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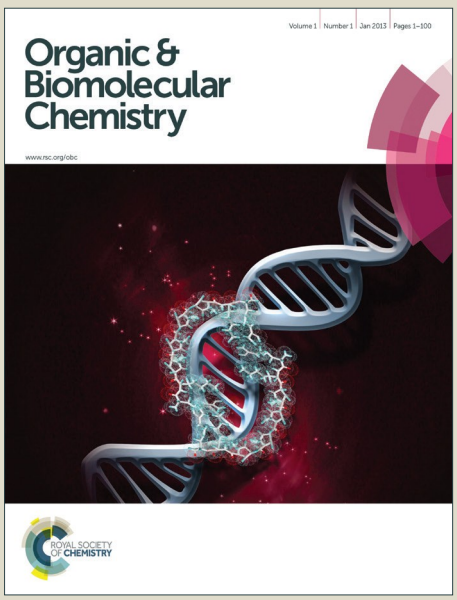

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ARTICLE

Concise synthesis of tubuphenylalanine and *epi*-tubuphenylalanine via diastereoselective Mukaiyama aldol reaction of silyl ketene acetal

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We have developed a straightforward and auxiliary-free synthetic route towards *t*Bu-tubuphenylalanine (*t*Bu-Tup) and *t*Bu-*epi*-tubuphenylalanine (*t*Bu-*epi*-Tup), which are the key components of tubulysins and their analogs. Lewis acid-mediated diastereoselective Mukaiyama aldol reaction using silyl ketene acetal and *N*-Boc-L-phenylalaninal provided γ-amino-β-hydroxyl-α-methyl esters, which were deoxygenated to γ-amino-α-methyl esters under Barton–McCombie deoxygenation conditions. Notably, the desired *t*Bu-Tup and *t*Bu-*epi*-Tup were obtained in good overall yields in four steps.

Introduction

Since Höfle and coworkers isolated natural tubulysins[1] from myxobacteria culture broth in 2000, great attention has been paid to their use as novel therapeutic antimitotic agents replacing taxol and epothilone. Indeed, tubulysins possess extraordinary potent antiproliferative activity and high selectivity in cancer (HL-60) versus normal (HUVEC) cells.[2] In particular, they exhibit picomolar IC_{50} values against multidrug-resistant human cancer cell lines such as P-glycoprotein-expressing KB-V1 cells.[3] Due to such promising biological activities and interesting structural complexity, many total and analog syntheses have been reported to date.

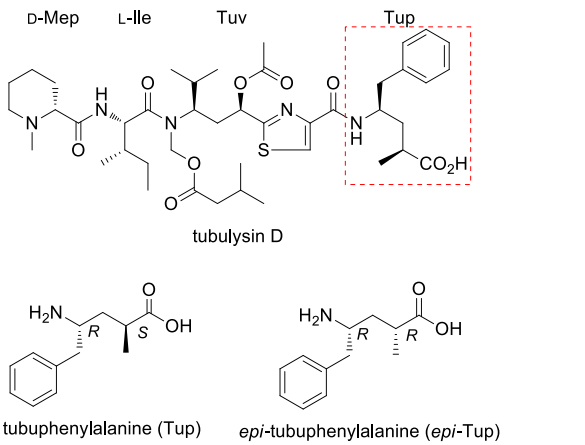


Fig. 1 The structure of tubulysin D, tubuphenylalanine, and *epi*-tubuphenylalanine.

Tubulysins are tetrapeptides composed of four amino acids; *N*-methyl-D-pipecolic acid (D-Mep), L-isoleucine (L-Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup)/tubutyrosine (Tut) (Fig. 1). In particular, Tuv and Tup/Tut are synthetically challenging unusual amino acids. According to structure-activity relationship (SAR) studies,[4] the N-terminus and Tuv unit should be relatively conserved for subnanomolar activity, whereas the C-terminal Tup/Tut has room for modification. This unit is considered to be a suitable position for novel derivatization, photoaffinity labeling, fluorescence tagging,[5] or folic acid conjugation.[6] Therefore, flexible stereoselective synthetic methods for C-terminal Tup and *epi*-Tup units are highly desirable. The prevailing methods for the synthesis of Tup or *epi*-Tup mostly include stereoselective hydrogenations and separation of the resulting diastereomers.[7] Other interesting synthetic methods describe a chiral aziridine ring-opening with a SAMP-hydrazone leading to Tup and a pseudoephedrine amide leading to *epi*-Tup,[8] diastereoselective methylation of a chiral lactame,[9] stereoselective alkylation[10] or aldol condensation[11] using Evans' auxiliary, diastereoselective Michael addition using a chiral *tert*-butanesulfinamide,[12] Ireland–Claisen rearrangement of allylic ester,[13] and the ring-opening of the epoxide derived from (−)-citronellol.[14] Although many synthetic methods have been reported to date, concise short synthetic methods for Tup and *epi*-Tup without using chiral auxiliaries are needed for the gram-scale synthesis and supply.

