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Research Article

Laser Desorption Mass Spectrometry of Synthetic Multiporphyrin Arrays

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ABSTRACT: Laser desorption mass spectrometry and matrix-assisted laser desorption mass spectrometry (MALDI-MS) have been investigated for the analysis of a set of synthetic compounds containing one, two, five, eight or nine porphyrins. Intact singly ionized molecule ions were observed for each compound and the spectra were readily interpretable. The use of a matrix of 4-hydroxy-α-cyano-cinnamic acid greatly diminished the extent of fragmentation. Examination of the resulting mass spectra provides insight into aspects of the MALDI process. The present results show that high molecular weight photochemically active materials that absorb strongly at the wavelength of laser illumination can be analysed effectively and that MALDI-MS is a powerful analytical tool for synthetic chemistry of porphyrin-based molecules with dimensions ranging to 10 nm. The strong molecule ions observed for the largest compounds investigated (Zn_8-octamer, Zn_9-nonamer) indicates that this method should be applicable to even larger porphyrin arrays. © 1997 by John Wiley & Sons, Ltd.

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KEYWORDS: laser desorption; matrix-assisted laser desorption; mass spectrometry; porphyrin; molecular photonic devices; nanoscale chemistry

1. INTRODUCTION

The quest for advanced materials had led to the synthesis of discrete molecules with nanometer-scale structures. We have been developing a molecular building block approach for the synthesis of multiporphyrin arrays [1], which has yielded light-harvesting arrays [2], a molecular photonic wire [3] and optoelectronic gate [4]. We now have extended this approach to structures containing up to nine porphyrins. The arrays are architecturally defined, have high solubility in organic solvents and contain porphyrins with precisely established metalation states. The success of synthesis in the nanoscale domain requires new characterization methods that are as rapid and incisive as existing methods are for analysing low molecular weight compounds.

Of the currently available techniques for assessing porphyrin-containing compounds of modest molecular weight, mass spectrometry has proved to be one of the most informative. ^{252}Cf Plasma desorption mass spectrometry is a superb analytical tool for characterizing porphyrin building blocks, but diminishes in utility quite rapidly with arrays containing more than three porphyrins [5]. Electrospray ionization mass spectrometry has been successfully applied to linear structures containing up; to eight porphyrins, albeit without information on the metalation state [6]. Laser desorption mass spectrometry has been applied primarily to the analysis of monomeric synthetic porphyrins, and in a few cases to dimeric or trimeric porphyrins. The bulk of these studies have been performed by laser irradiation of neat porphyrin films [7]. There is, however, a pressing need for mass spectrometric techniques that are equally effective for assessing the synthesis of larger multiporphyrin arrays.

In this paper we describe the analysis by laser desorption mass spectrometry of a set of compounds ranging from zinc tetraphenylporphyrin (ZnTPP) to an array containing nine zinc porphyrins. The newly developed technique of matrix-assisted laser desorption (MALDI-MS) has greatly extended the accessible mass range for investigating biomolecules such as proteins [8]. We find laser desorption mass

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spectrometry, both in the presence and absence of matrix, also to be a powerful analytical tool for monitoring the synthesis of large multiporphyrin arrays. In addition, examination of the resulting mass spectra provides insight into aspects of the MALDI process.

2. EXPERIMENTAL

Synthetic multiporphyrin arrays (dimer [1], pentamer [2], octamer [9] and nonamer [9] were prepared via a building block approach (Fig. 1) [1]. The Reaction products were purified by preparative size exclusion chromatography. Samples for mass spectrometric investigation were prepared by placing 1–2 μl of porphyrin solution ($< \sim 10^{-4}$ M in CH_2Cl_2) directly onto a stainless steel probe tip or onto a matrix of 4-hydroxy-α-cyano-cinnamic acid [10]. The matrix was deposited on the probe tip by placing 3 μl of a solution of 4-hydroxy-α-cyano-cinnamic acid in CH_2Cl_2/isopropyl alcohol (4:1) and allowing this solution to evaporate. The matrix is only slightly soluble in CH_2Cl_2. This two-layer technique is operationally simpler than the usual method of mixing and co-depositing a solution of matrix and analyte [10]. Time-of-flight mass spectra were obtained by irradiating the sample with 10 ns duration pulses of 355 nm wavelength light from a Nd(YAG) laser [11]. The spectra from 50 laser shots were added to obtain better statistics.

