Electronic Supplementary Information

Steps towards sustainable solid phase peptide synthesis: use and recovery of N-octyl pyrrolidone.

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Table of Contents
In vitro metabolism of NOP ................................................................. S2
Solubility tests ............................................................................. S17
Swelling tests .............................................................................. S19
Coupling reactions in solution phase ........................................ S21
Deprotection kinetic tests ............................................................. S28
SPPS of Aib-Enkephalin in Green Solvents .................................. S29
SPPS of linear Octreotide in Green Solvents ............................... S37
Green solvent mixtures ................................................................. S44
Recycling of linear Octreotide SPPS waste stream ....................... S44
In vitro metabolism of NOP

Table S1. NOP metabolites identified in rat and human liver microsomes by HPLC-HRMS

| Compound ID | Formula       | RT  (min) | Ion            | Observed mass (m/z) | Mass error (ppm) | Metabolic Reaction  | % of Parent response | Found in       |
|-------------|---------------|-----------|----------------|--------------------|------------------|---------------------|----------------------|----------------|
| NOP         | C₁₂H₂₃NO     | 5.56      | [M+H]^+        | 198.1849           | -1.8             | --                  | 100                  | HLM/RLM         |
| M1          | C₁₂H₂₁NO₂     | 3.19      | [M+H]^+        | 212.1642           | -1.6             | +O – H2             | 83.5                 | HLM/RLM         |
| M2          | C₁₂H₂₁NO₂     | 2.36      | [M+H]^+        | 212.1645           | -0.2             | +O – H2             | 6.0                  | HLM/RLM         |
| M3          | C₁₂H₂₁NO₂     | 2.44      | [M+H]^+        | 212.1642           | -1.5             | +O – H2             | 3.4                  | HLM/RLM         |
| M4          | C₁₂H₂₁NO₂     | 2.51      | [M+H]^+        | 212.1641           | -2.0             | +O – H2             | 7.7                  | HLM/RLM         |
| M5          | C₁₂H₂₁NO₂     | 2.56      | [M+H]^+        | 212.1641           | -2.3             | +O – H2             | 5.0                  | HLM             |
| M6          | C₁₂H₂₃NO₂     | 3.16      | [M+Na]^+       | 236.1615           | -2.5             | +O                  | 8.1                  | HLM/RLM         |
| M7          | C₁₂H₂₃NO₂     | 3.24      | [M+Na]^+       | 236.1616           | -1.9             | +O                  | 17.1                 | HLM/RLM         |
| M8          | C₁₂H₂₃NO₂     | 3.28      | [M+H]^+        | 212.1640           | -2.7             | +O – H2             | 6.3                  | HLM/RLM         |
| M9          | C₁₂H₂₃NO₂     | 4.40      | [M+H]^+        | 214.1795           | -3.0             | +O                  | 18.3                 | HLM/RLM         |
| M10         | C₁₂H₂₃NO₂     | 4.54      | [M+H]^+        | 214.1796           | -2.5             | +O                  | 28.9                 | HLM/RLM         |
| M11         | C₁₂H₂₃NO₂     | 4.75      | [M+H]^+        | 214.1796           | -2.7             | +O                  | 6.7                  | HLM             |
**Figure S1.** HPLC-HRMS trace of a RLM incubation of N-octyl pyrrolidone (NOP) at t=3h. Parent compound NOP and metabolites are reported together with their RT. At RT = 3.18 min, the most abundant metabolite (M1, with a mass shift of +14 with respect to NOP) and two minor metabolites (M6 and M7, with a mass shift of +16 with respect to NOP) co-elute. Extracted Ion Chromatograms (XIC) of co-eluting metabolites M1, M6 and M7 are reported in Figure S2.
Figure S2. Extracted Ion Chromatogram (XIC) of co-eluting metabolites M1, M6 and M7 in RLM at t=3h
Figure S3. Extracted Ion Chromatogram (XIC) of co-eluting metabolites M1, M6 and M7 in HLM at t=3h

- M6, 3.14
- M1, 3.19
- M7, 3.24
**Figure S4. a.** Low (upper) and high (lower) energy (HDMS$^2$) mass spectra for metabolite M1 (RT = 3.18 min) in RLM (incubation time: t=3h)

**Figure S4. b.** Low (upper) and high (lower) energy (HDMS$^2$) mass spectra for metabolite M1 (RT = 3.19 min) in HLM (incubation time: t=3h)
Figure S5. a. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M2 (RT = 2.35 min) in RLM (incubation time: t=3h)

Figure S5. b. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M2 (RT = 2.36 min) in HLM (incubation time: t=3h)
Figure S6. a. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M3 (RT = 2.44 min) in RLM (incubation time: t=3h)

Figure S6. b. Low energy mass spectrum for metabolite M3 (RT = 2.44 min) in HLM (incubation time: t=3h). Due to the low intensity of parent ion no high energy fragmentation was collected
Figure S7. a. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M4 (RT = 2.51 min) in RLM (incubation time: t=3h)

