Natural Product Synthesis

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Total Synthesis of Nominal Banyaside B: Structural Revision of the Glycosylation Site**

*Corinna S. Schindler, Louis Bertschi, and Erick M. Carreira**

The natural products that comprise the aeruginosin family of serine protease inhibitors[1] represent one of the seven main classes of cyanobacterial peptides.[2] They include the four glycopeptides, banyasides A[3a] (**1**) and B[3a] (**2**), suomilide,[3b] and spumigin HKVV,[3c] which incorporate a common, densely functionalized tricyclic azabicyclononane (Abn) core. The biological activity and their complexity render these as notable synthesis targets. These structures present two key challenges: access to the Abn core and installation of the side-chains adorning the periphery, which include lipids, cationic and anionic peptides, as well as glycoside appendages. We recently reported a nine-step synthesis of the Abn core.[4,5] Herein, we disclose studies focused on the side-chains. These studies have led to an approach for the installation of a hindered peptide along with O-sulfation, and glycosylation reactions. A key outcome of this work is the proposed revised structure for natural banyaside B (**3**), in which the glycoside is linked to the Abn core at the axial-C-9 OH (Figure 1).

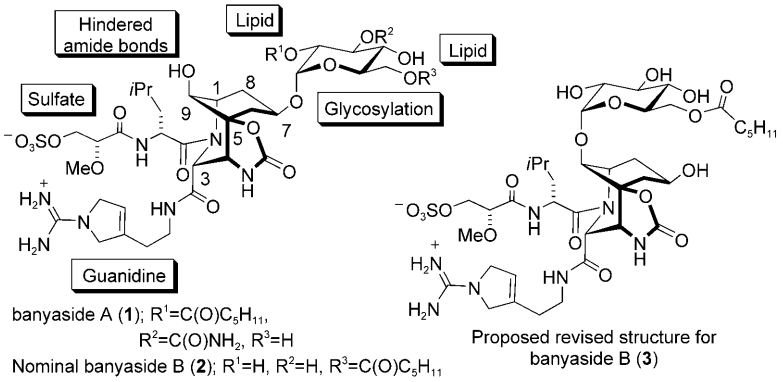
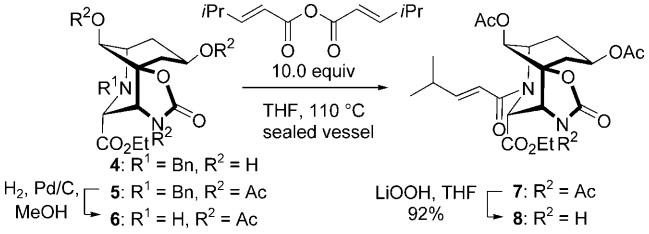


Figure 1. Azabicyclononanes of the aeruginosin family.

With a high-yielding route to the Abn core secured,[4] completion of the synthesis necessitated incorporation of peptides at the hindered secondary amine and C-3 carboxy group along with glycosylation at the C-7 position. Efforts to couple **4** (Scheme 1) and carboxybenzyl-D-leucine employing common reagents (e.g. EDC, DCC, BOPCl, BOP, DEPBT, PyBOP, HATU)[6] did not yield any product, and the starting secondary amine was either recovered or the corresponding C-7 or C-9 esters formed. Attempts at coupling under more harsh conditions were unsuccessful.[7] Traces of amide (< 5%) were observed (LCMS) following the procedure reported by Vedejs and Driver[8] with the symmetrical anhydride. Collectively, these results make it clear that the N-2 amine is sterically congested and rendered inaccessible. The steric effect is exacerbated by the substituents of the Abn core, which attenuate the reactivity of the embedded amine.



Scheme 1. Successful functionalization of the Abn *N*-2-amine. THF = tetrahydrofuran.

An approach was pursued in which simple carboxamides would be installed at the N-2 position under forcing conditions, and the adducts were subsequently elaborated to the desired D-leucine derivative. Amide formation at the N-2 position was effected by treatment of **6**[9] with 10.0 equivalents of 4-methylpent-2-enoic anhydride in tetrahydrofuran at 110 °C (Scheme 1).[10] Subsequent treatment of **7** with LiOOH in tetrahydrofuran led to selective oxazolidinone deprotection, forming **8** in 92% yield.

