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## Photochemistry of Conjugated Polyacetylenes. Photoreaction of 1,4-Diphenylbutadiyne with a Mixture of Olefins

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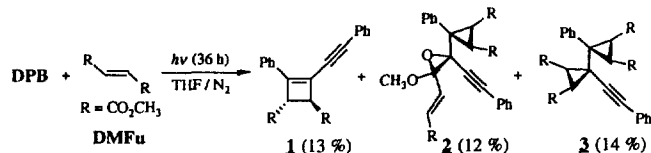
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Irradiation of 1,4-diphenylbutadiyne (DPB) with a mixture of electron-deficient and electron-rich olefins in deaerated tetrahydrofuran yields a 1:1 primary photoadduct between DPB and electron-deficient olefins. Irradiation of the primary photoadduct of DPB and dimethyl fumarate (DMFu) with various olefins such as DMFu, acrylonitrile (AN), and 2,3-dimethyl-2-butene (DMB) in deaerated tetrahydrofuran yields regiospecific 1:1 photoadducts. The electron-deficient olefins are more reactive than electron-rich olefins in the photoreaction which proceeds through excited triplet state.

### Introduction

The [2+2] photocycloaddition reaction of olefins has been extensively studied and the reaction mechanisms have been well understood. On the contrary, the photoreaction of acetylenes with olefins received relative little attention. There are a few reports of photocycloaddition reactions of acetylenes with olefins.<sup>1-8</sup> In most cases cyclobutene rings are formed, but cyclopropyl photoadducts as minor products were also observed.<sup>6-8</sup> When two alkene units are incorporated into a rigid molecule, such as cyclohexa-1,4-diene, the major photoproduct was the bicyclopentyl adduct.<sup>7,8</sup> From sensitizing and triplet quenching studies on the formation of cyclobutene photoproduct, the photoreaction is suggested to proceed through the lowest triplet excited state of acetylene. The formation of cyclopropyl photoproduct is proposed to be concerted.

We have previously reported interesting photoreactions of 1:1 1,4-diphenylbutadiyne (DPB) with several olefins to yield 1:1 and 1:2 photoadducts.<sup>9-11</sup> The photoreaction of DPB with dimethyl fumarate (DMFu) in deaerated THF solution at 300 nm yields one primary 1:1 photoadduct (**1**) and two secondary 1:2 photoadducts (**2** and **3**).<sup>11</sup>



Triplet quenching experiments showed that the photoreaction of DPB with DMFu to give **1** proceeds *via* triplet excited state of DPB and **2** and **3** are produced *via* singlet and triplet excited states of **1**, respectively. The photoadduct **2** very interestingly possessed a cyclopropane and an oxirane ring and a triplet carbene intermediate is proposed for **3** in a plausible mechanism.

In this investigation, we report regiospecific photocycloaddition reactions of DPB or **1** with electron-deficient and electron-rich olefins and the relative reactivity of some olefins.

### Results and Discussion

Irradiation of **1** with olefins in deaerated tetrahydrofuran at 300 nm yields 1:1 photoadducts and trace amount of **1'** (<1%), a configurational isomer of **1**. The structure of these adducts was determined by various physical methods such as UV, IR, NMR, and MS spectroscopy.

Some photoadducts are obtained when **1** is irradiated in deaerated tetrahydrofuran solutions of various olefins such as DMFu, AN, and DMB.

The UV spectra of **7** and **8** are quite different from those of **4**, **5**, and **6** which are very similar to each other indicating that these adducts have the same chromophore (Figure 1). The absorption maxima were slightly blue shifted in **4**, **5**, and **6** and considerably in **7** and **8**. IR spectra of **4** and **5** show a C $\equiv$ N stretching band while **6-8** show no acetylenic stretching band. The significant difference of IR spectra of **6** and **7** (or **8**) is the C=C stretching band at  $\sim 1600 \text{ cm}^{-1}$ . Mass spectra of all the photoproducts show molecular ion peaks indicating that all the products are formed by the ad-

