

Supplementary Information

# Biotechnological Transformation of Hydrocortisone into 16 $\alpha$ -Hydroxyprednisolone by Coupling *Arthrobacter simplex* and *Streptomyces roseochromogenes*

Odile Francesca Restaino <sup>1,\*</sup>, Simona Barbuto Ferraiuolo <sup>1</sup>, Addolorata Perna <sup>1</sup>, Marcella Cammarota <sup>1</sup>, Maria Giovanna Borzacchiello <sup>1</sup>, Antonio Fiorentino <sup>2</sup> and Chiara Schiraldi <sup>1</sup>

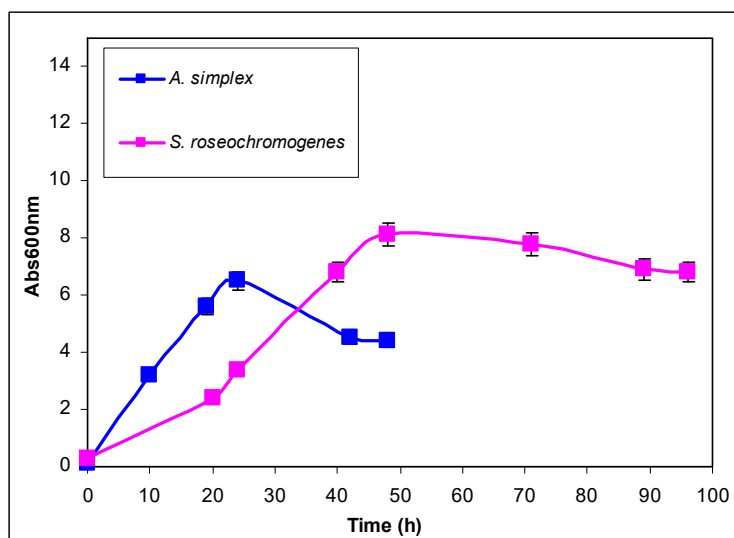
<sup>1</sup> Department of Experimental Medicine, Section of Biotechnology and Molecular Biology, University of Campania "Luigi Vanvitelli", Via De Crecchio 7, 80138 Naples, Italy; simona.barbutoferraiuolo@unicampania.it (S.B.F.); dolores.90@libero.it (A.P.); marcella.cammarota@unicampania.it (M.C.); mariagiovanna.borzacchiello@unicampania.it (M.G.B.); chiara.schiraldi@unicampania.it (C.S.)

<sup>2</sup> Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy; antonio.fiorentino@unicampania.it

