

## Synthesis of 6-Dimethylamino-9-[3'-(*O*-Methyl) (2*S*)-[UL-<sup>14</sup>C]-Tyrosinylamino)-3'-Deoxy-β-D-Ribofuranosyl] Purine

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### Summary

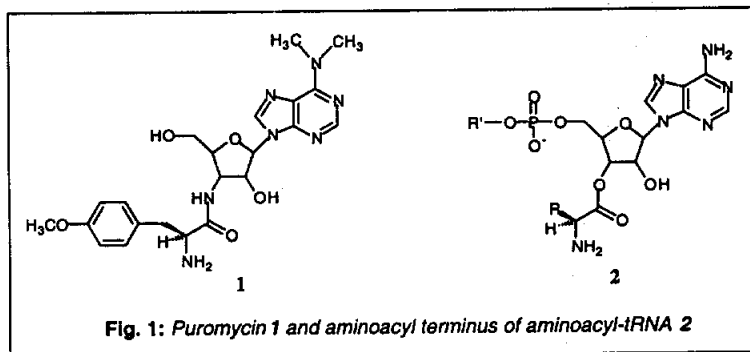
In order to investigate and further refine the mechanism of the unique cleavage activity of the 18 amino acid 2A region of the foot-and-mouth-disease virus (FMDV), the synthesis of <sup>14</sup>C-labelled puromycin is required. Puromycin is an inhibitor of protein synthesis and is an analogue of the terminal aminoacyl-adenosine portion of aminoacyl-tRNA. A short and expedient four step synthesis of 6-dimethylamino-9-[3'-(*O*-methyl) (2*S*)-[UL-<sup>14</sup>C]-tyrosinylamino)-3'-deoxy-β-D-ribofuranosyl] purine (<sup>14</sup>C-labelled puromycin) starting from (2*S*)-[UL-<sup>14</sup>C]-tyrosine is therefore described.

**Keywords:** puromycin, carbon-14, [UL-<sup>14</sup>C]-tyrosine, FMDV 2A region.

### Introduction

The naturally occurring aminoacyl nucleoside antibiotic puromycin **1** was isolated by Porter and co-workers<sup>1</sup> from *Streptomyces alboniger* in 1952. The structure of puromycin **1** was determined by degradation studies<sup>2</sup> and by total synthesis.<sup>3</sup> It has long been known that puromycin acts as an analogue of the terminal aminoacyl-adenosine portion of aminoacyl-tRNA **2** (Figure 1).<sup>4</sup> Puromycin inhibits protein synthesis by releasing nascent polypeptide chains before their synthesis is completed. It has been established that puromycin binds to the ribosome in competition with aminoacyl-tRNA at the A site.<sup>4</sup> The N-atom of the aminoacylamide moiety subsequently attacks the 3'-O ester carbonyl group of the peptidyl-tRNA at the P site of the ribosome to form a new amide bond. This reaction causes premature dissociation of polypeptide chains containing a C-terminal puromycin amide from the

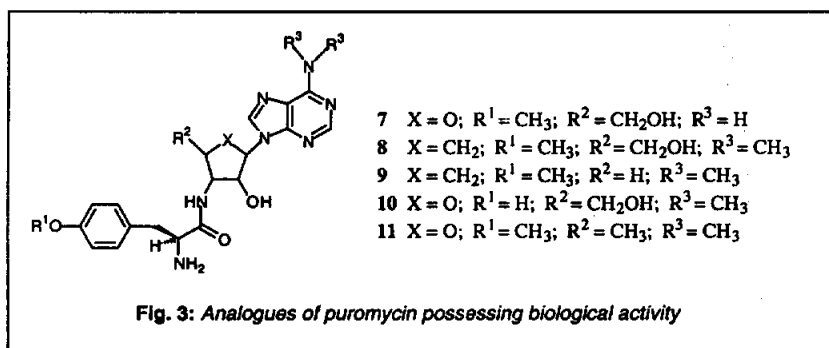
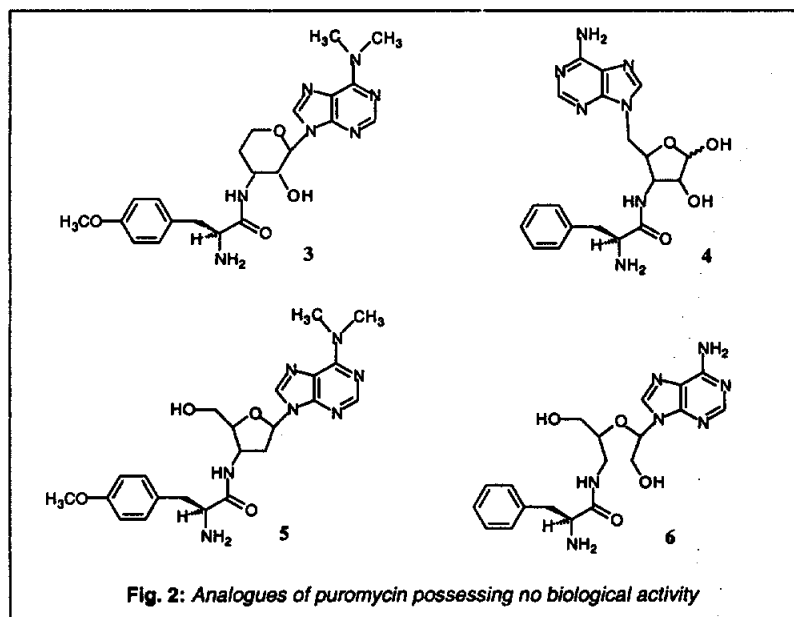
ribosome. The concept of A and P sites resulted from the use of puromycin to ascertain the location of peptidyl-tRNA. When peptidyl-tRNA is in the A site (before translocation), it can not react with puromycin.<sup>5,6</sup> Puromycin has been an invaluable tool in the investigation of the peptidyl transferase site.



