JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

N-Boc Deprotection and Isolation Method for Water-Soluble Zwitterionic Compounds

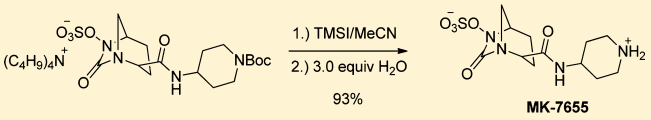
Zhijian Liu,*[,†] Nobuyoshi Yasuda,[†] Michael Simeone,[‡] and Robert A. Reamer[†]

[†]Process Chemistry, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065, United States

[‡]Chemical Process Development and Commercialization, Merck Manufacturing Division, P.O. Box 2000, Rahway, New Jersey 07065, United States

Supporting Information

ABSTRACT: A highly efficient TMSI-mediated deprotection and direct isolation method to obtain zwitterionic compounds from the corresponding *N*-Boc derivatives has been developed. This method has been demonstrated in the final deprotection/isolation of the β-lactamase inhibitor MK-7655 as a part of its manufacturing process. Further application of this process toward other zwitterionic compounds, such as dipeptides and tripeptides, has been successfully developed. Furthermore, a catalytic version of this transformation has been demonstrated in the presence of BSA or BSTFA.



Zwitterions are commonly encountered in biologically active compounds (Scheme 1) because of their unique properties, such as high solubility in aqueous media and high permeability in biological environments.[1] Unfortunately, the isolation of those compounds from aqueous solutions presents significant operational challenges to enable the removal of water. Although numerous industrial technologies have been developed to specifically address the isolation of these types of intermediates for commercial purposes,[2] the difficulty of this operation exponentially increases with scale, especially in the case that the zwitterionic target compounds are thermodynamically unstable. It would be ideal if the zwitterionic species could be isolated directly from the organic reaction medium without aqueous workup and/or extraction. Herein we report a TMSI-mediated deprotection of *N*-Boc amino acids that enables the direct isolation of zwitterionic products.

MK-7655 (**1**) is a β-lactamase inhibitor[3] that is currently in phase II studies in coadministration with Primaxin to treat serious infections by multidrug-resistant microorganisms.[4] MK-7655 is a zwitterionic species and is soluble only in water or aprotic polar solvents such as DMSO or NMP. In addition, the urea bond of **1** is highly reactive toward nucleophiles because of its highly strained bicyclic ring system, which is further activated by the *O*-sulfonate group.[5] Therefore, **1** possesses limited stability in an aqueous environment within a narrow pH window (pH 4−8).

MK-7655 was prepared by removal of the *N*-Boc group from the *N*-Boc tetrabutylammonium salt (**2**) (Scheme 2).[6] However, because of its instability and zwitterionic property, this deprotection of the *N*-Boc group from **2** and isolation of **1** were found to be very challenging, despite the broad usage of the Boc protecting group in organic syntheses.[7] Extensive studies of the Boc deprotection of **2** by our colleagues revealed that the only conditions suitable for this transformation were a combination of HBF_4OEt_2 and 2,2,2-trifluoroethanol. Under the optimal conditions, **1** could be isolated via aqueous workup and crystallized as the monohydrate from an isopropanol/water mixture in 68% yield on a laboratory scale (Scheme 2).[6] However, during further scale-up of this process, we encountered serious difficulties due to the instability and physical characteristics of **1**. As a result of these liabilities, alternative approaches to the Boc deprotection of **2** were examined in order to develop a simple and efficient deprotection/isolation protocol to obtain **1**.[8]

It is well-documented that Lewis acid-mediated deprotection of the *N*-Boc group can be achieved under mild conditions. For example, the *N*-Boc group can be easily converted to *N*-(CO_2TMS) using either TMSOTf or TMSI, and subsequent solvolysis/decarboxylation of *N*-(CO_2TMS) affords the corresponding deprotected amine under mild conditions (Scheme 3).[9] In addition, the *tert*-butyl halide generated from this system can be converted to isobutylene and hydrogen halide, which can be easily removed from the reaction system.

