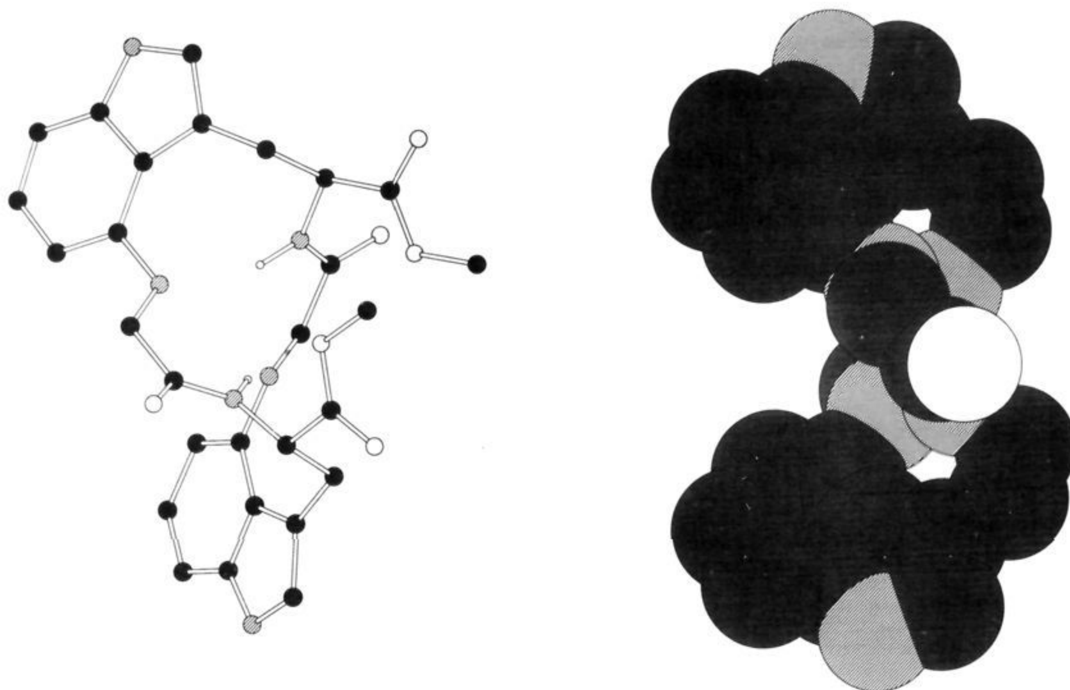


Figure 1. Plot of the X-ray crystal structure of **1** (solvent molecule omitted) (left); space-filling diagram concentrating on the helical macrocyclic region (right).

The proton NMR spectrum of **1** in chloroform shows a single set of sharp peaks with the amide NH signal at 10.2 ppm, about 4 ppm downfield of its normal range due to hydrogen bonding.[8] A strong NOE enhancement between the proton at the indole 5-position and the CH of the imine is also observed, consistent with the structure in Figure 1. The kinetics of unwinding the helix is beyond the range of dynamic NMR techniques, with no significant changes being seen in the spectrum between −55 and 65 °C in $CDCl_3$ and between 25 and 125 °C in DMSO-d_6. The ester groups at the asymmetric centers extend outward from the same face of the molecule and describe a deep groove in its surface; attempts to determine whether optical activity is conserved in the absence of these groups have proved unsuccessful due to difficulties associated with the radical decarboxylation of **1**. The indole rings reside in fixed planes inclined 21.7° to each other, and when viewed down the crystallographic *c* axis they define elliptical channels in which rows of disordered acetonitrile molecules are located.

Molecular modeling also reveals that the 18-membered macrocycle should include metals to form pseudotetrahedral helicates. Indeed there is evidence for the formation of a neutral copper(II) complex of **1** on standing with excess cupric acetate in dimethylformamide at room temperature. Chromatography on silica gives an olive green solid, which runs slightly behind the parent macrocycle and shows a strong M + 1 peak in the FAB mass spectrum at m/z 604, corresponding to **1** −2H + Cu. Work continues on the complexation properties of **1**.

