From the Ene Reaction of Nitrosocarbonyl Intermediates with 3-Methylbut-2-en-1-ol, a New Class of Purine N,O-Nucleoside Analogues

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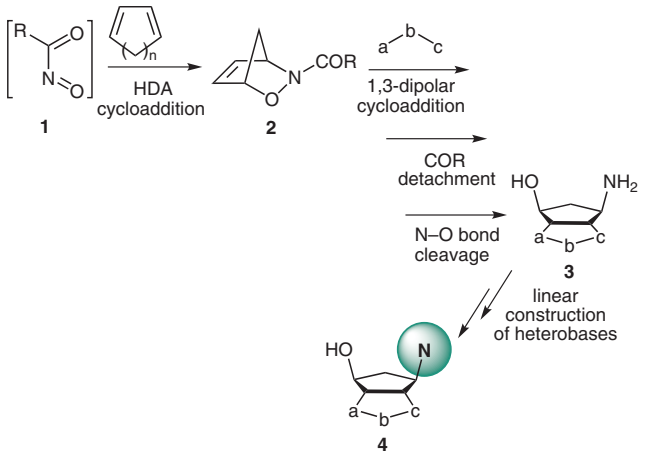
Abstract: Sterically encumbered nitrosocarbonyl derivatives of mesitylene or anthracene undergo ene reactions with 3-methylbut-2-en-1-ol to give the corresponding the 5-hydroxyisoxazolidine adducts in fair yields. According to the proposed mechanism, these heterocycles are derived from the anti-Markovnikov orientation of the ene reaction. The isoxazolidin-5-yl acetate derivatives are synthons of choice for the preparation of N,O-nucleoside analogues containing purine rings by means of the Vorbrüggen protocol.

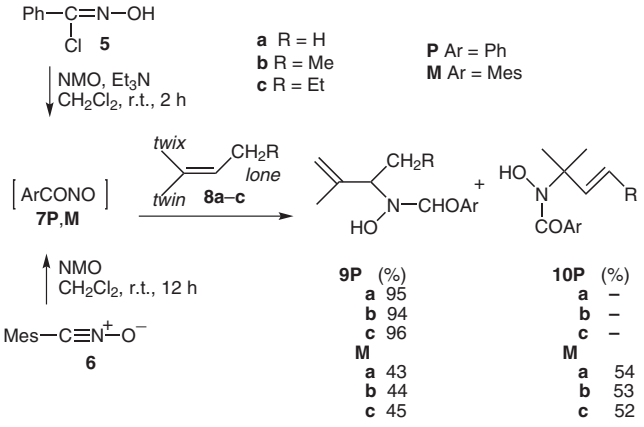
Key Words: nucleosides, ene reactions, heterocycles, cyclization

The synthesis of carbocyclic nucleosides[1] through the chemistry of nitrosocarbonyl intermediates has been extensively investigated by many research groups.[2] The generation of nitrosocarbonyl intermediates **1** by mild oxidation of nitrile oxides with tertiary amine *N*-oxides offers an efficient alternative route to hetero-Diels–Alder cycloadducts **2** with various substituents (Scheme 1).[3] Cycloadducts **2** are easily converted into 1,3-aminols **3**, which are useful synthons for the linear construction of purine and pyrimidine nucleosides analogues **4**.[4] Some of these nucleosides have been found to show moderate activity against human herpes viruses (types 1 and 2) and varicella viruses.[5] Recently, anthryl derivatives have been shown to be active against the human papilloma virus, with no cellular toxicity at the doses tested.[6]



Scheme 1

Applications of nitrosocarbonyl compounds in syntheses of biologically active molecules are well known.[7] Although nitrocarbonyl intermediates are powerful enophiles,[8] no applications of their ene reactions in syntheses of nucleosides have been reported. We found that nitrosocarbonyl intermediates undergo clean ene reactions with trisubstituted olefins **8**. Allylic hydrogens on the more-congested side of the alkene are abstracted exclusively (the *cis*-effect). The ene reaction of *N*-oxobenzamide (**7P**; Ar = Ph) proceeds with a Markovnikov orientation, whereas with the more-sterically encumbered 2,4,6-trimethyl-*N*-oxobenzamide (**7M**; Ar = Mes), the Markovnikov directing effect is eliminated and comparable *twix* and *lone* abstractions are observed (Scheme 2).[9]



