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Ring-Modified Analogues and Molecular Dynamics Studies To Probe the Requirements for Fungicidal Activities of Malayamycin A and Its *N*-Nucleoside Variants

Olivier Loiseleur,*,‡ Dougal Ritson,† Mafalda Nina,‡ Patrick Crowley,⊥ Trixie Wagner,§ and Stephen Hanessian*,†

Syngenta, Crop Protection Research, Schwarzwaldallee 215, CH-4002 Basel, Switzerland, Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG45 6EY, United Kingdom, Department of Chemistry, Université de Montréal, C. P. 6128, Succ. Centre-Ville, Montréal, P.Q., Canada, H3C 3J7, Novartis Institutes for Biomedical Research, Novartis Pharma AG, Lichtstrasse 35, CH-4056 Basel, Switzerland

olivier.loiseleur@syngenta.com; stephen.hanessian@umontreal.ca

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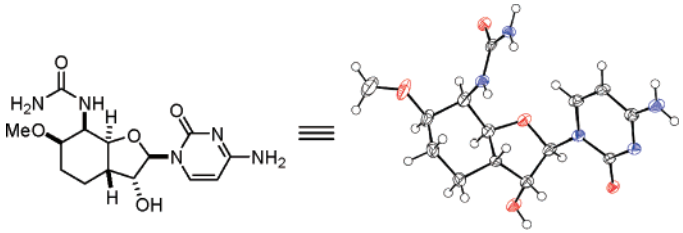

The importance of functional group orientations and the integrity of the bicyclic perhydrofuran core of malayamycin A and two equally active *N*-nucleoside analogues as fungicides were investigated. Two analogues **10** and **11**, representing a THP-truncated and a bicyclic aza-variant, were synthesized and found to be inactive. Molecular dynamics studies on malayamycin A and analogues were performed to highlight the importance of properly orientating the urea and methyl ether groups.

Introduction

Malayamycin A (**1**, Figure 1) is a naturally occurring *C*-nucleoside discovered at Syngenta Crop Protection Laboratories. It was isolated from the soil organism *Streptomyces malaysiensis*, and its structure was determined by extensive NMR studies and degradative work.[1] Further production of malayamycin A by fermentation delivered the novel desmethyl malayamycin A (**2**), which was isolated as a minor metabolite.[2] Within the class of nucleosides, only a handful of naturally occurring *N*- and *C*-pyrimidine nucleosides feature a bicyclic perhydrofuran motif rather than the commonly encountered monocyclic pentofuranosyl or hexofuranosyl core. Octosyl acid A[3] **3** and ezomycin A2[4] **4** are representative of such bicyclic *N*-nucleosides, while ezomycin B1[5] **5** is a *C*-nucleoside equivalent (Figure 1). Malayamycin A is the most recent entry in this group. In line with the ezomycins that have been reported to exhibit antifungal and antibacterial activities,[6] malayamycin A displays broad spectrum activity against phytopathogenic fungi in the greenhouse,[7] with a yet unknown mode of action. In contrast to the ezomycins, malayamycin A has a lower molecular

* Address correspondence to this author.
‡ Syngenta, Crop Protection Research & Technology.
⊥ Syngenta, Jealott's Hill International Research Centre.
† Université de Montréal.
§ Novartis Institutes for Biomedical Research, Novartis Pharma AG.

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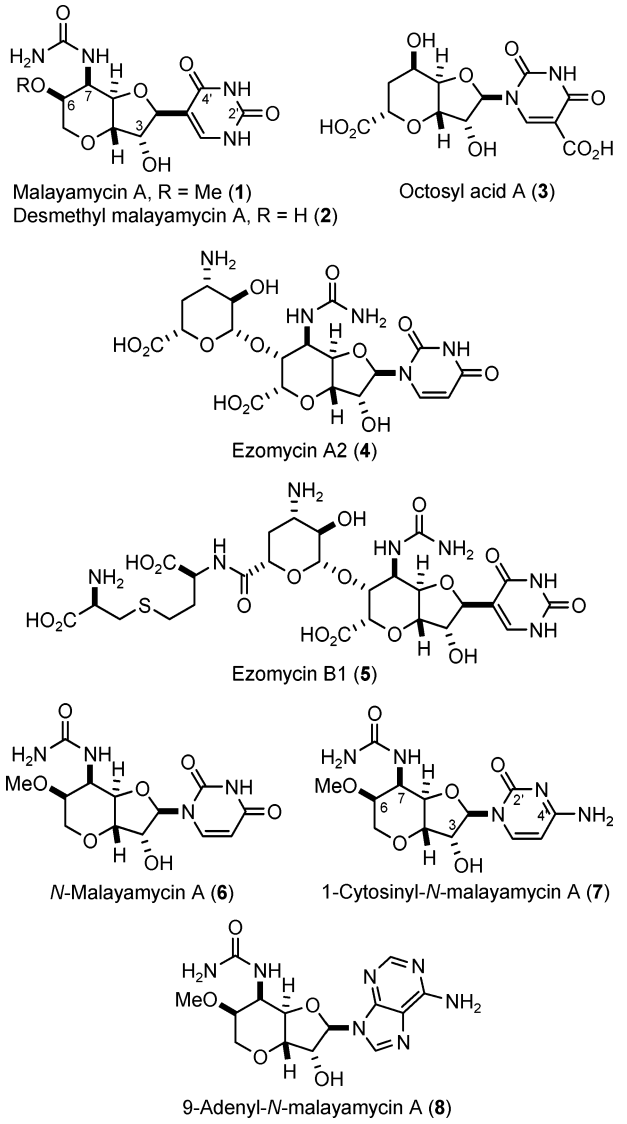

FIGURE 1. Structures of *N*- and *C*-nucleosides with a bicyclic dioxa perhydrofuropyran motif.

weight and does not contain carboxylic acid functions, which makes it a more appropriate lead for crop protection. Recently, the proposed structure and absolute stereochemistry of **1** were confirmed by a stereocontrolled total synthesis.[8] By analogy with the naturally occurring *N*- and *C*-ezomycins, *N*-malayamycin A (**6**) and the related pyrimidine and purine congeners (**7** and **8**, Figure 1) were synthesized via a synthetic route allowing for anomeric diversity.[9] Biological screening revealed the 1-cytosinyl-*N*-nucleoside **7** to be equivalent to malayamycin A in terms of fungicidal activity. On the other hand, related pyrimidine and purine congeners including *N*-malayamycin A (**6**) and 9-adenyl-*N*-malayamycin A (**8**) were either weak or completely inactive.

Results and Discussion

With this knowledge in hand, it was deemed appropriate to gain some information regarding the importance for biological activity of the functional groups displayed on the tetrahydropyran ring in **7**. The urea group, which is commonly shared with the ezomycins, proved to be required as functional group

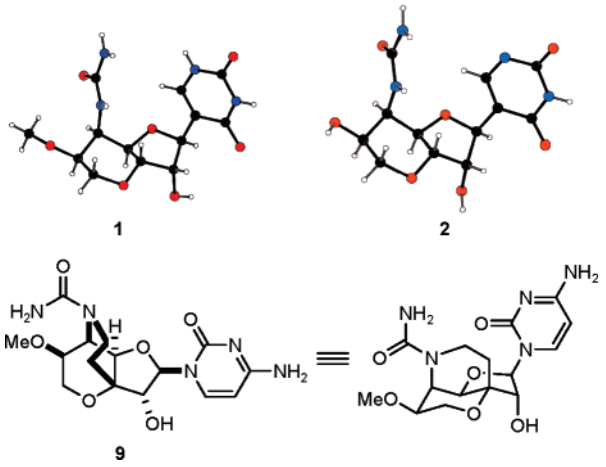

FIGURE 2. Ball-and-stick models (PLATON) of malayamycin A hydrate (**1**) and desmethyl malayamycin A hydrate (**2**) based on X-ray data. The structure-based designed tricyclic 1-cytosinyl- *N*-malayamycin, **9**.

modification at this site resulted in loss of fungicidal activity.[9a] We next turned our attention to the three-dimensional orientation of the urea onto the perhydrofuropyran scaffold. In the course of our synthetic efforts we obtained X-ray quality crystals of **1** and **2** from mixtures of ethanol/water (**1**) and isopropanol/water (**2**) after slow evaporation.[10] The three-dimensional crystal structures as depicted in Figure 2 revealed several interesting features. The *trans*-fused furanoside in the perhydrofuropyran bicyclic system is locked in the C_3-*endo* puckering mode. Accordingly, the $C_{5'}$-substituted pyrimidinone adopts an *anti*

(7) Malayamycin A **1** and all the analogues were tested against three foliar fungal diseases of plants. The compounds were diluted in reverse osmosis water to a final concentration of 100 ppm in water (1 mg of compound in a final volume of 10 mL) immediately before use. TWEEN 20 (registered trade mark, at a final concentration of 0.05% by volume) was added with the water to improve retention of the spray deposit. The compounds were applied to the foliage of the test plants grown on an artificial, cellulose based growing medium, by spraying the plant to maximum droplet retention. Tests were carried out against *Stagonospora Nodorum* (LEPTNO), *Blumeria graminis f.sp. tritici* (ERYSGT), and *Puccinia triticina* (PUCCRT) on wheat. Two replicates, each containing three plants, were used for each treatment. The plants were inoculated with either a calibrated fungal spore suspension or a "dusting" with dry spores 6 h (ERYSGT) or 1 day (PUCCRT and LEPTNO) after chemical application. The plants were then incubated under high humidity conditions (except those inoculated with *Blumeria graminis f.sp. tritici*) and put into an appropriate environment to allow infection to proceed until the disease was ready for assessment. The time period between chemical application and assessment varied from six to nine days according to the disease and environment. However, each individual disease was assessed after the same time period. The level of disease present (the percentage leaf area covered by actively sporulating disease) was assessed visually and the assessed values for all replicates were averaged to provide mean disease values. For malayamycin A **1** and 1-cytosinyl-*N*-malayamycin A **7** there was 0% fungal infection present for all three fungal pathogens, indicating 100% control. Desmethyl malayamycin A **2** gave 100% control of LEPTNO and PUCCRT, but 0% control of ERYSGT. The C_6-methoxy epimer **42** gave 70% control of PUCCRT and 0% of LEPTNO and ERYSGT. All the other analogues tested gave 0% control of all three diseases.