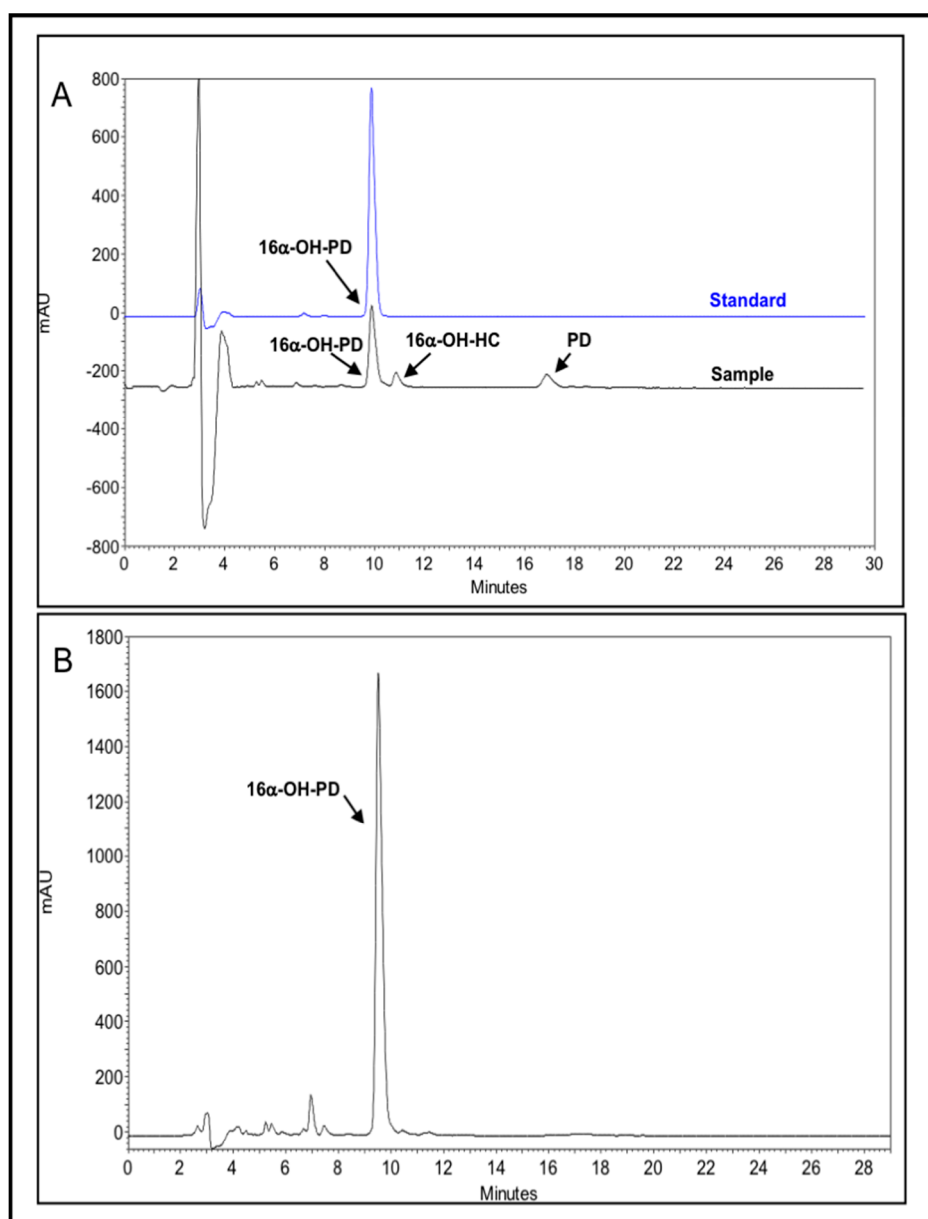
\* Correspondence: odilefrancesca.restaino@unicampania.it; Tel.: +39-81-5667666. Fax: +39-81-5667546

**Table S1.** Shake flask experiments of *A. simplex*: initial and final glucose concentrations on GYA or GEM III N media, at pH 6.0 or 7.0, at 26 °C or at 30 °C.

