Supporting Information

Bicyclic [3.3.0]-Octahydrocyclopenta[*c*]pyrrolo Antagonists of Retinol Binding Protein 4: Potential Treatment of Atrophic Age-Related Macular Degeneration and Stargardt Disease

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Supporting Information

Table of Contents

General Chemistry Information

All reactions were performed under a dry atmosphere of nitrogen unless otherwise specified. Indicated reaction temperatures refer to the reaction bath, while room temperature (rt) is noted as 25 °C. Commercial grade reagents and anhydrous solvents were used as received from vendors and no attempts were made to purify or dry these components further. Removal of solvents under reduced pressure was accomplished with a Buchi rotary evaporator at approximately 28 mm Hg pressure using a Teflon-linked KNF vacuum pump. Thin layer chromatography was performed using 1" x 3" AnalTech No. 02521 silica gel plates with fluorescent indicator. Visualization of TLC plates was made by observation with either short wave UV light (254 nm lamp), 10% phosphomolybdic acid in ethanol or in iodine vapors. Preparative thin layer chromatography was performed using Analtech, 20 × 20 cm, 1000 micron preparative TLC plates. Flash column chromatography was carried out using a Teledyne Isco CombiFlash Companion Unit with RediSep® Rf silica gel columns. If needed, products were purified by reverse phase chromatography, using a Teledyne Isco CombiFlash Companion Unit with RediSep® Gold C18 reverse phase column. Proton NMR spectra were obtained either on 300 MHz Bruker Nuclear Magnetic Resonance Spectrometer or 500 MHz Bruker Nuclear Magnetic Resonance Spectrometer and chemical shifts Bruker Nuclear Magnetic Resonance Spectrometer and chemical shifts (δ) are reported in parts per million (ppm) and coupling constant (*J*) values are given in Hz, with the following spectral pattern designations: s, singlet; d, doublet; t, triplet, q, quartet; dd, doublet of doublets; m, multiplet; br, broad. Tetramethylsilane was used as an internal reference. Melting points are uncorrected and were obtained using a MEL-TEMP Electrothermal melting point apparatus. Mass spectroscopic analyses were performed using positive mode electron spray ionization (ESI) on a Varian ProStar LC-MS with a 1200L quadrapole mass spectrometer. High pressure liquid chromatography (HPLC) purity analysis was performed using a Varian Pro Star HPLC system with a binary solvent system A and B using a gradient elusion [A, H_2O with 0.05% trifluoroacetic acid (TFA); B, CH_3CN with 0.05% TFA] and flow rate = 1 mL/min, with UV detection at 223 nm. All final compounds were purified to ≥95% purity and these purity levels were measured by a Varian Pro Star HPLC system. The following Varian Pro Star HPLC methods were used to establish compound purity:

A) Phenomenex C18(2) column (3.0 × 250 mm); mobile phase, A = H_2O with 0.05% TFA and B = CH_3CN with 0.05% TFA; gradient: 0–90% B (0.0–20.0 min); UV detection at 254 nm.

B) Phenomenex C18(2) column (3.0 × 250 mm); mobile phase, A = H_2O with 0.05% TFA and B = CH_3CN with 0.05% TFA; gradient: 0–100% B (0.0–20 min); UV detection at 254 nm.

RBP4 *In Vivo* PD (Serum RBP4 Measurements) Information

Whole blood was drawn into a centrifuge tube and was allowed to clot at room temperature for 30 minutes followed by centrifugation at 2000g for 15 minutes at 4°C to collect serum. Serum RBP4 was measured using the RBP4 dual ELISA kit (Enzo Life Sciences, Farmingdale, NY) following the manufacturer's instructions.

In Vivo PK Information and Data

Rat PK Studies

The objective of this study was to determine the plasma pharmacokinetics of compound **33**. Male Sprague Dawley rats were administered a single or 7-day repeat dose administration of the test article by oral gavage (po) dose route.

TEST ARTICLE AND VEHICLE INFORMATION:

IV dosing vehicle:	3% DMA/45% PEG300/12% ethanol/40% sterile water
PO dosing vehicle:	2% Tween 80 in 0.9% saline
Dose formulation:	The dose formulation was prepared by the step-wise addition (in the order listed) of the individual components of the vehicle to a weighed quantity of test compound in a volume that yielded the desired final concentration. Each formulation was prepared by mixing a weighed quantity of test compound with the appropriate volume of vehicle.
Handling:	Room temperature.
Stability:	Dose formulations were prepared fresh on each day of dosing; considered stable through use for dosing.
Disposition:	Unused dose formulations were discarded after dosing.

TEST SYSTEM:

Species and strain:	Rat; Sprague Dawley, with indwelling jugular vein cannula.
Supplier:	Charles River (Raleigh, NC).
Age on Day 0:	Approximately 8 weeks.
Weight on Day 0:	332–368 g.
Number on study:	Males – 9.

HUSBANDRY:

Housing:	One animal per cage in solid-bottom cages on stainless steel racks.
Bedding:	Hardwood bedding (P.J. Murphy Forest Products, Corp.; Montville, NJ).
Room Environment:	Temperature of 70–73 °F & relative humidity of 32%–57%.

Fluorescent lighting with illumination 12 hours per day.

Feed and Water: Certified rodent diet 2018C (Harlan, Madison, WI) Provided ad libitum. Water supply provided ad libitum via automatic watering system.

Enrichment: No consumable enrichment was provided.

Acclimation Period: One day. Prior to study start, the animals were observed for general health and acceptability for use.

EXPERIMENTAL DESIGNS

Table 2. Group Assignment, Dosing, and Sample Collection Timepoints for Single Dose PK study.

Group	Test Compound	Route	Dose (mg/kg)	Dose Conc. (mg/mL)	Dose Vol. (mL/kg)	No. of Animals	Plasma Collection Timepoints (hr)
1	**33**	IV	2.0	0.4	5	3	Pre-dose, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48
2	**33**	PO	5.0	0.5	10	3	Pre-dose, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48

Table 3. Group Assignment, Dosing, and Sample Collection Timepoints for Repeat 7-Day QD Dosing Study.

Group	Test Compound	Dose (mg/kg)	Dose Conc. (mg/mL)	No. of Animals	Plasma Collection Timepoints (hr)		
					Day 1	Day 2-6	Day 7
1[a]	**33**	5	1.0	3	Pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48	NA[c]	NA[c]
2[b]	**Vehicle**	0	0	3	Pre-dose, (-1), 0.25, 0.5, 1, 2, 4, 8, 12, 24	Pre-dose	Pre-dose, (-1), 0.25, 0.5, 1, 2, 4, 8, 12, 24
3[b]	**33**	5	1.0	3	Pre-dose	Pre-dose	Pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48

[a]Animals received a single dose administration on Day 1.
[b]Animals received a 7-day repeat dose administration on Days 1-7.
[c] NA = no samples collected.

Dosing:

Dose Routes:	single intravenous (IV; tail vein) oral gavage (PO)
Dose Volume:	IV – 5 mL/kg PO – 10 mL/kg, as a slow bolus based on the most recent body weight
Clinical Signs:	Animals were observed at dosing and blood sample collection.

Collections for Plasma Drug Level and Biomarker Determinations:

Blood Intervals:	See Table 1.
Blood Volume:	0.1 mL/sample. Blood samples were placed on ice upon collection.
Blood Collection Site:	Jugular vein catheter
Blood Collection Tube:	K_2EDTA

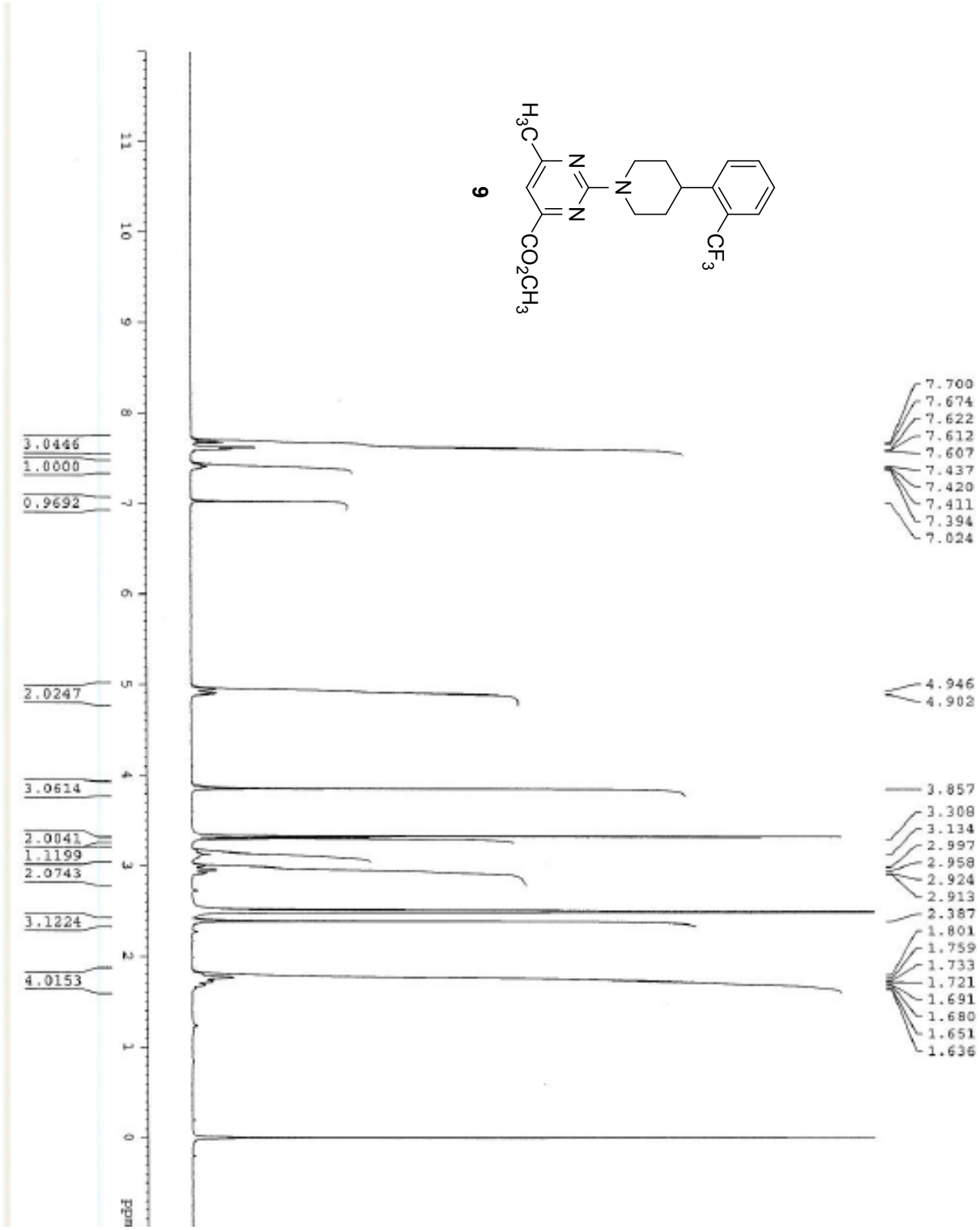
EUTHANASIA:

Euthanasia Intervals and Method: Following the last blood collection. CO_2 asphyxia.

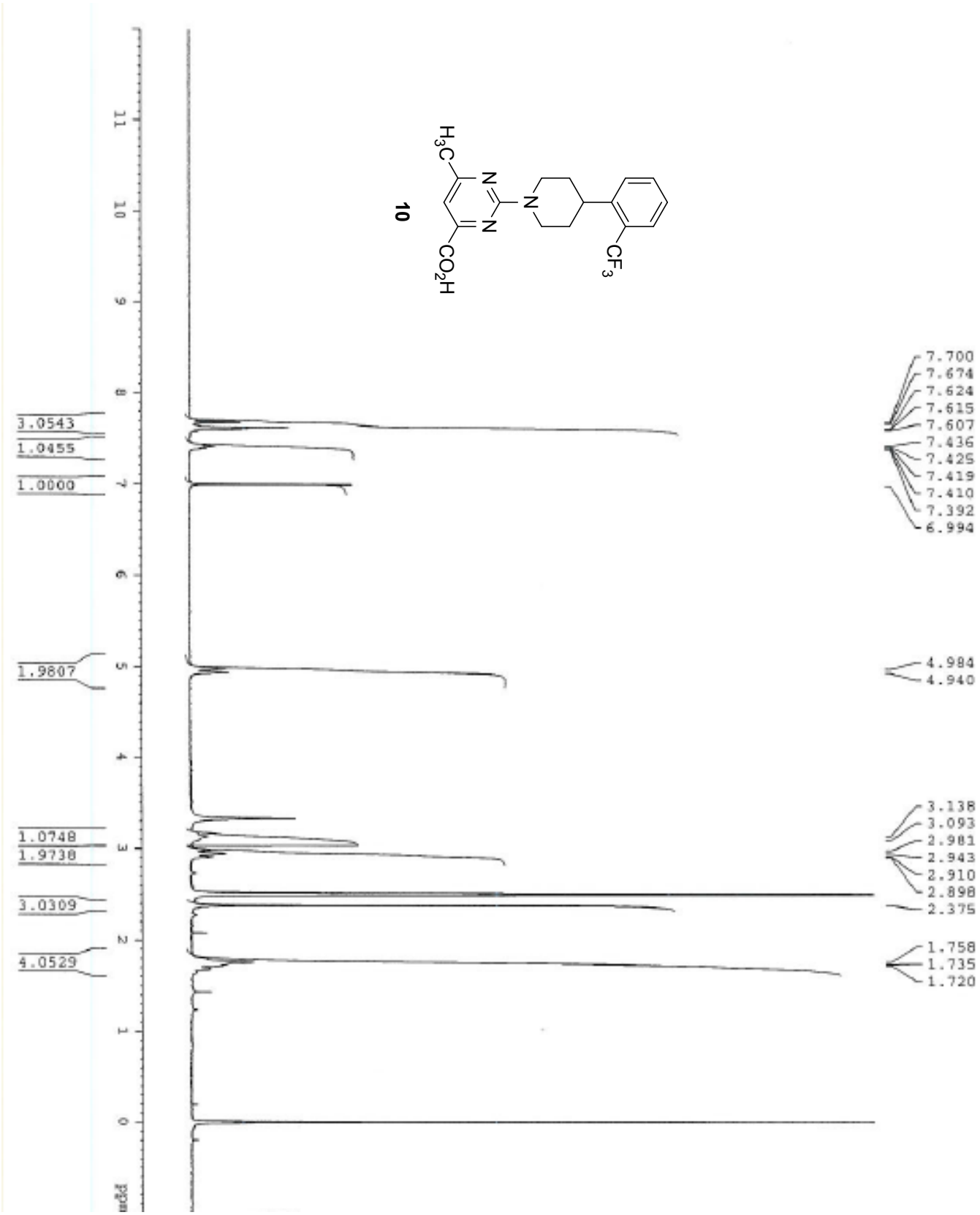
Drug Level Analyses and Pharmacokinetics (PK): Plasma samples were analyzed for drug levels using a discovery grade HPLC/MS/MS method. Plasma drug concentration versus time data were subjected to pharmacokinetic analysis using WinNonlin® (Version 4.1; Pharsight Corp.; Cary, NC). Data were subjected to non-compartmental analysis using WinNonlin® Model 200 (for extravascular administration); a uniform weighting factor was applied to each data set. T_{max} and C_{max} values were determined directly from the data. AUC_{last} and AUC_{INF} values were calculated using the log/linear trapezoidal (IV dose) or linear up/log down trapezoidal (PO dose). Half-life values were calculated from the first order rate constant associated with the observed terminal portion of the plasma drug concentration versus time curve, as estimated by regression analysis, using a minimum of three non-zero time points after Tmax. The regression with the largest adjusted R^2 (square of the correlation coefficient) was selected as the best fit. Regression fits with an adjusted R^2 of less than 0.800 were considered to be unreliable and were excluded from inclusion in the tabulation of kinetic parameters. Mean values and standard deviations for the pharmacokinetic parameters were calculated using WinNonlin.

