Synthesis of quaternary ammonium salts containing a polyprenyl substituent

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Quaternization of triethylamine, 4-dimethylaminopyridine, and *N*-methylimidazole with moraprenyl bromide gives the corresponding quaternary ammonium salts.

Key words: moraprenol, moraprenyl bromide, quaternization, tertiary amines, quaternary ammonium salts, isoprenoids, polyprenols.

Lately, genetic therapy is under intensive development. One of the problems of this field is a search for methods of specific and efficient introduction of genetic material into the target cells. In particular, penetration of various compounds into cells is assisted by positively charged lipids. Cationic lipids containing quaternary ammonium groups are used for transfection of various biologically active substances (nucleosides, polynucleotides, peptides, hormones, *etc*.) into the cells of animal origin and plant protoplasts.[1,2] The study of antitumor activity of cationic lipids revealed that the presence of a tetraalkyl quaternary ammonium group is crucial for such an activity. Pyridinium derivatives are yet more active.[3] Compounds containing *N*-methylimidazole group also display high antitumor and antimutagenic activity.[3]

Tetraalkylammonium derivatives, used as detergents in liposomes,[4,5] are of wide application among positively charged lipids. Polyamine lipophilic derivatives proved fairly efficient in the DNA transfection to eucariotic cells.[6,7] Glycerol lipophilic derivatives are under intensive studies, since they display inhibitory activity against neoplastic cells growth, suppress replication of the human immune deficiency virus.[8,9] It turned out that most known cationic lipids are toxic for cells upon increase in their concentration in liposome drugs. A search for nontoxic compounds of this class is under way, therefore, the synthesis and study of new positively charged lipids remain an actual trend of bioorganic chemistry.

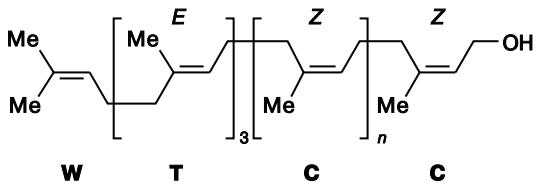
Growing interest to isoprenoid derivatives related to the study of posttranslational prenylation of proteins,[10,11] as well as low toxicity of polyisoprenoids with respect to susceptible cell cultures[12] prompted us to study possible ways for the synthesis of polyprenyl derivatives bearing a positive charge to be used in liposomology. Earlier, schemes for the synthesis of secondary and tertiary polyprenylamines have been developed.[13] It was shown that these compounds display antitumor and antiviral activity, as well as are interferon inductors.[14,15]

There are known only separate examples of the synthesis of quaternary ammonium polyprenyl derivatives. According to the method described earlier,[16] hepta- and nonaprenyltrimethylammonium iodides were obtained. It was found that poly-*Z*(*cis*)-heptaprenyltrimethylammonium iodide is an efficient mediator of transfection to eucariotic cells[17] in contrast to poly-*E*(*trans*)-nonaprenyltrimethylammonium iodide. We have developed a method for the synthesis of moraprenyltriethylammonium chloride allowing us to preserve *Z*-configuration of the terminal α-isoprene unit of the starting polyprenols. It was found that triethylamine is efficiently quaternized in the presence of moraprenol and phosphorus oxychloride.[18]

The present work is devoted to the study of quaternization of tertiary amines, *viz.*, 4-dimethylaminopyridine, triethylamine, and *N*-methylimidazole, in order to develop versatile method for the preparation of quaternary salts containing a moraprenyl substituent.

Results and Discussion

The most available polyprenols are plant prenols present in natural objects as a mixture of oligomerhomologs. Polyprenols from leaves of mulberry tree *Morus alba*, the so-called moraprenol (Mor—OH, WT_3C_{6-8}—OH, **1**), which is a mixture of C_{50}-, C_{55}-, and C_{60}-oligomerhomologs[19] in the ratio 1 : 6 : 4, were used as a prenol component.



