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Synthesis of the Antibiotically Active Part of Agrocin 84

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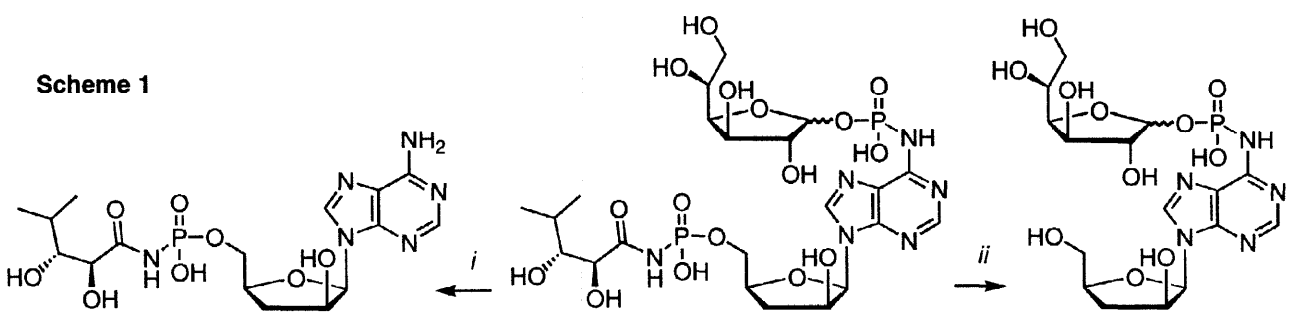
Abstract. Phosphitylation of bis-*O*-silylated *threo*-2,3-dihydroxy-4-methylpentanamide and condensation of the resulting *N*-acylphosphorodiamidite with 2'-*O*-acetyl-3'-deoxyarabinoadenosine led, after oxidation and deprotection, to the isolation of the title compound.

Nearly two decades ago, Kerr *et al.*[1] reported that the biological control of crown gall disease can be achieved by inoculation of susceptible plants with the non-pathogenic strain 84 of *Agrobacterium radiobacter.* Structural elucidation revealed that the controlling agent, so-called agrocin 84 (**1**), consists of the 3'-deoxy-D-arabinoadenosine core unit in which the N^6-exocyclic amino function of the adeninyl and HO-5' of arabinosyl units are connected *via* phosphoramidate bonds to the anomeric centre of D-glucofuranose and D-*threo*-2,3-dihydroxy-4-methylpentanamide, respectively. Structure-activity studies showed that fragment **2**, formed by brief heating (see Scheme 1) of agrocin 84 (**1**), exhibits a non-specific antibiotic activity for pathogenic strains. Moreover, subjection of agrocin 84 (**1**) to mild basic conditions gives fragment **3** displaying no antibiotic activity. However, the N^6-D-glucofuranosylphosphoramidate function in **3** proved to be essential for strain specificity of the antibiotic. Thus far, synthetic efforts in preparing agrocin 84 (**1**) have been limited to analogues of fragment **3** in which adenosine, instead of 3-deoxyarabinoadenosine, is either linked *via* a P-N(6)-phosphoramidate bond to HO-6 of methyl-β-D-glucopyranoside[2] or, as in agrocin 84 (**1**), to HO-1 (β) of D-glucofuranose.[3]

Scheme 1



2 **1** **3**

Reagents and conditions. *i.* 110 °C, pH 7.5, 10 min; *ii.* 25 °C, 1M NH_4OH, 3 h.

