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SYNTHESIS OF CONJUGATES BASED ON CHLORIN AND ISOSTEVIOL BUILDING BLOCKS

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Conjugates containing ent-beyerane carbocyclic frameworks on the periphery of a porphyrin macrocycle were prepared by acylation of chlorin e_6 derivatives with hydroxyl and amino groups using ent-16-ketobeyeran-19-oic acid chloride (diterpenoid isosteviol).

Key words: isosteviol, *ent*-beyerane, chlorin e_6, amino-chlorin, hydroxy-chlorin.

Chlorophyll *a* derivatives such as chlorin e_6 amides and pyropheophorbide *a* in addition to many others are currently being investigated as photosensitizers for photodynamic therapy of oncological and viral diseases. Several of them are the active ingredients in preparations that are already used in clinical practice [1]. We have used the diterpenoid isosteviol (*ent*-16-ketobeyeran-19-oic acid, **1**), which exhibits various types of physiological activity (hypotensive and antihypertensive effects [2], inhibition of oxidative phosphorylation [3], reduction of ATP-activity of certain phosphotases and oxidases [4]), to modify amino- and hydroxychlorins. The biological activity of isosteviol and its derivatives is due mainly to their interaction with cell membranes [2-4]. Model experiments showed that certain isosteviol derivatives can facilitate the transport of amino acids through membranes [5, 6]. Thus, it can be surmised that introducing onto the periphery of the chlorin macrocycle an isosteviol moiety, which is a functionalized *ent*-beyerane carbocyclic diterpenoid framework, will facilitate better interaction of the synthesized conjugate with cell membranes. This will increase the photosensitizing effect and could result in the preparation of new biologically active compounds.

We used an acylation reaction to introduce the *ent*-beyerane carbocyclic framework onto the periphery of the chlorin ring. The acylating agent was isosteviol acid chloride (**2**), which was prepared using thioyl chloride and isosteviol (**1**) [7] (Scheme 1). Amino and hydroxy groups that were convenient for acylation were introduced onto the periphery of the chlorin macrocycle using opening of the exocycle of methylpheophorbide *a* (**3**) [8-11] to synthesize amino- and hydroxychlorins (**4**-**6**). Opening of the exocycle by ethylenediamine in combination with amidation of the ester substituent in the 17-position by the same diamine produced a chlorin (**10**) with two amino groups [12].

Hydroxy and amino groups of the chlorins (**4**-**6** and **10**) were acylated by isosteviol acid chloride (**2**) by refluxing in THF in the presence of an excess of triethylamine (Scheme 1). PMR spectra of all three compounds (**6**-**8**) showed resonances at strong field for protons of the isosteviol moiety, in particular 3H singlets for methyls. Furthermore, PMR spectra of **7** and **8** exhibited an additional broad triplet for the amide that was formed by acylation of the amine by **2**. Using diaminochlorin **10** as the substrate for the acylation and a two-fold molar excess of **2** produced in 30% yield a chlorin with two diterpenoid moieties (**11**) (Scheme 1). PMR spectra of **11** had two additional broad triplets of amides that were formed by acylation of diaminochlorin with **2** when compared with the spectrum of starting **10**. Furthermore, a doubled set of singlets for methyls of the isosteviol moieties was observed at strong field in the PMR spectrum of **11**. The results confirmed the presence of two diterpenoid substituents bonded through an amide bond.