The functionalization of **8** was subsequently envisioned.[11,12] Treatment of **8** with catalyst $[Mn(dpm)_3]$/$PhSiH_3/O_2$ gave a 1:1 mixture of lactones **9** and **13** (79% yield). After their separation, configurational assignment was effected with 2D NOESY.[13] Lactones **9** and **13** were each subjected to lactone opening with amine **10**,[14] whereupon alcohols **11** and **12** were obtained in 85% and 81% yield, respectively.

[*] C. S. Schindler, L. Bertschi, Prof. Dr. E. M. Carreira
ETH Zürich, HCI H335, 8093 Zurich (Switzerland)
Fax: (+41) 44-632-1328
E-mail: carreira@org.chem.ethz.ch

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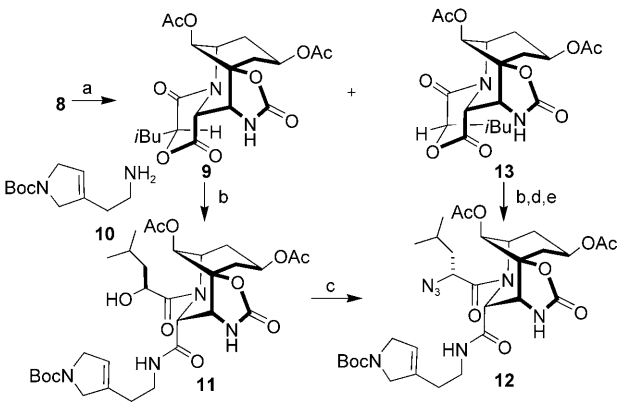
The key point in the synthesis required conversion of alcohol **11** into azide **12** (Scheme 2). Neither the use of DEAD/dppa[15] nor DEAD/HN_3 provided desired **12**, instead



Scheme 2. a) $[Mn(dpm)_3]$, $PhSiH_3$, O_2, EtOH, 23 °C, 79% (**9**/**13**, 1:1); b) **10** (1.0 equiv), CH_2Cl_2, 23 °C, 85%; c) 1. MsCl (1.1 equiv), Et_3N, CH_2Cl_2, 23 °C; 2. NaN_3 (1.5 equiv), DMF, 23 °C, 81% d) 1. MsCl (1.1 equiv), Et_3N, CH_2Cl_2, 23 °C; 2. LiCl (1.1 equiv), DMF, 23 °C, 78%; e) NaN_3 (1.5 equiv), DMF, 23 °C, 89%. $Mn(dpm)_3$ = tris(2,2,6,6-tetramethyl-3,5-heptanedionato)manganese(III), Ms = mesyl, Ac = acetyl, Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide.

leading to recovery of **11**. Mesylation of **11** followed by exposure to NaN_3 gave **12** in 81% yield (2 steps). The diastereomeric alcohol was converted into **12** by a double-inversion procedure that proceeds through the chloride and provides a stereoconvergent approach to **12**.

Azide reduction with aqueous PPh_3 led to formation of diketopiperazine **15** and amine **14** as a 1:1 mixture. Treatment of **12** with Pd/C also gave **15** as the sole product. The use of PMe_3 was found to be critical in furnishing **14**. However, when the amine product was subjected to peptide coupling, **15** was again isolated. Attention was then focused on methods that enabled rapid coupling. With anhydride **16**, adduct **17** was formed in 70% yield. Tripeptide **17** underwent deprotection ($LiOH{\cdot}H_2O$/aq. THF/MeOH) to afford the diol **18** in 95% yield (Scheme 3).

