Application Note

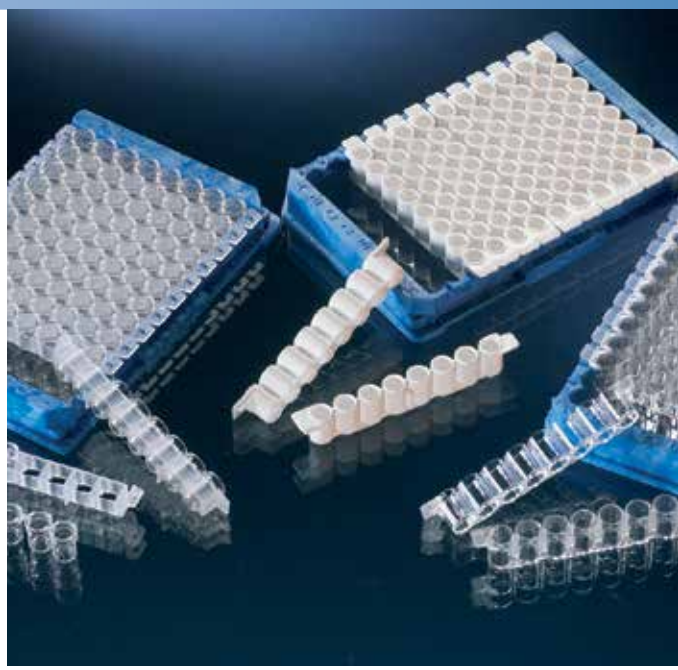
Coupling of Oligosaccharides to Thermo Scientific Nunc CovaLink NH Modules

Key Words

Thermo Scientific™ Nunc™ CovaLink™ NH Modules, solid phase, ELISA, activation with NHS/EDC, carbohydrates, oligosaccharides, binding of activated lactose.

Goal

The goal of this application note is to describe the use of carbohydrates covalently immobilized and detected on the Nunc CovaLink NH Modules. This is exemplified by the Erythrina corallodendron – lactose interaction 5.



The interest in carbohydrates has increased. One of the main reasons for this is due to the results obtained through research in cell communication, e.g. between leucocytes and in bacterial adhesion.

From that point of view carbohydrates are remarkable information molecules compared to peptides and nucleotides.

If, for instance, two identical monosaccharides are combined, 11 different disaccharides can be built. Whereas, if two identical aminoacids are combined, only one dipeptide can be formed, and with four different monosaccharides 35,560 unique tetrasaccharides can be created. It is no wonder then that carbohydrates by nature have been assigned a role in communication within living creatures [1,2].

The diversity of polysaccharides has, however, been a problem in the search for biologically relevant carbohydrate structures for use in the struggle against infection and cancer. Not only is it difficult to collect enough material for examination, it has also been difficult to perform carbohydrate chemistry.

However, synthetically made oligosaccharides have become a potential tool in the search for active structures. The technology now has evolved to a level comparable to that employed in peptide and nucleotide synthesis, hence a wide range of synthetic saccharides are now commercially available.

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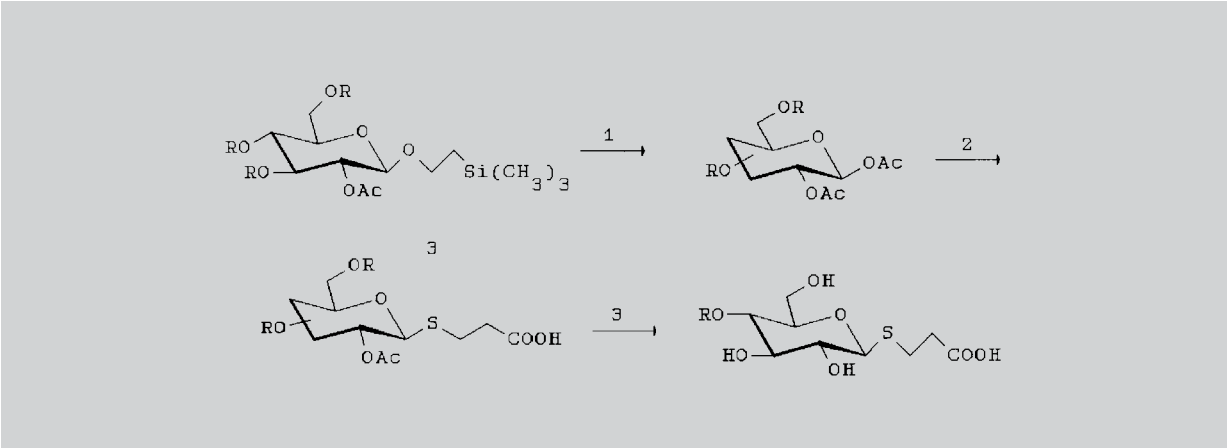
Synthesis of oligosaccharide utilizing the 2-(Tri-methylsilyl) -ethyl (TMSEt) group for anomeric protection.



