Supporting Information for

Biocatalytic oxidation of alcohols using galactose oxidase and a manganese (III) activator for the synthesis of islatravir

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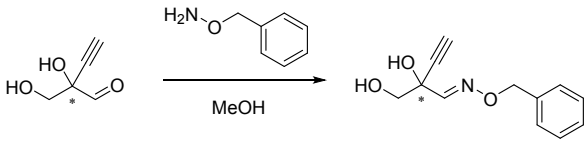
S1 Material and analytical methods

GOase-1 and GOase-2 refer to GALO-104 and GALO-105, respectively, which are the commercial names of these enzymes available for purchase from Codexis. These have been previously described as GOase Rd10bb and GOase Rd12bb, respectively.[1] GOase variants M_1 and M_{3-5} were purchased from Prozomix. PANK-102, ACK-103, PPM-045, PNP-102 and DERA-103 were prepared by Codexis and are commercially available from Codexis.[1] Sucrose phosphorylase was prepared by Codexis as previously reported.[1] Bovine catalase (wild-type) was purchased from Sigma Aldrich, and horseradish peroxidase (HRP, wild type, purified, PEO-301) was obtained from Toyobo.

2-Ethynylglycerol was prepared as described previously.[1] The other alcohols were commercially available and used as received. The oxidants used for HTE screening were purchased from commercial sources, except $K_3[Mn(C_2O_4)_3]$, which was synthesized using a literature procedure.[2] The enzymatic reactions were conducted using UPLC grade water, unless otherwise noted. NMR spectra were primarily obtained on a 400 MHz Bruker AVANCE III and 500 MHz Bruker UltraShield spectrometer. SFC (supercritical fluid chromatography) data were obtained on a Waters ACQUITY UPC^2 instrument. To enable the determination of the ee of 2-ethynylglyceraldehyde, the previously reported derivatization of the aldehyde with $BnONH_2$ was adapted.[1] An aliquot of the reaction mixture containing the desired 2-ethynylglyceraldehdye was added to a 5 g/L solution of $BnONH_2 \cdot HCl$ in MeOH, to form an oxime (Scheme S1). Following a 16 h age time, the oxime samples were analyzed by chiral SFC using a CHIRALPAK AD-3 column as previously described.[1] The side products (e.g. formic acid, 2-ethynylglyceric acid, unreacted 2-ethynylglycerol) were detected by ^{1}H NMR spectroscopy by sampling a measured aliquot of the reaction mixture into a solution of D_2O containing maleic acid or ^{t}BuOH as internal standards. Note: some of the Mn-containing mixtures were filtered prior to NMR analysis to improve the peak shape.



Scheme S1 Derivatization of 2-ethynylglyceraldehyde for SFC analysis

S2 High throughput experimentation (HTE)

HTE chemical oxidant screening procedure, 400 µL Assay.

1) Preparation of stock solutions. 300 mg GOase-1 was dissolved in 10 mL 1 mM $CuSO_4$ solution; 250 mg Bovine catalase was dissolved in 5 mL H_2O; 50 mg HRP was dissolved in 2.5 mL H_2O; 0.1 mmol chemical oxidant was dissolved or suspended in 0.6 mL H_2O.

2) HTE screening procedure. To a 96-well screening plate was added 120 μL 1.0 M 2-ethynylglycerol solution (in 0.56 M sodium phosphate pH 7 buffer), 80 μL GOase-1 solution and 40 μL catalase solution. Finally, chemical oxidant solution and H_2O were added (see Table S1 for details). The plate was sealed with a gas-permeable membrane, incubated with vigorous shaking (800 rpm). The reaction mixture was filtered. 20 μL filtrate was subjected to oxime derivatization (Scheme S1) for chiral SFC analysis to determine ee. 50 μL filtrate was sampled and mixed with maleic acid in D_2O (0.5 wt%) or *tert*-butanol in D_2O (1.0 v/v%) for quantitative ^1H NMR spectroscopy to determine assay yield.

Abbreviations used in table S2:

(4-PyMe$^+$Cl$^-$)4-Por Mn(III)Cl = Manganese(III) 5,10,15,20-tetra(4-pyridyl)-21H,23H-porphine chloride tetrakis(methochloride) (CAS 125565-45-9)

$(NH_4)_5[Fe(C_6H_4O_7)_2]$ = Ammonium iron(III) citrate (CAS 1185-57-5)

(Por)Fe(III)Cl = 5,10,15,20-Tetraphenyl-21H,23H-porphine iron(III) chloride (CAS 16456-81-8)

(Por)Mn(III)Cl = 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese(III) chloride (CAS 32195-55-4)

(R,R)-(Salen)MnCl = (R,R)-(−)-N,N′-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III) chloride, R,R-Jacobsen's catalyst

BQ = 1,4-benzoquinone

$C_6O_6K_2$ = Dipotassium rhodizonate

CF_3CPO = trifluoromethylcyclopropyl peroxide

CPO = cyclopropyl peroxide

Fe(III) Pc Cl = Iron(III) phthalocyanine chloride

Iron(III) phthalocyanine-4,4′,4″,4‴-tetrasulfonic acid = Iron(III) phthalocyanine-4,4′,4″,4‴-tetrasulfonic acid, compound with oxygen monosodium salt hydrate

$K_2ON(SO_3)_2$ = Potassium nitrosodisulfonate

Mn(III) Pc Cl = Manganese(III) phthalocyanine chloride

Table S1. Volumes of oxidant stock solution and H_2O in dosing procedure.

Oxidant Loading	Oxidant stock solution (0.83 M) / µL	H_2O / µL
0%	0	160
10%	12	148
50%	60	100
100%	120	40

Table S2. Results of the screening of chemical oxidants.

Oxidant	Loading	Absolute Peak Area (S)	Absolute Peak Area (R)	Absolute Peak Area (R+S)	*ee*
(4-PyMe$^+$Cl$^-$)4-Por Mn(III)Cl	10%	0	3842	3842	N.A.
(4-PyMe$^+$Cl$^-$)4-Por Mn(III)Cl	50%	10352	153280	163633	87.3%
$(HOCH_2CH_2S)_2$	10%	0	0	0	N.A.
$(HOCH_2CH_2S)_2$	50%	0	4884	4884	N.A.
$(Me_2NCS_2)_2$	10%	0	0	0	N.A.
$(Me_2NCS_2)_2$	50%	0	0	0	N.A.
$(NH_4)_2Ce(NO_3)_6$	10%	18551	197509	216060	82.8%
$(NH_4)_2Ce(NO_3)_6$	50%	0	0	0	N.A.
$(NH_4)_2Ce(NO_3)_6$	100%	0	0	0	N.A.
$(NH_4)_2S_2O_8$	10%	37546	472232	509778	85.3%
$(NH_4)_2S_2O_8$	50%	33221	302652	335873	80.2%
$(NH_4)_2S_2O_8$	100%	26860	177719	204580	73.7%
$(NH_4)_3Fe(oxalate)_3$	10%	5380	7922	13302	19.1%
$(NH_4)_3Fe(oxalate)_3$	50%	36134	433646	469779	84.6%
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	10%	5006	21972	26978	62.9%
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	50%	0	111377	111377	N.A.
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	100%	5366	9533	14899	28.0%
$(PhSeO)_2O$	10%	0	23994	23994	N.A.
$(PhSeO)_2O$	50%	8491	82861	91352	81.4%
(Por)Fe(III)Cl	10%	0	27579	27579	N.A.
(Por)Fe(III)Cl	50%	0	0	0	N.A.
(Por)Mn(III)Cl	10%	0	26955	26955	N.A.
(Por)Mn(III)Cl	50%	4961	66814	71775	86.2%
(R,R)-(Salen)MnCl	10%	0	40032	40032	N.A.
(R,R)-(Salen)MnCl	50%	11784	137725	149509	84.2%
(Salen)Mn(III)Cl	10%	0	39991	39991	N.A.
(Salen)Mn(III)Cl	50%	4458	60004	64462	86.2%
0.25 $Fe_4[Fe(CN)_6]_3$	10%	0	38637	38637	N.A.
0.25 $Fe_4[Fe(CN)_6]_3$	50%	0	15625	15625	N.A.
0.5 $Na_2Cr_2O_7$	10%	0	0	0	N.A.
0.5 $Na_2Cr_2O_7$	50%	9678	92197	101876	81.0%
0.5 $Fe_2(oxalate)_3$	10%	0	10319	10319	N.A.
0.5 $Fe_2(oxalate)_3$	50%	0	0	0	N.A.
0.5 $Fe_2(oxalate)_3$	100%	0	0	0	N.A.
2,6-Cl_2 indophenol Na	10%	8880	55477	64357	72.4%

2,6-Cl_2 indophenol Na	50%	5368	6916	12284	12.6%
2-Butanone Peroxide	10%	25894	231462	257356	79.9%
2-Butanone Peroxide	50%	0	0	0	N.A.
$3Na_2WO_4 \cdot 9WO_3$	10%	0	0	0	N.A.
$3Na_2WO_4 \cdot 9WO_3$	50%	0	7407	7407	N.A.
4-AcNH-TEMPO	10%	0	34347	34347	N.A.
4-AcNH-TEMPO	50%	0	28206	28206	N.A.
4-AcNH-TEMPO	100%	0	40411	40411	N.A.
4-OH-TEMPO	10%	0	34656	34656	N.A.
4-OH-TEMPO	50%	0	25911	25911	N.A.
4-OH-TEMPO	100%	0	18632	18632	N.A.
$AgNO_3$	10%	31772	429377	461149	86.2%
$AgNO_3$	50%	0	0	0	N.A.
BQ	10%	4732	9706	14438	-5.0%
BQ	50%	24897	3197	28095	-77.2%
BQ	100%	48338	4289	52627	-83.7%
BzOOBz	10%	5454	4260	9714	-12.3%
BzOOBz	50%	26192	240725	266917	80.4%
$C_6O_6K_2$	10%	6504	5934	12438	-4.6%
$C_6O_6K_2$	50%	0	0	0	N.A.
CF_3CPO	10%	16674	236628	253303	86.8%
CF_3CPO	50%	12379	143000	155379	84.1%
CH_3COOOH	10%	5816	52761	58576	80.1%
CH_3COOOH	50%	0	0	0	N.A.
$Co(acac)_3$	10%	0	14141	14141	N.A.
$Co(acac)_3$	50%	0	13506	13506	N.A.
$Co(III)(dmgH)_2(py)Cl$	10%	0	46752	46752	N.A.
$Co(III)(dmgH)_2(py)Cl$	50%	0	0	0	N.A.
$Co(III)(en)_3Cl_3$	10%	0	16406	16406	N.A.
$Co(III)(en)_3Cl_3$	50%	0	14518	14518	N.A.
CoF_3	10%	14899	158689	173588	82.8%
CoF_3	50%	21805	228757	250562	82.6%
CPO	10%	4094	49579	53673	84.7%
CPO	50%	0	58739	58739	N.A.
CrO_2	10%	0	12811	12811	N.A.
CrO_2	50%	13959	161007	174966	84.0%
CsI_3	10%	8822	55054	63876	72.4%
CsI_3	50%	0	0	0	N.A.
CsI_3	100%	0	0	0	N.A.
Cumene OOH	10%	0	0	0	N.A.
Cumene OOH	50%	17591	179039	196631	82.1%
Dess-Martin	10%	0	34451	34451	N.A.
Dess-Martin	50%	141448	0	141448	-N.A.
Dichloroisocyanuric sodium	10%	0	9794	9794	N.A.
Dichloroisocyanuric sodium	50%	0	9077	9077	N.A.
Dichloroisocyanuric sodium	100%	0	9568	9568	N.A.
$Fc^+BF_4^-$	10%	0	0	0	N.A.
$Fc^+BF_4^-$	50%	0	9781	9781	N.A.
$Fc^+PF_6^-$	10%	7611	64405	72016	78.9%

$Fc^{+}PF_6^{-}$	50%	0	0	0	N.A.
$Fe(acac)_3$	10%	6713	18594	25307	46.9%
$Fe(acac)_3$	50%	6831	13820	20651	33.8%
Fe(III) Pc Cl	10%	8980	0	8980	-N.A.
Fe(III) Pc Cl	50%	23835	218254	242089	80.3%
$Fe(OEt)_3$	10%	0	28840	28840	N.A.
$Fe(OEt)_3$	50%	0	26307	26307	N.A.
$Fe(OTs)_3$	10%	0	67759	67759	N.A.
$Fe(OTs)_3$	50%	17024	210428	227452	85.0%
$Fe_2(SO_4)_3$	10%	0	17492	17492	N.A.
$Fe_2(SO_4)_3$	50%	0	0	0	N.A.
$Fe_2(SO_4)_3$	100%	0	5761	5761	N.A.
Fe_3O_4	10%	0	23731	23731	N.A.
Fe_3O_4	50%	0	13791	13791	N.A.
FeF_3	10%	7352	60822	68174	78.4%
FeF_3	50%	0	33856	33856	N.A.
$FePO_4$	10%	0	26972	26972	N.A.
$FePO_4$	50%	0	0	0	N.A.
FK 102 Co(III) TFSI	10%	11135	128732	139866	84.1%
FK 102 Co(III) TFSI	50%	0	17190	17190	N.A.
FK 269 Co(III) TFSI	10%	0	4129	4129	N.A.
FK 269 Co(III) TFSI	50%	16228	262909	279137	88.4%
H_2O_2	10%	0	29524	29524	N.A.
H_2O_2	50%	0	25916	25916	N.A.
hemin	10%	0	19310	19310	N.A.
hemin	50%	0	174287	174287	N.A.
hemin	100%	0	12762	12762	N.A.
$HgCl_2$	10%	10166	67373	77539	73.8%
$HgCl_2$	50%	35147	421111	456258	84.6%
IBX py	10%	30540	88698	119238	48.8%
IBX py	50%	0	17711	17711	N.A.
Iron(III) phthalocyanine-4,4′,4″,4‴-tetrasulfonic acid	10%	25043	298477	323520	84.5%
Iron(III) phthalocyanine-4,4′,4″,4‴-tetrasulfonic acid	50%	12636	147472	160109	84.2%
Iron(III) phthalocyanine-4,4′,4″,4‴-tetrasulfonic acid	100%	0	14978	14978	N.A.
K_2IrCl_6	10%	13160	90899	104058	74.7%
K_2IrCl_6	50%	17757	84361	102118	65.2%
K_2IrCl_6	100%	75233	54903	130137	-15.6%
K_2MnO_4	10%	8962	79064	88026	79.6%
K_2MnO_4	50%	0	0	0	N.A.
$K_2ON(SO_3)_2$	10%	0	25187	25187	N.A.
$K_2ON(SO_3)_2$	50%	0	0	0	N.A.
K_2PtCl_6	10%	0	26769	26769	N.A.
K_2PtCl_6	50%	3795	55047	58842	87.1%
$K_2S_2O_8$	10%	0	0	0	N.A.
$K_2S_2O_8$	50%	39878	365388	405266	80.3%
$K_3Fe(CN)_6$	10%	18380	200108	218489	83.2%
$K_3Fe(CN)_6$	50%	10500	110953	121452	82.7%
$K_3Fe(CN)_6$	100%	0	44508	44508	N.A.

$K_3Mn(CN)_6$	10%	0	0	0	N.A.
$K_3Mn(CN)_6$	50%	8518	44730	53248	68.0%
$KClO_3$	10%	0	31025	31025	N.A.
$KClO_3$	50%	6918	99018	105936	86.9%
KIO_3	10%	0	9986	9986	N.A.
KIO_3	50%	12505	224420	236925	89.4%
KIO_4	10%	0	6755	6755	N.A.
KIO_4	50%	18809	10193	29001	-29.7%
$KMnO_4$	10%	24702	215227	239930	79.4%
$KMnO_4$	50%	5753	5176	10930	-5.3%
Lauroyl peroxide	10%	0	28548	28548	N.A.
Lauroyl peroxide	50%	6370	59537	65908	80.7%
$LiMn_2O_4$	10%	4131	49248	53378	84.5%
$LiMn_2O_4$	50%	16205	301519	317724	89.8%
$LiMnO_2$	10%	0	0	0	N.A.
$LiMnO_2$	50%	0	9536	9536	N.A.
m-CPBA	10%	0	27916	27916	N.A.
m-CPBA	50%	0	0	0	N.A.
$MeReO_3$	10%	0	0	0	N.A.
$MeReO_3$	50%	0	0	0	N.A.
$Mn(acac)_3$	10%	19435	203835	223269	82.6%
$Mn(acac)_3$	50%	0	0	0	N.A.
Mn(III) Pc Cl	10%	12984	97169	110152	76.4%
Mn(III) Pc Cl	50%	7089	30747	37836	62.5%
$Mn(OAc)_2$	10%	13970	110070	124040	77.5%
$Mn(OAc)_2$	50%	13382	95158	108540	75.3%
$Mn(OAc)_3$	10%	51923	731670	783593	86.7%
$Mn(OAc)_3$	50%	19575	205626	225201	82.6%
$Mn(OAc)_3$	100%	15929	148849	164779	80.7%
Mn(oxalate)	10%	7565	43280	50844	70.2%
Mn(oxalate)	50%	28851	300284	329136	82.5%
Mn_2O_3	10%	9006	56483	65490	72.5%
Mn_2O_3	50%	6846	28636	35482	61.4%
MnF_3	10%	36474	498590	535064	86.4%
MnF_3	50%	6392	6361	12753	-0.2%
MnO_2	10%	19249	206432	225681	82.9%
MnO_2	50%	23393	305432	328824	85.8%
MnO_2	100%	28314	346099	374413	84.9%
MnO_2 99.9%	10%	0	31622	31622	N.A.
MnO_2 99.9%	50%	11357	199031	210388	89.2%
MnO_2 activated	10%	0	5596	5596	N.A.
MnO_2 activated	50%	11967	204692	216659	89.0%
$MnSO_4$	10%	11701	83771	95472	75.5%
$MnSO_4$	50%	21364	188827	210191	79.7%
Na_2CO_3 1.5H_2O_2	10%	5330	0	5330	-N.A.
Na_2CO_3 1.5H_2O_2	50%	9950	0	9950	-N.A.
Na_2MoO_4	10%	0	0	0	N.A.
Na_2MoO_4	50%	0	0	0	N.A.
$Na_2S_2O_8$	10%	6651	0	6651	-N.A.

$Na_2S_2O_8$	50%	40926	365507	406433	79.9%
Na_2WO_4	10%	0	0	0	N.A.
Na_2WO_4	50%	13140	233157	246297	89.3%
$Na_3PO_4 \cdot 12WO_3$	10%	0	39794	39794	N.A.
$Na_3PO_4 \cdot 12WO_3$	50%	0	4034	4034	N.A.
$Na_4W_{10}O_{32}$	10%	3510	43260	46770	85.0%
$Na_4W_{10}O_{32}$	50%	0	0	0	N.A.
$NaBO_3$	10%	25036	247678	272714	81.6%
$NaBO_3$	50%	5809	13399	19208	39.5%
$NaBrO_3$	10%	0	47224	47224	N.A.
$NaBrO_3$	50%	0	37169	37169	N.A.
$NaBrO_3$	100%	0	25431	25431	N.A.
$NaClO_2$	10%	4696	5732	10428	9.9%
$NaClO_2$	50%	24381	0	24381	-N.A.
$NaClO_2$	100%	0	0	0	N.A.
$NaClO_4$	10%	28700	368970	397670	85.6%
$NaClO_4$	50%	0	23393	23393	N.A.
NaFe(EDTA)	10%	0	0	0	N.A.
NaFe(EDTA)	50%	0	14748	14748	N.A.
$NaONO_2$	10%	0	28888	28888	N.A.
$NaONO_2$	50%	0	10483	10483	N.A.
$NaVO_3$	10%	0	7637	7637	N.A.
$NaVO_3$	50%	0	0	0	N.A.
$NaVO_3$	100%	0	0	0	N.A.
$NH_4Fe(SO_4)_2$	10%	0	12966	12966	N.A.
$NH_4Fe(SO_4)_2$	50%	28107	323002	351108	84.0%
NiO_2	10%	0	9289	9289	N.A.
NiO_2	50%	8287	75278	83564	80.2%
nPr_4RuO_4	10%	35308	398582	433890	83.7%
nPr_4RuO_4	50%	8997	0	8997	-N.A.
Oxone	10%	0	27664	27664	N.A.
Oxone	50%	0	0	0	N.A.
Ph_3COOH	10%	6049	5185	11235	-7.7%
Ph_3COOH	50%	38284	450777	489061	84.3%
$PhI(OAc)_2$	10%	0	0	0	N.A.
$PhI(OAc)_2$	50%	0	0	0	N.A.
PhSSPh	10%	0	13075	13075	N.A.
PhSSPh	50%	6844	74674	81518	83.2%
$PyH^+Br_3^-$	10%	0	0	0	N.A.
$PyH^+Br_3^-$	50%	0	19966	19966	N.A.
$Ru(NH_3)_6Cl_3$	10%	0	0	0	N.A.
$Ru(NH_3)_6Cl_3$	50%	0	44702	44702	N.A.
$RuCl_3$	10%	0	0	0	N.A.
$RuCl_3$	50%	0	7815	7815	N.A.
TBA oxone	10%	0	0	0	N.A.
TBA oxone	50%	0	0	0	N.A.
tBuONO	10%	0	0	0	N.A.
tBuONO	50%	5976	85326	91302	86.9%
tBuOOAc	10%	0	291826	291826	N.A.

tBuOOAc	50%	22442	237940	260382	82.8%
tBuOOBz	10%	14557	200822	215379	86.5%
tBuOOBz	50%	0	39487	39487	N.A.
tBuOOH	10%	24924	284263	309187	83.9%
tBuOOH	50%	37342	253442	290784	74.3%
tBuOOH	100%	21019	206974	227993	81.6%
tBuOOtBu	10%	5143	25378	30521	66.3%
tBuOOtBu	50%	7213	22629	29842	51.7%
tBuOOtBu	100%	5438	28409	33848	67.9%
Urea H_2O_2	10%	19236	168490	187726	79.5%
Urea H_2O_2	50%	6961	27557	34519	59.7%
$VO(acac)_2$	10%	25191	288911	314102	84.0%
$VO(acac)_2$	50%	0	0	0	N.A.
$VOSO_4$	10%	0	9746	9746	N.A.
$VOSO_4$	50%	0	10472	10472	N.A.
$VOSO_4$	100%	0	13824	13824	N.A.
WO_3	10%	129047	111290	240337	-7.4%
WO_3	50%	0	0	0	N.A.
ZnO_2	10%	17143	234055	251198	86.4%
ZnO_2	50%	0	0	0	N.A.

Table S3. Results of the screening of selected chemical oxidants.

Oxidant	Loading	Absolute Peak Area	ee (R:S)	AY (by ^{1}H NMR)	Conversion (by ^{1}H NMR)
HRP		582354	88.2%	46%	62%
MnF_3	10%	535064	86.4%	43%	59%
$Mn(OAc)_3$	10%	583593	86.7%	48%	56%
$Na_2S_2O_8$	10%	469779	84.6%	31%	48%
FK 102 Co(III) TFSI	50%	397670	85.6%	37%	46%
tBuOOAc	50%	260382	82.8%	36%	46%
Ph3C-OOH	50%	489061	84.3%	31%	42%
$(NH_4)_2S_2O_8$	10%	509778	85.3%	39%	41%
Fe(III) Pc-tetrasulfonic acid Na salt	10%	323520	84.5%	21%	40%
$Mn(acac)_3$	50%	461149	86.2%	28%	40%
MnO_2	100%	374413	84.9%	19%	36%
Jacobsen's Mn-salen catalyst	50%	253303	86.8%	31%	36%
$PhMe_2C$-OOH	10%	351108	84.0%	22%	33%
tBuOOH	10%	309187	83.9%	19%	31%
K_2PtCl_6	10%	131002	80.6%	12%	30%
Mn_2O_3	50%	272714	81.6%	20%	29%
$Mn(OAc)_2$	50%	257356	79.9%	24%	27%
$KMnO_4$	10%	239930	79.4%	13%	26%
$(PhCOO)_2$	50%	266917	80.4%	17%	23%
$Fc^+PF_6^-$	50%	314102	84.0%	21%	23%

CoF_3	50%	250562	82.6%	16%	21%
Fe(III) Pc Cl	50%	242089	80.3%	16%	20%
$K_3Fe(CN)_6$	10%	218489	83.2%	17%	17%
Mn(III) Pc Cl	50%	187726	79.5%	12%	16%
FeF_3	50%	173588	82.8%	13%	15%
K_2IrCl_6	10%	28982	74.7%	8%	10%
4-OH-TEMPO	10%	34656	65.5%	6%	6%
None		30695	72.0%	3%	4%

S3 Preparation of 2-ethynylglycerol solution

For the oxidation reactions reported in this manuscript, a stock solution of 2-ethynylglycerol in sodium phosphate buffer was used. First, the 2-ethynylglycerol was first prepared as a monosodium salt. 2-ethynylglycerol (153 g) was dissolved in 600 mL isopropylalcohol. A 25% solution of sodium methanolate (2 eq.) was added dropwise over 1 hour under N_2. The resulting thick beige slurry was aged for a further 1 hour. Then, the slurry was filtered, and the cake washed twice with isopropylalcohol, then twice with 2-MeTHF, then dried under an N_2 flow. ^{1}H NMR (500 MHz, D_2O): δ 3.67, d, J = 11 Hz (2H); δ 3.62, d, J = 11 Hz (2H).