Figure S7. b. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M4 (RT = 2.51 min) in HLM (incubation time: t=3h)
**Figure S8.** Low energy mass spectrum for metabolite **M5** (RT = 2.56 min) in HLM (incubation time: t=3h). Due to the low intensity of parent ion, no high energy fragmentation was collected.
Figure S9. a. Low (upper) and high (lower) energy (HDMS<sup>E</sup>) mass spectra for metabolite M6 (RT = 3.16 min) in RLM (incubation time: t=3h)

Figure S9. b. Low (upper) and high (lower) energy (HDMS<sup>E</sup>) mass spectra for metabolite M6 (RT = 3.16 min) in HLM (incubation time: t=3h). Due to the low intensity of parent ion, no high energy fragmentation was collected
Figure S10. a. Low (upper) and high (lower) energy (HDMS$_E$) mass spectra for metabolite M7 (RT = 3.24 min) in RLM (incubation time: t=3h)

Figure S10. b. Low (upper) and high (lower) energy (HDMS$_E$) mass spectra for metabolite M7 (RT = 3.24 min) in HLM (incubation time: t=3h)
Figure S11. a. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M\textbf{8} (RT = 3.28 min) in RLM (incubation time: t=3h)

Figure S11. b. Low (upper) and high (lower) energy (HDMS\textsuperscript{E}) mass spectra for metabolite M\textbf{8} (RT = 3.28 min) in HLM (incubation time: t=3h). Due to the low intensity of parent ion, no high energy fragmentation was collected
Figure S12. a. Low (upper) and high (lower) energy (HDMS$^E$) mass spectra for metabolite M9 (RT = 4.40 min) in RLM (incubation time: t=3h)

Figure S12. b. Low (upper) and high (lower) energy (HDMS$^E$) mass spectra for metabolite M9 (RT = 4.40 min) in HLM (incubation time: t=3h)
Figure S13. a. Low energy mass spectrum for metabolite M10 (RT = 4.53 min) in RLM (incubation time: t=3h). Due to the low intensity of parent ion, no high energy fragmentation was collected

Figure S13. b. Low (upper) and high (lower) energy (HDMS²) mass spectra for metabolite M10 (RT = 4.54 min) in HLM (incubation time: t=3h)
**Figure S14.** Low (upper) and high (lower) energy (HDMS²) mass spectra for metabolite M11 (RT = 4.75 min) in HLM (incubation time: t=3h). Due to the low intensity of parent ion, no high energy fragmentation was collected.

**Figure S15.** Low (upper) and high (lower) energy (HDMS²) mass spectra for metabolite parent compound NOP (RT = 5.55 min) in RLM (incubation time: t=3h)
Solubility tests

Table S2. Solubilization efficacy of Fmoc-AA(PG)-OH amino acids and coupling reagents mixtures in green solvents

| Solvent | Mixture Fmoc-AA(PG)-OH + coupling reagents (A-E) |
|---------|--------------------------------------------------|
| Gly     | +A | +B | +C | +D | +E |
| Ala     | +A | +B | +C | +D | +E |
| Aib     | +A | +B | +C | +D | +E |
| NOP     | +A | +B | +C | +D | +E |
| NCP     | +A | +B | +C | +D | +E |
| NBnP    | +A | +B | +C | +D | +E |

Solubilization monitored at 0.2 M concentration unless 0.1 M is specified.

Representative examples of solubility of Fmoc-amino acids in pyrrolidones in presence of selected coupling reagent combinations

Figure S16. Fmoc-Aib-OH (1 eq) after 5 minutes stirring in 1 mL NOP (left), NCP (center) or NBnP (right) mixed with a) DIC/Oxyma Pure® (1 eq); b) HOBT/DIC (1 eq); c) COMU/DIPEA (1 eq)

Figure S17. Fmoc-Phe-OH (1 eq) after 5 minutes stirring in 1 mL NOP (left), NCP (center) or NBnP (right) mixed with a) DIC/Oxyma Pure® (1 eq); b) HOBT/DIC (1 eq); c) COMU/DIPEA (1 eq)
Figure S18. Fmoc-Cys(Trt)-OH (1 eq) after 5 minutes stirring in 1 mL NOP (left), NCP (center) or NBnP (right) alone (a) or mixed with b) DIC/Oxyma Pure® (1 eq); c) COMU/DIPEA (1 eq). In cases a) and c), the mixture in NBnP was further diluted to 0.1M to allow complete dissolution, as reported in the main text.
## Swelling tests

### Table S3. Physical parameters of resins evaluated for swelling tests

| Resin          | Bead size (μm) | Bead size (mesh) | Loading (mmol g⁻¹) | Cross-linking (%) |
|----------------|----------------|------------------|--------------------|-------------------|
| PS-Wang        | 75-150         | 100-200          | 1.1                | 1                 |
| PS-Trt-Cl      | 37-75          | 200-400          | 1.85               | 1                 |
| PS-RinkAmide   | 500-560        | 35-37            | 0.4-0.7            | 1                 |
| TG-Wang        | 90             | 170              | 0.20               | -                 |
| TG-RinkAmide   | 100-200        | 75-150           | 0.23               | -                 |
| CM-Wang        | 150-500        | 35-100           | 0.5-1.2            | -                 |
| CM-RinkAmide   | 150-500        | 35-100           | 0.4-0.6            | -                 |

