Supporting Information

Structure of (–)-Neodysidenin from *Dysidea herbacea*. Implications for Biosynthesis of 5,5,5-Trichloroleucine Peptides

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Marfey's Analysis of L-leucine and D,L-leucine: Standard amino acids (50 mM) were prepared as aqueous solutions and N_{α}-(1-fluoro-2,4-dinitrophenyl-5)-L-alanine amide (Marfey's reagent: Marfey, P. *Carlsberg. Res. Commun.* **1984**, *49*, 591-596) was prepared as an acetone solution (1% w/v). HPLC analysis was carried out using a Rainin HPXL binary pump HPLC system on a Microsorb-MV^{TM} C_{18} column (3μ, 100 × 4.6 mm) at a flow rate of 2.0 mL/min, UV monitoring at λ 340 nm, and gradient elution with 10% to 60% CH_3CN: 0.05 M aqueous triethylammonium phosphate (TEAP, pH 3.2) over 40 min.

L-Leucine: A stirred aqueous solution of L-leucine solution (100 μL, 5 μmol) in a 'V'-shaped 1.0 mL vial was treated with excess Marfey's reagent (200 μL) and 1.0 M $NaHCO_3$ (40 μL) and heated to 40 °C for 1 hour. The mixture was cooled over 20 minutes and treated with 2M HCl (20 μL). HPLC analysis of the L-leucine Marfey's derivative (10 μL injection, 13.9 nmol) showed elution of the L-leucine derivative at a retention time of 18.9 minutes.

D,L-Leucine: The above conditions were used for the preparation of the D,L-leucine Marfey's derivative. Injection of 10 μL (1.39 nmol) of Marfey's-D,L-leucine-derivative gave peaks with a retention times of 18.9 min for the L-derivative (UV detector response: 6.81 mAU.s.nmol^{-1}) and 23.1 min for the D- derivative (6.67 mAU.s.nmol^{1}), respectively.

A. Hydrolysis of (–)-Neodysidenin - Determination of C-2 and C-7 Configurations

(–)-Neodysidenin (0.4 mg, 700 nmol) was hydrolyzed with 6M HCl (1 mL) at 110 °C for 12 h. Solvent was removed under a stream of N_2 and the sample was redissolved in H_2O (1.0 mL), extracted with EtOAc (2 × 1 mL). The H_2O-soluble fraction was concentrated, dissolved in AcOH (50 μL) and treated with Zn dust (5 mg). The heterogeneous mixture was stirred vigorously for 6 hours at 60 °C, followed by dilution with AcOH (0.5 mL). Saturated aqueous H_2S was added to precipitate Zn salts (ZnS) and the mixture centrifuged. The aqueous supernatant was concentrated, taken up with H_2O (13 μL) and treated with excess Marfey's reagent and $NaHCO_3$ aq. (scaled, total volume 51

μL). HPLC analysis under the same conditions (10 μL injection) gave a peak corresponding to L-leucine Marfey's derivative(19.6 min, 15.1 mAU.sec). A coinjection of the latter sample (13 μL) with D,L-leucine Marfey's derivative (2 μL of a 1.38 mM solution) gave an increase in the peak area of L-leucine, only. The overall yield of L-leucine over the three steps (acid hydrolysis, Zn reduction and Marfey's derivitization) was estimated as 8.8 nmol (~1.3%) from (–)-neodysidenin.

B. Ozonolysis-Hydrolysis of Neodysidenin - Determination of C-13 Configuration by Capillary Electrophoresis.

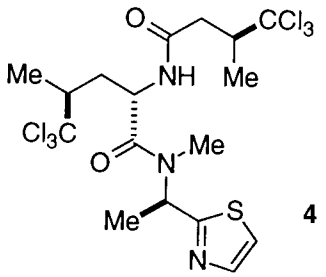
A solution of **4** (~200μg) in MeOH (0.5 mL) at -78° C was treated with ozone-saturated MeOH (3 mL) for 15 min. Excess ozone was removed by sparging with N_2 and the solution was treated with H_2O_2 (30% v/v, 2 drops) and 88% HCOOH (88% v/v, 2 drops) and warmed to 40° C for 30 min. The mixture was concentrated to dryness and the residue hydrolyzed (6M HCl, 100° C, 10 h) followed by partitioning with EtOAc. The two partitions were treated separately as described below.