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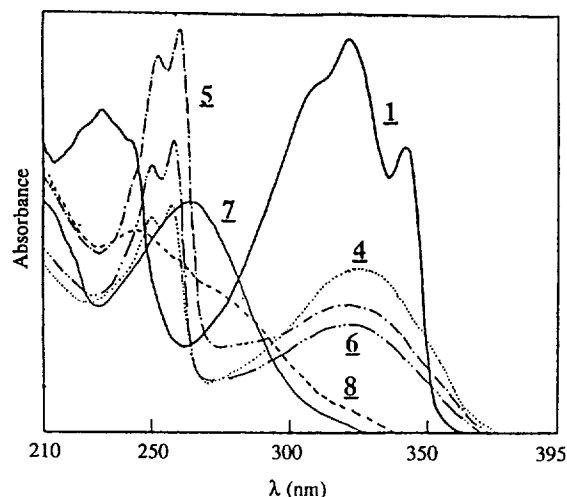
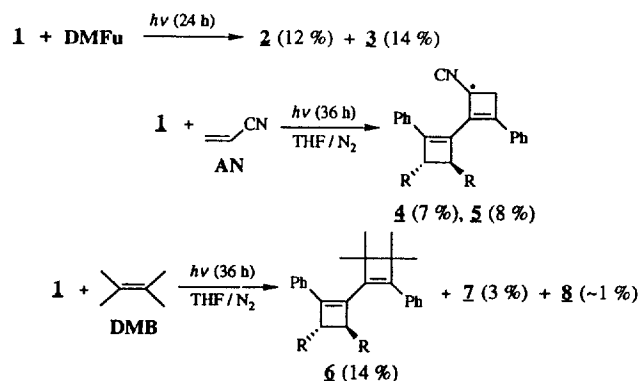
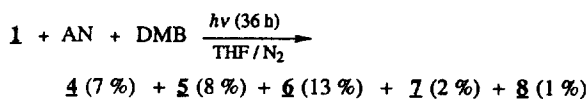
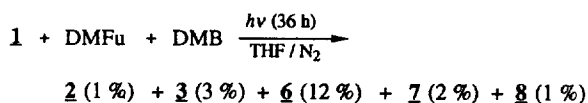


Figure 1. UV absorption spectra of photoproducts 4-8 in MeOH.



dation of one olefin molecule. In  $^{13}\text{C}$ -NMR spectra of these adducts except **2** and **3**, two  $\text{sp}$  hybridized carbons disappeared and two  $\text{sp}^2$  carbons are generated instead indicating the reaction site to be one of the  $\text{C}\equiv\text{C}$  triple bonds. Coupling patterns and chemical shifts of **7** and **8** are quite different from those of **6** and indicate that **7** and **8** are the secondary photoproducts showing two isolated uncoupled olefinic protons and two new  $\text{sp}^2$  carbons. Photolysis of pure **6** in THF results in the formation of **7** and **8**, and spectral data suggest splitting one of the cyclobutene rings.

To compare the reactivity of olefins with **1**, deaerated THF solutions of **1** and mixture of olefins (DMFu-DMB, AN-DMB) were irradiated to obtain similar 1:1 photoadducts. The chemical yields of **2** and **3** are very low compared to that of DPB and DMFu photoreaction and the major photoproduct is formed between the remaining triple bond in **1** and DMB or AN suggesting that triplet state of **1** reacts with AN and DMB before rearranging into carbenoids.



Irradiation of DPB with olefin mixture in THF yields pri-

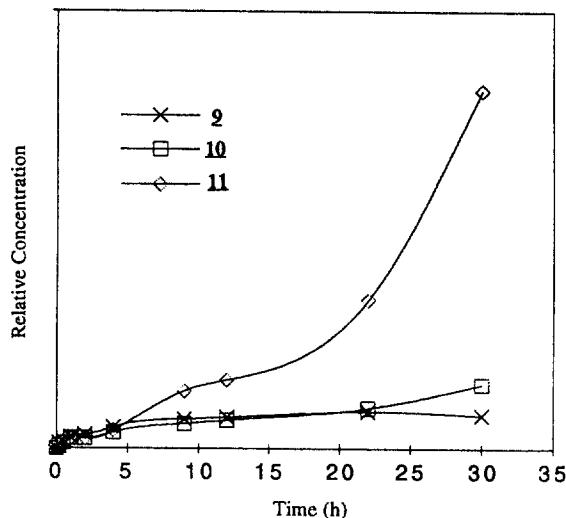
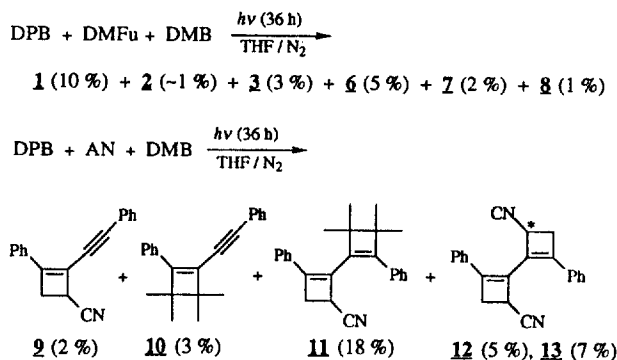


