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Synthesis of N-protected ^{14}C-Labelled (2E)-5-amino-5-methylhex-2-enoic Acid Analogues

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SUMMARY

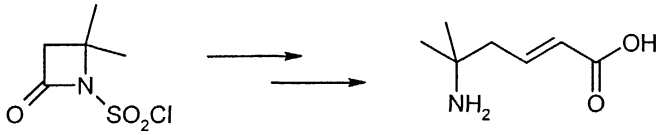
A novel method of preparing N-protected (2E)-5-amino-5-methylhex-2-enoic acids has been developed based on the synthesis of 3-methyl-3-amino-butanol. The method was used to synthesise ^{14}C-labelled compounds of (1) via synthesis of triethylphosphono[1-^{14}C]acetate.

Key words: Amino acids, Amino alcohols, Amino aldehydes, ^{14}C, Enzymes.

INTRODUCTION

A wide range of peptidomimetics have been synthesised by Novo Nordisk A/S in order to test their ability to release growth hormone. In this synthesis, (2E)-5-amino-5-methylhex-2-enoic acid (1) is an important building block in their total synthesis[1]. The goal was to find an inexpensive and readily scalable method towards (1), which also could be applied for radiolabelling of (1) for Absorption-Distribution-Metabolism-Excretion (ADME) studies.

The original method has its origin in the formation of 4,4-dimethyl-2-oxo-azetidine-1-sulfonyl chloride [2, 3] (2). Hydrolysis and Boc protection of the amine functionality furnishes N-(tert-butoxycarbonyl)-4,4-dimethyl-2-oxoazetidine which can be ring opened by utilisation of aqueous base. Subsequent reduction and oxidation of 3-tert-butoxycarbonylamino-3-methylbutanoic acid provides 3-tert-butoxycarbonylamino-3-methylbutanal[1,4], which leads to (2E)-5-(N-Boc)-amino-5-methylhex-2-enoic acid in two steps[1]. Unfortunately, the method has its limitations towards the use of other N-protection groups and is not suitable for large-scale preparation.



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Scheme 1: Original Strategy for (1)

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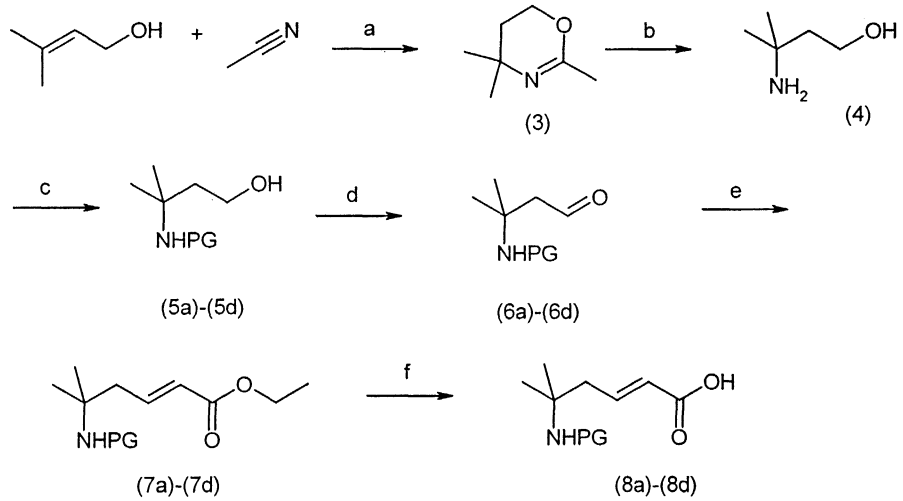
We identified 3-methyl-3-aminobutanol (4) as the key intermediate for the synthesis, which could lead to 3-tert-butoxycarbonylamino-3-methylbutanal (6a) in two steps. 3-Methyl-3-aminobutanol has been synthesised via a difficult high pressure, high temperature addition of ammonia (700psi/150°C/16h) to 3,3-dimethylacrylic acid, forming 3-amino-3-methylbutyric acid, which is reduced to its alcohol via $LiAlH_4$ [5] and addition of ammonia to isoprene di-hydrochloride[6] at high pressure.

RESULTS AND DISCUSSION

We now present a novel synthetic approach to (2E)-5-amino-5-methylhex-2-enoic acid, which has its origin in the Ritter type formation of 2,4,4-trimethyl-5,6-dihydro-4H-[1,3]oxazine [7,8,9,10,11] (1). Other protecting groups can easily be introduced by this new method.



