Supporting Information

On the Substrate Specificity of Dehydration by Lacticin 481 Synthetase

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General procedure for LctM assays with truncated LctA analogs

The truncated substrate (5–10 μg) was dissolved in sterile Millipore water (20 μL) and 5 μL of solution was used for the assay. The peptide solution was incubated with buffer (2 μL, 500 mM Tris.HCl, 100 mM $MgCl_2$, 250 μg/mL, pH 7.5, 25 ^oC), 100 mM ATP (1 μL) and sterile Millipore water (8 μL). His-LctM (1.0 mg/mL, 4 μL) was added to the buffered peptide solution. The assay mixture was incubated at 25 ^oC for 5 to 12 h. Assay products were analyzed by MALDI-TOF MS in one of two ways. In the first method 1 μL of the assay mixture was mixed with 9 μL of sinapic acid matrix (prepared in 30% CH_3CN, containing 0.1% TFA) and 5 μL was spotted directly on the target plate. A second procedure involved acidification of the assay mixture with 5 μL of 5% TFA (final pH ~2). The acidified solution was then loaded onto a C18 zip-tip, and the assay product eluted with 8 μL of α-hydroxycinnamic acid matrix (prepared in 50% CH_3CN, containing 0.1% TFA) of which 5 μL was spotted on the MALDI plate.

LctM assay with His_6-LctA(1-37)-CNMNTWA. MALDI-TOF MS calcd 6876 (M), 6858 (M^+ - H_2O), 6840 (M^+ - 2 H_2O), 6822 (M^+ - 3 H_2O); Found, 6863 (M^+ -H_2O), 6846 (M^+ - 2 H_2O and M^+ - 3 H_2O + O), 6828 (M^+ - 3 H_2O).

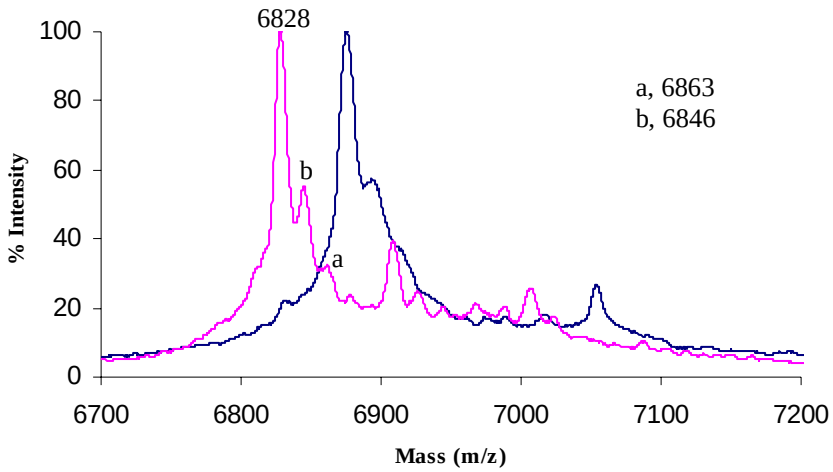

Figure S1. The assay is shown in purple and the control in blue.

LctM assay with His_6-LctA(1-37)-CNMN<u>Propyl-Ser</u>WA. MALDI-TOF MS calcd 6904 (M), 6886 (M^+ - H_2O), 6868 (M^+ - 2 H_2O), 6850 (M^+ - 3 H_2O); Found, 6904 (M), 6886 (M^+ – H_2O), 6868 (M^+ - 2 H_2O), 6851 (M^+ - 3 H_2O).

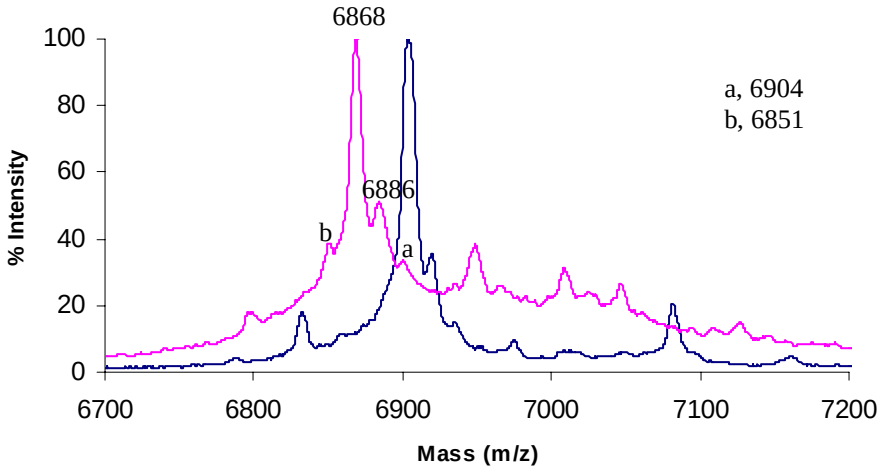

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Figure S2. The assay is shown in purple and the control in blue.

LctM assay with His_6-LctA(1-37)-CNMN<u>Isopropyl-Ser</u>WA. MALDI-TOF MS calcd 6904 (M), 6886 (M^+ - H_2O), 6868 (M^+ - 2 H_2O), 6850 (M^+ - 3 H_2O); Found, 6905 (M), 6888 (M^+ – H_2O), 6870 (M^+ - 2 H_2O).

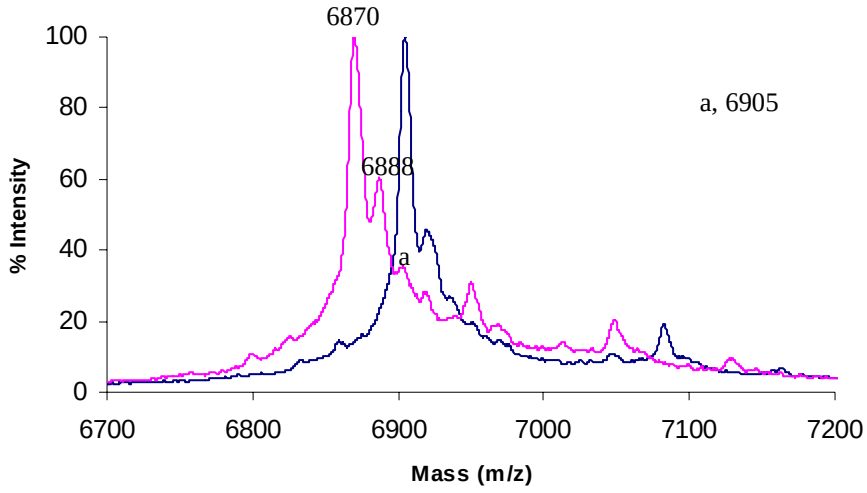
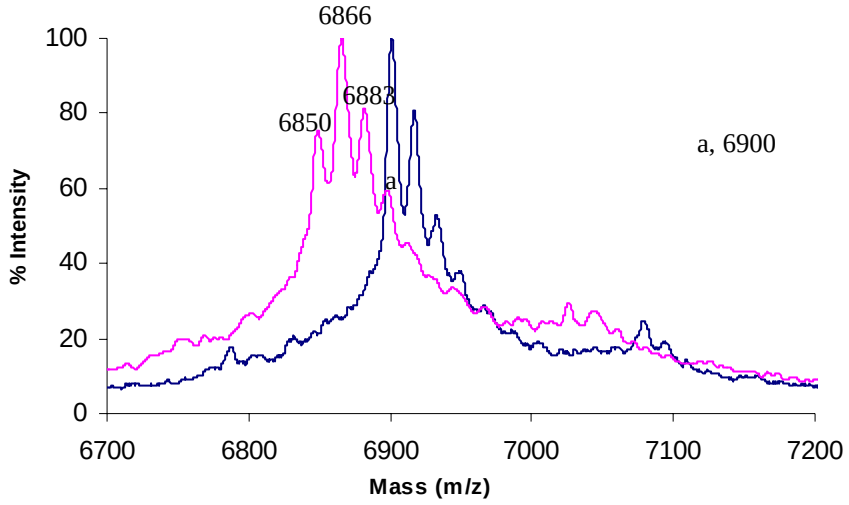
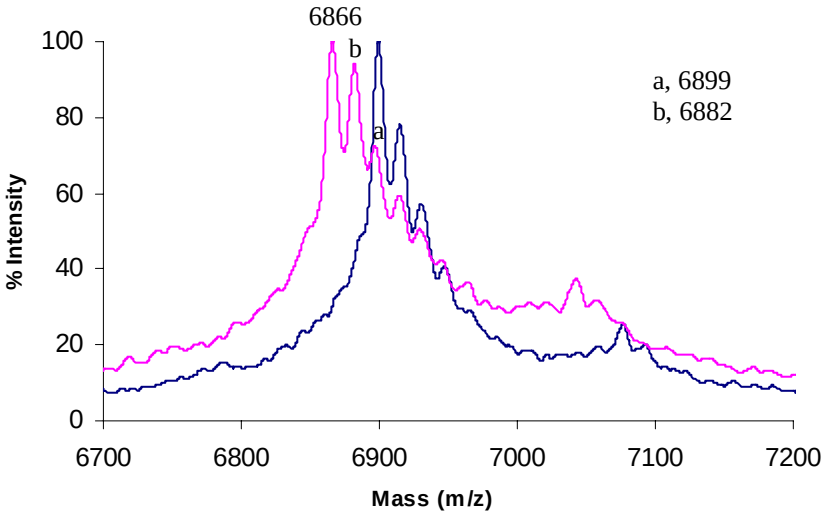


Figure S3. The assay is shown in purple and the control in blue.

LctM assay with His_6-LctA(1-37)-CNMN(*E*)-<u>Allyl-Thr</u>WA. MALDI-TOF MS calcd 6902 (M), 6884 (M^+ - H_2O), 6866 (M^+ - 2 H_2O), 6848 (M^+ - 3 H_2O); Found, 6900 (M), 6883 (M^+ – H_2O), 6866 (M^+ - 2 H_2O with an underlying peak from M^+ - 3 H_2O + O due to an oxidized impurity in the starting peptide, M + O), 6850 (M^+ – 3 H_2O). **LctM assay with His_6-LctA(1-37)-CNMN<u>Z-Allyl-Thr</u>WA.** MALDI-TOF MS calcd 6902 (M), 6884 (M^+ - H_2O), 6866 (M^+ - 2 H_2O), 6848 (M^+ - 3 H_2O); Found, 6899 (M), 6882 (M^+ – H_2O with an underlying peak from M^+ - 2 H_2O + O due to an oxidized impurity in the starting peptide, M + O), 6866 (M^+ - 2 H_2O).



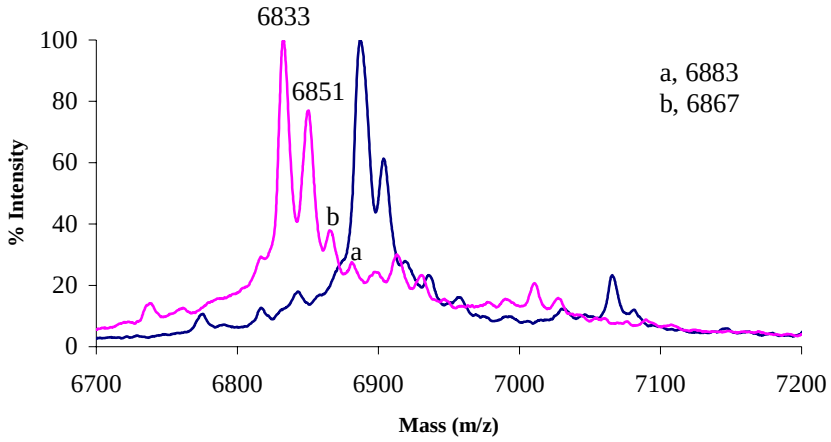
A



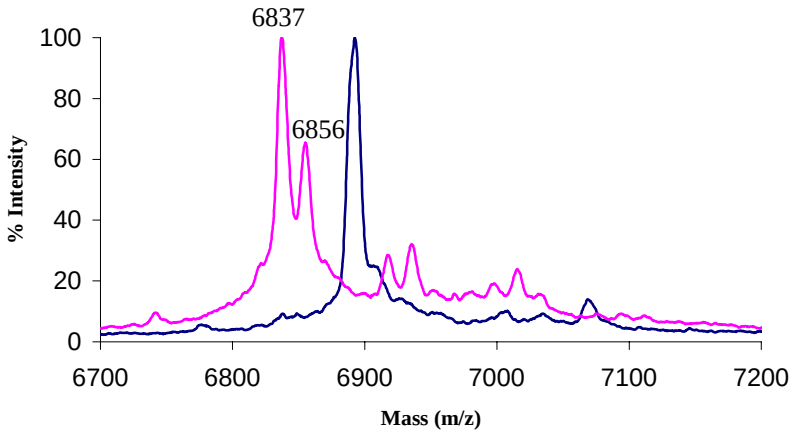
B

Figure S4. The assays are shown in purple and the control in blue. A) His_6-LctA(1-37)-CNMN(*E*)-Allyl-ThrWA; B) LctM assay with His_6-LctA(1-37)-CNMN(*Z*)-Allyl-ThrWA.

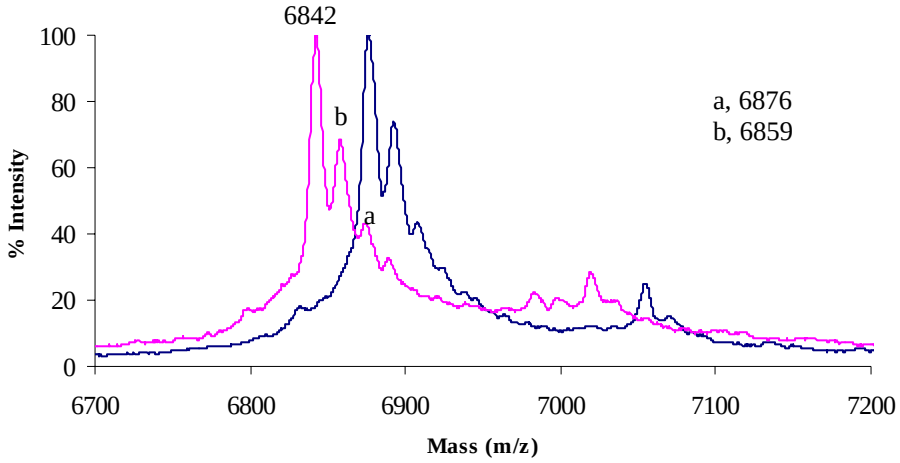
LctM assay with His_6-LctA(1-37)-CNMNEthynyl-SerWA. MALDI-TOF MS calcd 6886 (M), 6868 (M^+ - H_2O), 6850 (M^+ - 2 H_2O), 6832 (M^+ - 3 H_2O); Found, 6883 (M), 6867 (M^+ – H_2O), 6851 (M^+ - 2 H_2O with an underlying peak from M^+ - 3 H_2O + O from an oxidized impurity in the starting peptide, M + O), 6833 (M^+ - 3 H_2O).



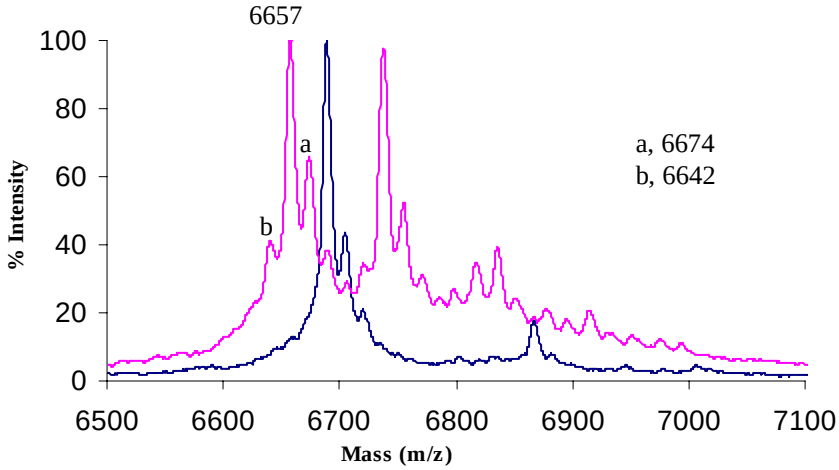
LctM assay with His_6-LctA(1-37)-CNMN<u>Vinyl-Ser</u>WA. MALDI-TOF MS calcd 6888 (M), 6870 (M^+ - H_2O), 6852 (M^+ - 2 H_2O), 6834 (M^+ - 3 H_2O); Found, 6856 (M^+ - 2 H_2O), 6837 (M^+ - 3 H_2O).



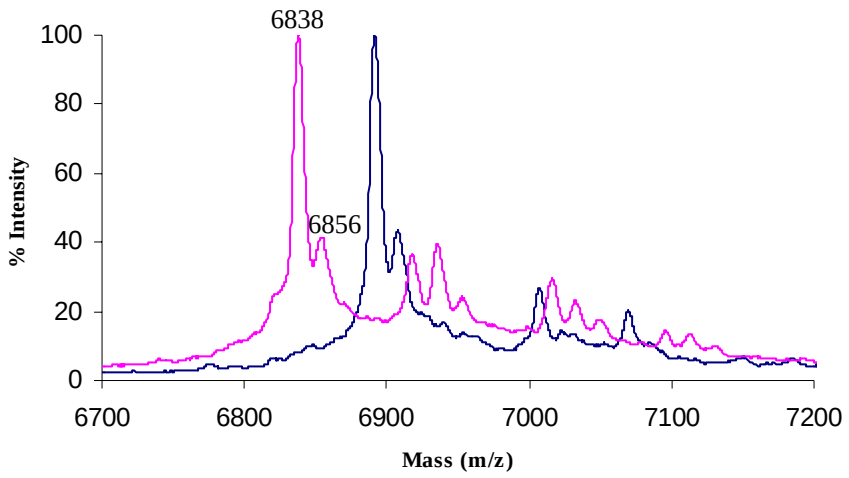
LctM assay with His_6-LctA(1-37)-CNMN<u>Allo-Thr</u>WA. MALDI-TOF MS calcd 6876 (M), 6858 (M^+ - H_2O), 6840 (M^+ - 2 H_2O), 6822 (M^+ - 3 H_2O); Found, 6876 (M), 6859 (M^+ - H_2O with an underlying peak from M^+ - 2 H_2O + O from an oxidized impurity in the starting peptide, M + O), 6842 (M^+ - 2 H_2O).



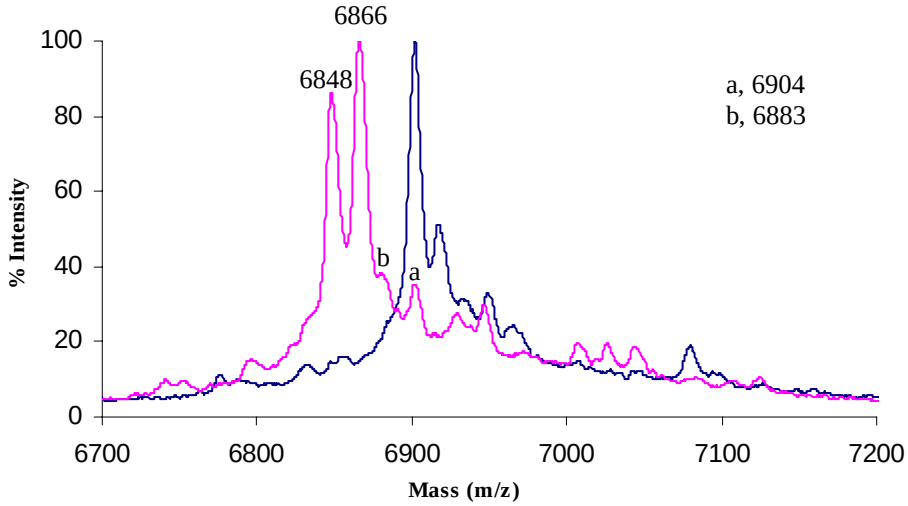
LctM assay with His_6-LctA(1-37)-CNMNβ^2-SerA. MALDI-TOF MS calcd 6690 (M), 6672 ($M^+ - H_2O$), 6654 ($M^+ - 2\ H_2O$), 6636 ($M^+ - 3\ H_2O$); Found, 6691(M), 6674 ($M^+ - H_2O$ and $M^+ - 2\ H_2O + O$), 6657 ($M^+ - 2\ H_2O$), 6642 ($M^+ - 3H_2O$).



