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Synthesis of hybrid peptides from unnatural aminoacids

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Hexa peptide **10** has been prepared by the condensation of tetrapeptide acid and dipeptide amine by using standard coupling reagents EDCI, HOBT and DIPEA. In the same method, terta peptide **8** has also been prepared from the acid of dipeptide **6** and amine salt of dipeptide **7** in dichloromethane. Dipeptide **5** has been prepared from the unnatural amino acid which is prepared from D-Glucose and Xyloses. NMR spectral data of peptides indicates presence of secondary structure in peptides.

Keywords: Unnatural Aminoacids, Coupling reactions, Peptides, Secondary structure

Peptides are carriers of a variety of functions in living organisms. They act as neurotransmitters, neuro modulators, hormones, paracrine factors, cytokines and antigens, and influence essentially all vital physiological processes *via* inter- and intra-cellular communication and by signal transduction mediated through various classes of receptors[1,2]. Peptides are generally poor drug candidates due to their limitations, characterized by fast hydrolytic cleavage, poor penetration of membranes, rapid photolytic degradation, conformational instability and unfavourable pharmacokinetics[3]. Numerous methods have been examined to overcome these problems and to improve the bioavailability of native and synthetic peptides, but these have met with only limited success to date[4]. For these reasons much effort has been expended to find ways to replace biologically active parts of peptides with non-peptide structures, termed peptidomimetics, in the hope of obtaining orally active entities[5]. The scope of research in this field has expanded rapidly and at present constitutes an important approach to drug design and discover[6,7]. One of the possible strategies in the development of peptide mimetic agents, besides the popular bio iso steric amide bond replacements and mimetics of peptide secondary structure, is the incorporation of unnatural α-amino acid derivatives. Conformationally restricted, non-proteinogenic α-amino acids have a great potential in elucidating the bioactive conformation of peptides. It must be emphasized that there are only a few amino acid analogs that facilitate suitable and predictable restrictions of conformational flexibility without drastically changing the stereo-electronic properties of the initial peptide[8]. In order to retain biological activity, constraints must affect the conformation of the backbone and simultaneously enable crucial side-chain interactions with the receptor[1,6]. Incorporation of unnatural α-amino acids can specifically restrict the rotation of N^{α}- C^{α}, C^{α}-C(O), C(O)-NH bonds and side-chain conformations by covalent or non covalent steric interactions[1,6]. There are many examples, ranging from simple α-methylated amino acids to proline mimetics and various unsaturated α,β-amino acids[9]. Unnatural α-amino acids incorporated into biologically active peptides and proteins modify their activity, stability, bioavailability and binding specificity[1,6]. Furthermore, incorporation of unnatural amino acids into various enzymes has been used to evaluate protein folding, protein function and signal transduction[10-12]. For this reason, incorporation of constrained amino acids and related compounds into peptides is a critical component of efforts to understand the proteome and its relation to life, health and disease. The desire to develop enantiometrically pure unnatural α-amino acids, given the importance of the geometric distribution of side chain functional groups, pushes further not only the barriers of asymmetric synthesis but also the invention and development of enantioselective chromatographic separation methods that enable adequate separation of the amino acid enantiomers[13-15]. Inspired by the biological profile of peptides and in continuation of work on biological active molecules[16-25] we synthesized some novel peptides from unnatural amino acids developed from the R-allose and S-xylose.

Experimental Details

Commercial grade reagents were used as supplied. Solvents, except of analytical reagent grade, were dried and purified according to literature procedures when necessary. Reaction progress and homogeneity of the compounds were checked by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates from Merck and compounds visualized either by exposure to UV light or dipping in 1% aqueous potassium permanganate solution. Silica gel chromatographic columns (60–120 mesh) were used for separations. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using 2 mL cell with a path length of 1 dm with $CHCl_3$ or $CDCl_3$ as solvent. Melting points were recorded using Fisher-Johns apparatus. All melting points are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (500 MHz for ^1H and 75 MHz for ^{13}C). Chemical shifts are reported in δ (ppm) against TMS as internal reference and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H mass spectrometer.

