CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200501374

Self-Assembly of Amylin(20–29) Amide-Bond Derivatives into Helical Ribbons and Peptide Nanotubes rather than Fibrils

Ronald C. Elgersma,[a] Tania Meijneke,[a] George Posthuma,[b] Dirk T. S. Rijkers,[a] and Rob M. J. Liskamp*[a]

Abstract: Uncontrolled aggregation of proteins or polypeptides can be detrimental for normal cellular processes in healthy organisms. Proteins or polypeptides that form these amyloid deposits differ in their primary sequence but share a common structural motif: the (anti)parallel β sheet. A well-accepted approach for interfering with β-sheet formation is the design of soluble β-sheet peptides to disrupt the hydrogen-bonding network; this ultimately leads to the disassembly of the aggregates or fibrils. Here, we describe the synthesis, spectroscopic analysis, and aggregation behavior, imaged by electron microscopy, of several backbone-modified amylin(20–29) derivatives. It was found that these amylin derivatives were not able to form fibrils and to some extent were able to inhibit fibril growth of native amylin(20–29). However, two of the amylin peptides were able to form large supramolecular assemblies, like helical ribbons and peptide nanotubes, in which β-sheet formation was clearly absent. This was quite unexpected since these peptides have been designed as soluble β-sheet breakers for disrupting the characteristic hydrogen-bonding network of (anti)parallel β sheets. The increased hydrophobicity and the presence of essential amino acid side chains in the newly designed amylin(20–29) derivatives were found to be the driving force for self-assembly into helical ribbons and peptide nanotubes. This example of controlled and desired peptide aggregation may be a strong impetus for research on bionanomaterials in which special shapes and assemblies are the focus of interest.

Keywords: amyloids · helical structures · peptides · protein modifications · self-assembly

Introduction

Uncontrolled peptide/protein aggregation leading to precipitation of proteins is a major cause of a number of diseases[1] for which there is no therapy available as yet. The most well-known diseases of these diseases are Alzheimer's disease,[2] Parkinson's disease,[3] transmissible spongiform encephalopathies (scrapie, bovine spongiform encephalopathy (BSE), and Creutzfeldt–Jakob disease),[4] and diabetes type II.[5] The latter is characterized by deposits of islet amyloid polypeptide (IAPP) in the form of amylin fibrils which are present in the pancreatic islets. Since peptide–peptide, peptide–protein, and protein–protein interactions are ubiquitous, it is fair to expect that, in the future, diseases will be uncovered where protein aggregation is a (co)causitive factor, especially when locally high peptide/protein concentrations favor possible intermolecular aggregation, as is the case for the example with insulin.[6] Insights into the mechanism of aggregation by structure–aggregation–activity studies, might not only shed more light on the structural parameters, which play key roles in these processes, but also lead to compounds capable of interfering with aggregation.[7–9]

For interference with amyloid formation by inhibition of β sheets, small molecules including small peptides and peptidomimetics are naturally preferred as possible future drugs for inhibition of fibril growth or resolubilization of fibrils in aggregation diseases and considerable activity is currently taken place in this area of research.[7–9] Although we have shown that mutation of a single amide bond, into the corresponding ester, peptoid, or *N*-butylated amino acid residue, at position 28 of human IAPP(20–29)[10] was able to inhibit amyloid formation,[10a] these modified human IAPP(20–29)

[a] R. C. Elgersma, T. Meijneke, Dr. Ir. D. T. S. Rijkers, Prof. Dr. R. M. J. Liskamp
Department of Medicinal Chemistry
Utrecht Institute for 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. G. Posthuma
Department of Cell Biology
Center for Electron Microscopy
University Medical Center, 3508 GA Utrecht (The Netherlands)

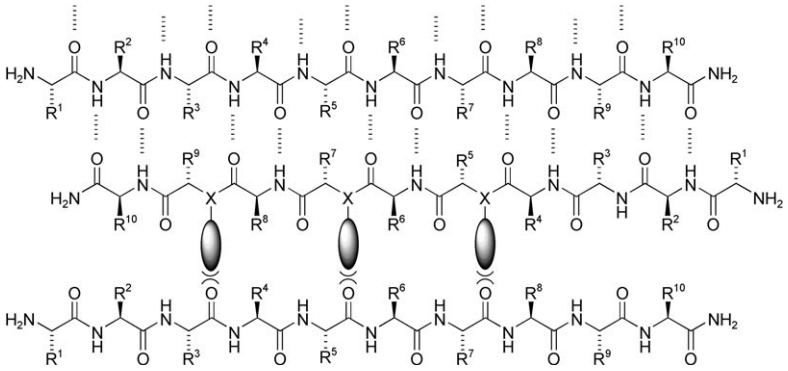
derivatives were not, however, able to inhibit/retard fibril formation of *full-length* amylin (37 residues)[11,12] or to resolubilize preformed amylin fibrils.[10b] However, amide bond modification at *alternate* positions has been used successfully by Meredith and co-workers with (Aβ(16–22): Ac-Lys-Leu-Val-Phe-Phe-Ala-Glu-NH_2) peptides[13] in order to obtain aggregation inhibitors. Therefore, we have designed amylin-(20–29) derivatives in which the amide bonds at positions 24, 26, and 28 have been modified by *N*-butylation or by incorporation of peptoid- or ester-bond moieties. Thus, at crucial positions the N*H* hydrogen-bond donors of the amide bond are no longer present and bulky substituents have been introduced on to the nitrogen atom. Alternatively, N*H* hydrogen-bond donors were replaced by oxygen atoms through the preparation of suitable depsipeptides. These newly designed amylin derivatives did not form amyloid fibrils but surprisingly gave rise to the formation of helical ribbons and peptide nanotubes by self-assembly.

In general, these and other backbone modifications assume a central position in our research program.[14] Backbone modifications are both very important and highly interesting because the peptide side chains are maintained for molecular recognition, while changes in the geometrical constraints (φ, ψ, and ω dihedral angles, absence or presence of hydrogen-bond donors/acceptors) are crucial for the resulting supramolecular assembly. It will be the balance between these two structural moieties—side chains versus backbone—in peptides and peptidomimetics that will determine the outcome with respect to the biological activity and/or material properties.

There are many examples of controlled and desired protein aggregation mechanisms in living organisms. If these protein aggregation mechanisms, especially those involved in certain diseases, lead to typical morphological changes, they may have a significant impact on research into bionanomaterials,[15] in which special shapes and assemblies are within the focus of interest. The described amylin derivatives open up promising possibilities for the further study of the process of aggregation as well as for carrying out structure–activity-relationship studies in order to design bionanomaterials based on self-assembly of amyloid-derived peptides.

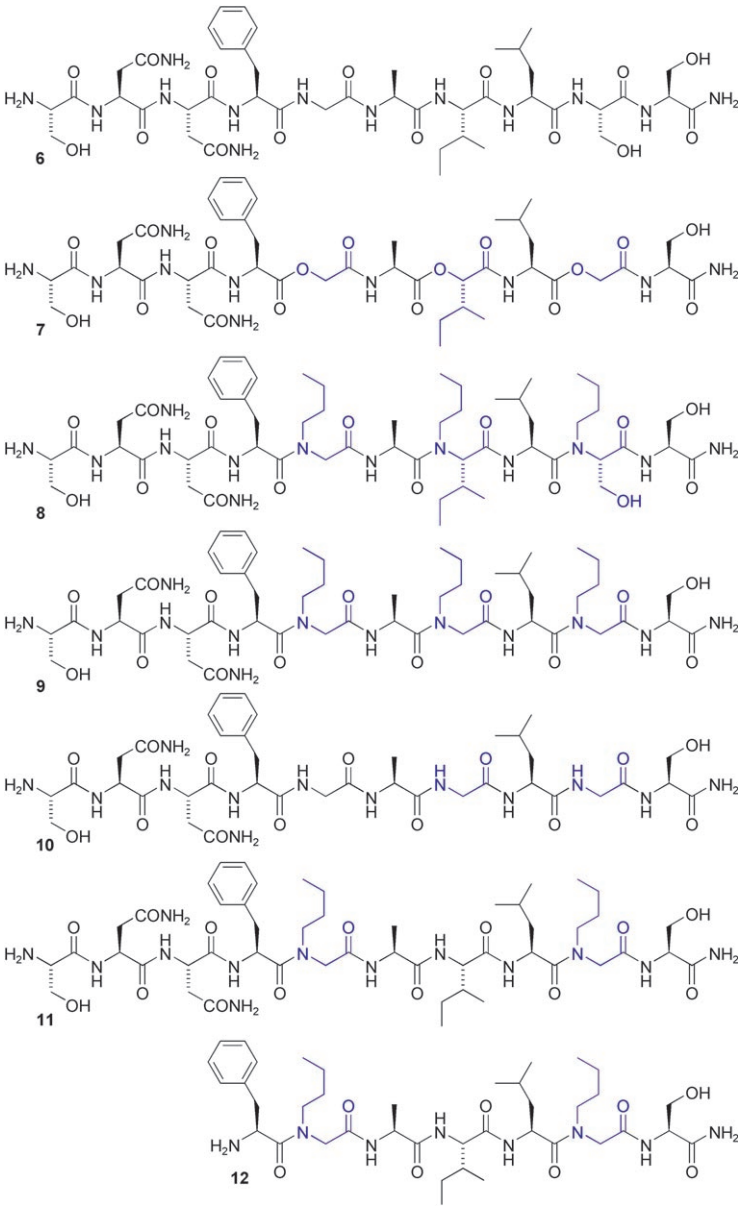
Results and Discussion

Rationale for design: Amyloid fibrils ultimately result from the assembly of antiparallel oriented peptides, in which the amide bonds of the peptide backbone can form an ideal hydrogen-bonding network (Scheme 1, top).[16] In general, the formation of (anti)parallel β-pleated sheets is responsible for a decreased solubility of many proteins. However, by using a simple model as a basis for preventing or at least interfering with this hydrogen-bond formation (Scheme 1, bottom), we, and others, have shown that specifically designed peptides are capable of delaying and/or inhibiting β-sheet formation and thus fibril formation.[9,10a] In principle, such peptides, denoted as β-sheet-breaker peptides, could be used as a new approach for therapeutic intervention in amyloid formation. Along these lines, we found that a single amino acid substitution in the amylin(20–29) sequence, namely, Ser28→*N*Nle (*N*Nle = *N*-butylglycine), was responsible for complete inhibition of fibril formation and also delayed fibril formation of native amylin(20–29).[10a] However, preformed fibrils of native amylin(20–29) or full-length amylin did not redissolve in the presence of amylin(20–29)Ser28→*N*Nle. This contrasted with results described in the literature with *N*-methylated Aβ(16–22) and Aβ(1–40).[13] Therefore, the successful approach of Meredith and co-workers, in which a peptide backbone was modified at three alternate amide bonds to obtain a potent antiamyloidogenic peptide that could redissolve preformed Aβ(1–40) fibrils,[13] was applied to our amyloid peptide model, amylin-(20–29). At three alternate positions[17] (Scheme 1) the amide bonds of the native peptide **6** were replaced by ester moieties (depsipeptides), *N*-butylated amino acid residues, *N*-butylated glycine residues (peptoids) by preparation of human IAPP(20–29) derivatives **7**–**12** (Scheme 2). In the cases of the peptide–peptoid hybrids **9**, **11**, and **12**, the influence of the α-amino acid side chains on the amyloidogenic character of the peptide was also investigated.



Scheme 1. Rationale for the design of β-sheet-breaker peptides based on the amylin(20–29) sequence.