^{1}H NMR Spectral Data:

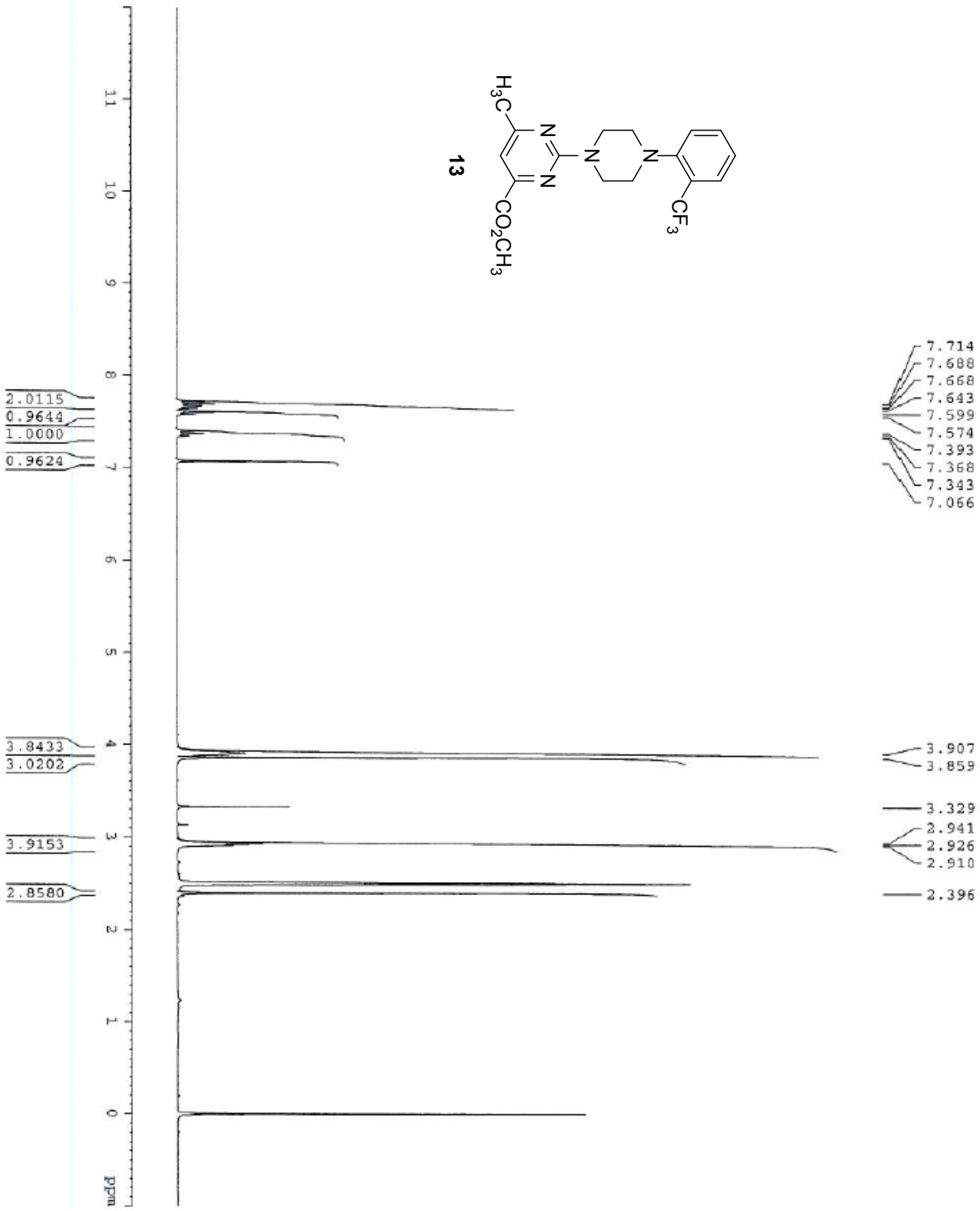
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **9**



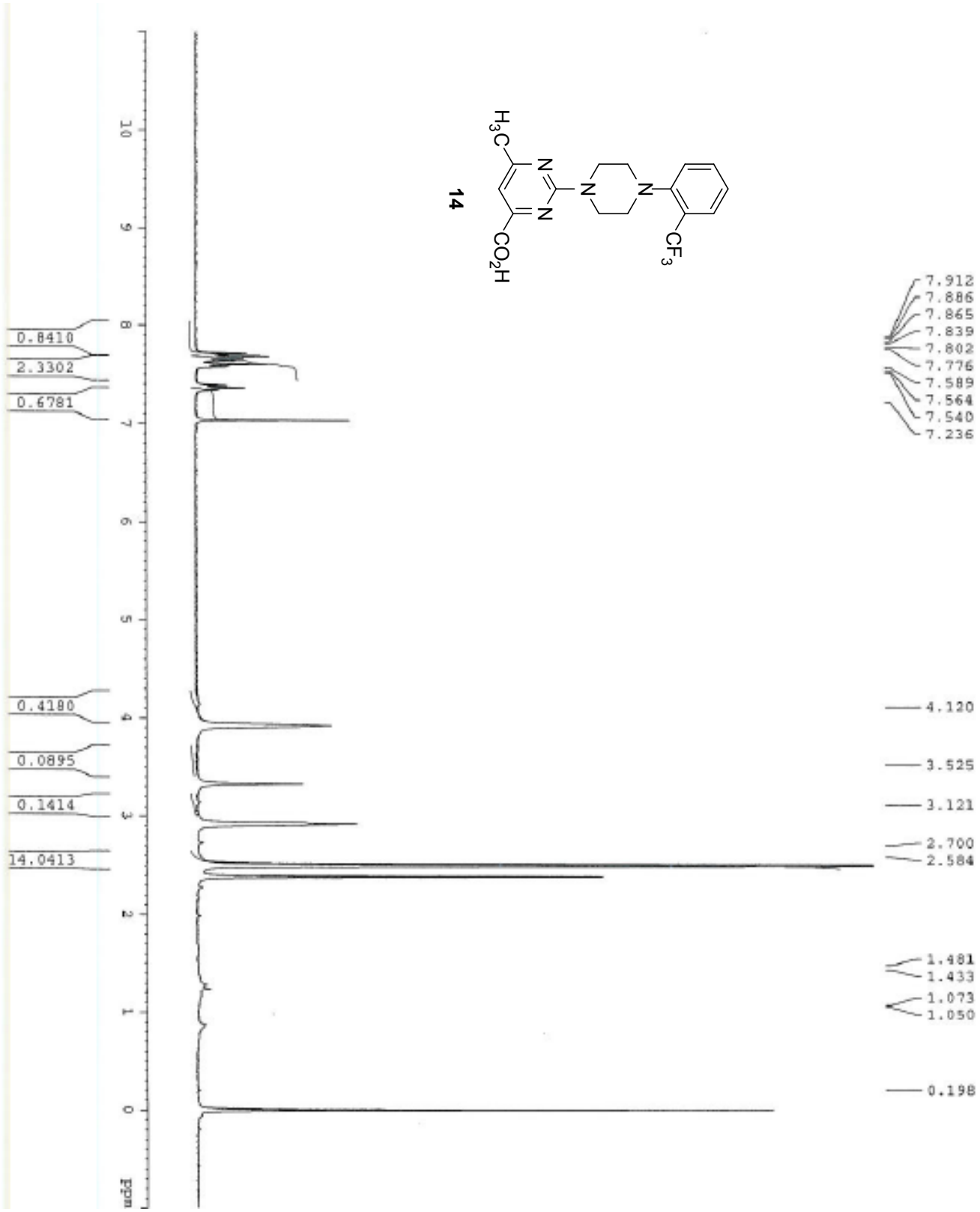
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **10**



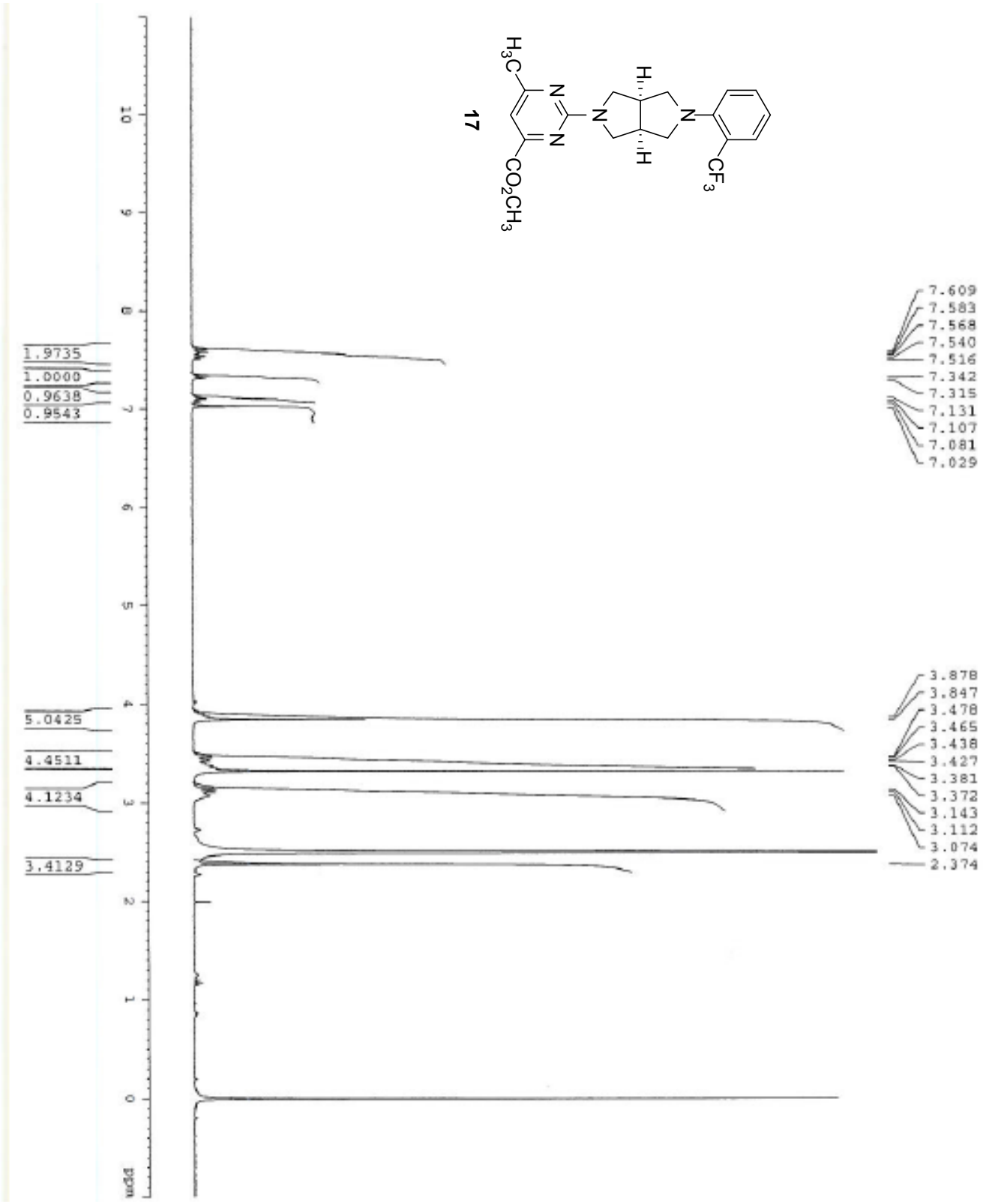
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **13**



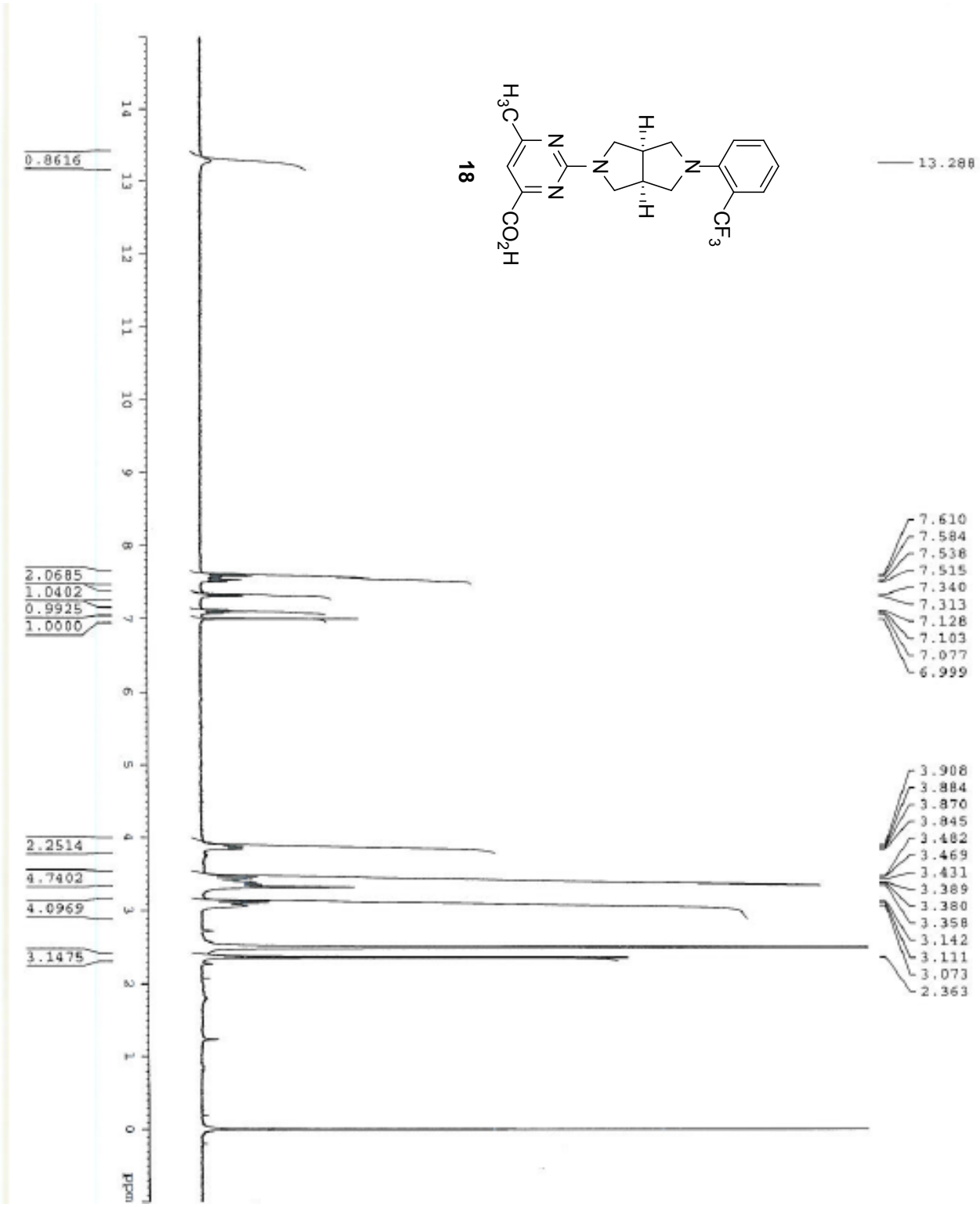
^{1}H NMR (300 MHz, DMF): Analogue **14**



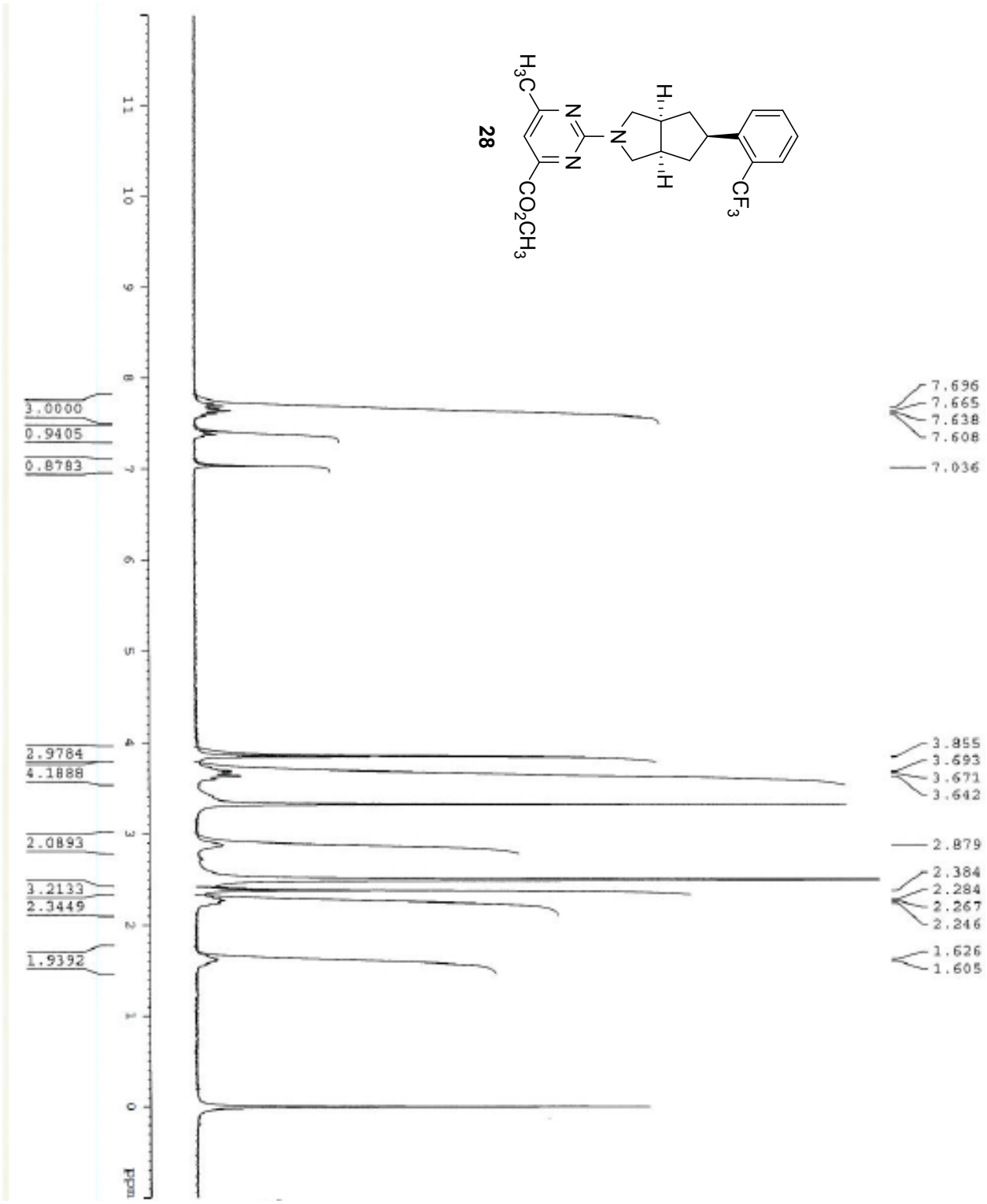
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **17**



