nature portfolio

Peer Review File

Rapid amide ligation between α-halo acylsilanes and amines under aqueous conditions

Corresponding Author: Professor Wenbo Liu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This manuscript describes an interesting amide formation between primary amines and alpha-brome acylsilanes. This is a different approach and contributes to the growing field of alternative mechanisms for amide formation using acyl main group species. It differs from prior work but building the formal oxidant (in this case the alpha-bromide) into the acyl donor, rather than using a hydroxylamine or similar activated amine.

There is therefore some novelty to this report, so from a purely mechanistic point of view there is merit to publication. As currently written however, there are considerable issues that preclude recommending acceptance at this time.

1) It is not a Click reaction – it requires KF and NEt3. I realise that the Cu-mediated alkyne azide cycloaddition is considered a Click reaction, but it is not – and Sharpless would be the first to concede that point. A true click reaction does not require any external reagents.

2) Its advertised as proceeding in water – its compatible with aqueous conditions but not pure water or buffer. This is a different case and readers should not be mislead. (A minor point – why is the glassware oven dried if water is added?)

3) I like the mechanism, but it's hard to make a case for the utility of this reaction in the current data set. Everything presented would be better accomplished with a standard NHS ester – the reactivity and product profiles would be similar. In principle, I could imagine some attractive applications, such as trapping a specific lysine residue at an active site – although I understand that getting such a bulky TMS acylgroup in there will be challenging.

4) I'm not convinced by the kinetic measurements. This is a very high rate constant. It may be correct but it should be test by a reaction at far lower concentration. A rate of 0.7 M-1 s-1 would imply high conversion at near equimolar stoichiometry even at 1 millimolar or lower.

5) This work raises the question of whether or not other alpha-halo acyl groups, such as acylboronates, exhibit this reactivity. The paper would be greatly strengthen by testing those as well.

Reviewer #2

(Remarks to the Author)
• What are the noteworthy results?
The authors describe a unique and mechanistically intriguing way to couple amines to form amide products, using alpha-bromoacylsilanes as coupling partners. The scope of the method is quite broad with respect to the amine coupling partners and is applicable to small molecules, natural products, amino acids, protein coupling reactions, and nylon polymer formation.

• Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

While the methodology described is mechanistically interesting, it is unclear what advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters. On reading this manuscript, it was unclear if any products that were synthesized could not have been just as easily prepared via a conventional coupling reaction.

While the scope is broad with respect to amine coupling partners, there are limitations on the acyl donor side. Alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine, which places a limit on the substrate scope. Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic acids/activated esters that are used in traditional amide bond forming coupling reactions.

The modification of protein lysine residues by activated esters (e.g. NHS esters) is well known, and it is unclear what advantages are offered by the described method. Further, the described new method for protein modification is limited by the use of 4:1 DMSO:ammonium acetate buffer, as such a high proportion of DMSO will denature proteins, rendering useless the coupled protein products.

Overall, there is a need to establish the niche that will be filled by using alpha-bromoacylsilanes as coupling partners. Direct comparisons to NHS ester chemistry would be useful.

• Does the work support the conclusions and claims, or is additional evidence needed?

The mechanism proposed by the authors involves a 1,2-silyl shift to produce an alpha-silylamide intermediate. This is proposed largely based on the isolation of 3-Int from a reaction performed without KF (Fig 2D). While this mechanism is plausible, the isolation of 3-Int does not prove that it is an intermediate that forms under the reaction conditions. It would seem necessary to rule out the formation of ketene intermediates, which of course are well known intermediates for amide bond forming reactions with amines. Ketenes could be formed via an elimination mechanism which is well precedented to produce strained alkenes, e.g. https://pubs.acs.org/doi/full/10.1021/acs.chemrev.0c01011

• Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

The authors report a 2nd order rate constant, but they should not report more than a half-life based on the data presented. Their data (Fig 2B) follows ~zero order behavior. This may be an artifact of using sampling rather than more precise in situ monitoring of kinetics. To extract a 2nd order rate constant from pseudo first order behavior, data should be collected at varied concentrations, and a plot of k(obs) vs concentration should show a linear plot with a zero intercept.

• Is the methodology sound? Does the work meet the expected standards in your field?
Yes
• Is there enough detail provided in the methods for the work to be reproduced?
Yes

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I have reviewed the first version of this manuscript, and have read the responses and changes made. Although I still have some concerns about the broad applicability, I can see reasonable case for occasions where this unique chemistry may be of valuable. I am therefore, in principle, supportive of publication.

However, and unless this is addressed properly I withdraw my support, I still do not think the kinetics are properly done. If the reaction was really 0.7 M-1 s-1, with the setup of 10 equiv of amine and 1 equiv of the acyl silane, at a starting concentration of 0.1 M silane, the reaction should be fully completed in less than 10 seconds. This doesn't seem to be the case, and I believe the authors have calculated instead a pseudo-first order rate Iwhich would be measured as s-1). This needs to be corrected before further review.

Reviewer #2

(Remarks to the Author)
In a prior review of this manuscript, it was pointed out that the methodology described is mechanistically interesting, but it was unclear what advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters. It was also pointed out that the scope is limited on the acyl donor side as alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine,
which places a limit on the substrate scope. Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic acids/activated esters that are used in traditional amide bond-forming coupling reactions.

In their response, the authors have provided an example of N-methylaniline coupling that fails with an NHS ester, which is an important point to make. They provide a second demonstration of selective mono-functionalization, which is not compelling since they compare a mono-activated substrate (requiring synthesis) to a simpler diacid. Additionally, they show that lysozyme (a protein with disulfides but without free thiols) can be selectively functionalized 7 times, and they speculate that this could not be accomplished with NHS ester chemistry by citing literature but without running the comparison. Likely, with a protein containing free cysteine, both methods will modify thiols from a kinetic perspective to give labile thioesters.

Overall, the authors make a stronger case, especially with the N-methylaniline, but the point still holds that their applications are rather niche.

It was also pointed out that the described new method for protein modification is limited by the use of 4:1 DMSO:ammonium acetate buffer, as
such a high proportion of DMSO will denature proteins. To this point, the authors have shown that a reduced proportion of DMSO (1:6) is sufficient for lysozyme modification. This is a satisfactory response.

In terms of the mechanism, it was pointed out that a ketene mechanism had not been ruled out. The authors run two additional experiments, showing observation of 3-Int and the lack of reaction when aniline is used as nucleophile vs low yielding reaction when benzylamine is nucleophile. The experiments suggest a role for amine, but do not actually rule out a ketene mechanism, as the amine base may initiate the elimination reaction, producing intermediate silylamine, which may undergo addition to the ketene. Addition of TMS-dimethylamine to ketene itself is known (https://doi.org/10.1016/S0022-328X(00)88613-1).

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I appreciate the authors correcting the rate constant calculation and now clearly reporting the biphasic conditions under the initially reported experimental protocol. The reaction at 1:1 stoichiometry at 10 micromolar (20% conversion) is the more convincing data. This seems to be missing from the SI. If this is added, I support publication of this manuscript.

Reviewer #2

(Remarks to the Author)
In two prior reviews of this manuscript, it was pointed out that the methodology described is interesting, but it was unclear what *major* advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters, especially given that alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine,
which places a limit on the substrate scope. Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic acids/activated esters that are used in traditional amide bond-forming coupling reactions.

In further response, the authors have provided the example of acylation of cycloleucine, which was unsuccessful with NHS chemistry in their hands. However, cycloleucine acylations with other reagents (acid chlorides, mixed anhydrides) are known in the literature. The authors have also discussed that phenols react with NHS esters, suggesting chemoselectivity that was not otherwise available. However, numerous examples of acylations of amines in preference to phenolic groups are well known.

Regarding mechanism, it was pointed out in a previous review that the authors experiments suggest a role for amine, but do not rule out a ketene mechanism. Their response that phenol does not react (point #1), and their response regarding lack of reactivity in dry THF (point #4) do not really add new information since amine is absent in these experiments. In point #2, they discuss that the mechanism is leaving group dependent, but it is unclear how that provides evidence for either mechanism. Finally (point #3) the authors provide an experiment where benzylketene is independently generated; this would be compelling, except that monoalkylketenes are unstable! (see Tidwell, Science of Synthesis, 23.14, 2006). Thus, reacting an phenylethylacylchloride for 14 hours would consume starting material, but not to give stable benzylketene.

Publication in a more specialized journal is advised.

Response to Referees

Reviewer Comments to Author:

Reviewer: 1

Comments:

This manuscript describes an interesting amide formation between primary amines and alpha-brome acylsilanes. This is a different approach and contributes to the growing field of alternative mechanisms for amide formation using acyl main group species. It differs from prior work but building the formal oxidant (in this case the alpha-bromide) into the acyl donor, rather than using a hydroxylamine or similar activated amine. There is therefore some novelty to this report, so from a purely mechanistic point of view there is merit to publication. As currently written however, there are considerable issues that preclude recommending acceptance at this time.

Response: We are grateful to the reviewer for the positive comments on this work. Your encouragement is very important for us to continue our research. We have made revisions to the manuscript in accordance with your suggestions.

Comments:

1. It is not a Click reaction – it requires KF and NEt_3. I realise that the Cu-mediated alkyne azide cycloaddition is considered a Click reaction, but it is not – and Sharpless would be the first to concede that point. A true click reaction does not require any external reagents.

Response: Thank you for your insightful comments. We have changed the word" Click-type" in the title to "Rapid". We hope that this new title can more precisely describe our work. Meanwhile, we have removed the word "click" in the manuscript whenever applicable.

2. Its advertised as proceeding in water – its compatible with aqueous conditions but not pure water or buffer. This is a different case and readers should not be mislead. (A minor point – why is the glassware oven dried if water is added?)

Response: Thank you for your critical comments. We agree with you that "in pure water" and "under aqueous conditions" are different. To more precisely describe our work, we have changed the word "in water" to "under aqueous conditions" in the title.

Regarding the minor point (running the aqueous reactions with oven dried glassware), as a tradition in our laboratory, we always place the clean glassware in the oven before use. We have multiple

projects running in the lab. Some projects are sensitive to water, which require dry glassware. Therefore, to make things easier, we did not intentionally distinguish this project from others in terms of the glassware storage. Nevertheless, we agree with you that there is no need to run the reaction in oven-dried glassware. Control experiments show that using a reaction flask that was not oven-dried produces the identical result with that oven-dried.

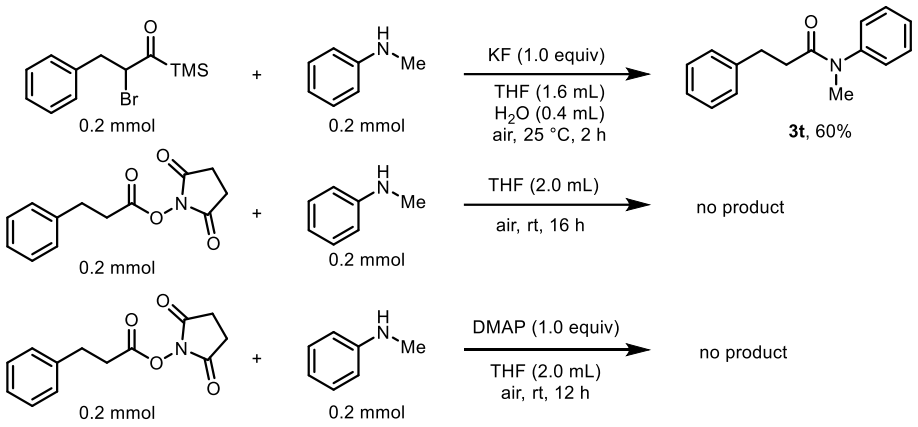
3. I like the mechanism, but it's hard to make a case for the utility of this reaction in the current data set. Everything presented would be better accomplished with a standard NHS ester – the reactivity and product profiles would be similar. In principle, I could imagine some attractive applications, such as trapping a specific lysine residue at an active site – although I understand that getting such a bulky TMS acylgroup in there will be challenging.

Response: Thank you for your critical comments and great advice. We are aware that NHS ester reacting with amine to afford an amide is a remarkable and robust reaction, which has found broad applications in numerous fields even in drug-antibody conjugation. It is not our purpose to completely replace this classical reaction.

