JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Peptide Bond Formation of Amino Acids by Transient Masking with Silylating Reagents

Wataru Muramatsu* and Hisashi Yamamoto*

Cite This: J. Am. Chem. Soc. 2021, 143, 6792–6797
Read Online
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A one-pot peptide bond-forming reaction has been developed using unprotected amino acids and peptides. Two different silylating reagents, $HSi[OCH(CF_3)_2]_3$ and MTBSTFA, are instrumental for the successful implementation of this approach, being used for the activation and transient masking of unprotected amino acids and peptides at *C*-termini and *N*-termini, respectively. Furthermore, CsF and imidazole are used as catalysts, activating $HSi[OCH(CF_3)_2]_3$ and also accelerating chemoselective silylation. This method is versatile as it tolerates side chains that bear a range of functional groups, while providing up to >99% yields of corresponding peptides without any racemization or polymerization.

Amide bond-forming reactions are of paramount importance in the pharmaceutical industry.[1] Owing to a rapid growth in the global peptide therapeutics market, peptide bond-forming reactions have recently garnered significant attention as they play a pivotal role in peptide synthesis.[2] To date, the elongation of peptide chains is generally accomplished by iterative operations, namely: (1) the *N*-protection of amino acid **A**, which serves as a nucleophile to form **P−A**; (2) the activation of the carboxyl group on **P−A** with peptide coupling reagents to form **P−A−X**; (3) amidation with **B−P′** to form **P−A−B−P′**; and (4) deprotection to form **A−B−P′** (Scheme 1a).[3] Multiple purification operations are necessitated for the elongation of the peptide chains. If the targeted peptides are racemized, cumbersome manipulations using high performance liquid chromatography (HPLC) are further required, preventing the mass synthesis of peptides at low costs. During peptide synthesis on an industrial scale,[4] several metric tons of organic solvents are required to separate stereoisomers. Clearly, this makes peptide synthesis not only expensive, but also harmful to the environment as well.[5] Therefore, efficient, practical, and simple protocols have been a long-standing pursuit in academia and industry, along with sophisticated peptide coupling reagents that can be used in peptide synthesis to completely avoid racemization.[3]

Over the past quarter century, one-pot syntheses have been extensively developed due to their high applicability toward green and sustainable chemistry.[6] The one-pot strategy has been widely adopted in organic synthesis and used to efficiently furnish a number of natural products and pharmaceuticals.[6,7] Owing to the features of one-pot syntheses, such as reduced waste, labor, time, energy, solvent use, and cost,[6] we believe that incorporating an environmentally benign one-pot strategy into peptide manufacturing is desirable (Scheme 1b).

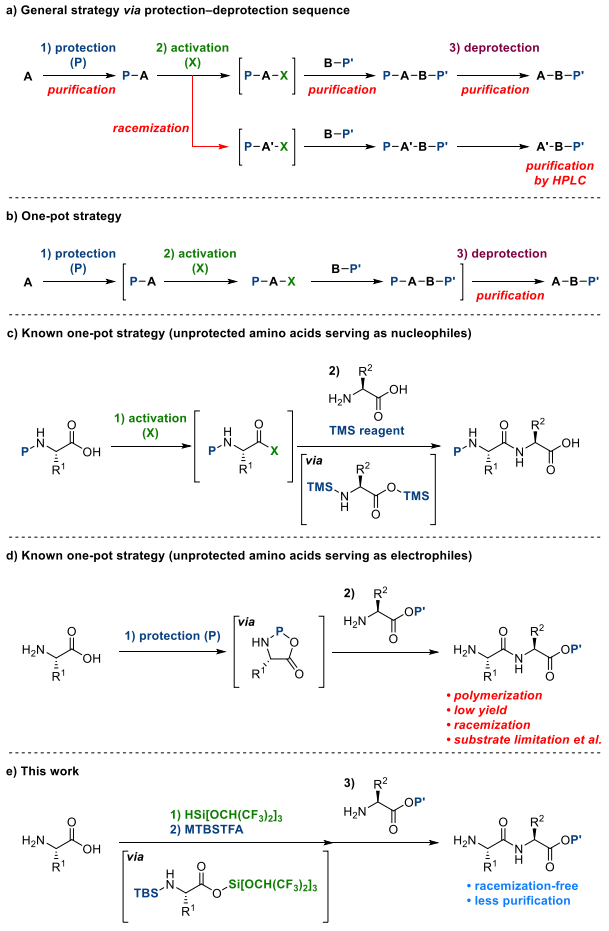
Peptide syntheses using unprotected amino acids as nucleophilic components have been frequently reported (Scheme 1c).[8] Recently, Kurasaki and co-workers determined that sterically demanding unprotected *N*-methylamino acids can be used for peptide synthesis.[8l] Remarkably, Fuse and co-workers changed the operation assembly from batch to flow for rapid peptide synthesis.[8j] However, concerns regarding racemization and potential health hazards[9] still remain, when using peptide coupling reagents. Conversely, reports of peptide bond-forming reaction utilizing unprotected amino acids as electrophilic components are limited (Scheme 1d). Although reports of formation of peptide bonds proceeding through the formation of heterocyclic (mostly five-membered) intermediates in bidentate protection/activation systems have been few, the low and uncontrolled reactivity of the heterocyclic intermediates has led to low yields, substrate limitation, racemization, and polymerization.[10] Consequently, there is room for improvement regarding the aforementioned problems vexing current one-pot approaches (Scheme 1b−d) and the classical step-by-step approach (Scheme 1a). Herein, we perform one-pot peptide bond-forming reactions by implementing transient masking with two different silylating reagents (Scheme 1e).

