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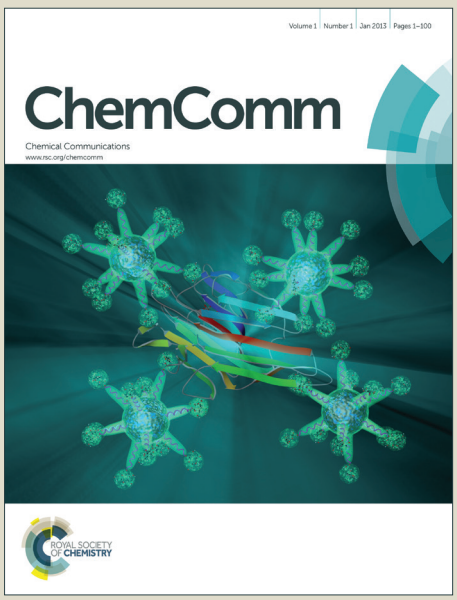

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COMMUNICATION

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

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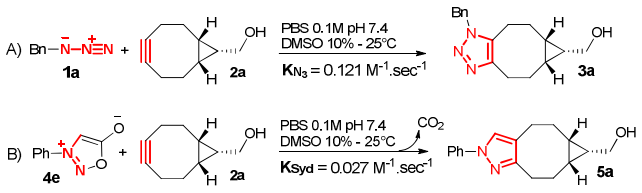
New sydnones derivatives have been synthesized and screened for their capacity to undergo fast copper-free cycloaddition reaction with bicyclo-[6.1.0]-nonyne. The influences of substitution in position *N*-3 and *C*-4 of sydnones have been particularly studied leading to the identification of highly reactive partners for bio-orthogonal ligation reactions.

Among the described bio-orthogonal reactions, the strain promoted azide-alkyne cycloaddition reaction (SPAAC) remains one of the most popular click tool for in vitro as well as in vivo ligation applications.[1] To improve the kinetics of SPAAC, efforts have been more focused on the development of new strained cycloalkynes[2] than on the discovery of new dipoles. Nitrones,[3] tetrazines[4] and nitrile oxides[5] are today the main reaction partners for cycloalkynes which can successfully replace azide compounds. Recently, our group has identified sydnones as a new interesting dipole partner for the Cu-catalyzed cycloaddition reaction with terminal alkynes.[6] Following this work, we investigated the copper-free version of this reaction. During the progress of our work, J. W. Chin et al published the strain promoted cycloaddition reaction of phenylsydnones with bicyclo-[6.1.0]-nonyne (BCN, **2a**).[7] The determined rate constant was 0.054 $M^{-1}.sec^{-1}$ which is in the same range than SPAAC therefore stimulating the pursuit of our investigations.

An interesting structural feature of sydnones is the possibility to vary the reactivity of the dipole by adding appropriate substitutions in position 3 and 4 of the mesoionic ring. Here we described our attempts to improve the strain promoted sydnone-alkyne cycloaddition (SPSAC) by exploring the influence of the structure of sydnones on the kinetics of the reaction.

Preliminary experiments indicated that, under our reaction conditions (1.5 equiv. of BCN, pH 7.4 in PBS buffer), phenylsydnone **4e** is more than 4 times less reactive than regular azides such as benzyl azide **1a** (scheme 1).



Scheme 1 Strain promoted 1, 3 - dipolar cycloaddition of benzyl azide versus phenyl sydnone with BCN. Reactions conditions: [dipoles] = 100 µM, [BCN] = 150 µM, PBS 0.1 M (pH 7.4) containing 10% DMSO. Kinetic constants were calculated from initial velocity values (yields < 10%).

We therefore synthesized a series of sydnones bearing various substituents in position 3 and 4 according to known procedures.[8] The SPSAC reactions of sydnones with BCN were carried out at room temperature in PBS containing 10% DMSO (w.w) and kinetics were followed for 2 hours by HPLC using an internal standard (Table S1). Rate constants were determined under initial rate conditions.

