Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1837–1842

Transformation of the amyloidogenic peptide amylin(20–29) into its corresponding peptoid and retropeptoid: Access to both an amyloid inhibitor and template for self-assembled supramolecular tapes

Ronald C. Elgersma,[a] Gwenn E. Mulder,[a] John A. W. Kruijtzer,[a] George Posthuma,[b] Dirk T. S. Rijkers[a] and Rob M. J. Liskamp[a,*]

[a]*Department of Medicinal Chemistry & Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands*
[b]*Department of Cell Biology, Center for Electron Microscopy, University Medical Center, 3508 GA Utrecht, The Netherlands*

Received 12 December 2006; revised 12 January 2007; accepted 13 January 2007
Available online 24 January 2007

Abstract—The highly amyloidogenic peptide sequence of amylin(20–29) was transformed into its corresponding peptoid and retropeptoid sequences to design a novel class of β-sheet breaker peptides as amyloid inhibitors. This report describes the synthesis of the chiral peptoid building block of L-isoleucine, the solid phase synthesis of the peptoid and retropeptoid sequences of amylin(20–29), and the structural analysis of these amylin derivatives in solution by infrared spectroscopy, circular dichroism, and transmission electron microscopy. It was found that the peptoid sequence did not form amyloid fibrils or any other secondary structures and was able to inhibit amyloid formation of native amylin(20–29). Although the retropeptoid did not form amyloid fibrils it had only modest amyloid inhibitor properties since supramolecular tapes were formed.

Uncontrolled protein aggregation which leads to the formation of amyloid fibrils and amyloidogenic plaques is a major cause of cell degeneration resulting in cell death in many well-known incurable diseases such as Alzheimer's Disease, Parkinson's Disease, and late onset diabetes (type II diabetes).[1] The latter disease is characterized by the aggregation of human islet amyloid polypeptide (hIAPP) in the insulin producing islet β-cells.[1c] Islet amyloid polypeptide, also known as amylin, is a peptide of 37 amino acid residues, and from structure–activity relationship studies it is known that the (20–29) core region is highly amyloidogenic and rapidly forms amyloid fibrils via a cross β-sheet topology.[2] Based on the seminal papers of Tjernberg et al.[3] and Soto et al.,[4] respectively, in which they describe the design of soluble β-sheet mimics as amyloid fibril inhibitors,[5] we[6] and others[7] used the amylin(20–29) region as a template to design backbone-modified amylin derivatives that are able to inhibit fibril formation of either the (20–29) sequence or full length amylin (Fig. 1).

Previously, we have shown that backbone-modified amylin(20–29) derivatives are promising inhibitors of amyloid formation of native amylin(20–29).[6] Backbone-modified amylin(20–29) derivatives in which one or three amide bonds have been replaced by α-hydroxy acids, *N*-butyl amino acids, *N*-butyl glycines (norleucine peptoid) or β-aminoethane sulfonamides[8] have been designed and successfully synthesized.

Among these β-sheet breaker peptides, we have shown that replacement of an amide bond by a norleucine peptoid derivative is the most effective approach to obtain amyloid inhibitors of amylin(20–29).[6a] Based on these results we designed and synthesized the peptoid and retropeptoid sequences of amylin(20–29) as shown in Figure 2, to study their properties as β-sheet breaker peptidomimetics.

Peptoids[9] are N-alkylated glycine derivatives in which the amino acid side chains are shifted from the α-carbon to the α-amino functionality. A variety of applications in medicinal chemistry[10a] as protease resistant

Keywords: Amyloid; Peptoid; Peptidomimetics; β-Sheet breaker; Self-assembly.
* Corresponding author. Tel.: +31 30 253 7396; fax: +31 30 253 6655; e-mail: R.M.J.Liskamp@pharm.uu.nl