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n = 5—7

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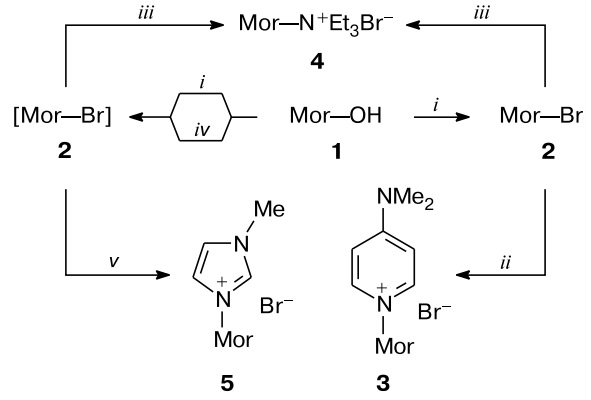
Two alternative approaches were used for the synthesis of polyprenol derivatives bearing a positive charge: with the isolation of a mixture of intermediate polyprenyl bromides (further, moraprenyl bromide) and without their isolation (Scheme 1). Using the first approach, moraprenyl bromide (**2**) was converted into 4-dimethylamino-1-moraprenylpyridinium (**3**) and moraprenyltriethylammonium bromides (**4**).

Scheme 1



Reagents: *i*. Me_3SiBr[20]; *ii*. DMAP; *iii*. Et_3N; *iv*. PBr_3; *v*. 1-methylimidazole.

Earlier, we have developed a method for the synthesis of moraprenyl bromide (**2**)[20] by the reaction of bromotrimethylsilane (Me_3SiBr) with moraprenol (**1**), allowing us to preserve *Z*-configuration of the α-isoprene unit. To obtain salt **3** (see Scheme 1, *ii*), we accomplished the reaction of bromide **2** with a three-fold excess of 4-dimethylaminopyridine (DMAP) in the benzene—acetonitrile mixture, 2 : 3. The reaction was completed within 62 h at 60 °C in the dark under argon (Ar). The target product **3** was obtained in 52% yield. The structure of **3** was confirmed by the ^{1}H NMR spectrum, which contained no signal at δ 3.98 (CH_2—Br) and did contain signals for the =CH—C$\underline{H}_2$N groups at δ 4.62 (α-*Z*-isomers) and δ 4.66 (α-*E*-isomers), the ratio of the signal integral intensities being 25 : 1.

To obtain moraprenyltriethylammonium bromide (**4**), the reaction of **2** with 2.5-fold excess of triethylamine (see Scheme 1, *iii*) was carried out in the benzene—acetonitrile mixture, 1 : 1 (36 h, 45 °C, Ar, in the dark), which led to salt **4** in 51% yield. The structure of **4** was confirmed by the NMR spectroscopic data: the ^{1}H NMR spectrum contained no signal for the starting moraprenyl bromide at δ 3.98 (CH_2—Br) and did contain signals for the =CH—C$\underline{H}_2$N groups at δ 3.67 (α-*Z*-isomers **4**) and δ 3.75 (α-*E*-isomers **4**). The NMR spectroscopic data showed the absence of the products of allylic rearrangement, the ratio α-*Z*- : α-*E*-isomers **4** was 6 : 1.

To study a possibility of an increase in the *Z*-stereoselectivity of the process, a method for the synthesis of **4** without isolation of the intermediate polyprenyl bromides **2** has been developed taken triethylamine as an example. Bromides **2** were generated *in situ* using PBr_3 or Me_3SiBr (see Scheme 1, *i*, *iv*).

In the first case (see Scheme 1, *iv*, *iii*), 1.2 equiv. of PBr_3 were added to a solution of moraprenol (**1**) in heptane (−80 °C). After the reaction mixture was quenched (see Experimental), Et_3N was added and it was stirred for 96 h at 20 °C in the dark (Ar). The ^{1}H NMR spectrum of salt **4** contained no signal for the CH_2OH groups of the starting compound **1** at δ 4.05, rather it contained signals characteristic of the =CH—C$\underline{H}_2$N groups for α-*Z*-isomers **4** at δ 3.67 and α-*E*-isomers **4** at δ 3.75. The yield of salts **4** was 58%, with the ratio α-*Z*- : α-*E*-isomers (7 : 1) remaining virtually the same.

In the second case (see Scheme 1, *i*, *iii*), a solution of moraprenol in heptane was treated with 3 equiv. of Me_3SiBr at −80 °C and kept with Et_3N for 72 h at 60 °C in the dark (Ar). As a result, the yield of salt **4** increased to 77% and the fraction of α-*E*-isomers decreased to 10%.

