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A Scalable Synthesis of 1-Cytosinyl-*N*-malayamycin A: A Potent Fungicide

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Abstract:

A stereocontrolled synthesis of 1-cytosinyl-*N*-malayamycin A, an *N*-analogue of the naturally occurring malayamycin A with fungicidal activity, is reported. The approach was designed to rely solely on substrate control for introduction of the required stereochemistry, avoiding the use of chiral reagents or auxiliaries. Formation of the *N*-nucleoside was achieved through the activation of a thioglycoside, proceeding via sulfonium and thionium intermediates. Ring closure metathesis was used to build the bicyclic perhydrofuropyran heterocycle.

Introduction

Malayamycin A (**1**, Figure 1) is a naturally occurring *C*-nucleoside discovered at Syngenta Crop Protection Laboratories in Jealott's Hill, U.K. It was isolated from the soil organism *Streptomyces malaysiensis*, and its structure was determined by extensive NMR studies and degradative work.[1] Only a handful of *N*- and *C*-nucleosides (**1** and **5**−**7**, Figure 1) feature a bicyclic perhydrofuran motif[2] rather than the commonly encountered monocyclic pentofuranosyl and hexofuranosyl core.[3] Malayamycin A is the most recent entry in this group. In line with the ezomycins that exhibit antifungal and antibiotic activities,[2b,f] malayamycin A is a potent fungicide even though it displays a lower molecular weight. Recently, the proposed structure and absolute stereochemistry of **1** were confirmed by a stereocontrolled total synthesis.[4,5] By analogy with the naturally occurring *N*- and *C*-ezomycins, it was of interest to consider *N*-malayamycin A (**2**) and the related pyrimidine and purine congeners (**3**, **4** Figure 1) as potential targets. The availability of such analogues with modified heterocyclic aglycones would provide an opportunity to test their biological activities, since the corresponding *C*-malayamycins are presently not available. Accordingly, a synthetic route to *N*-malayamycins allowing for anomeric diversity was developed.[6,7] Biological screening then showed the 1-cytosinyl-*N*-nucleoside **3** to be

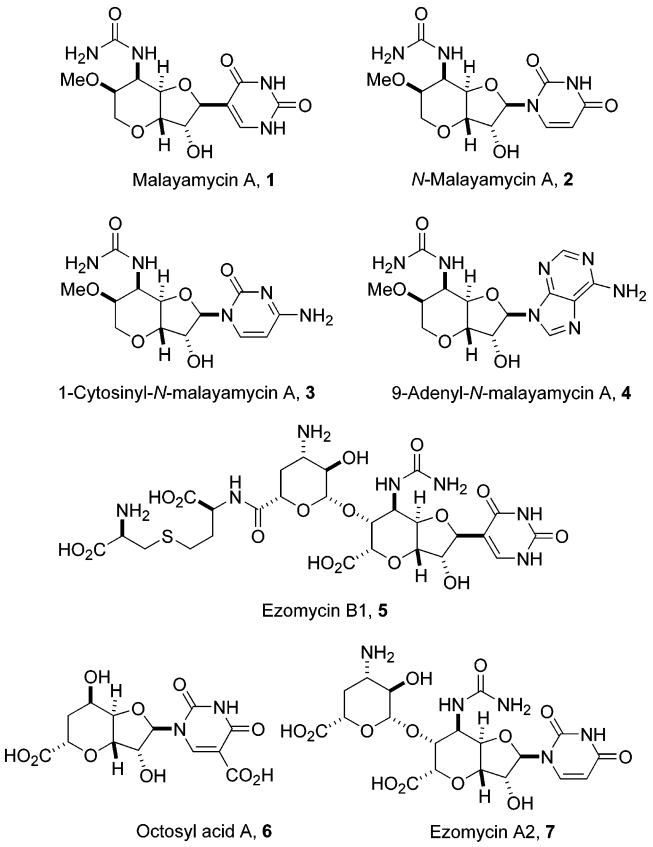

Figure 1. **Structures of *N*- and *C*-nucleosides with a bicyclic dioxa perhydrofuropyran motif.**

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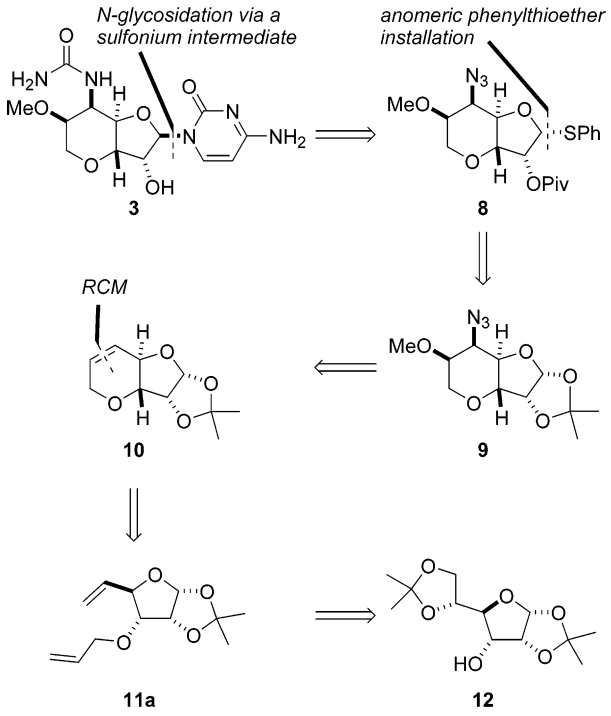
Scheme 1. **Retrosynthetic analysis of 3**



equivalent to malayamycin A in terms of fungicidal activity. However, further exploration of the efficacy of this compound was hampered by the supply of material, which could be delivered by synthesis. This stood in contrast to malayamycin A, which is supplied in sufficient amounts by fermentation. Consequently, we set out to revise our first generation approach to *N*-malayamycins. Herein we report full details of a process allowing for the delivery of sufficient amounts of material for the advanced evaluation of 1-cytosinyl-*N*-malayamycin A (**3**) as a fungicide in crop protection.