Biosynthetically, Tup unit has been proposed to derive from phenylalanine unit by a nonribosomal peptide synthetase (NRPS)–polyketide synthase (PKS) hybrid system. Müller and co-workers identified the biosynthetic gene cluster associated with the tubulysin biosynthesis in *A. disciformis* An d48 in

2004[15] and in *Cystobacter* sp. SBCb004 in 2010.[16] They proposed that β-ketoacylsynthase (KS), acyltransferase (AT), β-ketoacylreductase (KR), C-methyltransferase (CMT), β-hydroxydehydratase (DH), enoyl reductase (ER) and thioesterase (TE) domains are involved in the formation of Tup unit. Presumably, a β-ketoacyl condensation to the phenylalanine unit would be followed by sequential reductions (Fig. 2). We were inspired by this biosynthetic consideration, and therefore, in order to develop novel direct synthetic methods for *t*Bu-Tup (**1a**) and *t*Bu-*epi*-Tup (**1b**), we envisioned diastereoselective Mukaiyama aldol condensation of silyl ketene acetal and *N*-Boc-L-phenylalaninal, leading to the *β*-hydroxy-*γ*-amino acids (**2**); the subsequent deoxygenation of *β*-hydroxyl group would provide *t*Bu-Tup (**1a**) and *t*Bu-*epi*-Tup (**1b**) in a concise manner (Scheme 1).

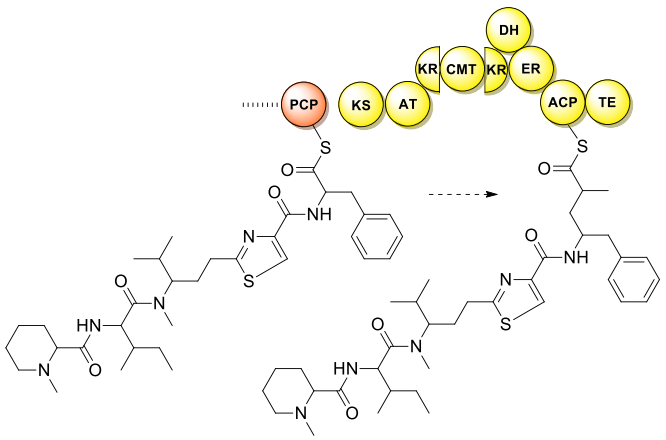
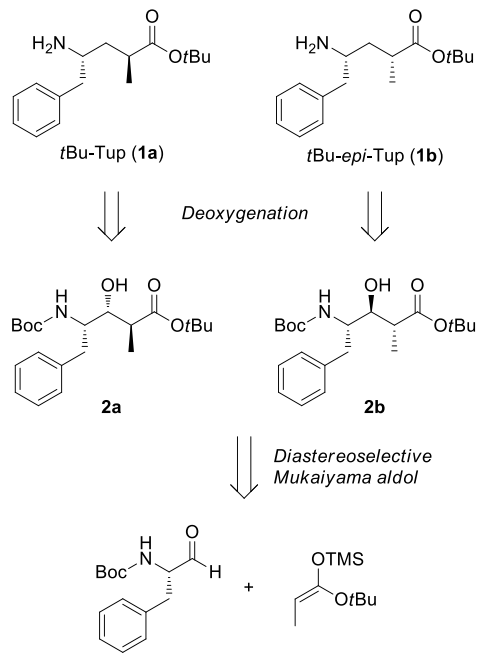


Fig. 2 Presumed biosynthetic pathway of Tup unit.



Scheme 1 Retrosynthetic analysis of *t*Bu-Tup (**1a**) and *t*Bu-*epi*-Tup (**1b**) synthesis.

Results and Discussion

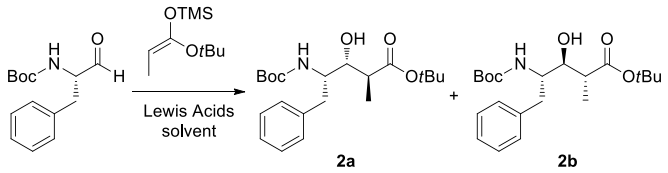
In 1973, Mukaiyama first discovered $TiCl_4$-mediated aldol reactions[17] of silyl enol ethers and aldehydes,[18] which allowed the drawbacks of classical aldol reactions to be overcome. This renowned Mukaiyama aldol reaction has been extensively studied over several decades for various catalysts. Later, silyl enol ethers could be replaced with silyl ketene acetals, leading to the synthesis of *β*-hydroxy esters. In order to develop an auxiliary-free direct synthetic pathway towards Tup and *epi*-Tup, we focused our attention on diastereoselective Mukaiyama aldol reactions of *α*-amino aldehydes and silyl ketene acetals leading to *γ*-amino esters. We chose a commercially available *N*-Boc-L-phenylalaninal and *O*-trimethylsilyl ketene acetal derived from *tert*-butyl propionate as a substrate. We screened various Lewis acids to optimize suitable conditions for the Mukaiyama aldol reactions of *O*-trimethylsilyl ketene acetal and *N*-Boc-L-phenylalaninal (Table 1). Several Lewis acids mediated Mukaiyama aldol reactions smoothly at −78 °C, and provided the desired *γ*-amino-*β*-hydroxy-*α*-methyl ester **2a** and **2b**. Only 2,3-*anti*-aldol adducts were isolated and identified. Their stereochemistry was also clearly determined (*vide infra*). Ti-based catalysts such as $TiCl_4$ and $TiCl(OiPr)_3$ produced **2b** as a major product in rather low yields with good diastereoselectivity (Table 1, entries 1 and 2). When Et_2AlCl was used as a Lewis acid, the diastereoselectivity for **2b** was similar, however, the yield was still low (22%, entry 3). ZnI_2 was an effective Lewis acid, which exclusively afforded the **2b** and TMS-protected aldol adduct **2b'** in 70% combined yield (Scheme 2). Interestingly, a catalytic amount of Bu_2Mg also provided 2,3-*anti*-3,4-*anti* product **2b** and **2b'** in 61% combined yield. Presumably, the TMS of the silyl ketene acetal was delivered to the aldehyde carbonyl oxygen during aldol addition and formed the TMS-protected aldol adduct **2b'**. Therefore, after the aldol reaction was completed, TMS-protected aldol adduct **2b'** was treated with tetrabutylammonium fluoride (TBAF), HF-pyridine, or diluted HCl at 0 °C for 15 min. The deprotection of the TMS group resulted in the formation of **2b** in 71% yield for TBAF, 74% yield for HF-pyridine, and 76% yield for diluted HCl, respectively.