Puromycin is known to have antimicrobial,<sup>3,7</sup> antitumour<sup>3,5,6</sup> and antimalarial<sup>1,3</sup> properties. However, its therapeutic use has been avoided due to its inherent toxicity.<sup>8</sup> Many analogues of puromycin have been synthesised in order to decrease its toxicity and enhance its biological activity, and have provided a greater understanding of the structural requirements that are necessary for its biological activity. Studies<sup>9</sup> have shown that both the amino acid side chain and the aminonucleoside motifs are required. The amino acid must be of the L-configuration and, the amino group of the amino acid must be unsubstituted. The nucleoside amino group must be at the 3'-position of the ribofuranosyl ring and it is also evident that the nature of the amino acid side chain is important. Here, *O*-alkyl tyrosine and phenylalanine moieties are known to be most active. Replacement of the ribofuranosyl ring by a 3-aminopentapyranose moiety **37**, or, attachment of the *N*<sup>6</sup>-dimethyladeninyl unit at the 5'-carbon of the ribose moiety rather than the anomeric 1'-centre **4**,<sup>10</sup> affords inactive compounds (Figure 2). 2'-Deoxypuromycin **5**<sup>11</sup> and 2',3'-*seco* analogues **6**<sup>12</sup> are ineffective as antimicrobial agents (Figure 2). Nevertheless the removal of either the *N*-methyl groups on the dimethylamino moiety **7**,<sup>13</sup> the *O*-atom in the furanosyl ring (to give carbocyclic analogues **8** and **9**),<sup>14</sup> the aryl methoxy ether group in the amino acid moiety **10**,<sup>15</sup> or, the hydroxymethyl group (5'-OH) **11**,<sup>14</sup> result in biologically active compounds (Figure 3).

<sup>14</sup>C-Labelled puromycin and other <sup>14</sup>C-labelled puromycin analogues (e.g. <sup>14</sup>C-prolyl analogue) are required to probe the molecular details of the mechanism of translation and the unique translation product self-cleavage activity of the 18 amino acid 2A region of the foot-and-mouth disease virus (FMDV).<sup>16</sup> The refinement of proposed structural models for co-translational aphtho- and cardio- virus polypeptide cleavage where hydrolysis appears to be mediated by a ribosomally bound 2A-polypeptidyl-tRNA molecule at its own 3'-O acyl adenosyl ester linkage,<sup>17</sup> is now an

important objective in understanding viral replication and the control of eukaryotic gene expression.

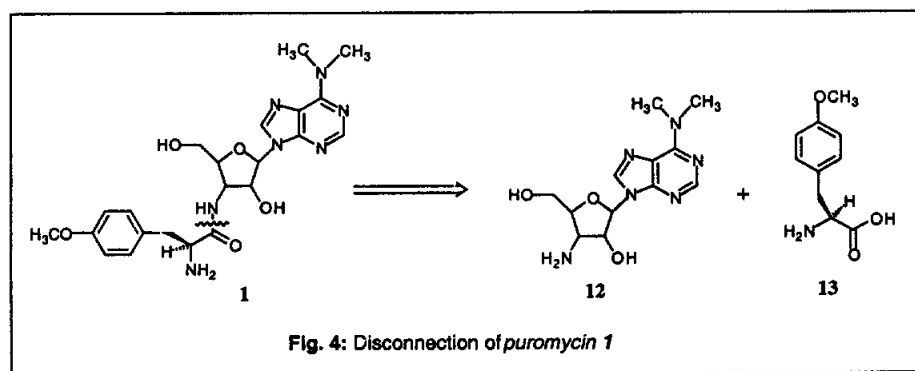


<sup>3</sup>H- and <sup>14</sup>C- Radiolabelled puromycin have been previously synthesised,<sup>5,18</sup> and each synthesis introduces the radiolabel into the *O*-methyl (methoxy) group of the tyrosine-derived moiety. The <sup>3</sup>H-puromycin isotopomer was prepared by methylating *N*-Cbz-(2*S*)-tyrosine with <sup>3</sup>H-dimethyl sulfate, and the resulting tritiated *N*-Cbz-*O*-methyl-(2*S*)-tyrosine was condensed with puromycin aminonucleoside 12 to give the required amide. The synthesis of the <sup>14</sup>C-puromycin was prepared by coupling puromycin aminonucleoside 12 with *N*-Cbz-(2*S*)-tyrosine hydrazide to give the

corresponding tyrosyl analogue of puromycin. The product was then *O*-methylated using 10 eq. of  $^{14}\text{CH}_2\text{N}_2$  which was itself synthesised from the *N*- $^{14}\text{C}$ -methyl-*N*-nitroso-*p*-toluenesulfonamide ( $^{14}\text{C}$ -Diazald). The reagents used to introduce the radiolabel are toxic and were prepared from carcinogenic precursors. Moreover, vast excesses of volatile radiolabelled alkylating agents were used to effect methylation. Therefore, it was necessary to devise a 'safer' synthesis which would be more efficient in the utilisation of the radiolabelled precursor. It was also important in probing the self-cleavage of viral translation product to devise a route suitable for the synthesis of radiolabelled puromycin analogues, including those containing other radionuclei, *e.g.*  $^3\text{H}$ ,  $^{35}\text{S}$  and  $^{125}\text{I}$ , in their amino acid moieties. Here we describe a useful synthesis of  $^{14}\text{C}$ -puromycin starting from commercially available (2*S*)-[UL- $^{14}\text{C}$ ]-tyrosine.

