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Synthesis and biological evaluation of enantiomeric rhamnose analogues of the antitumour agent spicamycin — is the mode of action by modification of N-linked glycoproteins?

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Abstract

The synthesis of both enantiomers of dodecyl rhamnospicamycin **2a** and **2b**, a rhamnose analogue of the naturally occurring combinatorial library spicamycin **1**, are derived from L-rhamnose and methyl α-D-mannopyranoside, respectively. The L-(+)-enantiomer **2a** containing an L-rhamnose fragment is shown to be highly cytotoxic towards human myeloma cells with an IC_{50}=120 nM, whereas the D-(−)-enantiomer **2b**, based on a D-mannose structure, shows no significant cytotoxicity. The analogue **16**, in which the nucleotide base fragment has been replaced by a simple methoxy group, has no cytotoxicity. Initial studies towards clarifying the mechanism of anti-cancer action of spicamycin analogues are reported.

1. Introduction

Spicamycin **1**, an antitumour antibiotic isolated from *Streptomyces alanosinicus* 879-MT_3,[1] is a family of compounds containing a fatty acid linked to glycine which is conjugated to a 4-aminopyranosylamine which itself is connected through the anomeric amine by a very rare nucleoside link to the N-6 amino group of adenine.[2,3] The members of the spicamycin group differ only by the constituent fatty acids conjugated to the glycine side chain. Spicamycin has potent antitumour activity; a wide range of analogues with variation in the structure of the side chain connected to the 4-amino group of the sugar has been prepared by semi-synthetic procedures from degradation of the natural material.

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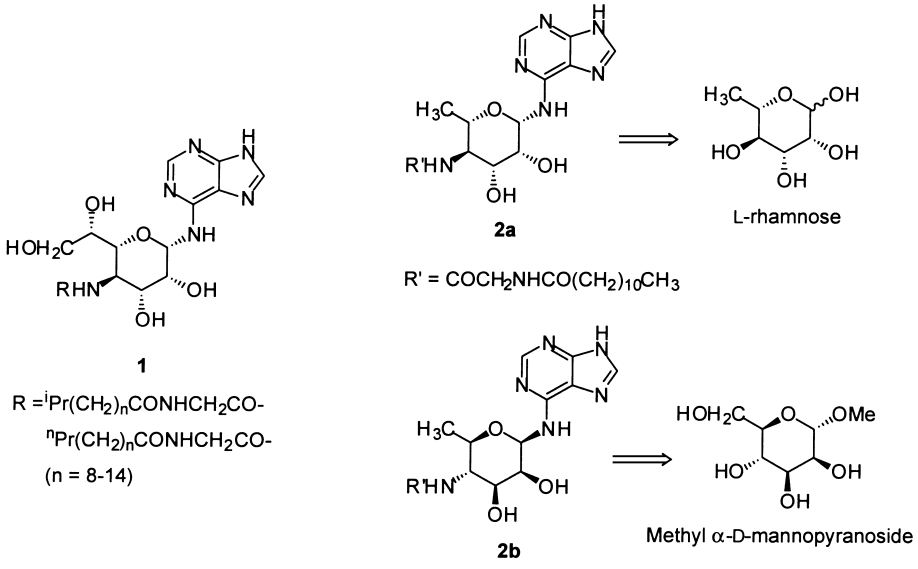


From these structure–activity studies[4,5] based on the variation of the side chain unit, it was found that the dodecanoyl derivative **1** (R=dodecanoyl) had antitumour activity against human gastric cancer SC-9 superior to that of mitomycin C, which is clinically used for many kinds of solid tumours.[6] Another semi-synthetic analogue of **1** with a tetradecadienoyl side chain, KRN 5500, even more potently inhibits the growth of certain tumour lines in vitro and displays marked in vivo activity in Colo 205 colon carcinoma xenografts.[7–9] KRN5500, which is in clinical trials in the USA, has marked in vitro activity against human B-chronic lymphocytic leukemia cells concomitant with decreased expression of p27(kip1) and cleavage of caspase-3.[10] Because of the unique structure of spicamycin, KRN5500 appears to have a different mode of action from other antitumour drugs, with non-overlapping toxicities; studies indicate that KRN5500 is expected to be a suitable candidate for combination chemotherapy with cisplatin, carboplatin or etoposide for non-small-cell lung cancer.[11]

Although such a biologically active natural product containing lipids, carbohydrates, amino acids and nucleoside bases in one structure is an attractive target for synthesis, there is only one report of a model approach to a total synthesis of spicamycin **1** in a study of the palladium-catalysed coupling of glycosyl-amines with 6-chloropurines.[12] It has been suggested that the mode of action of spicamycin may involve modification of the processing of N-linked glycoproteins and that the sugar component in **1** may resemble swainsonine.[13] The absolute configuration of **1** was unambiguously established by X-ray crystallographic analysis of a fragment containing the sugar moiety;[14] its carbohydrate component is a seven carbon sugar analogue of L-mannose and is thus closely related to L-rhamnose, whereas swainsonine is related to D-mannose. The synthesis of other analogues containing a different carbohydrate unit than is present in the natural product may allow a clarification of some aspects of the mechanism of action involved. This paper reports:

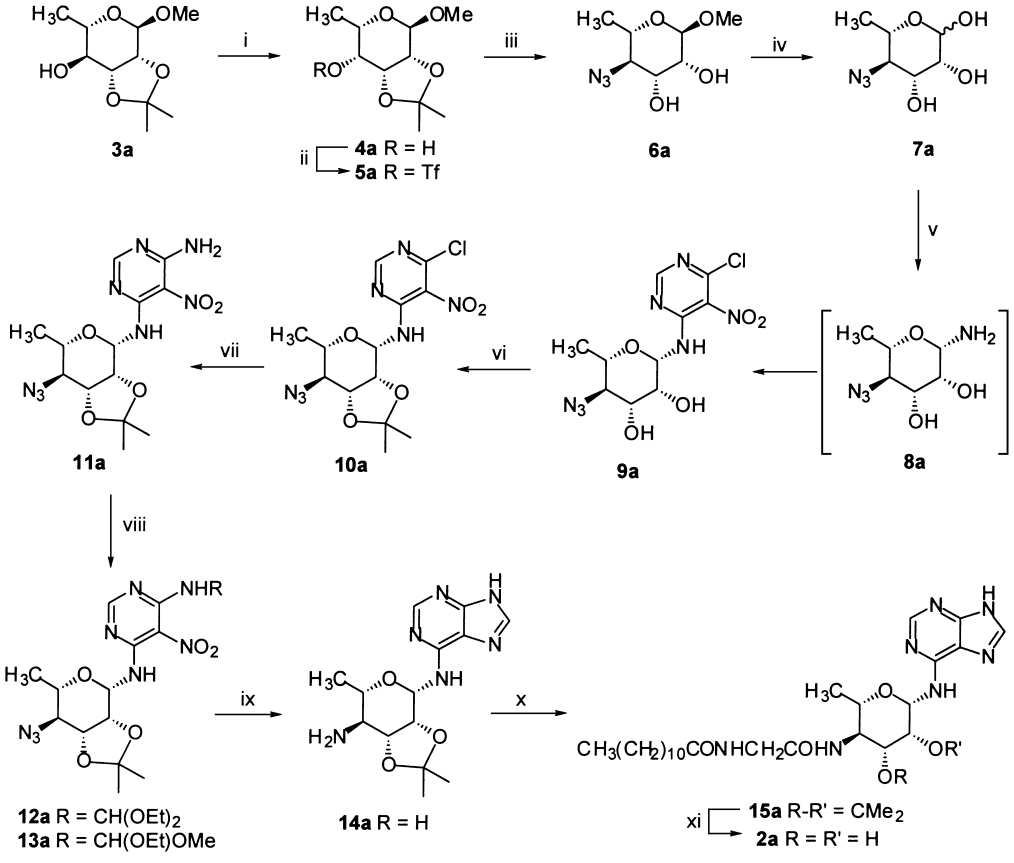
(i) the synthesis of both enantiomers of the rhamnospicamycin analogues **2a** [from L-rhamnose, and very closely related to the natural product **1**] and **2b** [methyl α-D-mannopyranoside, and thus more closely structurally related to swainsonine]; and

(ii) their cytotoxicity against human myeloma cells.

A preliminary report of some of this work has appeared.[15]

2. Synthesis

The synthesis of L-(+)-rhamnospicamycin **2a** requires conversion of the C-4 hydroxyl group in L-rhamnose to a nitrogen functional group with retention of configuration, introduction of the adenine moiety at the anomeric position of the sugar, and attachment of the acyl amino acid chain at the nitrogen group in C-4 (Scheme 1). Although there is a choice about the order in which the fragments are joined to the sugar moiety, it was originally intended to use an azide as a masked amine at C-4 while the adenine group was attached to the anomeric position amine; the azide could then be converted to an amine for conjugation to generate a potential library of rhamnospicamycin analogues.



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

The unprotected C-4 hydroxyl group acetonide **3a**, prepared from L-rhamnose in 87% overall yield,[16] underwent oxidation by pyridinium chlorochromate (PCC) in dichloromethane to give the corresponding ketone; subsequent reduction of the ketone with sodium borohydride gave the inverted alcohol **4a** (95% yield from **3a**) as previously described.[17] By minor modifications of the previous procedure[18] the alcohol **4a** was converted to the triflate **5a** which with sodium azide in dimethylformamide, followed by cleavage of the acetonide with aqueous trifluoroacetic acid (TFA), gave the L-*rhamno*azide **6a** in 60% yield from **4a** (58% yield from **3a**). More vigorous hydrolysis of **6a** with aqueous TFA at reflux removed

the anomeric protecting group to give the azidolactols **7a** in 86% yield as an anomeric mixture, α:β ratio of 3:2.

No products could be identified from the reaction of the lactol **7a** with adenine, so that an anomeric nitrogen was introduced by treatment of **7a** with aqueous ammonia to give the β-pyranosylamine **8a**. The amine **8a** was difficult to purify and was used in situ without any attempts at purification. All attempts to introduce the adenine moiety directly by addition of 6-chloropurine to **8a** were unsuccessful; it is likely that some procedure using palladium catalysis would be necessary.[12] Pyranosylamines such as **8a** are well know to form dimeric amines; such species were isolated as the sole products from reactions of **8a** with chloropurine under more forcing conditions. It was thus necessary to react the anomeric amine **8a** with a much more reactive pyrimidine electrophile, and then to develop the synthesis of the adenine fragment subsequently. Reaction of **8a** with 4,6-dichloro-5-nitropyrimidine in the presence of triethylamine gave the pyrimidine **9a** in 20% yield from **7a**. For ease of the later transformations, the *cis*-diol unit in **9a** was protected as the more easily manipulated acetonide **10a** in 70% yield.