Conversely, BF_3•OEt_2 in CH_3CN gave both **2a** and **2b** with 51:49 d.r. in 62% combined yield (entry 4). Such a change in selectivity was rather interesting because the majority of Lewis acids preferred the formation of 2,3-*anti*-3,4-*anti* aldol adduct **2b**. This result prompted us to study BF_3•OEt_2-mediated Mukaiyama aldol reaction in various solvents. When toluene was used as a solvent at low temperature (−78 °C), the formation of **2a** and **2b** increased to 67% with 44:56 d.r. (entry 5). The best result in terms of the formation of 2,3-*anti*-3,4-*syn* aldol adduct **2a** was observed under the conditions of BF_3•OEt_2 in CH_2Cl_2 (entries 9−11). BF_3•OEt_2-mediated reactions in CH_2Cl_2 gave 2,3-*anti*-3,4-*syn* aldol adduct **2a** as a major product, regardless of the employed equivalent of *O*-trimethylsilyl ketene acetal. The combined yield increased up to 82% (entry 10).

We were also interested in catalytic Mukaiyama aldol reactions using chiral and achiral Lewis acid catalysts. However, the Mukaiyama aldol reaction of *O*-trimethylsilyl ketene acetal

and *N*-Boc-L-phenylalaninal required stoichiometric amount of Lewis acids except Bu_2Mg. Chiral Lewis acid catalysts such as $Cu(OTf)_2$-BOX, ZnI_2-PyBOX, $Zr(OEt)_4$-BINOL and $Ti(O\text{-}iPr)_4$-BINOL were not effective and provided **2b** in low yields (0–15%). In addition, Mukaiyama aldol reactions using catalytic amount of achiral Lewis acids were not effective either: 20 mol% of $BF_3{\bullet}OEt_2$ provided **2b** and **2b'** in 1% and 2% yields, respectively, and 88% of *N*-Boc-L-phenylalaninal was recovered after 24 h at –78 °C. Only catalytic amount of Bu_2Mg (5 mol%) afforded **2b** and **2b'** in good yield.

Table 1. Diastereoselective Mukaiyama aldol reaction of silyl ketene acetal and *N*-Boc-L-phenylalaninal.

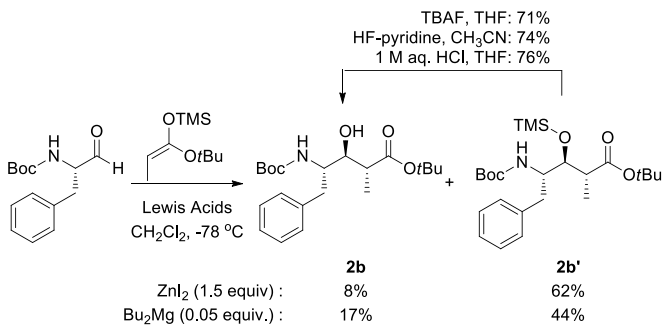


Entry	Conditions (equiv.)	Equiv. of silyl ketene acetal	Yield [a] (%) (**2a**/**2b** ratio)[b]
1	$TiCl_4$ (1.5), CH_2Cl_2, −78 °C, 2 h	1.5	36 (7/93)
2	$TiCl(OiPr)_3$ (1.5), CH_2Cl_2, −78 °C, 18 h	1.5	17 (0/100)
3	Et_2AlCl (1.5), CH_2Cl_2, −78 °C, 18 h	1.5	22 (0/100)
4	$BF_3{\bullet}OEt_2$ (1.5), CH_3CN, -40 °C, 2 h	2.0	62 (51/49)
5	$BF_3{\bullet}OEt_2$ (1.5), toluene, −78 °C, 2 h	1.5	67 (44/56)
6	$BF_3{\bullet}OEt_2$ (1.5), *i*PrOH, −40 °C, 2 h	2.0	NR[c]
7	$BF_3{\bullet}OEt_2$ (1.5), THF, −78 °C, 2 h	2.0	67 (45/55)
8	$BF_3{\bullet}OEt_2$ (1.5), [bmim][BF_4], 0 °C, 1.5 h	2.0	23 (33/67)
9	$BF_3{\bullet}OEt_2$ (1.2), CH_2Cl_2, −78 °C, 2 h	1.5	72 (54/46)
10	$BF_3{\bullet}OEt_2$ (1.5), CH_2Cl_2, −78 °C, 2 h	2.0	82 (51/49)
11	$BF_3{\bullet}OEt_2$ (1.5), CH_2Cl_2, −78 °C, 2 h	3.0	75 (49/51)

[a] Isolated yield.