Scheme 2

In pursuing our researches on ene reactions, we extended our studies to other trisubstituted olefins, particularly allylic alcohols **8** (R = OH). We selected 3-methylbut-2-en-1-ol (**8d**) as a model compound for our investigations of the selectivity of the outcome of the ene reactions of nitrosocarbonyl compounds bearing sterically demanding substituents. Unexpectedly, we obtained the isoxazolidines **11**, derived from anti-Markovnikov addition to the alcohol **8**. Isoxazolidines **11** are valuable synthons in the preparation of new N,O-nucleoside pyrimidine analogues **12** through the Vorbrüggen protocol (Scheme 3).[10]

To explore the reactivity of isoxazolidines **11**, we extended the synthesis to some representative purine-type heterobases. 2,4,6-Trimethylbenzonitrile oxide (**6**) was added to a stirred solution of *N*-methylmorpholine *N*-oxide (1.1 equiv) in dichloromethane in the presence of an

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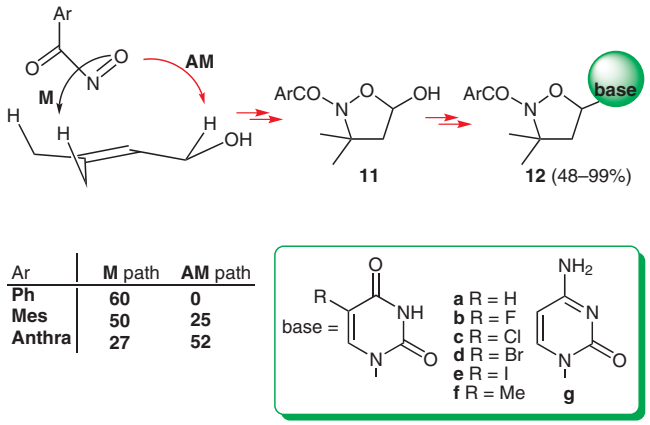
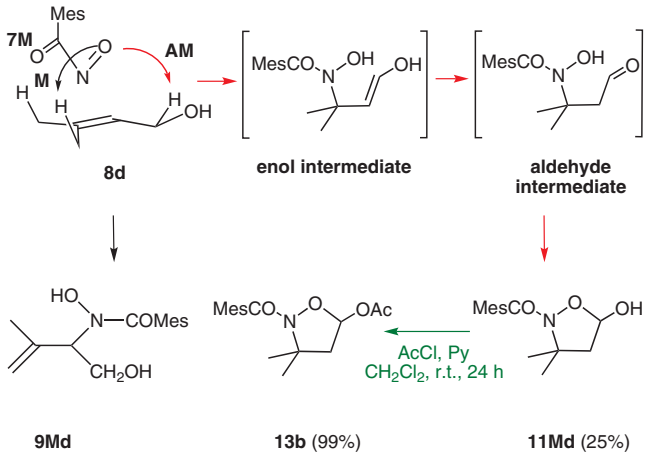


Scheme 3

excess (5 equiv) of 3-methylbut-2-en-1-ol (**8d**) at room temperature. After stirring the mixture overnight and column chromatographic separation, we isolated the ene adducts **9Md** and **11Md** in 50% and 25% yield, respectively.[10] Adduct **9Md** is formed by addition of the nitrosocarbonyl **7M** at the less-substituted carbon atom of the allylic alcohol **8d** by the Markovnikov pathway,



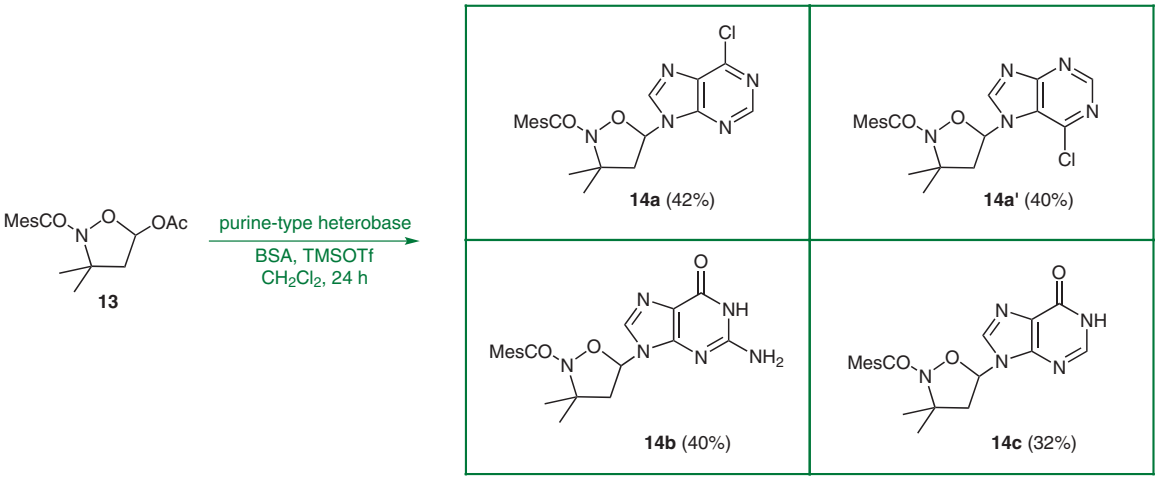
Scheme 4 (M = Markovnikov path, AM = anti-Markovnikov path)

