ChemMatrix, a Poly(ethylene glycol)-Based Support for the Solid-Phase Synthesis of Complex Peptides

Fayna García-Martín,[†] Martina Quintanar-Audelo,[†] Yésica García-Ramos,[†] Luis J. Cruz,[†] Catherine Gravel,[‡] Robert Furic,[‡] Simon Côté,[‡] Judit Tulla-Puche,*[,†] and Fernando Albericio*[,†,§]

Barcelona Biomedical Research Institute, Barcelona Science Park, University of Barcelona, Josep Samitier 1, 08028-Barcelona, Spain, Matrix Innovation Inc., 1450 City Councillors, Suite 230, Montreal, Québec, Canada, and Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1, 08028-Barcelona, Spain

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CM (ChemMatrix) resin is a new, totally poly(ethylene glycol) (PEG)-based resin, made exclusively from primary ether bonds and, therefore, highly chemically stable. It exhibits good loading and is user-friendly because of its free-flowing form upon drying. It performs excellently for the preparation of hydrophobic, highly structured, and poly-Arg peptides, as compared to polystyrene (PS) resins. In the most striking example, stepwise solid-phase assembly of the highly complex β-amyloid (1−42) peptide resulted in a crude material of 91% purity. In contrast, literature procedures using PS or PEG−PS-based resins for this peptide required convergent approaches, additional time-consuming steps, or both. In addition to the difficulties of its synthesis, characterization of the β-amyloid (1−42) peptide as a monomer is also a challenge, and methods for characterization by HPLC and MALDI-TOF have also been developed.

Introduction

Solid-phase synthesis[1,2] is currently the method of choice for peptide synthesis, in which a stepwise strategy is used for small and medium peptides, or a convergent strategy is used for large peptides.[3−10] This methodology has been also extended to the synthesis of small molecules.[11−13] The success of the solid-phase approach is closely tied to the performance of the solid support. Classically, polystyrene (PS)-based solid supports have been the most used;[14−16] however, PS is limited by its hydrophobicity, thus prompting the evaluation of more hydrophilic supports. On the basis of the early work of Mutter,[17] the first commercially available poly(ethylene glycol) (PEG)−PS resins were developed independently in the mid-1980s by Zalipsky, Albericio, and Barany (PEG−PS)[18−20] and Bayer and Rapp (TentaGel).[21,22] These systems can be synthesized either by reaction of preformed oligooxyethylenes with aminomethylated polystyrene beads (PEG−PS) or by graft polymerization on polystyrene beads (TentaGel). More recently, other PEG−PS-based resins have been developed and commercialized. For example, Hudson and co-workers[23] have developed Champion I and II (NovaGel), which are prepared from PEG linear blocks and from branched PEG chains, respectively. In addition, Argogel has been prepared by PEG attachment through branched diol supports.[24] The primary objective of grafting PEG onto PS was the combination of a hydrophobic PS core with hydrophilic PEG chains in the same support. Due to the unique conformational flexibility of PEG chains, PEG−PS resins are compatible with both polar and nonpolar solvents.[15]

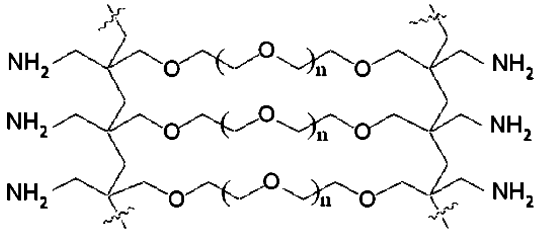

Figure 1. Chemical structure of aminomethyl CM resin.

More hydrophilic PEG-based resins were then developed, including those containing a small amount of polystyrene or polyamide, made by Meldal and co-workers,[15,25,26] or acrylate with polymerizable vinyl groups, in the work of Barany and Kempe.[27−29]

Finally, totally PEG-based resins were independently developed by Meldal and co-workers[30,31] and Côté.[32] The optimal properties of the PEG are due to the vicinal arrangements of carbon−oxygen bonds throughout the chain, which provoke that the PEG assumes helical structures with gauche interactions between the polarized bonds.[15] PEG can exhibit three different helical arrangements: the first, largely hydrophobic; the second, of intermediate hydrophobicity; and the third, hydrophilic. The amphiphilic nature of PEG makes the resin well-solvated in both polar and nonpolar solvents.[15]

Herein is described the first report on the use of a total PEG-based resin (CM resin, Figure 1) for the synthesis of complex peptides. The peptides chosen as models were an

* To whom correspondence should be addressed. E-mail: albericio@pcb.ub.es, jtulla@pcb.ub.es.

[†] Barcelona Biomedical Research Institute, Barcelona Science Park, University of Barcelona.

[‡] Matrix Innovation Inc.

[§] Department of Organic Chemistry, University of Barcelona.

