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„First Total Synthesis of the Nematicidal Cyclododecapeptide Omphalotin A Using Novel Racemization-free Triphosgene-mediated Couplings on Solid Phase"

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The first synthesis of the linear precursor peptide was accomplished with *N*-methyl isoleucine (residue 8) as first amino acid attached to the resin:

Synthesis outline for the linear dodecapeptide OmA(9-8) with HPLC purities for intermediate peptides:

1.) Coupling MeVal -> MeIle: BTC, 2,5 h, chloranil test negative.
2.) Coupling Sar -> MeVal: BTC, 4 h, chloranil test negative.
3.) Coupling MeVal -> Sar: BTC, 3 h, chloranil test negative.
4.) Coupling MeVal -> MeVal: BTC, 3 h, chloranil test negative, area of prod. peak: 96 %.
5.) Coupling Ile -> MeVal: HOAt, 16 h, chloranil test slightly positive, area of prod. peak: 80 %.
    2nd coupling HOAt, 20 h, chloranil test negative.
6.) Coupling MeVal -> Ile: BTC, 1 h, Kaiser test negative, area of prod. peak: 95%
7.) Coupling Trp -> MeVal: HOAt, 18 h, chloranil test slightly positive, area of prod. peak: 93 %.
8.) Coupling Sar -> Trp: BTC, 1 h, Kaiser test negative, area of prod. peak: 93 %.
9.) Coupling MeIle -> Sar: BTC, 1 h, chloranil test negative, area of prod. peak: 96 %.
10.) Coupling Val -> MeIle: HOAt, 16 h, chloranil test positive, area of prod. peak: 85%.
    2nd coupling HOAt, 15 h, chloranil test negative.
11.) Coupling Sar -> Val: BTC, 1 h, area of prod. peak: 93 %.

(all HPLC-purities [area of prod. peak] for Fmoc-deprotected peptide, 214 nm)

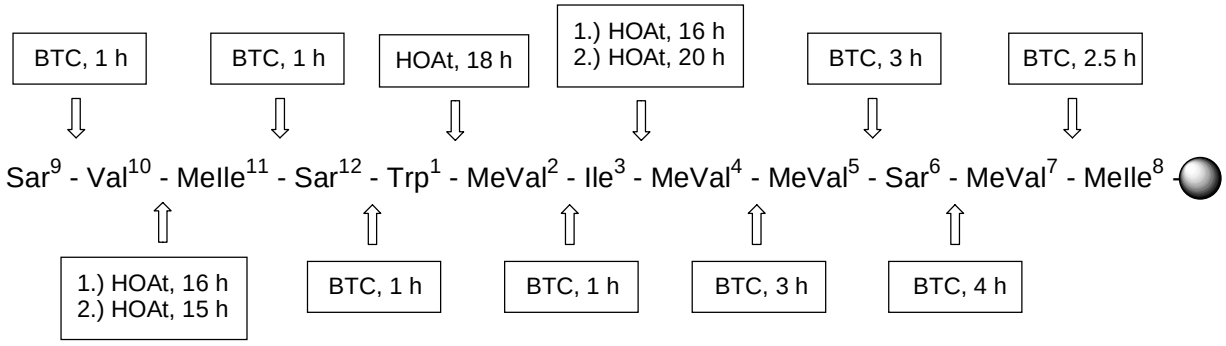

Fig. 1: Graphical synthesis outline for the linear dodecapeptide OmA(9-8).

The efficience of the BTC-coupling is illustrated by the purity of the permethylated pentapeptide Fmoc-OmA(4-8) obtained by single BTC-couplings:

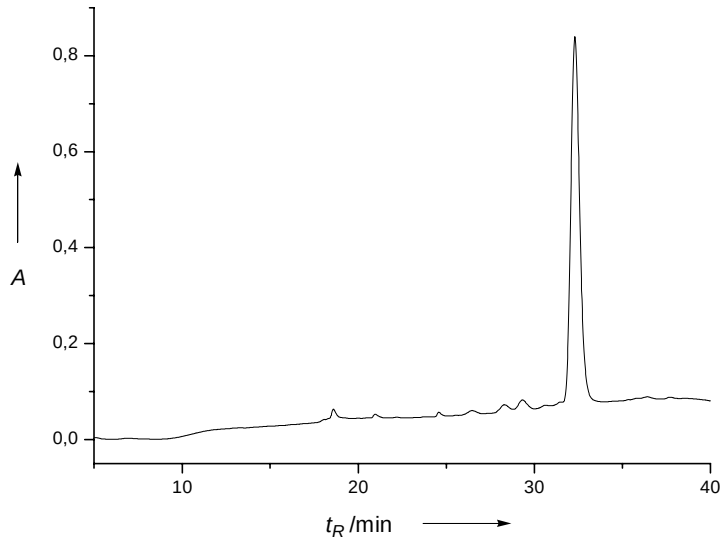

Fig. 2: HPLC-Chromatogram of the permethylated pentapeptide Fmoc-OmA(4-8) (Fmoc-MeVal-MeVal-Sar-MeVal-MeIle-OH), synthesized exclusively by the novel BTC-method. Single couplings were employed for the attachment of each amino acid. Even the very difficult coupling of Fmoc-MeVal to MeVal is was quantitative according to chloranil test after only one coupling cycle.