We here report for the first time a convenient route to the synthesis of the biologically active fragment **2.** A straightforward preparation of target compound **2** is hampered by two main factors. First of all, the P-O(5') bond is readily cleaved (*cf.* conversion of **1** into **3**) under mild basic conditions. Secondly, N→O phosphoryl

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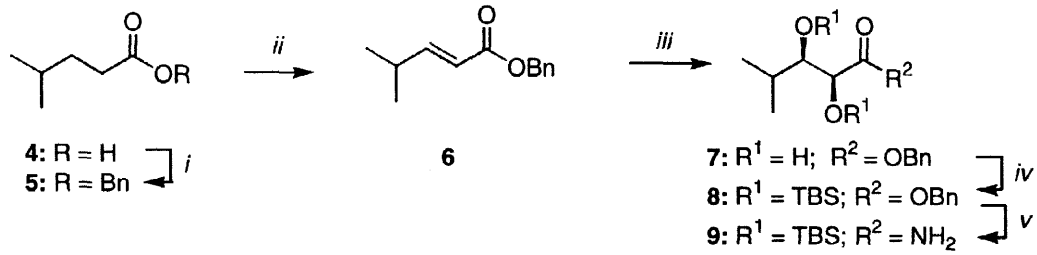
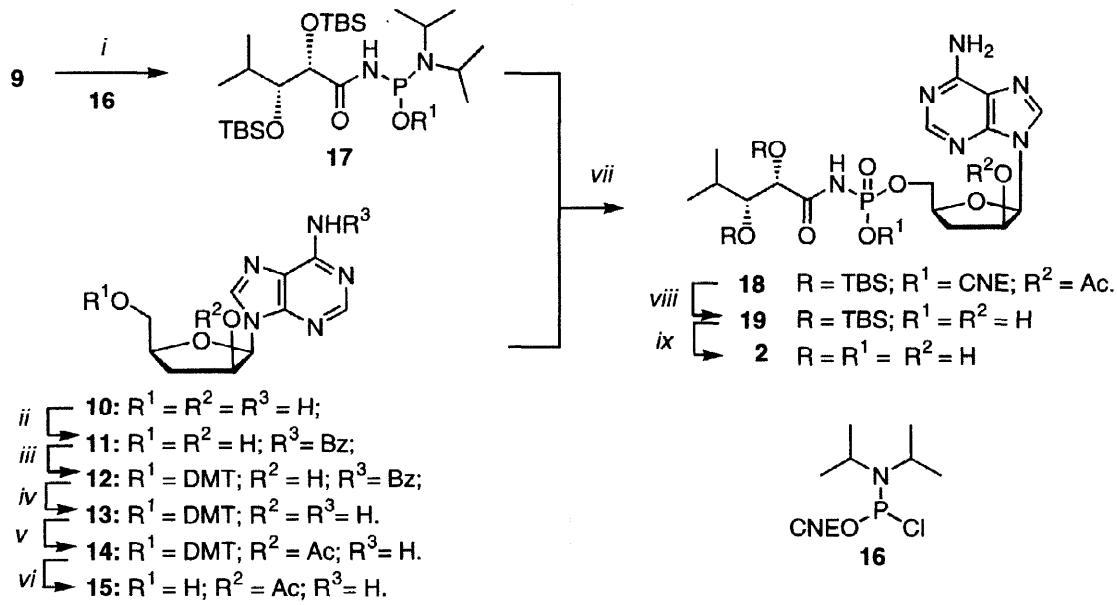
Scheme 2



Reagents and conditions: *i* DCC, DMAP, BnOH, 89 %; *ii* (a) LDA, PhSeBr (b) H_2O_2, 74%; *iii* AD-mix-β, 90%; *iv* TBS-Tf, 2,6-lutidine, CH_2Cl_2, 0 °C, 6 h, 85%; *v.* (a) H_2, Pd/C, *t*-BuOH/THF, 10 min; (b) BOP,DIPEA, NH_3, *t*-BuOH/THF, 20 min, 78 % (two steps) ;

migration of the *N*-acylphosphoramidate bond in **2** readily occurs[1] under mild acidic conditions. It was anticipated that protection of the diol function in the 2(S), 3(R)-dihydroxy-4-methylpentanamide unit with *tert*-butyldimethylsilyl (TBS) groups, as in compound **9** in Scheme 2, would not only be compatible with **2**, but also with the removal of the base labile groups required for transient protection of HO-2' in the 3'-deoxyarabinoadenosine as well as the *N*-acylphosphoramidate function. The preparation of key intermediate **9** is depicted in Scheme 2, and commences with the esterification of commercially available 4-methylpentanoic acid (**4**). Thus, condensation of **4** with benzyl alcohol under the influence of DCC/DMAP gave the benzyl ester **5**, which was converted into the corresponding *E*-alkene derivative **6** involving phenyl selenation followed by