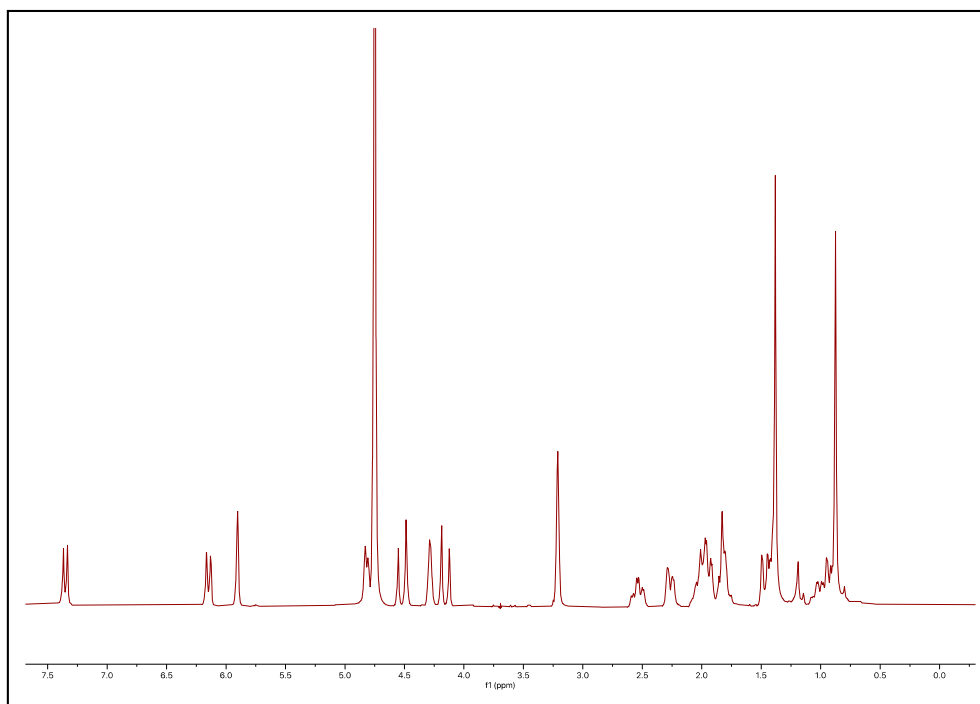
Glucose(g/L)	26 °C		30 °C	
	0 h	48 h	0 h	48 h
<b>GYA pH 6</b>	30.0	8.1	30.0	4.2
<b>GYA pH 7</b>	30.1	8.2	30.1	6.0
<b>GEM III N pH 6</b>	12.0	3.6	12.1	1.7
<b>GEM III N pH 7</b>	12.0	3.8	12.1	1.3



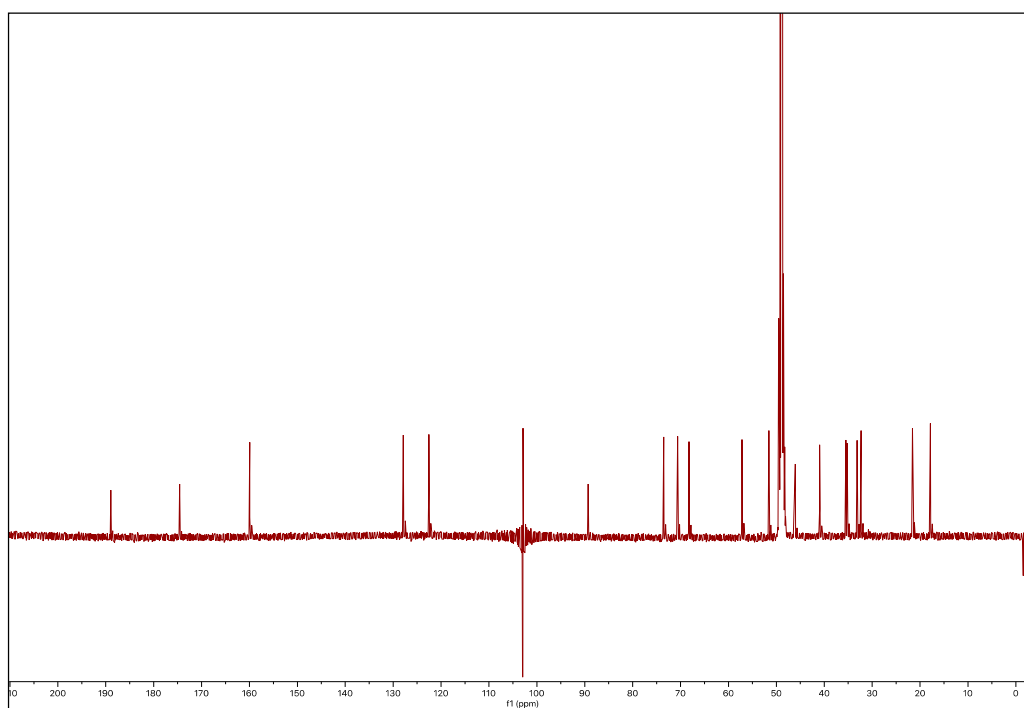
**Figure S1.** Growth curves of *A. simplex* and *S. roseochromogenes* in shake flask experiments on GYA and GEM III N media, respectively, at pH 6.0 and at 26 °C with initial addition of 0.1 g·L<sup>-1</sup> of 16 $\alpha$ -OH-HC and of PD, respectively.



