Example of Sacrificial Unit Using Two Different Click Reactions in Coupling and Decoupling (CAD) Chemistry

Roman Bielski, Zbigniew J. Witczak and Donald Mencer

Abstract An example of a specific coupling and decoupling (CAD) chemistry is described. It takes advantage of propargyl acrylate as a sacrificial unit (SU). The addition of a selected compound representing a molecular unit equipped with an azide functionality to the terminal triple bond of the SU and another compound acting as a molecular unit equipped with a thiol functionality to the conjugated double bond of the SU proceeded at very good yields. The construct containing two molecular units can be decoupled using a few different reactions and the decoupling can take place at two positions.

A few years ago, we committed two articles [1, 2] in which we emphasized the growing importance of decoupling of connected molecular units and a need for developing novel cleaving techniques. The point is that there are many circumstances when it is necessary to disconnect two (or more) components of a larger construct. They include the need for effective methods to:

- terminate chemo or radiotherapy,
- decouple moieties that were introduced to enable the employment of specific analytical procedures,
- decouple molecular units from the surface,
- decouple the final product of the synthesis from the solid support.

While there are magnificent methods of coupling various molecular units, the number of good decoupling methodologies is rather limited. There is a clear need

R. Bielski (✉) · Z.J. Witczak (✉)
Department of Pharmaceutical Sciences, Nesbitt School of Pharmacy, Wilkes University, 84 W. South Street, Wilkes-Barre, PA 18707, USA
e-mail: bielski1@verizon.net

Z.J. Witczak
e-mail: zbigniew.witczak@wilkes.edu

D. Mencer
Department of Chemistry and Biology, Wilkes University, 84 W. South Street, Wilkes-Barre, PA 18707, USA

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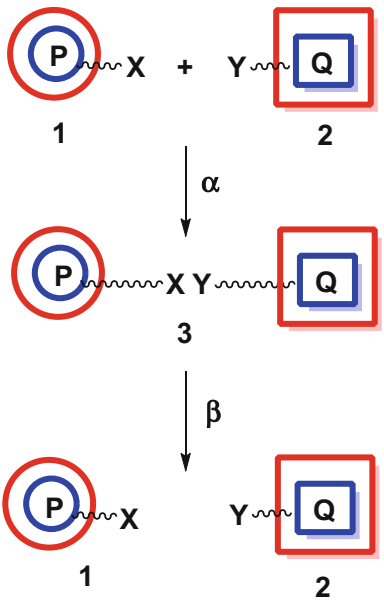
for more and better chemistries applicable to disconnecting molecular units. Very often we know in advance that at a certain point we will have to disconnect the coupled units. In such a case, we should design the whole process wisely. We proposed a general approach to such situations which we call coupling and decoupling (CAD). It seems that the CAD chemistry should become an important tool of bioorthogonal chemistry, a wonderful concept introduced several years ago by Carolyn Bertozzi [3–5].

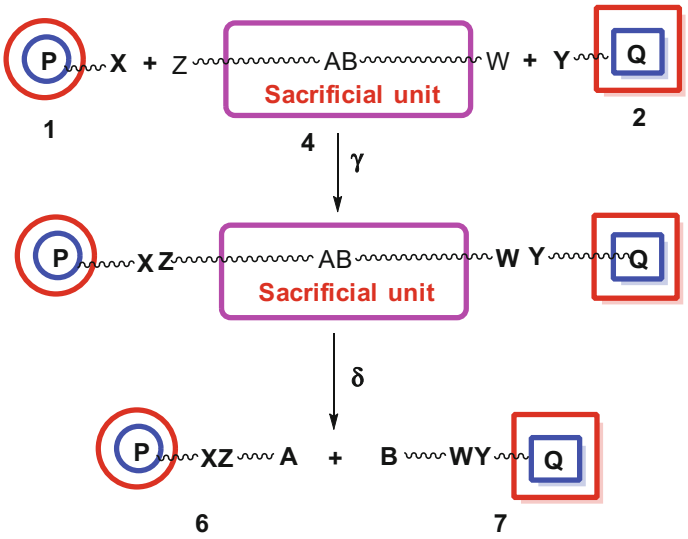
The coupling chemistry should belong to the category of click chemistry [6, 7]. The decoupling chemistry should be as simple and easy as possible and designed for a specific circumstance. For example, experiments performed in vivo and in the laboratory may require very different chemistries.

