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SYNTHESIS OF HUMAN M BLOOD GROUP ANTIGENIC GLYCOPEPTIDE

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Abstract: A first total synthesis of N-terminal glycoheptapeptide of human glycophorin AM was accomplished by solution phase peptide condensation utilizing the tetrasaccharide-linked amino acid building blocks designed for Fmoc strategy.

Glycophorin A is a major transmembrane sialoglycoprotein found in human erythrocyte. Fifteen Ser/Thr residues in the extracellular domein (1-50 amino acid region) are glycosylated with the tetrasaccharide containing two sialic acids (1). The polymorphic N-terminal pentapeptide sequences correspond to the MN blood group epitopes.[2,3,4]

In connection with our interest in synthetic approaches to such highly O-glycosylated proteins as Glycophorin A, we recently reported syntheses of glycooligopeptides carrying dimeric or trimeric sialosyl Tn epitope [α-D-Neu*p*5Ac-(2→6)-D-Gal*p*NAc] as the carbohydrate branching.[5,6] We describe herewith a total synthesis of the N-terminal heptapeptide of glycophorin AM by employing the more complex tetrasaccharide units (1).

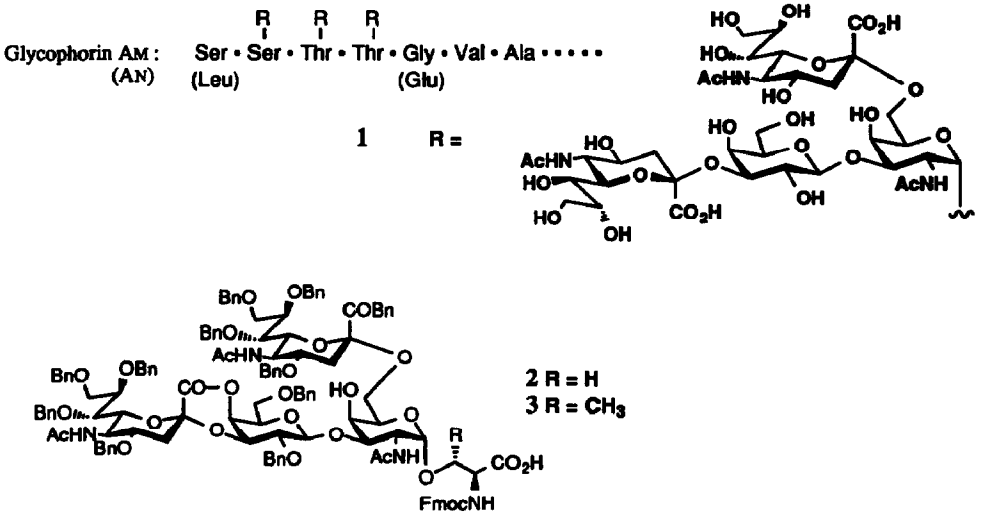

Fig 1

As reported in the previous paper,[7] we have designed and synthesized the protected tetrasaccharide-serine and -threonine conjugates 2 and 3 via stereocontrolled glycosylations by employing Gal*p*N3, Gal*p*, Ser/Thr, and Neu*p*5Ac synthons. We first describe a model experiment in order to find out a mild condition for the conversion of sialyl lactone into sialic acid. We

prepared a simpler disaccharide as the prototype lactone compound and studied the condition for lactone hydrolysis.