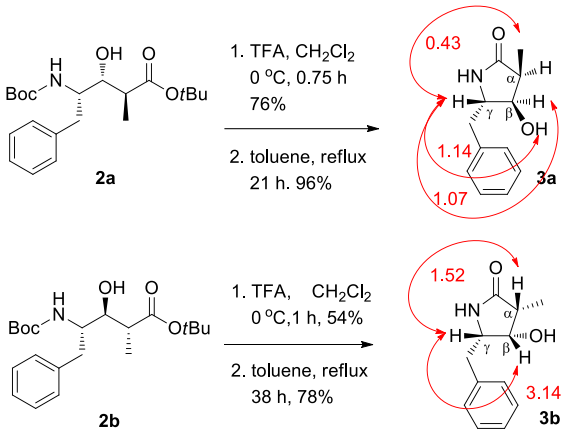
[b] determined by chiral HPLC.

[c] No reaction.



Scheme 2 Retrosynthetic analysis of *t*Bu-Tup (**1a**) and *t*Bu-*epi*-Tup (**1b**) synthesis.

To define the stereochemistry of the γ-amino-β-hydroxy-α-methyl esters **2a** and **2b**, following purification by flash column chromatography, **2a** and **2b** were subjected to *N*-Boc-deprotection and a cyclization sequence to yield lactam **3a** and **3b** (Scheme 3). Subsequently, the stereochemistry of lactam **3a** and **3b** was clearly determined by serial NOE experiments, which supported the presented stereochemical assignment: when the γ-*H* was irradiated, the lactam **3a** did not show an increment in signal between α-*H* and γ-*H*, suggesting a *trans* CH_α-CH_γ relationship. Similarly, the same experiment with the lactam **3b** showed a signal increment of 1.52%, suggesting a *cis* CH_α-CH_γ relationship. These observations confirmed the 2,3-*anti*-3,4-*syn* configuration of ester **2a** and the 2,3-*anti*-3,4-*anti* configuration of ester **2b** (Scheme 3). Additionally, α-CH_3, α-*H*, and β-*H* of lactam **3a** and **3b** were irradiated, and the collected data reconfirmed the current stereochemical assignments (see supplementary information).

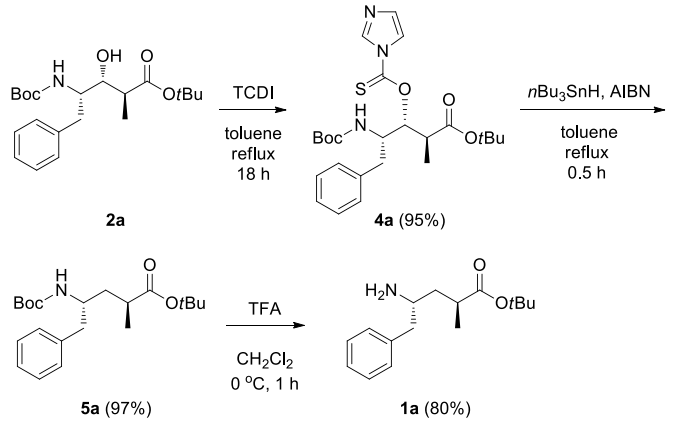


Scheme 3 Determination of the relative stereochemistry of **2a** and **2b**.

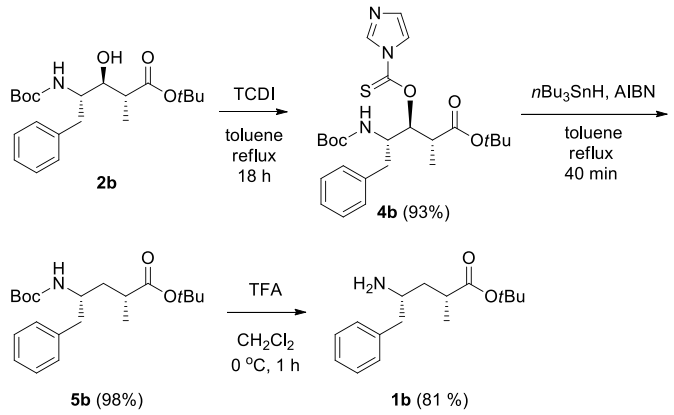
Next, ester **2a** and **2b** were subjected to further synthetic steps. The secondary hydroxyl groups of the Mukaiyama aldol products **2a** and **2b** were removed by the Barton–McCombie deoxygenation procedure.[19] Firstly, **2a** was treated with 1,1'-thiocarbonyldiimidazole (TCDI) and converted to thiocarbamate **4a** in 95% yield (Scheme 4). Subsequent treatment with Bu_3SnH in the presence of a catalytic amount of AIBN afforded the *N*-Boc-protected amino ester **5a** in 97% yield. Following selective deprotection of the *N*-Boc group of **5a** in the presence of the *tert*-butyl ester group provided the desired *t*Bu-Tup (**1a**) in 80% yield. Similarly, **2b** was treated with TCDI and the resulting thiocarbamate **4b** was converted to **5b** under the conditions of Bu_3SnH and AIBN (Scheme 5). Subsequently, the *N*-Boc-protecting group was selectively deprotected to yield *t*Bu-*epi*-Tup (**1b**) in 81% yield. NMR spectra of *t*Bu-Tup (**1a**) and *t*Bu-*epi*-Tup (**1b**) are almost identical depending on the conditions used. These behave very similarly with respect to NMR spectroscopy and chromatography. This issue has also been previously reported in the literature.[8]

Conclusions

In summary, we explored Lewis acid-catalyzed diastereoselective Mukaiyama aldol condensation using commercially available *O*-trimethylsilyl ketene acetal and *N*-Boc-L-phenylalaninal. We developed an auxiliary-free direct synthetic pathway leading to *t*Bu-Tup and *t*Bu-*epi*-Tup, which were synthesized in 31% and 40% overall yields, respectively, in only four to five steps. This is a very concise, practical, and high-yielding synthetic route, and the method can be easily applied to the multi gram-scale synthesis of tubulysins and their analogs.



