Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2014

Supporting Information for:

4-Halogeno-Sydnones for Fast Strain Promoted Cycloaddition with Bicyclo-[6.1.0]-nonyne.

Lucie Plougastel,[a] Oleksandr Koniev,[b] Simon Specklin, [a] Elodie Decuypere,[a] Christophe Créminon,[c] David Buisson, [a] Alain Wagner, [b] Sergii Kolodych* [b] and Frédéric Taran*[a]

[a] CEA, iBiTecS, Service de Chimie Bioorganique et de Marquage, Gif sur Yvette, F-91191, France. Fax: (+33) 169087991; Tel: (+33) 169082685; E-mail: frederic.taran@cea.fr

[b] Laboratory of Functional Chemo–Systems UMR 7199, 74 route du Rhin, 67401 Illkirch, France.

[c] CEA, iBiTecS, Service de Pharmacologie et d'Immunoanalyse, Gif sur Yvette, F-91191, France.

Table of Contents

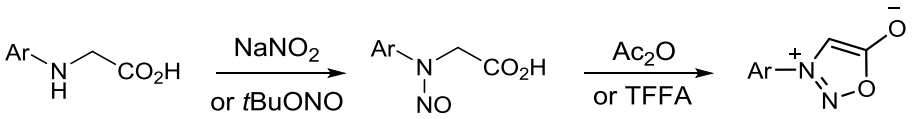
General p2
Synthesis and analytical data of sydnones p2
Stability of sydnones p15
Stability and reactivity of sydnones in blood plasma p16
Kinetic Studies p17
Competitive reaction between chlorosydnone **4q** and azide **1b** with BCN p24
Hammet plot p26
Protein labelling p26

• General

Organic solvents (Aldrich) were used without further purification. Purifications of reactions products were carried out by flash chromatography using Merck silica gel (40–63 μm). Infrared spectra (IR) were obtained on a Perkin Elmer system 2000 FTIR spectrophotometer and are reported as wavelength numbers (cm^{-1}). Infrared spectra were collected by preparing a KBr pellet containing the title compound. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), …. Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Electrospray mass spectra were obtained using an ESI/TOF Mariner Mass Spectrometer. Unless otherwise noted, all other commercially available reagents and solvents were used without further purification.

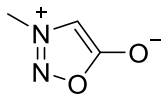
• Synthesis and analytical data of sydnones

Sydnones were prepared by nitrosylation/cyclodehydration of arylglycines (scheme S1) with some modifications of the original method.[1]



Scheme S1. General route to sydnones from aryl-glycines

3-methyl-1,2,3-oxadiazol-3-ium-5-olate (4a)[2]



$C_3H_4N_2O_2$
MW: 100.08 $g.mol^{-1}$
Yellow oil
Yield: 31%

To a stirred solution of sarcosine (8.90 g, 100 mmol) in 17 mL of concentrated hydrochloric acid, and 25 mL of water at 0 °C was added dropwise a solution of sodium nitrite (13.8 g, 200 mmol) in water (50 mL) over a period of 40 min. The solution was warmed gradually to ambient temperature. The aqueous solution was then extracted continuously with ether (75 mL) for 48 h and the ethereal extract was dried over $MgSO_4$ before evaporation of the ether to obtain 3.90 g (33.0 mmol, mixture of syn/anti-isomers : 1/1) of the nitroso intermediate compound. A solution of the later in acetic anhydride (30 mL) was heated to reflux for 5 min: color changes from colorless to green and eventually yellow were observed during this period. The reaction mixture was cooled and the excess of acetic anhydride was removed under *vacuum.* 3.08 g (31.0 mmol, 31%) of compound **4a** were isolated.

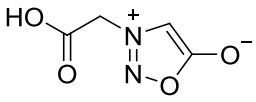
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.34 (s, 1H), 4.04 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.4, 95.9, 39.5.

IR (NaCl, cm^{-1}): 3488, 3138, 3030, 2953, 2096, 1852, 1726, 1418, 1388, 1198, 1156, 1088, 1062, 948, 870, 728, 616.

LCMS (ESI) *m/z*: 145 $[M+H]^+$.

3-(carboxymethyl)-1,2,3-oxadiazol-3-ium-5-olate (4b)



$C_4H_4N_2O_4$
MW: 144.09 $g.mol^{-1}$
Brown solid
Yield: 59%

To a stirred solution of iminodiacetic acid (6.70 g, 50.0 mmol) in 8.4 mL of concentrated hydrochloric acid, and 12.5 mL of water at 0 °C was added dropwise a solution of sodium nitrite (6.90 g, 100 mmol) in water (25.0 mL) over a period of 40 min. The solution was warmed gradually to ambient temperature. The aqueous solution was then extracted continuously with ether (40 mL) for 48 h and the ethereal extract was dried over $MgSO_4$ before evaporation of the ether to obtain 3.80 g (23.5 mmol) of the intermediate nitroso compound. A solution of the later in acetic anhydride (21.0 mL) was heated to reflux for 20 min. The reaction mixture was cooled and the excess of acetic anhydride were removed under *vacuum*. 30 mL of water were added to the residue and the solution was washed twice with CH_2Cl_2. The aqueous layer was then evaporated. 2.00 g (13.8 mmol, 59%) of compound **4b** were isolated.

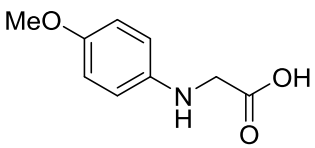
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.90 (s, 1H), 5.34 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 172.1, 167.1, 98.8, 54.5.

LCMS (ESI) *m/z*: 145 $[M+H]^+$.

HRMS (ESI): *m/z* calcd for $C_4H_3N_2O_4$ $(M-H^+)$: 143.0093; found: 143.0092.

2-[(4-methoxyphenyl)amino]acetic acid (6c)



$C_9H_{11}NO_3$
MW: 181.19 $g.mol^{-1}$
Beige solid
Yield: 57%

To a suspension of *p*-anisidine (18.5 g, 150 mmol) and sodium acetate (14.8 g, 180 mmol) in ethanol (50 mL) was added ethyl chloroacetate (22.1 g, 180 mmol). The mixture was refluxed for 7 h, left overnight at room temperature and poured into crushed ice. The formed precipitate was filtrated and dried. To the intermediate ester in water (75 mL) was added sodium hydroxide (6.58 g, 165 mmol) and the mixture was refluxed for 30 min. After cooling, the reaction mixture was acidified to pH 2 by dropwise addition of a 4 M solution of HCl. The resulting precipitate was filtered and washed with water. The crude product was purified by recrystallization from a mixture of ethanol/water 8/2. 15.4 g (85.1 mmol, 57%) of compound **6c** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.71 (m, 2H), 6.51 (m, 2H), 3.73 (s, 2H), 3.63 (s, 3H).

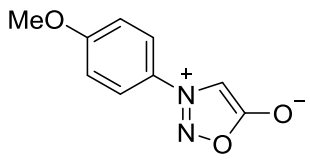
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 173.3, 151.4, 142.8, 114.9 (2C), 113.5 (2C), 55.7, 45.8.

IR (KBr, cm^{-1}): 2957, 1561, 1394, 1314, 1259, 1234, 1194, 1181, 1169, 1104, 1032, 1005, 989, 885, 869, 835, 8917, 803.

LCMS (ESI): *m/z*: 182 $[M+H]^+$, 136 $[M-CO_2+H]^+$.

Mp.: 151–153 °C.

3-(4-methoxyphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4c)



$C_9H_8N_2O_3$
MW: 192.17 g.mol^{-1}
Light purple solid
Yield: 50%

To a mixture of compound **6c** (7.25 g, 40.0 mmol) in anhydrous THF (80 mL) was added *t*-butyl nitrite (4.54 g, 44.0 mmol). The mixture was stirred at room temperature for 30 min and CDI (7.13 g, 44.0 mmol) was added. After 1 h stirring at room temperature, EtOAc was added and the reaction was quenched with an aqueous solution of HCl 1M and the aqueous layer was extracted with EtOAc. The organic layers were combined and washed with a saturated solution of NaCl before being dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallization from EtOAc and hexane. 3.87 g (20.1 mmol, 50%) of compound **4c** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.64 (m, 2H), 7.06 (m, 2H), 6.68 (s, 1H), 3.88 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.0, 162.4, 127.7, 122.62 (2C), 115.1 (2C), 93.4, 55.8.

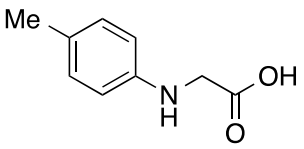
IR (NaCl, cm^{-1}): 3114, 1761, 1609, 1514, 1452, 1310, 1269, 1179, 1086, 1026, 947, 857, 828.

LCMS (ESI): *m/z*: 193 $[M+H]^+$.

Mp.: 123–125 °C.

HRMS (ESI): *m/z* calcd for $C_9H_9N_2O_3$ $(M+H^+)$: 193.0613; found: 193.0605.

2-[(4-methylphenyl)amino]acetic acid (6d)



$C_9H_{11}NO_2$
MW: 165.19 g.mol^{-1}
Beige solid
Yield: 42%

To a suspension of *p*-toluidine (16.1 g, 150 mmol) and sodium acetate (14.8 g, 180 mmol) in ethanol (50 mL) was added ethyl chloroacetate (22.1 g, 180 mmol). The mixture was refluxed for 7 h, left overnight at room temperature and poured into crushed ice. The formed precipitate was filtrated and dried. To the intermediate ester in water (75 mL) was added sodium hydroxide (6.58 g, 165 mmol) and the mixture was refluxed for 30 min. After cooling, the reaction mixture was acidified to pH 2 by dropwise addition of a 4 M solution of HCl. The resulting precipitate was filtered and washed with water. The crude product was purified by recrystallization from a mixture ethanol/water 8/2. 10.5 g of compound **6d** (63.6 mmol, 42%) were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.87 (d, *J* = 8.4 Hz, 2H), 6.44 (d, *J* = 8.4 Hz, 2H), 3.72 (s, 2H), 2.12 (s, 3H).

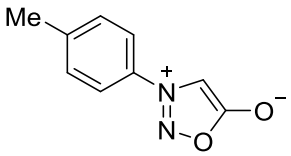
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 173.2, 146.3, 129.7 (2C), 124.9, 112.6 (2C), 45.3, 20.5.

IR (KBr, cm^{-1}): 2968, 2076, 2021, 1906, 1202, 1321, 1273, 1240, 1191, 1173, 1108, 1035, 1022, 1000, 981, 944, 903, 877, 864, 820.

LCMS (ESI): *m/z*: 166 $[M+H]^+$, 120 $[M-CO_2+H]^+$.

Mp.: 119–121 °C.

3-(4-methylphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4d)



$C_9H_8N_2O_2$
MW: 176.18 g.mol^{-1}
Beige solid
Yield: 69%

To a mixture of compound **4a** (4.96 g, 30.0 mmol) in anhydrous THF (60 mL) was added *t*-butyl nitrite (3.40 g, 33.0 mmol). The mixture was stirred at room temperature for 30 min and CDI (5.35 g, 33.0 mmol) was added. After 1 h stirring at room temperature, EtOAc was added and the reaction was quenched with an aqueous solution of HCl 1M. The aqueous layer was extracted with EtOAc and the organic layers were combined, washed with a saturated solution of NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallization from EtOAc and hexane. 3.67 g of compound **4d** (14.1 mmol, 69%) were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.59 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 6.71 (s, 1H), 2.46 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.0, 143.2, 132.4, 130.7 (2C), 121.0 (2C), 93.4, 21.3.

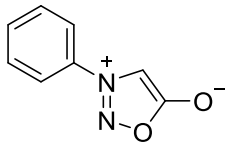
IR (NaCl, cm^{-1}): 3139, 1878, 1752, 1602, 1509, 1447, 1351, 1311, 1292, 1227, 1175, 1083, 1010, 970, 947, 857, 817.

LCMS (ESI): *m/z*: 177 $[M+H]^+$.

Mp.: 146–148 °C.

HRMS (ESI): *m/z* calcd for $C_9H_9N_2O_2$ $(M+H^+)$: 177.0664; found: 177.0659.

3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4e)



$C_8H_6N_2O_2$
MW: 162.15 g.mol^{-1}
White solid
Yield: 49%

To a vigorously stirred suspension of *N*-phenyl glycine (21.5 g, 142 mmol) in 10% aqueous HCl (142 mL) at 0 °C was added dropwise a solution of $NaNO_2$ (9.80 g, 142 mmol) in water (142 mL) over a period of 40 min. The resulting mixture was stirred at room temperature under argon for 14 h. The product was collected by filtration, washed with a small amount of methanol and dried to obtain 22.8 g (127 mmol) of the intermediate nitroso compound. The later was stirred at 100 °C for 3 h in acetic anhydride (150 mL) and the resulting solution was concentrated under *vacuum*. The residue was triturated with water (100 mL) and the precipitate was collected by filtration. The crude product was purified by recrystallization from methanol. 11.3 g (70.0 mmol, 49%) of compound **4e** were isolated.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 7.94 (dd, *J* = 8.1 Hz, *J* = 1.5 Hz, 2H), 7.78 (s, 1H), 7.77–7.67 (m, 3H).

^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 168.5, 132.4, 130.2 (2C), 126.6, 121.6 (2C), 94.9.

