Electronic Supplementary Information:

One-pot Isomerization-Cross Metathesis-Reduction (ICMR) Synthesis of Lipophilic Tetrapeptides

Mouhamad Jida\(^1\)*, Cecilia Betti,\(^1\) Peter Schiller,\(^2\) Dirk Tourwé\(^1\) and Steven Ballet\(^1\)*

\(^1\)Department of Organic Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
\(^2\)Department of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, QC, H2W 1R7, Canada.

mouhamad.jida@vub.ac.be and sballet@vub.ac.be

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[Diagram showing the synthesis process]

Lipophilic opioid tetrapeptide TIPP and DIPP derivatives
1. General information

All standard amino acids were obtained from commercial sources (Sigma Aldrich and Chemp-Impex); solvents used were reagent grade purity. Thin layer chromatography (TLC) was performed on plastic sheet pre-coated with silica gel 60F_{254} (Merck, Darmstadt, Germany) using specified solvent systems. Data collection was done with Masslynx software. Analytical RP-HPLC was performed using an Agilent 1100 Series system (Waldbonn, Germany) with a SUPELCO Discovery BIO Wide Pore® (Bellefonte, PA, USA) RP C-18 column (15 cm x 2.1 mm, 3 µm). Flow rates of 1 mL/min were used and detection was done at 215 nm. The solvent system consisted of 0.1% TFA in water and 0.1% TFA in acetonitrile. The gradient consisted of a 20 min run from 3 to 97% or 30 to 70% acetonitrile at a flow rate of 0.3 mL/min. Preparative HPLC was performed on a Gilson apparatus and controlled with the software package Unipoint. The reversed phase C18-column (SUPELCO Discovery BIO Wide Pore 25 cm x 21.2 mm, 10 µm) was used under the same conditions as the analytical RP-HPLC, but with a flow rate of 20 mL/min. A purity of more than 95% was determined for all compounds by analytical RP-HPLC using the conditions described above. HRMS data was recorded with a Micromass QTOF-micro system using electrospray (ESP) ionization (positive or negative ion mode). Mass spectra were recorded with a LCMS-MS triple-quadrupole system. Visualization of the products on TLC plates was realized using UV light (254 nm), KMnO_4 spray. Reactions were performed using a Biotage® Initiator+ SP Microwave Synthesizer. All commercial reagents and solvents were used without further purification.

Figure 1. The Biotage® initiator+ SP Wave synthesizer
2. Solution-phase peptide synthesis of (3a-h) and (4a-h)

**Peptide synthesis of (3a-h) and (4a-h) in solution: (Coupling, Boc Deprotection, Saponification, Allylation, Debenzylation and ICMR reactions)**

**Coupling:**
To amino acid (AA). TFA or HCl salt (1 equiv.) in EtOAc was added DIEA (2 equiv.), T3P (2 equiv.) and the corresponding Boc-AA-OH or Butenoic acid (1 equiv) was dissolved in EtOAc. The mixture was stirred for 2 h, and then extracted with water, citric acid and brine. The organic layer was dried over MgSO₄, and after filtration, the residue was evaporated to afford the desired peptide as a white solid in good purity determined by RP-HPLC and LC-MS. No further purification was necessary.

**Boc Deprotection:**
Boc-protected peptide was dissolved in a mixture of TFA/CH₂Cl₂ : 50/50. The reaction was stirred for 2 h, and the mixture was evaporated. Purified TFA salts were used in the next reactions after precipitation in Et₂O and filtration (Characterized by RP-HPLC and LCMS). No further purification was necessary.

**Saponification:**
Benzyl ester (1 equiv.) was dissolved in THF/H₂O : 80/20 (0.2 M), and LiOH (1.5 equiv.) was added. The reaction was monitored by RP-HPLC, and after 2 h, it was complete. The mixture was poured into 1:1 EtOAc-H₂O (5 mL) then was acidified to pH 4 by 1N HCl, and extracted with EtOAc (3 x 10 mL). The organic layers were combined and washed with brine (10 mL). The organic phase was evaporated to afford the corresponding peptide in good yields as yellow oil (determined by RP-HPLC and LC-MS). No further purification was necessary.