### Table S4. Calculations of standard deviations for the swelling measurements of the reported resins

#### PS-Wang resin

|            | Swell 1 | Swell 2 | Swell 3 | Swelling (mean value) | Standard deviation |
|------------|---------|---------|---------|-----------------------|--------------------|
| DMF        | 5,6     | 5,5     | 5,6     | 5,6                   | 0,05               |
| NOP        | 5,4     | 5,6     | 5,6     | 5,5                   | 0,09               |
| NCP        | 5,1     | 5       | 5,2     | 5,1                   | 0,08               |
| NBnP       | 2,3     | 2,1     | 2,4     | 2,3                   | 0,12               |
| NBP        | 4,3     | 4,4     | 4,7     | 4,5                   | 0,17               |
| NOP/DMC 80:20 | 5,1 | 5,3     | 5,2     | 5,2                   | 0,08               |
| DMC        | 3,2     | 3,4     | 3,2     | 3,3                   | 0,09               |

#### PS-Trt-Cl resin

|            | Swell 1 | Swell 2 | Swell 3 | Swelling (mean value) | Standard deviation |
|------------|---------|---------|---------|-----------------------|--------------------|
| DMF        | 3,1     | 3,3     | 3,3     | 3,2                   | 0,12               |
| NOP        | 3,4     | 3,5     | 3,2     | 3,4                   | 0,12               |
| NCP        | 3,5     | 3,6     | 3,6     | 3,6                   | 0,05               |
| NBnP       | 1,4     | 1,7     | 1,6     | 1,6                   | 0,12               |
| NBP        | 5       | 4,9     | 5       | 5,0                   | 0,05               |
| NOP/DMC 80:20 | 3,1 | 3,1     | 2,9     | 3,0                   | 0,09               |
| DMC        | 3,3     | 3,2     | 3,5     | 3,3                   | 0,12               |

#### PS-Rink Amide resin

|            | Swell 1 | Swell 2 | Swell 3 | Swelling (mean value) | Standard deviation |
|------------|---------|---------|---------|-----------------------|--------------------|
| DMF        | 3,3     | 3       | 3,4     | 3,2                   | 0,17               |
| NOP        | 1,4     | 1,7     | 1,6     | 1,6                   | 0,12               |
| NCP        | 1,5     | 1,5     | 1,6     | 1,5                   | 0,05               |
| NBnP       | 1,5     | 1,7     | 1,6     | 1,6                   | 0,08               |
| NBP        | 2,1     | 2       | 2       | 2,0                   | 0,05               |
|                 | Swell 1 | Swell 2 | Swell 3 | Swelling (mean value) | Standard deviation |
|----------------|---------|---------|---------|-----------------------|--------------------|
| **TG-Wang resin** |         |         |         |                       |                    |
| DMF            | 6,2     | 5,8     | 6,1     | 6,0                   | 0,17               |
| NOP            | 1,7     | 1,6     | 1,8     | 1,7                   | 0,08               |
| NCP            | 2,3     | 2,6     | 2,5     | 2,5                   | 0,12               |
| NBnP           | 1,1     | 1,4     | 1,4     | 1,3                   | 0,14               |
| **TG-Rink Amide** |         |         |         |                       |                    |
| DMF            | 6,3     | 6       | 6,4     | 6,2                   | 0,17               |
| NOP            | 4,2     | 4,5     | 4,1     | 4,3                   | 0,17               |
| NCP            | 3,3     | 3,4     | 3,4     | 3,4                   | 0,05               |
| NBnP           | 4,2     | 3,9     | 3,9     | 4,0                   | 0,14               |
| **CM-Wang resin** |         |         |         |                       |                    |
| DMF            | 4,3     | 4,5     | 4,5     | 4,4                   | 0,09               |
| NOP            | 1,4     | 1,2     | 1,5     | 1,4                   | 0,12               |
| NCP            | 1,5     | 1,7     | 1,7     | 1,6                   | 0,09               |
| NBnP           | 1,6     | 1,4     | 1,5     | 1,5                   | 0,08               |
| **CM-Rink Amide** |         |         |         |                       |                    |
| DMF            | 8,0     | 7,7     | 7,8     | 7,8                   | 0,12               |
| NOP            | 3,5     | 3,3     | 3,5     | 3,4                   | 0,09               |
| NCP            | 6,0     | 6,1     | 6,1     | 6,1                   | 0,05               |
| NBnP           | 6,7     | 6,9     | 6,6     | 6,7                   | 0,12               |
Coupling reactions in solution phase
Chromatograms referred to selected entries of Table 6 (main text) are reported below. Peaks of target Z-Phg-Pro-NH$_2$, Z-D-Phg-Pro-NH$_2$ and starting Z-Phg-OH (if still present) are considered in the spectra.

