Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 5935–5939

Aspects of investigating scrambling in the synthesis of porphyrins: different analytical methods

Christian B. Nielsen* and Frederik C. Krebs

Polymer Department, Risø National Laboratory, Frederiksborgvej 399, DK-4000 Roskilde, Denmark

Received 13 April 2005; revised 16 June 2005; accepted 22 June 2005
Available online 11 July 2005

Abstract—Herein, we discuss the analyses and quantification of the different components in porphyrin mixtures, prepared from *p*-anisaldehyde, *p*-tolualdehyde, and 5-(4-bromophenyl)-dipyrromethane with acid catalysis, using NMR and HPLC. The advantages and disadvantages of these analytical methods are emphasized. Due to the similar size of a bromine atom and a methyl group it was possible to grow crystals suitable for X-ray crystallographic studies from a mixture of porphyrins, where the 4-position of the *meso*-phenyl rings was either substituted with methyl groups or bromine atoms. We also show that X-ray studies are inferior to NMR analysis for determining the components in a porphyrin mixture.

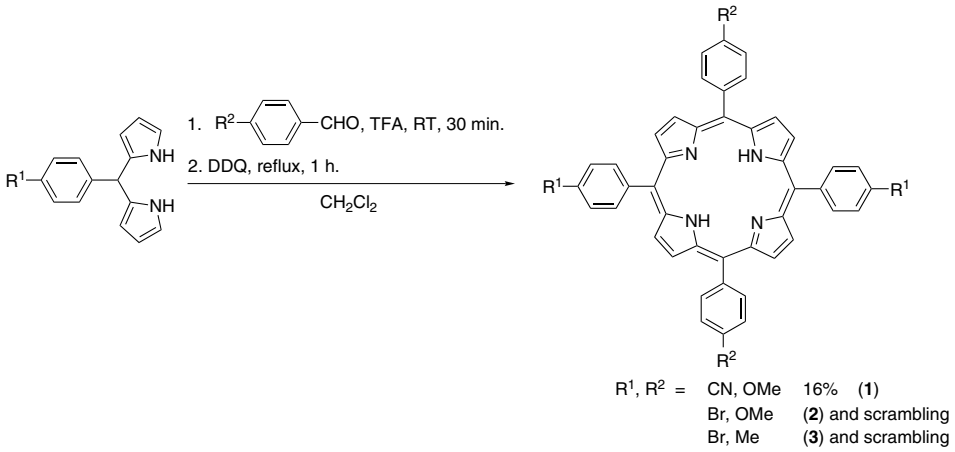
The preparation of tetra *meso*-substituted porphyrins is an active research area as such porphyrins are used as molecular building blocks in areas such as molecular electronics.[1,2] Several methods exist for the preparation of porphyrins with up to four different *meso*-substitutents but common to all of these methods is that a porphyrinogen is formed, which is then oxidized to the desired porphyrin. Acidolysis of the porphyrinogen can lead to scrambling resulting in the isolation of a mixture of up to six different porphyrins.[3] Successful attempts have been reported in the literature to suppress the scrambling by using electron deficient, orthoesters,[4] sterically hindered dipyrromethanes,[5] or dipyrromethane itself, in the synthesis of porphyrins.[5,6] A method has also been published, where carbinols of dipyrromethanes were condensed with themselves to give the corresponding porphyrinogens, which were then oxidized to the porphyrins.[7–9] This procedure resulted in low or undetectable scrambling of the porphyrins discussed. In perspective, the conditions that have been developed for suppressing scrambling in porphyrin synthesis seem to be highly dependent on the porphyrin in question and reliable detection and quantification of the different porphyrins present in a mixture is important for further investigations of scrambling in porphyrinogens.