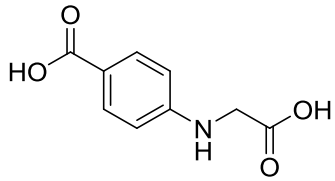
IR (NaCl, cm^{-1}): 3110, 1760, 941, 735.

LCMS (ESI) *m/z*: 163 $[M+H]^+$.

Mp.: 131–133 °C. `

HRMS (ESI): *m/z* calcd for $C_8H_7N_2O_2$ $(M+H^+)$: 163.0508; found: 163.0501.

4-[(carboxymethyl)amino]benzoic acid (6f) [3]



$C_9H_9NO_4$
MW: 195.17 g.mol^{-1}
White solid
Yield: 64%

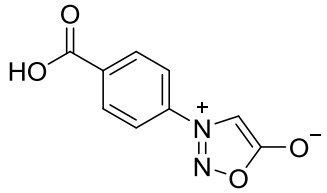
To a solution of sodium chloroacetate (2.33 g, 20.0 mmol) in water (20 mL) was added 4-aminobenzoic acid (2.74 g, 20.0 mmol). The resulting mixture was stirred at reflux for 10 h. After cooling at room temperature the precipitate was filtered and washed with water. Purification was peformed by recrystallization from ethanol. 2.50 g (12.8 mmol, 64%) of compound **6f** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.67 (d, *J* = 8.7 Hz, 2H), 6.66 (br. s., 1H), 6.57 (d, *J* = 8.7 Hz, 2H), 3.87 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 172.1, 167.5, 152.1, 131.0, 117.6 (2C), 111.2 (2C), 44.1.

LCMS (ESI) *m/z*: 196 $[M+H]^+$.

3-(4-carboxyphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4f) [3]



$C_9H_6N_2O_4$
MW: 207.16 g.mol^{-1}
White solid
Yield: 44%

To a vigorously stirred suspension of *N*-aryl amino acid **6f** (1.58 g, 8.10 mmol) in 10% aqueous HCl (8.1 mL) at 0 °C was added dropwise a solution of $NaNO_2$ (0.56 g, 8.10 mmol) in water (8.10 mL) over a period of 40 min. The resulting mixture was stirred at room temperature under argon for 14 h. The precipitate was collected by filtration, washed with a small amount of methanol and dried to obtain 1.54 g (6.90 mmol, 85%) of the intermediate nitroso compound. The later (1.30 g, 5.80 mmol) was stirred at 100 °C for 3 h in acetic anhydride (6.7 mL). The resulting solution was concentrated under *vacuum*. The residue was triturated with water (10 mL) and the precipitate was collected by filtration. The crude product was purified by recrystallization from methanol. 622 mg (3.00 mmol, 52%) of compound **4f** were isolated.

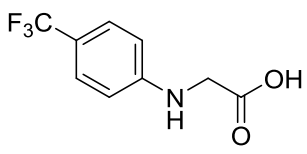
^{1}H NMR (400 MHz, DMF-d_7, δ ppm): 8.32 (d, *J* = 8.5 Hz, 2H), 8.19 (d, *J* = 8.5 Hz, 2H), 7.88 (s, 1H).

^{13}C NMR (100 MHz, DMF-d_7, δ ppm): 169.7, 167.1, 138.9, 135.7, 132.3 (2C), 123.1 (2C), 96.1.

LCMS (ESI) *m/z*: 207 $[M+H]^+$.

Mp.: 232–234 °C.

2-{[4-(trifluoromethyl)phenyl]amino}acetic acid (6g)



$C_9H_8F_3NO_2$
MW: 219.16 g.mol^{-1}
Beige solid
Yield: 30%

To a solution of *p*-trifluoromethylaniline (322 mg, 2.00 mmol) in Methanol (25 mL) at 0 °C were added NaOAc (328 mg, 4.00 mmol), glacial acetic acid (0.460 mL, 8.00 mmol), glyoxylic acid monohydrate (276 mg, 3.00 mmol) and $NaCNBH_3$ (126 mg, 2.00 mmol). The solution was warmed slowly to rt over 2 h. The mixture was filtered through a plug of SiO_2 and washed with a solution of acetic acid 1% in EtOAc. Brine was added and the aqueous layer was extracted twice with EtOAc. The organic layers were combined, dried over $MgSO_4$

and concentrated under *vacuum*. Purification was performed by recrystallization from a mixture ethanol/water 8/2. 130 mg (0.59 mmol, 30%) of compound **6g** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.38 (d, *J* = 8.5 Hz, 2H), 6.72–6.57 (m, 3H), 3.87 (s, 2H), 3.59–3.14 (br. s, 1H).

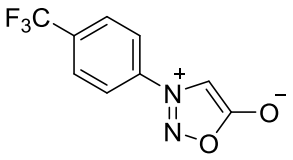
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 172.1, 151.3, 126.2 (2C), 125.3 (q, *J* = 267 Hz), 115.8 (q, *J* = 32 Hz), 111.6 (2C), 44.1.

IR (KBr, cm^{-1}): 3421, 2899, 1901, 1731, 1616, 1585, 1538, 1491, 1441, 1412, 1319, 1281, 1242, 1188, 1164, 1151, 1110, 1062, 1005, 926, 825.

LCMS (ESI) m/z: 220 $[M+H]^+$.

Mp.: 141–143 °C.

3-[4-(trifluoromethyl)phenyl]-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4g)



$C_9H_5F_3N_2O_2$
MW: 230.15 g.mol^{-1}
Orange solid
Yield: 70%

To a mixture of compound **6g** (50.0 mg, 0.228 mmol) in anhydrous THF (0.5 mL) was added *t*Butyl nitrite (0.030 mL, 0.251 mmol). The mixture was stirred at room temperature for 30 min and TFAA (0.030 mL, 0.251 mmol) was added. After 1 h stirring at room temperature, EtOAc was added and the reaction was quenched with a saturated solution of $NaHCO_3$. The aqueous layer was extracted with EtOAc and the organic layers were combined, dried over $MgSO_4$ and evaporated. 37.0 mg (0.161 mg, 70%) of compound **4g** were obtained.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.92 (m, 4H), 6.84 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 168.8, 137.3, 134.7 (q, *J* = 34 Hz), 127.7 (2C), 123.0 (q, *J* = 273 Hz), 122.1 (2C), 94.0.

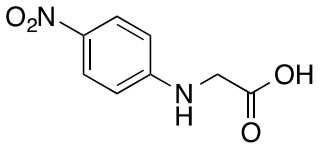
IR (NaCl, cm^{-1}): 3131, 2921, 2351, 1900, 1785, 1732, 1715, 1682, 1651, 1645, 1634, 1615, 1520, 1463, 1429, 1354, 1328, 1244, 1181, 1162, 1117, 1070, 1023, 1012, 951, 862, 850.

LCMS (ESI) *m/z*: 231 $[M+H]^+$.

Mp.: 131–133 °C.

HRMS (ESI): *m/z* calcd for $C_9H_6F_3N_2O_2$ $(M+H^+)$: 231.0381; found: 231.0374.

2-[(4-nitrophenyl)amino]acetic acid (6h)



$C_8H_8N_2O_4$
MW: 196.16 g.mol^{-1}
Yellow solid
Yield: 61%

To a suspension of 4-nitroaniline (13.8 g, 100 mmol) in water (100 mL) was added chloroacetic acid (18.9 g, 200 mmol). The mixture was refluxed overnight and the resulting precipitate was filtered and successively washed with water and with a mixture of hexane/diethyl ether. The solid was dissolved in an aqueous solution of NaOH 6 M and the resulting solution was washed with EtOAc. The organic layer was extracted with a solution of NaOH 6 M and the combined aqueous extracts were acidified at 0 °C to pH 2 with an HCl solution. The resulting precipitate was filtered, washed with water and dried. 11.9 g (60.7 mmol, 61%) of compound **8a** were obtained.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 12.80 (br. s, 1H), 8.00 (d, *J* = 9.2 Hz, 2H), 7.44 (t, *J* = 6.1 Hz, 1H), 6.66 (d, *J* = 9.2 Hz, 2H), 3.98 (d, *J* = 6.1 Hz, 2H).

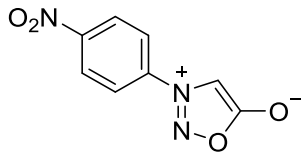
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 171.5, 154.3, 136.4, 126.1 (2C), 111.3 (2C), 44.1.

IR (KBr, cm^{-1}): 3364, 3099, 1737, 1601, 1532, 1495, 1466, 1438, 1407, 1362, 1291, 1180, 1143, 1111, 993, 916, 843.

LCMS (ESI): *m/z*: 197 $[M+H]^+$.

Mp.: 224–226 °C.

3-(4-nitrophenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4h)



$C_8H_5N_3O_4$
MW: 207.14 g.mol^{-1}
Orange solid
Yield: 57%

To a mixture of compound **6h** (0.981 g, 5.00 mmol) in anhydrous THF (10 mL) was added *t*-butyl nitrite (0.567 g, 5.50 mmol. The mixture was stirred at room temperature for 30 min and TFAA (1.16 g, 5.50 mmol) was added. After 1 h stirring at room temperature, EtOAc was added and the reaction was quenched with a saturated solution of $NaHCO_3$. The resultant precipitate was filtered and washed with EtOAc. 15.4 g (85.1 mmol, 57%) of compound **4h** were obtained.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.53 (m, 2H), 8.23 (m, 2H), 7.97 (s, 1H).

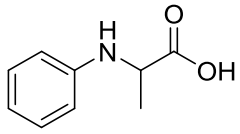
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 166.3, 149.4, 136.5, 125.5 (2C), 123.2 (2C), 95.8.

IR (KBr, cm^{-1}): 3120, 1903, 1780, 1621, 1595, 1537, 1459, 1434, 1346, 1316, 1294, 1243, 1180, 1122, 1109, 1094, 1019, 1011, 952, 862, 847.

LCMS (ESI): *m/z*: 208 $[M+H]^+$.

Mp.: 188–190 °C.

2-(phenylamino)propanoic acid (6i) [3]



$C_9H_{11}NO_2$
MW: 165.19 g.mol^{-1}
White solid
Yield: 65%

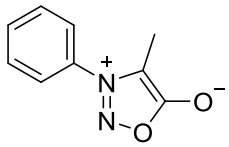
A mixture of bromobenzene (3.15 g, 20.0 mmol), CuI (380 mg, 2 mmol), DL-alanine (2.70 g, 30.0 mmol), potassium phosphate monohydrate (13.8 g, 60.0 mmol), deanol (6.00 mL) and water (20.0 mL) was vigorously stirred at 90 °C for 40 h. The mixture was cooled to room temperature and poured into crushed ice. A 6M solution of HCl was added dropwise to pH 4-5 and th mixure was extracted with EtOAc. The organic layers were combined, washed with brine and water, dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallization from H_2O/Methanol. 2.15 g (13.0 mmol, 65%) of compound **6i** were isolated.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 7.10–7.04 (m, 2H), 6.57–6.53 (m, 3H), 3.93 (q, *J* = 7.0 Hz, 1H), 3.44 (s, br, 1H), 1.37 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 175.9, 147.8, 128.8 (2C), 116.2, 112.4 (2C), 50.9, 18.1.

IR (NaCl, cm^{-1}): 3061, 2980, 2932, 2774, 2725, 2670, 2357, 1559, 1491, 1452, 1414, 1390, 1357, 1334, 1304, 1288, 1259, 1125, 1089, 1058, 1024, 958, 907, 846, 775.

4-methyl-3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4i) [3]



$C_9H_8N_2O_2$
MW: 176.17 g.mol^{-1}
White solid
Yield: 58%

To a vigorously stirred suspension of *N*-phenylalanine **6i** (1.64 g, 9.90 mmol) in 10% aqueous HCl (10 mL) at 0 °C was added dropwise a solution of $NaNO_2$ (824 mg, 11.9 mmol) in water (10 mL) over a period of 40 min. The resulting mixture was stirred at room temperature under argon for 14 h. The product was collected by filtration, washed with a small amount of methanol and dried to obtain 1.10 g (5.70 mmol) of the nitroso intermediate compound. The later was stirred at 100 °C for 3 h in acetic anhydride (10 mL) and the resulting solution was concentrated under *vacuum*. The residue was triturated with water (100 mL) and the precipitate was collected by filtration. The crude product was purified by recrystallization from methanol. 604 mg (5.70 mmol, 58%) of compound **4i** were isolated.

^{1}H NMR (400 MHz, CDCl$_3$, δ ppm): 7.76–7.63 (m, 3H), 7.55 (dd, *J* = 7.5 Hz, *J* = 1.5 Hz, 2H), 2.18 (s, 3H).

^{13}C NMR (100 MHz, CDCl$_3$, δ ppm): 168.8, 134.0, 132.2, 130.2 (2C), 124.5 (2C), 105.2, 8.0.

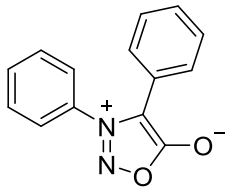
IR (NaCl, cm^{-1}): 3436, 3067, 3021, 2930, 2363, 1964, 1903, 1803, 1770, 1743, 1593, 1485, 1456, 1371, 1295, 1244, 1063, 1015, 882, 768, 719, 689, 637.

LCMS (ESI) m/z: 177 $[M+H]^+$, 178 $[M+2H]^+$.

Mp.: 91–93 °C.

HRMS (ESI): *m/z* calcd for $C_9H_9N_2O_2$ $(M+H^+)$: 177.0664; found: 177.0658.

