

Supplementary Information

Aqueous Micelles as Catalytic Nanoreactors for Dephosphorylation Reactions

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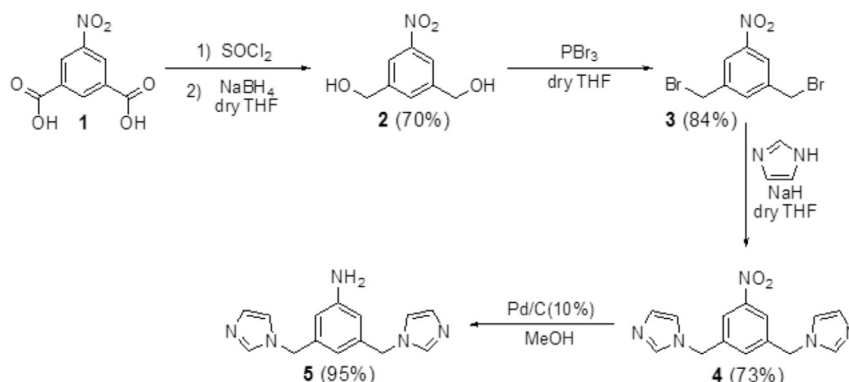
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Blm synthesis and characterization data

The novel imidazole derivative, 3,5-bis((1*H*-imidazol-1-yl)methyl)aniline (BIm), was obtained through the synthetic route of Scheme S1, using 5-nitroisophthalic acid (**1**) as starting material. The detailed procedures and conditions for obtaining compounds **2-5** of the route, as well as the characterization data, are presented sequentially.

All reagents and solvents were of the best available analytical grade from commercial sources and used as received. Tetrahydrofuran (THF) was dried by distillation from sodium/benzophenone and used fresh. Silica-gel Si 60-F254 (Merck) was used for thin layer chromatography. Purifications were carried out by recrystallization using commercial grade solvents and by column chromatography on silica-gel 60-200 mesh 60A (Acros). Melting points were determined with an Olympus BX50 microscope equipped with a Mettler Toledo FP-82 Hot Stage. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded with a Varian Mercury Plus spectrometer operating at 400 and 100.6 MHz, respectively.

3,5-Bis(hydroxymethyl)nitrobenzene (**2**): 5-nitroisophthalic acid (2 g, 9.47 mmol) was dissolved in 10 mL of thionyl chloride and refluxed for 24 h; the remaining SOCl₂ was removed by distillation and the solid, isophthalic acid dichloride, was kept under reduced pressure for 2 h, and then dissolved in 15 mL of dry THF. To this solution, an ice-cooled slurry of sodium borohydride (1.07 g, 28.3 mmol) in 20 mL of dry THF was added dropwise under argon flow. The reaction mixture was warmed to room temperature and stirred for 20 h under argon. Afterwards, 15 mL of water were added slowly and carefully (due to gas release, which was stopped with addition of HCl 5% solution). The aqueous phase was extracted with sodium acetate solution; the organic phase was dried with sodium sulfate and THF was evaporated, yielding a white solid **2** in 70% (1.21 g, 6.61 mmol); m.p. 90.3-91.7 °C (lit. 91-92 °C);¹ ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.61 (s, 4H, -CH₂OH), 5.52 (broad, 2H, -OH), 7.70 (s, 1H, Ar), 8.04 (s, 2H, Ar); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 61.60, 119.03, 130.42, 144.89, 147.85.



Scheme S1. Route for BIm synthesis.

