SUPPORTING INFORMATION
for
The Synthesis and Optical Properties of Two-photon Absorbing GFP-type Probes

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Methyl 2-(1-ethoxyethyliden)amino ethanoate 1

Previously characterized and prepared in the literature.[1,2]

(*E*)-methyl 2-((ethoxy(phenyl)methylene)amino)acetate 2

Previously characterized and prepared in the literature.[3] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.342 (t, 3H, J= 7.0 Hz); 3.691 (s, 3H); 4.074 (s, 2H); 4.311 (q, 2H, J= 7.0 Hz); 7.309 (m, 2H); 7.393 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.141 ($-CH_2CH_3$); 51.763 ($-CH_2-$); 51.999 ($-OCH_3$); 61.848 ($-CH_2CH_3$); 127.638 (Ar); 128.386 (Ar); 129.638 (Ar); 131.633 (Ar); 164.257 (N=C); 171.750 (C=O).

General procedure for the preparation of chromophores

In a small 4-mL vial equipped with a stirbar, imidate (**1** or **2**) (1.89 mmol) and benzaldehyde (1.89 mmol) were dissolved in a minimum amount of toluene (1 mL). With vigorous stirring, the sealed mixture was heated at 70 ºC overnight in an oil bath. The solvent was removed under reduced pressure and the resulting chromophore purified using column chromatography (silica gel, ethyl acetate/hexanes).

(*Z*)-4-benzylidene-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (HC)

Previously characterized and prepared in the literature, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.134 (2H, d); 7.409 (3H, m); 7.137 (1H, s); 4.387 (2H, s); 3.776 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.493 ($-CH_3$); 41.233 ($-CH_2-$); 52.779 ($-OCH_3$); 128.199 (Ar); 128.687 (Ar); 130.263 (Ar); 132.201 (Ar); 133.940 (Ar-CH=C); 137.980 (C-4); 161.263 (C-2); 167.969 (C=O); 170.014 (C-5).

(*Z*)-4-(4-methylbenzylidene)-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (MC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.035 (2H, d); 7.229 (2H, d); 7.130 (2H, s); 4.394 (2H, s); 3.783 (3H, s); 2.387 (3H, s); 2.333 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.501 ($-CH_3$); 21.677 ($Ar-CH_3$); 41.272 ($-CH_2-$); 52.782 ($-OCH_3$); 128.521 (Ar-CH=C); 129.533 (Ar); 131.303 (C-4); 132.285 (Ar); 137.286 (Ar);

140.943 (Ar); 160.561 (C-2); 168.044 (C=O); 170.085 (C-5).

(*Z*)-4-(4-bromobenzylidene)-2-methyl-1-(methoxycarbonylmethyl)-1-imidazol-5-one (BrC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.007 (2H, d), 7.542 (2H, d), 7.046 (1H, s), 4.388 (2H, s), 3.786 (3H, s); 2.328 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.534 ($-CH_3$); 41.258 ($-CH_2-$); 52.827 ($-OCH_3$); 124.832 (Ar); 126.587 (Ar-CH=C); 131.946 (Ar); 132.887 (C-4); 133.530 (Ar); 138.443 (Ar); 161.761 (C-2); 167.909 (C=O); 169.870 (C-5). HRMS (ES): m/z (M+H) calcd. for $C_{14}H_{13}BrN_2O_3$, 337.0182; found 337.0174.

(*Z*)-4-(4-dimethylaminobenzylidene)-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (DMC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.064 (2H, d); 7.112 (1H, s); 6.702 (2H, d); 4.394 (2H, s); 3.769 (3H, s); 3.053 (6H, s); 2.311 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.329 (-CH3); 39.966 ($-N(CH_3)_2$); 41.248 ($-CH_2-$); 52.596 ($-OCH_3$); 111.694 (Ar-CH=C); 122.100 (Ar); 129.706 (Ar); 133.948 (C-4); 134.299 (Ar); 151.624 (Ar); 157.536 (C-2); 168.244 (C=O); 169.956 (C-5) HRMS (ES): m/z (M+H) calcd. for $C_{16}H_{19}N_3O_3$, 324.1324; found 324.1322.

(*Z*)-4-(4-nitrobenzylidene)-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (NC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.288 (2H, d); 8.232 (2H, d); 7.083 (1H, s); 4.403 (2H, s); 3.794 (3H, s); 2.366 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.646 ($-CH_3$); 41.286 ($-CH_2-$); 52.924 ($-OCH_3$); 123.713 (Ar); 124.188 (Ar); 140.151 (Ar-CH=C-); 140.700 (C-4); 147.839 (Ar); 164.080 (C-2); 167.744 (C-5); 169.645 (C-2). HRMS (ES): m/z (M+H) calcd. for $C_{14}H_{13}N_3O_5$, 303.27; found 304.0923.

(*Z*)-4-(4-t-butoxybenzylidene)-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (^{t}BuOC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.067 (2H, d, J_o= 8.4 hz); 7.111 (1H, s); 7.021 (2H, d, J_o= 8.4 hz); 4.384 (2H, s); 3.768 (3H, s); 2.318 (3H, s); 1.387 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.455 ($-CH_3$); 29.991 ($-C(CH_3)_3$); 41.244 ($-CH_2-$); 52.745 ($-OCH_3$); 79.450 ($-C(CH_3)_3$); 123.382 (Ar); 128.135 (Ar-CH=C); 128.764 (Ar); 133.402 (Ar); 136.665 (C-4); 157.971 (Ar); 160.317 (C-2); 168.038 (C=O); 170.014 (C-5). HRMS (ES): m/z (M+H) calcd. for $C_{18}H_{22}N_2O_4$, 324.1324; found 324.1322.

(*Z*)-4-(3,5-dibromobenzylidene)-2-methyl-1-(methoxycarbonylmethyl)imidazol-5-one (DiBrC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.219 (2H, d, J_m= 1.6 hz); 7.635 (1H, s, almost t); 6.892 (1H, s); 4.376 (2H, s); 3.780 (3H, s); 2.347 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.546 (-CH3); 41.240 ($-CH_2-$); 52.859 ($-OCH_3$); 122.989 (Ar); 124.007 (Ar); 133.192 (Ar); 135.027 (Ar-CH=C); 137.335 (Ar); 139.803 (C-4);

163.178 (C-2); 167.755 (C=O); 169.537 (C-5).

(*Z*)-4-(4-dimethylaminobenzylidene)-2-phenyl-1-(methoxycarbonylmethyl)imidazol-5-one (BC)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.170 (2H, d); 7.741 (2H, dd); 7.503 (3H, m); 7.269 (1H, s); 6.711 (3H, d); 4.514 (2H, s); 3.712 (3H, s); 3.067 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 40.033 (-$N(CH_3)_2$); 43.067 (-CH_2-); 52.606 (-OCH_3); 111.724 (Ar-CH=C); 122.278; 128.233; 128.900; 130.872; 131.434; 134.208 (C-4); 134.909; 151.857 (C-2); 168.552 (C=O); 170.923 (C-5)

(*Z*)-4-(4-nitrobenzylidene)-2-phenyl-1-(methoxycarbonylmethyl)imidazol-5-one (NPC)

^{1}H NMR (400 MHz, $CHCl_3$, δ): 3.748 (s, 3H, -OCH_3); 4.558 (s, 2H, -CH_2-); 7.411 (m, 3H, Ar-H); 7.568 (m, 2H, Ar-H); 7.972 (m, 2H, Ar-H)

References

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