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Stereochemical Assignment and First Synthesis of the Core of Miharamycin Antibiotics

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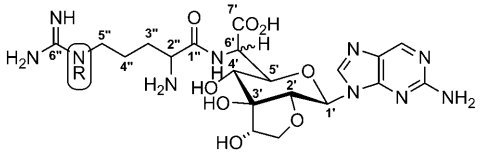
Abstract: The relative configuration at C-6′ of nucleoside antibiotic miharamycin A has been elucidated by NMR spectroscopy and proved to be *S*. The total synthesis of miharamycin B has also been investigated, which has led to the unprecedented construction of its core. The bicyclic sugar moiety has been elaborated by means of a SmI_2-based keto–alkyne coupling. Elongation of its C-6 position towards a bicyclic sugar amino acid and conversion into a suitable glycosyl donor enabled efficient N-glycosylation with 2-aminopurine to take place to afford the nucleosidic part of miharamycin B. Final peptide coupling with arginine afforded the skeleton of miharamycin B. Unfortunately, attempts to deprotect this scaffold failed to afford the complex nucleoside antibiotic.

Keywords: antibiotics · natural products · nucleosides · structure elucidation · total synthesis

Introduction

Complex nucleoside antibiotics comprise an extensive array of natural products that combine the structural features of nucleosides, higher monosaccharides, disaccharides, peptides and lipids.[1] They exhibit a variety of biological activities including antitumor, antiviral, antibacterial and antifungal properties.[1,2] While an increasing number of compounds of this class has been accessed by total synthesis,[3] there are still notable targets that have been approached but not yet synthesized. Amongst them, miharamycins A and B, isolated forty years ago from *Streptomyces miharaensis* SF-489, display strong activity against the rice blast disease caused by *Pyricularia oryzae*.[4] The structure of miharamycins was partially elucidated twenty-five years ago with the help of extensive spectroscopic and chemical degradation studies.[5]



miharamycin A (**1**): R = OH
miharamycin B (**2**): R = H

Miharamycins were found to be made up of an unusual higher monosaccharide component, appended to an N-terminal amino acid residue at C-6′, and a purine nucleobase connected at N^9. However, the absolute configuration at C-6′ for both antibiotics remains undetermined. Although considerable effort has been devoted to the total synthesis of miharamycins because of their unique and complex structure,[6] none of these attempts has yet been successful.

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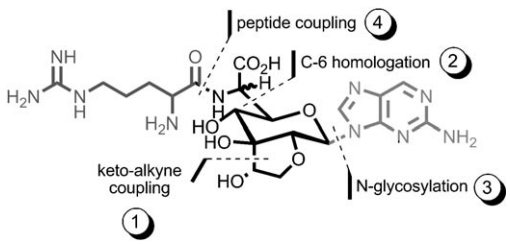
Results and Discussion

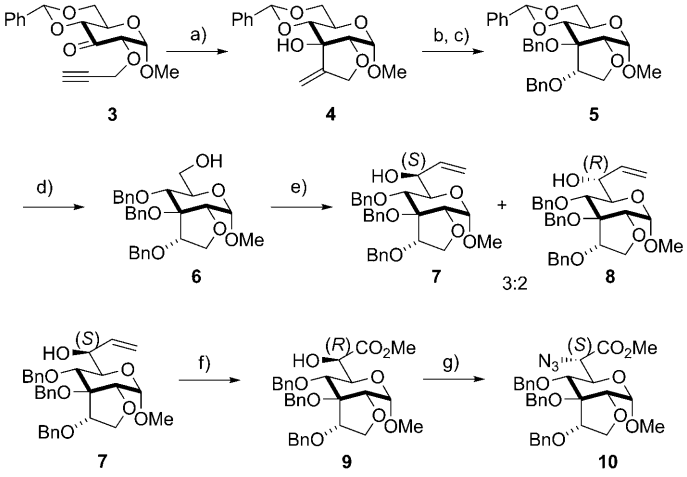
Determination of the relative configuration at C-6′ of miharamycin A: A prerequisite to the synthetic work was the confirmation of the relative structure of miharamycins and the determination of their configuration at C-6′. This was investigated by NMR spectroscopy, combining complementary standard 2D methods at 500 and 700 MHz (see the Supporting Information). The assignment of the ^{1}H and ^{13}C NMR spectroscopic resonance signals collected for miharamycin A supported the structure proposed by Seto.[5] Moreover, three key features allowed the non-ambiguous deduction of an *S* configuration for C-6′: a small $J_{H5'H6'}$ value (1.8 Hz, gauche-type major orientation), a large $J_{H5'C7'}$ value (>8 Hz, anti-type major orientation, C-7′ being the carboxylic carbon atom) and the existence of a H-1′/H-2″ NOE (associated distance ca. 4 Å). These three experimental observations can only be accommodated by the *S* configuration of the C-6′ stereogenic centre, along with the existence of a major conformer in which the lateral chain is folded towards the aminopurine moiety (see the Supporting Information).[7] Although no sample of miharamycin B has been available to us for NMR spectroscopic studies, it is highly probable that miharamycins A and B share the same *S* configuration at C-6′ since both antibiotics have been isolated from the same microorganism and *S*-configured natural amino acids are dominant in members of the peptidyl nucleoside family of antibiotics.[1]

Chemical synthesis: We then concentrated our efforts towards the total synthesis of miharamycin B, which incorporates an easily available L-arginine moiety. The synthetic challenge of miharamycin B stands in its unique elongated bicyclic sugar moiety and in the presence of an unusual 2-aminopurine nucleobase, which suggests the four disconnections listed in Scheme 1. Tactics harvested in previously completed complex nucleoside antibiotics syntheses usually place the sugar moiety construction early in the synthesis and the N-glycosylation step before the peptide coupling due to the Lewis basicity of the amide bond that can sometimes interfere with glycosylation conditions.[1] We, therefore, relied on the following strategy: 1) construction of the bicyclic carbohydrate unit, 2) elaboration of the amino acid at C-6′, 3) nucleoside synthesis and 4) peptide coupling followed by final deprotection (Scheme 1). Protection of hydroxyl and amino groups as benzyl ethers and *O*-benzyl carbamates respectively should ensure a clean final deprotection step under rather neutral conditions.



Scheme 1. Key disconnections planned for the synthesis of the miharamycin B framework.

As part of a program exploiting samarium(II) iodide methodology[8] to access and elucidate the framework of natural products displaying unusual sugar-like moieties,[9] we first investigated the synthesis of the bicyclic sugar moiety of miharamycin (Scheme 2). This bicyclic sugar moiety can

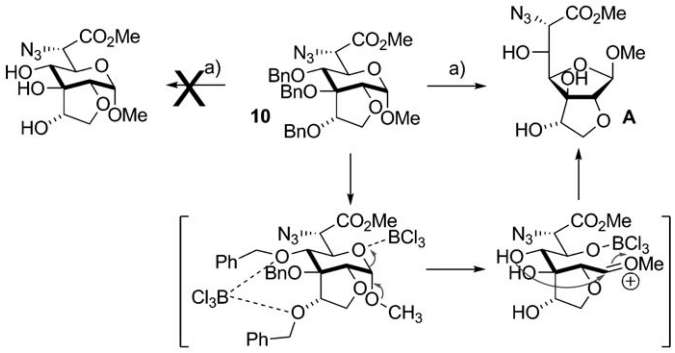


Scheme 2. Synthesis of azido ester **10**. a) SmI_2 (0.1 M in THF), HMPA, *t*BuOH, THF, 94%; b) O_3, −78°C, Me_2S, CH_2Cl_2 then $NaBH_4$, EtOH, 0°C, 84% over two steps; c) BnBr, NaH, DMF, 95%; d) $LiAlH_4/AlCl_3$, Et_2O/CH_2Cl_2 2:1, 50°C, 88%; e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C then CH_2=CHMgBr, THF, −78°C, 65% over 2 steps; f) O_3, −78°C, Me_2S, CH_2Cl_2 then $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, *t*BuOH/H_2O/2-methyl-but-2-ene 2:2:1 then MeI, $KHCO_3$, DMF, 65% over three steps; g) Tf_2O, pyridine, CH_2Cl_2, −78°C then NaN_3, DMF, 82% over 2 steps.

