Natural Products

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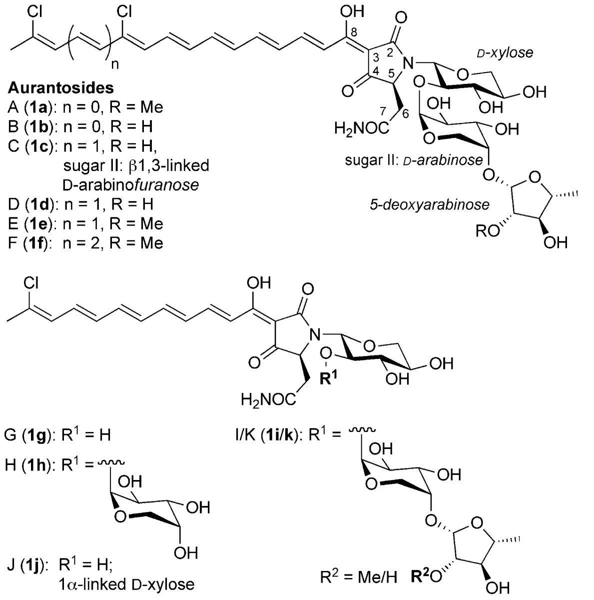
Total Synthesis of Aurantoside G, an *N*-β-Glycosylated 3-Oligoenoyltetramic Acid from *Theonella swinhoei*

*Markus Petermichl, Sebastian Loscher, and Rainer Schobert**

Abstract: *The first synthesis of a natural N-glycosylated 3-acyltetramic acid is reported. Aurantoside G (**1g**), a deep-red metabolite of the marine sponge Theonella swinhoei, is highly delicate in the pure state. It features a chlorinated dodecapentaenoyl side chain at an L-asparagine-derived tetramic acid, the ring nitrogen atom of which is linked to a β-configured D-xylose. The side chain was built through consecutive Wittig and HWE reactions and used to N-acylate the amino group of an asparaginate that had already been N-xylosylated through a Fukuyama–Mitsunobu reaction. This N-acylation step fixes the β-configuration of the xylose, which is essential for the antifungal activity, but only if the sugar carries bulky, electron-rich protecting groups such as PMB. In the final step, the heterocycle was closed quantitatively through a basic Lacey–Dieckmann condensation of an entirely unprotected precursor.*

Natural 3-acyltetramic acids (3-acylpyrrolidine-2,4-diones) are hybrid polyketide/amino acid metabolites that are produced by bacteria, molds, fungi, and sponges, and which show a high incidence and broad spectrum of biological activities.[1] Owing to the flexibility of the polyketide biosynthesis machinery, they come in many structural variants and complexities. Some of them include sugar residues. While the majority of these compounds are O-glycosylated at the end of the 3-acyl side-chain, such as the epicoccamides[2] and ancorinosides,[3] others feature C-glycosylation at this position, like the aflastatins,[4] or N-glycosylation at the lactam nitrogen atom, as in the aurantosides[5] and rubrosides.[6] Only a few total syntheses of glycotetramates, and none of N-glycosylated tetramic acids, have been reported, so far.[7] All known aurantosides A–K (**1a**–**k**) have been isolated as orange-red pigments from lithistid marine sponges of the genera *Theonella* (**1a**,[5a,b] **1b**,[5a] **1g**–**i**,[5d] **1j**),[5e] *Homophymia* (**1c**),[5c] *Siliquariaspongia* (**1d**–**f**),[5f] or *Melophlus* (**1k**).[5g] They share an asparagine-derived tetramic acid that carries a mono- or dichlorinated polyenoyl residue at C-3, which is N-glycosylated with either D-xylose or di- and trisaccharides comprised of D-xylose, D-arabinose, and 5-deoxyarabinofuranose. Some aurantosides show a distinct antifungal effect that is not clearly related to their structures. Aurantosides A, B, E and I are efficacious against *Candida albicans* (wildtype) at minimal inhibitory concentrations (MIC) of 1 μg mL^{-1} or below, while aurantosides D, G and K show MIC values of between 2 and 10 μg mL^{-1}. The aurantosides F, H, and J are virtually inactive and aurantoside C has not been tested yet. The mode of antifungal action might be related to that of the polyene macrolides of the nystatin type. The few tests for antimicrobial activity have mostly been disappointing.



