

BIOSYNTHESIS OF VINEOMYCINS A<sub>1</sub> AND B<sub>2</sub>

NOBUTAKA IMAMURA, KATSUMI KAKINUMA and NOBUO IKEKAWA\*

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology,  
Nagatsuta, Midori-ku, Yokohama, 227, Japan

HARUO TANAKA and SATOSHI ŌMURA\*

School of Pharmaceutical Sciences, Kitasato University and  
The Kitasato Institute, Minato-ku, Tokyo, 108, Japan

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Biosynthetic studies of the antibacterial and antitumor antibiotics vineomycins A<sub>1</sub> (**1**) and B<sub>2</sub> (**2**), produced by *Streptomyces matensis* subsp. *vineus*, were carried out by labeling experiments with [1-<sup>13</sup>C]- and [1,2-<sup>13</sup>C<sub>2</sub>]sodium acetate followed by <sup>13</sup>C NMR spectroscopy. The results show that the benz[a]anthraquinone chromophore of **1** is derived from a decacetate metabolite with decarboxylation at the carboxyl end and that **2** is formed *via* C-C bond cleavage of **1**. Isolation of rabelomycin from the fermentation broth of the same strain suggests a close biosynthetic relationship among the simple benz[a]anthraquinone antibiotics such as rabelomycin, tetrangomycin, aquayamycin, a C-glycosylated benz[a]anthraquinone, and vineomycins. These biosynthetic data prompted us to reconsider the previously published structure of the antibiotic SS-228Y, which has now been revised.

Vineomycins (A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>) produced by *Streptomyces matensis* subsp. *vineus* are new antibiotics, active against Gram-positive bacteria and Sarcoma 180 solid tumor in mice.<sup>1)</sup> The structures of vineomycins A<sub>1</sub> (**1**)<sup>2)</sup> and B<sub>2</sub> (**2**)<sup>3)</sup> have recently been reported, the former being a new type of glycosylated benz[a]anthraquinone (Fig. 1). Thus far, seven antibiotics which have a benz[a]anthraquinone skeleton similar to the chromophore of **1** have been described, *i.e.*, aquayamycin (**3**),<sup>4)</sup> rabelomycin (**4**),<sup>5)</sup> ochromycinone,<sup>6)</sup> tetrangulol,<sup>7)</sup> tetrangomycin,<sup>7)</sup> yonomycin<sup>8)</sup> and SS-228Y (**5**).<sup>9)</sup> All of them with the exception of **5**, which is elaborated by a marine microorganism,<sup>10)</sup> have been reported to have a methyl group at C-3 and an oxygen functionality at C-1. The biosynthesis of these antibiotics is likely to be similar to that of the chromophore of **1** since coproduction of **4** was observed in the culture of the vineomycin producer *Streptomyces matensis* subsp. *vineus* as described in the Experimental section.

As no biosynthetic studies on the benz[a]anthraquinone antibiotics have appeared and the chromophore of **1** and **2** suggests a close structural biosynthetic relationship, we initiate an investigation of the biosyntheses of **1** and **2** by means of the <sup>13</sup>C labeling technique.

Fig. 1. The structures of vineomycins and related compounds.

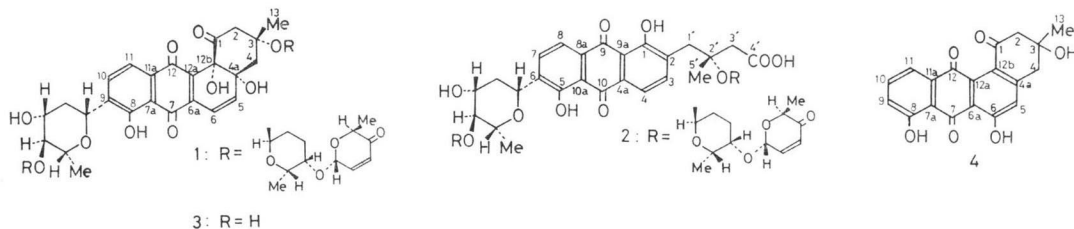
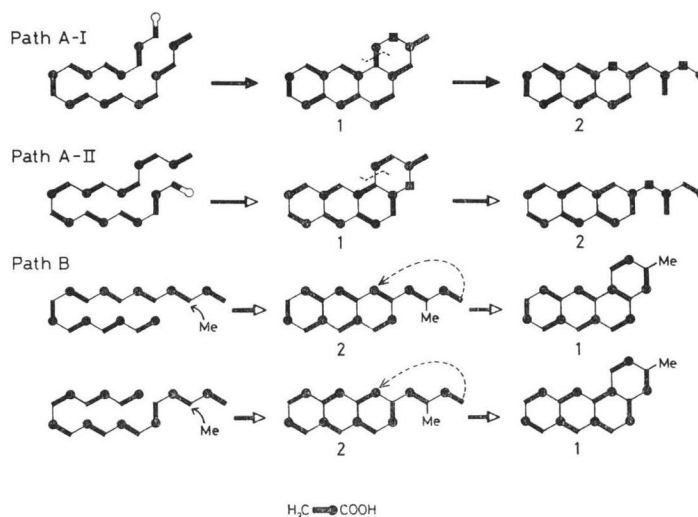