***Diphenyl-1,2,3λ⁵-oxadiazol-3-ylium-5-olate (4j)*[4]**



$C_{14}H_{10}N_2O_2$
MW: 238.25 g.mol^{-1}
Yellow solid
Yield: 22%

A mixture of phenylsydnone (200 mg, 1.23 mmol), phenylboronic acid (301 mg, 2.47 mmol), $Pd(OAc)_2$ (2.61 mg, 0.03 mmol) and K_2CO_3 (341 mg, 2.47 mmol) in DMF (4.0 mL) was stirred at 90 °C in the dark overnight. After cooling at room temperature, water was added and the reaction mixture was extracted with EtOAc. The organic layers were combined before being dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (SiO_2, heptane/ethyl acetate 9/1). 64.0 mg (0.27 mmol, 22%) of compound **4j** were isolated.

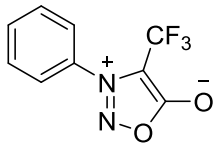
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.64–7.70 (m, 1H), 7.55–7.61 (m, 2H), 7.47–7.51 (m, 2H), 7.29 (s, 5H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.1, 134.7, 132.2, 130.2 (2C), 128.7 (2C), 127.4 (2C), 125.0 (2C), 124.4, 107.9.
It should be noted that the ^{13}C signal of C4 is not visible.

LCMS (ESI): *m/z*: 239 $[M+H]^+$.

Mp.: 175–177 °C.

***5-hydroxy-3-phenyl-4-(trifluoromethyl)-1,2,3λ⁵-oxadiazol-3-ylium (4k)*[5]** $C_9H_5F_3N_2O_2$
MW: 230.15 g.mol^{-1}
Light yellow solid
Yield: 59%



To a stirring suspension of phenyliodosydnone **4p** (60.4 mg, 0.20 mmol) and copper iodide (38.1 mg, 0.2 mmol) in DMF (1.0 mL) was added methyl fluorosulfonyldifluoroacetate (0.13 mL, 1.0 mmol) under a nitrogen atmosphere and the reaction was heated at 80 °C for 20 h. The reaction was filtered, brine was added and the solution was extracted with EtOAc, the organic layers were combined and washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (SiO_2, heptane/ethyl acetate 8.5/1.5). 27.0 mg (0.12 mmol, 59%) of compound **4k** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.76 (m, 1H), 7.67 (m, 2H), 7.62–7.57 (m, 2H).

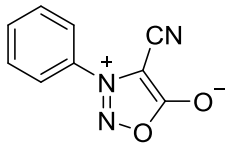
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.2, 133.8, 133.4, 130.3 (2C), 124.7 (2C), 119.5 (q, J = 267 Hz).
It should be noted that the ^{13}C signal of C4 is not visible.

LCMS (ESI): *m/z*: 231 $[M+H]^+$.

Mp.: 107–109 °C.

HRMS (ESI): *m/z* calcd for $C_{11}H_9F_3N_3O_2$ $(M+CH_3CN+H^+)$: 272.0647; found: 272.0650.

4-cyano-3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4l)



$C_9H_5N_3O_2$
MW: 187.16 g.mol^{-1}
Light brown solid
Yield: 45%

To a mixture of phenylsydnone **4e** (100 mg, 0.62 mmol) in anhydrous THF (1 mL) was added dropwise a solution of *n*BuLi (1.6 M in hexanes, 587 µL, 0.94 mmol) at -78 °C. After 30 min at -78 °C, a solution of toluenesulfonyl cyanide (225 mg, 0.50 mmol) in THF (240 µL) was added. The temperature was slowly increased to room temperature over 1 h and the mixture was stirred overnight. The reaction was quenched with a saturated solution of NH_4Cl and the aqueous layer was extracted with EtOAc. The organic layers were combined before being dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (SiO_2, Heptane/Ethyl acetate 9/1). 33.0 mg (0.28 mmol, 45%) of compound **4l** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.83–7.78 (m, 3H), 7.76–7.69 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6, 134.0, 133.3, 130.8 (2C), 123.0 (2C), 107.8.
It should be noted that the ^{13}C signal of C4 is not visible.

LCMS (ESI): *m/z*: 189 $[M+H]^+$.

Mp.: 142–144 °C.

HRMS (ESI): *m/z* calcd for $C_{11}H_9N_4O_2$ $(M+CH_3CN+H^+)$: 229.0726; found: 229.0723.

*General procedure **A1** for chlorination of sydnones*
To a stirred solution of sydnone (0.50 mmol, 1 eq) in acetic acid (1.2 mL) was added *N*-chlorosuccinimide (2 eq). The mixture was stirred at room temperature for 6 h. EtOAc was added and the mixture was evaporated. The crude product was purified by flash chromatography to afford the chlorinated compound.

*General procedure **A2** for chlorination of sydnones*
To a stirred solution of sydnone (206.2 mg, 1 mmol) in a mixture dioxane/HCl 1M 2/1 (12 mL) was added dropwise a solution of sodium hypochlorite 10% (1.23 mL, 1 mmol). The mixture was stirred at room temperature for 4 h. The mixture was poured in a solution of $Na_2S_20_3$ 20%. EtOAc was added and the aqueous layer was extracted 3 times with EtOAc.

The organic layers were combined, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel column to afford the chlorinated compound.

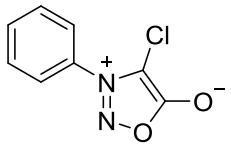
*General procedure **B** for bromination of sydnones*

To a stirred solution of sydnone (0.50 mmol, 1 eq) in acetic acid (1.2 mL) was added *N*-bromosuccinimide (1.1 eq). The mixture was stirred at room temperature for 2h and 5 mL of water was added. The resulting precipitate was isolated by filtration to afford the brominated compound.

*General procedure **C** for iodination of sydnones*[6]

To a stirred solution of sydnone (0.50 mmol, 1 eq) in acetic acid (1.2 mL) was added *N*-iodosuccinimide (1.1 eq). The mixture was stirred at room temperature for 30 min and 5 mL of water was added. The resulting precipitate was isolated by filtration to afford the iodinated compound.

4-chloro-3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4m)



$C_8H_5ClN_2O_2$
MW: 196.59 g.mol^{-1}
Light green solid
Yield : 65%

The title compound **4m** was obtained in 65% yield from *N*-chlorosuccinimide (133 mg, 1.00 mmol) and phenylsydnone **4e** (81.1 mg, 0.50 mmol) according to general procedure **A1**. The crude product was purified by flash chromatography (SiO_2, Ethyl acetate/Heptane 3/7) to afford the chlorinated compound.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.76–7.62 (m, 5H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.9, 133.0, 132.7, 130.1 (2C), 124.3 (2C).
It should be noted that the ^{13}C signal of C4 is not visible.

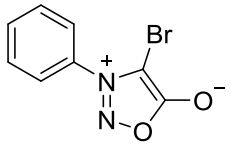
IR (NaCl, cm^{-1}): 3069, 1903, 1786, 1748, 1499, 1466, 1432, 1350, 1222, 1181, 1040, 1027 934, 879.

LCMS (ESI): *m/z*: 197 [M(^{35}Cl)+H]$^+$, 199 [M(^{37}Cl)+H]$^+$.

Mp.: 115–117 °C.

HRMS (ESI): *m/z* calcd for $C_8H_6ClN_2O_2$ (M+H$^+$): 197.0118; found: 197.0112.

4-bromo-3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4n)



$C_8H_5BrN_2O_2$
MW: 241.04 g.mol^{-1}
Orange solid
Yield: 71%

The title compound **4n** was obtained in 71% yield from *N*-bromosuccinimide (107 mg, 0.50 mmol) and phenylsydnone **4e** (81.1 mg, 0.50 mmol) according to general procedure **B**.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.75–7.60 (m, 5H).

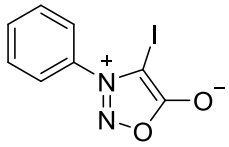
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 165.7, 134.0, 132.8, 130.2 (2C), 124.8 (2C), 84.2.

LCMS (ESI): *m/z*: 241 $[M(^{79}Br)+H]^+$, 243 $[M(^{81}Br)+H]^+$.

Mp.: 137–139 °C.

HRMS (ESI): *m/z* calcd for $C_8H_6BrN_2O_2$ $(M+H^+)$: 240.9613; found: 240.9607.

4-iodo-3-phenyl-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4o)



$C_8H_5IN_2O_2$
MW: 288.04 g.mol^{-1}
Beige solid
Yield: 65%

The title compound **4o** was obtained in 65% yield from *N*-iodosuccinimide (124 mg, 0.55 mmol) and phenylsydnone (81.1 mg, 0.50 mmol) according to general procedure **C**.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.77–7.55 (m, 5H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 168.9, 135.3, 132.7, 130.2 (2C), 125.2 (2C), 50.7.

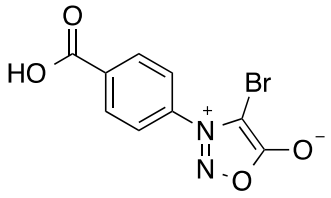
IR (KBr, cm^{-1}): 3066, 2622, 1927, 1897, 1882, 1769, 1720, 1591, 1498, 1491, 1461, 1420, 1332, 1313, 1295, 1203, 1176, 1159, 1074, 1035, 1020, 966, 927, 862, 849.

LCMS (ESI): *m/z*: 289 $[M+H]^+$.

Mp.: 161–163 °C.

HRMS (ESI): *m/z* calcd for $C_8H_6IN_2O_2$ $(M+H^+)$: 288.9474; found: 288.9476.

4-bromo-3-(4-carboxyphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4p)



$C_9H_5BrN_2O_4$
MW: 285.05 g.mol^{-1}
Orange solid
Yield: 44%

The title compound **4p** was obtained in 44% yield from *N*-bromosuccinimide (97.9 mg, 0.55 mmol) and carboxyphenylsydnone **4f** (103 mg, 0.50 mmol) according to general procedure **B**.

^{1}H NMR (400 MHz, Acetone-d_6, δ ppm): 8.39 (d, *J* = 8.6 Hz, 2H), 8.03 (d, *J* = 8.6 Hz, 2H).

^{13}C NMR (100 MHz, Acetone-d_6, δ ppm): 166.1, 166.0, 138.3, 135.3, 132.1 (2C), 126.7 (2C), 85.9.

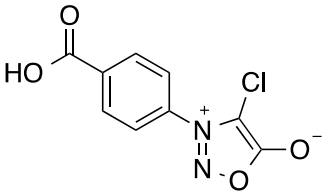
IR (KBr, cm^{-1}): 2820, 2087, 1942, 1904, 1850, 1743, 1606, 1591, 1509, 1445, 1423, 1335, 1317, 1290, 1205, 1127, 1114, 1035, 1018, 974, 942, 881, 858, 820, 808.

LCMS (ESI): *m/z*: 285 [M(^{79}Br)+H]$^+$, 287 [M(^{81}Br)+H]$^+$.

Mp.: 117–119 °C (decomp.).

HRMS (ESI): *m/z* calcd for $C_9H_6BrN_2O_4$ (M+H$^+$): 284.9511; found: 284.9505.

3-(4-carboxyphenyl)-4-chloro-1,2,3λ^5-oxadiazol-3-ylium-5-olate (4q)



$C_9H_5ClN_2O_4$
MW: 240.60 g.mol^{-1}
Orange solid
Yield: 40%

The title compound **4q** was obtained in 40% yield from sodium hypochlorite 10% (1.23 mL, 2.00 mmol) and carboxyphenylsydnone **4f** (206 mg, 1.00 mmol) according to general procedure **A2**. The crude product was purified by flash chromatography (SiO_2, DCM/Methanol 99/1, 1% AcOH) to afford the chlorinated compound.

^{1}H NMR (400 MHz, MeOD, δ ppm): 8.30 (m, 2H), 7.87 (m, 2H).

^{13}C NMR (100 MHz, MeOD, δ ppm): 169.2, 166.0, 162.5, 138.7, 132.2 (2C), 126.1 (2C).

IR (KBr, cm^{-1}): 3315, 2927, 2549, 1791, 747, 1691, 1607, 1510, 1455, 1425, 1346, 1320, 1290, 1219, 1176, 1128, 1113, 1043, 1018, 182, 972, 945, 886, 859, 808.

LCMS (ESI): *m/z*: 241 [M(^{35}Cl)+H]$^+$, 243 [M(^{37}Cl)+H]$^+$.

Mp.: 159–161 °C.

HRMS (ESI): *m/z* calcd for $C_{11}H_9ClN_3O_4$ (M+H+CH_3CN^+): 282.0282; found: 282.0272.

• Stability of sydnones

General procedure for measuring the stability of sydnones.
To 900 µL of phosphate buffered saline (PBS, 100 mM) was added 89 µL of DMSO, 10 µL of the solution of benzamide (internal standard, 100 mM in DMSO) and 1 µL of the solution of sydnone (100mM in DMSO). The resulting mixture was immediately injected in HPLC and the ratio of benzamide versus sydnone peak areas was calculated. The second injection was carried out after 2 h of incubation at 25 °C. Stability level was evaluated by comparing the initial sydnone/benzamide peak area ratio with the peak area ratio after incubation.
All sydnones showed no observable degradation.

