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Synthesis of (2'*Z*,6'*E*) and (2'*E*,6'*E*)-[1'-^{13}C] Ubiquinone 3 by means of (2*E*,6*E*) and (2*Z*,6*E*)-[1-^{13}C] Farnesyltrimethyltin Reagents

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SUMMARY

(2*Z*,6*E*) and (2*E*,6*E*)-[1-^{13}C] farnesates were obtained by a Horner reaction of the geranylacetone with triethyl phosphono [1-^{13}C] acetate treated with NaH. Reduction of these esters with $LiAlH_4/AlCl_3$ gave the corresponding isomeric farnesols which were separated by column chromatography. Each isomer was brominated with PBr_3 to provide the corresponding bromo derivatives which were then reacted with Me_3SnLi to afford the farnesyltrimethyltins. The latter were coupled with 2,3-dimethoxy-5-methyl-1,4-benzoquinone in the presence of $BF_3.OEt_2$ followed by oxidation with Ag_2O to give rise to (2'*Z*,6'*E*) and (2'*E*,6'*E*)-5,6-dimethoxy-2-farnesyl-3-methyl-[1'-^{13}C] 1,4-benzoquinone.

Key-words: (2*E*,6*E*)-[1-^{13}C] Farnesol, (2*Z*,6*E*)-[1-^{13}C] farnesol, (2*E*,6*E*)-[1-^{13}C] farnesyltrimethyltin, (2*Z*,6*E*)-[1-^{13}C] farnesyltrimethyltin, (2'*Z*,6'*E*)-[1'-^{13}C] Ubiquinone 3, (2'*E*,6'*E*)-[1'-^{13}C] Ubiquinone 3, Horner reaction

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INTRODUCTION

Studies of the affinity of chainless/derivatives of ubiquinones in the photosynthetic bacterial reaction centers showed that the double bond of the polyprenyl chain nearest the quinonic ring is important in the anchoring of these cofactors in their sites (1). In order to evaluate the importance of the geometry of the $\Delta^{1'}$ double bond of the side chain we developed a synthesis of (2'*Z*,6'*E*) and (2'*E*,6'*E*)-5,6-dimethoxy-2-farnesyl-3-methyl-[1'-^{13}C] 1,4-benzoquinones.