Glycosyl donor **19** was prepared and exposed to acceptor **18** in the presence of Me_3SiOTf (0.1 equiv), leading to the formation of a diastereomeric mixture of glycopeptide **20** in 75% yield (α/β ratio 2:1). These diastereomers were separated following deprotection (tris(dimethylamino)sulfonium difluorotrimethylsilicate, TASF) and thus afforded **21** (92% yield, Scheme 4). The pyrrolidine was deprotected with trifluoroacetic acid (TFA) and then allowed to react with **22** to install the protected guanidine and furnish **23** in 69% yield. The primary alcohol was subjected to O-sulfation ($SO_3{\cdot}$py) to afford **24**, which in turn was deprotected to give **2**.

Careful comparison and analysis of the NMR spectroscopic data for synthetic **2** to that reported for the natural banyaside B revealed significant differences of up to 3 ppm in the ^{13}C NMR chemical shifts (Figure 2). Thus, the ^{13}C shifts for the anomeric carbon atom as well as the C-7 carbon atom on the Abn core of synthetic **2** differed by $\delta \approx 2$ ppm. The ^{13}C shifts for the C-8 carbon atom

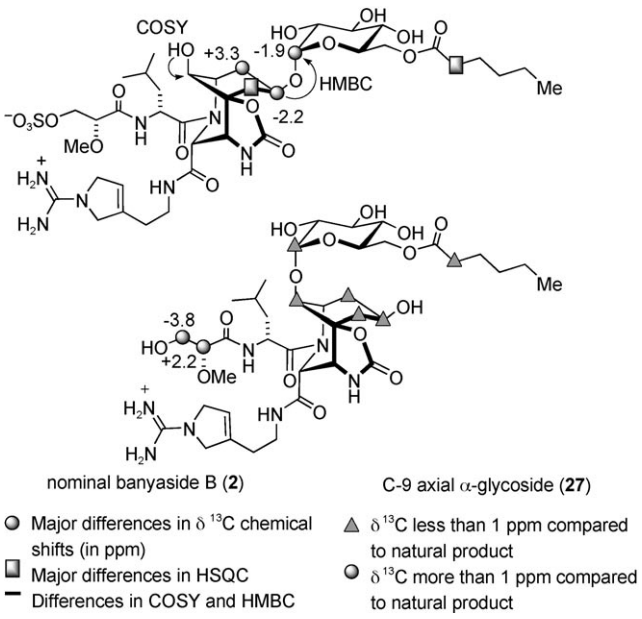
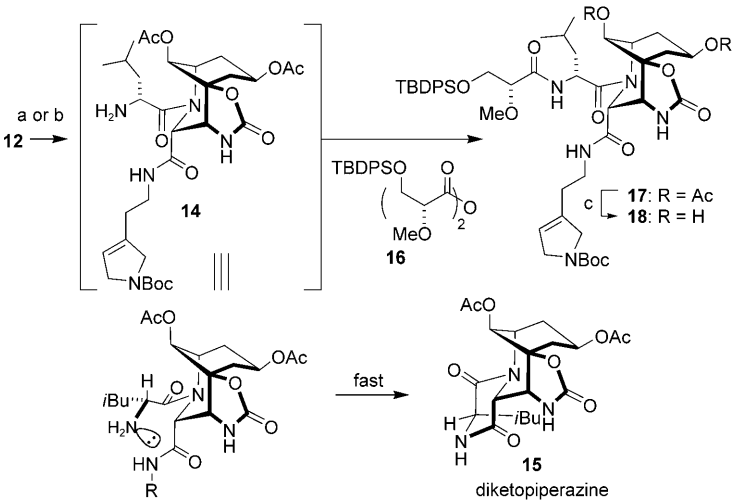