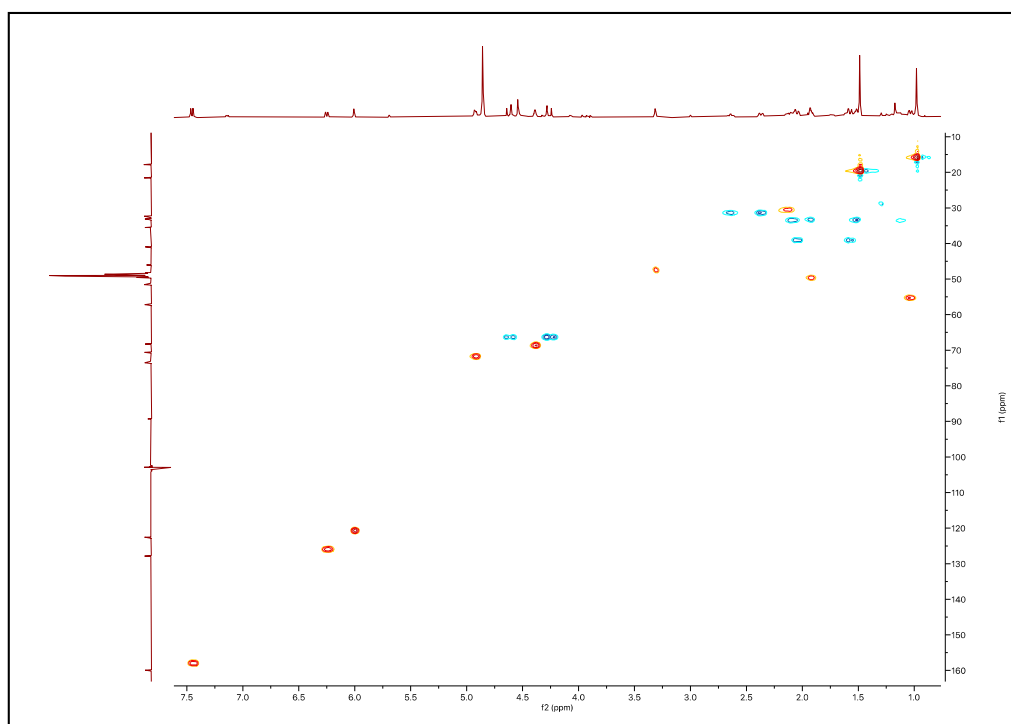
**Figure S2.** Overlaid HPLC chromatograms of the 16 $\alpha$ -OH-PD standard (blue line) and of the sample of 0.1 g·L<sup>-1</sup> HC bioconversion (black line) obtained by coupling *A. simplex* and *S. roseochromogenes* in whole cell experiments at pH 6.0 and at 26 °C (A). HPLC chromatogram of the purified 16 $\alpha$ -OH-PD (B). All the peaks are indicated by the arrows.



