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Diimine Reduction of C=C Double Bonds: Scope and Limitations of the Application to Solid-Phase Peptide Synthesis

M. Isabel García-Aranda,[a] Rosario González-Muñiz,[a] M. Teresa García-López,[a] and M. Jesús Pérez de Vega*[a]

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Experiments have been performed to prove the applicability and efficacy of 2-nitrobenzenesulfonohydrazide in the on-resin reduction of C=C double bonds of peptide derivatives. The method is useful for the two solid-phase peptide synthesis strategies, Fmoc/*t*Bu and Boc/Bz, which is compatible with common protecting groups and, in some instances, with other functionalities of interest in the preparation of highly elaborated peptide derivatives/peptidomimetics. The utility of the method has been demonstrated by the clean reduction of a C=C-bridged 11-mer cyclic peptide derivative to its hydrocarbon C–C analogue.

Introduction

The enormous advances experienced during the last few years in solid-phase synthesis have allowed the implementation of a wide range of organic reactions in the solid phase. However, reduction and hydrogenation are among the issues still to be solved by this methodology due to the difficulty of adapting heterogeneous catalysis to solid-phase procedures.[1] There are only a few examples of the use of homogeneous catalysis. One of them is the employment of Wilkinson's catalyst under mild hydrogen pressure,[2,3] but, even if hydrogenation takes place in solid phase, it does not always work well.[4] The use of soluble non-cross-linked polystyrene has also been reported for the partial hydrogenation of an acetylene bond on 5% $Pd/BaSO_4$ as catalyst.[5] However, until now, the method that has proven to be most efficient for olefin reduction in the solid phase is the in situ generation of diimine from sulfonohydrazides. Thus, in 1998 Lacombe et al. reported the solid-phase diimine reduction of olefins by using diimine generated in situ from benzenesulfonohydrazide in DMF at 100 °C.[6] The use of a modified reagent, triisopropylbenzenesulfonohydrazide, has also been reported to reduce cross-linked peptides with olefin bridges.[7] However, this reagent, used in solution, only worked for *cis* isomers, as reported by Vederas and coworkers for some oxytocin analogues.[8] Very recently, Buszek and Brown described an improved method for the reduction of C–C multiple bonds in solid phase by using diimine generated from 2-nitrobenzenesulfonohydrazide (NBSH) and triethylamine at room temperature.[4] The procedure is operationally simple, and the reagent works efficiently under very mild conditions with different non-peptide substrates. Labile linked resins such as PS-diethylsilyl ethers and PS-dihydropyrans were stable under the reaction conditions. These authors described an extensive study; however, its applicability to peptide synthesis still remains to be explored.

The increasing interest in ring-closing metathesis (RCM) as a tool for the synthesis of constrained hydrocarbon-bridged cyclic peptides has promoted our interest in implementing a method for the on-resin reduction of the generated C=C double bond to give more flexible saturated hydrocarbon bridges.[9,10] Therefore, we decided to explore whether the reduction of C–C multiple bonds by using NBSH is compatible with common solid-phase peptide synthesis (SPPS) strategies. Accordingly, we designed a set of experiments to evaluate the compatibility of the method with (a) different amino acid residues in the molecule, (b) different amino acid side-chain and N-terminal protecting groups and (c) different commonly used solid supports.

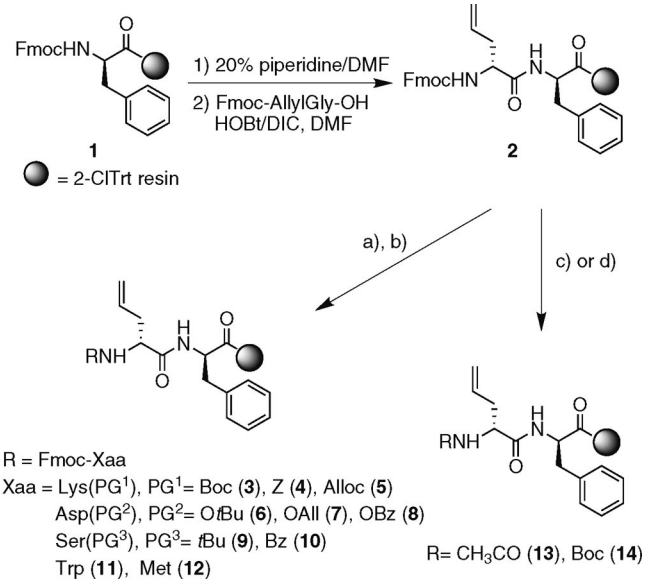
In addition, the stability under the reaction conditions of other functionalities susceptible to reduction, but of interest in the elaboration of highly modified peptide derivatives, has also been explored. Finally, the method has been applied to the reduction of an RCM-generated double-bond macrocyclic peptide to the corresponding hydrocarbon-bridged analogue.

Results and Discussion

To study points (a) and (b) tripeptide derivatives **3**–**12** containing Lys(PG^1) [PG^1 = Boc (**3**), Z (**4**) and Alloc (**5**)], Asp(PG^2) [PG^2 is O*t*Bu (**6**), OAll (**7**) and OBz (**8**)],

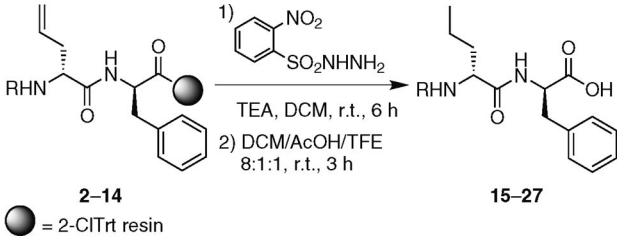
[a] Instituto de Química Médica, CSIC,
Juan de la Cierva, 3, 28006 Madrid, Spain
Fax: +34-915644853
E-mail: pdevega@iqm.csic.es

Ser(PG^3) [PG^3 = *t*Bu (**9**) and Bz (**10**)], Trp (**11**) and Met (**12**) were prepared in solid phase from Fmoc-AllylGly-Phe-resin **2** as common starting material (Scheme 1). In addition, to explore the compatibility of the reduction method with different N-terminal protecting groups, the Fmoc moiety of the dipeptide derivative **2** was replaced with Ac and Boc to give the analogues **13** and **14**, respectively. 2-Chlorotrityl resin was initially selected as solid support as the cleavage conditions allow the protecting groups of the constituent amino acids to remain unaltered in most cases.



Scheme 1.

Treatment of **2**–**14** with diimine generated from NBSH[11] under the conditions of Buszek and Brown[4] for 6 h led to the complete conversion of the AllylGly moiety into the norvaline (Nva) residue (Scheme 2). Assays trying to decrease the reaction time were unsuccessful. After cleavage from the resin and purification, the resulting reduced analogues **15**–**27** were obtained. Their yields are reported in Table 1.



Scheme 2.

As expected, simultaneous reduction of the Alloc and OAll protecting groups of the Lys and Asp side-chains in **5** and **7** also occurred to give compounds **18** and **20**. However, all the other side-chain protecting groups remained unaltered (Table 1).

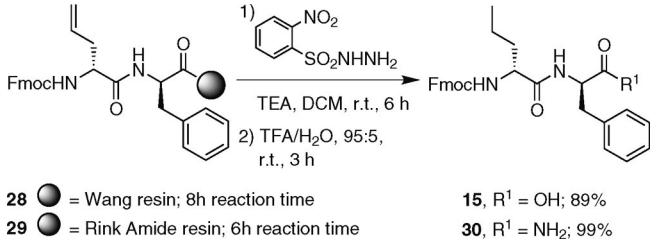
Table 1. Reduced derivatives **15**–**27** prepared by Scheme 2.

	R	Isolated yield [%]
15	Fmoc	86
16	Fmoc-Lys(Boc)	98
17	Fmoc-Lys(*Z*)	83
18	Fmoc-Lys(COOPr)	95
19	Fmoc-Asp(O*t*Bu)	91
20	Fmoc-Asp(OPr)	90
21	Fmoc-Asp(OBz)	77
22	Fmoc-Ser(*t*Bu)	78
23	Fmoc-Ser(Bz)	87
24	Fmoc-Trp	60
25	Fmoc-Met	75
26	Ac	98
27	Boc	97

Because the Fmoc/*t*Bu strategy is one of the most widely used in SPPS, special attention was paid to determining whether partial N-terminal Fmoc deprotection also occurred under the reduction conditions. Fortunately, except for the case of the Fmoc-Trp derivative **11**, in which 25% of the Fmoc-deprotected norvaline (Nva) analogue **24** was formed, Fmoc deprotection of the N-terminal residues was less than 15% or even undetectable (compound **3**), as determined by HPLC–MS of the corresponding reduced tripeptides.