Scheme 3



Reagents and conditions: *i.* DIPEA, CH_2Cl_2, 100%; *ii.* (a) TMS-Cl, Pyridine, 20 min; (b) BzCl, Pyridine, 2 h; (c) $NH_3 \cdot H_2O$, Pyridine 0 °C 30 min; *iii.* DMT-Cl, Pyridine, 4h; *iv.* NH_3/MeOH, 60 h; *v.* Ac_2O, Pyridine, 4 °C, 32 h; *vi.* DCA/CH_2Cl_2, $HSnBu_3$, 0 °C, 30 min; *vii.* (a) 1-MTT (6 eq.), CH_3CN, 1 h; (b) I_2 (0.2 M in pyridine)/THF/H_2O, 0 °C; *viii.* $NH_3 \cdot H_2O$/MeOH (2/1), 15 h; *ix.* 1M TBAF in DMF, 6 min.

oxidation[4]. Sharpless asymmetric dihydroxylation[5] of **6** afforded **7** (95% ee), the *threo* configuration of which was firmly established by its conversion into known *threo*-2,3-dihydroxy 4-methylpentanoic acid {$[\alpha]_D$ +12.8 (c 0.36, EtOH); $[\alpha]_D$ lit.[1] +13.6}. Silylation of **7** with *tert*-butyldimethylsilyl triflate (TBS-Tf) in the presence

of 2,6-lutidine proceeded smoothly[6] to yield the fully protected derivative **8**. Transformation of **8** into the required building unit **9** could be realized most effectively by executing the following three-step one-pot procedure. Thus, reductive debenzylation of **8**, and subsequent *in situ* activation of the free carboxylic acid group with BOP[7] in the presence of diisopropylethylamine (DIPEA), followed by bubbling dry gaseous ammonia through the reaction mixture gave **9** in a yield of 39% over the seven steps.

The 2'-*O*-acetyl-3'-deoxyarabinoadenosine building block **15** was readily accessible (Scheme 3) from known[8] 3'-deoxyarabinoadenosine (**10**) by the following well established protecting group manipulations. Tritylation of the N^6-benzoyl derivative **11**, prepared from **10** by the Jones *N*-acylation procedure[9], with 4,4'-dimethoxytrityl chloride (DMT-Cl) followed by ammonolysis of the N^6-benzoyl group of **12** led to the partially protected derivative **13**. Selective *O*-acylation[10] of **13** with acetic anhydride at 4 °C gave, after detritylation of **14** with dichloroacetic acid (DCA) in the presence of the effective cation scavenger tributyltin hydride, the 2'-*O*-acetyl-3'-deoxyarabinoadenosine **15** in an overall yield of 33% (based on **10**).

In line with our previous experiences[11], introduction of the *N*-acylphosphoramidate bond between the two building units **9** and **15** could be readily accomplished *via* the two-step phosphitylation protocol depicted in Scheme 3. Thus, phosphitylation of **9** with the bifunctional reagent **16** in the presence of DIPEA gave, after purification by silica gel chromatography, the monofunctional phosphorodiamidite **17** in a near quantitative yield. Selective *O*-phosphitylation of **15** with **17** using an excess of the rather acidic activating reagent 1-methyltetrazoline-5-thione[12] (1-MTT) gave, after *in situ* oxidation of the transiently formed *N*-acylphosphoramidite intermediate with iodine in aqueous pyridine, the fully protected compound **18**[13] in a near quantitative yield. Removal of the two base-labile protecting groups (*i.e.* acetyl and cyanoethyl) with aqueous ammonia for 15 h at 20 °C yielded, as gauged by ^{31}P NMR spectroscopy, the partially deprotected product **19**. Subsequent desilylation of **19** with tetrabutylammonium fluoride (TBAF) in dry DMF for 6 min, followed by