(a) *Aqueous Partition.* The aqueous-soluble fraction from the hydrolysate and samples of standard *N*-methyl-L-alanine and *N*-methyl-D,L-alanine (Sigma) were separately converted to their corresponding Marfey's derivatives, as described above for leucine standards, and aliquots of each were diluted 1:5 with the electrophoresis running buffer (100 mM aqueous sodium borate, containing 200 mM sodium dodecyl sulfate, pH 8.5). Electrophoresis of the Marfey's derivative solutions was carried out using a variant known as micellar electrokinetic capillary chromatography (MECC, Tran, A. D.; Blanc, T.; Leopold, E. J. *J. Chromatogr.* **1990**, *516*, 241-249). Separations were achieved using a Hewlett Packard ^{3D}CE instrument coupled to a diode array detector (monitoring at λ 340 nm) with a 50 μm × 40 cm capillary (20 kV, temperature, 25° C). The column was preconditioned before each run with 1.0 M NaOH, followed by running buffer, 15 min each. Samples were injected using the pressure method (50 mbar, 10 s) and run over 30 minutes with running buffer. The *N*-methyl-L-alanine and *N*-methyl-D-alanine Marfey's derivative standards eluted at 11.2 and 11.5 minutes, respectively, with baseline resolution. The hydrolysate-Marfey's derivative gave a peak which co-eluted with authentic *N*-methyl-D-alanine Marfey's derivative.

(b) EtOAc-Soluble Fraction: The ethyl acetate-soluble fraction was concentrated to dryness, dissolved in MeOH and treated with excess diazomethane in ether, diluted with EtOAc (5 mL) and a portion (1 mL) concentrated and redissolved in EtOAc (200 μL) to