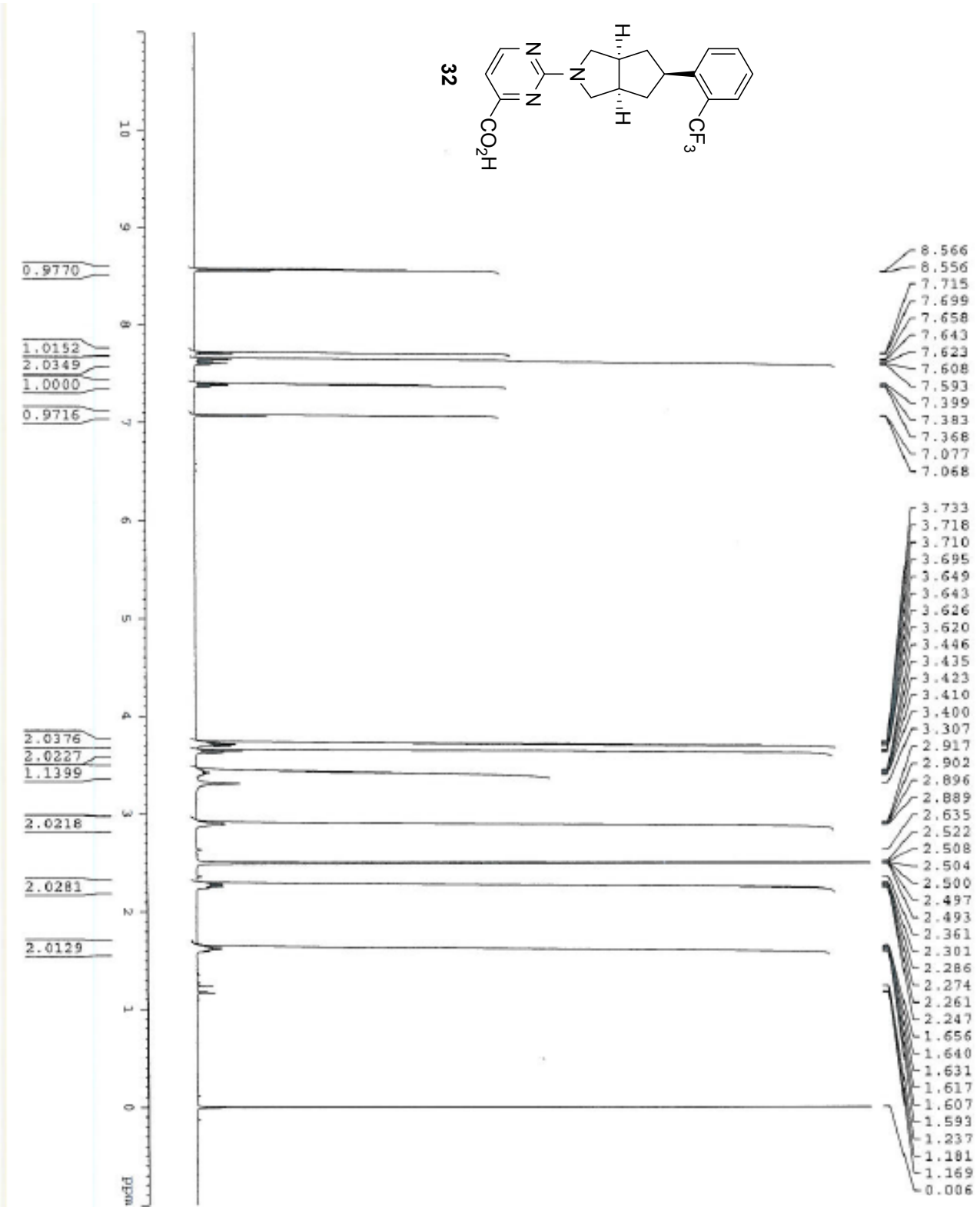
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **18**



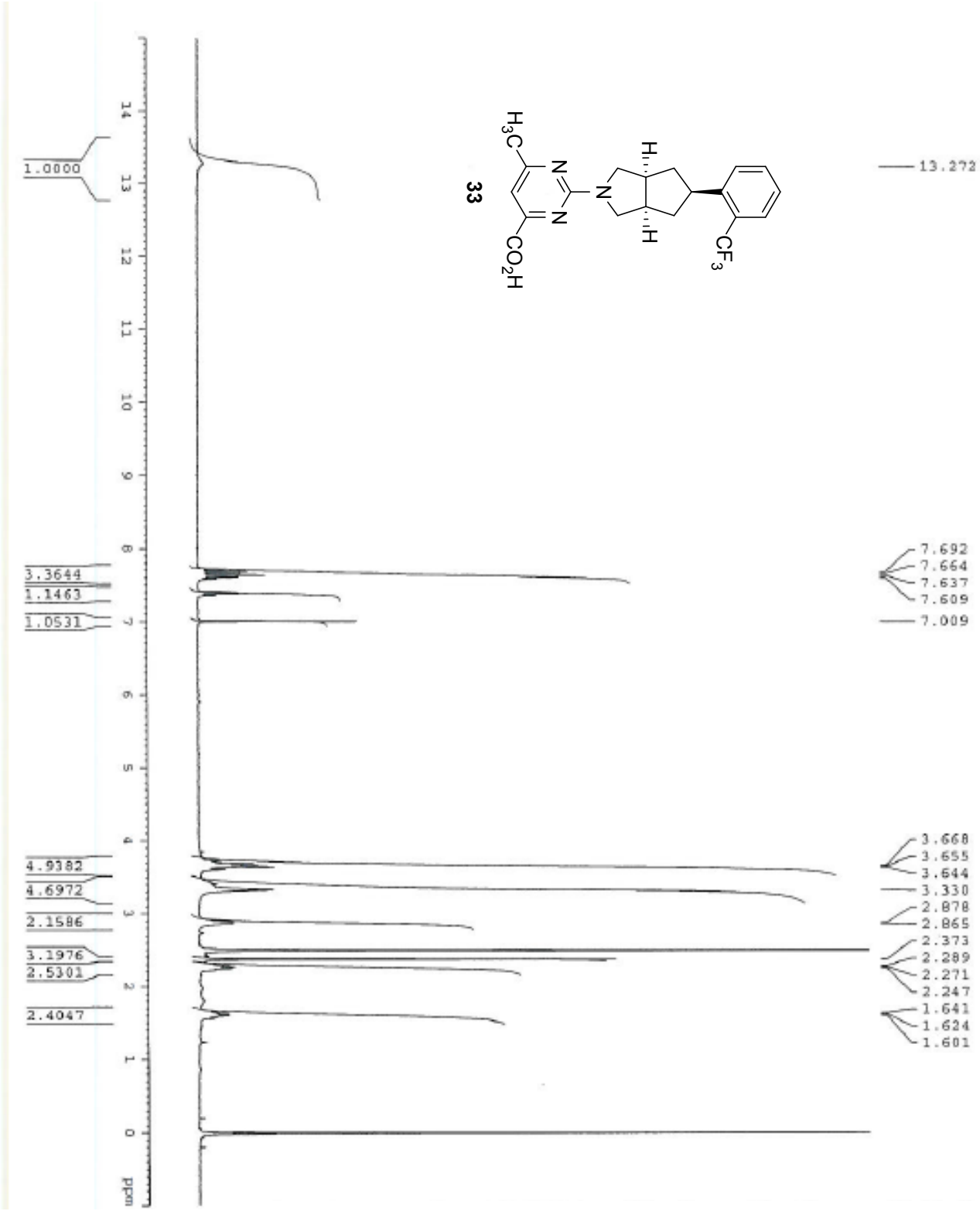
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **28**



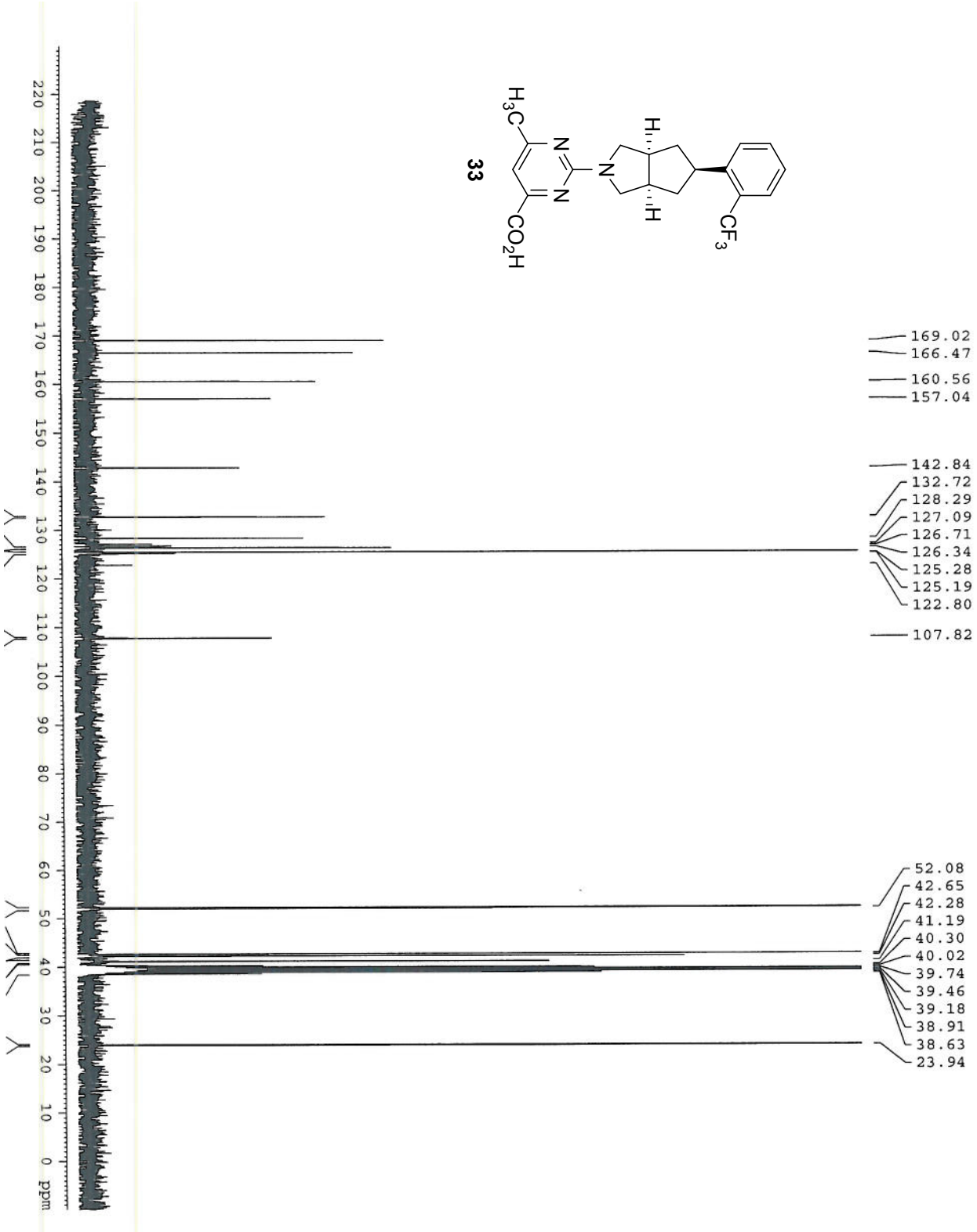
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **32**



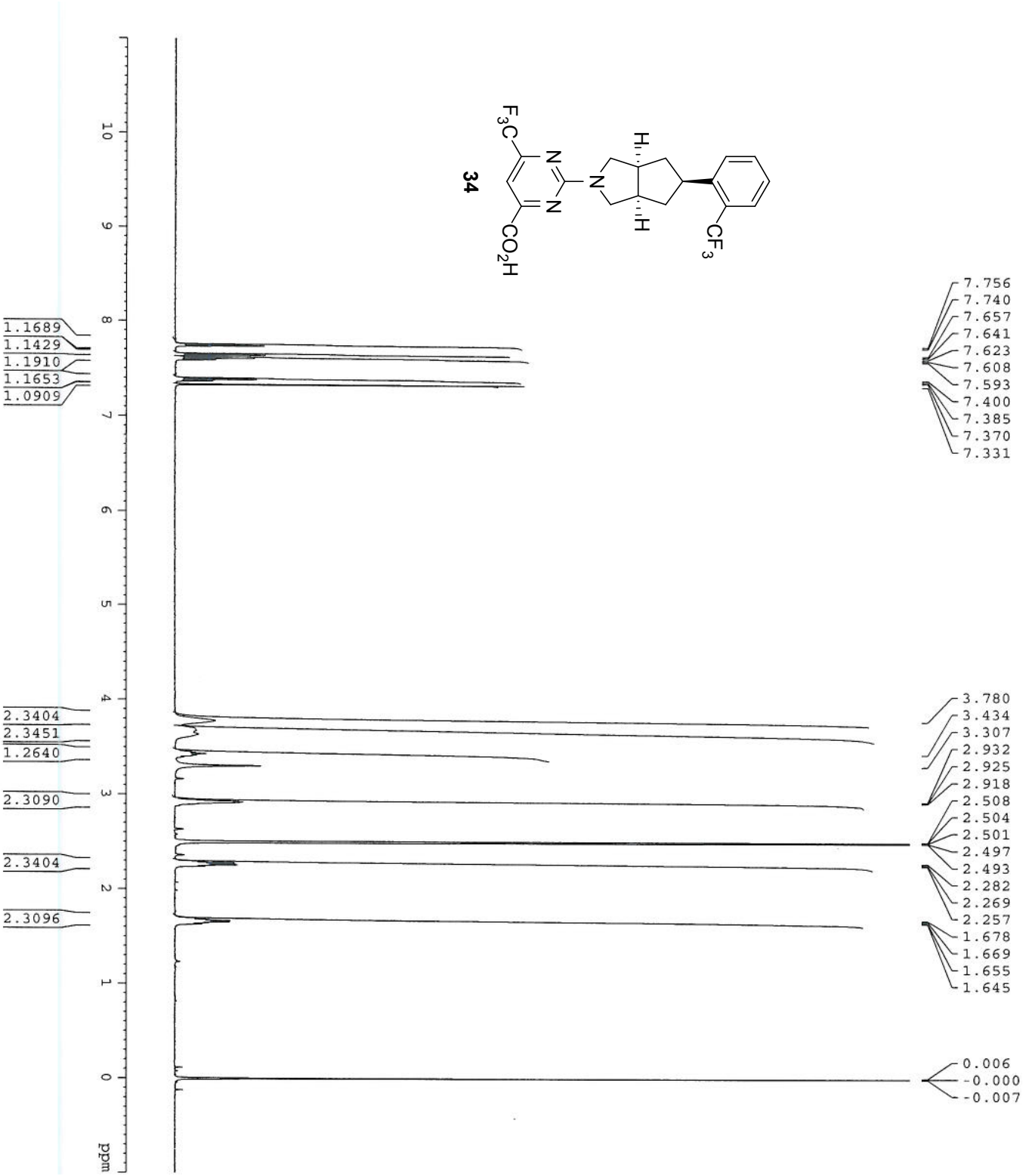
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **33**



