

Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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Supporting Information

Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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Abbreviations

DME = 1,2-dimethoxyethane

EtOAc = Ethyl acetate

ϵ_r = Reichardt's value

IPA = Isopropanol

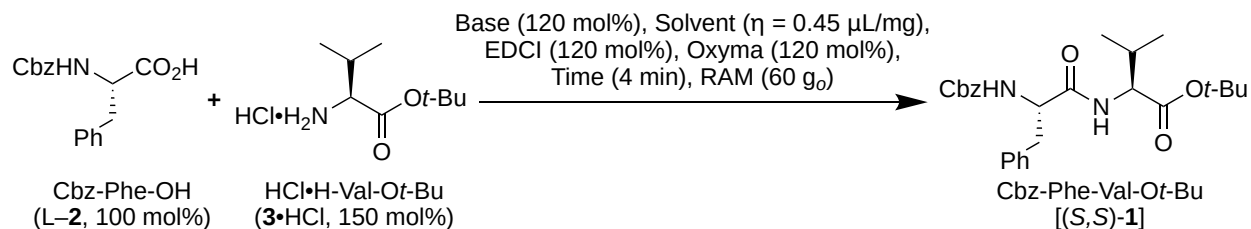
PCC = Propylene carbonate

MTBE = Methyl *tert*-butyl ether

NMP = *N*-methyl pyrrolidinone

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Equation S1. Model dipeptide synthesis with RAM to evaluate effects of liquid and bases on conversion.

Table S1. Influence of base on conversion in the synthesis of dipeptide **[(S,S)-1]** with the RAM at 60 g_o for 4 min using DME and NBP as solvents ($\eta = 0.45 \mu\text{L}/\text{mg}$).

Base	pK _a ^a	Dissolution rate constant in DMF, ^b 100 °C (mol/cm ² ·s)	Solvent / [$\eta = 0.45 (\mu\text{L}/\text{mg})$]	Viscosity / cP, 25°C ^c	% Conversion
Et ₃ N	10.72	Miscible	DME	0.42	92
KHCO ₃	6.35	9.6×10^{-9}			89
NaHCO ₃	6.35	5.5×10^{-9}			83
NaH ₂ PO ₄	2.16	N/A			66
Et ₃ N	10.72	Miscible	NBP	4	87
NaHCO ₃	6.35	5.5×10^{-9}			78
K ₂ CO ₃	10.33	110×10^{-9}			70

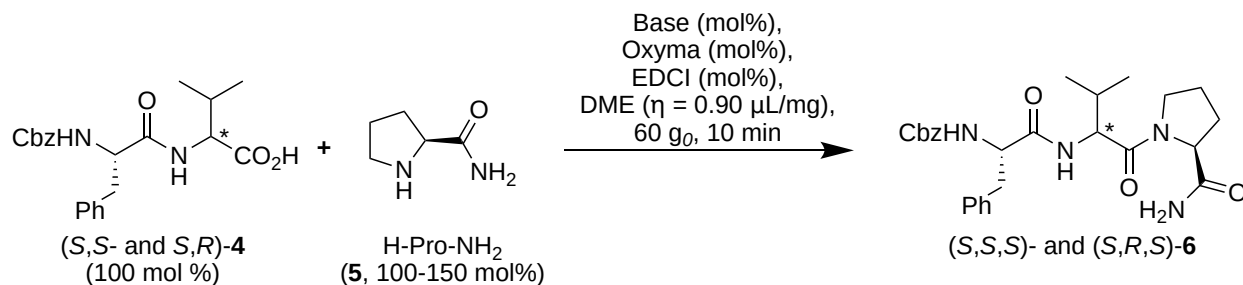
^apK_a of conjugate acids Et₃N⁺H, H₂CO₃, H₃PO₄, and KHCO₃ per reference [1].

^b Per reference [2].

^cPer references [3] and [4].

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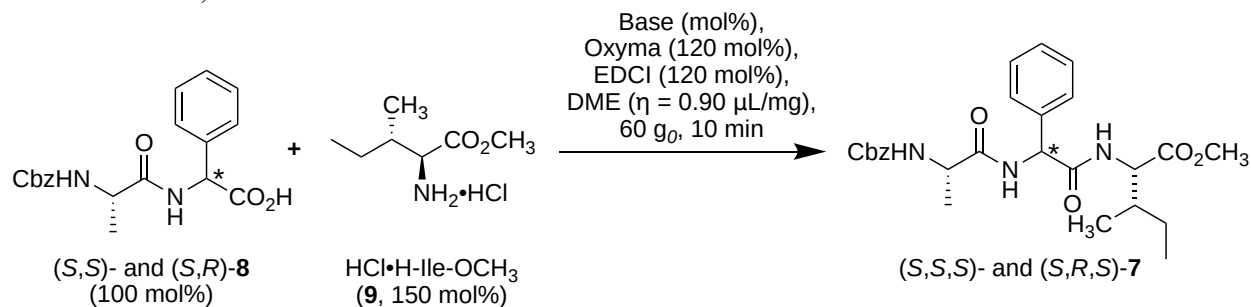
Equation S2. Model tripeptide synthesis: Cbz-Phe-L- and D-Val-Pro-NH₂ [(S,R,S)- and (S,S,S)-6].

Table S2. Conversion and epimerization in the synthesis of Cbz-Phe-L- and D-Val-Pro-NH₂ {(S,R,S)- and (S,S,S)-6 [$R_t = 7.47$ min and 7.12 min respectively]} from (S,S)- and (S,R)-4 ($R_t = 7.81$ min) as assessed by HPLC (Method A, $\lambda = 214$ nm).

Dipeptide acid	H-Pro-NH ₂ (mol%)	EDCI (mol%)	Oxyma (mol%)	Et ₃ N (mol%)	Conversion (%)	Epimer (%)
Cbz-Phe-Val-OH	150	100	120	0	92	6.6
Cbz-Phe-Val-OH	150	120	100	0	90	6.8
Cbz-Phe-Val-OH	150	120	120	0	95	7.2
Cbz-Phe-Val-OH	120	120	120	0	96	9.1
Cbz-Phe-Val-OH	100	120	120	0	97	10.0
Cbz-Phe-Val-OH	150	120	120	120	91	13.6
Cbz-Phe-D-Val-OH	150	120	120	120	92	4.8

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Equation S3. Model tripeptide synthesis: Cbz-Ala-L- and D-Phg-Ile-OMe [(S,R,S)- and (S,S,S)-**7**]

Table S3. Conversion and epimerization study (method E/F, $\lambda = 205 \text{ nm}$) for the synthesis of Cbz-Ala-L- and D-Phg-Ile-OMe [(S,R,S)- and (S,S,S)-**7** [$R_t = 11.5/13.3$ and $11.2/12.7 \text{ min}$ respectively]] as assessed by HPLC.

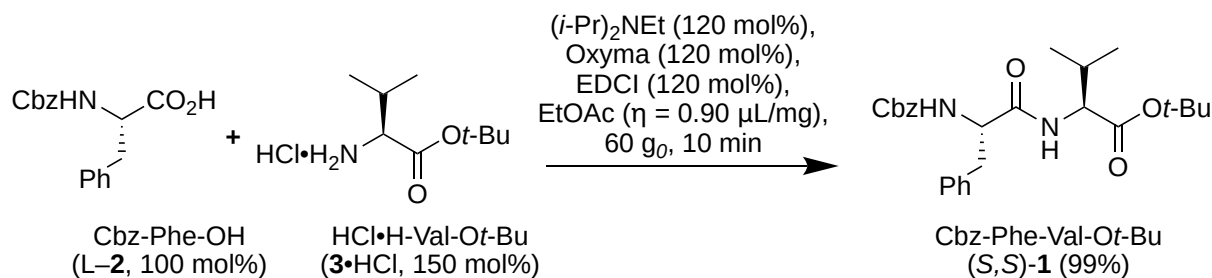
Dipeptide acid	Base ^a	pK _a	Epimer (%)	Conversion (%) ^b
Cbz-Ala-Phg-OH	(<i>i</i> -Pr) ₂ EtN	11.40	1.53	100
Cbz-Ala-Phg-OH	Et ₃ N	10.72	0.97	77
Cbz-Ala-Phg-OH	2,4,6-Collidine	7.43	3.09	45
Cbz-Ala-Phg-OH	4-Me-morpholine	7.38	5.87	52
Cbz-Ala-Phg-OH	NaH ₂ PO ₄ (400 mol%)	2.16	0.88	81
Cbz-Ala-Phg-OH	NaH ₂ PO ₄	2.16	0.68	26
Cbz-Ala-D-Phg-OH	(<i>i</i> -Pr) ₂ EtN	11.30	1.06	88
Cbz-Ala-D-Phg-OH	Et ₃ N	10.72	0.88	84

^a pK_a refers to conjugate acids: Et₃N⁺H, 2,4,6-Collidinium, H₃PO₄ and 4-Me-morpholinium per reference [1] and (*i*-Pr)₂EtN⁺H per reference [5].

^b Conversion was assessed using HPLC (method A, 214 nm): Cbz-Ala-L- and D-Phg-Ile-OMe {(S,R,S)- and (S,S,S)-**7** [$R_t = 8.4 \text{ min}$]} Cbz-Ala-L-Phg-OH ($R_t = 6.9 \text{ min}$).

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Equation S4. RAM Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu) to (S,S)-1 using DME.

Table S4. RAM Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu): Reagents & PMI, using DME.

	Reagent	Number of times used	Mass or Volume (g or mL)	Density (g/mL)	Mass used (g)
Reaction	Cbz-Phe-OH	1	1.00	-	1.00
	HCl·H-Val-Ot-Bu	1	1.05	-	1.05
	Oxyma	1	0.570	-	0.570
	$(i\text{-Pr})_2\text{NEt}$	1	0.520	-	0.520
	DME	1	3.45	0.87 ^a	3.00
Purification	EtOAc	1	10	0.894 ^b	8.9
	H ₂ O	1	5	1.00	5
	1 N HCl	3	5	1.02 ^c	15
	Saturated aqueous NaHCO ₃	5	5	1.03 ^d	26
	Brine	5	5	1.15 ^e	29
Total mass (g)		89.9			
Mass product (g)		1.50			
Yield (%)		99			
PMI		59.9 ^f			
PMI (without purification)		4.1 ^f			

^aDensity of DME at 298.15K and atmospheric pressure per reference [6].

^bDensity of EtOAc at 298.15K and atmospheric pressure per reference [7].

^cDensity of 1N HCl at 298.15K and atmospheric pressure per reference [8].

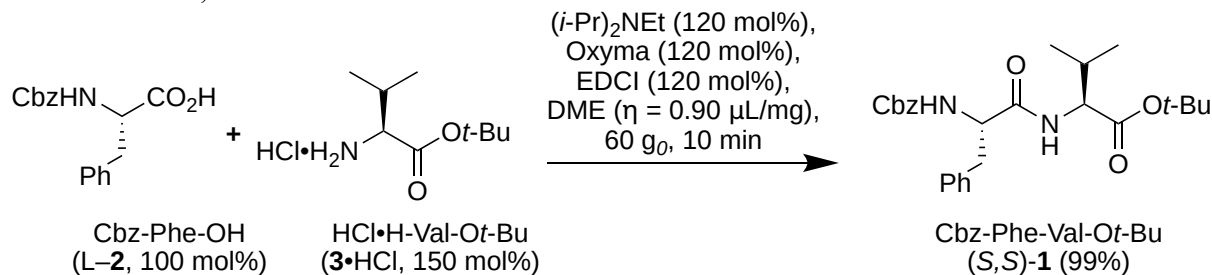
^dDensity saturated aqueous NaHCO₃ at 298.15K and atmospheric pressure per reference [9].

^eDensity of brine at 298.15K and atmospheric pressure per reference [10].

^fPMI was calculated using the following formula: $PMI = \sum \text{Mass Of Materials} / \text{Mass Of Isolated Product}$.

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Equation S5. RAM Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu) to (S,S)-1 using EtOAc.

Table S5. RAM Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu): Reagents & PMI, using EtOAc.

Step	Reagent	Number of times used	Mass or Volume (g or mL)	Density (g/mL)	Mass used (g)
Reaction	Cbz-Phe-OH	1	1.00	-	1.00
	HCl·H-Val-Ot-Bu	1	1.05	-	1.05
	Oxyma	1	0.570	-	0.570
	$(i\text{-Pr})_2\text{NEt}$	1	0.520	-	0.520
	EtOAc	1	3.45	0.894 ^a	3.11
Purification	EtOAc	1	10	0.894 ^a	9
	H ₂ O	1	5	1.00	5
	1 N HCl	3	5	1.02 ^b	15
	NaHCO ₃	5	5	1.03 ^c	26
	Brine	5	5	1.15 ^d	29
Total mass (g)		90.0			
Mass product (g)		1.36			
Yield (%)		90			
PMI		66.2 ^e			
PMI (without purification)		4.6 ^e			

^a Density of EtOAc at 298.15K and atmospheric pressure per reference [7].

^b Density of 1N HCl at 298.15K and atmospheric pressure per reference [8].

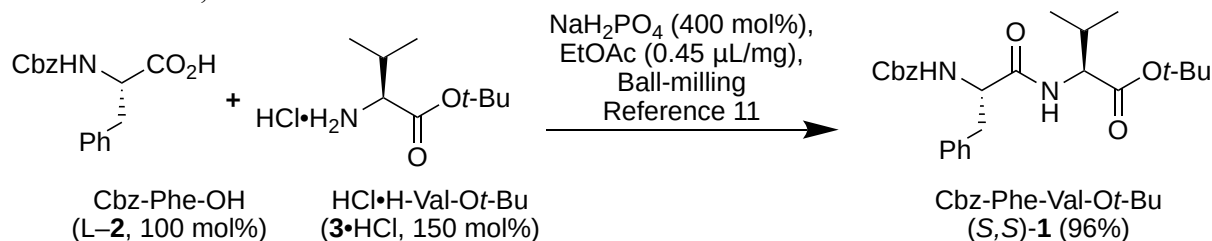
^c Density saturated aqueous NaHCO₃ at 298.15K and atmospheric pressure per reference [9].

^d Density of brine at 298.15K and atmospheric pressure per reference [10].

^e PMI was calculated using the following formula: $PMI = \frac{\sum \text{Mass Of Materials}}{\text{Mass Of Isolated Product}}$.

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Equation S6. Previously Reported Ball-Milled Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu) to (S,S)-1.¹¹

Table S6. Ball-Milled Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu): Reagents & PMI.^a

	Reagent	Number of times used	Mass or Volume (g or mL)	Density (g/mL)	Mass used (g)
Reaction	Cbz-Phe-OH	1	0.83	-	0.83
	HCl·H-Val-Ot-Bu	1	0.87	-	0.87
	Oxyma	1	0.47	-	0.47
	NaH ₂ PO ₄	1	1.32	-	1.32
	EtOAc	1	1.80	0.894 ^c	1.62
Purification	EtOAc "recovered" ^b	1	15	0.894 ^c	14
	H ₂ O "recovered" ^b	1	15	1.00	15
	EtOAc extract	2	15	0.894 ^c	27
	1 N HCl	2	15	1.02 ^d	30
	1 N NaOH	2	15	1.04 ^e	31
	Brine	1	15	1.15 ^f	17
Total mass (g)		139.1			
Mass product (g)		1.207 ^a			
Yield (%)		96 ^a			
PMI		115.5 ^g			
PMI (without purification)		4.2 ^g			

^a Values extracted for the supporting information page 7 as per reference [11]

^b Values were inferred as they were not specified in the supporting information page 7 of reference [11].

^c Density of EtOAc at 298.15K and atmospheric pressure per reference [7].

^d Density of 1N HCl at 298.15K and atmospheric pressure per reference [8].

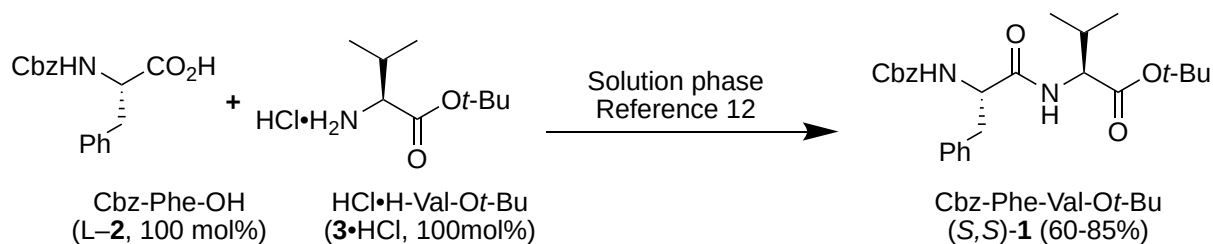
^e Density of 1N NaOH at 298.15K and atmospheric pressure per reference [8].

^f Density of brine at 298.15K and atmospheric pressure per reference [10].

^g PMI was calculated using the following formula: $PMI = \frac{\sum \text{Mass Of Materials}}{\text{Mass Of Isolated Product}}$.

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Equation S7. Previously Reported Solution-Phase Coupling (Cbz-Phe-OH + HCl·H-Val-Ot-Bu) to (S,S)-1.¹²

Table S7. Previously Reported Solution-Phase (Cbz-Phe-OH + HCl·H-Val-Ot-Bu): Reagents & PMI.^a

	Reagent	Number of times used	Mass or Volume (g or mL)	Density (g/mL)	Mass used (g)
Reaction	Cbz-Phe-OH	1	1.03	-	1.03
	HCl·H-Val-Ot-Bu	1	0.72	-	0.72
	BOP	1	1.52	-	1.52
	(<i>i</i> -Pr) ₂ NEt	1	1.34	-	1.34
	CH ₂ Cl ₂	1	10	1.34 ^a	13
Purification	EtOAc	1	25	0.894 ^b	22
	1M KHSO ₄	3	20	>1.04 ^c	62
	10% Na ₂ CO ₃	2	20	~ 1.11 ^d	44
	Brine	1	20	1.15 ^e	23
	Flash chromatography	N/A			
Total mass (g)		170.2			
Yield (%)		[60,85]			
Mass product (g)		[0.939, 1.33]			
PMI ^f		[127.9,181.2] ^g			
PMI (without purification)		[13.5,19.2] ^g			

^a Density of CH₂Cl₂ at 298.15K and atmospheric pressure per reference [13].

^b Density of EtOAc at 298.15K and atmospheric pressure per reference [7].

^c Density of KHSO₄ at 298.15K and atmospheric pressure per reference [14].

^d Density of 10% Na₂CO₃ at 298.15K and atmospheric pressure per reference [15].

^e Density of brine at 298.15K and atmospheric pressure per reference [10].

^f Without considering solvent used in chromatography reported in supporting information per reference [12].

^g PMI was calculated using the following formula: $PMI = \sum \text{Mass Of Materials} / \text{Mass Of Isolated Product}$.

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Experimental

General

Unless specified, solvents and reagents were purchased from commercial suppliers (MilliporeSigma, Oakwood, Combi-blocks) and used without further purification. Ethyl cyanohydroxyiminoacetate (Oxyma Pure®, “Oxyma”) was purchased from Combi-blocks Inc. San Diego, California, USA. Thin layer chromatography (TLC) was performed on glass plates coated with silica gel (Merck 60 F254 plates) and visualized by UV absorbance or staining with ninhydrin, potassium permanganate, and bromocresol green solutions. Melting points were obtained on a Büchi melting point B-540 apparatus and are uncorrected. Accurate mass measurements were performed on an LC-MSD instrument in electrospray ionization (ESI-TOF) mode at the Université de Montréal Mass Spectrometry Regional Centre and are reported as $[M]^+$ or $[M + H]^+$ ions. Nuclear magnetic resonance spectra ($^1H/^{13}C/^{19}F$) were recorded on Bruker instruments (700/175, 400/100 or 500/125/470 MHz). Chemical shifts are reported in parts per million (ppm) and coupling constant J values in Hertz (Hz). Spectroscopic 1H and decoupled $^{13}C\{^1H\}$ NMR experiments were recorded at room temperature (298 K) in $CDCl_3$ (7.26/77.16 ppm), CD_3OD (3.31/49.03 ppm), CD_3CN (1.94/118.26 ppm) and D_2O (4.79 ppm) using tetramethyl silane (TMS) as reference. Free induction decays (FIDs) were respectively processed into spectral data using TopSpin® or MestReNova software. Abbreviations are used to describe peak multiplicities: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quadruplet), m (multiplet), and br (broad). Signals of minor isomeric protons and carbons are respectively reported in brackets and parentheses.

Unless specified otherwise, LC-MS analyses were assessed on an Agilent 1100 series instrument coupled with an Agilent quadrupole 6110 detector using analytical HPLC on a $5\ \mu m \times 50\ mm \times 4.6\ mm$ C_{18} Phenomenex Gemini column with a flow rate of 0.80 mL/min at 30 °C. The mobile phase consisted of solvent I, H_2O containing 0.1% formic acid (FA) and solvent II, CH_3CN containing 0.1% FA or III CH_3OH containing 0.1% FA. The elution gradients were as follows: Method A, 10% (0-1 min), 10%-90% (1-9 min), 90% (9-10 min), and 10% (10-12 min) of II in I; Method B, 30% (0-1 min), 30%-95% (1-9 min), 95% (9-10 min), and 30% (10-12 min) of II in I; Method C, 50% (0-1 min), 50%-90% (1-9 min), 90% (9-10 min), and 50% (10-12 min) of III in I; Method D, 50% (0-1 min), 50%-90% (1-9 min), 90% (9-10 min), and 50% (10-12 min) of II in I.

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Analyses to measure diastereomeric ratios (d.r.) of tripeptides (*S,R,S*)- and (*S,S,S*)-**6** and **7** as well as dipeptide (*S,R*)- and (*S,S*)-**10** were ascertained by LC-MS on an Agilent 1260 infinity instrument coupled with an Agilent TOF 6224 detector using analytical HPLC on a 5 $\mu\text{m} \times 50 \text{ mm} \times 4.6 \text{ mm}$ C₁₈ Phenomenex Gemini column with a flow rate of 0.80 mL/min at 30 °C eluting with elution gradients of Method E, 30% (0-1 min), 30%-70% II (1-17 min), 70% (17-18 min), and 30% (18-20 min) of II in I and Method F, 30% (0-1 min), 30%-50% (1-17 min), 70% (17-18 min), and 30% (18-20 min) of II in I.

Chiral SFC-UV-MS was performed on an Agilent 1260 infinity instrument coupled with an Agilent quadrupole 6120 detector using the appropriate Chiralpak column. Method G employed a Chiralpak AD-H 4.6 mm $\times$ 250 mm $\times$ 5 μm column at 30 °C using 80% CO₂ and 20% isopropanol (IPA) as modifier with a 3 mL/min flow rate over 15 min under 150 bar of pressure. Method H employed a Chiralpak OJ-H 4.6 mm $\times$ 250 mm $\times$ 5 μm column at 30 °C using 90% CO₂ and 10% methanol as modifier with a 3 mL/min flow rate over 15 min under 150 bar of pressure.

Cbz-Phe-Val-Ot-Bu [(*S,S*)-1]

In a 20 mL vial, a mixture of Cbz-Phe-OH (**L-2**, 1.00g, 3.34 mmol, 100 mol%), HCl•H-Val-Ot-Bu (**L-3**, 1.05 g, 5.01 mmol, 150 mol%), Oxyma (570 mg, 4.01 mmol, 120 mol%), and (*i*-Pr)₂NEt (518 mg, 4.01 mmol, 120 mol%) in DME (3.45 mL, $\eta = 0.90 \text{ }\mu\text{L/mg}$) was manually agitated, and the suspension was treated with EDCI (769 mg, 4.01 mmol, 120 mol%). After sealing and standing for 1 min, the vial was mixed in the RAM at 60 g₀ for 10 min, when > 99% conversion was ascertained by LC-MS analysis (Method A, $\lambda = 214 \text{ nm}$): Cbz-Phe-OH ($R_t = 7.75 \text{ min}$); dipeptide (*S,S*)-**1** ($R_t = 10.05 \text{ min}$), which appeared on TLC as a less polar spot [$R_f = 0.31$ (1:4 EtOAc/hexanes, visualized using UV light)]. The reaction mixture was partitioned between EtOAc (10 mL) and water (5 mL) with agitation for 2 min and transferred to a separating funnel. The organic phase was washed with 1 N HCl (3 $\times$ 5 mL), sat. aq. NaHCO₃ (5 $\times$ 5 mL), and brine (5 mL), dried over Na₂SO₄, filtered, and evaporated to afford Cbz-Phe-Val-Ot-Bu [(*S,S*)-**1**, 1.50 g, 99%] as colorless foam: $R_t = 10.05 \text{ min}$ (Method A, $\lambda = 214 \text{ nm}$); $R_f = 0.31$ (1:4 EtOAc/hexanes, visualized using UV light). ¹H NMR (400 MHz, CDCl₃) δ 7.36-7.15 (m, 10H), 6.51 (br d, 1H, $J = 7.8 \text{ Hz}$), 5.52 (d, 1H, $J = 8.1 \text{ Hz}$), 5.08 (s, 2H), 4.58-4.45 (m, 1H), 4.37 (dd, 1H, $J = 8.5, 4.6 \text{ Hz}$), 3.13-3.02 (m, 2H), 2.16-2.03 (m, 1H), 1.45 (s, 9H), 0.86 (d, 3H, $J = 6.9 \text{ Hz}$), 0.84 (d, 3H, $J = 7.0 \text{ Hz}$); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 170.8, 170.5, 156.0, 136.4, 136.3, 129.4, 128.7, 128.6, 128.2, 128.1, 127.0, 82.0, 67.0, 57.6, 56.3, 38.5, 31.5, 28.1, 18.8, 17.8. HRMS (ESI-TOF) m/z

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$[M+H]^+$ calcd. for $[C_{26}H_{35}N_2O_5]^+$, 455.2540 found 455.2541. Employing Et_3N (406 mg, 4.01 mmol, 120 mol%) instead of $(i\text{-}Pr)_2NEt$ in the protocol described above gave Cbz-Phe-Val-*Ot*-Bu [(*S,S*)-**1**, 1.46 g, 95%] as colorless foam. Employing EtOAc (3.45 mL, $\eta = 0.90 \mu\text{L}/\text{mg}$) instead of DME in the protocol described above gave Cbz-Phe-Val-*Ot*-Bu [(*S,S*)-**1**, 1.36 g, 90%].

Cbz-Phe-D-Val-*Ot*-Bu [(*S,R*)-**1**]

Employing the protocol described for the synthesis of Cbz-Phe-Val-*Ot*-Bu, the reaction of Cbz-Phe-OH (**2**, 750 mg, 2.51 mmol, 100 mol%) and $HCl \cdot H\text{-D-Val-}Ot\text{-Bu}$ (**D-3**, 788 mg, 3.76 mmol, 150 mol%) afforded Cbz-Phe-D-Val-*Ot*-Bu [(*S,R*)-**1**, 1.10 g, 97% yield] as colorless foam: $R_t = 10.06$ min (Method A, $\lambda = 214$ nm): $R_f = 0.31$ (1:4 EtOAc/hexanes, visualized using UV light): 1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.17 (m, 10H), 6.35 (br d, $J = 8.2$ Hz, 1H), 5.47 (br d, $J = 7.13$ Hz, 1H), 5.10-5.03 (m, 2H), 4.57-4.41 (m, 1H), 4.35 (dd, $J = 8.6, 4.5$ Hz, 1H), 3.13-3.00 (m, 2H), 2.07-1.95 (m, 1H), 1.45 (s, 9H), 0.76 (d, 3H, $J = 7.2$ Hz), 0.74 (d, 3H, $J = 7.0$ Hz); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 170.7, 170.6, 155.9, 136.5, 136.3, 129.3, 128.8, 128.6, 128.2, 128.1, 127.1, 82.1, 67.1, 57.6, 56.5, 38.8, 31.4, 28.1, 18.8, 17.7. HRMS (ESI-TOF) m/z $[M+Na]^+$ calcd. for $[C_{26}H_{34}N_2O_5Na]^+$, 477.2358 found 477.2360. Examination of the diastereotopic methyl doublets at 0.86 and 0.84 ppm and 0.76 and 0.74 ppm for (*S,S*)- and (*S,R*)-Cbz-Phe-Val-*Ot*-Bu [(*S,S*)- and (*S,R*)-**1**] during incremental addition of 1% of the latter indicated a $> 99:0.5$ d.r. for the former.

Cbz-Phe-Val-OH [(*S,S*)-**4**]

To a solution of dipeptide (*S,S*)-**1** (1.00 g, 2.20 mmol, 100 mol%) in CH_2Cl_2 (2 mL), trifluoroacetic acid (2 mL) was added. The reaction mixture was stirred for 6 h, when TLC showed complete disappearance of the starting ester and appearance of a more polar product [$R_f = 0.40$ (1:9 CH_3OH/CH_2Cl_2 , visualized using UV light and bromocresol green)]. The volatiles were evaporated under reduced pressure to a residue which was sequentially treated and evaporated with CH_3OH (2×10 mL), EtOAc (2×10 mL) and ether (2×10 mL) to afford acid (*S,S*)-**4** (870 mg, 98%) as a beige powder which was used without further purification: $R_t = 7.87$ min (Method A, $\lambda = 214$ nm); $R_f = 0.4$ (1:9 CH_3OH/CH_2Cl_2 , visualized using UV light and bromocresol green).

Alternatively, a solution of dipeptide (*S,S*)-**1** (1.27 g, 2.79 mmol, 100 mol%) in 4 M HCl in 1,4-dioxane (4 mL) was stirred for 4 h, when a white precipitate appeared and TLC showed complete disappearance of the starting ester and appearance of a more polar product [$R_f = 0.40$ (1:9 CH_3OH/CH_2Cl_2 , visualized using UV light and bromocresol green)]. The volatiles were

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evaporated under reduced pressure to a residue which was sequentially treated and evaporated with 1,4-dioxane (2×2 mL), ether (2×5 mL), CH_3OH (3×5 mL) and ether (2×5 mL) to afford acid (*S,S*)-**4** (1.11 g, quant.) as a beige powder which exhibited identical chromatographic properties as above and was used without further purification.

Cbz-Phe-D-Val-OH [(*S,R*)-4**]**

Employing the protocol described for the preparation of acid (*S,S*)-**4**, ester (*S,R*)-**1** (500 mg, 1.10 mmol, 100 mol%) was solvolized using TFA in DCM to afford Cbz-Phe-D-Val-OH [(*S,R*)-**4**, 429 mg, 98% yield] as beige oil (429 mg, 98% yield): $R_t = 7.87$ min (Method A, $\lambda = 214$ nm); $R_f = 0.4$ (1:9 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, visualized using UV light and bromocresol green).

Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-6**]**

Employing the protocol described for the preparation of dipeptide (*S,S*)-**1** without base, acid (*S,S*)-**4** (100 mg, 0.19 mmol, 100 mol%) and proline amide (**5**, 38.3 mg, 0.23 mmol, 120 mol%) were coupled to afford Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 127 mg, 99% yield] as beige solid: $R_t = 7.12$ min (Method A, $\lambda = 214$ nm); $R_f = 0.24$ (100% EtOAc, visualized using UV light and bromocresol green); mp = 167-170 °C (lit.¹⁶ 168-170 °C).

Cbz-Phe-D-Val-Pro-NH₂ [(*S,R,S*)-6**]**

Employing the protocol described for the preparation of dipeptide (*S,S*)-**1** without base, acid (*S,R*)-**4** (100 mg, 0.19 mmol, 100 mol%) and proline amide (**5**, 38.3 mg, 0.23 mmol, 120 mol%) were coupled to afford Cbz-Phe-D-Val-Pro-NH₂ [(*S,R,S*)-**6**, 127 mg, 99% yield] as beige solid: $R_t = 7.47$ min (Method A, $\lambda = 214$ nm); $R_f = 0.24$ (100% EtOAc, visualized using UV light and bromocresol green).

Cbz-Ala-Phg-Ile-OMe [(*S,S,S*)-7**]**

Employing the protocol described for the preparation of dipeptide (*S,S*)-**1**, acid (*S,S*)-**8** (89.4 mg, 0.25 mmol, 100 mol%) and HCl•H-Ile-OMe (**9**, 68.4 mg, 0.38 mmol, 150 mol%) were coupled to give Cbz-Ala-Phg-Ile-OMe [(*S,S,S*)-**7**, 113 mg, 93% yield] as white solid: $R_f = 0.39$ (1:1 EtOAc/hexanes, visualized using UV light and KMnO_4); $R_t = 8.50$ min (Method A, $\lambda = 214$ nm); $R_f = 0.74$ (7:3 EtOAc/hexanes, visualized using UV light and KMnO_4); $R_f = 0.39$ (50% EtOAc in hexanes, visualized using UV light and KMnO_4); ^1H NMR (500 MHz, CDCl_3) δ 7.60 (br d, 1H, $J = 6.8$ Hz), 7.36-7.26 (m, 7H), 7.26-7.22 (m, 3H), 7.01 (br d, 1H, $J = 7.9$ Hz), 5.86 (br d, 1H, $J = 7.4$ Hz), 5.74 (br d, 1H, $J = 7.3$ Hz), 5.10-5.03 (m, 2H), 4.54-4.52 (m, 1H), 4.50-4.43 (m, 1H), 3.57 (s, 3H), 1.89-1.81 (m, 1H), 1.41-1.31 (m, 4H), 1.18-1.09 (m, 1H), 0.89-0.83 (m, 6H);

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$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ 172.3, 171.9, 169.8, 156.1, 137.3, 136.5, 129.0, 128.6, 128.4, 128.2, 128.1, 127.2, 67.0, 57.0, 52.1, 50.6, 38.0, 25.3, 19.3, 15.5, 11.6. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_6]^+$, 484.2448 found 484.2450.

Cbz-Ala-D-Phg-Ile-OMe [(S,R,S)-7]

Employing the protocol described for the preparation of dipeptide (S,S)-1, acid (S,R)-8 (89.4 mg, 0.25 mmol, 100 mol%) and $\text{HCl}\cdot\text{H-Ile-OMe}$ (9, 68.4 mg, 0.38 mmol, 150 mol%) were coupled to afford Cbz-Ala-D-Phg-Ile-OMe [(S,S,S)-7, 115 mg, 95% yield] as white solid: R_t = 8.64 min (Method A, λ = 214 nm); R_f = 0.70 (7:3 EtOAc/hexanes, visualized using UV light and KMnO_4). ^1H NMR (500 MHz, CDCl_3) δ 7.62 (br d, 1H, 6.0 Hz), 7.38-7.27 (m, 7H), 7.26-7.22 (m, 3H), 7.04 (br d, 1H, J = 8.2 Hz), 5.88 (br d, 1H, J = 7.2 Hz), 5.73 (br d, 1H, J = 6.6 Hz), 5.13 (d, 1H, J = 12.0 Hz), 5.07 (d, 1H, J = 11.9 Hz), 4.55 (dd, 1H, J = 8.8, 5.3 Hz), 4.47-4.38 (m, 1H), 3.64 (s, 3H), 1.78-1.71 (m, 1H), 1.36 (d, 3H, J = 7.0 Hz), 1.23-1.15 (m, 1H), 0.97-0.87 (m, 1H), 0.73 (dd, 3H, J = 7.4, 7.4 Hz), 0.64 (d, 3H, J = 6.9 Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ 172.3, 172.0, 169.8, 156.2, 138.1, 136.4, 128.9, 128.6, 128.3, 128.2, 127.0, 67.1, 56.9, 56.5, 52.3, 50.6, 38.0, 24.9, 18.9, 15.2, 11.4. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_6]^+$, 484.2448 found 484.2447.

Cbz-Ala-D-Phg-OH [(S,R)-8]

Employing the protocol described for the preparation of acid (S,S)-4 using 4 M HCl in 1,4-dioxane, dipeptide (S,R)-12 (500 mg, 1.21 mmol, 100 mol%) was solvolized to afford Cbz-Ala-D-Phg-OH [(S,R)-8, 432 mg, 99% yield] as beige powder: R_t = 6.93 min (Method A, λ = 214 nm); R_f = 0.24 (1:9 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, visualized using UV light and bromocresol green).

Cbz-Ala-Phg-OH [(S,S)-8]

Employing the protocol described for the preparation of acid (S,S)-4 using 4 M HCl in 1,4-dioxane, dipeptide ester (S,S)-12 (1.50, 3.64 mmol, 100 mol%) was solvolized to give Cbz-Ala-Phg-OH [(S,S)-8, 1.28 g, 99% yield] as beige powder: R_t = 6.87 min (Method A, λ = 214 nm); R_f = 0.24 (1:9 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, visualized using UV light and bromocresol green).

Cbz-D-Phg-Pro-NH₂ [(R,S)-10]

Employing the protocol described for the preparation of dipeptide (S,S)-1 without base, Cbz-D-Phg-OH (D-11, 250 mg, 0.88 mmol, 100 mol%) and H-Pro-NH₂ (5, 150 mg, 1.36 mmol, 150 mol%) were coupled to give Cbz-D-Phg-Pro-NH₂ [(R,S)-10, 332 mg, 99% yield] as white foam: R_f = 0.22 (100% EtOAc, visualized using UV light and KMnO_4); R_t = 6.36 min (Method F,

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$\lambda = 205$ nm). ^1H NMR (500 MHz, CDCl_3) 0.95:0.05 mixture of amide isomers δ 7.43-7.38 (m, 2H), 7.38-7.21 (m, 8H), 6.93 (br s, 1H), [6.50-6.46 (m, 1H)], 6.39-6.32 (m, 1H), 6.05-5.94 (m, 1H), [5.86 (br s, 1H), 5.75 (br s, 1H)], 5.49-5.40 (m, 1H), 5.08-4.97 (m, 2H), 4.53-4.44 (m, 1H), 3.78-3.70 (m, 1H), [3.67-3.62 (m, 1H), 3.57-3.53 (m, 1H)], 3.16-3.08 (m, 1H), [3.06-2.96 (m, 1H)], 2.23-2.12 (m, 1H), 2.04-1.92 (m, 1H), 1.89-1.69 (m, 2H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ (173.9) 173.8, 169.6, 155.9 (155.7), 136.2, 135.8, 129.2, 128.8, 128.5, 128.1, 128.0, 67.0, (60.9) 60.4 (60.0), (57.8) 57.5, (56.9), (47.3), 47.0, (29.7) 28.4, (24.8) 24.5. HRMS (ESI-TOF) m/z $[\text{M}+\text{Na}]^+$ calcd. for $[\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_4\text{Na}]^+$, 404.1580 found 404.1581.

Cbz-Phg-Pro-NH₂ [(*S,S*)-10]

Employing the protocol described for the preparation of dipeptide (*S,S*)-1 without base, Cbz-Phg-OH (**L-11**, 250 mg, 0.88 mmol, 100 mol%) and H-Pro-NH₂ (**5**, 150 mg, 1.36 mmol, 150 mol%) were coupled to give Cbz-Phg-Pro-NH₂ [(*S,S*)-10, 311 mg, 99% yield] as white powder: $R_f = 0.26$ (100 % EtOAc, visualized using UV light and KMnO_4); $R_t = 5.71$ min (Method F, $\lambda = 205$ nm). ^1H NMR (500 MHz, CDCl_3) 0.9:0.15 mixture of amide isomers δ 7.43-7.25 (m, 10H), [6.99-6.90 (m, 1H)], 6.72-6.57 (m, 1H), 6.38-6.30 (m, 1H), [6.31-6.25 (m, 1H)], 6.11-5.83 (m, 1H), 5.48 (d, 1H, $J = 7.6$ Hz), [5.43 (d, 1H, $J = 6.3$ Hz), 5.29-5.21 (m, 1H)], 5.12-5.07 (m, 1H), 5.05-5.00 (m, 1H), 4.63-4.50 (m, 1H), [4.12-4.08 (m, 1H), 3.78-3.68 (m, 1H)] 3.60-3.51 (m, 1H), [3.45-3.35 (m, 1H)], 3.23-3.11 (m, 1H), 2.25-2.13 (m, 1H), 2.03-1.74 (m, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ (173.9) 173.4, 169.8 (169.4, 156.1) 155.7, 136.6, 136.3 (136.2, 136.1, 129.4) 129.2 (129.1, 128.7) 128.5, 128.2, (128.10) 128.08, (128.02) 128.97, (67.1) 67.0, (60.4, 60.2) 60.0, (57.9), 57.5, (57.4) 57.0, (47.3) 47.1 (47.0, 31.6) 27.7, 24.9 (22.1). HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{21}\text{H}_{24}\text{N}_3\text{O}_4]^+$, 382.1761 found 382.1761.

Cbz-Ala-D-Phg-Ot-Bu [(*S,R*)-12]

Employing the protocol described for the preparation of dipeptide (*S,S*)-1, Cbz-Ala-OH (**13**, 500 mg, 2.24 mmol, 100 mol%) and $\text{HCl}\cdot\text{H-D-Phg-Ot-Bu}$ (**D-14**, 697 mg, 3.36 mmol, 150 mol%) were coupled to afford Cbz-Ala-D-Phg-Ot-Bu [(*S,R*)-12, 915 mg, 99%] as white powder: $R_t = 9.13$ min (Method A, $\lambda = 214$ nm); $R_f = 0.32$ (3:7 EtOAc/hexanes, visualized using UV light). mp = 113-116 °C. ^1H NMR (400 MHz, CDCl_3) δ 7.41-7.24 (m, 11H), 5.68 (br d, 1H, $J = 7.7$ Hz), 5.45 (d, 1H, $J = 7.2$ Hz), 5.16-5.03 (m, 2H), 4.48-4.33 (m, 1H), 1.37 (s, 9H), 1.34 (d, 3H, $J = 7.1$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 171.7, 169.7, 156.1, 137.0, 136.4, 128.8, 128.5, 128.2,

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128.12, 128.05, 127.0, 82.7, 67.0, 57.0, 50.4, 27.8, 18.7. HRMS (ESI-TOF) m/z $[M+Na]^+$ calcd. for $[C_{23}H_{28}N_2O_5Na]^+$, 435.1890 found 435.1887.

Cbz-Ala-Phg-Ot-Bu [(S,S)-12]

Employing the protocol described for the preparation of amide (S,S)-1, Cbz-Ala-OH (**13**, 1.00 g, 4.48 mmol, 100 mol%) and HCl•H-Phg-Ot-Bu (**L-14**, 1.39 g, 6.72 mmol, 150 mol%) were coupled to provide Cbz-Ala-Phg-Ot-Bu [(S,S)-**12**, 1.81 g, 98%] as white powder: R_t = 9.09 min (Method A, λ = 214 nm); R_f = 0.32 (3:7 EtOAc/hexanes, visualized using UV light); mp = 97.0-99.5 °C. 1H NMR (400 MHz, $CDCl_3$) δ 7.36-7.23 (m, 11H), 5.58 (d, 1H, J = 7.7 Hz), 5.42 (d, 1H, J = 7.3 Hz), 5.09-5.00 (m, 2H), 4.46-4.35 (m, 1H), 1.41-1.34 (m, 12H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 171.7, 169.6, 156.0, 136.9, 136.3, 128.8, 128.5, 128.24, 128.16, 128.1, 127.1, 82.7, 66.9, 57.1, 50.4, 27.9, 18.9. HRMS (ESI-TOF) m/z $[M+Na]^+$ calcd. for $[C_{23}H_{28}N_2O_5Na]^+$, 435.1890 found 435.1879. Employing Et_3N (544 mg, 5.38 mmol, 120 mol%) instead of (*i*-Pr) $_2NEt$ in the same protocol gave peptide (S,S)-**12** as white powder (1.76 g, 95%). Examination of the diastereotopic methyl group carbon signals at 18.9 and 18.7 ppm of Cbz-Ala-Phg-Ot-Bu [(S,S)-**12**] during incremental addition of 1% of Cbz-Ala-D-Phg-Ot-Bu [(S,R)-**12**] indicated > 99:1 d.r. of the former.

Fmoc-Trp(Boc)-ODmb [(S)-21]

In a 20 mL vial, a mixture of Fmoc-Trp(Boc)-OH (**L-15**, 1.00 g, 1.90 mmol, 100 mol%), 2,4-dimethoxybenzyl alcohol (383 mg, 2.28 mmol, 120 mol%) and Oxyma (324 mg, 2.28 mmol, 120 mol%) was treated with DME (2.20 mL, η = 0.90 μ L/mg) and (*i*-Pr) $_2NEt$ (295 mg, 2.28 mmol, 120 mol%). The reaction mixture was manually agitated, and the suspension was treated with EDCI (437 mg, 2.28 mmol, 120 mol%). After sealing and standing for 1 min, the vial was mixed in the RAM at 60 g θ for 10 min, when appearance of less polar spot was observed by TLC [R_f = 0.30 (2:3 EtOAc/hexanes, visualized under UV light)]. The reaction mixture was partitioned between EtOAc (10 mL) and water (5 mL) and transferred to a separating funnel. The organic phase was washed with 1 N HCl (3 $\times$ 5 mL), sat. aq. $NaHCO_3$ (5 $\times$ 5 mL), and brine (5 mL), dried over Na_2SO_4 , evaporated under reduced pressure to a residue which was transferred using Et_2O (2 $\times$ 5 mL) onto a pad of silica gel (5.0 g, 3 cm/5 cm; packed with Et_2O) on a sintered glass filter. After washing the pad with hexanes (3 $\times$ 22 mL), elution with 1:1 EtOAc/hexanes (4 $\times$ 22 mL) and evaporation of the collected fractions under reduced pressure afforded ester (S)-**21** as white foam (1.27 g, 99% yield): R_f = 0.30 (2:3 EtOAc/hexanes, visualized under UV light). 1H NMR

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(400 MHz, CDCl₃) δ 8.13 (br d, 1H, $J = 7.48$ Hz), 7.76 (br d, 2H, $J = 7.48$ Hz), 7.58-7.51 (m, 3H), 7.46 (s, 1H), 7.35-7.27 (m, 3H), 7.25-7.20 (m, 1H), 7.12 (br d, 1H, $J = 8$ Hz), 6.60 (br s, 1H), 6.65-6.40 (m, 2H), 5.55 (br d, 1H, $J = 8.1$ Hz), 5.14 (br s, 2H), 4.98 (ddd, 1H, $J = 18.7, 12.5, 6.3$ Hz), 4.84-4.77 (m, 1H), 4.39 (dd, 1H, $J = 10.6, 7.5$ Hz), 4.34 (dd, 1H, $J = 10.6, 7.5$ Hz), 4.23-4.17 (m, 1H), 3.81-3.78 (m, 6H), 3.35-2.99 (m, 2H), 1.66 (s, 9H); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 171.7, 161.6, 159.1, 156.5, 155.8, 149.6, 143.9, 143.8, 141.3, 135.4, 131.6, 130.7, 127.7, 127.14, 127.13, 125.2, 124.5, 124.3, 122.7, 120.0, 119.0, 115.8, 115.3, 115.1, 104.2, 98.6, 83.7, 76.8, 83.7, 70.0, 67.2, 63.2, 55.43, 55.39, 55.3, 47.2, 28.2, 27.9. HRMS (ESI-TOF) m/z [M+K]⁺ calcd. for [C₄₀H₄₀N₂O₈K]⁺, 715.2416 found 715.2396. Employing DME instead of EtOAc in the same protocol gave ester (*S*)-**21** (125 mg, 99%) as colorless foam. Analysis by chiral-SFC-MS (Method G) and incremental addition of Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**, $R_t = 6.04$ min] in Fmoc-Trp(Boc)-ODmb [(*S*)-**21**, $R_t = 10.72$ min] indicated that the d.r. of the latter was > 99.5:0.5.

Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**]

Employing the protocol described for the preparation of ester (*S*)-**21**, Fmoc-D-Trp(Boc)-OH (**D-15**, 100 mg, 0.19 mmol, 100 mol%) was esterified with 2,4-dimethoxybenzyl alcohol (38.3 mg, 0.23 mmol, 120 mol%) to afford Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**, 26 mg, 98% yield] as white foam that exhibited identical spectral properties as the enantiomer.