The dried sodium salt (27.7 g) was dissolved in 150 mL water. The pH was adjusted to pH 7.0 using ortho-phosphoric acid. The total volume was adjusted to 200 mL with water. This yields a 1 M solution of 2-ethynyl glycerol in 0.56 M sodium phosphate. This stock solution was stored for use directly in the biocatalytic oxidation reactions.

S4 Gram-scale biocatalytic oxidation

GOase-2 (225 mg), bovine catalase (250 mg) were each hydrated with 5 mL water and slowly shaken until dissolved. 125 uL of a 0.1 M aqueous solution of $CuSO_4$ was added to the GOase solution. The activator, if used, was also hydrated with 5 mL water (HRP, 100 mg, 6 wt%) or $Mn(OAc)_3$ (69 mg for 2 mol%). In a 100 mL EasyMax vessel equipped with an overhead stirrer, sparger and flow controller, water (22 mL) was added with antifoam 204 (20 µL). The reaction mixture was sparged with air at 50 standard cubic centimeters per minute (sccm) and stirred at 100 rpm, and the temperature set to 20 °C. The enzyme solutions were added to the vessel (GOase, then activator, then catalase). 12.9 mL of the 1 M stock solution of ethynylglycerol/buffer was added to the reaction mixture. The reaction was stirred at 600 rpm. Throughout the reaction, a pH meter dosing unit added aliquots of 5 N NaOH to maintain a pH 7.0. Periodic samples were taken using an attached EasySampler: 20 µL of the reaction mixture was diluted into 1.6 mL

of a 5 g/L $BnONH_2 \cdot HCl$ solution in MeOH to quench and derivatize the aldehyde. These samples were analyzed by chiral SFC to determine aldehyde yield and conversion.

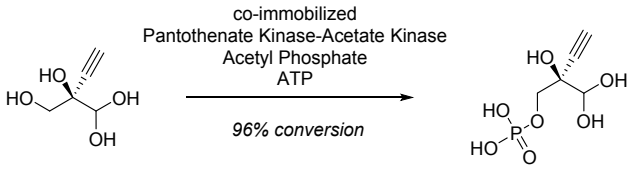
Comparison of impurities profiles on scale-up

Using the above conditions with 10 mol% $Mn(OAc)_3$ vs 6 wt% HRP, *ca.* 10% of 2-ethynylglyceric acid was observed with both activators (conversion measured by ^1H NMR spectroscopy), although less formic acid was observed with $Mn(OAc)_3$ than HRP (2.9% *vs* 5%, respectively).

S5 Subjection of $Mn(OAc)_3$-containing streams to downstream enzymatic cascade

Phosphorylation step

The immobilization and reaction procedures followed those described in reference 1, except acetate kinase was not immobilized here.



Immobilization of pantothenate kinase on IMAC resin: Immobilization of Nuvia IMAC Ni-charged resin (3 mL based on settled volume) was added to a filter funnel and washed three times with water (3 × 15 mL) holding for ten minutes at each wash. The resin was washed one time with binding buffer (3 × 15 mL; 500 mM sodium chloride, 50 mM sodium phosphate, 15 mM imidazole, pH 8.0). In a 50 mL falcon tube evolved pantothenate kinase (PANK-102) (0.750 g) lyophilized powder was dissolved in 30 mL binding buffer. The washed resin was charged to the tube and the solution was rotated on rotary mixer for 18 h at room temperature. The resin was filtered and washed four times with binding buffer (4 × 15 mL) and three times with potassium PIPES buffer (2 × 15 mL, 50 mM, pH 6.5) and used directly in the reaction. The contents were slurried to 20 mL with 35 mM PIPES solution (pH 6.5) and 10 mL was transferred to two separate 15 mL falcon tubes. The contents were allowed to settle at 4 °C. The contents of one falcon tube were utilized in the subsequent reaction.

Synthesis of (R)-2-ethynylglyceraldehyde 3-phosphate: To a 50 mL reactor (*R*)-2-ethynylglyceraldehyde solution (29.3 mL of a 258 mM aqueous solution, 7.56 mmol), aqueous magnesium chloride solution (1 M, 0.33 mL, 0.3 mmol), acetyl phosphate diammonium salt (89 wt%, 1.849 g, 9.45 mmol), and adenosine triphosphate disodium salt hydrate (ATP, 42 mg, 0.076 mmol) were added. The pH was adjusted to 6.4 using 5 N KOH, and resin prepared with immobilized PANK-102 was added. Acetate kinase (ACK-103)

lyophilized powder (5 mg) was added. The reaction was stirred for 20 hours with pH maintained at 6.4 using 5 N KOH. After 20 hours, the reaction was judged to be complete (96% conversion) by LC (Figure S1) following the derivatization procedure in reference 1. The resin was filtered and the filtrate was collected. The aqueous filtrate provided (*R*)-2-ethynylglyceraldehyde 3-phosphate solution for further reaction in API step.

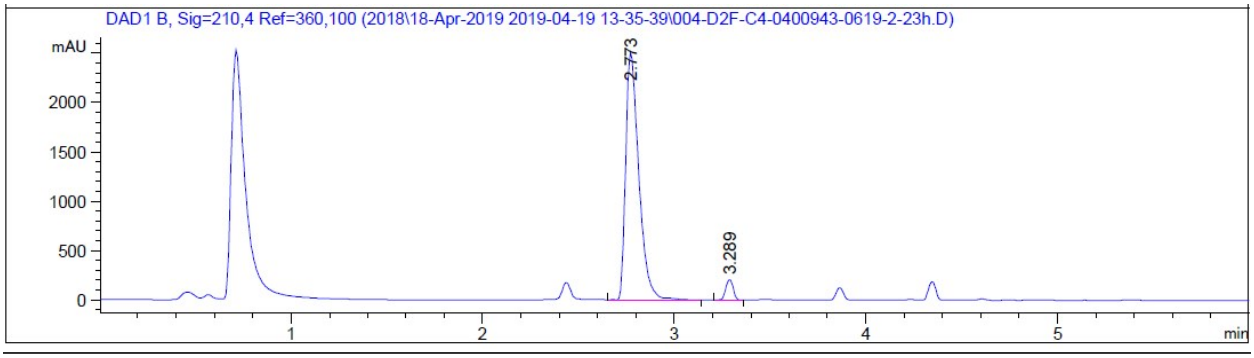
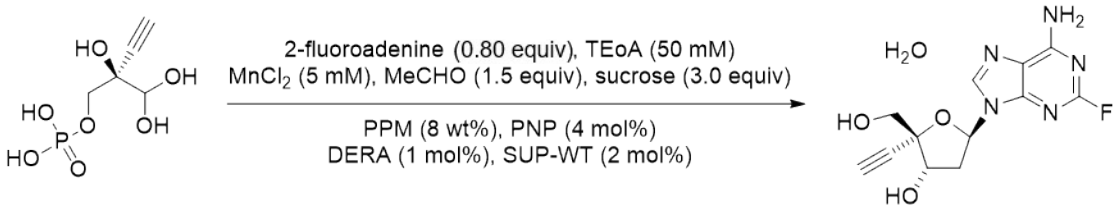


Figure S1 LC chromatogram of phosphorylation reaction (2.773 mins = pdt, 3.289 mins = SM).

API Step



The procedure described in reference 1 was followed. A solution of (*R*)-2-ethynylglyceraldehyde 3-phosphate (0.115 M, 2.38 mmol, 20.7 mL) was added to a 50 mL glass vessel equipped with overhead stirring. Triethanolamine (0.206 g, 1.38 mmol) was added and the pH of the solution was adjusted to 7.65 with 250 µL of 8 M KOH, before charging manganese dichloride tetrahydrate (72.2 mg, 0.365 mmol) and sucrose (2.85 g, 8.33 mmol). The pH of the solution was adjusted to 7.52 using 150 µL of 8 M KOH and the 4 enzymes were added while stirring at 300 rpm: DERA-103 (5.0 mg, 1 wt% vs (*R*)-2-ethynylglyceraldehyde 3-phosphate); PPM-045 (90 mg, 18 wt% vs (*R*)-2-ethynylglyceraldehyde 3-phosphate); PNP-102 (20 mg, 4 wt% vs (*R*)-2-ethynylglyceraldehyde 3-phosphate); SP-WT (10 mg, 2 wt% vs (*R*)-2-ethynylglyceraldehyde 3-phosphate). Once the enzymes dissolved, 2-fluoroadenine (292 mg, 1.90 mmol) was added as a slurry in 16.5 mL in DI water. The temperature of the reaction was increased to 35 °C, and acetaldehyde (40% in isopropyl alcohol; 0.485 mL, 3.57 mmol) was added, before adjusting the pH to 7.58 with 200 µL of 5 M KOH. The reactor was sealed, and the suspension was stirred at 35 °C for 20 h, at which time the LCMS indicated 98% conversion of 2-fluoroadenine. The suspension was cooled

to 5 °C for 60 min and filtered, rinsing with cold water (5 mL x 3). The off-white solid was suction dried in air to give MK-8591 monohydrate (0.509 g, 1.64 mmol, 86% yield vs 2-fluoroadenine, 69% yield vs (*R*)-2-ethynylglyceraldehyde 3-phosphate). All analytical data match those previously reported.[3]

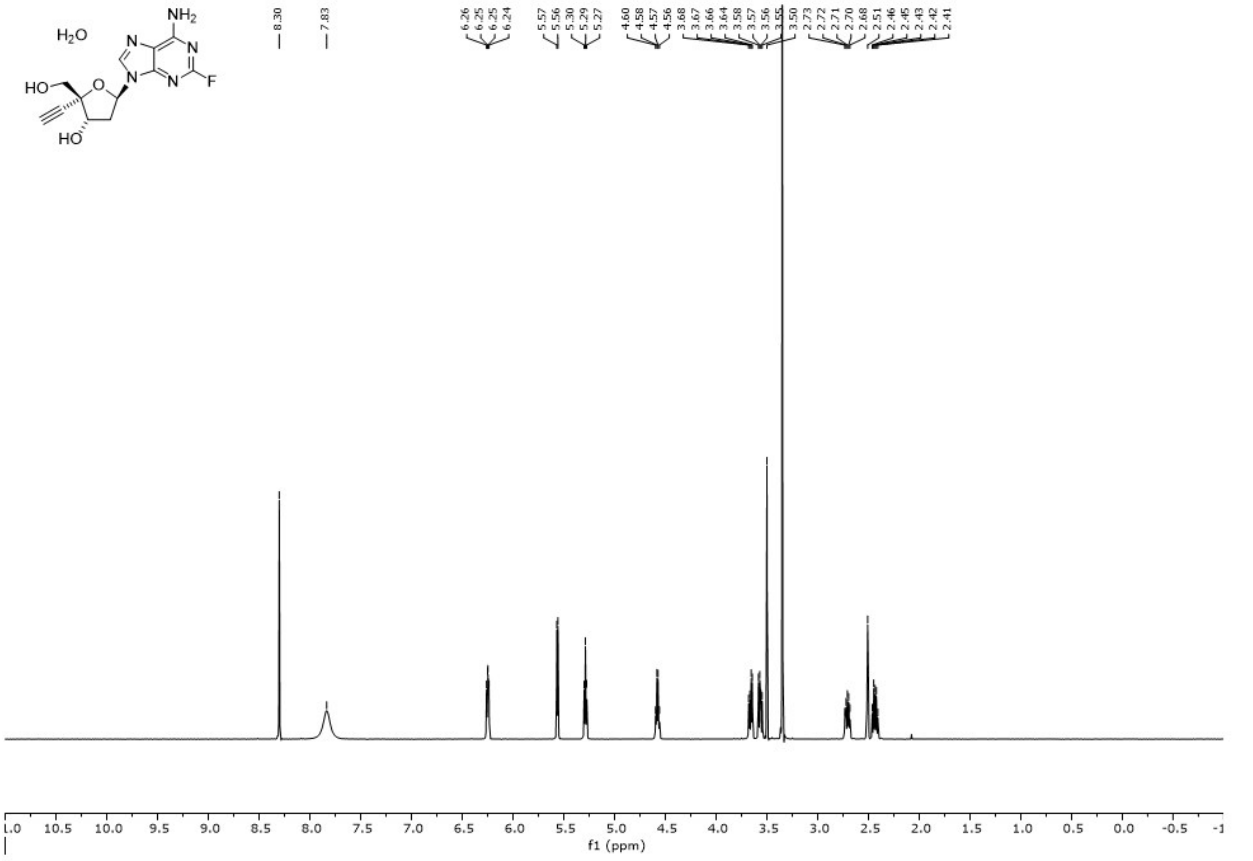


Figure S2 [1]H NMR spectrum of islatravir.

S6 Procedure for activation period studies

Reactions probing the activation period using Mn(III) additives were conducted in Whatman polypropylene 24 well 10 mL plates on a Eppendorf ThermoMixer C. For a typical reaction, the reaction volume was 2 mL. The temperature of the ThermoMixer was set to 25 °C. Stock solutions of catalase (50 mg / mL) and GOase (45 mg / ml) were prepared in water. To the reaction wells, 1080 µL water was added, followed by 200 µL of each of the GOase and catalase stock solutions. The block was set to shake at 800 rpm. To the shaking wells, 3 µL of 0.1 M $CuSO_4$ solution was added to each reaction well. 516 µL of the substrate/buffer solution was added. The required amount of $Mn(OAc)_3$ (e.g. 2.77 mg $Mn(OAc)_3 \cdot 2H_2O$ for 2 mol%), or other activator, was added to the relevant wells directly as a solid. The reaction was profiled by sampling 10 µL aliquots into 800 uL of a 5 g/L solution of $BnONH_2 \cdot HCl$ in MeOH to obtain the yield and ee of

aldehyde *via* SFC (see derivatization procedure above). Final conversions were obtained by NMR spectroscopy. The enzyme stock solutions were freshly prepared before each reaction.

S7 Initial rates *vs* $K_3[Mn(C_2O_4)_3]$ loading

Figure S3 shows similar initial rates across a range of loadings from 1 to 10 mol% $K_3Mn(C_2O_4)_3$.

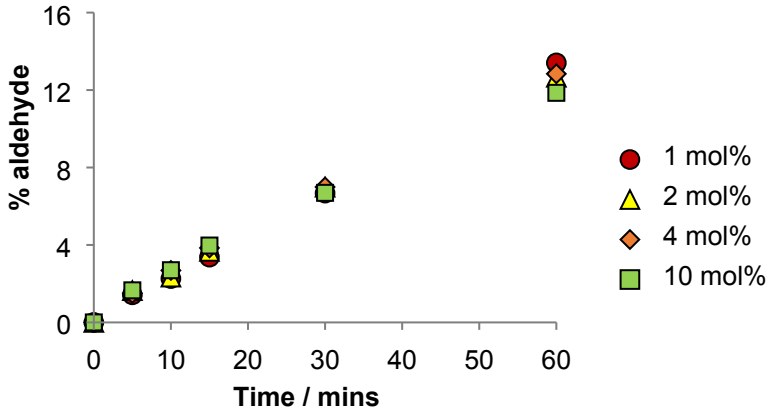


Figure S3 Initial period for desymmetrizing oxidation of 2-ethynylglycerol (0.258 M) using $K_3Mn(C_2O_4)_3$ at loadings of 1, 2, 4 and 10 mol% as activators for GOase-2 (14 wt%). Catalase loading = 14 wt%.

S8 Procedure for using $Mn(OAc)_3$ as an activator for alternative substrates/GOase variants

Reactions were conducted in Whatman polypropylene 24 well 10 mL plates on a Eppendorf ThermoMixer C. The volume of reactions was 2 mL, and most reactions (furfuryl alcohol, 5-(hydroxymethyl)furfural, benzyl alcohol, 4-chlorobenzylalcohol and 4-methoxybenzylalcohol) used 15 mg/mL substrate loading. Cinnamyl alcohol was conducted at 7.5 mg/mL substrate. The temperature of the ThermoMixer was set to 25 °C. The required GOase variant was prepared as a stock solution, and 0.1 M $CuSO_4$ was added to the stock (0.55 µmol $CuSO_4$ added per mg GOase in the stock). To the reaction wells, water was added (volume calculated such that the total reaction volume after all subsequent stock solution additions is 2 mL), then 200 uL of a 1 M solution of pH 7.0 sodium phosphate buffer. Next, GOase stock solution (amount depends upon loading) and catalase stock solution (14 wt% relative to substrate) were added to the reaction wells. The plate was shaken at 800 rpm. The required amount of $Mn(OAc)_3$ (5 mol% relative to substrate) was added to the relevant wells directly as a solid. HRP was added as a stock solution (9 mg / mL stock solution; 3 wt% added to the reaction relative to the substrate). The substrate (e.g. 30 mg) was added as a 150 mg/mL

solution in water or DMSO (for solubility purposes). For each substrate, the reactions with no activator, HRP or $Mn(OAc)_3$ were run in parallel. The enzyme stock solutions were freshly prepared before each reaction. The plate was sealed with an oxygen permeable membrane, and shook at 800 rpm at 25 °C. For entries 2-6, the reactions were profiled at selected time points by sampling aliquots into NMR tubes containing 500 µL D_2O with maleic acid as an internal standard. 50 µL d_6-DMSO was added to improve the solubility when necessary. For entry 1, due to peaks overlapping with maleic acid, 100 uL of each reaction mixture was sampled into a vial containing preweighed trimethoxybenzene (TMB) as an alternative internal standard, and the mixture diluted in d_6-DMSO. Overoxidation to the carboxylic acids was not observed. NMR spectra are shown in Figures S4-S22. 'Entry' refers to the entry in Table 1 in the main text, and SM (#) and product (*) and other peaks (*e.g* solvent) are labeled in the first and/or second spectrum of the series. Fufural (entry 1),[4] benzaldehyde (entry 3),[5] 4-anisaldehyde (entry 4),[5] 4-chlorobenzaldehyde (entry 5)[6] and cinnamaldehyde (entry 6)[7] were assigned by comparison with literature data. 2,5-furandicarboxaldehyde (entry 2) was assigned by comparison with an authentic commercial sample in D_2O with H_2O and maleic acid (Figure S7).

Control experiments were undertaken with each substrate to test for background oxidation under the aerobic conditions. For each substrate, three reactions were conducted: 1) no additive 2) 5 mol% $CuSO_4$ and 3) 5 mol% $Mn(OAc)_3$. Each substrate was dissolved in 0.1 M pH 7.0 sodium phosphate buffer, the additive (if used) was added, and the plate was sealed with an oxygen permeable membrane, and shook at 800 rpm at 25 °C for the desired reaction time (similar to the times in Table 1 in the main text): furfuryl alcohol, 24 h; 5-hydroxymethylfurfural, 18 h; benzyl alcohol, 3 h; 4-methoxybenzyl alcohol, 18 h; 4-chlorobenzyl alcohol, 18 h; cinnamyl alcohol, 3h. The reactions were sampled for NMR spectroscopy by sampling 200 uL of the reaction mixture into 500 uL d_6-DMSO containing trimethoxybenzene (TMB) as internal standard. No evidence for oxidation under these conditions was observed by NMR spectroscopy (Figures S23 – S40).

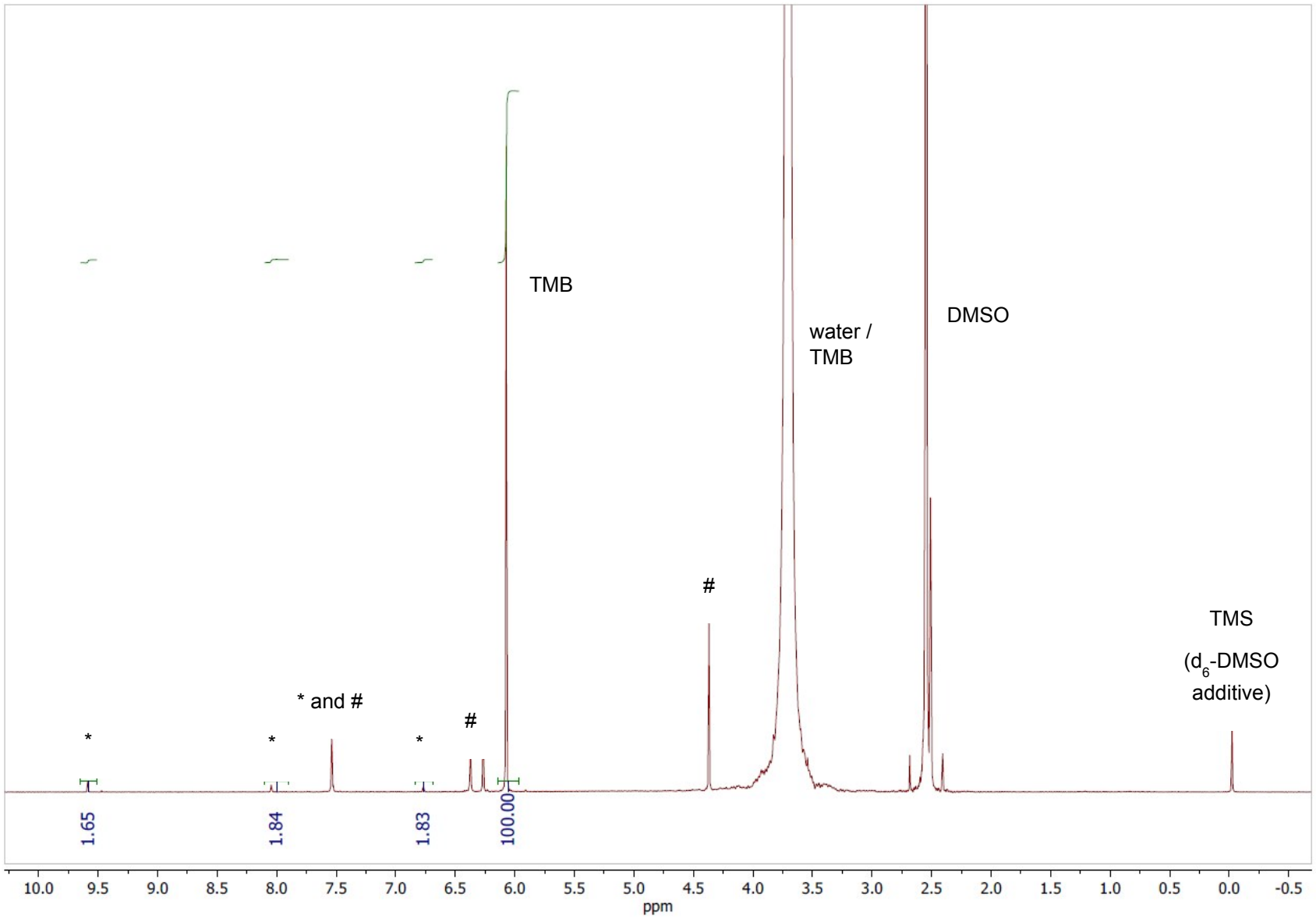


Figure S4 Table 1 Entry 1, no activator. # = SM, * = product.