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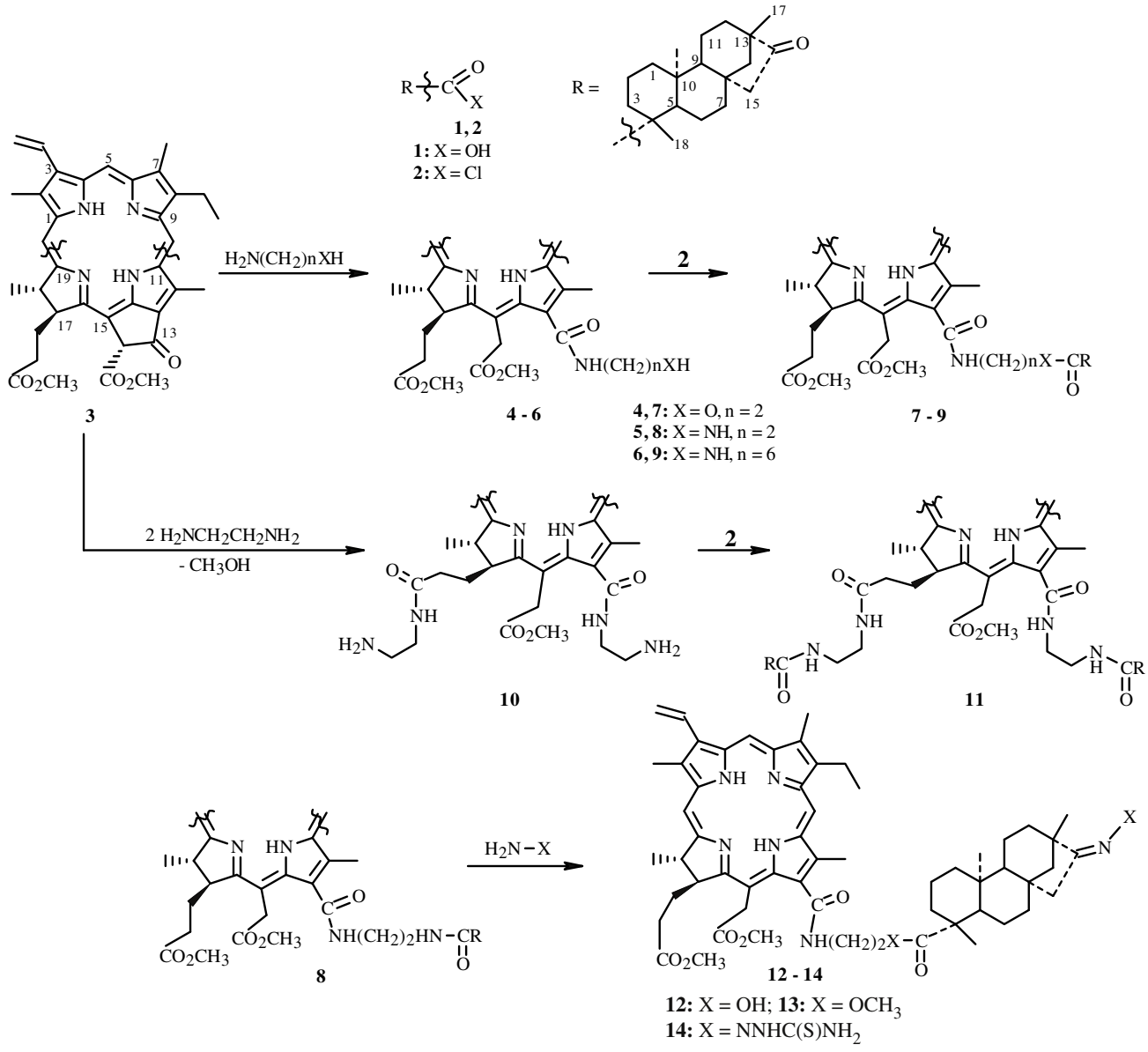

Scheme 1

The added isosteviol moiety can undergo further chemical transformations. For example, the ketone of the isosteviol moiety was reacted to produce several imine derivatives (**12**-**14**) (Scheme 1), the PMR spectra of which had resonances for the isosteviol and chlorin portions that were analogous to those of these same portions in the spectrum of the starting chlorin conjugate with isosteviol (**5**). Furthermore, PMR spectra of the resulting imine derivatives of isosteviolchlorins showed resonances for hydroxyl (for **12**, 2.19 ppm, 1H, br.s, OH-16′), methoxyl (for **13**, 3.76 ppm, 3H, s, CH_3O-21′), and thiosemicarbazone (for **14**, 8.23 ppm, 1H, br.s, NH-16′).

Thus, we synthesized chlorins containing on the periphery one and two diterpenoid isosteviol moieties, showed that further modification of the isosteviol moiety conjugated to the chlorin ring is possible, and synthesized several imine derivatives.

EXPERIMENTAL

Isosteviol acid chloride (**2**) was prepared by reacting isosteviol (**1**) and thionyl chloride [6]; methylpheophorbide *a* (**3**), from blue-green alga spirulina as before [12]. Amides **4**, **5**, and **6** were synthesized by the literature methods [8-11].