Fig. 2. Possible biosynthetic patterns of the chromophores of vineomycin A<sub>1</sub> (1) and B<sub>2</sub> (2).

The structural relationship of **1** and **2** implies that either the chromophore of **1** or that of **2** is the precursor of the other one. Thus, the chromophore of **1** may be formed first and then converted to that of **2** by ring opening, or the chromophore of **2** may be biosynthesized first from a polyacetate and a C<sub>1</sub> donor and then it is converted to the chromophore of **1** by ring closure, as shown in Fig. 2 (pathway A or B, respectively). The former hypothesis seems more likely than the latter one since the chromophore of **1** is presumed to be derived from a simple polyacetate molecule, while pathway B requires a more complex process. If A is the correct pathway carbon 19 of the chromophore of **1** suggests loss of one carbon atom from a putative polyacetate molecule containing 20 carbon atoms, probably by decarboxylation *via* either pathway A-I or A-II (Fig. 2). To clarify the biosynthetic pattern, feeding experiments were undertaken by addition of either [1-<sup>13</sup>C]- or [1,2-<sup>13</sup>C<sub>2</sub>]sodium acetate to a growing culture of *Streptomyces matensis* subsp. *vineus*. The resultant <sup>13</sup>C-enriched antibiotic was extracted from the culture and purified by repeated column chromatography and preparative thin-layer chromatography on silica gel. Since the yield of labeled **1** with [1,2-<sup>13</sup>C<sub>2</sub>]acetate as precursor was insufficient, the biosynthetic pathway of **2** was analyzed first by <sup>13</sup>C NMR experiments.

The <sup>13</sup>C NMR spectrum of labeled **2** with [1,2-<sup>13</sup>C<sub>2</sub>]acetate showed enriched signals of all 19 carbons in the chromophore of **2**, indicating that **2** is derived from a decacetate. Sixteen signals were flanked by satellite signals due to the <sup>13</sup>C-<sup>13</sup>C splitting of doubly-labeled acetate units incorporated intact and the remaining 3 singlet signals ( $\delta_c$  44.4, 161.5 and 174.0 ppm) were obviously enriched by <sup>13</sup>C incorporation because their signal heights were *ca.* two to three times higher than those of the non-enriched glycoside moieties. These singlet signals suggest cleavages of at least two bonds of [<sup>13</sup>C-<sup>13</sup>C]acetate units during the biosynthesis of the chromophore of **2**. To reveal the sites of the cleavages, the full <sup>13</sup>C NMR assignment of the chromophore of **2** was carried out. The characteristic chemical shifts in the proton noise decoupled spectrum and the multiplicities in the off-resonance spectrum permitted the assignment of five carbon signals, *i.e.*, one methyl carbon ( $\delta_c$  23.2 ppm, C-5'), one non-protonated oxygen-bearing sp<sup>3</sup> carbon ( $\delta_c$  77.2 ppm, C-2'), one carboxylic acid carbon ( $\delta_c$  174.0 ppm, C-4') and two overlapping quinone carbonyl carbons ( $\delta_c$  187.7 ppm, C-9 and C-10). Since the anthraquinone nucleus of **2** is C<sub>2</sub>-symmetrical, the carbon signals of the nucleus tend to appear as pairs, and once one of the paired signals

Table 1.  $^{13}\text{C}$  NMR assignment of the chromophore of **1** and the results of feeding experiments.

