Click Chemistry Hot Paper

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Ultrafast Click Chemistry with Fluorosydnones

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Abstract: *We report the synthesis and reactivity of 4-fluorosydnones, a unique class of mesoionic dipoles displaying exquisite reactivity towards both copper-catalyzed and strain-promoted cycloaddition reactions with alkynes. Synthetic access to these new mesoionic compounds was granted by electrophilic fluorination of σ-sydnone Pd^{II} precursors in the presence of Selectfluor. Their reactions with terminal and cyclic alkynes were found to proceed very rapidly and selectively, affording 5-fluoro-1,4-pyrazoles with bimolecular rate constants up to $10^4\,M^{-1}\,s^{-1}$, surpassing those documented in the literature with cycloalkynes. Kinetic studies were carried out to unravel the mechanism of the reaction, and the value of 4-fluorosydnones was further highlighted by successful radiolabeling with [^{18}F]Selectfluor.*

Click chemistry and bioorthogonal reactions are exquisitely suited for tracking biomolecules in their native environment, and are therefore central to advance our understanding of complex biological processes. As a result, tremendous efforts have been deployed by the scientific community to enlarge the arsenal of available bioorthogonal reactions that meet the stringent requirements of rate, selectivity, and biocompatibility.[1] The copper-catalyzed (CuAAC) and strain-promoted (SPAAC) azide–alkyne cycloaddition reactions are possibly the most commonly used click reactions for in vitro as well as in vivo ligation applications.[2] Nitrones,[3] tetrazines,[4] and diazo groups[5] are also suitable dipole partners for strained cycloalkynes, but with the exception of tetrazines (k values up to $10^3\,M^{-1}\,s^{-1}$),[6] strain-promoted cycloadditions involving cycloalkyne partners display lower second-order rate constants, which typically range from 10^{-2} to $30\,M^{-1}\,s^{-1}$. Recently, our group has identified sydnones as a new class of dipoles for Cu-catalyzed cycloaddition with terminal alkynes, leading to 1,4-pyrazoles with complete control over regioselectivity; the reaction was coined CuSAC (copper-catalyzed sydnone–alkyne cycloaddition).[7] The copper-free version of this reaction was reported by the group of J. W. Chin[8] and by J. M. Murphy and co-workers.[9] In their work, sydnones were found to react more slowly than azides with cyclic alkynes, thereby limiting applicability as a bioorthogonal ligation substrate. At the same time, we reported[10] that the presence of a halogen in the 4-position has a beneficial impact on the rate of the reaction, with the order of reactivity correlating with halogen electronegativity (Cl > Br > I). In the best case, 4-chlorosydnones were ten times more reactive than azides when reacted with bicyclo[6.1.0]nonyne (BCN, k values up to $1.6\,M^{-1}\,s^{-1}$),[10] and were found to be competent substrates for terminal alkynes under Cu catalysis.[11] With these data in mind, we aimed to develop a novel class of sydnones with the most electronegative element, fluorine, in the 4-position. We anticipated that these sydnones might serve as superior substrates for bioorthogonal ligation; moreover, the cycloaddition of 4-fluorosydnones with terminal alkynes would provide a new synthetic route to 5-fluoro-1,4-pyrazoles of medicinal and agrochemical relevance. Herein, we report the synthesis of 4-fluorosydnones, their ability to afford a large collection of 5-fluoro-1,4-pyrazoles, and their exceptional properties in the context of bioorthogonal chemistry (Scheme 1).

Although several reports have described the synthesis and use of 4-chloro-, 4-bromo-, and 4-iodosydnones,[12] no data are available concerning the preparation of 4-fluorosydnones. Preliminary studies focused on the direct electrophilic fluorination of 4-*H*-sydnones but this process proved unsuccessful.[13] The demonstration that aryl–fluorine bond formation can be induced by reductive elimination from high-valent Pd^{IV} complexes[14] led us to consider this strategy as a possible route towards 4-fluorosydnones. This approach requires the formation of stable sydnone palladium complexes **2** with a C–Pd σ bond. Inspired by this work, we synthesized complexes **2a** and **2b** by reaction of 4-bromo- and 4-iodo-sydnone **1a** and **1b** with zerovalent $Pd(dba)_2$ and triphenylphosphine at room temperature.[15] In addition, the new bipyridine σ-sydnonyl Pd complexes **2c**–**2g** were obtained upon gentle