The optimized route to access H-L-Phe-L-Ala-O*t*Bu **1** from H-L-Phe-OH and H-L-Ala-O*t*Bu[11] is outlined in Table 1. We originally proposed the addition of $HSi[OCH(CF_3)_2]_3$[12] for the formation of the silyl ester of H-L-Phe-OH, followed by the transient masking of the amino group on H-L-Phe-OH using *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA).[13] The subsequent peptide bond-forming reaction with H-L-Ala-O*t*Bu afforded the desired dipeptide **1** in 99% yield without any observation of epimerization or side reactions,[14] such as polymerization (99.7:0.3 dr and >99.9:1 er; entry 1). On a gram scale (7.5 mmol), the peptide bond-forming reaction proceeded with essentially identical yield and purity as for that

Received: March 9, 2021
Published: April 30, 2021

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792−6797

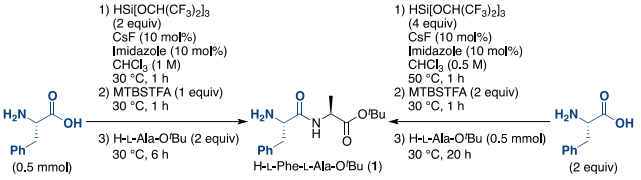
Scheme 1. Strategies for the Elongation of Peptide Chains



conducted on a 0.50 mmol scale (>99% yield; entry 1). In the optimized study, we found that CsF and imidazole catalysts play major roles in accelerating chemoselective silylation on the carboxyl group of H-L-Phe-OH, owing to the in situ generation of imidazole−Si[OCH(CF$_3$)$_2$]$_3$ from CsF, imidazole, and HSi[OCH(CF$_3$)$_2$]$_3$.[15] In fact, when HSi[OCH(CF$_3$)$_2$]$_3$ was simultaneously added with MTBSTFA, **1** was obtained in high yield (95% yield, 99.6:0.4 dr, >99.9:1 er, entry 2). Although the optimized protocol can be applied in the absence of CsF and imidazole, the reaction takes twice as long (entries 3 and 4). In the absence of HSi[OCH(CF$_3$)$_2$]$_3$, no amide bond formation is observed (entry 5). In the absence of MTBSTFA, the yield of **1** significantly decreases (entry 6). An aminosilane catalyst served as a Lewis acid to refine the peptide bond formation using *N*-protected amino acids in a previous study;[12] however, it seemed incompatible with unprotected amino acid in the present study (entry 7).

Furthermore, the applicability of several other catalysts for the activation of hydrosilanes was evaluated.[16] Contrary to expectation, they were ineffective compared to the combination of CsF and imidazole catalysts (entries 8−12). The use of toluene in place of CHCl$_3$ led to a similar outcome (entries 13−21). Additionally, HSi(OCH$_2$CF$_3$)$_3$ was less reactive than HSi[OCH(CF$_3$)$_2$]$_3$ (entry 22). Several alternatives for TBS protection, such as *tert*-butyldimethylsilyl-*N*-phenylbenzimidate (TBSBEZA) and *N*,*O*-bis(*tert*-butyldimethylsilyl)acetamide (BTBSA), can be used instead of MTBSTFA (entries 23−26). However, neither *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA; used for TMS protection) nor *N*-methyl-*N*-triethylsilyltrifluoroacetamide (MTESTFA; used for TES protection) could give higher yields than that achieved when using MTBSTFA (entries 27 and 28).[13] This is consistent with the generally recognized relative stability of O−Si or N−Si bonds (TBS > TES > TMS).[17] When H-L-Phe-OH (2 equiv) was reacted with H-L-Ala-O*t*Bu under the optimal reaction conditions, a longer reaction time (20 h) was required to furnish **1** in 91% yield with a small amount of unpredictable byproduct, H-L-Ala-L-Ala-O*t*Bu (**2b**) (entry 29).[18] This is likely because of the unexpected coupling of H-L-Ala-O*t*Bu with H-L-Ala-OH. This was induced by the cleavage of *t*Bu group from H-L-Ala-O*t*Bu during the long reaction, which occurs owing to the acidity of HOSi[OCH(CF$_3$)$_2$]$_3$; consequently, the yield obtained in entry 29 is slightly lower than that in entry 1.

Table 1. One-Pot Peptide Bond-Forming Reaction of H-L-Phe-OH with H-L-Ala-O*t*Bu[a]



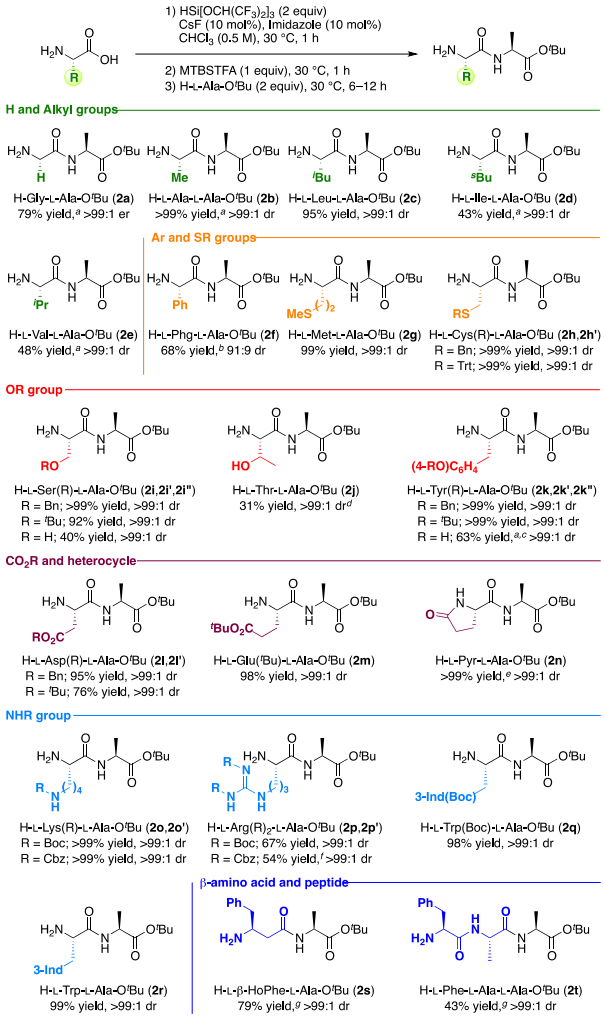
entry	variation from the "standard" conditions	yield of **1** (%)	dr, er of **1**
1	none	99 (>99)[b]	>99:1
2[c]	none	95	>99:1
3	no CsF/imidazole	80	>99:1
4	no CsF/imidazole, 12 h	>99	>99:1
5	no HSi[OCH(CF$_3$)$_2$]$_3$	0	–
6	no MTBSTFA	<1	–
7[d]	PMBNHSi[OCH(CF$_3$)$_2$]$_3$, instead of CsF/imidazole	82	>99:1
8	(PPh$_3$)$_3$RhCl, instead of CsF/imidazole	56	>99:1
9	(PPh$_3$)$_3$RuCl$_2$, instead of CsF/imidazole	58	>99:1
10	Co$_2$(CO)$_8$, instead of CsF/imidazole	65	>99:1
11	B(C$_6$F$_5$)$_3$, instead of CsF/imidazole	72	>99:1
12	Et$_3$N, instead of CsF/imidazole	82	>99:1
13	no solvents, instead of CHCl$_3$	81	>99:1
14	toluene, instead of CHCl$_3$	94	>99:1
15	cyclohexane, instead of CHCl$_3$	96	>99:1
16	DCM, instead of CHCl$_3$	80	>99:1
17	CPME, instead of CHCl$_3$	81	>99:1
18	2-Me-THF, instead of CHCl$_3$	43	>99:1
19	AcOEt, instead of CHCl$_3$	67	>99:1
20	MeCN, instead of CHCl$_3$	21	>99:1
21	DMF, instead of CHCl$_3$	23	>99:1
22	HSi(OCH$_2$CF$_3$)$_3$, instead of HSi[OCH(CF$_3$)$_2$]$_3$	28	>99:1
23	TBSBEZA, instead of MTBSTFA	88	>99:1
24[e]	BTBSA, instead of MTBSTFA	66	>99:1
25	TBSCl, instead of MTBSTFA	88 (90)[f]	>99:1
26	TBSOTf, instead of MTBSTFA	71 (94)[f]	>99:1
27	MSTFA, instead of MTBSTFA	10	>99:1
28	MTESTFA, instead of MTBSTFA	30	>99:1
29	none	91	>99:1

