

# Acid Hydrolysis of Neutral Glycosphingolipids

Thesis submitted in fulfillment of the degree of Doctorate of Philosophy

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## **Attestation of Authorship**

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made.

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## Abstract

Blood group glycolipids are important tools in the study of microbial receptor interactions and other biological phenomena. Presently blood group glycolipids of interest are isolated from biological samples. However, all glycolipids are not readily available due to the low frequency of some phenotypes in the general population. The ability to acquire the rare glycolipids from the degradation of common glycolipids would be a useful alternative to trying to obtain the molecules from biological sources.

This research set out to establish the ability of blood group glycolipids to be degraded into useful glycolipids in a controlled manner by acid hydrolysis and possibly metal catalysis. The initial experiments investigated the degradation/hydrolysis of the more readily available glycolipid globoside with a range of salts and acids to establish degradation concepts such as; temperature, type of acid, acid concentration, and the role of metal ions in glycolipid degradation. These concepts then led to a series of degradation experiments with the blood group glycolipids Le<sup>b</sup> and ALe<sup>b</sup>. These glycolipids were incubated with a range of acid concentrations and varying temperatures. Thin layer chromatography separation and chemical and immunochemical staining were the main methods used to identify the products of degradation.

It was established that metal ions were not directly involved in the catalysis of glycolipids in the short-term, however some metal ions were indirectly implicated in their degradation due to their ability to form acid solutions. Acid hydrolysis was established as the principle mechanism for glycan chain degradation. In general it was found that the glycan chain primarily lost its fucose groups (in no particular order) and was then followed by sequential degradation of the remaining glycan chain. The glycan chain also appeared to have a protective function on the ceramide moiety. Degradation of globoside established a simple sequential pathway of glycan chain reduction from the non-reducing end. Blood group glycolipids ALe<sup>b</sup> and Le<sup>b</sup> first lost their fucose side groups followed by sequential reduction of the glycan chain. Although not fully controllable, degradation of Le<sup>b</sup> was able to produce Le<sup>a</sup>, Le<sup>d</sup> and Le<sup>c</sup>. In contrast degradation of ALe<sup>b</sup> did not produce any Le<sup>a</sup> or Le<sup>d</sup>. Instead A-type 1 and two novel A-like structures, 'linear A' and 'GalNAc-Le<sup>a</sup>' were generated. Le<sup>c</sup> was only produced from ALe<sup>b</sup> in extremely acidic conditions.

This research established the ability to generate, by acid hydrolysis, a range of rare and "unnatural" novel glycolipids from more commonly available structures. It is of interest that the so-called unnatural glycolipids obtained from the acid hydrolysis of ALe<sup>b</sup> may, in theory, occur naturally in the acid environment of the stomach, and as such could have the

potential to be implicated in disease. It is probable that by applying the principles learned here, a range of novel and natural structures suitable for use in the study of biological interactions can be obtained.

## Abbreviations

ALe<sup>b</sup>, A Lewis b, GalNAc $\alpha$ 1-3Gal(Fuc $\alpha$ 1-2) $\beta$ 1-3GlcNAc(Fuc $\alpha$ 1-4) $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

A-type 1, GalNAc $\alpha$ 1-3Gal(Fuc $\alpha$ 1-2) $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

A-type 2, GalNAc $\alpha$ 1-3Gal(Fuc $\alpha$ 1-2) $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

B-type 1, Gal $\alpha$ 1-3Gal(Fuc $\alpha$ 1-2) $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

B-type 2, Gal $\alpha$ 1-3Gal(Fuc $\alpha$ 1-2) $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Cer, Ceramide

Fuc, L-fucose

G-4-4, globoside, GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Gal, D-galactose

GalNAc, N-acetyl galactosamine

GalNAc-Le<sup>a</sup>, GalNAc $\alpha$ 1-3Gal $\beta$ 1-3GlcNAc(Fuc $\alpha$ 1-4) $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

GC, Gas Chromatography

Glc, D-glucose

GlcNAc, N-acetyl glucosamine

Le<sup>a</sup>, Lewis a, Gal $\beta$ 1-3GlcNAc(Fuc $\alpha$ 1-4) $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Le<sup>b</sup>, Lewis b, Gal(Fuc $\alpha$ 1-2) $\beta$ 1-3GlcNAc(Fuc $\alpha$ 1-4) $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Le<sup>c</sup>, Lewis c / Type 1 precursor, Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Le<sup>d</sup>, Lewis d / H type 1, Gal(Fuc $\alpha$ 1-2) $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Linear A, GalNAc $\alpha$ 1-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer

Man, D-mannose

MS, Mass Spectrometry

NMR, Nuclear Magnetic Resonance

TLC, Thin Layer Chromatography