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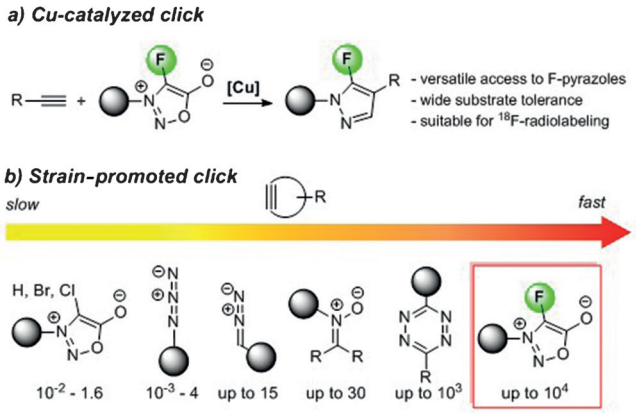
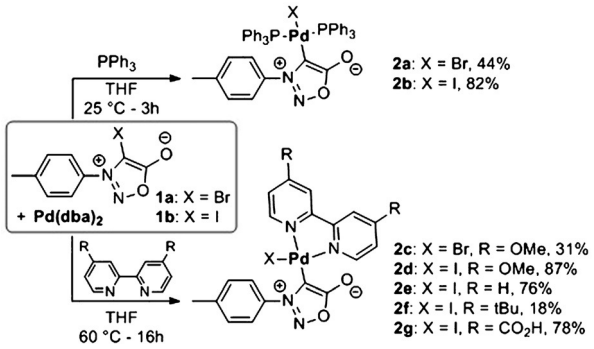


Scheme 1. Fluorosydnones as coupling partners for ultrafast click reactions with alkynes. a) CuSAC reactions for the synthesis of fluoropyrazoles. b) Reaction rates reported for cycloadditions with cycloalkynes. All rate constants are second order ($M^{-1}s^{-1}$).

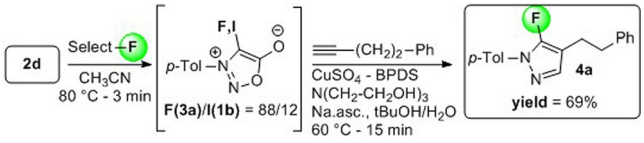


Scheme 2. Synthesis of sydnone palladium complexes **2**. dba = dibenzylideneacetone.

heating at 60 °C in THF (Scheme 2). All Pd^{II} complexes were isolated and fully characterized.[13]

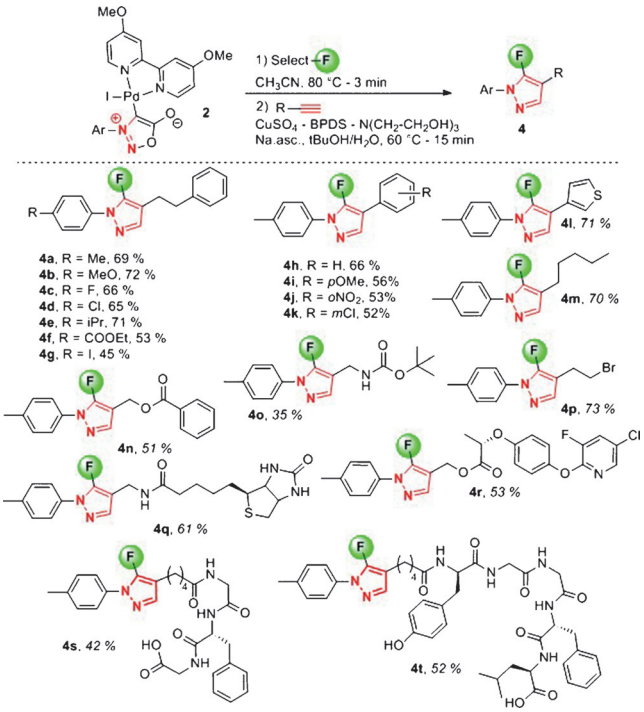
The Pd complexes **2a**–**2g** were then subjected to electrophilic fluorination. After extensive optimization,[13] Selectfluor was identified as the sole reagent capable of affording the desired 4-fluorosydnone **3a**. As anticipated, the efficiency of the fluorination process was found to be highly dependent on the structural features of the Pd complex. The phosphine complexes **2a** and **2b** did not react (see the Supporting Information, Table S1, entries 1 and 2), but selected bipyridine Pd complexes underwent successful fluorination to give **3a** (Table S1, entries 4, 5, 8, 11, and 12). Upon reductive elimination, the putatively formed high-valent $Pd^{IV}F$ species derived from **2c** led exclusively to C–Br bond formation; in contrast, C–F bond formation was observed in preference to C–I bond formation when **2d** and **2e** were subjected to fluorination (Table S1, entries 4 and 5). Complex **2d**, which features a methoxy-substituted bipyridine ligand, was the best substrate in terms of both yield and selectivity for C–F bond formation. The optimized reaction conditions thus entail reacting **2d** with 2 equiv of Selectfluor in CH_3CN at 80 °C for 3 min.