[a]Percentages represent isolated yields. dr's and er's were determined using HPLC. [b]Yield obtained via the gram-scale synthesis of **1**. [c]HSi[OCH(CF$_3$)$_2$]$_3$ was added simultaneously with MTBSTFA. [d]PMBNHSi[OCH(CF$_3$)$_2$]$_3$ (3 mol %) was added. [e]BTBSA (0.5 equiv) was used. [f]With Et$_3$N (1 equiv).

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792−6797

With the optimal reaction conditions in hand, a wide variety of unprotected and partially protected amino acids serving as electrophiles were treated with H-L-Ala-O*t*Bu (Scheme 2). H-

Scheme 2. Scope of Electrophiles[h]



[a]Silylation using $HSi[OCH(CF_3)_2]_3$ was performed at 50 °C. [b]All processes were performed at room temperature. [c]$HSi[OCH(CF_3)_2]_3$ (3 equiv) was added. [d]$HSi[OCH(CF_3)_2]_3$ (3 equiv) and MTBSTFA (2 equiv) were added. [e]$HSi[OCH(CF_3)_2]_3$ (1 equiv) was added and silylation using MTBSTFA was omitted. [f]NMR yield.[22] [g]No solvents were used. [h]Percentages represent isolated yields. er was determined using HPLC after *N*-Boc-protection of **2a** was performed. dr's were determined by ^{1}H NMR.

Gly-OH, H-L-Ala-OH, and H-L-Leu-OH led to satisfying yields of the corresponding dipeptides **2a**–**c** without any loss of stereochemical integrities. Amino acids possessing a secondary or relatively bulky side chain (e.g., Ile, Val, Phg, and Arg) provided the desired products (**2d**–**f**, **2p**, and **2p′**, respectively) in only moderate yields, which were unsatisfactory due to incomplete conversions. Shibasaki and co-workers previously reported that the use of conventional coupling reagents in the amidation of Bz-L-Val-OH caused extensive epimerization (53:47 dr).[19] Thus, our method is attractive not only because it does not cause epimerization, but also because it allows the protection/deprotection sequence to proceed via a one-pot protocol. The mild reaction conditions tolerate a broad range of functional groups (OR and SR) and afford diverse desired dipeptides **2g**–**i**, **2k**, **2i′**, and **2k′** in excellent yields. Different ester, heterocyclic, and NHR groups were also incorporated into the corresponding dipeptides **2l**–**r**, **2l′**, and **2o′**, which were isolated in high yields. Notably, the protocol can accommodate reactions using amino acids bearing free hydroxy groups and H-L-Trp-OH, which furnish **2i″**, **2j**, **2k″**, and **2r** in 31–99% yield without side reactions or the loss of their stereochemical integrities. These results suggest that our method may be applicable to a peptide coupling using most amino acids bearing free amino groups such as H-L-Lys-OH and H-L-Arg-OH. Unfortunately, the corresponding coupling products could not be purified by normal silica-gel chromatography due to their extremely high polarity. Modest racemization was observed in the synthesis of **2f** because H-L-Phg-OH and its derivatives are among the most easily racemized unnatural amino acids.[14,20,21] Furthermore, the electrophilic scope can be expanded to include unprotected *β*-amino acids and dipeptides. The success of our one-pot method would lead to its prospective application in peptide bond formation using not only *N*-/*C*-termini protecting-group-free oligopeptides but also proteins.

A broad variety of amino acid esters, serving as nucleophilic counterparts, smoothly participated in this process (Scheme 3). When amino acid esters comprising H or alkyl side chains were employed with H-L-Phe-OH, the corresponding dipeptides **3a**–**3d** were successfully furnished in 95–98% yields. Subsequently, our attention turned to the applicability of amino acid esters bearing an array of functional groups for formation of peptide bonds with several unprotected and partially protected amino acids as electrophilic components. It was observed that H-L-Phe-O*t*Bu, H-L-Met-O*t*Bu, H-L-Cys(Trt)-O*t*Bu, H-L-Ser(*t*Bu)-OH, and H-L-Tyr(*t*Bu)-O*t*Bu, possessing aromatic, sulfide, or ether side chains, smoothly transformed into **3e**, **3g**–**3i**, and **3k** in excellent yields with >99:1 dr.

