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Synthesis of Muramyl dipeptide Analogs by Incorporation of 3,3,3-Trifluoroalanine

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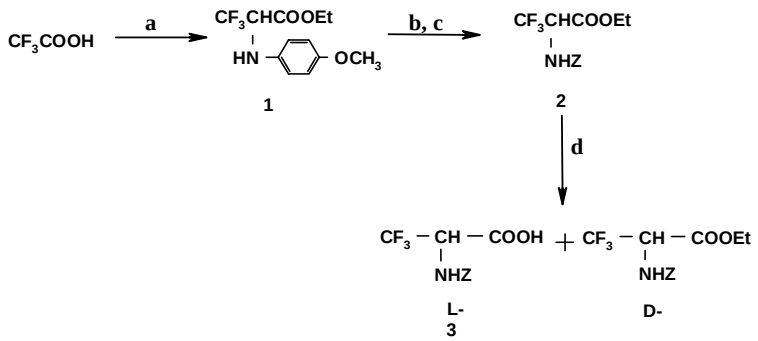
Abstract: Carbobenzoxy-L-3, 3, 3-trifluoroalanine was synthesized and it was incorporated into MDP for replacement of L-alanine.

Keywords: Muramyl dipeptide, 3, 3, 3-trifluoroalanine, adjuvant, peptide.

N-Acetylmuramyl-L-alanyl-D-isoglutamine (Muramyl dipeptide, MDP), the smallest immunoactive fragment of the cell wall peptidoglycans, which exhibits diverse biological activities such as adjuvant property, enhancement of host defence ability against microbial infection as well as antiviral and antitumor potency[1, 2]. In the last decades, studies on the synthesis of analogs and the relationship between structure and activity of MDP analogs have emerged since its first synthesis in 1975[1, 3, 4]. Because of the potential biological activity of organofluorine compounds, the incorporation of the fluorine atom into molecules has recently been studied extensively. Herein we wish to report the synthesis and resolution of 3,3,3-trifluoroalanine (*i.e.* trifluoromethylglycine, TFMGly) and its introduction into MDP in place of alanine in peptide moiety.

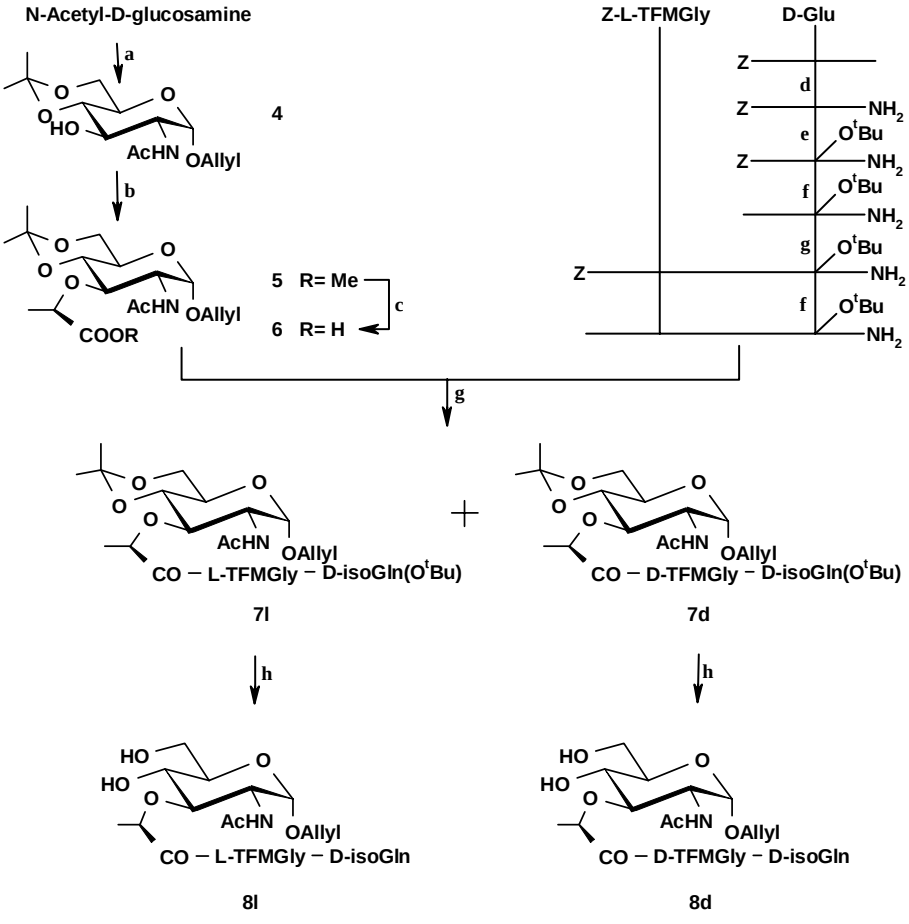
Scheme 1



Reagents and conditions: a. ref. 5; b. CAN, CH_3CN/H_2O, 0°C; c. ZCl, K_2CO_3, H_2O/EtOAc; d. subtilisin, H_2O/CH_3COCH_3, pH 7.8.

