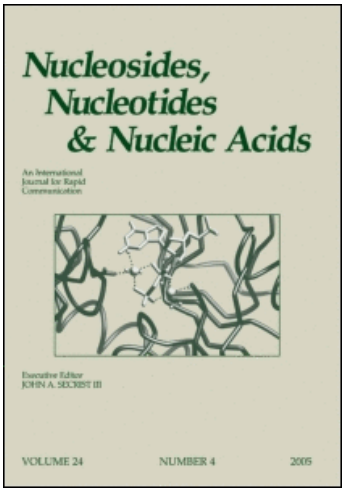

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A Practical Synthesis of The Antibiotic Toyocamycin

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NOTE

A PRACTICAL SYNTHESIS OF THE ANTIBIOTIC TOYOCAMYCIN

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Abstract

The nucleoside antibiotic toyocamycin was synthesized by condensation of the silylated 4-amino-6-bromo-5-cyanopyrrolo[2,3-d]pyrimidine with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose, followed by debromination and deblocking.

Introduction

Our synthesis of sangivamycin derivatives as potential inhibitors of protein kinases[1] required the large-scale preparation of the precursor toyocamycin. Although several methods for the synthesis of toyocamycin related nucleosides were available, they did not readily lend themselves for scale-up, primarily because of the difficulties encountered in coupling the sugar to pyrrolo[2,3-d]pyrimidines. Thus, ribosidation by the fusion procedure[2] provided protected toyocamycin in only 9% yield. While glycosidation of the sodium salt[3] of pyrrolo[2,3-d]pyrimidines significantly improved the yield of the coupling reaction, the requirement for specifically protected α-ribofuranosyl chloride is inconvenient. In this communication we show that the reaction of commercially available 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose with silylated 4-amino-6-bromo-5-cyano-[2,3-d]pyrimidine in the presence of trimethylsilyl trifluoromethylsulfonate[4] provided protected 6-bromotoyocamycin in high yield.

Chemistry

2-Amino-5-bromo-3,4-dicyanopyrrole[5,6] (**1**), 4-amino-6-bromo-5-cyanopyrrolo-[2,3-d]pyrimidine[2] (**2**) and 4-acetamido-6-bromo-5-cyanopyrrolo[2,3-d]-pyrimidine[2] (**3**) were prepared by the reported procedures. The coupling reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribofuranose with silylated 4-acetamido-6-bromo-5-cyano-pyrrolo[2,3-d]pyrimidine (**3**) was monitored by TLC which showed two products during the initial stages of the reaction. As the reaction progressed, the lower R_f