Fmoc-Lys(Boc)-OCH₂CH=CH₂ (**22**)

In a 20 mL vial, Fmoc-Lys(Boc)-OH (**16**, 3.02 g, 6.45 mmol, 100 mol%), Oxyma (1.28 g, 9.02 mmol, 140 mol%), allyl alcohol (0.66 mL, 0.56 g, 9.67 mmol, 150 mol%), (*i*-Pr)₂NEt (1.12 mL, 0.83 g, 6.45 mmol, 100 mol%) and EtOAc (7.2 mL, $\eta = 1.0$ μ L/mg) were mixed manually with a spatula and treated with EDCI (1.48 g, 7.73 mmol, 120 mol%). The vial was sealed and mixed in the RAM at 80 g₀ for 12 min, when 100% conversion of the starting amino acid ($R_t = 6.9$ min) to allyl ester **22** ($R_t = 8.3$ min) was ascertained by LC-MS analysis ($\lambda = 280$ nm, Method B). The reaction mixture was transferred to a separating funnel and diluted with EtOAc (75 mL). The organic layer was washed successively with water (3 $\times$ 75 mL), 0.1 N HCl (3 $\times$ 75 mL), sat. aq. NaHCO₃ (3 $\times$ 75 mL), and brine (50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield Fmoc-Lys(Boc)-OCH₂CH=CH₂ (**22**, 3.20 g, 98%) as a yellowish foam of 95% purity $\lambda = 280$ nm, Method B): $R_f = 0.30$ (50% EtOAc in hexanes, visualized by UV light). ¹H NMR (400 MHz, CD₃OD) δ 7.80 (d, 2H, $J = 7.5$ Hz), 7.67 (t, 2H, $J = 6.9$ Hz), 7.42-7.36 (m, 2H), 7.33-7.27 (m, 2H), 5.98-5.87 (m, 1H), 5.33 (dd, 1H, $J = 17.3, 1.6$ Hz), 5.21 (dd, 1H, $J = 10.5,$

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1.4 Hz), 4.65-4.59 (m, 2H), 4.42-4.30 (m, 2H), 4.27-4.14 (m, 2H), 3.07-2.98 (t, 2H, $J = 6.4$ Hz), 1.89-1.79 (m, 1H), 1.75-1.65 (m, 1H), 1.53-1.46 (m, 2H), 1.46-1.38 (overlapping m 2H and s 9H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD) δ 173.8, 158.7, 158.6, 145.3, 145.2, 142.6, 133.4, 128.8, 128.2, 128.2, 126.3, 126.2, 120.9, 118.6, 79.9, 68.0, 66.7, 55.5, 48.4, 41.0, 32.2, 30.5, 28.8, 24.2. HRMS (ESI-TOF) m/z $[\text{M}+\text{Na}]^+$ calcd. for $[\text{C}_{29}\text{H}_{36}\text{N}_2\text{O}_6\text{Na}]$, 531.2466 found 531.2470.

H-D-Val-ODmb [(R)-24]

In a 20 mL vial, Fmoc-D-Val-OH (D-17, 1.00 g, 2.95 mmol, 100 mol%), EDCI (678 mg, 3.54 mmol, 120 mol%), Oxyma (838 mg, 5.90 mmol, 200 mol%) and 2,4-dimethoxybenzyl alcohol (646 mg, 3.84 mmol, 130 mol%) were ground with a spatula, suspended in EtOAc (4.4 mL, $\eta = 1.0$ $\mu\text{L}/\text{mg}$), and treated with (*i*-Pr) $_2\text{NEt}$ (1.04 mL, 896 mg, 5.90 mmol, 200 mol%). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g θ for 12 min and partitioned between H $_2$ O (10 mL) and EtOAc (50 mL). The organic phase was washed successfully with H $_2$ O (2 x 10 mL), 0.5 M aq. KHSO_4 (3 x 10 mL), sat. aq. NaHCO_3 (3 x 10 mL), and brine (10 mL), dried over Na_2SO_4 , filtered, and concentrated under reduce pressure to provide Fmoc-D-Val-ODmb [(R)-23] as orange foam: $R_f = 0.29$ (1:4 EtOAc/hexanes, visualized under UV light and with ninhydrin) of 93% purity {LC-MS, Method A, $\lambda = 254$ nm, $R_t = 11.12$ min; $m/z = 512.2$ $[\text{M}+\text{Na}]^+$ }; HRMS (ESI-TOF) m/z $[\text{M}+\text{NH}_4]^+$ calcd. for $[\text{C}_{29}\text{H}_{40}\text{N}_3\text{O}_6]^+$, 507.2490 found 507.2489.

In a 20 mL vial, Fmoc-D-Val-ODmb [(R)-23 from above, 3.54 mmol, 82% purity] was treated with a solution of 5 vol% of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in EtOAc (5.00 mL, 1.00 mmol, 40 mol%). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g θ for 12 min, diluted with EtOAc to a volume of 100 mL, and extracted with ice-cooled 0.05 M aq. KHSO_4 (3 x 10 mL). The combined aqueous phase was collected in an ice-cooled Erlenmeyer flask, adjusted to pH 9 using 50% aq. NaOH , and extracted with EtOAc (3 x 25 mL). The combined organic extractions was dried over Na_2SO_4 , filtered, and concentrated under reduce pressure to provide H-D-Val-ODmb [(R)-24] as pale-yellow oil (564 mg, 72% yield from Fmoc-D-Val-OH (D-17): $R_f = 0.58$ (1:9 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, visualized with UV light and ninhydrin); $R_t = 10.99$ min [$> 99\%$ purity (Method A, $\lambda = 254$ nm)]. ^1H NMR (400 MHz, CD_3CN) δ 7.24 (d, 1H, $J = 8.5$ Hz), 6.54 (d, 1H, $J = 2.4$ Hz), 6.49 (dd, 1H, $J = 2.5, 8.4$ Hz), 5.09 (d, 1H, $J = 11.7$ Hz), 4.99 (d, 1H, $J = 11.8$ Hz), 3.80 (s, 3H), 3.79 (s, 3H), 3.16 (d, 1H, $J = 5.2$ Hz), 1.91-1.80 (m, 1H), 1.62 (br s, 2H), 0.88 (d, 3H, $J = 6.9$ Hz), 0.83 (d, 3H, $J = 7.0$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz,

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CD₃CN) δ 176.6, 162.5, 160.1, 132.5, 117.6, 105.4, 99.3, 62.4, 60.9, 56.2, 56.1, 33.3, 19.5, 17.6.

HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₁₄H₂₁NO₄Na]⁺: 290.1363, found: 290.1364.

H-L-Val-ODmb [(S)-24] was prepared by the same protocol used to synthesize H-D-Val-ODmb [(R)-24] employing Fmoc-L-Val-OH (L-17, 1.00 g, 2.95 mmol, 100 mol%), EDCI (678 mg, 3.54 mmol, 120 mol%), Oxyma (838 mg, 5.90 mmol, 200 mol%), 2,4-dimethoxybenzyl alcohol (646 mg, 3.84 mmol, 130 mol%) and (*i*-Pr)₂NEt (1.04 mL, 896 mg, 5.90 mmol, 200 mol%) in EtOAc (4.4 mL, η = 1.0 μ L/mg) to give Fmoc-L-Val-ODmb [(S)-23, 3.54 mmol, R_f = 0.29 (1:4 EtOAc/hexanes, visualized using UV light and ninhydrin)] of 90% purity {LC-MS, Method A, λ = 254 nm, R_t = 11.07 min.; m/z = 512.2 [M+Na]⁺; HRMS (ESI-TOF) m/z [M+NH₄]⁺ calcd. for [C₂₉H₄₀N₃O₆]⁺, 507.2490 found 507.2501}. Treatment with a solution of 5 vol% DBU in EtOAc (5.00 mL, 1.00 mmol, 40 mol%) and aqueous workup provided H-L-Val-ODmb [(S)-24] as pale-yellow oil [239 mg, 75% yield from Fmoc-L-Val-OH (L-17)]: R_f = 0.58 (1:9 CH₃OH/CH₂Cl₂, visualized with UV light and ninhydrin); R_t = 10.98 min [$> 99\%$ purity (Method A, λ = 254 nm)]. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₁₄H₂₁NO₄Na]⁺, 290.1363 found 290.1366.

Boc-Leu-NH₂ [(S)-25]

Employing the protocol described for the preparation of dipeptide (S,S)-1 with a modified purification procedure detailed below, Boc-Leu-OH (L-18, 2.00 g, 8.65 mmol, 100 mol%) and NH₄Cl (694 mg, 13.0 mmol, 150 mol%) were coupled to afford Boc-Leu-NH₂ [(S)-25] as white solid. After the reaction, the mixture was evaporated under reduced pressure, and the residue was partitioned between EtOAc (15 mL) and water (2 mL). The organic layer was washed with sat. aq. NaH₂PO₄ (3 $\times$ 5 mL), sat. aq. NaHCO₃ (3 $\times$ 5 mL), and brine (5 mL), dried over Na₂SO₄, filtered and concentrated to a residue, which crystallized from EtOAc on addition of CH₂Cl₂ when cooling to 4 °C; white needles (1.64 g, 84% yield) were collected by filtration: R_f = 0.31 (100 % EtOAc, visualized using KMnO₄). ¹H NMR (500 MHz, CDCl₃) showed a 85:15 mixture of carbamate isomer δ 6.49 (br s, 1H), [6.38 (br s, 1H), 6.04 (br s, 1H)], 5.96 (br s, 1H), [5.41 (br s, 1H)], 5.19-5.00 (m, 1H), 4.24-4.09 (m, 1H), [4.08-3.90 (m, 1H)], 1.73-1.66 (m, 1H), 1.65-1.60 (m, 1H), 1.52-1.46 (m, 1H), 1.42 (s, 9H), 0.93 (d, 3H, J = 6.6 Hz), 0.92 (d, 3H, J = 6.5 Hz); ¹³C {¹H} NMR (125 MHz, CDCl₃) δ 175.7, 156.0, 80.2, 52.7, 41.4, 28.4, 24.9, 23.1, 22.0. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₁₁H₂₂N₃O₃Na]⁺, 253.1523, found 253.1511.

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Boc-Lys(COCF₃)-NH₂ [(S)-26]

Employing the protocol described for the preparation of dipeptide (S,S)-1 with a modified purification procedure detailed below, Boc-Lys(COCF₃)-OH (L-19, 500 mg, 1.46 mmol, 100 mol%, prepared according lit.¹⁷) and NH₄Cl (117 mg, 2.19 mmol, 150 mol%) were coupled to afford Boc-Lys(COCF₃)-NH₂ [(S)-26] as white solid. After the reaction, the mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc (10 mL) and water (2 mL). The organic layer was washed with water (2 mL), sat. aq. NaH₂PO₄ (2 × 5 mL), sat. aq. NaHCO₃ (2 × 5 mL), and brine (5 mL). On standing, a white precipitate formed in the organic layer which was cooled to 4 °C for 18 h. White crystalline solid (474 mg, 95% yield) was isolated after filtration, washing with ice cold EtOAc (3 × 3 mL) and drying under vacuum: *R*_f = 0.45 (100 % EtOAc, visualized using KMnO₄). ¹H NMR (500 MHz, CD₃OD) as a 1:5.25 mixture of carbamate isomers δ 4.05-3.96 (m, 1H), [3.96-3.88 (m, 1H)], 3.30-3.26 (m, 2H), 1.81-1.71 (m, 1H), 1.67-1.54 (m, 3H), 1.49-1.35 (m, 2H), 1.44 (s, 9H); ¹⁹F NMR (470 MHz, CD₃OD) δ -73.4; ¹³C{¹H} NMR (125 MHz, CD₃OD) as a mixture of carbamate isomers δ (178.3) 178.0, 159.0 (q, *J* = 36.7 Hz), 157.9, (157.4), 117.6 (q, *J* = 286.6 Hz), (81.6), 80.6, 57.1, (55.7), 40.5, 33.0, 29.4, 28.7, 24.2. HRMS (ESI-TOF) *m/z* [M+Na]⁺ calcd. for [C₁₃H₂₂N₃O₄F₃Na]⁺, 364.1455 found 364.1442.

H-D-Leu-NHCH₂CCH (28)

In a 20 mL vial, Fmoc-D-Leu-OH (D-20, 1.00 g, 2.83 mmol, 100 mol%), EDCI (649 mg, 3.39 mmol, 120 mol%), and Oxyma (681 mg, 4.79 mmol, 170 mol%) were ground with a spatula into a powder, suspended in EtOAc (3 mL, η = 1.0 μL/mg), and treated with propargylamine (220 μL, 189 mg, 3.39 mmol, 120 mol%). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g₀ for 8 min. The homogeneous solution was partitioned between H₂O (20 mL) and EtOAc (100 mL). The organic phase was washed successfully with H₂O (2 x 20 mL), 0.5 M aq. KHSO₄ (3 × 20 mL), sat. aq. NaHCO₃ (3 × 20 mL), and brine (20 mL), dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Analysis of the resulting beige solid (976 mg) showed Fmoc-D-Leu-NHCH₂CCH [27, *R*_f = 0.53 (2:3 EtOAc/hexanes, visualized using UV light and ninhydrin)] of 87% purity {LC-MS, Method C, λ = 254 nm, *R*_t = 9.06 min; *m/z* = 391.1 [M+H]⁺} containing 13% of recovered Fmoc-D-Leu-OH {*R*_t = 9.41, Method C; *m/z* = 376.1 [M+Na]⁺}. Employment of THF as reaction solvent gave Fmoc-D-Leu-NHCH₂CCH (27, 495 mg,

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90% yield) in >99% conversion as pale beige, solid: ^1H NMR (400 MHz, CD_3CN) δ 7.84 (d, 2H, $J = 7.5$ Hz), 7.67 (dd, 2H, $J = 3.2, 7.4$ Hz), 7.42 (t, 2H, $J = 7.2$ Hz), 7.34 (dt, 2H, $J = 1.3, 7.3$ Hz), 6.97 (br s, 1H), 5.92 (br d, 1H, $J = 7.3$ Hz), 4.39 (dd, 1H, $J = 7.0, 10.7$ Hz), 4.31 (dd, 1H, $J = 7.5, 10.1$ Hz), 4.23 (t, 1H, $J = 6.9$ Hz), 4.04 (dd, 1H, $J = 8.5, 15.4$ Hz), 3.91 (dd, 2H, $J = 2.0, 5.6$ Hz), 2.41 (t, 1H, $J = 2.5$ Hz), 1.63 (sept., 1H, $J = 6.7$ Hz), 1.52 (d, 1H, $J = 6.7$ Hz), 1.50 (d, 1H, $J = 5.6$ Hz), 0.93 (d, 3H, $J = 6.6$ Hz), 0.89 (d, 3H, $J = 6.4$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3CN) δ 173.2, 157.1, 145.2, 142.2, 128.7, 128.7, 128.1, 126.2, 121.0, 81.3, 71.7, 67.2, 54.5, 48.1, 41.7, 29.1, 25.5, 23.3, 21.7. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_3]^+$, 391.2016 found 391.2010. **Fmoc-Leu-NHCH₂CCH [(S)-27]**, 476 mg, 86%] was prepared from Fmoc-L-Leu-OH (**L-20**) in THF using the same reaction conditions to prepare Fmoc-D-Leu-NHCH₂CCH [(R)-27]. Analysis by chiral-SFC-MS (Method H) and incremental addition of Fmoc-D-Leu-NHCH₂CCH [(R)-27, $R_t = 8.48$ min] in Fmoc-Leu-NHCH₂CCH [(S)-27, $R_t = 3.61$ min] indicated that the e.r. of the latter was > 99.5:0.5.

In a 20 mL vial, Fmoc-D-Leu-NHCH₂CCH [(R)-27 from above, 200 mg, 0.51 mmol, 87% purity) was treated with a solution of 5 vol% DBU in EtOAc (0.60 mL, 0.20 mmol). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g_o for 12 min, diluted with EtOAc to reach a volume of 100 mL, and extracted with 0.5 M aq. KHSO₄ (3 $\times$ 5 mL). The combined aqueous layers was adjusted to pH 9 using 50% aq. NaOH and extracted with EtOAc (3 $\times$ 25 mL). The combined organic extractions was dried over Na₂SO₄, filtered, and concentrated under reduce pressure to provide H-D-Leu-NHCH₂CCH (**28**) as pale-yellow oil [75 mg, 87% yield from Fmoc-D-Leu-OH (**D-20**): $R_f = 0.32$ (1:9 CH₃OH/CH₂Cl₂, visualized with ninhydrin). ^1H NMR (400 MHz, CD_3CN) δ 7.54 (br s, 1H), 3.92 (dd, 2H, $J = 2.5, 6.0$ Hz), 3.24 (dd, 1H, $J = 4.4, 9.7$ Hz), 2.40 (t, 1H, $J = 2.5$ Hz), 1.80-1.67 (m, 1H), 1.55 (br s, 2H), 1.52 (ddd, 1H, $J = 4.4, 9.3, 13.6$ Hz), 1.27 (ddd, 1H, $J = 5.1, 9.8, 14.7$ Hz), 0.93 (d, 3H, $J = 6.6$ Hz), 0.90 (d, 3H, $J = 6.6$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3CN) δ 176.6, 81.8, 71.4, 54.2, 45.1, 28.8, 25.4, 23.7, 21.7. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_9\text{H}_{17}\text{N}_2\text{O}]^+$, 169.1335 found 169.1334.

H-Trp(Boc)-ODmb (29)

In a 20 mL vial, Fmoc-Trp(Boc)-ODmb [(S)-21, 1.00 g, 1.48 mmol, 100 mol%] was treated with DME (1.95 mL), diethylamine (1.08 g, 14.8 mmol, 1000 mol%) and DBU (22.5 mg, 0.15 mmol, 40 mol%). After sealing and standing for 1 min, the vial was mixed with the RAM at 60 g_o for 10 min, when TLC showed complete disappearance of the starting material and appearance of

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a more polar spot [$R_f = 0.71$ (1:9 CH₃OH/CH₂Cl₂, visualized using UV light and ninhydrin)]. The volatiles were removed under reduced pressure. The residue was partitioned between EtOAc (10 mL) and water (10 mL). The organic phase was washed with 1N KHSO₄ (3 × 5 mL), sat. aq. NaHCO₃ (3 × 5 mL), water (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered, and evaporated to afford a residue which was partitioned between CH₃CN (5 mL) and hexanes (10 mL). The bottom layer was washed with hexanes (2 × 10 mL) and concentrated under reduced pressure to a residue which was deposited on a pad of silica gel (3 × 3 × 5 cm) wet packed with CH₂Cl₂. The pad was washed with CH₂Cl₂ (5 × 22 mL) and with 10% EtOAc in CH₂Cl₂ (22 mL). Elution with EtOAc (7 × 22 mL) and evaporation of the collected fractions under reduced pressure afforded H-Trp(Boc)-ODmb (**29**) as viscous yellow oil (658 mg, 98%): $R_f = 0.71$ (1:9 CH₃OH/CH₂Cl₂, visualized using UV light and ninhydrin); $R_t = 5.83$ min (Method A, $\lambda = 214$ nm). HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₂₁H₂₄N₃O₄]⁺, 455.2178 found 455.2196.

o-Nps-Ala-Trp(Boc)-ODmb [(*S,S*)-**30**]

Employing the protocol described for the preparation of dipeptide (*S,S*)-**1** without base, *o*-Nps-Ala-OH (**35**, 150 mg, 0.55 mmol, 105 mol%) and H-Trp(Boc)-ODmb (**29**, 237 mg, 0.52 mmol, 100 mol%) were coupled to afford *o*-Nps-Ala-Trp(Boc)-ODmb [(*S,S*)-**30**, 381 mg, 98% yield] as white powder: $R_f = 0.46$ (2:3 EtOAc/hexanes, visualized using UV light and KMnO₄). ¹H NMR (400 MHz, CDCl₃) δ 8.11 (br d, 1H, $J = 7.7$ Hz), 7.98 (dd, 1H, $J = 7.6, 1.6$ Hz), 7.75 (dd, 1H, $J = 7.8, 1.3$ Hz), 7.59-7.55 (m, 1H), 7.54-7.50 (m, 1H), 7.48-7.42 (m, 1H), 7.50 (br s, 1H), 7.33-7.27 (m, 1H), 7.23-7.18 (m, 1H), 7.09-7.03 (m, 1H), 6.89 (br d, 1H, $J = 7.7$ Hz), 6.44-6.39 (m, 2H), 6.12 (br d, 1H, $J = 7.9$ Hz), 5.08 (d, 1H, $J = 12.4$ Hz), 5.05 (d, 1H, $J = 12.4$ Hz), 4.80-4.73 (m, 1H), 4.11-4.03 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.16 (dd, 1H, $J = 14.9, 5.6$ Hz), 3.07 (dd, 1H, $J = 14.9, 5.9$ Hz), 1.65 (s, 9H), 1.29 (d, 3H, $J = 7.1$ Hz); ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 171.1, 170.7, 161.5, 159.0, 149.5, 147.6, 135.2, 133.7, 133.5, 132.8, 131.5, 130.6, 130.4, 125.5, 124.5, 124.2, 122.6, 118.8, 115.5, 115.3, 114.8, 104.1, 98.4, 83.8, 63.2, 55.34, 55.32, 53.3, 52.7, 28.1, 27.3, 19.3. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₃₄H₃₈N₄O₁₁SNa]⁺, 733.2150 found 733.2141. Employing 1 g of *o*-Nps-Ala-OH (**35**) in the same protocol gave dipeptide (*S,S*)-**30** (1.4 g, 94% yield) as beige powder.

Fmoc-Arg(Pbf)-Gly-OEt (**31**)

Employing the protocol described for the preparation of dipeptide (*S,S*)-**1**, Fmoc-Arg(Pbf)-OH (**36**, 1.00 g, 1.54 mmol, 100 mol%) and HCl•H-Gly-OEt (**37**, 323 mg, 2.31 mmol, 150 mol%)

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were coupled to furnish Fmoc-Arg(Pbf)-Gly-OEt [(*S*)-**31**, 1.01 g, 89% yield] as white powder: R_f = 0.40 (100 % EtOAc, visualized using UV light). ^1H NMR (500 MHz, CDCl_3) δ 7.71 (d, 2H, J = 7.5 Hz), 7.69-7.76 (m, 1H), 7.58-7.50 (m, 2H), 7.34 (t, 2H, J = 7.4 Hz), 7.25-7.18 (m, 2H), 6.53-6.35 (m, 2H), 6.28-6.10 (m, 2H), 4.42-4.34 (m, 1H), 4.34-4.25 (m, 2H), 4.16-4.07 (m, 3H), 4.02 (dd, 1H, J = 17.5, 5.5 Hz), 3.88 (dd, 1H, J = 17.5, 5.0 Hz), 3.44-3.28 (m, 1H), 3.27-3.09 (m, 1H), 2.94-2.82 (m, 2H), 2.58 (s, 3H), 2.50 (s, 3H), 2.06 (s, 3H), 2.00-1.88 (m, 1H), 1.78-1.68 (m, 1H), 1.68-1.57 (m, 2H), 1.42 (s, 6H), 1.20 (t, 3H, J = 7.1 Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ 173.0, 170.2, 158.9, 156.6, 143.9, 143.8, 141.25, 138.4, 132.6 (br), 132.4, 127.7, 127.1, 125.2, 124.8, 120.0, 117.7, 86.5, 67.2, 61.5, 54.2, 47.1, 43.2, 41.3, 40.2 (br), 30.0, 28.6, 25.3 (br), 19.4, 18.0, 14.1, 12.5. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{38}\text{H}_{48}\text{N}_5\text{O}_8\text{S}]^+$, 734.3218 found 734.3208.

Fmoc-Aib-Aib-Ot-Bu (**32**)

In a 20 mL vial, a mixture of Fmoc-Aib-OH (**38**, 200 mg, 0.615 mmol, 100 mol%), $\text{HCl}\cdot\text{H-Aib-Ot-Bu}$ (**39**, 147 mg, 0.92 mmol, 150 mol%), Oxyma (191 mg, 1.23 mmol, 300 mol%), and Et_3N (93.3 mg, 0.92 mmol, 200 mol%) in DME (0.90 mL, η = 1.0 $\mu\text{L}/\text{mg}$) was manually agitated and treated with EDCI (191 mg, 1.23 mmol, 200 mol%). After sealing and standing for 1 min, the vial was mixed in the RAM at 80 g ϕ for 40 min, when > 94% conversion was ascertained by LC-MS analysis (Method A, λ = 214 nm): Fmoc-Aib-OH (**38**, R_t = 8.47 min); peptide **32** (R_t = 10.45 min). The reaction mixture was partitioned between EtOAc (30 mL) and water (15 mL) with agitation for 2 min, transferred to a separating funnel. The organic phase was washed with 1 N HCl (3 $\times$ 15 mL), sat. aq. NaHCO_3 (3 $\times$ 15 mL), and brine (15 mL), dried over Na_2SO_4 , filtered, and evaporated to afford Fmoc-Aib-Aib-Ot-Bu (**32**, 235 mg, 82%) as white solid: R_f = 0.47 (2:3 EtOAc/hexanes, visualized using UV light). ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, 2H, J = 7.2 Hz), 7.60 (d, 2H, J = 7.3 Hz), 7.40 (t, 2H, J = 7.6 Hz), 7.31 (t, 2H, J = 7.2 Hz), 6.90 (s, 1H), 5.51 (s, 1H), 4.39 (d, 2H, J = 6.7 Hz), 4.21 (t, 1H, J = 6.8 Hz), 1.53 (s, 11H), 1.45 (s, 10H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 174.0, 173.4, 155.1, 144.1, 141.5, 127.9, 127.2, 125.2, 120.1, 81.9, 66.7, 57.1, 56.9, 47.4, 28.0, 25.5, 24.3. HRMS (ESI-TOF) m/z $[\text{M}+\text{Na}]^+$ calcd. for $[\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_5\text{Na}]^+$, 489.2359 found 489.2354.

H-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**33**)

In a 20 mL vial, H-D-Leu-NHCH₂CCH (**28**, 202 mg, 1.20 mmol, 100 mol%), Fmoc-D-Glu(*t*-Bu)-OH (**40**, 510 mg, 1.20 mmol, 100 mol%), EDCI (276 mg, 1.44 mmol, 120 mol%) and

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Oxyima (289 mg, 2.03 mmol, 170 mol%) were suspended in EtOAc (1.3 mL, $\eta = 1.0 \mu\text{L}/\text{mg}$). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g₀ for 8 min, and the reaction mixture was partitioned between H₂O (20 mL) and EtOAc (100 mL). The organic phase was washed successfully with H₂O (2 x 20 mL), 0.5 M aq. KHSO₄ (3 x 20 mL), sat. aq. NaHCO₃ (3 x 20 mL) and brine (20 mL), dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Analysis of the resulting beige solid (180 mg) showed Fmoc-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [**41**, 690 mg, $R_f = 0.27$ (2:3 EtOAc/hexanes, visualized with UV light and ninhydrin)] of 84% purity {LC-MS, Method A, $\lambda = 254 \text{ nm}$, $R_t = 9.96 \text{ min}$,; $m/z = 598.3$ [M+Na]⁺} containing 16% of recovered Fmoc-D-Glu(*t*-Bu)-OH [**40**, $R_t = 9.38 \text{ min}$, Method A; $m/z = 448.1$ [M+Na]⁺]: HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₃₃H₄₂N₃O₆]⁺, 576.3068 found 576.3073.

In a 20 mL vial, Fmoc-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**41** from above, 400 mg, 0.69 mmol, 84% purity) was treated with a solution of 5 vol% DBU in EtOAc (1.2 mL, 0.40 mmol, 58 mol%). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g₀ for 12 min, diluted with EtOAc to reach a volume of 100 mL, and extracted with ice-cooled 0.05 M aq. KHSO₄ (3 x 5 mL). The combined aqueous phase was collected in an ice-cooled Erlenmeyer flask, adjusted to pH 9 using 50% aq. NaOH, and extracted with EtOAc (3 x 25 mL). The combined organic extractions was dried over Na₂SO₄, filtered, and concentrated under reduce pressure to provide H-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**33**) as pale-yellow oil [194 mg, 80% from Fmoc-D-Glu(*t*-Bu)-OH (**40**): $R_f = 0.52$ (1:9 CH₃OH/CH₂Cl₂, visualized with ninhydrin). ¹H NMR (400 MHz, CDCl₃) δ 7.60 (br d, 1H, $J = 7.6 \text{ Hz}$), 6.51 (br s, 1H), 4.41-4.32 (m, 1H), 4.05 (ddd, 1H, $J = 2.8, 5.5, 17.9 \text{ Hz}$), 3.98 (dd, 1H, $J = 2.4, 5.1, 17.5 \text{ Hz}$), 3.43 (dd, 1H, $J = 5.1, 7.4 \text{ Hz}$), 2.35 (t, 2H, $J = 7.3 \text{ Hz}$), 2.21 (t, 2H, $J = 2.4 \text{ Hz}$), 2.17-2.04 (m, 1H), 1.90-1.70 (m, 1H), 1.70-1.55 (m, 2H), 1.53 (br s, 2H), 1.45 (s, 9H), 0.96 (d, 3H, $J = 6.3 \text{ Hz}$), 0.92 (d, 3H, $J = 6.1 \text{ Hz}$); ¹³C{¹H} NMR (100 MHz, CD₃CN) δ 175.7, 173.5, 173.0, 81.3, 80.7, 71.7, 55.3, 52.1, 41.9, 32.5, 31.1, 29.1, 28.3, 25.5, 23.4, 21.7. HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₁₈H₃₂N₃O₄]⁺, 354.2387 found 354.2383.

Fmoc-D-Thr(*t*-Bu)-D-Val-ODmb [(*R,R*)-34**]** was prepared using the protocol to synthesize and purify diastereomer (*R,S*)-**34** using Fmoc-D-Thr(*t*-Bu)-OH (**42**, 158 mg, 0.50 mmol, 100 mol%), H-D-Val-ODmb [(*R*)-**24**, 202 mg, 0.75 mmol, 150 mol%], EDCI (192 mg, 1.00 mmol, 100 mol%), and Oxyima (213 mg, 1.50 mmol, 300 mol%) in EtOAc (0.80 mL, $\eta = 1.0 \mu\text{L}/\text{mg}$). Analysis of the light orange foam showed Fmoc-D-Thr(*Ot*-Bu)-D-Val-ODmb [(*R,R*)-**34**, $R_f = 0.22$ (1:4

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EtOAc/hexanes, visualized with UV light and ninhydrin)] of 99% purity {LC-MS, Method D, $\lambda = 254$ nm, $R_t = 10.58$ min; $m/z = 669.2$ $[M+Na]^+$ containing recovered Fmoc-D-Thr(*t*-Bu)-OH {**42**, $R_t = 5.93$, Method D; $m/z = 420.1$ $[M+Na]^+$ }. Purification of 190 mg of the residue by filtration on a silica gel pad (4 x 4 cm) wet packed with hexanes eluting with hexanes (3 $\times$ 25 mL), 20% EtOAc in hexanes (3 $\times$ 25 mL), and 40% EtOAc in hexanes (3 $\times$ 25 mL). Evaporation of the latter fractions gave Fmoc-D-Thr(*t*-Bu)-D-Val-ODmb [(*R,R*)-**34**, 166 mg, 95%] as transparent oil: 1H NMR (400 MHz, $CDCl_3$) δ 7.85 (br d, 1H, $J = 8.2$ Hz), 7.76 (d, 2H, $J = 7.7$ Hz), 7.60 (d, 2H, $J = 7.4$ Hz), 7.40 (tt, 2H, $J = 1.4, 7.7$ Hz), 7.31 (tt, 2H, $J = 0.9, 7.2$ Hz), 7.26-7.21 (m, 1H), 6.51-6.42 (m, 2H), 6.04 (br d, 1H, $J = 4.9$ Hz), 5.22 (d, 1H, $J = 11.6$ Hz), 5.10 (d, 1H, $J = 11.8$ Hz), 4.51 (dd, 1H, $J = 4.2, 8.5$ Hz), 4.42-4.33 (m, 2H), 4.27-4.20 (m, 2H), 4.21-4.13 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.28-2.18 (m, 1H), 1.30 (s, 9H), 1.09 (d, 3H, $J = 6.3$ Hz), 0.94 (d, 3H, $J = 6.9$ Hz), 0.91 (d, 3H, $J = 7.0$ Hz); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 171.5, 169.7, 161.6, 159.2, 156.2, 144.1, 143.9, 141.4, 141.4, 131.8, 127.8, 127.2, 125.3, 120.1, 120.1, 116.4, 104.0, 98.6, 75.7, 67.1, 67.0, 62.6, 58.4, 57.7, 55.5, 55.4, 47.3, 30.9, 28.3, 19.2, 17.5, 16.2. HRMS (ESI-TOF) m/z $[M+H]^+$ calcd. for $[C_{37}H_{47}N_2O_8]^+$, 647.3327 found 647.3347.

Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*R,S*)-**34**]

In a 20 mL vial, Fmoc-D-Thr(*t*-Bu)-OH (**42**, 198 mg, 0.50 mmol, 100 mol%), H-L-Val-ODmb [(*S*)-**24**, 134 mg, 0.50 mmol, 100 mol%], EDCI (192 mg, 1.00 mmol, 200 mol%), and Oxyma (213 mg, 1.50 mmol, 298 mol%) were suspended in EtOAc (0.75 mL, $\eta = 1.0$ μ L/mg). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g ϕ for 8 min. The reaction mixture was partitioned between H₂O (10 mL) and EtOAc (50 mL). The organic phase was washed successfully with H₂O (2 x 10 mL), 0.5 M aq. KHSO₄ (3 $\times$ 10 mL), sat. aq. NaHCO₃ (3 $\times$ 10 mL), and brine (10 mL), dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Analysis of the light orange foam showed Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*R,S*)-**34**, $R_f = 0.22$ (1:4 EtOAc/hexanes, visualized with UV light and ninhydrin)] of 99% purity {LC-MS, Method D, $\lambda = 254$ nm, $R_t = 10.72$ min; $m/z = 669.2$ $[M+Na]^+$ containing Fmoc-D-Thr(*t*-Bu)-OH {**42**, $R_t = 5.93$, Method D; $m/z = 420.1$ $[M+Na]^+$ }. Purification of 185 mg of the residue by filtration on a silica gel pad (4 x 4 cm) wet packed with hexanes eluting with hexanes (3 $\times$ 25 mL), 20% EtOAc in hexanes (3 $\times$ 25 mL), and 40% EtOAc in hexanes (3 $\times$ 25 mL). Evaporation of the latter fractions gave Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*R,S*)-**34**, 164 mg, 94%] as transparent oil: 1H

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NMR (400 MHz, CDCl₃) δ 7.76 (d, 2H, J = 7.4 Hz), 7.62 (d, 1H, J = 8.5 Hz), 7.61 (d, 2H, J = 7.3 Hz), 7.40 (tt, 2H, J = 1.5, 7.4 Hz), 7.31 (dt, 2H, J = 1.0, 7.4 Hz), 7.49-7.21 (m, 1H), 6.48-6.43 (m, 2H), 6.05 (bd, 1H, J = 4.6 Hz), 5.19 (d, 1H, J = 11.6 Hz), 5.11 (d, 1H, J = 11.6 Hz), 4.60 (dd, 1H, J = 4.2, 8.2 Hz), 4.43-4.33 (m, 2H), 4.30-4.15 (m, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 2.30-2.17 (m, 1H), 1.28 (s, 9H), 1.07 (d, 3H, J = 6.3 Hz), 0.99 (d, 3H, J = 6.7 Hz), 0.92 (d, 3H, J = 6.7 Hz); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 171.5, 168.9, 161.6, 159.2, 156.1, 144.1, 143.9, 141.4, 141.4, 131.8, 127.8, 127.2, 125.3, 120.1, 120.1, 116.3, 104.1, 98.6, 75.7, 67.0, 66.6, 62.7, 58.5, 57.6, 55.5, 55.4, 47.3, 31.5, 28.2, 19.3, 17.5, 16.9. HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₃₇H₄₇N₂O₈]⁺, 647.3327 found 647.3347.

Fmoc-D-Thr(*t*-Bu)-D-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*R*)-**43**]

In a 20 mL vial, Fmoc-D-Thr(*t*-Bu)-D-Val-ODmb [(*R*)-**34**, 300 mg, 0.46 mmol, 99% purity] was treated with a solution of 5 vol% TFA in CH₂Cl₂ (3 mL, 2.02 mmol, 436 mol%). The vial was sealed. The reaction mixture was mixed using the RAM at 80 g₀ for 4 min. The neon pink solution was diluted with CH₂Cl₂ (5 mL), treated with K₂CO₃ (279 mg, 2.02 mmol, 439 mol%) giving gas evolution, followed by H₂O (5 mL), and agitated until two pale yellow layers were observed at pH 8. The aqueous layer was separated and adjusted to pH 2-3 using 0.5 M aq. KHSO₄ and extracted with CH₂Cl₂ (3 × 5 mL). The organic layer and extractions were combined, dried over Na₂SO₄, filtered, and concentrated under reduce pressure to a pale-yellow foam (209 mg), which was shown to contain Fmoc-D-Thr(*t*-Bu)-D-Val-OH [(*R*)-**45**, R_f = 0.39 (1:9 CH₃OH/CH₂Cl₂, visualized with UV light and ninhydrin)] of > 99% purity {LC-MS, Method A, λ = 254 nm, RT = 11.03 min.; m/z = 520.3 [M+Na]⁺}; HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₂₈H₃₇N₂O₆]⁺, 497.2646 found 497.2646.

In a 4 mL borosilicate vial, H-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**33**, 50 mg, 0.14 mmol, 100 mol%), Fmoc-D-Thr(*t*-Bu)-D-Val-OH [(*R*)-**45**, 70 mg, 0.14 mmol, 100 mol%], EDCI (54 mg, 0.28 mmol, 200 mol%), and Oxyma (60 mg, 0.42 mmol, 300 mol%) were suspended in EtOAc (0.24 mL, η = 1.0 μ L/mg). The vial was sealed. After agitation with the RAM at 80 g₀ for 40 min, the reaction mixture was partitioned between H₂O (10 mL) and EtOAc (50 mL). The organic phase was washed successfully with H₂O (2 x 10 mL), 0.5 M aq. KHSO₄ (3 × 10 mL), sat. aq. NaHCO₃ (3 × 10 mL), and brine (10 mL), dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Analysis of the resulting beige solid (108 mg) showed Fmoc-D-Thr(*t*-Bu)-D-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*R*)-**43**, R_f = 0.31 (1:1 EtOAc/hexanes, visualized using UV light

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and ninhydrin), d.r. > 96:4 as determined by LC-MS [Method D, λ = 254 nm, R_t = 8.24 min and 10.21 min for (*R*)- and (*S*)-**43**] of 61% purity. Purification of 83 mg by flash chromatography¹⁸ on silica gel eluting with a gradient of 25 to 75% EtOAc in hexanes gave tetrapeptide (*R*)-**43** {75 mg, 78% from Fmoc-D-Thr(*t*-Bu)-D-Val-ODmb [(*R*)-**45**]} as a beige solid of 97.5% purity {LC-MS, Method A, λ = 254 nm, R_t = 11.41 min containing 2.5% of Fmoc-D-Thr(*t*-Bu)-L-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*S*)-**43**] R_t = 11.66 min}: ¹H NMR (700 MHz, CDCl₃) showed a 85:15 isomeric mixture δ 7.77 (d, 2H, J = 7.7 Hz), [7.71 (dd, 2H, J = 3.3, 5.5 Hz)], 7.59 (dd, 2H, J = 4.5, 7.0 Hz), [7.53 (dd, 2H, J = 3.2, 5.7 Hz)], 7.40 (dt, 2H, J = 1.9, 7.4 Hz), 7.33 (br s, 1H), 7.31 (t, 2H, J = 7.4 Hz), 7.28 (br d, 1H, J = 5.2 Hz), 6.97 (br t, 1H, J = 5.0 Hz), 6.93 (bd, 1H, J = 7.7 Hz), 5.84 (br d, 1H, J = 4.9 Hz), 4.50-4.38 (m, 3H), 4.32-4.27 (m, 1H), 4.26-4.18 (m, 2H), 4.17 (t, 1H, J = 4.0 Hz), 4.13 (t, 1H, J = 5.6 Hz), 4.07 (ddd, 1H, J = 1.8, 5.7, 17.3 Hz), 4.07 (ddd, 1H, J = 2.4, 4.9, 17.6 Hz), 2.51-2.43 (m, 1H), 2.38-2.30 (m, 1H), 2.28-2.21 (m, 1H), 2.16 (t, 1H, J = 2.3 Hz), 1.95-1.88 (m, 1H), 1.87-1.80 (m, 1H), 1.71-1.61 (m, 3H), 1.43 (s, 9H), 1.29 (s, 9H), 1.14 (d, 3H, J = 6.4 Hz), 1.00 (d, 3H, J = 6.7 Hz), 0.95 (d, 3H, J = 7.0 Hz), 0.93 (d, 3H, J = 6.7 Hz), 0.89 (d, 3H, J = 6.1 Hz); ¹³C{¹H} NMR (175 MHz, CDCl₃) δ 173.2, 172.0, 171.9, 171.2, 171.1, 156.5, 143.9, 143.7, 141.5, 141.5, 132.6, 128.0, 127.2, 125.2, (131.0), 120.2, (128.9), 81.4, 79.9, 71.2, 68.3, 67.3, 66.7, 60.4, 59.5, 54.5, 52.0, 47.3, 40.2, 38.9, 32.5, 30.5, 29.3, 29.1, 28.4, 28.2, 26.8, 25.0, 23.4, 21.5, 19.7, 18.0. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₄₆H₆₅N₅O₉Na]⁺, 854.4675 found 854.4664.

Fmoc-D-Thr(*t*-Bu)-L-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*S*)-**43**]

Employing the protocol for the synthesis and purification of diastereomer (*R*)-**45**, Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*S*)-**34**, 300 mg, 0.46 mmol, 99% purity] was treated with a 5 vol% TFA in CH₂Cl₂ (3 mL, 2.02 mmol, 436 mol%) to afford Fmoc-D-Thr(*t*-Bu)-L-Val-OH [(*S*)-**45**] as pale-yellow foam (190 mg): R_f = 0.45 (1:9 CH₃OH/CH₂Cl₂, visualized with UV light and ninhydrin) of > 99% purity {LC-MS, Method A, λ = 254 nm, R_t = 11.17 min; m/z = 520.3 [M+Na]⁺}; HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₂₈H₃₇N₂O₆]⁺, 497.2646 found 497.2651. Without further purification, Fmoc-D-Thr(*t*-Bu)-L-Val-OH [(*S*)-**45**, 70 mg, 0.14 mmol, 100 mol%] and H-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**33**, 49 mg, 0.14 mmol, 100 mol%) were coupled and purified as described for the synthesis of tetrapeptide (*R*)-**43** using EDCI (54 mg, 0.28 mmol, 200 mol%), and Oxyma (60 mg, 0.42 mmol, 300 mol%) in EtOAc (0.24 mL, η = 1.0 μ L/mg) to

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provide tetrapeptide (*S*)-**43** as beige solid (100 mg of 62% purity and > 97:3 d.r. as determined by LC-MS, Method D, $\lambda = 254$ nm, $R_t = 10.21$ min): $R_f = 0.31$ (1:1 EtOAc/hexanes, visualized with UV light and ninhydrin). Purification of 50 mg of the residue by flash chromatography¹⁸ on silica gel eluting with a gradient of 25 to 75% EtOAc in hexanes gave tetrapeptide (*S*)-**43** {43 mg, 75% yield from Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*S*)-**34**] as a beige solid of 98.5% LC-MS purity, Method A, $\lambda = 254$ nm, $R_t = 11.66$ min, containing 1.5% of (*R*)-**43**, $R_t = 11.41$ min}: ¹H NMR (400 MHz, CD₃CN) showed a 3:2 isomeric mixture δ 7.83 (d, 2H, $J = 7.4$ Hz), 7.68 (t, 2H, $J = 8.1$ Hz), 7.46-7.38 (m, 3H), 7.38-7.31 (m, 3H), 7.21 (br d, 1H, $J = 8.3$ Hz), 6.96 (br t, 1H, $J = 5.2$ Hz), 5.86 (br d, 1H, $J = 7.0$ Hz), 4.42-4.27 (m, 2H), 4.27-4.19 (m, 3H), 4.18-4.10 (m, 1H), 4.04-3.96 (m, 1H), 3.90 (dd, 1H, $J = 2.2, 5.9$ Hz), [3.94 (dd, 1H, $J = 2.2, 5.9$ Hz)], 3.90-3.84 (m, 1H), 3.79 (dd, 1H, $J = 2.4, 5.5$ Hz), [3.75 (dd, 1H, $J = 2.4, 5.5$ Hz)], 2.38 (t, 1H, $J = 2.5$ Hz), 2.35-2.28 (m, 2H), 2.15-2.00 (m, 2H), 1.91-1.80 (m, 1H), 1.71-1.58 (m, 3H), 1.42 (s, 9H), 1.25 (s, 9H), 1.02 (d, 6H, $J = 6.4$ Hz), 0.99 (d, 3H, $J = 6.9$ Hz), 0.91 (d, 3H, $J = 6.0$ Hz), 0.84 (d, 3H, $J = 6.0$ Hz); ¹³C{¹H} NMR (100 MHz, CD₃CN) δ 174.0, 173.2, 172.8, 172.1, 171.3, 156.7, 145.5, 144.8, 142.2, 142.1, 128.73, 128.70, 128.2, 128.1, 126.3, 126.2, 121.0, 81.31, 81.30, 76.2, 71.8, 68.3, 67.4, 61.4, 59.4, 54.9, 52.6, 48.1, 40.8, 32.4, 30.8, 29.3, 28.4, 28.3, 26.9, 25.6, 23.6, 21.4, 19.7, 19.1, 17.9. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₄₆H₆₅N₅O₉Na]⁺, 854.4675 found 854.4665.

Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**]

In a 20 mL vial, Fmoc-Lys(Boc)-D-Leu-ODmb [(*R*)-**47**, 300 mg, 0.410 mmol) was treated with a solution of 5% TFA in DCM (3 mL, $\eta = 10$ mL/g). The vial was sealed and mixed in the RAM at 80 *g₀* for 3 min, when 100% conversion of the starting ester (*R*)-**47** ($R_t = 9.0$ min) to Fmoc-Lys(Boc)-D-Leu-OH [(*R*)-**51**, $R_t = 7.4$ min] was ascertained by LC-MS analysis ($\lambda = 280$ nm, Method B). The reaction mixture was treated with solid K₂CO₃ (50 mg) with gas evolution. After evaporation of the reaction mixture, the residue was digested with MeOH and filtered onto 5-10-micron particle retention filter paper. The filter cake was washed with MeOH. The filtrate and washings were combined and evaporated to afford acid (*R*)-**51** as red foam (230 mg) of 97 % purity (LC-MS analysis at $\lambda = 280$ nm, Method B).

In another 20 mL vial, Fmoc-Nle-D-Trp(Boc)-ODmb (**46**, 280 mg, 0.352 mmol) was treated with a 5 vol% solution of DBU in THF (1.20 mL, $\eta = 3$ mL/g), and mixed in the RAM at 80 *g₀* for 6 min, when LC-MS analysis ($\lambda = 280$ nm, Method B) showed complete consumption of

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carbamate **46** ($R_t = 10.3$ min). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (10 mL), and washed with water (3×10 mL) and brine (10 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to provide 278 mg of yellow oil, which was shown by LC-MS ($\lambda = 280$ nm, Method B) to contain H-Nle-D-Trp(Boc)-ODmb (**52**, $R_t = 4.4$ min) contaminated with dibenzofulvene ($R_t = 8.7$ min). Without further purification, the yellow oil (278 mg) was transferred to a 20 mL vial, treated with acid (*R*)-**51** (215 mg, 0.370 mmol, 105 mol%), Oxyma (70 mg, 0.493 mmol, 140 mol%), and EtOAc (0.8 mL, $\eta = 2.0$ $\mu\text{L}/\text{mg}$), followed by EDCI (81 mg, 0.423 mmol, 120 mol%). The vial was sealed and mixed in the RAM at 80 g θ for 12 min, when LC-MS analysis ($\lambda = 280$ nm, Method B) indicated 98% consumption of amine **52** ($R_t = 4.4$ min). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (10 mL), washed with water (3×10 mL), 0.1 N HCl (3×10 mL), sat. aq. NaHCO_3 (3×10 mL), and brine (10 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to provide a yellowish foam (364 mg), which was shown by LC-MS ($\lambda = 280$ nm, Method B) to contain Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**] of 83% purity. The foam was chromatographed¹⁸ on silica gel using 50% EtOAc in hexanes. Evaporation of the collected fractions afforded tetrapeptide (*R*)-**44** as white solid (320 mg, 80%): $R_f = 0.35$ (1:1 EtOAc/hexanes, visualized under UV light). ^1H NMR (400 MHz, CD_3OD) δ 8.03 (d, 1H, $J = 8.2$ Hz), 7.75 (d, 2H, $J = 7.6$ Hz), 7.59-7.47 (m, 3H), 7.44 (d, 1H, $J = 9.5$ Hz), 7.35 (t, 2H, $J = 7.4$ Hz), 7.28-7.21 (m, 3H), 7.20-7.14 (m, 1H), 6.98 (d, 1H, $J = 8.3$ Hz), 6.46-6.33 (d, 1H, $J = 2.8$ Hz), 6.40-6.35 (dd, 1H, $J = 8.4, 2.4$ Hz), 5.15-4.93 (m, 2H), 4.76 (dd, 1H, $J = 8.3, 5.9$ Hz), 4.33-4.23 (m, 3H), 4.13 (dd, 1H, $J = 10.4, 6.7$ Hz), 4.03 (q, 2H, $J = 7.5$ Hz), 3.76 (s, 3H), 3.76 (s, 3H), 3.24 (dd, 1H, $J = 15.0, 6.0$ Hz), 3.09 (dd, 1H, $J = 14.8, 8.4$ Hz), 3.04-2.97 (m, 2H), 1.77-1.55 (m, 6H), 1.64 (s, 9H), 1.49-1.39 (m, 3H), 1.41 (s, 9H), 1.35-1.28 (m, 2H), 1.15-1.00 (m, 4H), 0.96-0.87 (dd, 6H, $J = 15.6, 6.0$ Hz), 0.71 (t, 3H, $J = 6.5$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD) δ 175.4, 174.6, 174.2, 172.9, 162.9, 160.2, 158.5, 158.4, 151.0, 145.3, 145.1, 142.6, 136.7, 132.1, 131.6, 128.8, 128.2, 128.1, 126.3, 126.2, 125.44, 125.39, 123.7, 120.9, 119.9, 117.1, 116.2, 105.5, 99.2, 84.9, 79.9, 68.1, 63.7, 56.7, 56.0, 55.8, 54.7, 54.0, 53.6, 48.3, 41.1, 32.6, 30.5, 28.8, 28.4, 28.1, 26.0, 24.1, 23.4, 23.2, 21.8, 14.2. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd. for $[\text{C}_{63}\text{H}_{83}\text{N}_6\text{O}_{13}]^+$, 1131.5965 found 1131.6013. Analysis by chiral-SFC-MS (Method H) and incremental addition of Fmoc-Lys(Boc)-Leu-Nle-D-Trp(Boc)-ODmb [(*S*)-**44**, $R_t = 4.57$ min] in Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**, $R_t = 3.49$ min] indicated a d.r. of $> 97.5:2.5$ for the latter.