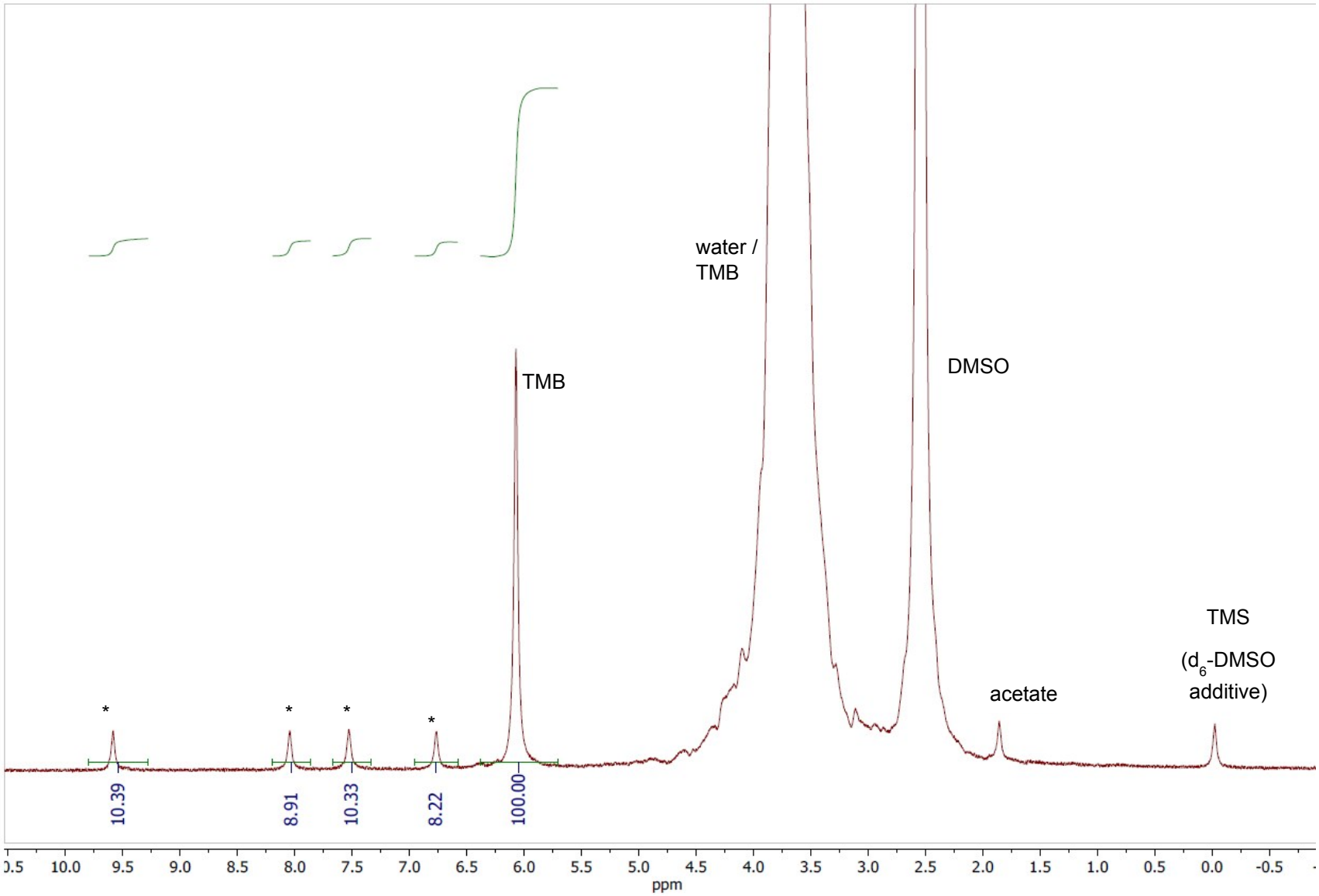


Figure S5 Table 1 Entry 1, $Mn(OAc)_3$. * = product.

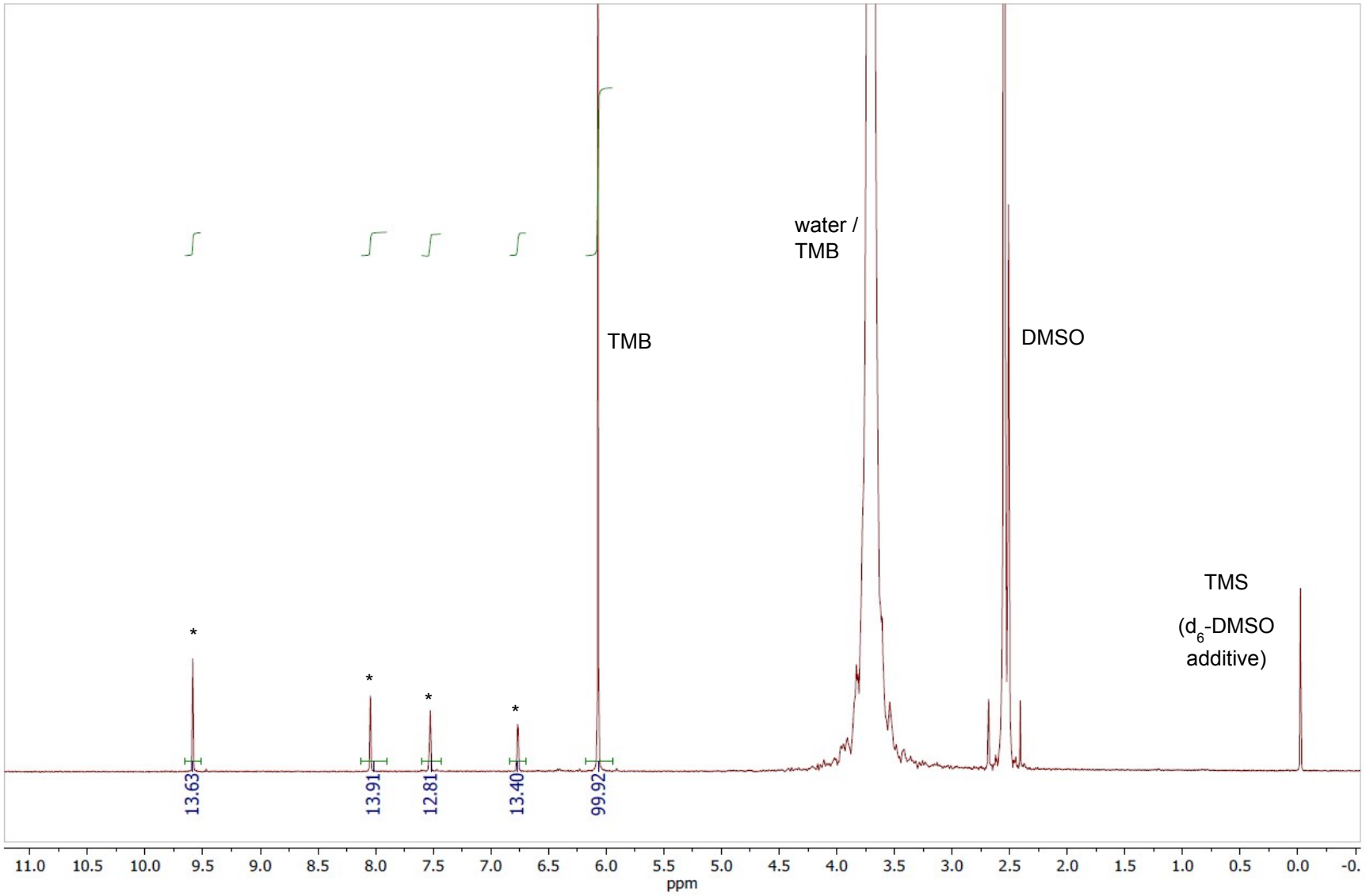


Figure S6 Table 1 Entry 1, HRP. * = product.

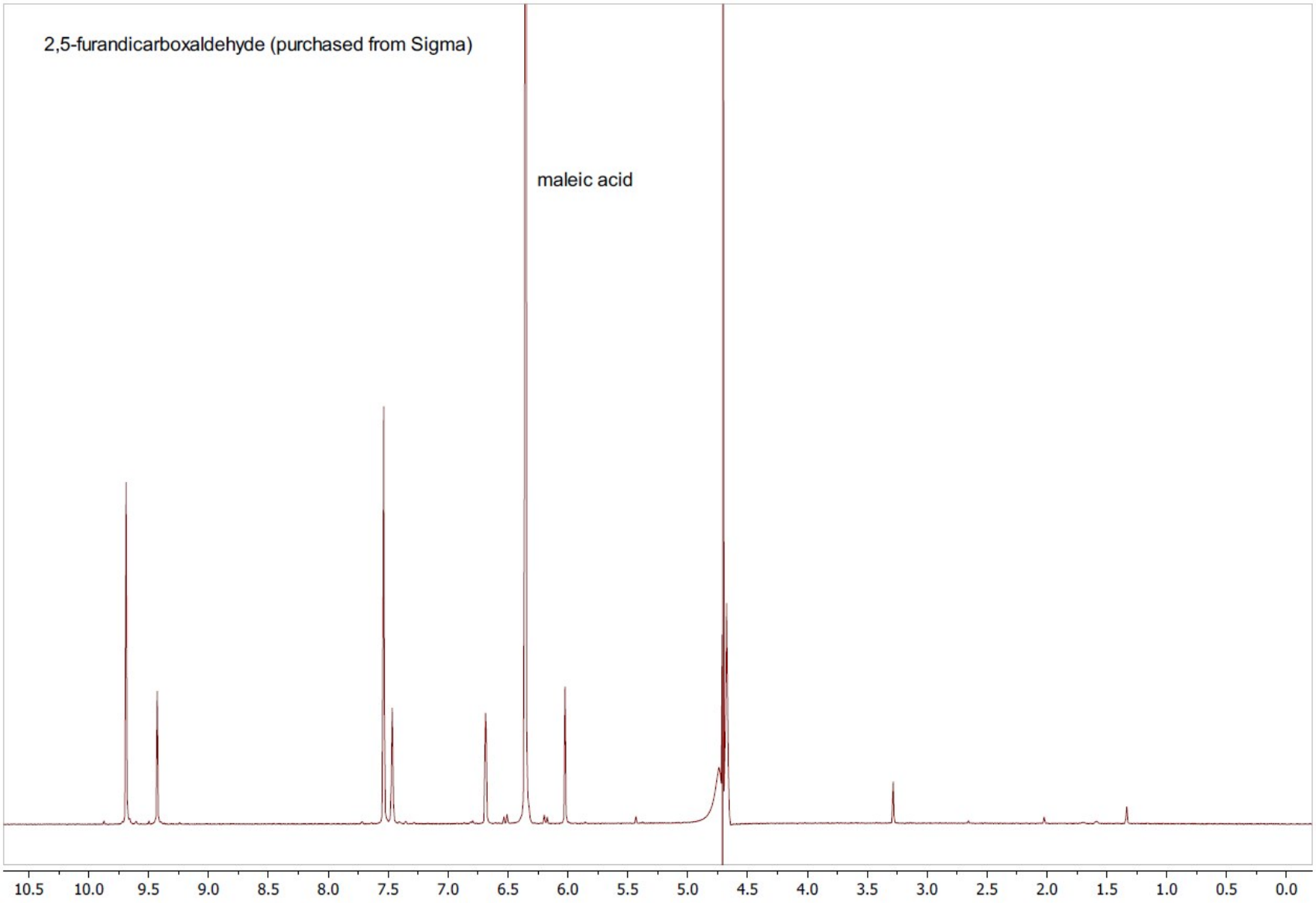


Figure S7 2,5-furandicarboxaldehyde (entry 2 product), purchased from Sigma Aldrich, in water (4.8 ppm) with maleic acid additive

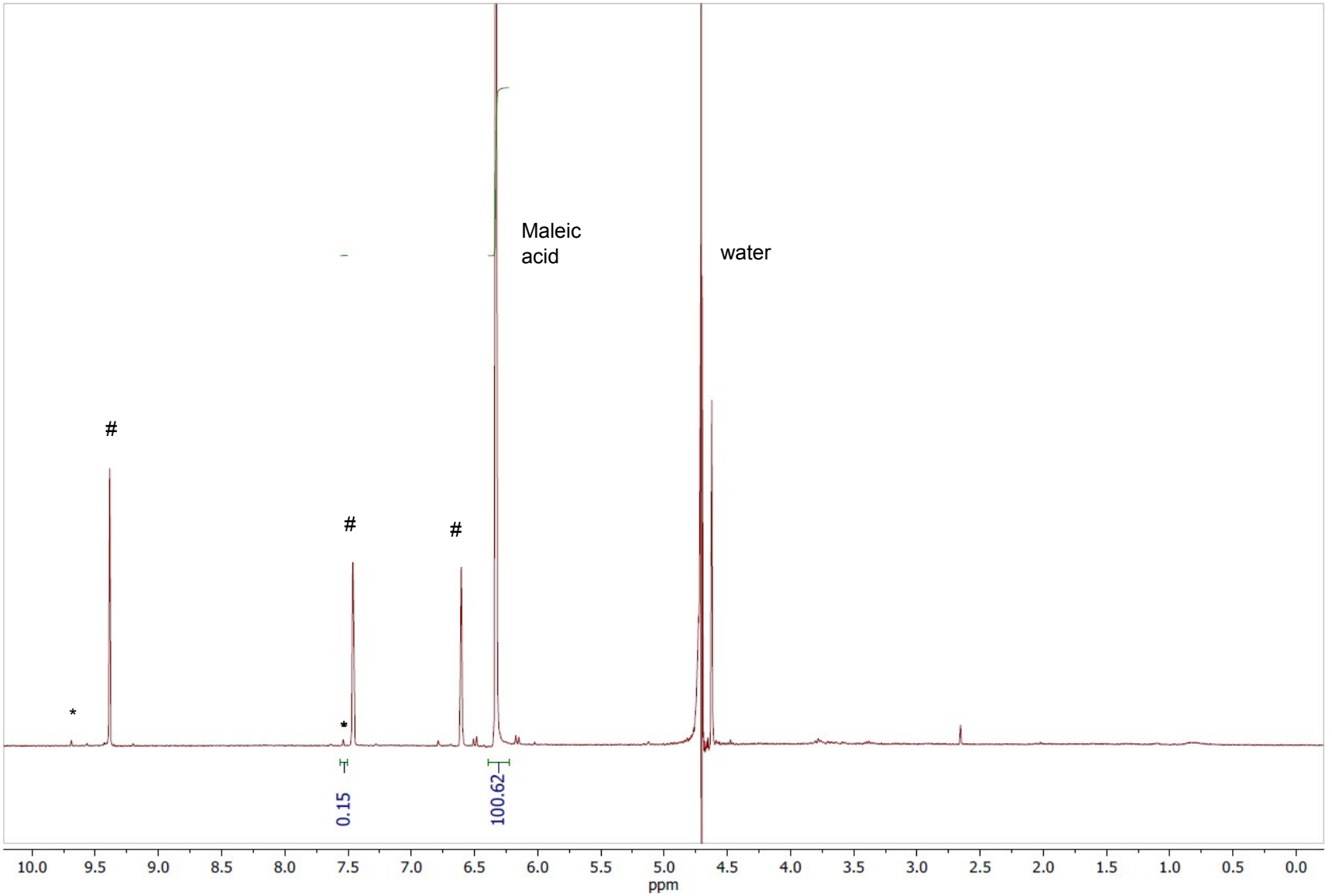


Figure S8 Table 1 Entry 2, no activator. # = SM, * = product.

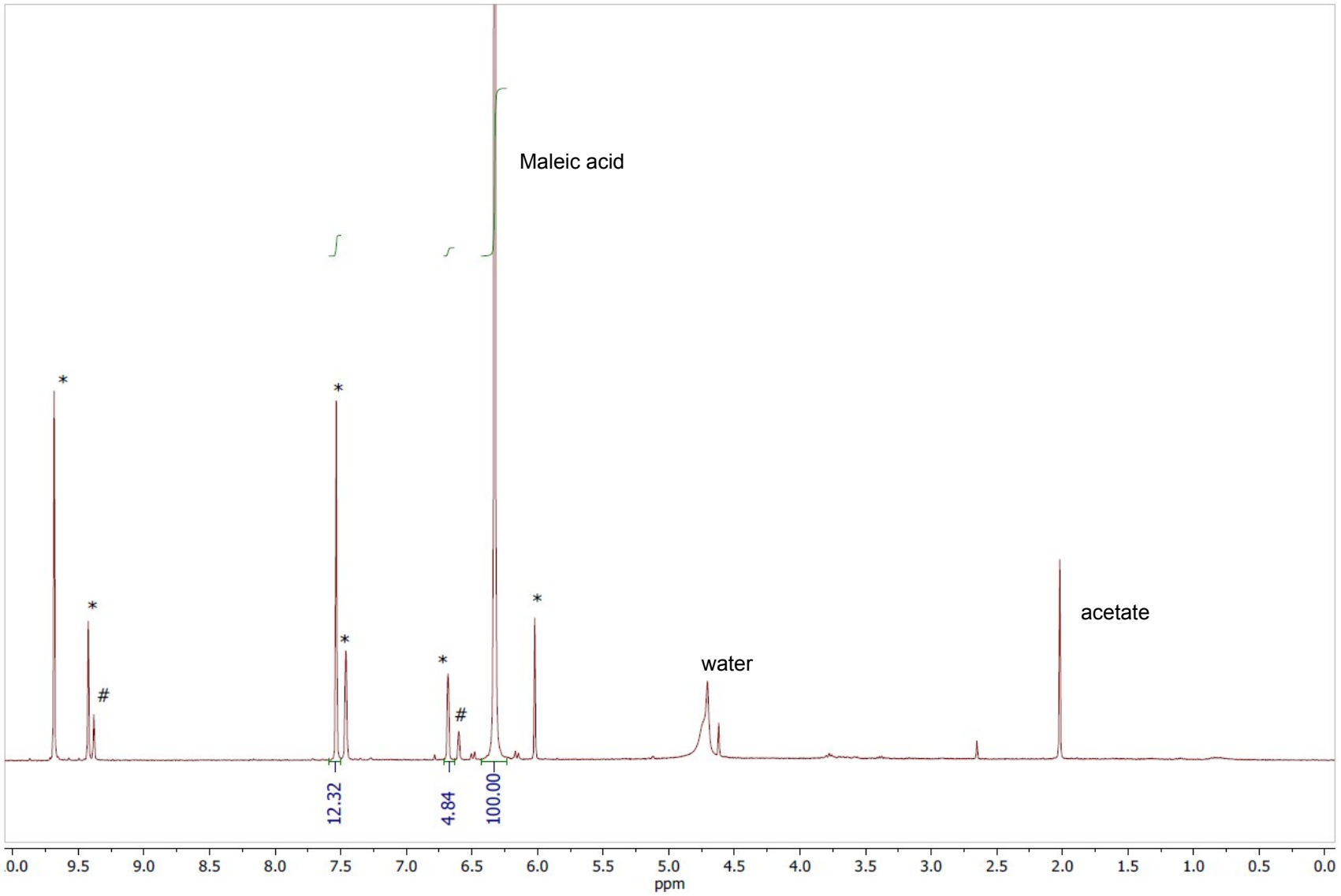


Figure S9 Table 1 Entry 2, $Mn(OAc)_3$. # = SM, * = product.

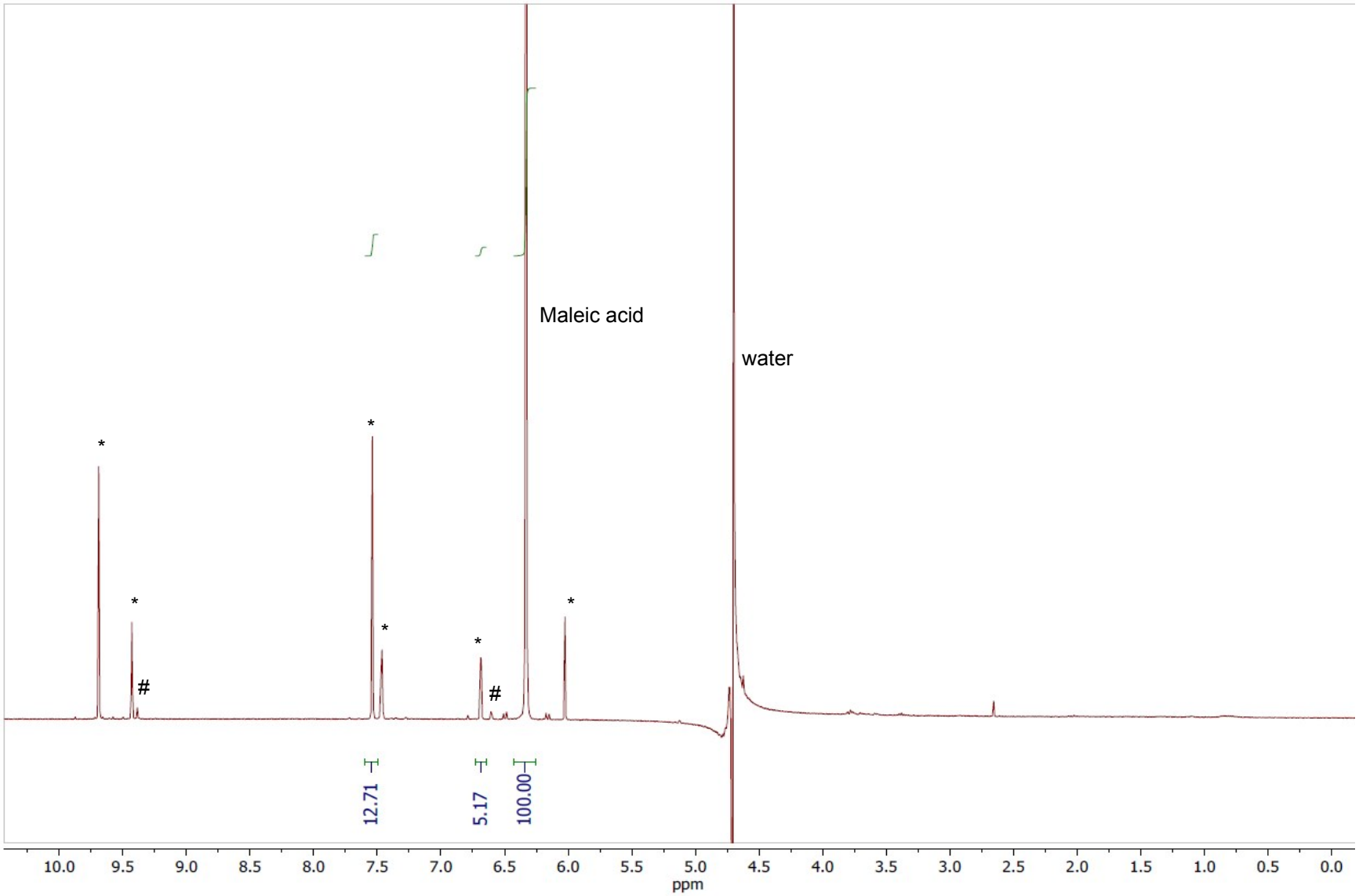


Figure S10 Table 1 Entry 2, HRP. # = SM, * = product.

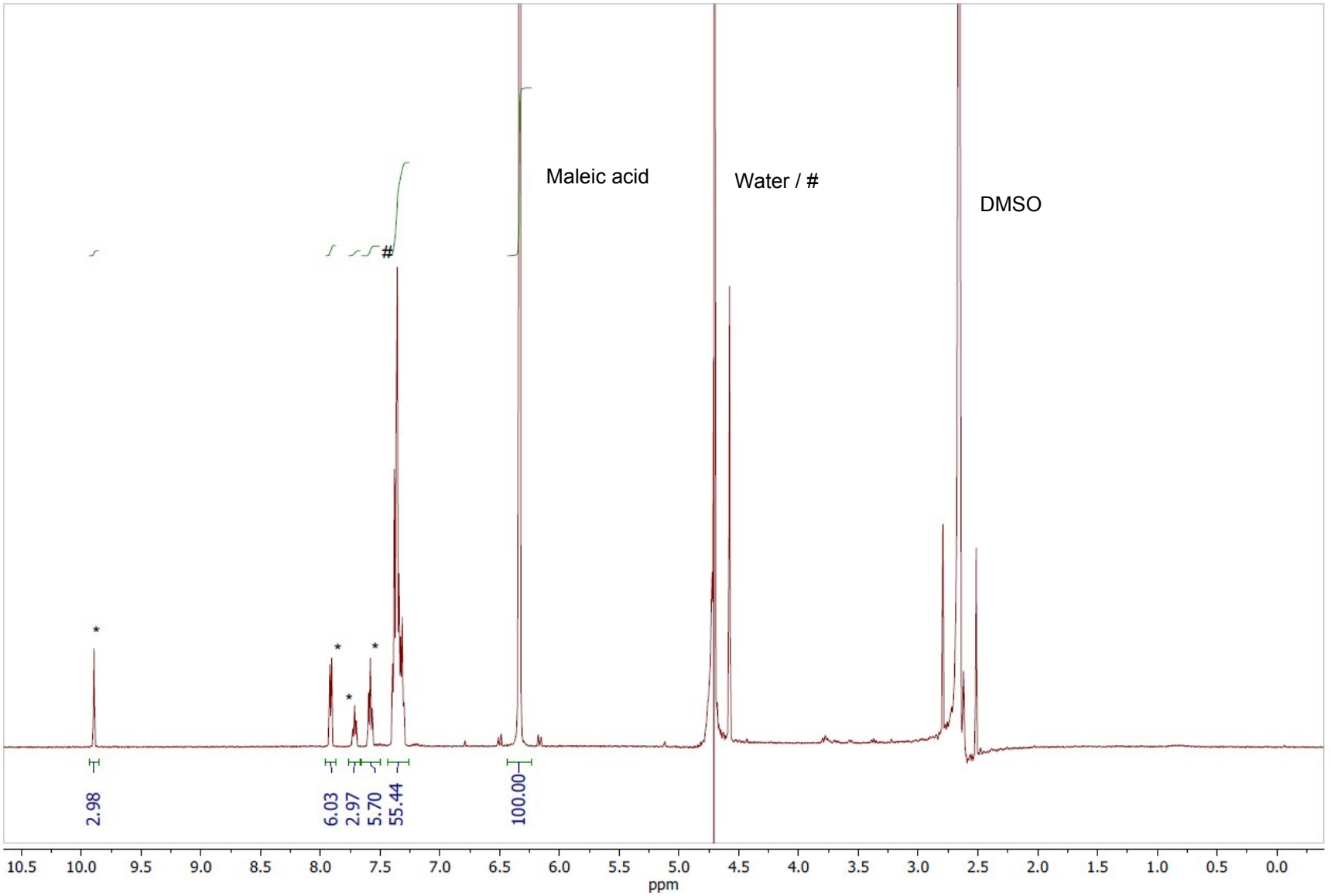


Figure S11 Table 1 Entry 3, no activator. # = SM, * = product.