Because detachment from the solid support was not observed during the reduction, the stability of Trt can be assumed when used as a protecting group of the side-chains.

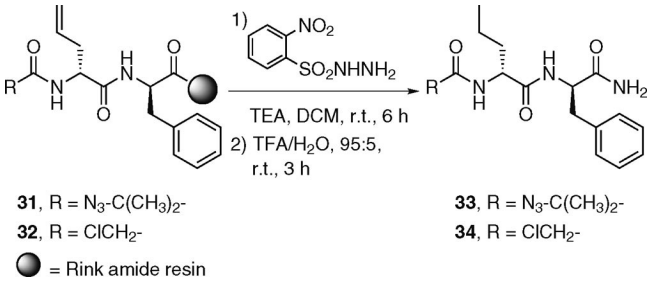
To study point (c) the reduction of Fmoc-AllylGly-Phe with diimine on Wang and Rink Amide resins (compounds **28** and **29**) was undertaken under similar conditions to those used with the 2-ClTrt support (Scheme 3). Compared with the reduction of derivative **2**, a longer reaction time (8 h) was required to complete the reduction on the Wang resin.



Scheme 3.

Bearing in mind the possibility of preparing highly modified peptide derivatives through the introduction of cyclization points for click chemistry,[12] we thought it of interest to check the stability of the C≡C triple bond and azide functionalities towards the reaction conditions and to consider the possibility of reducing double bonds in their presence. Thus, Fmoc-PropargylGly-Phe anchored to 2-ClTrt resin was subjected to the standard reduction condi-

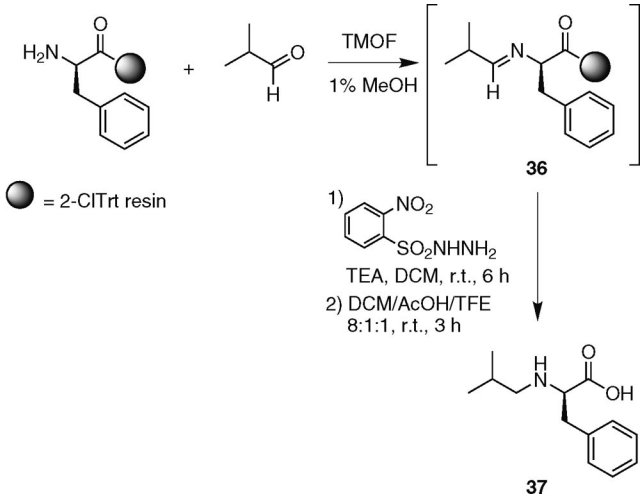
tions and was recovered unaltered in a 70% yield (by HPLC). After a double treatment with NBSH, the expected compound **15** was formed in only about 50% yield (HPLC–MS data), even though Buszek and Brown reported total reduction under these conditions for non-peptide substrates.[4] On the other hand, removal of the Fmoc group from *N*-protected derivative **29** followed by coupling with α-azidoisobutyric acid (N_3-Aib) gave the azide derivative **31**. Reduction of **31** led to 80% conversion into the norvaline derivative **33**, with the azide group unaltered, isolated in 70% yield (Scheme 4). The behaviour of both the C≡C triple bond and the azide function towards reduction by this method could make possible click chemistry after diimine reduction of the C=C double bond.



Scheme 4.

In addition, the *N*-chloroacetyl derivative **32** was prepared by *N*-Fmoc deprotection of **29** and subsequent reaction with 2-chloroacetyl chloride[13] (Scheme 4). These derivatives have special interest for us as they are key intermediates in the synthesis of 4-alkyl-4-carboxy-2-azetidinones from amino acids.[13,14] On this occasion Rink Amide resin was used as solid support to avoid early cleavage due to the sensitivity of the 2-ClTrt resin to the acidic media. Treatment of **32** with NBSH under the standard reduction conditions led to reduced **34**, with the chloromethyl function unaltered, in only 60% yield (by HPLC). The partial formation of byproducts due to the nucleophilic substitution of the chloride by the reagent (9%) and hydrogenolysis of the acetyl group (7%) was detected by HPLC–MS. These results indicate that highly reactive halogenated derivatives seem not to be fully compatible with the reaction conditions.

In an effort to further extend the scope of the reaction, the good results obtained for the reduction of C=C double bonds prompted us to try to reduce the C=N double bonds. It is known that reductive amination is of special interest in the synthesis of ψ[CH_2-NH] pseudo-peptides[15] and that it is a practical procedure to access *N*-alkyl-amino acids.[16] In our particular case, this application has the additional interest of affording key intermediates in the above-mentioned synthesis of 4-alkyl-4-carboxy-2-azetidinones from amino acids.[13,14] Thus, after removal of the Fmoc group from compound **1**, the resulting amine was treated with isobutyraldehyde to generate the corresponding imine intermediate under the conditions previously optimized in our group.[17] Then reduction under the standard diimine reaction conditions was attempted (Scheme 5). HPLC of the cleaved raw material showed a low percentage of only 9% of the desired product **37**. Although it has been reported that this procedure is less useful for inherently polar bonds (N=N, C=O), a different explanation could account for the low yield in the C=N reduction. It could be possible that the hydrazine competes with the amino acid for the aldehyde because the formation of the imine is a reversible process. Attempts to improve the conversion by changing the reaction conditions and using the three different solid supports indicated were unsuccessful, thus hampering the application of this method to reductive amination reactions.

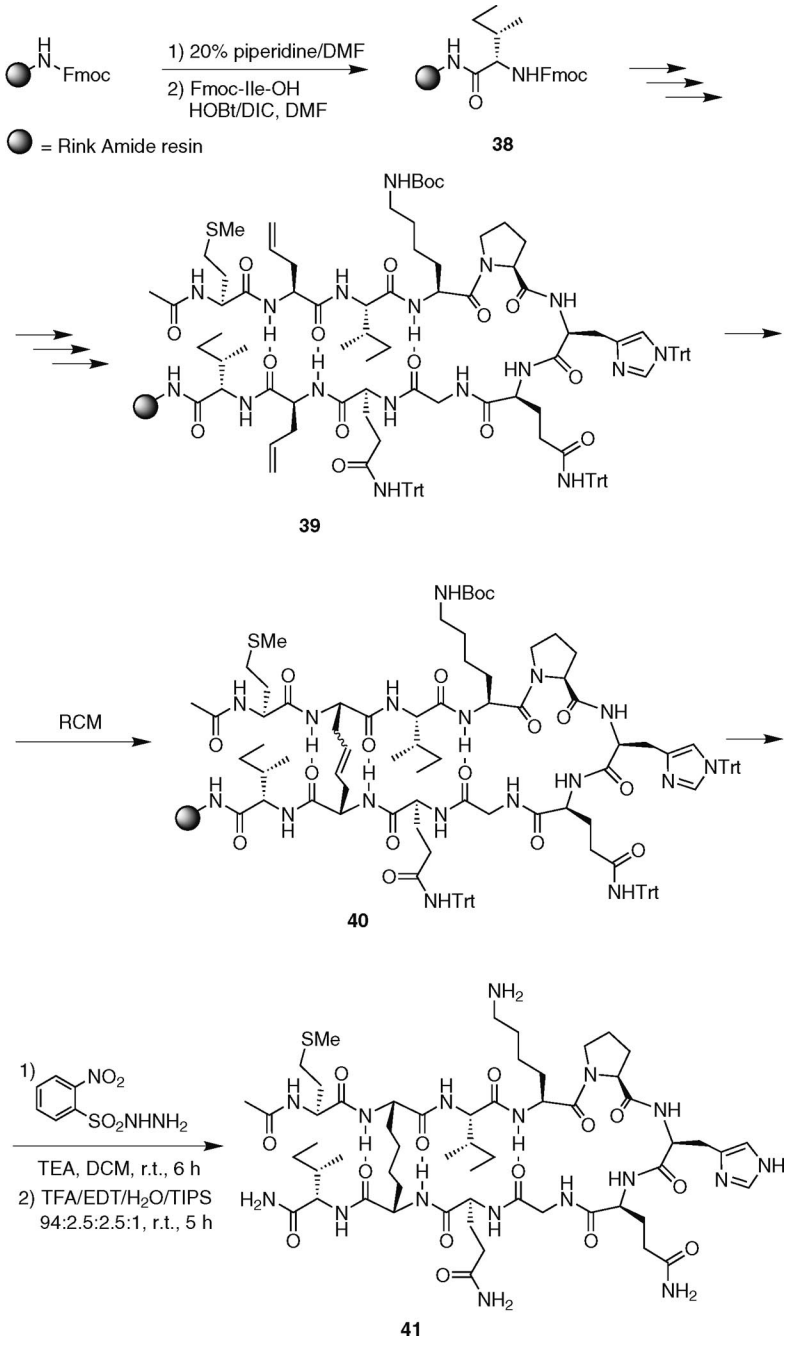


Scheme 5.

