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Synthesis of Fluoro-containing Muramyl Dipeptide Analogs

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Abstract: Twelve Fluoro-containing muramyl dipeptide analogs with perfluoroalkyl at C1 of sugar moiety or C-terminal of peptidyl chain were synthesized. © 1998 Published by Elsevier Science Ltd. All rights reserved.

N-Acetylmuramyl-L-analyl-D-isoglutamine(MDP) was shown to be the minimal structure of peptidoglycan from the bacterial cell wall required essentially for immunoadjuvant activity and a number of other immunogenic and pharmacological activities[1-4]. In a previous paper we reported that some fluoroalkylated MDP analogs exhibited a promising immune-activity[5]. In continuation of our studies on exploring the relationship between the biologic activity and chemical structure of MDP analogs, we now design and synthesize a set of new fluoro-containing MDP analogs bearing the lipophilic substituents at the C1 position of sugar moiety or the C-terminal of peptidyl chain. The information gleaned from the previous studies on SAR of MDP analogs indicated that the introduction of a lipophilic substituent into MDP may cause various important effects on its biological activity by increasing the adjuvant activity and decreasing the pyrogenicity which is one of the major side effects of MDP[6].

Considering the synthesis of fluoro-containing MDP analogs, a need for the incorporation of perfluoroalkyl into the molecules of MDP analogs led us to utilize allyl as protecting group for C_1-hydroxyl of sugar skeleton or C-terminal carboxyl of the peptidyl chain. As depicted in scheme 1, the synthesis began with the preparation of allyl 2-acetamido-2-deoxy-4,6-isopropylidene-D-glucopyranoside (**1**)[7] using a modification of literature's procedure[8]. The product **1** thus obtained showed that α-anomer was dominant as identifying by the H-signal of NMR spectrum at about δ4.69 ($J_{1,2}$=3.32Hz) for the prevailing α-anomer.

As it has been reported previously[9,10]for the condensation of racemic 2-chloropropionic acid with benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside, the corresponding 3-O-(D-1-carboxyethyl) derivative was shown to be the product in preponderant amount as compared to the 3-O-(L-1-carboxyethyl) derivative. Using the similar procedure, condensation of **1** with DL-2-chloropropionic acid followed by esterification of the resulting mixture, separation by silica gel chromatography revealed that 3-O-(D-1-carboxyethyl) methyl ester derivative **2** was the only product. We further verify the configuration of **2** using a literature procedure[11] as shown in scheme 2. It has been reported that trifluoromethanesulfonate of (S)-lactate reacted with 3-O-glucopyranose gave diastereospecifically muramic acid derivative in high yield. Based on this fact, condensation of **1** with trifluoromethanesulfonate of (S)-lactate followed by methylation and separation gave product **2'**. It was shown to be virtually identical with **2** by comparison of their mp., $[\alpha]_D$, MS as well as the data of elemental analysis.

This paper is dedicated to the late Professor Yu Wang.

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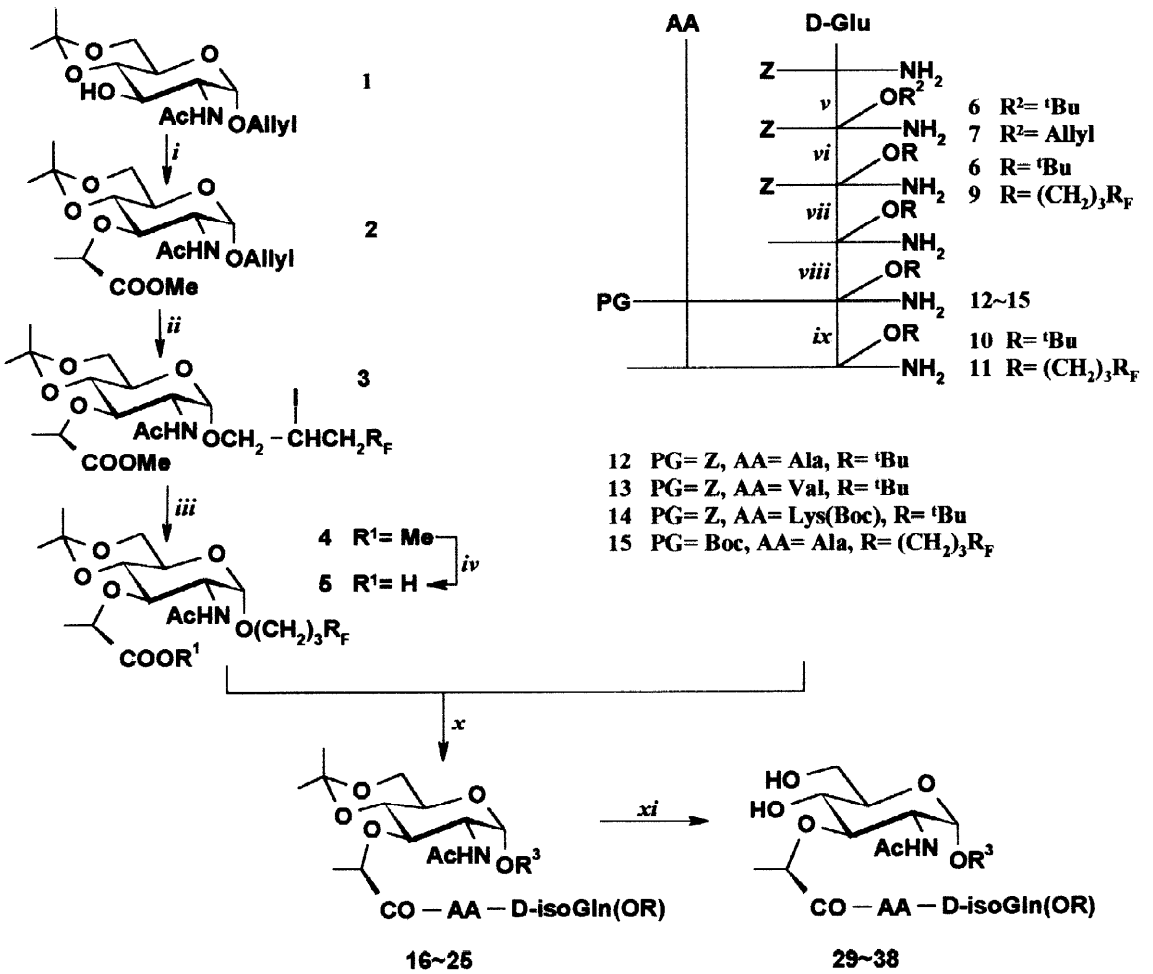
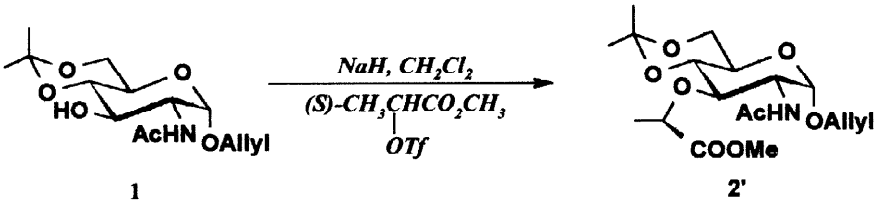