The purpose of the present work is to illustrate how to quantify the distribution of scrambled porphyrins and also present an X-ray study of the mixture of porphyrins obtained from the synthesis of a *meso*-bromo-phenyl and -tolyl substituted porphyrin. We have focused on the use of ^{1}H NMR, MALDI-TOF, and HPLC techniques for quantifying the outcome of porphyrin syntheses. These methods are discussed by applying them to the product distribution obtained from porphyrin syntheses using 5-(4-bromophenyl)-dipyrromethane, *p*-anisaldehyde, and *p*-tolualdehyde. We also demonstrate that a pure non-scrambled porphyrin is obtained when the electron deficient 5-(4-cyanophenyl)-dipyrromethane is reacted with *p*-anisaldehyde in accordance with previous results.

The compounds isolated from the porphyrin synthesis (Scheme 1) were characterized by NMR spectroscopy, which revealed that **1** was isolated without the presence of scrambling products in accordance with expectations as we had used an electron deficient dipyrromethane. However, **2** and **3** crystallized along with their scrambling products, which are clearly seen from the NH-part (21*H* and 23*H* protons) of the ^{1}H NMR spectrum (see Fig. 1). MALDI-TOF of the porphyrin mixture resulting from the synthesis of **3** is depicted in Figure 2 and we identified molecular masses corresponding to porphyrins with zero, one, two, and three bromine

Keywords: Analysis of porphyrin mixtures; Analytical techniques; NMR; HPLC; X-ray crystallography.
* Corresponding author. Tel.: +45 46774798; fax: +45 46774791; e-mail: christian.b.nielsen@gmail.com

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.06.123



Scheme 1. Syntheses of the *trans*-A_2B_2 substituted porphyrins discussed in the present work.

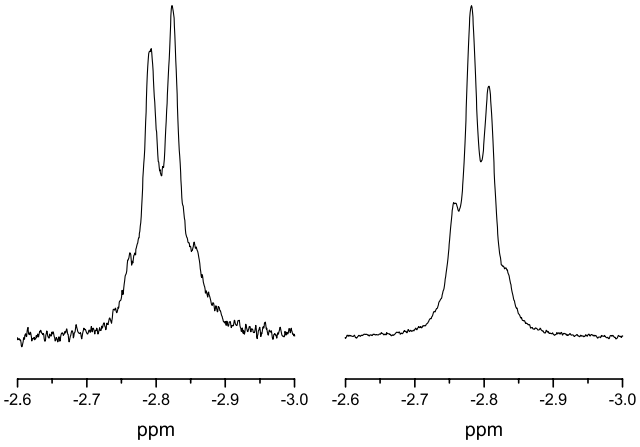


Figure 1. ^{1}H NMR spectra of the NH region of **2** with scrambling products (left) and **3** with scrambling products (right).

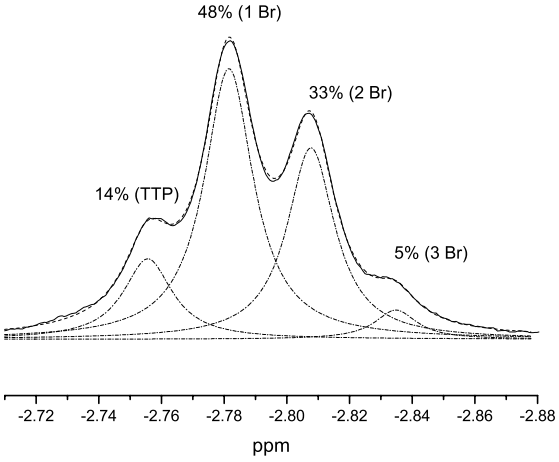


Figure 3. Four Lorentzian functions were fitted to the NH region of the ^{1}H NMR spectrum recorded for **3** with scrambling products.