Synthesis of the peptides: Unfortunately, direct solid-phase synthesis of the depsipeptide **7** and the *N*-butylated peptide **8** gave unsatisfactory results.[10b] It was thought that introduction of the complete building blocks comprising the depsipeptide moiety and the peptoid moiety, that is, Fmoc-Ala-Ilec-OH (**2**; Fmoc = 9*H*-fluoren-9-ylmethoxycarbonyl, Ilec = 2*S*-hydroxy-3*S*-methylpentanoic acid) and Fmoc-Ala-



Scheme 2. Structures of the amylin(20–29) derivatives synthesized in this study.

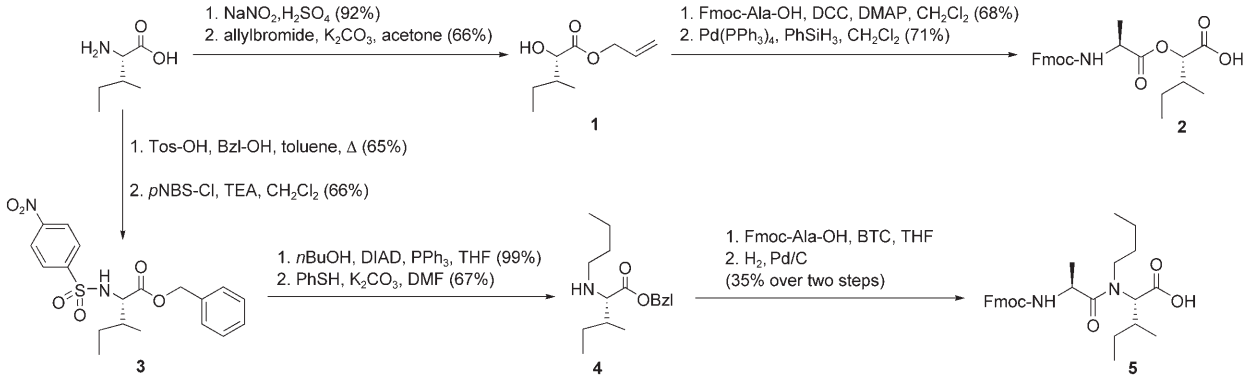
(*N*Bu)Ile-OH (**5**), respectively, would be more successful (Scheme 3).

For the synthesis of Fmoc-Ala-Ilec-OH (**2**), the L-α-hydroxy acid equivalent of L-isoleucine was first synthesized by diazotization of the amino acid according to the method of Shin et al.[18a] followed by esterification with allylbromide; α-hydroxy acid allyl ester **1** was obtained in 61% overall yield. Fmoc-Ala-OH was coupled to this with DCC/DMAP in 68% yield[18c] and the allyl ester was removed with Pd-$(Ph_3P)_4$ in the presence of phenylsilane as a scavenger[19] to give the Fmoc-protected dipeptide acid **2** in 71% yield.

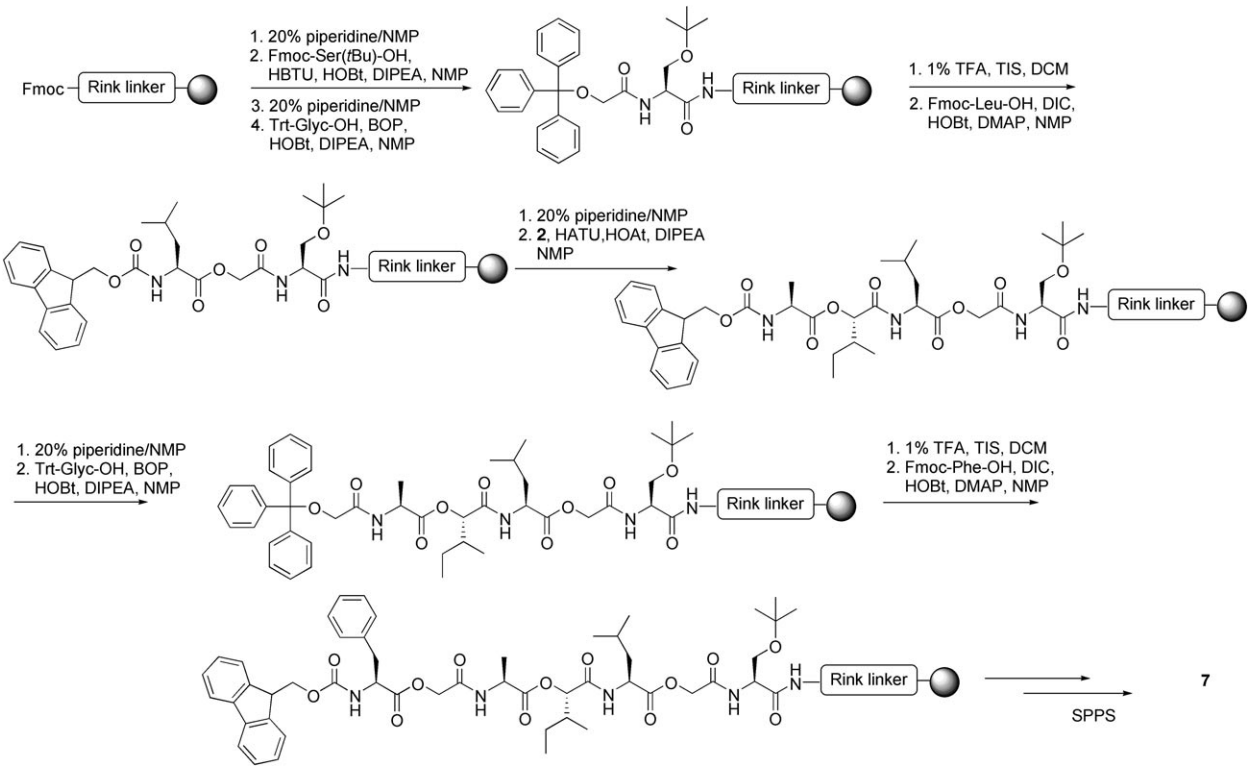
For *N*-butylation of isoleucine, the corresponding benzyl ester (65% yield) was synthesized and this was followed by introduction of the sulfonamide moiety with *p*-nitrobenzenesulfonyl chloride in the presence of TEA as base to give **3** in 66% yield. After a Mitsunobu reaction[20] with 1-butanol/PPh_3/DIAD in THF, with a nearly quantitative yield,[21] and removal of the *p*-nitrobenzenesulfonyl moiety by thiophenolate, the secondary amine **4** was obtained in 67% yield. Coupling of Fmoc-Ala-OH to H-*N*(Bu)Ile-OBzl was very difficult due to severe steric hindrance. Coupling reagents 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDCI)/7-aza-1-hydroxy-1*H*-benzotriazole (HOAt),[22] *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU)/HOAt/TEA,[23] and neat *sym*-collidine/HATU[24] did not give the desired dipeptide. However, the use of triphosgene, as described by Falb et al.,[25] via the in situ acid chloride gave—after hydrogenolysis—the required Fmoc-Ala-(*N*Bu)Ile-OH (**5**) building block in 35% (over two steps).

Peptides **6** and **10** were synthesized automatically by using Fmoc/*t*Bu solid-phase peptide-synthesis protocols.[26] Peptide–peptoid hybrids **9**, **11**, and **12** were assembled on the solid phase as described by Kruijtzer et al.[27]

The synthesis of depsipeptide **7** is depicted in Scheme 4. After treatment of Fmoc-Ser(*t*Bu)-NH-Rink amide ArgoGel with piperidine to remove the Fmoc group, *O*-tritylglycolic acid was coupled with BOP/HOBt/DIPEA in NMP. This coupling was complete after 2 h according to the Kaiser test[28] and the trityl group was then removed by diluted



Scheme 3. Synthesis of depsidipeptide Fmoc-Ala-Ilec-OH (**2**) and *N*-butylated dipeptide Fmoc-Ala-*N*(Bu)Ile-OH (**5**). DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-(*N*,*N*-dimethylamino)pyridine, Tos = toluene-4-sulfonyl, Bzl = benzyl, *p*NBS-Cl = 4-nitrobenzenesulfonyl chloride, TEA = triethylamine, DIAD = diisopropyl azodicarboxylate, THF = tetrahydrofuran, DMF = *N*,*N*-dimethylformamide, BTC = bis(trichloromethyl)carbonate = triphosgene.



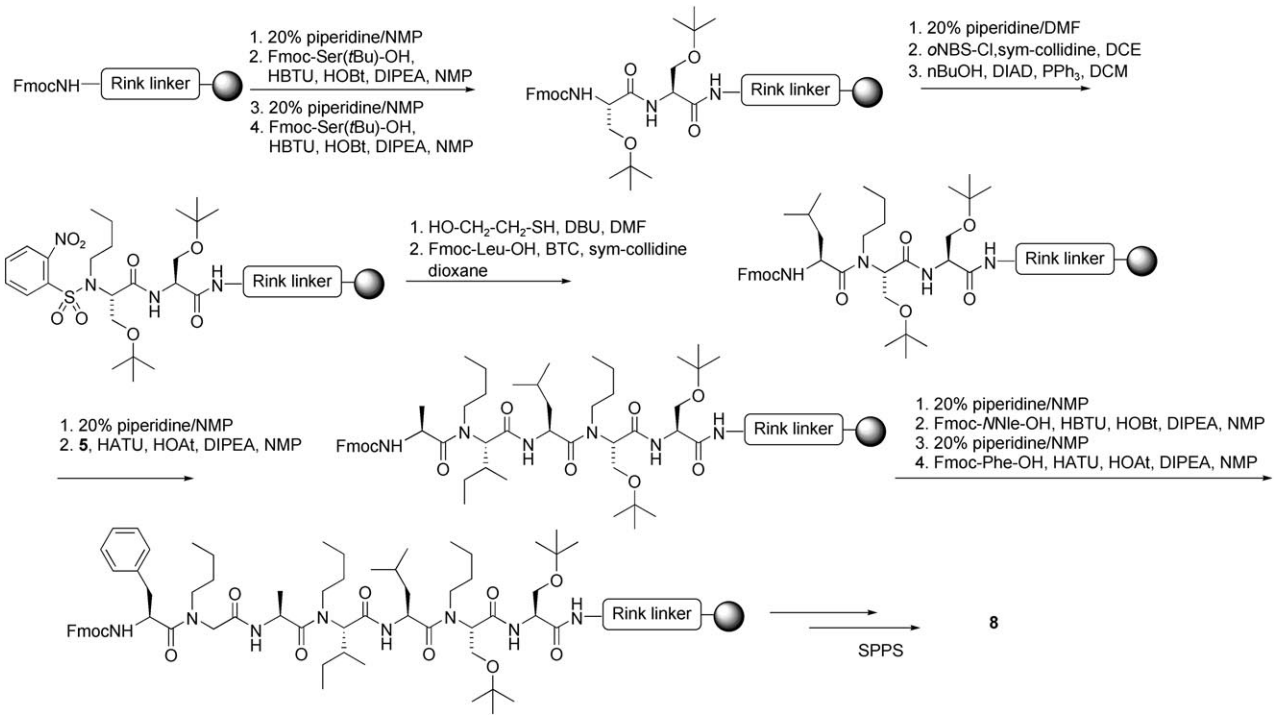
Scheme 4. Solid-phase synthesis of depsipeptide **7**. Rink = 4-[(2′,4′-dimethoxyphenyl)aminomethyl]phenoxy, NMP = *N*-methylpyrrolidone, HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt = *N*-hydroxybenzotriazole, DIPEA = *N*,*N*-diisopropylethylamine, Trt = trityl = triphenylmethyl, Glyc = glycolic acid, BOP = benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate, TFA = trifluoroacetic acid, TIS = triisopropylsilane, DCM = dichloromethane, DIC = *N*,*N*′-diisopropylcarbodiimide, SPPS = solid-phase peptide synthesis.