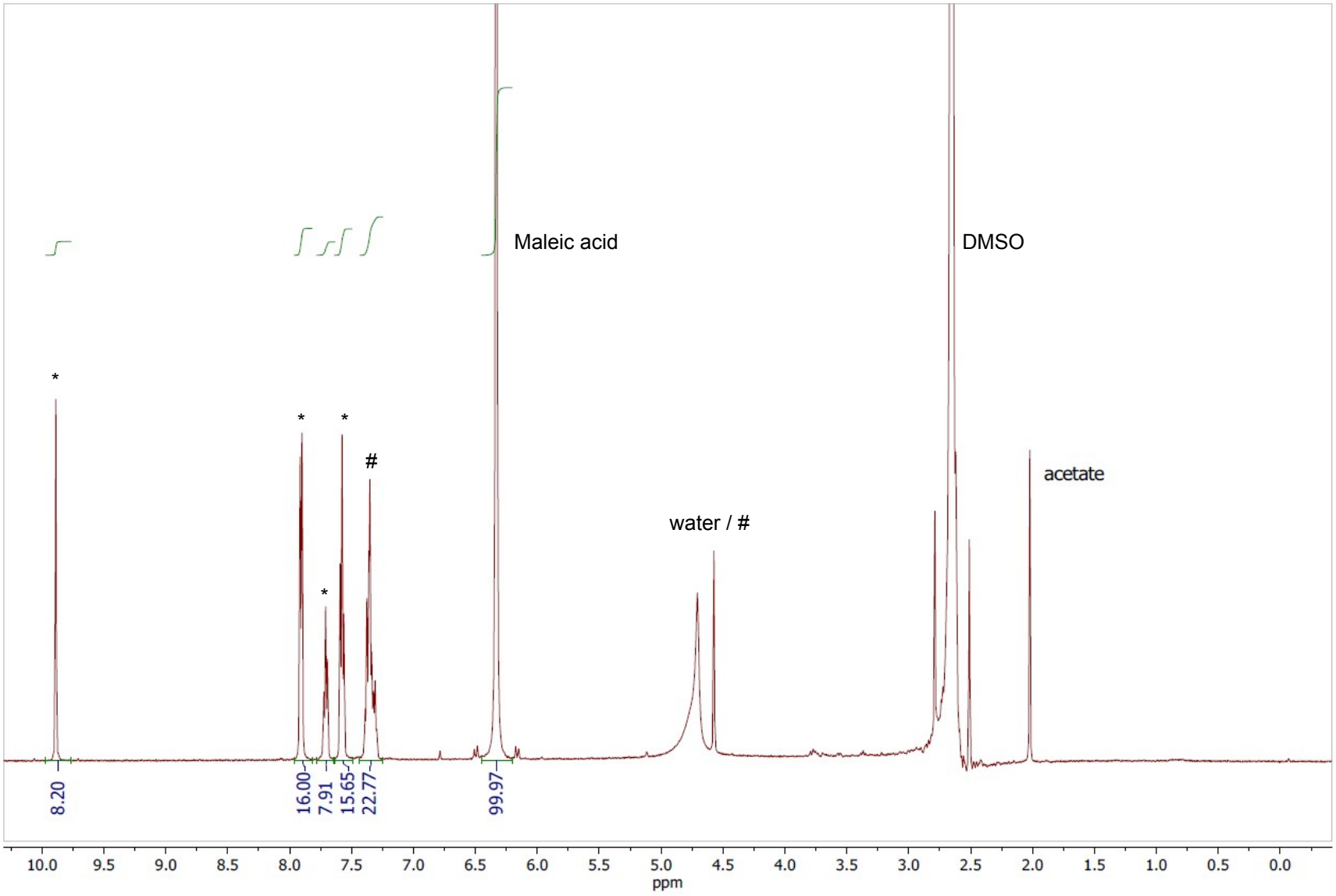


Figure S12 Table 1 Entry 3, $Mn(OAc)_3$. # = SM, * = product.

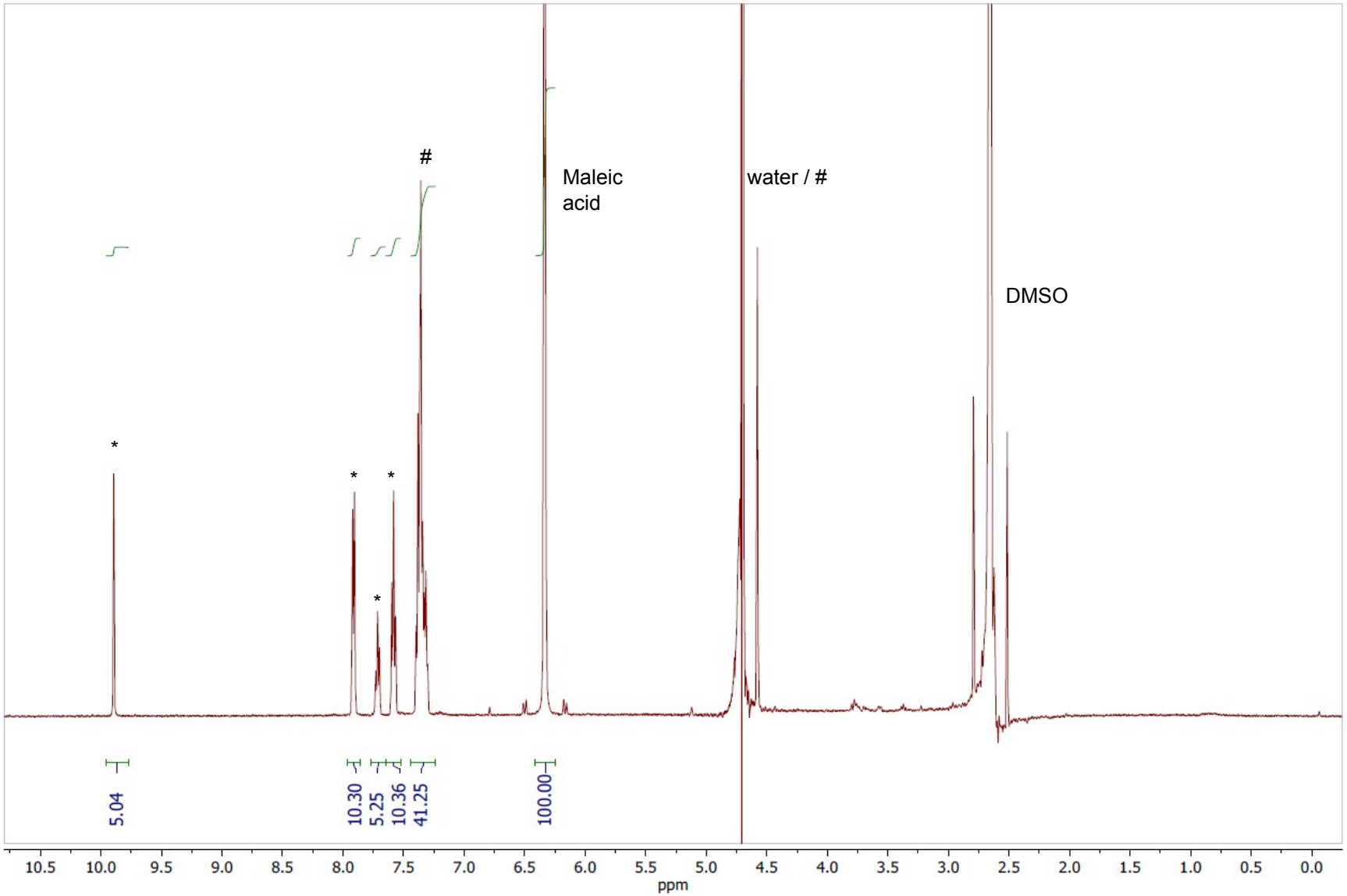


Figure S13 Table 1 Entry 3, HRP. # = SM, * = product.

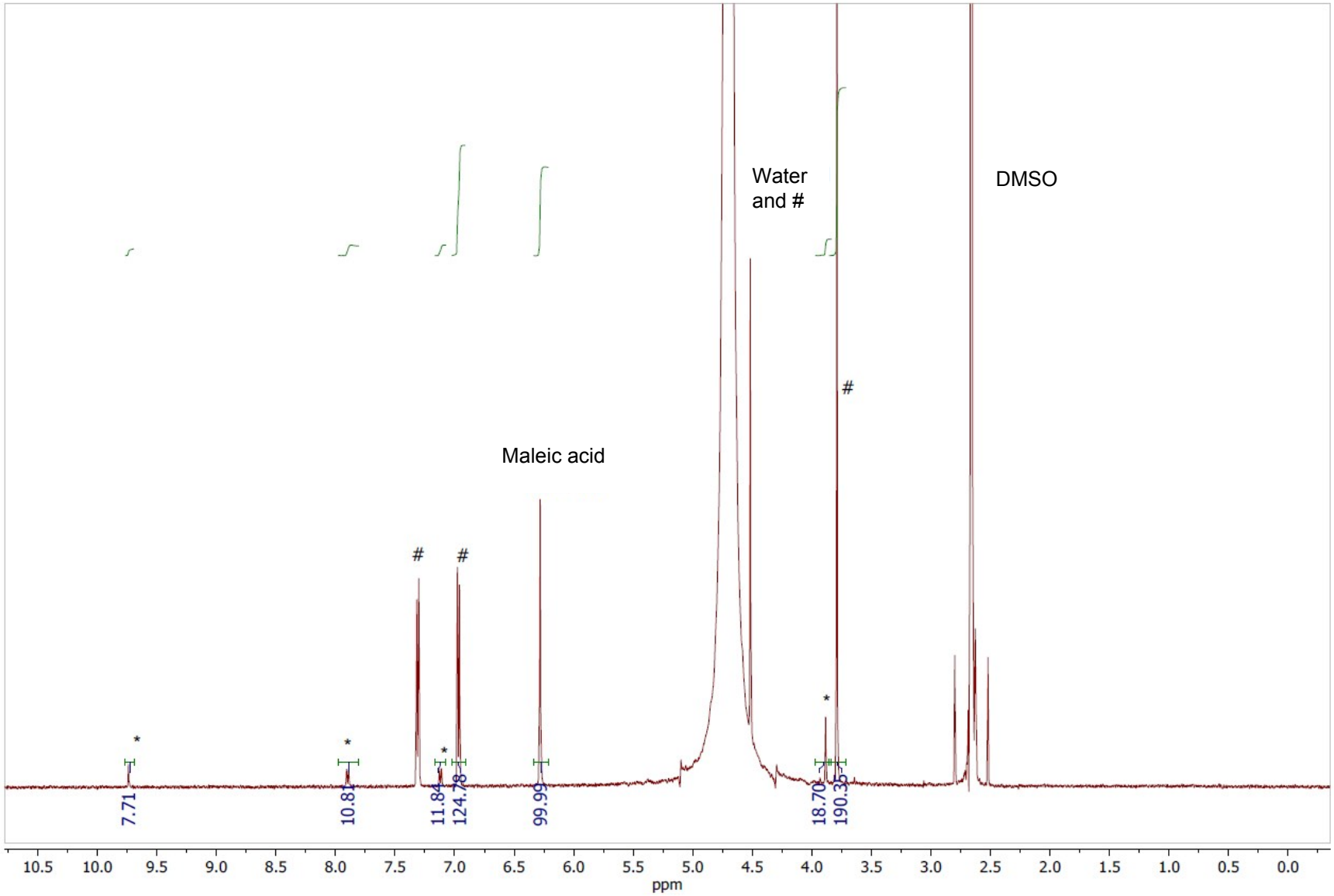


Figure S14 Table 1 Entry 4, no activator. # = SM, * = product.

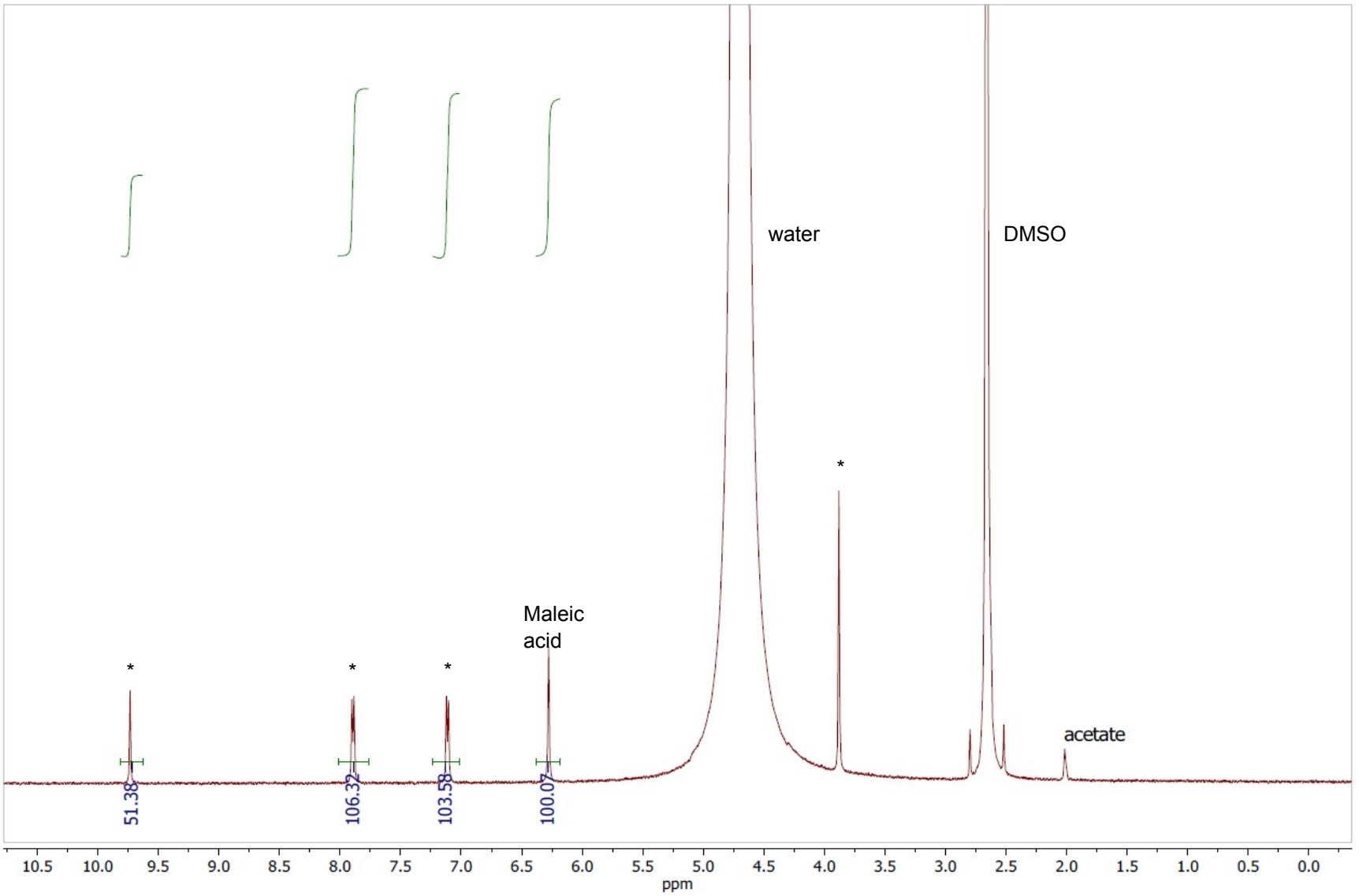


Figure S15 Table 1 Entry 4, $Mn(OAc)_3$. * = product.

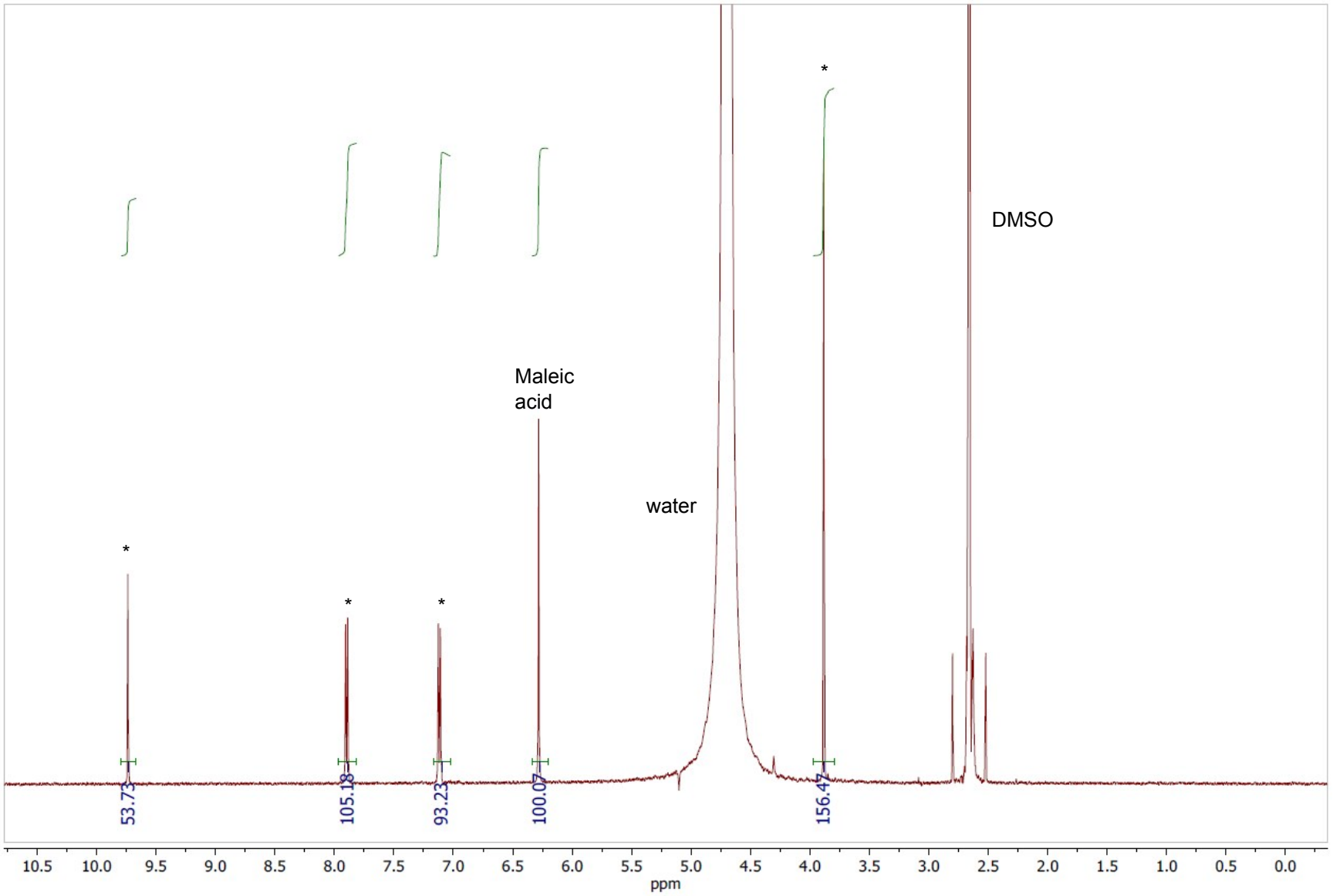


Figure S16 Table 1 Entry 4, HRP. * = product.

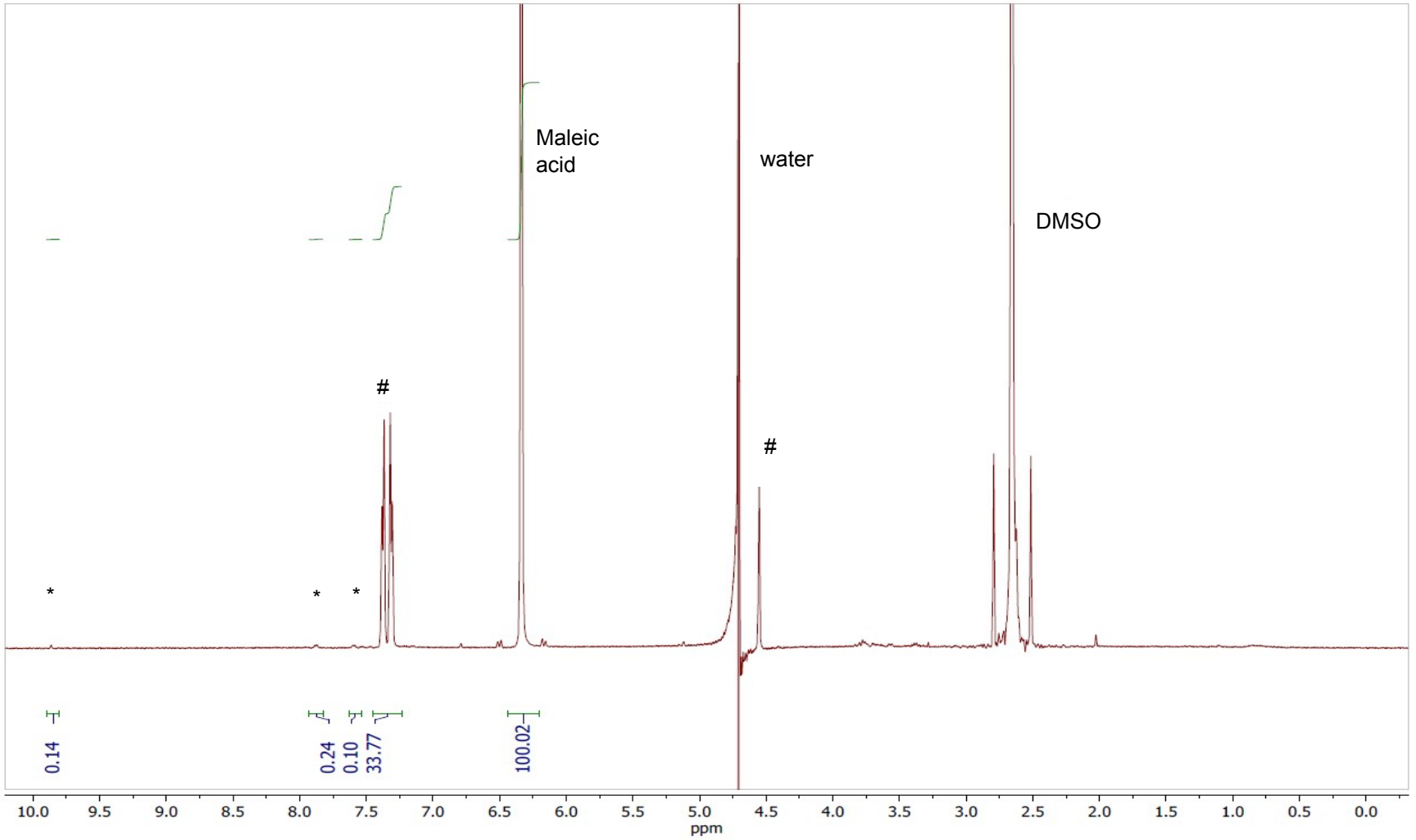


Figure S17 Table 1 Entry 5, no activator. # = SM, * = product.