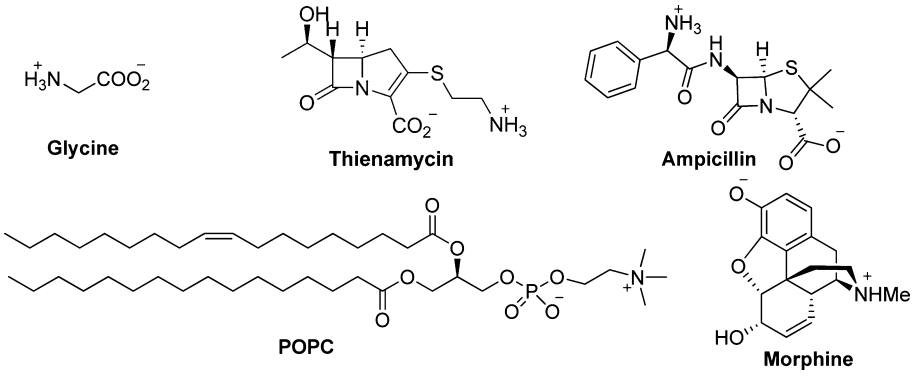
We envisioned that this approach would be suitable for the deprotection of **2** to afford **1** under mild conditions, and direct isolation of **1** would be possible as a result of its zwitterionic property. Therefore, a variety of mainly silicon-based Lewis acids were studied for the deprotection of **2**, and the results are summarized in Table 1. We noticed that the Lewis acidity of TMSX has a big influence on this transformation. For example, treatment of **2** with 1.1 equiv of TMSCl in MeCN at 0 °C gave no reaction at all (entry 1). Only 35% conversion was achieved with TMSBr under identical conditions (entry 2). Full conversion was obtained with TMSOTf, but numerous impurities were quickly generated over the course of the reaction (entry 3). To our delight, when **2** was treated with TMSI in MeCN, full conversion was obtained in less than 30

Received: October 9, 2014
Published: November 7, 2014

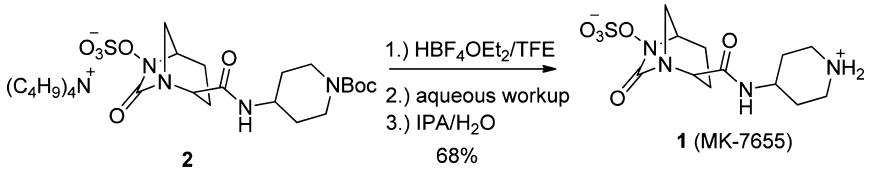
ACS Publications © 2014 American Chemical Society

dx.doi.org/10.1021/jo502319z | J. Org. Chem. 2014, 79, 11792−11796

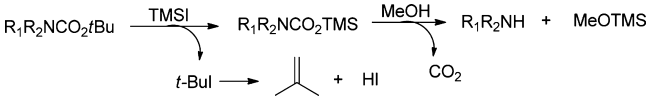
Scheme 1. Important Biologically Active Zwitterionic Compounds



Scheme 2. Deprotection of Penultimate 2 with HBF_4OEt_2 and Isolation of 1



Scheme 3. TMSI-Mediated Deprotection of the *N*-Boc Group

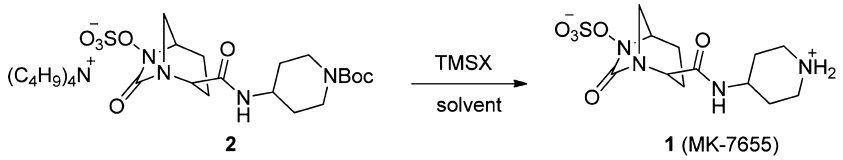


min at 0 °C with minimal generation of impurities. The deprotection of **2** with TMSI could be accomplished in several solvents (entries 4–7), with MeCN and methylene chloride being the best choices for this transformation. MeCN was selected for further development from an environmental point of view. The optimal amount of TMSI was studied (entries 4 and 8–11), and 1.25 equiv was found to be the most suitable for this transformation (entry 10). It is interesting to point out that 1 equiv of TMSI is sufficient for this transformation (entry 9), which suggests that silylation of the sulfonate group of **2** is not a competing pathway.