## Results and Discussion

The obvious disconnection of puromycin **1** gives the readily available nucleoside puromycin aminonucleoside **12** and (*O*-methyl) (2*S*)-tyrosine **13** (Figure 4), which will be required in uniformly labelled form.

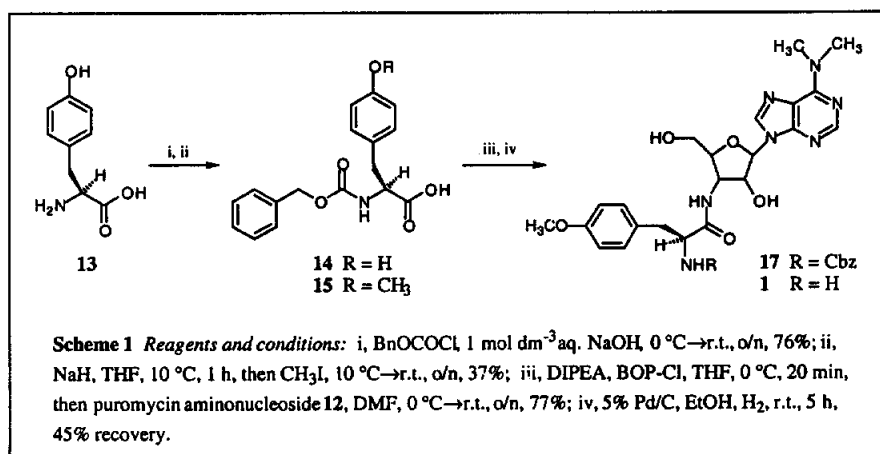


Thus, the synthesis would involve the coupling of an appropriately activated carboxy group of an *N*-protected labelled *O*-methyl tyrosine analogue and puromycin aminonucleoside, followed by deprotection.

Treatment of (2*S*)-tyrosine **13** with benzyl chloroformate in the presence of 1 mol dm<sup>-3</sup> aqueous NaOH solution gave the expected *N*-Cbz protected tyrosine derivative **14** in 76% yield (Scheme 1).<sup>19</sup>

*O*-Alkylation of tyrosine was carried out following the method of Mendelson and co-workers.<sup>20</sup> Although the reaction was successful on the 100 mg scale, when the reaction was attempted on a small scale (*ca.* 50 mg), as would be required for the radiolabelled synthesis, no alkylation was observed when using dry DMF as the solvent. The reaction appeared to be very sensitive to the presence of small amounts

of water. When the tyrosine derivative **14** was dissolved in dry THF and was treated with 1 equivalent of NaH and then after 1 h with methyl iodide the desired product **15** and unreacted phenol **14** were isolated after the appropriate workup, but no methyl ester was formed. Purification by flash silica chromatography afforded some of the unreacted phenol **14** (47%) as well as the required *O*-methylated tyrosine **15** as a white solid in 37% yield {mp 105–107 °C (lit.,<sup>21</sup> 106–107 °C);  $[\alpha]_D +11.1$  (*c* 2.7 in ethanol) (lit.,<sup>21</sup> +12 in 95% alcohol)}. When 5 equivalents of methyl iodide were used in the reaction, alkylation of both the phenolate and carboxylate anions of *N*-Cbz (2*S*)-tyrosine **14** occurred and the corresponding ester **16** was isolated in 64% yield. This ester could be hydrolysed using 1 mol dm<sup>-3</sup> aqueous NaOH solution to give the corresponding free acid **15** in quantitative recovery.



Treatment of the acid **15** with DIPEA and *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)<sup>22</sup> followed by the addition of puromycin aminonucleoside afforded the *N*-Cbz puromycin **17** in 77% yield as a white solid {(HRMS: found  $[M + H]^+$ , 606.2699. C<sub>30</sub>H<sub>36</sub>N<sub>7</sub>O<sub>7</sub> requires 606.2676)}. Finally deprotection of the *N*-benzyloxycarbonyl moiety using catalytic hydrogenolysis gave puromycin **1** with a moderate conversion, *ca.* 50–55%. Some unreacted starting material and an over hydrogenated product, resulting from partial hydrogenation of the adenine moiety were also evident. It was noticed that the latter product was formed when either an excess of 5% Pd/C catalyst was used or, prolonged reaction times were employed. Reducing the quantity of catalyst or the reaction time retarded the formation of the hydrogenated by-product. The crude puromycin **1** was purified by reversed phase HPLC using gradient reverse phase conditions, to give pure puromycin **1** as a white solid in 45% yield. This material gave the expected analytical and spectral data.

