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# Synthetic Tumor-Associated Glycopeptide Antigens

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**Glycopeptides with $T_N$ antigen (GalNAc)Ser/Thr and T-antigen structures (βGall-3GalNAc)Ser/Thr, described as tumor-associated antigens, were synthesized and coupled to bovine serum albumin. Alternatively, synthetic methods for the construction of β-anomeric analogues of the $T_N$ and T-antigen glycopeptides were developed, aiming at antigenic structures having a varied stereochemistry of the linkage between the carbohydrate and the peptide moiety. As a further type of potential tumor-associated antigen, fucosyl-chitobiose asparagine glycopeptides were synthesized, deprotected, and coupled to bovine serum albumin. The chemical methods developed now make the complex sensitive glycoprotein partial structures accessible in analytically pure form and in preparative amounts.**

## Introduction

Carbohydrate structures of the outer cell membrane glycoproteins are suspected to exhibit decisive functions in intercellular communication and in the regulation of cell growth (*1*). In agreement with this molecular mechanistic aspect, it has been found that abnormal cell growth and tumor development parallel a dramatic change in the structures of membrane glycoproteins (*2*). The altered glycoproteins of the tumor cell membranes seem to be tumor-associated antigens. In this sense, Springer and colleagues reported in a number of articles that glycoproteins of the $T_N$ and T-antigen type represent tumor-associated antigen structures (*3*) (Fig. 1). According to their results, these structures occur in exposed form only in epithelial tumors, whereas they are not present in normal cells of the same tissues (*4*).

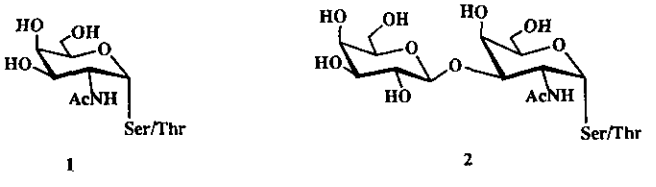


FIGURE 1. Glycoproteins of the $T_N$ (1) and T-antigen (2).

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## Synthetic Glycopeptides with $T_N$ and T-Antigen Structure

With the aim of making related glycopeptides available for immunological evaluations, we have elaborated selective syntheses of compounds with such tumor-associated antigen structures (*5*). The coupling of the synthetic glycopeptide antigens to bovine serum albumin was achieved by applying a special carbodiimide procedure in water without introducing an artificial linker component to give the conjugates 3 and 4 (Fig. 2). According to the carbohydrate analysis data, the conjugate 3 contains an average of 25 synthetic glycopeptide units coupled to the protein, whereas conjugate 4 carries 38 T-antigen glycopeptide units per protein molecule. The synthetic antigens 3 and 4 have the particular advantage that they are not microheterogeneous in the carbohydrate part. The microheterogeneity of glycoproteins isolated from bio-

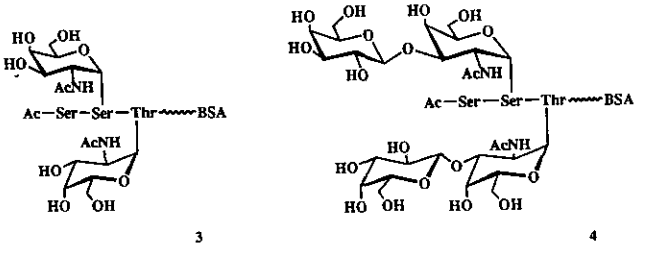


FIGURE 2. Structures of the synthetic antigens 3 and 4.

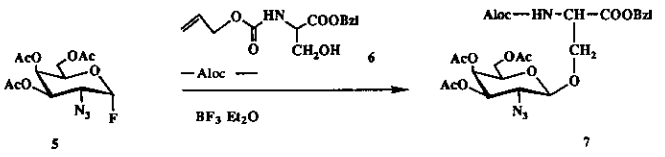


FIGURE 3. Reaction of glycosyl fluoride (5) with allyloxycarbonyl serine benzyl ester (6) to produce a glycoconjugate with galactosamine β-glycosidically linked to serine or threonine (7).

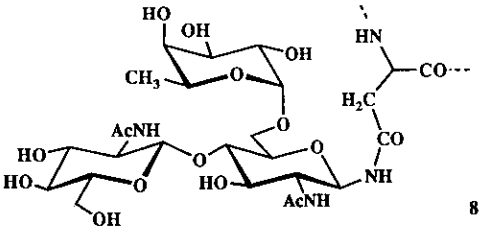


FIGURE 4. An *N*-glycoprotein fucosylated in the core region.

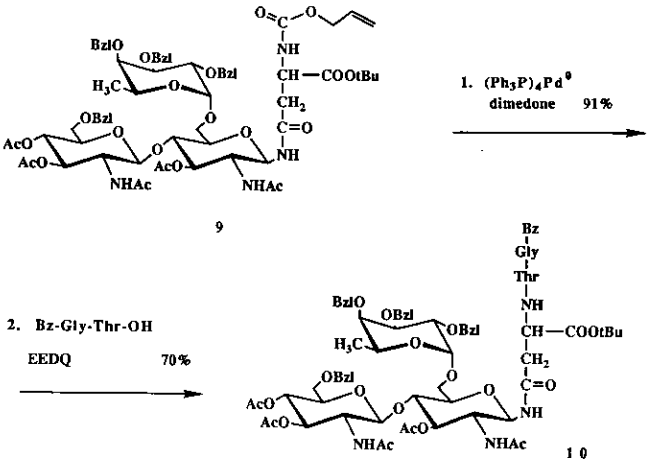


FIGURE 5. Synthesis of a branched fucosylated chitobiosyl amine.

logical sources often causes serious difficulties in immunological applications and leads to irreproducible effects.