Diaminochlorin (**10**) was prepared from methylpheophorbide *a* (**3**) as before [9]. PMR spectra were recorded on Bruker Avance 600 (600 MHz) and AMX-400 (400 MHz) instruments; mass spectra, on a Dynamo Maldi Tof (Finnigan) instrument; IR spectra in KBr disks, on a Specord M-80 instrument. TLC was carried out on Sorbfil plates; column chromatography, over silica gel (La Chema, 100-400 mesh).

Chlorin e_6 13-*N*-[2-(*O*-Isosteviol)hydroxyethyl]amide-15,17-dimethyl Ester (7). A solution of the 13-*N*-(2-hydroxyethyl)-amide-15,17-dimethyl ester of chlorin e_6 (700 mg, 1.05 mmol) in THF (15 mL) was treated with isosteviol acid chloride (350 mg, 1.04 mmol) and triethylamine (0.5 mL), refluxed for 1.5 h [TLC, eluent CCl_4:$(CH_3)_2CO$, 4:1], diluted with $CHCl_3$ (100 mL), rinsed of triethylamine with water, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 30:1] to afford **7** (36 mg, 12%, 30% conversion). IR spectrum (KBr, cm^{-1}): 1740 (νC=O ester), 1638 (amide-I), 1610 (chlorin band), 1526 (amide-II).

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 9.83 (1H, s, H-10), 9.76 (1H, s, H-5), 8.93 (1H, s, H-20), 8.10 [1H, dd, J = 11.8, 7.8, H-3(1)], 6.68 [1H, br.t, J = 4.4, NH-13(1) (amide)], 6.38 [1H, br.d, J = 12, H-3(2) (*trans*)], 6.21 [1H, br.d, J = 7.6, H-3(2) (*cis*), CH_2-15(1)], 5.56 (1H, d, J = 12.8), 5.27 (1H, d, J = 12.4), 4.49-4.56 (1H, m, H-18), 4.38-4.47 (1H, m, H-17), 4.13-4.21 [2H, m, CH_2-8(1), CH_2-13(2), CH_2-13(3)], 3.68-3.76 (2H, m), 3.46-3.53 (2H, m), 3.86 [3H, s, CH_3-15(3)], 3.64 [3H, s, CH_3-17(4)], 3.59 [3H, s, CH_3-12(1)], 3.51 [3H, s, CH_3-2(1)], 3.37 [3H, s, CH_3-7(1)], 2.52-2.58 [2H, m, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.36-2.48 (2H, m), 1.79-1.84 (2H, m), 1.74 [3H, t, J = 5, CH_3-8(2)], 1.74 [3H, d, J = 4.8, CH_3-18(1)], 1.22 [3H, s, CH_3-19′ (isosteviol)], 0.85 [3H, s, CH_3-17′ (isosteviol)], 0.68 [3H, s, CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 2.16-2.22 (2H, m), 1.77-1.84 (2H, m), 1.59-1.63 (2H, m), 1.47-1.58 (4H, m), 1.36-1.40 (2H, m), 1.22-1.28 (4H, m), 0.88-1.08 (2H, m), −1.69 (1H, br.s, NH-I), −1.72 (1H, br.s, NH-III). MS (*m*/*z*): 967.0 $[M]^+$.

Chlorin e_6 13-*N*-[2-(*N*-Isosteviol)aminoethyl]-amide-15,17-dimethyl Ester (8). A solution of the 13-*N*-(2-aminoethyl)-amide-15,17-dimethyl ester of chlorin e_6 (300 mg, 0.45 mmol) in THF (10 mL) was treated with isosteviol acid chloride (155 mg, 0.46 mmol) and triethylamine (0.3 mL), refluxed for 1.5 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (100 mL), rinsed of triethylamine with water, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 20:1] to afford **8** (226 mg, 52%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1736 (νC=O ester),1653 (amide-I), 1601 (chlorin band), 1507 (amide-II).