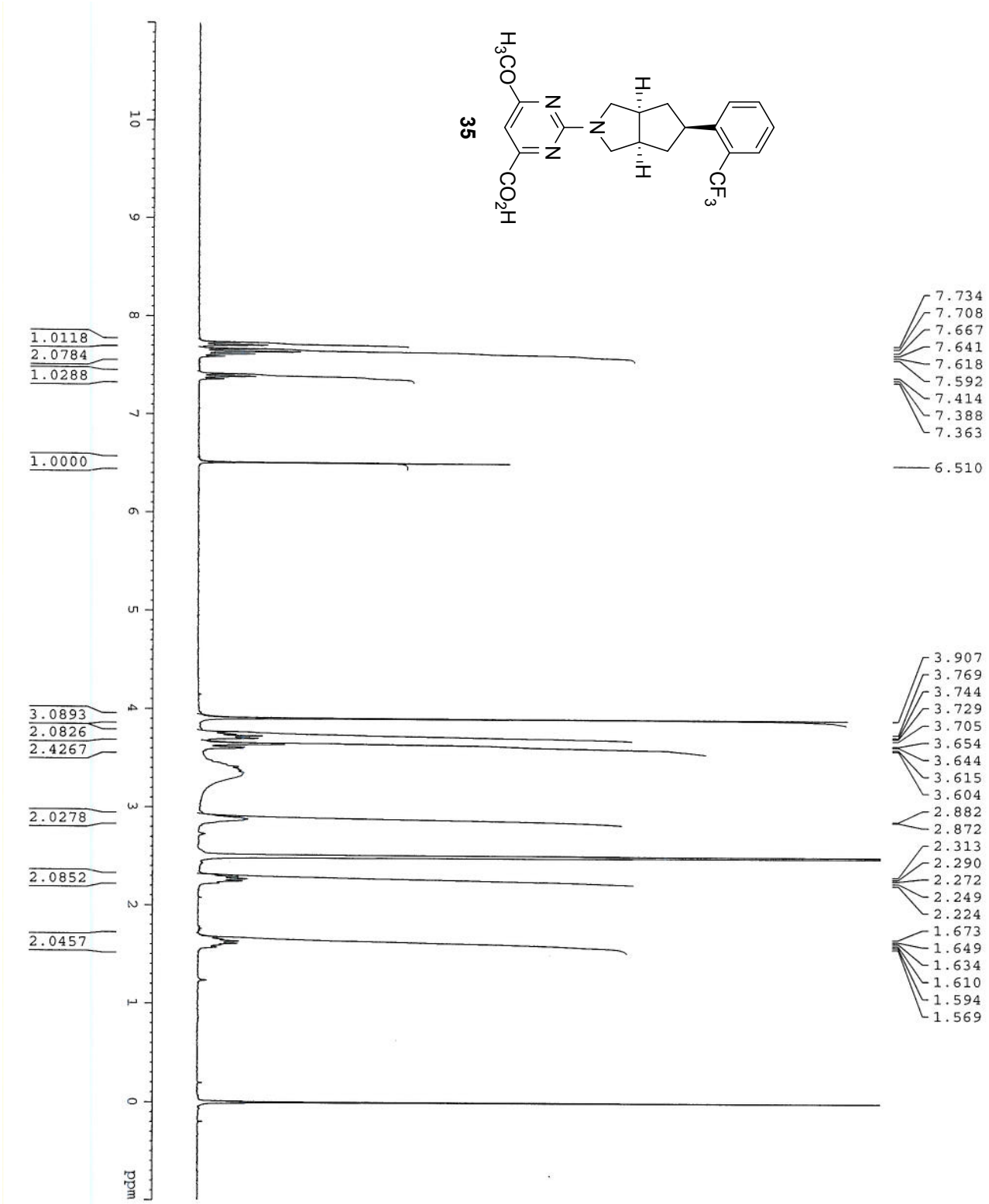
^{13}C NMR (300 MHz, DMSO-d_6): Analogue **33**



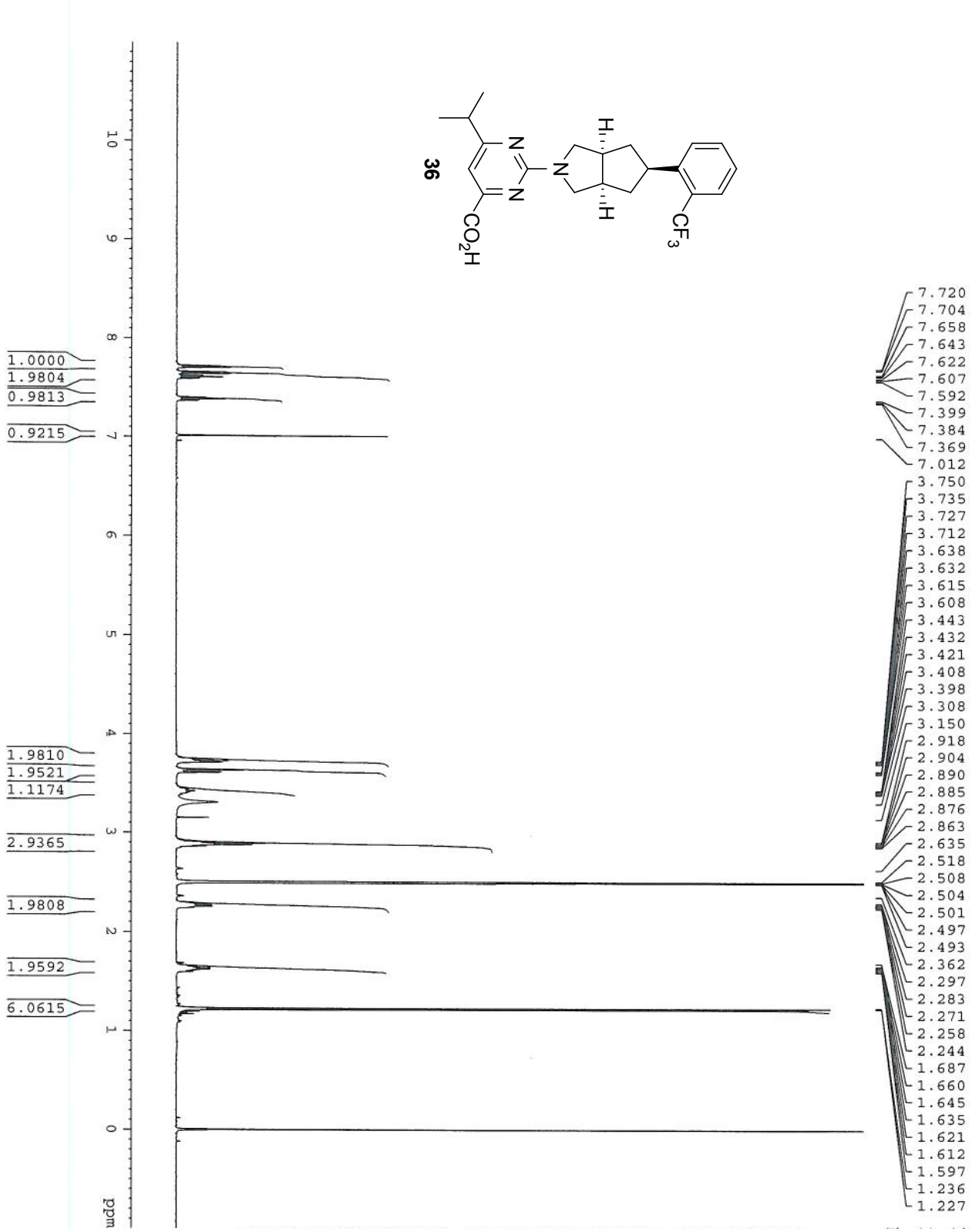
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **34**



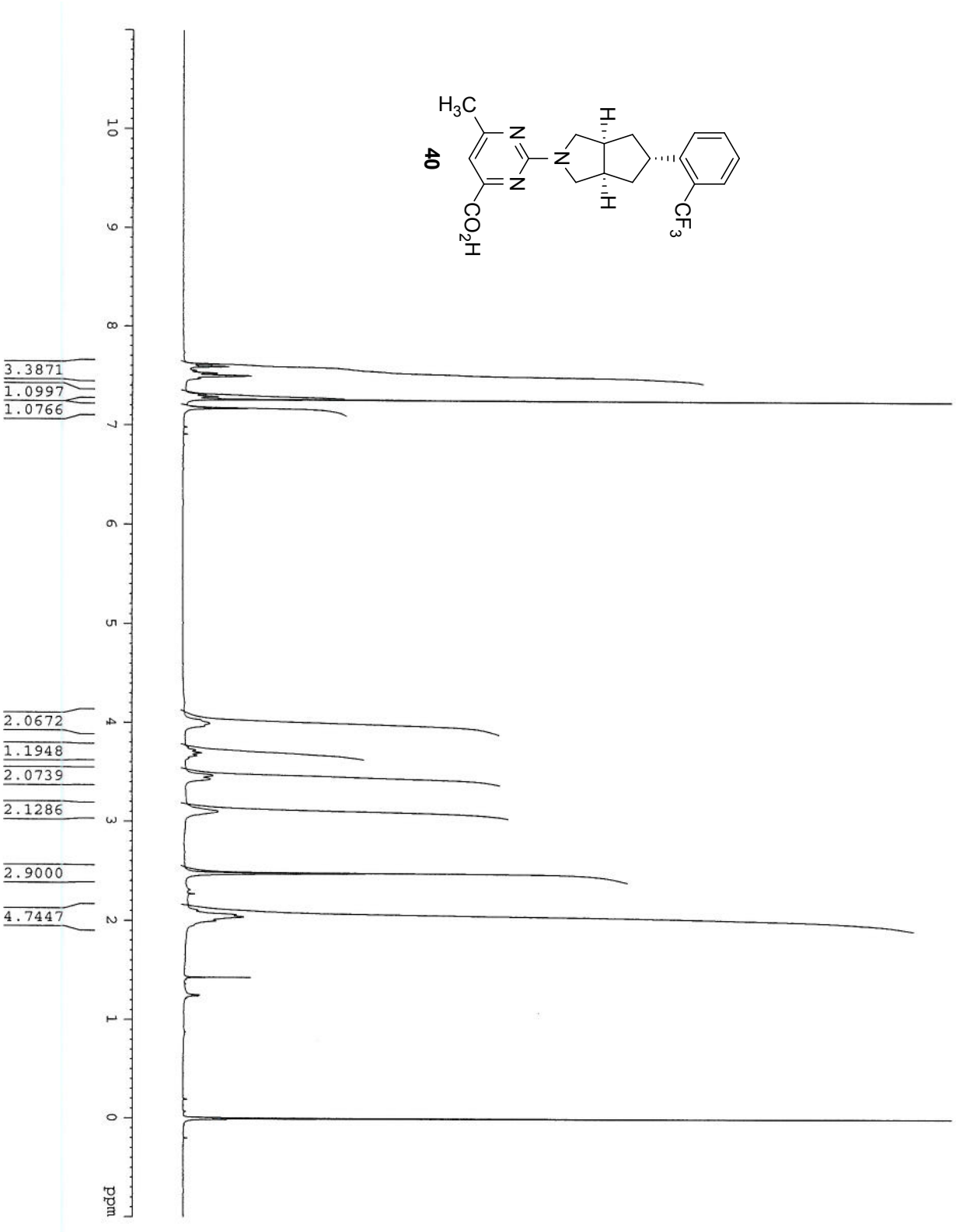
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **35**



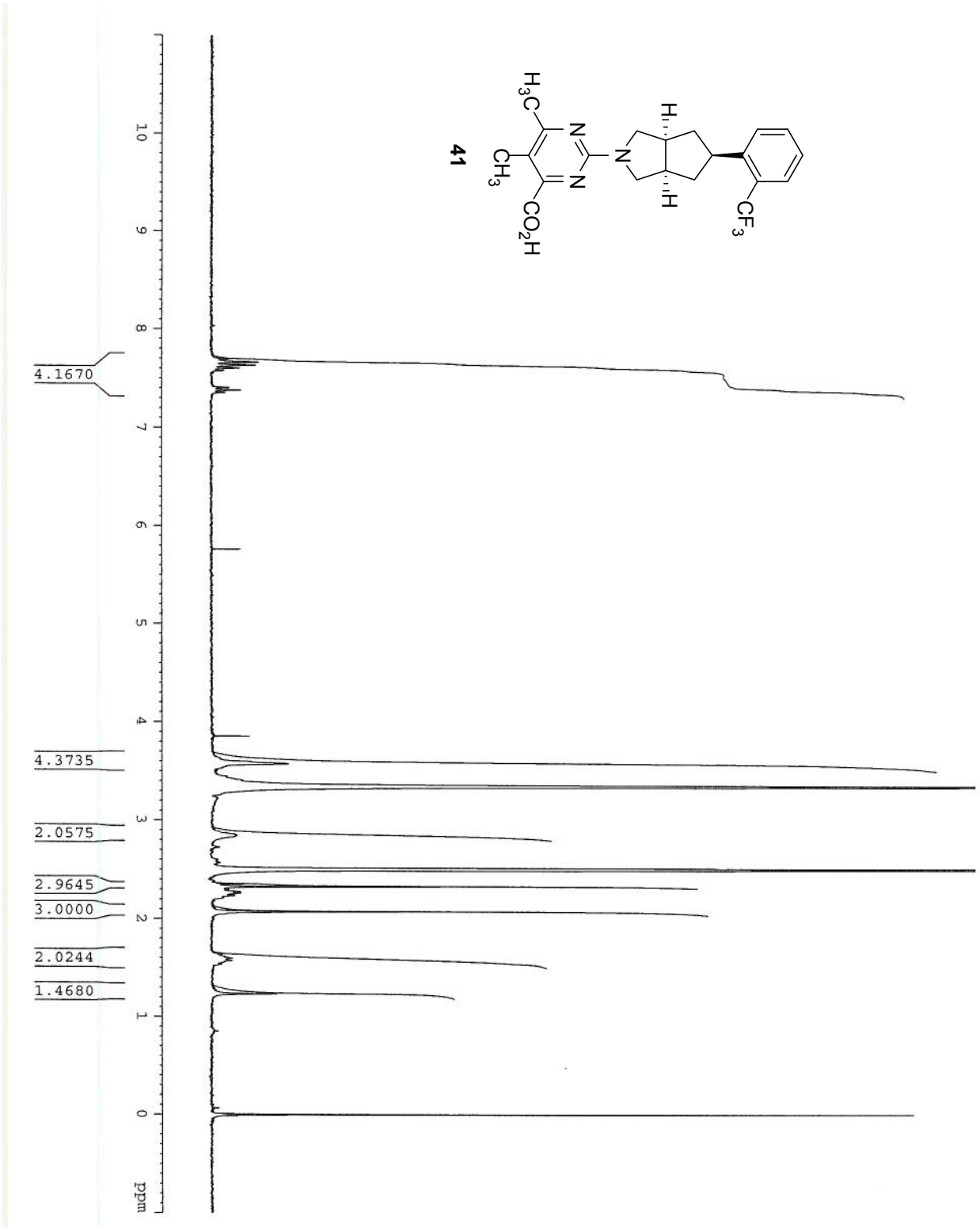
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **36**



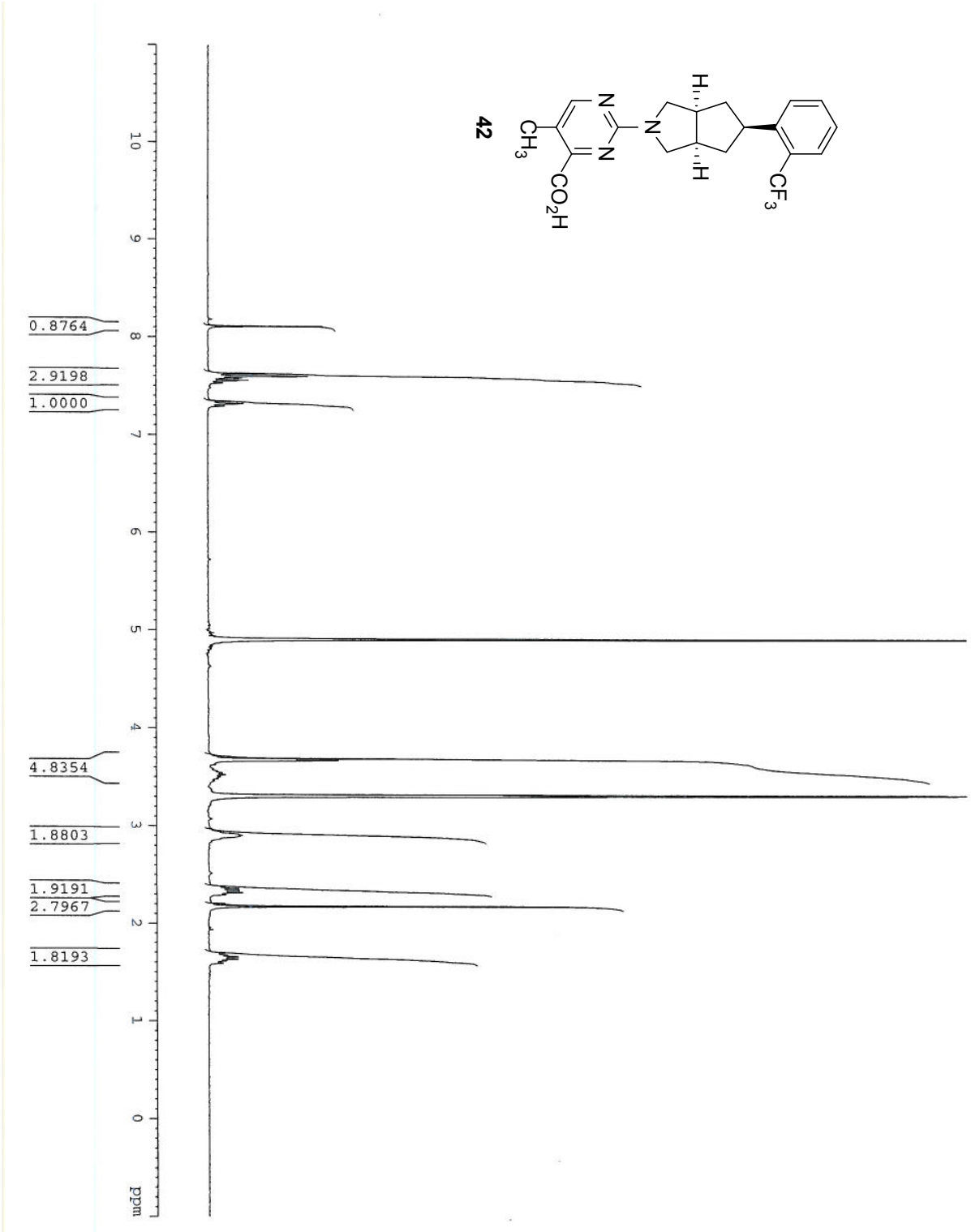
^{1}H NMR (300 MHz, $CDCl_3$): Analogue **40**