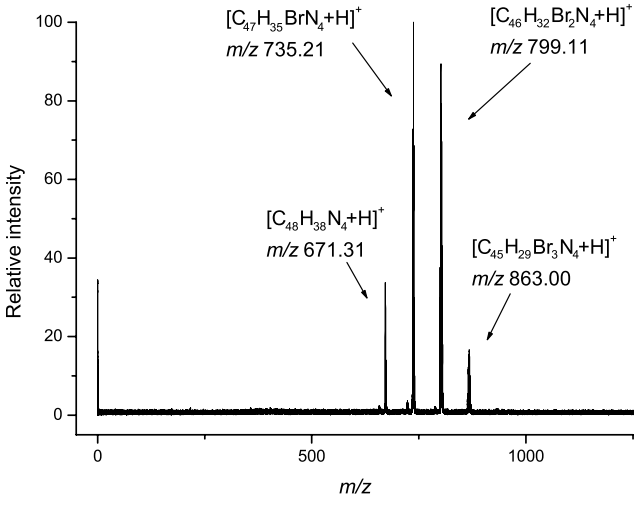
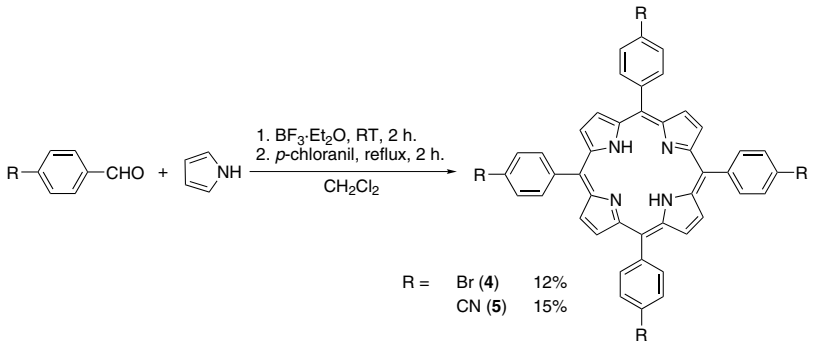


Figure 2. MALDI-TOF of **3** with scrambling products.

atoms. MALDI-TOF of the mixture of **2** (not shown) revealed porphyrins with one, two, and three bromine atoms.

The NH region in the ^{1}H NMR spectrum of the mixture of porphyrins resulting from the synthesis of **2** and **3** can be fitted with four Lorentzian functions as shown in Figure 3. From the MALDI-TOF experiments, we know that the mixture of **3** contained tetra-tolylporphyrin (TTP). In independent experiments, we recorded the ^{1}H NMR spectra of TTP and a tetra bromo-substituted porphyrin and found that the NH protons had chemical shifts of −2.76 and −2.86 ppm, respectively. Bromine substitution thus causes an upfield shift of the NH protons. Using these results, we determined the distribution of the bromine-containing porphyrins in the scrambling mixture of **3**: TTP occurred in 14% and the porphyrins containing one, two, and three bromine atoms occurred in 48%, 33%, and 5%, respectively, in the mixture. A similar analysis of a porphyrin mixture has been described,[10] where the NH-signal in the ^{1}H NMR spectrum was used to determine whether multiple products were obtained from a porphyrin synthesis (the porphyrin mixture investigated in Ref. 10 had iodine and hydrogen as substituents on the *meso*-phenyl rings instead of Me and Br as in **2**). However, the analysis carried out in Ref. 10 was only quantitative and the



Scheme 2. Syntheses of the symmetrical porphyrins discussed in the present work.

distribution of porphyrins was not determined. In order to investigate the limitations of using the resonances from the NH protons in the ^{1}H NMR spectrum of a porphyrin mixture, we first looked at mixtures of TPP (tetraphenylporphyrin) and TTP prepared simply by mixing pure TTP and TPP mixtures. However, the NH protons in these two porphyrins had almost identical chemical shift values so it was not possible to deduce anything about the composition of these mixtures. Similarly, the NH protons in **5** (Scheme 2) had a chemical shift of −2.87 ppm being almost identical to the value of the NH protons in **4** (−2.86). Apparently, the electron accepting or donating properties of the *meso*-substituent is not the only factor that determines the chemical shift of the NH resonances as the cyano group is a considerably stronger electron acceptor than bromine.

