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Synthesis and shift-reagent-assisted full NMR assignment of the bacterial (Z_8 , E_2 , ω)-undecaprenol

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Abstract

The repeating isoprene unit is a fundamental biosynthetic motif. Repetitive structure presents challenges both for synthesis and for structural characterization. In this synthesis of the (Z_8 , E_2 , ω)-undecaprenol of prokaryotic glycobiology, we exemplify solutions to these challenges. Allylation of sulfone-derived carbanions controlled the stereochemistry, and its proof-of-structure was secured with $\text{Eu}(\text{hfc})_3$ complexation to disperse the overlaid resonances of its ^1H NMR spectrum.

Graphical Abstract

The synthesis of (Z_8 , E_2 , ω)-undecaprenol is accomplished with proof-of-structure secured with $\text{Eu}(\text{hfc})_3$ complexation to disperse the overlaid resonances of ^1H NMR spectrum.

The repeating isoprene is a fundamental motif in biosynthesis. Although this motif is perhaps more closely identified in modern chemistry as the precursor to secondary metabolites—as exemplified by the oligoisoprene-derived terpenoids—the ancient and fundamental importance of the repeating isoprene is attested to by its presence in essential primary metabolites. These metabolites are exemplified by the ubiquinones, and by the prenol lipids used for the transport of the saccharide motifs of glycobiology.¹ A notable prenol of bacterial glycobiology is (Z_8 , E_2 , ω)-undecaprenol **1** [(2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*Z*,34*E*,38*E*)-3,7,11,15,19,23,27, 31,35,39,43-undecamethyl-2,6,10,14,18,22,26,30,34,38,42-tetratetracontadecaen-1-ol] as shown in Fig.

1A. In bacteria this undecaprenol (also known as “bactoprenol”) forms a small (in terms of the number of molecules, and as its phosphate and diphosphate derivatives) steady-state pool² that is recycled constantly to sustain the simultaneous delivery during growth of the disaccharide motif used for cell-wall biosynthesis, of the polysaccharide motifs of the bacterial surface, and (in Gram-negative bacteria) of the lipopolysaccharide of the outer leaflet of its outer membrane. The absolute necessity to sustain this dynamic pool is underscored by the direct correlation between chemical structures that interfere with the recycling of this undecaprenol, and opportunities for new antibacterial discovery.³

The bacterial (Z_8 , E_2 , ω)-undecaprenol is not commercially available. Moreover, as a consequence of the small size of the pool of the (Z_8 , E_2 , ω)-undecaprenol-containing primary metabolites its direct isolation from bacteria, in meaningful (multi-mg) quantities, is challenging.⁴ The absolute rarity of undecaprenol-containing entities is a barrier to experimental studies. Accordingly, considerable effort has been made toward the identification of surrogate prenols for (Z_8 , E_2 , ω)-undecaprenol that are available in larger quantities and that retain biological recognition, are more easily experimentally handled in vitro, or that incorporate reporter groups for the purpose of assay.^{5–9} Two syntheses of bactoprenol (**1**) have been reported, one as communication in 1986¹⁰ and in 2008 the second as a solid-phase synthesis.¹¹ Notwithstanding these syntheses, the undecaprenol used in all synthetic efforts directed toward Lipid II (undecaprenyl-diphospho-*N*-acetyl glucosamyl-*N*-acetylmuramoyl pentapeptide), the pivotal undecaprenol-containing intermediate in peptidoglycan biosynthesis,¹² is the more available (Z_7 , E_3 , ω)-undecaprenol **2**^{6–8, 13} isolated from plants.¹⁴ Indeed, the interchangeability of the two in some biological assays has led to repeated representations in the literature of Lipid II as a structure derived from undecaprenol **2** (CAS 26296-50-4), rather than the true bactoprenol, **1** (CAS 88200-23-1). The demonstrated value of synthetic Lipid II analogs (notably with fluorophore incorporation into the peptide stem,⁷ prenol,^{6, 8} and both⁶ segments of Lipid II) opens the challenge of the spectroscopic validation of the prenol structure, as its repeating isoprene motif overlays a nearly identical spectroscopic signature. In support of our studies toward practical access to polyprenol structures, we reported the synthesis of (Z_4 , E_2 , ω)-heptaprenol (**3**).¹⁵ This prenol is an alternative in certain biological assays to the more complex and scarcer bactoprenol.⁹ A key discovery made during the course of our synthesis was the advantageous chemical-shift dispersion in its ¹H and ¹³C{¹H} NMR spectra provided by Eu(hfc)₃ (Europium tris[3-(heptafluoropropyl hydroxymethylene)-(+)-camphorate]). This discovery enabled the unambiguous structural assignment of this heptaprenol. Here, we describe the total synthesis of bactoprenol (**1**) and the full NMR assignment of its structure using Eu(hfc)₃ chemical-shift dispersion. Moreover, we establish Eu(hfc)₃ chemical-shift dispersion as a general means of structural validation of complex polyprenol structures by the comparative NMR analysis of the (Z_7 , E_3 , ω)-undecaprenol (**2**) of plants.