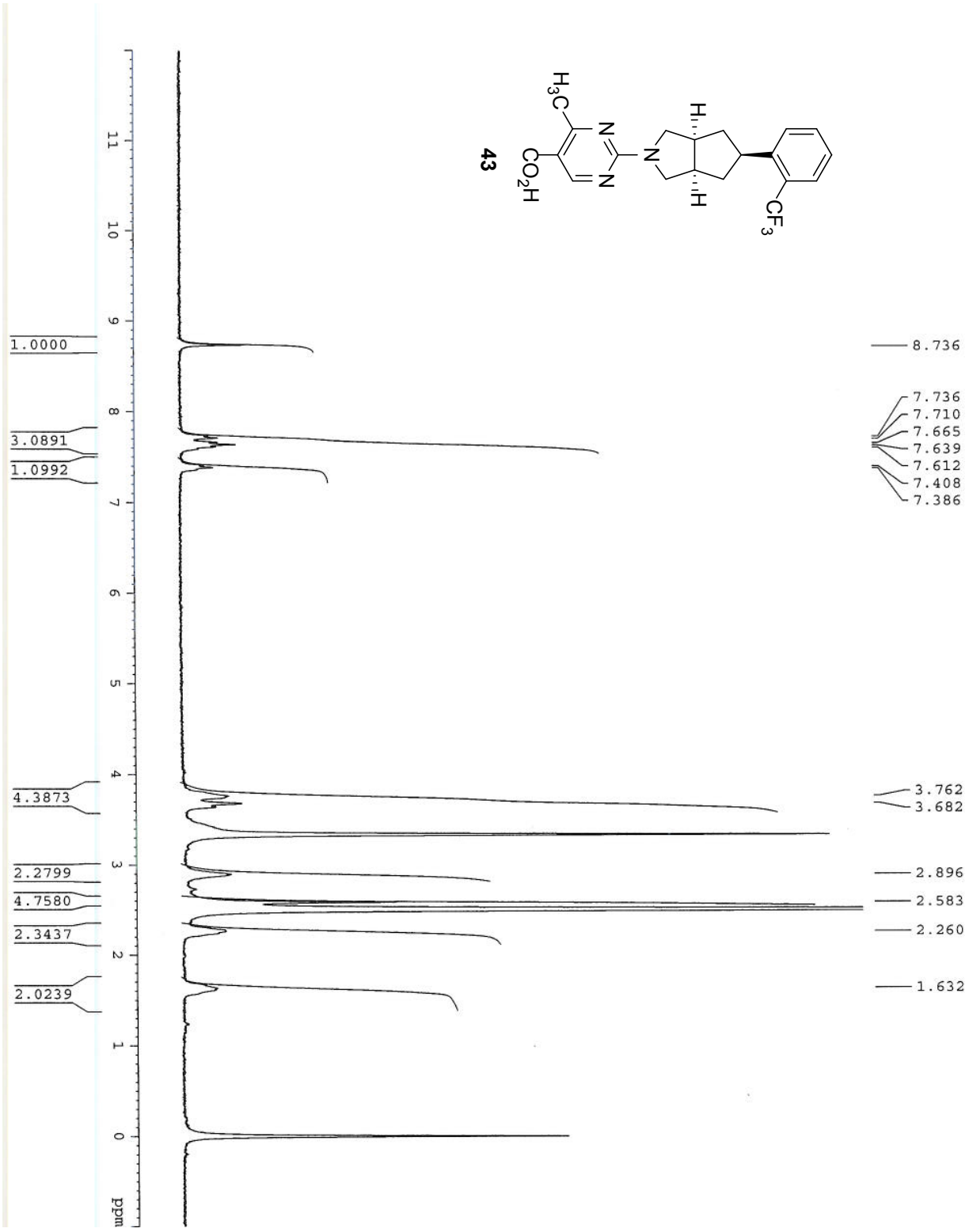
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **41**



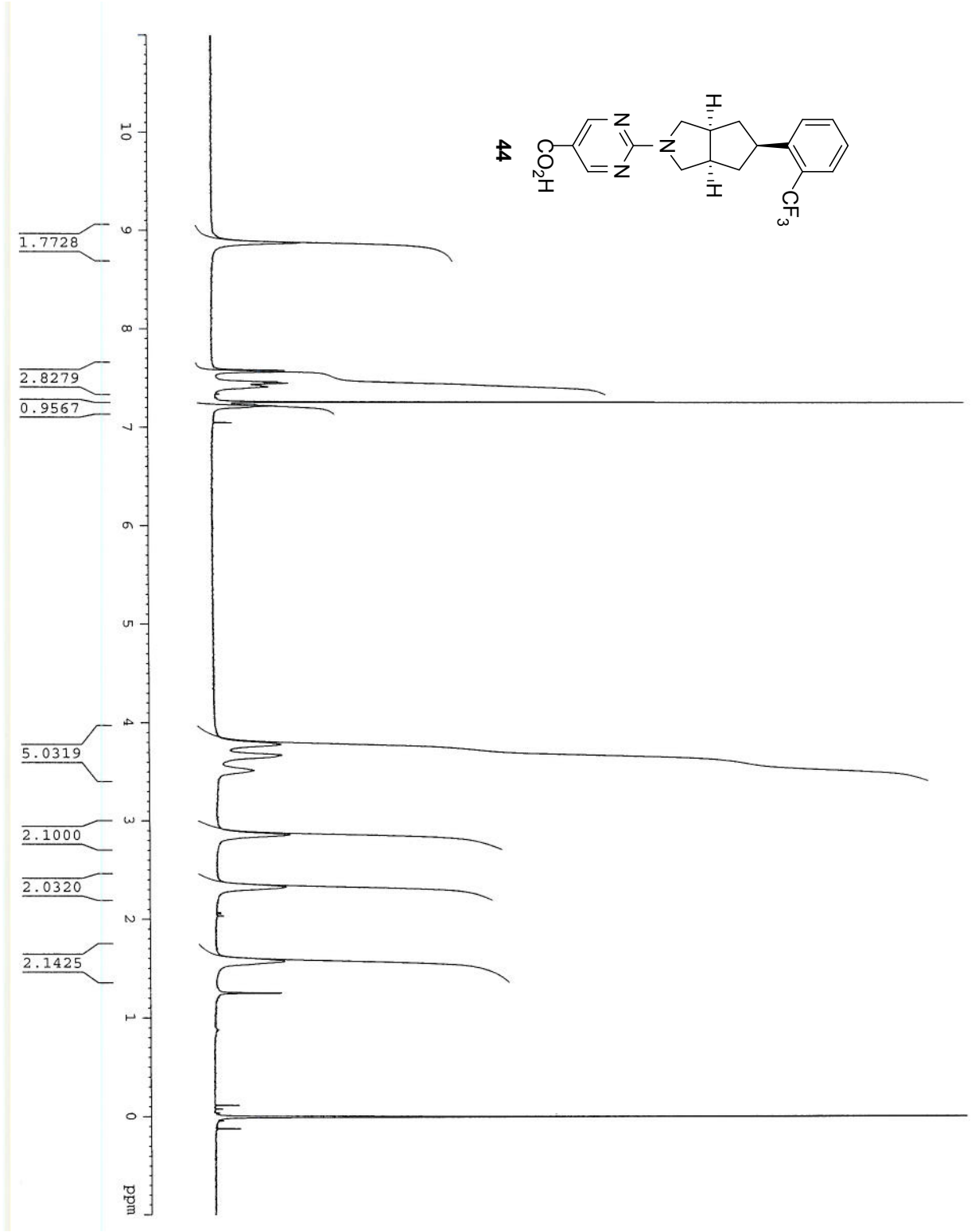
^{1}H NMR (300 MHz, CD_3OD): Analogue **42**



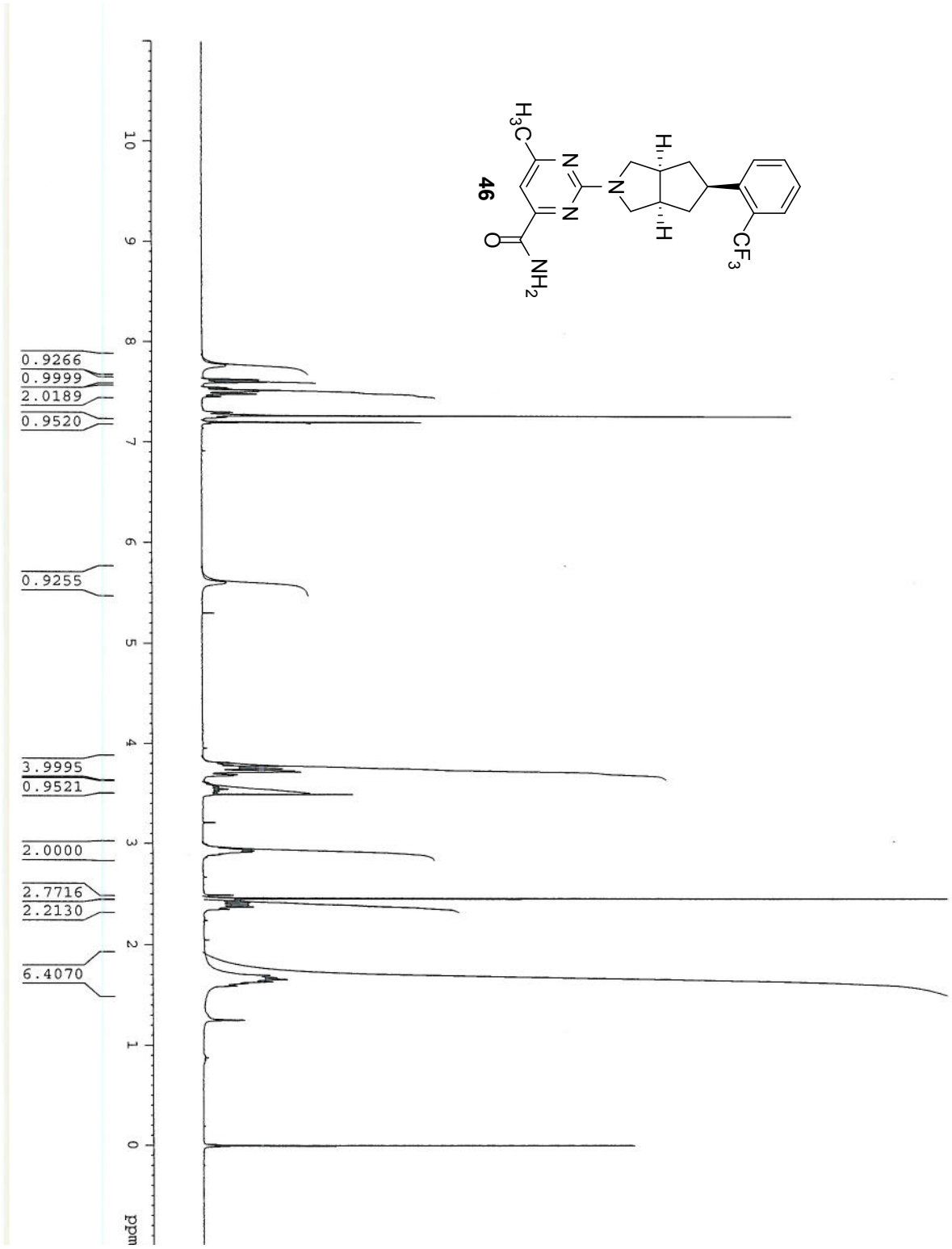
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **43**



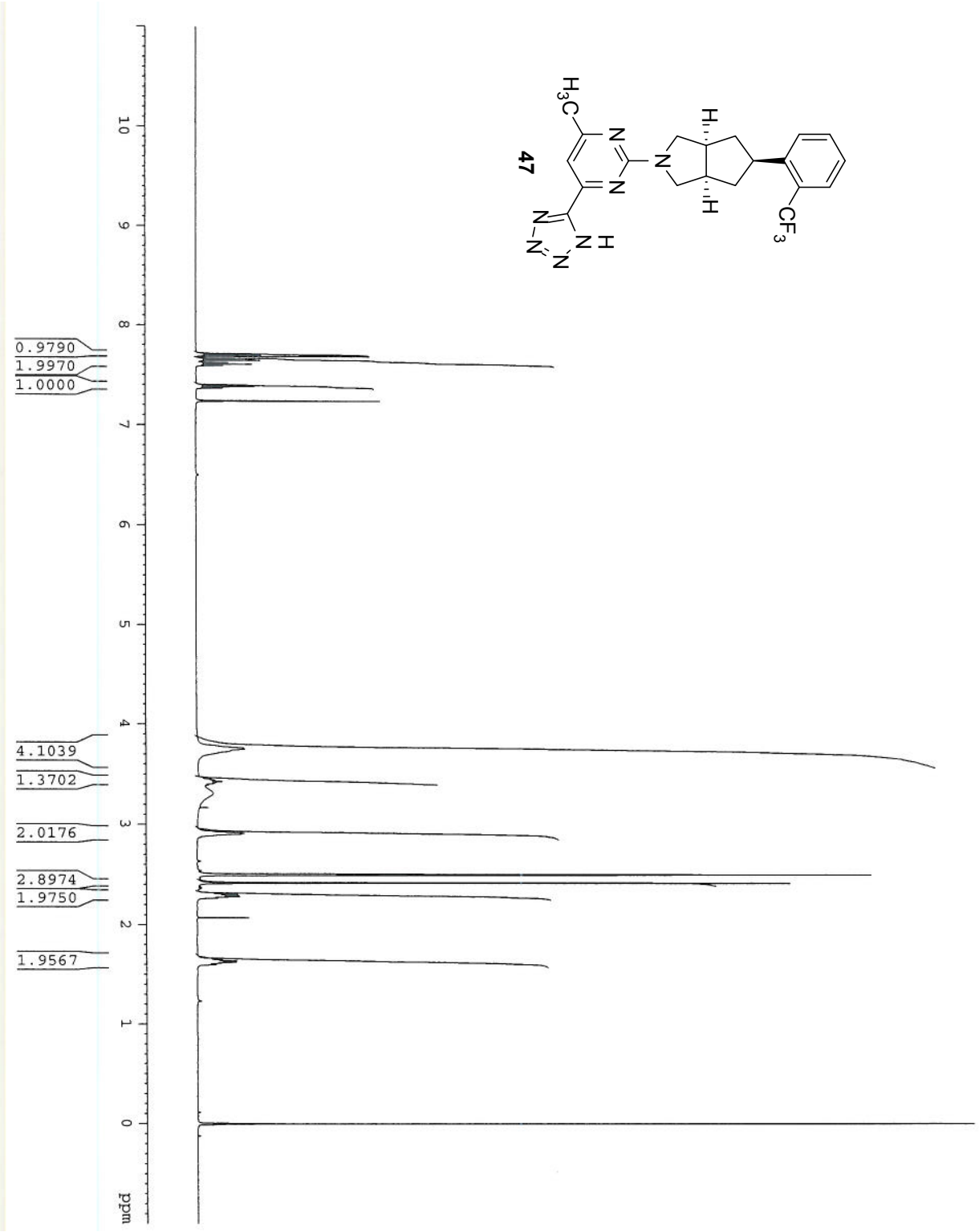
^{1}H NMR (500 MHz, $CDCl_3$): Analogue **44**



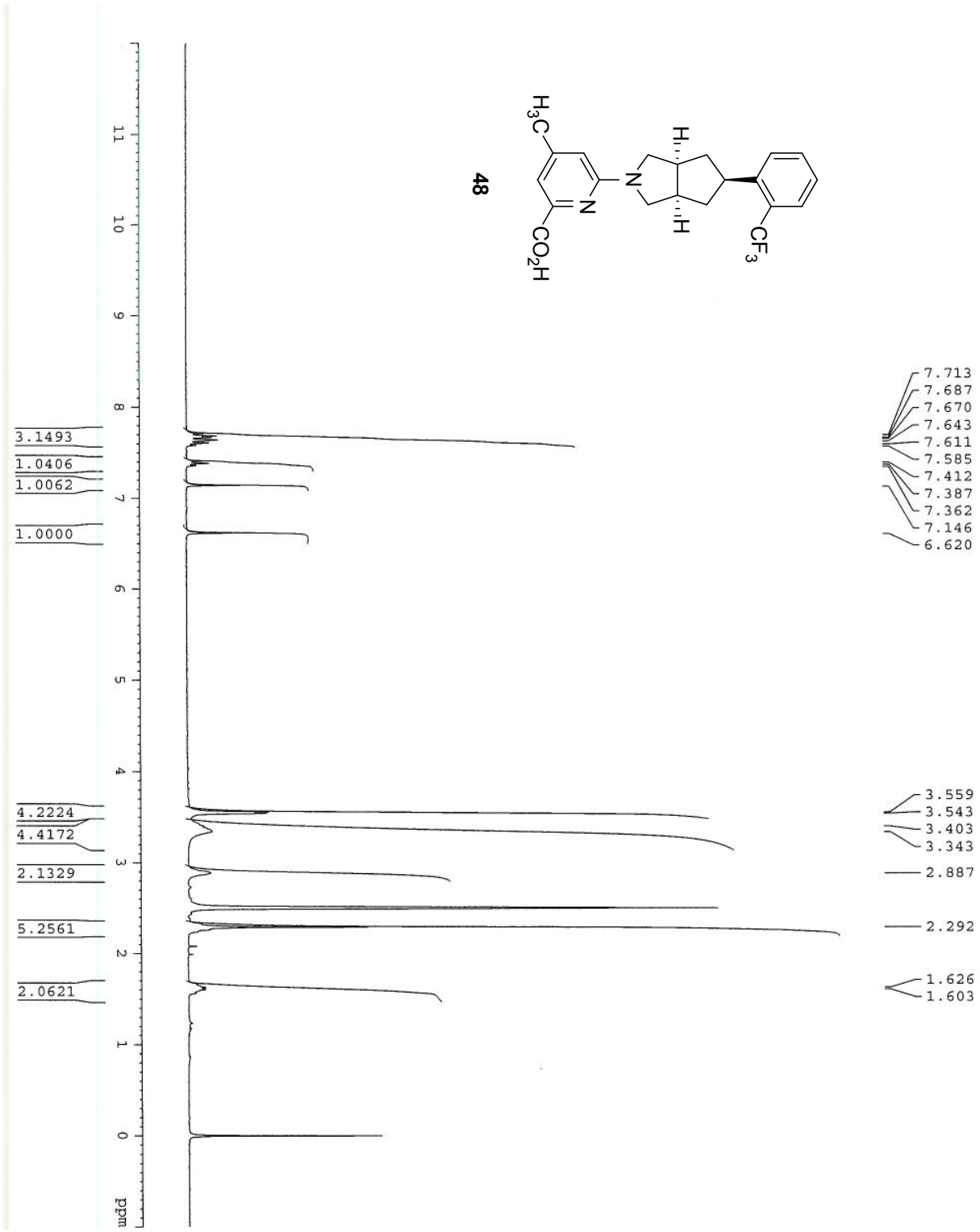
^{1}H NMR (300 MHz, $CDCl_3$): Analogue **46**