• Stability and reactivity of sydnones in blood plasma

*Evaluation of the stability of sydnones **4p** and **4q** in blood plasma.*

To 900 µL of blood plasma were added 40 µL of DMSO, 50 µL of benzamide solution (internal standard, 100 mM in DMSO) and 10 µL of sydnone solution (100 mM in DMSO). A 100 µL aliquot of the resulting solution was added to 900 µL of MeCN and centrifuged. The resulting MeCN solution was injected in HPLC and the ratio of sydnone versus benzamide peak areas was calculated. The remaining plasma solution was incubated at 25 °C for 12 h and then the second aliquot was treated in the same manner and injected in HPLC. Stability level was evaluated by comparing the initial sydnone/benzamide peak area ratio with the peak area ratio after incubation. Compounds **4p** and **4q** showed no observable degradation in blood plasma during 12 h at 25 °C (Figure S1).

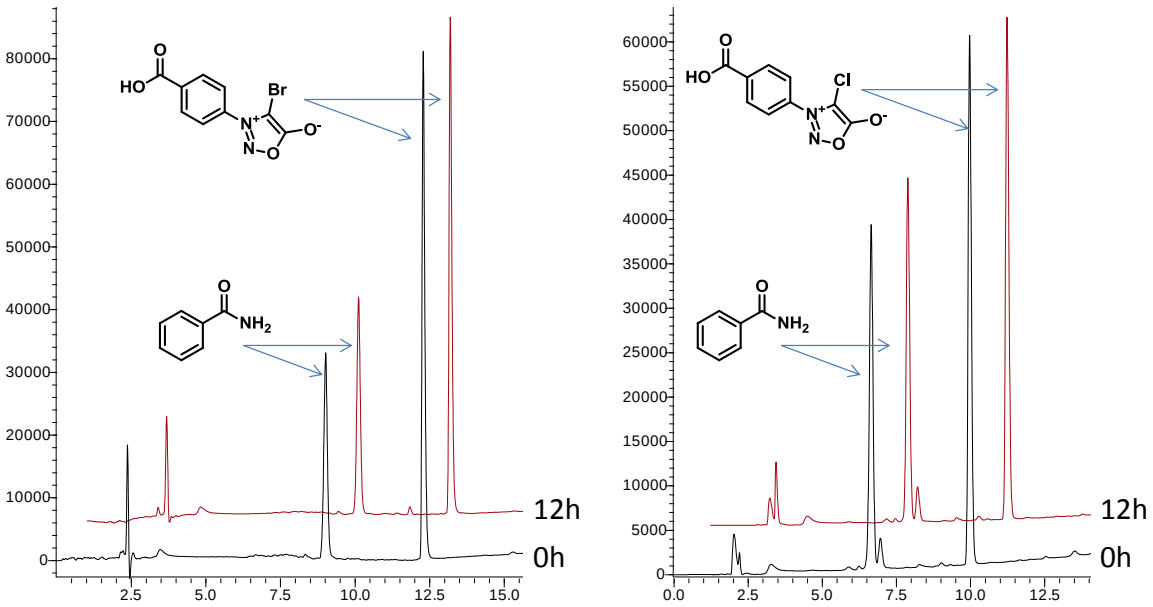


Figure S1. Stability of **4p** and **4q** in blood plasma.

*Evaluation of the reactivity of sydnones **4p** and **4q** in blood plasma.*

Reactions of sydnones **4p** and **4q** with BCN were carried out in blood plasma/DMSO (9:1) mixtures at 1 mM concentration of sydnones and 1.5 mM concentration of BCN using the following procedure:

To 900 µL of blood plasma was added 25 µL of DMSO, 50 µL of the solution of benzamide (internal standard, 100 mM in DMSO) and 10 µL of the solution of sydnone (100 mM in DMSO). A 100 µL aliquot of the resulting solution was added to 900 µL of MeCN and centrifuged. The resulting MeCN solution was injected in HPLC and the ratio of sydnone versus benzamide peak areas was calculated. To the remaining plasma solution was added 13.5 µL of the solution of BCN (100 mM in DMSO). The mixture was incubated at 25 °C for 1 h and then the second aliquot was treated in the similar manner. Conversion rate was evaluated by comparing the initial normalized sydnone peak area with the peak area after incubation. Compound **4p** showed 87% conversion into the coupling product (Figure S2). Compound **4q** showed 93% conversion into the coupling product (Figure S3).

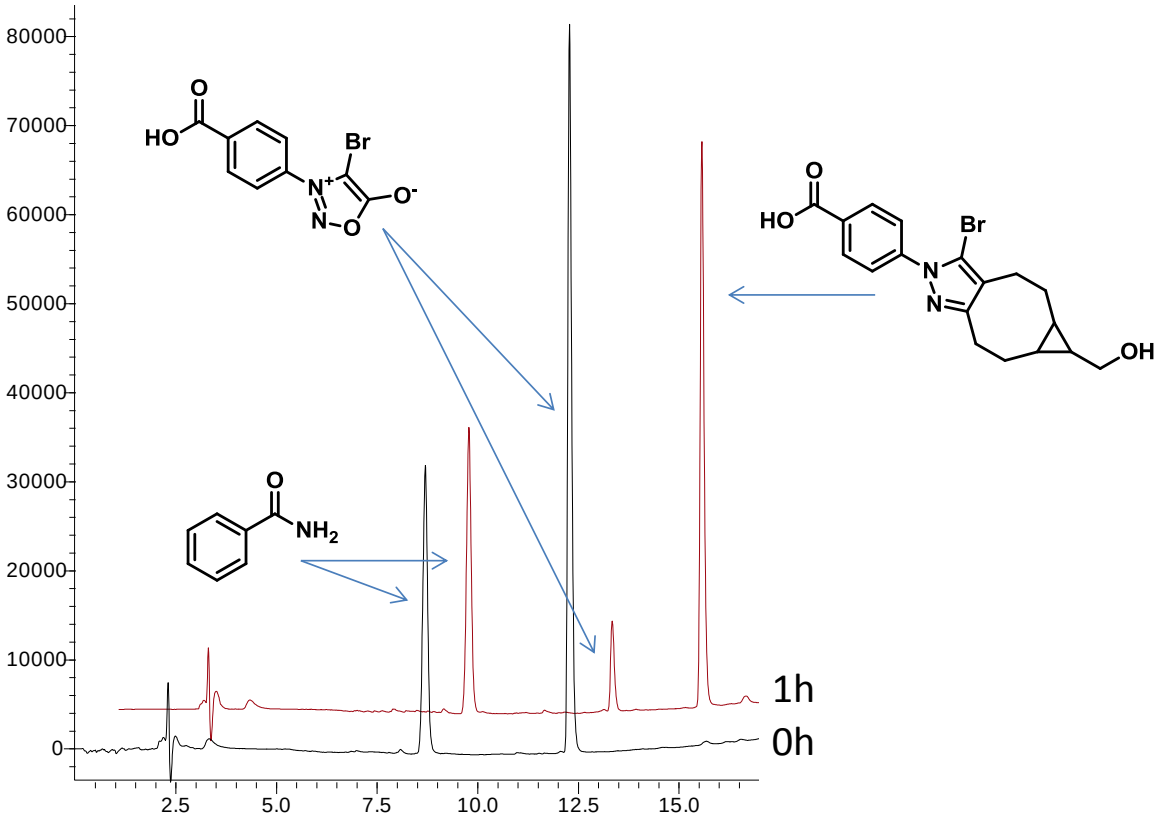


Figure S2. Reactivity of the compound **4p** in blood plasma.

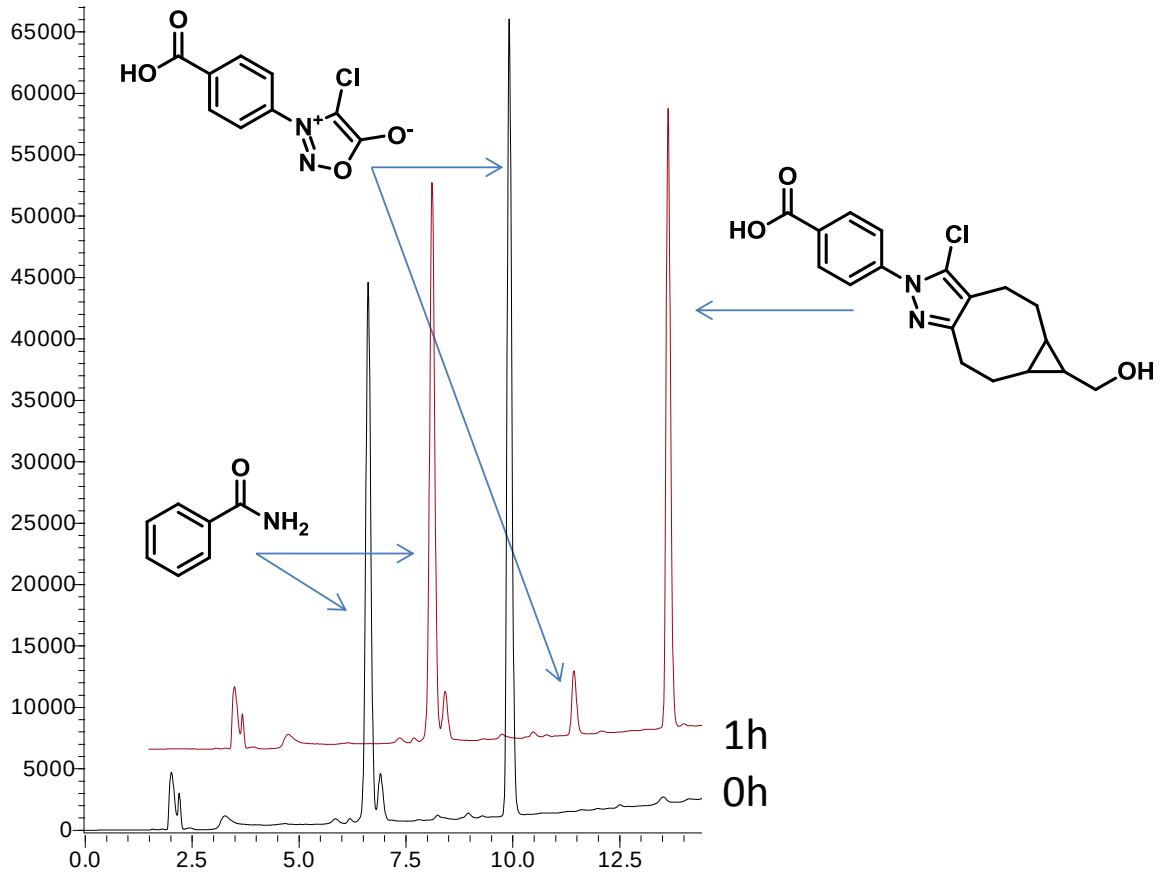


Figure S3. Reactivity of the compound **4q** in blood plasma.

• Kinetic Studies

Reactions of sydnones **4d**, **4e**, **4f**, **4g**, **4h**, **4i**, **4m**, **4n**, **4o**, **4p** and **4q** with BCN were carried out in PBS/DMSO (9:1) mixtures at 100 μM concentration of sydnones and 150 μM concentration of BCN using the following procedure:
To 900 μL of phosphate buffered saline (PBS, 100mM) was added 87.5 μL of DMSO, 10 μL of the solution of benzamide (internal standard, 100 mM in DMSO), 1 μL of the solution of sydnone (100mM in DMSO) and 1.5 μL of the solution of BCN (100mM in DMSO). The reaction mixture was injected in HPLC every 30 min and the conversion was followed by measuring the normalized sydnone peak area.

Reactions of sydnones **4a**, **4b**, **4c**, **4j**, **4k** and **4l** with BCN were carried out in PBS/DMSO (9:1) mixtures at 1 mM concentration of sydnones and 1.5 mM concentration of BCN using the following procedure:
To 900 μL of phosphate buffered saline (PBS, 100mM) was added 25 μL of DMSO, 50 μL of the solution of benzamide (internal standard, 100 mM in DMSO), 10 μL of the solution of sydnone (100mM in DMSO) and 15 μL of the solution of BCN (100mM in DMSO). The reaction mixture was injected in HPLC every 30 min and the conversion was followed by measuring the normalized sydnone peak area.

