Bioorg. Med. Chem. Lett. 129 (2025) 130390

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tunable spontaneous release of a carboxylic acid via a β-eliminative cleavable linker☆

Brenno Masina [a], Nicola R.F. Knowles [b], Ronald T. Raines [a,*]

[a] Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[b] Harvard–MIT Program in Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

ARTICLE INFO

This Letter is dedicated to Professor Bryan C. Dickinson, winner of the 2025 Tetrahedron Young Investigator Award.

Keywords:
Carboxylic acid
Drug delivery
β-Elimination
Ester
Self-immolative linker

ABSTRACT

Self-cleavable linkers offer controlled payload release without the need for external stimuli, making them valuable for applications in chemical biology and clinical settings. In this work, we expand the scope of the β-eliminative cleavable linkers developed by Santi and coworkers to include the release of carboxylic acids. We demonstrate that the half-life of the ensuing ester conjugates can be controlled by varying electron-withdrawal using a pendant aryl sulfone moiety. The nascent ester group hydrolyzes readily in mouse serum but is stable in human serum. This system offers a tunable, autonomous release mechanism that could enable the delivery of new payloads in therapeutic contexts.

Introduction

A fundamental aspect of drug conjugates is their use of a cleavable linker to transiently link a carrier or targeting moiety to a chemotherapeutic agent.[1] Most drug conjugate systems have relied on linkers that are cleaved via exogenous conditions such as pH,[2] light,[3] or the presence of a reactant[4] or endogenous enzyme.[3] Such reliance on external stimuli necessarily leads to operational complexity and can limit the practical applications of these conjugates.

Self-cleavable linkers, in contrast, are of interest because of their ability to release payloads in a manner that is independent of external stimuli. An especially elegant self-cleavable linker system has been developed by Santi and coworkers.[5] In their conjugates, payloads containing a primary or secondary amino group are elaborated as a carbamic acid that is released via a base-catalyzed β-elimination reaction (Scheme 1). The immolation rate of these molecules is controlled by the Brønsted acidity of the hydrogen that is β to the carbamyl group. This acidity is affected by the electronic properties of a substituted phenyl sulfone, which acts as a "modulator" group that is also installed on the β carbon. For example, greater electron-withdrawal decreases the pK_a of the labile proton and increases the rate of the β-elimination reaction. This tunability allows for the conjugate half-life to be adjusted from minutes to years. Self-cleavable linkers based on this reaction have been exploited for the sustained release of small-molecule drugs from a PEG carrier[6] and hydrogels (including a GLP-1 receptor agonist),[7] offering advantageous pharmacokinetics. To date, however, the utility of this β-eliminative linker has been demonstrated only for payloads bearing an amino group or a modified phenyl group.[5,8] The expansion of this autonomous self-cleavable linker to accommodate other functional groups would enable its applicability to a wider range of molecules and drugs.

Carboxylic acid groups are present in approximately 12% of bioactive molecules in the medicinal chemistry literature,[9] making them one of the most useful handles for drug conjugates. Moreover, the pK_a of a carboxylic acid is similar to that of a carbamic acid (e.g., HOC(*O*)CH_3 has pK_a = 4.8, whereas HOC(*O*)NH_2 has pK_a = 3.9), suggestive of similar efficacy for carboxylates and carbamates as leaving groups. In this work, we sought to test this equivalence and potentially expand the scope of functional groups that can be delivered with the β-eliminative linker system to include carboxylic acids.

☆ This article is part of a Special issue entitled: 'Bryan Dickinson' published in Bioorganic & Medicinal Chemistry Letters.

* Corresponding author.
E-mail address: rtraines@mit.edu (R.T. Raines).

https://doi.org/10.1016/j.bmcl.2025.130390
Received 19 April 2025; Received in revised form 29 July 2025; Accepted 25 August 2025
Available online 27 August 2025

Results and discussion

To demonstrate the controlled release of a carboxylic acid, we chose a model payload that can be detected easily. Specifically, we deployed a rhodamine B-based fluorophore that we synthesized via alkylation of rhodamine piperazine amide **1** (Scheme 2), which was accessed from rhodamine B as described previously.[10] Similar rhodamine-based fluorophores retain their fluorescence properties at different pHs,[11] which is desirable for our analyses.