Scheme 4 Synthesis of *t*-butyltubuphenylalanine (**1a**).



Scheme 5 Synthesis of *t*-butyl-*epi*-tubuphenylalanine (**1b**).

Experimental

General Remarks

All reactions were performed in oven-dried glassware under positive Ar pressure with magnetic stirring, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless-steel cannula. TLC was performed on 0.25 mm E. Merck silica gel 60 F_{254} plates and visualized under UV light (254 nm) or by staining with cerium ammonium molybdenate (CAM), *p*-anisaldehyde, or ninhydrin. Flash chromatography was performed on E. Merck 230−400 mesh silica gel 60. All reagents were purchased from commercial suppliers, and used without further purification unless otherwise noted. Solvents were distilled from proper drying agents (CaH_2 or Na wire) under Ar atmosphere at 760 mm Hg. All moisture- and/or oxygen-sensitive solids were handled and stored in a glove box under N_2. NMR spectra were recorded on Varian Unity 400 instruments at 24 °C. Chemical shifts are expressed in ppm relative to TMS (^{1}H, 0 ppm) or solvent signals: $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.2 ppm), or DMSO-d_6 (^{1}H, 2.50 ppm; ^{13}C, 39.5 ppm); coupling constants are expressed in Hz. High resolution mass spectra electrospray ionization (HRMS-ESI) was obtained on an Agilent technologies 6220 TOF LC/MS spectrometer. Chiral HPLC analysis for determination of diastereomeric ratio (*d.r.*) was performed on an Agilent 1100 system (G1379A micro vacuum degasser, G1311A quaternary pump, G1329A ALS autosampler, and G1314A VWD detector) equipped with a chiral Lux 5u Cellulose-1 column (5 µm, 1000 Å , 4.6 × 250 mm). Solvents were eluted at a flow rate of 1 mL/min at room temperature using a binary solvent system (solvent A: Hexane, solvent B: isopropanol, 99% A over 20 min) with UV detection at 214 nm.

(4*S*)-*tert*-Butyl 4-((*tert*-butoxycarbonyl)amino)-3-hydroxy-2-methyl-5-phenylpentanoate (2a and 2b) (entry 10)

N-Boc-L-phenylalaninal (30 mg, 120 µmol) was dissolved in anhydrous CH_2Cl_2 (0.6 mL), and the solution was cooled to –78 °C. A solution of silyl ketene acetal (48.6 mg, 240 µmol, 2.0 equiv.) in anhydrous CH_2Cl_2 (0.6 mL) and BF_3•OEt_2 (23 µL, 180 µmol, 1.5 equiv) was added separately. The reaction mixture was stirred for 2 h at –78 °C, diluted with CH_2Cl_2 (1 mL), quenched with saturated aqueous $NaHCO_3$ (0.8 mL), and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (6:1 hexane:EtOAc) to afford alcohol **2a** and **2b** as white solids (37.1 mg, combined yield 82%, **2a**:**2b** 51:49). The diastereomers **2a** and **2b** were separated by column chromatography (6:1 hexane:EtOAc) and used for further reactions, respectively.

2a: TLC: R_f 0.65 (2:1, hexane/EtOAc). $[\alpha]_D^{22.8}$ = +2.4 (c 1.04, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30–7.27 (m, 2H), 7.22–7.20 (m, 3H), 4.61 (d, *J* = 9.6 Hz, 1H), 3.96 (m, 1H), 3.59 (m, 1H), 3.53(d, *J* = 7.2 Hz, 1H), 2.94 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.83 (m, 1H), 2.57 (m, 1H), 1.47 (s, 9H), 1.33 (s, 9H), 1.25 (d, *J* = 7.6 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.9, 155.4, 138.2, 129.8, 128.6, 126.5, 81.8, 79.4, 75.5, 53.5, 42.2, 35.6, 28.5, 28.3, 14.9. HRMS (ESI) *m/z* calcd for $C_{21}H_{33}NO_5$ 379.2359, found 379.2360.

2b: TLC: R_f 0.68 (2:1, hexane/EtOAc). $[\alpha]_D^{23.8}$ = –32.4 (c 0.9, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30–7.18 (m, 5H), 4.90 (d, *J* = 9.6 Hz, 1H), 3.92 (dd, *J* = 16.4, 8.4 Hz, 1H), 3.58 (dd, *J* = 8.4, 4.0 Hz, 1H), 3.47 (d, *J* = 4.0 Hz, 1H), 2.96–2.85 (m, 2H), 2.48 (m, 1H), 1.44 (s, 9H), 1.39 (s, 9H), 1.06 (d, *J* = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.2, 155.9, 138.6, 129.6, 128.6, 126.5, 81.6, 79.5, 72.7, 53.0, 43.7, 39.2, 28.6, 28.3, 14.2. HRMS (ESI) *m/z* calcd for $C_{21}H_{33}NO_5$ 379.2359, found 379.2355.