Scheme 1[a]

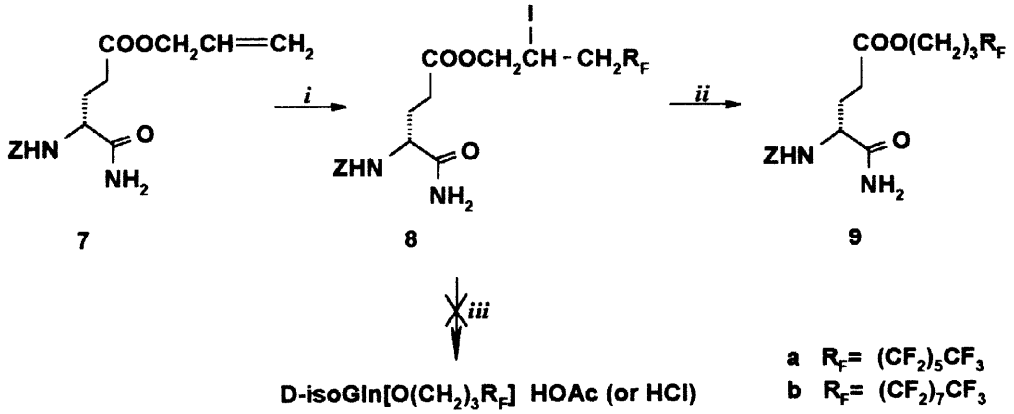
[a]**Reagents**: *i.* a. NaH, DL-$CH_3CHClCOOH$, dioxane, 70°C; b. K_2CO_3, MeI, DMF, r.t.; *ii.* R_FI, $NaHCO_3/Na_2S_2O_4$, H_2O/CH_3CN, r.t.; *iii.* H_2, Pd/C, NaOAc, MeOH, r.t.; *iv.* 0.5N NaOH, EtOH, 40°C; *v.* for R= ᵗBu: ᵗBuBr, K_2CO_3, BTEAC, DMA, 55°C; for R= Allyl: Cs_2CO_3, CH_2=$CHCH_2Br$, DMF, r.t.; *vi.* see Scheme 3; *vii.* H_2, Pd/C, AcOH, MeOH, r.t.; *viii.* for **12~14**: HBBTU, DIEA, DMF,r.t.; for **15**: Boc-Ala-NCA, NMM, DMF, r.t.; *ix.* H_2, Pd/C for Z, TFA for Boc; *x.* HBBTU, DIEA, DMF,r.t.; *xi.* TFA, r.t..



Scheme 2

The introduction of the perfluoroalkyl chain (R_F=$(CF_2)_5CF_3$ or $(CF_2)_7CF_3$) was carried out via sulfinatodehalogenation[12]. Condensation of **2** with perfluoroalkyl iodide by initiating with $Na_2S_2O_4$ to afford the adducts **3**. Removal of iodine atom by reduction of **3** with Zn/HOAc was unsatisfactory because the

isopropylidene protecting group will be susceptible to acidic condition. Therefore catalytic hydrogenation (Pd/C, MeOH, NaOAc)was found to be effective for the removal of iodine from **3**.



Scheme 3[a]

[a]**Reagents**: *i.* R_FI, $NaHCO_3/Na_2S_2O_4$, H_2O/CH_3CN, r.t.; *ii.* 5atm H_2, Pd/C, Et_2NH, EtOH, EtOAc, r.t.; *iii.* H_2, Pd/C, HOAc or HCl, solvent.

Scheme 3 illustrated the preparation of Z-D-isoglutamine γ-perfluoroalkyl ester. Condensation of Z-D-isoglutamine allyl ester **7** with perfluoroalkyl iodide using the similar procedure as described above for the perfluoroalkylation at C_1 of sugar to give **8**. Attempt to deiodination of **8** by similar condition as used for obtaining **4** from **3** or by transfer hydrogenolysis[13]resulted in failure. Removal of iodine from **8** was performed by catalytic hydrogenation in ethyl acetate containing 1.25eq of Et_2NH and 10eq of EtOH over Pd/C under pressure[14]. The synthesis of dipeptides (**12~14**) given in scheme 1 proceeded in good yield by N-deprotection of **6** with catalytic hydrogenolysis(Pd/C) and coulping to Z-amino acids using a novel coupling reagent, O-benzotriazol-1-yl-N, N, N', N'-bis(tetramethylene)uronium hexafluorophosphate (HBBTU)[15]. Dipeptide **15** was prepared by N-deprotection of **9** with trifluoromethanesulfonic acid and then coupled with Boc-alanine N-carboxyanhydride (Boc-Ala-NCA)[16].

