Inhibition of Amyloid Fibril Formation of Human Amylin by N-Alkylated Amino Acid and α-Hydroxy Acid Residue Containing Peptides

Dirk T. S. Rijkers,[a] Jo W. M. Höppener,[b] George Posthuma,[c] Cornelis J. M. Lips,[b] and Rob M. J. Liskamp*[a]

Abstract: Amyloid deposits are formed as a result of uncontrolled aggregation of (poly)peptides or proteins. Today several diseases are known, for example Alzheimer's disease, Creutzfeldt – Jakob disease, mad cow disease, in which amyloid formation is involved. Amyloid fibrils are large aggregates of β-pleated sheets and here a general method is described to introduce molecular mutations in order to achieve disruption of β-sheet formation. Eight backbone-modified amylin derivatives, an amyloidogenic peptide involved in maturity onset diabetes, were synthesized. Their β-sheet forming properties were studied by IR spectroscopy and electron microscopy. Modification of a crucial amide N*H* by an alkyl chain led to a complete loss of the β-sheet forming capacity of amylin. The resulting molecular mutated amylin derivative could be used to break the β-sheet thus retarding β-sheet formation of unmodified amylin. Moreover, it was found that the replacement of this amide bond by an ester moiety suppressed fibrillogenesis significantly. Introduction of N-alkylated amino acids and/or ester functionalities—leading to depsipeptides—into amyloidogenic peptides opens new avenues towards novel peptidic β-sheet breakers for inhibition of β-amyloid aggregation.

Keywords: aggregation · amyloid · peptides · peptidomimetics · protein modifications

Introduction

Peptide – protein and protein – protein interactions are vital in cellular processes of healthy organisms, but they can also be detrimental or lethal to the organism as has been shown in diseases where there is a uncontrolled aggregation leading to accumulation of for example protein fibrils, amyloidogenic plaques.[1] The most well-known diseases in this respect are Alzheimer's disease,[2] and transmissible spongiform encephalopathies involving prions (scrapie, BSE and Creutzfeldt – Jakob Disease).[3]

[a] Prof. Dr. R. M. J. Liskamp, Dr. Ir. D. T. S. Rijkers
Department of Medicinal Chemistry
Utrecht Institute for Pharmaceutical Sciences
Faculty of Pharmaceutical Sciences
Utrecht University, P.O. Box 80082
3508 TB Utrecht (The Netherlands)
Fax: (+31) 30 253 6655
E-mail: r.m.j.liskamp@pharm.uu.nl

[b] Dr. J. W. M. Höppener, Prof. Dr. C. J. M. Lips
Department of Internal Medicine and Endocrinology
University Medical Center
3508 AB Utrecht (The Netherlands)

[c] Dr. G. Posthuma
Department of Cell Biology
Center for Electron Microscopy, University Medical Center
3508 GA Utrecht (The Netherlands)

Less known, but of increasing impact in view of the aging population and obesity in the Western world is maturity onset diabetes (diabetes type II).[4] The protein involved in this disease is amylin also known as *i*slet *a*myloid *p*oly*p*eptide (IAPP). It is a 37 amino acid residue peptide hormone produced by islet β-cells of the pancreas and it is co-secreted with insulin.[5] Amyloid deposits of fibrillar amylin in the pancreatic islets are present in the vast majority of patients with non-insulin-dependent diabetes mellitus (diabetes type II).[6] These fibrillar assemblies are cytotoxic for islet β-cells and are associated with the depletion of these cells which accompanies the progression of the disease.[7]

Structure – activity studies have shown that amino acid residues 20 – 29 that is Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser of amylin are crucial for amyloid formation.[8, 9] Moreover, a proline scan of this decamer has demonstrated that substitution of serine at position 28 by a proline residue efficiently reduces the capacity for amyloid fibril formation.[10]

We are interested in the design and synthesis of soluble β-sheet mimetics that interfere with amyloid fibril formation by inhibiting the assembly of β-pleated sheet containing molecules. This assembly leads to a decreased solubility, gel formation and/or aggregation.

The design approach we wanted to follow—in order to interfere with the formation of β-sheets, thereby acting as β-sheet breakers[11]—was to come up with compounds which would prevent *further* aggregation once a β-sheet has already

  0947-6539/02/0818-4285 $ 20.00+.50/0

started to form and/or which are destabilizing the β-sheet once the compounds are incorporated; thus they would act as "mutations". In a more molecular way we wanted our molecular constructs to be able to form hydrogen bonds with the β-chain of a starting or growing β-sheet; at the same time they should be incapable of accepting a subsequent β-chain for example as a result of their inability to form hydrogen bonds either by the absence of a hydrogen bond donor/acceptor or by a turn-like structure preventing proper alignment.

The results presented here show that site-specific alkylation[12] is a more general and effective approach than merely N-methylation for disruption of β-sheet formation. In addition, so far, substitution of an amide functionality by an ester moiety has not been described as an approach for access to new β-sheet breaker peptide derivatives as inhibitors for amyloid fibrillogenesis.

