Pergamon

Tetrahedron Letters, Vol. 35, No. 30, pp. 5347-5350, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01057-9

SYNTHESIS OF BENZOCHLORIN IMINIUM SALTS WITH IMPROVED PHOTOSENSITIZING PROPERTIES

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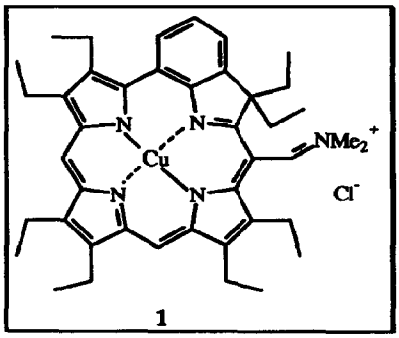
Abstract: *Treatment of a nickel benzochlorin iminium salt with conc. sulfuric acid generates the free base analogs and their sulfonated derivatives. Insertion of zinc lead to the formation of metallo-benzochlorins with improved photodynamic activities.*

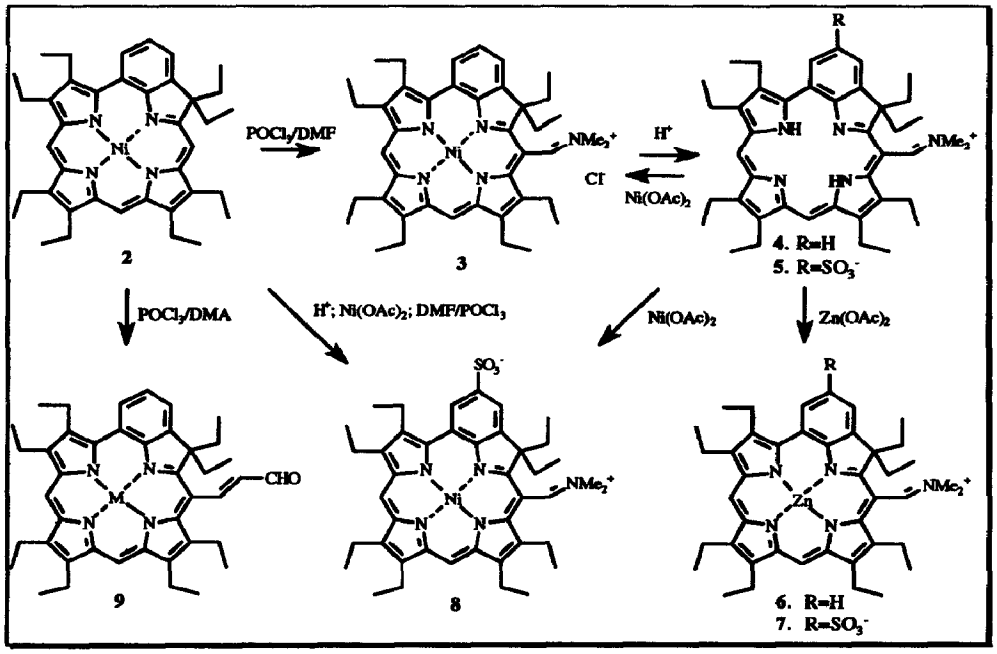
We have reported that the copper octaethylbenzochlorin iminium salt **1** demonstrated unexpected oxygen dependent photoactivity following irradiation with light from either an Alexandrite laser or a xenon arc lamp.[1] We also reported that this activity suggested a use for **1** as a photosensitizer in photodynamic therapy. This is a treatment modality in which a sensitizer is selectively localized in neoplastic tissue and the target site then irradiated with light at a wavelength at which the sensitizer absorbs. The subsequent generation of species such as superoxide, hydroxyl radicals and/or singlet oxygen could lead to irreversible cell damage and hence to necrosis of the lesion. In more recent studies it was proposed that the photochemical process did not involve singlet oxygen generation (Type II mechanism) but rather an electron transfer (Type I mechanism).[2] It was also reported that the presence of both the copper and the iminium functionality of **1** are necessary for photodynamic activation.[3] In this latter study however, only nickel and copper complexes were evaluated. We reasoned that the preparation of the metal-free analog of **1**, or indeed of metallo-derivatives such as zinc would result in a longer lived triplet excited state which may react by a Type II mechanism rather than a Type I mechanism. Since Type II mechanisms involve energy transfer rather than electron transfer, the absorbing molecule is regenerated and can then absorb more photons to produce additional singlet oxygen. Assuming that the sensitizer is not itself destroyed by singlet oxygen, lower concentrations may be used to generate an equivalent amount of oxidative damage.