Any synthetic approach to the aurantosides has to address the issues of their inherent instability,[5e] and the necessity to control the β-configuration of the N-glycosidic linkage. The latter seems to play a role in the antifungal effect, which is apparent from the activity difference between the epimers **1g** and **1j**. We now developed a route to aurantoside G (**1g**), the key steps of which should also be applicable to the syntheses of its more complex congeners. Our retrosynthetic approach is outlined in Scheme 1. To avoid problems associated with the polarity and metal-chelating propensity of 3-acyltetramic acids, this functionality was to be generated only in a final base-induced Lacey–Dieckmann[8] cyclization of the fully functionalized N-(D-xylosyl)-*N*-(β-ketotetradecapentaenoyl)asparaginate. The latter should be prepared by aminolysis of thioester **2**, as the source of the entire 3-acyl side-chain, with methyl *N*-D-xylosylasparaginate (**3**) according to a method reported by Ley et al.[9] We intended to build thioester **2** through HWE olefination of the known β-ketophosphonate **4** with the chlorinated tetraenal **5**, which should be accessible through a Wittig olefination of carboethoxymethylenetriphenylphosphorane with the aldehyde 3-chlorobut-(2*Z*)-enal, ideally prepared in situ through oxida-

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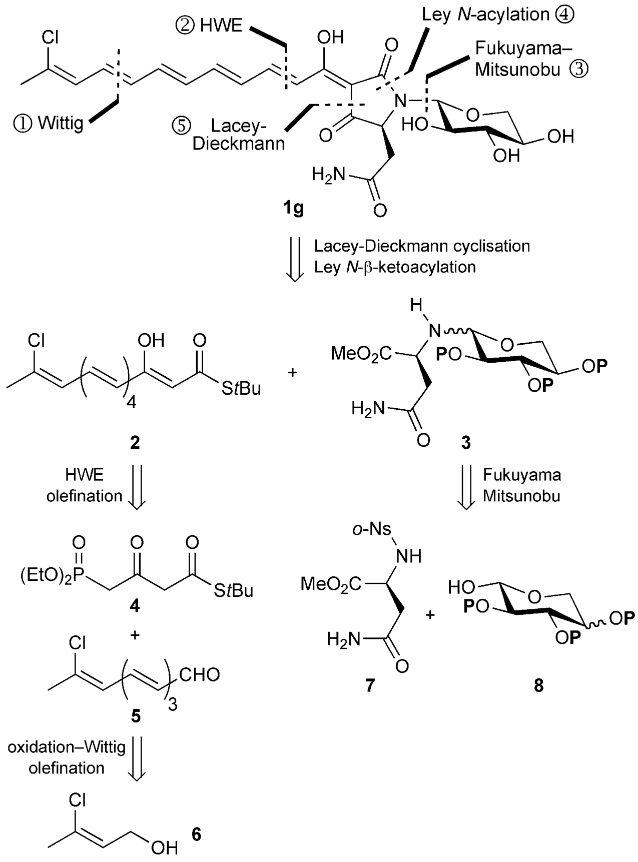
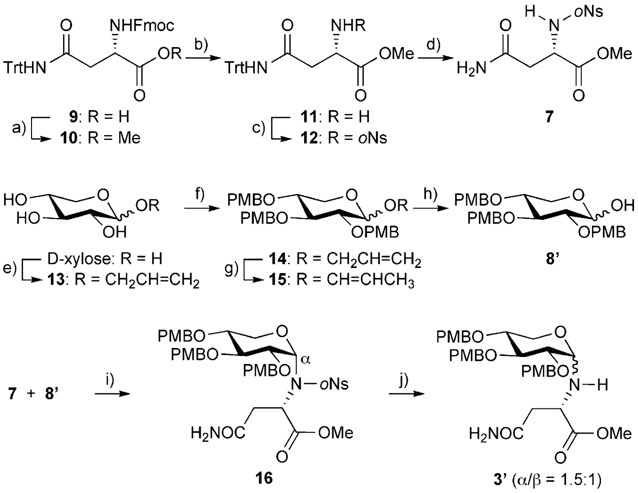


Scheme 1. Retrosynthetic approach to aurantoside G (**1g**). *o*Ns = *o*-$(O_2N)C_6H_4SO_2$.



Scheme 2. Synthesis of **3′**. Reagents and conditions: a) K_2CO_3, MeI, DMF, 0 °C→RT, 1 h; 99%. b) piperidine, DMF, RT, 1 h; 99%. c) *o*NsCl, NEt_3, CH_2Cl_2, 0 °C→RT, 16 h; 92%. d) F_3CCO_2H, CH_2Cl_2, 0 °C→RT, 1 h; 89%. e) allylic alcohol, $BF_3 \times OEt_2$, reflux, 16 h; 77%. f) PMBCl, NaH, DMF, 0 °C→RT, 72 h; 86%. g) KO*t*Bu, DMF, 70 °C, 2 h. h) 0.1 M HCl, acetone, reflux, 1 h; 87% over 2 steps. i) DIAD, PPh_3, THF, −78 °C→RT, 16 h; 69%. j) DIPEA, PhSH, DMF, RT, 4 h; 77%. DMF = *N*,*N*-dimethylformamide, DIAD = diisopropyl azodicarboxylate, DIPEA = *N*,*N*-diisopropylamine, Fmoc = fluorenylmethyloxycarbonyl, Trt = triphenylmethyl.

