SYNTHESES OF PROTOPORPHYRIN-IX REGIOSELECTIVELY CARBON-13 LABELLED AT THE ALPHA-VINYL CARBONS

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SUMMARY

A method for transformation of readily available beta-vinyl 99% carbon-13 enriched derivatives of protoporphyrin-IX dimethyl ester (3-5) into the less accessible alpha-vinyl labelled isomers (6-8) is described. The procedure involves thallium(III) promoted vinyl carbon rearrangement, and proceeds through 2,2-dimethoxyethyl (e.g. 10), formylmethyl (e.g. 11), 2-hydroxyethyl (e.g. 12) and 2-chloroethyl (e.g. 13) porphyrins; the rearranged vinyl groups are regenerated from 2-chloroethyl in the last step by treatment with base. No evidence of vinyl carbon scrambling in the sequence is observed, and spectroscopic data of the products are given.

Key Words: Carbon-13 labelling; vinyl groups; protoporphyrin-IX; thallium(III) rearrangement.

INTRODUCTION

Deuterium labelled derivatives of heme, the iron(II) complex of protoporphyrin-IX, have proven to be invaluable in NMR studies of structure/function relationships in heme proteins (1), and in characterization of the phenomenon of heme orientational heterogeneity in native and reconstituted heme proteins (2). Such studies with deuterium labelled compounds are, however, limited to deuterium NMR, or to proton NMR studies of paramagnetic heme proteins because the resonances being studied must be hyperfine shifted from those of the protein envelope.

In order to avoid these difficulties and allow NMR studies of diamagnetic heme proteins, we have recently begun syntheses of protoporphyrins bearing heavily enriched carbon-13 labels in selected positions. It has been shown that the important vinyl groups in protoporphyrin-IX dimethyl ester (1) can be 99% carbon-13 enriched at the

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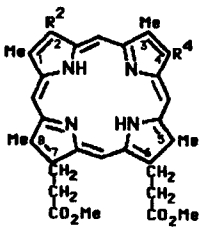
beta-carbons by way of a Wittig reaction on diformyldeuteroporphyrin-IX dimethyl ester (2) (3,4) [to give compound (3)], and this work was extended to afford regioselectively labelled porphyrins (4) and (5) (4). Attempts to obtain alpha-vinyl labelled protoporphyrins-IX (6)-(8) were thwarted by low yields in the obvious approach, Friedel-Crafts acylation of the iron(III) complex of deuteroporphyrin-IX (9), followed by borohydride reduction to hematoporphyrin and dehydration (4). In the present paper we describe an efficient route to compounds (6)-(8) which involves thallium(III) promoted rearrangement of the readily available beta-vinyl labelled porphyrins (3)-(5) (5).

RESULTS AND DISCUSSION

The doubly labelled protoporphyrin-IX dimethyl ester (3) was treated with three equivalents of thallium(III) trinitrate trihydrate (Aldrich) and gave the 2,4-bis-(2,2-dimethoxyethyl)-porphyrin (10). This porphyrin was hydrolyzed in tetrahydrofuran using aqueous hydrochloric acid to give the corresponding dialdehyde (11). Brief treatment with sodium borohydride at 0°C followed by esterification with diazomethane resulted in formation of the bis(2-hydroxyethyl)-porphyrin (12) in 87% yield from (3). Treatment with thionyl chloride gave the 2,4-bis-(2-chloroethyl)-porphyrin (13), and the vinyl groups were regenerated by stirring at 105°C in 3% aqueous potassium hydroxide-pyridine solution. The propionic acid side chains were re-esterified with diazomethane and afforded compound (6).

A similar sequence of reactions was performed on compound (4) to afford (7), and likewise, (5) gave (8). Spectroscopic analysis (Experimental) revealed that there had been no scrambling of the vinyl carbons in the reaction sequence, and that the transformation takes place with total rearrangement of the two-carbon vinyl substituents. Figure 1 shows the proton NMR spectrum of the vinyl region in compound (7); NMR studies with the corresponding reconstituted heme proteins will be described elsewhere.



