Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Supporting Information

An improved stereodivergent and practical syntheses of α- and β-pseudouridine

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Optimization of conditions for the cyclisation step

10 mg (unless indicated otherwise) *(S)*-**14** was stirred in MeOH containing the indicated water% and acid additives (total solvent volume 0.25 mL) for the indicated temperatures and times. The mixture was evaporated below the reaction temperature on a high vacuum rotavap then the residue was mixed with ethanol and evaporated again to remove traces of acid. The residue was dissolved in DMSO-d_6 and ^{1}H NMR was recorded.

Table S1. Optimization of conditions for one-pot deprotection-cyclisation of (*S*)-**14**.

Entry	T / ºC	time/h	water%	[acid]/M	Ψ%	α-Ψ%	acid
1	40	17	30	heterogeneous	3.2	16.5	Amberlyst-15
2	55	17	30	heterogeneous	8.5	29.1	Amberlyst-15
3	40	17	10	1.2	8.8	27.2	TFA
4	55	16	30	3.6	12.7	1.5	HCl
5	90	3	30	1.2	23.8	13.1	HCl
6	25	23	10	1.2	38.6	61.4	HCl
7	55	17	10	1.2	39.8	54.2	TFA
8	55	17	10	1.2+0.26	40.3	17.7	HCl+TFA
9	40	16	30	0.6	41.0	55.3	HCl
10	50	2.5	30	1.2	42.0	48.7	HCl
11	40	16	30	1.2	47.6	43.3	HCl
12	70	3	30	1.2	50.5	26.3	HCl
13	55	16	30	1.2	52.9	25.9	HCl
14	40	16	30	3.6	53.2	20.2	HCl
15	55	16	30	0.6	57.8	30.6	HCl
16	50	24	10	1.2	62.9	13.8	HCl
17	40	20	10	1.2	63.3	17.7	HCl
18$^{\$}$	40	17	10	1.2+0.26	75.8	15.0	HCl+TFA
19*	40	17	10	1.2+0.26	70.0	22.2	HCl+TFA
20$^{\#}$	40	17	10	1.2+0.26	63.7	19.1	HCl+TFA
21	40	1	10	1.2	29.0	67.6	HCl
22	55	1	30	1.2	38.2	53.8	HCl
23	25	4.5	20	1.2	15.5	55.6	HCl

$^{\$}$20 mg scale, *100 mg scale, $^{\#}$500 mg scale

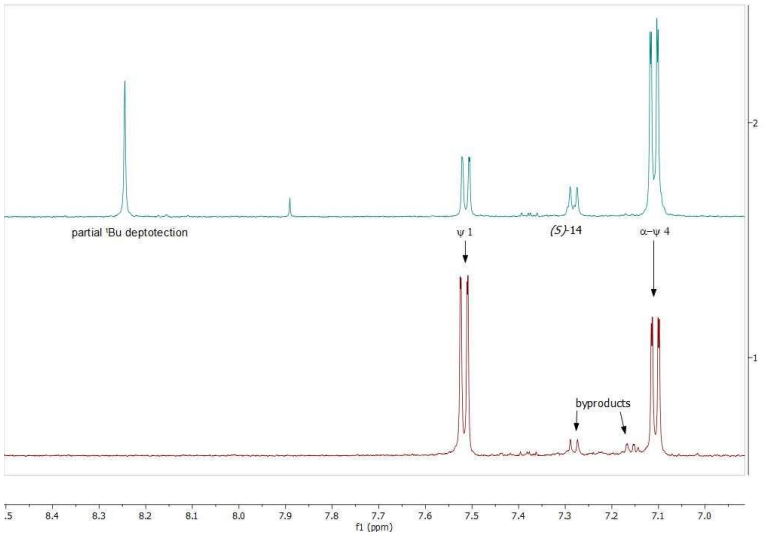


Figure S1. An example of a ^{1}H NMR spectrum from the optimization studies with the peaks of interest annotated.

The Ψ% and *α*-Ψ% values were calculated from the integrals of starting materials, intermediates, products, and byproducts as below and refer to the percentage of the compound in the crude mixture.

8.27 ppm: partial *t*Bu deprotection; 7.52 ppm: Ψ **1**;7.25 ppm: intermediate ***(S)*-14**; 7.32 and 7.16 ppm: byproducts; 7.11 ppm; *α*-Ψ **4**

NMR studies on one-pot deprotection-cyclisation for *α*-pseudouridine synthesis

A 10 mL microwave vial was charged with diol ***(S)*-14** (50 mg), MeOH (1.125 mL) and concentrated aq. HCl (0.125 mL), then capped with a rubber septum. The mixture was stirred at room temperature and at the indicated time points 200 µL aliquots were mixed with 4 mL EtOH and evaporated to dryness on a high vacuum rotavap at room temperature. The residue was dissolved in DMSO-d_6 and ^{1}H NMR spectrum was recorded.