The synthesis of radiolabelled puromycin **1b** was carried out in an identical manner to the synthesis of the non-labelled puromycin **1**. Radiolabelled *N*-Cbz puromycin **17b** was prepared from (2*S*)-[UL- $^{14}$ C]-tyrosine in an overall yield of 13% (with an overall radiochemical yield of 7% and a final specific activity of 109  $\mu$ Ci/mmol). The final deprotection of the *N*-benzyloxycarbonyl moiety was achieved using the previously optimised conditions for catalytic hydrogenolysis and gave the desired radiolabelled puromycin **1b**. Purification by reversed phase HPLC, as above for the unlabelled product, gave pure  $^{14}$ C-labelled puromycin **1b** (specific activity 98  $\mu$ Ci/mmol) (Figure 5), for which all spectroscopic data was consistent with the corresponding unlabelled compound.

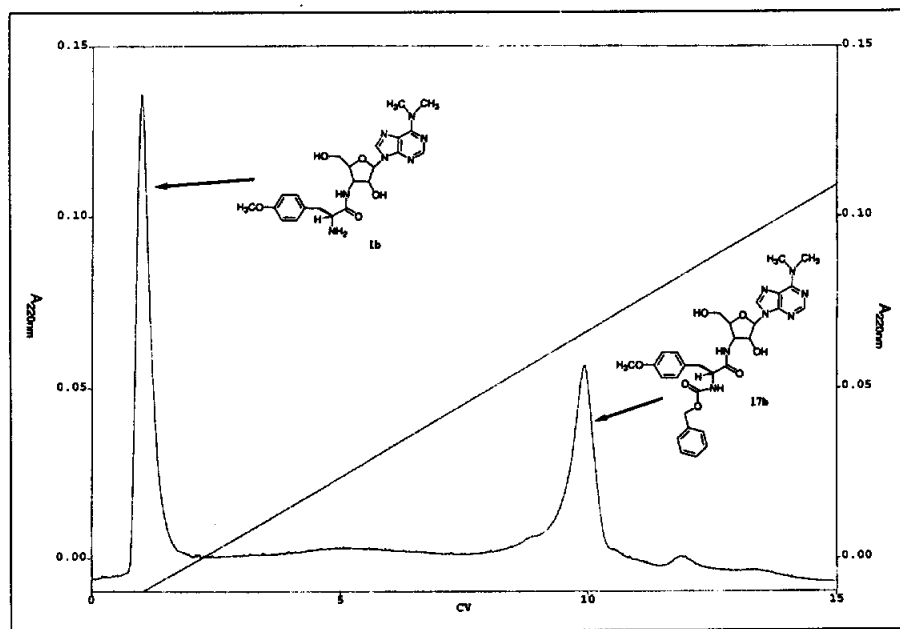


Fig. 5: HPLC trace of purification of radiolabelled puromycin **1b**

Experiments are in progress utilising the radiolabelled puromycin **1b** to probe events within the ribosome and further refine the mechanism of FMDV 2A activity.

### Experimental

NMR spectra were recorded on a Bruker AC-300 spectrometer ( $^1$ H, 300 MHz;  $^{13}$ C, 75.4 MHz), Bruker AM-300 spectrometer ( $^1$ H, 300 MHz;  $^{13}$ C, 75.4 MHz), Bruker DX-500 spectrometer ( $^1$ H, 500 MHz;  $^{13}$ C, 125.8 MHz), a Varian Gemini spectrometer ( $^1$ H, 200 MHz;  $^{13}$ C, 50.3 MHz), a Varian Gemini spectrometer ( $^1$ H,

300 MHz;  $^{13}\text{C}$ , 75.4 MHz) and a Varian Unity Plus 500 spectrometer ( $^1\text{H}$ , 500 MHz;  $^{13}\text{C}$ , 125.6 MHz).  $^1\text{H}$ -NMR spectra were referenced internally to  $(\text{C}^2\text{H}_3)_2\text{SO}$  ( $\delta$  2.47) or  $^2\text{HOH}$  ( $\delta$  4.68).  $^{13}\text{C}$ -NMR spectra were referenced to  $(\text{C}^2\text{H}_3)_2\text{SO}$  ( $\delta$  39.70).  $J$  values are given in Hz. Melting points were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity Ltd. AA-1000 polarimeter using 5 cm path length cells at room temperature and are given in units of  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Mass spectra were recorded on a VG AutoSpec and VG ZabSpec. Major fragments are given as percentages of the base peak intensity. Solvents and common reagents were purified according to the method of Perrin and Armarego.<sup>23</sup> Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV254), and compounds were visualised by UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid or aqueous potassium permanganate. (2*S*)-Tyrosine was purchased from Calbiochem-Novabiochem (UK) Ltd (Beeston, Nottingham). [ $\text{UL-}^{14}\text{C}$ ]-Tyrosine was purchased from Sigma-Aldrich (Poole, Dorset, UK). All other chemicals were of analytical grade or were recrystallised or redistilled before use.