3. RESULTS

Figure 2 compares the high mass portion of the mass spectrum obtained from ZnTPP prepared as a neat film (right) with that obtained from ZnTPP deposited on a matrix (left). The peak corresponding to the singly charged intact molecule ion [($M^{\bullet+}$ and/or $(M + H)^+$] is the largest peak in both spectra. The available resolution does not permit a distinction to be made between $M^{\bullet+}$ or (M + H). For the neat ZnTPP sample, two large fragment ion peaks (marked with arrows) are present in the mass spectrum. These fragment ions arise from the loss of one or two phenyl moieties. For the sample of ZnTPP with matrix, the same two fragment ion peaks are also present in the mass spectrum but with much lower relative intensities.

Figure 3 compares the mass spectrum obtained from the Zn_9-nonamer prepared as a neat film (right) with that of Zn_9-nonamer deposited on a matrix (left). The dominant peak in the spectrum of the neat porphyrin nonamer arises from a fragment ion with m/z = 732 (marked with an arrow). This peak likely corresponds to a fragment consisting of a single porphyrin subunit (molecular weight = 728 u), and is also observed as an intense peak in the spectra of the octamer and the pentamer. The ratio between the intensity of this fragment and the intact Zn_9-nonamer is much larger in the case of ablation from a neat film compared with ablation from the matrix, i.e. the use of the matrix reduces considerably the amount of fragmentation.

Figure 4 compares the high mass regions of the mass spectra obtained from four prophyrin arrays prepared as neat films (right) with the spectra obtained from these arrays deposited on a matrix (left). All the spectra were obtained at the same irradiance. For each sample, the singly charged intact molecule ion ($M^{\bullet+}$ and/or $(M + H)^+$, was readily detected and its mass determined [12]. The absence of multiply charged ions of the larger arrays is notable given the large number of readily ionizable groups (i.e. zinc porphyrins). No significant demetalation of zinc porphyrins was observed. For the neat porphyrins, especially for the Zn_9-nonamer and Zn_8-octamer, there is a step function change in the background level at the molecule ion. The higher background level at lower masses arises from fragmentation of ions in the acceleration region of the mass spectrometer and indicates a more extensive fragmentation of the porphyrin ions from the neat film.

As demonstrated in Figs 2–4, the amount of fragmentation was very extensive in the neat samples. The fragmentation patterns obtained from the samples deposited on the matrix mirrored those of the neat samples, though the extent of fragmentation was much less. Although the total yield of ions was greater from the neat samples, most of these ions were fragments. The samples deposited on the matrix, on the other hand, gave a lower yield of ions, but the fraction of intact molecule ions was higher. The effect of a matrix in decreasing the amount of fragmentation has previously been shown in a comparison between laser desorption of neat material and matrix-assisted laser desorption for quaternary phosphonium salts [13] oligometallocenes [14] and vitamin B_{12} [15] as well as in the first demonstration of MALDI-MS by Karas and coworkers using amino acids and short peptides [16].

The present results demonstrate the utility of

MALDI-MS for analysing multiporphyrin arrays. We have also applied this method to the successful analysis of arrays containing combinations of free base and zinc porphyrins [1]. In some cases, small quantities of higher molecular weight impurities (consistent with the addition of one, two or three porphyrins) were detected in the mass spectra of pentamers and larger arrays, arising from side reactions in the synthesis of the arrays. The mass accuracy (0.1%) and resolution (100–200 full width at half maximum) of MALDI-MS for multiporphyrin arrays are, while lower than those for peptides, sufficient to identify these impurities. This method provides rapid analysis of small amounts of samples and is ideally suited for evaluating the success of synthetic reactions. MALDI-MS provides