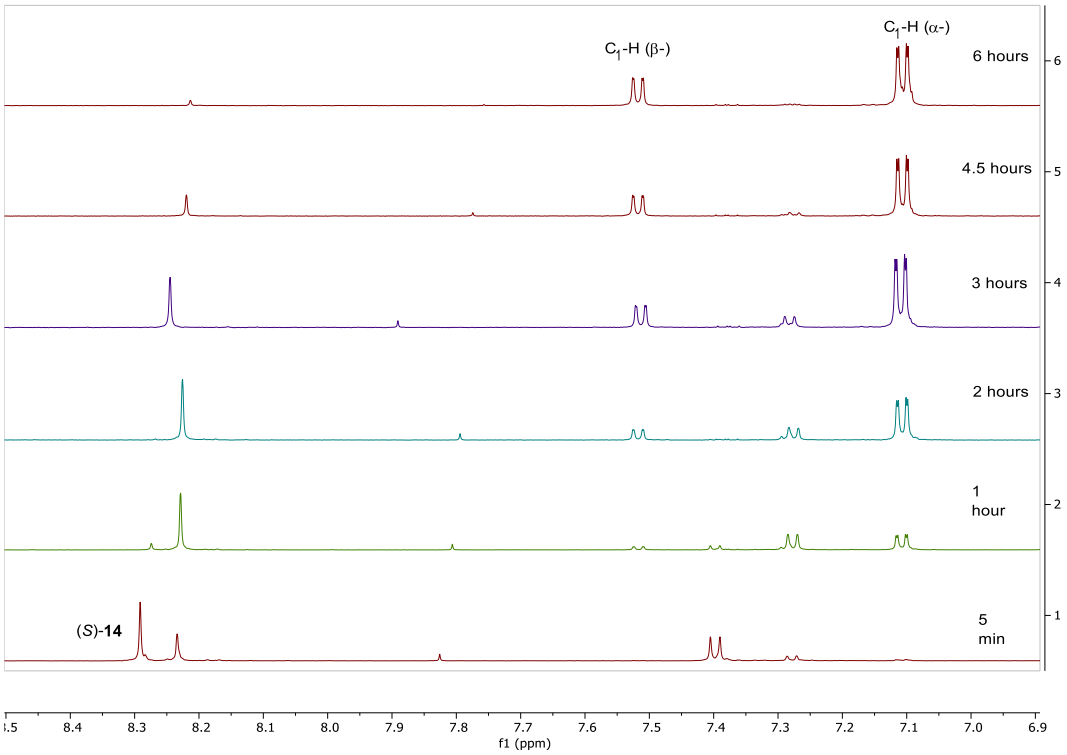


Figure S2. ^{1}H NMR kinetics of the HCl-mediated cyclisation of ***(S)*-14** at room temperature

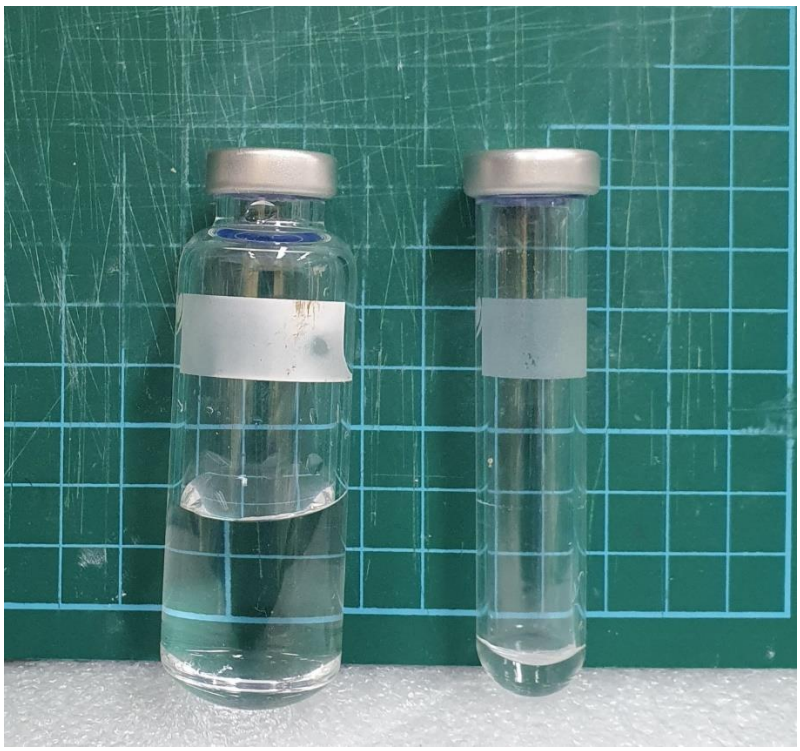
Effect of size of reactor vessels on the product composition from the one-pot deprotection-cyclisation process

We note that the products composition of the acid-mediated one-pot deprotection-cyclisation process is sensitive to the surface area-volume of the reactor vessels. Herein, we made a calculation to analyze the impact of the size of the reactor vessel used in this study on the outcome of the reaction.