Results and Discussion

Synthesis Plan. Our strategy was designed to rely upon substrate-based stereocontrols only. To achieve this goal, the retrosynthesis outlined in Scheme 1 was envisaged. Ring closure metathesis[8] of an appropriate diene **11a** prepared from commercially available[9] 1,2,5,6-di-*O*-isopropylidene-α-D-allofuranose **12** would generate the unsaturated perhydrofuropyran **10**, which would undergo regio- and stereoselective functionalization to install the azido and methoxy groups in a *cis*-relationship (**9**). An anomeric phenylthio group would then be installed and used to introduce the cytosine en route to the target molecule. The activation of thioglycosides with thiophilic agents as a means to prepare bicyclic nucleosides was previously exploited.[2l,3c,10] A major challenge, however, was to maintain the integrity of the *trans*-fused and relatively strained bicyclic core during the exchange of a thiophenyl group to a purine or pyrimidine moiety.

Synthesis of the Perhydrofuropyran Motif 9. Our efforts first revolved around the stereocontrolled construction of the *trans*-fused bicyclic dioxa core. The synthesis of **9** commenced with the known 1,2,5,6-di-*O*-ispropilydene-α-D-allofuranose **12**, which is available in two steps from diacetone-D-glucose or can be obtained from a commercial supplier.[9] Allylation of the C_3 hydroxyl[11] followed by regioselective removal of the 5,6-isopropylidene group delivered diol **13** in 84% yield. This diol was transformed into the corresponding di-*O*-mesylate, from which the bis-olefin **11a** was obtained by treatment with NaI, in readiness for a ring-closing metathesis. The NaI mediated conversion of the intermediate di-*O*-mesylate to an olefin was initially performed in diethyl ketone at 100 °C, which represents the classical conditions for this type of reaction.[12] However, the presence of diethyl ketone caused the undesired formation of up to 15% of **11b** alongside **11a**. The separation of **11b** required a chromatographic purification step. This *trans*-acetalisation phenomenon was suppressed by switching to *N*,*N*-dimethylacetamide (DMA) as the solvent, and a simple distillation then became sufficient to provide analytically pure **11a**. In addition, the yield of **11a** in DMA (92%) was superior to the same reaction performed in diethyl ketone, even when taking into account the formation of **11b** in the mass balance. Ring closure metathesis (RCM) of **11a** using the Grubbs first generation catalyst[8,13] gave the tricyclic olefin **10** in 85% yield. Purification of **10** could again be performed by distillation. In their studies of the application of the Grubbs RCM reaction to synthesize carbohydrates, van Boom and co-workers[14] reported a 63% yield for the formation of **10** at a substrate concentration of 0.02 M in order to minimize oligomerization. Independently, we had performed the same cyclisation at a concentration of 0.05 M and a catalyst loading of 3.5 mol %. On a 30 g scale the yield was consistently over 80%. At higher dilutions, the yield was improved to over 90% but at the expense of practicality. At concentrations higher than 0.05 M formation of oligomers became significant and the yield decreased significantly. Treatment of **10** with *N*-bromosuccinimide (NBS) in aqueous THF followed by NaOH[15] gave the epoxide **14b**. Subsequent opening with sodium azide in 2-methoxyethanol afforded the desired azide **15b** regioisomer in 48% yield (3 steps). Inversion of configuration was achieved by oxidation with the Dess−Martin periodinane reagent[16] to **16**, reduction with $NaBH_4$, and methylation to give the fully functionalized dihydropyran subunit **9** (Scheme

(7) Hanessian, S.; Marcotte, S.; Huang, G.; Crowley, P. J.; Loiseleur, O. Patent app. No. WO 2005005432, January 20, 2005.
(8) For pertinent reviews, see (a) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117. (b) Grubbs, R.; Chang, S. *Tetrahedron* **1998**, *54*, 4413. (c) Armstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 371. (d) Fürstner, A.; Picquet, M.; Bruneau, C.; Dixneuf, P. H. *Chem. Commun*. **1998**, 1315. (e) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2037.
(9) 1,2,5,6-Di-*O*-isopropylidene-α-D-allofuranose (**12**) is available at Carbopharm Gmbh, Industriestrasse 10, A-8502 Lannach, Austria (www.carbopharm.com).
(10) For related methods, see (a) Knapp, S.; Shieh, W.-C. *Tetrahedron Lett.* **1991**, *32*, 3627. (b) Knapp, S.; Shieh, W.-C.; Jaramillo, C.; Trilles, R. V.; Nandan, S. R. *J. Org. Chem*. **1994**, *59*, 946.
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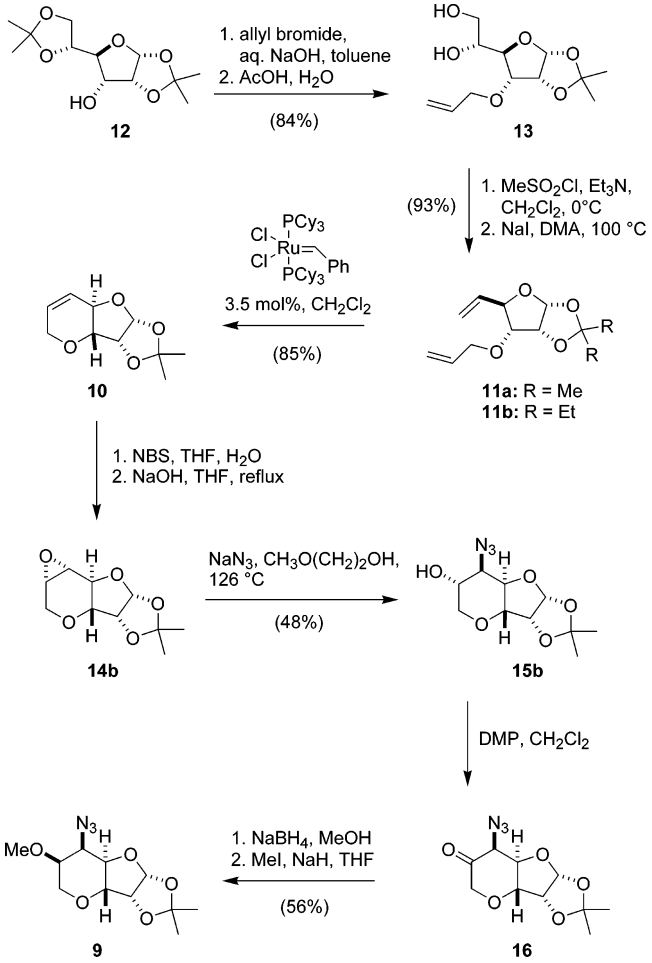
Scheme 2. **Synthesis of the perhydrofuropyran 9**



2). Overall, this 11-step sequence was carried out on a 100 g scale and required only three purifications by chromatography (one for the intermediate **15b** and two for the conversion of **16** to **9**).

