# Two New Resveratrol Tetramers from *Upuna borneensis*

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**Phytochemical investigation of an acetone extract of *Upuna borneensis* (Dipterocarpaceae) resulted in the isolation of two new resveratrol tetramers, upunaphenols O (1) and P (2). The structures were elucidated by spectroscopic analysis including NMR experiments.**

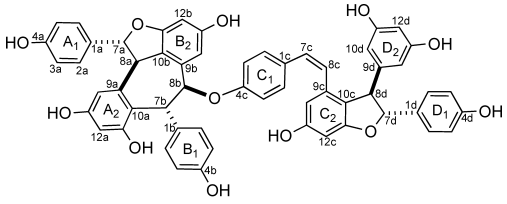
**Key words** *Upuna borneensis*; Dipterocarpaceae; resveratrol oligomer; upunaphenol

During the course of our research on the isolation and identification of bioactive polyphenols in Dipterocarpaceaeous plants, we have previously reported the structural variety of resveratrol oligomers in *Upuna borneensis*.[1—7] Further detailed examination of the acetone extract of stem yielded two new resveratrol tetramers, upunaphenols O (**1**) and P (**2**). The structure of isolates **1** and **2** were elucidated by means of 2D NMR techniques such as $^1$H–$^1$H shift correlation spectroscopy (COSY), $^{13}$C–$^1$H COSY, and $^1$H–$^{13}$C heteronuclear multiple-bond correlation (HMBC), and the stereostructures were proposed by analysis of the nuclear Overhauser spectroscopy (NOESY) spectra.

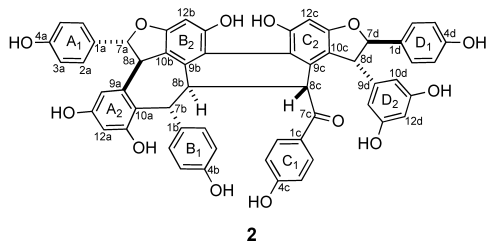
## Results and Discussion

Upunaphenols O (**1**) ($[\alpha]_D^{25}$ +84°), and P (**2**) ($[\alpha]_D^{25}$ +180°) were purified from an acetone-soluble stem segment of *U. borneensis* by column chromatography over silica gel, Sephadex LH-20, octadecyl silica (ODS), and PTLC (preparative TLC).

Upunaphenol O (**1**), obtained as a pale yellow solid, showed positive reaction to the Gibbs reagent. The molecular formula of $C_{56}H_{42}O_{12}$ was established by an $[M+H]^+$ ion peak at *m*/*z* 907.2732 in the electron spray ionization (ESI)-MS together with the NMR spectral data, which suggested that **1** is a resveratrol tetramer and bears 36 degrees of unsaturation. Acetylation of **1** yielded a nona-acetate (ESI-MS: *m*/*z* 1307.3484 $[M+Na]^+$), suggesting that **1** bears nine phenolic hydroxyl groups. The $^1$H- and $^{13}$C-NMR spectral data together with $^1$H–$^1$H COSY, $^{13}$C–$^1$H COSY and HMBC spectra (Fig. 1, Table 1) showed the presence of *ortho*-coupled aromatic protons assignable to three 4-hydroxylphenyl groups (rings $A_1$, $B_1$ and $D_1$), a 4-oxygenated phenyl group (ring $C_1$), three sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring (rings $A_2$, $B_2$ and $C_2$), and a 3,5-dihydroxyphenyl group ($D_2$). The NMR spectral data also disclosed the presence of two sets of aliphatic signals characteristic for 2,3-diaryldihydrobenzofuran moieties (H-7a/H-8a; H-7d/H-8d),[8] two coupled aliphatic methine protons (H-7b/H-8b), *cis*-coupled olefinic protons (H-7c/H-8c), and nine phenolic hydroxyl groups ($\delta_H$ 7.97—8.64). Considering the molecular formula, the remaining three oxygens can be allocated to ether linkage. In the HMBC spectrum (Fig. 1), significant $^3J$ correlations were observed between H-7a/C-2a(6a), H-14a/C-8a, H-7b/C-2b(6b), H-14b/C-8b, H-7c/C-2c(6c), H-8c/C-14c, H-7d/C-2d(6d), and H-8d/C-10d(14d), indicating that rings $A_1$, $A_2$, $B_1$, $B_2$, $C_1$, $C_2$, $D_1$, and $D_2$ are attached at C-7a, C-8a, C-7b, C-8b, C-7c, C-8c, C-7d, and C-8d, respectively. Further correlations observed between H-8a/C-11b, H-7b/C-11a, and H-8d/C-11c supported