(3R)-methyl 3-((3aR,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-3-((3R)-3-((3aR,6R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-3-(pivaloyloxyamino)propanamido)propanoate, 5

A solution of **1** (0.500 g, 0.1 mmol) in THF/MeOH/H_2O (5 mL, 3:1:1) was treated with LiOH (0.100 g, 0.4 mmol) at 0°C to RT. After 1 h, the *p*H value adjusted to 2-3 with aq 1N HCl solution at 0°C and the mixture was extracted with EtOAc (2×10 mL). The organic layer was dried (anhyd. Na_2SO_4) and concentrated to give **2** (0.400 g, 78%) as a white solid which was used for next reaction without further purification.

A solution of acid **2** (0.300 g,0.07 mmol), HOBt (0.04 g,0.02 mmol) and EDCI (0.60 g, 0.38 mmol) in CH_2Cl_2 5 mL was stirred at 0°C under nitrogen atmosphere for 15 min, treated sequentially with salt **4** prepared from compound **3** and DIPEA (0.06 mL,0.038 mmol) and stirred for 8 h. The reaction mixture was quenched with aq. saturated NH_4Cl solution (20 mL). After 10 min it was diluted with $CHCl_3$ (3×10 mL) and washed with water (20 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was dried (anhyd. Na_2SO_4), concentrated and the residue was purified by column chromatography (60-120 mesh silica gel, 1.2% MeOH in $CHCl_3$) to afford **5** (0.309 g, 0.05 mmol, 65%) as a white solid. m. p. 196-198°C. $[\alpha]_D$ -41.0 (*c* 0.36, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.17 (bs, -NH), 5.89 (d, 1H, *J* = 2.9 Hz, -C_1H), 5.87 (d, 1H, *J* = 2.9 Hz, -C_1H), 5.25 (bs, -NH), 4.44 (d, 2H, *J* = 1.9 Hz, 2× -C_4H), 4.35 (d, 2H, *J* = 2.9 Hz, 2× -C_2H), 4.27 (d, 1H, *J* = 2.9 Hz, -C_3H), 4.11- 3.97 (m, 1H, -CH-N), 3.88 (d, 1H, *J* = 3.9 Hz, -C_3H), 3.74 (s, 3H, -OCH_3), 3.46 (s, 3H, -OCH_3), 3.36 (s, 3H, -OCH_3), 3.38-3.26 (m, 1H, -CH-N), 2.7(2H,d,*J* = 2.2Hz,CH_2), 2.5(2H,d,*J* = 2.2 Hz,CH_2), 1.44, 1.43 (2s, 6H, 2× -CH_3), 1.42 (s, 9H, -Boc), 1.28, 1.26 (2s, 6H, 2× -CH_3); ^{13}C NMR (150 MHz, $CHCl_3$): δ 173.2, 172.5, 156.2, 112.1, 112.0, 105.0, 104.7, 85.2, 84.5, 81.5, 81.4, 81.1, 79.6, 79.5, 76.3, 76.0, 53.0, 46.4, 43.4, 28.3, 26.9, 26.8, 26.4, 26.3; HRMS: *m/z* $[M+Na]^+$ Calcd for $C_{28}H_{46}N_2O_{13}Na$: 631.2795. Found: 631.2789.

(6R,10R,14R,18R)-methyl6,14,18-tris((3aR,6R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-10-((3aR,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2,2-dimethyl-3,8,12,16-tetraoxo-4-oxa-5,9,13,17-tetraazaicosan-20-oate, 8

A solution of ester **5** (0.100 g, 0.016 mmol) in THF/MeOH/H_2O (5 mL, 3:1:1) was treated with LiOH (0.100 g, 0.4 mmol) at 0°C to RT. After 1 h, the *p*H value adjusted to 2-3 with aq 1N HCl solution at 0°C and the mixture was extracted with EtOAc (2×10 mL). The organic layer was dried (anhyd. Na_2SO_4) and concentrated to gave **6** (0.087 g, 89%) as a white solid which was used for next reaction without further purification.