#### (2*S*)-*N*-Benzyloxycarbonyl tyrosine 14

To a stirred solution of (2*S*)-tyrosine 13 (181 mg, 1.00 mmol) dissolved in 1 mol  $\text{dm}^{-3}$  aqueous sodium hydroxide solution (2  $\text{cm}^3$ ) at 0 °C was added benzylchloroformate (140  $\text{mm}^3$ , 1.2 mmol) in five portions over a period of 15 min with vigorous stirring. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was then extracted with ethyl acetate (2 x 10  $\text{cm}^3$ ). The aqueous phase was carefully acidified with 6 mol  $\text{dm}^{-3}$  aq. HCl and then extracted with ethyl acetate (3 x 15  $\text{cm}^3$ ). The latter organic phases were combined, washed with brine (15  $\text{cm}^3$ ), dried ( $\text{MgSO}_4$ ) and the solvents removed under reduced pressure to give the urethane 14 as a colourless oil which was used in the next step without any further purification (0.24 g, 76%), mp 95–98 °C (lit.<sup>19</sup> 101 °C);  $[\alpha]_{\text{D}} +10.5$  (*c* 5 in acetic acid) (lit.<sup>19</sup> +11.1 in acetic acid);  $\delta_{\text{H}}$ [200 MHz;  $(\text{C}^2\text{H}_3)_2\text{SO}$ ] 2.64–3.25 (2 H, m,  $\text{CH}_2\text{C}_6\text{H}_4\text{OH}$ ), 4.04–4.15 (1 H, m,  $\alpha\text{-H}$ ), 4.98 (2 H, s,  $\text{PhCH}_2$ ), 6.65 [2 H, d,  $J$  7.7, Ar-H *meta*, (Tyr)], 7.04 [2 H, d,  $J$  8.2, Ar-H *ortho*, (Tyr)], 7.06–7.38 (5 H, m, Ar-H), 7.59 (1 H, d,  $J$  8.5, NH) and 9.23 (1 H, s,  $\text{CO}_2\text{H}$ );  $\delta_{\text{C}}$ [50.3 MHz;  $(\text{C}^2\text{H}_3)_2\text{SO}$ ] 36.2 ( $\text{CH}_2\text{C}_2\text{H}_2\text{OH}$ ), 56.3 ( $\alpha\text{-C}$ ), 65.7 ( $\text{PhCH}_2$ ), 115.5 [Ar-CH *meta*, (Tyr)], 128.1, 128.3, 128.5, 128.9 and 130.6 (Ar-CH), 137.6 (Ar-C quaternary), 156.5 (CO, urethane), 156.6 [Ar-C quaternary *para*, (Tyr)] and 174.1 (CO, acid).

#### (2*S*)-*N*-Benzyloxycarbonyl [ $\text{UL-}^{14}\text{C}$ ]-tyrosine 14b

(2*S*)-*N*-Benzyloxycarbonyl [ $\text{UL-}^{14}\text{C}$ ]-tyrosine 14b was prepared in an identical manner to that described above, using a mixture of (2*S*)-[ $\text{UL-}^{14}\text{C}$ ]-tyrosine 13b (total activity 50  $\mu\text{Ci}$ , specific activity 181  $\mu\text{Ci}/\text{mmol}$ ) and (2*S*)-tyrosine (50 mg,

0.276 mmol). The urethane **14b** was isolated as a colourless oil (63 mg, 72%) with a measured radiochemical content of 26.7  $\mu\text{Ci}$  (53% radiochemical yield and a specific activity of 134  $\mu\text{Ci}/\text{mmol}$ ).  $m/z$  (EI) 315 (11%,  $\text{M}^+$ ), 107 (100,  $\text{PhCH}_2\text{O}^+$ ), 91 (74,  $\text{PhCH}_2^+$ ) and 77(26,  $\text{Ph}^+$ ).

#### (*O*-Methyl) (2*S*)-*N*-benzyloxycarbonyl tyrosine **15**

To a stirred solution of (2*S*)-*N*-benzyloxycarbonyl tyrosine **14** (100 mg, 0.32 mmol) in dry THF (3  $\text{cm}^3$ ) at 10 °C was added sodium hydride (60% in mineral oil, 29 mg, 0.73 mmol). The reaction mixture was stirred for 1 h at 10 °C and then methyl iodide (20  $\text{mm}^3$ , 0.32 mmol) was added. After stirring for a further 3 h at 10 °C, a solution of ice-water (10  $\text{cm}^3$ ) and ethyl acetate (15  $\text{cm}^3$ ) were added. The aqueous phase was separated and washed with ethyl acetate (2 x 10  $\text{cm}^3$ ), carefully acidified with 6 mol  $\text{dm}^{-3}$  aq. HCl and then re-extracted with ethyl acetate (3 x 10  $\text{cm}^3$ ). The latter organic extracts were washed with brine (10  $\text{cm}^3$ ) and then dried ( $\text{MgSO}_4$ ). The solvent was removed under reduced pressure to give an off-white solid which was purified by flash silica chromatography using 98%  $\text{CH}_2\text{Cl}_2$ -MeOH as the eluant to give some unreacted starting material **14** (35 mg) and the desired compound **15** as a white solid (39 mg, 37%), mp 105–107 °C (lit.,<sup>21</sup> 106–107 °C);  $[\alpha]_{\text{D}} +11.1$  ( $c$  2.7 in ethanol) (lit.,<sup>21</sup> +12 in 95% alcohol);  $\delta_{\text{H}}$ [200 MHz;  $(\text{C}^2\text{H}_3)_2\text{SO}$ ] 2.72–3.07 (2 H, m,  $\text{CH}_2\text{C}_6\text{H}_2\text{OCH}_3$ ), 3.73 (3 H, s,  $\text{CH}_2\text{C}_6\text{H}_4\text{OCH}_3$ ), 4.09–4.20 (1 H, m,  $\alpha$ -H), 4.99 (2 H, s,  $\text{PhCH}_2$ ), 6.85 [2 H, d,  $J$  8.2, Ar-H *meta*, (Tyr)], 7.19 [2 H, d,  $J$  8.0, Ar-H *ortho*, (Tyr)], 7.31–7.33 (5 H, m, Ar-H) and 7.59 (1 H, d,  $J$  8.4, NH);  $\delta_{\text{C}}$ [50.3 MHz;  $(\text{C}^2\text{H}_3)_2\text{SO}$ ] 35.9 ( $\text{CH}_2\text{C}_6\text{H}_4\text{OCH}_3$ ), 55.2 ( $\text{OCH}_3$ ) 56.1 ( $\alpha$ -C), 65.5 ( $\text{PhCH}_2$ ), 113.9 [Ar-CH *meta*, (Tyr)], 127.8, 128.0, 128.6, 130.0 and 130.4 (Ar-CH), 137.3 (Ar-C quaternary), 156.3 (CO, urethane), 158.1 [Ar-C quaternary *para*, (Tyr)] and 173.7 (CO, acid);  $m/z$  (EI) 329 (5%,  $\text{M}^+$ ), 121 (100,  $\text{CH}_2\text{C}_6\text{H}_4\text{OCH}_3^+$ ), 91 (37,  $\text{PhCH}_2^+$ ) and 77(8,  $\text{Ph}^+$ ).