Mercury salt-promoted glycosylation[8] of **5**, prepared in 3 steps from p-methoxyphenyl β-D-galactopyranoside[9] (*1*. acetone, $CuSO_4$, p-TsOH, 24h, 68%; 2. BnBr, NaH, THF, 60°, 20h, 98%; *3*. 80%CF_3CO_2H aq, CH_2Cl_2, 0°, 2h, 95%), with **4**[5,8] afforded **6** (69%), which on desulfurization (Ph_3SnH, AIBN, PhH, reflux, 6h) gave rise to a mixture of **7** (64%) and **8** (25%). The compound **7** was readily converted into **8** by treatment with DBU in THF (96%). Hydrogenation of **7** [20%$Pd(OH)_2$-C, 80%MeOH aq, 5days] gave **9** (94%). Similarly **8** was debenzylated in 80%THF aq. to give **10** (89%). The lactone **10** was stable in neutral condition even after heating at 80° in D_2O for 23h, while the carboxylic acid **9** decomposed under the same condition due to autocatalysis. However, by an addition of 4eq.$NaHCO_3$ to the D_2O solution (0.05M, pH 7.5-8) the lactone ring of **10** was slowly hydrolyzed [42% (48h); 63% (120h); 75% (160h); 80% (215h); 92% (340h); ~100% (570h)] to exhibit the ^{1}H-NMR signals identical with those of **9**. On the basis of these observations concerning the properties of sialyl galactose lactone, we considered that the requisite cleavage of lactone ring would be performed after deprotection of synthetic glycooligopeptide. It is noteworthy that similar lactone in a synthetic sialyl LeX derivative has readily been hydrolyzed on standing in D_2O at 25° for 2h under neutral condition.[10]

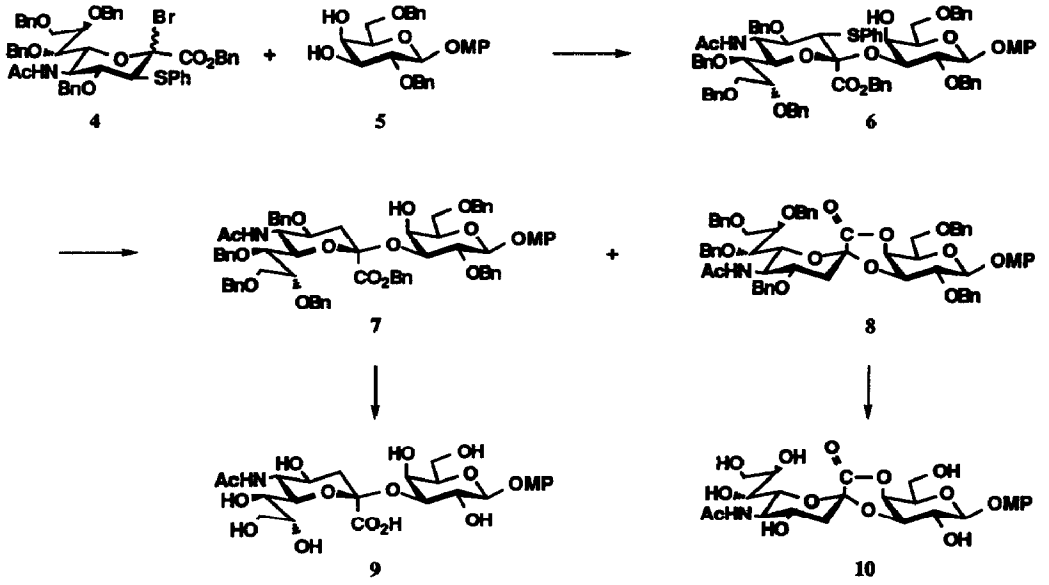

Fig 2

Synthetic program for the N-terminal heptapeptide of glycophorin AM was designed based on the Fmoc methodology and executed as summarized in Fig 3. With EEDQ or IIDQ as the coupling agent in CH_2Cl_2, the peptide chain was elongated. IIDQ was used when the amino components carried the carbohydrate branchings. Fmoc group was cleaved by treatment with morpholine. It is to be noted that the reaction between **2** and **20** afforded a high condensation yield (81%) in spite of the presence of the unfavorable bulkiness in both reaction components.