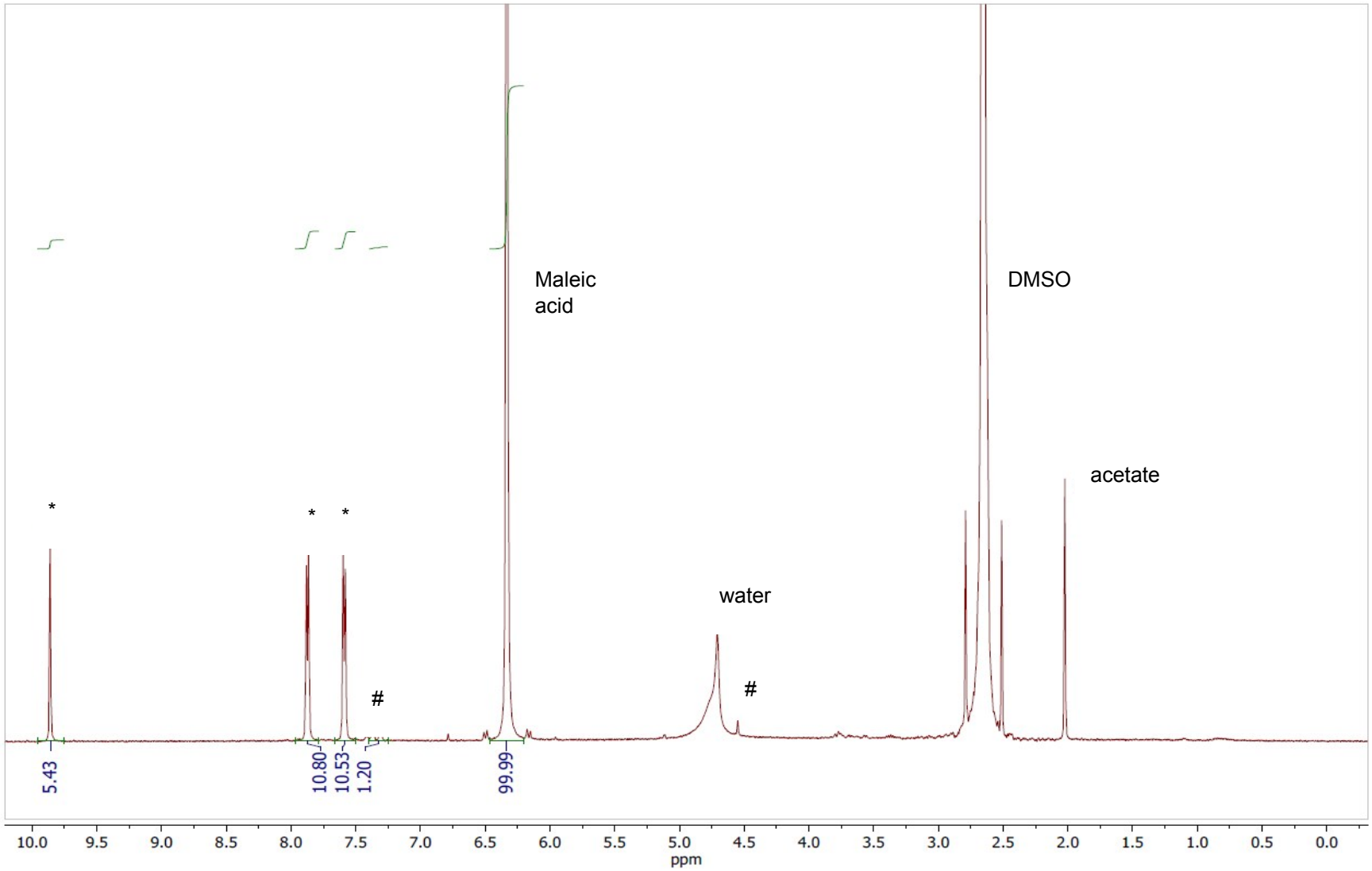


Figure S18 Table 1 Entry 5, $Mn(OAc)_3$. # = SM, * = product.

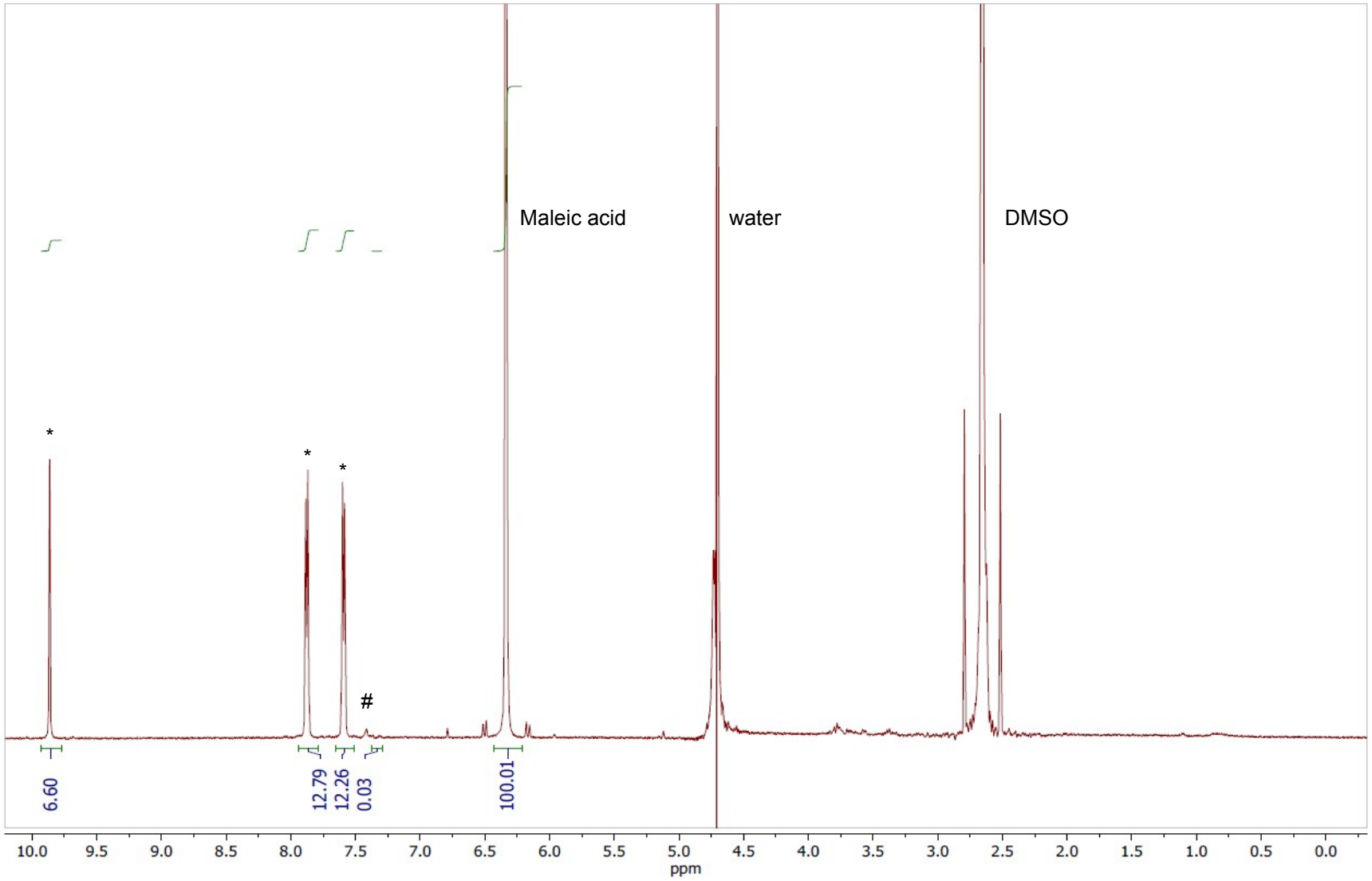


Figure S19 Table 1 Entry 5, HRP. # = SM, * = product.

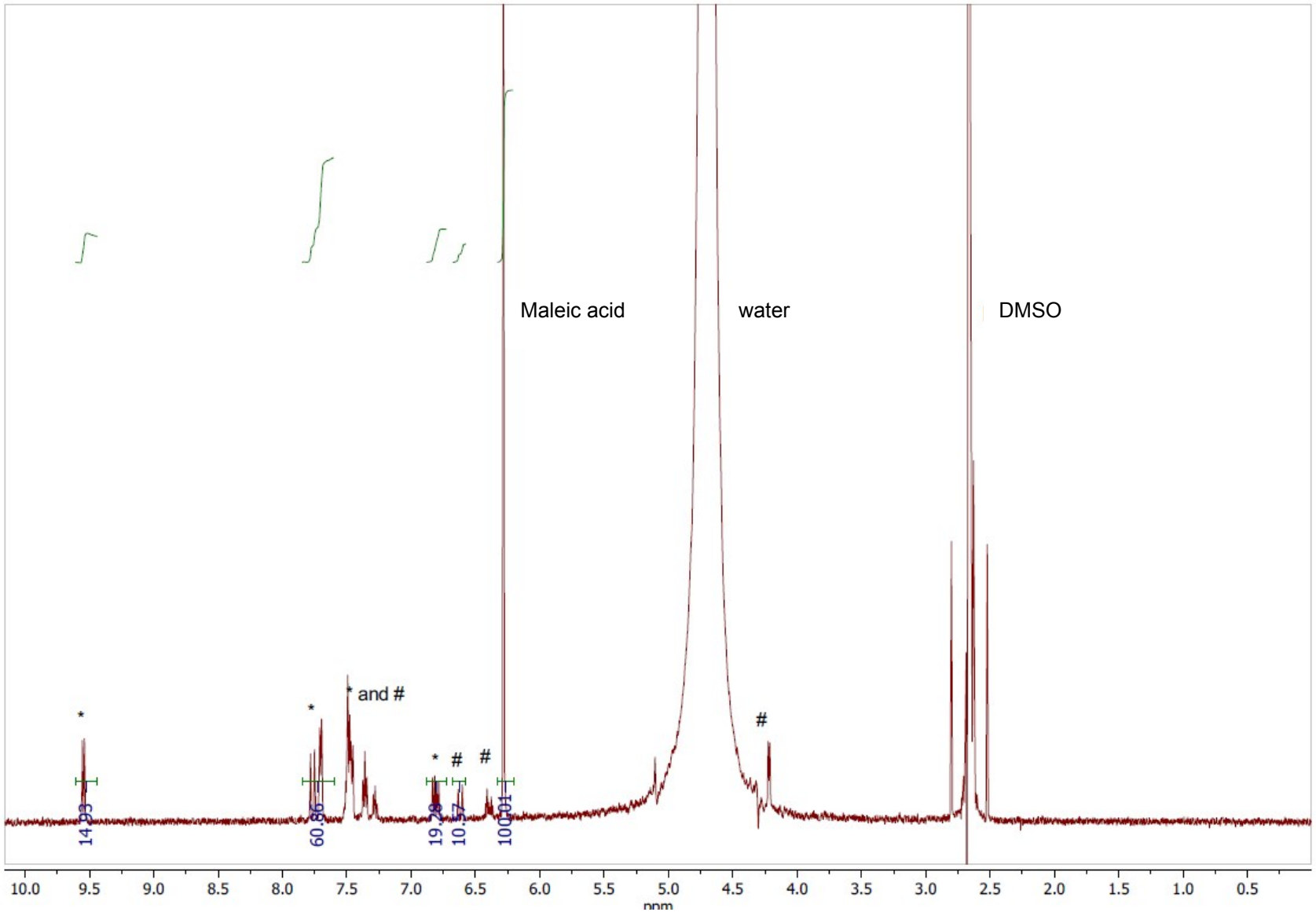


Figure S20 Table 1 Entry 6, no activator. # = SM, * = product.

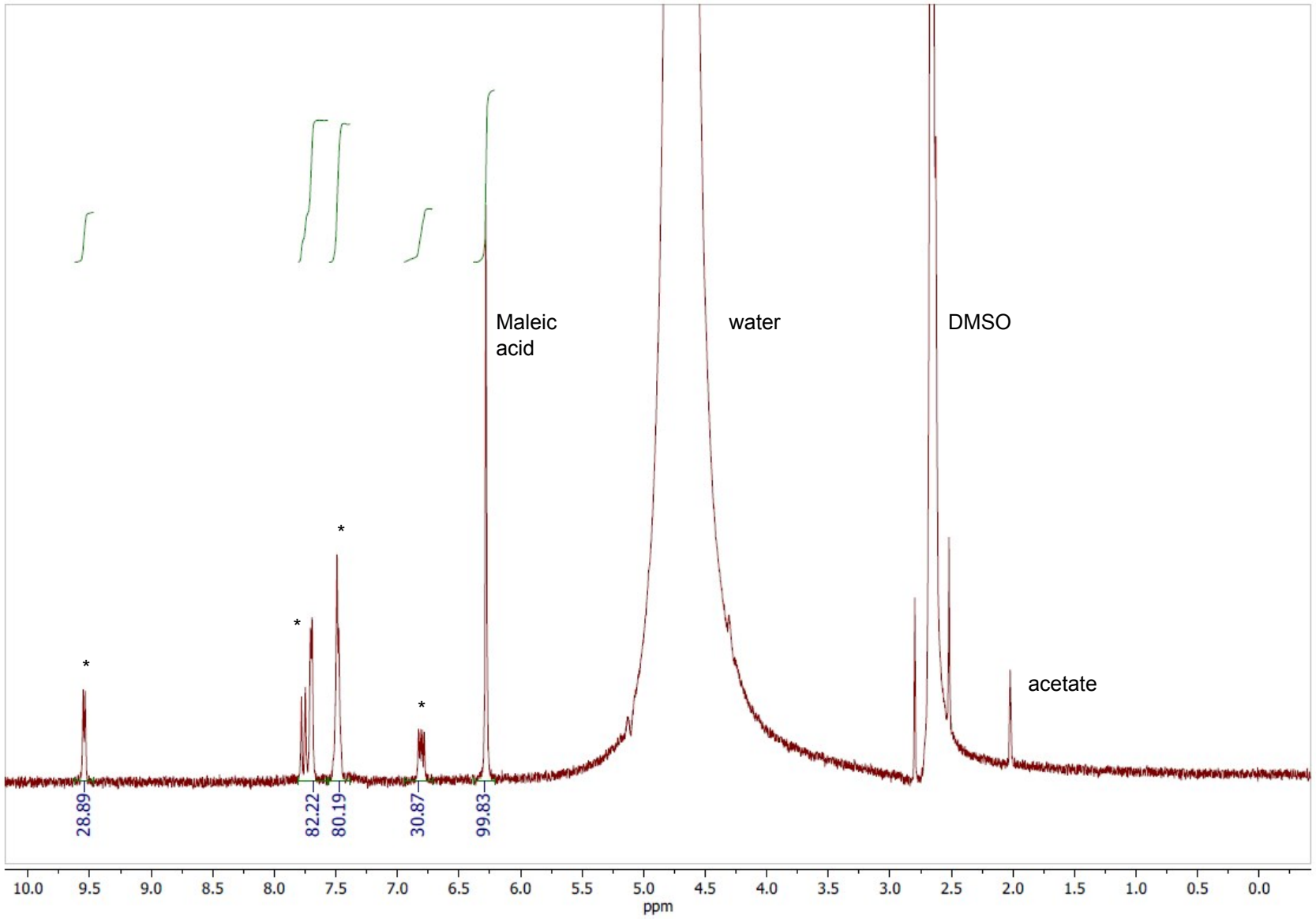


Figure S21 Table 1 Entry 6, $Mn(OAc)_3$. * = product.

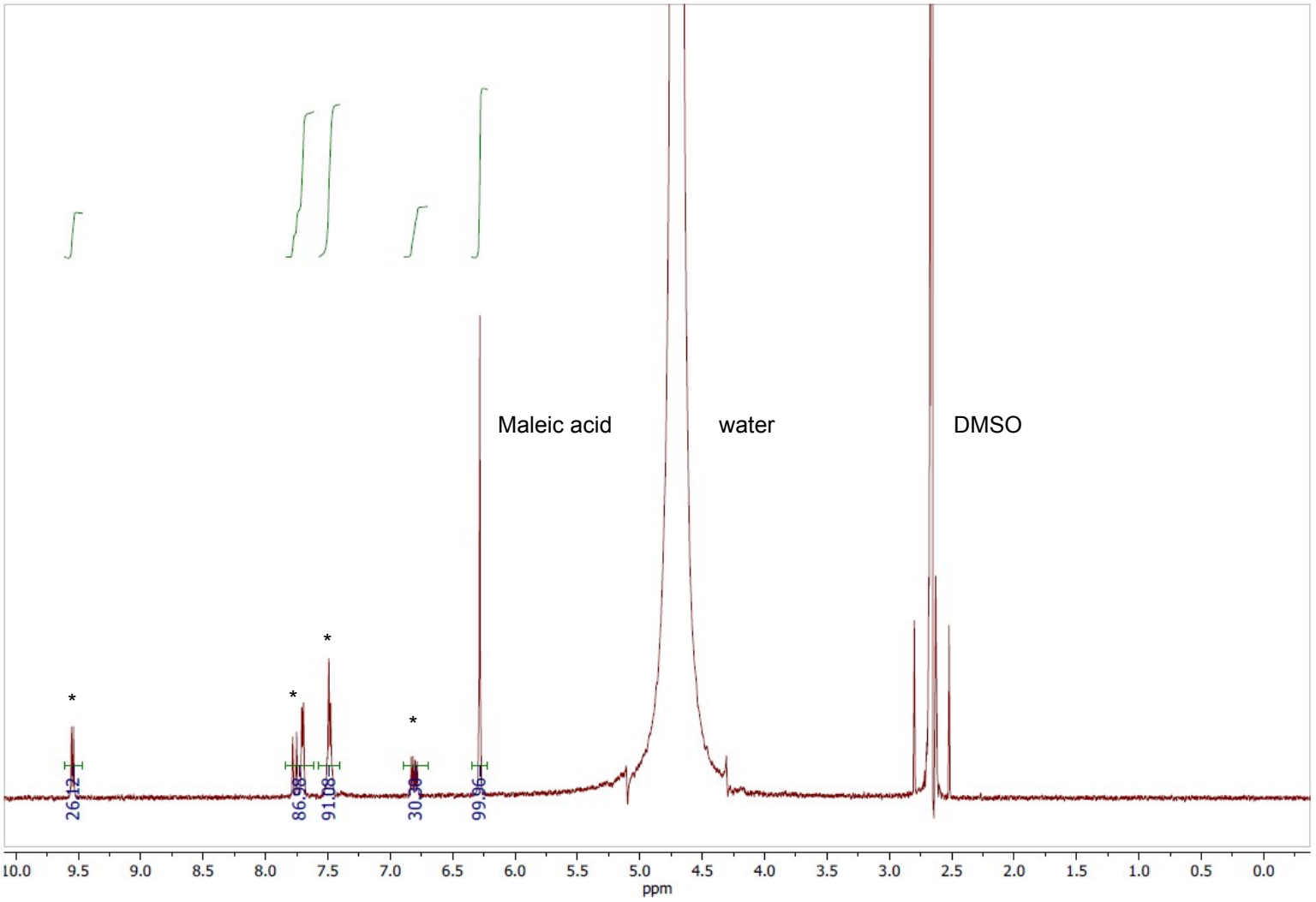


Figure S22 Table 1 Entry 6, HRP. * = product.

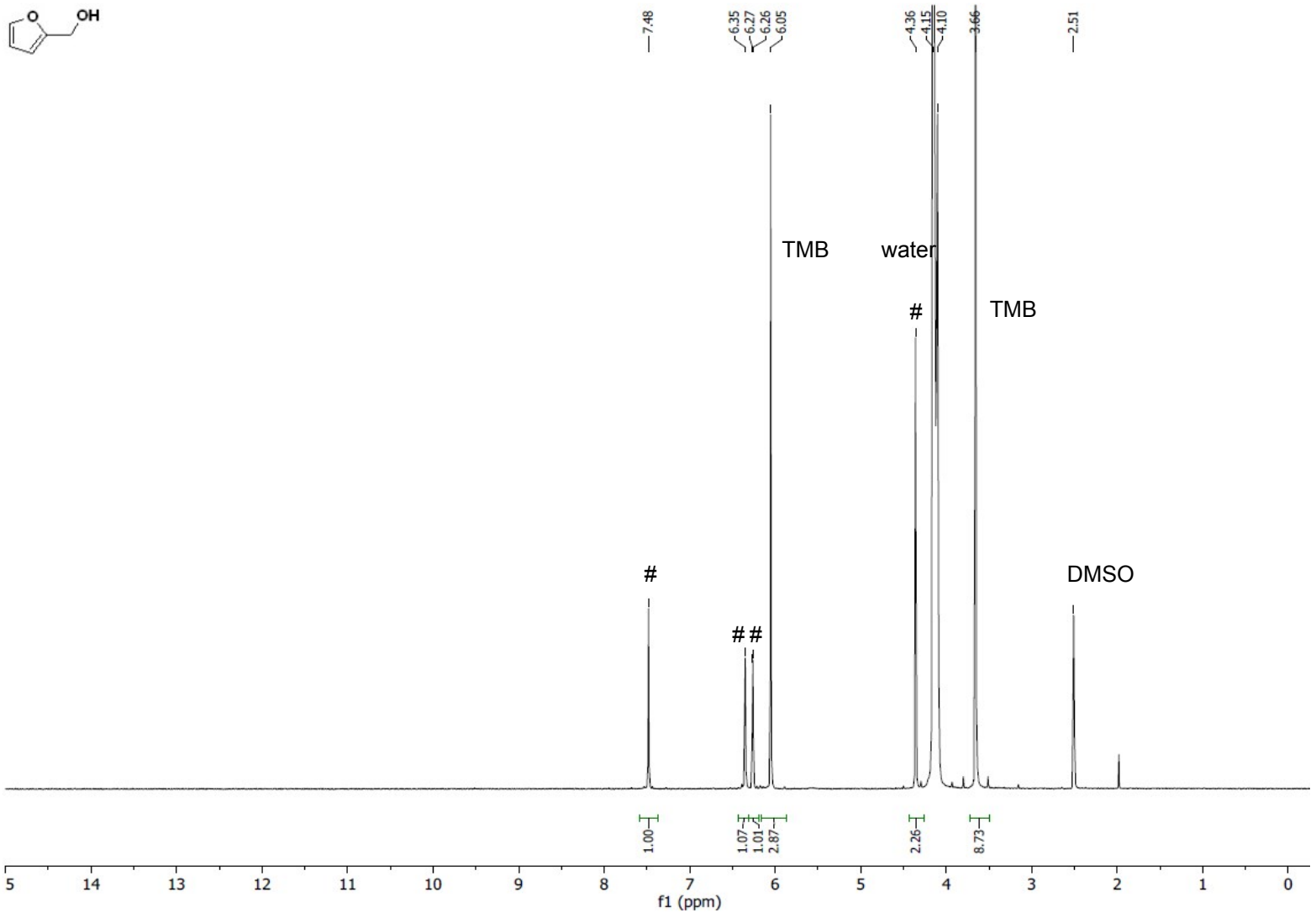


Figure S23 Control experiment for entry 1. # = SM.