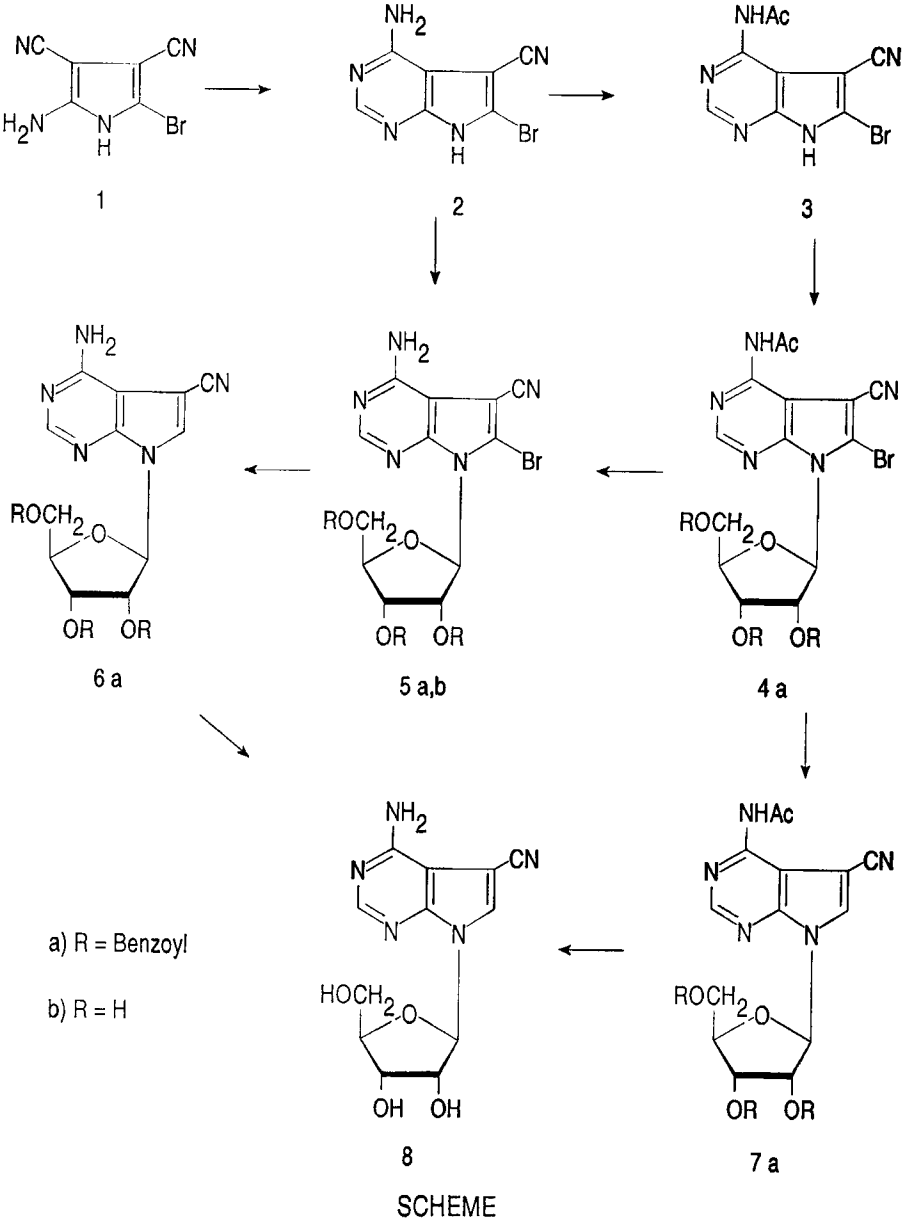
component, presumably an isomeric nucleoside, gradually diminished in amount and at the end of the reaction only the less polar product was present in the reaction mixture. When coupling proceeded in the presence of excess sugar, another high-R_f product was formed, tentatively identified by NMR as a diribofuranosyl nucleoside. The coupling procedure was further simplified by the use of silylated 4-amino-6-bromo-5-cyano-pyrrolo[2,3-d]pyrimidine (**2**). Using this intermediate, formation of the diribosyl by-product was not observed, even when excess sugar was used. Debromination of the protected nucleosides was carried out by hydrogenation over Pd/C in dioxane and in the presence of triethylamine, followed by deprotection by methanolic ammonia to give toyocamycin (**8**). This approach was chosen because the original procedure[2], which debrominates the deblocked nucleoside by hydrogenation in ethanol, is hindered by the poor solubility of both the starting material and the product. Assignment of the site of ribosidation in **5b** and **8** as N-7 was made on the basis of their ultraviolet spectra[2]. The structure of the nucleoside **8** was confirmed by a rigorous comparison with an authentic sample of toyocamycin[2].

Conversion of toyocamycin into sangivamycin was accomplished by the reported method[2].

Experimental

All melting points, taken in open capillary tubes in a Mel-Temp apparatus, are uncorrected. Elemental analyses were performed by Robertson Laboratory, Morison, N.J. Thin-layer chromatography was carried out using Analtech Uniplate GF 250 silica gel plates (EM Reagents). EM Science silica gel-60 (230-400 mesh) was used for flash column chromatography. Infrared and UV spectra were recorded on Perkin-Elmer 457 and 710 B spectrophotometers using KBr pellets and on a Cary 14 spectrophotometer, respectively. ^{1}H NMR spectra were recorded on a Varian 390 spectrometer, and chemical shifts are given in ppm using tetramethylsilane as internal standard. Solvents were evaporated under diminished pressure at bath temperatures below 35° C.

