EurJOC European Journal of Organic Chemistry

SHORT COMMUNICATION

DOI: 10.1002/ejoc.201300358

Studies of Reversible Conjugate Additions

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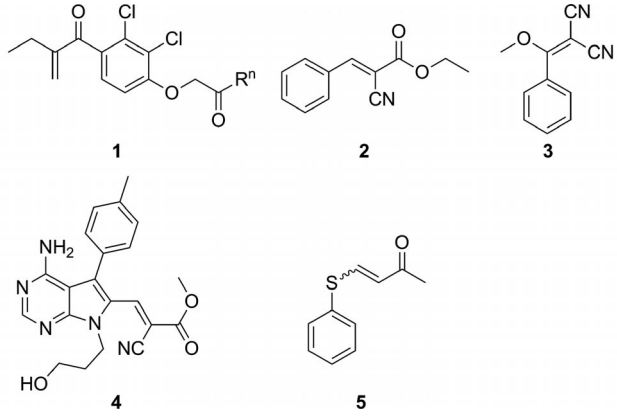
Keywords: Sulfur / Reversible reactions / Michael addition / Combinatorial chemistry

Benzalcyanoacetamides were designed and synthesized as reversible thiol conjugate addition acceptors. These thia-conjugate additions can rapidly and reversibly achieve equilibrium under aqueous conditions at neutral pH. Kinetic studies show that electron-withdrawing groups at the 4-position of the phenyl ring of the benzalcyanoacetamides promote the conjugate addition at equilibrium. Dynamic thiol exchange of these conjugate acceptors is faster than singly activated α,β-unsaturated carbonyl compounds. These thia-conjugate additions can be assembled as potentially useful components in dynamic combinatorial chemistry.

Introduction

Dynamic combinatorial chemistry (DCC) employs reversible covalent bonds to create equilibrium mixtures of interchanging components that create larger assemblies,[1] often with the goal of templating the receptors to specific guest molecules.[2] The spontaneous multicomponent assembly of large molecules with clefts and cavities avoids the effort involved in multistep synthesis. Recently, the thia-Michael addition has been used to construct dynamic combinatorial libraries (DCLs), but its utility is limited by slow exchange and other competitive reactions at physiological pH.[3] Thus, improving the kinetics of exchange could make this reaction another tool to exploit in DCC.

An example of the use of the thia-Michael addition comes from Greaney, for which he sought to conjugate glutathione.[4] The exchange of components in his conjugate acceptor reactions took minutes to hours to achieve equilibrium at pH 8.0. He used singly activated conjugate acceptors, such as common α,β-unsaturated carbonyls **1** (R^n: hydroxy group or small collection of amino groups). Interestingly, in the 1960s, conjugate acceptors derived from active methylene acceptor units were studied and found to rapidly add to thiols in an equilibrium process.[5] For example, equilibrium constants ranging from 17 to 1300 M^{-1} for thiol addition to 2-cyanoacrylates **2** were reported. Further, Bernasconi studied methoxybenzylidenemalononitrile (**3**) as a conjugate acceptor for thiolates in DMSO and found equilibrium constants ranging from 5 to 1410 M^{-1}.[6] Most recently, Taunton created covalent-modifying enzyme inhibitors for selective reaction with active-site cysteine residues by using conjugate acceptors derived from active methylene unit **4**.[7] Because thiol addition was found to occur in these three studies in an equilibrium process, it is apparent that the exchange of different thiols and conjugate acceptors should occur; yet, this was not studied. Recently, our group reported the mechanistic details of a reversible and rapid exchange of thiols with β-sulfido-α,β-unsaturated carbonyl compounds **5** in organic and aqueous media.[8] In this study, component exchange was proven. With these results in mind, we set out to verify that rapid component exchange of thiols and conjugate acceptors derived from active methylene species would occur at neutral pH, and we sought to study the substituent effects thereof. This study verifies the utility of this reaction for ultimate use in DCC.



Results and Discussion

Our work focused upon the reversible conjugate addition of thiols to benzalcyanoacetamides in CD_3CN/D_2O solutions at pD 7.0. First, we found that the addition of thiols to benzalcyanoacetamides occurred faster than we could re-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201300358.

