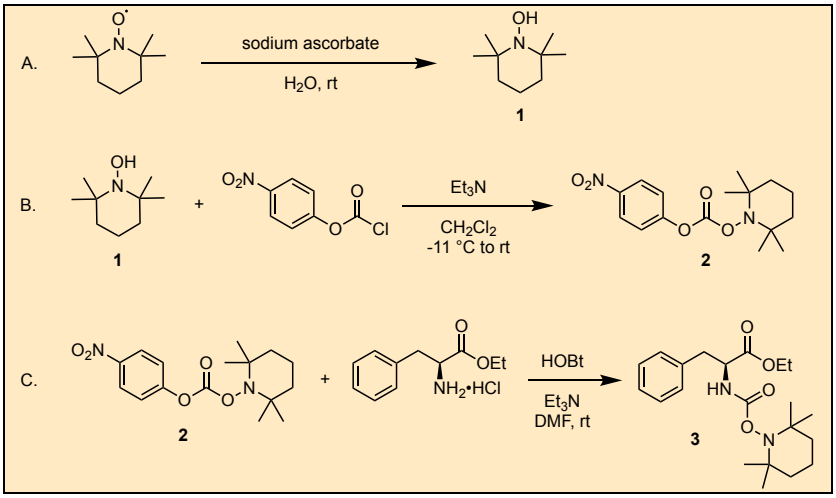
Organic Syntheses

Synthesis of 4-Nitrophenyl (2,2,6,6-Tetramethylpiperidin-1-yl) Carbonate (NPTC) for *N*-Protection of L-Phenylalanine Ethyl Ester

Joseph R. Lizza and Peter Wipf*[1]

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Checked by Kensuke Miura, Hiroaki Itoh, and Masayuki Inoue



Procedure (Note 1)

A. *2,2,6,6-Tetramethylpiperidin-1-ol* (**1**). A 500-mL Erlenmeyer containing a magnetic stir bar (PTFE-coated, 140 mm x 10 mm, polygon-type) and a temperature probe is charged with sodium ascorbate (25.4 g, 125 mmol, 2.0 equiv) (Note 2) and deionized water (180 mL), affording a pale-yellow solution (Figures 1A and 1B). The stirring is slowly increased until a stable vortex is established (Note 3). An initial temperature reading is taken and the probe is removed (Note 4) before freshly ground TEMPO (10.0 g, 62.7 mmol, 1.0 equiv) (Note 5) is added in one portion (Figure 1C). The probe is

DOI: 10.15227/orgsyn.097.0096 Published on the Web 7/10/2020

reinserted and the red suspension is stirred vigorously at ambient temperature (Figure 1D). Once the red solids are fully converted to a white precipitate, and internal temperature no longer increases (*ca.* 15 min) (Notes 6 and 7) (Figure 1E), CH_2Cl_2 (100 mL) (Note 8) is added and stirring is continued for 10 min.

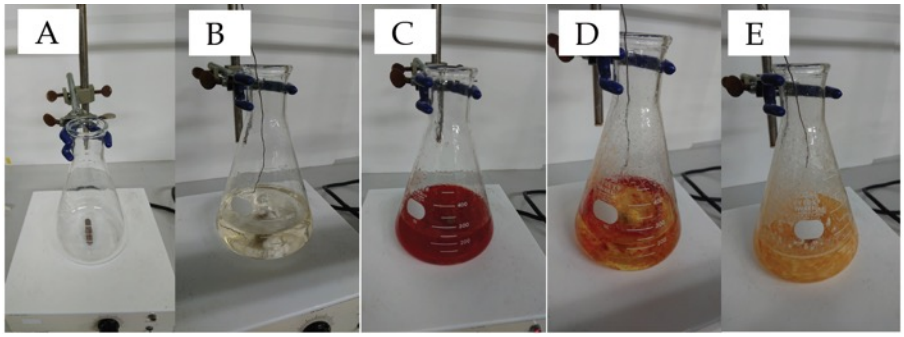


Figure 1. A) Reaction set-up B) after addition of sodium ascorbate, C) after addition of TEMPO, D) at partial conversion to 1, and E) after full conversion to 1 (photos provided by Checkers)

The stir bar is removed, and the biphasic mixture is transferred to a 500- mL separatory funnel. The organic layer is separated and the aqueous layer is extracted with CH_2Cl_2 (100 mL) (Note 9) (Figures 2A, 2B). The combined organic layers are dried with Na_2SO_4 (45 g) (Figure 2C) and decanted into a 500-mL 3-necked flask (the central neck: 29/32 joint, side necks: 15/25 joint) containing a stir bar (35 mm PTFE-coated, egg-shaped). An additional aliquot of CH_2Cl_2 (25 mL) is used to wash the drying agent and flask (Note 10). This solution is used in the next step without further purification.

B. *4-Nitrophenyl (2,2,6,6-tetramethylpiperidin-1-yl) carbonate* (**2**, "NPTC"). A 3-necked flask fitted with a temperature probe, a 100-mL pressure-equalizing addition funnel (15/25 joint), a connecting adaptor (upper outer joint 29/32, lower inner joint 15/25), and a drying-tube (15/25 joint) filled with anhydrous $CaSO_4$ is mounted inside a cooling bath (Note 11) (Figure 3A). After addition of the solution from step A (Figure 3B), dry-ice and brine (Note 12) are added to the bath, and the solution is stirred until a stable internal temperature of -11 ± 2 °C is achieved. The addition funnel is charged with 4-nitrophenyl chloroformate (14.6 g, 69.5 mmol, 1.1 equiv) (Note 13) as a solution in CH_2Cl_2 (55 mL) (Note 14) (Figures 4A and 4B).

Triethylamine (18.0 mL, 127 mmol, 2.0 equiv) (Note 15) is added *via* syringe through the drying-tube port.

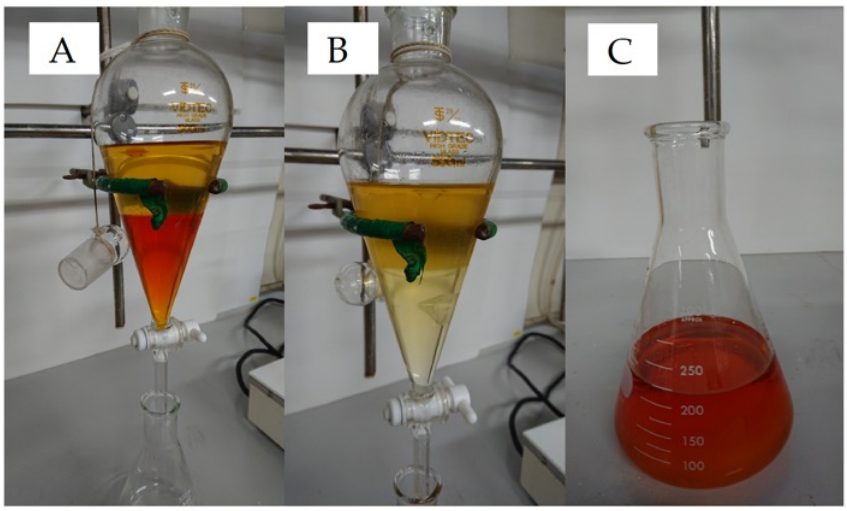


Figure 2. A) First extraction of 1 (CH_2Cl_2), B) second extraction, and C) combined organic layers with crude 1 over Na_2SO_4 (photos provided by Checkers)

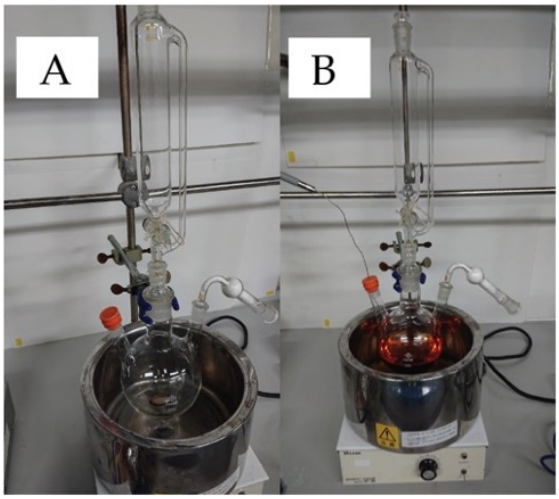


Figure 3. Reaction set-up A) before, and B) after addition of solution from step A (photos provided by Checkers)

The drying-tube is reattached and the chloroformate solution is added. The addition rate is continually adjusted to maintain an internal temperature of −11 ± 2 °C (*ca*. 30 min) (Note 16) (Figure 4B). After the addition of the reagent is complete, the addition funnel is washed with CH_2Cl_2 (Note 17) and the cooling bath is removed (Note 18) (Figure 4C). The reaction mixture is stirred for an additional 90 min (Notes 19 and 20) (Figure 4D).

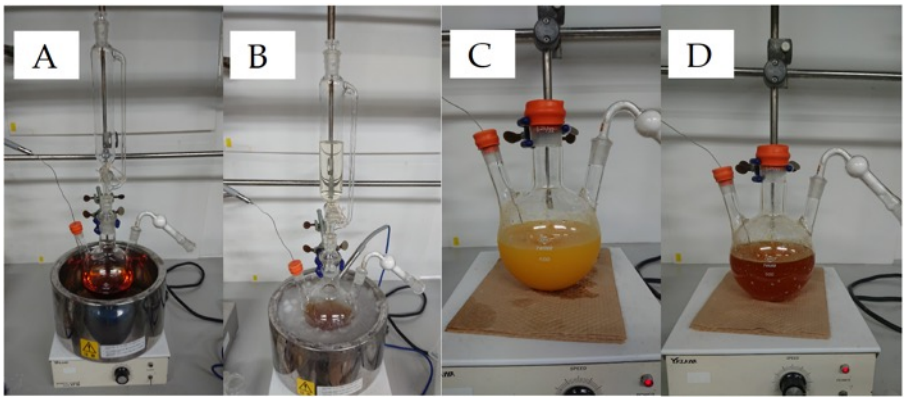


Figure 4. Reaction set-up A) before addition of dry ice/brine mixture, B) cooled reaction mixture during addition of the chloroformate, C) appearance after removal of the cooling-bath, and D) after stirring (90 min) (photos provided by Checkers)

The drying-tube, temperature probe, and stir-bar are removed, and the contents of the flask are transferred to a 1-L separatory funnel. Additional CH_2Cl_2 (50 mL) is used to wash the flask. A saturated aqueous solution of Na_2CO_3 (250 mL) is then added, and the contents of the separatory funnel are shaken vigorously (Figure 5A). The organic layer is separated and the orange aqueous layer is extracted with CH_2Cl_2 (1 x 100 mL). The combined organic layers are washed sequentially with a saturated aqueous solution of Na_2CO_3 (2 x 250 mL), water (150 mL), and brine (200 mL) (Note 21) (Figures 5B and 5C). After addition of solid Na_2SO_4, (75 g) to the organic layer (Figure 5D) and filtration through a fritted vacuum funnel (coarse) (Note 22) (Figures 6A and 6B), the red-orange solution is concentrated on a rotary evaporator (bath temperature 30 °C, 20 mmHg) to produce a yellow solid.