4-Acetamido-6-bromo-5-cyano-7-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine (**4a**) A mixture of dry 4-acetamido-6-bromo-5-cyanopyrrolo[2,3-d]-pyrimidine (**3**, 9.0 g, 32 mmol), xylene (40 mL), hexamethyldisilazane (40 mL) and chlorotrimethylsilane (0.5 mL) was heated and stirred at reflux temperature for 10 hr. The solution was evaporated to an oily residue, which was co-evaporated three times with dry toluene (3 x 40 mL), and dried at 40° C (0.01 mm Hg) for 1 hr. A solution of silylated **3** and 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribofuranose (10.0g, 19.8 mmol) in 1,2-dichloroethane (500 mL) was cooled to 0° C, and a solution of trimethylsilyl trifluoromethanesulfonate (10 mL, 40 mmol) in 1,2-dichloroethane (30 mL) was added dropwise with stirring during 15 min. The resulting mixture was stirred at room temperature for 1 hr and at 80-85° C for 20 hr. It was then cooled to 0° C and diluted with methylene chloride (200 mL), poured into a stirred mixture of sodium hydrogen carbonate (20 g), ice and water, and filtered. The filtered unreacted pyrrolopyrimidine base (2.5 g) was washed with methylene chloride (50 mL), and the combined filtrate washed gently (to avoid emulsion) with water and dried ($MgSO_4$). The solvent was removed by evaporation to give a foamy solid, which was taken up in chloroform (50 mL) and poured onto a short column of silica gel. The column was eluted with ethyl acetate/petroleum ether (1/4) to remove impurities and further elution with ethyl



SCHEME

acetate/petroleum ether (1/1) yielded the product as a light colored foam (17.5 g, 90%). R_f= 0.3 (chloroform/ethyl acetate, 4/1); IR (KBr) ν_{max} 3300 (NH), 2200 (CN), 1720 (C=O), 1600 (C=O, amide), 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.15 (3H, s, N-Ac), 4.85 (3H, m), 6.15 (2H, d, J=3.00 Hz), 6.30 (1H, m, H-1'), 7.25 (9H, m, aromatic), 7.70 (6H, m, aromatic), 8.40 (1H, s, H-2), 8.80 (1H, br. s, NH). Anal. Calcd for: $C_{35}H_{26}BrN_5O_8$: C,58.01; H,3.59; N,9.66. Found: C,57.99; H,3.60; N,9.73.

4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (5a) 4-Amino-6-bromo-5-cyanopyrrolo[2,3-d]-pyrimidine (2, 5.0 g, 21 mmol) was sequentially silylated with hexamethyldisilazane (30 mL) and chlorotrimethylsilane (0.5 mL) as described for the preparation of **4a** and dissolved together with dry 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribofuranose (10.0 g, 19.8 mmol) in anhydrous 1,2-dichloroethane (300 mL). The solution was cooled to 0° C and a solution of trimethylsilyl trifluoromethanesulfonate (8 mL, 32 mmol) in 1,2-dichloroethane (25 mL) was added dropwise and with stirring during 15 min. The reaction mixture was stirred under N_2 at room temperature for 1 hr; at 75 - 80° C for 18 hr, and at reflux for a further 3 hr. The deep brown solution was cooled to 0° C, diluted with methylene chloride (200 mL) and poured into a stirred mixture of ice, water, and sodium hydrogen carbonate (14.0 g, 115 mmol). The mixture was filtered and unreacted **2** was washed with methylene chloride (50 mL) to recover 1.5 g of **2**. The combined filtrate was washed with water (2x 50 mL), dried ($MgSO_4$), and evaporated to a brown foam. Chromatography of this foam on a short column of silica gel using ethyl acetate/toluene (1/5) gave the product (8.8 g, 88 %), which was recrystallized from toluene. MP 180-2° C; R_f = 0.35 (ethyl acetate/chloroform, 1/4), IR (KBr) ν_{max} 3350 (NH), 2200 (CN), 1720 (CO, ester), 1600, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.85 (3H, m), 5.65 (2H, br.s, NH_2), 6.20 (1H, d, J=3.5 Hz, H-1'), 6.60 (2H, m), 7.30 (9H, m, aromatic), 7.85 (6H, m, aromatic), 8.15 (1H, s, H-2).
Anal. Calcd for: $C_{33}H_{24}N_5O_7Br$: C, 57.94; H,3.80; N,10.24. Found: C,58.04; H,3.76; N,10.04.

