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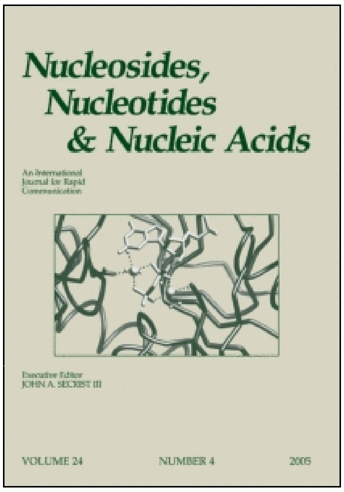

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TOTAL SYNTHESIS OF THE NATURALLY OCCURRING ANTIBIOTIC TOYOCAMYCIN USING NEW AND IMPROVED SYNTHETIC PROCEDURES

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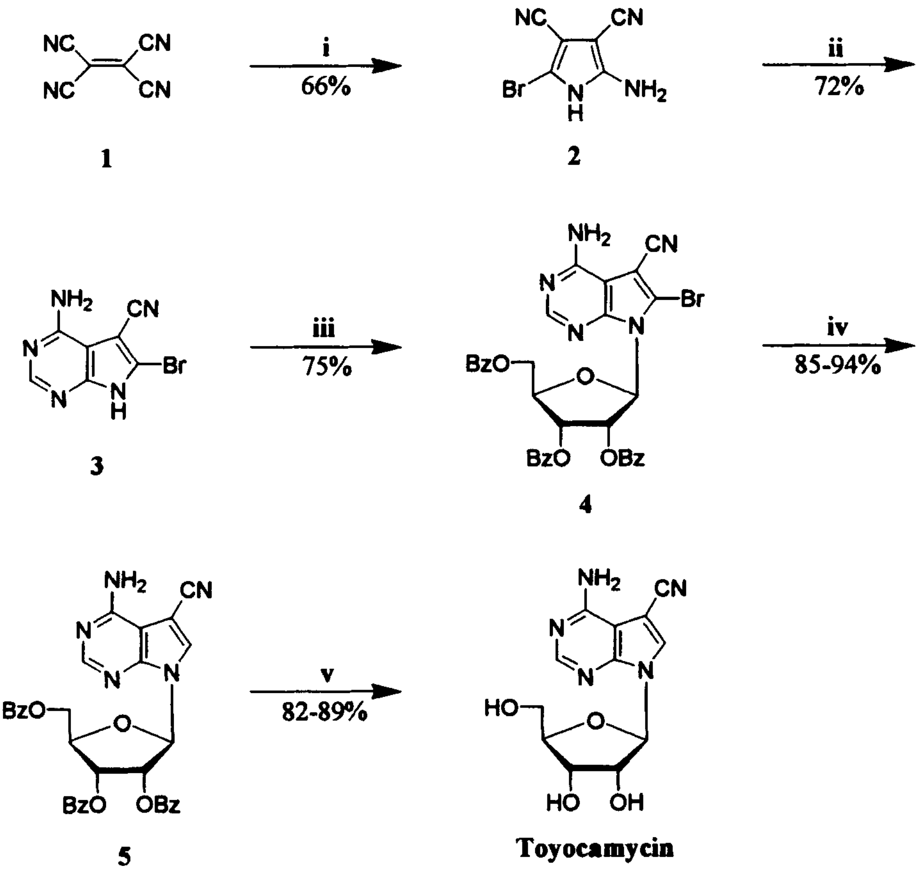
ABSTRACT: Starting with commercially available tetracyanoethylene, we describe a more efficient and higher yielding synthesis of toyocamycin with regards to convenience, overall yield, and total reaction time than those syntheses previously reported.

INTRODUCTION

Toyocamycin was first isolated from *Streptomyces toyocaensis* in 1956.[1] The total synthesis of this naturally occurring nucleoside antibiotic has been previously reported[2-4] by several investigators using different synthetic procedures. Though successful, these synthetic procedures have been limited by low yields, long reaction times, or a difficult work up. Since toyocamycin is a key intermediate in the synthesis of analogs containing the pyrrolo[2,3-*d*]pyrimidine heterocycle,[5-8] we now report a more efficient and higher yielding total synthesis of toyocamycin, than previously reported, using new and improved procedures.

