Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 12

SYNTHESIS OF [^{119m}Sn]-MESOPORPHYRIN IX DICHLORIDE

Jon F. Denissen

Biotransformation Section, Drug Metabolism Department, Abbott Laboratories, Abbott Park, IL 60064 U.S.A.

SUMMARY

Tin mesoporphyrin IX dichloride (Sn-MPCl$_2$) is a heme oxygenase inhibitor of current clinical interest for the treatment of neonatal hyperbilirubinemia. The synthesis of [^{119m}Sn]-MPCl$_2$ for drug metabolism and disposition studies is reported. [^{119m}Sn]-MPCl$_2$ was prepared in 60% radiochemical yield by metalation of the porphyrin nucleus of mesoporphyrin IX dihydrochloride with tin(II)-119m acetate. The product had a specific activity of 43.4 mCi/mmol and a radiochemical purity of 99%, as determined by radio-HPLC analysis.

KEY WORDS: [^{119m}Sn]-Mesoporphyrin IX dichloride, mesoporphyrin IX, Sn-119m, hyperbilirubinemia.

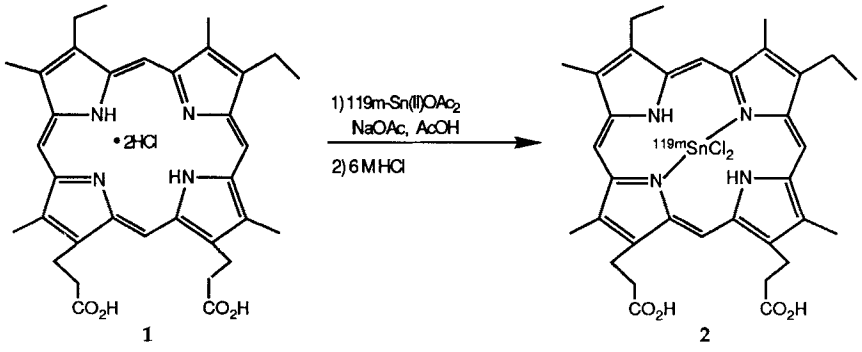
INTRODUCTION

The metalloporphyrin tin mesoporphyrin IX dichloride (Sn-MPCl$_2$, 2) is a potent, competitive inhibitor of heme oxygenase,[1] the rate-limiting enzyme in the degradation of heme to bilirubin. This compound has been shown to be effective in decreasing plasma bilirubin levels in both adult and neonatal animals and humans and is under current evaluation as an alternative to phototherapy in the treatment of neonatal hyperbilirubinemia (infantile jaundice).[2,3a-c] Although tin(IV) metalloporphyrin complexes can only be demetalated under very rigorous conditions,[4] it was desired nonetheless to investigate the metabolism and distribution of Sn-MPCl$_2$ with special attention given to the possibility of oxidative degradation of the tetrapyrrole ring, which could potentially result in the release of inorganic tin species.[5] To this end, labelled Sn-MPCl$_2$ was prepared by metalation of the porphyrin nucleus of mesoporphyrin IX dihydrochloride (1) with tin(II)-119m acetate as shown in Scheme I. The metastable isotope of tin-119 is a gamma-energy emitter possessing a half life of ca. 250 days, making it a suitable label for short-term biotransformation studies.

0362–4803/90/121421–06$05.00

Received May 19, 1990
Revised July 9, 1990

SCHEME I.