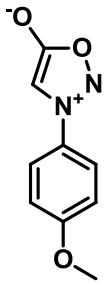
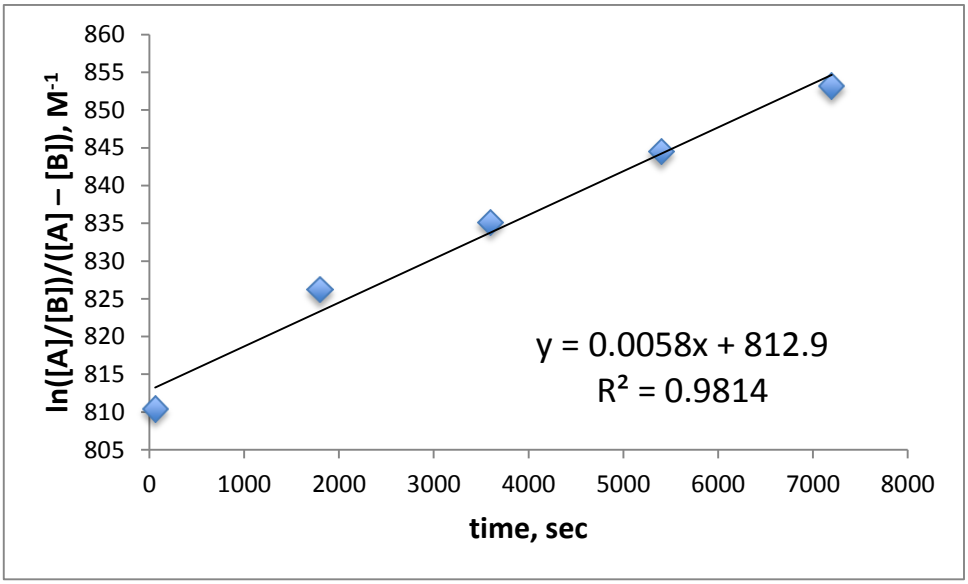
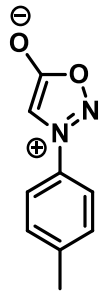
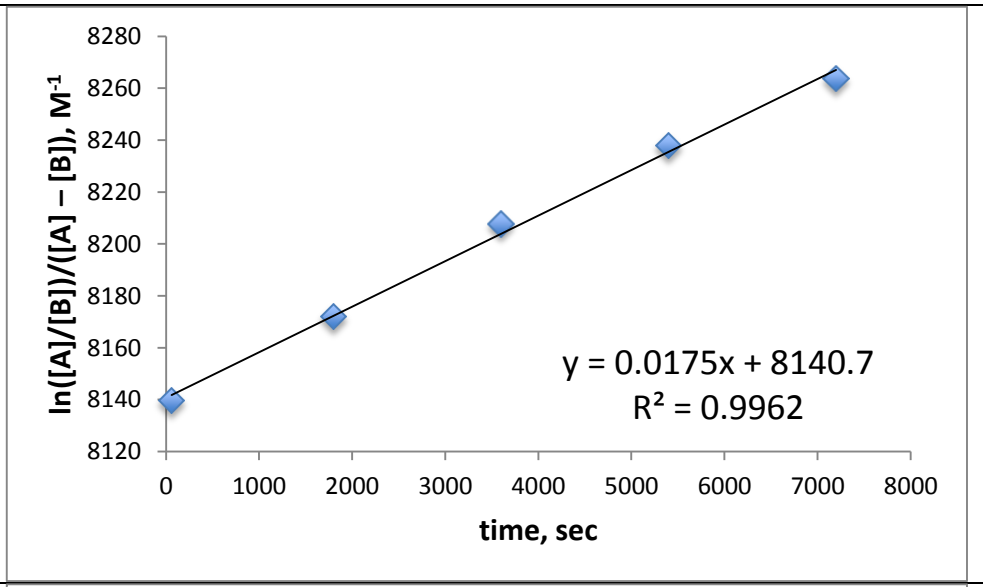
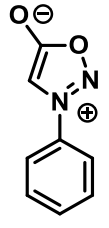
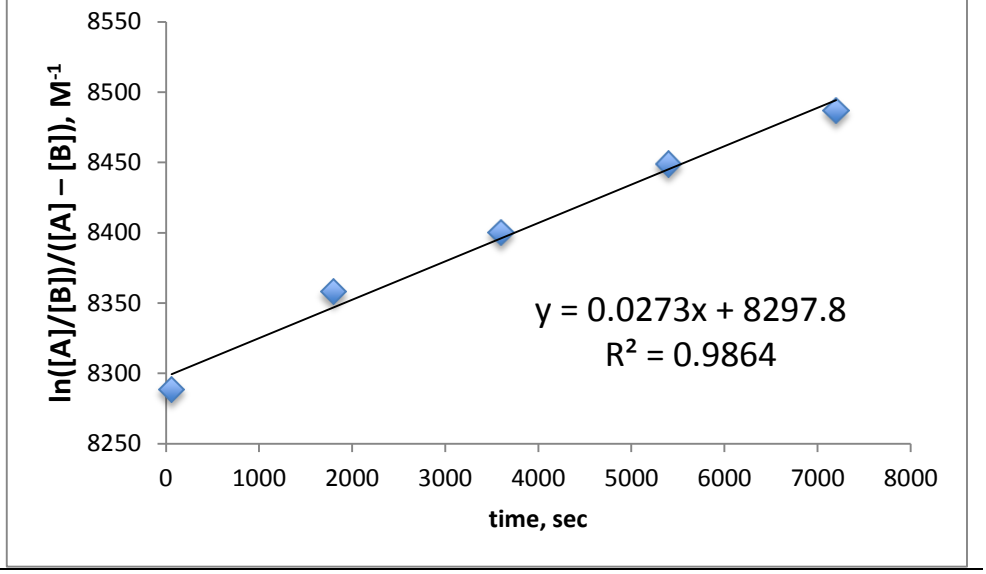
Second order reaction rate was determined by plotting ln([A]/[B])/([A] – [B]) versus time and analyzing by linear regression (**Equation S1**). Second order rate constant corresponds to the determined slope.

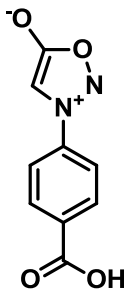
$$\frac{\ln\left(\frac{[A]}{[B]}\right)}{[A]-[B]} = kt + const$$

Equation S1. [A] – concentration of sydnones (M); [B] – concentration of BCN (M); t – reaction time (sec); k – reaction rate ($M^{-1}\cdot sec^{-1}$)

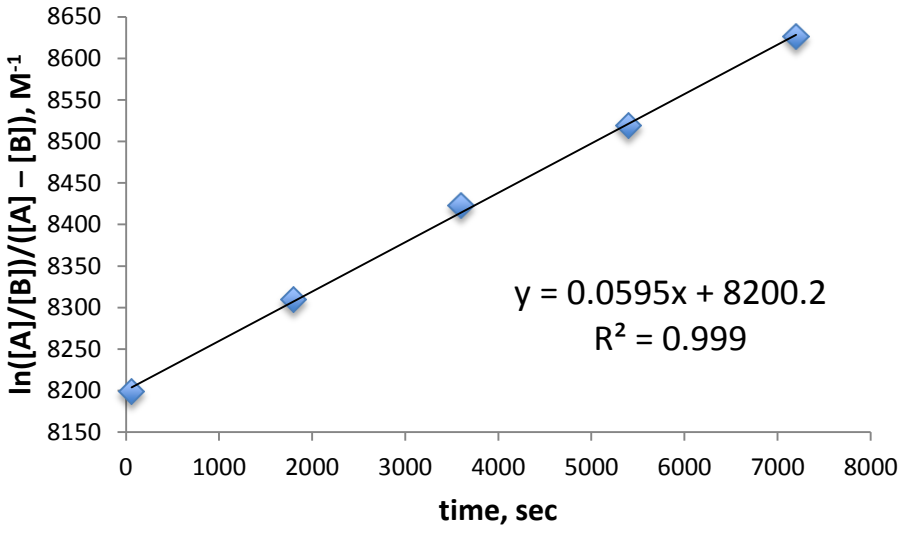
Linear regression curves for the reactive sydnones are illustrated in **Table S1**.

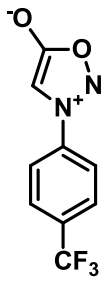
Table S1. Linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time

 **4c**	
 **4d**	
 **4e**	

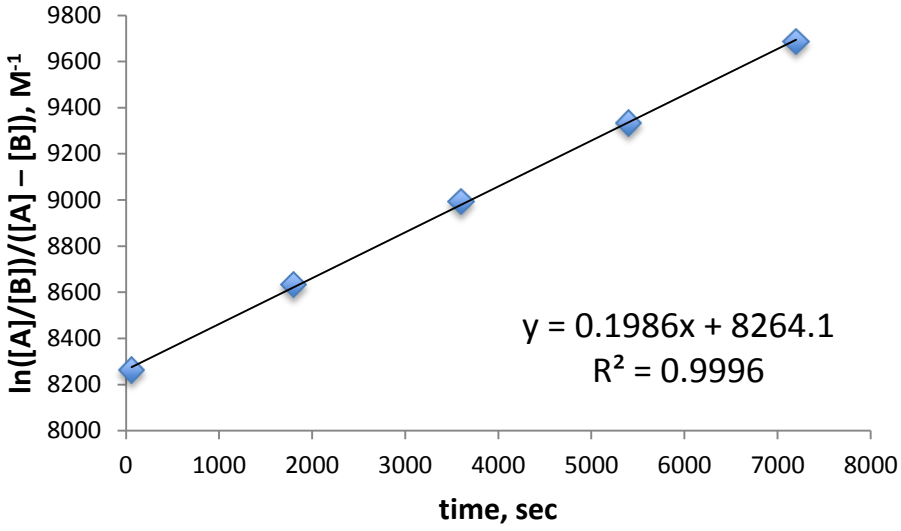


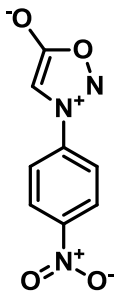
4f


ln([A]/[B])/([A] – [B]), M-1
time, sec
y = 0.0595x + 8200.2
R² = 0.999

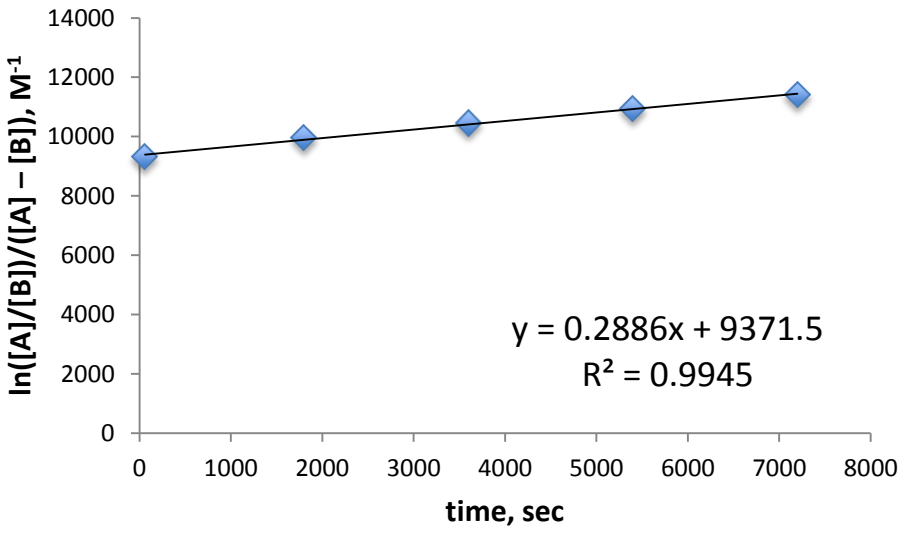


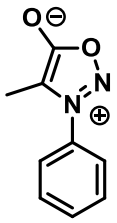
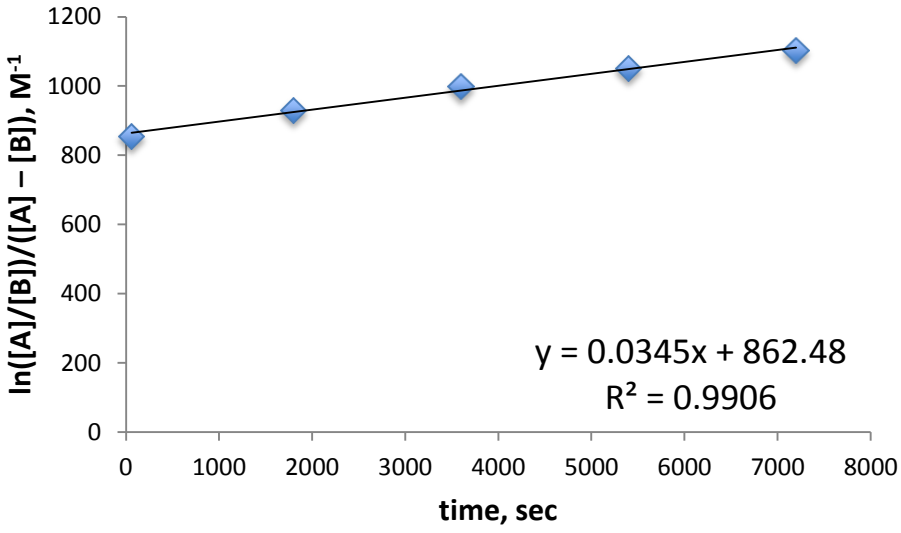
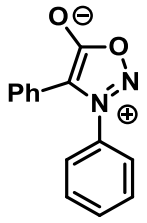
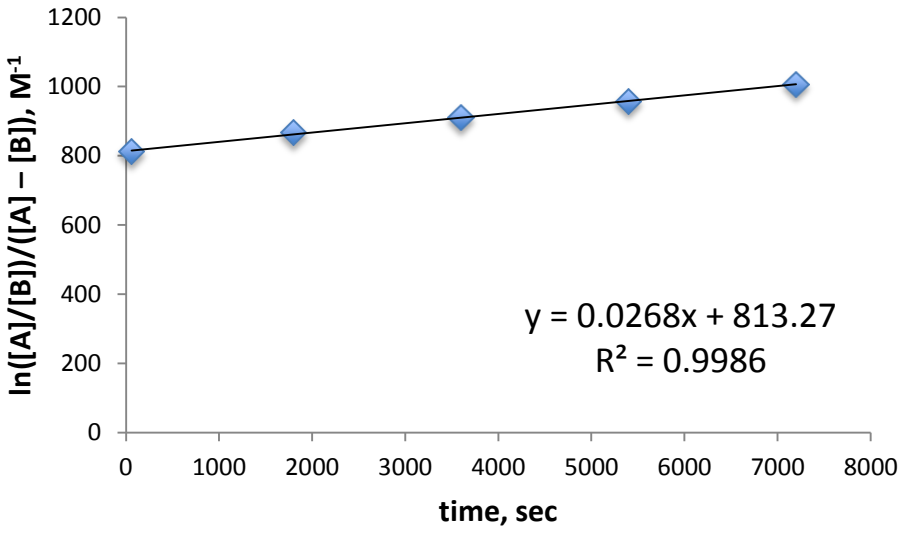
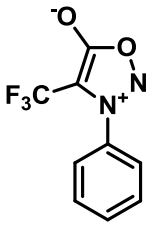
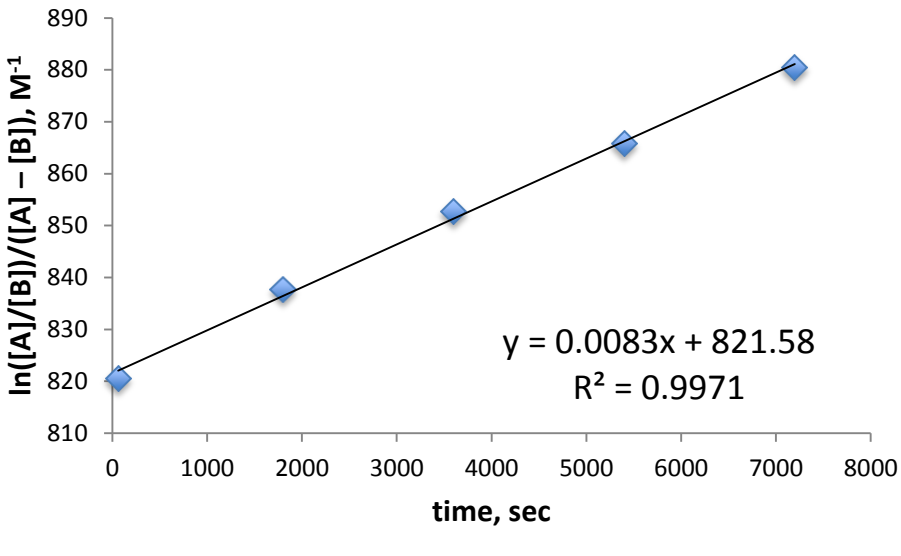
4g