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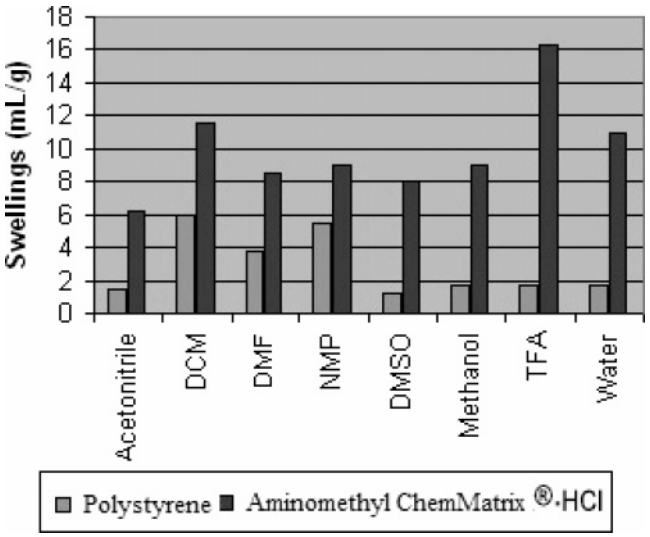

Figure 2. Swelling of polystyrene and CM resins in different solvents.

artificial decameric model of repetitive sequence; a synthetic vaccine of 38 residues; a complex polyarginine containing peptide with 21 residues; and the well-known, synthetically challenging β-amyloid (1−42) peptide.

Results and Discussion

Physical Properties. CM resin, which is a 100% PEG-based resin (Figure 1),[32] exhibits loadings comparable to PS resins (up to 1.0 mmol/g). The resin becomes free-flowing upon drying, thus facilitating its weighing and resulting in minimal loss during transfer. Furthermore, it is easily filtered and washed with almost any solvent. For the final step in preparing the resin for a synthesis, the resin is preferably washed with methanol (or CH_2Cl_2) then directly transferred to the reaction vessel to be dried under vacuum overnight.

CM resin swells well in almost any kind of solvent (Figure 2), even in polar solvents such as CH_3CN, DMSO or MeOH, which do not swell PS resins. CM resin swells better than PS in DMF and CH_2Cl_2, which are the most appropriate solvent for PS. Other solvents commonly used for PS resin, such as THF, are also very compatible with CM resin, which also swells in aqueous buffers, allowing the preparation and on-resin screening of libraries.[33]

CM is chemically stable to a broad range of solvents, including acids and bases, with the exception of strong Lewis acids. The broad compatibility of CM was demonstrated in IR studies of the resin after overnight treatment in the corresponding solvent at room temperature (Figure 3).

When CM resin is successively shrunk, swelled, and reshrunk by using different types of solvents, no notable deterioration of the beads is observed by microscopy. This indicates that in contrast to other resins, which normally fracture during similar treatments, CM resin is not affected by osmotic shock.

Comparison of CM and Polystyrene Resins. The pioneering work of Deber and Felix,[34] Kent,[35] Merrifield,[36] and Sheppard[37−40] led to the realization that certain peptide sequences, including highly hydrophobic structures, are exceedingly difficult to synthesize. The observed problems have been attributed to peptide aggregation and poor resin solvation, as well as possible association of the peptide with the resin matrix.[37] The performance of CM and polystyrene resins in the preparation of a set of synthetically challenging peptides was compared. The set comprised a decameric peptide of repetitive sequence, β-amyloid (1−42), and two