1

We here report that nickel octaethylbenzochlorin iminium salt **3**, prepared by reaction of nickel octaethylbenzochlorin **2**[4] with Vilsmeier reagent can be demetallated by prolonged treatment with

concentrated sulfuric acid. Two products were formed. The first was eluted from a silica gel column with 8% methanol/dichloromethane and the second, with 12% methanol/ dichloromethane. The first fraction was shown to be the expected metal-free analog **4** by ^{1}H NMR spectroscopy where resonances for the inner -NH protons were observed at δ= 5.99ppm as expected due to the ring electron withdrawing properties of the iminium group. Of particular interest was the UV/vis spectrum of **4** which included two major absorption bands at 795nm (Q band) and 387nm (Soret band), both of similar intensity. Thus, these benzochlorin iminium salts behave as typical chlorins when metallated, i.e. incorporation of a metal results in a blue shift of the Q band, rather than as benzochlorins where the opposite trend is observed.[5] The second fraction **5** gave a similar ^{1}H NMR spectrum to that of **4** except that one benzenoid proton resonance was absent while the remaining two exhibited a very weak long range coupling. We have previously reported that this is consistent with the presence of a sulfonate group in the *para*-position.[5] In the present case, mass spectrometry data were also consistent with the formulation of the product as iminium salt **5**.



Treatment of **4** with nickel (II)acetate regenerated **3** in good yield while the corresponding sulfonated analog **8** (λ_{max} 778nm) could be prepared either by nickel insertion into the sulfonated free-base iminium salt **5** or by sequential treatment of the nickel salt **2** with sulfuric acid, nickel acetate and Vilsmeier reagent, thus providing chemical proof of the structures of these derivatives. Of greater importance for the generation of potential photosensitizing agents was the preparation of the zinc

derivatives **6** and **7** of the free-base and sulfonated iminium salts **4** and **5** respectively. Both were easily prepared in good yield by treatment of the free-base iminium salts with zinc acetate. As expected, in each case zinc insertion was accompanied by a blue shift of the Q band in the visible spectrum, from 795nm to 738nm in the case of zinc octaethylbenzochlorin iminium salt **6** and from 795nm to 721nm in the case of the sulfonated analog **7**.

Attempts were also made to prepare iminium salts with visible absorptions further red shifted by using the modified Vilsmeier reagent generated from phosphorus oxychloride and dimethyl amino-acrolein. Reaction of the nickel benzochlorin **2** with this reagent generated the expected iminium complex, as evidenced by the appearance of a visible absorption band at 816nm, red shifted due to increased conjugation. However all attempts to isolate and purify the product were accompanied by rapid hydrolysis of the iminium salt to the 2-formylvinyl derivative **9**. Evidence for this transformation included a shift in the Q band to 732nm and the presence, in the ^{1}H NMR spectrum of a resonance (doublet) attributable to the formyl proton at δ= 9.55ppm.[6]

In vitro and *in vivo* studies with the free-base and zinc derivatives **4**, **5** and **6** are currently in progress and will be reported elsewhere. However it is encouraging to note that a determination of the intracellular concentrations of each sensitizer needed to cause a 50% decrease in the viability of mouse leukemic L1210 cells upon irradiation were: **1**: 2100μM; **4**: 13.5μM; **5**: 20μM; **6**: 385μM.[7] Thus our original premise that removal of the coordinated copper should increase the potential for photodamage appears to be correct with the free-base iminium salts **4** and **5** some 20 times as active as the copper salt **1**.

ACKNOWLEDGEMENTS: This research was supported in part by DHHS grant P01 CA48733.