**1**



**2**

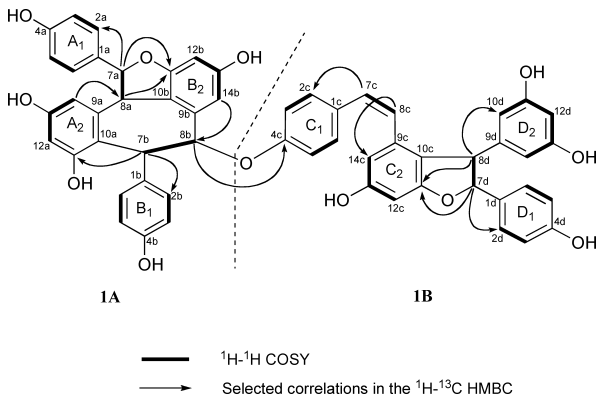



Fig. 1. Selected Correlations in 2D NMR of **1**

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Table 1. $^{1}$H- and $^{13}$C-NMR Spectral Data of **1** and **2**

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1a | | 133.5 | | 133.8 |
| 2a(6a) | 7.42 (d, 8.4) | 130.4 | 7.24 (d, 8.4) | 128.2 |
| 3a(5a) | 6.94 (d, 8.4) | 116.5 | 6.78 (d, 8.4) | 116.1[f] |
| 4a | | 158.49[i] | | 158.1[g] |
| 7a | 5.78 (d, 9.5) | 93.5 | 6.10 (d, 4.2) | 90.0 |
| 8a | 5.32 (d, 9.5) | 52.1 | 4.78 (d, 4.2) | 52.0 |
| 9a | | 141.4 | | 142.1 |
| 10a | | 117.9 | | 121.1 |
| 11a | | 158.1[e] | 7.93 (br s) | 158.5 |
| 12a | 6.27 (br s)[a] | 102.1 | 6.06 (d, 2.2) | 102.4 |
| 13a | | 156.8 | | 156.4[h] |
| 14a | 6.27 (br s)[a] | 106.6 | 6.55 (br s) | 105.2 |
| 1b | | 134.0 | | 137.6 |
| 2b(6b) | 6.82 (d, 8.6) | 130.7[d] | 6.89 (d, 8.4) | 131.2 |
| 3b(5b) | 6.52 (d, 8.6) | 115.1 | 6.67 (d, 8.4) | 115.4 |
| 4b | | 155.9 | | 156.4[h] |
| 7b | 5.33 (d, 5.8) | 47.1 | 4.04 (d, 12.8) | 45.9 |
| 8b | 5.41 (d, 5.8) | 83.0 | 3.54 (d, 12.8) | 46.4 |
| 9b | | 137.4 | | 132.8 |
| 10b | | 118.2 | | 124.4 |
| 11b | | 160.9 | | 156.9 |
| 12b | 6.03 (d, 2.2) | 96.9 | 6.32 (s) | 97.2 |
| 13b | | 158.54[i] | | 152.7 |
| 14b | 5.91 (d, 2.2) | 109.5 | | 115.5 |
| 1c | | 130.6[e] | | 127.2 |
| 2c(6c) | 7.02 (d, 8.6) | 130.6[e] | 6.56 (d, 8.6) | 131.1 |
| 3c(5c) | 6.79 (d, 8.6) | 116.8 | 6.39 (d, 8.6) | 114.9 |
| 4c | | 158.0 | | 161.3 |
| 7c | 6.21 (d, 13.2) | 130.7[d] | | 195.0 |
| 8c | 6.03 (d, 13.2) | 126.5 | 4.48 (s) | 46.3 |
| 9c | | 136.8 | | 133.9 |
| 10c | | 120.0 | | 122.2 |
| 11c | | 162.5 | | 160.6 |
| 12c | 6.31 (d, 2.2) | 96.7 | 6.54 (s) | 98.9 |
| 13c | | 159.2 | | 155.2 |
| 14c | 6.25 (d, 2.2) | 108.4 | | 116.8 |
| 1d | | 133.7 | | 133.7 |
| 2d(6d) | 7.11 (d, 8.4) | 127.9 | 7.32 (d, 8.6) | 127.9 |
| 3d(5d) | 6.84 (d, 8.4) | 116.1 | 6.93 (d, 8.6) | 116.1[f] |
| 4d (OH) | 8.47 (br s) | 158.1[e] | | 158.1[g] |
| 7d | 5.32 (d, 5.3) | 93.8 | 5.32 (d, 6.2) | 94.3 |
| 8d | 4.04 (d, 5.3) | 57.1 | 4.54 (d, 6.2) | 58.4 |
| 9d | | 146.8 | | 145.0 |
| 10d(14d) | 6.05 (d, 2.2) | 106.8 | 5.75 (d, 2.0) | 107.0 |
| 11d(13d) | | 159.5 | | 159.1 |
| 12d | 6.18 (t, 2.2) | 101.9 | 5.52 (t, 2.0) | 101.5 |
| OH groups | 8.64 (br s, OH-4a) | | | |
| | 8.03 (br s, OH-11a) | | | |
| | 8.10 (br s, OH-13a)[b] | | | |
| | 7.97 (br s, OH-4b) | | | |
| | 8.10 (br s, OH-13b)[b] | | | |
| | 8.37 (br s, OH-13c) | | | |
| | 8.13 (br s, OH-11d(13d)) | | | |