The *syn*-relationship of the azido and the methoxy groups in **9** was readily confirmed by coupling constants in ^{1}H NMR spectroscopy data. In addition, the establishment of the desired configuration at C_6 and C_7 was reinforced by the observation of strong NOE enhancements between H_{7a} and H_7 in **15b** and H_{7a} and both H_7 and H_6 in **9** (Figure 2).

Our initial efforts to functionalize the endocyclic double bond in **10** relied on a direct epoxidation to **14b** with *m*CPBA followed by *trans*-diaxial ring opening with the azide ion (Scheme 3). However, this protocol led to the regioisomeric azide **15a**.[5,6] Evidently, epoxidation had occurred from the

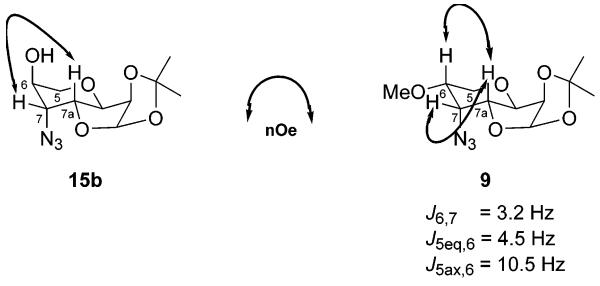
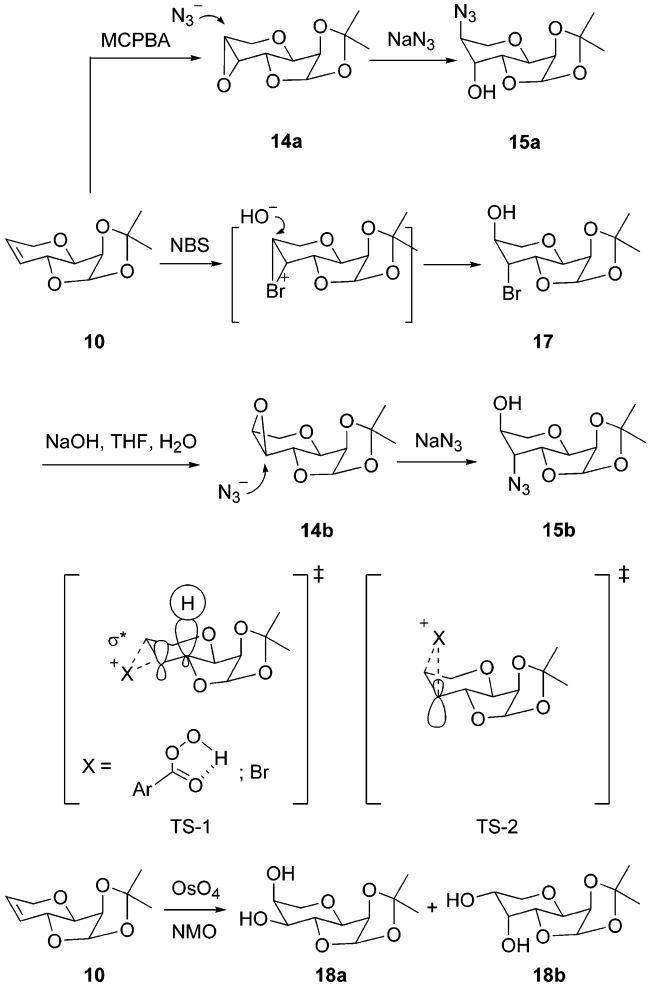


Figure 2. **NOE correlations (NOESY) and coupling constant data for 15 and 9.**

Scheme 3

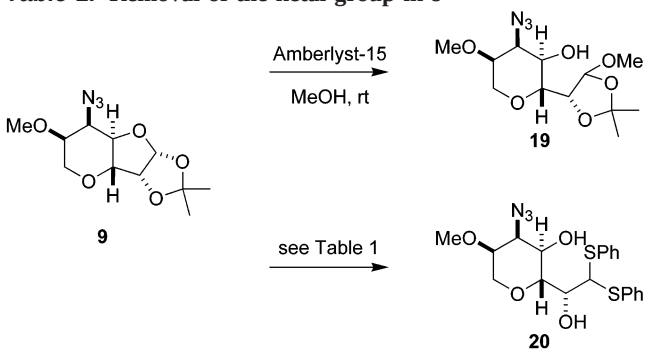


endo face of the bicyclic motif. This preference may be rationalized by considering the favorable electron-donating ability of the C−H σ bond with the developing antibonding orbital in the transition state model TS-1 leading to the observed epoxide.[17] Approach of the peracid from the opposite side as depicted in TS-2 would not benefit from the same stereoelectronic effect. Likewise, treatment with NBS in aqueous THF gave the *endo* bromonium ion, which underwent *trans*-diaxial attack by a hydroxide ion leading to the diaxial bromohydrin **17**. Subsequent treatment with base generated the correct regioisomeric epoxide **14b** (Scheme 3). Interestingly, dihydroxylation of **10** under Upjohn conditions (OsO_4, NMO)[18] was nonselective, providing a 1:1 mixture of diols **18**.

Introduction of the Cytosine Moiety. The stage was now set for the crucial introduction of the cytosine base. Removal of the 2,3-*O*-isopropylidene protecting group was investigated first. Attempts to deprotect model substrate **10** under a variety of acidic conditions led to decomposition of the constrained bicyclic dioxa core with concomitant disappear-

(16) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem.* Soc. **1991**, *113*, 7277. For a safe preparation of the reagent, see: (c) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537.
(17) Cieplak, A. S. *J. Am. Chem. Soc.* **1981**, *103*, 4540.
(18) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973.