The stereocontrolled generation of a novel hydrogen-bonded helix is of particular interest from a number of perspectives. There are analogies to the restriction of conformational equilibria of biologically active cyclic peptides by transannular hydrogen bonding,[9] and repetition of the same H-bonding motif would make possible the characterization of extended, helically wound, conformationally rigid macromolecules of controlled chirality. The helical backbone self-assembles through molecular events which involve recognition, as do metallohelicates,[2-4] and elaboration to analogous, extended systems would also make possible the spontaneous organization of substituents in the helical periphery.

(8) We observe amide NH signals in a range of precursor and related compounds between 6.4 and 6.6 ppm in $CDCl_3$.

(9) For a recent example, see: Rizo, J.; Koerber, S. C.; Bienstock, R. J.; Rivier, J.; Hagler, A. T.; Gierasch, L. M. *J. Am. Chem. Soc.* **1992**, *114*, 2852. Rizo, J.; Koerber, S. C.; Bienstock, R. J.; Rivier, J.; Gierasch, L. M.; Hagler, A. T. *J. Am. Chem. Soc.* **1992**, *114*, 2860.

Acknowledgment. This work benefited from support by the SERC and Fisons Pharmaceuticals.

Supplementary Material Available: Full details of the determination of the crystal structure, tables of atom coordinates, bond lengths, bond angles, torsion angles, anisotropic temperature factors, and the numbering scheme and a view down the crystallographic *c* axis of a 4 × 10 unit cell region of the crystal of compound **1** (9 pages); table of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

Photochemical Functionalization of Polymer Surfaces and the Production of Biomolecule-Carrying Micrometer-Scale Structures by Deep-UV Lithography Using 4-Substituted Perfluorophenyl Azides

Mingdi Yan,[†] Sui Xiong Cai,[†] M. N. Wybourne,[‡] and John F. W. Keana*[,†]

Departments of Chemistry and Physics
University of Oregon, Eugene, Oregon 97403

Received October 8, 1992

Polymer,[1] silica,[2] and graphite[3] surface modification by the introduction of functional groups has been the subject of intensive research toward the development of resist materials,[4] biosensors,[5] and biomaterials.[6] Recently, surface modification has been combined with photolithography to spatially direct the synthesis of peptides or oligonucleotides[7] and the immobilization of biopolymers.[8] Most of the surface modification processes involve sequential treatment of surfaces with chemical reagents.[8] Few studies have employed azides as surface modification reagents.[9] We now report the surface modification of polymers with *N*-

[†] Department of Chemistry.
[‡] Department of Physics.

(1) Braybrook, J. H.; Hall, L. D. *Prog. Polym. Sci.* **1990**, *15*, 715–734.
(2) Bhatia, S. K.; Hickman, J. J.; Ligler, F. S. *J. Am. Chem. Soc.* **1992**, *114*, 4432–4433.

0002-7863/93/1515-814$04.00/0 © 1993 American Chemical Society

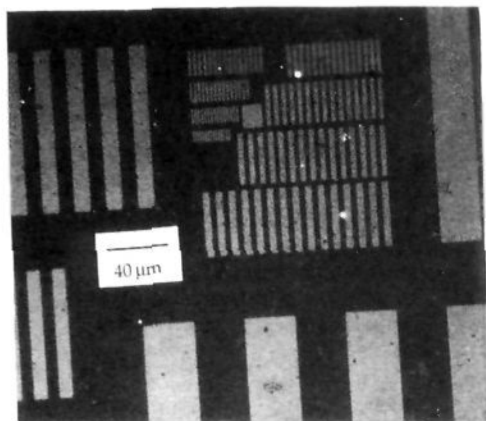
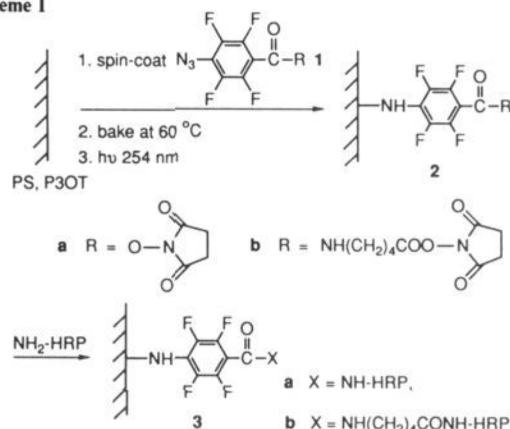


