Journal of Photoscience (2002), Vol. 9(2), pp. 178–181

The Synthetic Approaches to Modify Methyl (Pyro)pheophorbide a

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Pyropheophorbide and pheophorbide-photosensitizers as chlorin analogues are promising new compounds for PDT because the chlorin analogues are activated with much longer red light at > 670nm and produce less long-term normal tissue phototoxicity than Photofrin. The various chlorin derivatives can be obtained by modifying peripheral substituted group among which meso-H, vinyl group and exocyclic ring are the most active positions. These characteristics prompted us to introduce various groups for constructing modified pyropheophorbide and pheophorbide a compounds. A stereospecific introduction of various double bonds at 3-position was performed to methylpheophorbide a to give a long hydrophobic moiety and cyclic derivatives. Chlorin-C_{60} dyad and chlorin- C_{60}–porphyrin triad also were easily prepared by the reaction of terminal aldehyde of methyl pyropheophorbide a. For the reaction on meso δ-position, bromination and Vismeier formylation can occur. N,N-dimethylaminoacrolein also reacted on δ-position and was cyclized to isobacteriochlorin, but other modification has not been succeeded. Exocyclic keto function was also modified to give purpurin derivatives, bicyclic and spiro compounds. In this presentation we report a series of modified pyropheophorbide and pheophorbide a derivatives.

Key words : photodynamic therapy, porphyrins, chlorines, (pyro)pheophorbide a, photosensitizer, *Spirulina*, tumor

INTRODUCTION

Lack of selectivity between normal and tumor tissues largely limited the use and efficiency of chemotherapeutic drugs in clinical therapy.

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Photodynamic therapy (PDT) is other kind of medical treatment which employs light and drugs, tumor-localizing photosensitizer, to bring about a cytotoxic or modifying effect to cancerous or otherwise unwanted tissue.

In this therapy, the properties of tumor localization of porphyrin-based analogues and photochemical generation of reactive oxygen

species are combined with precise delivering of laser-generated light to produce a treatment that offers selective tumorcidal action.

Although many porphyrin derivatives are widely used as photosensitizer in photodynamic therapy, so far Photofrin (a hematoporphyrin derivatives) is the only drug that has been approved worldwide for the treatment of a variety of cancers because of some drawback associated with Photofrin and other porphyrin-based photosensitizer, such as purified form, retention in skin and their weak absorption in the long-wavelenghth region. So, the emphasis for development of new drugs has been concentrated on molecular design, chemical synthesis and biological action for porphyrin derivatives.

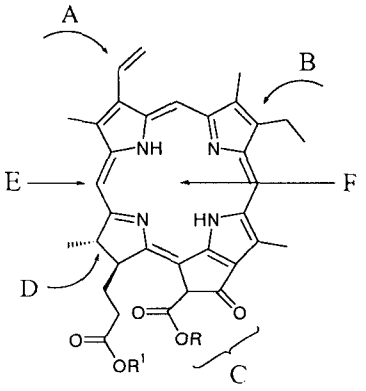
In recent years natural chlorin pigments, which stand in marked contrast to symmetric porphyrin pigment due to substantially stabilized S_1 energies, a strong Qy absorption band, and unique redox reactivates, were chosen to construct chlorin and bacteriochlorin based dyads with variable geometry and distances between the chromophores [1] .

MATERIALS AND METHODS

For our studies, methyl pyropheophorbide a (MPPa) or methyl pheophorbide a (MPa) were used as staring materials. MPa was obtained from alga *Spirulina maxima* extracts with methanol and 5% sulfuric acid. MPPa was transformed via decarbonylation of MPa with reflux in organic base solvent.

The 3-vinyl group of MPP a was reacted with OsO_4 in base condition followed by glycol cleavage, using sodium periodate on silica gel, to give the methyl 3-formyl chlorin. 3-Acetyl chlorin was obtained by brominaton, hydroxylation and oxidation by tetrapropylammonium perruthenate and N-methyl-morpholine to give keto acetal 3.

The Grignard reactions were performed on these acetyl and formyl chlorin to give various alkene derivatives. As we expected all the alkenes can be controlled to give pure trans isomers by elaborating temperature or by using trifluoromethene sulfonic acid.



Chemically labile positions

The Grignard reaction on acetyl chlorin with straight chain and subsquent dehydration reaction gave a full mixture of olefins, but with cyclic Grignard reagent such as cyclopentyl, cyclohexyl and cycloheptyl gave the only product after dehydration, the terminal olefin.