(1) $R^2 = R^4 = CH{=}CH_2$

(2) $R^2 = R^4 = CHO$

(3) $R^2 = R^4 = CH{=}\overset{*}{C}H_2$

(4) $R^2 = CH{=}\overset{*}{C}H_2$, $R^4 = CH{=}CH_2$

(5) $R^2 = CH{=}CH_2$, $R^4 = \overset{*}{C}H{=}CH_2$

(6) $R^2 = R^4 = \overset{*}{C}H{=}CH_2$

(7) $R^2 = \overset{*}{C}H{=}CH_2$, $R^4 = CH{=}CH_2$

(8) $R^2 = CH{=}CH_2$, $R^4 = \overset{*}{C}H{=}CH_2$

(9) $R^2 = R^4 = H$

(10) $R^2 = R^4 = \overset{*}{C}H_2CH(OMe)$

(11) $R^2 = R^4 = \overset{*}{C}H_2CHO$

(12) $R^2 = R^4 = \overset{*}{C}H_2CH_2OH$

(13) $R^2 = R^4 = \overset{*}{C}H_2CH_2Cl$

* = 99% carbon-13 enrichment

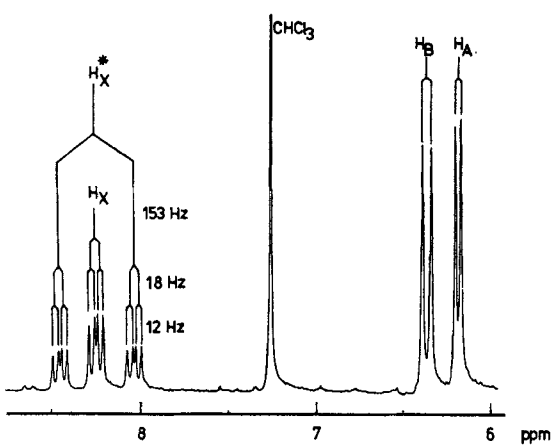


Figure 1: 360 MHz proton NMR spectrum of the vinyl ABX pattern in 2-[$^{13}C_1$]-vinyl protoporphyrin-IX dimethyl ester (7).

EXPERIMENTAL

General: Melting points, which are uncorrected, were measured on a Thomas/Bristoline microscopic hot-stage apparatus. Electronic absorption spectra were measured on a Hewlett-Packard 8450A spectrophotometer. Proton NMR spectra were obtained at 360 MHz on a Nicolet NT-360 spectrometer. Chemical shifts are reported relative to $CHCl_3$ at 7.260 ppm. Monitoring of reactions by thin-layer chromatography was performed on

cut strips (approx. 2 cm by 6 cm) of E. Merck silica gel 60 F254 precoated (0.25 mm thickness) plastic-backed sheets. Column chromatography was carried out using E. Merck neutral alumina (70-230 mesh); the alumina was deactivated with either 6% H_2O (Brockmann Grade III) or 15% H_2O (Brockmann Grade V) before use.

2,4-Bis-(1-^{13}C-2,2-dimethoxyethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin, (10). Doubly beta-vinyl ^{13}C-labeled protoporphyrin-IX dimethyl ester (3) (434 mg) in 100 mL dichloromethane and 15 mL methanol was treated with a solution of thallium(III) trinitrate trihydrate (124 mg) (Aldrich) in 35 mL methanol. After stirring for 12 min at 40° C, and after cooling, sulfur dioxide gas was bubbled through the solution for 1 min. Concentrated hydrochloric acid was added to this mixture and stirred for 2 min. The supernatant was decanted and the precipitated thallium(I) salts were washed with dichloromethane (150 mL). The combined organic layer was washed three times with 150 mL water and, after filtration, was evaporated to dryness under vacuum. The residue was used immediately in the next reaction, but for NMR a small sample was crystallized from dichloromethane/hexane, mp 154-155° C (on block at 140°C), [Lit.(5) mp approx 230°C (dec), unlabelled]. Proton NMR, ppm, -3.80 (br s, 2H, NH), 3.28 (t, 4H, CH_2CO), 3.46 (s, 6H, Me), 3.47 (s, 12H, $CH(OC\underline{H}_3)_2$), 3.66 (s, 6H, OMe), 3.67 (s, 6H, Me), 4.27 and 4.34 (each dd, 4H, J = 4.1, 127.15 Hz, $^{13}C\underline{H}_2CH(OCH_3)_2$), 4.43 (t, 4H, $C\underline{H}_2CH_2CO$), 5.15 (m, 2H, $C\underline{H}(OCH_3)_2$), 10.09, 10.11, 10.16, 10.17 (each s, 1H, meso H).

