Bioorganic & Medicinal Chemistry Letters 21 (2011) 5808–5811

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of β-functionalized Temoporfin derivatives for an application in photodynamic therapy

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ARTICLE INFO

Article history:
Received 27 July 2010
Revised 27 July 2011
Accepted 29 July 2011
Available online 8 August 2011

Keywords:
Photodynamic therapy (PDT)
Photosensitizer
Photocytotoxicity
Temoporfin
Chlorins

ABSTRACT

The synthesis of novel β-functionalized derivatives of the clinically used photosensitizer Temoporfin has been achieved by nucleophilic addition reactions to a corresponding diketo chlorin. The β-substituted dihydroxychlorin products exhibit a strong absorption in the red spectral region, a high singlet oxygen quantum yield, and were found to be highly effective in in vitro assays against HT-29 tumor cells.

Photodynamic therapy (PDT) is a well established modality for selective destruction of malignant cells. After administration of the photosensitizer it is locally activated with laser light. Thus, cytotoxic singlet oxygen is produced resulting in cell damage or cell death.[1] The first generation sensitizers were based on a porphyrin core system but their efficacy is limited by the weak absorbance in the red spectral region. However, chlorins and other second generation sensitizers possess significantly stronger absorptions at longer wavelengths, thus increasing both the efficacy and the depth of effect.[2]

Chlorins can principally be obtained either by reduction or oxidation of one of the β-pyrrolic double bonds of porphyrins. For instance, simple β-unsubstituted chlorins are synthesized by reduction with in situ formed diimide.[3] However, the resulting chlorins are oxidation-susceptible and are often not easily separable from the starting porphyrin and by-products.[4] A β-unsubstituted chlorin is Temoporfin (Foscan®) which carries *meta*-hydroxyphenyl groups in *meso*-positions (Fig. 1). It is a clinically approved photosensitizer in Europe for the palliative treatment of head and neck cancer. An example for the oxidative functionalization of the porphyrin double bond is the *cis*-dihydroxylation. Although expensive and very toxic, osmium tetroxide has been established as the standard oxidizing agent for the synthesis of such β-dihydroxy-substituted chlorins.[5] Other reagents proved insufficient. We here report the 'osmium free' synthesis of novel β-substituted Temoporfin derivatives[6] by nucleophilic addition reactions to a corresponding diketo chlorin. In addition, in vitro studies were carried out to preliminarily assess the PDT efficacy of these new chlorins against HT-29 tumor cells.

Our synthetic strategy is related to a route developed by Crossley and co-workers.[7] In the present case, this approach involved preparation of the common dicarbonyl precursor **5**. Its synthesis was accomplished in four steps starting from known porphyrin **1**[8] (Scheme 1). First, selective mononitration and copper complex-

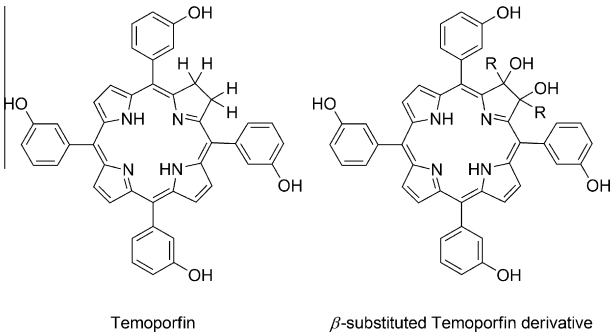

Figure 1. Structure of Temoporfin and its novel derivatives.

