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ORIGINAL ARTICLE

Biosynthesis of 4-aminoheptose 2-epimers, core structural components of the septacidins and spicamycins

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Septacidins and spicamycins are acylated 4-aminoheptosyl-β-*N*-glycosides produced by *Streptomyces fimbriatus* and *S. alanosinicus*, respectively. Their structures are highly conserved, but differ in the stereochemistry of the 4-aminoheptosyl residues. The origin of this stereochemistry is unknown, but is presumably because of the difference in their biosynthetic pathways. We have synthesized the septacidin 4-aminoheptose to verify the difference between septacidin and spicamycin. Isotopic enrichment studies were undertaken using *S. fimbriatus*, and show that the septacidin heptose is derived from the pentose phosphate pathway. This indicates conserved pathways leading to the biosynthesis of 4-amino-4-deoxy-L-*gluco*-heptose or 4-amino-4-deoxy-L-*manno*-heptose.
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INTRODUCTION

The septacidins (**1**) and spicamycins (**2**) are unique but structurally related natural products produced by fermentation of diverse actinomyces species.[1,2] Both are differentiation inducers of leukemia cells, and a modified spicamycin (KRN5500) is in clinical trials for neuropathic pain suppression.[3,4] In 2011, the US Food and Drug Administration designated KRN5500 a Fast Track Drug Development Program.[5] Both series of compounds are comprised of four structural motifs: (1) a 4-deoxy-4-amino-L-heptose sugar; (2) a 6-*N*-glycosidically linked adenine group; (3) an amide-linked glycine; and (4) a variety of *N*-glycyl-linked acyl groups (Figure 1). To date, very little is known about their biosynthesis, although it is interesting for two reasons. First, the heptosyl *N*-glycosidic linkage is reported to be a to primary amine group, an uncommon structural feature that is found in only few natural products, and which implies the presence of potentially unusual *N*-glycosyltransferase biosynthetic enzymes. Second, the 4-deoxy-4-amino-L-heptosyl sugar motifs are reported to be *gluco*-heptose for septacidin, but *manno*-heptose for spicamycin.[1,2,6–8] This crucial difference in the stereochemistry at the 2′-position has marked implications on the potential biosynthetic pathways for septacidins and spicamycins. Either the presence of a unique 2-epimerase functionality is implied or the two different aminoheptoses may be biosynthesized by entirely diverse pathways. Moreover, this simple change in the stereochemistry at one position has implications on the relative biological activities of septacidins and spicamycins.

With these questions in mind, we first sought to confirm the reported stereochemistry for the carbohydrate motifs. The absolute configuration of septacidin has been tentatively assigned by Agahigian *et al.*[6] Septacidin was acid hydrolyzed to the aminoheptose, which was subsequently converted to an aminohexose by cleavage of the C6–C7 bond by periodate oxidation. A proton NMR study of the methyl glycoside tetraacetate derivative of this aminohexose was used to assign it as 4-amino-4-deoxy-L-*gluco*-pyranose, but unfortunately this was only undertaken at low resolution with a low field (60 MHz) NMR instrument.[6]

On the basis of this work, the absolute configuration of spicamycin was inferred to be 6-(4-amino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine.[7] More recently, anicemycin, an analog of spicamycin (Figure 1), has been isolated from an unrelated *Streptomyces* strain. The relative configuration has been shown by MS and NMR to be the same as spicamycin,[8] but unfortunately the absolute configuration was not determined. The absolute configuration of spicamycin has also been determined by X-ray crystallography,[7] but this has not yet been completed for the septacidins or anicemycins.

The spicamycin/septacidin series of aminosugar antibiotics have only been addressed by total synthesis on one previous occasion by Chida's group. These workers accessed spicamycin from myo-inositol by a lengthy route involving resolution of a non-symmetrical derivative, introduction of nitrogen with inversion of configuration, oxidative ring opening, two-carbon homologation, one-carbon

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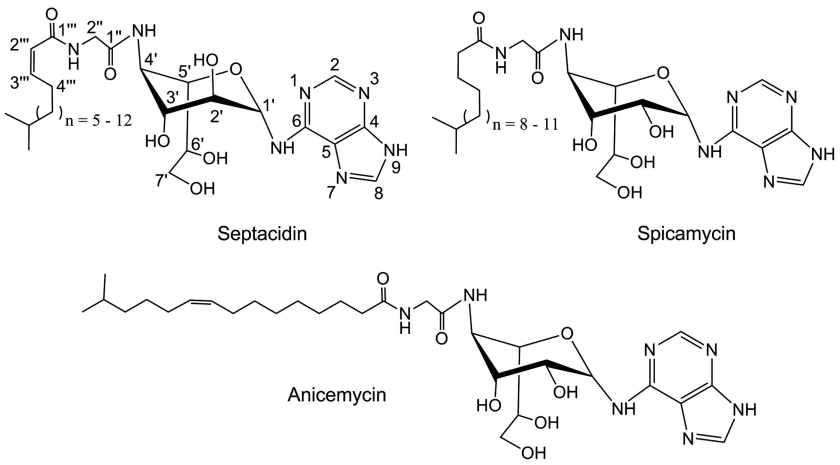


Figure 1 Structures of the septacidins (top left) and spicamycins (top right). Note the difference in the core 4-aminoheptose stereochemistry at C-2, in the identity of *N*-linked fatty acids. Anicemycin (lower) is a spicamycin analog with a specific unsaturated *N*-acyl group. The numbering system is as described previously.[8]

dehomologation and eventual introduction of the *N*-glycoside.[9] The coupling constants ($J_{H1,H2} < 1$ Hz, $J_{H2,H3} = 3.2$ Hz, $J_{H3,H4} = 9.4$ Hz, $J_{H4,H5} = 9.4$ Hz) observed in the ^{1}H NMR spectrum of this synthetic compound supported the β-*manno*-heptose configuration. Model D-series, β-D-*manno*-heptose and β-D-*gluco*-heptose analogs, of spicamycin and septacidin have also been reported by Chida *et al.*[10]

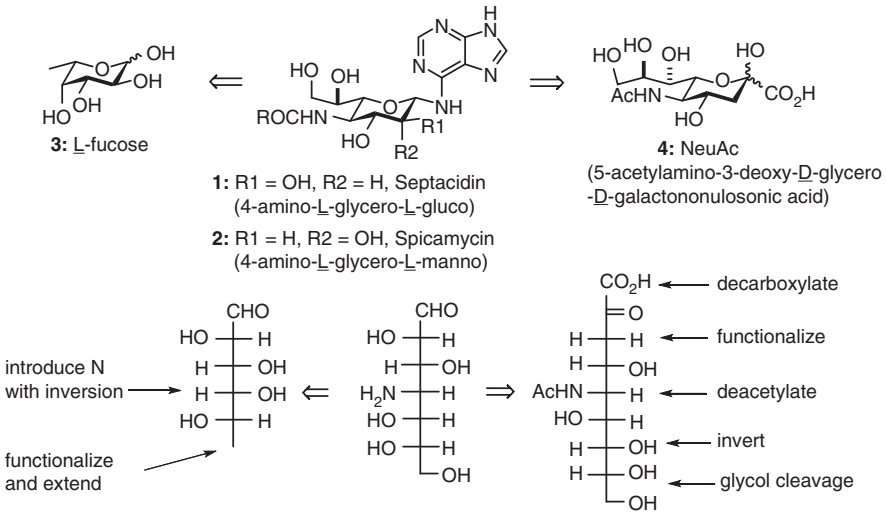
The synthesis of the first lower homologs of both spicamycin and septacidin has been described in the literature by two groups who adopted largely analogous routes. Thus, Acton and co-workers[11] prepared 7-nor-septacidin analogs from L-fucose by incorporation of nitrogen at the 4-position, with inversion of configuration and installation of the *N*-glycoside, whereas Mons and Fleet[13] obtained 7-nor-spicamycin analogs from L-rhamnose through azide displacement of an activated hydroxyl group, with inversion of configuration and eventual installation of the *N*-glycoside.[12]

