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Synthesis, Ion Complexation Study, and 3D-Structural Analysis of Peptides Based on Crown-Carrier, C^{α}-Methyl-L-DOPA Amino Acids

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Selected series of terminally protected model peptides to the hexamer level, based on four novel, crown-ether containing C^{α}-methyl-L-DOPA (L-Mdp) amino acid residues, namely L-Mdp[15-C-5], L-Mdp[18-C-6], L-Mdp[benzo-24-C-8] and L-Mdp[(*S*)-Binol-20-C-6], combined with either L-Ala or L-Ala/Aib or Gly/Aib, were synthesized by solution methods. An ESI-MS analysis of their alkali metal cation complexation ability was carried out. Their FTIR absorption, ^{1}H NMR, ECD, and VCD spectroscopic properties suggest that all of these crowned amino acids are strong inducers of (left-handed) β-turns and 3_{10}-helical structures.

Introduction

Peptides are ideally suited to serve as spacers and templates in studies of probe···probe interactions and supramolecular chemistry because of the commercial availability and chirality of their amino acid building blocks, the potential control of their secondary structures, and the relative ease of their synthesis and purification/characterization procedures.[1–5] In this connection, synthetic C^{α}-trisubstituted α-amino acids bearing crown-ether side-chain moieties have been shown to be of relevant interest for the preparation of ion-selective molecular receptors and artificial ion channels. They can be easily assembled in structurally well-defined, nanometer-scale, architectures of bis-crown as well as polymeric crown compounds on a peptide framework.[4–6] In the past few years, a series of crown-carrier derivatives of L-DOPA,[4–16] L-Lys,[17] or L-Glu,[18] have been synthesized for the construction of specific peptide receptors for alkali metal, ammonium and di-ammonium ions. Application of this concept to C^{α}-tetrasubstituted α-amino acids should result in more defined and predictable peptide structures, as these residues are known to be able to explore a very limited amount of the conformational space available to C^{α}-trisubstituted α-amino acids, and to strongly induce folded, turn and helical conformations in short peptide backbones.[2,3,19–29] Accordingly, we have previously described the synthesis of Bip[20-C-6],[30,31] the first crown-carrier, axially dissymmetric, C^{α}-tetrasubstituted α-amino acid having a 2,2′,6,6′-tetrasubstituted-1,1′-biphenyl frame. However, we were unable to prepare this amino acid in enantiomerically pure state because of extensive racemization occurring at several stages of the synthesis. Therefore, only limited structural information on some of its peptide derivatives could be obtained.[31] Later on, other groups synthesized indane-based,[32] as well as C^{α}-hydroxymethyl-serine-based,[33,34] achiral, C^{α}-tetrasubstituted α-amino acid derivatives with various crown-ether side chains. As for 3D-structural analyses, however, only two X-ray diffraction studies of single-crowned amino acids and doubly crowned 2,5-dioxopiperazines were performed.[33,34] Furthermore, these investigations, albeit of some interest, could not afford any valuable information on the preferred conformations of these residues due to the very short main-chain length of the compounds examined.

In this paper, we wish to present our detailed results on synthesis in solution, alkali metal ion complexation, and conformational analysis by FT-IR absorption, ^{1}H NMR, EDC (electronic circular dichroism), and VCD (vibrational circular dichroism) techniques of peptides based on novel crown-carrier, C^{α}-tetrasubstituted, α-amino acids with α-carbon chirality (instead of axial chirality), namely L-Mdp[15-C-5],[35] L-Mdp[18-C-6],[35] L-Mdp[benzo-24-C-

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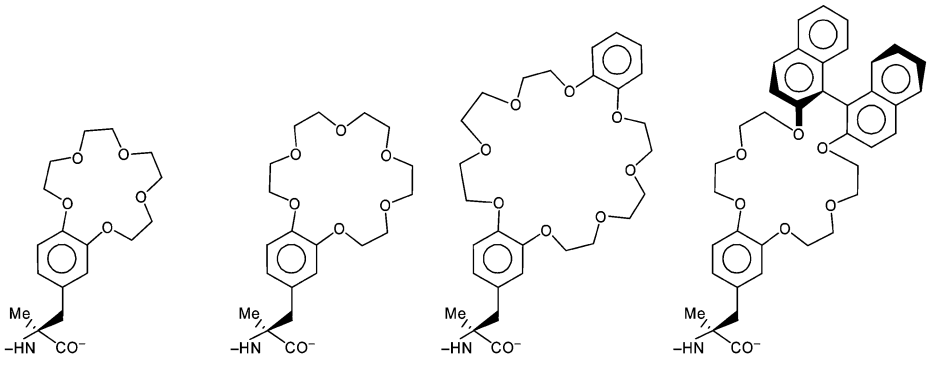


L-Mdp[15-C-5] L-Mdp[18-C-6] L-Mdp[benzo-24-C-8] L-Mdp[*(S)*-Binol-20-C-6]

(a) L-Mdp[15-C-5] / L-Ala / Aib series*

Boc-L-Mdp[15-C-5]-OMe	**1a****
Boc-Aib-L-Mdp[15-C-5]-L-Ala-OMe	**3a**
Fmoc-L-Ala-Aib-L-Mdp[15-C-5]-L-Ala-OMe	**4a**
Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe	**6a**

(b) L-Mdp[15-C-5] / Gly / Aib series

Boc-Aib-L-Mdp[15-C-5]-Gly-OMe	**3b**
Boc-{Aib-L-Mdp[15-C-5]-Gly}$_2$-OMe	**6b**

(c) L-Mdp[18-C-6] / L-Ala series

Boc-L-Mdp[18-C-6]-OMe	**1c**
Boc-L-Mdp[18-C-6]-L-Ala-OMe	**2c**
Fmoc-L-Ala-L-Mdp[18-C-6]-L-Ala-OMe	**3c**
Fmoc-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe	**4c**
Boc-L-Mdp[18-C-6]-(L-Ala)$_2$-L-Mdp[18-C-6]-L- Ala-OMe	**5c**
Fmoc-{L-Ala-L-Mdp[18-C-6]-L-Ala}$_2$-OMe	**6c**

(d) L-Mdp[18-C-6] / L-Ala / Aib series

Boc-Aib-L-Mdp[18-C-6]-L-Ala-OMe	**3d**
Fmoc-L-Ala-Aib-L-Mdp[18-C-6]-L-Ala-OMe	**4d**
Boc-{Aib-L-Mdp[18-C-6]-L-Ala}$_2$-OMe	**6d**

(e) L-Mdp[benzo-24-C-8] / L-Ala / Aib series

Boc-L-Mdp[benzo-24-C-8]-OMe	**1e**
Boc-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe	**3e**
Fmoc-L-Ala-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe	**4e**
Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe	**6e**

(f) L-Mdp[*(S)*-Binol-20-C-6] / L-Ala series

Z-L-Mdp[*(S)*-Binol-20-C-6]-OMe	**1f**
Z-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala-OMe	**2f**
Fmoc-L-Ala-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala-OMe	**3f**
Z-(L-Ala)$_2$-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala-OMe	**4f**
Z-L-Mdp[*(S)*-Binol-20-C-6]-(L-Ala)$_2$-L-Mdp[*(S)*-Binol-20-C-6]-L- Ala-OMe	**5f**
Fmoc-{L-Ala-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala}$_2$-OMe	**6f**

(g) L-Mdp[*(S)*-Binol-20-C-6] / L-Ala / Aib series

Boc-Aib-L-Mdp[*(S)*-Binol-20-C-6]-OH	**2g**
Boc-Aib-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala-OMe	**3g**
Fmoc-L-Ala-Aib-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala-OMe	**4g**
Boc-{Aib-L-Mdp[*(S)*-Binol-20-C-6]-L-Ala}$_2$-OMe	**6g**

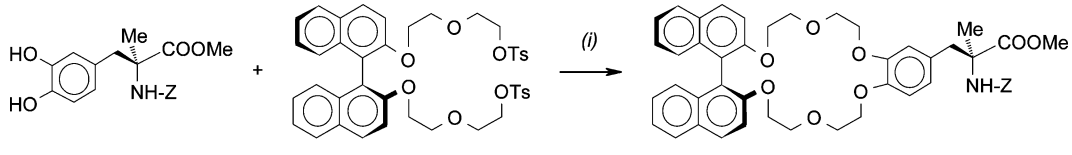
Scheme 1. Chemical structures of the crown-carrier C^{α}-methyl-L-DOPA residues, and list of amino acid derivatives and peptides discussed in this work. * Aib = α-aminoisobutyric acid, Binol = 2,2′-dihydroxy-1,1′-binaphthyl, Boc = *tert*-butyloxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl, Mdp = C^{α}-methyl-DOPA (α-methyl-β-3,4-dihydroxyphenylalanine), OMe = methoxy, Z = benzyloxycarbonyl. ** In each entry the number indicates how many amino acid residues characterize the primary structure of the peptide, while the letter indicates the type of peptide series.

8],[35] and L-Mdp[(*S*)-Binol-20-C-6],[36] prepared from L-Mdp following a route established by Voyer and co-workers[4–16] in the case of L-DOPA, combined with either L-Ala or L-Ala/Aib or Gly/Aib residues, to the hexamer level. The amino acids and peptides examined in this study are listed in Scheme 1. Preliminary reports of part of this work have been published.[37,38]

Results and Discussion

Synthesis

The syntheses and characterizations of the starting, Boc/OMe terminally protected, crowned α-amino esters **1a**, **1c**, and **1e** (Scheme 1), and the corresponding *N*-Boc α-amino acids Boc-L-Mdp[15-C-5]-OH, Boc-L-Mdp[18-C-6]-OH, and Boc-L-Mdp[benzo-24-C-8]-OH have been previously reported.[35] In addition, Z-L-Mdp[(*S*)-Binol-20-C-6]-OMe (**1f**)[36] was prepared in 60% yield by double alkylation with cyclization of the catechol function of Z-L-Mdp-OMe, using Cs_2CO_3 in DMF (dimethylformamide) as a base and (–)-(*S*)-Binol[$(OCH_2CH_2)_2OTs]_2$ [2,2′-bis(5-tosyloxy-3-oxa-1-pentyloxy)-1,1′-binaphthyl][39] as the alkylating agent (Scheme 2). The terminally protected Z-L-Mdp-OMe reagent was prepared in 67% yield by treatment of H-L-Mdp-OMe with Z-OSu [1-(benzyloxycarbonyloxy)succinimide] in acetonitrile, followed by reaction with pyrrolidine in dichloromethane in view of the selective *O*-deacylation of the side



Z-L-Mdp-OMe (-)-(*S*)-Binol[$O(CH_2CH_2)_2OTs]_2$ Z-L-Mdp[*(S)*-Binol-20-C-6]-OMe **1f**

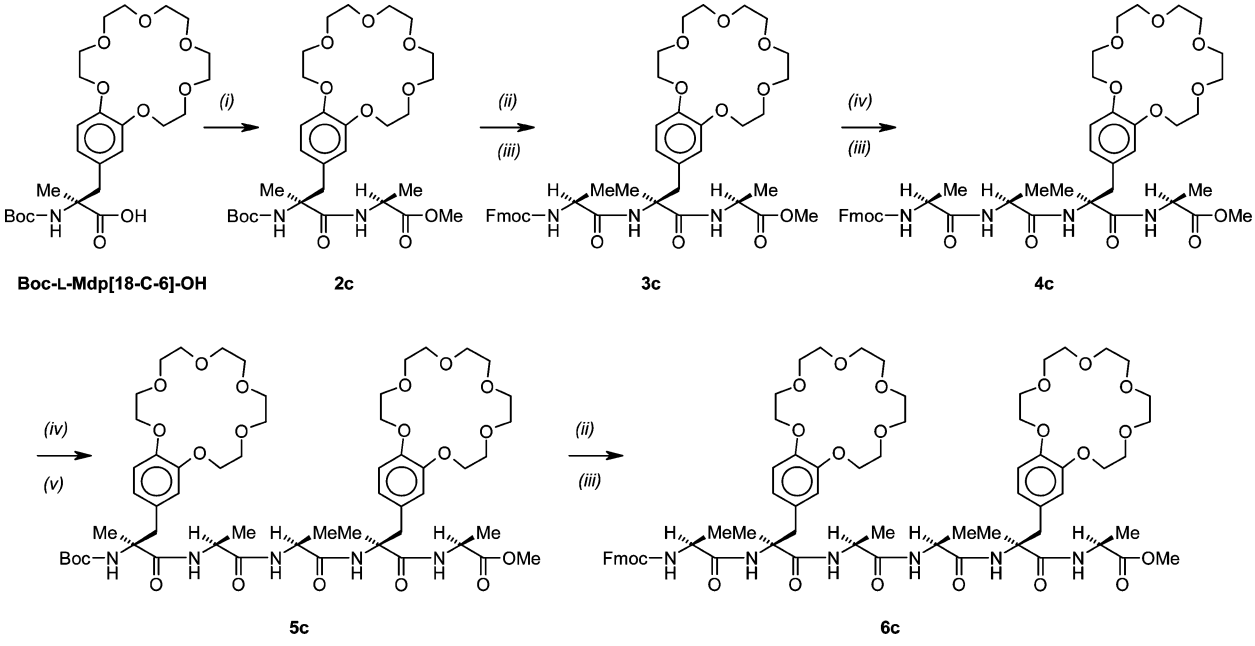
Scheme 2. Synthesis of Z-L-Mdp[(*S*)-Binol-20-C-6]-OMe (**1f**) (*i*) Cs_2CO_3, DMF, 60 °C, 20 h.

product Z-Mdp[*O*-Z,*O*-H]-OMe, in a similar manner as previously reported.[35] Saponification of **1f** in 1 N NaOH/MeOH (methanol) gave its *C*-deprotected derivative Z-L-Mdp[(*S*)-Binol-20-C-6]-OH in 60% yield.

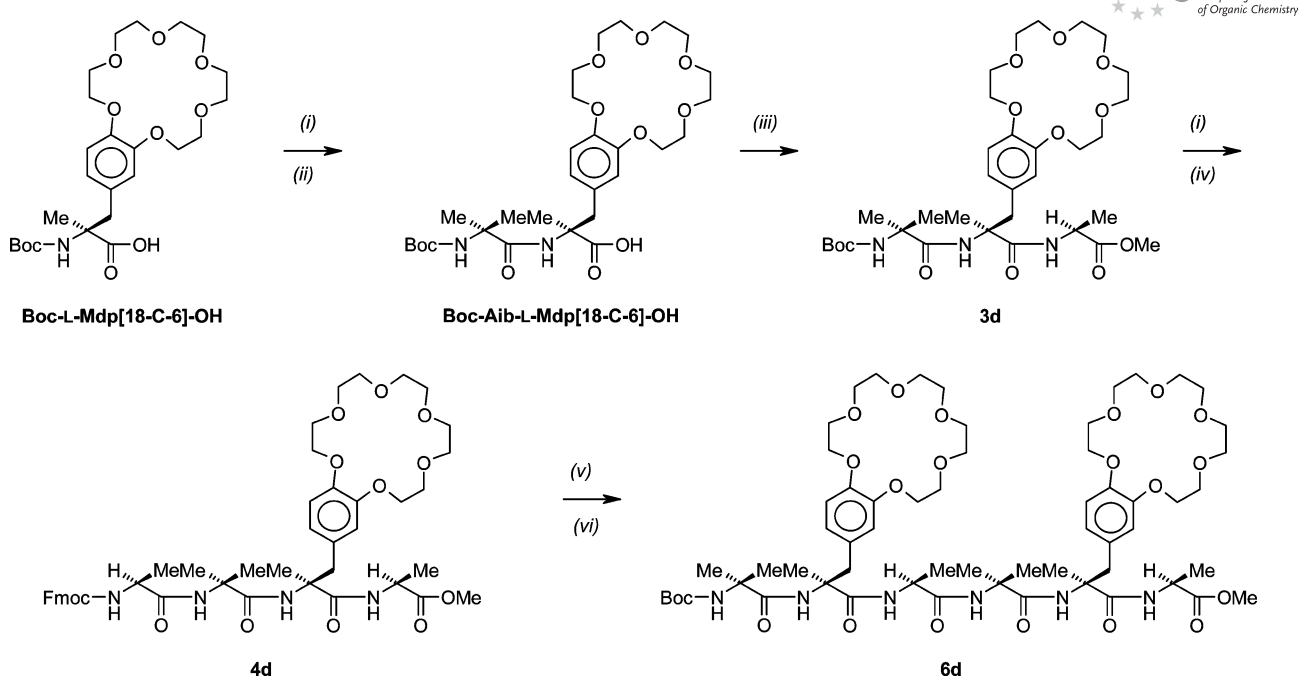
Peptides based on these crowned amino acids, combined with either L-Ala, or L-Ala/Aib, or Gly/Aib to hexamer level (in which two crowned Mdp residues are located at the *i* and *i*+3 positions of the sequence) were prepared by solution methods. The urethane-protected *N*-carboxyanhydrides (UNCAs)[40,41] Fmoc-L-Ala-NCA, Boc-Gly-NCA, and Boc-Aib-NCA were used for coupling at the N-terminus of either the crowned Mdp or the Aib residue, while couplings of both H-Gly-OMe and H-L-Ala-OMe at the C-terminus of the *N*-protected crowned Mdp residues were performed by the EDC [*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide]/HOAt (1-hydroxy-7-aza-1,2,3-benzotriazole)[42] activation method. These methods are known to be efficient in difficult steps involving sterically demanding, C^{α}-tetrasubstituted, α-amino acids.[43]

As for the L-Mdp[18-C-6]/L-Ala (**c**) series (Scheme 3), coupling of Boc-L-Mdp[18-C-6]-OH with H-L-Ala-OMe in the presence of an excess of NMM (*N*-methylmorpholine) by the EDC/HOAt method gave **2c**, which was *N*-deprotected in TFA (trifluoroacetic acid)/CH_2Cl_2 and then coupled with Fmoc-L-Ala-NCA in THF (tetrahydrofuran), in the presence of an excess of DIEA (*N*,*N*-diisopropylethylamine), to afford the tripeptide Fmoc-L-Ala-L-Mdp[18-C-6]-L-Ala-OMe **3c**, as previously reported.[35] Coupling of Fmoc-L-Ala-NCA at the deprotected N-terminus of **3c** (the UNCA method, although not strictly required for such an Ala-Ala coupling, was used for convenience), gave Fmoc-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe **4c** in 64% yield. Stepwise synthesis was pursued by coupling Boc-L-Mdp[18-C-6]-OH at the deprotected N-terminus of **4c** by the EDC/HOAt method to give Boc-L-Mdp[18-C-6]-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe **5c** (51% yield), and then Fmoc-L-Ala-NCA at the deprotected (TFA/CH_2Cl_2) N-terminus of **5c** to furnish the hexapeptide Fmoc-{L-Ala-L-Mdp[18-C-6]-L-Ala}$_2$-OMe **6c** in 64% yield. In a similar manner for the L-Mdp[(*S*)-Binol-20-C-6]/L-Ala (**f**) series, coupling of Z-L-Mdp[(*S*)-Binol-20-C-6]-OH with H-L-Ala-OMe by the EDC/HOAt method gave Z-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe **2f** (30% yield), which was *N*-deprotected by hydrogenolysis and then coupled with Fmoc-L-Ala-NCA in the presence of DIEA to give the tripeptide Fmoc-L-Ala-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (**3f**) (50% yield). Coupling of Z-L-Ala-OH at the deprotected (Et_2NH/CH_3CN/CH_2Cl_2) N-terminus of **3f** by the EDC/HOBt (1-hydroxy-1,2,3-benzotriazole)[44] method gave Z-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (**4f**) (47% yield). Coupling of Z-L-Mdp[(*S*)-Binol-20-C-6]-OH at the deprotected (H_2/Pd-C) N-terminus of **4f** by the EDC/HOAt method furnished Z-L-Mdp[(*S*)-Binol-20-C-6]-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (**5f**) (41% yield), and coupling of Fmoc-L-Ala-NCA at the deprotected (H_2/Pd-C) N-terminus of **5f** afforded the hexapeptide Fmoc-{L-Ala-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala}$_2$-OMe (**6f**) (35% yield).

