enzyme. The low pK_a is presumably caused by shielding of the His from bulk solvent. This is similar to the situation observed for papain in which the pK_a of active site His-159 is about 4 in derivatives of papain in which the negatively charged sulfur of Cys-25 is neutralized by acylation.[18] In native papain, the pK_a of His-159 is near 8.[18]

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NMR Solvent Peak Suppression with a Soft-Pulse Nonlinear Excitation Sequence

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Considerable effort has gone into designing NMR radio-frequency pulse sequences that suppress a strong but unwanted resonance by avoiding its excitation.[1-11] The best-known applications for these frequency-selective excitation sequences involve ^1H NMR studies of the H_2O-exchangeable protons of nucleic acids and proteins. Here alternative methods for reducing the H_2O signal, such as saturation by selective preirradiation or use of ^2H_2O as a solvent, fail because they also reduce or eliminate the signals of interest.

The frequency response of an ideal solvent peak suppression sequence would show a flat region of minimal excitation close to the H_2O signal, flanked by broad bands of maximal excitation free from frequency-dependent phase variations. This combination of properties is elusive. For example, the "jump-return" pulse sequence[2] gives good phase behavior, but the range of suppression is narrow and the excitation bands are not flat. The popular 1331 sequence[4,5] gives a broad suppression band but delivers maximal excitation over a relatively narrow band of frequencies, with considerable phase variations. Recent suggestions increase the excitation bandwidths but induce even larger phase gradients.[6-10]

It has recently been shown[11] that a frequency response close to the ideal can be attained by taking advantage of the nonlinear response obtained with large flip angles. The new class of sequences is called NERO (nonlinear excitation rejecting on-resonance). In initial trials,[11] difficulty was encountered in solutions of high H_2O concentration, for reasons still not entirely clear. We now demonstrate that this is not an inherent property of the NERO pulse sequences by reporting good solvent peak suppression in aqueous solution together with flat excitation free from phase gradients. We also present a new member of this class, NERO-2

$$(48)-\overline{110}-(115)-\overline{130}-240-(360)-240-\overline{130}-(115)-\overline{110}-(48) \quad \text{(I)}$$

which is a time-symmetric sequence of six rectangular pulses and five delays of free precession, the latter given by the intervals in parentheses. Overbars indicate pulses 180° out of phase. This soft-pulse sequence is used by adjusting the radio-frequency field intensity such that the Rabi frequency is twice the frequency difference between the solvent resonance and the center of the desired excitation band. Pulse durations are then calculated as usual in terms of the flip angles given above. The precession delays in parentheses have been given as rotating-frame precession angles at the center of the excitation band—therefore, in this case, the time units are twice those of the pulses. The delay at the beginning of the sequence is only necessary when the sequence is used in combination with other pulses, in which case NERO-2 behaves just as a single 90° pulse about the x axis for those spins within the excitation bands.

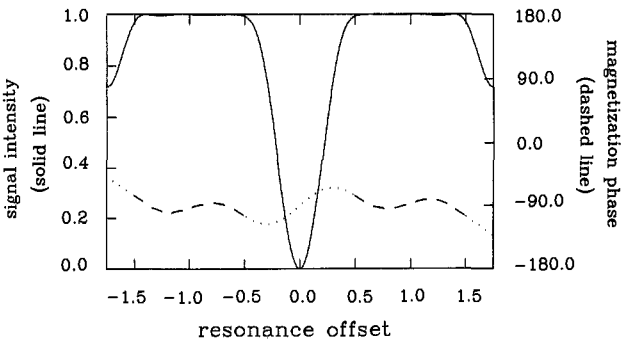

Figure 1. Theoretical frequency dependence of the intensity (solid line) and phase (dashed line) of transverse magnetization excited by NERO-2. The frequency units are normalized to the offset between solvent null and excitation band midpoint.

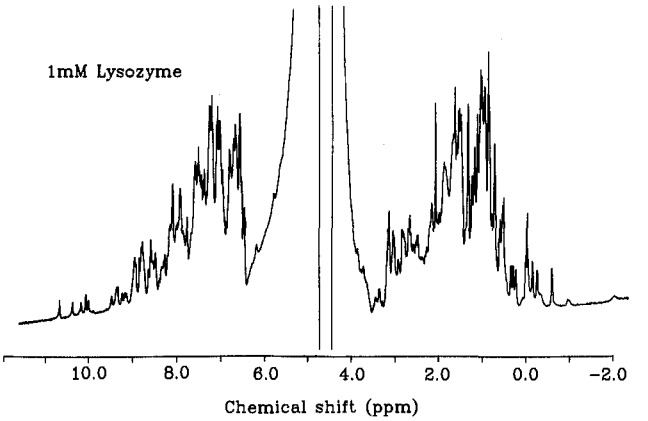

Figure 2. 400-MHz proton spectrum of 1 mM hen egg-white lysozyme in 90% H_2O and 10% ^2H_2O at 57 °C obtained in 100 transients on a Bruker AM-400 with NERO-2. The pulse sequence intervals in (I) were 76.3, (159.7), 90.2, 166.7, (500.0), 166.7, 90.2, (159.7), 76.3, and (66.7) μs in chronological order (precession delays in parentheses, initial delay omitted). The "trim pulse" had a duration of 3 μs.