whereas the anti-Markovnikov route gives an enol intermediate that readily converts into the corresponding aldehyde; this, in turn, cyclizes to give the required isoxazolidine **11Md** (Scheme 4).

The acetyl derivative **13**, when prepared by standard procedures[11] in nearly quantitative yield, was found to be identical to an authentic sample.[10] The Vorbrüggen protocol can be applied to silylated heterobases or to commercially available heterobases in the presence of a silylating agent.[12] We synthesized purine derivatives from commercially available heterobases in the presence of trimethylsilyl *N*-(trimethylsilyl)ethanecarboximidate (BSA) as a silylating agent and trimethylsilyl triflate as a promoter.[13] The acetylated isoxazolidine **13** was added under nitrogen at room temperature to a solution of the selected heterobase (2 equiv) and BSA (2 equiv) in dichloromethane, and the solution became clear after boiling for two hours. The mixture was then cooled to 0 °C on ice, trimethylsilyl triflate was added, and the mixture was refluxed overnight (Scheme 5). Purification by column chromatography gave the desired compound **14a–c** as white solids.

The nucleoside analogues **14a–c** were isolated as racemic mixtures in fair yields (32–42%), and their structures were confirmed by analysis and spectroscopic studies. Each of the reactions gave a single product, except in the case of 6-chloropurine, which gave an approximately 1:1 mixture of the isomeric products **14a** and **14a′** through coupling at either the N7 or the N9 nitrogen atom of the purine ring. This behavior of the 6-chloropurine is frequently displayed in coupling reactions that involve the nucleophilic properties of this heterocyclic ring.[14]

In the ^{1}H NMR spectra of nucleosides **14a–c**, signals corresponding to the *N*-(mesitylcarbonyl)isoxazolidine moiety were easily detectable and they were found in the expected ranges, as previously described.[10] Substitution of the acetate group with a heterocyclic ring in the 5-position showed a smooth effect on the corresponding chemical shifts. The new representative signals correspond to the purine moieties. The results obtained with 6-chloropu-



Scheme 5

rine deserve special comment, because two isomeric products, **14a** and **14a′**, were obtained in which the purine ring is linked to the isoxazolidine unit at the N7 or N9 atom, respectively. The ^{1}H NMR spectra confirmed the presence of the 6-chloropurine ring in both products. In **14a**, signals for the N=CH singlets of the purine ring occur at δ = 8.60 and 8.73, whereas in **14a′**, the same signals occur at δ = 8.78 and 8.91, respectively. However, the spectroscopic pattern is very similar for the two regioisomers, and ^{1}H–^{13}C long-range correlation NMR experiments were unable to distinguish between the two isomers.[14] A definitive characterization was achieved by means of X-ray analysis of isomer **14a**, in which the purine ring is linked to the isoxazolidine moiety through the N7 atom (Figure 1; for full details, see the Supporting Information). As a consequence, the structure of **14a′** was defined as shown in Scheme 5.

