A highly cytotoxic L-rhamnose analogue of the antitumour agent spicamycin

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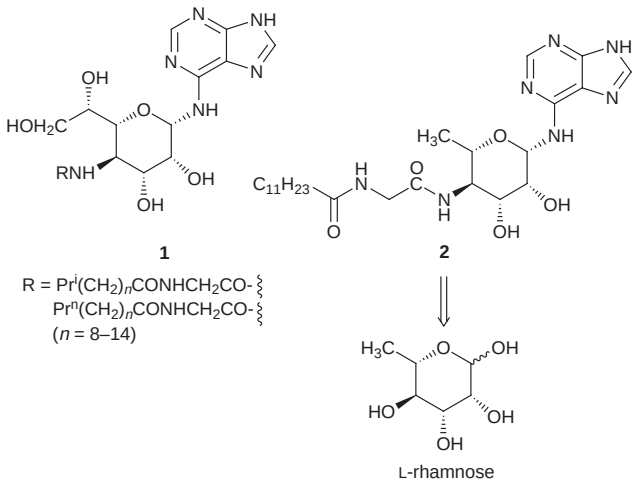
Rhamnospicamycin 2, a rhamnose analogue (containing adenine, a carbohydrate, an amino acid and a fatty acid) of the naturally occurring combinatorial library spicamycin 1, is prepared from L-rhamnose and shown to be highly cytotoxic towards human myeloma cells (IC_{50} = 120 nM).

Nucleoside analogues linked to a sugar through the NH_2 group of adenine are very rare.[1] Spicamycin **1**, an antitumour



antibiotic isolated from *Streptomyces alanosinicus*,[2] is a naturally occurring combinatorial library of fatty acids linked through glycine to a seven carbon sugar and then through the anomeric position of the carbohydrate to the amino group of adenine.[3] The potential of spicamycin as a new class of antitumour agent[4] stimulated structure activity studies[5] that found that the dodecanoyl derivative **1** (R = decanoyl) had antitumour activity against human gastric cancer SC-9 superior to that of mitomycin C, which is clinically used for many kinds of tumours.[6] A semi-synthetic analogue of spicamycin **1** with a tetradecadienoyl side chain[7] potently inhibits the growth of certain human tumour lines *in vitro* and displays marked *in vivo* activity in Colo 205 colon carcinoma xenografts;[8] it will shortly be available for Phase 1 clinical trials in the USA. Exposure of cells to spicamycin at low concentrations of the drug alters glycoprotein processing and induces some accumulation of oligomannosides; while the molecular mechanism for the activity of spicamycin needs to be clarified, the effects of the compound are consistent with a prominent early effect on the enzymatic machinery or organelles important for proper glycoprotein processing and emphasise the novelty of this agent's likely mechanism of action.[9]

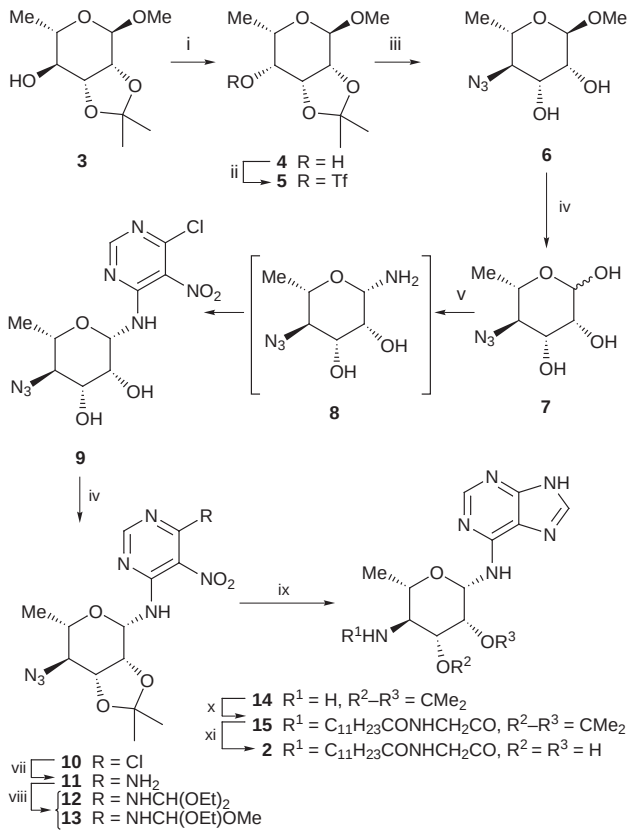
Spicamycin **1** contains a nucleoside base, a carbohydrate, an amino acid and a lipid fragment and thus provides an interesting target for synthesis, and for constructing a wide range of combinatorial libraries from suitable building blocks in which of these components may be varied. No studies on the total synthesis of spicamycin have been reported, all modifications to the structure arising from semi-synthetic procedures from degradation of the natural material. The absolute configuration of the carbohydrate component in **1** was determined by X-ray crystallographic analysis;[10] **1** is a seven carbon sugar analogue of L-mannose, and is thus closely related to L-rhamnose. The total synthesis of closely related carbohydrate analogues of spicamycin would allow the importance of structural features of the carbohydrate to be determined and provide materials for the elucidation of the mechanism of activity of this class of antitumour agent.