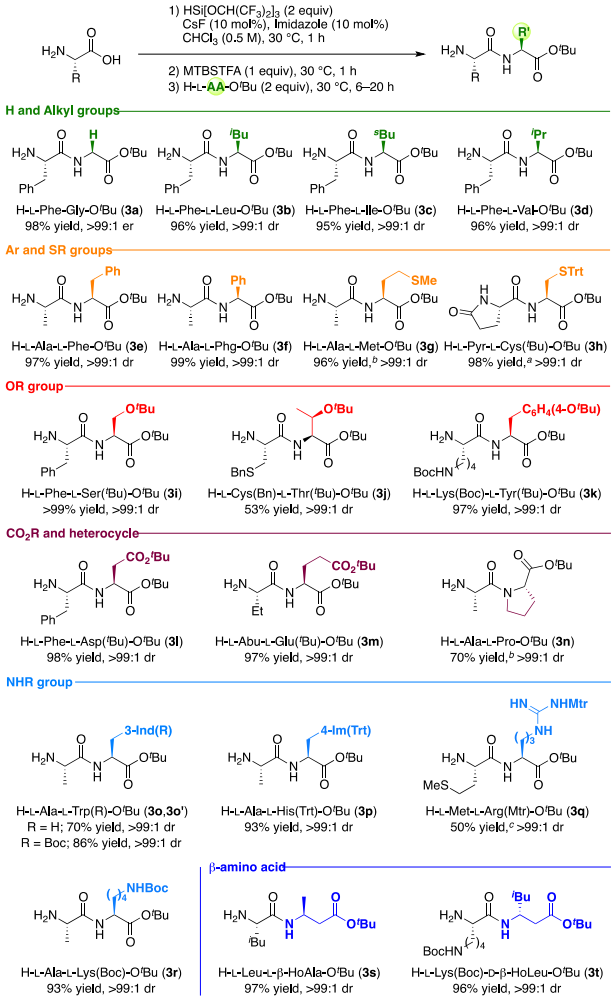
Optimum reaction conditions allowed the use of highly sensitive amino acid toward racemization (H-L-Phg-O*t*Bu) and the reaction between sterically hindered amino acids, thereby affording **3f** and **3j** in 99% and 53% yield, respectively, without any observation of epimerization or side reactions. Amino acids comprising ester, amide, and amine groups, such as Asp, Glu, Trp, His, Arg, and Lys, and heterocyclic amino acid (Pro) also tolerated the reaction conditions, giving high yields of **3l**–**3r** and **3o′**. Finally, we demonstrated that the use of *β*-amino acid esters as nucleophilic components gave desired peptides **3s** and **3t** in 97 and 96% yield, respectively, without the occurrence of epimerization or side reactions.

Thus, the presented method generates peptides in high yields without any problems.[14] Although isolating them from the remaining nucleophilic components by silica-gel column chromatography alone is occasionally difficult, substrates such as H-L-Ala-O*t*Bu and H-L-Val-O*t*Bu can be easily removed with an ordinary oil rotary vacuum pump due to their relatively low boiling points. Furthermore, we have confirmed that amino acid esters having relatively high boiling points, such as H-L-Asp(*t*Bu)-O*t*Bu, also can be removed using an oil diffusion pump to help purification of the products.

Possible mechanisms are proposed in Figure 1. With $HSi[OCH(CF_3)_2]_3$ serving as a silane-based coupling reagent, the one-pot peptide bond-forming reaction could produce the targeted peptides via two possible pathways. The major pathway (blue route) begins with the in situ generation of silylimidazole from $HSi[OCH(CF_3)_2]_3$ due to the presence of CsF and imidazole as catalysts.[14] Silylimidazoles are among the most powerful protective silyl donors, reacting chemoselectively with

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792–6797

Scheme 3. Scope of Nucleophiles[d]



[a]Silylation using $HSi[OCH(CF_3)_2]_3$ was performed at 50 °C. [b]$HSi[OCH(CF_3)_2]_3$ (1 equiv) was added and silylation using MTBSTFA was omitted. [c]H-L-Arg(Mtr)-O*t*Bu was added in a glovebox.[23] [d]Percentages represent isolated yields. er was determined using HPLC after *N*-Boc-protection of **3a** was performed. dr's were determined by ^{1}H NMR.

alcohols and carboxylic acids in the presence of amines.[24] The resulting silylimidazole undergoes chemoselective silylation with amino acids forming silyl esters **A** (and/or **A′**). Next, the silylation of **A** (and/or **A′**) using MTBSTFA followed by the amidation of disilyl intermediates **B** (and/or **B′**) may afford the corresponding precursors **C**.[25] Finally, the removal of TBS group by silica-gel chromatography can produce the target peptides. Alternatively, in the minor pathway (pink route), $HSi[OCH(CF_3)_2]_3$ reacts directly with amino acids forming **A** (and/or **D**, **A′**, **D′**). Subsequently, the silylation of **A** (and/or **D**, **A′**, **D′**) using MTBSTFA followed by the amidation of **B** (and/or **E**, **B′**, **E′**) may afford the desired peptides via the formation of precursors **C** (and/or **F**).[26]

In summary, two silylating reagents with different properties were effectively used to circumvent undesired racemization during peptide bond formation and to address several challenges facing conventional peptide synthesis, such as the reduction of waste, labor, time, energy, solvent use, and cost. Thus, we were able to develop a general one-pot protocol for peptide bond formation of unprotected or partially protected amino acids and peptides. Given the operational simplicity of this approach, and by offering newly designed peptides with high optical purity and broad applicability to pharmaceutically relevant scaffolds, we expect this method to be widely adopted by academics and industrial researchers.