Similarly prepared:

2-(1-^{13}C-2,2-Dimethoxyethyl)-4-(2,2-dimethoxyethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8- tetramethylporphyrin. From (4), mp 158-159°C. Proton-NMR, ppm, -3.47 (br s, 2H, NH), 3.29 (m, 4H, CH_2CO), 3.47, 3.49, 3.66, 3.68 (each s, 9H, 9H, 6H, 6H, Me, and OMe), 4.38 (dd, 2H, J = 5.34 and 127.28 Hz, $^{13}C\underline{H}_2CH(OCH_3)_2$), 4.38 (d, 2H, J = 5.36 Hz, $C\underline{H}_2CH(OCH_3)_2$), 4.43 (t, 4H, $C\underline{H}_2CH_2CO$), 5.17 (t, 2H, J = 5.35 Hz, $C\underline{H}(OCH_3)_2$), 10.10, 10.12, 10.18, 10.19 (each s, 1H, meso H).

2-(2,2-Dimethoxyethyl)-4-(1-^{13}C-2,2-dimethoxyethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin. From (5), mp 154-155°C. Proton-

NMR, ppm, -3.73 (br s, 2H, NH), 3.29 (t, 4H, CH_2CO), 3.47 (s, 6H, Me), 3.49 (s, 12H, $CH(OC\underline{H}_3)_2$), 3.66 (s, 6H, OMe), 3.68 (s, 6H, Me), 4.39 (dd, 2H, J = 4.79 and 127.10 Hz, $^{13}C\underline{H}_2CH(OCH_3)_2$), 4.39 (d, 2H, J = 4.80 Hz, $C\underline{H}_2CH(OCH_3)_2$), 4.43 (t, 4H, $C\underline{H}_2CH_2CO$), 5.17 (t, 2H, J = 4.80 Hz, $C\underline{H}(OCH_3)_2$), 10.09, 10.12, 10.18, 10.19 (s, 1H, meso H).

2,4-Bis-(1-^{13}C-2-formylmethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin, (11). The foregoing 2,4-diacetalporphyrin (10) was dissolved in tetrahydrofuran containing water (3 mL) and conc. hydrochloric acid (1.5 mL). The mixture was refluxed for 5 min at 70° C, cooled briefly, and diluted with dichloromethane (100 mL). The organic phase was then washed with 100 mL water. To the aqueous phase, 50 mL of brine was added and this was extracted twice with dichloromethane (100 mL). The dichloromethane phases were combined and washed twice with water (100 mL). The solvent was evaporated to give a dark purple residue which was immediately carried through the next procedure. A portion of the product (3 mg) was recrystallized (dichloromethane/hexane) to give purple prisms, mp 143-144°C. Proton-NMR, ppm, -3.67 (br s, 2H, NH), 3.29 (m, 4H, CH_2CO), 3.63, 3.65, 3.66, 3.69 (each s, 3H, 6H, 6H, 3H, Me, and OMe), 4.43 (m, 4H, $C\underline{H}_2CH_2CO$), 5.13 (d, 4H, J = 128.27 Hz, $^{13}C\underline{H}_2CHO$), 9.957, 9.99, 10.146 (br), 10.161 (each s, 1H, meso H), 10.260 (td, 1H, J_{C13-H} = 26.53Hz, CHO), 10.268 (td, 1H, J_{C13-H} = 26.64 Hz, CHO).

Similarly prepared:

2-(1-^{13}C-2-Formylmethyl)-4-(2-formylmethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyporphyrin. Mp 146-147°C. Proton-NMR, ppm, -3.80 (br s, 2H, NH), 3.27 (m, 4H, CH_2CO), 3.596, 3.60, 3.61, 3.63, 3.66 (each s, 3H, 3H, 3H, 3H, 6H, Me, and OMe), 4.39 (t, 4H, $C\underline{H}_2CH_2CO$), 5.05 (d, 2H, J = 128.14 Hz, $^{13}C\underline{H}_2CHO$), 5.05 (s, 2H, $C\underline{H}_2CHO$), 9.87, 9.88, 10.07 (each s, 1H, meso H), 10.088 (d, 1H, J = 4.05 Hz as long range ^{13}C-H coupling, meso H), 10.228 (t, 1H, J = 2.14 Hz, CHO), 10.229 (td, 1H, J_{C13-H} = 25.74 Hz, CHO).