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.01.042

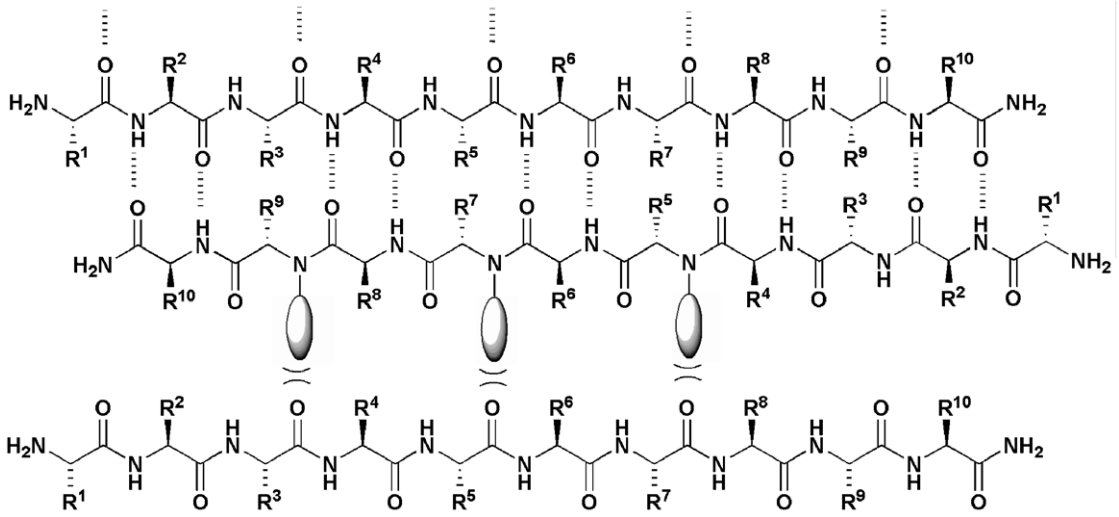


Figure 1. Rationale for design of β-sheet breaker peptides based on the amylin(20–29) sequence. Incorporation of sterical hindrance or removal of essential hydrogen bond donors will disrupt the hydrogen bond network of the (anti)parallel β-sheet and further growth is arrested.