be considered the equivalent of a ring-opened 3′-keto compound and should be accessible by cyclisation of the corresponding α-propargyloxy ketone **3**. When treated with SmI_2 in the presence of hexamethylphosphoramide (HMPA) and *t*BuOH, ketone **3** smoothly afforded the desired exoalkene **4** through a 5-*exo*-dig ketyl–alkyne cyclisation.[10] Other approaches to the bicyclic carbohydrate core of miharamycins have also been reported more recently.[11] Further, ozonolysis of the olefin followed by $NaBH_4$ reduction, occurring exclusively from the *exo* face of the bicyclic system, furnished the desired kinetic diol, which was then benzylated to afford the tricyclic acetal **5**. Construction of the sugar amino acid core was then studied. Beside non-carbohydrate-based approaches that condense Garner's aldehyde as a masked amino acid,[12] several methods are available to homologate the C-6 position of a sugar pyranoside and construct the amino acid. Although stereocontrolled ethynylation of a dialdosugar has been reported,[13] we focused on a chain extension methodology, which used the vinyl group as a synthetic equivalent of the carboxylic acid functionality; this led to a lower degree of stereocontrol to obtain both epimers at C-6′ for further SAR studies. Regioselective benzylidene opening yielded primary alcohol **6**, which was further oxidized under

Swern conditions to give the aldehyde; this was then directly engaged in the alkylation step with vinyl magnesium bromide to afford the diastereomeric allylic alcohols (*S*)-**7** and (*R*)-**8** in a 3:2 ratio. The newly established C-6′ *S* configuration of miharamycin A and the required double inversion at C-6 to introduce the amino acid moiety prompted us to start from the *S*-configured alcohol **7**. Ozonolysis of allylic alcohol **7** and further oxidation to the carboxylic acid with $NaClO_2$ followed by esterification with iodomethane yielded the C-6 (*R*)-methyl ester **9**. Triflation of the free OH group and subsequent displacement with sodium azide provided the desired C-6 (*S*)-azido ester **10** as an oil (Scheme 2).

To firmly establish the stereochemistry of the azido ester **10** at C-6 (Scheme 3), the synthesis of the corresponding triacetylated azido ester was envisioned for X-ray crystallography purposes. Swapping hydroxyl protecting groups while keeping the azido group as a masked amine required the initial removal of the benzyl groups in the presence of BCl_3 at low temperature.[14] Unfortunately, under these conditions, a fused bistetrahydrofuran derivative **A** was isolated as the sole product, the structure of which was confirmed by X-ray crystallography (Figure 1).[15]



Scheme 3. Proposed mechanism for the skeleton rearrangement of azidoester **10**. a) BCl_3 (1 M in CH_2Cl_2), CH_2Cl_2, −78 to 0 °C, 65 %.

This skeleton rearrangement could be tentatively explained by a BCl_3-induced endocyclic cleavage of the pyranosidic ring to form an acyclic oxonium ion followed by an intramolecular nucleophilic attack of the hydroxyl group at the 4-position leading to an inversion of configuration of the anomeric methoxy group. This problematic ring contraction should be addressed because the key N-glycosylation step requires similar Lewis acidic conditions. The azido ester moiety of compound **10** was not implicated in this transformation and its *S* configuration was confirmed at this point.

Introduction of 2-aminopurine, an atypical nucleobase,[16] was then investigated and required preliminary conversion of the sugar amino acid precursor into a suitable glycosyl donor. Acetolysis of the anomeric methoxy group in azido ester **10** was optimised (Ac_2O, 5 % conc. H_2SO_4 in AcOH, low temperature) to minimize pyranosidic ring contraction and led to the corresponding glycosyl donor **11** in 39 % yield.[17] In a first approach, N-glycosylation with 2-aminopurine under classical Vorbrüggen conditions[18] that require persilylation of the nucleobase was studied. Unfortunately, all attempts to glycosylate 2-aminopurine as its bis-silylated *N*-acetyl derivative by using Czernecki[6a] and Garner's[6b] procedures ($SnCl_4$, $(CH_2Cl)_2/CH_3CN$, reflux and trimethylsilyl trifluoromethanesulfonate (TMSOTf), $(CH_2Cl)_2$, reflux, respectively) reported with glucose peracetate failed and led either to decomposition or recovery of the glycosyl donor with no trace of the expected nucleoside.[19] Hence, the more reactive 6-chloro-2-aminopurine was used as a masked 2-aminopurine. Coupling of the bis(trimethylsilyl) *N*-acetyl derivative of 6-chloro-2-aminopurine **12** with the glycosyl donor **11** under Vorbrüggen's conditions was examined (Scheme 4, Table 1).

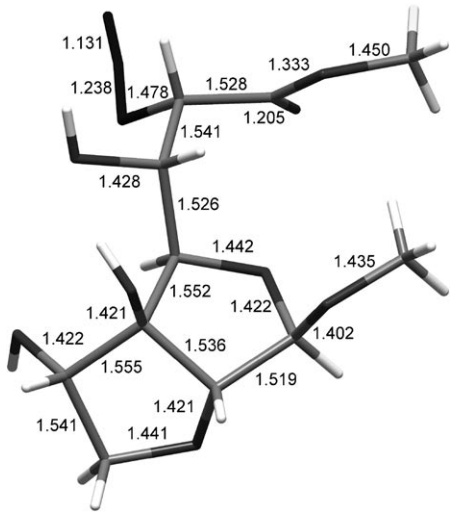
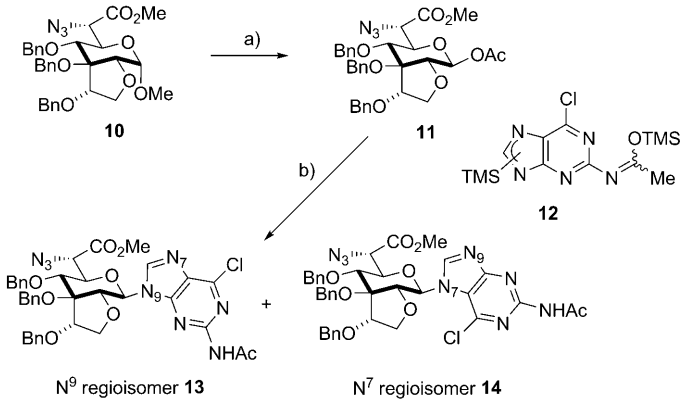


Figure 1. X-ray structure of the fused tetrahydrofuran derivative **A**.

Glycosylation of purines is rarely regiospecific and usually produces mixtures of N^9 and N^7 products despite numerous studies aimed at finding ways to maximize N^9 glycosylation.[21] While Garner[6b] devised conditions leading mainly to the thermodynamic N^9 regioisomer starting from glucose

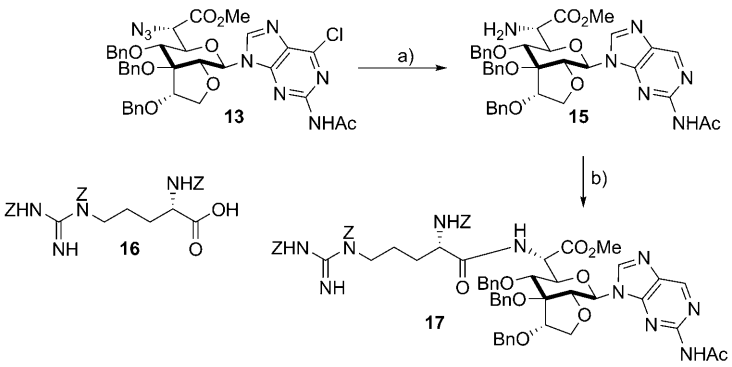


Scheme 4. N-glycosylation step. a) Ac_2O, H_2SO_4 5 % in AcOH, −20 to 0 °C, 39 %; b) **12**, TMSOTf, solvent and temperature (see Table 1).