Table 1 Protected Muramyl Dipeptide Analogs **16~25**

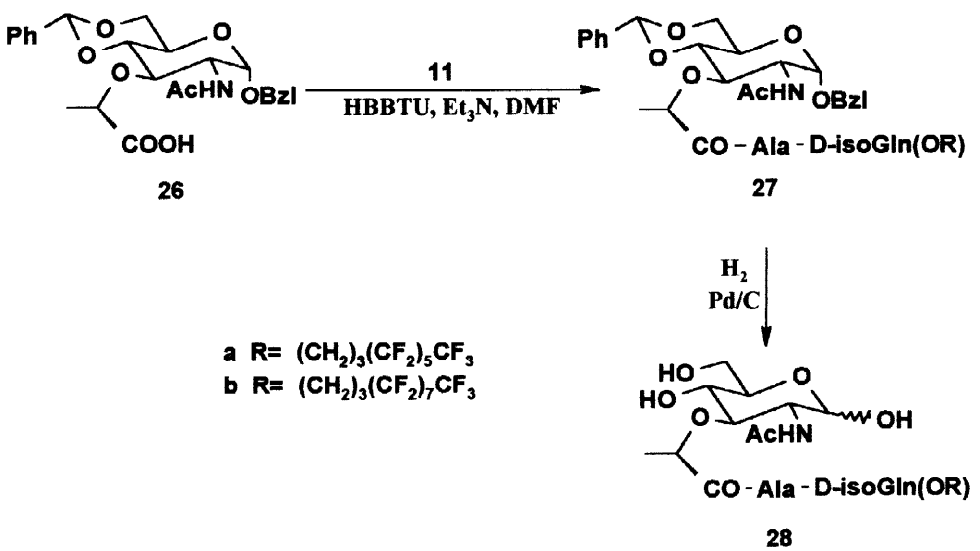
Compd	AA	R^3	R
16	Ala	$(CH_2)_3(CF_2)_5CF_3$	^tBu
17	Ala	$(CH_2)_3(CF_2)_7CF_3$	^tBu
18	Val	$(CH_2)_3(CF_2)_5CF_3$	^tBu
19	Val	$(CH_2)_3(CF_2)_7CF_3$	^tBu
20	Lys(Boc)	$(CH_2)_3(CF_2)_5CF_3$	^tBu
21	Lys(Boc)	$(CH_2)_3(CF_2)_7CF_3$	^tBu
22	Ala	$(CH_2)_3(CF_2)_5CF_3$	$(CH_2)_3(CF_2)_5CF_3$
23	Ala	$(CH_2)_3(CF_2)_5CF_3$	$(CH_2)_3(CF_2)_7CF_3$
24	Ala	$(CH_2)_3(CF_2)_7CF_3$	$(CH_2)_3(CF_2)_5CF_3$
25	Ala	$(CH_2)_3(CF_2)_7CF_3$	$(CH_2)_3(CF_2)_7CF_3$

Finally, the condensation of the muramic acid derivative **5** with the N-deprotected dipeptides **12-14** was accomplished using HBBTU to afford compounds **16~25** respectively (Table 1). The compounds bearing both the perfluoroalkyl groups at the γ-carboxyl of D-isoglutamine and the C_1 position of sugar were prepared by condensation of the N-deprotected dipeptide **15** with **5** using the same reagent. The subsequent removal of the isopropylidene in **16~25** and *tert*-butyl group **16~21** was carried out using trifluoroacetic acid to yield the desired MDP analogs **29~38** (Table 2).

Table 2 The Final Products **29~38**

Compd	AA	R^3	R
29	Ala	$(CH_2)_3(CF_2)_5CF_3$	H
30	Ala	$(CH_2)_3(CF_2)_7CF_3$	H
31	Val	$(CH_2)_3(CF_2)_5CF_3$	H
32	Val	$(CH_2)_3(CF_2)_7CF_3$	H
33	Lys(TFA)	$(CH_2)_3(CF_2)_5CF_3$	H
34	Lys(TFA)	$(CH_2)_3(CF_2)_7CF_3$	H
35	Ala	$(CH_2)_3(CF_2)_5CF_3$	$(CH_2)_3(CF_2)_5CF_3$
36	Ala	$(CH_2)_3(CF_2)_5CF_3$	$(CH_2)_3(CF_2)_7CF_3$
37	Ala	$(CH_2)_3(CF_2)_7CF_3$	$(CH_2)_3(CF_2)_5CF_3$
38	Ala	$(CH_2)_3(CF_2)_7CF_3$	$(CH_2)_3(CF_2)_7CF_3$

Compounds **28a-b** were prepared by the condensation of **26**[9] with **11** followed by catalytic hydrogenolysis (Scheme 4).



Scheme 4

EXPERIMENTAL

General. Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AM 300MHz or AMX 600MHz spectrometer and are reported as parts-per-million(ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used: singlet(s), doublet(s), triplet(t), quartet(q), multiplet(m), broad(br). Mass spectra were taken with HP5890A, and VG QUATTRO mass spectrometers. Elemental analyses for carbon, hydrogen and nitrogen were determined on a MOD-1106 elemental analyzer. Optical rotations were determined using a Perkin-Elmer 241 MC polarimeter. Flash column chromatography was performed with 300-400 meshes silica gel, and analytical thin layer chromatography was performed on precoated silica gel plates (60F-254) with the systems (v/v) indicated. Solvents and reagents were purified by standard methods as necessary.

Allyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-(methoxycarbonyl)ethyl)-α-D-glucopyranoside (2)

A solution of 15.12g (50mmol) of **1** in 800ml of dry dioxane was treated under stirring with 27.2g of 80% NaH dispersion in oil at 90°C, and stirred for 1hr. The solution was cooled to 70°C and 21.44ml (250mmol) of DL-2-chloropropionic acid was added dropwise. The stirring was continued for 2hr, the reaction mixture was cooled to r.t. and then treated with excess water carefully until the effervescence was over. The solvents were removed *in vacuo*, the residue was dissolved in 900ml of water and extracted with ether (250ml) and the aqueous phase was acidified to pH2 with cold 6N HCl. The product which precipitated was taken up in ethyl acetate (200ml×3) and the extract was washed with water, dried over $MgSO_4$, and evaporated to give an oil. The above oil was dissolved in DMF(400ml), K_2CO_3 (109g) and MeI (23.4ml) were added subsequently. After stirring at rt. for 23hr., the reaction mixture was filtered and the solid was washed with ethyl acetate. The combined filtrate and washings was evaporated to dryness and the residue was distributed between ethyl acetate and water. The aqueous layer was extracted once with ethyl acetate. The combined organic phase was washed subsequently with 10% $Na_2S_2O_3$, brine and water, dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography to give 11.8g (60.7%) as white solid, mp 68-72°C. R_f = 0.60 (AcOEt-petroleum ether=1:1). $[\alpha]^{20}_D$ +138.3°(*c* 1.0, $CHCl_3$). EIMS: 388([M+1]$^+$, 60%). ^{1}HNMR($CDCl_3$) δ7.36(d, NH), 5.86(1H, m, C=CH-C), 5.27(1H, d, J=3.55Hz, H-1), 5.26 (1H, ddd, J= 17.28, 1.60Hz, $=CH_2$), 5.18 (1H, dd, J=10.37, 1.38Hz, $=CH_2$), 4.48(1H, q, J=7.04Hz, α-H of Lact.) , 4.11, 3.98 (1H×2, 2m, $C=CCH_2$), 3.77(3H, s, OCH_3), 3.86-3.59(6H, m, H-2,3,4,5,6,6'), 2.04(3H, s, Ac), 1.50,1.40 (3×3H, 2s, Me×3). Anal. calcd for $C_{18}H_{29}NO_8$: C, 55.80; H, 7.54; N, 3.62. Found : C, 55.59; H, 7.53; N, 3.61.