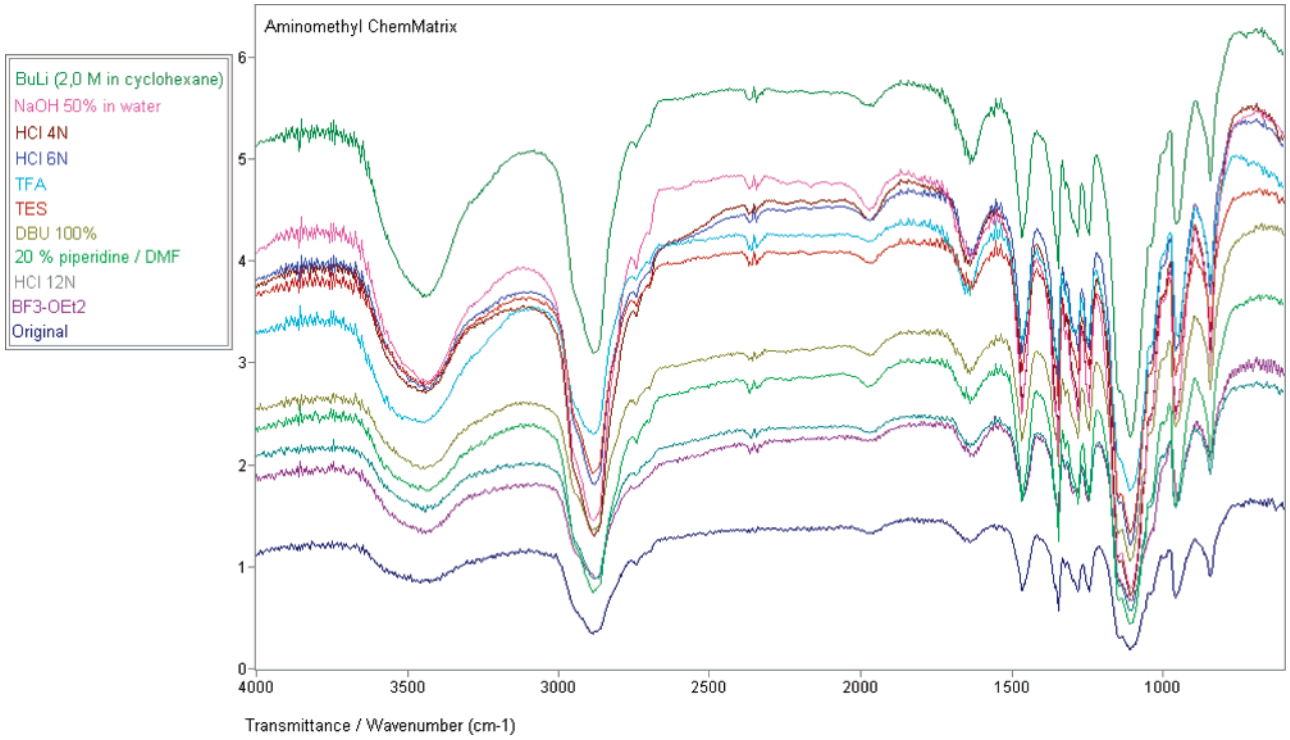

Figure 3. IR spectra of aminomethyl CM resin taken in a KBr mixture: (a) BuLi (2.0 M solution in cyclohexane); (b) NaOH, 50% in water; (c) HCl, 4 N; (d) HCl, 6 N; (e) TFA; (f) TES; (g) DBU, 100%; (h) piperidine−DMF (1:4); (i) HCl, 12 N; (j) $BF_3 \cdot OEt_2$, 100%; (k) untreated aminomethyl CM resin.

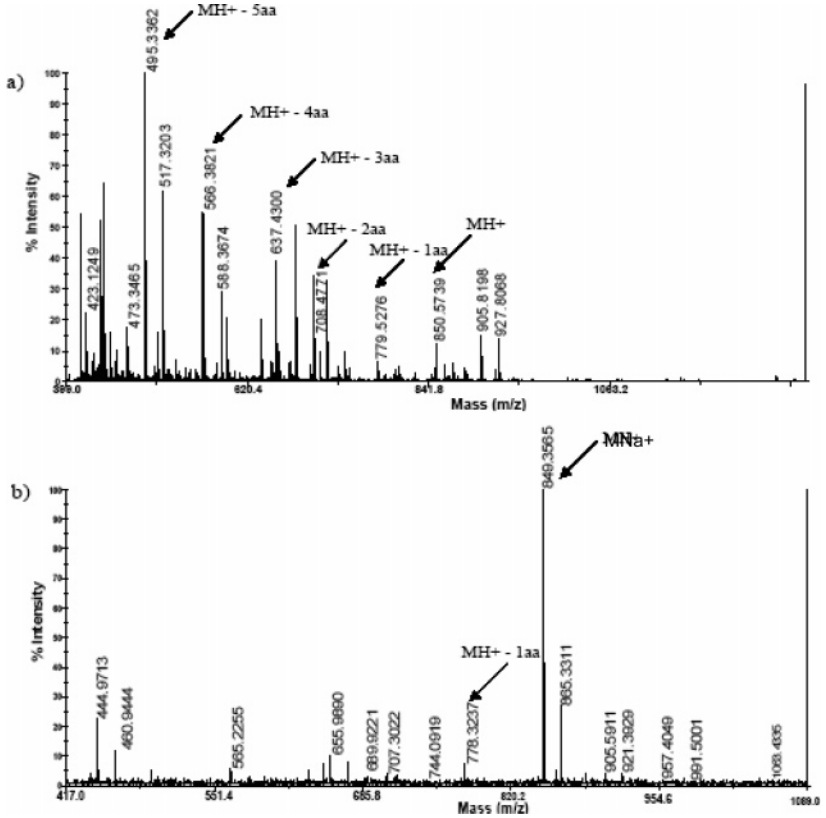

Figure 4. MALDI-TOF of a decameric model of repetitive sequence on (a) polystyrene resin and (b) CM resin.

peptides containing difficult sequences from our own research programs.

Synthesis of the Decameric Model Peptide of Repetitive Sequence. Oligo(aminoacyl) sequences are known to pose synthesis complications, including slow coupling rates as well as incomplete deprotections that typically start after the addition of the third residue and are most pronounced between the fifth and eighth residues.[36,41−49] For syntheses involving Fmoc chemistry, the final products include families of oligomers and Fmoc-protected oligomers. A number of approaches, including changes in reaction times, reagents, solvents, and the addition of chaotropes, have been explored to optimize the deprotection and the coupling steps. More substantial improvements have been made by altering the type of solid support used.[37,41,47,48] For the case at hand, MALDI-TOF spectra reveal that CM resin afforded a very clean crude product (Figure 4b), as compared to that obtained with PS resin (Figure 4a), for which the major product was the pentapeptide, and the desired product was present only in minor amounts.

Synthesis of Bacuma. Bacuma is a 38-amino acid that is currently under development as a synthetic vaccine. In addition to its large size, its sequence is also challenging due to steric hindrance of protecting groups used for consecutive positions in the sequences such as QNWT and KKQYIK. Furthermore, other fragments also contain hydrophobic sequences, such as LAFL and FIGI. All of these factors contribute to the difficulty encountered in our laboratory upon attempting the solid-phase synthesis of Bacuma. Three syntheses have been performed on different supports. The first synthesis, carried out with Fmoc-Rink-MBHA resin, was stopped after 27 cycles because the purity at that stage of a sample of the intermediate peptide obtained after TFA cleavage was <8% according to HPLC/MS (Figure 5a). HPLC/MS analysis of material corresponding to a second synthesis, carried out on CTC resin, showed that the target peptide was not present and that the synthesis could not proceed further than cycle 30 (eight residues before the final) (Figure 5b). On the other hand, a synthesis carried out on Fmoc-Rink-CM resin generated crude material of remarkable purity for a peptide of this size, as shown by HPLC and MALDI-TOF (Figure 5c).