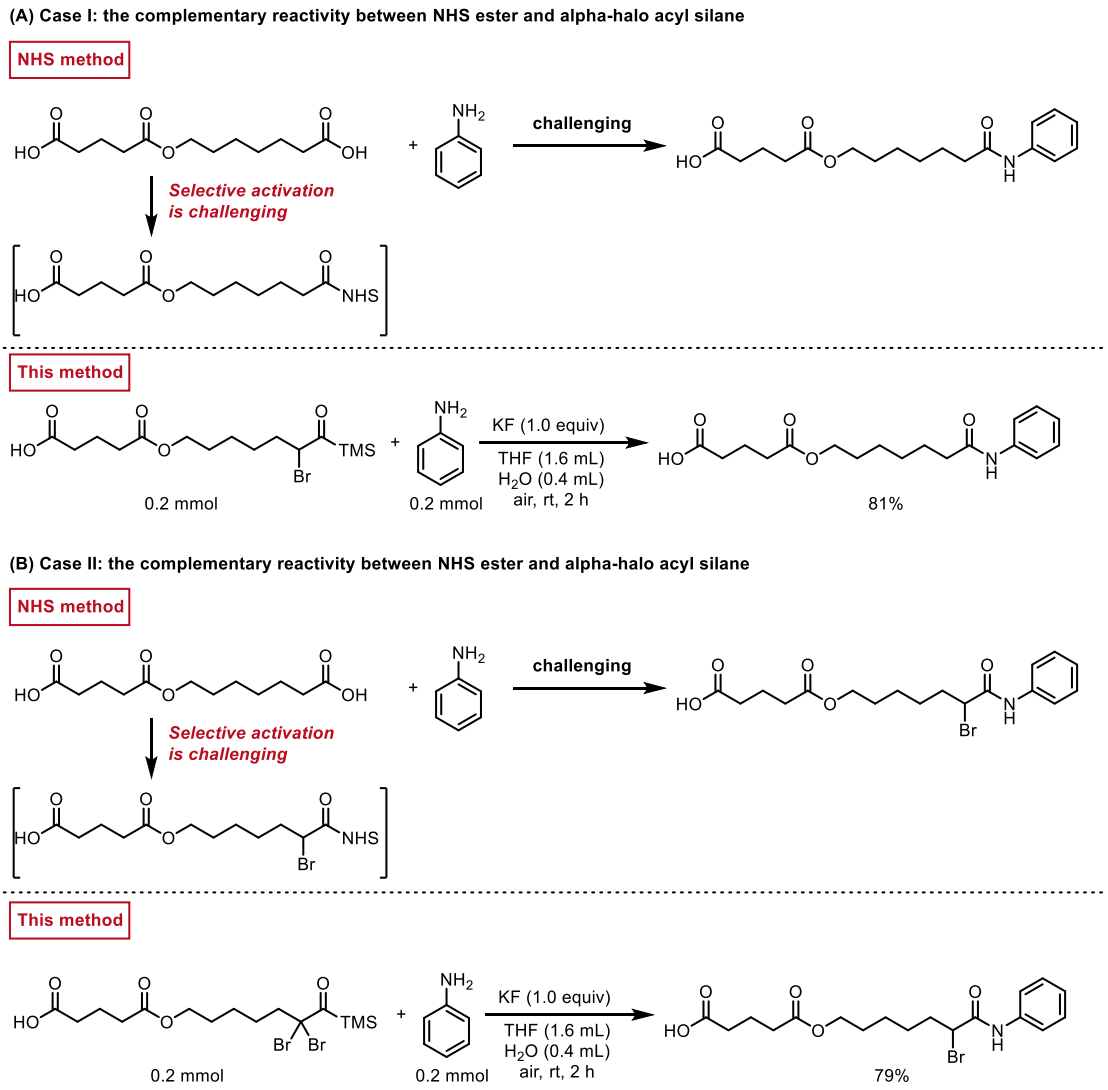
However, the reactivity and selectivity of our alpha-halo acyl silane with amine are different from NHS esters with amines. It has been reported that NHS esters can react with alcohol and other nucleophiles (please see the discussion in Kalkhof, S.; Sinz, A. Chances and pitfalls of chemical cross-linking with amine-reactive N-hydroxysuccinimide esters. Analytical and Bioanalytical Chemistry 2008, 392, 305-312). Therefore, in the protein modification, in principle, the serine, cysteine, tyrosine, glutamic acid may be modified if the target is the lysine modification. In our case, we have performed control experiments, alcohols, carboxylic acids, and phenols cannot react with alpha-halo acyl silanes at all. The high selectivity of alpha-halo acyl silane indeed provides advantages over NHS esters.

We performed the following control experiments to further demonstrate the advantages of this alpha-halo acylsilanes over NHS esters:

(1) When N-methyl aniline, a weakly nucleophilic and bulky reagent, was used under standard conditions, the target product **3t** was obtained in 60% yield. However, with the NHS ester as the acyl donor, even in organic solvent, no product **3t** was detected. Notably, adding a stoichiometric amount of the nucleophilic DMAP also failed to produce **3t**.



(2) When using the NHS ester method, it's usually challenging to selectively functionalize one carboxyl group without affecting the other when multiple non-identical carboxylic acids are present. However, our method can provide an alternative method to form an amide without affecting the carboxylic acid group, as demonstrated in the example below. This further indicates that the method is orthogonal to traditional carboxylic acid/amine couplings. Moreover, α-bromo amides are extremely important compounds. Introducing a bromine atom at the α-position of one carboxyl group selectively in presence of multiple carboxylic acids to access an α-bromo amide is usually difficult. Nevertheless, this can be easily achieved using this method.



(3) NHS esters are capable of reacting with nucleophilic groups (such as thiols and hydroxyl groups) in addition to amino groups, potentially leading to impure labeled products (Kalkhof, S.; Sinz, A. Chances and pitfalls of chemical cross-linking with amine-reactive N-hydroxysuccinimide esters. Analytical and Bioanalytical Chemistry 2008, 392, 305-312). In contrast, this method selectively reacts with amino groups without reacting with other nucleophilic groups, effectively avoiding product impurity issues. For example, when 120 equivalents of alpha-Br acyl silane was employed to react with lysozyme, mass spectrometry only detected the number of lysine plus terminal amino modification (+7 modifications), indicating that α-halo acylsilanes are highly selective for amines (Figure S10).

The above points convincingly suggest the complementary reactivity profiles of our method with the classical NHS ester/amine coupling approach.

Furthermore, capturing specific lysine residues at active sites represents a highly meaningful application, particularly for advancing covalent drug development. In this revised study, we first validated the method's potential for developing lysine-targeted probes. By introducing alkynyl groups into cell lysates using this method, which was followed by performing a CY5-azide click reaction, we successfully labeled specific small proteins, as visualized by gel imaging (Figure S24c). The results also demonstrated compatibility with diverse protein lysis approaches, including direct RIPA lysis and PBS-based lysis. These findings underscore its applicability in lysine-targeted covalent probe development, biomarker identification in pathological cells, and covalent inhibitor design. *Notably, we think that selectively labelling different amines with acyl silanes by modulating the steric hindrance is a brilliant idea (Thank you very much!)*. We are actively exploring covalent inhibitor development using α-halo acylsilanes, and further progress will be reported in subsequent studies.

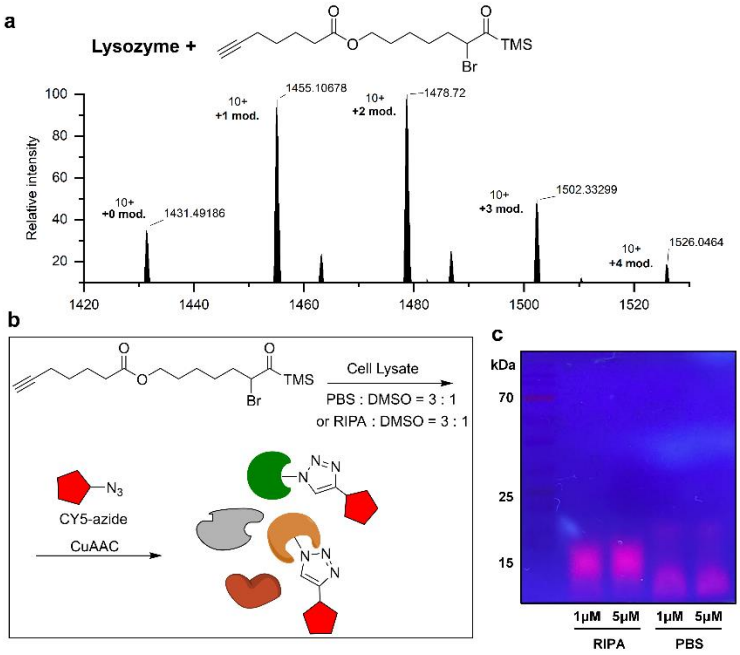


Figure S24. (a) Coupling of lysozyme to alkyne bearing substrates. (7.0 equiv), (b) CY5 labeling of proteins in cell lysates. (c) Gel imaging after CY5 labeling.

Notably, NHS esters have been reported to exhibit poor stability in weakly alkaline environments (e.g., RIPA cell lysis buffers, pH 7.4-8.0) (Mädler, S.; Bich, C.; Touboul, D.; Zenobi, R. Chemical Cross-linking with NHS Esters: A Systematic Study on Amino Acid Reactivities. J. Mass Spectrom. **2009**, 44 (5), 694-706). In contrast, our method maintains efficacy even at low concentrations under

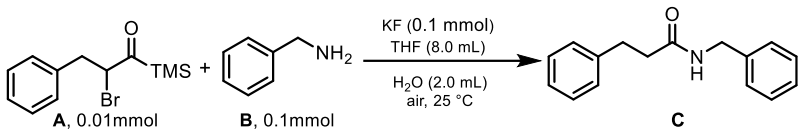
these conditions (Figure S19c), demonstrating significant advantages for protein labeling in lysates. Overall, the reaction system developed in this study shows different and complementary reactivity profiles compared to traditional NHS ester/amine couplings.

4. I'm not convinced by the kinetic measurements. This is a very high rate constant. It may be correct but it should be test by a reaction at far lower concentration. A rate of 0.7 M-1 s-1 would imply high conversion at near equimolar stoichiometry even at 1 millimolar or lower.

Response: Thank you for the critical comments and suggestions. The rate constant we measured was indeed determined at a concentration of 1.0 mM (not 0.1 M). The experimental details can be found in the supporting information (SI).

We realized that the initial way we presented the kinetic data in Figure 2 was a little confusing. We're sorry for that and have corrected the figure in a clearer style. In addition, we redetermined the rate constant within the half-life period. The rate constant is determined to be 0.611 $M^{-1}s^{-1}$ for 1.0 mM within the half-life, which is consistent with our previous value.

1.0 mM



Under an ambient atmosphere, benzylamine (10.7 mg, 0.1 mmol, 10.0 equiv), tetrahydrofuran (8.0 mL), H_2O (2.0 mL), KF (5.8 mg, 0.1 mmol, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (2.8 mg, 0.01 mmol, 1.0 equiv) were added to an oven-dried round bottom flask (25.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [$\mathbf{A}_0$] - [**C**] versus time (sec) were plotted (**Figure S1**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.611 $M^{-1}\cdot s^{-1}$.

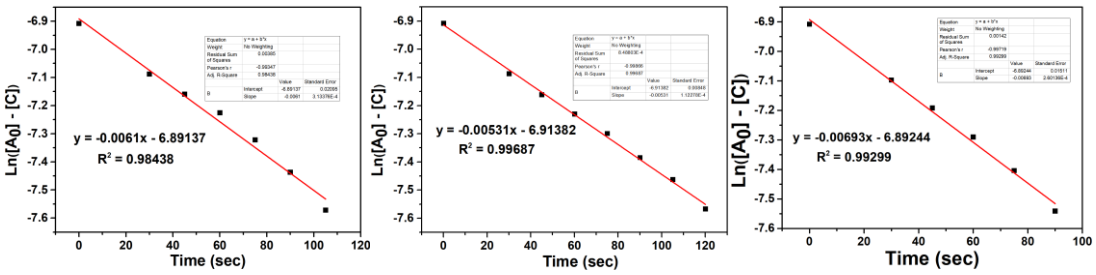


Figure S1. Pseudo-first order kinetics

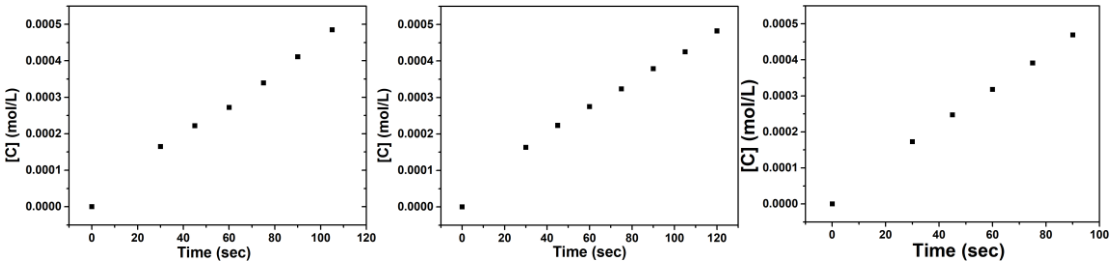
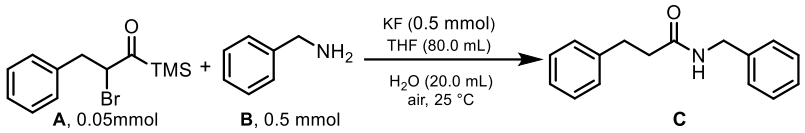


Figure S2. Product vs time (sec)

Subsequently, we also measured a rate constant of 0.973 $M^{-1}\cdot s^{-1}$ for 0.5 mM within the half-life.