**Allylation:**
A mixture of free carboxylic acid peptide derivatives (1 equiv.), K₂CO₃ (2 equiv), Bu₄NI (0.1 equiv), and alkyl bromide (1.1 equiv.) in DMF (0.05 M) was stirred at r.t. for 18 h. After the addition of a 1 M aqueous solution of KHSO₄ (25 mL), the product was extracted with ethyl acetate (3 x10 mL). The organic phase was washed with a saturated aqueous solution of NaHCO₃ (10 mL), an aqueous saturated solution of NaCl (20 mL), and then dried over Na₂SO₄.
Evaporation of the solvent afforded the corresponding ester as yellow oil with excellent purity determined by RP-HPLC and LC-MS. The peptide was purified by RP-HPLC on a Supelco Discovery BIO wide pore preparative C18 column in good overall yield and was >95% pure as determined by analytical RP-HPLC and, the structure of pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry.

**One-pot tandem Isomerization/Cross-Metathesis/Reduction/Cleavage:**
The purified tetrapeptides 3 or 4 (0.1 mmol) (Figure 2) was transferred into a sealed dry vial containing 3 mL of dry CH$_2$Cl$_2$ then, 1-hexene (0.4 mmol) and Umicore M2 catalyst (0.01 mmol) were added consecutively. The solution was heated at 50 °C in a sealed vial for 24 h. Rh(I)-catalysed homogeneous hydrogenation using Umicore M2 (0.01 mmol) in the presence of Et$_3$SiH (1 mmol) effected quantitative reduction of alkene at 50 °C in CH$_2$Cl$_2$ for 48 hours. After completion of the reaction (monitored by HPLC), the solvent was removed by evaporation under reduced pressure then the crude product was used directly in the next step without purification. Deprotection of tBu- and boc-protected terapeptide (precursors of 3a-h) was achieved in a mixture of TFA/CH$_2$Cl$_2$:50/50 for 2h. The benzyl ester (precursors of 4a-h) was removed by hydrogenation. 20% Pd/C (0.04 mmol) was added to a solution of amino-ester TFA salt (0.4 mmol) in MeOH (10 mL). The reaction was stirred under H$_2$-atmosphere at room temperature for 24 h. After filtration through Celite and concentration under reduced pressure, the desired product was obtained in good purity as a white solid. The peptides were isolated by filtration and purified by RP-HPLC on a Supelco Discovery BIO wide pore preparative C18 column in moderate overall yield and was >95% pure as determined by analytical RP-HPLC and, the structure of pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry. This reaction conducted to the formation of seven isolated peptides of (3a-h) and seven isolated peptides of (4a-h) (Figure 3).

**Benzyl-ester deprotection:**
To obtain the final products (4a-h) after the ICMR reactions, the benzyl ester was removed by hydrogenation. 20% Pd/C (0.1 equiv.) was added to a solution of amino-ester TFA salt (1 equiv.) in MeOH (0.1 M). The reaction was stirred under H$_2$-atmosphere at room temperature for 24 h. After filtration through Celite and concentration under reduced pressure, the desired product was
obtained in good purity as a white solid. The resulted peptides were purified by RP-HPLC in excellent purity as determined by analytical RP-HPLC and, the structure of pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry.

Figure 2. Peptide synthesis of tetrapeptides 3 and 4 in solution using T3P as coupling reagent
Figure 3. Synthesis of tetrapeptides (3a-h) and (4a-h) by One-pot tandem isomerization/cross-metathesis/reduction/deprotection.

Peptide Characterization. Boc-Tyr(tBu)-Tic-Phe-Phe-O-All (3). HPLC (standard gradient): $t_{ret} = 21.20$ min. ESI-HRMS [M + H$^+$]: $m/z = 831.4319$ (calculated for C$_{49}$H$_{58}$N$_4$O$_8$: 831.4327).

H-Tyr-Tic-Phe-Phe-O-propyl (3a). HPLC (standard gradient): $t_{ret} = 15.62$ min. ESI-HRMS [M + H$^+$]: $m/z = 677.3311$ (calculated for C$_{40}$H$_{44}$N$_4$O$_6$: 677.3334).

H-Tyr-Tic-Phe-Phe-O-butyl (3b). HPLC (standard gradient): $t_{ret} = 16.18$ min. ESI-HRMS [M + H$^+$]: $m/z = 691.3502$ (calculated for C$_{41}$H$_{46}$N$_4$O$_6$: 691.3940).

H-Tyr-Tic-Phe-Phe-O-pentyl (3c). HPLC (standard gradient): $t_{ret} = 16.86$ min. ESI-HRMS [M + H$^+$]: $m/z = 705.3643$ (calculated for C$_{42}$H$_{48}$N$_4$O$_6$: 705.3647).