DISCUSSION

The total synthesis of toyocamycin is illustrated in Scheme 1. The synthesis of 2-amino-5-bromo-3,4-dicyanopyrrole (**2**) from tetracyanoethylene (**1**) in 33% yield was first reported by Middleton et al.[9] Later, Swayze et al.[10] reported a 66% yield of the same pyrrole by simply changing the solvent system and reaction temperature. The synthesis of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**3**) from compound **2** was first reported by Tolman et al.[3] In this one-pot reaction, compound **2** was heated with formamidine acetate in 2-ethoxyethanol at reflux temperature (135° C) for 36 h to obtain a 51% yield of compound **3**. Recently, a more efficient and higher yielding synthesis was reported by Porcari and Townsend.[11] Compound **2** was heated with triethylorthoformate in acetonitrile at reflux temperature for 1 h to obtain an imino ether intermediate. This intermediate was heated at 105° C with saturated ethanolic ammonia in a sealed reaction vessel for 6 h to obtain a 72% yield of compound **3**. In the synthesis of toyocamycin, the key step is the regio- and stereospecific glycosylation of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**3**). In the first reported synthesis[3] of toyocamycin, it was rationalized that electron withdrawing substituents at C-4 would facilitate the fusion condensation reaction of the heterocycle with the acetylated sugar to obtain the correct regio- (N-7) and stereoisomer (β) of the nucleoside. The reported[3] acid catalyzed fusion of 4-acetamido-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine, 6-bromo-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine, and 4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose afforded the desired protected nucleosides in 9%, 31%, and 28% yield, respectively. To overcome the low yields, Sharma et al.[4] approached this glycosylation step by modifying a procedure developed by Vorbruggen et al.[12] Briefly, compound **3** or 4-acetamido-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine was silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in xylene at reflux temperature for 10 h. After the workup, the silylated intermediates were dissolved in dichloroethane and reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (TBAR) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) over a temperature range of 0° C to reflux for no less than 21 h. A 61% yield (88% yield calculated from unrecovered starting material) of protected 6-bromotoyocamycin (**4**) and a 75% yield (90% yield calculated from unrecovered starting material) of protected 4-acetamido-6-bromotoyocamycin were obtained. To overcome the long reaction times, we were able to accomplish this glycosylation step in high yields and short reaction times by first silylating 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**3**) with 2 equivalents of *N,O*- bis(trimethylsilyl)acetamide (BSA) in dry acetonitrile under argon at room temperature.



[a]Reagents: i) Ref. 10; ii) Ref. 11; iii) BSA, CH_3CN then TBAR, TMSOTf, 80° C; iv) Ref. 4 or 10% Pd/C, NH_4CO_2H, EtOH, reflux; v) Ref. 4 or NH_3, MeOH.

SCHEME 1.[a] Total synthesis of toyocamycin.

The reaction mixture remained a suspension, but a change in consistency was observed. Within 15 min the reaction was complete and 1 equivalent of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was added. With the addition of 3 equivalents of trimethylsilyl trifluoromethanesulfonate (TMSOTf) under argon at room temperature, the suspension quickly (5 min) became a yellow solution and thin layer chromatography showed the disappearance of starting material and the appearance of product. After 10 min, the reaction mixture was stirred at 80° C for 3 h to drive the reaction to completion. A simple workup afforded 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (4) in 75% yield. Debromination of compound 4 has been accomplished in high yields using hydrogen gas,[4] however we opted to use a simple method which does not require the use of hydrogen gas. Debromination was accomplished by heating a mixture of compound 4, ammonium formate, and 10% palladium on charcoal in ethanol at reflux temperature for 3 h. Again a simple workup afforded 4-amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo-[2,3-d]-pyrimidine (5) in 85% yield. Compound 5 was deprotected using saturated methanolic ammonia under standard conditions to obtain toyocamycin in 82% yield. In comparison to the synthetic procedure reported by Sharma et al,[4] in which they obtain toyocamycin from compound 2 in an overall yield of 26% after a total reaction time of 97 h, we report the synthesis of toyocamycin from compound 2 in an overall yield of 38% after a total reaction time of 21.5 h.

In conclusion, we described a more efficient and higher yielding synthesis of toyocamycin with regards to convenience, overall yield, and total reaction time than those syntheses previously reported.

EXPERIMENTAL

General Procedures. Reaction mixtures were evaporated at 60° C under reduced pressure (water aspirator) using a Buchi R-151 rotary evaporator. Melting points (uncorrected) were obtained on a Laboratory Devises Mel-Temp melting point apparatus. Thin layer chromatography used Analtech GHLF SiO_2 prescored plates. Developed TLC plates were visualized under ultraviolet light (254 nm). Proton magnetic resonance (^1H NMR), carbon magnetic resonance (^{13}C NMR), and distortionless enhancement by polarization transfer (DEPT) spectra were obtained with a Brucker Avance DPX 300 or DRX 500 spectrometer (solutions in $CDCl_3$ or DMSO-d_6) with the chemical shifts reported in parts per million downfield from tetramethylsilane as the internal standard. UV spectra were obtained with a Kontron UVIKON 860 ultraviolet spectrometer. Elemental analysis were performed by the Analytical Laboratory, Department of Chemistry, University of Michigan, Ann Arbor, MI.