0.5 mM



Under an ambient atmosphere, benzylamine (53.5 mg, 0.5 mmol, 10.0 equiv), tetrahydrofuran (80.0 mL), H_2O (20.0 mL), KF (29.0 mg, 0.5 mmol, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (14.2 mg, 0.05 mmol, 1.0 equiv) were added to an oven-dried round bottom flask (250.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [A_0] - [C] versus time (sec) were plotted (**Figure S3**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.973 $M^{-1}\cdot s^{-1}$.

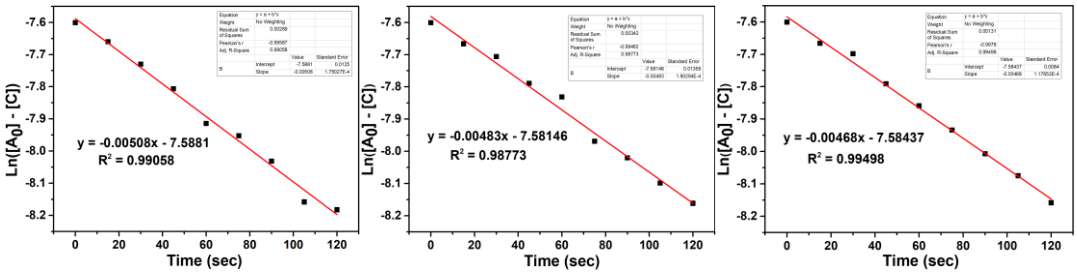


Figure S3. Pseudo-first order kinetics

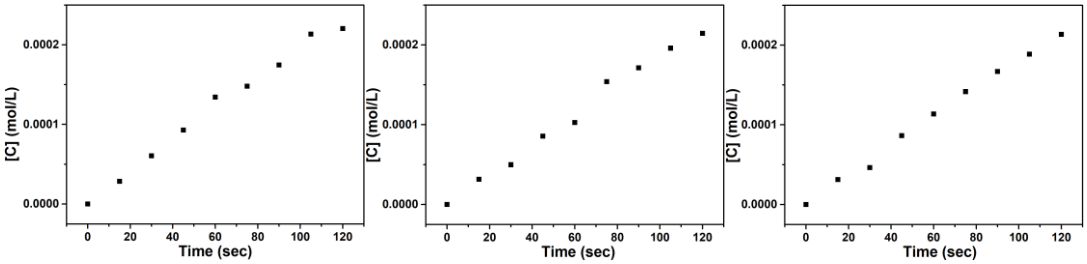
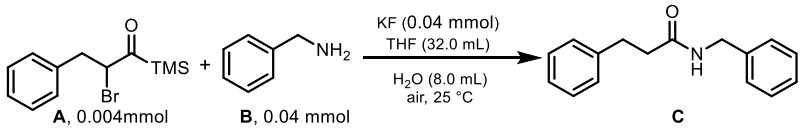


Figure S4. Product vs time (sec)

Finally, we measured a rate constant of 0.861 $M^{-1}\cdot s^{-1}$ for 0.1 mM within the half-life.

0.1 mM



Under an ambient atmosphere, benzylamine (1.0 M, 40.0 mL, 10.0 equiv), tetrahydrofuran (32.0 mL), H_2O (8.0 mL), KF (1.0 M, 40.0 mL, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (0.1 M, 40.0 mL, 1.0 equiv) were added to an oven-dried round bottom flask (100.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [A_0] - [C] versus time (sec) were plotted (**Figure S5**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.861 $M^{-1} \cdot s^{-1}$.

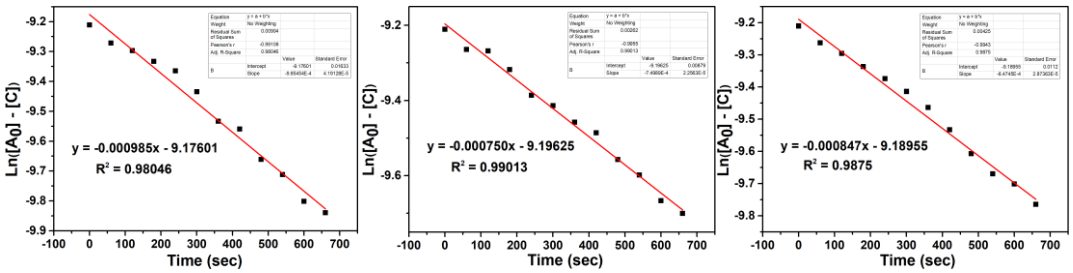


Figure S5. Pseudo-first order kinetics

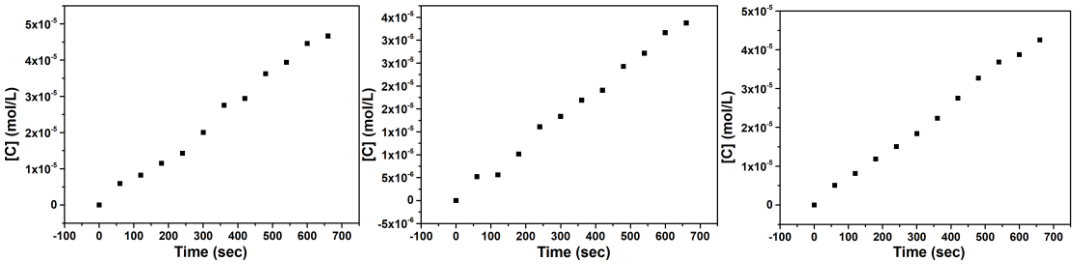
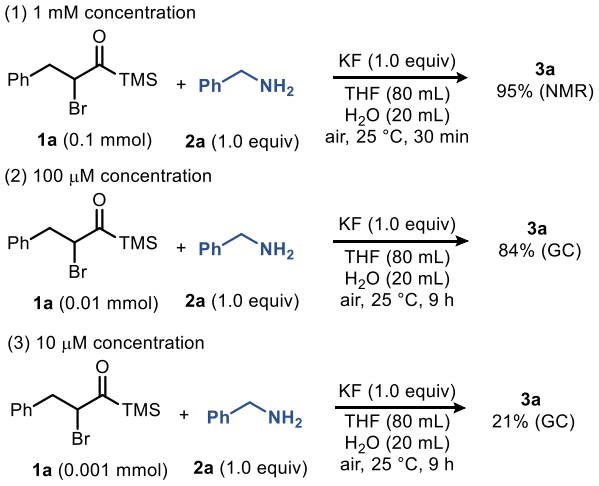


Figure S6. Product vs time (sec)

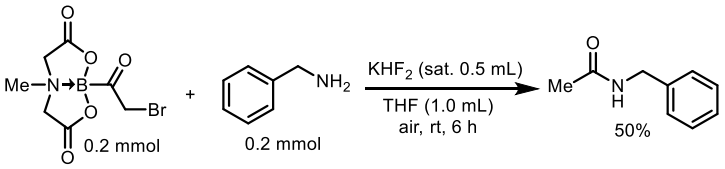
Additionally, at 1 mM, the reaction was completed in 30 minutes with 95% yield at 25 °C. Upon further dilution to 0.1 mM, the reaction furnished the product in 84% yield in 9 hours at 25 °C. When both reactants were diluted to 10 μM, the product was delivered in 21% yield after 9 hours.



These experiments indeed demonstrate that the rate constant is very high.

5. This work raises the question of whether or not other alpha-halo acyl groups, such as acylboronates, exhibit this reactivity. The paper would be greatly strengthen by testing those as well.

Response: Thank you for your valuable questions. As you suggested, we tested the reaction between α-bromoacyl (MIDA)boronate and amine. We are glad to find that the amide can indeed be detected in 50% yield. We are currently carrying out systematic optimization of the reaction. Relevant research results will be reported in detail in subsequent work.



Reviewer #2 (Remarks to the Author):

- What are the noteworthy results?

The authors describe a unique and mechanistically intriguing way to couple amines to form amide products, using alpha-bromoacylsilanes as coupling partners. The scope of the method is quite broad with respect to the amine coupling partners and is applicable to small molecules, natural products, amino acids, protein coupling reactions, and nylon polymer formation.

Response: We are grateful to the reviewer for the positive comments on this work. Your encouragement is very important for us to continue our research.

- Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

While the methodology described is mechanistically interesting, it is unclear what advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters. On reading this manuscript, it was unclear if any products that were synthesized could not have been just as easily prepared via a conventional coupling reaction.

Response: Please see below for the comparison with traditional activated ester method.

While the scope is broad with respect to amine coupling partners, there are limitations on the acyl donor side. Alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine, which places a limit on the substrate scope. Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic

acids/activated esters that are used in traditional amide bond forming coupling reactions.

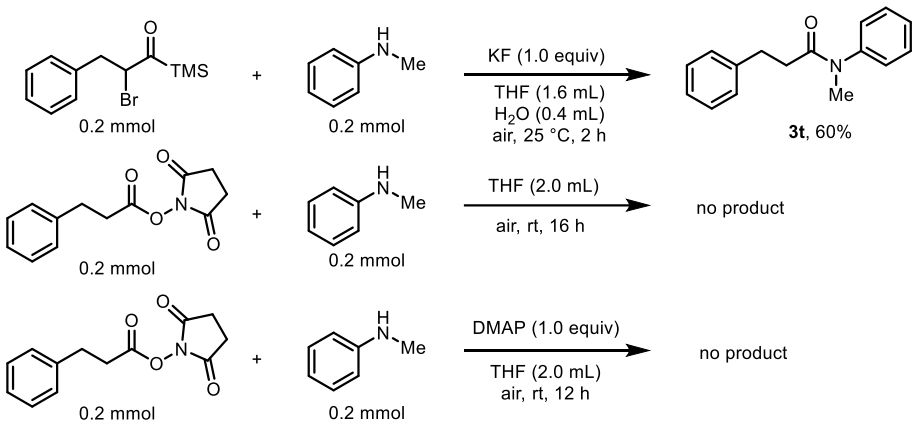
Response: Thank you for your valuable comments. We agree with you that compared to carboxylic acids, alpha-halo acyl silanes are less readily available. However, this new functionality provides different and complementary reactivity profiles from traditional carboxylic acid/amine couplings. please see below for the comparison with traditional activated ester method.

The modification of protein lysine residues by activated esters (e.g. NHS esters) is well known, and it is unclear what advantages are offered by the described method. Further, the described new method for protein modification is limited by the use of 4:1 DMSO:ammonium acetate buffer, as such a high proportion of DMSO will denature proteins, rendering useless the coupled protein products.

Response: Thank you for your critical comments.

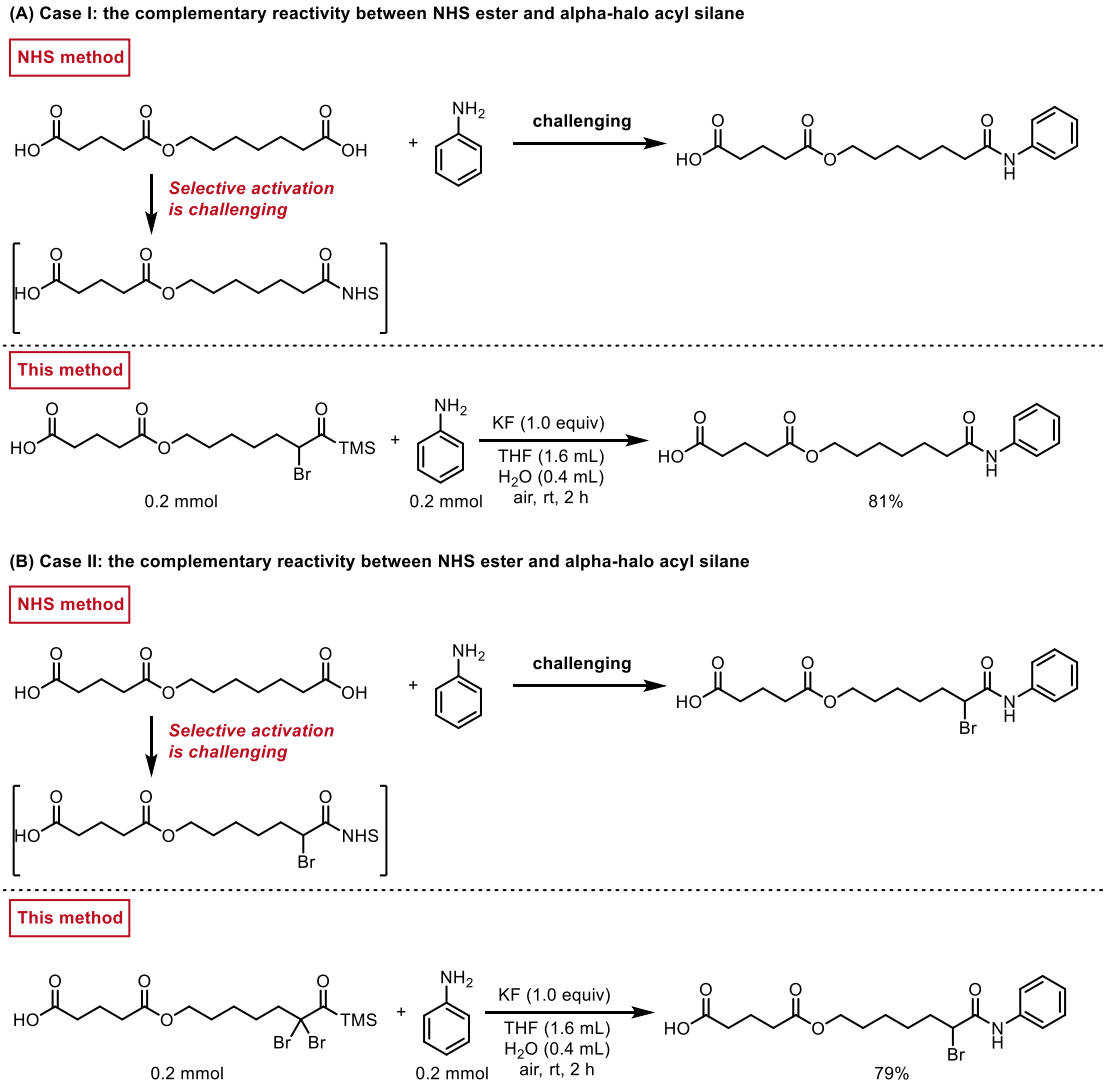
This amide ligation method shows the following advantages compared to activated esters. To facilitate the discussion, we take NHS esters as an example.