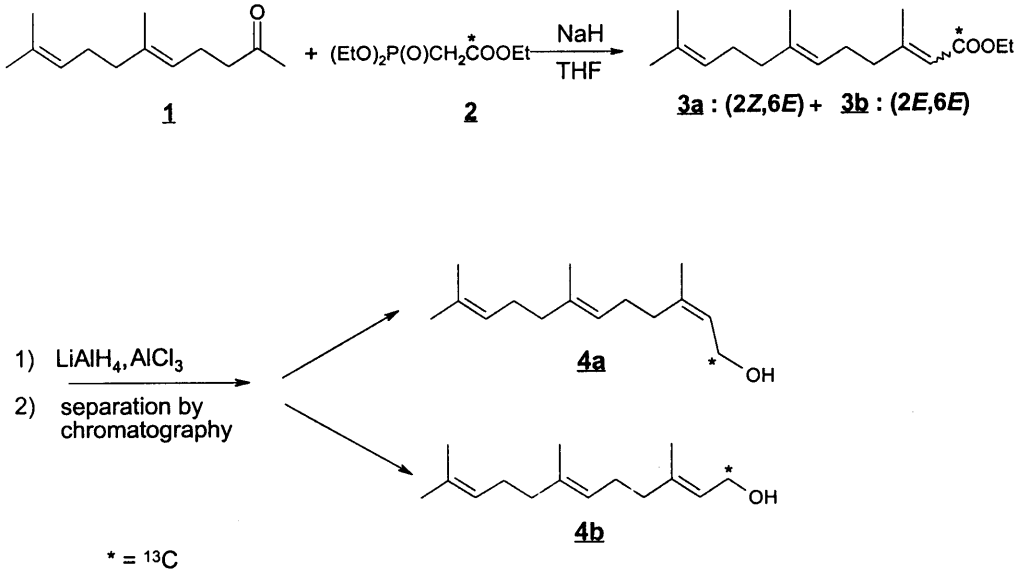
RESULTS AND DISCUSSION

The elegant total synthesis of ubiquinones reported by Rüttimann and Lorenz (2) is based on the construction of the quinone ring by a Diels Alder reaction followed by the attachment of the desired polyprenyl side chain. We recently applied this method to the preparation of ubiquinones regiospecifically labelled with ^{13}C in the quinone ring (3,3a,4). However the low overall yields we obtained on the millimole scale led us to look for another scheme in order to label with [^{13}C] the farnesyl side chain of ubiquinone 3. We considered that the Lewis acid (BF_3) catalyzed allylation of quinones with allyltin reagents first developed by Y. Naruta (5,6,10) might be the method of choice for the synthesis of ubiquinones **7a** and **7b** labelled with ^{13}C at C-1 of the side chain (schemes 2 and 3). This strategy involves the following steps (schemes 1 to 3).

A-*(2Z,6E) [1-^{13}C] Farnesol: 4a and (2E,6E) [1-^{13}C] Farnesol: 4b synthesis:*

The Horner reaction between geranylacetone **1** and triethyl phosphono [1-^{13}C] acetate **2** afforded a mixture of (2*Z*,6*E*) and (2*E*,6*E*) esters **3a**:**3b** (45/55 proportion) in 70% yield

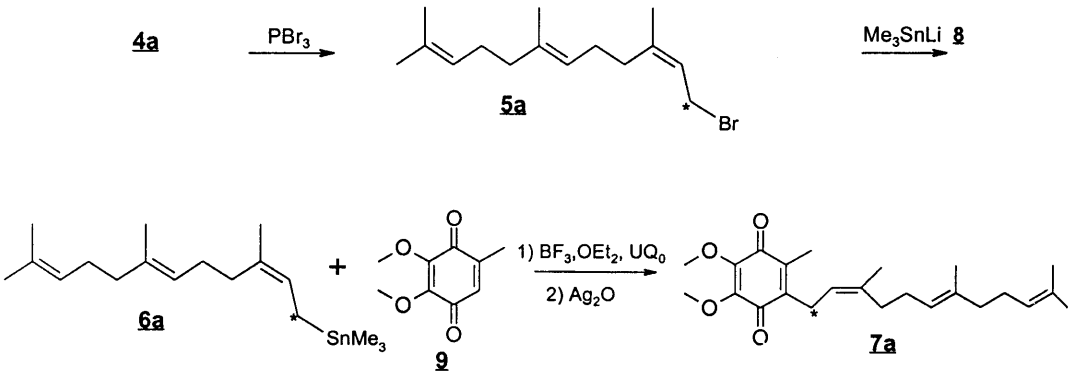
(7). Their reduction with $LiAlH_4/AlCl_3$ (yield: 94%) gave the isomers **4a** and **4b** (8) which were separated by column chromatography (scheme 1).



scheme 1

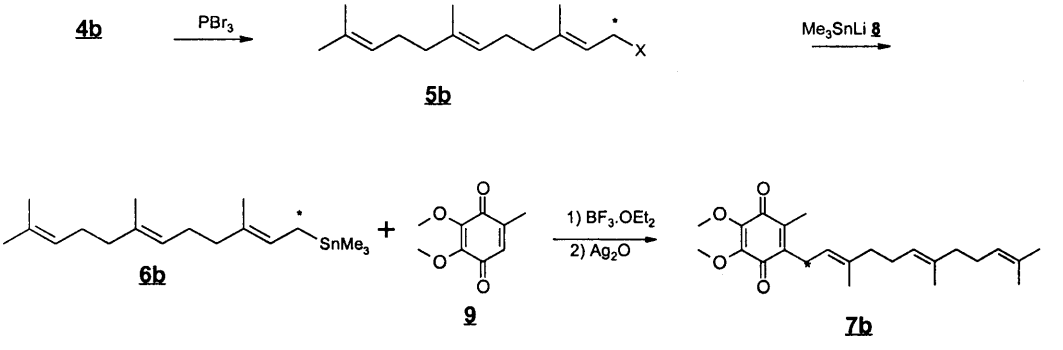
B-*(2'Z,6'E) [1'-^{13}C] Ubiquinone 3:7a and (2'E,6'E) [1'-^{13}C] Ubiquinone 3:7b synthesis:*