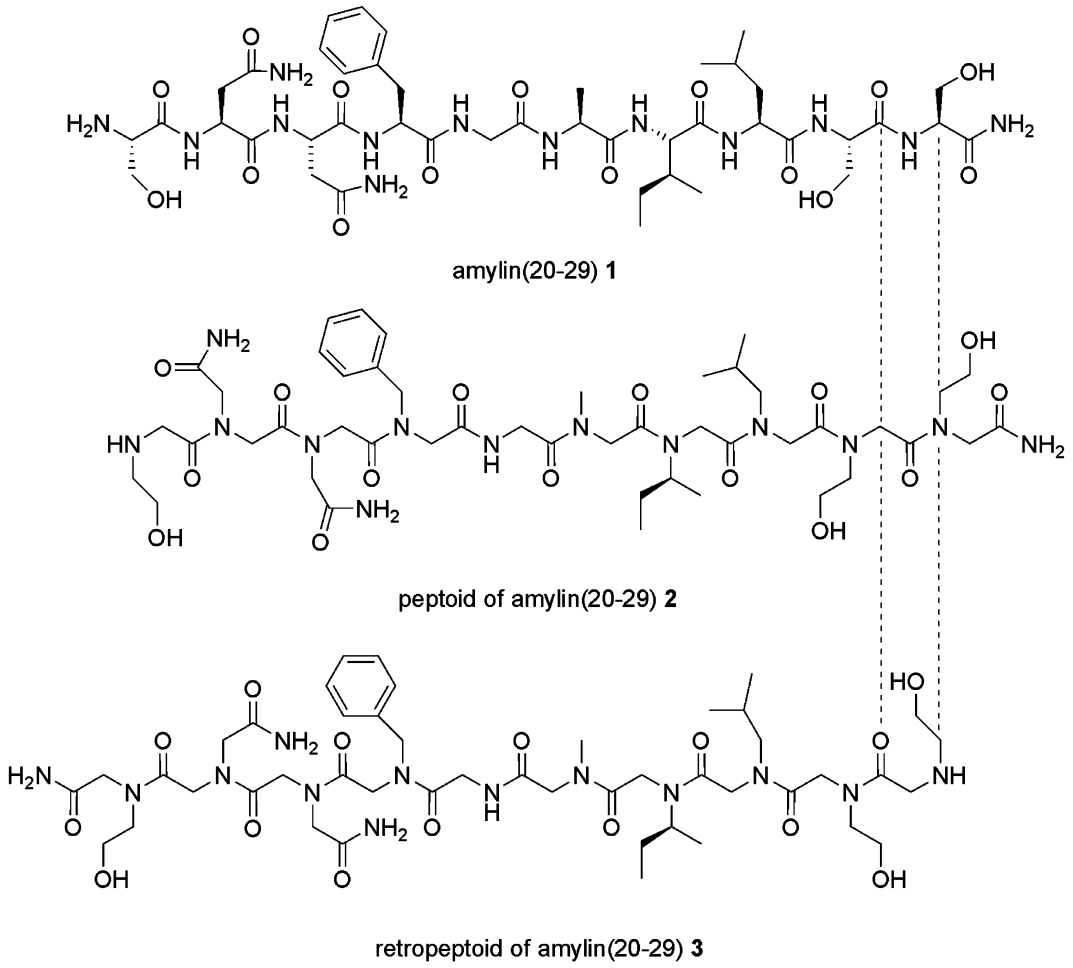


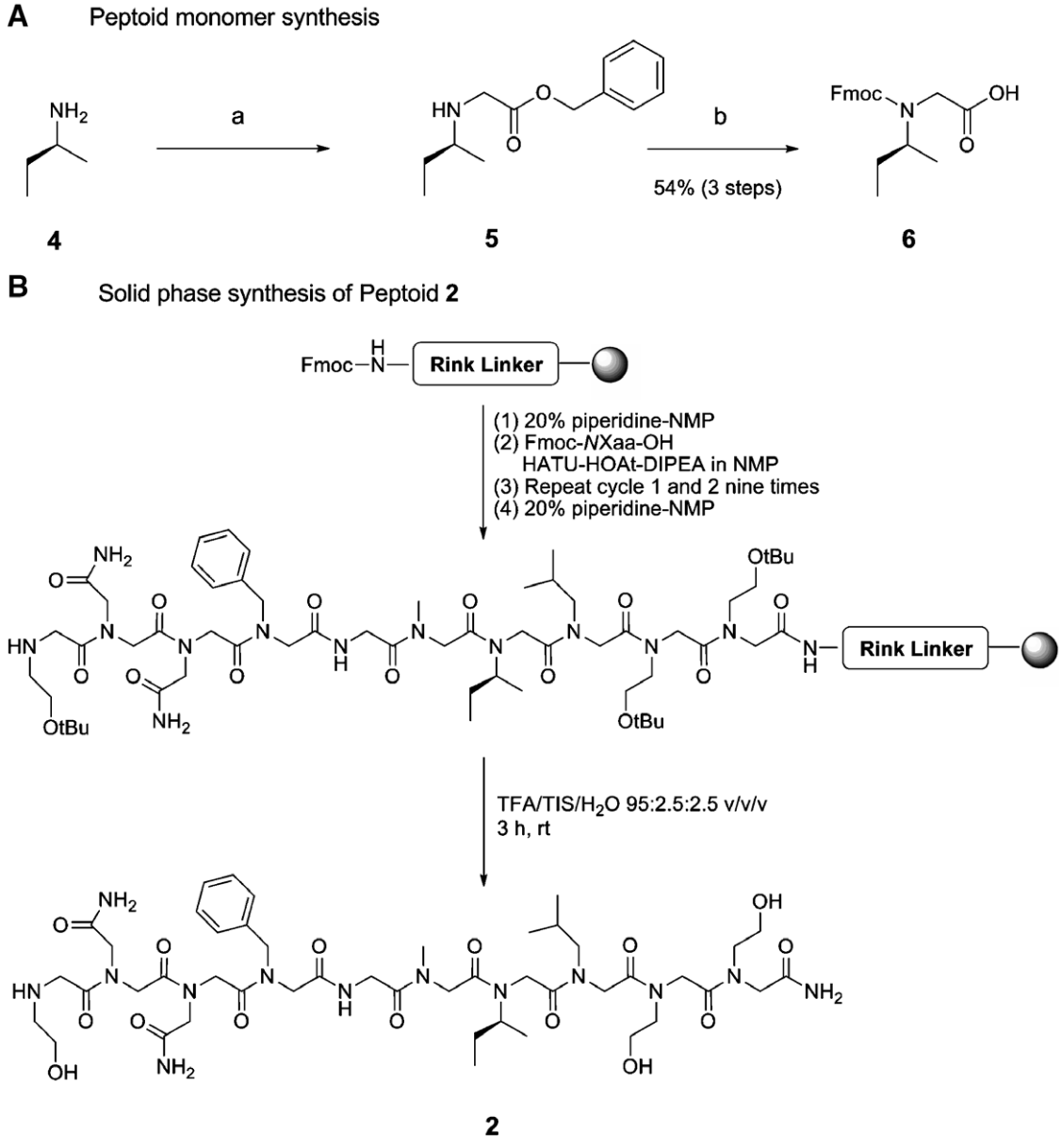
Figure 2. Amino acid sequence of native amylin(20–29) **1** and the corresponding peptoid **2** and retropeptoid **3** derivatives of amylin(20–29).