2-(2-Formylmethyl)-4-(1-^{13}C-2-formylmethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin. Mp 148-150°C. Proton-NMR, ppm, -3.72 (br s, 2H, NH), 3.28 (m, 4H, CH_2CO), 3.62, 3.64, 3.645, 3.65 (unresolved s, 12H, 6H, Me, and OMe), 4.41 (t, 4H, $C\underline{H}_2CH_2CO$), 5.09 (d, J = 128.11Hz, $^{13}C\underline{H}_2CHO$), 5.09 (s, 2H,

CH_2CHO), 9.93, 9.96, 10.117 (each s, 1H, meso H), 10.116 (br d, 1H, J = 2.83 Hz as long range ^{13}C-H coupling, meso H), 10.248 (td, 1H, J_{13-H} = 25.82 Hz, CHO), 10.256 (t, 1H, J = 2.13 Hz, CHO).

2,4-Bis(1-^{13}-C-2-hydroxyethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin, (12). The foregoing porphyrin residue (11) was dissolved in 100 mL of tetrahydrofuran/methanol/dichloromethane (30:25:45) and was treated at 0° C with sodium borohydride (1 g) in 30 mL ice cold methanol. The mixture was stirred for 10 min before acetic acid (3 mL) was added to quench the excess borohydride. After dilution with chloroform (100 mL), the organic mixture was washed three times with 200 mL of water, and evaporated to dryness. The residue was briefly treated with ethereal diazomethane, and after careful evaporation, the re-esterified product was chromatographed on neutral alumina (Brockmann Grade V), eluting with 1% methanolic chloroform. The dark purple major band was isolated and evaporated to give a crystalline solid. After recrystallization from dichloromethane/hexane the overall yield from (3) was 398.5 mg (87%), obtained as deep purple prisms, mp 226-227°C [Lit.(5) mp 225-226°C, unlabelled]. The carbon-13 NMR spectrum showed a single enhanced resonance at 30.05 ppm in the proton decoupled mode. Proton-NMR, ppm, -3.73 (br s, 2H, NH), 3.29 (m, 4H, CH_2CO), 3.65, 3.66 (each s, 9H, 9H, Me, and OMe), 4.30 (td, 4H, J = 127 Hz, $^{13}CH_2CH_2OH$), 4.41 (t, 4H, CH_2CH_2CO), 4.47 (distorted t, 4H, CH_2CH_2OH), 10.07, 10.09, 10.095, 10.10 (s, 4H, meso H).

Similarly prepared:

2-(1-^{13}C-2-Hydroxyethyl)-4-(2-hydroxyethyl)-6,7-bis-(2-methoxy-carbonylethyl)-1,3,5,8-tetramethylporphyrin. Mp 215-217°C. Proton-NMR, ppm, -3.75 (br s, 2H, NH), 3.28 (m, 4H, CH_2CO), 3.65, 3.66 (each s, 9H, 9H, Me, and OMe), 4.28 (td, 2H, J = 126 Hz, $^{13}CH_2CH_2OH$), 4.33 (t, 2H, CH_2CH_2OH), 4.41 (t, 4H, CH_2CH_2CO), 4.47 (distorted t, 4H, CH_2CH_2OH), 10.07, 10.09, 10.10 (s, 4H, meso H).