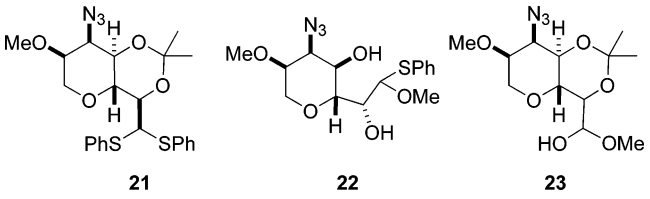
Table 1. **Removal of the ketal group in 9**



entry	H^+	PhSH (equiv)	solvent	temp	yield (%)
1	Amberlyst-15	3	CH_2Cl_2	5 °C	56[a]
2	Amberlyst-15	6	CH_2Cl_2	5 °C	84
3	Amberlyst-15	3	CH_3OH	5 °C to rt	0[b]
4	Montmorillonite K10	3	CH_2Cl_2	5 °C to rt	0
5	Nafion NR 50	3	CH_2Cl_2	5 °C to rt	0

[a] Isolation of 31% of **21**. [b] Formation of **22** (22%) and **23** (25%).

byproducts:

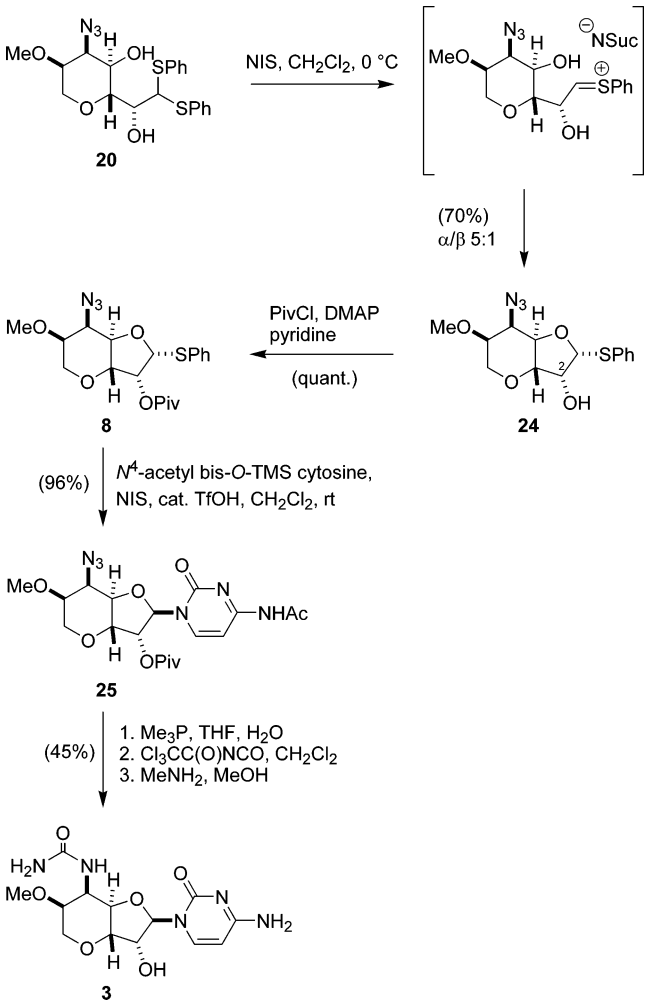


ance of the vinylic protons according to ^{1}H NMR spectroscopy. Consequently, we turned our attention to **9**, in which the double bond had been functionalized. Treatment with Amberlyst-15 in MeOH cleanly afforded the mixed acetal **19** (Table 1). We then anticipated that the addition of benzenethiol to this acid-mediated furanoside opening might facilitate the cleavage of the acetal, while delivering directly diphenyl dithioacetal **20**, a key intermediate in our first generation synthesis of *N*-malayamycins.[6] Indeed, treatment of **9** with 3 equiv of benzenethiol in the presence of Amberlyst-15 suspended in CH_2Cl_2 delivered **20** in 56% yield along with 31% of the cyclic acetal **21** (entry 1, Table 1). Increasing the stoichiometry in benzenethiol to 6 equiv afforded **20** in 84% yield (entry 2).

The choice of Amberlyst to mediate the dithioacetal formation was critical to its success. Indeed, use of Montmorillonite K10 or Nafion NR 50 did not promote the desired conversion. Competition between PhSH and MeOH was a problem when MeOH was used as the solvent instead of CH_2Cl_2 and led to the isolation of the mixed acetals **22** and **23**. Cyclization of **20** via an iodo sulfonium intermediate triggered by *N*-iodosuccinimide (NIS) followed by the ensuing thionium ion intermediate provided directly the bicyclic phenylthio glycoside **24** in 70% yield, predominantly as the α-anomer (Scheme 4).

To ensure a stereocontrolled introduction of the cytosinyl unit, we chose to protect the C_2-hydroxyl as a pivalate (**8**). Activation of the phenylthio group with NIS and triflic acid[19]

Scheme 4. **Stereocontrolled introduction of the pyrimidine and completion of the synthesis**



in the presence of N^4-acetyl bis-*O*-TMS cytosine led to **25** in 96% yield as the only detectable anomer, as a result of pivalate participation.[10b,20] Reduction of the azido group in **25** under modified Staudinger conditions,[21] followed by treatment with trichloroacetyl isocyanate[10b] and deprotection with methylamine gave 1-cytosinyl-*N*-malayamycin A **3**, as a colorless solid. The overall sequence could be easily reproduced on a gram scale.

Conclusions

We have described a process for the preparation of *N*-malayamycin analogue **3** that proceeds in 4.5% yield over 18 steps.[22] The key carbocyclization step to the bicyclic perhydrofuropyran intermediate involved a Grubbs ring closure metathesis, which proceeded in excellent yield. Regioselective functionalization of the bicyclic olefin was achieved through stereocontrolled epoxidation and diaxial opening with an azide ion. The formation of the bicyclic

(19) Veeneman, G. H.; van Leuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.
(20) Sato, S.; Nunomura, S.; Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 4097.
(21) (a) Vaultier, M.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763. (b) Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635.
(22) First generation route to **3**: 25 steps, 2% yield (ref 6).