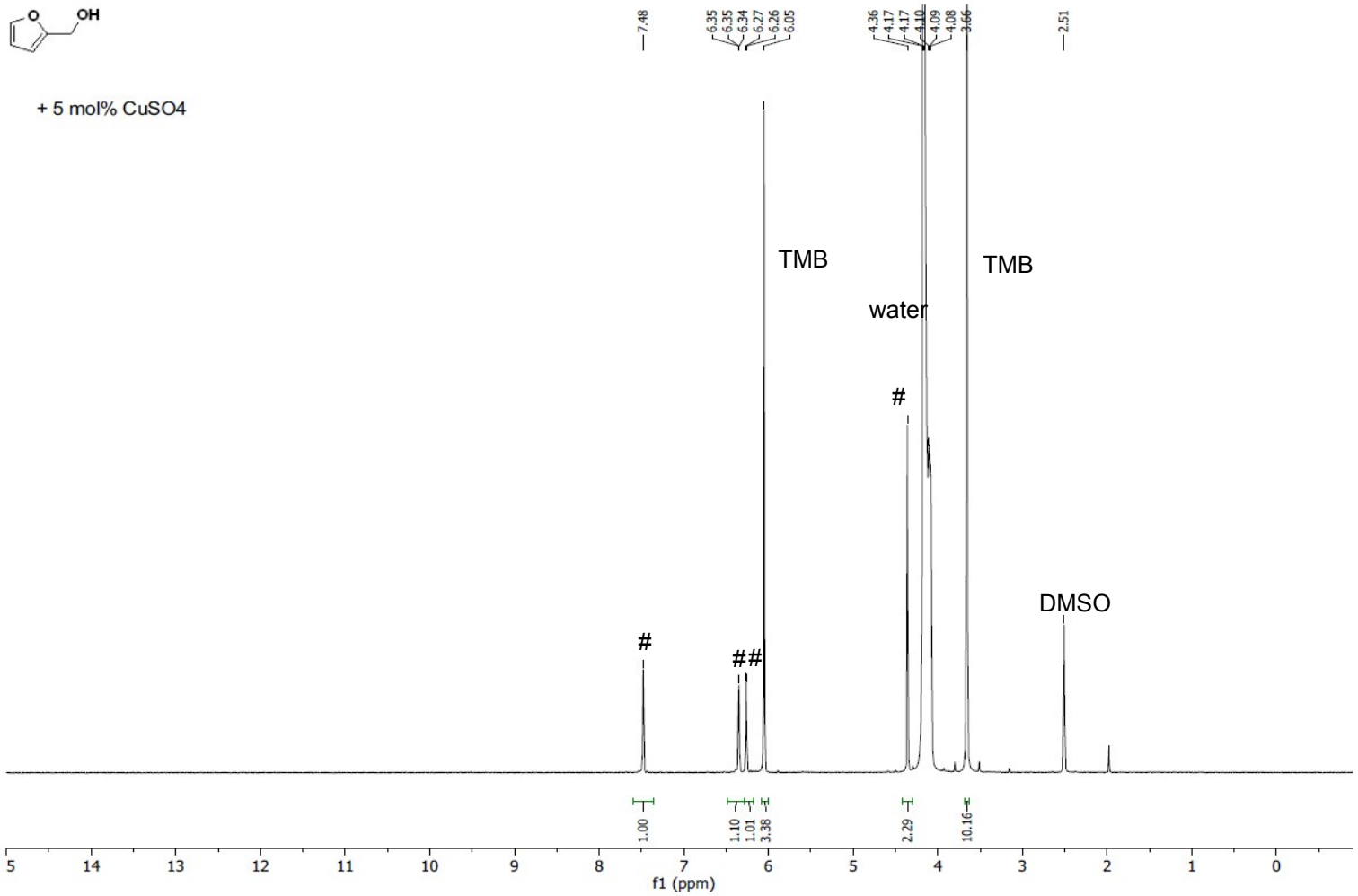


Figure S24 Control experiment for entry 1. # = SM.

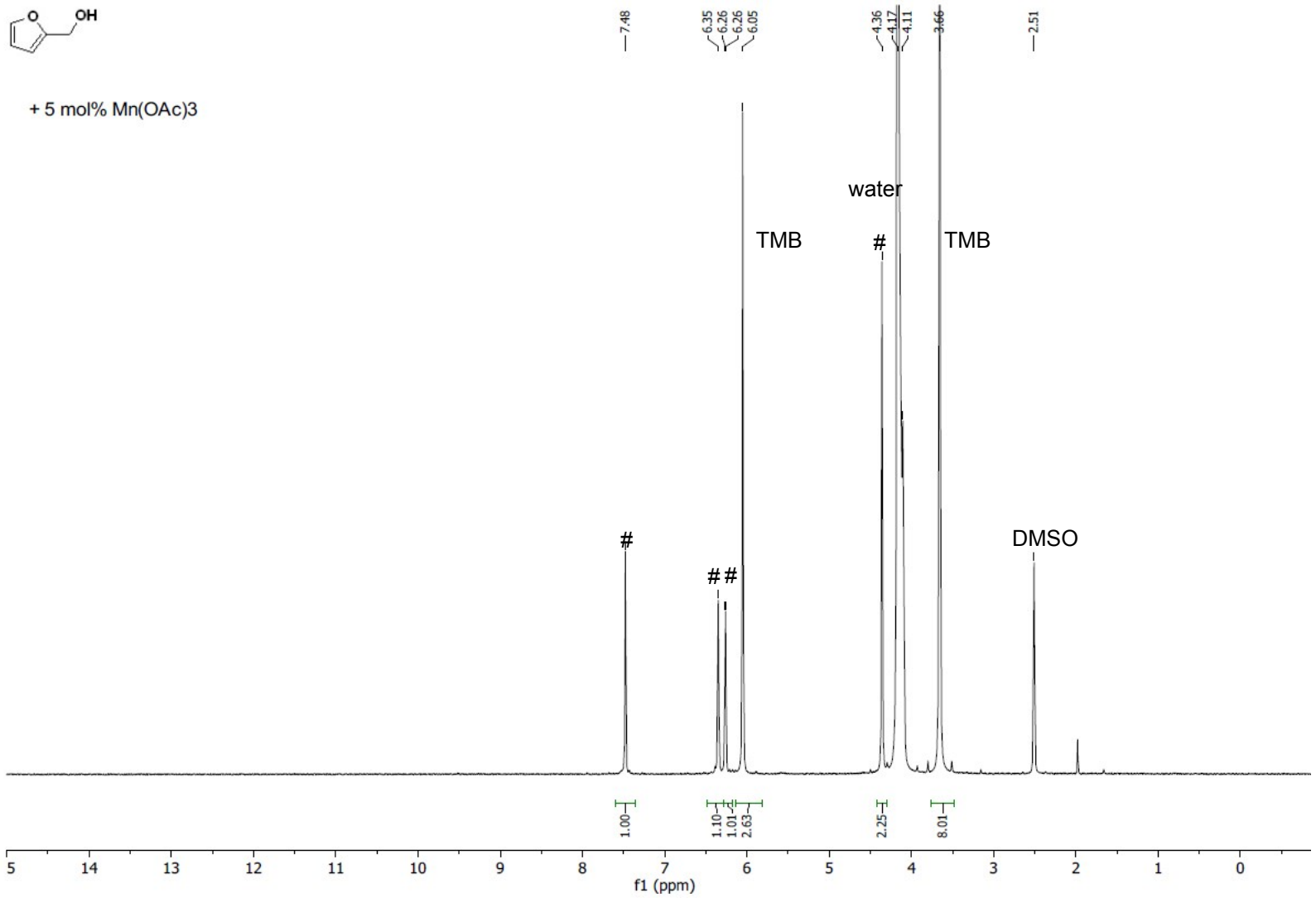


Figure S25 Control experiment for entry 1. # = SM.

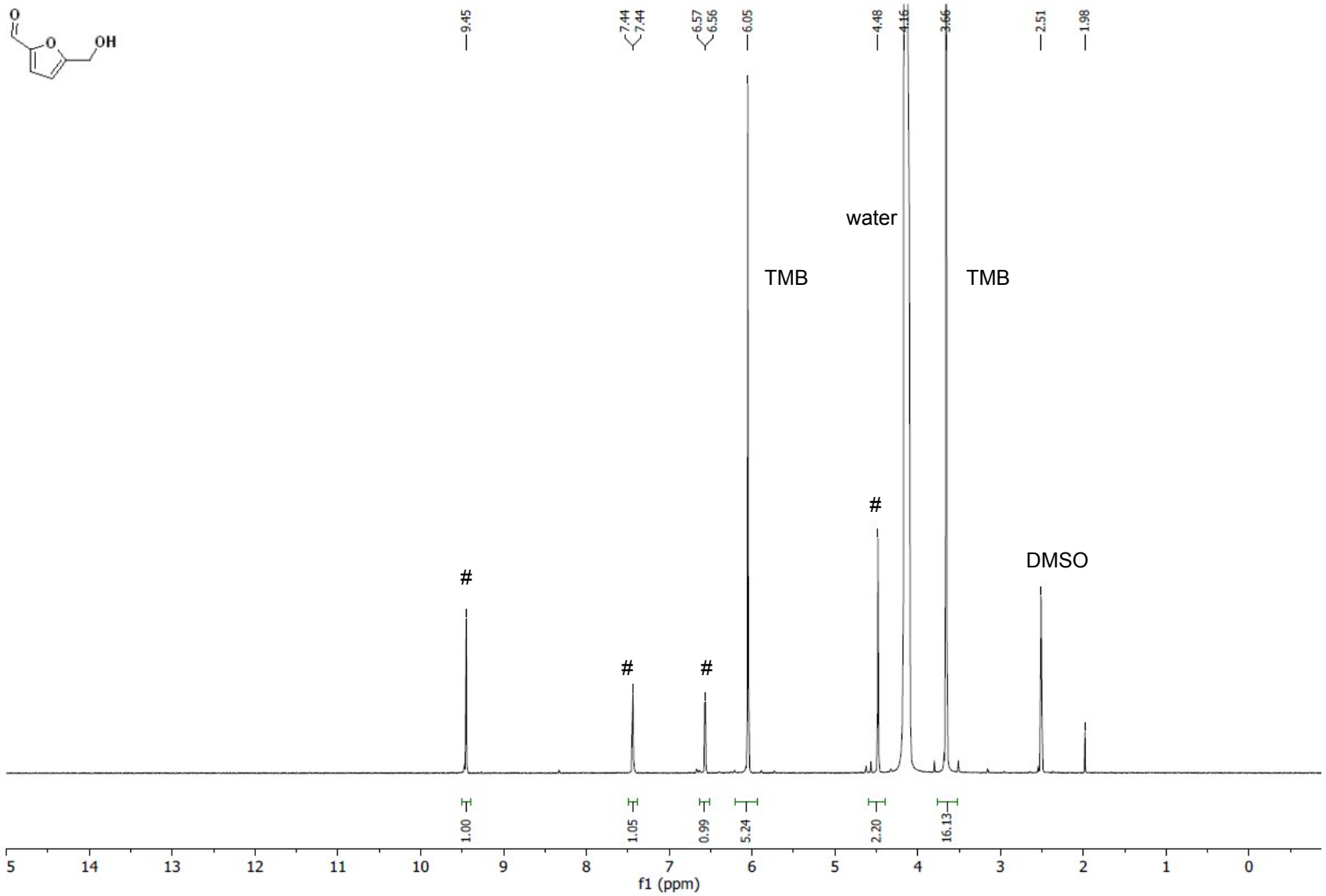


Figure S26 Control experiment for entry 2. # = SM.

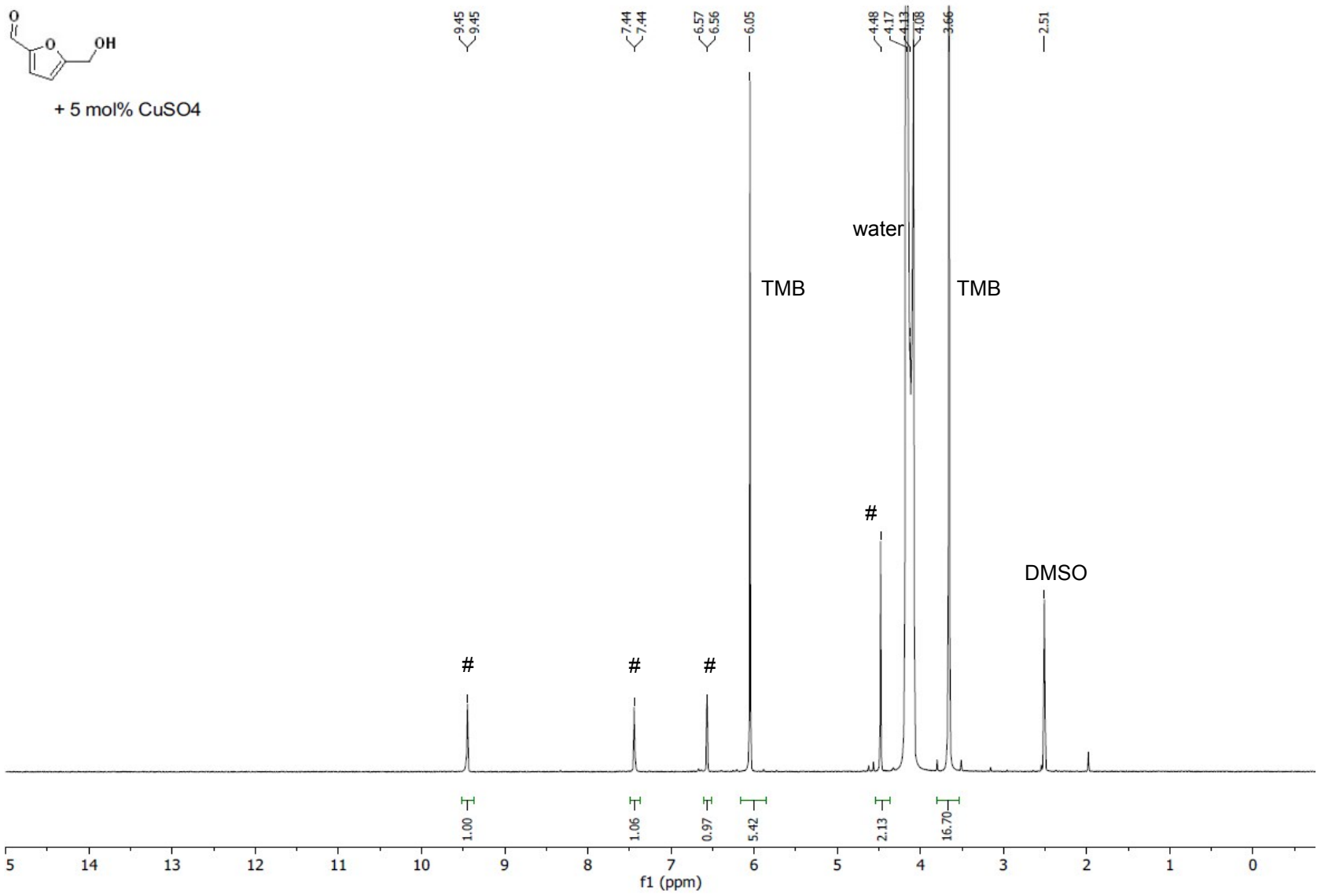


Figure S27 Control experiment for entry 2. # = SM.

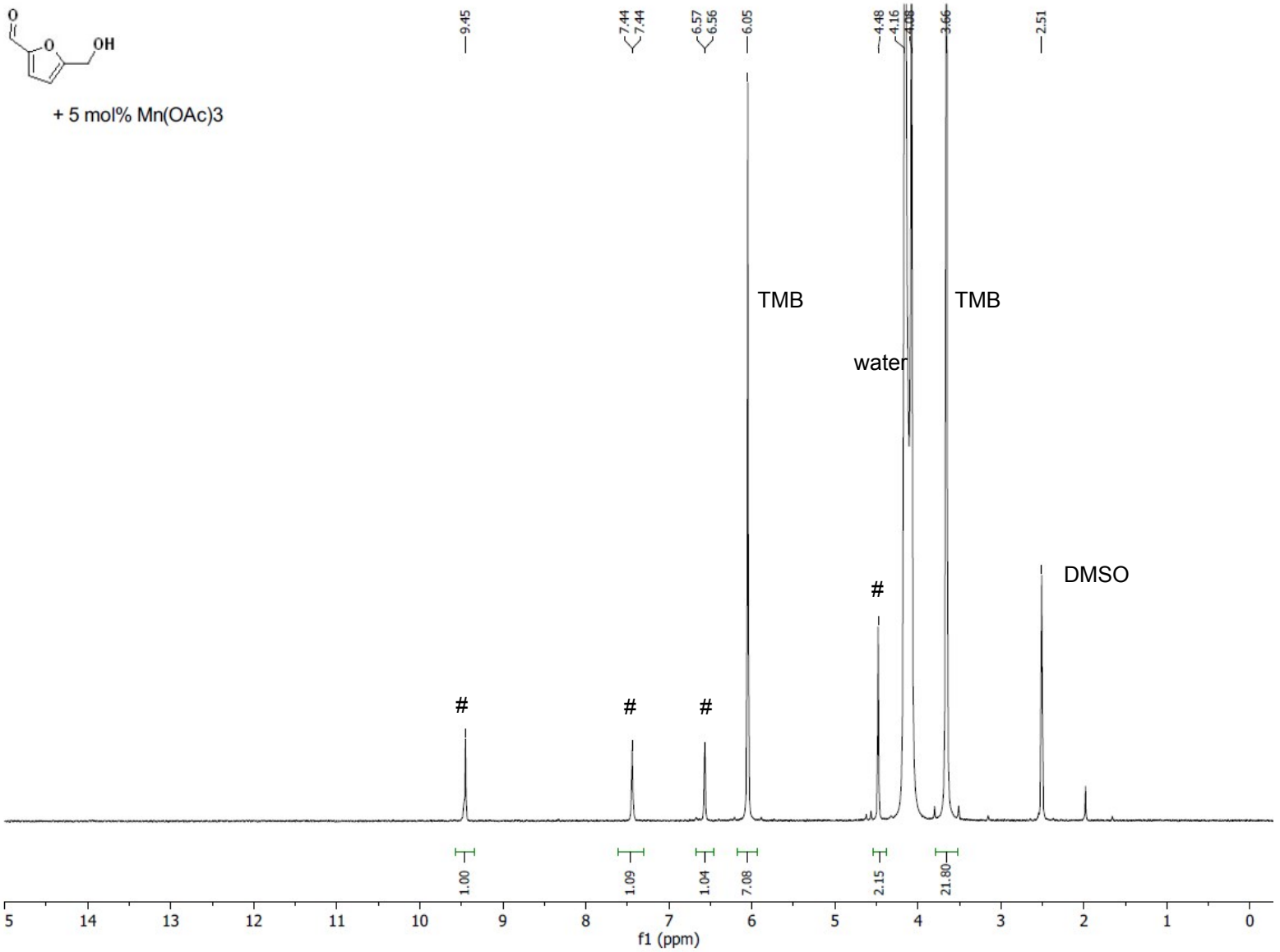


Figure S28 Control experiment for entry 2. # = SM.

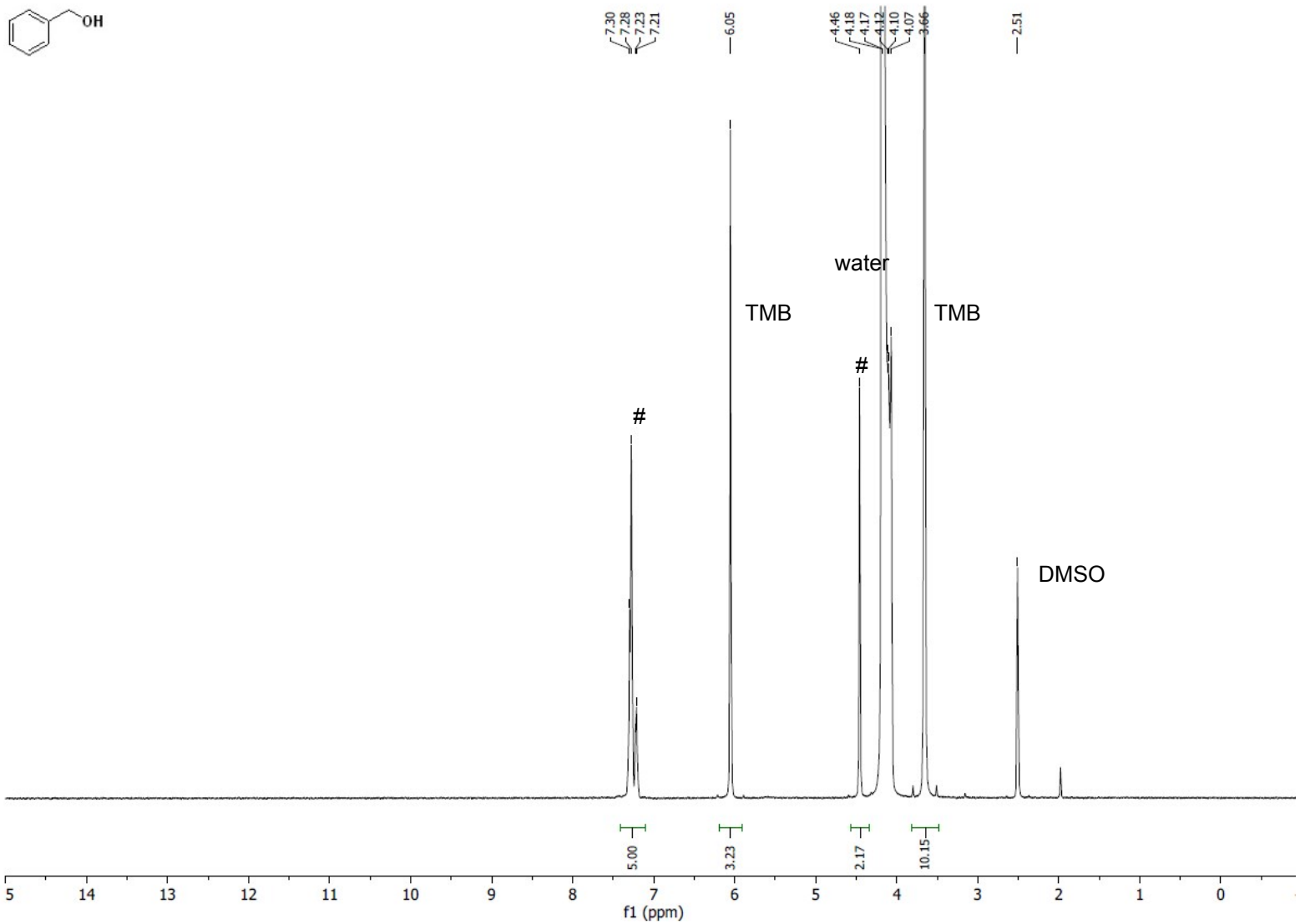


Figure S29 Control experiment for entry 3. # = SM.

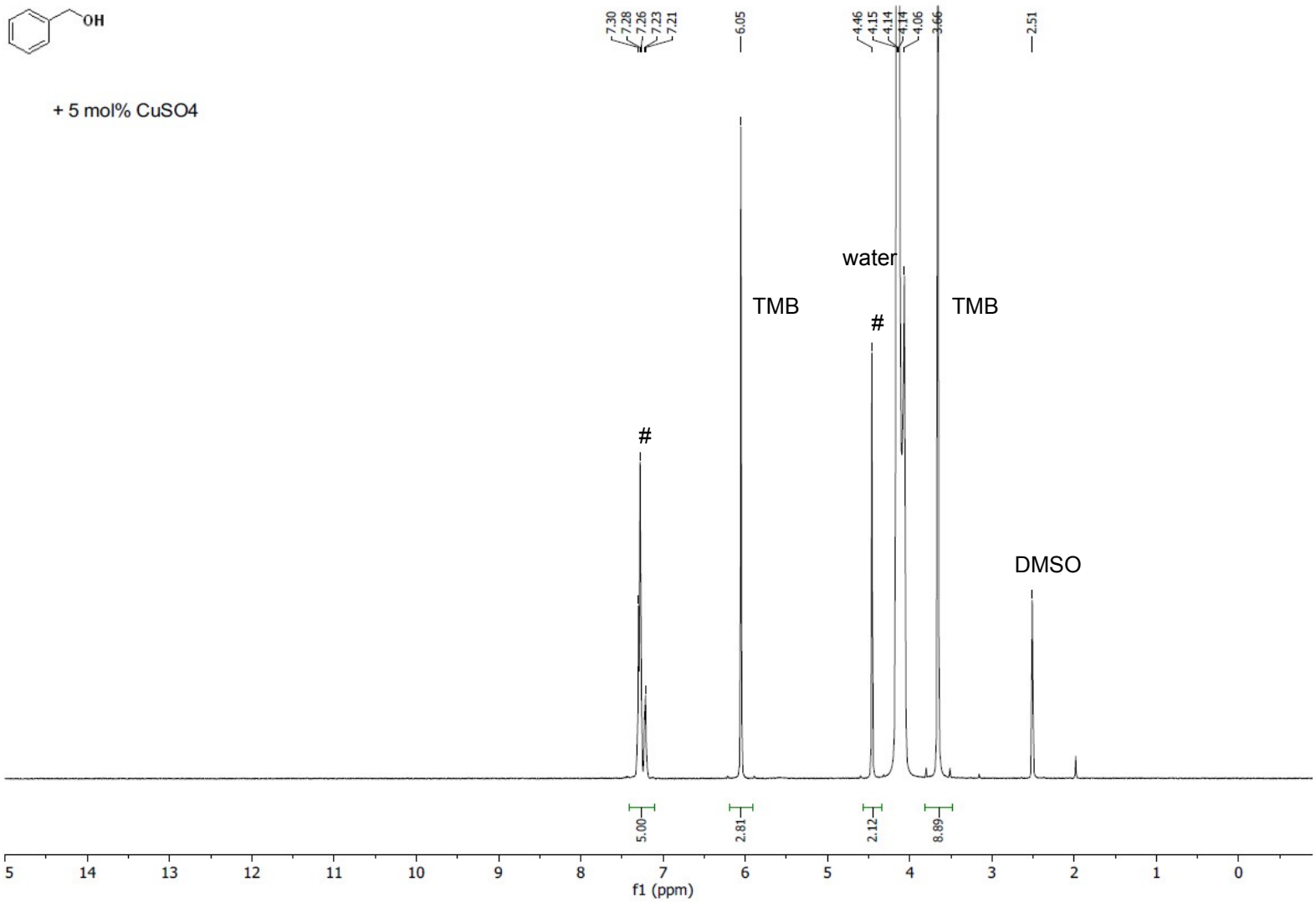


Figure S30 Control experiment for entry 3. # = SM.

