N,O-Nucleosides from Ene Reaction of (Nitrosocarbonyl)mesitylene with Crotyl Alcohol: Selectivity, Scope, and Limitations

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Abstract The (nitrosocarbonyl)mesitylene intermediate undergoes an ene reaction with crotyl alcohol, affording two regioisomeric adducts in fair yields. The sterically demanding (nitrosocarbonyl)mesitylene slightly shifts the C2/Markovnikov orientation towards a C3/anti-Markovnikov pathway, affording a 5-hydroxyisoxazolidine that serves as a synthon for the preparation of N,O-nucleoside analogues through the Vorbrüggen protocol. The selectivity of the ene reaction is discussed in the light of C=C bond polarization and steric effects. The structures of the N,O-nucleosides are assigned and discussed on the basis of spectroscopic observations and X-ray analysis.

Key words N,O-nucleosides, ene reaction, nitroso carbonyls, crotyl alcohol, Vorbrüggen protocol

The ene reactions of aromatic nitrosocarbonyl intermediates 1 (Scheme 1) with allylic alkoxy olefins were investigated and the synthesis of N,O-nucleoside analogues through the Vorbrüggen protocol was proposed.1 The Chiacchio and Romeo groups recently described the synthesis of truncated phosphonated C-1′-branched N,O-nucleosides as HIV inhibitors or inducers of apoptosis based on 1,3-dipolar cycloadditions of nitrones with vinyl acetate, followed by coupling with silylated nucleobases.2 The same approach was also followed by the research groups of Bortolini and Maiuolo for the synthesis of antiproliferative drugs.3 The 1,3-dipolar cycloaddition synthetic strategy is a valuable method for spacer substitution in classical nucleosides. Furthermore, the introduction of a side chain led to several branched nucleosides which were found to be potential antitumor or antiviral agents,4 and an interesting cytotoxicity and apoptotic activity was also observed.5 Our alternative for the synthesis of N,O-nucleosides relies upon the mild oxidation of aromatic nitrile oxides with tertiary amine N-oxides to generate the nitrosocarbonyl intermediates 1 and the ene reaction with allylic alcohols such as 3-methylbut-2-en-1-ol (Scheme 1).6 3-Methylbut-2-en-1-ol can be regarded as a trisubstituted allylic alkoxy olefin, and the allylic hydrogens on the more congested side of the alkene are exclusively abstracted (the ‘cis effect’), thus resembling singlet oxygen behavior.

Scheme 1 Ene reaction of nitrosocarbonyls 1 with 3-methylbut-2-en-1-ol and synthesis of isoxazolidine nucleosides

The canon (a general rule) for these reactions is that they proceed straightforwardly to the ene adducts in accordance with the prevailing HOMO(olefin)–LUMO(nitrosocarbonyl) interaction, somewhat enforced by the polarization of the C=C bond induced by the slightly electron-withdrawing group CH2OH.7 (Nitrosocarbonyl)benzene 1P (P = phenyl) follows a Markovnikov (M) orientation and preferentially...
absorbs the twix hydrogens over the lone ones. With the more sterically demanding (nitrosocarbonyl)mesitylene (1M; M = mesityl), the Markovnikov directing effect is diminished and twix and lone abstraction are comparable (Scheme 1).8

The anti-Markovnikov (AM) pathway becomes the preferred one when the bulkier anthracene aromatic ring replaces mesitylene in the nitrosocarbonyl structure 1A (A = anthryl).1 The anti-Markovnikov route leads to enol formation and subsequent cyclization to the isoxazolidine 1sox, resembling the structures obtainable through nitrogen additions to vinyl acetate derivatives (Scheme 1). By adapting the Vorbrüggen protocol, a library of N,O-nucleosides N,O-N was prepared from commercially available uracils and purines.1,9

On pursuing our research in nitrosocarbonyl ene reactions, we extended the studies to another allylic alcohol, the disubstituted crotyl alcohol, which was allowed to react in the presence of the mesitylene nitrosocarbonyl 1M. The products of the ene reaction were characterized and the structures were assigned, allowing a closer examination of the selectivity outcome and disclosing new synthetic applications.

