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An Entry of the Chemoselective Sulfo-Click Reaction into the Sphere of Nucleic Acids

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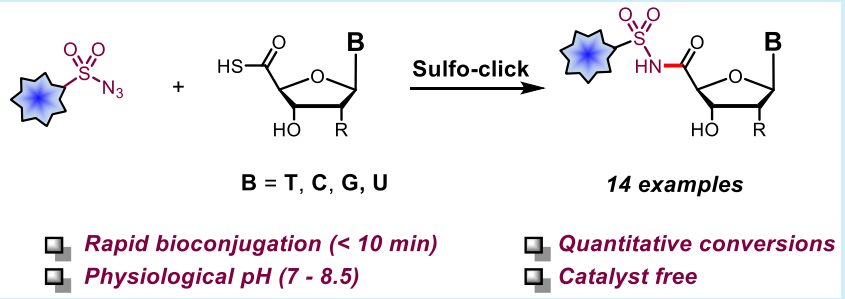

ABSTRACT: We report here the first example of a sulfo-click conjugation reaction to be applied to modified nucleosides. The reaction, which proceeds rapidly ($k \sim 2.0 \times 10^{-1}$ M^{-1} s^{-1} at 25 °C) under aqueous biocompatible conditions in the ribo- and deoxyribonucleoside series, affords the corresponding conjugated products in excellent yields. Furthermore, we demonstrate the orthogonality of the reaction with the copper-catalyzed azide−alkyne click reaction (CuAAC) by performing a one-pot dual labeling of a nucleoside carrying two orthogonal azido groups.

Site-specific bioconjugation and labeling of biomolecules to different partners (i.e., fluorophores, vectors, affinity tags, etc.) have become essential for understanding and imaging cellular processes.[1] In particular, the unique advantages of biocompatible click reactions that combine bioorthogonality, efficiency, and chemoselectivity have provided important advances for the development of new diagnostic and therapeutic applications. While many bioorthogonal ligations have been applied to proteins,[2] lipids,[3] saccharides,[4] and DNA,[5] the site-specific 5′ modification of ribo- and deoxyribonucleosides is rather limited to classical coupling reactions[6] or click-derived methodologies[7] that cannot support biocompatible conditions (Figure 1). The selective coupling of different labels to nucleosides is however important to increase their biological activity, drug resistance, or bioavailability but also for imaging purposes.[8]

The reaction between azides and thioacids also known as thioacid-azide ligation (TAL) is an emergent surrogate click reaction that upon a stepwise addition−cycloaddition−retro [3+2] cycloaddition sequence leads to an amide linkage generating elemental sulfur and dinitrogen as the sole byproducts. First described as a side reaction in 1980,[9] it was only in 2003 that Williams and co-workers highlighted its potential as an original amidation reaction in a neutral or basic medium.[10] While the use of aliphatic azides requires heating at 60 °C for several hours, it has been demonstrated that electron-deficient azides[11] readily react at rt. In 2010, in their study pertaining to the site-specific conjugation of peptides,

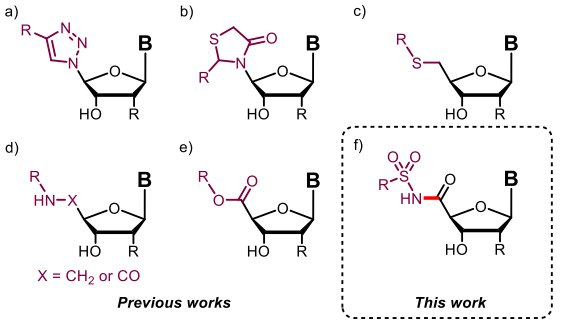

Figure 1. Known 5′ nucleoside coupling reactions.