1 2

EXPERIMENTAL

General Aspects. Tin-119m acetate (7 mCi, 18.48 mg Sn) in acetic acid (7.7 mL) was obtained from Amersham International, which estimated the material to contain 10-15% of the desired tin(II)-119m acetate species. Mesoporphyrin IX dihydrochloride was obtained from Porphyrin Products, Logan, Utah U.S.A. All other reagents and solvents were purchased commercially and used without further purification. Liquid scintillation counting was peformed with an LKB-Wallac 1214 Rackbeta Excel liquid scintillation counter and Packard Insta-Gel scintillation cocktail. Instrument window settings were optimized about the tin-119m isotope energy spectrum and an efficiency correction ("quench") curve was calculated from six aliquots of tin-119m acetate reagent of equal radioactivity containing progressively larger amounts of mesoporphyrin IX as quenching agent. A nearly linear curve of ca. 90% counting efficiency was observed and used unedited for DPM calculations. Proton NMR spectra were recorded on a Nicolet QE 500 (500 MHz) instrument in acetone-d_6. Mass spectra were recorded on a Kratos MS50 (FAB) instrument. Radiochemical purity determinations were performed on a high-performance liquid chromatography (HPLC) system consisting of a Waters 6000A solvent delivery system, a Rheodyne 7125 injector, a Kratos Spectroflow 757 variable wavelength absorbance detector, a Radiomatic Flo-one Beta Model IC radioactive flow detector and a Hamilton PRP-1 (10 μm) 4.1 x 250 mm column. A flow rate of 2.0 mL/min and a detector wavelength of 405 nm were standard. Tin mesoporphyrin product was eluted from the column with 0.02 M tetrabutylammonium hydroxide (pH 11.6)/acetonitrile (67.5:32.5), which was then followed by a switch in mobile phase to 0.02 M tetrabutylammonium hydroxide (pH 11.6)/acetonitrile/methanol (20:50:30) at ca. 14.3 minutes in order to elute mesoporphyrin IX starting material.[6] Tin mesoporphyrin and mesoporphyrin dihydrochloride samples were dissolved in 0.02 M tetrabutylammonium hydroxide for HPLC analysis and were protected from light during all ensuing manipulations to reduce the possibility of photodegradation.

Metalation of Mesoporphyrin IX Dihydrochloride.[7] A solution of 7.0 mCi (0.379 mCi/mg, 0.155 mmol total tin, 0.023 mmol maximum tin(II)-119m (15% maximum estimated by Amersham)) of tin-119m acetate in 7.7 ml (2.4 mg Sn/mL) of acetic acid and 6.8 mg (0.05 mmol) of sodium acetate trihydrate were stirred under nitrogen at 70° C for 30 min in a 10-mL round bottom flask equipped with a magnetic stir bar, septum, and nitrogen inlet and outlet. Mesoporphyrin IX dihydrochloride (14.9 mg, 0.023 mmol) was suspended in 0.5 mL of acetic acid and added, via syringe, to the reaction solution under red light. Two 0.5-mL acetic acid rinses were performed to ensure the complete transfer of starting material to the reaction mixture, which was then heated to 100° C and stirred under nitrogen in the dark for 2.5 hours.

Complete metalation was assured by the addition of ca. 2 mg (0.009 mmol) of tin(II)-acetate (Alfa) as a 0.5 mL acetic acid suspension and the reaction mixture was allowed to cool to 60° C over 20 min. Hydrochloric acid (6 M, 26 µL, 3.3 equiv.) solution was added to effect ligand exchange and the reaction mixture was stirred at 60° C for 1 hour. The resulting suspension was then allowed to stand at room temperature overnight in the dark in order to aggregate the fine product crystals. The solid product was vacuum filtered through a medium porosity fritted glass filter funnel and rinsed successively with acetic acid (2 x 1 mL), 6 M HCl (2 x 1 mL), 0.5 M HCl (2 x 1 mL), isopropanol (1.5 mL) and ether (2 x 1 mL) under red light. After 19 hours of vacuum drying, 11.0 mg (63%) and 633 µCi (60%) of [^{119m}Sn]-$MPCl_2$ was obtained as a magenta solid having a specific activity of 43.4 mCi/mmol (58 µCi/mg, 97% of theoretical specific activity based on a tin(II)-119m/total tin-119m ratio of 0.15).