For the L-Mdp[18-C-6]/L-Ala/Aib (**d**) series (Scheme 4), the starting amino acid derivative Boc-L-Mdp[18-C-6]-OH was first *N*-deprotected in TFA/CH_2Cl_2 to give the free amino acid trifluoroacetate salt, which was successfully acylated at its N-terminus using Boc-Aib-NCA to afford the dipeptide Boc-Aib-L-Mdp[18-C-6]-OH, and then, after coupling with H-Ala-OMe by the EDC/HOAt method, to furnish the tripeptide Boc-Aib-L-Mdp[18-C-6]-L-Ala-OMe (**3d**).[35] As mentioned above, coupling of Fmoc-L-Ala-NCA



Scheme 3. Synthesis of the L-Mdp[18-C-6]/L-Ala peptide series **2c**–**6c**. (*i*) HCl·H-L-Ala-OMe, NMM, EDC, HOAt, CH_2Cl_2, 0 °C to room temp. (*ii*) TFA/CH_2Cl_2, 1:3, 0 °C to room temp. (*iii*) Fmoc-L-Ala-NCA, DIEA, THF, 0 °C to room temp. (*iv*) 10% (v/v) Et_2NH/CH_3CN, room temp. (*v*) Boc-L-Mdp[18-C-6]-OH, EDC, HOAt, CH_2Cl_2, 0 °C to room temp.



Scheme 4. Synthesis of the L-Mdp[18-C-6]/L-Ala/Aib peptide series **3d**–**6d**. (*i*) TFA/CH_2Cl_2, 1:3, 0 °C to room temp. (*ii*) Boc-Aib-NCA, DIEA, THF, 0 °C to room temp. (*iii*) HCl·H-L-Ala-OMe, NMM, EDC, HOAt, CH_2Cl_2, 0 °C to room temp. (*iv*) Fmoc-L-Ala-NCA, DIEA, THF, 0 °C to room temp. (*v*) 10% (*v*/*v*) Et_2NH/CH_3CN, room temp. (*vi*) Boc-Aib-L-Mdp[18-C-6]-OH, EDC, HOAt, CH_2Cl_2, 0 °C to room temp.

at the deprotected N-terminus of **3d** gave Fmoc-L-Ala-Aib-L-Mdp[18-C-6]-L-Ala-OMe (**4d**) in 73% yield. At that stage, a 2+4 segment condensation (instead of a stepwise coupling) was adopted: the dipeptide Boc-Aib-L-Mdp[18-C-6]-OH, structurally not prone to racemization at its C-terminal C^{α}-tetrasubstituted α-amino acid through activation, was coupled with the *N*-deprotected (Et_2NH/CH_3CN) **4d** by the EDC/HOAt method to afford the desired hexapeptide Boc-{Aib-L-Mdp[18-C-6]-L-Ala}$_2$-OMe **6d** in 40% yield.

The same strategy of segment condensation was applied to the other series of peptides: L-Mdp[15-C-5]/L-Ala/Aib (**a**), L-Mdp[15-C-5]/Gly/Aib (**b**), L-Mdp[benzo-24-C-8]/L-Ala/Aib (**e**), and Z-L-Mdp[(*S*)-Binol-20-C-6]/L-Ala/Aib (**g**). Briefly, the crude free amino acid trifluoroacetate salt, resulting from *N*-deprotection (TFA/CH_2Cl_2) of Boc-L-Mdp[15-C-5]-OH, was acylated at its N-terminus with Boc-Aib-NCA to afford the dipeptide Boc-Aib-L-Mdp[15-C-5]-OH in 67% yield. This compound was coupled with a large excess of either H-L-Ala-OMe or H-Gly-OMe by the EDC/HOAt method to furnish the tripeptides **3a** (53% yield) and **3b** (70% yield), respectively. Acylation at the deprotected N-terminus of **3a** with Fmoc-L-Ala-NCA gave Fmoc-L-Ala-Aib-L-Mdp[15-C-5]-L-Ala-OMe **4a** in 56% yield, and 2+4 segment condensation of Boc-Aib-L-Mdp[15-C-5]-OH with *N*-deprotected **4a** by the EDC/HOAt method afforded the hexapeptide Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe **6a** in 45% yield. On the other hand, for the L-Mdp[15-C-5]/Gly/Aib (**b**) series, the tripeptide Boc-Aib-L-Mdp[15-C-5]-Gly-OMe (**3b**), structurally not prone to racemization, allowed its activation in a (3+3) segment condensation step. Accordingly, **3b** was saponified in 1 N NaOH/MeOH to give Boc-Aib-L-Mdp[15-C-5]-Gly-OH in 89% yield (crude). Another portion of **3b** was *N*-deprotected (TFA/CH_2Cl_2) and the two tripeptide segments were coupled by the EDC/HOAt method to afford the hexapeptide Boc-{Aib-L-Mdp[15-C-5]-Gly}$_2$-OMe (**6b**) in 29% yield. For the (**e**) series, the crude free amino acid trifluoroacetate salt resulting from *N*-deprotection (TFA/CH_2Cl_2) of Boc-L-Mdp[benzo-24-C-8]-OH was acylated at its N-terminus with Boc-Aib-NCA in THF to afford the dipeptide Boc-Aib-L-Mdp[benzo-24-C-8]-OH in 50% yield. This compound was coupled with a large excess of H-L-Ala-OMe by the EDC/HOAt method to give a mixture of tripeptide Boc-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (**3e**) (*ca* 85–90% yield) and 1-chloromethyloxy-7-aza-1,2,3-benzotriazole ($ClCH_2OAt$).[35] This mixture could be used directly in the next steps of *N*-deprotection and then acylation with Fmoc-L-Ala-NCA to give **4e** in 75% overall yield after purification by chromatography. Finally, 2+4 segment condensation of Boc-Aib-L-Mdp[benzo-24-C-8]-OH with *N*-deprotected **4e** by the EDC/HOAt method gave the hexapeptide Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (**6e**) in 53% yield. For the (**g**) series, the crude free amino acid resulting from *N*-deprotection (by hydrogenolysis with H_2/Pd-C) of Z-L-Mdp[(*S*)-Binol-20-C-6]-OH was acylated at its N-terminus with Boc-Aib-NCA to afford the dipeptide Boc-Aib-L-Mdp[(*S*)-Binol-20-C-6]-OH (**2g**) in 72% yield. This compound was coupled with a large excess of H-L-Ala-OMe by the EDC/HOAt method to furnish the tripeptide Boc-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (**3g**) in 68% yield. Acylation of *N*-deprotected (TFA/CH_2Cl_2) **3g** with Fmoc-

L-Ala-NCA gave Fmoc-L-Ala-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (**4g**) in 62% yield, and 2+4 segment condensation of **2g** with *N*-deprotected **4g** by the EDC/HOAt method gave the hexapeptide Boc-{Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala}$_2$-OMe (**6g**) in 32% yield.

ESI-MS Screening of Alkali Metal Ion Complexation

Electrospray-ionization mass spectroscopy (ESI-MS) was used for the screening of alkali metal ion (Li^+, Na^+, K^+, Rb^+, Cs^+) complexation by the crowned tripeptides Boc-Aib-L-Mdp[15-C-5]-L-Ala-OMe (**3a**), Boc-Aib-L-Mdp[18-C-6]-L-Ala-OMe (**3d**), Boc-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (**3e**) and the corresponding hexapeptides Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe (**6a**), Boc-{Aib-L-Mdp-[18-C-6]-L-Ala}$_2$-OMe (**6d**), Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (**6e**). In the emerging ESI-MS technique, also recently applied to complexation by bis(crown ethers),[45–47] the complexes that are observed in the gas phase reflect the types and distributions of those present in solution.[48–51]

The first set of experiments was carried out with 9:1 CH_3CN/H_2O solutions containing each tripeptide (0.1 mM)/-hexapeptide (0.1 mM) couple (**3a**,**6a**; or **3d**,**6d**; or **3e**,**6e**) and an equimolar mixture of the five alkali metal chlorides, each in twofold excess (0.2 mM). For the mono-crowned tripeptides, major mass peaks arising from the 1:1 ion-crown (tripeptide) complexes were observed, with neither peaks originating from 1:2 ion–crown complexes nor $[M + H]^+$ peaks arising from uncomplexed crowns being present. The selective cation binding ability was obtained for each tripeptide by dividing the respective peak height by that of the most intense base peak (Figure 1). The observed sequences Na^+ (100) > K^+ (74) > Li^+ (48) > Rb^+ (40) > Cs^+ (23) for [15-C-5]-**3a**, K^+ (100) > Rb^+ (38) > Na^+ (11) ≈ Cs^+ (11) > Li^+ (0) for [18-C-6]-**3d**, and Rb^+ (100) > Cs^+ (91) > K^+ (83) > Na^+ (23) > Li^+ (8) for [benzo-24-C-8]-**3e** are very close to the published, corresponding sequences for *N*-Z-protected [15-C-5]- and [18-C-6]-crowned C^{α}-tetrasubstituted α-amino esters,[33,34] as well as [15-C-5]-, [18-C-6]-

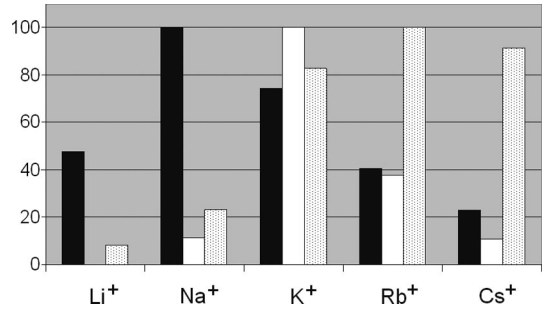


Figure 1. Histogram showing the selective cation binding ability of the mono-crowned tripeptides Boc-Aib-L-Mdp[15-C-5]-L-Ala-OMe (**3a**) (black), Boc-Aib-L-Mdp[18-C-6]-L-Ala-OMe (**3d**) (white) and Boc-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (**3e**) (dotted) vs. Li^+, Na^+, K^+, Rb^+, and Cs^+ ions.

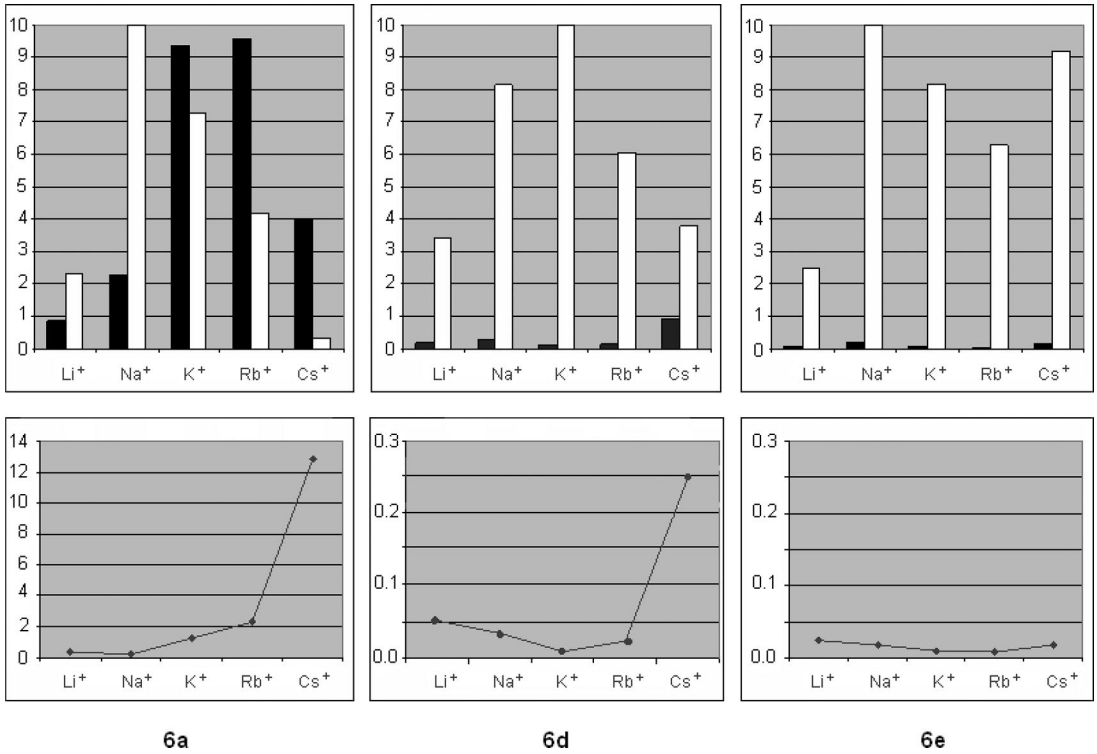


Figure 2. Histograms (upper part) showing the percentage of mono-charged 1:1 (black) vs. the double-charged 2:1 (white) ion–hexapeptide complexes of the doubly crowned hexapeptides Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe (**6a**), Boc-{Aib-L-Mdp[18-C-6]-L-Ala}$_2$-OMe (**6d**), Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (**6e**) with Li^+, Na^+, K^+, Rb^+, and Cs^+ ions, and plots (lower part) of the corresponding ratios *r* = 1:1/2:1 complexes.

and [quino/benzo-24-C-8]-quino/benzocrown ethers,[52] all determined by the FAB-MS or the ESI-MS method.

For the doubly crowned hexapeptides, the mass spectra recorded under the above conditions were difficult to analyse, because of the occurrence of a variety of 2:1 mixed ions-hexapeptide complexes (Li^+/Na^+, Li^+/Rb^+, Li^+/Cs^+, Na^+/K^+, Na^+/Rb^+, Na^+/Cs^+, ...) (results not shown). Therefore, we switched to a second set of experiments in which 9:1 CH_3CN/H_2O solutions for each tripeptide (0.1 mM)-hexapeptide (0.1 mM) couple (**3a**,**6a**; or **3d**,**6d**; or **3e**,**6e**) and *only one alkali metal chloride* in large excess (1 mM) were injected. Using this approach, the *r* ratio between the mono-charged 1:1 and the double-charged 2:1 ion-hexapeptide complexes could be compared for each of the five alkali metal cations and for each hexapeptide **6a**, **6d** and **6e** (Figure 2).

It clearly appears that the *r* ratio depends on both the size of the alkali metal cation and the size of the crown ether. Indeed, while *r* is low (<0.025) for the bis[benzo-24-C-8] peptide **6e** whatever the cation, it is higher than 1 for the bis[15-C-5] peptide **6a** vs. only the biggest cations K^+ (1.3), Rb^+ (2.3) and Cs^+ (12.9), as compared to Li^+ (0.4) and Na^+ (0.2). For the medium-sized crown-ether bis[18-C-6] peptide **6d** the *r* ratio is low for Li^+ (0.05), Na^+ (0.03), K^+ (0.01) and Rb^+ (0.02), but is ca. 10-fold increased for Cs^+ (0.2), known for a long time to be able to bind as a sandwich complex between two 18-crown-6 ligands.[53] Similar bis-crown effects, determined either potentiometrically or by means of extraction methods, have been previously reported in several cases involving bis(azacrown ethers),[54] bis(benzocrown ethers),[55,56] bis(benzoazacrown ethers),[57] and as a highlight reference, the bis-crown ether peptides reported by Voyer and co-workers.[4,9]

Altogether, our ESI-MS results strongly suggest the occurrence of cooperativity between the two crown-ether side chains at *i* and *i*+3 positions of the hexapeptide main chains, as expected for 3_{10}-helical secondary structures. The bis-crown effect is especially pronounced for the hexapeptide **6a** which contains the smallest [15-C-5] crown ether, but it also occurs for **6d** with the middle-size [18-C-6] crown ether in the presence of the largest cation Cs^+. For the alkali metal cations of smaller size, this effect is probably disfavored in our experiments by the presence of a large excess of alkali metal chloride, favoring the formation of 2:1 ion-hexapeptide complexes as soon as the relative sizes of the host-guest dyad are compatible.

Conformational Analysis

We studied the conformational preferences of the seven, terminally protected, crown-carrier, L-Mdp peptide series in structure supporting solvents ($CDCl_3$, MeOH, 2,2,2-trifluoroethanol = TFE) by use of FTIR absorption, ^1H NMR, ECD, and VCD spectroscopic techniques.

In part A of Figure 3, the FTIR absorption curves in the amide A (N–H stretching) region of the L-Mdp[(*S*)-Binol-20-C-6]/L-Ala peptide series (**1f**–**6f**) in $CDCl_3$ solution are reported, while part B of Figure 3 shows the curves of the three highest oligomers (**3g**, **4g**, and **6g**) of the L-Mdp[(*S*)-Binol-20-C-6]/L-Ala/Aib series in the same solvent. In these two typical Mdp series an intense band below 3400 cm^{-1}, associated with H-bonded -CONH- groups,[58–60] is already present at the trimer level. Relative to the band at about 3425 cm^{-1}, assigned to free (solvated) -CONH- groups, the band below 3400 cm^{-1} is remarkably more intense in the **3g** oligomer, characterized by two C^{α}-methylated residues (Aib and Mdp), than in the **3f** oligomer (with one Mdp only). Upon main-chain elongation, the relative intensity of the low-frequency band increases, although irregularly (depending upon the nature of the added amino acid, whether C^{α}-methylated or not). A correspondent shift of the absorption maximum to lower frequency is also clearly visible. In the concentration range 0.1 mM–10 mM the spectra of the

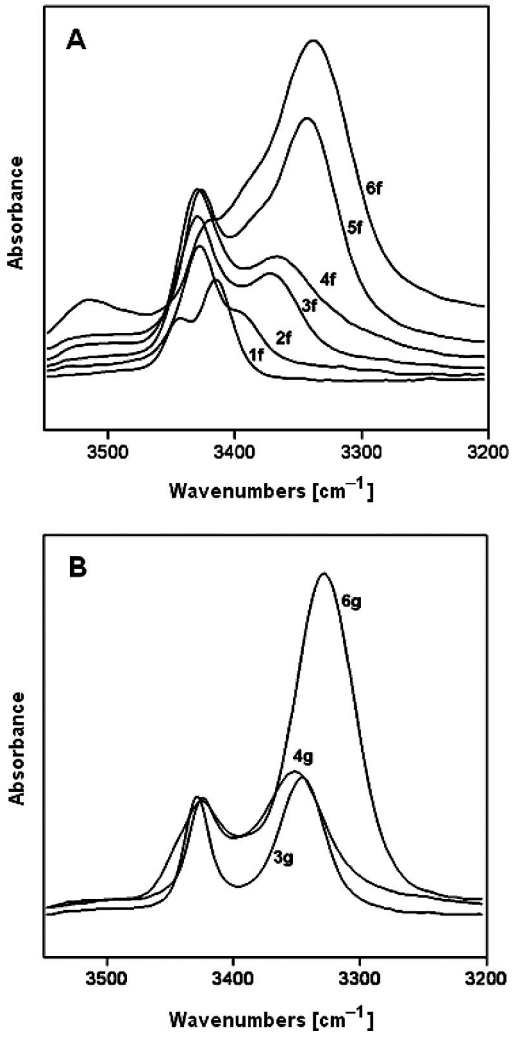


Figure 3. FTIR absorption spectra in the N–H stretching region of the L-Mdp[(*S*)-Binol-20-C-6]/L-Ala peptide series **1f**–**6f** (**A**) and the L-Mdp[(*S*)-Binol-20-C-6]/L-Ala/Aib series **3g**, **4g**, and **6g** (**B**) in $CDCl_3$ solution (peptide concentration: 1 mM).

peptides do not change remarkably (not shown), thereby suggesting the absence of self-association and consequently that the observed C=O···H–N H-bonding is essentially of the intramolecular type. From our FTIR absorption analysis it is also quite evident that the contribution of the L-Mdp (and Aib) residues to the stability of the folded structures adopted by all of the oligopeptides studied in this work is much more significant than that of either L-Ala or Gly.