Table 1. TMSOTf-mediated N-glycosylation of persilylated N^2-acetyl 6-chloropurine **12** with glycosyl donor **11**.

Entry[a]	Solvent	T [°C]	Reaction t [h]	**14/13** (N^7/N^9)[b]	Yield [%]
1	CH_3CN	65	3	1:0	55
2	$(CH_2Cl)_2/CH_3CN$	85	3	8:1	53
3	$(CH_2Cl)_2$	85	3	2:1	43
4	$(CH_2Cl)_2$	85	16	2:1	40
5	$(CH_2Cl)_2$	85	16	_[c]	_[c]
6	$PhCH_3$	110	4	1:4[d]	32
7	$PhCH_3$	85	4	1:3	58

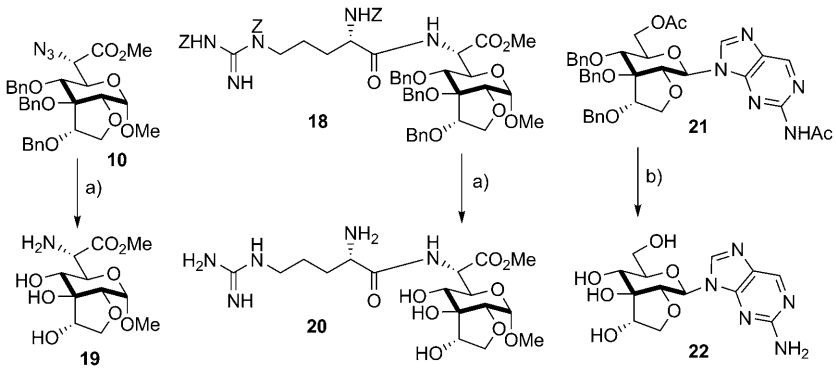
[a] All reactions were carried out on a 0.05 mmol scale of sugar substrate and yielded β-anomers exclusively. [b] The regioselectivity (N^7/N^9 ratio) of the N-glycosylation was determined by HMBC.[20] [c] Decomposition of the nucleobase and the glycosyl donor. [d] Deacetylation of the purine nucleobase was observed.



Scheme 5. Synthesis of the skeleton of miharamycin B. a) H_2, 10% Pd/C, Et_3N, EtOAc; b) **16**, isobutyl chloroformate, Et_3N, THF, −20°C, 70% over 2 steps.

peracetate, the same conditions applied to the perbenzylated glycosyl donor **11** afforded the kinetic N^7 regioisomer **14** as the major product (Table 1, entries 3 and 4). Since pyranosidic ring contraction was observed either during BCl_3-mediated deprotection of benzyl ethers or during acetolysis of the corresponding peracetylated methyl glycoside, benzyl groups had to be kept and glycosylation conditions (solvent, temperature) had to be finely tuned to favour the N^9 regioisomer. Non-polar solvents and high reaction temperatures usually lead to the thermodynamic N^9 compound as the major regioisomer,[22] but such harsh conditions (prolonged reaction times, temperatures above 100°C) resulted in decomposition of the nucleobase and of the glycosyl donor **11**. Finally, glycosylation performed with TMSOTf in toluene at 85°C for 4 h gave the best yield and N^9/N^7 ratio of the corresponding β-*N*-nucleoside **13** (entry 7). Noteworthy is the exclusive β-nucleoside formation observed in all cases, which, in the absence of neighbouring-group participation, can be tentatively explained by the steric hindrance of the α face of the pyranosidic ring due to the presence of the bulky tetrahydrofuran ring. We next moved to the peptidyl coupling step that required preliminary reduction of the azido group in nucleoside **13**. This reduction step was further exploited to also remove the chlorine atom on the nucleobase and concomitantly unmask the 2-aminopurine moiety. Optimised hydrogenation (Pd/C) of **13** cleanly afforded the tribenzylated amino ester **15**, which was directly engaged in the peptide coupling reaction without purification. While classical peptidyl coupling conditions (1,3-dicyclohexylcarbodiimide, 1-hydroxybenzotriazole) gave unsatisfactory yields, activation of the *N*-benzyloxycarbonyl-protected L-arginine **16**[23] with isobutyl chloroformate[24] resulted in the formation of the core of miharamycin B **17** in 70% yield (Scheme 5).

Unfortunately, removal of the protecting groups to afford miharamycin B proved to be highly problematic (Scheme 6). While hydrogenolysis (Pd/C) of the Z groups present on the L-arginine moiety seemed to occur smoothly, the benzyl groups present on the bicycle proved reluctant to undergo hydrogenolysis under these conditions. Deprotection of model compounds was thus explored. Successful hydrogenolysis under similar conditions of nucleobase-free bicyclic sugar **10** and sugar amino acid **18** was observed to afford trihydroxylated structures **19** and **20**, respectively, but the nucleoside **21** was totally inert to hydrogenolysis performed with 10% Pd/C, Pd black, Pearlman catalyst at 30 psi or catalytic hydrogen transfer, a behaviour also reported with other benzylated nucleosides.[25] Nevertheless, hydrogenolysis of nucleoside **21** under pressure (400 psi) with 10% Pd/C afforded the fully deprotected nucleoside **22** after acetamide and ester hydrolysis. When these hydrogenolysis conditions (H_2, 400 psi, 10% Pd/C, AcOH, 8 h) were applied to protected miharamycin **17**, removal of the Z group on the arginine moiety was observed as well as removal of all but one of the benzyl groups. Iteration of this process to convert the remaining monobenzylated compound led to decomposition of the fully debenzylated molecule.



Scheme 6. Debenzylation attempts on model compounds. a) H_2 (30 psi), 10% Pd/C, glacial AcOH, 2.5 h; b) H_2 (400 psi), 10% Pd/C, glacial AcOH, 8 h then NH_4OH/MeOH 1:1, 60°C, 24 h, 86%.

Conclusion

We have confirmed the structure of miharamycin A and elucidated for the first time its stereochemistry at C-6′. NMR spectroscopic conformational analysis further suggested a folding of the arginine appendage above the sugar ring towards the 2-aminopurine nucleobase. Total synthesis of miharamycin B was also undertaken and has led to the first synthesis of the core of miharamycins **20**, including construction of its unique bicyclic sugar amino acid moiety as well as the regio- and stereoselective introduction of the atypical 2-aminopurine nucleobase. Unfortunately, final deprotection of this core proved reluctant to all conditions used. Nevertheless, this work has revealed and solved unexpected synthetic problems (ring contraction, N-glycosylation) arising from the unique structure of the miharamycins. The results presented herein could be helpful for the construction of related complex nucleoside natural products, such as amipurimycin,[26] a synthetic target under investigation,[27] but still to be reached by total synthesis.

Experimental Section

NMR spectroscopic studies: NMR spectroscopic experiments in D_2O solution were carried out at 500 and 700 MHz and at 288 and 298 K. A concentration of ca. 18 mM of miharamycin A was used. A complete assignment of the ^{1}H NMR resonance signals of miharamycin A was achieved on the basis of TOCSY, HMQC and HMBC experiments. NMR spectroscopic experiments were also performed in a mixture of H_2O/D_2O 90:10 to detect the NH resonance. In this case, a sample concentration ca. of 7 mM was used and the experiments were recorded at 500 MHz, 290 K and with the Watergate pulse sequence for water suppression.