The results indicated in Table 1 clearly showed a strong influence of the substituents nature both in position 3 and 4 on the kinetics of the cycloaddition. Structural motifs increasing the partial positive charge at the *N*-3 atom of the sydnone ring were found beneficial to the reaction: whereas *N*-alkyl sydnones were found inactive in our reaction conditions (Table 1, entries 1 and 2), *N*-aryl sydnones bearing strong electron withdrawing groups reacted up to 10 times faster than *N*-phenyl sydnone **4e** (compared entries 6-8 with entry 5 in Table 1). Good correlation between SPSAC rate constants and the Hammet σ values was obtained (ρ = 1.35, Figure S5). The presence of a halogen in position 4 induced the strongest positive impact in the kinetics of the reaction. The increase of the sydnone reactivity toward BCN appeared correlated to the electro negativity of the halogen (Cl > Br> I, entries 14-16, Table 1). However, all other electron

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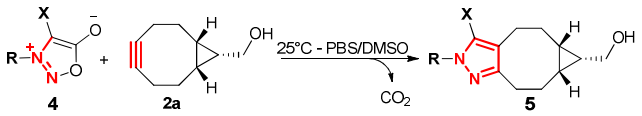
withdrawing groups tested in this study were found prejudicial to the reaction (entries 11-13, Table 1).

Table 1 Influence of the sydnone structure on the SPSAC reaction.[a]



Entry	4	R	X	K_{Syd} [b]	K_{Syd}/K_{N3} [c]
1	**4a**	CH_3	H	no reaction	-
2	**4b**	CH_2CO_2H	H	no reaction	-
3	**4c**	$pOMeC_6H_4$	H	0.006 ± 0.001	**0.05**
4	**4d**	$pMeC_6H_4$	H	0.032 ± 0.001	**0.27**
5	**4e**	C_6H_5	H	0.027 ± 0.002	**0.22**
6	**4f**	$pCO_2HC_6H_4$	H	0.059 ± 0.001	**0.49**
7	**4g**	$pCF_3C_6H_4$	H	0.199 ± 0.002	**1.64**
8	**4h**	$pNO_2C_6H_4$	H	0.289 ± 0.012	**2.29**
9	**4i**	C_6H_5	CH_3	0.018 ± 0.002	**0.14**
10	**4j**	C_6H_5	C_6H_5	0.027 ± 0.001	**0.22**
11	**4k**	C_6H_5	CF_3	0.008 ± 0.001	**0.07**
12	**4l**	C_6H_5	CN	0.003 ± 0.001	**0.02**
13	**4m**	C_6H_5	Cl	0.872 ± 0.034	**7.21**
14	**4n**	C_6H_5	Br	0.592 ± 0.021	**4.89**
15	**4o**	C_6H_5	I	0.306 ± 0.008	**2.53**
16	**4p**	$pCO_2HC_6H_4$	Br	0.798 ± 0.065	**6.60**
17	**4q**	$pCO_2HC_6H_4$	Cl	1.593 ± 0.034	**13.16**

[a] reactions were conducted with 100 µM of sydnones and 150 µM of BCN in PBS 0.1 M (pH 7.4) containing 10% DMSO; [b] $M^{-1}.sec^{-1}$; [c] rate constant ratio calculated from the value obtained with benzyl azide.

According to our results the simple presence of a Cl atom in position 4 of the sydnone induced a 30-fold increase of the SPSAC kinetics (compare entry 5 with 14 in Table 1). We then tried to combine this halogen effect with appropriate substitutions in the *N*-aryl group of sydnones. Unfortunately, chlorosydnones bearing strong electron withdrawing groups like pNO_2-phenyl in position 3 were found difficult to isolate. We however succeeded in synthesizing sydnone **4q** bearing a carboxylic group useful for further anchoring to proteins. Sydnone **4q** is a stable compound which reacts more than 10 times faster than azides (entry 18, Table 1).