Figure 4 illustrates the results of the $CDCl_3$/DMSO NH proton titrations of two representative Mdp hexapeptides (**6b** and **6g**) using 400 MHz NMR spectroscopy. DMSO is extremely efficient in H-bonding to urethane and peptide NH groups.[61,62] The urethane NH proton (NH^1) is known to be upfield shifted (< 6 ppm) with respect to the peptide NH protons in $CDCl_3$ solution.[59] All other NH proton resonances were attributed on the basis of their peak multiplicities and ROESY experiments. In this analysis, we noted that: (*i*) The NH^1 proton chemical shift is extremely dependent on the DMSO percentage (it moves upfield significantly). (*ii*) The NH^3–NH^6 proton chemical shifts are remarkably insensitive to the DMSO addition. (*iii*) The sensitivity of the NH^2 proton chemical shift is intermediate. We do not attribute the limited sensitivity of the crown-carrier Mdp NH^2 chemical shift induced by the perturbing solvent to the participation of this NH group in the intramolecular H-bonding scheme of the folded hexapeptide, but rather to a substantial inaccessibility of the solvent promoted by the bulkiness and chemical characteristics of the ring-substituted Mdp side-chain and its position in the peptide sequence.[38]

Taken together, our FTIR absorption and ^{1}H NMR results favor the conclusion that the longest oligomers of the seven Mdp-based peptide series tend to fold into well developed β-turns and 3_{10}-helices (indeed in these ordered secondary structures neither the NH^1 nor the NH^2 group is expected to be involved in intramolecular H-bonds).[21] This outcome is not surprising in view of the well established conformational propensities of C^{α}-methylated α-amino acids.[2,3,19–29]

ECD spectroscopy in the far-UV region (190–250 nm), where the $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions of chiral peptide chromophores are seen, is extensively exploited to analyse the secondary structures of conformationally restricted oligopeptides and high molecular weight polypeptides and proteins.[63,64] Three typical L-Mdp hexapeptides examined (**6a**, **6d**, and **6e**) display a very strong and negative ECD band at about 205 nm accompanied by a weak, broad, and negative band centerd in the vicinity of 230 nm (Figure 5). Below 195 nm the ECD spectra become positive. The ratios between the intensities of the weak and strong negative bands are in the range 0.2–0.3. The shape of these ECD patterns is reminiscent of that characteristic of right-handed 3_{10}-helices,[65–67] but the intensities are significantly higher

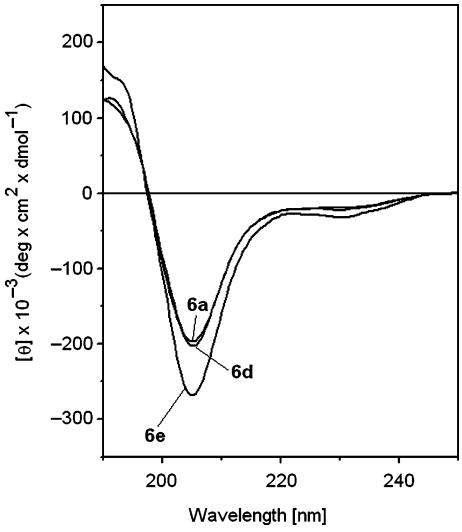


Figure 5. ECD spectra in the 190–250 nm region of Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe (**6a**), Boc-{Aib-L-Mdp[18-C-6]-L-Ala}$_2$-OMe (**6d**), and Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (**6e**) in MeOH solution (peptide concentration: 1 mM).

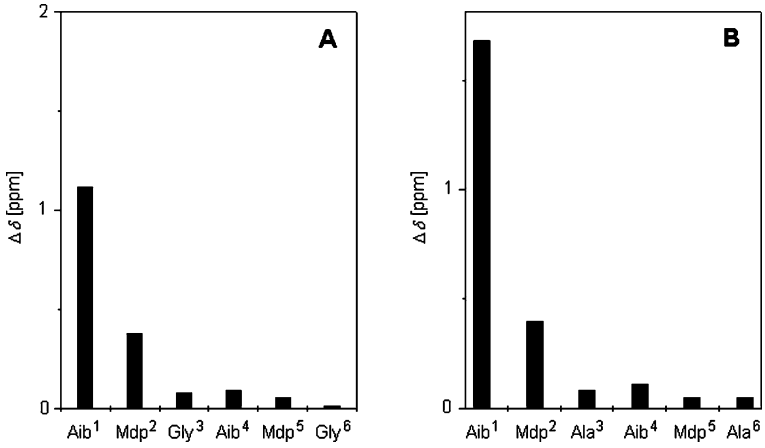


Figure 4. Histograms showing the variations of NH proton chemical shifts in the ^{1}H NMR spectra of Boc-{Aib-L-Mdp[15-C-5]-Gly}$_2$-OMe (**6b**) (**A**) and Boc-{Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala}$_2$-OMe (**6g**) (**B**) upon addition of 11% DMSO (v/v) to the $CDCl_3$ solution (peptide concentration: 1 mM).

in these ring-substituted L-Mdp peptides. Therefore, on the basis of the ECD spectra only, we cannot exclude a dominant contribution of the Mdp aromatic chromophores in this spectral region, covering that of the peptide chromophores.[64,68] For this same reason, we do not discuss the ECD spectra of peptides **6c**, **6f**, and **6g**, heavily influenced by contributions of electronic transitions arising from the fluorenyl[69,70] and binaphthyl[71,72] chromophores characteristic of the Fmoc *N*-protecting group (**6c** and **6f**) and the crowned side chain (**6g**), respectively. ECD spectra for peptide **6a**, recorded in a halogenated hydrocarbon (CH_2Cl_2) from 250 nm to 215 nm (not transparent below 215 nm), and in a 9:1 CH_3CN/H_2O solvent mixture containing either 1 mM NaCl or CsCl, do not markedly vary from that shown in Figure 5 for the same peptide. Therefore, we are inclined to exclude any dramatic solvent or bis-crown effect on the preferred conformation of our peptides.

We checked this preliminary conclusion by VCD spectroscopy in the amide I and amide II regions, a technique that is insensitive to the presence of aromatic chromophores in the peptide molecule. The IR absorption and VCD spectra of a typical hexapeptide (**6e**) are shown in Figure 6. In the IR absorption spectrum, the amide I band is broad and shifted to a relatively high frequency (maximum at ca. 1675 cm^{-1}), which suggests some conformational disorder but is inconsistent with an unordered structure. The amide II region is more complex, having a shoulder at ca. 1530 cm^{-1} followed by stronger and sharper features clustered around 1500 cm^{-1}, the latter of which probably arise from the side-chain modes. The 1530 cm^{-1} band additionally has the expected relative intensity for an amide II as compared to the amide I band. Another strong feature occurs at 1450 cm^{-1} which is likely due to methyl and methylene group deformations.

The VCD spectrum is quite complex. The amide I region is characterized by a very weak positive band which possibly is a part of a couplet, but the sign is somewhat undeterminate. By contrast, the amide II region has a very strong positive band followed to lower energy by a strong (positive) couplet corresponding to the sharper side-chain bands at ca. 1500 cm^{-1}. The relative sign and magnitude of the amide I and II VCD bands are consistent with a 3_{10}-helical structure with a left-handed screw sense.[73,74] The relatively low magnitude and breadth of the bands suggest some dynamic equilibrium with partially distorted (frayed) helices.

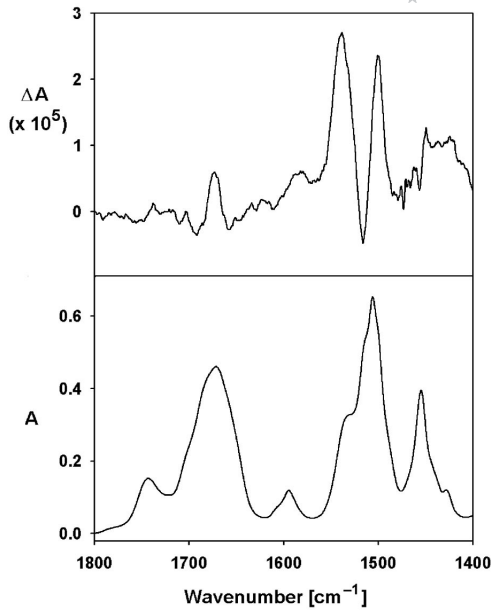


Figure 6. IR absorption (bottom) and VCD (top) spectra in the amide I and amide II regions for Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (**6e**) in $CDCl_3$ solution (peptide concentration: 8 mM). Both spectra are corrected for solvent baselines, but the smaller features ($\Delta A \approx 0.5 \times 10^{-5}$) in the VCD, as well as the positive drift in the amide II region, as the frequency approaches 1500 cm^{-1}, are likely to be due to baseline fluctuations arising from various sources.

Conclusions

In this paper we described solution synthesis and chemical characterization of seven series of *N*- and *C*-protected peptides (to the hexapeptides) generated by incorporating one or two L-Mdp guest residues, each decorated with a crown-ether carrier in their side chains, into guest peptides based on Gly, L-Ala and/or Aib residues. Four different types of crowns with diverging sizes were exploited.

A mass spectrometry analysis of alkali metal cation complexation revealed the onset of cooperativity between the two identical crown-ether moieties located at positions 2 and 5 of each hexameric peptide sequence. The rank order of binding ability is that expected on the basis of the relative alkali metal cation and crown ether dimensions.

By using a combination of FTIR absorption, ^1H NMR, ECD, and VCD techniques we were able to show that the longest peptides of each series tend to fold into *left*-handed β-turns and 3_{10}-helices, strongly promoted by L-Mdp (despite the occurrence in most sequences of one or two L-Ala residues). This outcome is not surprising as Mdp is a member of the class of C^{α}-*methylated*, γ-*branched* α-amino acids, well known to induce strong helicogenicity and a screw sense opposite to that of their corresponding C^{α}-unmethylated counterparts.[24,29] The nature of the side-chain crown-ether substituent does not appear to play any significant role on peptide preferred conformation. We are confident that the longest peptides discussed in this paper will nicely complement the L-DOPA based peptides proposed earlier by Voyer and co-workers,[4–16] as the two side chains of the crown-ether Mdp residues, introduced at positions *i* and *i*+3 of the sequence, will locate one on top of the other in the ternary 3_{10}-helix with a favorable opportunity for cooperative cation binding.

Experimental Section

Synthesis of Peptides: Melting points were measured with a Mettler apparatus with a final temperature raise of 3 °C/min or by means of a capillary tube immersed in an oil bath (Tottoli apparatus,

Büchi) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker WM300 spectrometer operating at 300 MHz and 77 MHz, respectively, the solvent $CDCl_3$ (^{1}H: δ = 7.27 ppm, ^{13}C: δ = 77.00 ppm) being used as the internal standard. Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. Mass spectra (electrospray mode) were measured with a Hewlett–Packard HP5989MS spectrometer. Elemental analyses were performed by the C.N.R.S. Service of Microanalyses at Gif-sur-Yvette (France). The optical rotations were measured in a 1-dm thermostatted cell with a Perkin–Elmer 241 polarimeter, with an accuracy of 0.3%. Analytical or preparative TLC and preparative column chromatography were performed on Kieselgel F 254 and Kieselgel 60 (0.040–0.063 mm) (Merck), respectively, with the following eluant systems: 3% MeOH/97% CH_2Cl_2 (*I*); 5% MeOH/95% CH_2Cl_2 (II); 10% MeOH/90% CH_2Cl_2 (III); 20% MeOH/80% CH_2Cl_2 (IV); CH_2Cl_2/MeOH/EtOAc (ethyl acetate)/1-BuOH (butanol)/AcOH (acetic acid)/H_2O 8:2.5:3:1:4:0.5 (V); EtOAc/1-BuOH/AcOH/H_2O, 1:1:1:1 (VI). UV light (λ = 254 nm) and/or ninhydrin spray allowed visualization of the spots after TLC runs for all compounds. Except when mentioned, all starting materials and solvents were obtained from commercial suppliers and were used as received. The ditosylate (–)-(*S*)-Binol[$(OCH_2CH_2)_2OTs]_2$ was prepared according to Cram and co-workers[39] The syntheses and characterizations of the derivatives Boc-L-Mdp[15-C-5]-OMe (**1a**), Boc-L-Mdp[15-C-5]-OH, Boc-L-Mdp[18-C-6]-OMe (**1c**), Boc-L-Mdp[18-C-6]-OH, Boc-L-Mdp[benzo-24-C-8]-OMe (**1e**), and Boc-L-Mdp[benzo-24-C-8]-OH, as well as the peptides Boc-L-Mdp[18-C-6]-L-Ala-OMe **2c**, Fmoc-L-Ala-L-Mdp[18-C-6]-L-Ala-OMe (**3c**), Boc-Aib-L-Mdp[18-C-6]-OH, and Boc-Aib-L-Mdp[18-C-6]-L-Ala-OMe (**3d**), have been reported previously.[35]

***N*-Boc Deprotection (General Procedure A):** TFA (1:3 to 1:2 v/v) was added to an ice-cold solution of *N*-Boc-amino acid or *N*-Boc-peptide in CH_2Cl_2. The solution was magnetically stirred from 0 °C to room temperature for 3–4 h and then evaporated in vacuo. The residue was repeatedly dissolved in CH_2Cl_2 and the solution evaporated in vacuo at 40 °C. The residue was repeatedly triturated with Et_2O (diethyl ether) and the solvents evaporated from the mixture in vacuo. The crude product was used in the next step without further purification.

***N*-Fmoc Deprotection (General Procedure B):** Diethylamine (1:9 *v*/*v*) was added to a solution of *N*-Fmoc-peptide in CH_3CN. The solution was magnetically stirred at room temperature for 4 h and then evaporated in vacuo. The residue was repeatedly dissolved in CH_2Cl_2 and/or CH_3CN, and the solution evaporated in vacuo at 40 °C. The crude product (mixture of *N*-deprotected peptide and dibenzofulvene) was used in the next step without further purification.

***N*-Z Deprotection (General Procedure C):** A large excess of 10% Pd/C under argon was added to a solution of *N*-Z-peptide in MeOH/THF , and then a 1 N HCl solution (ca. 2 equiv. mol/mol) was added. The mixture was magnetically stirred under H_2 at room temperature for 3 h, filtered through Celite and evaporated in vacuo. The crude product was used in the next step without further purification.

Treatment of the Chromatographic Fractions (General Procedures D and E): In order to remove traces of 1-BuOH and AcOH, as well as mineral impurities extracted from silica gel, possibly present in the compounds separated by column chromatography or preparative TLC with eluants (V) and/or (VI), the chromatographic samples were treated in the following manner. The solution containing the desired product, resulting from column chromatography or from extraction of a silica gel TLC band, was evaporated to dryness in vacuo at 40 °C. The residue was repeatedly evaporated in vacuo at 40 °C after addition of CH_2Cl_2 and H_2O (for the removal of 1-BuOH). The residue was then solubilized in CH_2Cl_2 (ca. 150 mL) and the solution was washed successively either with 0.5 N HCl (100 mL) and H_2O (2 × 100 mL) (general procedure D), or with 0.5 N HCl (100 mL), H_2O (100 mL), 5% $NaHCO_3$ (2 × 100 mL) and H_2O (2 × 100 mL) (general procedure E), dried ($MgSO_4$), filtered, and evaporated in vacuo at 40 °C.

Boc-Aib-L-Mdp[15-C-5]-OH: The amino acid derivative Boc-L-Mdp[15-C-5]-OH[35] (0.968 g; 2.06 mmol) was *N*-deprotected in CH_2Cl_2 (15 mL) and TFA (5 mL) according to general procedure A. The obtained crude TFA·H-L-Mdp[15-C-5]-OH (not characterized) was treated with Boc-Aib-NCA (1.419 g; 6.19 mmol) and DIEA (1.430 mL; 8.25 mmol) in THF (10 mL) at 50–55 °C for 3 d. The solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 (150 mL). The solution was washed with 0.5 N HCl (2 × 100 mL), H_2O (2 × 100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was chromatographed on a 2.3 × 55 cm column of silica gel with eluant (V). The combined solution of the product-containing fractions was treated by general procedure D to afford 0.765 g (67%) of pure Boc-Aib-L-Mdp[15-C-5]-OH as a solid. R_f = 0.19 (V). ^{1}H NMR ($CDCl_3$): δ = 7.00 [s, 1 H, NH Mdp], 6.72 [d (partly masked), $J \approx$ 8.1 Hz, 1 H, ArH^6 Mdp], 6.71 [s (br), 1 H, ArH^2 Mdp], 6.67 [d (br), $J \approx$ 8.1 Hz, 1 H, ArH^5 Mdp], 6.09 [s (br), 1 H, COOH], 5.26 [s (br), 1 H, NH Aib], 4.07 [m, 4 H, OCH_2], 3.85 [m, 4 H, OCH_2], 3.72 [s (br), 8 H, OCH_2], 3.36 [d, J = 13.6 Hz, 1 H] and 3.20 [d, J = 13.6 Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.62 [s, 3 H, CH_3^β Mdp], 1.42 [s, 3 H] and 1.39 [s (masked), 3 H, CH_3^β Aib], 1.39 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 175.9, 174.4 [COOH Mdp and CO Aib], 154.9 [CO Boc], 148.6, 148.0 [C^{Ar}-O], 129.4, 123.0, 116.1, 113.6 [C^{Ar}], 80.2 [O-C Boc], 70.8, 70.7, 70.3, 69.5, 68.8 [OCH_2], 60.8 [C^α Mdp], 56.8 [C^α Aib], 41.1 [$ArCH_2^\beta$ Mdp], 28.2 [CH_3 Boc], 25.8 [CH_3^β Mdp], 25.0, 23.1 [CH_3^β Aib] ppm. $[\alpha]_{589}^{25}$ = +9, $[\alpha]_{578}^{25}$ = +9, $[\alpha]_{546}^{25}$ = +10, $[\alpha]_{436}^{25}$ = +26 (c = 0.2 in MeOH). $C_{27}H_{42}N_2O_{10}$ (554.622): calcd. C 58.47, H 7.63, N 5.05; found C 57.95, H 7.71, N 4.67.