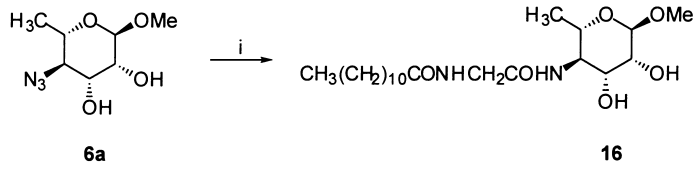
For elaboration of the pyrimidine to the purine nucleus, **10a** was treated with aqueous ammonia to give the corresponding aromatic amine **11a** in 97% yield. In order to develop the construction of the adenine it is necessary to reduce the nitro group in **11a**, preferably without affecting the azide functionality; in spite of extensive investigation of a large number of conditions for both catalytic and other reducing conditions, all attempts failed to identify any selectivity for the nitro group over the azide in **11a**. Complete reduction of the nitrogen functions in **11a** would result in a triamine which would be a troublesome intermediate for the regioselective introduction of the additional carbon necessary for the construction of the purine ring. Accordingly, prior to reduction, the additional carbon was introduced by reaction of **11a** with triethyl orthoformate to give, after work-up in the presence of methanol, a mixture of the compounds **12a** and **13a** in a ratio of 1:1 and a combined yield of 65%. Hydrogenation of the mixture of **12a** and **13a** in methanol in the presence of palladium on charcoal effected the reduction of the azide and nitro groups to the corresponding diamines which spontaneously cyclised to obtain the desired purine **14a** in 53% yield. The proposed β-stereochemistry at the anomeric position of compound **14a** was confirmed by the observed NOE enhancements of the proton at C-1 with the protons at C-2, C-3 and C-5.

Condensation of the amine **14a** with dodecanoylglycine[6] in dimethylformamide in the presence of *N*-hydroxysuccinimide and 1-(3-dimethylaminoproyl)-3-ethylcarbodiimide hydrochloride (EDCI) gave the glycopeptide **15a** in 71% yield. The structure of **15a** in terms of the site of attachment to the adenine nucleus was clearly confirmed by COSY, HMQC and HMBC experiments.

Finally, removal of the acetonide in **15a** with aqueous acetic acid afforded L-(+)-rhamnospicamycin **2a** in 80% yield. The ^{1}H NMR of **2a** in DMSO is temperature dependent — in contrast to the lack of such dependence of the ^{1}H NMR in CD_3OD — and may indicate some propensity for intramolecular bonding between the carbohydrate hydroxyl protons and the adenine nucleus. The addition of the conjugated glycine-fatty acid side chain renders the material soluble in organic solvents and easily purified by silica flash chromatography in organic solvents.

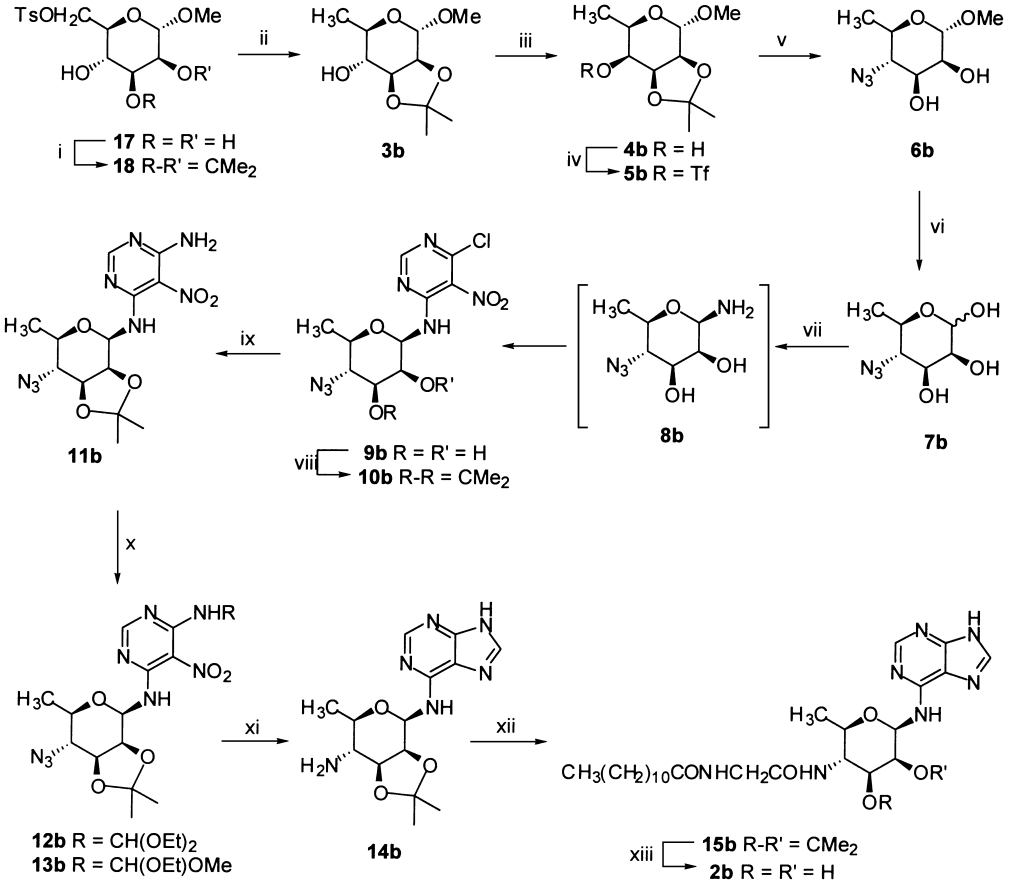
In order to investigate the influence of the nucleoside fragment in the activity of this class of antitumour agent, the methyl rhamnoside **16** was prepared from the azide **6a** (Scheme 2). Hydrogenation of **6a** in methanol in the presence of palladium over charcoal allowed isolation of the corresponding amine, which was coupled to dodecanoylglycine with EDCI and *N*-hydroxysuccinimide in dimethylformamide in the presence of triethylamine to afford the glycopeptide **16** in 79% overall yield from **6a**.

Because of suggestions that the mechanism of cytotoxicity might involve a structural resemblance to swainsonine, the enantiomer D-(−)-rhamnospicamycin **2b** containing a D-mannose moiety was prepared for biological evaluation from mannose. The tosylate **17**, prepared from methyl α-D-mannopyranoside,[19] was converted into the acetonide **18** by reaction with 2,2-dimethoxypropane and camphorsulfonic acid



Scheme 2. (i) H_2, Pd/C, MeOH, rt: then dodecanoylglycine, DMF, EDCI, *N*-hydroxysuccinimide, rt

in quantitative yield. Further reduction of **18** with sodium borohydride in dimethylsulfoxide at 85°C allowed the 6-deoxy sugar **3b**, the enantiomer of **3a**, to be obtained in 85% yield (Scheme 3). D-(−)-Rhamnospicamycin **2b** was then prepared from D-rhamnopyranoside **3b** by an identical sequence of steps to those used for the preparation of **2a**, involving double inversion in C-4 with introduction of an azide group, elaboration of the nucleoside base in C-1 and finally coupling with the alkanoylamino acid.



Scheme 3. (i) 2,2-Dimethoxypropane, CSA, acetone, rt; (ii) $NaBH_4$, DMSO, 85°C; (iii) PCC, molecular sieves 3 Å, CH_2Cl_2, rt; then $NaBH_4$, EtOH–H_2O, 0°C; (iv) Tf_2O, Py, CH_2Cl_2, −10°C; (v) NaN_3, DMF, rt; then TFA:H_2O 3:7, rt; (iv) TFA:H_2O 1:1, 1,4-dioxan, reflux; (vii) NH_3 (aq), 60°C; then 4,6-dichloro-5-nitropyrimidine, Et_3N, DMF, rt; (viii) $Me_2C(OMe)_2$, CSA, acetone, rt; (ix) NH_3 (aq), MeOH, 60°C; (x) $HC(OEt)_3$, 140°C; then MeOH, silica; (xi) H_2, Pd/C, MeOH, rt; (xii) dodecanoylglycine, DMF, *N*-hydroxysuccinimide, EDCI, rt; (xiii) 70% AcOH (aq), 60°C

The above sequences have severe problems in the synthesis of anything other than small amounts of material; it is likely that a relatively early introduction of the fatty acid side chain would provide intermediates which were easier to handle. However, the above sequences provided enough material for preliminary evaluation of the rhamnose analogues.

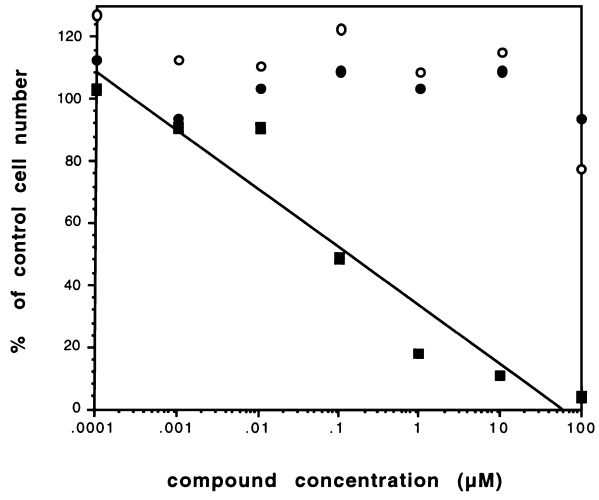


Figure 1. Effect of rhamnospicamycin analogues on HL60 cell viability. Cells were grown for 15 h in the presence of each compound at the concentrations shown and viability determined as described in the Experimental section; each point represents the average of duplicate cultures. L-Rhamnospicamycin **2a** (■); D-rhamnospicamycin **2b** (○); methyl glycoside **16** (•)

3. Biological evaluation of the rhamnospicamycin analogues

In preliminary experiments, L-rhamnospicamycin **2a** was found to be highly cytotoxic for cultured human myeloma HL60 cells with an IC_{50} for cell growth and viability of 120 nM (Fig. 1), a similar potency to that reported for the dodecanoyl derivative of spicamycin **1**. By contrast, the D-enantiomer **2b** showed only weak activity at the highest concentration tested (22% reduction in growth and viability at 100 μM). The analogue **16** (in which the adenine moiety is missing) was not found to be cytotoxic at any of the concentrations tested. These results show that the adenine fragment is crucial for cytotoxicity; any structural resemblance to swainsonine in the active series is not superficially obvious in that the D-analogue has no significant biological activity whereas the L-isomer is highly cytotoxic.

Initial effects of **2a** on N-linked glycoprotein processing were investigated. The effect of variation of the concentration of L-rhamnospicamycin **2a** appeared to have two effects on mannose incorporation in HL60 cells. The first at low concentrations (0.1–0.5 nM) provided an increase in mannose incorporation after a 16 h treatment with compound. At higher concentrations, mannose incorporation was inhibited in a concentration dependent manner (Fig. 2).

