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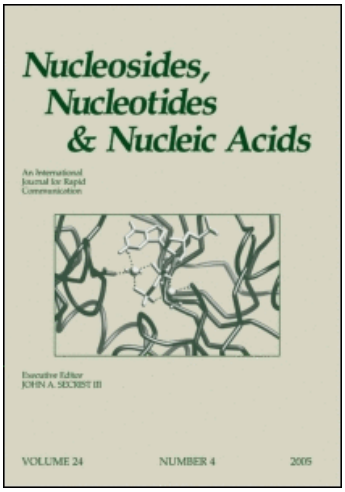

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NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1403-1406 (1997)

A STUDY TOWARDS TOTAL SYNTHESIS OF ANTIBIOTIC AGROCIN 84

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Abstract. A versatile synthetic route to compound **2** - an analog of the naturally occurring bacteriocin Agrocin 84 is described.

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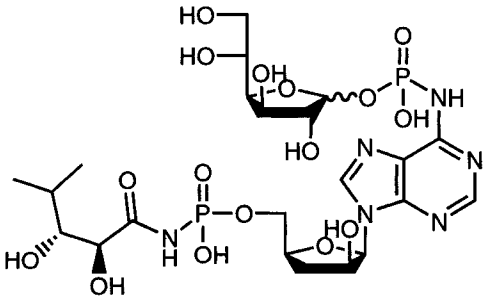
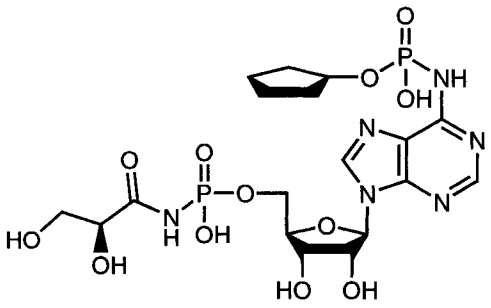
The naturally occurring antibiotic Agrocin 84 (**1**) acts with a high degree of specificity against strains of *Agrobacterium* which cause cancer in plants[1]. Agrocin 84, a N^6, 5'-*O*-diphosphorylated 9-(3'-deoxy-β-D-*threo*-pentafuranosyl)adenine derivative[2], interferes with DNA synthesis in bacterial cells[3]. The D-glucofuranosylphosphoramidate at the exocyclic amino group of adenine is responsible for cellular uptake of **1**. On the other hand, the toxicity of Agrocin 84 is determined by *N*-(D-*threo*-2,3-dihydroxy-4-methylpentanoyl)phosphoramidate attached to the 5'-hydroxyl of the deoxyarabinose residue[1].



Agrocin 84 (1)



Model compound (2)

With the objective to gain more insight into the formation of the phosphoramidate linkages, we here report the preparation of model compound **2**.

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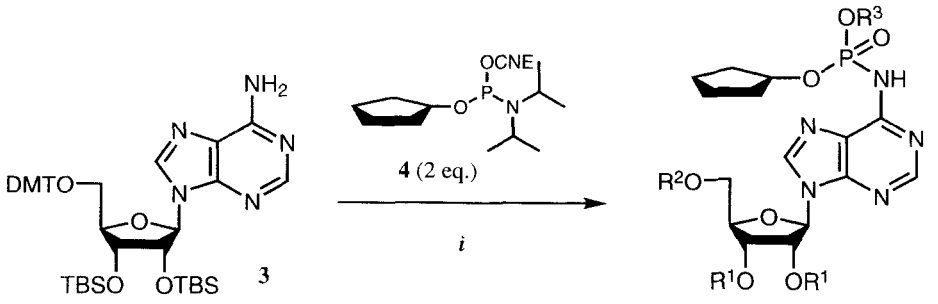
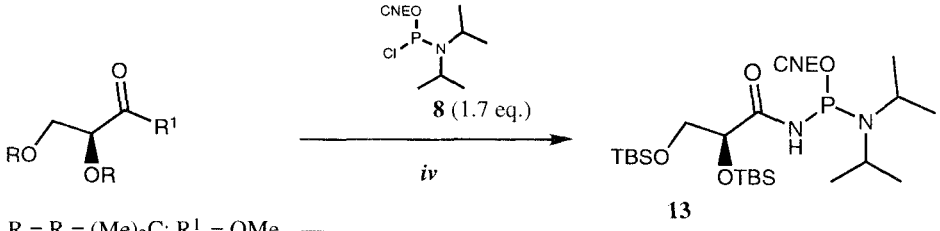


Reagents and conditions:
i (a) 1*H*-tetrazole (2 eq.), CH_3CN/CH_2Cl (1/1), 10 min (b) tBuOOH, 10 min, 73%; *ii* 2% DCA/CH2Cl, $HSnBu_3$ (1.2 eq.), 0 °C, 50 min, 95% .