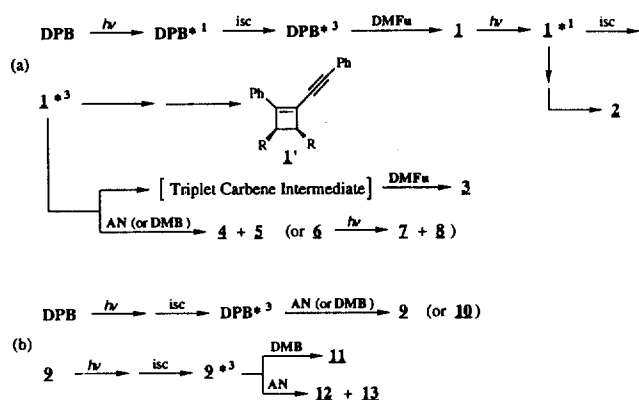
Figure 2. Relative concentration change of **9**, **10**, and **11**.

mary 1:1 (**1**, **9**, and **10**) and secondary 1:2 photoadducts (**2**, **3**, **6**, **7**, **8**, **11**, **12**, and **13**).



The reaction of DPB-AN-DMB mixture solution shows less olefin selectivity than that of DPB-DMFu-DMB, and the 1:1 photoadducts **9** and **10** are obtained in almost equal amounts. The concentration of **10** increased linearly but **9** reaches a maximum and decreases on extended irradiation and **11** increases sharply at this point indicating that the major precursor of **11** is **9** (Figure 2). Irradiation of **9** with DMB yields **11** very efficiently (>70% yield in 24 hours) but photoreaction of **10** with AN proceeded very slowly and does not proceed at all when AN/**10** concentration ratio is below 10. These reactivity and selectivity differences can be explained applying the frontier orbital theory<sup>12</sup> which states that the energy differences between HOMO and LUMO of the reacting species are decisive in rate determination. The energy difference between reactive radicals and electron-deficient olefins is smaller than that of radicals and electron-rich olefins and consequently electron-deficient olefins are more reactive.

A plausible mechanism for the photoreaction of DPB (or **1**) with olefins is proposed in Figure 3. The concentration of olefins did not affect the number of products formed but is the most important factor for the product ratio for the reaction. When DPB-mixed olefin system is irradiated, 1:2 adducts including cyclopropane rings between DPB and DMFu (or DMB) are obtained in very small amounts indica-



**Figure 3.** The plausible reaction mechanisms of DPB (or 1) with olefins. (a) DPB-DMFu-DMB and 1-DMB (or AN) and (b) DPB-AN-DMB systems.

ting that triplet state of 1 or 9 probably reacts fast with AN and DMB before rearranging into carbenoids. The reactivities of olefins are in the order of DMFu $\gg$ DMB and AN $\gg$ DMB.

## Experimental

### Instruments

$^1\text{H}$ -NMR spectra were recorded on a AM-300 spectrometers with chemical shifts being referenced against TMS as an internal standard or the residual proton of the solvent  $\text{CDCl}_3$ .  $^{13}\text{C}$ -NMR spectra were run on a AM-300 spectrometer with  $\text{CDCl}_3$  as solvent and internal standard. The chemical shifts ( $\delta$ ) are reported in ppm. UV spectra were recorded on a Shimadzu 3100 spectrophotometer. Mass (MS) spectra were determined at 70 eV with a Hewlett-Packard 5985A GC/MS interface by the electron impact (EI) method. FT-IR spectra were recorded on a Bomem MB-100 spectrometer in a NaCl cell or KBr pellet. High-performance liquid chromatography (HPLC) was performed on a Waters Associates Model 244 liquid chromatograph (Mildford, MA) equipped with a Model 6000A solvent delivery system, Model 440 UV absorbance detector fixed at 254 nm, Model R401 differential refractometer, and Model U6K universal injector. Lichrosorb Si-60 were used for quantitative and preparative analyses.

