

Functional Characterization of TtnD and TtnF Unveiling New Insights into Tautomycetin Biosynthesis

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Supporting Information

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TABLE S1. Targeted gene inactivation by the REDIRECT technology in *S. griseochromogenes*

Gene	Primers ^a	Cosmid ^b	Strain
$\Delta ttnD$	<i>ttnDF1</i> : 5'-AATGAAGCGACTCAAGGATCTCCGCGAGTACCTGGCGGTGATTCCGGGGATCCGTCGACC-3' <i>ttnDR1</i> : 5'-TGTCAGGCAAGGAGCTCGATGACCCGCTGCCGCACCGGCTCTGTAGGCTGGAGCTGCTTC-3'	pBS13025	SB13013
$\Delta ttnF$	<i>ttnFF1</i> : 5'-GGTGACGAGCACACGAAGCGAGACGGATCTGACCGGCCGATTCCGGGGATCCGTCGACC-3' <i>ttnFR1</i> : 5'-CGTCAGTCGATCCACTCCGGGCGGCTCGAGCCCCGCGCACTGTAGGCTGGAGCTGCTTC-3'	pBS13026	SB13014

^aUnderlined letters represent the nucleotide homologous to the DNA regions internal to targeted genes.

^bpBS13025 and pBS13026 are based on cosmid pBS13009 that carries a part of the *ttn* cluster.¹

TABLE S2. Southern analysis confirming the genotypes of mutant Strains^a

Strains	Gene targeted	Probe	Fragment replaced (bp)	Restriction Site used	Signal Size (kbp)	
					WT	mutant
SB13013	$\Delta ttnD$	2200-bp <i>Bam</i> HI fragment from pBS13027 ^b	1380	<i>Xho</i> I	3.63	2.29
SB13014	$\Delta ttnF$	1370-bp <i>Bam</i> HI fragment from pBS13028 ^b	1440	<i>Xho</i> I	3.06	3.53

^aSee Figures S1 and S2 for details.

^bpBS13027 and pBS13028 were constructed by subcloning *Bam*HI fragments containing *ttnD* and *ttnF*, respectively, from cosmid pBS13012 that carries a part of the *ttn* cluster¹ into the same site of pUC18.²

TABLE S3. Expression constructs for complementation to the $\Delta ttnD$ and $\Delta ttnF$ mutants

Mutant strain	Gene mutated	Primers used to make the expression constructs ^a	Construct ^b	Complemented strain
SB13013	$\Delta ttnD$	<i>ttnDFP3</i> : 5'-CCAATGCATGAAGCGACTCAAGGAT-3' <i>ttnDP3</i> : 5'-GCTCTAGATCAGGCAAGGAGCTCGAT-3'	pBS13029	SB13015
SB13014	$\Delta ttnF$	<i>ttnFFP3</i> : 5'-GCAATGCATATGACGAGCACACGAAGCG-3' <i>ttnFRP3</i> : 5'-GCTCTAGATCAGTCGATCCACTCCGG-3'	pBS13030	SB13016

^aThe *ttnD* and *ttnF* genes were amplified with the primers listed (*Nsi*I and *Xba*I restriction sites are underlined), respectively, from cosmid pBS13012.¹

^bThe PCR-amplified *ttnD* and *ttnF* genes from pBS13012 were digested with *Nsi*I and *Xba*I and cloned into the same sites of pBS6027³ to afford pBS13029 and pBS13030, respectively, in which the expression of *ttnD* and *ttnF* is under the control of the *Erme** promoter.

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- Sambrook, J. E.; Fritsch, E. F.; Maniatis, T. *Molecular cloning: a Laboratory Manual*, 3rd Ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2000.
- Li, W.; Ju, J.; Rajski, S. R.; Osada, H.; Shen, B. *J. Biol. Chem.* **2008**, 283, 28607-28617.

FIGURE S1. Inactivation of *ttnD* in *S. griseochromogenes* by gene replacement using the REDIRECT Technology. (A) Construction of the $\Delta ttnD$ gene replacement mutant and restriction maps of *S. griseochromogenes* wild-type and SB13013 mutant strains showing the predicted fragment sizes upon *Xho*I digestion. (B) Southern analysis of the wild-type (lane 5) and SB13013 (lanes 2, 3 and 4 are three individual isolates) digested genomic DNAs and using the 2198-bp *Bam*HI fragment as a probe. Lane 1, molecular weight standard.