Similar effects are seen with shorter incubation times (0.5–1 h) where concentrations of **2a** lower than 100 nM stimulate mannose incorporation (Fig. 3). After 8 h incubation there is a significant reduction in cellular incorporation of mannose. These results compare favourably with previous data showing increased mannose incorporation in HL60 cells treated with spicamycin **1** at or below the IC_{50} for cell growth.[13] However, at higher concentrations of **2a** that are still below the IC_{50} for cell growth, a dramatic decrease in mannose uptake is observed. The reasons for the discrepancy between these results using **2a** and previously published data with spicamycin **1**[13] are unclear. The concentration dependent effects of **2a** were confirmed by studying the interaction of mannose-specific lectins with rhamnospicamycin treated HL60 cells using flow cytometry. Concentrations of **2a** of 10 nM showed an increase in lectin binding sites, whilst higher concentrations (100 nM) caused a significant reduction (results not shown).

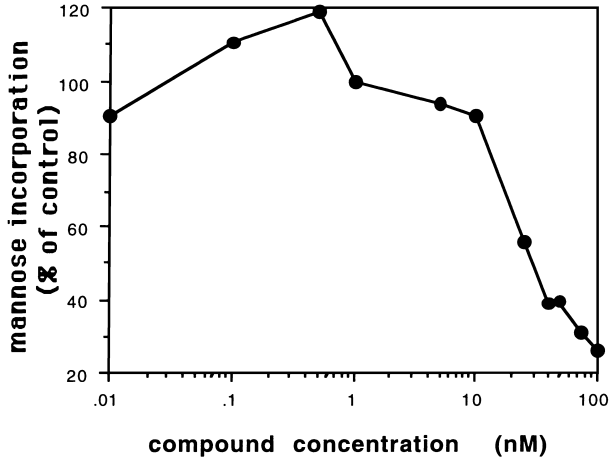


Figure 2. Effect of concentration L-rhamnospicamycin **2a** on mannose uptake over 16 h. HL60 cells were cultured for 16 h in the presence of various concentrations of **2a** and then pulsed for 2 h with ^{3}H-mannose as described in the Experimental section

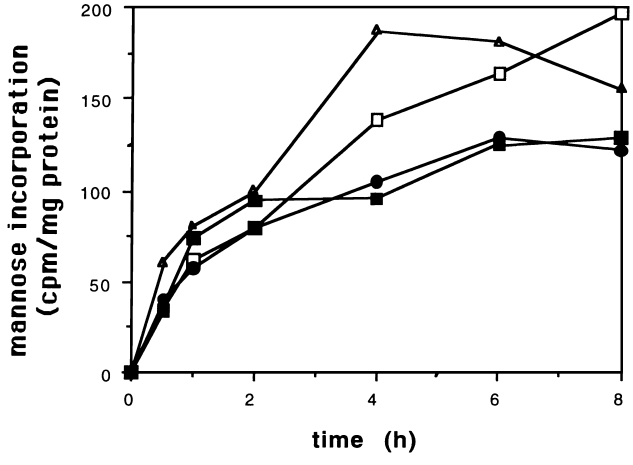


Figure 3. Effect of rhamnospicamycin **2a** concentration and time on mannose incorporation in HL60 cells. Cells were cultured for various times in the presence of **2a** and ^{3}H-mannose and the incorporated radioactivity determined as described in the Experimental section. (□), control, no **2a**; (△), 10 nM **2a**; (■), 50 nM **2a**; (•), 100 nM **2a**

4. Summary

This paper reports the details of the synthesis of enantiomeric rhamnose analogues of spicamycin. It shows that the diol side chain in the 7-carbon sugar which is a mimic of L-mannose is unimportant in that replacement of the diol by methyl to give **2a** does not have any significant reduction on the cytotoxicity. There are structural features in the sugar moiety that are important for biological activity since the enantiomer **2b** which resembles D-mannose in the sugar unit is effectively inactive. The lack of cytotoxicity in **16** demonstrates that the adenine moiety is also necessary. By contrast to spicamycin **1**, the rhamnose analogue appears to have an effect additional to the proposed swainsonine-like increase in glycoprotein *N*-glycan mannosylation.[13] This effect is more analogous to that observed with tunicamycin treatment, leading to a reduction in glycoprotein *N*-glycans and points to a mode of action that is multifactorial. Since spicamycin **1** is known to be metabolised in vivo, it may be that metabolites of rhamnospicamycin **2a** are responsible for this additional activity.

This work confirms that this class of compound is likely to be an interesting set of materials but the

present synthetic route needs significant modification in order to provide access to reasonable quantities of materials for evaluation and for convincing studies on the mechanism of action of such compounds.

5. Experimental

Melting points were recorded on a Kofler hot block and are corrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz) or a Bruker AM 500 (500 MHz) spectrometer. ^{13}C Nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz), a Bruker AC 200 (50 MHz) or a Bruker AM 500 (125 MHz) spectrometer and multiplicities were assigned using the DEPT sequence. All chemical shifts are quoted on the δ-scale. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; dd, double–doublet; ddd, double–double–doublet; t, triplet; q, quartet; dq, double–quartet; m, multiplet; br, broad; app, apparent. Infra-red spectra were recorded on a Perkin–Elmer 1750 FT IR spectrophotometer. Mass spectra were recorded on a VG platform (APCI, positive or negative as stated) or a VG autospec spectrometer or VG 20-250 spectrometer (chemical ionisation [NH_3, CI] as stated). Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis service of the Dyson Perrins Laboratory. Thin layer chromatography (TLC) was carried out on plastic or aluminium sheets coated with $60F_{254}$ silica, and plates were developed using a spray of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures.

5.1. *Methyl 6-deoxy-2,3-*O*-isopropylidene-α-*L*-talopyranoside* ***4a***

The title compound (5.97 g, 93% over two steps) $[\alpha]_D^{21}$ −54.2 (*c* 1.41, MeOH) {lit.[18] $[\alpha]_D^{21}$ −43.8 (*c* 1.62, $CHCl_3$)} was prepared from **3a** (6.39 g, 29.3 mmol), as previously described.[18]

Following essentially the same procedure,[18] the enantiomeric compound **3b** (4.32 g, 19.82 mmol) gave methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-talopyranoside **4b** (4.03 g, 93% over two steps) as a colourless oil; $[\alpha]_D^{21}$ +51.7 (*c* 1.32, MeOH); {lit.[20] $[\alpha]_D^{21}$ −48.9 (*c* 1.3, MeOH)}; ν_{max} (film) 3517 (OH), 2836 (OMe) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.34 (3H, d, *J*=6.6 Hz, H_3-6), 1.39, 1.59 (2×3H, 2×s, $C(CH_3)_2$), 2.20 (1H, d, *J*=6.7 Hz, OH), 3.41 (3H, s, OMe), 3.56 (1H, t, *J*=5.5 Hz, H-4), 3.84 (1H, q, *J*=6.6 Hz, H-5), 4.03 (1H, d, *J*=6.3 Hz, H-2), 4.21 (1H, dd, *J*=5.0, 6.2 Hz, H-3), 4.94 (1H, s, H-1); δ_C (50 MHz, $CDCl_3$) 16.53 (q, C-6), 25.09, 25.67 (2×q, $C(CH_3)_2$), 55.02 (q, OMe), 64.22, 66.80, 72.95, 73.17 (4×d, C-2, C-3, C-4, C-5), 98.50 (d, C-1), 109.38 (s, $C(CH_3)_2$); *m/z* (APCI+) 241 (MNa^+, 100%); HRMS *m/z* found: 236.149597 (MNH_4^+); $C_{10}H_{22}NO_5$ requires: 236.149798.

5.2. *Methyl 4-azido-4-deoxy-α-*L*-rhamnopyranoside* ***6a***

The title compound (1.05 g, 62% over two steps) mp 81–82°C (acetone/hexane) {lit.[18] 79–83°C (ether/hexane)} was prepared from the alcohol **4a** (2.01 g, 9.22 mmol) as previously described.[18]

Following essentially the same procedure,[18] the alcohol **4b** (2.04 g, 9.36 mmol) yielded the corresponding enantiomeric azide **6b** (1.2 g, 63% over three steps) as a white crystalline solid; mp 81–82°C (acetone/hexane); $[\alpha]_D^{21}$ +128.0 (*c* 0.72, MeOH) {lit.[21] 81.5–82.5°C, $[\alpha]_D^{27}$ +127 (*c* 1.0, MeOH)}; ν_{max} (film) 3368 (OH), 2836 (OMe), 2114 (N_3) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.36 (3H, d, *J*=6.2 Hz, H_3-6),

2.55 (2H, br s, 2×OH), 3.28 (1H, t, *J*=9.9 Hz, H-4), 3.36 (3H, s, OMe), 3.58 (1H, ddd, *J*=6.1, 6.2, 10.0 Hz, H-5), 3.83 (1H, dd, *J*=3.4, 9.8 Hz, H-3), 3.91 (1H, dd, *J*=1.6, 3.4 Hz, H-2), 4.69 (1H, d, *J*=1.4 Hz, H-1); δ_C (50 MHz, $CDCl_3$) 18.13 (q, C-6), 54.97 (q, OMe), 65.55, 66.61, 70.07, 70.42 (4×d, C-2, C-3, C-4, C-5), 100.72 (d, C-1); *m/z* (APCI−) 202 ($M-H^-$, 45%), 122 (100%); found: C, 41.31; H, 6.42; N, 20.28; $C_7H_{13}N_3O_4$ requires: C, 41.38; H, 6.45; N, 20.68%.

5.3. 4-Azido-4-deoxy-α-L-rhamnopyranose **7a**

A 1:1 mixture of trifluoroacetic acid:water (84 ml) was added to a stirred solution of azide **6a** (1.81 g, 8.93 mmol) in 1,4-dioxan (5.4 ml) and the reaction mixture was heated to reflux. TLC (ethyl acetate:hexane, 7:3) after 15 h showed no starting material (R_f 0.5) and a new product (R_f 0.15). The reaction mixture was cooled and concentrated in high vacuo. The residue was pre-adsorbed onto silica gel and purified by flash column chromatography (hexane:ethyl acetate, 4:6) to afford 4-azido-4-deoxy-α-L-rhamnopyranose (mixture of anomers α:β, 2:1) **7a** (1.46 g, 86%) as a white crystalline solid; mp 117–118.5°C (acetone/hexane) {lit.[18] 119–120°C (ethyl acetate/hexane), $[\alpha]_D^{21}$ −17.5 (initial), −57.6 (equilibrium) (*c* 1.07, MeOH)}; ν_{max} (film) 3392 (OH), 2114 (N_3) cm^{-1}; δ_H (500 MHz, CD_3CN+D_2O) 1.21 (3H, d, *J*=6.2 Hz, H_3-6 α-anomer), 1.24 (3H, d, *J*=5.8 Hz, H_3-6 β-anomer), 3.16 (1H, ddd, *J*=5.7, 5.9, 9.8 Hz, H-5 β-anomer), 3.19 (1H, t, *J*=9.6 Hz, H-4 β-anomer), 3.25 (1H, t, *J*=10.0 Hz, H-4 α-anomer), 3.52 (1H, dd, *J*=3.2, 9.4 Hz, H-3 β-anomer), 3.66 (1H, ddd, *J*=6.1, 6.2, 10.1 Hz, H-5 α-anomer), 3.70 (1H, dd, *J*=1.8, 3.3 Hz, H-2 α-anomer), 3.73 (1H, d, *J*=3.3 Hz, H-2 β-anomer), 3.74 (1H, dd, *J*=3.3, 10.1 Hz, H-3 α-anomer), 4.59 (1H, s, H-1 β-anomer), 4.96 (1H, d, *J*=1.7 Hz, H-1 α-anomer); δ_C (50 MHz, D_2O+dioxan) 18.10 (2×q, C-6), 65.26, 65.74, 67.63, 69.96, 70.80, 71.50, 72.97 (8×d, C-2, C-3, C-4, C-5), 94.28, 94.78 (2×d, C-1); *m/z* (APCI−) 188 ($M-H^-$, 15%), 101 (100%); found: C, 38.37; H, 5.87; N, 21.87; $C_6H_{11}N_3O_4$ requires: C, 38.10; H, 5.86; N, 22.21%.