Of course, when the coupling process can be reversed at the desired moment, there is no reason not to use the "reversible" reactions. For example, if we can use the formation of ester as a coupling process and its hydrolysis as a decoupling process, we should. Unfortunately, such approach is usually impossible, particularly when the molecular units comprise of a variety of functional groups. Scheme 1 shows the use of "reversible" reactions.

Our methodology (CAD) asks for employing a multifunctional compound that we call a sacrificial unit (SU). The Scheme 2 shows the concept. The sacrificial unit **4** is equipped with two, Z and W, (different) functionalities both capable of forming click products. The two click reactions should not be the same. Additionally, somewhere within the SU (between the functionalities capable of clicking), a cleavable moiety (AB, sacrificial functionality) must be present. After connecting the clickable functionalities Z and W to molecular units (**1** and **2**), the formed construct **5** contains both units. After performing the necessary chemistry and



Scheme 1 Reversible reactions applicable to coupling and decoupling



Scheme 2 The use of sacrificial units in the CAD chemistry

accomplishing the desired goals, the molecular units are decoupled by splitting the cleavable moiety (AB).

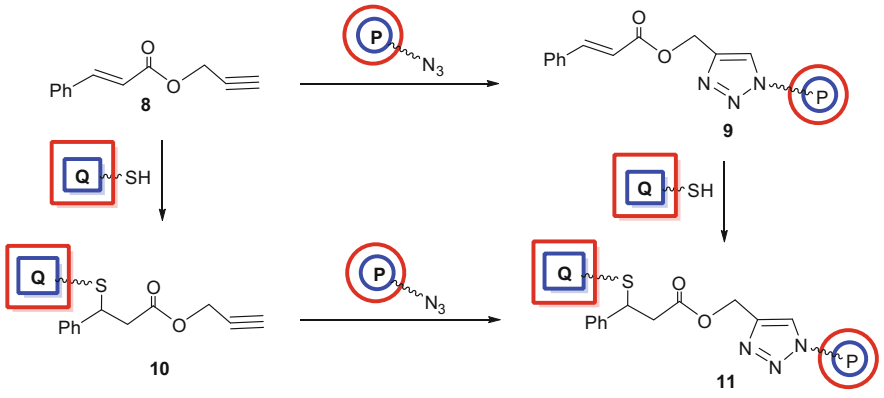
In most cases, the coupling chemistry is much less demanding because almost always it can be performed in the laboratory. The decoupling must often take place in vivo. However, many decoupling procedures can be performed in the lab as well. Then, many more processes are applicable. The only important requirement is that the molecular units or at least one of them are not degraded by the decoupling procedure.

Since the publication of our paper introducing the CAD concept, several new, effective methods and approaches to the CAD chemistry have been added to the existing repertoire. We find particularly interesting novel decoupling processes. Recently, scientists from the Granada University in Spain [8] offered wonderful examples of a well-designed CAD chemistry. They take advantage of a vinyl sulfonate-based coupling (Michael addition to vinyl sulfonate). To accomplish the decoupling, they use a nucleophilic substitution of sulfonate acting as a leaving group. Another approach to the CAD chemistry was offered by the Xavier group [9]. They use the Huisgen addition of azide to the terminal triple bond as a coupling process and very successfully hydrolyze an amide in the decoupling process. Professor Unciti-Bronceta's group from Edinburgh describes spectacular results of decoupling using metallic palladium [10]. Another very interesting methodology for decoupling has been described by scientists from the Peking University who employ kinases both in vitro and in vivo [11].

