Supporting Information

Antiangiogenic Activity and Chemical Derivatization of the Neurotoxic Acetogenin, Annonacin, Isolated from *Asimina triloba*

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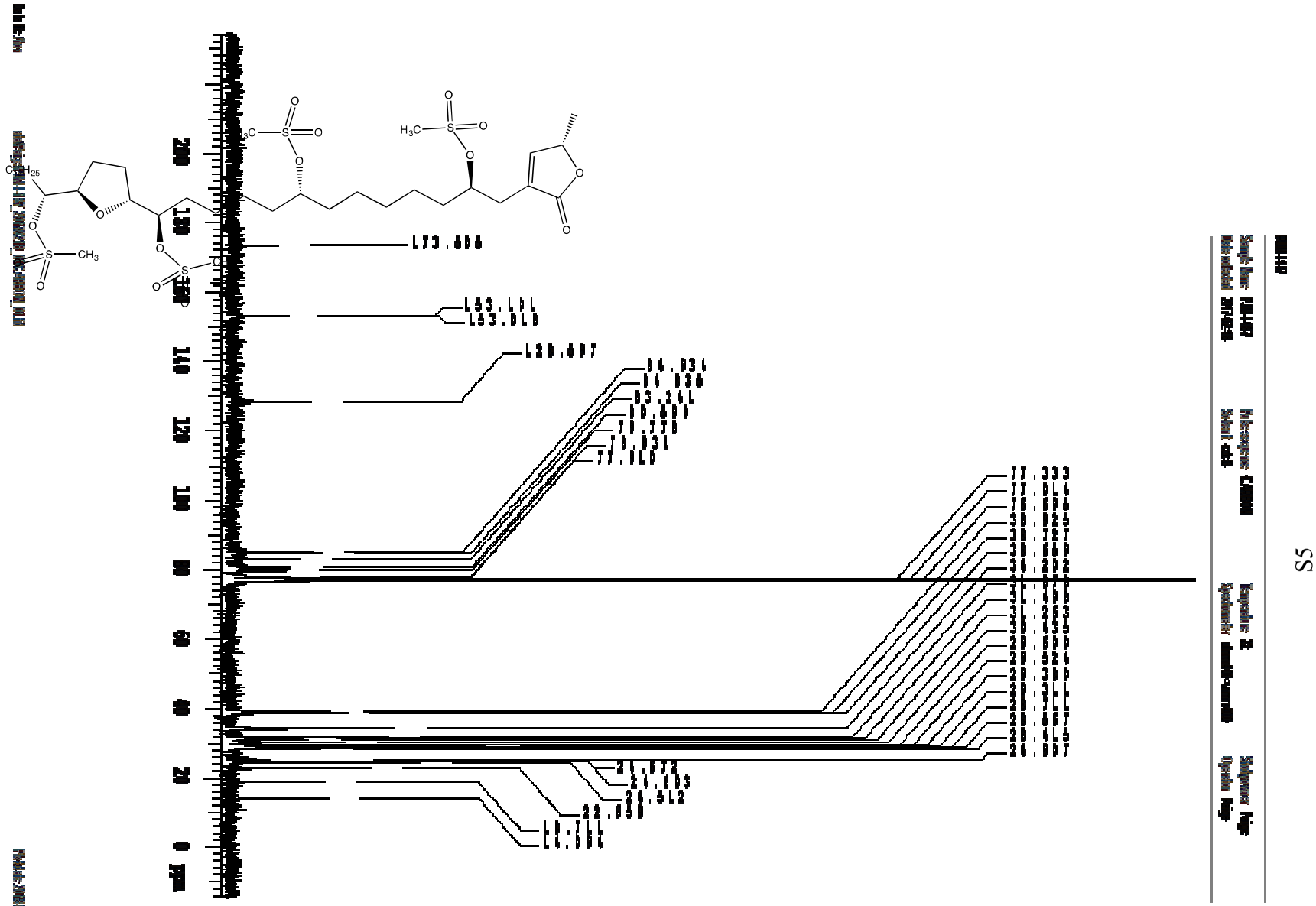
Rat Aortic Ring (RAR) Bioassays of 1, 3, and 5. RAR assays were conducted by the NCI Clinical Pharmacology group in Bethesda, MD, USA.[1] Animal care and use within the NIH/NCI Intramural Research Program was approved by the Animal Care and Use Committee (ACUC) and was conducted according to the NIH Policy and Procedure 3040-2. Female 6-8 week old Sprague-Dawley rats were euthanized by asphyxiation with CO_2 followed by removal of the thoracic aorta. With a dissecting microscope, 1 mm thick rings were then cut. Next, 250 μL of Matrigel Growth Factor Reduced Basement Membrane Matrix (Corning, Corning, NY, USA) was plated in a 24-well plate (Corning, Corning, NY, USA) and left to set for 1 h. Rings were plated in the wells with an additional 250 μL of Matrigel and incubated overnight in endothelial cell basal medium (EBM-II) containing VEGF (Lonza, Walkersville, MD, USA). Medium was replaced the following day with EBM-II without VEGF and containing compounds **1**, **3**, or **5**. Rings were incubated for 5 days and imaged. The pixel density of each image was quantified by employing a gray-scale analysis of background versus light pixels (outgrowths) as determined by the NIH ImageJ program and PhotoShop. The bar graphs show percent microvessel growth relative to growth of the untreated rings, and are derived from the relative pixel density of the images. The bar graphs were generated using Prism 7 software. Comparisons were made using Student's *t*-test with $P < 0.05$ as the criterion for statistical significance. The data were represented as the means ± SEM.