N-nucleoside was achieved by activation of a thioglycoside, proceeding sequentially via sulfonium and thionium intermediates. The present route proved to be scalable and could solve the supply problem, allowing an extensive exploration of the efficacy of **3** as a fungicide for crop protection.

Experimental Section

(*R*)-1-[(3a*R*,5*R*,6*R*,6a*R*)-6-(Allyloxy)-tetrahydro-2,2-dimethylfuro[2,3-*d*][1,3]dioxol-5-yl]ethane-1,2-diol (13). In a reactor equipped with a mechanical stirrer, a solution of **12** (100 g, 384 mmol) in 1.6 L of CH_2Cl_2 was treated sequentially with allyl bromide (50 mL, 591 mmol), aqueous NaOH (50%, 1.6 L), and tetrabutylammonium iodide (14.2 g, 38.4 mmol). After stirring vigorously at 23 °C for 5 h, the aqueous layer was extracted with *tert*-butylmethyl ether (TBME) (500 mL × 2). The organic extracts were combined, washed with aq. satd. NH_4Cl (1 L + addition of solid NH_4Cl for neutralization of NaOH), dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by recrystallization in hexane afforded 106.3 g (92%) of the desired allyl ether as colorless crystals, which were engaged in the next reaction. R_f 0.51 (SiO_2, AcOEt); mp 36.0−37.5 °C.

A solution of the above material (106 g, 353 mmol) in AcOH−H_2O 75:25 v/v (700 mL) was kept at 23 °C for 15 h and then concentrated in vacuo at 30 °C. TBME (300 mL) and aq. satd. $NaHCO_3$ (30 mL) were added to the residue. The aqueous layer was extracted with TBME (200 mL × 3). The organic extracts were combined, washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo to give 84 g (91%) of the diol **13** as a colorless solid, which did not require further purification. R_f 0.11 (SiO_2, AcOEt); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.01−5.92 (m, 1 H), 5.79 (d, 1H, J = 4.0 Hz), 5.34 (dq, 1H, J = 18.5, 1.0 Hz), 5.27 (dd, 1H, J = 10.0, 1.0 Hz), 4.64 (t, 1H, J = 4.0 Hz), 4.26 (ddt, 1H, J = 12.0, 5.3, 1.0 Hz), 4.12−4.02 (m, 3H), 3.93 (dd, 1H, J = 9.0, 4.0 Hz), 3.74 (dd, 1H, J = 11.0, 5.0 Hz), 3.70 (dd, 1H, J = 11.0, 4.5 Hz), 2.40−1.80 (br s, 2H), 1.58 (s, 3H), 1.37 (s, 3H).

(3a*R*,5*R*,6*R*,6a*R*)-6-(Allyloxy)-tetrahydro-2,2-dimethyl-5-vinylfuro[2,3-*d*][1,3]dioxole (11a). To a solution of **13** (83 g, 319 mmol) and triethylamine (111 mL, 798 mmol) in 400 mL of CH_2Cl_2 at 0 °C under an atmosphere of argon was added dropwise methanesulfonyl chloride (54 mL, 702 mmol) (strongly exothermic). After 30 min at 0 °C, water (200 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (200 mL × 2). The organic extracts were combined, washed with aq. satd. $NaHCO_3$ (50 mL), dried over $MgSO_4$, and concentrated in vacuo to give the bis-mesylate as a pale yellowish oil, which was engaged in the next reaction. R_f 0.33 (SiO_2, 1:1 hexane/AcOEt).

A solution of the above material in 1.5 L of *N*,*N*-dimethylacetamide under an atmosphere of argon was treated with NaI (243 g, 1.59 mol). The resulting suspension was stirred at 100 °C for 2.5 h, whereas a dark-brown coloration developed gradually. The reaction mixture cooled to room temperature was diluted with water (1 L) and treated with aq. satd. $Na_2S_2O_3$ (1.5 L). After stirring for 1 h, hexane (1 L) was added. The phases were separated, and the aqueous layer was extracted with hexane (500 mL × 2). The combined organic extracts were washed with brine (500 mL × 3), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by Kugelrohr distillation under reduced pressure to afford 67 g of diene **11a** (93%) as a colourless oil. R_f 0.76 (SiO_2, 1:1 hexane/AcOEt); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97−5.80 (m, 2H), 5.78 (d, 1H, J = 4.2 Hz), 5.44 (dt, 1H, J = 16.5, 1.2 Hz), 5.30 (dq, 1H, J = 16.5, 1.0 Hz), 5.27 (dt, 1H, J = 10.0 1.0 Hz), 5.22 (dq, 1H, J = 10.0 1.2 Hz), 4.61 (t, 1H, J = 4.0 Hz), 4.42 (dd, 1H, J = 8.8, 7.0 Hz), 4.18 (ddt, 1H, J = 13.5, 5.5, 1.0 Hz), 4.11 (ddt, 1H, J = 13.5, 6.0, 1.0 Hz), 3.52 (dd, 1H, J = 8.8, 4.0 Hz), 1.60 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.9, 134.4, 118.7, 118.1, 107.3, 103.7, 82.2, 79.0, 77.7, 71.7, 26.8, 26.5.

(2*R*,3*R*,3b*R*,7a*R*)-2,2-Dimethyl-3a,5,7a,8a-tetrahydro-3b*H*-[1,3]dioxolo[4,5]furo[3,2-*b*]pyran (10). A solution of **11a** (30 g, 133 mmol) and Grubbs first generation catalyst (3.83 g, 4.65 mmol) in 2.6 L of CH_2Cl_2 saturated with argon was refluxed under a slight flow of argon for 8 h. After completion of the reaction as shown by TLC (SiO_2, hexanes/AcOEt, 3.1), the reaction mixture was washed with water (600 mL), dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by filtration over a pad of SiO_2 (elution with CH_2Cl_2) followed by Kugelrohr distillation under reduced pressure afforded 22.4 g of **10** (85%) as a pale brown solid. R_f 0.60 (SiO_2, AcOEt); mp 64−65 °C; $[\alpha]^{20}_D$ +17.7 (*c* 3.3 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (d, 1H, J = 8.1 Hz), 5.83 (d, 1H, J = 3.5 Hz), 5.64 (d, 1H, J = 2.1 Hz), 4.63 (t, 1H, J = 3.6 Hz), 4.38 (s, 1H), 4.40 (s, 2 H), 3.26 (dd, 1H, J = 8.1, 3.9 Hz), 1.53 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 127.3, 126.3, 113.2, 105.6, 78.9, 76.1, 69.4, 68.6, 26.0, 25.8; MS (ESI) 199.1 $[M + H]^+$.