To confirm the relative stereochemistries, an authentic sample of the septacidin glycone was prepared, starting from a neuramic acid thioglycoside. In principle, the enantiomer of septacidin should be available from 4-amino-4-deoxy-D-glucose, which can be obtained by degradation of the aminoglycoside antibiotic apramycin or by substitution at the 4-position of D-galactose, followed by one-carbon homologation at the 6-position. Alternatively, functionalization of L-fucose at the 6-position by a C–H activation process[14] followed by homologation and application of the Acton and Fleet approach should afford septacidin (Scheme 1). Ultimately, however, we selected a third approach starting from the readily available *N*-acetylneuraminic acid by simple removal of carbons 1 and 9, inversion at C-7 and functionalization at C-3 (Scheme 1). This approach was particularly attractive in view of recent improvements to the decarboxylation[15] of *N*-acetylneuraminic acid reported by the Gervay-Hague's group[16] with modifications by the Wong laboratory,[17] and because of our earlier experience with application of the Barton decarboxylation reaction[18] to the ulosonic acid glycosides.[19]

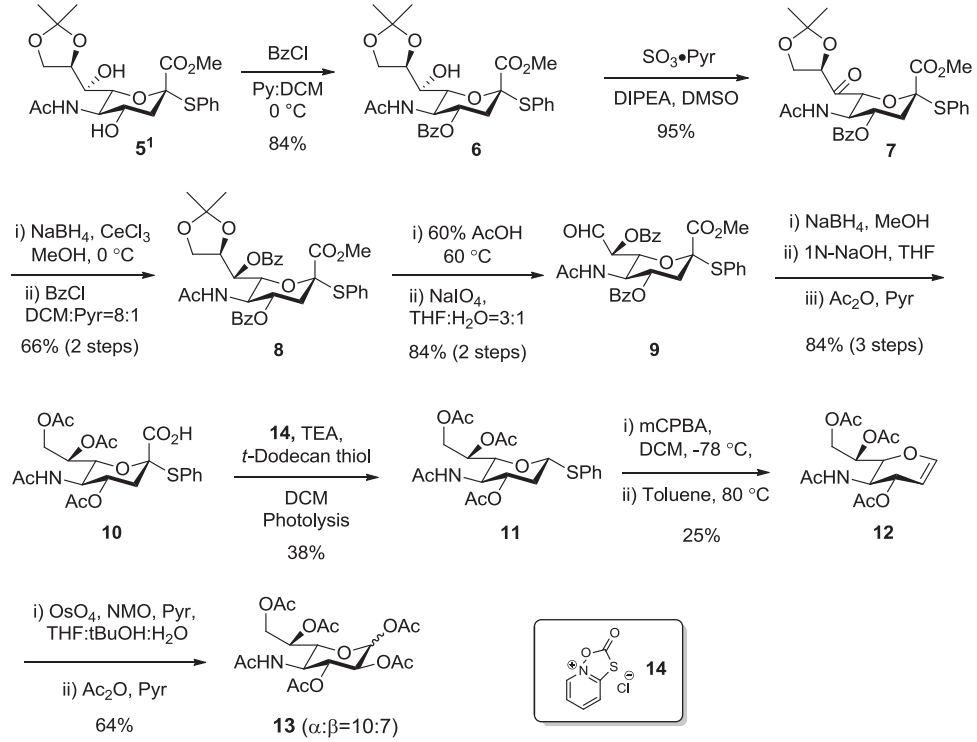
In the event, reaction of the known neuraminic acid derivative **5**[20] with benzoyl chloride at 0 °C enabled selective esterification on the less hindered 4-hydroxyl group, leaving the 7-hydroxyl group free for inversion. Direct inversion of **6** by the Mitsunobu protocol, or by triflation followed by displacement with various nucleophiles, was not productive; therefore, an oxidation reduction sequence was undertaken. Among the several oxidation procedures investigated, the Parikh–Doering protocol[21,22] with the sulfur trioxide–pyridine complex in dimethylsulfoxide was most satisfactory and gave ketone **7** in 95% yield. Stereoselective reduction of **7** was best achieved with the Luche reagent[23] in methanol at 0 °C, enabling, after benzoylation, isolation of the 7-epi-sialoside **8** in 66% yield with a selectivity of 17:1. Removal of the acetonide under standard conditions followed by treatment with sodium metaperiodate then afforded the aldehyde **9** in 84% yield. Reduction with sodium borohydride, saponification of the benzoate groups and acetylation gave the octulosonic acid derivative **10** in 84% yield. Application of the Barton decarboxylation procedure[17] using *tert*-dodecanethiol[24,25] as the hydrogen atom source lead to the isolation of the 2-deoxy-β-thioglycoside **11** in 38% yield and a minor amount of the α-isomer. Oxidation of **11** with *meta*-chloroperoxybenzoic acid at −78 °C gave a glycosyl sulfoxide, which on heating to 80 °C in toluene underwent elimination to provide the glycal **12**, albeit in only 25% yield for the two steps. Finally, reaction of the glycal **12** with catalytic osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide followed by acetylation gave the protected septacidin glycone **13** in 64% yield, with excellent selectivity for the desired *gluco*-isomer consistent with the precedent (Scheme 2).

The naturally occurring aminoheptoses were obtained by acid hydrolysis of native septacidin and spicamycin. This required a two-step lysis treatment, the first a methanolysis step to de-*N*-acylate the adenosyl-aminoheptose core (Figure 2), and a second aqueous acid hydrolysis to recover the free aminoheptoses. We noted that attempted strong acid hydrolysis of the adenosyl-aminoheptoses leads predominantly to a 1,7-anhydro-4-aminoheptose, presumably via a 1,7-dehydration reaction (Supplementary Data).

The free 4-aminoheptoses from septacidin and spicamycin, and that from the synthetic septacidin glycone **13** were derivatized to form aldononitrile acetate derivatives (peracetylated aldononitriles (PAANs)) suitable for analysis by GC/MS.[26] To aid in the EI-MS fragmentation assignments (necessary for the later isotopic enrichment experiments), we also included 4-amino-D-glucose in this study, obtained from hydrolysis of apramycin. The authentic 4-amino-L-*gluco*-heptose PAAN from the synthetic standard eluted at 17.4 min. This coeluted with the 4-amino-L-heptose PAAN from hydrolyzed native septacidin (Figure 3). In contrast with this, the 4-aminoheptose derivative from spicamycin eluted at 17.7 min. The three samples had identical EI-MS spectra, giving rise to stable ions that predominantly arise from C3–C4 (*m/z* 288, 168, 128 and 84) and C4–C5 (*m/z* 241, 199 and 139) cleavage either side of



Scheme 1 Retro-synthesis of the 4-amino-4-deoxy-L-*gluco*-pyranose core heptose of septacidins from L-fucose and *N*-acetylneuraminic acid.



Scheme 2 Synthesis of an authentic septacidin glycone starting from neuramic acid thioglycoside 5.

the *N*-acetyl group, and from a minor loss of the C7 carbon unit, as discussed below.

Biosynthesis studies

Having established that the aminoheptose sugar from native septacidin is 4-amino-*gluco*-heptose, we sought to apply this information to understanding its biosynthetic pathway. The biosynthesis of higher (long-chain) sugars generally involves the ligation of 2 or 3 carbon units (via the pentose phosphate pathway) or by the chain extension of pre-existing hexosyl or pentosyl intermediates.[27,28] To determine which, *Streptomyces fimbriatus*, cultures were grown on glucose-containing medium and metabolically labelled by the introduction of 11 different ^{13}C-enriched biosynthetic precursors to the cultures after 3 days. After a further 5 days of growth, the cultures were acidified and the labelled cells were harvested by centrifugation. The labelled septacidins were extracted from cell pellets with butanol, and evaporated to dryness. The incorporation of ^{13}C into the septacidins was verified by MALDI-time-of-flight/MS (MALDI-TOF/MS). The samples were treated with the methanolic HCl to give adenine-heptose, plus a mixture of fatty acids and glycyl-fatty acids as their methyl esters. After partitioning between chloroform and water, the ^{13}C-labelled adenine-heptoses in the aqueous phase were analyzed by MALDI-TOF/MS to confirm the presence of label (Figure 2). The chloroform-extracted acyl component was analyzed by electron

impact GC/MS. The labelled adenine-septoses were further hydrolyzed with aqueous trifluoroacetic acid (2 M, 100 °C, 30 min) to give free adenine and heptose (4-amino-4-deoxyheptose). This was confirmed by peracetylation of the labelled heptoses and analysis by GC/MS and MALDI-TOF/MS. To analyze the positional incorporation of ^{13}C, the labelled heptoses were converted to aldononitrile acetate derivatives (PAANs) and analyzed by electron impact GC/MS.[26]