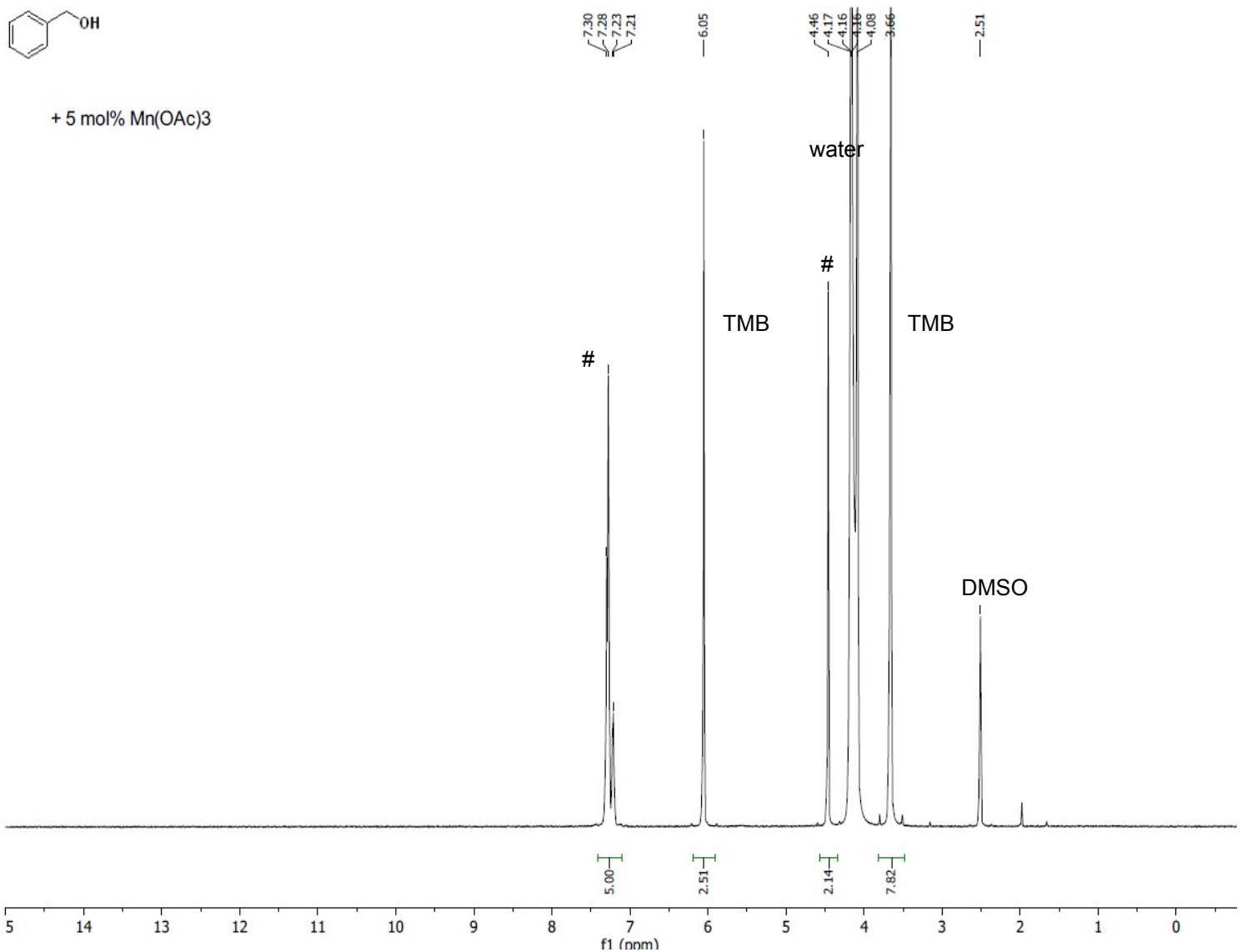


Figure S31 Control experiment for entry 3. # = SM.

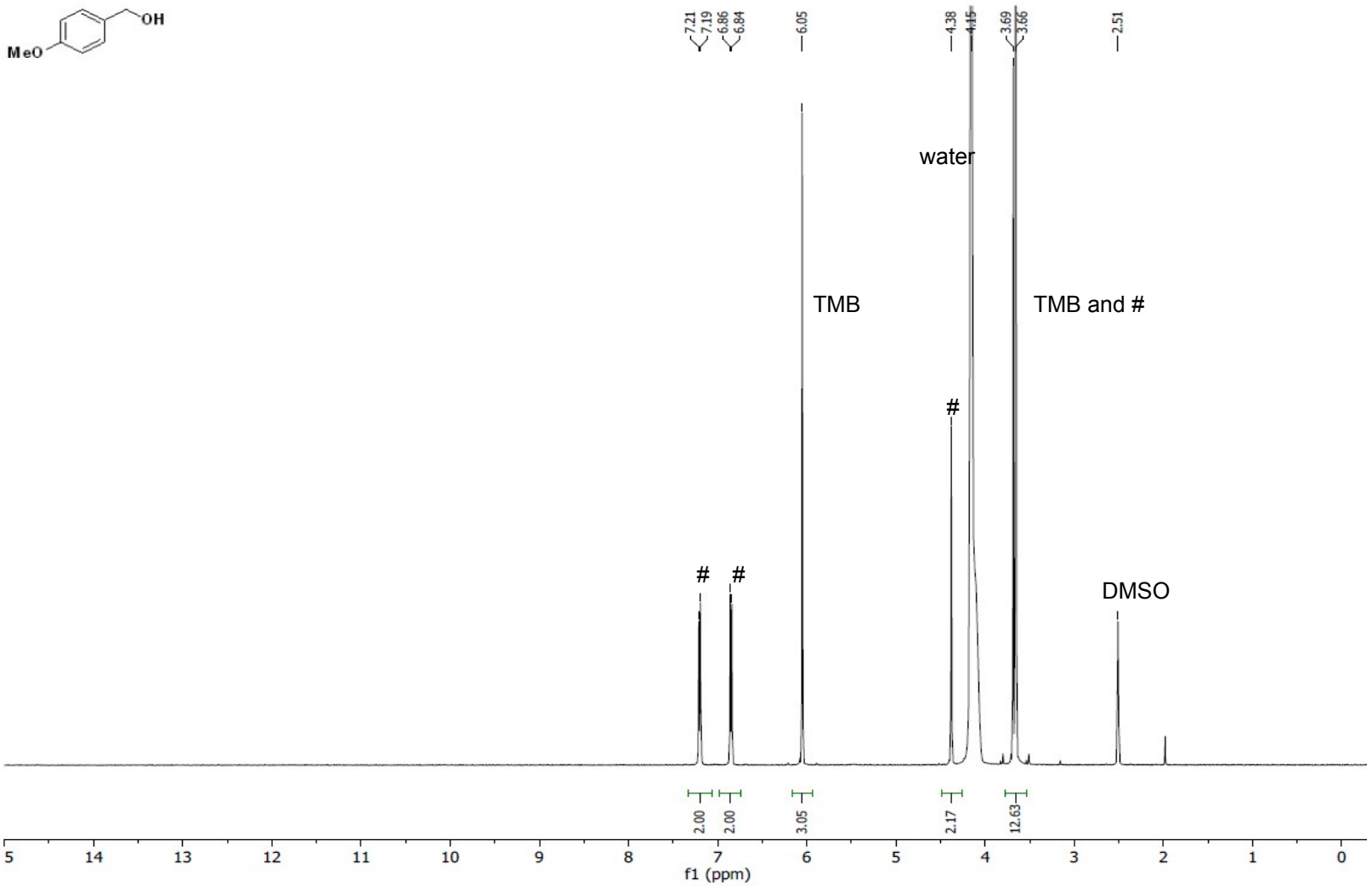


Figure S32 Control experiment for entry 4. # = SM.

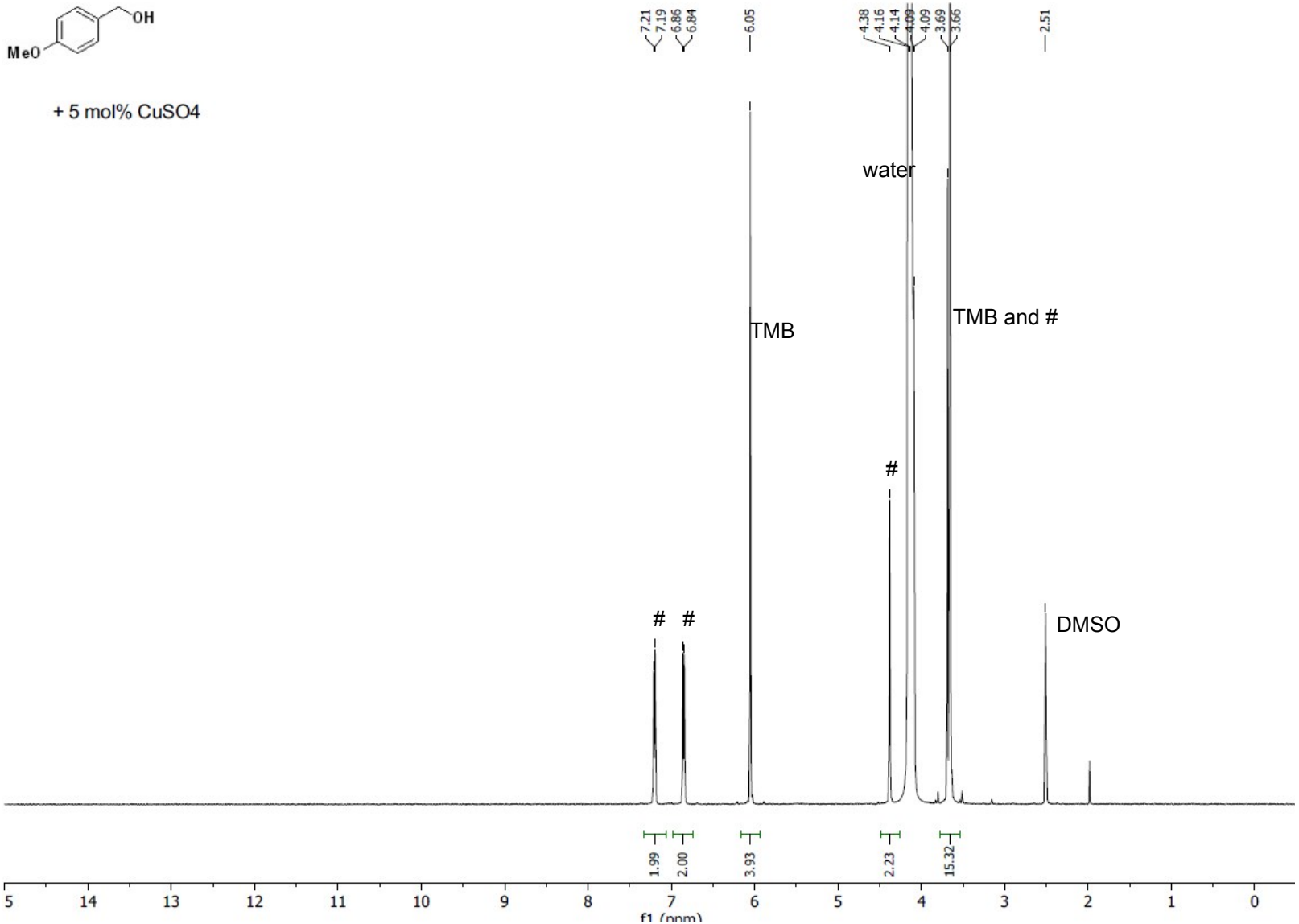


Figure S33 Control experiment for entry 4. # = SM.

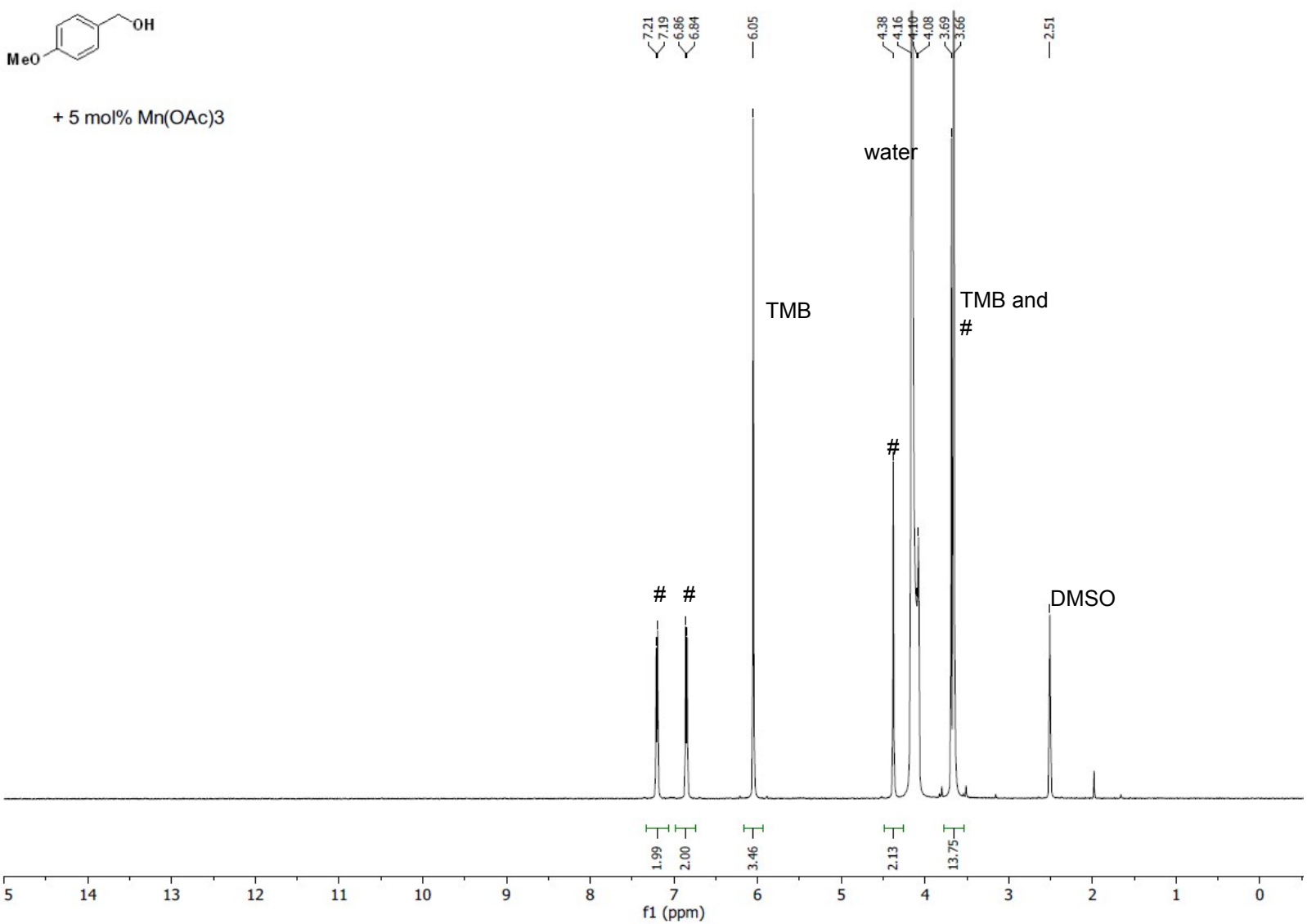


Figure S34 Control experiment for entry 4. # = SM.

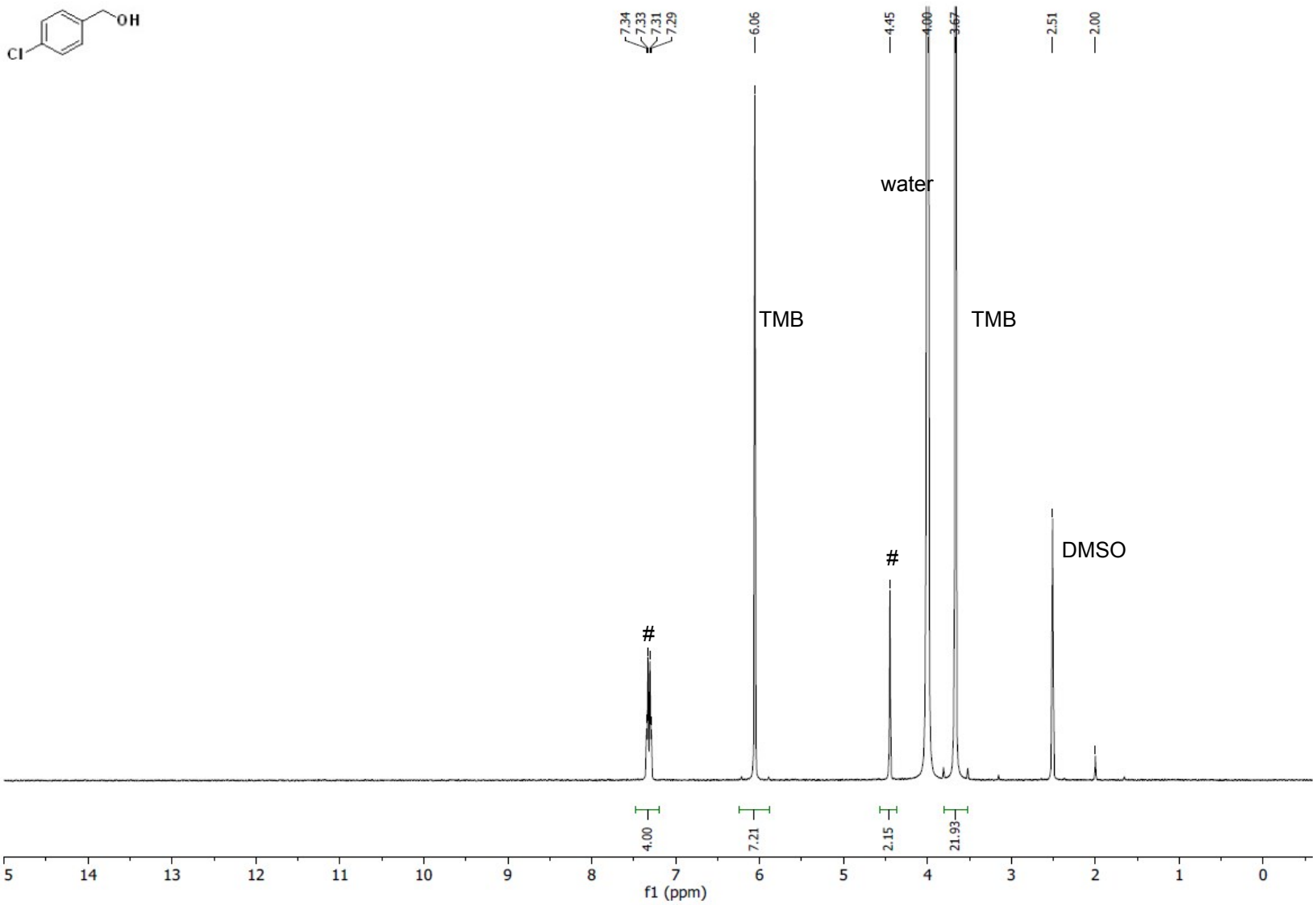


Figure S35 Control experiment for entry 5. # = SM.

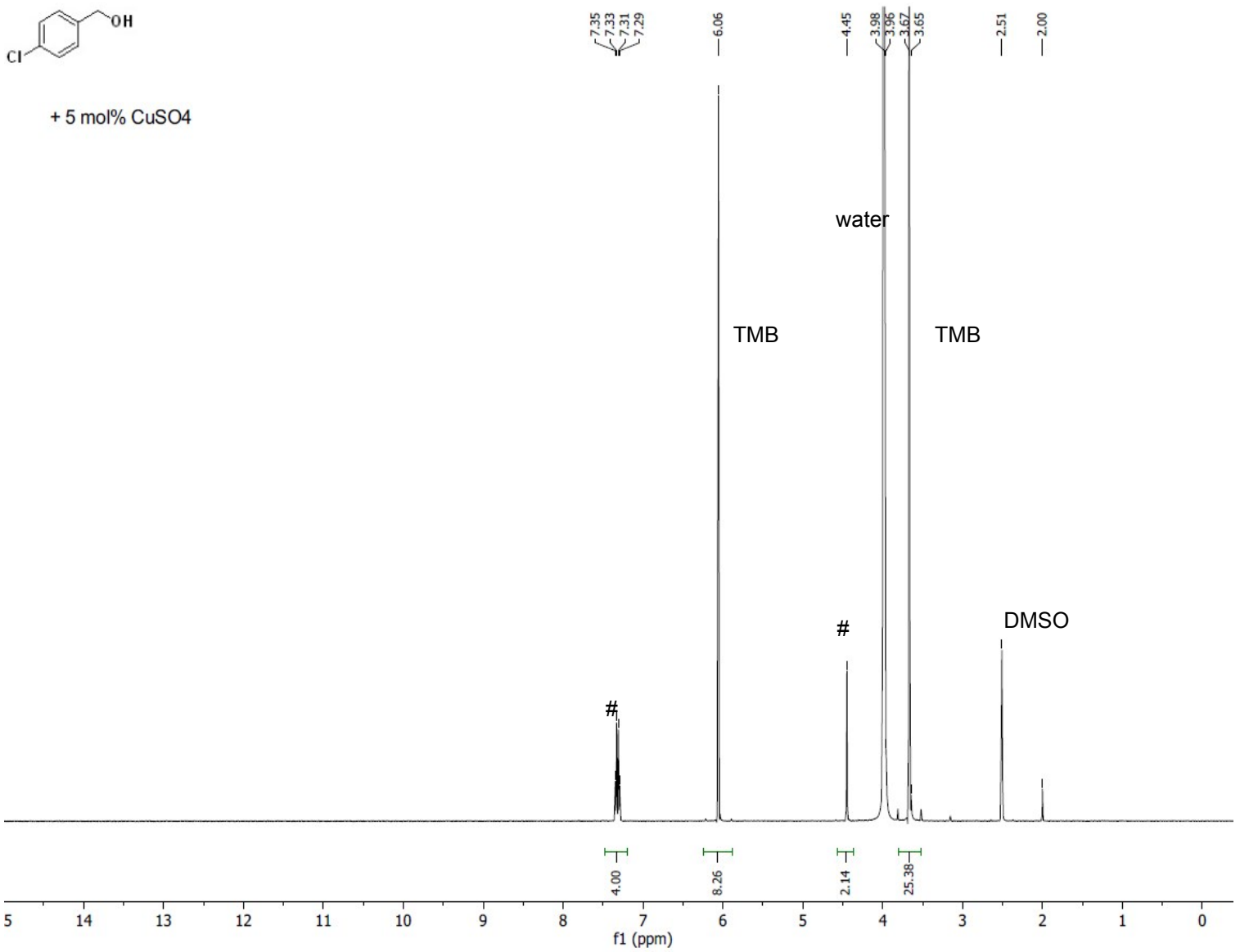


Figure S36 Control experiment for entry 5. # = SM.