2-(2-Hydroxyethyl)-4-(1-^{13}C-2-hydroxyethyl)-6,7-bis(2-methoxy-carbonylethyl)-1,3,5,8-tetramethylporphyrin. Mp 216-218°C. Proton-NMR, ppm, -3.74

(br s, 2H, NH), 3.28 (t, 4H, CH_2CO), 3.62, 3.63, 3.65, 3.66 (each s, 6H, 6H, 3H, 3H, Me, and OMe), 4.29 (td, 2H, J = 126 Hz, $^{13}C\underline{H}_2CH_2OH$), 4.29 (t, 2H, $C\underline{H}_2CH_2OH$), 4.39 (t, 4H, $C\underline{H}_2CH_2CO$), 4.44 (distorted t, 4H, $CH_2C\underline{H}_2OH$), 10.04, 10.05, 10.058, 10.07 (each s, 1H, meso H).

2,4-Bis-(1-^{13}C-2-chloroethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin, (13). To 345 mg bis(2-hydroxyethyl)porphyrin (12), was added dichloromethane (100 mL), dimethylformamide (30 mL), and anhydrous potassium carbonate (11 g), followed by dropwise addition of thionyl chloride (10 mL). The mixture was stirred for 9.5 h at 25° C. The solution was cautiously poured into dichloromethane (300 mL) and water (300 mL). The organic phase was washed three times with saturated aqueous sodium bicarbonate (300 mL), water (300 mL), and evaporated to dryness. Trace amounts of dimethylformamide were removed under high vacuum (0.01 torr) at 70° C. After brief treatment with ethereal diazomethane and solvent evaporation, the residue was purified by chromatography on neutral alumina (Brockmann Grade III, elution with 20% toluene in dichloromethane). The major band was isolated to give a 62% (224 mg) yield after evaporation and crystallization from dichloromethane/hexane, mp 183-184.5°C (on block at 170°C), [Lit.(6) mp 216-217°C, unlabelled], as deep red prisms. The proton-decoupled carbon-13 NMR spectrum showed a single enhanced signal at 30.18 ppm. Proton-NMR, ppm, -3.79 (br s, 2H, NH), 3.29 (m, 4H, CH_2CO), 3.65, 3.66 (each s, 6H, 12H, Me, and OMe), 4.32 (m, 4H, CH_2Cl), 4.42 (t, 4H, $C\underline{H}_2CH_2CO$), 4.51 (md, 4H, J = 124.56 Hz, $^{13}CH_2CH_2Cl$), 10.01, 10.09, 10.11 (each s, 2H, 1H, 1H, meso H).

Similarly prepared:

2-(1-^{13}C-2-Chloroethyl)-4-(2-chloroethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin. Mp 181.5-182.5°C. Proton-NMR, ppm, -3.75 (br s, 2H, NH), 3.29 (m, 4H, CH_2CO), 3.65, 3.66, 3.67, 3.68 (each s, 6H, 3H, 6H, 3H, Me, and OMe), 4.33 (m, 4H, CH_2Cl), 4.42 (t, 4H, $C\underline{H}_2CH_2CO$), 4.53 (td, 2H, J = 7.63 and 133.75 Hz, $^{13}CH_2CH_2Cl$), 4.54 (t, 2H, J = 7.68 Hz, CH_2CH_2Cl), 10.02, 10.03, 10.11, 10.12 (each s, 1H, meso H).

2-(2-Chloroethyl)-4-(1-^{13}C-2-chloroethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin. Mp 195-196°C. Proton-NMR, ppm, -3.79 (br s, 2H, NH), 3.29 (t, 4H, CH_2CO), 3.64, 3.65, 3.67 (each s, 3H, 9H, 6H, Me, and OMe), 4.31 (m, 4H, CH_2Cl), 4.41 (t, 4H, $C\underline{H}_2CH_2CO$), 4.49 (td, 2H, J = 7.55 and 132.38 Hz, $^{13}CH_2CH_2Cl$), 4.50 (t, 2H, J = 7.48 Hz, CH_2CH_2Cl), 9.99, 10.08, 10.09, 10.10 (s, 1H, meso H).