(2*R*,3*R*,3b*R*,6*R*,7*R*,7a*R*)-7-Azido-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5]furo[3,2-*b*]pyran-6-ol (15b). To a solution of **10** (45.6 g, 230 mmol) in THF−H_2O (1:1 v/v, 600 mL) cooled by ice bath was added NBS (51.0 g, 286 mmol). After 10 min, the ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. The mixture was quenched with aq. satd. $Na_2S_2O_3$ (380 mL) and stirred for 20 min. The phases were separated, and the aqueous layer was extracted with EtOAc (300 mL × 3). The combined organic extracts were dried over $MgSO_4$, filtrated, and concentrated in vacuo. The resulting brown crystalline solid was engaged in the next reaction. R_f 0.52 (SiO_2, AcOEt).

A solution of the above residue in THF (550 mL) was treated with aq. 1 N NaOH (550 mL) and refluxed for 1 h. The mixture was then poured into H_2O (300 mL) and extracted with EtOAc (300 mL × 2). The combined organic layer was washed with brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo to give 48.8 g of the epoxide **14b** as a yellowish crystalline solid, which did not require further purification and which was engaged in the next reaction. R_f 0.46 (SiO_2, AcOEt).

The above epoxide **14b** was dissolved in 2-methoxyethanol (500 mL), and sodium azide (17.9 g, 276 mmol) was

added. The mixture was heated at 130 °C for 2 h, diluted with brine (300 mL) and water (100 mL), and extracted with EtOAc (300 mL × 3). The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash silica gel chromatography (cyclohexane/EtOAc 4:1 to 2:1, gradient) to afford 28.5 g of azide **15b** (48%) as a pale yellowish crystalline solid. R_f 0.19 (SiO_2, 1:1 cyclohexane/AcOEt); mp 115.0−117.0 °C; $[\alpha]^{20}_D$ +42.5 (*c* 2 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (d, 1H, J = 3.4 Hz), 4.66 (t, 1H, J = 3.6 Hz), 4.34 (t, 1H, J = 2.3 Hz), 4.31 (dd, 1H, J = 9.8, 2.3 Hz), 3.90−3.77 (two m, 3 H), 3.54 (dd, 1H, J = 9.8, 4.2 Hz), 2.30 (d, 1H, J = 6.8 Hz), 1.58 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 113.8, 104.3, 76.3, 75.0, 72.0, 69.7, 68.6, 68.5, 26.1, 25.9; LRMS (ESI): 258.1 $[M + H]^+$.

(2*R*,3*R*,3b*R*,6*S*,7*R*,7a*R*)-7-Azido-6-methoxy-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5]furo[3,2-*b*]pyran (9). To a solution of **15b** (28.5 g, 111 mmol) in CH_2Cl_2 (500 mL) at water bath controlled room temperature was added Dess−Martin periodinane (56.5 g, 133 mmol). The mixture was stirred for 2.5 h. Aq. satd. $NaHCO_3$ (200 mL) and $Na_2S_2O_3$ (200 mL) were then added sequentially. The mixture was stirred for 20 min, and the phases were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with water (300 mL), dried over $MgSO_4$, and concentrated in vacuo to give 20.1 g of ketone **16** as a yellowish resin, which was engaged in the next reaction. R_f 0.38 (SiO_2, 75:25 cyclohexane/AcOEt).

To a solution of the above ketone **16** in MeOH (400 mL) at 0 °C was added $NaBH_4$ (4.46 g, 118 mmol). The resulting mixture was stirred at room temperature for 1 h and then concentrated in vacuo without heating. The residue was taken up with EtOAc (400 mL) and washed with brine (400 mL). The aqueous layer was extracted with EtOAc (350 mL × 2). The organic extracts were combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash silica gel chromatography (cyclohexane/EtOAc 4:1 to 2:1, gradient) afforded 16.4 g of the desired alcohol as a colourless oil, which was engaged in the next reaction. R_f 0.42 (SiO_2, 75:25 cyclohexane/AcOEt); mp 113.0−115.0 °C.

To a solution of the above alcohol in THF (200 mL) at 0 °C under an atmosphere of argon was added NaH (60% suspension, 5.08 g, 127 mmol). After stirring for 1 h at 0 °C, MeI (7.89 mL, 127 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1.5 h and was quenched by adding satd. NH_4Cl (300 mL). The mixture was extracted with EtOAc (250 mL × 3). The combined organic layer was washed with brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash silica gel chromatography (cyclohexane/EtOAc, 6:1 to 4:1, gradient) to afford **9** (16.9 g, 56%) as a colorless oil, which crystallized upon conservation at 4 °C. R_f 0.41 (SiO_2, 1:1 cyclohexane/AcOEt); mp 90.5−92.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 (d, 1H, J = 4.0 Hz), 4.62 (t, 1H, J = 4.0 Hz), 4. 58 (t, 1H, J = 3.2 Hz), 3.98 (dd, 1H, J = 10.5, 4.5 Hz), 3.86 (dd, 1H, J = 9.5, 3.2 Hz), 3.65 (dd, 1H, J = 9.5, 4.0 Hz), 3.62 (t, 1H, J = 10.5 Hz), 3.58 (ddd, 1H, J = 10.5, 4.5, 3.2 Hz), 3.45 (s, 3H); 1.58 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 107.3, 105.8, 76.2, 76.1, 74.8, 73.6, 66.1, 59.0, 57.5, 26.3, 26.0; MS (ESI) 244 $[M + H - N_2]^+$.