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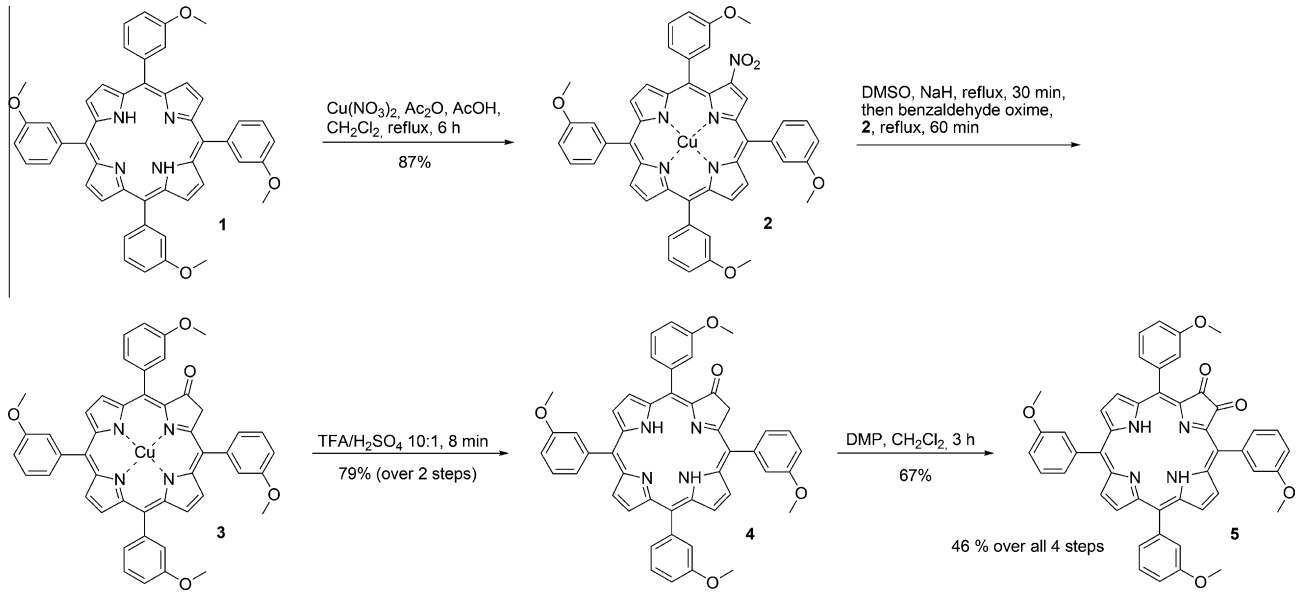
doi:10.1016/j.bmcl.2011.07.113



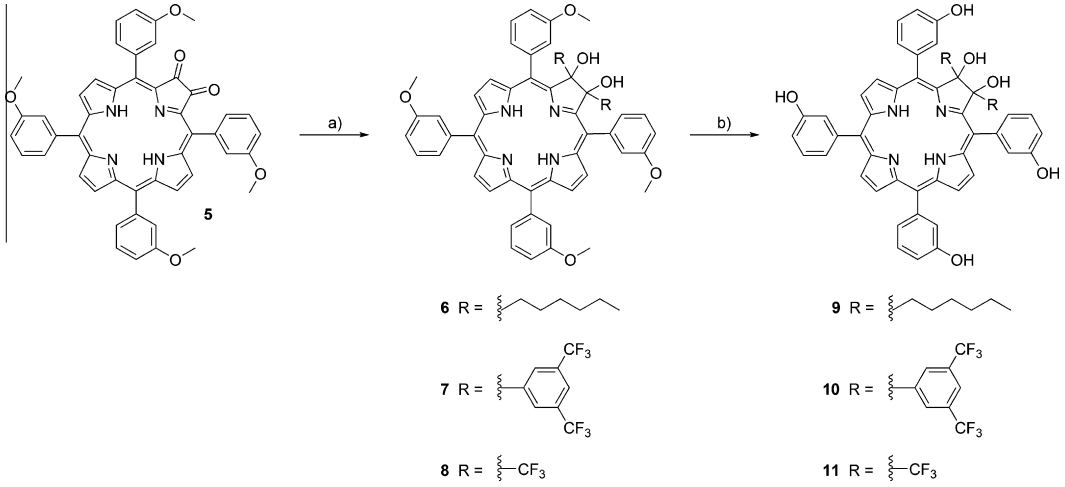
Scheme 1. Synthesis of precursor-chlorin **5**.

ation of porphyrin **1** was achieved in one step using $Cu(NO_3)_2$ in a mixture of acetic anhydride and acetic acid.[9] The nitroporphyrin copper(II)-complex **2** was obtained in 87% yield and subsequently converted to the 2-hydroxyporphyrin derivative **3** by treatment with benzaldoxime in a solution of sodium methyl-sulfinylmethanide.[10] The following decomplexation of copper(II)-porphyrin **3** using concentrated sulfuric acid in dichloromethane failed[11] and led to sulfonated products. Instead, a 10:1 mixture of trifluoroacetic acid and sulfuric acid afforded the copper-free *meta*-methoxyphenyl-substituted porphyrin derivative **4** in 79% yield over two steps. Finally, oxidation using Dess-Martin periodinane (DMP)[11] yielded the diketo chlorin **5** in 67% yield. Thus, porphyrin **1** was efficiently converted to the diketo chlorin **5** in four steps with an overall yield of 46% (Scheme 1).