We used this approach for the synthesis of 1-methyl-3-moraprenylimidazole derivative **5** (see Scheme 1, *i*, *v*), which was obtained by the reaction of moraprenol (**1**) with Me_3SiBr with subsequent addition of 3 equiv. of 1-methylimidazole (18 h at 60 °C (Ar) in the dark). Work-up of the reaction mixture gave rise to product **5** in 90% yield. Its structure was confirmed by the ^{1}H NMR spectrum, which showed the absence of the signal for the CH_2OH groups of the starting moraprenol (**1**) at δ 4.05 and the presence of the signals for the =CH—C$\underline{H}_2$N groups of α-*Z*-isomers **5** at δ 4.67 and α-*E*-isomers **5** at δ 4.70. The ratio of integral intensities of the latter was 24 : 1.

The studies performed showed that the reaction of moraprenyl bromide with tertiary amines leads to the corresponding moraprenyl-substituted quaternary ammonium salts. A developed synthetic method without isolation of rather labile moraprenyl bromide allowed us to significantly increase the yield of moraprenyltriethylammonium bromide (**4**) with certain suppression of the *Z*-*E*-isomerization of the α-isoprene unit. Under conditions developed, heterocyclic amines are quaternized with α-*Z*-stereoselectivity of ~96%.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) and Bruker WM-500 (500.13 MHz for ^{1}H and 125.7 MHz for ^{13}C) spectrometers. Chemical shifts are given relatively to Me_4Si. The reaction progress and isolation of products were monitored by TLC on plates (5×2 cm) with the bound layer of silica gel (Silica Gel 60, Merck, Germany) in the following systems: $CHCl_3$—MeOH—H_2O, 60 : 25 : 4 v/v (A) and $CHCl_3$—MeOH—H_2O, 40 : 10 : 1 v/v (B).

Unsaturated compounds were visualized in the iodine vapors with subsequent treatment with 50% aq. sulfuric acid and charring. Centrifugation was carried out for 10 min at 7000 rpm.

4-Dimethylamino-1-moraprenylpyridinium bromide (3). 4-Dimethylaminopyridine (18.3 mg, 150 μmol) was added to a solution of moraprenyl bromide (**2**) (44 mg, 53 μmol) in the benzene—acetonitrile mixture (2 : 3, 0.5 mL), the reaction mixture was stirred at 60 °C in the dark (Ar). After 62 h, the solvents were evaporated, the residue was treated with heptane (2 mL), shaken, a precipitate was separated by centrifugation. The supernatant was concentrated to dryness, diluted with heptane (1.5 mL), and subjected to another centrifugation. The target product was extracted with acetonitrile (2×1.5 mL, 1 mL). The combined extracts were concentrated to dryness at 20 °C. The yield of salt **3** was 28 mg (52 %), R_f 0.52 (B). ^{1}H NMR (500.13 MHz, $CDCl_3$—CD_3OD, 4 : 1 (v/v)), δ: 1.47 (s, 12 H, Me *E*-units); 1.54 (s, 21 H, Me *Z*-units); 1.80 (s, 3 H, Me—C(3) *Z*-isomers); 1.85—1.93 (m, 36 H, CH_2); 2.03 (m, 4 H, $H_2C(5)$ and CH_2— W-units); 3.11 (s, 6 H, Me—N); 4.62 (d, 1.92 H, $H_2C(1)$ *Z*-isomers, J = 7.6 Hz); 4.66 (d, 0.08 H, $H_2C(1)$ *E*-isomers, J = 7.6 Hz); 4.98 (m, 10.2 H, =CH); 5.21 (t, 1 H, HC(2) *Z*-isomers, J = 7.6 Hz); 6.46 (d, 2 H, HC(3′), HC(5′)), J = 5.0 Hz); 7.91 (d, 2 H, HC(2′), HC(6′), J = 5.0 Hz). ^{13}C NMR (125.7 MHz), δ: 15.7 (Me *E*-units); 17.4 (*cis*-Me W-units); 23.2 (Me *Z*-units); 23.6 (Me—C(3) *Z*-isomers); 24.6 (*trans*-Me W-units); 26.2 (CH_2—CH= *Z*-units); 26.4 (CH_2—CH= *E*-units); 32.0 (CH_2–C(Me)= *Z*-units); 39.5 (CH_2–C(Me)= *E*-units); 39.8 (C(4)); 40.0 (Me—N); 54.9 (C(1)) *Z*-isomers); 108.0 (C(3′), C(5′)); 116.9 (C(2) *Z*-isomers); 124.2 (=CH *Z*-units); 124.8 (=CH *E*-units); 128.6 (Me—C= W-units); 134.7 (Me—C= *E*-units); 135.1 (Me—C= *Z*-units); 136.5 (C(3)); 141.2 (C(2′), C(6′)), 156.3 (C(4′)).