General methods: Melting points were determined with a Büchi B-510 capillary apparatus and are uncorrected. Optical rotations were measured at (20±2)°C with a Perkin–Elmer Model 241 digital polarimeter, by using a 10 cm, 1 mL cell. Mass spectra (CI (ammonia) and FAB) were obtained with a JMS-700 spectrometer. Elemental analysis were performed by the service d′analyse de l′Université Pierre et Marie Curie, 75252 Paris cedex 05 (France). ^{1}H NMR spectra were recorded at 400 MHz with a Brüker DRX 400 for solutions in $CDCl_3$, [D_6]DMSO or D_2O at room temperature. Assignments were confirmed by COSY experiments. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), brs (broad singlet) etc. ^{13}C NMR spectra were recorded at 100.6 MHz with a Brüker DRX 400 spectrometer. Assignments were confirmed by the J-mod technique, HMQC and HMBC. Reactions were monitored by TLC on a precoated plate of Silica Gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with H_2SO_4 10% in EtOH or with 0.2% w/v cerium sulfate and 5% ammonium molybdate in 2 M H_2SO_4. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

Tricyclic exoalkene 4: A suspension of samarium(II) iodide (170 mL of a 0.1 M solution in THF, 17.28 mmol), HMPA (13.8 mL, 79.49 mmol) and *t*BuOH (2.2 mL, 22.80 mmol) were stirred at room temperature under argon. A solution of ketone **3** (2.2 g, 6.91 mmol) in dry THF (100 mL) was added by syringe and the resulting mixture was stirred at room temperature for 45 min under argon. At this point, TLC analysis indicated the complete consumption of starting material and the formation of a single compound. Dilute HCl (20 mL, 0.1 M) was added and the reaction mixture was then filtered through a Celite plug. The reaction mixture was concentrated under reduced pressure and the residue obtained was then diluted with CH_2Cl_2 (250 mL) and water (75 mL). The aqueous layer was further extracted with CH_2Cl_2 (3×150 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue obtained was purified by flash chromatography (cyclohexane/EtOAc 2:1) to yield the alkene **4** (2.1 g, 95%) as a white solid. R_f=0.40 (cyclohexane/EtOAc 1:1); m.p. 125–127°C (ether/cyclohexane); $[\alpha]_D$=+151.0 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ=7.55–7.41 (m, 5H; H arom.), 5.87 (t, 1H, $J_{8'a,9a}$=$J_{8'a,9b}$=2.5 Hz; H-8′a), 5.58 (s, 1H; H-7), 5.10 (t, 1H, $J_{8'b,9a}$=$J_{8'b,9b}$=2.5 Hz; H-8′b), 4.84 (d, 1H; $J_{1,2}$=5.5 Hz; H-1), 4.72 (m, 2H; H-9a, H-9b), 4.36 (dd, 1H; $J_{6a,5}$=5.0, $J_{6a,6b}$=10.0 Hz; H-6a), 4.16 (d, 1H, $J_{2,1}$=5.5 Hz; H-2), 4.08 (d, 1H, $J_{4,5}$=10.0 Hz; H-4), 3.95 (dt, $J_{5,6a}$=5.0 Hz, 1H, $J_{5,4}$=$J_{5,6b}$=10.0 Hz; H-5), 3.77 (t, 1H, $J_{6b,5}$=$J_{6b,6a}$=10.0 Hz; H-6b), 3.41 (s, 3H; OCH_3), 2.60 ppm (s, 1H; OH); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C): δ=147.5 (C-8), 137.2 (C arom., quat.), 129.2, 128.4, 126.2 (5CH arom.), 108.4 (C-8′), 102.6 (C-7), 100.1 (C-1), 83.7 (C-2), 82.8 (C-4), 76.3 (C-3), 73.2 (C-9), 69.2 (C-6), 59.7 (C-5), 55.5 ppm (OCH_3); elemental analysis calcd (%) for $C_{17}H_{20}O_6$: C 63.74, H 6.29; found: C 63.48, H 6.29.

Bicycle 5: Alkene **4** (0.75 g, 2.34 mmol) was dissolved in CH_2Cl_2 (120 mL) and cooled to −78°C (note: this reaction cannot be performed on a large scale (above 1 g) since monitoring of the reaction (detection of the blue coloration) is difficult and results in a partial reaction or overoxidation of the product). Ozone was bubbled through the reaction mixture until a blue coloration persisted (typically 8 min). Dimethylsulfide (0.05 mL) was added and the mixture was allowed to warm to room temperature over a period of 1 h. The solvent was then removed under reduced pressure and the crude ketone was used in the next step without further purification. Sodium borohydride (98 mg, 2.58 mmol) was added carefully to a solution of the crude ketone in ethanol (50 mL) at 0°C. The reaction mixture was allowed to reach room temperature. After 1 h, TLC analysis indicated the complete consumption of starting material and the formation of a single compound. Methanol (50 mL) was added, the solvent was removed under reduced pressure and the residue was coevaporated with methanol (3×25 mL). Purification by flash column chromatography (cyclohexane/EtOAc 2:1 1:1) afforded the corresponding diol (0.64 g, 84% over two steps) as a white crystalline solid. R_f=0.20 (cyclohexane/EtOAc 1:1); m.p. 167–169°C (CH_2Cl_2/cyclohexane); $[\alpha]_D$=+94 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ=7.58–7.41 (m, 5H; H arom.), 5.55 (s, 1H; H-7), 4.92 (d, 1H, $J_{1,2}$=5.5 Hz; H-1), 4.42 (dd, 1H, $J_{9a,8}$=4.5, $J_{9a,9b}$=9.5 Hz; H-9a), 4.40 (d, 1H, $J_{OH,8}$=11.5 Hz; OH), 4.35 (dd, 1H, $J_{6a,5}$=5.0, $J_{6a,6b}$=10.0 Hz; H-6a), 4.25 (dt, 1H, $J_{5,6a}$=5.0, $J_{5,4}$=$J_{5,6b}$=10.0 Hz; H-5), 4.21 (dd, 1H, $J_{8,9a}$=4.5, $J_{8,OH}$=11.5 Hz; H-8), 4.10 (d, 1H, $J_{2,1}$=5.5 Hz; H-2), 3.99 (d, 1H, $J_{9b,9a}$=9.5 Hz; H-9b), 3.92 (d, 1H, $J_{4,5}$=10.0 Hz; H-4), 3.68 (t, 1H, $J_{6b,5}$=$J_{6b,6a}$=10.0 Hz; H-6b), 3.58 (s, 3H; OCH_3), 2.63 ppm (s, 1H; OH); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C): δ=137.0 (C arom., quat.), 129.5, 128.4, 126.5 (5CH arom.), 103.0 (C-7), 99.2 (C-1), 83.3 (C-4), 88.9 (C-2), 79.9 (C-3), 78.5 (C-9), 77.1 (C-8), 69.9 (C-6), 60.5 (C-5), 55.8 ppm (OCH_3); elemental analysis calcd (%) for $C_{16}H_{20}O_7$: C 59.25, H 6.22; found: C 59.19, H 6.22.

