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Chlorin e₆ 13¹:15²-Anhydride: A Key Intermediate in Conjugation Reactions of Chlorin e₆**

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Abstract

Since the patent for the photodynamic therapy agent Talaporfin (mono-*L*-aspartylchlorin e₆) was issued in 1987, confusion has existed regarding which of the three carboxylic acid groups in the chlorophyll degradation product, chlorin e₆ (**1**), is modified in standard amino acid type conjugations (using DCC or EDC and an organic base) with amino acids and other biomolecules. Here it is shown that the site of conjugation is the central 15²-carboxylic acid, such reactions proceeding in numerous examples via a 13¹,15²-anhydride for which a high resolution X-ray structure is reported. Conjugation with eight oxygen and nitrogen nucleophiles, in every case, afforded the 15²-conjugate, reinforcing the earlier conclusion that Talaporfin is the 15²-aspartyl conjugate of chlorin e₆ and suggesting that reports of 17³-conjugation of chlorin e₆ using stoichiometric peptide coupling procedures should be subjected to further scrutiny.

Keywords

Talaporfin; chlorin e₆; anhydride; amino acid conjugation; LS-11

Photodynamic therapy (PDT) is a binary cancer therapy that relies on the selective uptake of a photosensitizer in tumor tissues, followed by generation of singlet oxygen and/or other cytotoxic species upon irradiation with light of appropriate wavelength.^[1–3] Photofrin® (porfimer sodium) had been commercially developed and approved in more than 40 countries as a first generation photosensitizer. Problems associated with low absorption of light within the “therapeutic window” (600–800 nm) and its slow clearance from skin have resulted in residual patient photosensitivity.^[4] Second generation photosensitizers, such as mono-(*L*)-aspartylchlorin e₆, (aka Talaporfin, NPe6, MACE, LS-11) have been used in advanced-stage PDT clinical trials. Talaporfin is prepared by treatment of the tricarboxylic acid chlorin e₆ (**1**) with aspartic acid in presence of an organic base and a peptide coupling agent (e.g. DCC). It has a strong and characteristic solvent-dependent chlorin-type

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CCDC 1046544-1046547 contain the supplementary crystallographic data for the low-temperature structure determinations in this paper. These data can be obtained, free of charge, from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

absorption at 666 nm and is able to generate cytotoxic singlet oxygen in high yields upon irradiation, with rapid clearance from normal tissues.^[4-5]

A patent search identifies Talaporfin (LS-11) as the 17³-aspartyl derivative of chlorin e₆ (**2**) though the option for a mixture with other regioisomers was left open.^[6] This is unlikely however because high-field proton NMR spectroscopy of the tetramethyl ester of LS-11 shows no (<5%) evidence of isomeric impurities. In 1998 a thorough 2D NMR study was published claiming that Talaporfin is actually the 15²-regioisomer (**3**) of chlorin e₆ (**1**).^[7] The conclusions from this paper were largely ignored, possibly because they were counter-intuitive from a mechanistic standpoint since the propionic side chain in chlorin e₆ is potentially the most reactive and, of the three carboxylic acid side chains, the least liable to steric hindrance. As a result, most papers between 1998 and 2007 assumed Talaporfin to be the 17³-aspartyl derivative (**2**). The identity of Talaporfin remained a matter of conjecture and the distributors remained silent from 1998 to 2007 on the critically important structural issue raised by Gomi et al.^[7] Recently, our group reported the unambiguous syntheses of 13¹-, 15²- and 17³-aspartyl regioisomers (**2-4**) of Talaporfin, as their tetramethyl esters, and definitively confirmed that Talaporfin is the 15²-regioisomer (**4**);^[8] other conjugations were also shown to occur preferentially at the 15² position.^[9] We were also able to obtain the X-ray structure of the tetramethyl ester of Talaporfin, conclusively placing the aspartic acid on the 15² position.^[8] Considering the fact that chlorin e₆ possesses no less than three carboxylic acid functional groups, all of which are able to undergo amino-acid coupling reactions, the unexpected attachment of the amino acid to the 15² position required an explanation.

