reactions were measured by incubation of the enzyme with the inhibitors, periodic removal of portions which were then diluted 25-fold, addition of $[1\text{-}^{14}C]$IPP,[15] and measurement of residual isomerase activity using the acid-lability assay.[14,16] Control experiments without addition of the inhibitors established that the enzyme lost less than 1% of its initial catalytic activity. The results are given in Table I.

As expected for an active-site-directed process, IPP protected isomerase from inhibition. In the presence of 1.0 μM IPP, the rates of inactivation by 0.10 μM FIPP and 0.22 μM FDMAPP decreased by 42% and 37%, respectively. With 2 μM IPP in the buffer, the rate of inactivation by 5.0 μM NIPP decreased by 75%.[17]

We examined whether the inhibition was through covalent modification of the enzyme or slow release of a tightly bound inhibitor. Isomerase (15–60 μg, SA 0.3–0.6 μmol min^{-1} mg^{-1}) was incubated at 37 °C with $[4\text{-}^3H_2]$FIPP and $[1\text{-}^3H]$FDMAPP in 100 μL of buffer under conditions that resulted in >98% loss of catalytic activity. The solutions were then diluted to a final volume of 2 mL and treated as described in Table II. The samples were concentrated with a microconcentrator (Centricon-10 Amicon Corp., MW cutoff 10000 daltons). The radioactivity of concentrate (100 μL) and filtrate (1.9 mL) was measured. The concentrate was repeatedly diluted to 2 mL and reconcentrated until the radioactivity in the filtrate dropped to background levels. The results in Table II show that radioactivity from the fluorinated analogues remains with the enzyme under the denaturing conditions of 6 M urea, 70% ethanol/water, and 0.5% SDS at 100 °C for 90 s. Radioactivity is, however, released when the isomerase–inhibitor adducts are treated with high levels of BME under denaturing conditions in buffer containing 0.5% SDS. Furthermore, we found that catalytic activity could be restored to inactivated isomerase by thiol reagents. To restore activity, excess inhibitor was removed from a sample of isomerase inactivated by FIPP and FDMAPP by microconcentration. A portion of each sample was treated for 20 min at 37 °C with buffer containing 100 mM DTT. Control samples treated in a similar manner in buffer without DTT had no detectable activity, while those treated with high levels of DTT regained 10% of their original activity.[18]

Our data clearly support inactivation of isomerase by FIPP and FDMAPP through an active-site-directed covalent modification of the enzyme. The enzyme–adduct complexes are stable to a variety of conditions that denature the protein. Presumably covalent modification involves displacement of the allylic fluorides in the inhibitors by a nucleophile(s) in the catalytic site by S_N2 or S_N2' processes to generate catalytically inactive adducts.[19] Although we have not demonstrated covalent attachment for NIPP, the behavior of ammonium inhibitor is similar to the allylic fluorides, including partial restoration of activity upon treatment of inhibited enzyme with DTT. Alternatively, NIPP may function as a transition state or reactive intermediate analogue of a carbocation that binds extremely tightly.[17] Regardless of the nature of the inhibition, the fluoro and ammonium pyrophosphate analogues are impressive inhibitors of isomerase. Work is in progress to determine the mechanisms of the inhibition reactions including the nature of the covalent attachments.

Acknowledgment. We thank E. Bruenger and H. C. Rilling for assistance with the purification of isomerase and Andy Woodside for a sample of FDMAPP. This study was supported by the National Institutes of Health, GM 21328.

Registry No. IPP, 358-71-4; FIPP, 99282-16-3; FDMAPP, 99282-17-4; NIPP, 96555-67-8; isopentyl diphosphate isomerase, 9033-27-6.

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[15] Available from Amersham.

[16] In the same buffer $K_M^{IPP} = 1.1 \pm 0.3$ μM and $K_M^{DMAPP} = 0.9 \pm 0.2$ μM.

[17] Similar conclusions were recently reported for NIPP by Reardon and Abeles. We thank Professor Abeles for a preprint of his results. Reardon, J. E.; Abeles, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 4078–4079.

[18] Activity could also be recovered when isomerase irreversibly inhibited by NIPP was treated with DTT.