entities,[10b] antimicrobial agents,[10c] inhibitors of protein–protein interactions,[10d] and artificial protein mimics[10e] have been described in the literature. Peptoids are non-chiral peptidomimetics, except for the chirality of any proline residues or β-carbon atoms of isoleucine and threonine. Moreover, the absence of hydrogen bond donors in the peptide backbone (except for glycine) and the increased flexibility of the backbone due to the presence of tertiary amides which induce ciscoid conformations should abrogate the tendency to form (anti)parallel β-sheets. Translation of a peptide sequence may result either in a peptoid (direct translation) or in a

retropeptoid (retrosequence) as is shown in Figure 2. In the retropeptoid series, the relative orientation of the carbonyl groups to the side chains is maintained and this better resemblance to the parent peptide may be responsible for the slightly better biological activities of retropeptoids.[11b]

The required Fmoc-protected peptoid building blocks were synthesized according to the protocols as described by Kruijtzer et al.[11] Chiral Fmoc–*N*Ile-OH[12] was synthesized as follows: (*S*)-(+)-2-aminobutane was reacted with benzyl bromoacetate in the presence of 1 equiv triethylamine in dichloromethane as solvent. Then, the benzyl ester was treated with Pd/C in a hydrogen atmosphere and the resulting N-alkylated glycine derivative was treated with Fmoc–ONSu in the presence of triethylamine as base to protect the secondary amine resulting in Fmoc–*N*Ile-OH **6** in 54% overall yield (Scheme 1A).

The solid phase synthesis of the peptoid **2** and retropeptoid **3** was carried out on 0.25 mmol scale on a Tentagel Rink Amide resin[13] using the Fmoc/^{t}Bu protocols as was described earlier (Scheme 1B).[11b] Coupling reactions were carried out in the presence of HATU/



Scheme 1. Reagents and conditions: A: (A) benzylbromoacetate, TEA, DCM; (B) 1—H_2, 10% Pd/C, 2—Fmoc–ONSu, TEA, 54% yield over 3 steps. B: Solid phase synthesis of peptoid **2**.[15,16]

Table 1.

Peptide	ESI-MS mass found (calcd) $[M+H]^+$	HPLC analysis t_R (min)[16]	Yield (%) after HPLC purification
Amylin (20–29) (**1**)	1008.55 (1008.50)	17.66	nd
Peptoid (**2**)	1050.50 (1050.55)	16.65	20 mg (8)
Retropeptoid (**3**)	1050.15 (1050.55)	16.53	31 mg (12)

HOAt/DIPEA[14] to ensure complete acylation of the sterically hindered secondary amines (Scheme 1B). After completion of the synthesis, the peptoid derivatives were deprotected and detached from the resin by treatment with TFA in the presence of TIS and H_2O as scavengers.[15] Finally, compounds **2** and **3** were purified by preparative HPLC and characterized by mass spectrometry (Table 1).[16]