#### (*O*-Methyl) (2*S*)-*N*-benzyloxycarbonyl [ $\text{UL-}^{14}\text{C}$ ]-tyrosine **15b**

(*O*-Methyl) (2*S*)-*N*-benzyloxycarbonyl [ $\text{UL-}^{14}\text{C}$ ]-tyrosine **15b** was prepared in an identical manner to that described above, using (2*S*)-*N*-benzyloxycarbonyl [ $\text{UL-}^{14}\text{C}$ ]-tyrosine **14b** (63 mg, 0.2 mmol, specific activity 134  $\mu\text{Ci}/\text{mmol}$ , total activity 26.7  $\mu\text{Ci}$ ), to give an off-white solid which was purified by flash silica chromatography using 94%  $\text{CH}_2\text{Cl}_2$ -MeOH as the eluant to give some unreacted starting material **14b** (21 mg), and the desired compound **15b** as a white solid (17 mg, 26%), with a measured radiochemical content of 7.3  $\mu\text{Ci}$  (27% radiochemical yield and a specific activity of 140  $\mu\text{Ci}/\text{mmol}$ ), mp 104–107 °C (lit.,<sup>29</sup> 104–109 °C, for the non-radiolabelled compound);  $m/z$  (EI) 329 (7%,  $\text{M}^+$ ), 121 (94,  $\text{CH}_2\text{C}_6\text{H}_4\text{OCH}_3^+$ ), 107 (100,  $\text{PhCH}_2\text{O}^+$ ), 91 (98,  $\text{PhCH}_2^+$ ) and 77(24,  $\text{Ph}^+$ ).



**Methyl [(*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl] tyrosinate ester 16**

To a stirred solution of (2*S*)-*N*-benzyloxycarbonyl tyrosine **14** (100 mg, 0.32 mmol) in dry THF (3 cm<sup>3</sup>) at 10 °C was added sodium hydride (60% in mineral oil, 29 mg, 0.73 mmol). The reaction mixture was stirred for 1 h at 10 °C and then methyl iodide (99 mm<sup>3</sup>, 1.59 mmol) was added. The reaction mixture was stirred for 3 h at 10 °C and then extracted with ethyl acetate (3 x 10 cm<sup>3</sup>). The combined organic extracts were successively washed with 10% citric acid solution (15 cm<sup>3</sup>), 10% NaHCO<sub>3</sub> solution (15 cm<sup>3</sup>), water (15 cm<sup>3</sup>) and then dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to give the product **16** as an off-white solid which was used in the next step without any further purification (67 mg, 64%),  $\delta_{\text{H}}$ [200 MHz; (C<sup>2</sup>H<sub>5</sub>)<sub>2</sub>SO] 2.63–2.99 (2 H, m, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.59 (3 H, s, CO<sub>2</sub>CH<sub>3</sub>), 3.76 (3 H, s, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 4.13–4.22 (1 H, m,  $\alpha$ -H), 4.99 (2 H, s, PhCH<sub>2</sub>), 6.87 [2 H, d, *J* 8.0, Ar-H *meta*, (Tyr)], 7.20 [2 H, d, *J* 8.5, Ar-H *ortho*, (Tyr)], 7.22–7.39 (5 H, m, Ar-H) and 7.58 (1 H, d, *J* 8.5, NH);  $\delta_{\text{C}}$ [50.3 MHz; (C<sup>2</sup>H<sub>5</sub>)<sub>2</sub>SO] 36.1 (CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 52.3 (CO<sub>2</sub>CH<sub>3</sub>), 55.4 (OCH<sub>3</sub>) 56.4 ( $\alpha$ -C), 65.8 (PhCH<sub>2</sub>), 114.2 [Ar-CH *meta*, (Tyr)], 128.0, 128.1, 128.3, 130.1 and 130.6 (Ar-CH), 137.5 (Ar-C quaternary) 156.6 (CO, urethane), 158.1 [Ar-C quaternary *para*, (Tyr)] and 173.1 (CO, ester).