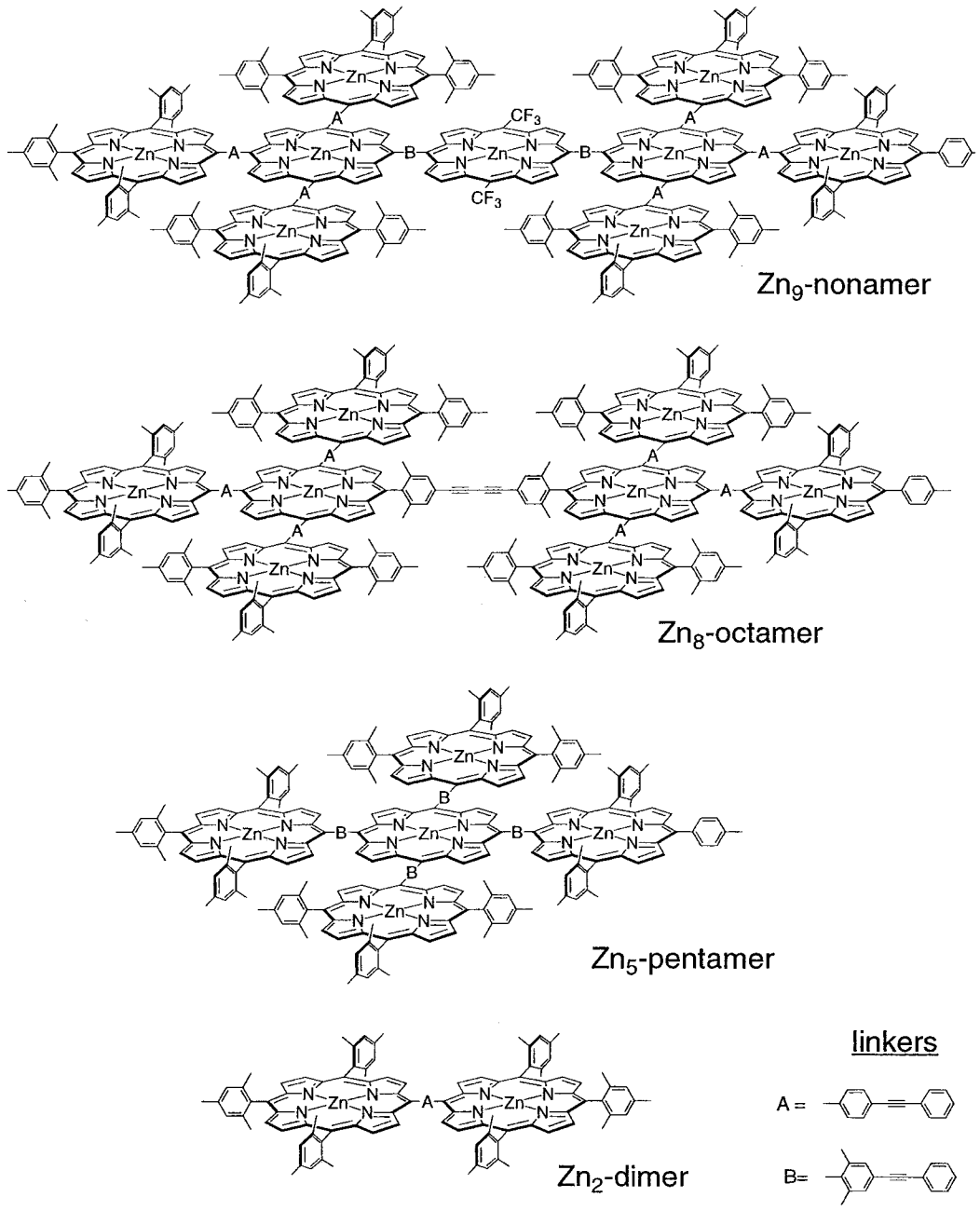

Fig. 1. Structures of the multiporphyrin arrays.

valuable information concerning side-reaction products and is an ideal feedback tool for optimizing the synthesis and purification of the desired product.