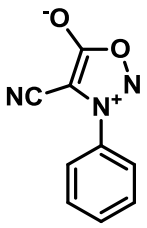

ln([A]/[B])/([A] – [B]), M-1
time, sec
y = 0.1986x + 8264.1
R² = 0.9996



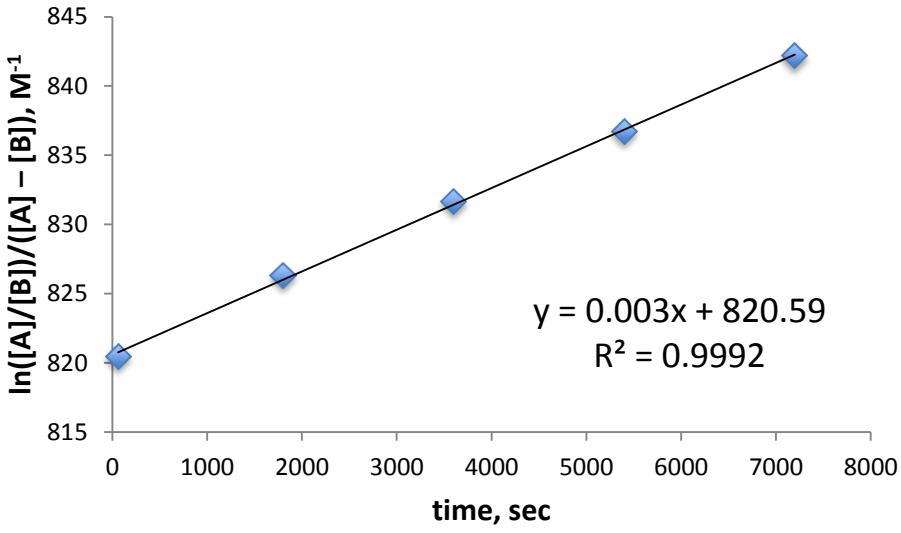
4h

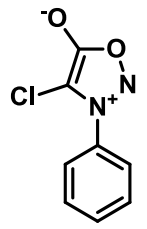

ln([A]/[B])/([A] – [B]), M-1
time, sec
y = 0.2886x + 9371.5
R² = 0.9945

 **4i**	
 **4j**	
 **4k**	

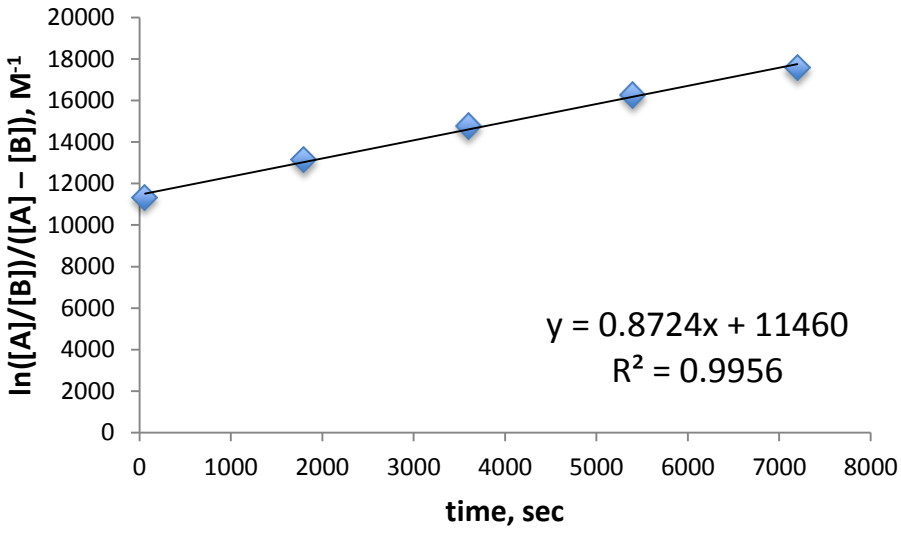


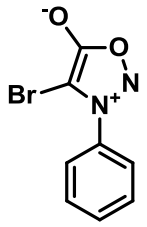
4l


ln([A]/[B])/([A] – [B]), M-1
845
840
835
830
825
820
815
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 0.003x + 820.59
R² = 0.9992

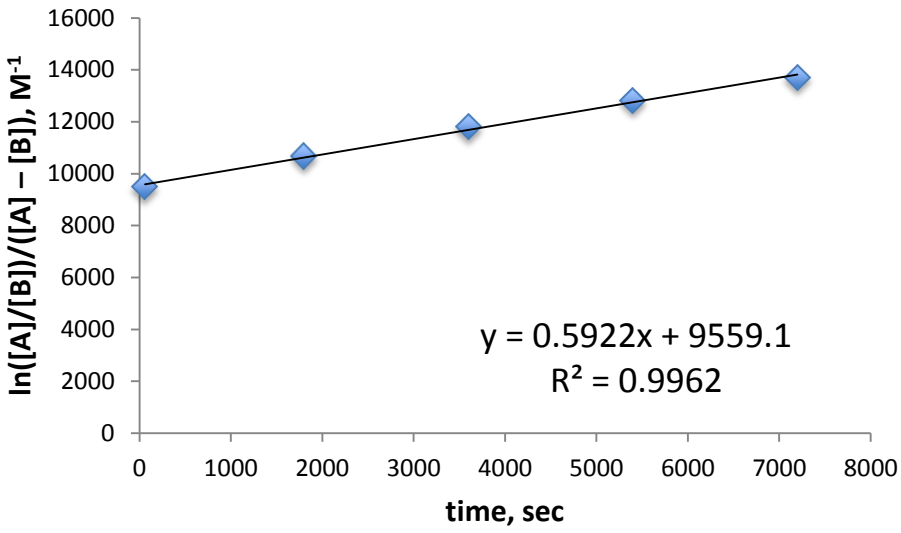


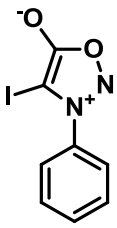
4m


ln([A]/[B])/([A] – [B]), M-1
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 0.8724x + 11460
R² = 0.9956

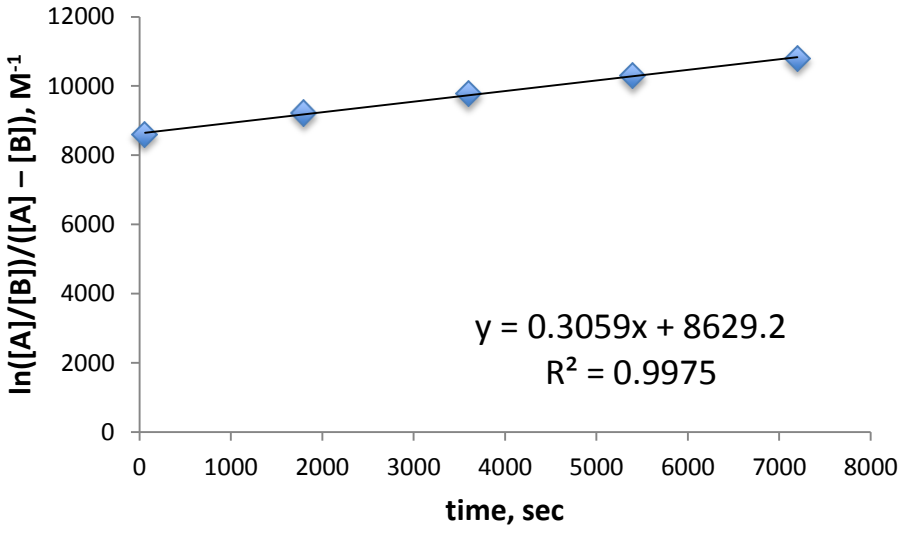


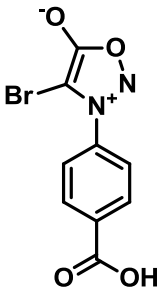
4n


ln([A]/[B])/([A] – [B]), M-1
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 0.5922x + 9559.1
R² = 0.9962

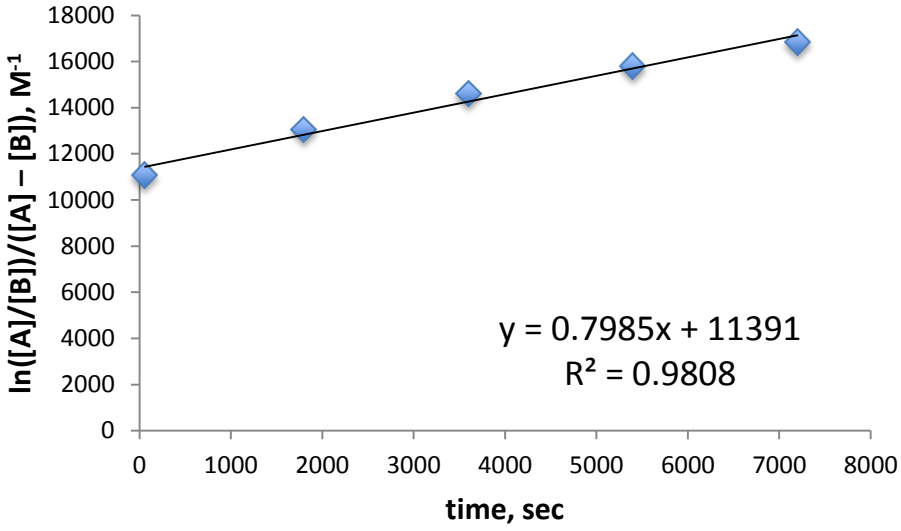


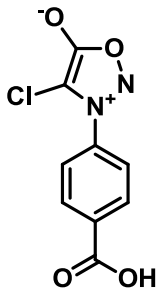
4o


ln([A]/[B])/([A] – [B]), M-1
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 0.3059x + 8629.2
R² = 0.9975

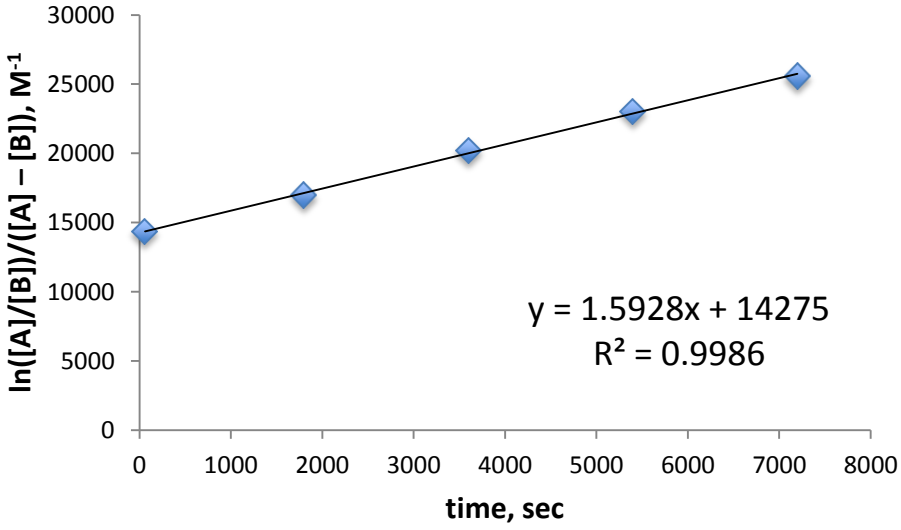


4p


ln([A]/[B])/([A] – [B]), M-1
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 0.7985x + 11391
R² = 0.9808



4q


ln([A]/[B])/([A] – [B]), M-1
30000
25000
20000
15000
10000
5000
0
0
1000
2000
3000
4000
5000
6000
7000
8000
time, sec
y = 1.5928x + 14275
R² = 0.9986

• Competitive reaction between chlorosydnone 4q and azide 1b with BCN followed by NMR

To a solution of sydnone **4q** (237 µL, 90.0 µmol, 1 eq) in CD_3OD were successively added a solution of 1-(azidomethyl)-4-chlorobenzene (263 µL, 90.0 µmol, 1 eq) in CD_3OD and a solution of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN) (100 µL, 90.0 µmol, 1 eq) in MeOD. The reaction was monitored by ^{1}H-NMR.

