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A Scalable Synthesis of a Hydroxamic Acid LpxC Inhibitor

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ABSTRACT: A short and scalable synthesis of chiral hydroxamic acid **1**, a LpxC inhibitor, is described. This work discloses a novel diastereoselective addition of 2-nitropropane-generated lithium salt to *tert*-butanesulfinimine. Moreover, the impurity profiles of reactions giving oily products and practical means of purifying these products are discussed in detail.

INTRODUCTION

The increasing number of multidrug-resistant bacterial pathogens calls for urgent needs for developing new molecules treating bacterial infectious diseases with novel mechanisms.[1] The enzyme LpxC (UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-GlcNAc deacetylase) catalyzes the biosynthesis of lipopolysaccharides contained in Gram-negative bacterial membranes and has been identified as a pharmaceutical target.[2,3] Hydroxamic acids are an important class of LpxC inhibitors.[4] Here we report a scalable synthesis of a new LpxC inhibitor, hydroxamic acid derivative **1**.

The original synthesis from medicinal chemistry is shown in Scheme 1. Racemic β-nitro-valine **4** was prepared in a two-step sequence and coupled with benzoic acid derivative **7** to afford **8**. Following a reduction to **9** and protection to **10**, the desired enantiomer **11** was yielded by chiral preparative separation. Removal of the protecting group and installation of hydroxylamine afforded the desired **1**.

This synthesis involving a late-stage chiral separation is not economical for multikilogram manufacture. An asymmetric version would be more efficient for this purpose. We were successful in achieving an asymmetric synthesis of **1** and developing the new synthesis to a practical process for scale-up.

The asymmetric synthesis is outlined in Scheme 2. Condensation of ethyl glyoxylate **2** with (*S*)-(−)-*tert*-butanesulfinamide **12** followed by diastereoselective addition with 2-nitropropane lithium salt and cleavage of the chiral auxiliary produced the desired enantiomer **15**. Coupling amine **15** with acid **7** gave **16**, which was reduced and then treated with hydroxylamine to generate **1**.

RESULTS AND DISCUSSION

The synthesis began with the known condensation of ethyl glyoxylate **2** with (*S*)-(−)-*tert*-butanesulfinamide **12**[5] (Scheme 3). The reported condensation conditions utilize activated molecular sieves in CH_2Cl_2 at room temperature, and the reaction takes up to 42 h. We found that these conditions could be replaced with $MgSO_4$ in toluene at elevated temperature (56 °C), which resulted in a 20-h reaction time. After concentration to a ∼85% solution in toluene, the crude *N*-sulfinylimino ester **13** was used for the next step without further purification.

Davis et al. have reported high diastereoselective additions of Grignard reagents to sulfinylimino ester **13**[5] to access α-amino acids. Inspired by this work, we decided to explore the addition of a 2-nitropropane-generated anion to sulfinylimino ester **13** to access the desired synthetic intermediate **14**. Even though it could be a straightforward way to access chiral vicinal diamines in general, such a type of additions has not been reported to the best of our knowledge. To our delight, we achieved such an addition with high diastereoselectivity (98:2) by using 0.4 equiv of LiHMDS and 1.2 equiv of 2-nitropropane at −20 °C in THF. The full conversion of the reaction by using a substoichiometric amount of base supports the assumption that it might be an interesting case of a base-catalyzed addition reaction;[6] namely the newly formed adduct in the event served as additional base to deprotonate the rest of 2-nitropropane for reaction completion. The stereochemistry of **14** was assigned by comparison with an authentic sample on chiral HPLC and is consistent with the prediction on the basis of the open-transition-state model depicted by Davis.[7] The reaction also proceeded with formation of the elimination byproduct **18** (at 8% by HPLC area) and other unidentified impurities, the yield of which was estimated to be ∼40%, and was purified after removal of the chiral auxiliary. Our preliminary studies indicated that either a higher amount of LiHMDS or a higher reaction temperature led to more elimination byproduct **18**, while a lower amount of base or a lower reaction temperature resulted in an incomplete reaction. Using potassium *tert*-butoxide as base also triggered the addition but gave lower selectivity. Using triethylamine as base led to the decomposition of **13**.