PAMPA Assay of 1. PAMPA assay was conducted by Creative Bioarray, Shirley, NY, USA. Stock solutions (10 mM) of the positive controls, testosterone and methotrexate used in the assay, were prepared in DMSO or acetonitrile. A stock solution of **1** was prepared in DMSO (10 mM) and further diluted with PBS (pH 7.4) whereby the final concentration of the test compound was 10 μM. A solution (1.8% w/v) of lecithin in *n*-dodecane was then prepared and the mixture

was sonicated to ensure complete dissolution. The lecithin/dodecane mixture (5 mL) was pipetted into each acceptor plate well (top compartment) of a Multiscreen-IP Filter Plate (Merk-Millipore MAIPN4550, 0.45 μm) and contact between the pipette tip and membrane was avoided. Within 10 min after application of the artificial membrane, PBS solution (300 μL, pH 7.4) was added to each well of the acceptor plate. Then, 300 μL of each of the test compound-containing solutions was added to each well of the donor plate (bottom compartment) in triplicate. The acceptor plate was then placed, slowly and carefully, onto the donor plate whereby the underside of the membrane was in contact with the test compound-containing solutions in all wells. The plate lid was replaced followed by incubation at 25 ˚C (16 h) at 60 rpm. After incubation, aliquots of each well of the acceptor and donor plates were transferred into a 96-well plate. Into each well was added a methanol solution (200 μL) of alprazolam (100 nM), labetalol (200 nM) and ketoprofen (2 μM), as internal controls. The plate was then covered and vortexed at 750 rpm (100 sec) followed by centrifugation at 3,220 g (20 min). The compound concentrations were determined using a Shimadzu LC system and an API 4000 MS instrument with an ESI interface and Gemini 3 μM NX-C^{18} 1 10 Å (50 x 2 mm) column (25 ˚C). The chromatographic conditions consisted of : mobile phase (phase A), water (0.1% ammonium hydroxide solution); (phase B) methanol (0.1% ammonium hydroxide solution, Gradient: 0-0.3 min, 5% B; 0.3-1.2 min, 5-100% B 1.2-1.5 min, 100% B; 1.5-1.6 min, 100-5% B; 1.6-2.0 min, 5% B; injection volume, 5 μL; elution rate, 0.6 mL/min; MS ion source, turbospray; ionization model, ESI; scan type, MRM; collision gas, 10 L/min; curtain gas, 30 L/min; nebulization gas, 50 L/min; auxiliary gas, 50 L/min; temperature, 500 ˚C; ion spray voltage, +5500 V (positive MRM)/ -4500 V (negative MRM).

[1]Luzzio, F. A.; Mayorov, A. V.; Ng, S. S. W.; Kruger, E. A. Figg, W. D. *J. Med. Chem.* 2003, *46*,3793-3799.

^{1}H NMR spectra of (1*R*,6*R*,12*R*)-13-((*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)-1-((2*R*,5*R*)-5-((*R*)-1 ((methylsulfonyl)oxy) tridecyl)tetrahydrofuran-2-yl)tridecane-1,6,12-triyl trimethanesulfonate (2)



^{13}C NMR spectra of (1*R*,6*R*,12*R*)-13-((*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)-1-((2*R*,5*R*)-5-((*R*)-1 ((methylsulfonyl)oxy) tridecyl)tetrahydrofuran-2-yl)tridecane-1,6,12-triyl trimethanesulfonate (2)