TFA. The resulting free hydroxy group was coupled to Fmoc-Leu-OH with DIC/HOBt/DMAP in NMP[29] and the coupling yield (76 %) was determined by an Fmoc determination.[30] Next, Fmoc-group removal was effected with piperidine and dipeptide building block **2** was introduced with HATU/HOAt/DIPEA.[23] After deprotection of the resulting product, *O*-trityl glycolic acid was coupled. Finally, removal of the trityl group was followed by attachment of Fmoc-Phe-OH through a carbodiimide-mediated coupling[18c] in 74 % yield. Three additional deprotection/coupling cycles completed the solid-phase synthesis and, after cleavage and simultaneous deprotection with TFA, depsipeptide **7** was obtained in an overall yield of 33 %.

Synthesis of the *N*-butylated-Gly24, -Ile26, and -Ser28 peptide **8** began with the preparation of Fmoc-Ser(*t*Bu)-Ser(*t*Bu)-NH-Rink amide ArgoGel resin (Scheme 5). After switching of the Fmoc group for an *o*NBS group, the resulting sulfonamide *N*H group could be subjected to a Mitsunobu reaction in a site-specific *N*-alkylation method.[31] This reaction was monitored by the bromophenol blue (BPB) test.[32] Then, the *o*NBS group was removed by treatment with β-mercaptoethanol in the presence of DBU as base. The resulting secondary amine was treated with Fmoc-Leu-OH/BTC in *sym*-collidine/dioxane as described by Falb et al.,[25] with modifications described by Jung and co-workers.[33] The coupling efficiency (70 %) was calculated from an Fmoc determination.[30] After Fmoc removal, dipeptide **5** was coupled, with HATU/HOAt as the coupling reagents in the presence of DIPEA as base in NMP. Five additional deprotection/coupling cycles completed the solid-phase synthesis and, after cleavage and simultaneous deprotection, peptide–peptoid hybrid **8** was obtained in an overall yield of 31 %.

Amyloid fibril formation: Native amylin(20–29) (**6**; 10 mg mL^{-1}) was dissolved in 0.1 % TFA/H_2O and this led to rapid gel formation. The presence of amyloid fibrils was verified by transmission electron microscopy (TEM; data not shown)[11,12] and β-sheet formation was confirmed by the amide I absorption at approximately 1630 cm^{-1} in the Fourier transform infrared (FTIR) spectrum (Table 1), which was in agreement with literature data and our earlier experiments.[10a,13]

The influence of substitution of three alternate amide bonds by ester moieties on amyloid fibril formation was studied with depsipeptide **7**. A solution of this peptide also formed a gel, a result that was rather unexpected since we had previously found that a *single* amide-bond replacement (on position 28) significantly postponed gel formation as compared to that with **6**.[10a] However, typical amyloid fibrils were *not* observed by TEM, a result that was confirmed by FTIR spectroscopy since the absorption at 1625–1630 cm^{-1}, typical for β-sheets, was absent.[34] Instead of amyloid fibrils, large helical ribbons[35] and even tubelike supramolecular



Scheme 5. Solid-phase synthesis of *N*-butylated peptide **8**. *o*NBS-Cl = 2-nitrobenzenesulfonyl chloride, DCE = 1,2-dichloroethane, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Table 1. Physicochemical properties of the amylin derivatives.

Peptide	Gelation	Fibrils	Morphology	FTIR [cm^{-1}]
6	yes: < 10 min, turbid	yes	length: ∞; width: 10 nm	1631 (s), 1670 (m)
7	yes: < 10 min, turbid	no	helical tapes; length: 7.4 μm; width: 170 nm	1665 (s), 1642 (m), 1740 (w)
8	no: clear solution	no	helical tapes; length: 1.5 μm; width: 250 nm	1673 (s), 1637 (m)
9	no: clear solution	no	–	1669 (s)
10	no: clear solution	no	–	1664 (s)
11	no: clear solution	no	–	1677 (s), 1642 (m)
12	no: clear solution	no	–	1678 (s)
13	yes: < 10 min, turbid	yes	length: ∞; width: 10 nm	1629 (s)

structures[36] were observed (Figure 1). Although never described for nor studied in depsipeptides, this morphology of helical ribbons has been observed before,[35] as intermediate nanostructures in the self-assembly of amyloid fibrils[37] or as final-stage folding assemblies.[38]

As expected, peptide **8** with three *N*-butylated amino acid residues formed a clear solution in 0.1% TFA/H_2O and gel formation did not occur. The *N*-alkylated peptide **8** was designed as a water-soluble β-sheet mimic to disrupt any β-sheet formation, according to the model in Scheme 1. Fibril formation, as judged by TEM and FTIR spectroscopy, was completely absent. Surprisingly, despite the absence of any visible gel formation, helical ribbons, similar to those formed by depsipeptide **7**, were also observed by TEM in this case (Figure 2).

Similarily, peptide–peptoid hybrid **9** rapidly dissolved in 0.1% TFA/H_2O and, here too, gel formation was absent. In addition, no amyloid fibrils could be detected by either TEM or FTIR spectroscopy. In contrast to depsipeptide **7** and *N*-alkylated peptide hybrid **8**, however, this amylin derivative did *not* assemble into helical supramolecular structures. In order to obtain some insight into the molecular basis of this behavior, peptide **10** was prepared, in which Ile26 and Ser28 were replaced by a glycine residue. This derivative might shed some light on the role of the side chain and/or amide in the formation of supramolecular assemblies. It was found earlier that replacement of Ser28 by glycine, as in peptide **13** (Table 2), did not affect fibril formation.[10a] However, the additional replacement of Ile26 by glycine completely abrogated fibril formation. Apparently, side-chain-to-side-chain interactions also play an important role in the formation of amyloid fibrils (**6** versus **9** and **10**) or other self-assembled structures (**7** and **8**).[39]

Inhibition of aggregation behavior: The aggregation behavior of native amylin(20–29) (**6**) was monitored in the pres-

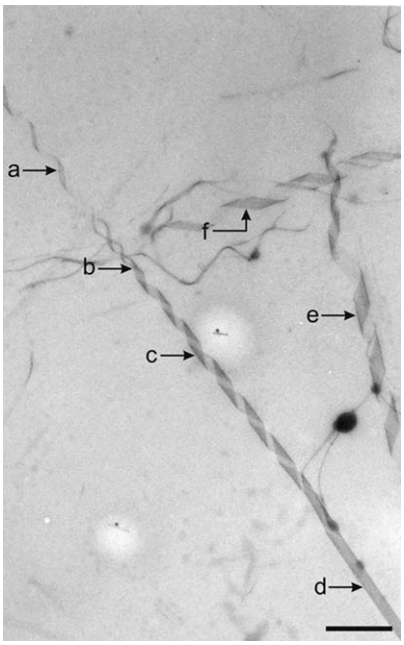


Figure 1. TEM image of amylin(20–29) derivative **7**. Scale bar: 1 μm. Arrow a: single strand; arrow b: two strands starting to intertwine; arrow c: helical ribbon; arrow d: (closed) peptide tube; arrows e and f: two helical tapes with pitches of 500 and 330 nm, respectively.

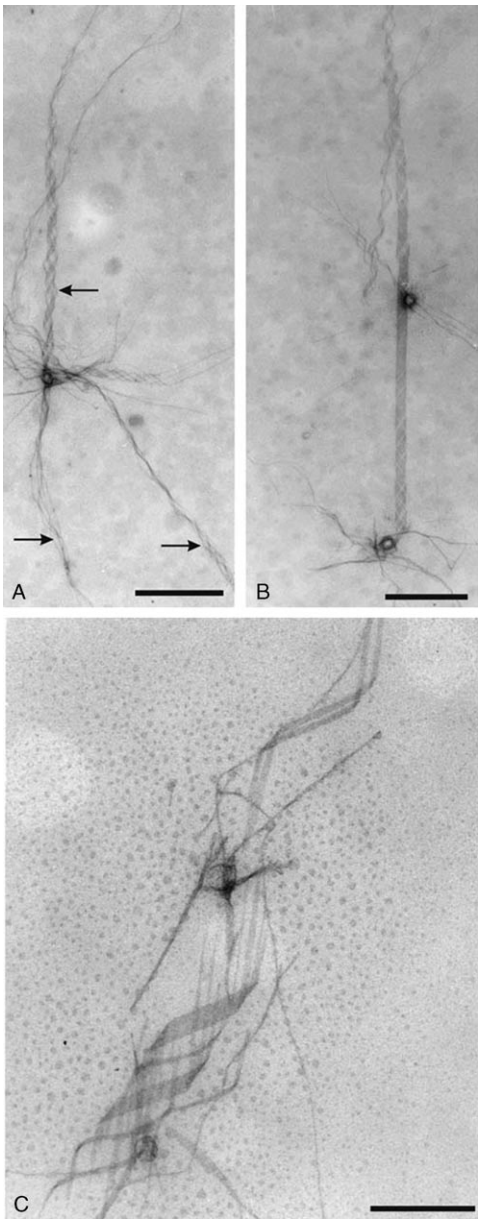


Figure 2. TEM images of amylin(20–29) derivative **8**. Scale bar: 2 μm (A and B), 0.5 μm (C). A) The arrows point at the filaments of which a ribbon consists; B) an almost closed peptide tube; C) enlargement of the joining of 5–7 filaments to form a peptide nanotube.

ence or absence of a potential inhibitory sequence. A stock solution of **6** was diluted in phosphate buffer at pH 7.4 and the increase of turbidity was monitored at 400 nm, as shown in Figure 3. Peptide **6** instantly increased the turbidity of the solution and, after 30 min, a plateau was reached. In the presence of an equal amount (w/w) of depsipeptide **7**, a small lag phase was observed and reaching the plateau was postponed to 60 min (Figure 3 A). This plateau was also approximately 20% lower than that observed in the absence of **7**. In case of the trialkylated peptide **8** with **6**, the lag phase was absent and the plateau was now reached after 45 min and was only slightly lower (10%) than the plateau reached by **6**. Peptides **7** and **8** did not increase the turbidity of the solution by themselves (data not shown), but they were not able to inhibit fibril formation of amylin(20–29) (see above). This may be the result of their tendency to form other supramolecular assemblies, that is, helical ribbons. As expected, the peptoid–peptide hybrid **9** inhibited fibril formation of **6** by almost 85%. These results were confirmed by electron microscopy of the amylin(20–29)/inhibitor mixtures. In an aged (2 weeks) mixture of peptide **6** with **7** or **8**, fibrils were observed. By contrast, in a mixture of **6** and peptoid–peptide hybrid **9**, fibrils remained absent (data not shown).

Our previously synthesized β-sheet breaker peptides based on amylin(20–29)[10a] in which the serine residue at position 28 was replaced by either glycolic acid, proline, or *N*-butyl glycine were also evaluated in this assay. As was described earlier, the Ser28→Glyc modification in amylin(20–29) (**14**, Table 2) did not prevent fibril formation completely. This peptide also did not inhibit fibril formation of **6** in this turbidity assay (Figure 3B). However, amylin(20–29) derivatives **15** (Ser28→Pro) and **16** (Ser28→*N*Nle) significantly inhibited fibril formation of **6**, since both mutations were responsible for the absence of any fibril formation in this assay. These observations were also confirmed by electron microscopy and no fibrils were observed when amylin(20–29)Ser28→Pro (**15**) or amylin(20–29)Ser28→*N*Nle (**16**) were mixed with native amylin(20–29) (data not shown).