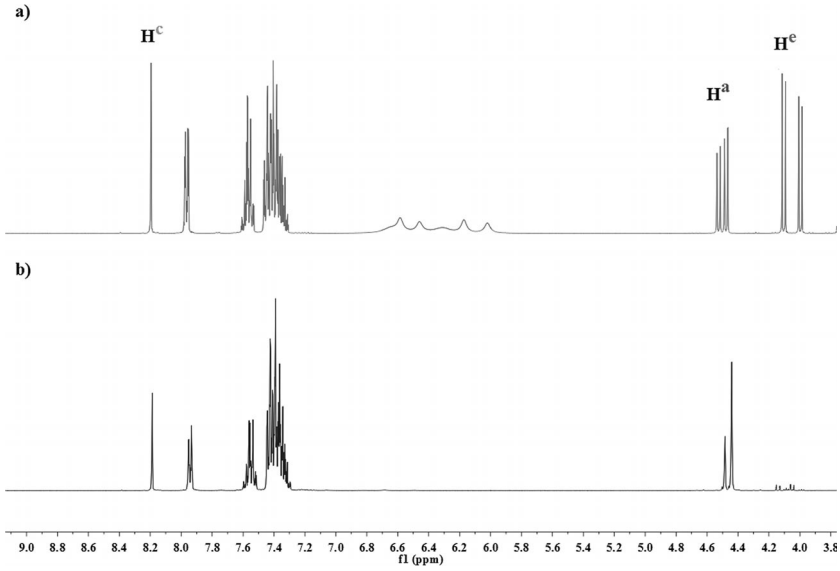
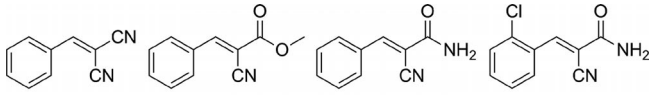


Figure 1. (a) Partial ^{1}H NMR spectrum of **8** upon the addition of 2-ME in CD_3CN (1 mL). The peaks corresponding to H^a, H^c and H^e were integrated in order to determine the equilibrium constants. (b) ^{1}H NMR spectrum 15 min after the addition of D_2O (100 μL). The α hydrogen (H^e) was deuterated by D_2O in this solvent system, so the peaks at 4.1 ppm disappeared and peaks for adjacent H^a/H^b changed to singlets.

cord a ^{1}H NMR spectrum. Further, although addition was clear in the NMR spectra, the products could not be isolated, but rather only the starting reactants were isolable. Therefore, the complexes must dissociate upon dilution during the workup procedure.

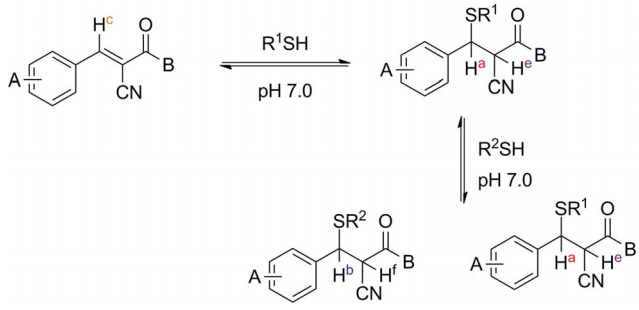
To study the conjugate addition itself and the reactivity of various conjugate acceptors, we first studied the addition of 2-mercaptoethanol (2-ME) to benzalmalononitrile (**6**), methylbenzalcyanoacetate (**7**), and benzalcyanoacetamide (**8**) by using ^{1}H NMR spectroscopy in CD_3CN. An equilibrium constant for the addition of 2-ME to **8** was measured as 161 M^{-1} through integration of the H^a and H^e resonances (Figure 1, a). Upon addition of D_2O, the α hydrogen atoms disappeared within 15 min (Figure 1, b).



6 **7** **8** **9**

However, within 30 min, the same reaction with the use of 2-ME and **6** in an acetonitrile/water (5:6) mixture led to hydrolysis to give malonitrile, which thereby limits the utility of **6**. Similarly, after 12 h, **7** also showed significant hydrolysis to methyl cyanoacrylate. However, the conjugate acceptor derived from cyanoacetamide (**8**) was stable to hydrolysis, and thus, our subsequent studies concentrated on these derivatives, although methyl cyanoacrylate was stable enough to be used in some studies.

After confirming reversible conjugate addition of a single thiol, we moved to testing component exchange in a CD_3CN/D_2O (5:6) mixture at pD 7.0. This solvent mixture proved optimal for keeping all the components soluble while maximizing the amount of water. Scheme 1 presents the basic idea in which different thiols exchange at the β position of the conjugate acceptors, and different conjugate acceptors (differing in the A and B groups) likewise exchange.



Scheme 1. Dynamic exchange of different thiols as well as differently substituted 2-cyanoacrylates.