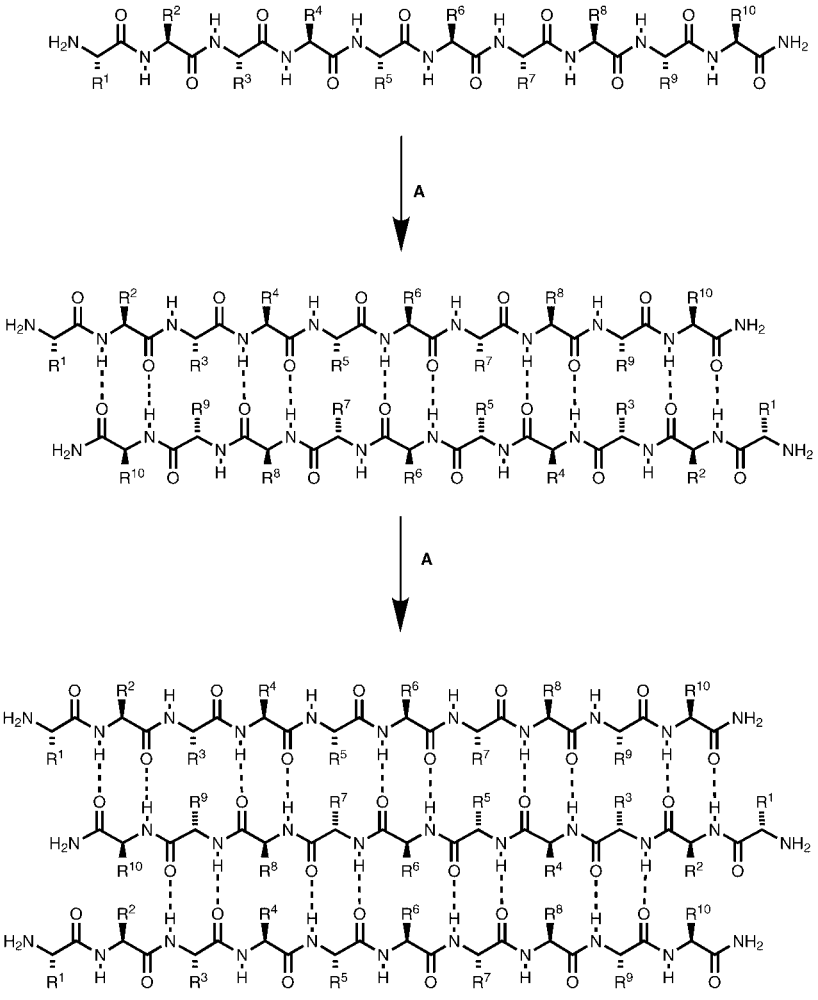
Results and Discussion

In this study, eight derivatives of human amylin(20–29) were designed and synthesized with a modification at position 28 (Ser) to study the effect on gelation that is amyloid fibrillogenesis due to the absence of hydrogen bond donor/acceptor or enhancement of the probability of β-turn formation (Figures 1 and 2).

A solution of native amylin(20–29) **1** gelled within 10 min. Fibrils were formed as was demonstrated by electron microscopy (EM) and Fourier transform infrared spectroscopy (FTIR) which is in agreement with the literature[8, 10, 13] (Table 1). It was found that the size of the side chain of amino acid 28 (Ser) did not affect gel and fibril formation since peptides **2** (28: Gly) and **3** (28: Nle = norleucine), with no side chain and a larger side chain, respectively, led to equal fast gelation as **1** (28: Ser) (Table 1). The fibrils of these peptides were 6–10 nm width, rigid and unbranched as was observed by EM (Figure 3). Moreover, FTIR spectra showed (Table 1) the typical absorbance of β-sheets at 1625 and 1633 cm^{-1}.[14]

However, when L-serine 28 was changed to D-serine, leading to peptide **4**, gelation was delayed for at least 2 h. Apparently, the three-dimensional orientation of the side chain (D-versus L-configuration) is of greater importance than its bulkiness and capable of delaying gel formation. Although D-amino acids are known to induce type II (II′) β-turns, the presence of the NH hydrogen bond donor of this amino acid still was sufficient to result in gel formation including fibril formation after aging of the solution as was evidenced by EM and FTIR (Table 1).

Substitution of the backbone NH at position 28 by an oxygen atom was achieved by the preparation of depsipeptide **5** (Scheme 1), of which gelation was even further delayed and did not take place within 24 h. This remarkable postponement of gel formation (144 times slower than **2**) points at the importance for gelation of the N-H hydrogen bond donor at this position. More importantly, FTIR clearly showed the reduced tendency of fibril formation, since the typical β-sheet band at 1625 cm^{-1} is almost absent. Furthermore, a strong absorbance at 1645 cm^{-1} was observed, indicating a random conformation. Electron microscopy, however, revealed the presence of only few scattered fibrillar structures. The strong FTIR absorbance is probably mainly the result of hydrophobic interactions since the FTIR absorbance at 1625–1633 cm^{-1} of the *inter*molecular hydrogen bonds in antiparallel β-sheets and/or fibrils is absent. Aging of the samples (three months) did not result in different FTIR spectra. Gel formation in this case is not a result of fibril formation but probably due to aspecific hydrophobic interactions.