LctM assay with His_6-LctA(1-37)-CNMN<u>Ethyl-Ser</u>WA. MALDI-TOF MS calcd 6890 (M), 6872 ($M^+ - H_2O$), 6854 ($M^+ - 2\ H_2O$), 6836 ($M^+ - 3\ H_2O$); Found, 6856 ($M^+ - 2\ H_2O$), 6838 ($M^+ - 3\ H_2O$).



LctM assay with His_6-LctA(1-37)-CNMN<u>Propynyl-Ser</u>WA. MALDI-TOF MS calcd 6900 (M), 6882 (M^+ - H_2O), 6864 (M^+ - 2 H_2O), 6846 (M^+ - 3 H_2O); Found, 6904 (M), 6883 (M^+ – H_2O), 6866 (M^+ - 2 H_2O), 6848 (M^+ – 3 H_2O).

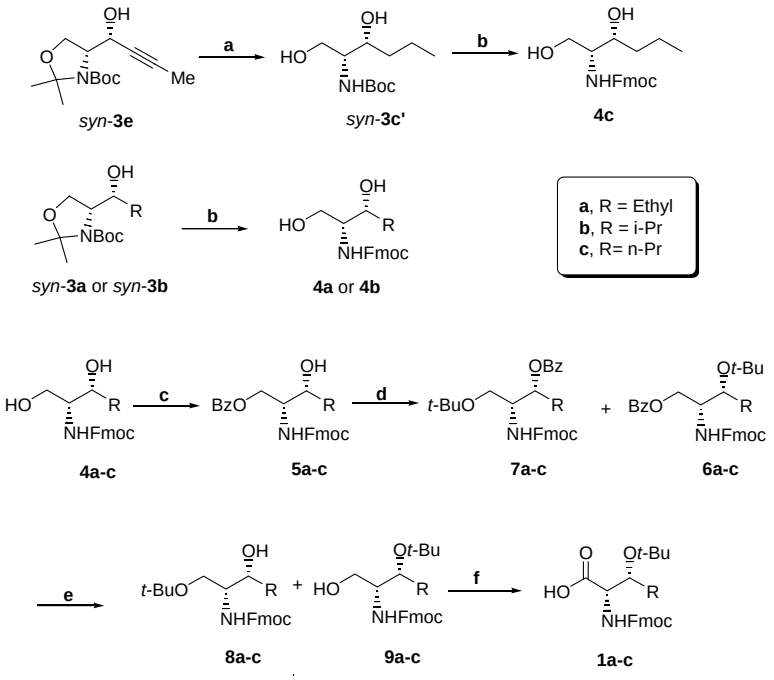


Synthesis of Thr Analogs

NOTE: The compound numbering in this supporting information is independent of the numbering in the text of the communication.

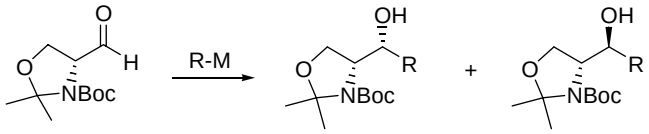
Synthesis of Amino Acids 1a-c.

Scheme 1.[a]



[a] (a) $Pd(OH)_2/C$, EtOAc, rt. 98%. (b) (1) 4 N HCl-dioxane, MeOH, rt. (2) FmocCl, $NaHCO_3$, THF/H_2O (2:1), **4a**, 93.5%; **4b**, 88%; **4c**, 71%. (c) BzCl, pyr. CH_2Cl_2, 0 ^oC, **5a**, 90%; **5b**, 95%; **5c**, 91%. (d) isobutylene, conc. H_2SO_4, CH_2Cl_2, **6a**+**7a**, 82% (95%); **6b**+**7b**, 85%, (93%); **6c**+**7c**, 83% (94%). (e) DIBAl-H, CH_2Cl_2, -78^oC, **9a**, 70%; **8a**, 20%; **9b**, 63%; **8b**, 21%; **9c**, 70%; **8c**, 16%. (f) Jones reagent, acetone, 0 ^oC to rt., **1a**, 91%; **1b**, 92%; **1c**, 93%.

Table 1. Addition of organozinc and Grignard reagents to D-Garner aldehyde **2**.



2 *syn*-**3** *anti*-**3**

Entry	RM	Solvent	3, yield (%)[a]	Syn/anti[b]	e.e.[e]
1	Et_2Zn	toluene	**3a**, 72	14:1	>99:1
2	i-Pr_2Zn	toluene	**3b**, 68	>19:1[c]	>99:1
3	1-PropynylMgBr, CuI	THF, Me_2S	**3e**, 95	16:1	>99:1
4	Ethynyltrimethyl-silane, EtMgBr, CuI	THF, Me_2S	**3d′**, 82	20:1[d]	>99:1

[a] isolated yield. [b] the *syn*/*anti* ratio was determined by ^{1}H NMR spectroscopy. [c] *anti* diastereoisomer was not detected by ^{1}H NMR spectroscopy. [d] Conditions according to reference [1].[e] Determined from Fmoc protected diols **4a-b** and **10d-e** by SFC using a chiral stationary phase (see following pages).

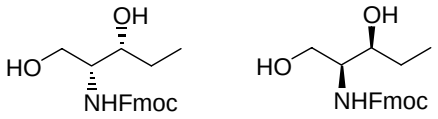
In related work, chelation controlled addition of organozinc reagents to (*S*)-*N*,*N*-dibenzyl-OBO-serinal has been shown to proceed with good *syn*-selectivity.[2] Use of Et_2Zn and *i*-Pr_2Zn and Garner aldehyde provides higher selectivities and yields than addition of the corresponding Grignard reagents to Garner aldehyde derivatives in which the dimethyl substituents of the acetonide in Garner's aldehyde are replaced by a cyclohexyl ring (*syn*/*anti* = 9:1, yield 57% for Et; *syn*/*anti* = 6:1, 85%, for *i*-Pr).[3] In a recently reported study it was shown that addition of vinylzirconium reagents to Garner aldehyde catalyzed by $ZnBr_2$ also provides *syn* products with good selectivity.[4]

The relative configuration of compounds *syn*-**3b** and *syn*-**3d** were deduced by optical rotation producing data consistent with literature reports.[1,3,5] The relative stereochemistry of compounds *syn*-**3a**, *syn*-**3c** and *syn*-**3e** were confirmed by X-ray diffraction of compounds **4a** and **5c** (shown on pages S13 and S19).
The possibility of some racemization of the Garner aldehyde during the reaction with the organometallic reagents was investigated by chiral SFC analysis shown on the following pages. Racemic standards were obtained by preparation of the enantiomeric series using the same methodology as in Scheme 1 and Table 1 but starting with L-Garner aldehyde.

Representative Supercritical Fluid Chromatography (SFC) to Determine the Stereochemical Purity of Compounds 4a-b.

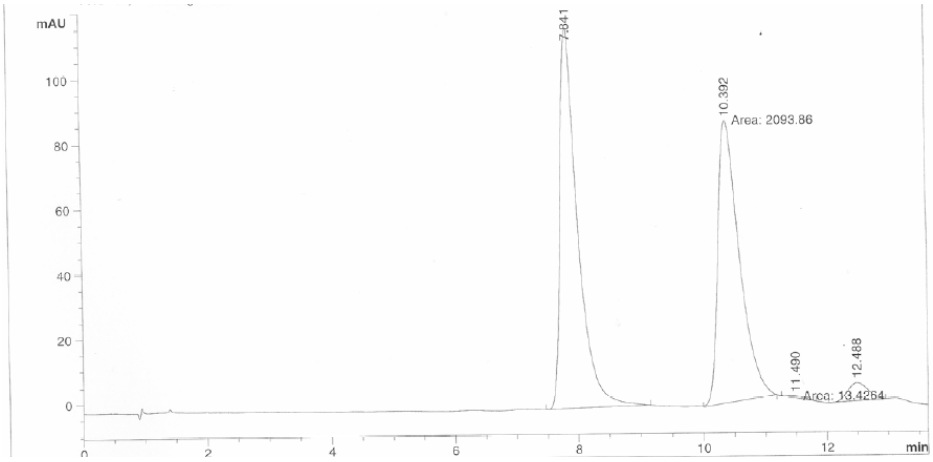
SFC conditions for diol **4a**:

Chiralcel OD column (Daicel chemical industries, 25 cm x 4.6 mm I.D.), 125 psi, 3 mL/min, 40 ^{o}C. Mobile phase: CO_2/15% MeOH.

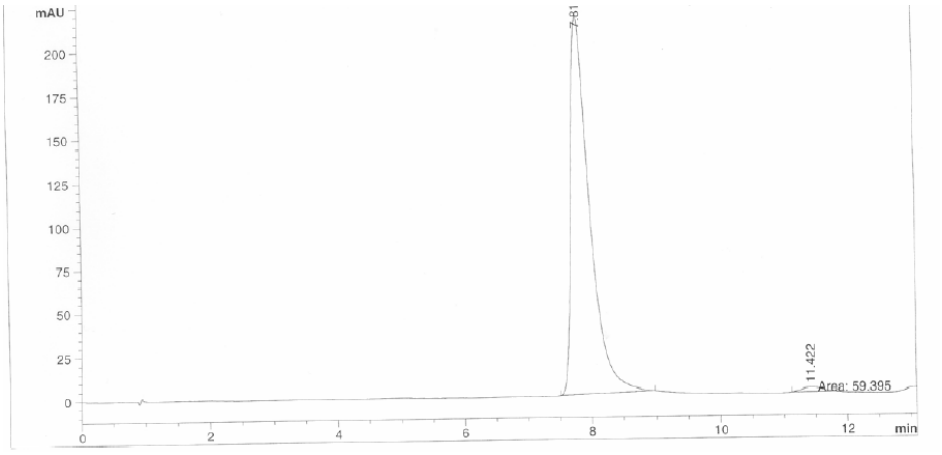


4a **4a'**

SFC for a mixture of **4a** and **4a′** (**4a**, Rt = 7.84 min; **4a′** = 10.39 min):



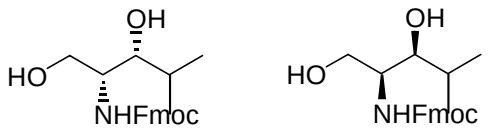
SFC for diol **4a** obtained through addition of Et_2Zn to Garner aldehyde (**4a**, Rt = 7.81 min)



No enantiomer **4a′** was found by SFC for sample **4a**. The e.r. of **4a** is therefore estimated as > 99:1.

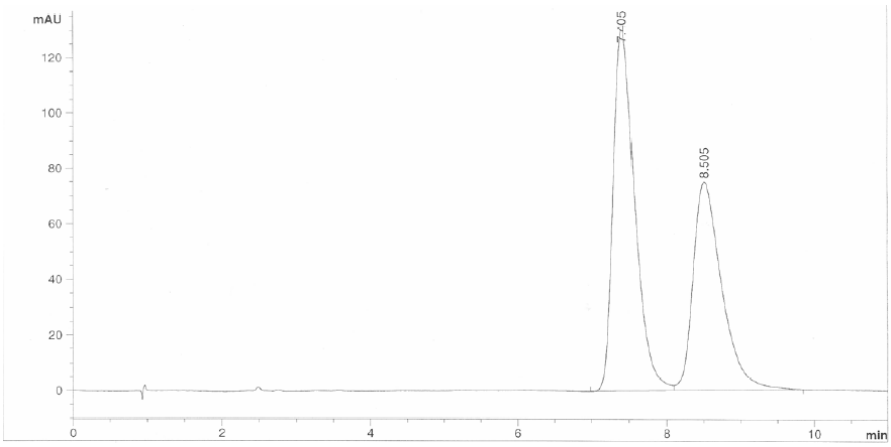
SFC conditions for diols **4b**

Chiralpak AD column (Daicel chemical industries, 25 cm x 4.6 mm I.D.), 125 psi, 3 mL/min, 40 ^{o}C, 220 nm. Mobile phase: CO_2/15% MeOH.

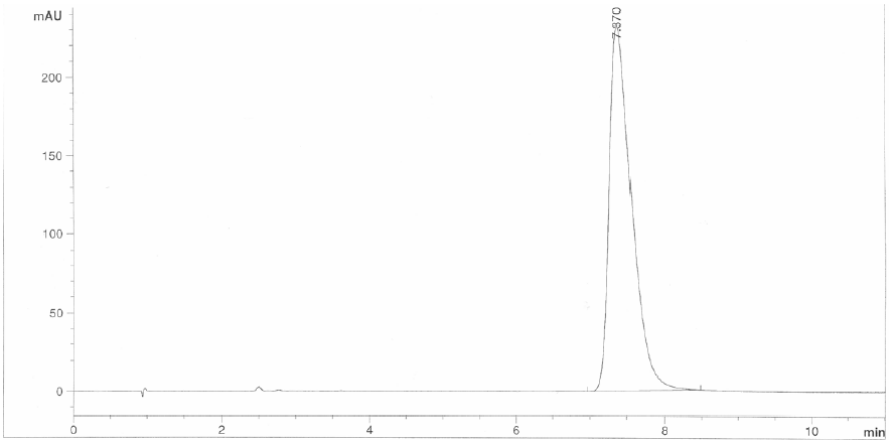


4b **4b'**

SFC for a racemic mixture of **4b** and **4b′** (**4b**, Rt = 7.40 min; **4b′**, Rt = 8.50 min).



SFC for diol **4b** (Rt = 7.37 min)



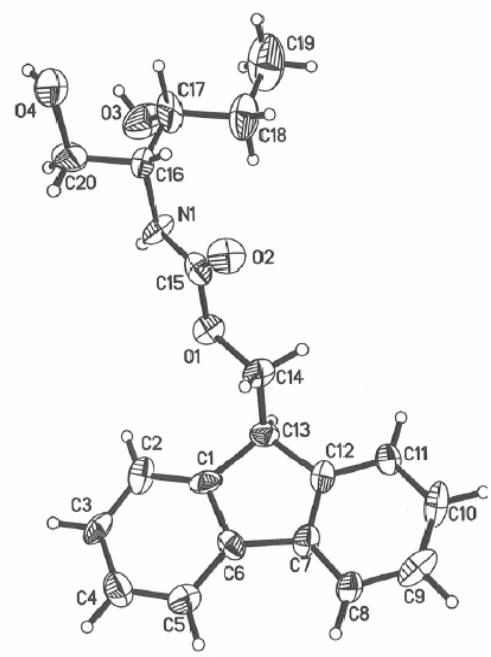
No enantiomer **4b′** was detected by SFC for sample **4b** prepared by as shown in Table 1 and Scheme 1. The e.r. of **4b** is therefore estimated as > 99:1.

Synthetic Procedures and Spectroscopic Characterization of Intermediates in the Synthesis of 1a-c

(4*R*)-4-((1*R*)-1-Hydroxy-propyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*syn*-3a). To a solution of D-Garner aldehyde **2** (2.29 g, 10 mmol) in toluene (100 mL) was added dropwise Et_2Zn (1.0 M in hexanes, 40 mL) at –78 ^oC. After being stirred at the same temperature for 10 min, the reaction mixture was warmed up to 0 ^oC and stirred for 7 h. Saturated aqueous NH_4Cl was added. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (Hexane / EtOAc = from 7:1 to 3:1, R_f = 0.4) to give a mixture of *syn* and *anti* **3a** as an oil (1.865 g, 72%, *syn*/*anti* = 14:1, determined by ^1H NMR). *Syn* **3a:** $[\alpha]_D^{25}$ = 32.8^o (*c* 0.78, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.93 – 3.79 (m, 4H), 3.62 (br, 1H), 1.57 (s, 3H), 1.47 (s, 13H), 1.35 (m, 1H), 0.99 (t, *J* = 7.0 Hz, 3H).

((1*R*, 2*R*)-2-Hydroxy-1-hydroxymethyl-butyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (4a). To a solution of compounds *syn*-**3a** and *anti*-**3a** (1.674g, 6.463 mmol) in MeOH (80 mL) was added dropwise 4 N HCl-dioxane (27.3 mL) over 20 min at 0 ^oC. The reaction mixture was then stirred at room temperature for 1.5 h. The solvent was removed, and the residue was redissolved in MeOH and concentrated again. The resulting sticky oil was dissolved in THF/H_2O (2:1, 81 mL). FmocCl (2.579 g, 9.049 mmol) was added at 0 ^oC, followed by $NaHCO_3$ (1.264 g, 15.511 mmol). The reaction mixture was stirred at room temperature for 1.5 h, and was diluted with EtOAc. The resulting mixture was washed with water and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 1:2, R_f = 0.4) to give compound **4a** (2.06 g, 93.5%) as a white solid. mp 133 –135 ^oC; $[\alpha]_D^{25}$ = -16.6^o (c 0.74, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 5.49 (d, *J* = 8.4 Hz, 1H), 4.46 (dd, *J* = 10.4 Hz, 6.4 Hz, 1H), 4.40 (dd, *J* = 10.4, 6.8 Hz, 1H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.84 – 3.79 (m, 3H), 3.67 – 3.64 (m, 1H), 2.77 (d, *J* = 2.8 Hz, 1H), 2.59 (t, *J* = 5.2 Hz, 1H), 1.50 (m, 2H), 0.94 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.0, 143.8, 141.29, 141.26, 127.7, 127.0, 125.0, 119.9, 73.9, 66.7, 64.8, 54.2, 47.2, 27.0, 10.0; IR (thin film) 3400 (br), 1694, 1514, 1450 cm^{-1}; MS *m/z* (ESI) 342 ($M^+ + H^+$), 179, 120; HRMS Calcd for $C_{20}H_{24}NO_4$ ($M^+ + H^+$): 342.1705. Found: 342.1705.

ORTEP drawing of the X-ray crystallographic structure of **4a.**



Benzoic acid (2*R*, 3*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-pentyl ester (5a). To a solution of compound **4a** (1.60 g, 4.692 mmol) in CH_2Cl_2 (80 mL) was added BzCl (0.71 mL, 5.630 mmol) at 0 ^oC, followed by pyridine (1.15 mL, 14.076 mmol). After being stirred for 1 h, the reaction mixture was diluted with EtOAc, and washed with water, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 2:1, R_f = 0.3) to give product **5a** (1.880 g, 90%) as a white solid. mp 128 – 130 ^oC; $[\alpha]_D^{25}$ = 4.2^o (*c* 1.325, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.0 Hz, 2H), 7.75 (d, *J* = 7.0 Hz, 2H), 7.59 – 7.55 (m, 3H), 7.44 – 7.37 (m, 4H), 7.29 (t, *J* = 7.0 Hz, 2H), 5.29 (d, *J* = 9.5 Hz, 1H), 4.55 (dd, *J* = 11.0 Hz, 7.5 Hz, 1H), 4.42 (d, *J* = 6.5 Hz, 2H), 4.32 (dd, *J* = 11.0 Hz, 6.5 Hz, 1H), 4.19 (t, *J* = 6.5 Hz, 1H), 4.04 (q, *J* = 7.5 Hz, 1H), 3.68 (t, *J* = 6.0 Hz, 1H), 2.62 (br, 1H), 1.55 (m, 2H), 0.97 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 167.0, 156.5, 143.81, 143.76, 141.3, 133.4, 129.7, 129.5, 128.5, 127.7, 127.0, 125.0, 119.9, 71.1, 66.8, 63.9, 52.9, 47.2, 26.6, 10.1; IR (thin film) 3433 (br), 1718, 1521, 1450 cm^{-1}; MS *m/z* (ESI) 446 ($M^+ + H^+$), 224 (100); HRMS Calcd for $C_{27}H_{28}NO_5$ ($M^+ + H^+$): 446.1967. Found: 446.1955.