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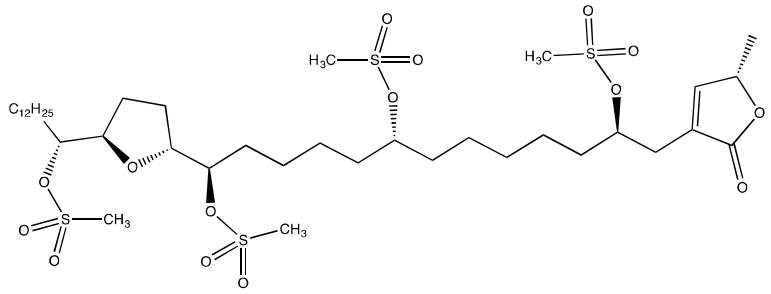
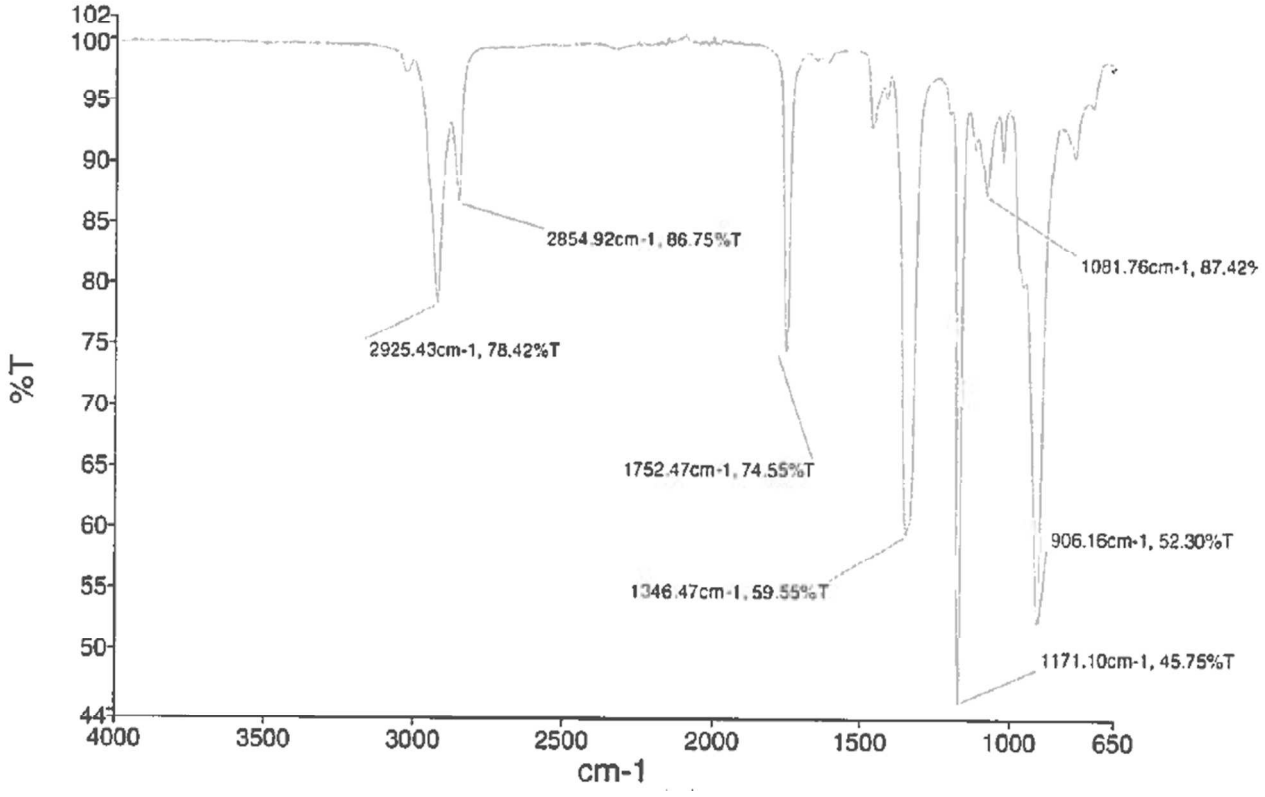
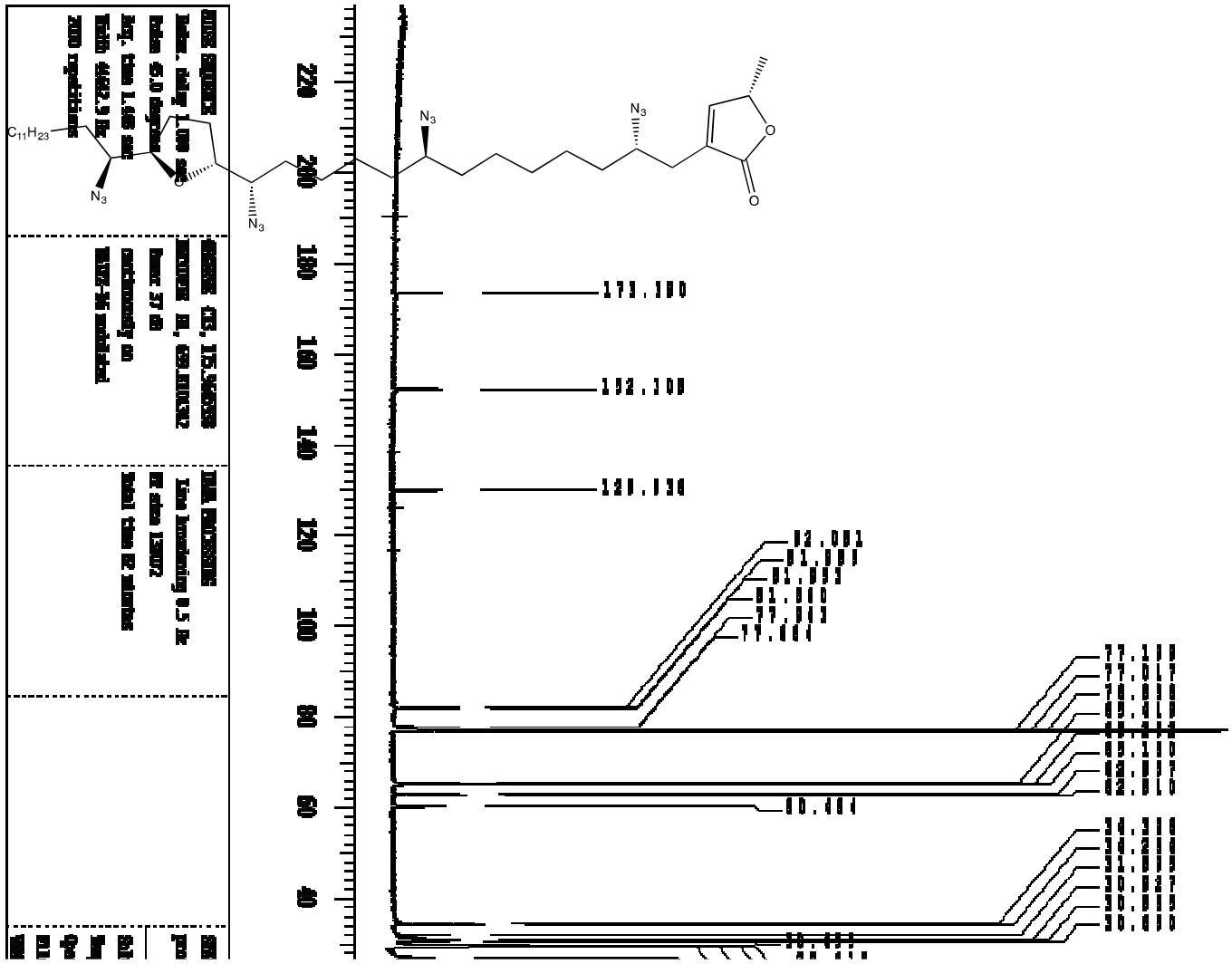