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Fmoc-Lys(Boc)-Leu-Nle-D-Trp(Boc)-ODmb [(S)-44] was synthesized from Fmoc-Lys(Boc)-Leu-ODmb [(S)-47, 302 mg, 0.413 mmol, 100 mol%) and Fmoc-Nle-D-Trp(Boc)-ODmb (**46**, 250 mg, 0.316 mmol, 100 mol%) and purified according to the protocols described for tetrapeptide (*R*)-**44** to afford white solid (298 mg, 83% from (S)-**47**): $R_f = 0.35$ (1:1 EtOAc/hexanes, visualized under UV light). ^1H NMR (400 MHz, CD_3OD) δ 8.05 (d, 1H, $J = 8.3$ Hz), 7.79 (d, 2H, $J = 7.5$ Hz), 7.66 (d, 2H, $J = 7.5$ Hz), 7.53 (d, 1H, $J = 7.7$ Hz), 7.47 (s, 1H), 7.38 (t, 2H, $J = 7.5$ Hz), 7.34-7.24 (m, 3H), 7.20 (t, 1H, $J = 7.6$ Hz), 7.06 (d, 1H, $J = 8.3$ Hz), 6.47 (d, 1H, $J = 2.3$ Hz), 6.41 (d, 1H, $J = 8.3$ Hz), 5.07 (q, 2H, $J = 12.0$ Hz), 4.81-4.77 (m, 1H), 4.42-4.29 (m, 3H), 4.29-4.17 (m, 2H), 3.98 (s, 1H), 3.77 (s, 6H), 3.27-3.20 (m, 1H), 3.10 (dd, 1H, $J = 14.7, 8.9$ Hz), 3.02 (t, 2H, $J = 6.8$ Hz), 1.74-1.53 (m, 6H) 1.61 (s, 9H), 1.48-1.39 (m, 3H), 1.42 (s, 9H), 1.37-1.28 (m, 2H), 1.17-0.98 (m, 4H), 0.87 (dd, 6H, $J = 14.2, 6.5$ Hz), 0.72 (t, 3H, $J = 7.0$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD) δ 175.3, 174.4, 174.0, 172.8, 163.0, 160.3, 158.7, 158.6, 151.0, 145.3, 145.2, 142.6, 136.8, 132.2, 131.6, 128.8, 128.2, 126.3, 125.4, 123.7, 121.0, 119.9, 117.2, 117.1, 116.2, 105.5, 99.2, 84.8, 79.9, 68.1, 63.8, 56.8, 56.0, 55.8, 54.4, 53.7, 53.6, 48.4, 41.4, 41.0, 32.9, 32.6, 30.6, 28.8, 28.7, 28.5, 28.1, 25.9, 24.2, 23.5, 23.4, 21.9, 14.3. HRMS (ESI-TOF) m/z $[\text{M}+\text{Na}]^+$ calcd. for $[\text{C}_{63}\text{H}_{82}\text{N}_6\text{O}_{13}\text{Na}]^+$, 1153.5832 found 1153.5823.

Fmoc-Nle-D-Trp(Boc)-ODmb (46)

In a 20 mL vial, Fmoc-D-Trp(Boc)-OH (**D-15**, 2 g, 3.80 mmol, 100 mol%), Oxyma (0.648 g, 4.56 mmol, 120 mol%), 2,4-dimethoxybenzyl alcohol (0.703 g, 4.18 mmol, 110 mol%), (*i*-Pr)₂NEt (0.73 mL, 0.54 g, 4.18 mmol, 110 mol%) and 2-MeTHF (4.2 mL, $\eta = 1.0$ $\mu\text{L}/\text{mg}$) were mixed with a spatula and subsequently treated with EDCI (0.801 g, 4.18 mmol, 110 mol%). The vial was sealed and mixed in the RAM at 80 g₀ for 12 min, when 89% conversion of amino acid **D-15** ($R_t = 9.2$ min) to Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**, $R_t = 10.6$ min] was ascertained by LC-MS analysis ($\lambda = 280$ nm, Method B). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (50 mL), and washed with water (3×50 mL), 0.1 N HCl (3×50 mL), sat. aq. NaHCO_3 (3×50 mL), and brine (30 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure yielding ester (*R*)-**21** (2.16 g) as a yellowish foam of 86% purity (LC-MS analysis at $\lambda = 280$ nm, Method B) contaminated with acid **D-15**. Without further purification, the mixture was added to a 20 mL vial, treated with a 20% solution of piperidine in THF (6.5 mL, 3 mL per g of foam), and mixed in the RAM at 80 g₀ for 12 min, when LC-MS analysis ($\lambda = 280$ nm, Method A) showed complete consumption of starting carbamate (*R*)-**21** ($R_t = 10.6$ min). The reaction

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mixture was transferred to a 125 mL Erlenmeyer flask using a minimal amount of acetonitrile, precipitated with 1:1 MeCN/water (40 mL), and filtered onto a 22-micron pore-size membrane. The filter cake was washed until white using a 1:1 MeCN/water (3×20 mL). The filtrate and washings were transferred to a separating funnel and extracted with EtOAc (40 mL). The organic phase was washed with water (40 mL), sat. aq. NaHCO_3 (40 mL) and brine (20 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to afford H-D-Trp(Boc)-ODmb [(*R*)-**29**, 1.49 g, $R_t = 6.7$ min] as a yellow oil of 75% purity ($\lambda = 280$ nm, Method A) contaminated with dibenzofulvene ($R_t = 10.7$ min) and dibenzofulvene piperidine adduct ($R_t = 5.8$ min) as major impurities. Without further purification, amine (*R*)-**29** (1.49 g) was transferred to a 20 mL vial, treated with Fmoc-Nle-OH (**50**, 1.22 g, 3.44 mmol), Oxyma (0.559 g, 3.93 mmol), and 2-MeTHF (3.96 mL, $\eta = 1.0$ $\mu\text{L}/\text{mg}$), followed by EDCI (0.691 g, 3.61 mmol). The vial was sealed and mixed in the RAM at 80 g_0 for 12 min, when LC-MS analysis ($\lambda = 280$ nm, Method A) indicated complete consumption of the starting amine (*R*)-**29** ($R_t = 6.7$ min). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (50 mL), and washed with water (3×50 mL), 0.1 N HCl (3×50 mL), sat. aq. NaHCO_3 (3×50 mL), and brine (30 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to provide Fmoc-Nle-D-Trp(Boc)-ODmb (**46**, 2.71 g) as a yellowish foam of 79% purity assessed by LC-MS analysis ($\lambda = 280$ nm, Method B). The residue was dissolved in Et_2O (40 mL), treated until turbid with hexanes (20 mL) and evaporated to a reduced volume (ca. 30 mL), which was filtered. The precipitate was washed with ether and dried to give dipeptide **46** (1.92 g, 74 % yield from acid **D-15**) as white solid of 96% purity ($\lambda = 280$ nm, Method B): $R_f = 0.30$ (3:7 EtOAc/hexanes, visualized under UV light). ^1H NMR (400 MHz, CD_3CN) δ 8.06 (d, 1H, $J = 8.2$ Hz), 7.82 (d, 2H, $J = 7.6$ Hz), 7.63 (d, 2H, $J = 7.6$ Hz), 7.53 (d, 1H, $J = 7.7$ Hz), 7.45 (s, 1H), 7.40 (t, 2H, $J = 7.5$ Hz), 7.30 (tdd, 3H, $J = 7.1, 4.9, 2.4$ Hz), 7.22 (td, 1H, $J = 7.5, 1.1$ Hz), 7.10 (d, 1H, $J = 8.3$ Hz), 6.95 (d, 1H, $J = 8.1$ Hz), 6.50 (d, 1H, $J = 2.4$ Hz), 6.43 (dd, 1H, $J = 8.3, 2.4$ Hz), 5.85 (d, 1H, $J = 8.2$ Hz), 5.04 (s, 2H), 4.77 (td, 1H, $J = 8.2, 5.4$ Hz), 4.36-4.22 (m, 2H), 4.19 (d, 1H, $J = 7.4$ Hz), 4.04-3.92 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.21 (ddd, 1H, $J = 14.9, 5.4, 1.1$ Hz), 3.08 (ddd, 1H, $J = 14.9, 8.3, 0.9$ Hz), 1.61 (s, 9H), 1.54 (d, 1H, $J = 6.5$ Hz), 1.42 (dd, 1H, $J = 14.6, 7.1$ Hz), 1.26-1.04 (m, 4H), 0.79 (t, 3H, $J = 7.1$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3CN) δ 172.9, 172.4, 162.5, 160.0, 156.9, 150.5, 145.2, 145.0, 142.1, 136.3, 132.1, 131.3, 128.7, 128.1, 126.2, 125.44, 125.37, 123.5, 121.0, 119.9, 116.9,

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116.5, 116.1, 105.4, 99.3, 84.7, 67.3, 63.4, 56.3, 56.0, 55.8, 53.1, 48.0, 32.9, 28.3, 27.9, 23.0, 14.2.

HRMS (ESI-TOF) m/z $[M+H]^+$ calcd. for $[C_{46}H_{52}N_3O_9]^+$, 790.3698 found 790.3692.

Fmoc-Lys(Boc)-D-Leu-ODmb [(R)-47]

In a 20 mL vial, Fmoc-D-Leu-OH (D-20, 2 g, 5.66 mmol, 100 mol%), Oxyma (0.965 g, 6.79 mmol, 120 mol%), 2,4-dimethoxybenzyl alcohol (1.14 g, 6.79 mmol, 120 mol%), (*i*-Pr)₂NEt (1.08 mL, 0.80 g, 6.23 mmol, 110 mol%) and 2-MeTHF (5.3 mL, η = 1.0 μ L/mg) were mixed and subsequently treated with EDCI (1.19 g, 6.23 mmol, 110 mol%). The vial was sealed and mixed in the RAM at 80 g₀ for 12 min, when 98% conversion of acid D-20 (R_t = 7.5 min) to Fmoc-D-Leu-ODmb [(R)-48, R_t = 9.5 min) was ascertained by LC-MS analysis (λ = 280 nm, Method B). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (50 mL), and washed with water (3 $\times$ 50 mL), 0.1 N HCl (3 $\times$ 50 mL), sat. aq. NaHCO₃ (3 $\times$ 50 mL), and brine (30 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure yielding ester (R)-48 (2.91 g) as yellowish foam of 91 % purity (λ = 280 nm, Method B) contaminated with acid D-20 and another impurity (R_t = 9.1 min). Without further purification, the mixture was added to a 20 mL vial, treated with a 20% solution of piperidine in THF (8.7 mL, 3 mL per g of foam), and mixed in the RAM at 80 g₀ for 12 min, when LC-MS analysis (λ = 280 nm, Method B) showed complete consumption of carbamate (R)-48 (R_t = 9.5 min). The reaction mixture was transferred to a 125 mL Erlenmeyer flask using a minimal volume of acetonitrile, precipitated with 1:1 MeCN/water (40 mL), and filtered onto a 22-micron pore-size membrane. The filter cake was washed until white using a 1:1 MeCN/water (3 $\times$ 20 mL). The filtrate and washings were transferred to a separating funnel and extracted with EtOAc (40 mL). The organic phase was washed with water (40 mL), sat. aq. NaHCO₃ (40 mL) and brine (20 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to afford H-D-Leu-ODmb [(R)-49, 1.45 g, R_t = 5.3 min] as yellow oil of 68 % purity (λ = 280 nm, Method A) contaminated with dibenzo fulvalene (R_t = 10.7 min) and dibenzo fulvalene piperidine adduct (R_t = 4.6 min) as major impurities. Without further purification, amine (R)-49 (1.45 g) was transferred to a 20 mL vial, treated with Fmoc-Lys(Boc)-OH (16, 2.54 g, 5.41 mmol, 105 mol%), Oxyma (0.879 g, 6.18 mmol, 120 mol%), and 2-MeTHF (6 mL, η = 1.0 μ L/mg), followed by EDCI (1.09 g, 5.67 mmol, 110 mol%). The vial was sealed and mixed in the RAM at 80 g₀ for 12 min, when LC-MS analysis (λ = 280 nm, Method B) indicated complete consumption of amine (R)-49 (R_t = 5.3 min). The reaction mixture was transferred to a separating funnel, diluted with EtOAc (50 mL), and washed with

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water (3 × 50 mL), 0.1 N HCl (3 × 50 mL), sat. aq. NaHCO₃ (3 × 50 mL), and brine (30 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to provide Fmoc-Lys(Boc)-D-Leu-ODmb [(*R*)-**47**, 3.52 g] as yellowish foam of 82% purity assessed by LC-MS analysis (λ = 280 nm, Method B). The foam was dissolved in Et₂O (40 mL), treated until turbid with hexanes (20 mL) and evaporated to a reduced volume (ca. 30 mL), which was filtered. The precipitate was washed and dried with ether to give dipeptide (*R*)-**47** (3.05g, 81 % from acid D-**20**) as a white solid of 96% purity (λ = 280 nm, Method B): R_f = 0.4 (1:1 EtOAc/hexanes, visualized under UV light). ¹H NMR (400 MHz, CD₃OD) δ 7.80 (d, 2H, J = 7.5 Hz), 7.66 (dd, 2H, J = 7.4, 4.2 Hz), 7.39 (t, 2H, J = 7.3 Hz), 7.31 (td, 2H, J = 7.5, 1.2 Hz), 7.16 (d, 1H, J = 8.3 Hz), 6.49 (d, 1H, J = 2.4 Hz), 6.43 (dd, 1H, J = 8.4, 2.3 Hz), 5.13-4.97 (m, 2H), 4.46-4.31 (m, 3H), 4.21 (t, 1H, J = 6.8 Hz), 4.11 (dd, 1H, J = 8.7, 5.3 Hz), 3.78 (s, 3H), 3.74 (s, 3H), 3.01 (t, 2H, J = 6.8 Hz), 1.76-1.55 (m, 5H), 1.48-1.27 (m, 4H), 1.42 (s, 9H), 0.88 (dd, 6H, J = 8.90, 6.00 Hz); ¹³C {¹H} NMR (100 MHz, CD₃CN) δ 173.4, 173.0, 162.5, 160.0, 157.1, 145.2, 145.1, 142.1, 132.2, 128.7, 128.2, 128.1, 126.2, 121.0, 117.2, 105.4, 99.3, 79.0, 67.4, 63.0, 56.3, 56.0, 51.8, 48.1, 41.2, 40.7, 32.8, 30.3, 28.7, 25.5, 23.5, 23.1, 21.8. HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₄₁H₅₄N₃O₉]⁺, 732.3855 found 732.3851.

Fmoc-Lys(Boc)-Leu-ODmb [(*S*)-47**]** was synthesized from Fmoc-Leu-OH (L-**20**, 2.00 g, 5.66 mmol, 100 mol%) and purified according to the protocols described for dipeptide (*R*)-**47** to afford 2.83g (79 % yield from L-**20**) of white solid of 90% purity (λ = 280 nm, Method B): R_f = 0.4 (5:5 EtOAc/hexanes, visualized under UV light). ¹H NMR (400 MHz, CD₃CN) δ 7.83 (d, 2H, J = 7.5 Hz), 7.67 (dd, 2H, J = 7.5, 4.3 Hz), 7.42 (t, 2H, J = 7.5 Hz), 7.37-7.30 (m, 2H), 7.20 (d, 1H, J = 8.3 Hz), 6.83 (d, 1H, J = 7.7 Hz), 6.53 (d, 1H, J = 2.4 Hz), 6.47 (dd, 1H, J = 8.4, 2.3 Hz), 5.91 (d, 1H, J = 7.9 Hz), 5.27 (s, 1H), 5.11-4.96 (m, 2H), 4.43-4.28 (m, 3H), 4.22 (t, 1H, J = 7.1 Hz), 4.07-3.98 (m, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.98 (m, 2H), 1.71-1.50 (m, 5H), 1.43-1.26 (m, 4H) 1.38 (s, 9H), 0.86 (dd, 6H, J = 15.2, 6.5 Hz); ¹³C {¹H} NMR (100 MHz, CD₃CN) δ 173.5, 173.0, 162.5, 160.1, 157.1, 145.2, 145.1, 142.1, 132.3, 128.7, 128.1, 126.2, 121.0, 117.1, 105.4, 99.3, 79.0, 67.3, 63.1, 56.3, 56.1, 55.6, 51.9, 48.1, 41.2, 40.7, 32.7, 30.3, 28.7, 25.5, 23.4, 23.0, 21.9. HRMS (ESI-TOF) m/z [M+H]⁺ calcd. for [C₄₁H₅₄N₃O₉]⁺, 732.3855 found 732.3849.

HCl•H-Tyr(*t*-Bu)-Aib-Aib-Phe-Leu-NH₂ (**54**)

Boc-Tyr(*t*-Bu)-Aib-Aib-Phe-Leu-NH₂ (62, 20 mg, 0.41 mmol, 100 mol%) was treated with 4 M HCl in 1,4-dioxane (5.2 mL) and stirred at room temperature for 12 h. The volatiles were

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evaporated under reduced pressure to a residue that was twice dissolved in MeOH (0.5 mL), precipitated with 1:4 pentane:Et₂O (10 mL), and collected by centrifugation. After drying under vacuum, hydrochloride **54** (17 mg, 100%) was obtained as white powder: R_f = 0.32 (1:9 CH₃OH/CH₂Cl₂, visualized using ninhydrin). ¹H NMR (500 MHz, D₂O) δ 7.44-7.35 (m, 2H), 7.34-7.27 (m, 3H), 7.21-7.15 (m, 2H), 6.93-6.87 (m, 2H), 4.60-4.55 (m, 1H), 4.35-4.23 (m, 1H), 4.19-4.14 (m, 1H), 3.25-3.08 (m, 4H), 1.65-1.48 (m, 3H), [1.44 (s, 3H)], 1.38 (s, 3H), 1.35 (s, 3H), [1.33 (s, 3H)], 1.32 (s, 3H), 1.27 (s, 3H), 0.94-0.83 (m, 6H); ¹³C{¹H} NMR (125 MHz, D₂O) δ 177.11, 177.05, 175.7, 173.5, 168.5, 155.2, 136.1, 131.0, (129.4), (129.2), 129.1, 128.9, 127.3, 125.3, 115.8, 56.9, 56.8, 55.3, 54.1, 52.0, (39.9), 39.5, 36.1, 35.9, **24.6**, 24.3, 24.1, (23.8), 23.7, 23.1, 22.2, 20.3. HRMS (ESI-TOF) m/z [M-Cl]⁺ calcd. for [C₃₂H₄₇N₆O₆]⁺, 611.3552 found 611.3541.

HCl·H-Leu-NH₂ (**55**)

Carbamate (*S*)-**25** (1.00 g, 4.34 mmol, 100 mol%) was treated with 4 M HCl in 1,4-dioxane (5.0 mL) and stirred at room temperature for 18 h, when a white precipitate formed. The volatiles were evaporated under reduced pressure to a residue which was dissolved in MeOH (0.5 mL), precipitated with Et₂O (10 mL) and collected by centrifugation thrice. After drying under vacuum, hydrochloride **55** (720 mg, quant.) was isolated as white powder: R_f = 0.25 (1:9 CH₃OH/CH₂Cl₂, visualized using KMnO₄). Hydrochloride **55** was used without further purification.

HCl·H-Phe-Leu-NH₂ (**57**)

In a 20 mL vial, a mixture of HCl·H-Leu-NH₂ (**55**, 300 mg, 2.30 mmol, 100 mol%), Boc-Phe-OH (917 mg, 3.46 mmol, 150 mol%), Oxyma (655 mg, 4.61 mmol, 200 mol%), and Et₃N (233 mg, 2.30 mmol, 100 mol%) in DME (2.8 mL, η = 0.90 μ L/mg) was manually agitated. The suspension was treated with EDCI (663 mg, 3.46 mmol, 150 mol%). After sealing and standing for 1 min, the vial was mixed in the RAM at 80 g₀ for 15 min, when > 99% conversion was ascertained by LC-MS analysis (Method A, λ = 214 nm): Boc-Phe-OH (R_t = 8.27 min); Boc-Phe-Leu-NH₂ (**56**, R_t = 7.57 min), which appeared on TLC as a less polar spot [R_f = 0.42 (2:3 EtOAc/hexanes, visualized using UV light)]. The reaction mixture was partitioned between EtOAc (10 mL) and water (5 mL) with agitation for 2 min and transferred to a separating funnel. The organic phase was washed with 1 N HCl (2 $\times$ 5 mL), sat. aq. NaHCO₃ (6 $\times$ 5 mL), brine (5 mL), dried over Na₂SO₄, filtered, and evaporated to afford 722 mg of an off white solid, Boc-Phe-Leu-NH₂ (**56**): R_t = 7.57 min (Method A, λ = 214 nm). Without further purification, dipeptide **56** was treated with 4 M HCl in 1,4-dioxane (2.0 mL) and stirred at room temperature for 10 h, when a

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white precipitate was observed. The volatiles were evaporated under reduced pressure. The residue was dissolved in MeOH (0.8 mL), precipitated with Et₂O (12 mL), and collected by centrifugation thrice. After drying under vacuum, hydrochloride **57** (682 mg, 95% from hydrochloride **55**) was isolated as white solid: R_f = 0.61 (1:9 CH₃OH/CH₂Cl₂, visualized using KMnO₄). ¹H NMR (400 MHz, D₂O) δ 7.42-7.34 (m, 3H), 7.29-7.24 (m, 2H), 4.32-4.37 (m, 1H), 4.27-4.22 (m, 1H), 3.19 (d, 2H, J = 7.4 Hz), 1.60-1.49 (m, 3H), 0.89 (d, 3H, J = 8.0 Hz), 0.85 (d, 3H, J = 8.0 Hz); ¹³C{¹H} NMR (100 MHz, D₂O) δ 176.2, 168.9, 133.5, 129.4, 129.2, 128.0, 54.1, 52.2, 39.9, 36.8, 24.2, 21.9, 20.8. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₁₅H₂₃N₃O₂Na]⁺, 278.1863 found 278.1861.

HCl•H-Aib-Phe-Leu-NH₂ (**59**)

Employing a modification of the protocol for the synthesis and purification of HC•H-Phe-Leu-NH₂ (**57**), Boc-Aib-OH (60 mg, 0.29 mmol, 100 mol%) and hydrochloride **57** (97 mg, 0.31 mmol, 100 mol%) were coupled using Oxyma (50 mg, 0.35 mmol, 120 mol%), *i*-Pr₂EtN (46 mg, 0.35 mmol, 100 mol%) and EDCI (113 mg, 0.59 mmol, 200 mol%) in DME (419 μ L, η = 0.90 μ L/mg) with agitation in the RAM at 80 g₀ for 20 min to afford Boc-Aib-Phe-Leu-NH₂ (**58**) as white solid (108 mg, 79%): R_f = 0.29 (100% EtOAc, visualized using KMnO₄). Without further purification, tripeptide **58** was treated with 4 M HCl in 1,4-dioxane (2 mL) and stirred at room temperature for 4 h, when TLC showed consumption of the starting material and appearance of a more polar spot [R_f = 0.15 (1:9 CH₃OH/CH₂Cl₂, visualized using ninhydrin)]. The volatiles were evaporated. The residue was triturated with 1:9 CH₃OH/Et₂O (2 mL), 1:9 CH₃OH/MTBE (3 $\times$ 2 mL) and 1:9 CH₃OH/Et₂O (2 mL) to afford hydrochloride **59** as white powder (92 mg, 78% from hydrochloride **57**): R_f = 0.15 (1:9 CH₃OH/CH₂Cl₂, visualized using ninhydrin). ¹H NMR (500 MHz, CD₃OD) δ 8.31-8.20 (m, 1H), 7.34-7.23 (m, 4H), 7.22-7.17 (m, 1H), 4.79 (dd, 1H, J = 10.6, 5.5 Hz), 4.45-4.39 (m, 1H), 3.23 (dd, 1H, J = 13.8, 5.1 Hz), 3.00 (dd, 1H, J = 13.8, 10.5 Hz), 1.76-1.57 (m, 3H), 1.58 (br s, 3H), 1.38 (br s, 3H), 0.95 (d, 3H, J = 6.5 Hz), 0.91 (d, 3H, J = 6.4 Hz); ¹³C{¹H} NMR (125 MHz, CDCl₃) δ 177.2, 173.3, 173.1, 138.4, 130.4, 129.5, 127.8, 58.2, 56.3, 53.0, 42.2, 38.5, 25.9, 24.2, 24.0, 23.5, 22.0. HRMS (ESI-TOF) m/z [M-Cl]⁺ calcd. for [C₁₉H₃₁N₄O₃]⁺, 363.2391 found 363.2387.

HCl•H-Aib-Aib-Phe-Leu-NH₂ (**61**)

Employing the protocol described for the preparation of hydrochloride **59**, Boc-Aib-OH (30.6 mg, 0.15 mmol, 100 mol%) was coupled to HCl•H-Aib-Phe-Leu-NH₂ (**59**, 60 mg, 0.15

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mmol, 100 mol%) to afford a white solid, Boc-Aib-Aib-Phe-Leu-NH₂ (**60**, 53 mg): R_f = 0.29 (100% EtOAc, visualized using KMnO₄), which was converted to HCl•H-Aib-Aib-Phe-Leu-NH₂ (**60**, 45 mg, 61% from hydrochloride **59**) as white solid: ¹H NMR (500 MHz, CD₃OD) δ 7.28-7.22 (m, 4H), 7.21-7.16 (m, 1H), 4.50-4.43 (m, 2H), 3.30 (dd, 1H, J = 13.9, 4.9 Hz), 3.15 (dd, 1H, J = 13.9, 10.3 Hz), 1.76-1.69 (m, 2H), 1.62-1.56 (m, 11H), 1.38 (br s, 3H), 1.23 (br s, 3H), 0.96 (d, 3H, J = 6.2 Hz), 0.94 (d, 3H, J = 6.2 Hz); ¹³C{¹H} NMR (125 MHz, CDCl₃) δ 177.2, 176.4, 174.3, 173.5, 139.1, 130.4, 129.5, 127.8, 58.5, 57.6, 57.0, 52.9, 42.0, 37.1, 25.8, 25.4, 24.5, 24.1, 23.8, 23.4, 22.2. HRMS (ESI-TOF) m/z [M-Cl]⁺ calcd. for [C₂₃H₃₈N₅O₄]⁺, 448.2918 found 448.2908.

Boc-Tyr(*t*-Bu)-Aib-Aib-Phe-Leu-NH₂ (**62**)

Employing a modification of the protocol described for the preparation of dipeptide (*S,S*)-**1**, Boc-Tyr(*t*-Bu)-OH (14 mg, 0.041 mmol, 100 mol%) was coupled to hydrochloride **61** (20 mg, 0.041 mmol, 100 mol%) with agitation in the RAM at 80 g₀ for 20 min. After aqueous workup, the residue was dissolved in CH₂Cl₂ (0.1 mL), precipitated with pentane (2 mL), and collected by centrifugation thrice as previously described.¹⁹ After drying under vacuum, Boc-Tyr(*t*-Bu)-Aib-Aib-Phe-Leu-NH₂ (**62**) was obtained as white powder (24 mg, 75%): R_f = 0.26 (100% EtOAc, visualized using KMnO₄). ¹H NMR (500 MHz, CDCl₃) δ 7.62 (d, 1H, J = 8.8 Hz), 7.49-7.35 (m, 4H), 7.28-7.24 (m, 1H), 7.22-7.12 (m, 2H), 7.11-7.06 (m, 2H), 7.02-6.98 (m, 2H), 6.35 (br s, 1H), 5.45 (br s, 1H), 4.95 (br s, 1H), 4.65-4.58 (m, 1H), 4.46-4.40 (m, 1H), 4.06-3.96 (m, 1H), 3.48 (dd, 1H, J = 14.5, 2.8 Hz), 3.14-3.01 (m, 2H), 2.98-2.91 (m, 1H), 1.96-1.87 (m, 1H), 1.87-1.69 (m, 3H), 1.51-1.47 (m, 3H), 1.46-1.42 (m, 9H), 1.42-1.40 (m, 3H), 1.38-1.34 (m, 9H), 1.33-1.29 (m, 3H), 1.20-1.15 (m, 3H), 0.96 (d, 3H, J = 1.7 Hz), 0.95 (d, 3H, J = 1.8 Hz); ¹³C{¹H} NMR (125 MHz, CDCl₃) δ 177.1, 176.7, 174.5, 172.2, 172.1, 156.7, 155.3, 138.4, 129.7, 129.3, 128.4, 126.7, 124.8, 82.1, 79.0, 57.8, 57.3, 57.0, 56.0, 51.2, 39.7, 36.5, 36.4, 29.0, 28.3, 27.6, 27.1, 25.1, 23.4, 23.1, 21.0. HRMS (ESI-TOF) m/z [M+Na]⁺ calcd. for [C₄₁H₆₂N₆O₈Na]⁺, 789.4521 found 789.4513.

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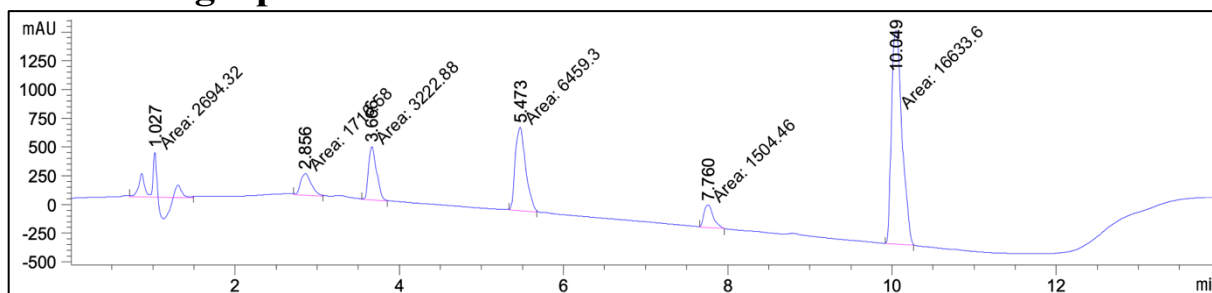
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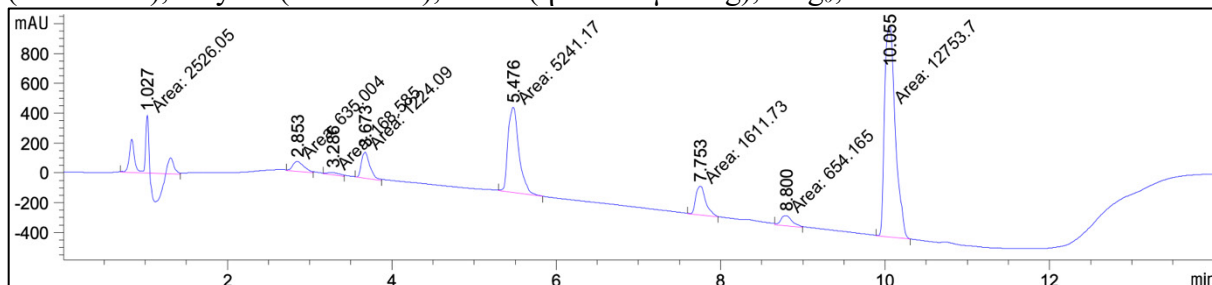
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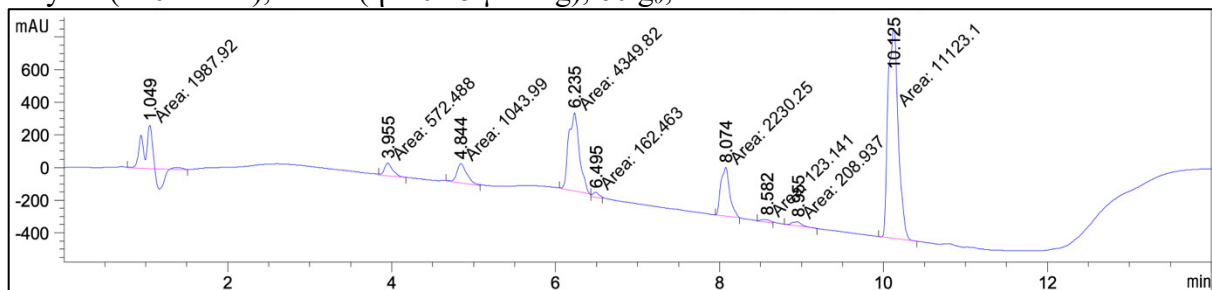
Chromatographic data



Chromatogram S1. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.05$ min (conversion 92%)], containing peaks for Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.47 min), *N*-acyl urea (3.67 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.45$ μ L/mg), 60 g₀, 4 min.



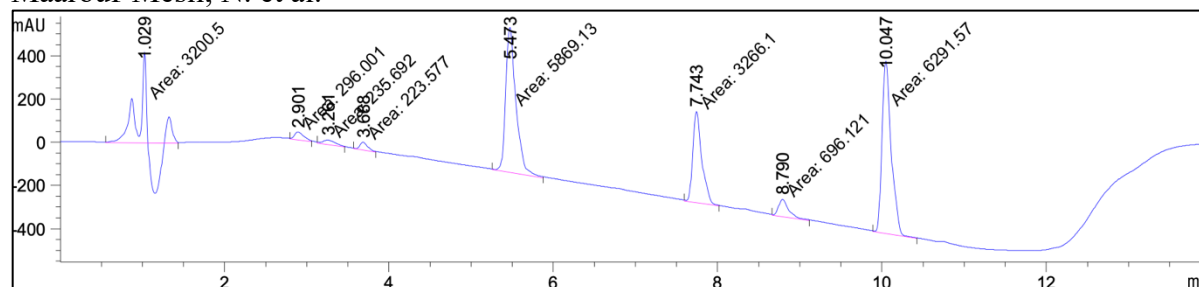
Chromatogram S2. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.06$ min (conversion 89%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.48 min), *N*-acyl urea (3.67 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), KHCO₃ (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.45$ μ L/mg), 60 g₀, 4 min.



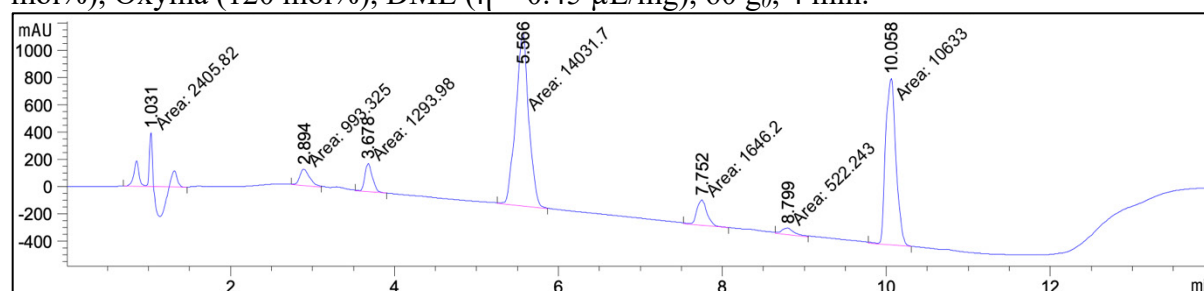
Chromatogram S3. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.13$ min (conversion 83%)], Cbz-Phe-OH (2, $R_t = 8.07$ min), Oxyma (6.24 min), *N*-acyl urea (3.96 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), NaHCO₃ (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.45$ μ L/mg), 60 g₀, 4 min.

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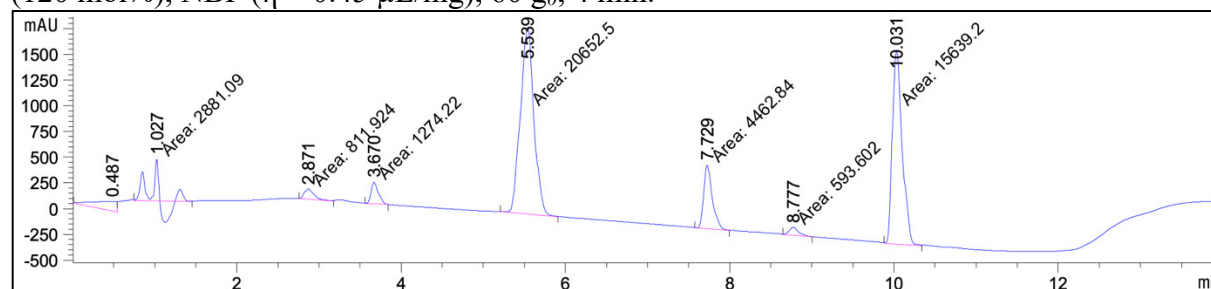
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Chromatogram S4. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.05$ min (conversion 66%)], Cbz-Phe-OH (L-2, $R_t = 7.74$ min), Oxyma (5.47 min), *N*-acyl urea (3.67 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), NaH_2PO_4 (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.45$ $\mu\text{L}/\text{mg}$), 60 g₀, 4 min.



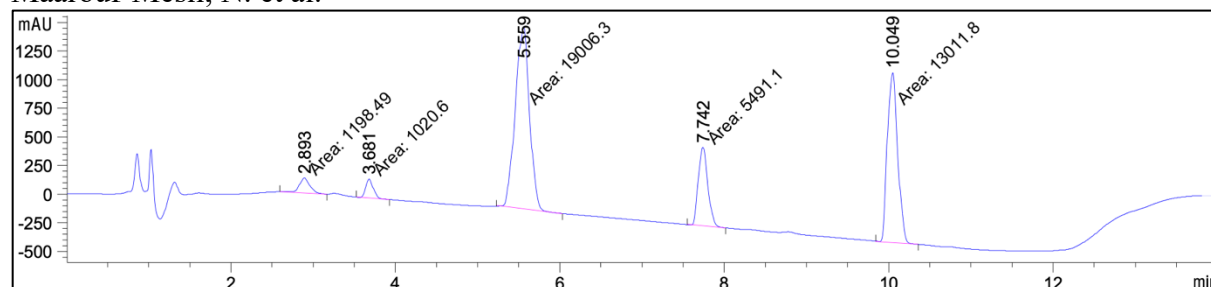
Chromatogram S5. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.06$ min (conversion 87%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.57 min), *N*-acyl urea (3.68 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et_3N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), NBP ($\eta = 0.45$ $\mu\text{L}/\text{mg}$), 60 g₀, 4 min.



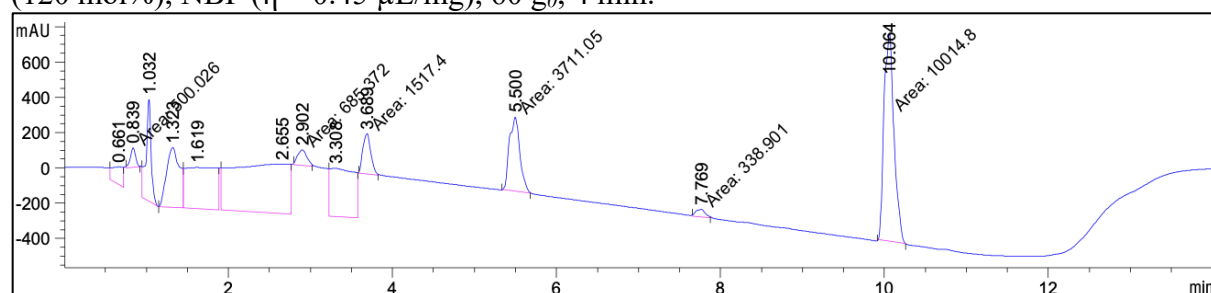
Chromatogram S6. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.06$ min (conversion 78%)], Cbz-Phe-OH (L-2, $R_t = 7.73$ min), Oxyma (5.54 min), *N*-acyl urea (3.67 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), NaHCO_3 (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), NBP ($\eta = 0.45$ $\mu\text{L}/\text{mg}$), 60 g₀, 4 min.

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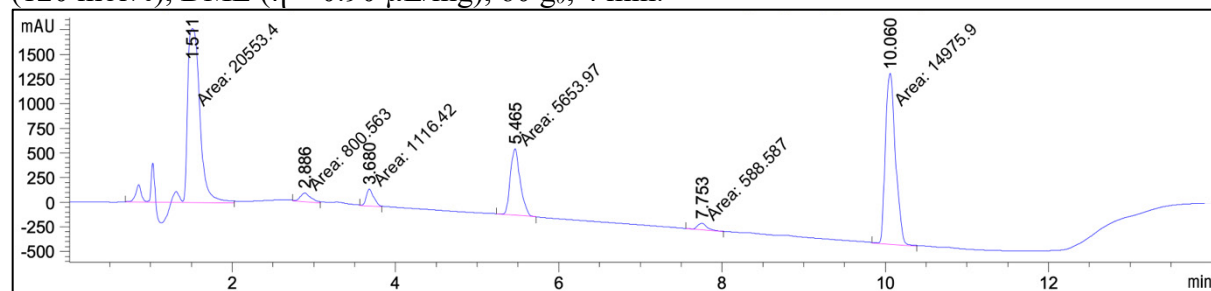
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Chromatogram S7. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.05$ min (conversion 70%)], Cbz-Phe-OH (L-2, $R_t = 7.74$ min), Oxyma (5.56 min), *N*-acyl urea (3.68 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), K_2CO_3 (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), NBP ($\eta = 0.45$ μ L/mg), 60 g ϕ , 4 min.



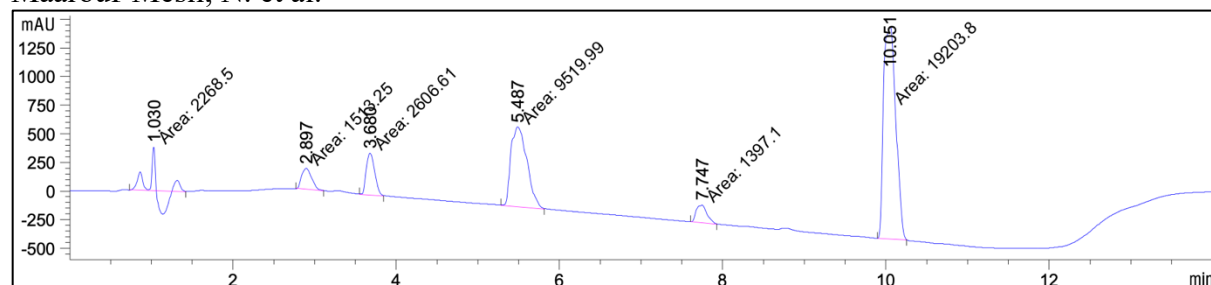
Chromatogram S8. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.07$ min (conversion 97%)], Cbz-Phe-OH (L-2, $R_t = 7.77$ min), Oxyma (5.50 min), *N*-acyl urea (3.69 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et_3N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g ϕ , 4 min.



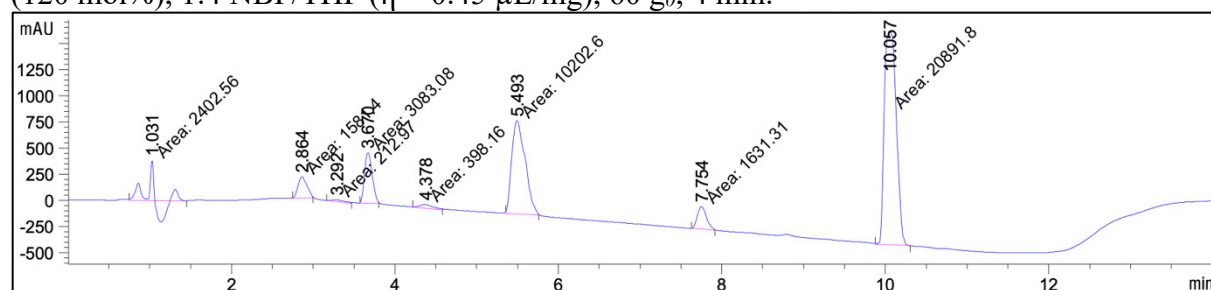
Chromatogram S9. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.06$ min (conversion 96%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.47 min), *N*-acyl urea (3.68 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et_3N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DMF ($\eta = 0.45$ μ L/mg), 60 g ϕ , 4 min.

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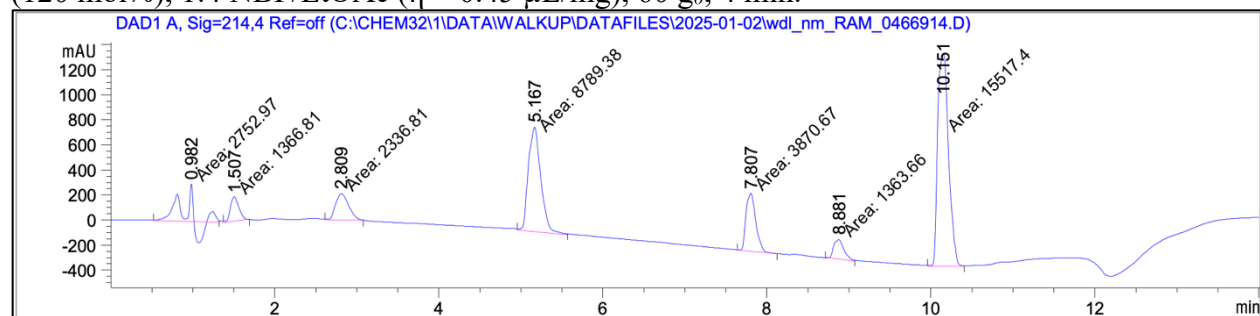
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Chromatogram S10. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.06$ min (conversion 94%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.49 min), *N*-acyl urea (3.68 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 NBP/THF ($\eta = 0.45$ μ L/mg), 60 g₀, 4 min.



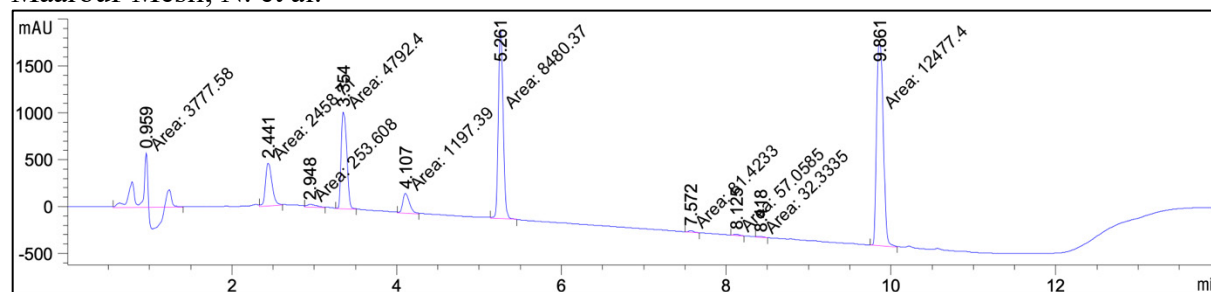
Chromatogram S11. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.05$ min (conversion 93%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.49 min), *N*-acyl urea (3.67 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 NBP/EtOAc ($\eta = 0.45$ μ L/mg), 60 g₀, 4 min.