In the context of a current project directed to the synthesis of constrained analogues of the 81–91 fragment of vascular endothelial growth factor (VEGF), we prepared macrocyclic peptide **40** from the corresponding bis(AllylGly) linear derivative **39** by generation of the C=C bridge by RCM (Scheme 6).[18] We then decided to attempt the on-resin reduction of the C=C bond of this peptide. Thus, treatment of **40** with NBSH under the described standard conditions resulted in the total reduction of this double bond, as evidenced from HPLC–MS and ^{1}H NMR experiments of the crude reaction products after cleavage from the resin. Byproducts in the crude reaction mixture correspond to oxidation analogues of compound **41** at the Met residue (HPLC–MS data). HPLC purification led to compound **41** in 12% total yield after 14 steps (Figure 1).

Conclusions

We have proved the versatility and efficacy of 2-nitrobenzenesulfonohydrazide as a useful reagent for the reduction of C=C bonds in solid-supported peptide derivatives. With simple peptides, the reduction proceeds under very mild conditions, preserving the protecting groups most com-



Scheme 6.

monly used in peptide synthesis and being highly compatible with different solid supports and with some other functionalities. Only the Fmoc group seems to be slightly sensitive to the reaction conditions, especially if prolonged reaction times are required. On the basis of these results it can be concluded that NBSH is compatible with the most common strategies used in SPPS: Fmoc/*t*Bu and Boc/Bz. The successful application of this methodology to the synthesis of a complex C–C-bridged macrocyclic peptide by reduction of the corresponding C=C analogue, previously prepared by ring-closing metathesis, increases the versatility of this method.

Experimental Section

General: All reagents were of commercial quality. Amino acids and HOBt were provided by NEOMPS, DIC by Fluka and the second-generation Hoveyda–Grubbs catalyst, *o*-nitrobenzenesulfonyl chloride and hydrazine hydrate by Aldrich. The chlorotrityl and Wang resins were supplied by GL Biochem Sanghai and the Rink Amide resin by Novabiochem. Solvents were dried and purified by standard methods. Analytical RP-HPLC was performed on a reversed-phase X-Bridge column (2.1 × 100 mm, 3.5 μm) at a flow rate of 0.25 mL/min and by using a diodo array UV detector. Mixtures of CH_3CN + 0.08% formic acid (solvent A) and H_2O + 0.1% formic acid (solvent B) were used as the mobile phase. For the purification

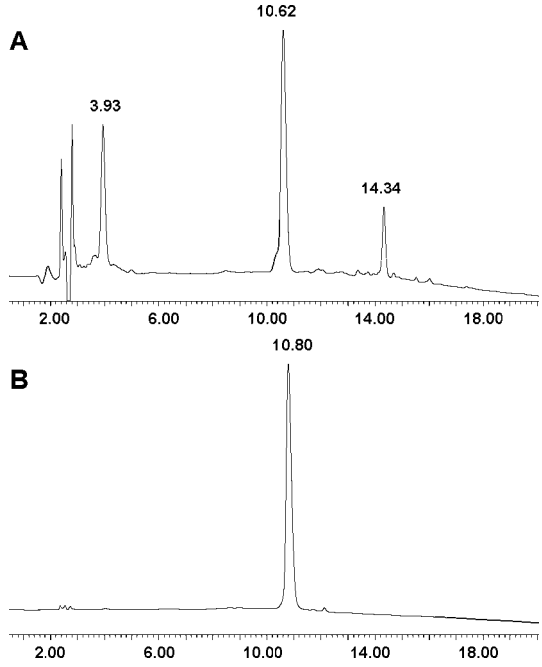


Figure 1. HPLC chromatograms of compound **41**. (A) Crude material and (B) pure compound after semi-preparative HPLC purification.

of compound **41**, a semi-preparative Waters 600 HPLC equipment was used equipped with an ACE 5 C18–300 column (10 × 250 mm). Mixtures of CH_3CN (solvent A) and H_2O + 0.05% TFA (solvent B) were used as the mobile phase. NMR spectra were recorded with a Bruker Avance 300 or a Varian Inova 400 spectrometer for experiments in DMSO (samples at 5–10 mM concentrations) and for compound **41** with a Bruker Avance 600 spectrometer, operating at a proton frequency of 600.13 MHz and equipped with a cryoprobe. To confirm the NMR peak assignments, COSY and HSQC experiments were performed. Electrospray mass spectra (ES-MS) were recorded in positive mode with a Waters HPLC–MS ZQ 2000 spectrometer. HRMS (EI+) was carried out with an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer. The resins were swollen in DCM/DMF/DCM/DMF (4 × 0.5 min each). All compounds were synthesized manually in parallel on resin according to the Fmoc/*t*Bu strategy. *o*-Nitrobenzenesulfonohydrazide was synthesized by using the method described in ref.[4]

General Coupling Procedure: The appropriate Fmoc-protected resin (100 mg, 0.034 mmol of Rink Amide, 0.033 mmol of Wang, 0.015 mmol of ClTrt), previously swollen, was treated with 20% piperidine in DMF (3 × 10 min) and washed with DMF/DCM/DMF/DCM/DMF (4 × 0.5 min each). Then a solution of the corresponding Fmoc-protected amino acid (3 equiv. for the 2-chlorotrityl resin and 1.5 equiv. for the other resins) in anhydrous DMF (1 mL), HOBt (3 equiv. for the 2-chlorotrityl resin and 1.5 equiv. for the other resins) and DIC (3 equiv. for the 2-chlorotrityl resin and 1.5 equiv. for the other resins) were added. The coupling reactions were allowed to proceed at room temperature overnight. When necessary, the coupling was repeated with a fresh portion of Fmoc-amino acid and the indicated coupling reagents. After complete coupling, the resins were drained and washed with DMF/DCM/DMF/DCM (5 × 0.5 min each). Coupling reactions to primary and secondary amines were monitored by the Kaiser ninhydrin and the chloranil test, respectively.

General Reduction Procedure: Et_3N (40 equiv.) was added to a solution of 2-nitrobenzenesulfonohydrazide (20 equiv.) in anhydrous DCM (2 mL). The mixture was immediately added to the resin under Ar. The reduction was completed after 6 h at room temp. Then the resin was drained and washed with DCM/DMF/[DMF/H_2O (1:1)]/THF/DCM (4 × 0.5 min each).

General Procedure for the Cleavage from the 2-Chlorotrityl Resin: After the reduction reaction according to the indicated procedure, the resin-bound derivative was treated with a mixture of AcOH/TFE/DCM (1:1:8) at room temp. for 3 h. After washing with DCM (5 × 1 mL), the filtrates were concentrated and lyophilized to give the corresponding derivatives **15**–**27** after purification through a normal-phase SPE cartridge [DCM/MeOH (99:1)].

General Procedure for the Cleavage from Rink Amide Resin and Wang Resin: After the reduction reaction according to the indicated procedure, the resin-bound derivative was treated with a mixture of TFA/H_2O (95:5) at room temp. for 5 h. After washing with DCM (5 × 1 mL), the filtrates were concentrated and lyophilized to give the corresponding products **30**, **33** and **34** after purification through a normal-phase SPE cartridge [DCM/MeOH (99:1)].