1-(1-carbomethoxy)ethyl trifluoromethanesulfonate

A solution of pyridine(1.7ml) in methylene chloride (70ml) was cooled to -22°C. Trifluoromethanesulfonic anhydride (3.36ml, 20mmol) was added, after 2min followed by the addition of methyl (S)-lactate (1.91ml, 20mmol) and the mixture was warmed to ambient temperature with a water bath, and stirred for 0.5hr. The mixture was then filtered, the solvent was evaporated, and the residue passed through a silica gel plug (~4cm) with petroleum ether (bp 30~60°C). After evaporation of the solvent, 3.5g (74.4%) of colorless liquid was

obtained. ^{1}HNMR(neat) δ5.09(1H, q, J=7Hz, α-H), 3.60(3H, s, $COOCH_3$), 1.50(3H, d, J=7Hz, CH_3CH). ^{19}FNMR(neat) δ-0.5.

Allyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-(methoxycarbonyl)ethyl)-α-D-glucopyranoside (2')

The solution of 301mg (1mmol) of **1** in 5.5ml of dry CH_2Cl_2 was cooled to 0°C under nitrogen atmosphere. To this cooled solution 26mg (1.08mmol) of NaH then was added. After the evolution of H_2 was over, a solution of 1-(1-carbomethoxy)ethyl trifluoromethanesulfonate (297mg, 1.26mmol) in dry CH_2Cl_2 (1.1ml) was dropped slowly into the mixture. Stirred at r.t. overnight. The reaction mixture was filtered, and the filtrate was diluted with CH_2Cl_2, then washed with cold water to neutral, dried over Na_2SO_4. Removal of the solvent *in vacuo* followed by flash chromatography gave white solid. mp 68-70°C. $[\alpha]^{20}_D$ +136.8°(*c* 0.56, $CHCl_3$). EIMS: 388([M+1]$^+$, 27%). Anal. calcd for $C_{18}H_{29}NO_8$: C, 55.80; H, 7.54; N, 3.62. Found: C, 55.68; H, 7.57; N, 3.86.

General procedure for the synthesis of compound 3

To a solution of **2** (1.16g, 3mmol) and R_FI (3.6mmol) in CH_3CN (3.6ml) was added 2.4ml of water followed by $Na_2S_2O_4$ (690mg, 3.96mmol) and $NaHCO_3$ (499mg 5.94mmol) portionwise. The mixture was stirred at rt. for 2hr. After removal of the solvents, the residue was distributed between ethyl acetate and water, and the aqueous phase was extracted with EtOAc. The combined organic layer was washed with brine and dried over Na_2SO_4. The crude product was purified by flash chromatography to give a syrup.

3a: yield 73%. R_f=0.43 (AcOEt-petroleum ether=1:2). $[\alpha]^{27}_D$ +62.2° (c 0.59, CH_3OH). ^{19}FNMR($CDCl_3$) δ3.81(3F,CF_3), 36.60(2F, m, CF_2), 44.84, 45.95, 46.69, 49.23(8F, 4s, $(CF_2)_4$). HRMS: calcd for $C_{24}H_{30}F_{13}INO_8$ ([M+1$^+$]): 834.0817, found: 834.0820.

3b: yield 69.5%. R_f=0.56 (AcOEt-petroleum ether=1:2). $[\alpha]^{27}_D$ +65.6° (c 0.50, CH_3OH). HRMS: calcd. for $C_{26}H_{29}F_{17}INO_8$: 933.0665, found: 933.0656.

General procedure for the synthesis of compound 4

The mixture of **3**(1mmol) and sodium acetate (1.7mmol) was hydrogenated for 2hr at rt. in the presence of 10% Pd/C (53mg). Filtered, and the filtrate was evaporated. The residue was distributed between 10% $Na_2S_2O_3$ and ethyl acetate. The aqueous phase was extracted once more and the combined organic phases were washed once with water and dried over Na_2SO_4. After filtration and concentration, the product was obtained.

4a: oil, yield 100%, R_f=0.40 (AcOEt:petroleum ether=1:2). $[\alpha]^{27}_D$ +62.8°(c 0.54, CH_3OH). ^{19}FNMR($CDCl_3$) δ3.86 (3F, CF_3), 37.40(2F,CF_2), 44.99,45.97,46.62,49.24(8F,$(CF_2)_4$). HRMS: calcd for $C_{24}H_{30}F_{13}NO_8$: 707.1764, found: 707.1791.

4b: white solid, yield 94.5%, mp 71-75°C. R_f=0.49(AcOEt:petroleum ether=1:2), $[\alpha]^{25}_D$ +61.3°(c 0.54, CH_3OH). HRMS: calcd. for $C_{26}H_{30}F_{17}NO_8$: 807.1700, found: 807.1714.

General procedure for the synthesis of compound 5

The solution of **4**(1mmol) in 7ml of EtOH was treated with 4ml of 0.5N NaOH solution at 40°C for 20min. After removal of solvents the residue was dissolved in H_2O and the solution was acidified with 1N HCl while

cooling. The mixture was extracted twice with ethyl acetate. The organic phase was washed with brine and water, dried (Na_2SO_4) and concentration to give white foam.

5a: yield 90.7%. $[\alpha]^{20}_D$ +66.7° (c 0.50, CH_3OH). ESIMS: 694($[M+1]^+$, 10%).

5b: yield 96.2%. $[\alpha]^{27}_D$ +56.2° (c 0.60, CH_3OH). ESIMS: 794($[M+1]^+$, 5%).

