Nitrones as dipoles for rapid strain-promoted 1,3-dipolar cycloadditions with cyclooctynes†

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Received (in College Park, MD, USA) 15th October 2009, Accepted 8th December 2009
First published as an Advance Article on the web 23rd December 2009
DOI: 10.1039/b921630h

Strain-promoted cycloadditions of nitrones with cyclooctynes (k_2 = 1.5 M^{-1} s^{-1} at 25 °C) are up to 25 times more rapid than comparable reactions of azides.

An emerging field in organic reaction methodology focuses on the development of chemical transformations suitable for applications in chemical biology.[1] Despite intense interest, few transformations display the appropriate selectivity, kinetics and biocompatibility required for tracking biological processes in living systems. While approaches based on the Staudinger ligation,[2] the Diels–Alder reaction,[3] various nucleophilic additions,[4] thiol-based reactions[5] and cross-metathesis[6] have been reported, most efforts have converged on [3 + 2] dipolar cycloadditions of azides with alkynes, which are rapid when promoted by potentially toxic[7] copper species.[8] Biocompatible copper-free variants of the "click" reaction with azides have been achieved by employing strained cyclooctynes[9] and subsequent difluoro-[10] or dibenzo-[11] activation of the cyclooctyne partner (Fig. 1A), yet even these biased reactions often take several hours to reach completion at ambient temperatures. Faster rates may be achieved by employing more reactive 1,3-dipoles such as nitrile oxides[12] or photochemically generated nitrile imines,[13] but the use of more common 1,3-dipoles such as nitrones[14] has not been described.

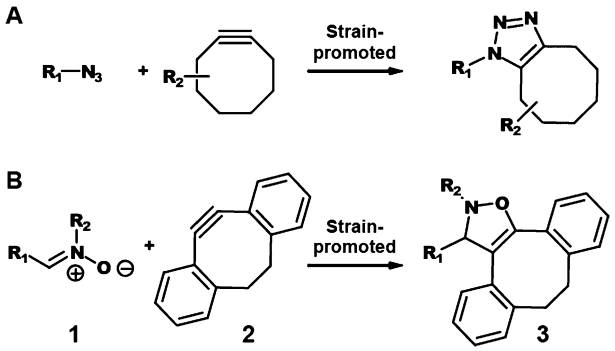

Fig. 1 (A) Strain-promoted [3 + 2] cycloadditions of azides and cyclooctynes. (B) Strain-promoted [3 + 2] cycloadditions of nitrones and cyclooctynes.

Having recently become interested in nitrones for use in Kinugasa reactions in aqueous media,[15] we speculated that these dipoles might provide faster intrinsic kinetics than azides for reactions with strained alkynes. Given the value of increasing reaction rates as a means for reducing the required concentration of labeling reagents for bioorthogonal reactions, we set out to evaluate the kinetics and generality of the nitrone cycloaddition with strained alkynes. Herein, we report that nitrones serve as rapid, selective and conveniently prepared 1,3-dipoles for cycloaddition with benzannulated cyclooctyne **2**[16] at ambient temperatures (Fig. 1B).

The nitrone scope was evaluated with respect to its conformational flexibility and electronics (Table 1).‡ Acyclic nitrones (**1a–k**) were prepared by micelle-promoted condensation of an aryl aldehyde and appropriate hydroxylamine.[17] Endocyclic nitrones **1l** and **1n** were prepared by oxidation of the amine[18] while **1m** was prepared using a straightforward one-pot reaction sequence.[19] Expectedly, acyclic aromatic nitrones bearing electron-withdrawing groups (entries 4–7) reacted more rapidly than the unsubstituted parent nitrone and those bearing electron-donating groups (entries 8–10). Second order rate constants for nitrones **1b** and **1i** were found to be 0.13 ± 0.01 M^{-1} s^{-1} and 0.088 ± 0.004 M^{-1} s^{-1}, respectively, in C_6D_6 at 25 °C (Table 2, entries 1 and 2). These rates are comparable with typical values for reactions of azides with difluorinated cyclooctyne (DIFO).[10] Encouragingly, endocyclic nitrones showed increased reactivity relative to their acyclic counterparts. Nitrone **1n**, which features benzylic activation and is contained within a rigid six-membered ring, appeared qualitatively to be the most rapid by TLC analysis and therefore warranted further quantitative study. The second order rate constant for the reaction of **1n** with **2** in C_6D_6 at 25 °C was determined to be 1.5 ± 0.1 M^{-1} s^{-1} by ^{1}H NMR (see ESI†). This additional rate acceleration most likely arises at least in part due to strain in the cyclic nitrone, making the reaction doubly strain-promoted. Put in context, this rate is about 20 times faster than the reaction of DIFO with benzyl azide[10] and approximately 25 times faster than the reaction of dibenzocyclooctyne with benzyl azide measured under our conditions (see ESI†).