With common precursor **5** in hand, the addition of nucleophilic agents was investigated. In a first experiment chlorin **5** was reacted with hexyl magnesium bromide affording the dialkylated chlorin **6** in 53% yield (Scheme 2). No mono-addition product was detected. Pleasingly, the desired double addition product was formed as a single diastereoisomer. Selective formation of the presumed *trans*-diol is believed to be favored due to steric reasons.[12]

We next focused on fluoro-substituted dihydroxy-chlorins for mainly two reasons: (i) Generally, fluorinated porphyrins exhibit higher triplet quantum yields[13] and singlet oxygen quantum yields, respectively, and (ii) the substitution with electron-withdrawing groups in β-position stabilizes the vicinal diol against pinacol–pinacolone rearrangement.[14] We therefore treated diketone **5** with 3,5-bis(trifluoromethyl)phenyl magnesium bromide and surprisingly only the two-fold addition product **7** was obtained. However, the use of the electron-deficient and very bulky Grignard reagent led to a reduced yield of only 44% (Scheme 2). In order to minimize the steric stress we inserted trifluoromethyl groups directly without any spacer by using the Ruppert–Prakash reagent CF_3SiMe_3/TBAF (Scheme 2).[15] This reaction afforded a mixture of the dihydroxychlorin and the corresponding trimethylsilyl ether. After treatment with additional TBAF the clean diol product **8** was isolated in 78% yield.[16]

Finally, chlorins **6**, **7**, and **8** were subjected to boron tribromide mediated cleavage of the phenolic methyl ethers. The corresponding *meta*-hydroxyphenyl-substituted products **9**, **10**, and **11** were obtained in yields of 57–87%.[17,18] These β-substituted chlorins were found to consist of a mixture of atropisomers because the



Scheme 2. Synthesis of Temoporfin derivatives. Reagents and conditions: (a) For the synthesis of product **6**: *n*-HexMgBr, THF, −45 °C, 3 h, 53%; for the synthesis of product **7**: $(CF_3)_2$PhMgBr, THF, −45 °C, 3 h, 44%; for the synthesis of product **8**: Me_3SiCF_3/TBAF, THF, −40 °C, 8 h, 78%. (b) **9**, **10**, **11** BBr_3, CH_2Cl_2, −50 °C, 16 h, 57–87%.

Table 1
Absorption data and $^1O_2$ yield of chlorins **9**, **10**, and **11**[a]

Sensitizer	λ_{max} ε	λ_{max} ε	λ_{max} ε	λ_{max} ε	λ_{max} ε	$^1O_2$[b]
9	418	518	544	600	653	0.97
	116800	10800	7400	4600	21900	
10	412	514	543	592	644	1.56
	213300	18300	17800	9000	25000	
11	407	518	547	599	653	1.10
	166900	15200	15600	7700	27600	

[a] Absorption spectra in acetone [λ_{max} in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$].
[b] $^1O_2$ yields (in EtOH) are given relative to $^1O_2$ quantum yield of Temoporfin.

meta-substituted aryl groups next to the oxidized pyrrolic subunit are hindered in their rotation and the substituents can point independently in the same or opposite directions.

We next investigated the photophysical properties of the synthesized dihydroxychlorins. In Table 1 the absorption data and the singlet oxygen yields (relative to $^1O_2$ quantum yield of Temoporfin) of the novel chlorins **9**, **10**, and **11** are summarized. Chlorins **9** and **11** possess a high extinction coefficient at 653 nm whereas the corresponding Q band of chlorin **10** is shifted to 644 nm. The singlet oxygen yields for compounds **9** and **11** are quite similar to that of Temoporfin (Table 1). For the trifluoromethyl-substituted chlorin **10** an increased relative quantum yield of 1.56 was observed (Table 1). Finally, the photocytotoxicity of sensitizers **9**, **10**, and **11** was evaluated in cell assays against human colon adenocarcinoma cells HT-29 (Fig. 2).[19] The assays were carried out after incubation for 24 h in 10% FCS containing medium and both the dark and the phototoxicity were determined at two different sensitizer concentrations (2 and 10 μmol). A laser with a wavelength of 652 nm at a dose rate of 50 J/cm^2 was used as the light source. The photodynamic activity was compared to the approved sensitizer Temoporfin. Chlorins **9** and **11** showed phototoxicity at both concentrations and exhibited a very similar level of activity as compared to Temoporfin. Chlorin **10** displayed a lower activity and was only effective at a concentration of 10 μmol. None of the tested sensitizers showed dark toxicity.