To further understand this transformation, a detailed NMR study was performed. It was observed that facile formation of the intermediate **2-N(CO_2TMS)** occurred upon treatment of **2** with TMSI in an NMR tube even at −20 °C (see Figure 1, NMR-2 in the Supporting Information). This TMS carbamate species underwent subsequent solvolysis/decarboxylation upon addition of water to form **1**, which precipitated from the reaction mixture (Scheme 4).[10]

In addition to mild reaction conditions and high-yield conversion, the TMSI-mediated deprotection of the *N*-Boc group from **2** provided the biggest advantage due to the zwitterionic characteristic of **1**. MK-7655 was directly crystallized from the reaction medium after a limited amount of water addition, rejecting both organic and inorganic impurities in the

Table 1. Screening of Silicon-Based Lewis Acids for Boc Deprotection of Compound 2

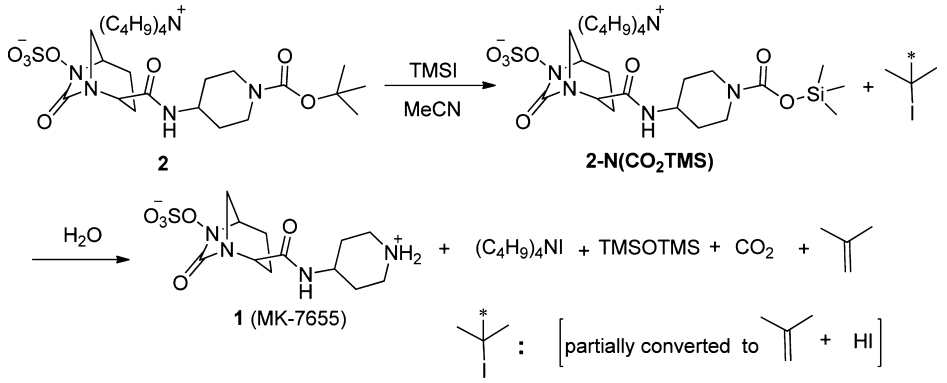


entry[a]	TMSX	solvent	equiv of Lewis acid	% conv.	% yield of **1**[b]
1	TMSCl	MeCN	1.1	n.r.	n.r.
2	TMSBr	MeCN	1.1	55	n.d.
3	TMSOTf	MeCN	1.1	100	n.d.[c]
4	TMSI	MeCN	1.1	100	91
5	TMSI	DCM	1.1	100	90
6	TMSI	EtOAc	1.1	93	82
7	TMSI	toluene	1.1	42	n.d.
8	TMSI	MeCN	0.8	98	91[d]
9	TMSI	MeCN	1.0	100	92[d]
10	**TMSI**	**MeCN**	**1.25**	**100**	**93**
11	**TMSI**	**MeCN**	**1.3**	**100**	**93**

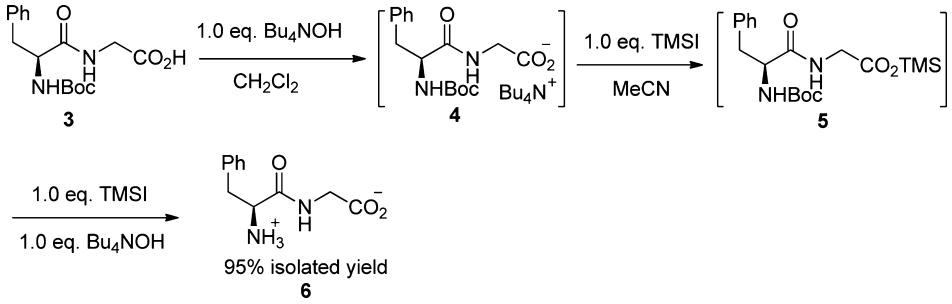
[a]The reaction volume was 6 mL/g at 0 °C under N_2, and water was used to quench the reaction when full conversion was obtained. [b]n.r. = no reaction; n.d. = not determined. [c]API degradation was observed. [d]Impurities were generated.