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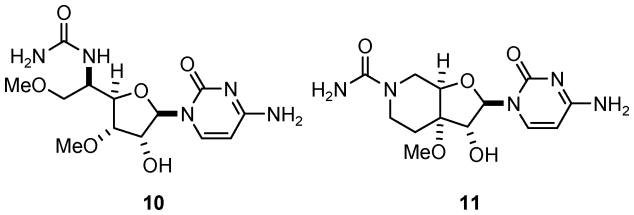


10 **11**

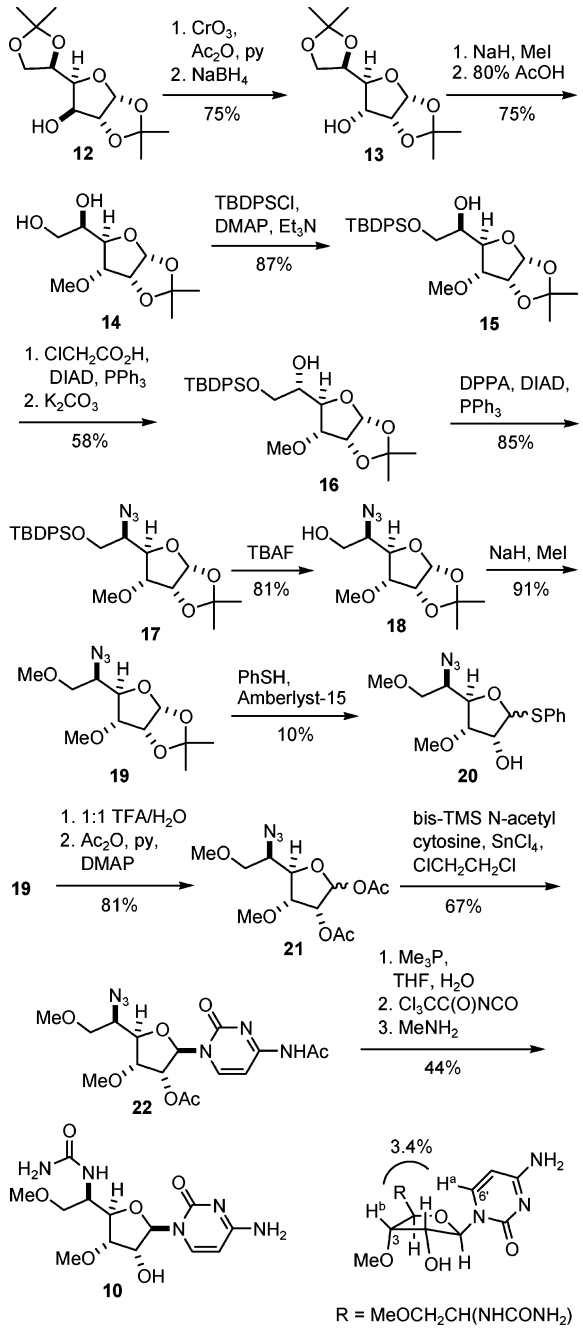
FIGURE 3. Truncated (**10**) and constrained (**11**) analogues of **7**.

orientation to the furanoside ring, with the $C_{2'}$-carbonyl group pointing away from the tetrahydrofuran ring oxygen.[11] The urea group adopts an orthogonal position, which bisects the plane of the chairlike tetrahydropyran ring of the bicyclic system with the carbonyl group oriented "outward". We utilized the three-dimensional functional characteristics shown in the crystal structures of **1** and **2** to derive the tricyclic *N*-cytosinyl analogue **9**, in which the axially orientated urea group was connected to the perhydrofuropyran core by an ethano bridge (Figure 2).

Preliminary superposition of a modeled structure optimized in vacuo with a semiempirical method over the X-ray structure of **1** showed excellent congruence with the urea orientation being locked by the ethano bridge. Accordingly, a synthetic route to **9** was developed[12] although biological testing showed **9** to be devoid of fungicidal activity. To further probe the orientations of the urea and methoxy groups in relation to the bicyclic core, we embarked on the synthesis of the monocyclic and bicyclic *N*-nucleosides **10** and **11** (Figure 3). In **10** we "relaxed" the urea and the adjacent C_6-methoxy groups by allowing them to adopt freely rotating preferred orientations in a truncated tetrahydropyran. Compound **11** represents a constrained analogue with a fixed urea orientation. The methoxy oxygen in **11** was intended to mimic the tetrahydropyran oxygen, possibly a H-bond acceptor.

Diacetone-D-glucose **12** was an appropriate starting material, possessing functional handles in all the relevant positions when compared to the target **10** (Scheme 1). Oxidation of **12** and reduction of the corresponding ketone with $NaBH_4$ in MeOH gave the known alcohol **13**[13] in 75% yield. The epimer (not shown) was recovered in 15% yield and as oxidation of **12** was essentially quantitative, the stereoselectivity of the hydride addition was reflected in the yields of the epimers. *O*-Methylation of **13** was performed by using NaH and MeI in THF and the resulting methyl ether was stirred in 80% AcOH in H_2O for 48 h to hydrolyze the distal acetonide. Attempts to selectively methylate the primary alcohol of **14** with the Meerwein salt ($Me_3O^+BF_4^-$)[14] were unsuccessful, so the primary alcohol was protected as the *tert*-butyldiphenylsilyl ether **15**.

SCHEME 1. Synthesis of Compound 10



Next, we had to install an azide group at C_5, later to be converted to the urea in **10**, but the secondary alcohol of **15** did not have the requisite stereochemistry. Hence, a double inversion was required and the first displacement was carried out under Mitsunobu conditions, using chloroacetic acid as the nucleophilic component.[15] Although the reaction was slow, the desired ester was recovered in reasonable yield after 4 days, and was then subjected to saponification with K_2CO_3 in MeOH to provide the inverted secondary alcohol **16**. The second inversion, and introduction of the azide group, was performed on **16** under Mitsunobu conditions in the presence of diphenyl phosphoryl azide in THF. Compared to **15**, the reaction was remarkably faster, giving the azide **17** in high yield after 18 h. Liberation of the primary alcohol of **17** with use of TBAF in THF gave

(10) Compounds **1** and **2** both show a modulated structure (see: Chapuis, G.; Schönleber A. *Chimia* **2001**, *55*, 523). To obtain coordinates suitable as starting model for the molecular dynamics simulations the modulated structures were approximated by the corresponding superstructures which revealed eight virtually identical molecular conformations for compound **1** and four for compound **2**. Coordinates of those approximations will not be deposited as the modulated structure of compound **1** will be described in detail in a forthcoming paper (see also: Loiseleur, O.; Wagner, T.; Schönleber, A.; Petricek, V. Deutsche Gesellschaft für Kristallographie (DGK) 15. Jahrestagung, 2007 Bremen, conference abstracts).

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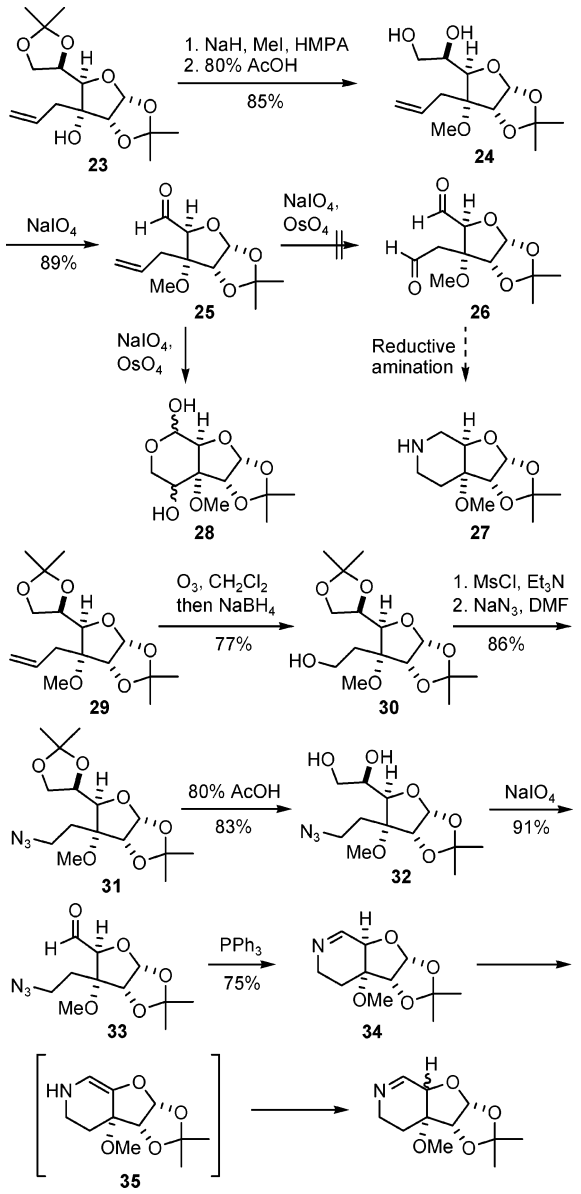
18 as a colorless, crystalline solid from which single-crystal X-ray analysis could be performed, thereby confirming that the sequence of inversions had occurred as intended.[16] *O*-Methylation of the alcohol in **18** was carried out under standard conditions to give **19**, which then had to be manipulated so that the anomeric position could be readily activated during the nucleobase coupling step. For this purpose thioglycosides have been used in conjunction with thiophilic reagents to good effect.[9a,c,d,17] The acetonide **19** was stirred with thiophenol and Amberlyst-15 in CH_2Cl_2[9d,12] leading to a very low yield (10%) of the thioglycoside **20** in addition to several other reaction products. Since other methods such as PhSH/BF_3·OEt_2, PhSH/TFA, and PhS-TMS/ZnI_2[18] gave similar results, we then turned to a Vorbrüggen coupling.[19] Hence, **19** was stirred in 50% aqueous TFA for 2 h and the corresponding diol was acetylated to give **21** as a 1:1 mixture of anomeric acetates. Subsequent treatment with 4-(*N*-trimethylsilyl)acetamido-2-(trimethylsilyloxy)-pyrimidine[17a,b] in the presence of $SnCl_4$ in dichloroethane gave the *N*-nucleoside **22** in good yield. Finally, reduction of the azide group under Staudinger conditions,[20] urea formation, and final deprotection[9d,12] gave the desired compound **10**. The determination of the stereochemistry at the anomeric position was secured by NOE experiments. Irradiation of the cytosinyl proton $C_{6'}$-H^a gave a good enhancement (3.4%) of the proton C_3-H^b to confirm that the nucleobase was in the β-position (Scheme 1).