ii →
5: R^1 = OTBS; R^2 = DMT; R^3 = OCNE
6: R^1 = OTBS; R^2 = H; R^3 = OCNE.
15: $R^1 = R^2 = R^3$ = H

Scheme 1



9: R = R = $(Me)_2C$; R^1 = OMe
10: R = R = $(Me)_2C$; R^1 = NH_2
11: R = R = H; R^1 = NH_2
12: R = R = TBS; R^1 = NH_2

i, *ii*, *iii*

Reagents and conditions:
i NH3/MeOH, 2 h; *ii* Dowex-H^+/ MeOH, 3 h; *iii* TBS-Cl, imidazole, DMF, 24 h 75% over the 3 steps; *iv* DIPEA (5 eq.), 2 h, 60%.

Scheme 2

It was envisaged that target molecule **2** could be prepared by sequential introduction of the N^6-phosphoramidate[4] and 5'-(*N*-acylphosphoramidate)[5] functions at an appropriately protected adenosine derivative *via* phosphoramidite chemistry.

To this end, we first prepared phosphoramidite **4** (δ_p 147.0 ppm) by phosphitylation of cyclopentanol with phosphochloridite **8** and triethylamine. Reaction of 2',3'-di-(*O-tert*-Butyldimethylsilyl)-5'-*O*-(4,4'-dimethoxytrityl)adenosine[6] (**3**) with phosphoramidite **4** in the presence of 1*H*-tetrazole and subsequent oxidation with *t*BuOOH afforded, after purification on Sephadex LH-20 column, homogeneous phosphoramidate **5** (δ_p - 1.9 ppm) in 73% yield. Removal of 5'-DMT-group in **5** with 2% dichloroacetic acid in the presence of the cation scavenger $HSnBu_3$ gave, after silica gel column chromatography, partially protected N^6-phosphorylated nucleoside **6** in an excellent yield .

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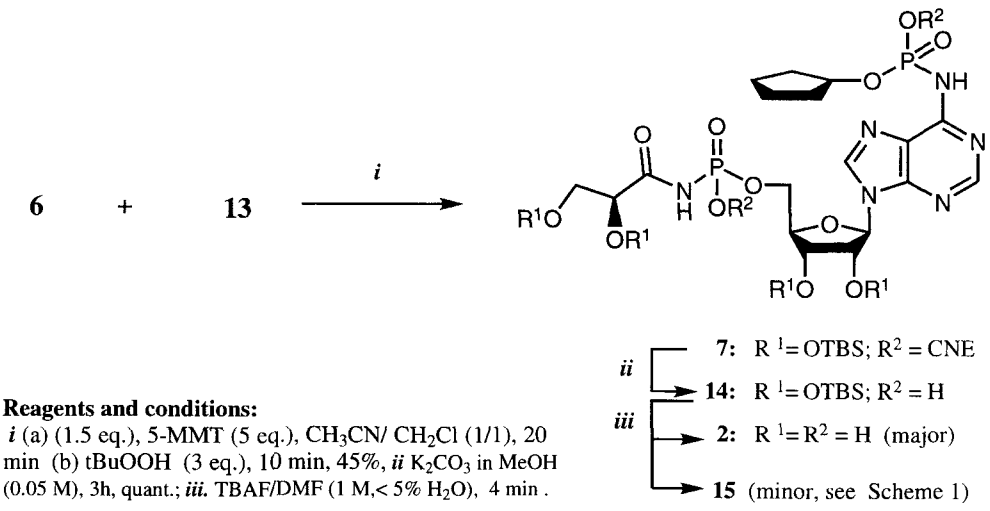

Reagents and conditions:
i (a) (1.5 eq.), 5-MMT (5 eq.), CH_3CN/ CH_2Cl (1/1), 20 min (b) tBuOOH (3 eq.), 10 min, 45%, *ii* K_2CO_3 in MeOH (0.05 M), 3h, quant.; *iii.* TBAF/DMF (1 M,< 5% H_2O), 4 min .

Scheme 3

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Next, the introduction of the *O*-(*N*-acylphosphoramidate) group at the 5'-OH of **6** was undertaken. In order to achieve this goal, we first prepared the phosphitylating reagent **13** starting from the commercially available L-glyceric acid derivative **9** (Scheme 2). Thus, ammonolysis of methyl ester **9** was followed by cleavage of the isopropylidene group in **10** with Dowex-H^+/MeOH, and then silylation of the resulting α,β-dihydroxyamide **11**, afforded (*S*)-2,3-O-di(*t*-butyldimethylsilyloxy)propamide (**12**) in 75% yield based on **9**. Phosphitylation[5] of **12** with reagent **8** in the presence of DIPEA[7] gave **13** (δ_P 118.0, 116.6 ppm) in 60% yield. 5-Mercapto-1-methyltetrazole[8] (5-MMT)-assisted phosphitylation[9,5] of **6** with reagent **13**, followed by oxidation of the intermediate phosphoramidite with *t*BuOOH, and purification (silica gel column chromatography/gel filtration on Sephadex LH-20), gave the fully protected target compound[10] **7** in 45% yield.