To this end we proposed^[8] that the 13¹:15²-anhydride (**5**) of chlorin e₆ could be a key intermediate in this conjugation reaction, presumably by DCC mediation of a dehydration reaction between the 13¹ and 15² carboxylic acids prior to involvement of the amino acid nucleophile, which subsequently attacks the more electrophilic aliphatic 15²-carbonyl rather than the aromatic nuclear carbonyl of the anhydride. In this paper we report a number of regiochemical conjugations to chlorin e₆, showing that they all result in attachment to the 15² position, and describe the synthesis and definitive structural identification of the 13¹,15²-anhydride as a key intermediate in these reactions of chlorin e₆.

We began by treatment of chlorin e₆ (**1**), obtained from *Spirulina pacifica* alga,^[10] with DCC and then methanol. The resulting monomethyl ester was then treated with diazomethane to give chlorin e₆ trimethyl ester (**6**). The diazomethane treatment enables efficient purification and crystallization of the corresponding esters. The X-ray structure of this compound is shown in Figure 1. The methyl propionate and methyl substituents at the 17 and 18 positions are confirmed to be mutually *trans*. The two independent molecules in the asymmetric unit differ in the conformations of the three ester substituents and of the ethyl group at the 8 position.

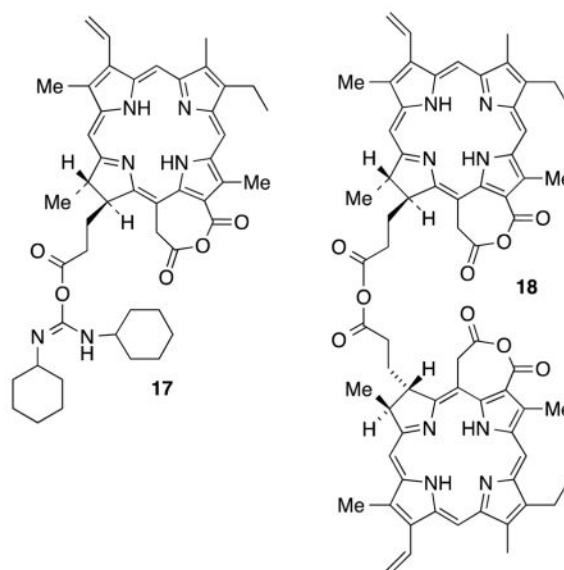
When the same reaction was done using ethoxide/ethanol in place of the methanol, a monoethyl-dimethyl ester was obtained after diazomethane treatment, and ¹H NMR spectroscopy (Figure 2) clearly showed this to be the 15²-ethyl ester (**7**). In Figure 2 the three methoxyl resonances of **6** appear at 4.27, 3.79, and 3.65 ppm and have previously been

assigned^[8] to the 13¹, 15², and 17³ resonances, respectively. The product of the ethoxide reaction clearly shows the peak at 3.79 ppm to be absent, with new ethyl resonances appearing at 1.24 (t) and 4.28 (q) ppm.

The same process was repeated, but using EDC in place of DCC, with a number of amines, alcohols and a thiol, each replacing the methanol in the initial reaction. In all cases, ¹H NMR spectroscopy (Table 1; for spectra see SI) indicated reaction at the 15²-carbonyl, and a typical X-ray structure of the monopropionamide product **8** (Figure 3) corroborated this conclusion. Four independent molecules exhibit a wide variation in conformation of the substituents, most notably in the ethyl group at C8 and in the monopropionamide. ¹H NMR assignments were based on the known, and fairly constant shifts of the OMe protons in the corresponding 13¹-, 15²- and 17³-methyl esters. All of the compounds (**7–14**) possessed a ~4.2–4.3 ppm peak characteristic of the 13¹-OMe and also lacked the ~3.79 ppm peak which is typical for the 15²-OMe. The only exception was compound (**11**) in which the peak at 3.80 ppm is assigned to the methyl in the aryl thiol. In the case of the synthesis of the benzyl-dimethyl ester **14** (45% yield) a 21% yield of the corresponding 15²,17³-dibenzyl-13¹-methyl ester **15** was also obtained. Figure 4 shows the X-ray structure of this bis-conjugate **15** after treatment with diazomethane to facilitate purification and crystallization. As with the other structures, the four independent molecules exhibit considerable conformational variability, particularly in the benzyl substituents.