Measured in acetone-$d_6$ at 300 MHz ($^{1}$H-NMR) and 75 MHz ($^{13}$C-NMR). *a*—*h*) Overlapping. *i*) Interchangeable.

the links between C-8a/C-10b, C-7b/C-10a, and C-8d/C-10c, respectively. Additional cross peaks observed between H-7a/C-11b, H-8b/C-4c, and H-7d/C-11c supported the presence of ether linkage (C-7a/O/C-11b, C-8b/O/C-4c C-7d/O/C-11c). The planar structure of upunaphenol O, that included two dihydrobenzofuran rings, has been concluded to be **1**. The stereostructure of **1** was determined by analysis of the NOESY spectrum with the assistance of computer-aided

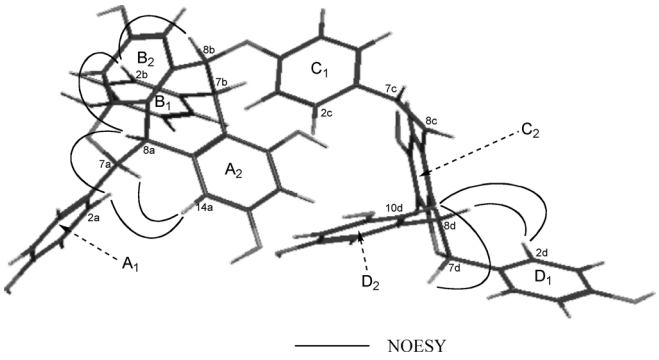


Fig. 2. Selected NOEs Observed in the NOESY Experiment with **1** Model of **1** Drawn by Pcmodel 9.1 Molecular Modeling Program

molecular modeling (Fig. 2). A 3D structure of **1** was generated with the Pcmodel 9.1 molecular modeling program software, using the MMFF94 force field (MM2 type) for energy minimization.[9] The *trans* orientations of H-7a/H-8a and H-7d/H-8d on the dihydrobenzofuran rings were confirmed by the distinctive NOEs between H-7a/H-14a, H-14a/H-2a(6a), H-8a/H-2a(6a), H-7d/H-10d(14d), H-2d(6d)/H-10d(14d) and H-8d/H-2d(6d). The strong NOE (H-14a/H-2a(6a)) and the large coupling constant (H-7a/H-8a: $J$=9.5 Hz) supported the *trans* diequatrial orientation of two aromatic rings (rings $A_1$ and $A_2$) and *trans* diaxial orientation of H-7a/H-8a. Significant NOE between H-8a/H-2b(6b) indicates that ring $B_1$ and H-8a are of *syn* orientation. The *syn*-orientation of H-8b and ring $B_1$ was deduced based on the cross peak for H-8b/H-2b(6b) in the spectrum. From these results, the relative configuration of six asymmetric carbons in **1**, C-7a, C-8a, C-7b, C-8b, C-7d, and C-8d, were determined as *rel-R*, *R*, *R*, *S*, *R*, and *R*, respectively. The structure can be regarded as a complex product composed of two resveratrol dimer units **1A** and **1B** [unit **1A**: resveratrols A—B (resveratrol A: ring $A_1$-C-7a-C-8a-ring $A_2$); unit **1B**: resveratrols C—D]. The structures of **1A** and **1B** are identical with resveratrol dimers, ampelopsin A and (*Z*)-$\varepsilon$-viniferin, respectively. Both compounds have also been isolated from the same material.[3] The both dimers are then presumed to be precursors of **1**.

