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Peptide Bond Modification. I. Simple and Efficient Method of Boc-GlyΨ[CH(OH)CH_2]Gly-OH Synthesis

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Peptides belong to biopolymers that play one of the key role in living organisms. Some of them posses regulatory functions like peptide hormones (*e.g.* insulin and oxytocin) and some play a role in signal transduction like neurotransmitters (*e.g.* peptides like endorphine and enkephalin). Peptides *in vivo* undergo fast enzymatic degradations and this process is beneficial for organisms because final degradation products are non-toxic molecules-amino acids, but on the other hand short lifetimes of peptides in organism exclude their wide usage as drugs (for example half-lifetime of leucine-enkephalin in blood is about few minutes [1]). The best solution solving that problem came from so called "peptidomimetics". The peptidomimetics are derivatives of peptides in which certain parts were chemically modified to achieve enhanced activity, higher selectivity, longer lifetime and better transportation profile. The most used modifications consist of: a side chain modification of amino acid residues, change of configuration of amino acid and modification of amide bond [2]. One of the most interesting peptide bond modification – hydroxyethylene peptide bond surrogate – can be achieved by replacement of the amide bond -CO-NH- by -CH(OH)-CH_2-. It mimics a structure of an amide bond transition state occurring during the hydrolysis in which both C- and N-atom posses sp^3 hybridization [3]. This kind of structure ensures strong binding with active site of proteolytic enzymes like HIV-protease, enkephalinase, renin but what is even more important it cannot be hydrolyzed, so it can serve as an effective inhibitor. There are many papers devoted to studies of enzyme inhibitors containing the hydroxyethylene peptide bond surrogate [4–8]. Synthetic studies of the hydroxyethylene peptide bond surrogate have started with isolation of natural inhibitors of pepsin, compounds containing statines. These inhibitors contain β-hydroxy-γ-amino acids as an element mostly important for the biological activity [9].

Most of syntheses of the hydroxyethylene moiety have used γ-lactone derivatives as a key element [10–12]. One of the most important procedures has been based on a reaction between N-protected amino aldehydes and suitable donor synthon like anion

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of ethyl propiolate, followed by hydrogenation and lactonization [10]. Other methods have used either Grignard reagent obtained from (2S)-3-benzyloxy-1-bromo-2-methylpropane and amino aldehyde [11] or dimethylsulfonium methylide and amino aldehyde followed by reaction with appropriate malonate [12]. There are also methods utilizing derivatization of chiral γ-lactone (see Fig. 1) obtained previously from glutamic acid [14].

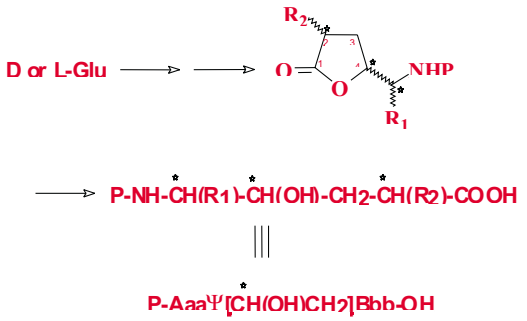

Figure 1. Preparation of a hydroxyethylene peptide bond surrogates.

In this paper we present simple and efficient synthesis of both enantiomers of t-butyloxycarbonyl-protected pseudodipeptide (R)- or (S)-Boc-GlyΨ[CH(OH)-CH_2]Gly-OH. The strategy developed in this work is outlined in Fig. 2.

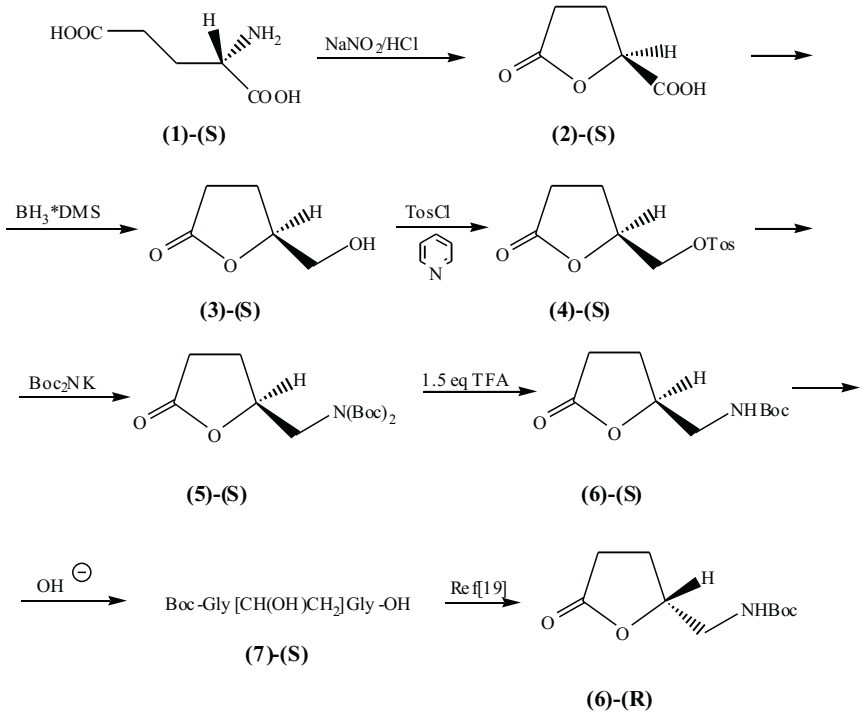

Figure 2. Synthetic scheme.