We carried out the CuSAC with the crude reaction mixture as the separation of 4-iodosydnone **1b** and 4-fluorosydnone **3a** proved challenging. Perfect chemo- and regioselectivity led exclusively to 5-fluoro-1,4-pyrazole **4a**, which was isolated in 69 % overall yield from **2d**; no traces of 5-iodo-1,4-pyrazole or other side products were detected (Scheme 3).



Scheme 3. Preparation of fluoropyrazole **4a** from Pd complex **2d**. BPDS = bathophenanthroline disulfonate, Na.asc. = sodium ascorbate.

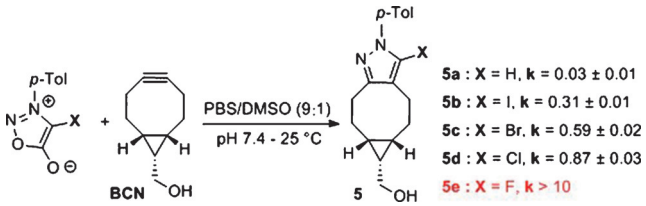
Encouraged by this result, a series of Pd complexes were prepared from both electron-rich and electron-poor 3-aryl-4-iodosydnones.[13] These sydnone Pd^{II} precursors were subjected to the two-step fluorination–CuSAC procedure described in Scheme 3. The results demonstrate the generality of the method; the procedure showed high functional-group tolerance, and the reactions proceeded in less than 15 min (Scheme 4). 5-Fluoro-1,4-pyrazoles are important synthetic



Scheme 4. Scope of the two-step reaction leading to 5-fluoro-1,4-pyrazoles.

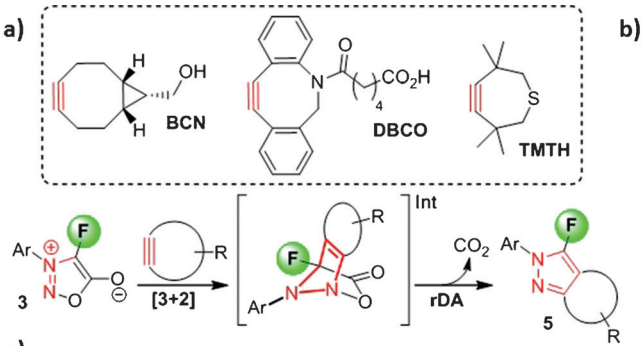
targets and building blocks for pharmaceutical research and drug development,[16] but their synthesis remains challenging and suffers from significant limitations, such as low scope, low yields, and lack of control over regioselectivity.[17] Our approach represents a general and efficient route to these fluorinated heterocycles.

Having established that 4-fluorosydnones are responsive to click chemistry under Cu catalysis, we were tantalized by the prospect of developing a copper-free variant with strained cycloalkynes for potential applications in bioorthogonal chemistry. Preliminary experiments were highly encouraging; fluorination of **2d** with Selectfluor followed by rapid filtration and treatment with 1.5 equiv of BCN (10 mM) in a MeOH/H_2O mixture afforded the fluorinated cycloadduct **5e** in quantitative yield after a few seconds at room temperature. We evaluated the rate constant of the reaction by HPLC monitoring and compared the kinetic data with those obtained for other halogenated sydnones. The results confirmed the dramatic increase in the reaction rate upon introduction of the fluorine atom in the 4-position of the sydnone core (Scheme 5).