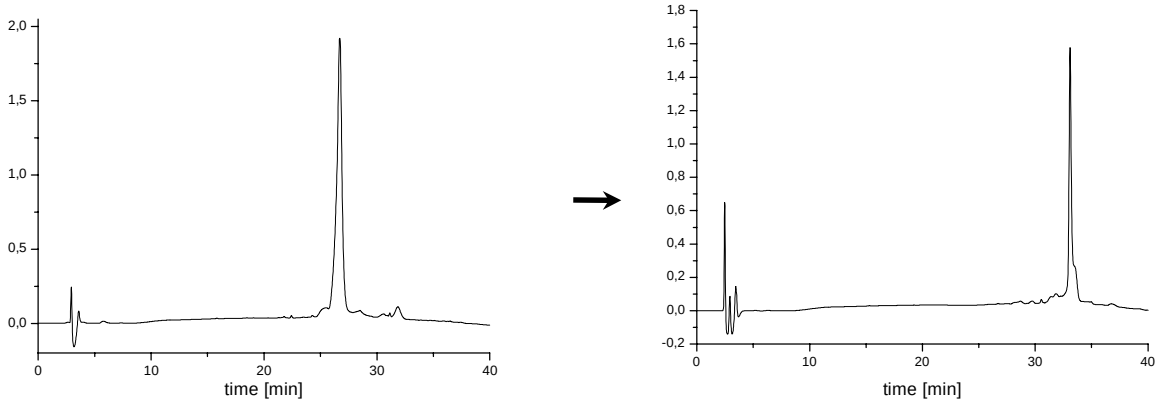

Fig. 3: HPLC of crude linear dodecapeptide OmA(9-8) (pure enantiomer) and its cyclization product. The cyclization via activation of *N*-methyl amino acids leads to partial racemization (up to 30 %, see table 1).

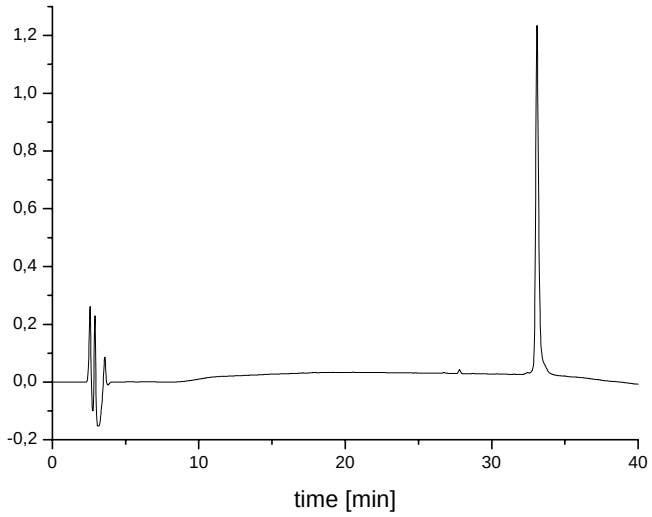

Fig. 4: Omphalotin A from linear dodecapeptide OmA(9-8) via activation of C-terminal L-MeIle^{8} after purification by preparative HPLC-MS. The slight shoulder at about t_R = 34 min indicates the presence of [D-MeIle^{8}]-Omphalotin A even in the purified product (see table 1).

Table 1: Content of D-MeVal and D-MeIle in natural Omphalotin A and in synthetic products from dodecapeptide with C-terminal MeIle[8]. As the low values found in the linear dodecapeptide indicate, no epimerization occurs during peptide synthesis with BTC-activation. After cyclization, the content of D-MeIle rises to 15.4 %. With two MeIle residues being present in Omphalotin A, and MeIle[8] being the only one which can possibly epimerize during cyclization, this value must be doubled in order to give the approximate content of D-MeIle in sequence position 8. Thus, the final product, purified by preparative HPLC, contains about 12-14 % of [D-MeIle[8]]-Omphalotin A.

	D-MeVal	D-MeIle	D-MeIle[8] (C-term. residue of cyclization)
natural product	0.86 %	1.76 %	-
linear dodecapeptide OmA(9-8)	0.66 %	0.97 %	-
OmA (crude product, from OmA(9-8))	0.76 %	15.4 %	ca. 30 %
OmA (purified, from OmA(9-8))	0.31 %	6.9 %	ca. 12-14 %

As these results show, the BTC-coupling itself does not cause significant racemization. In order to prevent the formation of diastereomeric byproducts, further syntheses of linear precursor peptides of Omphalotin A werde started with sarcosine as first amino acid attached to the resin.