dx.doi.org/10.1021/jo502319z | J. Org. Chem. 2014, 79, 11792–11796

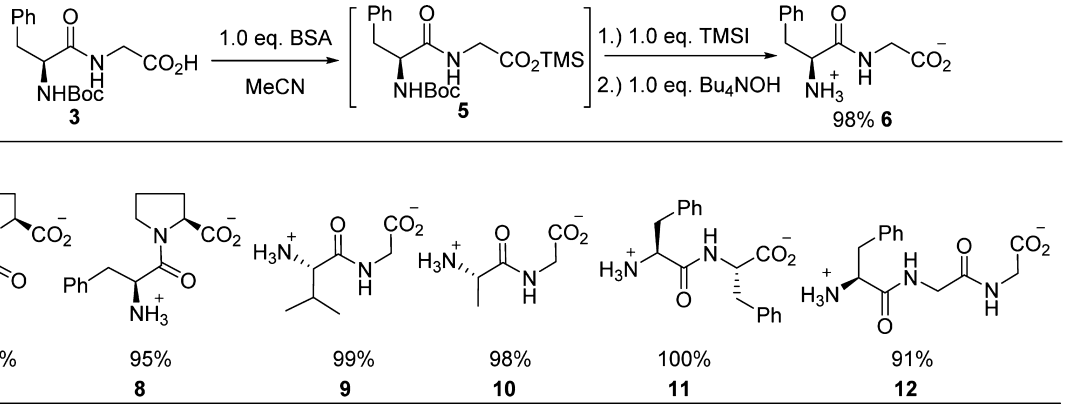
Scheme 4. TMSI as a Lewis Acid for Boc Deprotection



Scheme 5. TMSI-Mediated Boc Deprotection of a Dipeptide Salt



Scheme 6. Boc Deprotection of Peptides with BSA/TMSI



reaction mixture. For example, tetrabutylammonium iodide, which has very high solubility in MeCN,[11] was easily washed away during the isolation. Under our optimal conditions, **1** could be directly isolated in 93% yield by filtration. This method has been successfully demonstrated on a large scale.

This highly efficient Boc deprotection/isolation method could be applied to other zwitterionic substrates, such as peptides. Protection and deprotection are standard steps in most peptide syntheses. However, Boc as a protecting group has not been widely applied in peptide synthesis because of the relatively harsh conditions required for its removal.[12] In addition, because of their zwitterionic properties, the isolation and purification of peptides often encounter extra challenges. We proposed that our Boc deprotection/isolation protocol would be suitable for peptide synthesis and examined this hypothesis.

Boc-protected peptide tetrabutylammonium salt **4**, prepared from Boc-Phe-Gly-OH (**3**), was treated with TMSI in MeCN at room temperature. Full conversion required 2 equiv of TMSI since the first equivalent of TMSI was consumed for the generation of TMS ester **5**.[13] Consequently, a base was required to neutralize HI, and the desired zwitterionic product **6** could be directly isolated from the reaction mixture in 95% yield by filtration (Scheme 5).