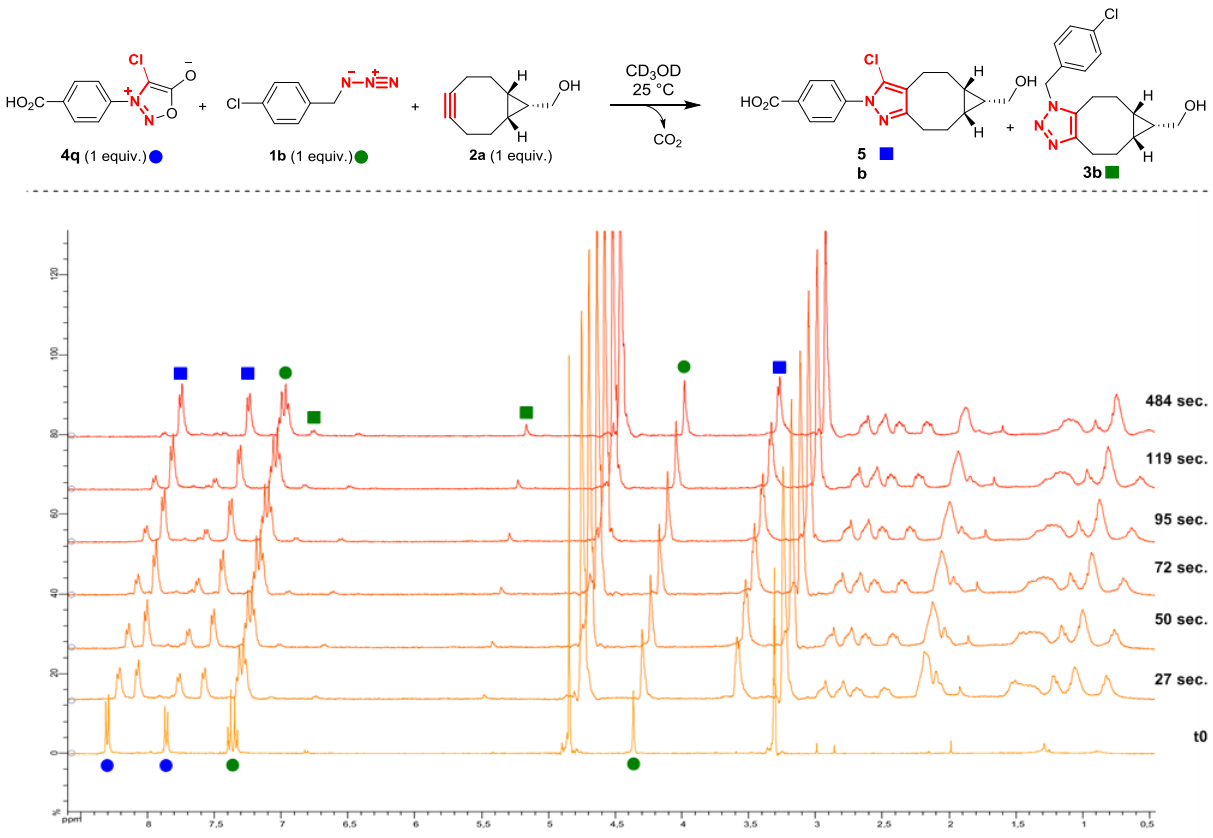
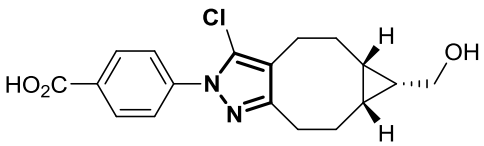


Figure S4. Competitive kinetic followed by ^{1}H-NMR.

4-[12-chloro-5-(hydroxymethyl)-10,11- diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-11-yl]benzoic acid (5b)



$C_{18}H_{19}ClN_2O_3$
MW: 346.11g.mol^{-1}
Light green solid
Yield: 63%

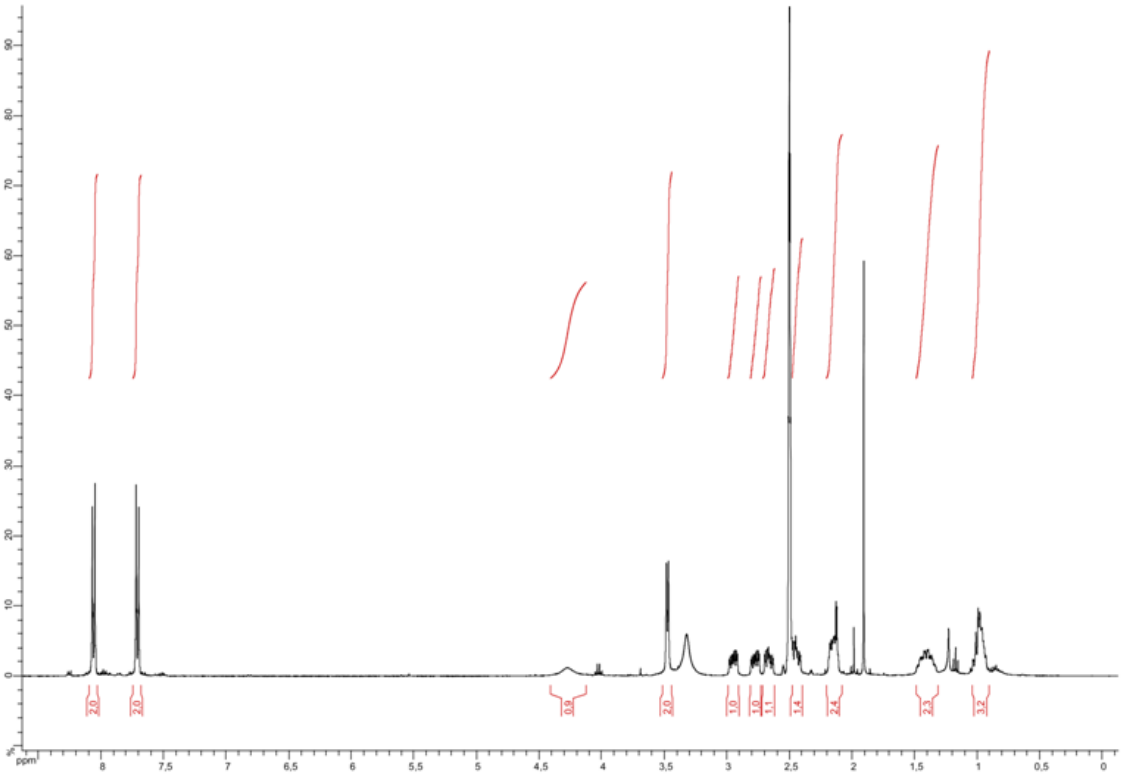
To a solution of sydnone **4q** (24.1 mg, 0.10 mmol) in methanol (1 mL) was added (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN) (15.0 mg, 0.10 mmol). The mixture was stirred 1 h at room temperature and methanol was evaporated under *vacuum*. The crude product was purified by preparative TLC (DCM/Methanol 95/5, 1% AcOH). 22.0 mg (0.06 mmol, 63%) of compound **5b** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.04 (d, *J* = 8.6 Hz, 2H), 7.69 (d, *J* = 8.6 Hz, 2H), 4.27 (br. s, 1H), 3.46 (d, *J* = 5.1 Hz, 2H), 2.99–2.88 (m, 1H), 2.81–2.71 (m, 1H), 2.70–2.60 (m, 1H), 2.48–2.38 (m, 2H), 2.18–2.08 (m, 2H), 1.48–1.30 (m, 2H), 1.05–0.89 (m, 2H).

^{13}C NMR (100 MHz, DMSO-d6, δ ppm): 166.5, 153.9, 141.4, 130.3 (2C), 129.4, 123.9, 123.6 (2C), 118.8, 57.3, 27.6, 23.0, 22.3, 22.2, 21.4, 19.7, 19.6.

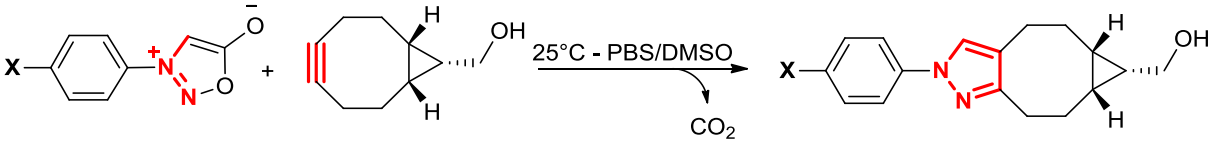
LCMS (ESI): *m/z*: 247 [M(^{35}Cl)+H]$^{+}$, 249 [M(^{37}Cl)+H]$^{+}$.

Mp.: 57–59 °C.



• Hammet plot

The σ values used in this study were from literature.[7]



X	K ($M^{-1}.sec^{-1}$)	σ	$Log.K_{(X)}/K_{(H)}$
H	0,027	1	
CH_3	0,032	-0,17	0,07378621
OCH_3	0,006	-0,27	-0,65321251
CO_2H	0,059	0,45	0,33948825
NO_2	0,289	0,78	1,02953408
CF_3	0,199	0,54	0,86748931

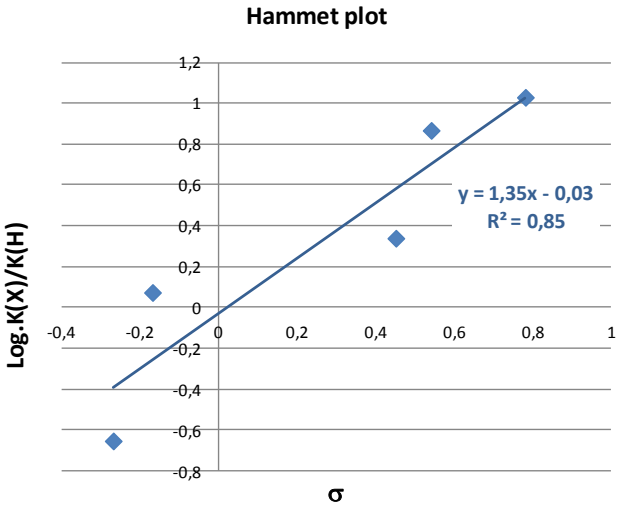


Figure S5. Hammet plot of the rate of the SPSAC reaction against σ Hammet constant.

The more electron withdrawing substituent X induces the faster cycloaddition reaction, ρ = 1.35

• Protein labelling

*Preparation of the activated ester of sydnone **4f** and **4e**:*

To a solution of *N*-hydroxysuccinimide (25 µL, 44.64 µmol, 1 eq) in DMF was added a solution of dicyclohexylcarbodiimide (25 µL, 44.64 µmol, 1 eq) in DMF and a solution of sydnone (250 µL, 44.64 µmol) in DMF. The mixture was reacted at room temperature during 1 h and was directly used for the conjugation with BSA protein.

Preparation of BSA-Sydnone conjugates:

A solution of BSA (250 µL, 72 nmol) in phosphate buffer (100 mM, pH 7.4) and 950 µL of borax buffer (0.05 M, pH 9.2) were added to the previous solution. The resulting mixture was incubated during 1 h at 37 °C and purified by molecular sieve chromatography using a 1.6 x 20 cm column packed Sephadex® G-25 medium gel (eluted with 5 mM potassium phosphate buffer pH 7.4).

29 residues of sydnone **4f** per BSA protein were conjugated according to MALDI TOF-MS analysis.

28 residues of sydnone **4q** per BSA protein were conjugated according to MALDI TOF-MS analysis.

Preparation of BSA-pyrazole conjugate:

A solution of BC*N*-POE3-NH-lissamine rhodamine B conjugate **2b** (51.2 µL, 130 nmol, 1 eq/sydnones) purchased from SynAffix, in methanol was added to a solution of the BSA-sydnone conjugate (50 µg/mL) in phosphate buffer (5 mM, pH 7.4) and reacted at room temperature. Sampling for electrophoresis and MALDI-TOF analysis were performed at 5 min, 15 min, 30 min, 1 h and 16 h.

Analysis of the coupling products:

MALDI-TOF-MS analyses were performed on a 4800 spectrometer MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA).

For the MALDI analysis of the chlorinated BSA-cycloadduct a pretreatment on a reverse-phase zip tip (OMIX® Tip C4, 10 µl, Varian) was performed to improve the signal strength.

5 min	15 min	30 min	1 h	16 h
11	16	19	20	23

Table S2. Average number of BCN conjugates linked to one BSA-SydnoneH according to MALDI TOF-MS.