Crude **14** was treated with hydrochloric acid in ethanol (prepared by addition of AcCl to ethanol) to provide **15**. Free amine **15** itself is an oil and cannot be purified by extractive workup. We found that concentrating the reaction solution followed by the addition of TBME that led to direct precipitation of **15** hydrochloric salt with some ammonium chloride was an efficient way to remove all organic impurities. Further removal of ammonium chloride by washing the product in IPAc solution with aqueous alkaline solution resulted in **15** in 20% yield over three steps, 97% chemical purity, and 99% ee by HPLC. Even though the overall isolation yield is modest (due to the addition step), the synthesis is operationally simple to access enantiomeric pure β-nitro-valine **15** and, hence, was adopted for pilot-plant manufacture.

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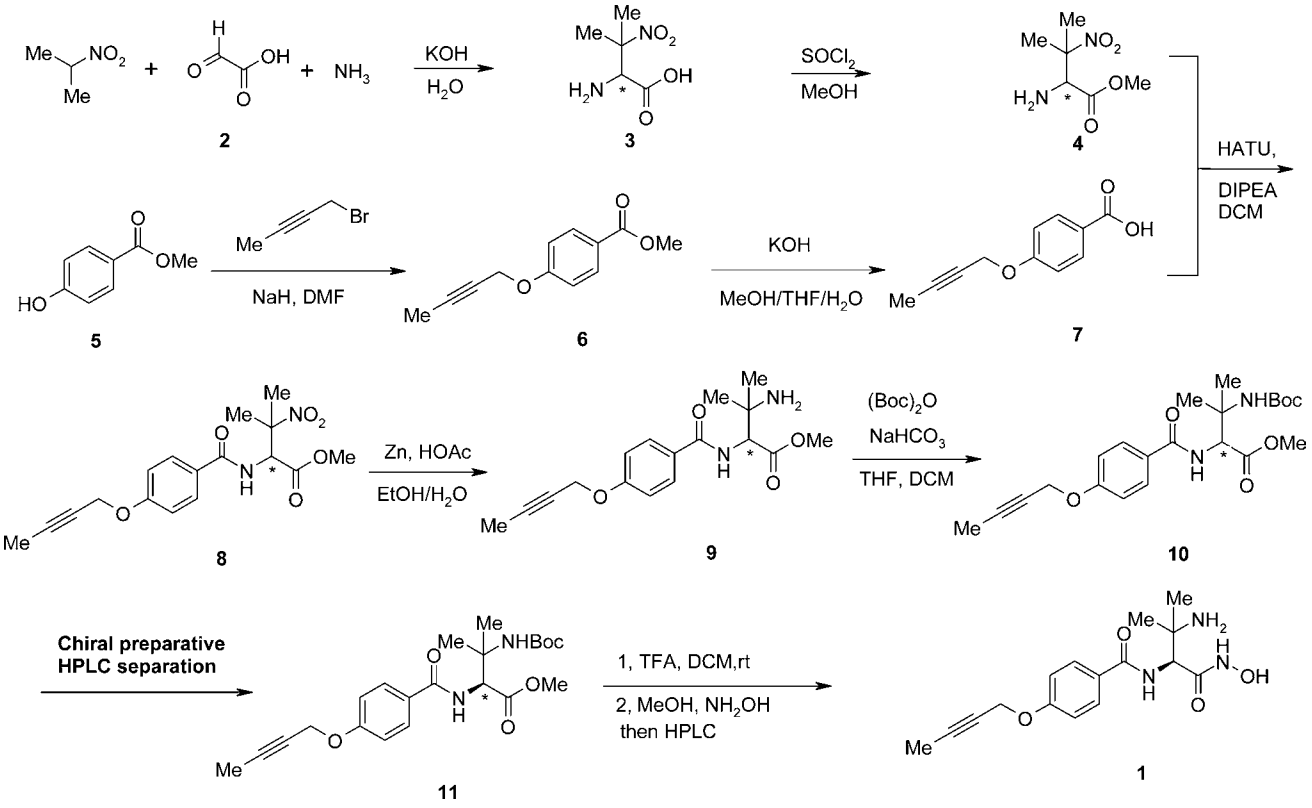
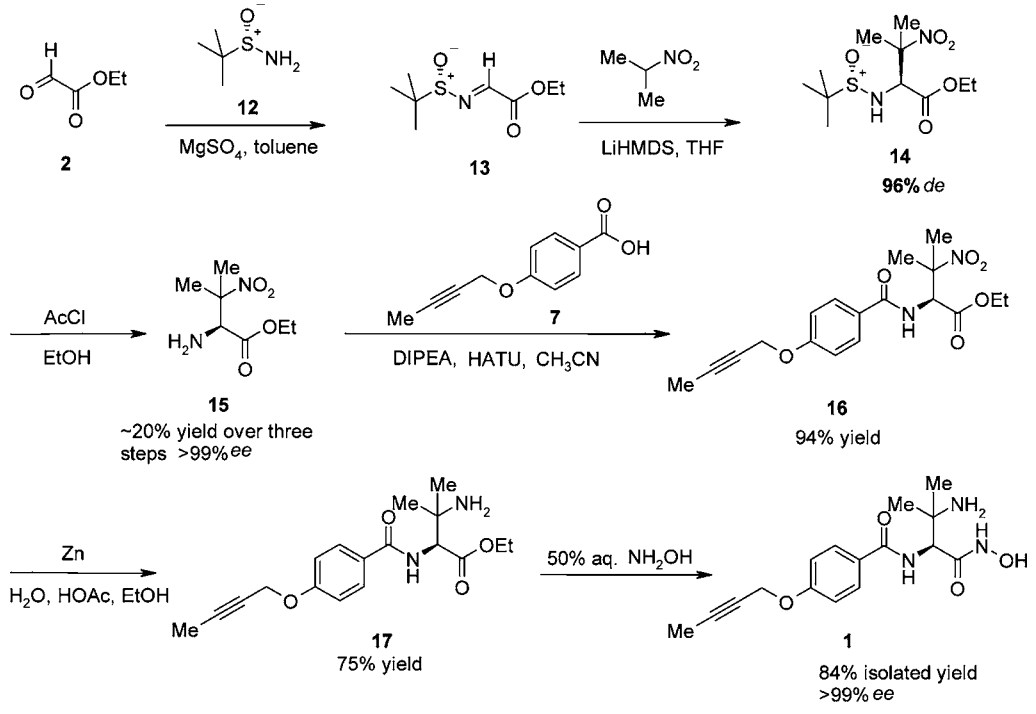
Scheme 1. Original synthesis