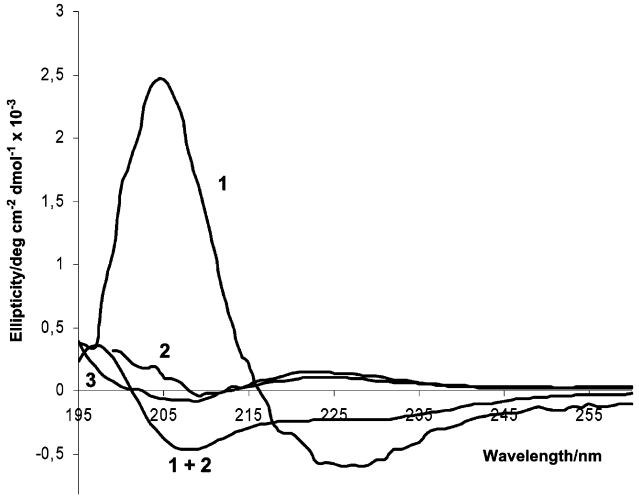


Figure 3. CD spectra of native amylin(20–29) **1** and the peptoid **2**, respectively, the retropeptoid **3** of amylin(20–29) at a concentration of 1 mg/mL in 0.1% TFA/H_2O and aged for 4 days at 4 °C prior to analysis. Trace **1** + **2**: an equal amount (1:1 w/w) of native amylin(20–29) **1** and peptoid **2** was mixed and dissolved in 0.1% TFA/H_2O (final concentration: 2 mg/mL). This mixture was stored for 4 days at 4 °C prior to analysis.

As a control, native amylin(20–29) **1** was prepared. This peptide slowly dissolved in 0.1% TFA/H_2O and rapidly formed an opalescent gel (within 10 min at a concentration of 10 mg/mL). Typical amyloid fibrils were visible by transmission electron microscopy (TEM).[6a,8] These fibrils consisted of a cross β-sheet topology as evidenced by Fourier transform infrared spectroscopy (FTIR) since the typical type I amide absorption was clearly visible at ν 1630 cm^{-1}. Moreover, circular dichroism spectroscopy (CD) showed the characteristic curve of a β-sheet with a minimum at λ 227 nm and a maximum at λ 205 nm (Fig. 3).

The peptoid derivative **2** rapidly dissolved in 0.1% TFA/H_2O (10 mg/mL) and the clear solution remained fluid for at least three weeks at 4 °C. An intense absorption at ν 1668 cm^{-1} was observed by FTIR while the 1630 cm^{-1} absorption was clearly absent indicating a random coil conformation. The absence of any secondary structure was independently confirmed by CD (Fig. 3) and TEM, since no amyloid fibrils or other aggregates were visible.

To test the efficiency of peptoid **2** as a β-sheet breaker, an equal amount (w/w) of native amylin(20–29) **1** was mixed with this peptoid analog and dissolved in 0.1% TFA/H_2O. The turbidity of the resulting solution was monitored in time (Fig. 4, curve 2).[17] This experiment showed that peptoid **2** was a good inhibitor of amyloid formation of **1**, since the turbidity of the solution was reduced to ~20% compared to a solution/gel of **1** (Fig. 4, curve 1). A mixture, containing an equal amount (w/w) of **1** and **2**, was dissolved in 0.1% TFA/H_2O and the obtained solution was analyzed by CD. As can be seen in Figure 3, this solution (trace 1 + 2) did not show the