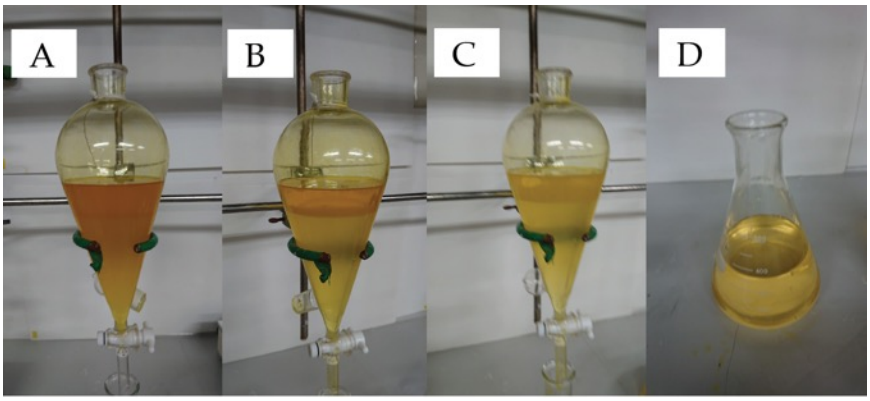


Figure 5. A) Extraction of crude 2 with CH_2Cl_2, B) base wash of combined organic layers, C) second base wash, D) organic layer over Na_2SO_4 (photos provided by Checkers)

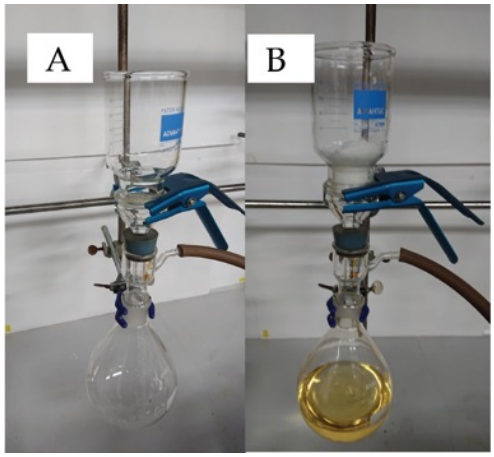


Figure 6 A) Set-up for filtration and B) filtration from drying agent (photos provided by Checkers)

The yellow solid is dried under high-vacuum for 30 min (ambient temp, 1-2 mmHg), and the residue (Figure 6A) is dissolved in acetone (250 mL) (Note 23) with swirling to give a reddish solution (Figure 6B). The flask is stoppered and placed in a freezer (–25 °C) for 16 h (Note 24).

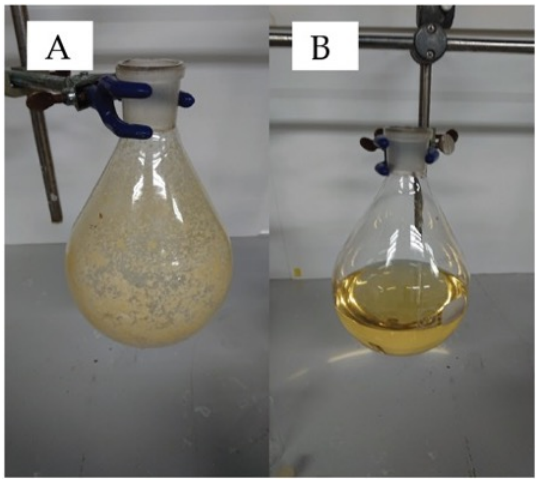


Figure 7. A) Crude 2 after brief drying in vacuo, and B) solution in acetone (photos provided by Checkers)

The crystals thus formed are collected by suction filtration on a 6-cm Kiriyama funnel (Note 25) (Figure 8A). The filter-cake is compressed (Note 26) and then washed with cold acetone (−25 °C, 2 x 25 mL) (Figure 8B). The crystallization is repeated two more times with the mother liquor (Note 27), and the crops are combined to give **2** ("NPTC") (15.1 g, 74%) (Note 28) as an off-white, hydrophobic, bench-stable, crystalline solid (Note 29) (Figure 8C).

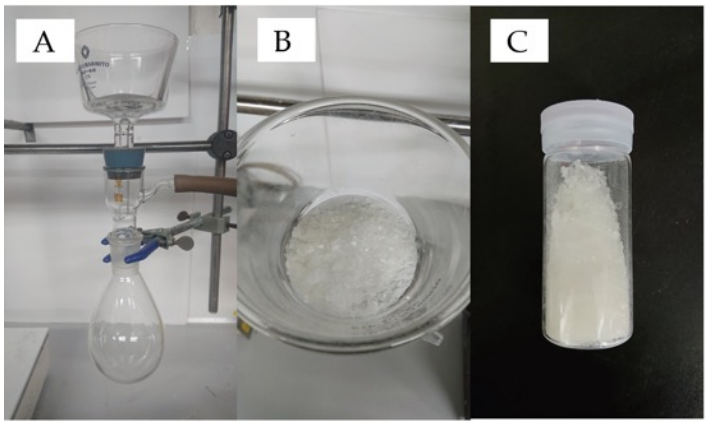


Figure 8. A) Suction filtration setup, B) the washed filter cake, and C) dried product 2 (photos provided by Checkers)

C. *Ethyl (((2,2,6,6-tetramethylpiperidin-1-yl)oxy)carbonyl)*-L-*phenylalaninate* (**3**). A three-necked 300-mL round-bottom flask (the central neck: 29/32 joint, side necks: 15/25 joint) containing a stir bar (30 mm, PTFE-coated, egg-shaped) and a temperature probe (Figure 9A) is charged with **2** (12.6 g, 38.7 mmol, 1.2 equiv), L-phenylalanine ethyl ester hydrochloride (7.50 g, 32.3 mmol, 1.0 equiv) (Note 30), and 1-hydroxybenzotriazole hydrate (2.48 g, 16.0 mmol, 0.5 equiv) (Notes 31 and 32) (Figure 9B). *N*,*N*-Dimethylformamide (DMF) (65 mL) (Note 7) is added, ensuring all solids are washed down the side of the flask. The main port and one side port are sealed with 29/32 and 15/25 rubber septa, respectively. Another side port is fitted with a drying-tube (anhydrous $CaSO_4$) (Figure 9C). The heterogeneous solution is stirred for 5 min (Notes 8 and 9) (Figure 9C).

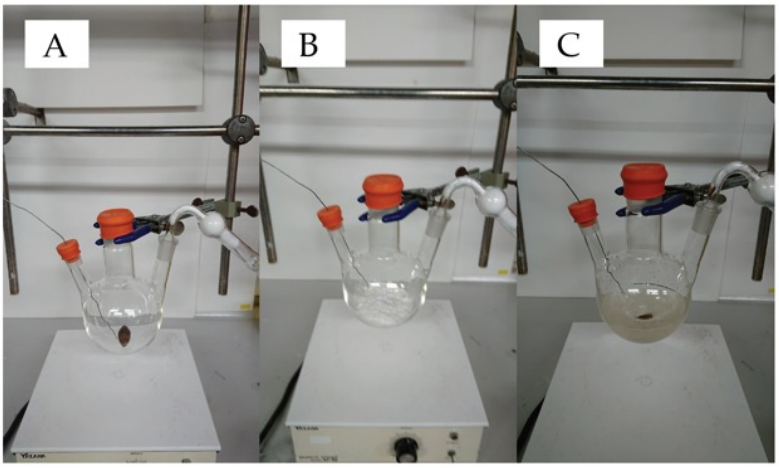


Figure 9. Reaction set-up A) before, and B) after addition of reagents, C) after solvent addition (photos provided by Checkers)

Triethylamine (11.5 mL, 8.26 g, 80.8 mmol, 2.5 equiv) is added in two portions over 2 min *via* syringe (Note 36) (Figure 10A). The reaction mixture is stirred at ambient temperature for 24 h and turns an orange-yellow color (Figure 10B). The reaction is diluted with 100 mL of toluene (Notes 37 and 38) (Figure 10C) and loaded onto a slurry-packed (toluene) column (ID 7 cm) containing basic alumina and topped with sand (Notes 39 and 40) (Figures 11A and 11B). The flask and alumina cake are washed with additional toluene (50 mL) (Figures 11C and 11D). The resulting light-tan filtrate is concentrated

on a rotary evaporator (bath temperature 40 °C, 780 mmHg to 40 mmHg) to afford a viscous liquid that begins to crystallize and is further dried on high vacuum for 12 h (ambient temp, 1-2 mmHg) to afford a tan solid.

