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Synthesis of Ketomethylene Amino Pseudopeptide Analogues via Reductive Amination of Glyoxals Derived from α-Amino Acids

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Abstract—The reductive amination of an amino acid derived glyoxal, with the free amino group of a protected amino acid or oligopeptide fragment, has been developed as a simple and efficient method for the preparation of ketomethylene amino pseudo-oligopeptide isosteres AaΨ($COCH_2NH$)Aa. Trichlorosilane–DMF is the reagent of choice for the reduction.

The importance of proteolytic enzymes in a wide spectrum of biological functions has created a need for more potent and specific inhibitors.[1] One class of useful and versatile inhibitors is the nonhydrolyzable ketomethylene oligopeptide analogues of natural substrates.[2] Ketomethylene pseudopeptides can be formed via the incorporation of a methylene unit in the peptide amide bond as in **A**,[2,3] or by a direct replacement of the -NH- function as in **B**[4–7] (Fig. 1). One potentially important difference between these two classes of inhibitors is that the aminoketone A retains the ability of hydrogen bond formation through the nonamide -NH- group.[8]

The aim of the work described here was to synthesize putative peptide-based inhibitors of proteinases involved in the processing of the Alzheimer's disease-associated amyloid precursor protein (APP). Our strategy was to replace each of the scissile peptide bonds, corresponding to the α-, β-, and γ-secretase cleavage sites in APP, with non-hydrolyzable isosteres. As part of this approach, we attempted the synthesis of ketomethylene amino-containing of type **A**, which span the P and P′ sites around the scissile amide bonds. This isostere, Ψ($COCH_2NH$), is believed to act as a transition state analogue in the inhibition of serine proteases. In a study of its use in inhibitors of the serine protease furin,[9] it was found to yield inhibitors more potent than those containing Ψ($COCH_2$), despite the consequent increase in spatial separation of amino side chains at positions P_1 and P_1'. Two methods are currently available for the synthesis of ketomethylene isosteres.[2] The first relies on the alkylation of an oligopetide amine by an amino acid derived chloromethyl ketone.[2,3] The second methodology is a modification of the Dakin–West procedure.[2,10,11] Unfortunately, both these methods involve conditions that cannot easily be adapted to peptide synthesis especially in the presence of sensitive protective groups.

We have devised a simple route to ketomethylene amino peptides of type **A** from *N*-protected amino acids **1** which is based on the use of novel *N*-protected amino glyoxals **3**[12,13] in reductive amination of selected amines (Scheme 1). The route is outlined in Scheme 1 and the results obtained with combinations of two Cbz-protected amino acids and four amines, the latter including a Phe-Val derivative, are summarised in Table 1. The *N*-protected amino acid was first transformed into the terminal diazoketone **2** using standard, well established methodology.[14,15] The diazoketone was then oxidised to the *N*-protected amino glyoxal **3** using dimethyldioxirane (DMD) in acetone as oxidant, a process that was rapid (ca. 10 min at 0 °C), quantitative and clean.

The acetone solvent was removed by rotary evaporation and replaced by dichloromethane, ethanol or dimethylformamide and to the resulting solution was added the

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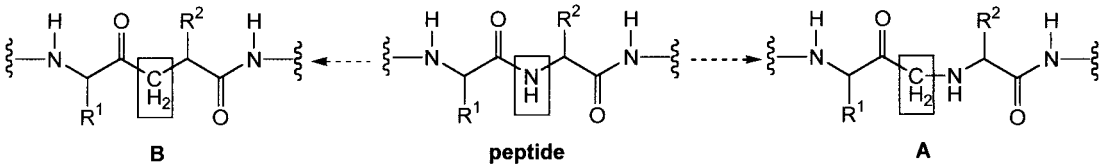
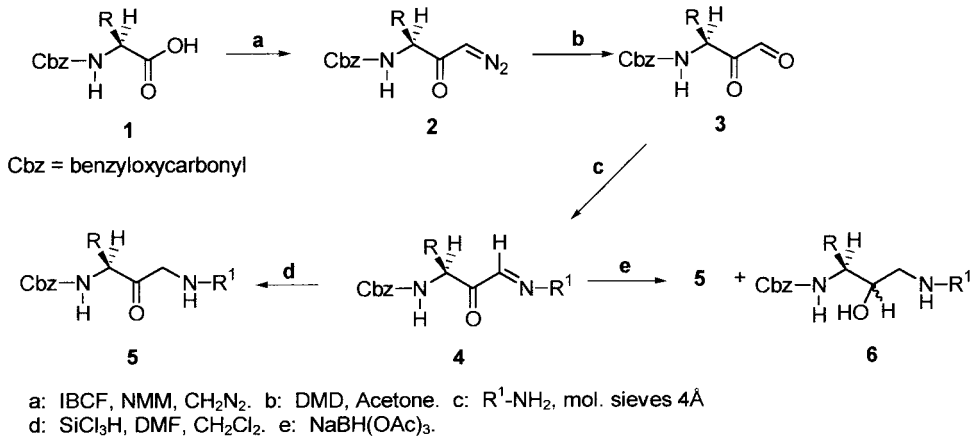
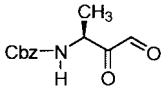
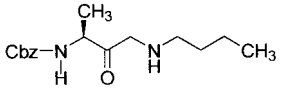
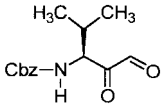
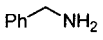
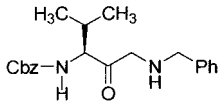
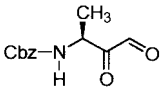
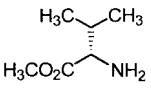
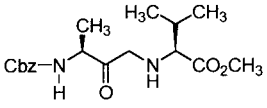
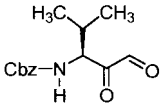
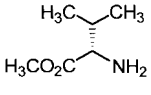
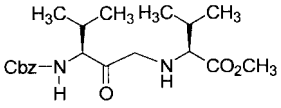
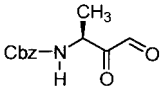
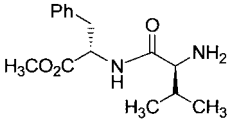
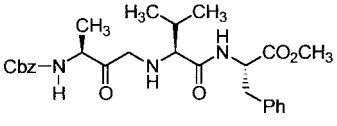


