New Phototriggers: Extending the *p*-Hydroxyphenacyl π,π* Absorption Range

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General Methods. Normal workup from an organic solvent involved drying over $MgSO_4$ and rotary evaporation. Melting points were obtained using a Thomas Hoover capillary melting point apparatus and are uncorrected. All NMR spectra were recorded on a Bruker QE 300 or Bruker DRX 400. All chemicals were used as purchased. All solvents were dried before use.

HPLC measurements were on a Rainin Dynamax SD-200 system consisting of two pumps, software controlled, an absorbance detector model UV-C, and a C18 3 μm 4.6 x 21 cm reverse phase column. All analysis employed a gradient elution with a flow rate of 1 mL/min. The gradient was run from 98% A to 70% A over 25 minutes; solvent systems were a 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B).

2-Bromo-4'-hydroxy-3'-methoxyacetophenone (6). To the solution of acetovanillone (9.97 g, 60 mmol) in 50 mL $CHCl_3$ was added cupric bromide (26.80 g, 120 mmol) in 50 mL of EA at room temperature. The solution was refluxed with vigorous stirring for 4 h. After the reaction mixture was filtered the solvent was removed in vacuo. The resulting brown solid was purified once with Norit-A in boiling benzene. The solvent of the colorless solution was removed in vacuo. The resulting light yellow oil was purified by silica gel column chromatography (CH_2Cl_2/EA = 40:1). After collection of appropriate fractions for the product the solvent was concentrated and gave light-yellow crystals of 2-bromo-4'-hydroxy-3'-methoxyacetophenone (**6**) (6.85 g, 47%): mp 79-80°C (lit.[1] 78-79°C); IR(KBr) 3428, 1667, 1589, 1515, 1464, 1451, 1430, 1298 cm^{-1}; 1H-NMR (400 MHz, acetone-d_6) τ 7.66 (dd, 1H, J = 8.32 Hz, J = 1.9 Hz), 7.60 (d, 1H, J = 1.9 Hz), 6.96 (d, 1H, J = 8.32 Hz), 4.68 (s, 2H), 3.94 (s, 3H); 13C-NMR (100 MHz, acetone-d_6) τ

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189.9, 152.5, 148.0, 126.9, 124.5, 115.1, 111.8, 55.9, 32.0; FAB-MS m/z (relative intensity) 247 (M+H, 90), 245 (M^+H, 93); exact mass calcd for $C_9H_{10}O_3Br$ (M+H): 244.9813 found: 244.9813; Anal. Calcd for $C_9H_9O_3Br$: C, 44.11; H, 3.70 Found: C, 44.32; H, 3.94

γ-O-(4-hydroxy-3-methoxyphenacyl) t-butyl N-t-boc L-glutamate (8a). To a solution of 2-bromo-4'-hydroxy-3'-methoxyacetophenone (**6**) (500 mg, 2.04 mmol) dissolved in 50 mL benzene, which was cooled to 7°C, was added 619 mg (2.04 mmol) N-t-boc-L-glutamic acid, γ-t-butyl ester. Then the addition of 1,8-diazabicyclo[4.3.0]undec-7-ene (DBU, 346 mg, 2.27 mmol) followed dropwise for 10 min. The reaction mixture was allowed to reach room temperature and after that the mixture was stirred overnight. Thin layer chromatography indicated, that the reaction was complete. The solvent was removed in vacuo and the crude product was purified by silica gel column chromatography (CH_2Cl_2/EA = 9:1). After collection of appropriate fractions for the product the solvent was concentrated and gave white crystals of γ-O-(4-hydroxy-3-methoxyphenacyl) t-butyl N-t-boc glutamate (**8a**) (540 mg, 57%): mp 43°C; $[\alpha]_D$ = -7.1 (c = 1.0, EA); IR(Film) 3369, 2979, 2937, 1732, 1694, 1592, 1518, 1455, 1429, 1392, 1368, 1278, 1159 cm^{-1}; UV-vis ($CHCl_3$) λ_{max} (ε) 302 (11131), 275 (16156); 1H-NMR (400 Mhz, $CDCl_3$) τ 7.50 (d, 1H, J = 1.85Hz), 7.46 (dd, 1H, J = 1.85 Hz, J = 8.25 Hz), 6.96 (d, 1H, J = 8.25 Hz), 5.32 (s, 2H), 5.22 (m, 1H), 4.26 (m, 1H), 3.95 (s, 3H), 2.59 (m, 2H), 2.26 (m, 1H), 2.03 (m, 1H), 1.48 (s, 9H), 1.45 (s, 9H); 13C-NMR (100 Mhz, $CDCl_3$) τ 190.9, 172.8, 171.7, 155.9, 151.6, 147.4, 127.4, 123.2, 114.5, 110.1, 82.6, 80.2, 66.2, 56.5, 53.8, 30.5, 28.7, 28.5, 28.4; FAB-MS m/z (relative intensity) 468 (M^+1, 8); exact mass calcd for $C_{23}H_{34}NO_9$ (M^+H): 468.2234 found: 468.2220