Fmoc-Nva-Phe-OH (15): Amorphous solid (6 mg, 86% yield). ^{1}H NMR (300 MHz,[D$_6$]DMSO): δ = 8.04 (d, J = 7.7 Hz, 1 H, NH Phe), 7.87 (d, J = 7.5 Hz, 2 H, Ar-H Fmoc), 7.70 (dd, J = 7.0 and 5.0 Hz, 2 H, Ar-H Fmoc), 7.40 (m, 3 H, 2 Ar-H Fmoc, 1 NH Nva), 7.30 (t, J = 7.4 Hz, 2 H, Ar-H Fmoc), 7.20 (m, 5 H, Ar-H Phe), 4.40 (m, 1 H, α-H Phe), 4.23 (m, 3 H, CH Fmoc, CH_2 Fmoc), 3.99 (m, 1 H, α-H Nva), 3.04 (dd, J = 13.8 and 5.0 Hz, 1 H, β-H Phe), 2.90 (dd, J = 13.8 and 8.4 Hz, 1 H, β-H Phe), 1.48 (m, 2 H, β-H Nva), 1.24 (m, 2 H, γ-H Nva), 0.84 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.8 (CO), 171.9 (CO), 155.8 (CO), 143.9 (Ar-C Fmoc), 143.7 (Ar-C Fmoc), 140.7 (Ar-C Fmoc), 137.5 (Ar-C Phe), 129.2 (Ar-CH Phe), 128.1 (Ar-CH Phe), 127.6 (Ar-CH Fmoc), 127.1 (Ar-CH Fmoc), 126.3 (Ar-CH Phe), 125.3 (Ar-CH Fmoc), 120.1 (Ar-CH Fmoc), 65.6 (CH_2 Fmoc), 54.3 (α-C Nva), 53.4 (α-C Phe), 46.7 (CH Fmoc), 36.7 (β-C Phe), 34.1 (β-C Nva), 18.6 (γ-C Nva), 13.6 (δ-C Nva) ppm. HPLC–MS: t_R = 5.85 min (gradient from 50 to 100% of solvent A in 15 min); m/z = 487.54 [M + 1]$^+$. $C_{29}H_{30}N_2O_5$ (486.56): calcd. C 71.59, H 6.21, N 5.76; found C 71.63, H 6.15, N 5.83.

Fmoc-Lys(Boc)-Nva-Phe-OH (16): Amorphous solid (11 mg, 98% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.00 (d, J = 7.2 Hz, 1 H, NH Phe), 7.87 (d, J = 7.5 Hz, 2 H, Ar-H Fmoc), 7.81 (d, J = 7.9 Hz, 1 H, NH Lys), 7.70 (dd, J = 7.1 and 4.1 Hz, 2 H, Ar-H Fmoc), 7.40 (m, 3 H, 2 Ar-H Fmoc, 1 NH Nva), 7.31 (m, 2 H, Ar-H Fmoc), 7.19 (m, 5 H, Ar-H Phe), 6.75 (t, J = 5.0 Hz, 1 H, ε-NH Lys), 4.36 (m, 1 H, α-H Phe), 4.23 (m, 4 H, 1 α-H Lys, 1 CH Fmoc, 1 CH_2 Fmoc), 3.94 (m, 1 H, α-H Nva), 3.02 (dd, J = 14.0 and 5.4 Hz, 1 H, β-H Phe), 2.87 (m, 3 H, 1 β-H Phe, 2 ε-H Lys), 1.55–1.50 (m, 4 H, 2 β-H Nva, 2 β-H Lys), 1.35–1.22 [m, 15 H, 9-$CO_2C(CH_3)_3$, 2 γ-H Nva, 2 γ-H Lys, 2 δ-H Lys], 0.79 (t, J = 7.1 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.1 (CO), 172.1 (CO), 171.7 (CO), 156.3 (CO), 155.9 (CO), 144.2 (Ar-C Fmoc), 144.1 (Ar-C Fmoc), 141.0 (Ar-C Fmoc), 137.9 (Ar-C Phe), 129.5 (Ar-CH Phe), 129.3 (Ar-CH Phe), 128.4 (Ar-CH Phe), 128.0 (Ar-CH Fmoc), 127.6 (Ar-CH Fmoc), 127.4 (Ar-CH Fmoc), 126.6 (Ar-CH Phe), 125.7 (Ar-CH Fmoc), 121.7 (Ar-CH Fmoc), 120.4 (Ar-CH Fmoc), 77.7 [-$CO_2C(CH_3)_3$], 66.0 (CH_2 Fmoc), 54.9 (α-C Nva), 53.9 (α-C Phe), 52.4 (α-C Lys), 47.0 (CH Fmoc), 40.1 (ε-C Lys), 37.0 (β-C Phe), 34.8 (β-C Nva), 32.0 (β-C Lys), 29.6 (δ-C Lys), 28.6 [-$CO_2C(CH_3)_3$], 23.3 (γ-C Lys), 18.7 (γ-C Nva), 14.1 (δ-C Nva) ppm. HPLC–MS: t_R = 7.67 min (gradient from 50 to

100% A in 15 min); *m*/*z* = 715.83 [M + 1]$^+$. $C_{40}H_{50}N_4O_8$ (714.36): calcd. C 67.21, H 7.05, N 7.84; found C 67.34, H 6.94, N 7.99.

Fmoc-Lys(Z)-Nva-Phe-OH (17): Amorphous solid (9 mg, 83% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.08 (d, *J* = 7.7 Hz, 1 H, NH Phe), 7.87 (d, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.79 (d, *J* = 8.1 Hz, 1 H, NH Lys), 7.69 (dd, *J* = 7.2 and 4.2 Hz, 2 H, Ar-H Fmoc), 7.45 (d, *J* = 8.4 Hz, 1 H, NH Nva), 7.39 (t, *J* = 7.3 Hz, 2 H, Ar-H Fmoc), 7.32–7.27 (m, 8 H, 2 Ar-H Fmoc, 5 Ar-H Z, 1 ε-NH Lys), 7.20 (m, 5 H, Ar-H Phe), 4.98 (s, 2 H, CH$_2$ Z), 4.40 (m, 1 H, α-H Phe), 4.26–4.21 (m, 4 H, 1 α-H Lys, 1 CH Fmoc, 2 CH$_2$ Fmoc), 3.94 (m, 1 H, α-H Nva), 3.05–2.84 (m, 4 H, 2 β-H Phe, 2 ε-H Lys), 1.53–1.22 (m, 10 H, 2 β-H Nva, 2 β-H Lys, 2 γ-H Nva, 2 γ-H Lys, 2 δ-H Lys), 0.79 (t, *J* = 7.1 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.1 (CO), 172.0 (CO), 171.9 (CO), 156.4 (CO), 156.3 (CO), 144.2 (Ar-C Fmoc), 144.1 (Ar-C Fmoc), 141.0 (Ar-C Fmoc), 137.8 (Ar-C), 137.6 (Ar-C), 129.4 (Ar-CH Phe), 128.7 (Ar-CH Phe), 128.5 (Ar-CH Z), 128.1 (Ar-CH Z), 128.0 (Ar-CH Fmoc), 127.4 (Ar-CH Z), 126.7 (Ar-CH Phe), 125.6 (Ar-CH Fmoc), 120.4 (Ar-CH Fmoc), 65.9 (CH$_2$ Fmoc), 65.5 (CH$_2$ Z), 54.9 (α-C Nva), 53.7 (α-C Phe), 52.4 (α-C Lys), 47.0 (CH Fmoc), 39.3 (ε-C Lys), 36.7 (β-C Phe), 34.8 (β-C Nva), 31.9 (β-C Lys), 29.5 (δ-C Lys), 23.2 (γ-C Lys), 18.7 (γ-C Nva), 14.1 (δ-C Nva) ppm. HPLC–MS: t_R = 7.87 min (gradient from 50 to 100% A in 15 min); *m*/*z* = 749.84 [M + 1]$^+$. $C_{43}H_{48}N_4O_8$ (748.35): calcd. C 68.97, H 6.46, N 7.48; found C 69.02, H 6.42, N 7.55.

Fmoc-Lys(COOPr)-Nva-Phe-OH (18): Amorphous solid (10 mg, 95% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.01 (d, *J* = 7.4 Hz, 1 H, NH Phe), 7.87 (d, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.81 (d, *J* = 8.1 Hz, 1 H, NH Lys), 7.70 (dd, *J* = 7.2 and 4.2 Hz, 2 H, Ar-H Fmoc), 7.45 (d, *J* = 8.7 Hz, 1 H, NH Nva), 7.40 (t, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.31 (t, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.19 (m, 5 H, Ar-H Phe), 7.04 (t, *J* = 5.25 Hz, 1 H, ε-NH Lys), 4.37 (m, 1 H, α-H Phe), 4.26–4.20 (m, 4 H, 1 α-H Lys, 1 CH Fmoc, 1 CH$_2$ Fmoc), 3.95 (m, 1 H, α-H Nva), 3.85 (t, *J* = 6.7 Hz, 2 H, CO$_2$C*H*$_2$CH$_2$CH$_3$), 3.03 (dd, *J* = 13.9 and 5.2 Hz, 1 H, β-H Phe), 2.96–2.88 (m, 3 H, 1 β-H Phe, 2 ε-H Lys), 1.54–1.22 (m, 12 H, 2 β-H Nva, 2 β-H Lys, 2 COCH$_2$C*H*$_2$CH$_3$, 2 γ-H Nva, 2 γ-H Lys, 2 δ-H Lys), 0.84 (t, *J* = 7.4 Hz, 3 H, CO$_2$CH$_2$CH$_2$C*H*$_3$), 0.80 (t, *J* = 7.3 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.2 (CO), 172.1 (CO), 171.8 (CO), 156.7 (CO), 156.3 (CO), 144.2 (Ar-C Fmoc), 144.1 (Ar-C Fmoc), 141.0 (Ar-C Fmoc), 137.1 (Ar-C Phe), 129.5 (Ar-CH Phe), 128.4 (Ar-CH Phe), 128.0 (Ar-CH Fmoc), 127.4 (Ar-CH Fmoc), 126.6 (Ar-CH Phe), 125.6 (Ar-CH Fmoc), 120.4 (Ar-CH Fmoc), 66.0 (CH$_2$ Fmoc), 65.4 (CO$_2$*C*H$_2$CH$_2$CH$_3$), 54.9 (α-C Nva), 53.4 (α-C Phe), 52.4 (α-C Lys), 47.0 (CH Fmoc), 39.3 (ε-C Lys), 37.0 (β-C Phe), 34.8 (β-C Nva), 31.9 (β-C Lys), 29.5 (δ-C Lys), 23.3 (γ-C Lys), 22.4 (CO$_2$CH$_2$*C*H$_2$CH$_3$), 18.7 (γ-C Nva), 14.1 (δ-C Nva), 10.6 (CO$_2$CH$_2$CH$_2$*C*H$_3$) ppm. HPLC–MS: t_R = 6.65 min (gradient from 50 to 100% A in 15 min); *m*/*z* = 701.83 [M + 1]$^+$. $C_{39}H_{48}N_4O_8$ (700.35): calcd. C 66.84, H 6.90, N 7.99; found C 66.88, H 6.85, N 8.15.