Moraprenyltriethylammonium bromide (4). ***A.*** Triethylamine (13 μL, 92 μmol) was added to a solution of moraprenyl bromide (**2**) (38.7 mg, 46.7 μmol) in the benzene—acetonitrile mixture (1 : 1, 0.2 mL) under argon, the mixture was stirred at 45 °C in the dark and after 36 h the solvents were evaporated to dryness. The residue was treated with heptane (1 mL), a precipitate formed was separated by centrifugation, a solution obtained was concentrated *in vacuo* of an oil pump to 0.7 mL. The target product was extracted with acetonitrile (3×1 mL), the combined extracts were concentrated to dryness at 20 °C. The yield of salt **4** was 22.6 mg (51%), R_f 0.7 (A). ^{1}H NMR (500.13 MHz, $CDCl_3$—CD_3OD, 4 : 1 (v/v)), δ: 1.23 (t, 9 H, Me—CH_2—N, J = 7.3 Hz); 1.48 (s, 12 H, Me *E*-units); 1.57 (s, 21 H, Me *Z*-units); 1.70 (s, 0.32 H, Me—C(3) *E*-isomers); 1.78 (s, 2.68 H, Me—C(3) *Z*-isomers); 1.80—2.10 (m, 40 H, CH_2); 3.20 (q, 6 H, Me—CH_2—N, J = 7.3 Hz); 3.67 (d, 1.72 H, $H_2C(1)$ *Z*-isomers, J = 7.6 Hz); 3.75 (d, 0.28 H, $H_2C(1)$ *E*-isomers, J = 7.6 Hz); 5.00 (m, 10 H, =CH); 5.09 (t, 0.14 H, HC(2) *E*-isomers, J = 7.6 Hz); 5.15 (t, 0.86 H, HC(2) *Z*-isomers, J = 7.6 Hz). ^{13}C NMR (125.7 MHz), δ: 7.8 (Me—CH_2—N); 16.0 (Me *E*-units); 17.7 (*cis*-Me W-units); 23.5 (Me *Z*-units); 24.0 (Me—C(3) *Z*-isomers); 25.7 (*trans*-Me W-units); 26.5 (CH_2—CH= *Z*-units); 26.8 (CH_2–CH= *E*-units); 32.3 (CH_2—C(Me)= *Z*-units); 32.8 (C(5)); 39.8 (CH_2—C(Me)= *E*-units); 40.4 (C(4)); 53.0 (Me—CH_2—N); 55.2 (C(1) *Z*-isomers); 55.9 (C(1) *E*-isomers); 109.9 (C(2) *E*-isomers); 110.3 (C(2) *Z*-isomers); 124.4—125.1 (=CH); 131.3 (Me—C= W-units); 135.1 (Me—C= *E*-units); 135.4 (Me—C= *Z*-units).