To confirm this result we decided to run a competitive cycloaddition reaction by mixing equimolar amounts of BCN **2a**, azide **1b** and chlorosydnone **4q** in CD_3OD, the reaction was followed by NMR analysis. Complete reaction was observed in few minutes leading to the pyrazole cycloadduct **5b** almost quantitatively; only traces of triazole **3b** were detected (Figure 1 and S4).

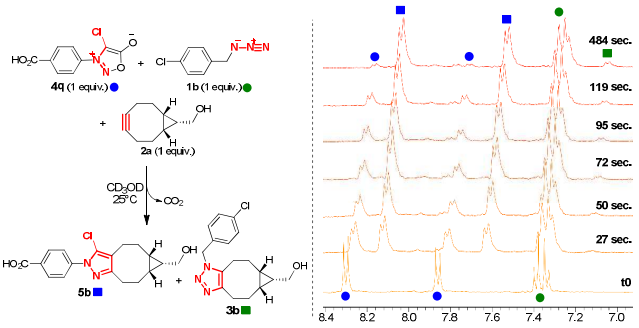


Fig. 1 ^1H NMR spectroscopic analysis of the competitive reaction between chlorosydnone **4q** and azide **1b** with BCN in CD_3OD at 25°C.

Sydnones are usually very stable mesoionic compounds, even in vivo.[9] Because the presence of a halogen in position 4 of the sydnone heterocycle might alter its reactivity towards nucleophiles, we checked the stability of sydnones **4p** and **4q.** Our results indicated perfect stability of these halogeno-sydnones and clean SPSAC reaction with BCN in biological media such as serum or in a 10 mM glutathione solution (Figures S1, S2 and S3). To demonstrate the advantage of using 4-chlorosydnones for bioconjugation, we investigated the application of this reaction for protein labeling. Sydnones **4f** and **4q** were first covalently attached to BSA (used as model protein) by standard peptide coupling procedures with an excess of reagents. Fluorescent labeling of solutions of the corresponding BSA-sydnone conjugates, used at 50 µg/mL, were then carried out with one equivalent per sydnone of the BCN-TAMRA conjugate **2b** (Figure 2).

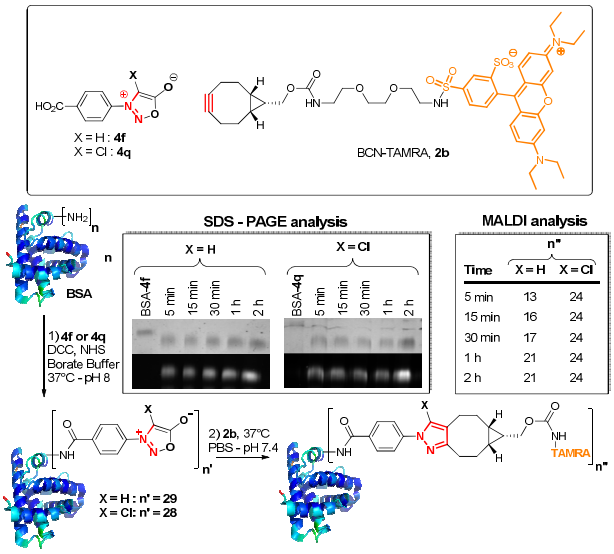


Fig. 2 Protein labelling by SPSAC. Structure of sydnones and BCN reactants.

The results obtained by SDS-PAGE and MALDI-TOF analysis confirmed the superior ability of 4-chlorosydnones to react with **2b** allowing an effective and fast labeling of the protein. Kinetic

data indicated that more than 85% of the 4-chlorosydnones on BSA were transformed into pyrazoles in less than 5 min (60% in 30 min for non-chlorinated sydnones).