H-Tyr-Tic-Phe-Phe-O-hexyl (3d). HPLC (standard gradient): $t_{ret} = 17.52$ min. ESI-HRMS [M + H$^+$]: $m/z = 719.3604$ (calculated for C$_{43}$H$_{50}$N$_4$O$_6$: 719.3803).

H-Tyr-Tic-Phe-Phe-O-heptyl (3e). HPLC (standard gradient): $t_{ret} = 17.78$ min. ESI-HRMS [M + H$^+$]: $m/z = 733.3970$ (calculated for C$_{44}$H$_{52}$N$_4$O$_6$: 733.3959).

H-Tyr-Tic-Phe-Phe-O-octyl (3f). HPLC (standard gradient): $t_{ret} = 18.89$ min. ESI-HRMS [M + H$^+$]: $m/z = 747.4130$ (calculated for C$_{45}$H$_{54}$N$_4$O$_6$: 747.4116).

H-Tyr-Tic-Phe-Phe-O-nononyl (3g). HPLC (standard gradient): $t_{ret} = 19.84$ min. ESI-HRMS [M + H$^+$]: $m/z = 761.4261$ (calculated for C$_{46}$H$_{56}$N$_4$O$_6$: 761.4272).

Allyl-CO-Tyr(Bn)-Tic-Phe-Phe-OBn (4). HPLC (standard gradient): $t_{ret} = 20.99$ min. ESI-HRMS [M + H$^+$]: $m/z = 883.4052$ (calculated for C$_{55}$H$_{58}$N$_4$O$_7$: 883.4066).
**Butyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4a).** HPLC (standard gradient): \( t_{\text{ret}} = 19.97 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 809.3922 \) (calculated for \( C_{49}H_{52}N_4O_7 \): 809.3909).

**Pentyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4b).** HPLC (standard gradient): \( t_{\text{ret}} = 20.45 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 845.3883 \) (calculated for \( C_{50}H_{54}N_4O_7Na \): 845.3885).

**Hexyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4c).** HPLC (standard gradient): \( t_{\text{ret}} = 21.00 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 837.4241 \) (calculated for \( C_{51}H_{56}N_4O_7 \): 837.4222).

**Heptyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4d).** HPLC (standard gradient): \( t_{\text{ret}} = 21.58 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 851.4379 \) (calculated for \( C_{52}H_{58}N_4O_7 \): 851.4378).

**Octyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4e).** HPLC (standard gradient): \( t_{\text{ret}} = 22.12 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 887.4330 \) (calculated for \( C_{53}H_{60}N_4O_7Na \): 887.4354).

**Nonyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4f).** HPLC (standard gradient): \( t_{\text{ret}} = 22.61 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 901.4492 \) (calculated for \( C_{54}H_{62}N_4O_7Na \): 901.4510).

**Decyl-CO-Tyr(Bn)-Tic-Phe-Phe-OH (4g).** HPLC (standard gradient): \( t_{\text{ret}} = 23.22 \text{ min.} \) ESI-HRMS \([M + H^+]\): \( m/z = 893.4896 \) (calculated for \( C_{55}H_{64}N_4O_7 \): 893.4848).

### 3. Solid-phase peptide synthesis of (5a-h), (6a-h) and (7a-h)

**Preparation of 6a-h (Pathway A)**

**Synthesis of (S)-O-Alkyl-N-Boc-tyrosine methyl ester:**

A mixture of Boc-(S)-tyrosine methyl ester (8.11 mmol), \( K_2CO_3 \) (16.1 mmol), \( Bu_4NI \) (0.81 mmol), and alkyl bromide (9.73 mmol) in DMF (25 mL) was stirred at r.t. for 18 h. After the addition of a 1 M aqueous solution of KHSO\(_4\) (25 mL), the product was extracted with ethyl acetate (3 x10 mL). The organic phase was washed with a saturated aqueous solution of NaHCO\(_3\) (10 mL), an aqueous saturated solution of NaCl (20 mL), and then dried over Na\(_2SO_4\). Evaporation of the solvent afforded amino ester as yellow oil with excellent purity determined by RP-HPLC and LC-MS (Figure 4).
Metathesis step: Synthesis of (S)-O-Alkenyl-N-Boc-tyrosine methyl ester:

To a solution of (S)-O-Allyl-N-Boc-tyrosine methyl ester (3 mmol) in dry CH$_2$Cl$_2$ (0.2 M) was added 1-hexene (12 mmol) and Grubbs' second generation catalyst (0.3 mmol). The solution was refluxed under a flow of inert gas for 24 h. After completion of the reaction (monitored by TLC), the solvent was removed by evaporation under reduced pressure then the crude product was purified by flash chromatography on silica (hexane/ethyl acetate, 8:2) to give the desired product in good purity (determined by RP-HPLC and LC-MS) as brown oil (Figure 5).