Benzyloxycarbonyl-D-isoglutamine tert-butyl ester (6)

Z-isoGln was dissolved in dimethylacetamide in the presence of benzyltriethylammonium chloride (BTEAC). Dried K_2CO_3 was then added followed by ^{t}BuBr and the mixture was stirred at 55°C for 24hr. After cooling, cold water (1000ml) was added to the reaction mixture, and the resulting solid precipitate was filtered by suction and dissolved in 150ml of ethyl acetate. The solution was washed twice with water, dried and concentrated to afford 3.28g (57.3%) of **6,** mp 134-135.5°C. $[\alpha]^{18}_D$ +5.1° (c 2.3, CH_3OH). lit.[17]: mp 140-142°C, $[\alpha]_D$ +4.3° (c 2, CH_3OH).

Synthesis of benzyloxycarbonyl-D-isoglutamine allyl ester (7)

To a stirred suspension of Z-isoGln(2.8g, 10mmol) and Cs_2CO_3(6.11g) in DMF(15ml) was added 8.8ml of allyl bromide. The mixture was stirred at rt. for 2.5hr. After removal of the solvent, the residue was distributed between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with brine and water, dried over Na_2SO_4 and evaporated. After recrystallization of the crude product from ethyl acetate, 2.77g (86.6%) of **7** was obtained, mp 120-122°C. R_f =0.23(petroleum ether-EtOAc=1:1). $[\alpha]^{20}_D$ +7.5°(c 2.1, $CHCl_3$). EIMS: 276($[M\text{-}CONH_2]^+$,11%), 91(Bzl^+, 100). ^{1}HNMR($CDCl_3$) δ7.33(5H, Ph), 6.41(1H, br, NH), 5.85 (3H, m, H_2N+ C=C**H**-CH_2), 5.30(1H,dd, J=17.29Hz, 1.43Hz, =CH_2), 5.23 (1H,dd, J=10.44Hz, 1.14Hz, =CH_2), 5.09 (2H, s, CH_2Ph), 4.57 (2H, d, J=5.63Hz, C=CH-C**H**$_2$), 4.29(1H, m, α-H), 2.50(2H,m, CH_2), 2.15,1.94 (2H, m, CH_2). Anal. calcd for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.75. Found: C, 60.08; H, 6.34; N, 8.90.

Synthesis of compound 8

The procedure for **3** was followed with **7** (3mmol), in 7.5ml CH_3CN, 3ml H_2O, R_FI(3.6mmol), $Na_2S_2O_4$(3.96 mmol) and $NaHCO_3$(6mmol). The crude product was purified by flash chromatography to give white solid.

8a: yield 50.8%, mp 125-127°C. R_f =0.45(acetone-petroleum ether=1:2). $[\alpha]^{22}_D$ +3.1°(c 1.2, $CHCl_3$). ESIMS: 767(MH^+, 100%). ^{1}HNMR($CDCl_3$) δ7.35(5H, s, Ph), 6.28(1H, br, ZNH), 5.68(2H, br, $CONH_2$), 5.10(2H, s, CH_2Ph), 4.35(4H,m,α-H+OCH_2CHI), 2.89(2H,m,CH_2R_F), 2.53(2H,m, γ-CH_2), 1.98(2H,m,β-CH_2). ^{19}FNMR ($CDCl_3$) δ3.76(3F,t, J=9.4Hz, CF_3,), 37.5(2F, m, CF_2), 44.9, 46.0, 46.5, 49.2(8F,$(CF_2)_4$). Anal. calcd for $C_{22}H_{20}F_{13}IN_2O_5$: C, 34.48; H, 2.63; N, 3.65. Found: C, 34.22; H, 2.43; N, 3.52.

8b: yield 49.3%, mp 138-140°C. R_f = 0.38(acetone-petroleum ether=1:2). $[\alpha]^{25}_D$ = +3.3° (c 1.1, $CHCl_3$). ESIMS: 868($[M+2]^+$,100%), 889($[M+Na]^+$, 30). ^{1}HNMR($CDCl_3$) δ7.35(5H, s, Ph), 6.23(1H,br, ZNH), 5.61 (2H, br, $CONH_2$), 5.11(2H, s, CH_2Ph), 4.35 (4H,m,α-H+OCH_2CHI), 2.89(2H,m,CH_2R_F), 2.52(2H, m, γ-CH_2), 1.98(2H, m,β-CH_2). ^{19}FNMR($CDCl_3$) δ3.73(3F, t, CF_3), 36.0(2F,CF_2), 44.6-49.2(12F,$(CF_2)_6$). Anal. calcd for $C_{24}H_{20}F_{17}IN_2O_5$: C, 33.28; H, 2.33; N, 3.23. Found: C, 33.31; H, 2.17; N, 3.11.

Synthesis of compound 9

Compound **8** (mmol) was dissolved in ethyl acetate (20~40ml) followed by the addition of 10% Pd/C (60mg), Et_2NH (1.25mmol) and anhydrous EtOH(10mmol). The mixture was hydrogenated at rt. under 5atms of H_2 atmosphere. After filtration and concentration, the residue was distributed between ethyl acetate and 10% $Na_2S_2O_3$ solution, the organic phase was washed to neutral with water, dried over Na_2SO_4 and concentrated. After flash chromatography, **9** was obtained as white solid.

9a: yield 70%, mp 116-118°C. R_f = 0.40(acetone-petroleum ether=1:2). $[\alpha]^{22}_D$ +2.2°(c 1, $CHCl_3$), ESIMS: 641($[M+1]^+$, 50%). ^{1}HNMR($CDCl_3$) δ7.33(5H, s, Ph), 6.25,5.54(2H, 2br, $CONH_2$), 5.63(1H, d, J= 7.21Hz, ZNH), 5.11(2H, s, CH_2Ph), 4.29(1H, m, α-H), 4.16(2H, t, J= 6.14Hz, $COOCH_2$), 2.56,2.47,2.18,1.95(8H, 4m, β-CH_2 + γ-CH_2 + $CH_2CH_2R_F$). ^{19}FNMR($CDCl_3$) δ3.78(3F, t, CF_3), 37.5(2F,CF_2), 45.0-49.2(8F,$(CF_2)_4$). Anal. calcd for $C_{22}H_{21}F_{13}N_2O_5$: C, 41.26; H, 3.31; N, 4.35. Found: C, 41.43; H, 3.23; N, 4.37.