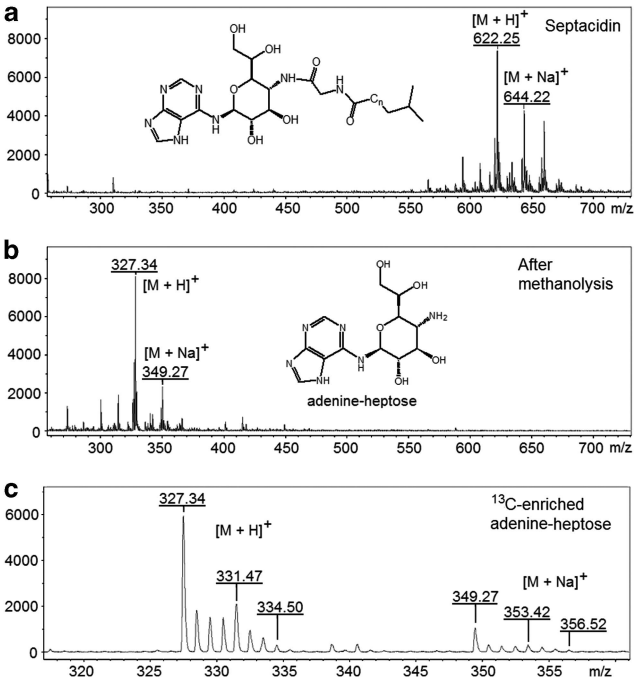


Figure 2 MALDI/MS of (**a**) septacidins, (**b**) adenine-heptose and (**c**) ^{13}C-enriched adenine-heptose.

Isotopic incorporations into the *N*-acylglycine structural motifs

The fatty acid methyl esters from septacidins eluted from the GC between 11 and 20 min as 11 major peaks, plus 8 other minor components. Seven major saturated, branched-chain fatty acids were assigned by the presence of characteristic *m/z* 74 and 87 MS ions, approximately corresponding to the eight major septacidins detected by LC/MS and by MALDI-TOF/MS (Supplementary Data).

Unsaturated fatty acids were also detected, as characterized by additional *m/z* 55 and 69 ions. Fourteen *N*-acylglycine methyl esters also eluted from the GC between 20 and 30 min, after the acyl esters. The major EI/MS fragment ions arising from these acylglycine methyl esters were *m/z* 144, 131 and 90, which are characteristic of this group of compounds.[29] Those with a saturated acyl chain gave *m/z* 131 as the largest fragment ion, formed by β-cleavage to the amide carbonyl and a McLafferty-type rearrangement. Noticeably, the septacidin acylglycine components with unsaturated acyl chains generated *m/z* 131 as only minor ions. This indicates that the double bond present in these compounds is positioned either α or β to the acyl carbonyl, thereby impeding the McLafferty rearrangement mechanism.

The odd-electron ion appearing at *m/z* 131 is very characteristic of α-unsubstituted, saturated *N*-acylglycine methyl esters, and hence contains the carbonyl and methylene carbons from the original glycine motif plus carbons 1 and 2 of the acyl chain (Scheme 3). Examination of the ^{13}C-enrichment into the *m/z* 131 ion showed that the major incorporation occurs from carbons 3 to 6 of [U-^{13}C]glucose (Supplementary Data). The ^{13}C-enrichment is less from [1-^{13}C]glucose- or [2-^{13}C]glucose precursors, and very little incorporation was observed from labelled acetates or pyruvate. Glycine is generally biosynthesized from serine, and the initial three-carbon precursor of serine and glycine, 3-phosphoglycerate, is derived from the glycolytic pathway, where it arises from carbons 4, 5 and 6 of glucose. Hence, this is consistent with the observed ^{13}C incorporations of carbons 3–6 from [U-^{13}C]glucose into the glycine motif of the septacidin *N*-acylglycines.

The *N*-acylglycine esters also generated minor fragment ions because of the carbon–carbon bond cleavage in the acyl backbone. Thus, the major GC peak at 25.9 min corresponds to the 16-carbon

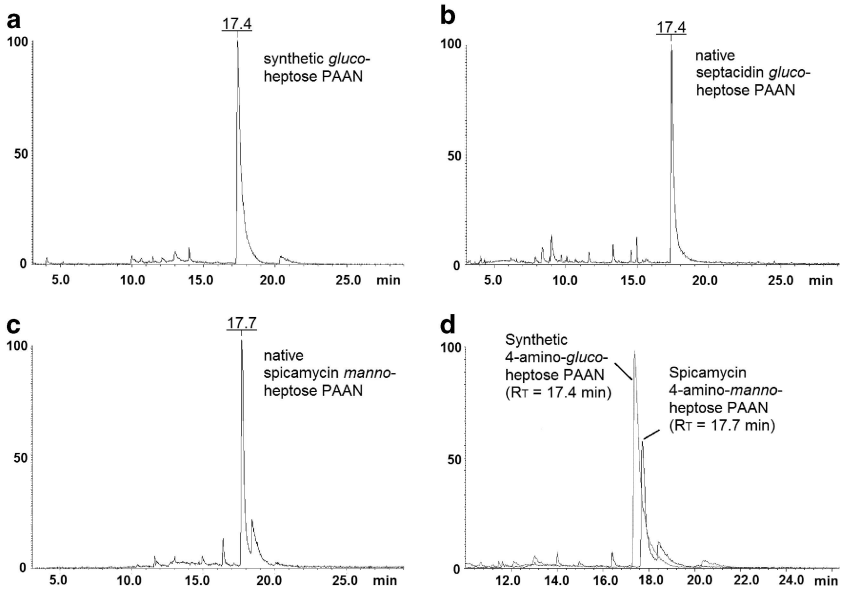
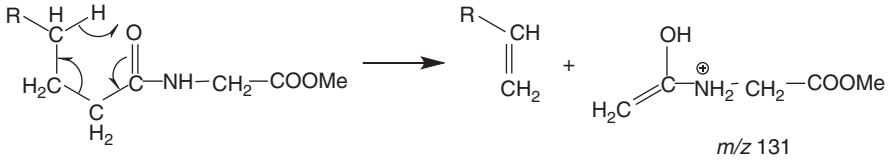


Figure 3 GC traces of 4-aminoheptosyl aldononitrile acetate derivatives: (**a**) Synthetic, $R_T = 17.4$ min; (**b**) from septacidin, $R_T = 17.4$ min; (**c**) from spicamycin, $R_T = 17.7$ min; and (**d**) overlaid chromatographs of synthetic and spicamycin PAANs. A full color version of this figure is available at *The Journal of Antibiotics* journal online.



Scheme 3 The *m/z* 131 fragment ion from McLafferty rearrangement of *N*-acylglycine methyl esters is comprised of two carbons from glycine, and two from the *N*-acyl chain.