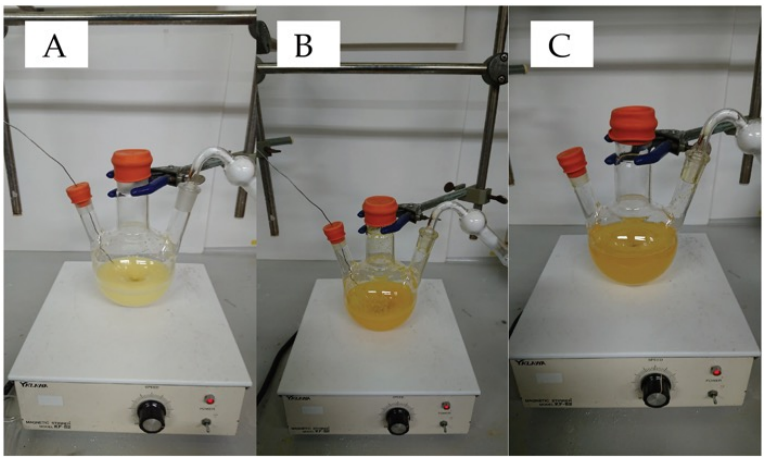


Figure 10. Reaction mixture A) after addition of triethylamine, B) after 24 h stirring, and C) after dilution with toluene (photos provided by Checkers)

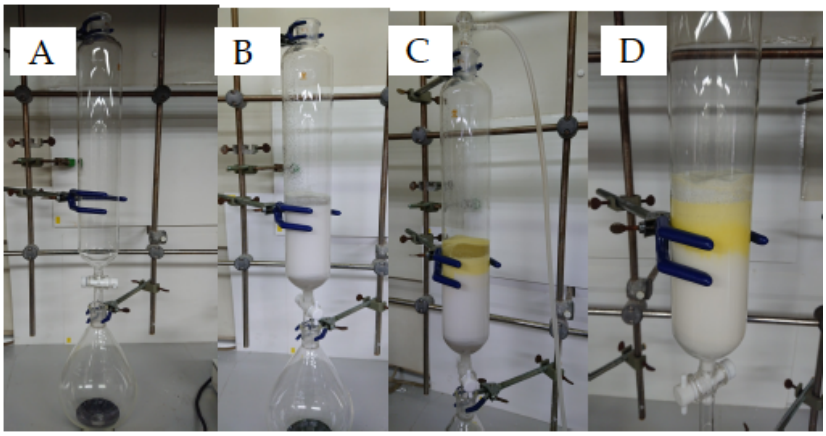


Figure 11. A) Column set-up, B) the column containing basic alumina and sand layers before addition of reaction mixture, C) during filtration using compressed air, and D) after filtration. The alumina bed retains 4- nitrophenol and other acidic or insoluble side products such as HOBt (photos provided by Checkers)

The solid is purified by chromatography on silica (Note 41) to afford the 2,2,6,6-tetramethylpiperidin-1-yloxycarbonyl ("Tempoc")-protected amino ester **3** (9.58 g, 77%) as a colorless solid (Note 42) (Figure 12). Excess **2** (3.19 g, 26%) is also recovered in the purification and can be reused. Ester **3** shows negligible racemization (Note 43).

Figure 12. Product 3 (photos provided by Checkers)

Notes

1. Prior to performing each reaction, a thorough hazard analysis and risk assessment should be carried out with regard to each chemical substance and experimental operation on the scale planned and in the context of the laboratory where the procedures will be carried out. Guidelines for carrying out risk assessments and for analyzing the hazards associated with chemicals can be found in references such as Chapter 4 of "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at https://www.nap.edu/catalog/12654/prudent-practices-in-the-laboratory-handling-and-management-of-chemical. See also "Identifying and Evaluating Hazards in Research Laboratories" (American Chemical Society, 2015), which is available via the associated website "Hazard Assessment in Research Laboratories" at https://www.acs.org/content/acs/en/about/governance/committees

/chemicalsafety/hazard-assessment.html. In the case of this procedure, the risk assessment should include (but not necessarily be limited to) an evaluation of the potential hazards associated with TEMPO, sodium ascorbate, methylene chloride, triethylamine, 4-nitrophenyl chloroformate, sodium potassium tartrate, acetone, sodium carbonate, 1-hydroxybenzotriazole, L-phenylalanine ethyl ester hydrochloride, *N*,*N*-dimethylformamide, ethyl acetate, toluene, alumina, and silica gel.

2. Sodium ascorbate (98%) was purchased from FUJIFILM Wako Pure Chemical Corporation as off-white crystals and was used as received (checkers). Sodium ascorbate (99%) was purchased from Alfa Aesar as off-white crystals and was used as received (submitters).
3. The submitters reported that when the reduction was performed in a round-bottomed flask with an egg-type stir-bar, the reaction proceeded more slowly. The Erlenmeyer provides better agitation and results in a shorter reaction time.
4. The submitters reported that when the thermocouple was left in the flask, reactant tended to accumulate on the probe, resulting in unreduced TEMPO.
5. TEMPO (98%) was purchased from FUJIFILM Wako Pure Chemical Corporation as a red crystalline solid which contained large chunks (checkers). TEMPO (99%) was purchased from Oakwood Products as a red crystalline solid which contained large chunks. The crystals were lightly ground with a mortar and pestle to provide a finer, more uniform reagent for the subsequent heterogeneous reaction (submitters).
6. The reduction was slightly exothermic, and the internal temperature increased. Temperature readings were conducted in 3-min intervals. TEMPO reduction was complete in ca. 15 min and was accompanied by a white precipitate with no further increase in internal temperature (Figure 13).

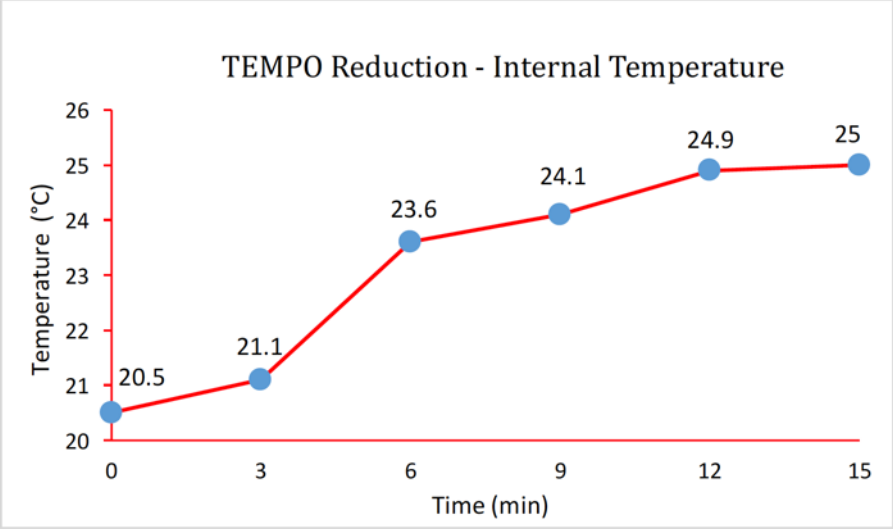


Figure 13. Temperature profile of the reduction of TEMPO with sodium ascorbate

7. In the TLC analysis of the crude product, the R_f values of the product **1** and TEMPO in hexane/EtOAc (4/1, v/v) were 0 and 0.55, respectively. The spot of the product **1** on the silica gel 60 F_{254} plate (TLC Silica gel 60 F_{254}, purchased from Merck KGaA) was visualized with UV light (254 nm) (Figure 14).

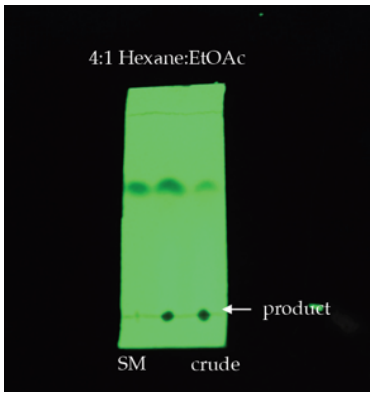


Figure 14. TLC analysis of the crude product in step A (photo provided by Checkers)

8. Methylene chloride (ACS grade) was purchased from Sigma Aldrich and used as received (checkers). Methylene chloride (ACS grade) was purchased from Fisher Scientific and used as received (submitters). Upon addition, the color changed to an orange homogeneous-appearing solution that separated into two phases when stirring stopped.
9. This 100 mL was first used to wash the Erlenmeyer flask.
10. This aliquot was also decanted into the round-bottomed flask.
11. The cooling bath used had a diameter of 21 cm and a height of 10 cm.
12. The dry-ice/brine mixture produced a cooling bath temperature of −16 ± 2 °C, which was maintained by periodic addition of dry-ice. Such aqueous sodium chloride baths are more efficient than salt/ice baths, provide stable temperatures, and are also safer than corresponding organic solvent/dry-ice counterparts.
13. 4-Nitrophenyl chloroformate (96%) was purchased from FUJIFILM Wako Pure Chemical Corporation as a white powder and was used as received (checkers). 4-Nitrophenyl chloroformate (96%) was purchased from Oakwood Products as a white powder and was used as received (submitters).
14. The reagent was charged into a 100-mL round-bottomed flask containing a stir-bar (2 cm length, PTFE-coated, egg-shaped). After addition of CH_2Cl_2 (35 mL), dissolution was complete in 10 min (endothermic, temperature not recorded). The solution was transferred to the addition funnel with CH_2Cl_2 (2 x 10 mL).
15. Triethylamine (99%) was purchased from FUJIFILM Wako Pure Chemical Corporation and was used as received (checkers). Triethylamine (99%) was purchased from Fisher Scientific and was used as received (submitters).
16. The external bath temperature was maintained at −16 ± 2 °C.
17. The walls of the addition funnel and chloroformate that crystallized at the tip and the wall of the adaptor (Figure 15) were washed into the flask with CH_2Cl_2 (10 mL). The addition funnel port was fitted with a rubber septum.

Figure 15. 4-Nitrophenyl chloroformate on the wall of the adaptor (photo provided by Checkers)

18. A crystalline solid was observed towards the end of the chloroformate addition, presumably triethylamine hydrochloride, which precipitates from CH_2Cl_2 at low temperatures. This precipitate undergoes dissolution upon warming.
19. The internal temperature slowly rose from −11 °C to 17 °C during this time (Figure 16).