The synthesis of **11** commenced with the known alcohol **23**,[21] which was methylated, and the desired diol **24** was unmasked by stirring in 80% AcOH/H_2O (Scheme 2). Oxidative cleavage of the vicinal diol was performed with sodium periodate to give the corresponding aldehyde **25** in high yield. Attempts to form the bis-aldehyde **26** that was to be utilized in a double reductive amination sequence to form the piperidine **27**, with $NaIO_4$ and catalytic OsO_4, resulted in the trapping of the intermediate diol as the hemiacetal **28**. Since ozonolysis of **25** was also unsuccessful, a second approach was investigated where the nitrogen, to be incorporated into the piperidine ring of **11**, would be installed first followed by azabicycle formation. We first considered an intramolecular "aza-Wittig" reaction. Hence, **29** was subjected to standard ozonolysis conditions followed by treatment with $NaBH_4$ to give **30**. Although a Mitsunobu reaction (DPPA, PPh_3, DIAD, THF) of the primary alcohol of **30** gave the corresponding primary azide **31** in 77% yield, we chose the higher yielding 2-step sequence of mesylation with subsequent azide displacement (86% over 2 steps). Again, aqueous AcOH was employed to remove the acetonide providing the vicinal diol **32**, which was subsequently cleaved with sodium periodate to give the aldehyde **33**. Although formation of the "aza-ylide" with PPh_3 in Et_2O and cyclization were successful, the resulting imine **34** underwent partial epimerization at C_7, possibly due to formation of the enamine intermediate **35**. An alternative approach based on an intramolecular S_N2 cyclization is shown in Scheme 3. Thus, the aldehyde **33** was reduced with $NaBH_4$ and the primary alcohol was activated as the mesylate

SCHEME 2. Synthesis of Compound 11



36. Upon catalytic hydrogenation of the azide group with Pd black in MeOH/Et_3N, facile ring closure took place to afford the expected bicyclic oxapiperidine. We had found in earlier studies that the robust *tert*-butylsulfonyl[22] (Bus) protecting group for amines could be removed under conditions compatible with *N*-nucleosides.[12] Consequently, the intermediate bicyclic amine was treated with *tert*-butylsulfinyl chloride, and the resulting *tert*-butylsulfinyl amide was oxidized with *m*-CPBA to the *tert*-butylsulfonyl amide **37**. Treatment of **37** with PhSH/Amberlyst-15 in CH_2Cl_2 gave the thioglycoside **38** directly in a 5:1 ratio of α:β anomers, respectively, rather than the expected diphenyl dithioacetal that had previously been observed in this series,[9a,d,12] albeit in modest yield. Protection of the alcohol as the pivaloyl ester and treatment with NIS/TfOH and bis-silylated cytosine gave the expected nucleoside **40** in 76% yield. The β-stereochemistry at the anomeric position was determined by ^{1}H NMR (5.95 ppm, $J = 0$ Hz). Removal of the Bus group of **40** was achieved with TfOH in the presence of anisole in CH_2Cl_2[22,23]

(16) See the Supporting Information, S46.

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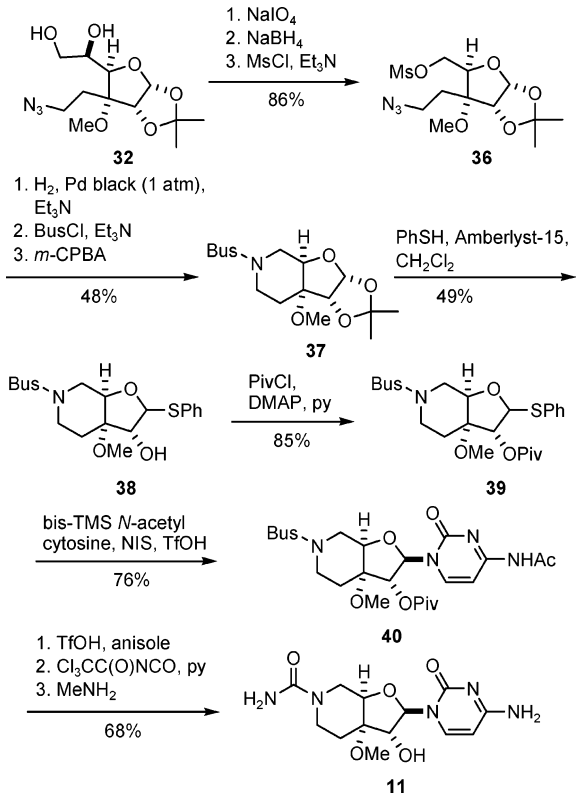
SCHEME 3. Synthesis of Compound 11



and the final sequence of urea installation and deprotection was carried out as before to give **11** in good yield. Not unexpectedly, the structurally simplified analogues **10** and **11** were also devoid of activity, joining a long list of other chemically modified congeners relegated to the ranks of inactive fungicides.[9a,d,12] Clearly the unique spatial arrangement of the urea and methoxy groups, in conjunction with the topology of the dioxabicyclic core structure in malayamycin or its *N*-nucleoside analogues **6** and **7**, are critical features for containing their potent fungicidal activity.

We then decided to study the preferred conformations of malayamycin A and congeners in vacuo using an ab initio RHF/3-21G* method and in water using molecular dynamics simulations. We chose the C_6-methoxy substituent as an entry point. To understand the functional importance of the methyl ether, we considered desmethyl malayamycin A **2** and a series of C_6-modified 1-cytosinyl-*N*-malayamycin analogues which had been prepared previously (Figure 4).[9a,d] Although not as active as malayamycin A **1**, desmethyl malayamycin A **2** had significant fungicidal activity, whereas the more bulky ethoxy-substituted congener **41** was inactive. The C_6-methoxy epimer **42** had much weaker fungicidal activity and the C_6-fluoro-substituted analogue **43** was inactive. This structure−activity pattern points to the possible contribution of an intermolecular hydrogen bond to C_6-OMe in the binding of malayamycin A at the active site. However, other effects might be at work.

At that stage, we probed the conformational features of 1-cytosinyl-*N*-malayamycin A (**7**) and derivatives **42** and **43**, and compared preferred conformations in vacuo and in water to develop a hypothesis for the optimal conformation required for activity. Although there is considerable evidence that many

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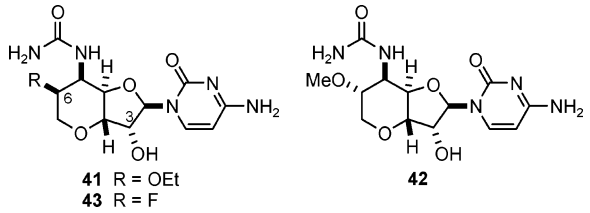

FIGURE 4. C_6 Analogues of 1-cytosinyl-*N*-malayamycin A (**7**).

ligands do not bind to proteins in their global energy minimum conformation in vacuo, many pharmacophore models have been based on such models in cases where no additional information is available.[24] The X-ray structure of **1** was used as a starting conformation to predict the conformational preferences of **7** in vacuo with use of MMFF, an empirical molecular mechanics method. Starting conformations for congeners **42** and **43** were derived from the X-ray structure and modified as appropriate. The Monte Carlo conformational analysis search technique was used to locate the conformational distribution of the active 1-cytosinyl-*N*-malayamycin A **7**, the inactive C_6-fluoro-*N*-malayamycin **43**, and the *epi*-C_6-methoxy-*N*-malayamycin **42**. The predicted global minimum and two local minima for each analogue were subsequently geometry optimized with the HF/3-21G* ab initio method (Figure 5).

Comparison of the crystal structure of malayamycin A with the global energy minimum conformation of **7** in vacuo shows that major topological features such as the dihedral angle for the pyrimidinone and the urea groups are mostly conserved, and that the tetrahydropyran ring is in a chair conformation (see Figure 5A). Not surprisingly, the rotational position of the pyrimidinone is well in line with that found in nucleotides, i.e., the base is *anti* with respect to the furanoside ring.[11] In the calculated global minimum, both the C_6-OMe and the O_1 atom are equatorially disposed in a symmetric fashion relative to the urea and the urea-NH interacts with C_6-OMe through an intramolecular hydrogen bond. On the other hand, the next local energy minimum corresponds to a conformation displaying an intramolecular hydrogen bond between the urea-NH, O_1 and the $C_{2'}$-carbonyl group of the now *syn*-oriented pyrimidinone. This energy minimum and the global minimum are almost *iso*-energetic. Accordingly, the related conformations should be equally available.

When C_6-OMe is removed from its position adjacent to the urea, as in the inactive C_6-fluoro-1-cytosinyl-*N*-malayamycin A (**43**) and the weakly active *epi*-C_6-methoxy-*N*-malayamycin **42**, this equilibrium is disrupted. Without the methoxy in proximity to the urea, the intramolecular hydrogen bond interaction of O_1 and the pyrimidinone with the urea now predominates and the base is locked in a *syn* orientation. For **43**, the lowest energy geometries in vacuo correspond to a tetrahydropyran ring in a twist boat and a chair conformation (see Figure 5B). For **42**, the lowest energy geometry corresponds to the chair conformation with an intramolecular H-bond of the urea-NH with the pyrimidinone $C_{2'}$-carbonyl (see Figure 5C). Thus, it appears that the derivatives **42** and **43** do not allow the urea group and the pyrimidinone to be oriented as in 1-cytosinyl-