((1*R*, 2*R*)-2-tert-Butoxy-1-hydroxymethyl-butyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (9a). To a solution of compound **5a** (1.025 g, 2.303 mmol) in CH_2Cl_2 (15 mL) was added

conc. H_2SO_4 (14 μL) at -10 °C, then isobutylene was bubbled through the solution for 5 min at the same temperature. The reaction was sealed and stirred for 2.5 days at room temperature. The reaction mixture was diluted with ethyl acetate, and the resulting mixture was washed with sat. aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.7) to give a mixture of compound **6a** and **7a** (945 mg, yield 82%, based on recovered starting material was 95%) as a foam, and recovered starting material **5a** (138 mg). The mixture of compounds **6a** and **7a** (1.421 g, 2.836 mmol) was dissolved in CH_2Cl_2 (50 mL) and the solution was cooled to –78 °C. DIBAl-H (1.0 M in hexane, 8.4 mL, 8.4 mmol) was added dropwise, and the reaction mixture was stirred at –78 °C for 3 h, and was quenched with MeOH at –78 °C. The resulting mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.4) to give product **9a** (786 mg, 70%) as a foam and by product **8a** (224 mg, 20%) as an oil.

Compound **9a:** $[\alpha]_D^{25}$ = -11.3° (*c* 1.185, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 2H), 5.27 (d, *J* = 9.0 Hz, 1H), 4.44 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.39 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.82 (q, *J* = 6.5 Hz, 1H), 3.70 – 3.62 (m, 3H), 2.72 (t, *J* = 6.5 Hz, 1H), 1.55 (m, 2H), 1.23 (s, 9H), 0.90 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.0, 143.9, 141.3, 127.6, 127.0, 125.1, 119.9, 74.2, 72.1, 66.7, 63.7, 53.7, 47.2, 28.7, 26.1, 10.1; IR (thin film) 3436 (br), 1705, 1505, 1450 cm^{-1}; MS *m/z* (ESI) 398 ($M^+ + H^+$), 342 (100); HRMS Calcd for $C_{24}H_{32}NO_4$ ($M^+ + H^+$): 398.2331. Found: 398.2328.

Compound **8a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.5 Hz, 1.5 Hz, 2H), 5.52 (d, *J* = 9.0 Hz, 1H), 4.42 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.38 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.85 (t, *J* = 7.0 Hz, 1H), 3.74 – 3.68 (m, 3H), 3.62 (dd, *J* = 8.5 Hz, 2.0 Hz, 1H), 1.55 (m, 1H), 1.45 (m, 1H), 1.20 (s, 9H), 0.95 (t, *J* = 7.0 Hz, 3H).

(2*S*, 3*R*)-3-tert-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-pentanoic acid (1a). To a solution of compound **9a** (450 mg, 1.133 mmol) in acetone (38 mL) was added dropwise Jones reagent (1 M, 3.4 mL) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was warmed to room temperature and stirred for another 4 h. The reaction mixture was quenched with isopropanol and stirred for 20 min. The resulting mixture was diluted with ethyl acetate and washed with water (20 mL). The aqueous layer was extracted with ethyl acetate, the

combined organic layers were washed with brine (10 mL x 2), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 20:1,Rf = 0.15) to give amino acid **1a** (422 mg, 91%) as a white solid. mp 135-137 ^oC; $[\alpha]_D^{25}$ = 29.6^o (*c* 0.945, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (dd, *J* = 7.5 Hz, 4.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 5.73 (d, *J* = 6.5 Hz, 1H), 4.46 – 4.38 (m, 3H), 4.25 (t, *J* = 7.0 Hz, 1H), 4.05 (m, 1H), 1.63 (m, 1H), 1.44 (m, 1H), 1.29 (s, 9H), 0.96 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.2, 143.8, 143.6, 141.3, 127.7, 127.0, 125.1, 120.0, 76.2, 72.2, 67.2, 56.6, 47.0, 28.3, 24.8, 10.3; IR (thin film) 3437, 3437–2360 (br), 1723, 1507, 1450 cm^{-1}; MS m/z (ESI) 434 ($M^+ + Na^+$), 412 ($M^+ + H^+$), 356 (100), 179 (60), 134 (70); HRMS Calcd for $C_{24}H_{30}NO_5$ ($M^+ + H^+$): 412.2124. Found: 412.2118.

(4*R*)-4-((1*R*)-1-Hydroxy-2-methyl-propyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*syn*-3b). To a solution of D-Garner aldehyde **2** (180 mg, 0.786 mmol) in toluene (8 mL) was added dropwise iPr_2Zn (1.0 M in toluene, 3 mL) at –78 ^oC. After being stirred at the same temperature for 10 min, the reaction was warmed to 0 ^oC and stirred for 5 h. Saturated aqueous NH_4Cl was added, the resulting mixture was extracted with diethyl ether, washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography on silica gel (Hexane/EtOAc = 5:1, R_f = 0.3) to give compound *syn*-**3b** (138 mg, 68%; ^1H NMR showed only *syn*-**3b** was formed) as a white solid. mp 84-86 ^oC (lit. 78–80 ^oC); $[\alpha]_D^{25}$ = 56.9^o (*c* 1.15, $CHCl_3$) (lit. $[\alpha]_D^{25}$ = 55.3^o (*c* 1.15 $CHCl_3$), reference 3); ^1H NMR (400 MHz, $CDCl_3$) δ 4.03 (br, 2H), 3.91 (dd, *J* = 9.2 Hz, 5.6 Hz, 1H), 3.74 (d, *J* = 9.2 Hz, 1H), 3.49 (d, *J* = 8.8 Hz, 1H), 1.64 (m, 1H), 1.61 (s, 3H), 1.48 (s, 12H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 93.8, 81.4, 78.5, 65.1, 60.6, 31.0, 28.3, 27.3, 24.3, 20.3, 14.2.

((1*R*, 2*R*)-2-Hydroxy-1-hydroxymethyl-3-methyl-butyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (4b). Compound **4b** (yield 88%) was prepared using the same procedure as for preparation of compound **4a**. mp 114-115 ^oC; $[\alpha]_D^{25}$ = -13.6^o (*c* 0.83, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.49 (d, *J* = 8.5 Hz, 1H), 4.46 (dd, *J* = 10.5 Hz, 6.5 Hz, 1H), 4.40 (dd, *J* = 10.5, 6.5 Hz, 1H), 4.20 (t, *J* = 6.5 Hz, 1H), 3.83 – 3.74 (m, 3H), 3.50 (dd, *J* = 8.0 Hz, 2.8 Hz, 1H), 2.88 (d, *J* = 3.5 Hz, 1H), 2.61 (t, *J* = 5.5 Hz, 1H), 1.70 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.7, 143.8, 141.28, 141.25, 127.6, 127.01,

126.98, 125.0, 119.9, 77.6, 66.6, 64.9, 52.4, 47.2, 30.8, 18.9, 18.8; IR (thin film) 3401 (br), 1699, 1515, 1450 cm^{-1}; MS *m/z* (ESI) 356 ($M^+ + H^+$), 179 (73), 134 (82); HRMS Calcd for $C_{21}H_{26}NO_4$ ($M^+ + H^+$): 356.1862. Found: 356.1855.

Benzoic acid (2*R*, 3*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-4-methyl-pentyl ester (5b). Compond **5b** (yield 95%) was prepared using the same procedure as for preparation of compound **5a**. $[\alpha]_D^{25}$ = 6.0° (*c* 0.725, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.5 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.59 – 7.54 (m, 3H), 7.45 – 7.37 (m, 4H), 7.29 (t, *J* = 7.0 Hz, 2H), 5.36 (d, *J* = 9.0 Hz, 1H), 4.53 (dd, *J* = 11.2 Hz, 7.5 Hz, 1H), 4.42 (d, *J* = 7.0 Hz, 2H), 4.32 (dd, *J* = 10.5 Hz, 6.5 Hz, 1H), 4.24 – 4.18 (m, 2H), 3.33 (d, *J* = 8.5 Hz, 1H), 2.82 (br, 1H), 1.77 (m, 1H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 167.0, 156.4, 143.83, 143.77, 141.3, 133.3, 129.7, 129.5, 128.4, 127.6, 127.0, 125.0, 119.9, 75.3, 66.7, 64.3, 51.1, 47.2, 30.6, 19.1, 18.9; IR (thin film) 3434 (br), 1722, 1515, 1450 cm^{-1}; MS *m/z* (ESI) 460 ($M^+ + H^+$), 238 (51); HRMS Calcd for $C_{28}H_{30}NO_5$ ($M^+ + H^+$): 460.2124. Found: 460.2105.

((1*R*, 2*R*)-2-tert-Butoxy-1-hydroxymethyl-3-methyl-butyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (9b). To a solution of compound **5b** (1.133 g, 2.468 mmol) in CH_2Cl_2 (16 mL) was added conc. H_2SO_4 (15 uL) at -10 °C, then isobutylene was bubbled through the solution for 5 min at the same temperature. The reaction was sealed and stirred for 4.5 days at room temperature. The reaction mixture was diluted with ethyl acetate, and the resulting mixture was washed with sat. aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.7) to give a mixture of compounds **6b** and **7b** (830 mg) as a foam, and recovered starting material **5b** (369 mg). The latter was treated with isobutylene using the same procedure as above. After being stirred for another 4.5 days, compounds **6b** and **7b** (247 mg) were obtained, along with starting material (105 mg). The overall yield of compounds **6b** and **7b** from this procedure was 85% (based on recovered starting material was 93%). The mixture of compounds **6b** and **7b** (830 mg, 1.612 mmol) were dissolved in CH_2Cl_2 (30 mL) and the solution was cooled to –78 °C. DIBAl-H (1.0 M in hexane, 4.8 mL, 4.8 mmol) was added dropwise, and the reaction mixture was stirred at the same temperature for 3 h, and was quenched with MeOH at –78 °C. The resulting mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc

= 4:1 R_f = 0.4) to give product **9b** (420 mg, 63%) as a foam and by product **8b** (140 mg, 21%) as an oil.

Compound **9b:** $[\alpha]_D^{25}$ = -9.8° (*c* 1.09, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.2 Hz, 2H), 7.62 (dd, *J* = 7.2 Hz, 4.4 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 2H), 5.55 (d, *J* = 8.0 Hz, 1H), 4.47 (dd, *J* = 10.4 Hz, 7.2 Hz, 1H), 4.32 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.82 (q, *J* = 7.2 Hz, 1H), 3.68 – 3.63 (m, 2H), 3.53 – 3.48 (m, 1H), 3.16 (dd, *J* = 7.2 Hz, 4.8 Hz, 1H), 1.94 (m, 1H), 1.26 (s, 9H), 0.95 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 143.84, 143.81, 141.2, 127.5, 127.0, 126.9, 125.0, 119.8, 74.0, 71.8, 66.7, 63.9, 51.4, 47.2, 32.8, 28.9, 19.0, 16.5; IR (thin film) 3436 (br), 1705, 1505, 1447 cm^{-1}; MS *m/z* (ESI) 418 ($M^+ + Li^+$), 412 ($M^+ + H^+$), 356 (100); HRMS Calcd for $C_{25}H_{33}NO_4$ ($M^+ + H^+$): 412.2488. Found: 412.2468.

Compound **8b:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.31 (td, *J* = 7.6 Hz, 1.2 Hz, 2H), 5.53 (d, *J* = 8.8 Hz, 1H), 4.42 (dd, *J* = 10.6 Hz, 7.6 Hz, 1H), 4.38 (t, *J* = 10.6 Hz, 6.8 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.90 (d, *J* = 9.6 Hz, 1H), 3.85(d, *J* = 0.8 Hz, 1H), 3.72 (dd, *J* = 9.4 Hz, 2.8 Hz, 1H), 3.58 (dd, *J* = 9.4 Hz, 2.0 Hz, 1H), 3.49 (d, *J* = 8.8 Hz, 1H), 1.70 (m, 1H), 1.19 (s, 9H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H).

(2*S*, 3*R*)-3-tert-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-4-methyl-pentanoic acid (1b). Amino acid **1b** (yield 92%) was prepared using the same procedure as for preparation of amino acid **1a**. mp 76–78 °C; $[\alpha]_D^{25}$ = 21.8° (*c* 0.805, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.78 (d, *J* = 7.5 Hz, 1H), 4.44 (m, *3*H), 4.26 (t, *J* = 7.0 Hz, 1H), 4.02 (m, 1H), 1.93 (m, 1H), 1.27 (s, 9H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.96 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.1, 143.9, 143.6, 141.3, 127.7, 127.0, 125.12, 125.09, 119.9, 74.3, 67.2, 54.8, 47.1, 28.4, 18.4; IR (thin film) 3436, 3436 – 2361 (br), 1723, 1507, 1450 cm^{-1}; MS *m/z* (ESI) 426 ($M^+ + H^+$), 370 (70), 179 (83), 148 (100); HRMS Calcd for $C_{25}H_{32}NO_5$ ($M^+ + H^+$): 426.2280. Found: 426.2282.

(4*R*)-4-((1*R*)-1-Hydroxy-prop-2-ynyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*syn*-3e). To a solution of CuI (6.30 g, 33 mmol) in THF (67 mL) and Me_2S (13.5 mL) at –78 °C was added dropwise 1-propynylmagnesium bromide (0.5 M in THF, 48 mL, 24 mmol). The resulting yellow milky solution was stirred at the same temperature for 30 min, and then stirred at –30 °C for 30 min. The reaction mixture was cooled to -78 °C, D-Garner aldehyde **2** (3.435 g, 15 mmol) in THF (30 mL) was added dropwise, and the reaction mixture was allowed

to warm to room temperature and stirred overnight. The reaction was quenched with sat. aqueous NH_4Cl and diluted with water. The aqueous phase was extracted with diethyl ether. The combined organic phase was washed with 0.5 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1, R_f = 0.2) to give compound *syn*-**3e** (3.809 g, 95%, *syn*/*anti* = 16:1, determined by ^{1}H NMR) as a sticky oil. $[\alpha]_D^{25}$ = 37.3^o (*c* 0.91, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.55 (br, 1H), 4.60 and 4.56 (rotamer) (2s, 1H), 4.05 – 4.02 (m, 1H), 3.93 (q, *J* = 6.5 Hz, 1H), 3.83 – 3.78 (m, 1H), 1.77 (s, 3H), 1.50 (s, 3H), 1.42 – 1.40 (m, 12H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 154.9, 94.5, 81.7, 81.4, 78.0, 66.0, 65.2, 62.4, 28.2, 27.0, 24.2, 3.5; IR (thin film) 3436 (br), 1698, 1475, 1394 cm^{-1}; MS *m/z* (ESI) 292 ($M^+ + Na^+$), 276 ($M^+ + Li^+$), 270 ($M^+ + H^+$), 214 (73), 156 (40); HRMS Calcd for $C_{14}H_{24}NO_4$ ($M^+ + H^+$): 270.1705. Found: 270.1715.

(4*R*)-4-((1*S*)-1-Hydroxy-prop-2-ynyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*anti*-3e). To a solution of D-Garner aldehyde **2** (100 mg, 0.437 mmol) in THF (5 mL) was added dropwise 1-propynylmagnesium bromide (0.5 M in THF, 1.4 mL) at –78 ^{o}C. The reaction was stirred at the same temperature for 30 min and then warmed to room temperatue and stirred for 4 h. The reaction was quenched with sat. aqueous NH_4Cl. The aqueous phase was extracted with diethyl ether. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1, R_f = 0.2) to give compound **3e** (84 mg, 60%, *anti*/*syn* = 9:1, determined by ^{1}H NMR) as a sticky oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.45 (br, 1H), 4.44 and 4.38 (rotamer) (2s, 1H), 4.03 – 3.75 (m, 3H), 1.80 and 1.77 (rotamer) (2s, 3H), 1.45 – 1.40 (m, 15H).

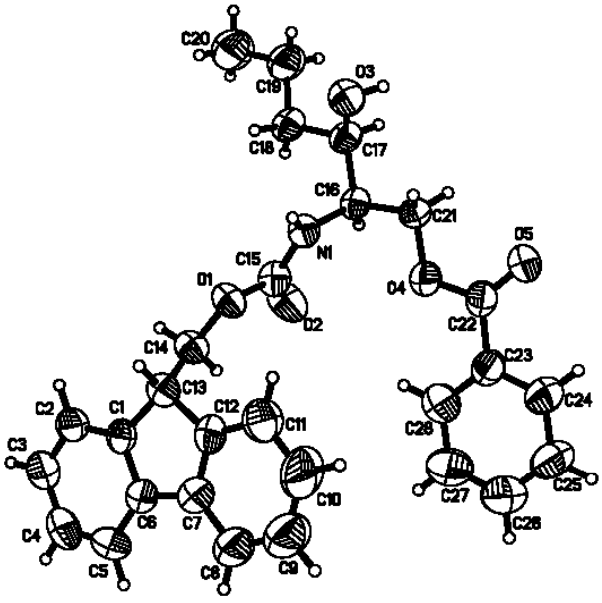
((1*R*, 2*R*)-2-Hydroxy-1-hydroxymethyl-pentyl)-carbamic acid tert-butyl ester (*syn*-3c). To a solution of compound *syn*-**3e** (1.063 g, 3.966 mmol) in MeOH (60 mL) was added $Pd(OH)_2$/C (20%, 421 mg, 0.793 mmol) at room temperature. Then H_2 was introduced into the flask. The reaction was stirred with a H_2-balloon overnight. The $Pd(OH)_2$ /C was filtered through a short cellite pad. The filtrate was concentrated to give compound *syn*-**3c** (907 mg, 98%) as a sticky yellow oil that was used for the next step without purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (d, *J* = 9.0 Hz, 1H), 3.89 (m, 1H), 3.72 – 3.54 (m, 4H), 1.49 – 1.38 (m, 13H), 0.89 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 79.5, 71.7, 64.5, 54.4, 36.1, 28.3, 18.7, 13.9.

((1*R*, 2*R*)-2-Hydroxy-1-hydroxymethyl-pentyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (4c). Compound **4c** (yield 71%) was prepared using the same procedure as for preparation

of compound **4a**. mp 94 - 95 ^{o}C; $[\alpha]_D^{25}$ = -15.7^{o} (*c* 0.76, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 5.56 (d, *J* = 8.5 Hz, 1H), 4.45 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.39 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.21 (t, *J* = 7.0 Hz, 1H), 3.92 (m, 1H), 3.77 (d, *J* = 4.5 Hz, 2H), 3.64 – 3.61 (m, 1H), 2.81 (br, 2H), 1.48 – 1.34 (m, 4H), 0.92 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.0, 143.8, 141.3, 127.7, 127.0, 125.0, 119.9, 71.9, 66.9, 64.6, 54.7, 47.2, 36.1, 18.7, 13.9; IR (thin film) 3401 (br), 3064, 1697, 1517, 1450 cm^{-1}; MS *m/z* (ESI) 356 ($M^+ + H^+$), 179 (40), 134 (53); HRMS Calcd for $C_{21}H_{26}NO_4$ ($M^+ + H^+$): 356.1862. Found: 356.1844.