0947-6539/02/0818-4286 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 18

Table 1. Gelation and fibril formation of the peptides.

Peptide	Mass $[M+H]^+$	Gelation[a]	Fibrils[b]	Morphology[b]	FTIR[c] [cm^{-1}]	Inhibition of gelation **1**:peptide 1:1 *w/w*
1	1008.51[d] 1008.50[e]	< 10 min	yes	infinite length width: 10 nm	1629(s); 1639(w); 1671(w)	no
		6 h[f]	yes	infinite length width: 10 nm	1629(s); 1639(w); 1671(w)	no
2	978.63[d] 978.50[e]	< 10 min	yes	infinite length width: 10 nm	1628(s); 1647(w); 1670(w)	no
3	1034.30[d] 1034.56[e]	< 10 min	yes	length: > 100 nm width: 6 nm	1631(s); 1644(w); 1669(w)	no
4	1008.38[d] 1008.50[e]	2 h	yes	infinite length width: 10 nm	1632(s); 1647(w); 1667(w)	not determined
5	979.45[d] 979.48[e]	24 h	suppressed	infinite length width: 10 nm	1629(w); 1645(s); 1672(s) 1747(s)	not determined
6	1064.64[d] 1064.57[e]	no	no	no	1626(w); 1642(s); 1674(s)	**1**+**6** (10 mg mL^{-1}): 2 h
7	1034.45[d] 1034.45[e]	no	no	small aggregates	1628(w); 1644(s); 1672(s)	**1**+**7** (10 mg mL^{-1}): 2 d **1**+**7** (5 mg mL^{-1}): 7 d
8	1018.53[d] 1018.78[e]	no	no	no	1626(w); 1642(s); 1674(s)	not determined

[a] 10 mg mL^{-1} in 0.1% TFA/H_2O at 20 °C; [b] viewed under EM at 10 mg mL^{-1} in 0.1% TFA/H_2O at 20 °C; [c] intensity of the IR signals, s: strong, m: moderate, w: weak; [d] found mass value; [e] calculated mass value; [f] 5 mg mL^{-1} in 0.1% TFA/H_2O at 20 °C.

