of the carborane icosahedron and is bonded to two carbon and three boron atoms at distances of 2.586 (9), 2.549 (7), 2.539 (10), 2.482 (8), and 2.488 (9) Å, respectively. The bonding face of the 7,8-dicarbolide ligand is planar to within 0.012 (9) Å, and the four-nitrogen plane of the OEP ligand is exact within experimental error (0.005 (4) Å). The latter completes the coordination about Zr at distances ranging from 2.209 (4) to 2.217 (4) Å. The zirconium lies 0.904 Å from the nitrogen plane and 2.096 Å from the C_2B_9 ligand face. The carborane ligand is tilted with respect to the porphyrin ring such that the normals to the two bonding faces form an angle of 4.9°. In the absence of any compelling electronic arguments, we ascribe this distortion to crystal packing effects.

We are currently studying the reactivity of this compound and are looking to extend this synthetic strategy to a variety of related porphyrin and carborane systems.

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Supplementary Material Available: Listings of details of the structure determination, including tables of crystal and data collection parameters, temperature factor expressions, positional parameters, intramolecular distances and angles, and least-squares planes (15 pages); listing of observed and calculated structure factors (24 pages). Ordering information is given on any current masthead page.

Second-Harmonic Generation from Hyperpolarizable Amphiphiles at Polymer–Polymer Interfaces

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To exhibit second-harmonic generation (SHG; frequency-doubling of light), and other useful second-order nonlinear optical (NLO) properties, a material must contain hyperpolarizable moieties that are arranged in noncentrosymmetric (i.e., oriented) fashion. Such an arrangement can occur in bulk samples that spontaneously form noncentrosymmetric crystals or liquid crystals or that have been "poled" in strong electric fields, or in oriented thin films, whether self-assembled or deposited from an aqueous/air interface.[1,2] Some problems observed with these current techniques include, in the preparation, use of expensive chemicals or apparatus (e.g., Langmuir–Blodgett trough); in the final material, physical delicateness, instability of molecular orientation over time, and intractability toward forming into a waveguide or other thin-film device. We report here the preparation, by a relatively inexpensive and simple spin-coating technique, of physically robust SHG-active samples in which amphiphilic hyperpolarizable molecules are permanently oriented and protected at the interface between solid layers of hydrophobic and hydrophilic polymers.

Hyperpolarizable dyes **1–3** were synthesized and characterized according to the literature.[3] Poly(ethylene-*co*-maleic acid) (PE*co*MA) solution was prepared by dissolving 10% w/w of commercial 1:1 ethylene/maleic anhydride copolymer (Aldrich Inc. "Tm 235°") in distilled water and filtering through a 0.45-μm membrane. Samples for SHG measurements were prepared thus (Figure 1): a few drops of PE*co*Me solution were placed on a circular glass cover slip (Fisher, 18 mm × 0.21 mm), which was then spun at 3000 rpm for 1 min and then dried in vacuo, to give a smooth and uniform clear coating typically 0.5 μm thick (Dektak profilometer). The film was then wetted with 5–10 drops of dye solution (0.3–0.9% w/w in 1:9 MeOH/toluene), spun dry, rinsed with toluene, and then dried by spinning and in vacuo. A clear $^{1}/_{2}$-μm layer of polystyrene ("PS"; Sp^2 Inc. "45,000 g/mol") was then applied in similar fashion from a filtered 10% w/w toluene solution.