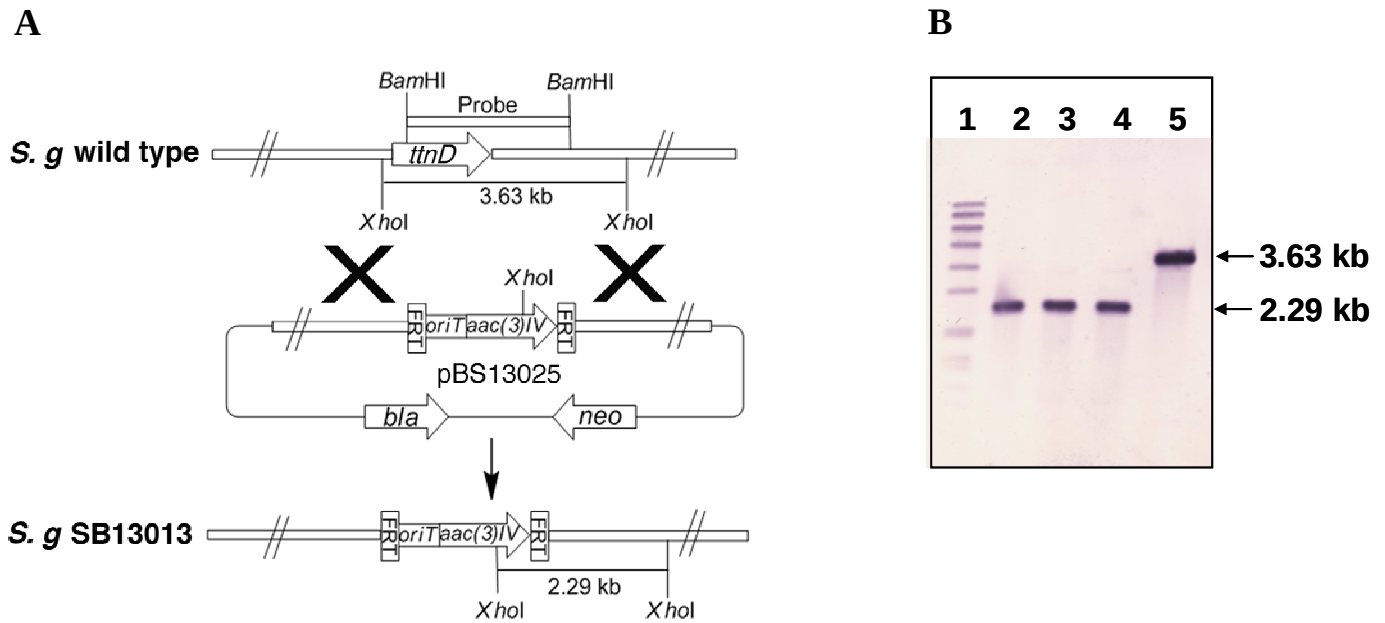
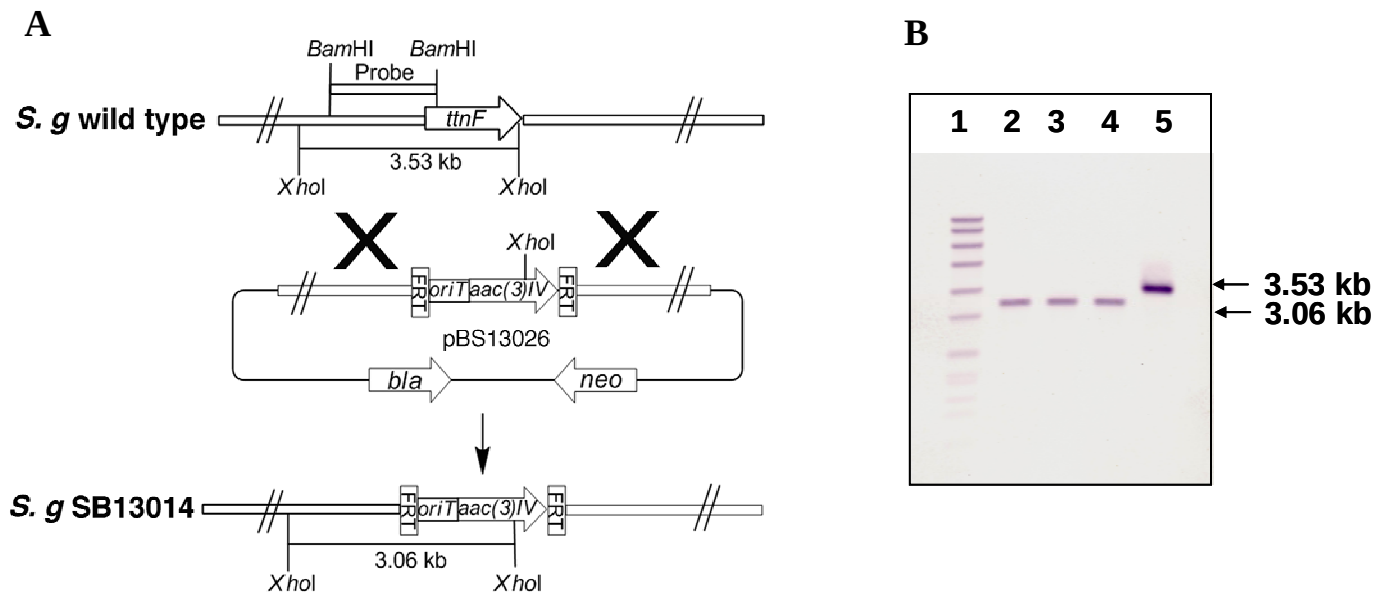


FIGURE S2. Inactivation of *ttnF* in *S. griseochromogenes* by gene replacement using the REDIRECT Technology. (A) Construction of the $\Delta ttnF$ gene replacement mutant and restriction maps of *S. griseochromogenes* wild-type and SB13014 mutant strains showing predicted fragment sizes upon *Xho*I digestion. (B) Southern analysis of the wild-type (lane 5) and SB13014 (lanes 2, 3 and 4 are three individual isolates) digested genomic DNAs and using the 1370-bp *Bam*HI fragment as a probe. Lane 1, molecular weight standard.