L-Glutamic acid was used as the starting chiral substrate for preparation of γ-lactone **2** *via* deamination/hydroxylation reaction [14]. So obtained lactone **2** was converted into hydroxymethyl derivative **3** by reduction using borane/dimethylsulfide complex [15]. The hydroxyl group of **3** was protected by tosyl moiety and compound **4** was formed. It is worth to mention that although **2** and **3** were a semisolid and oil, respectively, the tosyl derivative **4** was a solid easy to recrystallize. The conversion of tosyloxy group of **4** into "amino group precursor" has been previously achieved by reaction with azide followed by hydrogenation [14]. Because of problems with hydrogenation step (low yield, problems with product isolation) we decided to obtain "amino group precursor" *via* reaction of **4** with modified Gabriel reagent – potassium salt of di-*tert*-butyl-imidodicarbonate [17,18]. Such protected compound **5** (crystalline compound) can be used for preparation of either mono Boc-protected derivative **6** [18] and after ring opening pseudodipeptide **7** or can be converted into other pseudodipeptides *via* alkylation of C-2 of the lactone ring [7,12,16]. An advantage of the proposed methodology comes from a nature of the final product – the compound **5** is solid (simple purification) and additionally from the simplicity of the transformation **5** → **6** (it can be easily achieved by action of 1.5 equivalents of trifluoroacetic acid in methylene chloride) [18]. Besides these two facts it is worth to emphasize that the lactone **5** (or **6**) can be converted into another enantiomer by ring opening (basic hydrolysis) followed by its closure *via* Mitsunobu methodology [19].

Preparation of (4S)-γ-[N,N-di-*tert*-butyloxycarbonyl-aminomethyl]-γ-lactone (5). To a stirred solution of Boc_2NK (0.76g) in DMF (6 ml), (4S)-γ-toluenesulfonyloxymethyl-γ-lactone in DMF (2 ml) was added dropwise. The resulting mixture was heated to 50°C for 2 h. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate (50 ml) and brine (5 ml). Next, the organic phase was washed with 1% $KHSO_4$ (3×5 ml), 5% $NaHCO_3$ (2×5 ml) and brine (2×5 ml), and dried ($MgSO_4$). Filtration of the drying agent, removal of the solvent under reduced pressure and crystallization of remaining residue (ethyl acetate/hexane) yielded final product (0.32 g, 34%). IR (KBr) ν (cm^{-1}): 1779 (C=O, lactone), 1747 (C=O, urethane), 1697 (C=O, urethane); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.50 (s, 18H, *tert*-butyl), 1.8–2.4; 2.6–2.8 (m, 4H, CH_2-CH_2), 3.8–4.2 (m, 2H, CH_2-N), 4.8 (m, 1H, CH); m.p. = 76–79°C; $[\alpha]_{546}^{21}$ = 125 (c = 0.08, CH_2Cl_2).

Preparation of (4S)-γ-[N-*tert*-butyloxycarbonyl-aminomethyl]-γ-lactone (6). To a chilled (ice/water bath, 0°C) solution of (4S)-γ-[N,N-di-*tert*-butyloxycarbonyl-aminomethyl]-γ-lactone (**5**) (1.26 g) in 5 ml of methylene chloride 0.46 ml (1.5 equiv.) of trifluoroacetic acid in 5 ml of methylene chloride was added. The bath was removed and stirring was continued for additional 2 h at room temperature (TLC inspection revealed end of the reaction). The resulting solution was washed with 5% $NaHCO_3$ (2×2 ml), brine (2 ml), and dried ($MgSO_4$). Filtration of the drying agent, removal of the solvent under reduced pressure, yielded oil final product (0.78 g, 91%). IR (KBr) ν (cm^{-1}): 1777 (C=O, lactone), 1711 (C=O, urethane); ^{1}H-NMR

($CDCl_3$) δ (ppm): 1.50 (s, 9H, *tert*-butyl), 1.8–2.4; 2.6–2.8 (m, 4H, CH_2-CH_2), 3.2–3.6 (m, 2H, CH_2-N), 4.6 (m, 1H, CH), 5.1 (s, 1H, NH); $[\alpha]_{546}^{21}$ = 32.4 (c = 1.26, CH_2Cl_2).

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