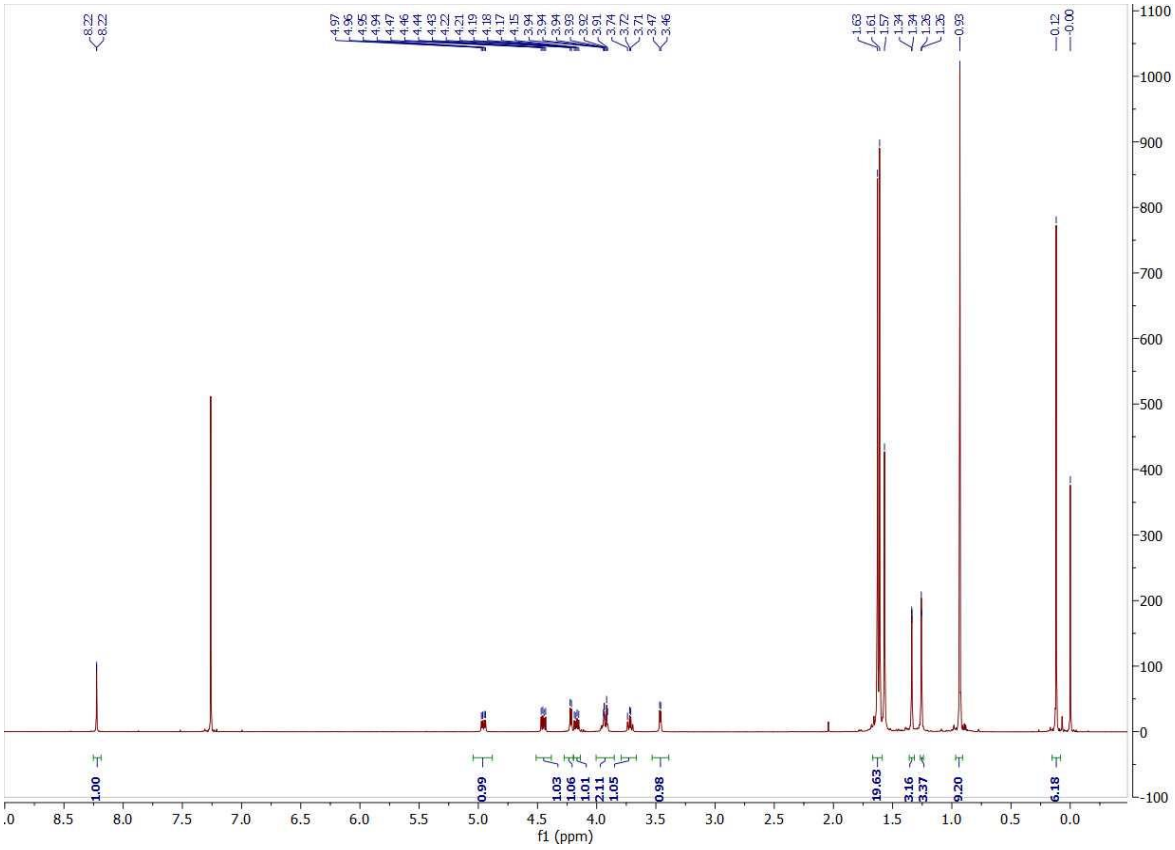
The relative heat transfer area was *roughly* estimated by measuring the radius (r) of the microwave reactor vials below and assuming a cylindrical reactor where heat transfer only occurs from the bottom ($r^2 \times \pi$) and the sides ($2 \times r \times \pi \times h$). The height (h) is the measured height of the reaction mixture. The reactor surface area (A) was divided by the solvent volume (A/V) to determine the relative heat transfer area in cm^2/mL. Thus, the surface area per unit volume of the reactor correlates well with the product composition (α-/β-anomers ratio) of the one-pot deprotection-cyclisation process. We believe that this data will aid in the planning of the scale up of this reaction.

$A = (2 \times r \times \pi \times h) + r^2 \times \pi$; and $V = r^2 \times \pi \times h$