Reduction of C=C double bonds (Alkene):

20% Pd/C (0.2 mmol) was added to a solution of amino-ester (2 mmol) in MeOH (10 mL). The reaction was stirred under H2-atmosphere at room temperature for 24 h. After filtration through Celite and concentration under reduced pressure, the desired product was obtained in good purity (determined by RP-HPLC and LC-MS) as a brown oil (Figure 6).
**Saponification:**
Methyl ester (1.78 mmol) was dissolved in THF/H₂O 80:20 (50 mL), and LiOH (2.67 mmol) was added. The reaction was monitored by RP-HPLC, and after 2.5 h, it was complete. The mixture was poured into 1:1 EtOAc-H₂O (50 mL) then was acidified to pH 4 with 1N HCl, and extracted with EtOAc (3 x 20 mL). The organic layers were combined and washed with brine (30 mL). The organic phase was dried over MgSO₄, and after filtration, the mixture was evaporated to afford the corresponding amino-acid in good yields as yellow or off white oil (determined by RP-HPLC and LC-MS) (Figure 7).
Solid-Phase synthesis of tetrapeptides (6a-h) via the standard Nα-Fmoc methodology on Phe-Wang resin (Pathway A):

The peptides (6a-h) were synthesized manually by Nα-Fmoc methodology on Phe-Wang resin (0.1 mmol scale) using O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU)/N-methylmorpholine (NMM) as the coupling reagents/mixtures. A 3-fold excess of the building blocks [Fmoc-Phe-OH, Fmoc-Tic-OH, and modified Boc-Tyr(O-Alkyl)-OH] and activating agents was applied in the presence of 3 mL of NMM (0.4 M in DMF). Fmoc-AA-OH and NMM in DMF were added to the swollen solid support, and the reaction mixture was shaken for 3 hours. The resin was washed three times with DMF, three times with i-PrOH and three times with CH₂Cl₂. Completion of the coupling was tested by means of the Kaiser or chloranil test. In case of a positive color test, the coupling was repeated until a negative test was obtained. The next amino-acid was consecutively coupled using the procedure described above. Fmoc deprotections were carried out by treating the resin twice (5 and 30 min) with 20% 4-methyl piperidine in DMF. Final cleavage of the peptide from the resin as well as the Boc side chain protection group removal was accomplished by treatment with TFA/Et₃SiH/water 60:35:5 for 2
hours. The peptide was isolated by filtration and purified by RP-HPLC on a Supelco Discovery BIO wide pore preparative C18 column in good overall yield and was >95% pure as determined by analytical RP-HPLC and, the structure of pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry (Figure 8).

Figure 8. Solid-Phase synthesis of tetrapeptides (6a-h) via the standard Nα-Fmoc methodology.

Peptide Characterization. H-Tyr(ethyl)-Tic-Phe-Phe-OH (6a’). HPLC (standard gradient): t_{ret} = 8.43 min. TLC R_f (EBAW), 0.73. ESI-HRMS [M + H^+]: m/z = 654.4064 (calculated for C_{33}H_{50}N_9O_5: 654.4086).

H-Tyr(propyl)-Tic-Phe-Phe-OH (6a). HPLC (standard gradient): t_{ret} = 15.41 min. TLC R_f (EBAW), 0.77. ESI-HRMS [M + H^+]: m/z = 677.3331 (calculated for C_{40}H_{43}N_4O_6: 677.3334).

H-Tyr(butyl)-Tic-Phe-Phe-OH (6b). HPLC (standard gradient): t_{ret} = 16.05 min. TLC R_f (EBAW), 0.78. ESI-HRMS [M + H^+]: m/z = 691.3466 (calculated for C_{41}H_{45}N_4O_6: 691.3940).

H-Tyr(pentyl)-Tic-Phe-Phe-OH (6c). HPLC (standard gradient): t_{ret} = 16.70 min. TLC R_f (EBAW), 0.79. ESI-HRMS [M + H^+]: m/z = 705.3622 (calculated for C_{42}H_{47}N_4O_6: 705.3647).