Upunaphenol P (**2**) was obtained as a pale yellow amorphous solid. The composition was deduced to be $C_{56}H_{40}O_{13}$ from the pseudo-molecular ion peak of $[M+H]^+$ at $m/z$ 921.2527 in the ESI-MS spectrum and the $^{13}$C-NMR spectrum which showed 56 carbon signals. Acetylation of **2** yielded a deca-acetate (ESI-MS: $m/z$ 1365 $[M+Na]^+$), suggesting the presence of 10 phenolic hydroxyl groups. A signal in the $^{13}$C-NMR spectrum ($\delta_C$ 195.0) showed the presence of a carbonyl group (C-7c) in the molecule. Analysis of the $^{1}$H- and $^{13}$C-NMR spectral data (Table 1), $^{1}$H–$^{1}$H, and $^{13}$C–$^{1}$H COSY and HMBC spectral data revealed four resveratrol units (resveratrols A—D) consisting of four 4-hydroxyphenyl groups (rings $A_1$—$D_1$), a 3,5-dioxygenated-1,2-tetrasubstituted benzene ring (ring $A_2$), two 3,5-dioxygenated-1,2,6-pentasubstituted benzene rings (rings $B_2$ and $C_2$), a 3,5-dihydroxy benzene ring (ring $D_2$), two set of mutually coupled aliphatic protons characterized 2,3-diaryldihydrobenzofuran moieties (H-7a/H-8a; H-7d/H-8d), and a sequence of three aliphatic protons (H-7b/H-8b/H-8c). Due to $^{3}J$ cross peaks in the HMBC spectrum (Fig. 3), these partial

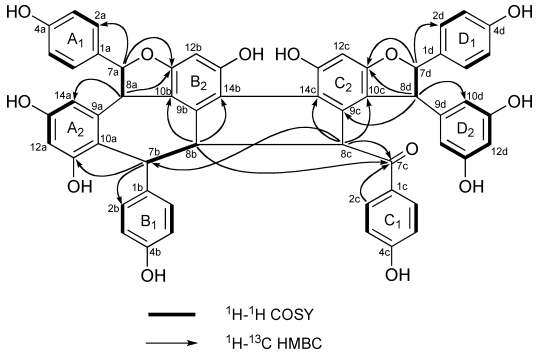


Fig. 3. Main Connectivities from the HMBC and $^1$H–$^1$H COSY Experiments with **2**

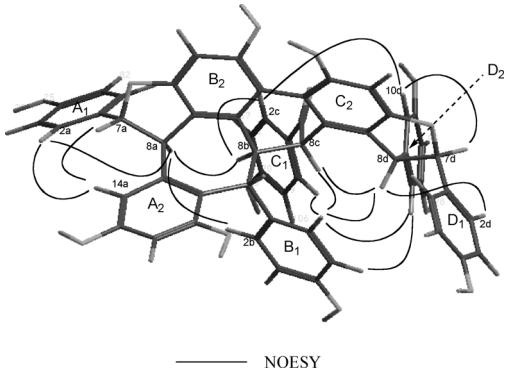


Fig. 4. Selected NOEs Observed in the NOESY Experiment with **2** Model of **2** Drawn by Pcmodel 9.1 Molecular Modeling program