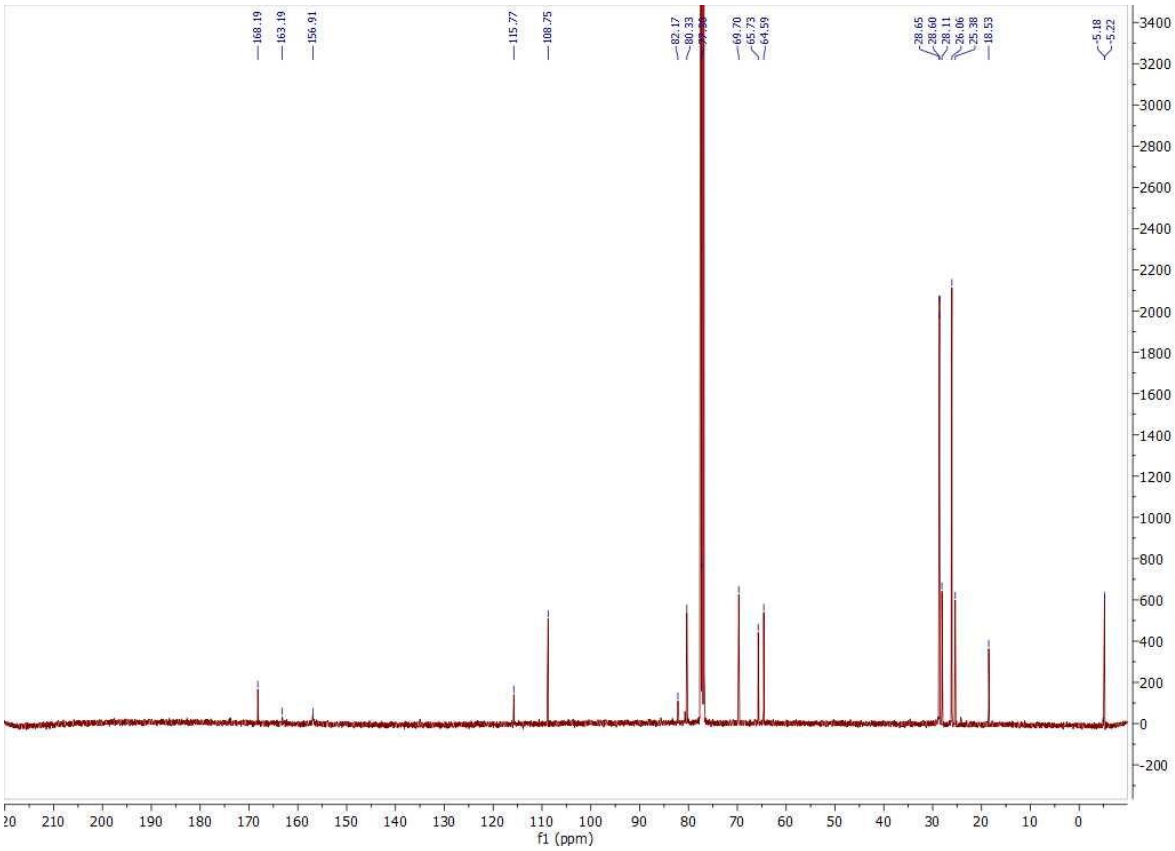
vial	reaction scale	r	h	V	A	**Relative heat transfer area (A/V)**
10 mL	20 mg	0.775 cm	0.85 cm	0.88 mL	6.02 cm^2	**6.84 cm^2/mL**
10 mL	100 mg	0.775 cm	2.8 cm	2.90 mL	15.51 cm^2	**5.35 cm^2/mL**
30 mL	500 mg	1.375 cm	3.1 cm	13.44 mL	32.70 cm^2	**2.43 cm^2/mL**



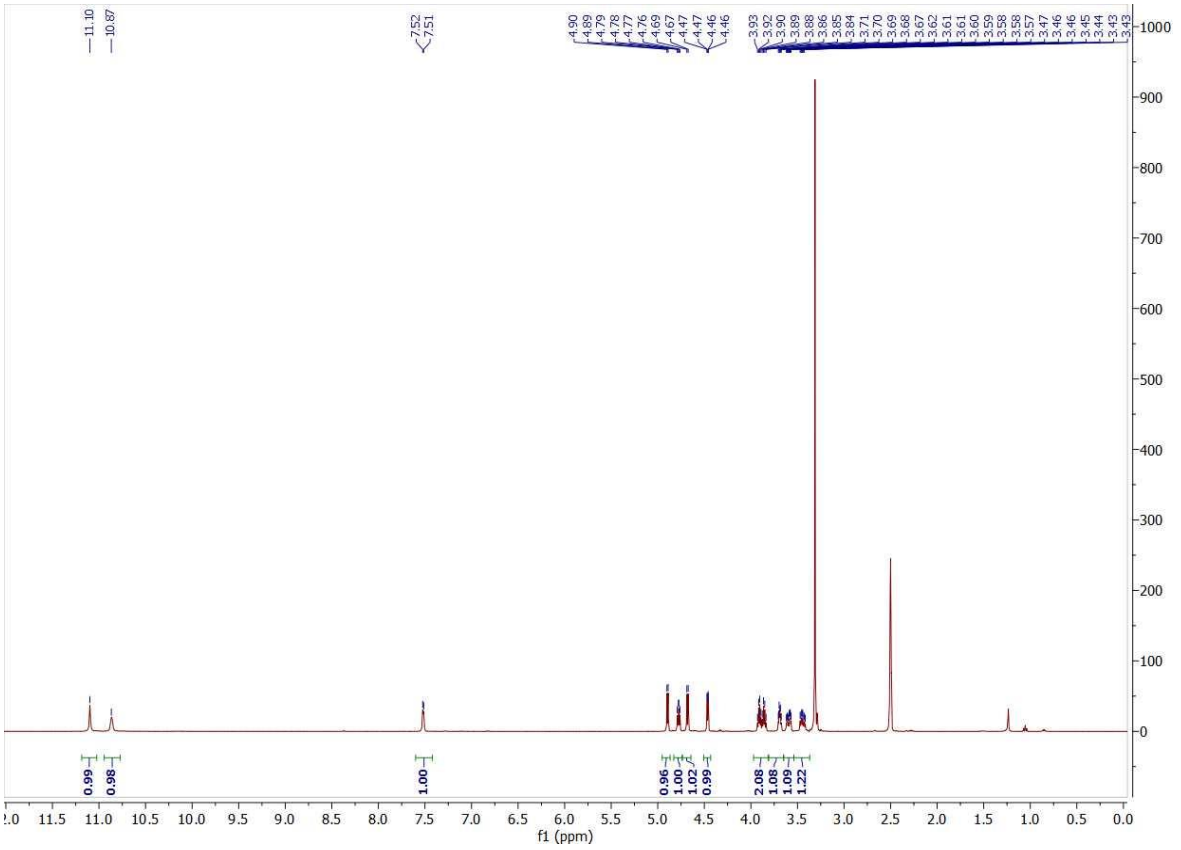
Reactor vessels used in this study. 30 mL (left) and 10 mL (right) microwave reactor vessel with crimp-seal cap. Solvent volumes for the 20 mg and 500 mg scale reactions are illustrated by water.