Benzoic acid (2*R*, 3*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-hexyl ester (5c). Compound **5c** (yield 91%) was prepared using the same procedure as for preparation of compound **5a**. mp 111 – 113 ^{o}C; $[\alpha]_D^{25}$ = 10.8^{o} (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.5 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 – 7.55 (m, 3H), 7.44 – 7.38 (m, 4H), 7.29 (t, *J* = 7.5 Hz, 2H), 5.30 (d, *J* = 9.5 Hz, 1H), 4.55 (dd, *J* = 11.0 Hz, 7.5 Hz, 1H), 4.42 (d, *J* = 7.0 Hz, 2H), 4.32 (dd, *J* = 11.0 Hz, 6.5 Hz, 1H), 4.20 (t, *J* = 7.0 Hz, 1H), 4.01 (q, *J* = 7.5 Hz, 1H), 3.79 (m, 1H), 2.60 (br, 1H), 1.56 (m, 1H), 1.50 – 1.45 (m, 2H), 1.38 (m, 1H), 0.93 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 167.0, 156.5, 143.84, 143.80, 141.3, 133.4, 129.8, 129.5, 128.5, 127.7, 127.0, 125.0, 120.0, 69.4, 66.8, 65.5, 63.9, 53.4, 47.2, 35.7, 18.9, 13.9; IR (thin film) 3430 (br), 3054, 1720, 1602, 1517 cm^{-1}; MS *m/z* (ESI) 460 ($M^+ + H^+$), 238 (48); HRMS Calcd for $C_{28}H_{30}NO_5$ ($M^+ + H^+$): 460.2124. Found: 460.2103.

ORTEP drawing of the X-ray crystallographic structure of **5c.**



((1*R*, 2*R*)-2-*tert*-Butoxy-1-hydroxymethyl-pentyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (9c). To a solution of compound **5c** (830 m g, 1.808 mmol) in CH_2Cl_2 (12 mL) was added conc. H_2SO_4 (12 μL) at -10 ^oC, then isobutylene was bubbled through the solution for 10 min at the same temperature. The reaction was sealed and stirred for 3 days at room temperature. The reaction mixture was diluted with ethyl acetate, and the resulting mixture was washed with sat. aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.7) to give a mixture of compounds **6c** and **7c** (776 mg, yield 83%, conversion yield 94%) as a foam, and recovered starting material **5c** (95 mg). The mixture of compounds **6c** and **7c** (760 mg, 1.476 mmol) were dissolved in CH_2Cl_2 (26 mL) and the solution was cooled to –78 ^oC. DIBAl-H (1.0 M in hexane, 4.57 mL, 4.57 mmol) was added dropwise, and the reaction mixture was stirred at the same temperature for 3 h, and was quenched with MeOH at –78 ^oC. The resulting mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.4) to give product **9c** (425 mg, 70%) as a foam and by product **8c** (97 mg, 16%) as an oil.

Compound **9c**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.23 (d, *J* = 9.0 Hz, 1H), 4.44 (dd, *J* = 11.0 Hz, 7.5 Hz, 1H), 4.39 (dd, *J* = 11.0, 7.0 Hz, 1H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.79 (q, *J* = 6.5 Hz, 1H), 3.73 – 3.64 (m, 3H), 1.57 – 1.55 (m, 1H), 1.47 – 1.44 (m, 1H), 1.36 – 1.30 (m, 2H), 1.23 (s, 9H), 0.92 (t, *J* = 7.5 Hz, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.9, 143.9, 141.2, 127.6, 126.9, 125.0, 119.9, 74.1, 70.1, 66.7, 63.2, 54.2, 47.1, 35.5, 28.7, 18.9, 14.2; IR (thin film) 3436 (br), 3067, 1704, 1609, 1505 cm^{-1}; MS *m/z* (ESI) 418 ($M^+ + Li^+$), 412 (40), 410 (30); HRMS Calcd for $C_{25}H_{34}NO_4$ ($M^+ + H^+$): 412.2488. Found: 412.2492.

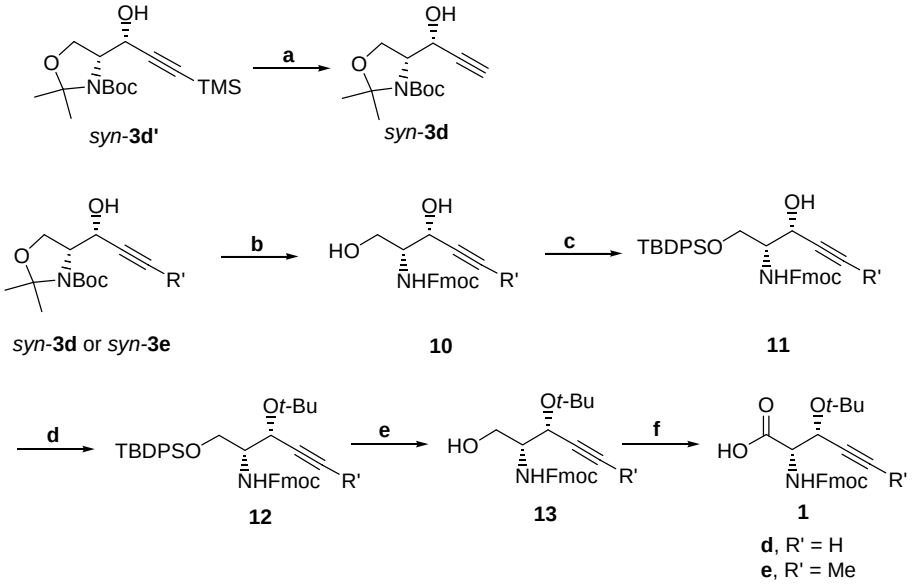
Compound **8c**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.54 (d, *J* = 8.5 Hz, 1H), 4.43 – 4.37 (m, 2H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.96 (m, 1H), 3.70 (d, *J* = 8.5 Hz, 2H), 3.64 – 3.60 (m, 2H), 1.53 (m, 1H), 1.44 – 1.39 (m, 3H), 1.20 (s, 9H), 0.94 (t, *J* = 7.5 Hz, 3H).

(2*S*, 3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid (1c). Amino acid **1c** (yield 93%) was prepared using the same procedure as for preparation of amino acid **1a.** mp 148-150 ^oC; $[\alpha]_D^{25}$ = 47.8^o (*c* 0.80, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (dd, *J* = 7.0 Hz, 5.0 Hz, 2H), 7.41 (td, *J* = 7.5 Hz, 2.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 5.73 (d, *J* = 6.5 Hz, 1H), 4.43 – 4.40 (m, 3H), 4.25 (t, *J* = 7.0 Hz, 1H), 4.13 (m, 1H),

1.55 (m, 1H), 1.46 (m, 1H), 1.33 – 1.28 (m, 11H), 0.93 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.2, 143.8, 143.6, 141.3, 127.7, 127.1, 125.1, 120.0, 70.7, 67.2, 56.9, 47.0, 33.7, 28.3, 19.1, 14.0; IR (thin film) 3437, 3437–2360 (br), 1724, 1512, 1451 cm^{-1}; MS *m/z* (ESI) 426 ($M^+ + H^+$), 370 (100), 179 (31), 148 (45); HRMS Calcd for $C_{25}H_{32}NO_5$ ($M^+ + H^+$): 426.2280. Found: 426.2277.

Synthesis of Amino Acids 1d and 1e.

Scheme 2.[a]

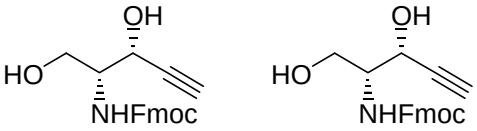


[a](a) TBAF, THF, 0 °C, 100%. (b) (1) 4 N HCl-dioxane, MeOH, (2) FmocCl, $NaHCO_3$, THF/H_2O (2:1), **10d**, 100%; **10e**, 83%. (c) TBDPSCl, imidazole, CH_2Cl_2, 0 °C, **11d**, 86%; **11e**, 80%. (d) isobutylene, conc. H_2SO_4, CH_2Cl_2, **12d**, 69%, (98%); **12e**, 64%, (94%). (e) HF·pyr., THF, rt., **13d**, 97%; **13e**, 95%. (f) Jones reagent, acetone, 0 °C to rt., **1d**, 93%; **1e**, 93%. **NOTE**: The enantiomers **10d'** and **10e'** were also prepared using the same methodology such that racemic mixtures of these compounds were available as standards for chrial SFC.

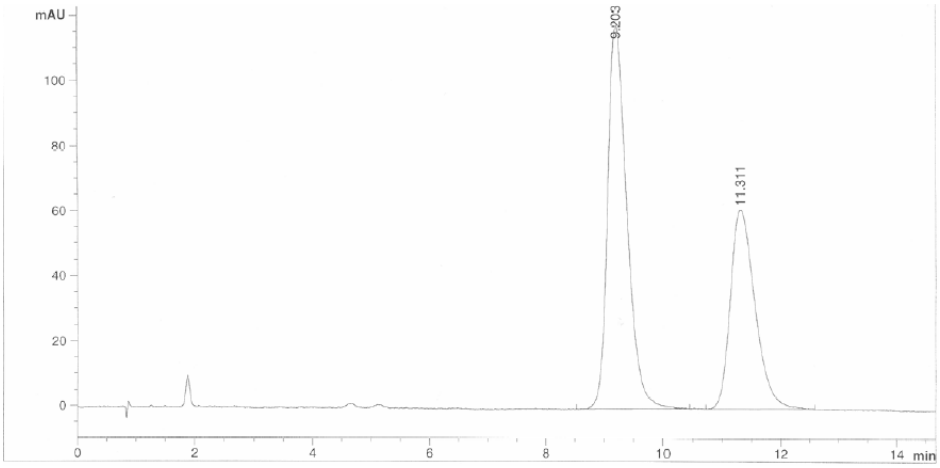
Supercritical Fluid Chromatography (SFC) to Determine the Stereochemical Purity of Compounds 10d-e.

SFC conditions for diols **4b**

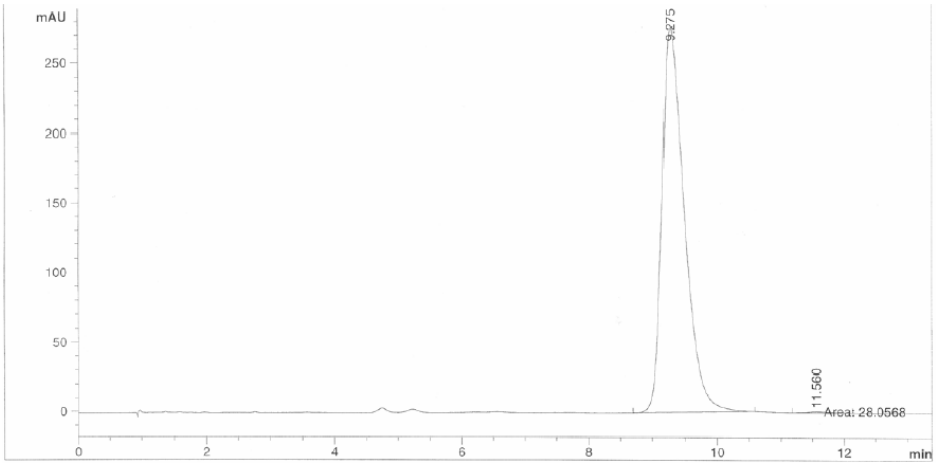
Chiralpak AD column (Daicel chemical industries, 25 cm x 4.6 mm I.D.), 125 psi, 3 mL/min, 40 °C, 220 nm. Mobile phase: CO_2/15% MeOH.



SFC for a mixture of **10d** and **10d′** (**10d**, Rt = 9.20 min; **10d′**, Rt = 11.31 min).



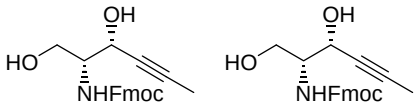
SFC for diol **10d** (**10d**, Rt = 9.27 min; **10d′**, Rt = 11.56 min)



From this data, the e.e. of diol **10d is** determined to be 99.1 %.

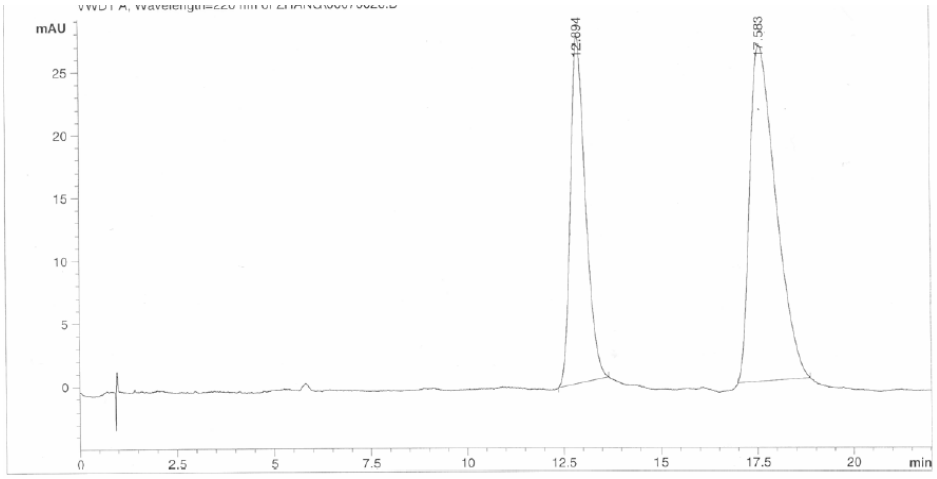
Conditions for SFC with compounds 10e and 10e':

Chiralpak AD column (Daicel chemical industries, 25 cm x 4.6 mm I.D.), 125 psi, 3 mL/min, 40 ^{o}C. Mobile phase: CO_2/13% MeOH.

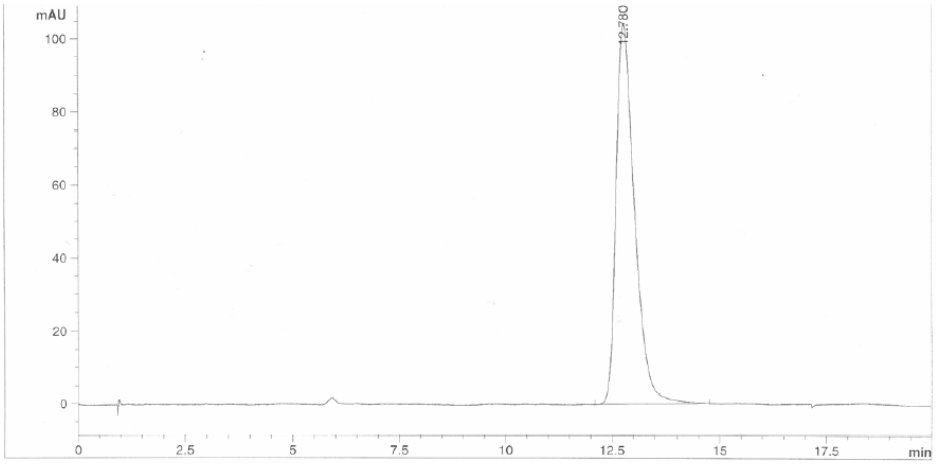


10e **10e'**

SFC of a mixture of **10e** and **10e′** (**10e**, Rt = 12.89 min; **10e′** = 17.58 min)



The SFC for diol **10e** (**10e**, Rt = 12.78 min)



No enantiomer **10e′** was detected by SFC in a sample of **10e** determined as shown in Table 1 and Scheme 2. The e.r. of **10e** is therefore estimated as > 99:1.

Experimental Procedures and Spectroscopic Characterization of Intermediates.

(4*R*)-4-((1*R*)-1-Hydroxy-3-trimethylsilanyl-prop-2-ynyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*syn*-3d′). EtMgBr (1.0 M in THF, 3 mL, 3 mmol) was added dropwise to a solution of (ethynyl)trimethylsilane (452 μL, 3.2 mmol) in THF (9 mL) at 0 °C. Then the resulting solution was heated to reflux and stirred for 1 h. The resulting Grignard compound was cooled to room temperature and transferred via canula to a solution of CuI (838 mg, 4.4 mmol) in THF (9 mL) and Me_2S (1.8 mL) at –78 °C over a period of 10 min at which time the solution turned into a milky yellow liquid. The resulting mixture was warmed to -30 °C and stirred for 30 min. Then the mixture was cooled to - 78 °C, and a solution of D-Garner aldehyde **2** (458 mg, 2 mmol) in THF (2 mL) was added. The mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was quenched with satd. NH_4Cl, and diluted with water. The aqueous layer was extracted with diethyl ether, the combined organic layers were washed with 0.5 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1 R_f = 0.2) to give a sticky oil *syn*-**3d′** (537 mg, 82%, *syn*/*anti* = 20:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.77 and 5.74 (rotamer) (2d, *J* = 5.0 Hz, 1H), 4.70 and 4.65 (rotamer) (2t, *J* = 4.5 Hz, 1H), 4.03 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 3.96 (m, 1H), 3.80 (m, 1H), 1.52 (s, 3H), 1.44 – 1.39 (m, 12H), 0.12 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 155.1, 104.3, 94.7, 90.5, 81.7, 66.6, 65.3, 62.3, 28.3, 27.2, 24.3, 0.30.

(4*R*)-4-((1*R*)-1-Hydroxy-prop-2-ynyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (*syn*-3d). To a solution of *syn*-**3d′** (4.62 g, 14.128 mmol) in THF (180 mL) was added dropwise TBAF (1.0 M in THF, 18.6 mL, 18.6 mmol) at 0 °C. After stirring for 15 min, the reaction was quenched with 0.5 N HCl, and the mixture was extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to give product *syn*-**3d** (3.6 g, 100%) as a white solid, which was used without further purification. mp 92 - 93 °C (lit.[1] 92.5 – 93.5 °C); $[\alpha]_D^{25}$ = 45.7° (c 1.02, $CHCl_3$) (lit. enantiomer,[1] $[\alpha]_D^{25}$ = -50.8° (c 0.945, $CHCl_3$)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.78 and 5.74 (rotamer) (2d, *J* = 4.8 Hz, 1H), 4.65 - 4.60 (m,1H), 4.05 – 4.03 (m, 1H), 3.98 – 3.93 (m, 1H), 3.85 – 3.80 (m, 1H), 3.25 and 3.23 (2s, 1H), 1.52 (s, 3H), 1.45 – 1.41 (m, 12H).

((1*R*, 2*R*)-2-Hydroxy-1-hydroxymethyl-but-3-ynyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (10d). Compound **10d** (yield 100%) was prepared using the same procedure as for preparation of compound **4a**. mp 116 - 118 ^{o}C; $[\alpha]_D^{25}$ = -2.7^{o} (*c* 1.075, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.64 (m, 2H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 8.0 Hz, 2H), 4.52 (m, 1H), 4.38 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.28 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.20 (t, *J* = 7.0 Hz, 1H), 3.80 – 3.74 (m, 2H), 3.66 (dd, *J* = 10.0 Hz, 6.0 Hz, 1H), 2.81 (d, *J* = 1.5 Hz, 1H); ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.9, 145.30, 145.27, 142.6, 128.7, 128.2, 126.3, 126.2, 120.9, 83.7, 79.4, 75.0, 67.9, 62.0, 61.7, 58.8; IR (thin film) 3400 (br), 2118, 1697, 1520, 1450 cm^{-1}; MS *m/z* (ESI) 338 ($M^+ + H^+$), 179 (100), 116 (83); HRMS Calcd for $C_{20}H_{20}NO_4$ ($M^+ + H^+$): 338.1392. Found: 338.1393.