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-bis-(1-^{13}C--vinyl)porphyrin, (6). The bis-(2-chloroethyl)porphyrin (13) (160 mg) in pyridine (75 mL) was refluxed under nitrogen for 30 min. To this mixture was added water (12 mL) and this was stirred at 105°C for 5 min, before being treated with 3% potassium hydroxide (13.5 mL) solution. The mixture was stirred under nitrogen for an additional 2.5 h at 105°C. After cooling, tetrahydrofuran (100 mL) and dichloromethane (100 mL) were added and the organic phase was washed three times with dilute hydrochloric acid (200 mL, 2M) and finally with water (200 mL). The organic layer was evaporated to dryness and treated briefly with diazomethane. After evaporation of solvent, the residue was chromatographed in the dark on neutral alumina (Brockmann Grade III, elution with 20% toluene in dichloromethane). Recrystallization from methanol/dichloromethane afforded the product (6) in 52.1% (74.1 mg) yield as reddish purple prisms, mp 219-220°C [Lit.(5) mp 228-229°C, unlabelled]. UV-VIS (CH_2Cl_2): λ_{max} 404 (ϵ 167,800), 504 (13,900), 540 (11,200), 576 (6,300), 630 nm (4,800). The carbon-13 NMR spectrum showed a single enhanced resonance at 130.21 ppm in the proton decoupled mode, which split into a doublet (J = 153.26Hz) in the proton coupled mode. Proton-NMR, ppm, -3.79 (br s, 2H, NH), 3.27 (t, 4H, CH_2CO), 3.60, 3.61, 3.66, 3.68, 3.69 (each s, 3H, 3H, 6H,3H,3H, Me, and OMe), 4.39 (t, 4H, $C\underline{H}_2CH_2CO$), 6.18 (d, 2H, J = 11.39 Hz, beta-vinyl CH), 6.36 (d, 2H, J = 17.82 Hz, beta-vinyl CH), 8.257, 8.262 (overlapping

ddd, J = 11.6, 17.7, and 153.26 Hz, alpha-vinyl ^{13}CH), 9.99, 10.03, 10.13, 10.16 (s, 1H, meso H).

Similarly prepared:

6,7-Bis(2-methoxycarbonyethyl)-1,3,5,8-tetramethyl-2-(1-^{13}C-vinyl)-4-vinylporphyrin, (7). Mp 217-218°C. UV-VIS (CH_2Cl_2): λ_{max} 404 (ϵ 160,000), 504 (13,800), 540 (11,100), 576 (6,500), 630 nm (5,000). Proton-NMR, ppm, -3.78 (br s, 2H, NH), 3.27 (t, 4H, CH_2CO), 3.60, 3.67, (each s, 6H, 12H, Me, and OMe), 4.38 (t, 4H, CH_2CH_2CO), 6.18 (d, 2H, J = 11.6 Hz, beta-vinyl CH), 6.36 (d, J = 17.11 Hz, beta-vinyl CH), 8.25 (ddd, 1H, J = 11.5, 17.75, 153.14 Hz, alpha-vinyl ^{13}CH), 8.25 (dd, 1H, J = 11.5 and 17.78Hz, alpha-vinyl CH), 9.99, 10.02, 10.12, 10.15 (s, 1H, meso H). In the proton decoupled carbon-13 NMR spectrum a single enhanced resonance was observed at 130.25 ppm, and this peak was split into a doublet, J = 153.16 Hz, in the proton coupled mode.

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinyl-4-(1-^{13}C-vinyl)porphyrin, (8). Mp 218-219°C. UV-VIS (CH_2Cl_2): λ_{max} 404 (ϵ 166,000), 504 (13,300), 540 (11,300), 574 (7,500), 630 nm (4,800). Proton-NMR, ppm, -3.77 (br s, 2H, NH), 3.27 (t, 4H, CH_2CO), 3.605, 3.61, 3.67, 3.68, 3.69 (each s, 3H, 3H, 6H, 3H, 3H, Me, and OMe), 4.39 (t, 4H, CH_2CH_2CO), 6.18 (d, 2H, J = 11.65 Hz, beta vinyl CH), 6.36 (d, 2H, J = 17.85 Hz, beta-vinyl CH), 8.26 (ddd, 1H, J = 10.7, 17.3, and 152.54 Hz, alpha-vinyl ^{13}CH), 8.26 (dd, 1H, J = 11.65 and 18.34 Hz, alpha-vinyl CH), 10.00, 10.03, 10.13,10.16 (s, 1H, meso H). The proton decoupled carbon-13 NMR spectrum showed an enriched singlet at 130.21 ppm, whereas the proton coupled resonance showed a doublet, J = 153.15 Hz.

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