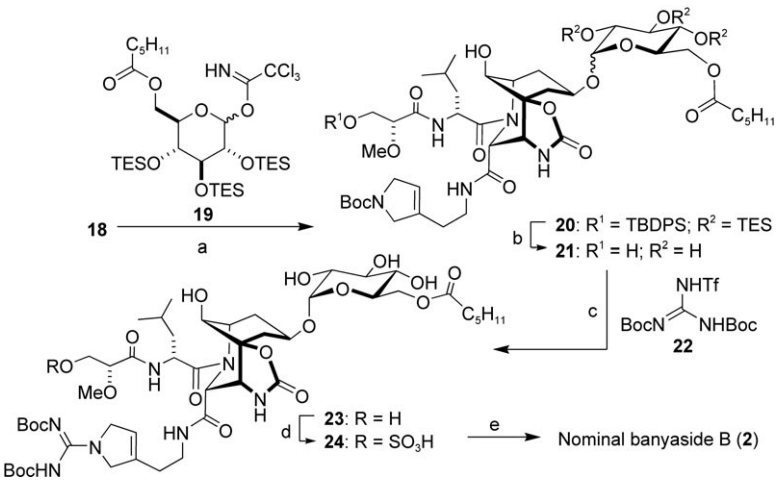
Figure 2. Comparison of nominal banyaside B (**2**) and C-9 axial α-glycoside (**27**) with the NMR spectroscopic data published for natural banyaside B.

of synthetic **2** were off by $\delta = +3.3$ ppm, while δ(C-7) were off by $\delta = -2.2$ ppm. Despite these discrepancies, the dehydropyrrole fragment as well as the D-leucine subunit compared well to the natural product.

Because the differences described above were localized about the C-7 glycosylation site in synthetic **2** (Figure 2), we speculated that the natural compound might more properly be described with the glycosidic linkage at C-9-OH (**3**).

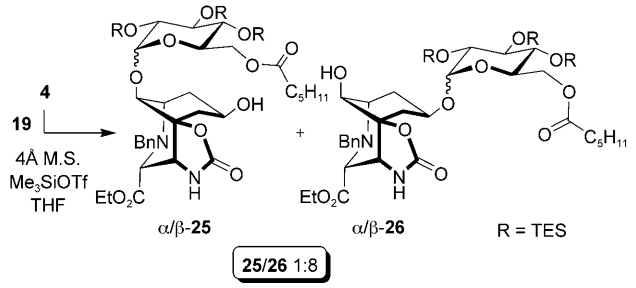


Scheme 3. a) H_2, Pd/C (10 mol%), MeOH, 95%; b) 1. PMe_3, aq. THF, 23 °C; 2. **16** (5.0 equiv), CH_2Cl_2, 23 °C, 70%; c) LiOH (3.0 equiv), THF, H_2O, MeOH, 23 °C, 95%. TBDPS = *tert*-butyldiphenylsilyl.



Scheme 4. a) **19** (1.0 equiv), Me_3SiOTf (0.1 equiv), 4 Å M.S., THF, 23 °C, 75 % (α/β, 2:1); b) TASF (5.0 equiv), DMF, 23 °C, 92 %; c) 1. F_3CCO_2H (10.0 equiv), CH_2Cl_2, 23 °C; 2. **22** (3.0 equiv), Et_3N, $CHCl_3$, 23 °C, 69 %; d) SO_3·py (1.0 equiv), CH_2Cl_2, 23 °C, 25 %; e) F_3CCO_2H/CH_2Cl_2 (9:1), 23 °C, 95 %. M.S. = molecular sieves, TES = triethylsilyl, TASF = tris(dimethylamino)sulfonium difluorotrimethylsilicate, Tf = trifluoromethanesulfonyl.

During the course of optimizing the glycosylation, we observed the equatorial-OH (C-7 OH) to be more-reactive than the axial-OH (C-9 OH). Thus, glycosylation of diol **4** with 1.0 equivalent of **19** furnished an 8:1 product mixture, assigned as the C-7 (**26**) and C-9 (**25**) monoglycosides, respectively (Scheme 5). Noteworthy, and diagnostic for the



Scheme 5. Attempted glycosylations of the banyaside core (**4**).

C-7 isomer, was HMBC correlations between the anomeric proton and Abn C-7 as well as a COSY cross-peak between the H-9 proton and the Abn 9-OH (Figure 2). The minor isomer (**25**) lacks a HMBC signal involving the anomeric proton and the Abn C-9 carbon atom, a feature also noted in the spectra for natural banyaside B. These results prompted us in turn to prepare the corresponding regioisomer of **20**, which was converted into **27**, following procedures described above.