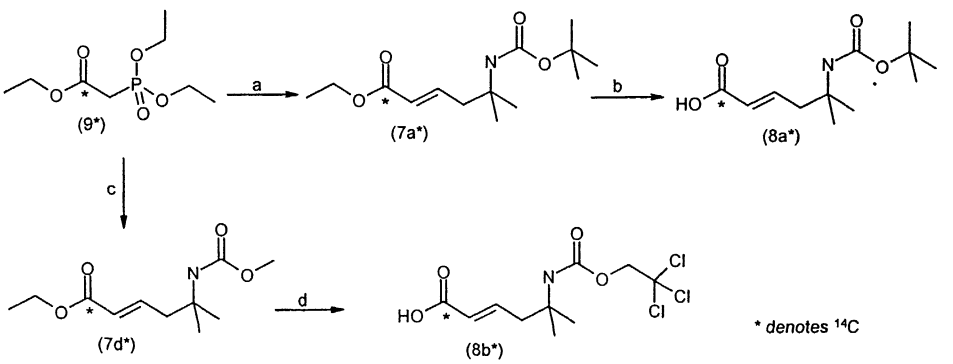
Scheme 2: Total Synthesis of N-protected (2E)-5-amino-5-methylhex-2-enoic Acids. (Reaction conditions: **a.** ref.12; **b.** 6M NaOH, 80°C, 18h; **c.** 2-PrOH/buffer pH 11, PG, 0-5°C then 25°C, 18h; **d.** DMSO, $Pyr.SO_3$ complex, $N(Et)_3$, 25°C, 18h; **e.** Triethylphosphonoacetate, Potassium-t-butoxide, toluene, 65°C, ½h; **f.** EtOH, 2M NaOH, reflux, 1h or Candida Antarctica immobilised., MIBK-H_2O, 60°C, 7 days).

Compound	PG	(%)Yield 5a-5d	(%)Yield 6a-6d	(%)Yield 7a-7d	(%)Yield 8a-8d
A	Boc	91	95	100	85
B	Troc	90	81	89	100
C	CBz	89	86	100	66
D	MeOCO	53	26	94	70

Compound (3) can be synthesised via condensation of acetonitrile and 3-methyl-but-2-en-1-ol. Until now, dihydro-1,3-oxazines have only found use in the formation of branched aldehydes and ketones.[12,13,14] However, 2,4,4-trimethyl-5,6-dihydro-4H-[1,3]oxazine can be converted to 3-methyl-3-aminobutanol (4) by addition of aqueous base. It was possible to increase the yield of 3-methyl-3-amino-butanol significantly. 3-Methyl-3-aminobutanol can be isolated or converted directly to its N-protected amino alcohol under Schotten-Baumann conditions. It is important to note that the OSu-esters must be applied in order to avoid interference with the OH-group of (3). With the exception of entry (5d) good yields of the N-protected amino alcohols were obtained.

We found that 3-(tert-butoxycarbonyl)amino-3-methylbutanol (5a) could be oxidised smoothly to the aldehyde applying the Moffat oxidation. This method was used for all N-protected amino alcohols. It was found that (6d) was unstable under these conditions and the yield was low.

Subsequent reaction with the Wittig-Horner reagent triethylphosphonoacetate and ester hydrolysis furnished (2E)-5-(N-PG)amino-5-methylhex-2-enoic acid (8a)-(8d). Triethylphosphonoacetate gave the (E) isomer as the only product. The ester hydrolyses of 7b and 7d had to be modified due to instability of the N protection group under basic conditions. Among different proteinases, lipases and esterases Candida Antarctica B (immobilised) gave the best results. The final yield of 8b and 8d varied from reasonable to excellent.



Scheme 3: Synthesis of ^{14}C-labelled (2E)-5-(N-Protected)-amino-5-methylhex-2-enoic acids. (Reaction conditions: **a.** i) potassium tert-butylate, THF, rt, 1½ h. ii) 3-(tert-butoxycarbonyl-aminomethyl)-3-methylbutanal, THF, rt, 23 h; **b.** dioxane, H_2O, LiOH, rt, 24 h; **c.** i) potassium tert-butoxide, toluene, 60°C. ii) triethyl phosphono[1-^{14}C]acetate, 65°C, 1 h. iii) 3-methoxycarbonylamino-3-methylbutanal, 65-75°C. **d.** i) NaOH, 2-propanol, H_2O reflux, 25 h. ii) $NaHCO_3$, succinimidyl-2,2,2-trichloroethyl carbonate, NaOH, H_2O.

The above shown method was used to synthesise the ^{14}C-labelled compounds of (1) with different protecting groups. The goal was to synthesise radiolabelled versions of 8a and 8b as shown in the scheme below. The key intermediate 9* was synthesised according to Dawson *et al.*[15]. Reaction of the triethylphosphono[1-^{14}C]acetate with base and the required protected aldehyde led to the desired intermediates. Again, only the (E) isomer was observed. Conventional ester hydrolyses of 7a* led to 8a*. Treatment of 7d* with base and succinimidyl-2,2,2-trichloroethyl carbonate afforded 8b*.