9b: yield 57%, mp 137.5-140°C. R_f =0.50(acetone-petroleum ether=2:3). $[\alpha]^{22}_D$ +3.5°(c 0.73, $CHCl_3$). FABMS: 741(MH^+, 18%). ^{1}HNMR($CDCl_3$) δ7.32(5H, s, Ph), 6.65,6.31 (2H, 2br, $CONH_2$), 5.72(1H, d, J= 6.42Hz, ZNH), 5.11 (2H, s, CH_2Ph), 4.32(1H, m, α-H), 4.18(2H, m, $COOCH_2$), 2.58,2.14,1.96(8H, m, β-CH_2 + γ-CH_2 + $CH_2CH_2R_F$). ^{19}FNMR($CDCl_3$) δ3.75(3F, t, J=8.2Hz, CF_3), 37.5(2F, m, CF_2), 45.0, 45.8, 46.4, 49.2 (12F,$(CF_2)_6$). Anal. calcd for $C_{24}H_{21}F_{17}N_2O_5$: C, 38.93; H, 2.86; N, 3.78. Found: C, 38.87; H, 2.46; N, 3.44.

General procedure for the synthesis of dipeptides 12~14

To a solution of **6**(2.35g, 7mmol) in 50ml of absolute methanol was added 376mg of 10% Pd/C and 1.4ml of glacial acetic acid. The mixture was hydrogenated at rt. for 4hr. The mixture was filtered and the filtrate was evaporated to dryness to afford D-isoGln(O^{t}Bu)•HOAc as a white solid in 94.4% yield.

1.60mmol of Z-AA and 1.68mmol of HBBTU were dissolved in 15ml of DMF, 0.56ml (3.2mmol) of DIEA was added followed by the addition of solution of D-isoGln(O^{t}Bu)•HOAc (393mg, 1.5mmol) in DMF (neutralized with 0.26ml of DIEA). The mixture was stirred at rt. for 4hr. After removal of the solvents, the residue was distributed between 5% $NaHCO_3$ and ethyl acetate. The aqueous layer was extracted once. The combined organic layers were washed successively with 5% $NaHCO_3$, brine, 0.5M citric acid, brine, 5% $NaHCO_3$, brine and water and dried over Na_2SO_4. The crude product was purified by recrystallization or flash chromatography.

12: Recrytallized from EtOAc-petroleum ether, yield 78.6%, mp 163-164.5°C. ESIMS: 408($[M+1]^+$, 75%). $[\alpha]^{25}_D$ -5.4°(c 1.5, CH_3OH). lit[17]: mp 164-166°C, $[\alpha]_D$ -3° (c 1.5 , CH_3OH).

13: Recrytallized from CH_3OH-EtOAc-petroleum ether, yield 75%, mp 213-214°C. R_f= 0.28 (AcOEt-petroleum ether=2:1). lit[18]:mp 210-211°C. EIMS: 362($[M-OBu^t]^+$, 1.1%), 335(8), 91(100). ^{1}HNMR (CD_3OD) δ7.50(5H,Ph), 5.28(2H, s, CH_2Ph), 4.55(1H, dd, α-H of D-isoGln), 4.00(1H, d, J=7.85Hz, α-H of Val,), 2.52,2.20,2.03(5H, 3m, γ-CH_2+β-CH_2+$CH(Me)_2$), 1.63(9H, s, ^{t}Bu), 1.18(6H, 2d, J=4.94,4.99Hz, $2CH_3$ of Val residue).

14: Purified by flash chromatography, yield 77.5%, mp 131.5-132.5°C. $[\alpha]^{22}_D$ +1.6°(c 1.9, CH_3OH). R_f=0.25 (EtOAc-petroleum ether=2:1). ESIMS: 565(MH^+, 22%). ^{1}HNMR(CD_3OD) δ7.50(5H, Ph), 5.28(2H, s, CH_2Ph), 4.55(1H, dd, α-H of D-isoGln), 4.22(1H, d, J=6.80Hz, α-H of Lys,), 3.22(2H, t), 1.64(18H, 2s, 2×^{t}Bu). Anal. calcd for $C_{28}H_{44}N_4O_8$: C, 59.75; H, 7.93; N, 9.90. Found: C, 59.56; H, 7.85; N, 9.92.

Synthesis of dipeptide 15

1 mmol of compound **9** was treated with 10ml of 1:10 CF_3SO_3H-TFA at rt. for 60min. After removal of the solvents, the residue was coevaporated with toluene for three times and dissolved in 15ml of DMF. The mixture was neutralized with NMM followed by addition of 1.2mmol of Boc-Ala-NCA. The reaction mixture was stirred at rt. for 60min. After the treatment as usual and purification by flash chromatography, **15** was obtained as a white foam.

15a: yield 83.6%. R_f= 0.25 (EtOAc-petroleum ether= 4:1). $[\alpha]^{20}_D$ -4.1°(c 1.1, CH_3OH). FABMS: 678 ($[M+1]^+$, 6%). ^{1}HNMR ($CDCl_3$) δ7.25(1H, d, J=7.68Hz, NH), 6.82,5.72(2H, 2br, $CONH_2$), 5.14(1H, d, J=5.69Hz, NH), 4.51(1H,m, α-H of D-isoGln), 4.17(2H, t, J= 6.04Hz, OCH_2), 4.11 (1H, q, J= 6.92Hz, α–H of Ala), 2.51,2.19, 1.99 (8H, 3m, β-CH_2+ γ-CH_2+ $CH_2CH_2R_F$), 1.42 (9H, s, ^{t}Bu), 1.36(3H, d, J=6.94Hz, CH_3 of Ala residue). Anal. calcd for $C_{22}H_{28}F_{13}N_3O_6$: C, 39.01; H, 4.17; N, 6.20. Found: C, 38.78;H, 3.98; N, 5.82.