The present paper shows a possible execution of the concept of the CAD sacrificial unit. We wanted the unit to be as non-expensive, simple, easy to synthesize,

and robust as possible. We selected propargyl ester of *E*-cinnamic acid as our first target. We expected the propargyl group to be a useful functionality capable of entering a click reaction with an azide group of one of molecular units to form a cyclic triazole. Additionally, the cinnamic acyl functionality can act as an acceptor of a thiol to form a Michael adduct (Scheme 3). While it is not a perfect Michael addition acceptor, we expected ester to be sufficiently effective for our purposes. Thus, we planned to employ a (click) Huisgen reaction between the terminal triple bond of the SU and the azide group of the molecular unit and a (click) Michael addition of the mercaptan present in the other molecular unit to a double bond of the conjugated ester functionality of the SU. The thioether **11** resulting from the Michael addition should be an object of a facile hydrogenation to decouple the unit connected to the thiol from the unit connected to the azide. Alternatively, the hydrolysis or LAH reduction of the ester functionality could result in decoupling at the cinnamic ester location.

The addition of azides to propargyl groups is very well established as a classical click chemistry reaction [7]. Almost equally well established as a click chemistry process is a Michael addition of thiols to α,β-unsaturated carbonyl (or sulfonyl) compounds [12]. Utilizing cinnamic esters as Michael acceptors is less common. Primo, esters are poorer Michael acceptors than corresponding aldehydes or ketones and, secundo, cinnamates are poorer Michael acceptors than corresponding esters without the benzene ring—acrylates. Our reasoning for employing a thiol addition to cinnamic ester was based on the fact that cinnamic esters are very non-expensive, easily available and there are very many substituted derivatives of cinnamic esters. Moreover, today there is a plurality of catalysts available for the Michael addition. They include such catalysts as Lewis acids [13–15], nucleophiles and bases [12, 16], fluorapatite [17], KF-alumina [18], organocatalysts [19], and even bifunctional catalysts [20]. There are examples of successful catalytic Michael additions to cinnamic acid derivatives. A few years ago, it has been shown that in the presence



Scheme 3 The possible use of propargyl cinnamate as the sacrificial unit

of iodine even cinnamic acids give excellent yields of Michael addition with thiols and other nucleophiles [21].

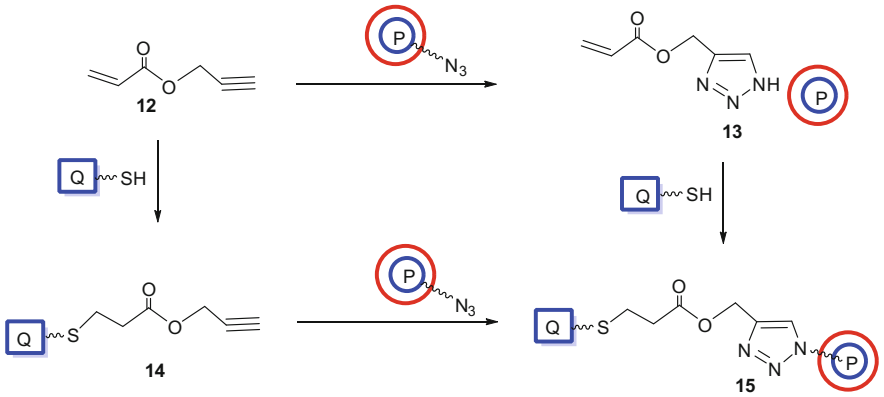
We selected a monosaccharide derivative, 1-azido-1-deoxy-2,3,4,6- tetraacetyl-β-D-glucopyranose, to act as a molecular unit equipped with the azide group. It is easily available and should be perfectly visible in the NMR spectrum both before and after the decoupling. We selected thiophenol as a moiety representing a molecular unit capable of adding to conjugated, unsaturated ester. Again, it has NMR (aromatic) signals easily distinguishable from aromatic signals of protons (and carbon atoms) of the cinnamic unit.