Synthesis outline for the linear dodecapeptide OmA(1-12) with HPLC purities for intermediate peptides:

1.) Coupling MeIle -> Sar: BTC, 3 h, chloranil test negative.
2.) Coupling Val -> MeIle: HATU, 16 h, chloranil test negative.
3.) Coupling Sar -> Val: BTC, 2,5 h, Kaiser test negative.
4.) Coupling MeIle -> Sar: BTC, 4 h, chloranil test negative, area of prod. peak: 98 %.
5.) Coupling MeVal -> MeIle: BTC 3 h, chloranil test slightly positive, peak area of pentapeptide: 15 %.
    2nd coupling BTC 3 h, chloranil test negative, no pentapeptide detectable.
6.) Coupling Sar -> MeVal: BTC, 3,5 h, chloranil test negative, no hexapeptide detectable.
7.) Coupling MeVal ->Sar: BTC, 3 h, chloranil test negative, area of prod. peak: 91 %.
8.) Coupling MeVal -> MeVal: BTC, 3,5 h, chloranil test slightly positive, peak area of octapeptide: 9 %.
    2nd coupling BTC 3,5 h, chloranil test negative, area of prod. peak: 91 %, no octapeptide detectable.
9.) Coupling Ile -> MeVal: HATU, 4 h, peak area of nonapeptide: 21 %.
    2nd coupling HATU, 4 h, chloranil test positive,
    3rd coupling HOAt, 6,5 h, no nonapeptide detectable.
10.) Coupling MeVal -> Ile: BTC, 6 h, no decapeptide detectable.
11.) Coupling Trp -> MeVal: HATU, 5 h, area of prod. peak: 84 %.

HPLC-purity of Fmoc-deprotected dodecapeptide OmA(1-12): 75 %

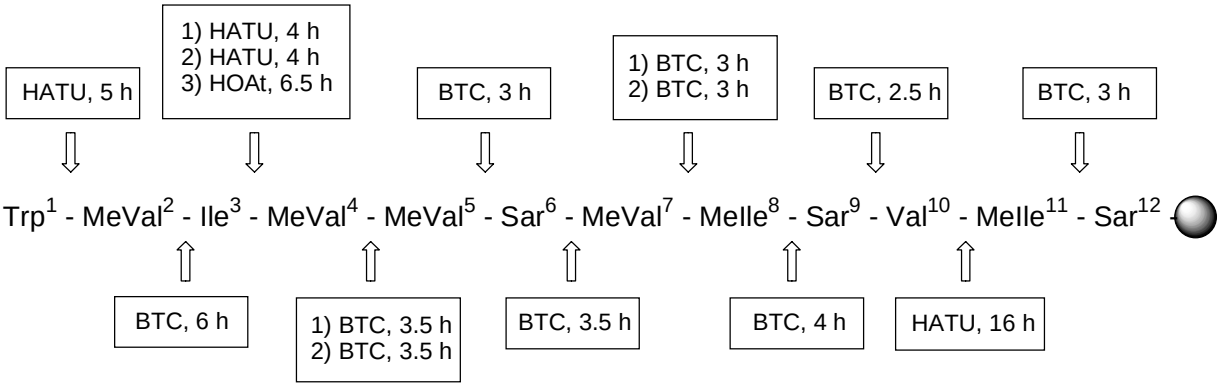

Fig. 5: Graphical synthesis outline for the linear dodecapeptide OmA(1-12).

Synthesis outline for the linear dodecapeptide OmA(10-9) with HPLC purities for intermediate peptides:

1.) Coupling MeIle -> Sar: BTC, 3 h, chloranil test negative.
2.) Coupling MeVal -> MeIle: BTC, 3 h, chloranil test negative.
3.) Coupling Sar -> MeVal: BTC, 2,5 h, chloranil test negative.
4.) Coupling MeVal -> Sar: BTC, 3 h, chloranil test negative, area of prod. peak: >98%.
5.) Coupling MeVal -> MeVal: BTC 3 h, chloranil test slightly positive.
2nd coupling BTC 3 h, chloranil test negative. area of prod. peak: > 95 %.
6.) Coupling Ile -> MeVal: HATU, 20 h, chloranil test slightly positive
2nd coupling HATU 3 h, chloranil test slightly positive
3rd coupling HOAt 16 h, chloranil test negative. area of prod. peak: ca. 90 %.
7.) Coupling MeV ->Ile: BTC, 3 h, area of prod. peak: 90 %.
8.) Coupling Trp -> MeV: HATU 4 h, area of prod. peak: 89 %.
9.) Coupling Sar -> Trp: BTC, 1 h, area of prod. peak: 86 %.
10.) Coupling MeIle -> Sar: BTC, 4 h, area of prod. peak: 83 %.
11.) Coupling Val -> MeIle: HATU, 5 h, area of prod. peak: 80 %.