γ-O-(4-hydroxy-3-methoxyphenacyl) L-glutamate (1a). A solution of γ-O-(4-hydroxy-3-methoxyphenacyl) t-butyl N-t-boc glutamate (**8a**) (497 mg, 1.06 mmol) in 10 mL trifluoroacetic acid (TFA) was cooled to 0°C and reacted for 4h with stirring. The resulting solution was concentrated by a rotary evaporator and residual solvent removal with a high vacuum pump. The crude product was extracted with a solution of H_2O/EA. The aqueous layer was collected and the water was lyophilized off to give a clear oil of γ-

O-(4-hydroxy-3-methoxyphenacyl) L-glutamate, trifluoroacetate salt (**1a**) (431 mg, 95.3%): $[\alpha]_D$ = +7.2 (c=1.0, H_2O); IR(Film) 3398, 3121, 2947, 2646, 1750, 1680, 1590, 1520, 1460, 1430, 1386, 1280, 1195 cm-1; UV-vis (H_2O) $\lambda_{max}(\varepsilon)$ 279 (9314), 307 (7933); ^{1}H-NMR (300 Mhz, D_2O) τ 7.35 (dd, 1H, J = 1.97 Hz, J = 8.37 Hz), 7.25 (d, 1H, J = 1.97 Hz), 6.78 (d, 1H, J = 8.37 Hz), 5.31 (s, 2H), 4.08 (m, 1H), 3.73 (s, 3H), 2.71 (m, 2H), 2.21 (m, 2H); ^{13}C-NMR (100Mhz, D_2O) τ 194.5, 174.0, 171.8, 152.0, 147.9, 126.3, 124.0, 115.5, 111.3, 67.1, 56.2, 52.3, 29.7, 25.3; FAB-MS (free amine) m/z (relative intensity) 312 (M+1, 35); exact mass calcd for $C_{14}H_{18}NO_7$ (free amine, M^+H): 312.1083 found: 312.1076

2-Bromo-3',5'-dimethoxy-4'-hydroxyacetophenone (7)[2]. To the solution of 3',5'-dimethoxy-4'-hydroxyacetophenone (5 g, 25 mmol) in 30 mL $CHCl_3$ was added cupric bromide (11.38 g, 51 mmol) in 30 mL of EA at room temperature. The solution was refluxed with vigorous stirring for 21 h. After the reaction mixture was filtered, the solvent was removed in vacuo. The resulting light-brown solid was purified by silica gel column chromatography (CH_2Cl_2/EA = 30:1). After collection of the appropriate fractions for the product the solvent was concentrated and gave white crystals of 2-bromo-3',5'-dimethoxy-4'-hydroxyacetophenone (**7**) (4.69 g, 68%): mp 126°C (lit.[7] 118-120°C); IR(KBr) 3379, 2955, 1695, 1684, 1669, 1612, 1589, 1578, 1518, 1460, 1426, 1385, 1334, 1271, 1224, 1190, 1155, 1124, 1109 cm^{-1}; ^{1}H-NMR (400 Mhz, acetone-d_6) τ 8.33 (s, 1H), 7.39 (s, 2H), 4.73 (s, 2H), 3.92 (s, 6H); ^{13}C-NMR (100 Mhz, acetone-d_6) τ 190.4, 148.5, 142.7, 125.9, 107.7, 56.7, 32.5; FAB-MS m/z (relative intensity) 275 (M+H, 37), 277 (M^+H, 36); exact mass calcd for $C_{10}H_{12}O_4Br$ (M+H): 274.9919 found 274.9891; Anal. Calcd for $C_{10}H_{11}O_4Br$: C, 43.66; H, 4.03 Found: C, 43.54; H, 3.90.

γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) t-butyl N-t-Boc L-glutamate (9a). To a solution of 2-bromo-3',5'-dimethoxy-4'-hydroxyacetophenone (**7**) (600 mg, 2.18 mmol) dissolved in 75 mL benzene, which was cooled to 7°C, was added 662 mg (2.18 mmol) N-t-boc-L-glutamic acid, γ-t-butyl ester. Then the addition of 1,8-diazabicyclo[4.3.0]undec-7-ene (DBU, 356 mg, 2.34 mmol) in 10 mL benzene followed dropwise for 10 min. The reaction mixture was allowed to reach room temperature and

after that the mixture was stirred overnight. Thin layer chromatography indicated, that the reaction was complete. The solvent was removed in vacuo and the crude product was purified by silica gel column chromatography (CH_2Cl_2/EA = 8:2). After collection of appropriate fractions for the product the solvent was concentrated and gave white crystals of γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) t-butyl N-t-boc glutamate (**9a**) (727 mg, 67%): mp 45°C; $[\alpha]_D$ = -6.9 (c = 1.0, EtOAc); IR (Kbr) 3420, 2979, 2934, 1739, 1717, 1695, 1609, 1518, 1459, 1425, 1369, 1324, 1254, 1220, 1161, 1115, 1049 cm^{-1}; UV-vis ($CHCl_3$) $\lambda_{max}(\varepsilon)$ 298 (15177), 239 (8053); ^{1}H-NMR (400 Mhz, $CDCl_3$) τ 7.20 (s, 2H), 6.08 (s, 1H), 5.34 (s, 2H), 5.20 (m, 1H), 4.27 (m, 1H), 3.97 (s, 6H), 2.61 (m, 2H), 2.27 (m, 1H), 2.03 (m, 1H), 1.49 (s, 9H), 1.46 (s, 9H); ^{13}C-NMR (100 Mhz, $CDCl_3$) τ 190.4, 172.4, 171.3, 155.4, 147.0, 140.4, 125.7, 105.1, 82.2, 79.7, 65.7, 56.5, 53.3, 30.1, 28.3, 28.1, 28.0; FAB-MS m/z (relative intensity) 498 (M^+1, 30); exact mass calcd for $C_{24}H_{36}NO_{10}$ (M+H): 498.2339 found: 498.2331.

γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) L-glutamate (2a). A solution of γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) t-butyl N-t-boc glutamate (**9a**) (723 mg, 1.45 mmol) in 10 ml trifluoroacetic acid (TFA) was cooled to 0°C and reacted for 4 h with stirring. The resulting solution was concentrated by a rotary evaporator and residual solvent removal with a high vacuum pump. The crude product was extracted with a solution of H_2O/EA. The aqueous layer was collected and the water was lyophilized off to give a clear oil of γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) L-glutamate, trifluoroacetate salt (**2a**) (650 mg, 98%): $[\alpha]_D$ = +9.0 (c = 0.9, H_2O); IR (Film) 3420, 3160, 2948, 2652, 1739, 1678, 1612, 1519, 1463, 1427, 1373, 1327, 1279, 1198, 1171 cm^{-1}; UV-vis (H_2O) $\lambda_{max}(\varepsilon)$ 304 (11730); ^{1}H-NMR (400 Mhz, D_2O) τ 6.81 (s, 2H), 5.23 (s, 2H), 4.07 (m, 1H), 3.66 (s, 6H), 2.72 (m, 2H), 2.22 (m, 2H); ^{13}C-NMR (100 Mhz, D_2O); □ = 193.9, 173.9, 171.9, 147.4, 140.9, 124.5, 105.6, 67.0, 56.3, 52.4, 29.7, 25.3; FAB-MS (free amine) m/z (relative intensity) 342 (M^+1, 100); exact mass calcd for $C_{15}H_{20}NO_8$ (free amine, M+H): 342.1189 found: 342.1193.