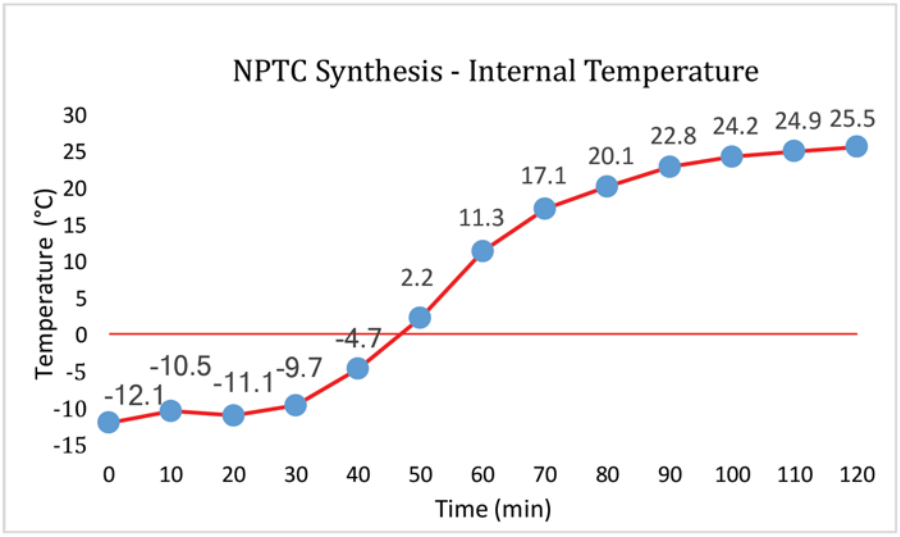


Figure 16. Temperature profile of the acylation of 1 with 4-nitrophenyl chloroformate. The cooling bath was removed at the 30-min mark

20. In the TLC analysis of the crude product, the R_f value of the product **2** in EtOAc/toluene (3/17, v/v) was 0.73. The spot of the product **2** on the silica gel 60 F_{254} plate (TLC Silica gel 60 F_{254}, purchased from Merck KGaA) was visualized with UV light (254 nm) (Figure 17).

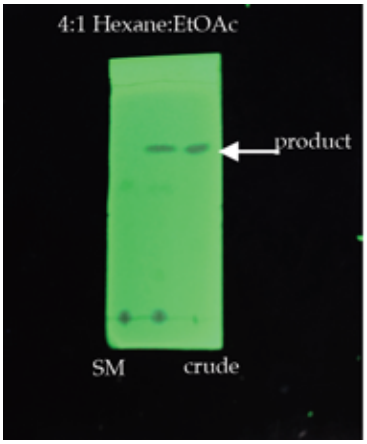


Figure 17. TLC analysis of the crude product in step B (photo provided by Checkers)

21. In one instance by the submitters, an emulsion formed at the organic/aqueous layer during the brine wash. The emulsion was dissolved with half-saturated Rochelle's salt (40 mL) and gentle swirling.
22. The drying agent was washed with CH_2Cl_2 and filtered (100 mL, then 50 mL).
23. Acetone (ACS grade) was purchased from Sigma Aldrich and used as received (checkers). Acetone (ACS grade) was purchased from Fisher Scientific and used as received (submitters).
24. The submitters reported that crystallization should begin within 2 h after the solution was placed in the freezer. If no crystals were observed, a glass stir-rod was inserted into the cold solution, removed, and allowed to dry. This rod was then used to scratch the sides of the flask which immediately caused a few crystals to form, and the flask was returned to the freezer.
25. The diameter of the Kiriyama funnel was chosen such that the crystals formed a dense filter cake, which facilitated proper washing.
26. The filter cake should be compressed well, ensuring that all adhering impurities were efficiently removed in the subsequent washings.

27. The mother liquor was transferred with acetone to a 500-mL round-bottomed flask, concentrated on a rotary evaporator (bath temperature 38 °C, 780 mmHg to 200 mmHg), and then transferred to a 300-mL round-bottomed flask and concentrated (about 130–140 mL) (Figure 18) on a rotary evaporator (bath temperature 38 °C, 780 mmHg to 200 mmHg). This concentrate was seeded with 5–10 mg of **2** from crop 1, allowed to cool to ambient temperature, and subsequently placed in the freezer (−25 °C) for 24 h. The crystals that precipitated were collected by suction filtration and washed with 35 mL of cold acetone (−25 °C). The process was repeated with concentration in a 300-mL round-bottomed flask (about 50 mL) and washing with 25 mL of cold acetone (−25 °C). The masses collected from crop 1, 2, and 3 were on 9 g, 5 g, and 1 g, respectively. The crystals from crop 1 were typically colorless to off-white and small, whereas the crystals from crops 2 and 3 were larger and pale-beige in color. Individual NMR analysis of the 3 crops showed pure **2** in all cases. The crystals were granulated with a PTFE-coated stir-bar retriever to provide a more uniform product before being combined. Afterwards, the crystal size and color were almost identical (Figure 19).

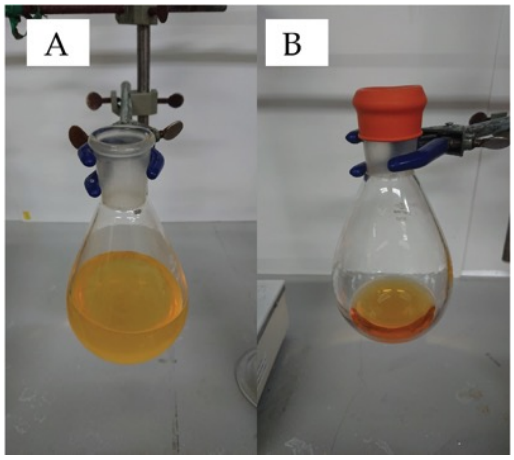


Figure 18. Reduction volumes and appearances of mother liquors from first and second crystallizations (photos provided by Checkers)

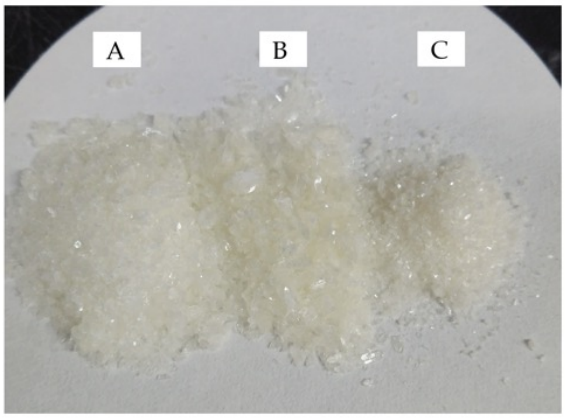


Figure 19. A) Crop 1, B) crop 2, and C) crop 3; after granulation (photos provided by Checkers)

28. Concentration of the mother liquor gave a red solid (2.56 g), which still contained **2** (TLC) (Figures 20A, 20B, and 20C); however, this material was not purified further. The major impurity was TEMPO, which tended to coelute with **2** on SiO_2 (hexane/EtOAc). Complete separation could be achieved by substitution of toluene for hexane.

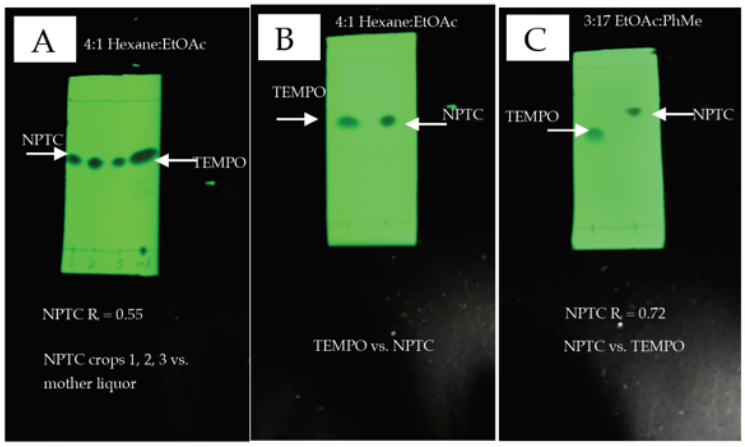


Figure 20. A) TLCs of product 2 (NPTC) crops 1, 2, and 3 vs. mother liquor, B) combined crops vs. TEMPO, C) NPTC vs. TEMPO resolved with EtOAc/toluene. Visualization with a 254 nm UV lamp (photos provided by Checkers)

29. When the reaction was carried out on a half-scale, 7.60 g (75%) of product **2** was obtained. The product exhibited the following properties: R_f 0.55 (4/1, hexane/EtOAc, v/v); mp 137–139 °C (dec.); IR (film) 2978, 2939, 1797, 1521, 1344, 1204, 1179, 914, 862 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.21 (s, 6 H), 1.24 (s, 6 H), 1.42 – 1.45 (m, 1 H), 1.55 – 1.74 (m, 5 H), 7.39 (dd, 2 H, *J* = 9.2, 2.3 Hz) 8.28 (dd, 2 H, *J* = 9.2, 2.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 16.7, 20.4, 31.5, 39.2, 60.9, 121.6, 125.2, 145.2, 153.9, 155.8; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}N_2O_5Na$ $[M+Na]^+$ 345.1421, found 345.1432. Quantitative NMR using a 1,3,5-trimethoxybenzene qNMR standard purchased from FUJIFILM Wako Pure Chemical Corporation (99.90%) indicated 98% purity. The submitters reported that other runs occasionally contained impurities that typically adsorb onto the crystal surface if the filter cake is not sufficiently compressed before the washing steps. These impurities have been previously identified as 4-nitrophenol and TEMPO. Both may be removed after all crops have been combined; the former by making a slurry by addition of 1.0 M aqueous Na_2CO_3, filtering, and washing with water; the latter by sublimation of the impurity under high-vacuum (ambient temperature, 1-5 mmHg).
30. L-Phenylalanine ethyl ester hydrochloride (99%) was purchased from FUJIFILM Wako Chemical Corporation as a fluffy white solid and was used as received (checkers). L-Phenylalanine ethyl ester hydrochloride (99%) was purchased from Sigma Aldrich as a fluffy white solid and was used as received (submitters).
31. *Caution*! HOBt is potentially explosive, especially in its anhydrous form. The submitters reported that HOBt hydrate (98% w/ 20% wt. water) has also been used on smaller scales with no detriment to yield. Anhydrous HOBt was used in the submitters' sequence without incident.
32. HOBt hydrate (97% w/ 11% wt. water) was purchased from FUJIFILM Wako Pure Chemical Corporation as a fine white powder and was used as received (checkers). Anhydrous HOBt (97%) was purchased from Amatek Chemical as a fine white powder and was used as received (submitters).
33. *N,N*-Dimethylformamide (ACS grade) was purchased from Sigma Aldrich and was used as received.
34. Dissolution of the solid was accompanied by a decrease in internal temperature (*ca.* 2.5 °C).
35. Stirring speed should not be vigorous to minimize the amount of reagent that may collect on the walls of the flask.