H-Tyr(hexyl)-Tic-Phe-Phe-OH (6d). HPLC (standard gradient): t_{ret} = 17.45 min. TLC R_f (EBAW), 0.79. ESI-HRMS [M + H^+]: m/z = 719.3822 (calculated for C_{43}H_{49}N_4O_6: 719.3803).
HTTyr(hexyl)-Tic-Phe-Phe-OH (6e). HPLC (standard gradient): \( t_{ret} = 18.21 \) min. TLC \( R_f \) (EBAW), 0.80. ESI-HRMS [M + H⁺]: \( m/z = 733.3943 \) (calculated for \( C_{44}H_{51}N_4O_6 + \)).

HTTyr(octyl)-Tic-Phe-Phe-OH (6f). HPLC (standard gradient): \( t_{ret} = 18.99 \) min. TLC \( R_f \) (EBAW), 0.80. ESI-HRMS [M + H⁺]: \( m/z = 747.4127 \) (calculated for \( C_{45}H_{53}N_4O_6 + \)).

HTTyr(nonyl)-Tic-Phe-Phe-OH (6g). HPLC (60% to 100% of CH₃CN as gradient): \( t_{ret} = 9.84 \) min. TLC \( R_f \) (EBAW), 0.81. ESI-HRMS [M + H⁺]: \( m/z = 761.4295 \) (calculated for \( C_{46}H_{55}N_4O_6 + \)).

HTTyr(decyl)-Tic-Phe-Phe-OH (6h). HPLC (60% to 100% of CH₃CN as gradient): \( t_{ret} = 10.88 \) min. TLC \( R_f \) (EBAW), 0.80. ESI-HRMS [M + H⁺]: \( m/z = 775.4421 \) (calculated for \( C_{47}H_{57}N_4O_6 + \)).

HTTyr(undecyl)-Tic-Phe-Phe-OH (6h'). HPLC (60% to 100% of CH₃CN as gradient): \( t_{ret} = 12.20 \) min. TLC \( R_f \) (EBAW), 0.82. ESI-HRMS [M + H⁺]: \( m/z = 789.4586 \) (calculated for \( C_{48}H_{59}N_4O_6 + \)).

Microwave-assisted solid-phase synthesis of tetrapeptides (5a-h) and (6a-h) via the tandem isomerization/cross-metathesis/reduction reactions (Pathway B) (See Table 3 and 4).

The linear tetrapeptides (5a-h) and (6a-h) were synthesized manually by Nα-Fmoc methodology on Phe-Wang resin (0.1 mmol scale) using \( O\)-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU)/ \( N\),\( N\)-diisopropylethylamine (DIEA) as the coupling reagents/mixtures. Fmoc-Phe-Wang resin was swollen in DMF (3 mL) for 30 min. In a separate 5 mL glass vial, a solution of Fmoc-protected amino acid (Fmoc-Xaa-OH, 3 equiv.), TBTU (3 equiv.) and DIEA (6 equiv.) in DMF (3 mL) was stirred for 10 min and added to the swollen resin. Under microwave irradiation (Biotage SP Wave, 75 °C, power was set at 400 W), the mixture was shaken for 5 min with Fmoc-Phe-OH, 5 min with Fmoc-Tic-OH, and 5 min with Boc-Tyr(tBu)-OH or Boc-Tyr(All)-OH. The next amino-acid was consecutively coupled using the procedure described above. After each coupling step, the resin was washed with DMF and the Fmoc protecting group was removed with 20% 4-methyl-piperidine in DMF for 20 min at room temperature without irradiation. Next, the resin was filtered and respectively washed by vortexing for 1 min with DMF (3 x 3 mL), MeOH (3 x 3 mL) and CH₂Cl₂ (3 x 3 mL) to afford the tetrapeptides 5 and 6 linked to Wang resin. For structure confirmation, a microcleavage of the peptide from the resin by treatment with TFA/CH₂Cl₂/TES/H₂O 60:35:2.5:2.5 for 2 hours, followed by LCMS analysis, gave the desired peptides 5 and 6 with excellent purity. After completion of the coupling, the
resin was transferred into a sealed dry vial containing 3 mL of dry CH₂Cl₂ then, 1-hexene (0.4 mmol) and Umicore M2 catalyst (0.01 mmol) were added consecutively. The solution was heated at 50 °C for 24 h. Rh(I)-catalysed homogeneous hydrogenation using Umicore M2 (0.01 mmol) in the presence of Et₃SiH (1 mmol) effected quantitative reduction of the resin-attached alkene at 50°C in CH₂Cl₂ for 48 hours. The resin was washed three times with DMF, three times with i-PrOH and three times with CH₂Cl₂. Final cleavage of the peptide from the resin as well as the Boc side chain protection group removal was accomplished by treatment with TFA/DCM/Et₃SiH/H₂O 60:35:2.5/2.5 for 2 hours. The peptides were isolated by filtration and purified by RP-HPLC on a Supelco Discovery BIO wide pore preparative C18 column in good overall yield and was >95% pure as determined by analytical RP-HPLC and, the structure of final pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry.