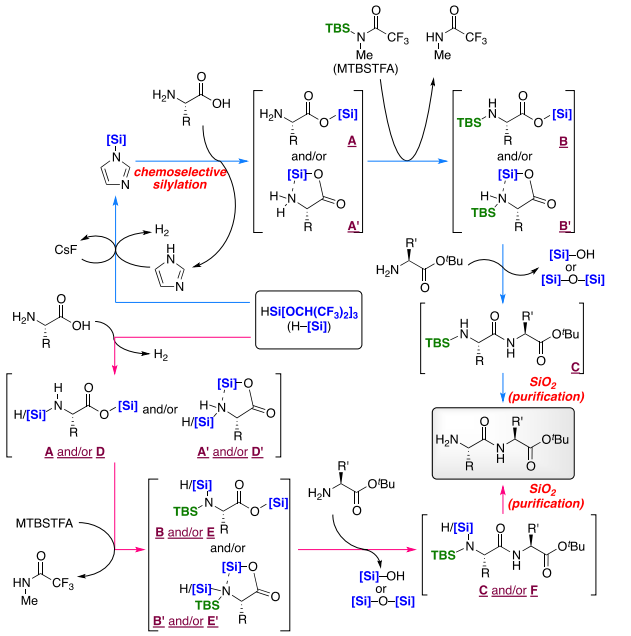


Figure 1. Proposed reaction mechanisms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c02600.

Experimental procedures, characterization data, ^{1}H and ^{13}C HPLC data, and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Wataru Muramatsu − *Molecular Catalyst Research Center, Chubu University, Kasugai, Aichi 487-8501, Japan;* orcid.org/0000-0001-7183-7981; Email: muramatsu@isc.chubu.ac.jp

Hisashi Yamamoto − *Molecular Catalyst Research Center, Chubu University, Kasugai, Aichi 487-8501, Japan;* orcid.org/0000-0001-5384-9698; Email: hyamamoto@isc.chubu.ac.jp

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c02600

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Support has been provided by Grant-in-Aid for Scientific Research S (JP17H06142) from Japan Society for the Promotion of Science (JSPS), Daiko Foundation, Takeda Science Foundation, and Sumitomo Chemical Co., Ltd., Japan. We would like to thank Yuuki Matsunaga for assistance with measurement using reversed-phase HPLC.

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792−6797

REFERENCES

(1) (a) Klebe, G. *Drug Design*; Springer: Berlin, Heidelberg, 2013. (b) Harrington, A.; Tal-Gan, Y. The Importance of Amide Protons in Peptide Drug Development. *Future Med. Chem.* **2019**, *11*, 2759−2763. (c) Kumari, S.; Carmona, A. V.; Tiwari, A. K.; Trippier, P. C. Amide Bond Bioisosteres: Strategies, Synthesis, and Successes. *J. Med. Chem.* **2020**, *63*, 12290−12358.

(2) (a) Srivastava, V. *Peptide-Based Drug Discovery: Challenges and New Therapeutics*; The Royal Society of Chemistry: London, 2017. (b) Grand View Research. *Peptide Therapeutics Market By Application (Cancer, Cardiovascular Disorder, Metabolic Disorder, Respiratory Disorder, Pain, Dermatology), By Type (Generic, Innovative) By Type of Manufacturers (In-house, Outsourced), And Segment Forecasts, 2018−2025*; Grand View Research: San Francisco, CA, 2017.

(3) (a) Albericio, F.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. New Trends in Peptide Coupling Reagents. *Org. Prep. Proced. Int.* **2001**, *33*, 203−303. (b) Valeur, E.; Bradley, M. Amide Bond Formation: Beyond the Myth of Coupling Reagents. *Chem. Soc. Rev.* **2009**, *38*, 606−631. (c) El-Faham, A.; Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. *Chem. Rev.* **2011**, *111*, 6557−6602.

(4) El-Faham, A.; Funosas, R. S.; Prohens, R.; Albericio, F. COMU: A Safer and More Effective Replacement for Benzotriazole-Based Uronium Coupling Reagents. *Chem. - Eur. J.* **2009**, *15*, 9404−9416.

(5) (a) Sheldon, R. A. Organic Synthesis; Past, Present and Future. *Chem. Ind. (London)* **1992**, 903−906. (b) Sheldon, R. A. Metric of Green Chemistry and Sustainability: Past, Present, and Future. *ACS Sustainable Chem. Eng.* **2018**, *6*, 32−48. (c) Rasmussen, J. H. Synthetic Peptide API Manufacturing: A Mini Review of Current Perspectives for Peptide Manufacturing. *Bioorg. Med. Chem.* **2018**, *26*, 2914−2918.

(6) (a) Yi, W.; Zeng, X.; Gao, S. Pot Economy Synthesis. In *Green Techniques for Organic Synthesis and Medicinal Chemistry*, 2nd ed.; Zhang, W., Cue, B. W., Eds.; Wiley: Hoboken, NJ, 2018; pp 407−439. (b) Zhang, W.; Yi, W.-B. *Pot, Atom, and Step Economy (PASE) Synthesis*; Spring Nature: Switzerland, AG, 2019. (c) Walji, A. M.; MacMillan, D. W. C. Strategies to Bypass the Taxol Problem. Enantioselective Cascade Catalysis, a New Approach for the Efficient Construction of Molecular Complexity. *Synlett* **2007**, *2007*, 1477−1489. (d) Hayashi, Y. Pot Economy and One-Pot Synthesis. *Chem. Sci.* **2016**, *7*, 866−880. (e) Eastgate, M. D.; Schmidt, M. A.; Fandrick, K. R. On the Design of Complex Drug Candidate Syntheses in the Pharmaceutical Industry. *Nat. Rev. Chem.* **2017**, *1*, No. 0016. (f) Kulkarni, S. S.; Wang, C.-C.; Sabbavarapu, N. M.; Podilapu, A. R.; Liao, P.-H.; Hung, S.-C. One-Pot" Protection, Glycosylation, and Protection−Glycosylation Strategies of Carbohydrates. *Chem. Rev.* **2018**, *118*, 8025−8104.