36. Addition of base to this concentrated solution resulted in a minor increase in internal temperature (*ca.* 2 °C). No cooling bath was used to absorb this exotherm.
37. Toluene (ACS grade) was purchased from Sigma Aldrich and used as received (checkers). Toluene (ACS grade) was purchased from Fisher Scientific and used as received (submitters).
38. In the TLC analysis of the crude product, the R_f value of the product **3** in toluene/EtOAc (17/3, v/v) was 0.43. The spot of the product **3** on the silica gel 60 F_{254} plate (TLC Silica gel 60 F_{254}, purchased from Merck KGaA) was visualized with UV light (254 nm) and ninhydrin reagent.

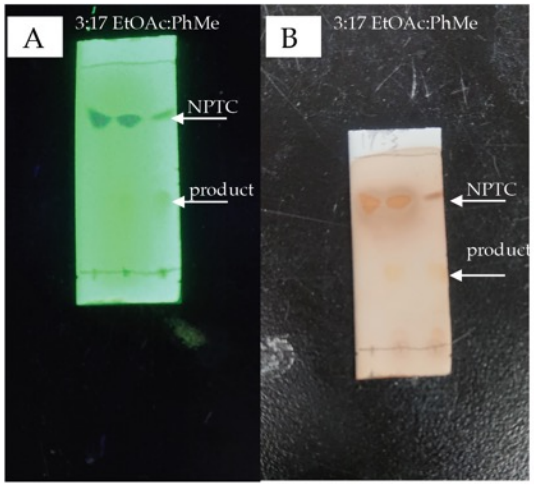


Figure 21. TLC analysis of the crude product in step C. Visualization with A) UV light (254 nm) and B) ninhydrin reagent (photos provided by Checkers)

39. Basic alumina (45–145 μM) was purchased from FUJIFILM Wako Pure Chemical Corporation and was used as received (checkers). Basic alumina, Brockmann grade I (50–200 μM) was purchased from Acros Organics and was used as received (submitters).
40. A column (ID 7 cm) was charged with basic alumina (550 g) that was wetted with toluene (*ca.* 350 mL). A 1.5-cm layer of sand was added, and the reaction mixture was loaded with the aid of a long-neck funnel. The crude product was washed through with toluene (1.0 L) using compressed air. Basic alumina effectively removed the 4-nitrophenol

byproduct and HOBt from the reaction mixture, thus avoiding the need for an aqueous work-up.

41. The crude was dissolved in toluene (15 mL) and loaded onto a slurry-packed (toluene) column (ID 7 cm) containing SiO_2 (0.040–0.050 mm, 400 g, purchased from Kanto Chemical). After loading, a 1-cm layer of sand was added to the top of the SiO_2 and toluene (500 mL) was eluted under positive air pressure. The solvent system was switched to 19/1 toluene/EtOAc (ACS grade purchased from Sigma Aldrich which was used as received (checkers), ACS grade purchased from Fisher Scientific which was used as received (submitters)), and fractions were taken in 200-mL bottles. Product **3** eluted first and was typically removed with 1.75 L of this mixture. After elution of product **3**, the solvent polarity was increased to 4/1 toluene/EtOAc, and elution of the product **3** (R_f 0.43, 17/3 toluene/EtOAc, v/v) was completed with an additional 1.2 L of this solvent mixture. Fractions 12 through 19 were combined, concentrated on a rotary evaporator (38 °C, 780 to 40 mmHg), and dried in vacuo (1-2 mmHg) at ambient temperature for 12 h.

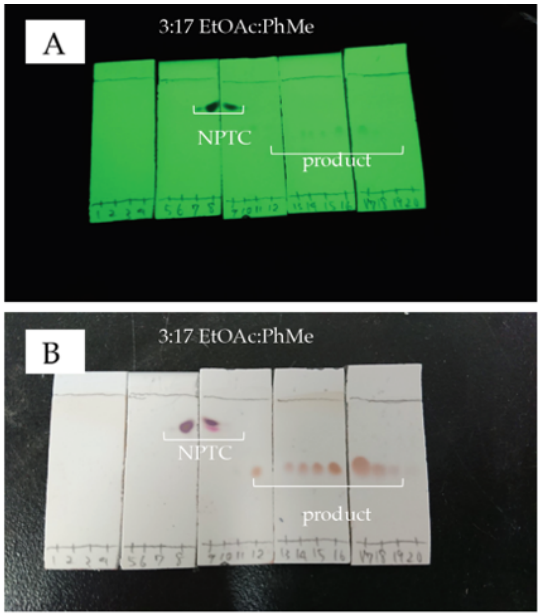


Figure 22. TLC analysis of the fractions. Visualization with A) UV light (254 nm) and B) ninhydrin reagent (photos provided by Checkers)

42. When the reaction was carried out on a half-scale, 4.85 g (78%) of product **3** was obtained. The product exhibited the following properties: $[\alpha]_D^{24}$ 1.92 (*c* 0.69, $CHCl_3$); R_f 0.43 (17/3, toluene/EtOAc, v/v); mp 81–83 °C; IR (film) 3309, 2968, 2933, 1722, 1500, 1455, 1365, 1253, 1183, 1032, 740, 696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.86 (s, 3 H), 1.03 (s, 3 H), 1.16 (s, 3H), 1.17 (s, 3 H), 1.24 (t, *J* = 7.6 Hz, 3 H), 1.36 – 1.49 (m, 3 H), 1.52 – 1.62 (m, 3 H), 3.12 – 3.19 (m, 2 H), 4.17 (q, *J* = 7.5 Hz, 2 H), 4.65 (dt, *J* = 7.5, 6.3 Hz, 1 H), 7.17 – 7.16 (m, 2 H), 7.22 – 7.30 (m, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.0, 16.6, 20.46, 20.49, 31.4, 31.6, 37.9, 39.6 (2C), 54.5, 60.6, 60.7, 61.3, 127.1, 128.5, 129.2, 135.8, 157.9, 171.4; HRMS (ESI) *m*/*z* calcd for $C_{21}H_{33}N_2O_4$ $[M+H]^+$ 377.2435, found 377.2430. Quantitative NMR using a 1,3,5-trimethoxybenzene qNMR standard purchased from FUJIFILM Wako Pure Chemical Corporation (99.90%) indicated 97% purity (checkers).
43. Racemization detection of **3** was conducted using Marfey's analysis: Compound **3** (0.025 mmol) was heated (105 – 110 °C) in 6 N aqueous HCl (5 mL) on an aluminum block in a sealed vessel for 24 h. After cooling of the solution to ambient temperature, the bulk of the HCl gas was removed by sparging with argon for 10 min. An aliquot of the mixture was transferred to a 10 mL round-bottomed flask and concentrated with heating (70 °C) under high-vacuum (780 mmHg to 40 mmHg) for 30 min. The white solid residue was dissolved in deionized water (0.500 mL) and the solution (0.060 mL) was transferred to a vial before being treated with a 36 mM solution of Marfey's reagent (1-fluoro-2-4-dinitrophenyl-5-L-alanine amide) in freshly distilled acetone (118 μL). The solution was made alkaline by addition of 1.0 M aqueous $NaHCO_3$ (48 μL). The vial was sealed and placed in an aluminum block at 40 °C for 1 h under exclusion of ambient light. During this time, the bright-yellow solution turned fluorescent orange. After cooling to ambient temperature, 2.0 N aqueous HCl (24 μL) was added and the sample was shaken before being passed through a micron-filter into a brown vial and kept refrigerated in the dark until analysis (no longer than 24 h) by HPLC. HPLC analysis was conducted on an Agilent 1200 HPLC system utilizing an Inertsil ODS-4 4.6 mm x 250 mm C18 column with a 5-micron particle size. Elution with water/acetonitrile (both containing 0.1% (v/v) trifluoroacetic acid) was conducted at a constant a flow rate of 1.0 mL/min using a linear gradient of 10–60% acetonitrile over 50 min and a 50-min acquisition time. The injection volume should be no larger

than 2 μL as larger volumes resulted in saturation of the detector. Under these conditions, the excess Marfey's reagent eluted first (t_R = 30.5 min) followed by the L-isomer (t_R = 38.2 min), the D-isomer (t_R = 40.8 min), and finally an unidentified peak (t_R = 41.3 min) (Figure 23). Standards were prepared using the aforementioned conditions of: Tempoc protected DL-PheOEt (synthesized correspondingly (*vida supra*) from the commercial DL-phenylalanine ethyl ester hydrochloride) and from commercial DL-PheOH and L-PheOEt· HCl. Amino acids and esters (98%) for standards were purchased from FUJIFILM Wako Pure Chemical Corporation and were used as received (checkers). Marfey's reagent (99%) was purchased from Sigma Aldrich as a yellow powder and used as received (checkers). Amino acids and esters (98%) for standards were purchased from Alpha Aesar and were used as received. Marfey's reagent (98%) was purchased from Sigma Aldrich as a yellow powder and used as received (submitters). The enantiomeric excess of compound **3** was determined to be 99% by the HPLC analysis, which indicated that negligible racemization occurred during the protection.