### Materials

1,4-Diphenylbutadiyne (DPB) was purchased from Aldrich Chemical Co. 2,3-Dimethyl-2-butene (DMB) and acrylonitrile (AN) were purchased from Aldrich Chemical Co. and purified by fractional distillation prior to use. Dimethyl fumarate (DMFu) from Aldrich Chemical Co. was used after recrystallization from methanol. Tetrahydrofuran was dried over sodium metal with benzophenone indicator followed by fraction distillation before use. Methylene chloride was dried over phosphorus pentoxide. Chromatographic and spectroscopic grade organic solvents (Merck) were used for normal phase HPLC and absorption spectra, respectively. Doubly distilled and deionized water was also used for reverse phase HPLC.

**Irradiation of 1,4-Diphenylbutadiyne (DPB) with Dimethyl Fumarate (DMFu).** 1,4-Diphenylbutadiyne (DPB, 4 mM) solution in tetrahydrofuran (500 mL) with dimethyl

fumarate (DMFu, 100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 36 hours, the reaction mixture was evaporated *in vacuo*. Unreacted DMFu and DPB was crystallized out in methanol. The photoproducts 1, 1-*cis*, 2, and 3 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 to 6/1/1 (v/v/v), in 13, 4, 12, and 14% yields, respectively. Compound 1-*cis*:  $^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.74–7.35 (m, 10H), 4.24/4.08 (d/d,  $J_{\text{cis}}=5.55$  Hz, 1H/1H), 3.77 (s, 3H), and 3.71 (s, 3H) ppm;  $^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  170.70, 170.08, 147.08, 132.50, 131.78, 129.32, 128.91, 128.49, 128.40, 126.04, 122.58, 118.38, 97.28, 83.71, 52.23, 52.18, 47.59, and 46.66 ppm; MS (70 eV)  $m/e$  346 ( $\text{M}^+$ , 28.9), 331 ( $\text{M}^+-\text{CH}_3$ , 1.6), 314 (64.6), 299 (6.8), 286 (76.3), 271 (2.4), 226 (51.5), 215 ( $\text{C}_{17}\text{H}_{11}$ , 100.0), and 202 (DPB $^+$ , 22.3%); UV (MeOH)  $\lambda_{\text{max}}$  343, 322, 310, and 243 nm; IR (NaCl) 3060–3025, 2952, 2195 (m), 1735, 1572, 1439, 1218, 1027, 759, and 691  $\text{cm}^{-1}$ .

**Irradiation of 1 with DMFu.** 1 (4 mM) solution in tetrahydrofuran (200 mL) with DMFu (100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 24 hours, the reaction mixture was evaporated *in vacuo*. Unreacted reactant 1 and the photoproducts 1-*cis*, 2, and 3 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 to 6/1/1 (v/v/v), in ~1, 12, and 14% yields, respectively.

**Irradiation of 1 with Acrylonitrile (AN).** 1 (4 mM) solution in tetrahydrofuran (200 mL) with acrylonitrile (AN, 100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor for 36 hours. After removal of unreacted AN and solvent under reduced pressure, the reaction mixture was subjected to column chromatography using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 (v/v/v) as an eluent. The photoproducts 4 and 5 were purified by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 4/1/1 (v/v/v) as an eluting solvents in 7 and 8%, respectively. Compound 4:  $^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_2$ )  $\delta$  7.27–7.16 (m, 10H), 4.22 (d,  $J'_{12}=18.8$  Hz, 1H), 4.19 (d,  $J'_{21}=1.88$  Hz, 1H), 3.95 (t,  $J_{12}=3.64$  Hz, 1H), 3.74 (s, 3H), 3.70 (s, 3H), and 3.22 (d,  $J_{21}=3.64$  Hz, 2H) ppm;  $^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.19, 171.17, 144.64, 142.83, 133.15, 132.50, 130.48, 129.26, 129.04, 128.09, 127.20, 127.10, 126.65, 119.40, 77.20, 52.40, 52.33, 48.58, 46.15, 32.45, and 26.53 ppm; MS (70 eV)  $m/e$  390 ( $\text{M}^+$ , 7.0), 367 (21.0), 339 (11.1), 308 (24.3), 280 ( $\text{M}^+-\text{C}_4\text{H}_7\text{O}_4$ , 100.0), 279 (39.1), 253 (42.6), 252 (46.5), 239 (26.4), 202 (DPB $^+$ , 21.7), 139 (32.2), 105 (24.4), and 77 (46.8%); UV (MeOH)  $\lambda_{\text{max}}$  322, 257, and 249 nm; IR (NaCl) 3111–3004, 2953, 2931, 2236 (w), 1737, 1722, 1438, 1200, 1174, and 699  $\text{cm}^{-1}$ . Compound 5:  $^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.27–7.16 (m, 10H), 4.18 (d,  $J'_{12}=1.88$  Hz, 1H), 4.03 (d,  $J'_{21}=1.88$  Hz, 1H), 3.89 (q,  $J_{12}=4.97$  Hz,  $J_{13}=2.37$  Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.28 (dd,  $J_{21}=4.54$  Hz,  $J_{23}=13.92$  Hz, 1H), and 3.15 (dd,  $J_{31}=2.68$  Hz,  $J_{32}=13.92$  Hz, 1H) ppm;  $^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.76, 171.13, 145.02, 143.04, 133.11, 132.48, 130.79, 129.32, 129.06, 128.16, 128.13, 127.00, 126.93, 126.80, 119.62, 77.19, 52.54, 52.44, 47.02, 46.44, 32.47, and 27.03 ppm; MS (70 eV)  $m/e$