15b: yield 82.2%, R_f= 0.25 (EtOAc-petroleum ether= 4:1). $[\alpha]^{20}_D$ -5.8° (c 1, CH_3OH). EIMS: 778($[M+1]^+$, 0.2%), 678(8.0), 562(32.8). ^{1}HNMR ($CDCl_3$) δ7.22 (1H, d, J=6.59Hz,NH), 6.81,5.69(2H, 2br, $CONH_2$), 5.13(1H, d, NH), 4.51(1H, m, α-H of D-isoGln), 4.17(2H, t, J=6.07Hz, $COOCH_2C$), 4.11 (1H, m, α-H of Ala), 2.53,2.19,1.99(8H, m, β-CH_2+γ-CH_2 of D-iGln+ $CH_2CH_2R_F$), 1.43(9H, s, ^{t}Bu), 1.36 (3H, d, J=6.81Hz, CH_3 of Ala residue,). Anal. calcd for $C_{24}H_{28}F_{17}N_3O_6$: C, 37.08; H, 3.63; N, 5.40. Found: C, 36.71; H, 3.31; N, 4.95.

General procedure for the synthesis of protected muramyl peptides 16~25

The carbobenzoxy groups in dipeptides **12~14** were deblocked following the similar procedure for the deprotection of **6** to give H-AA-D-isoGln(O^{t}Bu)• HOAc (**10**), and the *tert*-butyloxycarbonyl group in **15** was removed with TFA to give **11.**

General procedure: 0.2mmol of **5** and 95mg (0.24mmol) of HBBTU were dissolved in 3ml of DMF, the solution of above deblocked dipeptide **10** in 2ml of DMF (neutralized with DIEA) was added. The mixture was stirred at rt. until the completion by TLC track. The solvents were removed *in vacuo* and 5% $NaHCO_3$ was added. The product which precipitated was taken up in ethyl acetate (×3) and the organic phase was washed subsequently with 5% $NaHCO_3$, 0.5M cold citric acid, brine and water, dried over Na_2SO_4. The crude product was purified by flash chromatography to give white foam.

16: yield 81.7%, R_f= 0.44 (CH_2Cl_2-CH_3OH=20:1). $[\alpha]^{20}_D$ = +61.2° (c 0.4, CH_3OH). ESIMS: 950($[M+2]^+$, 100%). ^{1}HNMR(DMSO-d_6) δ7.00(1H, d, J=6.30Hz, NH of Ala), 6.95,5.47(2H, 2s, $CONH_2$), 6.34(1H, d, J=8.50Hz, NH of AcNH), 4.87(1H, d, J=3.80Hz, H-1), 4.42(1H, m, α-H of D-isoGln), 4.22(2H, m, α-H of Ala+H-2), 4.10(1H, q, J=6.60Hz, α-H of Lact.), 1.99(3H, s, Ac), 1.51-1.41(18H), 1.38(3H, d, J=6.80Hz, CH_3). Anal. calcd for $C_{35}H_{49}F_{13}N_4O_{11}$•$H_2O$: C, 43.48; H, 5.32; N, 5.80. Found: C, 43.40; H, 5.07; N, 5.36.

17: yield 76%, R_f= 0.69(CH_2Cl_2-CH_3OH=10:1). $[\alpha]^{22}_D$ +52.0°(c 0.49, CH_3OH). ESIMS: 1049($[M+1]^+$, 100%). ^{1}HNMR ($CDCl_3$) δ7.14(1H, d, J=7.78Hz, NH), 7.08(1H, d, J=6.15Hz, NH,), 7.01,5.66(2H, 2s, $CONH_2$), 6.47(1H, d, J=8.46Hz, NH), 4.86(1H, d, J=3.79Hz, H-1), 4.40(1H, m, α-H), 4.30(1H, q, J=6.81Hz, α-H of Lact.), 1.96(3H, s, Ac), 1.50-1.35(21H, ^{t}Bu+ $(CH_3)_2C$+ $2CH_3$). Anal. calcd for $C_{37}H_{49}F_{17}N_4O_{11}$•$H_2O$: C, 41.66; H, 4.82; N, 5.25. Found: C, 41.84; H, 4.54; N, 4.80

18: yield 61%, R_f= 0.54 (CH_2Cl_2-CH_3OH=15:1). $[\alpha]^{22}_D$ +57.9°(c 0.47, CH_3OH). ESIMS: 978($[M+2]^+$, 100%). ^{1}HNMR($CDCl_3$) δ7.41(1H, d, J=7.40Hz, NH), 6.94(1H, d, J=6.90Hz ,NH), 6.86,5.71(2H, 2s, $CONH_2$), 6.79

(1H, d, J=7.17Hz, NH), 5.04(1H, d, J=3.55Hz, H-1), 4.43(1H, m, α-H of D-isoGln), 4.30(1H, q, J=6.83Hz, α-H of Lact.), 1.52-1.36(18H, ^{t}Bu+$(CH_3)_2C$+CH_3 of Lact.), 1.00(6H, 2d, J=6.69Hz, $2CH_3$ of Val residue).

19: yield 69.8%, R_f= 0.55(CH_2Cl_2-CH_3OH=15:1). $[\alpha]^{22}_D$ +52.4°(c 0.5, CH_3OH). ESIMS: 1078([M+2]$^+$, 25%).

20: yield 97.5%, R_f= 0.54 (CH_2Cl_2-CH_3OH= 15:1). $[\alpha]^{22}_D$ +52.5° (c 0.47, CH_3OH). ESIMS: 1107([M+2]$^+$, 82%). ^{1}HNMR($CDCl_3$) δ7.49(1H, d, J=7.84Hz, NH), 7.04(1H, d, J=6.44Hz, NH), 6.99,5.71(2H, 2s, $CONH_2$), 6.66(1H, d, J=8.12Hz, NH), 4.99(1H, d, J=3.63Hz, H-1), 4.44(1H, m), 4.16(3H, m), 3.12(2H, d, J=6.05Hz), 1.51-1.33(2×^{t}Bu+$(CH_3)_2C$+CH_3 of Lact.).

21: yield 71.3%, R_f= 0.46(CH_2Cl_2-CH_3OH=15:1). $[\alpha]^{22}_D$ +52.9°(c 0.44, CH_3OH). ESIMS:1207([M+2]$^+$, 92%).

22: yield 89.8%, mp 77-82°C. R_f= 0.52 ($CHCl_3$-CH_3OH=15:1). $[\alpha]^{16}_D$ +38.0° (c 0.64, $CHCl_3$). FABMS: 1252(M$^+$, 100%). Anal. calcd for $C_{40}H_{46}F_{26}N_4O_{11}$: C, 38.35; H, 3.70; N, 4.47. Found: C, 38.48; H, 3.63; N, 4.46.