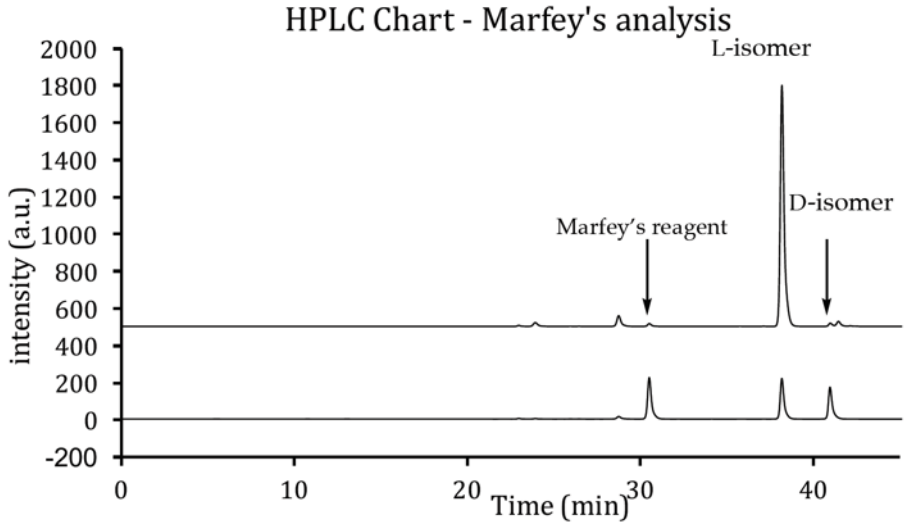


Figure 23. HPLC charts for determination of enantiomeric excess of compound 3. Marfey's analysis of synthesized compound (top) and standards (bottom)

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red "Caution Notes" within a procedure. It is important to recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

The procedures described in *Organic Syntheses* are provided as published and are conducted at one's own risk. *Organic Syntheses, Inc.*, its Editors, and its Board of Directors do not warrant or guarantee the safety of individuals using these procedures and hereby disclaim any liability for any injuries or damages claimed to have resulted from or related in any way to the procedures herein.

Discussion

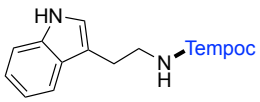
Protecting groups (PGs) play an important role in synthetic chemistry,[2] and although their use increases the number of steps in a given protocol,[3] they have proven essential for the syntheses of complex scaffolds such as carbohydrates,[4] polyfunctional pharmaceuticals,[5] and natural products.[6] In this regard, amines are one of the most commonly protected moieties due to their basicity, nucleophilicity, and coordinating ability - all of which can interfere with many synthetic transformations[7] and metal-mediated

processes.[8] Carbamates represent the most commonly used of amine PGs due to their ease of installation, robustness, and removal under mild conditions. In particular, *t*-butoxycarbonyl (Boc),[9] benzyloxycarbonyl (Cbz),[10] and 9-fluorenylmethoxy carbonyl (Fmoc),[11] are frequently encountered in synthetic[12] and peptide chemistry;[13] their ability to withstand a variety of reaction conditions, while concomitantly being cleavable in an orthogonal fashion, have made them a popular item in the organic chemist's toolbox.[14]

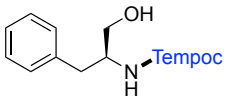
The first use of 2,2,6,6-tetramethylpiperidinylxoycarbonyl (Tempoc) as an amine protecting group was reported in the enantioselective total synthesis of (−)-cycloclavine.[15] This group proved essential to the synthesis since it was both stable to lithiation as well as readily removed by thermolysis. Following, the general utility of Tempoc as an amine protecting group was evaluated.[16] Specifically, with a straightforward method to obtain Tempoc transfer reagent **2** (NPTC), this compound's reactivity was explored across a range of diverse amines (Table 1), and removal was demonstrated using either *in situ* generated catalytic copper(I) species, or through thermolytic cleavage (Table 2). Furthermore, selective removal of Tempoc was found to be feasible in the presence of Boc- and Cbz-protected functionalities, and vice versa (Table 3). The current protocol on the *N*-protection of phenylalanine illustrates the utility of the Tempoc group for amino acid protection and peptide chemistry.

Table 1. Representative Scope of Tempoc-Protected Primary, Secondary, and Heterocyclic Amines

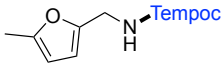
Primary Amines[a,b]



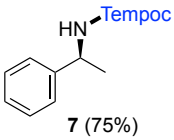
4 (92%)



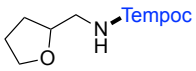
5 (72%)



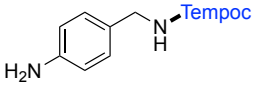
6 (92%)



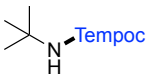
7 (75%)



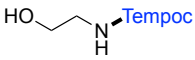
8 (93%)



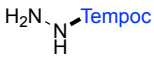
9 (93)%



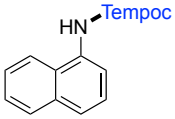
10 (32%)



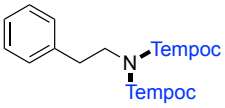
11 (94%)



12 (95%)[c]

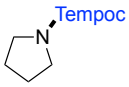


13 (-)

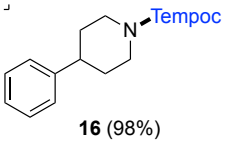


14 (90%)[d]

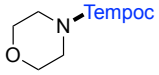
Secondary Amines[a,b]



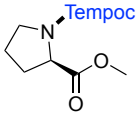
15 (91%)



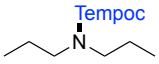
16 (98%)



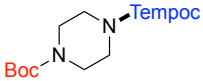
17 (98%)



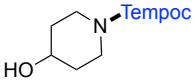
18 (87%)[e]



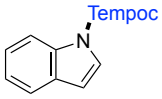
19 (56%)



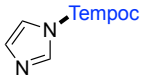
20 (91%)



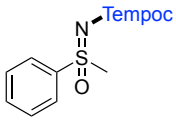
21 (87%)



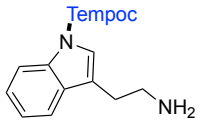
22 (94%)[f]



23 (91%)



24 (82%)[g]



25 (71%)[h]

[a]Reaction conditions: amine (1 equiv), NPTC (1.2 equiv), and Et_3N (3 equiv), in DMF (0.5 M), rt, 12 h; [b]Isolated yields; [c]NPTC(1 equiv), hydrazine [d]NaH as base (2.6 equiv), NPTC (2.4 equiv); [e]4 equiv of Et_3N; [f]NaH (1.3 equiv) as base. [g]NaH (1.1 equiv) as base; [h]NaH (1.3 equiv), 4 h, then NPTC (1.2 equiv)

Table 2. Copper-Mediated and Thermolytic Deprotection of Tempoc-Protected Amines

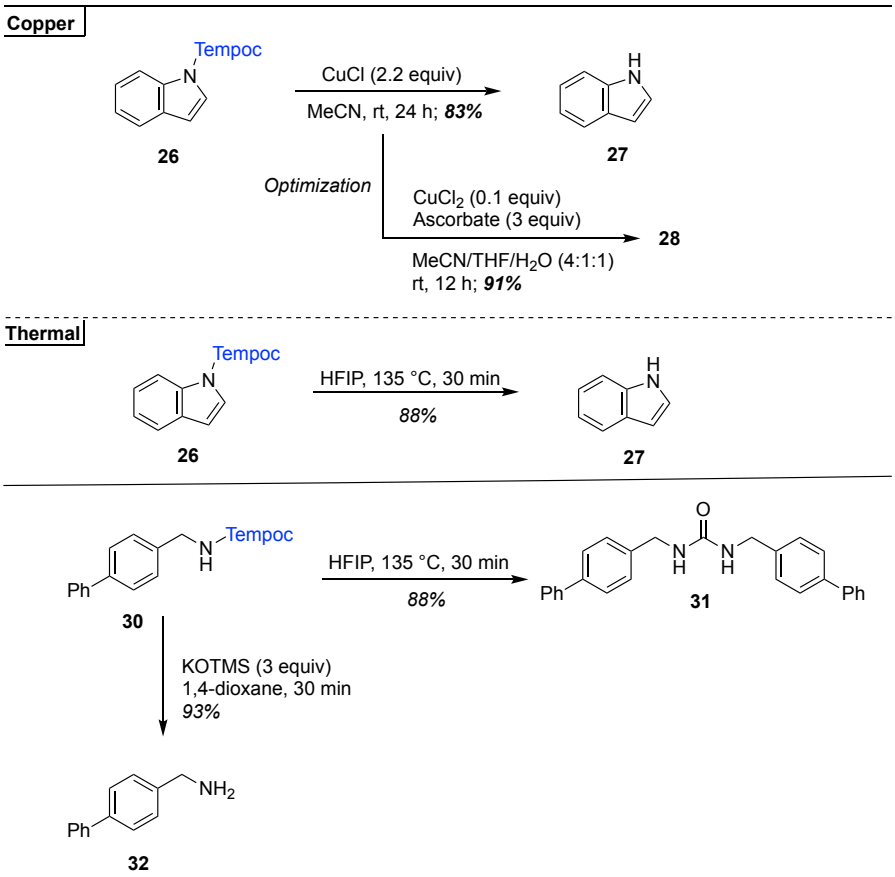
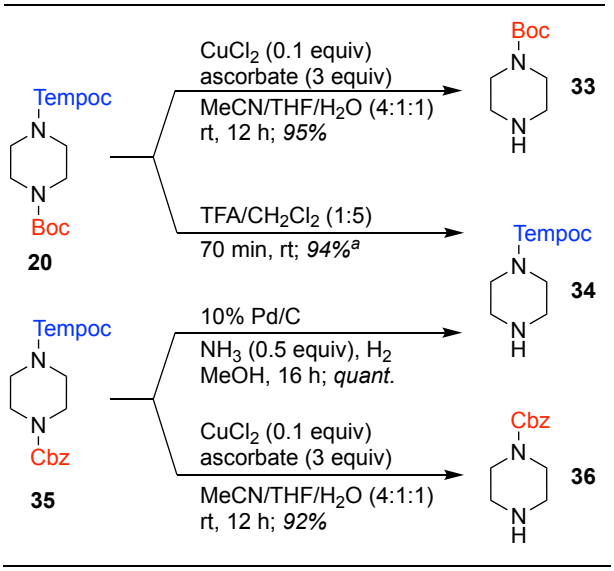


Table 3. Orthogonal Deprotection of Tempoc-, Boc-, and Cbz-, Amines



[a]Isolated as TFA salt.