Here we report the synthesis of the spicamycin analogue **2** from L-rhamnose and its cytotoxicity against human myeloma cells. The synthesis of rhamnospicamycin **2** requires introduction of nitrogen with retention of configuration at C-4 of L-rhamnose, following by elaboration at the anomeric position of the sugar and at the nitrogen group in C-4. Introduction of the nitrogen as an azide at C-4 permits flexibility in the approach to spicamycin analogues by allowing development of the amino acid side chain or the introduction of the adenine moiety at the anomeric position of rhamnose in either order. Here, the purine is introduced first, but there are clearly many approaches with opportunities for the development of libraries at different stages of the synthesis.

The acetonide **3**, prepared from L-rhamnose in 87% overall yield as previously described,[11] has only the hydroxy group at C-4 of rhamnose unprotected. Oxidation of **3** with PCC in CH_2Cl_2 and subsequent reduction of the resulting ketone with $NaBH_4$ afforded the inverted alcohol **4** in 95% yield over the two steps. Esterification of **4** with Tf_2O and pyridine in CH_2Cl_2 gave the triflate **5** in 82% as a relatively unstable oil. Treatment of **5** with NaN_3 in DMF, followed by cleavage of the acetonide with aqueous TFA, gave the 4-azidorhamnose derivative **6** [mp 81–82 °C (acetone–hexane) $[\alpha]_D^{21}$ −123.5 (*c* 0.72, MeOH)] in 60% yield over two steps (40% from rhamnose). More vigorous hydrolysis of **6** with aqueous TFA at reflux yielded the azido lactols **7**[12] in 86% as a mixture of anomers (α:β, 2:1).

The anomeric amine **8** was obtained from treatment of **7** with aqueous NH_3. All attempts to introduce the adenine moiety directly by addition of 6-chloropurine to **8** were unsuccessful; the principal products were dimeric amines derived from **8**. Accordingly, **8** was reacted with the more electrophilic 4,6-dichloro-5-nitropyrimidine (in the presence of Et_3N as base) to afford the pyrimidine derivative **9** [mp 142–144 °C; $[\alpha]_D^{21}$ +23.2 (*c* 0.59, MeOH)] in 20% yield from **7**, which was converted into the more easily manipulated acetonide **10** [mp 127–129 °C; $[\alpha]_D^{21}$ +50.9 (*c* 0.55, Me_2CO)] in 70% yield. Studies to improve the yield of this key step are in progress.

Reaction of **10** with aqueous NH_3 resulted in nucleophilic displacement of the chloride to give the corresponding amine **11** [mp 180–182 °C, $[\alpha]_D^{21}$ +3.5 (*c* 0.60, Me_2CO)] in 97% yield. Investigation of the activity of a range of reducing agents with the nitro azide **11** failed to identify any reagent with significant selectivity for the nitro group over the azide. Accordingly, the additional carbon necessary for purine formation was added to the free amino group in **11** prior to reduction; treatment of **11** with triethyl orthoformate gave, after work-up in the presence of MeOH, a mixture of the compounds **12** and **13** in a ratio of 1:1 in 65% yield. Hydrogenation of the azides **12** and **13** in MeOH in the presence of Pd/C effected the reduction of the azide and nitro groups to the corresponding diamines which sponta-



Scheme 1 *Reagents and conditions:* i, PCC, molecular sieves 3Å, CH_2Cl_2, then $NaBH_4$, EtOH–H_2O, 0 °C; ii, Tf_2O, Py, CH_2Cl_2, −10 °C; iii, NaN_3, DMF, then TFA–H_2O 3:7; iv, TFA–H_2O 1:1, 1,4-dioxane, reflux; v, NH_3 (aq), then 4,6-dichloro-5-nitropyrimidine, Et_3N, DMF; vi, $Me_2C(OMe)_2$, CSA, acetone; vii, NH_3 (aq), MeOH, 60 °C; viii, $HC(OEt)_3$, 140 °C, then MeOH, silica; ix, H_2, Pd/C, MeOH; x, dodecanoylglycine, DMF, *N*-hydroxysuccinimide, WSC·HCl; xi, 70% AcOH (aq), 60 °C

neously cyclised to give the purine **14**[13] [oil; $[\alpha]_D^{21}$ +25.4 (*c* 0.22, MeOH)] in 53% yield.

Condensation of the amine **14** with dodecanoylglycine[5] in DMF in the presence of *N*-hydroxysuccinimide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl) gave the glycopeptide **15** in 71% yield.[14] Finally, removal of the acetonide in **14** with aq. AcOH gave rhamnospicamycin **2**, mp 224–226 °C (decomp.) $[\alpha]_D^{21}$ +9.71 (*c* 0.35 MeOH) in 82% yield; rhamnospicamycin is columnable in organic solvents and easy to purify. The NMR of **2** in DMSO is temperature dependent (in contrast to the NMR in CD_3OD) and may indicate some propensity for intramolecular hydrogen bonding between the carbohydrate hydroxy protons and the adenine nucleus.[15]

In a preliminary study rhamnospicamycin **2** was found to be highly cytotoxic with an IC_{50} value for cell growth (assessed over 15 h in culture using human myeloma cells, HL60 cells) is 120 nM; thus **2** displays much the same potency as that reported for the dodecanoyl derivative of spicamycin **1**.