**6-Dimethylamino-9-[3'-(*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl tyrosinylamino]-3'-deoxy- $\beta$ -D-ribofuranosyl]purine; (*N*-Benzyloxycarbonyl puromycin) **17****

To a stirred solution of (*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl tyrosine **15** (25 mg, 0.075 mmol) at 0 °C in dry THF (1 mm<sup>3</sup>), under an atmosphere of argon, was added DIPEA (26 mm<sup>3</sup>, 0.15 mmol) and *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) (19.5 mg, 0.075 mmol). The solution was allowed to stir for 15 min at 0 °C and then a solution of puromycin aminonucleoside **12** (18 mg, 0.06 mmol) in dry DMF (1 cm<sup>3</sup>) was added. The reaction mixture was stirred for an additional 30 min at 0 °C and then allowed to warm to room temperature overnight. The resulting mixture was concentrated under reduced pressure and then redissolved in DCM, and successively washed with 10% citric acid solution (8 cm<sup>3</sup>), 10% NaHCO<sub>3</sub> solution (8 cm<sup>3</sup>), brine (10 cm<sup>3</sup>) and dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to give a colourless oil, which upon trituration with diethyl ether afforded the amide **17** as a white solid (35 mg, 77%), mp 196–197 °C (lit.,<sup>3</sup> 199–200 °C) (HRMS: found [M + H]<sup>+</sup>, 606.2699. C<sub>30</sub>H<sub>36</sub>N<sub>7</sub>O<sub>7</sub> requires 606.2676);  $\delta_{\text{H}}$ [500 MHz; (C<sup>2</sup>H<sub>5</sub>)<sub>2</sub>SO] 2.67–2.93 (1 H, m, 1 H of CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 2.89–2.94 (1 H, m, 1 H of CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.37 [6 H, s, N(CH<sub>3</sub>)<sub>2</sub>], 3.45–3.50 (1 H, m, 1 H of 5'-CH<sub>2</sub>), 3.64–3.70 (1 H, m, 1 H of 5'-CH<sub>2</sub>), 3.71 (3 H, s, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.91–3.95 (1 H, m, 4'-H), 4.29–4.34 (1 H, m,  $\alpha$ -H), 4.44–4.48 (1 H, m, 3'-H), 4.48–4.52 (1 H, m, 2'-H), 4.93 (2 H, m, PhCH<sub>2</sub>), 5.15 (1 H, t, *J* 5.5, 5'-OH), 5.98 (1 H, d, *J* 3.3, 1'-H), 6.06 (1 H, d, *J* 4.9, 2'-OH), 6.81 [2 H, d, *J* 9.0, Ar-H *meta*, (Tyr)], 7.18–7.24 [4 H, m, Ar-H *ortho*, (Tyr) and Ar-H *ortho*, (Cbz)], 7.25–7.33 [3 H, m, Ar-H *para*, (Cbz) and Ar-H

*meta*, (Cbz)], 7.39 [1 H, d,  $J$  8.5, NH (urethane)], 8.08 (1 H, d,  $J$  7.6, 3'-NH), 8.22 (1 H, s, 2-H) and 8.41 (1 H, s, 8-H);  $\delta_{\text{C}}$  [125.8 MHz; (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>SO] 37.1 (CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 37.8 [N(CH<sub>3</sub>)<sub>2</sub>], 50.3 (3'-C), 54.9 (OCH<sub>3</sub>) 56.3 ( $\alpha$ -C), 60.9 (5'-C), 65.1 (PhCH<sub>2</sub>), 73.0 (2'-C), 83.3 (4'-C), 89.3 (1'-C), 113.4 [Ar-CH *meta*, (Tyr)], 119.6 (5-C), 127.3 [Ar-CH *ortho*, (Cbz)], 127.6 [Ar-CH *para*, (Cbz)], 128.2 [Ar-CH *meta*, (Cbz)], 129.7 [Ar-C quaternary, (Tyr)], 130.2 [Ar-CH *ortho*, (Tyr)], 137.0 [Ar-C quaternary, (Cbz)], 137.8 (8-C), 149.6 (4-C), 151.8 (2-C), 154.3 (6-C), 155.7 (CO, urethane), 157.8 [Ar-C quaternary *para*, (Tyr)] and 171.8 (CO, amide);  $m/z$  (FAB) 628 (30%, [M + Na]<sup>+</sup>), 606 (81, [M + H]<sup>+</sup>) and 164 (100).

**6-Dimethylamino-9-[3'-(*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl [UL-<sup>14</sup>C]-tyrosinylamino)-3'-deoxy- $\beta$ -D-ribofuranosyl]purine 17b**

6-Dimethylamino-9-[3'-(*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl [UL-<sup>14</sup>C]-tyrosinylamino)-3'-deoxy- $\beta$ -D-ribofuranosyl]purine 17b (<sup>14</sup>C-labelled *N*-Cbz puromycin) was prepared in an identical manner to that described above, using (*O*-methyl) (2*S*)-*N*-benzyloxycarbonyl [UL-<sup>14</sup>C]-tyrosine 15b (17 mg, 0.052 mmol, specific activity 140  $\mu$ Ci/mmol, total activity 7.3  $\mu$ Ci). The amide was isolated as a white solid (21 mg, 67%), with a measured radiochemical content of 3.8  $\mu$ Ci (52% radiochemical yield and a specific activity of 109  $\mu$ Ci/mmol). <sup>1</sup>H NMR spectrum in (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>SO of the product 17b was shown to be identical to that for the unlabelled *N*-benzyloxycarbonyl puromycin 53.  $m/z$  (FAB) 644 (20%, [M + K]<sup>+</sup>), 628 (100, [M + Na]<sup>+</sup>) and 606 (23, [M + H]<sup>+</sup>).