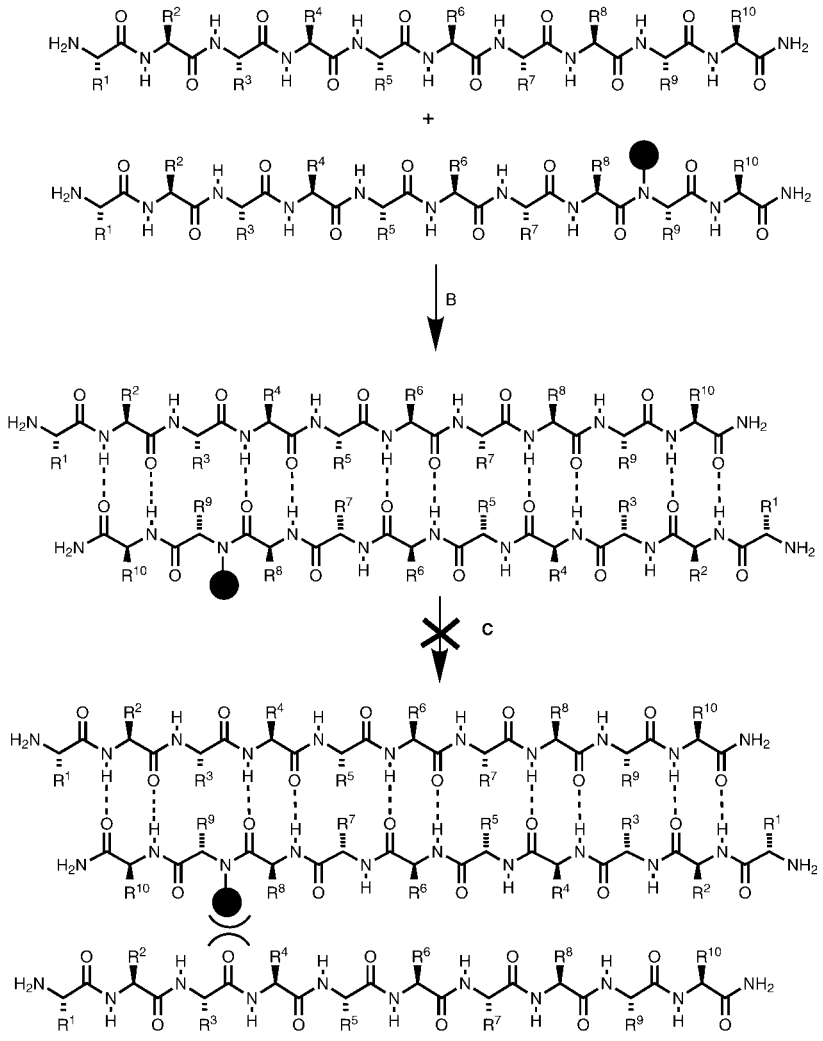


Figure 1. Rationale for the design of β-sheet breaker peptides based on human Amylin(20–29). For R^1–R^{10} see Figure 2. **A**: formation and further growth of an antiparallel β-sheet as occurs with unmodified amylin(20–29); **B**: a backbone-modified amylin(20–29) derivative is added that ultimately alters the hydrogen bond pattern as a result of either sterical hindrance or absence of an essential hydrogen bond as is shown in **C**: further growth of the antiparallel β-sheet is then prevented due to an improper hydrogen bonding network by the β-sheet breaker peptide.

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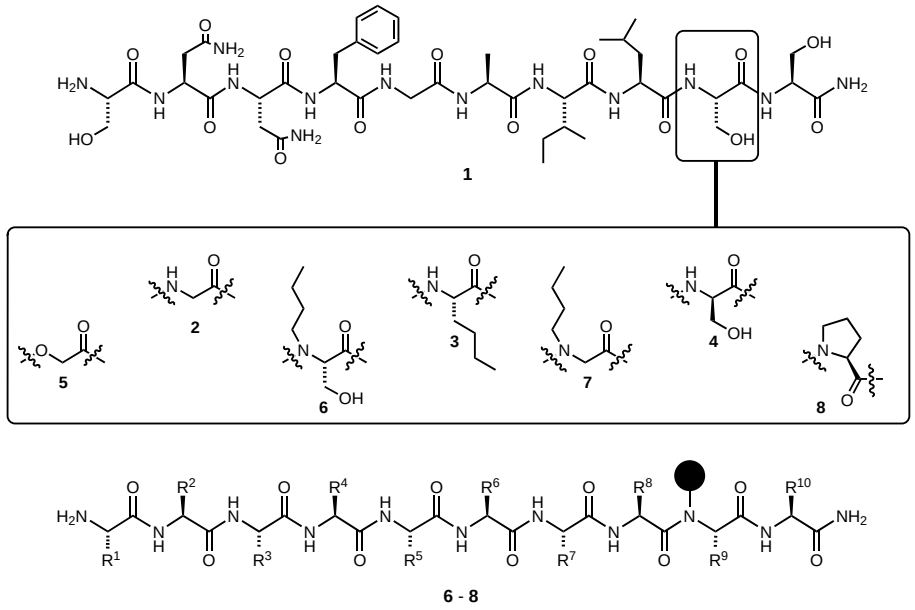

Figure 2. The designed amylin(20–29) derivatives as novel β-sheet breaker peptides.

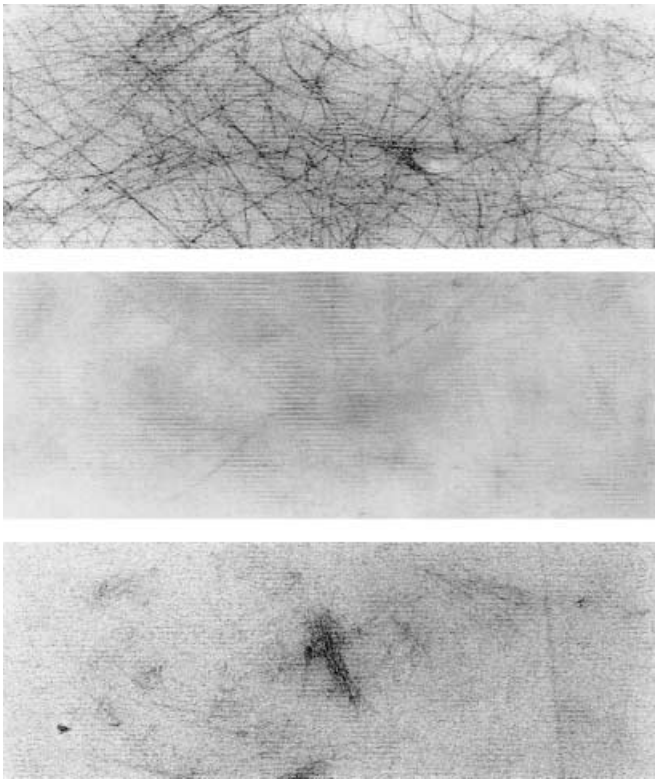

Figure 3. EM pictures of amylin peptides, A: **1;** B: **5** and C:**7**. Magnification: 20000 $\times$.

An alternative, albeit important approach to remove the N-H hydrogen bond donor, is alkylation of the amide nitrogen. Several studies from the literature exist in which N-methylated amino acids have been used to disrupt β-sheet formation. Site-specific N-alkylation and peptoid derivatives, however, allow us to introduce an alkyl chain of choice at any position in the peptide sequence, that is tuning steric bulk that is unfavorable for β-sheet packing. Therefore compounds **6** and **7** were prepared (Schemes 2 and 3)with an N-alkylated serine and an N-alkylated glycine (peptoid) residue, respectively.

