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Synthesis of an Oligonucleotide with a Terminal Puromycin

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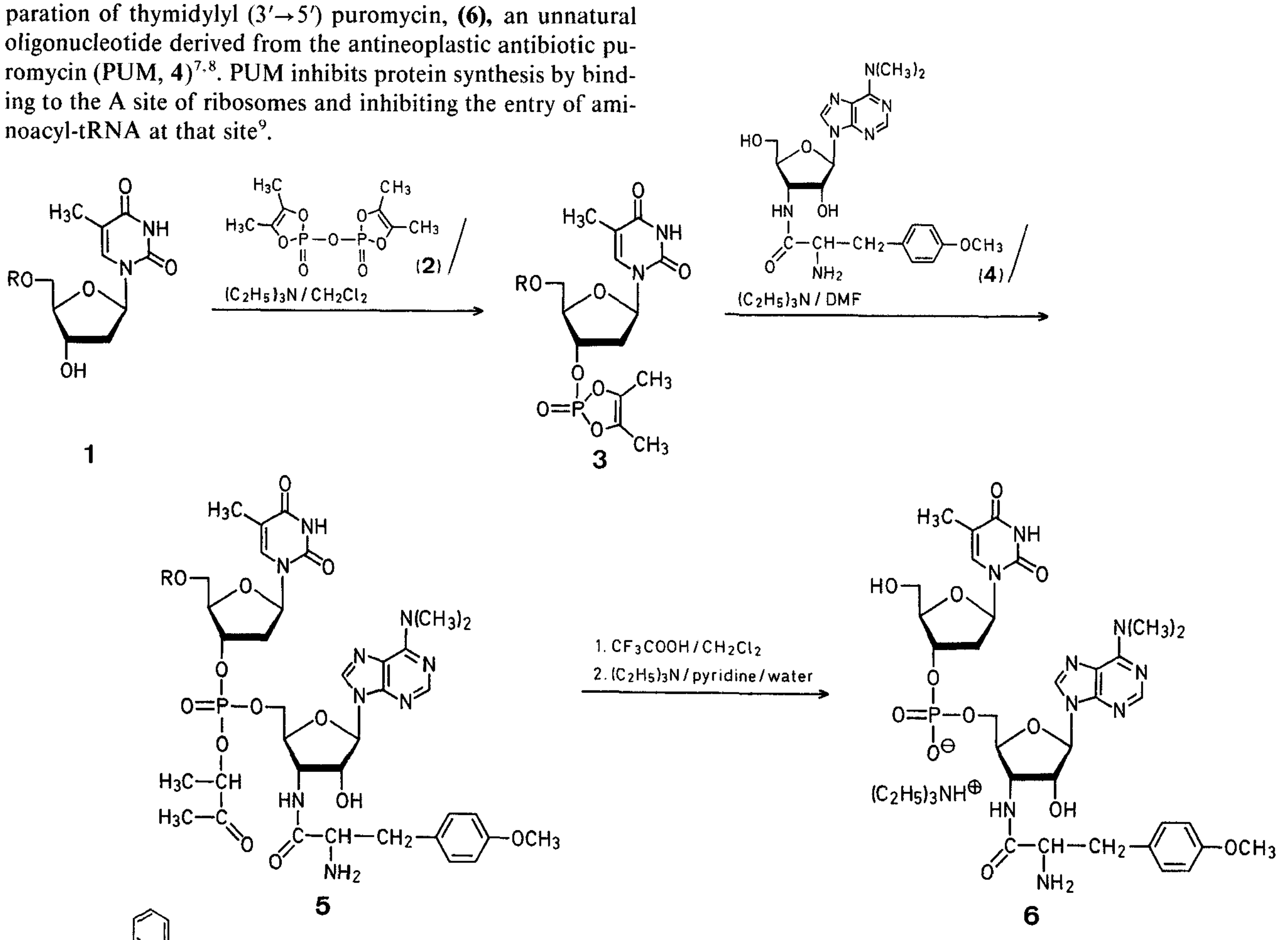
We have recently described the synthesis of oligonucleotide derivatives with membrane-recognition properties[1,2]. This was achieved by covalently binding the 1,2-diacyl-*sn*-glycerophosphoryl moiety (phosphatidyl group) to one of the hydroxy functions of the sugar. The resulting phosphatidylnucleosides and phosphatidyloligonucleotides[1,2] can be incorporated into liposomes[3] made from natural phospholipids, and in some cases, the synthetic liponucleotides themselves form bilayers and liposomes when dispersed in aqueous media. One of the goals of this research is to prepare membrane-compatible nucleotides derived from unnatural nucleosides[4,5,6].

However, many of the unnatural nucleosides do not withstand the conditions required to remove protective groups in oligonucleotide synthesis. This communication describes the preparation of thymidylyl (3'→5') puromycin, (**6**), an unnatural oligonucleotide derived from the antineoplastic antibiotic puromycin (PUM, **4**)[7,8]. PUM inhibits protein synthesis by binding to the A site of ribosomes and inhibiting the entry of aminoacyl-tRNA at that site[9].