The synthesis of the aryl sulfone linker precursors was achieved by following the route developed by Santi and coworkers.[5] To demonstrate the tunability of the half-life of our ester linker, we installed three different aryl sulfone modulator groups of varying electron-withdrawing ability in azido-alcohols **3A**–**3C**. Azido-alcohol **3D** lacked an aryl group. The fluorophore–linker conjugates were obtained by esterifying azido-alcohols **3A**–**3D** with carboxylic acid **2** via a Steglich esterification reaction (Scheme 2), which is often employed in the total synthesis of natural products.[12] The azido group in esters **4A**–**4D** enables their stable conjugation to carrier or targeting moieties in future applications.

The release of the fluorophore from the esters should proceed through a β-elimination reaction wherein the rate-determining step is the dissociation of a C—H bond β to the carboxyl group. Thus, the reaction is expected to follow a second-order kinetic equation with a first-order dependence on hydroxide ion concentration. Consequently, at constant pH, the reaction should follow pseudo-first-order kinetics with an observed kinetic constant (k_{obs}) independent of ester concentration. To determine the value of k_{obs} and the corresponding half-life ($t_{½} = \ln 2/k_{obs}$) of the β-elimination reactions, the release of the fluorophore from esters **4A**–**4D** was monitored by ultra-high performance liquid chromatography (UPLC) at pH 6.0, pH 7.0, and pH 8.0 and 37 °C. As expected, the concentration of the fluorophore conjugates decayed exponentially with time at all pHs (Fig. 1). Also consistent with expectations, the immolation of modulator-bearing esters **4A**–**4C** showed a strong rate-dependence on pH, with shorter half-lives at higher pH (Table 1). In contrast, the release of the fluorophore from modulator-free ester **4D** was much slower ($t_{½}$ = 120–130 h) and nearly pH agnostic. Because the acidity of its C—H bond is low, ester **4D** likely releases its fluorophore via ester bond hydrolysis. This change in mechanism is consistent with the rate of ester hydrolysis being nearly independent of pH in the regime of $6.0 \leq pH \leq 8.0$.[13]

The half-life of the modulator-bearing esters **4A**–**4C** varied with the identity of the installed modulator. For each ester at each pH, the half-life was in accord with the value of the Hammett sigma constant for the *para*-substituent on the phenyl ring of the aryl-sulfone (σ_p: –H = 0, –Cl = 0.23, –CF_3 = 0.54).[14] Specifically, the chloride-substituted ester **4B** displayed an ~2-fold half-life reduction compared to the unsubstituted ester **4A**, whereas the trifluoromethyl-substituted ester **4C** displayed an ~4-fold half-life reduction compared to the unsubstituted ester **4A**. This trend is consistent with half-life variations reported with carbamate linkers.[5a]

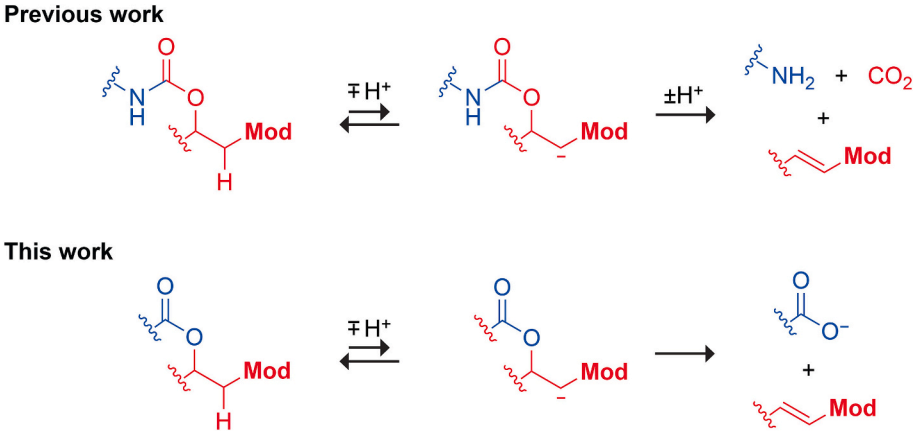
The half-life variation for each pH unit difference for the esters **4A**–**4C** was less than 10-fold, which indicates that the reaction order for hydroxide-ion concentration is <1. Therefore, the reaction kinetics are more complex in the ester system than in previously characterized carbamates.[5a] We hypothesized that one factor that could complicate the release kinetics is the pH-dependent protonation of the amino group in the pendant piperazinyl group of the fluorophore, which could influence the rate of the β-elimination reaction by decreasing the pK_a of the labile proton. Competition from ester hydrolysis, which has its own pH-dependency,[15] is another factor that could complicate the release kinetics.