(1) When N-methyl aniline, a weak nucleophilic and bulky reagent, was used under standard conditions, the target product **3t** was obtained in 60% yield. However, with the NHS ester as the acyl donor, even in organic solvent, no product **3t** was detected. Notably, adding a stoichiometric amount of the nucleophilic catalyst DMAP also failed to produce **3t**.



(2) The NHS ester method is usually challenging to selectively functionalize one carboxyl group without affecting the other when multiple non-identical carboxylic acids are present. However, our method can provide an alternative method to form an amide without affecting the carboxylic acid group, as demonstrated in the example below. This further indicates that the method is orthogonal

to traditional carboxylic acid/amine couplings. Moreover, α-bromo amides are extremely important compounds. Introducing a bromine atom at the α-position of carboxyl group in presence of multiple carboxylic acids to synthesize an α-bromo amide is usually difficult. Nevertheless, this can be easily achieved using this method.



(3) NHS esters are capable of reacting with nucleophilic groups (such as thiols and hydroxyl groups) in addition to amino groups, potentially leading to impure labeled products (Kalkhof, S.; Sinz, A. Chances and pitfalls of chemical cross-linking with amine-reactive N-hydroxysuccinimide esters. Analytical and Bioanalytical Chemistry 2008, 392, 305-312). In contrast, this method selectively reacts with amino groups without reacting with other nucleophilic groups, effectively avoiding product impurity issues. For example, even when 120 equivalents of alpha-Br acyl silane was employed to react with lysozyme, mass spectrometry only detected the number of lysine plus terminal amino modification (+7 modifications), indicating that α-halo acylsilanes are highly

selective for amines (Figure S10).

(4) Notably, NHS esters have been reported to exhibit poor stability in weakly alkaline environments (e.g., RIPA cell lysis buffers, pH 7.4-8.0) (Mädler, S.; Bich, C.; Touboul, D.; Zenobi, R. Chemical Cross-linking with NHS Esters: A Systematic Study on Amino Acid Reactivities. J. Mass Spectrom. **2009**, 44 (5), 694-706). In contrast, our method maintains efficacy even at low concentrations under these conditions (Figure S19c), demonstrating significant advantages for protein labeling in lysates. Overall, the reaction system developed in this study shows different and complementary reactivity profiles compared to traditional NHS ester/amine couplings.

Regarding the denature issue of protein in high portion of DMSO, we evaluated the reaction performance in various DMSO/water mixtures and demonstrated successful reaction progression even at a DMSO-to-water ratio of 1:6 (v/v) (Figure S26). Under these conditions, the protein can survive without denaturing. These results broadens the potential application scope of the methodology.

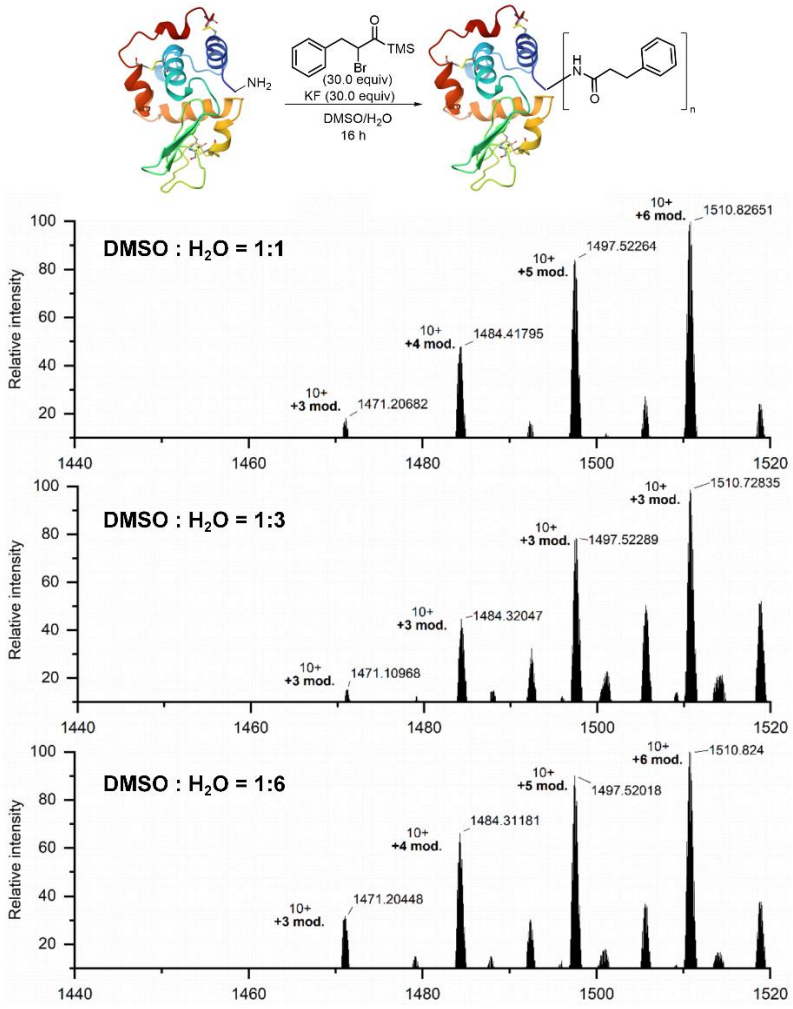


Figure S26. The reaction results of lysozyme with 1a under different ratios of DMSO/H_2O.

Additionally, using lysozyme as a model, we evaluated enzyme activity under different DMSO concentrations. Lysozyme retained approximately 70% activity after 30 minutes in 50% DMSO aqueous solution and maintained around 60% activity in 80% DMSO aqueous solution, This finding aligns with established literature, demonstrating that dimethyl sulfoxide (DMSO) exhibits reduced impact on enzymatic activity compared to common organic solvents like MeCN (Ingenbosch, K. N.; Vieyto-Nuñez, J. C.; Ruiz-Blanco, Y. B.; Mayer, C.; Hoffmann-Jacobsen, K.; Sanchez-Garcia, E. Effect of Organic Solvents on the Structure and Activity of a Minimal Lipase. J. Org. Chem. **2022**, 87 (3), 1669–1678). However, following modification with using **1a** by our method, enzymatic activity decreased to approximately 50%, likely due to modification groups occupying the enzyme's active site. If the modified groups are screened and the binding force between enzyme and substrate is strengthened, the enzyme activity may also be improved. These results suggested the method's

broad applicability in enzyme modification, enabling rapid functionalization while preserving partial enzymatic activity, which has great significance for the optimization of active enzymes.

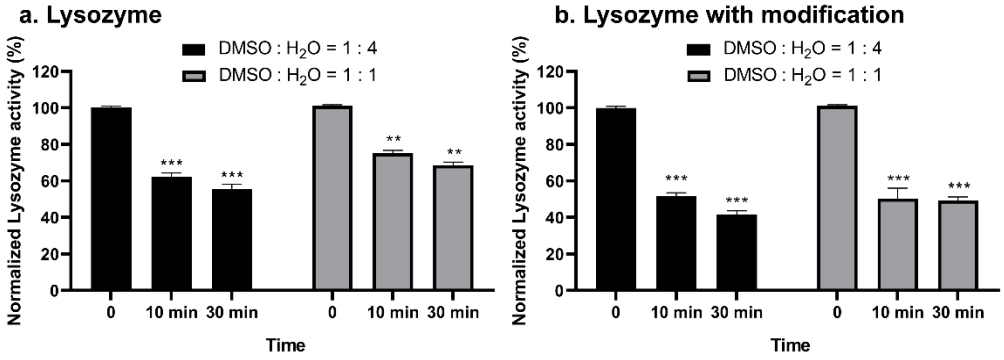


Figure S27. The lysozyme activity under different conditions was detected by ADP-Glo.

Overall, there is a need to establish the niche that will be filled by using alpha-bromoacylsilanes as coupling partners. Direct comparisons to NHS ester chemistry would be useful.

Response: Thank you for your critical comments. Based on your suggestion as well as the suggestions from referee 1, we have performed extensive comparison experiments between the alpha-halo acyl silane and activated carboxylic esters, which reveal that alpha-halo acyl silanes indeed offered different reactivities.

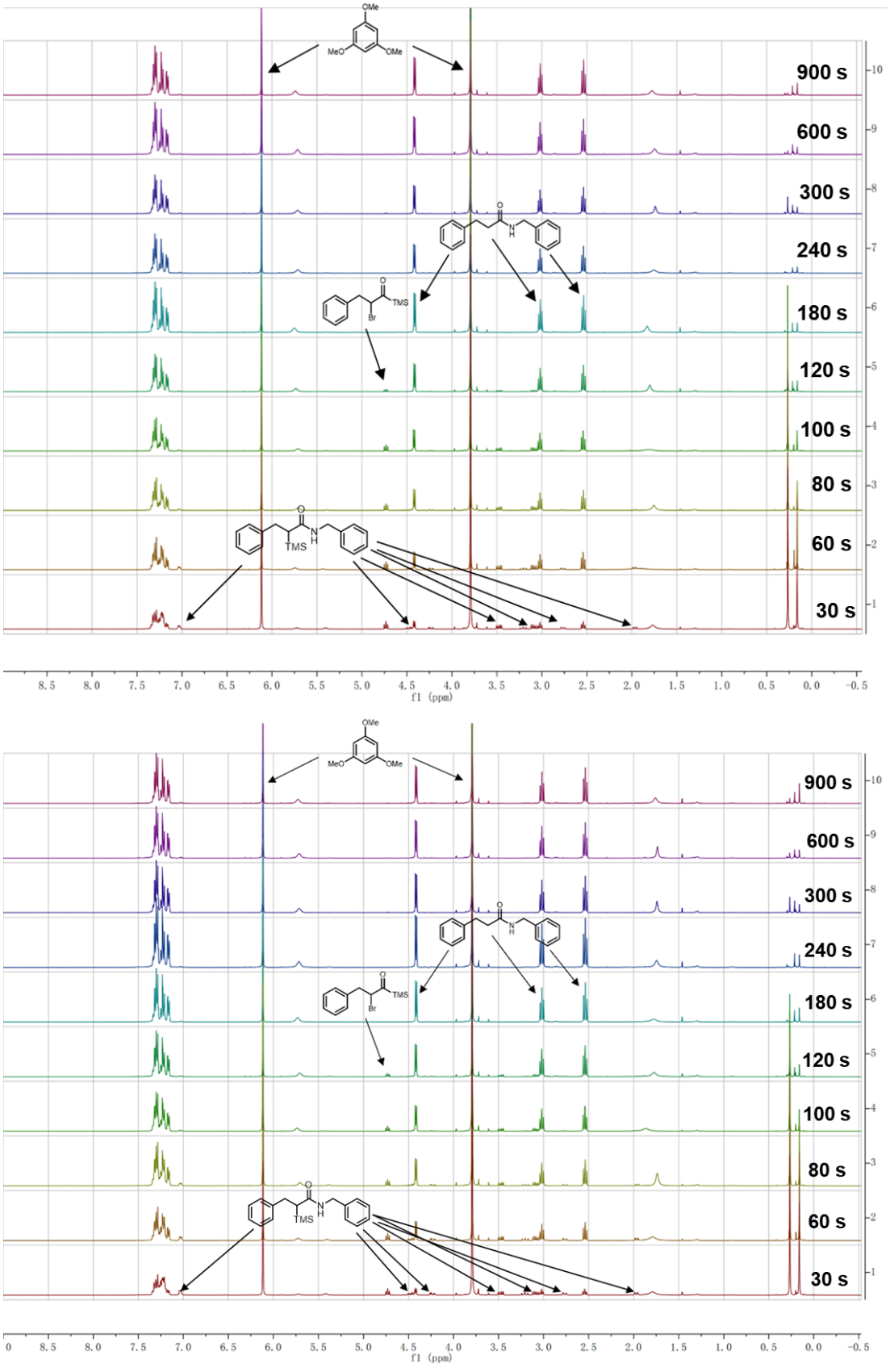
- Does the work support the conclusions and claims, or is additional evidence needed?