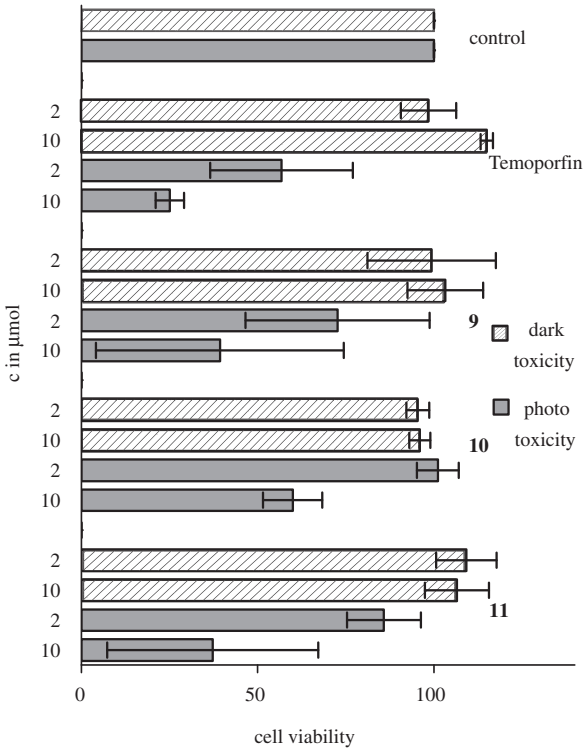


Figure 2. HT-29 cell assays.

In conclusion, an 'osmium free' strategy for the synthesis of β-functionalized Temoporfin derivatives is presented. The approach via a common diketo chlorin intermediate gives broad access to novel β-substituted dihydroxy-chlorins.[20] Alkyl-, aryl-, and trifluoromethyl groups could be used as nucleophiles and a clean double addition reaction was observed. Compared to Temoporfin, the β-substituted sensitizers possess a significantly increased chemical stability. They exhibit a comparatively strong absorption in the red spectral region, a high singlet oxygen quantum yield, and were highly effective in in vitro assays against HT-29 tumor cells.

Acknowledgments

The support of this research by the Arbeitsgemeinschaft industrieller Forschungsvereinigungen 'Otto-von-Guericke' e. V. (AiF) (Project: KF0579901UL7 and KF0249303UL7) is gratefully acknowledged.

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16. Typical procedure for the nucleophilic addition: A solution of 7,8-dioxo-5,10,15,20-tetrakis-(3-methoxyphenyl)-7,8-chlorin **5** (100 mg, 0.13 mmol) in dry tetrahydrofuran (7 mL) was cooled under an argon atmosphere to −40 °C. Trifluoromethyltrimethylsilane (350 μL, 2.66 mmol) and TBAF·3 H_2O (10 mg, 0.03 mmol) were added and the mixture was stirred for 8 h. In order to cleave the trimethylsilyl ether additional TBAF·3 H_2O (100 mg, 0.3 mmol) was added and the reaction mixture was stirred until TLC analysis showed complete conversion. Then, water (40 mL) and CH_2Cl_2 (50 mL) were added, the organic layer was separated, washed with water (40 mL), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography with CH_2Cl_2/EtOAc 99:1 as eluent. The compound 7,8-dihydroxy-5,10,15,20-tetrakis-(3-methoxyphenyl)-7,8-bis-(trifluoromethyl)-7,8-chlorin **8** was obtained after recrystallization from CH_2Cl_2/MeOH. (92 mg, 78%).
Compound **8**: mp 177 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.63-8.61 (m, 2H), 8.47

(s, 2H), 8.03–8.00 (m, 2H), 7.89–7.77 (m, 4H), 7.68–7.43 (m, 6H), 7.35–7.26 (m, 6H), 4.13–4.11 (m, 1H), 4.02–4.01 (m, 1H), 4.00–3.98 (br m, 3H), 3.98-3.97 (m, 3H), 3.91–3.89 (br m, 3H) 3.87–3.85 (m, 3H), −1.48–(−1.52) (br m, 2H); ^{13}C (126 MHz, $CDCl_3$) δ 159.02, 158.20, 153.63, 149.54, 149.17, 142.55, 141.55, 138.98, 138.82, 136.03, 133.24, 128.97, 128.48, 128.38, 127.80, 127.46, 126.91, 125.87, 125.09, 124.53, 124.46, 120.63, 119.81, 119.29, 115.40, 113.84, 111.57, 111.45, 55.61, 55.50; ^{19}F (376 MHz, $CDCl_3$) δ −73.92, −73.94, −74.12, −74.15; HRMS (ESI) *m/z* calcd for $C_{50}H_{39}F_6N_4O_6^+$ $(M+H)^+$ 905.2768. Found 905.2774.