(1*R*, 2*R*)-[1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-hydroxy-but-3-ynyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (11d). To a solution of compound **10d** (2.038 g, 6.047 mmol) in CH_2Cl_2 (150 mL) was added TBDPSCl (1.50 mL, 5.745 mmol) at room temperature, followed by imidazole (1.22 g, 18.14 mmol). After being stirred for 40 min, the reaction mixture was quenched with water and diluted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 2:1, R_f = 0.6) to give compound **11d** (2.98 g, 86%) as a foam. $[\alpha]_D^{25}$ = -5.6^{o} (*c* 0.785, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.0 Hz, 4H), 7.60 (t, *J* = 8.0 Hz, 2H), 7.47 – 7.40 (m, 8H), 7.30 (t, *J* = 7.0 Hz, 2H), 5.35 (d, *J* = 9.0 Hz, 1H), 4.76 (s, 1H), 4.44 (dd, *J* = 9.5 Hz, 7.5 Hz, 1H), 4.36 (dd, *J* = 9.5 Hz, 7.5 Hz, 1H), 4.24 (t, *J* = 6.5 Hz, 1H), 4.04 (m,1H), 3.94 (m, 1H), 3.87 (dd, *J* = 9.5 Hz, 6.0 Hz, 1H), 3.26 (br, 1H), 2.46 (s, 1H), 1.11 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 143.9, 143.7, 141.3, 135.5, 132.5, 132.4, 130.0, 127.8, 127.7, 127.0, 125.1, 125.0, 119.9, 82.0, 74.4, 67.1, 63.3, 63.1, 55.9, 47.0, 26.8, 19.1; IR (thin film) 3430 (br), 3305, 2118, 1707, 1512, 1450 cm^{-1}; MS m/z (ESI) 598 ($M^+ + Na^+$), 320 (10); HRMS Calcd for $C_{36}H_{38}NO_4Si$ ($M^+ + H^+$): 576.2570. Found: 576.2571.

(1*R*, 2*R*)-[2-*tert*-Butoxy-1-(*tert*-butyl-diphenyl-silanyloxymethyl)-but-3-ynyl]-carbarmic acid 9*H*-fluoren-9-ylmethyl ester (12d). Compound **12d** (yield 69%, conversion yield 98%) was prepared using the same procedure as for preparation of compound **6a**. $[\alpha]_D^{25}$ = -13.6^{o} (*c* 1.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.70 – 7.60 (m, 6H), 7.45 – 7.39 (m, 7H), 7.32 – 7.28 (m, 3H), 5.18 (d, *J* = 9.0 Hz, 1H), 4.60 (s, 1H), 4.41 (dd, *J* = 10.5 Hz,

7.5 Hz, 1H), 4.35 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.25 (t, *J* = 7.5 Hz, 1H), 3.96 (m, 1H), 3.84 – 3.76 (m, 2H), 2.34(s, 1H), 1.26 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.2, 144.0, 143.9, 141.3, 135.6, 133.2, 133.0, 129.8, 127.8, 127.7, 127.6, 127.0, 125.2, 125.1, 119.9, 83.7, 75.6, 73.2, 66.8, 61.8, 60.3, 56.7, 47.2, 28.0, 26.8, 19.2; IR (thin film) 3430 (br) 3441, 3305, 3069, 1724, 1506 cm^{-1}; MS *m/z* (ESI) 654 ($M^+ + Na^+$), 576 (10); HRMS Calcd for $C_{40}H_{46}NO_4Si$ ($M^+ + H^+$): 632.3196. Found: 632.3201.

(1*R*, 2*R*)-(2-*tert*-Butoxy-1-hydroxymethyl-but-3-ynyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (13d). To a solution of commercial HF·pyridine (4.52 mL) in THF (60 mL) was added pyridine (9.04 mL). A solution of compound 12d (1.9 g, 3.011 mmol) in THF (6 mL) was then added at 0 ^{o}C, and the resulting mixture was stirred at room temperature. After being stirred for 7 h, the reaction was diluted with ethyl acetate, and neutralized with sat. aqueous $NaHCO_3$. The aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with 1 N HCl, sat. aqueous $NaHCO_3$, and brine subsequently, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 2:1, R_f = 0.2) to compound 13d (1.15 g, 97%) as a foam. $[\alpha]_D^{25}$ = -2.7^o (*c* 1.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (t, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.0 Hz, 2H), 5.34 (d, *J* = 8.0 Hz, 1H), 4.49 (s, 1H), 4.45 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.38 (dd, *J* = 10.5, 6.5 Hz, 1H), 4.25 (t, *J* = 6.5 Hz, 1H), 3.90 – 3.78 (m, 3H), 2.46 (t, *J* = 6.5 Hz, 1H), 2.42 (s, 1H), 1.28 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.4, 143.82, 143.78, 141.3, 127.7, 127.0, 125.12, 125.07, 120.0, 83.2, 76.2, 74.0, 66.9, 62.0, 61.8, 55.8, 47.1, 27.9; IR (thin film) 3435 (br), 3303, 1708, 1510 cm^{-1}; MS *m/z* (ESI) 394 ($M^+ + H^+$), 338 (100); HRMS Calcd for $C_{24}H_{28}NO_4$ ($M^+ + H^+$): 394.2018. Found: 394.1999.

(2*S*, 3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxy carbonylamino)-pent-4-ynoic acid (1d). Amino acid **1d** (yield 93%) was prepared using the same procedure as for preparation of compound **1a**. mp 132-135 ^{o}C; $[\alpha]_D^{25}$ = -5.3^o (*c* 0.825, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.66 (m, 2H), 7.37 (t, *J* = 7.0 Hz, 2H), 7.29 (t, *J* = 7.0 Hz, 2H), 4.80 (s, 1H), 4.40 – 4.36 (m, 2H), 4.28 – 4.21 (m, 2H), 2.78 (s, 1H), 1.24 (s, 9H); ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.8, 145.30, 145.25, 142.6, 128.8, 128.2, 126.4, 126.3, 120.9, 76.9, 75.2, 68.3, 64.0, 32.7, 28.5, 23.7, 14.4; IR (thin film) 3435–2977 (br), 3435, 3303, 1715, 1604, 1514 cm^{-1}; MS *m/z* (ESI) 430 ($M^+ + Na^+$), 408 ($M^+ + H^+$), 352 (80), 179 (100), 130 (81); HRMS Calcd for $C_{24}H_{26}NO_5$ ($M^+ + H^+$): 408.1811. Found: 408.1829.

((1R, 2R)-2-Hydroxy-1-hydroxymethyl-pent-3-ynyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (10e). Compound **10e** (yield 83%) was prepared using the same procedure as for preparation of compound **4a**. mp 122–123 ^{o}C; $[\alpha]_D^{25}$ = 3.7^{o} (c 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 7.76 (d, J = 7.5 Hz, 2H), 7.65 (t, J = 7.5 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.28 (t, J = 7.5 Hz, 2H), 4.46 (m, 1H), 4.39 (dd, J = 10.5 Hz, 7.0 Hz, 1H), 4.26 (dd, J = 10.5, 7.0 Hz, 1H), 4.20 (t, J = 6.5 Hz, 1H), 3.77 – 3.72 (m, 2H), 3.66 – 3.62 (m, 1H), 1.77 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.9, 145.33, 145.24, 142.56, 142.54, 128.7, 128.1, 126.3, 126.2, 120.9, 82.4, 78.9, 67.9, 62.5, 61.9, 59.1, 13.9, 3.2; IR (thin film) 3400 (br), 2237, 1698, 1519, 1450 cm^{-1}; MS m/z (ESI) 374 ($M^+ + Na^+$), 352 ($M^+ + H^+$), 179 (100), 130 (90); HRMS Calcd for $C_{21}H_{22}NO_4$ ($M^+ + H^+$): 352.1549. Found: 352.1546.

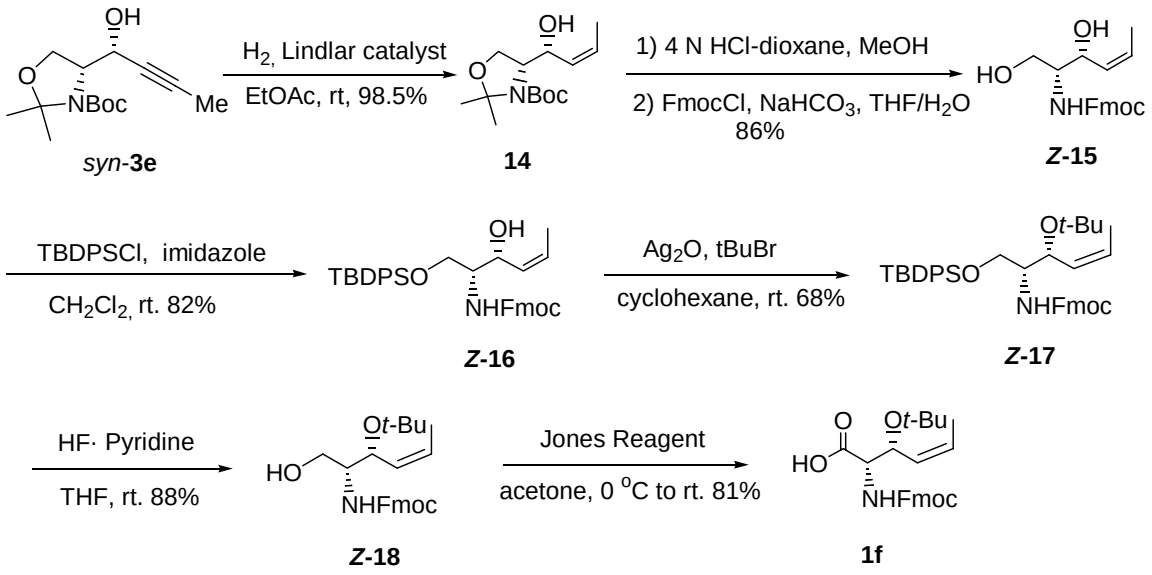
(1*R*, 2*R*)-[1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-hydroxy-pent-3-ynyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (11e). Compound **11e** (yield 80%) was prepared using the same procedure as for preparation of compound **11d**. mp 154 – 156 ^{o}C; $[\alpha]_D^{25}$ = -3.9^{o} (*c* 0.76, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.67 (d, *J* = 6.5 Hz, 4H), 7.59 (t, *J* = 8.0 Hz, 2H), 7.45 – 7.36 (m, 8H), 7.30 (t, *J* = 7.5 Hz, 2H), 5.29 (d, *J* = 8.5 Hz, 1H), 4.69 (s, 1H), 4.42 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.32 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.24 (t, *J* = 6.5 Hz, 1H), 3.93 – 3.91 (m, 2H), 3.83 (m, 1H), 1.18 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 143.9, 143.8, 141.26, 141.24, 135.5, 132.6, 132.5, 130.0, 127.9, 127.7, 127.0, 125.1, 125.0, 119.9, 82.6, 77.4, 67.0, 63.6, 56.2, 47.1, 26.8, 19.2, 3.6; IR (thin film) 3435 (br), 3071, 2248, 1710, 1513, 1450 cm^{-1}; MS *m/z* (ESI) 590 ($M^+ + H^+$), 512 (40), 179 (16); HRMS Calcd for $C_{37}H_{40}NO_4Si$ ($M^+ + H^+$): 590.2727. Found: 590.2730.

(1*R*, 2*R*)-[2-*tert*-Butoxy-1-(*tert*-butyl-diphenyl-silanyloxymethyl)-pent-3-ynyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (12e). Compound **12e** (yield 64%, conversion yield 94%) was prepared using the same procedure as for preparation of compound **6a**. $[\alpha]_D^{25}$ = -12.7^{o} (*c* 1.09, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.68 (m, 4H), 7.63 (dd, *J* = 12.0 Hz, 7.5 Hz, 2H), 7.44 – 7.37 (m, 8H), 7.32 – 7.29 (m, 2H), 5.14 (d, *J* = 9.0 Hz, 1H), 4.54 (m, 1H), 4.41 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.33 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.26 (t, *J* = 7.5 Hz, 1H), 3.93 – 3.91 (m, 1H), 3.81 (m, 2H), 1.78 (s, 3H), 1.25 (s, 9H), 1.10 (s, 9H); IR (thin film) 3442 (br) 3071, 1726, 1504 cm^{-1}; MS *m/z* (ESI) 668 ($M^+ + Na^+$), 646 ($M^+ + H^+$), 590 (62); HRMS Calcd for $C_{41}H_{48}NO_4Si$ ($M^+ + H^+$): 646.3353. Found: 646.3362.

(1*R*, 2*R*)-(2-*tert*-Butoxy-1-hydroxymethyl-pent-3-ynyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (13e). Compound **13e** (yield 95%) was prepared using the same procedure as for preparation of compound **13d**. $[\alpha]_D^{25} = 5.2^o$ (*c* 0.69, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (td, *J* = 7.5 Hz, 1.0 Hz, 2H), 5.36 (d, *J* = 7.0 Hz, 1H), 4.44 (m, 2H), 4.37 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.86 (t, *J* = 7.0 Hz, 1H), 3.80 – 3.78 (m, 2H), 2.79 (br, 1H), 1.82 (s, 3H), 1.31 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.4, 143.86, 143.82, 141.3, 127.6, 127.0, 125.09, 125.05, 119.9, 82.1, 78.2, 75.7, 66.8, 62.4, 62.3, 55.9, 47.1, 28.0, 3.6; IR (thin film) 3436 (br), 2232, 1709, 1510 cm^{-1}; MS *m/z* (ESI) 408 ($M^+ + H^+$), 407 (M^+), 352 (100); HRMS Calcd for $C_{25}H_{30}NO_4$ ($M^+ + H^+$): 408.2175. Found: 408.2179.

(2*S*, 3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hex-4-ynoic acid (1e). Compound **1e** (yield 93%) was prepared using the same procedure as for preparation of compound **1a**. mp 105-107 ^oC; $[\alpha]_D^{25} = 54.0^o$ (*c* 0.81, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 5.78 (d, *J* = 7.0 Hz, 1H), 4.77 (s, 1H), 4.46 (dd, *J* = 7.5 Hz, 4.0 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 2H), 4.26 (t, *J* = 7.5 Hz, 1H), 1.82 (s, 3H), 1.33 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.2, 143.74, 143.67, 141.3, 127.7, 127.1, 125.2, 120.0, 83.0, 77.4, 75.9, 67.4, 62.2, 58.7, 47.0, 27.9, 3.6; IR (thin film) 3441–2977 (br), 3441, 2232, 1726, 1513 cm^{-1}; MS *m/z* (ESI) 444 ($M^+ + Na^+$), 422 ($M^+ + H^+$), 366 (100), 179 (30), 144 (52); HRMS Calcd for $C_{25}H_{28}NO_5$ ($M^+ + H^+$): 422.1967. Found: 422.1972.

Synthesis of Amino Acid 1f.



(Z)-(4*R*)-4-((1*S*)-1-Hydroxy-but-2-enyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (14). To a solution of compound *syn*-**3e** (1.325 g, 4.926 mmol) in ethyl acetate (27 mL) was added Lindlar catalyst (622 mg) at room temperature. Then H_2 was introduced into the flask. The reaction was stirred with a H_2-balloon for 40 min. The Lindlar catalyst was filtered through a short cellite pad. The filtrate was concentrated to give compound **14** (1.315 g, 98.5%) as a clear oil that was used for the next step without purification. $[\alpha]_D^{25}$ = 49.0° (*c* 0.81, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.70 – 5.64 (m, 1H), 5.37 (t, *J* = 9.8 Hz, 1H), 4.63 (t, *J* = 8.5 Hz, 1H), 3.98 (m, 1H), 3.87 (m, 1H), 3.78 (m, 1H), 1.67 (dd, *J* = 7 Hz, 1.5 Hz, 3H), 1.55 (s, 3H), 1.47 – 1.45 (m, 12H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 154.9, 130.1, 128.6, 94.2, 81.3, 69.6, 64.5, 62.2, 28.3, 27.0, 24.1, 13.4; IR (thin film) 3436 (br), 1694, 1479, 1456, 1392 cm^{-1}; MS *m/z* (ESI) 294 ($M^+ + Na^+$); HRMS Calcd for $C_{14}H_{25}NO_4Na$ ($M^+ + Na^+$): 294.1681. Found: 294.1687.

(*Z*)-((1R, 2R)-2-Hydroxy-1-hydroxymethyl-pent-3-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*Z*-15). Compound *Z*-**15** (yield 86%) was prepared using the same procedure as for preparation of compound **4a**. $[\alpha]_D^{25}$ = -29.8° (*c* 1.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 5.65 – 5.59 (m, 2H), 5.46 (t, *J* = 9.0 Hz, 1H), 4.74 (m, 1H), 4.44 – 4.35 (m, 2H), 4.19 (t, *J* = 7.0 Hz, 1H), 3.74 – 3.67 (m, 3H), 3.27 – 3.21 (br, 2H), 1.66 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.1, 143.7, 141.22, 141.21, 129.2, 128.4, 127.6, 127.0, 125.0, 119.9,

67.1, 66.7, 63.3, 56.2, 47.1, 13.4; IR (thin film) 3401, 1698, 1517 cm^{-1}; MS *m/z* (ESI) 376 (M^+ + Na^+); HRMS Calcd for $C_{21}H_{24}NO_4$ (M^+ + H^+): 354.1705. Found: 354.1718.

(*Z*)-(1*R*, 2*R*)-[1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-hydroxy-pent-3-enyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*Z*-16). Compound *Z*-**16** (yield 82%) was prepared using the same procedure as for preparation of compound **11d**. $[\alpha]_D^{25}$ = -28.4° (*c* 0.74, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.0 Hz, 2H), 7.66 (d, *J* = 7.0 Hz, 4H), 7.61 (t, *J* = 7.0 Hz, 2H), 7.46 – 7.29 (m, 10H), 5.71 – 5.64 (m, 1H), 5.50 (td, *J* = 8.5 Hz, 1.5 Hz, 1H), 5.41 (d, *J* = 8.5 Hz, 1H), 4.87 (dd, *J* = 8.5 Hz, 3 Hz, 1H), 4.42 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.33 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.85 – 3.81 (m, 2H), 3.75 – 3.73 (m, 1H), 1.72 (d, *J* = 7.0 Hz, 3H), 1.10 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.5, 143.9, 143.8, 141.3, 135.56, 135.52, 132.6, 132.5, 130.00, 129.98, 129.5, 128.2, 127.88, 127.86, 127.7, 127.0, 125.15, 125.06, 120.0, 67.2, 66.9, 64.6, 55.9, 47.1, 26.8, 19.1, 13.5; IR (thin film) 3436 (br), 1704, 1589, 1511 cm^{-1}; MS *m/z* (ESI) 614 (M^+ + Na^+); HRMS Calcd for $C_{37}H_{42}NO_4Si$ (M^+ + H^+): 592.2883. Found: 592.2911.