[19] The observation that isomerase is inactivated by inhibitors of sulfhydryl groups has led to speculation that sulfhydryl groups are involved in catalysis.[20–24]

[20] Agranoff, B. W.; Eggerer, H.; Henning, V.; Lynen, F. *J. Biol. Chem.* **1960**, *235*, 326–332.

[21] Ogura, K.; Nishino, T.; Seto, S. *J. Biochem.* **1968**, *64*, 197–204.

[22] Shah, D. H.; Cleland, W. W.; Porter, J. W. *J. Biol. Chem.* **1965**, *240*, 1946–1956.

[23] Holloway, P. W.; Popjak, G. *Biochem. J.* **1968**, *106*, 835–840.

[24] Banthorpe, D. V.; Doonan, S.; Gutowski, J. A. *Arch. Biochem. Biophys.* **1977**, *184*, 381–390.

Synthesis of Aromatic Schiff Base Oligomers at the Air/Water Interface

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While built-up films of organic monolayers formed at the air–water interface (Langmuir–Blodgett films) have great potential for use in electronic devices,[1] their fragility is a serious limitation.[2] Work on polymerizable monolayers has produced more structurally stable films but has been limited to aliphatic materials.[3] A few studies on small molecules, such as anthracene[4] and porphyrins,[5] avoided using long alkyl chains. We report that aromatic Schiff base oligomers formed at the air–water interface when dialkylterephthalaldimines were spread onto acidic solutions of *p*-phenylenediamine. Built-up films of poly(*p*-phenyleneterephthalaldimine) (PPTA)[6] contained only a trace of aliphatic material, the original alkyl chains having served as disposable aids to surface layer formation. PPTA itself does not dissolve in known spreading solvents and cannot be spread on the water surface.

Surface pressure studies of dialkylterephthalaldimines indicated that they have sufficiently strong interactions with the water surface to spread on it.[7] The equilibrium spreading pressure (ESP) of liquid dihexylterephthalaldimine was 17.2 mN/m at 20 °C. The excess aldimine formed lenses on the surface. This ESP is slightly less than the 20.7 mN/m of ethyl tetradecanoate.[8] The pressure–area curves of dioctadecylterephthalaldimine indicated that while it might form a true monolayer at large surface areas, upon compression it collapsed to areas too small for a monolayer. The observed zero-pressure-extrapolated (zpe) areas[9] were temperature-dependent (0.38 ± 0.02 nm^2/molecule [sd of measurement, 2 determinations] at 5 °C, 0.34 ± 0.01 [2] at 10 °C, 0.24 ± 0.02 [9] at 20 °C, 0.17 ± 0.02 [2] at 30 °C, 0.14 ± 0.02 [2] at 40 °C). This was an equilibrium effect, not an artifact of spreading, since a film spread on a 40 °C subphase gave a zpe area of 0.34 nm^2/molecule if the subphase was cooled to 10 °C

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[1] Kuhn, H. *Pure Appl. Chem.* **1981**, *53*, 2105–2122. Vincett, P. S. *Thin Solid Films* **1980**, *68*, 135–171.

[2] Sagiv, J. *J. Am. Chem. Soc.* **1980**, *102*, 92–98. Pitt, C. W.; Walpita, L. M. *Thin Solid Films* **1980**, *68*, 101–127.

[3] See, for example: Lopez, E.; O'Brien, D. F.; Whitesides, T. H. *Biochem. Biophys. Acta* **1982**, *693*, 437–443. Folda, T.; Gros, L.; Ringsdorf, H. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 167–174. Tieke, B.; Lieser, G. *J. Colloid Interface Sci.* **1982**, *88*, 471–486. Fukuda, K.; Shibasaki, Y.; Nakahara, H. *Thin Solid Films* **1983**, *99*, 87–94 and references cited therein.

[4] Vincett, P. S.; Barlow, W. A.; Boyle, F. T.; Finney, J. A.; Roberts, G. G. *Thin Solid Films* **1979**, *60*, 265. Roberts, G. G.; McGinnity, T. M.; Barlow, W. A.; Vincett, P. S. *Thin Solid Films* **1980**, *68*, 223.

[5] Jones, R.; Tredgold, R. H.; Hodge, P. *Thin Solid Films* **1983**, *99*, 25–32.

