Tetrahedron Letters,Vol.27,No.30,pp 3521-3524,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

EFFICIENT METALLOPORPHYRIN SYNTHESIS UNDER MILD CONDITIONS USING N-BENZYLPORPHYRINS.

David K. Lavallee,* Adrian White,* Alan Diaz,* Jean-Paul Battioni and Daniel Mansuy****

(*) Chemistry Department, Hunter College of the City University of New York, 695 Park Ave., New York, New York 10021 (U.S.A.) and (**) Université René Descartes, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UA 400, 45 rue des Saints-Pères, 75270 Paris, (France)

Abstract: A general method has been developed for nearly quantitative synthesis of N-benzylporphyrins by use of benzyldiphenylsulfonium tetrafluoroborate. The N-benzylporphyrins (including natural, synthetic, water soluble and non-water soluble porphyrins) insert Cu(II), Co(II), Pd(II) and Ni(II) rapidly and then readily lose the N-benzyl group to give non-N-substituted metalloporphyrins. The reactions of Cu(II), Co(II) and Pd(II) are efficient under mild conditions compatible with biological preparations.

In order to prepare radiolabelled porphyrin complexes of metal nuclides with short half-lives for medical applications, we have sought more rapid and quantitative synthetic methods which would allow for insertion of nuclides at low concentration without subsequent separation. Three features of the chemistry of N-substituted porphyrins have led us to investigate the possibility that two steps of metalation of an N-substituted porphyrin and subsequent dealkylation could occur faster than one step metalation of a non-N-substituted porphyrin: 1) the N-substituted porphyrins react rapidly with metal ions, as much as 10^5 times faster than corresponding non-N-substituted porphyrins,[1] 2) the N-substituent of these complexes can be removed to produce the non-N-substituted metalloporphyrin,[2] and 3) the p-nitrobenzyl substituent is readily removed by nucleophiles, including water.[2] Until now, however, the only synthetic method for N-benzylporphyrins was limited to N-p-nitrobenzyl derivatives of meso-substituted porphyrins and does not proceed in high yield.[3] Herein we report that 1) the use of benzyldiphenylsulfonium salts provides a convenient, general method for synthesizing N-benzylporphyrins and 2) the reactions of N-benzylporphyrins with metal ions are fast, providing an efficient synthesis of metalloporphyrins.

Two methods provide yields of N-benzylporphyrins in excess of 90%. (Spectral characteristics are given in Table I. A typical preparation of N-BzHPP DME[4] follows). In the first, H_2PP DME and a 10% excess of benzyldiphenylsulfonium tetrafluoroborate, [5] (typically 2 x 10^{-3} to 10^{-2} M) were stirred at room temperature overnight. In the second, the reagents were combined at the same relative amounts (10^{-2} M, 250 mg of H_2PP DME and 175 mg of the sulfonium salt in CH_2Cl_2) in a glass tube with Teflon^{TM} stopper and heated at 110° C for 2h. In each procedure, the reaction mixture was neutralized with 1 M aqueous ammonia and extracted with water. The product was isolated by column chromatography using silica gel (or neutral alumina for tetraphenylporphyrin derivatives) with CH_2Cl_2 and CH_2Cl_2/CH_3CN mixtures as eluents. In both cases, the product before chromatography often appears pure by spectrophotometry and TLC and isolated yields of over 90% are typical.

Table 1. Spectral Characteristics of Some N-benzylporphyrins[a]

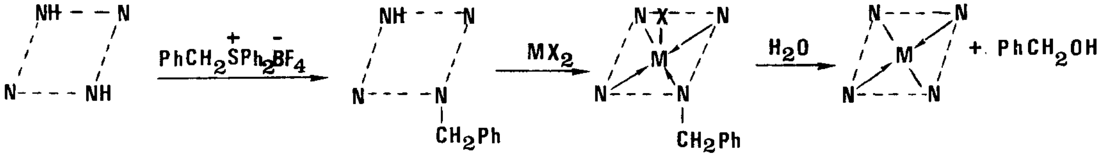
Porphyrin	UV-Visible Spectra: Maxima,nm (Log of Molar Absorbance)
N-benzylprotoporphyrin IX dimethyl ester[b,c]	417 (5.04), 511 (4.04), 544 (3.83), 593 (3.65), 627 (3.34), 651 (3.34)
N-benzyltetraphenylporphine	434 (5.59), 533 (4.11), 573 (4.34), 615 (3.85), 675 (3.82)
N-p-nitrobenzyltetrakis-(p-sulfonatophenyl)porphine^{4-}	435 (5.43), 540 (3.94), 584 (4.20), 610 (3.97), 672 (3.78)
N-benzyltetrakis(p-carboxyphenyl)porphine^{4-}	437 (5.28), 536 (3.96), 578 (4.12), 618 (sh), 678 (3.66)