4a and **4b** were brominated with PBr_3 to give quantitatively **5a** and **5b** which were reacted with Me_3SnLi to afford farnesyltrimethyltins **6a** and **6b** in 58 and 59% yields. Some experiments for the preparation of farnesyltrialkyltins on a mmole scale showed that better yields were obtained with Me_3SnLi than with $Bu_3SnMgCl$ and with farnesyl bromide than with farnesyl chloride. **6a** and **6b** were purified by chromatography on a reversed phase column, no decomposition was observed in contrast with previous results (10) using a silicagel column. Experiments directed at the introduction of the chain from crude farnesyltrialkyltins led to low yields, likely because of the presence of



scheme 2

a large proportion of $(Me_3Sn)_2$ which was identified by ^{119}Sn NMR (9). The coupling of **6a** and **6b** onto 2,3-dimethoxy-5-methyl-1,4-benzoquinone in the presence of $BF_3.OEt_2$ was followed by oxidation with Ag_2O to afford (2'*E*,6'*E*)-[1'-^{13}C] ubiquinone **7a** (scheme 2) and (2'*Z*,6'*E*)-[1'-^{13}C] ubiquinone 3 **7b** (scheme 3) in 88 and 89% yields respectively.



scheme 3

The geometry of the $\Delta^{1'}$ double bond of **7a** and **7b** was checked by ^{1}H NMR based on the shift of the δ of CH_3-C_3, which was observed at 1.72 ppm for the *Z* isomer and at 1.66 ppm for the *E* isomer (12) and the $\Delta^{1'}Z/\Delta^{1'}E$ proportion determined by HPLC was

96:4 for **7a** and 3:97 for **7b**. These results show that the quinone allylation with trialkylstannyl derivatives is a useful method to introduce [^{13}C farnesyl] side chains of quinones on the mmole scale.

EXPERIMENTAL:

General

Triethyl phosphono [1-^{13}C] acetate (isotopic enrichment: 99%) was obtained from *LEMAN* France. HPLC analysis were performed on a *Merck* system (Darmstadt), and the HPLC purifications on a *Dupont* system. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR at 75 MHz in $CDCl_3$ on a *Bruker* AM 400.

(2*Z*,6*E*)-3,7,11-Trimethyl-2,6,10-undeca-[1-^{13}C]-1-yl acetate 3a and (2*E*,6*E*)-3,7,11-trimethyl-2,6,10-undeca-[1-^{13}C] 1-yl acetate 3b:

NaH (0.46 g, 9.6 mmol) (50% dispersion in oil) was washed with THF (3x5 mL) and suspended in THF (10 mL). A solution of triethyl phosphono [1-^{13}C] acetate **2** (2 g, 8.9 mmol) in THF (2 mL) was added dropwise under stirring. After the hydrogen gas evolution subsided, a solution of geranylacetone **1** (1.55 g, 8 mmol) in THF (2 mL) was added and stirred for 1h. The reaction mixture was acidified with 1N HCl (20 mL) and extracted with diethyl ether (3x30 mL). The combined organic layers were washed with H_2O, dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by flash chromatography on silicagel with hexane/diethyl ether: 98:2 as eluent to give 1.48 g of a mixture of **3a**:**3b** in 45/55 (yield = 70%). **3a+3b:** ^{1}H NMR: 1.2 (td, CH_3-CH_2, ^{3}J= 7 Hz), 1.6 (br s, CH_3-C_{11}, CH_3-C_{12}), 1.65 (s, CH_3-C_7), 1.9-2.09 (m, CH_2-CH_2), 2.15 (s, CH_3-C_3), 4.1 (qd, CH_3-CH_2), 5.07, 5.15 (2t, H-C_{10}, H-C_6), 5.65 (br s, H-C_2);

^{13}C NMR: 13.7 *CH_3*-CH_2, 15.6 C_3, 17.3 C_7, 25.0, 26.8, C_5, C_9, 39.3, 40.6 C_4, C_8, 58.9 *CH_2*-CH_3, 115.4 C_2, 123.2 C_6, 123.9 C_{10}, 127.8 C_{11}, 130.8 C_7, 135.6 C_3, 166.1 C_1-*Z*, 166.7 C_1-*E*.