A Solution of **6** (0.087 g,0.014 mmol), HOBt (0.05 g,0.07 mmol) and EDCI (0.11 g,0.07 mmol) in dry CH_2Cl_2 (5 mL) was stirred at 0°C for 15 min and treated with salt **7** (0.090 g, 0.014 mmol) and DIPEA (0.03 mL) uner a nitrogen atmosphere for 5 h. Work-up as described for **5** and purification of the residue by column chromatography (60-120 mesh silica gel 3% MeOH in $CHCl_3$) afforded **8** (0.070 g, 45%) as a white solid m.p.230-232°C. $[\alpha]_D$ –54.2° (*c* 0.09, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.58 (bs, -NH), 7.55 (bs, -NH), 7.36 (bs, -NH), 6.03, 6.00, 5.96, 5.94 (4d, 1H, *J* = 3.1 Hz, 4× -C_1H), 5.53 (bs, -NH), 4.65-4.48 (m, 5H, 4× -C_4H, C_2H), 4.47 (d, 1H, *J* = 3.1 Hz, -C_2H), 4.43 (s, 3H, 3× -C_3H), 4.36 (d, 1H, *J* = 3.1 Hz, -C_2H), 4.10-4.01 (m, 1H, -CH-N), 3.99 (s, 1H, -C_3H,), 3.95 (d, 1H, *J* = 3.1 Hz, -C_2H), 3.91 (dd, 1H, *J* = 9.3, 5.2 Hz, -CH-N), 3.79 (dd, 2H, *J* = 7.2, 13.5 Hz, -CH-N), 3.77 (s, 3H, -OCH_3), 3.46, 3.45, 3.44, 3.39 (4s, 12H, 4× -OCH_3), 2.70(d, 2H, *J*=3.6Hz, CH_2), 2.62 (d, 2H, *J*=3.2Hz, CH_2), 2.58 (d, 2H,

J = 3.6 Hz, CH_2), 2.50 (d, 2H, *J* = 3.1Hz, CH_2), 1.48, 1.47, 1.46, 1.45 (4s, 12H, 4× -CH_3), 1.44 (s, 9H, -Boc), 1.31 (s, 3H, -CH_3), 1.30 (s, 9H, 3× -CH_3); ^{13}C NMR (150 MHz, $CHCl_3$): δ 175.2, 172.6, 150.4, 1118.8, 105.6, 83.6, 83.1, 80.2, 55.6, 51.2, 47.0, 45.6, 44.1, 36.5, 29.9, 27.0, 26.2; HRMS: *m/z* $[M+Na]^+$ Calcd for $C_{50}H_{80}N_4O_{23}Na$:1128.3538. Found: 1128.3525.

(6R,10R,14R,18R,22R,26R)-methyl 6,14,22-tris((3aR,6R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-10,18,26-tris ((3aR, 6S, 6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2,2-dimethyl-3,8,12,16,20,24-hexaoxo-4-oxa-5,9,13,17,21,25-hexaazaoctacosan-28-oate, 10

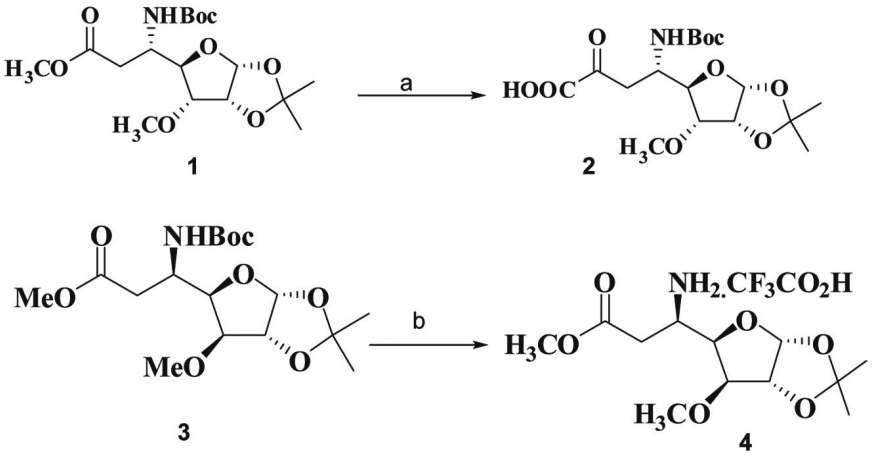
A solution of ester **8** (0.060 g, 0.005 mmol) in THF/MeOH/H_2O (5 mL, 3:1:1) was treated with LiOH (0.100 g, 0.4 mmol) at 0°C to RT. After 1 h, the *p*H value adjusted to 2-3 with aq 1N HCl solution at 0°C and the mixture was extracted with EtOAc (2×10 mL). The organic layer was dried (anhyd. Na_2SO_4) and concentrated to gave **9** (0.050 g, 84%) as a white solid which was used for the next reaction without further purification.