Synthesis of a Polyarginine Peptide. This peptide contains three consecutive Arg's at its N terminal. It is well-known that Arg-containing peptides are difficult to synthesize due to the high steric hindrance of the Pbf group used for Arg side-chain protection and, more importantly, the low coupling yield that results from δ-lactam formation.[3] Figure 6a shows that, although the desired product could be obtained via synthesis on Fmoc-Rink-MBHA resin, it was present only in minor amounts and was accompanied by many deletion peptides having masses that correspond to the absence of two and three Arg residues (Figure 6a). However, using Fmoc-Rink-CM resin, these deletions were not detected, and only one major peak, corresponding to the desired product, was obtained (Figure 6b).

Synthesis of β-Amyloid (1−42). β-Amyloid (1−42) is prone to aggregation, and the complications implied in its synthesis and characterization are well-documented. The assembly of this peptide or fragments of it has been attempted both in solution[50] and on solid phase using polystyrene,[51,52] Tentagel,[53] PEG−PS,[53,54] and Pepsyn K[55] as solid supports and using both Boc[50,51] and Fmoc[52−55] chemistries. Several strategies have been studied to overcome the difficulties of

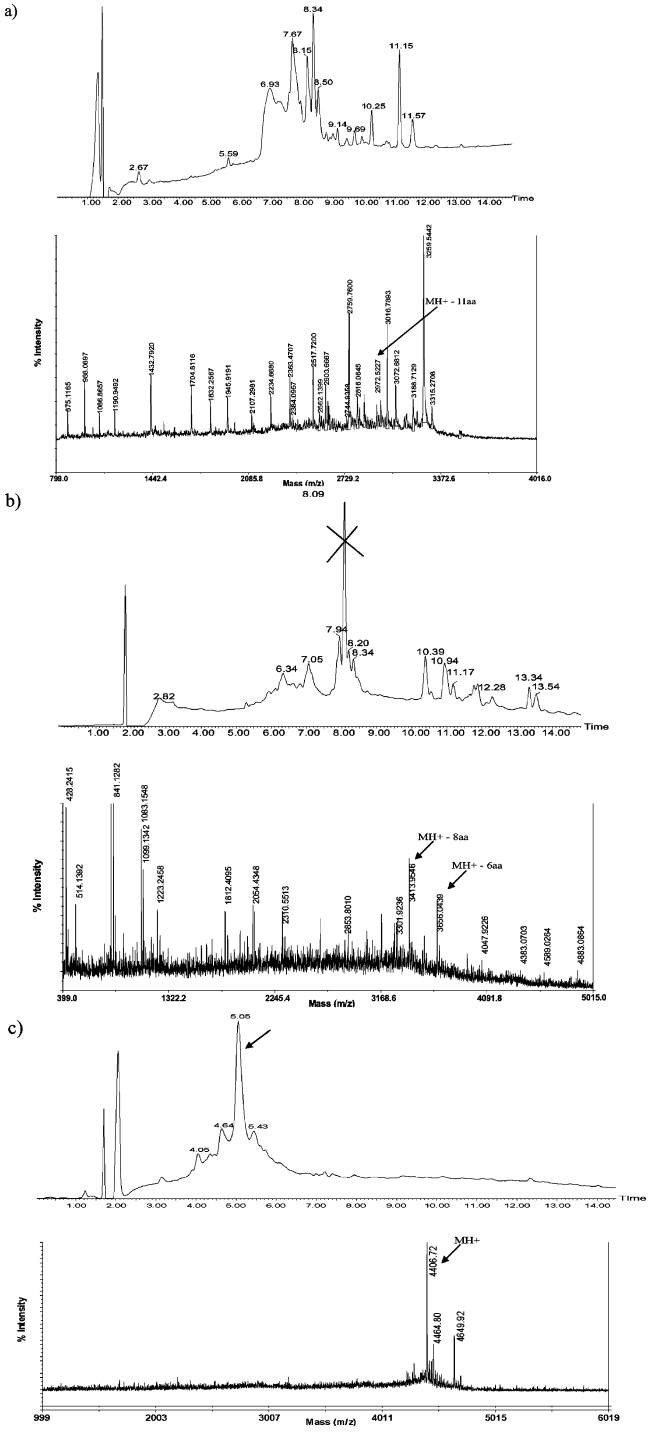

Figure 5. MALDI-TOF and HPLC at 220 nm of Bacuma on (a) Rink-MBHA resin, (b) CTC resin, and (c) CM resin. HPLC conditions: C-18 column; linear gradient 0.1% aqueous TFA/0.1% TFA in CH_3CN, from 7:3 to 1:4 over 15 min; flow rate of 1.0 mL/min.