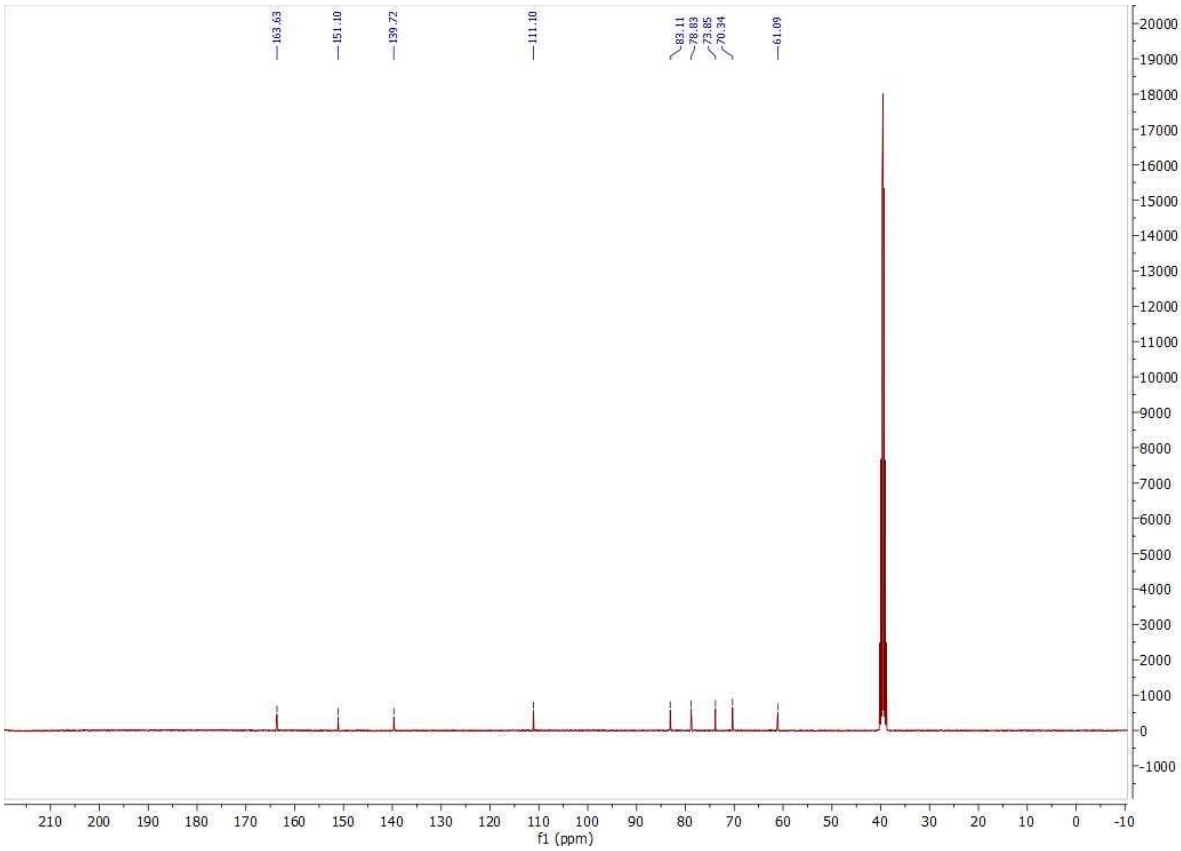
^{1}H NMR of *(S)*-**14** in $CDCl_3$



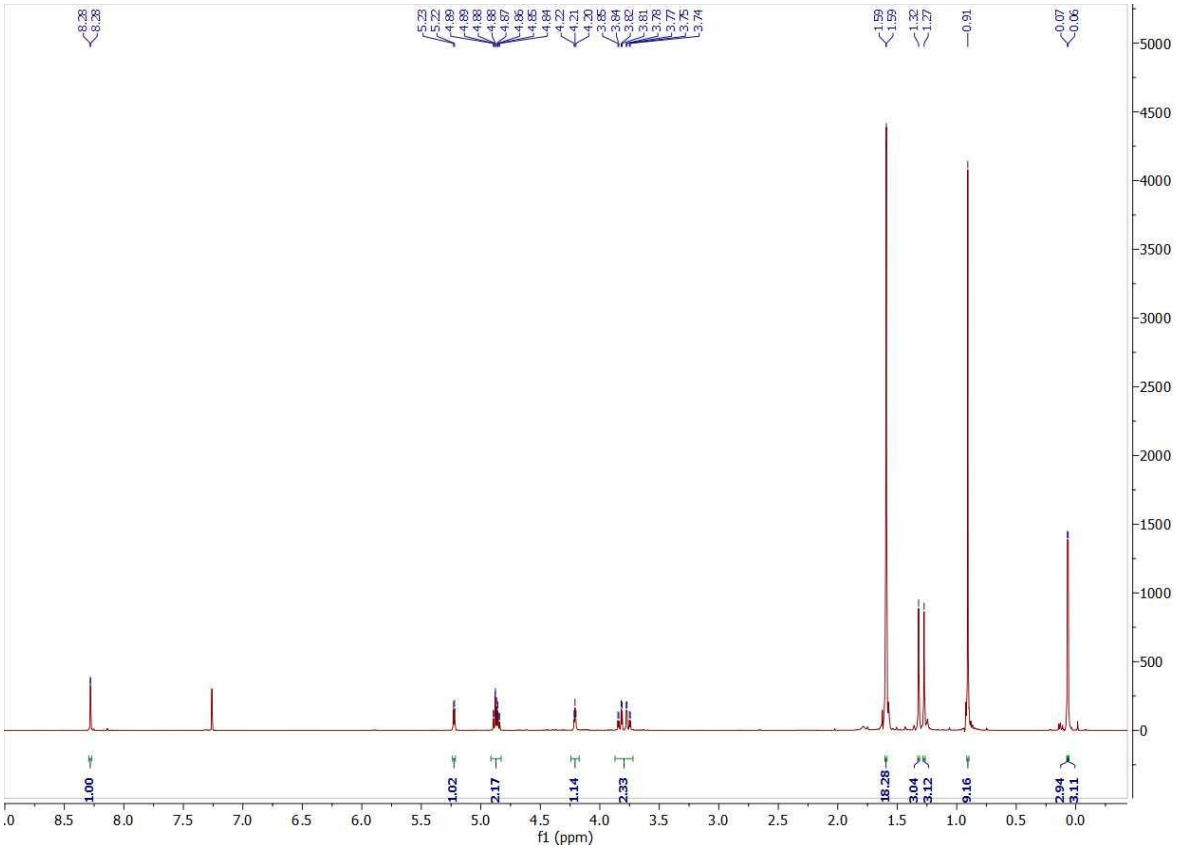