structures could be connected except for a bond for C-14b and C-14c. The established partial structure satisfied all the oxygen's function and accounted for 36 of the 37 required degrees of unsaturation, which suggested that the ring formation was required. The C-14b–C-14c bond was assumed after consideration of the molecular skeleton. The planar structure of **2** was thus characterized. The planar structure of **2** is similar to upunaphenol I except for a presence of a carbonyl group (C-7c) instead of an absence of a C–C bond (C-7c/C-10d) in upunaphenol I.[5] The relative configuration of **2** was determined by NOESY experiments (Fig. 4). All *trans* orientations of two 2,3-diaryldihydrobenzofuran rings were determined by the NOEs observed between H-7a/H-14a, H-14a/H-2a(6a), H-8a/H-2b(6b), H-7d/H-10d(14d), and H-8d/H-2d(6d). The relative *cis* disposition for H-8a, ring $B_1$, H-8b, ring $D_2$, and a 4-hydroxybenzoyl group (ring $C_1$) were inferred from NOEs for H-8a/H-2a(6a), H-8a/H-8b, H-8b/H-2c(6c), and H-2c(6c)/H-10d(14d). The results explains well the stereostructure of **2**, where all the asymmetric carbons are situated in the same manner as upunaphenol I.[5] Previously, we have reported the co-presence of resveratrol tetramers, upunaphenols B and H—J, (−)-hopeaphenol and stenophyllol A, in the same material, that could be derived from the main component, (−)-hopeaphenol.[3] Compound **2** is also presumed to have biogenetic relation with hopeaphenol.

## Experimental

The following instruments were used: optical rotations, JASCO P-1020 polarimeter; UV spectra, Shimadzu UV-2200 spectrophotometer (in MeOH solution); $^1$H- and $^{13}$C-NMR spectra, JEOL JNM LA-300 (chemical shift values are presented as $\delta$ values with tetramethylsilane (TMS) as internal standard); ESI-MS, Thermo Fisher Scientific LTQ Orbitrap instrument.

The following adsorbents were used for purification: analytical TLC, Merck Kieselgel 60 $F_{254}$ (0.25 mm); preparative TLC, Merck Kieselgel 60 $F_{254}$ (0.5 mm); column chromatography, Merck Kieselgel 60, Pharmacia Fine Chemicals AB Sephadex LH-20 and Fuji Silysia Chemical Chromatorex.

*Upuna borneensis* Sym. was cultivated in Bogor Botanical Garden, Bogor, Indonesia, and its stems were collected in May 2000 and identified by one of co-authors (D.D.). A voucher specimen (number DP-012) has been deposited in Gifu Pharmaceutical University.

Extraction and Isolation of Compounds (**1** and **2**): The extraction procedure was the same as that on our previous reports.[1—7] Fr. 7 ($CHCl_3$–MeOH, 9 : 1) was further subjected to Sephadex LH-20 CC (MeOH) to give eight fractions (Fr. 7a—Fr. 7h). Compounds **2** (17 mg) was purified from the fraction of Fr. 7e after purification by Sephadex LH-20 CC (MeOH) and PTLC (EtOAc–$CHCl_3$–MeOH–$H_2O$, 15 : 8 : 4 : 1). Fr. 8 ($CHCl_3$–MeOH, 8 : 1) was divided into seven parts (Fr. 8a—Fr. 8g) in the same way as that of Fr. 7. Sub fraction of Fr. 8f gave **1** (12 mg) after CC over ODS (MeOH–$H_2O$, 6 : 4).