HPLC-Radioassay of [^{119m}Sn]-$MPCl_2$. The radiochemical purity of the labelled product was determined by radio-HPLC (0.094 µCi, 1.63 µg injection) and found to be 99%. Product identity was confirmed by co-injection with unlabelled Sn-$MPCl_2$ standard to afford a single peak having a retention time of 6.3 min. A 1% radiochemical impurity was observed to elute in the column void volume at ca. 1.5 min and presumably arose from traces of unreacted tin-119m reagent that were not completely removed by the product wash sequence. The amount of unmetalated mesoporphyrin starting material present in the labelled product was found to be $\leq$3% by HPLC analysis (R_T = 20.2 min).

Mass Spectroscopy and ^{1}H NMR Analysis. The mass spectra of Sn-$MPCl_2$ standard and [^{119m}Sn]-$MPCl_2$ displayed similar fragmentation patterns (excluding sample size effects on peak intensities). Major intensities were observed at m/z 719 and m/z 836 for the unlabelled standard, consistent with the $(M-Cl)^+$ and $(M-Cl+(NBA-HCl))^+$ ions, respectively. The latter ion arises from the use of p-

nitrobenzyl alcohol (NBA, FW 153) as the FAB matrix, resulting in tin ligand substitution of NBA accompanied by the loss of HCl prior to ionization. Similarly, major intensities were observed at m/z 717 and m/z 834 for the labelled product. The uniform difference of 2 a.m.u. between major fragmentation peaks of labelled vs. unlabelled tin mesoporphyrin results from the use of enriched tin-118 for the commercial production of tin-119m radioisotope. Natural elemental tin consists mainly of tin-120 (33%) along with a lesser amount of tin-118 (24%).

The ^{1}H NMR spectrum of [^{119m}Sn]-$MPCl_2$ (57 µg in 0.5 mL acetone-d_6) was identical to that of the unlabelled standard (72 µg in 0.5 mL acetone-d_6). Despite prolonged drying in vacuo, ethyl ether and acetic acid were observed as minor impurities in the labelled product spectrum. ^{1}H NMR 500 MHz, acetone-d_6)[8] δ 2.04 (t, J=7.5 Hz, 6H, $2CH_2\underline{CH_3}$), 3.51 (t, J=7.5 Hz, 4H, $2CH_2COOH$), 3.90 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 3.95 (s, 6H, $2CH_3$), 4.4 (dq, J=7.5, 1.5 Hz, 4H, $2\underline{CH_2}CH_3$), 4.68 (t, J=7.5 Hz, 4H, $2\underline{CH_2}CH_2COOH$), 10.82 (s, 1H, meso H), 10.84 (s, 2H, 2 meso H), 11.12 (s, 1H, meso H).

DISCUSSION

Although a route enabling carbon-14 labelling of the methyl groups of the two mesoporphyrin ethyl sidechains can be devised from a deuterium labelling sequence described by Smith[9] (involving ozonolysis of the vinyl sidechains of protoporphyrin IX followed by Wittig addition of ^{14}C-label and diimide reduction of the regenerated labelled vinyl groups to ethyl groups), this route was not utilized because the catabolism of the tetrapyrolle ring portion of heme is well established.[5] Instead, future studies exploring the metabolic fate of the coordinating metal atom were made possible through the use of tin-119m as the radioactive label. The metalation reaction was conducted with the usual precautions taken for work with gamma-emitting isotopes (lead apron and lead gloves worn during manipulations, lead shielding of reaction apparatus, etc.) and was conducted under red light as a precaution against photodegradation, although mesoporphyrin is significantly more resistant to decomposition in light than are many other porphyrins (i.e. protoporphyrin). The tin-119m acetate reagent purchased from Amersham was estimated to contain 10-15% of the total radioactivity as the desired tin(II) oxidation state, with the remainder presumably consisting of the tin(IV) oxidation state. Therefore, the upper limit of 15% tin(II)-119m acetate, corresponding to 0.023 mmol, was used in the calculation of the reaction stoichiometry. An equimolar amount of mesoporphyrin IX dihydrochloride was combined with the labelled reagent in acetic acid and the mixture was heated under a nitrogen atmosphere. An inert atmosphere was used primarily to preserve the tin(II) reagent species, for the original tin(II) oxidation state in the porphyrin product is not stable in the