PMR spectrum (400 MHz, DMF-d_7, δ, ppm, J/Hz): 9.92 (1H, s, H-10), 9.88 (1H, s, H-5), 9.23 (1H, s, H-20), 9.01 [1H, br.t, J = 4.8, NH-13(1) (amide)], 8.40 [1H, dd, J = 18, 11.6, H-3(1)], 7.58 [1H, br.t, J = 5.4, NH-13(3) (amide)], 6.50 [1H, dd, J = 17.8, 1.8, H-3(2) (*trans*)], 6.21 [1H, dd, J = 11.6, 1.2, H-3(2) (*cis*), CH_2-15(1)], 5.70 (1H, d, J = 18.4), 5.43 (1H, d, J = 18.4), 4.72 (1H, br.quint, J = 7.2, H-18), 4.55 (1H, br.d, J = 8.8, H-17), 3.89 [2H, br.quint, J = 7.4, CH_2-8(1)], 3.64-3.76 [4H, m, CH_2-13(2), CH_2-13(3)], 3.78 [3H, s, CH_2-15(3)], 3.63 [3H, s, CH_3-7(4)], 3.60 [3H, s, CH_3-12(1)], 3.59 [3H, s, CH_3-2(1)], 3.38 [3H, s, CH_3-7(1)], 2.57 [2H, dd, J = 18.6, 3.8, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.28-2.42 (2H, m), 1.92-2.10 (2H, m), 1.73 [3H, t, J = 7.6, CH_3-8(2)], 1.73 [3H, d, J = 7.2, CH_3=18(1)], 1.26 [3H, s, CH_3-19′ (isosteviol)], 1.21 [3H, s, CH_3-17′ (isosteviol)], 0.90 [3H, s, CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 2.21-2.30 (3H, m), 1.98-2.05 (3H, m), 1.52-1.58 (2H, m), 1.38-1.49 (8H, m), 1.12-1.18 (2H, m), −1.60 (1H, br.s, NH-1), −1.91 (1H, br.s, NH-III). MS (*m*/*z*): 966.2 $[M]^+$.

Chlorin e_6 13-*N*-[6-(*N*-Isosteviol)aminohexyl]-amide-15,17-dimethyl Ester (9). A solution of the 13-*N*-(6-aminohexyl)-amide-15,17-dimethyl ester of chlorin e_6 (240 mg, 0.33 mmol) in THF (8 mL) was treated with isosteviol acid chloride (180 mg, 0.53 mmol) and triethylamine (0.5 mL), refluxed for 2 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (100 mL), rinsed of triethylamine with water, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [(eluent CCl_4:$(CH_3)_2CO$ 10:1] to afford **9** (85 mg, 23%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1740 (νC=O ester),1640 (amide-II), 1612 (chlorin band), 1525 (amide-I).

PMR spectrum (400 MHz, DMF-d_7, δ, ppm, J/Hz): 9.91 (1H, s, H-10), 9.87 (1H, s, H-5), 9.23 (1H, s, H-20), 8.89 [1H, br.t, J = 5.2, NH-13(1) (amide)], 8.39 [1H, dd, J = 17.6, 11.6, H-3(1)], 7.20 [1H, br.t, J = 5.4, NH-13(7) (amide)], 6.49 [1H, dd, J = 18, 1.2, H-3(2) (*trans*)], 6.19 [1H, dd, J = 11.6, 1.2, H-3(2) (*cis*), CH_2-15(1)], 5.74 (1H, d, J = 18.4), 5.41 (1H, d, J = 19.2), 4.72 (1H, br.quint, J = 7.2, H-18), 4.55 (1H, br.d, J = 8.8, H-17), 3.89 [2H, br.quint, J = 7.4, CH_2-8(1), CH_2-13(2), CH_2-13(3), CH_2-13(4), CH_2-13(5), CH_2-13(6), CH_2-13(7)], 3.75-3.85 (3H, m), 3.55-3.67 (2H, m), 3.18-3.28 (3H, m), 1.83-1.95 (4H, m), 3.78 [3H, s, CH_3-15(3)], 3.63 [3H, s, CH_3-17(4)], 3.59 [3H, s, CH_3-12(1)], 3.58 [3H, s, CH_3-2(1)], 3.38 [3H, s, CH_3-7(1)], 2.40 [2H, dd, J = 17.8, 3.8, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.23-2.36 (2H, m), 1.75-1.82 (2H, m),

1.73 [3H, t, J = 7.6, CH_3-8(2)], 1.73 [3H, d, J = 7.2, CH_3-18(1)], 1.13 [3H, s, CH_3-19′ (isosteviol)], 0.83 [3H, s, CH_3-17′ (isosteviol)], 0.76 [3H, s ,CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH_2-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 2.10-2.20 (2H, m), 1.82-1.94 (2H, m), 1.47-1.67 (4H, m), 1.23-1.38 (4H, m), 0.96-1.09 (6H, m), −1.63 (1H, br.s, NH-I), −1.95 (1H, br.s, NH-III). MS (*m*/*z*): 1022.3 $[M]^+$.

