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Synthesis and Anti-norovirus Activity of Pyranobenzopyrone Compounds

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Abstract

During the last decade, noroviruses have gained media attention as the cause of large scale outbreaks of gastroenteritis on cruise ships, dormitories, nursing homes, etc. Although noroviruses do not multiply in food or water, they can cause large outbreaks because approximately 10 - 100 virions are sufficient to cause illness in a healthy adult. Recently, it was shown that the activity of acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) enzyme may be important in norovirus infection. In search of anti-noroviral agents based on the inhibition of ACAT1, we synthesized and evaluated the inhibitory activities of a class of pyranobenzopyrone molecules containing amino, pyridine, substituted quinolines, or 7,8-benzoquinoline nucleus. Three of the sixteen evaluated compounds possess ED_{50} values in the low μM range. 2-Quinolylmethyl derivative **3A** and 4-quinolylmethyl derivative **4A** showed ED_{50} values of 3.4

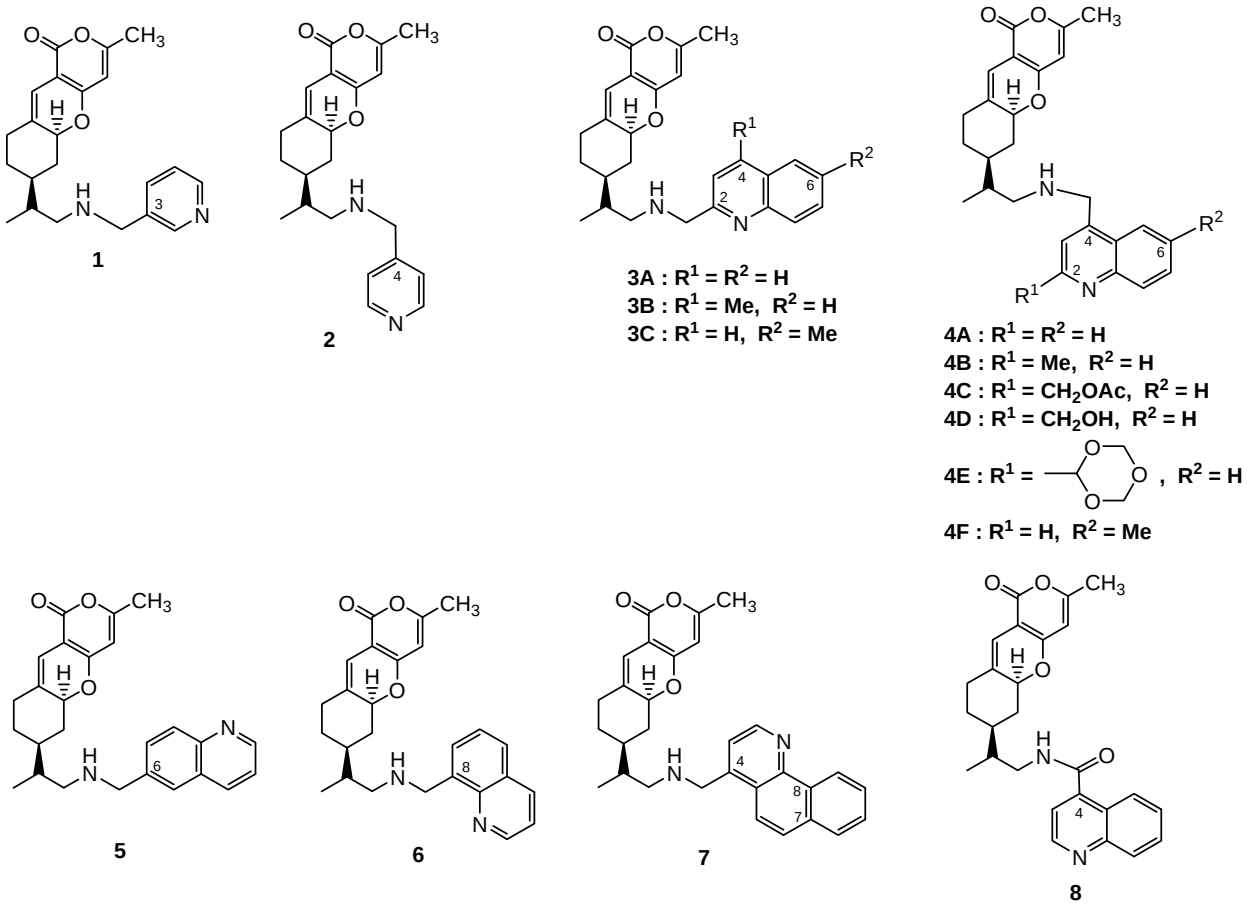
and 2.4 μM and TD_{50} values of >200 and 96.4 μM, respectively. The identified active compounds are suitable for further modification for the development of anti-norovirus agents.

Key words: acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1), caliciviruses, norovirus, anti-noroviruses, pyranobenzopyrones.