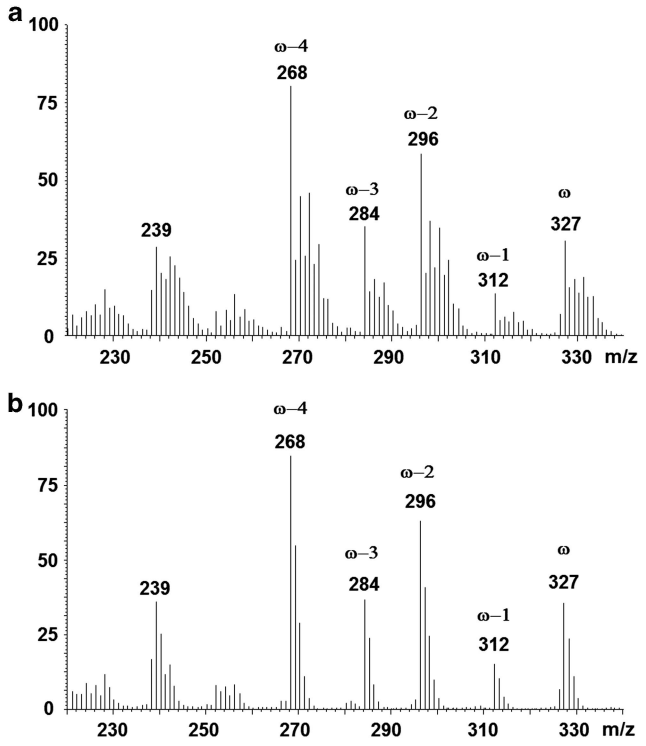


Figure 4 EI-MS spectra of the septacidin glycyl-C16-acyl Me esters, isotopically enriched from (**a**) [U-^{13}C]glucose and (**b**) [2-^{13}C]glucose precursors.

acylglycine chain of the major septacidin, and contained the fragment ions *m/z* 327 (ω), 312 ($\omega-1$), 296 ($\omega-2$), 284 ($\omega-3$) and 268 ($\omega-4$) (Figure 4). Similarly, the GC peaks at retention time 24.3 and 24.5 min are due to *N*-acylglycine esters with 15 acyl carbons, and it gives rise to the corresponding series of MS fragment ions at *m/z* 313 (ω), 298 ($\omega-1$), 282 ($\omega-2$), 270 ($\omega-3$) and 254 ($\omega-4$). These are 14 Da smaller than the ions from the GC peak at 25.9 min, due to the one less -CH_2- in the acyl chain. Isotopic enrichment from [U-^{13}C]glucose and [2-^{13}C]glucose was detected in all of these acyl group ions (Figure 4). The two-carbon unit incorporations from [U-^{13}C]glucose into these ions suggest that the biosynthesis occurs via acetyl-CoA. Moreover, the acyl $\omega-2$ and $\omega-4$ ions are more heavily labelled by [1-^{13}C]glucose, suggesting that labelled acetyl-CoA arising from [1-^{13}C]glucose is more selectively incorporated into the glycyl-fatty acids. This was also apparent from ^{13}C-acetate labelling, where [2-^{13}C]acetate gave greater enrichment than [1-^{13}C]acetate (Supplementary Data).

Isotopic incorporations into the 4-acetylamino-4-deoxy-L-heptose sugar

Following methanolysis of the isotopically labelled septacidin and washing with chloroform, the aqueous portions contained the *N*-glycosidically linked adenine-heptoses. These were acid hydrolyzed to the free aminoheptose, and analyzed as their aldononitrile acetate derivatives by GC/MS. Ion extraction of characteristic ion *m/z* 139 revealed a GC peak at 17.4 min corresponding to the aldononitrile acetate derivative of 4-amino-4-deoxy-L-glucoheptose. The EI/MS spectra are shown in Figure 5. Examination of the ^{13}C-labelled sugar components showed that seven fragment ions are useful for tracing isotopic incorporations. Ions *m/z* 241, 199, 139 and 97 originate from carbon 1 to 4 of the parent aminoheptose, and ions *m/z* 288, 228 and 168 originate from carbons 4 to 7 (Figure 5).

These ions were well resolved from neighboring peaks, allowing us to assess isotopomer incorporations into these carbons. The enrichment from [1-^{13}C]fructose, [2-^{13}C]fructose and [U-^{13}C]fructose was at relatively low level, and the isotopomer patterns were similar. By comparison, enrichment from the labelled glucose precursors was clearly apparent (Figure 5). Ions *m/z* 288 and 228 have less ^{13}C incorporated than *m/z* 241, 199 and 139 (Figure 5). This was also apparent for *m/z* 60. This suggests that the six glucose carbons are preferentially incorporated into the aminoheptose at carbon 1–4, rather than at carbons 4–7. Carbons 1 and 2 from [1-^{13}C]glucose and [2-^{13}C]glucose, respectively, are also incorporated into most of the fragment ions, but again enrichment was greater than carbon 1–4. Noticeably, the scrambling of ^{13}C isotopomers into all of these ions suggests that the glucose is first metabolized to small catabolites before being reincorporated into the aminoheptose backbone. This scrambling of labelled carbon atoms is characteristic of metabolism via the pentose phosphate recovery pathways, and is in contrast to an accompanying GC/MS peak due to microbial trehalose, where M + 6 ions clearly show the incorporation of [U-^{13}C]glucose or [U-^{13}C]mannose as intact six-carbon units (Supplementary Data). Noticeably, [U-^{13}C]xylose, ^{13}C-acetates and [3-^{13}C]pyruvate do not incorporate into the trehalose to any great extent. [U-^{13}C]mannose is also incorporated into the septacidins via 2- or 3- carbon precursors in a manner analogous to glucose. This leads to the conclusion that small labelled metabolites arising selectively from glucose (or mannose) are the metabolic precursors of the seven-carbon amino sugar. The low-level incorporation and scrambling of ^{13}C into septacidins from [1-^{13}C]acetate, [2-^{13}C]acetate and [3-^{13}C]pyruvate also tends to support this conclusion.

The known biosynthetic pathways for seven-carbon sugars are via the pentose phosphate recovery pathway intermediate sedoheptulose-7-P. In several Gram-negative bacteria, sedohepulose-7-P is converted to ADP-D-*glycero*-D-*manno*-heptose sugar nucleotide. A 6-epimerase activity (RfaD)[30] inverts the chirality at C-6 to generate ADP-β-L-*glycero*-D-*manno*-heptose, which is incorporated into bacterial lipopolysaccharide.[31,32] In Gram-positive bacteria, four enzymes convert D-sedoheptulose-7-P into GDP-D-*glycero*-α-D-*manno*-heptose,[33] which in *Campylobacter* can be further converted into GDP-6-deoxy-D-*manno*-heptose and GDP-6-deoxy-D-*altro*-heptose.[34,35] For the biosynthesis of septacidin and spicamycin, we also predict *N*-heptosyl glycosyltransferase, 4-*O*-oxidoreductase and 4-amidotransferase, glycosyltransferase activities and enzymes

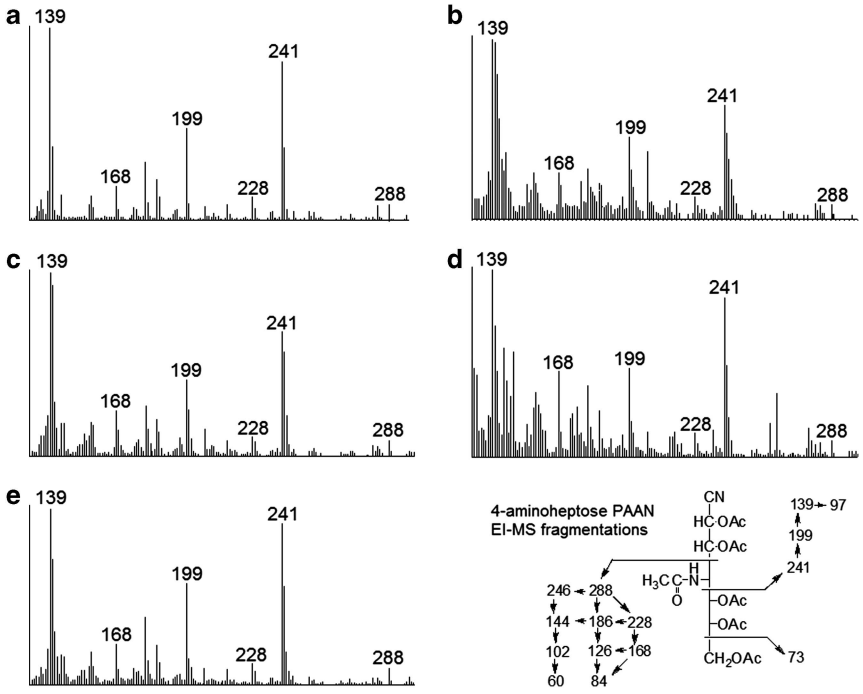
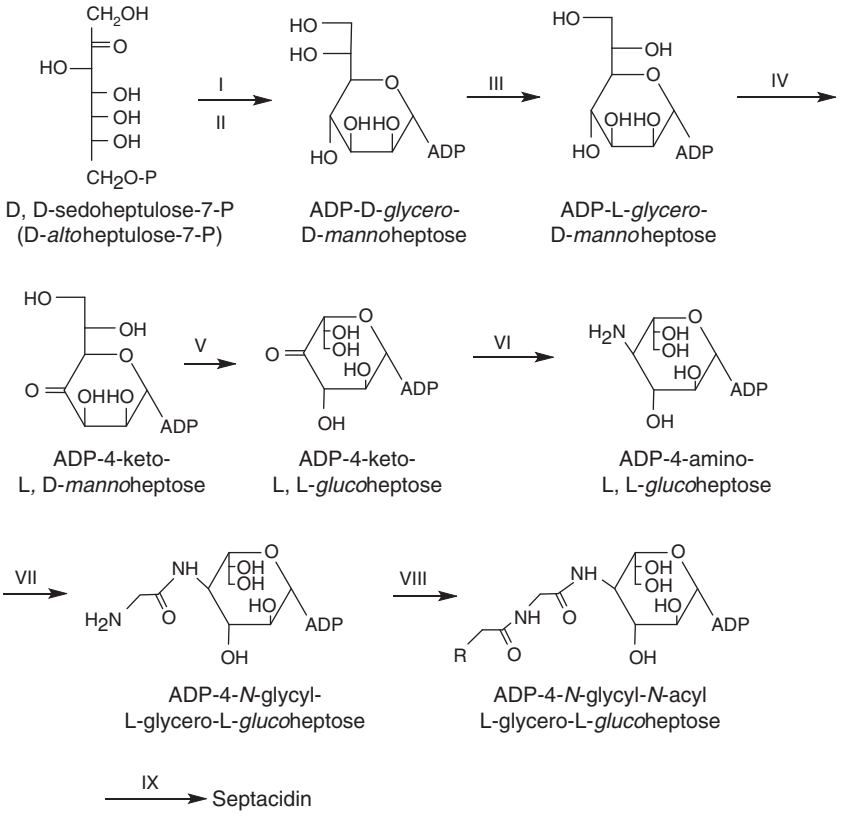