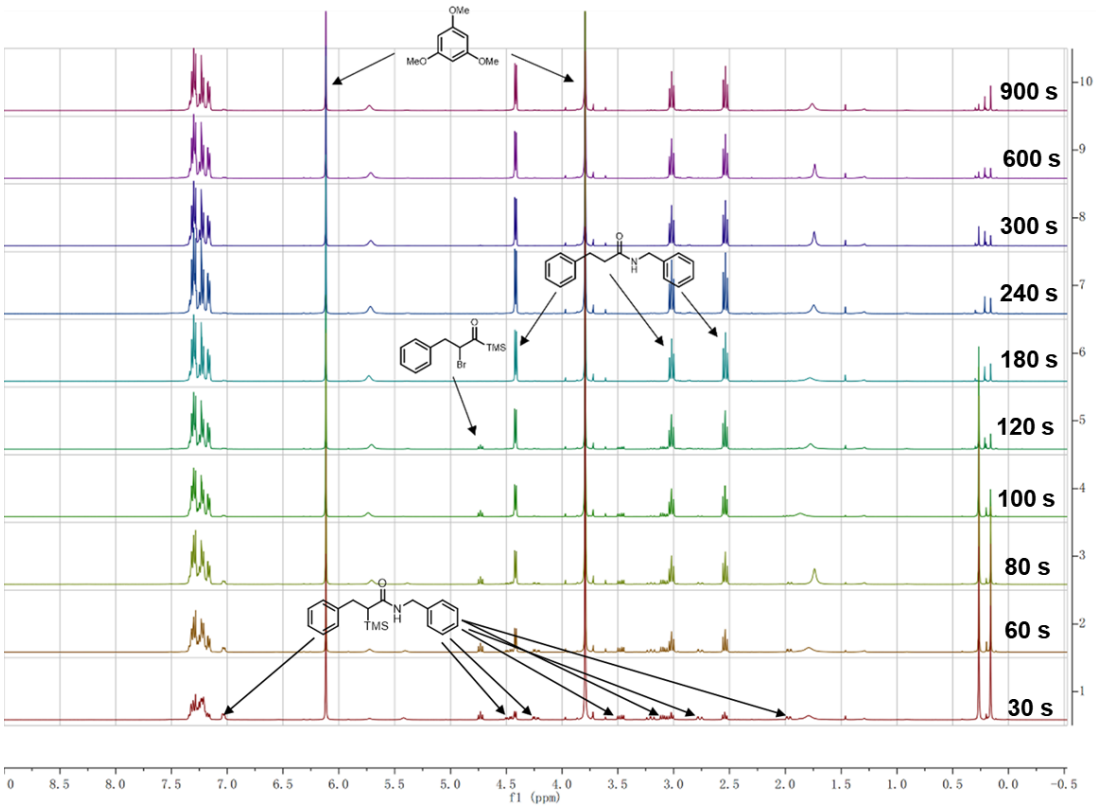
The mechanism proposed by the authors involves a 1,2-silyl shift to produce an alpha-silylamide intermediate. This is proposed largely based on the isolation of 3-Int from a reaction performed without KF (Fig 2D). While this mechanism is plausible, the isolation of 3-Int does not prove that it is an intermediate that forms under the reaction conditions. It would seem necessary to rule out the formation of ketene intermediates, which of course are well known intermediates for amide bond forming reactions with amines. Ketenes could be formed via an elimination mechanism which is well precedented to produce strained alkenes, e.g. https://pubs.acs.org/doi/full/10.1021/acs.chemrev.0c01011

Response: Thank you for your critical and valuable comments. This mechanistic proposal is not only based on isolating 3-Int from a KF-free reaction. Two key points have been added to the SI:

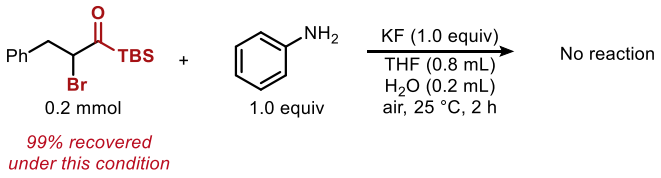
(1) Under standard conditions, by shortening the reaction time, 3-Int formation was observed via ^{1}H

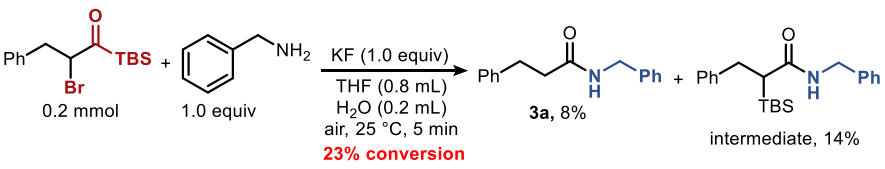
NMR. As the reaction proceeded, 3-Int gradually disappeared, yielding the desired product.





(2) To confirm that the reaction starts with amine reacting with the acyl silane, not the acyl silane with KF to form the ketene, we used more hindered TBS acyl silane and less reactive aniline to run the control experiments. No reaction occurred after 2 hours and the alpha-bromo acyl silane was completely recovered. However, when aniline was switched to benzylamine, 8% of the desired product and 14% of the intermediate formed within 5 minutes. This shows that the reaction is initiated by the amine reacting with acyl silane. If the ketene intermediate was formed, the alpha-bromo acyl silane cannot be recovered. Therefore, the possibility to generate a ketene can be ruled out. These control experiments also suggest that the reactivities of acyl silane and amines must be carefully balanced.



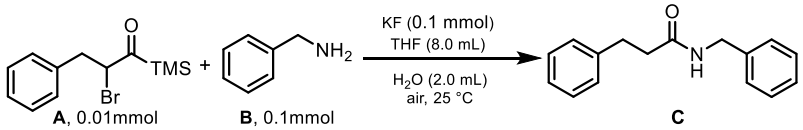


• Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

The authors report a 2nd order rate constant, but they should not report more than a half-life based on the data presented. Their data (Fig 2B) follows ~zero order behavior. This may be an artifact of using sampling rather than more precise in situ monitoring of kinetics. To extract a 2nd order rate constant from pseudo first order behavior, data should be collected at varied concentrations, and a plot of k(obs) vs concentration should show a linear plot with a zero intercept.

Response: Thank you for you great suggestions and comments. Following your suggestion, we redetermined the constant. The data all fall within the half-life period, and the plot shows a zero intercept. To measure the rate constant more accurately, we measured it not only at 1 mM but also at 0.5 mM and 0.1 mM concentrations.

1.0 mM



Under an ambient atmosphere, benzylamine (10.7 mg, 0.1 mmol, 10.0 equiv), tetrahydrofuran (8.0 mL), H_2O (2.0 mL), KF (5.8 mg, 0.1 mmol, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (2.8 mg, 0.01 mmol, 1.0 equiv) were added to an oven-dried round bottom flask (25.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [**A**$_0$] - [**C**] versus time (sec) were plotted (**Figure S1**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.611 $M^{-1}\cdot s^{-1}$.

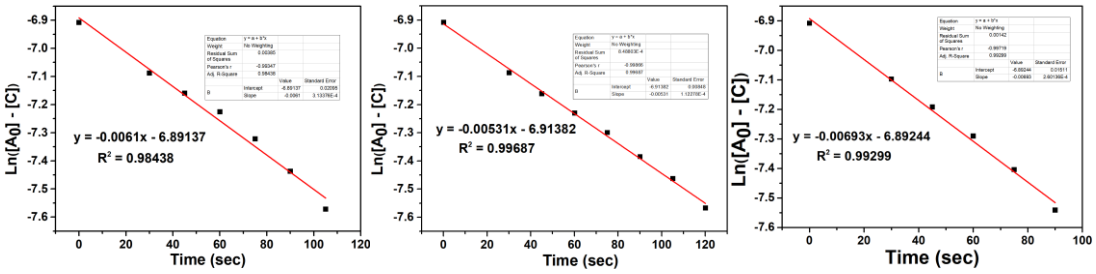


Figure S1. Pseudo-first order kinetics

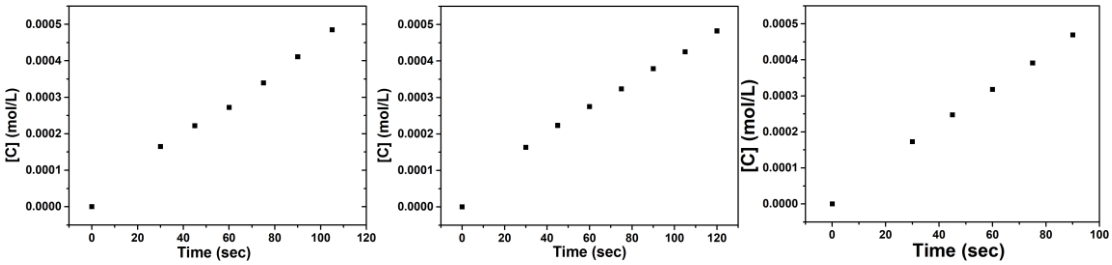
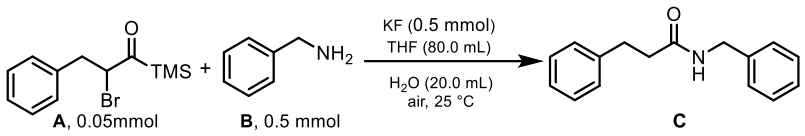


Figure S2. Product vs time (sec)

Subsequently, we also measured a rate constant of 0.973 $M^{-1} \cdot s^{-1}$ for 0.5 mM within the half-life.

0.5 mM



Under an ambient atmosphere, benzylamine (53.5 mg, 0.5 mmol, 10.0 equiv), tetrahydrofuran (80.0 mL), H_2O (20.0 mL), KF (29.0 mg, 0.5 mmol, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (14.2 mg, 0.05 mmol, 1.0 equiv) were added to an oven-dried round bottom flask (250.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [**A**$_0$] - [**C**] versus time (sec) were plotted (**Figure S3**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.973 $M^{-1} \cdot s^{-1}$.

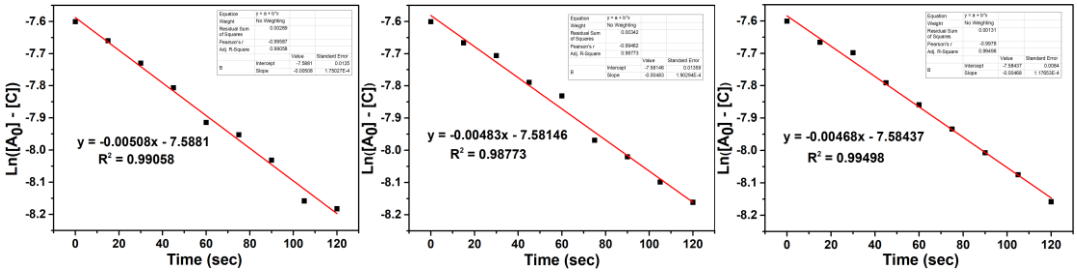


Figure S3. Pseudo-first order kinetics

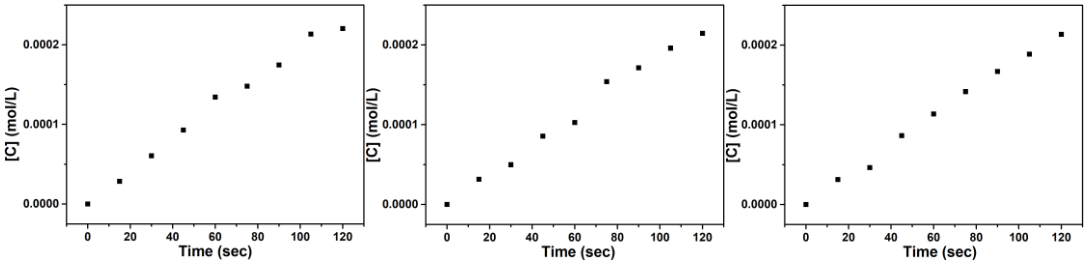
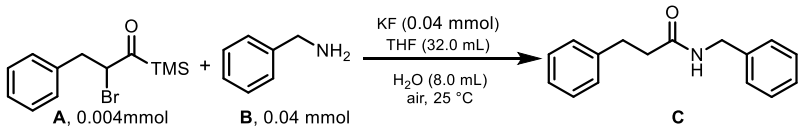


Figure S4. Product vs time (sec)

Finally, we measured a rate constant of 0.861 $M^{-1}{\cdot}s^{-1}$ for 0.1 mM within the half-life.