[6] Chemical Abstracts Nomenclature—poly(nitrilo-1,4-phenylenenitrilomethylidene-1,4-phenylene).

[7] Doubly distilled water, dual stage quartz still preceded by ion exchange, activated charcoal, and membrane filter; commercial "Lauda" film balance.

[8] Fukuda, K.; Ishii, Y. In "Shin Jikken Kagaku Koza (Lectures on New Experimental Chemistry)"; Iguchi, Iguchi, H., Ed.; Maruzen: Tokyo, 1975; Vol. 18, p 500.

[9] Maximum slope extrapolated to zero pressure, hereafter referred to as the zero extrapolated (zpe) area; compression rate, 0.08 (nm^2/molecule)/min; initial area: 3.0 nm^2/molecule.

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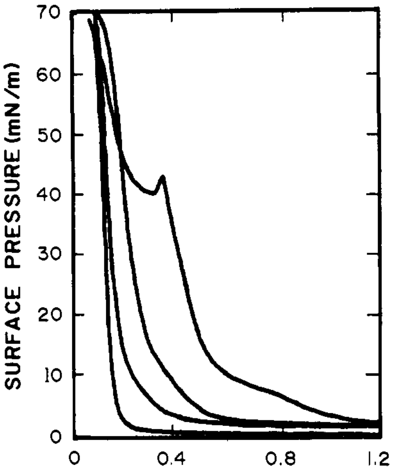

Figure 1. Surface pressure–area curves of dioctadecylterephthalaldimine on *p*-phenylenediamine solutions (pH 4.41 [HCl], 20 °C, spread from 0.026% solution in $CHCl_3$, initial area 3 nm^2/molecule, compression rate 0.08 (nm^2/molecule/min). *p*-Phenylenediamine concentrations in ascending order from bottom left: zero, 0.001 M, 0.0032 M, 0.01 M.

before compression. Likewise, a film spread at 10 °C gave a zpe area of 0.15 nm^2/molecule if the subphase temperature was first raised to 40 °C. The collapse pressures were high, 55–60 mN/m departure from maximum slope (65–70 mN/m peak pressure). The dodecyl homologue collapsed at 20–22 mN/m (zpe area = 0.14 ± 0.03 [2] nm^2/molecule 20 °C). In contrast, dioctadecylisophthalaldimine spread at low temperature gave zpe areas consistent with monolayer formation (0.48 nm^2/molecule at 5 °C, 0.53 ± 0.02 [2] at 10 °C). At 20 °C, this abruptly dropped to 0.265 ± 0.002 (4) nm^2/molecule; at 30 °C, the zpe area was 0.223 nm^2/molecule. The equilibrium situation for organic liquids spread on water is generally considered to be a monolayer with excess liquid collecting into lenses.[10] This description would apply to dihexylterephthalaldimine. In the case of the long-chain compounds,[11] the linearity and stiffness of the terephthalaldimine group coupled with its central placement either render the monolayer unstable to compression or favor the formation of island-type aggregates.

The spreading behavior of dioctadecylterephthalaldimine in the presence of diamine was investigated by measuring the surface pressure–area curves on pH 4.41 *p*-phenylenediamine subphases.[12] The lowest pressure curve in Figure 1 is of the aldimine on pH 4.41 water without diamine. It is essentially identical with those obtained on distilled water alone. As diamine was introduced the curves moved to higher surface pressures and a shoulder appeared in the curves measured on 0.01 M diamine. Although this shoulder could be due to hydrolysis of the aldimine, it did not appear in curves of a 1:1 (mol/mol) aldimine–octadecylamine mixture. Nor did it appear if the subphase was replaced with water either directly after spreading or after waiting 1 h from spreading the aldimine. We infer that the increase in surface pressure is due to adsorption of the *p*-phenylenediamine. Since the film balance uses a float system and the subphase was essentially identical on both sides of the float, we infer that *p*-phenylenediamine adsorbed more strongly to the spread film than to the bare air–water interface.