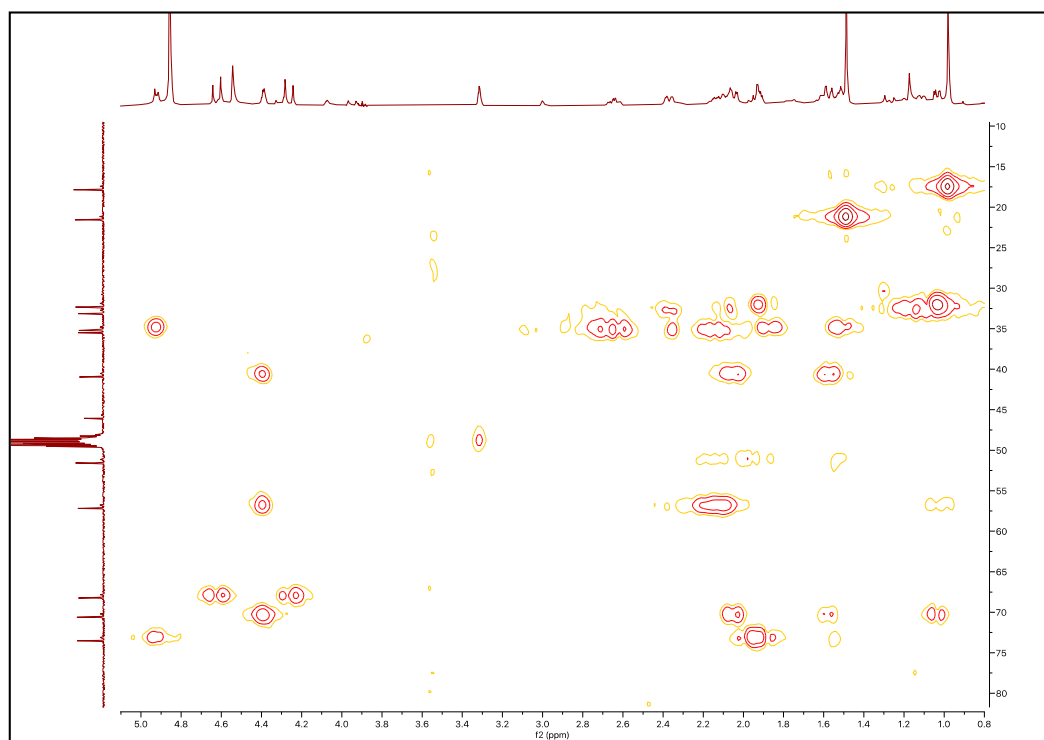
**Figure S3.**  $^1\text{H}$  NMR of  $16\alpha\text{-OH}$  prednisolone obtained by bioconversion of HC by coupling *A. simplex* and *S. roseochromogenes*.



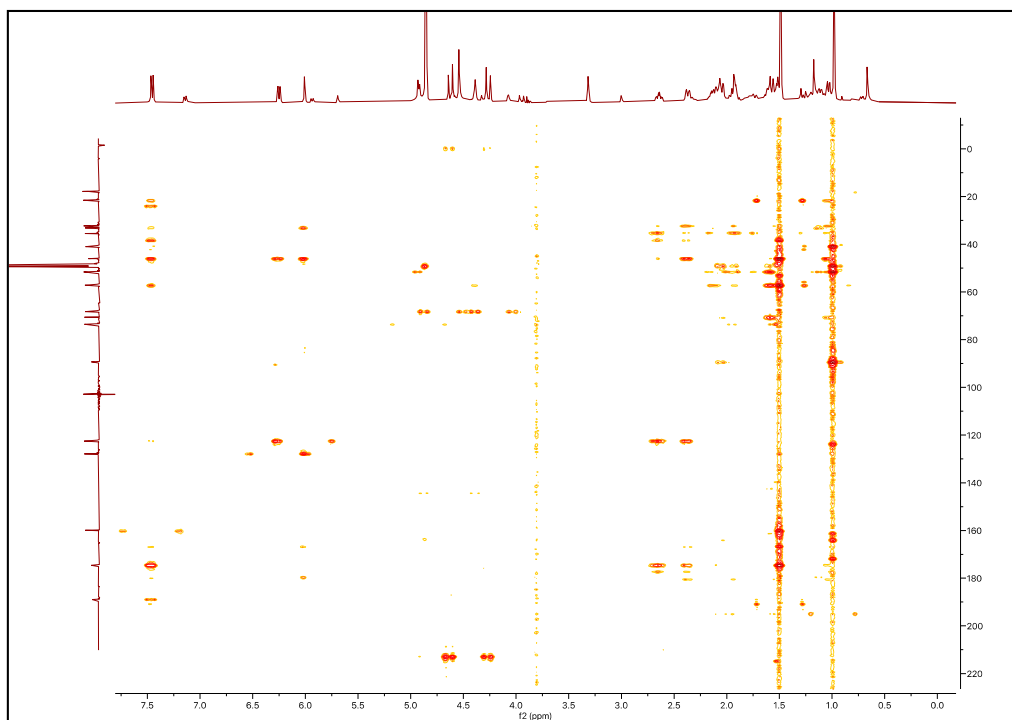
**Figure S4.**  $^{13}\text{C}$  NMR of  $16\alpha\text{-OH}$  prednisolone obtained by bioconversion of HC by coupling *A. simplex* and *S. roseochromogenes*.