399 ( $M^+$ , 7.0), 367 (21.6), 340 (18.9), 308 (24.3), 280 ( $M^+-C_4H_7O_4$ , 100.0), 279 (39.7), 253 (34.8), 239 (27.6), 202 (DPB $^+$ , 21.6), 139 (32.2), 105 (24.3), and 77 (46.8%); UV (MeOH)  $\lambda_{max}$  322, 257, and 249 nm; IR (NaCl) 3115-3012, 2934, 2850, 2237 (w), 1738, 1733, 1442, 1182, 1027, and 762  $cm^{-1}$ .

**Irradiation of 1 with 1,4-Dimethylbutene (DMB).** 1 (4 mM) solution in tetrahydrofuran (200 mL) with 1,4-dimethyl-2-butene (DMB, 100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor for 36 hours. The photoreaction mixture was concentrated *in vacuo* and filtered before HPLC separation. Photoproducts 6, 7 and 8 were isolated by normal phase HPLC using *n*-hexane/ethyl acetate/methylene chloride; 20/1/1 (v/v/v) as an eluent in 14, 3, ~1% yields, respectively. Compound 6:  $^1H$ -NMR (300 MHz,  $CDCl_3$ )  $\delta$  7.26-7.08 (m, 10H), 4.10 (d,  $J_{12}=1.88$  Hz, 1H), 3.96 (d,  $J_{21}=1.88$  Hz, 1H), 3.73 (s, 3H), 3.68 (s, 3H), 1.43 (s, 3H), 1.32 (s, 3H), 1.21 (s, 3H), and 1.20 (s, 3H) ppm;  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ )  $\delta$  172.07, 172.05, 148.83, 140.68, 139.13, 134.61, 133.79, 133.22, 128.09, 127.94, 127.80, 127.31, 126.96, 126.33, 52.22, 51.99, 48.54, 47.33, 46.53, 46.42, 23.68, 22.88, 22.75, and 22.65 ppm; MS (70 eV) *m/e* 430 ( $M^+$ , 29.7), 355 ( $M^+-C_6H_5$ , 15.2) 295 (24.5), 253 (19.5), 239 (11.7), 202 (DPB $^+$ , 19.8), 179 (29.6), 165 (36.8), 128 (36.9), 115 (51.5), 105 (23.1), 91 ( $C_7H_7^+$ , 100.0), 84 (DMB $^+$ , 88.3), 49 (59.0), and 41 (35.8%); UV (MeOH)  $\lambda_{max}$  323, 260, and 252 nm; IR (NaCl) 3116-3012, 2951, 2921, 1737, 1733, 1442, 1164, 1022, and 764  $cm^{-1}$ . Compound 7:  $^1H$ -NMR (300 MHz,  $CDCl_3$ )  $\delta$  7.40-7.15 (m, 10H), 6.41 (s, 1H), 5.69 (s, 1H), 3.49 (s-s, 3H-3H), 1.42 (s, 6H), and 1.15 (s, 6H);  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ )  $\delta$  165.95, 165.89, 153.13, 149.29, 147.72, 139.31, 136.86, 134.00, 128.82, 128.22, 128.14, 127.34, 126.43, 126.22, 123.38, 51.35, 51.26, 51.18, 47.01, 22.91, and 22.80 ppm; MS (70 eV) *m/e* 430 ( $M^+$ , 8.2), 415 (19.8), 355 ( $M^+-C_6H_5$ , 15.7), 295 (50.1), 252 (22.5), 215 (21.9), 202 (DPB $^+$ , 16.5), 179 (14.6), 165 (43.08), 152 (25.9), 128 (46.9), 115 (79.6), 105 (33.8), 91 ( $C_7H_7^+$ , 100.0), 77 (37.7), 59 (54.9), and 41 (19.1%); UV (MeOH)  $\lambda_{max}$  264 nm; IR (NaCl) 3094-3014, 2930, 2855, 1729, 1594, 1493, 1446, 1368, 1338, 1241, 1173, 1017, 769, and 764  $cm^{-1}$ . Compound 8:  $^1H$ -NMR (300 MHz,  $CDCl_3$ )  $\delta$  7.37-7.15 (m, 10H), 6.17 (s, 1H), 5.94 (s, 1H), 3.69 (s, 3H), 3.46 (s, 3H), 1.13 (s, 6H), and 1.04 (s, 6H) ppm;  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ )  $\delta$  165.95, 165.89, 153.13, 149.29, 147.72, 139.67, 139.31, 136.86, 134.00, 128.82, 128.22, 128.14, 127.34, 126.43, 126.22, 123.38, 51.35, 51.26, 51.18, 47.01, 22.91, and 22.80 ppm; MS (70 eV) *m/e* 430 ( $M^+$ , 6.9), 415 (15.6), 355 ( $M^+-C_6H_5$ , 25.7), 295 (55.7), 252 (28.8), 215 (36.4), 202 (DPB $^+$ , 28.2), 179 (30.5), 165 (58.1), 152 (31.0), 128 (66.5), 115 (85.2), 105 (58.6), 91 ( $C_7H_7^+$ , 100.0), 77 (43.4), 59 (55.7), and 41 (27.3%); UV (MeOH)  $\lambda_{max}$  244 nm; IR (NaCl) 3113-3010, 2947, 2871, 1730, 1610, 1439, 1265, 1174, 773, and 696  $cm^{-1}$ .