Protected Triester 5:

A solution of 5'-*O*-*p*-methoxytritylthymidine (**1**; 0.133 g, 0.26 mmol; dehydrated by evaporation from dry pyridine) in dichloromethane (1 ml) is added to a stirred dichloromethane solution (1 ml) of bis[1,2-dimethylethenylene] pyrophosphate[10] (**2**; 0.073 g, 0.26 mmol) containing triethylamine (0.04 ml). After 2 h at 25 °C, the solution is evaporated to give the cyclic phosphate **3** (moisture-sensitive). Puromycin dihydrochloride dihydrate ($\mathbf{4} \cdot 2\,HCl \cdot 2\,H_2O$; 0.150 g, 0.26 mmol) is treated with pyridine (3 ml) and the solution is evaporated (three times). The residue is dissolved in dimethylformamide (2 ml) and the solution is added to a dimethylformamide solution (1 ml) of the cyclic phosphate **3** containing triethylamine (0.16 ml) at 20 °C. After 10 h at 0 °C and 2 h at 20 °C, the solution is evaporated to give the protected triester **5**, which is dissolved in chloroform (3 ml) for application to preparative T.L.C. plates (two 20 x 20 cm precoated silica gel plates, PLC 60F-254, Merck Cat. No. 5766). The plates are developed (2×) with 7:3:1 ethyl acetate/acetone/water. The band containing **5** (detected under U.V.) is scraped and the silica is extracted with 3:1 chloroform/methanol (3 x 150 ml). The filtered solution is evaporated and the residue dried (3 h, 0.1 torr), to give **5**; yield: 0.201 g (70% based on puromycin).

$C_{56}H_{64}O_{14}N_9P \cdot 3\,H_2O$ calc.		C 57.38	H 5.98	N 10.76
(1172.2)	found	57.44	6.10	10.36



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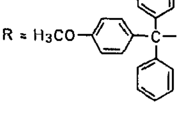

Thymidylyl(3'→5')puromycin (6):

A solution of protected triester **5** (0.197 g) in dichloromethane (40 ml) is cooled to 0 °C and added to a stirred 0.026 molar dichloromethane solution of trifluoroacetic acid (120 ml) at 0 °C. After 15 min at 0 °C, the solution is treated with pyridine (4.5 ml). The solution is evaporated and the residue is mixed with pyridine (3 ml), water (3 ml), and triethylamine (0.2 ml). The mixture is stirred at 25 °C for 2 h, and evaporated. The residue is treated with toluene (25 ml) and the solution is evaporated. The residue is dissolved in water (30 ml), the solution is filtered, and applied to a column of DEAE-cellulose ($HCO_3^{\ominus}$ form). The column is eluted with water (200 ml) to remove nucleoside-containing material, and then with a linear gradient of aqueous triethylammonium hydrogen carbonate (0.005-0.35 molar). The elution is monitored spectrophotometrically at 254 nm; the second peak contains the desired product **6**. The solution is evaporated to dryness and the residue is evaporated from methanol (3 × 50 ml) to remove excess buffer salt. The final methanol solution is concentrated (0.2 ml) and diluted with ether (2 ml) to precipitate **6**, which is collected and dried (2 h, 25 °C/0.5 torr); yield of PUM-nucleotide **6**: 60 mg (41% based on triester).

Analysis by H.P.L.C. showed only one peak. (Partisil 10/25 SAX column; gradient 0.01-0.05 molar KH_2PO_4; pH 4.5.)

Integration of ^{1}H-N.M.R. signals (in D_2O at 300 MHz) showed that thymidylyl (3'→5')puromycin was obtained as a ~1/1 mixture of the triethylammonium salt **6** and the zwitterion.

This material is dissolved in water (2 ml) and treated with triethylamine (20 μl). The solution is freeze-dried and the solid residue dried (25 °C/1 torr, 30 min) to give the pure triethylammonium salt **6**; $[\alpha]_D^{20}$: +29.0° (*c* 1.5, water); for puromycin **(4)**, $[\alpha]_D^{25}$: +30.0° (*c* 0.8, water).

$C_{38}H_{57}O_{12}N_{10}P \cdot 3H_2O$	calc.	C 49.02	H 6.82	N 15.05
(930.9)	found	48.93	6.67	14.45

U.V. (water, pH 4.5): $\lambda_{max} = 274$ nm ($\varepsilon = 27600$).

^{1}H-N.M.R.: $\delta = 1.16$ (t, $\underline{CH_3}CH_2N$); 1.64 (s, dT-CH_3); 3.10, 3.12 [s, s; PUM-$N(CH_3)_2$]; 3.68 (s, PUM-OCH_3); 5.7, 5.8 (m, m; C-1-H of sugar); 7.22 (s; thymine ring); 7.92, 8.16 ppm (s, s; purine ring); integration of the pertinent signals gave the expected ratios.

In 0.1 molar ammonium acetate buffer (pH 5.8) the PUM-nucleotide **6** is degraded by calf spleen phosphodiesterase (Boehringer) into 3'-thymidylic acid and PUM in the expected 1:1 molar ratio (analysis by H.P.L.C., Partisil 10/25 SAX column, 0.05 molar KH_2PO_4, pH 4.5). Degradation of **6** with snake venom phosphodiesterase is very slow compared to a natural dinucleotide. In natural oligonucleotides, the spleen diesterase initiates degradation at the free 5'-OH end of the chain and produces nucleoside-3'-phosphates and phosphorus-free nucleoside, while the venom diesterase initiates degradation at the free 3'-OH end of the chain and produces nucleoside-5'-phosphates and phosphorus-free nucleoside.

In a second approach to the synthesis of the PUM-nucleotide **6**, the primary α-amino group of PUM is protected by the benzyloxycarbonyl group. Condensation with the cyclic triester **3** gives the corresponding benzyloxycarbonyl derivative of **5** in 74% yield. The 3-oxo-2-butyl protective group is removed as before, followed by removal of the monomethoxytrityl and benzyloxycarbonyl protective groups by $H_2/Pd/C$ (10%) in 80% acetic acid. The same compound **6** is obtained by this route, but only in 10% yield as a result of depurination[11] by acetic acid during deprotection.

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