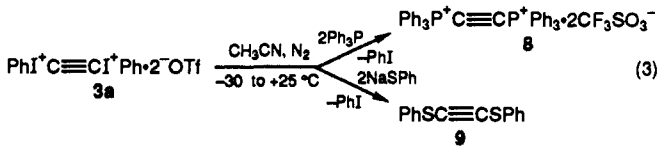
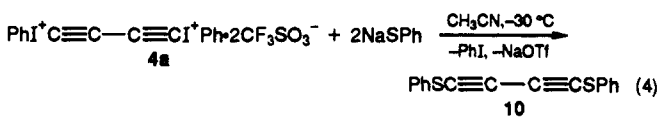
above −10 to −20 °C and hence *all* handling must be done at low temperatures.[13]

The bisiodonium adducts **3** and **4** were characterized by IR and multinuclear NMR spectroscopy, and where stable, (i.e., **3a**) by microanalysis.[12,13] Specifically, the infrared indicated only aromatic C–H stretching and absorptions highly characteristic of triflates and nonaflates.[14] The presence of the perfluoroalkanesulfonate groups was confirmed by ^{19}F NMR. In the case of **4a** a characteristic[9] conjugated C≡C absorption occurs at 2070 cm^{-1}. The ^1H NMR spectra had the typical 2:1:2 aromatic signals highly characteristic of the phenyl group in iodonium salts. Most important, the ^{13}C NMR spectra were all consistent with the proposed structures.

The title compounds **3** and **4** may be looked upon as novel, "stabilized" forms[15,16] of C_2 and C_4, respectively. Moreover, in analogy with the behavior of **1** toward nucleophiles[2] they should be premier C_2 and C_4 transfer agents and thereby serve as progenitros par exellence for difunctionalized acetylenes and diacetylenes.[9] Indeed, reaction of **3a** with 2 equiv of either Ph_3P or NaSPh in CH_3CN at −30 to +25 °C results in the corresponding difunctional acetylenes **8**[17] and **9**,[18] respectively (eq 3). Likewise, **4a** reacts with Ph_3P and NaSPh but the resulting diphosphonium-1,3-diyne is unstable and only product **10**[19] may be isolated (eq 4).





In conclusion, we have discovered a simple procedure for the preparation of the hitherto unknown diiodonium acetylenes **3** and diiodonium diacetylenes **4**. These adducts readily react with nucleophiles resulting in C_2 and C_4 transfer and the concomitant formation of difunctional acetylenes and difunctional 1,3-diynes, respectively. Further chemistry and uses of these novel diiodonium acetylenes will be the subject of future reports.

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(13) Compound **4a** was isolated by filtration at −50 °C under N_2 and, upon washing with *cold* ether, gave 85% of **4a** as a white microcrystalline solid that turned to a black tar between −15 and −10 °C. *All* spectra were obtained at −35 °C: IR (CH_2Cl_2) 3051, 2070 (C≡C), 1444, 1382, 1260, 1173, 1030 cm^{-1}; ^1H NMR (CD_3CN) δ 7.7–7.8 (m, 2 H), 7.9–8.0 (m, 1 H), 8.3–8.4 (m, 2 H); ^{13}C NMR (CD_3CN) δ 33.11 (C≡C), 84.01 (C≡C), 120.5 (quart, J_{CF} = 318 Hz, CF_3), 129.75, 131.90, 132.77, 134.47 (Ph); ^{19}F NMR (CD_3CN) δ −78.61 (s, CF_3). The corresponding nonaflate **4b** and tosylate and mesylate salts were too unstable to isolate and to obtain spectral data.

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(15) For other novel C_2 units, see: Hoffmann, R. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1593.

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(17) For **8**: 99% yield; mp 218–220 °C dec; IR (CCl_4) 3059, 1584, 1485, 1285, 1240, 1027 cm^{-1}; ^1H NMR (CD_3CN) δ 7.65–7.74 (m, 2 H), 7.75–7.84 (m, 2 H), 7.9–8.0 (m, 1 H); ^{31}P NMR ($CDCl_3$) δ 21.16; ^{19}F NMR (CD_3CN) δ −78.65 (s, CF_3); ^{13}C NMR (CD_3CN) δ 114.9 (m, C≡C), 130.4, 134.25, 135.95, 137.9 (all m, Ph). Anal. Calcd for $C_{40}H_{30}P_2F_6S_2O_6$: C, 56.74; H, 3.57; S, 7.57. Found: C, 56.39; H, 3.82; S, 7.63.

(18) For **9**: 66%, oil; IR (neat) 2957, 1579, 1477, 1439, 1298, 1072, 1023, 998, 899, 735, 687 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.2–7.4 (m, 3 H), 7.5–7.6 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 87.98 (C≡C), 126.03, 127.11, 129.28, 133.27 (Ph).