Figure 5 GC/MS of ^{13}C-enriched aminoheptose from septacidins. (**a**) Unlabelled control; (**b**) [U-^{13}C]Glc; (**c**) [2-^{13}C]Glc; (**d**) [U-^{13}C]Xyl; (**e**) [U-^{13}C]Man. Fragment ions are from ^{13}C-aminoheptosyl aldononitrile acetates (PAANs).



Scheme 4 Proposed nine-step biosynthetic pathway from sedo-heptulose-7-phosphate to septacidin. I. sedoheptulose-7-phosphate isomerase; II. D-*glycero*-β-D-*manno*-heptose-1-phosphate adenylyltransferase (RfaE); III. 6-epimerase (RfaD); IV. 4-oxidase; V. 3,5-epimerase; VI. 4-aminotransferase; VII. *N*-glycyltransferase; VIII. *N*-acyltransferase; IX. adenine *N*-glycosyltransferase.

involved in biosynthesis and transfer of *N*-acyl groups (Scheme 4). Assuming an L-heptose 2-epimerase that acts via an 1,2-enediol intermediate, this would suggest that the epimerization occurs on the free heptose sugar, before the formation of the adenine glycoside. The heptose (or 4-aminoheptose) would subsequently form an activated heptosyl sugar nucleotide (either L-*gluco*- or L-*manno*-), before a heptosyltransferase-catalyzed transfer to the adenine acceptor. This suggests that diverse biosynthetic pathways occur for septacidins or spicamycins after the 2-epimerase step, and will likely involve either L-*gluco*-heptosyl- or L-*manno*-heptosyl-specific enzymes. In keeping with other natural products, this probably occurs before the differential *N*-acylations and active cellular transport of the diversified septacidins or spicamycins.

METHODS

Chemicals used for culturing and chemical analysis were obtained from Sigma-Aldrich (St Louis, MO, USA). The septacidin-producing streptomyces strain, *S. fimbriatus* NRRL B-3175, was obtained from the Agricultural Research Service Microbial Collection housed at the NCAUR laboratory in Peoria, Illinois, USA. The strain was cultured as described below. Spicamycin (KRN5500) was obtained from the Developmental Therapeutics Program of the National Cancer Institute, National Institutes of Health (Bethesda, MD, USA). Isotopically enriched sugars were purchased from Omicron Bio chemicals (South Bend, IN, USA).

General methods

Solvents and other reagents for chemical synthesis were purchased from Aldrich Chemical (Milwaukee, WI, USA) and Tokyo Chemical Industry (Portland, OR, USA), and used without further purification. The reactions were monitored by TLC ($20 \times 20\,cm^2$; layer thickness, 0.25 mm; Silica Gel HL TLC Plates w/UV254; Sorbent Technologies, Norcross, GA, USA). Visualization was accomplished with UV light (at 254 nm) and exposure to sulfuric acid in methanol (5:95 (v v)) or ceric ammonium molybdate solution ($Ce(SO_4)_2$: 4 g; $(NH_4)_6Mo_7O_{24}$: 10 g; H_2SO_4: 40 ml; H_2O: 360 ml), followed by heating. Purifications were performed on silica gel columns (Sorbent Technologies, Standard Grade, 32–63 μm). ^1H NMR and ^{13}C NMR spectra were recorded with Agilent 600 (^1H: 600 MHz; ^{13}C: 150 MHz) and Varian-500S (^1H: 500 MHz; ^{13}C: 125 MHz) spectrometers; multiplicities are given as singlet (s), broad (br), doublet (d), double doublets (dd), triple doublets (td), septuplet (sept), triplet (t), quintet (q) or multiplet (m). Assignments in ^1H and ^{13}C NMR were made by first-order analysis of the spectra by using ACD/NMR processor software (Advanced Chemistry Development, Toronto, ON, Canada) and were verified by H −H COSY and ^{13}C-edited HSQC experiments. Residual solvent signals were used as an internal reference ($CDCl_3$: δ 7.26 p.p.m. ^1H NMR, δ 77.16 p.p.m. ^{13}C NMR). Specific optical rotations were measured on an Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA) with a path length of 10 cm. HR-MS ESI-TOF mass spectra were recorded using a Micromass LCT (Waters, Milford, MA, USA) instrument.

Culturing and metabolic labelling experiments

S. fimbriatus NRRL B-3175 was grown for 3 days in culture on glucose-containing medium (tryptone–yeast extract–glucose) and metabolically labelled after this time by the introduction of different ^{13}C-enriched biosynthetic precursors to the cultures. The enriched precursors used were: [1-^{13}C]glucose, [2-^{13}C]glucose, [U-^{13}C]glucose, [1-^{13}C]fructose, [2-^{13}C]fructose, [U-^{13}C]fructose, [1-^{13}C]mannose, [U-^{13}C]mannose, [U-^{13}C]xylose, [1-^{13}C]ribose, [U-^{13}C]erythrose, [3-^{13}C]pyruvate, [1-^{13}C]acetate or [2-^{13}C]acetate. After a further 5 days of growth, the cultures were acidified and the labelled cells were harvested by centrifugation. The isotopically enriched septacidins were extracted from the cell pellets with butanol, which was centrifuged and evaporated to dryness. The incorporation of ^{13}C into the septacidins was verified by MALDI-TOF/MS and NMR. The samples were treated with dry methanolic HCl (1 M, 60 °C, 1 h) to give adenine-septacidose, plus a mixture of fatty acids and glycyl-fatty acids as their methyl esters. After partitioning between chloroform and water, the ^{13}C-labelled adenine-septoses in the aqueous phase were analyzed by MALDI-TOF/MS to confirm the presence of label. The chloroform-extracted acyl component was analyzed by electron impact GC/MS. The labelled adenine-septoses were further hydrolyzed with aqueous trifluoroacetic acid (2 M, 100 °C, 30 min) to give free adenine and septacidose (4-amino-4-deoxyheptose). This was further confirmed by per-acetylation (acetic anhydride: pyridine 1:1 (v v), 60 °C, 30 min) of the labelled septacidoses and analysis by GC/MS and MALDI-TOF/MS. To analyze the positional incorporation of ^{13}C, the labelled septacidoses were converted to aldononitriles acetate derivatives (PAANs) and analyzed by electron impact GC/MS.