(all HPLC-purities [area of prod. peak] for Fmoc-protected peptides, λ = 214 nm)
HPLC-purity of Fmoc-deprotected dodecapeptide OmA(10-9): 80 %

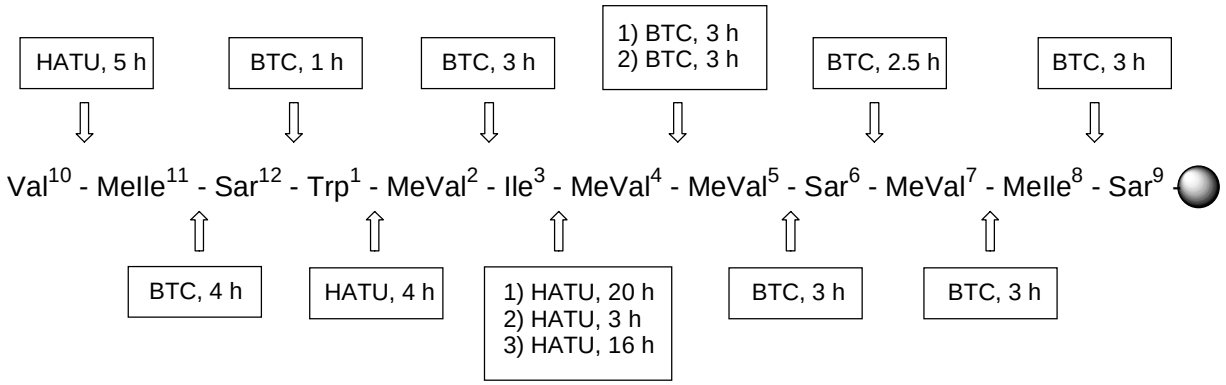

Fig. 6: Graphical synthesis outline for the linear dodecapeptide OmA(10-9).

Synthesis outline for the linear dodecapeptide OmA(7-6) with HPLC purities for intermediate peptides:

1.) Coupling MeVal -> Sar: BTC, 2,5 h, chloranil test negative.
2.) Coupling MeVal -> MeVal: BTC, 3 h, chloranil test negative.
3.) Coupling Ile -> MeVal: HATU, 3 h, chloranil test positive, peak area of tripeptide: 25 %.
    2nd coupling: HOAt, 16 h, chloranil test negative.
4.) Coupling MeVal -> Ile: BTC, 3 h, Kaiser test negative, no tetrapeptide detectable.
5.) Coupling Trp -> MeVal: HATU, 3 h, chloranil test slightly positive.
    2nd coupling HATU, 3 h, chloranil test negative, no pentapeptide detectable.
6.) Coupling Sar -> Trp: BTC, 2,5 h, Kaiser test negative, no hexapeptide detectable.
7.) Coupling MeIle ->Sar: BTC, 3 h, chloranil test negative, no heptapeptide detectable.
8.) Coupling Val -> MeIle: HATU, 4 h, chloranil test positive, peak area of octapeptide: 10 %.
    2nd coupling HOAt 16 h, chloranil test negative, no octapeptide detectable.
9.) Coupling Sar -> Val: BTC, 3 h, no nonapeptide detectable.
10.) Coupling MeIle -> Sar: BTC, 4 h, no decapeptide detectable.
11.) Coupling MeVal -> MeIle: BTC, 4 h, area of prod. peak (Fmoc-protected): 92 %.

HPLC-purity of Fmoc-deprotected dodecapeptide OmA(7-6): 90 %

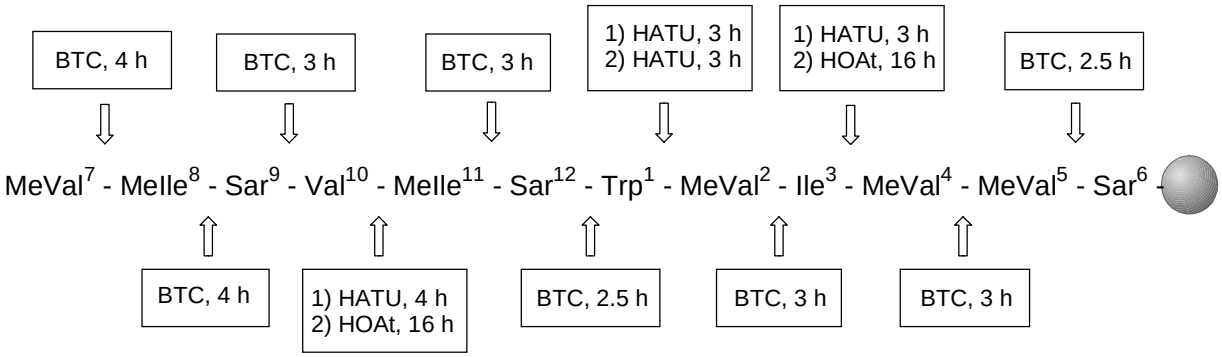

Fig. 7: Graphical synthesis outline for the linear dodecapeptide OmA(7-6).