EXPERIMENTAL

General methods: All chemicals and solvents (analytical grade) were purchased from Merck, Fluka, or Aldrich. Melting points were obtained from a Buchi 510 and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX 300 or a DRX 400 spectrometer, and chemical shifts (δ) are in ppm relative to TMS. Elemental analyses were obtained from Novo Nordisk Microlab. Elemental analysis of all unlabelled compounds was found acceptable. TLC-plates were from Merck. (Silica gel 60 F_{254}) and the spots were developed in 1g 4-methoxybenzaldehyde dissolved in 10ml 98% H_2SO_4, 10ml AcOH and 100ml EtOH. GC spectra were obtained from a Chrompack cp 9000 equipped with a WCOT fused silica, CP-sil 5 CB column. A FID detector was employed. Hydrogen was also used as carrier gas. A method was used by which the temperature was maintained at 150°C for 5min, and then raised with 25°C/min until 250°C, where it was maintained for 5 min. The 70 eV E.I. solid mass spectrum was recorded on a Finnigan MAT-TSQ 70 mass spectrometer using a temperature programmed solid probe. HPLC-analysis for the non ^{14}C-labelled compounds was recorded on a Lachrom HPLC-system with a DAD-detector. A RP C-18 Novo Nordisk (NN) (250 x 4 mm, 5 μm) column was used. Absorptions were recorded at UV 220nm, 0.9 ml/min and 35°C. Solvent 1: 175ml water and 1.1ml $N(Et)_3$ were mixed. The pH was adjusted to 3 with 10% conc. H_3PO_4. Water was added up to a total volume of 200ml followed by 1.8liter MeOH. Solvent 2: 950ml water and 5.5ml $N(Et)_3$ were mixed. The pH was adjusted to 3 with 10% conc. H_3PO_4. Water was added to a total volume of 1000ml followed by 1000ml MeCN. Solvent 3: 600ml water and 5.5ml $N(Et)_3$ were mixed. The pH was adjusted to 3 with 10% conc. H_3PO_4. Water was added up to a total volume of 700ml followed by 1300ml MeCN. HPLC-analyses and semi-preparative separations for the radiolabelled compounds were performed on a Merck HPLC system. Six different HPLC systems (A-G) were used. A: RP C-18 (NN) (250 x 4mm, 5μm.) using 20% MeCN (0.1% TFA) at 1.0ml/min. B: RP C-18 SymmetryPrep™ (150 x 19mm, 8μm.) using 40% MeCN (0.1% TFA) at 9.0ml/min. C: RP C-18 (NN) (250 x 4mm, 5μm.) using 60% MeCN (0.1% TFA) at 1.0ml/min. D: RP C-18 (NN) (250 x 4mm, 5μm.) using 50% MeCN (0.1% TFA) at 1.0ml/min. E: RP C-18 Hypersil BDS (250 x 4.6mm, 5μm) using 50% MeCN (0.1% TFA) at 0.9ml/min. F: Phenomenex Prodigy ODS (250 x 21.2mm,

10μm) using 40% MeCN at 20 ml/min. G: Hypersil BDS (250 x 4.6mm, 5μm) using 50% MeCN (0.1% TFA) at 0.9ml/min.

3-Amino-3-methylbutan-1-ol (4)

2,4,4-Trimethyl-5,6-dihydro-4H-[1,3]oxazine[12] (35g- 0.28Mol) was added to 6M NaOH (90ml) while stirring. Two phases were formed. The mixture was heated to 80°C and stirred overnight (normally 18h) at 80°C. The reaction mixture (now one phase) was cooled to room temperature and extracted with 3x 250ml DCM. The combined organic phase was washed with brine (300ml) and dried. Removal of solvent gave (27.7g - 96%) of (4) as colorless oil. The crude product was used without further purification. **GC data:** RT(2,4,4-Trimethyl-5,6-dihydro-4H-[1,3]oxazine)-2.60min; RT(4)-2.66min. **NMR** ($CDCl_3$): **^{1}H**: 3.84 (CH_2,m, 2H), 2.91(-OH,NH_2br.s,3H),1.59 (CH_2,t,2H), 1.21(2xCH_3,2s,6H) **^{13}C**: CH_2:60.39ppm; CH_2:50.93ppm; C:42.65ppm; 2xCH_3:31.00ppm. **SP/MS [M+H]**: 104.1.