(2*Z*,6*E*)-3,7,11-Trimethyl-2,6,10-dodecatrien-[1-^{13}C] 1-ol: ((2*Z*,6*E*) [1-^{13}C]-farnesol): 4a and (2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-[1-^{13}C] 1-ol: ((2*E*,6*E*)-[1-^{13}C] Farnesol): 4b:

To a suspension of $LiAlH_4$ (0.356 g, 9.37 mmol) and $AlCl_3$ (0.25 g, 1.87 mmol) in diethyl ether (15 mL) cooled at -30°C was added a mixture of **3a**+**3b** (0.99 g, 3.74 mmol) and stirrred for 15 min. The reaction mixture was hydrolysed with moist diethyl ether (20 mL) and with a saturated NH_4Cl solution (30 mL) and extracted with diethyl ether (3x30 mL). The combined organic extracts were washed with H_2O (30 mL), dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified on a silicagel column with hexane/ethyl acetate: 93:7 as eluent to give 0.238 g of **4a**, 0.145 g of **4a**+**4b** (75/25) and 0.395 g of **4b** (reduction yield: 94%).

4a: ^1H NMR: 1.24 (s, OH), 1.59 (s, CH_3-C_{11}, CH_3-C_{12}), 1.67 (s, CH_3-C_7), 1.71 (s, CH_3-C_3), 1.94-2.1 (m, 4 CH_2-CH_2), 4.09 (td, CH_2-C_1, ^3J= 6 Hz, $^1J(^{13}C, H)$= 142 Hz), 5.06 (m, H-C_{10}, H-C_6), 5.43 (t, H-C_2, ^3J= 6 Hz); ^{13}C NMR: 15.6, 15.9, CH_3-C_3, CH_3-C_7, 26.0 CH_3-C_{11}, 26.4, 25.3 C_5, C_9, 39.3, 39.4 C_4, C_8, 58.8 C_1, 123.5 C_2, 124 C_6, C_{10}, 131.1 C_{11}, 135.1 C_7, 139.5 C_3.

4b: ^1H NMR: 1.1 (m, OH), 1.59 (s, CH_3-C_{12}, CH_3-C_{11}), 1.67, 1.73 (2s, CH_3-C_7, CH_3-C_3), 1.93-2.14 (m, 4 CH_2-CH_2), 4.15 (dt, H-C_1, ^3J= 6 Hz, $^1J(^{13}C, H)$= 142 Hz), 5.07 (m, H-C_{10}, H-C_6), 5.4 (t, H-C_2, ^3J= 6 Hz); ^{13}C NMR: same spectra as **4a**; 59.2 C_1.

(2*Z*,6*E*)-[1-^{13}C] 1-Bromo-3,7,11-trimethyl-2,6,10-dodecatriene: ((2*Z*,6*E*)-[1-^{13}C] Farnesyl bromide): 5a:

To a solution of **4a** (0.23 g, 1.03 mmol) in diethyl ether (5 mL) containing 2 drops of pyridine cooled at -5°C was added PBr_3 (0.14 mL, 1.47 mmol) and the solution stirred for 2h. The reaction mixture was poured onto ice-water (20 mL) and extracted with diethyl ether (3x20 mL). The combined organic extracts were washed with brine (20 mL) and dried over $MgSO_4$ and evaporated to give 0.28 g of **5a** as an oil which was used in the next step, without further purification. ^{1}H NMR: 1.58 (s, CH_3-C_{11}), 1.59 (s, CH_3-C_{12}), 1.66 (s, CH_3-C_7), 1.75 (s, CH_3-C_3), 1.96-2.16 (m, CH_2-CH_2), 3.99 (dd, CH_2-C_1, ^{3}J= 8.4 Hz, ^{1}J(^{13}C, H) = 153 Hz), 4.77 (m, H-C_{10}, H-C_6), 5.39 (t, H-C_2, ^{3}J = 8.4 Hz). ^{13}C NMR: 16.2 CH_3-C_3, 17.2 CH_3-C_7, 26.4 CH_3-C_{11}, 28.6 C_1, 39.3 C_4, C_8, 120.3 C_2, 123.1 C_6, C_{10}, 131.1 C_{11}, 135.4 C_7, 143.3 C_3.