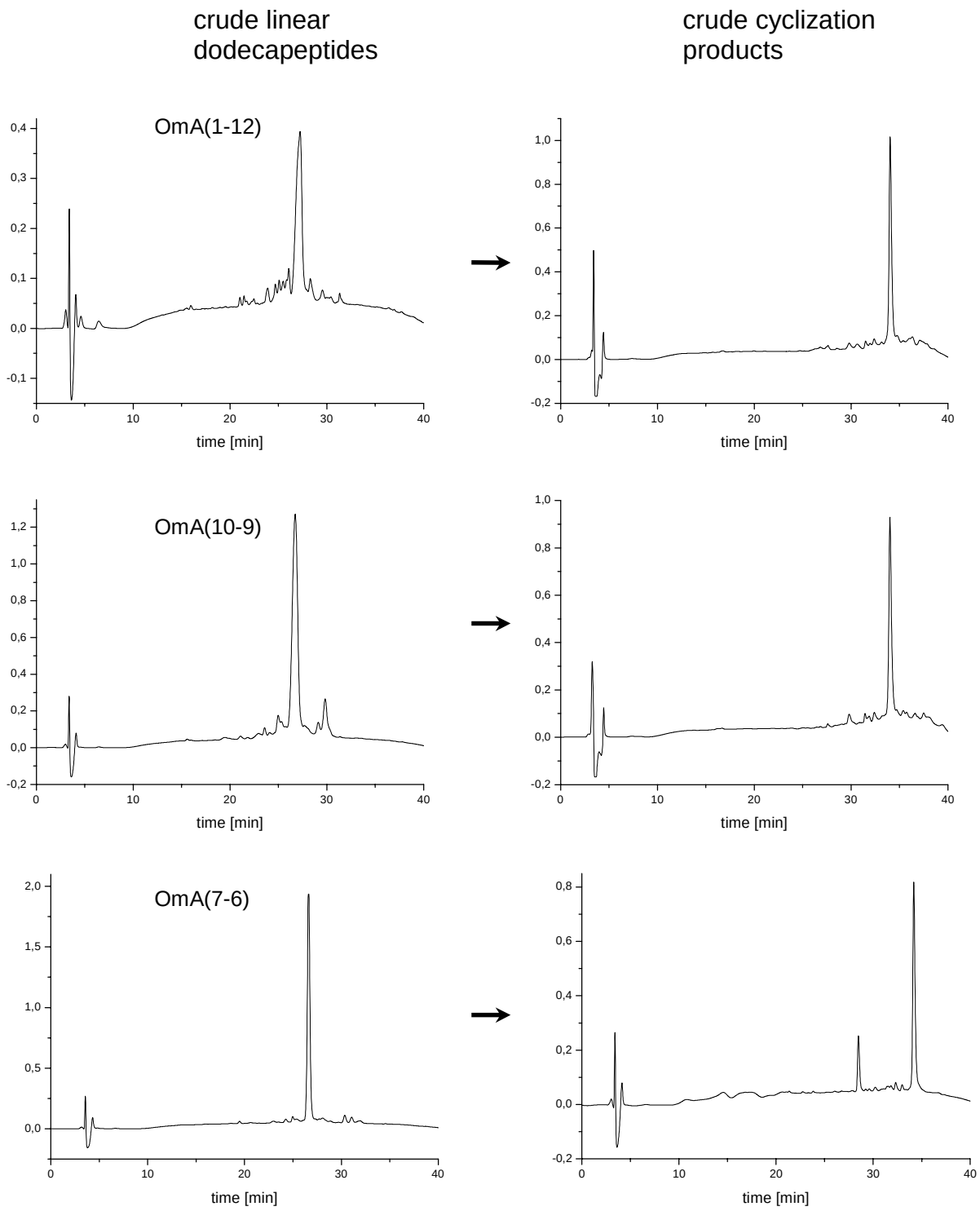

Fig. 8: Analytical chromatograms for linear dodecapeptides with *C*-terminal sarcosines (sequence position 6, 9 and 12) and their respective crude cyclization products (λ = 214 nm). No linear peptide can be found in the cyclization products, thus showing the efficiency of the employed EDCI/HOAt-cyclization protocol.