**Irradiation of 6.** 6 (4 mM) solution in tetrahydrofuran (50 mL) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor for 24 hours. The photoreaction mixture was concentrated *in vacuo*, and filtered before HPLC separation. Photoproducts 7 and 8 were isolated by normal phase HPLC.

**Irradiation of 1 with a Mixture of DMFu and DMB.**

1 (4 mM) solution in tetrahydrofuran (200 mL) with a mixture of DMFu-DMB (100 mM-100 mM) was deaerated by

nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 36 hours, the reaction mixture was evaporated *in vacuo*. Unreacted DMFu was crystallized out in methanol. The photoproducts 1-*cis*, 2, 3, 6, 7, and 8 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 to 6/1/1 (v/v/v), in ~1, 1, 3, 12, 2 and 1% yields, respectively.

**Irradiation of 1 with a Mixture of AN and DMB.** 1 (4 mM) solution in tetrahydrofuran (200 mL) with a mixture of AN-DMB (100 mM-100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 36 hours, the reaction mixture was evaporated *in vacuo*. Unreacted DMFu was crystallized out in methanol. The photoproducts 4, 5, 6, 7, and 8 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 4/1/1 to 20/1/1 (v/v/v), in 7, 8, 13, 2, and 1% yields, respectively.

**Irradiation of DPB with Mixture of DMFu and DMB.** DPB (4 mM) solution in tetrahydrofuran (200 mL) with a mixture of DMFu-DMB (100 mM-100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 36 hours, the reaction mixture was evaporated *in vacuo*. Unreacted DMFu was crystallized out in methanol. The photoproducts 1, 1-*cis*, 2, 3, 6, 7, and 8 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 to 6/1/1 (v/v/v), in 10.1, ~1, 3, 5, 2, and 1% yields, respectively.