Following the above procedure, the azide **6b** (1.77 g, 8.72 mmol) gave the D-azido lactols **7b** (1.41, 85%) as a white crystalline solid; mp 115–116°C (acetone/hexane) $[\alpha]_D^{21}$ +18.8 (initial), +64.6 (equilibrium) (*c* 1.11, MeOH) {lit.[21] 118–118.5°C (ethanol/*n*-pentane), $[\alpha]_D^{27}$ +21.6 (initial), +57.4 (30 min) (*c* 1.00, MeOH)}; ν_{max} (film) 3370 (OH), 2114 (N_3) cm^{-1}; δ_H (500 MHz, CD_3CN+D_2O) 1.21 (3H, d, *J*=6.2 Hz, H_3-6 α-anomer), 1.24 (3H, d, *J*=5.7 Hz, H_3-6 β-anomer), 3.15 (1H, ddd, *J*=5.8, 5.9, 9.9 Hz, H-5 β-anomer), 3.19 (1H, t, *J*=9.6 Hz, H-4 β-anomer), 3.25 (1H, t, *J*=10.0 Hz, H-4 α-anomer), 3.52 (1H, dd, *J*=3.2, 9.3 Hz, H-3 β-anomer), 3.65 (1H, ddd, *J*=6.2, 6.3, 10.1 Hz, H-5 α-anomer), 3.69 (1H, dd, *J*=1.7, 3.2 Hz, H-2 α-anomer), 3.72 (1H, d, *J*=3.5 Hz, H-2 β-anomer), 3.74 (1H, dd, *J*=3.3, 9.9 Hz, H-3 α-anomer), 4.59 (1H, s, H-1 β-anomer), 4.96 (1H, d, *J*=1.4 Hz, H-1 α-anomer); δ_C (125 MHz, D_2O+dioxan) 18.25, 18.31 (2×q, C-6), 65.26, 65.73, 67.63, 69.92, 70.77, 71.47, 72.93 (8×d, C-2, C-3, C-4, C-5), 94.19, 94.69 (2×d, C-1); *m/z* (APCI−) 188 ($M-H^-$, 100%); found: C, 38.10; H, 5.84; N, 22.02; $C_6H_{11}N_3O_4$ requires: C, 38.10; H, 5.86; N, 22.21%.

5.4. 6-(4′-Azido-4′-deoxy-β-L-rhamnopyranosylamino)-4-chloro-5-nitropyrimidine **9a**

4-Azido-4-deoxy-α-L-rhamnopyranose **7a** (585 mg, 3.09 mmol) was dissolved in aqueous ammonia (32% v/v, 29 ml) and the reaction mixture was heated to 60°C. TLC (ethyl acetate:methanol, 9:1) after 19 h indicated the absence of starting material (R_f 0.58) and the formation of a major product (R_f 0.14). The solvent was removed in vacuo and the crude product **8a** dissolved in dry DMF (17.5 ml). Then triethylamine (0.86 ml, 6.19 mmol) and 4,6-dichloro-5-nitropyrimidine (1.32 g, 6.81 mmol) were added, while stirring the mixture at room temperature. TLC (ethyl acetate:methanol, 9:1) after 30 min showed no starting material (R_f 0.14) and the formation of two new spots (R_f 0.77 and R_f 0.44). The reaction was

quenched with water, and the mixture stirred for a further 60 min. After adding some drops of 10% HCl, the mixture was extracted with CH_2Cl_2 (×3). The organic extracts were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was pre-adsorbed on silica gel and purified by flash column chromatography (ethyl acetate:hexane, 3:7) to afford the chloro-derivative **9a** (214 mg, 20% over two steps); mp 142–144°C; $[\alpha]_D^{21}$ +23.2 (*c* 0.59, MeOH); ν_{max} (film) 3378 (OH, NH), 2114 (N_3), 1584 (NO_2) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.26 (3H, d, *J*=5.9 Hz, H_3-6′), 3.29 (1H, t, *J*=9.7 Hz, H-4′), 3.34 (1H, ddd, *J*=6.0, 6.0, 9.8 Hz, H-5′), 3.70–3.98 (2H, m, OH), 3.73 (1H, dd, *J*=3.2, 9.5 Hz, H-3′), 3.87 (1H, dd, *J*=1.1, 3.2 Hz, H-2′), 5.58 (1H, dd, *J*=1.0, 8.6 Hz, H-1′), 8.21 (1H, d, *J*=8.3 Hz, NH), 8.45 (1H, s, H-3); δ_C (125 MHz, CD_3CN) 18.69 (q, C-6′), 65.62, 70.64, 72.64, 73.50, 79.02 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 128.60 (s, C-6), 155.51, 156.13 (2×s, C-1, C-5), 159.09 (d, C-3); *m/z* (APCI+) 346 and 348 (MH^+, 100 and 30%); HRMS *m/z* found: 346.067385 (MH^+); $C_{10}H_{13}N_7O_5Cl$ requires: 346.066670.

Following the above procedure, the compound **7b** (925 mg, 4.89 mmol) gave 6-(4′-azido-4′-deoxy-β-D-rhamnopyranosylamino)-4-chloro-5-nitropyrimidine **9b** (325 mg, 19% over two steps); mp 141–143°C; $[\alpha]_D^{21}$ −27.9 (*c* 0.34, MeOH); ν_{max} (film) 3370 (OH, NH), 2115 (N_3), 1590 (NO_2) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.28 (3H, d, *J*=5.9 Hz, H_3-6′), 3.31 (1H, t, *J*=9.5 Hz, H-4′), 3.37 (1H, ddd, *J*=5.8, 6.0, 9.8 Hz, H-5′), 3.73–3.90 (2H, m, OH), 3.75 (1H, dd, *J*=2.9, 9.1 Hz, H-3′), 3.89 (1H, dd, *J*=1.2, 3.1 Hz, H-2′), 5.60 (1H, dd, *J*=1.0, 8.6 Hz, H-1′), 8.22 (1H, d, *J*=8.5 Hz, NH), 8.47 (1H, s, H-3); δ_C (125 MHz, CD_3CN) 18.67 (q, C-6′), 65.58, 70.61, 72.62, 73.47, 79.00 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 128.52 (s, C-6), 155.48, 156.09 (2×s, C-1, C-5), 159.07 (d, C-3); *m/z* (APCI+) 346 and 348 (MH^+, 90 and 25%), 145 (100%); HRMS *m/z* found: 346.066461 (MH^+); $C_{10}H_{13}N_7O_5Cl$ requires: 346.066670.

5.5. 6-(4′-Azido-4′-deoxy-2′,3′-O-isopropylidene-β-L-rhamnopyranosylamino)-4-chloro-5-nitropyrimidine **10a**

2,2-Dimethoxypropane (1.12 ml) and camphorsulfonic acid (15 mg, 0.062 mmol) were added to a solution of the chloro-derivative **9a** (225 mg, 0.625 mmol) in acetone (AR grade, 11.25 ml) at room temperature. TLC (hexane:ethyl acetate, 1:1) after 16 h indicated the presence of starting material (R_f 0.32) and the formation of a major product (R_f 0.63) in a ratio 1:1. Solid sodium hydrogen carbonate was added and the mixture stirred for 1 h, filtered and concentrated in vacuo. The residue was redissolved in acetone (5.5 ml) and then 2,2-dimethoxypropane (0.55 ml) and camphorsulfonic acid (7 mg) were added again, while stirring the mixture at room temperature. After 15 h the reaction mixture was quenched as usual to give a residue which was pre-adsorbed on silica gel and purified by flash column chromatography (hexane:ethyl acetate, 85:15 → 65:35) to yield the acetonide **10a** (176 mg, 70% overall) as a white crystalline solid; mp 127–129°C; $[\alpha]_D^{21}$ +50.9 (*c* 0.55, acetone); ν_{max} (film) 3393 (NH), 2112 (N_3), 1584 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.34 (3H, d, *J*=5.9 Hz, H_3-6′), 1.42, 1.63 (2×3H, 2×s, $C(CH_3)_2$), 3.26 (1H, dd, *J*=6.9, 9.8 Hz, H-4′), 3.38 (1H, ddd, *J*=6.0, 6.1, 9.8 Hz, H-5′), 4.24 (1H, dd, *J*=5.3, 6.7 Hz, H-3′), 4.27 (1H, dd, *J*=2.4, 5.4 Hz, H-2′), 5.89 (1H, dd, *J*=2.0, 8.7 Hz, H-1′), 8.08 (1H, d, *J*=8.7 Hz, NH), 8.49 (1H, s, H-3); δ_C (125 MHz, $CDCl_3$) 18.40 (q, C-6′), 26.40, 28.08 (2×q, $C(CH_3)_2$), 66.11, 71.49, 73.25, 76.42, 78.08 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.32 (s, $C(CH_3)_2$), 127.84 (s, C-6), 155.08, 155.10 (2×s, C-1, C-5), 158.07 (d, C-3); *m/z* (APCI+) 386 and 388 (MH^+, 100 and 40%); HRMS *m/z* found: 386.099105 (MH^+); $C_{13}H_{17}N_7O_5Cl$ requires: 386.097970.