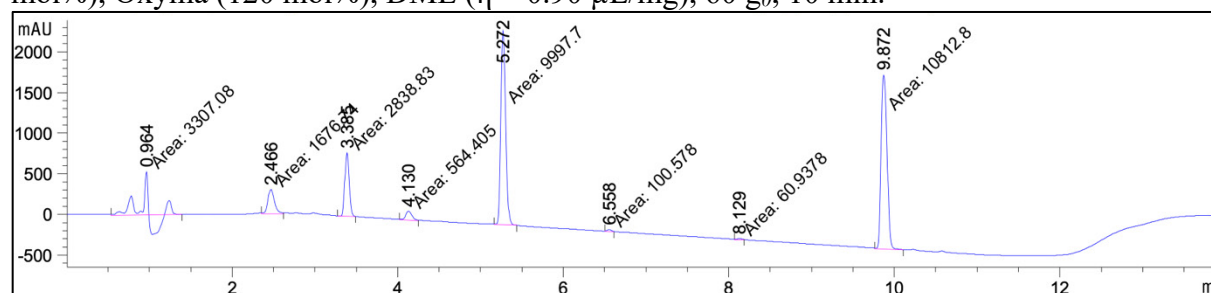
Chromatogram S12. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.15$ min (conversion 80%)], Cbz-Phe-OH (L-2, $R_t = 7.81$ min), Oxyma (5.17 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), without solvent, 60 g₀, 4 min.

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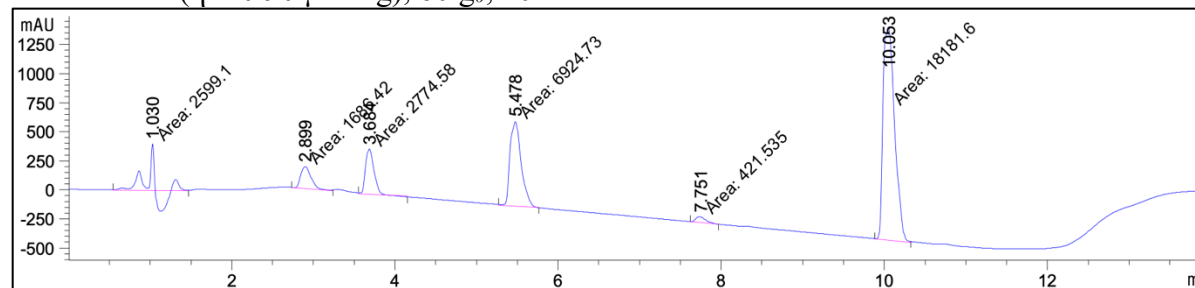
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Chromatogram S13. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(*S,S*)-1]: (*S,S*)-1 [$R_t = 9.86$ min (conversion 100%)], Cbz-Phe-OH (L-2, $R_t = 7.57$ min), Oxyma (5.26 min), *N*-acyl urea (3.35 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), (*i*-Pr)₂NEt (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



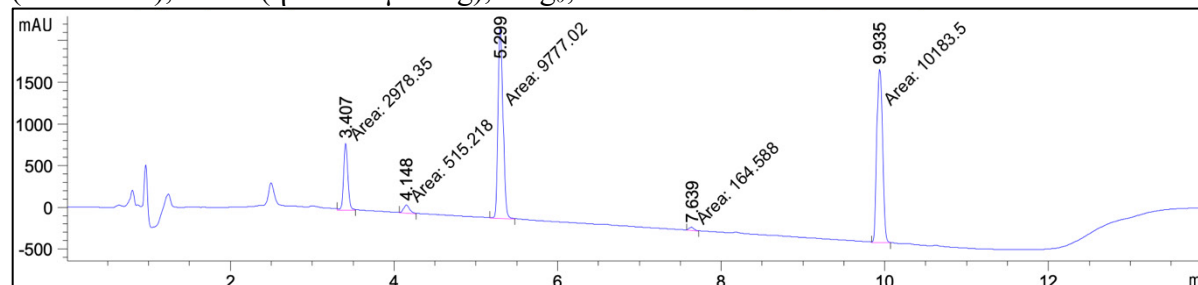
Chromatogram S14. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(*S,S*)-1]: (*S,S*)-1 [$R_t = 9.87$ min (conversion 100%)], Oxyma (5.27 min), *N*-acyl urea (3.38 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), (*i*-Pr)₂NEt (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 NBP/EtOAc ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



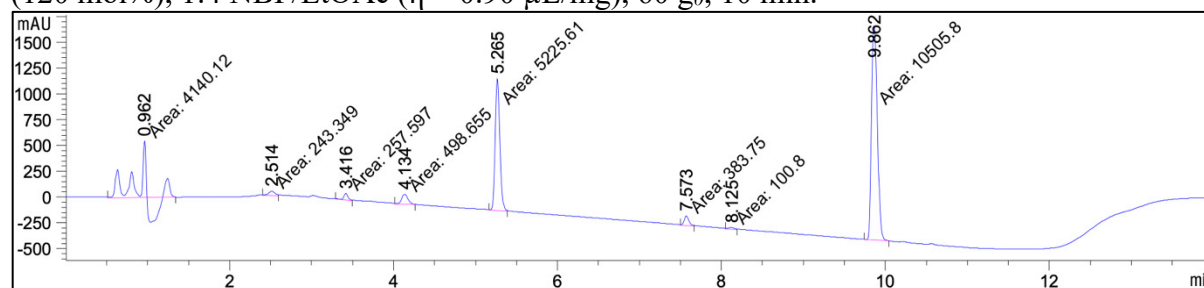
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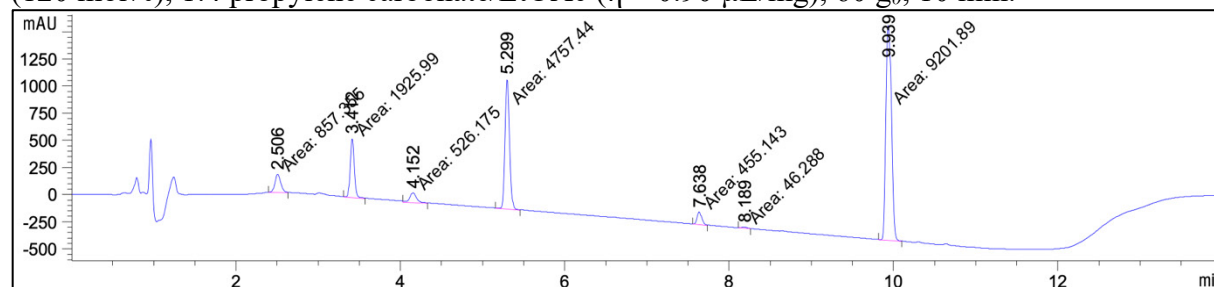
Chromatogram S15. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 10.05$ min (conversion 98%)], Cbz-Phe-OH (L-2, $R_t = 7.75$ min), Oxyma (5.48 min), *N*-acyl urea (3.68 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



Chromatogram S16. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 9.84$ min (conversion 98%)], Cbz-Phe-OH (L-2, $R_t = 7.64$ min), Oxyma (5.30 min), *N*-acyl urea (3.41 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 NBP/EtOAc ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



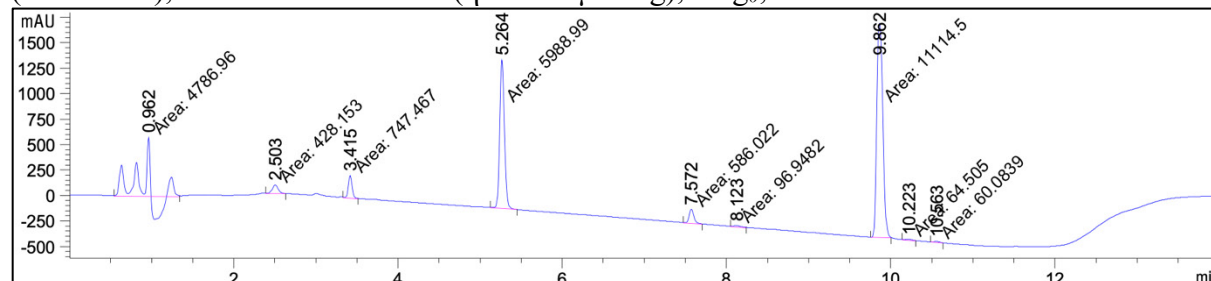
Chromatogram S17. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 9.86$ min (conversion 96%)], Cbz-Phe-OH (L-2, $R_t = 7.57$ min), Oxyma (5.27 min), *N*-acyl urea (3.42 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 propylene carbonate/EtOAc ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



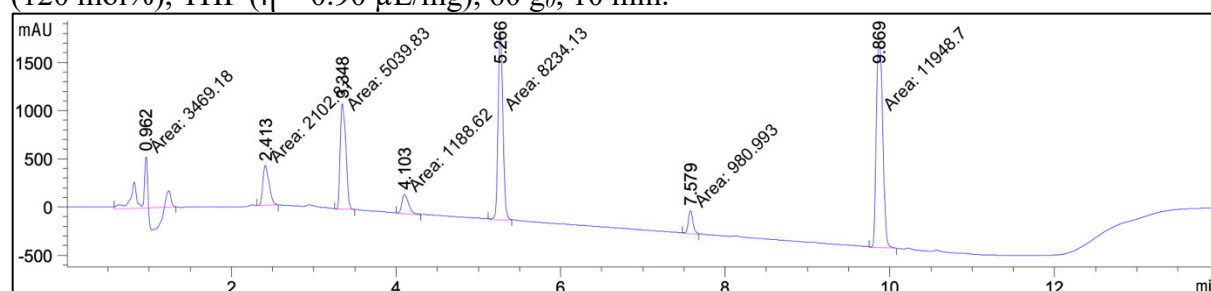
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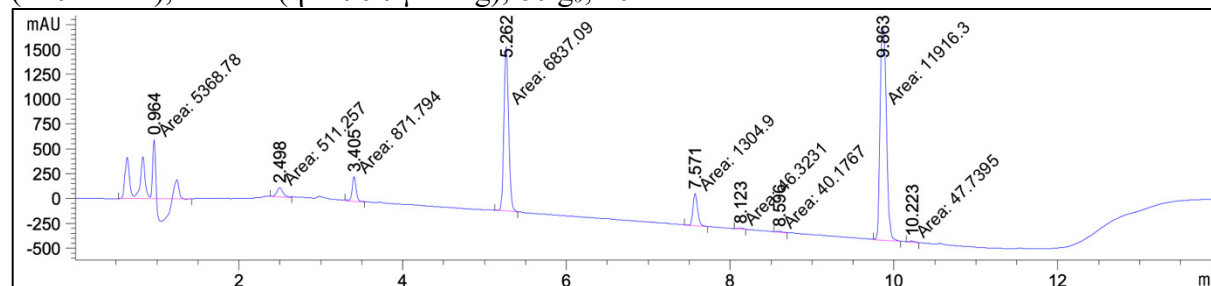
Chromatogram S18. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 9.94$ min (conversion 95%)], Cbz-Phe-OH (L-2, $R_t = 7.64$ min), Oxyma (5.30 min), *N*-acyl urea (3.41 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 1:4 sulfolane/EtOAc ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



Chromatogram S19. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 9.86$ min (conversion 95%)], Cbz-Phe-OH (L-2, $R_t = 7.57$ min), Oxyma (5.26 min), *N*-acyl urea (3.41 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), THF ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



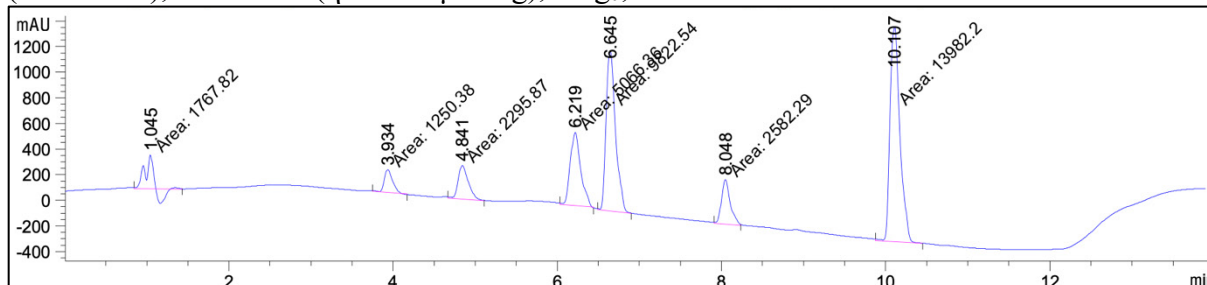
Chromatogram S20. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(S,S)-1]: (S,S)-1 [$R_t = 9.87$ min (conversion 92%)], Cbz-Phe-OH (L-2, $R_t = 7.58$ min), Oxyma (5.26 min), *N*-acyl urea (3.35 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), EtOAc ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



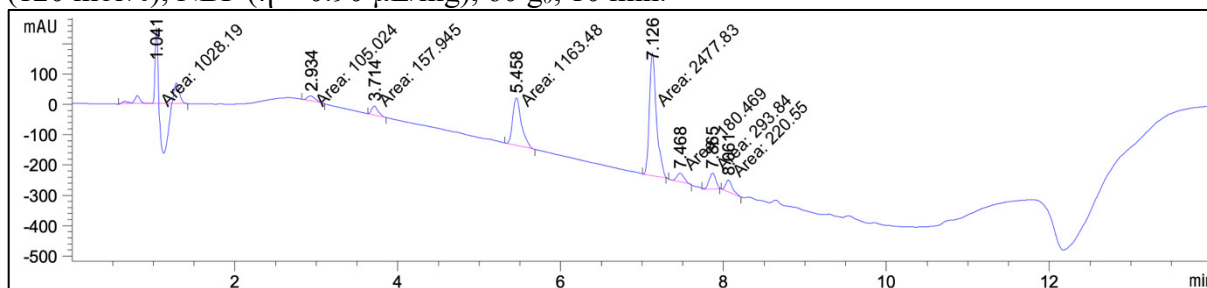
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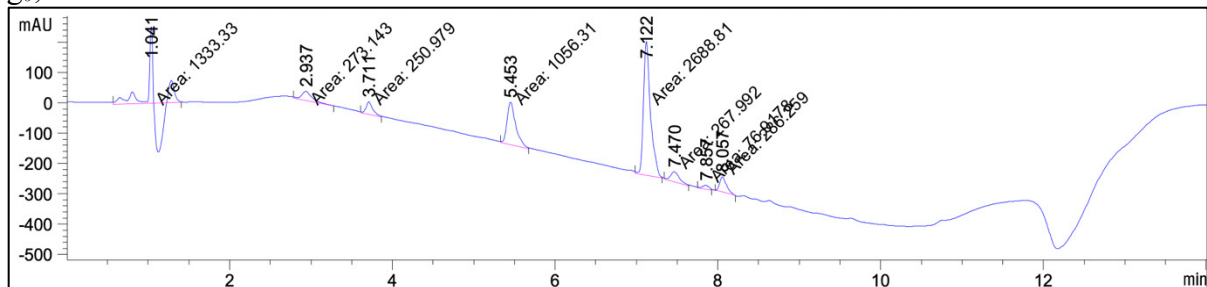
Chromatogram S21. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(*S,S*)-1]: (*S,S*)-1 [$R_t = 9.86$ min (conversion 90%)], Cbz-Phe-OH (L-2, $R_t = 7.57$ min), Oxyma (5.26 min), *N*-acyl urea (3.41 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), 2-MeTHF ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



Chromatogram S22. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Phe-OH (L-2) and HCl•H-Val-Ot-Bu (L-3) to provide Cbz-Phe-Val-Ot-Bu [(*S,S*)-1]: (*S,S*)-1 [$R_t = 10.11$ min (conversion 84%)], Cbz-Phe-OH (L-2, $R_t = 8.05$ min), Oxyma (6.65 min), *N*-acyl urea (3.93 min). Reaction conditions: HCl•Val-Ot-Bu (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), NBP ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



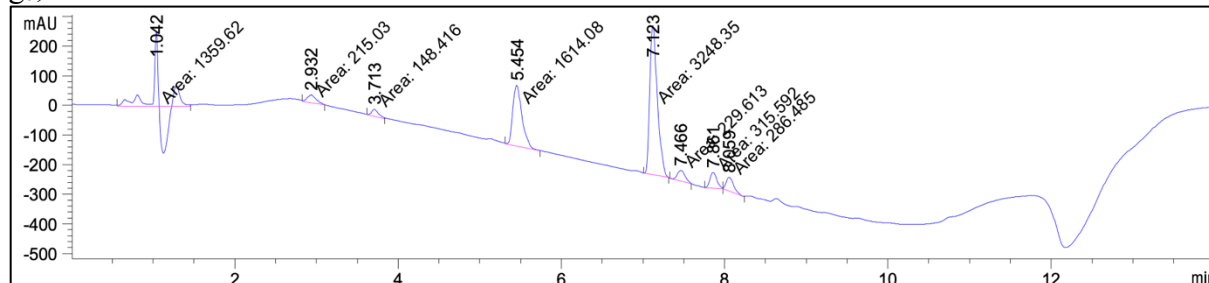
Chromatogram S23. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-4 and proline amide (5) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-6, 90% conversion]: diastereomer (*S,R,S*)-6 (6.8%, $R_t = 7.47$ min) and (*S,S,S*)-6 (93.2%, $R_t = 7.13$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (150 mol%), EDCI (120 mol%), Oxyma (100 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



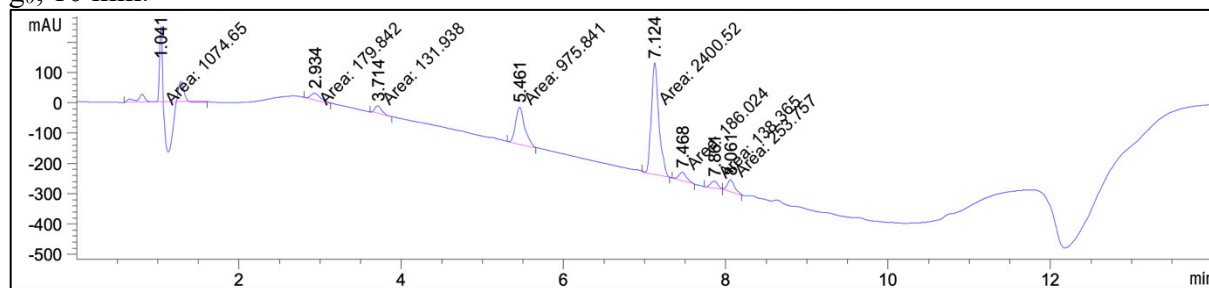
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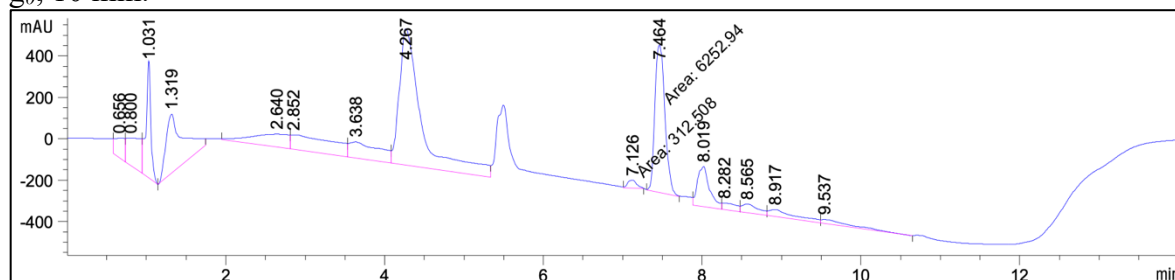
Chromatogram S24. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 96% conversion]: diastereomer (*S,R,S*)-**6** (9.1%, $R_t = 7.47$ min) and (*S,S,S*)-**6** (90.9%, $R_t = 7.13$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



Chromatogram S25. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 92% conversion]: diastereomers (*S,R,S*)-**6** (6.6%, $R_t = 7.47$ min) and (*S,S,S*)-**6** (93.4%, $R_t = 7.12$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (150 mol%), EDCI (100 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



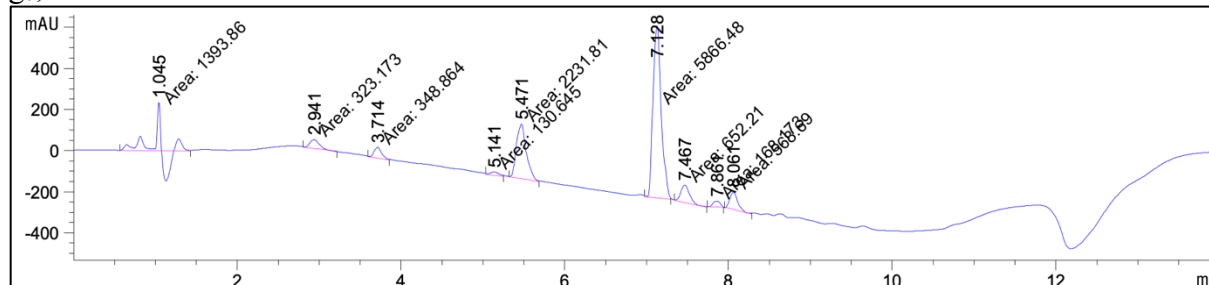
Chromatogram S26. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 95% conversion]: diastereomers (*S,R,S*)-**6** (7.2%, $R_t = 7.47$ min) and (*S,S,S*)-**6** (92.8%, $R_t = 7.12$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (150 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



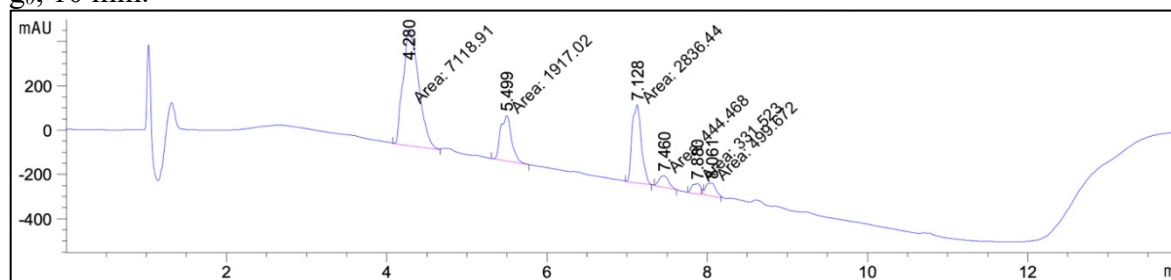
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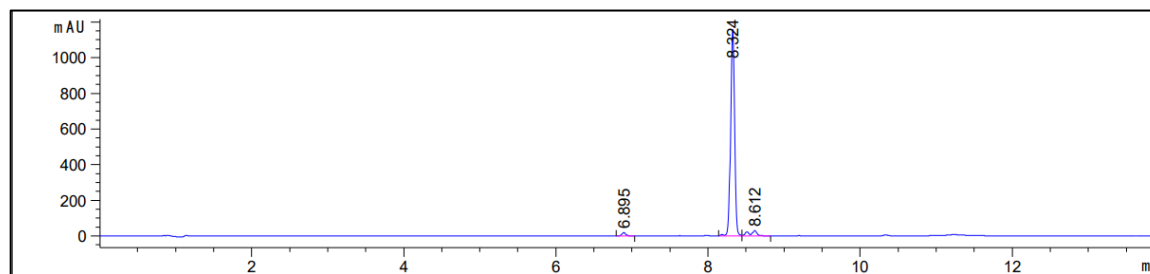
Chromatogram S27. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 95% conversion]: diastereomers (*S,R,S*)-**6** (7.2%, $R_t = 7.47$ min) and (*S,S,S*)-**6** (92.8%, $R_t = 7.12$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (150 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



Chromatogram S28. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 97% conversion]: diastereomers (*S,R,S*)-**6** (10%, $R_t = 7.47$ min) and (*S,S,S*)-**6** (90%, $R_t = 7.13$ min). Reaction conditions: Et₃N (0 mol%), Proline amide (100 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



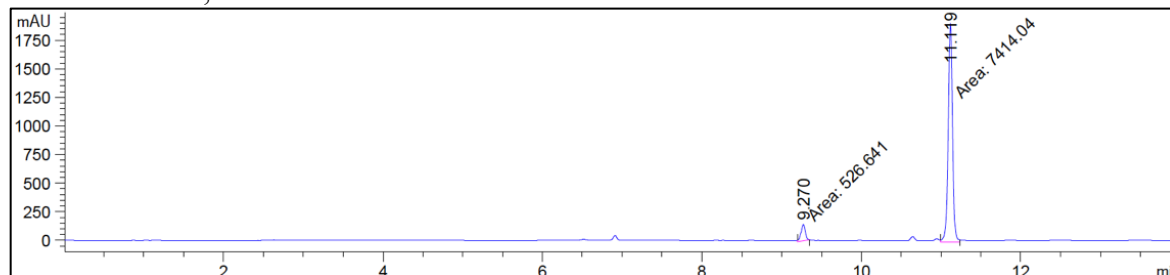
Chromatogram S29. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of acid (*S,S*)-**4** and proline amide (**5**) to provide Cbz-Phe-Val-Pro-NH₂ [(*S,S,S*)-**6**, 91% conversion]: diastereomers (*S,R,S*)-**6** (13.6%, $R_t = 7.46$ min) and (*S,S,S*)-**6** (86.4%, $R_t = 7.13$ min). Reaction conditions: Et₃N (120 mol%), Proline amide (150 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



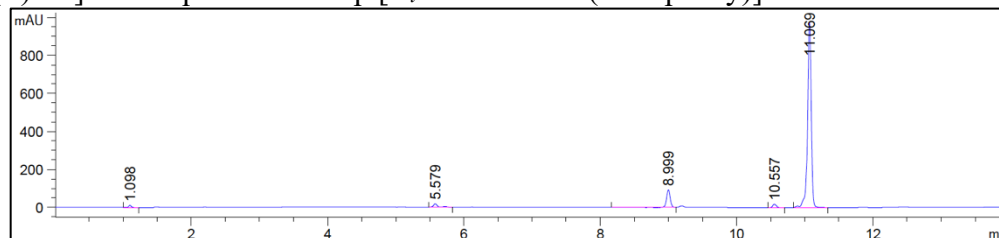
Chromatogram S30. LC-MS (Method B, $\lambda = 254$ nm) after aqueous workup of Fmoc-Lys(Boc)-OCH₂CH=CH₂ [**22** (94% purity), $R_t = 8.32$ min].

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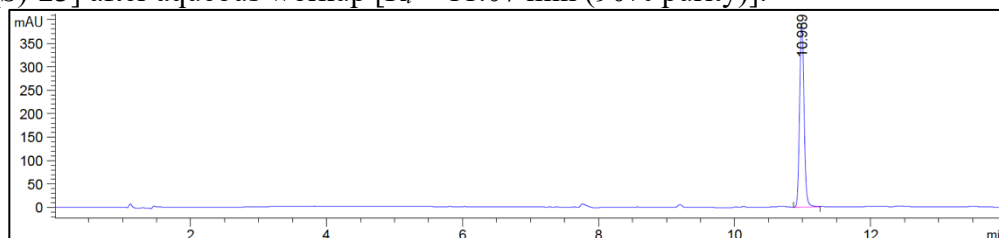
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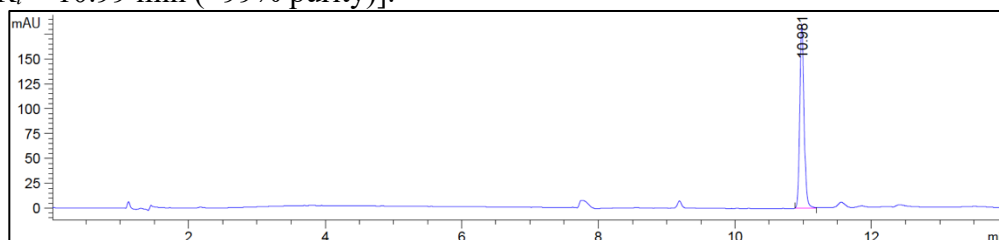
Chromatogram S31. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Val-ODmb [(*R*)-**23**] after aqueous workup [$R_t = 11.12$ min (93% purity)].



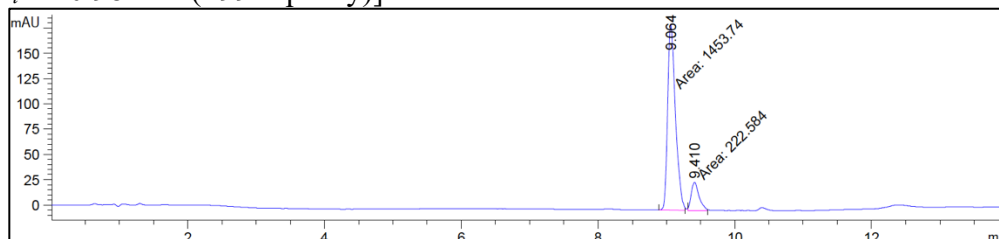
Chromatogram S32. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of Fmoc-L-Val-ODmb [(*S*)-**23**] after aqueous workup [$R_t = 11.07$ min (90% purity)].



Chromatogram S33. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of H-D-Val-ODmb [(*R*)-**24**, $R_t = 10.99$ min (>99% purity)].



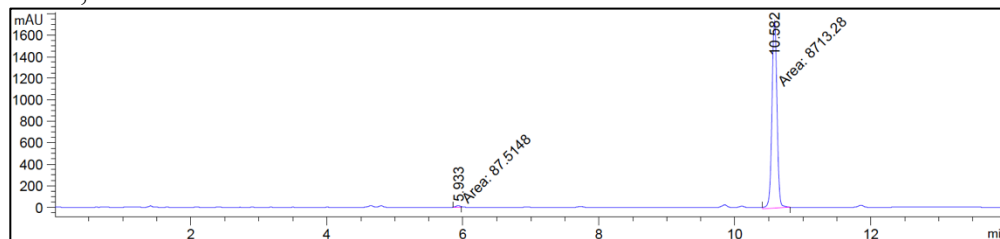
Chromatogram S34. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of H-L-Val-ODmb [(*S*)-**24**, $R_t = 10.98$ min (>99% purity)].



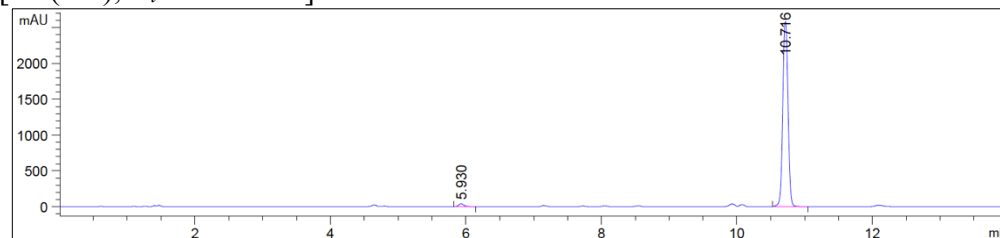
Chromatogram S35. LC-MS (Method C, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Leu-NHCH₂CCH (**27**, $R_t = 9.06$ min (87% purity)) containing recovered Fmoc-D-Leu-OH [**D-20** (13%), $R_t = 9.41$ min].

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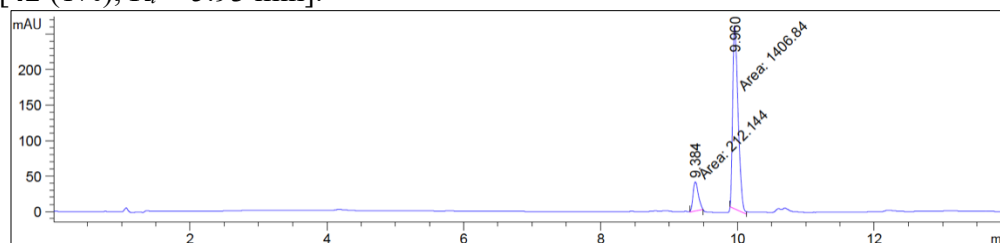
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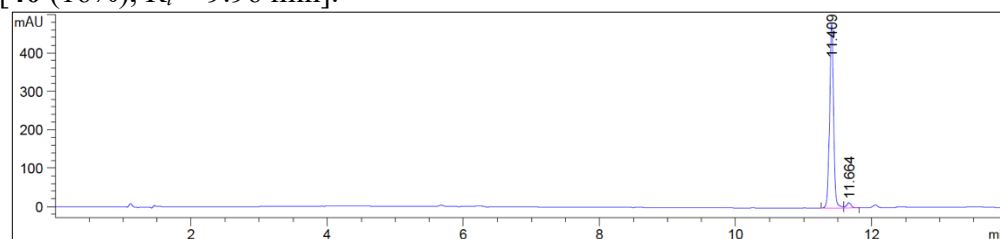
Chromatogram S36. LC-MS (Method D, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-D-Val-ODmb [(*R,R*)-**34**, $R_t = 10.58$ min (99% purity)] containing recovered Fmoc-D-Thr(*t*-Bu)-OH [**42** (1%), $R_t = 5.93$ min].



Chromatogram S37. LC-MS (Method D, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-L-Val-ODmb [(*R,S*)-**34**, $R_t = 10.58$ min (99% purity)] containing recovered Fmoc-D-Thr(*t*-Bu)-OH [**42** (1%), $R_t = 5.93$ min].



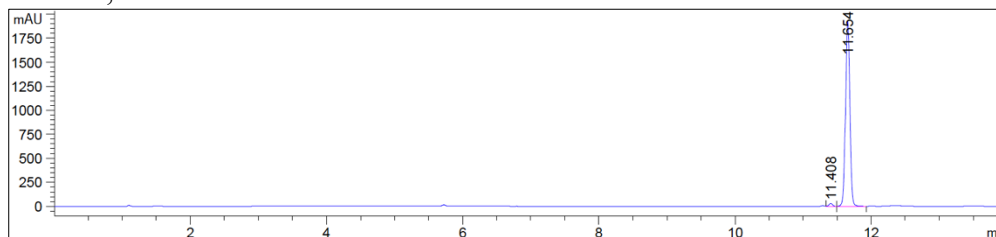
Chromatogram S38. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH (**41**, $R_t = 9.96$ min (84% purity)] containing recovered Fmoc-D-Glu(*t*-Bu)-OH [**40** (16%), $R_t = 9.96$ min].



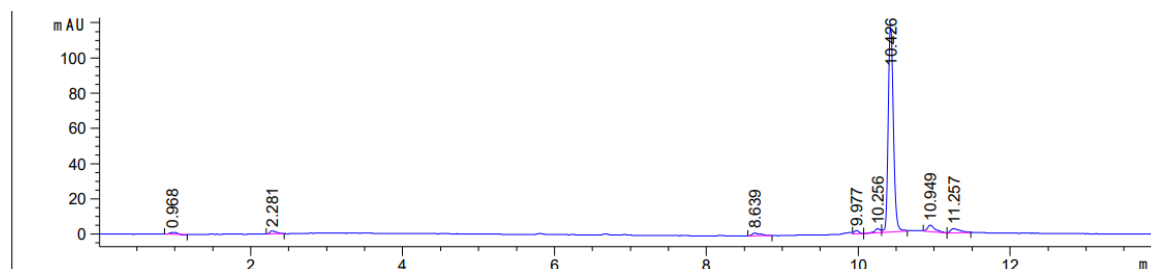
Chromatogram S39. LC-MS (Method A, $\lambda = 254$ nm) after purification on silica gel of Fmoc-D-Thr(*t*-Bu)-D-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*R*)-**43**, $R_t = 11.41$ min (97.5% purity)] containing (*S*)-**43** [2.5%, $R_t = 11.66$ min], d.r. 97.5:2.5.

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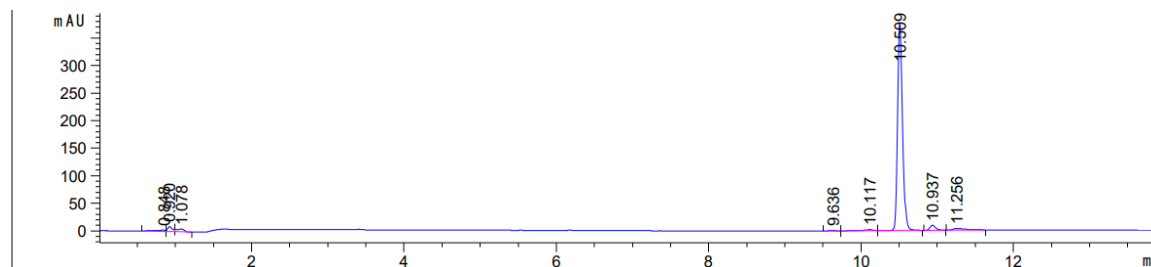
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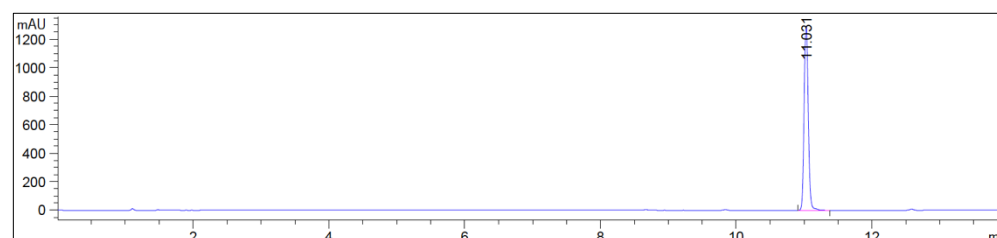
Chromatogram S40. LC-MS (Method A, $\lambda = 254$ nm) after purification on silica gel of Fmoc-D-Thr(*t*-Bu)-L-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*S*)-**43**, *R*_t = 11.41 min (1.5%) and 11.65 min (98.5%) for (*R*)- and (*S*)-**43**], d.r. 1.5:98.5.



Chromatogram S41. LC-MS (Method B, $\lambda = 280$ nm) after silica gel chromatography of Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44** (89% purity), *R*_t = 10.43 min].



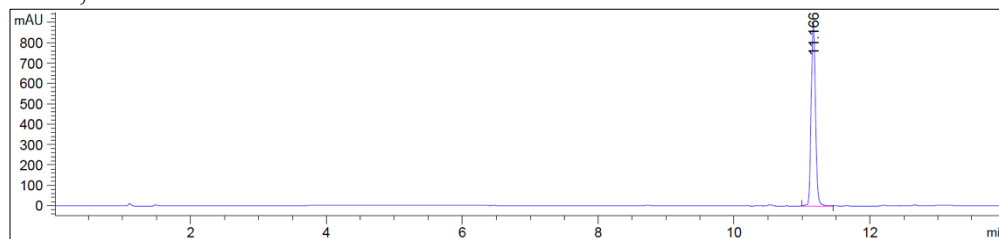
Chromatogram S42. LC-MS (Method B, $\lambda = 280$ nm) after silica gel chromatography of Fmoc-Lys(Boc)-Leu-Nle-D-Trp(Boc)-ODmb [(*S*)-**44** (97% purity), *R*_t = 10.51 min].



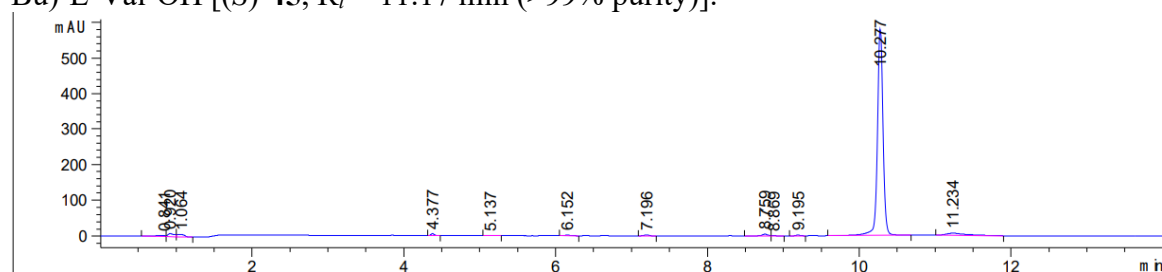
Chromatogram S43. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-D-Val-OH [(*R*)-**45**, *R*_t = 11.03 min (>99% purity)].

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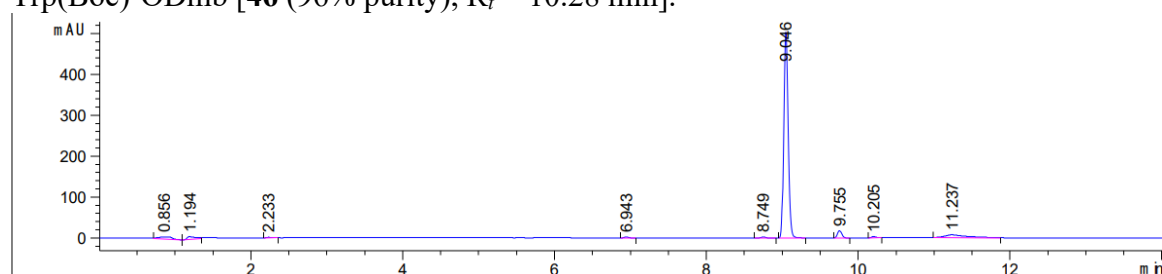
Maarouf-Mesli, N. et al.



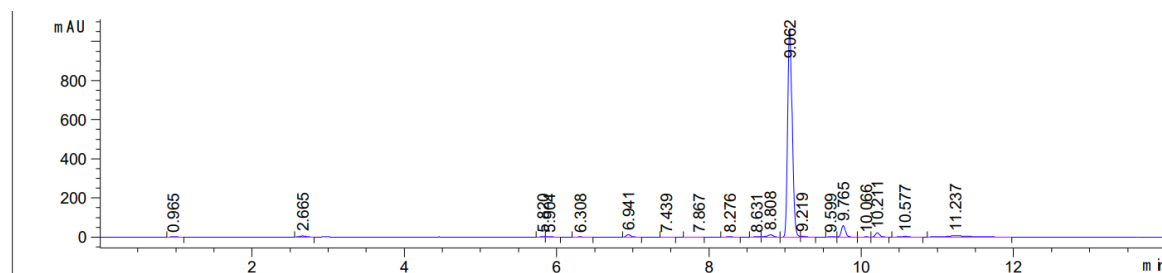
Chromatogram S44. LC-MS (Method A, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-L-Val-OH [(*S*)-**45**, $R_t = 11.17$ min (>99% purity)].



Chromatogram S45. LC-MS (Method B, $\lambda = 280$ nm) after precipitation of Fmoc-Nle-D-Trp(Boc)-ODmb [**46** (96% purity), $R_t = 10.28$ min].



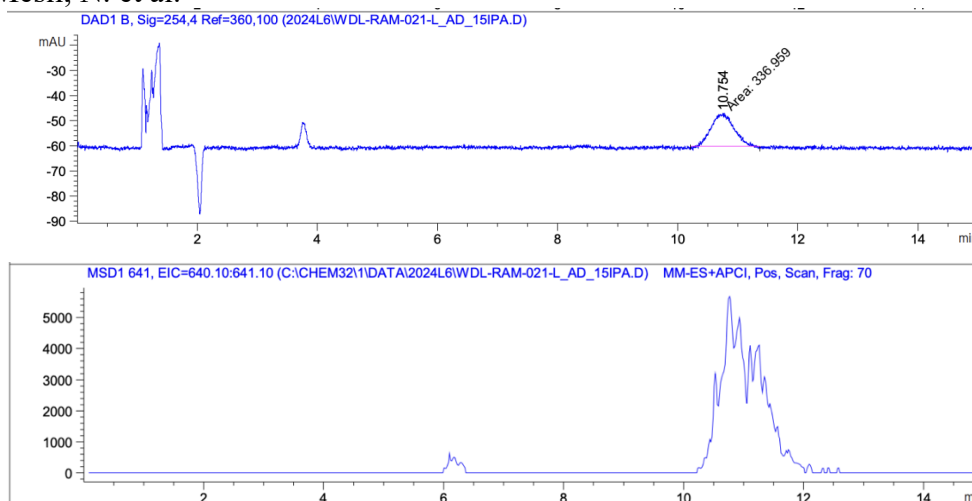
Chromatogram S46. LC-MS (Method B, $\lambda = 280$ nm) after precipitation of Fmoc-Lys(Boc)-D-Leu-ODmb [(*R*)-**47** (96% purity), $R_t = 9.06$ min].



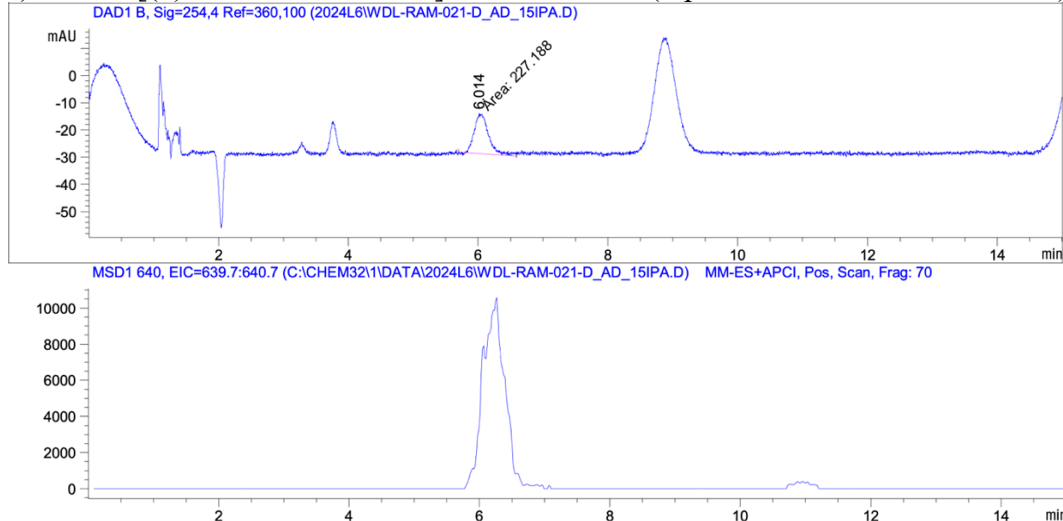
Chromatogram S47. LC-MS (Method B, $\lambda = 280$ nm) after precipitation of Fmoc-Lys(Boc)-Leu-ODmb [(*S*)-**47** (90% purity), $R_t = 9.05$ min].

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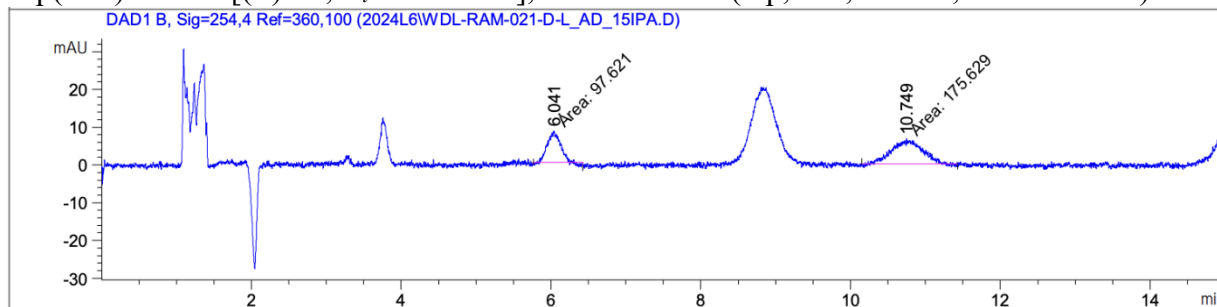
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Chromatogram S48. Chiral UV-MS-SFC chromatograms (Method G, $\lambda = 254$ nm) of Fmoc-Trp(Boc)-ODmb [(*S*)-**21**, $R_t = 10.75$ min], e.r. = 99.5:0.5 (top, UV; bottom, MS detection).