17. Procedure for the deprotection of methyl ethers: A solution of 7,8-dihydroxy-5,10,15,20-tetrakis-(3-methoxyphenyl)-7,8-bis-(trifluoromethyl)-7,8-chlorin **8** (80 mg, 0.09 mmol) in dry CH_2Cl_2 (30 mL) was cooled under an argon atmosphere to −50 °C. A boron tribromide solution in CH_2Cl_2 (1 M, 1.6 mL) was added dropwise over a period of 10 min. The reaction mixture was allowed to warm up slowly to room temperature and stirred for 18 h. Then water (100 mL) and ethyl acetate (100 mL) were added as well as sodium hydroxide solution 30% until neutral. The organic layer was separated, washed with water (2 × 100 mL), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography with CH_2Cl_2/MeOH 95:5 as eluent. Further purification was achieved by column chromatography with C_{18} reversed phase silica gel using MeOH/H_2O 95:5 as eluent. The title compound 7,8-dihydroxy-5,10,15,20-tetrakis-(3-hydroxyphenyl)-7,8-bis-(trifluoromethyl)-7,8-chlorin **11** was obtained after recrystallization from CH_2Cl_2/aq. MeOH. (65 mg, 87%).
Compound **11**: mp >300 °C; ^1H NMR (700 MHz, $(CD_3)_2CO$) δ 8.82–8.66 (m, 6H), 8.50 (s, 2H), 8.14–8.12 (m, 2H), 7.81–7.11 (m, 16H), 5.75–5.57 (m, 2H), −1.38 to −1.42 (m, 2H); ^{13}C NMR (176 MHz, $(CD_3)_2CO$) δ 156.35, 156.30, 156.05, 155.99, 155.46, 155.41, 153.44, 150.47, 150.39, 150.28, 150.19, 142.42, 142.39, 141.68, 141.61, 141.58, 141.51, 139.91, 139.87, 135.75, 132.86, 128.15, 128.12, 128.02, 127.96, 127.90, 127.52, 127.45, 126.21, 126.00, 125.53, 125.48, 125.43, 125.38, 125.33, 125.28, 125.23, 124.37, 124.30, 121.95, 121.39, 121.35, 121.16, 115.58, 115.52, 115.47, 115.40, 115.14, 112.52, 112.44, 112.28, 112.22, 90.4–89.8; ^{19}F (471 MHz, CD_3OD δ −75.02, −75.05; HRMS (ESI) *m/z* calcd for $C_{46}H_{31}F_6N_4O_6^+$ $(M+H)^+$ 849.2142. Found 849.2163.
18. It has recently been demonstrated that *meta*-hydroxy groups in related porphyrins can efficiently be glycosylated to increase solubility and amphiphilicity: Aicher, D.; Wiehe, A.; Stark, C. B. W. *Synlett* **2010**, 395.
19. Typical procedure for in vitro biological studies: HT-29 cells were grown in DMEM (cc-pro GmbH) supplemented with 10% heat-inactivated fetal calf serum (FCS, cc-pro GmbH), 1% penicillin (10000 IU) and streptomycin (10 000 μg/ml, cc-pro GmbH).
A photosensitizer stock solution (2 mM) was prepared in DMSO and was kept in the dark at 4 °C. Further dilution was performed in RPMI 1640 medium (without phenol red) supplemented with 10% FCS to reach a final photosensitizer concentration of 2 or 10 μM, respectively.
2×10^4 cells/well were seeded in micro plates and incubated for 24 h with fresh medium (RPMI without phenol red) containing 10% FCS with 2 or 10 μM of the photosensitizer. For photosensitization, cells were irridiated at room temperature with a 652 nm diode laser (Ceralas PDT 652, biolitec AG) at a fixed fluence rate of 100 mW/cm^2 (50 J/cm^2).
The cell viability was assessed using the XTT assay and the absorbance of the samples was measured with a spectrophotometer (Bio-Kinetics Reader EL312 e; Bio-Tek Instruments Inc.) at a wavelength of 490 nm. In order to measure reference absorbance (to measure non-specific readings) a wavelength of 630–690 nm was used.
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