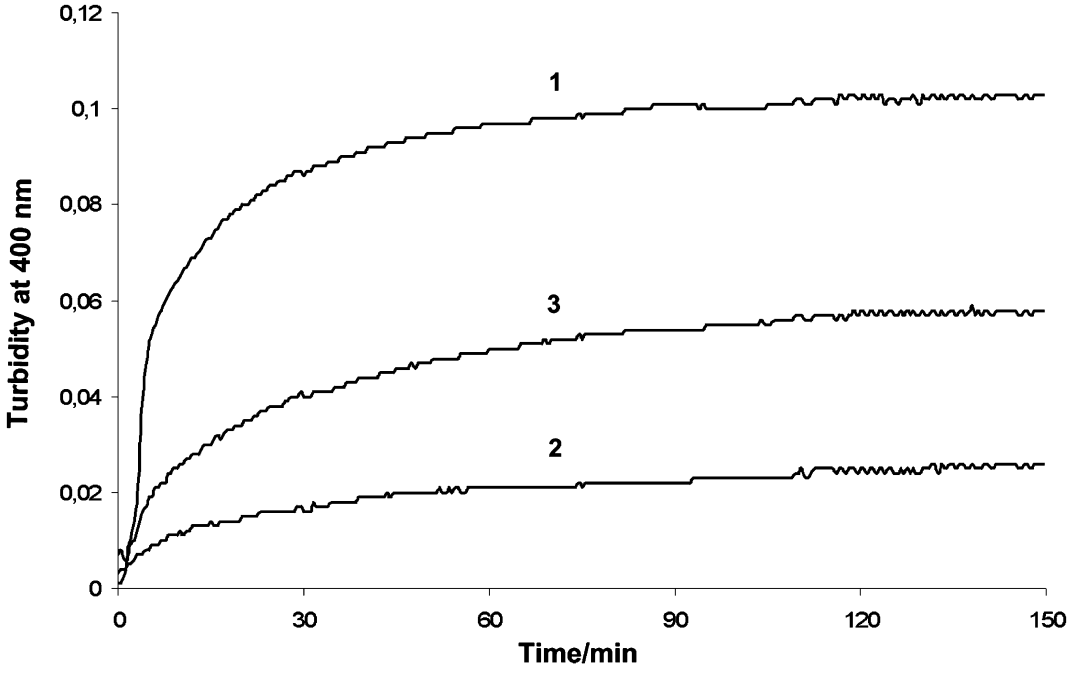


Figure 4. Aggregation curves. Curve 1, amylin(20–29) **1**; curve 2, amylin(20–29) **1** + peptoid **2** (1:1 w/w); curve 3, amylin(20–29) **1** + retropeptoid **3** (1:1 w/w).

typical β-sheet curve (trace 1), indicating that peptoid **2** was indeed able to inhibit/retard amyloid formation of amylin peptide **1**, at least for 4 days.

The retropeptoid **3** also rapidly dissolved in 0.1% TFA/H_2O to give a clear and fluid solution which was stable for at least three weeks at 4 °C. Despite the absence of any secondary structure as judged by FTIR and CD (Fig. 3), this derivative was only moderately active in inhibiting amyloid formation of peptide **1** (Fig. 4, curve 3) and the turbidity was reduced to only ~50%, which was quite unexpected.

Surprisingly, TEM analysis showed that retropeptoid **3** was able to form supramolecular assemblies like ribbons and tapes (Fig. 5A) and some of these progressed into closed nanotubes (Fig. 5B).

The formation of supramolecular folding assemblies was rather unexpected since nearly all hydrogen bond donors were absent. Apparently, the intrinsic self-assembly of **3** was driven by the correct side chain to carbonyl group orientation and thus hampered an efficient inhibition of amyloid formation of **1**. The morphology of the supramolecular folding assemblies was significantly different from typical amyloid fibrils as formed by **1**. The observed tapes had a diameter of ~40 nm and the formed nanotubes (diameter ~55 nm) had a right-handed helical twist and could be 4 μm long.