Figure S19. Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in DMF with COMU/DIPEA

| Product        | Rt (min) | Area (%) |
|----------------|---------|----------|
| Z-Phg-Pro-NH$_2$ | 18.208  | 99.0     |
| Z-D-Phg-Pro-NH$_2$ | 18.965  | 0.9      |
| Z-Phg-OH       | 23.988  | 0.1      |
**Figure S20.** Chromatogram of Z-Phg-Pro-NH₂, liquid phase synthesis in DMF with HOBt/DIC

![Chromatogram of Z-Phg-Pro-NH₂](image)

| Product          | Rt (min) | Area (%) |
|------------------|----------|----------|
| Z-Phg-Pro-NH₂    | 18.213   | 73.9     |
| Z-D-Phg-Pro-NH₂  | 18.973   | 11.3     |
| Z-Phg-OH         | 24.039   | 14.8     |

**Figure S21.** Chromatogram of Z-D-Phg-Pro-NH₂, liquid phase synthesis in DMF with DIC/OxymaPure®

![Chromatogram of Z-D-Phg-Pro-NH₂](image)

| Product          | Rt (min) | Area (%) |
|------------------|----------|----------|
| Z-Phg-Pro-NH₂    | 18.182   | 1.2      |
| Z-D-Phg-Pro-NH₂  | 18.937   | 76.5     |
| Z-Phg-OH         | 24.454   | 22.2     |
Figure S22. Chromatogram of Z-Phg-Pro-NH₂, liquid phase synthesis in NOP with DIC/OxymaPure®

| Product          | Rt (min) | Area (%) |
|------------------|----------|----------|
| Z-Phg-Pro-NH₂    | 18.203   | 93.7     |
| Z-D-Phg-Pro-NH₂  | 18.974   | 0.9      |
| Z-Phg-OH         | 24.321   | 5.3      |

Figure S23. Chromatogram of Z-Phg-Pro-NH₂, liquid phase synthesis in NOP with COMU/DIPEA

| Product          | Rt (min) | Area (%) |
|------------------|----------|----------|
| Z-Phg-Pro-NH₂    | 18.208   | 99.0     |
| Z-D-Phg-Pro-NH₂  | 18.965   | 0.9      |
| Z-Phg-OH         | 23.988   | 0.1      |
**Figure S24.** Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NOP with PyBOP/HOBt/DIPEA

![Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NOP with PyBOP/HOBt/DIPEA](image1)

| Product       | Rt (min) | Area (%) |
|---------------|----------|----------|
| Z-Phg-Pro-NH$_2$ | 18.363   | 69.7     |
| Z-D-Phg-Pro-NH$_2$ | 19.146   | 21.2     |
| Z-Phg-OH      | 24.539   | 9.1      |

**Figure S25.** Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NCP with DIC/OxymaPure®

![Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NCP with DIC/OxymaPure®](image2)

| Product       | Rt (min) | Area (%) |
|---------------|----------|----------|
| Z-Phg-Pro-NH$_2$ | 18.211   | 95.1     |
| Z-D-Phg-Pro-NH$_2$ | 18.980   | 1.1      |
| Z-Phg-OH      | 24.386   | 3.8      |
**Figure S26.** Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NCP with PyBOP/HOBt/DIPEA

| Product         | Rt (min) | Area (%) |
|-----------------|----------|----------|
| Z-Phg-Pro-NH$_2$| 18.366   | 60.9     |
| Z-D-Phg-Pro-NH$_2$| 19.150   | 19.0     |
| Z-Phg-OH        | 24.539   | 20.1     |

**Figure S27.** Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NCP with PyOxyma/DIPEA

| Product         | Rt (min) | Area (%) |
|-----------------|----------|----------|
| Z-Phg-Pro-NH$_2$| 18.351   | 81.1     |
| Z-D-Phg-Pro-NH$_2$| 19.137   | 1.9      |
| Z-Phg-OH        | 23.865   | 16.9     |
Figure S28. Chromatogram of Z-Phg-Pro-NH₂, liquid phase synthesis in NBnP with PyBOP/HOBt/DIPEA

| Product        | Rt (min) | Area (%) |
|----------------|----------|----------|
| Z-Phg-Pro-NH₂  | 18.638   | 67.8     |
| Z-D-Phg-Pro-NH₂| 19.500   | 32.2     |

Figure S29. Chromatogram of Z-Phg-Pro-NH₂, liquid phase synthesis in NBnP with PyOxyma/DIPEA

| Product        | Rt (min) | Area (%) |
|----------------|----------|----------|
| Z-Phg-Pro-NH₂  | 18.635   | 99.3     |
| Z-D-Phg-Pro-NH₂| 19.495   | 0.7      |
**Figure S30.** Chromatogram of Z-Phg-Pro-NH$_2$, liquid phase synthesis in NBnP with HOBr/DIC

![Chromatogram](image)

| Product              | Rt (min) | Area (%) |
|----------------------|----------|----------|
| Z-Phg-Pro-NH$_2$     | 18.622   | 89.9     |
| Z-D-Phg-Pro-NH$_2$   | 19.480   | 10.1     |
Deprotection kinetic tests
Deprotection kinetic tests in all investigated pyrrolidones revealed complete Fmoc removal in 2 minutes. A selected example in NOP is reported below.