0.1 mM



Under an ambient atmosphere, benzylamine (1.0 M, 40.0 mL, 10.0 equiv), tetrahydrofuran (32.0 mL), H_2O (8.0 mL), KF ((1.0 M, 40.0 mL, 10.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (0.1 M, 40.0 mL, 1.0 equiv) were added to an oven-dried round bottom flask (100.0 mL) with a magnetic bar. The reaction was monitored by GC-MS, using mesitylene as standards. The reactions were repeated 3 times.

Second-order kinetics

The reaction between benzylamine and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one is a bimolecular process that follows second-order kinetics. Reaction rates can be written as follows:

$$V = -\frac{d[A]}{dt} = k[A][B] = k_1[A]$$

$$[A] = [A_0] - [C]$$

$$\ln([A_0] - [C]) = -k_1 t + c$$

Logarithmic scale concentrations of [$\mathbf{A}_0$] - [**C**] versus time (sec) were plotted (**Figure S5**), and the slopes of their fitted curve were determined as k_1. From these, the second-order rate constant of this reaction was estimated as 0.861 $M^{-1}{\cdot}s^{-1}$.

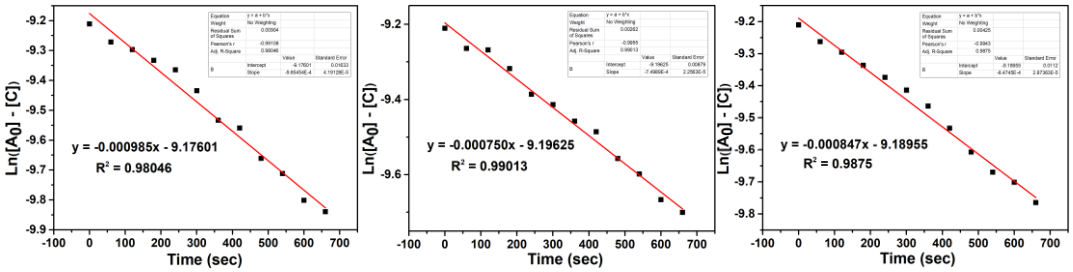


Figure S5. Pseudo-first order kinetics

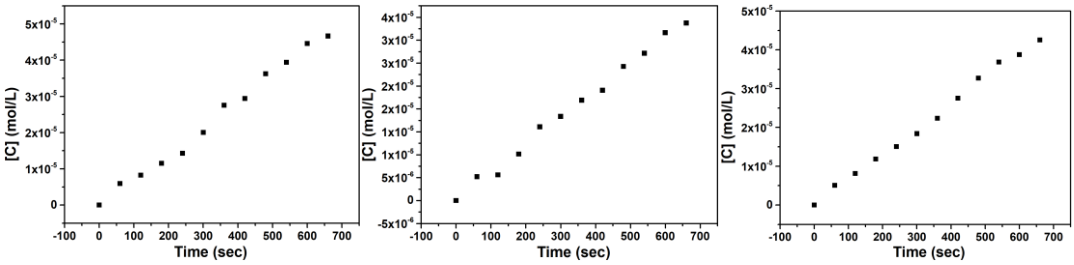


Figure S6. Product vs time (sec)

- Is the methodology sound? Does the work meet the expected standards in your field?

Yes

Response: Thank you for your valuable time to review our manuscript.

- Is there enough detail provided in the methods for the work to be reproduced?

Yes

Response: Thank you for your valuable time to review our manuscript.

Response to Decision Letter

Reviewer Comments to Author:

Reviewer: 1

Comments:

I have reviewed the first version of this manuscript, and have read the responses and changes made. Although I still have some concerns about the broad applicability, I can see reasonable case for occasions where this unique chemistry may be of valuable. I am therefore, in principle, supportive of publication.

However, and unless this is addressed properly I withdraw my support, I still do not think the kinetics are properly done. If the reaction was really 0.7 M-1 s-1, with the setup of 10 equiv of amine and 1 equiv of the acyl silane, at a starting concentration of 0.1 M silane, the reaction should be fully completed in less than 10 seconds. This doesn't seem to be the case, and I believe the authors have calculated instead a pseudo-first order rate l which would be measured as s-1). This needs to be corrected before further review.

Response:

Thank you for your constructive comments and suggestions. Based on your suggestions, we initially tested the reaction using 1.0 M benzylamine and 0.1 M acylsilane as starting materials. Under standard conditions (THF 0.8 mL, H_2O 0.2 mL, 1.0 M KF), the reaction was quenched rapidly in 10 s. The sample was analyzed by ^{1}H NMR. The assay yield of the product was calculated with 1,3,5-trimethoxybenzene (21.2 mg, 1.26 equiv) as an internal standard. By analyzing through ^{1}H NMR, we observed that the starting material (acyl silane) was indeed completely converted to the intermediate as well as some final products. (**Fig. S7**) This result suggest that under our standard conditions, the amine reacts with acyl silane very fast and the conversion of the alpha-silyl amide to the final amide is kind of slow.

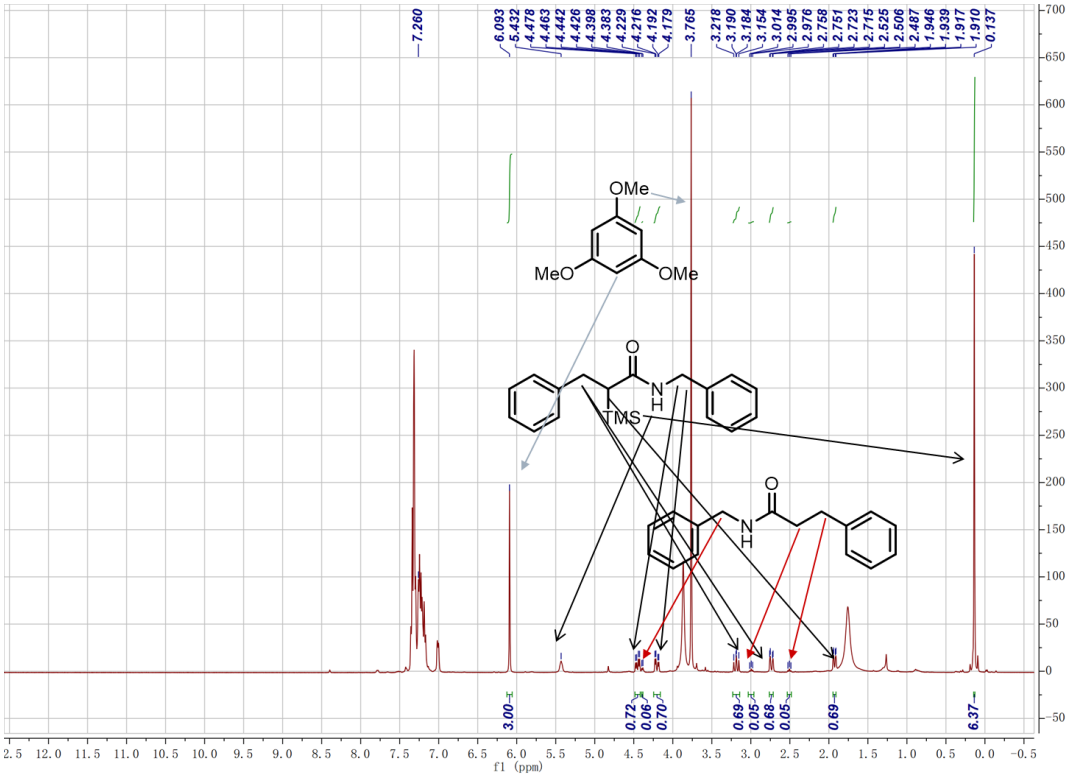
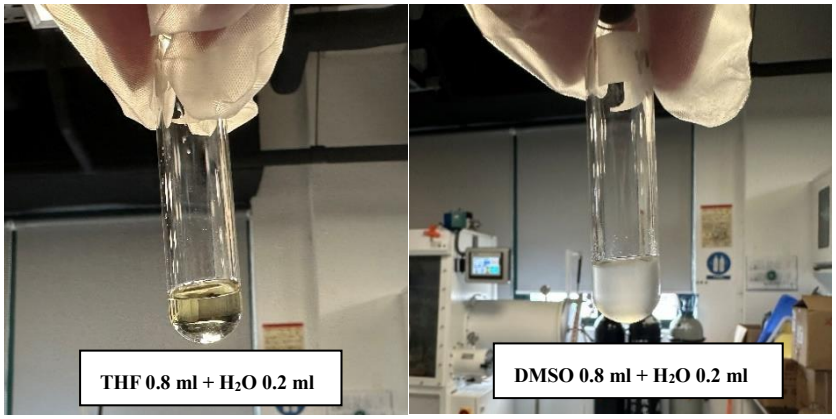


Figure S7. 1H NMR of the resulting mixtures for 3a, 3-Int and 1,3,5-trimethoxybenzene

We hypothesized that this outcome resulted from phase separation between the concentrated KF aqueous solution and THF (shown in the picture below), which reduced the effective KF concentration in the reaction system and prevented complete conversion of alpha-silyl intermediate to the amide product through desilylation. To address this issue, we changed THF by using DMSO, which resulted in a more miscible solution (shown in the picture below) and significantly increased the effective KF concentration.



With this change, the reaction achieved complete conversion of reactants to products within 10 seconds. Further testing at 5 seconds demonstrated that the reaction could reach

completion within this shorter timeframe (**Fig. S8**), consistent with our calculated rate constant.

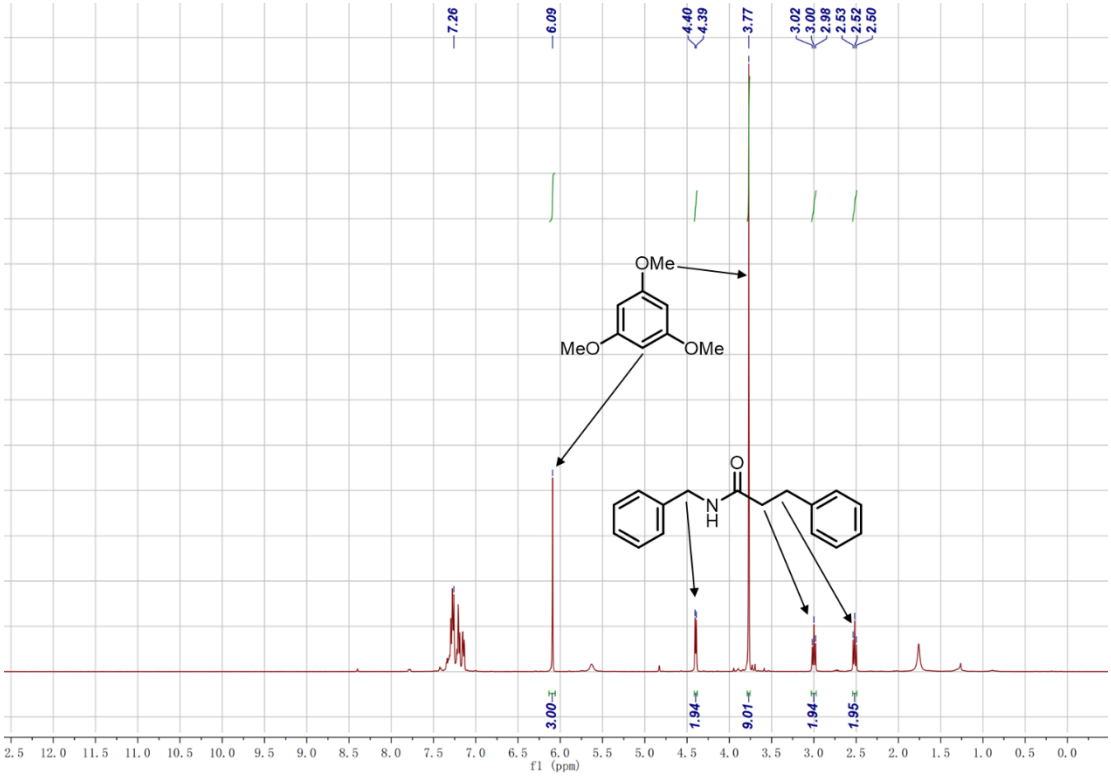


Figure S8. ^{1}H NMR of the resulting mixtures for **3a** and 1,3,5-trimethoxybenzene

Additionally, we sincerely apologize for the previous insufficiently clear description of the rate constant calculation, which may have led to misunderstandings. To address this, we have revised the relevant section (**SI: page S15-20**) for improved clarification.