presence of oxygen and autooxidation to tin(IV) is essentially unavoidable.[10] After 2.5 hours, unlabelled tin(II) acetate was added in an amount (0.009 mmol) large enough to insure complete metalation of the starting material yet not cause undue purification problems during the wash sequence. Hydrochloric acid (6 M) was added soon after, thereby replacing the two acetate ligands on the tin(IV) central atom with chloride. The wash sequence, designed to remove any unreacted reagent or starting material, and vacuum drying afforded a 60% radiochemical yield of product (633 µCi). The remainder of the radioactivity was presumably lost through the filter during the wash sequence due to the extremely fine nature of the product crystals and no attempt was made to recover this material. The final product specific activity (43.4 mCi/mmol) was 97% of the theoretical maximum specific activity calculated from a tin(II) content of 15% in the labelled tin acetate, thus verifying the accuracy of the estimate of content supplied by Amersham. [^{119m}Sn]-$MPCl_2$ product identity was confirmed by FABMS, ^{1}H NMR and HPLC coinjection with authentic standard. A 1% radiochemical impurity, presumably tin-119m acetate, and a $\leq$3% chemical impurity of mesoporphyrin starting material were observed.

CONCLUSION

A procedure for the preparation of tin mesoporphyrin IX dichloride bearing a Sn-119m radiolabel has been developed. This novel strategy for the labelling of the coordinating metal atom of this metalloporphyrin makes possible studies exploring the metabolism and disposition of this compound and may be applicable to the labelling of structurally analogous porphyrins of biological interest.

ACKNOWLEDGEMENT

The author would like to thank Mario Tiojanco and Arthur Alter of Abbott Laboratories for their valuable contributions to the development of this synthetic procedure.

REFERENCES AND NOTES

1. Drummond G.S., Galbraith R., Sardana M.K., Kappas A.- Arch. Biochem. Biophys. 255:64 (1987).
2. Galbraith R.A., Kappas A.- Hepatology 9:882 (1989).
3. For leading references on studies conducted with tin protoporphyrin IX, the less stable vinylic analog of tin mesoporphyrin, see: a) Kappas A., Drummond G.S., Manola T., Petmezaki S., Valaes T.- Pediatrics 81:485 (1988). b) Anderson K.E., Simionatto C.S., Drummond G.S., Kappas A.- J. Pharmacol. Exp. Ther. 228:327 (1984). c) Drummond G.S., Kappas A.- Science 217:1250 (1982).

4. Fuhrhop J.-H., Smith K.M.- Porphyrins and Metalloporphyrins, Smith K.M., Ed., Elsevier, Amsterdam, 1975, p. 629.
5. For a summary of heme catabolism, including protoporphyrin ring degradation and iron release, see: Smith E.L., Hill R.L., Lehman I.R., Lefkowitz R.J., Handler P., White A.- Principles of Biochemistry: Mammalian Biochemistry (Seventh Edition), McGraw-Hill, New York, 1983, pp 82-87.
6. Bauer J., Linton C., Norris B.- J. Chromatogr. 445:429 (1988).
7. This reaction is based upon a general procedure for the production of tin metalloporphyrins described in reference 4, p. 797.
8. For ^{1}H-NMR data on the analogous zinc mesoporphyrin in $CDCl_3$ see: Smith K.M., Abraham R.J., Pearson H.- Tetrahedron 38:2441 (1982).
9. Smith K.M., Fujinari E.M., Langry K.C., Parish D.W., Tabba H.D.- J. Am. Chem. Soc. 105:6638 (1983).
10. Reference 4, p. 179.