] which showed a preference for the South conformation for both cis (5b) and trans (5a) nucleoside analogues of 73 and 69%, respectively.\[17\] Furthermore, vibrational circular dichroism (VCD) was employed to study the conformation of 5a and 5b, as this technique offers the advantage that the experimental spectral data can be validated via comparison with in silico lowest energy conformations.\[18\] Also in this case, a preference for a South ring puckering was established for spirocyclic nucleosides analogues 5a and 5b. These results are supportive for extending novel constrained scaffolds 5a and 5b to create ligands for protein methyltransferase PRMT5, since several co-crystal structures with SAM (1) analogues sinefungin and dehydrosinefungin (4) demonstrate that South ring puckering is a common feature, whereas the ring conformation of sinefungin bound to other methyltransferases does not always adopt a South ring pucker.\[19\]

To demonstrate that novel 4'-spirocyclobutanes scaffolds (5a–5b) can be used to design SAM (1) competitive inhibitors of PMTs, we introduced a 2-aminoquinoline heterocycle at the cyclobutanol moiety, as this has been reported to be appropriate for expanding novel constrained scaffolds for a pharmacophore at the cyclobutanol group in compounds 27a and 27b significantly improved the activity compared to spirocyclic adenosine analogues 5a and 5b, resulting in a nearly 400-fold increase in potency for compound 27b (Table 1). These results clearly demonstrate that novel core scaffolds of type 5 are valuable building blocks for the preparation of target compounds for SAM (1) competitive inhibition of PRMT5.

In conclusion, we have developed a scalable and diastereoselective [2+2]-cycloaddition reaction using dichloroketene on 4'-exo-methylene furanos substrates, resulting in the formation of optically pure 4'-spirocyclic dichlorocyclobutanones. Calculations by DFT showed that the reaction proceeds via a

### Table 1. Inhibition potency of 5a–5b and 27a–27b for PRMT5-MEP50 complex measured at 10 nM PRMT5 concentration.

<table>
<thead>
<tr>
<th>R</th>
<th>Cmpd</th>
<th>IC\textsubscript{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>5a</td>
<td>3.52 ± 0.14</td>
</tr>
<tr>
<td>21b</td>
<td>5b</td>
<td>5.69 ± 0.22</td>
</tr>
<tr>
<td>27a</td>
<td>27a</td>
<td>31.1 ± 0.5</td>
</tr>
<tr>
<td>27b</td>
<td>27b</td>
<td>9.42 ± 0.3</td>
</tr>
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nonsynonymous concerted or a stepwise mechanism, which is controlled by the stereochemistry at the 3’-position of the sugar substrate. Dihydropyranotetraene scaffolds have been converted towards the corresponding 4’-spirocyclobutanones and these intermediates were found to be excellent substrates for a stereoselective Vorbrüggen glycosylation. This reproducible, high-yielding synthesis route enables access to megatran amounts of a novel class of 4’-spirocyclobutyl adenosine analogues in eight steps from commercially available sugar building blocks. A proof of concept was delivered that 4’-spirocyclic building blocks can be transformed into potent inhibitors for methyltransferase PRMTs.

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Conflict of interest

The authors declare no conflict of interest.

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