The peptide moiety in MDP was synthesized by Z/^{t}Bu strategy. Z-TFMGly was achieved starting from trifluoroacetic acid by several steps according to the known procedure[5]. The intermediate **1**, ethyl N-anisyl trifluoroalaninate (**Scheme 1**), was treated with ceric ammonium nitrate (CAN) in water-acetonitrile at 0°C, followed by protection with Z to give compound **2**[6]. Compound **2** can be successfully resolved by subtilisin to afford carbobenzoxy-L-3, 3, 3-trifluoroalanine **3** in a yield of 49%[10].

Scheme 2



Reagents and conditions: a. (i) allyl alcohol, AcCl, 70°C; (ii) $(CH_3)_2C(OEt)_2$, p-TsOH, acetone, 30°C; b. (i) NaH, DL-$CH_3CHClCOOH$, dioxane, 70°C; (ii) K_2CO_3, MeI, DMF, r.t.; c. 0.5N NaOH, EtOH; d. (i) trioxane, p-TsOH,toluene, reflux; (ii) NH_4OH, THF, r.t. ; e. ^{t}BuBr, K_2CO_3, BTEAC, DMA, 55°C; f. H_2, 10%Pd/C, AcOH, MeOH; g. HBPyU, NMM, DMF, r.t.; h. CF_3COOH, r.t.

Z-D-isoglutamine was synthesized from D-glutamic acid *via* formation and ring opening of oxazolidinone[7]. Treatment of Z-D-isoGln with *tert*-butyl bromide under the

J. Martinez's condition [7] afforded Z-D-isoGln(O^{t}Bu) in 57% yield. After deprotection of amino protecting group, isoGln(O^{t}Bu) was coupled with **3** using O-(benzotriazol-1-yl) -N, N, N′, N′-bis(tetramethylene)uronium hexafluorophosphate (HBPyU)[8]. A massive extent epimerization occured during this coupling, consequently a diastereomeric mixture of dipeptide Z-L,D-TFMGly-D-isoGln(O^{t}Bu) was obtained with the ratio of (L, D) to (D, D) being 1.57:1 determined by HPLC. The coupling yield only reached about 31%, which demonstrated the poor reactivity of TFMGly[9].

Allyl 2-acetamido-3-O-[(R)-1-carboxyethyl]-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside (**6**) was obtained in four steps starting from 2-acetamido-2-deoxy-α-D-glucopyranose in an overall yield of 35.2%. As shown in **Scheme 2**, 2-acetamido-2-deoxy-α-D-glucopyranose was converted to **4** by allylation of C_1-hydroxyl in the presence of AcCl and followed by protection of C_4 and C_6-dihydroxyls with isopropylidenyl group. Treatment of **4** with DL-2-chloropropionic acid followed by methylation afforded protected muramic acid **5** in 60.7% yield. Hydrolysis of **5** with aqueous sodium hydroxide gave product **6**. Compound **6** was subsequently coupled with amino-deprotected dipeptide to give diastereomeric mixture of **7l** and **7d**[11], which can be separated by column chromatography. After deprotection of **7l** and **7d** with CF_3COOH, the final products **8l** and **8d** were obtained respectively.

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10. Compound **3:** mp.101-104°C, R_f = 0.53 (EtOAc/Petroleum ether, 8:1), $[\alpha]^{18}_D$= +20.3 (c1.3, $CHCl_3$), EIMS: 277 (M^+), δ^1H ($CDCl_3$): 8.60 (1H, br, COOH), 7.35 (5H, s, Ph), 5.65 (br, NH), 5.27 (2H, s, CH_2Ph), 5.05 (1H, m, α-H). δ^{19}F ($CDCl_3$): -5.0ppm;
11. Typical procedure for preparation of **8**: To the solution of **15** (0.65 mmol) and HBPyU (0.65 mmol) dissolved in DMF (6 mL) were added NMM (1.7 mmol) and H-TFMGly-D-isoGln(O^{t}Bu) (0.433 mmol). The mixture was stirred overnight at room temperature. After removal of the solvent, the residue was partitioned between 5% sodium bicarbonate and ethyl acetate. The aqueous layer was extracted twice with ethyl acetate. The combined organic phases were washed subsequently with 5% sodium bicarbonate, aqueous citric acid, brine and water, dried over anhydrous sodium sulfate. The crude product was purified by silica gel column chromatography to give 147 mg of **7l** and 130 mg of **7d** (93.6% total

yield). Compound **7l** and **7d** were identified with FABMS, ^{1}HNMR and elemental analysis. Compound **7l:** mp.174-176°C, R_f = 0.60 (EtOAc/Petroleum ether, 9:1), $[\alpha]^{17}_D$= +48.4 (c 1.5, $CHCl_3$), FABMS: 683 ($[M+H]^+$); Compound **7d:** mp.107-109°C, R_f = 0.32 (EtOAc/Petroleum ether, 9:1), $[\alpha]^{17}_D$= +64.7 (c 0.9, $CHCl_3$), FABMS: 684 ($[M+2H]^+$). Calculated for $C_{29}H_{45}F_3N_4O_{11}$: C, 51.02, H, 6.64, N, 8.21. Found: C, 51.25, H, 6.69, N, 8.02 for **7l** and C, 51.05, H, 6.50, N, 7.95 for **7d**.

Compound **7** was treated with trifluoroacetic acid. The result compound was purified with reverse phase silica gel column chromatography to afford corresponding product **8**. FABMS of **8**: 587 ($[M+H]^+$) for **8l**, 587 ($[M+H]^+$) for **8d**.

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