5 min	15 min	30 min	1 h	16 h
24	24	24	24	24

Table S3. Average number of BCN conjugates linked to one BSA-SydnoneCl according to MALDI TOF-MS.

SDS-PAGE was performed on PhastGel™ GE Healthcare™ Gradient 10–15 gel using PhastGel™ GE Healthcare™ SDS buffer Strips. Fluorescence was visualized on Molecular Imager® VersaDoc™ MP 4000 system prior to staining with Coomassie Blue.

Results are indicated in figure S6 and S7.

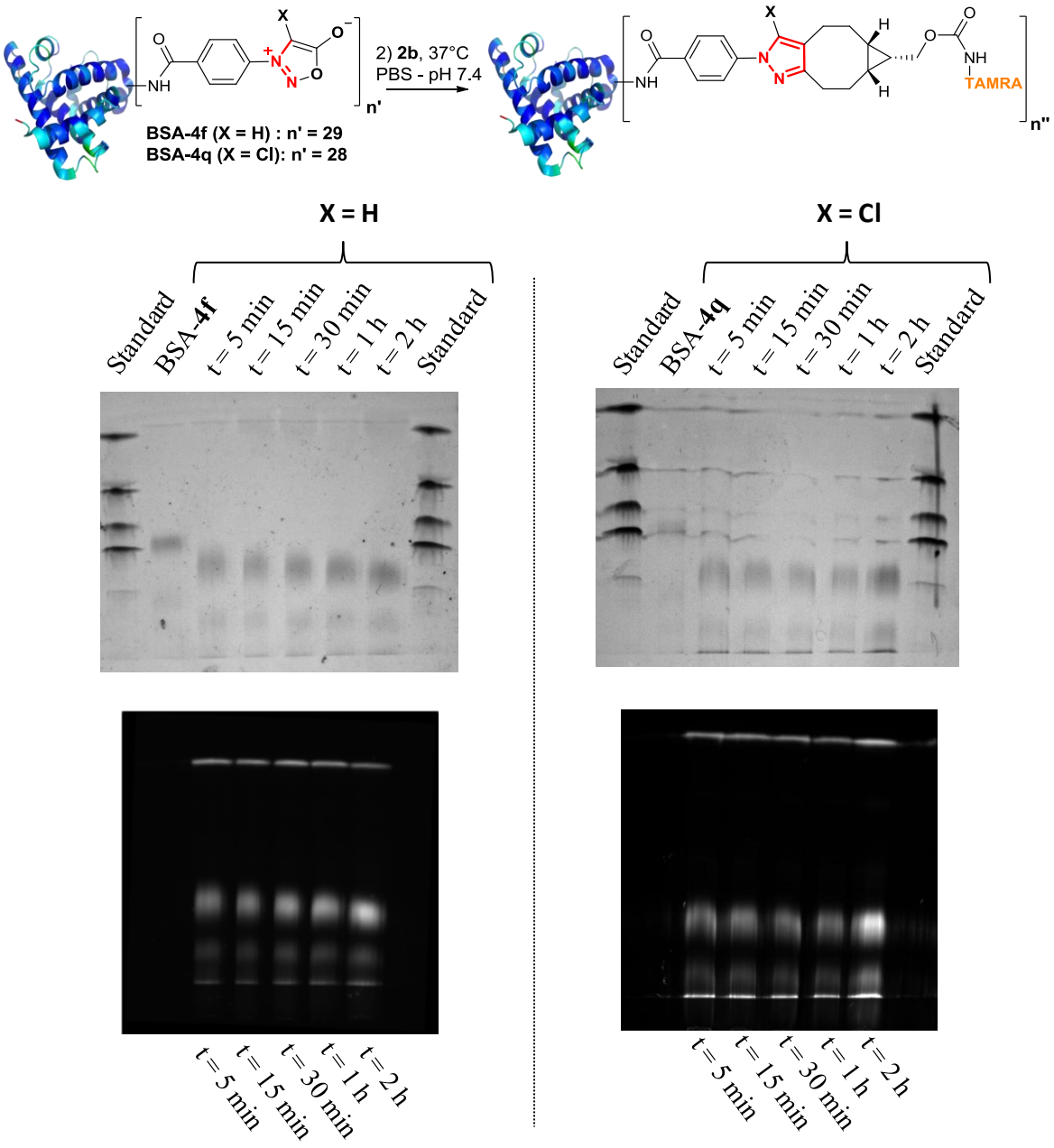
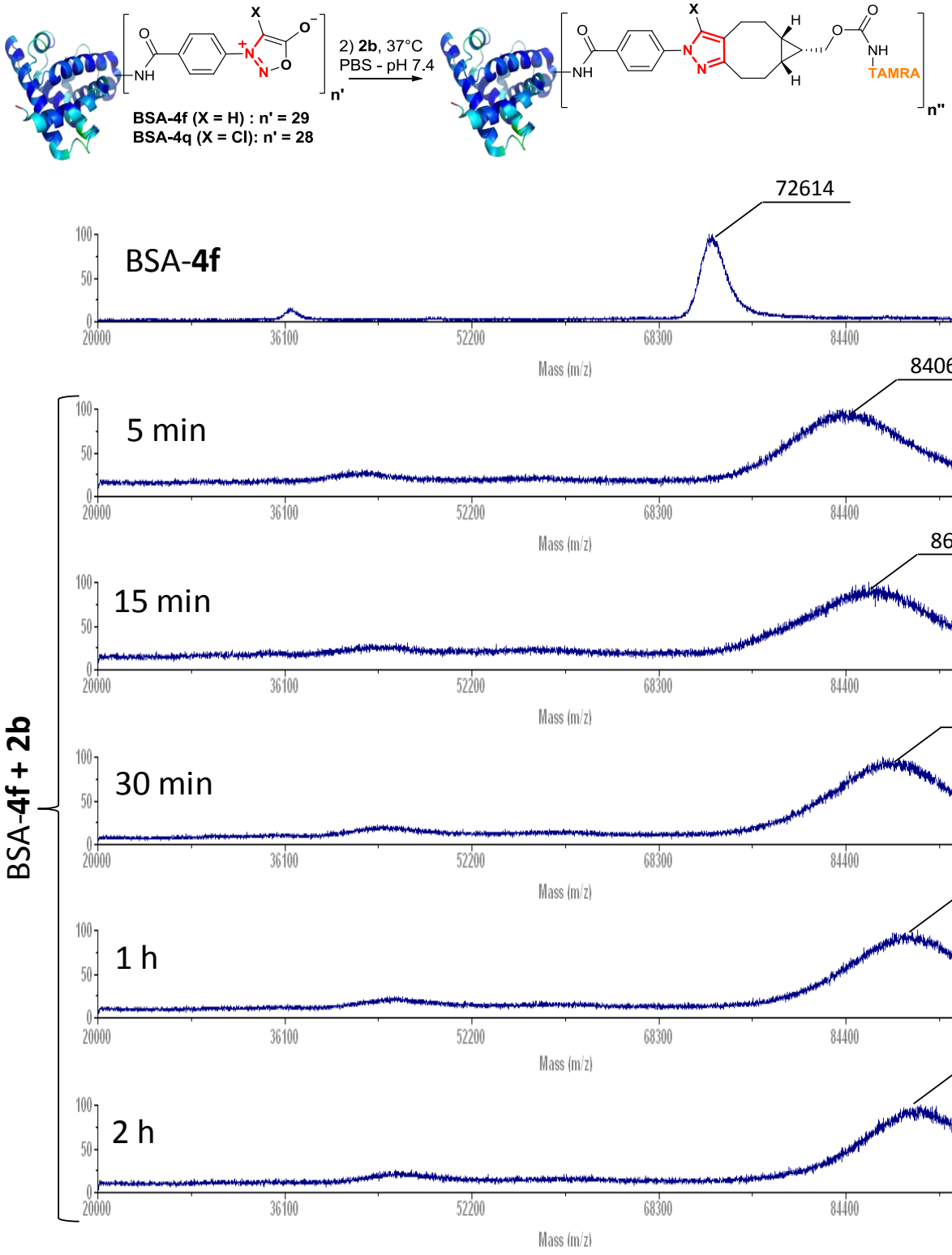


Figure S6. SDS-PAGE: the upper images show staining with Coomassie blue; the lower images show the gels visualized under ultraviolet irradiation.


2) 2b, 37°C
PBS - pH 7.4
BSA-4f (X = H) : n' = 29
BSA-4q (X = Cl): n' = 28
TAMRA
BSA-4f + 2b
BSA-4f
72614
5 min
84065
15 min
86337
30 min
87547
1 h
90560
2 h
90799
Mass (m/z)

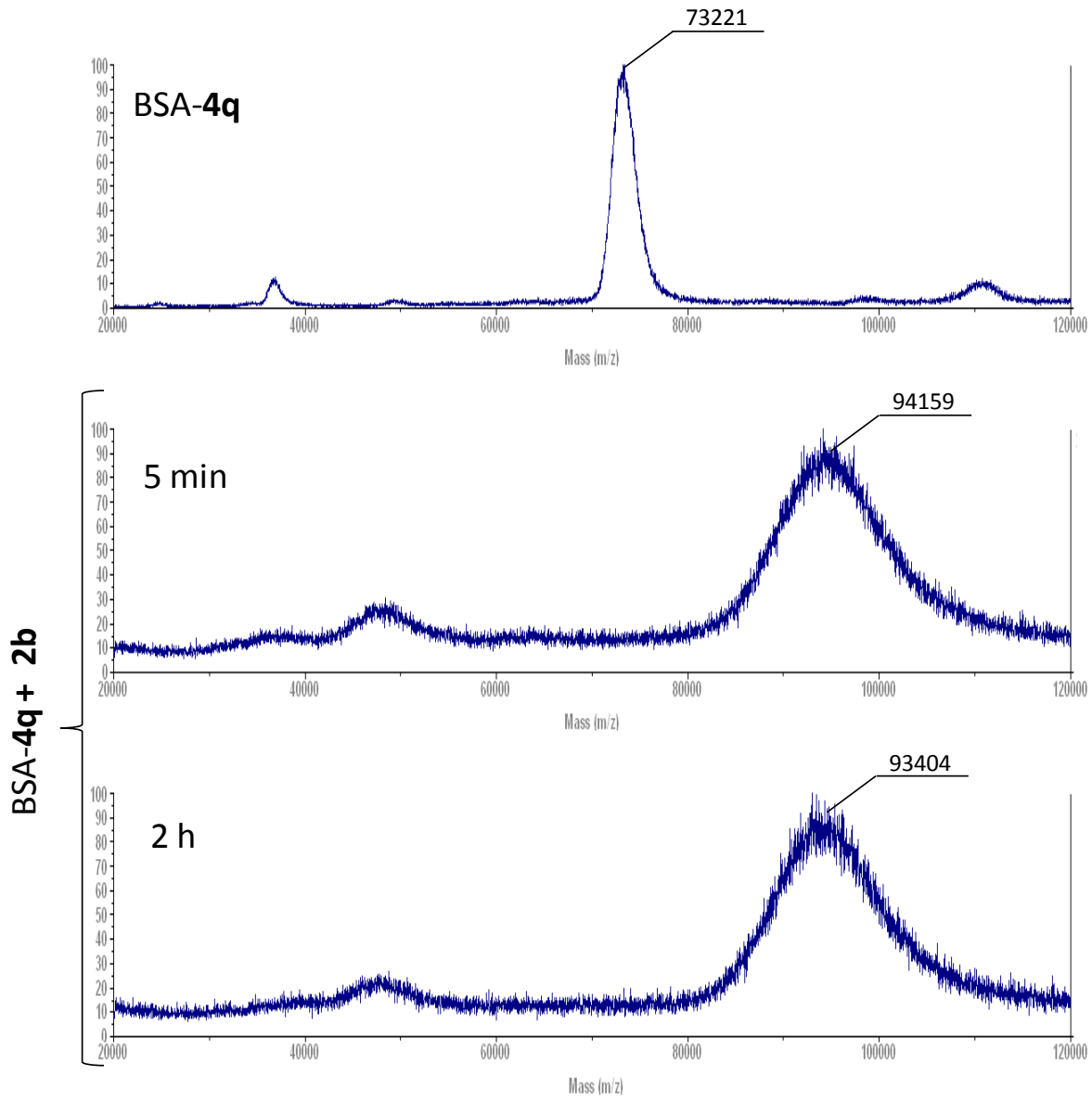


Figure S7. MALDI-TOF analyses of the labelling of the BSA-Sydnone conjugate.

References

[1] Earl, J. C.; Mackney, A. W. *J. Chem. Soc.* **1935**, 899.
[2] Kano, K.; Scarpetti, D.; Warner, J. C.; Anselme, J.-P.; Springer, J. P.; Arison, B. H. *Can. J. Chem.* **1986**, *64*, 2211–2219.
[3] Moustafa, M. A.; Gineinah, M. M.; Nasr, M. N.; Bayoumi, W. A. H. *Arch. Pharm.* **2004**, *337*, 427–433.
[4] Yang, Y.; Gong, H.; Kuang, C. *Synthesis* **2013**, *45*, 1469–1474.
[5] Foster, R. S.; Jakobi, H.; Harrity, J. P. A. *Org. Lett.* **2012**, *14*, 4858–4861.
[6] Brown, D. C.; Turnbull, K. *Synth. Commun.* **2013**, *43*, 3233–3237.
[7] C. Hansch, A. Leo, R. W. Taft, *Chem. Rev.* **1991**, *91*, 165.