The diastereoisomeric isoxazolidines deriving from formal anti-Markovnikov addition to the alcohols are precursors. The diastereoisomeric isoxazolidines deriving from the selectivity outcome and disclosing new synthetic applications were assigned, allowing a closer examination of the non-isolable aldehyde (Scheme 1). By adapting the Vorbrüggen protocol, a library of N,O-nucleosides N,O-N was prepared from commercially available uracils and purines.1,9

Addition of a dichloromethane (CH2Cl2) solution of mesitonitrile oxide (MNO) to a stirred solution of N-methylmorpholine N-oxide (NMO, 1.1 equiv) in CH2Cl2 in the presence of an excess (5 equiv) of crotyl alcohol (mixture of cis and trans) afforded, after standing overnight at r.t., the ene adducts 2 and 3 (Scheme 2), which were isolated by chromatographic separation of the reaction mixture in 50% and 23% yield, respectively.

The structures of the isolated ene adducts were determined by the corresponding analytical and spectroscopic data. In the 1H NMR spectrum of the ene adduct 2, the typical olefinic methylene signals are found as doublets at δ = 5.08 and 5.23, while the olefinic methine proton is typically found more deshielded at δ = 5.95 as a multiplet. The N–OH group is found strongly deshielded as a broad signal at δ = 10.40. The other signals are found in the expected ranges for the reported structure; in particular, the OH group resonates at δ = 3.34 as a doublet, also shown in the IR spectrum at 3152 cm–1. Compound 2 is derived from the addition of (nitrosocarbonyl)mesitylene (1M) to the hydroxymethyl substituted carbon atom C2 of the double bond of crotyl alcohol. Structure 2 belongs to the family of compounds obtained in previous work1,9 from 3-methylbut-2-en-1-ol in full accordance with a Markovnikov orientation.8,10 In the present case of crotyl alcohol, it is somewhat inappropriate to consider the carbon atom C2 the site of Markovnikov addition (C2/M addition), because of the 1,2-disubstitution of the C=C bond of the alcohol; however, this is useful and done for the sake of comparison with previous results.1,9

If the addition of the electrophilic (nitrosocarbonyl)mesitylene (1M) occurs at the C3 carbon atom, the product of the ene reaction is represented by the 5-hydroxyisoxazolidine 3; the primary adduct is the enol 4 that evolves into the non-isolable aldehyde 5, which undergoes cyclization to the stable hemiacetal 3 (Scheme 3). This type of 5-hydroxyisoxazolidine is usually prepared by reaction of hydroxamic acids and α,β-unsaturated aldehydes11 or through the ene protocol of nitrosocarbonyls and allylic alcohols.1 As in the previous cases,1,9 the origin of isoxazolidine 3 was undoubtedly attributed to the diminishing of the C2/M addition due to the more sterically demanding mesityl group that activates the C3/AM pathway.8,10

The structure of 3 was assigned on the basis of the corresponding analytical and spectroscopic data. All the NMR spectra indicate the presence of an inseparable mixture of diastereoisomers in a 2:1 ratio 2:1 (Schemes 2 and 3). In the case of the major diastereoisomer, the 1H NMR (CDCl3) spectrum indicates the presence of a deshielded proton at δ = 8.59 as a triplet (J = 4 Hz) coupled with two protons at δ = 2.03 and 2.51. The methyl group is found at δ = 1.03 as a doublet (J = 6 Hz), because of the coupling with the methine proton at δ = 3.98 (q, J = 6 Hz). The OH group is confirmed by the corresponding IR spectrum (strong band at 3284 cm–1). The 13C NMR spectrum presents a signal at δ = 98.3 resembling an acetal-type carbon atom. The structure assignment as shown in Scheme 2 was confirmed beyond any reasonable doubt from the X-ray analysis (Figure 1, see also Supporting Information).