(3-Hydroxy-1,1-dimethylpropyl)carbamic acid benzylester (5c)

60 ml of a buffer-pH 11.(Saturated $NaHCO_3$, adjusted with 4N NaOH to pH11) was added to a solution of 3-amino-3-methylbutan-1-ol (3.5g- 34mMol) in 60ml 2-PrOH.The reaction mixture was cooled to 0-5°C and N-(benzyloxycarbonyloxy)-succinimid (8.5g - 34mMol) was added in two portions with 10 minutes interval. The reaction mixture was stirred for 2 hours at 0-5°C. Stirring was maintained at room temperature for 16 hours. The reaction mixture was evaporated to remove 2-PrOH. The residue was diluted with 100ml water and extracted with 1x200ml and 1x 100ml MTBE. The combined organic phase was dried and removal of the solvent gave (5c) (7.2g - 89%) as colorless oil. The crude product was used without further purification. HPLC(Solvent1): RT values: cBz-OSu-2.61min;N-hydroxysuccinimid-2.40; 5c-2.75min. **NMR** ($CDCl_3$): **^{1}H**: 7.29-7.37 (Aromatic,m, 5H), 5.37 (NH,Broad signal, 1H), 5.04 (CH_2,s, 2H),3.77 (CH_2,t, 2H),1.9(OH,broad s,1H),1.87(CH_2,t,2H),1.35 (2xCH_3,s,6H). **^{13}C**: C=O:155.11; C:136.70; 2xCH:128.55; 2xCH:128.04; CH:128.02; CH_2:66.09; CH_2:59.39; C: 52.37; CH_2:43.10; 2xCH_3:27.40. **SP/MS M^+**: 237.2.

(3-Hydroxy-1,1-dimethyl-propyl)carbamic acid tert-butylester (5a)

Di-tert-butyl dicarbonate (2.1g-9.7mMol) was added to a stirred solution of 3-amino-3-methylbutan-1-ol **4** (1.0g- 9,7mMol) in 5ml THF and 5ml 2M NaOH. Stirring continued overnight. The reaction mixture was filtered, and 5ml water was added to the filtrate. The mixture was extracted with 2x20ml DCM. The combined organic phase was dried and removal of the solvent afforded (1,8g -91%) of (5a) as colorless oil. **GC data:** (4)-2,66min; Di-tert-butyl dicarbonate-5,07min; (5a)-6,03min. **NMR**($CDCl_3$): **^{1}H** according to Hansen

et al.[1] ^{13}C: CH_2:60.39ppm; CH_2:50.93ppm; C:42.65ppm; 2xCH_3:31.00ppm. **SP/MS [M+H]**: 204.2.

(3-hydroxy-1,1-dimethyl-propyl)carbamic acid 2,2,2-trichloro-ethylester(5b)

5b was synthesized according to the procedure for 5c. Carbonic acid 2,5-dioxopyrrolidin-1-yl ester- 2,2,2-trichloroethyl ester was applied. (3.5g-34mMol) 4 afforded (8.5g-90%) of (5b) as colorless oil. **GC data:** (4)-2,66min; (5b)-4,14min. **NMR**($CDCl_3$):^{1}**H**:5.67 (NH,broad s,1H), 4.67 (CH_2,s,2H), 3.83 (CH_2OH, m,3H), 1.91 (CH_2,m,2H), 1.79 OH,br,1H), 1.40(2xCH_3,s,6H). ^{13}**C**:C=O: 153.00; C: 95.9; CH_2: 74.01; CH_2: 59.41; C: 52.91; CH_2: 42.94; 2xCH_3: 27.1. **SP/MS** M^+ 278,1.

(3-hydroxy-1,1-dimethyl-propyl)carbamic acid ethyl ester(5d)

5d was synthesized according to the procedure for 5c. Succinimidyl ethyl ester carbonate was applied. (3.5g-34mMol) 4 afforded (2.94g-53%) of (5d) as colorless oil. **GC data:** (4)-2,66min; Succinimidyl ethyl ester-3,33+3,87min; (5d)-3,47min. **NMR**($CDCl_3$):^{1}**H**:5.38 (NH,s,1H), 3.79 (CH_2,t,2H), 3.61 (O-CH_3,s,3H), 2.08 (OH,s,1H), 1.87 (CH_2,t,2H), 1.35 (2xCH_3,s,6H). ^{13}**C**:C=O: 155.85; CH_2: 59.36; C: 52.27; CH_3: 51.51; CH_2: 43.32; CH_3: 27.26. **SP/MS [M+H]**: 162.1.