A solution of **9** (0.050 g, 0.004 mmol), HOBt (0.005 g,0.035 mmol) and EDCI (0.011 g, 0.0035 mmol) in dry CH_2Cl_2 (5 mL) was stirred at 0°C for 15 min and treated with salt 7 (0.050 g, 0.008 mmol) and DIPEA (0.03 mL) under nitrogen atmosphere for 5 h. Work-up as discribed for **5** and purification of the residue by column chromatography (60-120 mesh silica gel 3% MeOH in $CHCl_3$) afforded **10** (0.040 g, 55%) as a white solid m.p.243-245°C. $[\alpha]_D$ +27.8° (*c* 0.09, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (bs, -NH), 7.64 (bs, -NH) δ 7.56 (bs, 2× -NH), 7.36 (bs, -NH), 6.05 (s, 1H, -C_1H), 6.02 (s, 3H, 3× -C_1H), 5.98 (s, 1H, -C_1H), 5.96 (s, 1H, -C_1H), 5.54 (bs, -NH), 5.36 (bs, 2H, 2× -C_2H,), 5.30 (s, 1H, C_2H), 4.64-4.43 (m, 15H, 3× -C_2H, 6× -C_4H, 6× -C_3H), 4.35 (s, 1H, CH-N), 4.09-3.76 (m, 15H, 6× -CH_2-, 3×-CH-N), 3.76 (s, 3H, -OCH_3,), 3.46 (s, 9H, 3× -OCH_3), 3.44 (s, 6H, 2× -OCH_3), 3.39 (s, 3H, -OCH_3), 3.27 (d, 1H, *J* = 12.5 Hz, -CH-N), 3.14 (bs, 1H, -CH-N), 1.45 (s, 9H, -Boc), 1.29 (s, 18H, 6× -CH_3), 1.25 (s, 18H, 6×-CH_3); ^{13}C NMR (150 MHz,$CHCl_3$): δ 173.1, 172.3, 150.5, 111.4, 105.9, 83.6, 82.2, 81.6, 80.6, 57.5, 51.5, 47.9, 45.8, 44.2, 36.8, 29.5, 27.4, 26.2; HRMS: *m/z* [M+ Na^+] Calcd for $C_{72}H_{114}N_6O_{33}Na$: 1614.3453.Found: 1614.3439.

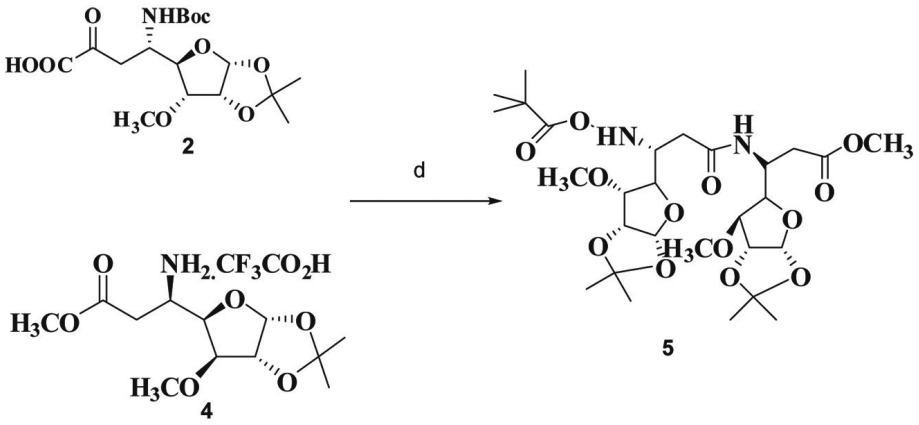
Results and Discussion

(S)-4-(tert-Butoxycarbonylamino)-4-((3aR,5R,6R, 6aR)-6-methoxy-2,2-dimethyl tetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-oxobutanoic acid **2** prepared from Glucose,(R)-methyl-3-amino-3-((3aR,5R, 6S, 6a R)-6-methoxy-2,2-dimethyl tetrahydrofur[2,3-d][1,3] dioxol-5-yl)propionate 2,2,2-trifluoroacetate **4** prepared from Xylose according to the literature procedure[26,27] (Scheme 1).