None of the β-sheet-breaker peptides based on amylin-(20–29) were able to inhibit fibril formation of full-length amylin(1–37) and preformed fibrils were also unaffected by the addition of peptide derivatives **7**–**9**, **11**, and **12**. Appa-

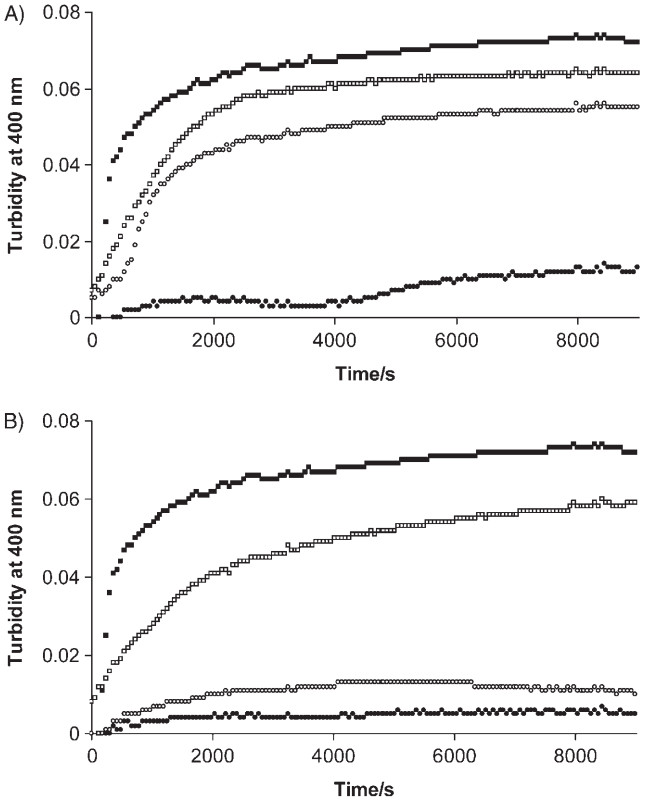


Figure 3. Aggregation curves. A) ■: **6** (10 mg mL^{-1}); □: **6+8** (10 mg mL^{-1}); ○: **6+7** (10 mg mL^{-1}); ●: **6+9** (10 mg mL^{-1}); B) ■: **6** (10 mg mL^{-1}); □: **6+14** (10 mg mL^{-1}); ○: **6+15** (10 mg mL^{-1}); ●: **6+16** (10 mg mL^{-1}).

Table 2. Sequences of the amylin derivatives synthesized in this study.

Peptide sequence	Mass $[M+H]^+$ found (calcd)	R_t [min]
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-NH_2 (**6**)	1008.65 (1008.50)	17.15
H-Ser-Asn-Asn-Phe-**Glyc**-Ala-**Ilec**-Leu-**Glyc**-Ser-NH_2 (**7**)	981.65 (981.45)	18.65
H-Ser-Asn-Asn-Phe-***N*Nle**-Ala-**(*N*Bu)Ile**-Leu-**(*N*Bu)Ser**-Ser-NH_2 (**8**)	1176.90 (1176.70)	21.40
H-Ser-Asn-Asn-Phe-***N*Nle**-Ala-***N*Nle**-Leu-***N*Nle**-Ser-NH_2 (**9**)	1090.85 (1090.63)	20.12
H-Ser-Asn-Asn-Phe-Gly-Ala-**Gly**-Leu-**Gly**-Ser-NH_2 (**10**)	922.60 (922.44)	15.77
H-Ser-Asn-Asn-Phe-***N*Nle**-Ala-Ile-Leu-***N*Nle**-Ser-NH_2 (**11**)	1090.80 (1090.27)	19.72
H-Phe-***N*Nle**-Ala-Ile-Leu-***N*Nle**-Ser-NH_2 (**12**)	688.85 (687.91)	22.48
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Gly**-Ser-NH_2 (**13**)	reference [10a]	n.d.[a]
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Glyc**-Ser-NH_2 (**14**)	reference [10a]	n.d.[a]
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Pro**-Ser-NH_2 (**15**)	reference [10a]	n.d.[a]
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-***N*Nle**-Ser-NH_2 (**16**)	reference [10a]	n.d.[a]

[a] n.d. = not determined.

rently, the highly amyloidogenic amylin(20–29) sequence is not optimal for efficient molecular recognition of amylin(1–37) fibrils, as recently described by Gazit and co-workers,[40] who found that amino acid residues at the N terminus are important for binding to full-length amylin.

Self-assembled ribbons and peptide nanotubes: Although peptides **7** and **8** were designed as β-sheet-breaker peptides for the disruption of the characteristic hydrogen-bond pattern (Scheme 2), they themselves form self-assembled structures that give rise to helical ribbons and peptide nanotubes. Several examples of self-assembly into helical ribbons/peptide nanotubes by designed β-sheet model peptides are described in the literature. However, according to FTIR data (see above) and CD spectroscopy (Figure 4), depsipeptide **7**

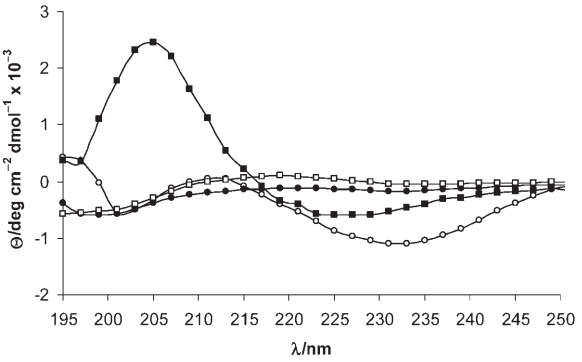


Figure 4. CD spectra of the amylin(20–29) derivatives. ■: **6**; □: **7**; ○: **8**; ●: **9**.

and *N*-alkylated peptide **8** do not form β sheets. Therefore, in our case, aggregation other than that responsible for the formation of β sheets must be now responsible for the formation of helical ribbons and peptide nanotubes. The increased hydrophobicity of peptides **7** and **8** might be a driving force for the self-assembly into the observed helical ribbons.

Replacement of a backbone amide with an ester moiety (depsipeptide **7**) eliminated the hydrogen-bond donor (N*H* group) and resulted in a weaker hydrogen-bond acceptor (ester carbonyl group).[41] However, the backbone conformation in the depsipeptide, in terms of φ and ψ dihydral angles, remained virtually unaffected and the *trans* ester conformation, as is the case in native amide bonds, was still strongly preferred.[41] Possibly, as a result of this amide to ester substitution, **7** did not aggregate into fibrils involving a hydrogen-bond pattern but formed (anti)parallel *β-sheet-like* tapes, which in turn self-assembled into helical ribbons due to the intrinsic chirality of the depsipeptide.

N-alkylation of three amide bonds in **8** or **9** also removed three N*H* hydrogen-bond donors. In contrast to the situation in **7**, the resulting tertiary amides significantly influenced the conformation of the backbone, since, for example, turns can be introduced by proline, *N*-alkyl amino acid, and *N*-alkyl glycine (peptoid) moieties and ciscoid conformations involving tertiary amide bonds are more preferred. Incorpo-

ration of these building blocks is a well-accepted approach to the design of β-sheet-breaker peptides.[9,10a,13,16] As was confirmed by FTIR spectroscopy from the absence of the 1625–1633 cm^{-1} peak, peptides **8** and **9** did not form β-sheet-aggregated fibers. The absence of β sheets was also confirmed by CD spectroscopy (Figure 4). However, here too, the increased hydrophobicity of peptide **8** might be responsible for the formation of the supramolecular assemblies shown in Figure 2. Absence of any supramolecular structures formed by peptide–peptoid hybrid **9** could be explained by the increased flexibility of this hybrid, as compared to trisalkylated peptide **8**, because the side chains on the α-carbon atoms in two amino acid residues are absent. This is corroborated by the observation that signals in the ^{1}H NMR spectrum of **9** occurred as doublets and broadening of the HPLC elution profiles was observed with the presence of peptoid moieties in the peptide backbone. Additionally, the absence of any secondary structure was confirmed by CD spectroscopy (Figure 4).

Morphology: In Figure 1, different folding stages of depsipeptide **7** can be observed. Arrow a points to a single strand and two strands intertwine at the position b to form a helical ribbon at position c. Ultimately, this self-assembly leads to the formation of a (closed) peptide tube at position d. Arrows e and f point to two helical tapes with different pitches (500 and 330 nm, respectively). A ribbon consists of several filaments, as is clearly shown in Figure 2 A (arrows). Figure 2 B shows an almost closed peptide tube and Figure 2 C is an enlargement of the joining of 5–7 filaments to form a peptide nanotube. The (almost) closed peptide tubes may be explored as insulation or as versatile precursors for nanowires.[36b,e,h] Alternatively, these tubes might lead to the formation of nanochannels. Multilayered peptides[42] may even provide adequate insulation to develop neuron mimics. Furthermore, nanochannels, -tubes, or -devices may be useful for drug delivery purposes.[15a,b]

Conclusion

We have found that by modifying the peptide amide linkage it is possible to dramatically alter the aggregation behavior of a peptide causing fibril formation involving β sheets. Synthetically challenging depsipeptides and *N*-alkylated peptides—both categories are not generally accessible as yet—as well as peptoid–peptide hybrids were designed and synthesized. Their behavior with respect to the formation of special supramolecular assemblies, that is, helical ribbons and peptide nanotubes, was quite unexpected and cannot be rationalized by assuming the formation of the common hydrogen-bonding pattern of β sheets. Also, subtle side-chain-to-side-chain interactions were found to play a decisive role in the formation of either fibrils or helical ribbons. The self-assembly of these modified amylin derivatives into structures other than amyloid fibrils makes them of high value for the design of peptide-based nanomaterials.

Experimental Section

Instruments and methods: The peptides were synthesized on an Applied Biosystems 433A peptide synthesizer. Analytical HPLC runs were carried out on a Shimadzu HPLC system and preparative HPLC runs were performed on a Gilson HPLC workstation. Liquid chromatography/electrospray-ionization mass spectrometry was measured on a Shimadzu LCMS-QP8000 single-quadrupole bench-top mass spectrometer operating in the 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. Circular dichroism spectra were measured on an OLIS RSM 1000 CD spectrometer. ^{1}H NMR spectra were recorded on a Varian G-300 (300 MHz) spectrometer and chemical shifts (δ) are given in ppm relative to tetramethylsilane (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 by using the attached proton test (APT) sequence. Retention factor (R_f) values were determined by thin-layer chromatography (TLC) on Merck precoated silica gel 60 F_{254} plates. Spots were visualized by UV quenching, ninhydrin, or Cl_2/*N*,*N*,*N*′,*N*′-tetramethyl-4,4′-diaminodiphenylmethand (TDM).[43] Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat and are uncorrected. Elemental analyses were done at the Kolbe Mikroanalytisches Labor (Mülheim an der Ruhr, Germany).

Chemicals and reagents: ArgoGel Fmoc-Rink-Amide resin functionalized with the 4-((2′,4′-dimethoxyphenyl)aminomethyl)phenoxyacetamido moiety (the Rink amide linker)[44] was used in all the syntheses. The coupling reagents HBTU[45] and BOP[46] were obtained from Biosolve. HATU[23] and HOAt[23] were obtained from Applied Biosystems. HOBt was from Advanced ChemTech and N^{α}-9-fluorenylmethyloxycarbonyl amino acids were obtained from MultiSynTech. The side-chain protecting groups were chosen as *tert*-butyl for serine and trityl for asparagine. Peptide-grade DCM, DCE, *tert*-butyl methylether (MTBE), NMP, and TFA and HPLC-grade acetronitrile were purchased from Biosolve. Piperidine, DMAP, DIPEA, TEA, and triphenylphosphine were obtained from Acros Organics. TIS, 1,2-ethanedithiol (EDT), and HPLC-grade TFA were obtained from Merck. DIAD, triphosgene, DIC, *p*NBS-Cl, and *o*NBS-Cl were purchased from Aldrich. Glycolic acid, *sym*-collidine, β-mercaptoethanol, and DBU were purchased from Fluka.