General Procedure for Photochemical Studies of 4'-Hydroxyphenacyl Derivatives. An NMR tube was charged with ca. 10 mg (ca. $4x10^{-5}$ mol) of the appropriate

photoprotected compound and 10 mol% of 1,2,3-benzene tricarboxylic acid as an internal standard in 2 mL CD_3CN/D_2O solvent. Irradiations were also done without the internal standard to determine if the standard affected the photoproduct distribution. HPLC analyses of the samples confirmed the ^1H NMR results for several of the caged derivatives.

Photolyses were performed on a merry-go-round apparatus equipped with 4 x 300 or 3500 nm lamps. The light output for the determination of quantum efficiencies was measured using the potassium ferrioxalate method.[3] All determinations of quantitative measurements were performed at least in triplicate. The data were submitted to linear least squares regression analysis using a Microcal Origin software program.

Isolation and Characterization of Additional Photoproducts after 100% Conversion

Into one NMR tube containing 2 mL of D_2O were introduced 27 mg (0.063 mmol) γ-O-(4-hydroxy-3-methoxyphenacyl) glutamate, trifluoroacetate salt (**1a**). The solution was deaerated for 10 min with argon and irradiated at 350 nm for 71h with 16 RPR-3500 lamps. 1H-NMR-spectra 300 Mhz were measured during irradiation to determine the time to reach complete conversion. Three additional photoproducts were isolated from the photolysed mixture by preparative TLC (CH_2Cl_2/EA = 9:1), using TLC plates silica gel 60 F_{254} pre-coated for preparative layer chromatography, 20x20 cm, layer thickness 2 mm from Merck.

Zone 1: R_f = 0.1, **Homovanillic acid (10).** ^1H-NMR (400 Mhz, D_2O) τ 6.80 (d, 1H, J = 1.76 Hz), 6.73 (d, 1H, J = 8.08 Hz), 6.64 (dd, 1H, J = 1.76 Hz, J = 8.08 Hz), 3.69 (s, 3H), 3.50 (s, 2H); ^{13}C-NMR (100 Mhz, D_2O) τ 177.4, 147.8, 144.3, 127.0, 122.7, 116.0, 114.1, 56.3, 40.3; FAB-MS m/z (relative intensity) 182 (M, 40); exact mass calcd for $C_9H_{11}O_4$ (M^+H): 183.0657 found: 183.0673

Zone 2: R_f = 0.25, **2-Hydroxyacetovanillone (11)[4]** IR (Film) 3389, 3274, 2960, 2928, 1674, 1588, 1516, 1464, 1428, 1396, 1282, 1202, 1174, 1135, 1093, 1039, 1016 cm^{-1}; ^1H-NMR (400 Mhz, D_2O) τ 7.27 (dd, 1H, J = 1.96 Hz, J = 8.23 Hz), 7.25 (d, 1H, J = 1.96 Hz), 6.72 (d, 1H, J = 8.23 Hz), 4.71 (s, 2H), 3.65 (s, 3H); ^1H-NMR (300 Mhz,

$CDCl_3$) τ 7.53 (d, 1H), 7.44 (dd, 1H), 6.98 (d, 1H), 4.83 (s, 2H), 3.98 (s, 6H); ^{13}C-NMR (125 Mhz, D_2O) τ 201.4, 153.0, 149.4, 128.3, 125.0, 116.9, 112,9, 66.4, 57.7; FAB-MS m/z (relative intensity) 183 (M^+1, 5); exact mass calcd for $C_9H_{11}O_4$ (M+H): 183.0657 found: 183.0659

Zone 3: R_f = 0.53, **Acetovanillone (12)[5].** IR (Film) 3286, 3010, 2962, 2941, 1656, 1573, 1519, 1468, 1451, 1418, 1296, 1219, 1191, 1160, 1119 cm^{-1}; ^{1}H-NMR (400 Mhz, D_2O) τ 7.48 (dd, 1H, J = 1.93 Hz, J = 8.35 Hz), 7.39 (d, 1H, J = 1.93 Hz), 6.83 (d, 1H, J = 8.35 Hz), 3.77 (s, 3H), 2.44 (s, 3H); 13C-NMR (100 Mhz, D_2O) τ 202.9, 151.2, 147.6, 129.8, 124.9, 115.3, 112.0, 56.2, 27.5; FAB-MS m/z (relative intensity) 167 (M+1, 20); exact mass calcd for $C_9H_{11}O_3$ (M+H): 167.0708 found: 167.0710.