this synthesis, such as the introduction of an oxidized Met-35 to disrupt hydrophobic interactions and the use of DMSO as a coupling cosolvent;[51] the use of DBU (1,8-diazabicyclo-[5,4,0]undec-7-en) to ensure complete Fmoc deprotection;[53] the introduction of Hmb (2-hydroxy-4-methoxybenzyl) backbone amide protection;[55] and finally, the use of an O−N intramolecular acyl migration reaction of the corresponding *O*-acyl isopeptide.[52,56] Synthesis of the β-amyloid (1−42) peptide using CM resin was carried out stepwise on an

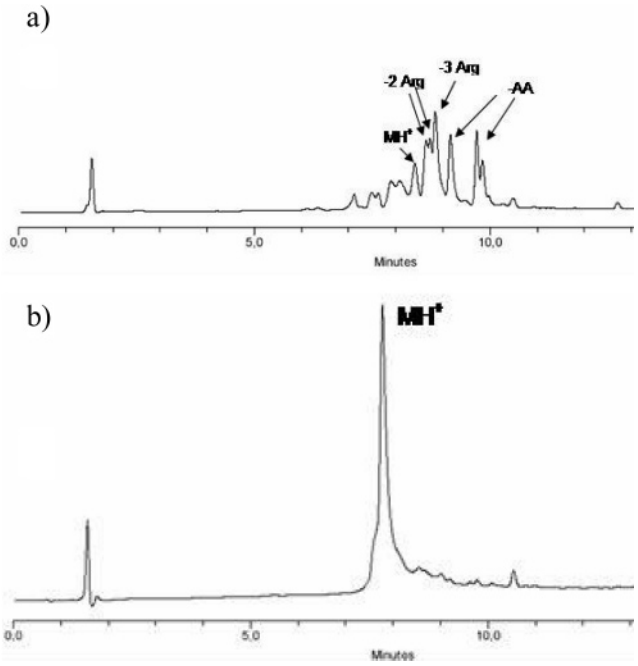

Figure 6. HPLC at 220 nm of Polyarginine peptide on (a) polystyrene resin and (b) CM resin. HPLC conditions: C-18 column, with a linear gradient of 0.1% aqueous TFA/0.1% TFA in CH_3CN, from 9:1 to 1:4 over 15 min; flow rate of 1.0 mL/min.

automatic synthesizer without apparent difficulties. Characterization of the peptide by HPLC and MALDI-TOF was the most difficult part, and new methods of sample preparation had to be developed. For MALDI-TOF, the matrix usually used is a solution of CHCA (α-cyano-4-hydroxycinnamic acid) in a 1:1 mixture of H_2O-CH_3CN in 0.1% TFA. However, under these conditions, the peptide aggregated and was not observed by MALDI-TOF. Thus, matrixes containing a greater concentration of TFA (1−50%) were assayed. Under these conditions, the peptide was clearly observed, especially at TFA concentrations of 10 and 30%. As shown in Figure 7a, it appears as the only peptide with masses higher than 2000 Da. In a first attempt to obtain an HPLC chromatogram of the β-amyloid (1−42) peptide using standard methodology, a sample of the peptide was dissolved in CH_3CN and injected into the HPLC, but no peak was observed. Next, a sample of the peptide was dissolved in DMSO,[31] but several peaks corresponding to the respective oligomers (dimer, trimer, and tetramer) were observed. Finally, a sample of the peptide was dissolved in HFIP and was directly injected into the HPLC. A single peak of 91% purity was obtained (Figure 7b), which was collected and characterized by MALDI-TOF to give the mass of the desired peptide. From these results, it can be concluded that using CM resin for β-amyloid synthesis circumvents the problem of peptide aggregation that normally arises when other resins are used, allowing synthesis without the need for new strategies or methods.

Conclusions

PEG-based resins such as CM offer a tremendous advantage over polystyrene resins for a broad range of applications. Furthermore, CM resin has all the advantages of PEG resins but without their loading limitations and, in some cases, poor