(2*R*,3*S*,4*S*)-*tert*-Butyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenyl-3-((trimethylsilyl)oxy)pentanoate (2b′)

N-Boc-L-phenylalaninal (75 mg, 300 µmol) and zinc iodide (144 mg, 450 µmol, 1.5 equiv.) were dissolved in anhydrous CH_2Cl_2 (0.5 mL) at –78 °C. After stirring at –78 °C for 5 min, a solution of silyl ketene acetal (152 mg, 750 µmol, 2.5 equiv.) in anhydrous CH_2Cl_2 (0.2 mL) was added. The reaction mixture was stirred at –78 °C for 1 h, diluted with CH_2Cl_2 (2 mL), quenched with saturated aqueous $NaHCO_3$ (1 mL) and H_2O (1 mL), and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (20:1 hexane:EtOAc → 6:1 hexane:EtOAc) to afford **2b′** (83.9 mg, 62%) as colorless sticky oil. TLC: R_f 0.69 (4:1, hexane/EtOAc). $[\alpha]_D^{25.3}$ = –78.7 (c 0.3, MeOH). ^{1}H-NMR (400 MHz, Benzene d_6): δ 7.25–7.23 (m, 2H), 7.18–7.04 (m, 3H), 4.91 (d, *J* = 10.0 Hz, 1H), 4.32 (m, 1H), 4.06 (d, *J* = 8.4 Hz, 1H), 2.85 (dd, *J* = 13.6, 8.4 Hz, 1H), 2.77 (dd, *J* = 8.4, 7.2 Hz, 1H), 2.70 (dd, *J* = 13.6, 5.6 Hz, 1H), 1.32 (s, 18H), 1.19 (d, *J* = 7.2 Hz, 3H), 0.20 (s, 9H). ^{13}C-NMR (100 MHz, Benzene d_6): δ 174.9, 156.0, 139.2, 130.2, 129.1, 127.0, 80.3, 79.2, 76.1, 53.2, 46.4, 41.0, 28.9, 28.6, 15.0, 1.4. HRMS (ESI) *m/z* calcd for $C_{24}H_{41}NO_5Si$ 451.2754, found 451.2754.

(2*R*,3*S*,4*S*)-*tert*-Butyl 4-((*tert*-butoxycarbonyl)amino)-3-hydroxy-2-methyl-5-phenylpentanoate (2b) (TMS deprotection)

Silyl ether **2b′** (14.2 mg, 31.0 µmol) was dissolved in tetrahydrofuran (0.78 mL). After cooling to 0 °C, 1 M aqueous HCl (0.16 mL) was added and the mixture was stirred at 0 °C for 0.25 h. The reaction mixture was diluted with CH_2Cl_2 (2 mL), treated with saturated aqueous $NaHCO_3$ (1 mL) at 0 °C, and extracted with CH_2Cl_2 (3 × 3 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (6:1 hexane:EtOAc) to afford **2b** (8.9 mg, 76%) as a white solid. The spectral data are identical to those of **2b** prepared by the method in entry 10 of table 1.

(2*S*,3*R*,4*S*)-*tert*-Butyl 3-((1*H*-imidazole-1-carbonothioyl)oxy)-4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (4a)

Alcohol **2a** (398 mg, 1.05 mmol) and 90% 1,1'-thiocarbonyldiimidazole (416 mg, 2.10 mmol, 2.0 equiv.) were dissolved in anhydrous toluene (10.5 mL) under Ar atmosphere. After heating at 60 °C for 18 h, the solvent was removed by rotary evaporation. The residue was purified by column chromatography (3:1 hexane:EtOAc) to afford thioester **4a** (487 mg, 95%) as a white solid. TLC: R_f 0.33 (2:1, hexane/EtOAc). $[\alpha]_D^{23.5}$ = –14.6 (c 1.05, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H), 7.59 (s, 1H), 7.30–7.26 (m, 2H), 7.23–7.17 (m, 3H), 7.03 (s, 1H), 5.97 (t, *J* = 5.6 Hz, 1H), 4.69 (d, *J* = 9.2 Hz, 1H), 4.45 (m, 1H), 3.02–2.97 (m, 2H), 2.79 (dd, *J* = 14.0, 8.4 Hz, 1H), 1.40 (s, 9H), 1.34 (s, 9H), 1.25 (d, *J* = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 183.7, 171.9, 155.1, 137.1, 136.8, 131.1, 129.2, 128.8, 127.1, 118.2, 84.8, 82.0, 80.1, 51.9, 42.1, 37.4, 28.5, 28.1, 13.1. HRMS (ESI) *m/z* calcd for $C_{25}H_{35}N_3O_5S$ 489.2297, found 489.2300.

(2*R*,3*S*,4*S*)-*tert*-Butyl 3-((1*H*-imidazole-1-carbonothioyl)oxy)-4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (4b)

Alcohol **2b** (455 mg, 1.20 mmol) and 90% 1,1'-thiocarbonyldiimidazole (476 mg, 2.40 mmol, 2.0 equiv.) were dissolved in anhydrous toluene (12 mL) under Ar atmosphere. After heating at 60 °C for 18 h, the solvent was removed by rotary evaporation. The residue was purified by column chromatography (3:1 hexane:EtOAc) to afford thioester **4b** (542 mg, 93%) as a white solid. TLC: R_f 0.35 (2:1, hexane/EtOAc). $[\alpha]_D^{22.6}$ = –55.5 (c 1.02, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H), 7.65 (s, 1H), 7.30–7.26 (m, 2H), 7.22–7.19 (m, 3H), 7.06 (s, 1H), 5.95 (dm, *J* = 8.4 Hz, 1H), 4.48–4.43 (m, 2H), 3.12–2.98 (m, 2H), 2.63 (m, 1H), 1.35 (s, 9H), 1.29 (d, *J* = 6.8 Hz, 3H), 1.25 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 184.3, 172.0, 155.5, 137.3, 137.0, 131.3, 129.4, 128.7, 127.0, 118.4, 86.2, 81.9, 80.1, 52.7, 43.4, 39.6, 28.3, 28.0, 14.2. HRMS (ESI) *m/z* calcd for $C_{25}H_{35}N_3O_5S$ 489.2297, found 489.2302.