In summary, we report new sydnone reactants which may represent an interesting new tool for bioorthogonal cycloadditions with strained alkynes. 4-chloro-sydnones were found to react with BCN more than 10 times faster than regular azides and more than 30 times faster than nonsubstitued sydnones. The high reactivity of chlorosydnones was applied to the fast and efficient fluorescent labeling of a model protein and should found some utility in particular for the labeling, or the sorting, of low-abundant proteins.

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Notes and references

1 See for example: (*a*) M. F. Debets, S. S. van Berkel, J. Dommerholt, A. T. J. Dirks, F. P. J. T. Rutjes, F. L. van Delft, Acc. Chem. Res. 2011, **44**, 805; (*b*) J. C. Jewett, E. M. Sletten, C. R. Bertozzi, *J. Am. Chem. Soc.* 2010, **132**, 3688; (*c*) S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* 2008, **320**, 664; (*d*) X. Ning, J. Guo, M. A. Wolfert, G-J. Boons, *Angew. Chem. Int. Ed.* 2008, **47**, 2253.

2 (*a*) M. King, R. Baati, A. Wagner, *Chem. Commun.* 2012, **48**, 9308; (*b*) J. Tummatorn, P. Batsomboon, R. J. Clark, I. V. Alabugin, G. B. Dudley, *J. Org. Chem*., 2012, 77, 2093; (*c*) M. R. Karver, R. Weissleder, S. Hilderbrand, *Angew. Chem. Int. Ed.*, 2012, **51**, 920; (*d*) G. de Almeida, E. M. Sletten, H. Nakamura, K. K. Palaniappan, C. R. Bertozzi, *Angew. Chem. Int. Ed.*, 2012, **51**, 2443; (*e*) J. C. Jewett, E. M. Sletten, C. R. Bertozzi, *J. Am. Chem. Soc.,* 2010, **132**, 3688; (*f*) J. A. Codelli, J. M. Baskin, N. J. Agard, C. R. Bertozzi, *J. Am. Chem. Soc.,* 2008, **130**, 11486; (g) E. M. Sletten, C. R. Bertozzi, *Org. Lett.,* 2008, **10**, 3097.

3 (*a*) X. Ning, R. P. Temming, J. Dommerholt, J. Guo, D. B. Ania, M. F. Debets, M. A. Wolfert, G.-J. Boons, F. L. van Delft, *Angew. Chem., Int. Ed.* 2010, **49**, 3065; (*b*) C. S. McKay, J. Moran, J. P. Pezacki, *Chem. Commun.* 2010, **46**, 931.

4 (*a*) K. Lang, L. Davis, S. Wallace, M. Mahesh, D. J. Cox, M. L. Blackman, J. M. Fox, J. W. Chin, *J. Am. Chem. Soc.* 2012, **134**, 10317; (*b*) N. K. Devaraj, S. Hilderbrand, R. Upadhyay, R. Mazitschek, R. Weissleder, *Angew. Chem. Int. Ed.* 2010, **49**, 2869.

5 (*a*) I. Singh, F. Heaney, *Chem. Commun.* 2011, **47**, 2706; (*b*) A. M. Jawalekar, E. Reubsaet, F. P. J. T. Rutjes, F. L. van Delft, *Chem. Commun.* 2011, **47**, 3198.

6 S. Kolodych, E. Rasolofonjatovo, M. Chaumontet, M-C. Nevers, C. Créminon, F. Taran, *Angew. Chem. Int. Ed.*, 2013, **52**, 12056.

7 S. Wallace, J. W. Chin, *Chem Sci.* 2014**, 5**, 1742.

8 For a review on sydnone synthesis, see: D. L. Browne, J. P. A. Harrity, *Tetrahedron*, 2010, **66**, 553. Detailed synthesis of 3- and 4-substituted sydnones and their corresponding references are indicated in SI.

9 (*a*) H. Wagner, J. B. Hill, *J. Med. Chem.* 1974, **17**, 1337; (*b*) J. B. Hill, R. E. Ray, H. Wagner, R. L. Aspinall, *J. Med. Chem.* 1975, **18**, 50.