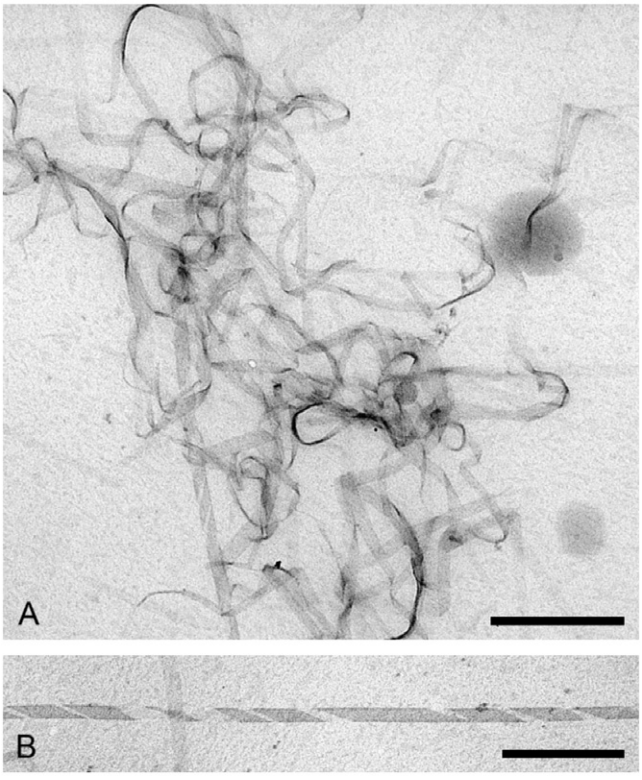


Figure 5. TEM image of retropeptoid **3**. (A) An aggregate of supramolecular ribbons and tapes; (B) enlargement of a ribbon progressing into a nanotube. Scale bar represents 500 nm in both figures.

In conclusion, we have shown that the peptoid and retropeptoid peptidomimetics of the amyloidogenic amylin(20–29) sequence are synthetically accessible in acceptable yields. These mimetics can be used as inhibitors of amyloid fibrillogenesis of amylin (20–29), showing that the peptoid is more potent than the retropeptoid. Removal of hydrogen bond donors in the backbone and the increased tendency to form ciscoid conformations may have led to the absence of any defined secondary structures of the (retro)peptoid in solution. Nevertheless, the retropeptoid was capable of forming supramolecular tapes, ribbons, and closed nanotubes via self-assembly, possibly driven by the correct side chain to carbonyl group orientation.

Acknowledgment

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

References and notes

1. (a) Westermark, P.; Wernstedt, C.; Wilander, E.; Sletten, K. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 827; (b) Cooper, G. J. S.; Willis, A. C.; Clark, A.; Turner, R. C.; Sim, R. B.; Reid, K. B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8628; (c) Höppener, J. W. M.; Ahrén, B.; Lips, C. J. M. *N. Engl. J. Med.* **2000**, *343*, 411; (d) Soto, C. *Nature Rev. Neurosci.* **2003**, *4*, 49.
2. Westermark, P.; Engström, U.; Johnson, K. H.; Westermark, G. T.; Betsholtz, C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5036.
3. Tjernberg, L. O.; Näslund, J.; Lindqvist, F.; Johansson, J.; Karlström, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1996**, *271*, 8545.
4. Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castaño, E. M.; Frangione, B. *Nat. Med.* **1998**, *4*, 822.
5. (a) Hughes, E.; Burke, R. M.; Doig, A. J. *J. Biol. Chem.* **2000**, *275*, 25109; (b) Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C. *Biochemistry* **2001**, *40*, 8237; (c) Yan, L.; Tatarek-Nossol, M.; Velkova, A.; Kazantzis, A.; Kapurniotu, A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2046.
6. (a) Rijkers, D. T. S.; Höppener, J. W. M.; Posthuma, G.; Lips, C. J. M.; Liskamp, R. M. J. *Chem. Eur. J.* **2002**, *8*, 4285; (b) Elgersma, R. C.; Meijneke, T.; Posthuma, G.; Rijkers, D. T. S.; Liskamp, R. M. J. *Chem. Eur. J.* **2006**, *12*, 3714.
7. Kapurniotu, A.; Schmauder, A.; Tenidis, K. *J. Mol. Biol.* **2002**, *315*, 339.
8. Elgersma, R. C.; Meijneke, T.; de Jong, R.; Brouwer, A. J.; Posthuma, G.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Biomol. Chem.* **2006**, *4*, 3587.
9. Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.
10. (a) Kruijtzer, J. A. W.; Nijenhuis, W. A. J.; Wanders, N.; Gispen, W. H.; Liskamp, R. M. J.; Adan, R. A. H. *J. Med. Chem.* **2005**, *48*, 4224; (b) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657; (c) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092; (d) Ruijtenbeek,

R.; Kruijtzer, J. A. W.; van de Wiel, W.; Fischer, M. J. E.; Flück, M.; Redegeld, F. A. M.; Liskamp, R. M. J.; Nijkamp, F. P. *Chem. Biol. Chem.* **2001**, *2*, 171; (e) Burkoth, T. S.; Beausoleil, E.; Kaur, S.; Tang, D.; Cohen, F. E.; Zuckermann, R. N. *Chem. Biol.* **2002**, *9*, 647.

