



## Introduction

Natural Pet. Health provided two lots of rapamycin to determine the chemical identification and organic impurity by  $^1\text{H}$  NMR spectroscopy. The information for the sample provided is listed in Table 1.

Table 1. Rapamycin sample received

Triclinic Sample Number	Compound	Additional Sample Information	Analysis Performed
TCL15544	Rapamycin	2 x 1 mg powder	$^1\text{H}$ NMR

## Result and conclusion

The sample received from Natural Pet. Health is identified as rapamycin, and contains trace amount of methanol impurity according to its comparison of  $^1\text{H}$  spectrum to literature data.

The full  $^1\text{H}$  NMR spectra of rapamycin sample (TCL15544) and pure rapamycin<sup>1</sup> from literature are displayed in Figure 1 and 2, respectively. When comparing two spectra, the chemical shifts (peak positions) and multiplicity (coupling) of rapamycin sample in the  $^1\text{H}$  NMR spectrum are consistent with those of pure rapamycin from the literature data, except for a negligible methanol impurity peak at 3.49 ppm (Figure 4).<sup>2</sup> Estimated methanol impurity is 0.056% (w/w) by NMR integrations.

## Experimental

### $^1\text{H}$ Nuclear Magnetic Resonance (NMR) Spectroscopy

The  $^1\text{H}$  NMR spectrum was acquired on Bruker 400 MHz spectrometer at Triclinic Labs. About 2 mg of a sample was dissolved in  $\text{CDCl}_3$  and the resulting solution was transferred into a 5-mm NMR tube for subsequent data acquisition. The spectrum was processed using TopSpin v3.2 and referenced to the chemical shift of the residual  $\text{CDCl}_3$  peak (7.26 ppm).

<sup>1</sup> Cholkar, K., Gunda, S., Earla, R. et al., Nanomicellar Topical Aqueous Drop Formulation of Rapamycin for Back-of-the-Eye Delivery, AAPS PharmSciTech, 16, 610–622 (2015).

<sup>2</sup> Nicholas R. Babij, Qiang Yang. Et al., NMR Chemical Shifts of Trace Impurities: Industrially Preferred Solvents Used in Process and Green Chemistry, Org. Process Res. Dev., 20, 3, 661–667 (2016).

Figure 1. Full  $^1\text{H}$  NMR spectrum of TCL15544 (rapamycin) in  $\text{CDCl}_3$ .