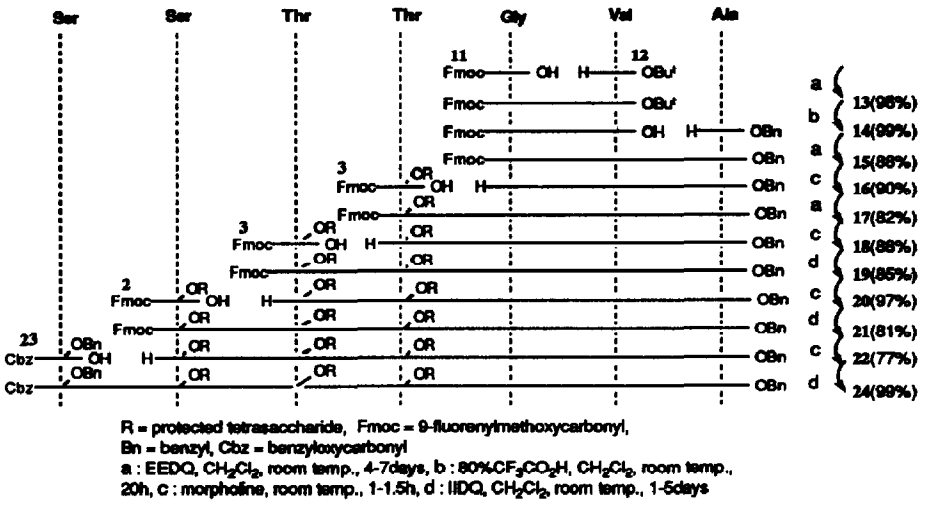

Fig 3

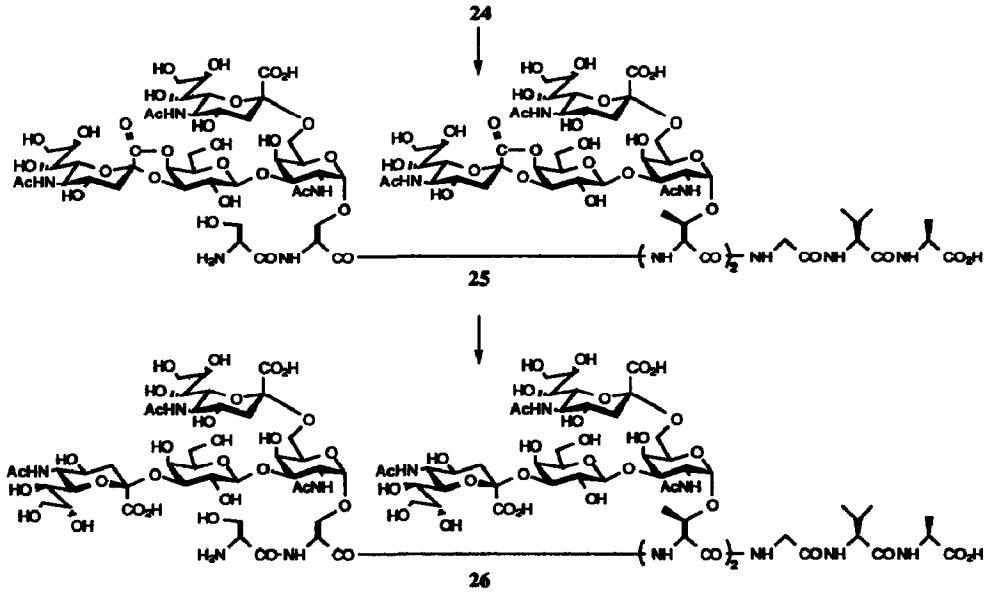

Fig 4

The protected heptapeptide-dodecasaccharide **24**[11] was hydrogenated with 20%Pd(OH)$_2$-C in 70% THF aq. to give **25**,[11] which was found to be stable as tri-lactone in D_2O after standing for several days at room temperature. By adding 20eq.$NaHCO_3$ into the D_2O solution (0.03M, pH7.5-8), hydrolysis of the lactone took place slowly as observed in the model study. After 11 days, the characteristic ^{1}H-signals assigned to the lactone (δ 2.59ppm for H-3β NeuAc and 5.25ppm for H-4 Gal) were no longer detectable. The product **26**[11] was isolated after gel permeation (Sephadex

LH-20) and ion exchange (Mono Q) chromatographies. More conveniently, when the hydrogenation of **24** was carried out in a mixture of 0.03M $NaHCO_3$ aq. and THF (3:7) for 11 days, **26** was exclusively produced and isolated quantitatively after gel permeation chromatography. The ^{1}H-NMR and FAB-MS data of the synthetic sample were compatible with the proposed structure. The carbohydrate branching structures including the configuration of Neu*p*5Ac glycosides were assigned primarily by the synthetic sequence and the reported spectra of the natural sialoglycoprotein fragments[12-15] were in good agreement with those of our synthetic sample.

