PYRROLOPYRIMIDINE NUCLEOSIDES 19.1 A TOTAL SYNTHESIS OF THE NUCLEOSIDE ANTIBIOTIC CADEGUOMYCIN [2-AMINO-7-(8-D-RIBOFURANOSYL)-PYRROLO[2,3-d]PYRIMIDIN-4-ONE-5-CARBOXYLIC ACID]

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Abstract. A total synthesis of 2-amino-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one-5-carboxylic acid has been accomplished and confirms the previous structural assignment for the nucleoside antibiotic cadeguomycin.

The isolation, characterization, and chemical synthesis of the pyrrolo[2,3-d]pyrimidine nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin, as well as their biological and chemotherapeutic activity, created considerable interest in the synthesis of compounds related to this class of adenosine analogs. The isolation and characterization of nucleoside Q6 and Q7 from certain t-RNA's prompted considerable interest in the synthesis as well as studies on the biological and chemotherapeutic activity of various guanosine-like pyrrolo[2,3-d]pyrimidine nucleosides. In the area of guanosine-like pyrrolo[2,3-d]pyrimidine nucleosides has been renewed by a recent report on the isolation and characterization of the new and novel nucleoside antibiotic cadeguomycin12,13 as 2-amino-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one-5-carboxylic acid (5). This antibiotic was reported13 to have inhibitory effects on transplantable animal tumors but no significant antimicrobial activity against bacteria and fungi. It has been assumed12 that this nucleoside antibiotic possessed the structure 5 solely on the basis of physicochemical properties. We now wish to report a total synthesis of the nucleoside 2-amino-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one-5-carboxylic acid (5, cadeguomycin).

We initiated two separate routes for the total synthesis of cadeguomycin, with each route being designed to ultimately afford the desired antibiotic. In the first route investigated, we envisaged the use of 2-amino-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one-5-carboxamide (4) as the immediate precursor of cadeguomycin. The synthesis of this precursor (4) was accomplished from the nucleoside antibiotic toyocamycin via a seven step sequence. However, the low yield (14%) from the last step, treatment of 2-amino-5-cyano-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (3) in concentrated ammonium hydroxide with hydrogen peroxide at < 20°, prompted us to investigate an alternate synthesis of 4. A successful alternative synthesis of 4 involved the conversion of 2-chloro-7-(8-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one-5-carboxamide (14), with liquid ammonia in a sealed steel reaction vessel (100°, 96 hrs), into 4 in 86% yield, mp 260.5-261.5° (dec., browning began > 228°), 1H-NMR (600 MHz, DMSO-d6): 10.95 (br s, 1H, N3-H), 7.55 (s, 1H, Cs-E), 9.54 and 7.10 (two br s, 2H, CONE2), 6.50 (br s, 2H, NE2) 5.90 (d, 1H, Cl-H, J1',2' = 6.4 Hz). The nucleoside 4 (15 mg) was then treated with 3N HCl at 80° for 44 hrs. The pH of the solution was adjusted to pH 8 by the addition of dilute ammonium hydroxide.
solution. The water was removed by lyophilization and the resulting white solid was isolated by HPLC (Whatman Partisil ODS-3, 0-20% MeOH/H₂O gradient, retention time 53 min.) in 7% yield. It was determined that this solid was identical to cadeguomycin (5) (See Table I). However, the low yield once again prompted us to investigate an alternate route for the preparation of cadeguomycin (5).

TABLE I

<table>
<thead>
<tr>
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<th>λ max, nm(ε)</th>
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<td>268(8650)</td>
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<td>270(6800)</td>
<td>295(4200)d</td>
<td>245(4500)k</td>
<td>234(7600)</td>
<td>290(4600)e</td>
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<tr>
<td>COOH</td>
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<td>272(6881)</td>
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<td>268(9175)e</td>
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<tr>
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<td>266(9314)</td>
<td>299(8658)f</td>
<td>272(7586)</td>
<td>229(21451)</td>
<td>0.53</td>
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A solution of the nucleoside 3 (50 mg 0.163 mmol) in 3.5 mL of 6N sodium hydroxide was heated at reflux for 3 hrs. Ethanol (40 mL) was added to the chilled reaction mixture (ice bath) which effected a precipitation of the sodium salt of 5. Decantation of the reaction
mixture furnished a thick syrup-like residue which was triturated with 40 mL of cold ethanol. The remaining solid was dissolved in water and the pH of the solution was adjusted to pH 4.0 using an ion-exchange resin (Amberlite, IR-120, H⁺-form). A suspension of the nucleoside 5 was decanted from the resin, the resin was washed with cold water (2x10 mL) and the liquid removed by lyophilization. The resulting white solid (30 mg) was extracted with 20 and then 10 mL of hot methanol. The methanol extracts were evaporated to dryness in vacuo to yield 19.4 mg (36.5%) of 2, which was dried over P₂O₅ at 80⁰/0.1 mm Hg for 15 hrs; mp 305-308⁰ (dec., browning began > 240⁰). A sample was recrystallized from a methanol:water(7:1/v:v) mixture and then dried under the same conditions as described above; mp 327-330⁰ (dec., browning began > 260⁰). ¹H-NMR(360 MHz, DMSO-d₆): 14.52 (s, 1H, COOH); 11.62 (br s, 1H, N₃-=H); 7.83 (s, 1H, C₆-H); 6.79 (br s, 2H, NH₂); 5.90 (d, 1H, C₁-H, J₁,₂ = 6.2 Hz); IR (KBr, cm⁻¹): 3350, 3440 (OH, NH); 1650 (CGOH) and essentially identical to the fingerprint region previously reported for cadeguomycin. The CIMS (CH₄) of the hexasilylated nucleoside 5 showed M and M-15 ions (758 and 743 m/z, respectively) which supported our structural assignment for the nucleoside 5. Characteristic peaks of the base series calculated for this structure were observed in GC EIMS (70 e.v.): B+188; B+131; B+128; B+116; B+100; B+74; B+58; B+30; B+13; B+2; B+1; B(409 m/z); B-14; and peaks related to the sugar moiety were identical to those previously reported; a peak for the molecular ion was not obtained under these conditions (EIMS).

The physicochemical data obtained for the nucleoside 5 proved to be very different from either the nucleoside 3 or the nucleoside 4, but essentially identical to the data reported for the naturally occurring cadeguomycin (Table 1). Therefore, this unequivocal synthesis of the nucleoside 5 and the comparison of physiochemical data for 5 and the reported values for cadeguomycin corroborates the previous structural assignment of cadeguomycin as 2-amino-7-(D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin- 4-one-5-carboxylic acid.

The synthetic pathway we have developed, vide supra, for the total synthesis of the nucleoside antibiotic cadeguomycin can now be used to obtain a number of specific analogs of cadeguomycin for more extensive biological and chemotherapeutic evaluations.

REFERENCES
16. Acceptable elemental analysis (C, H, N) have been obtained for all new compounds.

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