Isolation and Characterization of Additional Photoproducts after 100% Conversion. Into one NMR tube containing 2 mL of D_2O were introduced 30 mg (0.066 mmol) γ-O-(3,5-dimethoxy-4-hydroxyphenacyl) L-glutamate, trifluoroacatate salt (**2a**). The solution was deaerated for 10 min with argon and irradiated at 350 nm for 35 h with 26 RPR-3500 lamps. ^{1}H-NMR (400 Mhz) spectra were measured during irradiation to determine the time to reach complete conversion. Two additional photoproducts were isolated from the photolyzed mixture by preparative TLC (CH_2Cl_2/EA = 9:1), using TLC plates silica gel 60 F_{254} pre-coated for preparative layer chromatography, 20x20 cm, layer thickness 2 mm from Merck.

Zone 1: R_f = 0.2, **2,4'-dihydroxy-3',5'-dimethoxyacetophenone (13).** IR (Film) 3398, 2935, 2853, 1681, 1607, 1587, 1518, 1461, 1424, 1329, 1286, 1203, 1177, 1117 cm^{-1}; ^{1}H-NMR (400 Mhz, D_2O) δ7.18 (s, 2H), 4.88 (s, 2H), 3.81 (s, 6H); 13C-NMR (125 Mhz, D_2O) δ 203.9, 148.7, 139.4, 125. 8, 106.6, 65.9, 56.8; FAB-MS m/z (relative intensity) 213 (M+1, 12); exact mass calcd for $C_{10}H_{13}O_5$ (M+H): 213.0763 found: 213.0762

Zone 2: R_f = 0.48, **3',5'-dimethoxy-4'-hydroxyacetophenone (14)[6]**
IR(Film) 3359, 2924, 2856, 1728, 1658, 1650, 1604, 1573, 1517, 1465, 1457, 1418, 1336, 1284, 1255, 1199, 1184, 1110, 1084, 1028 cm^{-1}; ^{1}H-NMR (400 Mhz, D_2O) δ 7.19 (s, 2H), 3.79 (s, 6H), 2.47 (s, 3H); 13C-NMR (125 Mhz, D_2O) δ 202.3, 148.1, 141.0,

129.0, 107.7, 57.2, 26.6; FAB-MS m/z (relative intensity) 197 (M^+1, 7); exact mass calcd for $C_{10}H_{13}O_4$ (M+H): 197.0814 found: 197.0798

Phosphorescence Emission Studies. The phosphorescence measurements were performed in either ethylene glycol:H_2O (EG:W; 2:1) or ether:isopentane:ethanol (EPA; 5:5:2) glasses in a rotating cam double monochromator phosphoroscope illuminated with a 200 watt xenon-mercury lamp and detected with an IP28 photomultiplier. The solvents were HPLC or spectral grade. The samples were placed in a 0.1 mm quartz tube and cooled to 77K.

In a typical study, the phosphorescence spectrum of *p*-hydroxyphenacyl γ-aminobutyrate (18 - 36 mg, 0.05 - 0.10 mmol) was dissolved in 1.0 mL of EPA or EG:W and cooled to 77 K to form a clear glass. The phosphorescence emission and excitation spectra were measured for each sample. A blank of each solvent was checked for impurities.

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[1] a) Riegel, B.; Wittcoff, H. J. Am. Chem. Soc. **1946**, 68, 1913-1917
b) Koβmehl, G; Frohberg H.-C. Chem. Ber. **1986**, 119, 50-63

[2] Mischke, G. E. Acta Chem. Scand. **1973**, 27, 1355-1368

3.Hatchard, C. G.; Parker, C. A. *Proc. Roy. Soc. London* **1956**, *A-235*, 518-522.

[4] a) Euw, J. v.; Neher, R.; Reichstein, T.; Tait, S. A. S.; Tait, J. F.; Wettstein, A. Helv. Chim. Acta **1959**, 42, 1817-1829 b) Mühlenbeck, U.; Kortenbusch, A.; Barz, W. Phytochemistry **1996**, 42, 1573-1579

[5] spectroscopic data taken from acetovanillone (purchased from Aldrich) are identical to the data taken from zone 3

[6] Spectroscopic data taken from 3', 5'-dimethoxy-4'-hydroxyacetophenone (purchased from Aldrich) are identical to the data taken from zone 2