^{1}H NMR spectra of (*S*)-5-methyl-3-((2*S*,8*S*,13*S*)-2,8,13-triazido-13-((2*R*,5*R*)-5-((*S*)-1-azidotridecyl) tetrahydrofuran -2-yl)tridecyl)furan-2(5*H*)-one (3)



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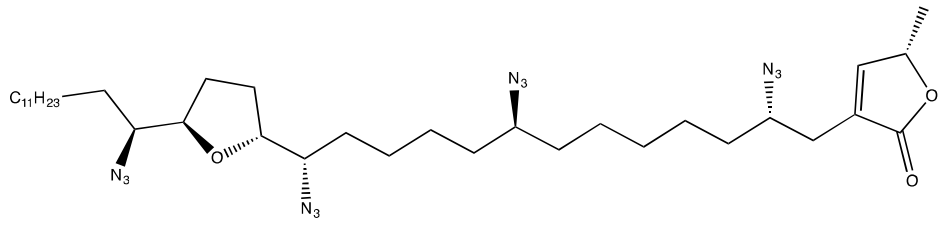
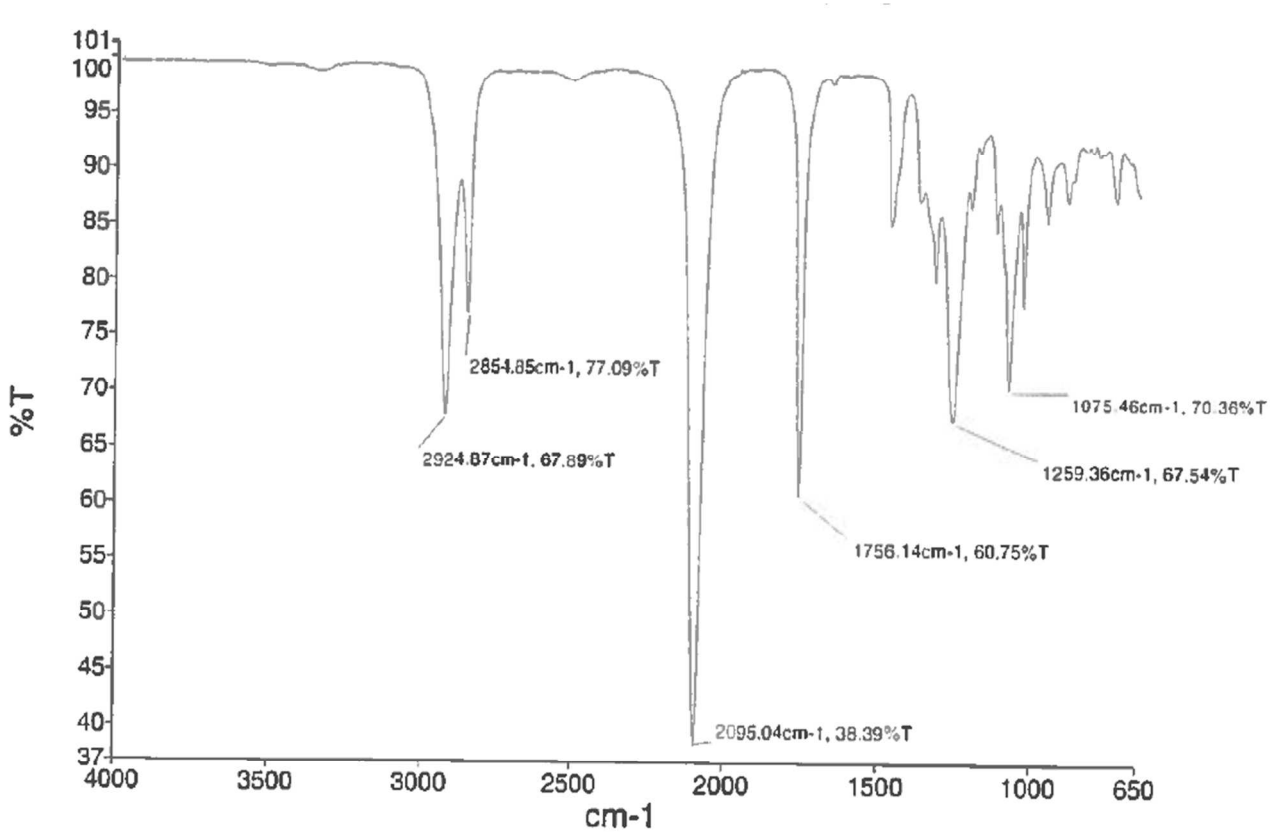
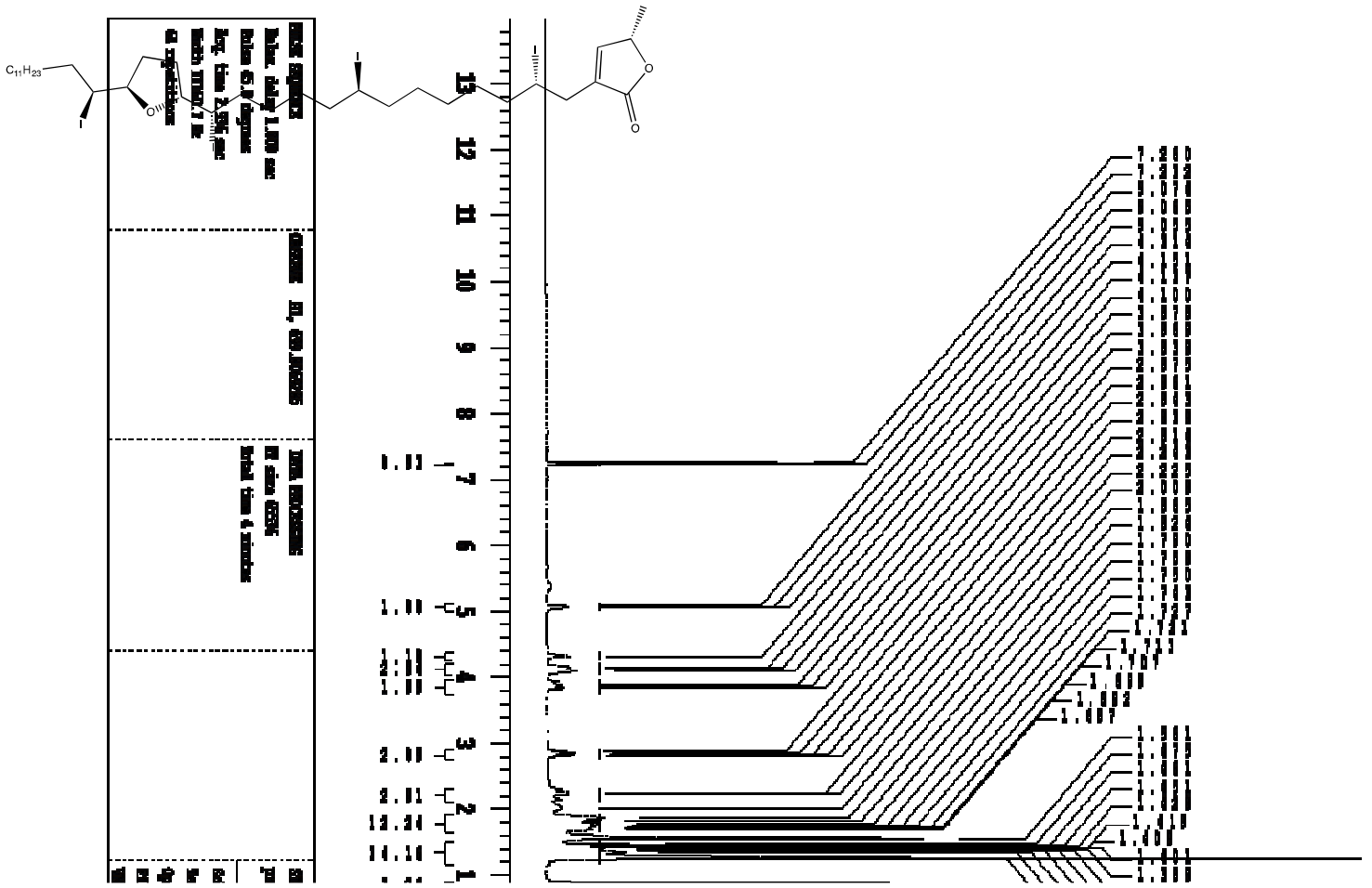
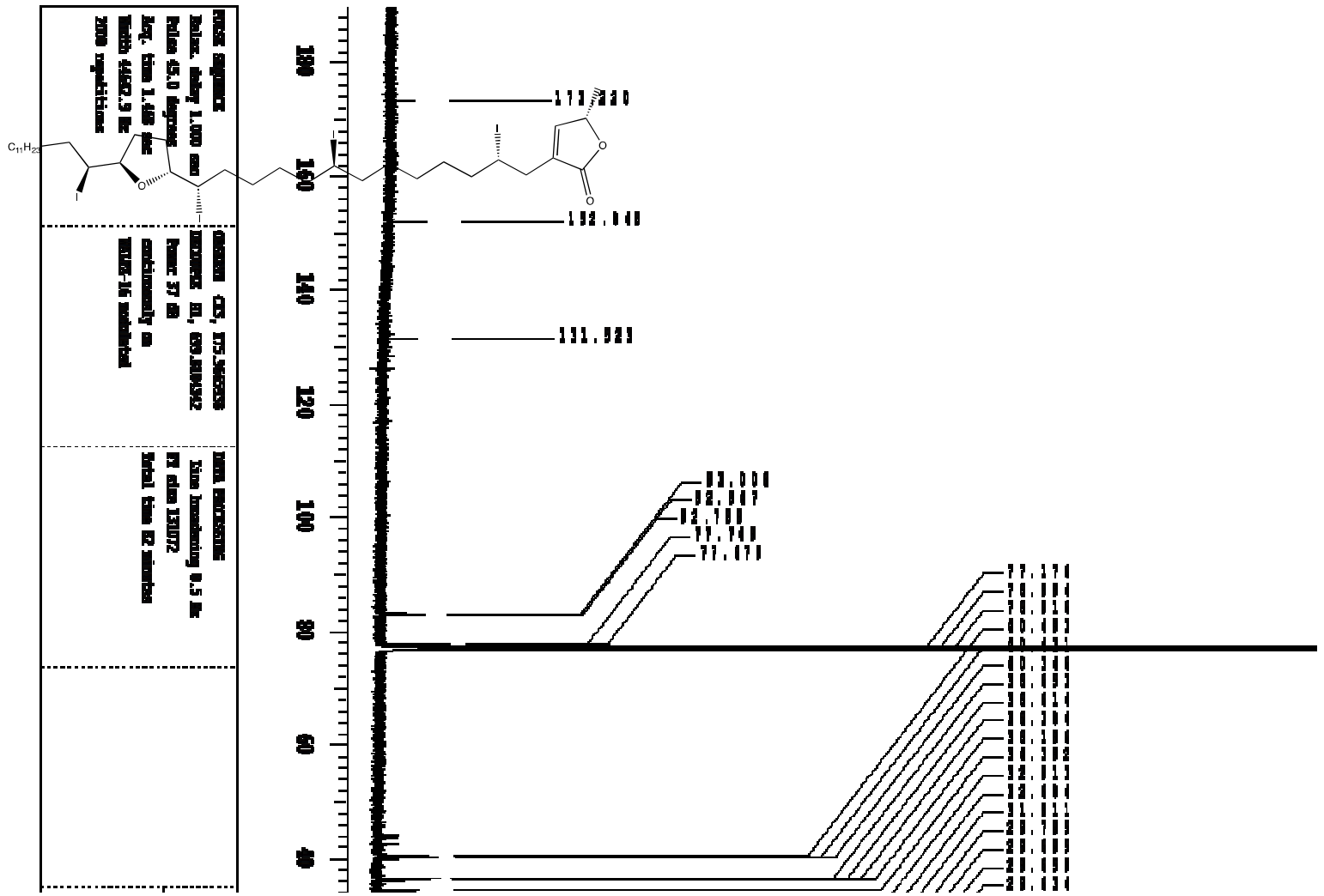