Synthesis and spectral assignments of 4-deoxy-4-amino-L-glycero-L-glucoheptose and intermediates

Compound ***6***. Methyl (phenyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-alpha-D-*galacto*-2-nonulopyranosid)onate (**6**). To a solution of compound **5**[1] (4.0 g, 8.8 mmol) in dichloromethane (80 ml) and pyridine (20 ml), benzoyl chloride (2.6 ml, 22.0 mmol) was added at 0 °C. The mixture was stirred for 1 h at 0 °C with monitoring of the reaction by TLC (hexane: EtOAc = 1:1, $R_f = 0.2$). The reaction was quenched with MeOH (10 ml) at 0 °C. Then, the reaction mixture was diluted with EtOAc, washed with H_2O, 1 N HCl, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane: EtOAc = 1:1–1:2) to give 4-*O*-benzoate **6** (4.12 g, 84%) as a white solid.

$[\alpha]_D^{RT}$ −43.5 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 7.98–7.97 (d, 2H, *J* = 7.3 Hz: Ar-*H*), 7.60–7.57 (m, 3H: Ar-*H*), 7.44–7.39 (m, 3H; Ar-*H*), 7.36–7.33 (t, 2H, *J* = 7.7, 7.4 Hz: Ar-*H*), 6.17–6.15 (d, 1H, *J* = 7.7 Hz: N*H*), 5.26–5.21 (td, 1H, *J* = 4.8, 6.2 Hz: H-4), 4.39 (br, 1H: 7-O*H*), 4.29–4.25 (q, 1H, *J* = 6.7 Hz: H-8), 4.11–4.05 (m, 2H: H-5, H-9_a), 3.99–3.97 (dd, 1H, *J* = 6.6, 1.9 Hz: H-9_b), 3.53 (s, 3H: COO*Me*), 3.47–3.46 (d, 1H, *J* = 7.3 Hz: H-7), 3.34–3.33 (dd, 1H, *J* = 1.2, 9.5 Hz: H-6), 2.98–2.95 (dd, 1H, *J* = 5.1, 7.7 Hz: H-3_{eq}), 2.20–2.16 (t, 1H, *J* = 12.2 Hz: H-3_{ax}), 1.89 (s, 3H: *Ac*NH), 1.36 (s, 3H: *Me*) and 1.25 (s, 3H: *Me*).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.8 (*C* = *O*), 169.0 (*C* = *O*), 167.7 (*C* = *O*), 136.9 (*Ar*), 134.0 (*Ar*), 130.0 (*Ar*), 129.9 (*Ar*), 128.8 (*Ar*), 128.7 (*Ar*), 108.8 (*C*Me_2), 87.3 (C2), 77.1 (C6), 74.7 (C8), 70.5 (C7), 70.1 (C4), 67.6 (C9), 52.5 (COO*Me*), 52.1 (C5), 37.8 (C3), 27.0 (C*Me_2), 25.5 (C*Me_2) and 23.2 (*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{28}H_{34}O_9NS$, $[M+H]^+$ 560.1954 found: 560.1975.

Compound ***7***. Methyl (phenyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-8,9-*O*-isopropylidene-7-oxo-2-thio-D-*glycero*-alpha-D-*galacto*-2-nonulopyranosid)onate (**7**). To a solution of compound **6** (5.5 g, 9.84 mmol) in DIPEA (diisopropylethylamine;40 ml) and dimethylsulfoxide (40 ml), $SO_3 \cdot$ pyridine (11.7 g, 73.8 mmol) was added at ambient temperature. The mixture was stirred for 1.5 h with monitoring of the reaction by TLC (toluene: EtOAc = 1:1, $R_f = 0.25$), diluted with EtOAc, washed with H_2O, 1 N HCl, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane: EtOAc = 2:1–1:1) to give 7-keto **7** (5.2 g, 95%) as a white solid.

$[\alpha]_D^{RT}$ 4.52 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 7.99–7.98 (m, 2H: Ar-*H*), 7.57–7.52 (m, 3H: Ar-*H*), 7.43–7.40 (m, 3H: Ar-*H*), 7.35–7.33 (m, 2H: Ar-*H*), 5.92–5.91 (d, 1H, *J* = 8.1 Hz: N*H*), 5.24–5.20 (td, 1H, *J* = 4.7, 6.6, 3.3, 3.4, 4.8: H-4), 4.99–4.96 (t, 1H, *J* = 7.3 Hz: H-8), 4.39–4.37 (t, 1H, J = 7.7, 8.1 Hz: H-9_a), 4.26–4.17 (m, 3H: H-5, H-6, H-9_b), 3.57(s, 3H: COO*Me*), 3.08–3.05 (dd, 1H, *J* = 4.7, 8.1 Hz: H-3_{eq}), 2.17–2.13 (t, 1H, J = 11.7 Hz: H-3_{ax}), 1.76 (s, 3H: *Ac*NH), 1.46 (s, 3H: *Me*) and 1.45 (s, 3H: *Me*).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 201.3 (*C* = *O*: C7), 170.7 (*C* = *O*), 167.9 (*C* = *O*), 166.4 (*C* = *O*), 136.7 (*Ar*), 133.7 (*Ar*), 130.4 (*Ar*), 129.9 (*Ar*), 129.1 (*Ar*), 129.0 (*Ar*), 128.7 (*Ar*), 128.4 (*Ar*), 111.0 (*C*Me_2), 87.7 (C2), 78.8 (C6),

78.5 (C8), 69.3 (C4), 66.1 (C9), 53.0 (COO*Me*), 51.0 (C5), 38.1 (C3), 25.8 (CMe_2), 25.7 (CMe_2) and 23.1 (*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{28}H_{31}NO_9SNa$, $[M+Na]^+$ 580.1617 found: 580.1617.

Compound ***8***. Methyl [phenyl (7S)-5-acetamido-4,7-*O*-dibenzoyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-alpha-D-*galacto*-2-nonulopyranosid]onate (**8**). To a solution of compound **7** (110 mg, 0.20 mmol) in MeOH (4 ml), $CeCl_3 \cdot 7H_2O$ (220 mg, 0.59 mmol) was added at 0 °C. The mixture was stirred for 1 h at 0 °C, and then $NaBH_4$ (11 mg, 0.30 mmol) was added. After stirring for 1 h at 0 °C with monitoring of the reaction by TLC (toluene: EtOAc = 2:3, $R_f = 0.15$), the reaction mixture was roughly concentrated in reduced pressure. Then, the reaction mixture was diluted with EtOAc, washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue (*S*:*R* > 17:1) was dissolved in dichloromethane (1.6 ml) and pyridine (0.4 ml), and then benzoyl chloride (72 µl, 0.62 mmol) was added. The mixture was stirred for 3 h at 0 °C with monitoring by TLC (hexane: EtOAc = 1:1, $R_f = 0.5$). Then, the reaction mixture was diluted with EtOAc, washed with H_2O, 1 N HCl, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 2:1–1:1) to give (*7S*)-*O*-benzoate **8** (90 mg, 66%) as a white solid.

$[\alpha]_D^{RT}$ −3.90 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 8.03–8.02 (d, 2H, *J* = 7.3 Hz; Ar-*H*), 7.99–7.97 (d, 2H, *J* = 7.4 Hz: Ar-*H*), 7.65–7.64 (d, 2H, *J* = 7.4 Hz: Ar-*H*), 7.57–7.54 (m, 2H: Ar-*H*), 7.44–7.39 (m, 5H: Ar-*H*), 7.37–7.34 (t, 2H, *J* = 7.7 Hz: Ar-*H*), 5.70–5.69 (d, 1H, *J* = 9.1 Hz: N*H*), 5.27–5.26 (dd, 1H, *J* = 3.3, 1.5 Hz: H-7), 5.09–5.04 (td, 1H, *J* = 4.4, 6.4 Hz: H-4), 4.72–4.69 (dd, 1H, *J* = 6.8, 5.5 Hz: H-8), 4.25–4.20 (q, 1H, J = 9.9 Hz: H-5), 3.94–3.92 (t, 1H, J = 7.9 Hz: H-9_b), 3.81–3.79 (dd, 1H, *J* = 3.0, 2.0 Hz: H-6), 3.33 (s, 3H: COO*Me*), 3.00–2.98 (dd, 1H, *J* = 4.9, 7.7 Hz: H-3_{eq}), 2.13–2.09 (t, 1H, *J* = 12.1 Hz: H-3_{ax}), 1.70 (s, 3H: *Ac*NH), 1.43 (s, 3H: CMe_2) and 1.42 (s, 3H: CMe_2).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.6 (*C* = *O*), 168.5 (*C* = *O*), 166.6 (*C* = *O*), 166.3 (*C* = *O*), 136.9 (*Ar*), 133.6 (*Ar*), 133.3 (*Ar*), 130.0 (*Ar*), 130.0 (*Ar*), 128.8 (*Ar*), 128.6 (*Ar*), 128.5 (*Ar*), 109.5 (*C*Me_2), 87.1 (C2), 77.3 (C6), 74.2 (C8), 73.3 (C7), 70.8 (C4), 66.4 (C9), 52.6 (COO*Me*), 51.3 (C5), 37.8 (C3), 26.5 (CMe_2), 26.1 (CMe_2) and 23.2 (*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{35}H_{37}NO_{10}SNa$, $[M+Na]^+$ 686.2036 found: 686.2025.