In conclusion, the Tempoc protecting group is effective for the protection of a variety of amines. The mild conditions required to install Tempoc using the easily-prepared, bench-stable, crystalline transfer reagent **3** (NPTC), and a facile deprotection through reductive cleavage with either catalytic copper(I), or under thermolytic conditions, allows for a range of applications in synthetic sequences. Moreover, resilience towards hydrogenolytic and acidic conditions allows for excellent orthogonality with Boc- and Cbz-protected amines. Consequently, the use of transfer reagent **3** (NPTC) allows for the introduction of an amine protecting group that provides a high degree of synthetic utility with respect to facilitating an efficient preparation of complex organic compounds.

References

1. Chevron Annex, 219 Parkman Av., Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, Email: pwipf@pitt.edu; ORCID: 0000-0001-7693-5863
2. Sartori, G.; Ballini, R.; Bigi, F.; Bosica, G.; Maggi, R.; Righi, P. *Chem. Rev.* **2004**, *104*, 199–250.
3. Huang, B.; Guo, L.; Jia, Y. *Angew. Chem. Int. Ed.* **2015**, *54*, 13599–13603.
4. Kumar, A.; Gannedi, V.; Rather, S. A.; Vishwakarma, R. A.; Ahmed, Q. N. *J. Org. Chem.* **2019**, *84*, 4131–4148.
5. Mannam, M. R.; Sankareswaran, S.; Gaddam, V. R.; Natarajan, S.; Kottapalli, R. P.; Kumar, P. *Org. Process Res. Dev.* **2019**, 23, 919–925.
6. Liu, H.; Chen, L.; Yuan, K.; Jia, Y. *Angew. Chem. Int. Ed.* **2019**, *58*, 6362–6365.
7. Orain, D.; Ellard, J.; Bradley, M. *J. Comb. Chem.* **2002**, *4*, 1–16.
8. Bernoud, E.; Lepori, C.; Mellah, M.; Schulz, E.; Hannedouche, J. *Catal. Sci. Technol.* **2015**, *5*, 2017–2037.
9. Varala, R.; Nuvula, S.; Adapa, S. R. *J. Org. Chem.* **2006**, *71*, 8283–8286.
10. Lebel, H.; Leogane, O. *Org. Lett.* **2006**, *8*, 5717–5720.
11. Ibrahim, T. S.; Tala, S. R.; El-Feky, S. A.; Abdel-Samii, Z. K.; Katritzky, A. R. *Synlett* **2011**, 2013–2016.
12. *Greene's Protective Groups in Organic Synthesis*; Wuts, P. G. M., Ed.; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2014.
13. Isidro-Llobet, A.; Álvarez, M.; Albericio, F. *Chem. Rev.* **2009**, *109*, 2455–2504.
14. Schelhaas, M.; Waldmann, H. *Angew. Chem. Int. Ed.* **1996**, *35*, 2056–2083.
15. (a) McCabe, S. R.; Wipf, P. *Angew. Chem. Int. Ed.* **2017**, *56*, 324–327. (b) McCabe, S. R.; Wipf, P. *Synthesis* **2019**, *51*, 213–224.
16. Lizza, J. R.; Bremerich, M.; McCabe, S. R.; Wipf, P. *Org. Lett.* **2018**, *20*, 6760–6764.

Appendix
Chemical Abstracts Nomenclature (Registry Number)

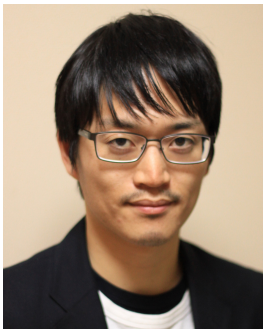
NPTC: 4-Nitrophenyl (2,2,6,6-tetramethylpiperidin-1-yl) carbonate; (2247828-39-1)
TEMPO: 1-Piperidinyloxy, 2,2,6,6-tetramethyl-; (2564-83-2)

Sodium ascorbate: L-Ascorbic acid, monosodium salt; (134-03-2)
Triethylamine: Ethanamine, *N*,*N*-diethyl-; (121-44-8)
4-Nitrophenyl chloroformate: Carbonochloridic acid, 4-nitrophenyl ester; (7693-46-1)
L-Phenylalanine ethyl ester hydrochloride: ethyl (2*S*)-2-amino-3-phenylpropanoate;hydrochloride; (3182-93-2)
1-Hydroxybenzotriazole: 1H-Benzotriazole, 1-hydroxy-; (2592-95-2)

Prof. Peter Wipf received a Ph.D. in 1987 from the University of Zürich under the guidance of Prof. Heinz Heimgartner. After 2.5 years as a Swiss NSF postdoctoral fellow with Prof. Robert E. Ireland at the University of Virginia, he joined the University of Pittsburgh, and was promoted to Distinguished Professor in 2004. Prof. Wipf received the NSF Presidential Faculty Fellowship in 1994, the Arthur C. Cope Scholar Award in 1998, the Ernest Guenther Award in the Chemistry of Natural Products in 2009, and the Humboldt Research Award in 2014. He is a Fellow of the AAAS (2002), the RSC (2004), and the ACS (2010). He also was the editor of volume 87 of *Organic Syntheses*, and he currently serves on the Board of Directors of *Organic Syntheses* and *Organic Reactions*, and as an Associate Editor for *ACS Med. Chem. Lett.*

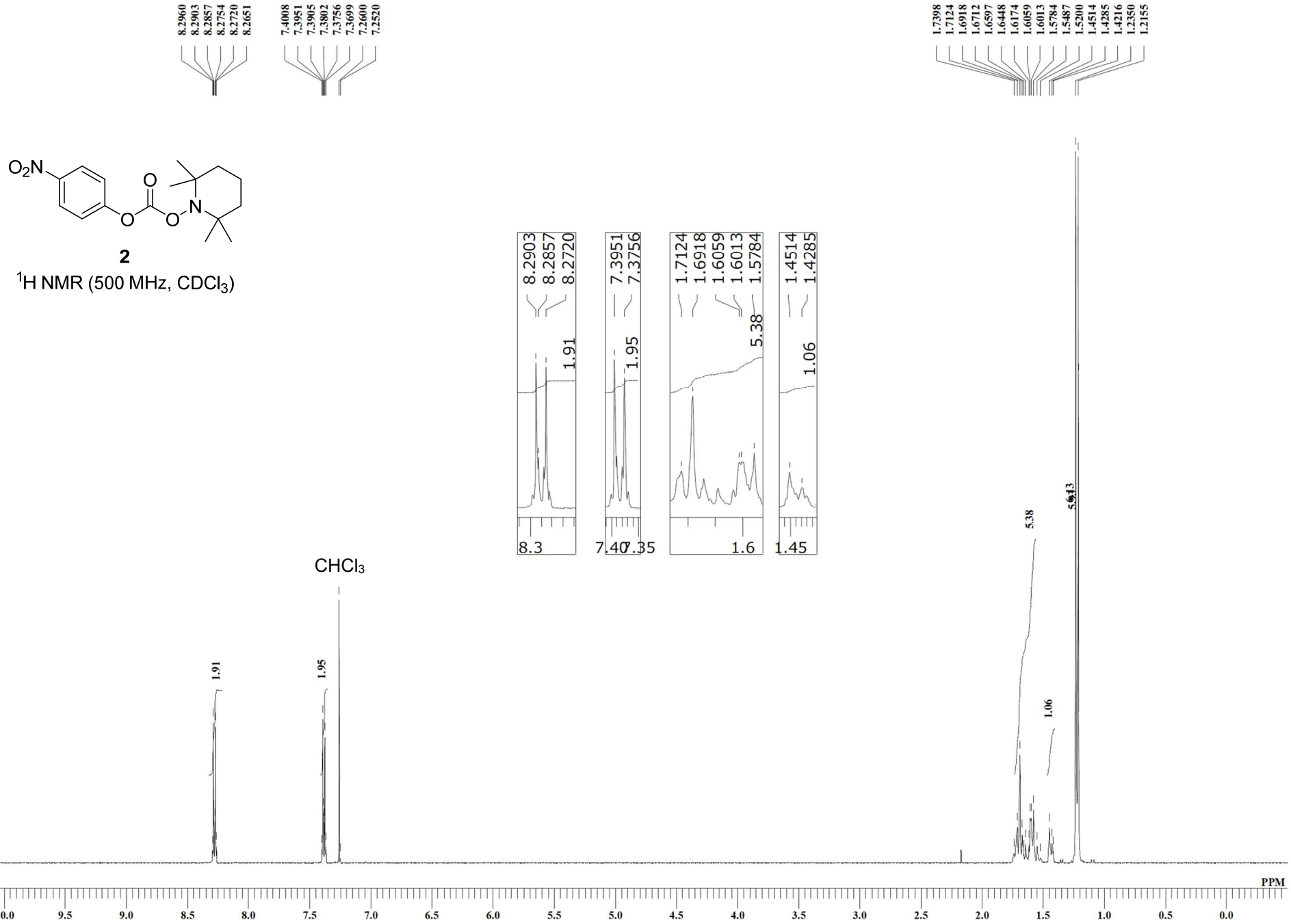
Joseph R. Lizza was born in 1984. In 2017 he received his B.S. in Chemistry and B.A. in Physics from Rowan University in New Jersey under the guidance of Dr. Gustavo Moura-Letts. There, his research centered on method development for the construction of biologically relevant molecular scaffolds. Joining the Wipf Research Group at the University of Pittsburgh in 2017, his current research concentrates on development of novel synthetic methods and the total synthesis of natural products.