Fmoc-Asp(O*t*Bu)-Nva-Phe-OH (19): Amorphous solid (9 mg, 91% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.11 (d, *J* = 7.5 Hz, 1 H, NH Phe), 7.87 (d, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.7 (d, *J* = 7.7 Hz, 1 H, NH Nva), 7.67 (m, 3 H, 2 Ar-H Fmoc, 1 NH Asp), 7.45 (t, *J* = 7.4 Hz, 2 H, Ar-H Fmoc), 7.29 (m, 2 H, Ar-H Fmoc), 7.22 (m, 5 H, Ar-H Phe), 4.36 (m, 2 H, 1 α-H Phe, 1 α-H Asp), 4.27–4.18 (m, 4 H, 1 α-H Nva, 1 CH Fmoc, 1 CH$_2$ Fmoc), 3.02 (dd, *J* = 13.9 and 5.3 Hz, 1 H, β-H Phe), 2.87 (dd, *J* = 13.9 and 8.8 Hz, 1 H, β-H Phe), 2.60 (dd, *J* = 16.2 and 4.7 Hz, 1 H, β-H Asp), 2.40 (dd, *J* = 16.2 and 9.6 Hz, 1 H, β-H Asp), 1.6–1.1 [m, 13 H, 2 β-H Nva, 2 γ-H Nva, 9 OC(C*H*$_3$)$_3$], 0.77 (t, *J* = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 172.8 (CO), 171.4 (CO), 170.3 (CO), 169.4 (CO), 155.8 (CO), 143.8 (Ar-C Fmoc), 143.7 (Ar-C Fmoc), 140.2 (Ar-C Fmoc), 137.5 (Ar-C Phe), 129.1 (Ar-CH Phe), 128.2 (Ar-CH Phe), 127.7 (Ar-CH Fmoc), 127.1 (Ar-CH Fmoc), 126.4 (Ar-CH Phe), 125.3 (Ar-CH Fmoc), 120.2 (Ar-CH Fmoc), 80.1 {Asp[*C*(CH$_3$)$_3$]}, 65.8 (CH$_2$ Fmoc), 53.5 (α-C Phe), 52.2 (α-C Nva), 51.3 (α-C Asp), 46.6 (CH Fmoc), 37.5 (β-C Asp), 36.6 (β-C Phe), 34.3 (β-C Nva), 27.7 [OC(*C*H$_3$)$_3$], 18.2 (γ-C Nva), 13.7 (δ-C Nva) ppm. HPLC–MS: t_R = 14.18 min (gradient from 20 to 100% A in 15 min); *m*/*z* = 658.81 [M + 1]$^+$. $C_{37}H_{43}N_3O_8$ (657.31): calcd. C 67.56, H 6.59, N 6.39; found C 67.65, H 6.48, N 6.50.

Fmoc-Asp(OPr)-Nva-Phe-OH (20): Amorphous solid (9 mg, 90% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.05 (d, *J* = 7.2 Hz, 1 H, NH Phe), 7.87 (d, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.82 (d, *J* = 8.1 Hz, 1 H, NH Nva), 7.68–7.58 (m, 3 H, 2 Ar-H Fmoc, 1 NH Asp), 7.40 (t, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.30 (t, *J* = 7.5 Hz, 2 H, Ar-H Fmoc), 7.19 (m, 5 H, Ar-H Phe), 4.40–4.35 (m, 2 H, 1 α-H Phe, 1 α-H Asp), 4.26–4.13 (m, 4 H, 1 α-H Nva, 1 CH Fmoc, 1 CH$_2$ Fmoc), 3.94 (t, *J* = 5.4 Hz, 2 H, OC*H*$_2$CH$_2$CH$_3$), 3.03 (dd, *J* = 13.6 and 4.8 Hz, 1 H, β-H Phe), 2.87 (dd, *J* = 13.6 and 8.7 Hz, 1 H, β-H Phe), 2.69 (dd, *J* = 16.5 and 4.8 Hz, 1 H, β-H Asp), 2.53 (m, 1 H, β-H Asp), 1.53 (m, 2 H, OCH$_2$C*H*$_2$CH$_3$), 1.44–1.34 (m, 2 H, β-H Nva), 1.22 (m, 2 H, γ-H Nva), 0.83 (t, *J* = 7.5 Hz, 3 H, OCH$_2$CH$_2$C*H*$_3$), 0.76 (t, *J* = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.7 (CO), 171.3 (CO), 170.2 (CO), 155.8 (CO), 143.7 (Ar-C Fmoc), 140.7 (Ar-C Fmoc), 137.5 (Ar-C Phe), 129.0 (Ar-CH Phe), 128.1 (Ar-CH Phe), 127.6 (Ar-CH Fmoc), 127.0 (Ar-CH Fmoc), 126.3 (Ar-CH Phe), 125.2 (Ar-CH Fmoc), 120.1 (Ar-CH Fmoc), 65.8 (CH$_2$ Fmoc), 65.6 (O*C*H$_2$CH$_2$CH$_3$), 53.4 (α-C Phe), 52.3 (α-C Nva), 51.1 (α-C Asp), 46.6 (CH Fmoc), 36.6 (β-C Phe), 36.2 (β-C Asp), 34.2 (β-C Nva), 21.4 (OCH$_2$*C*H$_2$CH$_3$), 18.2 (γ-C Nva), 13.6 (OCH$_2$CH$_2$*C*H$_3$), 10.2 (δ-C Nva) ppm. HPLC–MS: t_R = 13.61 min (gradient from 20 to 100% A in 15 min); *m*/*z* = 644.72 [M + 1]$^+$. $C_{36}H_{41}N_3O_8$ (643.29): calcd. C 67.17, H 6.42, N 6.53; found C 67.30, H 6.30, N 6.61.