In conclusion, we succeeded for the first time in a total synthesis of the glycosylated heptapeptide which carries three units of tetrasaccharide in parallel and corresponds to the human M blood group antigenic determinant in the erythrocyte membrane protein, glycophorin A.

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11. Physical data for the key compounds are given below. Values of δ_H, and $[\alpha]_D$ were measured at 23±2°C for the solution in D_2O and H_2O, respectively, unless noted otherwise. **9**: $[\alpha]_D$ -3.8° (c 0.2), δ_H 1.82(t, J 12.5Hz, H-3α NeuAc), 2.02(s, Ac), 2.77(dd, J 4.6, 12.5Hz, H-3β NeuAc), 3.80(s OMe), 5.04(d, J 7.6Hz, H-1 Gal), **10**: $[\alpha]_D$ -26.4° (c 0.5), δ_H 1.84(dd, J 11.6, 13.2Hz, H-3α NeuAc), 2.04(s, Ac), 2.62(dd, J 5.3, 13.2Hz, H-3β NeuAc), 3.80(s OMe), 5.06(d, J 8.3Hz, H-1 Gal), 5.38(d, J 4.0Hz, H-4 Gal), **24**: $[\alpha]_D$ +35.0° (c 0.4, $CHCl_3$), δ_H ($CDCl_3$) 0.79(d, J 6.4Hz, Me-Val), 0.82(d, J 6.9Hz, Me-Val), 1.02(br, 2 x Me-Thr), 1.20(d, J 5.9Hz, Me-Ala), 2.01[br, 3 x H-3β NeuAc(lactone)], 2.65(br, 3 x H-3β NeuAc), 4.94-5.18(m, CO_2CH_2Ph and H-4 Gal), FAB•MS (Pos) 6700.2 (M+1), **25**: δ_H 0.95(d, J 6.2Hz, Me-Val), 0.96(d, J 6.6Hz, Me-Val), 1.66(m, 3 x H-3α NeuAc), 1.79(brt, J 12.2Hz, 3 x H-3a NeuAc), 1.96, 1.98, 2.00, 2.02, and 2.03(5s, 9Ac), 2.59[dd, J 4.9, 13.0Hz, 3 x H-3β NeuAc(lactone)], 2.71(m, 3 x H-3β NeuAc), 5.25(brs, 3 x H-4 Gal), FAB•MS (Pos) 3411.6 (M+1), **26**: $[\alpha]_D$ +13.0° (c 0.2), δ_H 0.95(d, J 6.9Hz, Me-Val), 0.96(d, J 6.5Hz, Me-Val), 1.29(d, J 6.5Hz, Me-Thr), 1.33(d, J 7.3Hz, Me-Ala), 1.35(d, J 6.4Hz, Me-Thr), 1.68(m, 3 x H-3α NeuAc), 1.79(brt, J 12.2Hz, 3 x H-3α NeuAc), 1.98, 1.99, 2.00, and 2.02(4s, 9Ac), 2.72(m, 6 x H-3β NeuAc), 4.49(d, J 8.1Hz, 2 x H-1 Gal), 4.53(d, J 7.3Hz, H-1 Gal), 4.92(d, J 2.8Hz, H-1 GalNAc), 5.00(d, J 2.8Hz, H-1 GalNAc) FAB•MS (Neg) 3463.8 (M-1), 3484.9(M+Na-2), 3507.8(M+2Na-3), 3529.0(M+3Na-4).
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