Boc-Aib-L-Mdp[15-C-5]-L-Ala-OMe (3a): NMM (0.270 mL; 2.46 mmol) and then EDC (0.176 g; 0.92 mmol) was added to an ice-cold suspension of Boc-Aib-L-Mdp[15-C-5]-OH (0.307 g; 0.55 mmol), HCl·H-L-Ala-OMe (0.257 g; 1.84 mmol) and HOAt (0.167 g; 1.23 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was warmed to room temperature, magnetically stirred for 3 d, and then diluted with CH_2Cl_2 (150 mL). The organic phase was extracted with 0.5 N HCl (2 × 100 mL), H_2O (2 × 100 mL), 5% $NaHCO_3$ (2 × 100 mL), H_2O (2 × 100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product (0.289 g) was purified by preparative TLC on silica gel with eluant (V). The observed two main UV-positive bands in the silica gel were separated and extracted with several portions of a 1:1 MeOH:CH_2Cl_2 solution. The filtered solutions were treated by general procedure E to afford 0.041 g of the side product 1-(chloromethyl)oxy-7-aza-1,2,3-benzotriazole ($ClCH_2OAt$) resulting from substitution of the reaction solvent CH_2Cl_2 by HOAt, as previously observed in similar couplings,[35] and 0.187 g (53%) of pure tripeptide **3a** as a solid. R_f = 0.29 (V). ^{1}H NMR ($CDCl_3$): δ = 7.72 [d (br), $J \approx$ 6.2 Hz, 1 H, NH Ala], 6.73 [d, J = 8.1 Hz, 1 H, ArH^5 Mdp], 6.63 [d (br), $J \approx$ 1.8 Hz, 1 H, ArH^2 Mdp], 6.62 [dd (partly masked), $J \approx$ 8.4 and 1.8 Hz, 1 H, ArH^6 Mdp], 6.19 [s (br), 1 H, NH Mdp], 4.98 [s, 1 H, NH Aib], 4.52 [dq, $J \approx$ 7.1 and 7.1 Hz, 1 H, CH^α Ala], 4.07 [m, 4 H, OCH_2], 3.86 [m, 4 H, OCH_2], 3.72 [s, 8 H, OCH_2], 3.67 [s, 3 H, OCH_3], 3.53 [d, J = 14.3 Hz, 1 H] and 3.05 [d, J = 14.0 Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.46 [s, 3 H, CH_3^β Mdp], 1.46 [d, J = 7.3 Hz, 3 H, CH_3^β

Ala], 1.39 [s, 9 H, CH_3 Boc], 1.37 [s, 3 H] and 1.33 [s, 3 H, CH_3^{β} Aib] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.7, 173.5, 172.8 [CO Ala, CO Mdp and CO Aib], 155.1 [CO Boc], 148.5, 148.1 [C^{Ar}-O], 129.9, 123.7, 117.4, 113.5 [C^{Ar}], 80.9 [O-C Boc], 70.9, 70.42, 70.38, 69.53, 69.50, 69.2, 68.9 [OCH_2], 59.5 [C^{α} Mdp], 57.0 [C^{α} Aib], 51.9 [OCH_3], 48.3 [CH^{α} Ala], 39.0 [$ArCH_2^{\beta}$ Mdp], 28.1 [CH_3 Boc], 26.7 [CH_3^{β} Mdp], 24.8, 23.8 [CH_3^{β} Aib], 16.9 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –34, $[\alpha]_{578}^{25}$ = –36, $[\alpha]_{546}^{25}$ = –41, $[\alpha]_{436}^{25}$ = –73 (c = 0.22 in MeOH). $C_{31}H_{49}N_3O_{11}$ (639.726): calcd. C 58.20, H 7.72, N 6.57; found C 57.74, H 7.74, N 6.13.

Fmoc-L-Ala-Aib-L-Mdp[15-C-5]-L-Ala-OMe (4a): The peptide **3a** (0.140 g; 0.22 mmol) was *N*-deprotected in CH_2Cl_2 (6 mL) and TFA (2 mL) according to general procedure A. The crude TFA·H-Aib-L-Mdp[15-C-5]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.221 g; 0.65 mmol) and DIEA (0.114 mL; 0.65 mmol) in THF (2 mL) at room temperature for 4 d. The same work-up as for **3a** was applied. The crude product was purified by preparative TLC on silica gel with eluant (V). The separated band in the silica gel containing the desired product was extracted with several portions of MeOH/CH_2Cl_2, 1:1, and the filtered solution was treated by general procedure E to afford 0.101 g (56%) of pure **4a** as a solid. R_f = 0.37 (V). ^1H NMR ($CDCl_3$): δ = 7.76 [d, J = 7.5 Hz, 2 H, ArH Fmoc], 7.58 [d (br), $J \approx$ 6.9 Hz, 2 H, ArH Fmoc], 7.46 [d (br), $J \approx$ 6.9 Hz, 1 H, NH Ala], 7.40 [m (t-like), 2 H, ArH Fmoc], 7.29 [m (t-like), 2 H, ArH Fmoc], 6.71 [d, J = 7.9 Hz, 1 H, ArH^5 Mdp], 6.64 [s, 1 H, ArH^2 Mdp], 6.64 [s (masked), 1 H, NH Mdp], 6.63 [d (partly masked), $J \approx$ 7.5 Hz, 1 H, ArH^6 Mdp], 6.38 [s, 1 H, NH Aib], 5.69 [d (br), $J \approx$ 6.6 Hz, 1 H, NH Ala], 4.55 [dq, $J \approx$ 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.37 [m, 2 H, OCH_2 Fmoc], 4.19 [m (t-like), 1 H, CH Fmoc], 4.06 [m, 4 H, OCH_2], 3.95 [m (dq-like), 1 H, CH^{α} Ala], 3.85 [m, 4 H, OCH_2], 3.71 [s, 4 H, OCH_2], 3.69 [s, 4 H, OCH_2], 3.65 [s, 3 H, OCH_3], 3.59 [d, J = 14.1 Hz, 1 H] and 3.00 [d, J = 14.1 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.49 [s, 3 H, CH_3^{β} Mdp], 1.44 [d (partly masked), 3 H, CH_3^{β} Ala], 1.43 [s, 3 H] and 1.34 [s, 3 H, CH_3^{β} Aib], 1.28 [d, J = 6.9 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.9, 173.8, 173.0, 172.6 [2 CO Ala, CO Mdp and CO Aib], 156.5 [CO Fmoc], 148.4, 148.1 [C^{Ar}-O Mdp], 143.8, 143.6, 141.3, 129.9, 127.8, 127.1, 127.0, 125.0, 124.9, 123.7, 120.0, 117.3, 113.4 [C^{Ar} Mdp and C^{Ar} Fmoc], 70.8, 70.5, 70.3, 69.5, 69.2, 68.9 [OCH_2], 67.1 [CH_2 Fmoc], 60.0 [C^{α} Mdp], 57.3 [C^{α} Aib], 52.0 [OCH_3], 50.1 [CH^{α} Ala], 48.3 [CH^{α} Ala], 47.0 [CH Fmoc], 39.7 [$ArCH_2^{\beta}$ Mdp], 26.5 [CH_3^{β} Mdp], 24.6, 23.8 [CH_3^{β} Aib], 17.3, 17.0 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –28, $[\alpha]_{578}^{25}$ = –29, $[\alpha]_{546}^{25}$ = –38, $[\alpha]_{436}^{25}$ = –68 (c = 0.15, in MeOH). $C_{44}H_{56}N_4O_{12}\cdot 0.5H_2O$ (841.928): C 62.76, H 6.82, N 6.65; found C 62.67, H 6.92, N 6.26.

Boc-{Aib-L-Mdp[15-C-5]-L-Ala}$_2$-OMe (6a): The peptide **4a** (0.081 g; 0.09 mmol) was *N*-deprotected in CH_3CN (9 mL) and diethylamine (1 mL) according to general procedure B. The crude mixture of H-L-Ala-Aib-L-Mdp[15-C-5]-L-Ala-OMe and dibenzofulvene was treated with the dipeptide Boc-Aib-L-Mdp[15-C-5]-OH (0.054 g; 0.09 mmol), HOAt (0.026 g; 0.19 mmol), NMM (0.011 mL; 0.09 mmol) and EDC (0.028 g; 0.15 mmol) in CH_2Cl_2 (2 mL), at room temperature for 4 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product was chromatographed on a 1.5 × 30 cm column of silica gel and eluted successively with eluants (V) and (VI). The solution of the combined fractions containing the desired product was treated by general procedure E to afford 0.050 g (45%) of pure hexapeptide **6a** as a solid. R_f = 0.55 (VI). ^1H NMR ($CDCl_3$): δ = 7.75 [d, J = 7.0 Hz, 1 H, NH Ala], 7.72 [d (br., partly masked), 1 H, NH Ala], 7.64 [s, 1 H, NH Aib], 6.78 [d, J = 7.9 Hz, 1 H, ArH Mdp], 6.65 [s, 3 H, 2ArH Mdp and 1NH Mdp], 6.61–6.53 [m, 3 H, ArH Mdp], 6.23 [s (br), 1 H, NH Mdp], 5.16 [s (br), 1 H, NH Aib], 4.54 [dq, $J \approx$ 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.20 [dq (br), $J \approx$ 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.5–3.7 [m, 32 H, OCH_2], 3.69 [s, 3 H, OCH_3], 3.57 [d, J = 14.2 Hz, 1 H] and 3.09 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 2.98 [d, J = 14.1 Hz, 1 H] and 2.77 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.54 [s, 3 H, CH_3^{β} Mdp], 1.49 [d (partly masked), $J \approx$ 7.3 Hz, 3 H, CH_3^{β} Ala], 1.37 [d (partly masked), $J \approx$ 7.3 Hz, 3 H, CH_3^{β} Ala], 1.48 [s, 6 H], 1.30 [s, 3 H] and 1.25 [s, 6 H, CH_3^{β} Mdp and $4CH_3^{\beta}$ Aib], 1.34 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.5, 174.3, 173.8, 173.5, 173.3, 173.2 [CO Aib, Mdp and Ala], 156.0 [CO Boc], 149.0, 148.5, 148.2, 147.7 [C^{Ar}-O Mdp], 130.5, 128.0, 123.6, 122.9, 117.5, 116.0, 113.7, 113.4 [C^{Ar} Mdp], 81.3 [O-C Boc], 70.9, 70.8, 70.3, 69.5, 69.0, 68.8 [OCH_2], 59.5, 59.4 [C^{α} Mdp], 57.0, 56.9 [C^{α} Aib], 51.8 [OCH_3], 49.8, 48.3 [CH^{α} Ala], 43.0, 38.6 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 26.7, 26.0, 24.7, 23.9, 23.4, 22.7 [CH_3^{β} Mdp and CH_3^{β} Aib], 17.0, 16.3 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –53, $[\alpha]_{578}^{25}$ = –55, $[\alpha]_{546}^{25}$ = –67, $[\alpha]_{436}^{25}$ = –124 (c = 0.20, in MeOH). ESI^+ MS: m/z (%) = 1147.8 (73) $[M + H]^+$, 1169.8 (100) $[M + Na]^+$. $C_{56}H_{86}N_6O_{19}$ (1147.296): calcd. C 58.62, H 7.56, N 7.32; found C 58.73, H 7.85, N 6.64.

Boc-Aib-L-Mdp[15-C-5]-Gly-OMe (3b): A suspension of the dipeptide Boc-Aib-L-Mdp[15-C-5]-OH (0.302 g; 0.55 mmol), HCl·H-Gly-OMe (0.209 g; 1.66 mmol), HOAt (0.1519 g; 1.11 mmol), NMM (0.24 mL; 2.22 mmol) and EDC (0.159 g; 0.83 mmol) in CH_2Cl_2 (5 mL), was reacted under the same experimental conditions and work-up as for the synthesis of **3a**. The crude product (0.290 g) was chromatographed on a 1.5 × 36 cm column of silica gel and eluted successively with eluants (III) and (IV). The solution of the combined fractions containing the desired product was treated by general procedure E to afford 0.239 g (70%) of pure tripeptide **3b** as a solid. R_f = 0.24 (III). ^1H NMR ($CDCl_3$): δ = 7.81 [m (br), 1 H, NH Gly], 6.67–6.65 [m, 3 H, ArH Mdp], 6.34 [s (br), 1 H, NH Mdp], 5.18 [s, 1 H, NH Aib], 4.05 [s, 4 H, OCH_2], 4.05 [d (masked), 1 H] and 3.89 [d (partly masked), 1 H, CH_2 Gly], 3.84 [s, 4 H, OCH_2], 3.69 [s, 8 H, OCH_2], 3.66 [s, 3 H, OCH_3], 3.41 [d, J = 13.6 Hz, 1 H] and 3.00 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.46 [s, 3 H, CH_3^{β} Mdp], 1.34 [s, 15 H, CH_3 Boc and CH_3^{β} Aib] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.4, 173.2, 170.1 [CO Gly, CO Mdp and CO Aib], 155.3 [CO Boc], 148.6, 148.1 [C^{Ar}-O], 129.1, 132.2, 116.7, 113.5 [C^{Ar}], 80.7 [O-C Boc], 70.8, 70.3, 70.2, 69.4, 69.0, 68.8 [OCH_2], 59.7 [C^{α} Mdp], 56.9 [C^{α} Aib], 51.7 [OCH_3], 41.2 [CH^{α} Gly and $ArCH_2^{\beta}$ Mdp], 28.1 [CH_3 Boc], 25.4 [CH_3^{β} Mdp], 24.9, 23.9 [CH_3^{β} Aib] ppm. $[\alpha]_{589}^{25}$ = –18, $[\alpha]_{578}^{25}$ = –19, $[\alpha]_{546}^{25}$ = –23, $[\alpha]_{436}^{25}$ = –40 (c = 0.22, in MeOH). $C_{30}H_{47}N_3O_{11}$ (625.700): calcd. C 57.58, H 7.57, N 6.72; found C 57.59, H 7.64, N 6.11.

Boc-Aib-L-Mdp[15-C-5]-Gly-OH: A solution of 1 N NaOH (2 mL) was added to a solution of **3b** (0.090 g; 0.14 mmol) in MeOH (10 mL). The reaction mixture was magnetically stirred at room temperature for 4 h (with TLC monitoring), cooled by addition of ice, acidified by addition of 0.5 N HCl (50 mL), and extracted with CH_2Cl_2 (4 × 50 mL). The CH_2Cl_2 solution was washed with H_2O (2 × 50 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to afford 0.078 g (89%) of crude Boc-Aib-L-Mdp[15-C-5]-Gly-OH, which was used in the next coupling step (see **6b**) without further purification. R_f = 0.05 (III). ^1H NMR ($CDCl_3$): δ = 7.79 [m (br), 1 H, NH Gly], 6.78 [d, J = 8.1 Hz, 1 H, ArH^5 Mdp], 6.66 [s, 1 H, ArH^2 Mdp], 6.65 [d (partly masked), J = 8.0 Hz, 1 H, ArH^6 Mdp], 6.34 [s (br), 1 H, NH Mdp], 5.08 [s (br), 1 H, NH Aib], 4.09 [m, 4 H, 2 OCH_2], 4.04 [d (br), $J \approx$ 6.2 Hz, 1 H] and 3.95 [d (br), $J \approx$ 5.9 Hz, 1 H, CH_2 Gly], 3.90 [m, 4 H, OCH_2], 3.73 [m, 8 H, OCH_2], 3.32 [d, J = 13.2 Hz, 1 H] and 2.99 [d (br), $J \approx$ 12.5 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.55 [s, 3 H, CH_3^{β} Mdp], 1.42 [s, 3 H] and 1.39 [s (partly masked), 3 H, CH_3^{β} Aib], 1.38 [s, 9 H, CH_3 Boc] ppm.

Boc-{Aib-L-Mdp[15-C-5]-Gly}$_2$-OMe (6b): The peptide **3b** (0.087 g; 0.14 mmol) was *N*-deprotected in CH_2Cl_2 (6 mL) and TFA (2 mL) according to general procedure A. The crude TFA·H-Aib-L-Mdp[15-C-5]-Gly-OMe (not characterized) was treated with crude Boc-Aib-L-Mdp[15-C-5]-Gly-OH (0.078 g; 0.13 mmol), HOAt (0.035 g; 0.26 mmol), NMM (0.031 mL; 0.28 mmol) and EDC (0.037 g; 0.19 mmol) in CH_2Cl_2 (2 mL), at room temperature for 4 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product (0.081 g) was chromatographed on a 1.5 × 33 cm column of silica gel and eluted successively with eluants (V) and (VI). The solution of the combined fractions containing the desired product was treated by general procedure E to afford 0.041 g (29%) of pure hexapeptide **6b** as a solid. R_f = 0.40 (VI). ^{1}H NMR ($CDCl_3$): δ = 8.14 [m (t-like, br), 1 H, NH Gly], 7.79 [m (t-like, br), 1 H, NH Gly], 7.65 [s, 1 H, NH Aib], 6.78 [d, J = 7.9 Hz, 1 H, ArH Mdp], 6.78–6.55 [m, 5 H, ArH Mdp], 6.71 [s, 1 H, NH Mdp], 6.26 [s (br), 1 H, NH Mdp], 5.13 [s (br), 1 H, NH Aib],4.20 [dd, $J \approx$ 20.9 and 6.0 Hz, 1 H] and 3.95 [dd (partly masked), 1 H, CH_2 Gly], 4.10 [m, 6 H, OCH_2], 3.91 [m, 4 H, OCH_2], 3.9–3.7 [m, 22 H, OCH_2], 3.69 [s, 3 H, OCH_3], 3.58 [d, J = 13.6 Hz, 1 H] and 3.12 [d, J = 13.6 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 3.12 [d, J = 13.6 Hz, 1 H] and 2.84 [d, J = 13.9 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.55 [s, 3 H, CH_3^{β} Mdp], 1.48 [s, 3 H, CH_3^{β} Mdp], 1.45 [s, 3 H], 1.44 [s, 3 H], 1.34 [s, 3 H] and 1.33 [s, 3 H, $4CH_3^{\beta}$ Aib], 1.38 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 175.3, 175.0, 174.4, 173.3, 170.7, 170.5 [CO Aib, Mdp and Gly], 155.9 [CO Boc], 148.8, 148.4, 148.3, 147.7 [C^{Ar}-O Mdp], 130.4, 128.8, 123.6, 123.4, 117.8, 116.9, 113.4 [C^{Ar} Mdp], 81.6 [O-C Boc], 70.9, 70.5, 69.6, 69.2, 69.1, 68.9, 68.8 [OCH_2], 60.0, 59.4 [C^{α} Mdp], 57.4, 56.8 [C^{α} Aib], 51.8 [OCH_3], 44.5, 41.5 [CH^{α} Gly], 39.7, 39.3 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 26.6, 26.0, 24.5, 24.3, 24.0 [CH_3^{β} Mdp and CH_3^{β} Aib] ppm. $[a]_{589}^{25}$ = –80, $[a]_{578}^{25}$ = –81, $[a]_{546}^{25}$ = –96, $[a]_{436}^{25}$ = –175 (c = 0.20, in MeOH). ESI$^+$ MS: m/z (%) = 1119.8 (10) [M + H]$^+$, 1141.7 (100) [M + Na]$^+$. $C_{54}H_{82}N_6O_{19}$ (1119.244): calcd. C 57.94, H 7.38, N 7.51; found C 58.31, H 7.57, N 6.92.

Fmoc-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe (4c): The peptide **3c**[35] (0.168 g; 0.21 mmol) was *N*-deprotected in CH_3CN (18 mL) and diethylamine (2 mL) according to general procedure B. The crude mixture of H-L-Ala-L-Mdp[15-C-5]-L-Ala-OMe and dibenzofulvene was treated with Fmoc-L-Ala-NCA (0.144 g; 0.43 mmol) and DIEA (0.075 mL; 0.43 mmol) in THF (4 mL) at room temperature for 24 h. The same work-up as for **3a** was applied for extraction. The crude product was purified by preparative TLC on silica gel with eluant (V). The separated band in the silica gel containing the desired product was extracted with several portions of a 1:1 MeOH/CH_2Cl_2 solution, and the filtered solution was treated by general procedure E to afford 0.118 g (64%) of pure **4c** as a solid. R_f = 0.67 (IV), 0.49 (V). ^{1}H NMR ($CDCl_3$): δ = 7.74 [d, J = 7.4 Hz, 2 H, ArH Fmoc], 7.56 [d (br), $J \approx$ 7.4 Hz, 2 H, ArH Fmoc], 7.35 [m (t-like), 2 H, ArH Fmoc], 7.28 [m (split t-like), 2 H, ArH Fmoc], 7.10 [d, J = 7.2 Hz, 1 H, NH Ala], 7.05 [d (br), $J \approx$ 5.4 Hz, 1 H, NH Ala], 6.72 [s, 1 H, NH Mdp], 6.70 [d, J = 8.0 Hz, 1 H, ArH^5 Mdp], 6.64 [d, $J \approx$1.6 Hz, 1 H, ArH^2 Mdp], 6.61 [dd, J = 8.0 and ca. 1.6 Hz, 1 H, ArH^6 Mdp], 5.75 [d, J = 7.2 Hz, 1 H, NH Ala], 4.53 [dq, $J \approx$ 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.38 [m, 2 H, OCH_2 Fmoc], 4.16 [m, 3 H, $2CH^{\alpha}$ Ala and CH Fmoc], 4.07 [m, 4 H, OCH_2], 3.85 [m, 4 H, OCH_2], 3.7–3.6 [s, 12 H, OCH_2], 3.62 [s, 3 H, OCH_3], 3.38 [d, J = 13.8 Hz, 1 H] and 3.09 [d, J = 13.8 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.49 [s, 3 H, CH_3^{β} Mdp], 1.37 [d, J = 7.2 Hz, 3 H, CH_3^{β} Ala], 1.33 [d, J = 7.1 Hz, 3 H, CH_3^{β} Ala], 1.27 [d, J = 7.1 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.4, 173.1, 172.9, 171.5 [3 CO Ala and CO Mdp], 156.0 [CO Fmoc], 148.1, 147.7 [C^{Ar}-O Mdp], 143.7, 143.6, 141.2, 129.0, 127.7, 127.0, 124.9, 123.0, 119.9, 116.2, 113.1 [C^{Ar} Mdp and C^{Ar} Fmoc], 70.6, 70.5, 70.4, 69.4, 68.7, 68.6 [OCH_2], 66.8 [CH_2 Fmoc], 60.4 [C^{α} Mdp], 52.3 [OCH_3], 50.2, 48.3, 47.0 [3CH^{α} Ala and CH Fmoc], 40.5 [$ArCH_2^{\beta}$ Mdp], 23.9 [CH_3^{β} Mdp], 18.0, 17.7, 17.2 [CH_3^{β} Ala] ppm. $[a]_{589}^{25}$ = –61, $[a]_{578}^{25}$ = –63, $[a]_{546}^{25}$ = –73, $[a]_{436}^{25}$ = –128 (c = 0.22, in MeOH); $C_{45}H_{58}N_4O_{13}$·0.5H_2O (871.954): C 61.98, H 6.82, N 6.43; found C 61.83, H 6.56, N 6.25.