Fmoc-Asp(OBz)-Nva-Phe-OH (21): Amorphous solid (8 mg, 77% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.05 (d, *J* = 6.8 Hz, 1 H, NH Phe), 7.86 (m, 3 H, 2 Ar-H Fmoc, 1 NH Nva), 7.74–7.66 (m, 3 H, 2 Ar-H Fmoc, 1 NH Asp), 7.42–7.37 (m, 4 H, Ar-H Fmoc), 7.32 (m, 5 H, Ar-H Bz), 7.18 (m, 5 H, Ar-H Phe), 5.07 [s, 2 H, Asp(CH$_2$-Bz)], 4.40–4.36 (m, 2 H, 1 α-H Phe, 1 H α-H Asp), 4.26–4.19 (m, 4 H, 1 α-H Nva, CH Fmoc, CH$_2$ Fmoc), 3.02 (dd, *J* = 13.8 and 4.9 Hz, 1 H, β-H Phe), 2.89 (dd, *J* = 13.8 and 8.9 Hz, 1 H, β-H Phe), 2.68 (dd, *J* = 16.2 and 4.8 Hz, 1 H, β-H Asp), 2.49 (dd, *J* = 16.2 and 9.2 Hz, 1 H, β-H Asp), 1.6–1.40 (m, 2 H, β-H Nva), 1.20 (m, 2 H, γ-H Nva), 0.76 (t, *J* = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.7 (CO), 171.3 (CO), 170.2 (CO), 170.1 (CO), 155.2 (CO), 143.1 (Ar-C Fmoc), 140.1 (Ar-C Fmoc), 136.9 (Ar-C), 135.4 (Ar-C), 129.1 (Ar-CH Phe), 128.5 (Ar-CH Phe), 127.8 (Ar-CH Phe), 127.5 (Ar-H Bz), 127.4 (Ar-H Bz), 127.0 (Ar-CH Fmoc), 126.8 (Ar-H Bz), 125.7 (Ar-H Bz), 124.6 (Ar-CH Fmoc), 119.5 (Ar-CH Fmoc), 65.2 (CH$_2$ Fmoc), 65.1 [Asp(*C*H$_2$-Bz)], 52.8 (α-C Phe), 51.7 (α-C Nva), 50.5 (α-C Asp), 46.0 (CH Fmoc), 36.0 (β-C Phe), 35.6 (β-C Asp), 33.6 (β-C Nva), 17.6 (γ-C Nva), 13.1 (δ-C Nva) ppm. HPLC–MS: t_R = 8.63 min (gradient from 50 to 100% A in 15 min); *m*/*z* [M + 1]$^+$ = 692.73. $C_{40}H_{41}N_3O_8$ (691.29): calcd. C 69.45, H 5.97, N 6.07; found C 69.34, H 5.90, N 6.21.

Fmoc-Ser(*t*Bu)-Nva-Phe-OH (22): Amorphous solid (7 mg, 78% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.05 (d, *J* = 7.6 Hz,

1 H, NH Phe), 7.87 (d, J = 7.5 Hz, 2 H, Ar-H Fmoc), 7.78 (d, J = 8.2 Hz, 1 H, NH Nva), 7.70 (m, 2 H, Ar-H Fmoc), 7.40 (m, 3 H, 1 NH Ser, 2 Ar-H Fmoc), 7.30 (m, 2 H, Ar-H Fmoc), 7.18 (m, 5 H, Ar-H Phe), 4.35–4.22 (m, 5 H, 1 α-H Phe, 1 α-H Nva, 1 CH Fmoc, 2 CH_2 Fmoc), 4.10 (m, 1 H, α-H Ser), 3.03 (dd, J = 13.5 and 5.0 Hz, 1 H, β-H Phe), 2.87 (dd, J = 13.5 and 8.5 Hz, 1 H, β-H Phe), 1.55–1.39 (m, 2 H, β-H Nva), 1.22 (m, 2 H, γ-H Nva), 1.07 [s, 9 H, C(CH_3)], 0.79 (t, J = 6.9 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.0 (CO), 171.6 (CO), 169.8 (CO), 156.3 (CO), 144.2 (Ar-C Fmoc), 144.1 (Ar-C Fmoc), 141.1 (Ar-C Fmoc), 138.0 (Ar-C Phe), 129.5 (Ar-CH Phe), 128.4 (Ar-CH Phe), 128.0 (Ar-CH Phe), 127.4 (Ar-CH Fmoc), 126.6 (Ar-CH Phe), 125.7 (Ar-CH Fmoc), 120.5 (Ar-CH Fmoc), 73.2 [C(CH$_3$)], 66.1 (CH_2 Fmoc), 62.3 (β-C Ser), 55.8 (α-C Ser), 54.0 (α-C Phe), 52.5 (α-C Nva), 47.0 (CH Fmoc), 37.1 (β-C Phe), 35.0 (β-C Nva), 27.5 [C(CH$_3$)], 18.5 (γ-C Nva), 14.1 (δ-C Nva) ppm. HPLC–MS: t_R = 11.35 min (gradient from 40 to 100% A in 15 min); m/z = 630.72 [M + 1]$^+$. $C_{36}H_{43}N_3O_7$ (629.31): calcd. C 66.86, H 6.88, N 6.67; found C 66.75, H 6.96, N 6.75.

Fmoc-Ser(Bz)-Nva-Phe-OH (23): Amorphous solid (8 mg, 87% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.06 (d, J = 7.2 Hz, 1 H, NH Phe), 7.92 (d, J = 8.1 Hz, 1 H, NH Nva), 7.87 (d, J = 7.5 Hz, 2 H, Ar-H Fmoc), 7.70 (m, 2 H, Ar-H Fmoc), 7.62 (d, J = 8.3 Hz, 1 H, NH Ser), 7.39 (t, J = 7.6 Hz, 2 H, Ar-H Fmoc), 7.28–7.21 (m, 7 H, 2 Ar-H Fmoc, 5 Ar-H Bz), 7.19 (m, 5 H, Ar-H Phe), 4.45 [s, 2 H, Ser(CH_2-Bz)], 4.37–4.21 (m, 6 H, 1 α-H Phe, 1 α-H Nva, 1 α-H Ser, 1 CH Fmoc, 1 CH_2 Fmoc), 3.55 (m, 2 H, β-H Ser), 3.01 (dd, J = 13.8 and 5.3 Hz, 1 H, β-H Phe), 2.85 (dd, J = 13.8 and 8.4 Hz, 1 H, β-H Phe), 1.6–1.40 (m, 2 H, β-H Nva), 1.22 (m, 2 H, γ-H Nva), 0.78 (t, J = 7.1 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.1 (CO), 171.6 (CO), 169.6 (CO), 156.3 (CO), 144.2 (Ar-C Fmoc), 144.1 (Ar-C Fmoc), 141.1 (Ar-C Fmoc), 138.6 (Ar-C), 137.9 (Ar-C), 129.5 (Ar-CH Phe), 128.5 (Ar-CH), 128.5 (Ar-CH), 128.0 (Ar-CH), 127.8 (Ar-CH), 127.7 (Ar-CH), 126.7 (Ar-CH), 125.6 (Ar-CH Fmoc), 120.5 (Ar-CH Fmoc), 72.4 [Ser(CH$_2$-Bz)], 70.3 (β-C Ser), 66.2 (CH_2 Fmoc), 55.2 (α-C Ser), 53.8 (α-C Phe), 52.6 (α-C Nva), 47.0 (CH Fmoc), 37.0 (β-C Phe), 34.8 (β-C Nva), 18.6 (γ-C Nva), 14.1 (δ-C Nva) ppm. HPLC–MS: t_R = 11.49 min (gradient from 40 to 100% A in 15 min); m/z = 664.74 [M + 1]$^+$. $C_{39}H_{41}N_3O_7$ (663.29): calcd. C 70.57, H 6.23, N 6.33; found C 70.48 H 6.31, N 6.42.

Fmoc-Trp-Nva-Phe-OH (24): Amorphous solid (6 mg, 60% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.84 (s, 1 H, Ar-NH Trp), 8.06 (d, J = 8.1 Hz, 1 H, NH Phe), 7.84 (m, 3 H, 2 Ar-H Fmoc, 1 NH Nva), 7.67 (d, J = 7.6 Hz, 1 H, Ar-H Trp), 7.60 (t, J = 8.2 Hz, 2 H, Ar-H Fmoc), 7.52 (d, J = 8.7 Hz, 1 H, NH Trp), 7.41–7.15 (m, 10 H, 4 Ar-H Fmoc, 1 Ar-H Trp, 5 Ar-H Phe), 7.04 (t, J = 7.3 Hz, 1 H, Ar-H Trp), 6.94 (t, J = 7.3 Hz, 1 H, Ar-H Trp), 6.26 (s, 1 H, Ar-H Trp), 4.24 (m, 3 H, 1 α-H Phe, 1 α-H Nva, 1 α-H Trp), 4.12 (m, 3 H, 1 CH Fmoc, 1 CH_2 Fmoc), 3.13–2.88 (m, 4 H, 2 β-H Phe, 2 β-H Trp), 1.60–1.39 (m, 2 H, β-H Nva), 1.22 (m, 2 H, γ-H Nva), 0.80 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.1 (CO), 171.4 (CO), 171.0 (CO), 156.1 (CO), 144.1 (Ar-C Fmoc), 140.7 (Ar-C), 138.5 (Ar-C), 136.4 (Ar-C), 129.7 (Ar-CH Phe), 128.3 (Ar-CH), 128.0 (Ar-CH), 127.7 (Ar-CH), 127.4 (Ar-CH), 126.4 (Ar-CH Phe), 125.7 (Ar-CH Fmoc), 124.2 (Ar-CH), 121.8 (Ar-C), 121.1 (Ar-CH Trp), 120.4 (Ar-CH Fmoc), 119.1 (Ar-CH Trp), 111.6 (Ar-CH Trp), 110.5 (Ar-C), 66.0 (CH_2 Fmoc), 55.8 (α-C Phe), 54.5 (α-C Trp), 53.0 (α-C Nva), 46.9 (CH Fmoc), 37.2 (β-C Phe), 34.7 (β-C Nva), 28.1 (β-C Trp), 18.8 (γ-C Nva), 14.1 (δ-C Nva) ppm. HPLC–MS: t_R = 10.32 min (gradient from 40 to 100% A in 15 min); m/z = 673.76 [M + 1]$^+$. $C_{40}H_{40}N_4O_6$ (672.29): calcd. C 71.41, H 5.99, N 8.33; found C 71.52 H 5.85, N 8.47.