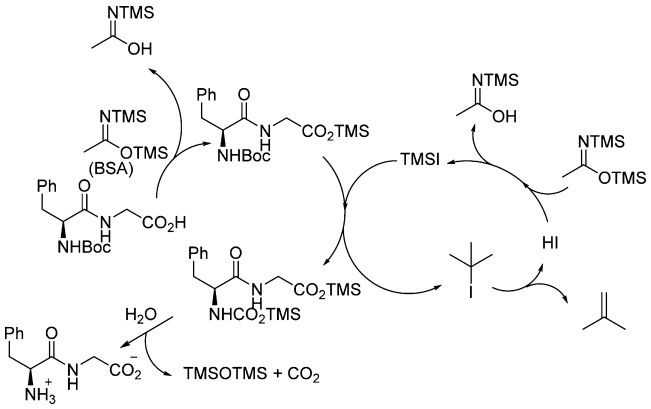
In order to simplify the deprotection/isolation sequence and reduce the amount of TMSI, **5** was prepared by treating **3** with the silylation reagent *N*,*O*-bis(trimethylsilyl)acetamide (BSA). Indeed, deprotection of **5**, prepared in situ from **3** with 1 equiv of BSA, proceeded smoothly upon the addition of 1 equiv of TMSI. The desired zwitterion **6** was directly isolated in 98% yield after neutralization of the reaction mixure with base (Scheme 6). A variety of *N*-Boc dipeptides were also deprotected using this methodology, with all of the substrates affording the corresponding dipeptides in excellent yields by direct crystallization. Furthermore, this protocol was expanded

dx.doi.org/10.1021/jo502319z | J. Org. Chem. 2014, 79, 11792−11796

to tripeptides. For example, Phe-Gly-Gly-OH was directly isolated in 91% yield from Boc-Phe-Gly-Gly-OH.

On the basis of our investigations with BSA in the Boc deprotection of peptides, we envisioned that it would be possible to regenerate TMSI from HI by reaction with excess BSA, since a stoichiometric amount of HI is generated from this transformation (Scheme 7). To test this hypothesis, **3** was

Scheme 7. Proposed Catalytic Cycle with the Combination of TMSI and BSA



treated with 2.2 equiv of BSA followed by the addition of 0.2 equiv of TMSI. Over 97% conversion was achieved after 2 days at room temperature, and **6** was directly isolated in 94% yield after the addition of water. The reaction time was shortened to 15 h by employing a more potent silylation reagent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Interestingly, the catalytic process had a similar generality and efficiency as the stoichiometric process (Scheme 8). Furthermore, this process could be applied back to *N*-Boc tetrabutylammonium salt **2**, and the corresponding compound MK-7655 (**1**) could be isolated in 75% yield. To the best of our knowledge, this is the first example of the deprotection of a Boc group with a catalytic amount of TMSI.

In summary, we have reported a highly efficient TMSI-mediated method for deprotection of Boc groups. This sets the stage for the direct isolation of zwitterionic compounds such as MK-7655 and peptides in high yields with excellent purities by simple filtration. In addition, it has been demonstrated that TMSI can be used as a catalyst in the presence of silylating agents.

EXPERIMENTAL SECTION

General Information. All commercially available chemicals were used without further purification. NMR spectra were recorded at 500 or 400 MHz for ^{1}H NMR and at 125 or 100 MHz for ^{13}C{^{1}H}NMR. Chemical shifts are reported in parts per million relative to the residual protic solvent for ^{1}H and the deuterated solvent for ^{13}C. TMSI, BSA, and BSTFA are commercially available compounds. Compound **2** was prepared according to a previously reported procedure.[6]

General Procedure for *N*-Boc Deprotection of Compound 2 with TMSI. Compound **2** (37.5 g, 54.3 mmol) was dissolved in MeCN, and the reaction mixture was cooled with a ice bath, after which TMSI (10.3 mL, 70.7 mmol) was added via addition funnel over 30 min between 0 and 5 °C. The resulting mixture was allowed to agitate for 1−2 h and then quenched with H_2O/MeCN (1:1, 6 mL) to afford a slurry. The slurry was warmed to room temperature and agitated for 12 h. Tetrabutylammonium acetate (13.6 mL, 13.6 mmol) was slowly added over 30 min to neutralize the excess acid. The slurry was agitated for 1 h and then filter to collect the solid. The solid was washed with MeCN/water (94:6, 60 mL × 4) to afford the crystalline product **1** (19.5 g, 95 wt %, 93% corrected yield).[8] The spectral data matched that of the previously reported compound.[6]