2-Hydroxy-3-methyl-pentanoic acid allyl ester (1): Isoleucine (5 g, 38 mmol) was dissolved in 2.5 N H_2SO_4 (25 mL) and cooled to 0 °C. A solution of $NaNO_2$ (3.9 g, 57.1 mmol) in H_2O (20 mL) was added dropwise over 1 h and the obtained reaction mixture was stirred for 2 h at 0 °C and then 16 h at room temperature. The reaction mixture was then extracted with diethyl ether (3 × 75 mL). The combined organic layers were washed with brine (50 mL) and dried with $MgSO_4$ and concentrated under reduced pressure. 2-Hydroxy-3-methyl-pentanoic acid was obtained as a colorless oil (4.63 g, 92 %): $[\alpha]_D^{20} = -13.9$ ($c = 1.0$ in $CHCl_3$; literature value:[18a] -21.6 ($c = 1.0$ in $CHCl_3$)); $R_f = 0.24$ (CH_2Cl_2/MeOH 95:5), 0.76 ($CHCl_3$/MeOH/AcOH 95:20:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.20$ (d, 1H, αCH), 2.02 (d, 1H, OH), 1.89 (m, 1H, βCH), 1.46–1.24 (m, 2H, γCH_2), 1.02 (d, 3H, γ′CH_3 Ilec), 0.92 ppm (t, 3H, δCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 179.2$, 74.6, 38.7, 23.6, 15.2, 11.6 ppm.

K_2CO_3 (5.2 g, 36.9 mmol, 1.5 equiv) and allylbromide (4.35 mL, 49.2 mmol, 2 equiv) were added to a solution of 2-hydroxy-3-methyl-pentanoic acid (3.35 g, 24.6 mmol) in acetone (100 mL). The obtained reaction mixture was stirred for 16 h at room temperature. The solvent was then evaporated in vacuo and the residue was redissolved in EtOAc (100 mL), washed with 5 % $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Allyl ester **1** was obtained as a colorless oil (2.80 g, 66 %): $R_f = 0.46$ (CH_2Cl_2/MeOH 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.98$–5.88 (m, 1H, CH allyl), 5.39–5.26 (dd, 2H, CH_2 allyl), 4.69 (d, 2H, CH_2 allyl), 4.11 (d, 1H, αCH), 2.77 (s, 1H, OH), 1.84 (m, 1H, βCH) 1.41–1.23 (m, 2H, γCH_2), 0.99 (d, 3H, γ′CH_3 Ilec), 0.90 ppm (t, 3H, δCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 174.7$, 131.4, 119.1, 74.7, 66.0, 39.1, 23.7, 15.4, 11.7 ppm.

2-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-propionyloxy]-3-methyl-pentanoic acid (Fmoc-Ala-Ilec-OH) (2): DCC (1.65 g, 8 mmol), Fmoc-

Ala-OH (2.63 g, 8 mmol), and DMAP (78 mg, 7.5 mol%) were added to a solution of allyl ester **1** (1.38 g, 8 mmol) in CH_2Cl_2 (75 mL). The obtained reaction mixture was stirred for 16 h at room temperature. Next, DCU was removed by filtration and the solvent was evaporated in vacuo. The residue was redissolved in EtOAc (150 mL) and the organic layer was washed with 1 N $KHSO_4$ (3×75 mL), 5% $NaHCO_3$ (3×75 mL), and brine (3×100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH 99:1) and Fmoc-Ala-Ilec-OAllyl was obtained as a white solid (2.48 g, 67%): M.p. = 72–75 °C; R_f = 0.60 (CH_2Cl_2/MeOH 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77–7.28 (m, 8H, arom. CH), 5.97–5.83 (m, 1H, CH allyl), 5.38–5.24 (m, 3H, CH_2 allyl/NH), 5.00 (d, 1H, αCH Ilec), 4.64 (d, 2H, OCH_2 allyl), 4.49 (t, 1H, αCH Ala), 4.37 (d, 2H, CH_2 Fmoc), 4.22 (t, 1H, CH Fmoc), 2.03/1.83 (2×s, 1H, βCH Ilec), 1.52 (d, 3H, βCH_3 Ala), 1.53–1.21 (br m, 2H, γCH_2 Ilec), 0.99 (d, 3H, γ′CH_3 Ilec), 0.91 ppm (q, 3H, δCH_3 Ilec); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.9, 168.9, 155.6, 143.9, 141.2, 131.4, 127.7, 127.0, 125.1, 119.9, 119.0, 76.9, 67.0, 66.0, 65.8, 49.4, 47.1, 39.1, 36.5, 24.4, 23.7, 18.6, 15.4, 11.7 ppm; EI-MS: (50 eV): *m/z* (%): 466.50 (52) $[M+H]^+$, 488.35 (100) $[M+Na]^+$.

Fmoc-Ala-Ilec-OAllyl (930 mg, 2 mmol) was dissolved in CH_2Cl_2 (25 mL) and purged with argon for 5 min. Phenylsilane (1.2 mL, 5 equiv) and a catalytic amount of $Pd^0(PPh_3)_4$ were then added under argon and the reaction mixture was stirred overnight at room temperature. Subsequently, the solvent was removed under reduced pressure and the residue was redissolved in EtOAc (50 mL). The organic layer was washed with 1 N $KHSO_4$ (2×25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. After purification by column chromatography (CH_2Cl_2/MeOH 92:8), **2** was obtained as a white foam (600 mg, 71%): M.p. = 131–133 °C; R_f = 0.46 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78–7.26 (m, 8H, arom. CH), 5.46 (d, 1H, NH), 4.97 (s, 1H, αCH Ilec), 4.45 (t, 1H, αCH Ala), 4.38 (d, 2H, CH_2 Fmoc), 4.21 (t, 1H, CH Fmoc), 2.05 (m, 1H, βCH Ilec), 1.49–1.21 (m, 5H, βCH_3 Ala/γCH_2 Ilec), 0.98 (d, 3H, γ′CH_3 Ilec), 0.90 ppm (t, 3H, δCH_3 Ilec); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.1, 171.9, 156.0, 143.6, 141.1, 133.9, 127.6, 126.9, 124.9, 119.8, 66.8, 49.7, 46.9, 36.2, 24.2, 17.9, 15.2, 11.4 ppm; EI-MS: (50 eV): *m/z* (%): 448.35 (100) $[M+Na]^+$; elemental analysis: calcd (%) for $C_{24}H_{27}NO_6$ (425.47): C 67.75, H 6.40, N 3.29; found: C 67.25, H 6.48, N 3.08.

3-Methyl-2-(4-nitrobenzenesulfonylamino)-pentanoic acid benzyl ester (3): H-Ile-OH (13.1 g, 100 mmol) was suspended in toluene (250 mL); benzylalcohol (12.9 mL, 125 mmol) and then TosOH·H_2O (20.9 g, 110 mmol) were added. The reaction mixture was refluxed for 16 h in a Dean–Stark apparatus and subsequently concentrated in vacuo. The residue was triturated with diethyl ether, filtered, and dried in a desiccator. The obtained tosylate was dissolved in EtOAc (150 mL); the organic layer was washed with a saturated solution of $NaHCO_3$ (100 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (75 mL) and then TEA (5.2 mL, 2 equiv) was added, followed by *p*NBS-Cl (4.1 g, 1 equiv). After being stirred for 16 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was redissolved in EtOAc (100 mL), washed with 1 N $KHSO_4$ (3×75 mL) and brine (50 mL), and dried (Na_2SO_4), and then the solvent was removed in vacuo. Compound **3** was obtained as a yellowish solid (6.10 g, 66%): M.p. = 79–83 °C; R_f = 0.55 (EtOAc/hexane 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 (d, 2H, arom. CH), 7.94 (d, 2H, arom. CH), 7.33 (m, 3H, arom. CH), 7.17 (m, 2H, arom. CH), 5.32 (d, 1H, NH), 4.92 (dd, 2H, CH_2 benzyl), 3.90 (q, 1H, αCH Ile), 1.88 (m, 1H, βCH Ile), 1.38–1.11 (m, 2H, γCH_2 Ile), 0.95–0.86 ppm (m, 6H, γ′CH_3/δCH_3 Ile); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 145.4, 134.5, 128.9, 128.6, 128.5, 128.3, 124.1, 67.4, 60.6, 38.4, 24.3, 15.5, 11.3 ppm.

2-Butylamino-3-methyl-pentanoic acid benzyl ester (4): A solution of sulfonamide **3** (4.42 g, 10.9 mmol) and PPh_3 (2.85 g, 10.9 mmol) in dry THF (25 mL) was cooled to −60 °C. *n*BuOH (1 mL) and then DIAD (2.15 mL) were added dropwise and the reaction mixture was allowed to react for 16 h. After removal of the solvent by evaporation, the obtained residue was dissolved in EtOAc. The precipitate was removed by filtration and the EtOAc layer was evaporated to dryness. After recrystallization, *p*NBS-*N*(Bu)Ile-OBzl was obtained in a nearly quantitative yield (5.0 g): M.p. = 87–90 °C; R_f = 0.62 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.00 (d, 2H, arom. CH), 7.87 (d, 2H, arom. CH), 7.34 (m, 3H, arom. CH), 7.18 (m, 2H, arom. CH), 4.88 (s, 2H, CH_2 benzyl), 4.27 (d, 1H, αCH Ile), 3.46–3.07 (m, 2H, *N*-CH_2 butyl), 1.93 (m, βCH Ile), 1.81–1.17 (m, 6H, CH_2 butyl (2×2H)/γCH_2 Ile), 0.97–0.86 ppm (m, 9H, CH_3 butyl/γ′CH_3/δCH_3 Ile); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.9, 149.6, 145.5, 134.4, 128.8, 128.6, 128.6, 128.4, 123.8, 66.8, 64.8, 45.6, 34.9, 32.6, 25.2, 20.2, 15.5, 13.5, 10.8 ppm.

K_2CO_3 (3.85 g) and then thiophenol (1.1 mL) were added to a solution of *p*NBS-*N*(Bu)Ile-OBzl (4.95 g) in DMF (40 mL) and the obtained reaction mixture was stirred for 90 min. The reaction mixture was then diluted by the addition of H_2O (80 mL) and the aqueous phase was extracted with diethyl ether (3×100 mL). The combined organic layers were washed with H_2O (2×60 mL), 5% $NaHCO_3$ (2×60 mL), and brine (60 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/MeOH 99:1) to obtain H-*N*(Bu)Ile-OBzl (**4**) as a slightly brownish oil (1.72 g, 67%): R_f = 0.41 (CH_2Cl_2/MeOH 97:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36 (m, 5H, arom. CH), 5.16 (s, 2H, CH_2 benzyl), 3.11 (d, 1H, αCH Ile), 2.59–2.37 (dm, 2H, *N*-CH_2 butyl) 1.67 (m, 1H, βCH Ile), 1.60–1.07 (m, 6H, CH_2 butyl (2×2H)/γCH_2 Ile), 0.90–0.84 ppm (m, 9H, CH_3 butyl/γ′CH_3/δCH_3 Ile); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.3, 135.9, 128.5, 128.4, 128.3, 66.2, 66.1, 48.3, 38.4, 32.3, 25.7, 21.9, 20.3, 15.5, 11.4 ppm; EI-MS: (50 eV): *m/z* (%): 278.25 (100) $[M+H]^+$.