**Figure S31.** Chromatogram of Fmoc-Phe-OH deprotection in NOP at t=0 (before base addition)

Legend: Piperidine = peak at 3.257 min; Fmoc-Phe-OH = peak at 15.273 min; NOP = peak at 16.536 min

**Figure S32.** Chromatogram of Fmoc-Phe-OH deprotection in NOP at t=2 minutes

Legend: Piperidine = peak at 3.250 min; H-Phe-OH = peak at 6.558 min; DBF-piperidine adduct = peak at 11.029 min; NOP = peak at 16.476 min; dibenzofulvene (DBF) = peak at 17.704 min
SPPS of Aib-Enkephalin in Green Solvents

**Figure S33.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NOP on PS-Wang resin

| Peptide    | Rt (min) | RRT | Area (%) |
|------------|----------|-----|----------|
| Des-Phe    | 15.680   | 0.74| 1.5      |
| Aib-Enkephalin | 21.233 | 1.00| 97.7     |
| Des-Aib    | 22.180   | 1.04| 0.8      |

**Figure S34.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NOP on PS-Trt-Cl resin

| Peptide            | Rt (min) | RRT | Area (%) |
|--------------------|----------|-----|----------|
| Aib-Enkephalin     | 20.604   | 1.00| 97.4     |
| Des-Aib            | 21.693   | 1.04| 1.0      |
| Des-Aib+tBu+TFA    | 22.252   | 1.08| 1.6      |
Figure S35. Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NCP on PS-Wang resin

| Peptide          | Rt (min) | RRT | Area (%) |
|------------------|----------|-----|----------|
| Des-Aib-Tyr      | 17.219   | 0.80| 1.0      |
| Aib-Enkephalin   | 21.550   | 1.00| 80.9     |
| Des-Aib          | 22.337   | 1.04| 10.5     |
| Aib-Enkephalin+TFA| 26.366   | 1.23| 7.6      |

Figure S36. Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NCP on PS-Trt-Cl resin
| Peptide          | Rt (min) | RRT | Area (%) |
|------------------|----------|-----|----------|
| Des-Phe-Aib      | 13.998   | 0.68| 4.8      |
| Des-Phe          | 15.663   | 0.76| 1.1      |
| Aib-Enkephalin   | 20.587   | 1.00| 88.9     |
| Des-Aib          | 21.649   | 1.04| 5.2      |

**Figure S37.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NBP on PS-Wang resin

| Peptide                      | Rt (min) | RRT | Area (%) |
|------------------------------|----------|-----|----------|
| Aib-Enkephalin               | 20.837   | 1.00| 89.7     |
| Des-Aib                      | 21.743   | 1.04| 6.3      |
| Des-Aib+Bu+TFA               | 22.458   | 1.08| 2.6      |
| Aib-Enkephalin+TFA           | 25.791   | 1.23| 1.4      |
**Figure S38.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in NBP on PS-Trt-Cl resin

| Peptide              | Rt (min) | RRT | Area (%) |
|----------------------|----------|-----|----------|
| Aib-Enkephalin       | 20.959   | 1.00| 91.6     |
| Des-Aib              | 21.834   | 1.04| 6.4      |
| Des-Aib+tBu+TFA      | 22.236   | 1.06| 2.0      |

**Figure S39.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in DMF on PS-Wang resin

| Peptide              | Rt (min) | RRT | Area (%) |
|----------------------|----------|-----|----------|
| Aib-Enkephalin       | 20.670   | 1.00| 84.0     |
| Des-Aib              | 21.535   | 1.04| 14.2     |
| Aib-Enkephalin+TFA   | 25.756   | 1.23| 1.8      |
**Figure S40.** Chromatogram of Aib-Enkephalin pentapeptide, manual SPPS in DMF on PS-Trt-Cl resin

| Peptide       | Rt (min) | RRT | Area (%) |
|---------------|----------|-----|----------|
| Aib-Enkephalin| 20.735   | 1.00| 88.1     |
| Des-Aib       | 21.567   | 1.04| 11.9     |

**Figure S41.** Mass spectrum of H₂N-Tyr-Aib-Aib-Phe-Leu-COOH (Aib-Enkephalin)
Figure S42. Mass spectrum of $\text{H}_2\text{N-Tyr-Aib-Aib-Phe-Leu-COOH} + \text{TFA}$ (Aib-Enkephalin+TFA)

Figure S43. Mass spectrum of $\text{H}_2\text{N-Tyr-Aib-Phe-Leu-COOH}$ (des-Aib)
Figure S44. Mass spectrum of H$_2$N-Tyr-Aib-Phe-Leu-COOH+tBu+TFA (des-Aib+tBu+TFA)

Figure S45. Mass spectrum of H$_2$N-Tyr-Aib-Aib-Leu-COOH (des-Phe)
Figure S46. Mass spectrum of H$_2$N-Aib-Phe-Leu-COOH (des-Aib-Tyr)

Figure S47. Mass spectrum of H$_2$N-Tyr-Aib-Leu-COOH (des-Phe-Aib)
SPPS of linear Octreotide in Green Solvents