Our first study involved the addition of 4-mercaptobenzoic acid to an equilibrated solution of 2-ME and 2-chlorobenzal cyanoacrylamide (**9**). The ^{1}H NMR spectrum taken after 0.5 h showed that the resonance of the product from the addition of 2-ME had decreased and that there was an increase in the resonance attributable to the product resulting from the addition of 4-mercaptobenzoic acid (Figure 2). We then changed the sequence of the addition of the thiols, adding 4-mercaptobenzoic acid first and 2-ME second. The ^{1}H NMR spectrum appeared identical, and this indicates that the same equilibrium mixture of products and starting thiols and conjugate acceptors results. Similarly, the two conjugate acceptors, methylbenzalcyanoacetate and benzalcyanoacetamide, were added in different sequences to 2-ME, and exactly the same ^{1}H NMR spectrum was recorded (see the Supporting Information). Equilibrium constants for the single addition of thiols are given in

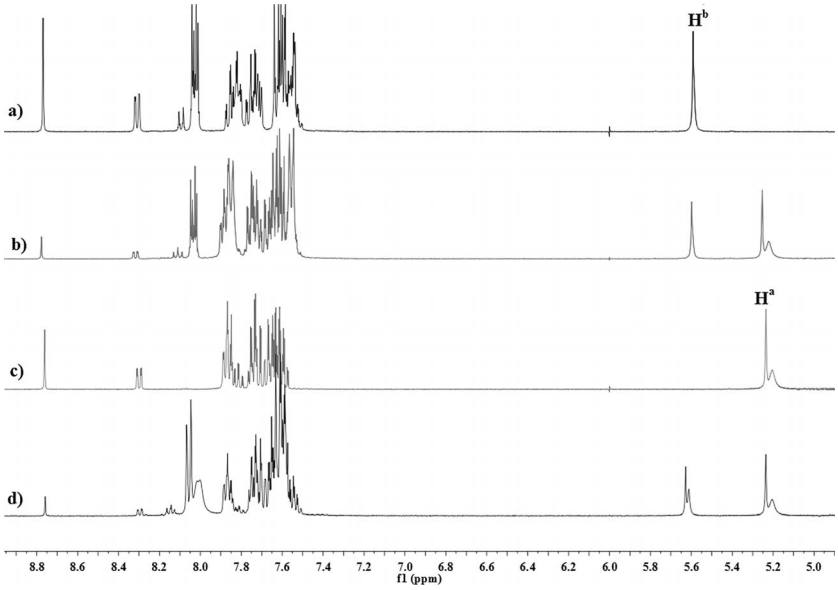
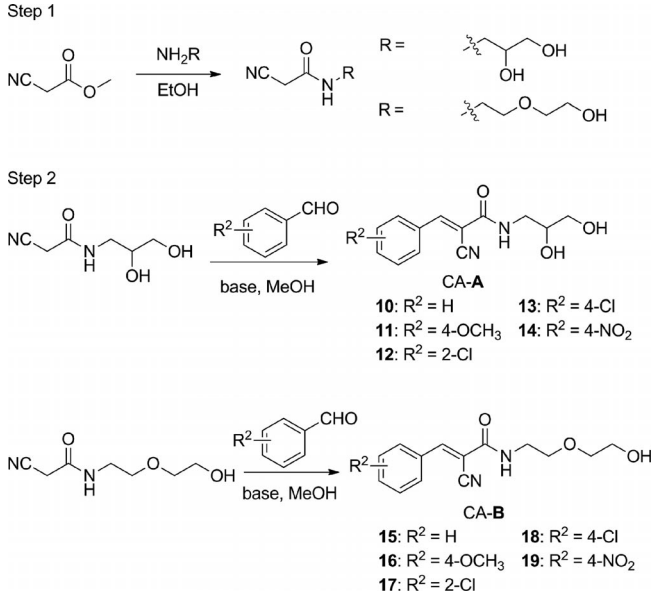


Figure 2. ^{1}H NMR spectra of reactions in CD_3CN/D_2O (5:6) in phosphate buffer, pD 7.0. (a) Partial spectrum of the reaction of **9** with 4-mercaptobenzoic acid. (b) Spectrum 30 min after the addition of 2-ME to (a). (c) Partial spectrum of the reaction of 2-ME with **9**. (d) Spectrum 30 min after the addition of 4-mercaptobenzoic acid to (c). Spectra (b) and (d) are essentially identical.

Table 1. The values suggest that electron-withdrawing substituents on the phenyl ring, such as chloride, favor the products at equilibrium.