Scheme 2. Asymmetric synthesis



Synthesis of acid **7** was achieved in a straightforward manner as shown Scheme 4. Following the propargylation of phenolic ester **5** with 1-bromo-but-2-yne in acetone, the reaction mixture containing intermediate **6** was filtered to remove inorganic salts and further treated with aqueous KOH solution for hydrolysis to **7**. Upon acidification of the reaction solution with aqueous HCl, the benzoic acid derivative **7** was precipitated out of the reaction and isolated in 91% overall yield and 98% purity by HPLC.

Amine **15** was then coupled with acid **7** using HATU in the presence of DIPEA to afford **16** and HOAt, tetramethylurea byproducts (Scheme 5). Compound **16** is an oil, and its easy

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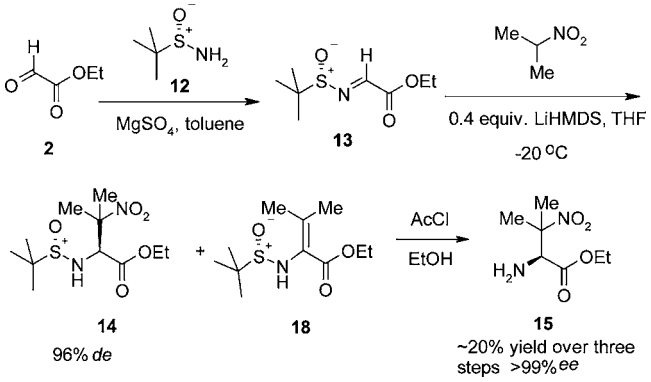
Scheme 3. Synthesis of amine 15



purification relies only on extractive workup. We found HOAt and tetramethylurea were removable by aqueous washing, whereas the intermediate **19** was not; thus, it was critical to use a slight excess of amine **15** to ensure its full consumption. All byproducts and impurities including HOAt, tetramethylurea, DIPEA, and excess amine **15** were removed by diluting the reaction mixture with IPAc and sequentially washing with aqueous hydrochloric acid solution (to remove DIPEA and excess amine **15**), aqueous sodium carbonate solution (to remove HOAt), and water. The product **16** was eventually isolated in 94% yield and over 95% purity by HPLC.