^{1}H NMR spectra of (*S*)-5-methyl-3-((2*S*,8*S*,13*S*)-2,8,13-triiodo-13-((2*R*,5*R*)-5-((*S*)-1-iodotridecyl) tetrahydrofuran -2-yl) tridecyl) furan-2(5*H*)-one (4)



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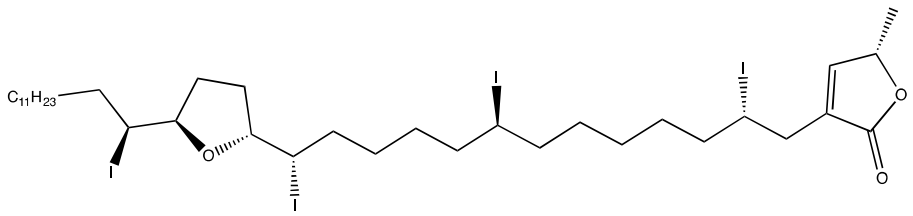

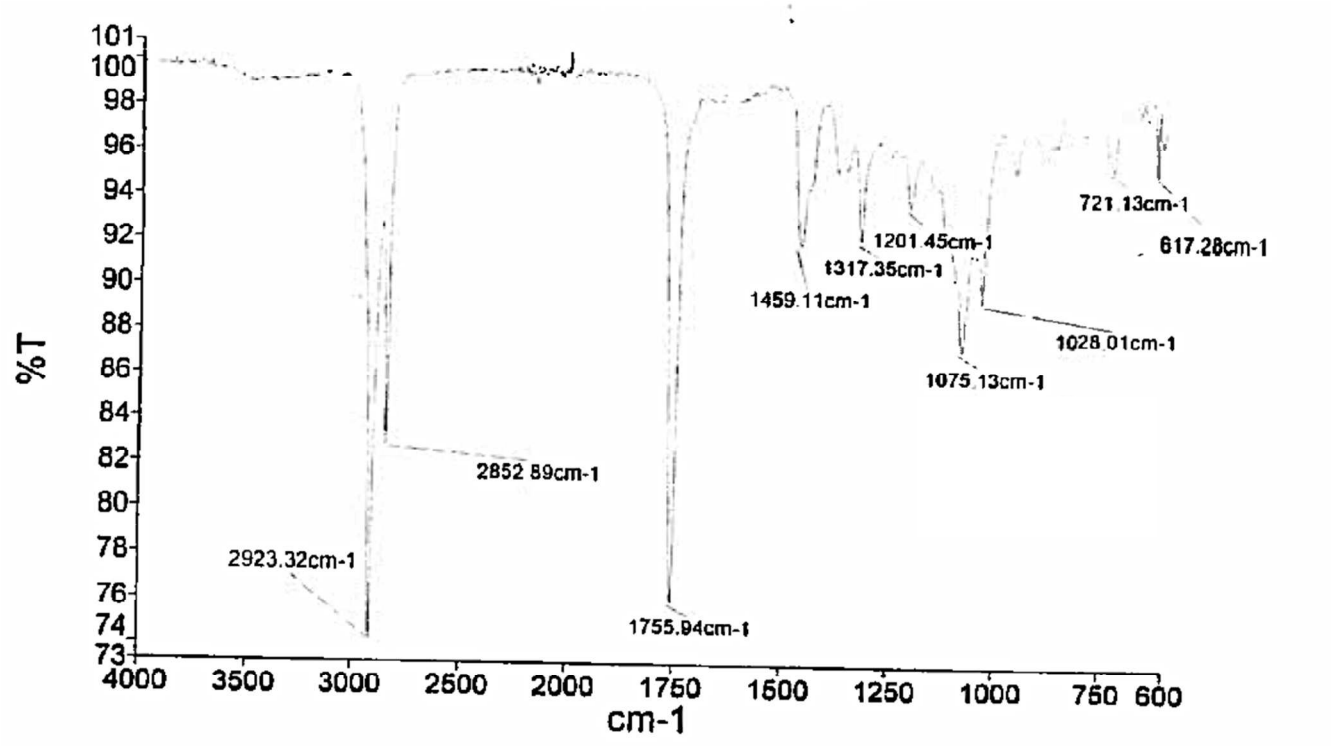

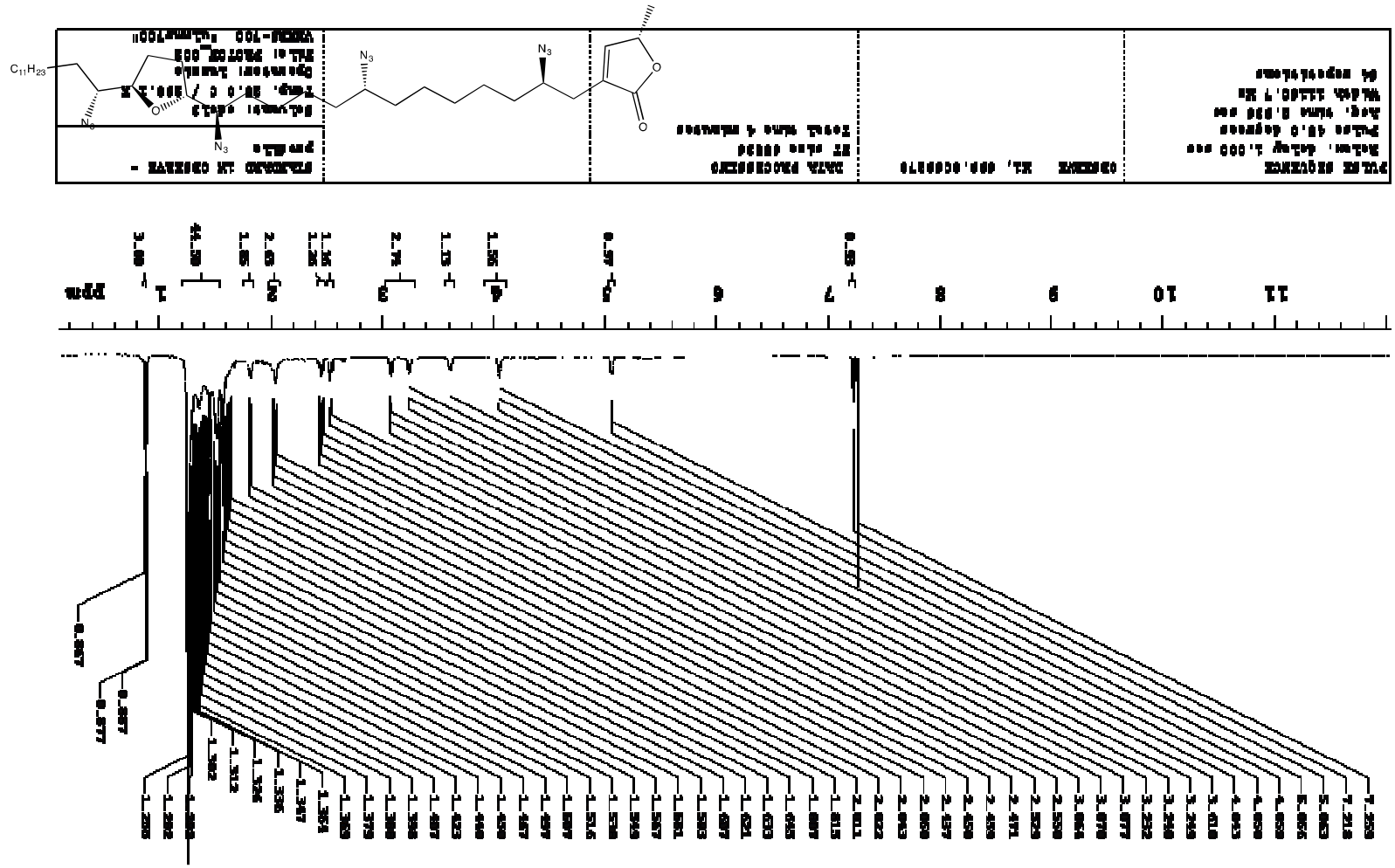

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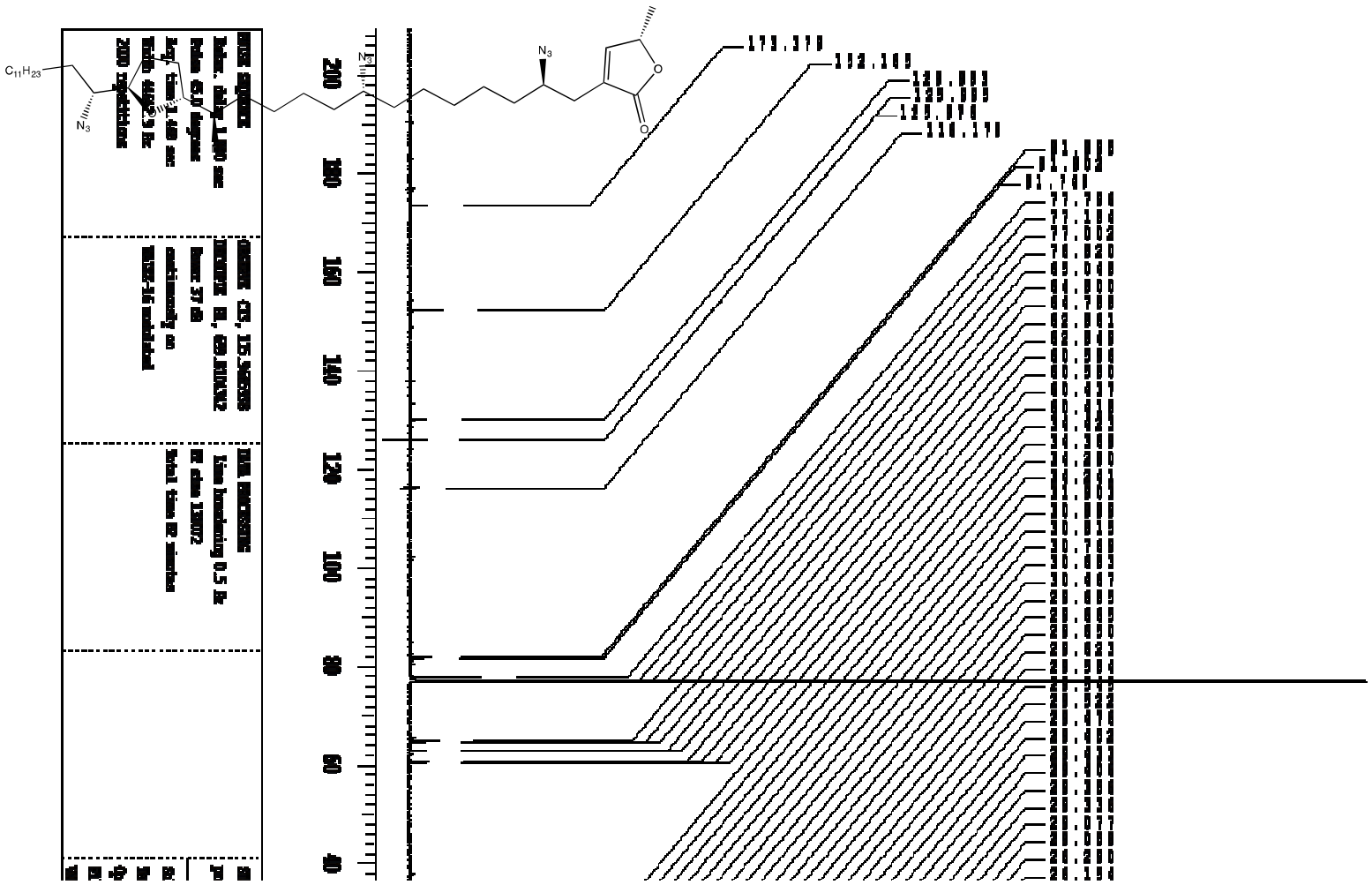

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