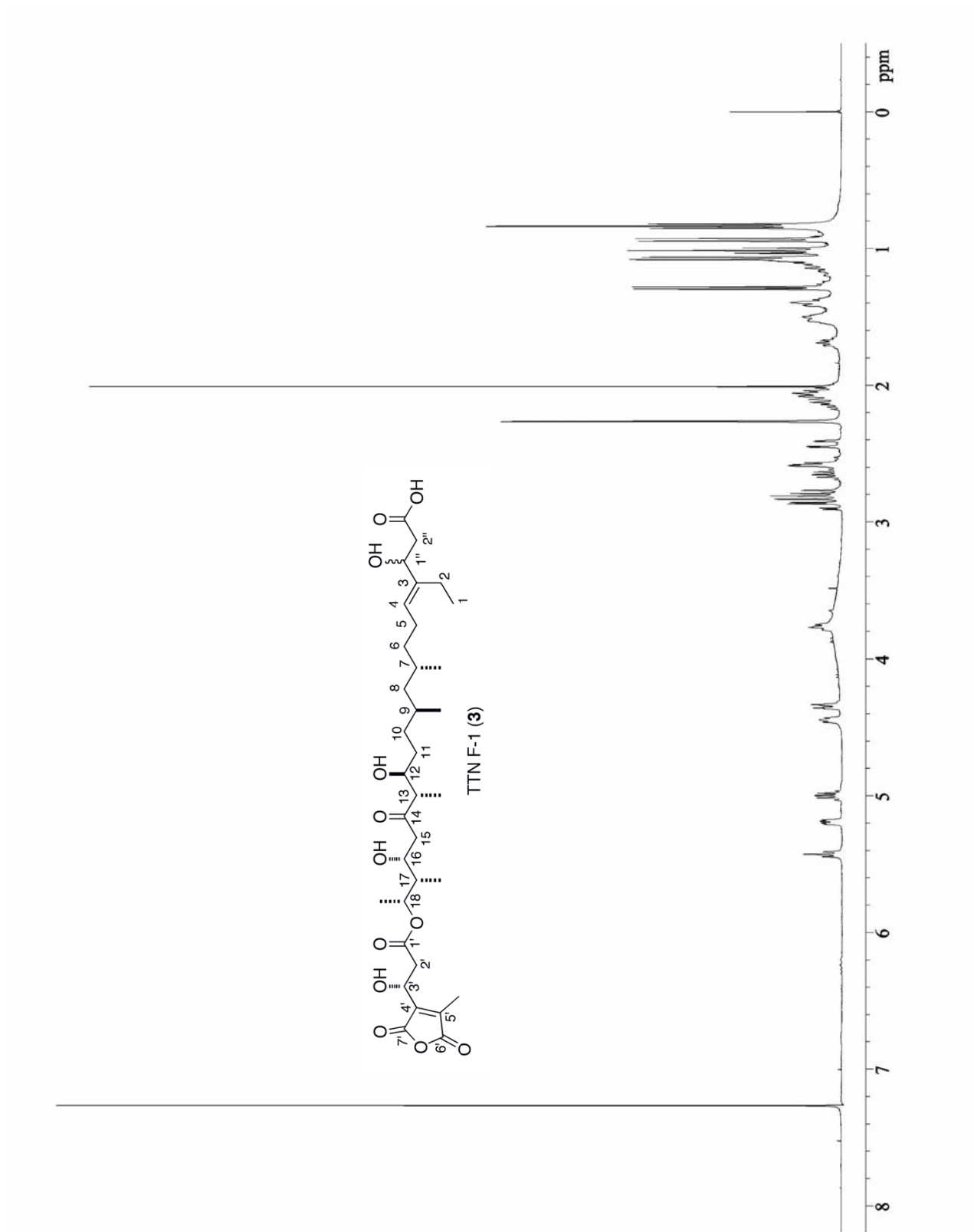


Figure S3. ^1H -NMR of TTN F-1 (3) in CDCl_3 .