Following established literature methods[1-3], we first determined the pseudo-first-order rate constant (K_1, s^{-1}) by plotting the logarithmic concentration of $[A_0]$–[C] against time (in seconds). The second-order rate constant (k, $M^{-1}\cdot s^{-1}$) was then estimated from K_1. Additionally, we have listed a comprehensive data table included k_1 and k values for each experimental set, along with the equation of the calculation method (**SI: page S15**). These updates aim to present the kinetic analysis more accurately.

(1). Noda, H.; Erős, G.; Bode, J. W. Rapid Ligations with Equimolar Reactants in Water with the Potassium Acyltrifluoroborate (KAT) Amide Formation. *J. Am. Chem. Soc.* **2014**, *136*, 5611-5614.

(2). Maji, B.; Breugst, M.; Mayr, H. N‐Heterocyclic Carbenes: Organocatalysts with Moderate Nucleophilicity but Extraordinarily High Lewis Basicity. *Angew. Chem. Int. Ed.*

2011, *50*, 6915-6919.

(3). Gordon, C. G.; Mackey, J. L.; Jewett, J. C.; Sletten, E. M.; Houk, K. N.; Bertozzi, C. R. Reactivity of Biarylazacyclooctynones in Copper-Free Click Chemistry. *J. Am. Chem. Soc*. **2012**, *134*, 9199-9208.

Reviewer: 2

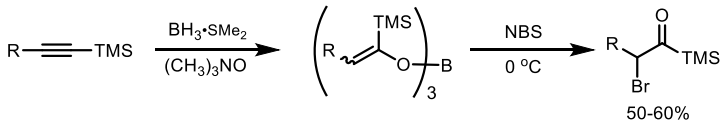
Comments:

In a prior review of this manuscript, it was pointed out that the methodology described is mechanistically interesting, but it was unclear what advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters. It was also pointed out that the scope is limited on the acyl donor side as alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine, which places a limit on the substrate scope.

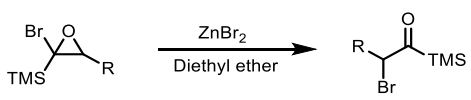
Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic acids/activated esters that are used in traditional amide bond-forming coupling reactions.

Response:

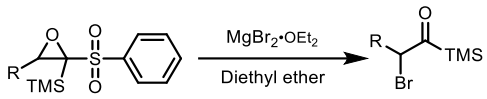
Thank you for pointing this out. The synthetic methods of α-bromoacylsilanes have also been constantly updated in recent years. In addition to alpha-bromination of acyl silane, there are also many other attractive methods. The following are other reported feasible synthetic methods:



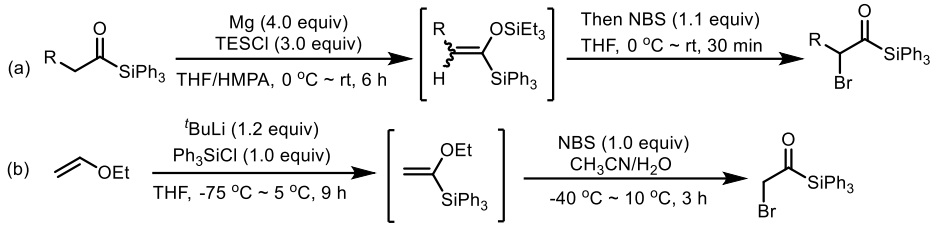
(a) Page, P. C. B.; Rosenthal, S. *Tetrahedron Lett*. **1986**, *27*, 5421; (b) Hooz, J.; Bridson, J. N. *Can. J. Chem*. **1972**, *50*, 2387.



Horiuchi, Y.; Taniguchi, M.; Oshima, K.; Utimoto, K. *Tetrahedron Lett*. **1995**, *36*, 5353.



Hewkin, C.T.; Jackson, R. F. W. *Tetrahedron Lett*. **1990**, 31, 1877.



Zhou, G. Li, Y.P.; Liu, Y.; He, X.-Q.; Liu, S.-S.; Shen, X. *J. Am. Chem. Soc*. **2025**, *147*, 15955.

All the above synthetic methods are based on the starting materials other than carboxylic acids, which are complementary to the carboxylic acid approaches.

In their response, the authors have provided an example of N-methylaniline coupling that fails with an NHS ester, which is an important point to make. They provide a second demonstration of selective mono-functionalization, which is not compelling since they compare a mono-activated substrate (requiring synthesis) to a simpler diacid. Additionally, they show that lysozyme (a protein with disulfides but without free thiols) can be selectively functionalized 7 times, and they speculate that this could not be accomplished with NHS ester chemistry by citing literature but without running the comparison. Likely, with a protein containing free cysteine, both methods will modify thiols from a kinetic perspective to give labile thioesters.

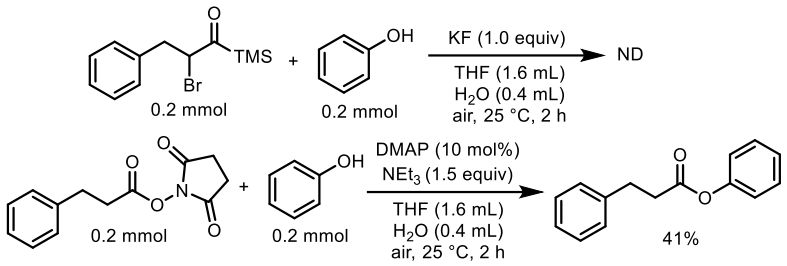
Overall, the authors make a stronger case, especially with the *N*-methylaniline, but the point still holds that their applications are rather niche.

Response:

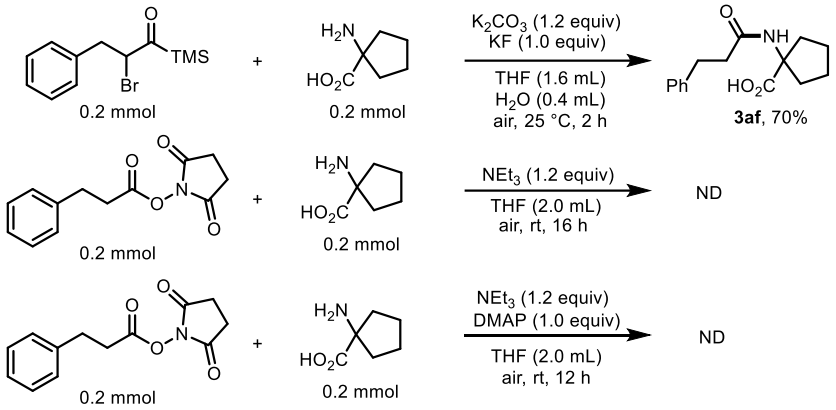
Thank you for your critical comments. Following several decades' optimization, the NHS ester does have a wide range of applications and it is not our intention to develop a new method to completely replace the classical NHS ester. Instead, we wish to disclose the unusual and novel transformations that has the potential applications in chemical biology and material science. Discovering new transformations is our primary focus currently. How to use this chemistry in a convincing way is our subsequent goal. Nonetheless, we indeed

observed some advantages that the NHS ester chemistry lacks：

(1) Compared to the NHS esters, this method does not react with phenol to form esters, whereas NHS esters do. This indicates that the method has better selectivity, as it will react solely with amines in the presence of phenolic hydroxyl groups, while NHS esters may produce ester by-products.



(2) In addition to *N*-methylaniline, we also found that under standard conditions, the target product **3af** can be obtained with a yield of 70% using cycloleucine as a nucleophile. However, when using the NHS ester activation strategy, the product **3af** was not detected even in organic solvents. It is worth noting that even with the addition of equivalent amounts of DMAP, **3af** was not detected.



In summary, the reaction demonstrates application potential due to its rapid kinetics, aqueous-phase compatibility, and high selectivity.

It was also pointed out that the described new method for protein modification is limited by the use of 4:1 DMSO:ammonium acetate buffer, as such a high proportion of DMSO will denature proteins. To this point, the authors have shown that a reduced proportion of DMSO (1:6) is sufficient for lysozyme modification. This is a satisfactory response.

Response:

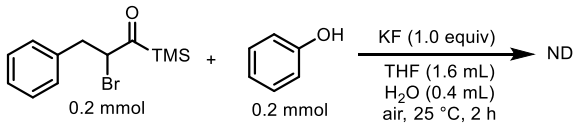
Thank you for your positive comments.

In terms of the mechanism, it was pointed out that a ketene mechanism had not been ruled out. The authors run two additional experiments, showing observation of 3-Int and the lack of reaction when aniline is used as nucleophile vs low yielding reaction when benzylamine is nucleophile. The experiments suggest a role for amine, but do not actually rule out a ketene mechanism, as the amine base may initiate the elimination reaction, producing intermediate silylamine, which may undergo addition to the ketene. Addition of TMS-dimethylamine to ketene itself is known (https://doi.org/10.1016/S0022-328X(00)88613-1).

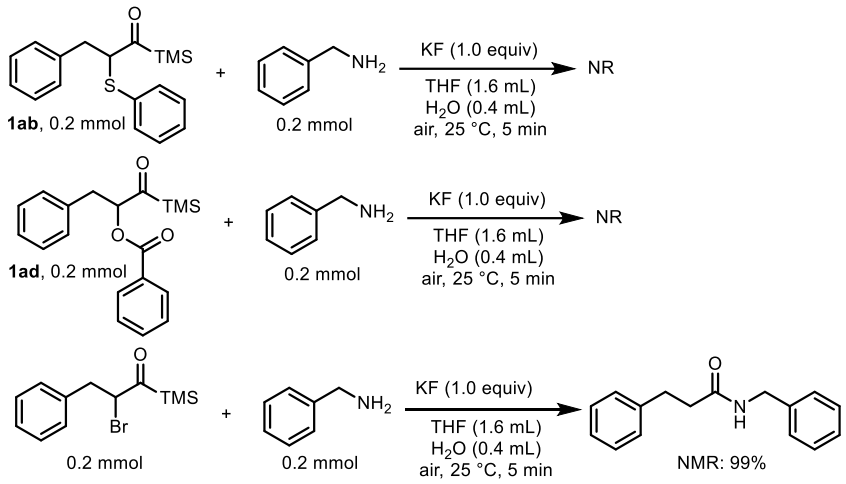
Response:

Thank you for your critical comments. To further elucidate the reaction mechanism, we conducted a series of control experiments to exclude the possibility of ketene intermediates.

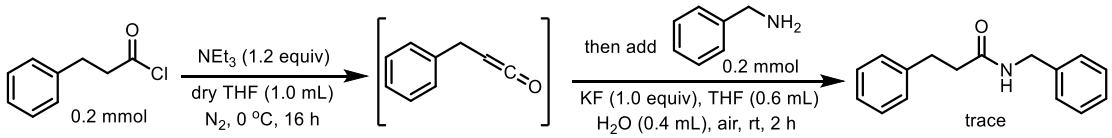
（1）Previous studies (Sheibani et al., *Synthesis*, **2006**, 3, 435-438) have demonstrated that ketene intermediates can react with phenol to form esters. However, under our reaction conditions, no ester byproducts were detected, suggesting that the transformation does not proceed via a ketene intermediate pathway.



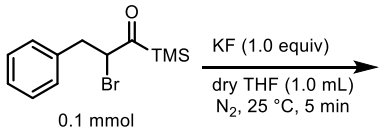
（2）Substitution of the bromine atom with other group under standard reaction conditions revealed that substrates **1ab** and **1ad** failed to yield the target product. Notably, no desilylation byproducts were detected, and the starting materials were recovered with 99% efficiency. These observations suggest that the KF-mediated desilylation of acylsilanes proceeds sluggishly under these conditions. In contrast, when α-bromo acylsilane was employed as the starting material, the desired product was obtained in 99% yield. This difference in reactivity strongly supports our proposed mechanism wherein amine reagents preferentially engage with acylsilanes to form α-silicon amide intermediates.