Chlorin e_6 13,17-*N*,*N*′-[2-(*N*,*N*′-Diisosteviol)aminoethyl]-diamide-15-methyl Ester (11). A solution of the 13,17-*N*,*N*′-(2-aminoethyl)-diamide-15-methyl ester of chlorin e_6 (180 mg, 0.26 mmol) in THF (7 mL) was treated with isosteviol acid chloride (240 mg, 0.71 mmol) and triethylamine (0.6 mL), refluxed for 2 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (100 mL), rinsed of triethylamine with water, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 2:1] to afford **11** (140 mg, 30%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1732 (νC=O ester), 1653 (amide-I),1601 (chlorin band), 1541 (amide-II).

PMR spectrum (400 MHz, DMF-d_7, δ, ppm, J/Hz): 9.90 (1H, s, H-10), 9.87 (1H, s, H-5), 9.22 (1H, s, H-20), 8.98 [1H, br.t, J = 4.8, NH-13(1) (amide)], 8.39 [1H, dd, J = 17.8, 11.6, H-3(1)], 7.98 [1H, br.t, J = 5.4, NH-13(3) (amide)], 7.57 [1H, br.t, J = 5, NH-17(3) (amide)], 7.23 [1H, br.t, J = 4.2, NH-17(5) (amide)], 6.50 [1H, dd, J = 17.8, 1.4, H-3(2) (*trans*)], 6.20 [1H, dd, J = 11.6, 1.2, H-3(2) (*cis*), CH_2-15(1)], 5.73 (1H, d, J = 18.8), 5.40 (1H, d, J = 18.8), 4.69 (1H, br.quint, J = 7.2, H-18), 4.49 (1H, br.d, J = 10, H-17), 3.89 [2H, br.quint, J = 7.4, CH_2-8(1)], 3.64-3.75 [4H, m, CH_2-13(2), CH_2-13(3)], 3.21-3.33 [4H, m, CH_2-17(4), CH_2-17(5)], 3.78 [3H, s, CH_3-15(3)], 3.60 [3H, s, CH_3-2(1)], 3.59 [3H, s, CH_3-2(1)], 3.38 [3H, s, CH_3-7(1)], 2.56 [2H, dd, J = 18.4, 3.6, CH_2-15′ (isosteviol)], 2.40 [2H, dd, J = 18.4, 3.2, CH_2-15″ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.60-2.68 (2H, m), 2.21-2.34 (2H, m), 1.72 [3H, t, J = 7.6, CH_3-8(2)], 1.73 [3H, d, J = 7.6, CH_3-18(1)], 1.25 [3H, s, CH_3-19′ (isosteviol)], 1.21 [3H, s, CH_3-19″ (isosteviol)], 1.05 [3H, s, CH_3-17′ (isosteviol)], 0.89 [3H, s, CH_3-17″ (isosteviol)], 0.83 [3H, s, CH_3-20′ (isosteviol)], 0.67 [3H, s, CH_3-20″ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′, CH_2-1″, CH_2-2″, CH_2-3″, CH-5″, CH_2-6″, CH_2-7″, CH-9″, CH_2-11″, CH_2-12″, CH_2-14″ (isosteviol)], 2.21-2.34 (3H, m), 1.93-2.10 (4H, m), 1.84-1.93 (3H, m), 1.77-1.83 (2H, m), 1.31-1.55 (12H, m), 1.09-1.18 (6H, m), 0.93-1.03 (6H, m), −1.61 (1H, br.s, NH-I), −1.94 (1H, br.s, NH-III). MS (*m*/*z*): 1297.3 $[M + H]^+$.

Chlorin e_6 13-*N*-[2-(*N*-Isostevioloxime)aminoethyl]-amide-15,17-dimethyl Ester (12). A solution of the 13-*N*-[2-(*N*-isosteviol)aminoethyl]-amide-15,17-dimethyl ester of chlorin e_6 (110 mg, 0.11 mmol) in pyridine (1.5 mL) was treated with hydroxylamine hydrochloride (110 mg, 1.6 mmol), refluxed for 1 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (50 mL), rinsed of pyridine with HCL solution (5%) and water until the rinsings were neutral, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 5:1] to afford **12** (55 mg, 49%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1736 (νC=O ester),1654 (amide-I), 1601 (chlorin band), 1522 (amide-II).