^{13}C NMR of *(S)*-**14** in $CDCl_3$



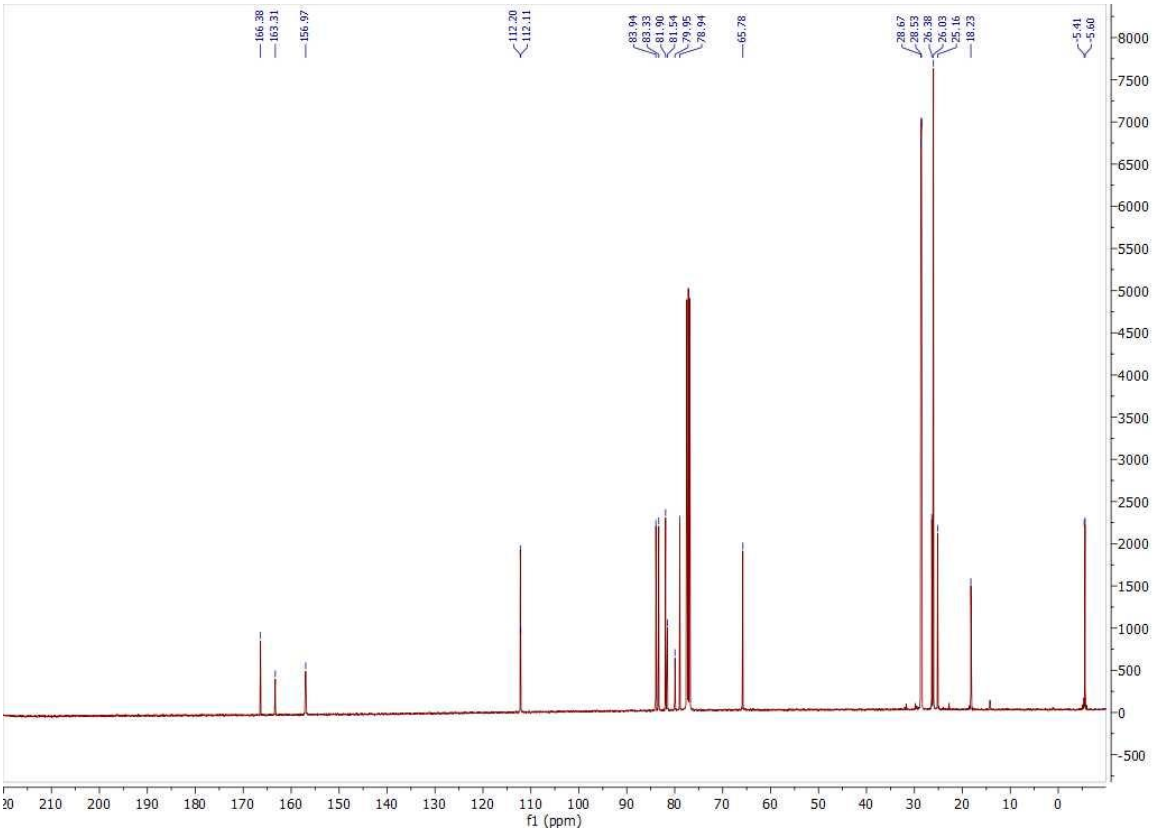
^{1}H NMR of *β*-pseudouridine **1** in DMSO-d_6