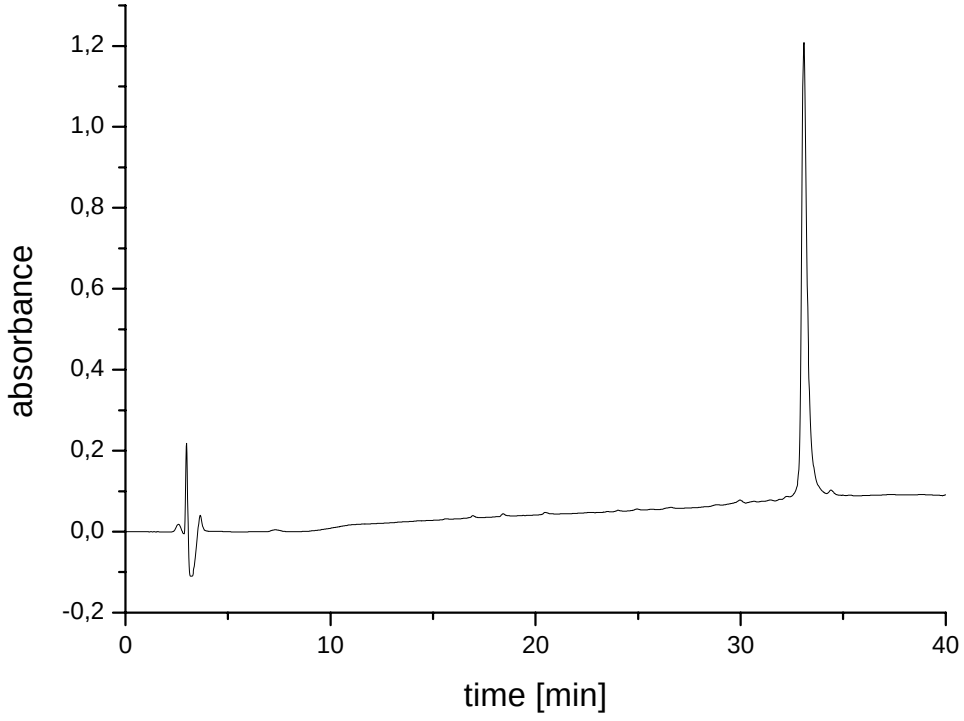

Fig. 9: Analytical Chromatogram ($\lambda = 214$ nm) of Omphalotin A purified by flash chromatography on silica. Synthetic Omphalotin A from linear dodecapeptides with C-terminal sarcosine is free of diastereomeric byproducts.

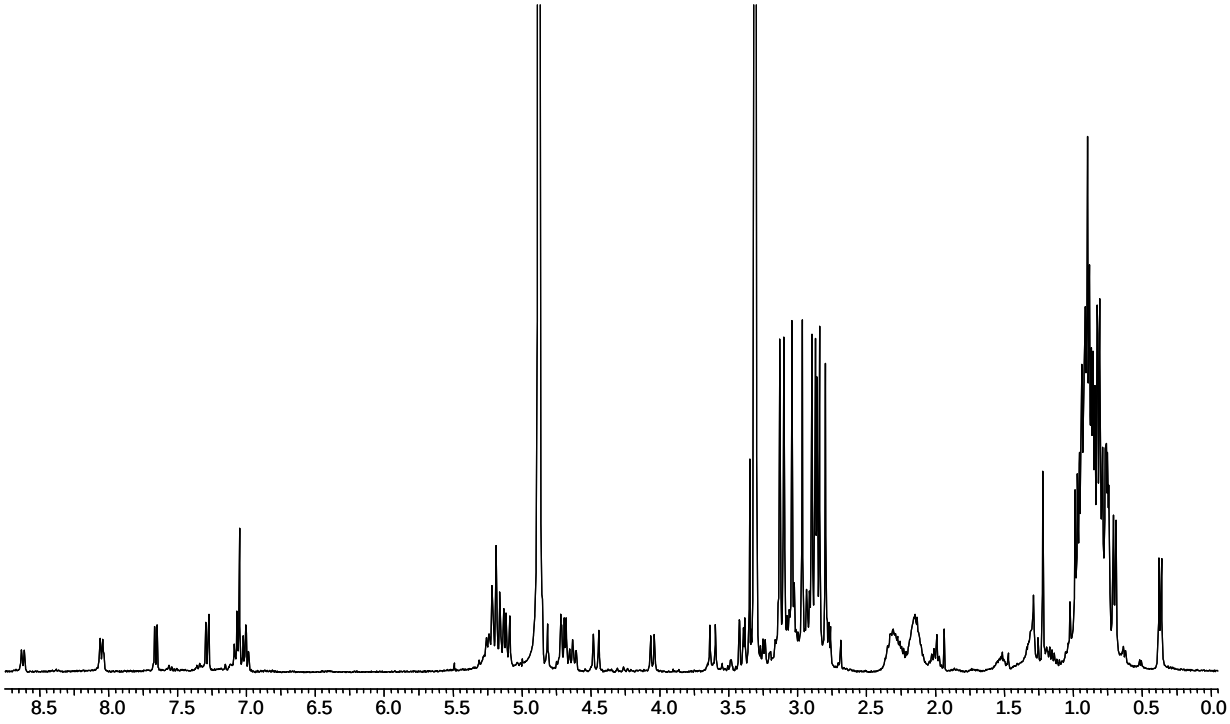

Fig. 10: ^{1}H-NMR (600 MHz, CD_3OD, 20 °C) of synthetic Omphalotin A.