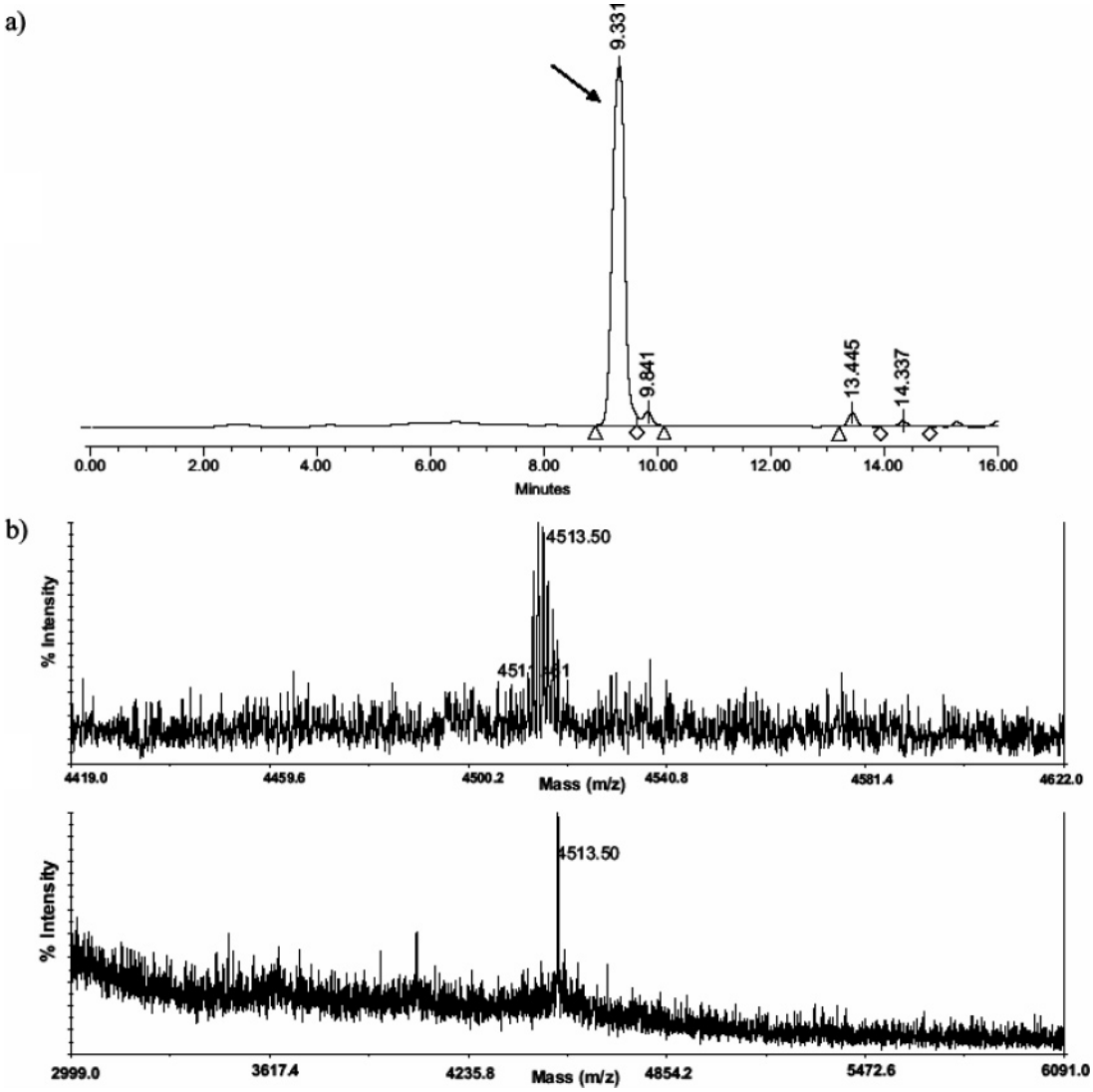

Figure 7. β-Amyloid peptide (1−42): (a) HPLC chromatogram of the crude β-amyloid dissolved in HFIP. HPLC conditions: C-8 column, with a linear gradient of 0.1% aqueous TFA/0.1% TFA in CH_3CN, from 4:1 to 2:3 over 15 min at 60 °C; flow rate of 1.0 mL/min. The title peptide is the main peak (t_R = 9.3 min); (b) MALDI-TOF of the crude.

chemical stability. Furthermore, due to its highly cross-linked matrix, it has unsurpassed mechanical stability. CM resin swells extremely well in all of the most common solvents and is, therefore, suitable for a broad range of organic chemistries. In addition, its compatibility with aqueous buffers allows its use for biochemical applications, such as on-resin screening of chemical libraries and in the development of affinity chromatography.[33] CM resin has performed extremely well, as compared to PS resins, in the solid-phase synthesis of hydrophobic, highly structured, and poly-Arg peptides. The positive results obtained for the synthesis of the β-amyloid (1−42) peptide should facilitate its production on a large scale for research on aberrant protein aggregation.

Experimental Section

General. Aminomethyl CM resin was from Matrix Innovation, Canada. IR spectra were run with a KBr pellet. HPLC was performed on a reversed-phase C_{18} column (4.6 × 150 mm, 5 μm), unless indicated otherwise. Linear gradients of 0.045% TFA and 0.036% in ACN were run at a flow rate of 1.0 mL/min. HPLC/MS was performed on a reversed-phase C_{18} column (3.9 × 150 mm, 5 μm) using aqueous (0.1%) formic acid and formic acid (0.07%) in CH_3CN as eluents.

Swelling Experiments. Resins (200 mg) were placed in a 3-mL syringe equipped with a 0.45-μm filter, treated with enough solvent to swell the resin, and allowed to stand for 5 min. The swollen resin was compressed with the piston until no more solvent could be extracted. The piston was pulled slowly until the resin recuperated its maximum volume in the syringe, and the volume of the resin was read (the void volume of the tip and the syringe was averaged to 0.15 mL). The swelling was calculated according to the following formula:

$$(\text{volume of the swelled resin} + 0.15\ \text{mL})/0.2\ \text{g} = x\ (\text{mL/g})$$

A deviation of <10% was noted for each solvent and for each resin.

Peptide Synthesis. 1. Automatic Synthesis. The peptide chains were elongated by means of an ABI 433A peptide synthesizer (Applied Biosystems, Foster City) employing standard Fmoc chemistry and 0.25 mmol FastMoc protocol.

Syntheses were conducted on a 0.1-mmol scale with a 10-fold excess of Fmoc-protected L-amino acids and 0.45 M HBTU, TBTU, or HCTU in the presence of HOBt in DMF as coupling reagents. Syntheses were carried out with a 15-min deprotection step with piperidine−DMF (1:4) and a 35-min coupling. After the assembly was complete, the peptide−resin was washed with CH_2Cl_2. Cleavage was performed as indicated in each section. Before liophylization, peptides were precipitated by adding cold *tert*-butylmethyl ether, the solution was decanted, and the solid was triturated with cold *tert*-butylmethyl ether, which was again decanted. This process was repeated twice.