(2*E*,6*E*)-[1-^{13}C] 1-Bromo-3,7,11-trimethyl-2,6,10-dodecatriene: ((2*E*,6*E*)-[1-^{13}C] Farnesyl bromide): 5b:

The reaction conditions are the same as described above. From 0.37 g of **4b**, 0.448 g of **5b** was obtained. ^{1}H NMR: 1.6 (br s, CH_3-C_{11}, CH_3-C_{12}), 1.67 (s, CH_3-C_7), 1.72 (s, CH_3-C_3), 1.94-2.14 (m, 4 CH_2-CH_2), 4.0 (dd, CH_2-C_1, ^{3}J = 8.4 Hz, ^{1}J(^{13}C, H) = 153 Hz), 5.1 (m, H-C_{10}, H-C_6), 5.5 (t, H-C_2, ^{3}J = 8.4 Hz). ^{13}C NMR: same spectra as **5a**; 29.4 C_1.

Trimethyltinlithium: 8 (12)

To a suspension of lithium (0.121g, 17.3 mmol) in THF (10 mL) cooled at 5°C was slowly added a solution of trimethyltinchloride (0.344 g, 1.73 mmol) in THF (2 mL) such that the temperature was below 5°C. After 3h with stirring at 5°C, the green

solution was filtered on glass wool under nitrogen atmosphere, washed with THF (8 mL) and directly used in the next step.

Trimethyl-((2*Z*,6*E*)-3,7,11-trimethyl-[1-^{13}C] 2,6,10-dodecatrienyl)-stannane: ((2*Z*,6*E*)-[1-^{13}C] Farnesyltrimethyltin): 6a:

To a solution of trimethyltinlithium (0.54 mmol) in THF (6 mL) prepared as described above and cooled at -60°C was added **5a** (0.128 g, 0.45 mmol). The solution was allowed to warm to room temperature with stirring for 2h. The reaction mixture was cooled again at -20°C, and cold saturated NH_4Cl solution (10 mL) was added. After extraction of the reaction mixture with diethyl ether (3x20 mL), the combined organic extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by chromatography on a reversed phase C18 with acetonitrile as eluent to give 0.14 g of **6a** as a colorless oil. (yield: 58%). ^{1}H NMR: 0.06 (m, CH_3-Sn, J^{117}Sn= 51 Hz, J^{119}Sn = 55 Hz), 1.60 (br s, CH_3-C_{12}, CH_3-C_{11}), 1.68 (br s, CH_3-C_7, CH_3-C_3), (dd, H-C_1, ^{1}J(H, ^{13}C) = 129 Hz, ^{3}J(H, H) = 8.6 Hz), 1.95-2.05 (m, 4 CH_2-CH_2), 5.12 (m, H-C_{10}, H-C_6), 5.27 (m, H-C_2); ^{13}C NMR: 9.8 CH_3-Sn, 12.1 C_1, 15.7 C_3, 17.4 C_7, 25.4 CH_3-C_{11}, 6.6, 26.8 C_5, C_9, 39.4 C_4, C_8, 120.4 C_2, 124.2 C_6, C_{10}, 129.6 C_{11}, 130.7 C_7, 134.3 C_3; ^{119}Sn NMR (C_6D_6): -2.2 (internal reference: $(CH_3)_4Sn$); -109.7 for $((CH_3)_3Sn)_2$.

Trimethyl-((2*E*,6*E*)-3,7,11-trimethyl-[1-^{13}C] 2,6,10-dodecatrienyl)-stannane: ((2*E*,6*E*)-[1-^{13}C] Farnesyltrimethyltin): 6b:

The reaction conditions were the same as described above, from 0.428 g of **5b**, 0.295 g of **6b** was obtained, yield: 59%. ^{1}H NMR: 0.05 (m, CH_3-Sn), 1.55 (s, CH_3-C_3), 1.59 (s, CH_3-C_7, CH_3-C_{12}), 1.67 (m, CH_2-C_1, CH_3-C_{11}), 1.9-.2.06 (m, 4 CH_2-CH_2), 5.07 (m, H-C_{10}, H-C_6), 5.3 (t, H-C_2, ^{3}J = 7 Hz); ^{13}C NMR: 12.2 C_1; same spectra as **6a**.