**Peptide Characterization.** *Propyl-CO-Tyr-Tic-Phe-Phe-OH (5a).* HPLC (standard gradient): t<sub>ret</sub> = 16.10 min. ESI-HRMS [M + H⁺]: m/z = 705.3298 (calculated for C₄₁H₄₄H⁺N₄O₇: 705.3282).

*Butyl-CO-Tyr-Tic-Phe-Phe-OH (5b).* HPLC (standard gradient): t<sub>ret</sub> = 17.09 min. ESI-HRMS [M + H⁺]: m/z = 719.3406 (calculated for C₄₂H₄₆H⁺N₄O₇: 719.3439).

*Pentyl-CO-Tyr-Tic-Phe-Phe-OH (5c).* HPLC (standard gradient): t<sub>ret</sub> = 17.39 min. ESI-HRMS [M + H⁺]: m/z = 733.3608 (calculated for C₄₃H₄₈H⁺N₄O₇: 733.3596).

*Hexyl-CO-Tyr-Tic-Phe-Phe-OH (5d).* HPLC (standard gradient): t<sub>ret</sub> = 18.08 min. ESI-HRMS [M + H⁺]: m/z = 747.3763 (calculated for C₄₄H₵₀H⁺N₄O₇: 747.3752).

*Heptyl-CO-Tyr-Tic-Phe-Phe-OH (5e).* HPLC (standard gradient): t<sub>ret</sub> = 19.10 min. ESI-HRMS [M + H⁺]: m/z = 761.3925 (calculated for C₄₅H₵₂H⁺N₄O₇: 761.3909).

*Octyl-CO-Tyr-Tic-Phe-Phe-OH (5f).* HPLC (standard gradient): t<sub>ret</sub> = 19.87 min. ESI-HRMS [M + H⁺]: m/z = 775.4070 (calculated for C₄₆H₵₄H⁺N₄O₇: 775.4066).

*Nonyl-CO-Tyr-Tic-Phe-Phe-OH (5g).* HPLC (standard gradient): t<sub>ret</sub> = 20.66 min. ESI-HRMS [M + H⁺]: m/z = 789.4257 (calculated for C₄₇H₵₆H⁺N₄O₇: 789.4222).

**Solid-phase synthesis of tetrapeptides (7a-h) via the tandem isomerization/cross-metathesis/reduction reactions (ICMR) (See Table 5).**