Boc-L-Mdp[18-C-6]-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe (5c): The peptide **4c** (0.095 g; 0.11 mmol) was *N*-deprotected in CH_3CN (9 mL) and diethylamine (1 mL) according to general procedure B. The crude mixture of H-(L-Ala)$_2$-L-Mdp[15-C-5]-L-Ala-OMe and dibenzofulvene was treated with Boc-L-Mdp[18-C-6]-OH[35] (0.071 g; 0.14 mmol), HOAt (0.038 g; 0.28 mmol), NMM (0.015 mL; 0.14 mmol) and EDC (0.040 g; 0.21 mmol) in CH_2Cl_2 (1.5 mL), at room temperature for 3 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (VI). The separated band in the silica gel containing the desired product was extracted with several portions of eluant (VI) and the filtered solution was treated by general procedure E to afford 0.064 g (51%) of pure pentapeptide **5c** as a solid. R_f = 0.49 (VI). ^{1}H NMR ($CDCl_3$): δ = 7.61 [d (br), $J \approx$ 5.7 Hz, 1 H, NH Ala], 6.99 [d, J = 7.4 Hz, 1 H, NH Ala], 6.84 [s (br), 1 H, NH Mdp], 6.83 [d, J = 8.2 Hz, 1 H, ArH^5 Mdp], 6.77 [d, J = 8.7 Hz, 1 H, ArH^5 Mdp], 6.70 [dd (partly masked), $J \approx$ 8.2 and ca. 1.8 Hz, 1 H, ArH^6 Mdp], 6.69 [d, $J \approx$ 1.8 Hz, 1 H, ArH^2 Mdp], 6.64 [dd, $J \approx$ 8.2 and ca. 1.8 Hz, 1 H, ArH^6 Mdp], 6.61 [d, $J \approx$ 1.8 Hz, 1 H, ArH^2 Mdp], 6.26 [d, J = 5.1 Hz, 1 H, NH Ala], 5.02 [s, 1 H, NH Mdp], 4.52 [dq, $J \approx$ 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.28 [dq, $J \approx$ 7.1 and 7.1 Hz, 1 H, CH^{α} Ala], 4.14 [m, 8 H, OCH_2], 4.10 [dq (partly masked), 1 H, CH^{α} Ala], 3.90 [m, 8 H, OCH_2], 3.74 [m, 8 H, OCH_2], 3.70 [m, 8 H, OCH_2], 3.69 [s, 4 H, OCH_2], 3.67 [m, 4 H, OCH_2], 3.66 [s, 3 H, OCH_3], 3.41 [d, J = 13.8 Hz, 1 H] and 3.11 [d, J = 13.9 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 2.98 [d, J = 14.1 Hz, 1 H] and 2.81 [d, J = 14.1 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.48 [s, 3 H, CH_3^{β} Mdp], 1.42 [s, 12 H, CH_3^{β} Mdp and CH_3 Boc], 1.41 [d (partly masked), $J \approx$ 7.2 Hz, 3 H, CH_3^{β} Ala], 1.35 [d, J = 7.2 Hz, 3 H, CH_3^{β} Ala], 1.27 [d, J = 7.3 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.0, 173.34, 173.30, 172.3, 172.1 [3 CO Ala and 2 CO Mdp], 155.6 [CO Boc], 148.8, 148.5, 148.3, 147.7 [C^{Ar}-O Mdp], 129.6, 127.5, 123.5, 122.9, 116.7, 116.0, 113.63, 113.57 [C^{Ar} Mdp], 81.3 [O-C Boc], 70.7, 70.51, 70.58, 69.7, 69.6, 69.5, 69.4, 69.1, 69.0, 68.9, 68.8 [OCH_2], 60.6, 60.0 [C^{α} Mdp], 52.1 [OCH_3], 50.2, 49.9, 48.2 [CH^{α} Ala], 43.7, 40.6 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 23.4, 22.6 [CH_3^{β} Mdp], 17.8, 17.1, 16.8 [CH_3^{β} Ala] ppm. $[a]_{589}^{25}$ = –60, $[a]_{578}^{25}$ = –61, $[a]_{546}^{25}$ = –74, $[a]_{436}^{25}$ = –132 (c = 0.20, in MeOH). ESI$^+$ MS: m/z (%) = 1158 (100) [M + Na]$^+$, 590.5 (78) [M + 2Na]$^{2+}$. $C_{55}H_{85}N_5O_{20}$ (1136.270): calcd. C 58.13, H 7.54, N 6.16; found C 57.94, H 7.48, N 5.68.

Fmoc-{L-Ala-L-Mdp[18-C-6]-L-Ala}$_2$-OMe (6c): The peptide **5c** (0.097 g; 0.09 mmol) was *N*-deprotected in CH_2Cl_2 (7.5 mL) and TFA (2.5 mL) according to general procedure A. The crude TFA·H-L-Mdp[18-C-6]-(L-Ala)$_2$-L-Mdp[18-C-6]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.116 g; 0.34 mmol) and DIEA (0.060 mL; 0.34 mmol) in THF (2 mL) at room temperature for 4 d. The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (VI). The separated band in the silica gel containing the desired product was extracted with several portions of eluant (IV) and the filtered solution was treated by general procedure E to afford 0.073 g (64%) of pure hexapeptide **6c** as a solid. R_f = 0.51 (IV), 0.61 (VI). ^{1}H NMR ($CDCl_3$): δ = 7.89 [d, J = 7.3 Hz, 1 H, NH Ala], 7.79 [d, J = 7.5 Hz, 1 H, ArH Fmoc],

7.77 [d, J = 7.4 Hz, 1 H, ArH Fmoc], 7.68 [d, J = 7.4 Hz, 1 H, ArH Fmoc], 7.53 [d, J = 7.4 Hz, 1 H, ArH Fmoc], 7.47–7.30 [m, 4 H, ArH Fmoc], 7.26 [d, $J \approx 6.1$ Hz, 1 H, NH Ala], 6.78 [d (partly masked), $J \approx 7$ Hz, 1 H, NH Ala], 6.77 [s, 1 H, NH Mdp], 6.75 [d (partly masked), $J \approx 7$ Hz, 1 H, NH Ala], 6.64 [d, J = 8.3 Hz, 1 H, ArH^5 Mdp], 6.59 [d (br), $J \approx 8$ Hz, 1 H, ArH^6 Mdp], 6.53 [s (br), 1 H, ArH^2 Mdp], 6.49 [s (br), 1 H, ArH^2 Mdp], 6.43 [d (br), $J \approx 8.2$ Hz, 1 H, ArH^6 Mdp], 6.35 [d, J = 8.1 Hz, 1 H, ArH^5 Mdp], 6.30 [s, 1 H, NH Mdp], 4.57 [dq, $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.37 [dq, $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.32 [m (partly masked), 1 H] and 4.15 [m (t-like), 1 H, OCH_2 Fmoc], 3.93 [dq (partly masked), 1 H, CH^{α} Ala], 4.5–3.6 [m, 41 H, OCH_2 and CH Fmoc], 3.67 [s, 3 H, OCH_3], 3.21 [m (br., q-like), $J \approx 7$ Hz, 1 H, CH^{α} Ala], 3.56 [d (partly masked), 1 H] and 3.02 [d, J = 13.8 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 2.88 [d, J = 14.0 Hz, 1 H] and 2.73 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.48 [s, 3 H, CH_3^{β} Mdp], 1.42 [s, 12 H, CH_3^{β} Mdp and CH_3 Boc], 1.41 [d (partly masked), $J \approx 7.2$ Hz, 3 H, CH_3^{β} Ala], 1.35 [d, J = 7.2 Hz, 3 H, CH_3^{β} Ala], 1.27 [d, J = 7.3 Hz, 3 H, CH_3^{β} Ala] ppm. 1.60 [s, 3 H, CH_3^{β} Mdp], 1.46 [d, J = 7.2 Hz, 3 H, CH_3^{β} Ala], 1.42 [d, J = 7.3 Hz, 3 H, CH_3^{β} Ala], 1.34 [d (partly masked), $J \approx 7$ Hz, 3 H, CH_3^{β} Ala], 1.33 [s, 3 H, CH_3^{β} Mdp], 1.17 [d, J = 7.3 Hz, 3 H, CH_3^{β} Ala]. ^{13}C NMR ($CDCl_3$): δ = 174.8, 174.1, 173.6, 172.8, 172.4, 172.0 [4 CO Ala and 2 CO Mdp], 157.7 [CO Fmoc], 148.9, 148.3, 147.6, 147.0 [C^{Ar}-O Mdp], 143.9, 142.9, 141.2, 130.1, 128.0, 127.3, 127.2, 125.0, 124.5, 123.4, 121.7, 120.1, 115.7, 115.2, 113.6, 112.1 [C^{Ar} Mdp and C^{Ar} Fmoc], 70.6, 70.5, 70.4, 70.3, 70.2, 70.1, 69.9, 69.5, 69.3, 69.2, 69.1, 68.8, 68.1 [OCH_2], 66.9 [CH_2 Fmoc], 59.8, 59.4 [C^{α} Mdp], 53.8 [CH Fmoc], 51.9 [OCH_3], 50.0, 48.9, 48.1, 46.7 [CH^{α} Ala], 45.1, 38.4 [$ArCH_2^{\beta}$ Mdp], 24.9, 22.5 [CH_3^{β} Mdp], 17.3, 17.1, 16.7, 15.7 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –66, $[\alpha]_{578}^{25}$ = –69, $[\alpha]_{546}^{25}$ = –81, $[\alpha]_{436}^{25}$ = –154 (c = 0.10, in MeOH). ESI$^+$ MS: m/z (%) = 1107 (100) [M – Fmoc + H]$^+$. $C_{68}H_{92}N_6O_{21}\cdot H_2O$ (1347.480): C 60.61, H 7.03, N 6.24; found C 60.68, H 6.74, N 5.96.

Fmoc-L-Ala-Aib-L-Mdp[18-C-6]-L-Ala-OMe (4d): The peptide **3d**[35] (0.166 g; 0.24 mmol) was N-deprotected in CH_2Cl_2 (7.5 mL) and TFA (2.5 mL) according to general procedure A. The crude TFA·H-Aib-L-Mdp[18-C-6]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.246 g; 0.73 mmol) and DIEA (0.127 mL; 0.73 mmol) in THF (2 mL) at room temperature for 5 d. The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (V). The separated band in the silica gel containing the desired product was extracted with several portions of eluant (IV) and the filtered solution was treated by general procedure E to afford 0.155 g (73%) of pure **4d** as a solid. R_f = 0.67 (IV), 0.33 (V). ^{1}H NMR ($CDCl_3$): δ = 7.74 [d, J = 7.5 Hz, 2 H, ArH Fmoc], 7.57 [d (br), $J \approx 7.4$ Hz, 1 H, ArH Fmoc], 7.56 [d (br), $J \approx 7.5$ Hz, 1 H, ArH Fmoc], 7.49 [d, J = 7.0 Hz, 1 H, NH Ala], 7.38 [m (t-like), $J \approx 7.3$ Hz, 2 H, ArH Fmoc], 7.31–7.24 [m (partly masked), 2 H, ArH Fmoc], 6.80 [s, 1 H, NH Mdp], 6.69 [d, J = 8.1 Hz, 1 H, ArH^5 Mdp], 6.64 [d, $J \approx 1.6$ Hz, 1 H, ArH^2 Mdp], 6.61 [dd, J = 8.1 and ca. 1.6 Hz, 1 H, ArH^6 Mdp], 6.42 [s, 1 H, NH Aib], 5.79 [d, J = 6.5 Hz, 1 H, NH Ala], 4.53 [dq, $J \approx 7.1$ and 7.1 Hz, 1 H, CH^{α} Ala], 4.41 [dd (br), $J \approx 10.5$ and 6.7 Hz, 1 H] and 4.35 [dd, J = 10.5 and 6.7 Hz, 1 H, OCH_2 Fmoc], 4.17 [m (t-like), $J \approx 6.6$ Hz, 1 H, CH Fmoc], 4.05 [m, 4 H, OCH_2], 3.95 [dq (br), $J \approx 6.8$ and 6.8 Hz, 1 H, CH^{α} Ala], 3.83 [m, 4 H, OCH_2], 3.7–3.6 [s, 12 H, OCH_2], 3.63 [s, 3 H, OCH_3], 3.59 [d (partly masked), 1 H] and 3.00 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.46 [s, 3 H, CH_3^{β} Mdp], 1.43 [d (partly masked), $J \approx 7.2$ Hz, 3 H, CH_3^{β} Ala], 1.42 [s, 3 H] and 1.32 [s, 3 H, CH_3^{β} Aib], 1.26 [d, J = 7.2 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.9, 173.6, 173.1, 172.7 [2 CO Ala, CO Aib and CO Mdp], 156.4 [CO Fmoc], 148.1, 147.7 [C^{Ar}-O Mdp], 143.8, 143.6, 141.2, 129.9, 127.7, 127.1, 127.0, 124.9, 124.8, 123.6, 119.9, 117.2, 113.3 [C^{Ar} Mdp and C^{Ar} Fmoc], 70.6, 70.53, 70.49, 70.44, 70.39, 69.5, 69.4, 69.0, 68.8 [OCH_2], 66.9 [CH_2 Fmoc], 59.9 [C^{α} Mdp], 57.2 [C^{α} Aib], 51.9 [OCH_3], 50.8, 48.3 [CH^{α} Ala], 46.9 [CH Fmoc], 39.4 [$ArCH_2^{\beta}$ Mdp], 26.5 [CH_3^{β} Mdp], 24.6, 23.7 [CH_3^{β} Aib], 17.1, 16.9 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –27, $[\alpha]_{578}^{25}$ = –28, $[\alpha]_{546}^{25}$ = –32, $[\alpha]_{436}^{25}$ = –56 (c = 0.25, in MeOH). $C_{46}H_{60}N_4O_{13}\cdot 0.5H_2O$ (885.980): calcd. C 62.35, H 6.94, N 6.32; found C 62.34, H 7.01, N 6.03.

Boc-{Aib-L-Mdp[18-C-6]-L-Ala}$_2$-OMe (6d): The peptide **4d** (0.133 g; 0.15 mmol) was N-deprotected in CH_3CN (9 mL) and diethylamine (1 mL) according to general procedure B. The crude mixture of H-L-Ala-Aib-L-Mdp[18-C-6]-L-Ala-OMe and dibenzofulvene was treated with the dipeptide Boc-Aib-L-Mdp[18-C-6]-OH[35] (0.091 g; 0.15 mmol), HOAt (0.041 g; 0.30 mmol), NMM (0.017 mL; 0.15 mmol) and EDC (0.044 g; 0.23 mmol) in CH_2Cl_2 (2 mL), at room temperature for 4 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product was chromatographed on a 1.5 × 29 cm column of silica gel and eluted successively with eluants (V) and (VI). The solution of the combined fractions containing the desired product was treated by general procedure E to afford 0.075 g (40%) of pure hexapeptide **6d** as a solid. R_f = 0.55 (VI). ^{1}H NMR ($CDCl_3$): δ = 7.73 [d, J = 7.0 Hz, 1 H, NH Ala], 7.68 [d (br) (partly masked), 1 H, NH Ala], 7.65 [s, 1 H, NH Aib], 6.79 [d, J = 7.9 Hz, 1 H, ArH Mdp], 6.68–6.56 [m, 6 H, 5ArH Mdp and 1NH Mdp], 6.19 [s (br), 1 H, NH Mdp], 5.38 [s, 1 H, NH Aib], 4.53 [dq, $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.25 [dq (br), $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.15–3.65 [m, 40 H, OCH_2], 3.69 [s, 3 H, OCH_3], 3.56 [d, J = 14.0 Hz, 1 H] and 3.08 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 2.94 [d, J = 14.0 Hz, 1 H] and 2.77 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.53 [s, 3 H, CH_3^{β} Mdp], 1.48 [d (partly masked), $J \approx 7.3$ Hz, 3 H, CH_3^{β} Ala], 1.36 [d (partly masked), $J \approx 7.3$ Hz, 3 H, CH_3^{β} Ala], 1.48 [s, 3 H], 1.46 [s, 3 H], 1.29 [s, 3 H], 1.27 [s, 3 H] and 1.09 [s, 3 H, CH_3^{β} Mdp and $4CH_3^{\beta}$ Aib], 1.33 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.6, 174.2, 173.8, 173.4, 173.30, 173.31 [CO Aib, Mdp and Ala], 155.9 [CO Boc], 148.8, 148.3, 147.9, 147.5 [C^{Ar}-O Mdp], 130.3, 127.8, 123.7, 122.8, 117.3, 115.8, 113.7, 113.3 [C^{Ar} Mdp], 81.3 [O-C Boc], 70.7, 70.6, 70.5, 70.4, 69.5, 69.4, 68.95, 68.91, 68.8 [OCH_2], 59.4, 59.3 [C^{α} Mdp], 57.3, 56.9 [C^{α} Aib], 51.8 [OCH_3], 49.8, 48.4 [CH^{α} Ala], 43.4, 38.7 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 26.6, 26.1, 24.8, 23.6, 23.4, 22.4 [CH_3^{β} Mdp and CH_3^{β} Aib], 17.0, 16.5 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –48, $[\alpha]_{578}^{25}$ = –49, $[\alpha]_{546}^{25}$ = –58, $[\alpha]_{436}^{25}$ = –104 (c = 0.24, in MeOH). ESI$^+$ MS: m/z (%) = 1235.8 (100) [M + H]$^+$, 1257.7 (100) [M + Na]$^+$. $C_{60}H_{94}N_6O_{21}$ (1235.400): calcd. C 58.33, H 7.67, N 6.80; found C 58.23, H 7.88, N 6.31.