Following the above procedure, the compound **9b** (325 mg, 0.942 mmol) afforded the enantiomeric acetonide **10b** (243 mg, 67%); mp 126–127°C; $[\alpha]_D^{21}$ −48.3 (*c* 0.59, acetone); ν_{max} (film) 3373 (NH), 2118 (N_3), 1580 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.34 (3H, d, *J*=6.1 Hz, H_3-6′), 1.43, 1.63 (2×3H, 2×s, $C(CH_3)_2$), 3.26 (1H, dd, *J*=6.8, 9.8 Hz, H-4′), 3.38 (1H, ddd, *J*=6.1, 6.1, 9.8 Hz, H-5′), 4.24 (1H, dd, *J*=5.4, 6.5 Hz, H-3′), 4.27 (1H, dd, *J*=2.5, 5.8 Hz, H-2′), 5.90 (1H, dd, *J*=2.1, 8.8 Hz, H-1′), 8.09

(1H, d, *J*=8.7 Hz, NH), 8.49 (1H, s, H-3); δ_C (50 MHz, $CDCl_3$) 18.24 (q, C-6′), 26.29, 27.99 (2×q, $C(CH_3)_2$), 66.11, 71.45, 73.27, 76.42, 78.09 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.42 (s, $C(CH_3)_2$), 127.95 (s, C-6), 155.35, 155.35 (2×s, C-1, C-5), 158.39 (d, C-3); *m/z* (APCI+) 386 and 388 (MH^+, 100 and 40%); HRMS *m/z* found: 386.097656 (MH^+); $C_{13}H_{17}N_7O_5Cl$ requires: 386.097970.

*5.6. 4-Amino-6-(4′-azido-4′-deoxy-2′,3′-*O*-isopropylidene-β-*L*-rhamnopyranosylamino)-5-nitropyrimidine* ***11a***

To a solution of the acetonide **10a** (136 mg, 0.353 mmol) in methanol (3 ml) was added aqueous ammonia (32% v/v, 10 ml) and the mixture was heated to 60°C. TLC (hexane:ethyl acetate, 1:1) after 16 h showed no starting material (R_f 0.63) and one product (R_f 0.12). The solvent was removed in high vacuo, the residue pre-adsorbed on silica gel and purified by flash column chromatography (ethyl acetate:hexane, 4:6) to yield the amine **11a** (126 mg, 97%) as a white crystalline solid; mp 180–182°C (ethyl acetate/*n*-hexane); $[\alpha]_D^{21}$ +3.5 (*c* 0.60, acetone); ν_{max} (film) 3425, 3350, 3300 (NH), 2109 (N_3), 1580 (NO_2) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.26 (3H, d, *J*=5.9 Hz, H_3-6′), 1.39, 1.60 (2×3H, 2×s, $C(CH_3)_2$), 3.34 (1H, dd, *J*=7.4, 10.0 Hz, H-4′), 3.40 (1H, ddd, *J*=5.8, 5.9, 10.0 Hz, H-5′), 4.29 (1H, dd, *J*=5.3, 7.4 Hz, H-3′), 4.31 (1H, dd, *J*=2.3, 5.3 Hz, H-2′), 5.95 (1H, dd, *J*=2.3, 8.8 Hz, H-1′), 6.95, 8.38 (2×H, 2×br s, $ArNH_2$), 8.04 (1H, s, H-3), 9.64 (1H, d, *J*=8.6 Hz, NH); δ_C (125 MHz, CD_3CN) 18.42 (q, C-6′), 26.57, 28.19 (2×q, $C(CH_3)_2$), 66.99, 71.63, 74.73, 76.90, 78.76 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.41 (s, $C(CH_3)_2$), 114.44 (s, C-6), 157.73, 160.17 (2×s, C-1, C-5), 160.56 (d, C-3); *m/z* (APCI+) 367 (MH^+, 100%); found: C, 42.85; H, 4.83; N, 30.59; $C_{13}H_{18}N_8O_5$ requires: C, 42.62; H, 4.95; N, 30.59%.

Following the above procedure, the acetonide **10b** (220 mg, 0.571 mmol) yielded the aromatic amine **11b** (190 mg, 91%) as a white crystalline solid; mp 180–182°C (ethyl acetate/*n*-hexane); $[\alpha]_D^{21}$ −4.2 (*c* 0.62, acetone); ν_{max} (film) 3425, 3340, 3300 (NH), 2108 (N_3), 1580 (NO_2) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.27 (3H, d, *J*=5.8 Hz, H_3-6′), 1.41, 1.62 (2×3H, 2×s, $C(CH_3)_2$), 3.36 (1H, dd, *J*=7.1, 10.1 Hz, H-4′), 3.42 (1H, ddd, *J*=5.7, 5.8, 9.8 Hz, H-5′), 4.30 (1H, dd, *J*=5.3, 7.1 Hz, H-3′), 4.33 (1H, dd, *J*=2.2, 5.2 Hz, H-2′), 5.96 (1H, dd, *J*=2.2, 8.7 Hz, H-1′), 6.97, 8.41 (2×H, 2×br s, $ArNH_2$), 8.06 (1H, s, H-3), 9.66 (1H, d, *J*=8.5 Hz, NH); δ_C (125 MHz, CD_3CN) 18.39 (q, C-6′), 26.55, 28.17 (2×q, $C(CH_3)_2$), 66.93, 71.59, 74.68, 76.84, 78.73 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.40 (s, $C(CH_3)_2$), 114.38 (s, C-6), 157.70, 160.07 (2×s, C-1, C-5), 160.53 (d, C-3); *m/z* (APCI+) 367 (MH^+, 100%); found: C, 42.66; H, 5.00; N, 30.52; $C_{13}H_{18}N_8O_5$ requires: C, 42.62; H, 4.95; N, 30.59%.

*5.7. 6-(4′-Azido-4′-deoxy-2′,3′-*O*-isopropylidene-β-*L*-rhamnopyranosylamino)-4-(diethoxymethyl)-amino-5-nitropyrimidine* ***12a*** *and 6-(4′-azido-4′deoxy-2′,3′-*O*-isopropylidene-β-*L*-rhamnopyranosyl-amino)-4-(ethoxymethoxymethyl)amino-5-nitropyrimidine* ***13a***

5.7.1. Method 1

A solution of the amine **11a** (100 mg, 0.30 mmol) in triethylorthoformate (5.4 ml) was heated to 140°C. TLC (ethyl acetate:hexane, 7:3) after 23 h showed traces of starting material (R_f 0.35) and one main product (R_f 0.65). The mixture was cooled and concentrated in high vacuo. The residue was pre-adsorbed on silica gel with methanol and purified by flash column chromatography (hexane:ethyl acetate, 85:15) to give the pyrimidine **12a** (42 mg, 30%) as a colourless oil; $[\alpha]_D^{21}$ −26.1 (*c* 0.51, $CHCl_3$); ν_{max} (film) 3360 (NH), 2111 (N_3), 1586 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.26 (6H, t, *J*=7.1 Hz, $CH(OCH_2CH_3)_2$), 1.35 (3H, d, *J*=6.1 Hz, H_3-6′), 1.42, 1.64 (2×3H, 2×s, $C(CH_3)_2$), 3.26 (1H, dd, *J*=7.6, 9.9 Hz, H-4′), 3.39 (1H, ddd, *J*=6.1, 6.1, 9.8 Hz, H-5′), 3.64–3.75 (4H, m, $CH(OCH_2CH_3)_2$), 4.22 (1H, dd, *J*=5.4, 7.5

Hz, H-3′), 4.26 (1H, dd, *J*=2.4, 5.4 Hz, H-2′), 5.95 (1H, dd, *J*=2.4, 8.7 Hz, H-1′), 6.38 (1H, d, *J*=6.5 Hz, C*H*(OEt)$_2$), 8.18 (1H, s, H-3), 9.30 (1H, d, *J*=6.4 Hz, N*H*CH(OEt)$_2$), 9.73 (1H, d, *J*=8.7 Hz, NH); δ_C (125 MHz, $CDCl_3$) 14.95 (2×q, CH(OCH$_2$*C*H$_3$)$_2$), 18.44 (q, C-6′), 26.40, 28.16 (2×q, C(*C*H$_3$)$_2$), 61.14, 61.25 (2×t, CH(O*C*H$_2$CH$_3$)$_2$), 66.38, 71.44, 73.59, 76.31, 78.16 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 99.51 (d, *C*H(OEt)$_2$), 111.09 (s, *C*(CH$_3$)$_2$), 113.26 (s, C-6), 156.17, 156.72 (2×s, C-1, C-5), 159.51 (d, C-3); *m/z* (APCI+) 469 (MH^+, 33%), 423 (M^+-OEt, 65%), 103 (100%); HRMS *m/z* found: 469.215200 (MH^+); $C_{18}H_{29}N_8O_7$ requires: 469.215921.

Further elution afforded the compound **13a** (47.5 mg, 35%) as a colourless oil; $[\alpha]_D^{21}$ −34.4 (*c* 0.59, $CHCl_3$); ν_{max} (film) 3361 (NH), 2111 (N_3), 1585 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.27 (6H, t, *J*=7.1 Hz, 2×CH(OCH$_2$C*H*$_3$)), 1.35 (6H, d, *J*=6.1 Hz, H$_3$-6′), 1.42, 1.64 (2×6H, 2×s, 2×C(C*H*$_3$)$_2$), 3.26 (2H, dd, *J*=7.6, 9.9 Hz, H-4′), 3.39 (2H, ddd, *J*=6.0, 6.1, 9.9 Hz, H-5′), 3.41, 3.42 (2×3H, 2×s, 2×OMe), 3.66–3.74 (4H, m, 2×CH(OC*H*$_2$CH$_3$)), 4.22 (2H, dd, *J*=5.3, 7.5 Hz, H-3′), 4.27 (1H, dd, *J*=2.4, 5.4 Hz, H-2′), 5.97 (2H, dd, *J*=2.4, 8.1 Hz, H-1′), 6.32 (1H, d, *J*=6.3 Hz, C*H*(OEt)(OMe)), 6.33 (1H, d, *J*=6.4 Hz, C*H*(OEt)(OMe)), 8.19 (2H, s, H-3), 9.28 (2H, d, *J*=6.3 Hz, 2×N*H*CH(OEt)(OMe)), 9.73 (2H, d, *J*=8.6 Hz, NH); δ_C (125 MHz, $CDCl_3$) 14.91 (2×q, CH(OCH$_2$*C*H$_3$)), 18.42 (2×q, C-6′), 26.39, 28.15 (4×q, 2×C(*C*H$_3$)$_2$), 52.00, 52.07 (2×q, 2×CH(OMe)), 61.51, 61.56 (2×t, CH(O*C*H$_2$CH$_3$)), 66.36, 71.43, 73.56, 76.30, 78.14 (10×d, C-1′, C-2′, C-3′, C-4′, C-5′), 100.20 (2×d, *C*H(OEt)(OMe)), 111.08 (2×s, *C*(CH$_3$)$_2$), 113.30 (2×s, C-6), 156.17, 156.69 (4×s, C-1, C-5), 159.53 (2×d, C-3); *m/z* (APCI+) 455 (MH^+, 5%), 423 (M^+-OMe, 100%); HRMS *m/z* found: 455.200981 (MH^+); $C_{17}H_{27}N_8O_7$ requires: 455.200271.