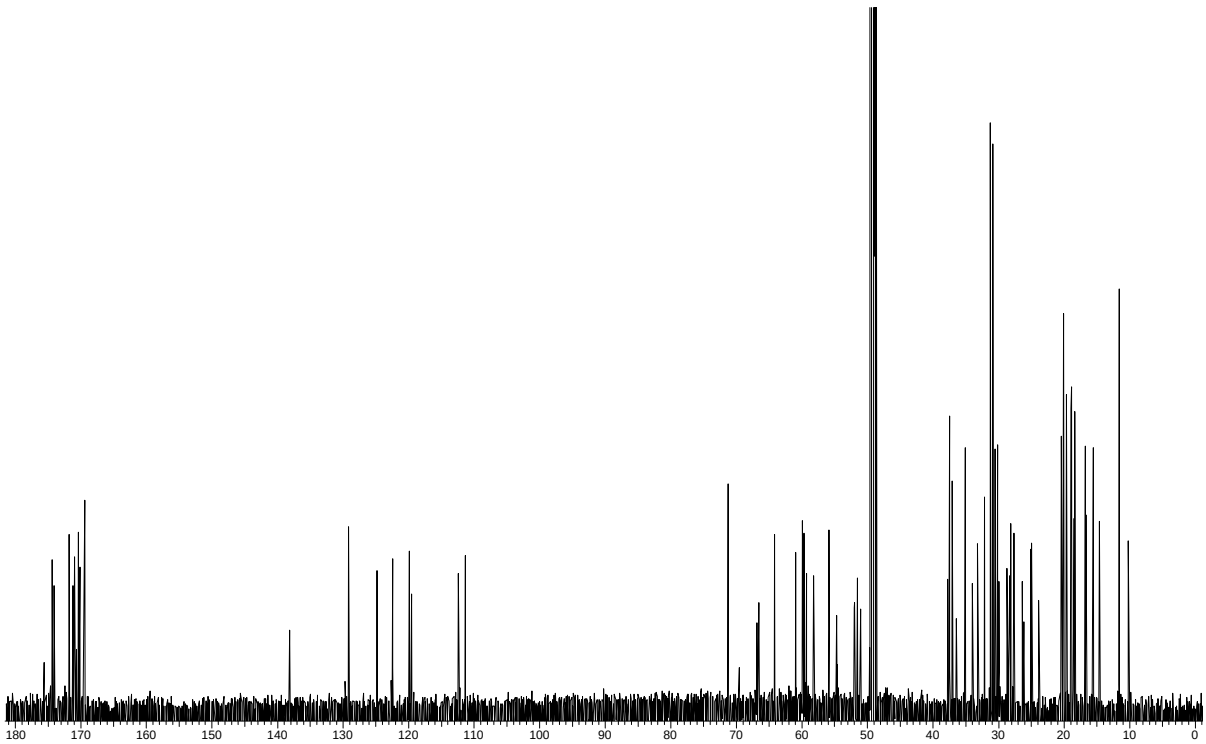

Fig. 11: ^{13}C-NMR (150 MHz, CD_3OD, 25 °C) of synthetic Omphalotin A.

NMR data of synthetic Omphalotin A:

^{1}H-NMR (600 MHz, CD_3OD, 20 °C): δ=8.61 (d, 1H, J=8.8 Hz, NH), 8.03 (d, 1H, J=8.3 Hz, NH), 7.66 (d, 1H, J=7.8 Hz, Ar-H), 7.29 (d, 1H, J=8.3 Hz, Ar-H), 7.07 (dd, 1H, J=8.8 Hz, Ar-H), 7.05 (s, 1H, Ar-H), 7.01 (dd, 1H, J=7.8, 8 Hz, Ar-H), 5.24 (dd, 1H, J=7.7 Hz, Trp^{1} α-CH), 5.21 (d, 1H, J=10.8 Hz, MeVal^{4} α-CH), 5.20 (d, 1H, J=10.8 Hz, MeVal^{5} α-CH), 5.18 (d, 1H, J=11.9 Hz, MeIle^{11} α-CH), 5.15 (d, 1H, J=10.8 Hz, MeVal^{7} α-CH), 5.10 (d, 1H, J=10.8 Hz, MeIle^{8} α-CH), 4.83 (d, 1H, J=16.5 Hz, Sar^{6} α-CH_2^{a}), 4.72 (m, 1H, Val^{10} α-CH), 4.70 (d, 1H, J=16.5 Hz, Sar^{9} α-CH_2^{a}), 4.63 (m, 1H, Ile^{3} α-CH), 4.46 (d, 1H, J=15.5 Hz, Sar^{12} α-CH_2^{a}), 4.06 (d, 1H, J=10.8 Hz, MeVal^{2} α-CH), 3.61 (d, 1H, J=16.5 Hz, Sar^{6} α-CH_2^{b}), 3.40 (d, 1H, J=16.5 Hz, Sar^{12} α-CH_2^{b}), 3.38 (d, 1H, J=16.5 Hz, Sar^{9} α-CH_2^{b}), 3.25 (m, 1H, Trp^{1} β-CH_2^{a}), 3.13 (s, 3H, MeIle^{11} N-CH_3), 3.10 (s, 3H, MeVal^{4} N-CH_3), 3.08 (m, 1H, Trp^{1} β-CH_2^{b}), 3.04 (s, 3H, Sar^{6} N-CH_3), 2.97 (s, 3H, Sar^{9} N-CH_3), 2.90 (s, 3 H, MeVal^{5} N-CH_3), 2.87 (s, 3H, MeVal^{7} N-CH_3), 2.86 (s, 3H, Sar^{12} N-CH_3), 2.84 (s, 3H, MeVal^{2} N-CH_3), 2.80 (s, 3H, MeIle^{8} N-CH_3), 2.36-2.21 (m, 3H, MeVal^{4,5,7} β-CH), 2.19-2.10 (m, 4H, MeVal^{2}, Ile^{3}, MeIle^{8,11} β-CH), 2.0 (m, 1H, Val^{10} β-CH), 1.52 (m, 2H, Ile^{3}, MeIle^{11} γ-CH_2^{a}), 1.31, 1.21 (m, 2H, MeIle^{8} γ-CH_2), 1.15 (m, 2H, Ile^{3}, MeIle^{11} γ-CH_2^{b}), 0.97-0.74 (m, 42H, C-CH_3), 0.70 (d, 3H, J=6.7 Hz, Val^{2} γ-CH_3), 0.38 (d, 3H, J=6.7 Hz, Val^{2} γ-CH_3).

^{13}C-NMR (150 MHz, CD_3OD, 25 °C): δ=174.5 (Ile^{3} CO), 174.4 (Val^{10} CO), 174.1 (Trp^{1} CO), 171.8 (MeVal^{4,5}, MeIle^{11} CO), 171.3 (MeIle^{8} CO), 171.0 (MeVal^{7} CO), 170.7 (MeVal^{2} CO), 170.4 (Sar^{9} CO), 170.2 (Sar^{12} CO), 169.4 (Sar^{6} CO), 138.2, 129.2, 124.9, 122.5, 119.9, 119.5, 112.4, 111.4 (indole-C), 66.9 (MeVal^{2} α-C), 61.0 (MeVal^{7} α-C), 60.0 (MeVal^{4} α-C), 59.7 (MeIle^{8} α-C), 59.4 (MeVal^{5} α-C), 58.2 (MeIle^{11} α-C), 55.9 (Val^{10} α-C), 54.7 (Ile^{3} α-C), 52.0 (Sar^{9} α-C, Sar^{12} α-C), 51.6 (Trp^{1} α-C), 51.1 (Sar^{6} α-C), 37.8 (Sar^{6} N-CH_3), 37.5 (Sar^{9} N-CH_3), 37.1 (Sar^{12} N-CH_3), 36.5 (Ile^{3} β-C), 33.2 (MeIle^{11} β-C), 32.1 (Val^{10} β-C), 32.0 (MeIle^{11} N-CH_3), 31.3 (MeVal^{4,5} N-CH_3), 31.1 MeIle^{8} N-CH_3), 30.9 (MeIle^{8} β-C), 30.6 (MeVal^{7} β-C), 30.2 (Trp^{1} β-C), 30.0 (MeVal^{7} N-CH_3), 29.8 (MeVal^{2} N-CH_3), 28.3 (MeVal^{2,4} β-C), 27.7 (MeVal^{5} β-C), 26.2 (Ile^{3} γ-CH_2), 25.1 (MeIle^{8} γ-CH_2), 25.0 (MeIle^{11} γ-CH_2), 20.4 (MeVal^{7} CH_3^{a}), 20.1 (MeVal^{4,5}, Val^{10} CH_3^{a}), 19.7 (MeVal^{2} CH_3^{a}), 18.9 (MeVal^{5}, Val^{10} CH_3^{b}), 18.5 (MeVal^{4} CH_3^{b}), 18.4 (MeVal^{7} CH_3^{b}), 18.2 (MeVal^{2} CH_3^{b}), 16.8 (MeIle^{8} γ-CH_3), 16.7 (MeIle^{11} γ-CH_3), 15.6 (Ile^{3} γ-CH_3), 11.7 (MeIle^{8} δ-CH_3), 11.6 (MeIle^{11} δ-CH_3), 10.2 (Ile^{3} δ-CH_3).