(2*S*,4*R*)-*tert*-Butyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (5a, P934)

Thioester **4a** (97.9 mg, 200 µmol) was dissolved in anhydrous toluene (1 mL). Bu_3SnH (64.6 µL, 240 µmol, 1.2 equiv.) and a solution of AIBN (6.57 mg, 40 µmol, 0.2 equiv.) in anhydrous toluene (1 mL) was added sequentially. The mixture was stirred at 120 °C for 30 min, quenched with water (1 mL), and extracted with CH_2Cl_2 (3 × 4 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (hexane → 10:1 hexane:EtOAc) to afford **5a** (70.1 mg, 97%) as a white solid. TLC: R_f 0.43 (4:1, hexane/EtOAc). $[\alpha]_D^{25.5}$ = +20.2 (c 1.14, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30–7.26 (m, 2H), 7.22–7.16 (m, 3H), 4.35 (d, *J* = 7.6 Hz, 1H), 3.87 (m, 1H), 2.84–2.72 (m, 2H), 2.47 (m, 1H), 1.82 (m, 1H), 1.42 (s, 9H), 1.40 (s, 9H), 1.39 (m, 1H, identified from HSQC), 1.10 (d, *J* = 7.6 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.6, 155.1, 138.0, 129.6, 128.3, 126.3, 80.2, 79.0, 49.8, 41.5, 37.8, 37.3, 28.4, 28.0, 17.7. HRMS (ESI) *m/z* calcd for $C_{21}H_{33}NO_4$ 363.2410, found 363.2404.

(2*R*,4*R*)-*tert*-Butyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (5b)

Thioester **4b** (294 mg, 600 µmol) was dissolved in anhydrous toluene (3 mL). Bu_3SnH (194 µL, 720 µmol, 1.2 equiv.) and a solution of AIBN (19.7 mg, 120 µmol, 0.2 equiv.) in anhydrous toluene (3 mL) was added sequentially. The mixture was stirred at 120 °C for 40 min, quenched with water (4 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (hexane → 10:1 hexane:EtOAc) to afford **5b** (213 mg, 98%) as a white solid. TLC: R_f 0.44 (4:1, hexane/EtOAc). $[\alpha]_D^{25.2}$ = –1.9 (c 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30–7.26 (m, 2H), 7.22–7.16 (m, 3H), 4.39 (d, *J* = 7.6 Hz, 1H), 3.85 (m, 1H), 2.83 (dd, *J* = 12.8, 4.4 Hz, 1H), 2.71 (dd, *J* = 12.8, 7.2 Hz, 1H), 2.35 (sextet, *J* = 6.8 Hz, 1H), 1.72 (m, 1H), 1.47 (m, 1H), 1.43 (s, 9H), 1.39 (s, 9H), 1.07 (d, *J* = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.5, 155.4, 138.1, 129.5, 128.3, 126.3, 80.2, 79.0, 50.7, 42.4, 38.1,

37.1, 28.4, 28.1, 17.7. HRMS (ESI) *m/z* calcd for $C_{21}H_{33}NO_4$ 363.2410, found 363.2410.

(2*S*,4*R*)-*tert*-Butyl 4-amino-2-methyl-5-phenylpentanoate (1a)

A solution of 20% TFA in anhydrous CH_2Cl_2 (3.69 mL) was added to a solution of **5a** (120 mg, 330 µmol) in anhydrous CH_2Cl_2 (0.4 mL) dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h. After the reaction was completed, the reaction mixture was diluted with CH_2Cl_2 (4 mL), and treated with saturated aqueous $NaHCO_3$ (4 mL) and 2 M NaOH (3.6 mL, pH 9). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (6 × 15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (20:1 CH_2Cl_2:MeOH) to afford deprotected amine **1a** (65.6 mg, 80%) as a pale yellow sticky oil. TLC: R_f 0.16 (20:1, CH_2Cl_2/MeOH). $[\alpha]_D^{25.2}$ = +14.3 (c 0.3, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.32–7.29 (m, 2H), 7.23–7.17 (m, 3H), 3.03 (m, 1H), 2.77 (dd, *J* = 13.6, 4.8 Hz, 1H), 2.57 (m, 1H), 2.47 (dd, *J* = 13.6, 8.8 Hz, 1H), 1.82 (ddd, *J* = 14.0, 10.4, 4.0 Hz, 1H), 1.43 (s, 9H), 1.40 (brs, 2H), 1.34 (ddd, *J* = 14.0, 9.2, 4.8 Hz, 1H), 1.14 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.9, 139.2, 129.3, 128.5, 126.3, 80.2, 50.7, 44.9, 42.4, 37.8, 28.1, 18.1. HRMS (ESI) *m/z* calcd for $C_{16}H_{25}NO_2$ 263.1885, found 263.1890.

(2*R*,4*R*)-*tert*-Butyl 4-amino-2-methyl-5-phenylpentanoate (1b).