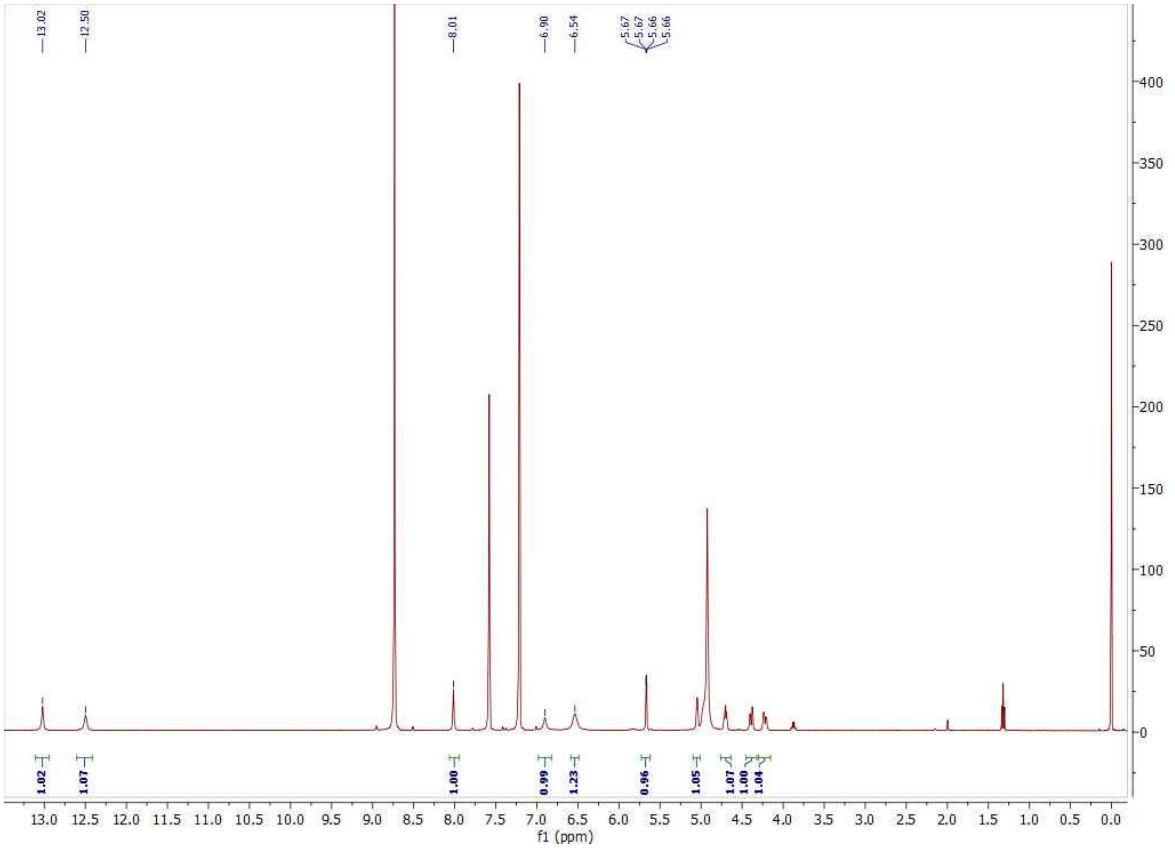
^{13}C NMR of *β*-pseudouridine **1** in DMSO-d_6