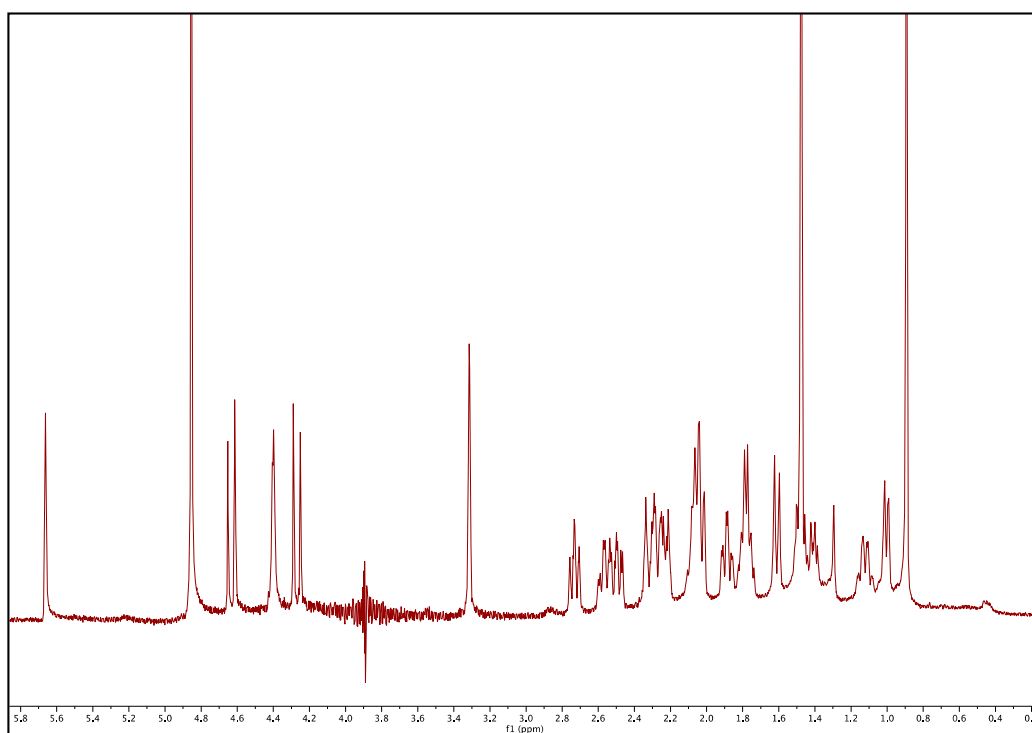
**Figure S5.** HSQC of 16 $\alpha$ -OH prednisolone obtained by bioconversion of HC by coupling *A. simplex* and *S. roseochromogenes*.



**Figure S6.** H2BC of 16 $\alpha$ -OH prednisolone obtained by bioconversion of HC by coupling *A. simplex* and *S. roseochromogenes*.



**Figure S7.** HMBC of  $16\alpha\text{-OH}$  prednisolone obtained by bioconversion of HC by coupling *A. simplex* and *S. roseochromogenes*.