Chemoselective reduction of the nitro group over the acetylenic function of **16** was achieved with zinc dust (<10 μm) in the presence of acetic acid. This is a known exothermic reaction. Our initial attempt by slow addition of acidic acid to the reaction mixture did not lead to control of the reaction heat. Hence, an inverse addition of the **16** solution in ethanol to the reaction mixture of zinc and acetic acid in ethanol was explored and proved to be efficient at controlling the reaction temperature below 5 °C. It is noteworthy that uncontrolled reaction temperature resulted in further reduction of the acetylenic function of product **17**.[8] The reduction was always accompanied by the formation of acid **20** (Scheme 6).

Even though we cannot completely rule out the pathway of hydrolysis of **17** to form **20**, it is more likely formed via reductive cleavage of the cyclized intermediate **22** which was identified in the reaction mixture by LC–MS (Scheme 7). We observed that **16** was quickly reduced to **21**, while its further reduction to **17** was slow, which opened up the competing pathway of cyclization to **22**. Therefore, more equivalents of zinc would increase the reaction rate of reducing **21** to **17** and disfavor its cyclization to **22** leading to **20**. Indeed, we observed that 10 equiv of zinc led to the **17**/**20** ratio of 94:6, while 5 equiv led to a ratio of 77:23 under otherwise identical conditions.

The acid impurity **20**, acetic acid, and zinc acetate were in any case removed by washing the crude product in IPAc solution with mixed aqueous alkali and EDTA tetrasodium salt solution. A subsequent acid–base wash eventually resulted in product **17** in 75% yield and 97% purity by HPLC. This treatment lowered the zinc content to less than 10 ppm.

Treatment of compound **17** with aqueous hydroxylamine in methanol gave a clean but slow reaction. It took 60 h for completion. Introducing 2 equiv of LiOH to the reaction dramatically increased the reaction rate to 3 h for completion. The crude product was purified by a sequence of salt formation and release to the free form to give **1** in 84% isolated yield, ≥98% purity, and ≥99% ee by HPLC (Scheme 8).

CONCLUSION

In summary, a short and scalable synthesis for producing hydroxamic acid derivative **1** is described. Diastereoselective addition of 2-nitropropane-derived lithium salt to a *tert*-butanesulfinimine is the key reaction in this approach. This in principle could serve as a general method for preparing chiral vicinal diamines which are quite prevalent in many molecules of biological interest. Further detailed studies of this reaction and an extension of the reaction scope are underway in our laboratory.

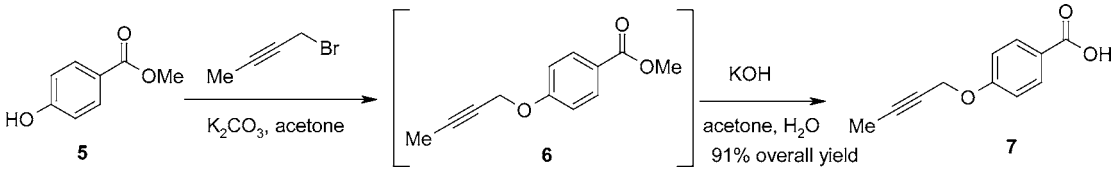
EXPERIMENTAL SECTION

NMR spectra were recorded on a Bruker 400 MHz spectrometer. HPLC analysis was performed using Agilent 1200, and the results were described as area %. LC–MS analysis was performed using Agilent 1200 and 6130 Quadrupole system.

(*E*)-(*S*)-2-Methyl-propane-2-sulfinylimino]-acetic acid ethyl ester (13). Under nitrogen atmosphere (*S*)-(−)-*tert*-butanesulfinamide **12** (1.45 kg, 11.96 mol) was added to a solution of ethyl glyoxylate **2** in toluene (2.40 L, 51 wt % in toluene, 12.06 mol) in a 20-L flask fitted with a mechanical stirrer and a condenser. After the addition, the mixture was heated to 50 °C, and a clear solution was obtained. $MgSO_4$ (2.88 kg, 23.93 mol) was added to the above solution, and the temperature was increased to 56 °C (internal temperature). The reaction mixture was stirred at 56 °C and monitored by HPLC. After 20 h almost all of the sulfinamide was consumed (<1% left). The reaction mixture was cooled to room temperature, filtered, and washed with toluene. The collected filtrate was concentrated to yield a yellow oil (2.67 kg, 85 wt % in toluene, measured by ^{1}H NMR). The crude *N*-sulfinylimino ester **13** (2.27 kg, 92%) was used as such for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.01 (s, 1 H), 4.38 (q, 2 H, J = 7.2 Hz), 1.39 (t, 3 H, J = 7.2 Hz), 1.28 (s, 9H).