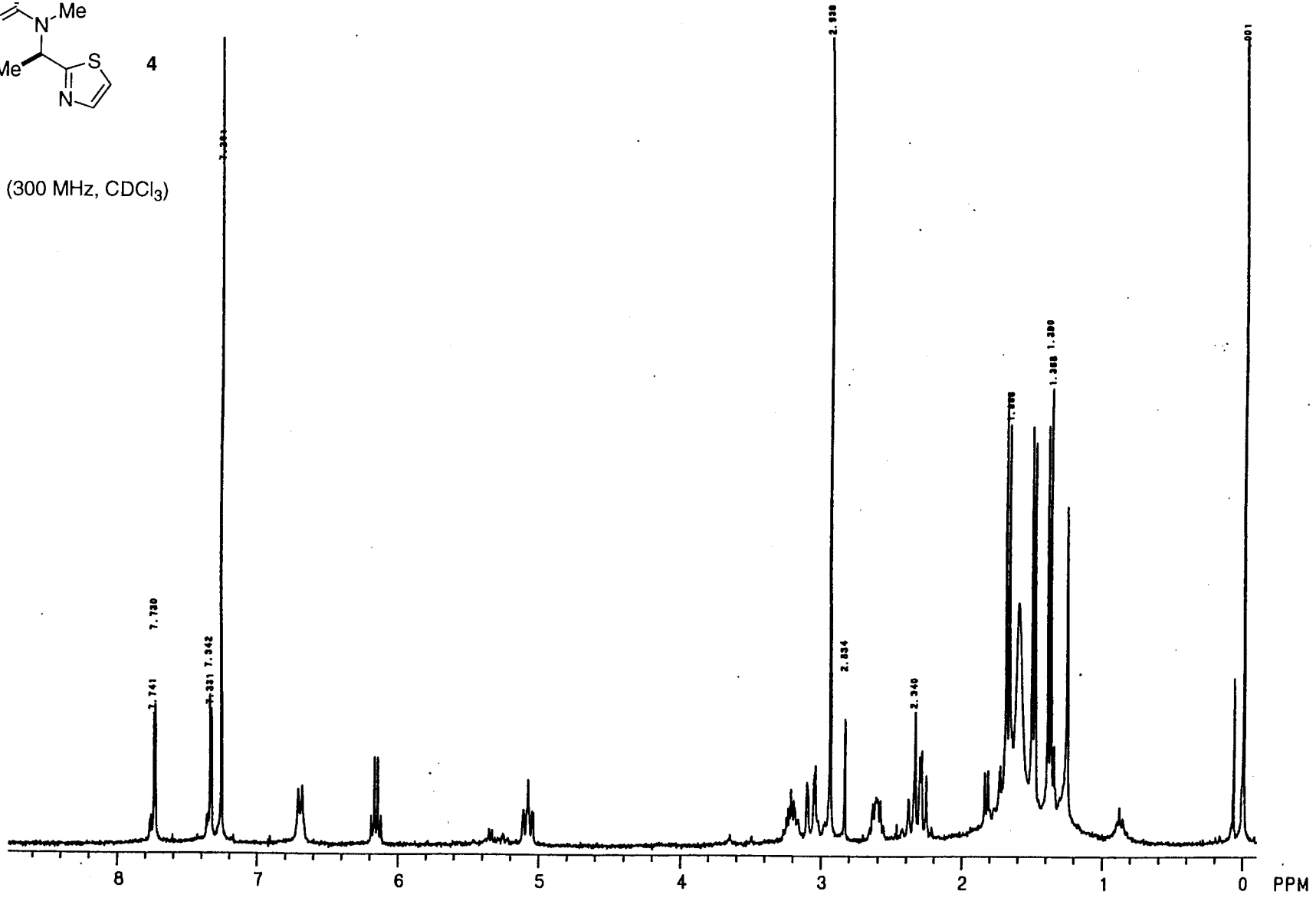
give the analyte solution. Standard solutions of methyl 4,4,4-trichloro-3-methylbutanoate, (±)-**6** and *R*-(+)-**6** were prepared by similar treatment of the corresponding acids (Brantley, S. E.; Molinski, T. F. *Organic Letters* **1999**, *1*, 2165-2167) with diazomethane. Chiral separation of the analyte solution was carried out by GCMS (Varian-Finnigan ion-trap MS, α-permethylated cyclodextrin capillary column, 0.25 mm × 30 m, 50° C for 5 min, then 50-180° C over 20 min. He flow rate 10 mL/min, split 2:1). Authentic *R*-(+)-**6** gave a single peak that eluted at 14.7 minutes. Injection of the analyte gave two peaks at 14.7 and 15.6 min with intensities of 1:12, respectively. Standard (±)-**6** also gave two peaks, but with equal intensity at the same retention times. Co-injection of equimolar aliquots of analyte and *R*-(+)-**6** gave the same two peaks, with double the intensity of the early eluting peak (14.7 minutes).

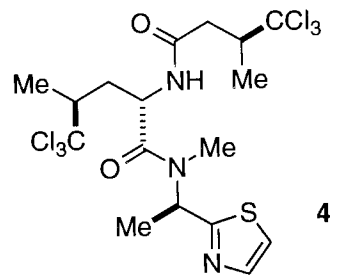
Repeated hydrolysis of **4** at higher temperature (6M HCl at 110° C, 12 h), followed by CH_2N_2 treatment gave a sample of **6** that was completely racemized.