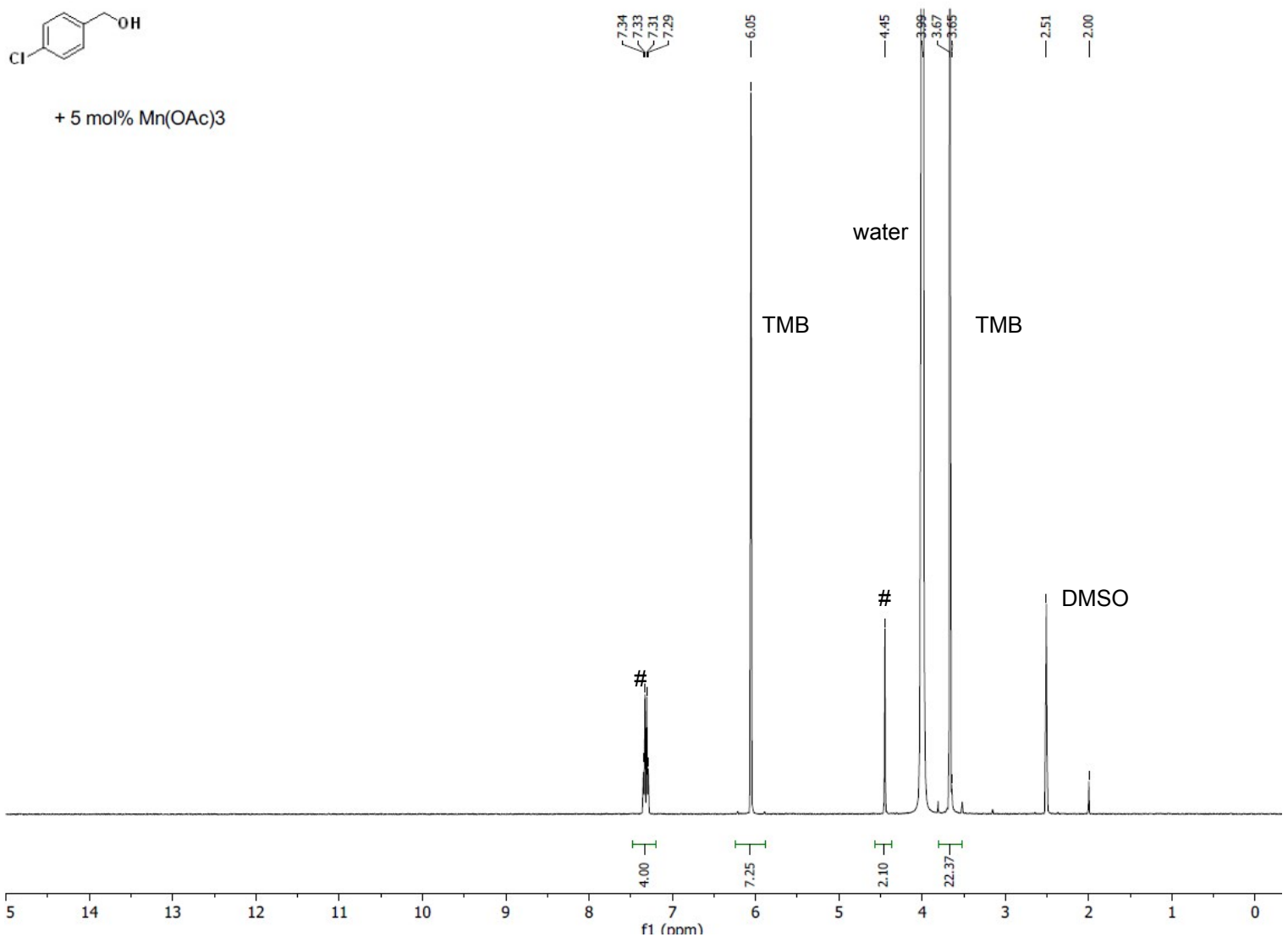


Figure S37 Control experiment for entry 5. # = SM.

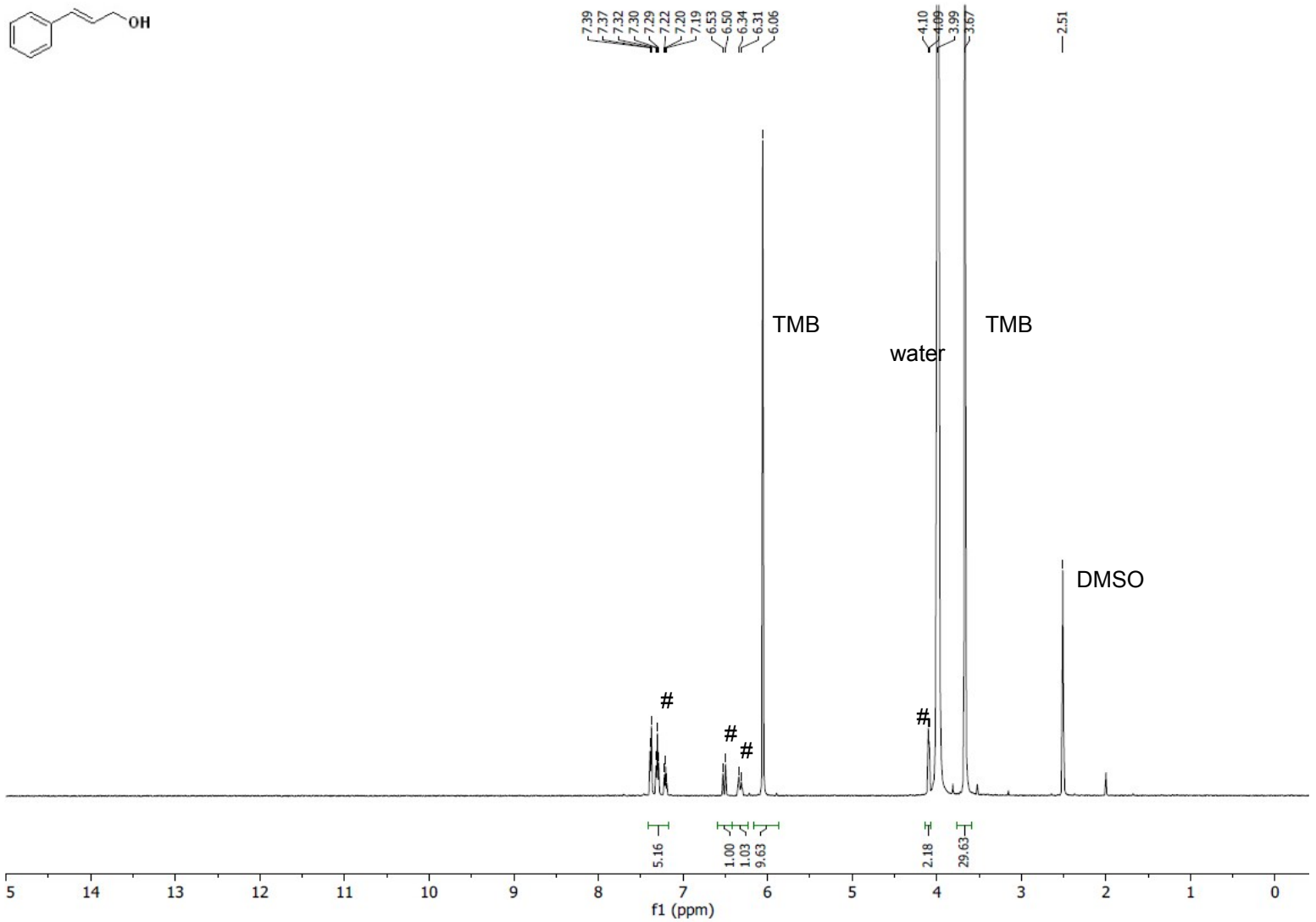


Figure S38 Control experiment for entry 6. # = SM.

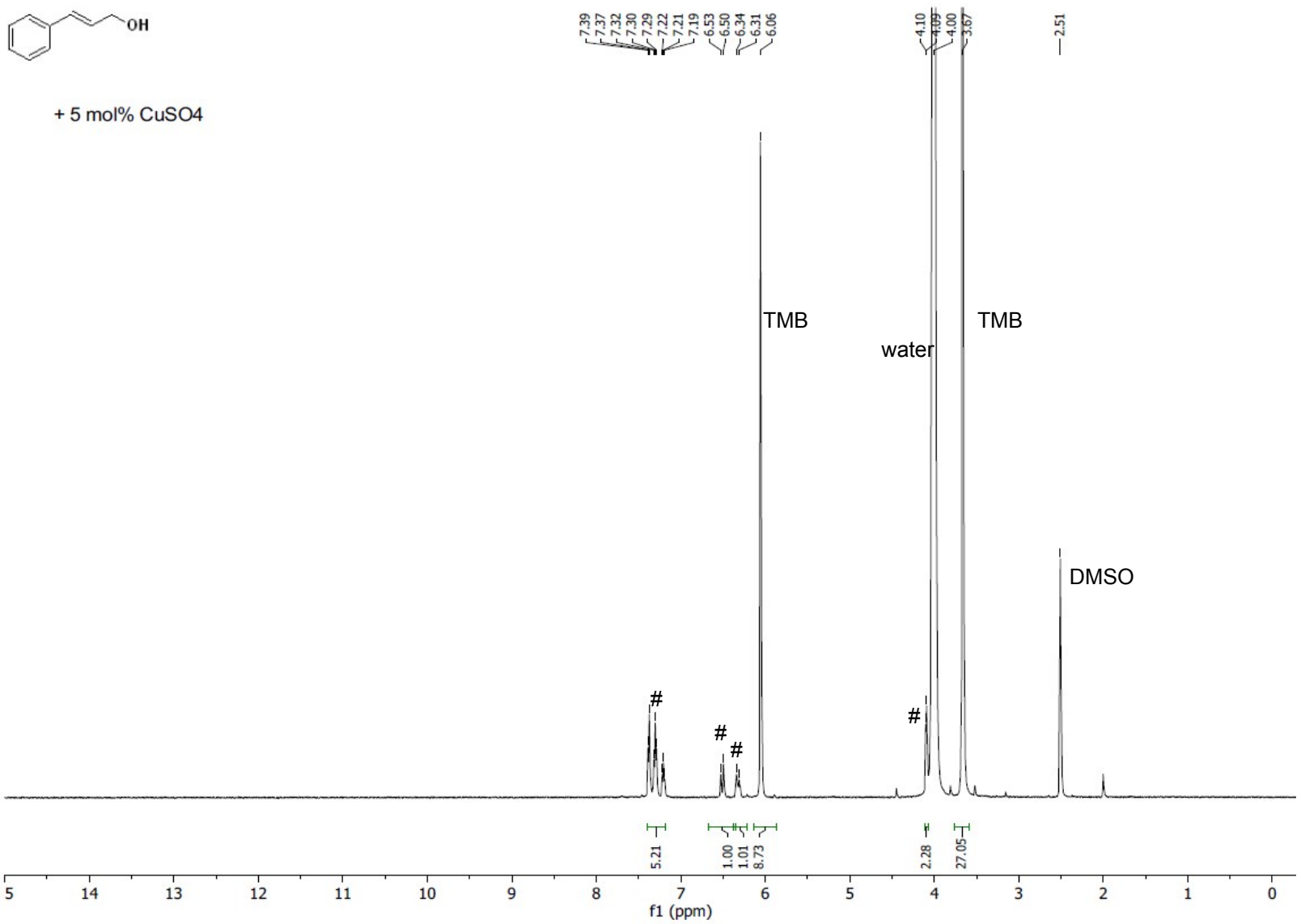


Figure S39 Control experiment for entry 6. # = SM.

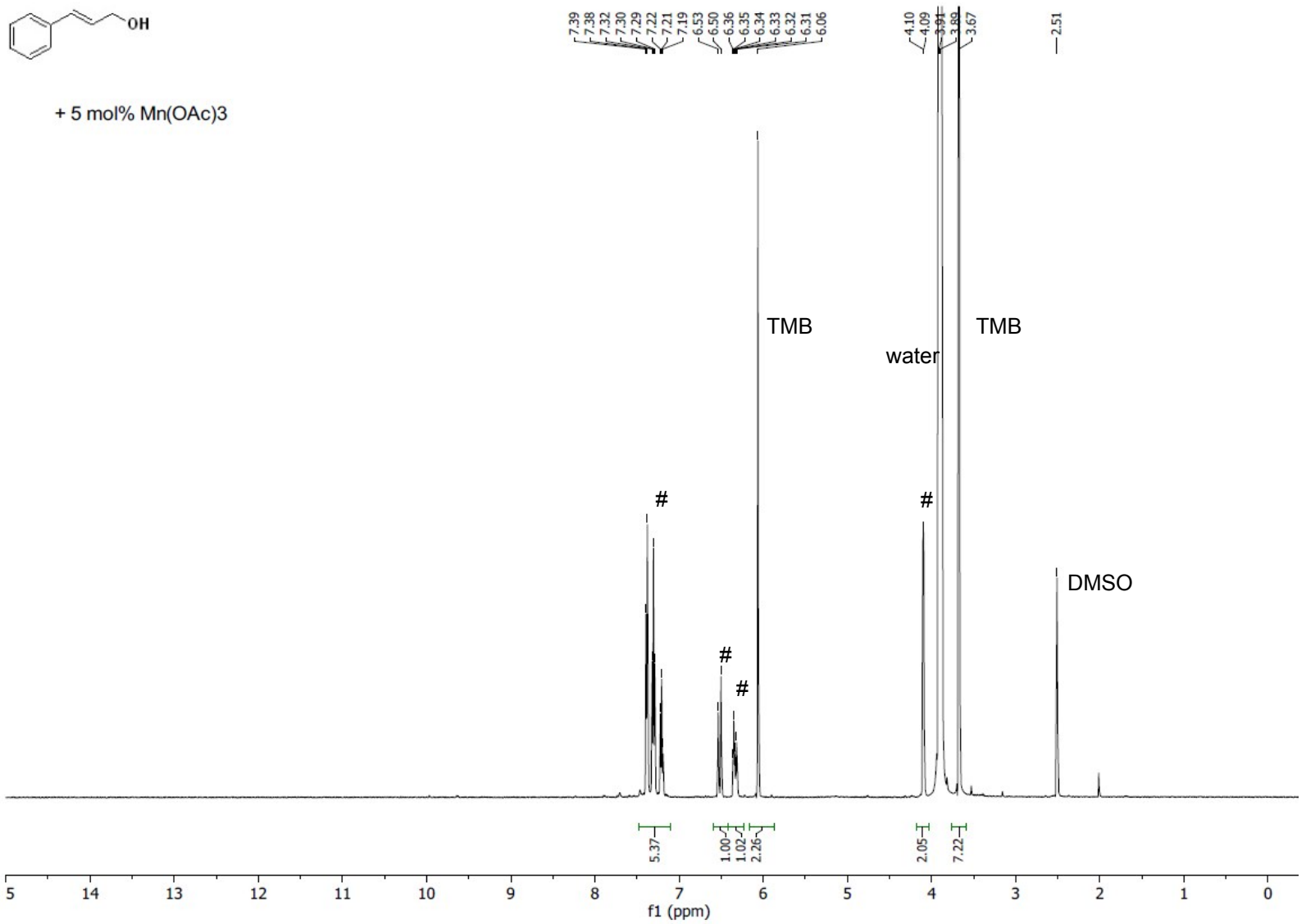


Figure S40 Control experiment for entry 6. # = SM.

S9 UV-Vis studies

The UV-Vis system used to record spectra consists in an OceanInsight DH-2000-BAL light source directly connected to an OceanInsight SQUARE ONE cuvette holder using threaded joints. The cuvette holder was directly connected to an OceanInsight FLAME-S-UV-VIS spectrometer. The spectra were recorded using OceanView 2. The exposure time was automatically adjusted to be under the saturation limit of the spectrometer. 10 scans were averaged to produce a spectrum. A background of the light source and a dark were taken before each experiment.

The buffer of the purified GOase-1 solution (section S10) was exchanged with 0.1M NaPi buffer, pH 7 using a 30 kDa molecular weight cut-off filter. The GOase concentration was checked using the absorption at 280 nm (ε_{280} = 126 488 $cm^{-1}.M^{-1}$ for $GOase_{Rd10BB}$) The GOase-1 solution was prepared by transferring 100 µL of a solution of GOAse buffer exchanged with NaPi, pH 7 (concentration: 404 mM) in a fused quartz Thorlabs sub-micro cuvette with a light path of 10 mm (Part #: CV10Q100S) containing 98 µL of NaPi buffer and the resulting solution was mixed using a pipette. 2 µL of 50 mM $CuSO_4$ stock was added and the final mixture was mixed by repeated pipetting. The final concentration of species in solution is 202 µM GOase, 0.5 mM Cu. The solution was aged for 1.5h after addition of $CuSO_4$.

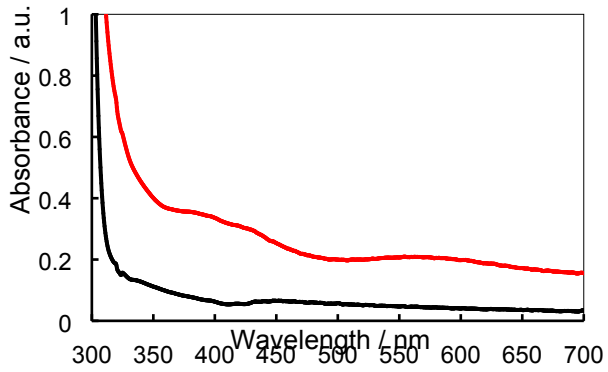


Figure S41 UV-Vis spectra of apo-GOase-1 (**---**), in-situ generated copper-bound $GOase^{semi}$ (**—**),

S10 GOase-1 purification by affinity chromatography

Purification of the proteins was performed by Evotec, USA. 4L *E. coli* culture expressing copper-free GOase-1 was lysed, clarified by resuspending into Buffer A. The suspension was clarified by centrifugation, loaded on the column and eluted with the buffer gradient, as described below.

Abbreviations

HEPES - (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

IMAC – immobilized metal-ion affinity chromatography

SEC – size-exclusion chromatography

Purification steps: IMAC/SEC

Mass: observed: 69,485 Da; calculated.: 69498 Da

Column: 25 mL His Trap FF

Buffer A: 50 mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole

Buffer B: 50 mM HEPES pH 7.5, 300mM NaCl, 500mM Imidazole

Gradient: 0% B over 15 CV, 0-100% B over 5 CV, 0% B for 10 CV

Pool: B10-F3; ,19.9mg/mL x 100 mL = 1395 mg

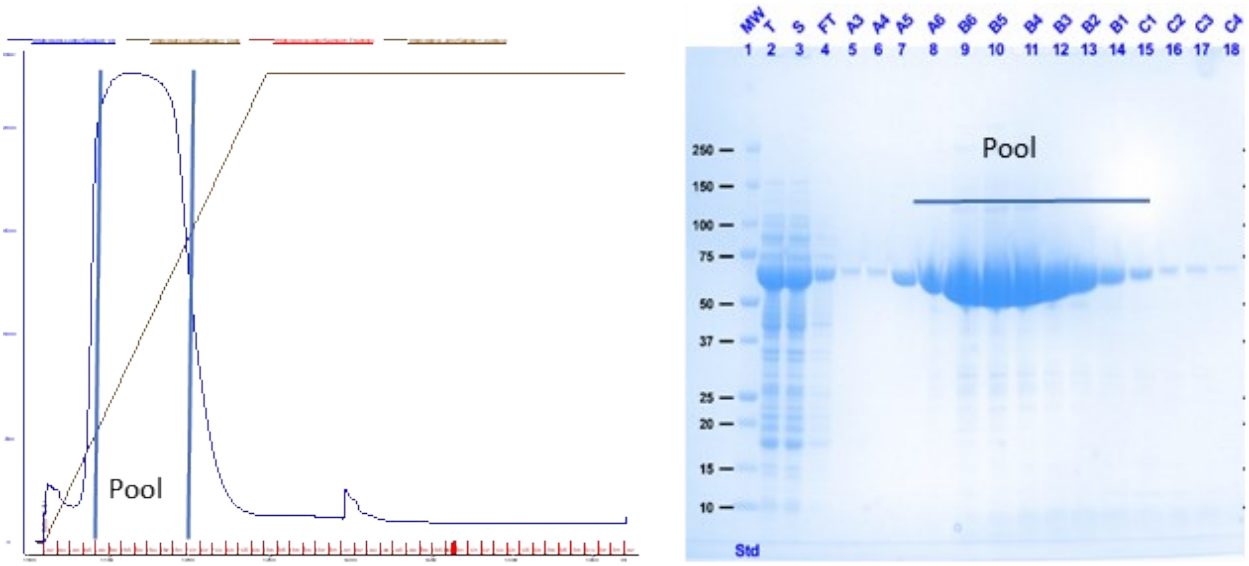


Figure S42 IMAC purification of GOase-1

SEC-purification

Column: 26/600 S200

SEC Buffer:50 mM HEPES pH 7.5

Sample:6 X 15 mL injection

Pool: 1,06 mg, aliquots of 25 mg/mL were stored at -80 ºC

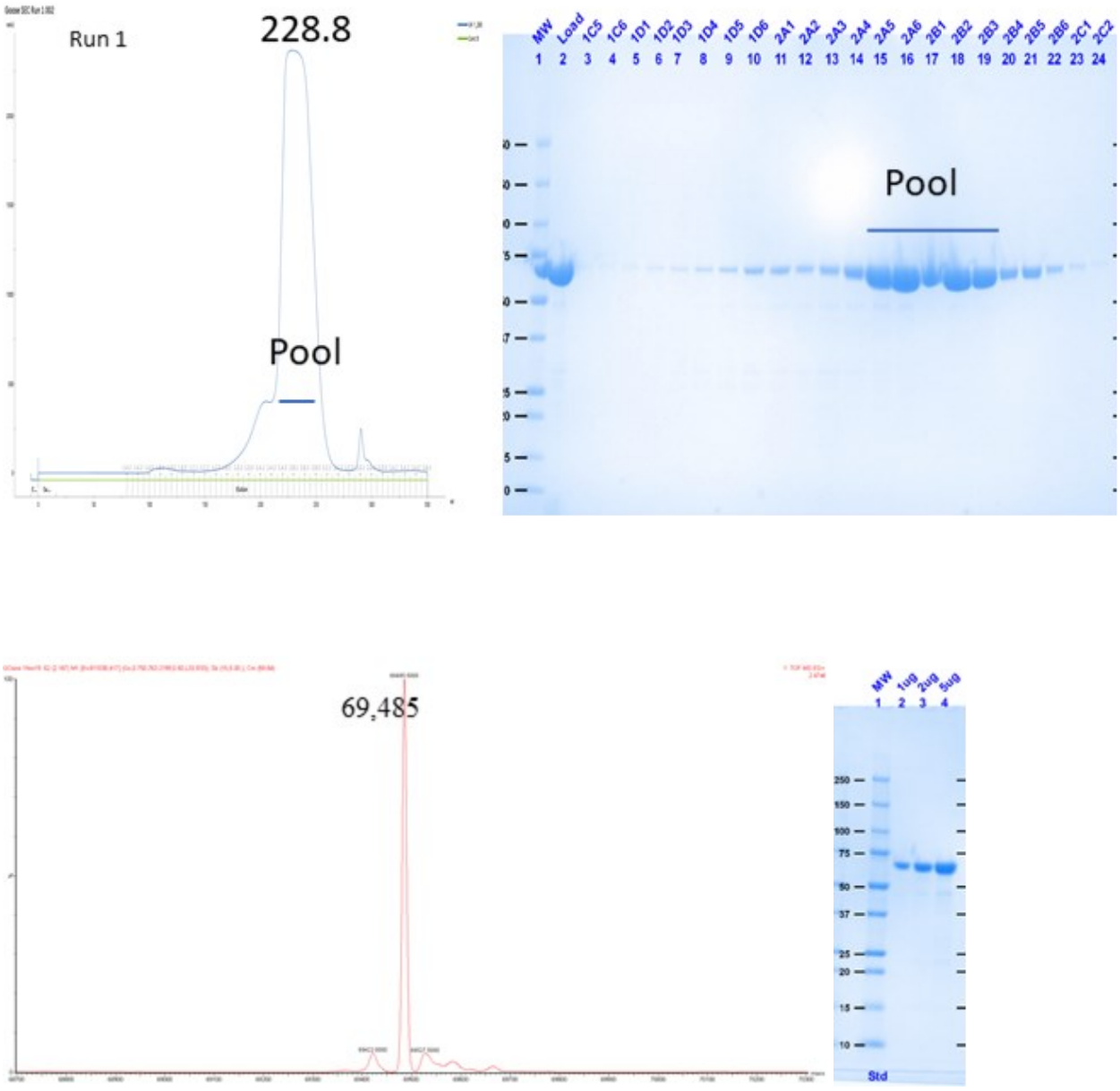


Figure S43 MS and SDS analysis of the purified GOase-1

S11 References

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