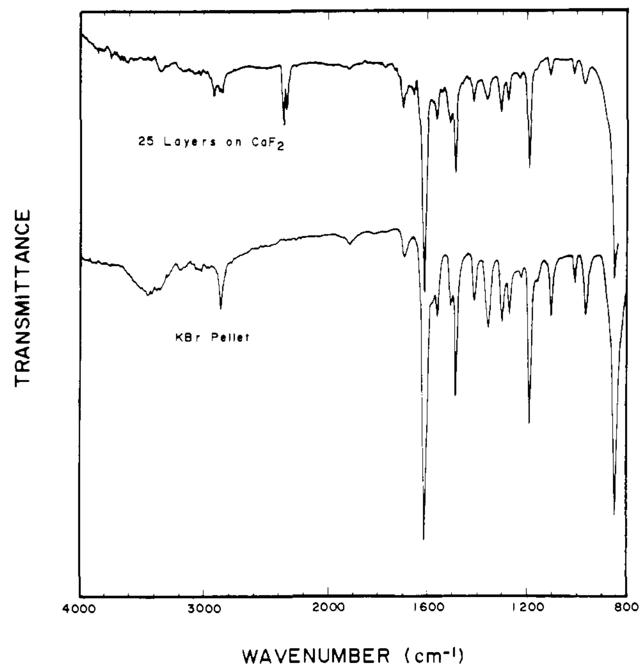

Figure 2. FTIR spectra (top) of built-up film made by reacting didodecylterephthalaldimine on 0.01 M *p*-phenylenediamine (overnight, pH 4.41, 20 °C, surface area 0.75 nm^2/molecule, surface pressure for building up film 10 mN/m for first 10 layers, remainder at 20 mN/m, 25 layers on CaF_2) and (bottom) of PPTA synthesized in *m*-cresol solution from terephthalaldehyde and *p*-phenylenediamine.[16b]

Reactions were performed with the surface area held constant.[13] In a typical reaction of dioctadecylterephthalaldimine, the surface pressure increased asymptotically over 4–5 h to 6–7 mN/m until instrument drift became dominant. Upon changing the subphase, this pressure dropped to zero suggesting a contribution due to soluble, surface-adsorbed species, probably phenylenediamine. Overnight is apparently long enough for equilibrium to be established. The surface pressure for the dodecyl homologue remained near zero for the duration of the reaction. While the collapse pressure of spread films of didodecylterephthalaldimine was 20 mN/m, that of the product film after changing the subphase was 50 mN/m. This film was stable for more than 16 h under an applied surface pressure of 23 mN/m.

Films could be built up only on well-cleaned, hydrophilic substrates and were Z-type. Deposition was achieved with CaF_2, quartz, silicon, germanium, and aluminum- and chromium-plated glass slides.[14] Deposition ratios were frequently in the range of 1.2–1.6.[15] The infrared spectra (Figure 2) of films built up on CaF_2 and of PPTA synthesized in solution[16] were almost identical. Most notable is the 1612-cm^{-1} C=N stretch band characteristic of PPTA. A trace of aliphatic material remains in this film, crudely estimated from intensity comparison to the original Schiff base to be 2% of the initial content. The octadecyl homologue left more, about 12%. Even though octadecylamine dissolves in acidic subphases,[17] the increased solubility of dodecylamine aids conversion. Some aldehyde is visible. The optical absorption

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(10) Adamson, A. W. "Physical Chemistry of Surfaces", 4th ed.; Wiley: New York, 1982; p 106.

(11) Melting points (corrected): dioctadecylterephthalaldimine, 74.5–75.5 °C; didodecylterephthalaldimine, 54.8–55.0 °C; dioctadecylisophthalaldimine, 61.2–61.3 °C. Synthesized by refluxing amine and dialdehyde in EtOH for 2 h and allowing it to cool overnight and then recrystallizing the precipitate from EtOH or MeOH.

(12) At this pH the diamine is almost entirely monoprotonated (pK_a 3.29, 6.08, from: "Lange's Handbook of Chemistry"; 12th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1979). The difference in pK_a for molecules in and out of monolayers has been estimated to be about 0.5 (Betts, J. J.; Pethica, B. A. *Trans. Faraday Soc.* **1956**, *52*, 1581).

(13) Typical reaction: terephthalaldimine spread from 0.026% chloroform solution to 0.75 nm^2/molecule on 0.01 M *p*-phenylenediamine, pH 4.41 (HCl), reaction overnight, subphase changed by passing 5 L of doubly distilled water under the monolayer. Continuous monitoring of subphase pH showed that it remained within 0.03 of the starting pH over the course of the reaction.