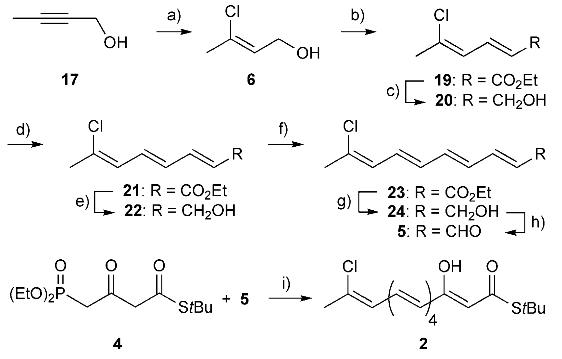
tion of alcohol **6**. The *N*-xylosylasparaginate **3** was to be prepared by a Fukuyama–Mitsunobu reaction of a tris-protected D-xylose **8** with N-nosylated methyl asparaginate **7**. It was unclear at this point whether the bulky *o*-nosyl group would give rise to α- or β-glycosylation and whether such a preferred configuration might be preserved throughout the synthesis.

We started out with the synthesis of the unknown methyl *N*-(*o*-nosyl)asparaginate **7** from commercially available bis-protected asparagine **9** in four steps and 80% overall yield.[10] The known intermediates, that is, the bisprotected methyl ester **10** and the α-aminoester **11**, were obtained for the first time in a pure crystalline form rather than as oils[11] (Scheme 2, top row).

It took some experimentation to identify *p*-methoxybenzyl (PMB) as the optimum protecting group for xylose. PMB as an electron-releasing group enables the Fukuyama–Mitsunobu reaction of D-xylose with the electron-poor, weakly nucleophilic amine **7**. Moreover, PMB is bulky enough to direct the subsequent N-acylation of **3** with **2** in favor of the β-configuration. The new PMB-protected xylose **8′** was prepared from D-xylose in four steps and 58% yield as a 1.7:1.0 mixture of α- and β-anomers. Selective 1-O-allylation[12] of D-xylose and subsequent benzylation of the resulting glycoside **13** with PMBCl furnished the fully protected xylose **14**. The allyl ether was cleaved[13] by first isomerizing it with base to the corresponding enol ether **15**, which was hydrolyzed right away with hydrochloric acid to leave building block **8′** (Scheme 2, middle row).

The Fukuyama–Mitsunobu reaction[14] of **8′** with sulfonamide **7** proceeded fast and with complete consumption of the starting materials. The N-nosylated product amine **16** was isolated in 69% yield as the pure α-anomer, as apparent from the NMR spectra. The chemical shifts of the C-1 of the xylose differed by ca. $\Delta\delta = 20$ ppm for α- and β-anomers, and the coupling constants $^3J_{HH}$ for 1-H were 3–4 Hz for the α-anomer and 7–8 Hz for the β-anomer. De-nosylation of **16** with thiophenol and Hünig's base afforded the key amine **3′** (**P** = PMB) in 77% yield, albeit as a 1.5:1.0 mixture of α- and β-anomers (Scheme 2, bottom).

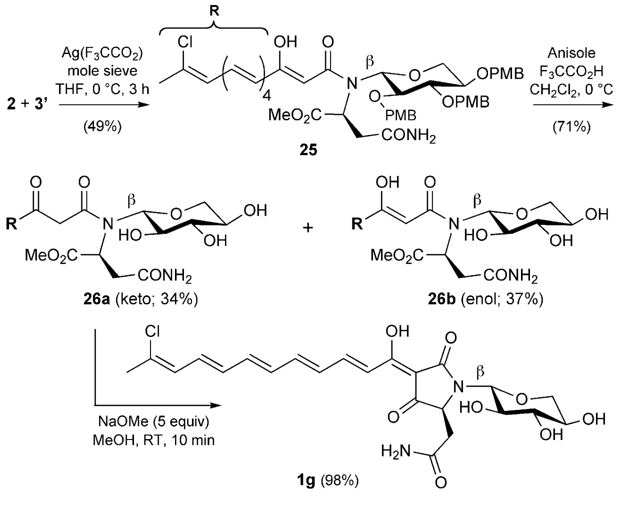
Next, thioester **2** was synthesized starting from 3-chlorobut-(2*Z*)-en-1-ol (**6**), which was prepared through the reaction of but-2-yn-1-ol (**17**) with Red-Al and NCS, according to an improved adaptation of the method of Poulter et al.[15] (Scheme 3). We began the stepwise chain elongation of alcohol **6** by employing a domino oxidation/Wittig olefination, as described by Taylor et al.,[16] using MnO_2 and carboethoxymethylenetriphenylphosphorane (**18**), since the corresponding aldehyde of **6** is rather delicate. The product ethyl dienoate **19** was reduced to the alcohol **20**, and the chain was lengthened in the same manner by domino oxidation/Wittig reaction with MnO_2 and ylide **18** to give the ethyl trienoate **21**. After a third sequence of reduction and domino oxidation/Wittig reaction, the ethyl tetraenoate **23** was obtained. It was reduced to the corresponding alcohol **24**, which was finally oxidized with MnO_2 to the desired aldehyde **5**. Because of its instability, even when kept under an inert gas atmosphere in a dark freezer at −20 °C, this aldehyde was submitted right away to a HWE reaction with Ley's *S*-*tert*-