The ^{13}C NMR shift for the anomeric carbon atom in **27** was found to be $\delta = 98.9$ ppm, which compares well with $\delta =$ 98.7 ppm for natural banyaside B. Indeed, **27** shows strong similarities and chemical shifts in the ^{13}C NMR spectra with the isolated natural product (Figure 2, triangles with $\Delta\delta <$ 1 ppm). The only major differences are in the D-leucine side-chain (circles, Figure 2, **27**), in line with the difference between the free primary OH and its O-sulfate (Figure 2, circles). Thus, upon O-sulfation of **23**, the ^{13}C shifts for the side-chain bearing the sulfate underwent a displacement from $\delta = 83.0$ to 79.8 ppm for the *C*HOMe carbon atom and $\delta = 61.6$ ppm to 66.3 ppm for the HO_3SOCH_2 carbon atom. Consequently, we proposed that natural banyaside B was the glycosylated α-anomer at axial-C-9 OH instead of the equatorial-C-7 OH as shown for **3** (Figure 1).

In order to provide additional corroborative evidence for the structural revision, glycoside **27** was subjected to the sulfation conditions described for **23**. Sulfated **27** displayed an identical retention factor by LCMS to that described by Pluotno and Carmeli[3a] for the natural product (YMC-Pack, ODS-A, isocratic MeOH/H_2O (1:1), EIC m/z 950.40, UV at 210 nm): $k' = 1.94$ (natural banyaside B), and $k' = 1.98$ (synthetic banyaside **3**). By contrast, the nominal banyaside **2** elicited $k' = 1.73$ (for details, see the Supporting Information).[3a]

In conclusion, the total synthesis of nominal banyaside B (**2**) has been completed in 23 steps, relying on the initially reported synthesis of the Abn core common to banyasides A (**1**) and nominal banyaside B (**2**), suomilide, and spumigin HKVV. The introduction of the three peptide side-chains proved to be challenging and in fact could not be realized following standard peptide coupling procedures, thereby clearly demarking the limitations of current methods for the installation of hindered peptide bonds. The total synthesis of nominal banyaside B (**2**) was realized by relying on a late-stage glycosylation and introduction of the guanidine subunit on the sensitive 2,5-dehydropyrrole. Finally, a key outcome of the investigations is the revision of the structure for banyaside B with the glycolipid appended at C-9.

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[1] For a recent review on the chemistry of the aeruginosins, see: K. Ersmark, J. R. Del Valle, S. Hanessian, *Angew. Chem.* **2008**, *120*, 1220; *Angew. Chem. Int. Ed.* **2008**, *47*, 1202.
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[6] EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochlo-ride, DCC = dicyclohexylcarbodiimide, BOPCl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, BOP = benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate, DEPBT = 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one, PyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, HATU = 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole.

[7] Details may be found in the Supporting Information. The use of Leuch's anhydride was also examined; see: H. R. Kricheldorf, *Angew. Chem.* **2006**, *118*, 5884; *Angew. Chem. Int. Ed.* **2006**, *45*, 5752.

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[9] Crystallographic data for **6**: $C_{18}H_{24}N_2O_9$, $M = 412.395$, monoclinic, space group $P2_1/n$, $a = 10.3693(2)$, $b = 10.3316(3)$, $c = 18.4211(4)$; $V = 1971.70(8)$ Å^3, $\rho_{calcd} = 1.389$ Mg m^{-3}, $T = 173$ K, reflections collected: 8319, independent reflections 4506 (R(int) = 0.038), R(all) = 0.0635, wR(gt) = 0.0505. CCDC 779698 (**6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[10] Complete removal of the DCC-derived byproduct was found to be crucial for the successful preparation of **8**.

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[13] For details, see the Supporting Information.

[14] For the preparation of **10**, see: S. Hanessian, R. Margarita, A. Hall, S. Johnstone, M. Tremblay, L. Parlanti, *J. Am. Chem. Soc.* **2002**, *124*, 13342.

[15] DEAD = diethyl azodicarboxylate, dppa = diphenylphosphoryl azide.