Liskamp et al. unveiled the "sulfo-click" between a thioacid and a sulfonyl azide,[12] which was later used in various applications, including protein and carbohydrate modification by several research groups.[13] The reactions are generally complete within 10 min at room temperature in organic or aqueous media in the presence of a base. Interestingly, the sulfo-click is one of the few click reactions that can generate a native amide linkage[14] and does not require obtrusive bulky or

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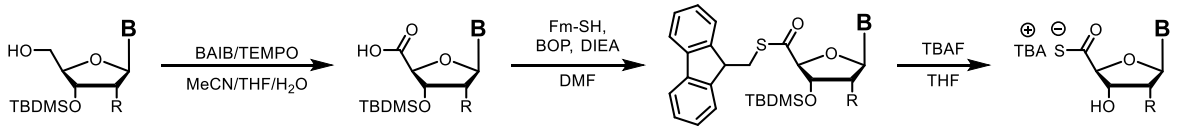
Scheme 1. Synthesis of 4′-Thioacid Nucleoside Derivatives



1a: **B** = T; R = H
1b: **B** = C^{Bz}; R = H
1c: **B** = G^{ibu}; R = H
1d: **B** =U; R = OTBDMS

2a: **B** = T; R = H, 77 %
2b: **B** = C^{Bz}; R = H, 71 %
2c: **B** = G^{ibu}; R = H, 86 %
2d: **B** =U; R = OTBDMS, 94 %

3a: **B** = T; R = H, 87 %
3b: **B** = C^{Bz}; R = H, 73 %
3c: **B** = G^{ibu}; R = H, 85 %
3d: **B** =U; R = OTBDMS, 94 %

4a: **B** = T; R = H, 70 %
4b: **B** = C^{Bz}; R = H, 59 %
4c: **B** = G^{ibu}; R = H, 93 %
4d: **B** =U; R = OH, quant.

hydrophobic reactive moieties compared to other click reactions (e.g., tetrazine, norbornene, cyclooctyne, etc.). Despite these interesting properties, the sulfo-click reaction has surprisingly never been applied to nucleic acid derivatives if we exclude the thioacid-azide ligation performed on a non-activated 3′-azido-3′-deoxythymidine with thioacetic and thiobenzoic acids at 60 °C for 36 h reported by Williams and co-workers.[10a]

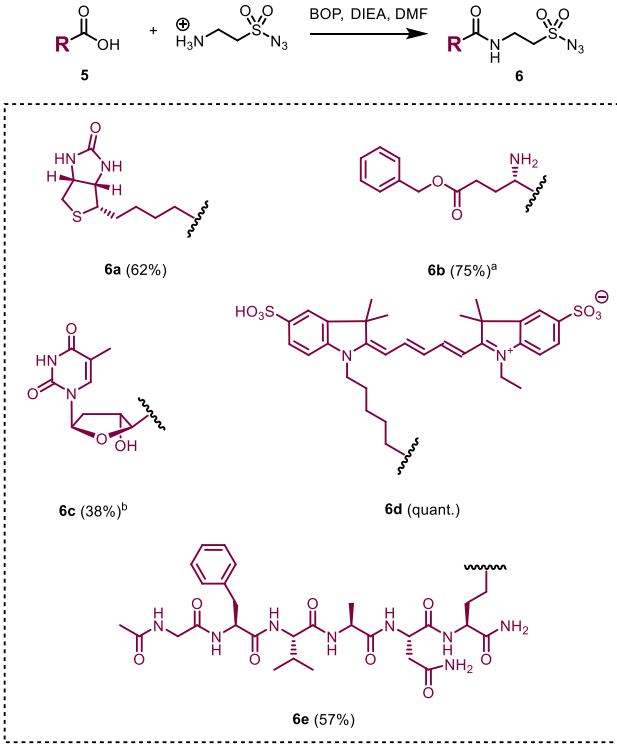
Considering our interest in the development of 5′-modified nucleoside derivatives for the formation of new internucleoside linkages[15] and the potential of the sulfo-click reaction to generate functional bioconjugates, we decided to explore its compatibility with nucleic acids by focusing on the introduction of the thioacid moiety at the 5′ position. We describe herein our endeavor and demonstrate that 4′-thioacid-modified deoxyribo- and ribonucleosides can be readily conjugated to various small molecules in water at very low concentrations.

The synthesis of 4′-thioacid nucleosides was developed through an efficient three-step sequence from 3′-*O*-TBDMS nucleosides (Scheme 1). 5′-Hydroxyl nucleosides **1a**−**d** were oxidized to carboxylic acids **2a**−**d**, respectively, using TEMPO in the presence of BAIB as a co-oxidant.[16] A coupling reaction with 9-fluorenylmethylthiol (Fm) generated corresponding thioesters **3a**−**d** in high yields. Previous works have reported the *in situ* generation of thioacids in the context of the sulfo-click reaction from base-sensitive thioesters.[13b,17] However, Fm thioesters have been shown to be stable in aqueous media at physiological pH. Therefore, we decided to generate unprotected 4′-thioacid nucleoside derivatives **4a**−**d** by treatment with TBAF in THF, leading to the desired compounds in the form of tetrabutylammonium (TBA) thiocarboxylate salts. To prevent possible hydrolysis of the thioacids under acidic[18] or basic[19] aqueous media, the thiocarboxylate nucleosides were purified as such by normal phase chromatography. It is noteworthy that all thiocarboxylate nucleoside salts were completely stable at −20 °C for months but are likely to degrade at rt.