2. Manual Synthesis. Manual solid-phase peptide elongation and other solid-phase manipulations were carried out in polypropylene syringes fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Fmoc removal was carried out with piperidine−DMF (1:4) (1 × 1 min, 2 × 10 min). Washings between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) using 10 mL of solvent/g of resin each time.

3. Previous Washings of CM Resin. Aminomethyl CM resin was washed before use as follows: MeOH (2 × 1 min), DMF (2 × 1 min), CH_2Cl_2 (3 × 1 min), TFA−CH_2Cl_2 (1:99) (3 × 1 min), DIEA−CH_2Cl_2 (1:19) (3 × 1 min), and CH_2Cl_2 (3 × 1 min).

Synthesis of a Decameric Model Peptide of Repetitive Sequence. 1. CTC Resin. The first amino acid was coupled manually as follows: CTC (0.1 mmol, 1.4 mmol/g) was washed with CH_2Cl_2 (3 × 1 min) and DMF (3 × 1 min) and swelled in CH_2Cl_2 for 15 min. Fmoc-aa-OH (4 equiv) and DIEA (5 equiv) were sequentially added to the resin, and the mixture was allowed to react for 90 min. Next, a capping step with MeOH (60 μL) was carried out. The rest of the synthesis was carried out automatically using HCTU as coupling reagent and DIEA as base. The complete peptide was cleaved from the support by using TFA−CH_2Cl_2 (1:99) (5 × 0.5 min). Characterization was made by MALDI-TOF (*m*/*z* calcd for $C_{35}H_{61}N_{11}O_{12}$, 826.95; found, 850.57 $[M + Na]^+$, 779.53 $[(M - aa) + Na]^+$, 708.48 $[(M - 2aa) + Na]^+$), 637.43 $[(M - 3aa) + Na]^+$, 566.38 $[(M - 4aa) + Na]^+$, 495.34 $[(M - 5aa) + Na]^+$).

2. CM Resin. The process was identical as before but using aminomethyl CM resin (0.1 mmol, 0.4 mmol/g) as solid support. Fmoc-Rink linker was incorporated with PyAOP−HOAt−DIEA (3:3:3:6). The first amino acid was incorporated manually using PyBOP−HOAt−DIEA (3:3:3:6) for 3 h, followed by an acetylation step. Synthesis was continued automatically using HCTU and DIEA as the coupling system. Cleavage was performed with TFA−TIS−H_2O (95:2.5:2.5) for 90 min. The peptide was characterized by MALDI-TOF (*m*/*z* calcd for $C_{35}H_{62}N_{12}O_{11}$, 826.95; found, 849.36 $[M + Na]^+$).

Synthesis of Bacuma Peptide. 1. Polystyrene. The first manual attempt at the synthesis of Bacuma was made on Fmoc-Rink-MBHA resin (1.08 mmol, 0.72 mmol/g). The coupling reactions were carried out with Fmoc-amino acids (4 equiv), HCTU (4 equiv), HOAt (4 equiv), and DIEA (12 equiv). After 27 cycles, a sample was taken and treated with TFA−TIS−H_2O (95:2.5:2.5). After the corresponding work-up, HPLC/MS analysis showed that the desired product was only a minor component of a complex mixture (t_R = 8.20 min, purity < 8%), and MALDI-TOF results revealed an incorrect mass (*m*/*z* calcd for $C_{131}H_{216}N_{36}O_{39}S$, 2949.57 found, 2972.48 $[M + Na]^+$). The synthesis was thus abandoned.

An automatic synthesis of Bacuma was carried out on CTC resin (0.1 mmol, 1.11 mmol/g). The first amino acid (4 equiv) was coupled manually using DIEA (12 equiv) in CH_2Cl_2. A capping step was carried out using MeOH and DIEA before Fmoc removal. The synthesis was continued in an automatic fashion using HCTU and DIEA as coupling system. Cleavage was performed with TFA−TIS−H_2O (95:2.5:2.5), followed by ether precipitation as explained in the general section. HPLC/MS analysis did not indicate the target peptide, and the synthesis was stopped after cycle 30 (eight residues before the final). MALDI-TOF (*m*/*z* calcd for $C_{201}H_{322}N_{50}O_{52}S_3$, 4363.34; *m*/*z* calcd for Bacuma − 6aa, $C_{169}H_{277}N_{41}O_{42}S_2$, 3619.44; found, 3658.02 $[M - 6aa + K]^+$; *m*/*z* calcd for Bacuma − 8aa $C_{160}H_{262}N_{38}O_{39}S$, 3374.14; found, 3414.96 $[M - 6aa + K]^+$).