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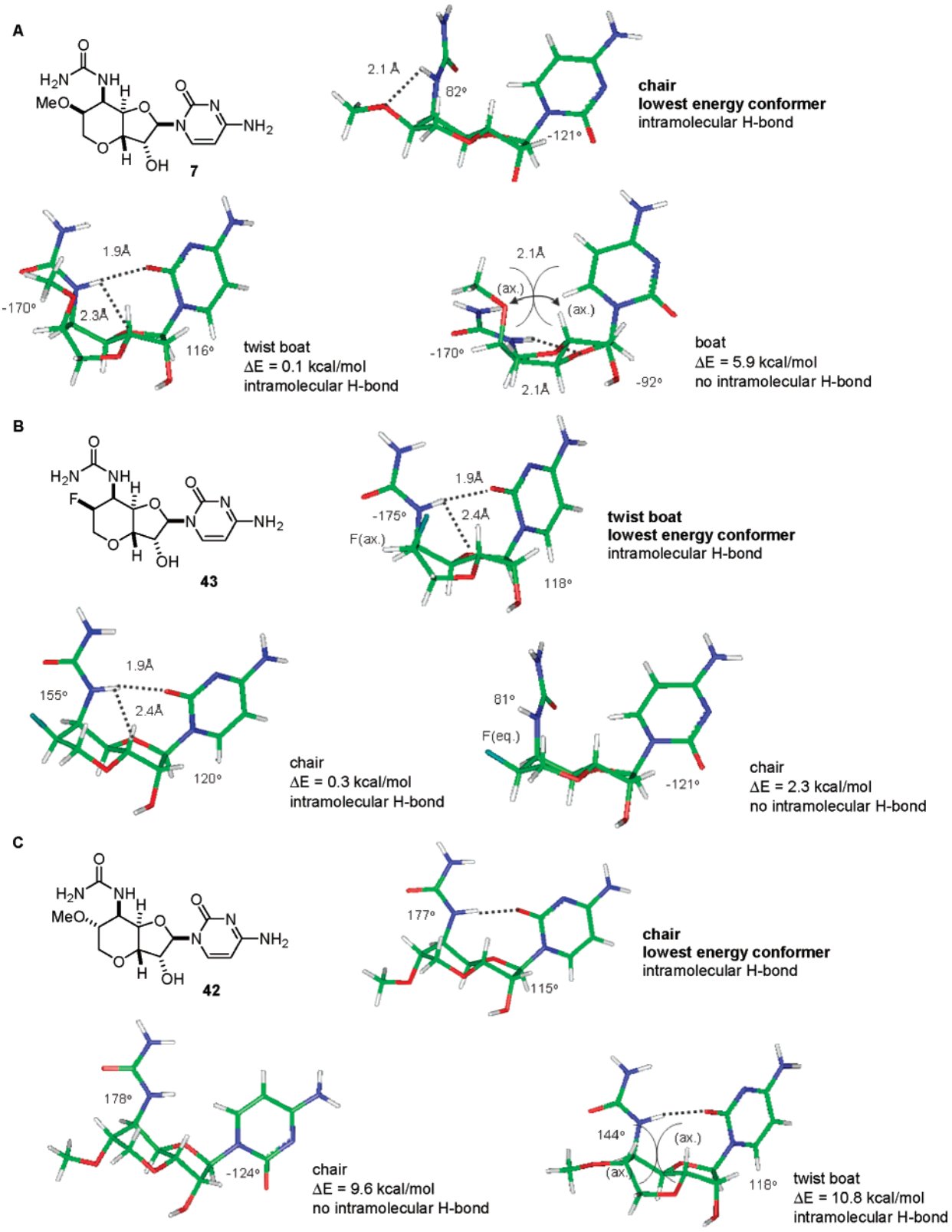


FIGURE 5. Conformational analysis of **7** (A), **43** (B), and **42** (C) in vacuo. Δ*E* is the internal energy difference between the global energy minimum and the local energy minimum geometries optimized with the RHF/3-21G* method.

N-malayamycin A **7**. Therefore, it is hypothesized that the orientation of the urea group and the pyrimidinone is important for activity and that this orientation is influenced by the C_6-methoxy group.

The barrier of rotation of the urea dihedral angle $C_{7a}-C_6-N-C(O)$ of 1-cytosinyl-*N*-malayamycin A (**7**) was assessed by an adiabatic dihedral driving calculation with the HF/3-21G* ab initio molecular orbital method (Figure 6). Figure 6 shows

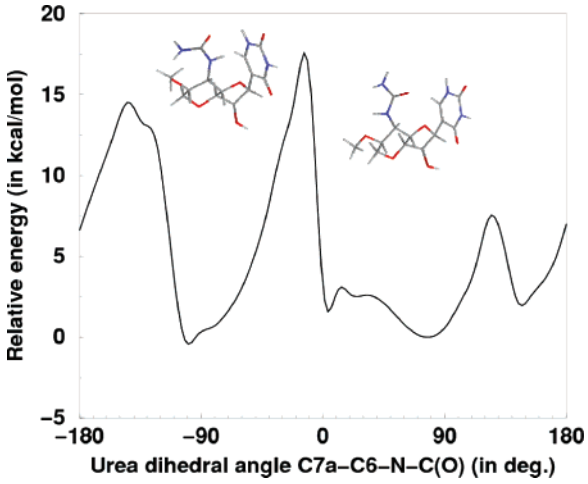
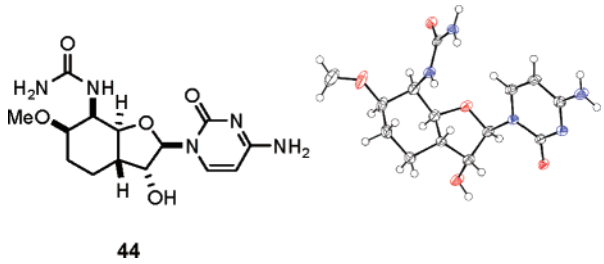


FIGURE 6. Ab initio HF/3-21G* torsional potential for rotation around the urea side chain of 1-cytosinyl-*N*-malayamycin A (**7**).

two key structures from the torsional curve for geometry optimizations with fixed urea dihedral angles. The two conformations were found equally energetic at this level of calculation, i.e., with the urea side chain oriented at 82° (similar to the X-ray structure) and at −103° (hydrogen bond between C_6-OMe and the urea-NH). Moreover, the torsional curve indicates that over the range from 0 to 120°, the energy differs by only 2 kcal·mol^{-1}. On the other hand, the calculations suggest that both equally energetic conformations are separated by 18 kcal·mol^{-1} free energies of activation and hence may not easily interconvert at room temperature.

Subsequently molecular dynamics simulations (MD) in water were performed on derivatives **7**, **42**, and **43** in order to predict the solvation effect on the sampled population of conformers and the urea side chain orientation, using the protocol described.[27] One major population for 1-cytosinyl-*N*-malayamycin A (**7**) accounting for 30% of the sampled conformations was observed during the MD simulation with an average conformation close to the lowest energy conformer in vacuo and an average urea dihedral angle $C_{7a}-C_6-N-C(O)$ of 85 ± 3°. For the *epi*-C_6-methoxy-*N*-malayamycin (**42**), the predominant population (52%) had an average urea dihedral angle of 148 ± 26°. For the C_6-fluoro-1-cytosinyl-*N*-malayamycin A (**43**), two major populations representing 30% and 27% of the overall population were sampled within 20 ns of MD with average dihedral angles of 127 ± 22° and 85 ± 3°, respectively. Overall the MD simulations analysis of the conformational space revealed the influence of the C_6-OMe on the orientation of the urea side chain.

To probe the influence of the oxygen atom $C_{3a}-O-C_5$ in the tetrahydropyran ring in *N*-malayamycin **7**, we prepared the *N*-cytosinyl carba-analogue **44** (Figure 7).[9a] X-ray quality crystals of **44** were obtained by slow evaporation from isopropanol, and the three-dimensional solid-state structure revealed no conformational differences compared to malayamycin A. Surprisingly, analogue **44** was devoid of fungicidal activity in spite of its virtually identical topology compared to the oxa-analogue **7**, which is equipotent to malayamycin A **1**. The poor tolerance to modifications of the oxygen incorporated in the



44

FIGURE 7. Structure of **44** in the crystal. Atomic displacement ellipsoids drawn at the 50% probability level, hydrogen atoms drawn as spheres of arbitrary radius (PLATON).

tetrahydropyran unit and the C_6-OMe delineates their importance for the biological activity of malayamycins.

Conclusion

In the course of our studies we have shown the importance of the substituent orientations and stereochemistry in a malayamycin-type core structure for maintaining fungicidal activity. The hypothesis that emerges from these studies is that in the topologically unique malayamycin core structure, a highly organized orientation of substituents is necessary for activity. In this regard, it is still noteworthy that activity was maintained in *N*-malayamycin and the corresponding *N*-cytosinyl analogues.[9c−e]

Experimental Section

1,2:5,6-Di-*O*-isopropylidene-D-allofuranose (13). To a dry flask was added CrO_3 (3.91 g, 39.1 mmol), under Ar atmosphere, containing anhydrous CH_2Cl_2 (70 mL), which was cooled to 0 °C.

(25) Kapur, B. M.; Allgeier, H. *Helv. Chim. Acta* **1968**, *51*, 89.

(26) Lee, J.-C.; Chang, S.-W.; Liao, C.-C.; Chi, F.-C.; Chen, C.-S.; Wen, Y.-S.; Wang, C.-C.; Kulkarni, S.-S.; Puranik, R.; Liu, Y.-H.; Hung, S.-C. *Chem. Eur. J.* **2004**, *10*, 399.

(27) Molecular dynamics simulations in explicit water were performed on 1-cytosinyl-*N*-malayamycin A (**7**) and congeners **42** and **43** with the molecular mechanics CHARMm software and CHARMM22 force field (Accelrys). The MD simulation used the continuum reaction field treatment of long-range SSBP module (Beglov; Roux *J. Chem. Phys.* **1994**, *100*, 9050) as implemented in the CHARMm module from InsightII (Accelrys). Force field parameters for the ligands consistent with the CHARMM22 force field were produced with the software WitNotP (Novartis Pharma). Three independent MD simulations were performed with the crystal structure of 1-cytosinyl-*N*-malayamycin A (**7**), and global minimum optimized geometries HF/3-21G* for **42** and **43** as starting conformations. A spherical region of 14 Å radius was simulated in atomic detail, including the inhibitor and 350 TIP3 water molecules for a total of 1101 atoms. This sphere size ensured that the solute has a solvation shell of explicit water at least 8 Å thick in all directions. The solvent outside this sphere was treated as a dielectric continuum with a dielectric constant of 80. The inner region has a dielectric of 1. Atomic partial charges in the inner region polarize the outer continuum, giving rise to a reaction field on each explicit atom, which is approximated here by a spherical harmonic expansion of order 15. Within the spherical region, electrostatic interactions were treated without any truncation by using a multipole approximation. Each simulation was run for 20 ns and trajectories were analyzed with an in-house clustering method derived from a previous work (Hamprecht, F. A.; Peter, C.; Daura, X.; Thiel, W.; van Gunsteren, W. F. *J. Chem. Phys.* **2001**, *114*, 2079). For each simulation, 10 000 structures were extracted from the last 10 ns of the trajectory at 1 ps intervals for analysis. The clustering was performed in Cartesian space. For each structure, a least-square translational and rotational fit was performed with the heavy atoms of residues 4−11 and the atom positional root-mean-square difference (RMSd) for this set of atoms was calculated. Terminal residues were not taken into account, as they tend to have more freedom of motion. The number of structures satisfying the similarity criterion set to RMSd ≤1 Å for the heavy atoms was determined in the pool of 10 000 structures. The structure with the highest number of neighbors (i.e. structures satisfying the similarity criterion) was taken as the central member of the first cluster. All structures belonging to the first cluster were removed from the pool. The number of neighbors was computed again with the remaining structures. The structure with the highest number of neighbors becomes the central member of the second cluster. The process is iterated until all structures were assigned to a cluster.