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3,5-Bis(bromomethyl)nitrobenzene (**3**): a solution of PBr_3 (1 mL, $d = 2.88 \text{ g mL}^{-1}$, 10.6 mmol) in 10 mL of dry THF was added dropwise under argon flow to a cooled solution of compound **2** (1.0 g, 5.46 mmol) in 10 mL of dry THF (acetone/ice/NaCl bath, temperature reached: $-10 \text{ }^\circ\text{C}$). The reaction mixture was stirred for 6 h at $-5 \text{ }^\circ\text{C}$ and then for 16 h at room temperature. The reaction mixture was poured into ice water, and THF was removed under reduced pressure. The aqueous phase was extracted with ethyl ether, and the organic phase was washed with saturated sodium bicarbonate and then with water, dried with sodium sulfate, and evaporated. The solid product was purified by chromatography on a silica gel column and eluted with a mixture of ethyl acetate and hexane 1:9, yielding the yellowish solid **3** in 84% (1.41 g, 4.56 mmol); m.p. 104.5-105.7 $^\circ\text{C}$ (lit. 103-104 $^\circ\text{C}$); $^1\text{H NMR}$ (400 MHz, acetone- d_6) δ 4.77 (s, 4H, $-\text{CH}_2\text{Br}$), 7.76 (s, 1H, Ar), 8.11 (s, 2H, Ar); $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3) δ 30.62, 123.67, 135.25, 140.35, 148.49.

3,5-Bis((1*H*-imidazol-1-yl)methyl)nitrobenzene (**4**): an ice-cooled solution of imidazole (0.88 g, 12.9 mmol) in 20 mL of dry THF was added dropwise to an ice-cooled slurry of sodium hydride (0.35 g, 14.6 mmol) in dry THF in a three-necked flask previously dried under inert atmosphere. Then reaction mixture was warmed to room temperature and stirred for 1 h. The mixture was cooled to $0 \text{ }^\circ\text{C}$, and compound **3** (1.0 g, 3.24 mmol) dissolved in 20 mL of dry THF was added dropwise. Reaction progress was followed by thin layer chromatography, and formation of a white precipitate indicated complete reaction. The reaction mixture was poured into a mixture of 100 mL of saturated NH_4Cl and 50 mL of saturated NaCl, and the product was extracted with chloroform. The organic phase was dried with sodium sulfate and removed under reduced pressure yielding the pale yellow solid **4** in 73% (0.67 g, 2.36 mmol); m.p. 166.1-168.8 $^\circ\text{C}$; $^1\text{H NMR}$ (400 MHz, acetone- d_6) δ 5.46 (s, 4H, $-\text{CH}_2\text{Im}$), 6.97 (s, 2H, Im), 7.17 (s, 2H, Im), 7.69 (s, 1H, Ar), 7.74 (s, 2H, Im), 8.06 (s, 2H, Ar); $^{13}\text{C NMR}$ (100.6 MHz, acetone- d_6) δ 49.87, 110.72, 120.12, 122.54, 130.51, 133.65, 138.47, 141.80.

3,5-Bis((1*H*-imidazol-1-yl)methyl)aniline (BIm, **5**): Pd/C 10% and a solution of **4** (1.69 g, 5.97 mmol) in 50 mL of methanol were added to a hydrogenator and the reaction was left under a hydrogen stream for 24 h. Afterwards, the reaction mixture was filtered on celite; methanol was evaporated and the solid obtained was recrystallized from 1,2-dichloroethane, yielding the white solid BIm in 95% (1.44 g, 5.68 mmol); m.p. 156.2-159.8 $^\circ\text{C}$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 4.99 (s, 4H, $-\text{CH}_2\text{Im}$), 5.22 (s,

2H, $-\text{NH}_2$), 6.25 (s, 2H, Ar), 6.30 (s, 1H, Ar), 6.89 (s, 2H, Im), 7.09 (s, 2H, Im), 7.66 (s, 2H, Im); $^{13}\text{C NMR}$ (100.6 MHz, DMSO- d_6) δ 49.59, 111.73, 113.61, 119.62, 128.54, 137.42, 138.74, 149.40; ESI-MS (M^+) m/z (%) calcd. for $\text{C}_{14}\text{H}_{16}\text{N}_5$ (monoprotonated BIm): 254.1; found: 254.3 (100).