4

^{1}H NMR (300 MHz, $CDCl_3$)





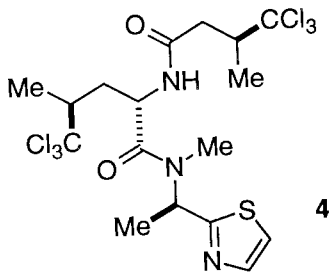
4

^{13}C NMR (125 MHz, $CDCl_3$)

200 150 100 50 0

ppm

COSY (300 MHz, $CDCl_3$)



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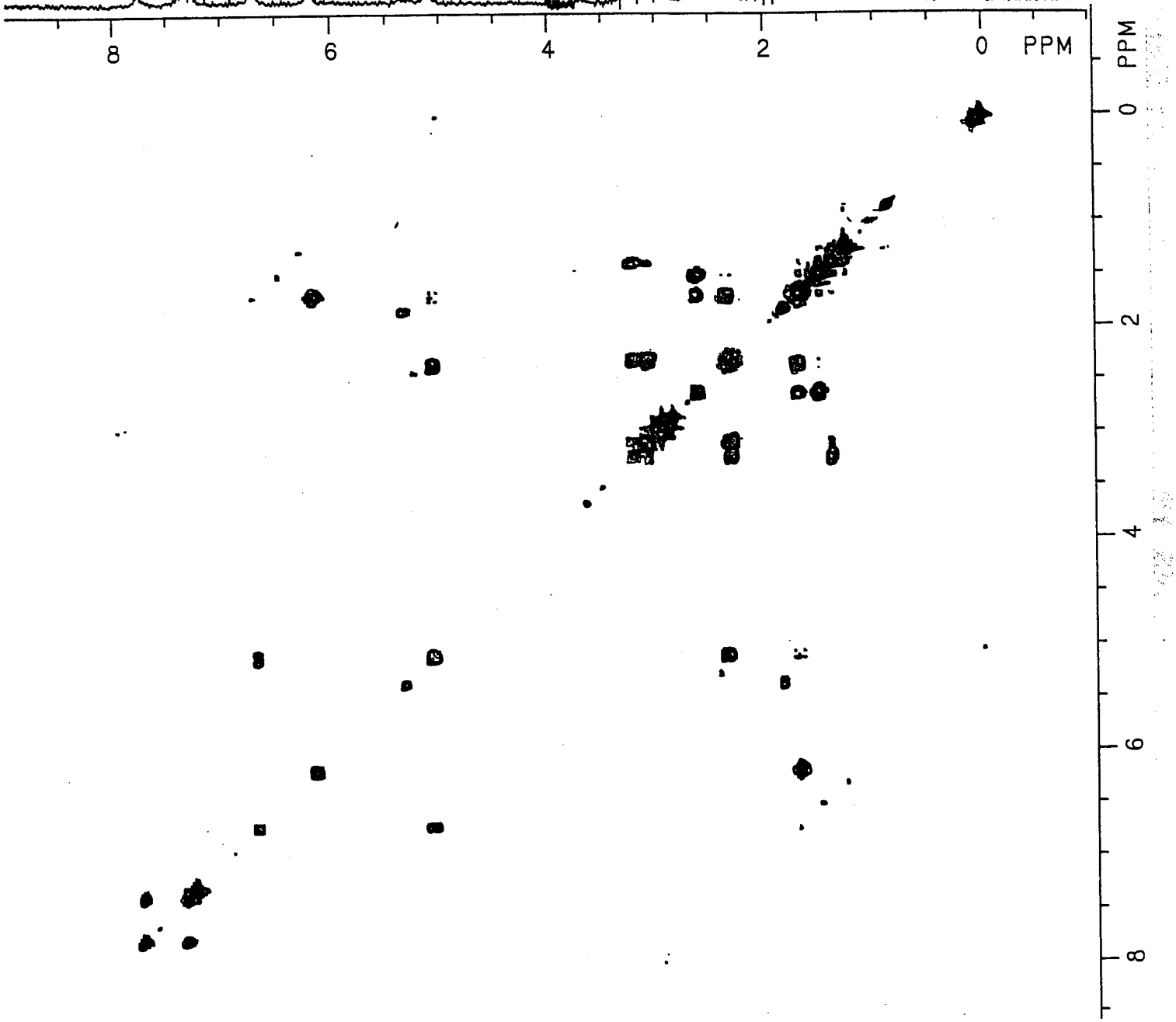