The previous syntheses of **1** used the consistency of the low-field one-dimensional ¹H NMR spectrum and mass spectrum for the proof of structure.^{10, 11} Synthetic protocols and intermediate characterization are available only for the solid-phase synthesis of Chang et al.¹¹ Our solution-phase synthesis of **1** follows the general strategy established by Sato et

al.¹⁰ as further refined¹⁵ in our previous synthesis of **3**, wherein a base-mediated reaction of the prenyl sulfone with the prenyl halide is used for prenyl homologation, and culminating in a reductive desulfonylation step. Thus, undecaprenol **1** was synthesized starting from commercial *E*, *E*-farnesol **4** using two cycles of sulfone formation and alkylation (using the nerol-derived *Z*₄-tetraprenyl bromide **5**¹⁵: the alkylation steps are highlighted in blue in Fig. 1B) and final global deprotection (using Li/EtNH₂ dissolving-metal reduction), in a 61% isolated yield following final chromatographic purification. The key building block **5** was constructed in 18 steps from nerol by our earlier method.¹⁵ Undecaprenol **1** was made in 26 convergent steps from nerol. In the course of the global deprotection, we observe as much as 4–15% impurities due to isomerization of the double bonds.

The NMR evaluation of **1** began with comparison of its ¹H NMR spectra obtained separately at 600 MHz in CDCl₃ (Fig. 2A) and C₆D₆ (Fig. 2B) as solvents. While the former solvent showed extensive overlap of resonances, partial resolution of these resonances was seen in C₆D₆. Careful titration of anhydrous¹⁶ Eu(hfc)₃ into C₆D₆ identified a mass ratio of 1:1 Eu(hfc)₃/**1** (or a molar ratio of 0.6:1) as optimal (Fig. 2C). Sequential NMR interrogation of this Eu(hfc)₃·**1** complex was undertaken. Homonuclear DQF-COSY and TOCSY correlation gave the ¹H connectivities. The ¹H–¹³C HSQC spectrum gave the ¹*J*¹H–¹³C correlations. The ¹³C-HMBC and ¹³C-HSQC-TOCSY spectra assigned the quaternary carbons, and were used to check that the connectivities established by the other spectra were correct. Finally, ROESY spectra analysis corroborated the *E* or *Z* stereochemistry of each prenyl unit.

The application of these analyses is exemplified. The TOCSY sub-spectrum correlation of ten of the twelve methyl resonances is shown in Fig. 3A. The two methyl resonances not shown in this figure are those of C-54 (δ 2.19) and C-55 (δ 3.11), which are outside of the range that is shown. The methyl resonances of the terminal ω-prenyl unit (CH₃-44, δ 1.58; CH₃-45, δ 1.69) were distinguished from the other methyl resonances by their mutual crosspeak and the crosspeaks to the methine CH-42 resonance (δ 5.25) and methylene CH₂-41 (δ 2.19) and CH₂-40 (δ 2.10) resonances. All crosspeaks that correlate with H-44 and H-45 are colored in green in Fig. 3A. The methyl resonance at δ 1.62 (CH₃-46) gave crosspeaks (colored in pink in Fig. 3A) with the ¹H resonances at δ 5.29 (CH-38), δ 2.21 (CH₂-37), and δ 2.12 (CH₂-36). In this way, each methyl resonance of each prenyl group was assigned (as shown for four methyl resonances of three prenyl groups, each colored differently in Fig. 3A).

The ¹*J* correlations of the HSQC spectrum (ESI) allowed interpretation of the ¹H–¹³C HMBC spectrum (Fig. 3B). Thus the quaternary C resonance at δ 131.21 was assigned to the C-43 carbon, as it gave crosspeaks to both the CH₃-44 and CH₃-45 methyl resonances (colored in green in Fig. 3B). The proton for CH₂-40 resonance (δ 2.10) correlated with the ¹³C resonances at δ 27.32 (CH₂-41), 40.30 (CH₂-40), 124.87, and 139.12. These last two resonances were assigned to CH-38 and C-39, respectively (colored in red). These CH-38 and CH-39 carbon resonances gave crosspeaks with the δ 1.62 methyl resonance, which is assigned as the methyl resonance (CH₃-46) of the neighboring prenyl unit. These HMBC correlations thus connect the two prenyl units (colored in green and red). The ROESY spectrum showed that the single correlation of the methyl resonance of CH₃-45 (δ 1.69) was to the methine resonance of CH-42, demonstrating that the two were *cis* relative to

each other. Likewise, the absence of a crosspeak between the resonances for CH-38 and CH₃-46 places the methyl as *trans* to the CH-38 hydrogen. Indeed, ROESY crosspeaks between the olefinic and methyl resonances were seen only for the *Z*-prenyl units (Fig. 3D). NMR analysis establishes unequivocally the structure of **1** as that of the natural product (*Z*₈, *E*₂, ω)-undecaprenol.