(1,1-dimethyl-3-oxo-propyl)carbamic acid benzylester (6c)

$N(Et)_3$ (15ml - 106mMol) was added to a stirred solution of (5c) (4.4g - 18.5mMol) in 30ml DMSO at room temperature,. Pyridin-SO_3-complex (5g - 31mMol) was dissolved in 30ml DMSO and added drop wise over 15min. (weakly exothermic). Stirring was maintained overnight. The next day, the reaction mixture was diluted with 60ml toluene and cooled to 15°C. Water (60ml) was carefully added (exothermic), and stirring was maintained for 20 min. The organic phase was separated and the aqueous phase extracted with toluene (60ml). The combined organic phase was washed with 0.5N $KHSO_4$ (2x50ml), 10% NaHCO3 (50ml), and with water (50ml). The organic phase was dried removal of the solvent afforded (6c) (3.8g- 86%) as brown oil. The crude product was used without further purification. **TLC:** Rt(5c)-0.4: Rt(6c)-0.6. EtOAc:Heptane 1:1. **NMR** ($CDCl_3$): ^{1}**H**: 9.77 (CHO,s,1H), 7.29-7.38 (Aromatic,m, 5H), 5.06 (CH_2,s, 2H), 4.93 (NH,Broad s, 1H), 2.87 (CH_2,s, 2H), 1.40 (2xCH_3,s,6H) ^{13}**C**:CHO:201.49; C=O: 154.78; C:136.38; 2xCH:128.57; CH:128.17; 2xCH:128.04; CH_2:66.39; CH_2: 52.25; C: 51.35; 2xCH_3:27.94. **SP/MS** M^+: 235.2.

(1,1-Dimethyl-3-oxo-propyl)carbamic acid tert-butylester (6a)

6a was synthesized according to the procedure for 6c. (145g-0.71Mol) 5a afforded (135g-95%) 6a as a yellow oil. **TLC:** Rt(5a)-0.2: Rt(6a)-0.65. EtOAc:Heptane 1:1.

NMR($CDCl_3$): **^{1}H** according to Hansen et al.[1] **^{13}C**: CHO: 202.23; NH-C=O: 154.91; C: 52.63; CH_2: 51.31; 3xCH_3: 28.78; 2xCH_3: 28.58. **SP/MS [M+H]**: 202.14.

(1,1-Dimethyl-3-oxo-propyl)carbamic acid 2,2,2-trichloro-ethylester(6b)

6b was synthesized according to the procedure for 6c. (1,0g-3.6mMol) 5b afforded (0,81g-81%) 6b as a yellow oil. **TLC:** Rt(5b)-0.15: Rt(6b)-0.5. EtOAc:Heptane 1:1. **NMR**($CDCl_3$):**^{1}H**:9.75 (CHO,s,1H), 5.10-5.25 (NH,broad s,1H), 4.70 (CH_2,s,2H), 2.91 (CH_2,d,2H), 1.79,1.40(2xCH_3,s,6H). **^{13}C**:CHO:200.65 ;HN-C=O: 151.50; C: 95.50; CH_2: 73.45; C: 52.26; C: 51.35; 2xCH_3: 27.74. **SP/MS [M+H]**: 277,59.

(1,1-dimethyl-3-oxo-propyl)carbamic acid methyl ester(6d)

6d was synthesized according to the procedure for 6c. (2.6g-16mMol) 5d afforded (0.7g-29%) 6d as a brown oil. **TLC:** Rt(5d)-0.2: Rt(6b)-0.57. EtOAc:Heptane 1:2. **NMR**($CDCl_3$):**^{1}H**:9.79 (CHO,s,1H), 4.93 (NH,s,1H), 3.63 (O-CH_3,s,3H), 2.86 (CH_2,s,2H), 1.40 (2xCH_3,s,6H). **^{13}C**:CHO: 201.55; C=O: 155.60; CH_2: 52.35; C: 51.27; CH_3: 51.73; 2xCH_3: 27.97. **SP/MS**: Thermally unstable.

Ethyl (2E)-5-(benzyloxycarbonylamino)-5-methylhex-2-enoate (7c)

Triethylphosphonacetate (1.6g - 7mMol) was added drop wise to a stirred mixture of potassium t-butoxide (0.8g - 7mMol) in 20ml toluene, heated to 60°C. Stirring was maintained at 60°C for ½h. 6c (1.5g - 6.4mMol) was then dissolved in 10ml toluene and added drop wise to the above mixture over 10 min. The reaction mixture was cooled to room temperature and stirring continued for additional 3h. The reaction mixture was further cooled on an ice bath to 0 to 5°C, and 20ml water was added drop wise while stirring. Stirring was maintained for further ½h. The phases were separated, and the aqueous phase extracted with 40ml toluene. The combined toluene phases were washed with 0.5N $KHSO_4$ (2x50ml), sat. NaHCO3 (50ml) and water (50ml). The organic phase was dried and removal of solvent gave (7c) (1.95g –100%) as yellowish oil. The crude product was used without further purification. **TLC**: RT(3) 0.3; RT(4) 0.6. EtOAc:Heptane 1:2. **NMR** ($CDCl_3$): **^{1}H**: 7.29-7.38 (Aromtic,m, 5H), 6.9 (=CHd.t, 1H), 5.86 (=CH,d, 1H), 5.05 (CH_2,s, 2H), 4.70 (NH,broad s, 1H), 4.19 (CH_2, q,2H), 2.64 (CH_2,d,2H), 1.32 (2xCH_3,s,6H), 1.29 (CH_3,t,3H) **^{13}C**:C=O:166.27; NHC=O:154.58; C=C:144.25; C:136.61; 2xCH:128.56; CH:128.09; 2xCH: 128.04; CH: 144.25; CH_2: 66.24; CH_2: 60.29; C: 52.68; CH_2: 42.40; 2xCH_3: 27.39; CH_3: 14.27. **SP/MS M^+**: 305.2.