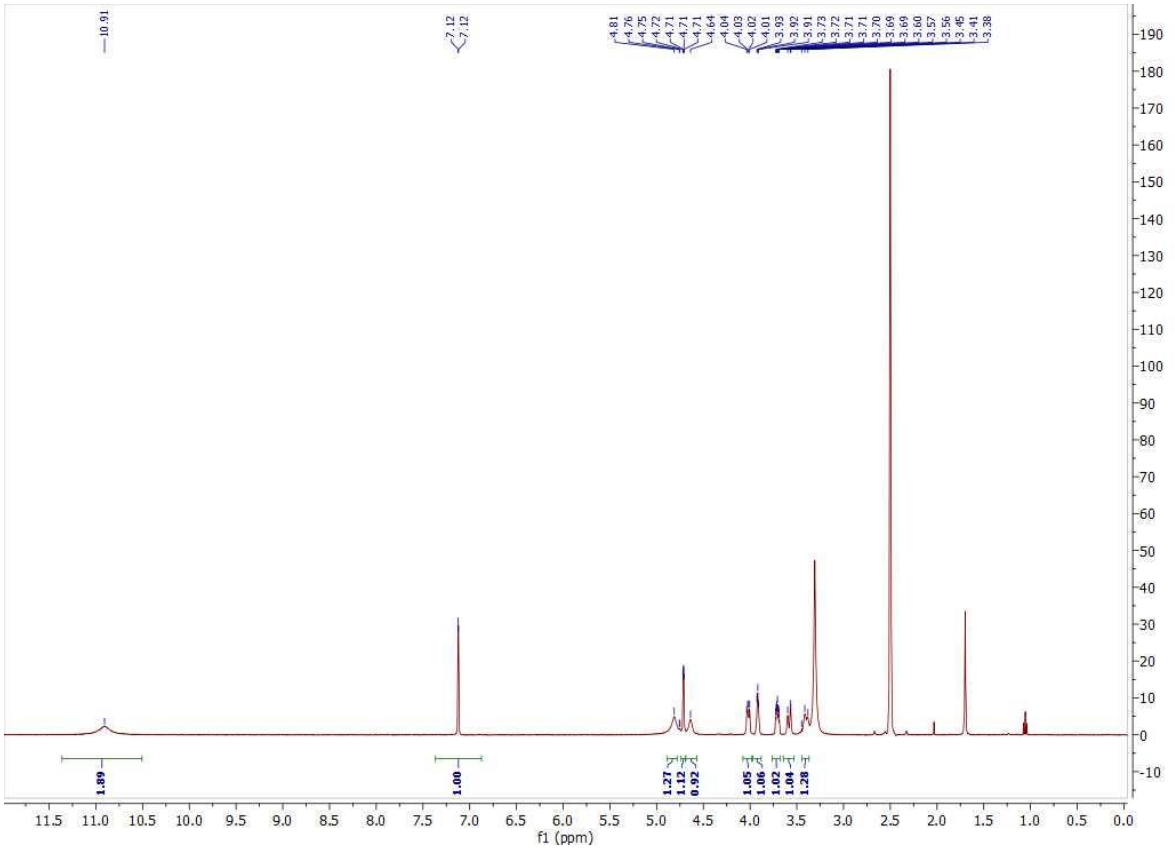
^{1}H NMR of **15** in $CDCl_3$



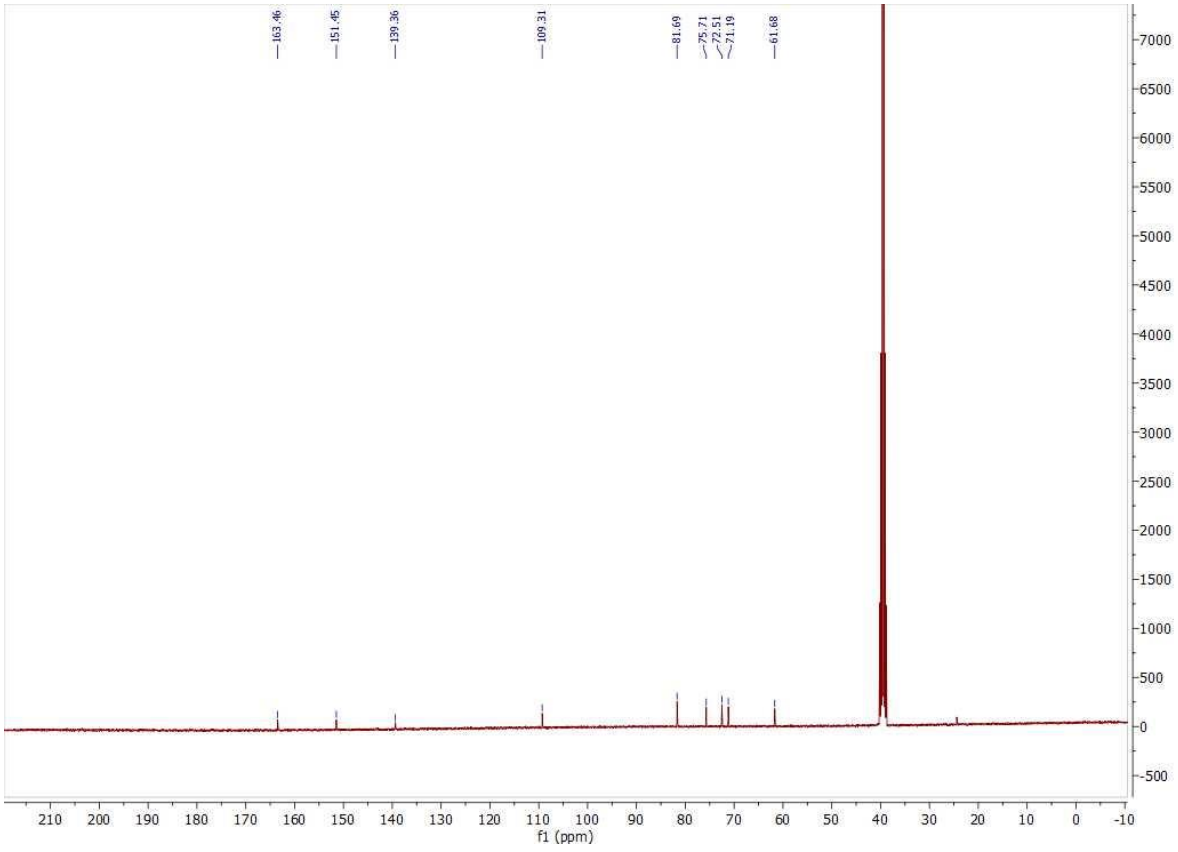
^{13}C NMR of **15** in $CDCl_3$



^{1}H NMR of α-pseudouridine **4** in pyridine-d_5



^{1}H NMR of α-pseudouridine **4** in DMSO-d_6



^{13}C NMR of α-pseudouridine **4** in DMSO-d_6