Then, a series of sulfonyl azide partners were synthesized in one step upon classical peptide coupling conditions by using the 2-aminoethanesulfonyl azide as a short linker.[20] Different classes of labels were selected to demonstrate the versatility of the sulfo-click reaction applied to nucleoside derivatives; these include biotin **6a**, an amino acid **6b**, a modified nucleoside **6c**, a fluorophore **6d**, and a peptide **6e** (Scheme 2).

A preliminary kinetic study run with 4′-thiocarboxylate uridine **4d** and sulfonyl azide thymidine **6c** showed that the reaction reached complete conversion within 10 min at rt under aqueous conditions [10 mM sulfonyl azide in a 25:75 aqueous 0.1 M $NaHCO_3$ buffer (pH 8.5)/NMP mixture] (see Figure S2). Under the aforementioned conditions, the sulfonyl azides previously synthesized (Scheme 2) were successfully

Scheme 2. Sulfonyl Azide Compounds Synthesized



[a]Yield over two steps after *N*-Boc deprotection. [b]Yield over two steps after 3′-*O*-TBDMS deprotection.

conjugated to all thioacetates **4a**−**d** leading to various 5′-labeled nucleosides in excellent yields, including biotin, which is often coupled to biomolecules as a tool for biological applications,[21] and the hydrosoluble fluorophore Cy 5.0, used for *in vivo* imaging (Scheme 3).[22] Similarly, 5′,5′-dinucleotides bearing a non-natural *N*-acyl sulfonamide linkage were also obtained in good to excellent yields. Remarkably, the sulfo-click reaction proceeded efficiently with sulfonyl azide **6b** demonstrating the orthogonality of the reaction in the presence of a free primary amine. Ultimately, we were able

Table 1. Kinetic Studies of the Sulfo-Click Reaction[a]

concentration (μM)	half-conversion (h)	quantitative conversion (h)
1000	0.6	∼5
100	2	∼20
10	7	∼40

[a]Reactions were performed at 37 °C with 1 equiv of the Cy 5.0 sulfonyl azide derivative and 2 equiv of 4′-thiocarboxylate thymidine in a mixture of aqueous 50 mM TEAA buffer (pH 7) and NMP (50% at 1 mM, 5% at 100 μM, and 0.5% at 10 μM).