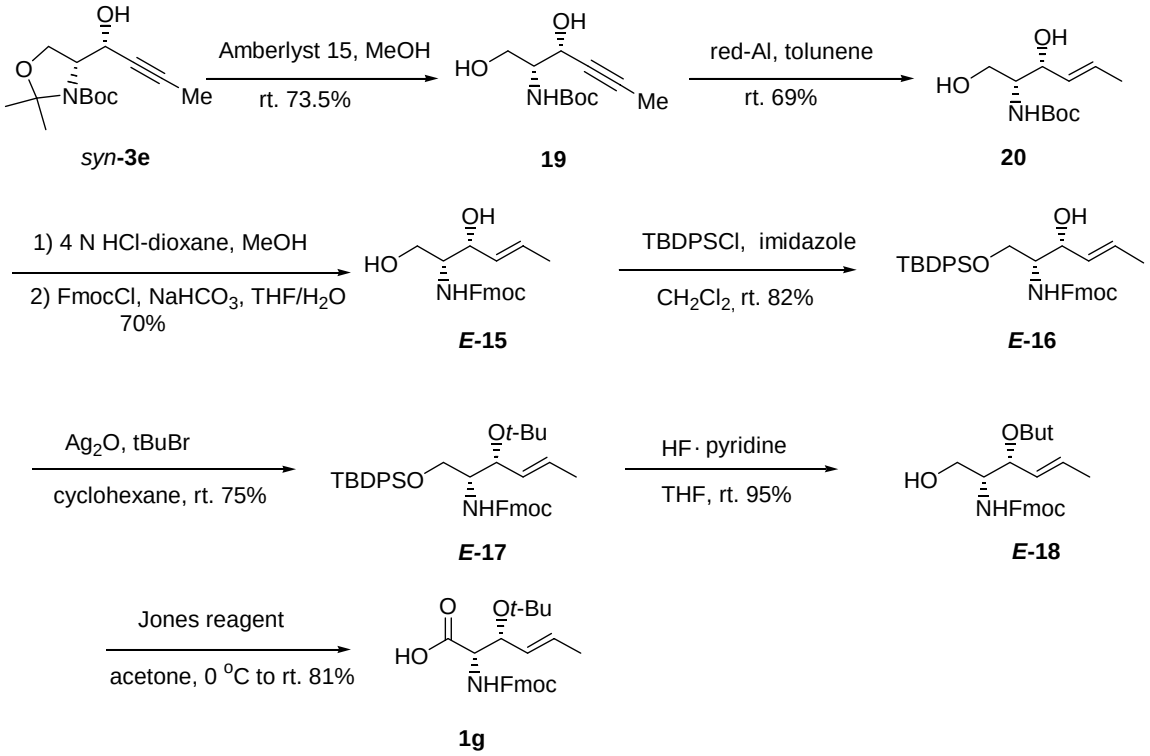
(*Z*)-(1*R*, 2*R*)-[2-*tert*-Butoxy-1-(*tert*-butyl-diphenyl-silanyloxymethyl)-pent-3-enyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*Z*-17). To a solution of compound *Z*-**16** (630 mg, 1.064 mmol) in cyclohexane (32 mL) was added Ag_2O (491mg, 2.138 mmol), followed by t-BuBr (362 μL, 3.192 mmol) at room temperature. The resulting suspension was stirred for 8 h at room temperature. After removal of solid and concentration, the residue was redissoved in cyclohexane (32 mL) and treated with Ag_2O (491 mg, 2.138 mmol) and *tert*-BuBr (362 μL, 3.192 mmol). The reaction mixture was stirred overnight. The solid was filtered, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 8:1, R_f = 0.3) to give compound *Z*-**17** (466 mg, 68%) as a foam, and recovered compound *Z*-**16** (166 mg, conversion 74%). $[\alpha]_D^{25}$ = -20.7° (*c* 0.21, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.68 (d, *J* = 7.5 Hz, 4H), 7.58 – 7.57 (m, 2H), 7.44 – 7.36 (m, 7H), 7.30 – 7.18 (m, 3H), 5.52 – 5.46 (m, 2H), 5.12 (d, *J* = 8.5 Hz, 1H), 4.74 (dd, *J* = 8.0 Hz, 3.0 Hz, 1H), 4.37 – 4.29 (m, 2H), 4.20 (t, *J* = 7.5 Hz, 1H), 3.75 – 3.64 (m, 3H), 1.69 (d, *J* = 5.5 Hz, 3H), 1.16 (s, 9H), 1.08 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.1, 144.0, 141.2, 135.6, 135.57, 135.2, 133.4, 133.2, 133.0, 129.7, 128.0, 127.8, 127.7, 127.66, 127.59, 127.0, 125.1, 124.2, 119.9, 74.0, 66.6, 65.4, 65.0, 62.3, 57.2, 47.2, 28.7, 26.9, 19.1, 13.3; IR (thin film) 3440, 1722,1609, 1589 cm^-

^{1}; MS *m/z* (ESI) 670 ($M^+ + Na^+$), 648 ($M^+ + H^+$), 574 (50); HRMS Calcd for $C_{41}H_{50}NO_4Si$ ($M^+ + H^+$): 648.3509. Found: 648.3500.

(*Z*)-(1*R*, 2*R*)-(2-*tert*-Butoxy-1-hydroxymethyl-pent-3-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*Z*-18). Compound *Z*-**18** (yield 88%) was prepared using the same procedure as for preparation of compound **13d**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.55 – 5.45 (m, 2H), 5.22 (br, 1H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.44 – 4.39 (m, 2H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.71 (s, 3H), 2.90 (s, 1H), 1.66 (d, *J* = 5.5 Hz, 3H), 1.21 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 143.9, 141.3, 131.6, 127.6, 127.0, 125.5, 125.0, 124.99, 119.9, 74.8, 67.6, 66.7, 63.2, 57.5, 56.6, 47.1, 28.5, 13.4; IR (thin film) 3436 (br), 1726, 1511 cm^{-1}; MS *m/z* (ESI) 432 ($M^+ + Na^+$), 410 ($M^+ + H^+$), 336 (50), 158 (100); HRMS Calcd for $C_{25}H_{32}NO_4$ ($M^+ + H^+$): 410.2331. Found: 410.2339.

(*Z*)-(2*S*, 3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hex-4-enoic acid (1f). Compound **1f** (yield 81%, foam) was prepared using the same procedure as for preparation of compound **1a**. $[\alpha]_D^{25}$ = 29.8° (*c* 1.14, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (t, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.70 – 5.62 (m, 2H), 5.34 (td, *J* = 10.5 Hz, 1.5 Hz, 1H), 5.00 (dd, *J* = 9.0 Hz, 4.0 Hz, 1H), 4.44 – 4.40 (m, 3H), 4.23 (t, *J* = 7.0 Hz, 1H), 1.63 (dd, *J* = 7.0 Hz, 1.5 Hz, 3H), 1.28 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.1, 143.7, 141.3, 127.7, 127.08, 127.06, 125.1, 125.0, 120.0, 67.2, 66.7, 58.2, 47.0, 28.2, 13.4; IR (thin film) 3431-2361 (br), 3431, 1738, 1732, 1478 cm^{-1}; MS *m/z* (ESI) 446 ($M^+ + Na^+$), 172 (100); HRMS Calcd for $C_{25}H_{30}NO_5$ ($M^+ + H^+$): 424.2124. Found: 424.2134.

Synthesis of Amino Acid 1g.



((1*R*, 2*R*)-2-Hydroxyl-1-hydroxymethyl-pent-3-ynyl)-carbamic acid *tert*-butyl ester (19). To a solution of compound *syn*-**3e** (1.53 g, 5.688 mmol) in MeOH (68 mL) was added Amberlyst 15 (192 mg) at room temperature. The resulting suspension was stirred overnight. After removal of the beads, the filtrate was concentrated to give compound **19** (956 mg, 73.5%) as a sticky oil which was used to do the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24 (d, *J* = 8.5 Hz, 1H), 4.57 (s, 1H), 3.83–3.77 (m, 3H), 3.42 (br, 2H), 1.84 (d, *J* = 2.0 Hz, 3H), 1.44 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.5, 82.6, 80.0, 65.4, 63.3, 62.5, 55.7, 28.2, 3.58; IR (thin film) 3390 (br), 2239, 1694 cm^{-1}; MS *m/z* (ESI) 252 ($M^+ + Na^+$), 230 ($M^+ + H^+$), 174 (100); HRMS Calcd for $C_{11}H_{20}NO_4$ ($M^+ + H^+$): 230.1392. Found: 230.1394.

(*E*)-((1*R*, 2*R*)-2-Hydroxyl-1-hydroxymethyl-pent-3-enyl)-carbamic acid *tert*-butyl ester (20). To a solution of red-Al (>65% wt% in toluene, 6.0 mL, 19.12 mmol) in toluene (6.0 mL) was added a solution of compound **19** (878 mg, 3.83 mmol) in toluene (6.0 mL) at 0 °C. The resulting mixture was stirred at room temperature for 2 days. The reaction was quenched with MeOH (3 mL) at 0 °C, then saturated aqueous potassium sodium tartrate was added. After being stirred at room temperature for 3 h, the reaction mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (Hexane/EtOAc = 1:2, R_f = 0.3) to give compound **20** (612 mg,

69%) as a foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74 – 5.69 (m, 1H), 5.47 (ddd, *J* = 15.0 Hz, 6.5 Hz, 1.5 Hz, 1H), 5.30 (d, *J* = 8.5 Hz, 1H), 4.29 (br, 1H), 3.81 (m, 1H), 3.71 (d, *J* = 4.0 Hz, 1H), 3.67 (t, *J* = 5.5 Hz, 2 H), 3.56 (m, 1H), 1.66 (d, *J* = 6.5 Hz, 3H), 1.39 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 130.3, 128.1, 79.6, 72.2, 63.3, 55.4, 28.2, 17.7; IR (thin film) 3392 (br), 1690, 1506 cm^{-1}; MS *m/z* (ESI) 254 ($M^+ + Na^+$), 232 ($M^+ + H^+$); HRMS Calcd for $C_{11}H_{22}NO_4$ ($M^+ + H^+$): 232.1549. Found: 232.1545.

(*E*)-((1R, 2R)-2-Hydroxy-1-hydroxymethyl-pent-3-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*E*-15). Compound ***E*-15** (yield 70%) was prepared using the same procedure as for preparation of compound **4a**. $[\alpha]_D^{25}$ = -9.0^o (*c* 0.81, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.79 – 5.73 (m, 1H), 5.54 – 5.50 (m, 2H), 4.43 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.38 – 4.35 (m, 2H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.78 (d, *J* = 3.5 Hz, 2H), 3.68 (m, 1H), 2.74 (s, 2H), 1.68 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.0, 143.7, 141.3, 130.0, 128.8, 127.7, 127.0, 125.0, 120.0, 72.7, 66.8, 63.7, 55.8, 47.1, 17.7; IR (thin film) 3402 (br), 1694, 1519 cm^{-1}; MS *m/z* (ESI) 376 ($M^+ + Na^+$), 354 ($M^+ + H^+$), 158 (100); HRMS Calcd for $C_{21}H_{24}NO_4$ ($M^+ + H^+$): 354.1705. Found: 354.1698.

(*E*)-(1*R*, 2*R*)-[1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-hydroxy-pent-3-enyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*E*-16). Compound ***E*-16** (yield 82%) was prepared using the same procedure as for preparation of compound **11d**. $[\alpha]_D^{25}$ = -12.1^o (*c* 0.66, $CHCl_3$);^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.67–7.64 (m, 4H), 7.61 (t, *J* = 7.5 Hz, 2H), 7.45–7.30 (m, 10H), 5.83–5.76 (m, 1H), 5.52 (dd, *J* = 15.5 Hz, 5.5 Hz, 1H), 5.42 (d, *J* = 9.0 Hz, 1H), 4.48 (m, 1H), 4.42 (dd, *J* = 10.5 Hz, 7.0 Hz, 1H), 4.31 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.87–3.81 (m, 2H), 3.75–3.72 (m, 1H), 2.87 (s, 1H), 1.71 (d, *J* = 6.5 Hz, 3H), 1.10 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.5, 143.8, 141.3, 135.5, 132.5, 132.4, 130.0, 128.7, 127.8, 127.7, 127.0, 125.1, 120.0, 72.8, 66.9, 65.0, 55.5, 47.1, 26.8, 19.2, 17.8; IR (thin film) 3437 (br), 1708, 1510 cm^{-1}; MS *m/z* (ESI) 592 ($M^+ + H^+$), 574 (100); HRMS Calcd for $C_{37}H_{42}NO_4Si$ ($M^+ + H^+$): 592.2883. Found: 592.2873.

(*E*)-(1*R*, 2*R*)-[2-*tert*-Butoxy-1-(*tert*-butyl-diphenyl-silanyloxymethyl)-pent-3-enyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*E*-17). Compound *E*-**17** (yield 75%) was prepared using the same procedure as for preparation of compound *Z*-**17**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.0 Hz, 2H), 7.69–7.63 (m, 4H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.43 – 7.36 (m, 7H), 7.31–

7.24 (m, 3H), 5.67–5.58 (m, 1H), 5.54 and 5.51 (rotamer) (d, *J* = 7.0 Hz, 1H), 5.12 (d, *J* = 7.5 Hz, 1H), 4.39 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.32–4.29 (m, 2H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.75–3.65 (m, 3H), 1.67 (d, *J* = 6.0 Hz, 3H), 1.17 (s, 9H), 1.08 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.1, 144.0, 141.3, 135.6, 133.4, 133.3, 132.3, 129.7, 127.72, 127.69, 127.6, 127.0, 126.7, 125.1, 120.0, 74.3, 70.4, 66.6, 62.3, 56.8, 47.2, 28.7, 26.8, 19.2, 17.8; IR (thin film) 3444, 1727, 1589, 1503 cm^{-1}; MS *m/z* (ESI) 648 ($M^+ + H^+$), 574 (100); HRMS Calcd for $C_{41}H_{50}NO_4Si$ ($M^+ + H^+$): 648.3509. Found: 648.3495.

(*E*)-(1*R*, 2*R*)-(2-*tert*-Butoxy-1-hydroxymethyl-pent-3-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*E*-18). Compound *E*-**18** (yield 95%) was prepared using the same procedure as for preparation of compound **13d**. $[\alpha]_D^{25}$ = -6.6° (*c* 0.245, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.68–5.63 (m, 1H), 5.50 (ddd, *J* = 15.5 Hz, 7.0 Hz, 1.5 Hz, 1H), 5.22 (d, *J* = 7.0 Hz, 1H), 4.44 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.36 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H), 4.25–4.21 (m, 2H), 3.70 – 3.68 (m, 3H), 2.86 (br, 1H), 1.69 (d, *J* = 6.0 Hz, 3H), 1.21 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.6, 143.9, 141.3, 131.3, 127.7, 127.0, 125.0, 119.9, 75.0, 72.5, 66.7, 63.2, 56.5, 47.2, 28.6, 17.8; IR (thin film) 3437 (br), 1712, 1505 cm^{-1}; MS *m/z* (ESI) 432 ($M^+ + Na^+$), 410 ($M^+ + H^+$), 336 (70), 158 (100); HRMS Calcd for $C_{25}H_{32}NO_4$ ($M^+ + H^+$): 410.2331. Found: 410.2339.

(*E*)-(2*S*, 3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hex-4-enoic acid (1g). Compound **1g** (yield 81%, foam) was prepared using the same procedure as for preparation of compound **1a**.$[\alpha]_D^{25}$ = 55.6° (*c* 0.77, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (m, 2H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.33 (t, *J* = 7.0 Hz, 2H), 5.74–5.68 (m, 1H), 5.64 (d, *J* = 7.0 Hz, 1H), 5.40 (ddd, *J* = 15.0 Hz, 7.0 Hz, 1.5 Hz, 1H), 4.59–4.57 (m, 1H), 4.46–4.36 (m, 3H), 4.25 (t, *J* = 7.5 Hz, 1H), 1.70 (d, *J* = 6.5 Hz, 3H), 1.28 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.0, 143.6, 141.3, 130.2, 127.7, 127.1, 125.11, 125.07, 120.0, 71.3, 67.1, 58.1, 47.0, 28.2, 17.8; IR (thin film) 3440–2977 (br), 3440, 1728, 1514 cm^{-1}; MS *m/z* (ESI) 446 ($M^+ + Na^+$), 424 ($M^+ + H^+$), 172 (100); HRMS Calcd for $C_{25}H_{30}NO_5$ ($M^+ + H^+$): 424.2124. Found: 424.2126.

General Procedure for Peptide Synthesis.

Peptides were synthesized on an automated peptide synthesizer by standard Fmoc chemistry. Preloaded Wang-resin (Fmoc-Ala-Wang resin, 0.1 mmol) and Fmoc-amino acids (0.4 mmol) were used. O-Benzotriazole-N,N,N′,N′,-tetramethyl-uroniumhexafluoro-phosphate (HBTU) was

used as coupling reagent. Both Fmoc-amino acids and HBTU were used in 3-fold excess relative to the resin. For the coupling of nonproteinogenic Fmoc-amino acids, a 2-fold excess relative to the resin was used. Piperidine (20% in DMF, 10 mL) was used as the deprotecting reagent. *N*-Methylmorpholine (NMM, 4.4% in DMF, 1.5 mL) was used with HBTU (4 equiv., relative to the resin) as the activating reagent. Resins were first swollen in DMF (6 mL) for 30 min. The Fmoc group was then removed with 20% piperidine in DMF (5 x 5 min, 6 mL). After deprotection, the resins were washed with DMF (9 x 30 s, 6 mL). Amino acids were activated and transferred into the reaction vessel for coupling (2 h). To reduce the possibility of racemization, the coupling of Fmoc-L-Cys-OH or nonproteinogenic amino acids was/were manually conducted with 1-hydroxybenzotriazole (HOBt, 1 equiv., relative to nonproteinogenic amino acids) and N,N′-diisopropylcarbodiimide (DIC, 1 equiv., relative to nonproteinogenic amino acids) in DMF (6 mL) for 2 h to 3 h. The completion of coupling was monitored by ninhydrin test. After each coupling, the resins were rinsed with DMF (9 x 30 s, 6 mL). In a final step, the N-terminal Fmoc was deprotected. Peptide cleavage from the resin was achieved with a mixture of TFA (6 mL), water (25 μL), ethanedithiol (EDT, 25 μL) with the drop-wise addition of triisopropylsilane (TIPS) until a colorless suspension, indicating consumption of the trityl cation, was obtained. After the suspension was stirred for 1 h at room temperature, the resin was filtered. The filtrate was concentrated to give crude peptide that was washed with cold diethyl ether, and lyophilized from 10% aqueous acetic acid after filtration through a 0.45 μm filter. The lyophilized crude peptides were purified by preparative RP-HPLC on a Vydac C18 column (2.2 cm x 25 cm) employing a water-acetonitrile solvent system. The standard HPLC gradient was 2-100% of solvent B over 45 min (A: H_2O, 0.1% TFA; B: CH_3CN 80% in H_2O v/v, 0.086% TFA). Peptides were detected by their absorbance at 220 nm.

CNMNβ²-SerA. RP-HPLC Rt = 12.9 – 14.9 min. ESI m/z calcd 652 (M); Found, 653 (M + H^+).

CNMNTWA. RP-HPLC Rt = 20.3 – 22.7 min. ESI m/z calcd 839 (M); Found, 839 (M), 840 (M + H^+).

CNMNAllo-ThrWA. RP-HPLC Rt = 20.4 – 22.1 min. ESI m/z calcd 839 (M); Found, 839 (M), 861(M + Na^+ - H).

CNMNβ²-SerWA. RP-HPLC Rt = 19.6 – 21.4 min. ESI m/z calcd 839 (M); Found, 839 (M), 840 (M + H^+).

CNMNEthyl-SerWA. RP-HPLC Rt = 20.8 – 23.1 min. ESI m/z calcd 853 (M); Found, 853 (M), 875 (M + Na^+- H).

CNMN<u>Vinyl-Ser</u>WA. RP-HPLC Rt = 20.3 – 22.7 min. ESI m/z calcd 851 (M); Found, 851 (M).

CNMN<u>Ethynyl-Ser</u>WA. RP-HPLC Rt = 20.4 – 22.7 min. ESI m/z calcd 849 (M); Found, 849 (M), 871 ($M + Na^+ - H$).