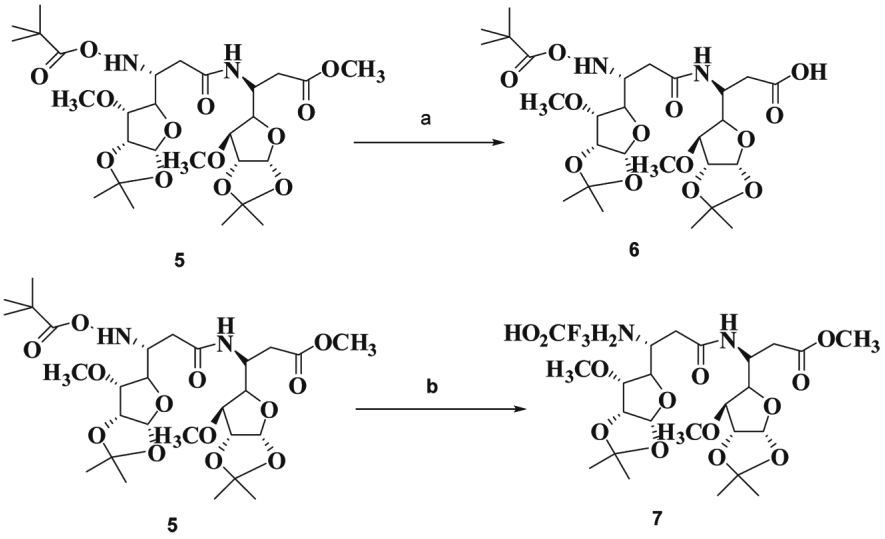
Dipeptide **5** of R-allose and S-xylose prepared by the condensation of unnatural amino acids **2** and **4** by using coupling reagents EDCI, HOBT in CH_2Cl_2 at 0°C to RT for 5 h (Scheme 2). Hydrolysis of dipeptide **5** with LiOH, THF and H_2O gave dipeptide acid **6**, which upon coupling with salt **7** prepared from dipeptide **5** by exposing with CF_3COOH and CH_2Cl_2 for 2 h furnished tetrapeptide **8** (Scheme 3 and Scheme 4). Likewise acid **9** upon coupling with salt **10** gave hexapeptide **11** by using coupling reagents EDCI, HOBT in CH_2Cl_2 at 0°C to RT for 5 h (Scheme 5 and Scheme 6). In NMR spectrum, appearance of NH proton signals indicates



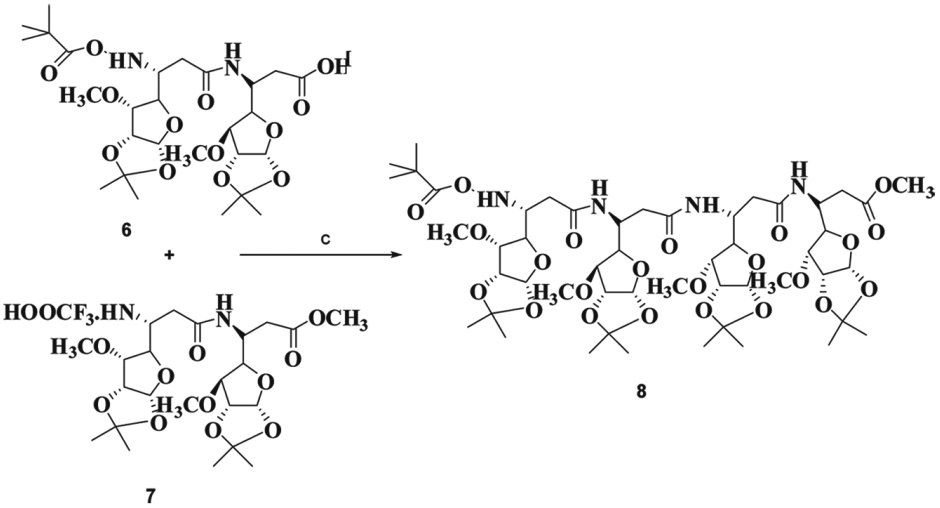
Scheme 1 — Reaction condition:(a) LiOH, THF, H_2O, MeOH, 0°C-RT, (b) TFA in CH_2Cl_2, 0°C-RT