Noroviruses, category B bioterrorism agents, are the leading cause of food- or water-borne gastroenteritis outbreaks. Studies have shown that noroviruses are responsible for approximate 90% of epidemic non-bacterial food-water-borne gastroenteritis outbreaks with an estimated 23 million cases annually in the US causing 50,000 hospitalizations and 300 deaths.[1-3] Norovirus belongs to the *Caliciviridae* family, and may infect an individual with as few as 10 viral particles. Outbreaks often occur in closed environments such as dormitories, cruise ships, hospitals, and care facilities. Caliciviruses (Family *Caliciviridae*) are small, non-enveloped RNA viruses of 27-35 nm in diameter. They possess a single-stranded, plus-sense genomic RNA of 7-8 kb, which encodes a nonstructural polyprotein, a major structural capsid protein of 58-80 kD (VP1), and a small basic protein (VP2).[4] Currently, there are no specific drugs for norovirus infection. Vaccine development for human noroviruses have faced challenges because noroviruses do not grow in cell culture, show high diversity, and immunity from heterologous strains do not seem to confer protection.[5] Furthermore, repeat infections in adults indicate that long-term immunity may be absent. Consequently there is an urgent need for the development of effective anti-noroviral drugs. The recent development of replicon-harboring cells for norovirus[6,7] has made possible the study of norovirus replication in cells. DNA microarray analysis of norovirus replicon-harboring cells (HG23) by an Affymetrix Gene Chip showed the

up regulations of acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) and other cholesterol modulating genes (> ±1.5 fold).[9] Moreover, commercially available ACAT inhibitors were shown to inhibit norovirus replication.[9] These findings suggest that cellular ACAT may be a potential therapeutic target for norovirus infection. Previously, we reported a class of pyranobenzopyrones that possesses ACAT inhibitory activity.[10] Hence, anti-norovirus activities of pyranobenzopyrones were investigated. Herein, we report the synthesis and anti-norovirus activity of a small library of sixteen pyranobenzopyrones containing amino, pyridine, quinoline, and 7,8-benzoquinoline nucleus (Figure 1). Among the pyranobenzopyrones, several compounds possess anti-norovirus activity in low micromolar concentrations *in vitro* with therapeutic index values of $\geq$ 40.

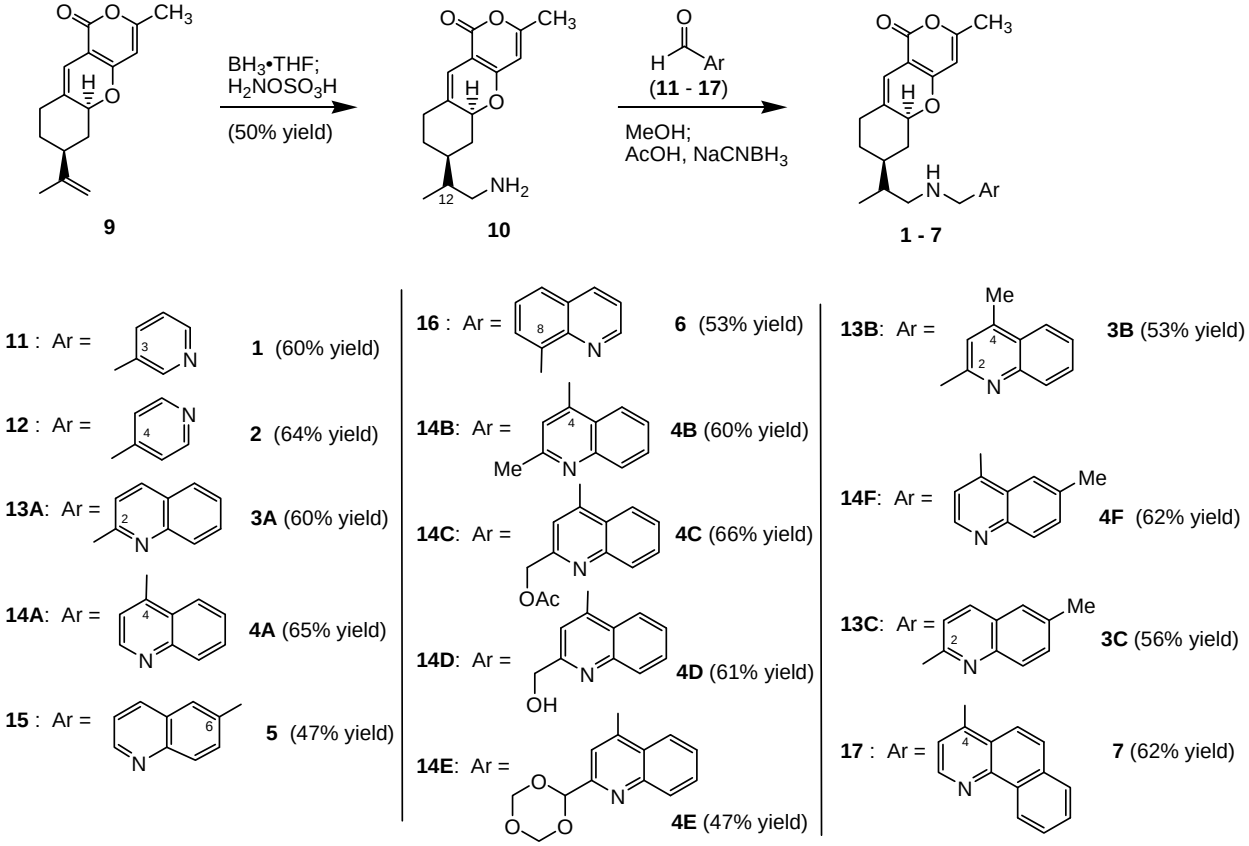
Figure 1. Synthesized and Bioevaluated Pyranobenzopyran Compounds 1 - 8.