In contrast to our synthesized (*Z*₈, *E*₂, ω)-undecaprenol bactoprenol **1**, the plant (*Z*₇, *E*₃, ω)-undecaprenol **2** was purchased. The same systematic strategy with Eu(hfc)₃ led to the full NMR assignment of (*Z*₇, *E*₃, ω)-undecaprenol **2**. The minor impurities present in **2** (presumably shorter prenols and/or undecaprenol isomers) did not interfere with our analysis. Although the overall appearance of the ¹H and ¹³C spectra of **2** is very similar to those of **1**, their ROESY spectra are notably different. Fig. 3D highlights the key difference: the presence of two *E*-prenyl units in **1** but three in undecaprenol **2** (box with dotted line in Fig. 3D). The *E* and *Z* prenyl are distinguished further by comparison of the ¹³C chemical shifts for the methyl resonances (C46, C47, C48, C49, C50, C51, C52, C53, C54) compared to the ¹³C chemical shifts of the methylene carbons (respectively, C5, C9, C13, C17, C21, C25, C29, C33, C37) that are covalently bonded to the same olefinic carbon. In the case of the *Z*-prenyls, the ¹³C chemical shifts for the methyl carbons (average value of δ 23.9) are deshielded by about 7.7 ppm compared to the ¹³C chemical shifts of the *E*-prenyls (δ 16.2). Conversely, the methylene chemical shift values are shielded by about 7.5 ppm for the *Z* isomers (δ 32.8) in comparison to the *E* isomers (δ 40.3). These same characteristics were observed previously for the betulaprenol-type polyprenols isolated from the leaves of *Ginkgo biloba*.¹⁴ These correlations, and the ROESY spectra, confirm that the point of difference between the two is the stereochemistry of one prenyl units (green boxes of Fig. 3D). The first complete NMR assignments for the two undecaprenols (as their Eu(hfc)₃ complex) are given in Table S1.

The efficient solution-phase convergent synthesis of **1** we report here (including the protocols and complete characterization of the synthetic intermediates, given in ESI) provides explicit guidance for future efforts toward complex polyprenol syntheses. Although our use of Eu(hfc)₃ as a shift reagent for the NMR assignment of achiral alkene-containing structure has precedent in the use of chiral shift reagent analysis of racemic alkene-containing structures,¹⁷ we emphasize (as have others^{18, 19}) that optimization of the choice of chiral-shift reagent, its stoichiometry, and of solvent can disperse effectively resonances with minimal line broadening. As both pure ¹H shift-pulse sequences²⁰ and computer-assisted methods for NMR spectra interpretation²¹ require a difference in chemical shifts, our decisive NMR differentiation of the two undecaprenol stereoisomers—one from bacteria, and one from plants—affirms the argument¹⁸ of a continuing relevance of the shift reagent to the *de novo* NMR assignment of complex chemical structure. The ability to prepare these valuable synthetic lipids with full spectral characterization opens opportunities for the exploration of their roles in the intricate biochemical pathways of living organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) The chemical structures of polyprenols **1–3**. (B) Linear chemical synthesis of (Z_8 , E_2 , ω)-undecaprenol **1**. Ts = *p*-toluenesulfonyl.

Figure 2. ^1H NMR spectra (600 MHz) of **1** (A) in CDCl_3 , (B) in C_6D_6 , and (C) in C_6D_6 , in the presence of $\text{Eu}(\text{hfc})_3$. The methyl signals are highlighted by the red bracket and asterisks.

Figure 3.

(A) Assignment of each prenyl unit (shown in a different color) using the TOCSY spectra of **1**. (B) Assignment of quaternary carbons and connectivity between two terminal prenyl units (colored in green and pink) of **1**. (C) Chemical structures of synthetic undecaprenol **1** and commercial plant undecaprenol **2** with atom numbering. (D) Side-by-side comparison of synthetic bacterial (**1**) and commercial plant sample (**2**) (800.13 Hz). The atoms in grey dots in panel C were used for exemplary HMBC interpretation in panel B. The atoms in green dots in panel C match green-boxed region in panel D.