Compound ***9***. A solution of compound **8** (415 mg, 0.63 mmol) in 60% AcOH (9 ml) was stirred at 60 °C for 2 h. After full consumption of starting material **8**, the reaction mixture was concentrated in reduced pressure, coevaporated with toluene three times and kept *in vacuo* for 1 h. Then, to a solution of resulting diol in tetrahydrofuran (4.5 ml) and H_2O (1.5 ml), $NaIO_4$ (535 mg, 2.5 mmol) was added. The reaction mixture was stirred for 12 h with monitoring by TLC (toluene: EtOAc = 1:1, $R_f = 0.3$, spots shows orange color with only heating), diluted with EtOAc, washed with H_2O, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane: EtOAc = 2:1–1:1) to give aldehyde **9** (310 mg, 84%) as a white solid.

$[\alpha]_D^{RT}$ −55.8 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 9.75 (s, 1H: C*H*O), 8.16–8.15 (d, 2H, *J* = 7.3 Hz: Ar-*H*), 8.01–7.99 (d, 2H, *J* = 7.4 Hz: Ar-*H*), 7.64–7.58 (m, 2H: Ar-*H*), 7.51–7.44 (m, 6H: Ar-*H*), 7.38–7.35 (m, 1H: Ar-*H*), 7.28–7.25 (m, 2H: Ar-*H*), 5.67–5.66 (d, 1H, *J* = 1.9 Hz: H-7), 5.64–5.63 (d, 1H, *J* = 9.5 Hz: N*H*), 5.17–5.12 (td, 1H, *J* = 4.8, 5.4, 4.0, 6.4, 4.8 Hz: H-4), 4.60–4.55 (q, 1H, *J* = 10.2, 10.0 Hz: H-5), 4.17–4.14 (dd, 1H, *J* = 1.8, 9.2 Hz: H-6), 3.53 (s, 3H: COO*Me*), 3.03–3.00 (dd, 1H, *J* = 4.8, 7.7 Hz: H-3_{eq}), 2.16–2.12 (t, 1H, *J* = 12.1, 12.5 Hz: H-3_{ax}) and 1.82 (s, 3H: *Ac*NH).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.8 (*C*HO), 170.7 (*C* = *O*), 168.4 (*C* = *O*), 166.9 (*C* = *O*), 165.8 (*C* = *O*), 136.8 (*Ar*), 133.9 (*Ar*), 133.8 (*Ar*), 130.4 (*Ar*), 130.4 (*Ar*), 130.0 (*Ar*), 130.0 (*Ar*), 128.9 (*Ar*), 128.7 (*Ar*), 128.7 (*Ar*), 87.1 (C2), 77.4 (C7), 77.3 (C6), 70.1 (C4), 53.0 (COO*Me*), 50.5 (C5), 37.9 (C3) and 23.3 (*Ac*NH).

HR-MS (ESI): *m/z* calcd for: $C_{31}H_{29}NO_9SNa$, $[M+Na]^+$ 614.1461 found: 614.1469.

Compound ***10***. To a solution of compound **9** (1.70 g, 2.88 mmol) in MeOH (15 ml), $NaBH_4$ (153 mg, 4.02 mmol) was added at 0 °C. The mixture was stirred for 1 h at 0 °C with monitoring of the reaction by TLC (toluene: EtOAc = 1:1, $R_f = 0.2$), diluted with EtOAc, washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in tetrahydrofuran (20 ml), followed by the addition of 1 N NaOH (20 ml). The reaction mixture was stirred for 3 h at an ambient temperature, quenched by 1 N HCl (solution color changes from pale yellow to transparent by the neutralization), concentrated and coevaporated with toluene three times. The residue was dissolved in acetic anhydride (10 ml) and pyridine (15 ml), stirred for 2 h at an ambient temperature with monitoring of the reaction by TLC (chloroform: MeOH = 5:1, $R_f = 0.5$), filtered by celite and concentrated. The residue was purified by column chromatography on silica gel (chloroform: MeOH = 10:1–5:1) to give carboxylic acid **10** (1.20 g, 84%) as a white solid.

$[\alpha]_D^{RT}$ −48.5 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 7.60–7.59 (d, 2H, *J* = 7.4 Hz: Ar-*H*), 7.38–7.36 (m, 1H: Ar-*H*), 7.33–7.31 (m, 2H: Ar-*H*), 6.56–6.55 (d, 1H, *J* = 9.2 Hz: N*H*), 5.08–5.07 (d, 1H, *J* = 7.7 Hz: H-7), 4.73–4.71 (td, 1H, *J* = 3.7, 5.4 Hz: H-4), 4.51–4.42 (m, 2H: H-$8_{a,b}$), 4.08–4.05 (q, 1H, *J* = 9.7, 9.9 Hz: H-5), 3.84–3.82 (d, 1H, *J* = 10.6 Hz: H-6), 2.75–2.72 (dd, 1H, *J* = 3.6, 8.0 Hz: H-3_{eq}), 2.07 (s, 3H: *Ac*), 2.02 (s, 3H: *Ac*), 1.95 (s, 3H: *Ac*), 1.90–1.86 (t, 1H, J = 12.3 Hz: H-3_{ax}) and 1.83 (s, 3H: *Ac*NH).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.1 (*C* = *O*), 171.6 (*C* = *O*), 171.2 (*C* = *O*), 171.0 (*C* = *O*), 136.7 (*Ar*), 130.0 (*Ar*), 128.9 (*Ar*), 86.2 (C2), 75.3 (C6), 71.9 (C7), 70.7 (C4), 61.8 (C8), 49.6 (C5), 37.4 (C3), 23.0 (*Ac*NH), 21.2 (O*Ac*) and 21.0 (O*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{22}H_{27}NO_{10}SNa$, $[M+Na]^+$ 520.1253 found: 520.1251.

Compound ***11***. To a solution of compound **10** (1.20 g, 2.40 mmol) and 1-oxa-2-oxo-3-thiaindolizinium chloride (2.30 g, 12.1 mmol) in dichloromethane (50 ml), triethylamine (2.7 ml, 19.3 mmol) was added at 0 °C under argon atmosphere. The mixture was stirred for 5 h at 0 °C, and then photolyzed (254 nm, Rayonnet photoreactor, Southern New England Ultraviolet Co., Branford, CT, USA) at 0 °C for 2 h. The reaction mixture was diluted with EtOAc, washed with H_2O, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue (α:β = 1:2 from ^1H NMR of crude mixture) was purified by column chromatography on silica gel (toluene: isopropanol = 40:1–30:1) to give β-thioglycoside **11** (527 mg, 38%) as a yellow solid.