The 5-hydroxy-isoxazolidine 3 obtained from the ene reaction of (nitrosocarbonyl)mesitylene (1M) with crotyl alcohol was used as a synthon to prepare N,O-nucleoside analogues by adapting the Vorbrüggen protocol12 as well as

other procedures for similar compounds.5,13 The diastereoisomeric acetyl derivatives 6a, b were prepared according to standard procedures (Scheme 4).1,14 The acetylated compounds were obtained in nearly quantitative yield, separated by column chromatography and fully characterized. The acetylated compounds 6a and 6b are diastereoisomers, each one obtained as a racemic mixture of enantiomers, isolated in 45% and 47% yields, respectively. The structures reported in Scheme 4 were assigned on the basis of the corresponding analytical and spectroscopic data and the diastereoisomeric outcome was determined by NOESY experiments. In the 1H NMR spectra (CDCl3), the presence of the acetyl groups is shown by two singlets at δ = 2.06 for 6a and at δ = 1.90 for 6b, while in the corresponding 13C NMR spectra two additional signals corresponding to C=O groups were both detected at δ = 169.0. The NOESY experiments allowed for the attribution of the reported stereochemistry (NOE correlations in red in Scheme 4). In the stereoisomer 6a, the proton at δ = 6.33 correlates with one of the two methylene protons at δ = 2.25, while the other one at δ = 2.72 correlates with the CH–N proton found at δ = 4.65. In the stereoisomer 6b, the proton at δ = 6.18 correlates with one of the two methylene protons at δ = 2.22, which displays a second correlation with the CH–N proton found at δ = 4.62; this relationship is also confirmed by the correlation between the methyl group at δ = 1.43 with the methylene proton at δ = 2.20 (Scheme 4).

To prepare the uracil derivatives, the Vorbrüggen protocol can be applied both on a previously silylated heterobase or on a commercial compound in the presence of silylating agents.12,15 In the cases at hand, we report the syntheses conducted on the commercial uracil base used in the presence of an in situ prepared silylating agent, bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as reaction promoters.16

The acetylated isoxazolidines 6a and 6b were added under nitrogen atmosphere at r.t. to a solution of uracil (2 equiv) and BSA (2 equiv) and the solutions became clear after boiling in CH2Cl2 for a couple of hours. The mixtures were then ice-cooled at 0 °C and TMSOTf (1 equiv) was added and the reaction mixtures refluxed overnight (Scheme 5). The desired compounds 7a, b were obtained as white solids separated by column chromatography and fully characterized. Nucleoside analogues 7a, b were isolated in good yields (37–49%) and their structures determined by analytical and spectroscopic data. The reactions were performed on a single diastereoisomer of the starting compound and gave mixtures of both diastereoisomers of the products belonging to the family of uracil derivatives in a nearly 1:1 ratio. Table 1 reports the yields, physicochemical data and the relevant 1H NMR spectroscopic data supporting the structural assignments given in Scheme 5.

The 1H NMR spectra of uracil derivatives 7a, b were typical for this type of compound, with doublets (J = 8 Hz) at δ = 5.69 and 7.70 (7a) and δ = 5.63 and 7.65 (7b), clearly related...
Synthesis of uracil isoxazolidine nucleoside analogues by the Vorbrüggen protocol

Table 1 Yields and Physicochemical and Spectroscopic Data of Compounds 7a,b

<table>
<thead>
<tr>
<th></th>
<th>mp (°C)a</th>
<th>Yield from 6a (%)</th>
<th>Yield from 6b (%)</th>
<th>IR (cm⁻¹) νmax</th>
<th>νC=O</th>
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<tr>
<td>7a</td>
<td>&gt;210</td>
<td>49</td>
<td>46</td>
<td>3164</td>
<td>1724, 1669</td>
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<tr>
<td>7b</td>
<td>&gt;210</td>
<td>47</td>
<td>37</td>
<td>3173</td>
<td>1702, 1637</td>
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1H NMR (δ, DMSO)

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<th>CH₂</th>
<th>CH–N</th>
<th>=CH–CO</th>
<th>O–CH–Ur</th>
<th>N=CH=</th>
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<tbody>
<tr>
<td>7a</td>
<td>2.29, 2.93 (m)</td>
<td>4.67 (d)</td>
<td>5.69 (d)</td>
<td>6.19 (t)</td>
<td>7.70 (d)</td>
</tr>
<tr>
<td>7b</td>
<td>2.50, 2.95 (m)</td>
<td>4.84 (m)</td>
<td>5.63 (d)</td>
<td>6.27 (d)</td>
<td>7.65 (d)</td>
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</table>

* From ethanol.