Scheme 5. Halogen effect on the strain-promoted alkyne–sydnone cycloaddition reaction. All rate constants are second order ($M^{-1}s^{-1}$).

It quickly became apparent that the rate of the reaction of 4-fluorosydnone **3a** with BCN was too high to be accurately measured by HPLC. We therefore investigated the kinetic profile of this reaction with stopped-flow techniques. The cycloaddition was monitored by absorption detection at 280 nm under pseudo-first-order conditions. Notably, two steps with opposite absorption amplitudes were clearly observed in the second time range.[13] The BCN concentration was varied, and the apparent rate constants were calculated. The pseudo-first-order rate constants k_{obs1} (s^{-1}) of the first step linearly vary with $[BCN]_{tot}$, suggesting that this step corresponds to the [3+2] cycloaddition event, which leads to a kinetic intermediate whose absorption at 280 nm experiences a hypochromic shift (loss of conjugation). The second step (hyperchromic shift at 280 nm with the restoring of the conjugation) was found to be independent of $[BCN]_{tot}$, which is in line with a retro-Diels–Alder (rDA) step concomitant with CO_2 extrusion. The bimolecular rate constant of the [3+2] cycloaddition ($k_{[3+2]}$) and the monomolecular rate constant of the rDA (k_{rDA}) step were calculated (Figure 1b). The time-dependent distribution diagrams of the species shown in Figure 1c contrast with the behavior of non-fluorinated sydnones, where the [3+2] cycloaddition step is likely to be rate-limiting.[9] In this case, the presence of the fluorine substituent facilitates the [3+2] cycloaddition, which becomes faster than the rDA step. The bimolecular rate constant $k_{[3+2]}$ for the reaction of **3a** with BCN was measured to be $42 \pm 4\ M^{-1}s^{-1}$, which indicates that 4-fluorosydnone **3a** is >1000 times more reactive than its non-fluorinated analogue and >300 times more reactive than azides. The use of more strained DBCO led to a significant rate enhancement particularly towards the [3+2] step ($900 \pm 300\ M^{-1}s^{-1}$) and, to a lesser extent, towards the rDA step. As expected, with the highly strained seven-member-ring sulfur-containing cycloalkyne TMTH,[18] both the [3+2] ($1500\ M^{-1}s^{-1}$) and the rDA ($0.98\ s^{-1}$) steps were accelerated. In line with our previous observations,[10] electron-withdrawing groups on the aryl unit of the sydnone partner (CF_3 for **3c**, F for **3b**, CH_3 for **3a**) increased the reactivity further, affecting the [3+2] step only;



b)

3	Ar	Alkyne	5	$k_{[3+2]}$ ($M^{-1}s^{-1}$)	k_{RDA} (s^{-1})
3a	pCH_3-C_6H_4	BCN	5e	42 ± 4	0.02 ± 0.01
3a	pCH_3-C_6H_4	DBCO	5f	900 ± 300	0.06 ± 0.01
3a	pCH_3-C_6H_4	TMTH	5g	1500 ± 300	0.98 ± 0.06
3b	pF-C_6H_4	TMTH	5h	3500 ± 1100	0.90 ± 0.10
3c	pCF_3-C_6H_4	TMTH	5i	12000 ± 2000	0.99 ± 0.02