Scheme 3. Synthesis of **2**. Reagents and conditions: a) (i) Red-Al, THF, 0 °C→RT, 16 h; (ii) NCS, THF, −78 °C→0 °C, 3 h; 82 %. b) MnO$_2$, Ph$_3$P=CHCO$_2$Et (**18**), CH$_2$Cl$_2$, reflux, 16 h; 63 %. c) DIBAL-H, CH$_2$Cl$_2$, −78 °C, 1 h; 75 %. d) MnO$_2$, **18**, CH$_2$Cl$_2$, reflux, 16 h; 77 %. e) DIBAL-H, CH$_2$Cl$_2$, −78 °C, 1 h; 94 %. f) MnO$_2$, **18**, CH$_2$Cl$_2$, reflux, 16 h; 60 %. g) DIBAL-H, CH$_2$Cl$_2$, −78 °C, 1 h; 77 %. h) MnO$_2$, CH$_2$Cl$_2$, RT, 3 h. i) NaH, THF, 0 °C, 3 h; 84 % over 2 steps. Red-Al = sodium bis(2-methoxyethoxy)aluminumhydride, THF = tetrahydrofuran, DIBAL-H = diisobutylaluminumhydride.

butyl 4-(diethylphosphono)-3-oxobutanethioate (**4**)[17] to afford the thioester **2** in 84 % yield (11 % over nine steps).

The aminolysis of thioester **2** with a 1.5:1.0 mixture of α- and β-anomers of methyl *N*-D-xylosylasparaginate **3′** in the presence of an excess of silver trifluoroacetate according to Ley's general method[7a,9] afforded the β-ketoamide **25** as a pure β-isomer in 49 % yield with respect to recovered unreacted **3′** (Scheme 4). We assume that only the β-anomer of **3′** enters into a reaction with **2** owing to steric shielding of the α-anomer by the PMB groups at the xylose, and that epimerization of the residual α-anomer is not fast enough under the reaction conditions. This epimerization definitely took place upon the recovery (extraction, concentration) of unreacted **3′**, which again yielded a mixture of both anomers, ready to be reacted once more. Several reaction cycles are necessary to completely convert **3′** into β-ketoamide **25**. Since all aurantosides except **1j** feature a β-xylose bearing a bulky arabinose at C-2, the above route to aurantoside G (**1g**) should also be applicable to the β-selective synthesis of these congeners. Cleavage of the PMB groups of **25** with trifluoroacetic acid afforded the unprotected N-glycosylated β-ketoamide **26** as a separable 1:1 mixture of keto and enol tautomers. Only the keto tautomer **26a** underwent Lacey–Dieckmann cyclization which, when stopped after 10 min reaction time, furnished the chemically and enantiomerically pure aurantoside G (**1g**) in virtually quantitative yield. The enol tautomer **26b** needed to be re-equilibrated with acid to give the initial 1:1 mixture of both epimers. Like other polyenoyltetramic acids such as β-lipomycin,[5e,7f] pure synthetic aurantoside G proved unstable and prone to decomposition, which thwarted further biological tests.



Scheme 4. Final steps to aurantoside G (**1g**): β-selective Ley N-(β-keto)acylation to give **25**, followed by deprotection and Lacey–Dieckmann cyclization.

In summary, the sponge metabolite aurantoside G (**1g**) was synthesized as the first example of an *N*-glycosylated 3-acyltetramic acid in 3.7 % overall yield. The β-configuration of the D-xylose residue, which is essential for its bioactivity, was fixed only in the course of the penultimate Ley-type N-acylation step. The β-selectivity is governed by the bulky PMB substituents on the xylose. The electron-releasing effect of the PMB residues was also exploited to promote the preceding Fukuyama–Mitsunobu glycosylation step. We expect this synthetic approach to be applicable also to the other aurantosides, which all bear bulky mono- or oligosaccharides at C-2 of a β-xylose. We also demonstrated that Lacey–Dieckmann cyclization may serve as the final step in the synthesis of even delicate tetramic acids, since it does not lead to racemization or interfere with extended conjugated polyene fragments or unprotected sugars.

Keywords: aurantosides · glycoconjugates · natural products · tetramic acids · total synthesis

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