23: yield 81.3%, mp 75-81°C. R_f= 0.44 ($CHCl_3$-CH_3OH=15:1). $[\alpha]^{16}_D$ +36.4°(c 0.51, $CHCl_3$). FABMS: 1353(MH$^+$, 2%). 461(100). Anal. calcd for $C_{42}H_{46}F_{30}N_4O_{11}$: C, 37.29; H, 3.43; N, 4.14. Found: C, 37.45; H, 3.19; N, 3.92.

24: yield 88.7%, mp 81-84°C. R_f= 0.50 ($CHCl_3$-CH_3OH= 15:1). $[\alpha]^{16}_D$ +34.7° (c 0.5, $CHCl_3$). FABMS: 1353 ([M+1]$^+$, 30%). Anal. calcd for $C_{42}H_{46}F_{30}N_4O_{11}$: C, 37.29; H, 3.43; N, 4.14. Found: C, 36.77; H, 3.12; N, 3.76.

25: yield 77.4%, mp 55-60°C. R_f= 0.41 ($CHCl_3$-CH_3OH=15:1). $[\alpha]^{16}_D$ +32.1°(c 0.34, $CHCl_3$). FABMS: 1452(M$^+$, 34%).

General procedure for the deprotection of 16~25

0.1mmol of compound was stirred with 2ml of TFA at rt. for 60min. After removal of the solvents, the residue was purified with flash chromatography (eluent:AcOH/CH_3OH/$CHCl_3$) to give the desired product.

29: yield 79%. R_f=0.60 ($CHCl_3$-CH_3OH-AcOH=10-2.5-0.25). $[\alpha]^{17}_D$ +59.6 °(c 0.2, CH_3OH). FABMS: 875 ([M+Na]$^+$, 8%), 853([M+1]$^+$, 27).

30: yield 83%, R_f=0.14($CHCl_3$-CH_3OH-AcOH=10:2:0.1). $[\alpha]^{21}_D$ +43.2°(c 0.44, CH_3OH). FABMS: 991 ([M+K]$^+$,36%), 953([M+1]$^+$, 73), 807(36).

31: yield 83.2%, R_f=0.28 ($CHCl_3$-CH_3OH=10:2). $[\alpha]^{19.5}_D$ +59.54°(c 0.26, CH_3OH). FABMS: 881([M+1]$^+$, 2%).

32: yield 93%, R_f=0.18($CHCl_3$-CH_3OH=10:2). $[\alpha]^{21}_D$ +50.8°(c 0.62, CH_3OH). FABMS: 1003 ([M+Na]$^+$, 4%).

33: yield73%, R_f=0.62(^{n}BuOH-AcOH-H_2O = 4:1:2). $[\alpha]^{17}_D$ +17.5°(c 0.8, CH_3OH). FABMS: 910([M+1]$^+$, 6%).

34: yield 80%, R_f=0.70(^{n}BuOH-AcOH-H_2O=4:1:2). $[\alpha]^{17}_D$ +48.4°(c 0.5, CH_3OH). FABMS: 1010([M+1]$^+$, 4.6%).

35: yield 80%, R_f=0.24 ($CHCl_3$-CH_3OH=10:1). $[\alpha]^{17}_D$ +53.4 °(c 0.3, CH_3OH). FABMS: 1235([M+Na]$^+$, 8%), 1213([M+1]$^+$,6%).

36: yield 80%, R_f=0.20 ($CHCl_3$-CH_3OH=10:1). $[\alpha]^{17}_D$ +35.3°(c 0.27, CH_3OH). FABMS: 1335([M+Na]$^+$, 13%), 1313([M+1]$^+$, 10).

37: yield 80%, R_f=0.20 ($CHCl_3$-CH_3OH=10:1). $[\alpha]^{17}_D$ +44.3 °(c 0.25, CH_3OH). FABMS: 1335($[M+Na]^+$, 6%), 1313($[M+1]^+$, 22).

38: yield 80%, R_f=0.17 ($CHCl_3$-CH_3OH=10:1). $[\alpha]^{17}_D$ +34.1°(c 0.25, CH_3OH). FABMS: 1435($[M+Na]^+$, 7%), 1412(M^+, 6).

Synthesis of compound 27

Following the foregoing general procedure for the preparation of **16**, after the reaction was completed, 5% $NaHCO_3$ was added, and the white precipitates were collected by filtration, dried and purified by column chromatography (eluted with $CH_3OH/CHCl_3$) to afford white solid.

27a: yield 85.5%, mp 251°C(dec.). R_f=0.51 ($CHCl_3$-CH_3OH =20:1). $[\alpha]^{22}_D$ +67.0° (0.24, 1/1 $CHCl_3$/ CH_3OH). FABMS: 1031($[M+1]^+$, 4%), 1053(10), 923(20), 462(100). Anal. calcd for $C_{42}H_{47}F_{13}N_4O_{11}$: C, 48.94; H, 4.60; N, 5.44. Found: C, 48.45; H, 4.47; N, 5.13.

27b: yield 94%, mp 257-258°C(dec.). R_f=0.55 ($CHCl_3$-CH_3OH=20:1). $[\alpha]^{22}_D$ +53.1°(c 0.21, 1:1 $CHCl_3$-CH_3OH). FABMS:1131($[M+1]^+$, 2%), 1032(22), 562(87). Anal. calcd for $C_{44}H_{47}F_{17}N_4O_{11}$: C, 46.73; H, 4.19; N, 4.95. Found: C, 47.28; H, 4.48; N, 4.58.

Procedure for the hydrogenation of 27

To the solution of **27** in HOAc was added 10% Pd/C and stirred at rt. and 1atm H_2 for 5 days. Filtered and the solid was washed with a little HOAc. The filtrate was evaporated *in vacuo* and the residue was purified by flash chromatography to give product **28**.

28a: yield 70.5%, R_f=0.38 ($CHCl_3$-CH_3OH=10:2). $[\alpha]^{19.5}_D$ +23.3°(c 0.2, CH_3OH). FABMS: 853($[M+1]^+$, 6%), 835(15), 462(100).

28b: yield 70%, R_f=0.41 ($CHCl_3$-CH_3OH =10:2). $[\alpha]^{21}_D$ +27.5°(c 0.24, CH_3OH). FABMS: 975($[M+Na]^+$, 8%), 562(42).

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