**Figure S8.**  $^1\text{H}$  NMR of prednisolone standard.

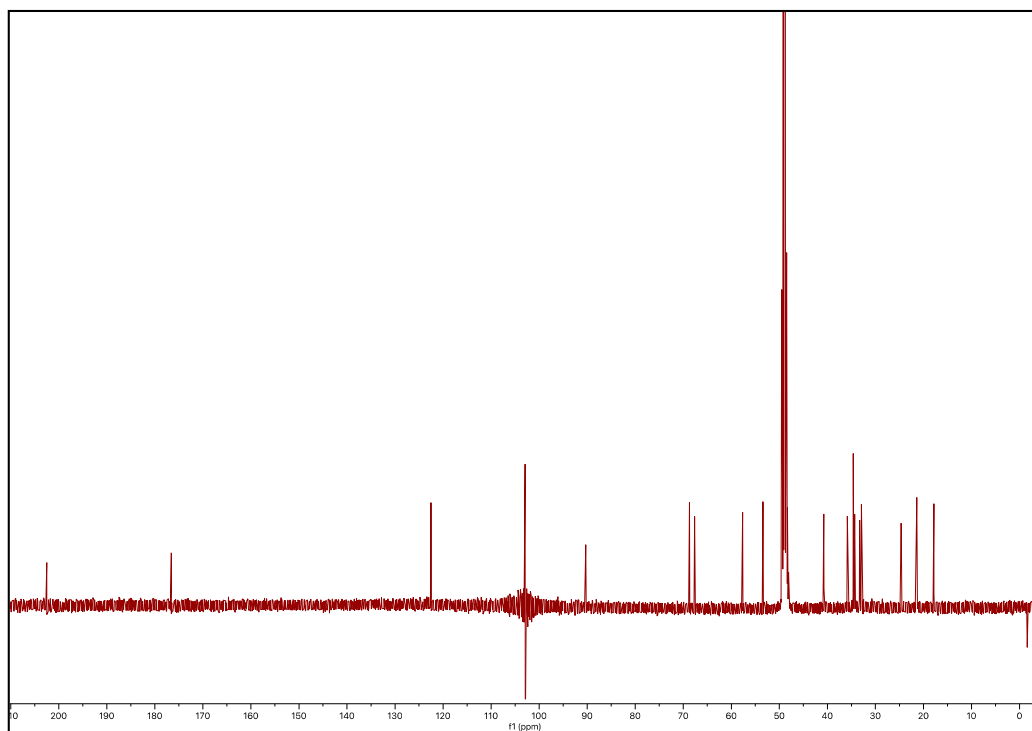


Figure S9. <sup>13</sup>C NMR of prednisolone standard.