Thus, from the structure alone it is not possible to determine whether the resonances for the NH protons will be different for two porphyrins. A (control) validation of the NH ^{1}H NMR method was also carried out by preparing mixtures with known concentrations of TTP and **4** followed by determining the composition of the mixtures by NMR. The correlation between the composition determined using the NMR method and the 'actual' composition is shown in Figure 4. A linear correlation with a slope of one is observed, as expected. This experiment also serves as an illustration of the concentration dependence of the chemical shift of the NH resonances: the concentration of **4** was varied from 0.40 to 2.77 mM and no change in the chemical shift was observed. Thus the most severe limitation in the NMR method is that it is not obvious from the structures when two different porphyrins will have NH resonances that are clearly distinguishable from each other. However, if the resonances can be clearly identified, the NMR method seems to be a powerful tool for determining the composition of a porphyrin mixture. Of course, the downfield region of the ^{1}H NMR spectra of **1**, **2**, and **3** can be used for looking at the scrambling products obtained from the oxidation of the porphinogens due to the simplicity of the spectra. In porphyrins with a more complex substitution pattern this part of the spectrum can become complicated, whereas in the upfield region above 0 ppm only the NH cavity protons have resonances. Thus if 2D NMR spectroscopy is to be avoided and only a 'simple' ^{1}H NMR spectrum is used for assessing isomer purity, this region of the spectrum appears to contain enough information.

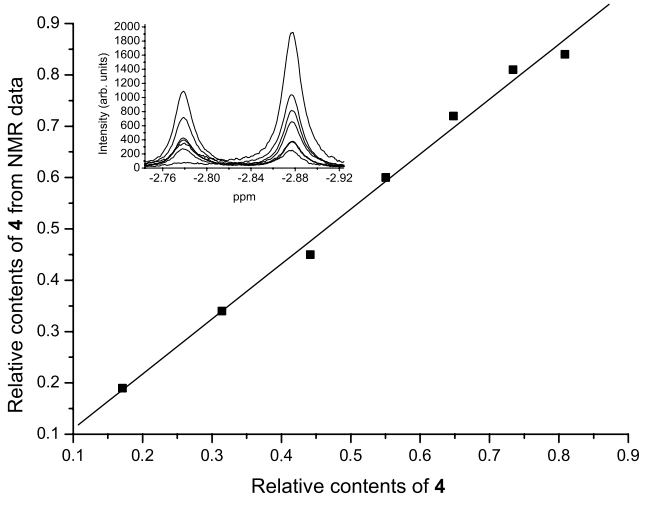


Figure 4. NH part of the ^{1}H NMR spectrum of mixtures of TTP and **4** (inset) and the correlation between the relative amount of **4** determined from NMR and the actual contents of **4** in the mixtures.

The MALDI-TOF spectrum of **2** (not shown) only revealed three components, whereas NMR clearly shows four components. To elucidate this discrepancy, we analyzed the mixture of **2** by HPLC using gradient elution with MeOH and THF. We monitored the absorbance at 420 nm as a function of the retention time and obtained the chromatogram depicted in Figure 5. By integrating the peaks in the chromatogram we determined the distributions of the porphyrins to be 9%, 46%, 39%, and 6% of the porphyrins with 0, 1, 2, and 3 bromine atoms, respectively, which is in good agreement with the product distribution from the NMR results. In reproducing the HPLC result, we noted that care should be taken with the solubility of the different porphyrins in the mixture, as we obtained different product distributions when some of the porphyrins had precipitated from the HPLC sample. As four components are observed in the mixture of **2** in both the NMR and HPLC results, we can conclude that the 'missing' porphyrin in the MALDI-TOF spectrum is due to insufficient ionization efficiency. We also tried to carry out HPLC analysis on **3** but we were unable to find a method that resulted in the detection of the different porphyrins present in the mixture of **3**. This is