(19) For **10**: 67%, oil; IR (neat) 3059, 2193 (C≡C), 1571, 1471, 1438, 1057, 1014, 997, 733, 686 cm^{-1}; ^1H NMR (CD_3Cl) δ 7.20–7.55 (m, Ph); ^{13}C NMR ($CDCl_3$) δ 71.72 (C≡C), 127.07 (C≡C), 127.41, 127.48, 129.03, 129.47 (Ph). This compound darkens and decomposes upon standing for several hours at room temperature.

Electron-Transfer Communication between a Redox Polymer Matrix and an Immobilized Enzyme: Activity of Nitrate Reductase in a Viologen–Acrylamide Copolymer

Itamar Willner,* Azalia Riklin, and Noa Lapidot

Department of Organic Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904, Israel

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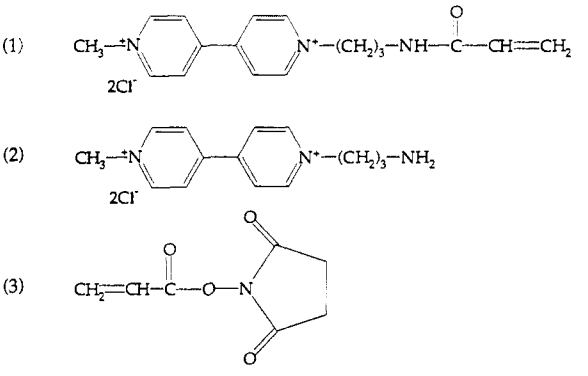
Long-range electron transfer to the active sites of oxidoreductase enzymes has been the subject of recent extensive research activities.[1-4] It has been shown that modification of the protein backbone by electron carriers allows electron transfer across the protein structure to the active site. Also, electrostatic association of glucose oxidase to a redox polyelectrolyte enabled electron-transfer communication between the redox polymer and the enzyme. Here we report on the long-range electron transfer to nitrate reductase through immobilization of the biocatalyst in a functionalized polymer matrix.

The monomer *N*-methyl-*N'*-(acrylamidopropyl)-4,4'-bipyridinium (**1**) is prepared by reacting *N*-methyl-*N'*-(aminopropyl)-4,4'-bipyridinium (**2**)[5] with *N*-(acryloyloxy)succinimide (**3**).[6] Nitrate reductase, EC 1.9.6.1 from *Aspergillus niger*, 0.2 mg, 0.1 unit, is polymerized[7] in an aqueous solution, pH = 7.5, that includes nitrate (7×10^{-3} M), **1** (113.3 mg), acrylamide (375 mg), and 20 mg of *N,N'*-methylenebis(acrylamide). The resulting



copolymer gel is washed thoroughly until no free **1** is detectable in the aqueous phase by dithionite reduction. The immobilized enzyme retains 55% of the native enzyme activity. By measuring the amount of **1** that is eliminated during the purification of the gel, we estimate the ratio **1**:acrylamide in the polymer gel to be ca. 1:35. Introduction of dithionite into an aqueous suspension of the functionalized gel beads results in the blue coloration of the gel pieces. Alternatively, this can be achieved by illuminating the gel beads in the presence of ruthenium(II) tris(bipyridine) ($Ru(bpy)_3^{2+}$) and ethylenediaminetetraacetic acid disodium salt ($EDTANa_2$), as sacrificial electron donor, at pH = 7.44. The blue color persists in the polymer for days, and no leakage of the blue color to the aqueous phase is observed. These observations imply that the bipyridinium functionalized acrylamide copolymer is reducible by dithionite and by the photochemical process.

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(5) Compound **2** was synthesized by reacting 4,4'-bipyridine with *N*-(*tert*-butoxycarbonyl)-3-amino-1-bromopropane followed by further alkylation with methyl iodide and hydrolysis of the dialkylated sali. All compounds gave satisfactory spectroscopic (^1H NMR) and microanalysis results. Full details on the experiments will be given in a later report.
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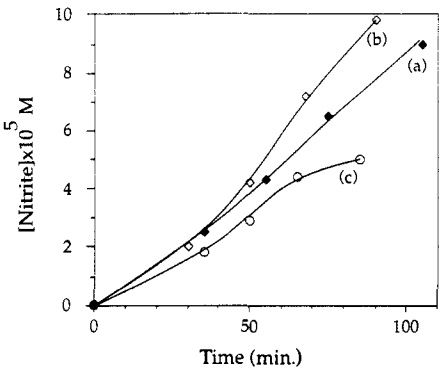

Figure 1. Rate of NO_3^- reduction in the assembly composed of nitrate reductase (0.023 unit, based on native enzyme) immobilized in the acrylamide (**1**) copolymer. In all experiments, $[NO_3^-] = 4.4 \times 10^{-3}$ M in 2.3 mL of Tris buffer, pH = 7.44. (a) Dark reduction in the presence of sodium dithionite, 9.4×10^{-3} M. (b) Through illumination in the presence of $Ru(bpy)_3^{2+}$ (5.5×10^{-5} M) and Na_2EDTA (4.4×10^{-3} M). (c) Through illumination in the presence of $Ru(bpy)_3^{2+}$ (5.5×10^{-5} M), Na_2EDTA (4.4×10^{-3} M), and added MV^{2+} (3.3×10^{-4} M), where the enzyme is immobilized in a nonfunctionalized acrylamide polymer.