Fmoc-Ala-*N*(Bu)Ile-OH (5): Fmoc-Ala-OH·H_2O (329 mg, 1 mmol) was coevaporated with chloroform and toluene (2×10 mL) and subsequently dissolved in dry THF (20 mL). Triphosgene (0.33 mmol) and *sym*-collidine (1.25 equiv) were added to this solution and the reaction mixture was stirred for 2 min to obtain the acid chloride. A solution of **4** (1 mmol) in dry THF (20 mL) was then added dropwise and the obtained reaction mixture was stirred for 1 h. After removal of the solvent, the residue was dissolved in EtOAc (50 mL) and subsequently washed with 1 N $KHSO_4$ (50 mL), 5% $NaHCO_3$ (50 mL), and brine (50 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in EtOH (25 mL), then $CHCl_3$ (2 mL) and subsequently Pd/C (100 mg) were added and the reaction mixture was stirred for 16 h in the presence of H_2. Subsequently, the reaction mixture was filtered over Hyflo and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5) and **5** was obtained as a white foam in 34% yield over two reaction steps (159 mg): R_f = 0.26 (EtOAc/hexane/AcOH 2:1:0.01); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78–7.30 (m, 8H, arom. CH), 5.67 (d, 1H, NH), 4.70 (t, 1H, αCH Ala), 4.38 (m, 2H, CH_2 Fmoc), 4.22 (t, 1H, CH Fmoc), 3.62–3.13 (m, 2H, *N*-CH_2 butyl), 2.47 (d, 1H, αCH Ile), 1.67 (t, 1H, βCH Ile), 1.49–1.08 (m, 6H, CH_2 butyl (2×2H, γCH_2 Ile), 1.01–0.88 ppm (m, 9H, CH_3 butyl/γ′CH_3/δCH_3 Ile); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.2, 172.3, 155.6, 143.8, 143.6, 141.2, 127.6, 127.0, 125.1, 119.9, 70.0, 68.4, 67.0, 50.1, 47.4, 47.0, 32.9, 31.4, 25.1, 19.9, 19.1, 16.0, 13.6, 10.9 ppm; EI-MS: (50 eV): *m/z* (%): 503.40 (100) $[M+Na]^+$; elemental analysis: calcd (%) for $C_{28}H_{36}N_2O_5$ (480.60): C 69.98, H 7.55, N 5.83; found: C 69.79, H 7.48, N 5.78.

Peptide synthesis (general procedure): Peptides **6** and **10** were synthesized by using the FastMoc protocol on a 0.25 mmol scale[26] on Argogel Fmoc-Rink-Amide resin to obtain the C-terminally amidated peptide.[44] 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 preactivated Fmoc amino acid (1.0 mmol) in the presence of DIPEA (2 equiv), 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) for 3 h. The peptides were precipitated with MTBE/hexane (1:1) at −20 °C and finally lyophilized from *tert*-butanol/H_2O (1:1).

Depsipeptide 7: Fmoc-Ser(*t*Bu)NH-Rink-Amide resin (0.10 mmol) was washed with CH_2Cl_2 (3×2 min, 10 mL) and NMP (3×2 min, 10 mL) and treated with 20% piperidine/NMP (3×8 min, 10 mL) to remove the Fmoc group. After washing the resin with NMP (3×2 min, 10 mL), CH_2Cl_2 (3×2 min, 10 mL), and NMP (3×2 min, 10 mL), Trt-Glyc-OH[10a] (127 mg, 0.40 mmol) was coupled to the α-amino group with BOP (177 mg, 0.40 mmol)/DIPEA (70 μL, 0.40 mmol) in NMP (10 mL). The

coupling was monitored by the Kaiser test[28] and was complete after 2 h. Subsequently, the resin was washed with NMP (3×2 min, 10 mL), CH_2Cl_2 (3×2 min, 10 mL), and NMP (3×2 min, 10 mL) and the trityl functionality was removed by treatment with TFA/TIS/CH_2Cl_2 (1:5:94, 5×2 min, 20 mL). After washing the resin with CH_2Cl_2 (5×2 min, 10 mL) and NMP (3×2 min, 10 mL), Fmoc-Leu-OH (141 mg, 0.40 mmol) was coupled to the α-hydroxy group with DIC (153 mg, 0.80 mmol)/HOBt (245, 1.6 mmol)/DMAP (49 mg, 0.40 mmol) in NMP (10 mL) for 16 h. The resin was washed with NMP (3×2 min, 10 mL) and CH_2Cl_2 (3×2 min, 10 mL) to remove the excess reagents; the coupling yield of Fmoc-Leu-OH, as calculated from an Fmoc determination,[30] was 76%. After removal of the Fmoc group, Fmoc-Ala-Ilec-OH (85 mg, 0.20 mmol) was coupled with HATU (76 mg, 0.20 mmol)/HOAt (27 mg, 0.20 mmol)/DIPEA (70 μL, 0.40 mmol) in NMP (10 mL). The coupling reaction was monitored by the Kaiser test and was complete after 90 min. The Fmoc group was removed by treatment with 20% piperidine in NMP and the resin was washed as described above. Trt-Glyc-OH was then coupled to the α-amino group with BOP/HOBt/DIPEA in NMP under the same conditions as those described earlier. Subsequently, the trityl group was removed by acid and Fmoc-Phe-OH was coupled to the primary hydroxy functionalityin the presence of DIC/HOBt/DMAP in NMP. The coupling yield was determined to be 74% (0.19 mmol g^{-1}). After this amino acid, the peptide sequence was completed as described in the general procedure.

***N*-Butylated-Gly24, -Ile26, and -Ser28 peptide 8**: Fmoc-Ser(*t*Bu)-Ser(*t*Bu)NH-Rink-Amide resin (0.20 mmol) was washed with CH_2Cl_2 (3×2 min, 10 mL) and NMP (3×2 min, 10 mL) and treated with 20% piperidine/NMP (3×8 min, 10 mL) to remove the Fmoc group. After washing the resin with DMF (5×2 min, 10 mL) and DCE (5×2 min, 10 mL) the α-amino functionality was treated with *o*NBS-Cl in the presence of *sym*-collidine as base in DCE (10 mL) for 2 h. After washing the resin with CH_2Cl_2 (6×2 min, 10 mL), the sulfonamide moiety was allowed to react with triphenylphosphine (319 mg), 1-butyl alcohol (222 μL), and DIAD (239 μL) in DCE (10 mL) for 3 h. The resin was then washed with CH_2Cl_2 (3×2 min, 10 mL) and DMF (6×2 min, 10 mL). The *o*NBS group was removed by treatment with 0.5 M 2-mercaptoethanol in DMF (5 mL) in the presence of DBU (182 μL) for 75 min. The resin was washed with DMF (6×2 min, 10 mL) and CH_2Cl_2 (3×2 min, 10 mL). Subsequently, Fmoc-Leu-OH was coupled to the *N*-butyl amine by using the BTC method.[25,33] First, Fmoc-Leu-Cl was prepared in situ by adding triphosgene (60 mg, 0.20 mmol) to a solution of Fmoc-Leu-OH in dioxane (10 mL) in the presence of *sym*-collidine (212 μL, 1.6 mmol) as base. After being stirred for 60 s, the reaction mixture was transferred to the resin and allowed to react for 1 h. This treatment was repeated once. The resin was then washed with DMF (3×2 min, 10 mL), CH_2Cl_2 (3×2 min, 10 mL), and DMF (3×2 min, 10 mL) and the yield was determined to be 70% (0.24 mmol g^{-1}). After capping of the resin with Ac_2O/DIPEA/HOBt in NMP (10 mL), the resin was treated with 20% piperidine/NMP to remove the Fmoc group and the resin was washed. Subsequently, Fmoc-Ala-*N*(Bu)Ile-OH (170 mg, 0.3 mmol) was coupled for 16 h by using HATU (133 mg, 0.35 mmol)/HOAt (48 mg, 0.35 mmol)/DIPEA (122 μl, 1.4 mmol) in NMP (10 mL). After coupling of this dipeptide, the synthesis was continued by coupling Fmoc-*N*Nle-OH with the peptoid-coupling protocol.[27] Finally, the synthesis was completed as described above in the general procedure.

Peptoid–peptide hybrids 9, 11, and 12: The Fmoc/*t*Bu-based solid-phase synthesis of peptoids and peptoid–peptide hybrids as described by Kruijtzer et al.[27] was used. In short, Fmoc-*N*Nle-OH was coupled to the α-amino group of the preceding amino acid in the presence of HBTU/HOBt and DIPEA in NMP for 45 min. After removal of the Fmoc group, the next amino acid residue was coupled to the secondary amine with HATU/HOAt/DIPEA in NMP for 90 min.

Peptide purification: The crude lyophilized peptides (30–60 mg) were dissolved in a minimum amount of 0.1% TFA in CH_3CN/H_2O (8:2) 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 mL min^{-1} by using a linear gradient of 100% buffer A → 100% buffer B in 60 min (buffer A: 0.1% TFA in H_2O; buffer B: 0.1% TFA in CH_3CN/H_2O 95:5). 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 1.0 mL min^{-1} by using a linear gradient 100% buffer A → 100% buffer B in 30 min.

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 by using the MacBioSpec program (Perkin–Elmer Sciex Instruments, Thornhill, ON, Canada). The values are given in Table 2.

Gelation experiments: Each peptide sample (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 checking 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 three weeks (10 μL) was placed on a carbon-coated copper grid. After 15 min, any excess of peptide was removed by washing the copper grid with a drop of demi-water. This was repeated five times. Finally, the samples were stained by 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 20000–100000 times.

Fourier transform infrared spectroscopy: A peptide gel/solution aged for three weeks (100 μL) was lyophilized and subsequently resuspended in D_2O (150 μL) and lyophilized. This treatment was repeated twice. The lyophilized peptides 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 spectrometer. 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.

Circular dichroism spectroscopy: CD spectra were measured at 1.0 nm intervals over the range 195–250 nm as the average of 20 runs and by using a spectral band width of 2.0 nm. Cuvettes of 0.5 mm thermostated at 20 °C were used, with the optical chamber continually flushed with dry N_2 gas. The spectra were measured in 0.1% TFA in H_2O. The concentrations (1 mg mL^{-1}) were determined on the basis of the calculated molecular mass of the purified lyophilized peptides. A peptide sample was dissolved in 0.1% TFA in H_2O and stored for 4 days at 4 °C prior to analysis.

Aggregation assay: The procedure for this assay was based on reference [47]. A stock solution (10 mM) of hIAPP(20–29) (**6**) in dimethylsulfoxide (DMSO) was prepared and this stock solution (25 μL) was added to an equimolar quantity of lyophilized inhibitor peptide (**7**–**11**). After immediate mixing, the obtained DMSO mixture was incubated for 15 min. The DMSO mixture was then diluted into a phosphate buffer (225 μL, pH 7.4, 100 mM NaCl, 1.8 mM NaH_2PO_4, 8.2 mM Na_2HPO_4) and the turbidity (absorbance at 400 nm) was measured over 150 min at room temperature. These aggregation assays were performed twice in three independent experiments. Turbidity measurements were performed on a Bio-TEK μQuant plate reader.

Acknowledgements

These investigations were supported by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO).

[1] a) E. H. Koo, P. T. Lansbury, Jr., J. W. Kelly, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9989–9990; b) J.-C. Rochet, P. T. Lansbury, Jr., *Curr. Opin. Struct. Biol.* **2000**, *10*, 60–88; c) E. Gazit, *Angew. Chem.* **2002**, *114*, 267–269; *Angew. Chem. Int. Ed.* **2002**, *41*, 257–259; d) C. Soto, *Nat. Rev. Neurosci.* **2003**, *4*, 49–60.

[2] a) G. G. Glenner, C. W. Wong, *Biochem. Biophys. Res. Commun.* **1984**, *122*, 1131–1135; b) J. Hardy, D. J. Selkoe, *Science* **2002**, *297*, 353–356.