Figure 1.



Scheme 1.

Table 1.

Entry	Glyoxal	Amine	Product	Time[a] (h)	Yield[b] (%)
a[c]				1	90
b[c]				1	90
c				4	72
d				4	65
e				6	62[d]

[a]Aldimine formation.
[b]Isolated yields after column chromatography.
[c]Condensation performed in dry CH_2Cl_2, followed by addition of DMF–$SiHCl_3$.
[d]Corrected for recovered aldimine which was isolated after column chromatography.

amine (Table 1) and 4Å molecular sieves. Aldimine formation 4 was complete in 1–6 h depending on the nature of the amine and the solvent. Reactions with *n*-butylamine and benzylamine were conducted in dichloromethane whereas ethanol (entries **c** and **d**) was a better solvent for reactions of the amino acid-derived amines. The condensation necessary to produce **e** was conducted in dimethylformamide. To complete the synthesis, the aldimine **4** was reduced. Sodium cyanoborohydride was the first reagent used for the reduction step due to its well documented application in reductive amination.[16,17] However, with our substrates, $NaCNBH_3$ was not sufficiently selective in that it produced a mixture of the desired aminoketone **5** and the corresponding amino alcohol **6**, the latter as a mixture of diastereoisomers. A similar lack of chemoselectivity was also observed when sodium triacetoxyborohydride[18,19] was employed as the reducing agent. However, the desired selectivity could be achieved through the use of trichlorosilane–dimethylformamide as the reducing agent.[20] The reduction proceeded smoothly† at 0 °C and no other products could be detected. Our methodology was tried with a number of amines and the results are summarized in Table 1. ^1H and ^{13}C NMR analysis of products **5c**–**5e** indicated that they were single diastereoisomers, confirming that racemisation did not occur at any step in the overall synthesis. The reaction times depended on the nature of the amino acid side chain R_1 and R_2. We are currently investigating the applicability of this method to resin bound peptides. When this is achieved, our methodology should be ideal for oligopeptide solid state synthesis and combinatorial applications.

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†A typical procedure for the reductive amination. **Cbz-l-Ala*W*($COCH_2NH$)-L-Val-OMe (5c)**. A solution of (*S*)-valine methyl ester (0.5 mmol, 74 mg) was added to a stirred solution of freshly prepared glyoxal **3c** (120 mg, 0.5 mmol) in ethanol (10 mL) containing molecular sieves (1 g). The mixture was stirred for 4 h under N_2 while the colour changes from colourless to bright yellow. At this stage the aldimine could be isolated in quantitative yield by filtration and removal of solvent under high vacuum. The crude imine was dissolved in CH_2Cl_2 (10 mL) under N_2. After cooling at 0 °C, Cl_3SiH (50 μL, 0.85 mmol) in DMF:CH_2Cl_2 (1:3, 3 mL) was added and the solution changed colour to light orange. The mixture was stirred for 4 h at this temperature, and MeOH (1 mL) was added. Saturated aqueous sodium bicarbonate was then added and any insoluble materials were removed by filtration. The aqueous layer was extracted with CH_2Cl_2 (2×20 mL). The organic layers were combined, dried and evaporated to afford the amine **5c** (120 mg, 72%). For further purification, the product was subjected to column chromatography on silica using ethyl acetate:hexane (20–40%). Compound **5c**: ^1H NMR ($CDCl_3$, 300 MHz) 0.93 (6H, d, (-CH_3)$_2$CH)), 1.25 (3H, d, Ala-CH_3), 1.98 (1H, m, (CH_3)$_2$C*H*), 3.02 (1H, d, Val-α-H), 3.61 (2H, q, -COC*H*$_2$NH-), 3.75 (3H, s, -OCH_3), 4.43 (1H, m, Ala-α-H), 5.05 5.01 (2H, dd, -C*H*$_2$O-), 5.77 (1H, d, -NH-), 7.24–7.34 (6H, m, -Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) 17.11, 17.49, 17.85, 30.38, 50.65, 52.71, 53.79, 67.14, 127.06–129.86, 135.19, 154.64, 206.17; Acc.Mass calculated 351.1913, found 351.1919.