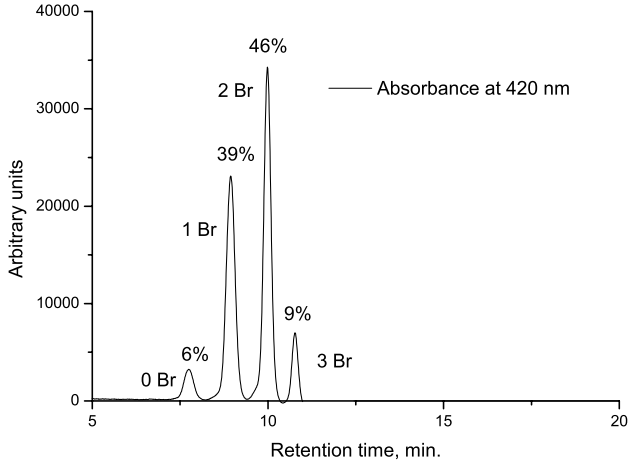


Figure 5. HPLC chromatogram of **2** with scrambling products.

ascribed to the lack of functional groups in **3** that can interact with the column stationary phase. In **2** there is a varying amount of methyl ether groups in the molecule, which can interact with the column stationary phase through polar interactions. No polar groups are present on the *meso*-substituent in **3**. This finding suggests a limitation of using HPLC for analyzing porphyrin mixtures. A further limitation of the HPLC method (using absorbance as the detection method) is that different porphyrins may have different extinction coefficients meaning that the distribution found from a HPLC study not only reflects the relative amount of the different porphyrins in the mixture but also differences in the extinction coefficients.

We were able to grow crystals suitable for X-ray crystallography of **3** but not of **2**, which is due to the similar van der Waals radii of the bromine atoms and the methyl groups.

It is only possible to obtain crystals from a porphyrin mixture when the varying functional groups in the molecular skeleton are of similar size. Single crystal X-ray crystallography is not, in general, a viable method for investigating scrambling in porphyrin synthesis. Crystal structures can be obtained from powder X-ray data, but this method is considerably more time consuming and is non-trivial. The purpose of including an X-ray study of **3** is to have complementary information to the ^{1}H NMR data as it was not possible to analyze the mixture of **3** using HPLC.

The X-ray studies on **3** revealed that the bromine/methyl positions in the asymmetric unit were clearly disordered exhibiting an electron density that was much lower than that of a bromine atom and much higher than that of a methyl group (Fig. 6). This was modeled as two overlying mutually exclusive substituents, one bromine and one methyl group that were refined freely with respect to the atomic position and having anisotropic *U*-values. The hydrogen atoms of the methyl group were fixed in a standard methyl geometry and were riding on the carbon atom. The sof was refined and this gave a slight over representation of the bromine atoms. The bond

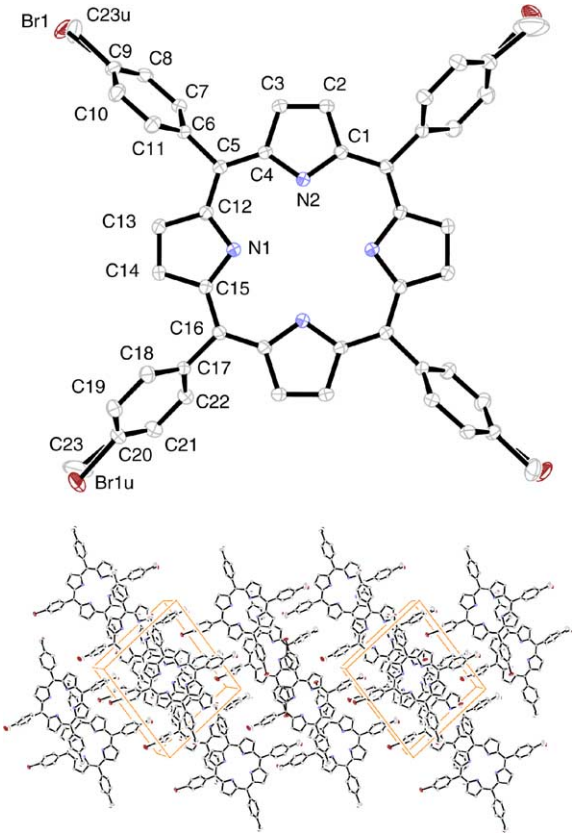


Figure 6. Structure and crystal packing (stereoview) of **3**.

lengths between the aromatic core and the substituents improved to reasonable values with C–Br bond distances of 1.8666(23) and 1.8624(24) Å, respectively, and C–C bond distances of 1.8624(24) and 1.5957(124), respectively. The C–C bond distances are perhaps a little larger than expected. This is ascribed to the difficulty of modeling a disordered carbon atom close to a heavy atom such as bromine. This is also reflected in the *U*-values, which are large for the methyl groups.