Ethyl (2E)-5 -(t-butyloxycarbonylamino)-5-methylhex-2-enoate (7a)

7a was synthesized according to the procedure for 7c. (133g-0.66Mol) 6a afforded (180g-100%) 7a as yellow oil. **HPLC**(Solvent2)**:** Rt(6a)-4,24min: Rt(7a)-19,18min. **NMR**($CDCl_3$): **^{1}H** according to Hansen et al.[1] **^{13}C**: RO-C=O: 166.56;HN-C=O: 154.60;

C=C:147.72; C=C:124.22; O-CH_2:60.71; C: 53.46; CH_2: 42.29; 3xCH_3: 28.77; 2xCH_3: 27.96; CH_3: 14.63. **Elementary analysis**: Calculated for $C_{14}H_{25}NO_4$: C,61.37; H,9.29; N,5.16. Found: C,60.59; H,9.32; N,5.24. **SP/MS [M+H]**: 272.30.

Ethyl (2E)-5 -(2,2,2-trichloro-ethoxycarbonylamino)-5-methylhex-2-enoate (7b)

7b was synthesized according to the procedure for 7c. (0.54-2mMol) 6b afforded (0.6g-89%) 7b as a yellow oil. **HPLC**(Solvent2): Rt(6b)-3,41min: Rt(7b)-26,74min. **NMR**($CDCl_3$):^{1}**H**:6.80-7.0 (=CH,d.t,1H), 5.80-5.95 (=CH,d,1H), 4.95 (NH,broad s,1H), 4.70 (CH_2,s,2H), 4.20 ((O-CH_2,q,2H)), 2.65 (CH_2,d,2H), 1.30(3xCH_3,s,9H). ^{13}**C**:RO-C=O: 166.56;HN-C=O: 152.90; C=C:144.13; C=C:125.20; C: 96.10; CH_2: 74.28; O-CH_2:60.71; C: 53.46; CH_2: 42.29; 2xCH_3: 27.64; CH_3: 14.63. **SP/MS M$^+$**: 346.05.

Ethyl (2E)-5 -(Methoxycarbonylamino)-5-methylhex-2-enoate (7d)

7d was synthesized according to the procedure for 7c. (48g-0.3Mol) 6d afforded (64.5g-94%) as orange oil. **TLC:** Rt(7)-0.3: Rt(8)-0.5. EtOAc:Heptane 1:1,developed with 4-Methoxybenzaldehyde developer (see experimental section) **NMR**($CDCl_3$):^{1}**H**:6.80-7.0 (=CH,d.t,1H), 5.80-6.95 (=CH,d,1H), 4.65 (NH,broad s,1H), 4.20 (O-CH_2,q,2H), 3.6 (O-CH_3,s,3H), 2.65 (CH_2,d,2H), 1.30(3xCH_3,s,9H). ^{13}**C**:COOH: 171,10; HN-C=O: 155.75; =CH: 147,30; C=: 124,27; O-CH_2: 60,30; O-CH_3: 52,87; C: 52,03; 2xCH_3: 27.76; -CH_3: 14,27. **SP/MS [M+H]**: 230,25.

(2E)-5-(Benzyloxycarbonylamino)-5-methylhex-2-enoic acid (8c)

7c (1.5g -4.9mMol) was dissolved in EtOH (12ml) and 2N NaOH (12ml). The mixture was heated to reflux and the temperature maintained for additional 1h. After completion of reaction, the mixture was cooled to room temperature and evaporated. The residue was diluted with 20ml water and washed with MTBE (20ml). The aqueous phase was acidified with 2N $KHSO_4$ to pH 3 and extracted with DCM (2x50ml). The combined organic phase was dried and removal of the solvent gave (8c) (0.9g - 66%) as a white solid. Mp: 98-100°C (re-crystallized isopropyl acetate and petrol ether (1:1)).

