Studies on Conjugates of Hydroxyl-Bearing Payloads: A Silyl Ether Strategy with Potential for Hydrolytic or Assisted Cleavage

W. Sean Fyvie, Cheng Guo, Peter R. Guzzo and David D. Manning*

Medicinal Chemistry Department, Curia Global, Inc., 26 Corporate Circle, Albany, NY 12212 USA

Over the course of working on a variety of complex drug conjugate programs within Curia, including antibody drug conjugates (ADCs), diverse ligand-drug conjugates (e.g. PROTACS), and liganded diagnostics (e.g. DOTA) we found that certain high interest drugs were often limited in the choice of a suitable functional group needed to connect the drug to the linker portion of the assemblage. Moreover, program objectives often dictated that the ideal drug conjugate required drug release at the biological site of action by means of cleavable linkers (**Figure 1**). The amino group is perhaps the most common functional group used to connect drug to linker as exemplified by ADCETRIS®. However, not all drugs of interest possess the requisite amino group necessary for connection. We found this especially true for many natural products, where hydroxyls are a common functional group encountered including poly-hydroxylated substrates. Absent an appropriate functional group on the drug, the only other option is for the drug to be derivatized to provide a suitable functional handle. This approach is normally undesired since the biological profile and likely the safety profile of the drug can change. Considering these challenges, we aimed to explore silicon ethers as a means to expand the arsenal of useful functional handles to both link and subsequently release hydroxyl-bearing drugs, particularly natural products.[1-5]

Figure 1. Illustration of Common ADC Drug-linker Conjugates

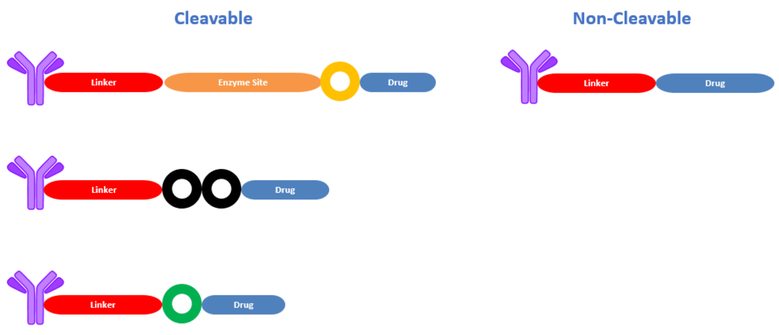
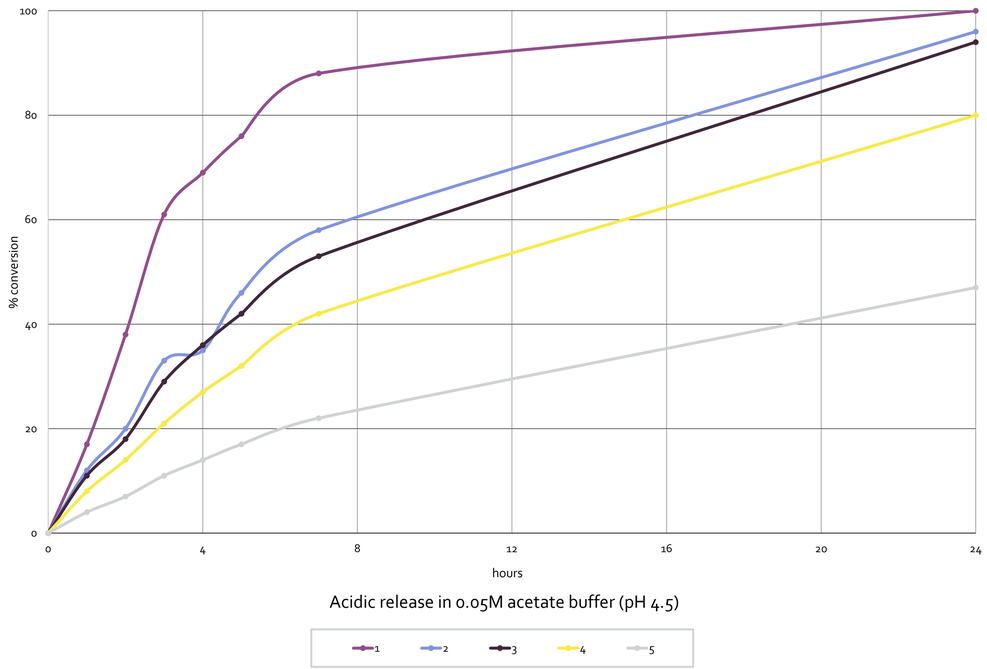


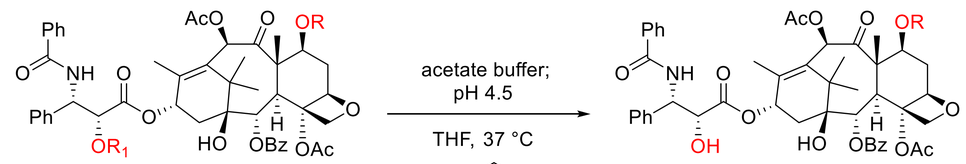
Figure 1. Circles represent a cleavable connection to the chemical linker. Enzymatic, disulfide and pH triggers are all known mechanisms to release drug in biological setting. Non-cleavable drug-linkers do not release the drug from the linker.