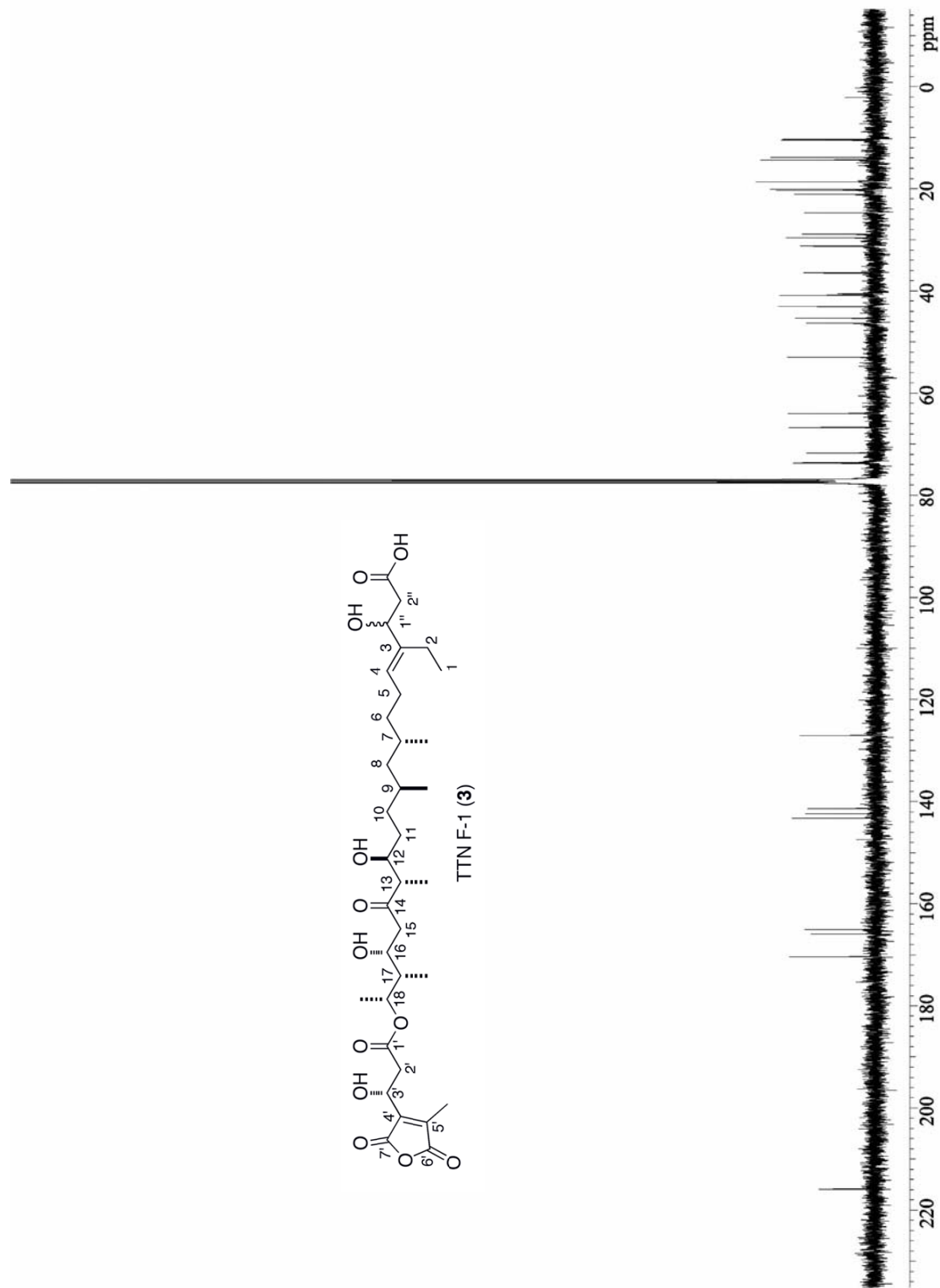


Figure S4. ^{13}C -NMR of TTN F-1 (3) in CDCl_3 .

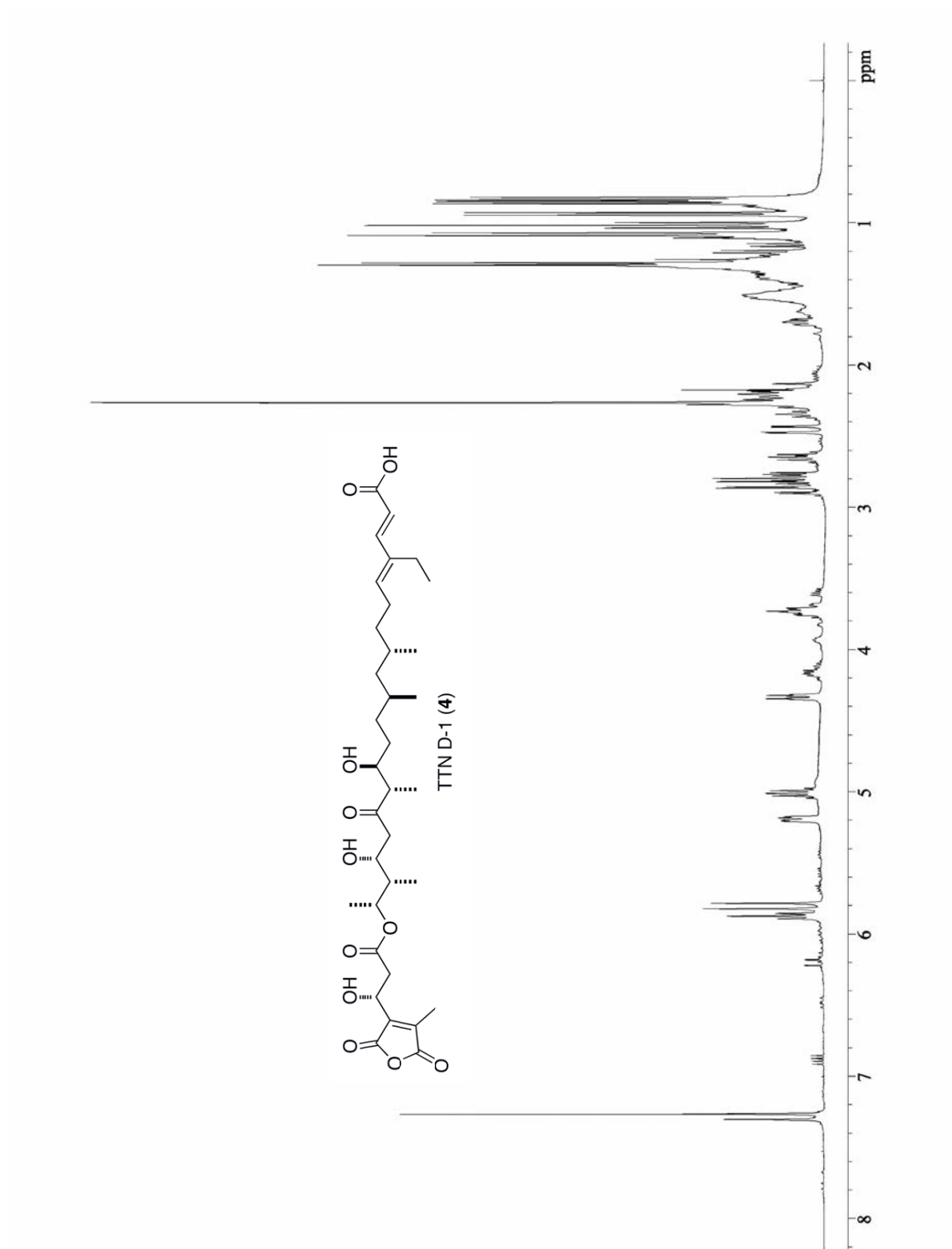


Figure S5. ^1H -NMR of TTN D-1 (4) in CDCl_3 .