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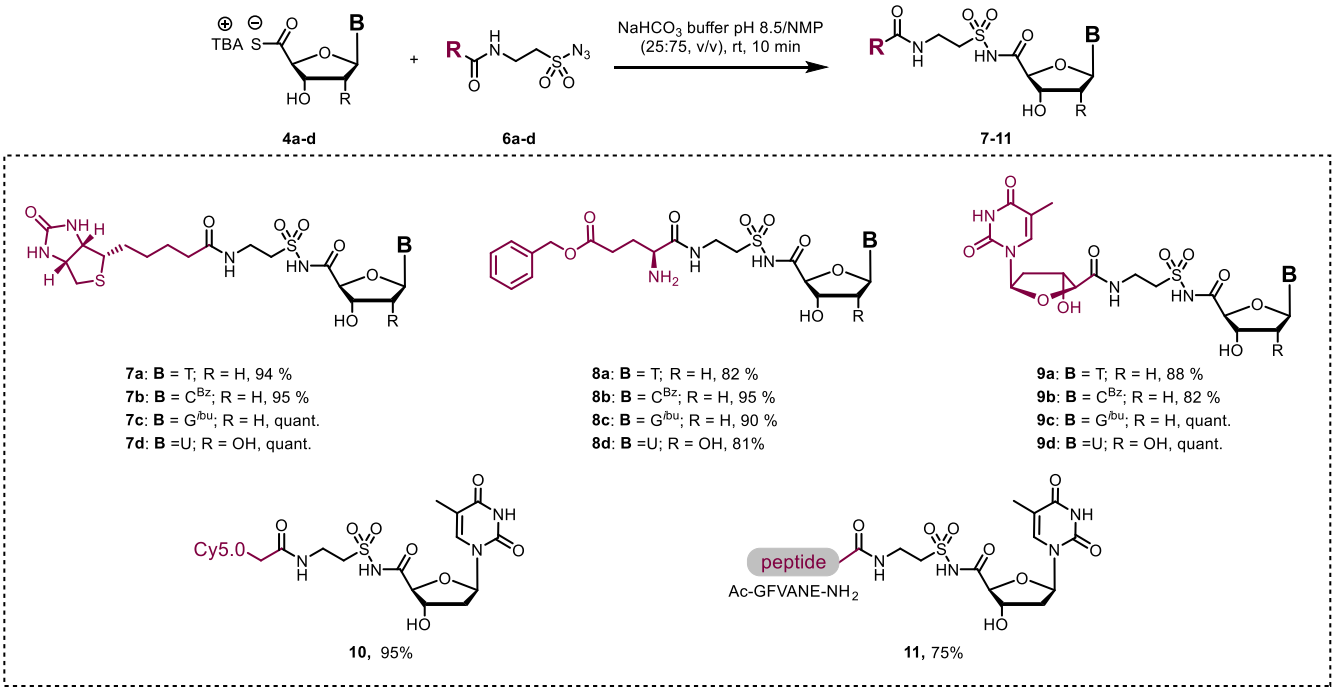
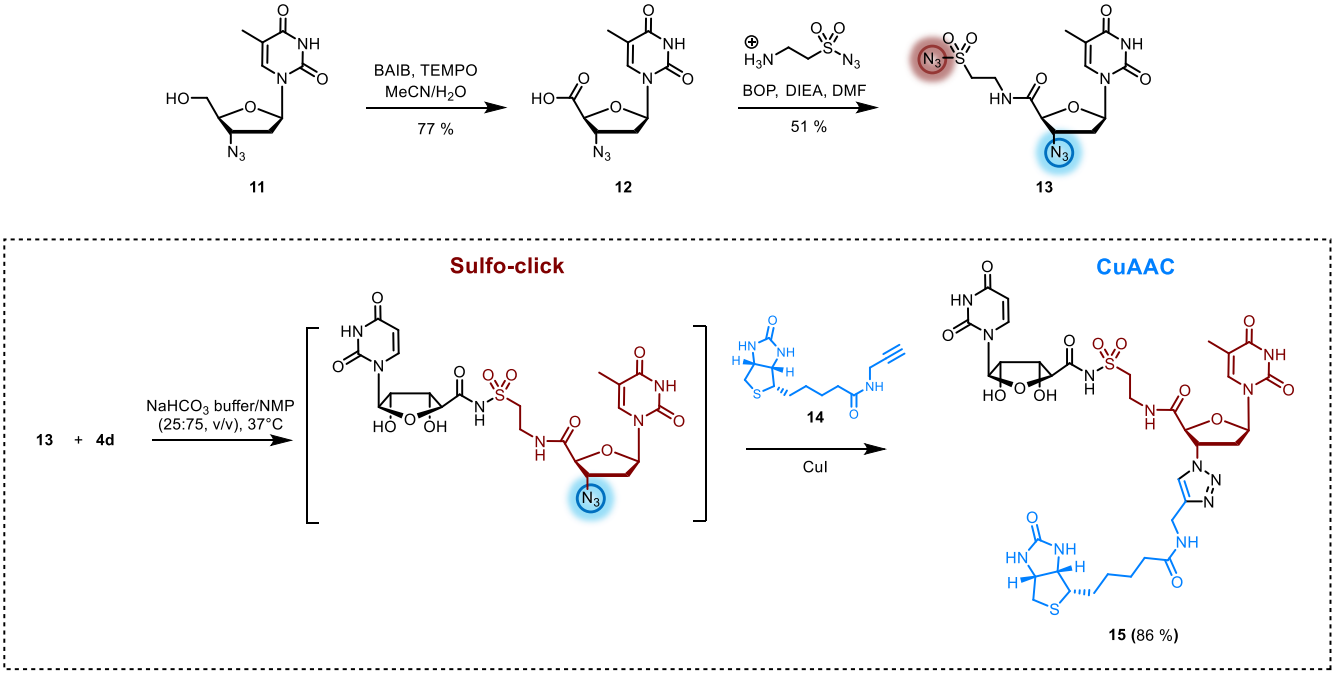
Scheme 3. Scope of the Sulfo-Click Applied to Nucleosides[a]



[a]Reactions were performed with 1 equiv of the sulfonyl azide derivative and 1.5 equiv of the 4′-thiocarboxylate nucleoside in a mixture of aqueous 0.1 M $NaHCO_3$ buffer (pH 8.5) and NMP [25:75 (v/v)].