Boc-Aib-L-Mdp[benzo-24-C-8]-OH: The amino acid derivative Boc-L-Mdp[benzo-24-C-8]-OH[35] (1.087 g; 1.67 mmol) was N-deprotected in CH_2Cl_2 (15 mL) and TFA (5 mL) according to general procedure A. The crude TFA·H-L-Mdp[benzo-24-C-8]-OH (not characterized) was treated with Boc-Aib-NCA (1.152 g; 5.02 mmol) and DIEA (1.170 mL; 6.70 mmol) in THF (10 mL) at 50–55 °C for 2 d. The solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 (150 mL). The solution was washed with 0.5 N HCl (2 × 100 mL), H_2O (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a 3 × 50 cm column of silica gel with eluant (III) and then (IV). The combined solution of the product-containing fractions was treated by general procedure D to afford 0.617 g (50%) of pure Boc-Aib-L-Mdp[15-C-5]-OH as a solid. R_f = 0.60 (IV), 0.58 (V). ^{1}H NMR ($CDCl_3$): δ = 7.64 [s, 1 H, NH Mdp], 6.93 [s (br), 5 H, 4ArH benzo and ArH^2 Mdp], 6.72 [d (br), $J \approx 7.9$ Hz, 1 H, ArH

Mdp], 6.67 [d (br), $J \approx 8.1$ Hz, 1 H, ArH Mdp], 5.66 [s (br), 1 H, NH Aib], 5.28 [s (br), 1 H, COOH], 4.15 [s (br), 6 H, OCH_2], 4.05 [s (br), 2 H, OCH_2], 3.86–3.62 [m, 16 H, OCH_2], 3.44 [d br, J = 13.2 Hz, 1 H] and 3.28 [d (br), J = 12.7 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.62 [s, 3 H, CH_3^{β} Mdp], 1.36 [s, 9 H, CH_3 Boc], 1.36 [s (masked), 3 H] and 1.26 [s, 3 H, CH_3^{β} Aib] ppm. ^{13}C NMR ($CDCl_3$): δ = 178.3 [COOH Mdp], 173.3 [CO Aib], 154.4 [CO Boc], 148.7 [C^{Ar}-O benzo], 148.2, 147.8 [C^{Ar}-O Mdp], 146.0, 133.7, 123.7, 122.5, 122.2, 116.6, 115.9, 115.2, 114.7 [C^{Ar}], 78.7 [O-C Boc], 69.5, 69.2, 68.9, 68.7, 68.5, 68.4, 68.2 [OCH_2], 61.9 [C^{α} Mdp], 56.1 [C^{α} Aib], 40.8 [$ArCH_2^{\beta}$ Mdp], 28.3 [CH_3 Boc], 25.5 [CH_3^{β} Mdp], 24.5, 23.9 [CH_3^{β} Aib] ppm. $[\alpha]_{589}^{25}$ = +8, $[\alpha]_{578}^{25}$ = +9, $[\alpha]_{546}^{25}$ = +11, $[\alpha]_{436}^{25}$ = +24 (c = 0.20, in MeOH). $C_{37}H_{54}N_2O_{13}\cdot 1.5H_2O$ (761.842): C 58.33, H 7.54, N 3.68; found C 58.51, H 7.19, N 3.57.

Boc-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (3e): A mixture of Boc-Aib-L-Mdp[benzo-24-C-8]-OH (0.308 g; 0.42 mmol), HCl·H-L-Ala-OMe (0.176 g; 1.26 mmol), HOAt (0.114 g; 0.84 mmol), NMM (0.200 mL; 1.82 mmol) and EDC (0.121 g; 0.63 mmol) in CH_2Cl_2 (5 mL) was reacted from 0 °C to room temperature for 4 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product (0.323 g) consisted of **3e** (ca. 85–90% yield), almost pure by ^{1}H and ^{13}C NMR, contaminated by the side product $ClCH_2OAt$ (as in the synthesis of **3a**), present in a ca. 5% (mol/mol) ratio (as determined by ^{1}H NMR). This mixture could be used in the next coupling step without further purification (vide infra). An aliquot of crude product obtained from another run was purified by preparative TLC on silica gel with eluant (V). The separated band in the silica gel containing the desired product was extracted with several portions of a 1:1 MeOH/CH_2Cl_2 solution, and the filtered solution was treated by general procedure E to afford a sample of pure **3e** as a solid. R_f = 0.38 (III), 0.65 (V). ^{1}H NMR ($CDCl_3$): δ = 7.71 [d (br), $J \approx 8.2$ Hz, 1 H, NH Ala], 6.89 [m, 4 H, ArH benzo], 6.74 [d, J = 8.0 Hz, 1 H, ArH^5 Mdp], 6.65 [d, $J \approx 1.8$ Hz, 1 H, ArH^2 Mdp], 6.63 [dd (partly masked), $J \approx 8.0$ and 1.8 Hz, 1 H, ArH^6 Mdp], 6.18 [s (br), 1 H, NH Mdp], 4.87 [s, 1 H, NH Aib], 4.54 [dq, J = 7.2 and 7.2 Hz, 1 H, CH^{α} Ala], 4.13 [m, 8 H, OCH_2], 3.90 [m, 8 H, OCH_2], 3.82 [s, 4 H, OCH_2], 3.81 [s, 4 H, OCH_2], 3.70 [s, 3 H, OCH_3], 3.56 [d, J = 13.9 Hz, 1 H] and 3.07 [d, J = 14.1 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.47 [s, 3 H, CH_3^{β} Mdp], 1.44 [d, J = 7.4 Hz, 3 H, CH_3^{β} Ala], 1.39 [s, 9 H, CH_3 Boc], 1.38 [s (partly masked), 3H] and 1.34 [s, 3H, CH_3^{β} Aib] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.6, 173.3, 172.8 [CO Ala, CO Mdp and CO Aib], 155.0 [CO Boc], 148.7 [C^{Ar}-O benzo], 148.1, 147.7 [C^{Ar}-O Mdp], 129.7, 125.6, 121.3, 121.2, 117.2, 114.0, 113.4 [C^{Ar}], 80.6 [O-C Boc], 70.9, 70.8, 69.63, 69.60, 69.4, 69.1, 69.0 [OCH_2], 59.4 [C^{α} Mdp], 56.8 [C^{α} Aib], 51.7 [OCH_3], 48.2 [CH^{α} Ala], 39.0 [$ArCH_2^{\beta}$ Mdp], 27.9 [CH_3 Boc], 26.5 [CH_3^{β} Mdp], 24.7, 23.7 [CH_3^{β} Aib], 16.7 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –25, $[\alpha]_{578}^{25}$ = –27, $[\alpha]_{546}^{25}$ = –30, $[\alpha]_{436}^{25}$ = –57 (c = 0.25, in MeOH). $C_{41}H_{61}N_3O_{14}\cdot 0.5H_2O$ (828.930): calcd. C 59.40, H 7.54, N 5.07; found C 59.67, H 7.81, N 4.61.

Fmoc-L-Ala-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (4e): The crude peptide **3e** containing ca. 5% (mol/mol) of $ClCH_2OAt$ (vide supra) (0.323 g; <0.39 mmol) was N-deprotected in CH_2Cl_2 (12 mL) and TFA (4 mL) according to general procedure A. The crude TFA·H-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.400 g; 1.19 mmol) and DIEA (0.210 mL; 1.21 mmol) in THF (3.5 mL) at room temperature for 6 d. The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel, eluted three times with an 1:1 mixture of CH_2Cl_2 and eluant (V). The separated band in the silica gel containing the desired product was extracted with several portions of a 1:1 MeOH/CH_2Cl_2 solution, and the filtered solution was treated by general procedure E to afford 0.320 g (75% overall yield from Boc-Aib-L-Mdp[15-C-5]-OH) of pure **4e** as a solid. R_f = 0.66 (V). ^{1}H NMR ($CDCl_3$): δ = 7.74 [d, J = 7.6 Hz, 2 H, ArH Fmoc], 7.56 [d, $J \approx 7.2$ Hz, 2 H, ArH Fmoc], 7.51 [d, J = 6.9 Hz, 1 H, NH Ala], 7.38 [m (t-like), $J \approx 7.2$ Hz, 2 H, ArH Fmoc], 7.27 [m (br., t-like), 2 H, ArH Fmoc], 6.87 [m, 4 H, ArH benzo], 6.81 [s, 1 H, NH Mdp], 6.69 [d, J = 8.0 Hz, 1 H, ArH^5 Mdp], 6.64 [d (br), 1 H, ArH^2 Mdp], 6.61 [d (br), $J \approx 8.0$ Hz, 1 H, ArH^6 Mdp], 6.38 [s, 1 H, NH Aib], 5.70 [d, J = 6.2 Hz, 1 H, NH Ala], 4.52 [dq, $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.36 [m, 2 H, OCH_2 Fmoc], 4.17 [m (t-like), $J \approx 6.9$ Hz, 1 H, CH Fmoc], 4.08 [m, 8 H, OCH_2], 3.94 [dq (br), $J \approx 6.9$ and 6.9 Hz, 1 H, CH^{α} Ala], 3.85 [m, 8 H, OCH_2], 3.77 [s, 4 H, OCH_2], 3.76 [s, 4 H, OCH_2], 3.62 [s, 3 H, OCH_3], 3.60 [d (partly masked), 1 H] and 3.02 [d, J = 13.9 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.46 [s, 3 H, CH_3^{β} Mdp], 1.43 [d (partly masked), $J \approx 7.2$ Hz, 3 H, CH_3^{β} Ala], 1.40 [s, 3H] and 1.33 [s, 3H, CH_3^{β} Aib], 1.26 [d, J = 6.9 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.9, 173.6, 173.0, 172.7 [2 CO Ala, CO Aib and CO Mdp], 156.4 [CO Fmoc], 148.8 [C^{Ar}-O benzo], 148.2, 147.8 [C^{Ar}-O Mdp], 143.7, 143.5, 141.1, 130.0, 127.6, 127.0, 126.9, 124.9, 124.8, 123.7, 121.4, 119.8, 117.4, 114.1, 113.5 [C^{Ar} Mdp and C^{Ar} Fmoc], 71.0, 70.9, 69.8, 69.7, 69.4, 69.2, 69.1 [OCH_2], 67.0 [CH_2 Fmoc], 59.8 [C^{α} Mdp], 57.1 [C^{α} Aib], 51.9 [OCH_3], 50.7, 48.3 [CH^{α} Ala], 46.9 [CH Fmoc], 39.2 [$ArCH_2^{\beta}$ Mdp], 26.5 [CH_3^{β} Mdp], 24.5, 23.5 [CH_3^{β} Aib], 17.1, 16.9 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –26, $[\alpha]_{578}^{25}$ = –27, $[\alpha]_{546}^{25}$ = –32, $[\alpha]_{436}^{25}$ = –54 (c = 0.21, in MeOH). $C_{54}H_{68}N_4O_{15}\cdot 0.5H_2O$ (1022.124): C 63.45, H 6.80, N 5.48; found C 63.41, H 7.01, N 5.39.

Boc-{Aib-L-Mdp[benzo-24-C-8]-L-Ala}$_2$-OMe (6e): The peptide **4e** (0.219 g; 0.22 mmol) was N-deprotected in CH_3CN (9 mL) and diethylamine (1 mL) according to general procedure B. The crude mixture of H-L-Ala-Aib-L-Mdp[benzo-24-C-8]-L-Ala-OMe and dibenzofulvene was treated with the dipeptide Boc-Aib-L-Mdp[benzo-24-C-8]-OH (0.156 g; 0.21 mmol), HOAt (0.058 g; 0.42 mmol), NMM (0.035 mL; 0.32 mmol) and EDC (0.062 g; 0.32 mmol) in CH_2Cl_2 (3 mL), at room temperature for 6 d under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product was chromatographed on a 1.5 × 29 cm column of silica gel, eluted successively with eluants (III) and (V). The solution of the combined fractions containing the desired product was treated by general procedure E to afford 0.170 g (53%) of pure hexapeptide **6e** as a solid. R_f = 0.35 (V). ^{1}H NMR ($CDCl_3$): δ = 7.72 [d, J = 6.8 Hz, 1 H, NH Ala], 7.66 [d (br., partly masked), 1 H, NH Ala], 7.62 [s, 1 H, NH Aib], 6.87 [m, 8 H, ArH benzo], 6.76 [d, J = 8.6 Hz, 1 H, ArH Mdp], 6.64–6.55 [m, 5 H, ArH Mdp], 6.50 [s, 1 H, NH Mdp], 6.19 [s (br), 1 H, NH Mdp], 5.36 [s, 1 H, NH Aib], 4.52 [dq, $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.22 [dq (br), $J \approx 7.2$ and 7.2 Hz, 1 H, CH^{α} Ala], 4.17–3.71 [m, 48 H, OCH_2], 3.68 [s, 3 H, OCH_3], 3.55 [d, J = 14.1 Hz, 1 H] and 3.07 [d, J = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 2.96 [d, J = 14.0 Hz, 1 H] and 2.70 [d, J = 13.8 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.52 [s, 3 H, CH_3^{β} Mdp], 1.48 [d (partly masked), $J \approx 7.3$ Hz, 3 H, CH_3^{β} Ala], 1.33 [d (partly masked), $J \approx 7.2$ Hz, 3 H, CH_3^{β} Ala], 1.45 [s, 3H], 1.44 [s, 3H], 1.24 [s, 3H], 1.22 [s, 3H] and 1.07 [s, 3H, CH_3^{β} Mdp and $4CH_3^{\beta}$ Aib], 1.28 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.6, 174.2, 173.7, 173.3 [CO Aib, Mdp and Ala], 155.9 [CO Boc], 148.85, 148.83 [C^{Ar}-O benzo], 148.9, 148.4, 148.0, 147.5 [C^{Ar}-O Mdp], 130.4, 127.9, 123.8, 122.9, 121.46, 121.42, 121.37, 117.5, 116.2, 114.2, 114.14, 114.08, 113.7, 113.4 [C^{Ar} Mdp], 81.1 [O-C Boc], 71.2, 71.1, 71.0, 69.9, 69.8, 69.44, 69.39, 69.36 [OCH_2], 59.44, 59.39 [C^{α} Mdp], 57.3, 56.8 [C^{α} Aib], 51.7 [OCH_3], 49.7, 48.4 [CH^{α} Ala], 43.2, 38.7 [$ArCH_2^{\beta}$ Mdp], 28.1 [CH_3 Boc], 26.6, 26.0, 24.7, 23.6, 23.4, 22.7 [CH_3^{β} Mdp and CH_3^{β} Aib], 17.0,

16.4 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –42, $[\alpha]_{578}^{25}$ = –43, $[\alpha]_{546}^{25}$ = –49, $[\alpha]_{436}^{25}$ = –92 (*c* = 0.20, in MeOH). ESI^+ MS: *m*/*z* (%) = 1507.9 (100) $[M + H]^+$, 1529.9 (25) $[M + Na]^+$. $C_{76}H_{110}N_6O_{25}$ (1507.688): calcd. C 60.54, H 7.35, N 5.57; found C 60.61, H 7.52, N 5.32.

Z-L-Mdp-OMe: The derivative HCl·H-L-Mdp-OMe[35] (7.80 g; 29.8 mmol) was dissolved in THF (80 mL) and MeCN (120 mL) was added. The solution was cooled on an ice bath. Triethylamine (4.90 mL; 35.8 mmol) was added and the mixture was stirred for 5 min before adding Z-OSu (8.90 g; 35.8 mmol). The mixture was stirred at 0 °C for 1 h, then at room temperature for 4 d. Solvents were removed under reduced pressure, and the resulting residue was taken up in EtOAc. The solution was washed successively with water, 0.5 N HCl (twice), then again with water. The organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel with eluant (I) to give two major fractions: i) Z-L-Mdp-OMe (5.67 g; 53%) as a colorless oil, R_f = 0.45 (III) and ii) a fraction containing a ca. 1:1 mixture of isomers of Z-Mdp[*O*-Z,*O*-H]-OMe (2.80 g; 19%), R_f = 0.86 (III). The fraction ii) was dissolved in CH_2Cl_2 (100 mL) and pyrrolidine (2.3 mL; 27.6 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (100 mL), 0.5 N HCl (100 mL) was added, and the mixture was stirred vigorously for 10 min. The organic phase was washed with 0.5 N HCl (three times), then with water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel with eluant (I) to give Z-L-Mdp-OMe (1.57 g), (overall 7.24 g; 67%). ^1H NMR ($CDCl_3$): δ = 7.36 [m, 5 H, ArH Z], 6.68 [d, *J* = 8.1 Hz, 1 H, ArH], 6.43 [d, *J* = 1.9 Hz, 1 H, ArH], 6.38 [dd, *J* = 8.1 and 1.9 Hz, 1 H, ArH], 5.81 [s (br), 1 H, ArOH], 5.66 [s (br), 1 H, ArOH], 5.53 [s (br), 1 H, NH], 5.17 [d, *J* = 12.2 Hz, 1 H] and 5.05 [d, *J* = 12.2 Hz, 1 H, $ArCH_2$ Z], 3.74 [s, 3 H, OCH_3], 3.23 [d, *J* = 13.3 Hz, 1 H] and 3.04 [d, *J* = 13.3 Hz, 1 H, $ArCH_2^{\beta}$], 1.60 [s, 3 H, CH_3^{β}] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.4 [C=O], 143.3, 143.0, [C^{Ar}-O], 128.6, 128.5, 128.3, 127.1, 122.3, 116.8, 115.2 [C^{Ar}], 66.6 [$ArCH_2$ Z], 60.9 [C^{α}], 52.7 [OCH_3], 41.3 [$ArCH_2^{\beta}$], 23.5 [CH_3^{β}] ppm. $[\alpha]_{589}^{25}$ = –24, $[\alpha]_{578}^{25}$ = –24, $[\alpha]_{546}^{25}$ = –31, $[\alpha]_{436}^{25}$ = –62 (*c* = 0.2, in MeOH). ESI^+ MS: *m*/*z* (%) = 382 (100) $[M + Na]^+$, 741 (18) $[2M + Na]^+$. $C_{19}H_{21}NO_6$ (359.336): calcd. C 63.50, H 5.89, N 3.90; found C 63.26, H 5.91, N 3.83.

Z-L-Mdp[(*S*)-Binol-20-C-6]-OMe (1f): A solution of Z-L-Mdp-OMe (3.20 g; 6.42 mmol) and Cs_2CO_3 (2.19 g; 6.74 mmol) in degassed MeOH (70 mL) was stirred under argon at 45 °C for 10 min and then evaporated to dryness in vacuo. DMF (5 mL) was added to the residue and the resulting mixture was evaporated to dryness under high vacuum at 45 °C in order to completely remove the residual MeOH. The residue was redissolved in DMF (150 mL) and the mixture heated at 60 °C under argon. A solution of (–)-(*S*)-Binol[$(OCH_2CH_2)_2OTs]_2$[39] (4.94 g; 6.42 mmol) in DMF (70 mL) was added dropwise over a 1 h period. The mixture was magnetically stirred at 60 °C for 16 h and then evaporated to dryness under high vacuum. The residue was taken up in CH_2Cl_2 (250 mL) and 5% $NaHCO_3$ (100 mL). The decanted CH_2Cl_2 solution was washed with 5% $NaHCO_3$ (2 × 150 mL), then with H_2O (3 × 150 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a column of silica gel with eluant (I) to afford 3.00 g (60%) of pure **1f** as a solid. R_f = 0.50 (*I*). ^1H NMR ($CDCl_3$): δ = 7.90 [m, 4 H, ArH Binol], 7.42–7.27 [m, 9 H, 4 ArH Binol and 5 ArH Z], 7.27–7.12 [m, 4 H, ArH Binol], 6.67 [d, *J* = 7.9 Hz, 1 H, ArH^5 Mdp], 6.54 [dd (partly masked), *J* ≈ 8.0 and 1.7 Hz, 1 H, ArH^6 Mdp], 6.52 [s (br), 1 H, ArH^2 Mdp], 5.52 [s (br), 1 H, NH], 5.17 [d, *J* ≈ 12.1 Hz, 1 H] and 5.10 [d, *J* ≈ 12.1 Hz, 1 H, CH_2 Z], 4.21 [m, 2 H, OCH_2], 4.04 [m, 2 H, OCH_2], 3.90 [m, 2 H, OCH_2], 3.81 [m, 2 H, OCH_2], 3.76 [s, 3 H, OCH_3], 3.68 [m, 4 H, OCH_2], 3.50 [m, 4 H, OCH_2], 3.36 [d (br), *J* ≈ 13.3 Hz, 1 H] and 3.11 [d, *J* = 13.7 Hz, 1 H, $ArCH_2^{\beta}$], 1.65 [s, 3 H, CH_3^{β}] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.1 [C=O], 154.6 [C=O Z], 154.3 [C^{Ar}-O Binol], 148.6, 148.0 [C^{Ar}-O Mdp], 136.5, 134.1, 129.4, 129.2, 128.5, 128.1, 127.8, 126.2, 125.4, 123.6, 122.6, 120.7, 120.6, 116.1, 116.0, 115.7, 113.7 [C^{Ar} Binol, Z, and Mdp], 70.2, 69.8, 69.7, 69.5 [OCH_2], 66.4 [CH_2 Z], 60.9 [C^{α}], 52.6 [OCH_3], 41.5 [$ArCH_2^{\beta}$], 23.6 [CH_3^{β}] ppm. $[\alpha]_{589}^{25}$ = –93, $[\alpha]_{578}^{25}$ = –97, $[\alpha]_{546}^{25}$ = –115, $[\alpha]_{436}^{25}$ = –269 (*c* = 0.10, in CH_2Cl_2). ESI^+ MS: *m*/*z* (%) = 808.4 (100) $[M + Na]^+$, 824.6 (9) $[M + K]^+$. $C_{47}H_{47}NO_{10}\cdot H_2O$ (803.870): calcd. C 70.22, H 6.14, N 1.74; found C 70.47, H 5.81, N 1.37.