(2'*Z*,6'*E*)-3,7,11-Trimethyl-[1'-^{13}C] 2,6,10-dodecatrienyl-5,6-dimethoxy-3-methyl-2,5-cyclohexadien-1,4-dione: ((2'*Z*,6'*E*)-[1'-^{13}C] Ubiquinone 3): 7a:

$BF_3.OEt_2$ (75 µL, 0.59 mmol) was added to a solution of **9** (35 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) cooled at -78°C. A solution of **6a** in CH_2Cl_2 (2 mL) was slowly added and stirred for 1h. The reaction mixture was acidified with 2N HCl (10 mL) and extracted with diethyl ether (3x20 mL). The combined extracts were evaporated, washed with H_2O (20 mL) and dried over saturated brine (20 mL). The organic phase was treated with Ag_2O (100 mg) for 1h with vigorous stirring, filtered and dried over $MgSO_4$. After evaporation of the organic layer, the crude product was purified on a silicagel column with hexane/ethyl acetate: 9:1 as eluent to give 52 mg of **7a** (yield= 89%). HPLC: column Partisil, eluent: hexane/diethyl ether: 95:5, flow rate: 1.5 mL/min, retention time: 25 min 35, **7a:7b**: 96/4; IR (NaCl): 2925, 2853, 1650, 1610, 1451, 1377, 1263, 1203, 1152, 1099; ^{1}H NMR: 1.58 (s, CH_3-$C_{12'}$), 1.61 (s, CH_3-$C_{11'}$), 1.66 (br s, CH_3-$C_{7'}$, CH_3-$C_{3'}$), 1.95-2.19 (m, 4 CH_2-CH_2), 2.0 (s, CH_3-C_3), 3.17 (dd, H-$C_{1'}$, ^3J = 6.9 Hz, $^1J(^{13}C, H)$ = 129 Hz), 3.96 (s, CH_3O-C_5), 3.97 (s, CH_3O-C_6), 4.91 (m, H-$C_{2'}$, ^3J = 3.6 Hz), 5.08, 5.15 (2m, H-$C_{6'}$, H-$C_{10'}$); ^{13}C NMR: 11.7 CH_3-C_3, 16.1 CH_3-$C_{7'}$, 25.0 CH_2-$C_{1'}$, 26.2 CH_3-$C_{11'}$, 39.4 $C_{4'}$, $C_{8'}$, 60.8 CH_3O, 118.7 $C_{2'}$, 123.6 $C_{6'}$, $C_{10'}$, 131 $C_{11'}$, 134.9 $C_{7'}$, 137.3 $C_{3'}$, 138.6 C_3, 141.4 C_2, 144 C_5, 144.1 C_6, 163.6 C_1, 164.5 C_4; HRMS: $C_{24}H_{34}O_4$: Calcd. 387.2488; Found: 387.2493; EI-MS: isotopic enrichment: 94%.

(2'*E*,6'*E*) -3,7,11-Trimethyl- [1'-^{13}C] 2,6,10-dodecatrienyl-5,6-dimethoxy-3-methyl-2,5-cyclohexadien-1,4-dione: ((2'*E*,6'*E*)-[1'-^{13}C] Ubiquinone 3): 7b:

The reaction conditions were the same as described above, from 0.23 g of **6b**, 0.275 g

of **7b** was obtained, yield: 88%. HPLC: same conditions as **7a**, retention time: 29 min 40, **7b**:**7a**: 97/3; IR (NaCl): 2924, 2853, 1740, 1649, 1611, 1450, 1380, 1264, 1203, 1152, 1100; ^{1}H NMR: 1.55 (s, CH_3-$C_{11'}$), 1.57 (s, CH_3-$C_{12'}$), 1.65 (s, CH_3-$C_{7'}$), 1.72 (s, CH_3-$C_{3'}$), 1.97-2.13 (m, 4 CH_2-CH_2), 2.0 (s, CH_3-C_3), 3.15 (dd, CH_2-$C_{1'}$, ^{3}J=7 Hz, ^{1}J(^{13}C, H)=135 Hz), 3.95 (s, CH_3O-C_5), 3.96 (s, CH_3O-C_6), 4.9 (t, H-$C_{2'}$, ^{3}J= 7 Hz), 5.05 (m, H-$C_{6'}$, H-$C_{10'}$); ^{13}C NMR: 25.1 CH_2-$C_{1'}$; same spectra as **7a**; HRMS: $C_{24}H_{34}O_4$: Calcd. 387.2488; Found: 387.2588; EI-MS: isotopic enrichment: 94%.

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