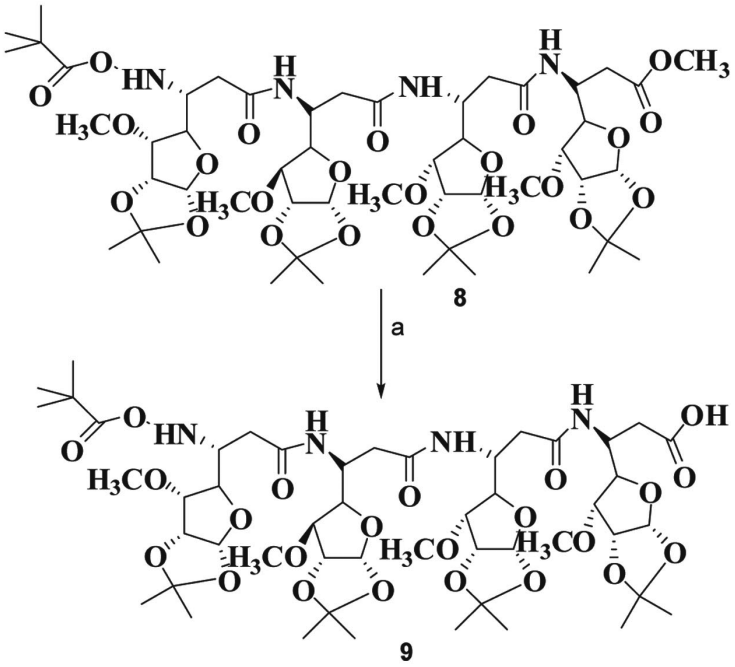
Scheme 2 — Reaction condition: (d) HOBt, EDCI, DIPEA, 0°C-RT



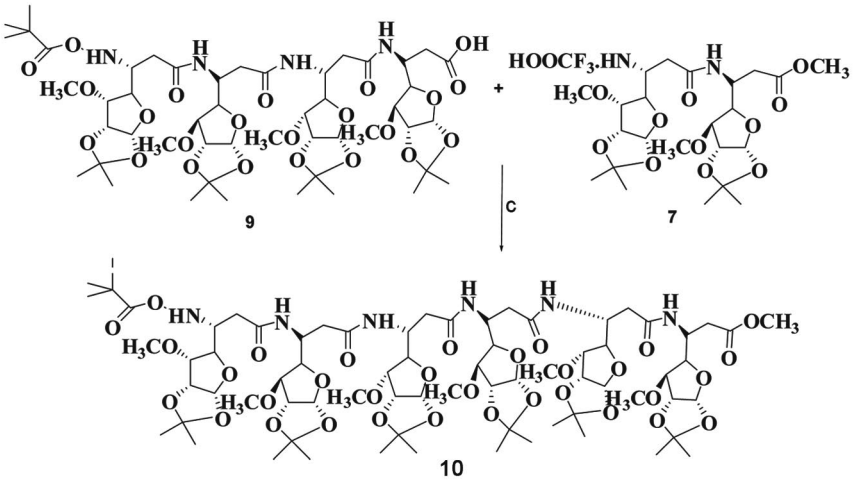
Scheme 3 — Reaction condition: (a) LiOH, THF, H_2O, MeOH, 0°C-RT, (b) TFA in CH_2Cl_2, 0°C-RT



Scheme 4 — Reaction condition: (c) HOBt, EDCI, DIPEA, 0°C-RT



Scheme 5 — Reaction condition: (a) LiOH, THF, H_2O



Scheme 6 — Reaction condition: (c) HOBt, EDCI, DIPEA, 0°C-RT

presence of secondary structure in synthesized peptides.

Conclusion

Di, tetra and hexa peptides synthesised from unnatural aminoacids R-allose and S-xylose by using standard coupling reagents, NMR spectral data of –NH protons at low field indictes presence of secondary structure in all these peptides. Furthur studies of these proteins are under progress.

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