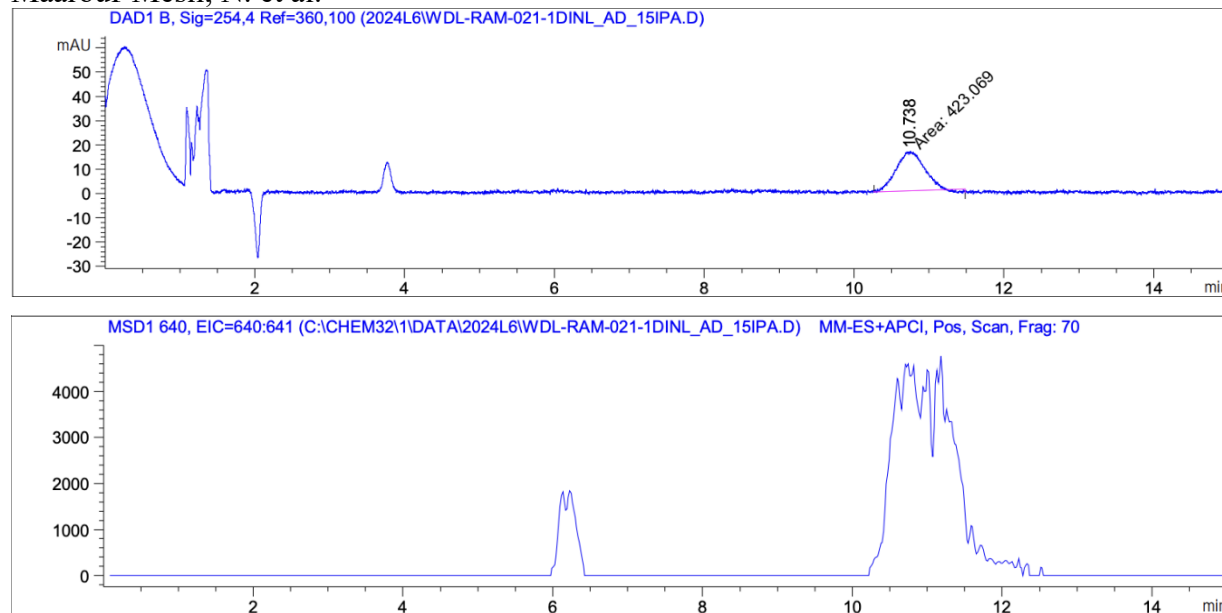
Chromatogram S49. Chiral UV-MS-SFC chromatograms (Method G, $\lambda = 254$ nm) of Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**, $R_t = 6.01$ min], e.r. = 99.5:0.5 (top, UV; bottom, MS detection).



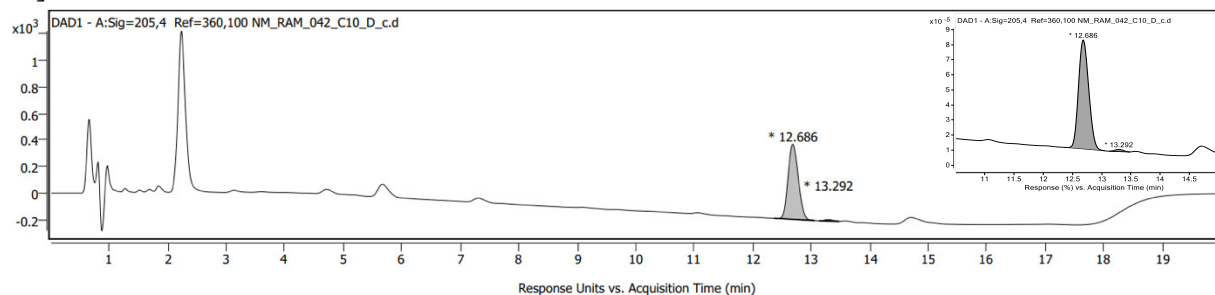
Chromatogram S50. Chiral UV-MS-SFC chromatogram (Method G, $\lambda = 254$ nm) of Fmoc-Trp(Boc)-ODmb [(*S*)-**21**, $R_t = 10.75$ min] and Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**, $R_t = 6.04$ min], e.r. = 99.5:0.5.

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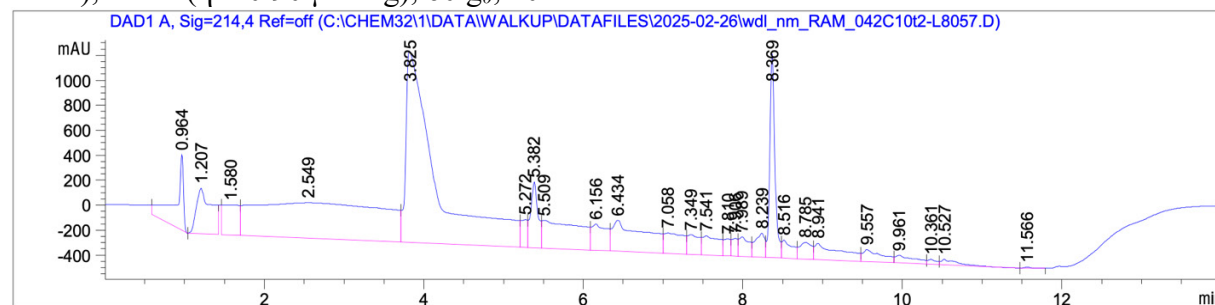
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Chromatogram S51. Chiral UV-MS-SFC chromatograms (Method G, $\lambda = 254$ nm) of Fmoc-Trp(Boc)-ODmb [(*S*)-**21**, $R_t = 10.74$ min] with addition of 1% of Fmoc-D-Trp(Boc)-ODmb [(*R*)-**21**].



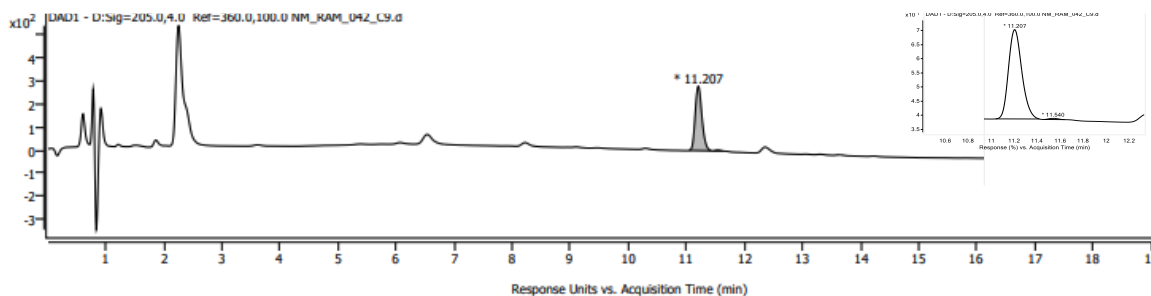
Chromatogram S52. LC-MS (Method F, $\lambda = 205$ nm) of Cbz-Ala-Phg-Ile-OMe [(*S,S,S*)-**7** 98.47%, $R_t = 12.7$ min] and Cbz-Ala-D-Phg-Ile-OMe [(*S,R,S*)-**7** 1.53%, $R_t = 13.3$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), (*i*-Pr)₂NEt (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



Chromatogram S53. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phg-OH [(*S,S*)-**8**] and HCl•H-Ile-OMe (**L-9**) to provide Cbz-Ala-Phg-Ile-OMe [(*S,S,S*)-**7**]: (*S,S,S*)-**7** [$R_t = 8.37$ min (conversion 100%)]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), (*i*-Pr)₂NEt (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.

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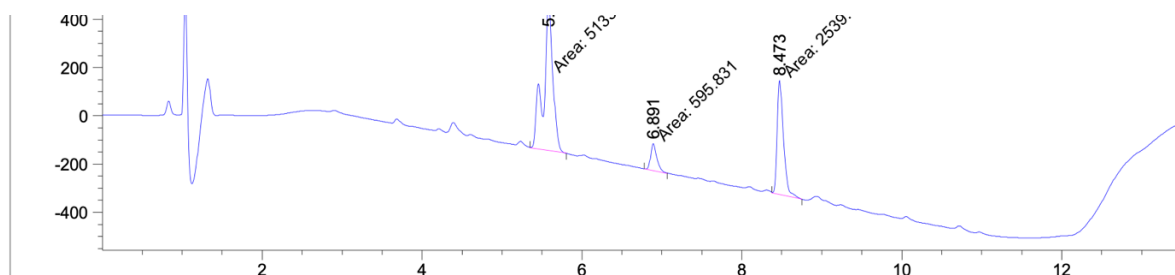
Maarouf-Mesli, N. et al.



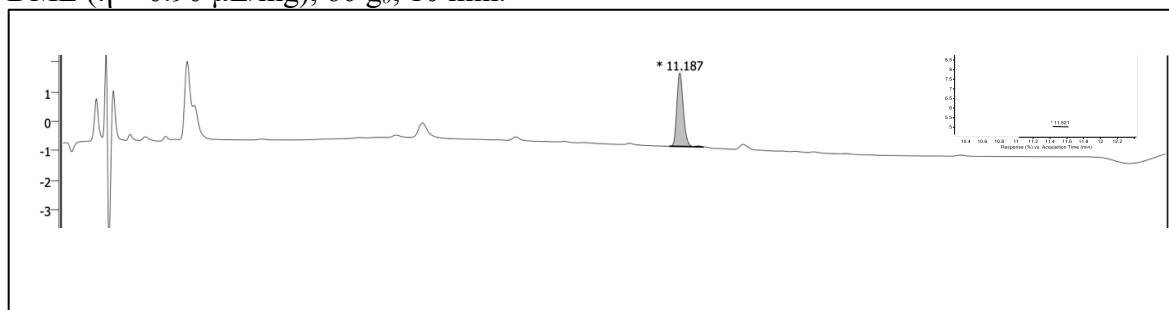
Chromatogram Peaks

RT	Area	Area Sum %
11.2	99.12%	
11.5	0.88%	

(*S,S,S*)-**7**
 99.12%, $R_t = 11.2$ min), Cbz-Ala-D-Phg-Ile-OMe [(*S,R,S*)-**7** 0.88%, $R_t = 11.5$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), NaH_2PO_4 (400 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



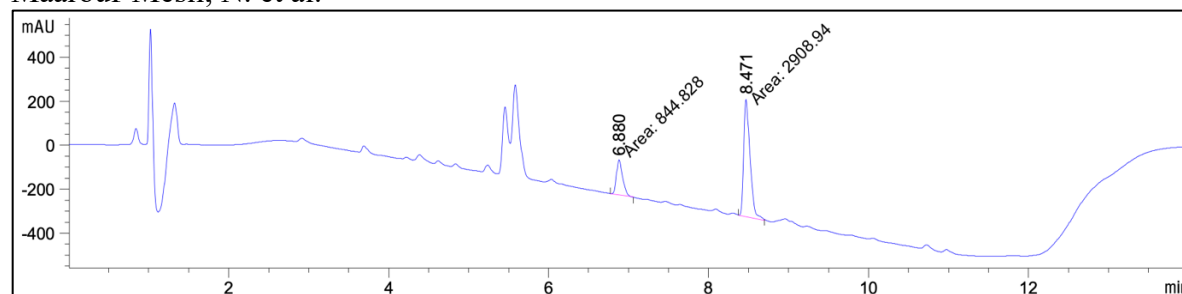
Chromatogram S55. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phg-OH [(*S,S*)-**8**] and HCl•H-Ile-OMe (**L-9**) to provide Cbz-Ala-Phg-Ile-OMe [(*S,S,S*)-**7**]: (*S,S,S*)-**7** [$R_t = 8.47$ min (conversion 81%)], Cbz-Ala-Phg-OH [(*S,S*)-**8**, $R_t = 6.89$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), NaH_2PO_4 (400 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



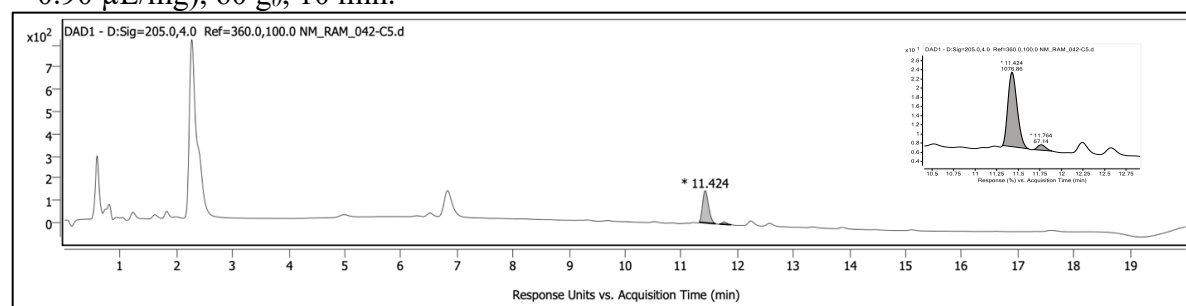
Chromatogram S56. LC-MS (Method E, $\lambda = 205$ nm) of Cbz-Ala-Phg-Ile-OMe (99.03%, (*S,S,S*)-**7**, $R_t = 11.2$ min), Cbz-Ala-D-Phg-Ile-OMe [(*S,R,S*)-**7** 0.97%, $R_t = 11.5$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), Et_3N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.

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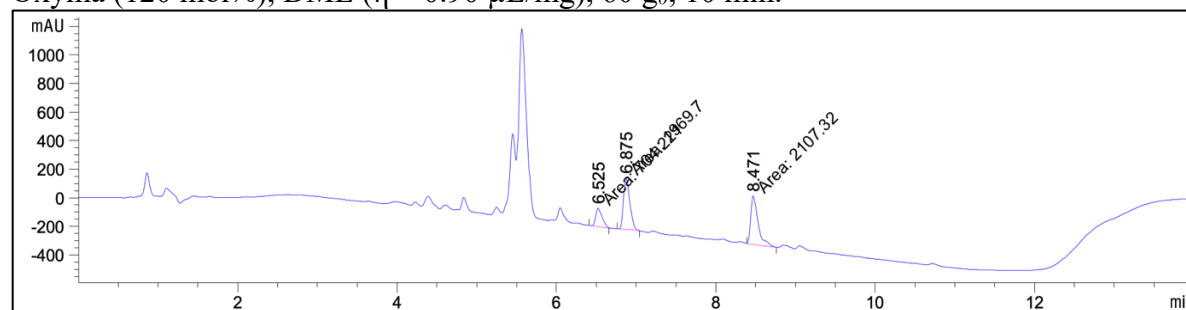
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Chromatogram S57. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phg-OH [(S,S)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-Phg-Ile-OMe [(S,S,S)-7]: (S,S,S)-7 [$R_t = 8.47$ min (conversion 77%)], Cbz-Ala-Phg-OH [(S,S)-8, $R_t = 6.88$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



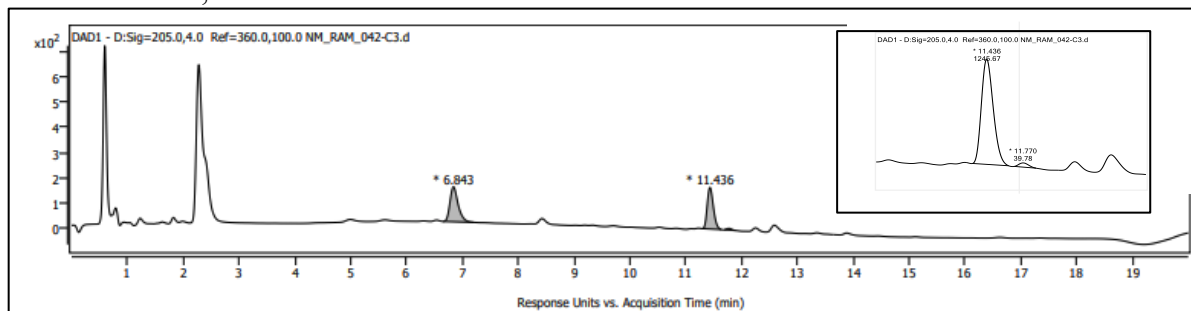
Chromatogram S58. LC-MS (Method E, $\lambda = 205$ nm) of Cbz-Ala-Phg-Ile-OMe [(S,S,S)-7] 94.13%, $R_t = 11.4$ min), Cbz-Ala-D-Phg-Ile-OMe [(S,R,S)-7] 5.87%, $R_t = 11.8$ min). Reaction conditions: HCl•H-Ile-OMe (150 mol%), *N*-methyl morpholine (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



Chromatogram S59. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phg-OH [(S,S)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-Phg-Ile-OMe [(S,S,S)-7]: (S,S,S)-7 [$R_t = 8.47$ min (conversion 52%)], Cbz-Ala-Phg-OH [(S,S)-8, $R_t = 6.88$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), *N*-methyl morpholine (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.

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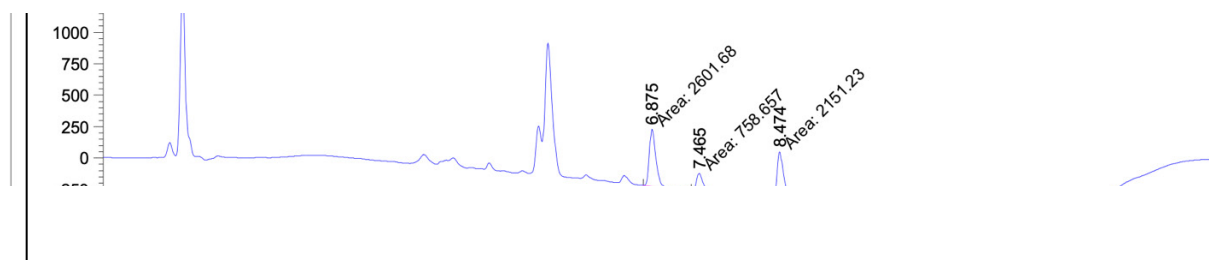
Maarouf-Mesli, N. et al.



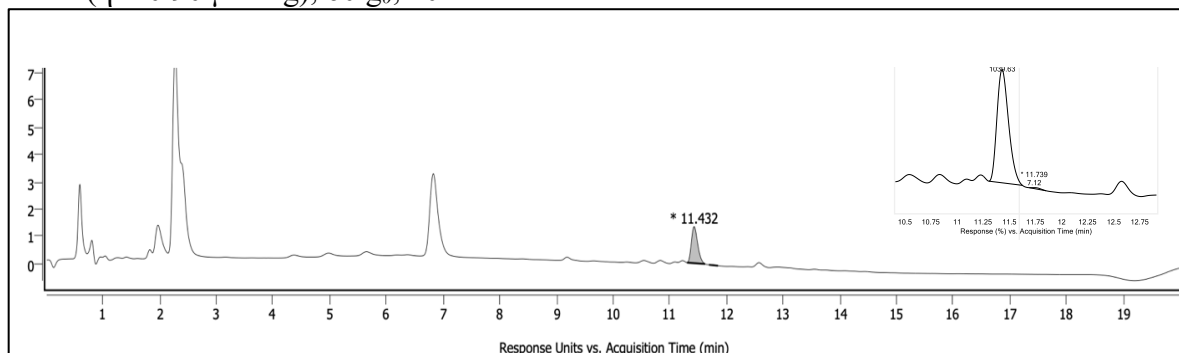
Chromatogram Peaks

RT	Area	Area Sum %
6.843		
11.436		

96.91%, $R_t = 11.4$ min], Cbz-Ala-D-Phe-Ile-OMe [(*S,R,S*)-7 3.09%, $R_t = 11.8$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), 2,4,6-collidine (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



Chromatogram S61. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phe-OH [(*S,S*)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-Phe-Ile-OMe [(*S,S,S*)-7]: (*S,S,S*)-7 [$R_t = 8.47$ min (conversion 45%)], Cbz-Ala-Phe-OH [(*S,S*)-8, $R_t = 6.88$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), 2,4,6-collidine (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.



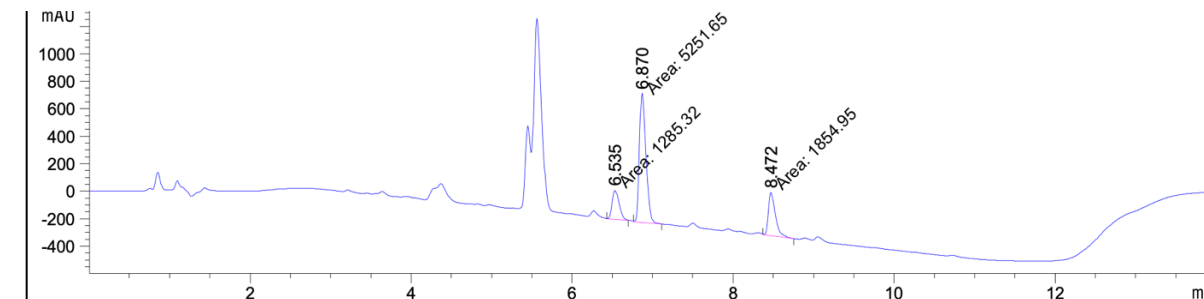
Chromatogram Peaks

RT	Area	Area Sum %
6.875		
7.465		
8.474		
11.432		

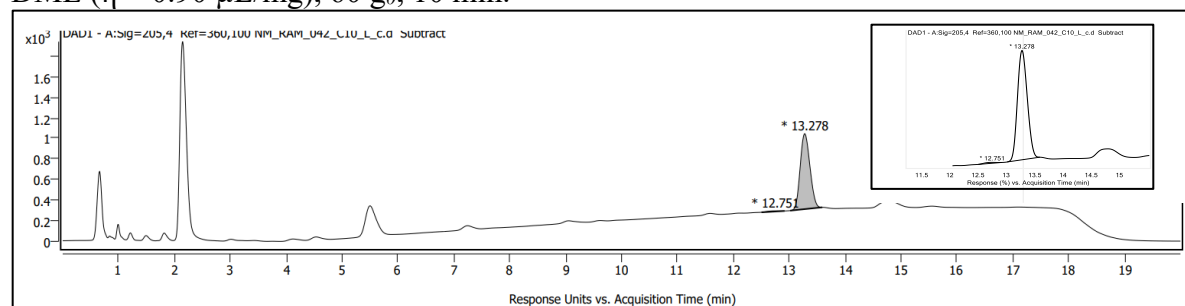
7, $R_t = 11.4$ min), Cbz-Ala-D-Phe-Ile-OMe (0.68% (*S,R,S*)-7, $R_t = 11.7$ min). Reaction conditions: HCl•H-Ile-OMe (150 mol%), NaH₂PO₄ (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g₀, 10 min.

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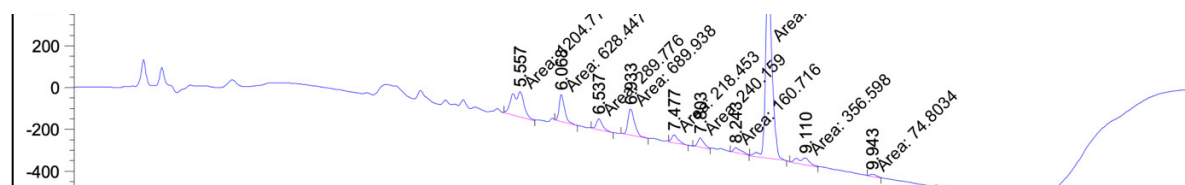
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Chromatogram S63. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phe-OH [(S,S)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-Phe-Ile-OMe [(S,S,S)-7]: (S,S,S)-7 [$R_t = 8.47$ min (conversion 84%)], Cbz-Ala-Phe-OH [(S,S)-8, $R_t = 6.87$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), NaH_2PO_4 (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g θ , 10 min.



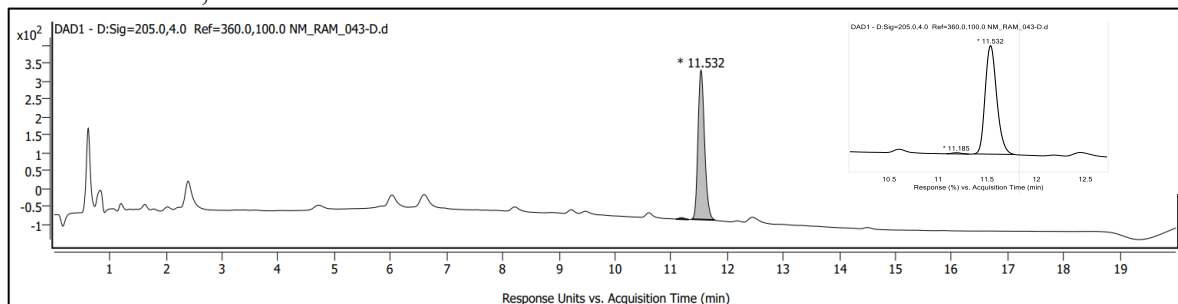
Chromatogram S64. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-Phe-Ile-OMe [(S,S,S)-7] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-Phe-Ile-OMe [(S,R,S)-7]: (S,R,S)-7 [$R_t = 13.3$ min (conversion 84%)], Cbz-Ala-Phe-Ile-OMe [(S,S,S)-7, $R_t = 12.8$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), (*i*-Pr) $_2$ EtN (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g θ , 10 min.



Chromatogram S65. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-D-Phe-OH [(S,R)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-D-Phe-Ile-OMe [(S,R,S)-7]: (S,R,S)-7 [$R_t = 8.64$ min (conversion 88%)], Cbz-Ala-D-Phe-OH [(S,R)-8, $R_t = 6.93$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), (*i*-Pr) $_2$ EtN (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ $\mu\text{L}/\text{mg}$), 60 g θ , 10 min.

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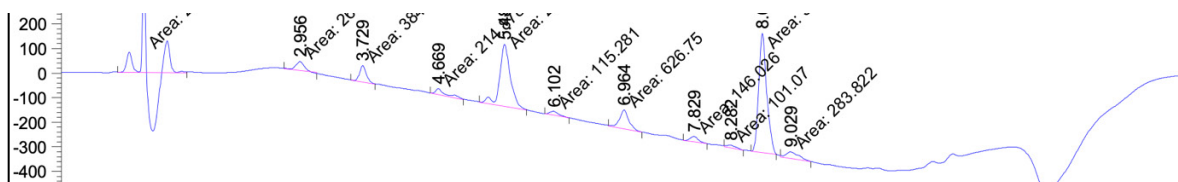
Maarouf-Mesli, N. et al.



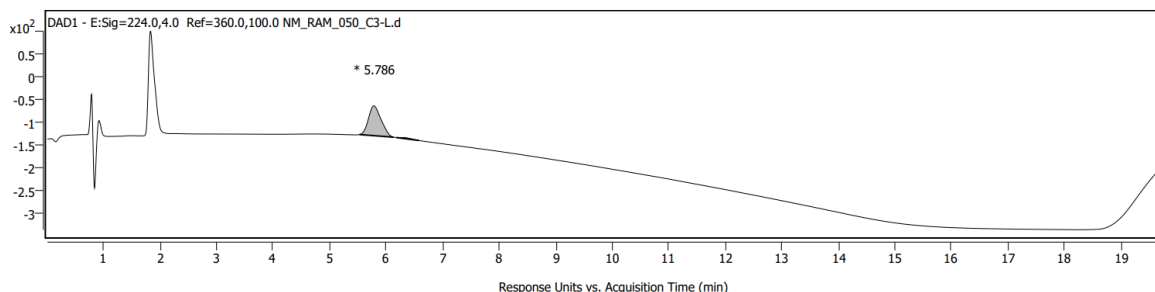
Chromatogram Peaks

RT	Area	Area Sum %
11.532	11.532	12%

(*S,R,S*)-7, $R_t = 11.5$ min), Cbz-Ala-Phg-Ile-OMe (0.88% (*S,S,S*)-7, $R_t = 11.2$ min). Reaction conditions: HCl•H-Ile-OMe (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.



Chromatogram S67. LC-MS (Method A, $\lambda = 214$ nm) for the coupling of Cbz-Ala-D-Phg-OH [(*S,R*)-8] and HCl•H-Ile-OMe (L-9) to provide Cbz-Ala-D-Phg-Ile-OMe [(*S,R,S*)-7]: (*S,R,S*)-7 [$R_t = 8.68$ min (conversion 84%)], Cbz-Ala-D-Phg-OH [(*S,R*)-8, $R_t = 6.96$ min]. Reaction conditions: HCl•H-Ile-OMe (150 mol%), Et₃N (120 mol%), EDCI (120 mol%), Oxyma (120 mol%), DME ($\eta = 0.90$ μ L/mg), 60 g₀, 10 min.

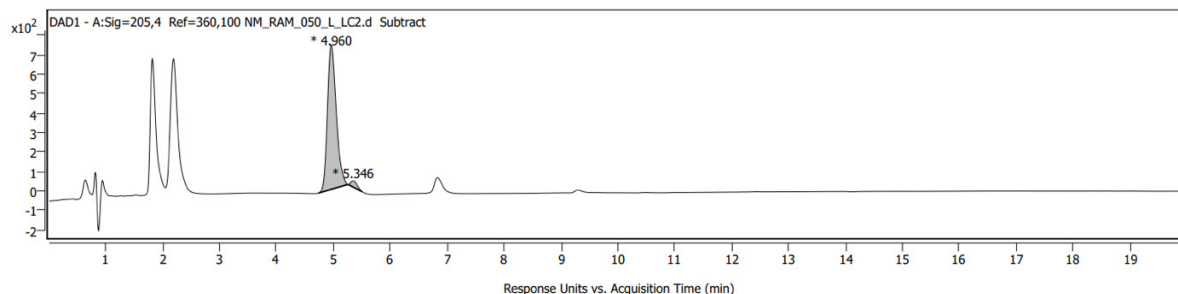


Chromatogram Peaks		
RT	Area	Area Sum %
5.8	1020	97.06
6.3	31	2.94

Chromatogram S68. LC-MS (Method E, $\lambda = 224$ nm) of Cbz-Phg-Pro-NH₂ (97.06%, (*S,S*)-10, $R_t = 5.8$ min), Cbz-D-Phg-Pro-NH₂ (2.94% (*S,R*)-10, $R_t = 6.3$ min). Reaction conditions: Cbz-Phg-OH (100 mol%), H-Pro-NH₂ (150 mol%), Oxyma (150 mol%), EDCI (120 mol%), DME (0.90 μ L/mg), RAM (60 g₀, 10 min).

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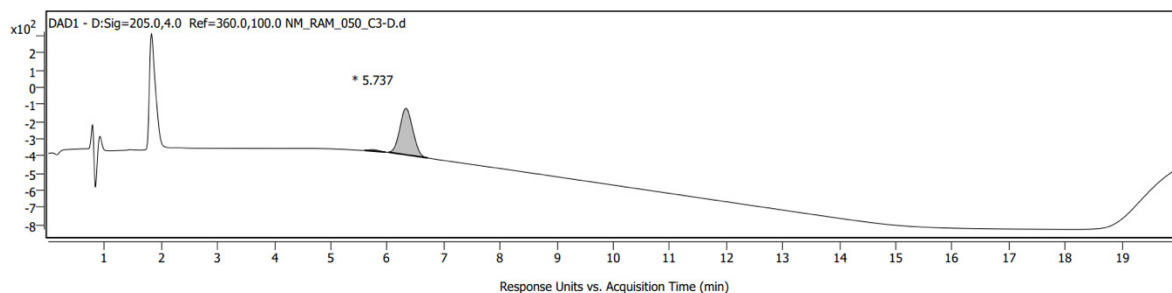
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Chromatogram Peaks

RT	Area	Area Sum %
5.0	8017	96.92
5.3	255	3.08

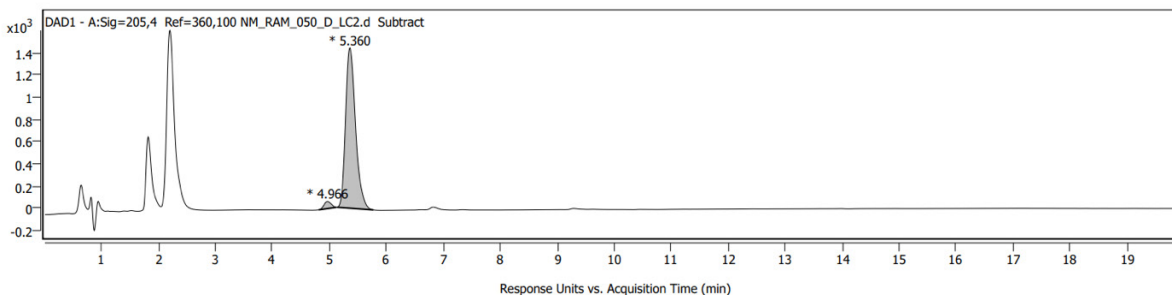
Chromatogram S69. LC-MS (Method E, $\lambda = 205$ nm) of Cbz-Phg-Pro-NH₂ (96.92%, (*S,S*)-**10**, $R_t = 5.0$ min), Cbz-D-Phg-Pro-NH₂ (3.08%, (*S,R*)-**10**, $R_t = 5.3$ min). Reaction conditions: Cbz-Phg-OH (100 mol%), H-Pro-NH₂ (150 mol%), Oxyma (120 mol%), EDCI (120 mol%), NBP (0.90 μ L/mg), RAM (60 g₀, 10 min).



Chromatogram Peaks

RT	Area	Area Sum %
5.7	101	2.30
6.3	4311	97.70

Chromatogram S70. LC-MS (Method E, $\lambda = 205$ nm) of Cbz-D-Phg-Pro-NH₂ (97.70%, (*S,R*)-**10**, $R_t = 6.3$ min), Cbz-Phg-Pro-NH₂ (2.30% (*S,S*)-**10**, $R_t = 5.7$ min). Reaction conditions: Cbz-D-Phg-OH (100 mol%), H-Pro-NH₂ (150 mol%), Oxyma (120 mol%), EDCI (120 mol%), DME (0.90 μ L/mg), RAM (60 g₀, 10 min).



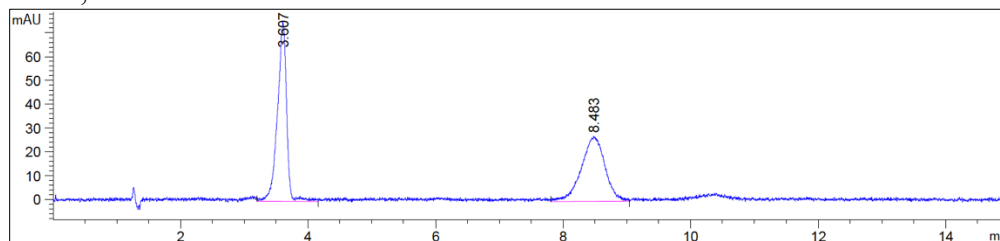
Chromatogram Peaks

RT	Area	Area Sum %
5.0	564	3.12
5.4	17539	96.88

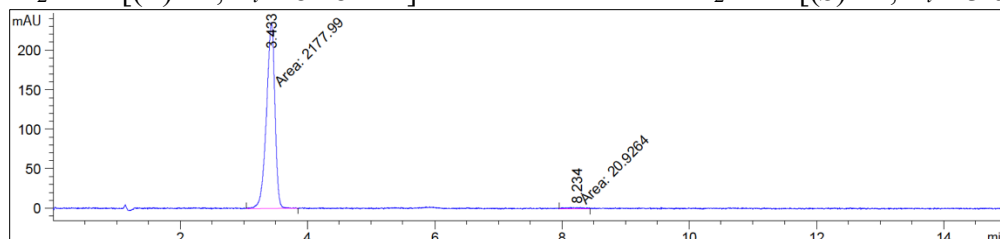
Chromatogram S71. LC-MS (Method E, $\lambda = 205$ nm) of Cbz-D-Phg-Pro-NH₂ (96.88%, (*S,R*)-**10**, $R_t = 5.4$ min), Cbz-Phg-Pro-NH₂ (3.12% (*S,S*)-**10**, $R_t = 5.0$ min). Reaction conditions: Cbz-D-Phg-OH (100 mol%), H-Pro-NH₂ (150 mol%), Oxyma (120 mol%), EDCI (120 mol%), NBP (0.90 μ L/mg), RAM (60 g₀, 10 min).

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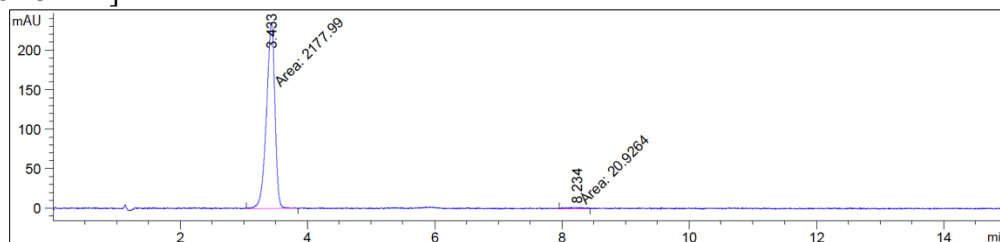
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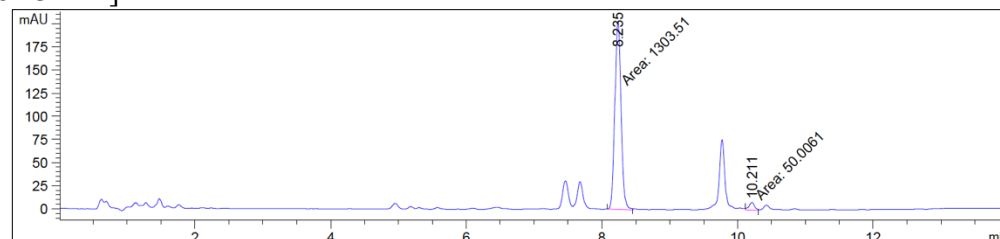
Chromatogram S72. Chiral UV-MS-SFC (Method H, $\lambda = 254$ nm) of a 1:1 mixture of Fmoc-D-Leu-NHCH₂CCH [(*R*)-**27**, $R_t = 8.48$ min] and Fmoc-Leu-NHCH₂CCH [(*S*)-**27**, $R_t = 3.61$ min].



Chromatogram S73. Chiral UV-MS-SFC (Method H, $\lambda = 254$ nm) of Fmoc-Leu-NHCH₂CCH [(*S*)-**27**, $R_t = 3.43$ min] containing a 1% incremental addition of Fmoc-D-Leu-NHCH₂CCH [(*R*)-**27**, $R_t = 8.23$ min].



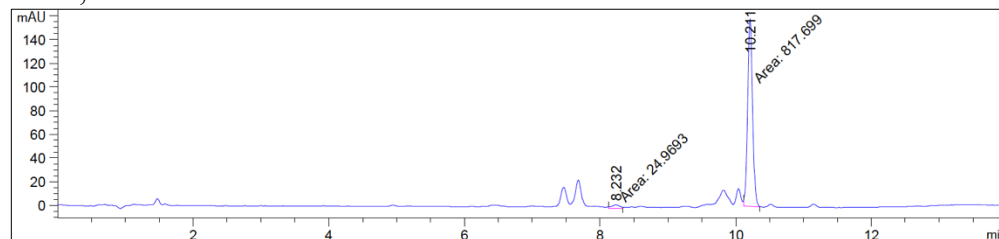
Chromatogram S74. Chiral UV-MS-SFC (Method H, $\lambda = 254$ nm) of Fmoc-Leu-NHCH₂CCH [(*S*)-**27**, $R_t = 3.43$ min] containing a 1% incremental addition of Fmoc-D-Leu-NHCH₂CCH [(*R*)-**27**, $R_t = 8.23$ min].



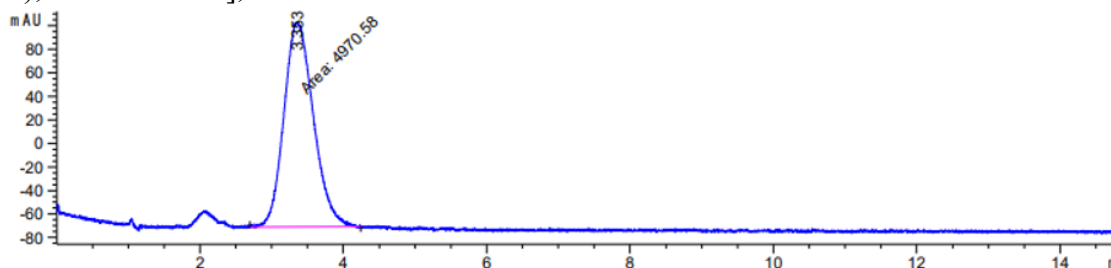
Chromatogram S75. LC-MS (Method D, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-D-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*R*)-**43** (96%), $R_t = 8.24$ min] containing [(*S*)-**43** (4%), $R_t = 10.21$ min], d.r. 96:4.

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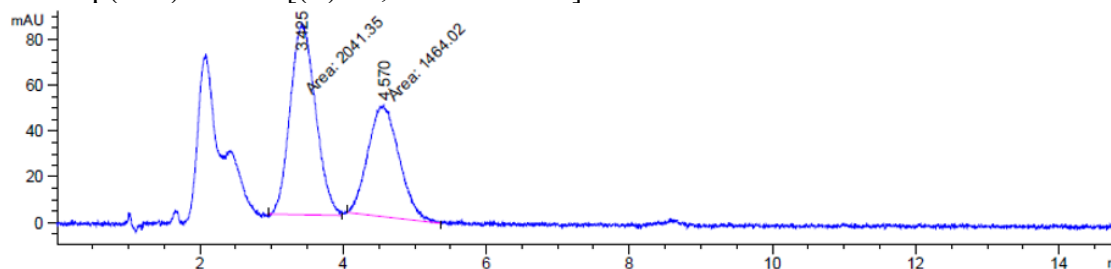
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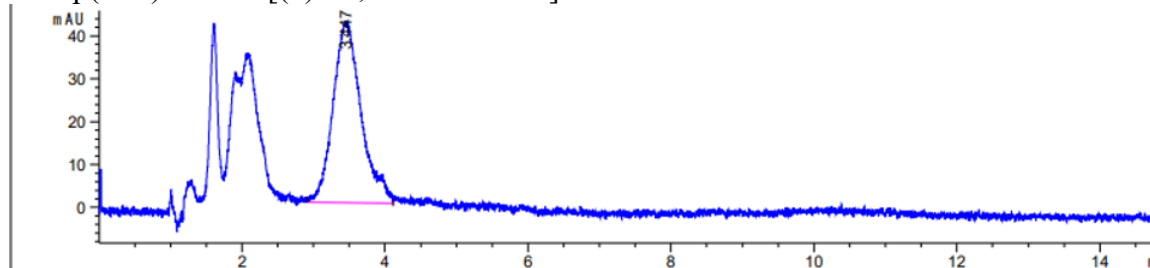
Chromatogram S76. LC-MS (Method D, $\lambda = 254$ nm) after aqueous workup of Fmoc-D-Thr(*t*-Bu)-L-Val-D-Glu(*t*-Bu)-D-Leu-NHCH₂CCH [(*S*)-**43** (97%), $R_t = 10.21$ min] containing [(*R*)-**43** (3%), $R_t = 8.24$ min], d.r. 97:3.



Chromatogram S77. Chiral SFC-UV-MS (Method H, $\lambda = 254$ nm) of Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**, $R_t = 3.49$ min].



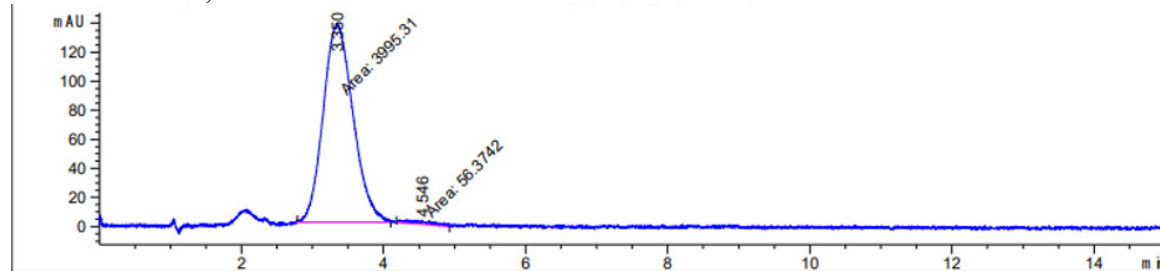
Chromatogram S78. Chiral SFC-UV-MS (Method H, $\lambda = 254$ nm) of a 1:1 mixture of Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**, $R_t = 3.49$ min] and Fmoc-Lys(Boc)-Leu-Nle-D-Trp(Boc)-ODmb [(*S*)-**44**, $R_t = 4.57$ min].



Chromatogram S79. Chiral SFC-UV-MS (Method H, $\lambda = 254$ nm) after aqueous workup of Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**, $R_t = 3.49$ min].

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Chromatogram S80. Chiral SFC-UV-MS (Method H, $\lambda = 254$ nm) of Fmoc-Lys(Boc)-D-Leu-Nle-D-Trp(Boc)-ODmb [(*R*)-**44**, $R_t = 3.49$ min] containing a 2.5% incremental addition of Fmoc-Lys(Boc)-Leu-Nle-D-Trp(Boc)-ODmb [(*S*)-**44**, $R_t = 4.57$ min].

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NMR Spectra

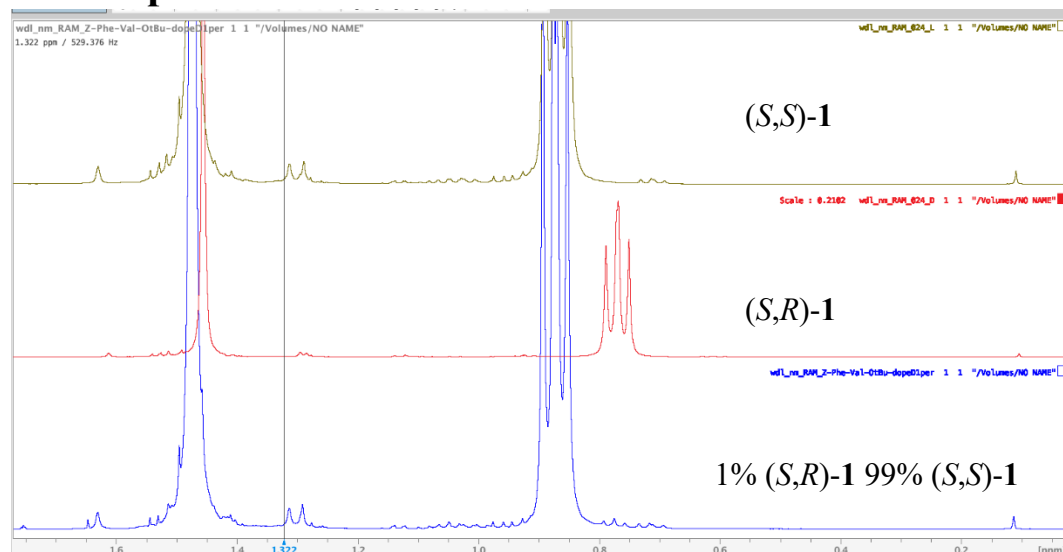


Figure S1. ¹H NMR spectra of (S,S)-1 (top), (S,R)-1 (middle), and 0.5% (S,R)-1 in 99% of (S,S)-1 (bottom).

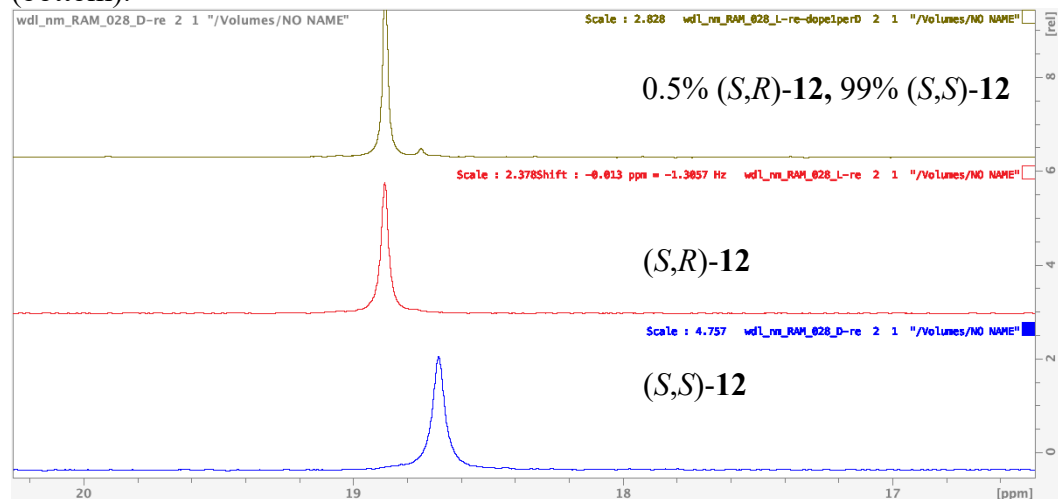


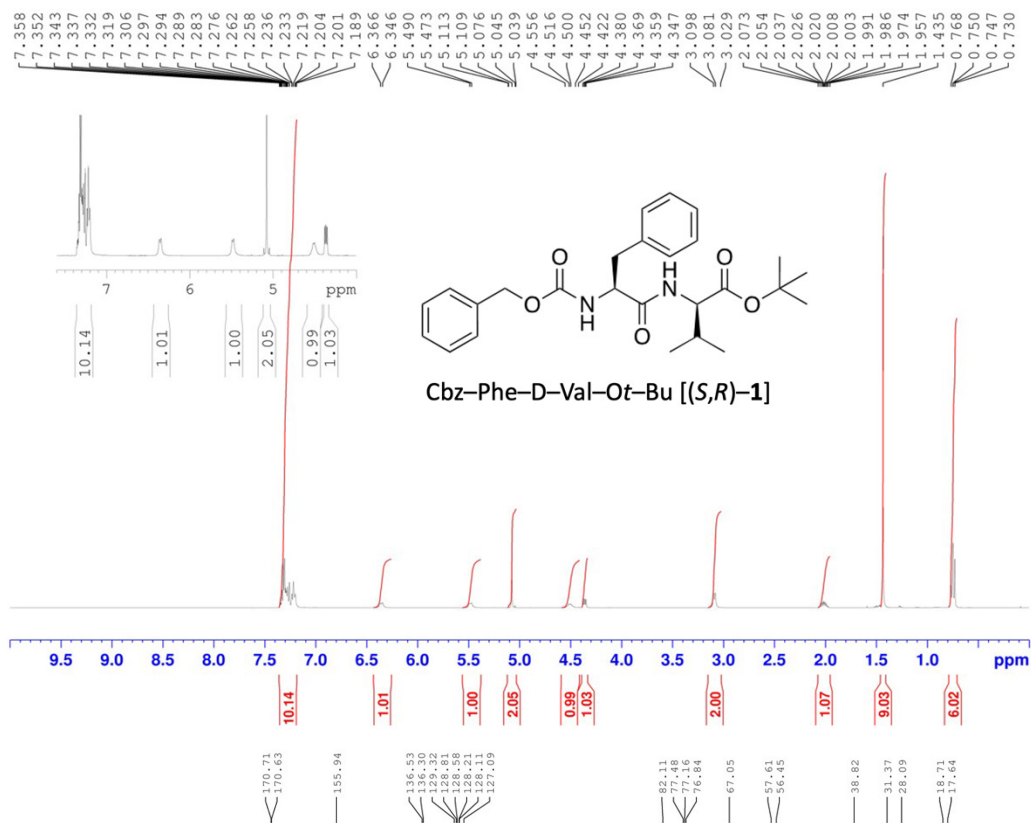
Figure S2. ¹H NMR spectra of 0.5% (S,R)-12 in 99% of (S,S)-12 (top), (S,R)-12 (middle), and (S,S)-12 (bottom).