(7) (a) Clarke, P. A.; Santos, S.; Martin, W. H. C. Combining Pot, Atom and Step Economy (PASE) in Organic Synthesis. Synthesis of Tetrahydropyran-4-ones. *Green Chem.* **2007**, *9*, 438−440. (b) Ishikawa, H.; Suzuki, T.; Hayashi, Y. High-Yielding Synthesis of the Anti-Influenza Neuramidase Inhibitor (−)-Oseltamivir by Three "One-Pot" Operation. *Angew. Chem., Int. Ed.* **2009**, *48*, 1304−1307. (c) Ishikawa, H.; Suzuki, T.; Orita, H.; Uchimaru, T.; Hayashi, Y. High-Yielding Synthesis of the Anti-Influenza Neuramidase Inhibitor (−)-Oseltamivir by Two "One-Pot" Sequences. *Chem. - Eur. J.* **2010**, *16*, 12616−12626. (d) DelMonte, A. J.; Fan, Y.; Girard, K. P.; Jones, G. S.; Waltermire, R. E.; Rosso, V.; Wang, X. Kilogram Synthesis of a Second-Generation LFA-1/ICAM Inhibitor. *Org. Process Res. Dev.* **2011**, *15*, 64−72. (e) ishikawa, H.; Honma, M.; Hayashi, Y. One-Pot High-Yielding Synthesis of the DPP4-Selective Inhibitor ABT-341 by a Four-Component Coupling Mediated by a Diphenylprolinol Silyl Ether. *Angew. Chem., Int. Ed.* **2011**, *50*, 2824−2827. (f) Mukaiyama, T.; Ishikawa, H.; Koshino, H.; Hayashi, Y. One-Pot Synthesis of (−)-Oseltamivir and Mechanistic Insights into the Organocatalyzed Michael Reaction. *Chem. - Eur. J.* **2013**, *19*, 17789−17800.

(8) (a) Hofmann, K.; Thompson, T. A.; Yajima, H.; Schwartz, E. T.; Inouye, H. Studies on Polypeptides. XIV. The Synthesis of Peptides Related to the N-Terminus of α-MSH and of the Corticotropins. *J. Am. Chem. Soc.* **1960**, *82*, 3715−3721. (b) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. The Use of Esters of N-Hydroxysuccinimide in Peptide Synthesis. *J. Am. Chem. Soc.* **1964**, *86*, 1839−1842. (c) Kricheldorf, H. E. Über die Silylierung von Aminosäuren und die Peptidsynthese mit Aminosäuretrimethylsilylestern. *Liebigs Ann. Chem.* **1972**, *763*, 17−38. (d) Hegarty, A. F.; McCarthy, D. G. Peptide Synthesis Using Unprotected Amino Acids and Novel Imidoyl Halide Reagents. *J. Am. Chem. Soc.* **1980**, *102*, 4537−4538. (e) Gagnon, P.; Huang, X.; Therrien, E.; Keillor, J. W. Peptide Coupling of Unprotected Amino Acids Through in situ *p*-Nitrophenyl Ester Formation. *Tetrahedron Lett.* **2002**, *43*, 7717−7719. (f) Katritzky, A. R.; Todadze, E.; Angrish, P.; Draghici, B. Efficient Coupling Involving Sterically Hindered Amino Acids. *J. Org. Chem.* **2007**, *72*, 5794−5801. (g) Brown, Z. Z.; Schafmeister, C. E. Exploiting an Inherent Neighboring Group Effect of α-Amino Acids to Synthesize Extremely Hindered Dipeptides. *J. Am. Chem. Soc.* **2008**, *130*, 14382−14383. (h) Meneses, C.; Nicoll, S. L.; Trembleau, L. Multigram-Scale Synthesis of Short Peptides via a Simplified Repetitive Solution-Phase Procedure. *J. Org. Chem.* **2010**, *75*, 564−569. (i) Noguchi, T.; Tehara, N.; Uesugi, Y.; Jung, S.; Imai, N. Convenient Peptide Synthesis without Protection of *C*-Terminals. *Chem. Lett.* **2012**, *41*, 42−43. (j) Fuse, S.; Masuda, K.; Otake, Y.; Nakamura, H. Peptide-Chain Elongation Using Unprotected Amino Acids in a Micro-Flow Reactor. *Chem. - Eur. J.* **2019**, *25*, 15091−15097. (k) Huang, Y.; Feng, W.-H. *N*,*O*-Bis(trimethylsilyl)acetamide/N-Hydroxysuccimide Ester (BSA/NHS) as Coupling Agents for Dipeptide Synthesis. *Chin. Chem. Lett.* **2016**, *27*, 357−360. (l) Kurasaki, H.; Nagaya, A.; Kobayashi, Y.; Matsuda, A.; Matsumoto, M.; Morimoto, K.; Taguri, T.; Takeuchi, H.; Handa, M.; Cary, D. R.; Nishizawa, N.; Masuya, K. Isosteary Mixed Anhydrides for the Preparation of N-Methylated Peptides Using *C*-Terminally Unprotected *N*-Methylamino Acids. *Org. Lett.* **2020**, *22*, 8039−8043.

(9) McKnelly, K. J.; Sokol, W.; Nowick, J. S. Anaphylaxis Induced by Peptide Coupling Agents: Lessons Learned from Repeated Exposure to HATU, HBTU, and HCTU. *J. Org. Chem.* **2020**, *85*, 1764−1768.

(10) (a) Burger, K.; Rudolph, M. Regiospecific Relations with Omega-Carboxy-Alpha-Amino Acids − Simple Synthesis for Aspartame. *Chem. Ztg.* **1990**, *114*, 249−251. (b) van Leeuwen, S. H.; Quaedflieg, P. J. L. M.; Broxterman, Q. B.; Liskamp, R. M. J. Synthesis of Amides from Unprotected Amino Acids by a Simultaneous Protection−Activation Strategy Using Dichlorodialkyl Silanes. *Tetrahedron Lett.* **2002**, *43*, 9203−9207. (c) van Leeuwen, S.H.; Quaedflieg, P.J.L.M.; Broxterman, Q.B.; Milhajlovic, Y.; Liskamp, R.M.J. The Synthesis of Amides and Dipeptides from Unprotected Amino Acids by a Simultaneous Protection Activation Strategy Using Boron Trifluoride Diethyl Etherate. *Tetrahedron Lett.* **2005**, *46*, 653−656. (d) Sabatini, M. T.; Boulton, L. T.; Sheppard, T. D. Borate Esters: Simple Catalysts for the Sustainable Synthesis of Complex Amide. *Sci. Adv.* **2017**, *3*, No. e1701028. (e) Sabatini, M. T.; Karaluka, V.; Lanigan, R. M.; Boulton, L. T.; Badland, M.; Sheppard, T. D. Protecting-Group-Free Amidation of Amino Acids Using Lewis Acid Catalysts. *Chem. - Eur. J.* **2018**, *24*, 7033−7043.