The striking effect of the matrix in reducing the extent of fragmentation raises questions concerning the mechanisms of MALDI [17,18]. At first glance, it may appear that the reduced fragmentation is caused by a lower energy deposition in the matrix/porphyrin sample compared with that in the neat porphyrin sample. This hypothesis appears incorrect, given the extinction coefficient at 355 nm in solution for the porphyrins (ε = 10 000 M^{-1} cm^{-1} per zinc porphyrin) and the matrix of 4-hydroxy-α-cyano-cinnamic acid (ε = 29 000 M^{-1} cm^{-1}). Assuming similar extinction coefficients in the solid, the absorption length ($l = m/(\varepsilon\varrho)$ where ϱ is the density of the solid and m is the molar mass) in neat porphyrins and 4-hydroxy-α-cyano-cinnamic acid are 680 nm and 65 nm, respectively. This means that the laser energy is deposited in a considerably smaller volume (higher energy density) in the matrix than in the case of the porphyrin. Thus the lower fragmentation (lower excitation) of the porphyrins ejected from the matrix is *not* caused by a lower energy density deposition in

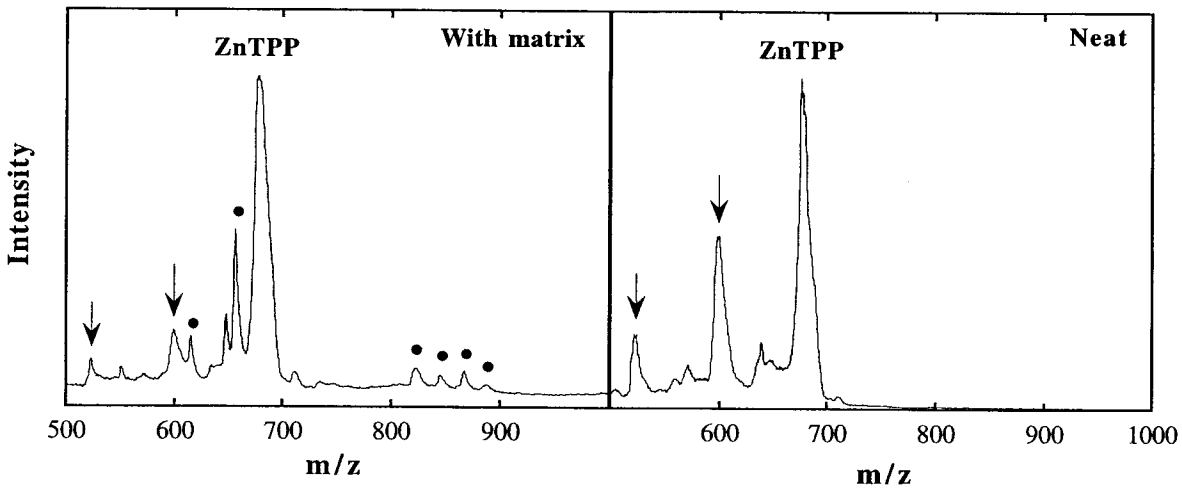

Fig. 2. Laser desorption mass spectra of ZnTPP obtained from a neat film (right) or deposited on a matrix (left). The spectra were obtained at the same irradiance. The peaks corresponding to matrix ions are marked with filled circles and the two largest fragment ion peaks are marked with arrows.