The linear tetrapeptides were synthesized manually by N<sup>a</sup>-Fmoc methodology on Phe-Wang resin (0.1 mmol scale) using O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
tetrafluoroborate (TBTU)/N-Methylmorpholine (NMM) as the coupling reagents/mixtures. A 3-fold excess of the building blocks [Fmoc-Phe-OH, Fmoc-Tic-OH, and Boc-Dmt(O-allyl)-OH] and activating agents was applied in the presence of 3 mL of NMM (0.4 M in DMF). Fmoc-AA-OH and NMM in DMF were added to the swollen solid support, and the reaction mixture was shaken for 3 hours. The resin was washed three times with DMF, three times with i-PrOH and three times with CH₂Cl₂. Completion of the coupling was tested by means of the Kaiser or chloranil test. In case of a positive color test, the coupling was repeated until a negative test was obtained. The next amino-acid was consecutively coupled using the procedure described above. Fmoc deprotections were carried out by treating the resin twice (5 and 30 min) with 20% 4-methyl-piperidine in DMF. After completion of the coupling, the resin was transferred into a sealed dry vial containing 3 mL of dry CH₂Cl₂ then, 1-hexene (0.4 mmol) and Grubbs' second generation catalyst (0.01 mmol) were added consecutively. The solution was heated at 50 °C in a sealed vial for 24 h. Rh(I)-catalysed homogeneous hydrogenation using Grubbs catalyst (0.01 mmol) in the presence of Et₃SiH (1 mmol) effected quantitative reduction of the resin-attached alkene at 50°C in CH₂Cl₂ for 48 hours. The resin was washed three times with DMF, three times with i-PrOH and three times with CH₂Cl₂. Final cleavage of the peptide from the resin as well as the Boc side chain protection group removal was accomplished by treatment with TFA/CH₂Cl₂/Et₃SiH/H₂O 60:35:2.5/2.5 for 2 hours. The peptides were isolated by filtration and purified by RP-HPLC (30% to 100% of CH₃CN as gradient) on a Supelco Discovery BIO wide pore preparative C18 column in good overall yield and was >95% pure as determined by analytical RP-HPLC. The structure of pure compounds was confirmed by high-resolution electrospray ionization (ESP) mass spectrometry. This reaction conducted to the formation of eight peptides: H-Dmt(propyl)-Tic-Phe-Phe-OH (12%); H-Dmt(butyl)-Tic-Phe-Phe-OH (11%); H-Dmt(pentyl)-Tic-Phe-Phe-OH (9%); H-Dmt(hexyl)-Tic-Phe-Phe-OH (9%); H-Dmt(heptyl)-Tic-Phe-Phe-OH (7%); H-Dmt(octyl)-Tic-Phe-Phe-OH (4%); H-Dmt(nonyl)-Tic-Phe-Phe-OH (traces); H-Dmt(decyl)-Tic-Phe-Phe-OH (traces).

**Peptide Characterization.** *H-Dmt-Tic-Phe-Phe-OH (7a).* HPLC (standard gradient): $t_{ret} = 13.74$ min. ESI-HRMS [M + H⁺]: $m/z = 663.3166$ (calculated for C₃₉H₄₂H⁺N₄O₆: 663.3177).

*H-Dmt(propyl)-Tic-Phe-Phe-OH (7b).* HPLC (standard gradient): $t_{ret} = 15.04$ min. TLC $R_f$(EBAW), 0.79. ESI-HRMS [M + H⁺]: $m/z = 705.3657$ (calculated for C₄₂H₄₈H⁺N₄O₆: 705.3647).
$H$-Dmt\((\text{butyl})\)-Tic-Phe-Phe-OH (7c). HPLC (standard gradient): $t_{\text{ret}} = 16.43$ min. TLC $R_f$ (EBAW), 0.80. ESI-HRMS [M + H$^+$]: $m/z = 719.3795$ (calculated for C$_{43}$H$_{50}$N$_4$O$_6$: 719.3803).

$H$-Dmt\((\text{pentyl})\)-Tic-Phe-Phe-OH (7d). HPLC (standard gradient): $t_{\text{ret}} = 17.11$ min. TLC $R_f$ (EBAW), 0.81. ESI-HRMS [M + H$^+$]: $m/z = 733.3958$ (calculated for C$_{44}$H$_{52}$N$_4$O$_6$: 733.3959).

$H$-Dmt\((\text{hexyl})\)-Tic-Phe-Phe-OH (7e). HPLC (standard gradient): $t_{\text{ret}} = 17.78$ min. TLC $R_f$ (EBAW), 0.82. ESI-HRMS [M + H$^+$]: $m/z = 747.4092$ (calculated for C$_{45}$H$_{54}$N$_4$O$_6$: 747.4116).

$H$-Dmt\((\text{heptyl})\)-Tic-Phe-Phe-OH (7f). HPLC (standard gradient): $t_{\text{ret}} = 18.49$ min. TLC $R_f$ (EBAW), 0.83. ESI-HRMS [M + H$^+$]: $m/z = 761.4235$ (calculated for C$_{46}$H$_{56}$N$_4$O$_6$: 761.4272).

$H$-Dmt\((\text{octyl})\)-Tic-Phe-Phe-OH (7g). HPLC (standard gradient): $t_{\text{ret}} = 19.22$ min. TLC $R_f$ (EBAW), 0.84. ESI-HRMS [M + H$^+$]: $m/z = 775.4414$ (calculated for C$_{47}$H$_{58}$N$_4$O$_6$: 775.4429).