Z-L-Mdp[(*S*)-Binol-20-C-6]-OH: 1 N NaOH (60 mL) was added to a solution of **1f** (1.16 g; 1.47 mmol) in MeOH (100 mL) and the mixture was stirred at 60 °C for 24 h. MeOH was evaporated in vacuo at 40 °C with portions of H_2O added. The resulting aqueous basic solution (ca. 150 mL) was cooled to 0 °C, acidified by addition of a large excess of 0.5 N HCl and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were washed with H_2O (2 × 150 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to afford 1.08 g (95%) of crude Z-L-Mdp[(*S*)-Binol-20-C-6]-OH as a solid, which was used in the next steps without further purification. R_f = 0.40 (III). ^1H NMR ($CDCl_3$): δ = 7.83 [m, 4 H, ArH Binol], 7.44–7.29 [m, 9 H, 4 ArH Binol and 5 ArH Z], 7.25–7.11 [m, 4 H, ArH Binol], 6.65 [d, *J* = 8.0 Hz, 1 H, ArH^5 Mdp], 6.60 [s (br), 1 H, ArH^2 Mdp], 6.56 [d (br), *J* ≈ 8.5 Hz, 1 H, ArH^6 Mdp], 5.47 [s (br), 1 H, NH], 5.16 [d, *J* ≈ 12.0 Hz, 1 H] and 5.09 [d, *J* ≈ 12.0 Hz, 1 H, CH_2 Z], 4.20 [m, 2 H, OCH_2], 4.03 [m, 2 H, OCH_2], 3.85 [m, 4 H, OCH_2], 3.65 [m, 4 H, OCH_2], 3.53 [s, 2 H, OCH_2], 3.46 [s, 2 H, OCH_2], 3.32 [d (br), 1 H] and 3.16 [d, *J* = 13.9 Hz, 1 H, $ArCH_2^{\beta}$], 1.63 [s (br), 3 H, CH_3^{β} Mdp] ppm. $[\alpha]_{589}^{25}$ = –73, $[\alpha]_{578}^{25}$ = –78, $[\alpha]_{546}^{25}$ = –72, $[\alpha]_{436}^{25}$ = –214 (*c* = 0.10, in CH_2Cl_2). ESI^+ MS: *m*/*z* (%) = 794.5 (100) $[M + Na]^+$, 810.4 (41) $[M + K]^+$.

Z-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (2f): NMM (0.320 mL; 2.92 mmol) and then EDC (0.210 g; 1.09 mmol) was added to an ice-cold suspension of Z-L-Mdp[(*S*)-Binol-20-C-6]-OH (0.564 g; 0.73 mmol), HCl·H-L-Ala-OMe (0.306 g; 2.19 mmol) and HOAt (0.199 g; 1.46 mmol) in THF (5 mL) and CH_2Cl_2 (5 mL). The reaction mixture was warmed to room temperature, magnetically stirred for 3 d, and then diluted with CH_2Cl_2 (150 mL). The same work-up as for **3a** was applied. The crude product was chromatographed on a column of silica gel with eluant (I) to afford 0.188 g (30%) of pure **2f** as a solid. R_f = 0.20 (*I*). ^1H NMR ($CDCl_3$): δ = 7.87 [m, 4 H, ArH Binol], 7.49–7.27 [m, 9 H, 4 ArH Binol and 5 ArH Z], 7.27–7.13 [m, 4 H, ArH Binol], 6.74 [d, *J* ≈ 8.0 Hz, 1 H, ArH Mdp], 6.69 [d, *J* ≈ 8.0 Hz, 1 H, ArH Mdp], 6.62 [d (br), 1 H, NH Ala], 6.60 [d, *J* ≈ 1.5 Hz, 1 H, ArH^2 Mdp], 5.25 [s (br), 1 H, NH Mdp], 5.15 [s, 2 H, CH_2 Z], 4.58 [dq, *J* ≈ 7.1 and 7.1 Hz, 1 H, CH^{α} Ala], 4.23 [m, 2 H, OCH_2], 4.05 [m, 2 H, OCH_2], 3.92 [m, 2 H, OCH_2], 3.85 [m, 2 H, OCH_2], 3.75 [s, 3 H, OCH_3], 3.69 [m, 4 H, OCH_2], 3.52 [m, 4 H, OCH_2], 3.30 [d, *J* = 13.9 Hz, 1 H] and 3.13 [d, *J* = 13.9 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.50 [s, 3 H, CH_3^{β} Mdp], 1.38 [d, *J* = 7.1 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.3, 173.0 [C=O Ala and Mdp], 155.0 [C=O Z], 154.3 [C^{Ar}-O Binol], 148.7, 148.0 [C^{Ar}-O Mdp], 136.2, 134.1, 129.4, 129.2, 128.9, 128.5, 128.3, 127.8, 126.2, 125.4, 123.6, 123.1, 120.7, 116.1, 113.8 [C^{Ar} Binol, Z, and Mdp], 70.2, 69.8, 69.7, 69.6, 69.4 [OCH_2], 66.8 [CH_2 Z], 60.2 [C^{α} Mdp], 52.4 [OCH_3], 48.3 [CH^{α} Ala], 41.1 [$ArCH_2^{\beta}$ Mdp], 24.0 [CH_3^{β} Mdp], 16.9 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –112, $[\alpha]_{578}^{25}$ = –116, $[\alpha]_{546}^{25}$ = –137, $[\alpha]_{436}^{25}$ = –298 (*c* = 0.10, in CH_2Cl_2). ESI^+ MS: *m*/*z* (%) = 879.6 (100) $[M + Na]^+$, 895.4 (14) $[M + K]^+$.

$C_{50}H_{52}N_2O_{11}\cdot H_2O$ (874.948): calcd. C 68.63, H 6.22, N 3.20; found C 68.96, H 6.06, N 3.18.

Fmoc-L-Ala-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (3f): The peptide **2f** (0.068 g; 0.08 mmol) was *N*-deprotected by hydrogenolysis over 10% Pd/C (0.043 g) in a mixture of MeOH (40 mL), THF (20 mL) and 1 N aqueous HCl (0.160 mL; 0.16 mmol) according to general procedure C. The crude HCl·H-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.100 g; 0.30 mmol) and DIEA (0.050 mL; 0.30 mmol) in THF (3 mL) at room temperature for 2 d. The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (I) to afford 0.049 g (50%) of pure **3f** as a solid. R_f = 0.24 (*I*). ^{1}H NMR ($CDCl_3$): δ = 7.86–7.70 [m, 6 H, 4 ArH Binol and 2 ArH Fmoc], 7.57–7.09 [m, 14 H, 8 ArH Binol and 6 ArH Fmoc], 6.84 [d (br), $J \approx 6.7$ Hz, 1 H, NH Ala^3], 6.66 [d (br), $J \approx 8.3$ Hz, 1 H, ArH^6 Mdp], 6.62 [d, $J \approx 8.1$ Hz, 1 H, ArH^5 Mdp], 6.61 [s (br), 1 H, ArH^2 Mdp], 6.44 [s (br), 1 H, NH Mdp], 5.38 [d (br), $J \approx 5.8$ Hz, 1 H, NH Ala^1], 4.51 [dq, $J \approx 7.1$ and 7.1 Hz, 1 H, CH$^\alpha$ Ala^3], 4.38 [dd, J = 7.1 Hz and 10.3 Hz, 1 H] and 4.27 [dd (broad), 1 H, CH_2O Fmoc], 4.17 [m, 4 H, Ar-CH Fmoc, OCH_2 and CH$^\alpha$ Ala^1], 3.99 [m, 2 H, OCH_2], 3.84 [m, 4 H, OCH_2], 3.68 [s, 3 H, OCH_3], 3.60 [m, 4 H, OCH_2], 3.46 [m, 4 H, OCH_2], 3.35 [d, J = 14.0 Hz, 1 H] and 3.14 [d, J = 13.9 Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.54 [s, 3 H, CH_3^β Mdp], 1.33 [d, J = 7.1 Hz, 6 H, CH_3^β Ala^{1,3}] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.2, 172.9, 171.8 [C=O Ala^{1,3} and Mdp], 156.2 [C=O Fmoc], 154.3 [C^{Ar}-O Binol], 148.6, 148.1 [C^{Ar}-O Mdp], 143.7, 143.6, 141.3, 134.1, 129.4, 129.2, 129.1, 127.8, 127.2, 126.3, 125.5, 125.1, 125.0, 123.7, 123.2, 120.6, 120.0, 116.1, 113.7 [C^{Ar} Binol, Fmoc, and Mdp], 70.2, 69.9, 69.8, 69.6, 69.5 [OCH_2], 67.2 [CH_2 Fmoc], 60.5 [C$^\alpha$ Mdp], 52.4 [OCH_3], 51.3, 48.4 [CH$^\alpha$ Ala^{1,3}], 47.0 [Ar-CH Fmoc], 40.9 [$ArCH_2^\beta$ Mdp], 24.0 [CH_3^β Mdp], 17.9 [CH_3^β Ala^{1,3}] ppm. $[\alpha]_{589}^{25}$ = –103, $[\alpha]_{578}^{25}$ = –110, $[\alpha]_{546}^{25}$ = –128, $[\alpha]_{436}^{25}$ = –266 (c = 0.10, in CH_2Cl_2). ESI$^+$ MS: m/z (%) = 1038.7 (100) [M + Na]$^+$, 1054.5 (30) [M + K]$^+$. $C_{60}H_{61}N_3O_{12}$ (1016.112): calcd. C 70.92, H 6.05, N 4.14; found C 70.89, H 6.29, N 4.05.

Z-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (4f): The peptide **3f** (0.125 g; 0.123 mmol) was *N*-deprotected in CH_3CN (9 mL)/CH_2Cl_2 (9 mL) and diethylamine (2 mL) according to general procedure B. To the crude mixture of H-L-Ala-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe and dibenzofulvene, were successively added Z-L-Ala-OH (0.055 g; 0.246 mmol), HOBt (0.034 g; 0.25 mmol) and THF (2.5 mL). The resulting suspension was magnetically stirred, cooled to ca. –10 °C (ice/salt bath), and EDC (0.047 g; 0.24 mmol) was added. The reaction mixture was stirred from –10 °C to room temperature for 3 d. The same work-up as for **3a** was applied. The crude product was purified by preparative TLC on silica gel with eluant (II) to afford 0.058 g (47%) of pure **4f** as a solid. R_f = 0.25 (II). ^{1}H NMR ($CDCl_3$): δ = 7.85 [m, 4 H, ArH Binol], 7.44–7.10 [m, 13 H, 8 ArH Binol and 5 ArH Z], 7.08 [d, $J \approx 7.3$ Hz, 1 H, NH Ala^4], 7.00 [d (br), 1 H, NH Ala^2], 6.73 [d, $J \approx 8.1$ Hz, 1 H, ArH^5 Mdp], 6.65 [d (br), $J \approx 7.9$ Hz, 1 H, ArH^6 Mdp], 6.64 [s (br), 2 H, ArH^2 Mdp and NH Mdp], 5.59 [d (br), $J \approx 7.1$ Hz, 1 H, NH Ala^1], 5.10 [d, $J \approx 12.1$ Hz, 1 H] and 5.04 [d, $J \approx 12.3$ Hz, 1 H, CH_2 Z], 4.54 [dq, $J \approx 7.1$ and 7.1 Hz, 1 H, CH$^\alpha$ Ala^4], 4.20 [m, 4 H, CH$^\alpha$ Ala^{1,2} and OCH_2], 4.03 [m, 2 H, OCH_2], 3.92 [m, 4 H, OCH_2], 3.70 [s, 3 H, OCH_3], 3.62 [m, 4 H, OCH_2], 3.53 [s (br), 4 H, OCH_2], 3.41 [d, J = 13.9 Hz, 1 H] and 3.13 [d, J = 13.9 Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.48 [s, 3 H, CH_3^β Mdp], 1.38 [d, J = 7.3 Hz, 3 H, CH_3^β Ala^4], 1.30 [d, J = 7.1 Hz, 6 H, CH_3^β Ala^{1,2}] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.4, 173.0, 172.8, 171.6 [C=O Ala^1, Ala^2, Ala^4 and Mdp], 155.9 [C=O Z], 154.2 [C^{Ar}-O Binol], 148.3, 147.9 [C^{Ar}-O Mdp], 134.0, 129.3, 129.1, 128.4, 128.2, 128.0, 127.7, 126.2, 125.3, 123.6, 123.3, 120.6, 120.5, 116.6, 116.1, 113.5 [C^{Ar} Binol, Z, and Mdp], 70.1, 70.0, 69.7, 69.6, 69.4, 69.3 [OCH_2], 67.0 [CH_2 Z], 60.4 [C$^\alpha$ Mdp], 52.2 [OCH_3], 50.3, 50.1, 48.3 [CH$^\alpha$ Ala^{1,2,4}], 40.7 [$ArCH_2^\beta$ Mdp], 23.8 [CH_3^β Mdp], 18.0, 17.7, 17.2 [CH_3^β Ala^{1,2,4}] ppm. $[\alpha]_{589}^{25}$ = –116, $[\alpha]_{578}^{25}$ = –123, $[\alpha]_{546}^{25}$ = –143, $[\alpha]_{436}^{25}$ = –293 (c = 0.10, in CH_2Cl_2). ESI$^+$ MS: m/z (%) = 1021.7 (79) [M + Na]$^+$, 1037.6 (14) [M + K]$^+$, 522.5 (100) [M + 2Na]$^{2+}$, 530.4 (15) [M + Na + K]$^{2+}$. $C_{56}H_{62}N_4O_{13}\cdot 2H_2O$ (1035.120): calcd. C 64.97, H 6.43, N 5.41; found C 64.85, H 6.17, N 5.13.

Z-L-Mdp[(*S*)-Binol-20-C-6]-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (5f): The peptide **4f** (0.047 g; 0.047 mmol) was *N*-deprotected by hydrogenolysis over 10% Pd/C (0.028 g) in a mixture of MeOH (40 mL), THF (20 mL) and 1 N aqueous HCl (0.080 mL; 0.080 mmol) according to general procedure C. The crude HCl·H-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (not characterized) was treated with Z-L-Mdp[(*S*)-Binol-20-C-6]-OH (0.045 g; 0.059 mmol), HOAt (0.016 g; 0.117 mmol), NMM (0.015 mL; 0.13 mmol) and EDC (0.017 g; 0.09 mmol) in THF (2.5 mL) and CH_2Cl_2 (2.5 mL), under the same experimental conditions as for **2f**. The same work-up as for **3a** was applied. The crude product was purified by preparative TLC on silica gel with eluant (II) to afford 0.031 g (41%) of pure **5f** as a solid. R_f = 0.28 (II). ^{1}H NMR ($CDCl_3$): δ = 7.84 [m, 8 H, ArH Binol], 7.47–7.060 [m, 22 H, 16 ArH Binol, 5 ArH Z and NH Ala], 7.04 [d, $J \approx 7.5$ Hz, 1 H, NH Ala^5], 6.82–6.55 [m, 7 H, 6 ArH Mdp^{1,4} and NH Mdp^4], 6.34 [d (br), 1 H, NH Ala], 5.46 [s, 1 H, NH Mdp^1], 5.12[d, $J \approx 12.5$ Hz, 1 H] and 5.06 [d, $J \approx 12.3$ Hz, 1 H, CH_2 Z], 4.58 [dq, $J \approx 7.1$ and 7.3 Hz, 1 H, CH$^\alpha$ Ala^5], 4.27 [m, 1 H, CH$^\alpha$ Ala], 4.21 [m, 5 H, CH$^\alpha$ Ala and OCH_2], 4.08–3.82 [m, 12 H, OCH_2], 3.68 [s, 3 H, OCH_3], 3.60–3.50 [m, 16 H, OCH_2], 3.49 [d (partly masked), 1 H] and 3.13 [d, J = 13.9 Hz, 1 H, $ArCH_2^\beta$ Mdp], 2.99 [d, J = 13.7 Hz, 1 H] and 2.89 [d, J = 13.9 Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.51 [s, 3 H, CH_3^β Mdp], 1.46 [s, 3 H, CH_3^β Mdp], 1.39 [d (partly masked), 3 H, CH_3^β Ala], 1.37 [d (partly masked), J = 7.1 Hz, 3 H, CH_3^β Ala^5], 1.28 [d (partly masked), 3 H, CH_3^β Ala] ppm. $[\alpha]_{589}^{25}$ = –104, $[\alpha]_{578}^{25}$ = –111, $[\alpha]_{546}^{25}$ = –127, $[\alpha]_{436}^{25}$ = –261 (c = 0.10, in CH_2Cl_2). ESI$^+$ MS: m/z (%) = 832.2 (100) [M + 2Na]$^{2+}$, 840.3 (18) [M + Na + K]$^{2+}$. $C_{94}H_{99}N_5O_{20}$ (1618.772): calcd. C 69.74, H 6.16, N 4.33; found C 69.76, H 6.39, N 4.35.

Fmoc-{L-Ala-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala}$_2$-OMe (6f): The peptide **5f** (0.017 g; 0.012 mmol) was *N*-deprotected by hydrogenolysis over 10% Pd/C (0.015 g) in a mixture of MeOH (8 mL), THF (4 mL) and 1 N aqueous HCl (0.020 mL; 0.020 mmol) according to general procedure C. The crude HCl·H-L-Mdp[(*S*)-Binol-20-C-6]-(L-Ala)$_2$-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.012 g; 0.034 mmol) and DIEA (0.010 mL; 0.060 mmol) in THF (2.5 mL) at room temperature for 3 d. The same work-up as for **3a** was applied. The crude product was purified by preparative TLC on silica gel with eluant (II) to afford 0.007 g (35%) of pure **6f** as a solid. R_f = 0.58 (II). ^{1}H NMR ($CDCl_3$): δ = 7.92–7.73 [m, 10 H, 8 ArH Binol from Mdp^{2,5} and 2 ArH Fmoc], 7.70–7.08 [m, 25 H, 16 ArH Binol from Mdp^{2,5}, 6 ArH Fmoc and 3 NH], 6.80–6.40 [m, 7 H, 6 ArH Mdp^{2,5} and 1 NH], 6.06 [m (br), 1 H, NH], 5.43 [m (br), 1 H, NH Ala^1], 4.60 [dq, $J \approx 7.3$ and 7.3 Hz, 1 H, CH$^\alpha$ Ala^6], 4.40–3.70[m, 22 H, 3 CH$^\alpha$ Ala^{1,3,4}, 8 OCH_2, CH_2O Fmoc and Ar-CH Fmoc], 3.67 [s, 3 H, OCH_3], 3.65–3.34 [m, 16 H, OCH_2], 3.34 [d (partly masked), 1 H] and 3.08 [d, J = 14.0 Hz, 1 H, $ArCH_2^\beta$ Mdp], 2.96 [d, J = 13.5 Hz, 1 H] and 2.80 [d (br), $J \approx 13.5$ Hz, 1 H, $ArCH_2^\beta$ Mdp], 1.60 [s, 3 H, CH_3^β Mdp], 1.46 [s, 3 H, CH_3^β Mdp], 1.44 [d (partly masked), $J \approx 7.1$ Hz, 3 H, CH_3^β Ala], 1.40 [d (partly masked), J = 6.8 Hz, 3 H, CH_3^β Ala], 1.33 [d (br., partly masked), 3 H, CH_3^β Ala], 1.21 [d (partly masked), J = 6.9 Hz, 3 H, CH_3^β Ala] ppm. ESI$^+$ MS:

m/z (%) = 912.3 (100) $[M + 2Na]^{2+}$, 920.3 (100) $[M + Na + K]^{2+}$, 928.3 (42) $[M + 2 K]^{2+}$. $C_{104}H_{108}N_6O_{21}\cdot 2H_2O$ (1813.984): calcd. C 68.86, H 6.22; found C 69.21, H 7.12.