Silicon ethers have the basic structure $R_1OSi\text{-}R_2R_3R_4$. As such, the silicon ether can be configured with a wide variety of substituents that can affect properties and is why silyl ethers have been heavily deployed in synthetic organic chemistry for the purpose of both selective protection and deprotection. In the case of bioconjugates where release of the drug is intended, we anticipated that we could exploit the tunable nature of the silyl ether to control the release of the drug through hydrolysis, a biorelevant release mechanism. For ADCs, hydrolytically labile linkers presumably cleave as part of endocytosis. Endosomes and lysosomes are reported to have increased acidic environments (pH ~4.5 – 6.2) relative to the systemic circulation (pH ~7.4). Previously, we had shown that the kinetics of release of the natural product vinblastine could be varied by manipulation of the silyl ether R-group (**Figure 2**).[1] Dimethylsilyl ether **3** had a $t_{1/2}$ of 3 hr whereas diphenylsilyl ether **5** exhibited a $t_{1/2}$ of approximately 24 hrs (acetate buffer, pH = 4.5). Compounds **1** and **3** were stable in 1:1 0.01 M PBS buffer/THF, pH = 7.4, 37 °C for 24 hr (data not shown).

Figure 2. Hydrolysis of Vinblastine Silyl Ethers



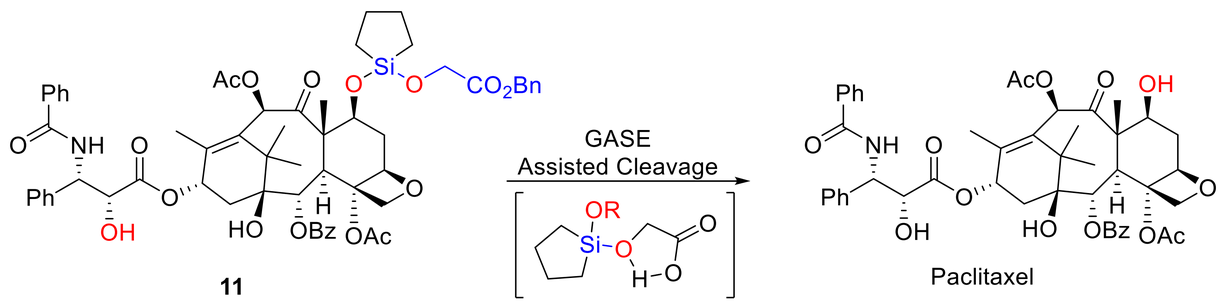
Scheme 1. Hydrolysis of Paclitaxel Silyl Ethers



R	Conditions	Result
6	1:1 [0.5 M Acetate buffer, pH 4.5]:THF, 37 °C, 20 h	No cleavage
7	1:1 [0.5 M Acetate buffer, pH 4.5]:THF, 37 °C, 20 h	88% cleavage to Paclitaxel
7	1:1 [0.01 M Acetate buffer, pH 4.5]: THF, 37 °C, 20 h	2% cleavage to Paclitaxel
8	1:1 [0.5 M Acetate buffer, pH 4.5]:THF, 37 °C, 20 h	major is **10**, no Paclitaxel formed

As part of our efforts to further understand the reactivity and scope of a silyl ether linker, we generated silyl ethers **6 – 8, 11** to model the behavior of a more elaborate drug conjugate linker**.** We reasoned that a silyl ether could cleave to release drug either by simple hydrolysis (**Scheme 1**) or by assisted cleavage (**Scheme 2**) through intramolecular H-bond activation of a glycolic acid silyl ether (GASE) derived from, for example, enzymatic cleavage of an ester or amide bond within the linker. Under buffered conditions, mono silyl ether **6** gave no cleavage to Paclitaxel after 20 hr in 0.5 M acetate buffer at 37 °C. In contrast, bis silyl ether **7** gave 88% of Paclitaxel under the same conditions. However, when the buffer strength was decreased to 0.01 M acetate only 2% cleavage was observed at the 20 hour mark. Compound **8**, doubly decorated with two silicon ethers, was devised to test hydrolytic sensitivity to steric environment of the drug attachment point for polyhydroxylated drugs. Under the stronger 0.5 M acetate conditions only mono-silyl ether hydrolysis was detected after 20 h. The identical but more sterically hindered silyl ether remained unreacted. Collectively, the rate of cleavage appears sensitive to both the steric environment of the linker attachment point, the buffer strength of a conjugate's environment and the nature of the R-groups attached to the silicon atom.