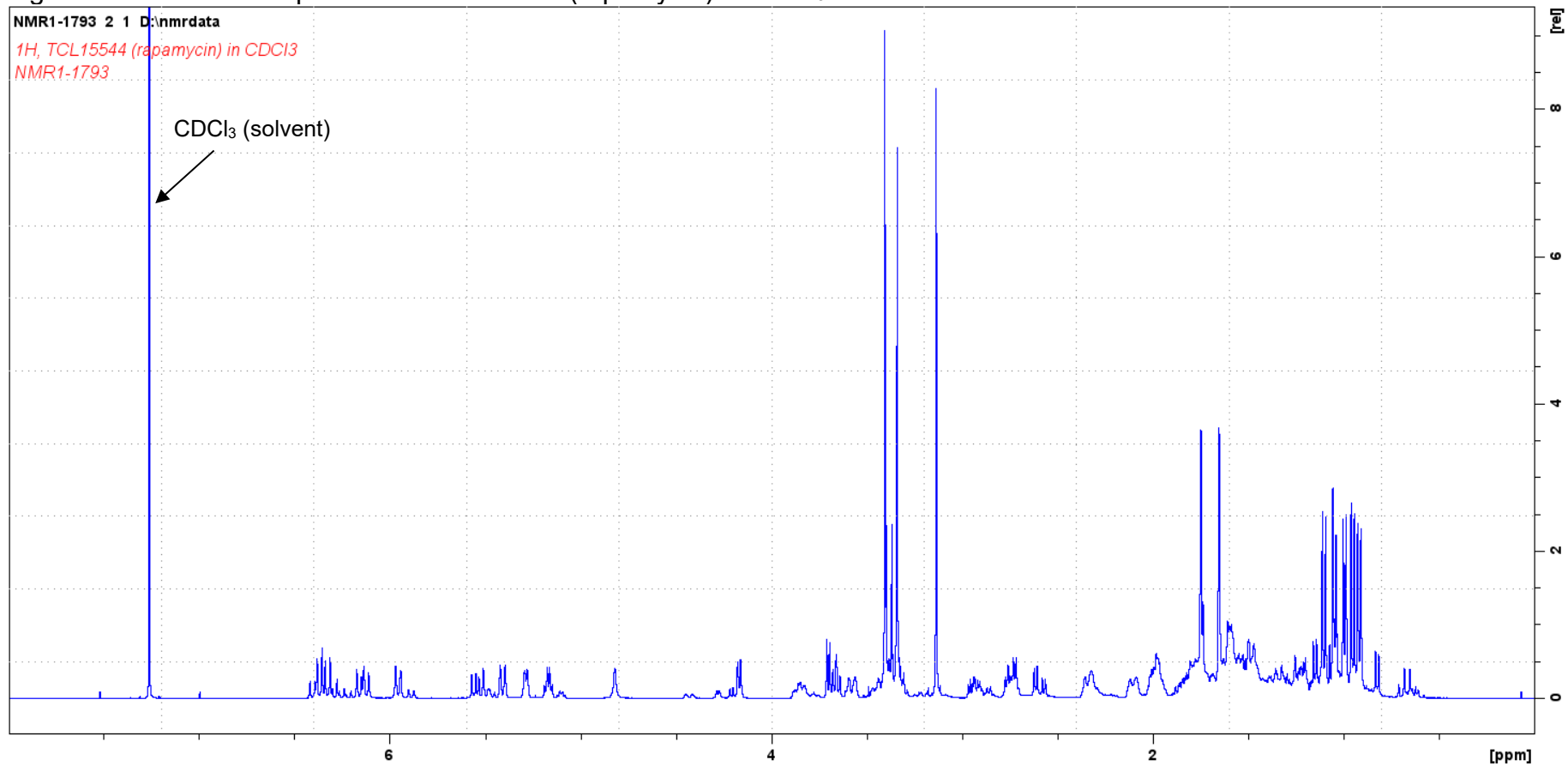


Figure 2. Literature  $^1\text{H}$  NMR spectrum of pure rapamycin in  $\text{CDCl}_3$ .<sup>1</sup>