BIm titration studies

Tables S1 and S2 present the data of BIm potentiometric titration in water and 0.01 mol L^{-1} CTAB micellar medium, respectively, both at $25 \text{ }^\circ\text{C}$. Plots of Vol_{KOH} vs. pH are given in Figures S1 and S2 for water and CTAB micellar medium, respectively; the fits of the profiles were performed using the program BEST7 and all the $\text{p}K_a$ values are presented in Table S3. In both aqueous and CTAB micellar medium, the aniline amino group is the first to be deprotonated with pH increase ($\text{p}K_{a1}$), and $\text{p}K_{a2}$ and $\text{p}K_{a3}$ refer to the imidazole groups. Using equations S1-S3, diagrams of BIm composition as a function of pH were plotted (in Figures S1 and S2, along with the respective titration curves, and the nomenclature of the species is with respect to the structures and equilibria of Scheme S2).

Table S1. Data of pH as a function of Vol_{KOH} obtained for $4 \times 10^{-3} \text{ mol L}^{-1}$ BIm potentiometric titration in aqueous medium, at $25 \text{ }^\circ\text{C}$ and $I = 0.1 \text{ mol L}^{-1}$ (KCl)

$\text{Vol}_{\text{KOH}} / \text{mL}$	pH	$\text{Vol}_{\text{KOH}} / \text{mL}$	pH	$\text{Vol}_{\text{KOH}} / \text{mL}$	pH
0	2.63	1	5.30	2.52	8.26
0.15	2.74	1.02	5.38	2.54	8.52
0.35	2.92	1.06	5.52	2.55	8.64
0.5	3.11	1.1	5.63	2.56	8.85
0.6	3.26	1.14	5.73	2.566	8.98
0.7	3.47	1.2	5.85	2.572	9.16
0.74	3.58	1.3	6.03	2.578	9.35
0.78	3.72	1.4	6.18	2.584	9.53
0.82	3.90	1.5	6.32	2.588	9.63
0.84	4.02	1.6	6.44	2.592	9.73
0.86	4.16	1.7	6.57	2.592	9.81
0.88	4.35	1.84	6.74	2.604	9.98
0.89	4.43	1.98	6.91	2.612	10.09
0.9	4.53	2.12	7.11	2.62	10.18
0.91	4.64	2.26	7.34	2.63	10.29
0.92	4.74	2.36	7.56	2.64	10.37
0.93	4.83	2.42	7.73	2.66	10.53
0.94	4.92	2.46	7.90	2.68	10.63
0.96	5.08	2.5	8.13	2.71	10.76
0.98	5.20	2.51	8.19		

Table S2. Data of pH as a function of Vol_{KOH} obtained for $4 \times 10^{-3} \text{ mol L}^{-1}$ BIm potentiometric titration in 0.01 mol L^{-1} CTAB aqueous micellar medium, at $25 \text{ }^\circ\text{C}$ and $I = 0.1 \text{ mol L}^{-1}$ (KCl)

$\text{Vol}_{\text{KOH}} / \text{mL}$	pH	$\text{Vol}_{\text{KOH}} / \text{mL}$	pH	$\text{Vol}_{\text{KOH}} / \text{mL}$	pH
0	2.81	0.81	5.47	2.29	8.06
0.15	2.94	0.87	5.61	2.31	8.23
0.3	3.13	0.93	5.74	2.33	8.43
0.45	3.44	0.99	5.85	2.35	8.91
0.5	3.60	1.05	5.94	2.356	9.07
0.55	3.82	1.11	6.03	2.362	9.25
0.56	3.89	1.17	6.11	2.368	9.46
0.58	4.04	1.25	6.22	2.374	9.66
0.6	4.21	1.33	6.32	2.38	9.78
0.62	4.42	1.41	6.41	2.384	9.90
0.63	4.51	1.51	6.53	2.388	9.94
0.64	4.61	1.61	6.65	2.392	9.99
0.65	4.70	1.71	6.78	2.4	10.14
0.666	4.84	1.81	6.90	2.408	10.22
0.682	4.95	1.91	7.05	2.418	10.32
0.698	5.04	2.01	7.21	2.432	10.46
0.714	5.13	2.11	7.41	2.446	10.56
0.73	5.20	2.17	7.55	2.46	10.64
0.75	5.29	2.23	7.76	2.48	10.75
0.77	5.35	2.27	7.95	2.5	10.84
0.81	5.47	2.28	8.01	2.52	10.91