Furthermore, serine 28 was replaced by a proline residue leading to **8**, which by virtue of the ring structure does not have an N-H available for hydrogen bonding. An additional feature of a proline moiety is the ability to promote a β-turn. Gel formation of these three amylin derivatives was not observed even after three months! Fibrils of peptides **6** and **8** could not be observed by EM. However, some largely unstructured aggregates, very different from the classical amyloid fibril morphology, were observed with **7** (Figure 3). FTIR was in agreement with the EM pictures since the strongest absorbance was at 1644 cm^{-1}; this indicates a random conformation (Table 1). This observation was in accordance with the literature data with respect to **8**.[8] Clearly, absence of gel formation was indicative for absence of fibril formation. It was shown that peptide–peptoid hybrid **7** was capable of inhibition of gel formation of native amylin(20–29), thereby acting as a β-sheet breaker peptide.[11]

In conclusion, we have shown that the presence of a single N-H hydrogen bond donor is crucial for gel formation and aggregation into fibrils of human amylin(20–29), which can be visualized by EM. At the basis of this fibril formation is the formation of β-sheets which can be observed by FTIR. However, merely replacement of the N-H by an oxygen (a hydrogen-bond acceptor) is not sufficient to suppress fibril formation completely, as was show by depsipeptide **5**.

Nevertheless, the absence of a single hydrogen bond donor rather than a putative induced β-turn is sufficient to retard amyloid fibrillogenesis. In order to obtain compounds which are capable of interfering with the formation of β-sheets the best approach seems to be site-specific alkylation of the amide nitrogen. This allows to probe which amide moiety of the amino acid sequence is the most sensitive to N-alkylation by introduction of steric bulk unfavorable for β-sheet packing.

Experimental Section

Instruments and methods: The peptides were synthesized on an Applied Biosystems 433A Peptide Synthesizer. Analytical and preparative HPLC runs were performed on a Gilson HPLC workstation. Electrospray ionization mass spectrometry was performed on a Micromass Platform II single quadrupole bench-top mass spectrometer operating in a positive ionization mode. Liquid chromatography electrospray ionization mass spectrometry was measured on a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode. Electron microscopy was performed on a Jeol 1200 EX transmission electron microscope. Fourier transform infrared spectra were measured on a BioRad FTS 6000 spectrophotometer. ^{1}H NMR spectra were recorded on

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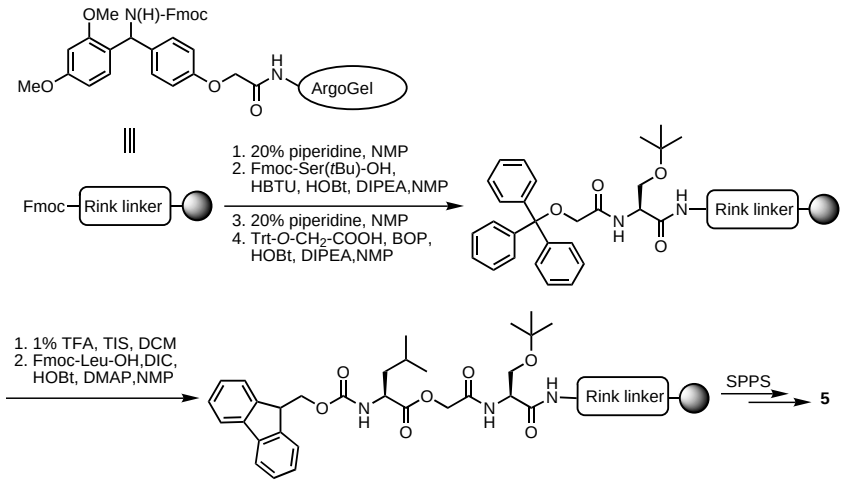
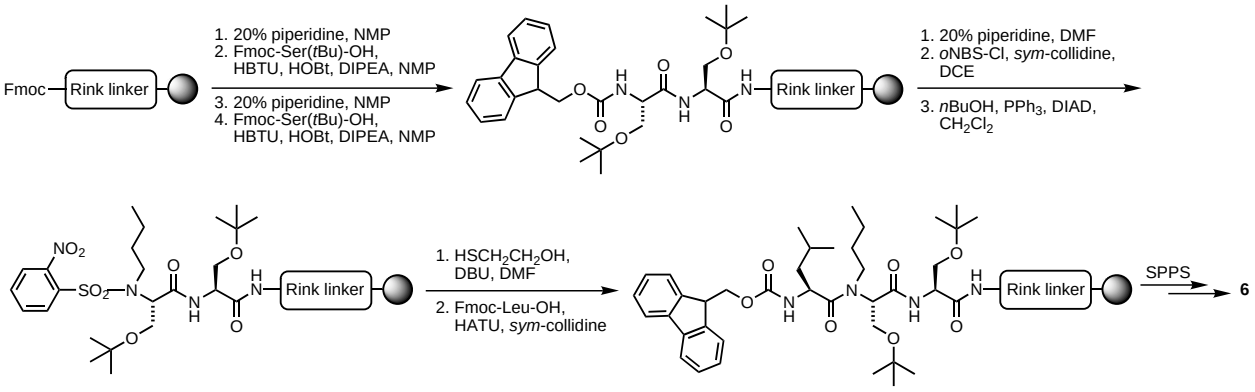