Scheme 5 Synthesis of uracil isoxazolidine nucleoside analogues by the Vorbrüggen protocol

To expand the synthetic applications of the Vorbrüggen protocol on the acetylated isoxazolines 6a,b and determine the scope and limitations of the method,19 we investigated the functionalization with the commercially available 6-chloropurine, being aware of the possible increasing to the uracil double bond, while both imide NH groups appeared highly deshielded at δ = 11.33 as singlets. The other signals are consistent for the isoxazolidine structure. The configurations of the two diastereoisomers 7a and 7b were assigned on the basis of NOESY experiments and the correlations are reported in red in Scheme 5. Compound 7a shows the uracil ring cis-related to the methyl group of the isoxazolidine ring, while in compound 7b the uracil is found trans to the methyl group. It must further be pointed out that the two products exist as racemic mixtures of enantiomers. The most intriguing fact in the synthesis of these nucleoside analogues is that both the diastereoisomers are obtained from each starting acetyl derivative: 6a gives a mixture of 7a and 7b; the same happens if the reaction is conducted starting from 6b (see yellow inset in Scheme 5). These results furnished some insights on the operating mechanism of the Vorbrüggen protocol on substrates 6 and deserve a discussion (vide infra). For this reason, for preparative purposes, the reaction can be conducted simply on a diastereoisomeric mixture of 6a,b, and products 7a and 7b can be separated once the reaction ends.

The uracil nucleoside analogues 7a and 7b could be submitted for biological evaluation without any further structural modifications, either for their antiviral behavior or, in other cases, to compare their activities with those of reported carbocyclic and heterocyclic structures.5,13,17,18
complexity of the reaction mixture. In fact, it is well known that the purine rings could give regioisomeric adducts at the N7 and N9 nitrogen atoms; if this is transferred to a substrate existing as a mixture of diastereoisomers, the stereochemical outcome increases its complexity.

The acetylated isoxazolines 6a, b were added under a nitrogen atmosphere at r.t. to a solution of 6-chloropurine (2 equiv) and BSA (2 equiv) and the solutions became clear after boiling in CH₂Cl₂ for a couple of hours. The mixtures were then ice-cooled at 0 °C and TMSOTf (1 equiv) was added and the reaction mixtures were refluxed overnight. Scheme 6 reports on the structures of the four possible products.

On the basis of previous observations, the reaction was conducted on the mixture of the diastereoisomeric acetylated compounds 6a, b to give a mixture of four possible diastereoisomeric products where the purine ring can be linked at the isoxazolidine moiety through the N7 and/or N9 nitrogen atoms. Two compounds 8a, b were obtained as white solids separated by column chromatography and characterized. The purine nucleoside analogues 8a, b were isolated in moderate yields (33% and 40%, respectively) and their structures were determined with analytical and spectroscopic data through a careful and detailed analysis in particular of the NMR data. Table 2 reports the yields, physicochemical data and the relevant ¹H NMR spectroscopic data supporting the structural assignments given in Scheme 6.

As reported, quite surprisingly, only two compounds were detected and separated from the reaction mixture. Both contain the 6-chloropurine rings coupled with the isoxazolidine moiety. The major task was the stereochemical assignment, since spectroscopic data alone give only a few suggestions on the relationship between the methyl group of the isoxazolidine ring and the purine ring. A definitive answer came from the X-ray crystal structure analysis of compound 8a; the structure 8aN7 was assigned. Combining information from the diffractometric analysis and the chemical shift data from the NMR spectra, it was possible to define the stereochemical outcome of this reaction (Figure 2).

