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A MILD METHOD FOR THE INTRODUCTION OF MAGNESIUM INTO BACTERIOPHEOPHYTIN *a*

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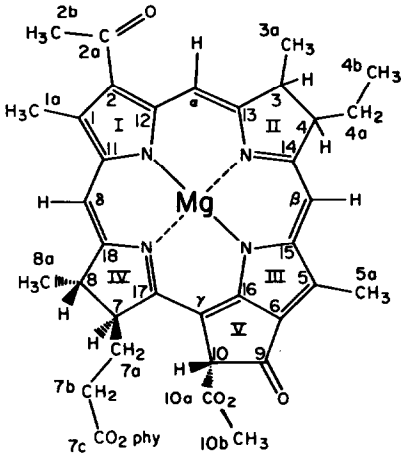
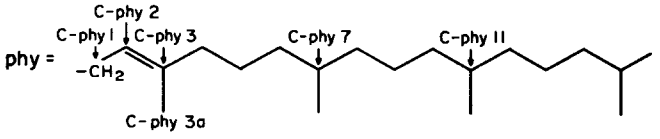
In conjunction with recent work concerning bacterial photosynthesis we have been engaged in the synthesis of several new derivatives of bacteriochlorophyll *a*, BChl *a*. The synthetic pathways leading to derivatives of BChl *a* often involve one or more steps requiring acidic conditions. However, at low pH the central magnesium atom of BChl *a* is expelled to yield bacteriopheophytin *a*, BPh *a*. The re-introduction of magnesium into the pheophytins of the photosynthetic pigments has remained until very recently a major problem in the preparation of derivatives of the magnesium containing pigments.[1] The principal source of this difficulty is the reactivity of the β keto ester in moderately strained ring V.





This functionality is particularly susceptible both to attack by nucleophiles on the carbonyl group at C-9 and oxidation at C-10 in the presence of strong base.[2] In addition, the methyl ketone at C-2 is vulnerable to nucleophilic attack. Nevertheless, in order to insert magnesium into chlorins and bacteriochlorins strong bases are necessary.[3]

Recently, Eschenmoser and his students prepared a versatile new reagent for the introduction of magnesium into pheophytins and their derivatives.[1] The reagent, iodomagnesium 2,6-di-tert-butyl-4-methylphenolate, metallates derivatives of the pheophytin *a* series rapidly at room temperature or slightly above, provided that the reaction is carried out in a solvent that does not strongly coordinate to magnesium. Higher temperatures are required to effect similar chemistry in the pheophytin *b* series. However, exposure of methyl bacteriopheophorbide *a* to this reagent in refluxing thiophene (80°) does not result in metallation of the macrocyclic ligand. The protons bonded to the pyrrolic nitrogen atoms of bacteriochlorins are apparently much less acidic than those of chlorins. Magnesium insertion is promoted by the addition of a somewhat stronger base, lithium 2,6-di-tert-butyl-4-methylphenolate, to the reaction mixture, followed by reflux at 80°. Nevertheless, approximately 60% of the methyl bacteriopheophorbide *a* is destroyed under these reaction conditions. This situation is undesireable when dealing with small amounts of scarce BPh *a* derivatives.

It occurred to us that the use of a strong, proton selective base in the presence of the Eschenmoser magnesium reagent under mild conditions might effect magnesium insertion without destroying the bulk of the bacteriochlorin. Ideally the desired base must be capable of proton transfer alone and remain completely non-nucleophilic. These requirements are satisfied by lithium 2,2,6,6-tetramethylpiperidide. The proton selective characteristics of this base have been examined extensively by Olofson.[4] We have found that a reagent consisting of equimolar amounts of this base and the Eschenmoser magnesium reagent metallates BPh *a* at room temperature. Reaction of BPh *a* with a 15-fold excess of this modified reagent for 30 minutes yielded 52% BChl *a*, and of equal importance returned 41% unchanged BPh *a*. The remaining few percent of altered pigment is easily separated from the product and starting material by column chromatography. The spectroscopic properties of both the BChl *a* and the residual BPh *a* are identical in every respect to those of authentic samples of BChl *a* and BPh *a* obtained from *R. spheroides*.

The molar excess of mixed reagent employed in this procedure is dictated both by the presence of acidic protons at four positions (2b, 10, NH) in BPh *a* and by competition between metallation and side product formation. Larger excesses of mixed reagent and/or longer reaction times result in significant amounts of side products, while smaller excesses and/or shorter reaction times yield less BChl *a*.