Carbon No. <sup>a</sup>	$\delta_{\text{C}}^{\text{b}}$	Carbon No. <sup>a</sup>	$\delta_{\text{C}}^{\text{b}}$
1	203.6*	11	118.4
2	48.0	12	181.0
3	78.8*	13	24.4
4	43.3	4a	81.2*
5	144.4	6a	137.3
6	116.2*	7a	112.9
7	187.1*	11a	129.3*
8	156.8*	12a	137.8*
9	137.6	12b	76.2
10	132.5*		

<sup>a</sup> The carbon numbering is as shown in Fig. 1.

<sup>b</sup> The signals with asterisk were labeled by  $[1-^{13}\text{C}]$ sodium acetate.

Table 2.  $^{13}\text{C}$  NMR assignment of the chromophore of **2** and the results of feeding experiments.

Carbon No. <sup>a</sup>	$\delta_{\text{C}}^{\text{b}}$	$J_{\text{CC}}$ Hz <sup>c</sup>	Carbon No. <sup>a</sup>	$\delta_{\text{C}}^{\text{b}}$	$J_{\text{CC}}$ Hz <sup>c</sup>
1	161.5	E.S. <sup>d</sup>	4a	131.5	55.2 <sup>e</sup>
2	134.9*	44.9	8a	131.7*	61.3 <sup>e</sup>
3	139.6	55.8 <sup>e</sup>	9a	115.2*	54.6 <sup>e</sup>
4	118.3*	55.2 <sup>e</sup>	10a	115.2	64.4
5	158.9*	64.4	1'	38.6	44.3
6	138.4	60.7	2'	77.2*	38.1
7	133.2*	60.7	3'	44.4	E.S. <sup>d</sup>
8	119.3	62.0 <sup>e</sup>	4'	174.0*	E.S. <sup>d</sup>
9	187.7	54.3 <sup>e</sup>	5'	23.2	39.6
10	187.7*	54.3 <sup>e</sup>			

<sup>a</sup> The carbon numbering is as shown in Fig. 1.

<sup>b</sup> The signals with asterisk were labeled by  $[1-^{13}\text{C}]$ sodium acetate.

<sup>c</sup> The coupling constants were obtained from the  $[1,2-^{13}\text{C}_2]$ sodium acetate feeding.

<sup>d</sup> Enriched carbon signal appeared as singlet.

<sup>e</sup> The coupling pair was confirmed by  $^{13}\text{C}$ - $\{^{13}\text{C}\}$  decoupling experiments.

is assigned, the other one of the pair can be assigned easily. The coupling constants and  $^{13}\text{C}$ - $\{^{13}\text{C}\}$  decoupling experiments of labeled **2** with  $[1,2-^{13}\text{C}_2]$ acetate further allowed us to assign all carbon signals as shown in Table 2. As a result, the remaining two  $^{13}\text{C}$ -enriched singlet signals at 161.5 and 44.4 ppm were attributed to C-1 and C-3', respectively. This indicates that the cleavages of acetate units take place at C-1, C-3' and C-4'. Since the C-4' signal was enriched, whereas those at C-1 and C-3' were not, by the  $[1-^{13}\text{C}]$ sodium acetate feeding (as summarized in Table 2), it was concluded that C-1 and C-3' were derived from the C-2 carbon of acetate. If C-3' and C-4' were derived from a single acetate molecule, the signals of C-3' and C-4' should be split by their coupling. It is, therefore, likely that C-1 and C-4' originated from the same acetate molecule provided that **2** is formed from a simple decacetate. In other words, the chromophore of **2** may be derived from that of **1** by the cleavage of the bond between C-12b and C-1 of **1** via pathway A-I (Fig. 2). This was further confirmed by the analysis of the  $^{13}\text{C}$  NMR spectrum of labeled **1**.