Recent results of investigations on tumor-associated immunodeterminant glycan structures suggest that the T-antigen disaccharide analogue with β-anomeric configuration at the penultimate galactosamine unit, rather than the natural T-antigen 2 itself, shows a tumor-associated reactivity (*6*). Therefore, we changed the concept of the glycopeptide syntheses and now aimed at structures containing galactosamine β-glycosidically linked to serine or threonine. The synthesis of glycoconjugates with these structural features was achieved, for example, by reacting the glycosyl fluoride with allyloxycarbonyl serine benzyl ester (Fig. 3).

In spite of the non-neighboring group active protection of the glycosyl donor 5, the β-anomeric conjugate is obtained selectively. The method is of fundamental importance for the projected syntheses of β-anomerically linked $T_N$ and T-antigen glycopeptide analogues. An alternative route for the synthesis of β-analogous $T_N$ and T-antigen glycopeptides consists in Koenigs-Knorr type reactions of the corresponding glycosyl bromides with serine and threonine derivatives with modified protecting group compositions. The efficiency of this concept could be demonstrated in principle by a β-selective conjugation of the T-antigen disaccharide with threonine.

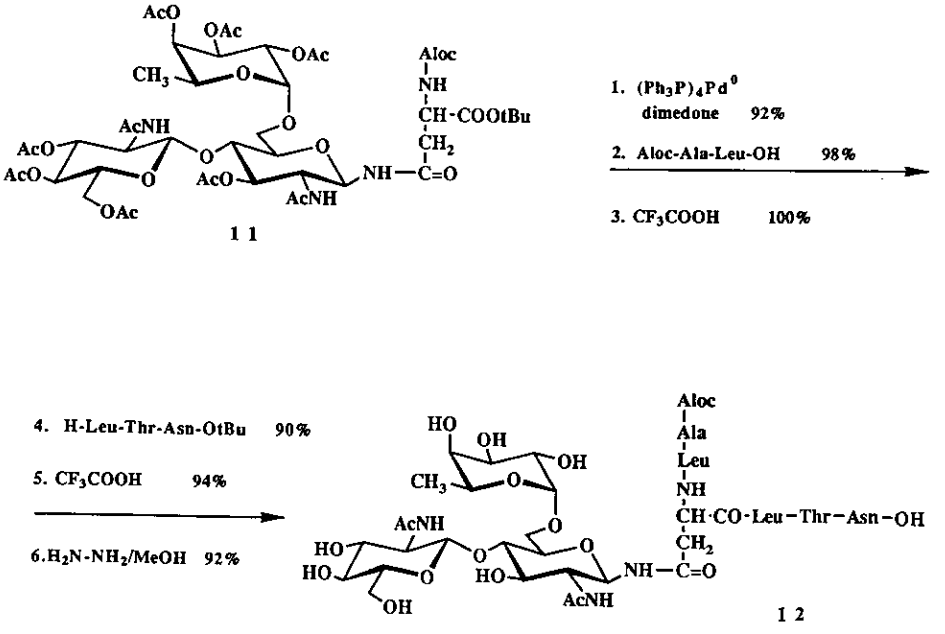


FIGURE 6. Synthesis of a saccharide asparagine conjugate.

## Fucosyl Chitobiose-Asparagine Glycopeptides

*N*-Glycoproteins fucosylated in the core region have also been suspected to be structures associated with tumor membranes (*7*). Therefore, glycopeptides of type 8 (Fig. 4) were selected as another goal in our syntheses of antigenic structures.

The synthesis of the branched fucosylated chitobiosyl amine requires some 20 steps. During the further processing of the trisaccharide asparagine conjugate 9 (Fig. 5), the allyloxycarbonyl (Aloc) protecting group was revealed to be a very efficient and selective tool (*8*). It is removable quite selectively and almost quantitatively by means of a palladium-catalyzed allyl transfer to the weakly basic morpholine. Subsequent chain extension yielded the trisaccharide tripeptide 10 (Fig. 5), a structure which is part of an influenza virus neuraminidase. The projected removal of the C-terminal *tert*-butyl ester protection, a precondition for the immunological evaluation of the synthetic glycopeptide, was shown to be impossible.

The treatment of compounds like 10 (Fig. 5) with trifluoroacetic acid resulted in complete cleavage of the fucoside bond. To overcome this serious problem in the synthesis of fucosylated glycopeptides, a new trisaccharide synthesis was elaborated. It aimed at the synthesis of a saccharide asparagine conjugate 11 (Fig. 6) that exclusively carries acetyl protecting groups in the carbohydrate moiety.

The synthesis of conjugate 11 requires almost 30 steps. The useful Aloc protecting method allowed a highly efficient and selective deblocking and N-terminal chain extension. The selective cleavage of the C-terminal *tert*-butyl ester of the resulting trisaccharide tripeptide using trifluoroacetic acid was now achieved without destruction of the fucoside bond. The selectively deblocked product could be condensed efficiently with a tripeptide ester to form the corresponding trisaccharide hexapeptide, a protected partial sequence of an envelope glycoprotein of a murine leukemia virus.

Further C-terminal deprotection and removal of the ester groups from the carbohydrate part delivered the free fucosyl chitobiose glycopeptide 12, which is interesting as a virus envelope structure as well as a potential tumor-associated antigen. Glycopeptide 12 was then coupled to bovine serum albumin by applying the method elaborated for the T-antigen glycopeptide. The conjugate obtained contains about 12% carbohydrate. It is currently being investigated for immunological applications.

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