Previous studies have shown that reduced methyl viologen, $MV^{\bullet+}$, reduces nitrate to nitrite in a reaction mediated by nitrate reductase.[8,9] In this system, $MV^{\bullet+}$ acts as a diffusing electron carrier to the enzyme's active site. We find that introduction of the **1** functionalized polymer beads that contain immobilized nitrate reductase (0.023 unit, based on native enzyme) to an aqueous solution, pH = 7.44, that includes nitrate, 4.4×10^{-3} M, and dithionite, 9.4×10^{-3} M, results in the formation of nitrite (Figure 1a). Similarly, illumination ($\lambda > 400$ nm) of a photosystem consisting of the enzyme (0.023 unit) immobilized in the functionalized polymer in an aqueous solution, pH = 7.44, $Ru(bpy)_3^{2+}$ (5.5×10^{-5} M), and $EDTANa_2$ (4.4×10^{-3} M) results in the reduction of nitrate to nitrite (Figure 1b). During both photochemical and chemical reduction reactions, the polymer appears dark blue (a color characteristic of 4,4'-bipyridinium radicals). All the components in the system are essential for the reduction (or photoreduction) of NO_3^-. Thus no nitrite is formed either in the absence of added nitrate or upon exclusion of the enzyme. Also, the reduction of NO_3^- is not directly affected by dithionite, and no formation of NO_2^- is observed when dithionite is added to nitrate reductase immobilized in a polyacrylamide polymer that is bare of the **1** copolymer. The results indicate that the polymer-anchored bipyridinium radical, formed by reduction of dithionite or through photochemical means, reduces the polymer-immobilized enzyme. We also observe that the rates of NO_2^- formation are similar for dithionite and the photosystem, and that MV^{2+} added to a nonfunctionalized acrylamide polymer that includes the biocatalyst, decreases slightly the rate of NO_3^- reduction, Figure 1c. These results suggest that the rate of NO_3^- reduction is limited by the process occurring at the biocatalyst active site. Laser flash photolysis experiments[10] in a photosystem composed of $Ru(bpy)_3^{2+}$ and polymer-immobilized nitrate reductase allow us to determine the electron-transfer rate constant, k_{et}, from the photoreduced polymer to the biocatalyst active site. The estimated value of this rate constant is $k_{et} = (9 \pm 3) \times 10^5$ s^{-1}.

A functional polymer–biocatalyst organization does not always result in an active assembly. Similar immobilization of glutathione reductase does not lead to an electron-transferring system, despite the fact that $MV^{\bullet+}$ does act as a diffusing electron carrier to this enzyme.[10] The lack of activity of glutathione reductase in the functionalized polymer is attributed to the protein crowded environment of the enzyme active site, which prevents direct electron-transfer communication with the polymer.

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Registry No. 1, 128269-89-6; **2**, 128269-88-5; **3**, 38862-24-7; NO_3^-, 14797-55-8; nitrate reductase, 9013-03-0; acrylamide, 79-06-1; *N,N'*-methylenebis(acrylamide), 110-26-9; 4,4'-bipyridine, 553-26-4; *N*-(*tert*-butoxycarbonyl)-3-amino-1-bromopropane, 128412-15-7.

Synthesis of the Ketodifluoromethylene Dipeptide Isostere

David B. Damon and Dennis J. Hoover*

Department of Medicinal Chemistry
Central Research Division, Pfizer Inc.
Groton, Connecticut 06340
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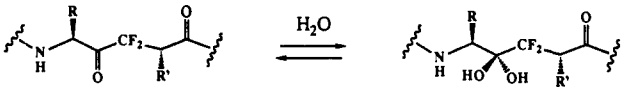
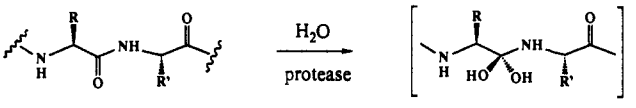
The inhibition of aspartic proteases such as human renin and HIV protease is a pressing problem in bioorganic chemistry. In this paper we present a solution to a critical element of this problem, the synthesis of the ketodifluoromethylene dipeptide isostere (I). This structure as the hydrate closely resembles II,

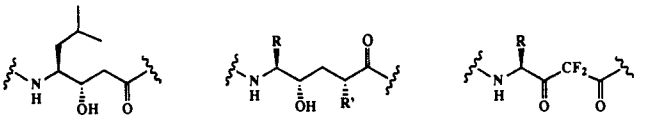


I, Ketodifluoromethylene Dipeptide Isostere



Protease Substrate II, Tetrahedral Intermediate

the putative tetrahedral intermediate in proteolytic cleavage of the peptide bond, differing only in substitution of difluoromethylene for tetrahedral nitrogen. Our interest in the synthesis of I has been driven by the vital contributions of more distant mimics of intermediate II, namely, statine and its derivatives,[1,2]



Statine Hydroxyethylene Dipeptide Isostere Difluorostatone

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