The  $^{13}\text{C}$  NMR signals of the chromophore of **1** were assigned as shown in Table 1 by a comparison with those of **3**.<sup>2)</sup> Since 9  $^{13}\text{C}$ -enriched signals were observed in the  $^{13}\text{C}$  NMR spectrum of **1** labeled with  $[1-^{13}\text{C}]$ acetate, it is likely that the chromophore of **1** is biosynthesized from a decacetate by decarboxylation. The labeling pattern of the chromophore of **1** enriched with  $[1-^{13}\text{C}]$ acetate was as expected from that of **2**, (pathway A-I). That is, the enriched signals of C-1, C-3, C-4a, C-6, C-7, C-8, C-10, C-11a and C-12a of **1** were quite compatible with the enrichment at C-4', C-2', C-2, C-4, C-10, C-5, C-7, C-8a and C-9a of **2**, respectively. On the other hand, although the minute amount of labeled **1** available from the  $[1,2-^{13}\text{C}_2]$ acetate experiment did not permit us to obtain all of the coupling constants, the coupling constants were estimated for at least two pairs, *i.e.*, between C-5 and C-6 ( $J_{\text{CC}}=67$  Hz) and between C-11 and C-11a ( $J_{\text{CC}}=63$  Hz). This suggests that the mode of elongation of the decacetate was the same as that of **2**. The site of decarboxylation was determined to be at C-2, because C-2 appeared as only

one enriched singlet signal, enrichment of which was confirmed by its peak height (2.1 times higher than that of the center peak of the C-4 triplet). The mode of elongation of the decacetate intermediate was further supported by the fact that the signals of C-1 ( $J_{CC}$  undetermined) and C-12b ( $J_{CC}=36$  Hz) were observed as triplets and the coupling constant of the latter was apparently different from that of C-12a ( $J_{CC}=60$ Hz). This indicates that C-12a and C-12b carbons are derived from different acetate molecules, and that C-1 and C-12b carbons originate from the same one.

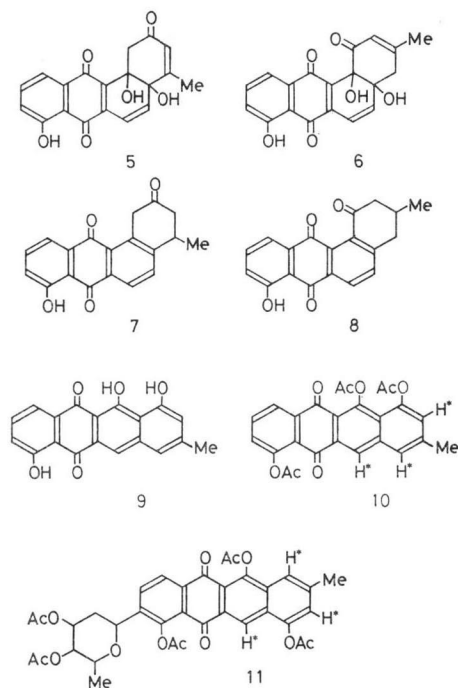
As a result, it was concluded that the biosynthesis of **1** and **2** proceeds *via* pathway A-I as shown in Fig. 2. Thus, **1** is biosynthesized from a decacetate whose starting acetate unit is C-13 and C-3, and the decarboxylation occurs at C-2. After the construction of the benz[a]anthraquinone skeleton, the bond between C-1 and C-12b is cleaved to give the chromophore of **2**. The finding that **4** was isolated together with **1** and **2**

from the same fermentation broth as described above suggests that all of the known benz[a]anthraquinone antibiotics are biosynthesized by a similar decacetate pathway. However, the biosynthesis of **5**, which is produced by a strain of *Chainia* isolated from a rather unique source of sea mud,<sup>10)</sup> cannot be explained by the afore mentioned pathway, because it has been reported to possess a 4-methyl-2-oxo-benz[a]anthraquinone skeleton. If this is the case, the biosynthesis of SS-228Y is presumably quite different from that of the other benz[a]anthraquinone antibiotics. This curious finding prompted us to reconsider the structure of **5**. We suggest that the structure of **5** be revised to **6** for the reasons stated below.

It was reported that hydrogenation of SS-228Y, followed by heating with *p*-toluenesulfonic acid, gave a dehydrated product, the structure of which was assigned as **7** in Fig. 3, mainly from the  $^1\text{H}$ - $\{^1\text{H}\}$ -NOE and lanthanide induced shift (LIS) experiments.<sup>9)</sup> The proximity of the aromatic proton on C-5 and a methine proton on C-4 was deduced from the enhancement of the aromatic proton signal upon saturation of the methine resonance. However, the methine proton signal was actually obscured by signals of some methylene protons in the  $^1\text{H}$  NMR spectrum. The NOE enhancement may therefore be due to saturation of a methylene proton instead. The location of a ketone functionality had been assigned by the LIS experiment. In the original study addition of a shift reagent  $\text{Eu}(\text{fod})_3$  induced downfield shifts of the signals at  $\delta_{\text{H}}$  2.5~3.1 ppm, but no details were described except for the one double-doublet signal of a proton of one methylene group adjacent to the above mentioned methine group. If the proposed structure **7** is correct, protons of both methylene groups (C-1 and C-3) should be similarly shifted downfield.