Table 1. Equilibrium constants for the reaction of two thiols with **7**, **8**, and **9** in CD_3CN/D_2O (5:6) at pD 7.0.

Compound	K [$\times 10^{-3}$ M^{-1}]	
	2-Mercaptoethanol	4-Mercaptobenzoic acid
7	0.09	0.06
8	0.26	0.12
9	1.06	0.25



Scheme 2. Synthesis of **10**–**14** and **15**–**19**.

Because our ultimate goal was to have reactions amenable to DCC in pure water, we set out to improve the water solubility of the conjugate acceptors. In this regard, we synthesized two derivatives of benzalcyanoacetamide in which the amide nitrogen atom was derived from 2-(2-aminoethoxy)ethanol and 3-aminopropane-1,2-diol (Scheme 2).

Table 2 shows the equilibrium constants for reactions of these compounds with 2-ME in CD_3CN/D_2O (5:6) at pD 7.0, as found by integration of the peaks in the ^{1}H NMR spectra. Although our ultimate goal was to use the conjugate acceptors in pure water (these compounds are soluble in 90:10 water/acetonitrile), to compare the activities of the conjugate acceptors with 2-ME and 4-mercaptobenzoic acid we used the previous solvent system. The conjugate additions were still fast and reversible such that the products could not be isolated. The trends in Table 2 reveal that in both studies, electron-withdrawing groups on the phenyl ring increase the equilibrium constants for conjugate addition, whereas electron-donating groups decrease

Table 2. Equilibrium constants for the reactions of **10**–**14** and **15**–**19** with 2-ME in CD_3CN/D_2O (5:6) at pD 7.0 in phosphate buffer.

Compound[a]	K [$\times 10^{-3}$ M^{-1}]	Deviation [%][b]
10	0.43	3
11	0.26	0
12	0.86	8
13	0.94	8
14	3.1	6
15	0.39	3
16	0.27	1
17	0.80	8
18	0.78	3
19	2.7	6

[a] See Scheme 2 for structures of the compounds. [b] Standard deviation.

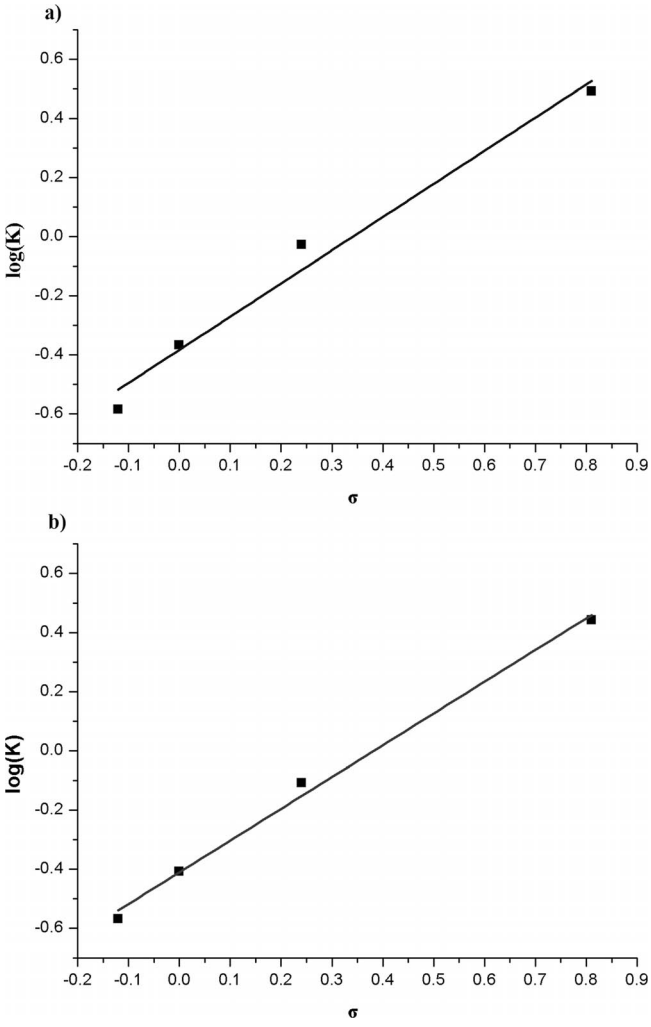


Figure 3. Hammett plot for (a) CA-**A** and (b) CA-**B**.

the values. Electron-withdrawing groups on the phenyl ring led to increased electrophilicity of the conjugate acceptor, and hence increased product formation at equilibrium. This was further revealed by examination of the Hammett plots (Figure 3). Although these plots appear to be slighted curved, the ρ values are near 1 (Table 3).