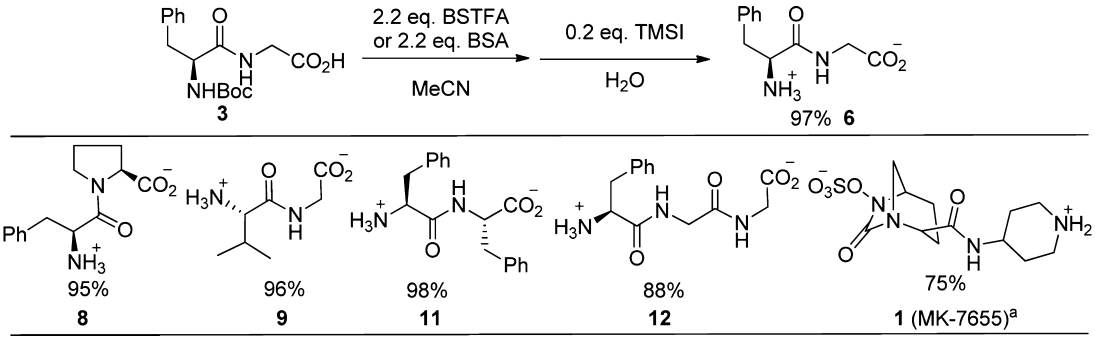
General Procedure for *N*-Boc Deprotection of Peptides with BSA/TMSI. To a slurry of *N*-Boc-peptide (1.00 g) in MeCN (6 mL) was added 1 equiv of BSA. The resulting clear solution was charged with 1 equiv of TMSI and stirred at ambient temperature for 30 min. Bu_4NOH solution (1 equiv, 1 M in MeOH) was added to afford a slurry, and then 3 equiv of water and methyl *tert*-butyl ether (6 mL) were added. The slurry was stirred for 2 h. The product was isolated by filtration of the slurry followed by a DCM wash to afford the desired peptide. Compounds **9**,[14] **10**,[15] **11**,[16] and **12**[17] are known compounds.

General Procedure for *N*-Boc Deprotection of Peptides with BSTFA and a Catalytic Amount of TMSI. To a slurry of *N*-Boc-peptide (1.00 g) in MeCN (6 mL) was added 2.2 equiv of BSTFA. The resulting clear solution was charged with 0.2 equiv of TMSI and stirred at ambient temperature for 15 h to achieve full conversion. Water (6 equiv) was added to afford a slurry, and then methyl *tert*-butyl ether (6 mL) was added. The slurry was stirred for 2 h. The product was isolated by filtration of the slurry followed by a DCM wash to afford the desired peptide.

*H-Phe-Gly-OH (**6**).* The indicated compound was obtained in 98% yield (0.73 g) as a white solid (mp 240−242 °C). ^{1}H NMR (400 MHz, D_2O) δ 2.98−3.10 (m, 2H), 3.38 (d, J = 17.3 Hz, 1H), 3.70 (d, J = 17.3 Hz, 1H), 4.08 (t, J = 7.3 Hz, 1H), 7.13−7.28 (m, 5H); ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 36.6, 43.1, 54.5, 127.8, 129.0, 129.3, 133.8, 168.9, 175.9; LC/MS (M 222.10) 223.12 (M + H$^+$).