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 9.70 (1H, s, H-10), 9.61 (1H, s, H-5), 8.81 (1H, s, H-20), 8.06 [1H, dd, J = 12, 8, H-3(1)], 6.95 [1H, br.t, J = 2.6, NH-13(1) (amide)], 6.93 [1H, br.t, J = 2.4, NH-13(3) (amide)], 6.34 [1H, br.d, J = 12, H-3(2) (*trans*)], 6.13 [1H, br.d, J = 8, H-3(2) (*cis*), CH_2-15(1)], 5.51 (1H, d, J = 12.4), 5.25 (1H, d, J = 12.4), 4.48 (1H, br.quint, J = 4.8, H-18), 4.39 (1H, br.d, J = 5.6, H-17), 3.81 [2H, br.quint, J = 5.2, CH_2-8(1)], 3.72-3.89 [4H, m, CH_2-13(2), CH_2-13(3)], 3.78 [3H, s, CH_3-15(3)], 3.62 [3H, s, CH_3-17(4)], 3.53 [3H, s, CH_3-12(1)], 3.49 [3H, s, CH_3-2(1)], 3.29 [3H, s, CH_3-7(1)], 3.01 [2H, dd, J = 12.2, 1.4, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.13-2.28 (2H, m), 1.81-1.91 (2H, m), 2.19 [1H, br.s, OH-16′ (isosteviol)], 1.73 [3H, t, J = 5.2, CH_3-8(2)], 1.72 [3H, d, J = 4.8, CH_3-18(1)], 1.29 [3H, s, CH_3-19′ (isosteviol)], 1.09 [3H, s, CH_3-17′ (isosteviol)], 0.92 [3H, s, CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 1.99-2.07 (2H, m), 1.76-1.81 (2H, m), 1.38-1.67 (8H, m), 1.10-1.25 (6H, m), −1.54 (1H, br.s, NH-I), −1.74 (1H, br.s, NH-III). MS (*m*/*z*): 981.3 $[M]^+$.

Chlorin e_6 13-*N*-[2-(*N*-Isosteviolmethoxime)aminoethyl]-amide-15,17-dimethyl Ester (13). A solution of the 13-*N*-[2-(*N*-isosteviol)aminoethyl]-amide-15,17-dimethyl ester of chlorin e_6 (130 mg, 0.13 mmol) in pyridine (2 mL) was treated with methoxylamine hydrochloride (130 mg, 1.6 mmol), refluxed for 1 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (50 mL), rinsed of pyridine with HCl solution (5%) and water until the rinsings were neutral, dried over anhydrous Na_2SO_4, evaporated at reduced pressure, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 10:1] to afford **13** (95 mg, 75%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1736 (νC=O ester),1653 (amide-I), 1605 (chlorin band), 1512 (amide-II).

PMR spectrum (400 MHz, DMF-d_7, δ, ppm, J/Hz): 9.91 (1H, s, H-10), 9.86 (1H, s, H-5), 9.23 (1H, s, H-20), 9.00 [1H, br.t, J = 5, NH-13(1) (amide)], 8.38 [1H, dd, J = 17.6, 11.6, H-3(1)], 7.54 [1H, br.t, J = 5.2, NH-13(3) (amide)], 6.48 [1H, dd, J = 17.6, 1.2, H-3(2) (*trans*)], 6.18 [1H, dd, J = 11.6, 0.8, H-3(2) (*cis*), CH_2-15(1)], 5.71 (1H, d, J = 19.2), 5.43 (1H, d,