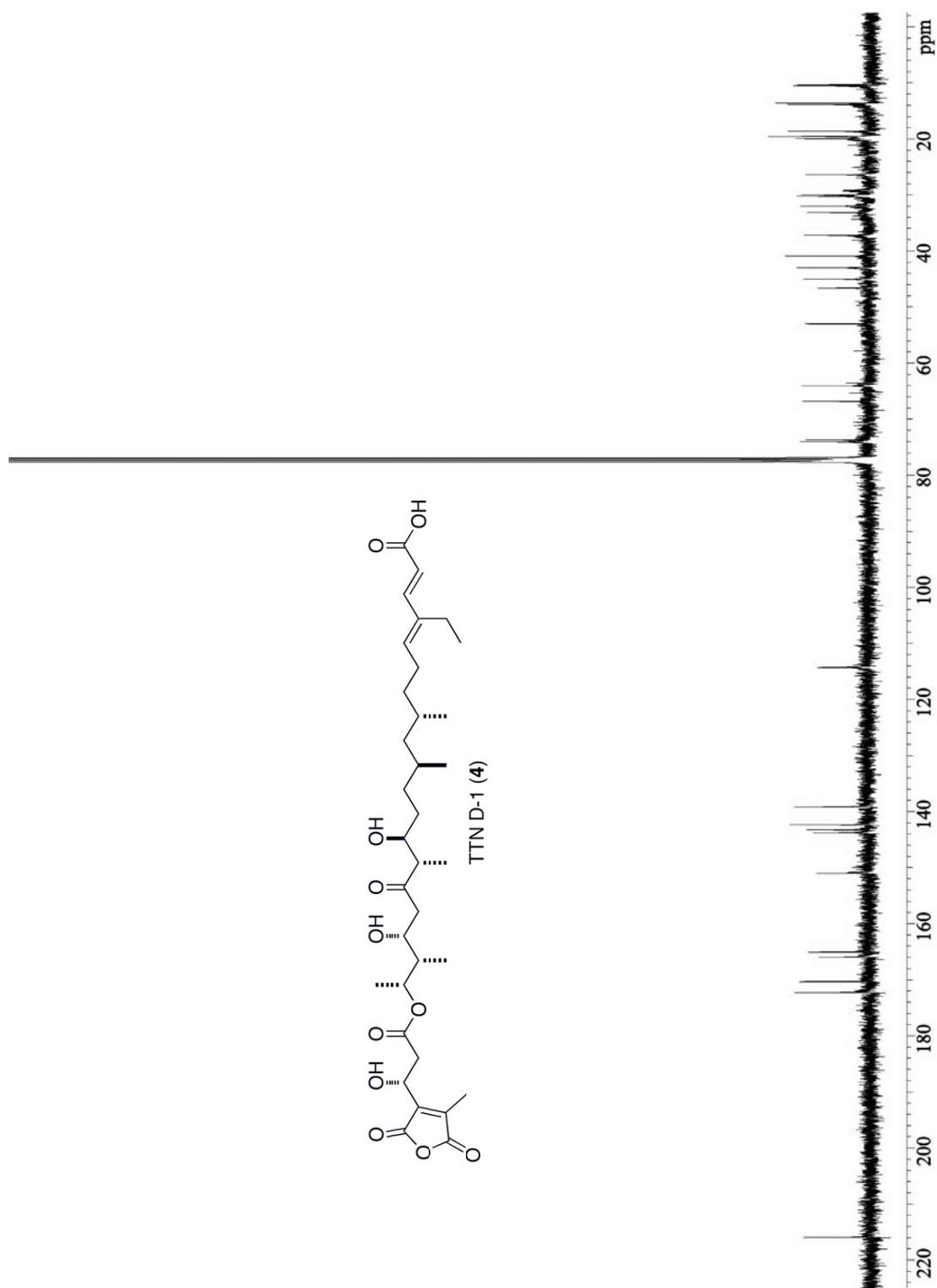


Figure S6. ^{13}C -NMR of TTN D-1 (4) in CDCl_3 .

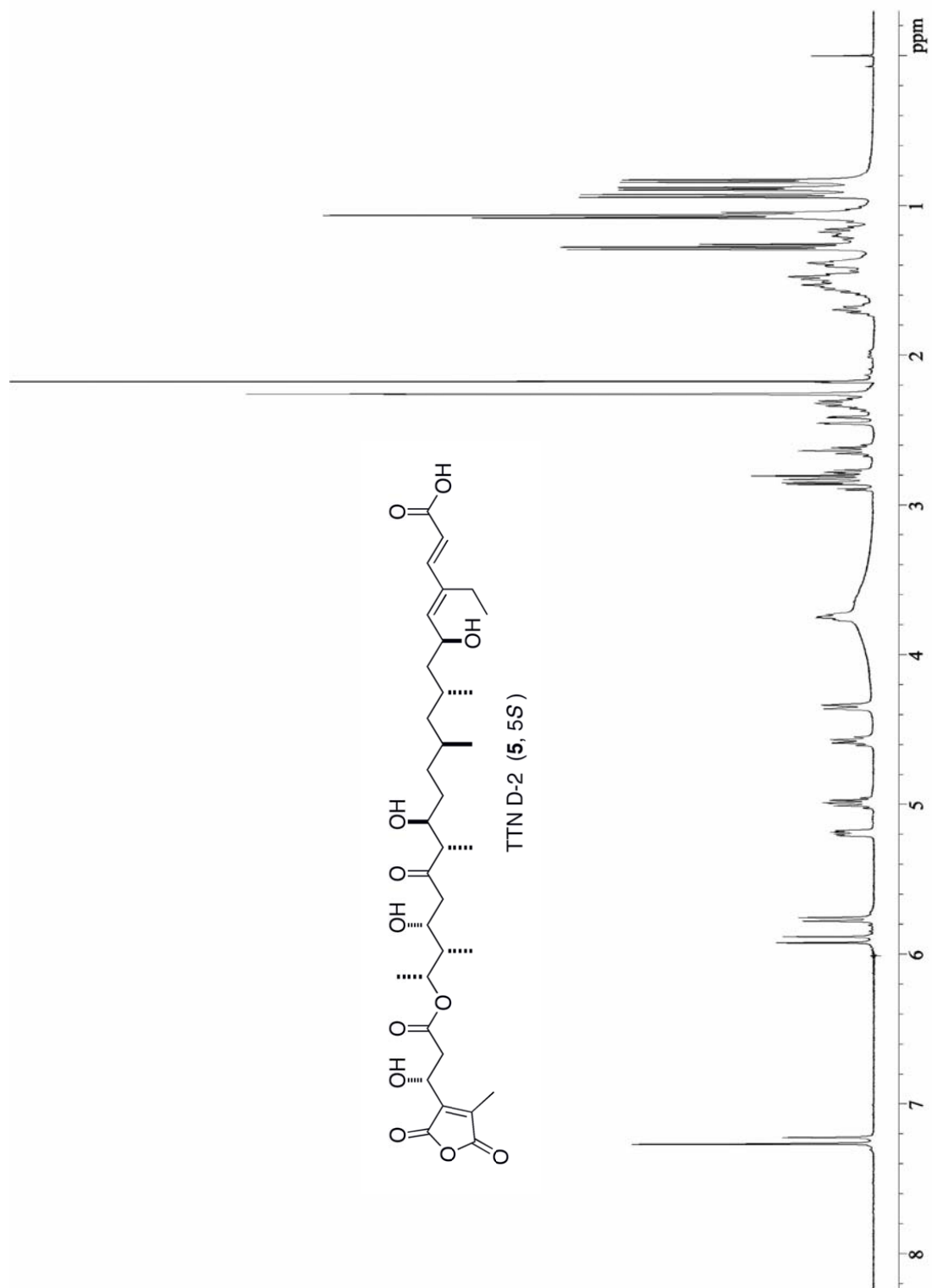
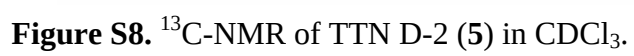