Figure 1. Micrometer-sized patterns taken under a fluorescence microscope (450–490 nm excitation; >510 nm emission) showing the surface modification of a PS film. Measurement of reflected white light intensities from the photographic film negative gave an average contrast between irradiated and nonirradiated regions of 4:1.

Scheme I



hydroxysuccinimide-functionalized (NHS) perfluorophenyl azides (PFPAs) **1a**[10] and **1b**[11] (Scheme I) by a simple spin-coating technique and photolysis. The combination of this method with photolithography produced well-defined micrometer-sized patterns as visualized by fluorescence microscopy.

A glass disk was spin-coated with a solution of 5 wt % polystyrene (PS) in xylene to form a ~0.5 μm thick film.[12] The film was then spin-coated with a solution of 0.5 wt % of **1a** or **1b** in nitromethane and baked at 60 °C for 20 min. Photolysis[13] of the film resulted in complete decomposition of the azido groups as indicated by FTIR[14] and covalent attachment of the NHS PFPA esters to the PS surface,[15] giving **2a** and **2b**, respectively, as indicated by the experiments outlined below. Since NHS-active esters react readily with primary amines to form amides,[16] a variety of primary amine-containing reagents including biomolecules may, in principle, be attached to the polymer surface by this method.

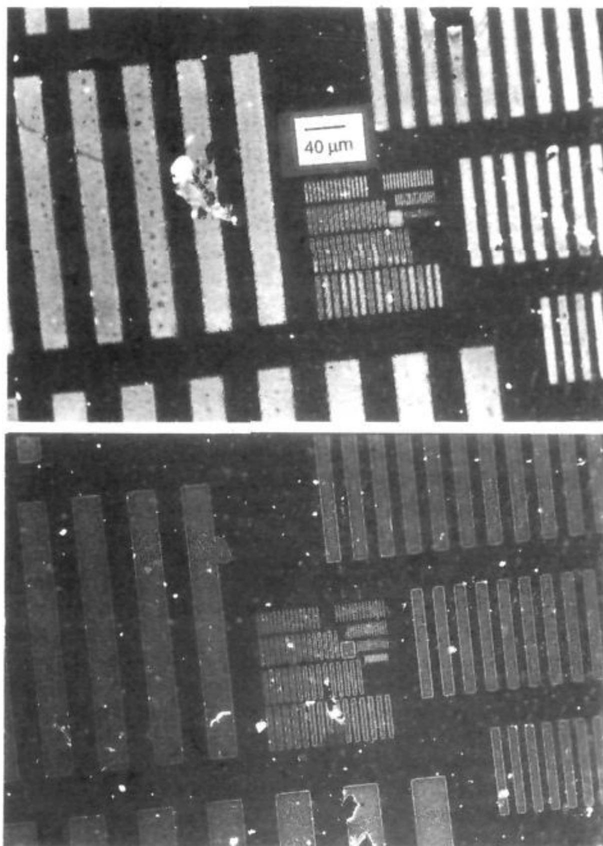


Figure 2. Micrographs of fluorescent protein formed by treating preformed PS patterns with (top) **1a** followed by photolysis and then *N*-(5-aminopentyl)biotinamide followed by fluorescein–avidin and (bottom) only fluorescein–avidin.