a) Satisfactory analyses and ^1H nmr spectra were obtained. b) Similar spectra are observed for N-benzyldeuteroporphyrin IX DME and N-benzylmesoporphyrin IX DME. c) the ^1H nmr spectrum shows 25% of each isomer: CH_2 (Bz) 4 sets of double doublets, -4 to -4.4 ppm.

The synthesis of metal complexes of PP DME using N-BzHPP DME on a 50 mg scale were run at 10^{-2} M and a two-fold excess of the appropriate chloride salt of the metal in refluxing methanol. Aliquots were periodically withdrawn and injected into cold methanol to quench the reaction. Products were obtained at the completion of reaction by extraction into CH_2Cl_2 after addition of water to the reaction mixture, filtration, and crystallization from CH_2Cl_2/CH_3CN mixtures. Reactions to form complexes of $TPPC_4$ were carried out at 90°C in 10^{-2} M acetate buffer (pH 6) or in 0.1 M borate buffer (pH 7-8). The tetrakis(p-carboxyphenyl)porphyrin complexes were isolated by acidifying the solution to pH 4 with acetic acid, filtration, washing with cold water and air drying. Products give the expected visible-uv spectra: those of PP DME are given in the literature[6a] and those of $TPPC_4{}^{4-}$ [6b] are very similar to corresponding TPP complexes[6c,d]. In the case of N-p-NO_2BzHTPPS$_4$, the product arising from the N-Bz group was extracted and identified by ^1H NMR spectroscopy to be p-nitrobenzylalcohol. Reactions at lower temperatures (15°C to 45°C) were carried out at 10^{-6} to 10^{-4} M in a thermostatted spectrophotometer.

The reaction of N-benzylporphyrins with metal ions such as Co(II), Cu(II), Ni(II) and Pd(II) (Scheme 1) results in the formation of the corresponding non-N-substituted metalloporphyrins at rates that are much faster (Table 2) than the corresponding rates for the direct formation of these complexes using non-N-substituted porphyrin precursors.[7] Reactions of Pd(II), Cu(II) and Co(II) are sufficiently rapid under mild conditions (pH 7.8, 0.1 M borate buffer, 40°C, 90% yields in one hour or less) for their use in the presence of proteins. Spectra taken during the reaction of N-benzylporphyrins with Cu(II) and Co(II) show only the N-benzylporphyrin complex and the non-N-substituted product, demonstrating that the rate limiting step is debenzylation. Data for the reaction with Cu(II) shown in Table 3 are consistent, since the rate constant is independent of [Cu(II)]. Since the solvent acts as nucleophile, the overall rate at a particular temperature is relatively independent of concentrations of all reactants. Also, the rates in methanol and in aqueous solution are comparable. The fact that the reaction is independent of metal ion concentration is especially advantageous for the use of radiolabels normally employed at very low concentrations (< 10^{-6}M).

Spectral overlay experiments with Ni(II) and Pd(II) (as $PdCl_4^{2-}$) show the rate determining step to be the complexation of the metal ion to form the initial N-benzylporphyrin complex (the observed spectra are those of the N-benzylporphyrin precursor and the non-N-substituted metalloporphyrin product.) In these cases, the reaction rate is not independent of metal ion concentration, but the overall reaction is still several orders of magnitude faster than the formation of the metalloporphyrins directly from the non-N-substituted porphyrin precursor. In these cases, however, a pseudo-first-order excess of the N-benzylporphyrin precursor must be used to obtain a highly predictable formation rate.



Scheme 1.