Figure S48. Chromatogram of linear Octreotide, manual SPPS in DMF

| Peptide                           | Rt (min) | RRT | Area (%) |
|-----------------------------------|----------|-----|----------|
| Cyclized Octreotide N,O shift     | 16.075   | 0.83| 1.4      |
| Cyclized Octreotide               | 17.093   | 0.88| 5.8      |
| Linear Octreotide + CO$_2$        | 18.573   | 0.97| 5.1      |
| Linear Octreotide                 | 19.314   | 1.00| 76.8     |
| Linear Octreotide + tBu           | 22.140   | 1.14| 9.0      |
| Linear Octreotide + tBu$_2$       | 24.459   | 1.26| 1.9      |
**Figure S49.** Chromatogram of linear Octreotide, manual SPPS in NBP

| Peptide                     | Rt (min) | RRT | Area (%) |
|-----------------------------|----------|-----|----------|
| Cyclized Octreotide         | 17.037   | 0.88| 2.4      |
| Linear Octreotide N,O-shift 1| 17.735   | 0.92| 0.7      |
| Linear Octreotide N,O-shift 2| 18.258   | 0.95| 4.2      |
| Linear Octreotide+CO₂        | 18.896   | 0.97| 4.8      |
| Linear Octreotide           | 19.371   | 1.00| 77.7     |
| Linear Octreotide+tBu       | 21.993   | 1.14| 7.2      |
| Linear Octreotide+tBu₂      | 23.891   | 1.26| 3.0      |
Figure S50. Chromatogram of linear Octreotide, manual SPPS in NOP

![Chromatogram](image)

| Peptide                      | Rt (min) | RRT | Area (%) |
|------------------------------|----------|-----|----------|
| Cyclized Octreotide          | 16.772   | 0.88| 0.7      |
| Linear Octreotide N,O-shift 1| 17.578   | 0.92| 1.0      |
| Linear Octreotide N,O-shift 2| 18.145   | 0.95| 5.2      |
| Linear Octreotide            | 19.019   | 1.00| 77.6     |
| Linear Octreotide+tBu        | 21.827   | 1.14| 11.7     |
| Linear Octreotide+tBu2       | 23.996   | 1.26| 3.8      |
**Figure S51.** Chromatogram of linear Octreotide, manual SPPS in NOP/DMC 80:20

| Peptide                      | Rt (min) | RRT | Area (%) |
|------------------------------|----------|-----|----------|
| Linear Octreotide N,O-shift 1| 18.170   | 0.92| 1.4      |
| Linear Octreotide N,O-shift 2| 18.668   | 0.95| 2.4      |
| Linear Octreotide+CO2        | 19.232   | 0.97| 21.6     |
| Linear Octreotide+CO2        | 19.742   | 1.00| 65.1     |
| Linear Octreotide+Boc        | 21.707   | 1.10| 1.7      |
| Linear Octreotide+tBu        | 22.261   | 1.14| 5.2      |
| Linear Octreotide+tBu2       | 23.348   | 1.18| 0.8      |
| Linear Octreotide+tBu2       | 24.164   | 1.26| 1.8      |
Figure S52. Chromatogram of linear Octreotide, automated SPPS in NOP/DMC 80:20

| Peptide               | Rt (min) | RRT | Area (%) |
|-----------------------|----------|-----|----------|
| Linear Octreotide N,O-shift 2 | 18.240   | 0.95| 2.0      |
| Linear Octreotide     | 19.428   | 1.00| 86.0     |
| Linear Octreotide+tBu | 22.051   | 1.14| 10.0     |
| Linear Octreotide+tBu2| 24.046   | 1.26| 2.0      |

Figure S53. Mass spectrum of linear Octreotide
Figure S54. Mass spectrum of linear Octreotide+CO$_2$

Figure S55. Mass spectrum of linear Octreotide+tBu
Figure S56. Mass spectrum of linear Octreotide+Boc

Figure S57. Mass spectrum of cyclic Octreotide
**Green solvent mixtures**

The viscosity was determined according to the following relation:\[^1\]

\[
\text{Viscosity} = \frac{\text{shear stress}}{\text{shear rate}}
\]

**Table S5.** NOP/DMC mixtures viscosity measurements at 25°C and relative plot at different ratios

| V% NOP | V% DMC | Viscosity (mPa·s 25°C) |
|--------|--------|------------------------|
| 100    | 0      | 6,6                    |
| 98,3   | 1,7    | 6,2                    |
| 96,4   | 3,6    | 5,6                    |
| 94,5   | 5,5    | 5,3                    |
| 92,1   | 7,9    | 4,9                    |
| 89,9   | 10,1   | 4,7                    |
| 87,6   | 12,4   | 4,5                    |
| 85     | 15,0   | 4,2                    |
| 82,1   | 17,9   | 4                      |
| 78,9   | 21,1   | 3,9                    |
| 70,2   | 29,8   | 3,3                    |
| 0      | 100    | 0,59                   |

**Recycling of linear Octreotide SPPS waste stream**

Five cases of linear Octreotide are compared, in order to determine the PMI of each process: i) conventional synthesis in DMF; ii) green synthesis in NOP; iii) green synthesis in NOP/DMC 80:20; iv) green synthesis in NOP with recycling of NOP (85%) and piperidine (95%); v) green synthesis in NOP/DMC 80:20 with recycling of NOP (85%), DMC (95%) and piperidine (95%).

To notice, piperidine involved in the formation of DBF-piperidine adduct was subtracted from the total recoverable piperidine volume.

SPPS of linear Octreotide was conducted applying always the same protocol, as reported in the Experimental Section (main text), apart from the employed solvent. In all five cases the total SPPS solvent consumption is considered to be the same, according to the protocol reported in the Experimental Section (main text). When the mixture NOP/DMC 80:20 was used, the ratio between the two solvents was maintained along all the synthetic steps.