Compound **1** (Upunaphenol O): A pale yellow solid; $[\alpha]_D^{25}$ +84° ($c$=0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 207 (4.91), 225sh (4.75), 285 (4.07) nm; positive ion ESI-MS $m/z$: 907.2732 $[M+H]^+$ (Calcd for $C_{56}H_{43}O_{12}$: 907.2749); $^1$H- and $^{13}$C-NMR spectral data [$^1$H (300 MHz), $^{13}$C (75 MHz), acetone-$d_6$], see Table 1; HMBC correlations, see Fig. 1 (selected) and H-2a(6a)/C-4a, C-7a; H-3a(5a)/C-1a, C-4a; OH-4a/C-3a(5a), C-4a; H-7a/C-9a; H-8a/C-1a, C-9a, C-10b; OH-11a/C-10a, C-11a, C-12a; H-12a/C-10a, C-11a, C-13a; OH-13a/C-12a, C-13a, C-14a; H-14a/C-10a, C-12a, C-13a; H-2b(6b)/C-4b, C-7b; H-3b(5b)/C-1b, C-4b; OH-4b/C-3b(5b), C-4b; H-7b/C-9a, C-10a, C-1b, C-9b; H-8b/C-10a, C-1b, C-9b, C-10b, C-14b; H-12b/C-10b, C-11b, C-13b, C-14b; OH-13b/C-12b, C-13b, C-14b; H-14b/C-10b, C-12b, C-13b; H-2c/C-4c, C-7c; H-3c(5c)/C-1c, C-4c; H-7c/C-9c; H-8c/C-1c, C-9c, C-10c; H-12c/C-10c, C-11c, C-13c, C-14c; OH-13c/C-12c, C-13c, C-14c; H-14c/C-10c, C-12c; H-2d(6d)/C-4d, C-7d; H-3d(5d)/C-1d, C-4d; OH-4d/C-3d(5d), C-4d; H-7d/C-9d; H-8d/C-9c, C-10c, C-1d; H-10d(14d)/C-11d(13d), C-12d; OH-11d(13d)/C-10d(14d), C-11d(13d), C-12d; H-12d/C-11d(13d); NOESY correlations: see Fig. 2 (selected) and H-3a(5a)/OH-4a; H-2a(6a)/H-7a; OH-11a/H-12a, H-7b; H-12a/OH-13a; OH-13a/H-14a; H-2b(6b)/H-7b; H-3b(5b)/OH-4b; H-8b/H-3c(5c); H-12b/OH-13b; OH-13b/H-14b, H-3c(5c); H-2c(6c)/H-10d(14d), OH-13c; H-8c/H-8d; H-12c/OH-13c; OH-13c/H-14c; H-2d(6d)/H-7d; H-3d(5d)/OH-4d; H-8d/H-10d(14d); H-10d(14d)/H-2c(6c), OH-11d(13d); OH-11d(13d)/H-12d.

**Acetylation of 1** A solution of **1** (1 mg) in pyridine (0.5 ml) containing $Ac_2O$ (0.1 ml) was kept at rt for 24 h. Workup in the usual manner and the purification of the resulting crude product (1 mg) by prep. TLC (*n*-hexane/EtOAc 1 : 1) afforded a nona-acetate (1 mg). A pale yellow solid; positive ion ESI-MS $m/z$: 1307.3484 $[M+Na]^+$ (Calcd for $C_{74}H_{60}O_{21}Na$: 1307.3519).

Compound **2** (Upunaphenol P): A pale yellow amorphous solid; $[\alpha]_D^{25}$ +180° ($c$=0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 225sh (4.50), 279 (3.96), 323 (3.52) nm; positive ion ESI-MS $m/z$: 921.2527 $[M+H]^+$ (Calcd for $C_{56}H_{41}O_{13}$: 921.2542); $^1$H- and $^{13}$C-NMR spectral data [$^1$H (300 MHz), $^{13}$C (75 MHz), acetone-$d_6$], see Table 1; HMBC correlations: see Fig. 3 (selected) and H-2a(6a)/C-4a, C-7a; H-3a(5a)/C-1a, C-4a; H-7a/C-1a, C-9a; H-8a/C-10a, C-10b; OH-11a/C-10a, C-11a; H-12a/C-10a, C-13a, C-14a; H-14a/C-10a, C-13a; H-2b(6b)/C-4b, C-7b ; H-3b(5b)/C-1b, C-4b; H-7b/C-9a, C-10a, C-1b; H-8b/C-1b, C-9b; H-12b/C-10b, C-11b, C-13b, C-14b; H-2c(6c)/C-4c; H-3c(5c)/C-1c, C-4c; H-8c/C-9c; H-12c/C-10c, C-11c, C-13c, C-14c; H-2d(6d)/C-4d, C-7d; H-3d(5d)/C-1d, C-4d; H-7d/C-1d, C-9d; H-8d/C-1d; H-10d(14d)/C-11d(13d), C-12d; NOESY correlations: see Fig. 4 (selected) and H-2a(6a)/H-7a; OH-11a/H-12a, H-7b; H-2b(6b)/H-7b; H-3c(5c)/H-10d(14d); H-2d(6d)/H-7d; H-8d/H-10d(14d).

**Acetylation of 2** Compound **2** (2 mg) was treated in the same way as **1** to afford a deca-acetate (2 mg). A pale yellow solid; positive ion ESI-MS $m/z$: 1363.3398 $[M+Na]^+$ (Calcd for $C_{76}H_{60}O_{23}Na$: 1363.3418).

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