B. A solution of moraprenol (314 mg, 410 μmol) in anhydrous heptane (0.2 mL) was cooled to −80 °C, followed by addition of a solution of PBr_3 (15 μL, 160 μmol) in heptane (0.2 mL). After heating to 20 °C, the mixture was shaken for 40 min, then diluted with heptane (10 mL), sequentially washed with 5% aq. $NaHCO_3$ (1 mL), H_2O (3×2 mL), and brine (2×2 mL), and dried with Na_2SO_4. The solution obtained was concentrated *in vacuo* to dryness at 20 °C, the residue (323.5 mg) was dissolved in the benzene—acetonitrile mixture (2 : 1, 0.5 mL), followed by addition of Et_3N (100 μL, 720 μmol) and stirring (Ar) for 96 h in the dark at 20 °C. A precipitate formed was separated by centrifugation and sequentially washed with the benzene—acetonitrile mixture (2 : 1, 3×2 mL) and heptane (2 mL). Combined solutions were concentrated to dryness at 20 °C, the residue was dissolved in heptane (5 mL), and the target product was extracted with acetonitrile (3×5 mL). The combined extracts were concentrated *in vacuo* to dryness at 20 °C. The yield of salt **4** was 221.3 mg (58%), R_f 0.7 (A). ^{1}H NMR (200.13 MHz, $CDCl_3$—CD_3OD, 5 : 1 (v/v)), δ: 1.21 (t, 9 H, Me—CH_2—N, J = 7.0 Hz); 1.48 (s, 12 H, Me *E*-units); 1.57 (s, 21 H, Me *Z*-units); 1.72 (s, 0.36 H, Me—C(3) *E*-isomers); 1.80 (s, 2.64 H, Me—C(3) *Z*-isomers); 1.80—2.10 (m, 40 H, CH_2); 3.22 (q, 6 H, Me—CH_2—N, J = 7.0 Hz); 3.67 (d, 1.76 H, $H_2C(1)$ *Z*-isomers, J = 8.0 Hz); 3.75 (d, 0.24 H, $H_2C(1)$ *E*-isomers, J = 8.0 Hz); 5.00 (m, 10 H, =CH); 5.15 (t, 1 H, HC(2), J = 8.0 Hz). ^{13}C NMR (50.3 MHz), δ: 7.7 (Me—CH_2—N); 16.0 (Me *E*-units); 17.6 (*cis*-Me W-units); 23.5 (Me *Z*-units); 24.0 (Me—C(3) *Z*-isomers); 25.8 (*trans*-Me W-units); 26.5 (CH_2—CH= *Z*-units); 26.7 (CH_2—CH= *E*-units); 32.2 (CH_2—C(Me)= *Z*-units); 32.7 (C(5)); 39.8 (CH_2—C(Me)= *E*-units); 40.2 (C(4)); 53.0 (Me—CH_2—N); 55.1 (C(1) *Z*-isomers); 55.8 (C(1) *E*-isomers); 109.9 (C(2) *E*-isomers); 110.2 (C(2) *Z*-isomers); 124.2—125.0 (=CH); 131.1 (Me—C= W-units); 135.0 (Me—C= *E*-units); 135.3 (Me—C= *Z*-units).

C. A solution of moraprenol (42.2 mg, 55 μmol) in anhydrous heptane (0.3 mL) was cooled to −80 °C (Ar), followed by addition of Me_3SiBr (20 μL, 151 μmol). The mixture was heated to 20 °C and kept for 2 h at this temperature, then it was cooled to 0 °C, and concentrated to dryness *in vacuo*. The residue was dissolved in the benzene—acetonitrile mixture (1 : 1, 0.2 mL), followed by addition of Et_3N (15 μL, 100 μmol) under argon. The reaction mixture was kept for 72 h with stirring at 60 °C in the dark, then the solvents were evaporated *in vacuo*, the residue was diluted with heptane (2.4 mL), shaken, a precipitate was separated by centrifugation. The solution was cooled to 0 °C and concentrated to 1 mL *in vacuo*, the target product was extracted with acetonitrile (2×1 mL, 0.5 mL). A combined extract was evaporated to dryness without heating. The yield of product **4** was 39.5 mg (77%), R_f 0.7 (A), R_f 0.5 (B). ^{1}H NMR (500.13 MHz, $CDCl_3$—CD_3OD, 4 : 1 (v/v)), δ: 1.20 (t, 9 H, Me—CH_2—N, J = 7.0 Hz); 1.47 (s, 12 H, Me *E*-units); 1.54 (s, 21 H, Me *Z*-units); 1.74 (s, 0.26 H, Me—C(3) *E*-isomers); 1.80 (s, 2.74 H, Me—C(3) *Z*-isomers); 1.80—2.00 (m, 40 H, CH_2); 3.20 (q, 6 H, Me—CH_2—N, J = 7.0 Hz); 3.67 (d, 1.82 H, $H_2C(1)$ *Z*-isomers, J = 7.6 Hz); 3.70 (m, 3 H, $H_2C(1)$ *E*-isomers + MeOH, H_2O); 4.90 (m, 10 H, =CH); 5.08 (m, 0.09 H, HC(2) *E*-isomers); 5.14 (t, 0.9 H, HC(2) *Z*-isomers, J = 8.0 Hz). ^{13}C NMR (125.7 MHz), δ: 7.2 (Me—CH_2—N); 15.9 (Me *E*-units); 17.6 (*cis*-Me W-units); 23.7 (Me *Z*-units); 24.2 (Me—C(3) *Z*-isomers); 26.0 (*trans*-Me W-units); 26.5 (CH_2—CH= *Z*-units); 26.6 (CH_2—CH= *E*-units); 32.0 (CH_2—C(Me)= *Z*-units); 32.4 (C(5)); 39.5 (CH_2—C(Me)= *E*-units); 39.8 (C(4)); 52.7