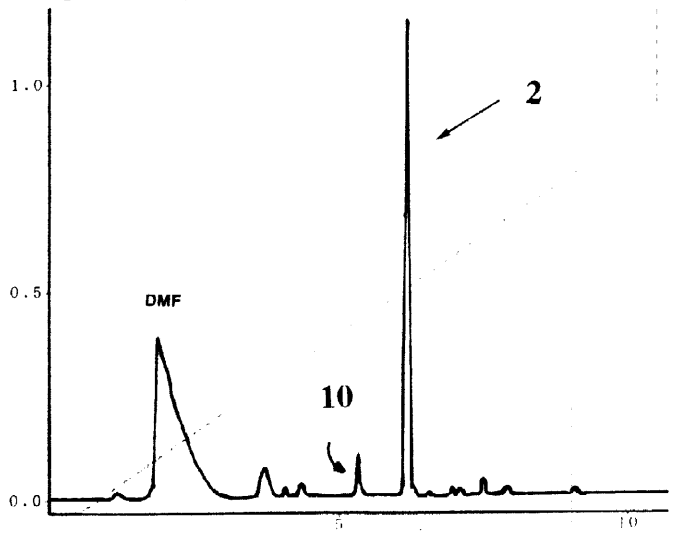


Figure 1. HPLC trace of crude **2**.

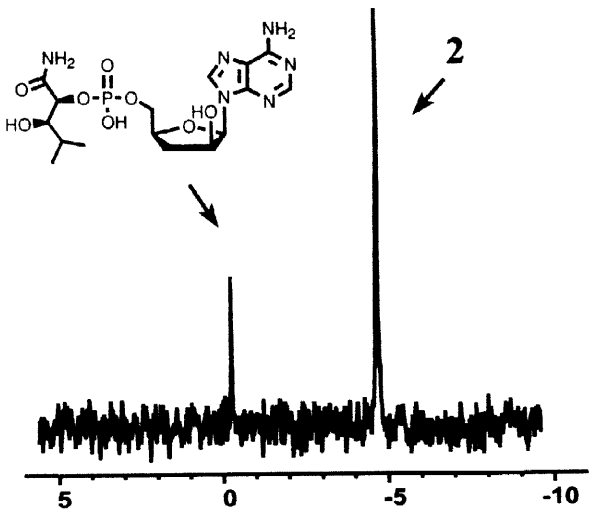


Figure 2. ^{31}P NMR spectrum of **2** after mild acid treatment

quenching with triethylammonium acetate (TEAA) buffer (pH 7, 1 M) afforded crude **2** which was *inter alia* contaminated (see Fig. 1) with a small amount (4.6%) of 3'-deoxyarabinoadenosine (**10**). Purification of the crude product by RP HPLC [(MeCN/TEAA (20 mM, pH 7.5)] gave homogeneous **2** in 35% yield. The homogeneity and structure of **2**, fully ascertained by analytical as well as spectroscopic data,[13] was also independently confirmed by the following chemical evidence. Subjection of **2** to H_2O-HOAc (9/1, v/v) for 2.5 h at ambient temperature led to a product having the same retention time (HPLC) and ESI-MS spectrum as the starting product. However, the ^{31}P NMR spectrum showed two distinct resonances (see Fig. 2). The major

resonance at δ_P -4.8 ppm coincided with the signal observed for **2** and the minor resonance at δ_P -0.2 ppm may be attributed to the earlier by Kerr *et al.*[1] proposed N→O phosphoryl migration product of **2.**

The results presented in this paper clearly show that the *N*-phosphorodiamidite derivative **17** is an effective building unit in the synthesis of the biologically active part of agrocin 84 (**1**). In addition, the partially protected compound **18** promises to be of great value in the future preparation of agrocin 84 (**1**) and analogs thereof.