11. (a) Kruijtzer, J. A. W.; Liskamp, R. M. J. *Tetrahedron Lett.* **1995**, *36*, 6969; (b) Kruijtzer, J. A. W.; Hofmeyer, L. J. F.; Heerma, W.; Versluis, C.; Liskamp, R. J. M. *Chem. Eur. J.* **1998**, *4*, 1570.
12. Fmoc–*N*Ile-OH (**6**) was purified using column chromatography (EtOAc/hexane/AcOH 50:50:1 → EtOAc/AcOH 100:1 v/v) fractions containing the pure product were collected and evaporated in vacuo followed by coevaporation with toluene to remove any residual AcOH. Compound **6** was obtained as a yellowish oil (1.29 g, 3.66 mmol, 54%). $[\alpha]_D^{20} + 1.5$ (*c* 1, $CHCl_3$); R_f (EtOAc/hexane/AcOH 60:40:1 v/v/v): 0.42; R_f (CH_2Cl_2/MeOH/AcOH 90:10:1 v/v/v): 0.40; the ^1H NMR spectrum clearly showed the presence of rotamers; δ_H (300 MHz, $CDCl_3$): 0.74–1.17 (m, 6H, $\gamma'CH_3$, δCH_3 Ile) 1.3–1.58 (m, 2H, γCH_2 Ile), 3.7–3.9 (m, 3H, βCH Ile, αCH_2), 4.1–4.3 (m, 1H, C*H* Fmoc), 4.4–4.6 (m, 2H, CH_2 Fmoc) 7.2–7.8 (m, 8H, Fmoc–C*H*); δ_c (75.5 MHz, $CDCl_3$): 175.9, 175.4, 156.7, 156.0, 143.9, 141.4, 127.6, 126.9, 125.2, 124.8, 119.9, 67.5, 53.6, 53.2, 47.3, 43.9, 43.1, 27.7, 27.4, 18.3, 18.0, 10.9; ESI-MS: calculated for $C_{21}H_{23}NO_4$: 353.42, found: 354.01 $[M+H]^+$.
13. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
14. Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.
15. *Peptide synthesis*. Peptoid **2** and retropeptoid **3** were synthesized on a 0.25 mmol scale on Tentagel Fmoc-Rink-Amide resin in order to obtain C-terminally amidated peptoids. The solid phase synthesis was carried out on an ABI 433A peptide synthesizer using the FastMoc protocol. Each synthetic cycle consisted of 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 preactivated peptoid building block, and a 6 min NMP wash. Peptoid building blocks were activated in situ with 1.0 mmol HATU/HOAt (0.36 M in NMP) in the presence of DIPEA (2.0 mmol). The peptoids were detached from the resin and deprotected by treatment with TFA/H_2O/TIS 95:2.5:2.5 v/v/v for 3 h. The peptoids were precipitated with MTBE-hexane 1:1 v/v at −20 °C and finally lyophilized form *tert*-butanol-H_2O 1:1 v/v.
16. *Purification and analysis*. The peptoids were purified by dissolving 50 mg crude material in a minimal amount of buffer A 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 using a linear gradient of buffer B (100% in 40 min) from 100% buffer A (buffer A: 0.1% TFA in CH_3CN/H_2O 5:95 v/v, buffer B: 0.1% TFA in CH_3CN/H_2O 95:5 v/v). The purity was determined 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 using a linear gradient of buffer B (100% in 20 min) from 100% buffer A (buffer A: 0.1% TFA in CH_3CN/H_2O 5:95 v/v; buffer B: 0.1% TFA in CH_3CN/H_2O 95:5 v/v). Compounds **1**–**3** and **6** were characterized by mass spectrometry. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Shimadzu LC MS QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode.
17. Ashburn, T. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 11012.