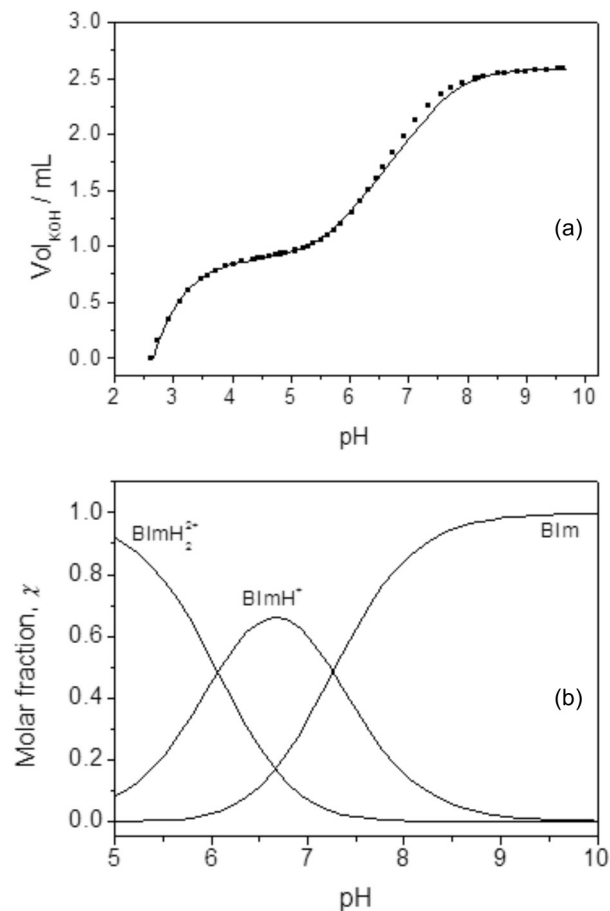


Figure S1. (a) Plot of Vol_{KOH} vs. pH for $4 \times 10^{-3} \text{ mol L}^{-1}$ BIm potentiometric titration in water at $25 \text{ }^\circ\text{C}$ and $I = 0.1 \text{ mol L}^{-1}$ (KCl). The solid line is the best fit obtained using BEST7. (b) BIm composition as a function of pH according to the $\text{p}K_a$ values obtained. The species BImH_3^{3+} is not shown.

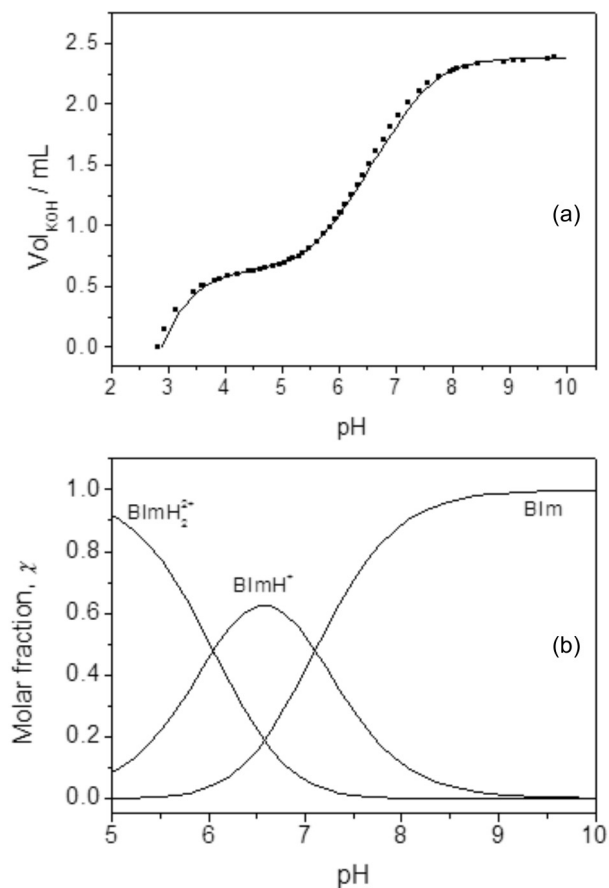
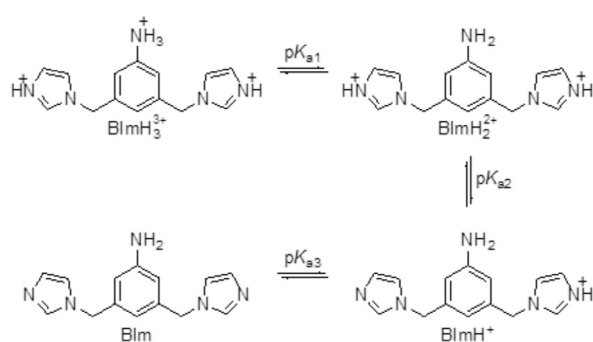