The versatility and covalent nature of this method are exemplified by the immobilization of horseradish peroxidase (HRP, Sigma) on PFPA-NHS-modified PS films by incubating the films **2a** and **2b** in a 50 μM solution of HRP in $NaHCO_3$ buffer (pH 8.2) at 25 °C for 3 h[17] followed by thorough rinsing with phosphate buffer (pH 7.0). The enzyme activity of the resulting immobilized HRP films **3a** and **3b** was determined spectrophotometrically at 420 nm and 25 °C in phosphate buffer using 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and hydrogen peroxide (1.8 mM ABTS/0.8 mM H_2O_2).[18] Provided that the immobilized HRP has the same activity as the native HRP,[19] the extent of immobilization of HRP was 0.5 ± 0.1 for **3a** and 1.0 ± 0.2 ng/mm^2 for the spacer-containing analogue **3b**,[20] indicating reasonable immobilization efficiencies[21] in these nonoptimized experiments.

Surface modification of the conducting polymer, poly(3-octylthiophene) (P3OT),[22] was accomplished in a similar manner. The extent of immobilization of HRP on PFPA-NHS-modified

(3) Delamar, M.; Hitmi, R.; Pinson, J.; Saveant, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 5883–5884.

(4) MacDonald, S. A.; Schlosser, H.; Ito, H.; Clecak, N. J.; Willson, C. G. *Chem. Mater.* **1991**, *3*, 435–442.

(5) Pantano, P.; Morton, T. H.; Kuhr, W. G. *J. Am. Chem. Soc.* **1991**, *113*, 1832–1833.

(6) Allcock, H. R.; Fitzpatrick, R. J.; Salvati, L. *Chem. Mater.* **1991**, *3*, 450–454.

(7) (a) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767–773. (b) Kiederowski, G. von *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 822–823.

(8) Rozsnyai, L. F.; Benson, D. R.; Fodor, S. P. A.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 759–761.

(9) Harmer, M. A. *Langmuir* **1991**, *7*, 2010–2012.

(10) Keana, J. F. W.; Cai, S. X. *J. Org. Chem.* **1990**, *55*, 3640–3647.

(11) *N*-Acylation of 5-aminopentanoic acid with 4-azido-2,3,5,6-tetrafluorobenzoyl chloride[10] gave the amide (mp 160–161 °C; HRMS calcd for $C_{12}H_{10}F_4N_4O_3$ 334.0687, found *m/z* 334.0710), which was then coupled with NHS in the presence of DCC to give succinimido 5-(4-azido-2,3,5,6-tetrafluorobenzamido)pentanoate (**1b**) (mp 93–95 °C; HRMS calcd for $C_{16}H_{13}F_4N_5O_5$ 431.0850, found *m/z* 431.0866).

(12) Cai, S. X.; Kanskar, M.; Wybourne, M. N.; Keana, J. F. W. *Chem. Mater.* **1992**, *4*, 879–884.

(13) Photolysis was carried out in a Rayonet photoreactor with 254-nm lamps for 5 min at ambient temperature under air.

(14) FTIR was performed with a control sample using a NaCl disk as the support.

(15) The reaction likely occurred via C–H bond insertion of the highly reactive nitrene intermediate derived from **1a** or **1b**. See ref 12; Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 5054–5067 and references cited therein.

(16) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839–1842.

(17) Brinkley, M. *Bioconjugate Chem.* **1992**, *3*, 2–13.

(18) Groome, N. P. *J. Clin. Chem. Clin. Biochem.* **1980**, *18*, 345–349.

(19) Nakane, P. K.; Kawaoi, A. *J. Histochem. Cytochem.* **1974**, *22*, 1084–1091.

(20) In control experiments, polymer films not spin-coated with PFPA were similarly baked, irradiated, and incubated with HRP solution. The resulting films showed no HRP activity.

P3OT films was 0.2 ± 0.1 with **3a** and 0.3 ± 0.1 ng/mm^2 with **3b**.[20]

Surface modification using PFPAs was combined with photolithography to generate micrometer-sized patterns. A PS film was spin-coated with a nitromethane solution of **1a**, baked, and irradiated[23] through a high-resolution photomask with a minimum feature size of 0.5 μm. The film was then dipped in nitromethane for 20 s, air-dried, and allowed to react with a solution of 5-(aminoacetamido)fluorescein in ethanol (4 mg/mL) at 25 °C for 1 h followed by thorough rinsing with ethanol. Figure 1 shows the resulting micrometer-sized patterns observed under a fluorescence microscope, further demonstrating this new surface modification strategy.[24] The smallest features (0.5 μm) are resolved though slightly broadened, probably owing to diffraction effects.