Anhydrous pyridine (11.8 mL, 11.5 g, 146 mmol) and Ac_2O (7.0 mL, 7.56 g, 74.0 mmol) were added followed by diacetone-D-glucose (6.00 g, 23.1 mmol), which was added portionwise over 10 min. After being stirred for 30 min the mixture was brought to room temperature and stirred a further 2 h. The black solution was poured into EtOAc (ca. 150 mL) and the mixture was filtered through silica washing with EtOAc. The solvents were removed and the residue pumped overnight.

The crude oil was dissolved in MeOH (20 mL) and $NaBH_4$ (1.30 g, 34.4 mmol) was added portionwise. After 3 h MeOH (50 mL) was added and the solution was concentrated. This process was repeated twice and the residue was dissolved in saturated NH_4Cl (50 mL), then extracted with EtOAc (3 × 75 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated and the oily residue was recrystallized from Et_2O−hexanes to give the product **13** as colorless plates. The mother liquors were purified by flash chromatography (hexanes−Et_2O, 1:1 then hexanes−EtOAc, 1:1), which gave the product **13** (4.49 g, 17.2 mmol, 75%) as a colorless solid. R_f 0.77 (hexanes−EtOAc, 1:1); mp 73 °C{lit.[13] mp 74−75 °C}; $[\alpha]^{25}_D$ + 39.8 (*c* 0.42, $CHCl_3$) {lit.[13] $[\alpha]^{25}_D$ + 37.8 (*c* 1.0, $CHCl_3$)}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.83 (d, J = 3.8 Hz, 1H), 4.64 (m, 1H), 4.33 (dd, J = 11.2, 6.4 Hz, 1H), 4.07 (m, 3H), 3.85 (dd, J = 8.5, 4.7 Hz, 1H), 2.57 (d, J = 8.4 Hz, 1H), 1.61 (s, 3H), 1.49 (s, 3H), 1.41 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.4, 109.5, 103.5, 79.2, 78.6, 75.1, 72.0, 65.4, 26.2, 26.1, 25.9, 24.9; LRMS (ESI) 261 (23%) $[M + H]^+$.

1,2-*O*-Isopropylidene-3-*O*-methyl-D-allofuranose (14). A solution of **13** (2.61 g, 10.0 mmol) in dry THF (60 mL), under Ar atmosphere, was cooled to 0 °C and NaH (60% dispersion in mineral oil, 600 mg, 15.0 mmol) was added portionwise. The mixture was allowed to warm to room temperature before MeI (900 μL, 2.05 g, 14.4 mmol) was added slowly. The reaction was stirred overnight at this temperature then cooled to 0 °C and a saturated solution of NH_4Cl (40 mL) was added. The product was extracted with Et_2O (3 × 50 mL) and the combined organic layers were washed with brine (40 mL), dried (Na_2SO_4), filtered, and concentrated.

The oily residue was stirred in AcOH/H_2O (4:1 v/v, 30 mL) for 2 days, then a saturated solution of $NaHCO_3$ (40 mL) was added and the mixture was neutralized by careful addition of solid $NaHCO_3$. After lypophilization brine (50 mL) was added and the solution was extracted with EtOAc (5 × 60 mL), then dried ($MgSO_4$), filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc−hexanes, 9:1 then 100% EtOAc), which gave the product **14** (1.76 g, 7.52 mmol, 75%) as a colorless solid. R_f 0.18 (100% EtOAc); mp 116 °C{lit.[25] mp 109−110 °C}; $[\alpha]^{25}_D$ +99.7 (*c* 1.1, $CHCl_3$) {lit.[25] $[\alpha]^{25}_D$ + 105.1 (*c* 1.0, $CHCl_3$)}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.81 (d, J = 3.6 Hz, 1H), 4.74 (dd, J = 3.9, 3.9 Hz, 1H), 4.08 (m, 2H), 3.83 (ddd, J = 10.2, 4.2, 1.5 Hz, 1H), 3.73 (m, 2H), 3.53 (s, 3H), 2.51 (br s, 2H), 1.61 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.8, 103.7, 78.7, 78.4, 76.5, 70.4, 62.6, 57.5, 26.3, 26.1; HRMS (ESI) calcd for $C_{10}H_{18}O_6Na$ [M + Na] 257.0996, found 257.0992.

6-*O*-*tert*-Butyldiphenylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-D-allofuranose (15). To a solution of **14** (823 mg, 3.52 mmol) in dry CH_2Cl_2 (18 mL), under Ar atmosphere, was added Et_3N (630 μL, 457 mg, 4.52 mmol), TBDPSCl (1.00 mL, 1.07 g, 3.89 mmol), and DMAP (cat) and the mixture was stirred at room temperature for 30 h. The mixture was washed with a saturated solution of NH_4Cl (2 × 15 mL), dried (Na_2SO_4), and filtered and the solvents were evaporated under vacuum. The product was purified by flash chromatography (hexanes−EtOAc, 4:1), which gave **15** (1.44 g, 3.05 mmol, 87%) as a colorless oil. R_f 0.54 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 42.4 (*c* 0.85, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.70 (m, 4H), 7.40 (m, 6H), 5.77 (d, J = 3.6 Hz, 1H), 4.69 (dd, J = 3.8, 3.8 Hz, 1H), 4.02 (m, 2H), 3.79 (dd, J = 8.3, 4.4 Hz, 1H), 3.75 (m, 2H), 3.42 (s, 3H), 2.59 (d, J = 2.7 Hz, 1H), 1.58 (s, 3H), 1.38 (s, 3H), 1.09 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 135.24, 135.21, 132.80, 132.76, 129.40, 129.37, 127.37, 127.36, 112.6, 103.6, 80.0, 77.2, 71.5, 64.0, 57.6, 26.5, 26.4, 26.2, 18.9; HRMS (ESI) calcd for $C_{26}H_{36}O_6SiNa$ [M + Na] 495.2173, found 495.2194.

6-*O*-*tert*-Butyldiphenylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-L-talofuranose (16). To a solution of **15** (1.44 g, 3.05 mmol) in dry toluene (38 mL), under Ar atmosphere, was added PPh_3 (1.61 g, 6.14 mmol) and chloroacetic acid (720 mg, 7.62 mmol), after which the solution was cooled to 0 °C. DIAD (1.20 mL, 1.23 g, 6.08 mmol) was added dropwise to the solution and the mixture was allowed to warm to room temperature. After 6 days the solvents were evaporated under vacuum and the product was purified by flash chromatography (hexanes−EtOAc, 4:1) to give the desired product. However, the material could not be obtained in a pure form so it was used in the next step.

The oily residue was stirred in MeOH (27 mL) containing K_2CO_3 (744 mg, 5.38 mmol) for 20 min. A saturated solution of NH_4Cl (50 mL) was added and the majority of the MeOH evaporated before the product was extracted with CH_2Cl_2 (3 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (100% CH_2Cl_2 to CH_2Cl_2−Et_2O, 24:1) provided the product **16** (848 mg, 1.80 mmol, 58%) as a colorless gum. R_f 0.38 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 27.4 (*c* 0.95, $CHCl_3$) {lit.[26] $[\alpha]^{25}_D$ + 35.6 (*c* 0.5, $CHCl_3$)}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.70 (m, 4H), 7.40 (m, 6H), 5.79 (d, J = 3.6 Hz, 1H), 4.70 (dd, J = 3.8, 3.8 Hz, 1H), 4.07 (dd, J = 9.0, 1.7 Hz, 1H), 3.90 (br s, 1H), 3.83 (m, 2H), 3.70 (dd, J = 10.1, 5.4 Hz, 1H), 3.52 (s, 3H), 2.33 (br s, 1H), 1.57 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 135.2, 132.7, 129.4, 127.4, 112.7, 103.9, 79.5, 77.0, 76.6, 69.3, 65.0, 58.1, 26.5, 26.2, 18.8; HRMS (ESI) calcd for $C_{26}H_{36}O_6SiNa$ [M + Na] 495.2173, found 495.2174.

5-Azido-6-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-D-allofuranose (17). A solution of **16** (804 mg, 1.70 mmol) in dry THF (18 mL), under Ar atmosphere, was cooled to 0 °C and PPh_3 (891 mg, 3.40 mmol) then DIAD (710 μL, 730 mg, 3.60 mmol) were added. Diphenyl phosphoryl azide (730 μL, 936 mg, 3.40 mmol) was added dropwise, and after stirring for 30 min the mixture was warmed to room temperature. The mixture was stirred overnight, then the solvents were evaporated under vacuum and the product was purified by flash chromatography (hexanes−EtOAc, 9:1). Chromatography was repeated (CH_2Cl_2−hexanes, 7:3) to give the product **17** (719 mg, 1.45 mmol, 85%) as a colorless oil. R_f 0.84 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 33.3 (*c* 0.45, $CHCl_3$); IR (neat) υ 2105; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.70 (td, J = 7.9, 1.6 Hz, 4H), 7.42 (m, 6H), 5.78 (d, J = 3.5 Hz, 1H), 4.66 (dd, J = 3.9, 3.9 Hz, 1H), 4.07 (dd, J = 8.7, 3.5 Hz, 1H), 3.90 (m, 1H), 3.77 (m, 2H), 3.66 (dd, J = 8.8, 4.4 Hz, 1H), 3.38 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H), 1.11 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 135.28, 135.23, 132.6, 132.5, 129.44, 129.40, 127.40, 127.36, 112.8, 103.6, 80.1, 76.5, 71.5, 64.1, 63.3, 57.6, 26.4, 26.3, 26.1, 18.8; LRMS (ESI) 498 (8%) $[M + H]^+$.