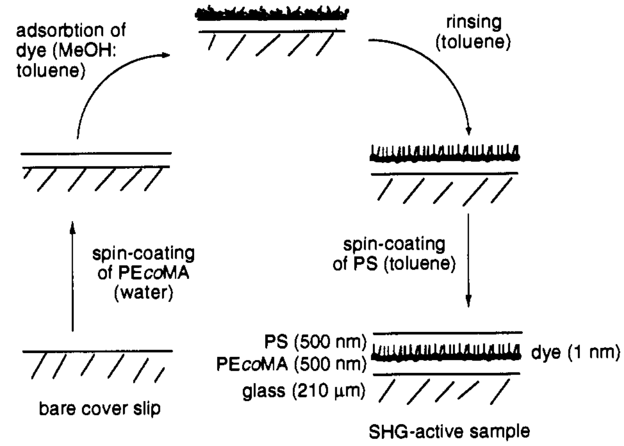
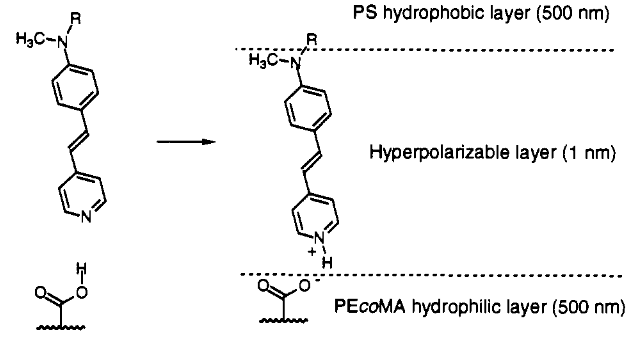


Figure 1. Preparation of samples for SHG testing.

Scheme I



1, R = CH_3
2, R = $(CH_2)_7CH_3$
3, R = $(CH_2)_{17}CH_3$

Scanning electron micrographs (75000×, Au + Pd coating), from above and in cross section, show the surface of PE*co*MA films to be very flat and smooth both before and after application of dye. The change in color from yellow to red-orange confirms protonation of the basic dye as it bonds (ionically) to the acidic hydrophilic polymer; this "chemisorbed" species tenaciously remains while excess free dye easily washes away with toluene. The absorbance of the final colored film at λ_{max} (470 nm) can be adjusted from 0.03 to 0.4 with increasing content of dye and/or methanol in the coloring solution. Contact angles θ of treated PE*co*MA films, measured on 1-μL sessile drops of distilled water using a home-made video apparatus within 4 s of application (PE*co*MA alone, 35–40°; **1** on PE*co*MA, 50–55°; **2** on PE*co*MA, 75–80°; **3** on PE*co*MA, 85–90°), indicate a "tails up" orientation of **2** and **3** even without overlaying polystyrene.

SHG measurements were made using s or p linear polarized light from a Q-switched Nd:YAG laser (1064 nm, 10-ns pulse

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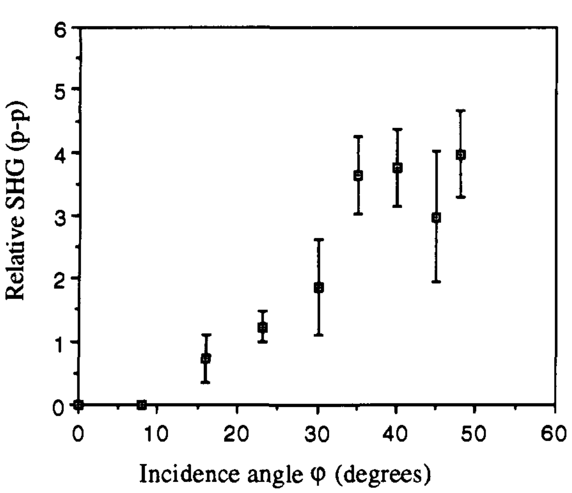

Figure 2. Relative SHG intensity (p–p) as a function of incidence angle φ (from the normal; up to limit imposed by sample holder) for PE*co*MA-**2**-PS sample (A_{470} = 0.12); several measurements.

duration, 5-mJ pulse energy). After the light passed through the sample at incidence angle φ (from normal to the sample surface), three filters and a polarizer were used to isolate either s- or p-polarized components of product 532 nm light, which were then quantitated with a photomultiplier (Hamamatsu R928); subsequent division by squared input intensities (measured with a photodiode) gave normalized average relative output intensities $I^{2\omega}_{\mathrm{p-p}}$ and $I^{2\omega}_{\mathrm{s-p}}$, each varying from pulse to pulse. With this apparatus, significant SHG could not be detected from samples prepared without dye (nonhyperpolarizable), or with **1** as the dye (nonoriented); however, SHG was clearly observed (signal/noise > 50) from PE*co*MA–**2**–PS and PE*co*MA–**3**–PS samples, varying with incidence angle φ as for monolayers prepared by Langmuir–Blodgett techniques (Figure 2);[4] SHG was also observed in samples prepared without PS overcoat, though these tended to cloud after several weeks in air. Surprisingly, SHG efficiency continued to increase with the amount of dye applied beyond the A_{470} = 0.02 for known[5] monolayers of similar compounds, up to 4-fold at A_{470} = 0.10–0.20 (beyond which the samples became visibly cloudy): evidently further dye molecules do not form an SHG-inactive centrosymmetric bilayer as expected, but maintain a common orientation as they associate with carboxylic groups a short way deeper in the PE*co*MA film.