This same methodology was also used for the surface modification of preformed polymer microstructures. A micrometer-scale pattern of PS, which had previously been fabricated on a silicon wafer using deep-UV lithography, was dipped in a nitromethane solution of **1a** for 10 s, baked, and photolyzed as before. The sample was then immersed in a solution of *N*-(5-aminopentyl)biotinamide in DMF (1 mg/0.2 mL) for 4 h and washed with DMF followed by ethanol. Taking advantage of the strong affinity of avidin for biotin,[25] fluorescein–avidin was attached to the surface by incubating the wafer in a solution of the fluorescent protein in pH 8.2 buffer (3.2 mg/0.5 mL) for 4 h. The micrometer-sized patterns and control shown in Figure 2 indicate the covalent attachment of the biotin–avidin–fluorescein assembly on the preformed PS microstructure.

This new surface modification strategy should find application in microelectronics and in the construction of novel micrometer-scale biosensors.

Acknowledgment. This work was supported by grants from the Office of Naval Research, the Oregon Resource Technology Development Corporation, and the National Institute of General Medical Sciences (GM 27137). We thank Dr. Gary Goncher of Tektronix Inc. for his help in carrying out the deep-UV exposure.

(21) An HRP molecule has a molecular weight of around 40000 and a radius of 2.67 nm in the hydrated state. (Steiner, H.; Dunford, H. B. *Eur. J. Biochem.* **1978**, *82*, 543–549.) Assuming a flat polymer surface, the surface coverage of a monolayer of HRP is 2.7 ng of HRP/mm^2.

(22) The nitrene insertion reaction probably involves CH bonds in the octyl side chain and retention of polymer conductivity. See: Cai, S. X.; Nabity, J. C.; Wybourne, M. N.; Keana, J. F. W. *J. Mol. Electron.* **1991**, *7*, 63–68.

(23) Photolysis was carried out in a KSM Karl Suss deep-UV contact aligner.

(24) As a control, a PS film without a spin-coating of NHS active ester **1a** was photolyzed, developed, and treated with 5-(aminoacetamido)-fluorescein. No fluorescent patterns were observed under the fluorescence microscope.

(25) Heitzmann, H.; Richards, F. M. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3537–3541.

Isolation and Structure Determination of 2-Amino-2-deoxyisochorismate: An Intermediate in the Biosynthesis of Anthranilate[†]

Anthony A. Morollo,*[,‡] M. G. Finn,[§] and Ronald Bauerle[‡]

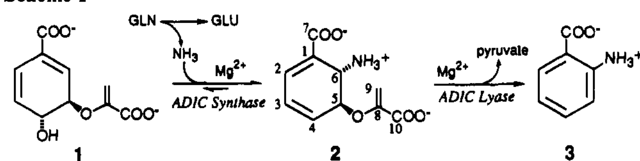
Department of Biology and the Molecular Biology Institute University of Virginia, Charlottesville, Virginia 22903 Department of Chemistry, University of Virginia Charlottesville, Virginia 22903

Received September 24, 1992

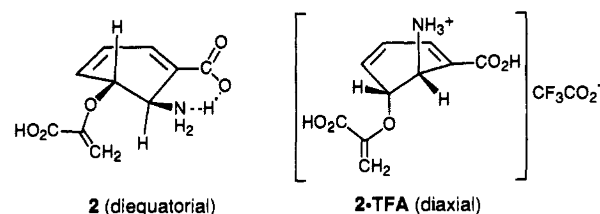
Anthranilate synthase (AS) catalyzes the initial reaction in the biosynthesis of tryptophan from chorismate[1] (Scheme I). The formation of anthranilate (*o*-aminobenzoate) **3** from chorismate

* Author to whom correspondence should be addressed. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22903.