(11) We chose amino acid *tert*-butyl esters as nucleophilic components because side reactions such as the self-intermolecular condensation or intramolecular cyclization of peptides can be avoided due to steric hindrance from the *tert*-butyl moiety, see: (a) Anderson, G. W.; Callahan, F. M. *t*-Butyl Esters of Amino Acids and Peptides and Their Use in Peptide Synthesis. *J. Am. Chem. Soc.* **1960**, *82*, 3359−3363. (b) Funasaki, N.; Hada, S.; Neya, S. Conformational Effects in Revered-Phase Liquid Chromatographic Separation of Diastereomers of Cyclic Dipeptides. *Anal. Chem.* **1993**, *65*, 1861−1867.

(12) Muramatsu, W.; Manthena, C.; Nakashima, E.; Yamamoto, H. Peptide Bond-Forming Reaction via Amino Acid Silyl Esters: New Catalytic Reactivity of an Aminosilane. *ACS Catal.* **2020**, *10*, 9594−9603.

(13) Mawhinney, T. P.; Madson, M. A. *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide Related *N*-*tert*-Butyldimethylsilyl Amides as Protective Silyl Donors. *J. Org. Chem.* **1982**, *47*, 3336−3339.

(14) Yang, Y. *Side Reactions in Peptide Synthesis*; Academic Press: Cambridge, MA, 2015.