**Irradiation of DPB with Mixture of AN and DMB.**

DPB (4 mM) solution in tetrahydrofuran (200 mL) with a mixture of AN-DMB (100 mM-100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 36 hours, the reaction mixture was evaporated *in vacuo*. The photoproducts 9, 10, 11, 12, and 13 were separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride; 3/1/1 to 6/1/1 (v/v/v), in 2, 3, 18, 5, and 7% yields, respectively. Compound 11:  $^1H$ -NMR (300 MHz,  $CDCl_3$ )  $\delta$  7.26-7.21 (m, 2H), 7.14-7.05 (m, 8H), 3.76 (dd,  $J_{12}=4.93$  Hz,  $J_{13}=2.49$  Hz, 1H), 3.22 ( $J_{21}=4.93$  Hz,  $J_{23}=13.8$  Hz, 1H), 3.14 (dd,  $J_{31}=2.49$  Hz,  $J_{23}=13.8$  Hz, 1H), 1.42 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), and 1.25 (s, 3H) ppm;  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ )  $\delta$  149.60, 142.38, 139.49, 134.53, 133.73, 128.69, 128.51, 127.93, 127.84, 127.51, 127.12, 126.53, 120.41, 48.23, 46.67, 32.52, 27.40, 23.66, 23.27, 22.98, and 22.92 ppm; MS (70 eV) *m/e* 346 ( $M^+$ , 28.9), 331 ( $M^+-CH_3$ , 1.6), 314 (64.6), 299 (6.8), 286 (76.3), 271 (2.4), 226 (51.5), 215 ( $C_{17}H_{11}$ , 100.0), and 202 (DPB $^+$ , 22.3%); UV (MeOH)  $\lambda_{max}$  324, 260, and 251 nm; IR (NaCl) 3096-3004, 2990-2860, 2238, 1492, 1458, 1369, 757, and 692  $cm^{-1}$ .

**Irradiation of 9 with DMB.** 9 (4 mM) solution in tetrahydrofuran (50 mL) with a mixture of DMB (100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 24 hours, the reaction mixture was evaporated *in*

*vacuo*. The photoproduct **11** was separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride: 8/1/1 (v/v/v) as an eluent to give **11** in 80 % yield.

**Irradiation of 10 with AN.** 10 (4 mM) solution in tetrahydrofuran (50 ml) with a mixture of AN (100 mM) was deaerated by nitrogen purging. Deaerated solution was irradiated with 300 nm UV light in a Rayonet Photochemical Reactor equipped with RUL 3000 Å lamps. After the irradiation for 24 hours, the reaction mixture was evaporated *in vacuo*. The photoproduct **11** was separated by column chromatography followed by HPLC using *n*-hexane/ethyl acetate/methylene chloride: 8/1/1 (v/v/v) as an eluent to give **11** in 20% yield.

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## A Study of the Retention Behavior of Proteins in High-Performance Liquid Chromatography(I): The Effect of Solvent and Temperature on Retention Behavior of Proteins in Reversed-Phase Chromatography

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The retention behavior of proteins was investigated by using reversed-phase chromatography (RPC), comparing to the retention behavior of small molecules in RPC. The evaluation was carried out on a SynChropak RP-P(C<sub>18</sub>) column with 0.1% aq. TFA-organic solvent modifier such as acetonitrile, isopropanol, and ethanol. The *Z* value (the number of solvent molecules required to displace the solute from the surface) was a general index for the characterization of protein retention as a function of organic concentration over a range of temperature between 5 and 70°C. Van't Hoff plots provided the basis for evaluating the enthalpic and entropic changes associated with the interaction between protein and the stationary phase. *Z* values did not change significantly at the range of temperature showing the consistent  $\Delta H^\circ$  and  $\Delta S^\circ$  values. From these investigation, it was concluded that the retention behavior of proteins in RPC was able to be predicted by the retention parameters applied to small molecules. Furthermore, myoglobin and hemoglobin in RPC as stated above showed a similar retention behavior regardless of their molecular weights.

### Introduction

In the 1970s, high-performance liquid chromatography (HPLC) has become a powerful technique widely used for the separation of large biological substances and for their purification. In particular, RPC has been the prime method of peptide analysis and purification, and has also become a widely used chromatographic tool for protein analysis.<sup>1-3</sup> According to Snyder *et al.* linear solvent strength (LSS) gradient elution theory developed for small molecules can also

be used to characterized the retention behavior of proteins even though there exist some differences between the RPC behavior of proteins and low-molecular-weight molecules.<sup>4-7</sup> It was suggested by Regnier *et al.* that the analysis of protein mixtures by RPC is complicated due to the nature of the solutions usually employed to affect high-resolution separation.<sup>8,9</sup> These conditions include low pH and the use of high concentrations of organic solvents as eluents, and both conditions are known to alter protein structure substantially. Karger *et al.* studied the changes in peak shape as a function