Sodium hydride (2.30 g, 45.35 mmol, 60% w/w) was added in portions to a solution of the diol (3.68 g, 11.35 mmol) in anhydrous DMF (40 mL) at 0°C whilst stirring. After 30 min, BnBr (8.10 mL, 68.09 mmol) was added at 0°C to the solution, which was then stirred for a further 2.5 h at room temperature. MeOH (30 mL) was added and the mixture was concentrated under reduced pressure. The residue was then diluted with ether (200 mL) and water (80 mL). The aqueous layer was extracted with ether (3×150 mL), the organic layers were combined, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 5:1) afforded the benzylated compound **5** (5.45 g, 95%) as a colourless oil. R_f=0.40 (cyclohexane/EtOAc 3:1); $[\alpha]_D$=+73.6 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ=7.48–7.24 (m, 15H; H arom.), 5.48 (s, 1H; H-7), 4.94 (d, 1H, $J_{1,2}$=5.8 Hz; H-1), 4.85 (d, 1H, J=12.2 Hz; CHPh), 4.77 (d, 1H, J=12.3 Hz; CHPh), 4.75 (d, 1H, J=12.2 Hz; CHPh), 4.66 (d, 1H, J=12.3 Hz; CHPh), 4.55 (dt, 1H, $J_{5,6a}$=5.1, $J_{5,4}$=$J_{5,6b}$=10.8 Hz; H-5), 4.38 (dd, 1H, $J_{8,9a}$=2.1, $J_{8,9b}$=4.9 Hz; H-8), 4.36–4.28 (m, 3H; H-6a, H-9a, H-9b), 4.27 (d, 1H; $J_{2,1}$=5.8 Hz; H-2), 4.11 (d, 1H, $J_{4,5}$=10.8 Hz; H-4), 3.67 (t, 1H, $J_{6b,5}$=$J_{6b,6a}$=10.8 Hz; H-6b), 3.46 ppm (s, 3H; OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C): δ=138.8, 138.6, 137.6 (C arom., quat.), 129.1–

126.2 (15 CH arom.), 102.1 (C-7), 99.5 (C-1), 86.0 (C-3), 82.6 (C-2), 81.9 (C-4), 81.5 (C-8), 75.6 (C-9), 72.6 (CH_2Ph), 70.0 (C-6), 67.0 (CH_2Ph), 59.3 (C-5), 55.2 ppm (OCH_3); HRMS (CIMS): *m/z*: calcd for $C_{30}H_{36}O_7N$: 522.2486 $[M+NH_4]^+$; found: 522.2480; elemental analysis calcd (%) for $C_{30}H_{32}O_7$: C 71.41, H 6.39; found: C 71.27, H 6.48.

Alcohol 6: $LiAlH_4$ (1.88 g, 49.54 mmol) was carefully added in three portions to a solution of compound **5** (5.0 g, 9.91 mmol) in a CH_2Cl_2/Et_2O mixture 1:1 (100 mL) at 0 °C under argon. After 10 min, the reaction mixture was warmed to 50 °C, and a solution of $AlCl_3$ (3.96 g, 29.73 mmol) in Et_2O (50 mL) was added dropwise under argon. After the reaction mixture had been stirred for 2.5 h, TLC analysis revealed complete consumption of the starting material. The reaction mixture was cooled to 0 °C and quenched by slow addition of EtOAc followed by water. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash column chromatography (cyclohexane/EtOAc 3:1 then 1:1) afforded alcohol **6** (4.38 g, 88 %) as a colourless oil. $R_f = 0.30$ (cyclohexane/EtOAc 1:1); $[\alpha]_D = +123.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.38$–7.27 (m, 15H; H arom.), 4.94 (d, 1H, $J_{1,2} = 5.9$ Hz; H-1), 4.95 (d, 1H, $J = 11.3$ Hz; CHPh), 4.78 (d, 1H, $J = 11.3$ Hz; CHPh), 4.67 (d, 1H, $J = 12.2$ Hz; CHPh), 4.66 (d, 1H, $J = 11.9$ Hz; CHPh), 4.63 (d, 1H, $J = 11.9$ Hz; CHPh), 4.57 (d, 1H, $J = 12.2$ Hz; CHPh), 4.38–4.30 (m, 4H; H-2, H-5, H-7, H-8a), 4.23 (dd, 1H, $J_{8b,7} = 6.1$, $J_{8b,8a} = 13.7$ Hz; H-8b), 4.17 (d, 1H, $J_{4,5} = 10.1$ Hz; H-4), 3.90 (ddd, 1H, $J_{6a,5} = 2.9$, $J_{6a,OH} = 4.9$, $J_{6a,6b} = 11.6$ Hz; H-6a), 3.83 (ddd, 1H, $J_{6b,5} = 3.7$, $J_{6b,OH} = 7.9$, $J_{6b,6a} = 11.6$ Hz; H-6b), 3.44 (s, 3H; OCH_3), 1.83 ppm (dd, 1H, $J_{OH,6a} = 4.9$, $J_{OH,6b} = 7.9$ Hz; OH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 139.0$, 139.0, 138.2 (C arom., quat.), 128.4–127.0 (15 CH arom.), 99.1 (C-1), 89.6 (C-3), 85.0 (C-7), 78.3 (C-2), 75.3 (C-8), 74.8 (C-4), 74.5 (CH_2Ph), 72.8 (CH_2Ph), 68.3 (C-5), 64.8 (CH_2Ph), 62.2 (C-6), 55.0 ppm (OCH_3); HRMS (CIMS): *m/z*: calcd for $C_{30}H_{38}O_7N$: 524.2643 $[M+NH_4]^+$; found: 524.2640; elemental analysis calcd (%) for $C_{30}H_{34}O_7$: C 71.13, H 6.76; found: C 70.85, H 7.01.