5-Azido-1,2-*O*-isopropylidene-3-*O*-methyl-D-allofuranose (18). To a solution of **17** (675 mg, 1.36 mmol) in dry THF (17 mL) was added slowly TBAF (1 M solution in THF, 2.10 mmol, 2.10 mL), and after the mixture was stirred for 1.5 h a saturated solution of NH_4Cl (20 mL) was added. The mixture was stirred for 15 min, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated. The product was then purified by flash chromatography (hexanes−Et_2O, 65:35 then hexanes−EtOAc, 1:1), which gave the product **18** (293 mg, 1.13 mmol, 81%) as a colorless solid. R_f 0.52 (hexanes−EtOAc, 1:1); mp 64−65 °C; $[\alpha]^{25}_D$ + 295.7 (*c* 0.35, $CHCl_3$); IR (neat) υ 3478, 2102; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.85 (d, J = 3.6 Hz, 1H), 4.76 (dd, J = 3.6, 3.6 Hz, 1H), 4.22 (dd, J = 8.8, 2.9 Hz, 1H), 4.02 (td, J = 5.4, 2.9 Hz, 1H), 3.88 (dd, J = 8.8, 4.3 Hz, 1H), 3.73 (d, J = 5.4 Hz, 2H), 3.53 (s, 3H), 1.61 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 113.0, 103.6, 79.6, 77.4, 71.2, 61.6, 58.7, 57.6, 26.5, 26.1; HRMS (ESI) calcd for $C_{10}H_{17}N_3O_5Na$ [M + Na] 282.1060, found 282.1061.

5-Azido-1,2-*O*-isopropylidene-3,6-di-*O*-methyl-D-allofuranose (19). A solution of **18** (290 mg, 1.12 mmol) in dry THF (7 mL) was cooled to 0 °C then NaH (60% dispersion in mineral oil, 72 mg, 1.79 mmol) was added portionwise. After the mixture was stirred for 30 min, MeI (140 μL, 319 mg, 2.24 mmol) was added dropwise, then the mixture was allowed to warm to room temperature and stirred overnight. A saturated solution of NH_4Cl (10 mL) was added and the product was extracted with Et_2O (3 × 10 mL), which was dried (Na_2SO_4), filtered, and concentrated. The oily residue was purified by flash chromatography (hexanes−EtOAc, 85:15), which gave the product **19** (272 mg, 1.00 mmol, 91%) as a colorless oil. R_f 0.70 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 107.6 (*c* 2.25, $CHCl_3$); IR (neat) *ν* 2099; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 5.81 (d, *J* = 3.6 Hz, 1H), 4.72 (dd, *J* = 4.1, 3.7 Hz, 1H), 4.10 (m, 1H), 4.02 (dt, *J* = 9.7, 3.5 Hz, 1H), 3.80 (dd, *J* = 8.7, 4.3 Hz, 1H), 3.52 (dd, *J* = 10.3, 3.9 Hz, 1H), 3.49 (s, 3H), 3.42 (dd, *J* = 10.3, 8.7 Hz, 1H), 3.41 (s, 3H), 1.61 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* (ppm) 113.0, 103.6, 79.6, 77.5, 76.7, 71.2, 61.6, 58.7, 57.6, 26.5, 26.1; HRMS (ESI) calcd for $C_{11}H_{19}N_3O_5$-Na [M + Na] 296.1217, found 296.1213.

5-Azido-1,2-di-*O*-acetyl-3,6-di-*O*-methyl-D-allofuranose (21). Compound **19** (84 mg, 0.31 mmol) was stirred in TFA/H_2O (1:1, v/v, 1 mL) for 2 h, a saturated solution of $NaHCO_3$ (3 mL) was added, and the mixture was neutralized with solid $NaHCO_3$. The product was extracted with EtOAc (3 × 5 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was used in the next step.

The oily residue was dissolved in anhydrous pyridine/anhydrous CH_2Cl_2 (2:1, 1.2 mL) and Ac_2O (290 μL, 313 mg, 3.07 mmol) then DMAP (cat.) were added. The mixture was stirred at room temperature overnight. After this time the solvents were evaporated and the crude material was purified by flash chromatography (hexanes−EtOAc, 4:1 to 7:3) to provide the product **21** (80 mg, 0.25 mmol, 81%) as a colorless oil and a 1:1 mixture of anomers. *β*-Anomer: R_f 0.68 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 47.4 (*c* 2.0, $CHCl_3$); IR (neat) *ν* 2133, 2100, 1749; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.12 (s, 1H), 5.31 (d, *J* = 3.9 Hz, 1H), 4.12 (m, 2H), 3.91 (m, 1H), 3.48 (dd, *J* = 10.3, 4.3 Hz, 1H), 3.38 (s, 3H), 3.37 (s, 3H), 3.31 (dd, *J* = 10.4, 7.9 Hz, 1H), 2.15 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* (ppm) 169.3, 168.7, 97.7, 80.4, 78.0, 72.8, 70.9, 62.0, 58.8, 58.5, 20.7, 20.3; LRMS (ESI) 258 (100%) $[M - OAc]^+$.

α-Anomer: R_f 0.58 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 58.5 (*c* 1.85, $CHCl_3$); IR (neat) *ν* 2134, 2101, 1749; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.39 (d, *J* = 4.6 Hz, 1H), 5.08 (dd, *J* = 6.7, 4.7 Hz, 1H), 4.24 (dd, *J* = 4.6, 3.4 Hz, 1H), 3.96 (dd, *J* = 6.6, 3.4 Hz, 1H), 3.75 (dt, *J* = 7.0, 4.8 Hz, 1H), 3.59 (dd, *J* = 10.2, 4.7 Hz, 1H), 3.50 (dd, *J* = 10.2, 7.0 Hz, 1H), 3.40 (s, 3H), 3.39 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* (ppm) 169.7, 169.6, 93.5, 83.0, 76.8, 71.5, 70.9, 61.6, 58.9, 58.6, 20.8, 20.2; LRMS (ESI) 258 (100%) $[M - OAc]^+$.

(2*R*,3*R*,4*R*,5*R*,1′′*R*)-Acetic Acid 2-(4′-Acetylamino-2′-oxo-2*H*-pyrimidin-1′-yl)-5-(1′′-azido-2′′-methoxyethyl)-4-methoxytetrahydrofuran-3-yl Ester (22). To a solution of crude bis-silylated *N*-acetyl cytosine[17b] (1.50 mmol of crude material) in anhydrous dichloroethane (2 mL), under Ar atmosphere, was added a solution of **21** (70 mg, 0.22 mmol) in anhydrous dichloroethane at room temperature. $SnCl_4$ (51 μL, 114 mg, 0.44 mmol) was added dropwise to the solution. Additional $SnCl_4$ (100 μL, 223 mg, 0.86 mmol) was added in 2 portions at 12 h intervals and the solution was stirred a further 12 h. A saturated solution of $NaHCO_3$ (5 mL) was added, and the product was extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. The crude material was purified by preparative thin layer chromatography (100% EtOAc) to provide the product **22** (61 mg, 0.15 mmol, 67%) as a colorless foam. R_f 0.44 (100% EtOAc); $[\alpha]^{25}_D$ + 92.7 (*c* 0.75, MeOH); ^{1}H NMR (400 MHz, CD_3OD) *δ* (ppm) 8.17 (d, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 5.93 (d, *J* = 3.4 Hz, 1H), 5.52 (dd, *J* = 5.5, 3.4 Hz, 1H), 4.18 (dd, *J* = 5.7, 5.7 Hz, 1H), 4.09 (m, 2H), 3.70 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.58 (dd, *J* = 10.0, 13.3 Hz, 1H), 3.43 (s, 3H), 3.39 (s, 3H), 2.20 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) *δ* (ppm) 171.3, 169.6, 162.9, 160.3, 155.9, 145.1, 96.5, 90.3, 80.4, 77.7, 73.4, 71.2, 61.2, 57.5, 22.8, 18.8; HRMS (ESI) calcd for $C_{16}H_{23}N_6O_7$ [M + H] 411.1623, found 411.1630.

(1′′*R*,2*R*,3*R*,4*R*,5*R*)-(1′′-[5-(4′-Amino-2′-oxo-2*H*-pyrimidin-1′-yl)-4-hydroxy-3-methoxytetrahydrofuran-2-yl]-2′′-methoxyethyl)urea (10). The *N*-glycoside **22** (19 mg, 0.046 mmol) was dissolved in THF (2 mL) and PMe_3 (1 M solution in THF, 170 μL, 0.17 mmol) was added. After the solution was stirred at room temperature for 5 h, H_2O (11 μL, 11 mg, 0.61 mmol) was added and the mixture was heated to reflux for 74 h. The solvents were removed and the residue was filtered through a plug of silica, washing with EtOAc then EtOAc−MeOH (7:3). The second fraction was collected, concentrated, and dried under vacuum overnight. The residue was dissolved in dry CH_2Cl_2 (1 mL) and trichloroacetyl isocyanate (23 μL, 36 mg, 0.18 mmol) was added under an Ar atmosphere at room temperature. LCMS showed the consumption of the intermediate amine after 1.5 h, so the solvents were evaporated and the residue was stirred in $MeNH_2$ 40 wt % in H_2O/MeOH (3:1, 0.6 mL) for 1 h. The solution was then lypophilized and the residue was purified by preparative thin layer chromatography ($CHCl_3$−MeOH, 4:1 to 7:3) to provide the product **10** (7 mg, 0.020 mmol, 44%) as a colorless solid. R_f 0.11 ($CHCl_3$−MeOH, 7:3); $[\alpha]^{25}_D$ −32.0 (*c* 0.35, MeOH); ^{1}H NMR (400 MHz, CD_3OD) *δ* (ppm) 7.66 (d, *J* = 7.5 Hz, 1H), 5.96 (d, *J* = 7.5 Hz, 1H), 5.93 (d, *J* = 6.0 Hz, 1H), 4.30 (dd, *J* = 5.8, 5.8 Hz, 1H), 4.03 (m, 2H), 3.84 (dd, *J* = 5.5, 3.4 Hz, 1H), 3.59 (dd, *J* = 9.4, 3.5 Hz, 1H), 3.47 (dd, *J* = 9.6, 3.5 Hz, 1H), 3.44 (s, 3H), 3.38 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) *δ* (ppm) 165.8, 159.9, 156.8, 140.8, 94.8, 89.3, 80.8, 79.8, 72.3, 71.1, 57.6, 56.5, 50.6; HRMS (ESI) calcd for $C_{13}H_{22}N_5O_6$ [M + H] 344.1565, found 344.1562.