HPLC(Solvent2): Rt(7c)-23,17min: Rt(8c)-6,81min. **NMR** ($CDCl_3$): ^{1}**H**:8.7 (COOH,br.s, 1H), 7.3-7.4 (aromatic,m, 5H), 7.01 (=CH,d.t, 1H), 5.87 (CH=,d, 1H), 5.06 (CH_2,,s, 2H), 4.73 (NH,broad s, 1H), 2.67 (CH_2,broad s,2H), 1.32 (2xCH_3,6H). ^{13}**C**:COOH:171.04; NHC=O:154.59; C=C:147.17; C:136.51; 2xCH:128.58; CH:128.14; 2xCH: 128.05; CH:123.84; CH_2: 66.33; C: 52.69; CH_2: 42.37; 2xCH_3: 27.47. **SP/MS M$^+$**: 277.2.

(2E)-5-(tert-butoxycarbonylamino)-5-methylhex-2-enoic acid (8a)

8a was synthesized according to the procedure for 8c. (268g-0,99Mol) 7a afforded (203g-85%) of 8a as white crystals. Mp 88-89°C (re-crystallized in isopropyl acetate and petrol

ether (1:1). **HPLC**(Solvent2): Rt(7a)-19,18min: Rt(8a)-5,67min. **NMR**($CDCl_3$): **^{1}H** according to Hansen et al.[1] **^{13}C:** HO-C=O: 171.59;HN-C=O: 154.60; C=C:147.72; C=C:124.21; C: 53.30; CH_2: 42.60; 3xCH_3: 28.77; 2xCH_3: 27.96. **SP/MS [M+H]**: 244.3.

(2E)-5-(2,2,2-trichloroethoxycarbonylamino)-5-methylhex-2-enoic acid (8c)

To a shaken solution of (7b) (120mg – 0.35mMol) in 18ml isobutyl-methyl-keton, and 2ml water was added 2ml Candida Anartica B (lipase immob. on Accurel EP100 load 86 KLO/g)- The reaction was shaken at 60°C until total conversion. The reaction took 10 days. The reaction mixture was filtered and evaporated to afford 13 (111mg – 100%) as yellow oil. Crystallization occurred after few days. Mp: 90-91°C (re-crystallized isopropyl acetate and petrol ether (1:1)). **HPLC**(Solvent3):Rt(7b)-7.20min; Rt(8b)-3.85min. **NMR**($CDCl_3$):**^{1}H**:6.80-7.0 (=CH,d.t, 1H), 5.80-6.95 (=CH,d,1H), 4.95 (NH,broad s,1H), 4.70 (CH_2,s,2H), 4.20, 2.65 (CH_2,d,2H), 1.30(2xCH_3,s,6H). **^{13}C**:HO-C=O: 169.40;HN-C=O: 150.74; C=C:144.95; C=C:122.20; C: 93.80; CH_2: 72.06; C: 51.20; CH_2: 40.08; 2xCH_3: 27.1. **SP/MS M$^+$**: 319,05.

(2E)-5-(Methoxycarbonylamino)-5-methylhex-2-enoic Acid (8d)

To a shaken solution of 7d (120mg – 0.52mMol) in 18ml isobutyl-methyl-keton, and 2ml water was added 3ml Candida Antarctica B (same as before). The reaction was shaken at 60°C until conversion. The conversion took 7 days. The reaction mixture was filtered and the filtrate evaporated affording 110mg of crude 8d as yellow oil. Filtration through a short silica gel 60 column with EtOAc and then MeOH followed by evaporation of solvent gave (75mg-70%) of 8d. **TLC:** Rt(8d)-0.1 (ethyl acetate): Rt(8d)-0.7. **HPLC**(Solvent3):Rt(7d)-3.67min; Rt(8d)-2.63min. **NMR**($CDCl_3$):**^{1}H**:6.80-7.0 (=CH,d.t, 1H), 5.80-6.95 (=CH,d,1H), 4.65 (NH,broad s,1H), 3.6 (O-CH_3,s,3H), 2.65 (CH_2,d,2H), 1.30(2xCH_3,s,6H). **^{13}C**:COOH: 171,10; HN-C=O: 155.75; =CH: 147,30; C=: 124,37; O-CH_3: 52,97; C:52,05; 2xCH_3: 27.77. **SP/MS [M+H]**: 202,32.

Ethyl (2E)-5-(tert.-Butoxycarbonylamino)-5-methyl[1-^{14}C]hex-2-enoate (7a*)

Triethylphosphono[1-^{14}C]acetate[15] was dissolved in dry THF (1ml) and potassium tert-butylate (81.64mg; 0.76mMol) was added. The mixture was allowed to react for 1½ hour at room temperature. 3-(Tert-butoxycarbonylaminomethyl)-3-methylbutanal (143.39mg; 0.71mMol) was dissolved in dry THF (1ml) and added to the stirred reaction mixture over 45 minutes. The reaction was allowed to continue for 23 hours at room temperature. The radiochemical conversion was >95%. HPLC (Systems A and C). The mixture was concentrated and the residue dissolved in 10ml 30%MeCN. TFA was added until pH ~ 5 and the solution purified by HPLC (System B). The relevant fractions were combined ($R_t \approx 13$ min.) and concentrated. Further purification was performed on a pre-activated (MeOH (10ml) followed by water (20ml)) Sep-Pak (C-18). The evaporated HPLC fractions were then

eluted with water (2x 10ml) and MeOH (6x 5ml). The MeOH fractions were analyzed by HPLC (System C) and fractions with radiochemical purity >95% were combined (9 mCi) and evaporated to dryness.