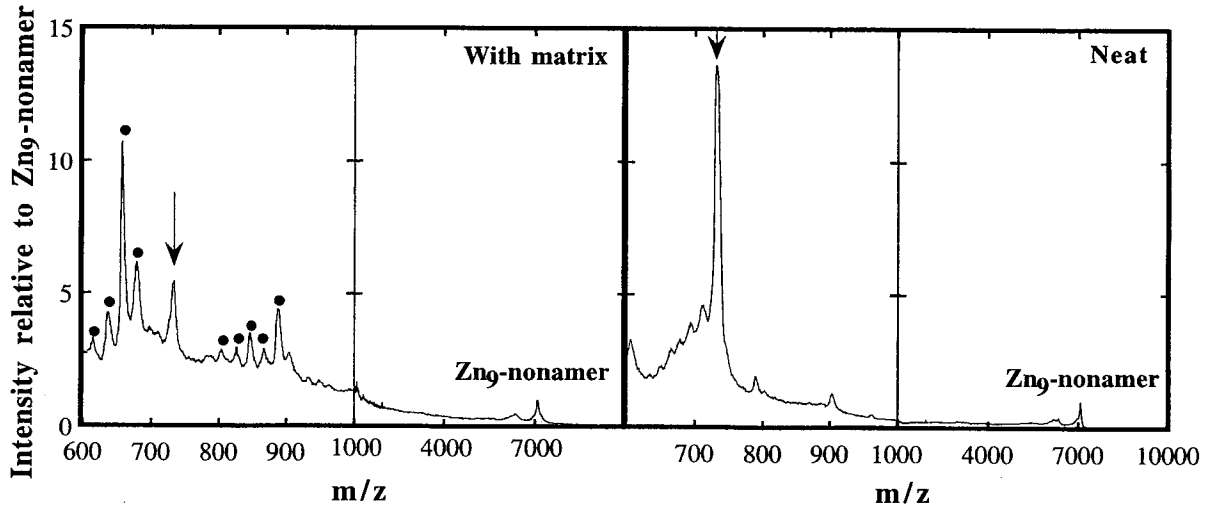

Fig. 3. Laser desorption mass spectra of the nonamer obtained from a neat film (right) or deposited on a matrix (left). All spectra were obtained at the same irradiance. The peaks corresponding to matrix ions are marked with filled circles and the main fragment ion (m/z = 732 u) is marked with an arrow. Note the change in the scale at m/z = 1000.