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References and Notes

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13. Compound **18** (2:1 mixture of diastereoisomers at phosphorus): ^{31}P (80.7 MHz, $CDCl_3$) -1.57, -1.63. ^{1}H NMR *^{1}H-resonances marked with asterisk showed apparent splitting due to presence of the minor P-diastereoisomer* (200 MHz, $CDCl_3$) δ 8.33 (s 1H H-8), 8.13* (s 1H H-2), 7.92* (br. d 1H NH J_{PH} 13), 6.45 (d 1H H-1' $J_{1'2'}$ 5.2); 5.72 (br. s 2H NH_2); 5.53 (m 1H H-2'); 4.44 (m 5H H-4', H-5', $CH_2C\underline{H}_2O$); 4.12* (d 1H H-2" *J* 3.6); 3.56 (dd 1H H-3" J_{32} 3.3, J_{34} 6.5); 2.63 (m 3H H-3'a, $C\underline{H}_2CH_2O$); 2.34 (m 1H H-3b); 1.84* (s 3H CH_3 Ac); 1.64 (br. m 1H H-4"); 0.98, 0.95, 0.93, 0.91 (m 24H H-5", CH_3C TBS); 0.12, 0.05, 0.04, -0.01 (s 12H CH_3Si). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.7 (CONH), 169.1 (C=O, Ac); 155.5 (C-6); 152.7 (C-2); 149.4 (C-4); 139.7 (C-8); 118.9 (C-5); 116.3 (CN); 84.3, 83.9 (C-1'); 78.6 (C-3"); 75.5, 75.4 (C-4', C-2"); 71.7, 71.6 (C-2'); 68.8, 68.4 (CH_2, CNE); 62.3, 62.1, 62.0 (C-5'); 32.3, 32.0 (C-3'); 30.7 (C-4"); 25.7, 25.5, 20.3, 19.8 (CH_3, tBu); 20.3 (CH_3, Ac); 19.8 (C-5") 19.6, 19.4 ($\underline{C}H_2CN$); 18.9 (C-5a"); 17.9 (C_q, tBu); -5.3, -5.0, -4.5 (CH_3, MeSi).
Compound **2**: ^{31}P (243 MHz, D_2O) -4.7. ^{1}H NMR (600 MHz, D_2O, NH_4^+- form) δ 8.53 (1H s H-8), 8.13 (1H s H-2), 6.26 (1H d H-1' $J_{1'2'}$ 5.2); 4.76 (m 1H H-2'); 4.41 (m 1H H-4'); 4.17 (m 2H H-2", H-5'); 4.09 (m 1H H-5'a); 3.44 (dd 1H H-3" J_1 1.3, J_2 9); 2.46 (ddd 1H H-3'a J_1 6.7, J_2 6.7, J_{gem} -13.4); 2.09 (ddd 1H H-3'b J_1 8.5, J_2 8.5, J_{gem} -13.3); 1.77 (m 1H H-4"); 0.92 (d 3H H-5" J_{54} 6.7); 0.83 (s 3H H-5"a J_{5a4} 6.9). ^{13}C NMR (151 MHz, D_2O, NH_4^+- form) δ 178.6 (C-1"); 157.8 (C-6); 151.2 (C-2); 149.3 (C-4); 142.5 (C-8); 119.2 (C-5); 86.0 (C-1'); 78.4 (C-3"); 77.8 (C-4', $^3J_{CP}$ 8.6); 73.0 (C-2", $^3J_{CP}$ 6.6); 71.5 (C-2'); 66.8 (C-5', $^2J_{CP}$ 4.3); 32.8 (C-3'); 30.6 (C-4"); 19.2, 18.9 (C-5", C-5a"). ESI-MS (m/z) 459.0 $(M-H)^-$.