For compound 8b, only the spectroscopic data are available to determine the structure. NOESY experiments gave successful answers on the stereochemistry of 8b, which was confirmed to belong to the trans series of the purine adducts, i.e. the purine ring and the methyl group are trans-related on the isoxazolidine moiety, as shown in Scheme 6, where the NOE correlations are indicated by red arrows. In the NMR spectrum we have also detected a minor component relative to 8b, not separable from the major; the spectroscopic (NMR) data are reported in Table 2. The signals do not correspond to hindered rotations, since we have verified the matter through variable temperature experiments, and no signal coalescence occurred in the spectra recorded.

Table 2 Yields and Physicochemical and Spectroscopic Data of Compounds 8a, b

<table>
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<tr>
<th></th>
<th>mp (°C)</th>
<th>Yield from 6a, b (%)</th>
<th>IR (cm⁻¹) νC=O</th>
<th>νC=N</th>
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<tr>
<td>8aN7</td>
<td>177–178</td>
<td>33</td>
<td>1663</td>
<td>1635</td>
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<tr>
<td>8aN9</td>
<td>–</td>
<td>ND b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8bN7</td>
<td>&gt;210</td>
<td>40 (mix)</td>
<td>1663</td>
<td>1624</td>
</tr>
<tr>
<td>8bN9</td>
<td></td>
<td></td>
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</table>

¹H NMR (δ, DMSO)

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<tr>
<th></th>
<th>CH₃</th>
<th>CH₂</th>
<th>CH–N</th>
<th>O–CH–Pur</th>
<th>CH=N</th>
<th>CH=N²</th>
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<tbody>
<tr>
<td>8aN7</td>
<td>1.55 (d)</td>
<td>3.21, 3.00 (m)</td>
<td>4.75 (m)</td>
<td>6.62 (t)</td>
<td>9.22 (s)</td>
<td>8.83 (s)</td>
</tr>
<tr>
<td>8aN9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8bN7</td>
<td>1.51 (d)</td>
<td>2.75, 3.55 (m)</td>
<td>5.10 (quin)</td>
<td>6.65 (d)</td>
<td>8.90 (s)</td>
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<tr>
<td>8bN9</td>
<td>1.06 (d)</td>
<td>2.75, 3.68 (m)</td>
<td>4.15 (m)</td>
<td>6.88 (d)</td>
<td>8.96 (s)</td>
<td>8.89 (s)</td>
</tr>
</tbody>
</table>

া From ethanol.

b ND = not detected.

c Methine proton from imidazole ring of 6-chloropurine.

d Methine proton from pyrimidine ring of 6-chloropurine.

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in DMSO. Compound 8b is made up of an inseparable mixture of two products, and we have tentatively assigned the reported structures on the basis of NMR observations.

In Table 2 the NMR signals related to compound 8b were separately assigned to the regioisomers 8bN7 (major) and 8bN9 (minor). This was done after comparison of the chemical shifts of the representative protons. These values for the 8bN7 regioisomer are more deshielded than those of the 8bN9 regioisomer, corresponding well with the observed values of solely compound 8aN7. Due to the lack of pure compounds available, these products were not used for biological evaluation.

We investigated the ene reactions of (nitrosocarbonyl)mesitylene, generated through the mild oxidative protocol with NMO, with crotyl alcohol, a 1,2-disubstituted allylic alcohol. The fast oxidation process of nitrile oxide to the nitrosocarbonyl intermediate prevents a possible side reaction, i.e. the addition of the electrophilic nitrile oxide to the reactive 1,2-disubstituted allylic alcohols. In fact, the alcohol employed is prone to undergo 1,3-dipolar cycloadDITION, being a slightly reactive dipolarophile. From the reaction mixtures, the presence of adducts between the aromatic nitrile oxides and crotyl alcohol were not observed in isolable amounts, even in the crude mixtures.

