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Chemoenzymatic Synthesis of the Human CD52 and CD24 Antigen Analogues

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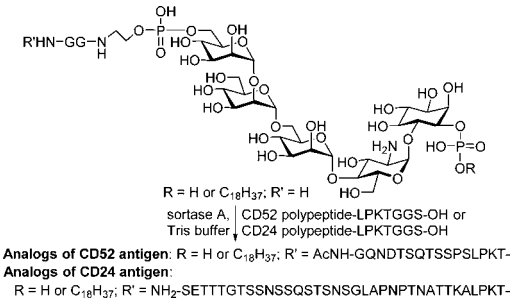
ABSTRACT



Analogs of the human CD52 and CD24 antigens carrying the common core structure of glycosylphosphatidylinositol (GPI) anchors and the intact polypeptide sequences of CD52 and CD24 were chemoenzymatically synthesized. CD52 and CD24 proteins were obtained by solid-phase peptide synthesis and then coupled to chemically synthesized GPI anchors under the influence of a bacterial enzyme, sortase A, to afford the target molecules in good yields.

The human CD52 and CD24 antigens are representative examples of glycosylphosphatidylinositol (GPI)-anchored proteins. These antigens play a vital role in many important biological processes, such as human immune recognition,[1] reproduction,[2−4] and tumor metastasis.[5−9] Moreover, they are also excellent models for the study of surface protein GPI anchorage to the extracellular membrane, a common and important phenomenon in eukaryotic species.[10−13] To gain insight into the biology of CD52 and CD24 antigens and to study protein GPI anchorage and related biological problems, it is necessary to have access to these molecules and their analogues or derivatives in sufficient quantity and purity, which is difficult to achieve through isolation and purification of natural products.

Several general synthetic strategies have been explored to address the challenge in accessing GPI-linked proteins. For instance, our group reported a convergent synthesis of GPI-linked peptides and glycopeptides via regioselective coupling of extensively protected synthetic GPI anchors to peptides/glycopeptides, which was followed by global deprotection.[14,15] The Bertozzi group[16] and the Seeberger group[17] described the synthesis of GPI-linked proteins via

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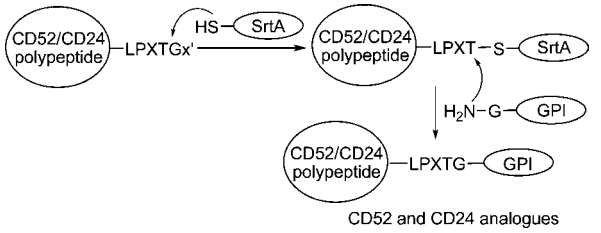
native chemical ligation (NCL) of Cys-containing GPI analogues and proteins, such as green fluorescent proteins and prion proteins. More recently, our group developed a novel synthetic strategy[18−20] that utilized sortase A (SrtA), a bacterial enzyme derived from *Staphylococcus aureus*,[21] to catalyze regioselective coupling of GPIs and peptides or glycopeptides, which may be widely applicable.

In this paper, the SrtA-based strategy was used for the synthesis of analogues of the CD52 and CD24 antigens, as outlined in Scheme 1. For this purpose, CD52 and CD24 proteins were designed to have the sorting signal LPXTG (X can be any amino acid) at the peptide *C*-terminus. SrtA can recognize the signal, react with it to break the peptide bond between T and G and form a reactive complex,[22] and finally link the carboxyl group of T to the amino group of G attached to the GPI anchor. GPI anchors utilized in this research were **1** and **2** (Figure 1) containing the intact GPI common core structure without and with a lipid chain on the inositol unit, respectively. In this case, the resultant GPI−protein conjugates can be used to investigate how the lipid chain may affect the structure and functions of GPI anchors and GPI-anchored proteins.