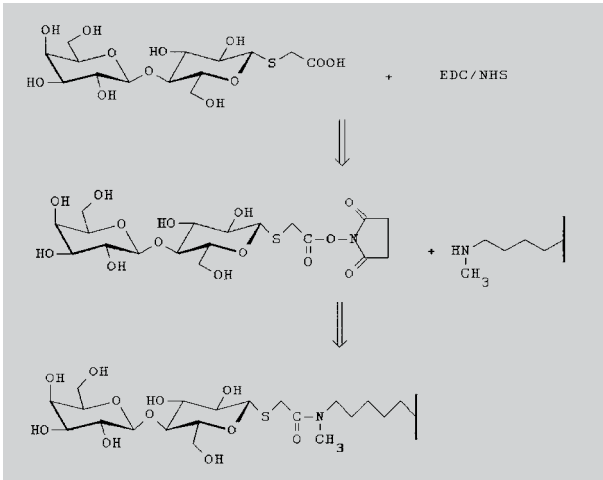
R = acryl group or »protected« sugar residues
A = acetyl group

1) Typically 3 equivalents of acetic anhydride and 1.1 equivalents of boron trifluoride-etherate in chloroform or toluene at room temperature for 1 hour. Purification by column chromatography [3].

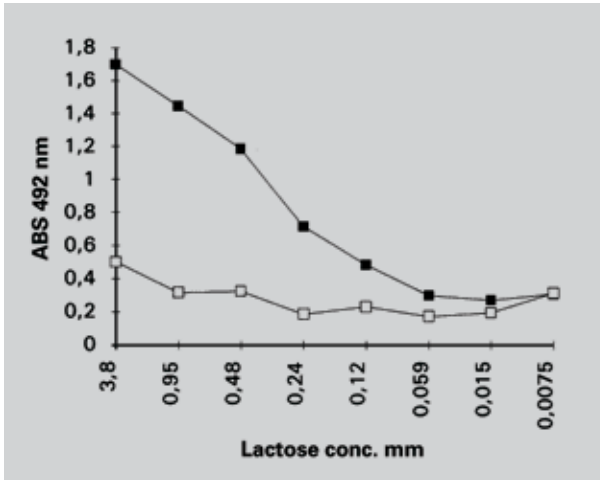
2) Mercaptopropionic acid (2.5-3 equivalents) and boron trifluoride-etherate (1.2 equivalents) in methylene chloride at 0°C [4]. Purification by column chromatography.

3) Deacetylation using sodium methoxide in methanol. Neutralization and chromatographic purification normally produces the deblocked sugar in quantitative yield.

Coupling to Nunc CovaLink NH



Binding of activated lactose on Nunc CovaLink NH



Coupling of sugar to Nunc CovaLink NH and detection of binding exemplified by the Erythrina corallodendron – lactose interaction 5.

Lactose activated (A-Lac) according to the method described above was used:

1. A 1:2 dilution of A-Lac was made in Nunc CovaLink NH wells. 100 µL 7.8 mm A-Lac in H_2O were added to wells in column 1. 50 µL H_2O were added to the rest of the wells, 50 µL were transferred from column 1 to column 2, etc. After mixing, 50 µL were disposed of from column 12. Finally NHS/EDC in aquous solution (25 µg each/well) was added to all wells (50 µL/well).
2. The plate was incubated on shaker at RT for 1.5 hour.
3. Blocked using PBS + 0.5% BSA overnight at 4°C (200 µL/well).
4. Rinsed with PBS + 0.5% BSA + 0.05% Triton X-100, 3 times 200 µL/well.
5. Biotinylated Erythrina corallodendron (10 µg/mL PBS + 0.5% BSA) corresponding to 0.36 µm regarding binding sites (100 µL/well).
6. Incubated for 2 hours at RT.
7. Rinsed using CovaBuffer (PBS + 2 M NaCl + 65 mm $MgSO_4 \cdot 2\ H_2O$ + 0.05% Tween 20) 3 times 200 µL/well.
8. Horseradish peroxidase conjugated Avidine added (1:1000 v/v in PBS + 0.5% BSA) (100 µL/well).
9. Incubated for 1 hour at RT.
10. Rinsed using CovaBuffer 3 times 200 µL/well.
11. Substrate (H_2O_2, OPD; 100 µL/well) 5 min. at RT; the reaction stopped by adding 1M H_2SO_4 (100 µL/well).
12. The results read at 492 nm.

References

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