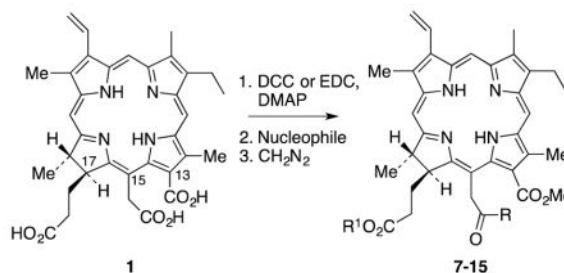
Definitive proof of the intermediacy of the anhydride **5** required isolation and characterization of it or a derivative during a coupling reaction, but in the absence of a nucleophile. Thus chlorin e₆ (**1**) was reacted with one equivalent of the peptide coupling reagent EDC in presence of DMAP.^[8] The proposed monocarboxylic acid anhydride product **5** was then treated with diazomethane under strictly anhydrous, nucleophile free conditions to facilitate the isolation of **16**. The methyl ester **16** thus generated was fully characterized by NMR, MALDI and UV-vis. Indeed, a crystal suitable for X-ray study was obtained and the structure is shown in Figure 5. The absolute configurations of the two chiral centers were confirmed, based on resonant scattering of the light atoms in CuK α radiation. Thus the configurations at C17 and C18 for all compounds in this series are both *S*, in agreement with starting material and the literature.^[11] Two independent molecules show considerable conformational difference, including the 7-membered ring of the anhydride.

When stoichiometric amounts of EDC or DCC and DMAP were used in attempts to form the anhydride **5**, additional dehydration reactions were evident. MALDI MS provided evidence for the existence of the novel compounds **17** and **18**, but as might be expected, such activated compounds tended to decompose upon chromatography or other attempts to isolate them.^[12] Compounds such as **17** and **18** tended to predominate when reactions with DCC were performed in the absence of the usual DMAP base. Formation of the 15²,17³-bis-aspartic acid conjugate of chlorin e₆ with DCC/DMAP and aspartic acid has also been reported.^[13]



Finally, the anhydride **16** was treated with L-aspartic acid dimethyl ester and triethylamine in the absence of a coupling agent, and gave the 15²-conjugate. Treatment with diazomethane gave the known tetramethyl ester of Talaporfin, in 75% yield from chlorin e_6 , and the product was identical in all respects with an authentic sample.

We conclude that conjugation of chlorin e_6 (**1**) with one equivalent a coupling agent such as DCC or EDC and a base such as DMAP should result in formation of the 15²-conjugate of chlorin e_6 via the corresponding 13¹,15²-anhydride; this has been shown to be categorically true for conjugations with aspartic acid, and in the numerous conjugations reported in this paper. A majority of reported conjugations of chlorin e_6 have either ignored the site of conjugation, or it has been assumed to give the 17³-conjugate^[14] in accord with the original patent^[6] and numerous subsequent publications, even including two of our own.^[13] Such studies and conclusions should be subjected to further scrutiny with regard to the regiochemical structure of the conjugate. We can visualize circumstances in which the usually presumed 17³-product might result from a conjugation reaction of chlorin e_6 , but this would be enhanced by use of an excess of DCC and proceed via doubly activated compounds such as **17** and **18**.