Figure 1 illustrates the theoretical performance of NERO-2, as predicted by numerical simulation of the Bloch equations in the absence of relaxation. The excited frequency bands are symmetric with respect to the solvent "notch" and are wide and flat, with the phase of excited transverse magnetization varying by only ±15°. These phase variations may be reduced further if different phases on either side of the solvent peak are acceptable, as shown elsewhere. Sequences based on linear response with comparable excitation bandwidths[6-10] have a similar overall duration, but they produce a phase difference of about 450° across the same range.

Figure 2 shows an experimental 400-MHz proton spectrum of a 1 mM solution of lysozyme in 90% H_2O and 10% ^2H_2O. The Bruker AM-400 spectrometer was modified by incorporation of a digital phase synthesizer to improve the accuracy of the phase shifts. The 180° pulse duration was 125 μs, thereby giving maximal excitation of frequency bands between 2.5 and 7.5 ppm upfield or downfield from water. The suppression of the water peak was satisfactory (about 300:1), and a large number of exchangeable NH proton resonances are visible. There are no

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apparent intensity distortions, and the phase of the whole enzyme spectrum could be corrected by the usual small linear frequency-dependent correction. *No base line correction of any kind was performed.* The "1331" sequence[4,5] produced hopelessly distorted base lines after phase correction under the same conditions.

To obtain this spectrum the usual precautions in obtaining solvent–peak suppressed spectra were followed. The probe was first detuned to avoid radiation damping and the water line shimmed carefully down to a width at half-height of about 1 Hz. The probe was retuned for the suppression experiment. A small "trim" pulse, with phase 270°, was added at the end of the sequence, and the suppression was optimized by empirical adjustment of this pulse together with slight variation of the two center pulses so as to correct for residual errors. However, we found that because of the absence of a large spectral phase correction, quite strong residual water signals could be tolerated as long as saturation of preamplifier or receiver were avoided. We conclude that at least on instruments that are equipped with digital phase shifters, stable radio-frequency amplifiers, and a resonance coil providing good radio-frequency homogeneity, NERO-2 may be the method of choice for suppressing the solvent peak without introducing large phase distortions.

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Polymerizable Dienoyl Lipids as Spectroscopic Bilayer Membrane Probes

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It is well known that dienoates and other α,β unsaturated carbonyl compounds are sensitive to solvent polarity.[1] Here we summarize the spectral properties of unpolymerized dienoyl lipids in isotropic media and molecular assemblies and demonstrate that the chromophore absorption maxima are sensitive to lipid chain packing as well as to media polarity. These characteristics provide a spectroscopic probe of the immediate environment of the chromophore in lipid bilayer membranes prior to polymerization.

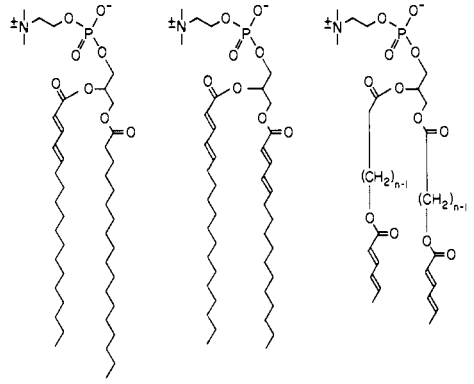
The monomeric amphiphilic membrane probes described here are also useful as polymerizable lipids[2] for the modification of bilayer physical properties, e.g., increasing colloidal stability[3] and decreasing membrane permeability.[4] Photopolymerization of reactive groups, e.g., methacryloyl,[5] dienoyl,[6] or styryl,[7] offers a general route to polymerized vesicles.

Table I. Absorption Maxima for Lipids in Isotropic and Smectic Media at 22 °C

	in CH_3CN		in H_2O	
lipid	λ_{max}, nm	log ϵ_{max}	λ_{max}, nm	log ϵ_{max}
1	260	4.32	260	4.20
2	260	4.63	260	4.49
3a	257	4.61	262	4.54
3b	257	4.56	242 (257)[a]	4.15 (4.56)[a]
4	260	4.69	237	4.48
5	260	4.75	232	4.52
6	257	4.70	260	4.54

[a] The values in the parentheses were determined at 40 °C.

Lipids form supramolecular bilayer structures when hydrated in aqueous buffers with close packing of the lipid chains. The area per lipid molecule is usually about 70 A^2.[8] In this study lipid dienoates based on three phosphatidylcholines **1**, **2**, and **3**,[3,9] a long chain sulfoethylamine **4**,[3] and a dialkyldimethylammonium salt **5**,[10] were prepared, and their absorption determined in organic solution and hydrated assemblies. A micelle-forming shorter chain compound **6**[10] was prepared for comparison.



The dienoylphosphatidylcholines (PC) **1** and **2** in acetonitrile solution absorb at 260 nm (Table I). The extinction coefficients for these compounds are 1.5–2 × 10^4 per dienoyl group per molecule. The lipid conformations in isotropic media minimize the ground-state interactions between the two chain chromophores. Bilayer assemblies of both the mono **1** and didienoyl PC **2** in water show absorption maxima at 260 nm, the same as observed in acetonitrile. Therefore, the two chromophores in **2** are not favorably arranged to form dimers or higher aggregates in the bilayer. Note also that the polarity of the chromophoric environment is similar in the bilayer and acetonitrile.

In contrast the absorption maxima of the chain terminal, disorbyl PC **3a**, is different in acetonitrile solutions (257 nm) and water assemblies (262 nm). This effect is similar to the absorption reported for sorbic acid in ethanol (254 nm) and hexane (261 nm)[11] and reflects the more hydrophobic nature of the interior of the bilayer, where the chromophore resides. Again in this case, as for **1** and **2**, the absorption spectra indicate that the chromo-

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