$[\alpha]_D^{RT}$ −17.0 (*c* 0.67, $CHCl_3$)

^1H NMR (600 MHz, $CDCl_3$): δ = 7.54–7.53 (m, 2H: Ar-*H*), 7.33–7.32 (m, 3H: Ar-*H*), 5.53–5.51 (d, 1H, *J* = 8.8 Hz: N*H*), 5.12–5.11 (m, 1H: H-6), 4.95–4.91 (td, 1H, *J* = 4.7, 5.9, 4.8, 5.8, 4.8 Hz: H-3), 4.69–4.66 (dd, 1H, *J* = 1.8, 9.9 Hz: H-1), 4.50–4.41 (m, 2H: H-$7_{a,b}$), 3.96–3.91 (q, 1H, *J* = 9.9 Hz: H-4), 3.56–3.54 (dd, 1H, *J* = 1.5, 9.1 Hz: H-5), 2.32–2.29 (ddd, 1H, *J* = 1.1, 3.6, 1.5, 6.3, 1.8, 3.3, 1.5 Hz: H-2_{eq}), 2.09 (s, 3H: *Ac*), 2.07 (s, 3H: *Ac*), 2.05 (s, 3H: *Ac*), 1.99 (s, 3H: *Ac*NH) and 1.85–1.79 (q, 1H, *J* = 11.8, 12.1, 12.1 Hz: H-2_{ax}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.3 (*C* = *O*), 170.9 (*C* = *O*), 170.8 (*C* = *O*), 170.7 (*C* = *O*), 133.0 (*Ar*), 132.6 (*Ar*), 129.0 (*Ar*), 128.3 (*Ar*), 82.2 (C1), 79.2, 71.6, 71.3, 61.9, 51.0 (C4), 36.6 (C2), 23.3 (*Ac*NH), 21.2 (O*Ac*), 21.1 (O*Ac*) and 21.0 (O*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{21}H_{27}NO_8SNa$, $[M+Na]^+$ 476.1355 found: 476.1358.

Compound ***12***. To a solution of compound **11** (50 mg, 0.11 mmol) in dichloromethane (1 ml), 77% *meta*-chloroperoxybenzoic acid (26 mg, 0.12 mmol) was added at −78 °C. The mixture was stirred for 7.5 h at −78 °C with monitoring of the reaction by TLC (toluene: isopropanol = 7.5:1,

$R_f = 0.2$), and then quenched by the addition of sat. aq. $NaHCO_3$ (1 ml) at −20 °C. The reaction mixture was diluted with EtOAc, washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was coevaporated by toluene (2 ml) two times and dried *in vacuo* for 1.5 h. The resulting solid was dissolved to dry toluene (2 ml) and stirred for 4 h at 90 °C under argon atmosphere. The reaction was monitored by TLC (EtOAc only, $R_f = 0.35$), diluted with EtOAc, washed with H_2O, sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane: EtOAc = 1:2–1:4) to give glycal **12** (9.4 mg, 25%) as a yellow syrup.

$[\alpha]_D^{RT}$ 16.0 (*c* 0.23, $CHCl_3$)

^1H NMR (500 MHz, $CDCl_3$): δ = 6.51–6.49 (d, 1H, *J* = 6.2 Hz: H-1), 5.69–5.68 (d, 1H, *J* = 9.2 Hz: N*H*), 5.39–5.35 (m, 1H: H-6), 5.09–5.07 (m, 1H: H-2), 4.91 (m, 1H: H-3), 4.55–4.52 (m, 2H: H-4, H-7_a), 4.33–4.30 (dd, 1H, *J* = 3.3, 5.9 Hz: H-5), 4.20–4.16 (dd, 1H, J = 4.0, 7.9, 4.4 Hz: H-7_b), 2.11 (s, 3H: *Ac*), 2.09 (s, 3H: *Ac*), 2.02 (s, 3H: *Ac*NH) and 1.99 (s, 3H: *Ac*).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.9 (*C* = *O*), 170.3 (*C* = *O*), 169.9 (*C* = *O*), 169.5 (*C* = *O*), 145.4 (C1), 99.6 (C2), 73.6 (C5), 67.9 (C6), 64.8 (C3), 61.9 (C7), 45.7 (C4), 23.5 (*Ac*NH), 21.2 (O*Ac*), 21.0 (O*Ac*) and 21.0 (O*Ac*).

HR-MS (ESI): *m/z* calcd for: $C_{15}H_{21}NO_8Na$, $[M+Na]^+$ 366.1165 found: 366.1180.

*Compound **13***. To a solution of compound **12** (9.4 mg, 27.4 μmol) in tetrahydrofuran (140 μl) and H_2O (30 μl), pyridine (5 μl) and freshly prepared OsO_4 (10 mg ml^{-1} tBuOH soln.: 140 μl) were added at 50 °C. The mixture was stirred for 1 h at 50 °C with monitoring of the reaction by TLC ($CHCl_3$: MeOH = 7.5:1, $R_f = 0.35$). Then, solvents were removed under reduced pressure and the residue was coevaporated with toluene two times. The resulting residue was dissolved in pyridine (300 μl) and Ac_2O (200 μl), stirred for overnight and concentrated. The residue was purified by column chromatography on silica gel (hexane: EtOAc = 1:2–0:1) to give *gluco*-hexaacetate **13** (8.1 mg, 64%, α:β = 10:7, Glc: Man = 25:1) as a yellow oil and an α, β-mixture.

$[\alpha]_D^{RT}$ −48.3 (*c* 0.54, $CHCl_3$: α, β = 10:7 mixture)

HR-MS (ESI): *m/z* calcd for: $C_{19}H_{27}NO_{12}Na$, $[M+Na]^+$ 484.1431 found: 484.1439.

*Compound **13α***. *^1H NMR (600 MHz, $CDCl_3$)*: δ = 6.34 (d, 1H, *J* = 3.7 Hz: H-1), 5.65–5.63 (d, 1H, *J* = 8.8 Hz: N*H*), 5.29–5.25 (t, 1H, *J* = 9.9 Hz; H-3), 5.12–5.10 (m, 1H: H-6), 5.05–5.03 (dd, 1H, *J* = 3.7, 6.6 Hz: H-2), 4.36–4.35 (m, 2H: H-$7_{a,b}$), 4.25–4.20 (q, 1H, *J* = 10.2 Hz: H-4), 4.03–4.01 (dd, 1H, *J* = 1.5, 9.9 Hz: H-5), 2.14 (s, 3H: *Ac*), 2.09 (s, 3H: *Ac*), 2.07 (s, 3H: *Ac*), 2.06 (s, 3H: *Ac*), 2.00 (s, 3H: *Ac*) and 1.99 (s, 3H: *Ac*NH).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.6 (*C* = *O*), 170.8 (*C* = *O*), 170.8 (*C* = *O*), 170.4 (*C* = *O*), 169.7 (*C* = *O*), 168.6 (*C* = *O*), 89.2 (C1), 73.3 (C5), 70.6 (C6), 69.7 (C3), 69.3 (C2), 61.3 (C7), 50.9 (C4), 23.3 (*Ac*NH), 21.2 (O*Ac*), 21.0 (O*Ac*), 21.0 (O*Ac*), 20.9 (O*Ac*) and 20.6 (O*Ac*).

*Compound **13β***. *^1H NMR (600 MHz, $CDCl_3$)*: δ = 5.64–5.63 (d, 1H, *J* = 9.1 Hz: N*H*), 5.62–5.61 (d, 1H, *J* = 8.1 Hz: H-1), 5.11–5.04 (m, 3H; H-2, H-3, H-6), 4.38–4.31 (m, 2 H: H-$7_{a,b}$), 4.21–4.16 (q, 1H, *J* = 9.9 Hz: H-4), 3.76–3.74 (dd, 1H, *J* = 1.8, 8.8 Hz: H-5), 2.12 (s, 3H: *Ac*), 2.10 (s, 3H: *Ac*), 2.07 (s, 3H: *Ac*), 2.05 (s, 3H: *Ac*), 2.03 (s, 3H: *Ac*) and 1.99 (s, 3H: *Ac*NH).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.3 (*C* = *O*), 170.9 (*C* = *O*), 170.9 (*C* = *O*), 170.5 (*C* = *O*), 169.2 (*C* = *O*), 169.1 (*C* = *O*), 92.0 (C1), 75.9 (C5), 72.6 (C3), 70.5 (C6), 70.1 (C2), 61.3 (C7), 50.9 (C4), 23.3 (*Ac*NH), 21.2 (O*Ac*), 21.0 (O*Ac*), 21.0 (O*Ac*), 20.8 (O*Ac*) and 20.7 (O*Ac*).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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NMR and MS spectral data for the products, and additional isotopic enrichment data are available via the internet.

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