(2*R*,3*R*,4*S*,5*S*)-4-Azido-tetrahydro-2-[(*R*)-1-hydroxy-2,2-bis(phenylthio)ethyl]-5-methoxy-2*H*-pyran-3-ol (20). To a solution of **9** (16.5 g, 60.8 mmol) in CH_2Cl_2 (400 mL) at 5 °C, PhSH (37.3 mL, 365 mmol) was added followed by Amberlyst-15 (16.5 g). The mixture was stirred at 5 °C for 4 h, then filtrated, quenched with aq. satd. $NaHCO_3$ (200 mL), and stirred for 15 min. After the separation of the two phases, the aqueous layer was extracted with CH_2Cl_2 (60 mL × 2). The combined organic layers were washed with brine (300 mL), dried with $MgSO_4$, filtrated, and concentrated in vacuo. Purification of the crude product by flash silica gel chromatography (cyclohexane/EtOAc, 9:1 to 7:3, gradient) afforded 22.1 g of dithioacetal **20** as a colorless oil (84%), which crystallized upon addition of seed crystals. R_f 0.46 (SiO_2, Et_2O); mp 93.0−94.5 °C; $[\alpha]^{20}_D$ +28.3 (*c* 0.79 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.26 (m, 10H), 4.88 (d, 1H, J = 2.2 Hz), 4.26 (br t, 1H), 3.91 (dd, 1H, J = 8.2, 2.2 Hz), 3.76 (t, 1H, J = 9.1 Hz), 3.69 (dd, 1H, J = 9.1, 3.6 Hz), 3.63 (ddd, 1H, J = 10.6, 4.8, 1.1 Hz), 3.41 (s, 3H), 3.34 (ddd, 1H, J = 10.6, 4.8, 3.2 Hz), 3.22 (t, 1H, J = 10.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.3, 129.1, 128.9, 128.0, 127.9, 76.0, 75.6, 72.7, 71.9, 63.2, 62.4, 56.9; HRMS (FAB) calcd for $C_{20}H_{23}N_3O_4S_2$ $[M^+]$ 433.1130, found 433.1132.

(2R,3*R*,3a*S*,6*S*,7*R*,7a*R*)-7-Azido-hexahydro-6-methoxy-2-(phenylthio)-2*H*-furo[3,2-*b*]pyran-3-ol (24). To a solution of **20** (13.8 g, 31.8 mmol) in dry CH_2Cl_2 (250 mL) at 0 °C and under an atmosphere of argon, NIS (7.52 g, 33.4 mmol) was added. After stirring at room temperature for 20 min, the mixture was quenched by adding aq. satd. $Na_2S_2O_3$ (300 mL) and then stirred for 10 min. Brine (200 mL) was added, and the two phases were separated. The aqueous layer was extracted with CH_2Cl_2 (100 mL × 2). The combined organic extracts were washed with brine (200 mL), dried with $MgSO_4$, filtrated, and concentrated in vacuo. Flash silica gel chromatography (cyclohexane/EtOAc 8:2 to 6:4, gradient) afforded 6.0 g of the α-anomer of phenylthio glycoside **24** (58%) as a colourless crystalline solid and 1.2 g of the β-anomer (12%) as a resin. At this stage, the two anomers of **24** could be combined and converted to the 1-cytosinyl-*N*-nucleoside **25** or converted separately to **25**. α-Anomer: R_f 0.22 (SiO_2, 80:20 Et_2O/cyclohexane); mp 131.0−132.5 °C; $[\alpha]^{20}_D$ +87.5 (*c* 0.76 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59−7.53 (m, 4H), 7.38−7.30 (m, 6H), 5.30 (s, 1H), 4.58 (br t, 1H), 4.34 (d, 1H, J = 4.8 Hz), 3.91 (dd, 1H, J = 10.5, 4.8 Hz), 3.88 (dd, 1H, J = 10.0, 2.5 Hz), 3.62 (dd, 1H, J = 10.0, 4.8 Hz), 3.58 (t, 1H, J = 10.5 Hz), 3.46 (ddd, 1H, J = 10.5, 4.8, 3.5 Hz), 3.43 (s, 3H), 2.35 (br s, 1H, OH); 2.50 (d, 1H, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.4, 130.9, 128.9, 127.1, 93.8, 76.2, 74.1, 73.9, 69.5, 65.6, 58.8, 57.4; HRMS (FAB) calcd for $C_{14}H_{18}N_3O_4S$ $[M + H]^+$ 324.1018, found 324.1008. β-Anomer: R_f 0.12 (SiO_2, 80:20 Et_2O/cyclohexane).

(2*R*,3*R*,3a*R*,6*S*,7*R*,7a*R*)-7-Azido-hexahydro-6-methoxy-2-(phenylthio)-2*H*-furo[3,2-*b*]pyran-3-yl Pivalate (8). To

the alcohol **24** (1.68 g, 5.2 mmol) in pyridine (30 mL), DMAP (3.18 g, 26.0 mmol) and pivaloyl chloride (1.60 mL, 13.0 mmol) were added and the mixture was stirred at room temperature for 1.5 h. Aq. satd. $NaHCO_3$ (100 mL) and TBME (100 mL) were added, and the two phases were separated. The aqueous layer was extracted with TBME (100 mL × 2). The combined organic extracts were washed with 0.1 N HCl (100 mL × 2) and aq. satd. $NaHCO_3$ (100 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification of the crude residue by flash silica gel chromatography (cyclohexane/EtOAc 95:5 to 92:8, gradient) afforded 2.11 g of pivalate **8** as a colourless resin (quantitative). α-Anomer: R_f 0.37 (SiO_2, 8:2 cyclohexane/AcOEt); $[\alpha]^{20}_D$ +187.3 (*c* 0.41 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51−7.27 (m, 5H), 5.90 (d, 1H, J = 3.4 Hz), 5.68 (t, 1H, J = 3.4 Hz), 4.62 (t, 1H, J = 3.2 Hz), 3.97 (dd, 1H, J = 10.0, 3.4 Hz), 3.92 (dd, 1H, J = 11.6, 4.4 Hz), 3.84 (dd, 1H, J = 10.0, 3.4 Hz), 3.61 (dd, 1H, J = 11.6, 10.0 Hz), 3.51 (m, 1H), 3.47 (s, 3H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 134.8, 131.6, 129.5, 127.8, 92.3, 76.8, 75.3, 73.9, 70.4, 66.0, 59.2, 57.9, 39.8, 27.6; HR-MS (FAB) calcd for $C_{19}H_{26}N_3O_5S$ $[M + H]^+$ 408.1601, found 408.1586.