Scheme 1. Solid-phase synthesis of depsipeptide **5**.

a Varian G-300 (300.1 MHz) spectrometer and chemical shifts are given in ppm (δ) relative to TMS. ^{13}C NMR spectra were recorded on a Varian G-300 (75.5 MHz) spectrometer and chemical shifts are given in ppm relative to $CDCl_3$ (77.0 ppm). The ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence. R_f values were determined by thinlayer chromatography (TLC) on Merck pre-coated silicagel 60 F_{254} glass plates. Spots were visualized by UV quenching, ninhydrin or Cl_2/TDM.[15] Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat (according to Dr. Tottoli) and are uncorrected. Elemental analyses were done by Kolbe Mikroanalytisches Labor (Mühlheim an der Ruhr, Germany).

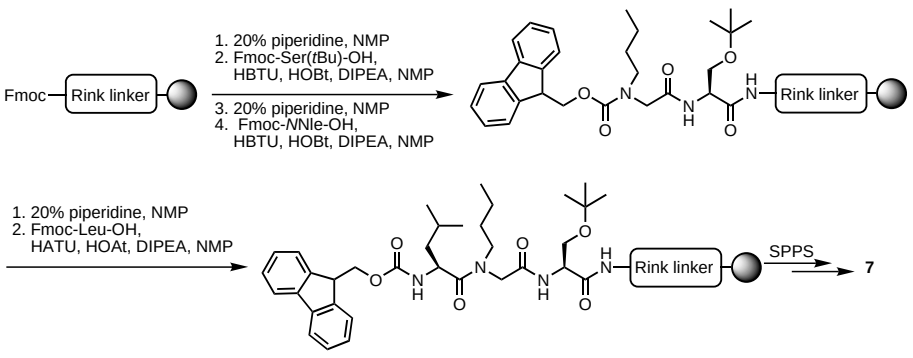
Chemicals and reagents: ArgoGel Rink-NH-Fmoc resin functionalized with a 4-((2′,4′-dimethoxyphenyl)aminomethyl)phenoxyacetamido moiety (Rink amide linker)[16] was used in all the syntheses. The coupling reagents tained from Advanced ChemTech. The side chain protecting groups were chosen as: *t*Bu: *tert*-butyl, for serine and Trt: trityl, for asparagine. Peptide grade dichloromethane (CH_2Cl_2), 1,2-dichloroethane (DCE), *tert*-butyl methyl ether (MTBE), *N*-methylpyrrolidone (NMP), trifluoroacetic acid (TFA) and HPLC grade acetronitrile were purchased from Biosolve. Piperidine, 4-(*N*,*N*-dimethylamino)pyridine (DMAP), *N*,*N*-diisopropylethylamine (DIPEA), *sym*-collidine, triethylamine (TEA) and triphenylphosphine were obtained from Acros Organics. Triisopropylsilane (TIS), 1,2-ethanedithiol (EDT) and HPLC grade TFA were obtained from Merck. Diisopropyl azodicarboxylate (DIAD), *N*,*N*′-diisopropylcarbodiimide (DIC) and 2-nitrobenzenesulfonyl chloride (*o*NBS-Cl) were purchased from Aldrich. Glycolic acid, β-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Fluka.

Peptide synthesis (general procedure): Peptides **1**–**4**, and **8** were synthesized using the FastMoc protocol on a 0.25 mmol scale[20] on Argogel Fmoc-Rink-amide resin to obtain C-terminally amidated peptides.[16] Each synthetic cycle consisted of N^{α}-Fmoc removal by a 10 min treatment with 20% piperidine in NMP, a 6 min NMP wash, a 45 min coupling step with 1.0 mmol of preactivated Fmoc amino acid in the presence of two equivalents DIPEA, and a 6 min NMP wash. N^{α}-Fmoc amino acids were activated in situ with 1.0 mmol HBTU/HOBt (0.36 M in NMP) in the presence of DIPEA (2.0 mmol). The peptides were detached from the resin and deprotected by treatment with TFA/H_2O/EDT)/TIS 85:8.5:4.5:2 *v/v/v/v* for 3 h. The peptides were precipitated with MTBE/



Scheme 2. Solid-phase synthesis of N-butylated Ser28 peptide **6**.

2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[17] and benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP)[18] were obtained from Richelieu Biotechnologies; *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU)[19] and 1-hydroxy-7-azabenzotriazole (HOAt)[19] were obtained from Applied Biosystems. *N*-Hydroxybenzotriazole (HOBt) and N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids were ob-



Scheme 3. Solid-phase synthesis of peptoid–peptide hydrid **7**.

hexane 1:1 *v/v* at −20 °C and finally lyophilized from *tert*-butyl alcohol/H_2O 1:1 *v/v*.