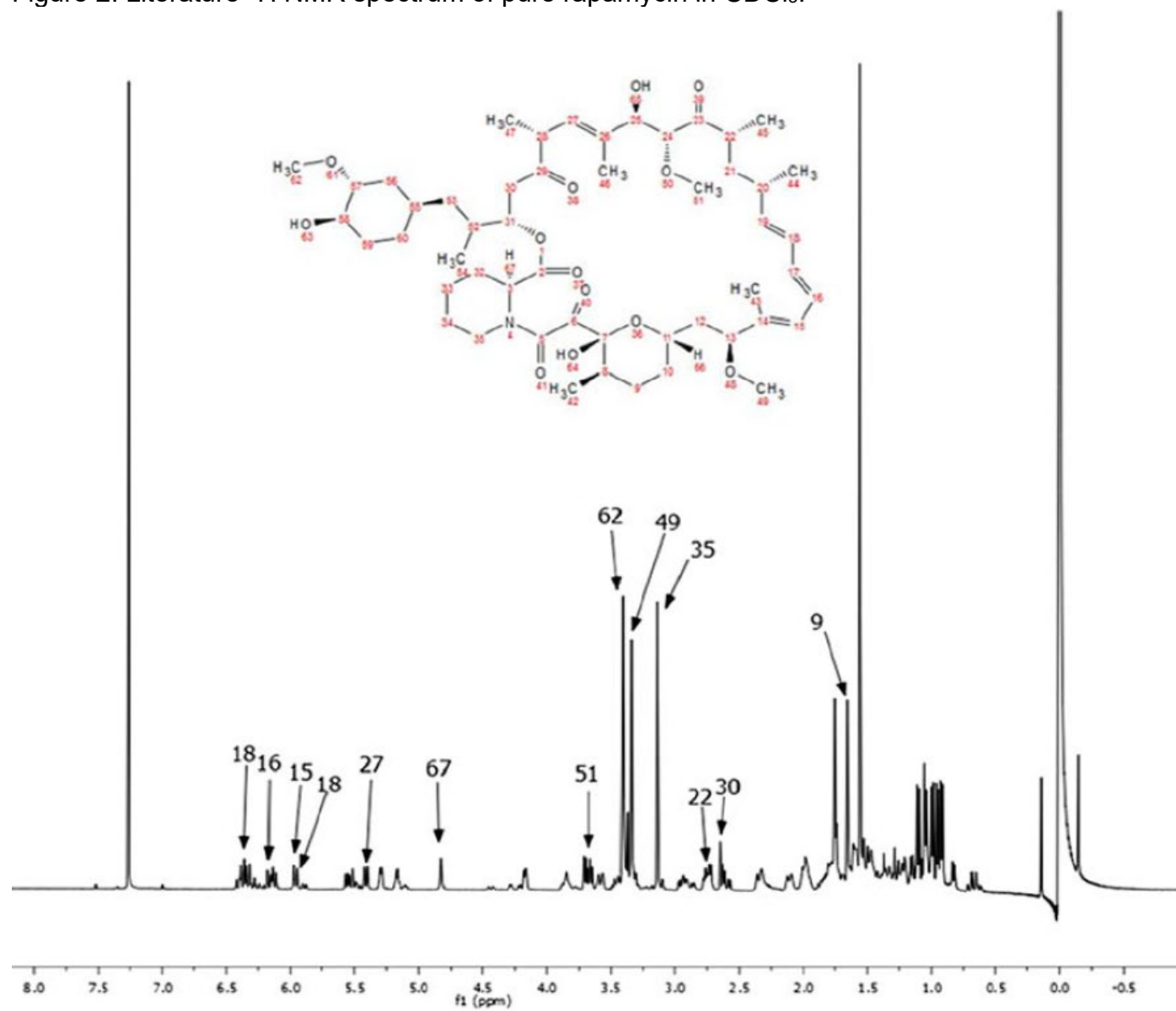


Figure 3. Expanded  $^1\text{H}$  NMR spectrum of TCL15544 (rapamycin) in  $\text{CDCl}_3$ .

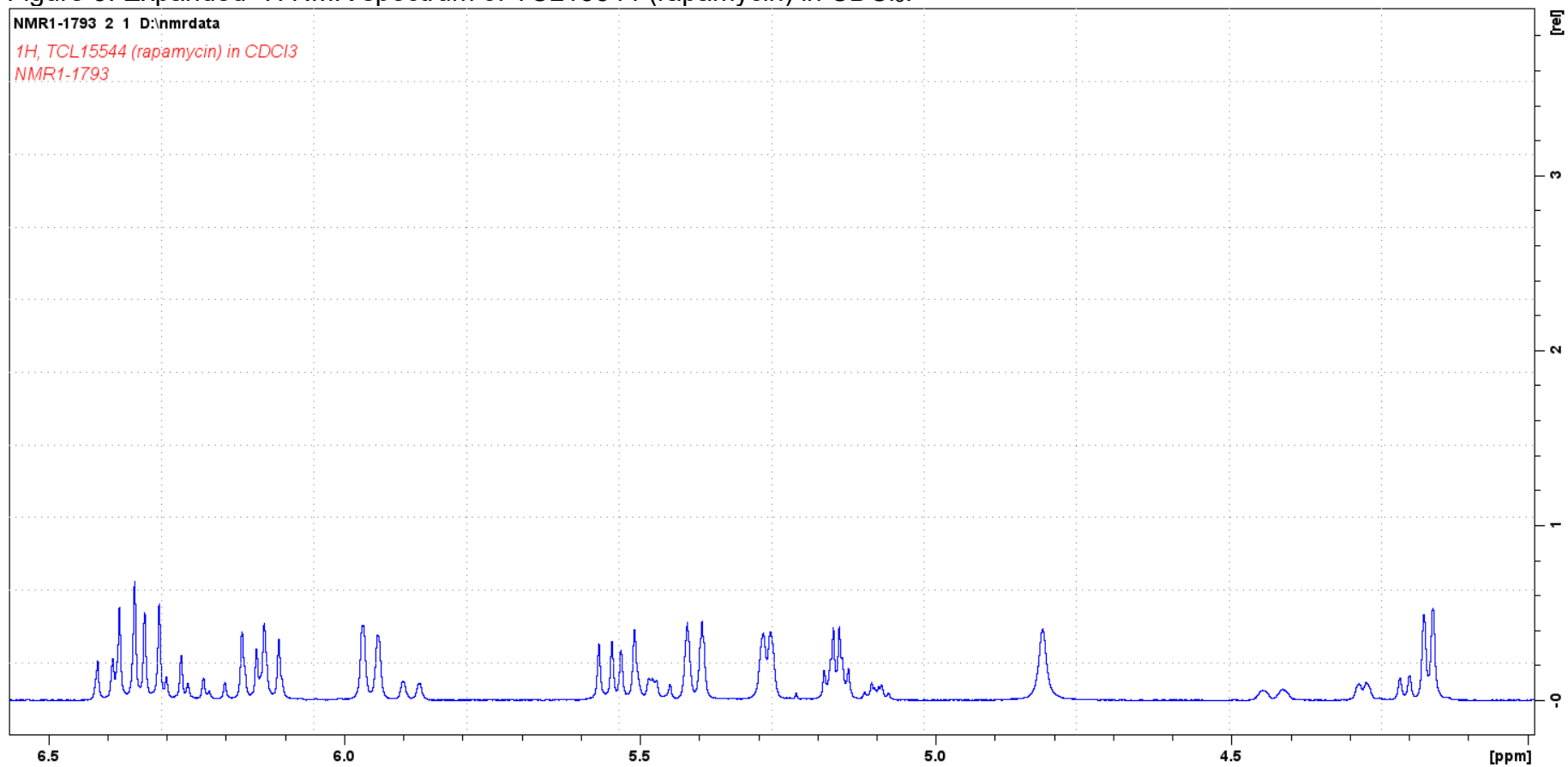


Figure 4. Expanded  $^1\text{H}$  NMR spectrum of TCL15544 (rapamycin) in  $\text{CDCl}_3$ .