Secondly, since the C-1 methylene protons of structure **7** must be greatly deshielded by the adjacent

Fig. 3. The structures of SS-228Y and related compounds.



aromatic ring and the C-2 carbonyl group, the  $^1\text{H}$  NMR chemical shifts of the C-1 methylene protons would be expected around  $\delta_{\text{H}}$  3.5 ppm as reported for  $\beta$ -tetralone ( $\delta_{\text{CH}_2}$  3.5 ppm).<sup>11)</sup> However, no signal was observed at the expected region in the published spectrum.<sup>9)</sup> This implies that there is no methylene group between the aromatic ring and the ketone.

Thirdly, the IR absorption peaks of the carbonyl groups of the dehydrated product were reported to be at 1700 (ketone), 1665 (non-chelated quinone) and 1630  $\text{cm}^{-1}$  (chelated quinone). As cited in the footnote of the original paper,<sup>9)</sup> the first absorption seems to be rather low for a non-conjugated carbonyl group in a six-membered ring. On the contrary, the frequency is quite reasonable when the carbonyl group is conjugated to the aromatic ring.

These considerations suggested a different structure **8** for the dehydrated product. We then realized that the structure **8** is identical with that reported for ochromycinone.<sup>9)</sup> This was confirmed by the consistency of the published data ( $^1\text{H}$  NMR, UV and IR data) of the dehydrated product of SS-228Y with those of ochromycinone.

The structure **9** assigned for the photochemically or thermally rearranged product, SS-228R, had been also deduced by the extensive  $^1\text{H}$ - $\{^1\text{H}\}$ -NOE experiments.<sup>9)</sup> Somewhat surprising, however, was the finding that the chemical shifts of the asterisked aromatic protons ( $\delta_{\text{H}}$  8.74, 7.80 and 7.37 ppm) of the SS-228R triacetate (**10**) were almost identical with those ( $\delta_{\text{H}}$  8.67, 7.74 and 7.34 ppm) of compound **11**, prepared from aquayamycin by  $\text{Ba}(\text{OH})_2$  treatment and acetylation,<sup>4)</sup> despite their distinct structural differences in the aromatic moieties.<sup>4,9)</sup> This fact would rather suggest their structural similarity.

Although we cannot rationalize the results of the NOE experiments on the rearranged product and its derivatives, the above discussions strongly suggest that the structure of SS-228Y should be revised to the biosynthetically acceptable **6**.

Therefore, all of the benz[a]anthraquinone skeletons known so far seem to be biosynthesized *via* pathway A-I. Concerning the biosynthesis of the rest of the molecule of **1** and **2**, the highly deoxygenated sugar moieties of **1** and **2** are presumably derived from glucose and the common pyran ring attached to the chromophore of **1**, **2** and **3** is considered to be biosynthesized also from glucose because a similar C-glycosylated moiety of an antibiotic granaticin was reported to be derived from glucose *via* a 6-deoxy-hex-4-ulose type intermediate.<sup>12)</sup>

## Experimental

Proton and carbon magnetic resonance spectra were recorded with a JEOL FX-90, FX-100 and/or FX-400 spectrometer, using deuteriochloroform as solvent. The chemical shifts are shown as  $\delta$  ppm downfield from tetramethylsilane as internal standard. IR Spectra were recorded with a Hitachi model 260-10 spectrophotometer. UV-Visible spectral data were obtained with a Shimadzu UV-200 double beam spectrophotometer. Mass spectral data were obtained with a Hitachi M-80 spectrometer. The  $^{13}\text{C}$ -labeled compounds were purchased from Merck, Sharp and Dohme, Canada Ltd.