To further study the reactive nature of endocyclic nitrones, the reaction of **1n** with **2** was performed in a variety of common deuterated solvents at ambient temperature and monitored by ^{1}H NMR (Table 3). At starting reaction concentrations of 100 mM, the reaction typically achieved 95% conversion to isoxazoline **3n** in less than 30 minutes, and in under 3 minutes for some solvents (entries 2 and 4).

In conclusion, the nitrone–cyclooctyne cycloaddition is well-positioned for development as a rapid metal-free, thermal

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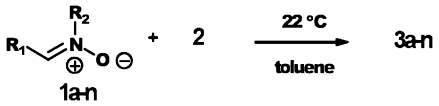
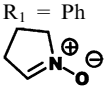
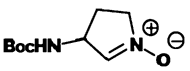
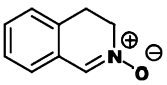
† Electronic supplementary information (ESI) available: Experimental procedures, kinetic data, spectroscopic characterization for all new compounds. See DOI: 10.1039/b921630h

Table 1 Nitrone scope and reaction times[a]



Entry	Nitrone			Product	Time/min	Yield[b] (%)
1	**1a**	R_1 = 4-$CO_2MeC_6H_4$	R_2 = Me	**3a**	44	84
2	**1b**	R_1 = Ph	R_2 = Me	**3b**	90	86
3	**1c**	R_1 = 4-$OMeC_6H_4$	R_2 = Me	**3c**	110	72
4	**1d**	R_1 = 4-$NO_2C_6H_4$	R_2 = Bn	**3d**	19	97
5	**1e**	R_1 = 4-$CO_2MeC_6H_4$	R_2 = Bn	**3e**	20	96
6	**1f**	R_1 = 4-BrC_6H_4	R_2 = Bn	**3f**	26	98
7	**1g**	R_1 = 4-CNC_6H_4	R_2 = Bn	**3g**	23	99
8	**1h**	R_1 = 4-MeC_6H_4	R_2 = Bn	**3h**	30	94
9	**1i**	R_1 = Ph	R_2 = Bn	**3i**	30	92
10	**1j**	R_1 = 4-$OMeC_6H_4$	R_2 = Bn	**3j**	62	95
11	**1k**	R_1 = Ph	R_2 = Ph	**3k**	30	96
12	**1l**			**3l**	75	97
13	**1m**			**3m**	40	>99[c]
14	**1n**			**3n**	2	>99

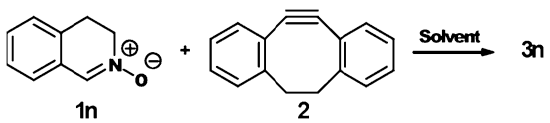
[a] Conditions: nitrone (100 mM); **2** (100 mM); 22 °C; toluene. [b] Isolated yield after column chromatography. [c] Single diastereoisomer observed.

Table 2 Second order rate constants, k_2 (M^{-1} s^{-1}), for reactions of nitrones and benzyl azide with **2**[a]

Entry	Substrate	k_2/M^{-1} s^{-1}
1	Nitrone **1b**	0.13 ± 0.01
2	Nitrone **1i**	0.088 ± 0.004
3	Nitrone **1n**	1.5 ± 0.1
4	Benzyl azide	0.062 ± 0.006

[a] Kinetic evaluation of nitrones: the appropriate substrate and **2** were pre-dissolved in C_6D_6 and mixed at equimolar concentrations of ~50 mM. Reactions were monitored by ^{1}H NMR at 25 °C and were repeated in triplicate (see ESI†).

Table 3 Solvent screen for reaction of **1n** with **2**[a]



Entry	Solvent	Reaction time for 95% conversion to **3n**/min
1	CD_3CN	8.6
2	C_6D_6	2.4
3	$CDCl_3$	33
4	d_8-THF	2.7
5	d_6-Acetone	7.7
6	d_6-DMSO	15

[a] Conditions: **1n** was added to **2** (both pre-dissolved, 100 mM, 1 : 1) in an NMR tube and monitored by ^{1}H NMR at 25 °C.

bioconjugation reaction. Endocyclic nitrone **1n**, in particular, displays fast reaction kinetics in a variety of solvents. Suitable modifications to make water-soluble reaction partners as well as the development of nitrone–alkyne bioconjugation reactions for applications in activity-based protein profiling are underway in our laboratories and will be reported in due course.

Notes and references

‡ Procedure for reaction of nitrones with **2** (Table 1). To a stirring solution of cyclooctyne **2** (10.2 mg, 0.05 mmol) in toluene (500 μL) was added nitrone (0.05 mmol). Reactions were stirred open to air and were monitored by thin layer chromatography for disappearance of the nitrone. Upon completion, the solvent was removed under reduced pressure and the crudes were purified by flash column chromatography to afford pure isoxazoline product.

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