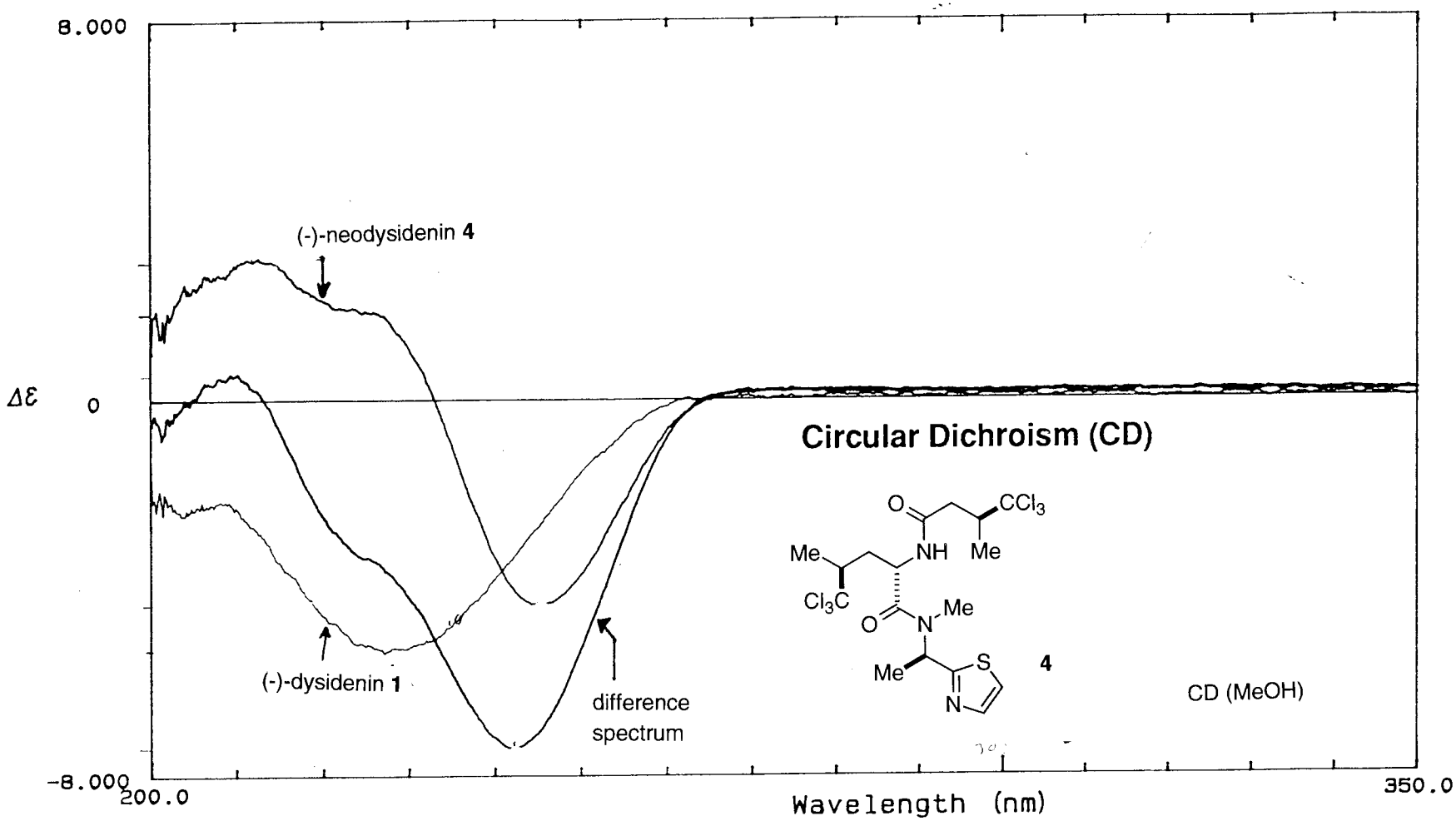

Dysid 3.72e-5M
Neodysidenin 6.67e-5M
Diff dydidenin - neodysidenin