While there are a number of steps in the synthesis that have yet to be optimised, we have shown that variation in the carbohydrate fragment of spicamycin may allow a wide range of novel materials with cytotoxic activity to be prepared. Spicamycin incorporates a nucleoside, a fatty acid, a sugar and an amino acid all into one component; the potent cytotoxicity with the possibility of a novel mode of action as an anti-cancer agent in regard to modification of glycoprotein processing, together with the opportunity to generate combinatorial libraries, makes this class of compound an attractive one for further synthetic study and biological evaluation of the mechanism.

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13 All new compounds have satisfactory HRMS or CHN microanalytical data; the anticipated β-stereochemistry at the anomeric position of **14** was confirmed by NOE enhancements between H-1 and the protons at H-2 (11.2%), H-3 (2%) and H-5 (13.8%).
14 The structure of **15** in terms of the anomeric configuration and of the site of attachment to the adenine nucleus was assigned on the basis of COSY, HMQC and HMBC experiments.
15 *Selected data* for **2**: δ_H(500 MHz, CD_3OD) 0.91 [3H, t, *J* 6.8, $CH_3(CH_2)_{10}CO$], 1.22 (3H, d, *J* 6.1, H_3-6′) 1.28–1.34 [16H, m, $CH_3(CH_2)_8CH_2$], 1.65 [2H, m, $CH_3(CH_2)_8CH_2CH_2CO$], 2.30 [2H, t, *J* 7.6, $CH_3(CH_2)_8CH_2CH_2CO$], 3.62 (1H, dq, *J* 6.1, 9.9, H-5′), 3.74 (1H, dd, *J* 3.5, 10.5, H-3′), 3.90 (2H, s, $RNHCH_2CONH$), 3.95 (1H, t, *J* 10.2, H-4′), 4.02 (1H, d, *J* 2.2, H-2′), 5.70 (1H, br s, H-1′), 8.19 (1H, s), 8.35 (1H, s); δ_H(500 MHz, [$^2H_6$]DMSO, 25 °C) 0.83 [3H, t, *J* 6.9, $CH_3(CH_2)_{10}CO$], 0.99 (3H, m, H_3-6′) 1.20–1.25 [16H, m, $CH_3(CH_2)_8CH_2$], 1.48 [2H, t, *J* 6.8, $CH_3(CH_2)_8CH_2CH_2CO$], 2.12 [2H, t, *J* 7.4, $CH_3(CH_2)_8CH_2CH_2CO$], 3.50–3.71 (4H, m), 3.68 (2H, d, *J* 5.7, $RNHCH_2CONH$), 3.80 (1H, s), 4.75 (1H, br s), 5.44, 5.55 (1H, 2 × br s), 7.15 (1H, br. s), 7.60 (1H, d, *J* 9.3), 7.99, 8.00 (1H, 2 × d, *J* 5.7), 8.21, 8.22 (1H, 2 × s), 8.27, 8.29 (1H, 2 × s); δ_H(500 MHz, [$^2H_6$]DMSO, 80 °C) 0.86 [3H, t, *J* 7.0, $CH_3(CH_2)_{10}CO$], 1.05 (3H, d, *J* 6.1, H_3-6′) 1.25–1.31 [16H, m, $CH_3(CH_2)_8CH_2$], 1.53 [2H, t, *J* 7.2, $CH_3(CH_2)_8CH_2CH_2CO$], 2.16 [2H, t, *J* 7.5, $CH_3(CH_2)_8CH_2CH_2CO$], 3.48 (1H, dq, *J* 6.1, 9.4, H-5′), 3.65 (1H, dd, *J* 2.9, 10.3, H-3′), 3.70 (1H, m), 3.72 (2H, d, *J* 5.5, $RNHCH_2CONH$), 3.86 (1H, d, *J* 2.1, H-2′), 5.00 (1H, br s), 5.73 (1H, br s, H-1′), 6.90 (1H, br s), 7.27 (1H, d, *J* 8.6), 7.60 (1H, br s), 8.11 (1H, s), 8.27 (1H, s); δ_C(125 MHz, [$^2H_6$]DMSO, 80 °C) 13.51 (q), 17.82 (q, C-6′), 21.74, 24.92, 28.37, 28.49, 28.54, 28.66, 28.71, 28.73, 31.01, 35.19 [10 × t, $CH_3(CH_2)_{10}CONHCH_2CONH$], 52.89, 70.00, 71.44, 71.86, 78.40 (5 × d, C-1′, C-2′, C-3′, C-4′, C-5′), 118.60 (s), 140.36 (d), 151.89 (d), 151.89 (s), 152.36 (s), 169.45, 172.55 (2 × s, 2 × C=O)

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