Scheme I



Scheme II



2 (diequatorial) 2•TFA (diaxial)

1 and glutamine has long been suggested to occur through an intermediate, *trans*-6-amino-5-[(1-carboxyethenyl)oxy]-1,3-cyclohexadiene-1-carboxylic acid, **2**,[2] commonly called 2-amino-2-deoxyisochorismate (ADIC), although a cis rather than trans stereochemistry has also been proposed.[3] Attempts to detect the accumulation of **2** during enzymatic turnover have heretofore been unsuccessful;[4] however, synthetic **2·TFA** has been shown to be a competent substrate for anthranilate formation by AS.[5] Additionally, the accumulation of a C5-*R*-lactyl aminocyclohexadiene has been demonstrated in enzymatic incubations using a C5-*R*-lactyl analog of chorismate as substrate, although the stereochemistry of the lactylaminocyclohexadiene was not defined.[6] Here we report the isolation of an aminated intermediate as the product of an enzymatic activity of the *Salmonella typhimurium* AS and present the formal structural identification of this intermediate as **2**.

The results of ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-COSY 2D NMR analysis of **2** are shown in Figure 1. The assignments are supported by ^{1}H,^{1}H correlations establishing the H2–H3–H4–H5–H6 and H9–H9′ spin systems, expected chemical shift values, and ^{1}H,^{13}C correlation to the carbon resonances. The coupling constant of 12.3 Hz measured for H5 and H6 is indicative of trans configuration at C5 and C6. The N-substituted carbon was identified from the proton-decoupled natural abundance ^{13}C NMR spectrum of a sample of **2** enriched in ^{15}N, which demonstrated splitting of the C6 resonance at 52.9 ppm into a doublet with J_{CN} = 6 Hz (Figure 1, inset). All data are consistent with the structure of **2** with stereochemistry as shown.

The ^{1}H NMR data obtained for **2** differ significantly from those reported for synthetic **2·TFA**,[5a] particularly the H6 coupling constant,[9] suggesting that either **2** and **2·TFA** exist in different

† Abbreviations used: AS, anthranilate synthase; TrpE, catalytic subunit of AS; ADIC, *trans*-6-amino-5-[(1-carboxyethenyl)oxy]-1,3-cyclohexadiene-1-carboxylic acid, commonly known as 2-amino-2-deoxyisochorismate; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

‡ Department of Biology and the Molecular Biology Institute, University of Virginia.

§ Department of Chemistry, University of Virginia.

(1) Bentley, R. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 307–384.

(2) (a) Levin, J. G.; Sprinson, D. B. *J. Biol. Chem.* **1963**, *239*, 1142–1150. (b) Srinivason, P. R. *Biochemistry* **1965**, *4*, 2860–2865.

(3) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980; pp 238–246.

(4) Walsh, C. T.; Liu, J.; Rusnak, F.; Sakaitani, M. *Chem. Rev.* **1990**, *90*, 1105–1129.

(5) (a) Policastro, P. P.; Au, K. G.; Walsh, C. T.; Berchtold, G. A. *J. Am. Chem. Soc.* **1984**, *106*, 2443–2444. (b) Teng, C. P.; Ganem, B. *J. Am. Chem. Soc.* **1984**, *106*, 2463–2464.

(6) Walsh, C. T.; Erion, M. D.; Walts, A. E.; Delany, J. J., III; Berchtold, G. A. *Biochemistry* **1987**, *26*, 4734–4745.

(7) The mutant AS complex, with a single amino acid substitution at HIS398 of the TrpE subunit (His → Met), was purified as described previously in the following: Caligiuri, M. G.; Bauerle, R. *J. Biol. Chem.* **1991**, *266*, 8328–8335. The preparation was homogeneous as determined by SDS–PAGE. The AS specific activity of the enzyme was ≈1% of the wild-type enzyme, assayed as described in the following: Bauerle, R.; Hess, J.; French, S. *Methods Enzymol.* **1987**, *142*, 366–386. The properties and kinetic behavior of this enzyme will be reported in more detail elsewhere (manuscript in preparation).

0002-7863/93/1515-816$04.00/0 © 1993 American Chemical Society