(Nitrosocarbonyl)mesitylene (1M) adds to crotyl alcohol affording adducts 2 and 3, which derive, respectively, from the preferred C2/M path or the competitive C3/AM route in the ene reaction (Scheme 7).1,8,9 The results are discussed in terms of two grounds: (a) polarization of the C=C bond and (b) steric effects.

In crotyl alcohol, the methyl and the hydroxymethylene groups are the substituents of the C=C bond and both concur to establish the polarization towards the C2 carbon atom as shown in Scheme 7.7 The methyl group (C4) is a modest donor, while hydroxymethylene (C1) is slightly electron withdrawing. As a consequence, the electron-rich C2 carbon atom is apt to receive the addition of the electrophilic nitrosocarbonyl intermediate. The subsequent abstraction of the allylic hydrogen on the methyl group leads to the Markovnikov (M) preference (adduct 2) of the ene reaction in accordance with the prevailing HOMO(olefin)−LUMO(nitrosocarbonyl) interaction. However, the reaction conducted with the sterically demanding (nitrosocarbonyl)me-
addends while in the TS of 1M the mesityl group is twisted out of the nitrosocarbonyl plane as usual, causing unfavorable steric crowding between its ortho methyl and the `trans' distal TME methyl as depicted in Figure 3. Moving to the case of crotyl alcohol, the evaluation of the steric effect is depicted in Figure 4. Taking into account that crotyl alcohol was used as a mixture of the cis and trans arrangements of the crotyl alcohol, the distorted mesitylene group of the enophile points the ortho methyl towards a simple hydrogen atom. Nevertheless, the crotyl alcohol contributes, although quantitatively less, in orienting the reaction towards the AM path significantly, despite the fact that the majority of the ene products result from the M route. The ene addition according to the C3/AM pathway is not equal to zero and the obtained results suggest the intrinsic relevance of the AM path. It results in enol formation and, although unstable, this intermediate immediately undergoes subsequent cyclization to the highly stable isoxazolidine structure. This can be the unexpected and powerful driving force able to somewhat compensate the electronic bias of the ene reaction and the weakness of steric effects.

5-Hydroxisoxazolidine 3, resembling structures obtainable through nitrene cycloadditions, is a useful synthon for the preparation of N,O-nucleoside analogues. By adapting the Vorbrüggen protocol12,19 as well as known procedures for similar compounds,5,13 we have prepared the uracil derivatives 7a,b and the 6-chloropurine derivatives 8a,b in good to excellent yields, even though in this last case the synthesis displays some deficiencies.

The stereochemical outcome of the functionalization reactions with in situ silylated heterobases according to the Vorbrüggen protocol clearly demonstrates that the triflate-promoted replacement of the acetoxy group with the heterobase in isoxazolidine 6 proceeds through the stabilized cation 6′ as shown in Scheme 9. Due to the flatness of cat-
ion \(6^\prime\), the addition of the silylated heterobase occurs on both faces of the isoxazolidine ring, producing mixtures of diastereoisomeric products. These products constitute a new class of nucleoside analogues with different heterobases, easily inserted on the isoxazolidine ring. Their potential in terms of biological activity is totally unexplored, but we believe could be promising. If we compare the new nucleoside structures with those of known compounds, both hetero- and carbocyclic, recently reported in the literature to be interesting adenosine receptor agonists.²¹

With the exception of compounds \(8a,b\), both the uracil derivatives \(7a,b\) were sent to the Southern Research Institute (SRI) of Birmingham (AL, USA) within the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAAF) program and tested against the Mycobacterium tuberculosis H\(37Rv\) strain in BACTEC 12B medium using the microplate Alamar Blue Assay.²² The minimum inhibition concentration (MIC \(\mu\)g/mL) were found >6.03 for all the compounds with \%Inh in the range 22–34. Further antiviral, antitumor, and apoptosis tests are currently under evaluation.