3-*O*-Methyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-prop-1′-enyl-D-allofuranose (29). The alcohol **23**[21] (3.67 g, 12.2 mmol) was dissolved in dry THF (75 mL), under an Ar atmosphere, and NaH (60% dispersion in mineral oil, 732 mg, 18.3 mmol) was added portionwise. The mixture was then refluxed gently for 1.5 h before being cooled to room temperature when HMPA (3 mL) and MeI (1.55 mL, 3.53 g, 24.9 mmol) were added. The mixture was heated to reflux for a further 2 h, then cooled to room temperature, and a saturated solution of NH_4Cl/ice (50 mL/20 g) was added. The product was extracted with Et_2O (3 × 50 mL), dried (Na_2SO_4), filtered, and concentrated to give an oily residue. Purification by flash chromatography (hexanes−Et_2O, 4:1) provided the product **29** (3.59 g, 11.4 mmol, 94%) as a very pale yellow oil. R_f 0.19 (hexanes−Et_2O, 4:1); $[\alpha]^{25}_D$ + 50.4 (*c* 0.54, $CHCl_3$) {lit.[21] $[\alpha]^{25}_D$ +20.0 (*c* 1.56, $CHCl_3$)}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 5.97 (m, 1H), 5.59 (d, *J* = 3.5 Hz, 1H), 5.20 (m, 2H), 4.43 (d, *J* = 3.6 Hz, 1H), 4.12 (m, 3H), 3.91 (m, 1H), 3.51 (s, 3H), 2.71 (dd, *J* = 15.0, 6.2 Hz, 1H), 2.30 (dd, *J* = 15.1, 7.7 Hz, 1H), 1.61 (s, 3H), 1.46 (s, 3H), 1.39 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* (ppm) 132.0, 118.3, 112.3, 109.4, 102.6, 83.2, 82.8, 81.7, 72.3, 68.2, 52.6, 33.7, 26.7, 26.13, 26.06, 25.1; LRMS (ESI) 315 (6%) $[M + H]^+$.

3-*O*-Methyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-(1′-hydroxypropyl)-D-allofuranose (30). Compound **29** (3.52 g, 11.2 mmol) was dissolved in CH_2Cl_2 (90 mL) and the solution was cooled to −78 °C. Ozone was bubbled through the solution until an excess was present and the mixture was allowed to stir for 10 min. The ozone was removed by sparging the solution with O_2, then $NaBH_4$ (3.90 g, 103 mmol) was added followed by careful addition of MeOH (40 mL). The solution was allowed to warm to room temperature, and was then stirred for 24 h. A saturated solution of NH_4Cl (50 mL) was added, and stirring was continued for 10 min, after which time the majority of the MeOH was removed under vacuum. Water (20 mL) was added and the aqueous layer was extracted with EtOAc (3 × 70 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography

(hexanes−EtOAc, 1:1) to provide the product **30** (2.73 g, 8.58 mmol, 77%) as a colorless, viscous oil. R_f 0.16 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 37.1 (*c* 0.75, $CHCl_3$); IR (neat) υ 3500; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.62 (d, J = 3.6 Hz, 1H), 4.54 (d, J = 3.5 Hz, 1H), 4.13 (m, 3H), 3.90 (m, 3H), 3.54 (s, 3H), 2.26 (br s, 1H), 2.09 (dt, J = 14.9, 6.5 Hz, 1H), 1.75 (dt, J = 15.0, 6.1 Hz, 1H), 1.60 (s, 3H), 1.44 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.6, 109.5, 102.5, 83.8, 83.5, 80.3, 72.5, 68.5, 58.0, 53.1, 31.8, 26.7, 26.1, 26.0, 25.0; HRMS (ESI) calcd for $C_{15}H_{26}O_7Na$ [M + Na] 341.1571, found 341.1572.

3-*O*-Methyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-(1′-azidopropyl)-D-allofuranose (31). Methanesulfonyl chloride (58 μL, 86 mg, 0.75 mmol) was added to a solution of **30** (200 mg, 0.63 mmol) and Et_3N (140 μL, 102 mg, 1.00 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C. After 1 h, water (5 mL) was added and the mixture was stirred for 10 min. The layers were separated and the organic phase was washed with a saturated solution of NH_4Cl (5 mL), dried (Na_2SO_4), filtered, and concentrated.

The residue was dissolved in dry DMF (4 mL) and NaN_3 (59 mg, 0.91 mmol) was added before the solution was heated to 90 °C. After 1 h the solution was cooled to room temperature and poured into brine (5 mL). The product was extracted with Et_2O (3 × 8 mL), which was then washed with H_2O (2 × 5 mL), dried (Na_2SO_4), and filtered, and the solvent was evaporated under vacuum. The crude material was purified by flash chromatography (hexanes−EtOAc, 9:1) to provide the product **31** (186 mg, 0.54 mmol, 86%) as a colorless oil. R_f 0.77 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ −16.2 (*c* 1.18, $CHCl_3$); IR (neat) υ 2099; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.63 (d, J = 3.4 Hz, 1H), 4.45 (d, J = 3.5 Hz, 1H), 4.16 (dd, J = 8.3, 5.9 Hz, 1H), 4.06 (d, J = 9.0 Hz, 1H), 4.00 (ddd, J = 9.0, 6.2, 5.9 Hz, 1H), 3.88 (dd, J = 8.4, 6.2 Hz, 1H), 3.57 (m, 2H), 3.50 (s, 3H), 2.09 (ddd, J = 14.7, 8.8, 5.9 Hz, 1H), 1.80 (m, 1H), 1.60 (s, 3H), 1.45 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.7, 109.6, 102.4, 83.2, 83.1, 80.9, 72.4, 68.5, 52.8, 45.8, 28.6, 26.6, 26.07, 26.05 25.1; HRMS (ESI) calcd for $C_{15}H_{25}N_3O_6Na$ [M + Na] 366.1635, found 366.1631.

3-*O*-Methyl-1,2-*O*-isopropylidene-3-*C*-(1′-azidopropyl)-D-allofuranose (32). The acetonide **31** (186 mg, 0.54 mmol) was stirred in AcOH/H_2O (4:1, v/v, 2 mL) for 3 days, then a saturated solution of $NaHCO_3$ (3 mL) was added and the mixture was neutralized by careful addition of solid $NaHCO_3$. The product was extracted with Et_2O (3 × 6 mL), dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (hexanes−EtOAc, 1:1) to provide the product **32** (135 mg, 0.45 mmol, 83%) as a colorless, viscous oil. R_f 0.18 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ + 61.7 (*c* 0.56, $CHCl_3$); IR (neat) υ 3467, 2100; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.67 (d, J = 3.6 Hz, 1H), 4.43 (d, J = 3.6 Hz, 1H), 4.02 (d, J = 8.8 Hz, 1H), 3.82 (dd, J = 11.0, 3.5 Hz, 1H), 3.76 (ddd, J = 8.8, 4.5, 3.5 Hz, 1H), 3.67 (dd, J = 10.9, 4.5 Hz, 1H), 3.57 (m, 2H), 3.49 (s, 3H), 2.39 (br s, 2H), 2.07 (ddd, J = 14.9, 8.8, 6.9 Hz, 1H), 1.92 (ddd, J = 15.1, 9.2, 6.2 Hz, 1H), 1.60 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.9, 103.0, 83.8, 82.2, 78.0, 69.4, 64.3, 52.9, 46.0, 29.0, 26.4, 26.1; HRMS (ESI) calcd for $C_{12}H_{21}N_3O_6Na$ [M + Na] 326.1323, found 326.1322.

(2*R*,3*R*,4*R*,5*R*)-Methanesulfonic Acid 3-(2′-Azidoethyl)-4,5-*O*-isopropylidene-3-methoxytetrahydrofuran-2-ylmethyl Ester (36). To a stirred solution of **32** (135 mg, 0.45 mmol) in CH_2Cl_2/MeOH/H_2O (1:1:1, 5 mL) at room temperature was added $NaIO_4$ (123 mg, 0.58 mmol). After 45 min, water (5 mL) was added and the product was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was then washed with water (5 mL), dried (Na_2SO_4), filtered, and concentrated, and the crude material was used directly in the next step.

The oily residue was dissolved in MeOH (4 mL) and $NaBH_4$ (29 mg, 0.77 mmol) was added at room temperature. The solution was stirred for 45 min then the solvent was evaporated under vacuum and MeOH (10 mL) was added. This procedure was repeated and the residue was dissolved in water (5 mL), then the product was extracted with CH_2Cl_2 (3 × 5 mL), dried (Na_2SO_4), filtered, and concentrated.

To a solution of the crude oil in anhydrous CH_2Cl_2 (4 mL), under Ar atmosphere, at 0 °C was added Et_3N (81 μL, 59 mg, 0.58 mmol) and methanesulfonyl chloride (39 μL, 57 mg, 0.50 mmol). After 1 h, water (5 mL) was added and the solution was stirred for 20 min. The product was extracted with CH_2Cl_2 (3 × 5 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by flash chromatography (hexanes−EtOAc, 7:3) to provide the product **36** (135 mg, 0.36 mmol, 86%) as a colorless oil. R_f 0.54 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ +45.6 (*c* 0.95, $CHCl_3$); IR (neat) υ 2100; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.74 (d, J = 3.6 Hz, 1H), 4.43 (d, J = 3.7 Hz, 1H), 4.41 (dd, J = 10.5, 1.6 Hz, 1H), 4.33 (m, 1H), 4.28 (dd, J = 10.4, 7.7 Hz, 1H), 3.44 (m, 5H), 3.09 (s, 3H), 1.94 (ddd, J = 14.9, 8.9, 6.3 Hz, 1H), 1.75 (ddd, J = 14.9, 8.9, 6.2 Hz, 1H), 1.59 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.9, 103.1, 82.5, 81.8, 77.9, 67.3, 52.7, 45.5, 37.5, 28.5, 26.4, 26.0; HRMS (ESI) calcd for $C_{12}H_{21}N_3O_7SNa$ [M + Na] 351.0992, found 351.0995.

(2*R*,3*R*,3a*R*,7a*R*)-2,3-*O*-Isopropylidene-3a-methoxy-6-(2′-methylpropane-2′-sulfonyl)octahydrofuro[2,3-*c*]pyridine (37). A suspension of **36** (135 mg, 0.38 mmol) and Pd black (18 mg) in MeOH/Et_3N (99:1, 5 mL) was degassed and an H_2 atmosphere (1 atms) was applied. After the solution was stirred for 1 h at room temperature, TLC indicated the consumption of starting material, so the suspension was filtered and the residue was washed with hot MeOH. The solvents were evaporated under vacuum and the residue was used directly in the next step.