**6-Dimethylamino-9-[3'-(*O*-methyl)(2*S*)-tyrosinylamino)-3'-deoxy- $\beta$ -D-ribofuranosyl] purine 1**

To a solution of *N*-Cbz puromycin 17 (5 mg, 8.26  $\mu$ mol) in ethanol (750 mm<sup>3</sup>) was added 5% palladium on carbon (0.5 mg) and the mixture was stirred under an atmosphere of hydrogen for 5 h. The catalyst was removed by filtration through a pre-washed Celite pad and the pad was washed with methanol (2 cm<sup>3</sup>) and warm water (3 cm<sup>3</sup>). The filtrate was concentrated under reduced pressure to give an off-white solid (recovery, 1.75 mg, 45%) which was purified by reversed phase HPLC on a Poros 10 R2 reverse-phase column (4.6 x 100 mm) [using gradient reverse-phase conditions, eluting with 100% (99.9% H<sub>2</sub>O/0.1% TFA) to 80% (99.9% CH<sub>3</sub>CN/0.5% H<sub>2</sub>O/0.05% TFA): 20% (99.9% H<sub>2</sub>O/0.1% TFA), at a flow rate of 1.5 cm<sup>3</sup> min<sup>-1</sup>, with the detector set at 220 nm]. The product 1 came through with the solvent front, and any unreacted *N*-Cbz puromycin 17 was collected with a retention volume of 8.5 column volumes. (HRMS: found [M + H]<sup>+</sup>, 472.2310. C<sub>22</sub>H<sub>30</sub>N<sub>7</sub>O<sub>5</sub> requires 472.2308);  $\delta_{\text{H}}$  (500 MHz; <sup>2</sup>H<sub>2</sub>O) 3.00 (1 H, dd,  $J$  13.6, 6.8, one of CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.19 (1 H, dd,  $J$  13.7, 10.5, one of CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.32 (1 H, dd,  $J$  12.9, 6.5, 1 H of 5'-CH<sub>2</sub>), 3.53 [6 H, br s, N(CH<sub>3</sub>)<sub>2</sub>], 3.61 (1 H, dd,  $J$  12.9, 11.7, 1 H of 5'-CH<sub>2</sub>), 3.75

(3 H, s, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 3.78–3.84 (1 H, m, 4'-H), 4.19 (1 H, dd, *J* 6.3, 9.5, α-H), 4.46 (1 H, dd, *J* 5.9, 8.1, 3'-H), 4.66 (1 H, dd, *J* 2.6, 5.8, 2'-H), 5.99 (1 H, d, *J* 2.6, 1'-H), 6.95 [2 H, d, *J* 8.5, Ar-H *meta*, (Tyr)], 7.17 [2 H, d, *J* 8.3, Ar-H *ortho*, (Tyr)], 8.28 (1 H, s, 2-H) and 8.39 (1 H, s, 8-H); δ<sub>C</sub>(125.8 MHz; <sup>2</sup>H<sub>2</sub>O) 38.6 (CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>), 43.1 [br, N(CH<sub>3</sub>)<sub>2</sub>], 53.1 (3'-C), 57.1 (OCH<sub>3</sub>), 58.0 (α-C), 62.7 (5'-C), 75.9 (2'-C), 85.0 (4'-C), 92.5 (1'-C), 117.1 [Ar-CH *meta*, (Tyr)], 122.1 (5-C), 129.0 [Ar-C quaternary, (Tyr)], 133.2 [Ar-CH *ortho*, (Tyr)], 142.0 (8-C), 148.2 (4-C), 150.2 (2-C), 151.6 (6-C), 161.6 [Ar-C *para*, (Tyr)] and 171.9 (CO, amide); *m/z* (ES) 494 (5%, [M + Na]<sup>+</sup>) and 472 (100, [M + H]<sup>+</sup>).

**6-Dimethylamino-9-[3'-(*O*-methyl)[UL-<sup>14</sup>C]-(2*S*)-tyrosinylamino]-3'-deoxy-β-D-ribofuranosyl] purine 1b**

6-Dimethylamino-9-[3'-(*O*-methyl) [UL-<sup>14</sup>C]-(2*S*)-tyrosinylamino]-3'-deoxy-β-D-ribofuranosyl] purine **1b** was prepared in an identical manner to that described above, using <sup>14</sup>C-labelled *N*-Cbz puromycin **17b** (5 mg, 8.26 μmol, specific activity 109 μCi/mmol, total activity 0.9 μCi). The crude material was purified by HPLC to give product **1b** (1.3 mg, 34%, specific activity 98 μCi/mmol, total activity 0.27 μCi, radiochemical yield 30%) and unreacted <sup>14</sup>C-labelled *N*-Cbz puromycin **17b**. For **1b**: *m/z* (FAB) 494 (4%, [M + Na]<sup>+</sup>), 472 (17, [M + H]<sup>+</sup>) and 337 (100, [M + H – H<sub>3</sub>COC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CH<sub>3</sub> + H]<sup>+</sup>).

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