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SELECTED SPECTROSCOPIC DATA:

4: ^{1}H NMR ($CDCl_3$), δ= 9.93 (s, 1H, CH=N); 8.72 (d, 1H, benzenoid-H); 8.47, 8.22 (s, 2H, *meso*-H); 7.78 (m, 2H, benzenoid-H); 5.98 (br s, 2H, -NH); 4.10 (s, 3H, N-CH_3); 3.50-3.00 (m, 12H, ring ethyl CH_2); 2.85, 2.60 (m, 4H, geminal ethyl CH_2); 2.75 (s, 3H, N-CH_3); 1.8-1.5 (m, 18H, ring ethyl CH_3); 0.15, -0.10 (t, 6H, geminal ethyl CH_3). UV/Vis (CH_2Cl_2) 388, 606, 798nm; ε ($M^{-1}cm^{-1}$) 52,310; 9,670; 49,000. Mass m/e 628. Anal. calcd for $C_{42}H_{55}N_5SO_4.H_2O$: C, 67.75; H, 7.66; N, 9.41. Found: C, 67.39; H, 7.49; N, 9.48.

5: ^{1}H NMR (MeOD), δ= 9.78 (s, 1H, CH=N); 9.62 (d, 1H, benzenoid-H); 8.50, 8.32 (s, 2H, *meso*-H); 8.60 (d, 1H, benzenoid-H); 6.00 (s, 2H, -NH); 4.20 (s, 3H, N-CH_3); 3.60-3.15 (m, 12H, ring ethyl CH_2); 3.00 (m, 2H, geminal CH_2); 2.78 (s, 3H, N-CH_3); 2.70 (m, 2H, geminal CH_2); 1.70-1.35 (m, 21H, ring ethyl CH_3); 0.15, -0.05 (t, 6H, geminal CH_3). UV/Vis (MeOH) 388, 610, 800nm; ε ($M^{-1}cm^{-1}$) 55,442; 9.887; 33,802. Mass m/e 708. Anal. calcd for $C_{42}H_{54}N_5S_2O_7Na$: C, 60.92; H, 6.57; N, 8.46. Found: C, 60.99; H, 6.46; N, 8.47.

6: ^{1}H NMR ($CDCl_3$), δ= 9.41 (s, 1H, CH=N); 8.31 (d, 1H, benzenoid-H); 7.98, 7.72 (s, 2H, *meso*-H); 7.65 (t, 1H, benzenoid-H); 7.58 (d, 1H, benzenoid-H); 3.87 (s, 3H, N-CH_3); 3.20-2.80 (m, 12H, ring ethyl CH_2); 2.70 (s, 3H, N-CH_3); 2.65-2.20 (m, 4H, geminal CH_2); 1.50-1.10 (m, 18H, ring ethyl CH_3); 0.05 (m, 6H, geminal CH_3). UV/Vis (CH_2Cl_2) 384, 449, 575, 740; ε ($M^{-1}cm^{-1}$) 65,083; 37,697; 7,720; 43,697. Mass m/e 691. Anal. calcd for $C_{42}H_{52}N_5SO_4Zn$: C, 64.00; H, 6.65; N, 8.88. Found: C, 64.42; H, 7.01; N, 9.00.

7: ^{1}H NMR (MeOD), δ= 9.35 (s, 1H, CH=N); 8.85 (d, 1H, benzenoid-H); 8.15, 8.11 (s, 2H, *meso*-H); 7.95 (d, 1H, benzenoid-H); 3.65 (s, 3H, N-CH_3); 3.20-3.00 (m, 12H, ring ethyl CH_2); 2.80 (s, 3H, N-CH_3); 2.80-2.30 (m, 4H, geminal CH_2); 1.52-1.10 (m, 18H, ring ethyl CH_3); 0.20, 0.10 (t, 6H, geminal CH_3). UV/Vis (MeOH) 382, 444, 722; ε ($M^{-1}cm^{-1}$) 55,901; 33,450; 36,482. Mass m/e 770. Anal. calcd for $C_{42}H_{51}N_5S_2O_7NaZn$: C, 56.66; H, 5.77; N, 7.87. Found: C, 56.39; H, 5.78; N, 7.44.

(*Received in USA* 9 *May* 1994; *revised* 27 *May* 1994; *accepted* 31 *May* 1994)