Comparison, for φ = 45°, of average $I^{2\omega}_{\mathrm{p-p}}$ (2.90 ± 0.75) and $I^{2\omega}_{\mathrm{s-p}}$ (0.120 ± 0.066) for PE*co*MA–**2**–PS (A_{470} = 0.12) enabled calculation[4] of a tilt angle (deviation from an average direction) of 21° (±3°), which compares very favorably with materials prepared by LB and other techniques.[2,4,6]

Besides providing one part of a permanent hydrophilic/hydrophobic interface for thermodynamically stable orientation of amphiphilic hyperpolarizable moieties, the PS layer can also act as a protective coating for the dye; as a substrate upon which to deposit further PE*co*MA–dye–PS layers for multiplied SHG efficiency across the laminate; and as a medium for longitudinal propagation/SHG of IR/visible light ("organic nonlinear waveguide"). Further physical characterization of nonlinear monolayers, multilayers, and waveguides is in progress.

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Tandem Stereochemistry of Peptidylglycine α-Monooxygenase and Peptidylamidoglycolate Lyase, the Two Enzymes Involved in Peptide Amidation

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Carboxy-terminal amidation is a prevalent post-translational modification essential to the bioactivity of many neuropeptides.[1,2] Recently, we and others have demonstrated that formation of peptide amides from their glycine-extended precursors is not catalyzed by a single enzyme, as previously thought, but is a two-step process resulting from the sequential action of two enzymatic activities.[3-8] The first enzyme, peptidylglycine α-monooxygenase (PAM, EC 1.14.17.3), catalyzes formation of the α-hydroxyglycine derivative of the glycine-extended precursor in a process dependent upon ascorbate, copper, and molecular oxygen.[3,4,9] The second enzyme, peptidylamidoglycolate lyase (PGL, EC 4.3.2.5), catalyzes breakdown of the α-hydroxyglycine derivative to produce the amidated peptide and glyoxylate.[3,4,8]

While it has been shown that the *pro-S* glycine hydrogen is stereospecifically abstracted during PAM catalysis,[10] the stereochemistry of enzymatic amidation remains unknown. Elucidation of the stereochemical course of the amidation process requires determination both of the configuration of the α-hydroxyglycine species produced from PAM-catalyzed oxygenation and of the stereospecificity of PGL-catalyzed dealkylation. These determinations are experimentally difficult due to the instability of α-hydroxyglycine derivatives under many conditions[8] and to the absence of an appropriate chromophore in typical amidation substrates. Yet, knowledge of the stereochemistry for the two enzymatic steps in amidation is essential for the design of pseudosubstrates and inhibitors directed selectively at PGL, and for exploring the very important question of whether α-hydroxyglycine peptides have a biological function.[3,8] We report herein a series of experiments which demonstrate that PAM and PGL exhibit tandem stereospecificities in carrying out the two requisite steps in carboxy-terminal amidation.

PAM and PGL (homogeneous on SDS–PAGE) were isolated from bovine pituitaries as described previously.[8] Incubation of purified PAM with the substrate TNP-D-Tyr-Val-Gly under standard reaction conditions[8] resulted in *complete* conversion of substrate to TNP-D-Tyr-Val-α-hydroxy-Gly, with no detectable formation of TNP-D-Tyr-Val-NH_2. Figure 1 shows HPLC traces of synthetic[11] and enzymatically-produced TNP-D-Tyr-Val-α-hydroxy-Gly. As is evident from panel A, the two diastereomers

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