(Me—CH_2—N); 54.9 (C(1) *Z*-isomers); 55.5 (C(1) *E*-isomers); 109.7 (C(2) *E*-isomers); 110.0 (C(2) *Z*-isomers); 123.0—124.8 (=CH); 130.8 (Me—C= W-units); 134.7 (Me—C= *E*-units); 135.0 (Me—C= *Z*-units).

1-Methyl-3-moraprenylimidazolium bromide (5). A solution of moraprenol (40.8 mg, 53.0 μmol) in anhydrous heptane (0.3 mL) was cooled to −80 °C, followed by addition of bromotrimethylsilane (20 μL, 151 μmol) (Ar). The mixture was heated to 20 °C, kept for 2 h, then cooled to 0 °C, and concentrated to dryness *in vacuo*. The residue was dissolved in the benzene—acetonitrile mixture (1 : 1, 0.2 mL), followed by addition of 1-methylimidazole (12 μL, 150 μmol) (Ar), and stirred for 18 h at 60 °C in the dark. The solvents were evaporated to dryness *in vacuo* at 20 °C, the residue was diluted with heptane (2 mL), shaken, a precipitate was separated by centrifugation. A solution was concentrated, the residue was dissolved in heptane (0.7 mL), the target product was extracted with acetonitrile (2×1 mL). A combined extract was concentrated to dryness at 20 °C. The yield of compound **5** was 43.8 mg (91%), R_f 0.5 (B). ^{1}H NMR (500.13 MHz, $CDCl_3$—CD_3OD, 4 : 1 (v/v)), δ: 1.44 (s, 9 H, Me *E*-units); 1.46 (s, 3 H, *cis*-Me W-units); 1.52 (s, 21 H, Me *Z*-units and *trans*-Me W-units); 1.67 (s, 0.12 H, Me—C(3) *E*-isomers); 1.70 (s, 3 H, Me—C(3) *Z*-isomers); 1.81 (m, 4 H, $H_2C(4)$); 1.90 (m, 36 H, CH_2); 2.00 (m, 2 H, CH_2 W-units); 2.07 (m, 2 H, $H_2C(5)$); 3.84 (s, 3 H, Me—N); 4.67 (d, 1.92 H, $H_2C(1)$ *Z*-isomers, J = 7.4 Hz); 4.70 (d, 0.08 H, $H_2C(1)$ *E*-isomers, J = 7.4 Hz); 4.95 (m, 10 H, =CH); 5.26 (t, 1 H, HC(2) *Z*-isomers, J = 7.3 Hz); 7.14 (s, 1 H, HC(4′)); 7.03 (s, 1 H, HC(5′)); 9.16 (s, 1 H, HC(2′)). ^{13}C NMR (125.7 MHz), δ: 15.7 (Me *E*-units); 17.3 (*cis*-Me W-units); 23.1 (Me *Z*-units); 23.5 (Me—C(3) *Z*-isomers); 25.7 (*trans*-Me W-units); 26.2 (CH_2—CH= *Z*-units); 26.4 (CH_2—CH= *E*-units); 31.7 (C(5)); 32.0 (CH_2—C(Me)= *Z*-units); 36.2 (Me—N); 39.5 (CH_2—C(Me)= *E*-units); 39.8 (C(4)); 47.0 (C(1) *Z*-isomers); 115.7 (C(2) *E*-isomers); 115.9 (C(2) *Z*-isomers); 121.4 (C(4′)), 123.4—124.8 (=CH); 124.5 (C(5′)); 134.7—136.2 (Me—C=); 136.4 (C(2′)).

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