Figure S7. ^1H -NMR of TTN D-2 (5) in CDCl_3 .



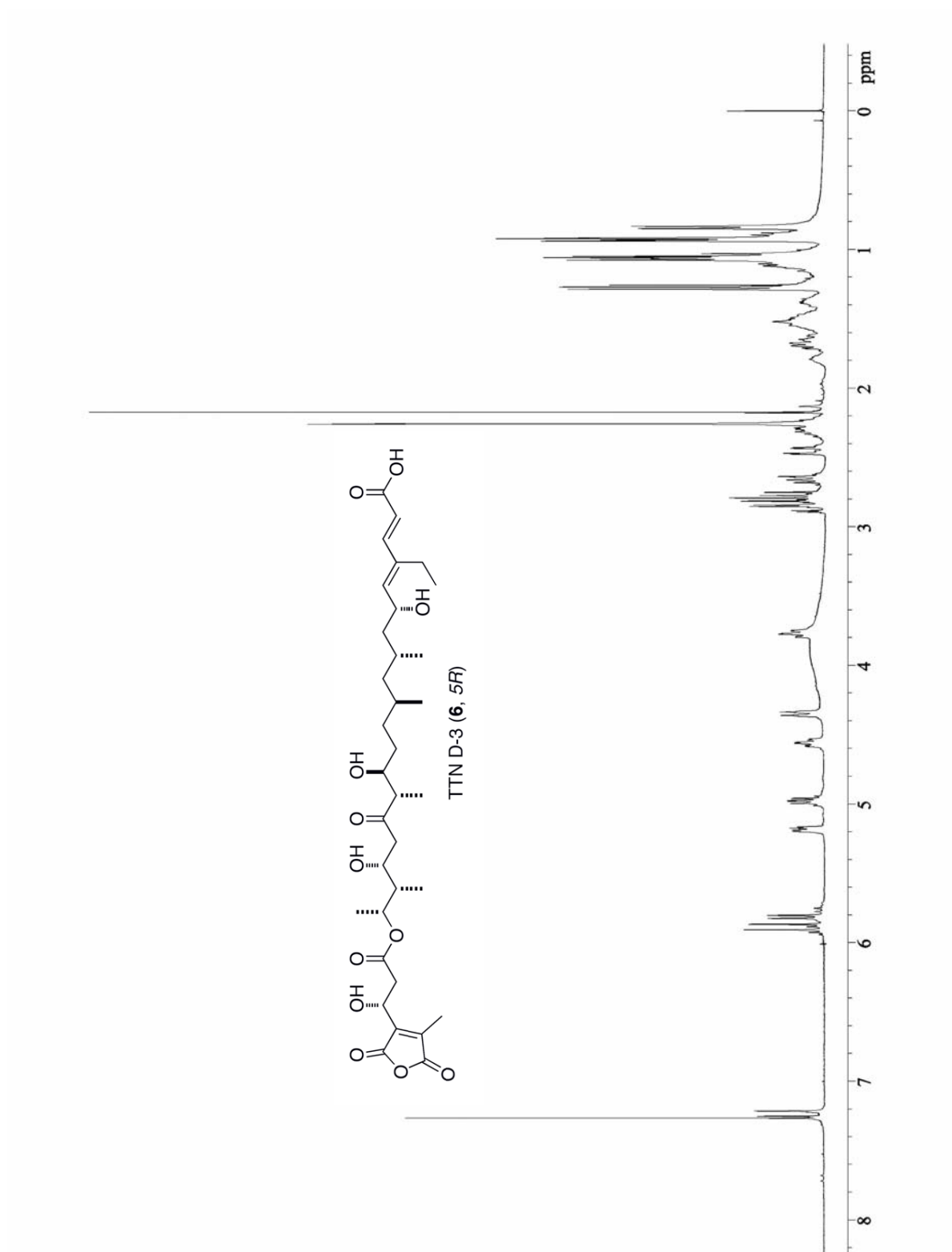


Figure S9. ^1H -NMR of TTN D-3 (**6**) in CDCl_3 .