Figure S2. (a) Plot of Vol_{KOH} vs. pH for $4 \times 10^{-3} \text{ mol L}^{-1}$ BIm potentiometric titration in 0.01 mol L^{-1} CTAB aqueous micellar medium at $25 \text{ }^\circ\text{C}$ and $I = 0.1 \text{ mol L}^{-1}$ (KCl). The solid line is the best fit obtained using BEST7. (b) BIm composition as a function of pH according to the $\text{p}K_{\text{a}}$ values obtained. The species BImH_3^{3+} is not shown.

Table S3. Summary of the $\text{p}K_{\text{a}}$ values obtained for BIm potentiometric titration in water and CTAB aqueous micellar medium, both at $25 \text{ }^\circ\text{C}$. Value of $\text{p}K_{\text{a}1}$ corresponds to the aniline group, and $\text{p}K_{\text{a}2}$ and $\text{p}K_{\text{a}3}$, to the imidazole groups

	Water	CTAB micellar medium
$\text{p}K_{\text{a}1}$	2.5	2.6
$\text{p}K_{\text{a}2}$	6.07	6.05
$\text{p}K_{\text{a}3}$	7.26	7.10



Scheme S2. Dissociation equilibria of BIm and designation of its four species.

$$\chi_{\text{BImH}_2^{2+}} = \frac{10^{-2\text{pH}}}{10^{-2\text{pH}} + 10^{-(\text{pH} + \text{p}K_{\text{a}2})} + 10^{-(\text{p}K_{\text{a}2} + \text{p}K_{\text{a}3})}} \quad (\text{S1})$$

$$\chi_{\text{BImH}^+} = \frac{10^{-(\text{pH} + \text{p}K_{\text{a}2})}}{10^{-2\text{pH}} + 10^{-(\text{pH} + \text{p}K_{\text{a}2})} + 10^{-(\text{p}K_{\text{a}2} + \text{p}K_{\text{a}3})}} \quad (\text{S2})$$

$$\chi_{\text{BIm}} = \frac{10^{-(\text{p}K_{\text{a}2} + \text{p}K_{\text{a}3})}}{10^{-2\text{pH}} + 10^{-(\text{pH} + \text{p}K_{\text{a}2})} + 10^{-(\text{p}K_{\text{a}2} + \text{p}K_{\text{a}3})}} \quad (\text{S3})$$