Allylic alcohols 7 and 8: Anhydrous DMSO (0.82 mL, 11.61 mmol) was added dropwise to a stirred solution of oxalyl chloride (0.84 mL, 9.67 mmol) in anhydrous CH_2Cl_2 (30 mL) at −78 °C under argon. After 15 min, a solution of alcohol **6** (0.98 g, 1.93 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise. After 1 h, dry Et_3N (2.16 mL, 15.48 mmol) was added and the reaction mixture was allowed to reach room temperature. After 1.5 h, water was added (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 80 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude aldehyde was coevaporated with toluene and used without further purification. Crude aldehyde was dissolved in dry THF (10 mL) and the solution cooled to −78 °C under argon. Vinyl magnesium bromide (9.65 mL, 9.65 mmol, 1 M in THF) was added dropwise to the solution and the reaction mixture was allowed to reach room temperature. After 1 h, the reaction mixture was quenched by the slow addition of a saturated aqueous NH_4Cl solution (10 mL) at 0 °C and was then diluted with ether (50 mL). The organic layer was separated, and the aqueous layer extracted with ether (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (cyclohexane/EtOAc 3:1 then 2:1) afforded the allylic alcohol (6*S*)-**7** (402 mg, 39 %) as a colourless oil. $R_f = 0.66$ (cyclohexane/EtOAc 1:1); $[\alpha]_D = +101.5$ ($c = 10$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.38$–7.29 (m, 15H; H arom.), 5.96 (ddd, 1H; $J_{7,6} = 5.1$, $J_{7,8b} = 10.6$, $J_{7,8a} = 17.3$ Hz; H-7), 5.33 (dt, 1H, $J_{8a,8b} = J_{8a,6} = 1.5$, $J_{8a,7} = 17.3$ Hz; H-8a), 5.23 (dt, 1H, $J_{8b,8a} = J_{8b,6} = 1.5$, $J_{8b,7} = 10.6$ Hz; H-8b), 4.99 (d, 1H, $J = 11.2$ Hz; CHPh), 4.94 (d, 1H, $J_{1,2} = 6.0$ Hz; H-1), 4.82 (d, 1H, $J = 11.2$ Hz; CHPh), 4.67 (d, 1H, $J = 11.8$ Hz; CHPh), 4.66 (s, 2H; CH_2Ph), 4.58 (d, 1H, $J = 11.8$ Hz; CHPh), 4.49–4.45 (m, 1H; H-6), 4.37 (dd, 1H, $J_{5,6} = 1.4$, $J_{5,4} = 10.2$ Hz; H-5), 4.34–4.19 (m, 5H; H-2, H-4, H-9, H-10a, H-10b), 4.01 (d, 1H, $J_{OH,6} = 9.1$ Hz; OH), 3.38 ppm (s, 3H; OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 139.0$ (C arom., quat.), 138.8 (C-7), 138.6, 138.3 (C arom., quat.), 128.4–127.1 (15 CH arom.), 115.3 (C-8), 99.3 (C-1), 90.0 (C-3), 85.3 (C-9), 78.1 (C-2), 75.3 (C-10), 75.0 (C-5), 74.2 (CH_2Ph), 72.9 (CH_2Ph), 70.5 (C-6), 70.0 (C-4), 64.8 (CH_2Ph), 55.1 ppm (OCH_3); HRMS (CIMS): *m/z*: calcd for $C_{32}H_{40}O_7N$: 550.2799 $[M+NH_4]^+$; found: 550.2803; elemental analysis calcd (%) for $C_{32}H_{36}O_7$: C 71.98, H 6.81; found: C 72.16, H 6.81; further elution afforded the allylic alcohol (6*R*)-**8** (268 mg, 26 %) as a colourless oil. $R_f = 0.59$ (cyclohexane/EtOAc 1:1); $[\alpha]_D = +94.2$ ($c = 10$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.39$–7.27 (m, 15H; H arom.), 6.00 (app ddd, 1H, $J_{7,6} = 6.3$, $J_{7,8a} = 10.4$, $J_{7,8b} = 17.0$ Hz; H-7), 5.35 (dt, 1H, $J_{8a,8b} = J_{8a,6} = 1.2$, $J_{8a,7} = 17.0$ Hz; H-8a), 5.26 (dt, 1H, $J_{8b,8a} = J_{8b,6} = 1.5$, $J_{8b,7} = 10.4$ Hz; H-8b), 4.97 (d, 1H, $J_{1,2} = 5.8$ Hz; H-1), 4.73 (d, 1H, $J = 10.9$ Hz; CHPh), 4.68 (s, 2H; CH_2Ph), 4.66 (d, 1H, $J = 12.0$ Hz; CHPh), 4.57 (d, 1H, $J = 12.0$ Hz; CHPh), 4.48 (d, 1H, $J_{2,1} = 5.8$ Hz; H-2), 4.46–4.42 (m, 2H; H-4, H-6), 4.22 (dd, 1H, $J_{10b,9} = 1.7$, $J_{10b,10a} = 8.7$ Hz; H-10b), 4.08 (dd, 1H, $J_{5,6} = 2.3$, $J_{5,4} = 11.7$ Hz; H-5), 3.44 (s, 3H; OCH_3), 2.68 ppm (d, 1H, $J_{OH,6} = 5.1$ Hz; OH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 138.7$, 138.1, 138.0, (C arom., quat.), 136.4 (C-7), 128.4–127.0 (15 CH arom.), 116.8 (C-8), 98.9 (C-1), 89.9 (C-3), 85.3 (C-9), 77.6 (C-2), 76.5 (C-5), 75.2 (C-10), 74.3 (CH_2Ph), 73.6 (C-4 or C-6), 72.9 (CH_2Ph), 70.1 (C-4 or C-6), 65.0 (CH_2Ph), 55.0 ppm (OCH_3); HRMS (CIMS): *m/z*: calcd for $C_{32}H_{40}O_7N$: 550.2799 $[M+NH_4]^+$; found: 550.2801; elemental analysis calcd (%) for $C_{32}H_{36}O_7$: C 71.98, H 6.81; found: C 71.90, H 6.63.

Hydroxy ester 9: Allylic alcohol (6*S*)-**7** (0.80 g, 1.50 mmol) was dissolved in CH_2Cl_2 (120 mL) and cooled to −78 °C. Ozone was bubbled through the reaction mixture until a blue colour persisted. Dimethylsulfide (0.1 mL) was added and the reaction mixture was allowed to reach room temperature. The solvent was evaporated and the crude aldehyde was used in the next step without further purification. $NaH_2PO_4 \cdot H_2O$ (2.69 g, 19.5 mmol) and $NaClO_2$ (2.44 g, 27 mmol) were added to a solution of the crude aldehyde in 2-methyl-but-2-ene (3 mL), *t*BuOH (6 mL) and water (6 mL). After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc (50 mL) and water (20 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The crude carboxylic acid obtained was directly engaged in the next step. To a solution of the crude carboxylic acid in DMF (8 mL) was added $KHCO_3$ (1.50 g, 15 mmol) followed by the slow addition of methyl iodide (0.79 mL, 12.75 mmol). After the reaction mixture had been stirred overnight, the solvent was removed and the crude residue diluted with Et_2O (80 mL) and water (30 mL). The aqueous layer was extracted with ether (3 × 80 mL) and the organic layers were combined, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 3:1) afforded the methyl ester (6*R*)-**9** (550 mg, 65 % over three steps) as a colourless oil; $R_f = 0.25$ (cyclohexane/EtOAc 2:1); $[\alpha]_D = +80.5$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.41$–7.32 (m, 15H; H arom.), 5.03 (d, 1H, $J = 11.2$ Hz; CHPh), 4.96 (d, 1H, $J_{1,2} = 5.8$ Hz; H-1), 4.86 (d, 1H, $J = 11.2$ Hz; CHPh), 4.82 (dd, 1H, $J_{5,6} = 1.6$, $J_{5,4} = 10.1$ Hz; H-5), 4.75 (d, 1H, $J = 11.9$ Hz; CHPh), 4.69 (s, 2H; CH_2Ph), 4.65–4.59 (m, 2H; H-6, CHPh), 4.41 (d, 1H, $J_{4,5} = 10.1$ Hz; H-4), 4.39–4.35 (m, 3H; H-2, H-8, H-9a), 4.28–4.24 (m, 1H; H-9a), 3.37 (s, 3H; OCH_3), 3.12 (s, 3H; OCH_3), 3.12 ppm (d, 1H, $J_{OH,6} = 7.6$ Hz; OH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 173.6$ (C=O), 138.9, 138.5, 138.1 (C arom., quat.), 128.3, −126.9 (15 CH arom.), 99.4 (C-1), 89.6 (C-3), 85.0 (C-2 or C-8), 77.8 (C-2 or C-8), 75.1 (C-9), 74.6 (CH_2Ph), 74.2 (C-4), 72.7 (CH_2Ph), 69.3 (C-5 and C-6), 64.7 (CH_2Ph), 54.9 (OCH_3), 52.2 ppm (OCH_3); HRMS (CIMS): *m/z*: calcd for $C_{32}H_{40}O_9N$: 582.2698 $[M+NH_4]^+$; found: 582.2694; elemental analysis calcd (%) for $C_{32}H_{36}O_9$: C 68.07, H 6.43; found: C 68.27, H 6.58.

Azido ester 10: Dry pyridine (1.40 mL, 17.28 mmol) was added to a solution of methyl ester (6*R*)-**9** (610 mg, 1.08 mmol) in dry CH_2Cl_2 (18 mL), followed by slow addition of Tf_2O (1.45 mL, 8.64 mmol) at −78 °C under argon. After the reaction mixture had been stirred for 90 min at room temperature, water (10 mL) and CH_2Cl_2 (20 mL) were added. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude triflate was directly engaged in the next step. Sodium azide (420 mg, 6.48 mmol) was added to a solution of the crude triflate in anhydrous DMF (12 mL) at room temperature. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was then diluted with ether (80 mL) and water (30 mL). The aqueous layer was extracted with ether (3 × 80 mL) and the organic layers were combined, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 4:1) afforded azido ester (6*S*)-**10** (522 mg, 82 % over two