The scale of the linear Octreotide SPPS was 0.22 mmol in all cases. The amount of the crude obtained was considered to be the same in all five cases (0.204 g), based on the amount of crude linear Octreotide isolated using the SPPS in NOP.
Stream of deprotection waste (including washings) and stream of coupling waste (including resin swelling and washings) were collected and distilled separately (see Experimental Section).

**PMI calculation for linear Octreotide SPPS**

*Process Mass Intensity (PMI)*$^\text{ii}$ is defined as the ratio between the total mass of materials and the mass of the isolated product and was calculated according to the following equation:

$$PMI = \frac{\sum \text{mass of materials}}{\text{mass of isolated product}}$$

Specifically, mass of materials includes:

- Starting materials: Fmoc-AA-OH, DIC, Oxyma Pure®, resin, cleavage cocktail (TFA+scavengers)
- Solvents (DMF or NOP or NOP/DMC 80:20, DCM for pre-cleavage resin washings and Et₂O for crude peptide precipitation)
- Base (piperidine)

Mass of starting materials, base, DCM and Et₂O are unvaried for all SPPS (Table S6), independently from the used solvent, while total mass of the used solvent (DMF or NOP or NOP/DMC 80:20) slightly varies according to their different densities (Table S7).

Total mass of materials employed in cases i), ii) and iv) (without recycling) is reported in Table S8.

**Table S6. Overview of starting materials, base, DCM and Et₂O that are unvaried for all SPPS (independently from the used solvent) and their total mass**

| Material                  | MW (g/mol) | d (g/mL) | eq | mmol | Volume (mL) | Mass (g) | repetitions | Total mass (g) |
|---------------------------|------------|----------|----|------|-------------|----------|-------------|----------------|
| Fmoc-Thr(tBu)-ol-Trt-PS resin |            |          | 1  | 0,22 | 0,2         | 0,2      | 1           | 0,2            |
| **Coupling**              |            |          |    |      |             |          |             |                |
| Fmoc-Cys(Trt)-OH          | 585,71     |          | 3  | 0,66 | 0,39        | 3        | 1           | 1,16           |
| Fmoc-Thr(tBu)-OH          | 397,43     |          | 3  | 0,66 | 0,26        | 1        | 1           | 0,26           |
| Fmoc-Lys(Boc)-OH          | 468,54     |          | 3  | 0,66 | 0,31        | 1        | 1           | 0,31           |
| Fmoc-D-Trp(Boc)-OH        | 526,28     |          | 3  | 0,66 | 0,35        | 1        | 1           | 0,35           |
| Fmoc-Phe-OH               | 387,43     |          | 3  | 0,66 | 0,26        | 1        | 1           | 0,26           |
| Fmoc-D-Phe-OH             | 387,43     |          | 3  | 0,66 | 0,26        | 1        | 1           | 0,26           |
| OxymaPure                 | 142,11     |          | 3  | 0,66 | 0,09        | 8        | 8           | 0,75           |
| DIC                       | 126,2      | 0,815    | 3  | 0,66 | 0,08        | 8        | 8           | 0,67           |
| **Deprotection**          |            |          |    |      |             |          |             |                |
| Piperidine                | 85,15      | 0,862    | 0,6| 0,52 | 16          | 8        | 16          | 8,28           |
| **Cleavage and precipitation** |           |          |    |      |             |          |             |                |
| TFA                       | 114,02     | 1,489    | 5,75| 8,56 | 1           | 8,56     |             |                |
| TIPS                      | 158,36     | 0,773    | 0,125| 0,10 | 1           | 0,10     |             |                |
| H₂O                       | 18,02      | 0,997    | 0,125| 0,12 | 1           | 0,12     |             |                |
| DCM                       | 84,93      | 1,325    | 2   | 2,65 | 3           | 7,95     |             |                |
| Et₂O                      | 102,17     | 0,725    | 25  | 18,13| 1           | 18,13    |             |                |
Table S7. Overview of the solvents used for SPPS with DMF, NOP and NOP/DMC 80:20 and their total mass for each SPPS

|                | MW (g/mol) | d (g/mL) | eq | mmol | Volume (mL) | Mass (g) | repetitions | Total mass (g) |
|----------------|------------|----------|----|------|-------------|----------|-------------|----------------|
| **DMF**        |            |          |    |      |             |          |             |                |
| DMF swelling    | 73,09      | 0,944    |    |      |             |          |             | 1,89           |
| DMF couplings   | 73,09      | 0,944    |    |      |             |          |             | 18,88          |
| DMF washings after couplings | 73,09 | 0,944 |    |      |             |          |             | 29,74          |
| DMF deprotection | 73,09 | 0,944 |    |      |             |          |             | 18,12          |
| DMF washings after deprotection | 73,09 | 0,944 |    |      |             |          |             | 33,98          |
| **NOP**        |            |          |    |      |             |          |             |                |
| NOP swelling    | 197,32     | 0,92     |    |      |             |          |             | 1,84           |
| NOP couplings   | 197,32     | 0,92     |    |      |             |          |             | 18,40          |
| NOP washings after couplings | 197,32 | 0,92 |    |      |             |          |             | 28,98          |
| NOP deprotection | 197,32 | 0,92 |    |      |             |          |             | 17,66          |
| NOP washings after deprotection | 197,32 | 0,92 |    |      |             |          |             | 33,12          |
| **NOP/DMC 80:20** | | | | | | | |
| NOP swelling    | 197,32     | 0,92     |    |      |             |          |             | 1,47           |
| NOP couplings   | 197,32     | 0,92     |    |      |             |          |             | 14,72          |
| NOP washings after couplings | 197,32 | 0,92 |    |      |             |          |             | 23,18          |
| DMC swelling    | 90,08      | 1,07     |    |      |             |          |             | 0,24           |
| DMC couplings   | 90,08      | 1,07     |    |      |             |          |             | 0,2568         |
| DMC washings after couplings | 90,08 | 1,07 |    |      |             |          |             | 4,11           |
| DMC deprotection | 90,08 | 1,07 |    |      |             |          |             | 7,70           |
| DMC washings after deprotection | 90,08 | 1,07 |    |      |             |          |             |                |