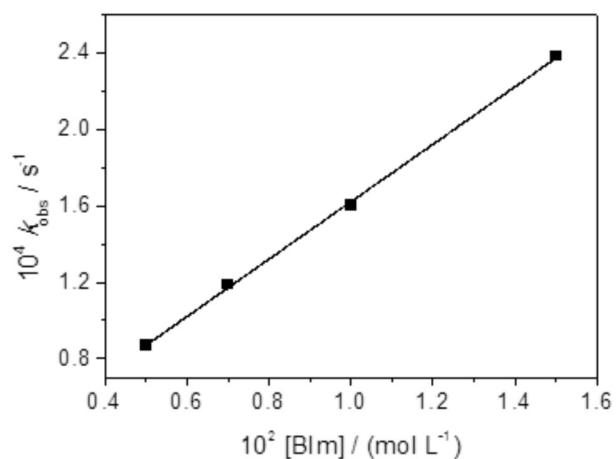
Kinetic data

Table S4. Data for the pH-rate profile of BIm (0.01 mol L⁻¹) reaction with DEDNPP (3.33 × 10⁻⁵ mol L⁻¹), at 25 °C

pH	$k_{\text{obs}} / \text{s}^{-1}$
3.00	9.86×10^{-6}
3.50	1.10×10^{-5}
3.99	1.05×10^{-5}
4.51	1.25×10^{-5}
5.07	1.99×10^{-5}
5.48	1.86×10^{-5}
5.74	2.52×10^{-5}
6.01	4.07×10^{-5}
6.52	7.24×10^{-5}
6.99	1.06×10^{-4}
7.51	1.27×10^{-4}
7.99	1.48×10^{-4}
8.58	1.60×10^{-4}
9.00	1.58×10^{-4}
9.46	1.60×10^{-4}

Table S5. Dependence of k_{obs} with respect to BIm concentration in the reaction with DEDNPP (3.33 × 10⁻⁵ mol L⁻¹), at pH 8.5 and 25 °C

[BIm] / (mol L ⁻¹)	$k_{\text{obs}} / \text{s}^{-1}$
0.005	8.68×10^{-5}
0.007	1.19×10^{-4}
0.010	1.60×10^{-4}
0.015	2.38×10^{-4}

**Figure S3.** Plot of k_{obs} vs. BIm concentration in the reaction with DEDNPP (3.33 × 10⁻⁵ mol L⁻¹), at pH 8.5 and 25 °C. The solid line represents the fit according to equation S4.

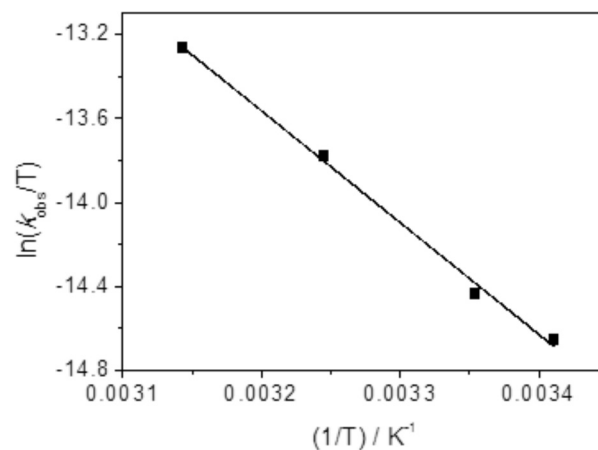
$$k_{\text{obs}} = k_0 + k_2[\text{BIm}] \quad (\text{S4})$$

Table S6. Kinetic parameters obtained from the linear fit of profile of Figure S3 with equation S4

k_0 / s^{-1}	$k_2 / (\text{L mol}^{-1} \text{s}^{-1})$
$(1.18 \pm 0.27) \times 10^{-5}$	$(1.50 \pm 0.03) \times 10^{-2}$

Table S7. Temperature effect on k_{obs} in the reaction of BIm (0.01 mol L⁻¹) with DEDNPP (3.33 × 10⁻⁵ mol L⁻¹), at pH 8.5

Temperature / K	$k_{\text{obs}} / \text{s}^{-1}$
293.15	1.26×10^{-4}
298.15	1.60×10^{-4}
308.15	3.20×10^{-4}
318.15	5.51×10^{-4}