*H-Ala-Pro-OH (**7**).* The indicated compound was obtained in 99% yield (0.64 g) as a white solid (mp 154−155 °C). ^{1}H NMR (1.5:1 rotamer ratio, asterisks denote minor rotamer peaks, 400 MHz, D_2O) δ 1.37* (d, J = 7.0 Hz, 1.2H), 1.44 (d, J = 7.0 Hz, 1.8H), 1.72−2.25 (m, 4H), 3.35−3.60 (m, 2H), 3.87* (q, J = 6.8 Hz, 0.39H), 4.15−4.19* (m, 0.39H), 4.22−4.26 (m, 1.2H); ^{13}C{^{1}H}NMR (asterisks denote minor rotamer peaks, 100 MHz, D_2O) δ 14.9, 15.3*, 22.0*,

Scheme 8. Use of a Catalytic Amount of TMSI for Boc Deprotection of Peptides and MK-7655



[a]1.1 equiv of BSA or BSTFA was applied.

dx.doi.org/10.1021/jo502319z | J. Org. Chem. 2014, 79, 11792−11796

24.9, 29.2, 31.4*, 47.2*, 47.3, 47.9, 48.1*, 61.8*, 62.1, 168.4, 169.2*, 178.6*, 179.0; LC/MS (M 186.10) 187.03 ($M + H^+$).

*H-Phe-Pro-OH (**8**).* The indicated compound was obtained in 95% yield (0.71 g) as a white solid (mp 104−106 °C). ^{1}H NMR (2.7:1 rotamer ratio, asterisks denote minor rotamer peaks, 400 MHz, D_2O) δ 1.51−1.55 (m, 2.16H), 1.64−1.70* (m, 0.77H), 1.70−1.89 (m, 0.74H), 2.08−2.15* (m, 0.27H), 2.87−2.95 (m, 1.04H), 3.07 (dd, J = 13.0, 7.5 Hz, 0.74H), 3.15−3.22 (m, 1.48 H), 3.26* (dd, J = 14.7, 4.8 Hz, 0.28H), 3.33−3.40 (m, 0.74H), 3.42−3.46* (m, 0.27H), 3.57−3.63* (m, 0.27H), 3.94 (dd, J = 9.9, 5.7 Hz, 0.73H), 4.17* (dd, J = 8.5, 5.8 Hz, 0.27H), 4.35* (dd, J = 8.9, 4.7 Hz, 0.27H), 7.10−7.31 (m, 5H); ^{13}C{^{1}H}NMR (asterisks denote minor rotamer peaks, 100 MHz, D_2O) δ 21.9, 24.6*, 29.2*, 30.9, 35.3*, 37.1, 47.0, 47.5*, 53.1, 53.2*, 61.8, 62.3*, 127.9*, 128.1, 129.1, 129.2, 129.6*, 133.5, 133.7*, 167.1*, 167.7, 178.2, 178.9*; LC/MS (M 262.13) 263.15 ($M + H^+$).

■ ASSOCIATED CONTENT

Supporting Information

NMR study of the reaction mechanism and spectra for all products. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: zhijian_liu@merck.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the following individuals from Merck Research Laboratories for analytic support: Hao Luo, Li Zhang, and Ping Zhuang. We also acknowledge John Limanto, Steven P. Miller, Joseph Lynch, Zheng Chen, Vincent Capodanno, Kevin Campos, Mark McLauglin, and Hao Chen for project support and suggestions.

■ REFERENCES

(1) (a) Liu, Y.; Zhang, J. *Chin. Med. J. (Engl. Ed.)* **2000**, *113*, 948−956. (b) Kahan, J. S.; Kahan, F. M.; Goegelman, R.; Currie, S. A.; Jackson, M.; Stapley, E. O.; Miller, T. W.; Miller, A. K.; Hendlin, D.; Mochales, S.; Hernandez, S.; Woodruff, H. B.; Birnbaum, J. *J. Antibiot.* **1979**, *32*, 1−12. (c) Novak, B.; Hudlicky, T.; Reed, J.; Mulzer, J.; Trauner, D. *Curr. Org. Chem.* **2000**, *4*, 343−362.