Fmoc-Met-Nva-Phe-OH (25): Amorphous solid (7 mg,75% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.00 (d, J = 7.3 Hz, 1 H, NH Phe), 7.86 (m, 3 H, 2 H Ar-H Fmoc, 1 H NH Nva), 7.70 (t, J = 6.3 Hz, 2 H, Ar-H Fmoc), 7.55 (d, J = 8.1 Hz, 1 H, NH Met), 7.40 (t, J = 7.4 Hz, 2 H, Ar-H Fmoc), 7.31 (t, J = 7.4 Hz, 2 H, Ar-H Fmoc), 7.18 (m, 5 H, Ar-H Phe), 4.34–4.07 (m, 6 H, 1 α-H Phe, 1 α-H Nva, 1 α-H Met, 1 CH Fmoc, 1 CH_2 Fmoc), 3.03 (dd, J = 13.9 and 5.1 Hz, 1 H, β-H Phe), 2.87 (dd, J = 13.9 and 8.2 Hz, 1 H, β-H Phe), 2.41 (m, 2 H, γ-H Met), 2.01 (s, 3 H, ε-H Met), 1.80 (m, 2 H, β-H Met), 1.55–1.41 (m, 2 H, β-H Nva), 1.22 (m, 2 H, γ-H Nva), 0.80 (t, J = 7.1 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.6 (CO), 171.3 (CO), 171.7 (CO), 155.9 (CO), 143.9 (Ar-C Fmoc), 143.7 (Ar-C Fmoc), 140.7 (Ar-C Fmoc), 137.7 (Ar-C Phe), 129.1 (Ar-CH Phe), 128.0 (Ar-CH Phe), 127.6 (Ar-CH Fmoc), 127.0 (Ar-CH Fmoc), 126.2 (Ar-CH Phe), 125.3 (Ar-CH Fmoc), 120.1 (Ar-CH Fmoc), 65.6 (CH_2 Fmoc), 53.8 (α-C Met), 53.6 (α-C Phe), 52.2 (α-C Nva), 46.6 (CH Fmoc), 36.7 (β-C Phe), 34.4 (β-C Nva), 31.7 (β-C Met), 29.7 (γ-H Met), 18.4 (γ-C Nva), 14.6 (ε-C Met), 13.7 (δ-C Nva) ppm. HPLC–MS: t_R = 9.98 min (gradient from 40 to 100% A in 15 min); m/z = 618.69 [M + 1]$^+$. $C_{34}H_{39}N_3O_6S$ (617.26): calcd. C 66.10, H 6.36, N 6.80; found C 66.22 H 6.25, N 6.98.

Ac-Nva-Phe-OH (26): Amorphous solid (5 mg, 98% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.05 (d, J = 7.8 Hz, 1 H, NH Phe), 7.88 (d, J = 8.3 Hz, 1 H, NH Nva), 7.21 (m, 5 H, Ar-H Phe), 4.37 (m, 1 H, α-H Phe), 4.23 (m, 1 H, α-H Nva), 3.03 (dd, J = 13.8 and 5.2 Hz, 1 H, β-H Phe), 2.88 (dd, J = 13.8 and 8.7 Hz, 1 H, β-H Phe), 1.79 (s, 3 H, $COCH_3$), 1.52 (m, 1 H, β-H Nva), 1.41 (m, 1 H, β-H Nva), 1.23 (m, 2 H, γ-H Nva), 0.82 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.2 (CO), 172.2 (CO), 169.4 (CO), 137.9 (Ar-C Phe), 129.5 (Ar-CH Phe), 128.5 (Ar-CH Phe), 126.7 (Ar-CH Phe), 53.8 (α-C Phe), 52.7 (α-C Nva), 36.9 (β-C Phe), 34.3 (β-C Nva), 22.8 (COCH_3), 18.8 (γ-C Nva), 14.0 (δ-C Nva) ppm. HPLC–MS: t_R = 9.98 min (gradient from 5 to 80% A in 15 min); m/z = 307.37 [M + 1]$^+$. $C_{16}H_{22}N_2O_4$ (306.16): calcd. C 62.73, H 7.24, N 9.14; found C 62.79 H 7.18, N 9.25.

Boc-Nva-Phe-OH (27): Amorphous solid (5 mg, 97% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.91 (d, J = 7.9 Hz, 1 H, NH Phe), 7.21 (m, 5 H, Ar-H Phe), 6.77 (d, J = 8.2 Hz, 1 H, NH Nva), 4.41 (m, 1 H, α-H Phe), 3.87 (m, 1 H, α-H Nva), 3.03 (dd, J = 13.8 and 5.0 Hz, 1 H, β-H Phe), 2.88 (dd, J = 13.8 and 8.6 Hz, 1 H, β-H Phe), 1.43 (m, 2 H, β-H Nva), 1.35 [s, 9 H, $COC(CH_3)_3$], 1.23 (m, 2 H, γ-H Nva), 0.79 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.2 (CO), 172.4 (CO), 155.5 (CO), 137.7 (Ar-C Phe), 129.5 (Ar-CH Phe), 128.5 (Ar-CH Phe), 126.7 (Ar-CH Phe), 78.4 [COC(CH$_3$)$_3$], 54.4 (α-C Nva), 53.5 (α-C Phe), 37.1 (β-C Phe), 34.5 (β-C Nva), 28.5 [COC(CH$_3$)$_3$], 18.9 (γ-C Nva), 14.0 (δ-C Nva) ppm. HPLC–MS: t_R = 13.67 min (gradient from 5 to 80% A in 15 min); m/z = 365.45 [M + 1]$^+$. $C_{19}H_{28}N_2O_5$ (364.20): calcd. C 62.62, H 7.74, N 7.69; found C 62.75 H 7.61, N 7.86.

Fmoc-Nva-Phe-NH$_2$ (30): Amorphous solid (16 mg, 99% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.59 (d, J = 8.2 Hz, 1 H, C_t-NH_2), 8.03 (m, 1 H, NH Phe), 7.91 (d, J = 7.4 Hz, 2 H, Ar-H Fmoc), 7.85 (d, J = 8.2 Hz, 1 H, C_t-NH_2), 7.72 (m, 2 H, Ar-H Fmoc), 7.44 (m, 3 H, 2 Ar-H Fmoc, 1 NH Nva), 7.35 (t, J = 7.4 Hz, 2 H, Ar-H Fmoc), 7.20 (m, 5 H, Ar-H Phe), 4.47 (m, 1 H, α-H Phe), 4.26 (m, 3 H, CH Fmoc, CH_2 Fmoc), 3.91 (m, 1 H, α-H Nva), 3.02 (dd, J = 13.7 and 4.7 Hz, 1 H, β-H Phe), 2.84 (dd, J =

13.8 and 8.8 Hz, 1 H, β-H Phe), 1.47 (m, 2 H, β-H Nva), 1.21 (m, 2 H, γ-H Nva), 0.84 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. HPLC–MS: t_R = 5.33 min (gradient from 50 to 100% of solvent A in 15 min); m/z = 486.5 $[M + 1]^+$. $C_{29}H_{31}N_3O_4$ (485.23): calcd. C 71.73, H 6.43, N 8.65; found C 71.63, H 6.30, N 8.32.