4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (4). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 4.1 g, 2.9 mL, 20 mmol) was added to a stirred suspension of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**3**) (2.4 g, 10 mmol) in dry acetonitrile (100 mL) at room temperature under argon. After 15 min, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5.0 g, 10 mmol) was added along with trimethylsilyl trifluoromethanesulfonate (TMSOTf, 6.7 g, 5.4 mL, 30 mmol). The suspension was stirred at room temperature for 10 min, during which time the suspension became a clear yellow solution. Under argon, the solution was heated to 80° C for 3 hr and then cooled. The solution was diluted with ethyl acetate (100 mL) and poured over saturated sodium bicarbonate (100 mL) at room temperature. The aqueous layer was separated and discarded and the organic layer was washed with brine (100 mL) and dried over magnesium sulfate. After filtration, the solvent was removed and the residue was dissolved in chloroform and eluted off a silica gel column (7 cm (d) x 9 cm (h)) using a solvent system of 1:9 ethyl acetate:chloroform. Fractions of 100 mL were collected and the UV containing fractions (TLC, 1:4 ethyl acetate:chloroform) whose R_f value was 0.35 were combined and evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and evaporated. The residue was dried in a vacuum oven at 65° C for 12 hr to obtain 5.1 g (75% yield) of pure **4**. MP 185-187° C (melted and resolidified between 110 - 160° C); R_f = 0.35 (1:4 ethyl acetate:chloroform); ^{1}H NMR ($CDCl_3$) δ 4.70 (1H, dd), 4.80 (1H, m), 4.92 (1H, dd), 5.66 (2H, s), 6.35 (1H, d), 6.48 (1H, t), 6.62 (1H, m), 7.41 (6H, m), 7.57 (3H, m), 7.93 (2H, m), 8.02 (4H, m), 8.26 (1H, s). Anal. Calcd for: $C_{33}H_{24}N_5O_7Br$: C, 57.94; H, 3.80; N, 10.24. Found: C, 58.13; H, 3.82; N, 10.41.

4-Amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (5). 4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (**4**) (2 g, 3 mmol), ammonium formate (1.9 g, 30 mmol) and 10% palladium on activated charcoal (200 mg) were suspended in ethanol (75 mL) and the suspension was heated at reflux temperature for 3 hr. The hot reaction mixture was filtered through Celite and washed with hot ethanol (100 mL). The solvent was removed and the residue was dissolved in chloroform and eluted from a silica gel column (5 cm (d) x 5 cm (h)) using a solvent system of 1:9 ethyl acetate:chloroform. Fractions of 100 mL were collected and the UV containing fractions (TLC, 1:4 ethyl acetate:chloroform) whose R_f value was 0.22 were combined and evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and evaporated. The residue was dried in a vacuum oven at 65° C for 12 hr to obtain 1.54 g (85% yield) of pure **5**. MP 174-176° C (melted and resolidified between 110 - 140° C); R_f = 0.22 (1:4 ethyl acetate:chloroform); ^{1}H NMR ($CDCl_3$) δ 4.72 (1H, dd), 4.83 (1H, m), 4.92 (1H, dd), 5.77 (2H, s), 6.16 (2H, m), 6.58 (1H, d), 7.3-7.7 (10H, m), 7.9-8.2 (6H, m), 8.32 (1H, s). Anal. Calcd for: $C_{33}H_{25}N_5O_7$: C, 65.67; H, 4.17; N, 11.60. Found: C, 65.48; H, 4.54; N, 11.54.

4-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (Toyocamycin). Methanolic ammonia (35 mL), saturated at 0° C, was added to a pressure bottle containing 4-amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**6**) (0.60 g, 1 mmol). The bottle was sealed and the reaction mixture was allowed to stir at room temperature for 8 h. The reaction vessel was cooled to 0° C, opened, and the solvent was evaporated to dryness. The residue was adsorbed onto silica gel and eluted from a silica gel column (3.5 cm (d) x 6 cm (h)) using a solvent system of 1:9 methanol:chloroform. Fractions of 20 mL were collected and the UV containing fractions (TLC, 1:9 methanol:chloroform) whose R_f value was 0.21 on TLC were combined and evaporated. The white residue was recrystallized from water to obtain 0.24 g (82% yield) of toyocamycin. MP 243-245° C (Lit.[2] MP 243° C), mixed MP with authentic sample of toyocamycin 243-245° C; R_f = 0.21 (1:9 methanol:chloroform); ^{1}H NMR (DMSO-d_6) δ 3.60 (2H, m, H-5′), 3.93 (1H, m), 4.10 (1H, m), 4.37 (1H, m), 5.21 (2H, m, 2 x OH), 5.48 (1H, d, OH), 6.06 (1H, d, H-1′), 6.93 (2H, bs, NH_2), 8.22 (1H, s, H-6), 8.45 (1H, s, H-2).); ^{13}C NMR (DMSO-d_6) 62.05 (CH_2), 71.04 (CH), 75.07 (CH), 83.83, 86.30 (CH), 88.57 (CH), 102.06, 116.18, 133.26 (CH), 150.97, 154.40 (CH), 157.88. UV [λ_{max} (ε)] (pH 1) 272 (11783), 234 (16020); (EtOH) 278 (13236), 231 (9077); (pH 11) 277 (16538); Anal. Calcd. For $C_{12}H_{13}N_5O_4{\cdot}0.25\ H_2O$: C, 48.73; H, 4.57; N, 23.68. Found: C, 48.81; H, 4.86; N, 23.26.

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