### Feeding Experiments

A loopful of mycelia and spores of *Streptomyces matensis* subsp. *vineus* grown on a KRAINSKY'S agar medium was transferred into a Sakaguchi flask containing 100 ml of a medium composed of 0.2% glucose, 0.5% peptone, 0.3% dried yeast, 0.5% meat extract, 0.5% NaCl and 0.3%  $\text{CaCO}_3$  (the pH was adjusted to pH 7.0 with 2 N NaOH before autoclaving). The flask was incubated at 27°C for 2 days on a reciprocal shaker to give a seed culture. The seed culture (2 ml aliquot) was transferred into 20 Sakaguchi flasks each containing 100 ml of a production medium (pH 7.0) composed of 1.0% glucose, 0.5% peptone, 0.3% dried yeast, 0.5% meat extract, 0.5% NaCl, 0.3%  $\text{CaCO}_3$  and 0.005% Adekanol LG-

109 (antifoaming agent, Adeka Co., Ltd.). After 1-day incubation at 27°C, 2 ml of 5.0% aq. solution of either [ $1\text{-}^{13}\text{C}$ ]sodium acetate (90 atom % enriched) or [ $1,2\text{-}^{13}\text{C}_2$ ]sodium acetate (90 atom % enriched) was added to the culture and then the incubation was continued for an additional 2 days. The resulting  $^{13}\text{C}$ -enriched antibiotics were extracted with ethyl acetate (2.0 liters) from the culture medium (2.0 liters) and purified by repeated silica gel column and preparative thin-layer chromatography as described previously.<sup>11</sup> The yields of labeled **1** and **2** were 5 mg, respectively from [ $1\text{-}^{13}\text{C}$ ]sodium acetate and 2 mg and 7 mg respectively from [ $1,2\text{-}^{13}\text{C}_2$ ]sodium acetate.

#### Isolation of **4**

The culture broth (60 liters) of *Streptomyces matensis* subsp. *vineus*, obtained by 3-day incubation in two 50-liter jars, was extracted with ethyl acetate (35 liters), and the extract was concentrated *in vacuo* to give a yellowish brown crude paste (5.2 g). The paste was subjected to column chromatography on 500 g of silica gel (Merck, 70~230 mesh, in a column of 5×50 cm) with chloroform - methanol (50:1~30:1) to give 1.2 g of vineomycin B<sub>2</sub> enriched fraction. Further column chromatography of this fraction on 200 g of silica gel (Merck, 70~230 mesh, in a column of 3×80 cm) with chloroform - ethyl acetate (5:2) gave a brown amorphous powder (352 mg). Thin-layer chromatography of the powder on silica gel 60 F<sub>254</sub> (Merck, 0.25 mm thickness) with chloroform - ethyl acetate (1:1) showed a spot corresponding to that of vineomycin B<sub>2</sub> and another yellowish spot which was slightly less polar than vineomycin B<sub>2</sub>. The less polar band was purified by preparative thin-layer chromatography on silica gel 60 F<sub>254</sub> (Merck, 0.5 mm thickness, 20×20 cm, 10 plates) developed three times using benzene - ethyl acetate (2:1) as solvent. The appropriate band was scraped and extracted with acetone. The filtered extract was evaporated to dryness to afford 50 mg of **4**, which was recrystallized from benzene - *n*-hexane and then from benzene - methanol to give 7 mg of yellowish needles; mp 189~190°C (dec.); MS: *m/z* 338 (M<sup>+</sup>); IR(KBr)  $\nu_{\text{C=O}}$  1703, 1680 and 1640 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\text{max}}$  228 ( $\epsilon$  27,100), 267 (27,800) and 432 nm (8,700); <sup>1</sup>H NMR (400 MHz)  $\delta$  1.46 (3H, s), 2.95 (1H, s), 2.97 (1H, s), 3.05 (2H, s), 6.94 (1H, s), 7.23 (1H, dd, *J*=8.3 and 1.3 Hz), 7.59 (1H, dd, *J*=7.7 and 1.3 Hz), 7.67 (1H, t, *J*=8.0 Hz), 11.61 (1H, s) and 12.24 ppm (1H, s); <sup>13</sup>C NMR (25.2 MHz)  $\delta$  29.4 (q), 44.5 (t), 53.8 (t), 71.6 (s), 115.4 (s), 117.0 (s), 120.0 (d), 122.3 (d), 124.3 (d), 129.1 (s), 135.8 (s), 138.0 (d), 138.0 (s), 151.9 (s), 162.1 (s), 163.9 (s), 183.9 (s), 192.6 (s) and 197.8 ppm (s).

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