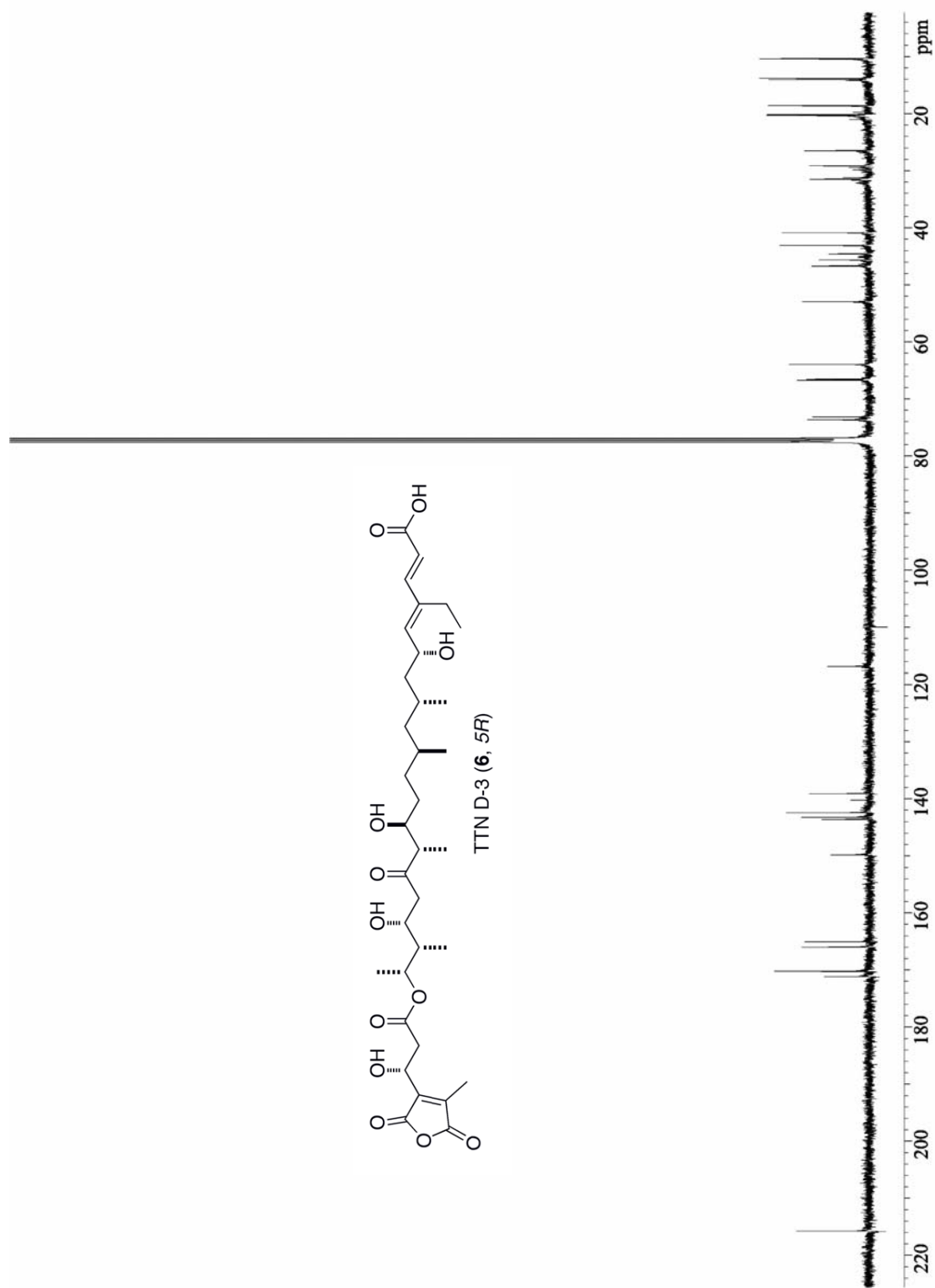


Figure S10. ¹³C-NMR of TTN D-3 (**6**) in CDCl₃.