Kensuke Miura was born in Akita, Japan. He graduated from the University of Tokyo in 2018 with a B.Sc in Pharmaceutical Sciences. He then continued his graduate studies at the same university under the supervision of Prof. Masayuki Inoue. His research interests are in the area of the total synthesis of natural products.

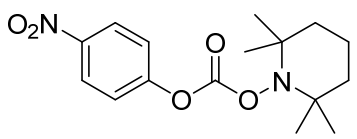


Hiroaki Itoh was born in Mie, Japan, in 1985. He received his B.Sc. degree in Pharmaceutical Sciences from the University of Tokyo in 2008, and he received his Ph.D. from the same university under the supervision of Prof. Masayuki Inoue. After working for FUJIFILM Corporation for two years, he was appointed as an assistant professor in the Graduate School of Pharmaceutical Sciences at the University of Tokyo. His research interests include the synthesis and chemical biology of biologically active natural products and their analogues with a particular focus on peptidic natural products and related molecules.

4-nitrophenyl (2,2,6,6-tetramethylpiperidin-1-yl) carbonate (NPTC, **2**)



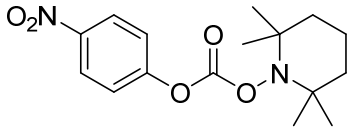
4-nitrophenyl (2,2,6,6-tetramethylpiperidin-1-yl) carbonate (NPTC, **2**)



2

^{13}C NMR (125 MHz, $CDCl_3$)

^{1}H qNMR for 4-nitrophenyl (2,2,6,6-tetramethylpiperidin-1-yl) carbonate (NPTC, **2**) with 1,3,5-trimethoxybenzene

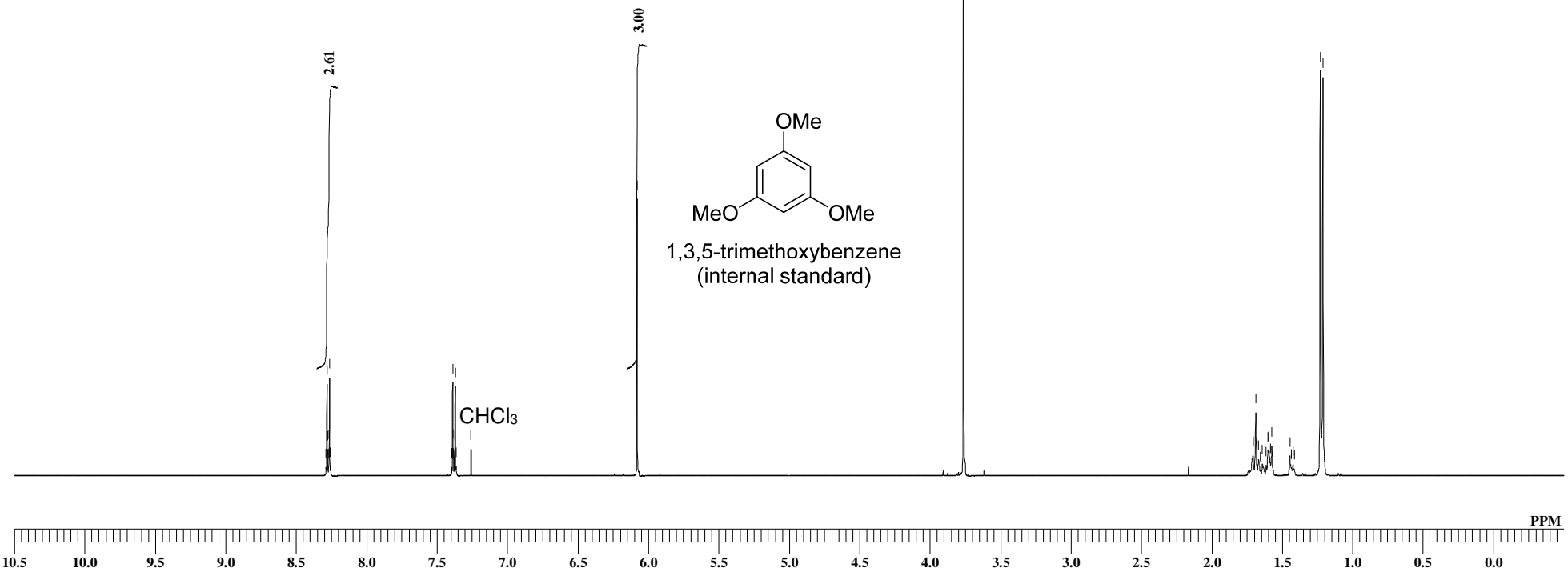


2

^{1}H NMR (500 MHz, $CDCl_3$)

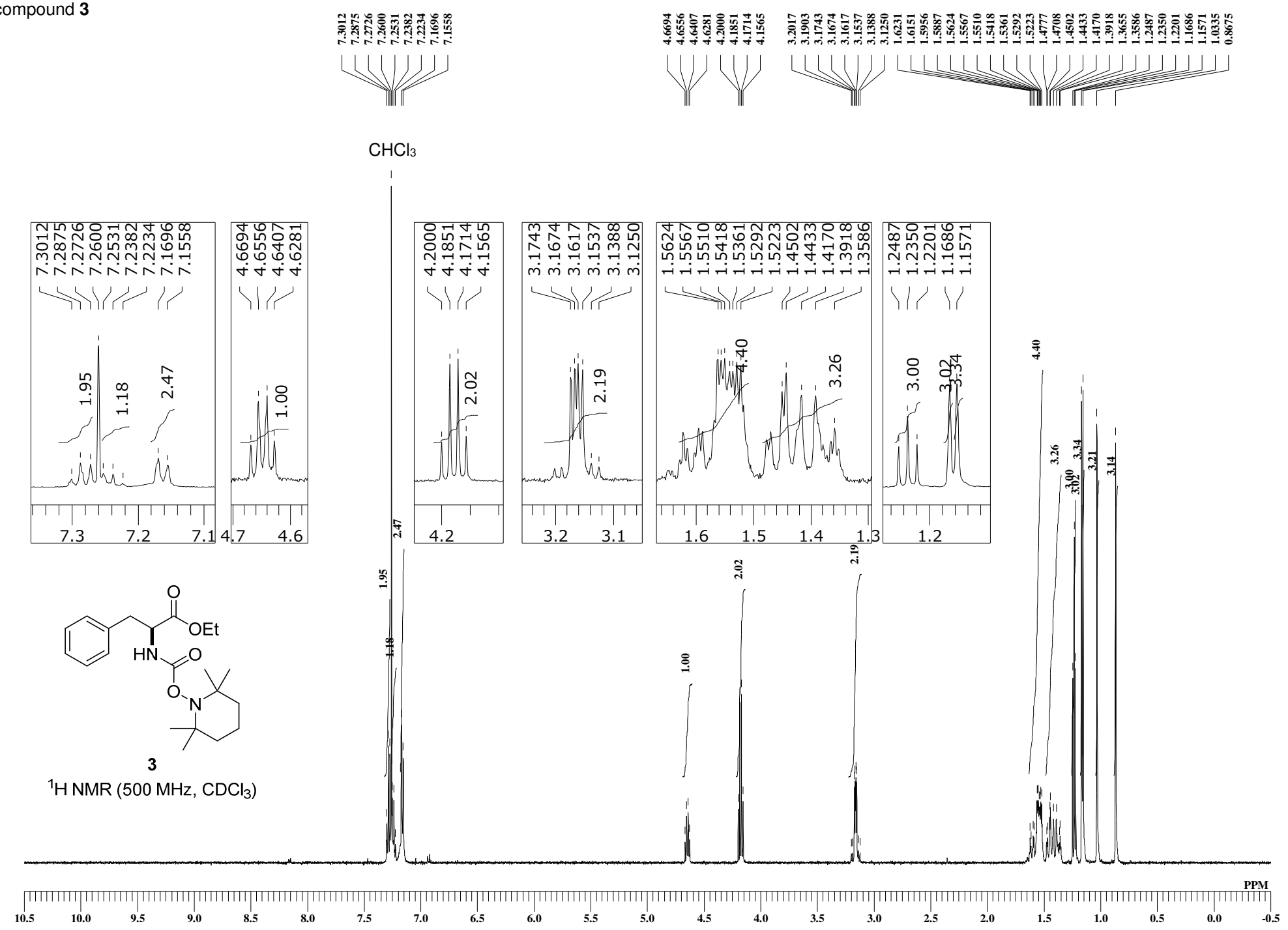
purity of **2**: 98%

	product (**2**)	internal standard (1,3,5-trimethoxybenzene)
molecular weight	322.36	168.19
amount	26.5 mg	10.4 mg (purity: 99.90%)
proton	2H	3H
area	2.61	3.00



1,3,5-trimethoxybenzene
(internal standard)

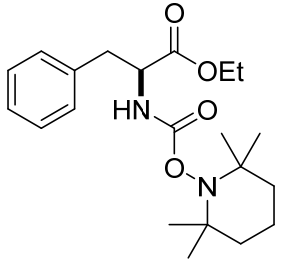
compound **3**



3

^{1}H NMR (500 MHz, $CDCl_3$)

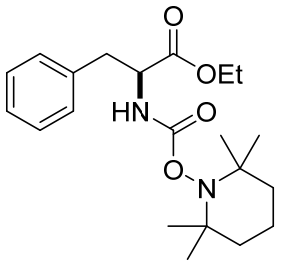
compound **3**



3

^{13}C NMR (125 MHz, $CDCl_3$)

^{1}H qNMR for compound **3** with 1,3,5-trimethoxybenzene

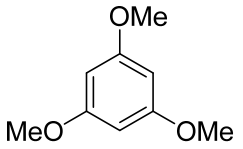


3

^{1}H NMR (500 MHz, $CDCl_3$)

purity of **3**: 97%

	product (**3**)	internal standard (1,3,5-trimethoxybenzene)
molecular weight	376.49	168.19
amount	22.0 mg	14.5 mg (purity: 99.90%)
proton	2H	3H
area	1.32	3.00



1,3,5-trimethoxybenzene
(internal standard)