J = 19.6), 4.72 (1H, br.quint, J = 7.2, H-18), 4.55 (1H, br.d, J = 8.4, H-17), 3.88 [2H, br.quint, J = 7.4, CH_2-8(1)], 3.66-3.75 [4H, m, CH_2-13(2), CH_2-13(3)], 3.78 [3H, s, CH_3-15(3)], 3.76 [3H, s, CH_3O-21′ (isosteviol methoxy)], 3.63 [3H, s, CH_3-17(4)], 3.60 [3H, s, CH_3-12(1)], 3.58 [3H, s, CH_3-2(1)], 3.37 [3H, s, CH_3-7(1)], 2.90 [2H, dd, J = 18.2, 3, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.26-2.43 (2H, m), 1.75-1.83 (2H, m), 1.73 [3H, d, J = 7.6, CH_3-18(1)], 1.72 [3H, t, J = 7.4, CH_3-8(2)], 1.22 [3H, s, CH_3-19′ (isosteviol)], 1.03 [3H, s, CH_3-17′ (isosteviol)], 0.90 [3H, s, CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 2.17-2.26 (2H, m), 1.97-2.11 (2H, m), 1.54-1.61 (3H, m), 1.30-1.43 (6H, m), 1.08-1.18 (3H, m), 0.78-0.88 (2H, m), −1.60 (1H, br.s, NH-I), −1.91 (1H, br.s, NH-III). MS (*m*/*z*): 996.6 $[M]^+$.

Chlorin e_6 13-*N*-[2-(*N*-Isostevioldthiosemicarbazone)aminoethyl]-amide-15,17-dimethyl Ester (14). A solution of the 13-*N*-[2-(*N*-isosteviol)aminoethyl]-amide-15,17-dimethyl ester of chlorin e_6 (140 mg, 0.14 mmol) in pyridine (2 mL) was treated with thiosemicarbazide hydrochloride (140 mg, 1.1 mmol), refluxed for 1 h [TLC, eluent CCl_4:$(CH_3)_2CO$ 4:1], diluted with $CHCl_3$ (50 mL), rinsed of pyridine with HCl solution (5%) and water until the rinsings were neutral, dried over anhydrous Na_2SO_4, evaporated, and chromatographed over silica gel [eluent CCl_4:$(CH_3)_2CO$ 5:1] to afford **14** (95 mg, 63%, 100% conversion). IR spectrum (KBr, cm^{-1}): 1736 (νC=O ester), 1653 (amide-I), 1603 (chlorin band), 1509 (amide-II).

PMR spectrum (400 MHz, DMF-d_7, δ, ppm, J/Hz): 9.91 (1H, s, H-10), 9.88 (1H, s, H-5), 9.24 (1H, s, H-20), 9.03 [1H, br.t, J = 4.2, NH-13(1) (amide)], 8.40 [1H, dd, J = 17.8, 11.8, H-3(1)], 8.23 [1H, br.s, NH-16′ (isosteviol)], 7.65 [1H, br.t, J = 4.8, NH-13(3) (amide)], 6.51 [1H, dd, J = 18, 1.2, H-3(2) (*trans*)], 6.21 [1H, dd, J = 11.8, 1.4, H-3(2) (*cis*), CH_2-15(1)], 5.70 (1H, d, J = 18.8), 5.44 (1H, d, J = 18.4), 4.72 (1H, br.quint, J = 6.6, H-18), 4.55 (1H, br.d, J = 9.2, H-17), 3.89 [2H, br.quint, J = 7.2, CH_2-8(1)], 3.65-3.76 [4H, m, CH_2-13(2), CH_2-13(3)], 3.77 [3H, s, CH_3-15(3)], 3.63 [3H, s, CH_3-17(4)], 3.60 [3H, s, CH_3-12(1)], 3.59 [3H, s, CH_3-2(1)], 3.38 [3H, s, CH_3-7(1)], 3.17 [2H, dd, J = 18.6, 2.6, CH_2-15′ (isosteviol), CH_2-17(1), CH_2-17(2)], 2.27-2.42 (2H, m), 1.75-1.85 (2H, m), 1.73 [3H, d, J = 7.2, CH_3-18(1)], 1.72 [3H, t, J = 7.4, CH_3-8(2)], 1.21 [3H, s, CH_3-19′ (isosteviol)], 1.12 [3H, s, CH_3-17′ (isosteviol)], 0.93 [3H, s, CH_3-20′ (isosteviol), CH_2-1′, CH_2-2′, CH_2-3′, CH-5′, CH_2-6′, CH_2-7′, CH-9′, CH_2-11′, CH_2-12′, CH_2-14′ (isosteviol)], 2.20-2.29 (2H, m), 1.96-2.03 (2H, m), 1.61-1.68 (2H, m), 1.37-1.57 (6H, m), 1.29-1.36 (2H, m), 1.14-1.20 (2H, m), 1.01-1.08 (2H, m), −1.60 (1H, br.s, NH-I), −1.91 (1H, br.s, NH-III). MS (*m*/*z*): 1040.9 $[M]^+$.

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