A solution of 20% TFA in anhydrous CH_2Cl_2 (3.29 mL) was added to a solution of **5b** (107 mg, 294 µmol) in anhydrous CH_2Cl_2 (0.36 mL) dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h. After the reaction was completed, the reaction mixture was diluted with CH_2Cl_2 (4 mL), and treated with saturated aqueous $NaHCO_3$ (4 mL) and 2 M NaOH (3.3 mL, pH 9). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (5 × 15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (20:1 CH_2Cl_2:MeOH) to afford deprotected amine **1b** (62.6 mg, 81%) as a yellow sticky oil. TLC: R_f 0.17 (20:1, CH_2Cl_2/MeOH). $[\alpha]_D^{25.6}$ = –29.8 (c 1.22, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.32–7.28 (m, 2H), 7.24–7.17 (m, 3H), 3.00 (m, 1H), 2.87 (dd, *J* = 13.2, 4.4 Hz, 1H), 2.53 (sextet, *J* = 7.2 Hz, 1H), 2.43 (dd, *J* = 13.2, 8.8 Hz, 1H), 1.74 (m, 1H), 1.50 (m, 1H), 1.45 (s, 9H), 1.22 (brs, 2H), 1.13 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.4, 139.4, 129.3, 128.5, 126.3, 80.0, 51.0, 45.1, 41.6, 37.9, 28.1, 17.4. HRMS (ESI) *m/z* calcd for $C_{16}H_{25}NO_2$ 263.1885, found 263.1887.

(3*S*,4*R*,5*S*)-5-Benzyl-4-hydroxy-3-methylpyrrolidin-2-one (3a)

A solution of 20% TFA in anhydrous CH_2Cl_2 (2.45 mL) was added to a solution of **2a** (83.1 mg, 219 µmol) dissolved in anhydrous CH_2Cl_2 (0.27 mL) dropwise at 0 °C. After the mixture was stirred at 0 °C for 0.75 h, the reaction mixture was diluted with CH_2Cl_2 (4 mL), and treated with saturated aqueous $NaHCO_3$ (4 mL) and 2 M NaOH (1.5 mL, pH 9). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (4 × 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (15:1 CH_2Cl_2:MeOH) to afford deprotected γ-amino-β-hydroxy-α-methyl ester (46.5 mg, 76%) as a white solid. The deprotected amine (45.8 mg, 164 µmol) was dissolved in anhydrous toluene (1.6 mL) and the mixture was stirred at 120 °C for 21 h. After the completion of the reaction, the solvent was removed by rotary evaporation and the residue was purified by column chromatography (EtOAc) to afford lactam **3a** (32.2 mg, 96%) as a white solid. TLC: R_f 0.42 (10:1, CH_2Cl_2/MeOH). $[\alpha]_D^{25.3}$ = –81.6 (c 0.31, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.35–7.32 (m, 2H), 7.26 (m, 1H), 7.20–7.18 (m, 2H), 5.43 (brs, 1H), 4.25 (m, 1H), 3.69 (m, 1H), 2.96 (dd, *J* = 13.2, 5.2 Hz, 1H), 2.66 (dd, *J* = 13.2, 8.8 Hz, 1H), 2.58 (m, 1H), 1.65 (d, *J* = 5.2 Hz, 1H), 1.20 (d, *J* = 7.6 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.9, 137.1, 129.0, 128.9, 127.0, 74.0, 62.4, 40.2, 40.0, 8.2. HRMS (ESI) *m/z* calcd for $C_{12}H_{15}NO_2$ 205.1103, found 205.1106.

(3*R*,4*S*,5*S*)-5-Benzyl-4-hydroxy-3-methylpyrrolidin-2-one (3b)

A solution of 20% TFA in CH_2Cl_2 (20 mL) was added to a solution of **2b** (85.9 mg, 226 µmol) dissolved in anhydrous CH_2Cl_2 (2 mL) dropwise at 0 °C. After the mixture was stirred at 0 °C for 1 h, quenched with 1 M NaOH (10mL), and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (20:1 CH_2Cl_2:MeOH) to afford deprotected γ-amino-β-hydroxy-α-methyl ester (33.9 mg, 54%) as a white solid. The deprotected amine (33 mg, 118 µmol) was dissolved in anhydrous toluene (2mL) and the mixture was stirred at 120 °C for 38 h. After the completion of the reaction, the solvent was removed by rotary evaporation and the residue was purified by column chromatography (20:1 CH_2Cl_2:MeOH) to afford lactam **3b** (18.8 mg, 78%) as a white solid. TLC: R_f 0.47 (10:1, CH_2Cl_2/MeOH). $[\alpha]_D^{23.6}$ = –71.2 (c 0.5, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.35–7.32 (m, 2H), 7.28–7.24 (m, 3H), 5.30 (brs, 1H), 4.29 (m, 1H), 3.85 (m, 1H), 3.02 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.87 (dd, *J* = 13.6, 8.8 Hz, 1H), 2.58 (m, 1H), 1.55 (d, *J* = 6.0 Hz, 1H), 1.23 (d, *J* = 7.6 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 178.1, 137.8, 129.2, 129.1, 127.1, 71.9, 59.4, 43.5, 35.5, 8.2. HRMS (ESI) *m/z* calcd for $C_{12}H_{15}NO_2$ 205.1103, found 205.1108.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [copies of ^{1}H & ^{13}C NMR spectra of **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a** and **5b**]. See DOI: 10.1039/b000000x/

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