The crude amine was dissolved in MeCN (5 mL) and Et_3N (210 μL, 152 mg, 1.50 mmol) then *tert*-butylsulfinyl chloride (1 M in CH_2Cl_2,490 μL, 0.49 mmol) was added at room temperature. After 2.5 h, water (5 mL) was added and stirring was continued for 10 min. The majority of the MeCN was removed under vacuum and brine (4 mL) was added. The product was extracted with EtOAc (3 × 8 mL), dried ($MgSO_4$), filtered, and concentrated. The excess triethylamine was removed by filtration through silica, washing with EtOAc−hexanes (4:1) after which the solvents were evaporated.

The orange syrup was dissolved in CH_2Cl_2 (4 mL) and *m*-CPBA (91 mg, 0.53 mmol) was added to the solution at room temperature. After the mixtue was stirred overnight, a saturated solution of Na_2SO_3 (4 mL) was added and stirring was continued for a further 30 min before the product was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (2 × 5 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by flash chromatography (hexanes−EtOAc, 65:35) to provide the product **37** (63 mg, 0.18 mmol, 48%) as a colorless solid. R_f 0.34 (hexanes−EtOAc, 1:1); mp 132 °C; $[\alpha]^{25}_D$ + 30.3 (*c* 1.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.77 (d, J = 3.7 Hz, 1H), 4.26 (d, J = 3.6 Hz, 1H), 3.95 (m, 2H), 3.62 (d, J = 2.8 Hz, 1H), 3.38 (s, 3H), 3.20 (dd, J = 14.5, 2.0 Hz, 1H), 3.07 (td, J = 13.1, 2.0 Hz, 1H), 1.96 (d, J = 4.4 Hz, 1H), 1.57 (s, 3H), 1.34 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.6, 103.8, 80.4, 78.2, 74.1, 61.0, 51.5, 44.8, 41.2, 26.3, 26.0, 25.1, 24.0; LRMS (ESI) 350 (100%) $[M + H]^+$.

(2*R*,3*R*,3a*R*,7a*R*)-3a-Methoxy-6-(2′-methylpropane-2′-sulfonyl)-2-phenylsulfanyloctahydrofuro[2,3-*c*]pyridin-3-ol (38). To a solution of **37** (171 mg, 0.49 mmol) and thiophenol (504 μL, 540 mg, 4.91 mmol) in anhydrous CH_2Cl_2 (12 mL), under an Ar atmosphere, at room temperature was added Amberlyst-15 (350 mg). The suspension was stirred for 10 days, filtered, washed with CH_2Cl_2, and concentrated. The crude material was purified by flash chromatography (hexanes−EtOAc, 3:2 to 1:1) to provide the product **38** (97 mg, 0.24 mmol, 49%) as a colorless solid and a 5:1 mixture of α:β-anomers, respectively. The minor β-anomer was not characterized. R_f 0.22 (hexanes−EtOAc, 1:1); mp 165 °C; $[\alpha]^{25}_D$ +161.7 (*c* 0.90, $CHCl_3$); IR (neat) υ 3459; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.53 (m, 2H), 7.25 (m, 3H), 5.68 (d, J = 3.5 Hz, 1H), 4.15 (m, 1H), 4.06 (s, 1H), 3.95 (br s, 1H), 3.65 (br s, 1H),

3.40 (s, 3H), 3.30 (dd, J = 14.5, 2.5 Hz, 1H), 3.14 (m, 1H), 2.81 (d, J = 4.6 Hz, 1H), 2.13 (d, J = 15.2 Hz, 1H) 1.63 (ddd, J = 15.0, 12.0, 4.4 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 135.1, 130.5, 128.5, 126.7, 92.4, 78.4, 74.1, 73.4, 61.1, 51.5, 45.5, 41.7, 25.2, 24.0; HRMS (ESI) calcd for $C_{18}H_{27}N_5O_5S_2$-Na [M + Na] 424.1223, found 424.1219.

(2*R*,3*R*,3a*R*,7a*R*)-2″,2″-Dimethylpropionic Acid 3a-Methoxy-6-(2′-methylpropane-2′-sulfonyl)-2-phenylsulfanyloctahydrofuro-[2,3-*c*]pyridin-3-yl Ester (39). The alcohol **38** (80 mg, 0.20 mmol) was dissolved in anhydrous pyridine/anhydrous CH_2Cl_2 (2:1, 3 mL), under an Ar atmosphere, and pivaloyl chloride (250 μL, 245 mg, 2.03 mmol) then DMAP (24 mg, 0.20 mol) were added at room temperature. After the mixture was stirred for 8 h, the solvents were evaporated under vacuum and the residue was dissolved in CH_2-Cl_2 (8 mL), which was washed with 0.1 M HCl (5 mL) then H_2O (5 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated then purified by flash chromatography (hexanes−EtOAc, 4:1) to provide the product **39** (82 mg, 0.17 mmol, 85%) as a colorless solid. R_f 0.89 (hexanes−EtOAc, 1:1); $[\alpha]^{25}_D$ +122.9 (c 3.0, $CHCl_3$); IR (neat) υ 1737; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.52 (dd, J = 7.9, 1.2 Hz, 2H), 7.28 (m, 3H), 5.72 (d, J = 4.6 Hz, 1H), 5.42 (d, J = 4.5 Hz, 1H), 4.10 (s, 1H), 4.03 (d, J = 4.6 Hz, 1H), 3.67 (d, J = 4.6 Hz, 1H), 3.26 (dd, J = 14.6, 2.0 Hz, 1H), 3.19 (s, 3H), 3.04 (m, 1H), 2.10 (d, J = 14.9 Hz, 1H), 1.59 (m, 1H) 1.39 (s, 9H), 1.35 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 176.6, 134.8, 130.6, 128.6, 126.9, 90.1, 77.8, 75.3, 73.3, 61.1, 52.1, 45.0, 41.4, 39.1, 26.9, 25.1, 24.0; HRMS (ESI) calcd for $C_{23}H_{35}NO_6S_2Na$ [M + Na] 508.1798, found 508.1791.

(2*R*,3*R*,3a*R*,7a*R*)-2″,2″-Dimethylpropionic Acid 2-(4′-Acetylamino-2′-oxo-2*H*-pyrimidin-1′-yl)-3a-methoxy-6-(2″-methylpropane-2″-sulfonyl)octahydrofuro[2,3-*c*]pyridin-3-yl Ester (40). Compound **39** (40 mg, 0.082 mmol) in anhydrous CH_2Cl_2 (1.4 mL) was added to a solution of the crude bis-silylated *N*-acetyl cytosine[17b] (0.57 mmol of crude material), under Ar atmosphere, in anhydrous CH_2Cl_2 (1 mL) at room temperature. NIS (74 mg, 0.33 mmol, lypophilized overnight) was added, then a solution of TfOH (10 μL, 17 mg, 0.11 mmol) in anhydrous CH_2Cl_2 (0.2 mL) was added dropwise. After 20 h, a saturated solution of $Na_2S_2O_3$ (4 mL) was added and the solution was stirred for 30 min before a saturated solution of $NaHCO_3$ (1.5 mL) was added. The product was extracted with EtOAc (4 × 8 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (3 mL), then stirred with a saturated solution of $Na_2S_2O_3$ (3 mL) for 20 min. The layers were separated and the organics washed with 0.1 M HCl (2 × 3 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by flash chromatography (hexanes−EtOAc, 4:1 to 1:4) to provide the product **40** (33 mg, 0.063 mmol, 76%) as a light orange solid. R_f 0.35 (100% EtOAc); mp >210 °C dec; $[\alpha]^{25}_D$ + 18.2 (c 1.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.89 (br s, 1H), 8.40 (d, J = 4.7 Hz, 1H), 7.55 (d, J = 4.8 Hz, 1H), 5.74 (s, 1H), 5.11 (s, 1H), 4.22 (d, J = 9.6 Hz, 1H), 3.97 (s, 1H), 3.67 (d, J = 8.8 Hz, 1H), 3.39 (dd, J = 9.5, 1.1 Hz, 1H), 3.20 (s, 3H), 3.10 (t, J = 8.1 Hz, 1H), 2.28 (s, 3H), 2.06 (d, J = 6.4 Hz, 1H), 1.41 (s, 9H), 1.39 (m, 1H), 1.32 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.9, 170.8, 162.9, 154.3, 143.6, 96.6, 91.5, 76.9, 76.5, 75.8, 61.1, 52.3, 44.8, 42.0, 38.5, 26.7, 26.3, 24.6, 24.1; LRMS (ESI) 529 (100%) [M + H]$^+$.

(2*R*,3*R*,3a*R*,7a*R*)-2-(4′-Amino-2′-oxo-2*H*-pyrimidin-1′-yl)-3-hydroxy-3a-methoxyhexahydrofuro[2,3-*c*]pyridine-6-carboxylic Acid Amide (11). To a solution of **40** (31 mg, 0.059 mmol) and anisole (96 μL, 95 mg, 0.88 mmol) in anhydrous CH_2Cl_2 (0.75 mL) was added TfOH (13 μL, 21 mg, 0.14 mmol) dropwise. When TLC indicated the disappearance of starting material, trichloroacetyl isocyanate (28 μL, 44 mg, 0.24 mmol) and anhydrous pyridine (10 μL, 10 mg, 0.12 mmol) were added dropwise. When LCMS indicated consumption of the intermediate amine, the solvents were removed under vacuum (without heating) and the residue was dissolved in 40 wt % $MeNH_2$ in H_2O/MeOH (2:1, 1.2 mL). After the solution was stirred for 3 days, the solvents were removed and the residue was purified by preparative HPLC to provide the product **40** (13 mg, 0.040 mmol, 68%) as a colorless solid. R_f 0.19 ($CHCl_3$−MeOH, 4:1); $[\alpha]^{25}_D$ + 107.5 (c 0.60, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.86 (d, J = 8.0 Hz, 1H), 6.14 (d, J = 7.7 Hz, 1H), 5.70 (s, 1H), 4.19 (d, J = 5.0 Hz, 1H), 4.14 (s, 1H), 4.08 (s, 1H), 3.74 (br s, 1H), 3.40 (br s, 4H), 3.15 (br s, 1H), 2.11 (d, J = 14.8 Hz, 1H), 1.29 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 159.6, 159.5, 147.1, 142.9, 93.2, 92.3, 78.0, 77.3, 75.5, 50.3, 42.9, 38.7, 24.4; HRMS (ESI) calcd for $C_{13}H_{20}N_5O_5$ [M + H] 326.1459, found 326.1456.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **13**−**19**, **21**, **22**, **10**, **29**−**32**, **36**−**40**, and **11** and X-ray crystallographic data for compounds **18** and **44**. This material is available free of charge via the Internet at http://pubs.acs.org.

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