The synthesis of pure terminal olefin was performed with the Witting type carbonyl methylenation by means of CH_2Br_2-Zn and $TiCl_4$ system from the acetyl, which was originated by Grignard reaction and subsequent oxidation of MPPd. The Grignard reaction on acetyl chlorin with ethylmagnesium bromide and 1-propynyl magnesium bromide followed by dehydration of

alcohol in benzene at 90°C gave a product having alkyne-substituted terminal olefin compound. If the Grignard reagent having a long alkyl chain, an unexpected benzoporphyrin type fused-ring compound was obtained along with the normal dehydrated product.

By treatment with BuLi, gem-dibromoalkene Zn-chlorins were rearranged to the corresponding 3-alkyne substituted derivative. Another conventional method for the synthesis of alkyne-substituted derivatives from ketone was attempted.

When ketone was treated with the onium salt of azaaromatics, 2-chloro-3-ethylbenzoxazolium tetrafluroborate, and triethylamine in dichlormethane at room temperature, the same alkyne derivatives were obtained.

Making use of two functional groups in E-ring of MPa to synthesize pyrazole-fused derivatives of MPPa was performed by treating phenylhydrazine with MPa in dichlormethane containing several drops of TFA. In this reaction phenylhydrazone was readily generated, but cyclization to pyrazole gave unexpectedly a decarbomethoxylated product. The reaction between MPa and urea with NaOBu in BuOH expecting pyrimidine-fused derivatives, but rearranged product purpurin-18 was generated instead [2].

Treatment of MPPa with thallium(III) nitrate gave aldehyde which was reacted with CBr_4/PPh_3 in anhydrous benzene for 12 hours, the dibromoalkene was isolated. The indole derivatives, 3-(3-indolyl)-3-devinyl derivative of MPPa, was obtained from the reaction of aldehyde with phenylhydrazine in AcOH followed by a subsequent rearrangement.

Although there are many reports for the synthesis of pyrroles, the Knorr method is extensively used for this purpose. Pederson et al reported the regioseslective synthesis of pyrroles by $NbCl_3$(DME) via the coupling of unsaturated imines or N,N-dimethylformamide [3].

When MPPa was treated with NaN_3 in methylenechloride with acid at 45°C, the rearrangement was completed to offer triazole ring at 3-position and lactam ring at E-ring..

The oximes of MPPa was prepared in the way that MPPa was treated with hydroxylamine hydrochloride and sodium hydroxide in EtOH to give a mixture of *Z,E*-oxime of pyropheophorbide a. The methylation on oximes with with 5% H_2SO_4 in MeOH, two isomers can be separated from each other by column. The oximes were subjected to Beckmann rearrangement in formic acid, resulted the unique lactams respectively.

The existence of three-membered carbon-nitrogen-oxygen ring has frequently been postulated in the older literatures mainly as a means of solving certain structural problems. In connection with our investigation of synthesis of derivatives of MPPa, we have observed oxaziranes when MPPa was reacted with NH_2OH.

On treatment MPPa with NH_2OH at high temperature the spiro oxazirane compounds were formed in good yield. After acylation with benzoyl chloride, the acylated products were obtained and easily identified.

Methyl Ni-3-(1-hydroxyethyl)-3-devinylpyropheophorbide a which was obtained by treatment with $Ni(AcO)_2$ in chloform, was reacted with 3-dimethylamino-acrolein (3-DMA) in the presence of phosphoryl chloride to give

mainly δ-acrolein chlorin, which can transformed into C_{60} dyad.

B ring of chlorin is also easily oxidized with OsO_4 to give vicinal diol, bacteriochlorin derivatives, which shifts Q-band to 770nm. The introduction of additional six-membered exocycle into the main macrocycle leads a further bathochromic shift to 818nm [4]

When chlorin was subjected to DDQ, lactone was formed on the D ring in moderate yield. This lactone was transformed smoothly to 18-hydroxy chlorin.

Most of metals can be cooperated into chlorin nucleus. According to the metals, PDT efficiency and the tumor cell selectivity differs. The strong fluorescence of chlorin compounds also serve an ideal tool for tumor diagnosis, Photodynamic Diagnosis (PDD).

RESULTS AND DISCUSSION

The Grignard reaction between carbonyl group and nucleophilic regent is an important method for introducing long chain alkyl group on the peripheral positions. Like other aromatic ring, the carbonyl group conjugated with chlorin chromophore on fused cyclopentane ring or on the newly introduced carbonyl position can take place this nucleophilic addition. Modified with long alkyl chain and heterocyclic groups helps the binding affinity to the Human Serum Albumin which leads high efficiency as new photosensitizers. Exocyclic functionality serves also various reactions to give chemically interested structures. The separation of structural isomers at the exocyclic derivatives possibly depends on the rigid macrocyclic backbones with firmly oriented saturated D ring. The new reactions on B ring and D ring opens possible new good candidate of photosensitizers with much longer absorption wavelength.

Metallochlorins also open wide door to the tumor cell selectivity and lead compounds for the next generation of PDD.

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