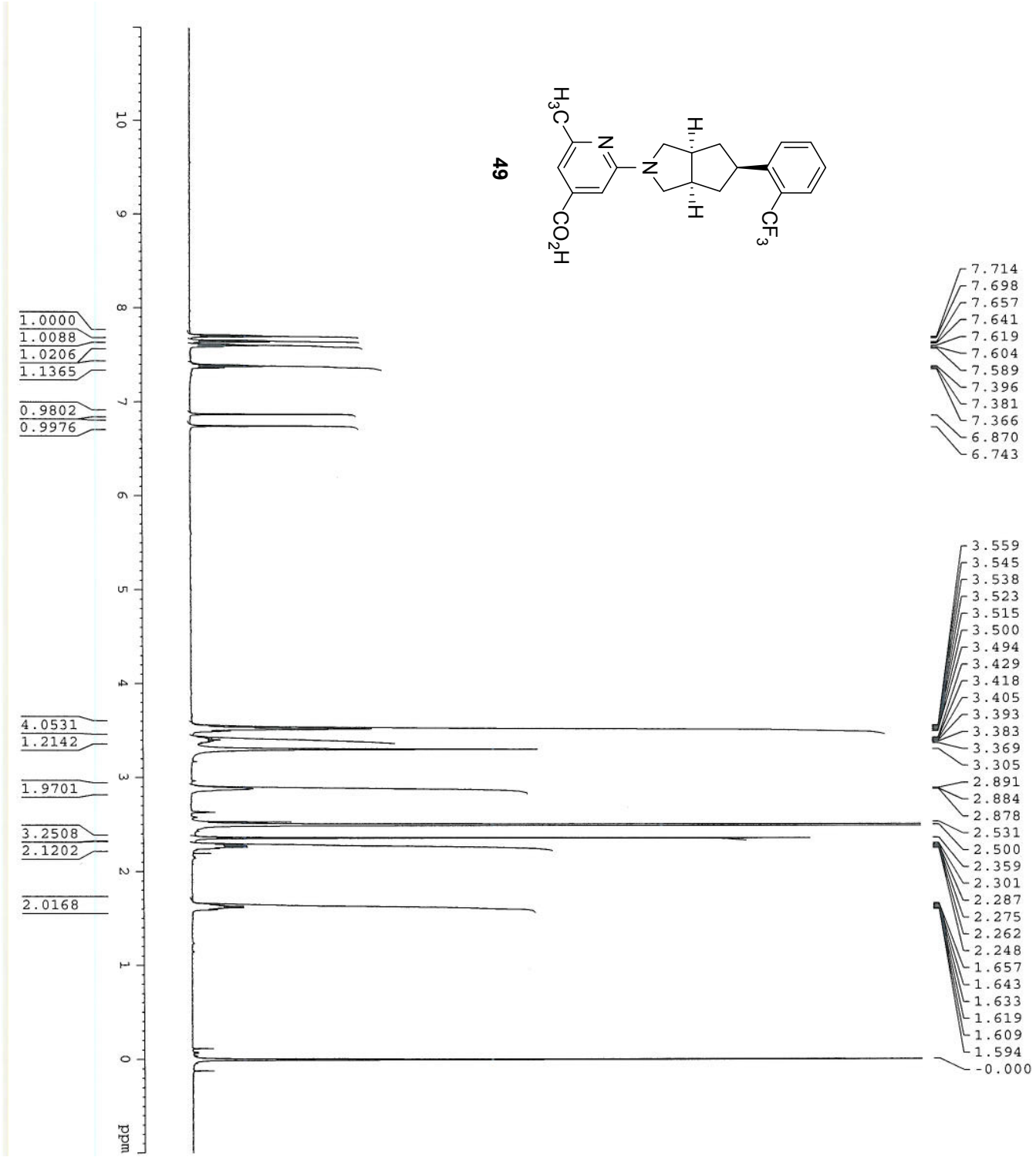
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **47**