steps) as a colourless oil; R_f=0.58 (cyclohexane/EtOAc 2:1); $[\alpha]_D$=+89.3 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ= 7.42–7.29 (m, 15H; H arom.), 5.02 (d, 1H, $J_{1,2}$=5.7 Hz; H-1), 5.01 (dd, 1H, $J_{5,6}$=2.5, $J_{5,4}$=10.3 Hz; H-5), 4.97 (d, 1H, J=10.0 Hz; CHPh), 4.74–4.67 (m, 3H; CH_2Ph, CHPh), 4.68 (d, 1H, J=12.3 Hz; CHPh), 4.53 (d, 1H, J=12.3 Hz; CHPh), 4.43–4.41 (m, 2H; H-2, H-6), 4.38 (dd, 1H, $J_{9a,8}$=5.5, $J_{9a,9b}$=8.9 Hz; H-9a), 4.36 (d, 1H, $J_{4,5}$=10.3 Hz; H-4), 4.31 (dd, 1H, $J_{8,9a}$=5.4, $J_{8,9b}$=1.7 Hz; H-8), 4.26 (dd, 1H, $J_{9b,8}$=1.7, $J_{9b,9a}$=8.9 Hz; H-9b), 3.56 (s, 3H; OCH_3), 3.29 ppm (s, 3H; OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=167.8 (C=O), 138.9, 138.0, 137.8 (C arom., quat.), 128.4–126.6 (15CH arom.), 99.6 (C-1), 89.8 (C-3), 85.6 (C-8), 77.2 (C-2), 75.3 (C-9), 74.4 (CH_2Ph), 73.7 (C-4), 72.7 (CH_2Ph), 68.9 (C-5), 64.5 (CH_2Ph), 63.1 (C-6), 55.5 (OCH_3), 52.0 ppm (OCH_3); HRMS (CIMS): m/z: calcd for $C_{32}H_{39}O_8N_4$: 607.2762 $[M+NH_4]^+$; found: 607.2761.

Bistetrahydrofuran A: Boron trichloride (3.4 mL, 3.40 mmol, 1 M in CH_2Cl_2) was added dropwise to a stirred solution of azido ester (6*S*)-**10** (100 mg, 0.17 mmol) in dry CH_2Cl_2 (4.5 mL) at −78 °C under argon. The reaction mixture was allowed to reach 0 °C over a period of 7 h, and was then quenched with a solution of CH_2Cl_2/MeOH 1:1 (12 mL) and concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 1:2) afforded the azido ester (6*S*)-**11** (35 mg, 65%) as a white crystalline solid. R_f=0.30 (cyclohexane/EtOAc 1:2); m.p. 82 °C (EtOAc); $[\alpha]_D$=−26.7 (c=1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=4.89 (s, 1H; H-1), 4.74 (d, 1H, $J_{5,4}$=5.6 Hz; H-5), 4.45 (t, 1H, $J_{8,9a}$=$J_{8,9b}$=5.6 Hz; H-8), 4.34–4.30 (m, 2H; H-4, H-6), 4.22 (s, 1H; H-2), 4.13 (dd, 1H, $J_{9a,8}$=5.6, $J_{9a,9b}$=9.5 Hz; H-9a), 3.88 (s, 3H; OCH_3), 3.84 (dd, 1H, $J_{9b,8}$=5.6, $J_{9b,9a}$=9.5 Hz; H-9b), 3.46 ppm (s, 3H; OCH_3), ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=170.0 (C=O), 108.9 (C-1), 92.9 (C-2), 90.7 (C-3), 79.5 (C-4), 78.8 (C-8), 74.2 (C-5), 74.1 (C-9), 63.4 (C-6), 56.4 (OCH_3), 53.3 ppm (OCH_3); HRMS (CIMS): m/z: calcd for $C_{11}H_{21}O_8N_4$: 337.1359 $[M+NH_4]^+$; found: 337.1343.

Glycosyl donor 11: A solution of sulfuric acid (5% in AcOH, 70 μL) was added dropwise at −20 °C to a solution of azido ester (6*S*)-**10** (150 mg, 254 mmol) in acetic anhydride (5 mL). The reaction mixture was stirred at −20 °C for 15 min and then neutralized by the slow addition of a $NaHCO_3$ saturated aqueous solution. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography (cyclohexane/CH_2Cl_2/EtOAc 8:1:1) afforded the glycosyl donor (6*S*)-**11** (61 mg, 39%) as a colourless oil. R_f= 0.44 (cyclohexane/EtOAc/CH_2Cl_2 4:1:1); $[\alpha]_D$=+23.9 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=7.37–7.22 (m, 15H; H arom.), 6.09 (d, 1H, $J_{1,2}$=7.7 Hz; H-1), 4.94 (d, 1H, J=9.7 Hz; CHPh), 4.79 (dd, 1H, $J_{5,6}$=2.4, $J_{5,4}$=9.7 Hz; H-5), 4.69 (s, 2H; CH_2Ph), 4.59–4.56 (m, 3H; CH_2Ph, CHPh), 4.49 (d, 1H, $J_{6,5}$=2.4 Hz; H-6), 4.41 (d, 1H, $J_{4,5}$=9.7 Hz; H-4), 4.28 (d, 1H, $J_{2,1}$=7.7 Hz; H-2), 4.22–4.20 (m, 2H; H-8, H-9a), 4.04 (br d, 1H, $J_{9b,9a}$=9.2 Hz; H-9b), 3.38 (s, 3H; OCH_3), 2.19 ppm (s, 3H; OAc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=167.4, 167.6 (2× C=O), 137.7, 137.4, 137.3 (C arom., quat.), 128.6–127.5 (15CH arom.), 93.5 (C-1), 90.1 (C-3), 84.6 (C-8), 77.5 (C-2), 74.8 (C-5), 74.6 (C-9), 73.8 (CH_2Ph), 72.8 (C-4), 72.6 (CH_2Ph), 65.0 (CH_2Ph), 63.1 (C-6), 52.3 (OCH_3), 21.1 ppm (OAc); HRMS (CIMS): m/z: calcd for $C_{33}H_{39}O_9N_4$: 635.2712 $[M+NH_4]^+$; found: 635.2719.