5.7.2. Method 2

A solution of the amine **11a** (189 mg, 0.516 mmol) in triethylorthoformate (9.2 ml) was heated to 140°C. TLC (ethyl acetate:hexane, 7:3) after 19 h showed traces of starting material (R_f 0.35) and one main product (R_f 0.65). The mixture was cooled and concentrated in high vacuo. The residue was pre-adsorbed on silica gel with ethanol and purified by flash column chromatography (hexane:ethyl acetate, 85:15) to give the compound **12a** (80 mg, 33%; 82% over recovered starting material) as a colourless oil, identical to the material above. Further elution (hexane:ethyl acetate, 4:6) gave recovered starting material **11a** (116 mg, 61%).

Following the above procedure described in method 1, the amine **11b** (190 mg, 0.519 mmol) gave the enantiomeric pyrimidine **12b** (50 mg, 20%) as a colourless oil; $[\alpha]_D^{21}$ +24.4 (*c* 0.98, $CHCl_3$); ν_{max} (film) 3362 (NH), 2111 (N_3), 1586 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.26 (6H, t, *J*=7.1 Hz, CH(OCH$_2$CH$_3$)$_2$), 1.35 (3H, d, *J*=6.1 Hz, H$_3$-6′), 1.42, 1.64 (2×3H, 2×s, C(C*H*$_3$)$_2$), 3.27 (1H, dd, *J*=7.6, 9.9 Hz, H-4′), 3.40 (1H, ddd, *J*=6.1, 6.1, 9.9 Hz, H-5′), 3.66–3.74 (4H, m, CH(OC*H*$_2$CH$_3$)$_2$), 4.23 (1H, dd, *J*=5.3, 7.5 Hz, H-3′), 4.27 (1H, dd, *J*=3.4, 5.3 Hz, H-2′), 5.98 (1H, dd, *J*=2.4, 8.7 Hz, H-1′), 6.39 (1H, d, *J*=6.5 Hz, C*H*(OEt)$_2$), 8.19 (1H, s, H-3), 9.31 (1H, d, *J*=6.4 Hz, N*H*CH(OEt)$_2$), 9.74 (1H, d, *J*=8.6 Hz, NH); δ_C (125 MHz, $CDCl_3$) 14.94 (2×q, CH(OCH$_2$*C*H$_3$)$_2$), 18.41 (q, C-6′), 26.39, 28.15 (2×q, C(*C*H$_3$)$_2$), 61.10, 61.21 (2×t, CH(O*C*H$_2$CH$_3$)$_2$), 66.35, 71.39, 73.54, 76.24, 78.13 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 99.45 (d, *C*H(OEt)$_2$), 111.07 (s, *C*(CH$_3$)$_2$), 113.21 (s, C-6), 156.13, 156.68 (2×s, C-1, C-5), 159.49 (d, C-3); *m/z* (APCI−) 467 ($M-H^-$, 15%), 421 (M^+-EtOH, 100%); HRMS *m/z* found: 469.215328 (MH^+); $C_{18}H_{29}N_8O_7$ requires: 469.215921. Further elution afforded the compound **13b** (92 mg, 39%) as a colourless oil; $[\alpha]_D^{21}$ +27.1 (*c* 0.93, $CHCl_3$); ν_{max} (film) 3362 (NH), 2111 (N_3), 1587 (NO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.26 (6H, t, *J*=7.1 Hz, 2×CH(OCH$_2$C*H*$_3$)), 1.35 (6H, d, *J*=6.1 Hz, H$_3$-6′), 1.41, 1.63 (2×6H, 2×s, 2×C(C*H*$_3$)$_2$), 3.25 (2H, dd, *J*=7.6, 9.8 Hz, H-4′), 3.38 (2H, ddd, *J*=6.1, 6.2, 9.8 Hz, H-5′), 3.40, 3.41 (2×3H, 2×s, 2×OMe), 3.65–3.73 (4H, m, 2×CH(OC*H*$_2$CH$_3$)), 4.21 (2H, dd, *J*=5.4, 7.2 Hz, H-3′), 4.26 (1H, dd, *J*=2.2, 5.2 Hz, H-2′), 5.96 (2H, dd, *J*=2.3, 8.7 Hz, H-1′), 6.31

(1H, d, *J*=6.3 Hz, C*H*(OEt)(OMe)), 6.33 (1H, d, *J*=6.4 Hz, C*H*(OEt)(OMe)), 8.18 (2H, s, H-3), 9.28 (2H, d, *J*=6.3 Hz, 2×N*H*CH(OEt)(OMe)), 9.73 (2H, d, *J*=8.5 Hz, NH); δ_C (125 MHz, $CDCl_3$) 14.89 (2×q, CH(OCH$_2$*C*H$_3$)), 18.39 (2×q, C-6′), 26.37, 28.14 (4×q, 2×C(*C*H$_3$)$_2$), 51.95, 52.03 (2×q, 2×CH(OMe)), 61.47, 61.52 (2×t, CH(O*C*H$_2$CH$_3$)), 66.32, 71.37, 73.52, 76.23, 78.10 (10×d, C-1′, C-2′, C-3′, C-4′, C-5′), 100.13 (2×d, *C*H(OEt)(OMe)), 111.05 (2×s, *C*(CH$_3$)$_2$), 113.24 (2×s, C-6), 156.11, 156.65 (4×s, C-1, C-5), 159.52 (2×d, C-3); *m/z* (APCI+) 455 (MH^+, 3%), 423 (M^+-OMe, 100%); HRMS *m/z* found: 455.199673 (MH^+); $C_{17}H_{27}N_8O_7$ requires: 455.200271.

5.8. 6-(4′-Amino-4′-deoxy-2′,3′-O-isopropylidene-β-L-rhamnopyranosylamino)-9H-purine **14a**

5.8.1. Method 1

To a solution of the compound **12a** (87 mg, 0.186 mmol) in methanol (5.1 ml) was added 10% Pd/C (23 mg) and the mixture was stirred at room temperature under a hydrogen atmosphere. TLC (ethyl acetate:hexane, 7:3) after 24 h indicated complete conversion of starting material (R_f 0.65) to a baseline product. The mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (chloroform:methanol, 9:1) to yield the protected purine **14a** (32 mg, 53%) as a colourless oil; $[\alpha]_D^{21}$ +25.4 (*c* 0.22, MeOH); ν_{max} (film) 3340 (NH_2, NH), 1615 (C=C Ar) cm^{-1}; δ_H (500 MHz, CD_3OD) 1.24 (3H, d, *J*=6.2 Hz, H$_3$-6′), 1.41, 1.58 (2×3H, 2×s, C(C*H*$_3$)$_2$), 2.61 (1H, dd, *J*=8.3, 9.7 Hz, H-4′), 3.40 (1H, ddd, *J*=6.2, 6.2, 9.7 Hz, H-5′), 4.00 (1H, dd, *J*=5.3, 8.2 Hz, H-3′), 4.36 (1H, dd, *J*=2.4, 5.3 Hz, H-2′), 6.04 (1H, br s, H-1′), 8.18 (1H, s), 8.35 (1H, s); δ_H (500 MHz, DMSO-d_6) 1.12 (3H, d, *J*=6.1 Hz, H$_3$-6′), 1.34, 1.50 (2×3H, 2×s, C(C*H*$_3$)$_2$), 2.42 (1H, t, *J*=8.6 Hz, H-4′), 3.25 (1H, ddd, *J*=6.1, 6.1, 9.3 Hz, H-5′), 3.34 (2H, br s, NH_2), 3.94 (1H, dd, *J*=5.4, 7.8 Hz, H-3′), 4.26 (1H, m, H-2′), 5.95 (1H, br s, H-1′), 8.26 (1H, s), 8.31 (1H, s); δ_C (125 MHz, CD_3OD) 18.15 (q, C-6′), 26.79, 28.61 (2×q, C(*C*H$_3$)$_2$), 57.99, 74.19, 75.46, 77.79, 81.23 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.33 (s, *C*(CH$_3$)$_2$), 119.02 (s), 142.13 (d), 152.86 (s), 153.53 (d), 154.15 (s); *m/z* (APCI−) 319 ($M-H^-$, 100%); HRMS *m/z* found: 321.167035 (MH^+); $C_{14}H_{21}N_6O_3$ requires: 321.167514.

5.8.2. Method 2

To a solution of the compounds **12a** and **13a**, ratio 6:4 (115 mg), in methanol (6.7 ml) was added 10% Pd/C (30 mg) and the mixture was stirred at room temperature under a hydrogen atmosphere. TLC (ethyl acetate:hexane, 7:3) after 24 h indicated complete conversion of starting material (R_f 0.65) to a baseline product. The mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (chloroform:methanol, 9:1) to yield the compound **14a** (42 mg, 53%) as a colourless oil, identical to the material above.

Following the above procedure described in method 2, the compounds **12b** and **13b**, ratio 1:2 (130 mg), yielded 6-(4′-amino-4′-deoxy-2′,3′-*O*-isopropylidene-β-D-rhamnopyranosylamino)-9*H*-purine **14b** (48 mg, 54%) as a colourless oil; $[\alpha]_D^{21}$ −26.0 (*c* 0.65, MeOH); ν_{max} (film) 3351 (NH_2, NH), 1619 (C=C Ar) cm^{-1}; δ_H (500 MHz, CD_3OD) 1.27 (3H, d, *J*=6.2 Hz, H$_3$-6′), 1.43, 1.60 (2×3H, 2×s, C(C*H*$_3$)$_2$), 2.64 (1H, dd, *J*=8.2, 9.7 Hz, H-4′), 3.40 (1H, ddd, *J*=6.2, 6.2, 9.7 Hz, H-5′), 4.03 (1H, dd, *J*=5.2, 8.1 Hz, H-3′), 4.38 (1H, dd, *J*=2.4, 5.2 Hz, H-2′), 6.06 (1H, br s, H-1′), 8.21 (1H, s), 8.38 (1H, s); δ_C (125 MHz, CD_3OD) 18.14 (q, C-6′), 26.79, 28.59 (2×q, C(*C*H$_3$)$_2$), 57.99, 74.11, 75.45, 77.84, 81.13 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 111.35 (s, *C*(CH$_3$)$_2$), 119.01 (s), 142.08 (d), 152.84 (s), 153.54 (d), 154.15 (s); *m/z* (APCI−) 319 ($M-H^-$, 100%); HRMS *m/z* found: 321.166338 (MH^+); $C_{14}H_{21}N_6O_3$ requires: 321.167514.