^{1}H NMR (300 MHz, DMSO-d_6): Analogue **48**

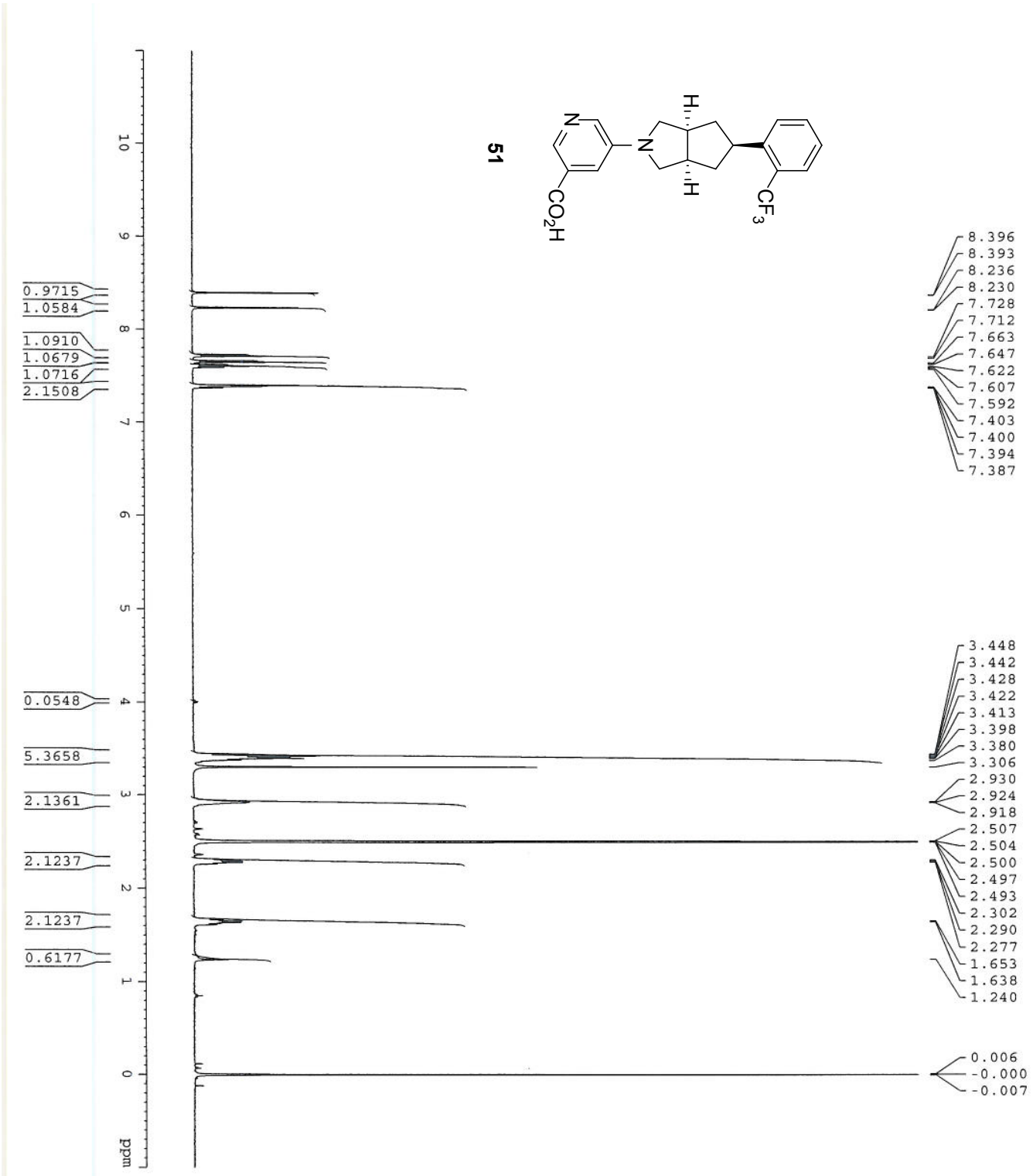


^{1}H NMR (500 MHz, DMSO-d_6): Analogue **49**

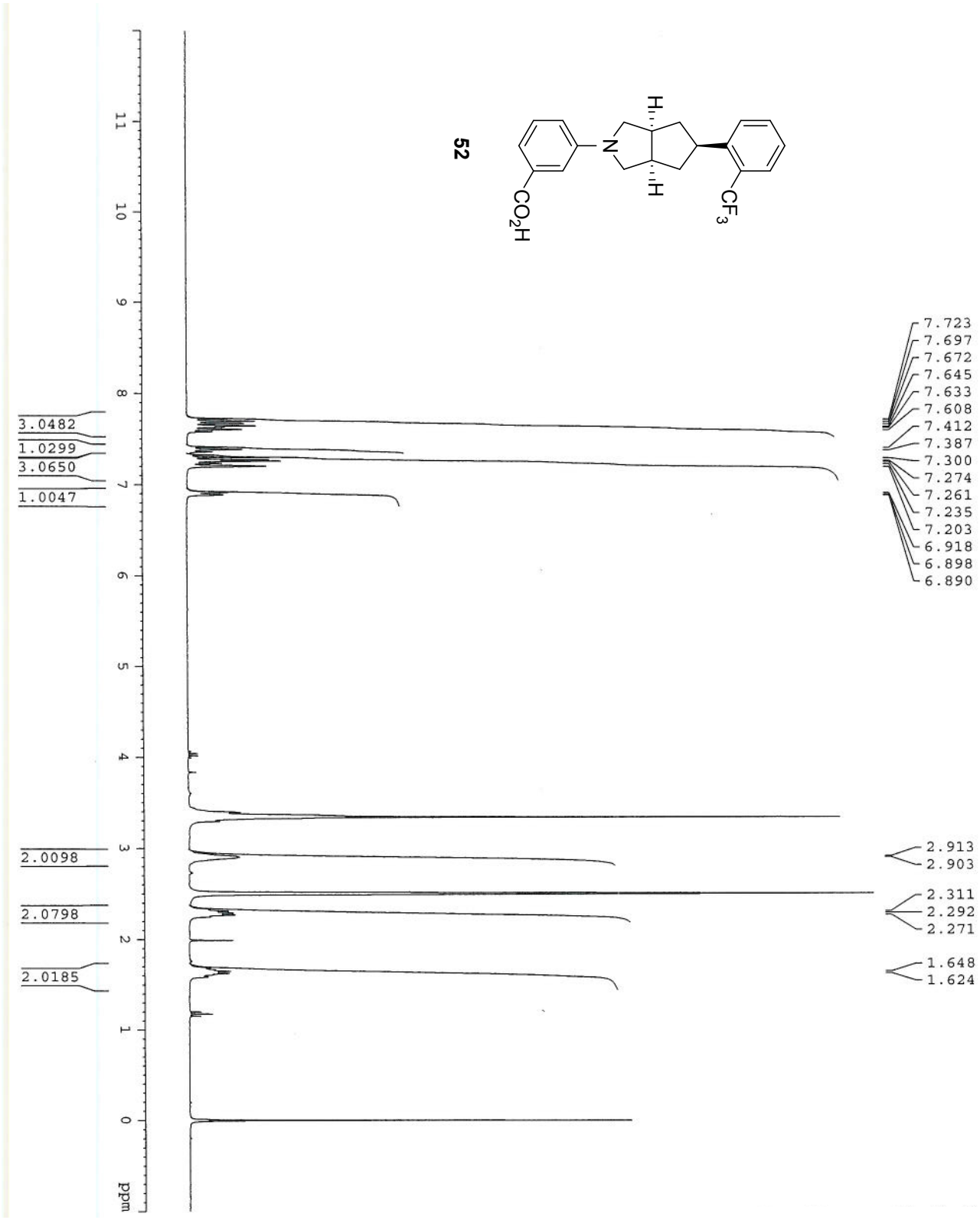


^{1}H NMR (300 MHz, $CDCl_3$): Analogue **50**

^{1}H NMR (500 MHz, DMSO-d_6): Analogue **51**



^{1}H NMR (300 MHz, DMSO-d_6): Analogue **52**



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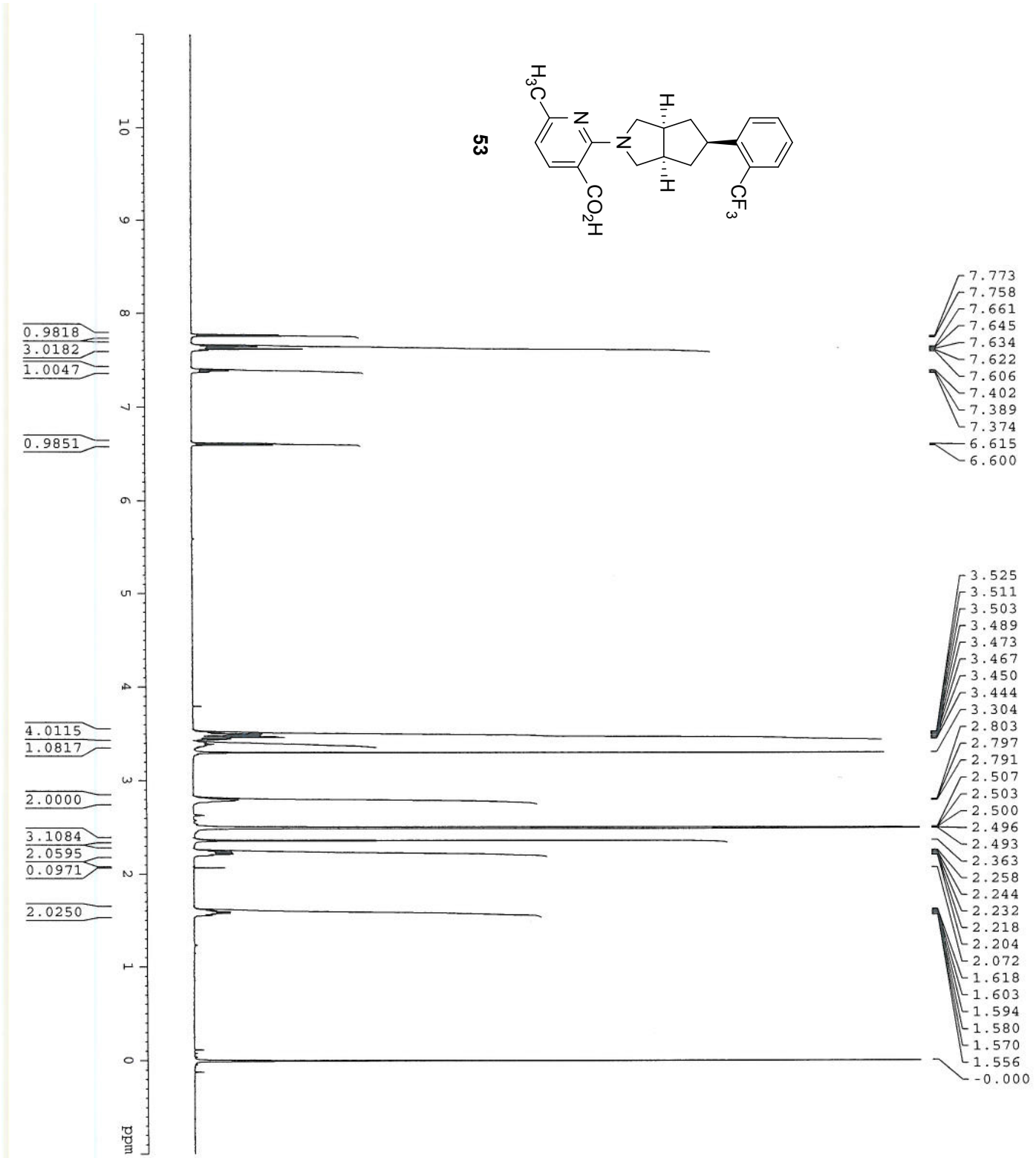
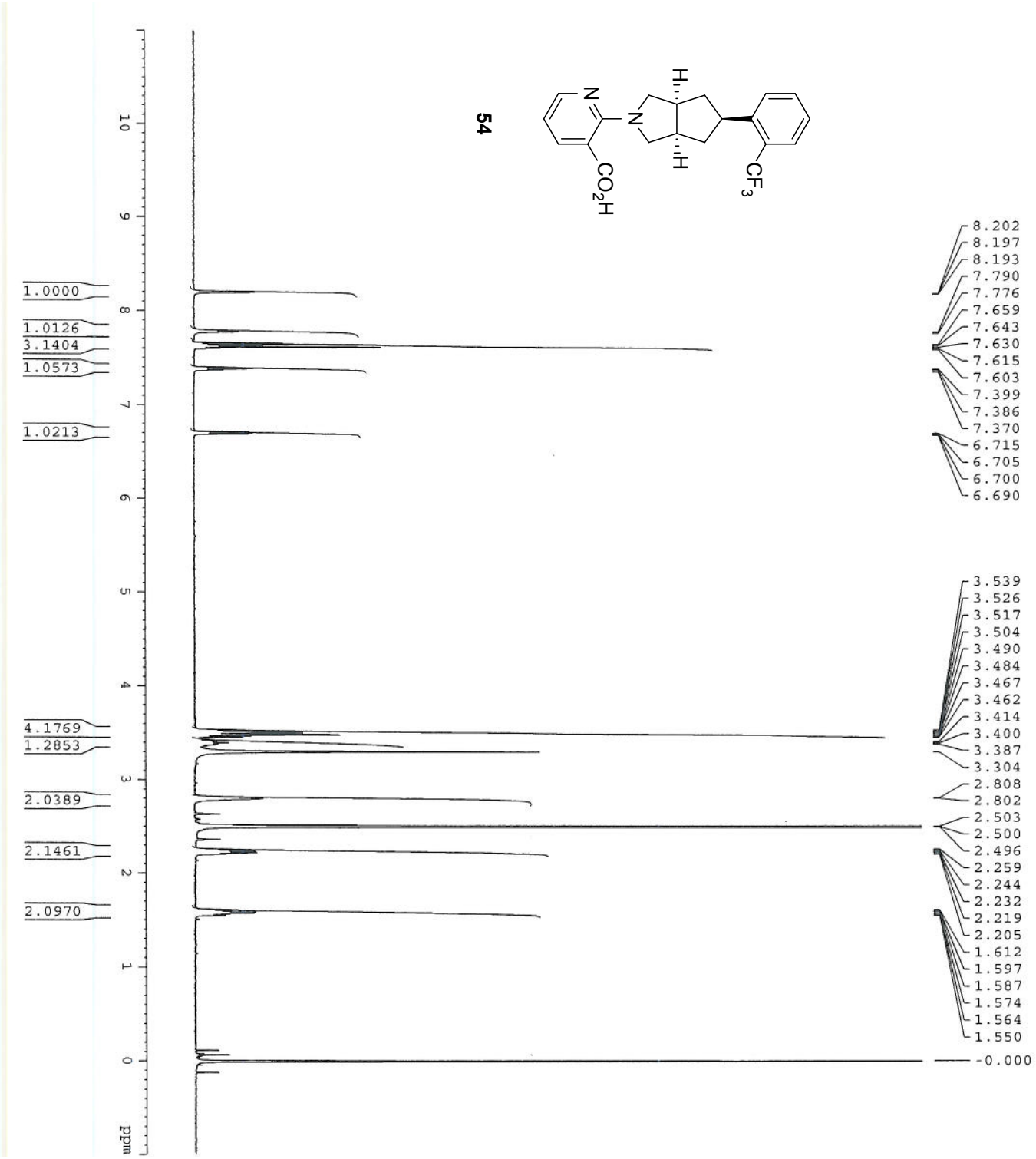
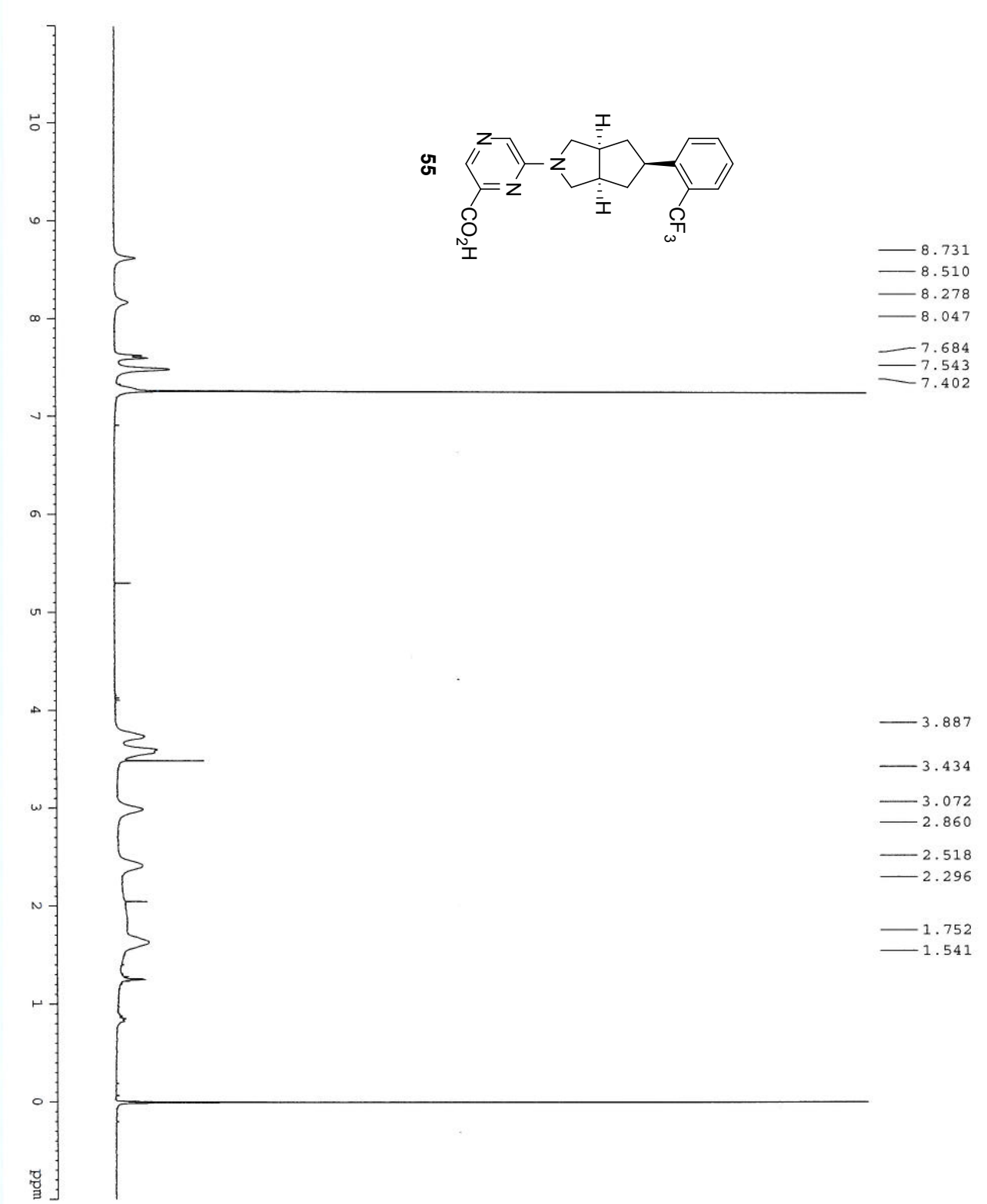


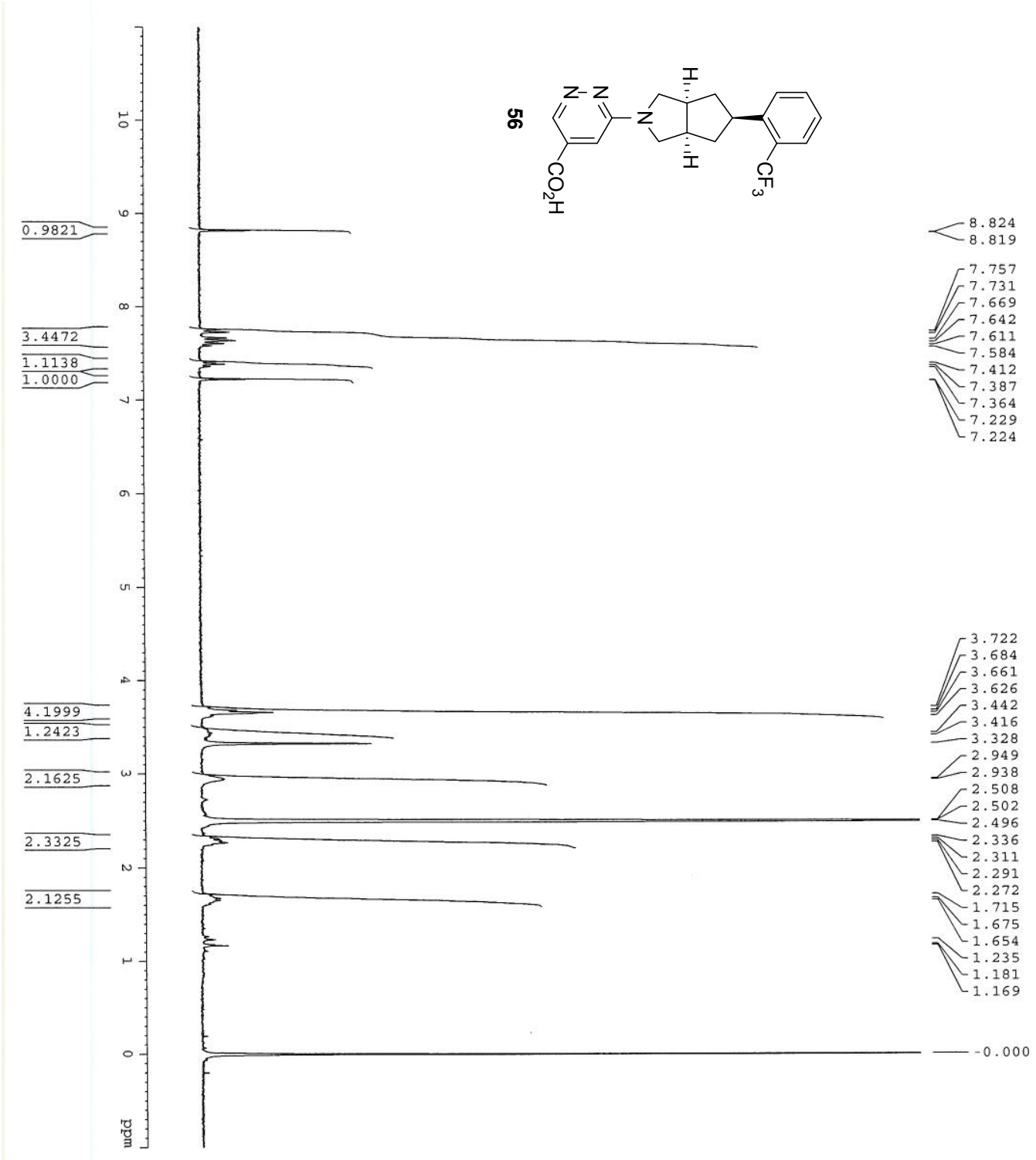
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **54**



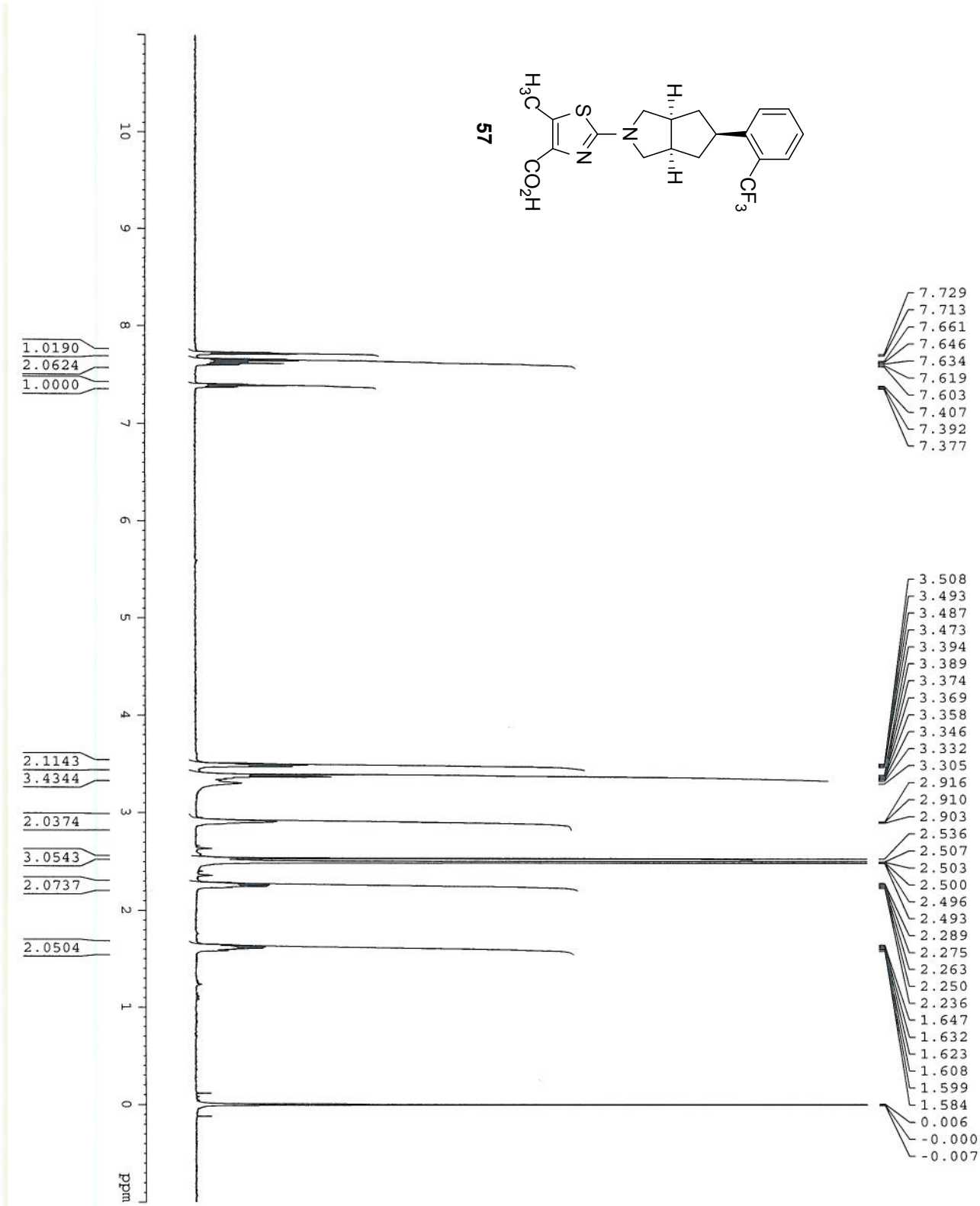
^{1}H NMR (300 MHz, $CDCl_3$): Analogue **55**



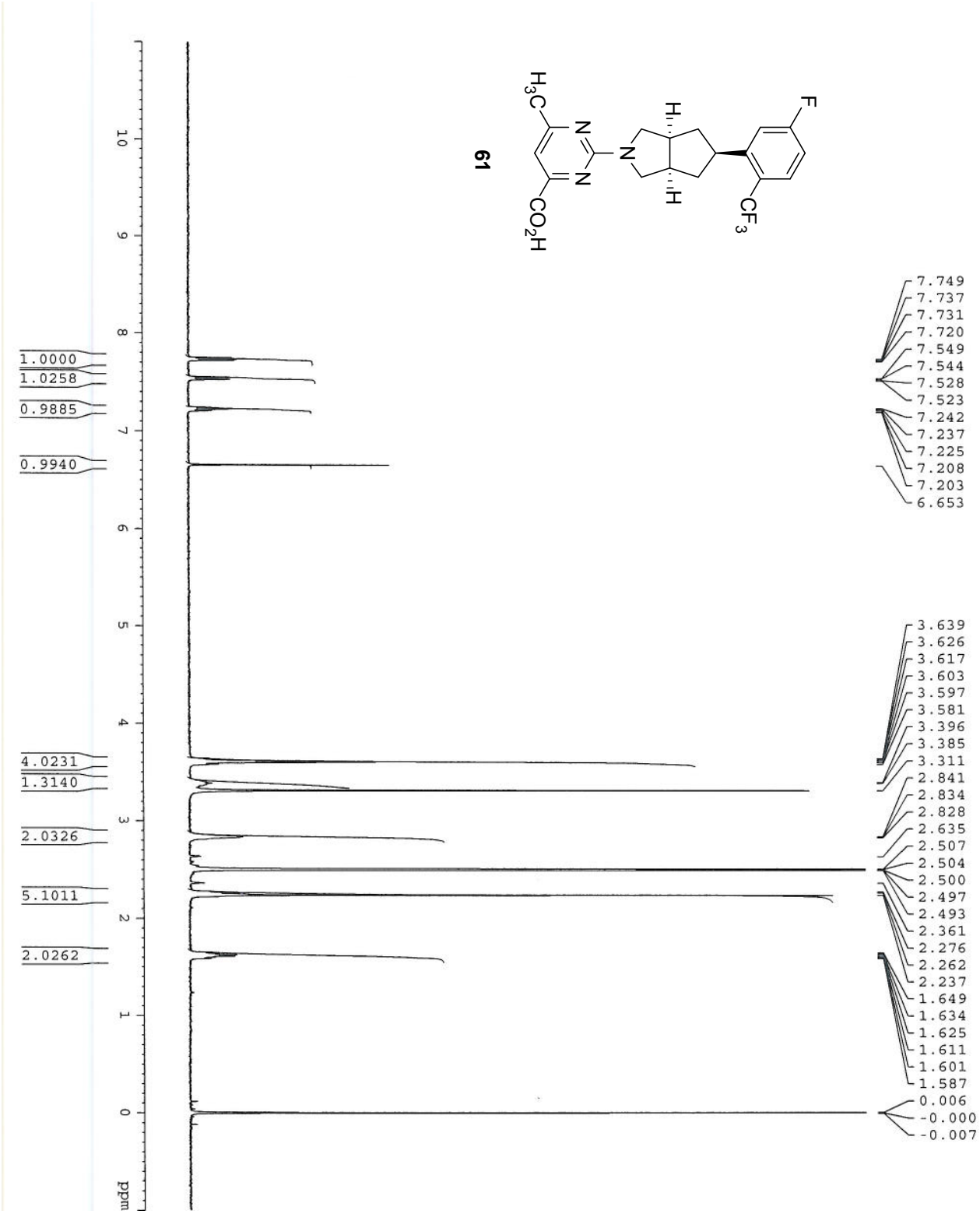
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **56**