Experimental Section

Synthesis of anhydride **16**. Chlorin e_6 (**1**) (100 mg, 0.17 mmol), EDC (32 mg, 0.17 mmol) and DMAP (9.0 mg, 0.07 mmol) were dissolved in CH_2Cl_2 (1.5 ml) and stirred for 2 h under N_2 at room temperature. Excess ethereal diazomethane was then added to the mixture and after 30 min stirring it was evaporated and chromatographed on a silica gel thick layer plate, eluting with DCM/MeOH (10:1). The brown product (**15**) was collected (62 mg, 63%) and crystallized from DCM:hexane (1:3). M.p. >260 °C. MALDI-TOF calcd. for $\text{C}_{35}\text{H}_{36}\text{N}_4\text{O}_5$: 592.2686 or 593.2764 ($\text{M}+\text{H}$)⁺; found 593.2750 ($\text{M}+\text{H}$)⁺. UV-Vis (DMSO): λ_{max} nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 411 (97600), 507 (9500), 542 (10300), 615 (7000), 669 (33100); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ (ppm) 9.57 (1H, s), 9.29 (1H, s), 8.49 (1H, s), 7.92 (1H, dd, $J=18.0$, 11.6 Hz), 6.31 (1H, d, $J=18.3$ Hz), 6.16 (1H, d, $J=12.0$ Hz), 5.40 (2H, m), 4.53 (1H, d, $J=9.5$ Hz), 4.38 (1H, m), 4.16 (1H, m), 3.72-3.67 (8H, m), 3.37 (3H, s), 3.19 (3H, s), 2.63 (1H, m), 2.34 (1H, m), 2.01 (1H, m), 1.74 (3H, d, $J=6.1$ Hz), 1.69 (3H, t, $J=7.6$ Hz), -0.40 (2H, br).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbek M, Moan J, Peng Q. *J Natl Cancer Inst.* 1998; 90:889–905. [PubMed: 9637138]
2. Pandey, RK.; Zheng, G. *The Porphyrin Handbook*. Kadish, KM.; Smith, KM.; Guillard, R., editors. Vol. 6. Academic Press; Boston: 2000. p. 157-230.
3. Vicente MGH. *Current Medicinal Chemistry. Anti-Cancer Agents.* 2001:175–194. [PubMed: 12678766]
4. Spikes, JD.; Bommer, JC. *Chlorophylls*. Scheer, H., editor. CRC Press; Boston: 1996. p. 1181-1204.
5. Song LMW, Wang KK, Zinsmeister AR. *Cancer.* 1998; 82:421–427. [PubMed: 9445202]
6. Bommer, JC.; Ogden, BF. US Patent. 4,693,885.. 1987.
7. Gomi S, Nishizuka T, Ushiroda O, Uchida N, Takahashi H, Sumi S. *Heterocycles.* 1998; 48:2231–2243.
8. Hargus JA, Fronczek FR, Vicente MGH, Smith KM. *Photochem Photobiol.* 2007; 83:1006–1015. [PubMed: 17880493]
9. Jinadasa RGW, Hu X, Vicente MGH, Smith KM. *J Med Chem.* 2011; 54:7464–7476. [PubMed: 21936519]
10. Smith KM, Goff DA, Simpson DJ. *J Am Chem Soc.* 1985; 107:4946–4954.
11. Fleming I. *Nature.* 1967; 216:151–152.
12. Jiao, L. PhD Dissertation. Louisiana State University; Baton Rouge: 2008. p. 194
13. a) Roberts WG, Shiao FY, Nelson JS, Smith KM, Berns MW. *J Natl Cancer Inst.* 1988; 80:330–336. [PubMed: 2965763] b) Roberts WG, Smith KM, McCullough JL, Berns MW. *Photochem Photobiol.* 1989; 49:431–438. [PubMed: 2727082]
14. Giuntini, F.; Boyle, R.; Sibrian-Vazquez, M.; Vicente, MGH. *Handbook of Porphyrin Science*. Ferreira, GC.; Kadish, KM.; Smith, KM.; Guillard, R., editors. Vol. 27. World Scientific Publishing Co; Singapore: 2014. p. 373-379.

