A Convergent Synthesis of the Amyloid Protein of Alzheimer's Disease

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The presence in the hippocampus of extracellular proteinaceous deposits known as amyloid plaques is a characteristic of Alzheimer's disease (AD).[1-3] The βA4 protein (Figure 1), which is the primary constituent of the AD amyloid plaque, is very difficult to solubilize and purify.[4,5] We report herein a chemical synthesis of this protein by a fragment coupling strategy which was designed to allow the purification of the synthetic product and to be adaptable to a large scale. The convergence of this route also makes it practical to synthesize isotope-labeled analogs that are required for structural studies.[6-8]

Stepwise solid-phase peptide synthesis suffers from the inherent problem that amino acid deletion impurities accumulate during the synthesis and are extremely difficult to remove from the desired product.[9,14] This problem is especially worrisome in the case of insoluble proteins such as βA4. Two of the three reports of the stepwise solid-phase synthesis of βA4[10-12] state that the crude synthetic material could not be purified, or its purity analyzed, by reverse-phase high-performance liquid chromatography (RPHPLC).[10,12] The convergent solid-phase fragment coupling approach[13-17] combines the practicality of the solid-phase method with the advantages of a solution-phase fragment coupling strategy.[18] The deletion impurities are removed at an early stage by RPHPLC purification of the protected peptide fragments. Consequently, a high-resolution final purification step is not required.

The protected peptide fragments **1**-**4** (Figures 2 and 3) (the standard one-letter amino acid codes are used: A(Ala), D(Asp), E(Glu), F(Phe), G(Gly), H(His), I(Ile), K(Lys), L(Leu), M(Met), N(Asn), Q(Gln), R(Arg), S(Ser), V(Val), Y(Tyr). Protected amino acids are designated as X'. The following protected amino acids were used: E(Bzl), K(Cl-Z), R(Mts), H(Bom), Y(2,6-Cl_2-Bzl), S(Bzl). D1 was protected as the *tert*-butyl ester, and D7 and D23 were protected as the cyclohexyl esters) were synthesized on the Kaiser oxime resin, purified by RPHPLC, and characterized by amino acid analysis, ^{1}H NMR, and fast atom bombardment mass spectrometry (FABMS).[13,14,19,20] The sequences of protected fragments **1** and **3** were determined by tandem FAB-collision induced dissociation MS.[21] Fragment **4** was deprotected[22] and sequenced by Edman degradation. Fragment **5** was assembled on the Kaiser resin by stepwise coupling of residues 10-17,[23] followed by coupling of fragment **1** (1.2 equiv) using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) activation (Figure 2).[13] The resin-bound product was cleaved[15,16] to provide **5**, which was purified

H_2N-D^{1}AEFRHDSG^{9}YEVHHQKL^{17}VFFAEDVG^{25}SNKGAIIG^{33}LMVGGVVIA^{42}-CO_2H

Figure 1.

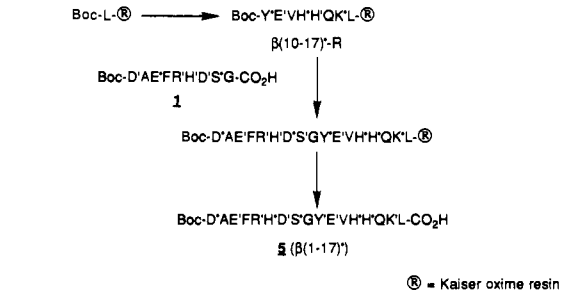

Figure 2.

Figure 3.