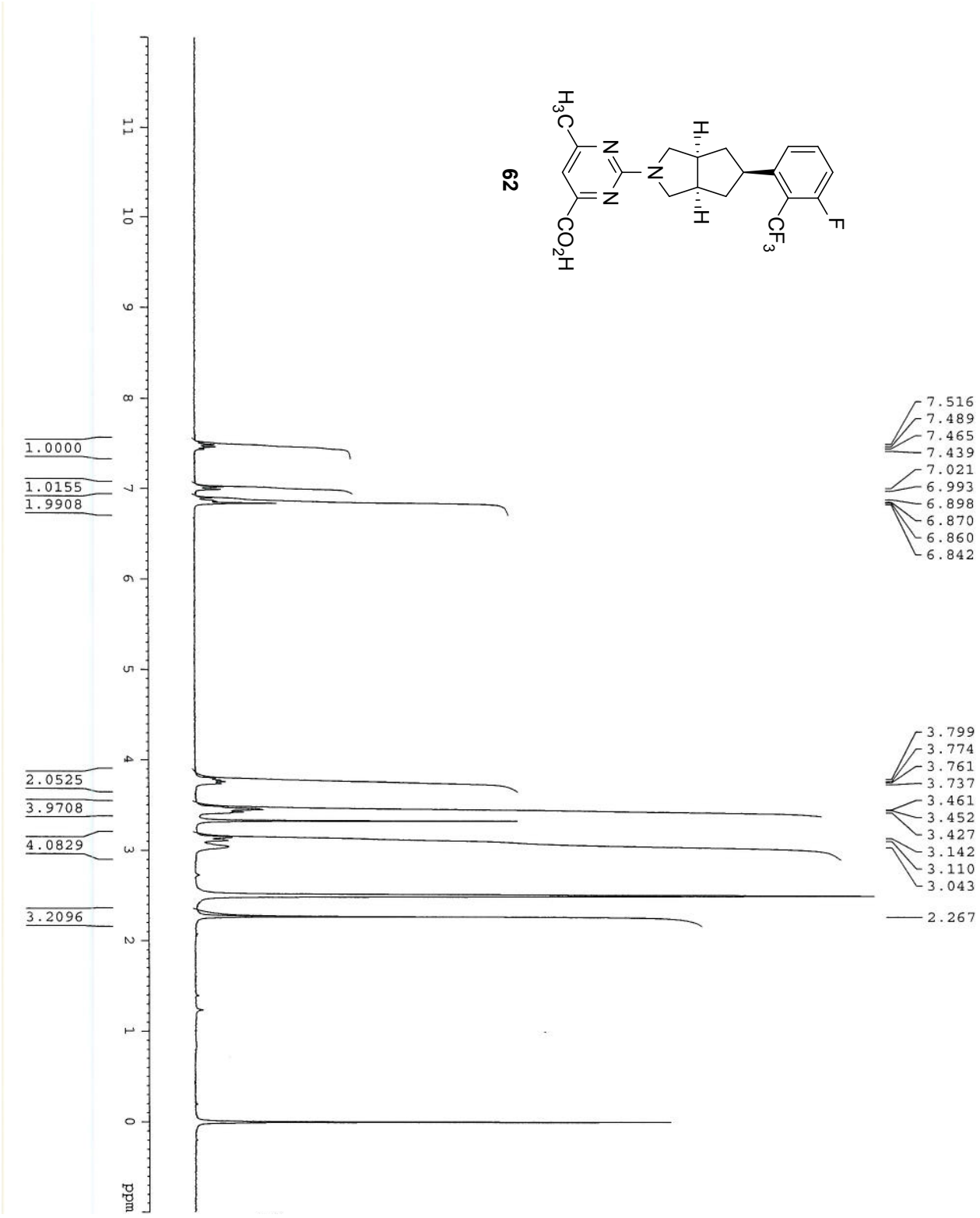
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **57**



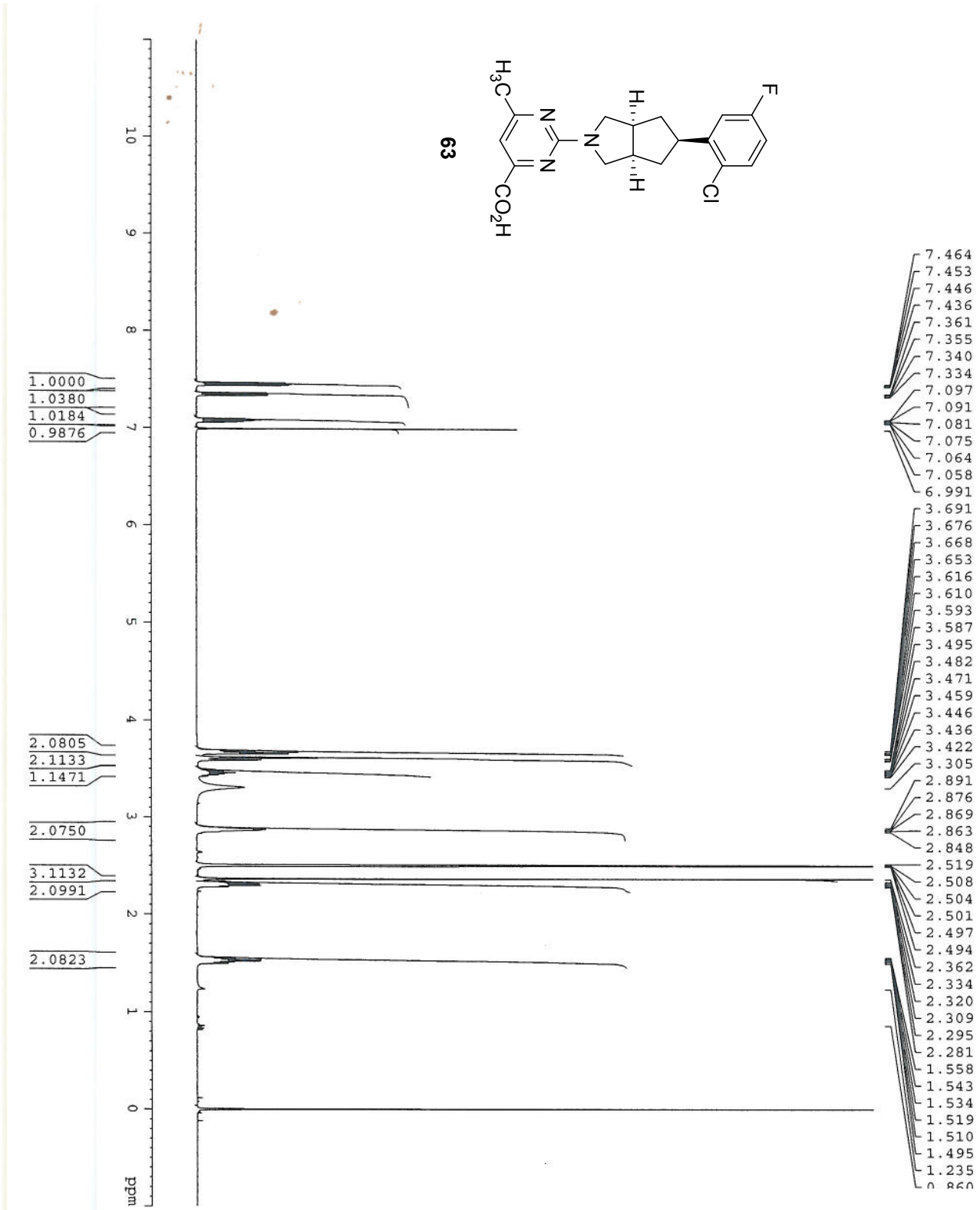
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **61**



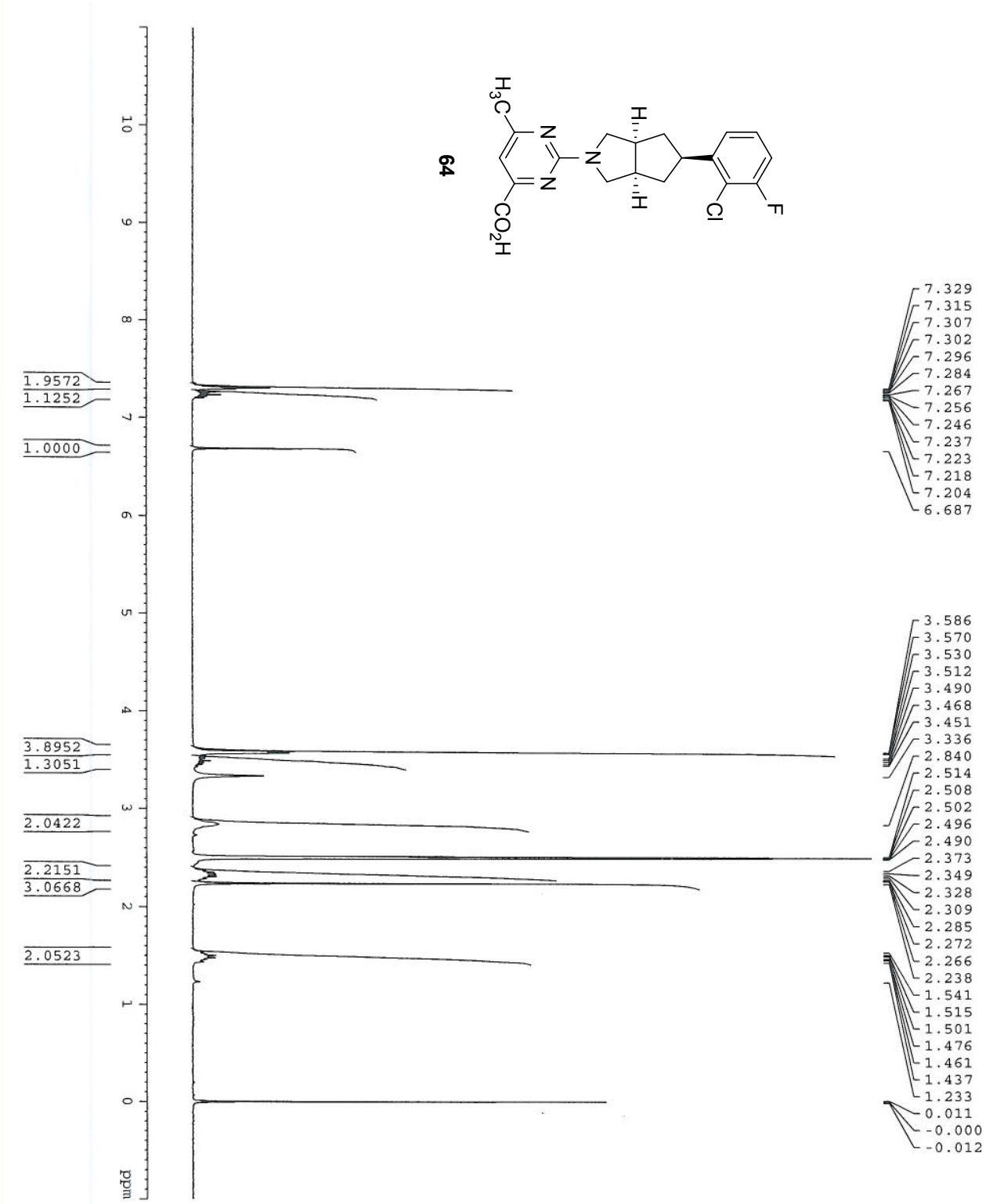
^{1}H NMR (300 MHz, DMSO-d_6): Analogue **62**



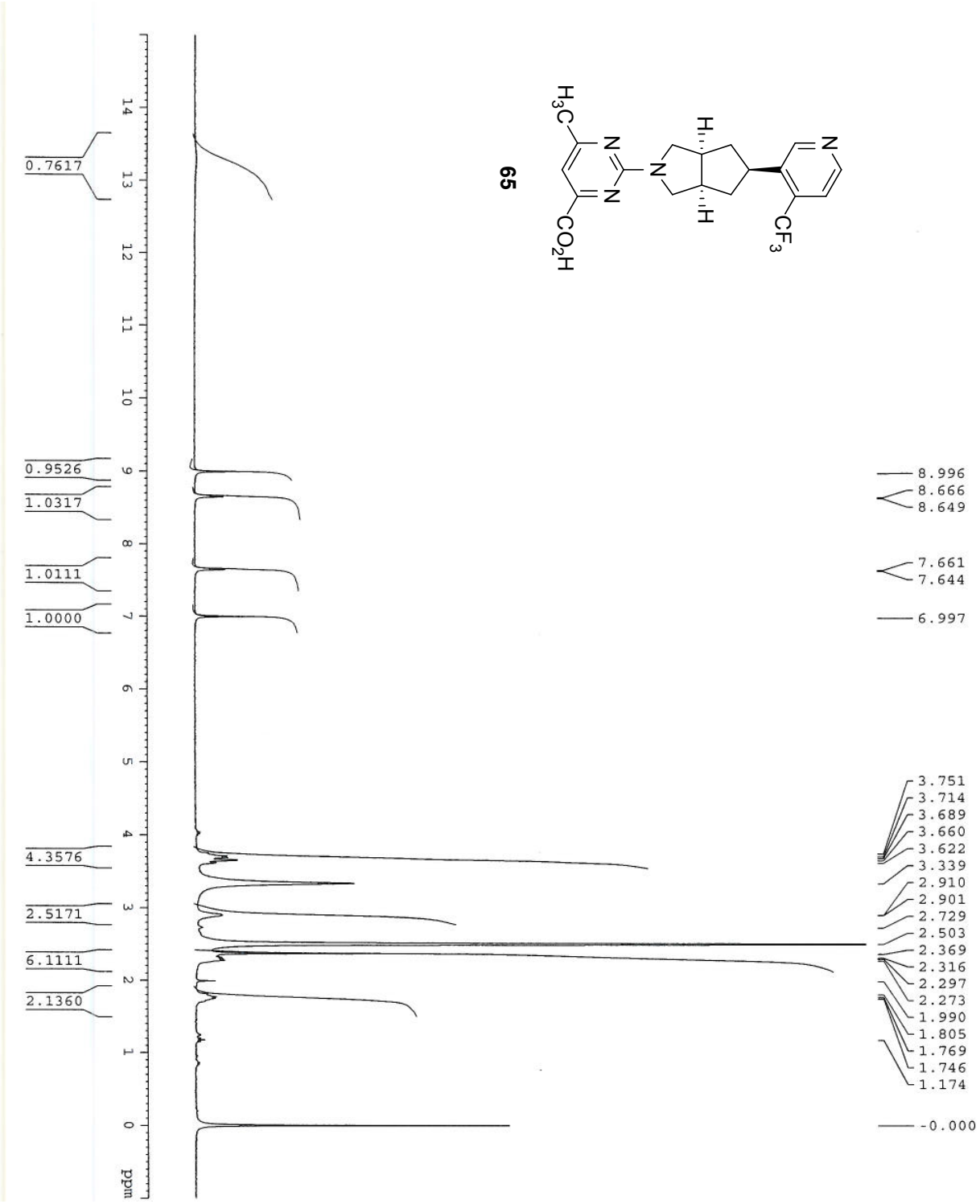
^{1}H NMR (500 MHz, DMSO-d_6): Analogue **63**



^{1}H NMR (300 MHz, DMSO-d_6): Analogue **64**



^{1}H NMR (300 MHz, DMSO-d_6): Analogue **65**



^{1}H NMR (300 MHz, DMSO-d_6): Analogue **66**