[3] a) T. M. Dawson, V. L. Dawson, *Science* **2003**, *302*, 819–822; b) M. J. Volles, P. T. Lansbury, Jr., *Biochemistry* **2003**, *42*, 7871–7878.
[4] a) S. B. L. Ng, A. J. Doig, *Chem. Soc. Rev.* **1997**, *26*, 425–432; b) S. B. Prusiner, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13363–13383; c) S. W. Liebman, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9098–9100.
[5] a) P. Westermark, C. Wernstedt, E. Wilander, K. Sletten, *Biochem. Biophys. Res. Commun.* **1986**, *140*, 827–831; b) G. J. S. Cooper, A. C. Willis, A. Clark, R. C. Turner, R. B. Sim, K. B. M. Reid, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8628–8632; c) J. W. M. Höppener, B. Ahrén, C. J. M. Lips, *N. Engl. J. Med.* **2000**, *343*, 411–419.
[6] M. R. Nilsson, C. M. Dobson, *Protein Sci.* **2003**, *12*, 2637–2641.
[7] a) M. A. Findeis, *Biochim. Biophys. Acta* **2000**, *1502*, 76–84; b) R. Kayed, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman, C. G. Glabe, *Science* **2003**, *300*, 486–489; c) C. M. Dobson, *Science* **2004**, *304*, 1259–1262.
[8] a) M. Pappolla, P. Bozner, C. Soto, H. Shao, N. K. Robakis, M. Zagorski, B. Frangione, J. Ghiso, *J. Biol. Chem.* **1998**, *273*, 7185–7188; b) L. J. Twyman, D. Allsop, *Tetrahedron Lett.* **1999**, *40*, 9383–9394; c) P. Kuner, B. Bohrmann, L. O. Tjenberg, J. Näslund, G. Huber, S. Celenk, F. Grüninger-Leitch, J. G. Richards, R. Jakob-Roetne, J. A. Kemp, C. Norstedt, *J. Biol. Chem.* **2000**, *275*, 1673–1678; d) B. Bohrmann, M. Adrian, J. Dubochet, P. Kuner, F. Müller, W. Huber, C. Norstedt, H. Döbeli, *J. Struct. Biol.* **2000**, *130*, 232–246; e) N. Reixach, E. Crooks, J. M. Ostrech, R. A. Houghten, S. E. Blondelle, *J. Struct. Biol.* **2000**, *130*, 247–258; f) T. Klabunde, H. M. Petrassi, V. B. Oza, P. Raman, J. W. Kelly, J. C. Sacchettini, *Nat. Struct. Biol.* **2000**, *7*, 312–321; g) M. B. Pepys, J. Herbert, W. L. Hutchinson, G. A. Tennent, H. J. Lachmann, J. R. Gallimore, L. B. Lovat, T. Bartfal, A. Alanine, C. Hertel, T. Hoffmann, R. Jakob-Roetne, R. D. Norcross, J. A. Kemp, K. Yamamura, M. Suzuki, G. W. Taylor, S. Murray, D. Thompson, A. Purvis, S. Kolstoe, S. P. Wood, P. N. Hawkins, *Nature* **2002**, *417*, 254–259; h) H. Razavi, S. K. Palaninathan, E. T. Powers, R. L. Wiseman, H. E. Purkey, N. N. Mohamedmohaideen, S. Deechongkit, K. P. Chiang, M. T. A. Dendle, J. C. Sacchettini, J. W. Kelly, *Angew. Chem.* **2003**, *115*, 2864–2867; *Angew. Chem. Int. Ed.* **2003**, *42*, 2758–2761; i) O. B. Wallace, D. W. Smith, M. S. Deshpande, C. Polson, K. M. Felsenstein, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1203–1206; j) P. Rzepecki, L. Nagel-Steger, S. Feuerstein, U. Linne, O. Molt, R. Zadmard, K. Aschermann, M. Wehner, T. Schrader, D. Reisner, *J. Biol. Chem.* **2004**, *279*, 47497–47505; k) Q. Li, J.-S. Lee, C. Ha, C. B. Park, G. Yang, W. B. Gan, Y.-T. Chang, *Angew. Chem.* **2004**, *116*, 6633–6639; *Angew. Chem. Int. Ed.* **2004**, *43*, 6331–6335; l) E. Morais-de-Sá, P. J. B. Pereira, M. J. Saraiva, A. M. Damas, *J. Biol. Chem.* **2004**, *279*, 53483–53490; m) Z. Wang, L. Wang, W. L. Klein, G. R. J. Thatcher, D. L. Venton, *J. Med. Chem.* **2004**, *47*, 3329–3333; n) M. Pickhardt, Z. Gazova, M. von Bergen, I. Khlistunova, Y. Wang, A. Hascher, E.-M. Mandelkow, J. Biernat, E. Mandelkow, *J. Biol. Chem.* **2005**, *280*, 3628–3635; o) F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy, G. M. Cole, *J. Biol. Chem.* **2005**, *280*, 5892–5901; p) I. Dolado, J. Nieto, M. J. Saraiva, G. Arsequell, G. Valencia, A. Planas, *J. Comb. Chem.* **2005**, *7*, 246–252; q) S. M. Johnson, H. M. Petrassi, S. K. Palaninathan, N. N. Mohamedmohaideen, H. E. Purkey, C. Nichols, K. P. Chang, T. Walkup, J. C. Sacchettini, K. B. Sharpless, J. W. Kelly, *J. Med. Chem.* **2005**, *48*, 1576–1587; r) N. S. Green, T. R. Foss, J. W. Kelly, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 14545–14550; s) A. D. Ferrão-Gonzales, B. K. Robbs, V. H. Moreau, A. Ferreira, L. Juliano, A. P. Valente, F. C. L. Almeida, J. L. Silva, D. Foguel, *J. Biol. Chem.* **2005**, *280*, 34747–34754.
[9] a) L. O. Tjenberg, J. Näslund, F. Lindqvist, J. Johansson, A. R. Karlström, J. Thyberg, L. Terenius, C. Nordstedt, *J. Biol. Chem.* **1996**, *271*, 8545–8548; b) J. Ghanta, C.-L. Shen, L. L. Kiessling, R. M. Murphy, *J. Biol. Chem.* **1996**, *271*, 29525–29528; c) C. Soto, M. S. Kindy, M. Baumann, B. Frangione, *Biochem. Biophys. Res. Commun.* **1996**, *226*, 672–680; d) C. Soto, E. M. Sigurdsson, L. Morelli, R. A. Kumar, E. M. Castono, B. Frangione, *Nat. Med.* **1998**, *4*, 822–826; e) J. Chabry, B. Caughey, B. Chesebro, *J. Biol. Chem.* **1998**, *273*, 13203–13207; f) M. A. Findeis, G. M. Musso, C. C. Arico-Muendel, H. W. Benjamin, A. M. Hundal, J.-J. Lee, J. Chin, M. Kelly, J. Wakefield, N. J. Hayward, S. M. Molineaux, *Biochemistry* **1999**, *38*, 6791–6800; g) E. Hughes, R. M. Burke, A. J. Doig, *J. Biol. Chem.* **2000**, *275*, 25109–25115; h) C. Soto, R. J. Kascsak, G. P. Saborío, P. Aucouturier, T. Wisniewski, F. Prelli, R. Kascsak, E. Mendez, D. A. Harris, J. Ironside, F. Tagliavini, R. I. Carp, B. Frangione, *Lancet* **2000**, *355*, 192–197; i) T. L. Lowe, A. Strzelec, L. L. Kiessling, R. M. Murphy, *Biochemistry* **2001**, *40*, 7882–7889; j) C. Heténi, T. Körtvélyesi, B. Penke, *Bioorg. Med. Chem.* **2002**, *10*, 1587–1593; k) J. R. Heal, G. W. Roberts, G. Christie, A. D. Miller, *ChemBioChem* **2002**, *3*, 86–92; l) A. Kapurniotu, A. Schmauder, K. Tenidis, *J. Mol. Biol.* **2002**, *315*, 339–350; m) L. A. Scrocchi, Y. Chen, S. Waschuk, F. Wang, S. Cheung, A. A. Darabie, J. McLaurin, P. E. Fraser, *J. Mol. Biol.* **2002**, *318*, 697–706; n) B. Permanne, C. Adessi, G. P. Saborio, S. Fraga, M.-J. Frossard, J. Van Dorpe, I. Dewachter, W. A. Banks, F. Van Leuven, C. Soto, *FASEB J.* **2002**, *16*, 860–862; o) C. W. Cairo, A. Strzelec, R. M. Murphy, L. L. Kiessling, *Biochemistry* **2002**, *41*, 8620–8629; p) G. Zhang, M. J. Leibowitz, P. J. Sinko, S. Stein, *Bioconjugate Chem.* **2003**, *14*, 86–92; q) F. Formaggio, A. Bettio, V. Moretto, M. Crisma, C. Toniolo, Q. B. Broxterman, *J. Pept. Sci.* **2003**, *9*, 461–466; r) T. Oishi, K.-I. Hagiwara, T. Kinumi, Y. Yamakawa, M. Nishijima, K. Nakamura, H. Arimoto, *Org. Biomol. Chem.* **2003**, *1*, 2626–2629; s) T. Yamashita, Y. Takahashi, T. Takahashi, H. Mihara, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4051–4054; t) C. Adessi, M.-J. Frossard, C. Boissard, S. Fraga, S. Bieler, T. Ruckle, F. Vilbois, S. M. Robinson, M. Mutter, W. A. Banks, C. Soto, *J. Biol. Chem.* **2003**, *278*, 13905–13911; u) R. J. Chalifour, R. W. McLaughlin, L. Lavoie, C. Morisette, N. Tremblay, M. Boulé, P. Sarazin, D. Stéa, D. Lacombe, P. Tremblay, F. Gervais, *J. Biol. Chem.* **2003**, *278*, 34874–34881; v) M. Mutter, A. Chandravarkar, C. Boyat, J. Lopez, S. Dos Santos, B. Mandal, R. Mimna, K. Murat, L. Patiny, L. Saucede, G. Tuchscherer, *Angew. Chem.* **2004**, *116*, 4267–4273; *Angew. Chem. Int. Ed.* **2004**, *43*, 4172–4178; w) S. Gilead, E. Gazit, *Angew. Chem.* **2004**, *116*, 4133–4136; *Angew. Chem. Int. Ed.* **2004**, *43*, 4041–4044; x) W. Hosia, N. Bark, E. Liepinsh, A. Tjenberg, B. Persson, D. Hallén, J. Thyberg, J. Jahansson, L. Tjenberg, *Biochemistry* **2004**, *43*, 4655–4661; y) Y. Fu, J. Bieschke, J. W. Kelly, *J. Am. Chem. Soc.* **2005**, *127*, 15366–15367.
[10] a) D. T. S. Rijkers, J. W. M. Höppener, G. Posthuma, C. J. M. Lips, R. M. J. Liskamp, *Chem. Eur. J.* **2002**, *8*, 4285–4291; b) D. T. S. Rijkers, J. W. M. Höppener, G. Posthuma, C. J. M. Lips, R. M. J. Liskamp in *Peptide Revolution: Genomics, Proteomics & Therapeutics: Proceedings of the 18th American Peptide Symposium* (Eds.: M. Chorev and T. K. Sawyer), Kluwer Academic Publishers, Dordrecht, The Netherlands, **2003**, pp. 773–774.
[11] P. Westermark, U. Engstrom, K. H. Johnson, G. T. Westermark, C. Betsholtz, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 5036–5040.
[12] For other regions of human amylin that form fibrillar assemblies, see: a) M. R. Nilsson, D. P. Raleigh, *J. Mol. Biol.* **1999**, *294*, 1375–1385; b) K. Tenidis, M. Waldner, J. Berhagen, W. Fischle, M. Bergmann, M. Weber, M.-L. Merkle, W. Voelter, H. Brunner, A. Kapurniotu, *J. Mol. Biol.* **2000**, *295*, 1055–1071; c) E. T. A. S. Jaikaran, C. E. Higham, L. C. Serpell, J. Zurdo, M. Gross, A. Clark, P. E. Fraser, *J. Mol. Biol.* **2001**, *308*, 515–525; d) R. Azriel, E. Gazit, *J. Biol. Chem.* **2001**, *276*, 34156–34161.
[13] a) D. J. Gordon, K. L. Sciarretta, S. C. Meredith, *Biochemistry* **2001**, *40*, 8237–8245; b) D. J. Gordon, S. C. Meredith, *Biochemistry* **2003**, *42*, 475–485.
[14] For examples, see: peptidosulfonamides: a) M. C. F. Monnee, M. F. Marijne, A. J. Brouwer, R. M. J. Liskamp, *Tetrahedron Lett.* **2000**, *41*, 7991–7995; b) R. de Jong, D. T. S. Rijkers, R. M. J. Liskamp, *Helv. Chim. Acta* **2002**, *85*, 4230–4243; c) R. M. J. Liskamp, J. A. W. Kruijtzer, *Mol. Diversity* **2004**, *8*, 79–87; urea peptidomimetics: d) A. Boeijen, R. M. J. Liskamp, *Eur. J. Org. Chem.* **1999**, 2127–2135; e) A. Boeijen, J. van Ameijde, R. M. J. Liskamp, *J. Org. Chem.* **2001**, *66*, 8454–8462; peptoids: f) J. A. W. Kruijtzer, L. J. F. Hofmeyer, W. Heerma, C. Versluis, R. M. J. Liskamp, *Chem. Eur. J.* **1998**, *4*, 1570–1580; peptoid ureas: g) J. A. W. Kruijtzer, D. J. Lefeber,