5.9. 6-[4′-Deoxy-4′-(dodecanoylglycyl)amino-2′,3′-O-isopropylidene-β-L-rhamnopyranosylamino)-9H-purine **15a**

To a stirred solution of the compound **14a** (25 mg, 0.078 mmol) and dodecanoylglycine (22 mg, 0.086 mmol) in dimethylformamide (2.6 ml) was added *N*-hydroxysuccinimide (4.5 mg, 0.039 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (22.4 mg, 0.117 mmol) at room temperature. TLC (chloroform:methanol, 9:1) after 15 h showed complete conversion of starting material (R_f 0.1) to a single product (R_f 0.26). The mixture was concentrated in high vacuo and the residue purified by flash column chromatography (chloroform:methanol, 95:5) to afford the compound **15a** (31 mg, 71%) as a white powder; mp 165–168°C; $[\alpha]_D^{21}$ +6.3 (*c* 0.59, $CHCl_3$); ν_{max} (film) 3290 (NH), 1652, 1644 (RCONH) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.86 (3H, t, *J*=6.8 Hz, C*H*$_3$(CH$_2$)$_{10}$CO-), 1.23 (19H, br s, H$_3$-6′, CH$_3$(C*H*$_2$)$_8$CH$_2$CH$_2$CO-), 1.39 (3H, s, C*H*$_3$CCH$_3$), 1.63 (5H, s, CH$_3$(CH$_2$)$_8$C*H*$_2$CH$_2$CO-, CH$_3$CC*H*$_3$), 2.26 (2H, t, *J*=7.5 Hz, CH$_3$(CH$_2$)$_8$CH$_2$C*H*$_2$CO-), 3.70 (1H, m, H-5′), 3.81 (1H, q, *J*=9.0 Hz, H-4′), 4.00 (1H, dd *J*=5.0, 16.3 Hz, RNHC*H*$_2$CONH-), 4.06 (1H, dd, *J*=4.9, 16.6 Hz, RNHC*H*$_2$CONH-), 4.29 (1H, m, H-3′), 4.34 (1H, m, H-2′), 6.06 (1H, br s, H-1′), 6.94 (1H, br s, NH-purine), 7.19 (1H, br s, RN*H*CH$_2$CONH-), 7.50 (1H, d, *J*=8.4 Hz, RNHCH$_2$CON*H*-), 8.01 (1H, s), 8.43 (1H, s); δ_C (125 MHz, $CDCl_3$) 14.07 (q), 18.15 (q, C-6′), 22.63, 25.68 (2×t), 26.52, 28.00 (2×q, C(*C*H$_3$)$_2$), 29.23, 29.28, 29.34, 29.47, 29.57, 29.66, 31.84, 36.23 (8×t), 43.74 (t, RCH$_2$CONH*C*H$_2$CONH-), 55.11 (d, C-4′), 71.41 (d, C-5′), 74.13 (d, C-2′), 76.05 (d, C-1′), 77.18 (d, C-3′), 110.45 (s, *C*(CH$_3$)$_2$), 119.81 (s), 139.31 (d), 150.42 (s), 152.09 (d), 153.45 (s), 169.91, 174.52 (2×s, 2×C=O); *m/z* (APCI−) 594 and 596 (M+Cl$^-$, 45 and 15%), 558 (M−H$^-$, 100%); HRMS *m/z* found: 560.355172 (MH$^+$); $C_{28}H_{46}N_7O_5$ requires: 560.356043.

Following the above procedure, the compound **14b** (50 mg, 0.156 mmol) gave the glycopeptide **15b** (64 mg, 73%) as a white solid; mp 164–166°C; $[\alpha]_D^{21}$ −8.1 (*c* 0.73, $CHCl_3$); ν_{max} (film) 3293 (NH), 1652, 1644 (RCONH) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.84 (3H, t, *J*=6.7 Hz, C*H*$_3$(CH$_2$)$_{10}$CO-), 1.21 (19H, br s, H$_3$-6′, CH$_3$(C*H*$_2$)$_8$CH$_2$CH$_2$CO-), 1.37 (3H, s, C*H*$_3$CCH$_3$), 1.61 (5H, s, CH$_3$(CH$_2$)$_8$C*H*$_2$CH$_2$CO-, CH$_3$CC*H*$_3$), 2.25 (2H, t, *J*=7.4 Hz, CH$_3$(CH$_2$)$_8$CH$_2$C*H*$_2$CO-), 3.71 (1H, m, H-5′), 3.82 (1H, q, *J*=8.7 Hz, H-4′), 4.01 (1H, dd *J*=4.4, 15.3 Hz, RNHC*H*$_2$CONH-), 4.07 (1H, dd, *J*=4.4, 15.3 Hz, RNHC*H*$_2$CONH-), 4.30 (1H, m, H-3′), 4.33 (1H, m, H-2′), 6.05 (1H, br s, H-1′), 6.97 (1H, br s, NH-purine), 7.47 (1H, br s, RN*H*CH$_2$CONH-), 7.80 (1H, d, *J*=5.9 Hz, RNHCH$_2$CON*H*-), 8.01 (1H, s), 8.42 (1H, s); δ_C (125 MHz, $CDCl_3$) 14.05 (q), 17.93 (q, C-6′), 22.59, 25.66 (2×t), 26.50, 27.98 (2×q, C(*C*H$_3$)$_2$), 29.19, 29.25, 29.32, 29.44, 29.53, 29.55, 31.81, 36.16 (8×t), 43.66 (t, RCH$_2$CONH*C*H$_2$CONH-), 55.00 (d, C-4′), 71.38 (d, C-5′), 74.07 (d, C-2′), 75.91 (d, C-1′), 76.99 (d, C-3′), 110.40 (s, *C*(CH$_3$)$_2$), 119.67 (s), 139.44 (d), 150.24 (s), 151.97 (d), 153.31 (s), 169.96, 174.53 (2×s, 2×C=O); *m/z* (APCI−) 594 and 596 (M+Cl$^-$, 15 and 3%), 558 (M−H$^-$, 100%); HRMS *m/z* found: 560.358349 (MH$^+$); $C_{28}H_{46}N_7O_5$ requires: 560.356043.

5.10. 6-[4′-Deoxy-4′-(dodecanoylglycyl)amino-β-L-rhamnopyranosylamino)-9H-purine **2a**

A solution of the acetonide **15a** (25 mg, 0.045 mmol) in a 7:3 mixture of acetic acid:water (3 ml) was stirred at 60°C. TLC (chloroform:methanol, 8:2) after 6 h showed no starting material (R_f 0.70) and one major product (R_f 0.21). The solvent was removed under reduced pressure and the residue purified by flash column chromatography (chloroform:methanol, 85:15) to give L-rhamnospicamycin **2a** (19 mg, 82%) as a white powder; mp 224–226°C (decomposition); $[\alpha]_D^{21}$ +9.71 (*c* 0.35, MeOH); ν_{max} (film) 3283 (NH, OH), 1633 (RCONH) cm^{-1}; δ_H (500 MHz, CD_3OD) 0.91 (3H, t, *J*=6.8 Hz, C*H*$_3$(CH$_2$)$_{10}$CO-), 1.22 (3H, d, *J*=6.1 Hz, H$_3$-6′) 1.28–1.34 (16H, m, CH$_3$(C*H*$_2$)$_8$CH$_2$CH$_2$CO-), 1.65 (2H, m, CH$_3$(CH$_2$)$_8$C*H*$_2$CH$_2$CO-), 2.30 (2H, t, *J*=7.6 Hz, CH$_3$(CH$_2$)$_8$CH$_2$CH$_2$CO-), 3.62 (1H, ddd, *J*=6.1, 6.1, 9.9 Hz, H-5′), 3.74 (1H, dd, *J*=3.5, 10.5 Hz, H-3′), 3.90 (2H, s, RNHCH$_2$CONH-), 3.95 (1H,

t, J=10.2 Hz, H-4′), 4.02 (1H, d, J=2.2 Hz, H-2′), 5.70 (1H, br s, H-1′), 8.19 (1H, s), 8.35 (1H, s); δ_H (500 MHz, DMSO-d_6 at T=25°C) 0.83 (3H, t, J=6.9 Hz, CH_3(CH$_2$)$_{10}$CO-), 0.99 (3H, m, H$_3$-6′) 1.20–1.25 (16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO-), 1.48 (2H, t, J=6.8 Hz, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO-), 2.12 (2H, t, J=7.4 Hz, CH$_3$(CH$_2$)$_8$CH$_2$CH_2CO-), 3.50–3.71 (4H, m), 3.68 (2H, d, J=5.7 Hz, 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 Hz), 7.99, 8.00 (1H, 2×d, J=5.7 Hz), 8.21, 8.22 (1H, 2×s), 8.27, 8.29 (1H, 2×s); δ_H (500 MHz, DMSO-d_6 at T=80°C) 0.86 (3H, t, J=7.0 Hz, CH_3(CH$_2$)$_{10}$CO-), 1.05 (3H, d, J=6.1 Hz, H$_3$-6′), 1.25–1.31 (16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO-), 1.53 (2H, t, J=7.2 Hz, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO-), 2.16 (2H, t, J=7.5 Hz, CH$_3$(CH$_2$)$_8$CH$_2$CH_2CO-), 3.48 (1H, ddd, J=6.1, 6.1, 9.4 Hz, H-5′), 3.65 (1H, dd, J=2.9, 10.3 Hz, H-3′), 3.70 (1H, m), 3.72 (2H, d, J=5.5 Hz, RNHCH_2CONH-), 3.86 (1H, d, J=2.1 Hz, 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 Hz), 7.60 (1H, br s), 8.11 (1H, s), 8.27 (1H, s); δ_C (125 MHz, DMSO-d_6 at T=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$_2$CONH-), 42.50 (t, RCH$_2$CONHCH$_2$CONH-), 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); *m/z* (APCI−) 554 and 556 (M+Cl$^-$, 70 and 21%), 518 (M−H$^-$, 45%), 176 (100%); HRMS *m/z* found: 520.326784 (MH$^+$); $C_{25}H_{42}N_7O_5$ requires: 520.324743.