We expected that the stability of the fluorophore-conjugate in an aqueous solution could be enhanced by controlling temperature and pH. To test this hypothesis, we incubated ester **4A** in a solution of pH 5.0 at 4 °C. After three weeks, only 10% of the conjugate was cleaved, indicating that similar ester constructs could be stored in solution for a prolonged period.

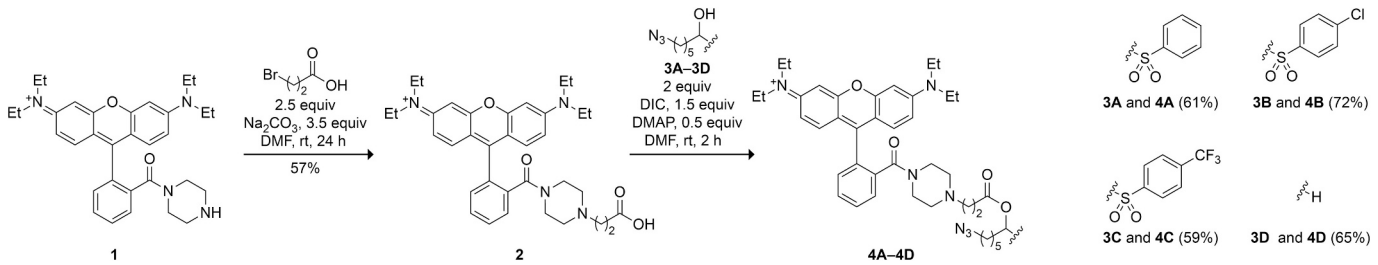
An important factor for the biological viability of our ester linker is its stability in mouse and human sera, which contain esterases.[3,16] Thus, the stability of our conjugates in serum could differ substantially from their stability in buffered solutions. Moreover, the esterase activity in human and mouse sera differs significantly. Some ester prodrugs hydrolyze 100 to 1000-fold more rapidly in mouse serum than in human serum.[3,16] We assessed the stability of esters **4A** and **4D** in serum. These conjugates differ by the modulator being absent in ester **4D** but present in ester **4A**. We hypothesized that the modulator could sterically shield the proximal ester group from enzymatic degradation. UPLC analyses demonstrated that both ester **4A** and ester **4D** were completely hydrolyzed after 1 h at 37 °C in mouse serum (Fig. S1). Likewise, modulator-free ester **4D** suffered complete hydrolysis after 1 h in human serum. In contrast, modulator-bearing ester **4A** was stable in human serum, displaying a half-life of 24 h, which is twofold greater than the half-life for carboxylic acid release via β-elimination at pH 7.0 (Table 1). These data are consistent with the aryl sulfonyl group of ester **4A** imposing steric hindrance to human esterases. If desired, the steric hindrance could be increased by installing additional functional groups (e.g., methyl groups) near the ester moiety, potentially slowing both enzymatic and nonenzymatic hydrolysis and thereby ceding more control of carboxylic acid release to the β-elimination route (Scheme 1).

Conclusions

We have expanded the β-eliminative cleavable linker developed by



Scheme 1. Putative mechanism for the spontaneous cleavage of the carbamate developed by Santi et al.[5] and ester developed herein. Mod = substituted phenyl sulfone.



Scheme 2. Synthetic route to esters **4 A–4D**.