R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 5335–5338; peptoid sulfonamides: h) J. van Ameijde, R. M. J. Liskamp, *Tetrahedron Lett.* **2000**, *41*, 1103–1106; alkene dipeptide isosteres: i) K. M. Bol, R. M. J. Liskamp, *Tetrahedron Lett.* **1991**, *32*, 5401–5404; j) K. M. Bol, R. M. J. Liskamp, *Tetrahedron* **1992**, *48*, 6425–6438.

[15] a) S. Zhang, D. M. Marini, W. Hwang, S. Santoso, *Curr. Opin. Chem. Biol.* **2002**, *6*, 865–871; b) K. Rajagopal, J. P. Schneider, *Curr. Opin. Struct. Biol.* **2004**, *14*, 480–486; c) M. A. Balbo Block, S. Hecht, *Angew. Chem.* **2005**, *117*, 7146–7149; *Angew. Chem. Int. Ed.* **2005**, *44*, 6986–6989; d) D. E. Wagner, C. L. Philips, W. M. Ali, G. E. Nybakken, E. D. Crawford, A. D. Schwab, W. F. Smith, R. Fairman, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12656–12661.

[16] a) J. M. Griffiths, T. T. Ashburn, M. Auger, P. R. Costa, R. G. Griffin, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1995**, *117*, 3539–3546; b) R. Tycko, *Biochemistry* **2003**, *42*, 3151–3159; c) S. A. Jayasinghe, R. Langen, *J. Biol. Chem.* **2004**, *279*, 48420–48425.

[17] It was shown previously that proline residues at these positions lead to inhibition of amyloid formation: D. F. Moriarty, D. P. Raleigh, *Biochemistry* **1999**, *38*, 1811–1818. Recently, it was shown that the incorporation of pseudoproline (oxazolidine) dipeptide derivatives at these positions prevents premature folding into β sheets and this enables the solid-phase synthesis of full-length amylin: A. Abedini, D. P. Raleigh, *Org. Lett.* **2005**, *7*, 693–696.

[18] a) I. Shin, M.-R. Lee, J. Lee, M. Jung, W. Lee, J. Yoon, *J. Org. Chem.* **2000**, *65*, 7667–7675. Recently, a general method for the synthesis of the L-α-hydroxy acid equivalents of the 20 naturally occurring amino acids has been described: b) S. Deechongkit, S.-L. You, J. W. Kelly, *Org. Lett.* **2004**, *6*, 497–500; c) O. Kuisle, E. Quiñoá, R. Riguera, *J. Org. Chem.* **1999**, *64*, 8063–8075.

[19] M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé, A. Loffet, *Tetrahedron Lett.* **1995**, *36*, 5741–5744.

[20] O. Mitsunobu, *Synthesis* **1983**, 1–28.

[21] J. F. Reichwein, R. M. J. Liskamp, *Tetrahedron Lett.* **1998**, *39*, 1243–1246.

[22] S.-Y. Han, Y.-A. Kim, *Tetrahedron* **2004**, *60*, 2447–2467.

[23] a) L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398; b) L. A. Carpino, . A. El-Faham, C. A. Minor, F. Albericio, *J. Chem. Soc. Chem. Commun.* **1994**, 201–203.

[24] L. A. Carpino, D. Ionescu, A. El-Faham, *J. Org. Chem.* **1996**, *61*, 2460–2465.

[25] F. Falb, T. Yechezkel, Y. Salitra, C. Gilon, *J. Pept. Res.* **1999**, *53*, 507–517.

[26] C. G. Fields, D. H. Lloyd, R. L. Macdonald, K. M. Otteson, R. L. Noble, *Pept. Res.* **1991**, *4*, 95–101.

[27] Reference [14f].

[28] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595–598.

[29] J. W. van Nispen, J. P. Polderdijk, H. M. Greven, *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 99–100.

[30] J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lambros, R. C. Makofske, C.-D. Chang, *Int. J. Peptide Protein Res.* **1979**, *13*, 35–42.

[31] a) S. C. Miller, T. S. Scanlan, *J. Am. Chem. Soc.* **1997**, *119*, 2301–2302; b) reference [21]; c) J. F. Reichwein, B. Wels, J. A. W. Kruijtzer, C. Versluis, R. M. J. Liskamp, *Angew. Chem.* **1999**, *111*, 3906–3910; *Angew. Chem. Int. Ed.* **1999**, *38*, 3684–3687.

[32] V. Krchnák, J. Vágner, P. Šafár, M. Lebl, *Collect. Czech, Chem. Commun.* **1988**, *53*, 2542–2548.

[33] a) B. Thern, J. Rudolph, G. Jung, *Tetrahedron Lett.* **2002**, *43*, 5013–5016; b) B. Thern, J. Rudolph, G. Jung, *Angew. Chem.* **2002**, *114*, 2401–2403; *Angew. Chem. Int. Ed.* **2002**, *41*, 2307–2309.

[34] a) S. Krimm, J. Bandekar, *Adv. Protein Chem.* **1986**, *38*, 181–364; b) K. J. Halverson, I. Suholeiki, T. T. Ashburn, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1991**, *113*, 6701–6703; c) T. T. Ashburn, M. Auger, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1992**, *114*, 790–791; d) M. Jackson, H. H. Mantsch, *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 95–120; e) J. Kubelka, T. A. Keiderling, *J. Am. Chem. Soc.* **2001**, *123*, 12048–12058; f) H. Hiramatsu, Y. Goto, H. Naiki, T. Kitagawa, *J. Am. Chem. Soc.* **2004**, *126*, 3008–3009.

[35] a) A. Aggeli, I. A. Nyrkova, M. Bell, R. Harding, L. Carrick, T. C. B. McLeish, A. N. Semenov, N. Boden, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11857–11862; b) S. Zhang, D. M. Marini, W. Hwang, S. Santoso, *Curr. Opin. Chem. Biol.* **2002**, *6*, 865–871; c) C. W. G. Fishwick, A. J. Beevers, L. M. Carrick, C. D. Whitehouse, A. Aggeli, N. Boden, *Nano Lett.* **2003**, *3*, 1475–1479; d) W. J. Goux, L. Kopplin, A. D. Nguyen, K. Leak, M. Rutkofsky, V. D. Shanmuganandam, D. Sharma, H. Inouye, D. A. Kirschner, *J. Biol. Chem.* **2004**, *279*, 26868–26875; e) S. Matsumura, S. Uemura, H. Mihara, *Chem. Eur. J.* **2004**, *10*, 2789–2794; f) J. H. Jung, J. A. Rim, S. J. Lee, S. S. Lee, *Chem. Commun.* **2005**, 468–470; g) A. M. Tamburro, A. Pepe, B. Bochicchio, D. Quaglino, I. P. Ronchetti, *J. Biol. Chem.* **2005**, *280*, 2682–2690.

[36] a) M. F. Perutz, J. T. Finch, J. Berriman, A. Lesk, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5591–5595; b) M. Reches, E. Gazit, *Science* **2003**, *300*, 625–627; c) K. Lu, J. Jacob, P. Thiyagarajan, V. P. Conticello, D. G. Lynn, *J. Am. Chem. Soc.* **2003**, *125*, 6391–6393; d) S. Ray, D. Haldar, M. G. B. Drew, A. Banerjee, *Org. Lett.* **2004**, *6*, 4463–4465; e) M. Yemini, M. Reches, J. Rishpon, E. Gazit, *Nano Lett.* **2005**, *5*, 183–186; f) C. Zhan, P. Gao, M. Liu, *Chem. Commun.* **2005**, 462–464; g) N. Kol, L. Adler-Abramovich, D. Barlam, R. Z. Shneck, E. Gazit, I. Rousso, *Nano Lett.* **2005**, *5*, 1343–1346.

[37] a) D. M. Marini, W. Hwang, D. A. Lauffenburger, S. Zhang, R. D. Kamm, *Nano Lett.* **2002**, *2*, 295–299; b) W. Hwang, D. M. Marini, R. D. Kamm, S. Zhang, *J. Chem. Phys.* **2003**, *118*, 389–397.

[38] a) H. A. Lashuel, S. R. LaBrenz, L. Woo, L. C. Serpell, J. W. Kelly, *J. Am. Chem. Soc.* **2000**, *122*, 5262–5277; b) S. Deechongkit, E. T. Powers, S.-L. You, J. W. Kelly, *J. Am. Chem. Soc.* **2005**, *127*, 8562–8570.

[39] For isoleucine residues, see: a) A. D. Williams, E. Portelius, I. Kheterpal, J.-t. Guo, K. D. Cook, Y. Xu, R. Wetzel, *J. Mol. Biol.* **2004**, *335*, 833–842; for phenylalanine residues, see: b) S. M. Tracz, A. Abedini, M. Driscoll, D. P. Raleigh, *Biochemistry* **2004**, *43*, 15901–15908; c) Y. Porat, Y. Mazor, S. Efrat, E. Gazit, *Biochemistry* **2004**, *43*, 14454–14462; d) O. S. Maki, E. Atkins, P. Sikorski, J. Johansson, L. C. Serpell, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 315–320; e) D. M. Chung, Y. Dou, P. Baldi, J. S. Nowick, *J. Am. Chem. Soc.* **2005**, *127*, 9998–9999; for general information, see: f) J. Gsponer, U. Haberthür, A. Cafisch, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5154–5159.

[40] Y. Mazor, S. Gilead, I. Benhar, E. Gazit, *J. Mol. Biol.* **2002**, *322*, 1013–1024.

[41] S. Deechongkit, P. E. Dawson, J. W. Kelly, *J. Am. Chem. Soc.* **2004**, *126*, 16762–16771.

[42] C. Boettcher, B. Scade, J.-H. Fuhrtop, *Langmuir* **2001**, *17*, 873–877.

[43] E. von Arx, M. Faupel, M. J. Bruggen, *J. Chromatogr.* **1976**, *120*, 224–228.

[44] H. Rink, *Tetrahedron Lett.* **1987**, *28*, 3787–3788.

[45] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillesen, *Tetrahedron Lett.* **1989**, *30*, 1927–1930.

[46] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron* **1975**, *31*, 1219–1222.

[47] T. T. Ashburn, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1993**, *115*, 11012–11013.

Received: November 4, 2005
Published online: March 10, 2006