Boc-Aib-L-Mdp[(*S*)-Binol-20-C-6]-OH (2g): Z-L-Mdp[(*S*)-Binol-20-C-6]-OH (1.04 g; 1.35 mmol) was *N*-deprotected by hydrogenolysis over 10% Pd/C (0.302 g) in a mixture of MeOH (60 mL) and THF (60 mL) according to general procedure C. The crude H-L-Mdp[(*S*)-Binol-20-C-6]-OH (not characterized) was treated with Boc-Aib-NCA (1.028 g; 4.48 mmol) and DIEA (0.780 mL; 4.48 mmol) in THF (7.5 mL) at 50–55 °C for 4 d. The solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 (150 mL). The solution was washed with 0.5 N HCl (2 × 100 mL), H_2O (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a column of silica gel with eluant (III) to afford 0.890 g (72%) of pure **2g** as a solid. R_f = 0.25 (III). ^{1}H NMR ($CDCl_3$): δ = 7.85 [m, 4 H, ArH Binol], 7.44–7.27 [m, 4 H, ArH Binol], 7.27–7.07 [m, 5 H, ArH Binol and NH Mdp], 6.72 [s, 3 H, ArH Mdp], 5.23 [s (br), 1 H, NH Aib], 4.22 [m, 2 H, OCH_2], 4.03 [m, 2 H, OCH_2], 3.92 [m, 4 H, OCH_2], 3.76 [s, 3 H, OCH_3], 3.67 [m, 4 H, OCH_2], 3.53 [s (br), 4 H, OCH_2], 3.39 [d, *J* = 13.7 Hz, 1 H] and 3.23 [d, *J* = 13.7 Hz, 1 H, $ArCH_2^{\beta}$], 1.63 [s, 3 H, CH_3^{β} Mdp], 1.44 [s, 3 H] and 1.40 [s (masked), 3 H, CH_3^{β} Aib], 1.40 [s, 9 H, CH_3 Boc] ppm. ^{13}C NMR ($CDCl_3$): δ = 174.3, 171.2 [C=O Mdp and Aib], 154.7 [C=O Boc], 154.2 [C^{Ar}-O Binol], 148.3, 147.7 [C^{Ar}-O Mdp], 133.9, 129.3, 129.1, 127.7, 126.2, 125.3, 123.6, 122.9, 120.5, 116.1, 113.5 [C^{Ar} Binol and Mdp], 79.8 [O-C Boc], 70.02, 69.96, 69.7, 69.3, 69.2 [OCH_2], 60.8 [C^{α} Mdp], 56.7 [C^{α} Aib], 40.8 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 25.7 [CH_3^{β} Mdp], 24.9, 22.9 [CH_3^{β} Aib] ppm. $[\alpha]_{589}^{25}$ = –112, $[\alpha]_{578}^{25}$ = –118, $[\alpha]_{546}^{25}$ = –136, $[\alpha]_{436}^{25}$ = –279 (*c* = 0.10, in CH_2Cl_2). ESI$^+$ MS: *m/z* (%) = 845.9 (100) $[M + Na]^+$, 862.0 (16) $[M + K]^+$. $C_{47}H_{54}N_2O_{11}\cdot H_2O$ (840.934): calcd. C 67.12, H 6.71, N 3.33; found C 67.14, H 6.62, N 3.82.

Boc-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (3g): NMM (0.200 mL; 1.82 mmol) and then EDC (0.130 g; 0.68 mmol) was added to an ice-cold suspension of **2g** (0.378 g; 0.46 mmol), HCl·H-L-Ala-OMe (0.192 g; 1.37 mmol) and HOAt (0.124 g; 0.91 mmol) in THF (5 mL) and CH_2Cl_2 (5 mL). The reaction mixture was warmed up to room temperature, magnetically stirred for 3 d, and then diluted with CH_2Cl_2 (150 mL). The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (I) to afford 0.281 g (68%) of pure **3g** as a solid. R_f = 0.27 (*I*). ^{1}H NMR ($CDCl_3$): δ = 7.85 [m, 4 H, ArH Binol], 7.76 [d (br), *J* = 7.8 Hz, 1 H, NH Ala], 7.45–7.27 [m, 4 H, ArH Binol], 7.27–7.10 [m, 4 H, ArH Binol], 6.74 [d, *J* = 8.3 Hz, 1 H, ArH^5 Mdp], 6.68 [d (br), *J* ≈ 8.3 Hz, 1 H, ArH^6 Mdp], 6.65 [s (br), 1 H, ArH^2 Mdp], 6.22 [s (br), 1 H, NH Mdp], 4.94 [s, 1 H, NH Aib], 4.56 [dq, *J* = 7.1 and 7.1 Hz, 1 H, CH^{α} Ala], 4.22 [m, 2 H, OCH_2], 4.05 [m, 2 H, OCH_2], 3.94 [m, 4 H, OCH_2], 3.71 [s, 3 H, OCH_3], 3.67 [m, 4 H, OCH_2], 3.55 [s (br), 4 H, OCH_2], 3.64 [d (masked), 1 H] and 3.10 [d, *J* = 13.8 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.49 [s, 3 H, CH_3^{β} Mdp], 1.46 [d, *J* ≈ 7.1 Hz, 3 H, CH_3^{β} Ala], 1.42 [s, 9 H, CH_3 Boc], 1.42 [s (masked), 3 H] and 1.35 [s, 3 H, CH_3^{β} Aib] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.6, 172.9 [C=O Aib, Mdp and Ala], 155.1 [C=O Boc], 154.3 [C^{Ar}-O Binol], 148.5, 148.1 [C^{Ar}-O Mdp], 134.1, 129.4, 129.2, 127.8, 125.4, 123.7, 120.7, 117.6, 116.2, 116.1, 113.8 [C^{Ar} Binol and Mdp], 81.0 [O-C Boc], 70.21, 70.18, 69.94, 69.87, 69.80, 69.6 [OCH_2], 59.7 [C^{α} Mdp], 57.1 [C^{α} Aib], 52.0 [OCH_3], 48.5 [CH^{α} Ala], 41.1 [$ArCH_2^{\beta}$ Mdp], 28.2 [CH_3 Boc], 26.9 [CH_3^{β} Mdp], 24.9, 23.9 [CH_3^{β} Aib], 17.1 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –126, $[\alpha]_{578}^{25}$ = –133, $[\alpha]_{546}^{25}$ = –153 $[\alpha]_{436}^{25}$ = –299 (*c* = 0.10 in CH_2Cl_2). ESI$^+$ MS: *m/z* (%) = 930.5 (100) $[M + Na]^+$, 946.5 (17) $[M + K]^+$. $C_{51}H_{61}N_3O_{12}\cdot H_2O$ (926.038): calcd. C 66.14, H 6.86, N 4.54; found C 66.61, H 8.81, N 4.76.

Fmoc-L-Ala-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (4g): The peptide **3g** (0.121 g; 0.13 mmol) was *N*-deprotected in CH_2Cl_2 (10 mL) and TFA (5 mL) according to general procedure A. The crude TFA·H-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe (not characterized) was treated with Fmoc-L-Ala-NCA (0.135 g; 0.40 mmol) and DIEA (0.070 mL; 0.40 mmol) in THF (5 mL) at room temperature for 6 d. The same work-up as for **3a** was applied. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (I) to afford 0.091 g (62%) of pure **4g** as a solid. R_f = 0.30 (*I*). ^{1}H NMR ($CDCl_3$): δ = 7.85 [m, 4 H, ArH Binol], 7.75 [d, *J* ≈ 7.5 Hz, 2 H, ArH Fmoc], 7.57 [d, *J* ≈ 7.5 Hz, 2 H, ArH Fmoc], 7.45–7.27 [m, 9 H, 4 ArH Binol, 4 ArH Fmoc and NH Ala^4], 7.27–7.10 [m, 4 H, ArH Binol], 6.71 [d, *J* = 8.3 Hz, 1 H, ArH^5 Mdp], 6.66 [d (br), *J* ≈ 8.3 Hz, 1 H, ArH^6 Mdp], 6.64 [s (br), 1 H, ArH^2 Mdp], 6.50 [s (br), 1 H, NH Mdp], 6.36 [s, 1 H, NH Aib], 5.70 [d (br), *J* ≈ 6.0 Hz, 1 H, NH Ala^1], 4.58 [dq, *J* ≈ 7.1 and 7.1 Hz, 1 H, CH^{α} Ala^4], 4.38 [m, 2 H, OCH_2 Fmoc], 4.21 [m, 3 H, OCH_2 and CH Fmoc], 4.06–3.89 [m, 7 H, 3 OCH_2 and CH^{α} Ala^1], 3.66 [s, 3 H, OCH_3], 3.63 [m, 4 H, OCH_2], 3.52 [s (br), 4 H, OCH_2], 3.60 [d (masked), 1 H] and 3.06 [d, *J* = 13.9 Hz, 1 H, $ArCH_2^{\beta}$ Mdp], 1.50 [s, 3 H, CH_3^{β} Mdp], 1.46 [d, *J* ≈ 7.3 Hz, 3 H, CH_3^{β} Ala], 1.44 [s, 3 H] and 1.36 [s, 3 H, CH_3^{β} Aib], 1.29 [d, *J* ≈ 7.1 Hz, 3 H, CH_3^{β} Ala] ppm. ^{13}C NMR ($CDCl_3$): δ = 173.7, 172.8, 172.5 [C=O Aib, Mdp and Ala], 156.2 [C=O Fmoc], 154.4 [C^{Ar}-O Binol], 148.5, 148.1 [C^{Ar}-O Mdp], 143.9, 143.6, 141.3, 134.1, 130.1, 129.5, 129.4, 129.2, 127.8, 127.2, 127.1, 126.3, 125.5, 125.1, 125.0, 123.9, 123.7, 123.6, 120.8, 120.7, 120.0, 117.7, 116.2, 116.1, 113.8 [C^{Ar} Binol, Mdp and Fmoc], 70.2, 70.1, 69.88, 69.84, 69.7, 69.5 [OCH_2], 67.3 [CH_2 Fmoc], 60.1 [C^{α} Mdp], 57.3 [C^{α} Aib], 52.0 [OCH_3], 48.3 [CH^{α} Ala], 47.4 [CH Fmoc], 39.7 [$ArCH_2^{\beta}$ Mdp], 26.7 [CH_3^{β} Mdp], 24.6, 23.8 [CH_3^{β} Aib], 17.3 [CH_3^{β} Ala] ppm. $[\alpha]_{589}^{25}$ = –123, $[\alpha]_{578}^{25}$ = –129, $[\alpha]_{546}^{25}$ = –149 $[\alpha]_{436}^{25}$ = –295 (*c* = 0.10 in CH_2Cl_2). ESI$^+$ MS: *m/z* (%) = 573.6 (100) $[M + 2Na]^{2+}$, 581.4 (62) $[M + Na + K]^{2+}$, 1123.9 (85) $[M + Na]^+$, 1139.8 (27) $[M + K]^+$. $C_{64}H_{68}N_4O_{13}\cdot H_2O$ (1119.232): calcd. C 68.68, H 6.30, N 5.01; found C 68.64, H 6.13, N 5.03.

Boc-{Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala}$_2$-OMe (6g): The peptide **4g** (0.083 g; 0.076 mmol) was *N*-deprotected in CH_3CN (9 mL)/CH_2Cl_2 (9 mL) and diethylamine (2 mL) according to general procedure B. The crude mixture of H-L-Ala-Aib-L-Mdp[(*S*)-Binol-20-C-6]-L-Ala-OMe and dibenzofulvene was treated with the dipeptide **2g** (0.078 g; 0.094 mmol), HOAt (0.021 g; 0.15 mmol) and EDC (0.022 g; 0.11 mmol) in CH_2Cl_2 (2 mL)/THF (1 mL), at room temperature for 4 d, under the same experimental conditions and work-up procedure as for the synthesis of **3a**. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (II) to afford 0.028 g (32%) of pure **6g** as a solid. R_f = 0.47 (II). ^{1}H NMR ($CDCl_3$): δ = 7.88 [m, 8 H, ArH Binol], 7.75 [d (partly masked), 1 H, NH Ala^6], 7.70 [d (br), 1 H, NH Ala^3], 7.60 [s, 1 H, NH $Mdp^{2,5}$ or Aib^4], 7.45–7.10 [m, 16 H, ArH Binol], 6.78–6.56 [m, 6 H, ArH $Mdp^{2,5}$], 6.52 [s (br), 1 H, NH $Mdp^{2,5}$ or Aib^4], 6.39 [s (br), 1 H, NH $Mdp^{2,5}$ or Aib^4], 5.07 [s (br), 1 H, NH Aib^1], 4.56 [dq, *J* ≈ 7.0 and 7.0 Hz, 1 H, CH^{α} Ala^6], 4.20 [m, 5 H, 2 OCH_2 and CH^{α} Ala^3], 4.04 [m, 4 H, OCH_2], 3.94–3.75 [m, 8 H, OCH_2], 3.71 [s, 3 H, OCH_3], 3.70–3.40 [m, 16 H, OCH_2], *ca* 3.60 [d (masked), 1 H] and 3.15 [d, *J* = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp^5], 2.99 [d, *J* = 14.0 Hz, 1 H] and 2.82 [d, *J* = 14.0 Hz, 1 H, $ArCH_2^{\beta}$ Mdp^2], 1.56 [s, 3 H, CH_3^{β} Mdp], 1.52 [d (partly masked), *J* ≈ 7.3 Hz, 3 H, CH_3^{β} Ala^6], 1.49 [s, 3 H, CH_3^{β} Mdp], 1.40 [d, *J* ≈ 7.3 Hz, 3 H, CH_3^{β} Ala^3], 1.34 [s, 9 H, CH_3 Boc], 1.37 [s, 3 H], 1.28 [s, 3 H], 1.27 [s, 3 H] and 1.20 [s, 3 H, CH_3^{β} $Aib^{1,4}$] ppm. ^{13}C NMR ($CDCl_3$): δ

= 174.4, 173.9, 173.6, 173.5, 173.3 [C=O Aib, Mdp and Ala], 155.8 [C=O Boc], 154.46, 154.37, 154.32 [C^{Ar}-O Binol], 149.0, 148.6, 148.4, 147.7 [C^{Ar}-O Mdp], 134.1, 130.8, 129.5, 129.2, 127.8, 126.4, 126.3, 125.5, 123.7, 123.2, 120.8, 120.7, 117.5, 116.4, 116.2, 116.1, 116.0, 114.0 [C^{Ar} Binol and $Mdp^{2,5}$], 81.5 [O-C Boc], 70.2, 70.1, 69.9, 69.5 [OCH_2], 59.8, 59.4 [C^{α} $Mdp^{2,5}$], 57.4, 56.9 [C^{α} $Aib^{1,4}$], 51.9 [OCH_3], 50.1, 48.5 [CH^{α} $Ala^{3,6}$], *ca* 43–39 (br, $ArCH_2^{\beta}$ $Mdp^{2,5}$], 28.3 [CH_3 Boc], 26.6, 26.2 [CH_3^{β} $Mdp^{2,5}$], 24.7, 24.2, 23.8, 22.7 [CH_3^{β} $Aib^{1,4}$], 17.1, 16.4 [CH_3^{β} $Ala^{3,6}$] ppm. $[a]_{589}^{25}$ = –121, $[a]_{578}^{25}$ = –124, $[a]_{546}^{25}$ = –145 $[a]_{436}^{25}$ = –308 (*c* = 0.12, in CH_2Cl_2). ESI^+ MS: *m*/*z* (%) = 865.0 (100) $[M + 2Na]^{2+}$, 873.1 (57) $[M + Na + K]^{2+}$, 1707.3 (20) $[M + Na]^+$, 1723.0 (6) $[M + K]^+$. $C_{96}H_{110}N_6O_{21}$ (1683.888): calcd. C 68.47, H 6.58, N 4.99; found C 68.35, H 6.61, N 4.93.

ESI-MS Ion Complexation Studies: Mass spectrometric analyses were performed with a quadrupole mass spectrometer Hewlett–Packard HP5989MS equipped with an electrospray ionization source. The voltage difference between the ESI needle and the counter electrode was 3.5 kV, and the heated capillary was held at 120 °C. A cone voltage of 35 V was chosen in order to minimize fragmentation and optimise ion intensity. The spectra were recorded from 100 to 1800 *m*/*z* in the positive ion mode. For mass scale calibration, a solution of sodium dodecyl sulfate was used, with characteristic ion adducts at 311.13, 599.26 and 887.40. The solutions analyzed were injected at 4 μL/min. They contained a sample of each tripeptide (0.1 mM)-hexapeptide (0.1 mM) couple **3a**–**6a**, or **3d**–**6d** or **3e**–**6e** in a 9:1 CH_3CN/H_2O solution (v/v), combined with either (first set of experiments) a solution of a mixture of LiCl (0.2 mM), NaCl (0.2 mM), KCl (0.2 mM), RbCl (0.2 mM), and CsCl (0.2 mM) in a 9:1 CH_3CN/H_2O (v/v) solution, or (second set of experiments) a solution of a single alkali metal chloride (1 mM) in a 9:1 CH_3CN/H_2O (v/v) solution. Purities of the purchased alkali metal chlorides were 98–99%.

FTIR Absorption: The FTIR absorption spectra were recorded with a Perkin–Elmer model 1720X spectrophotometer, nitrogen-flushed, equipped with a sample shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% D) was purchased from Aldrich. Solvent (baseline) spectra were recorded under the same conditions.

Nuclear Magnetic Resonance: The ^1H NMR spectra for conformational analysis were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in deuteriochloroform (99.96% D, Merck) and deuterated dimethyl sulfoxide ([D_6]DMSO, 99.96%, Acros) with tetramethylsilane as the internal standard.

Circular Dichroism: The CD spectra were obtained with a Jasco J-710 dichrograph. Cylindrical fused quartz cells of 10, 1.0, 0.2 and 0.1 mm path length (Hellma) were used. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity ($deg \times cm^2 \times dmol^{-1}$). Spectrograde MeOH and CH_3CN (Aldrich), freshly distilled CH_2Cl_2 and mQ H_2O were used as solvents.

Vibrational Circular Dichroism: The hexapeptide **6e** was dissolved in $CDCl_3$ at 16 and 8 mM concentrations. The resulting solutions were placed in a refillable IR cell (Specac) with CaF_2 windows separated by a 500 μm teflon spacer. IR absorption spectra were obtained on a Digilab (Varian) FTS-60A FTIR spectrometer with a DTGS detector at 4 cm^{-1} resolution, averaging 256 scans. Dispersive VCD spectra were recorded on an instrument described at length in the literature[75] for the same samples in the amide I and II regions with 10 cm^{-1} resolution, as an average of 8 scans. Baselines were corrected in both cases by identically obtained scans of just the solvent in the same cell. Due to high absorbance of the more concentrated sample, only data for the 12 mg/mL sample are reported, but spectra for the major features were consistent for both.

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