All melting points are uncorrected. Elemental analyses were done on an elemental analyzer. \(^1\)H and \(^13\)C NMR spectra were recorded on a 300 MHz spectrometer (solvents specified). Chemical shifts are expressed in ppm relative to internal tetramethylsilane. IR spectra (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; relative retention factors \(R_f\) (Nujol) were measured. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; \(R_f\) values are reported in brackets. (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; IR spectra (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; IR spectra (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; IR spectra (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H\(60\) and GF\(254\), respectively; IR spectra (Nujol) were recorded on a spectrophotometer.

**Benzamidine 2 and 1,2-Isoxazolidine 3 by Ene Reaction of (Nitroso-carbonyl)mesitylene (1M) with Crotly Alcohol**

NMO (8.75 g, 1.5 equiv) was added to a stirred, ice-cooled solution of crotly alcohol (21 mL, 5 equiv) in CH\(_2\)Cl\(_2\) (200 mL). A solution of mesitonitrile oxide (MNO; 8.01 g, 50 mmol) in CH\(_2\)Cl\(_2\) (200 mL) was added dropwise and the reaction mixture was allowed to stir at r.t. for 24 h. After dilution with an equivalent volume of CH\(_2\)Cl\(_2\), the organic phase was washed with H\(_2\)O and dried over anhydrous Na\(_2\)SO\(_4\). After filtration, the solvent was evaporated and the reaction mixture was separated by column chromatography; affording the ene adducts \(2\) and \(3\), the latter as an inseparable mixture of diastereoisomers in the ratio 2:1. In the NMR data of \(3\) the signals of the minor diastereoisomer are reported in brackets.

**N-Hydroxy-N-(1-hydroxybut-3-en-2-yl)-2,4,6-trimethylbenzamide (2)**

Yield: 6.2 g (50%); white crystals (EtOH); mp 137–140 °C.

IR (Nujol): 3152 (OH), 1619 (C=O) cm\(^{-1}\).

**5-Hydroxy-2-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoxazolidine (3)**

Yield: 2.77 g (23%); white crystals (EtOH); mp 134–137 °C.

IR (Nujol): 1758, 1634 (C=O) cm\(^{-1}\).

**5-Acetoxy-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoxazolidine (6a,b)**

Ac\(_2\)O (2.2 equiv), DMAP (0.34 equiv), and Et\(_3\)N (2.2 equiv) were added to a stirred, ice-cooled solution of \(3\) (1.26 g, 5.06 mmol) in anhydrous CH\(_2\)Cl\(_2\) (150 mL). The reaction mixture was allowed to stir at r.t. for 24 h. After dilution with an equivalent volume of CH\(_2\)Cl\(_2\), the organic phase was washed with a saturated solution of Na\(_2\)CO\(_3\) and dried over anhydrous Na\(_2\)SO\(_4\). After filtration, the solvent was evaporated and an oily residue was obtained, corresponding to a mixture of the two diastereoisomers \(6a,b\). The acetyl derivatives \(6a\) and \(6b\) were then separated by column chromatography and fully characterized.

**1,2-Isoxazolidine 6a**

Yield: 0.57 g (45%); white crystals (EtOH); mp 92–94 °C.

IR (Nujol): 3284 (OH), 1639 (C=O) cm\(^{-1}\).

**1,2-Isoxazolidine 6b**

Yield: 0.59 g (47%); yellowish oil.

IR (Nujol): 1760, 1669 (C=O) cm\(^{-1}\).
5-[2,4(1H,3H)-Dioxopirrimidin-1-yl]-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoazolidine (7a,b)

A solution of uracil (0.20 g, 1.78 mmol, 2 equiv) and BSA (2 equiv) in benzoyl)-1,2-isoxazolidine (7a,b)

Yield: 0.15 g (49% from 6a); white solid (EtOH); mp >210 °C (dec.).

IR (Nujol): 3173 (NH), 1702, 1637 (C=O) cm–1.


Nucleoside 7b

Yield: 0.144 g (47% from 6a), 0.11 g (37% from 6b); white solid (EtOH); mp > 210 °C (dec.).

IR (Nujol): 3173 (NH), 1702, 1637 (C=O) cm–1.