Table S8. Total mass of materials employed for SPPS without recycling

|                | DMF | NOP | NOP/DMC 80:20 |
|----------------|-----|-----|--------------|
| ∑ starting materials\(^a\) (g) | 13,0 | 13,0 | 13,0 |
| ∑ solvents\(^b\) (g) | 128,7 | 126,1 | 131,4 |
| ∑ base (g) | 8,3 | 8,3 | 8,3 |
| **Total (g)** | 150,0 | 147,4 | 152,7 |

\(^a\)Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure\(^\circ\), resin, cleavage cocktail (TFA+scavengers)
\(^b\)Solvents include DCM for pre-cleavage resin washings and Et\(_2\)O for crude peptide precipitation
When solvents (NOP or NOP/DMC 80:20) and base (piperidine) were recycled (cases iii and v), final PMI was calculated by subtracting the mass of recovery materials from the mass of used materials.

\[
PMI \text{ (for SPPS with recycling)} = \frac{\sum \text{mass of materials} - \sum \text{mass of recovered materials}}{\text{mass of isolated product}}
\]

Table S9 depicts the amount of total recovered mass of NOP or NOP/DMC 80:20 and piperidine when the recycling approach was employed. PMI calculations for all SPPS (cases i-v) are reported in Table 9 in the main body text and in Table S10.

**Table S9.** Total mass of solvents (NOP or NOP/DMC 80:20) and piperidine employed for SPPS and their recovered mass

| SPPS solvent | NOP | NOP/DMC 80:20 |
|--------------|-----|---------------|
| \(\sum \) NOP (g) | 100,0 | 80,0 |
| \(\sum \) NOP recycled (g) | 85,0 | 68,0 |
| \(\sum \) DMC (g) | - | 25,3 |
| \(\sum \) DMC recycled (g) | - | 24,0 |
| \(\sum \) base (g) | 8,28 | 8,3 |
| \(\sum \) base recycled (g) | 7,72 | 7,72 |
| **Total solvents + base recycled (g)** | **92,7** | **99,7** |

**Table S10.** PMI for linear Octreotide SPPS processes

| SPPS solvents | DMF | NOP | NOP* + recycling | NOP/DMC 80:20 | NOP/DMC 80:20 + recycling |
|---------------|-----|-----|-------------------|---------------|---------------------------|
| \(\sum \) starting materials\(^a\) (g) | 13.0 | 13.0 | 13.0 | 13.0 | 13.0 |
| \(\sum \) solvents\(^b\) (g) | 128.7 | 126.1 | 41.1 | 131.4 | 39.3 |
| \(\sum \) base (g) | 8.3 | 8.3 | 0.6 | 8.3 | 0.6 |
| **PMI\(^c\)** | 735 | 722 | 268 | 748 | 256 |

\(^a\) Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure\(^c\), resin, cleavage cocktail (TFA + scavengers)

\(^b\) Solvents include DCM for pre-cleavage resin washing and Et\(_2\)O for crude peptide precipitation

\(^c\) PMI = \(\sum m\) (starting materials) + \(\sum m\) (solvents) + \(\sum m\) (base) / \(m\) (crude linear Octreotide product)

Purities of distilled NOP, DMC (from coupling stream waste), piperidine or DMC/piperidine mixture was assessed > 95% from \(^1\)H NMR (Figures S58-59). Recovered NOP, DMC and piperidine was reused as such.
Figure S58. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of NOP recovered from distillation processes

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) 3.35 (t, $J = 7.0$ Hz, 2H), 3.24 (t, $J = 7.4$ Hz, 2H), 2.36 (t, $J = 8.1$ Hz, 2H), 2.04 – 1.93 (m, 2H), 1.54 – 1.43 (m, 2H), 1.18 – 1.30 (m, 10H), 0.86 (t, $J = 6.8$ Hz, 3H).
Figure S59. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of piperidine/DMC mixture recovered from distillation process of deprotection waste stream of linear Octreotide SPPS in NOP/DMC

$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) DMC: 3.75 (s, 6H); piperidine: 2.76 (s, 4H), 1.50 (s, 6H). DMC and piperidine are in a 1:0.86 V/V ratio corresponding to 1:0.75 mol/mol ratio.
G. Schramm, A practical approach to Rheology and Rheometry 2nd Edition by Gebruder HAAKE GmbH, 2000, p.15

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