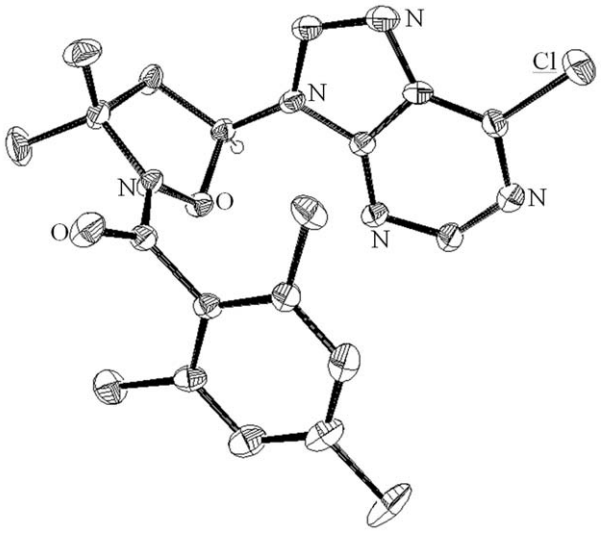


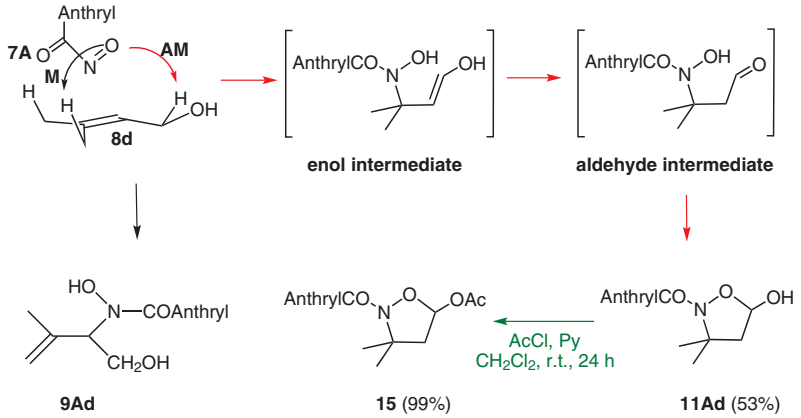
Figure 1 ORTEP plot of the compound **14a** with atom labeling (ellipsoids at 25% probability). Hydrogen atoms are omitted for clarity.

The guanine nucleoside **14b** is characterized by a single proton attributable to the guanine ring at δ = 7.88, whereas that of the NH_2 group is found at δ = 6.47 and the NH group is highly deshielded at δ = 10.25. In the hypoxanthine adduct **14c**, the heterobase CH protons appear as singlets at δ = 8.05 and 8.28, whereas the NH appears as a broad singlet at δ = 12.42.

Products containing fluorescent chromophores can be useful as chemical probes for various biological targets, and the field of imaging has received a great deal of attention in relation to its applications in achieving a better understanding of *in vivo* mechanisms.[15] As a result, new fluorophores and their applications are constantly being developed.[16] Moreover, fluorescent polyaromatic groups can be active through their ability to establish π–π stacking interactions with themselves[17] or with DNA intercalators;[18] these mechanisms occur in various cases, depending on the biological target.[15b,19]

We have previously reported an ene reaction of another typical stable nitrile oxide,[20] anthracene-9-carbonitrile oxide, prepared from the anthracene-9-carbaldehyde oxime by the procedure reported in literature.[21] The solid nitrile oxide was added in a portionwise manner to a stirred solution of allylic alcohol **8d** (5 equiv) in dichloromethane containing *N*-methylmorpholine *N*-oxide (2 equiv), and the mixture was stirred for 48 hours at room temperature. The two expected ene products **9Ad** and **11Ad** (Ar = 9-anthryl) were isolated in 28% and 53% yield, respectively (Scheme 6). Compound **9Ad** is formed by addition of the nitrosocarbonyl **7A** through the Markovnikov pathway, whereas the isoxazolidine **11Ad** is derived from the preferred anti-Markovnikov pathway. The synthesis was optimized, and crystal structures of both the ene adducts were obtained. The structure of the isoxazolidine **11Ad** has already been reported,[10] and X-ray analysis of ene compound **9Ad** confirmed the spectroscopic structural assignments (for the ORTEP view, see the Supporting Information).

The acetyl derivative **15** was prepared according to standard procedures[11] in nearly quantitative yield, and it was found to be identical to an authentic sample previously prepared.[10] The compound was subjected to a Vorbrüggen-type reaction with adenine by means of the protocol described above. When acetylated isoxazolidine **15** was add-



Scheme 6

ed to a solution of adenine (2 equiv) and BSA (2 equiv) in dichloromethane under nitrogen at room temperature, the solution became clear after boiling for two hours. The mixture was then cooled to 0 °C on ice, trimethylsilyl triflate was added, and the mixture was refluxed overnight (Scheme 7). Simple column chromatographic purification gave the desired compound **16** as a white solid in 36% yield.

Scheme 7

Adduct **16** is a high-melting compound (mp > 205 °C) and its IR spectrum showed the presence of an NH_2 group (ν_{NH2} = 3375, 3321 cm^{-1}). Its ^1H NMR spectrum confirmed the presence of the adenine ring, the N=CH singlets of the purine ring being found at δ = 8.22 and 8.46.