1H NMR (300 MHz, CDCl3): δ = 6.74 (s, 2 H, Ph), 6.18 (d, J = 5 Hz, 1 H, O-CH-O), 4.62 (quin, J = 6 Hz, 1 H, N-CH), 2.53 and 2.19 (m, 1 H + 1 H, CH2), 2.22 and 2.20 (s, 3 H + 6 H, CH3), 1.90 (s, 3 H, CH3CO), 1.43 (d, J = 6 Hz, 3 H, CH2).

13C NMR (75 MHz, CDCl3): δ = 169.0 (C=O), 168.8 (C=O), 138.4, 134.4, 133.6, 132.1, 127.9 (Arom.), 95.6 (CH-O), 50.2 (CH-N), 42.1 (CH3). 21.1, 21.0, 20.8, 19.3, 18.9 (CH3).


5-(6-Chloro-7H-purin-7-yl)-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoazolidine (8a,b)

A solution of 6-chloropurine (0.32 g, 2.06 mmol, 2 equiv) and BSA (2 equiv) in anhydrous CH2Cl2 (20 mL) was refluxed under N2 for 15–20 min until it became clear. It was then cooled to r.t. before a solution of isoxazolidines 6a or 6b (0.26 g, 0.89 mmol) in CH2Cl2 (10 mL) was added dropwise. The mixture was cooled to 0 °C and TMSOTf (0.16 mL, 1 equiv) was added. The reaction mixture was refluxed under stirring overnight and finally quenched with a saturated solution of NaHCO3 (pH 7). The mixture was diluted with an equivalent volume of CH2Cl2 and washed with H2O, before it was finally dried over Na2SO4. From the residue, nucleosides 7a and 7b were isolated after column chromatography (CHCl3 and CHCl3/MeOH) and fully characterized.

Nucleoside 8a

Yield: 0.13 g (33% from 6a,b); white solid (EtOH); mp 177–178 °C.

IR (Nujol): 1663 (C=O), 1635 (C=N) cm–1.


Nucleoside 8b

Yield: 0.16 g (40% from 6a,b); white solid (EtOH); mp >210 °C (dec.).

IR (Nujol): 1663 (C=O), 1624 (C=N) cm–1.

1H NMR (300 MHz, DMSO): δ = 9.22 (s, 1 H, CH=N), 8.83 (s, 1 H, CH=), 6.86 (s, 1 H, Ph), 6.70 (s, 1 H, Ph), 6.62 (t, J = 6 Hz, O-CH-N), 4.75 (m, 1 H, CH-N), 3.21 and 3.00 (m, 1 H + 1 H, CH2), 2.11 and 1.94 (s, 6 H + 3 H, CH3), 1.53 (d, J = 6 Hz, 3 H, CH2).

13C NMR (75 MHz, DMSO): δ = 162.0 (C=O), 152.3, 148.1 (C=N), 142.6, 137.7, 133.4, 133.2, 127.6, 127.5 (Arom.), 84.8 (CH-N), 52.6 (CH3), 20.6, 19.4, 19.1, 18.3 (CH3).


Biological Tests

Primary screen (dose response): determination of a 90% inhibitory concentration (IC90). The initial screen was conducted against Mycobacterium tuberculosis H37Rv (ATCC 27294) in BACTEC 12B medium by using the Microplate Alamar Blue Assay (MABA). The compounds were tested in ten twofold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The IC90 value of 0.19 μg/mL is considered ‘active’ for antitubercular activity. For further information, consult the URL http://www.taaaf.org.

X-ray Crystallography

A summary of the crystal data, data collection, and structures refinement of compounds 3 and 8aN7 are given in Tables S1–S3 of the Supporting Information. The structures are solved by direct methods; non-hydrogen atoms were refined anisotropically, and hydrogen atoms, located from the difference Fourier synthesis, were refined isotropically. 1980 CCDC 964611 and 964612 contain the supplementary.


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crystallographic data for 3 and 8aN7, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Supporting Information

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