α-Azidoisobutyryl-Nva-Phe-NH$_2$ (33): Amorphous solid (9 mg, 70% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.88 (d, J = 8.3 Hz, 1 H, NH Phe), 7.75 (d, J = 8.1 Hz, 1 H, NH Nva), 7.42 (s, 1 H, C_t-NH$_2$), 7.21 (m, 5 H, Ar-H Phe), 7.07 (s, 1 H, C_t-NH$_2$), 4.42 (m, 1 H, α-H Phe), 4.16 (m, 1 H, α-H Nva), 2.97 (dd, J = 13.8 and 5.1 Hz, 1 H, β-H Phe), 2.78 (dd, J = 13.8 and 9.0 Hz, 1 H, β-H Phe), 1.54 (m, 2 H, β-H Nva), 1.37 (s, 3 H, β-H Aib), 1.36 (s, 3 H, β-H Aib), 1.24–1.09 (m, 2 H, γ-H Nva), 0.80 (t, J = 7.3 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 172.7 (CO), 171.6 (CO), 171.1 (CO), 137.7 (Ar-C Phe), 129.3 (Ar-CH Phe), 128.0 (Ar-CH Phe), 126.3 (Ar-CH Phe), 63.9 (α-C Aib), 53.5 (α-C Phe), 53.0 (α-C Nva), 37.7 (β-C Phe), 33.7 (β-C Nva), 24.2 (β-C Aib), 18.6 (γ-C Nva), 13.6 (δ-C Nva) ppm. HPLC–MS: t_R = 12.63 min (gradient from 5 to 80% A in 15 min); m/z = 375.47 $[M + 1]^+$. $C_{18}H_{26}N_6O_3$ (374.21): calcd. C 57.74, H 7.00, N 22.44; found C 57.65 H 6.92, N 22.58.

Chloroacetyl-Nva-Phe-NH$_2$ (34): Amorphous solid (6 mg, 53% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.24 (d, J = 7.8 Hz, 1 H, NH Nva), 8.05 (d, J = 8.3 Hz, 1 H, NH Phe), 7.32 (s, 1 H, C_t-NH$_2$), 7.21 (m, 5 H, Ar-H Phe), 7.07 (s, 1 H, C_t-NH$_2$), 4.41 (m, 1 H, α-H Phe), 4.21 (m, 1 H, α-H Nva), 4.10 (d, J = 13.0 Hz, 1 H, CH$_2$Cl), 4.04 (d, J = 13.0 Hz, 1 H, CH$_2$Cl), 2.99 (dd, J = 13.8 and 5.0 Hz, 1 H, β-H Phe), 2.78 (dd, J = 13.8 and 9.4 Hz, 1 H, β-H Phe), 1.54–1.15 (m, 4 H, 2 β-H Nva, 2 γ-H Nva), 0.79 (t, J = 7.2 Hz, 3 H, δ-H Nva) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 172.8 (CO), 170.9 (CO), 165.8 (CO), 137.9 (Ar-C Phe), 129.1 (Ar-CH Phe), 128.1 (Ar-CH Phe), 126.3 (Ar-CH Phe), 53.7 (α-C Phe), 52.8 (α-C Nva), 42.6 (CH$_2$Cl), 37.4 (β-C Phe), 34.2 (β-C Nva), 18.3 (γ-C Nva), 13.7 (δ-C Nva) ppm. HPLC–MS: t_R = 10.63 min (gradient from 5 to 80% A in 15 min); m/z = 340.47 $[M + 1]^+$. $C_{16}H_{22}ClN_3O_3$ (339.13): calcd. C 56.55, H 6.53, N 12.37; found C 56.58, H 6.20, N 12.52.

Ac-cycle(2→10)-Met^1-Nva^2-Ile^3-Lys^4-Pro^5-His^6-Gln^7-Gly^8-Gln^9-Nva^{10}-Ile^{11}-NH$_2$ (41): After the corresponding standard couplings to resin-bound linear peptide **39**, this resin (150 mg, 0.03 mmol) was swollen with DCM/DMF (4 × 0.5 min each). Then, successive washings with a 0.8 M solution of the chaotropic agent LiCl in DMF and with DMF/DCM (4 × 0.5 min each) were performed. This procedure was repeated once more. The reaction mixture was purged with Ar, and a solution of second-generation Hoveyda–Grubbs catalyst (5 mg, 0.01 mmol) in DCE (4 mL) was added. The mixture was heated at 65 °C and gently stirred for 24 h. The addition was repeated twice (24 h each time). Then washings with DCE/DMF (3 × 0.5 min each) were carried out, and a solution of 300 equiv. of anhydrous DMSO in DMF was added. After 12 h at room temp., the resin was washed with DMF/DCM/MeOH (5 × 0.5 min each) to give resin **40**. This resin was reduced acording to the general procedure described above to the resin-bound macrocyclic peptide **41**, which was cleaved with TFA/EDT/H$_2$O/TIPS (94:2.5:2.5:1) at room temp. for 5 h. The filtrates were concentrated, and the product was precipitated from diethyl ether and purified by semi-preparative HPLC (15% A for 2 min, linear 15–45% A/B gradient for 25 min). Compound **41** (6 mg, 12% total yield) was obtained as an amorphous solid. *cis*/*trans* Lys-Pro rotamer ratio (1:4). ^{1}H NMR [*trans* rotamer, 600 MHz, H$_2$O/D$_2$O (9:1)]: Met^1: δ = 8.43 (1 H, NH), 4.38 (1 H, α-H), 2.59 (1 H, γ-H), 2.54 (1 H, γ-H), 2.09 (3 H, ε-H), 1.99 (2 H, β-H); Nva^2: δ = 8.41 (1 H, NH), 4.30 (1 H, α-H), 1.69 (2 H, β-H), 1.26 (1 H, γ-H), 1.21 (1 H, γ-H); Ile^3: δ = 8.37 (1 H, NH), 4.06 (1 H, α-H), 1.83 (1 H, β-H), 1.49 (1 H, γ-H), 1.22 (1 H, γ-H), 0.88 (3 H, γγ′-H), 0.87 (3 H, δ-H); Lys^4: δ = 8.43 (1 H, NH), 4.49 (1 H, α-H), 3.01 (2 H, ε-H), 1.77 (2 H, β-H), 1.70 (2 H, δ-H), 1.48 (2 H, γ-H); Pro^5: δ = 4.46 (1 H, α-H), 3.84 (1 H, δ-H), 3.60 (1 H, δ-H), 2.22 (1 H, β-H), 1.88 (2 H, β-H and γ-H); His^6: δ = 8.70 (1 H, NH), 4.52 (1 H, α-H), 3.22 (2 H, β-H); Gln^7: δ = 8.27 (1 H, NH), 7.69 (1 H, ε-H), 6.99 (1 H, ε-H), 4.35 (1 H, α-H), 2.23 (2 H, γ-H), 2.08 (1 H, β-H), 1.95 (1 H, β-H); Gly^8: δ = 8.60 (1 H, NH), 4.04 (1 H, α-H), 3.86 (1 H, α-H); Gln^9: δ = 8.41 (1 H, NH), 7.62 (1 H, ε-H), 6.97 (1 H, ε-H), 4.45 (1 H, α-H), 2.36 (2 H, γ-H), 2.08 (1 H, β-H), 1.91 (1 H, β-H); Nva^{10}: δ = 8.70 (1 H, NH), 4.18 (1 H, α-H), 1.74 (1 H, β-H), 1.58 (1 H, β-H), 1.26 (1 H, γ-H), 1.21 (1 H, γ-H); Ile^{11}: δ = 8.46 (1 H, NH), 4.11 (1 H, α-H), 1.83 (1 H, β-H), 1.52 (1 H, γ-H), 1.21 (1 H, γ-H), 0.94 (3 H, γγ′-H), 0.87 (3 H, δ-H) ppm. ^{13}C NMR (*trans* rotamer, 150 MHz, D$_2$O; only aliphatic carbon signals are shown): Met^1: δ = 55.6 (α-C), 32.9 (β-C), 31.9 (γ-C), 16.8 (ε-C); Nva^2: δ = 55.9 (α-C), 33.2 (β-C); Ile^3: δ = 61.3 (α-C), 38.1 (β-C), 27.5 (γ-C), 17.4 (γγ′-C), 12.8 (δ-C); Lys^4: δ = 54.6 (α-C), 41.9 (ε-C), 32.5 (β-C), 29.3 (δ-C), 24.6 (γ-C); Pro^5: δ = 62.9 (α-C), 50.7 (δ-C), 32.2 (β-C), 27.2 (γ-C); His^6: δ = 56.7 (α-C), 29.1 (β-C); Gln^7: δ = 55.4 (α-C), 33.6 (γ-C), 29.8 (β-C); Gly^8: δ = 44.8 (α-C); Gln^9: δ = 54.8 (α-C), 33.6 (γ-C), 30.3 (β-C); Nva^{10}: δ = 56.9 (α-C), 33.7 (β-C); Ile^{11}: δ = 60.7 (α-C), 38.3 (β-C), 27.2 (γ-C), 17.3 (γγ′-C), 12.6 (δ-C) ppm. HPLC–MS: t_R = 10.68 min (gradient from 5 to 60% of solvent A in 15 min); m/z = 631.25 $[M + 2 H]^{2+}$. HRMS (EI+): calcd. for $C_{56}H_{93}N_{17}O_{14}S$ 1259.6809 $[M]^+$; found 1259.6816.

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