These results show that isoxazolidine substrates, properly derivatized as their acetates, can be used in syntheses of a variety of N,O-nucleoside analogues. The novel isoxazolidine synthons can be easily prepared through ene reactions of nitrosocarbonyl intermediates bearing sterically encumbered aromatic substituents that drive ene additions to allylic alcohols along the anti-Markovnikov path (Figure 2). Because the addition of the nitrosocarbonyl moiety occurs on the more-substituted carbon atom of the allylic alcohol **8d**, the primary adducts are enolic forms that evolve into a nonisolable aldehydes that, in turn, cyclize to form the corresponding hemiacetals **11** (Scheme 4 and 6) as final and isolable compounds.

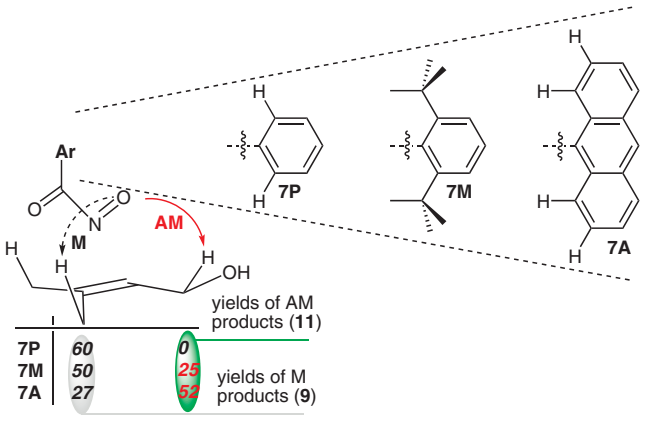


Figure 2

We have recently reported that crowding around the nitroso group slows the Markovnikov approach of 2,4,6-trimethylbenzonitrile oxide (**7M**) to a trisubstituted ethene and compensates somewhat for the Markovnikov electronic bias. Figure 3 shows the same features in the case of the allylic alcohol **8d**. From the point of view of the Markovnikov approach, it seems quite clear that the hydroxy group does not play any role in directing the addition of the nitrosocarbonyl intermediates in the transition state (M path, top view). The steric clashes that operate in the Markovnikov case shift the addition to the anti-Markovnikov path that leads to enol formation and subsequent cyclization to an isoxazolidine structure.[10]

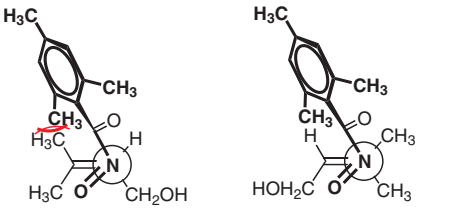


Figure 3 (M = Markovnikov, AM = anti-Markovnikov)

The structures of the 5-hydroxyisozaxolidines **11M** and **11Ad** resemble those that are obtained through addition of nitrones to vinyl ether derivatives, and they constitute useful synthons for the preparation of N,O-nucleoside analogues. By adapting the Vorbrüggen protocol,[22] as well as other known procedures for similar compounds,[23] we have reported syntheses of a selection of uracil derivatives, which we obtained in good-to-excellent yields.[10] However, the yields of the purine adducts were somewhat lower than expected on the basis of previous observations, and were generally below 50%. In view of planned future applications of this synthetic method, it is important to elucidate the limitations of the protocol beyond the scope of the current research.

One possible explanation of the low yields that were obtained when purine heterobases were used comes from the proposed mechanism for the Vorbrüggen-type coupling of these heterocycles with the isoxazolidine intermediate in comparison to the case when uracil is used. As soon as uracil is silylated by the BSA, the triflate anion triggers nucleophilic attack on the stabilized isoxazolidine cation generated from compounds **11** by loss of acetate, which is a good leaving group. In the case of 6-chloropurine, BSA silylation is somewhat less efficient and more complex, because many silylated species can be present in the reaction mixture.

The coupling reaction might be more difficult when other functional groups are present on the purine rings, as in the cases of guanine and adenine. An amino group can be easily involved in other tautomeric equilibria or silylation processes. Aware of these facts, we tried to modify our coupling protocol by increasing or decreasing the proportions of BSA or the triflate, but these experiments proved unsuccessful. Changing the reaction temperature or time did not greatly affect the reaction yields, and the reported conditions are, indeed, optimal for the Vorbrüggen glycosidation reactions of our isoxazolidine derivatives.

Future developments in synthetic methods for the preparation of N,O-nucleoside analogues are currently being pursued by varying both the allylic alcohol substrate and the substituents on the nitrosocarbonyl compound to permit the introduction of other functionalities onto the isoxazolidine ring.

The Vorbrüggen protocol has been shown to provide a robust method for the attachment of heterobases to activated isoxazolidine substrates. We believe that its application, with minor experimental adjustments, might be successful with other heterocyclic rings in various specific biological targets.