**Synthesis of $H$-Tyr(hepten-2-yl)-Tic-Phe-Phe-OH (via Wang resin) (See Scheme 2, pathway A).**

The peptide was synthesized manually via standard solid phase peptide synthesis using Wang resin as solid support (214 mg, 0.15 mmol, 1 eq) and N\(^\text{\wedge}\)-Fmoc or N\(^\text{\wedge}\)-Boc protected amino acids. The coupling reactions were performed with 3 eq of amino acid, 3 eq of TBTU (144 mg, 0.45 mmol) and 9 eq of DIPEA (223 µl, 1.35 mmol) in DMF, for 1.5 h. Fmoc deprotections were realized by means of 20% 4-Methyl-piperidine in DMF (5 + 15 min). Boc-Tyr(Ohepten-2-yl)-OH was used as last amino acids in sequence in order to have the fully deprotected peptide after the cleavage from the resin. The cleavage of the protected peptide from the resin was achieved using a mixture TFA/TES/H$_2$O 95:2.5:2.5 v/v for 3h. After evaporation of the cleavage mixture and precipitation in cold Et$_2$O a mixture of the crude desired peptide (40%) and $H$-Tyr-Tic-Phe-Phe-OH (60%) was obtained.

Due to the removal of the Tyrosine alkyl chain during the acidic cleavage the peptide was prepared using the 2-chlorotrityl resin as solid support, as milder condition are required for the peptide cleavage.

**Synthesis of Boc-Tyr(hepten-2-yl)-Tic-Phe-Phe-OH (via 2-chlorotrityl resin) (See Scheme 2, Pathway B).**

The peptide was synthesized manually via standard solid phase peptide synthesis using 2-chlorotrityl resin as solid support (230 mg, 0.15 mmol) and N\(^\text{\wedge}\)-Fmoc or N\(^\text{\wedge}\)-Boc protected amino acids. The first amino acid was anchored on the resin by a substitution reaction performed with 2 eq of Fmoc-Phe-OH (116 mg, 0.3 mmol) and 4 eq of DIPEA (100 µL, 06 mmol) in DCM and
subsequently the unreacted chlorotrityl groups were endcapped with methanol. The coupling reactions were performed with 3 eq of amino acid, 3 eq of TBTU (144 mg, 0.45 mmol) and 9 eq of DIPEA (223 µL, 1.35 mmol) in DMF, for 1.5 h. Fmoc deprotections were realized by means of 20% 4-Methyl-piperidine in DMF (5 + 15 min). The cleavage of the protected peptide from the resin was achieved using a mixture of 1% TFA in DCM for 1h. After evaporation of the cleavage mixture the crude peptide was obtained.

**Hydrogenation of double bond of Boc-Tyr(hepten-2-yl)-Tic-Phe-Phe-OH (See Scheme 2, Pathway B).**

The crude peptide Boc-Tyr(hepten-2-yl)-Tic-Phe-Phe-OH (70 mg, 0.084 mmol) was dissolved in 10 mL of degassed MeOH. Pd/C was added and the reaction mixture was stirred at r.t. under H₂ atmosphere (atmospheric pressure) for 3h. The suspension was filtrated over celite and the solvent was removed in vacuo, to afford 69 mg of crude as orange waxy oil.

**Boc deprotection of Boc-Tyr(heptyl)-Tic-Phe-Phe-OH (See Scheme 2, Pathway B).**

The crude peptide Boc-Tyr(heptyl)-Tic-Phe-Phe-OH (69 mg, 0.084 mmol) was dissolved in 10 mL of TFA/DCM 1:4 v/v mixture and stirred at rt for 2h. The reaction mixture was evaporated and the crude peptide was purified by semipreparative HPLC (50-100% acetonitrile in 20 min) to afford 31 mg of pure H-Tyr(heptyl)-Tic-Phe-Phe-OH. (Yield: 50%)
4. Analytical RP-HPLC spectrum of the mixture 3a-h and 5a-h

Analytical RP-HPLC of the mixture (5a-h) obtained, after cleavage from the resin, via the tandem isomerization/cross-metathesis/reduction reactions on solid-phase. The gradient consisted of a 20 min run from 30 to 70% acetonitrile at a flow rate of 0.3 mL/min.

Analytical RP-HPLC of the mixture (3a-h) obtained, via the tandem isomerization/cross-metathesis/reduction/deprotection reactions in solution. The gradient consisted of a 20 min run from 3 to 97% acetonitrile at a flow rate of 0.3 mL/min.
5. $^1$H NMR Spectra of 6e: (250 MHz, (CD$_3$)$_2$SO)