Carbohydrate Derived Azides as Substrates for Cellobiose Phosphorylase from *Clostridium Thermocellum*

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Cellobiose phosphorylase from *Clostridium thermocellum* was examined for its ability to catalyze the synthesis of cellobiose derivatives with glucose-1-phosphate as the donor and azido analogues of glucose as acceptors. We have prepared the 2-azido-2-deoxy, 3-azido-3-deoxy and 6-azido-6-deoxy analogues of glucose as analogues of the reducing glucose moiety, as well as the diastereoisomer allose with a view to producing novel oligosaccharides for pharmaceutical applications.

In this manner, 6-azido-deoxy-cellobiose was synthesized in good yield and was shown to be identical with a sample prepared by purely organic-chemical synthesis. To confirm the substrate property of 6-azido-6-deoxy-glucose, we have demonstrated the enzyme-catalyzed cleavage of 6-azido-6-deoxy-cellobiose into glucose 1-phosphate and 6-azido-6-deoxy-glucose. By contrast, the 6'-azido-6'-deoxy-cellobiose, expected to yield 6-azido-6-deoxy-glucose-1-phosphate and glucose upon enzyme-catalyzed cleavage, was not a substrate of cellobiose phosphorylase from *Clostridium thermocellum*. Attempts also failed to incorporate 3-azido-3-deoxy-glucose or allose in place of the reducing glucose moiety of cellobiose.

Interestingly, cellobiose phosphorylase seems to be able to incorporate a further 6-azido-6-deoxy-glucose unit into 6-azido-deoxy-cellobiose as acceptor to form a trisaccharide. The structural characterization of this product is currently in progress.