4-Amino-5-cyano-7-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (2,3,5-tri-O-benzoyltoyocamycin) (6a) A solution of **5a** (8.2 g, 12.0 mmol) and triethylamine (8 mL) in anhydrous dioxane (550 mL) was stirred at room temperature under atmospheric pressure H_2 for 5 hr in the presence of palladium on charcoal (10 %, 2 g). The mixture was filtered, the catalyst washed with chloroform (50 mL), and the combined filtrate evaporated. The residue was taken up in chloroform (300 mL), washed with water, dried ($MgSO_4$), and the solvent removed by evaporation to give **6a** as a white foam (6.8 g, 94%), which crystallized from methanol/ethanol. MP 169-70° C; R_f = 0.2 (ethyl acetate/chloroform, 1/4); IR (KBr) ν_{max} 3300 (NH), 2200 (CN), 1720 (CO), 1595, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.7 (3H, m), 5.85 (2H, br. s, NH_2), 6.15 (2H, d, J= 3.1, Hz), 6.5 (1H, d, J= 3.2 Hz, H-1'), 7.3 (9H, m, aromatic), 7.60 (1H, s, H-6), 7.90 (6H, m, aromatic), 8.20 (1H, s, H-6).
Anal. Calcd for: $C_{33}H_{25}N_5O_7$: C,65.67; H,4.17; N,11.60. Found: C,65.52; H,4.32; N,11.54.

4-Acetamido-5-cyano-7-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine (7a) A solution of the 6-bromo derivative **4a** (12.0 g, 16.6 mmol) and triethylamine (12 mL, 82 mmol) in anhydrous dioxane (300 mL) was stirred in the presence of palladium on charcoal (10%, 2 g) at room temperature under H_2 for 5 hr. The mixture was filtered and the solids washed with chloroform (50 mL) and the combined filtrate evaporated. The residue was dissolved in chloroform, washed with water, dried (Na_2SO_4) and evaporated to a light-colored foamy solid (9.8 g, 91%). R_f = 0.2 (chloroform/ethyl acetate, 4/1); IR (KBr) ν_{max} 3300 (NH), 2195 (CN),

1710 (C=O), 1600 (C=O, amide), 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (3H, s, N-Ac), 4.65 (3H, m), 6.10 (2H, m), 6.50 (1H, d, J= 3.50 Hz, H-1'), 7.40 (9H, m, aromatic), 7.80 (1H, s, H-6), 7.95 (6H, m, aromatic), 8.45 (1H, br. s, NH), 8.55 (1H, s, H-2). Anal. Calcd for: $C_{35}H_{27}N_5O_8$: C,65.11; H,4.22; N,10.85. Found: C,64.98; H,4.01; N,10.75.

4-Amino-6-bromo-5-cyano-7-(ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (6-Bromotoyocamycin (5b) A saturated solution of ammonia in anhydrous methanol containing **4a** (1.4 g, 1.9 mmole) was stirred at room temperature for 24 hr and evaporated. The residue was dried under reduced pressure (0.01 mm Hg) which was washed with ether-MeOH and crystallized from water to give 625 mg (87%) of **5b**. MP 247-49° C (Lit.[2] MP 245-50° C); IR (KBr) ν_{max} 3450-2800 (NH, OH), 2230 (CN), 1610 cm^{-1}; UV (EtOH) λ_{max} 282, (pH 1) 282, 232, (pH 11) 283 nm.

4-Amino-5-cyano-7-(ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (Toyocamycin, 8)

a) A solution of ammonia in dry methanol (800 mL, saturated at 0° C) was added to **6a** (15 g, 24.8 mmol) and the mixture stirred for 24 hr at room temperature in a stoppered vessel. The resulting solution was evaporated and the residue dried under reduced pressure (0.01 mm Hg). The residue was triturated with ethyl ether (2 x 50 mL), filtered and crystallized from water to give 6.2 g (89 %) of **8**. MP 240 - 2° C (Lit[2] MP 243 ° C), mixture MP of **8** with an authentic sample of toyocamycin 240 - 3° C. ; R_f = 0.7 (methanol/ethyl acetate, 1/4); IR (KBr) ν_{max} 3400 - 2800 (NH, OH), 2205 (CN), 1600 cm^{-1}; UV (EtOH) λ_{max} 289 (s), 279, 232, (pH 1) 271, 231, (pH 11) 285 nm.

b) Using the same conditions, treatment of compound **7a** (10.0 g, 15.5 mmol) with methanolic ammonia (400 mL) gave 3.85 g (82 %) of toyocamycin (**8**).

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