Scheme 1. General Design for the Synthesis of CD52 and CD24 Analogues



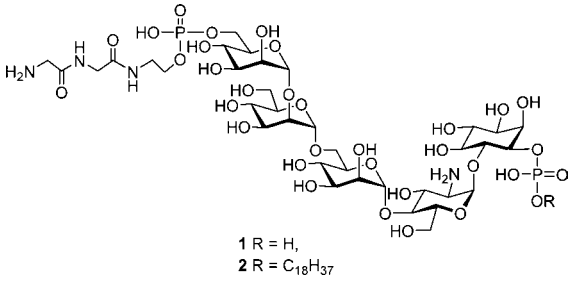
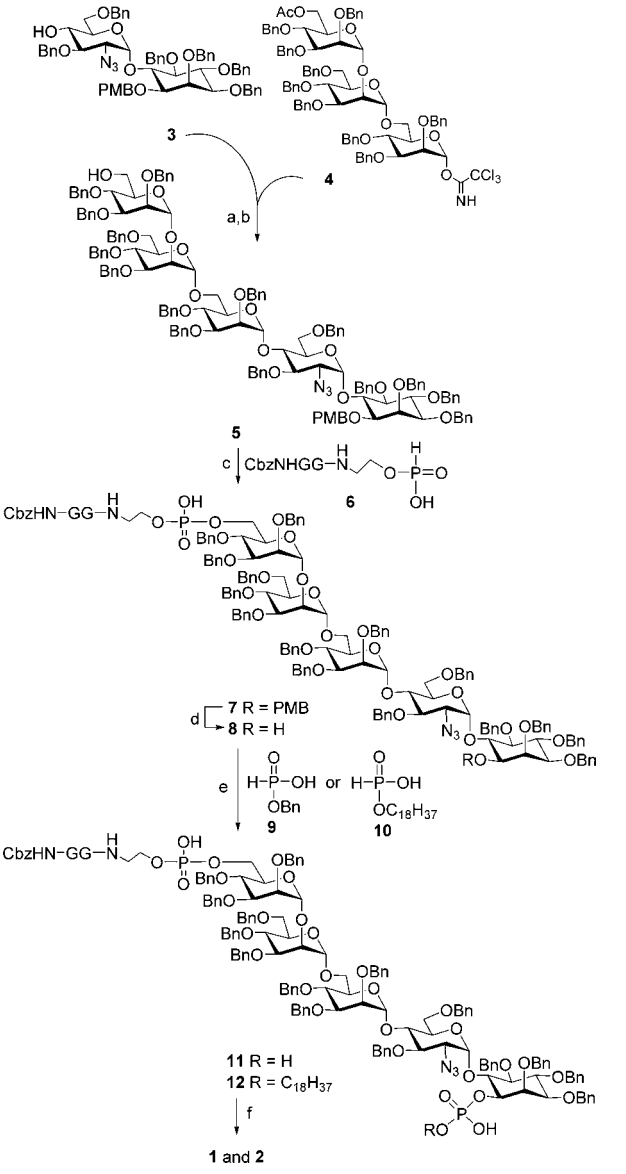


Figure 1. GPI anchors **1** and **2** used to synthesize CD52 and CD24 analogues.

The synthesis of GPIs **1** and **2** is depicted in Scheme 2. Glycosylation of pseudodisaccharide **3** with trimannosyl donor **4**, prepared according to reported procedures,[23,24] was α-selective to give a pseudopentasaccharide that was deacetylated under basic conditions to afford **5** in a 76% yield over two steps. The coupled DEPT NMR spectrum of **5** proved the α-configuration of all mannosyl residues judging from H1−C1 coupling constants. Phosphorylation of **5** was achieved by the one-pot two-step *H*-phosphonate method,[25] including reaction of **5** with **6** in the presence of pivaloyl chloride and thereafter oxidation of

Scheme 2. Synthesis of GPI Anchors **1** and **2**[a]



[a] Reaction conditions: (a) TMSOTf, Et_2O, −40 °C; (b) NaOMe, CH_2Cl_2/MeOH, 76% (over two steps); (c) PivCl, pyridine, rt; then I_2, pyridine/H_2O, 74%; (d) 5% TFA in CH_2Cl_2, 69%; (e) (1) PivCl, pyridine, rt; (2) I_2, pyridine/H_2O, 70−72%; (f) H_2, 10% $Pd(OH)_2$/C, 76−88%.