CNMN<u>Propyl-Ser</u>WA. RP-HPLC Rt = *20.8 – 23.2* min. ESI m/z calcd 867 (M); Found, 867 (M), 868 ($M + H^+$).

CNMN<u>Isopropyl-Ser</u>WA. RP-HPLC Rt = *20.6 – 22.8* min. ESI m/z calcd 867 (M); Found, 867 (M), 868 ($M + H^+$).

CNMN<u>Propynyl-Ser</u>WA. RP-HPLC Rt = *20.4 – 22.2* min. ESI m/z calcd 863 (M); Found, 863 (M).

CNMN(<u>*E*)-Allyl-Ser</u>WA. RP-HPLC Rt = *20.4 – 22.3* min. ESI m/z calcd 865 (M); Found, 865 (M).

CNMN(<u>*Z*)-Allyl-Ser</u>WA. RP-HPLC Rt = *25.7 - 28.6* min. ESI m/z calcd 865 (M); Found, 865 (M).

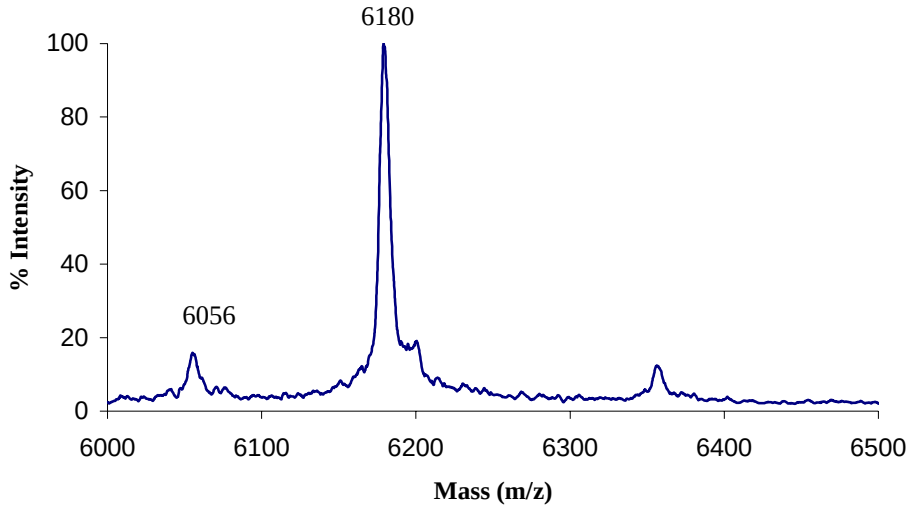
General procedure for purification of His-LctA (1-37) thioesters.

BL21(DE3) cells carring the plasmid pET15b containing an insert encoding the His-LctA(1-37)-intein-CBD fusion protein were grown in 3 L of LB (Amp. 100 μg/mL) until $OD_{600\ nm}$ = 0.6 – 0.7. Protein expression was induced with IPTG (1 M, 1.95 mL, 0.65 mM final), and the cells were grown for an additional 6 h at 25 ^oC. At the end of induction the cells were harvested by centrifugation at 18.5 k*g* for 15 min at 4 ^oC, and the cell pellet (~ 10 g) was resuspended in 20 mL of cell lysis buffer (20 mM Tris, 500 mM NaCl, 0.1% Triton X-100, 1 mM EDTA, pH 7.5 at 4 ^oC) and lysed by sonication at Amp 75, 5 s on, 9.9 s off for 15 – 20 min on ice. The lysate was centrifuged at 27 k*g* for 30 min, and the supernatant containing the fusion protein was bound to a capped pre-equilibrated chitin column (20 mL) at 4 ^oC for 2 h with gentle shaking. The column was subsequently washed with column buffer (20 mM HEPES, 500 mM NaCl, 1 mM EDTA, pH 7.2 at 4 ^oC) until the OD_{280} of the flow through was less than 0.01. At this point the column was washed with three column volumes of cleavage buffer (100 mM HEPES, 200 mM NaCl, 50 mM MESNa, 1 mM EDTA, pH 7.75 at 4 ^oC). Alternatively, for overnight intein mediated cleavage of the truncated His-LctA mutants and generation of the MES thioester, the column was equilibrated with 2 column volumes of cleavage buffer for 12 h at 4 ^oC. The eluate containing the peptide thioester was concentrated by Amicon YM1 membrane and lyophilized after acidification with 0.1% TFA. The lyophilized peptide thioester was purified by RP-HPLC on a C4 column (Vydac)

employing a water-acetonitrile solvent system (the standard HPLC gradient was 2-100% of solvent B over 45 min. A: H_2O, 0.1% TFA; B: CH_3CN 80% in H_2O, v/v 0.086% TFA. Peptides were detected by their absorbance at 220 nm) and analyzed by MALDI-TOF MS.

His-LctA(1-37)MES

Preparative RP-HPLC on C4 column Rt: 24.8 – 27.0 min. MALDI-TOF MS calcd 6181 (M). Found: 6180 (M^+).

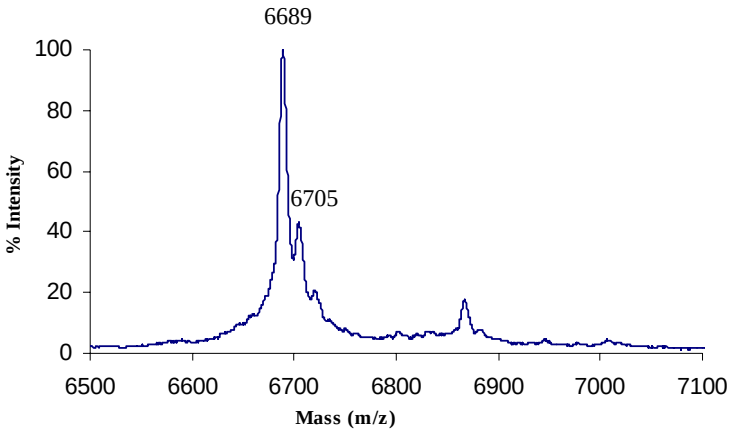


Note: For all MALDI-MS, the y-axis shows relative intensity.

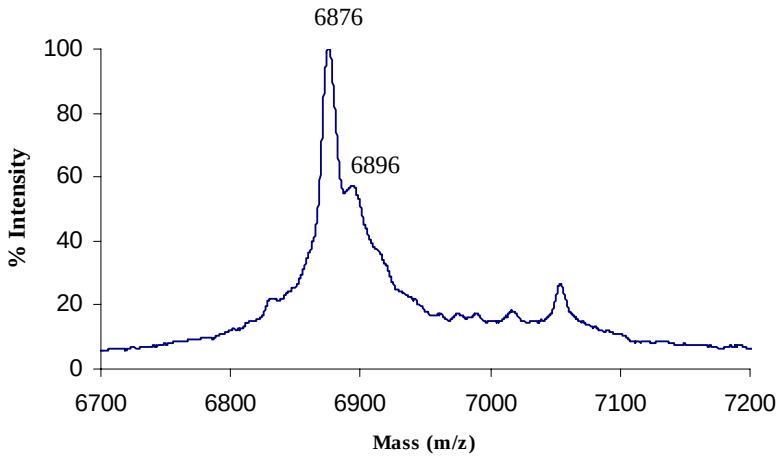
General Procedure for Ligation of His_6-LctA(1-37)MES with Synthetic Peptides.

Synthetic peptides (2-5 mg) that were previously purified by C18 RP-HPLC were dissolved in a minimum volume of ligation buffer (typically 160 – 250 μL) containing 100 mM HEPES, 200 mM NaCl, 50 mM MESNa, pH 7.75 at 4 ^oC. For hydrophobic peptides, the precipitation of the peptides was observed, lowering the peptide concentration; addition of DMSO (10 - 20 μL) lead to greater solubility and reduced precipitation. Then a solution of His_6-LctA(1-37)MES thioester in ligation buffer (60 μL) was added to the peptide solution to obtain a final concentration of 0.5 ~ 1.0 mM of the thioester. The pH was adjusted to 7.6 ~ 7.8 with 1 N NaOH, and ligation allowed for 12 – 16 h at 4 ^oC with gentle shaking. The crude ligation products were analyzed by MALDI-TOF MS for completeness of the reaction prior to acidification with 5% TFA. Briefly, 5 μL of the ligation buffer mixture was mixed with 9 μL of sinapic acid matrix (prepared in 30% CH_3CN, containing 0.1 TFA) and 1 μL of the mixture was spotted on the MALDI plate. The

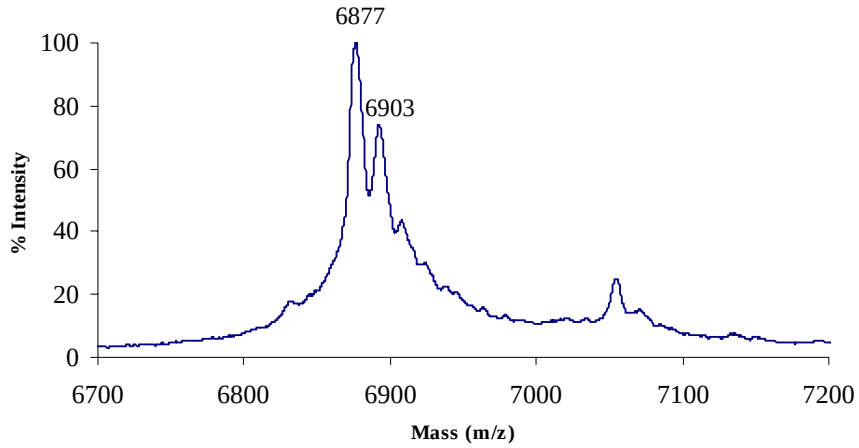
acidified ligation mixture was incubated with 60 – 70 mM TCEP for 30 min at 25 ^{o}C prior to purification by C4 analytical RP-HPLC. For the heterogeneous phase ligation, DMSO was added to the ligation mixture until a clear solution was obtained after the reaction was quenched with 50 μL of 5% TFA. TCEP was then added and the resulting mixture was incubated at 25 ^{o}C for 30 min. Fractions containing the ligation products from C4 RP-HPLC (The standard gradient HPLC was 2-100% of solvent B over 45 min. A: H_2O, 0.1% TFA; B: CH_3CN 80% in H_2O, v/v 0.086% TFA; peptides were detected by their absorbance at 220 nm) were analyzed by MALDI-TOF MS. **His$_6$-LctA(1-37)-CNMN$\underline{\beta^2}$-S$\underline{\text{er}}$A.** RP-HPLC Rt = 29.8 – 30.7 min. MALDI-TOF MS calcd 6690 (M); Found, 6689 (M), 6705 (M^+ + O).



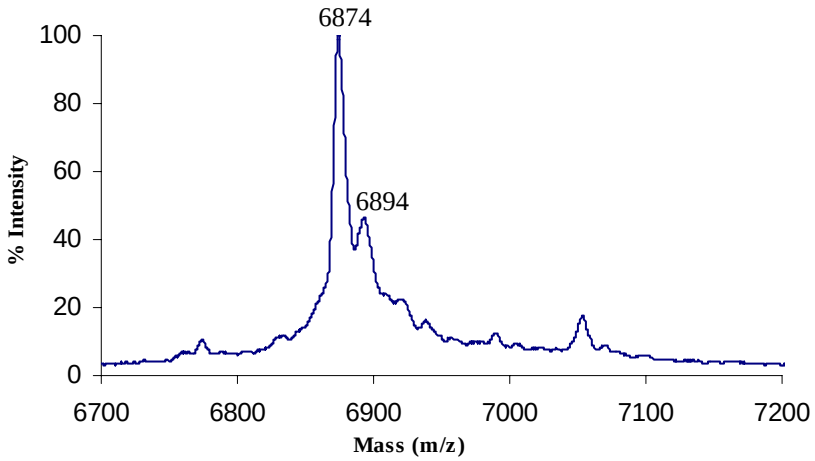
His$_6$-LctA(1-37)-CNMN$\underline{\text{T}}$WA. RP-HPLC Rt = 29.7 – 30.4 min. MALDI-TOF MS calcd 6876 (M); Found, 6876 (M).



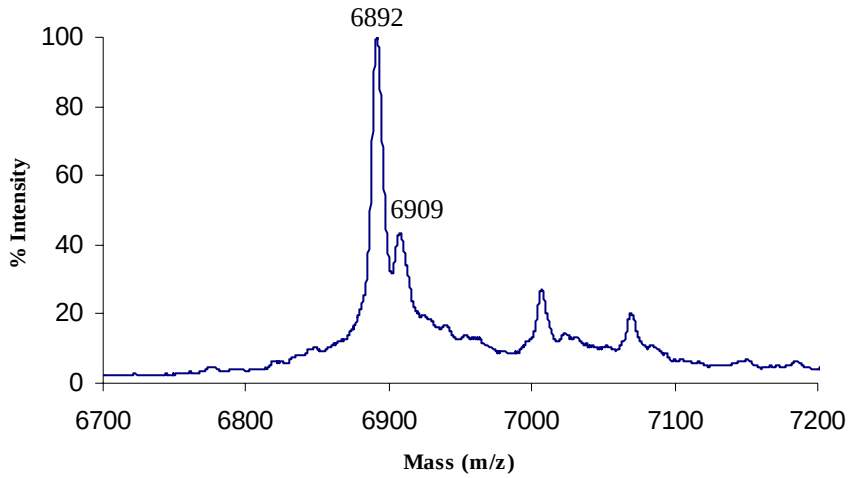
His_6-LctA(1-37)-CNMN<u>Allo-Thr</u>WA. RP-HPLC Rt = 29.48 – 30.22 min. MALDI-TOF MS calcd 6876 (M); Found, 6877 (M), 6893 (M^+ + O).



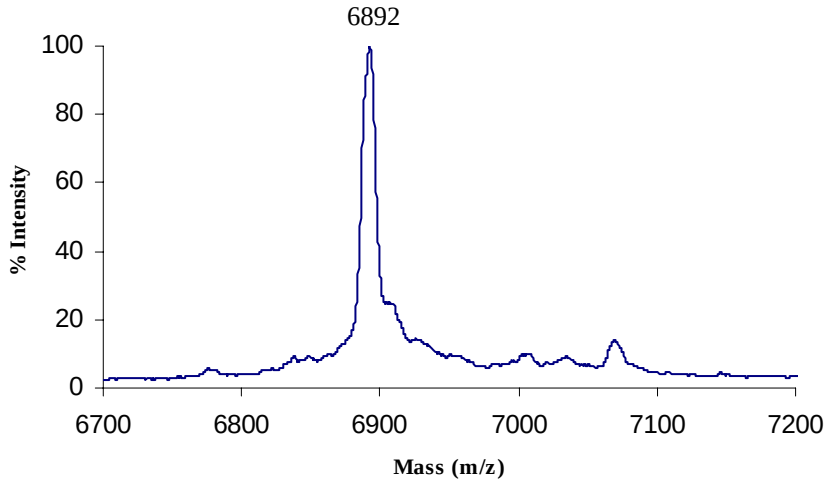
His_6-LctA(1-37)-CNMN<u>β^2-Ser</u>WA. RP-HPLC Rt = 28.7 – 29.3 min. MALDI-TOF MS calcd 6876 (M); Found, 6874 (M), 6894 (M^+ + O).



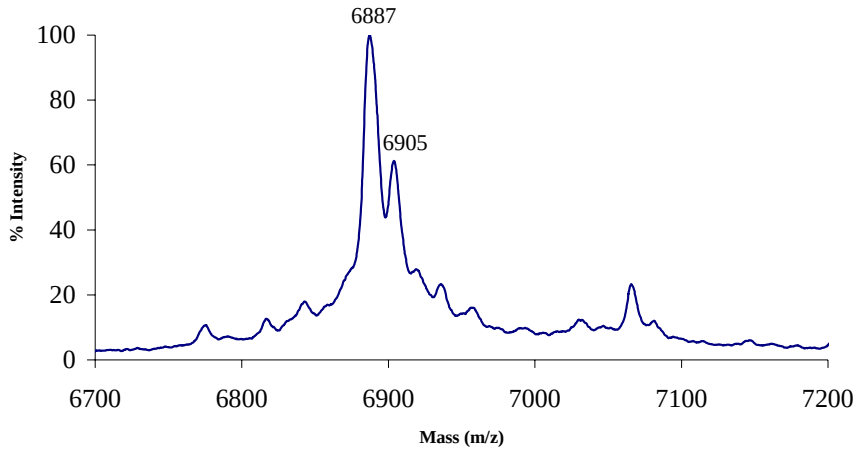
His_6-LctA(1-37)-CNMN<u>Ethyl-Ser</u>WA. RP-HPLC Rt = 29.43 – 30.02 min. MALDI-TOF MS calcd 6890 (M); Found, 6892 (M), 6909 (M^+ + O).



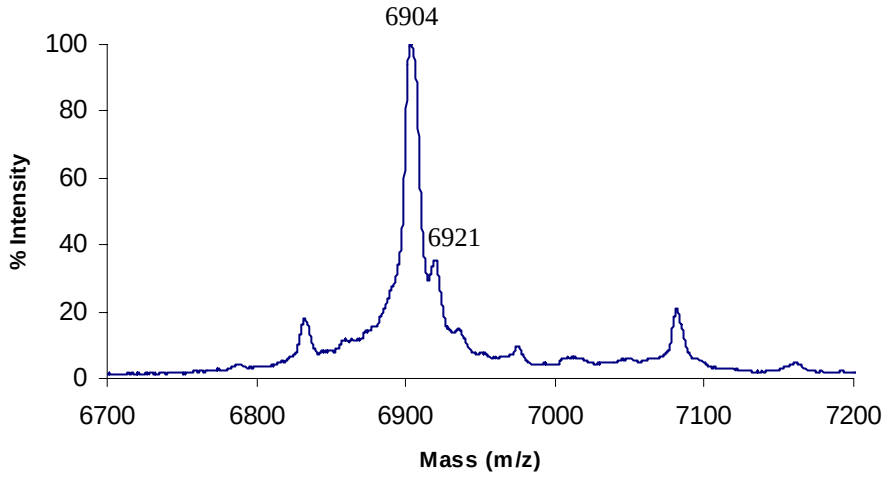
His_6-LctA(1-37)-CNMN<u>Vinyl-Ser</u>WA. RP-HPLC Rt = 28.8 – 29.7 min. MALDI-TOF MS calcd 6888 (M); Found, 6892 (M).



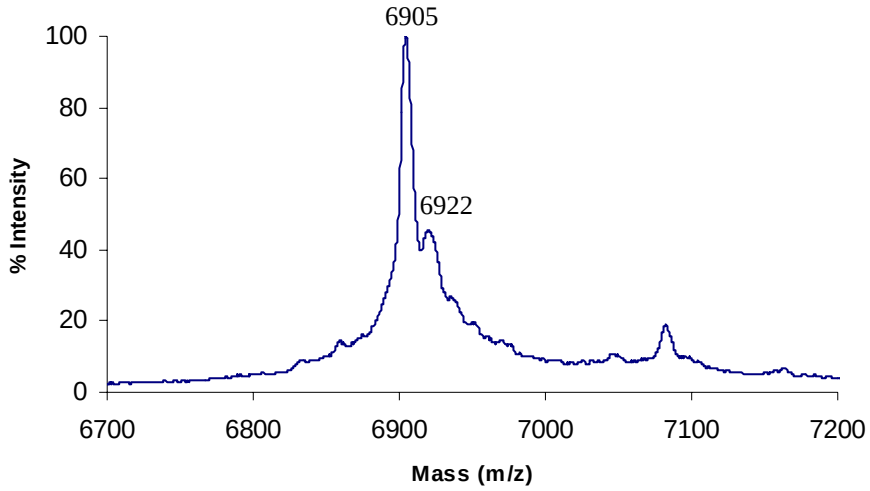
His_6-LctA(1-37)-CNMN<u>Ethynyl-Ser</u>WA. RP-HPLC Rt = 29.20 – 30.01 min. MALDI-TOF MS calcd 6886 (M); Found, 6887 (M), 6905 (M^+ + O).