(*S*)-3-Methyl-2-((*S*)-2-methyl-propane-2-sulfinylamino)-3-nitrobutyric Acid Ethyl Ester (14). 2-Nitropropane (485.5 mL, 5.40 mol) was slowly added to a mechanically stirred and cold LiHMDS solution (−20 °C) (1.82 L, 1 M in THF, 1.82 mol). During the addition, the internal temperature was kept below −15 °C. Stirring was continued for 30 min after the addition, and a solution of *N*-sulfinylimino ester **13** (1.10

Scheme 4. Synthesis of acid 7



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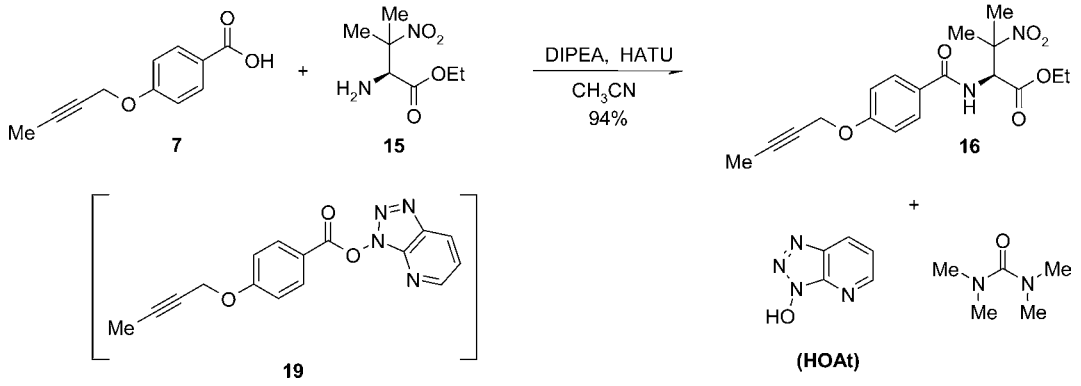
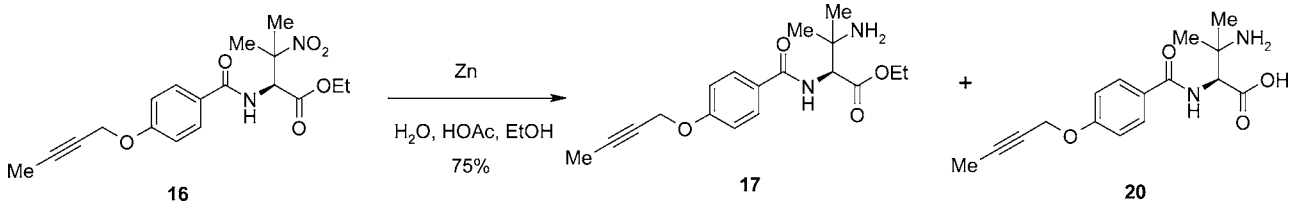
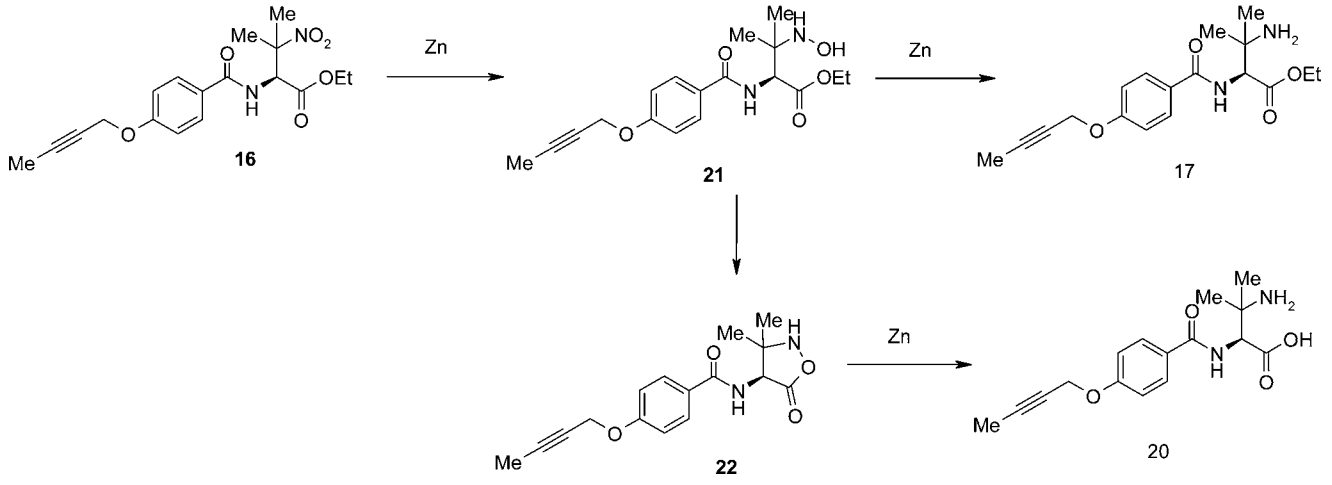
Scheme 5. Coupling of amine 15 and acid 7