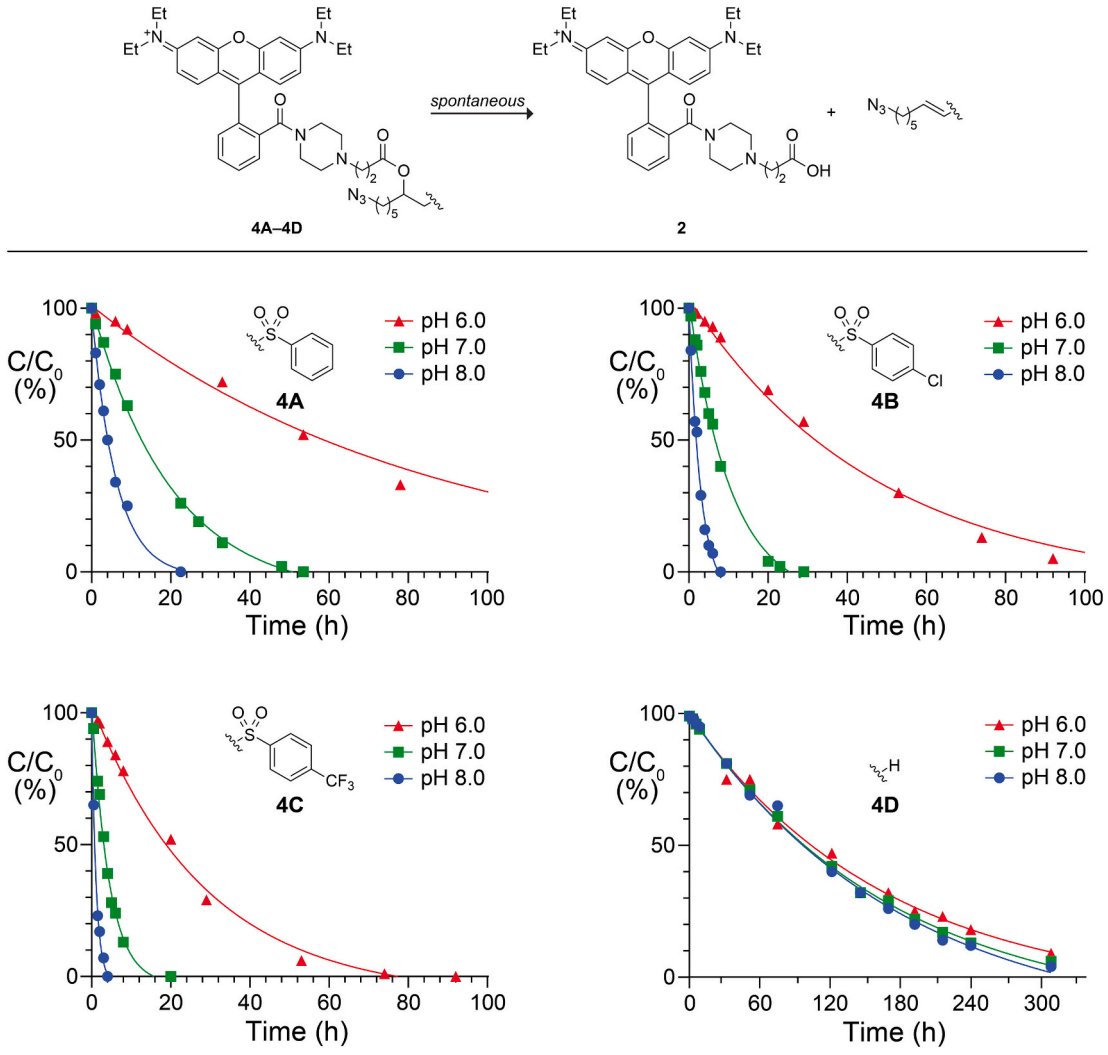


Fig. 1. Graphs of esters **4 A–4D** remaining intact as a function of incubation time at 37 °C in 100 mM sodium phosphate buffer, pH 6.0 or 7.0, or 100 mM Tris–HCl buffer, pH 8.0. Values of k_{obs} and $t_{½}$ are listed in Table 1.

Santi and coworkers to enable the incorporation and delivery of carboxylic acids via an ester linkage. We demonstrated that the half-life of this linker is tunable via the installation of an electron-withdrawing modulator moiety, allowing for control over the kinetics of payload release independent of external agents. Finally, we showed that conjugates bearing a modulator moiety are stable at pH 5.0 and 4 °C and resist ester hydrolysis in human serum. Hence, β-eliminative linkers could be viable options for the tunable delivery of bioactive carboxylic acids[9] in humans.

CRediT authorship contribution statement

Brenno Masina: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Nicola R.F. Knowles:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Ronald T. Raines:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

Table 1
Observed rate constant and half-life for fluorophore release from esters **4 A**–**4D** at pH 6.0, 7.0, and 8.0 and 37 °C.