Following the above procedure, the compound **15b** (62 mg, 0.111 mmol) gave D-rhamnospicamycin **2b** (43 mg, 75%) as a white solid; mp 218–220°C (decomposition); $[\alpha]_D^{21}$ −14.3 (*c* 0.53, MeOH); ν_{max} (film) 3282 (NH, OH), 1634 (RCONH) cm^{-1}; δ_H (500 MHz, DMSO-d_6 at T=25°C) 0.83 (3H, t, J=7.0 Hz, CH_3(CH$_2$)$_{10}$CO-), 0.99 (3H, m, H$_3$-6′) 1.22–1.26 (16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO-), 1.48 (2H, t, J=6.7 Hz, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO-), 2.12 (2H, t, J=7.4 Hz, CH$_3$(CH$_2$)$_8$CH$_2$CH_2CO-), 3.50–3.72 (4H, m), 3.69 (2H, d, J=5.7 Hz, RNHCH_2CONH-), 3.81 (1H, s), 4.77 (1H, br s), 5.51, 5.55 (1H, 2×br s), 7.10 (1H, br s), 7.61 (1H, d, J=9.2 Hz), 8.00, 8.01 (1H, 2×d, J=5.7 Hz), 8.21, 8.23 (1H, 2×s), 8.27, 8.30 (1H, 2×s); δ_H (500 MHz, DMSO-d_6 at T=45°C) 0.84 (3H, t, J=7.0 Hz, CH_3(CH$_2$)$_{10}$CO-), 1.02 (3H, d, J=6.1 Hz, H$_3$-6′) 1.22–1.28 (16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO-), 1.50 (2H, t, J=6.9 Hz, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO-), 2.14 (2H, t, J=7.5 Hz, CH$_3$(CH$_2$)$_8$CH$_2$CH_2CO-), 3.50 (1H, m), 3.62 (1H, dd, J=3.0, 10.4 Hz, H-3′), 3.67–3.74 (1H, m), 3.71 (2H, d, J=5.6 Hz, RNHCH_2CONH-), 3.83 (1H, s), 5.32 (1H, br s), 5.65 (1H, br s), 7.05 (1H, br s), 7.49 (1H, d, J=9.1 Hz), 7.87, 7.88 (1H, 2×d, J=5.6 Hz), 8.19 (1H, s), 8.29 (1H, s); δ_C (125 MHz, DMSO-d_6 at T=45°C) 13.95 (q), 18.10 (q, C-6′), 22.11, 25.24, 28.74, 28.77, 28.89, 29.00, 29.05, 29.08, 31.34, 35.33 (10×t, CH$_3$(CH_2)$_{10}$CONHCH$_2$CONH-), 42.49 (t, RCH$_2$CONHCH$_2$CONH-), 52.76, 70.13, 71.49, 72.16, 77.95 (5×d, C-1′, C-2′, C-3′, C-4′, C-5′), 119.40 (s), 140.76 (d), 152.05 (s), 152.18 (d), 152.46 (s), 169.60, 172.72 (2×s, 2×C=O); *m/z* (APCI−) 554 and 556 (M+Cl$^-$, 70 and 30%), 518 (M−H$^-$, 40%), 176 (100%).

*5.11. Methyl 4-deoxy-4-(dodecanoylglycyl)amino-β-*L*-rhamnopyranoside* ***16***

To a solution of the azide **6a** (72 mg, mmol) in methanol (1.7 ml) was added 10% Pd/C (15 mg) and the mixture was stirred at room temperature under a hydrogen atmosphere. TLC (ethyl acetate:hexane, 7:3) after 18 h showed complete conversion of starting material (R_f 0.50) to a baseline product. The mixture was filtered through Celite and the solvent removed under reduced pressure to give a white solid. This crude product was dissolved in dry dimethylformamide (5 ml) and then dodecanoylglycine (100 mg, 0.392 mmol), *N*-hydroxysuccinimide (102 mg, 0.534 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (20.5 mg, 0.178 mmol) were added at room temperature. TLC (chloroform:methanol, 9:1) after 18 h showed complete conversion of starting material (baseline) to a single product (R_f 0.3). The solvent was concentrated in high vacuo and the residue was pre-adsorbed

on silica gel and purified by flash column chromatography (chloroform:methanol, 96:4) to afford the compound **16** (116 mg, 79% over two steps) as a white solid; mp 144–146°C; $[\alpha]_D^{21}$ −41.05 (*c* 0.475, MeOH); ν_{max} (film) 3390 (NH, OH), 1636 (RCONH) cm^{-1}; δ_H (500 MHz, CD_3OD) 0.92 (3H, t, *J*=6.8 Hz, C*H*$_3$(CH$_2$)$_{10}$CO-), 1.20 (3H, d, *J*=6.3 Hz, H$_3$-6′) 1.31–1.33 (16H, m, CH$_3$(C*H*$_2$)$_8$CH$_2$CH$_2$CO-), 1.63 (2H, m, CH$_3$(CH$_2$)$_8$C*H*$_2$CH$_2$CO-), 2.28 (2H, t, *J*=7.5 Hz, CH$_3$(CH$_2$)$_8$CH$_2$C*H*$_2$CO-), 3.33 (3H, s, OMe), 3.66 (1H, dddd, *J*=6.0, 6.1, 6.1, 10.1 Hz, H-5′), 3.75 (1H, dd, *J*=3.2, 10.4 Hz, H-3′), 3.79 (1H, dd, *J*=1.7, 3.2 Hz, H-2′), 3.85 (2H, s, RNHC*H*$_2$CONH-), 3.92 (1H, t, *J*=10.4 Hz, H-4′), 4.62 (1H, d, *J*=1.5 Hz, H-1′); δ_C (125 MHz, CD_3OD) 13.97 (q), 17.92 (q, C-6′), 22.14, 25.19, 28.73, 28.77, 28.90, 29.01, 29.06, 29.09, 31.35, 35.17 (10×t, CH$_3$(*C*H$_2$)$_{10}$CONHCH$_2$CONH-), 42.12 (t, RCH$_2$CONH*C*H$_2$CONH-), 52.55 (d, C-4′), 54.07 (q, OMe), 67.24, 68.07, 69.40 (3×d, C-2′, C-3′, C-5′), 101.02 (d, C-1′), 169.29, 172.50 (2×s, 2×C=O); *m/z* (APCI−) 451 and 453 ($M+Cl^-$, 100 and 35%), 415 ($M-H^-$, 15%); HRMS *m/z* found: 417.296967 (MH^+); $C_{21}H_{41}N_2O_6$ requires: 417.296462.

5.12. *Methyl 2,3*-O-*isopropylidene-6*-O-*tosyl-α*-D-*mannopyranoside* ***18***

Methyl 6-*O*-tosyl-α-D-mannopyranoside **17** (12.6 g, 36.2 mmol) was dissolved in a mixture of acetone and 2,2-dimethoxypropane (200 ml, 9:1) and the resulting solution was adjusted to pH 4 using camphorsulfonic acid. The mixture was stirred for 2 h at room temperature when TLC (ethyl acetate:hexane, 8:2) showed no starting material (R_f 0.14) and the formation of a single product (R_f 0.58). Solid sodium hydrogen carbonate was added and the mixture stirred for 1 h, filtered and concentrated in vacuo to afford the acetonide **18** (14 g, quant.) as a foam; $[\alpha]_D^{21}$ +13.2 (*c* 1.03, MeOH); ν_{max} (film) 3446 (OH) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.33, 1.48 (2×3H, 2×s, C(C*H*$_3$)$_2$), 2.45 (3H, s, *p*-C*H*$_3$C$_6$H$_4$-), 2.86 (1H, d, *J*=5.0 Hz, OH), 3.34 (3H, s, OMe), 3.58–3.63 (3H, s, OMe), 3.73 (1H, ddd, *J*=3.9, 3.9, 9.4 Hz, H-5), 4.10 (1H, s), 4.11 (1H, s), 4.29 (2H, d, *J*=3.5 Hz, H$_2$-6), 4.84 (1H, s H-1), 7.34 (2H, d, *J*=8.2 Hz, Ar), 7.81 (2H, d, *J*=8.2 Hz, Ar); δ_C (50 MHz, $CDCl_3$) 21.48 (q, *p*-*C*H$_3$C$_6$H$_4$-), 25.85, 27.70 (2×q, C(*C*H$_3$)$_2$), 55.06 (q, OMe), 68.03, 68.55, 75.42, 78.22 (4×d, C-2, C-3, C-4, C-5), 69.29 (t, C-6), 98.28 (d, C-1), 109.85 (s, *C*(CH$_3$)$_2$), 128.13, 130.04 (2×d, Ar), 132.98, 145.17 (2×s, Ar); *m/z* (APCI+) 411 (MNa^+, 100%), 389 (MH^+, 32%); HRMS *m/z* found: 389.126851 (MH^+); $C_{17}H_{25}O_8S$ requires: 389.127015.

5.13. *Methyl 2,3*-O-*isopropylidene-α*-D-*rhamnopyranoside* ***3b***

To a stirred solution of the tosylate **18** (3 g, 7.73 mmol) in dimethylsulfoxide (75 ml) was carefully added sodium borohydride (877 mg, 23.19 mmol) and the mixture was heated to 85°C. TLC (hexane:ethyl acetate, 7:3) after 3 h showed absence of starting material (R_f 0.14) and the formation of a single product (R_f 0.25). After cooling to room temperature, the reaction mixture was quenched by addition of an excess of saturated solution of ammonium chloride until effervescence ceased. The solvent was removed in high vacuo, the residue pre-adsorbed onto silica gel and purified by flash column chromatography (hexane:ethyl acetate, 8:2) to yield the 6-deoxy-derivative **3b** (1.43 g, 85%) as a colourless oil; $[\alpha]_D^{21}$ +12.8 (*c* 1.09, MeOH) {lit.[22] $[\alpha]_D^{21}$ +13.2 (*c* 2, EtOH)}; ν_{max} (film) 3447 (OH), 2834 (OMe) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.31 (3H, d, *J*=6.3 Hz, H$_3$-6), 1.35, 1.52 (2×3H, 2×s, C(C*H*$_3$)$_2$), 2.80 (1H, br s, OH), 3.38 (3H, s, OMe), 3.41 (1H, m, H-4), 3.63 (1H, ddd, *J*=6.2, 6.3, 9.2 Hz, H-5), 4.06 (1H, t, *J*=7.0 Hz, H-3), 4.12 (1H, d, *J*=5.9 Hz, H-2), 4.85 (1H, s, H-1); δ_C (125 MHz, $CDCl_3$) 17.44 (q, C-6), 26.09, 27.95 (2×q, C(*C*H$_3$)$_2$), 54.90 (q, OMe), 65.67, 74.37, 75.70, 78.37 (4×d, C-2, C-3, C-4, C-5), 98.11 (d, C-1), 109.47 (s, *C*(CH$_3$)$_2$); *m/z* (CI, NH_3) 219 (MH^+, 75%), 204 (M^+-H_2O, 80%), 100 (100%); HRMS *m/z* found: 219.122491 (MH^+); $C_{10}H_{19}O_5$ requires: 219.123249.

5.14. Enzyme assays

HL60 cells were grown in RPMI medium (Life Technologies Ltd, Paisley, UK) and seeded at 1.5×10^5 cells per ml in 24 well culture plates. Compounds **2a**, **2b** and **16** were added at various concentrations and the cultures incubated at 37°C for 15 h. Cells were harvested, washed, treated with 0.05% trypan blue and the number of viable cells counted using a hemocytometer. For studies on the effects of concentration of rhamnospicamycin **2a** on mannose incorporation, HL60 cells were seeded at 10^5 cells/ml and incubated for 16 h at 37°C in the presence of 0.01 to 100 nM concentrations of spicamycin. Cells were then incubated with ^{3}H-mannose (Amersham, 5 μCi/ml) for 2 h at 37°C, washed with PBS, acid fixed and the cell protein solubilised with 0.5 M NaOH as previously described.[23] The soluble products were analysed for radioactivity by scintillation counting and protein content. The effect of spicamycin on mannose incorporation at various times was performed by seeding HL60 cells at 10^5 cells/ml and incubating with 10, 50 and 100 nM concentrations of **2a** in the presence of 5 μCi/ml ^{3}H-mannose. At intervals of 0.5 to 8 h the cells were washed, acid fixed and the radioactivity and protein content measured as described above.

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