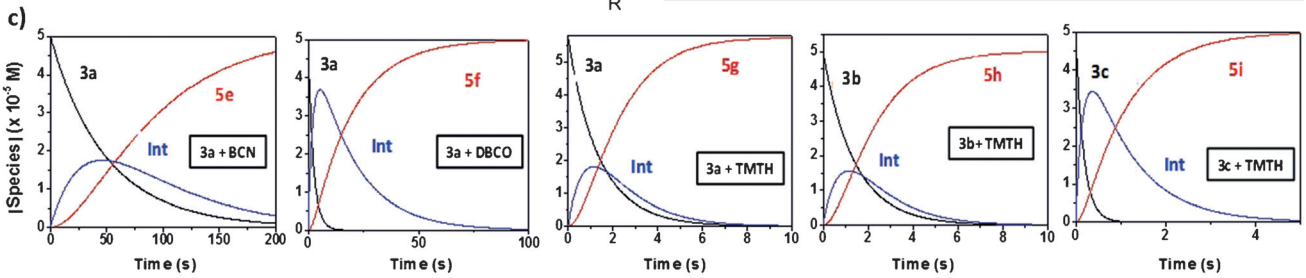
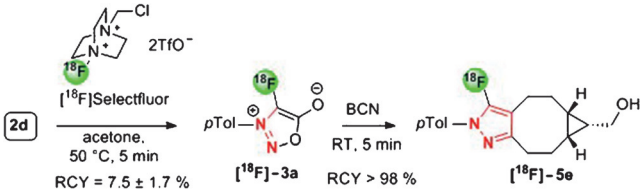


Figure 1. Kinetic studies. Experiments were carried out at 25 °C in PBS buffer containing 30% DMSO, sydnone (50 μM), and cycloalkyne (0.5–5 mM). a) Structures of the cycloalkynes used in this study and the reaction under investigation. b) Calculated rate constants. c) Time-dependent distribution diagrams of relevant compounds for [sydnone] = 50 μM and [cycloalkyne] = 0.5 mM. DBCO = dibenzocyclooctyne, TMTH = tetramethylthiacycloheptyne.

a sequential increase in $k_{[3+2]}$ from $1500\,M^{-1}s^{-1}$ (**3a**) to $3500\,M^{-1}s^{-1}$ (**3b**) and $12000\,M^{-1}s^{-1}$ (**3c**) was observed, while k_{rDA} remained constant (ca. 0.9–1.0 s^{-1}). Fluorosydnone **3c** thus reacts with TMTH with a rate constant greater than $10^4\,M^{-1}s^{-1}$, which represents, to the best of our knowledge, the fastest reaction involving a strained cycloalkyne.

Strain-promoted click reactions offer the opportunity for ^{18}F radiolabeling and have encouraged the development of a range of click radiochemical reactions with azides and tetrazines for ^{18}F incorporation into peptides and other biomolecules.[19] Encouraged by these results, we next explored the use of 4-fluorosydnones as a prosthetic group for ^{18}F incorporation. The radiosynthesis of [^{18}F]-**3a** was successfully carried out by direct ^{18}F fluorination of **2d** with [^{18}F]Selectfluor bis(triflate), a reagent prepared according to our previously described protocol.[20] Gratifyingly, the addition of a freshly prepared solution of electrophilic [^{18}F]Selectfluor bis(triflate) to Pd^{II} complex **2d** led to the formation of [^{18}F]-**3a** in $7.5 \pm 1.7\%$ radiochemical yield (RCY). The crude mixture was added to BCN and stirred at room temperature. Clean and complete conversion into the desired cycloadduct [^{18}F]-**5e** was observed after 5 min (Scheme 6).[13] This proof of concept highlights the possibility of using 4-fluorosydnone as a novel prosthetic group for the preparation of ^{18}F radiotracers.



Scheme 6. Preparation and use of [^{18}F]sydnone **3a**.

In conclusion, this study has provided a wealth of information on the reactivity of a new class of mesoionic dipoles, 4-fluorosydnones. These dipoles are within reach by C–F bond-forming reductive elimination from bipyridine-ligated Pd^{II} complexes and Selectfluor. Fluorosydnones are exceptionally reactive dipoles that are converted into a range of 5-fluoro-1,4-pyrazoles upon cycloaddition with alkynes under Cu catalysis. These reactions benefit from total control over regioselectivity, offering a useful synthetic route to fluorine-containing heterocycles that are of importance in medicinal and agrochemical chemistry. One striking feature of 4-fluorosydnones is their ability to undergo cycloaddition with strained cycloalkynes with reaction rates surpassing those documented in the literature for these dipolarophiles. Kinetic studies enabled the determination of the rate constants for the two individual, sequential steps of this reaction (cycloaddition, retro-Diels–Alder reaction). Finally, we have established that ^{18}F-labeled fluorosydnones are emerging as potential prosthetics for the ^{18}F labeling of (bio)molecules under remarkably mild reaction conditions.

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