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(20) Fragment **2**: amino acid analysis L 1.5 (1 calcd), M 1.0 (1), V 1.0 (1), G 1.0 (1); FABMS (calcd 518.2) 519.3 (M + H)$^+$, 541.3 (M + Na)$^+$; ^{1}H NMR (DMSO) δ 8.32 (t, 1 H, *J* = 6, GlyNH), 8.05 (m, 1 H, MetNH), 7.8 (d, 1 H, *J* = 9, ValNH), 7.09 (d, 1 H, *J* = 9, LeuNH), 4.5 (m, 1 H, MetαH), 4.25 (dd, 1 H, *J* = 6, 9, ValαH), 4.0 (m, 1 H, LeuαH), 3.85 (dd, 1 H, *J* = 6, 18, GlyαH), 3.75 (dd, 1 H, *J* = 6, 18, GlyαH), 2.5 (m, 2 H, Metγ H), 2.09 (s, 3 H, $MetSCH_3$), 2.02 (m, 2 H), 1.85 (m, 2 H), 1.75 (m, 1 H, Valβ H), 1.45 (s, 9 H, Boc), 0.92 (m, 12 H, Val and Leu CH_3); tandem MS-MS[21] confirms sequence. Fragment **5**: amino acid analysis D 1.9 (2), E/Q 3.0 (3), S 0.9 (1), G 1.1 (1), H 2.6 (3), R 1.0 (1), A 1.0 (1), Y 0.8 (1), L 1.0 (1), F 1.0 (1), K 0.9 (1); plasma desorption MS (PDMS) (calcd 3442.5) 3445.9 (M + H)$^+$; Edman degradation of deprotected β1-17[22] confirmed sequence.

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(23) All couplings were double couplings with acetylation (10 equiv of acetic anhydride) performed after the second coupling. β(10-17)'-Ⓡ (Figure 2) was cleaved[22] and analyzed by RPHPLC in order to verify its purity (>95%). The purified peptide β(10-17)' was characterized by PDMS [(calcd 1808.7) 1811.3 (M + H)$^+$, 833.3 (M + Na)$^+$] and ^{1}H NMR (DMSO, amide protons and other assigned resonances only): δ 9.14 (s, 1 H, His(Bom)2H), 9.12 (s, 1 H, His(Bom)2H), 8.44 (m, 2 H), 8.32 (d, 1 H, *J* = 7.5), 8.12 (d, 1 H, *J* = 9.0), 8.04 (m, 2 H), 7.73 (d, 1 H, *J* = 8.6), 7.18 (d, 2 H, *J* = 9.6), 6.95 (s, 1 H, His(Bom)4H), 6.94 (d, 1 H, *J* = 10.3, Tyr(Cl_2Bzl)NH), 6.85 (s, 1 H, His(Bom)4H), 5.67 (s, 2 H, His(Bom)), 5.66 (s, 2 H, His(Bom)), 5.15 (s, 2 H, Tyr(Cl_2Bzl)), 5.06 (s, 4 H, Glu(Bzl) and Lys(Cl-Z)), 4.55 (s, 2 H, His(Bom)), 4.53 (s, 2 H, His(Bom)), 1.27 (s, 9 H, Boc), 0.83 (d, 3 H, *J* = 7.2, Val or Leu CH_3), 0.79 (d, 3 H, *J* = 7.2, Val or Leu CH_3), 0.75 (d, 6 H, *J* = 7.2, Val or Leu CH_3's).

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by RPHPLC.[20] The yield of **5** based on fragment **1** was 59%. We have purified ca. 1 g of fragment **5** by RPHPLC.

The C-terminal five amino acids of the βA4 protein were coupled on the Merrifield resin in a stepwise manner.[24] Sequential couplings of fragments **2**–**4** were performed using our standard conditions (1.2–1.5 equiv of fragment, BOP activation, 23 °C)[13] in yields of >95%, 70–90%, and 80–90%, respectively.[25] The coupling of **5** to the resin-bound 25-mer β(18–42)′-Ⓡ was accomplished in 55–85% yield via four successive room-temperature coupling reactions, each using a progressively smaller amount of fragment **5** (2.3 equiv total) (coupling of the protected 25-mer β(1–25)′[13] to β(26–42)′-Ⓡ was unsuccessful). This final coupling is the only one in the synthesis in which epimerization at the C-terminus of the soluble fragment is possible. Model studies suggest that approximately 8% of the purified product is the diastereomer of βA4 containing D-Leu at position 17.[14,26] Epimerization can be reduced by running the final coupling at 4 °C.[14,26]