Scheme 4. Bioorthogonality of One-Pot Double Click Conjugation



to implement the sulfo-click on hexapeptide Ac-GFVANE-NH_2, which underlined the potential of the reaction on larger molecules. All reactions were monitored by RP-HPLC analysis, which confirmed the complete conversion of the sulfonyl azide within the first 10 min. Following these results, we next evaluated the rate of the sulfo-click reaction at low concentrations by monitoring the formation of *N*-acyl sulfonamide **10** (Table 1).

The data demonstrated that the reaction still occurred rapidly even at a concentration of 1 mM, reaching completion within 5 h. Interestingly, under more dilute conditions (100 and 10 μM), the ligation still occurred albeit at a slower rate. All in all, these results demonstrated the applicability of the sulfo-click reaction at low concentrations. The longer reaction times were not detrimental as both the thioacid and the sulfonyl azide are stable in aqueous media under physiological conditions for dozens of hours. Kinetic analysis unveiled a

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second-order kinetic constant (k = 2.0 × 10^{-1} M^{-1} s^{-1} at 25 °C, and k = 2.4 × 10^{-1} M^{-1} s^{-1} at 37 °C), which is greater than that determined earlier in organic media (k = 5.7 × 10^{-3} M^{-1} s^{-1} at 21 °C)[10b] and comparable to those of the Staudinger ligation ($k \sim 10^{-4}$ to 10^{-3} M^{-1} s^{-1}) or the SPAAC ($k \sim 10^{-2}$ to 1 M^{-1} s^{-1}).[23] These comparisons are made on orders of magnitude knowing that the experimental conditions differed.

Encouraged by these results, we decided to evaluate the possibility to carry out a one-pot sulfo-click/CuAAC sequence on a substrate carrying two azido groups. On the basis of the high reactivity of the electron-deficient sulfonyl azide, we assumed a complete orthogonality of the two azido groups. We thus designed a modified nucleoside bearing both a sulfonyl azide and an aliphatic azide at the 5′ and 3′ positions, respectively. Its synthesis started with commercially available azidothymidine, which was first oxidized using the TEMPO/BAIB conditions described above, leading to carboxylic acid **12**. A coupling reaction between **12** and 2-aminoethanesulfonyl azide using the BOP reagent in the presence of DIEA allowed the formation of the corresponding diazido-thymidine **13**. The latter was then subjected to a one-pot double-click sulfo-click/CuAAC conjugation sequence (Scheme 4). First, the sulfo-click was performed at 37 °C with a slight excess of 4′-thiocarboxylate thymidine **4a** in a $NaHCO_3$ buffer (0.1 M, pH 8.5)/NMP mixture (1:4), resulting in a successful chemoselective and quantitative conjugation after 10 min [determined by RP-HPLC (see Figure S1)]. CuI, DIEA, and commercially available biotin alkyne **14** were then added leading to the quantitative formation of the desired bis-conjugate **15** after 30 min (86% isolated yield). This result demonstrated that both reactions could be run sequentially and highlighted the tunability of the azido groups for dual conjugation.

In summary, we report here the first application of the sulfo-click chemistry on nucleoside derivatives. A straightforward sequence was developed to access deoxyribo- and ribonucleosides bearing a 4′-thioacetate group, allowing the successful implementation of the sulfo-click conjugation to this particular class of biomolecules. Quantitative reactions were observed within 10 min at a concentration of 10 mM. The transformation is rapid (k = 2.0 × 10^{-1} M^{-1} s^{-1}) under biocompatible conditions even at low micromolar concentrations. Moreover, we were able to implement the method to a one-pot sequential sulfo-click/CuAAC to selectively introduce two conjugations starting from a diazido precursor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00265.

Experimental synthetic procedures and characterizations with detailed descriptions of TAL kinetic studies (PDF)

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Notes

The authors declare no competing financial interest.

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