**Figure S4.** Eyring plot for the reaction of BIm (0.01 mol L⁻¹) with DEDNPP (3.33 × 10⁻⁵ mol L⁻¹), at pH 8.5. Enthalpy of activation was obtained from the Eyring equation (equation S5), and entropy of activation was calculated from equations S6 and S7, at 25 °C.

$$\ln\left(\frac{k_{\text{obs}}}{T}\right) = \ln\left(\frac{k_{\text{B}}}{h}\right) + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{R} \left(\frac{1}{T}\right) \quad (\text{S5})$$

$$\Delta G^{\ddagger} = -RT \ln\left(\frac{k_{\text{c}} h}{k_{\text{B}} T}\right) \quad (\text{S6})$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \quad (\text{S7})$$

where k_{B} is the Boltzmann constant, $1.38 \times 10^{-23} \text{ J K}^{-1}$, h , the Planck constant, $6.63 \times 10^{-34} \text{ J s}$, and R , the molar gas constant, $8.31 \text{ J K}^{-1} \text{ mol}^{-1}$.

Table S8. Data for the pH-rate profiles of the reactions of BIm with DEDNPP ($3.33 \times 10^{-5} \text{ mol L}^{-1}$) in 0.01 mol L^{-1} CTAB and 0.04 mol L^{-1} SDS micellar media, at 25°C

CTAB (0.01 mol L^{-1} BIm)		SDS (0.005 mol L^{-1} BIm)	
pH	$k_{\text{obs}} / \text{s}^{-1}$	pH	$k_{\text{obs}} / \text{s}^{-1}$
5.96	6.26×10^{-5}	6.00	1.19×10^{-5}
6.50	1.62×10^{-4}	6.50	2.62×10^{-5}
7.01	3.86×10^{-4}	7.00	7.05×10^{-5}
7.49	5.94×10^{-4}	7.50	1.19×10^{-4}
7.98	6.91×10^{-4}	8.00	1.91×10^{-4}
8.55	7.94×10^{-4}	8.50	2.40×10^{-4}
8.99	8.49×10^{-4}	9.00	2.98×10^{-4}

Table S9. Dependence of k_{obs} with respect to the surfactant concentration in the reactions of BIm (0.01 mol L^{-1}) with DEDNPP ($3.33 \times 10^{-5} \text{ mol L}^{-1}$) in CTAB and SDS micellar media, both at pH 8.5 and 25°C

CTAB		SDS	
[CTAB] / (mol L^{-1})	$k_{\text{obs}} / \text{s}^{-1}$	[SDS] / (mol L^{-1})	$k_{\text{obs}} / \text{s}^{-1}$
0	1.60×10^{-4}	0	1.60×10^{-4}
1.0×10^{-5}	1.45×10^{-4}	8.0×10^{-4}	2.20×10^{-4}
5.0×10^{-5}	1.57×10^{-4}	8.0×10^{-3}	6.12×10^{-4}
1.0×10^{-4}	1.70×10^{-4}	9.0×10^{-3}	6.93×10^{-4}
5.0×10^{-4}	2.58×10^{-4}	1.0×10^{-2}	7.10×10^{-4}
2.0×10^{-3}	4.27×10^{-4}	1.3×10^{-2}	7.15×10^{-4}
5.0×10^{-3}	5.44×10^{-4}	1.8×10^{-2}	7.07×10^{-4}
7.0×10^{-3}	7.02×10^{-4}	2.5×10^{-2}	6.16×10^{-4}
9.0×10^{-3}	7.52×10^{-4}	5.0×10^{-2}	4.85×10^{-4}
1.0×10^{-2}	7.29×10^{-4}	1.0×10^{-1}	3.13×10^{-4}
1.1×10^{-2}	7.92×10^{-4}		
1.5×10^{-2}	8.11×10^{-4}		
2.0×10^{-2}	7.68×10^{-4}		
2.5×10^{-2}	7.65×10^{-4}		
5.0×10^{-2}	6.70×10^{-4}		
1.0×10^{-1}	5.37×10^{-4}		