The use of N-benzylporphyrins provides the first feasible way to obtain radiolabelled metalloporphyrins using submicromolar concentrations of metal ions in aqueous solution at mild temperatures. This method has recently been applied to the formation of ^{67}Cu metalloporphyrins using porphyrin-antibody conjugates in buffered, aqueous solution at 40°C.[6]

Table 2. **Reaction Times and Yields for Formation of Metalloporphyrins From N-benzylporphyrins.**

Porphyrin	Metal	Completion	Yield	Porphyrin	Metal	Completion	Yield
N-benzylprotoporphyrin IX dimethyl ester[a]	Co(II)[b]	20 min	93%	N-benzyltetrakis-(p-carboxyphenyl) porphine[c]	Co(II)[b]	2 min	98%
	Cu(II)	10 min	93%		Cu(II)	2 min	98%
	Ni(II)	10 min	82%				
	Pd(II)	10 min	92%		Pd(II)	10 min	91%

a) In refluxing CH_3OH. b) The initial species formed is a Co(II) complex which is rapidly oxidized to the Co(III) complex. c) In 0.01 M acetate buffer, pH 6, 90°C.

Table 3. **Rate Constants for the Formation of $Cu(II)TPPS_4$ from $N\text{-}p\text{-}NO_2\text{-}BzHTPPS_4$.**

H_2O					CH_3OH			
pH	T, °C	$[Cu^{2+}]$	[N-Bzpor]	k_{obs}, $s^{-1} \times 10^4$	T, °C	$[Cu^{2+}]$	[N-Bzpor]	k_{obs}, $s^{-1} \times 10^4$
5.7	15	5.5×10^{-4}	4.1×10^{-5}	0.27 ∓ 0.02	15	2.1×10^{-3}	2.2×10^{-5}	2.2 ∓ 0.2
5.6	27	5.5×10^{-4}	4.1×10^{-5}	3.0 ∓ 0.4	27	2.1×10^{-3}	2.2×10^{-5}	5.6 ∓ 0.8
5.8	27	3.0×10^{-4}	2.1×10^{-4}	3.3 ∓ 0.1	27	2.1×10^{-3}	4.3×10^{-5}	5.4 ∓ 0.2
5.8	27	3.0×10^{-4}	4.1×10^{-6}	4.1 ∓ 0.2	27	2.1×10^{-3}	8.6×10^{-6}	5.6 ∓ 0.4
5.6	45	5.5×10^{-4}	2.1×10^{-5}	30.2 ∓ 1.9	45	2.1×10^{-3}	2.2×10^{-5}	35.0 ∓ 1.1

Acknowledgements. We are indebted to Professor Marc Julia and members of his research group for providing initial advice concerning the preparation and reactions of sulfonium salts. We are grateful for the generous support of the National Cancer Institute (CA25427), the Institute of General Medical Sciences (RR08176) and the CNRS of France. This project was made possible by a grant of the NATO scientific exchange program (568/83).

1. S. Funahashi, Y. Yamaguchi and M. Tanaka, **Bull. Chem. Soc. Jpn.,** (1984), 57, 204 and ref.

2. D. Kuila and D. K. Lavallee, **Inorg. Chem.,** (1984), 23, 3987 and ref. therein.

3. H.J. Callot, J. Fischer and R. Weiss, **J. Amer. Chem. Soc.,** (1982), 104, 1272

4. Abbreviations: N-benzylprotoporphyrin IX dimethyl ester, N-BzHPP DME, N-benzyltetraphenylporphine, N-BzHTPP, N-benzyltetrakis(p-carboxyphenyl)porphine, $N\text{-}BzTPPC_4$, N-p-nitrobenzyltetrakis(p-sulfonatophenyl)porphine, $N\text{-}p\text{-}NO_2BzTPPS_4$.

5. B. Badet and M. Julia, **Tetrahedron Letters,** (1979), 13, 1101.

6. a) J.E. Falk and R.S. Nyholm in "Current Trends in Heterocyclic Chemistry," A. Albert, et. al., eds., Academic Press, New York, (1958), 130, b) λ, nm (log ϵ): $CuTPPC_4$, 413 (5.13), 538 (3.85); $PdTPPC_4$, 415 (5.16), 522 (4.16); $CoTPPC_4Cl$, 430 (5.11), 547 (4.03), c) A. Wohlberg and J. Manassen, **J. Amer. Chem. Soc.,** (1970), 92, 2982, d) D.W. Thomas and A.E. Martell, **Archiv. Biochem. and Biophys.,** (1958), 76, 286.

7. D.K. Lavallee, **Coor. Chem. Rev.,** (1985), 61, 55.

8. J. Mercer-Smith, S. Figard, D.K. Lavallee and Z. Svitra, **J. Nucl. Med.,** (1985), **26**, 437, and in press.

(Received in France 2 June 1986)