Depsipeptide 5: a) Preparation of *O*-tritylglycolic acid: Trt-Cl (5.79 g, 20.73 mmol) was mixed with $SOCl_2$ (1 mL, 13.7 mmol) in CH_2Cl_2 (100 mL) to convert any residual tritylalcohol into the corresponding chloride. After 1 h of stirring the reaction mixture was evaporated in vacuo and coevaporated with CH_2Cl_2 to remove any residual $SOCl_2$. Glycolic acid (1.67 g, 22 mmol) and TEA (11.12 mL, 80 mmol) were dissoved in CH_2Cl_2 (50 mL) and cooled on ice. Trt-Cl was dissolved in CH_2Cl_2 (50 mL) and added dropwise to the cooled solution. The reaction mixture was stirred for 1 h on ice followed by 16 h at room temperature. Subsequently, the reaction mixture was acidified with 1N $KHSO_4$ to a final pH of 3. The resulting organic layer was subsequently washed with brine (2 × 30 mL) dried with $MgSO_4$ and concentrated under reduced pressure. The resulting oil slowly crystallized. *O*-Tritylglycolic acid was purified by recrystallization from CH_2Cl_2/hexane (6.16 g, 88 %). M.p. 131–133 °C; $R_f = 0.15$ (CH_2Cl_2/MeOH 9:1 *v/v*), 0.54 ($CHCl_3$/MeOH/AcOH 95:20:3 *v/v/v*), 0.88 ($CHCl_3$/MeOH/25 % NH_4OH 60:45:20 *v/v/v*); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.46-7.44$ (m, 6H; arom Trt), 7.33–7.28 (m, 9H; arom Trt), 3.88 (s, 2H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.23$ (C=O), 142.87 (qC arom), 128.46 (CH arom), 128.07 (CH arom), 127.40 (CH arom), 87.73 (qC), 62.02 (CH_2); EI-MS (50 eV): *m/z* (%): 317.15 (100) $[M-H]^+$; elemental analysis calcd (%) for $C_{21}H_{18}O_3$ (318.13): C 79.22, H 5.70; found: C 78.98, H 5.80.

b) Incorporation into the peptide: *O*-Tritylglycolic acid (318 mg, 1 mmol) was coupled for 2 h to H-Ser(*t*Bu)-NH-Rink-amide resin (0.25 mmol) with BOP (442 mg, 1 mmol)/HOBt (153 mg, 1 mmol) and DIPEA (348 μL, 2 mmol) in NMP (10 mL). The trityl functionality was removed by treatment with TFA/TIS/CH_2Cl_2 1:5:94 *v/v/v* (6 × 1 min). Triisopropylsilane was added to scavenge the tritylcation. Fmoc-Leu-OH (353 mg, 1 mmol) was coupled to the primary hydroxyl group with DIC (313 μL, 2.0 mmol)/HOBt (612 mg, 4.0 mmol)/DMAP (122 mg, 1.0 mmol) in NMP (10 mL) for 16 h.[21] The coupling yield of Fmoc-Leu-OH as calculated from an Fmoc determination was 70 %.[22] The synthesis of depsipeptide **5** was completed as is described in the general procedure.

N-Butylated Ser28 peptide 6: N-Butylation of the amino functionality of Ser28 was carried out by using our site specific alkylation method.[23] Fmoc-Ser(*t*Bu)-Ser(*t*Bu)-NH-Rink-amide resin (0.25 mmol) was treated with 20 % piperidine in DMF (3 × 8 min, 10 mL) and subsequently washed with DMF (5 × 10 mL) and CH_2Cl_2 (3 × 10 mL). The resulting amino functionality was reacted for 2 h with *o*NBS-Cl (337 mg, 1.25 mmol) in DCE (10 mL) with *sym*-collidine (400 μL, 2.50 mmol) as the base. After washing with CH_2Cl_2 (6 × 10 mL) the resulting sulfonamide was treated with triphenylphosphine (399 mg, 1.25 mmol), 1-butyl alcohol (278 μL, 2.50 mmol) and DIAD (299 μL, 1.25 mmol) in DCE (10 mL) during 90 min. Subsequently, the resin was extensively washed with CH_2Cl_2 (3 × 10 mL) and DMF (6 × 10 mL). The *o*NBS-functionality was removed by treatment with 0.5 M β-mercaptoethanol in DMF (5.0 mL, 2.50 mmol) in the presence of DBU (227 μL, 1.25 mmol) during 30 min. After washing with DMF (6 × 10 mL) and CH_2Cl_2 (3 × 10 mL), the secondary amine was coupled to Fmoc-Leu-OH (537 mg, 1.25 mmol) using HATU (578 mg, 1.25 mmol) as a coupling reagent in neat *sym*-collidine (10 mL) for 16 h.[24] After washing the resin with DMF (5 × 10 mL), CH_2Cl_2 (5 × 10 mL), 2-propanol (3 × 10 mL) and diethyl ether (3 × 10 mL), the coupling yield was determined by an Fmoc determination and was found to be 82 %.[22] The synthesis of the peptide was completed as described in the general procedure mentioned above.