The synthetic product was cleaved from the resin and deprotected using HF.[27,28] The crude product contained three major components: βA4, β18–42, and β26–42. This mixture produced a broad, unresolved peak on RPHPLC, supporting the proposal that the C-terminus of βA4 determines its solubility[19] and demonstrating the difficulty of separating deletion impurities which would be produced in a stepwise synthesis.[14] However, the low molecular weight impurities from this fragment synthesis (β18–42 and β26–42) were completely removed by gel-filtration HPLC (Waters μ-styragel-HT) in hexafluoroisopropyl alcohol. The recovery of material from this column was quite high. The gel-purified material was then rechromatographed by RPHPLC to remove minor side products, including β1–37 (a small amount (ca. 5%) was present in the crude peptide) and benzylated βA4 which was, in some cases, produced during the deprotection step.[27,28] Purified synthetic βA4 (purity >90% by MS) was characterized by laser desorption MS and amino acid analysis. The sequence was confirmed by Edman degradation.[29]

Operationally, the synthesis of βA4 in our laboratory involves eight coupling reactions: four amino acid couplings followed by four fragment couplings. The overall chemical yield is 42% from the C-terminal resin-bound amino acid as compared to ca. 66% for an efficient (99% per coupling) stepwise synthesis. However, the material synthesized by the fragment coupling approach can be easily purified because it is devoid of deletion impurities. The economy of this convergent strategy makes it possible to produce multimilligram quantities of isotope-labeled analogs and sequence analogs of the βA4 protein for studies of the structure of AD amyloid plaque.

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(24) β(38–42)′-Ⓡ (Ⓡ = Merrifield resin, Figure 3) was synthesized by double couplings with acetylation after the second coupling. The peptide was cleaved[27,28] and analyzed by FABMS (β38–42 (calcd MW = 457.2) 458.2 (M + H)$^+$) and amino acid analysis (G 1.0 (1), V 2.1 (2), I 1.2 (1), A 1.0 (1)) to verify its composition and purity (>95%). β(26–42)′-Ⓡ was cleaved, deprotected, and sequenced by Edman degradation.

(25) Fragment coupling yields were determined by amino acid analysis and quantitative ninhydrin analysis of the resin-bound product.

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(29) Characterization of synthetic βA4: laser desorption MS (MW = 4514.1) 4515.0 (M + H)$^+$; amino acid analysis D/N 4.1 (4 calcd), E/Q 3.9 (4), S 1.9 (2), G 6.3 (6), H 2.4 (3), R 0.8 (1), A 3.9 (4), Y 0.8 (1), V 4.2 (6), M 1.1 (1), I 2.0 (3), L 2.0 (2), F 2.8 (3), K 1.8 (2). Edman degradation of the crude product confirmed the sequence through 36 cycles, with preview at the fragment junctions, as expected.

Simultaneous Coordination of a Ketone by Two Main-Group Lewis Acids†

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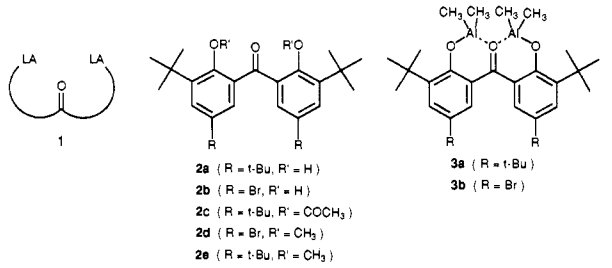
The carbonyl group of ketones and aldehydes is one of the most important functional groups in organic chemistry. Its intrinsically high reactivity can be enhanced by forming electrophilically activated complexes with Lewis acids.[2] In principle, simultaneous coordination by two or more Lewis acids could bring about multiple electrophilic activation, leading to even greater increases in reactivity and useful stereochemical effects.[3,4] However, it has not yet been established that ketones and aldehydes are basic enough to accommodate two main-group Lewis acids at the same time.[5,7] To test this possibility, we decided to synthesize a ketone **1** incorporating two nearby sites of Lewis acidity (LA). The carbonyl oxygen can interact with one site of Lewis acidity at a time, or it can interact symmetrically or unsymmetrically with both at once. We have found that simultaneous symmetric coordination is enthalpically feasible and that it has chemically significant consequences.



Suitable frameworks for juxtaposing a ketone and two sites of Lewis acidity were provided by 2,2′-dihydroxybenzophenones **2a**[8]

† Dedicated with affection and respect to Professor Frank H. Westheimer on the occasion of his 80th birthday.

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