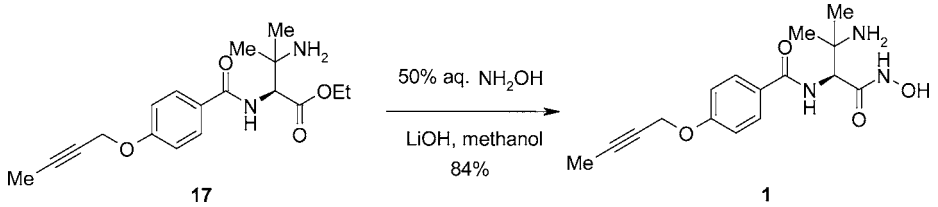
Scheme 6. Reduction of 16



Scheme 7. Reduction pathways



Scheme 8. Completion of the synthesis



kg, 85 wt % in toluene, 4.54 mol) in THF (4.5 L) was slowly added to the above solution in 80 min, and the internal temperature was maintained at about −20 °C. After the addition, stirring was continued for 60 min, and saturated aqueous NH_4Cl solution (3 L) was added to the cold reaction mixture followed by the addition of water (1 L). Ten minutes later, EtOAc (1.5 L) was added to the above mixture, and the layers were separated. The organic layer was washed with 10% brine (2 L × 1). The collected organic layer was concentrated to yield the crude product containing **14**/diastereomer of **14**/**18** (~98/2/7, 1.10 kg in total), which was used as such in the next step.

(*S*)-2-Amino-3-methyl-3-nitrobutyric Acid Ethyl Ester (15). Acetyl chloride (1.2 L, 16.82 mol) was slowly added to a mechanically stirred and cooled (0 °C) 10-L flask containing anhydrous ethanol (4.2 L). During the addition, the internal temperature was kept below 25 °C. After the addition, the mixture was stirred for 30 min at 25 °C.

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The crude sulfonamide **14** (1.40 kg) in EtOAc (1.4 L) was poured into the prepared HCl solution. Stirring was continued for 60 min, and the solvent was concentrated. MTBE (4.9 L) was added to the residue, and the solid was filtered after vigorous stirring. The filter cake was washed with MTBE and dried under vacuum to yield the hydrochloride salt of **15** as a white solid (370 g, >99.0% ee, ~20% yield over three steps) containing NH_4Cl impurity. ^1H NMR ($CDCl_3$, 400 MHz): δ 4.89 (s, 1 H), 4.30 (m, 2H), 1.95 (s, 3H), 1.88 (s, 3H), 1.29 (t, 3 H, J = 7.2 Hz). ^{13}C NMR (DMSO, 100 MHz): δ 166.0, 87.5, 62.7, 57.5, 24.4, 22.4, 13.7. ESI-MS: m/z = 191 $[M + 1]^+$.

4-But-2-ynyloxy-benzoic Acid (7). To a reactor was sequentially charged **5** (227.2 g, 1.49 mol), K_2CO_3 (247.1 g, 1.79 mol), acetone (1.15 L), and 1-bromo-but-2-yne (198.6 g, 1.49 mol) at RT under nitrogen atmosphere. The reaction mixture was heated to reflux for 6 h. After completion the reaction mixture was cooled to 25 °C. The inorganic salt in the reaction mixture was filtered and washed with acetone (300 mL).