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the resultant intermediate with iodine. Subsequently, **7** was treated with 5% trifluoroacetic acid in dichloromethane to get rid of the *p*-methoxybenzyl (PMB) protecting group, thereby exposing the inositol 1-*O*-position. Once again, the *H*-phosphonate method was used to install phosphoric acid and phospholipid moieties to **8** by means of **9** and **10**. Both reactions proceeded smoothly to give **11** and **12** in good yields. Finally, **11** and **12** were globally deprotected under a H_2 atmosphere in THF, MeOH, and H_2O (2:1:1) by using 10% $Pd(OH)_2/C$ as the catalyst to generate the desired GPI anchors **1** and **2**, ready for SrtA-catalyzed coupling to proteins. All synthetic intermediates involved in the synthesis and the final products were characterized with NMR and mass spectroscopies.

In the meantime, small proteins **13** and **14** containing the sorting signal and the intact polypeptide sequences of the CD52[26] and CD24[1] antigens were prepared by solid-phase peptide synthesis. To achieve GPI–protein coupling, GPIs **1**/**2** and proteins **13**/**14** were incubated with SrtA at 37 °C in Tris–HCl buffer (0.3 M, pH 7.5) containing 150 mM NaCl, 5 mM $CaCl_2$, and 0.5 mM mercaptoethanol (Scheme 3). The peptide, GPI, and SrtA concentrations were 0.5 mM, 2.5 mM, and 30 μM, respectively. Here, GPIs were used in relatively large excess (5 equiv) in order to suppress the reverse reaction. After 24 h of incubation, the reactions were quenched with 0.1% trifluoroacetic acid and then subjected to HPLC analysis and separation. It was observed that the reactions afforded the target molecules **15**, **16**, **17**, and **18** in 45%, 48%, 69%, and 51% yields, respectively. The reaction products were positively identified by MALDI-TOF MS. The calculated molecular weights of **15**, **16**, **17**, and **18** were 2815, 4564, 3067, and 4816, and their observed *m*/*z* values were 2814 (negative mode), 4563 (negative mode), 3068 (positive mode), and 4817 (positive mode), respectively. Furthermore, due to the presence of a lipid chain in the structure of **17** and **18**, these two conjugates ought to be more lipophilic than **15** and **16**. Indeed, it was found that while the HPLC retention time for **15** and **16** on a C-18 column eluted with 5–20% aqueous CH_3CN was 20.9 and 24.0 min, respectively, **17** and **18** were retained by the C-18 column under the same conditions and could not be washed out even by a higher concentration of CH_3CN. The HPLC retention time for **17** and **18** on a C-8 column eluted with 10%-80% aqueous CH_3CN was 31.9 and 31.2 min, respectively. These results provided additional proof to verify that in the presence of SrtA GPIs **1** and **2** were indeed coupled to proteins **13** and **14** to afford **15**–**18**.

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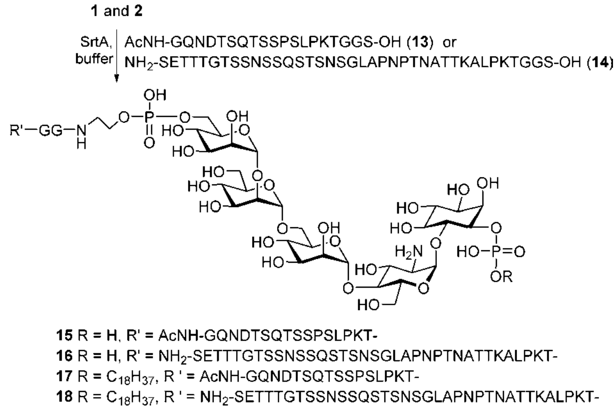
Scheme 3. SrtA-Mediated Synthesis of CD52 and CD24 Analogues



In brief, several analogues of the human CD52 and CD24 antigens containing the common core of GPI anchors and the intact peptide sequences of CD52 and CD24 antigens were synthesized via SrtA-catalyzed ligation of synthetic GPI anchors and proteins. These CD52 and CD24 analogues are useful not only for the study of CD52 and CD24 but also for structural, functional, and various other biological studies of GPIs and GPI-anchored proteins. Furthermore, this work has proved that SrtA could accept intact GPIs as substrates for ligation with proteins, demonstrating that the SrtA-based synthetic strategy may be generally useful for other GPI-anchored proteins as well.

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Supporting Information Available. Experimental procedures, ^{1}H, ^{13}C, and ^{31}P NMR spectra of intermediates involved in GPI synthesis, and mass spectra of the key intermediates and the final products. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.