We are currently planning biological assays to test the inhibitory activity of our products against some viruses and to elucidate the apoptotic behavior of our N,O-nucleoside analogues. On request, the purine derivatives **14a**–**c** and **16** were sent to the Southern Research Institute (Birmingham, AL) as part of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility program and they were tested against the *Mycobacterium tuberculosis H37Rv* in BACTEC 12B medium by using the microplate Alamar Blue assay.[24] The minimum inhibition concentrations were found greater than 6.25 μg/mL for all the compounds, with inhibition indices (%Inh) in the range 22–25.

In conclusion, we have shown that sterically encumbered nitrosocarbonyl derivatives of mesitylene and anthracene undergo ene reactions with 3-methylbut-2-en-1-ol to give the corresponding 5-hydroxyisoxazolidine adducts in fair yields. These heterocycles derive from the anti-Markovnikov orientation of the ene reaction, according to the proposed mechanism. The isoxazolidin-5-yl acetates are synthons of choice for the preparation, by means of the Vorbrüggen protocol, of N,O-nucleoside analogues containing purine rings.

All melting points are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer, available in our department. IR spectra (Nujol mulls for solid compounds, neat for oils) were recorded on a PerkinElmer RX-1 FTIR instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 spectrometer in the specified deuterated solvents. Chemical shifts are expressed in ppm (δ) relative to TMS as internal standard. Column chromatography and TLC were performed on silica gel 60 (0.063–0.200 mm) (Merck) with cyclohexane–EtOAc (9:1 to 5:5) as the eluent. Medium-pressure LC was performed on a Biotage FMP apparatus equipped with KP-SIL columns; the eluent was cyclohexane–EtOAc (9:1 to 7:3). The identity of samples from different experiments was confirmed by mixed-mp studies and by superimposition of IR spectra. X-ray crystallographic analysis was conducted at the Centro Grandi Strumenti of the University of Pavia by using an Enraf Nonius CAD4 diffractometer with graphite-monochromated Mo Kα radiation (see Supporting Information for details).

3-Methylbut-2-en-1-ol (**8d**, 99%) was purchased from chemical suppliers. 2,4,6-Trimethylbenzonitrile oxide (**6**) was prepared by oxidation of 2,4,6-trimethylbenzaldoxime with bromine.[22] Anthracene-9-carbaldehyde oxime was purchased from chemical suppliers.

Ene Reactions of Nitrosocarbonyls 7M and 7A with 3-Methylbut-2-en-1-ol (8d); General Procedure

NMO (1.8 g, 1.3 equiv) was added an ice-cooled stirred soln of enol **8d** (99%; 5 equiv) in CH_2Cl_2 (200 mL). A soln of 2,4,6-trimethylbenzonitrile oxide (2.0 g, 12.4 mmol) or anthracene-9-carbonitrile oxide (2.0 g, 9 mmol) in CH_2Cl_2 (100 mL) was added dropwise, and the mixture was stirred at r.t. for 24 h. After dilution with an equal volume of CH_2Cl_2, the organic phase was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography to give the corresponding ene adduct.[10]

2-Acyl-3,3-dimethyl-1,2-isoxazolidin-5-yl Acetates 13 and 15; General Procedure

AcCl (2.2 equiv), DMAP (0.34 equiv), and Et_3N (2.2 equiv) were added to an ice-cooled stirred soln of isoxazolidinol **11** (4.78 mmol) in anhyd CH_2Cl_2 (50 mL), and the mixture was stirred at r.t. for 24 h. The mixture was then diluted with CH_2Cl_2 (50 mL), and the organic phase was washed with sat. aq $NaHCO_3$ (25 mL), dried (Na_2SO_4), and concentrated to give a solid product.[10]

Nucleoside Derivatives 14; General Procedure

A soln of the appropriate heterobase (2 equiv) and TMS-N=C(Me)OTMS (2 equiv) in anhyd CH_2Cl_2 (50 mL) was refluxed under N_2 for 15–20 min until it became clear. It was then cooled to r.t. and a soln of isoxazolidine **13** (0.20 g, 0.65 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The mixture was cooled to 0 °C and TMSOTf (0.12 mL, 1 equiv) was added. The resulting mixture was refluxed and stirred overnight, and then the reaction was quenched with sat. aq $NaHCO_3$ (pH 7). The mixture was diluted with an equal volume of CH_2Cl_2, washed with H_2O (2 × 20 mL), dried (Na_2SO_4), filtered, and concentrated to give a residue that was purified by column chromatography.