A typical procedure is as follows: The preparation was performed in a glovebox filled with dry nitrogen. A grignard reagent was prepared from magnesium (24.0 mg, 1.0 mmol), methyl iodide (62 μℓ, 1.0 mmol), and dry ether (1 mℓ). A solution of 2,6-di-*tert*-butyl-4-methylphenol (242 mg, 1.1 mmol) in dry thiophene[5] (15 mℓ) was deoxygenated by bubbling dry helium (99.99%) through the solution. The solution of the phenol was stirred vigorously and protected from light. The entire Grignard solution was pipetted all at once into the phenol solution. The resulting mixture was allowed to stir for 15 minutes. A solution of lithium 2,2,6,6-tetramethylpiperidide, prepared by dissolving the corresponding amine[6] (170 μℓ, 1.0 mmol) in ether (1 mℓ) followed by careful addition of n-butyllithium (0.5 mℓ of 1.6 M solution in hexane, 0.8 mmol), was syringed into the reaction mixture. After 5 minutes of stirring, a solution of BPh *a*[7] (60.0 mg, 0.068 mmol) in dry thiophene (∿5 mℓ), deoxygenated with dry helium, was syringed into the reagent mixture. The reaction proceeded for 30 minutes at room temperature. The resultant dark green solution was poured all at once into pH 4.5 phosphate buffer. Extraction of the aqueous mixture with ether, followed by washing of the extract 3 times with additional buffer, drying over anhydrous sodium sulfate, and evaporation of the solvent yielded a bluish-purple residue. This residue was chromatographed on a 8 cm x 30 cm column of powdered sugar, elution with 0.5% n-propanol in petroleum ether. A single pink band eluted, followed by a single blue band. Collection of the two components resulted in 32.0 mg (52%) of BChl *a* and 24.5 mg (41%) of BPh *a*. Spectral Data - Bchl *a*: ^{1}H nmr (220 MHz, 5% pyridine-d_5 in benzene-d_6) δ 9.72 (s, α), 8.48 (s, β), 8.25 (s, δ), 6.35 (s, 10-H), 5.38 (m, phy-2), 4.60 (m, phy-1), 4.14-3.62 (m, 3,4,7,8-H), 3.49 (s, 10a-CH_3), 3.47 (s, 1-CH_3), 3.20 (s, 5-CH_3), 2.83 (s, 2-CH_3), 2.76-1.95 (m, 7a, 7b, 4a-H), 1.81 (t, J = 8 Hz, 4b-CH_3), 1.56 (d, J = 7 Hz, 3,8-CH_3), 1.27-0.90 (m, phytol), 0.79 (m, phy-3,7,11,15-CH_3); electronic transition spectrum (in ether) λ_{max}, (ε) 774 (94170), 720 sh (12240), 576 (21650), 535 sh (3760),

390 (53670), 359 (79100). BPh *a*: ^{1}H nmr (220 MHz, chloroform-d) δ 9.03 (s, α), 8.54 (s, β), 8.43 (s, δ), 6.10 (s, 10-H), 5.20 (m, phy-2), 4.48 (m, phy-1), 4.26 (m, 3,4-H or 7,8-H), 4.03 (m, 3,4-H or 7,8-H), 3.84 (s, 10a-H), 3.49 (s, 1-CH_3), 3.46 (s, 5-CH_3), 3.16 (s, 2-CH_3), 2.60-2.00 (m, 7a, 7b, 4a-H), 1.89 (t, J = 8 Hz, 4b-CH_3), 1.73 (d, J = 7 Hz, 3 or 8-CH_3), 1.68 (d, J = 7 Hz, 3 or 8-CH_3), 1.38-0.87 (m, phytol), 0.81 (m, phy-3,7, 11,15-CH_3); electronic transition spectrum (in ether) λ_{max}, (ε) 749 (49120), 677 (6930), 524 (19300), 490 sh (3880), 384 (44700), 357 (80560).

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5) Thiophene (Aldrich gold label) was purified by refluxing over potassium metal for 4 hours, followed by distillation, and storage over Linde 3A molecular sieves.

6) 2,2,6,6-Tetramethylpiperidine was purified by refluxing over potassium hydroxide for 1 hour , followed by distillation, deoxygenation with dry nitrogen, and storage over Linde 3A molecular sieves.

7) BPh *a* was prepared by treatment of an ether solution of BChl *a* with a few drops of trifluoroacetic acid, followed by aqueous wash to remove the acid, drying over anhydrous sodium sulfate, evaporation of the solvent, and precipitation from CH_2Cl_2 - petroleum ether. The BChl *a* was obtained from *R. spheroides* by the procedure of H. H. Strain and W. A. Svec, "The Chlorophylls", (L. P. Vernon and G. R. Seely, Eds.), p. 59, Academic Press, New York, 1966.