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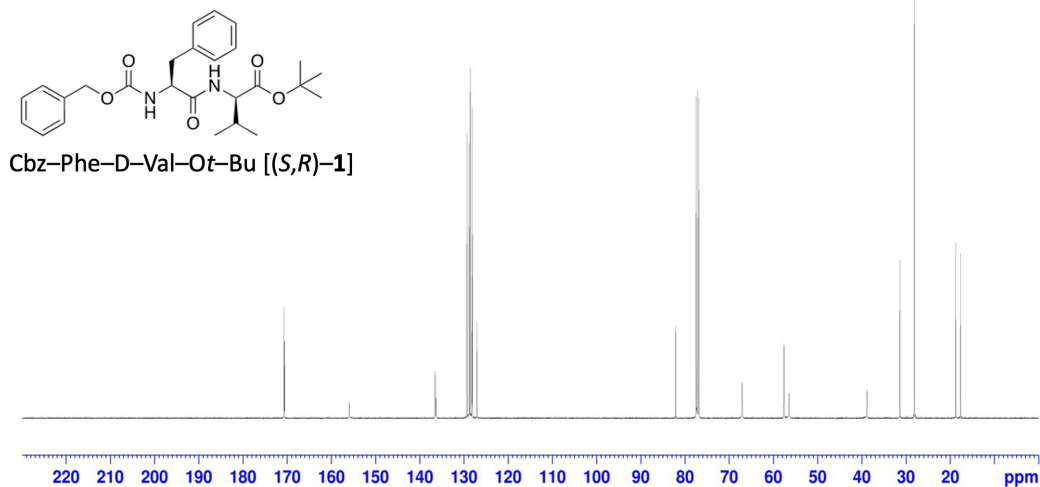
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

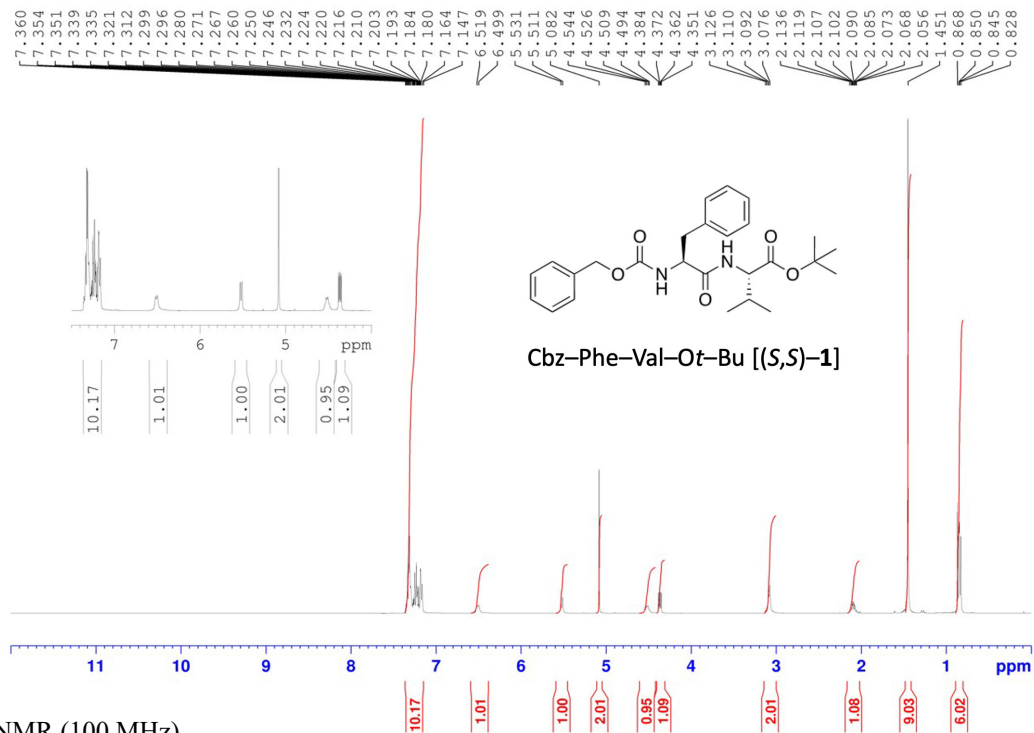


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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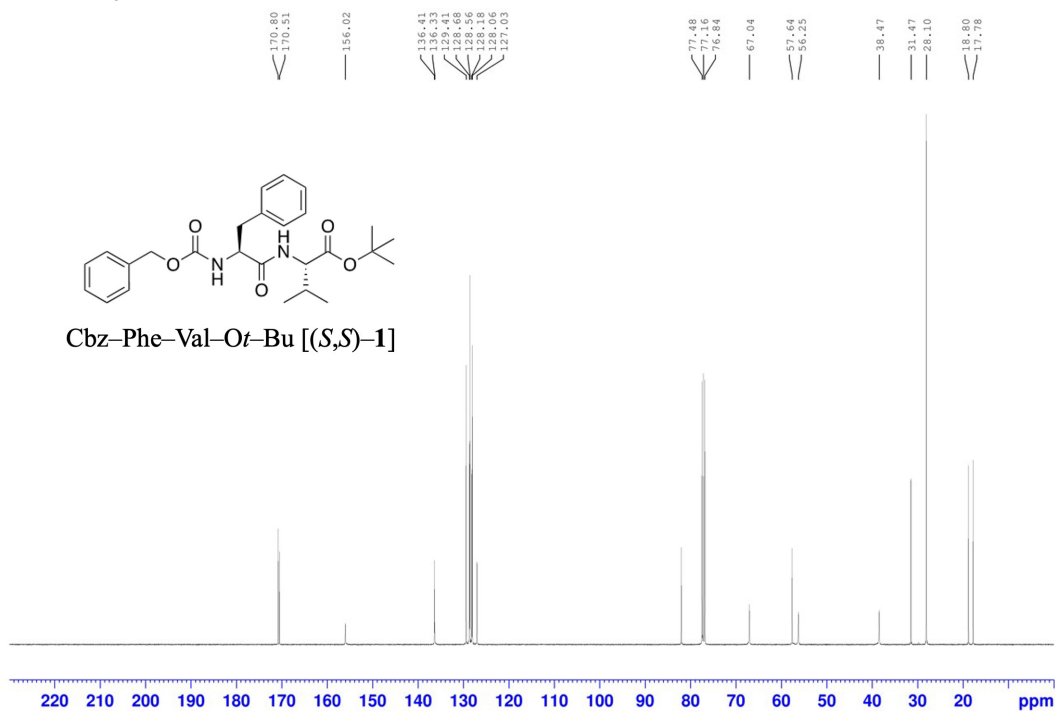
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

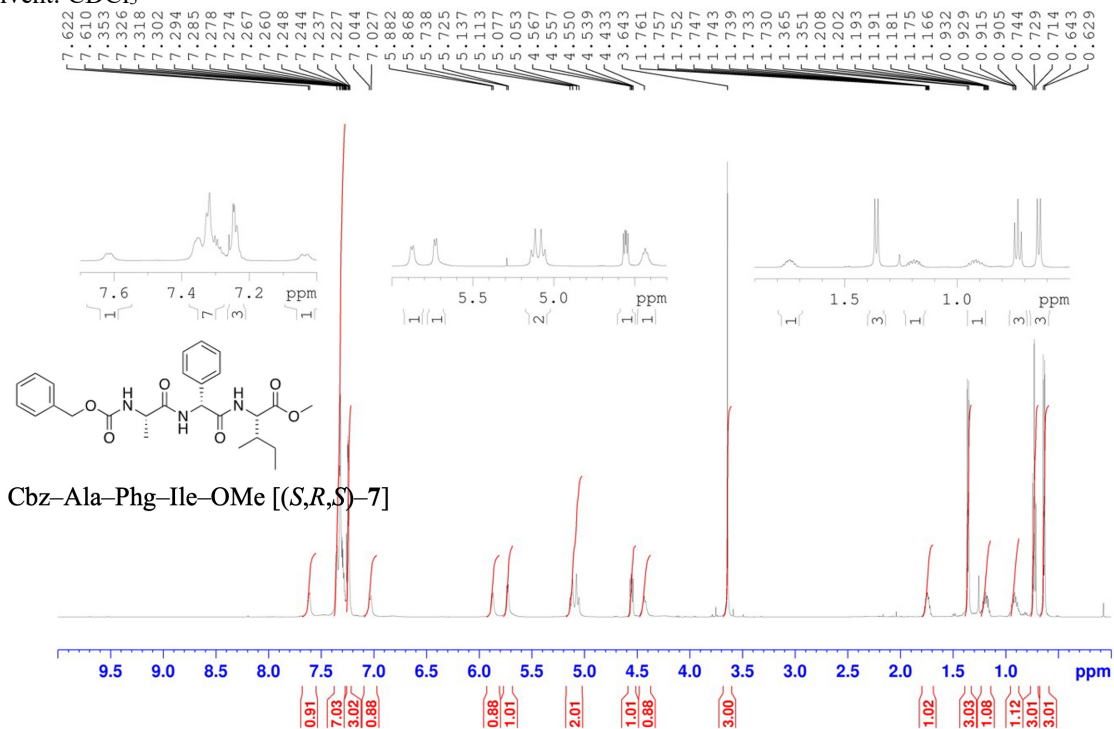


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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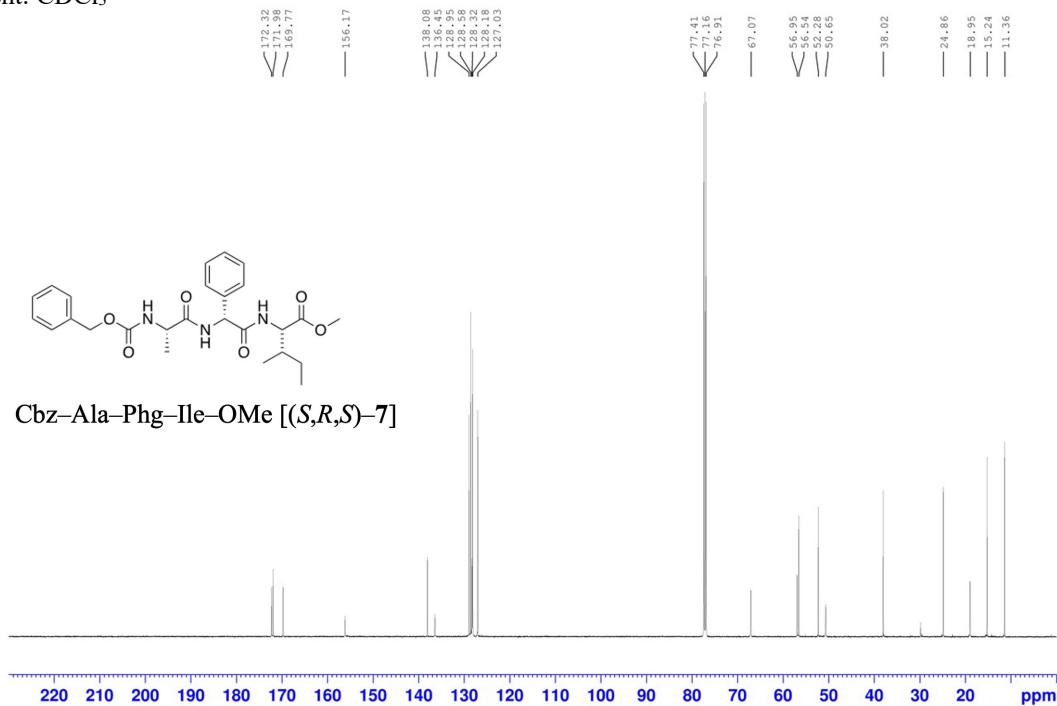
^1H NMR (500 MHz)

Solvent: CDCl_3



^{13}C NMR (125 MHz)

Solvent: CDCl_3

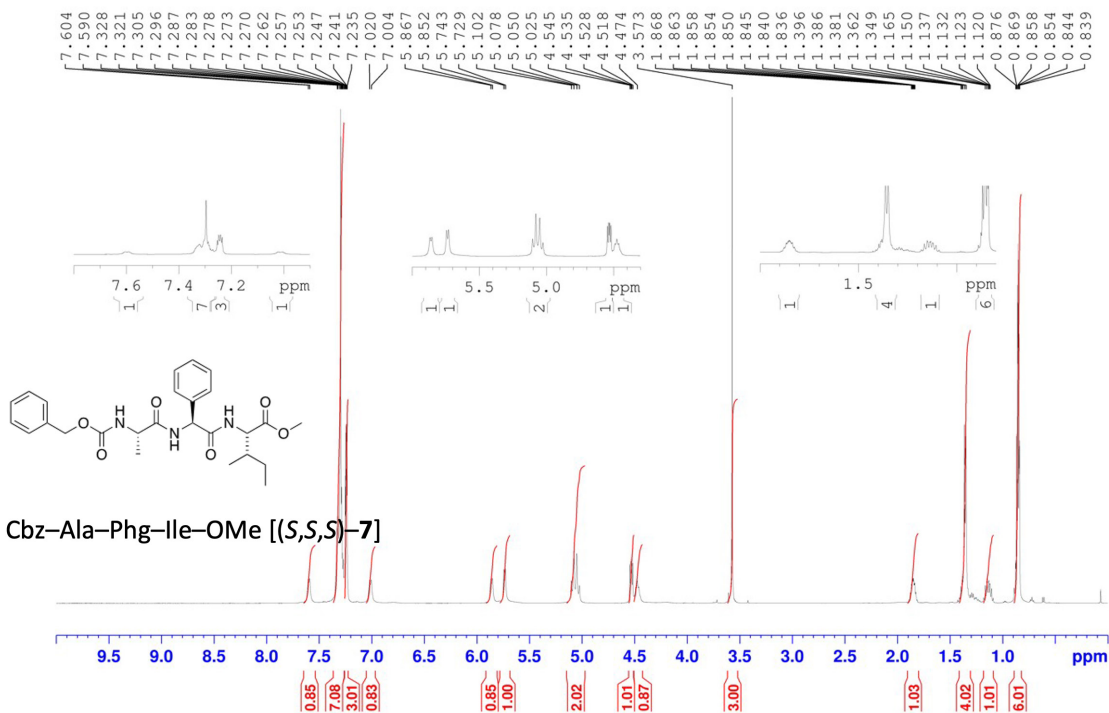


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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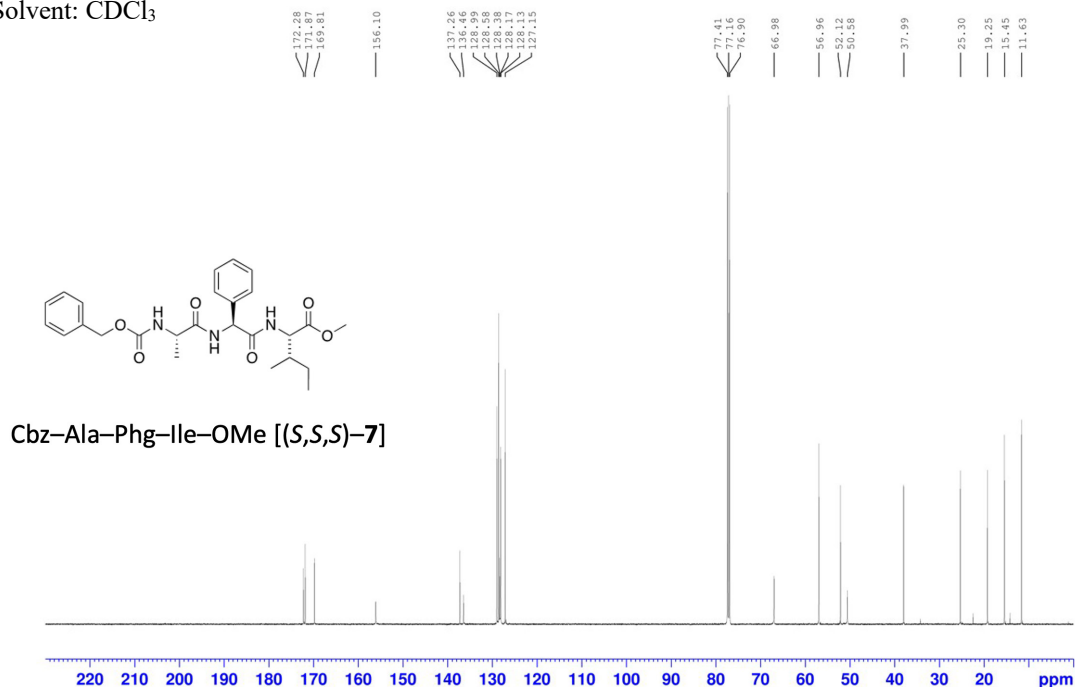
^1H NMR (500 MHz)

Solvent: CDCl_3



^{13}C NMR (125 MHz)

Solvent: CDCl_3

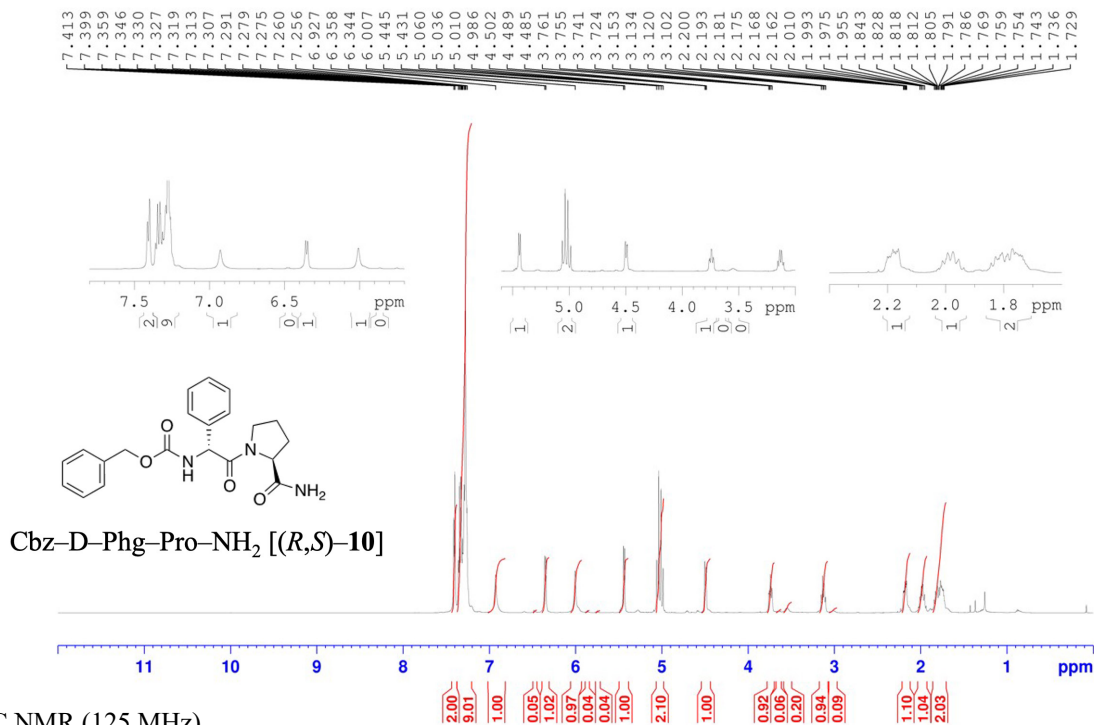


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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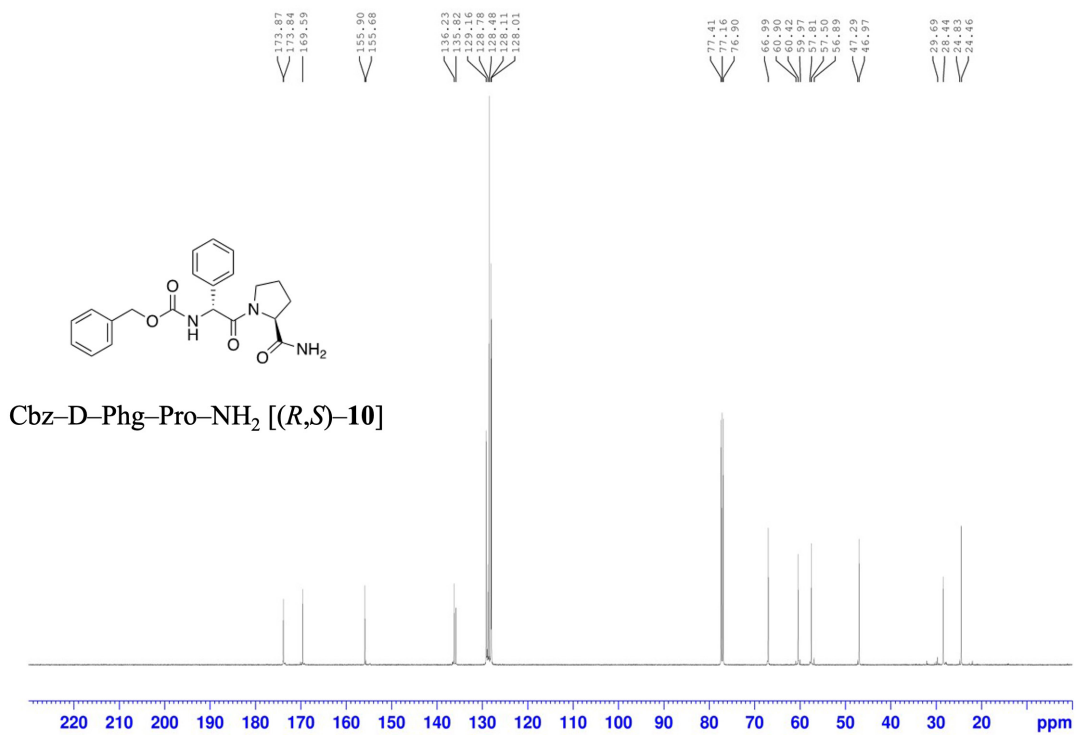
^1H NMR (500 MHz)

Solvent: CDCl_3



^{13}C NMR (125 MHz)

Solvent: CDCl_3

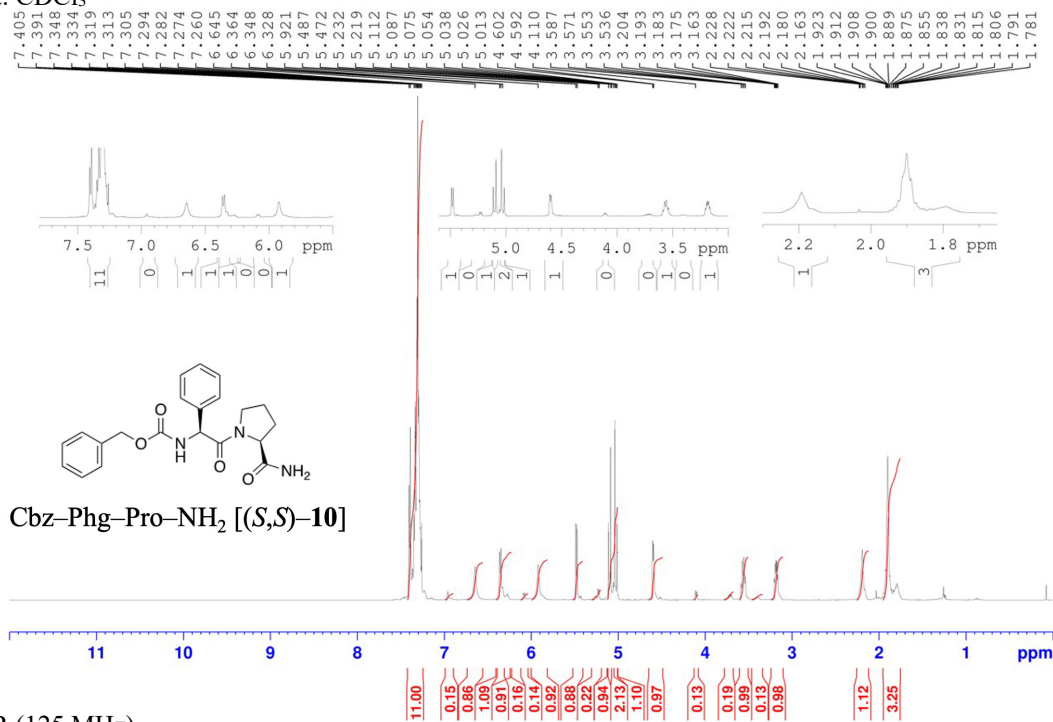


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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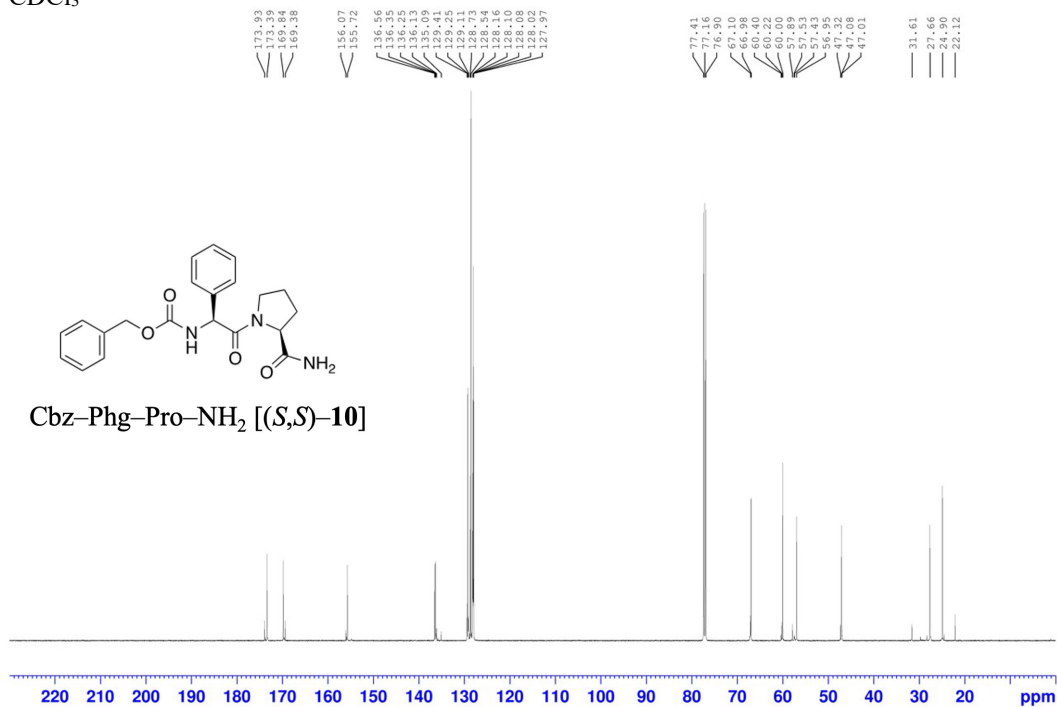
^1H NMR (500 MHz)

Solvent: CDCl_3



^{13}C NMR (125 MHz)

Solvent: CDCl_3

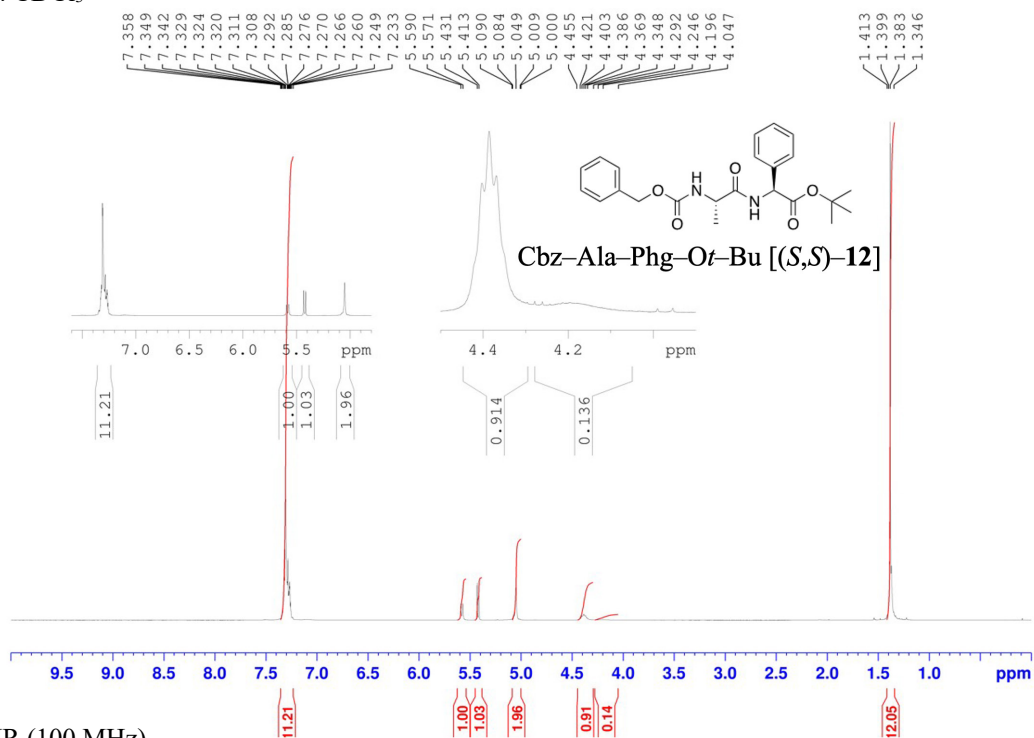


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

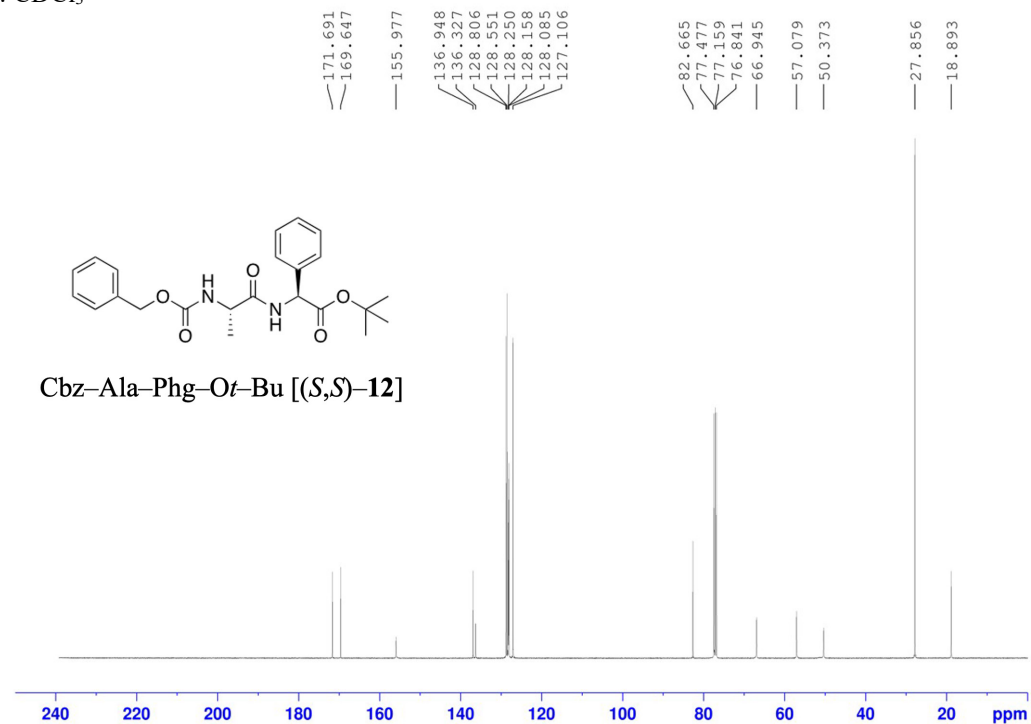
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

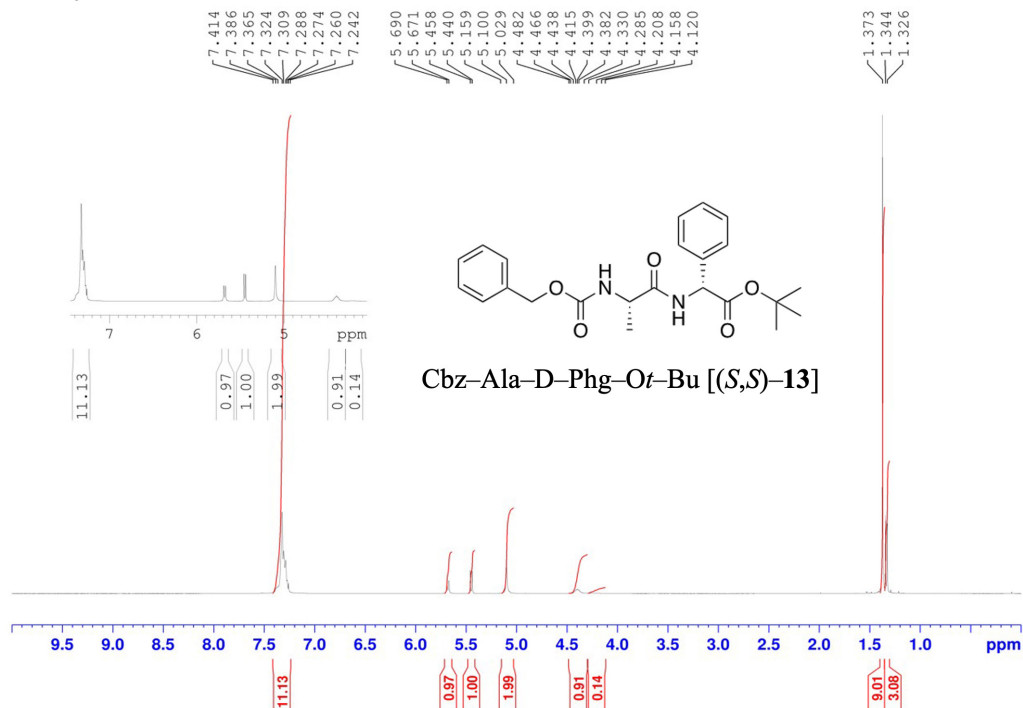


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

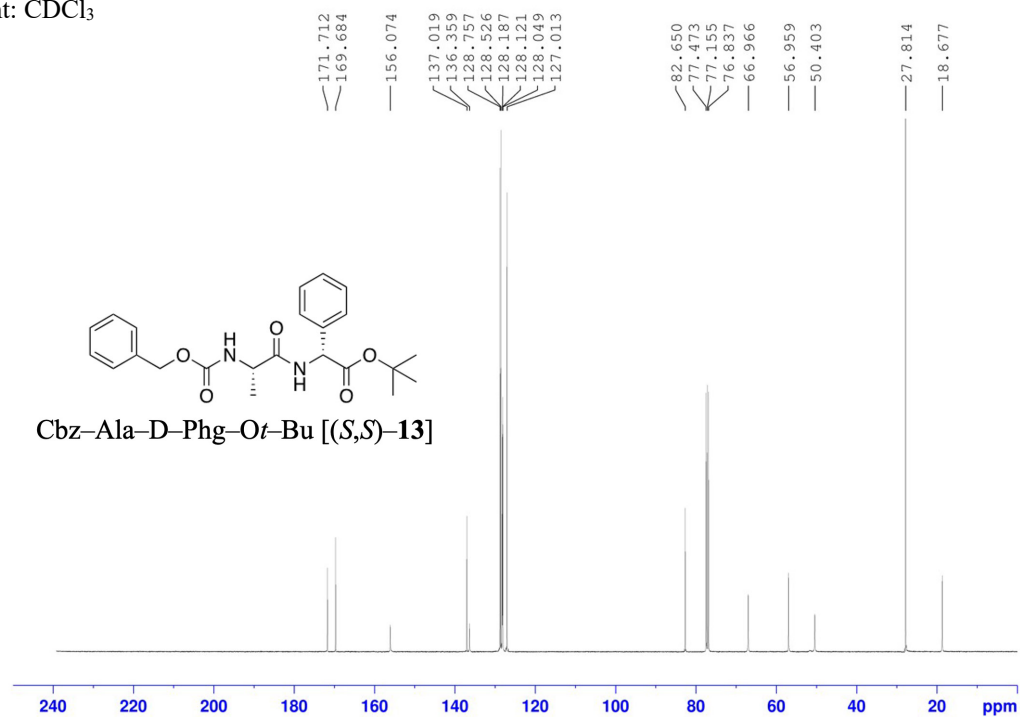
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

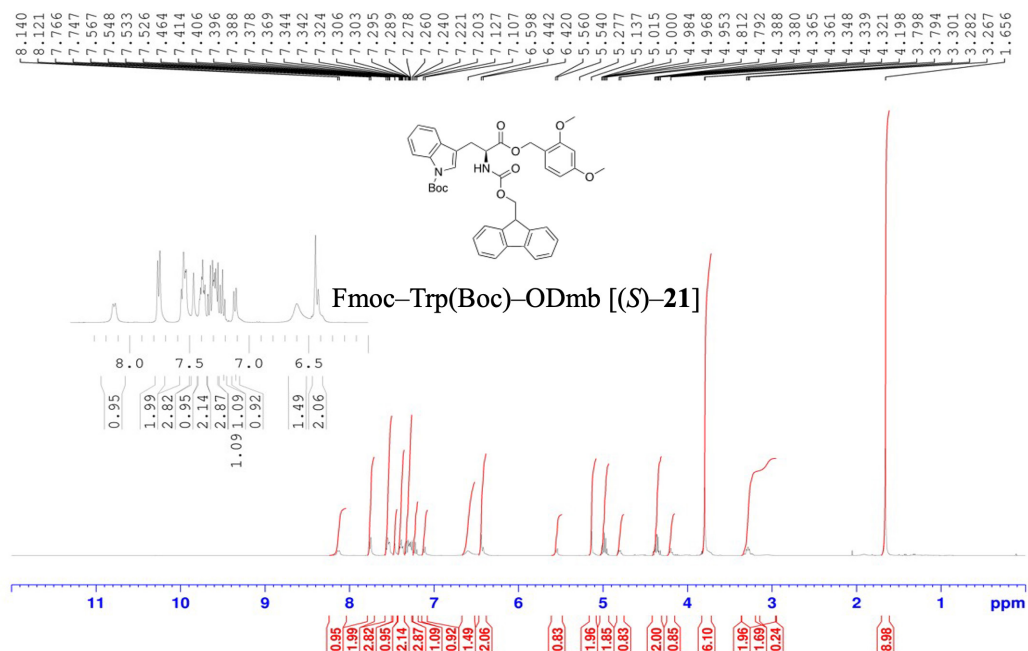


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

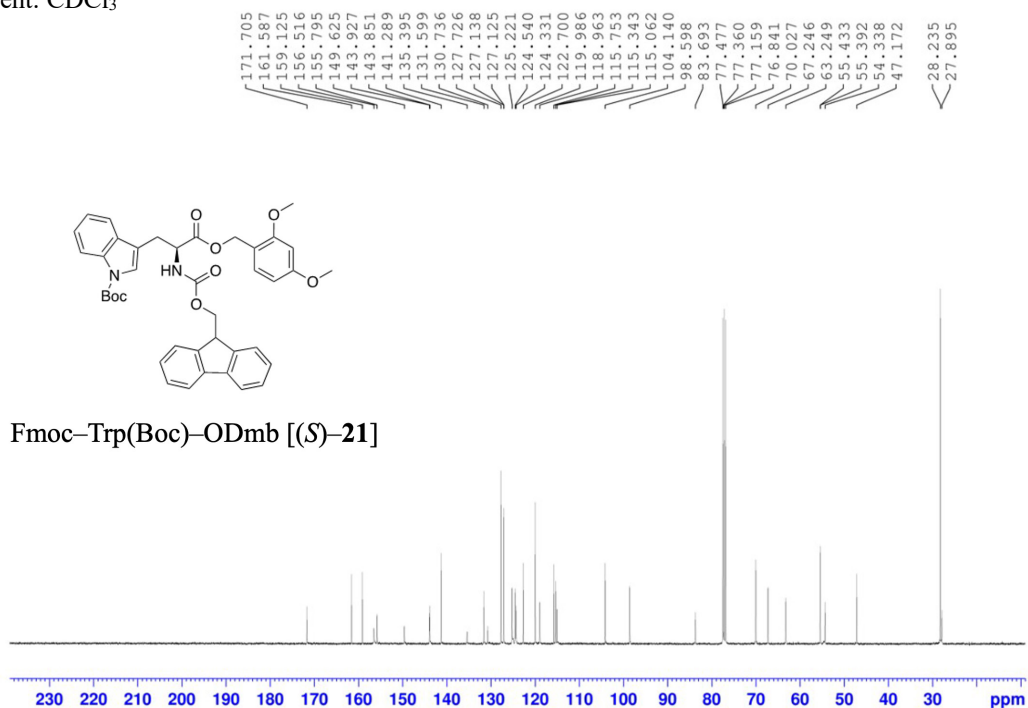
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

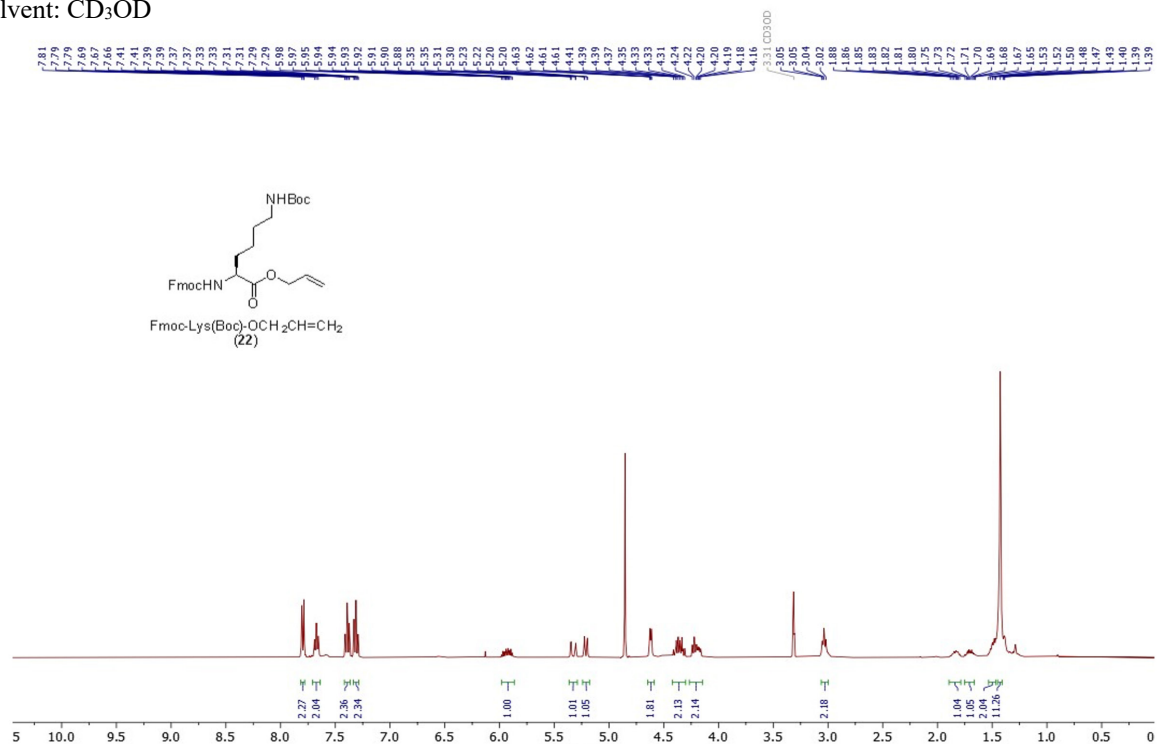


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

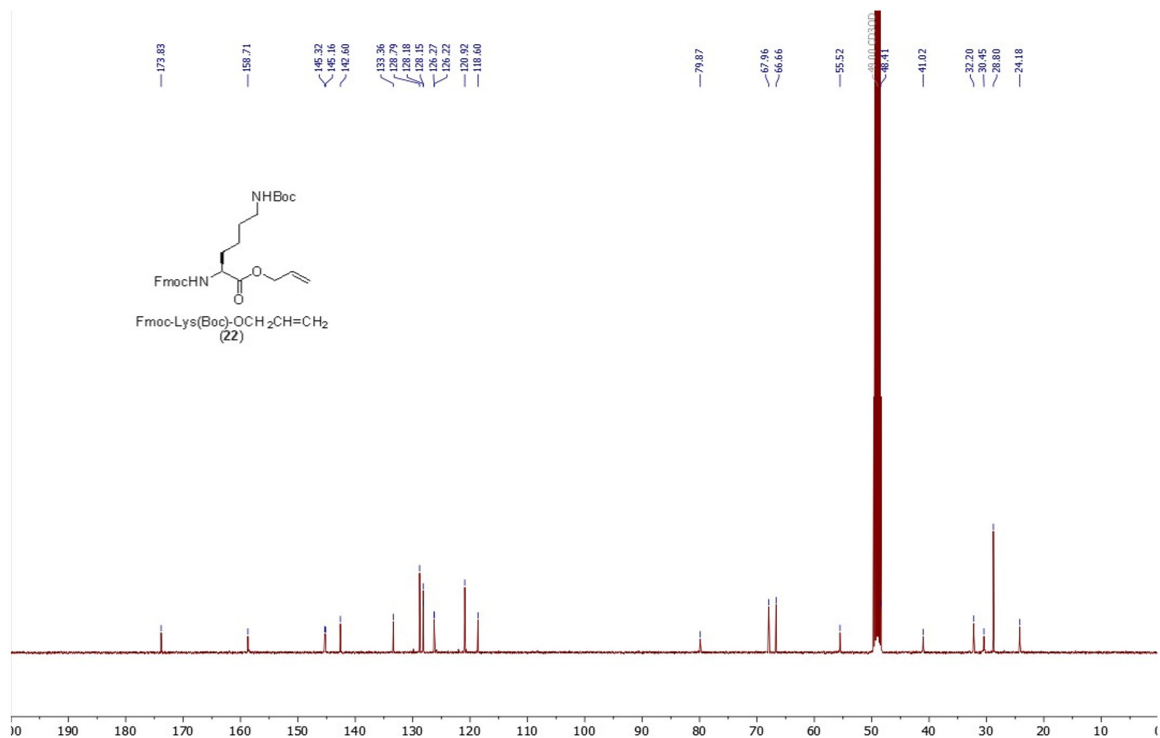
^1H NMR (400 MHz)

Solvent: CD_3OD



^{13}C NMR (100 MHz)

Solvent: CD_3OD



Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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^1H NMR (400 MHz)

Solvent: CD_3CN

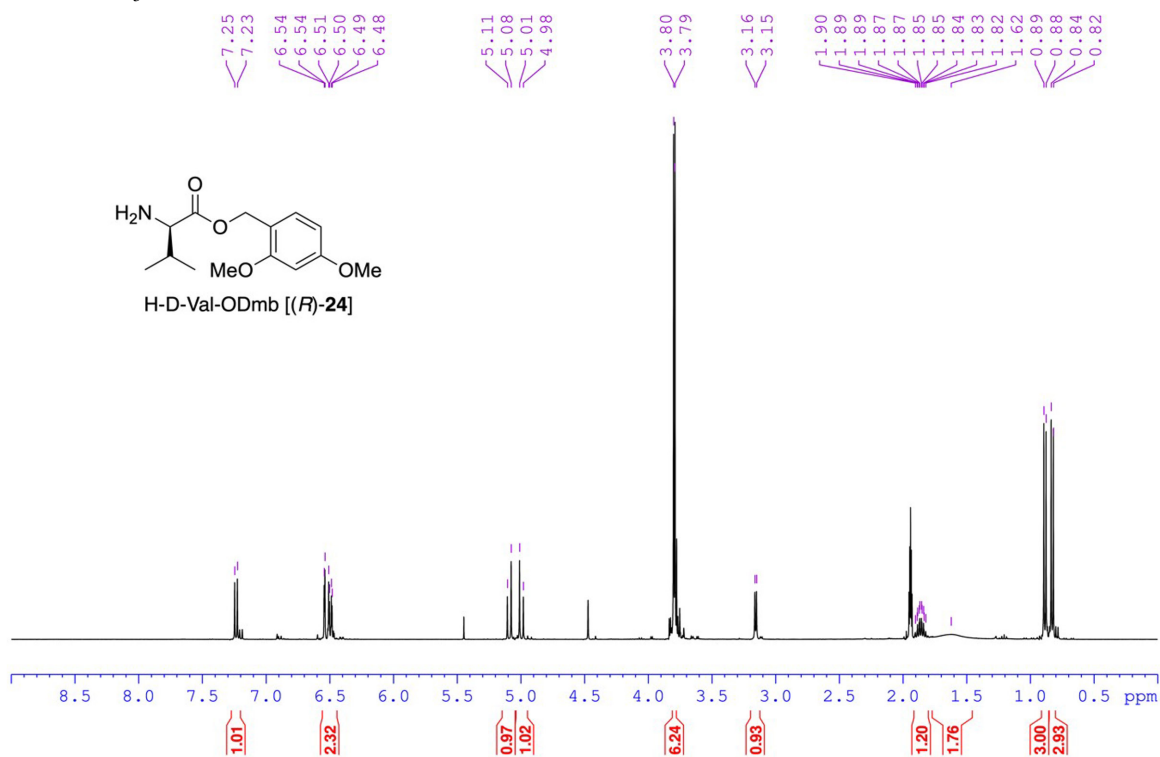
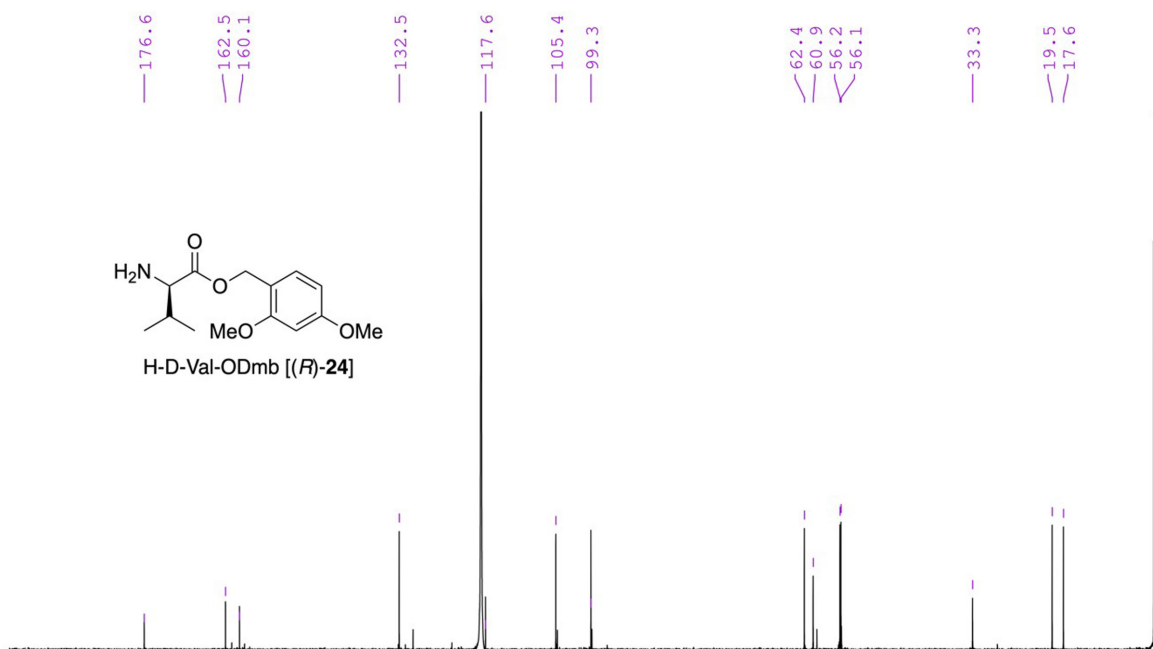


Figure 21: ^1H NMR (400 MHz, CD_3CN) spectra of (R)-24 in TopSpin.