(14) CaF_2, quartz, and silicon substrates were freshly cleaned in H_2SO_4–40% H_2O_2 (4:1) and rinsed well with doubly distilled water; Al- and Cr-plated slides were heated in air at 400 °C for 20 min; germanium was cleaned first with 10% HF and then with NH_4OH, H_2O_2, H_2O (1:1:4).

(15) The ratio of the change in the area of the film on the water surface at constant pressure during dipping of the substrate to the surface area of the substrate.

(16) Morgan, J. W. Japan Patent Application 51-138800, 1976. Suematsu, K.; Takeda, J. *J. Syn. Org. Chem. Jpn.* **1983**, *41*, 972–984.

(17) Gaines, G. L., Jr. "Insoluble Monolayers at the Air/Water Interface"; Wiley: New York, 1966; p 154.

maximum shifted from 400 to 435 nm, depending on diamine concentration and pH, the longer wavelength implying higher molecular weight. This condensation reaction probably occurs by hydrolysis of the starting Schiff base and subsequent formation of the more stable aromatic Schiff base.

Two main points derive from this work. First, alkyl chains were used as disposable aids to form reactive surface layers. The general criterion for spreading is that the attraction for the subphase surface be strong enough to overcome bulk cohesion.[18] Thus, in dialkyl terephthalaldimines we have spreadable monomers. No polymer was obtained when terephthalaldehyde solution was used since the terephthalaldehyde quickly crystallized. After formation of the surface layer and reaction with subphase diamine, the alkylamine, being no longer needed and water-soluble, is easily removed. Second, although aliphatic Schiff bases are too unstable to be formed in significant quantities at the air–water interface,[19] aromatic Schiff bases are stable. By combining these points, we produced built-up films, not obtainable by conventional LB techniques, of a polymer whose known heat resistance[20] is far higher than that of Langmuir–Blodgett materials used to date.

Acknowledgment. Thanks are due to H. Nakahara for instruction in film balance and Langmuir-Blodgett techniques.

Registry No. PPTA (SRU), 28157-08-6; dihexylterephthalaldimine, 90468-36-3; dioctadacylterephthalaldimine, 18312-95-3; (dioctadecylterephthaldimine)·(*p*-phenylenediamine) (copolymer), 99309-25-8; (didodecylterephthalaldimine)·(*p*-phenylenediamine) (copolymer), 99309-26-9; dioctadecylisophthalaldimine, 99309-24-7.

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[18] Reference 17, pp 138–139.

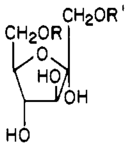
[19] Valenty, S. J. *Macromolecules* **1978**, *11*, 1221–1228.

[20] 10% weight loss at 460 °C, see: Lee, H.; Stoffey, D.; Neville, K. "New Linear Polymers"; McGraw-Hill: New York, 1967; p 320.

Additions and Corrections

Anomerization of Furanose Sugars and Sugar Phosphates [*J. Am. Chem. Soc.* **1985**, *107*, 2448–2456]. JOHN PIERCE, ANTHONY S. SERIANNI, and ROBERT BARKER*

Page 2450, Chart I: In the lower most structure (a generic *keto*-furanose), the letter R should be replaced by CH_2OR. The structure would then appear as



Hartree–Fock MO Theoretical Approach to Aromaticity. Interpretation of Hückel Resonance Energy in Terms of Kinetic Energy of π Electrons [*J. Am. Chem. Soc.* **1985**, *107*, 1161–1165]. HIROSHI ICHIKAWA* and YUKIKO EBISAWA

Page 1165: In the vertical axis of Figure 2, "KRE" should read "–KRE".

Bimetallic Solvated Metal Atom Dispersed Catalysts. New Materials with Low-Temperature Catalytic Properties [*J. Am. Chem. Soc.* **1984**, *106*, 2721–2722]. KENNETH J. KLABUNDE* and YUZO IMIZU

Page 2721: Table I, 2.3% Co has an initial rate for 1-C_4 hydrogenation of 0.0029 (not 0.029).