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792−6797

(15) Horner, L.; Mathias, J. Chemoselektive Mono- und Disilyletherbildung aus Tertiären und Sekundären Silanen. *J. Organomet. Chem.* **1985**, *282*, 155−174.
(16) (a) Birkofer, L.; Ritter, A. The Use of Silylation in Organic Syntheses. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 417−429. (b) Corriu, R. J. P.; Moreau, J. J. E. Alcoolyse Selective D'organosilanes Catalysee par un Complexe du Rhodium. *J. Organomet. Chem.* **1976**, *114*, 135−144. (c) Parks, D. J.; Piers, W. E. Tris(pentafluorophenyl)boron-Catalyzed Hydrosilation of Aromatic Aldehydes, Ketones, and Esters. *J. Am. Chem. Soc.* **1996**, *118*, 9440−9441. (d) Blackwell, J. M.; Foster, K. L.; Beck, V. H.; Piers, W. E. $B(C_6F_5)_3$-Catalyzed Silation of Alcohols: A Mild, General Method for Synthesis of Silyl Ethers. *J. Org. Chem.* **1999**, *64*, 4887−4892. (e) Parks, D. J.; Blackwell, J. M.; Piers, W. E. Studies on the Mechanism of $B(C_6F_5)_3$-Catalyzed Hydrosilation of Carbonyl Functions. *J. Org. Chem.* **2000**, *65*, 3090−3098. (f) Rubin, M.; Schwier, T.; Gevorgyan, V. Highly Efficient $B(C_6F_5)_3$-Catalyzed Hydrosilylation of Olefins. *J. Org. Chem.* **2002**, *67*, 1936−1940. (g) Harrison, D. J.; McDonald, R.; Rosenberg, L. Borane-Catalyzed Hydrosilylation of Thiobenzophenone: A New Route to Silicon−Sulfur Bond Formation. *Organometallics* **2005**, *24*, 1398−1400. (h) Rendler, S.; Oestreich, M. Conclusive Evidence for an S_N2-Si Mechanism in the $B(C_6F_5)_3$-Catalyzed Hydrosilylation of Carbonyl Compounds: Implications for the related Hydrogenation. *Angew. Chem., Int. Ed.* **2008**, *47*, 5997−6000. (i) Lee, P. T. K.; Skjel, M. K.; Rosenberg, L. Borane-Catalyzed Si−H Activation Routes to Polysilanes Containing Thiolato Side Chains. *Organometallics* **2013**, *32*, 1575−1578. (j) Hermeke, J.; Mewald, M.; Oestreich, M. Experimental Analysis of the Catalytic Cycle of the Borane-Promoted Imine Reduction with Hydrosilanes: Spectroscopic Detection of Unexpected Intermediates and a Refined Mechanism. *J. Am. Chem. Soc.* **2013**, *135*, 17537−17546. (k) Greb, L.; Tamke, S.; Paradies, J. Catalytic Metal-Free Si−N Cross-Dehydrocoupling. *Chem. Commun.* **2014**, *50*, 2318−2320. (l) Fang, H.; Oestreich, M. Reductive Deamination with Hydrosilanes Catalyzed by $B(C_6F_5)_3$. *Angew. Chem., Int. Ed.* **2020**, *59*, 11394−11398.
(17) (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999. (b) Davies, J. S.; Higginbotham, C. L.; Tremeer, E. J.; Brown, C.; Treadgold, R. C. Protection of Hydroxy Groups by Silylation: Use on Peptide Synthesis and as Lipophilicity Modifiers for Peptides. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3043−3048. (c) Nelson, T. D.; Crouch, R. D. Selective Deprotection of Silyl Ethers. *Synthesis* **1996**, *1996*, 1031−1069.
(18) This protocol is applicable to the synthesis of peptides as shown in Scheme 2 and 3. However, it is a bit troublesome because optimized equivalents of $HSi[OCH(CF_3)_2]_3$ are required for each reaction.
(19) Noda, H.; Furutachi, M.; Asada, Y.; Shibasaki, M.; Kumagai, N. Unique Physicochemical and Catalytic Properties Directed by the B_3NO_2 Ring System. *Nat. Chem.* **2017**, *9*, 571−577.
(20) (a) Ishihara, K.; Ohara, S.; Yamamoto, H. 3,4,5-Trifluorobenzeneboronic Acid as an Extremely Active Amidation Catalyst. *J. Org. Chem.* **1996**, *61*, 4196−4197. (b) Smith, G. G.; Sivakua, T. Mechanism of the Racemization of Amino Acids. Kinetics of Racemization of Arylglycines. *J. Org. Chem.* **1983**, *48*, 627−634. (c) Stroud, E. D.; Fife, D. J.; Smith, G. G. A Method for the Determination of the pKa of the α-Hydrogen in Amino Acids Using Racemiation and Exchange Studies. *J. Org. Chem.* **1983**, *48*, 5368−5369. (d) Elsawy, M. A.; Hewage, C.; Walker, B. Racemization of *N*-Fmoc Phenylglycine under Mild Microwave-SPPS and Conventional Stepwise SPPS Conditions: Attempts to Develop Strategies for Overcoming This. *J. Pept. Sci.* **2012**, *18*, 302−311. (e) Popovic, S.; Bieräugel, H.; Detz, R. J.; Kluwer, A. M.; Koole, H. A. A.; Streefkerk, D. E.; Hiemstra, H.; van Maarseveen, J. H. Epimerization-Free C-Terminal Peptide Activation. *Chem. - Eur. J.* **2013**, *19*, 16934−16937. (f) Fuse, S.; Mifune, Y.; Nakamura, H.; Tanaka, H. Total Synthesis of Feglymycin based on a Linear/Convergent Hybrid Approach Using Micro-Flow Amide Bond Formation. *Nat. Commun.* **2016**, *7*, 13491. (g) Liang, C.; Behnam, M A. M.; Sundermann, T. R.; Klein, C. D. Phenylglycine Racemization in Fmoc-Based Solid-Phase Peptide Synthesis: Stereochemical Stability is Achieved by Choice of Reaction Conditions. *Tetrahedron Lett.* **2017**, *58*, 2325−2329.
(21) We previously reported that **2e** and its epimer can be easily separated by normal phase silica-gel column chromatography, see: Muramatsu, W.; Tsuji, H.; Yamamoto, H. Catalytic Peptide Synthesis: Amidation of *N*-Hydroxyimino Esters. *ACS Catal.* **2018**, *8*, 2181−2187.
(22) Yield of **2p′** was calculated from ^{1}H NMR spectra data because TBSOH could not be removed from the mixture.
(23) H-L-Arg(Mtr)-O*t*Bu was added in a glovebox because it is insoluble in $CHCl_3$.
(24) (a) Simchen, G.; Heberle, J. 1-(Trimethylsilyl)imidazole, TMSIM. In *Silylating Agents*, 2nd ed.; Simchen, G., Heberle, J., Eds.; Fluka Chemie AG: Buchs, 1995; pp 40−42. (b) Grissom, J. W.; Gunawardena, G.; Gimisis, T.; Cismas, C. *N*-(Trimethylsilyl)imidazole. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: Hoboken, NJ, 2007. (c) Horning, M. G.; Moss, A. M.; Horning, E. C. A New Method for the Separation of the Catecholamines by Gas-Liquid Chromatography. *Biochim. Biophys. Acta, Gen. Subj.* **1967**, *148*, 597−600. (d) Richard, B. M.; Manno, J. E.; Manno, B. R. Gas Chromatographic Determination of Ethambutol. *J. Chromatogr.* **1974**, *89*, 80−83. (e) Gerlach, M.; Jutzi, P.; Stasch, J.-P.; Przuntek, H. Syntheses und Pharmakologische Eigenschaften von Silylierten Dopaminen und 4.4-Diphenylpiperidinen. *Z. Naturforsch., B: J. Chem. Sci.* **1983**, *38B*, 237−242. (f) Bernardi, P.; Dembech, P.; Fabbri, G.; Ricci, A.; Seconi, G. A General and Convenient Procedure for the Synthesis of *N*-Alkylarylamines and *N*-Alkylheteroarylamines by Electrophilic Amination of Cuprates with *N*-Alkylhydroxylamines. *J. Org. Chem.* **1999**, *64*, 641−643. (g) Joubert, J.; Roussel, S.; Christophe, C.; Billard, T.; Langlois, B. R.; Vidal, T. Trifluoroacetamides from Amino Alcohols as Nucleophilic Trifluoromethylating Reagents. *Angew. Chem., Int. Ed.* **2003**, *42*, 3133−3136.
(25) Orthosilicic acids and alcohols (ROH) are known to be easily generated by the hydrolysis of $HOSi(OR)_3$ and $(RO)_3SiOSi(OR)_3$ under acidic or basic conditions. HFIP would be recovered quantitatively by the hydrolysis of $HOSi[OCH(CF_3)_2]_3$ and $[(CF_3)_2CHO]_3SiOSi[OCH(CF_3)_2]_3$. HFIP purified by distillation can be reused for the preparation of $HSi[OCH(CF_3)_2]_3$. The challenge is currently under investigation, see: (a) Froberger, C. Notes- Synthesis of Tetra(perfluoroalkoxy)silanes. *J. Org. Chem.* **1960**, *25*, 311−312. (b) Ciriminna, R.; Fidalgo, A.; Pandarus, V.; Beland, F.; Ilharco, L. M.; Pagliaro, M. The Sol−Gel Route to Advanced Silica-Based Materials and Recent Applications. *Chem. Rev.* **2013**, *113*, 6592−6620. (c) Igarashi, M.; Matsumoto, T.; Yagihashi, F.; Yamashita, H.; Ohhara, T.; Hanashima, T.; Nakao, A.; Moyoshi, K.; Sato, K.; Shimada, S. Non-Aqueous Selective Synthesis of Orthosilicic Acid and Its Oligomers. *Nat. Commun.* **2017**, *8*, 140.
(26) We tried to determine the intermediates by ^{1}H NMR measurement. Unfortunately, reasonable data could not be obtained because NMR samples were suspended or separated into two layers. However, considering the results of present and previous studies (ref 12), we presume that the proposed reaction mechanism is not too far from the actual one. To clarify the details of the reaction mechanism, we understand that comprehensive studies including computational studies are essential. We will try them in the future.

https://doi.org/10.1021/jacs.1c02600
J. Am. Chem. Soc. 2021, 143, 6792−6797