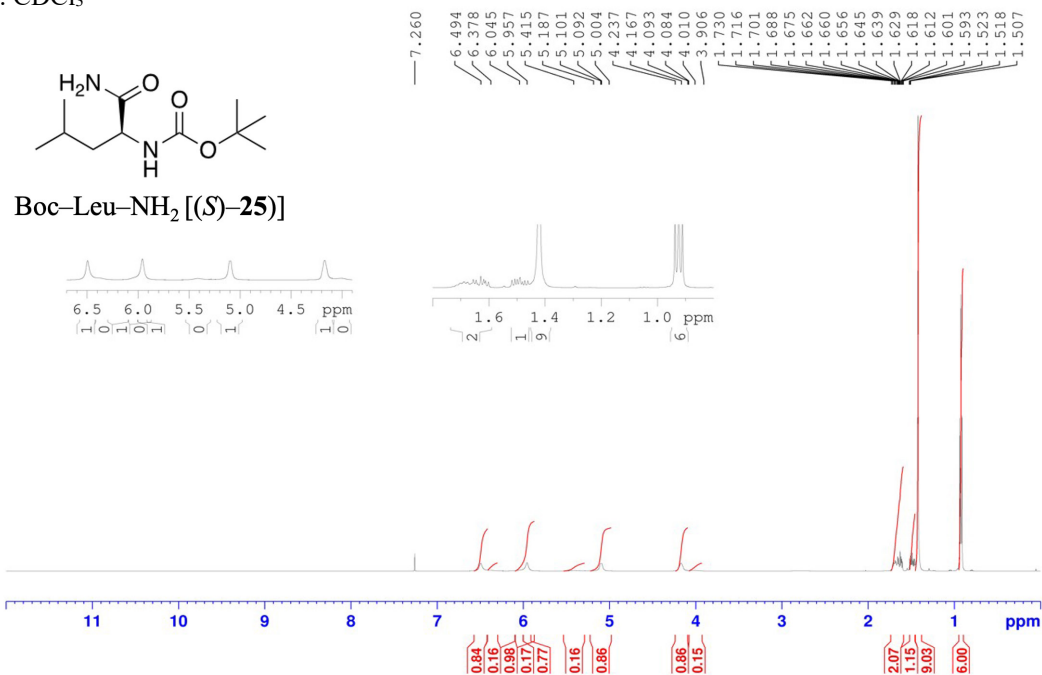


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

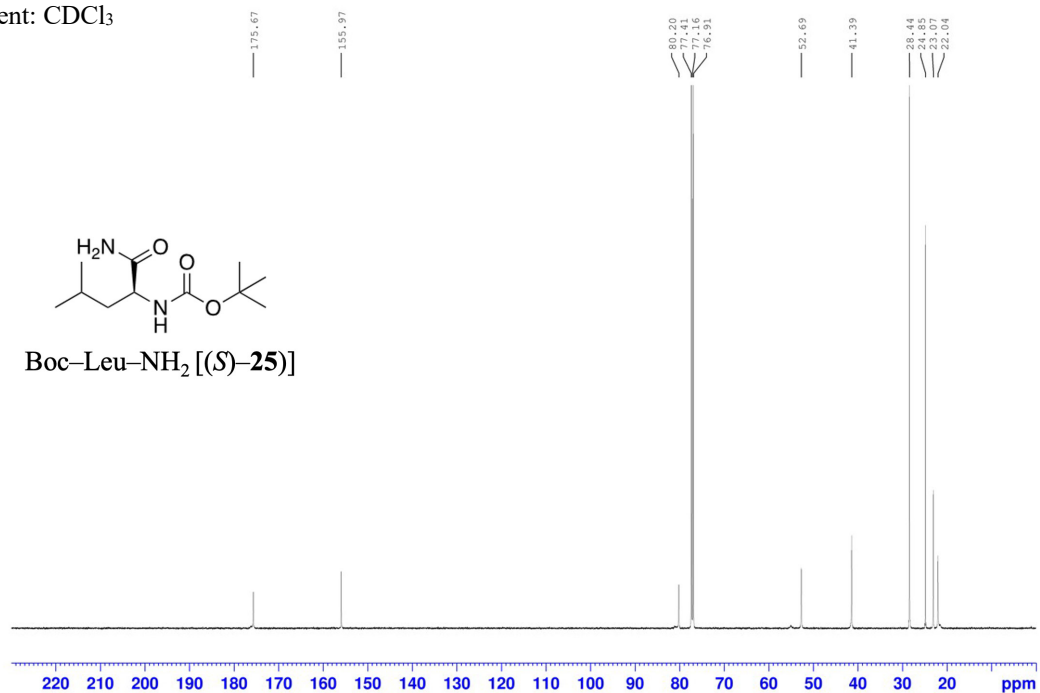
^1H NMR (500 MHz)

Solvent: CDCl_3



^{13}C NMR (125 MHz)

Solvent: CDCl_3

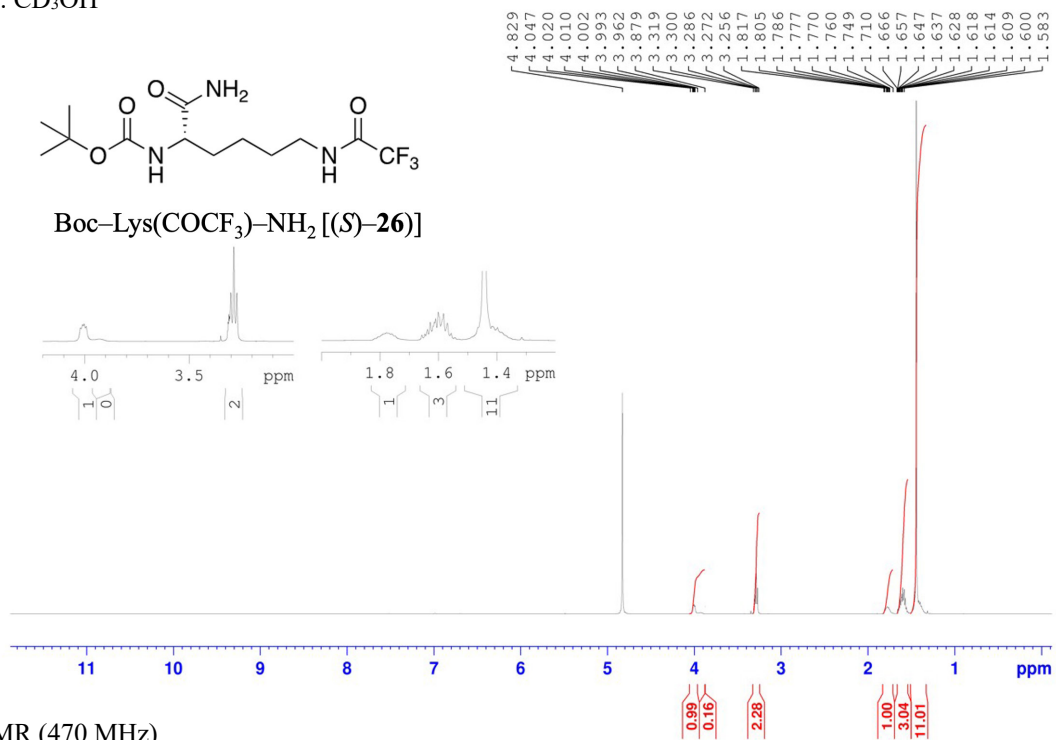


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

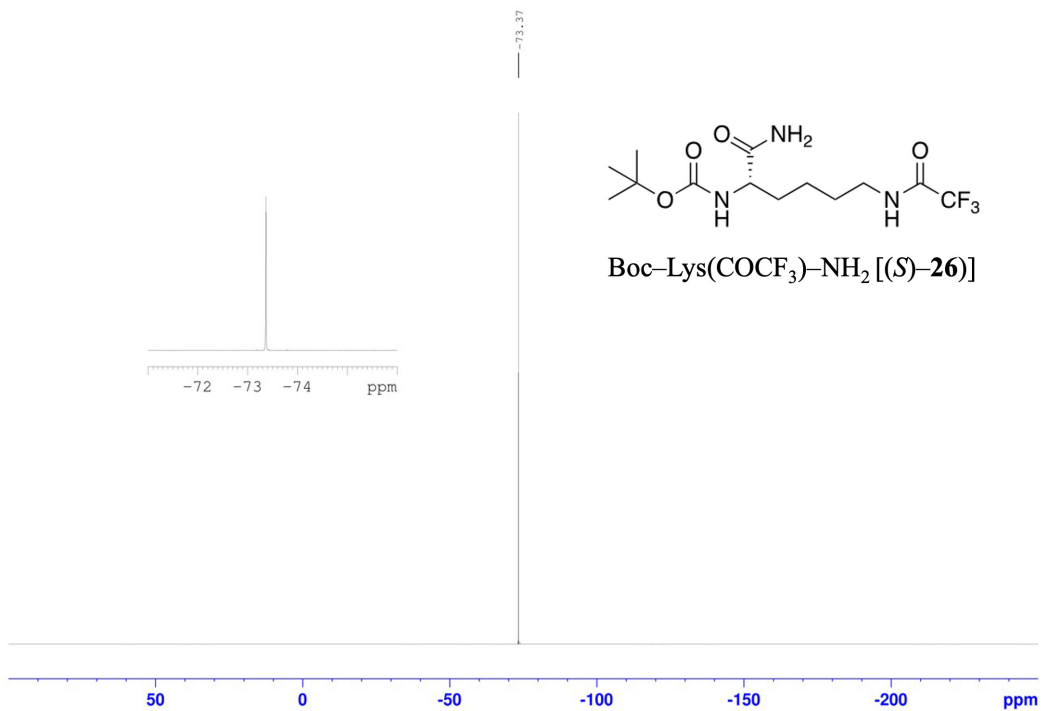
^1H NMR (500 MHz)

Solvent: CD_3OH



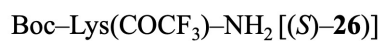
^{19}F NMR (470 MHz)

Solvent: CD_3OD



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Solvent: CD₃OD

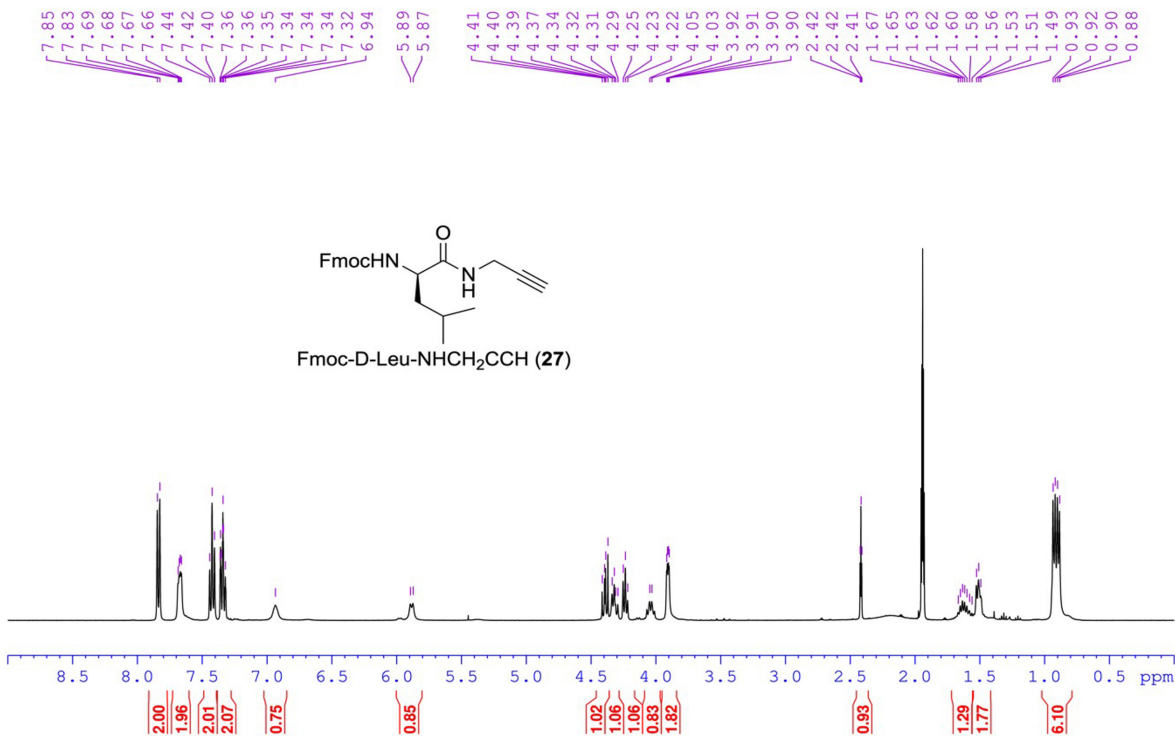


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

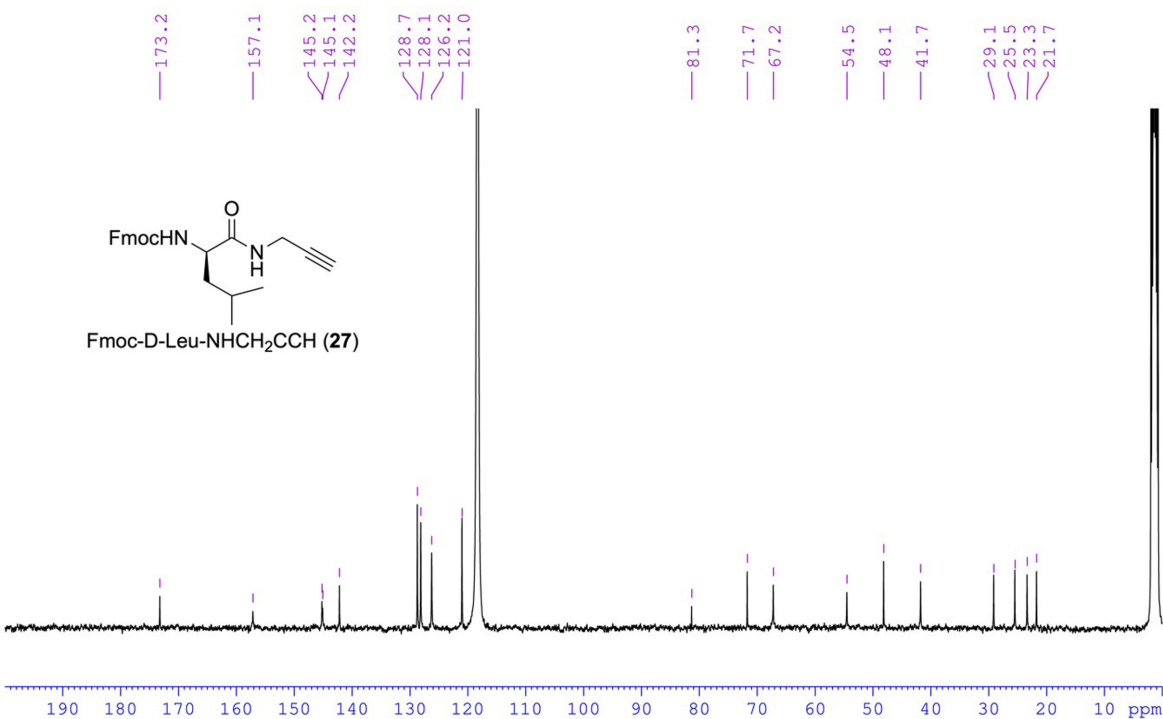
^1H NMR (400 MHz)

Solvent: CD_3CN



^{13}C NMR (100 MHz)

Solvent: CD_3CN

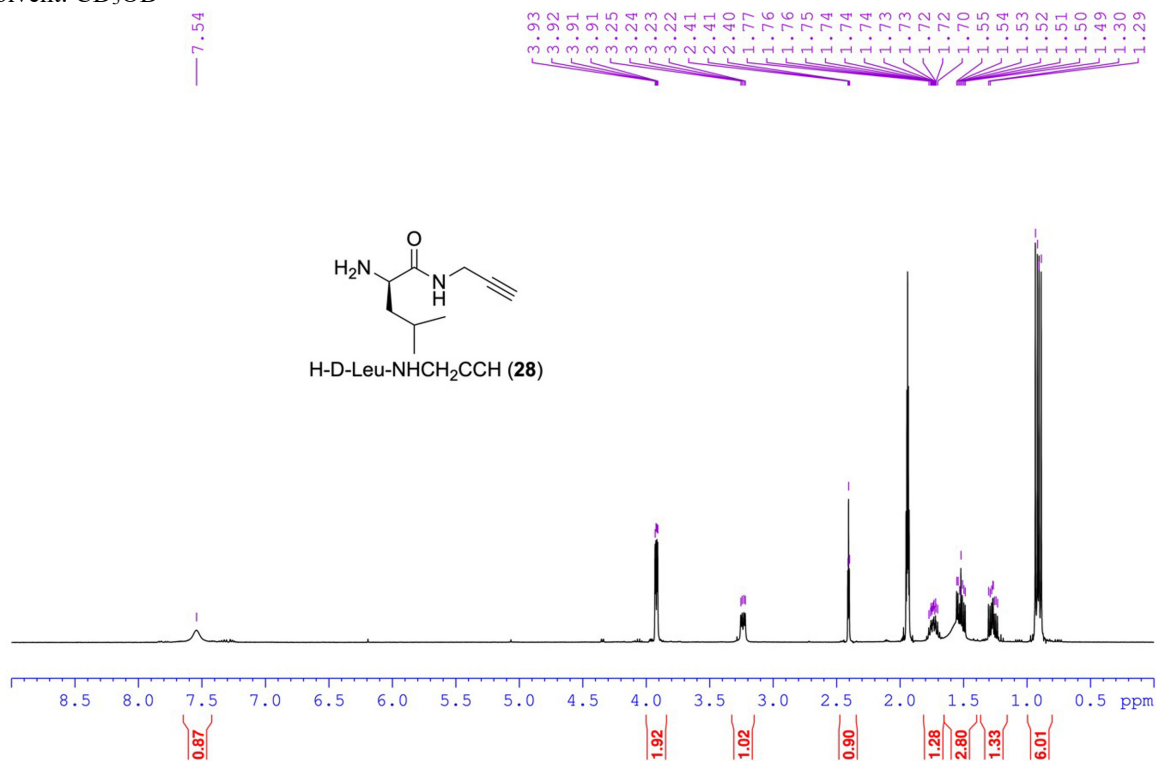


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

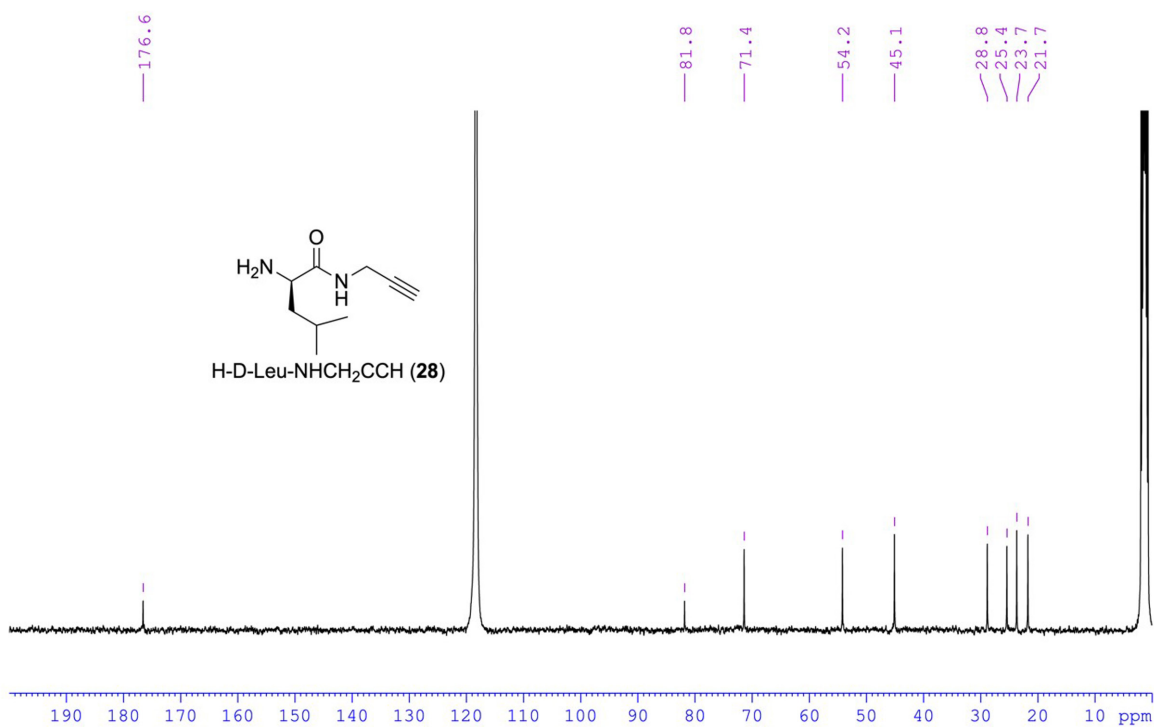
^1H NMR (400 MHz)

Solvent: CD_3OD



^{13}C NMR (100 MHz)

Solvent: CD_3OD

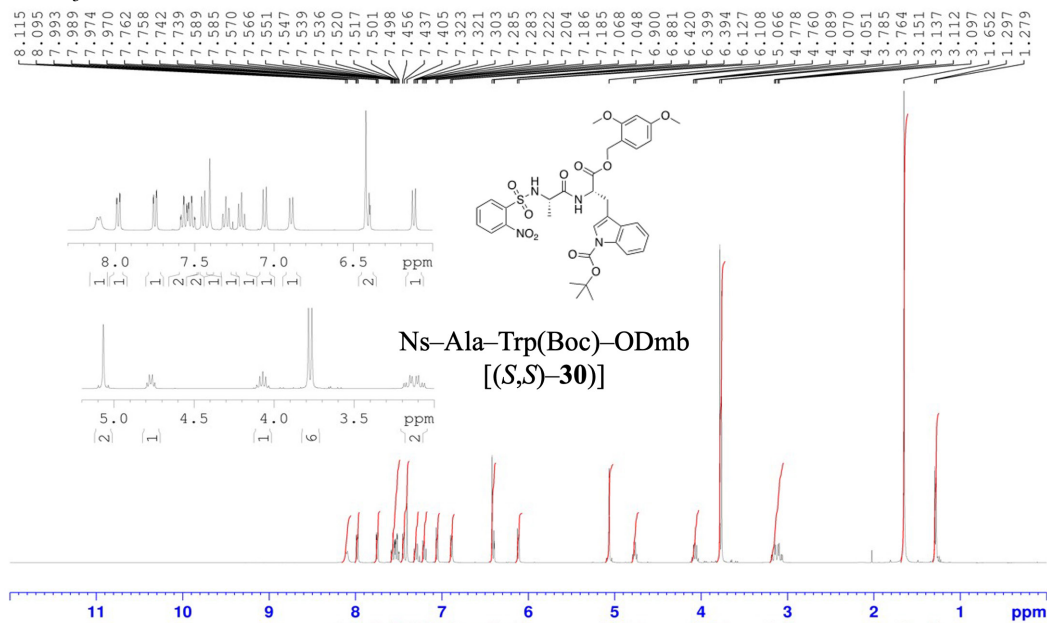


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

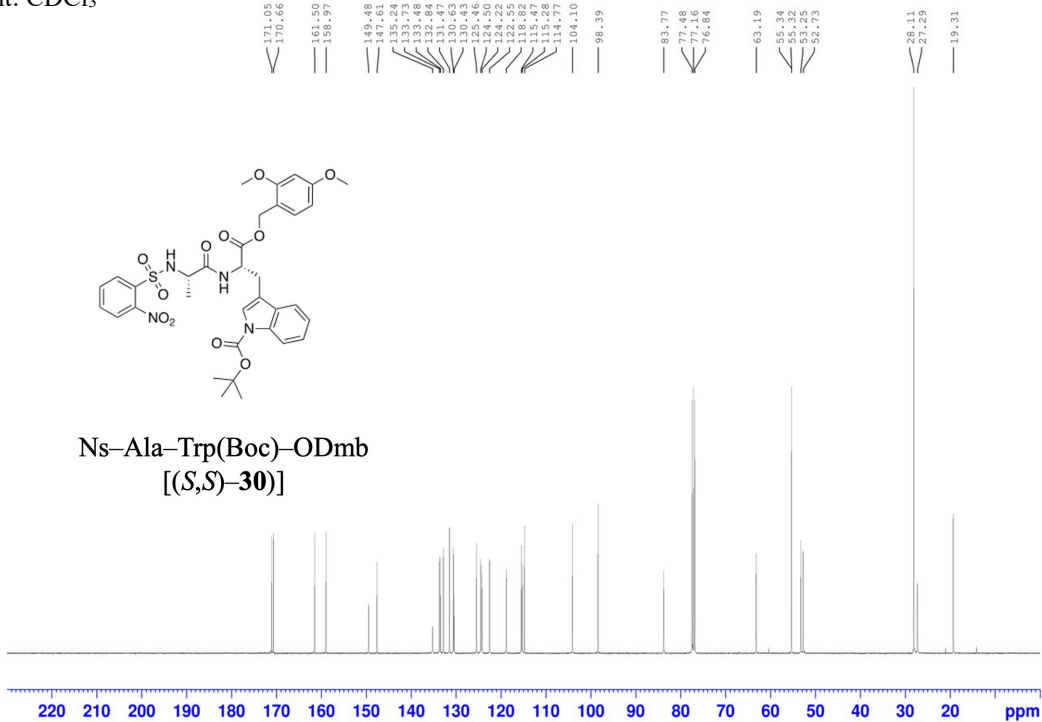
^1H NMR (500 MHz)

Solvent: CDCl_3



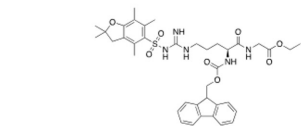
^{13}C NMR (125 MHz)

Solvent: CDCl_3

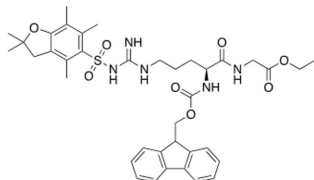


Maarouf-Mesli, N. et al.

Solvent: CDCl₃

Fmoc-Lys(Pbf)-Gly-OEt (**31**)

Solvent: CDCl₃

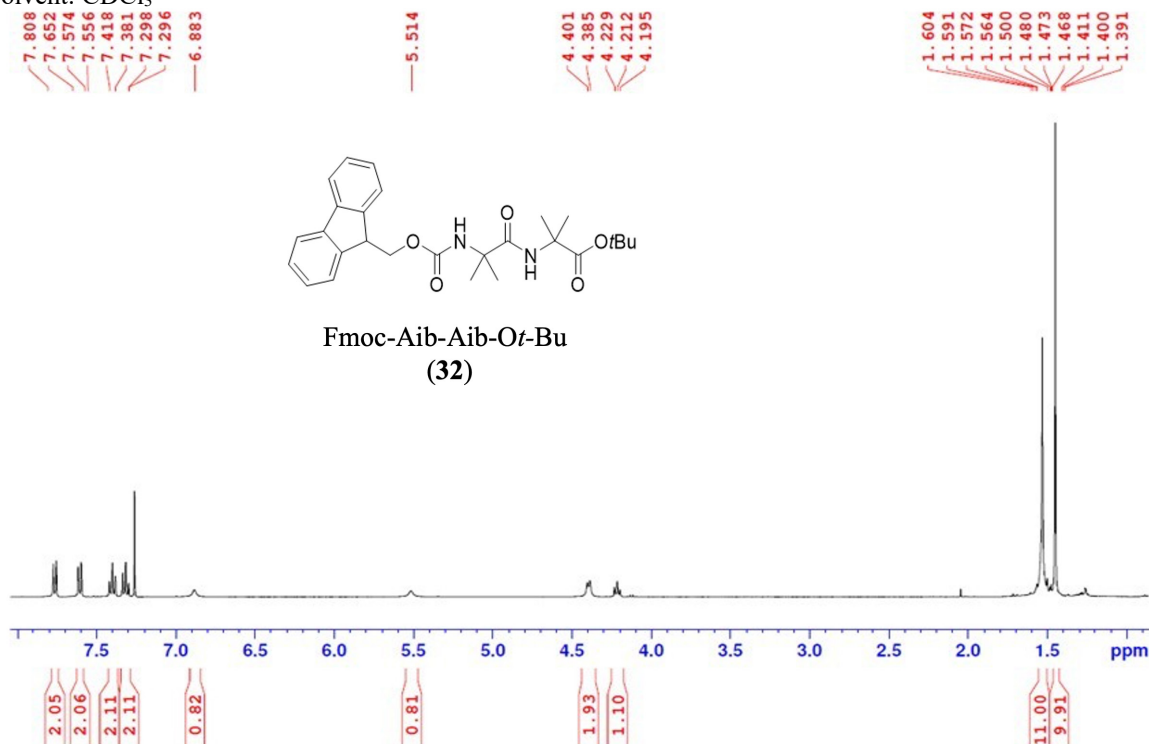
Fmoc-Lys(Pbf)-Gly-OEt (**31**)

Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

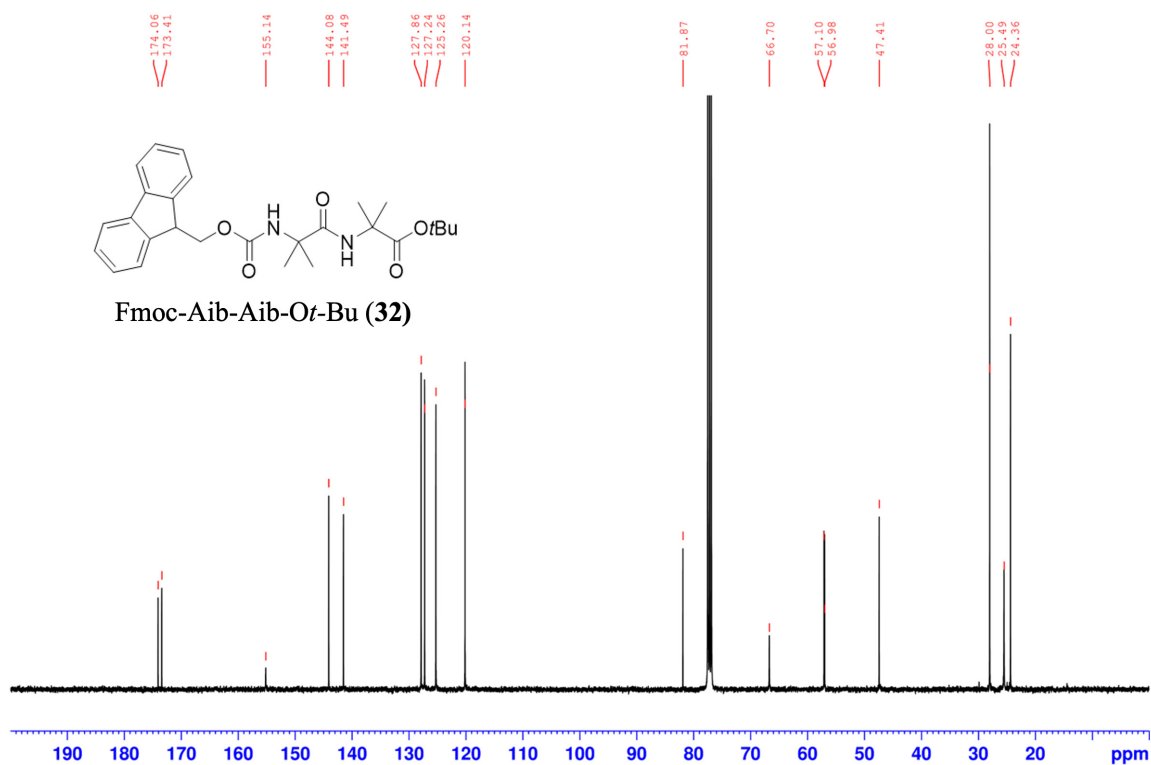
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

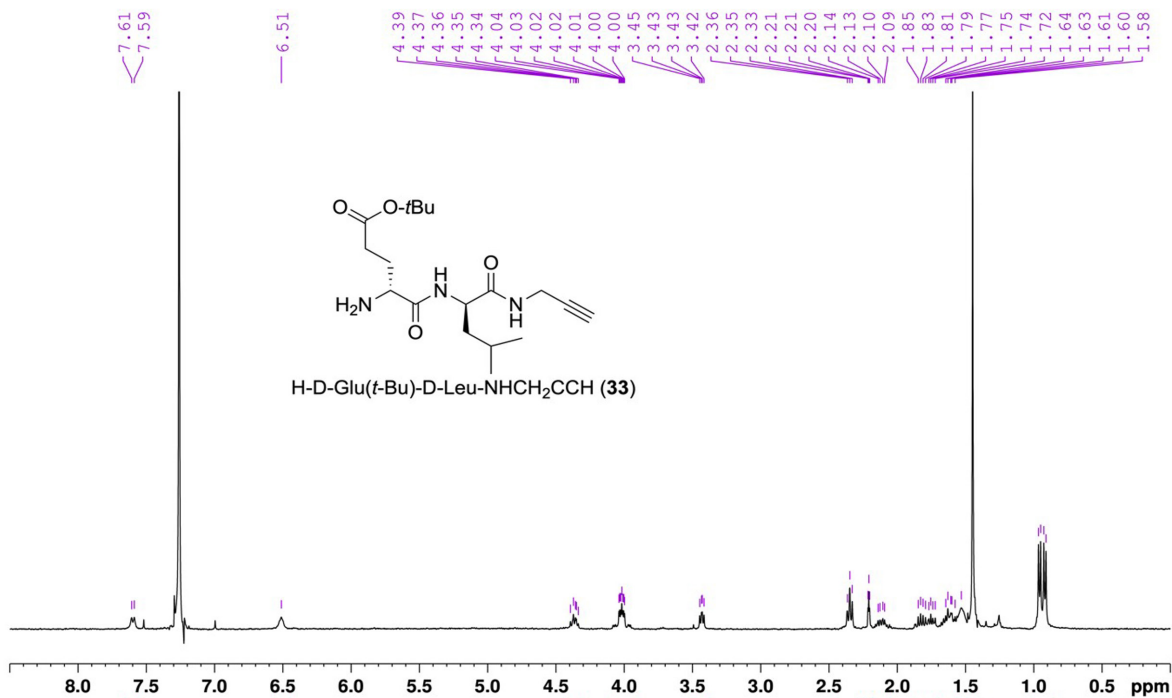


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

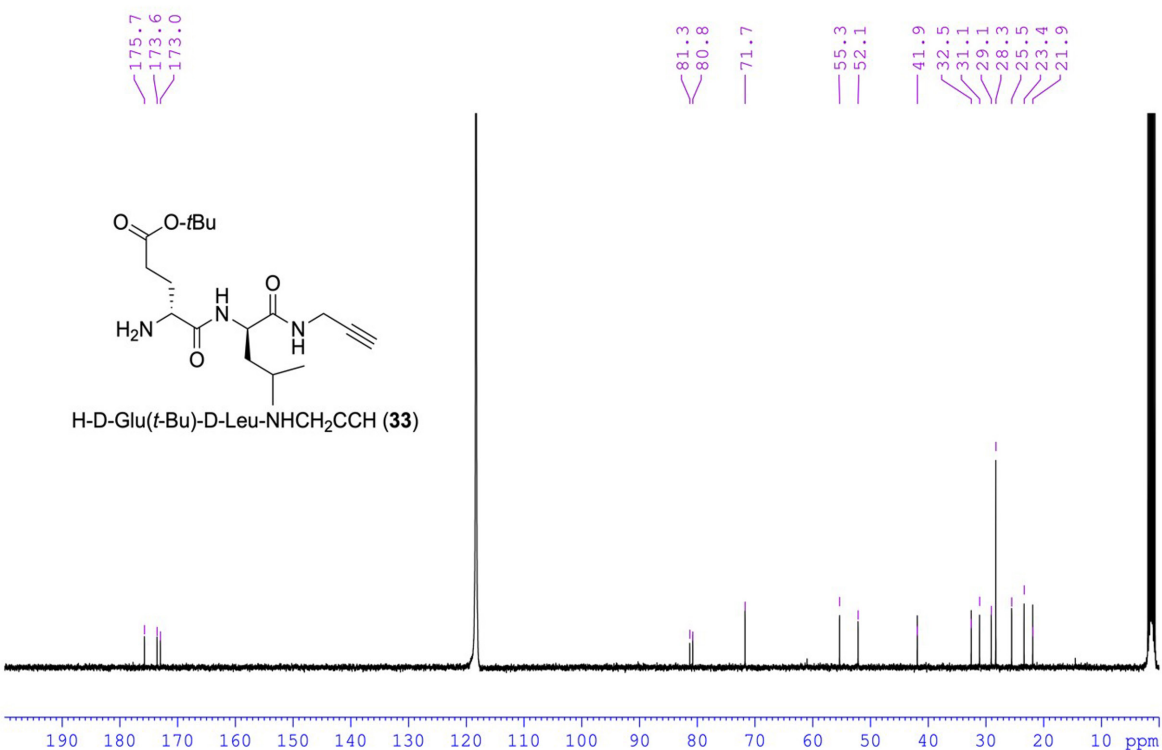
^1H NMR (400 MHz)

Solvent: CD_3CN



^{13}C NMR (100 MHz)

Solvent: CD_3CN

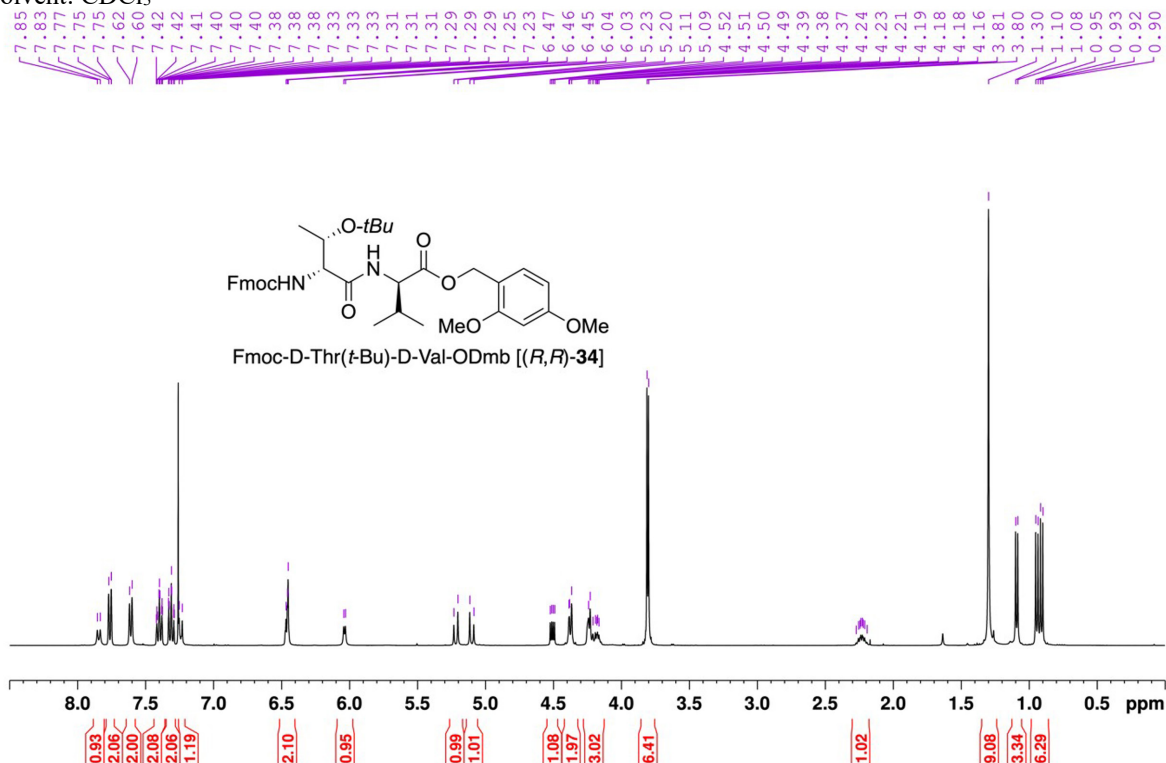


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

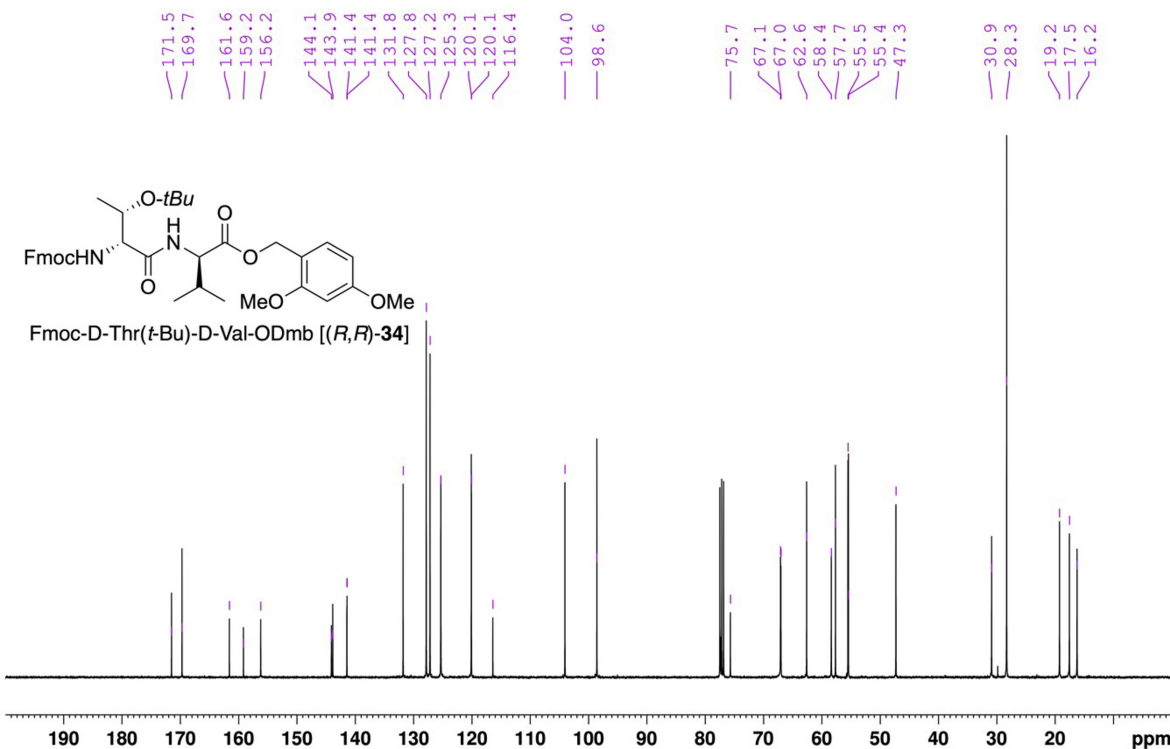
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