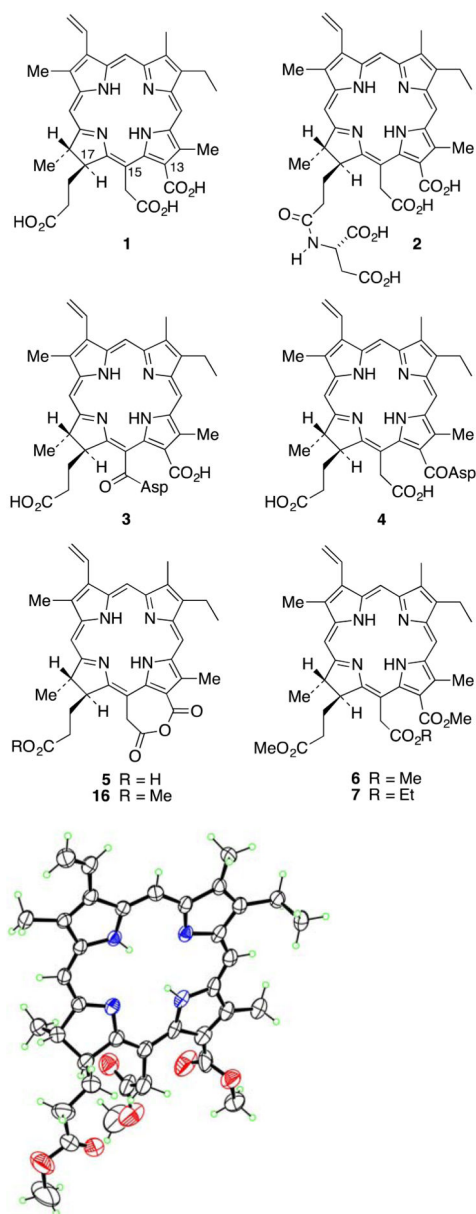


Figure 1.
X-Ray structure of chlorin e_6 trimethyl ester (**6**); one of two independent molecules, shown with 50% ellipsoids.

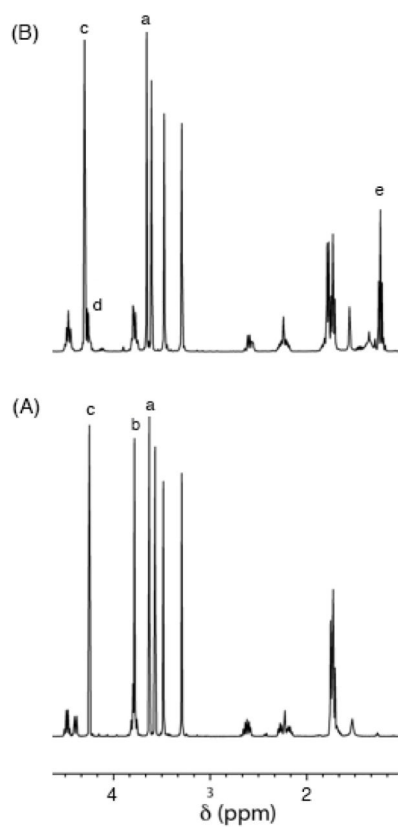


Figure 2. Proton NMR spectra, in CDCl₃, of (A) chlorin e₆ trimethyl ester (**6**), and (B) chlorin e₆ 15²-ethyl ester 13¹,17³-dimethyl ester (**7**). Assignments **a** 17³-OMe, **b** 15²-OMe, **c** 13¹-OMe, **d** 15²-OCH₂CH₃, **e** 15² OCH₂CH₃