The β-anomer of **24** was converted to the β-anomer of **8** in 85% yield under identical experimental conditions.

(2*R*,3*R*,3a*R*,6*S*,7*R*,7a*R*)-7-Azido-2-(4′-acetylamino-2′-oxo-3′,4′-dihydro-2*H*-pyrimidin-1′-yl)-6-methoxy-3-pivaloyloxy-hexahydrofuro[3,2-*b*]pyran (25). To a solution of **8** (1.22 g, 3.0 mmol), *N*-4-acetyl bis-*O*-TMS cytosine (2.14 g, 7.2 mmol), and NIS (1.62 g, 7.2 mmol) in CH_2Cl_2 (60 mL) at room temperature TfOH (625 μL, 7.2 mmol) was added over a period of 15 min. After stirring for 6 h, additional portions of NIS (0.68 g, 3.0 mmol) and TfOH (260 μL, 3.0 mmol) were added, and the mixture was stirred for 6 h. The mixture was quenched by adding a saturated solution of $Na_2S_2O_3$ (100 mL) and then stirred for 15 min. After filtration over a pad of Hyflo, the two phases were separated. The aqueous layer was extracted with CH_2Cl_2 (50 mL × 2). The combined organic extracts were washed with aq. satd. $NaHCO_3$ (100 mL) and dried with $MgSO_4$. After concentration in vacuo, the crude product was purified by flash silica gel chromatography (EtOAc/MeOH, 100:1 to 99:1, gradient) to afford 1.29 g of **25** as colourless resin (96%). R_f 0.53 (SiO_2, 9:1 AcOEt/MeOH); $[\alpha]^{20}_D$ +98.8 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.1 (br s, 1H), 8.15 (d, 1H, J = 7.5 Hz), 7.48 (d, 1H, J = 7.5 Hz), 5.99 (s, 1H), 5.32 (d, 1H, J = 4.5 Hz), 4.70 (br t, 1H), 3.95 (dd, 1H, J = 10.6, 4.3 Hz), 3.87 (dd, 1H, J = 10.0, 2.8 Hz), 3.77 (dd, 1H, J = 10.0, 4.5 Hz), 3.58 (t, 1H, J = 10.6 Hz), 3.56 (ddd, 1H, J = 10.6, 4.3, 2.5 Hz), 3.46 (s, 3H), 2.25 (s, 3H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 170.9, 162.9, 154.3, 143.8, 96.8, 91.1, 76.6, 75.9, 72.3, 71.6, 65.8, 58.8, 57.6, 38.7, 29.4, 26.9; LC−MS *m*/*z* (relative intensity): 450 (M^+, 100).

The β-anomer of **8** was converted to **25** in 52% yield under identical experimental conditions.

(2*R*,3*R*,3a*S*,6*S*,7*R*,7a*R*)-[2-(4′-Amino-2′-oxo-2*H*-pyrimidin-1′-yl)-3-hydroxy-6-methoxy-hexahydrofuro[3,2-*b*]pyran-7-yl]-urea (3) (1-Cytosinyl-*N*-malayamycin A). To a solution of azide **25** (450 mg, 1.0 mmol) in anhydrous THF (30 mL) saturated with argon was added Me_3P (2.50 mL, 1 M solution in toluene, 2.5 mmol) at room temperature. After stirring for 30 min, water (150 μL) was added. The resulting mixture was refluxed for 40 min and then concentrated. The residue was dried under reduced pressure (0.1 mmHg) for 2 h and dissolved in dry CH_2Cl_2 (20 mL). To this solution was added trichloroacetyl isocyanate (480 μL, 4.0 mmol) at room temperature under argon atmosphere. After stirring for 1 h, CH_2Cl_2 was removed and the residue was dissolved in MeOH (25 mL) and 40% $MeNH_2$ in H_2O (25 mL) and stirred at room temperature over 3 days. Evaporation of the mixture and purification by flash silica gel chromatography (CH_2Cl_2/MeOH/25% aq. NH_4OH, 100:15:1.5 to 100:33:3.3, gradient) afforded 154 mg of 1-cytosinyl-*N*-malayamycin A **3**, as a pale colourless solid (45%). R_f 0.32 (SiO_2, 70:30:3 CH_2Cl_2/MeOH/25% aq. NH_4OH); $[\alpha]^{20}_D$ +91.7 (*c* 0.06 in MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 (d, 1H, J = 7.5 Hz), 5.87 (d, 1H, J = 7.5 Hz), 5.67 (s, 1H), 4.97 (m, 1H), 4.19 (d, 1H, J = 4.3 Hz), 4.05 (dd, 1H, J = 10.6, 2.3 Hz), 3.96 (dd, 1H, J = 11.6, 5.4 Hz), 3.66 (dd, 1H, J = 10.6, 5.3 Hz), 3.50 (m, 1H), 3.37 (s, 3H), 3.35 (m, 1H); ^{1}H NMR (500 MHz, D_2O) δ 7.56 (d, 1H, J = 7.5 Hz), 6.00 (d, 1H, J = 7.5 Hz), 5.73 (s, 1H), 4.96 (br s, 1H), 4.33 (d, 1H, J = 5.3 Hz), 4.10−4.02 (m, 2H), 3.86−3.79 (m, 1H), 3.65 (dd, 1H, J = 10.5, 5.0 Hz), 3.51 (app. t, 1H, J = 11.2 Hz), 3.39 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.7, 161.3, 156.9, 140.7, 94.8, 94.7, 76.9, 74.1, 73.5, 72.6, 66.6, 55.8, 48.8; FAB-MS *m*/*z* (relative intensity) 364 ($M + Na^+$, 35), 342 ($M + H^+$, 18), 325 ($M^+ - NH_2$, 8).

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