(2E)-5-(tert-Butyloxycarbonylamino)-5-methyl[1-^{14}C]hex-2-enoic acid (8a*)

Dioxane (1.0 ml), water (0.5ml), and LiOH (4.79mg; 0.20mMol) were added to ethyl (2E)-5-(tert.-Butoxycarbonylamino)-5-methyl[1-^{14}C]hex-2-enoate (9 mCi), and the mixture was stirred over night at room temperature. The radiochemical conversion was ~ 93% according to HPLC (System D). The mixture was evaporated to dryness and the residue suspended in DCM (3ml) and water (0.5ml). 0.1M $NaHSO_4$ (1.3ml) was added. The separated organic layer was evaporated to dryness. Radiochemical yield: 4.5mCi. Radiochemical purity: >95% by HPLC (System D).

5-(N-methoxycarbonyl)-amino-5-methyl[1-^{14}C]-hex-2-enoic acid ethylester (7d*)[16]

Potassium t-butoxide (820mg, 7.31mMol) and dry toluene (10ml) were heated at 60°C under nitrogen. Triethylphosphono[1-^{14}C]acetate (1322 mg, 5.85 mMol) was dissolved in dry toluene (5ml) and added drop wise over 45 minutes. The solution was heated at 65°C for 1 hour. 3-Methoxycarbonylamino-3-methylbutanal (931mg, 5.85mMol) in dry toluene (5.5ml) was added drop wise over 45 minutes. After heating at 65-75°C for 1 hour a cloudy, viscous solution was formed. Further Potassium t-butoxide was added in small portions until 90% conversion. TLC analysis (silica, hexane:acetone:MeOH (50:50:2)). The reaction mixture was cooled and water (10ml) was added. The separated toluene phase was washed with 0.5M $KHSO_4$ (10ml), sat. NaHCO3 (10ml) and water (10ml), dried and concentrated to yellow oil. The oil was re-dissolved in DCM (10ml) and applied to a flash silica column (16x 5.5cm diameter, packed in DCM). Elution with DCM (750ml), DCM:Et_2O (97:3. 500ml), DCM:Et_2O (93:7, 100ml) gave product containing fractions which were pooled and concentrated to slightly yellow oil. Radiochemical yield: 174.3mCi (910.2mg, 3.94mMol), 67.4%. Radiochemical purity: 94.8% by HPLC (System E). 97.5% by TLC (silica, DCM:Et_2O (5:1)).

5-(N-Troc)-amino-5-methyl[1-^{14}C]hex-2-enoic acid (8b*)[16]

A solution of 5-(N-methoxycarbonyl)-amino-5-methyl[1-^{14}C]-hex-2-enoic acid ethylester (876.8mg, 3.80mMol, 167mCi) in 2-PrOH (3.5ml) was added drop wise over 15 minutes to 4.5 M NaOH (6.75ml), stirred at room temperature. The reaction mixture was heated at reflux under N_2 for 25 hours. HPLC analysis (System G) showed 83.6% of the "amino-acid" and 16.4% impurity. The reaction mixture was cooled to ambient temperature and 2-PrOH was removed. The resulting solution was cooled to 0-5°C and $NaHCO_3$ (737mg, 8.8mmol) dissolved in water (5ml) was added. The pH was adjusted to pH 9.5 with 10M HCl. Succinimidyl-2,2,2-trichloroethyl carbonate was added in six portions over 21 hours (a total of

2211.7mg, 7.61mmol). The pH of the solution was again adjusted to pH 7 using 4M HCl. The pH was re-adjusted to 9.5 with 2M NaOH and the solution washed with MTBE (50ml). The MTBE phase was extracted with water pH 10. The combined aqueous phase was adjusted to pH 2 with 4M HCl and the product extracted with MTBE (2x 40ml). The organic phase was washed with brine (20 ml) and the brine solution re-extracted with MTBE (2x 10ml). The individual MTBE solutions were dried and purified individually by HPLC (System F). The product from all HPLC runs was combined and concentrated to remove MeCN. The obtained solution was extracted from the aqueous phase with EtOAc (1x 80ml, 1x 20ml). Drying and removal of EtOAc gave colorless oil. Radiochemical yield: 107.4 mCi (696.6mg, 2.17mmol), 64.3%. Radiochemical purity: 94.6% by HPLC (System G). 96.2% by TLC (silica, ethyl acetate).

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