Thus, commercially available cinnamoyl chloride was reacted with propargyl alcohol to give the quantitative yield of expected ester (^{1}H NMR; 400 MHz, $CDCl_3$, δ 2.43 ppm, t, 1H, J = Hz, acetylenic; 4.72 ppm, d, 2H; methylene; 6.36 ppm, d, 2H, J = 3.5 Hz, 7.3 ppm, m, 3H, aromatic; 7.4 ppm, m, 2H, aromatic; 7.64 ppm, d, 2H, cinnamic). The reaction of the triple bond of propargyl ester with peracetylated glucose equipped with the azido group at C-1 proceeded smoothly in the presence of copper sulfate and ascorbic acid to give the addition product at a yield of 83% (after column chromatography). The presence of a sharp singlet at 7.85 ppm (^{1}H NMR: 400 MHz, $CDCl_3$, δ 1.80 ppm, s, 3H, Ac; 1.96, s, 3H, Ac; 2.00, s, 3H, Ac; 2.02, s, 3H, Ac; 3.95, m, 1H; 4.08, pd, 1H; 4.23, pd, 1H; 5.18, m, 1H; 5.3, m, 2H; 5.38, m, 2H; 5.83, d, 1H, C–1; 6.38, d, 1H, cinnamic; 7.3, m, 3H; 7.44, m, 2H; 7.66, d, 1H; 7.85, s, 1H, triazole) clearly indicated the formation of the cyclic triazole.

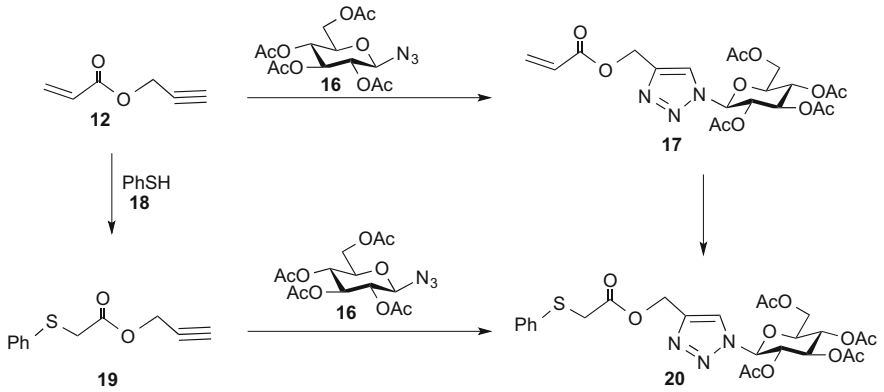
The purified cycloaddition product was reacted with various thiols (thiophenol, acetylcysteine, thiosalicylic acid, 2-thiazoline-2-thiol) in the presence of a catalyst (triethylamine {TEA}, ethyldiisopropylamine) in dichloromethane or chloroform at room or elevated temperatures. While the reagent slowly disappeared, we were not able to isolate satisfactory quantities of the expected addition products. The same can be said about the reaction between propargyl cinnamate and phenylthiol in the presence of TEA. No addition product could be isolated. Surprisingly, even reaction of cyclic triazole containing cinnamic ester with phenylthiol in the presence of iodine gave no addition product. However, it must be noted that trying to prove the generality of the method, we performed our reactions in a solvent (dichloromethane or chloroform) while it is recommended [21] to run the iodine catalyzed Michael addition in a solvent-free system.

In the light of these results, we decided to abandon the cinnamic ester and replace it with a similar compound but a substantially better Michael acceptor, commercially available propargyl acrylate **12**. It still contains a terminal triple bond (capable of reacting with the azide functionality), but the double bond is conjugated to the ester moiety only, and not to the aromatic ring (Scheme 4).