At this stage, attention was focused on the removal of the protective groups in **7** to furnish the target molecule **2**. First, the fully protected phosphoramidate **7** (δ_P -1.6, -2.4 ppm) was quantitatively converted with methanolic potassium carbonate into decyanoethylated derivative **14** (δ_P -4.3, -5.4), as gauged by ^{31}P NMR. Desilylation proceeded smoothly under the action of fluoride ion (TBAF/DMF). Analysis of the reaction mixture by RP HPLC[11] revealed the presence of two UV absorbing products (ratio 1/7). The two compounds were readily separated by RP HPLC and characterized by NMR-spectroscopy and mass-spectrometry. The structure of the major product, isolated in 70% yield as triethylammonium salt[12], was in comlete agreement with that of the target molecule **2**. On the other hand the analytical data of the minor product (11 %) were in accordance with those of nucleoside **15** lacking 5'-phosphoramidate substituent[12] .

In conclusion, the successful synthesis of Agrocin 84 analog **2** *via* a phosphoramidite approach is presented. The application of the latter methodology to the total synthesis of Agrocin 84 is now in progress.

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7. Abbreviations used: CNE, 2-cyanoethyl; DMAP, 4-dimethylaminopyridine; DIPEA, *N,N*-diisopropylethylamine; DMT, 4,4'-dimethoxytrityl; TBS, *tert*-butyldimethylsilyl; DCA, trichloroacetic acid; TEA, triethylamine; TEAA, triethylammonium acetate; 5-MMT, 5-mercapto-1-methyltetrazole.
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10. ^{31}P NMR δ -1.6, -2.4 ppm, *m/z* (electrospray) 1445.4 $(M+H)^+$, 1167 $(M+Na)^+$.
11. Analytical HPLC was performed at 40 °C on LiChrospher[4,22] 100 RP-18 column (Merck, 5 μm, 4.6 x 250 mm) using linear gradient from 0 to 50% of buffer B (0.05 M TEAA in 1:1 MeCN/water) in buffer A (0.05 M TEAA in 5% aq. MeCN); R_t 9.8 min (compound **2**), 13.8 min (compound **15**). Preparative HPLC was done at 20 °C on Econosphere[4,22] C-18 column (AllTech, 10 μm, 250 x 22.5 mm) using linear gradient from 0 to 30% of B (0.02 M TEAA in 1:1 MeCN/water) in buffer A (0.05 M TEAA in 5% aq. MeCN)
12. It is noteworthy that sodium salt of compound **2** could be prepared by passing a solution of **2** in water through a pad of Dowex-Na^+ resin but found to be unstable and decomposed slowly releasing N-phosphorylated adenosine **15**. Compound **2**: (TEA^+-salt): ^{1}H-COSY NMR (600 MHz, /D_2O) δ 8.51 (1H, s H-arom), 8.41 (1H, s, H-arom), 6.14 (1H, d, H-1', $J_{1'2'}$ 5.8), 4.44 (1H, dd, H-3', J_1 3.7, J_2 5.1), 4.34 (1H, m, H-4'), 4.14 (3H, m, H-5', H-2''), 3.74 (2H, m, AA'X, H-3''), 3.17 (6, q, CH_2, TEA^+ J 7.3) 1.64 (6H, m, cyclopentyl), 1.46 (2H, m, cyclopentyl), 1.24 (9, t, CH_3, TEA^+ J 7.3). MS (electrospray, negative mode) *m/z* 581 $(M-H)^-$, 603 $(M-2H+Na)^-$. Compound **15** (Na^+-salt): ^{1}H-COSY NMR (300 MHz, D_2O) δ 8.44 (1H, s H-arom), 8.40 (1H, s, H-arom), 6.11 (1H, d, H-1', $J_{1'2'}$ 6.1), 4.45 (1H, dd, H-3', J_1 3.5, J_2 5.2), 4.31 (1H, m, H-4'), 3.89 (2H, m, AA'X, H-5'), 1.57 (8H, m, cyclopentyl). MS (electrospray) *m/z* 438 $(M+Na)^+$, 460 $(M-H+2Na)^+$, 482 $(M-2H+3Na)^+$.

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