2. CM Resin. The synthesis was identical to that described above, but using aminomethyl CM resin (0.1 mmol, 0.4 mmol/g). The linker was coupled manually using Fmoc-Rink linker (3 equiv), PyBOP (3 equiv), HOAt (3 equiv), and DIEA (9 equiv) in DMF. Before Fmoc removal, an acetylation step was performed to block any possible free amino groups. The first amino acid was coupled manually, using HCTU (3 equiv), HOAt (3 equiv), and DIEA (9 equiv). The rest of the synthesis was carried out on the synthesizer, using HCTU and DIEA as the coupling system. Before cleavage, the resin was acetylated [Ac_2O−DIEA−DMF (10:5:85)] for 70 min. Cleavage was carried out using TFA−TIS−H_2O (95:2.5:2.5) for 90 min. The peptide was characterized by analytical HPLC (t_R = 12.19 min, 52.33%) and by HPLC/MS and MALDI-TOF (*m*/*z* calcd for $C_{203}H_{324}N_{50}O_{53}S_3$, 4405.35; found, 4406.72 $[M + H]^+$).

Synthesis of a Poly-Arginine Peptide. 1. Polystyrene Resin. Fmoc-Rink AM linker (3 equiv) was coupled on MBHA resin (0.15 g, 0.68 mmol/g). Peptide chain elongation was carried out on an automatic synthesizer, using TBTU as coupling reagent in the presence of HOBt. Cleavage was performed with TFA−TIS−H_2O (95:2.5:2.5) for 3 h to ensure complete removal of the various Pbf protecting groups. The peptide was characterized by HPLC (t_R = 7.90 min, 6%) and HPLC/MS (*m*/*z* calcd for $C_{35}H_{63}N_{13}O_{10}$, 2590.42; found, 1296.1 $[M + H]^+/2$, 864.6, $[M + H]^+/3$, 648.8 $[M + H]^+/4$, 519.4 $[M + H]^+/5$).

2. CM Resin. The process was carried out as in the case of polystyrene resin, but using aminomethyl CM resin (0.22 g, 0.45 mmol/g) having been previously incorporated with Fmoc-Rink AM linker using HOBt and DIPCDI (3 equiv) in DMF. Characterization was made using HPLC (t_R = 7.80 min, 68%) and HPLC/MS (*m*/*z* calcd for $C_{35}H_{63}N_{13}O_{10}$, 2590.42; found, 1297.1 $[M + H]^+/2$, 865.1, $[M + H]^+/3$, 649.1 $[M + H]^+/4$, 519.6 $[M + H]^+/5$).

Synthesis of the β-Amyloid (1−42) Peptide on CM Resin. The synthesis was carried out automatically except

for the incorporation of both the linker and the first amino acid, which were coupled manually on CM resin (0.12 g, 0.58 mmol/g resin). AB linker was incorporated with HBTU−HOBt−DIEA−DMF (3:3:3:9) for 90 min. The first amino acid was coupled to the handle by formation of an ester bond by means of Fmoc-L-Ala-OH−DIPCDI−HOAt−DMAP (5:5:5:0.5) in CH_2Cl_2 overnight at room temperature.[57] The 4(4-nitrobenzyl) pyridine test for alcohol determination[58,59] indicated free hydroxyl groups were still present, and a 2-h recoupling step was necessary. Next, acetylation of the N-terminal group was carried out using Ac_2O−DIEA (50 equiv each) in DMF for 15 min. The rest of the chain assembly was carried out automatically. Cleavage was performed with TFA−TIS−H_2O−EDT (95:2:2:1) for 90 min. After lyophilization, a modification of the disaggregating protocol developed by Zagorski et al.[60] was applied. This method consists of a first stage in which the peptide is dissolved in neat TFA to obtain the monomeric form. In a second stage, the acid is removed by evaporation, dissolved in HFIP so as to maintain the peptide as a monomer, and evaporated. This last step was repeated twice more, and an aliquot dissolved in HFIP was injected into the HPLC. Characterization by HPLC (t_R = 9.33 min, 91%) and MALDI-TOF (*m*/*z* calcd for $C_{203}H_{312}N_{55}O_{60}S_1$, 4512.28; found, 4513.50 $[M + H]^+$) confirmed the desired product.

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References and Notes

(1) Preliminary reports of portions of this work were presented at the 19th American Peptide Symposium, June 2005, La Jolla (CA).

(2) Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1982**, *247*, 977−983. The following additional abbreviations are used: AB linker, 3-(4-hydroxymethylphenoxy) propionic acid; Ac_2O, acetic anhydride; Boc, *tert*-butyloxycarbonyl; CHCA, α-cyano-4-hydroxycinnamic acid; CH_3CN, acetonitrile; CM, ChemMatrix; CTC, chlorotrityl chloride (Barlos) resin; DBU, 1,8-diazabicyclo[5,4,0]undec-7-en; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; EDT, 1,2-ethanedithiol; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 1-[bis(dimethylamino)methylene]-1H-benzotriazolium hexafluorophosphate 3-oxide; HCTU, 1-[bis(dimethylamino)methylene]-6-chloro-1*H*-benzotriazolium hexafluorophosphate 3-oxide; HOAt, 1-hydroxy-7-azabenzotriazole(3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine); HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MALDI, matrix-assisted laser desorption ionization; MBHA, *p*-methylbenzydrylamine; MeOH, methanol; MS, mass spectrometry; PEG, poly(ethylene glycol); PS, polystyrene solid support; PyAOP, 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)-phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-oxytris (pyrrolidino) phosphonium hexafluorophosphate; TBTU, 1-[bis-(dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide; TFA, trifluoroacetic acid; TIS, triisopropylsilane; TOF, time-of-flight. Amino acid symbols denote L-configuration unless indicated otherwise. All reported solvent ratios are expressed as v/v, unless otherwise stated.

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