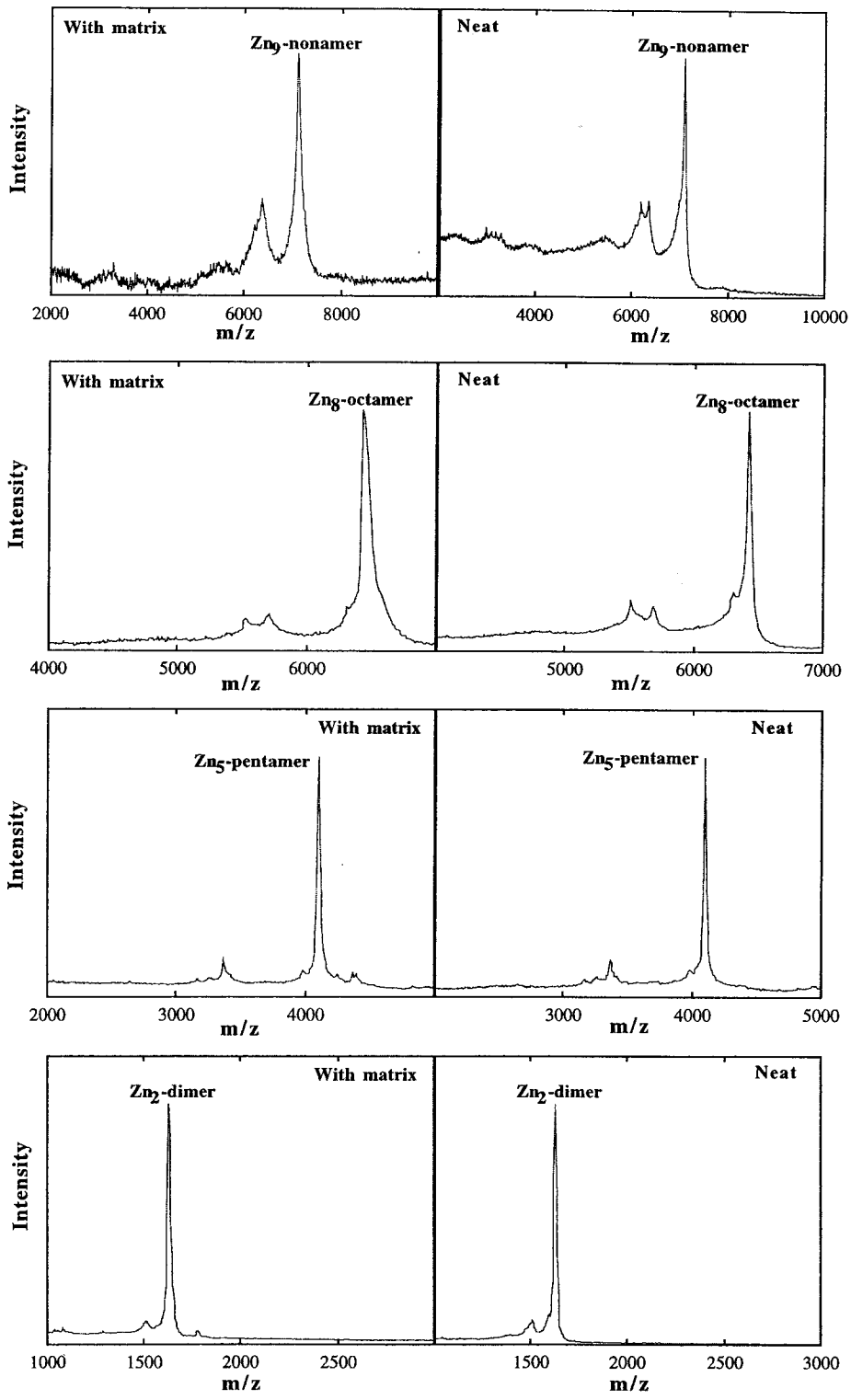

Fig. 4. Laser desorption mass spectra of four multiporphyrin arrays obtained from neat films (right) or deposited on matrices (left). All the spectra were obtained at the same irradiance. The structures of the multiporphyrin arrays are given in Fig. 1. In the pentamer, the methyl groups of linker B point towards the innermost porphyrin. In the nonamer the methyl groups of linker B point away from the innermost porphyrin.

the solid matrix/porphyrin sample immediately after the laser pulse and prior to ejection. The larger energy density in the matrix solid can, on the other hand, cause faster volatilization with decreased energy transfer to the porphyrins [17].

Elucidating the mechanism of the MALDI process is important for further refinement of this technique in support of synthetic chemistry of large molecular structures. There are several possible explanations for the production of less excited and consequently less fragmented analyte molecules compared with desorption from the neat material. (i) The heavier porphyrins may be more efficiently cooled in collisions with the lighter and more abundant matrix molecules [19]. In the volatilization of the neat porphyrins, the porphyrins themselves will act as a matrix, albeit much heavier than the cinnamic acid derivative. (ii) The smaller matrix molecules may be more readily volatilized than the larger porphyrins, i.e. their sublimation energy may be lower. When the porphyrins are imbedded in a large excess of matrix, the sublimation energy of the porphyrin–matrix mixture is approximately that of the matrix. The porphyrin molecules may be entrained in the stream of ejected matrix molecules and acquire less vibrational excitation than in the case of ejection from a neat porphyrin solid. (iii) The ionization mechanism may be different for the two preparations (e.g. protonation versus multiphoton ionization) leading to different levels of excitation. (iv) Radiative energy relaxation may occur at different rates in the two preparations. Work is currently in progress to ascertain the relative importance of the possibilities outlined above. Because the porphyrins appear to act as their own matrices they constitute an interesting system for studying the desorption mechanism.

4. SUMMARY

MALDI-MS is a powerful analytical tool in the synthetic chemistry of multiporphyrin arrays with dimensions ranging from 4 nm (dimer) to 10 nm (nonamer). High molecular weight, photochemically active materials that absorb strongly at the laser wavelength can be analysed. The strong molecule ion observed for each of the compounds investigated, including the octamer and nonamer, augurs well for the application of this method to even larger structures.

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