Table 3. Reaction conditions and ρ-values for the equilibrium constants taken from Table 2.

Structure[a]	ρ	K_H	r[b]
CA-**A**	1.12	414	0.97
CA-**B**	1.07	388	0.99

[a] See Scheme 2 for structures of the compounds. [b] Correlation coefficient.

After confirming that the reaction between 2-ME and the new conjugate acceptors was a rapid equilibrium process, we again set out to explore dynamic thiol and conjugate acceptor exchange. After equilibrium was reached between 2-ME and **10**–**14** and **15**–**19**, a second thiol was added. Three thiols were explored: 4-mercaptobenzoic acid, 3-mercaptopropane-1,2-diol, and *N*-acetyl-L-cysteine. For example, the ^{1}H NMR spectrum of **17** with 4-mercaptobenzoic acid was recorded 30 min after the addition of 2-ME, and it showed the product of the second thiol conjugate addition with diminished product from the first thiol addition (Figure 4). The spectra obtained were the same irrespective of the order of addition. Similar results were found for the other two thiols (see the Supporting Information). The results indicate dynamic component exchange for all the thiols studied with both conjugate acceptors.

Conclusions

Benzalcyanoacetamides rapidly and reversibly add thiols in mixtures of acetonitrile and water at neutral pH. Hammett plots reveal that electron-withdrawing groups at the 4-position of the phenyl ring facilitate conjugate addition. The dynamic nature of these conjugate addition re-

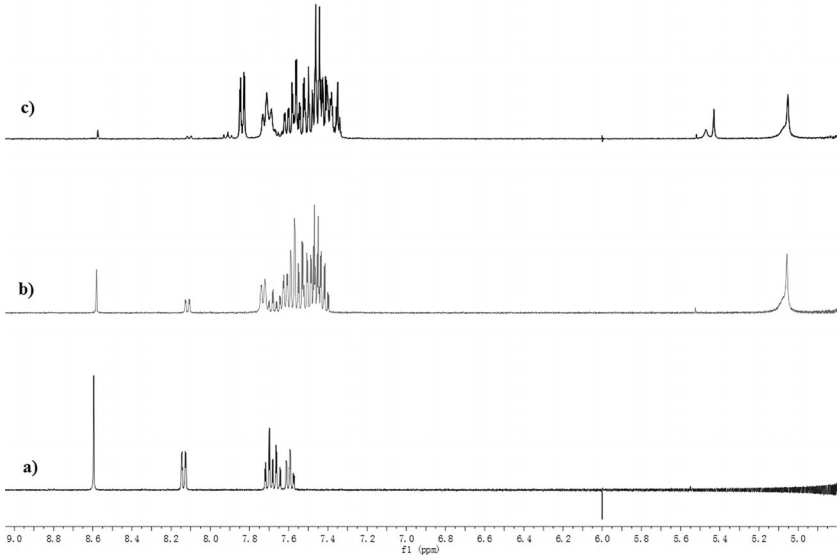


Figure 4. ^{1}H NMR spectra of dynamic thiol exchange in CD_3CN/D_2O (5:6) in phosphate buffer. (a) Partial spectrum of **17**. (b) Spectrum 15 min after the addition of 4-mercaptobenzoic acid to (a). (c) Spectrum 30 min after the addition of 2-ME to (b).

actions implies that they are amenable for use in DCC and that they are faster than thia-conjugate addition reactions to singly-activated α,β-unsaturated carbonyl compounds.

Experimental Section

General: The synthetic route to compounds **10**–**19** is displayed in Scheme 2 for a variety of derivatives. The synthesis involved two steps: First, the hydrophilic amines were allowed to react with methylcyanoacetate, followed by a classic Knoevenagel reaction involving base-induced condensation on the aldehydes.[9] Cyanoacetamides were synthesized by previously reported procedures. Details about the synthesis and characterization of all new compounds are available in the Supporting Information.

Supporting Information (see footnote on the first page of this article): Experimental details, dynamic exchange experiments, and copies of the ^{1}H NMR and ^{13}C NMR spectra for compounds **10**–**19**.

Acknowledgments

Thanks for financial support are due to the U. S. National Science Foundation (NSF) (CHE-1212971) and the Welch and China Scholarship Council. The authors thank Dr. Gururaj Joshi for helpful discussions.

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Received: March 8, 2013
Published Online: July 4, 2013

