I. Introduction

High-resolution NMR spectroscopy has become an invaluable technique in the study of biopolymers and of their constituents. Using $^1$H or $^{13}$C nuclei as probes information can be obtained about primary structures, conformations and intermolecular interactions of biomolecules in solution. In particular, the possibility to record spectra of underivatized compounds in aqueous solutions allows to afford further insight into the way of action of biomolecules under physiological conditions. For general reviews of high-resolution NMR spectroscopy in the study of biological systems the reader is referred to the recent books of BERLINER and REUBEN (1978, 1980), JARDETZKY and ROBERTS (1981), and SHULMAN (1979).

In 1977 we introduced the application of high-resolution $^1$H-NMR spectroscopy as a new method for structure elucidation of carbohydrate chains present in glycoproteins. These studies have shown that sialylated carbohydrate chains can completely be characterized with regard to the sialic acid residues. In this chapter relevant $^1$H- and $^{13}$C-NMR parameters of free and glycosidically bound sialic acids will be discussed.
II. $^1$H-NMR Spectroscopy

1. N-Acetyl- and N-Glycolyneuraminic Acid

As early as 1968 60 MHz $^1$H-NMR spectra were published of Neu5Ac in $^2$H$_2$O (CHAPMAN et al. 1968, KIMURA and TSURUMI 1968, BLIX and JEANLOZ 1969). Such a spectrum is given in Fig. 1. It shows a singlet belonging to the acetamido methyl protons at $\delta$ 2.05. Two broad signals are found at $\delta$ 3.7 and $\delta$ 3.9 comprising most of the skeleton protons. Although the investigators realized that this technique could be very promising for biochemical purposes, hardly any structural information can be obtained from this low-resolution NMR spectrum.

The recent development of NMR spectrometers operating at higher magnetic fields (up to 11.7 Tesla, equivalent to a frequency of 500 MHz for protons) together with the advances in computer capabilities have led to an enormous improvement in spectral resolution and in sensitivity. This progress is excellently reflected in the 500 MHz $^1$H-NMR spectrum of Neu5Ac in $^2$H$_2$O, as presented in Fig. 2. The spectrum consists of two subspectra belonging to the $\alpha$- and $\beta$-anomer of Neu5Ac. These anomers occur in a molar ratio of 7 : 93, respectively (JAQUES et al. 1977, HAVERKAMP et al. 1978, DABROWSKI et al. 1979, BEAU et al. 1980, FRIEBOHIN et al. 1980 a, b, HAVERKAMP et al. 1982). The 500 MHz $^1$H-NMR spectrum given in Fig. 2 allows the assignment of several of the signals for the $\alpha$-anomer of Neu5Ac. The spectrum of the $\beta$-anomer could completely be interpreted (BROWN et al. 1975, BEAU et al. 1980, HAVERKAMP et al. 1982). Chemical shifts and coupling constants are summarized in Table 1. It should be noted that the chemical shifts of Neu5Ac protons are pH-dependent. For the $\beta$-anomer the signals shift upfield upon increasing the pH from 2 to 7, e.g. for H3eq from $\delta$ 2.313 to $\delta$ 2.208 and for H3ax from $\delta$ 1.880 to $\delta$ 1.827 (see Table 1) (HAVERKAMP et al. 1982).