6-Chloro-9-[2-(mesitylcarbonyl)-3,3-dimethylisoxazolidin-5-yl]-9*H*-purine (14a)

Pale-yellow solid; yield: 0.11 g (42%); mp 192–194 °C (EtOAc).

IR (Nujol): 1630, 1589, 1537, 1210, 1078, 979, 722 cm^{-1}.

^{1}H NMR (300 MHz, acetone-d_6): δ = 1.79 and 1.92 (s, 6 H, $2CH_3$), 1.99, 2.17, and 2.26 (s, 9 H, $3CH_3$–Ar), 3.17 (dd, J = 14 and 8 Hz, 1 H, CH_2), 3.52 (dd, J = 14 and 5 Hz, 1 H, CH_2), 6.65 (s, 1 H, Ph), 6.70 (dd, J = 7 and 4 Hz, 1 H, CH), 6.78 (s, 1 H, Ph), 8.60 and 8.73 (s, 2 H, CH purine ring).

^{13}C NMR (75 MHz, acetone-d_6): δ = 19.1, 19.5, 21.4, 25.8, 26.8, 48.5, 64.2, 83.6, 121.3, 128.8, 128.9, 133.3, 134.1, 134.9, 135.3, 139.0, 151.6, 153.1, 168.2.

Anal. Calcd for $C_{20}H_{22}ClN_5O_2$ (399.87): C, 60.07; H, 5.55; N, 17.51. Found: C, 59.89; H, 5.51; N, 17.49.

6-Chloro-7-[2-(mesitylcarbonyl)-3,3-dimethylisoxazolidin-5-yl]-7*H*-purine (14a′)

Pale-yellow solid; yield: 0.10 g (40%); mp 217–219 °C (EtOAc–EtOH).

IR (Nujol): 1631, 1588, 1537, 1211, 1079, 979, 722 cm^{-1}.

^{1}H NMR (300 MHz, acetone-d_6): δ = 1.62 and 1.78 (s, 6 H, $2CH_3$), 1.96, 2.18, and 2.26 (s, 9 H, $3CH_3$–Ar), 3.24 (dd, J = 14 and 7 Hz, 1 H, CH_2), 3.45 (dd, J = 14 and 4 Hz, 1 H, CH_2), 6.44 and 6.79 (s, 2 H, Ph), 6.90 (dd, J = 7 and 4 Hz, 1 H, CH), 8.78 and 8.91 (s, 2 H, CH purine ring).

^{13}C NMR (75 MHz, acetone-d_6): δ = 18.6, 19.5, 21.4, 26.6, 26.9, 48.5, 63.6, 85.2, 122.5, 128.4, 128.8, 133.7, 134.9, 139.0, 148.0, 153.4, 160.1, 164.2.

Anal. Calcd for $C_{20}H_{22}ClN_5O_2$ (399.87): C, 60.07; H, 5.55; N, 17.51. Found: C, 60.10; H, 5.51; N, 17.49.

2-Amino-9-[2-(mesitylcarbonyl)-3,3-dimethylisoxazolidin-5-yl]-1,9-dihydro-6*H*-purin-6-one (14b)

Pale-yellow solid; yield: 0.10 g (40%); mp 213–217 °C (EtOAc).

IR (Nujol): 3313, 3150, 1691, 1626, 1529, 1223, 1094, 973, 778 cm^{-1}.

^1H NMR (300 MHz, DMSO): δ = 1.63 and 1.86 (s, 6 H, $2CH_3$), 1.93 and 2.16 (s, 9 H, $3CH_3$-Ar), 2.89 and 3.08 (dd, *J* = 14 and 6 Hz, 2 H, CH_2), 6.20 (t, *J* = 6 Hz, 1 H, CH), 6.47 (br s, 2 H, exch. D_2O), NH_2], 6.70 and 6.78 (s, 2 H, Ph), 7.88 (s, 1 H, CH), 10.25 (br s, 1 H, NH, exch. D_2O).

^{13}C NMR (75 MHz, DMSO): δ = 18.0, 18.5, 20.6, 24.9, 25.8, 46.3, 62.7, 80.3, 116.8, 127.5, 132.7, 133.0, 133.8, 135.1, 137.3, 151.2, 153.7, 156.6, 165.6.

Anal. Calcd for $C_{20}H_{24}N_6O_3$ (396.44): C, 60.59; H, 6.10; N, 21.20. Found: C, 60.53; H, 5.99; N, 20.99.