To the above filtrate was added a solution of KOH (133.7 g, 2.38 mol) in water (1.4 L). The mixture was stirred at 40 °C. After completion (1 h), 1.5 L of aqueous HCl (6 N) was slowly added while the internal temperature was kept below 25 °C. The white solid formed was filtered and washed with water (to pH = 7) and acetone and dried under vacuum to give **7** (258 g, 91% over 2 steps). ^1H NMR (DMSO, 400 MHz): δ 7.90 (d, 2 H, J = 8.8 Hz), 7.05 (d, 2 H, J = 8.8 Hz), 4.83 (d, 2 H, J = 2.0 Hz), 1.89 (s, 3 H). ^{13}C NMR (DMSO, 100 MHz): δ 166.9, 161.0, 131.2, 123.4, 114.6, 84.0, 74.3, 56.1, 3.1. ESI-MS: m/z = 189 $[M - 1]^+$.

(*S*)-2-(4-But-2-ynyloxy-benzoylamino)-3-methyl-3-nitrobutyric Acid Ethyl Ester (16). A suspension of **15** hydrochloric salt (100 g) in IPAc (2.5 L) was washed with NaOH solution (1 L × 1, 1 M), followed by water (1 L × 1). Then the IPAc layer was concentrated to give **15** as a light-yellow oil (61.3 g, yield 73%). The oil was dissolved in acetonitrile (760 mL), and **7** (50.8 g, 0.268 mmol) and HATU (101.7 g, 0.268 mol) were added and then DIPEA (69.1 g, 0.535 mol) at ~22 °C. After completion at about 19 h (intermediate **19** was completely consumed), IPAc was added. The solution was sequentially washed with aqueous HCl (1 M, 0.8 L × 2), water (1.3 L), aqueous Na_2CO_3 solution (11 wt %, 370 mL), and water (1.3 L × 2). The organic phase was concentrated to give **16** as an oil (91.3 g, >99% ee, yield 94%). ^1H NMR (DMSO, 400 MHz): δ 8.79 (d, 1 H, J = 9.2 Hz), 7.88 (d, 2 H, J = 8.8 Hz), 7.06 (d, 2 H, J = 8.8 Hz), 5.59 (d, 1 H, J = 9.2 Hz), 4.83 (d, 2 H, J = 2.4 Hz), 4.12 (m, 2 H), 1.82 (t, 3 H, J = 2.4 Hz), 1.66 (s, 3 H), 1.56 (s, 3 H), 1.15 (t, 3 H, J = 7.2 Hz). ^{13}C NMR (DMSO, 100 MHz): δ 168.3, 166.7, 160.1, 129.7, 125.9, 114.3, 88.7, 83.9, 74.3, 61.4, 56.9, 56.0, 25.0, 21.1, 13.8, 3.1. ESI-MS: m/z = 363 $[M + 1]^+$.