Peptoid–peptide hybrid 7: The synthesis of the peptoid monomer Fmoc-norleucine (Fmoc-*N*Nle-OH) was carried following the procedure of Kruijtzer et al.[25] Fmoc-*N*Nle-OH: m.p. 113–115 °C; $R_f = 0.36$ (CH_2Cl_2/MeOH 9:1 *v/v*), 0.69 ($CHCl_3$/MeOH/AcOH 95:20:3 *v/v/v*); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.77-7.24$ (br m, 8H; arom Fmoc), 4.57–4.43 (m, 2H; CH_2 Fmoc), 4.26–4.18 (m, 1H; CH Fmoc), 4.00/3.86 (ds, 2H; α-CH_2), 3.30–3.15 (dt, 2H; β-CH_2), 1.50–1.10 (br m, 2 × 2H; γ-CH_2/δ-CH_2), 0.87 (dt, 3H; ε-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.19$ (CO-OH), 156.68 (O-CO-NH), 143.81 (qC arom), 141.23 (qC arom), 127.61 (CH arom), 126.99 (CH arom), 124.76 (CH arom), 119.88 (CH arom), 67.42 (CH_2 Fmoc), 48.87 (α-CH_2), 47.19 (CH Fmoc), 30.20 (β-CH_2), 25.37 (CH_2), 19.82 (CH_2), 13.75 (ε-CH_3); EI-MS (50 eV): *m/z* (%): 376.05 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{21}H_{23}NO_4$ (353.17): C 71.37, H 6.56, N 3.96; found: C 71.40, H 6.63, N 3.93.

Fmoc-*N*Nle-OH was coupled to H-Ser(*t*Bu)-NH-Rink-amide resin (0.25 mmol)in the presence of HBTU (378 mg, 1.0 mmol)/HOBt (158 mg, 1.0 mmol) and DIPEA (354 μL, 2 mmol) in NMP (10 mL) for 45 min. After removal of the Fmoc group, Fmoc-Leu-OH (353 mg, 1.0 mmol) was coupled to the resulting secondary amine with HATU (380 mg, 1.0 mmol)/HOAt (136 mg, 1.0 mmol) and DIPEA (354 μL, 2 mmol) in NMP (10 mL) for 90 min. After this coupling step the synthesis was completed as described in the general procedure.

Peptide purification:[26] The crude lyophilized peptides (30–60 mg) were dissolved in a minimum amount of 0.1 % TFA in CH_3CN/H_2O 8:2 *v/v* and loaded onto an Adsorbosphere XL C8 HPLC column (90 Å pore size, 10 μm particle size, 2.2 × 25 cm). The peptides were eluted with a flow rate of 10.0 mL min^{-1} using a linear gradient of buffer B (100 % in 60 min) from 100 % buffer A (buffer A: 0.1 % TFA in H_2O, buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 *v/v*). The purities were evaluated by analytical HPLC on an Adsorbosphere XL C8 column (90 Å pore size, 5 μm particle size, 0.46 × 25 cm) at a flow rate of 1 mL min^{-1} using a linear gradient of buffer B (100 % in 30 min) from 100 % buffer A (buffer A: 0.1 % TFA in H_2O; buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 *v/v*).

Peptide characterization: The peptides were characterized by mass spectrometry. The mass of each analogue was measured and the observed monoisotopic $[M+H]^+$ values were correlated with the calculated $[M+H]^+$ values using MacBioSpec (Perkin Elmer Sciex Instruments, Thornhill, Ontario, Canada).

Gelation experiments: Each peptide (10 mg) was dissolved in 0.1 % TFA/H_2O (1 mL) at 25 °C. The aggregation state was determined by eye at regular time intervals by tilting the test tube and check if the solution still flowed. If no flow was observed, gelation was said to have taken place.

Transmission electron microscopy: A peptide gel/solution aged for one week (10 μL) was placed on a carbon coated copper grid. After 2 min, any excess was removed by blotting the copper grid on a drop of demi-water. Finally, the samples were stained with methylcellulose/uranyl acetate and dried in air. The samples were visualized under a Jeol 1200 EX transmission electron microscope operating at 60 kV. The magnification ranged from 20000 to 100000 ×.

Fourier transform infrared spectroscopy: A peptide gel/solution aged for one week (250 μL) was lyophilized and subsequently resuspended in D_2O (100 μL) and lyophilized. Lyophilization from D_2O was repeated twice. The lyophilized peptide were dried over P_2O_5 in high vacuum for 24 h. A peptide sample was mixed with KBr and pressed into a pellet. IR spectra were recorded on a BioRad FTS6000. The optical chamber was flushed with dry nitrogen for 5 min before data collection started. The interferograms from 1000 scans with a resolution of 2 cm^{-1} were averaged and corrected for H_2O and KBr.

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Received: May 13, 2002 [F4086]