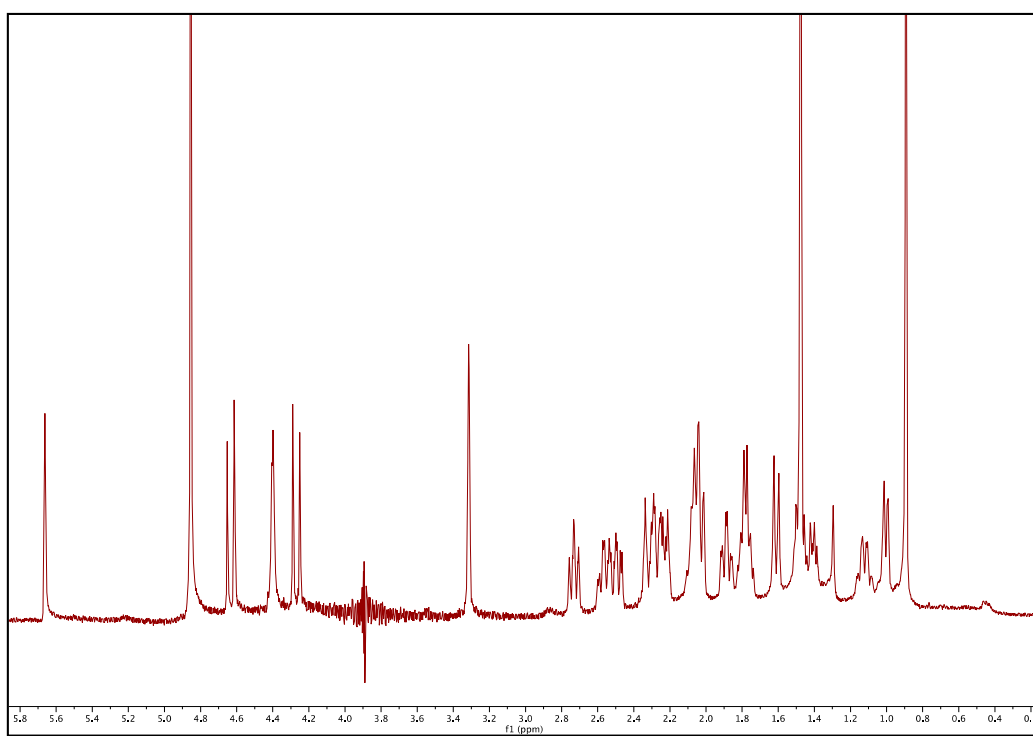
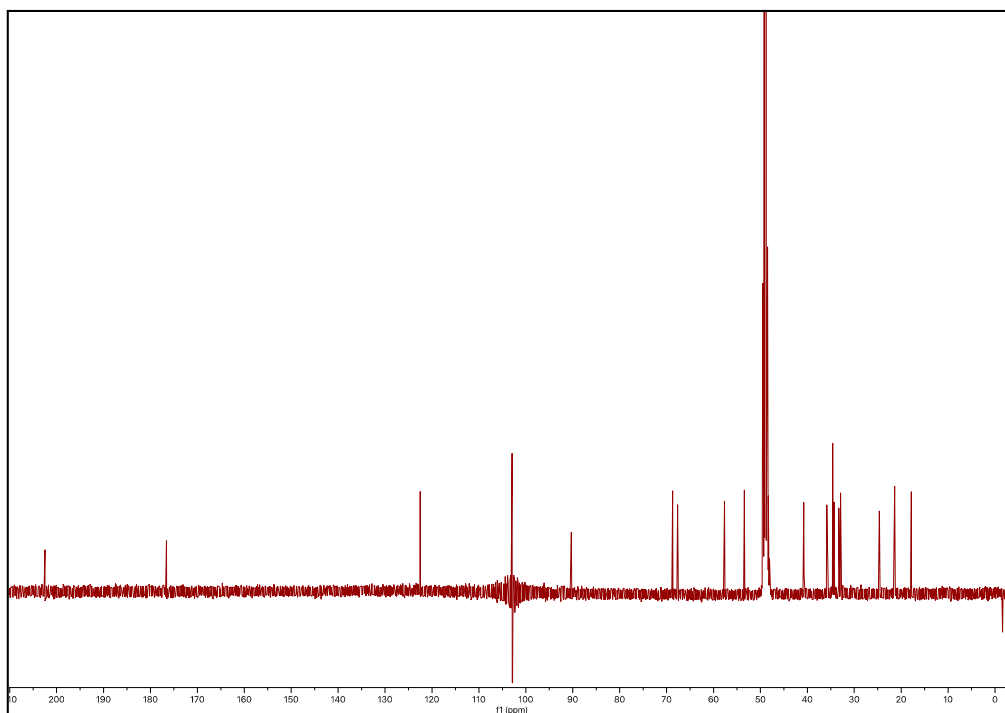


Figure S10. <sup>1</sup>H NMR of hydrocortisone standard.



**Figure S11.** <sup>13</sup>C NMR of hydrocortisone standard.