(*S*)-2-(4-But-2-ynyloxy-benzoylamino)-3-methyl-3-aminobutyric Acid Ethyl Ester (17). A stirred mixture of Zn dust (<10 μm) (17.9 g, 276 mmol) in EtOH (50 mL) and H_2O (3.3 mL) was cooled to −10 °C in a 250-mL flask under nitrogen atmosphere, followed by addition of HOAc (31.4 mL, 552 mmol) in one portion. The resulting mixture was cooled to −10 °C, and a solution of **16** (9.3 g, 25.7 mmol) in EtOH (50 mL) was slowly added while the internal temperature was maintained below 5 °C (the addition rate was controlled so as to maintain an internal temperature below 5 °C). After addition, the mixture was cooled to −10 °C (internal temperature) and stirred at this temperature until **16** was fully consumed. The reaction was then warmed to 0 °C and stirred for 10 h until full conversion of intermediate **21**. The reaction mixture was filtered and concentrated to remove EtOH. To the concentrated mixture was added IPAc (203 mL). The resulting mixture was cooled to ~22 °C, stirred for 10 min, and filtered. The filtrate was cooled to 0 °C with stirring, and combined aq EDTA tetrasodium salt and NaOH solution (0.25 M for EDTA tetrasodium salt and 4 M for NaOH, 148 mL) was added at such a rate that the internal temperature was kept below 22 °C (the pH value was checked to make sure it was 10−11; if not, more solution was added to reach this pH value). The separated IPAc layer was washed with a combined EDTA tetrasodium salt and NaOH solution (0.25 M for EDTA tetrasodium salt and 1 M for NaOH, 102 mL × 2), followed by water (62 mL × 1). The IPAc layer was concentrated to 20 mL and extracted with 1 M HCl aqueous solution (30.5 mL × 1). To the aqueous phase was added IPAc (204 mL). The mixture was stirred and cooled to 0 °C.To the mixture was added 1 M NaOH solution (39.6 mL) at such a rate that the internal temperature was maintained below room temperature. The two phases were separated, and the organic phase was washed with water (62 mL) and concentrated (internal temperature 40 °C, 50 mbar) to give **17** as an oil (6.4 g, yield: 75%, HPLC purity by area normalization: 97.4%). ^1H NMR (DMSO, 400 MHz): δ 8.09 (d, 1 H, J = 8.0 Hz), 7.84 (d, 2 H, J = 8.8 Hz), 7.03 (d, 2 H, J = 8.8 Hz), 4.85 (d, 2 H, J = 2.0 Hz), 4.32 (d, 1 H, J = 7.6 Hz), 4.12 (m, 2 H), 1.82 (t, 3 H, J = 2.0 Hz), 1.19 (t, 3 H, J = 7.2 Hz), 1.13 (s, 3H), 1.10 (s, 3 H). ^{13}C NMR (DMSO, 100 MHz): δ 171.0, 166.3, 159.8, 129.3, 126.6, 114.3, 83.8, 74.3, 62.0, 60.1, 56.0, 51.4, 28.2, 27.9, 14.1, 3.1. ESI-MS: m/z = 333 $[M + 1]^+$.

***N*-((*S*)-2-Amino-1-hydroxycarbamoyl-2-methylpropyl)-4-but-2-ynyloxy-benzamide (1).** A three-necked 250-mL round-bottom flask was charged with **17** (10.0 g, 30.12 mmol), and methanol (100 mL). The solution was cooled to 0−5 °C, and 50% aqueous hydroxylamine (22.13 mL, 361.4 mmol) was added followed by $LiOH{\cdot}H_2O$ (2.5 g, 60.24 mmol). The reaction mixture was stirred at 0−5 °C. HPLC of an aliquot of the reaction mixture after 3 h at 0−5 °C indicated completion of the reaction. Water (100 mL) was added to the reaction mixture at 0−5 °C. The pH of the mixture was adjusted to ~8.5 with 6 N HCl. The mixture was concentrated under vacuum. To the residue were added water (50 mL) and 6 N HCl until the pH of the solution was ~6. The solution was transferred to a 1-L, three-necked round-bottom flask. Aqueous NaOH (4 N) was slowly added until the pH of the mixture was 8.5. The product precipitated out as an oil. The mixture was heated at 45 °C for 0.5 h. The mixture was slowly cooled to ~22 °C and stirred at ~22 °C for 1 h. The off-white solid was filtered and dried under vacuum to give **1** (8.05 g, yield 84%, HPLC purity by area normalization 98.7%). ^1H NMR (DMSO, 400 MHz): δ 7.82 (d, 2 H, J = 8.4 Hz), 7.01 (d, 2 H, J = 8.4 Hz), 4.80 (d, 2 H, J = 2.4 Hz), 4.26 (d, 1 H, J = 8 Hz), 1.82 (t, 3 H, J = 2.4 Hz), 1.08 (s, 3 H), 0.99 (s, 3 H); ^{13}C NMR (DMSO, 100 MHz): δ 167.2, 165.7, 159.7, 129.2, 126.8, 114.2, 83.8, 74.4, 58.0, 55.9, 51.8, 28.5, 27.2, 3.1. ESI-MS: m/z = 320 $[M + 1]^+$.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

LiHMDS, lithium bis(trimethylsilyl)amide; THF, tetrahydrofuran; TBME, *tert*-butyl methyl ether; IPAc, isopropyl acetate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; HOAt, 1-hydroxy-7-azabenzotriazole; EDTA, ethylenediaminetetraacetic acid

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(7) The stereochemistry was confirmed by comparison with an authentic sample after transformation to **15**.
(8) The reduction of acetylene to alkene in **17** by zinc was confirmed by LC−MS.