Table S10. Values of k_{obs} as a function of BIm concentration in the reaction with DEDNPP ($3.33 \times 10^{-5} \text{ mol L}^{-1}$) in CTAB (0.01 mol L^{-1}) and SDS (0.01 mol L^{-1}) micellar media, at pH 9.0 and 25°C

CTAB		SDS	
[BIm] / (mol L^{-1})	$k_{\text{obs}} / \text{s}^{-1}$	[BIm] / (mol L^{-1})	$k_{\text{obs}} / \text{s}^{-1}$
9.20×10^{-4}	1.45×10^{-4}	9.20×10^{-4}	1.01×10^{-4}
3.68×10^{-3}	3.67×10^{-4}	3.68×10^{-3}	3.13×10^{-4}
5.52×10^{-3}	4.84×10^{-4}	5.52×10^{-3}	4.28×10^{-4}
7.36×10^{-3}	6.23×10^{-4}	7.00×10^{-3}	5.01×10^{-4}
1.00×10^{-2}	8.49×10^{-4}	1.00×10^{-2}	6.74×10^{-4}

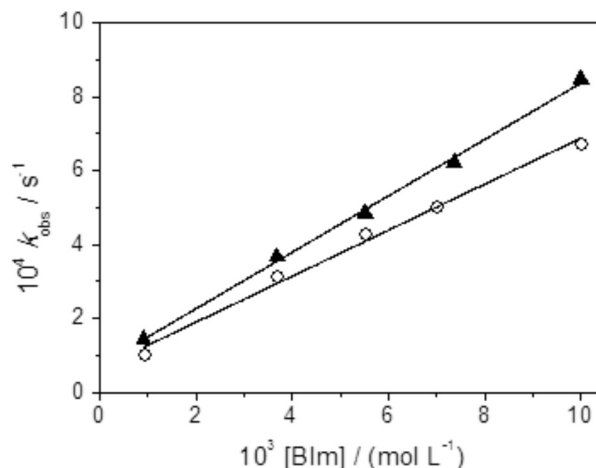


Figure S5. Effect of BIm concentration upon k_{obs} in the reaction with DEDNPP ($3.33 \times 10^{-5} \text{ mol L}^{-1}$) in CTAB (\blacktriangle) and SDS (\circ) media. In both cases, [surfactant] = 0.01 mol L^{-1} , pH 9.0 and 25°C .

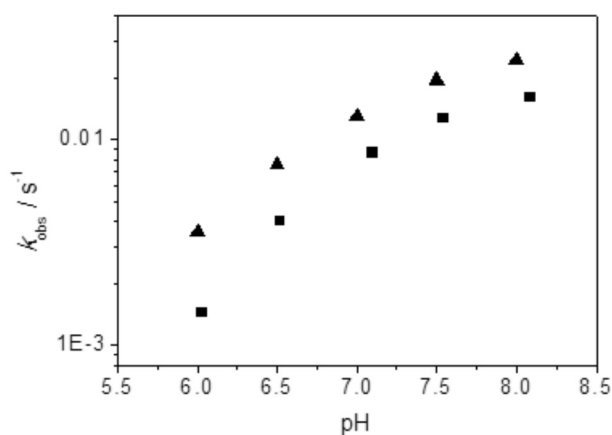


Figure S6. pH-rate profiles for the reactions of DEDNPP ($3.33 \times 10^{-5} \text{ mol L}^{-1}$) with imidazole (1 mol L^{-1}) in water (\blacksquare), obtained from the literature (reference 8 in the article), and in 0.01 mol L^{-1} CTAB medium (\blacktriangle), both at 25°C .

References

- Chhikara, B. S.; Kumar, N.; Tandon, V.; Mishra, A. K.; *Bioorg. Med. Chem.* **2005**, *13*, 4713.
- Rensing, S.; Arendt, M.; Springer, A.; Grawe, T.; Schrader, T.; *J. Org. Chem.* **2001**, *66*, 5814.