Propargyl acrylate was reacted with peracetylated glucose azide **16** in methylene chloride in the presence of copper (II) acetate/ascorbic acid to give the expected, crystalline click product (cyclic triazole) **17** (Scheme 5). The NMR (^{1}H) spectrum contained a singlet at 7.85 ppm which is characteristic of the proton attached to the triazole ring, four singlets representing protons of acetyl groups and typical carbohydrate signals. Additionally, it contained olefinic signals of the unreacted acrylic



Scheme 4 The possible use of propargyl acrylate as the sacrificial unit



Scheme 5 The example of using propargyl acrylate as the sacrificial unit

double bond. The yield was 88%. The purified product was further reacted with phenylthiol **18** in the presence of tetramethylguanidine (TMG) in chloroform to produce the expected addition product **20**. The reaction mixture was worked up and purified using a column chromatography to give the expected sulfide in 78% yield. The ^{1}H NMR spectrum indicated the presence of both phenyl and glucose signals (^{1}H NMR: 400 MHz, $CDCl_3$, δ 1.79 ppm, s, 3H, Ac; 1.97, s, 3H, Ac; 2.00, s, 3H, Ac; 2.02, s, 3H, Ac; 2.58, t, 2H, "acrylic" methylene; 3.1, broad t, 2H, "acrylic" methylene; 3.92, m, 1H; 4.08, pd, 1H; 4.14, pd, 1H; 5.18, s + m, 2H + 1H, "propargyl" methylene; 5.35, t, 2H; 5.81, t, 1H; 7.12–7.32, m, 5H, aromatic; 7.82, s, 1H, triazole).

The same product was synthesized when a one-pot approach was used. Thus, propargyl acrylate was reacted with a small excess of phenylthiol in methylene chloride in the presence of catalytic amounts of tetramethylguanidine (TMG). After

overnight stirring of the reaction mixture at room temperature, the TLC indicated a disappearance of the starting material. The mixture was treated with 1-azido-1-deoxy-2,3,4,6-tetraacetyl-β-D-glucopyranose and equimolar amounts of copper (II) acetate and ascorbic acid. The color of the reaction mixture changed after about 1 h. The stirring was continued for 3 more days. The work up (aqueous hydrogen carbonate solution, extraction, drying) followed by chromatography gave the crystalline product in 73% yield. The reaction of propargyl acrylate with tetraacetyl glucose azide {in the presence of Cu (I)} followed by the addition of phenylthiol and TMG gave the same product.

There are a few possible ways of decoupling the molecular unit (P) connected to the alkyl (propargyl) part of the SU from the molecular unit (Q) coupled to the acyl (acrylic) part of the SU. The decision which one is the most convenient must be based on the structure of the molecular units P and Q. One option is to hydrolyze the ester functionality provided that the applied conditions will not degrade the molecular units (or at least one of them). In our case of acetylated glucose, most methods will hydrolyze not only (modified) acrylic ester but also the acetate groups present in the carbohydrate unit. Usually, such deprotection will not be of any concern since decoupling is usually performed after all necessary steps had been already performed. Alternatively, one can achieve decoupling at the same position by reducing the ester functionality with lithium aluminum hydride. Of course, no other ester functionalities including acetates will survive. The LAH treatment will reduce carbon sulfur bonds as well but in most cases, it does not matter. The real question is if the molecular units will survive the treatment.

Another point of possible decoupling is the sulfur atom. It seems that some of the methods applicable to decoupling at the sulfur atom require milder conditions. Useful hydrogenation methods include the hydrogenation with Raney nickel and utilizing tributyltin hydride. Most other applicable methods take advantage of transition metal-mediated reactions [22] or sodium and lithium [23, 24].

The experiment using the methods described above and other possible chemistries enabling the decoupling is still performed. The results of this research will be published elsewhere.

1 Conclusion

In the described procedures we used peracetylated glucose azide and phenyl mercaptan as examples of molecular units. Of course, they are to represent much larger units such as proteins or polysaccharides. We expect that practically any molecule can replace peracetylated glucose and a phenyl group provided that they are equipped with the thiol and azide functionalities. The described methodology seems robust and applicable to a variety of molecular units. Moreover, all the starting materials, intermediates, and final products should be safe and applicable to many circumstances. We believe that the methodology is very easy to simple, employs non-expensive, easy available reactants, and the procedures are very simple.

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