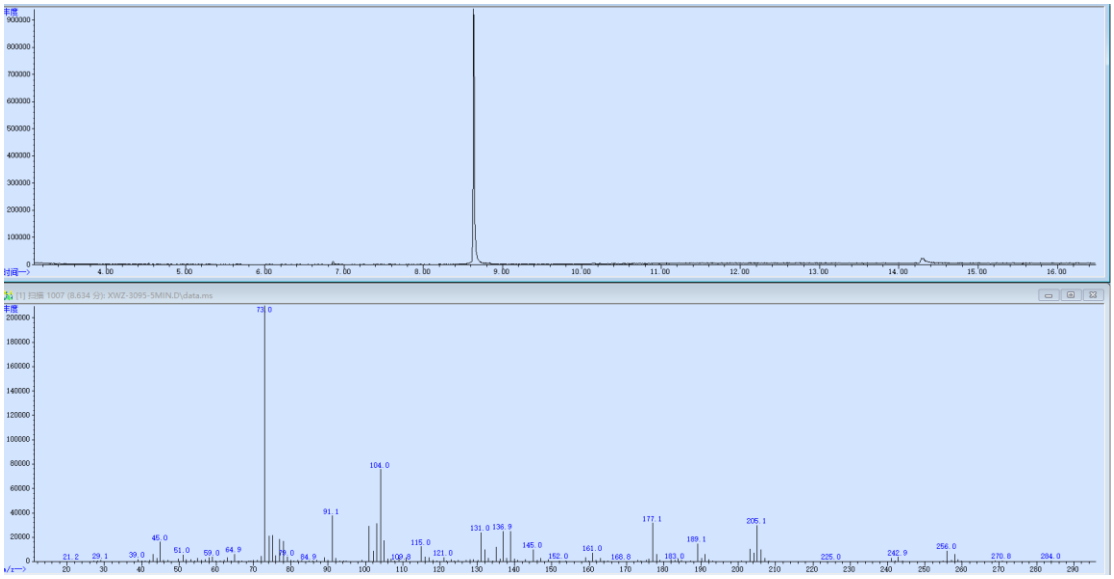
（3）It is well-recognized that ketene is highly water sensitive. Given that our reaction system contains water in substantial excess relative to benzylamine (the nucleophilic reagent), we believed that efficient conversion of ketene to amide in high yield would be challenging under these conditions. To verify this hypothesis, we prepared the ketene substrate following literature procedures (a) Tidwell, T. T. *Sci. Synth.* **2006**, *23*, 569-678; (b) Baigrie, L. M.; Seiklay, H. R.; Tidwell, T. T. *J. Am. Chem. Soc*. **1985**, *107*, 5391. When the prepared ketene was subjected to our standard reaction conditions, only trace amount of the target amide product was obtained. This control experiment with a ketene intermediate prepared by other established approach strongly suggests that our reaction does not involve a ketene intermediate.



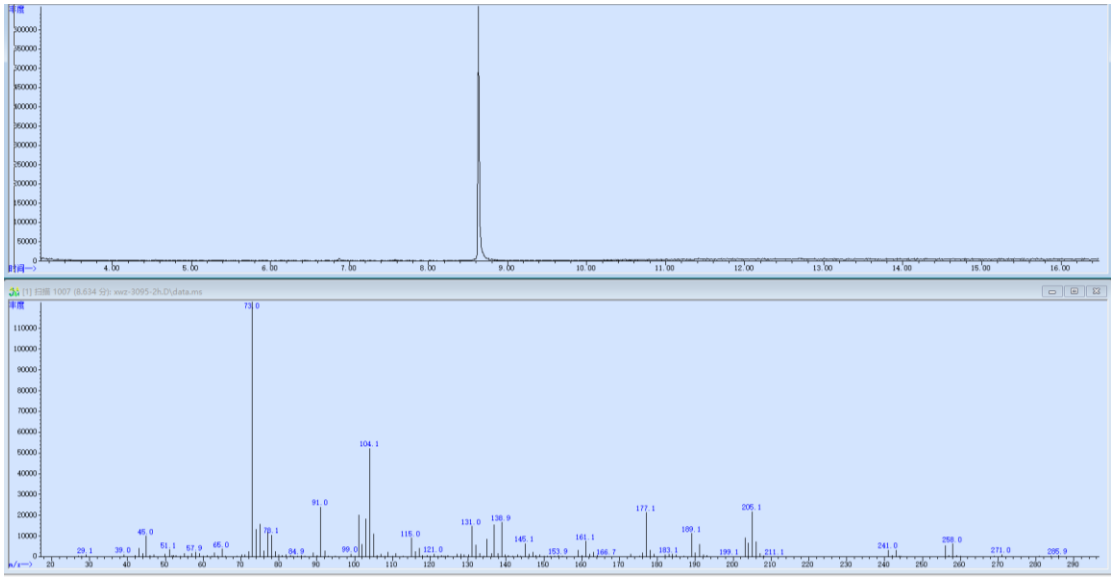
（4）To further eliminate the possibility of ketene mechanism, we reacted α – bromo acylsilane with potassium fluoride in an ultra-dry THF solvent and inert gas atmosphere, and monitored its dynamic changes over time using GC-MS technology. The results showed that the conversion of the substrate was still extremely low even after 24 h. This further confirms that the mechanistic hypothesis of the intermediate of ketene in this reaction pathway is unlikely.



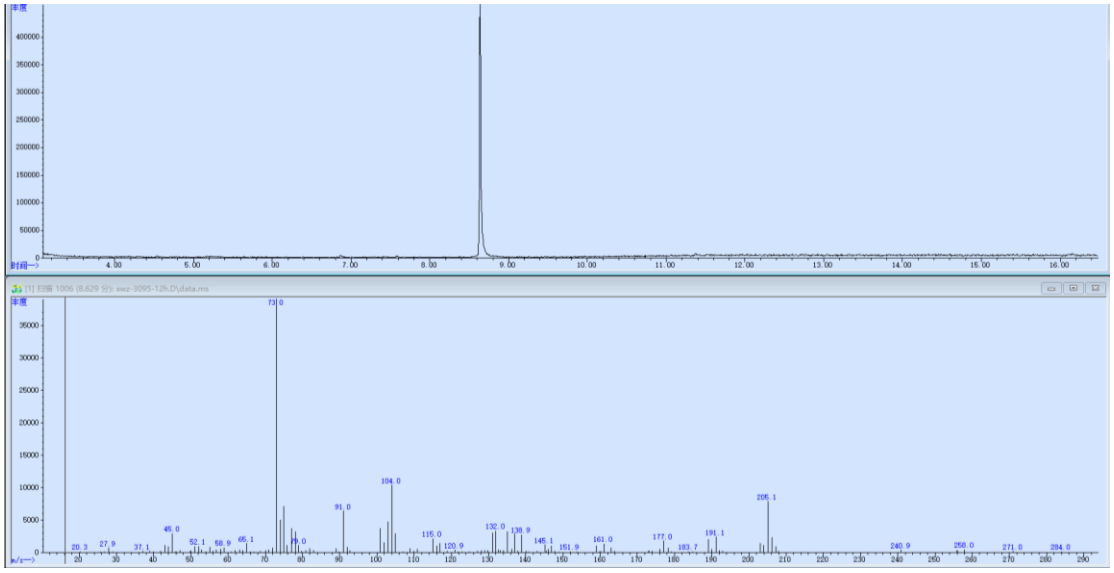
5 min



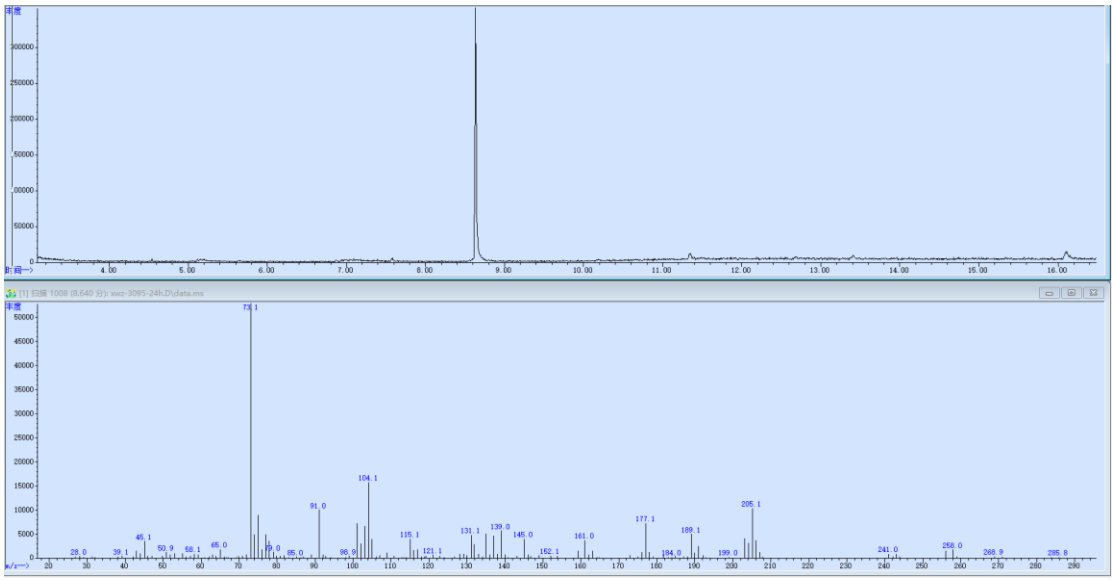
2 h



12 h



24 h



Collectively, these experimental results demonstrate that our reaction is initiated by the nucleophilic addition of amine towards acylsilane rather than the fluoride-promoted desilylation.

Response Letter

Reviewer Comments to Author:

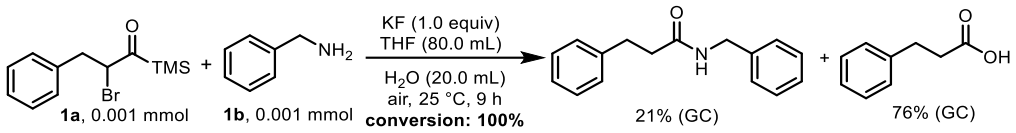
Reviewer: 1

Comments:

I appreciate the authors correcting the rate constant calculation and now clearly reporting the biphasic conditions under the initially reported experimental protocol. The reaction at 1:1 stoichiometry at 10 micromolar (20% conversion) is the more convincing data. This seems to be missing from the SI. If this is added, I support publication of this manuscript.

Response: Thank you for your valuable advice. We have added the reaction data obtained under 10 μM, 1:1 stoichiometric conditions to the Supporting Information. Note that under the 10 μM condition, the extremely low concentration of amine cannot compete with water to react with the electrophile, thereby leading to carboxylic acid as the major product.

10 μM concentration



Under an ambient atmosphere, benzylamine (0.01 M, 100.0 μL, 1.0 equiv), tetrahydrofuran (80.0 mL), H_2O (20.0 mL), KF (0.01 M, 100.0 μL, 1.0 equiv), and 2-bromo-3-phenyl-1-(trimethylsilyl)propan-1-one (0.01 M, 100.0 μL, 1.0 equiv) were added to an oven-dried round bottom flask (250.0 mL) with a round-bottom flask. The reaction was stirred at 25 °C for 9 h. The reaction was monitored by GC-MS, using 1,3,5-trimethoxybenzen as an internal standard.

Reviewer: 2

Comments:

In two prior reviews of this manuscript, it was pointed out that the methodology described is interesting, but it was unclear what *major* advantage it will hold over traditional coupling reactions involving carboxylic acids or activated esters, especially given that alpha-bromoacylsilanes must be synthesized under oxidizing conditions using bromine,

which places a limit on the substrate scope. Acylsilanes themselves are not especially readily available starting materials, and are less attractive starting materials relative to common carboxylic acids/activated esters that are used in traditional amide bond-forming coupling reactions.

In further response, the authors have provided the example of acylation of cycloleucine, which was unsuccessful with NHS chemistry in their hands. However, cycloleucine acylations with other reagents (acid chlorides, mixed anhydrides) are known in the literature. The authors have also discussed that phenols react with NHS esters, suggesting chemoselectivity that was not otherwise available. However, numerous examples of acylations of amines in preference to phenolic groups are well known.

Regarding mechanism, it was pointed out in a previous review that the authors experiments suggest a role for amine, but do not rule out a ketene mechanism. Their response that phenol does not react (point #1), and their response regarding lack of reactivity in dry THF (point #4) do not really add new information since amine is absent in these experiments. In point #2, they discuss that the mechanism is leaving group dependent, but it is unclear how that provides evidence for either mechanism. Finally (point #3) the authors provide an experiment where benzylketene is independently generated; this would be compelling, except that monoalkylketenes are unstable! (see Tidwell, Science of Synthesis, 23.14, 2006). Thus, reacting an phenylethylacylchloride for 14 hours would consume starting material, but not to give stable benzylketene.

Publication in a more specialized journal is advised.

Response: Thank you for your critical comments. Your suggestions have significantly improved the manuscript and helped us to understand this reaction more deeply.
We are aware that our method can not replace the coupling of carboxylic acid and amine to form the amide; but our discovery contributes a new reaction to build amides. We have demonstrated the synthetic applications of this method in terms of small molecule

synthesis, late-stage functionalization of drugs and cyclic peptide, the bioconjugation of proteins and polymer synthesis. Although other approaches indeed exist to do these things, we believe that the community still welcomes a new method. We will keep polishing this method towards compelling applications in future.