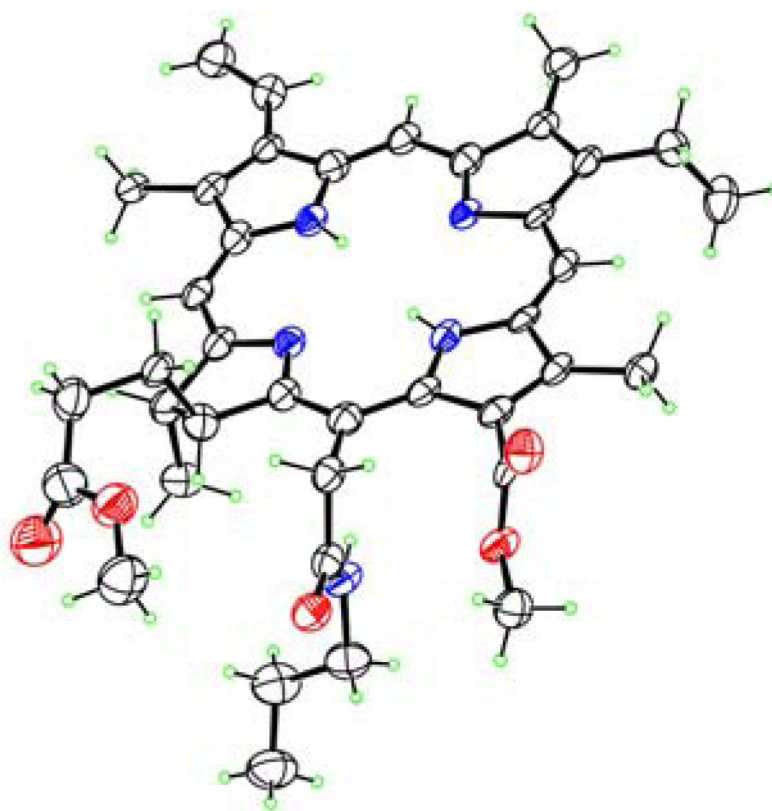


Figure 3.
X-Ray structure of the 15²-monopropanamide-13¹,15²-dimethyl ester conjugate (**8**) of chlorin e₆, one of four independent molecules, shown with 50% ellipsoids.

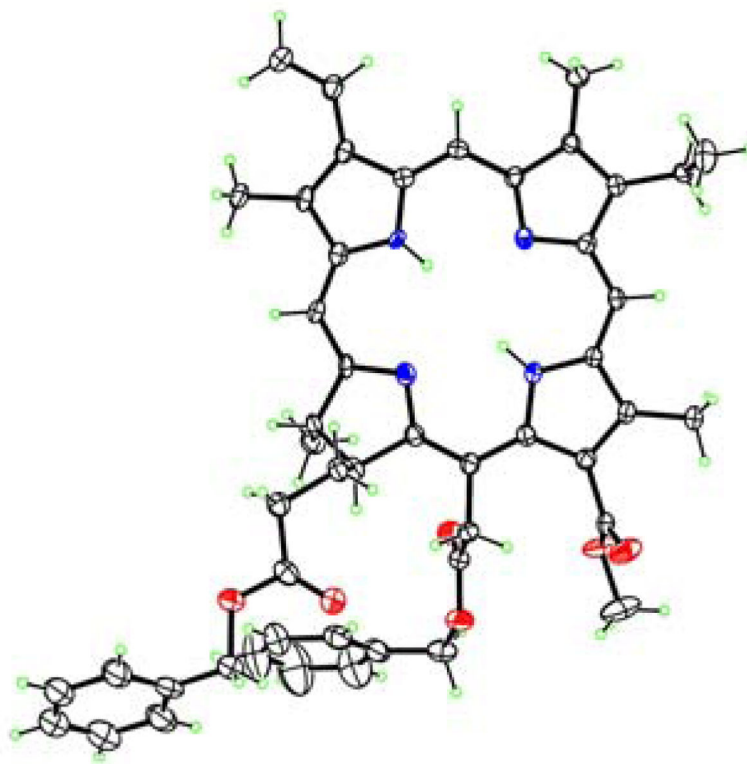


Figure 4. X-Ray structure of the chlorin e₆ 15²,17³-dibenzyl ester-13¹-methyl ester (**15**); one of four independent molecules, shown with 50% ellipsoids.



Figure 5. X-Ray structure (absolute configuration) of the chlorin e₆ 13¹:15²-anhydride 17³-methyl ester (**16**); one of two independent molecules, shown with 50% ellipsoids.

Proton NMR chemical shifts (400 MHz, CDCl₃) of methoxyl groups in products from the reaction of chlorin e₆ anhydride (**16**) with nucleophile, followed by diazomethane treatment.

Table 1

Product	R	R ¹	13 ¹ -OMe (ppm)	15 ² -OMe (ppm) ^[c]	17 ³ -OMe (ppm) ^[c]
6	OMe	Me	4.27	3.79	3.65
7	OEt	Me	4.29	-	3.66
8	NH(CH ₂) ₂ CH ₃	Me	4.29	-	3.61
9	NHCH(CH ₃) ₂	Me	4.29	-	3.60
10	NH(CH ₂) ₂ OH	Me	4.35	-	3.60
11	SPh ₂ Me	Me	4.27	-	3.60
12	OPh	Me	4.30	-	3.70
13	O(CH ₂) ₂ CHMe ₂	Me	4.28	-	3.64
14	OCH ₂ Ph	Me	4.17	-	3.62
15	OCH ₂ Ph	OCH ₂ Ph	4.17	-	-

“-” indicates not observed.