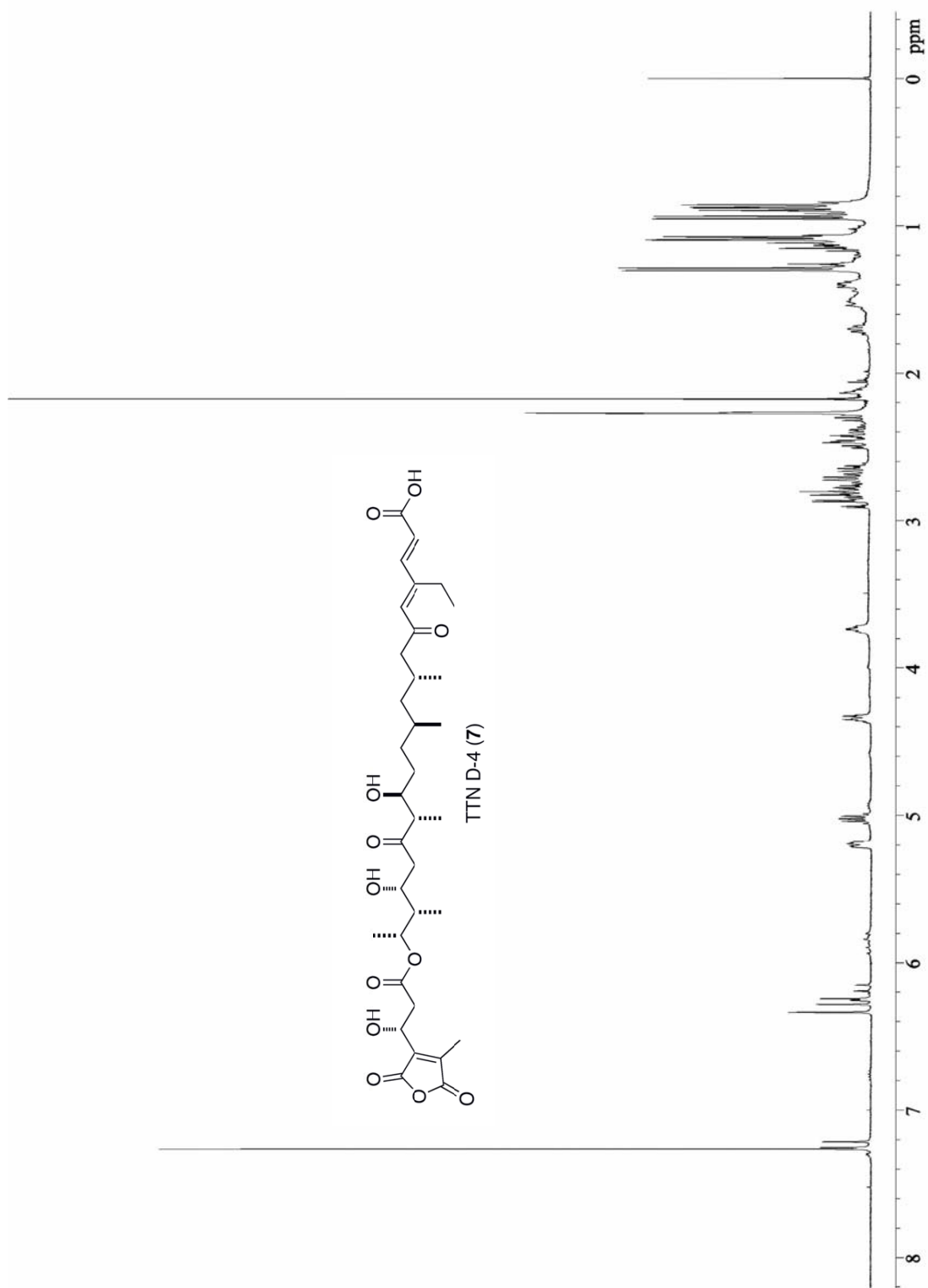


Figure S11. ^1H -NMR of TTN D-4 (7) in CDCl_3 .

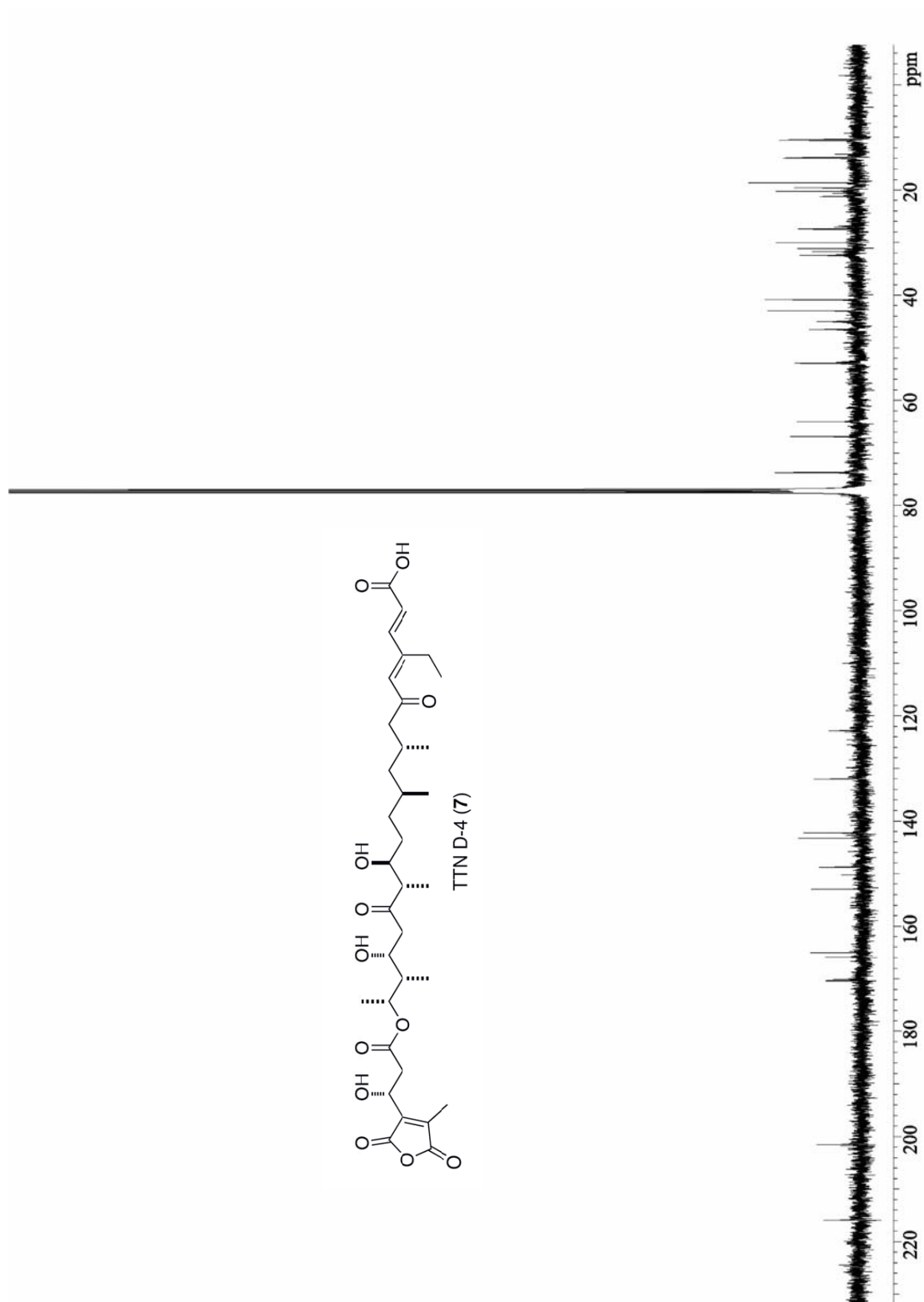


Figure S12. ^{13}C -NMR of TTN D-4 (7) in CDCl_3 .