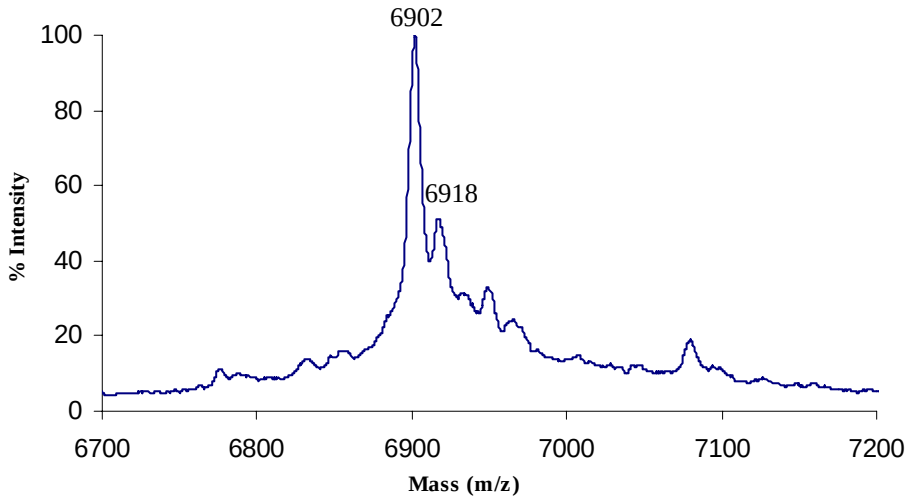
His_6-LctA(1-37)-CNMN<u>Propyl-Ser</u>WA. RP-HPLC Rt = 29.79 – 30.49 min. MALDI-TOF MS calcd 6904 (M); Found, 6904 (M), 6921 (M^+ + O).



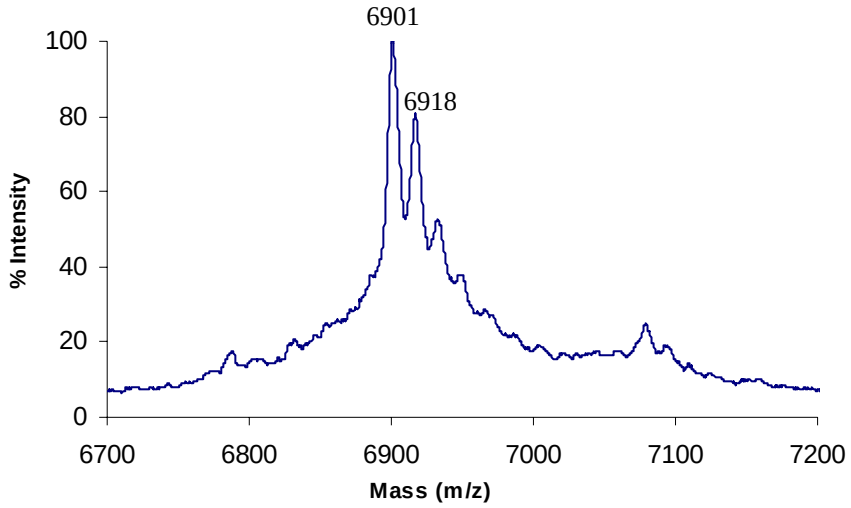
His_6-LctA(1-37)-CNMN<u>Isopropyl-Ser</u>WA. RP-HPLC Rt = 29.70 – 30.31 min. MALDI-TOF MS calcd 6904 (M); Found, 6905 (M), 6922 (M^+ + O).



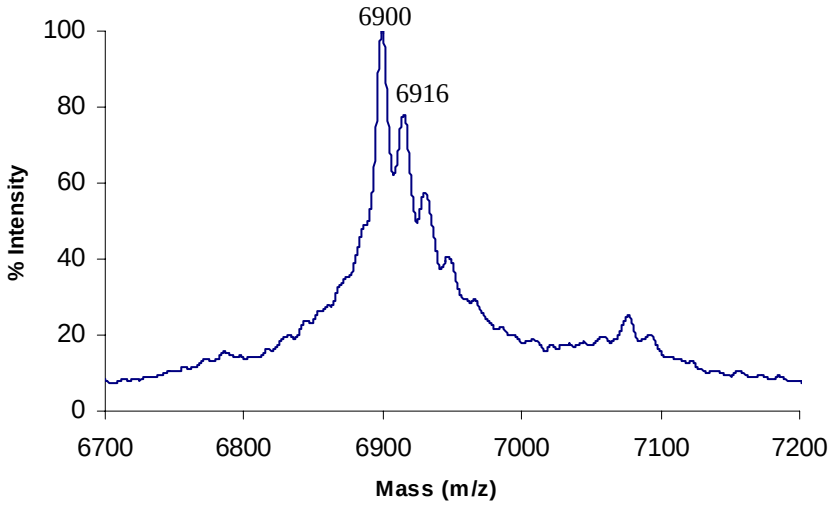
His_6-LctA(1-37)-CNMN<u>Propynyl-Ser</u>WA. RP-HPLC Rt = 29.78 – 30.39 min. MALDI-TOF MS calcd 6900 (M); Found, 6902 (M), 6918 (M^+ + O).



His_6-LctA(1-37)-CNMN(*E*)-Allyl-SerWA. RP-HPLC Rt = 29.06 – 29.99 min. MALDI-TOF MS calcd 6902 (M); Found, 6901 (M), 6918 (M^+ + O).



His_6-LctA(1-37)-CNMN(*Z*)-Allyl-SerWA. RP-HPLC Rt = 28.50 – 29.99 min. MALDI-TOF MS calcd 6902 (M); Found, 6900 (M), 6916 (M^+ + O).



Determination of the position of dehydration

The peptides that underwent only two dehydrations were further investigated to determine whether it was the non-proteinogenic amino acid at position 42 that was not accepted by the enzyme. For this purpose, the LctM product was subjected to treatment with cyanogens bromide. This reagent cleaves C-terminal to methionines resulting in the case of the LctA analogs in three fragments: the hexa-His tag terminating in a homoserine lactone, LctA2-40 also terminating in a

homoserine lactone, and a short tetrapeptide (see Figure S5). Analysis of the CNBr reaction using MALDI-MS was then used to distinguish whether the two-fold dehydration took place at Thr33 and Ser35 and not at the unnatural amino acid at position 42 because Thr33/Ser35 are in a different fragment upon CNBr cleavage. Note that in addition to catalyzing the dehydrations, LctM also catalyzes the cyclization reaction that adds Cys38 to Dhb33.[6] For the LctA analogs in this study this cyclization activity was verified by reaction with PHMB which showed that no free thiols were present in the reaction product (see Li et al[7] for a discussion of this protocol).

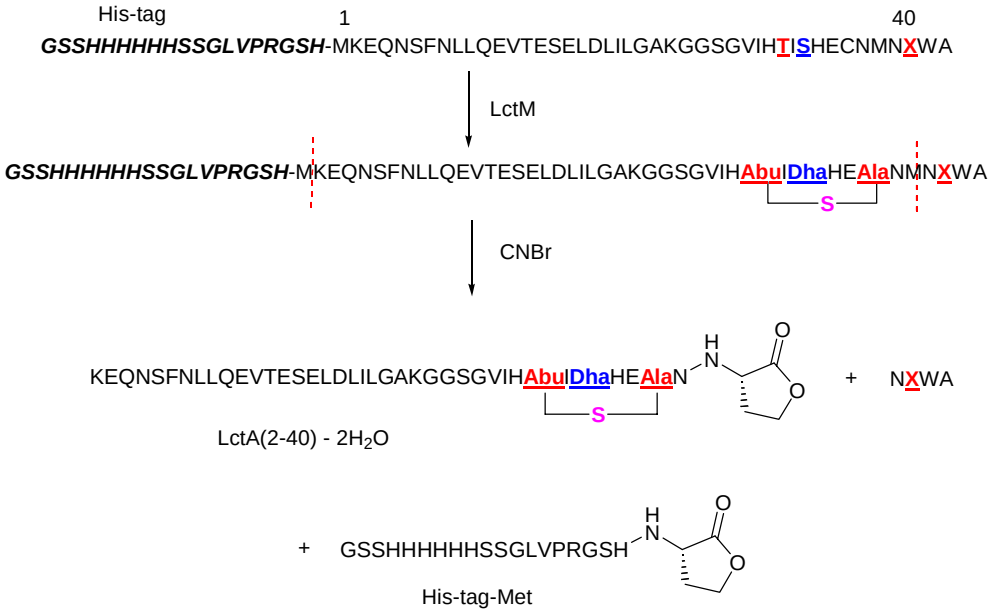


Figure S5. Strategy used to determine the site of dehydration.

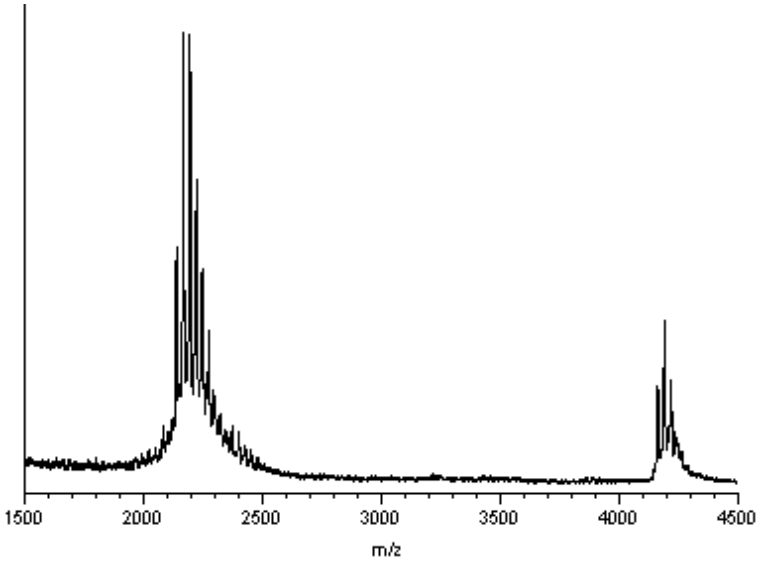
General Procedure for cleavage of dehydrated LctA analogs His_6-LctA(1-37)CNMN<u>Propyl-Ser</u>WA and His6-LctA(1-37)CNMN<u>Isopropyl-Ser</u>WA with CNBr.

The dehydrated substrate (15 μL) resulting from the assay of His_6-LctA(1-44)S42<u>Propyl-Ser</u> or His_6-LctA(1-44)<u>S42Isopropyl-Ser</u> with LctM was purified using a zip-tip eluting with 50% CH_3CN, 40% H_2O, 10% aqueous (3%) TFA. After the concentration, the resulting pure dehydrated peptide was treated with CNBr (10 μL, 50 mg/mL in 70% HCOOH) in the dark overnight. The reaction mixture was concentrated and 5 μL of water was added. Then 3 μL of the solution was placed into an eppendorf tube, followed by 5 μL of matrix (α-cyano-4-hydroxycinnamic acid supersolution in 50% CH_3CN, 40% H_2O, 10% aqueous (3%) TFA) , and then the sample was monitored by MALDI-TOF.

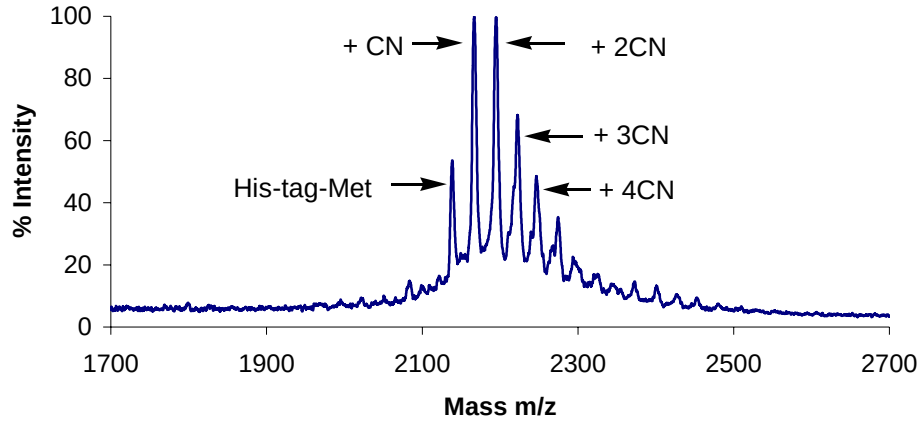
Cleavage of two-fold dehydrated His_6-LctA(1-37)CNMN<u>Propyl-Ser</u>WA with CNBr.

MALDI-TOF MS calcd His_6-Met, 2133; LctA(2-40) – 2 H_2O, 4159; Found, His_6-Met, 2139, 2167 (+ CN), 2195 (+ 2 CN), 2222 (+ 3 CN), 2246 (+ 4 CN); LctA(2-40)-2 H_2O, 4162, 4190 (+ CN), 4217 (+ 2 CN). Note that in addition to the cleavage observed at the two Met residues, CNBr treatment also resulted in cyanylation of the peptides (Fig S6). We tentatively assign these cyanylations to His residues in the His-tag as well as cyanylation of the two His in LctA2-40.

A.



B.



C.

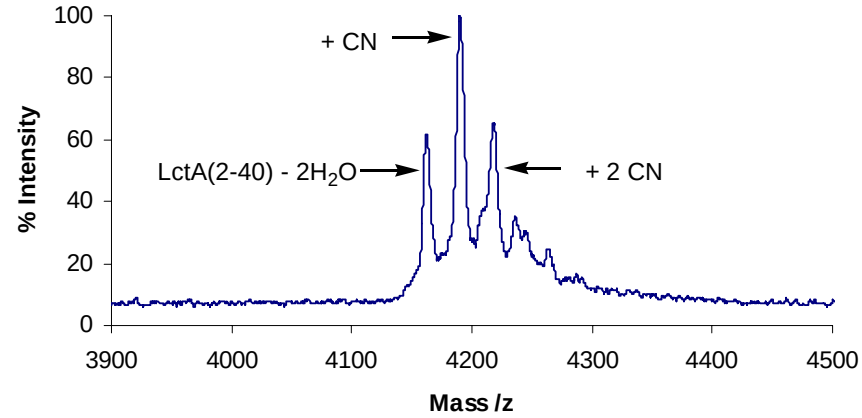
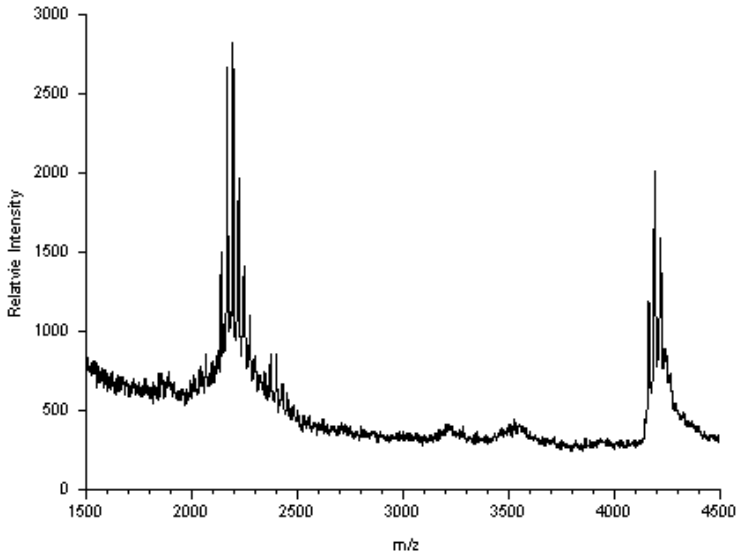


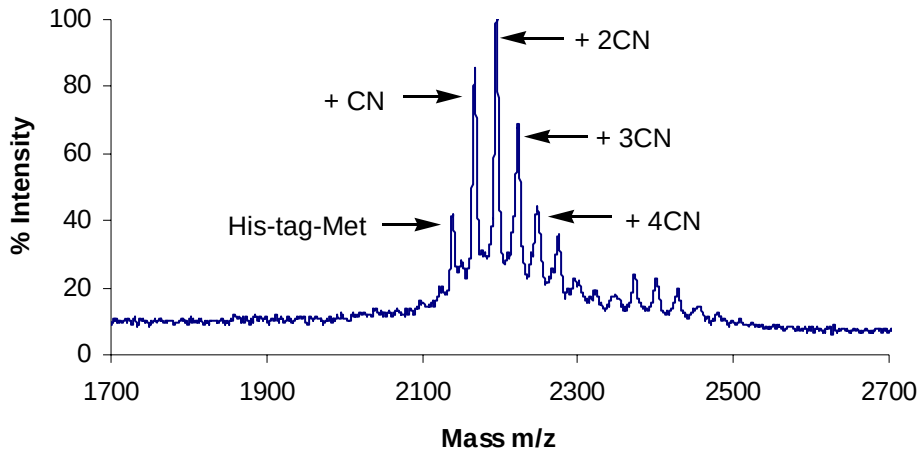
Figure S6. His_6-LctA(1-37)CNMNPropyl-SerWA treated with LctM and then CNBr. (A). Crude product. (B) zoom in on N-terminal fragment. (C) zoom in on LctA2-40 fragment.

Cleavage of two-fold dehydrated His_6-LctA(1-37)CNMN<u>Isopropyl-S</u>erWA with CNBr. The same procedure was followed as described for the peptide incorporating propylSer. MALDI-TOF MS calcd His_6-Met, 2133; LctA(2-40) – 2 H_2O, 4159; Found, His_6-Met, 2139, 2167 (+ CN), 2195 (+ 2 CN), 2222 (+ 3 CN), 2249 (+ 4 CN); LctA(2-40)-2 H_2O, 4163, 4190 (+ CN), 4218 (+ 2 CN).

A.



B.



C.

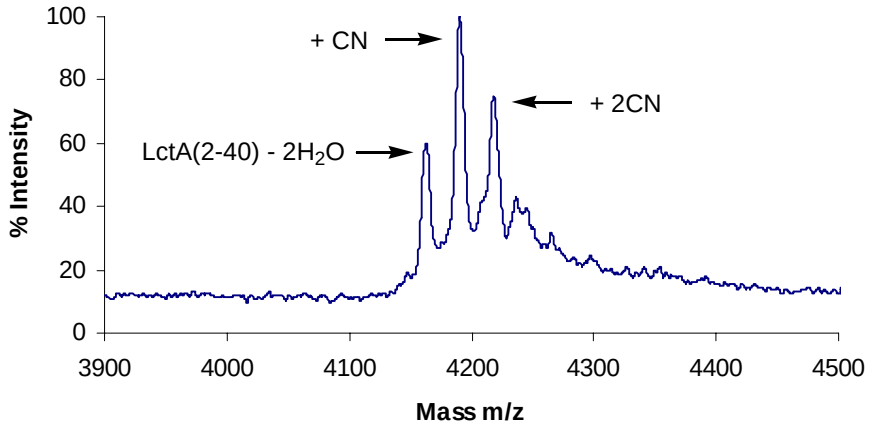


Figure S7. His_6-LctA(1-37)CNMNisopropyl-SerWA treated with LctM and then CNBr. (A). Crude product. (B) zoom in on N-terminal fragment. (C) zoom in on LctA2-40 fragment.

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