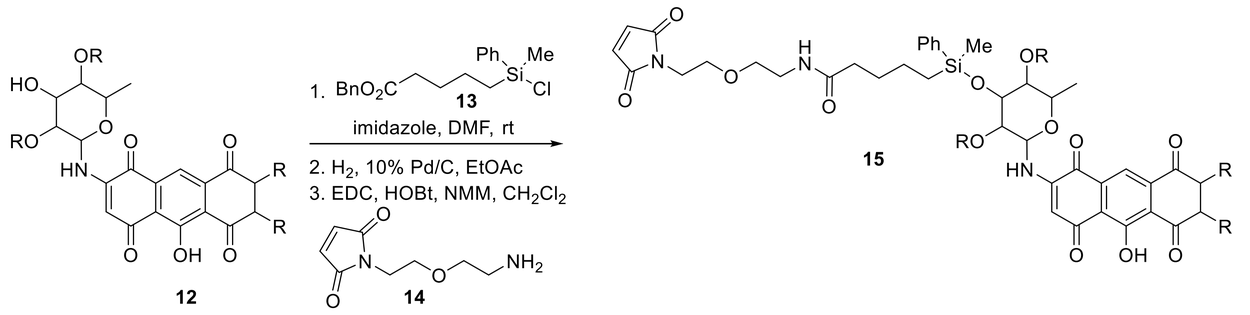
Scheme 2. Assisted Cleavage of Glycolic Acid Silyl Ethers (GASE)



ID	Conditions	Result
11	Balloon H_2, Pd/C, 1:1 [0.05 M PBS, pH 7.4]:THF, RT	100% cleavage to Paclitaxel @ 1.8 h
	Balloon H_2, Pd/C, 1:1 [0.01 M PBS, pH 7.4]:THF, RT	100% cleavage to Paclitaxel @ 2 h
8	Balloon H_2, Pd/C, 1:1 THF, RT	100% cleavage to Paclitaxel @ 2 h

Assisted silicon ether cleavage could offer a complementary pathway to alter the kinetics of drug release. Simulating this pathway, compound **11** was subjected to hydrogenolysis. Complete cleavage to Paclitaxel was observed at the first measured timepoint (2 hr). The most hydrolytically resistant bis-ether **8** also delivered full conversion to Paclitaxel under similar conditions. Silicon ether reagents which did not possess the same possibility of intramolecular activation did not present any cleavage during normal synthetic manipulations. For example, the poly-hydroxylated drug-linker reagent **15** (**Scheme 3**) was prepared under similar hydrogenation conditions but posed no cleavage concerns upon generation of the carboxylic acid in step 2.

Scheme 3. Preparation of Drug-Linker 15



In conclusion, we have demonstrated that silyl ethers have potential to serve as pH sensitive, tunable linkers and that GASE-assisted cleavage can augment the release of silyl ether-linked payloads otherwise resistant to pH cleavage alone. We anticipate future work to investigate enzymatic cleavage with a fully elaborated bioconjugate.

References

1. P. R. Guzzo, D. D. Manning, W. S. Fyvie and C. Guo; *Medicinal Chemistry GRC*, **2014** Colby-Sawyer College, NH
2. M. C. Finniss, K. S. Chu, C. J. Bowerman, J. C. Luft, Z. A. Haroon and J. M. DeSimone; *Med. Chem. Comm.* **2014**, *5*, 1355 – 1358.
3. R. V. Kolakowski, K. T. Haelig, K. K. Emmerton, C. I. Leiske, J. B. Miyamoto, J. H. Cochran, R. P. Lyon, P. D. Senter and S. C. Jeffrey; *Angew. Chem. Int. Ed.* **2016**, 55(28), 7948-79-51.
4. A. Dal Corso, M. Frigoli, M. Prevosti, M. Mason, R. Bucci, L. Belvisi, L. Pignataro and C. Gennari; *ChemMedChem* **2022**, 17, e202200279.
5. D. Wei, Y. Mao, H. Wang, S. Qu, J. Chen, J. Li, B. Jian and H. Chen; *Chinese Chemical Letters* **2023**, *34*, 108091