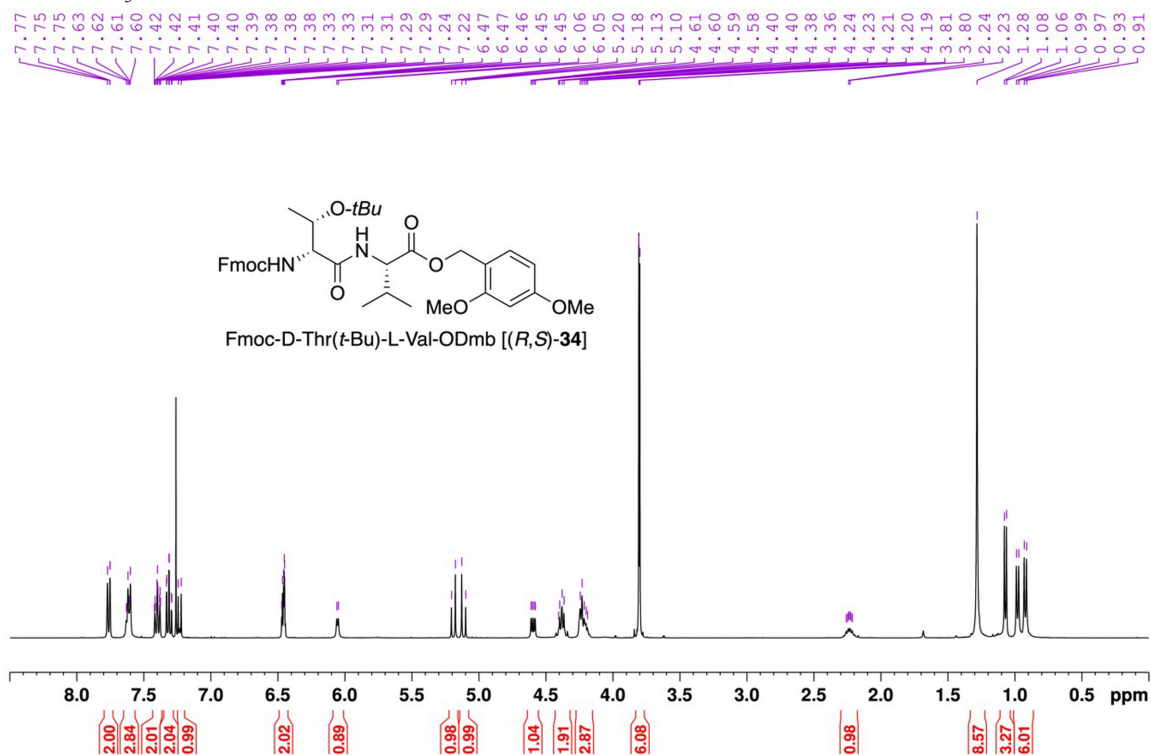


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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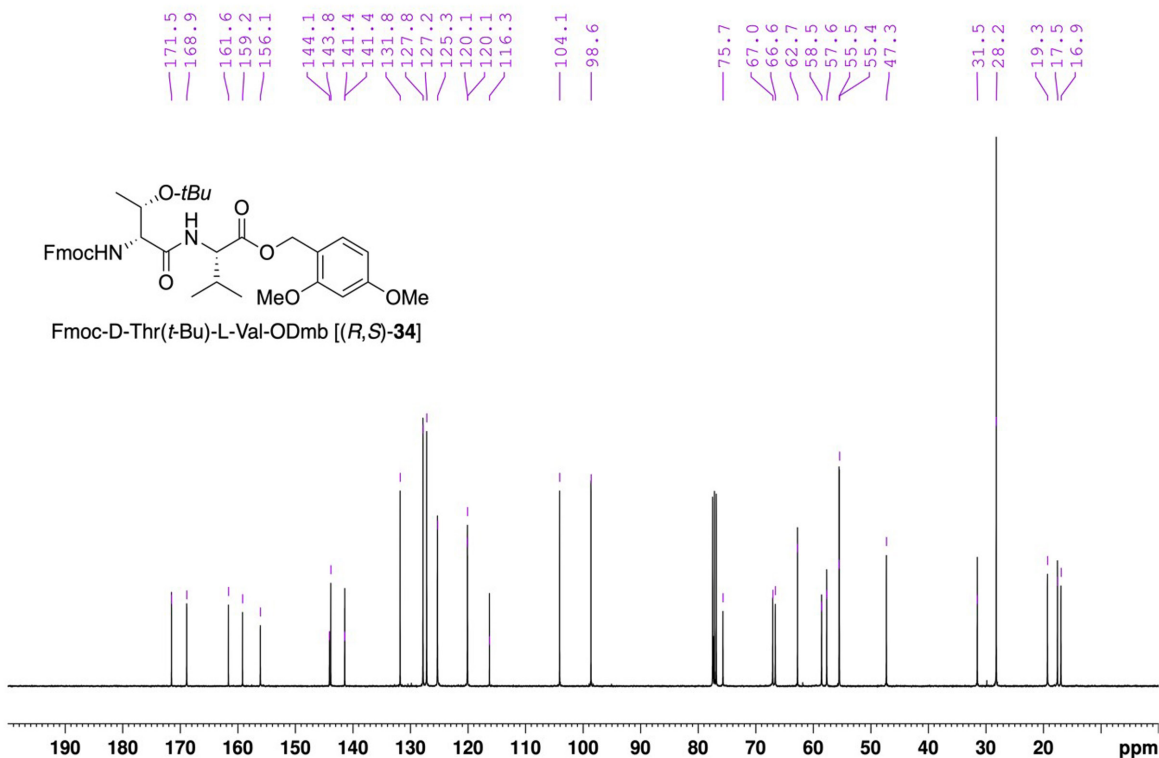
^1H NMR (400 MHz)

Solvent: CDCl_3



^{13}C NMR (100 MHz)

Solvent: CDCl_3

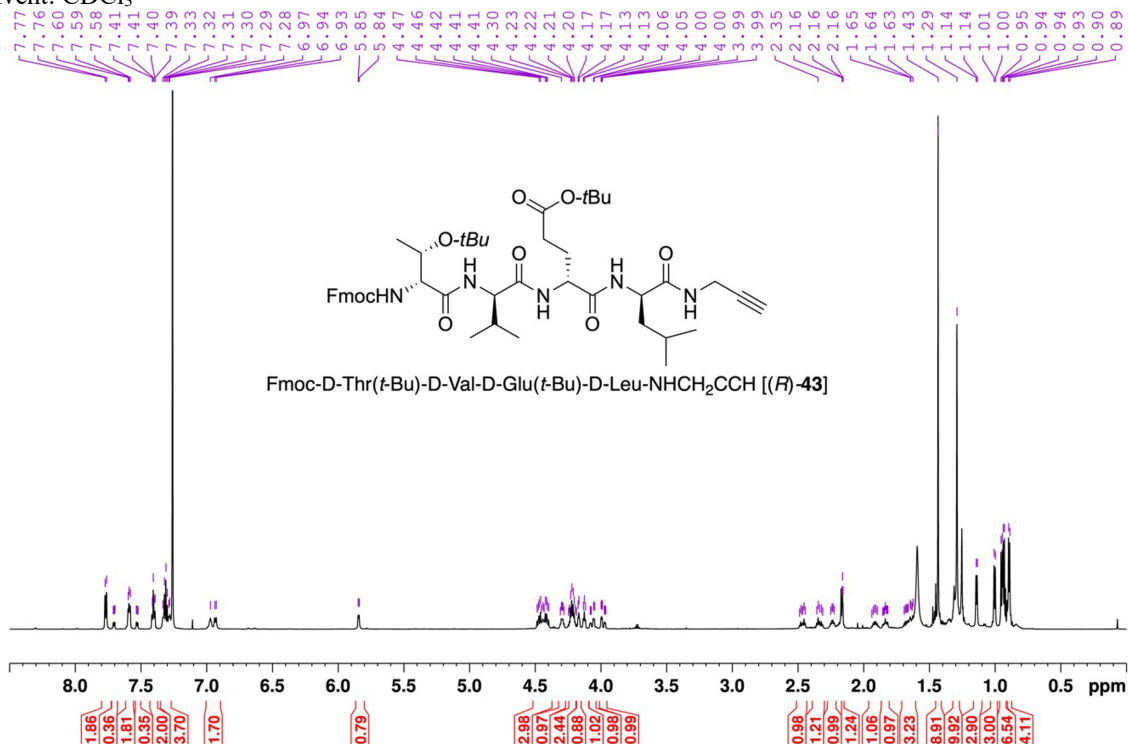


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

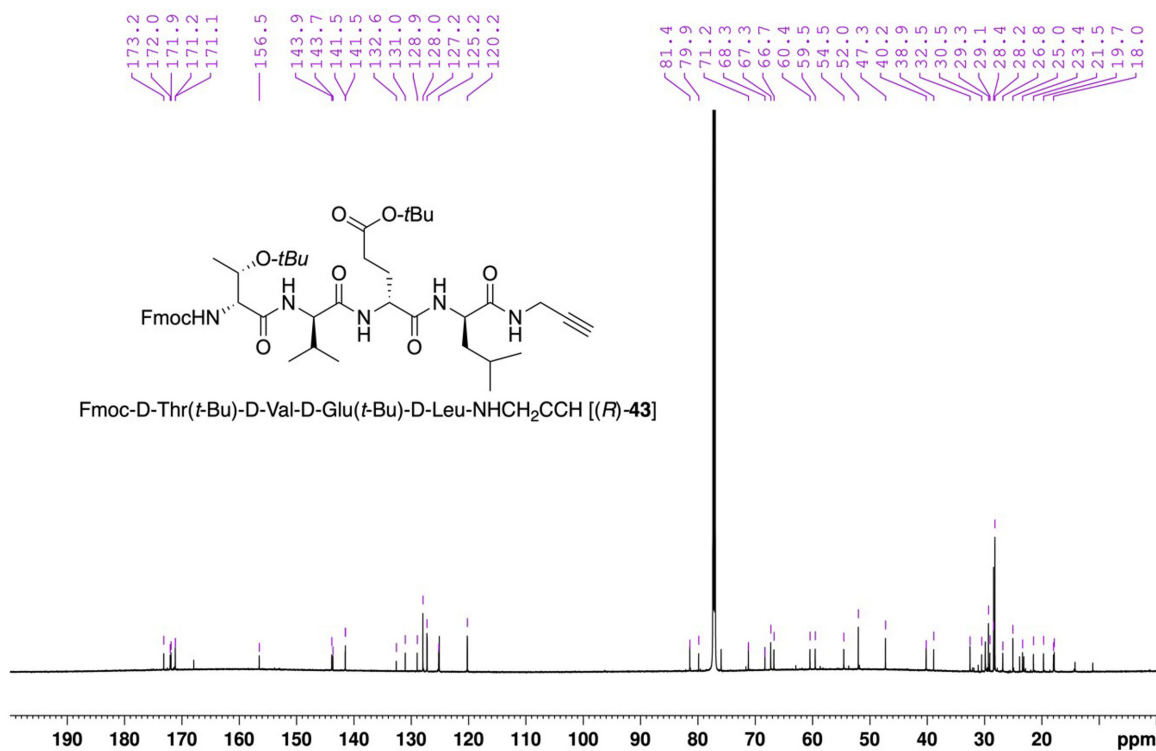
^1H NMR (700 MHz)

Solvent: CDCl_3



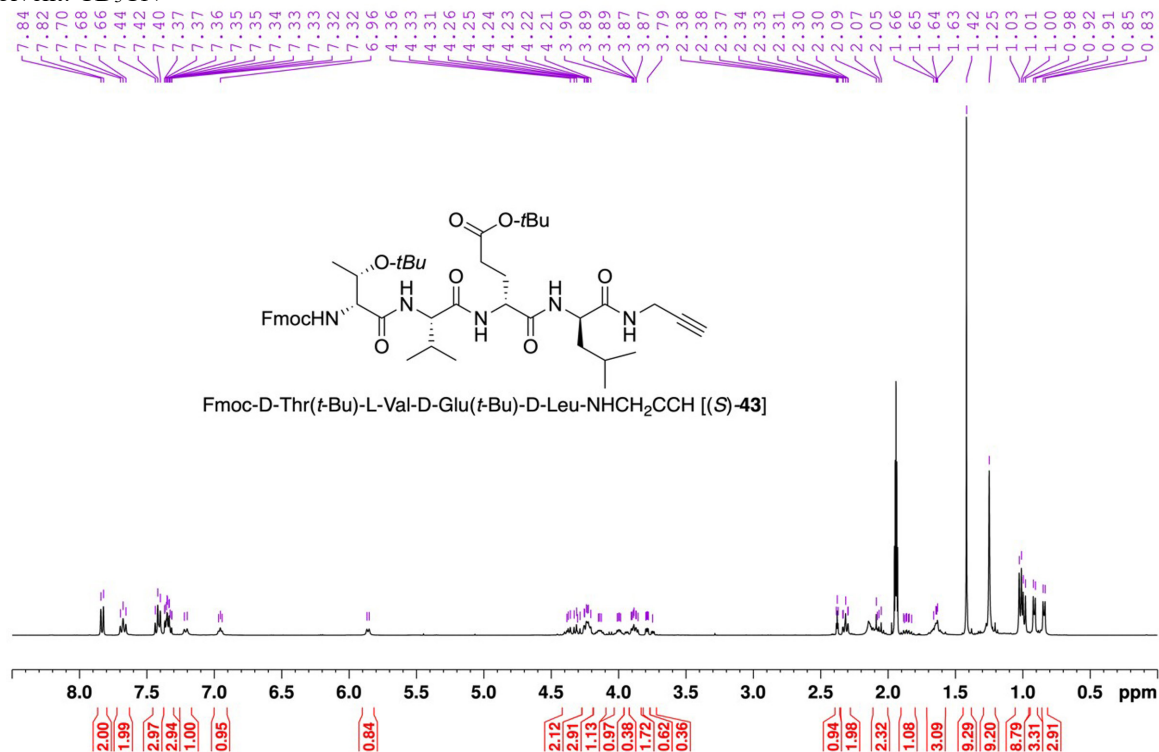
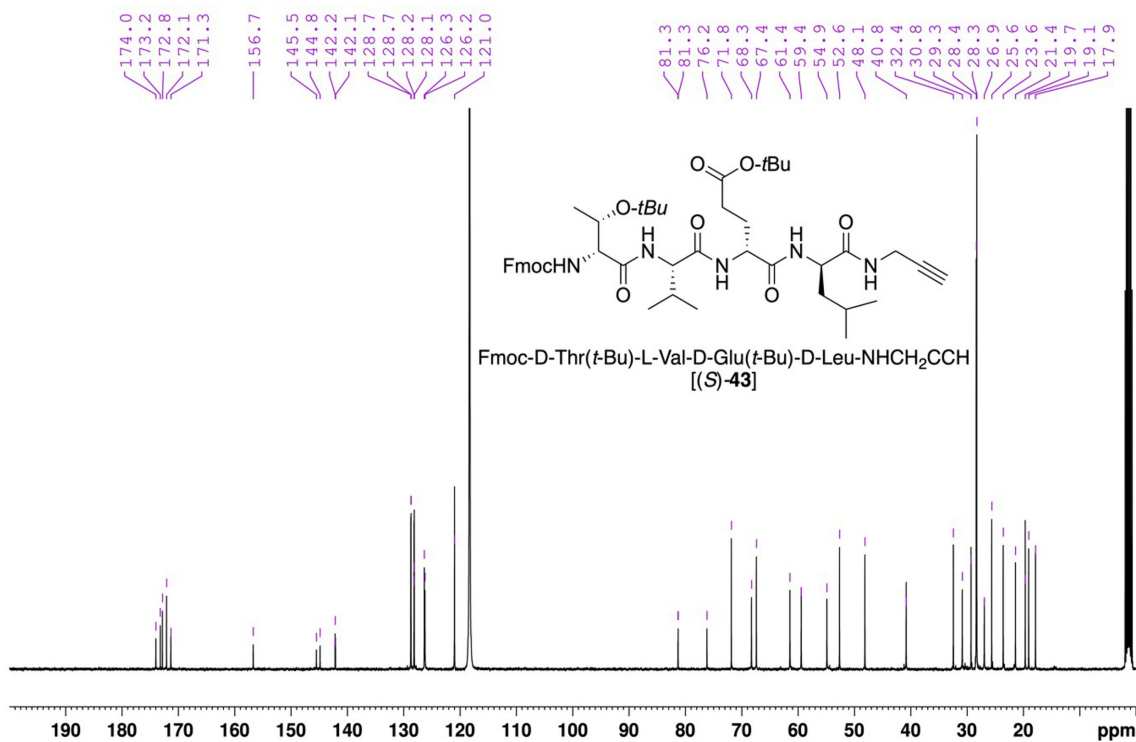
^{13}C NMR (175 MHz)

Solvent: CDCl_3



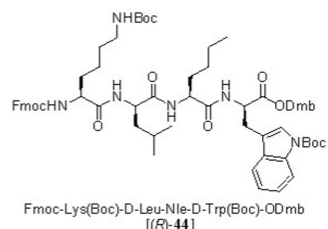
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Solvent: CD₃CN

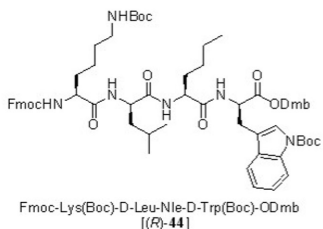
Solvent: CD₃CN

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Solvent: CD₃OD



Solvent: CD₃OD

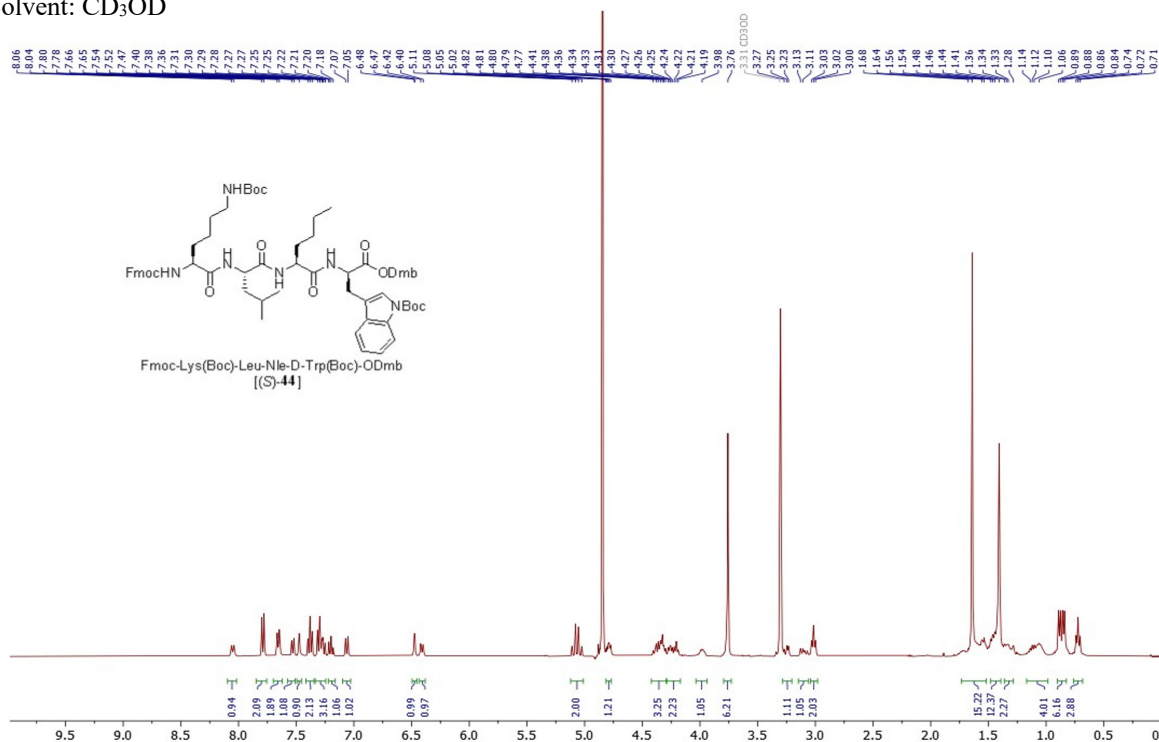


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

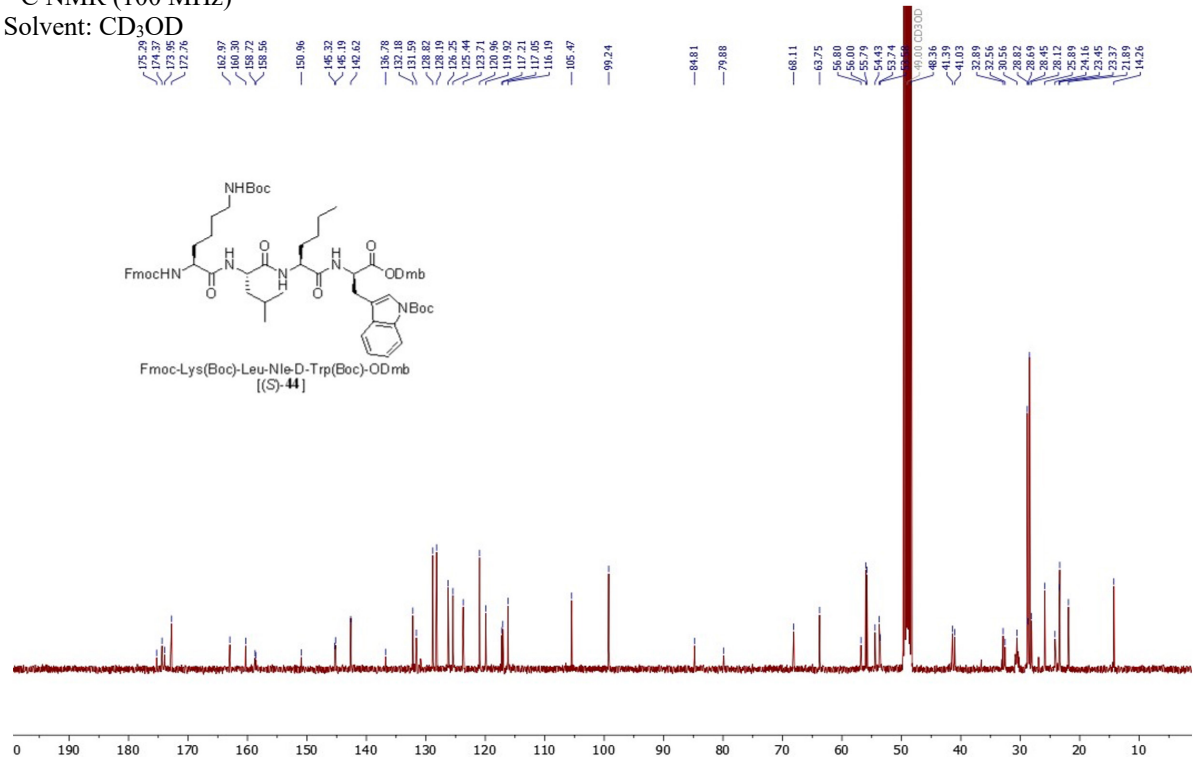
^1H NMR (400 MHz)

Solvent: CD_3OD



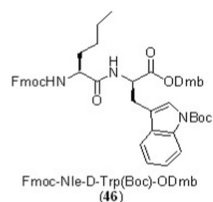
^{13}C NMR (100 MHz)

Solvent: CD_3OD

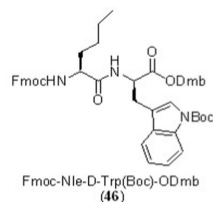


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Solvent: CD₃CN



Solvent: CD₃CN

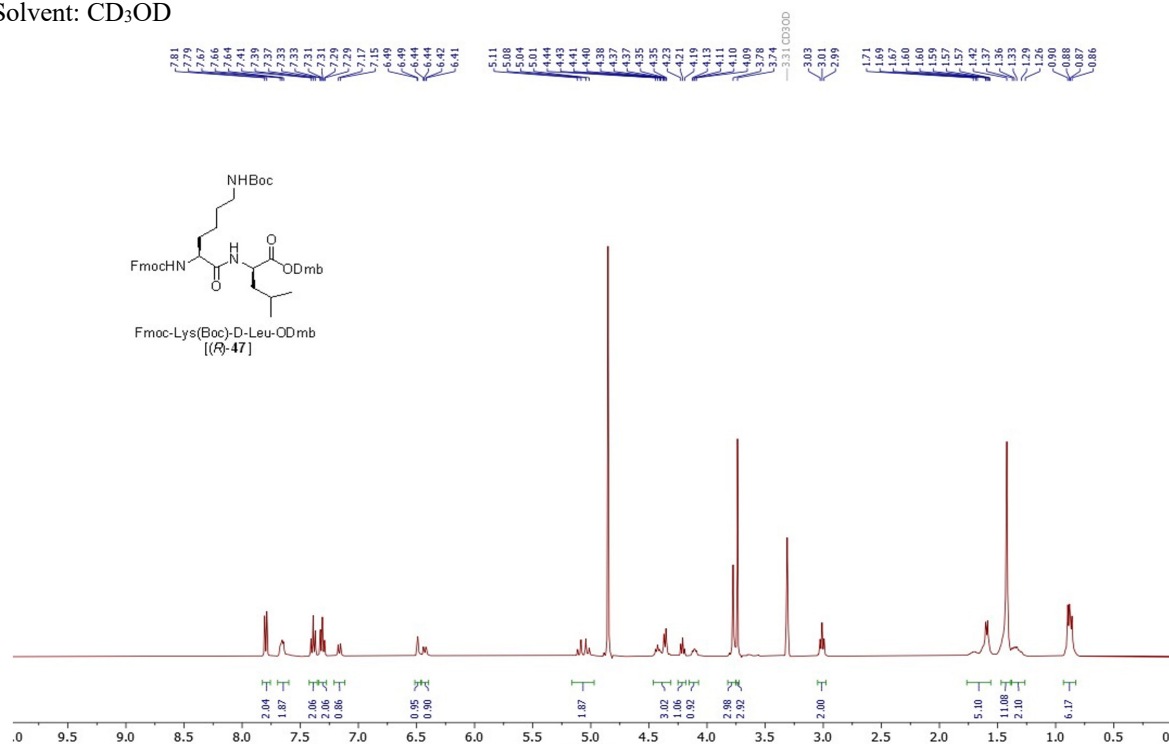


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

Maarouf-Mesli, N. et al.

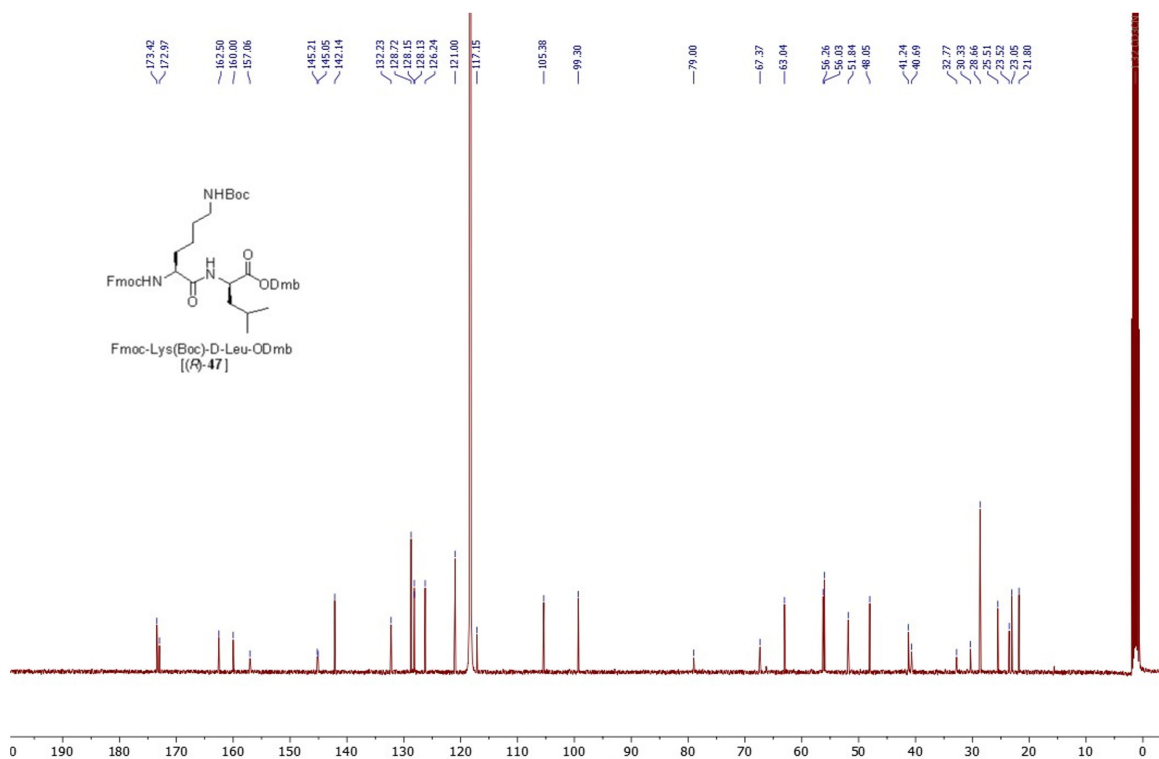
^1H NMR (400 MHz)

Solvent: CD_3OD



^{13}C NMR (100 MHz)

Solvent: CD_3OD

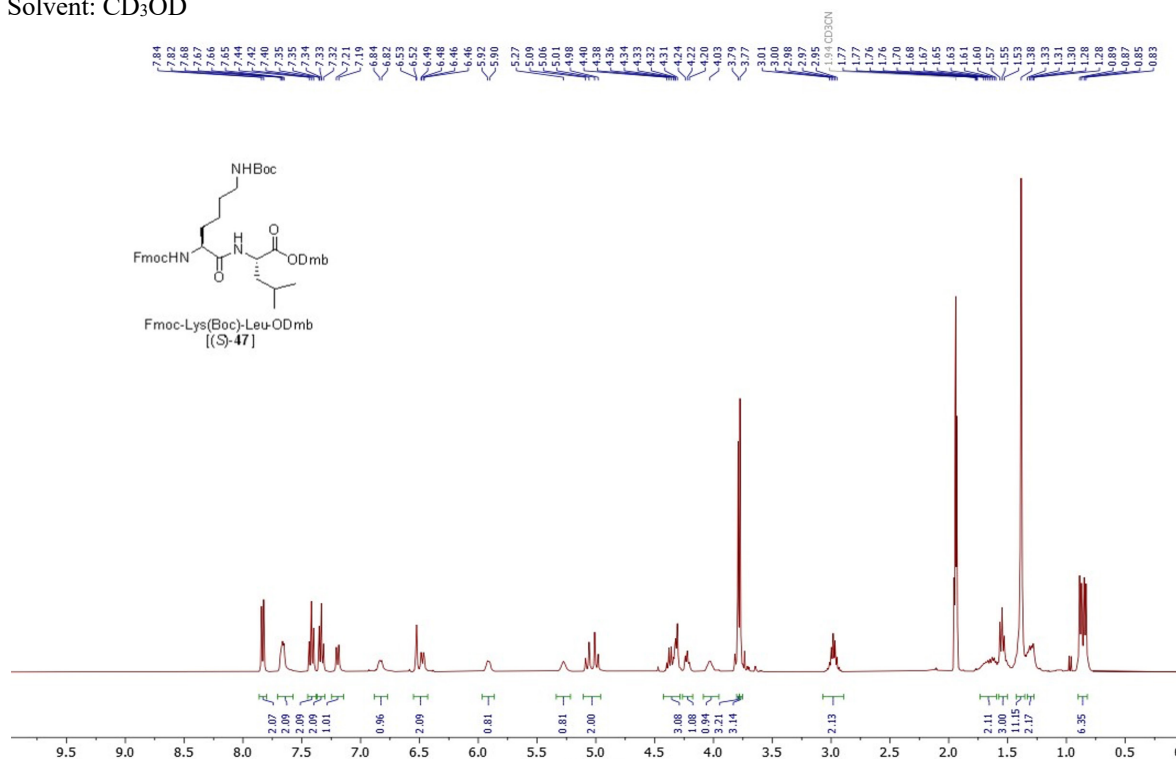


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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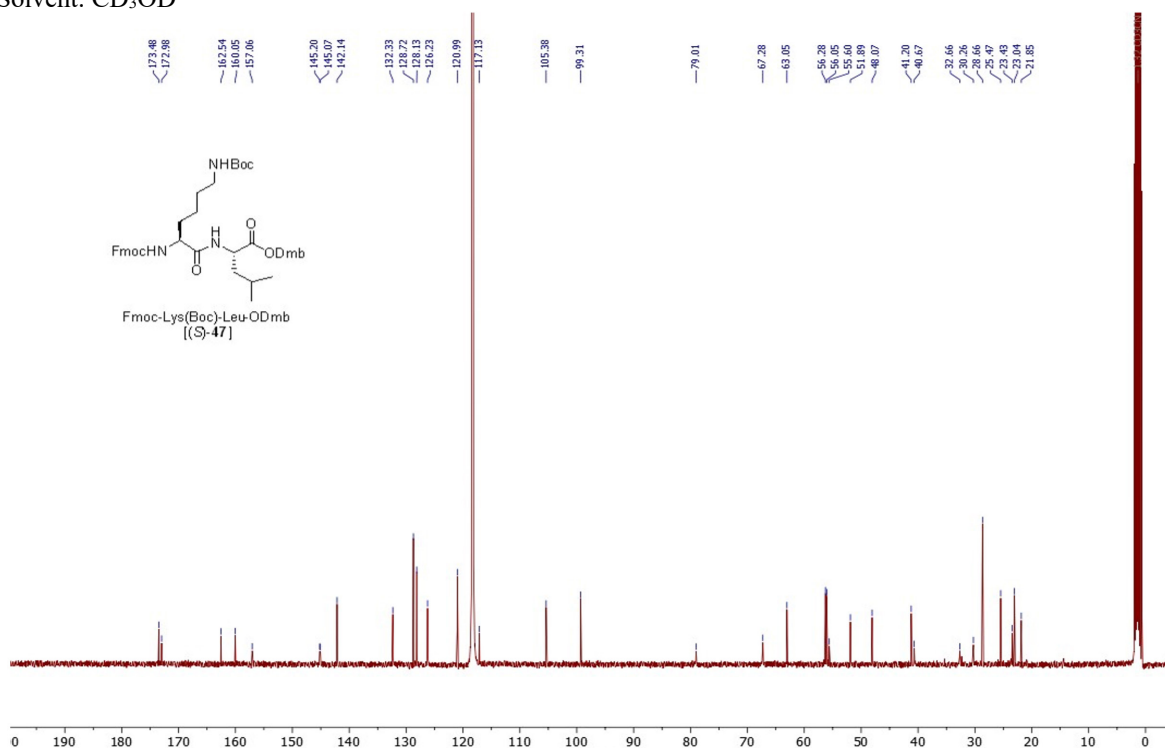
^1H NMR (400 MHz)

Solvent: CD_3OD



^{13}C NMR (100 MHz)

Solvent: CD_3OD

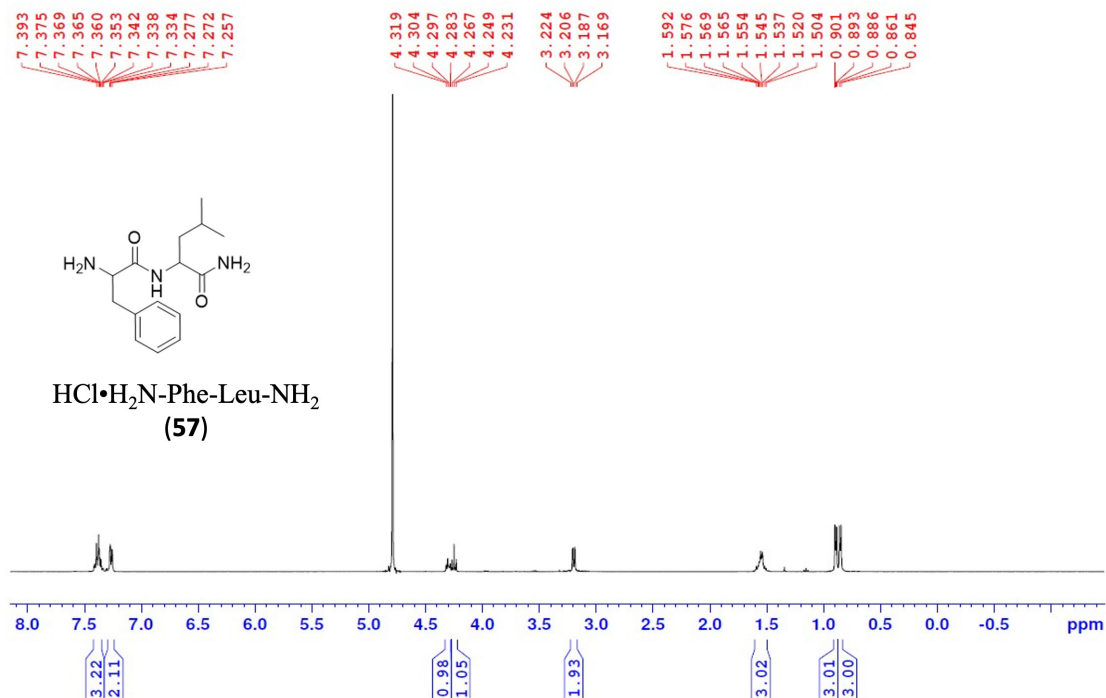


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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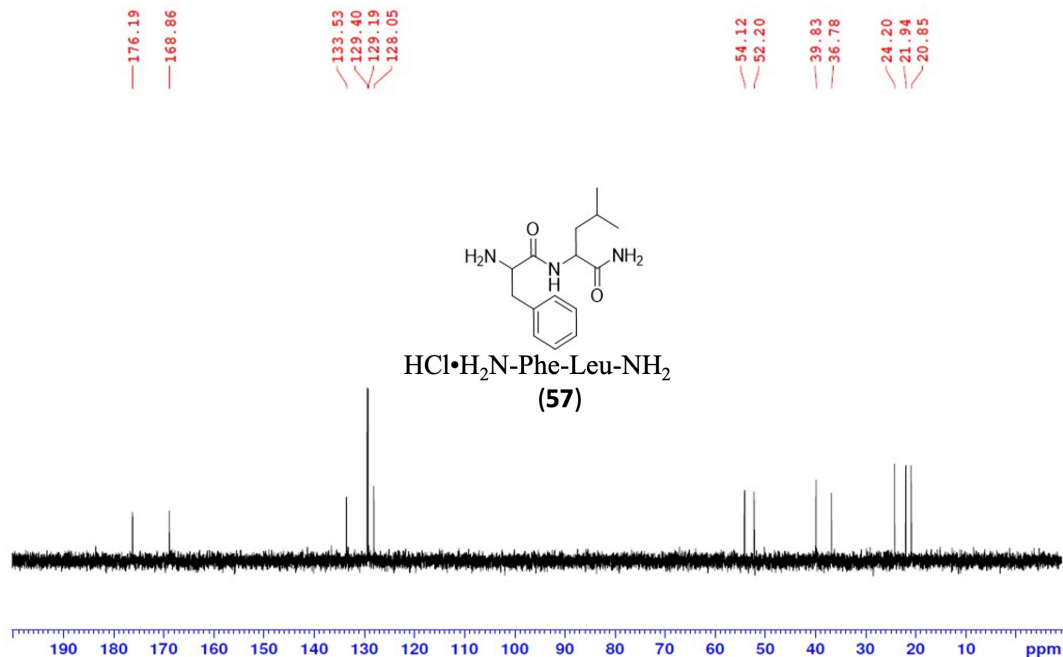
^1H NMR (400 MHz)

Solvent: D_2O



^{13}C NMR (100 MHz)

Solvent: D_2O

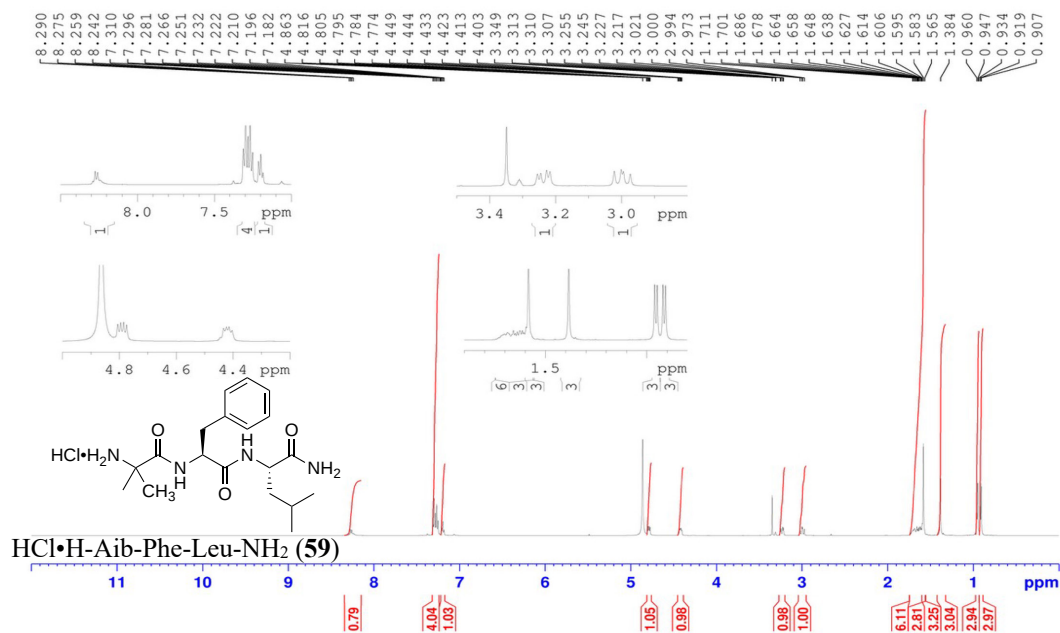


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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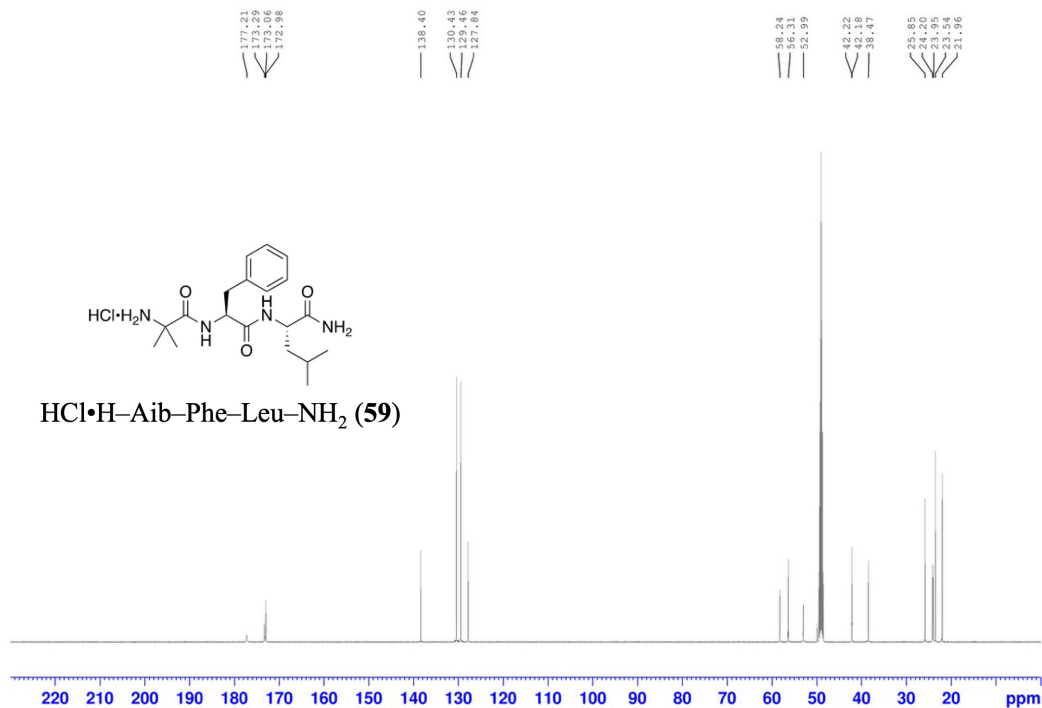
^1H NMR (500 MHz)

Solvent: CD_3OD



^{13}C NMR (125 MHz)

Solvent: CD_3OD

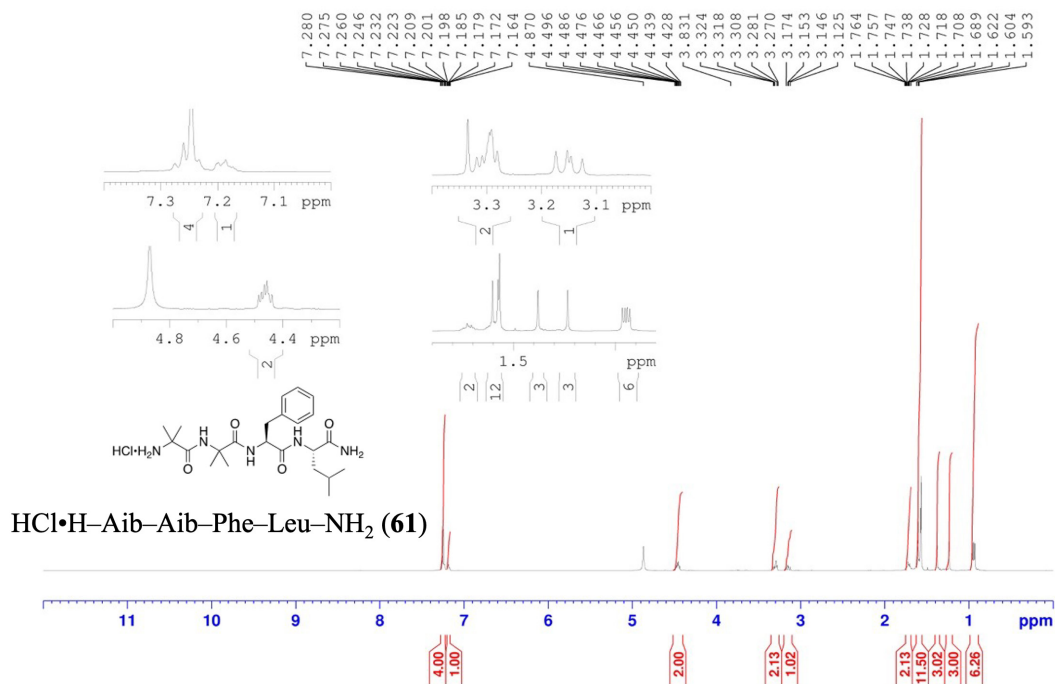


Solvent-Less Environmentally Benign Amino Ester, Amide, and Peptide Synthesis Enabled by Resonant Acoustic Mixing

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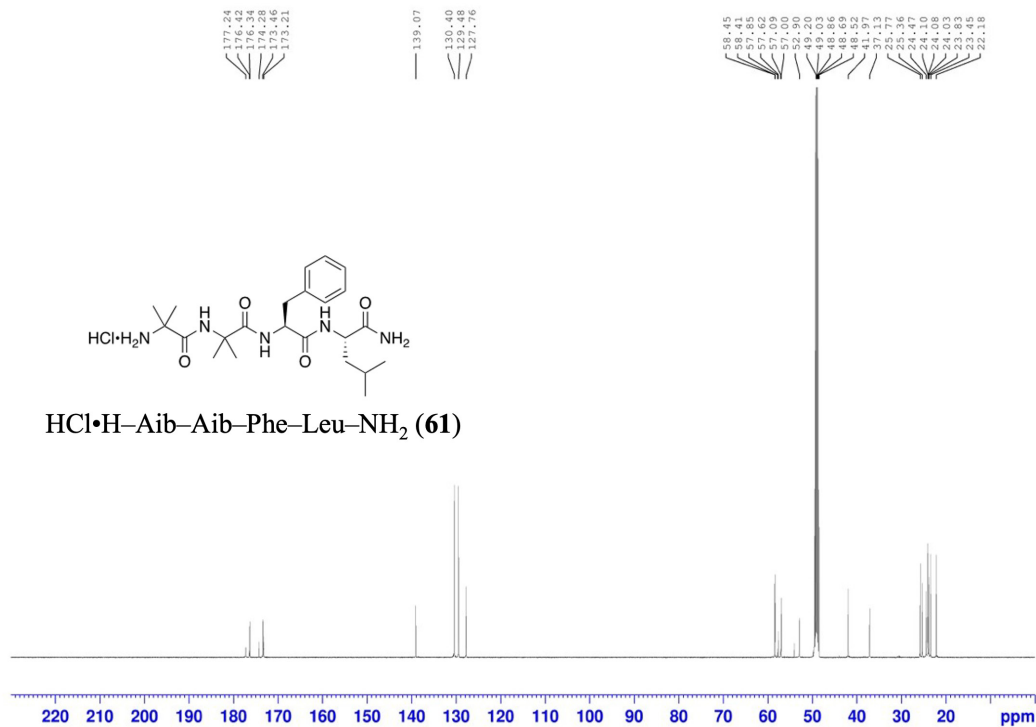
^1H NMR (500 MHz)

Solvent: CD_3OD



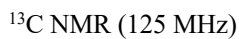
^{13}C NMR (125 MHz)

Solvent: CD_3OD



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Solvent: CDCl₃



Solvent: CDCl₃