The different analysis techniques give rise to differences in the estimated bromine and methyl content for **3** as shown in Table 1.

From this it is clear that while both NMR and HPLC gives similar results, as seen from the data for **2**, the X-ray method, however, overestimates the bromine content in **3**. The X-ray method is most likely limited due to at least two different factors. Firstly it is difficult to cor-

Table 1. A list of the bromine and methyl content obtained for **2** and **3** from each method of analysis

Technique	Br	Me/OMe
Compound **2**		
NMR	1.59	2.41
HPLC	1.58	2.42
Compound **3**		
NMR	1.29	2.71
X-ray	2.11	1.89

rectly model the occupancy when a heavy atom is close to a light atom. This is also reflected in the relatively long C–C bond length obtained. Secondly the result of the single crystal X-ray analysis is on one single crystal selected by the experimenter. The possibility of the crystals being more well-formed when the bromine methyl ratio is close to one cannot be excluded. Since the experimenter has a tendency to pick well-formed crystals this bias could be problematic. If a more accurate bromine methyl ratio should be obtained through the use of scattering techniques, neutron scattering might be considered as a more reliable method.

In conclusion, we have described how a to perform an analysis of porphyrin mixtures and determined the distribution of porphyrins arising from porphyrin synthesis using NMR and HPLC analysis. Also the limitations of these methods have been addressed. These relatively simple methods can be applied in a routine fashion to investigate in more detail the mechanisms responsible for scrambling in asymmetric porphyrin synthesis by analyzing the obtained product distribution. We have carried out X-ray crystallographic studies on a mixture of porphyrins obtained from condensing 5-(4-bromophenyl)-dipyrromethane with *p*-tolulaldehyde.

Supplementary data

Crystallographic data for the structural analysis of **3** has been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 276494). Copies of this information can be obtained free of charge via, www.ccdc.cam.ac.uk. Experimental procedures for the synthesis and for the X-ray analysis are provided as supplementary information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.06.123.

References and notes

1. Ashkenasy, G.; Cahen, D.; Cohen, R.; Shanzer, A.; Vilan, A. *Acc. Chem. Res.* **2002**, *35*, 121–128.
2. Yoon, D. H.; Lee, S. B.; Yoo, K. H.; Kim, J.; Lim, J. K.; Aratani, N.; Tsuda, A.; Osuka, A.; Kim, D. *J. Am. Chem. Soc.* **2003**, *125*, 11062–11064.
3. Geier, G. R.; Lindsey, J. S. *Tetrahedron* **2004**, *60*, 11435–11444.
4. Fox, S.; Hudson, R.; Boyle, R. W. *Tetrahedron Lett.* **2003**, *44*, 1183–1185.
5. Littler, B. J.; Ciringh, Y.; Lindsey, J. S. *J. Org. Chem.* **1999**, *64*, 2864–2872.
6. Cammidge, A. N.; Ozturk, O. *Tetrahedron Lett.* **2001**, *42*, 355–358.
7. Rao, P. D.; Dhanalekshmi, S.; Littler, B. J.; Lindsey, J. S. *J. Org. Chem.* **2000**, *65*, 7323–7344.
8. Rao, P. D.; Littler, B. J.; Geier, G. R.; Lindsey, J. S. *J. Org. Chem.* **2000**, *65*, 1084–1092.
9. Geier, G. R.; Callinan, J. B.; Rao, P. D.; Lindsey, J. S. *J. Porphyrins Phthalocyanines* **2001**, *5*, 810–823.
10. Lee, C. H.; Li, F. R.; Iwamoto, K.; Dadok, J.; Bothnerby, A. A.; Lindsey, J. S. *Tetrahedron* **1995**, *51*, 11645–11672.