9-[2-(Mesitylcarbonyl)-3,3-dimethylisoxazolidin-5-yl]-1,9-dihydro-6*H*-purin-6-one (14c)
Pale-yellow solid; yield: 0.08 g (32%); mp 175–177 °C (MeOH).

IR (Nujol): 3425, 1686, 1635, 1545, 1210, 1090, 953, 777 cm^{-1}.

^1H NMR (300 MHz, DMSO): δ = 1.65 and 1.84 (s, 6 H, $2CH_3$), 1.87, 2.15, and 2.17 (s, 9 H, $3CH_3$-Ar), 2.94 and 3.22 (dd, *J* = 14 and 7 Hz, 2 H, CH_2), 6.45 (t, *J* = 7 Hz, 1 H, CH), 6.66 and 6.77 (s, 2 H, Ph), 8.05 and 8.28 (s, 2 H, CH hypox ring), 12.42 (br s, 1 H, NH).

^{13}C NMR (75 MHz, DMSO): δ = 17.9, 18.5, 20.6, 24.9, 25.8, 46.4, 62.7, 81.2, 114.7, 127.5, 132.6, 133.1, 137.3, 138.5, 144.9, 148.3, 156.4, 165.7.

Anal. Calcd for $C_{20}H_{23}N_5O_3$ (381.42): C, 62.98; H, 6.08; N, 18.36. Found: C, 63.10; H, 6.11; N, 18.40.

9-[2-(9-Anthrylcarbonyl)-3,3-dimethylisoxazolidin-5-yl]-9*H*-purin-6-amine (16)
A soln of adenine (2 equiv) and TMSN=C(Me)OTMS (2 equiv) in anhyd CH_2Cl_2 (50 mL) was refluxed under N_2 for 15–20 min until it became clear. The mixture was cooled to r.t. and a soln of isoxazolidine **15** (0.20 g, 0.65 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The resulting soln was cooled to 0 °C and TMSOTf (0.12 mL, 1 equiv) was added. The mixture was refluxed with stirring overnight and then the reaction was quenched with sat. aq $NaHCO_3$ (pH 7). The resulting mixture was diluted with an equal volume of CH_2Cl_2, washed with H_2O (2 × 20 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography to give a yellowish solid; yield: 0.10 g (36%); mp > 205 °C (EtOH).

IR (Nujol): 3375, 3321, 1664, 1596, 1560, 1354, 975, 885, 730 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 2.05 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 3.00 (dd, *J* = 14 and 7 Hz, 1 H, H–CH), 3.47 (dd, *J* = 14 and 3 Hz, 1 H, HC–H), 5.62 (br s, 2 H, NH_2), 6.10 (dd, *J* = 7 and 3 Hz, 1 H, O–CH–NH), 7.45 (m, 4 H, arom.), 7.86 (d, *J* = 9 Hz, 1 H, arom.), 8.01 (m, 4 H, arom.), 8.22 (s, 1 H, CH=N), 8.46 (s, 1 H, CH=N).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.3, 30.8, 40.7, 63.1, 82.5, 119.9, 124.0, 124.4, 125.3, 125.4, 126.5, 127.1, 127.3, 127.5, 128.1, 128.6, 130.9, 137.8, 152.9, 155.1, 165.8.

Anal. Calcd. for $C_{25}H_{22}N_6O_2$ (438.47): C, 68.48; H, 5.06; N, 19.17. Found: C, 68.49; H, 5.05; N, 19.20.

Primary Screen (Dose Response): Determination of 90% Inhibitory Concentration
The initial screen was conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the microplate Alamar Blue assay. The compounds were tested in ten twofold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The 90% inhibitory concentration (IC90) is defined as the concentration that produces a 90% reduction in fluorescence relative to controls. This value is determined from the dose–response curve by using a curve-fitting program. An IC90 value of ≤10 μg/mL is considered to be 'Active' in terms of antitubercular activity. For further details, see http://www.nih.gov.

X-ray Crystallography
The crystal data, data collection, and structural refinements for compounds **9Ad** and **14a** are summarized in Table S3 of the Supporting Information. The structures were solved by direct methods. Nonhydrogen atoms were refined anisotropically, and hydrogen atoms, located from the difference Fourier synthesis, were refined isotropically.[25,26]

Crystallographic data for compounds **9Ad** (CCDC 921636) and **14a** (CCDC 921635) have been deposited and can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(1223)336033; E-mail: deposit@ccdc.cam.ac.uk; Web site: www.ccdc.cam.ac.uk/conts/retrieving.html.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis. Included are crystal structure analyses for compounds **9Ad** and **14a** and copies of the ^1H and ^{13}C NMR spectra of all the synthetized compounds.

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