Modulator	pH	k_{obs} (s^{-1})	$t_{½}$ (h)
	6.0	3.3×10^{-6}	59
$-SO_2C_6H_5$ (**4 A**)	7.0	14×10^{-6}	14
	8.0	45×10^{-6}	4.3
	6.0	58×10^{-7}	33
$-SO_2C_6H_4$-*p*-Cl (**4B**)	7.0	26×10^{-6}	7.3
	8.0	94×10^{-6}	2.1
	6.0	97×10^{-7}	20
$-SO_2C_6H_4$-*p*-CF_3 (**4C**)	7.0	63×10^{-6}	3.1
	8.0	24×10^{-5}	0.80
	6.0	1.5×10^{-6}	130
–H (**4D**)	7.0	1.5×10^{-6}	130
	8.0	1.6×10^{-6}	120

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

B.M. was supported by a scholarship from Spontefide SA (Lugano, Switzerland). N.R.F.K. was supported by an IMES Thompson (1933) Fellowship (MIT). This work was supported by grant R35 GM148220 (NIH).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2025.130390.

References

1. (a) Greenwald RB, Choe YH, McGuire J, Conover CD. *Adv Drug Deliv Rev*. 2003;55:217–250.(b) Bargh JD, Isidro-Llobet A, Parker JS, Spring DR. *Chem Soc Rev*. 2019;48:4361–4374.
2. Hamann PR, Hinman LM, Hollander I, et al. *Bioconjug Chem*. 2002;13:47–58.
3. Bahar FG, Ohura K, Ogihara T, Imai T. *J Pharm Sci*. 2012;101:3979–3988.
4. Versteegen RM, Rossin R, ten Hoeve W, Janssen HM, Robillard MS. *Angew Chem Int Ed*. 2013;125:14362–14366.
5. (a) Santi DV, Schneider EL, Reid R, Robinson L, Ashley GW. *Proc Natl Acad Sci USA*. 2012;109:6211–6216.(b) Schneider EL, Robinson L, Reid R, Ashley GW, Santi DV. *Bioconjug Chem*. 2013;24:1990–1997.
6. (a) Schneider EL, Ashley GW, Dillen L, et al. *Eur J Pharm Biopharm*. 2015;93:254–259.(b) Schneider EL, Hearn BR, Pfaff SJ, et al. *Bioconjug Chem*. 2016;27:2534–2539.(c) Machinaga N, Ashley GW, Reid R, et al. Transl. Vis. *Sci Technol*. 2018;7:21.(d) Fontaine SD, Hann B, Reid R, Ashley GW, Santi DV. *Cancer Chemother Pharmacol*. 2019;84:729–738.(e) Fontaine SD, Ashley GW, Houghton PJ, et al. *Cancer Res*. 2021;81:1076–1086.
7. (a) Hearn BR, Fontaine SD, Pfaff SJ, et al. *J Control Release*. 2018;278:74–79.(b) Schneider EL, Reid R, Parkes DG, Lutz TA, Ashley GW, Santi DV. *Domest Anim Endocrinol*. 2020;70, 106373.
8. For an alternative β-eliminative linker strategy, see: a Ji X, De La Cruz LKC, Pan Z, Chittavong V, Wang B. *Chem Commun*. 2017;53:9628–9631. For an alternative β-eliminative linker strategy, see:.b Ji X, Pan Z, Li C, et al. *J Med Chem*. 2019;28:3163–3168.c Li Z, Wang Y, Liu M, et al. *J Med Chem*. 2023;66:14583–14596.
9. Ertl P, Altmann E, McKenna JM. *J Med Chem*. 2020;63:8408–8418.
10. Serbian I, Hoenke S, Kraft O, Csuk R. *Med Chem Res*. 2020;29:1655–1661.
11. Nguyen T, Francis MB. *Org Lett*. 2003;5:3245–3248.
12. Tsakos M, Schaffert ES, Clement LL, Villadsen NL, Poulsen TB. *Nat Prod Rep*. 2015;32:605–632.
13. (a) Fife TH, Singh R, Bembi R. *J Organomet Chem*. 2002;67:3170–3183.(b) Rayne S, Forest K. Flavour. *Fragr J*. 2016;31:385–394.
14. Hansch C, Leo A, Taft RW. *Chem Rev*. 1991;91:165–195.
15. (a) Fort JJ, Mitra AK. *Int J Pharm*. 1990;59:271–279.(b) Jensen E, Bundgaard H. *Int J Pharm*. 1991;71:117–125.(c) Kahns AH, Buur A, Bundgaard H. *Pharm Res*. 1993;10:68–74.
16. Liederer BM, Borchardt RT. *J Pharm Sci*. 2005;94:2198–2206.