Nucleoside 13: *N*,*O*-bis(trimethylsilyl)acetamide (30 μL, 123 μmol) was added to a suspension of 2-acetamido-6-chloropurine (13 mg, 61 μmol) in dry 1,2-dichloroethane (1 mL) under argon. The mixture was heated to 80 °C for 45 min and then evaporated to dryness to afford crude compound **12**. This residue was then dissolved in dry toluene (0.5 mL) and a solution of the glycosyl donor **11** (25 mg, 41 μmol) in dry toluene (0.5 mL) was added, followed by the slow addition of TMSOTf (59 μL, 328 μmol) under argon. The reaction mixture was stirred at 85 °C for 4 h, cooled to room temperature and then diluted with CH_2Cl_2 (10 mL). The organic layer was neutralized with a $NaHCO_3$ saturated aqueous solution (2×5 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 3:1 then 2:1) afforded the N^9 nucleoside **13** (13.5 mg, 43%) as a colourless oil. R_f=0.63 (cyclohexane/EtOAc 1:1); $[\alpha]_D$=+25 (c=0.4 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=8.05 (s, 1H; H-8), 7.91 (br s, 1H; NH), 7.30–7.19 (m, 15H; H arom.), 6.08 (d, 1H, $J_{1',2'}$=8.9 Hz; H-1′), 4.95 (d, 1H, J=9.9 Hz; CHPh), 4.86 (br d, 1H, $J_{2',1'}$=8.9 Hz; H-2′), 4.79 (dd, 1H, $J_{5',6'}$=2.3, $J_{5',4'}$=9.8 Hz; H-5′), 4.74 (d, 1H, J=11.2 Hz; CHPh), 4.70 (d, 1H, J=11.2 Hz; CHPh), 4.58 (d, 1H, J=11.3 Hz; CHPh), 4.57–4.55 (m, 2H; H-4′, CHPh), 4.52 (d, 1H, J=11.3 Hz; CHPh), 4.37 (d, 1H, $J_{6',5'}$=2.3 Hz; H-6′), 4.27 (d, 1H, $J_{8',9'a}$=3.5 Hz; H-8′), 4.45 (dd, 1H, $J_{9'a,8'}$= 3.5, $J_{9'a,9'b}$=9.5 Hz; H-9′a), 4.04 (d, 1H, $J_{9'b,9'a}$=9.5 Hz; H-9′b), 3.36 (s, 3H; OCH_3), 2.41 ppm (s, 3H; NHAc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=170.5, 167.2 (2C=O), 152.6 (C-2 or C-6), 152.2 (C-4), 151.7 (C-2 or C-6), 142.5 (C-8), 137.5, 137.1, 137.0 (C arom., quat.), 128.7–127.4 (15CH arom.), 128.1 (C-5), 89.86 (C-3′), 85.1 (C-8′), 82.8 (C-1′), 77.4 (C-2′ or C-5′), 76.9 (C-2′ or C-5′), 74.8 (CH_2Ph), 74.7 (C-9′), 73.5 (CH_2Ph), 72.7 (C-4′), 65.4 (CH_2Ph), 63.1 (C-6′), 52.6 (OCH_3), 25.1 ppm (NHAc); HRMS (FAB): m/z: calcd for $C_{38}H_{37}O_8N_8$ $Na^{35}Cl$, $C_{38}H_{37}O_8N_8$ $Na^{37}Cl$: 791.2321 $[M+Na]^+$, 793.2291; found: 791.2328, 793.2301; further elution (cyclohexane/EtOAc 1:1 then 1:2) afforded the N^7 nucleoside **14** (4.5 mg, 15%) as a colourless oil. R_f=0.16 (cyclohexane/EtOAc 1:1); $[\alpha]_D$=+35 (c=0.3 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=8.45 (s, 1H; H-8), 8.05 (br s, 1H; NH), 7.41–7.30 (m, 15H; H arom.), 6.54 (d, 1H, $J_{1',2'}$=8.8 Hz; H-1′), 5.05 (d, 1H, J=10.1 Hz; CHPh), 4.87 (dd, 1H, $J_{5',6'}$= 2.2, $J_{5',4'}$=9.7 Hz; H-5′), 4.81 (d, 1H, J=11.0 Hz; CHPh), 4.76 (d, 1H, J= 11.0 Hz; CHPh), 4.66–4.55 (m, 5H; H-2′, H-4′, CHPh, CH_2Ph), 4.50 (d, 1H, $J_{6',5'}$=2.2 Hz; H-6′), 4.39 (d, 1H, $J_{8',9'a}$=3.3 Hz; H-8′), 4.35 (dd, 1H, $J_{9'a,8'}$=3.3, $J_{9'a,9'b}$=9.4 Hz; H-9′a), 4.19 (d, 1H, $J_{9'b,9'a}$=9.4 Hz; H-9′b), 3.47 (s, 3H; OCH_3), 2.63 ppm (s, 3H; NHAc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=171.0, 167.1 (2C=O), 163.2 (C-4), 152.6 (C-2 or C-6), 145.8 (C-8), 143.9 (C-2 or C-6), 137.5, 136.6, 136.8 (C arom. quat.), 128.8–127.4 (15CH arom.), 119.1 (C-5), 90.0 (C-3′), 84.9 (C-8′), 83.0 (C-1′), 77.5 (C-2′), 77.3 (C-5′), 74.7 (CH_2Ph), 74.4 (C-9′), 73.6 (CH_2Ph), 72.7 (C-4′), 65.5 (CH_2Ph), 63.1 (C-6′), 52.7 (OCH_3), 25.2 ppm (NHAc); HRMS (FAB): m/z: calcd for $C_{38}H_{37}O_8N_8$ $Na^{35}Cl$, $C_{38}H_{37}O_8N_8$ $Na^{37}Cl$: 791.2321 $[M+Na]^+$, 793.2291; found: 791.2317, 793.2277.

Protected miharamycin 17: 10% Pd/C (18 mg) and Et_3N (5 μL) were added to a solution of the N^9 nucleoside **13** (18 mg, 45 μmol) in EtOAc (1 mL). The solution was degassed three times and the air replaced by H_2. After stirring for 4 h at room temperature, the mixture was filtered through a Rotilabo Nylon 0.45 μm filter and the solvent evaporated under reduced pressure. The crude amine **15** was directly engaged in the next step. Triethylamine (16 μL, 115 μmol) was added at −20 °C under argon to a solution of protected L-arginine **16** (53 mg, 92 μmol) in anhydrous THF (1 mL) followed by slow addition of isobutylchloroformate (12 μL, 92 μmol). This solution was stirred for 45 min at −20 °C and a solution of the crude amine in dry THF (1 mL) was added at −20 °C under argon. After the reaction mixture had been stirred for 2 h at −20 °C under argon, the reaction was quenched by the addition of CH_2Cl_2/MeOH 1:1 (1 mL) and the solvent removed under reduced pressure. Purification by flash chromatography (CH_2Cl_2/EtOAc/MeOH 8:2:0.1) afforded the core of miharamycin B **17** (20 mg, 70% over two steps) as a colourless oil. R_f=0.24 (CH_2Cl_2/EtOAc/MeOH 1:1); $[\alpha]_D$=+8 (c=0.2 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=9.42, 9.30, 8.96 (br s, 3H; 3×NH), 8.87 (s, 1H; H-6), 8.80 (s, 1H; H-8), 7.40–7.21 (m, 30H; H arom.), 6.02 (d, 1H, $J_{1',2'}$=8.7 Hz; H-1′), 5.95 (d, 1H, $J_{NH,H6'}$= 8.3 Hz; NH), 5.20 (d, 1H, J=12.2 Hz; CHPh), 5.16 (d, 1H, J=12.2 Hz; CHPh), 5.13–4.97 (m, 7H; H-2′, H-6′, CHPh, 2CH_2Ph), 4.79–4.57 (m, 7H; H-4′, H-5′, CHPh, 2CH_2Ph), 4.39–2.29 (m, 1H; H-2“), 4.30 (d, 1H, $J_{8',9'a}$=3.2 Hz; H-8′), 4.22 (dd, 1H, $J_{9'a,8'}$=3.2, $J_{9'a,9'b}$=9.4 Hz; H-9′a), 4.04 (d, 1H, $J_{9'b,9'a}$=9.4 Hz; H-9′b), 3.98–3.75 (m, 2H; H-5”a, H-5“b), 3.53 (s, 3H; OCH_3), 2.19 (s, 3H; NHAc), 1.82–1.48 (m, 4H; H-3”a, H-3“b, H-4”a, H-4“b); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ=171.8, 171.0, 168.8, 163.5 (4×C=O), 160.8 (C=NH), 156.4, 155.8 (2×C=O), 152.9 (C-2), 152.2 (C-4), 149.8 (C-6), 143.0 (C-8), 137.7, 137.5, 137.0, 136.4, 136.3, 134.5 (C arom., quat.), 131.1 (C-5), 128.8–127.2 (30CH arom.), 89.9 (C-3′), 84.6 (C-8′), 83.0 (C-1′), 77.5 (C-5′ or C-4′), 76.3 (C-2′), 74.7 (CH_2Ph), 74.2 (C-9′), 73.6 (C-4′ or C-5′), 73.8 (CH_2Ph), 68.9 (CH_2Ph), 67.1 (CH_2Ph), 66.8 (CH_2Ph), 65.2 (CH_2Ph), 54.5 (C-2”), 52.6 (OCH_3), 52.4 (C-6′), 44.3 (C-5“), 29.7 (C-3” or C-4“), 25.2 (C-3” or C-4“), 24.9 ppm (NHAc); HRMS (FAB): m/z: calcd for $C_{68}H_{70}O_{15}N_{10}Na$: 1289.4914 $[M+Na]^+$; found: 1289.4920.

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