(2) (a) *Membrane Handbook*; Ho, W. S., Sirkar, K. K., Eds.; Chapman & Hall: New York, 1992. (b) Sheth, J. P.; Qin, Y.; Sirkar, K. K.; Baltzis, B. C. J. *J. Membr. Sci.* **2003**, *211*, 251−261.

(3) (a) Davies, J. *Science* **1994**, *264*, 360−362. (b) Drawz, S. M.; Bonomo, R. A. *Clin. Microbiol. Rev.* **2010**, *23*, 160−201.

(4) Blizzard, T. A.; Chen, H.; Kim, S.; Wu, J.; Bodner, R.; Gude, C.; Imbriglio, J.; Young, K.; Park, Y.-W.; Ogawa, A.; Raghoobar, S.; Hairston, N.; Painter, R. E.; Wisniewski, D.; Scapin, G.; Fitzgerald, P.; Sharma, N.; Lu, J.; Ha, S.; Hermes, J.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 780−785.

(5) Gordon, E. M.; Ondetti, M. A.; Pluscec, J.; Cimarusti, C. M.; Bonner, D. P.; Sykes, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 6053−6060.

(6) Mangion, I. K.; Ruck, R. T.; Rivera, N.; Huffman, M. A.; Shevlin, M. *Org. Lett.* **2011**, *13*, 5480−5483.

(7) Wuts, P. G. M.; Greene, T. W. In *Protecting Groups in Organic Synthesis*, 3rd ed.; Greene, T. W., Wuts, P. G. M., Eds.; John Wiley & Sons: New York, 1999.

(8) Miller, S. P.; Zhong, Y.-L.; Liu, Z.; Simeone, M.; Yasuda, N.; Limanto, J.; Chen, Z.; Lynch, J.; Capodanno, V. *Org. Lett.* **2014**, *16*, 174−177.

(9) (a) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495−496. (b) For review of the use of TMSI, see: Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225−2277. (c) Sakaitani, M.; Ohfune, Y. *Tetrahedron lett.* **1985**, *26*, 5543−5546. (d) Jung, M. E.; Lyster, M. A. *J. Chem. Soc., Chem. Commun.* **1978**, 315−316.

(10) For the detailed NMR study and full spectrum, see the Supporting Information.

(11) Note: tetrabutylammonium iodide has very high solubility in MeCN (0.5 g/mL).

(12) (a) Kates, S. A.; Albericio, F. *Solid-Phase Synthesis: A Practical Guide*; Marcel Dekker: New York, 2000. (b) Nomizu, M.; Inagaki, Y.; Yamashita, T.; Ohkubo, A.; Otaka, A.; Fujii, N.; Roller, P. P.; Yajima, H. *Int. J. Pept. Protein Res.* **1991**, *37*, 145−152. (c) Schnölzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. *Int. J. Pept. Protein Res.* **1992**, *40*, 180−193.

(13) Morita, T.; Okamoto, Y.; Sakurai, H. *Tetrahedron Lett.* **1980**, *21*, 835−838.

(14) Lagrille, O.; Taillades, J.; Boiteau, L.; Commeyras, A. *Eur. J. Org. Chem.* **2002**, 1026−1032.

(15) (a) Atkinson, C. E.; Aliev, A. E.; Motherwell, W. B. *Chem.—Eur. J.* **2003**, *9*, 1714−1723. (b) Durupthy, O.; Coupé, A.; Tache, L.; Rager, M.-N.; Maquet, J.; Coradin, T.; Steunou, N.; Livage, J. *Inorg. Chem.* **2004**, *43*, 2021−2030.

(16) Fransson, R.; Botros, M.; Sköld, C.; Nyberg, F.; Lindeberg, G.; Hallberg, M.; Sandström, A. *J. Med. Chem.* **2010**, *53*, 2383−2389.

(17) Mayer, C.; Hilvert, D. *Eur. J. Org. Chem.* **2013**, 3427−3431.