The synthesis of pyranobenzopyrones **1** – **7** stems from a reductive amination reaction[11] of amine **10** and various aldehydes, **11** – **17** as depicted in Scheme 1. Initially, amine **10** was prepared via a four-step sequence of reactions starting from pyranobenzopyrones **9** by hydroboration-hydroxylation reaction followed by mesylation, displacement with sodium azide, and reduction with H_2/Pd.[10] The synthesis was simplified by a one-pot hydroboration-amination reaction of **9** with BH_3•THF followed by hydroxylamine-O-sulfonic acid[12] in 50% yield (Scheme 1). A mixture of two diastereomers in a ratio of 1:1 resulted at the newly created carbon center C12 from the hydroboration reaction indicated by its ^{1}H and ^{13}C NMR spectra. The diastereomers are separable by HPLC but not silica gel column chromatograph. Alkylation of

amine **10** with 1 equivalent each of aldehydes **11** – **17** separately in methanol followed by sodium cyanoborohydride afforded amines **1** – **7**, respectively. Yields of compounds **1** - **7** range from 47 – 66%. Pyridinecarboxaldehydes, various substituted quinolinecarboxaldehydes, and 7,8-benzoquinoline-4-carboxaldehyde (**17** or 4-azaphenanthrene-1-carboxaldehyde) were used in the reductive amination reaction, and functional groups such as primary alcohol, ester, and trioxane are stable under the reaction conditions.

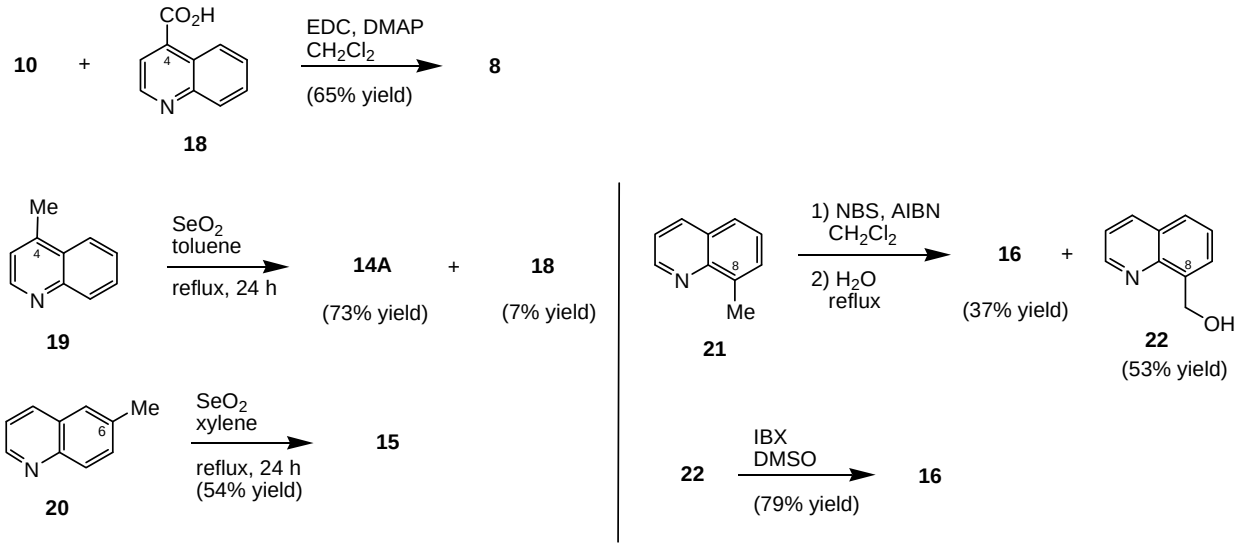
Scheme 1. Synthesis of Compounds 1 - 7.



Amide **8** was synthesized from the coupling reaction of quinoline-4-carboxylic acid (**18**), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and amine **10** in 65% yield (Scheme 2). Aldehydes **11**, **12**, and **13A** and carboxylic acid **18** along with various methylquinolines were

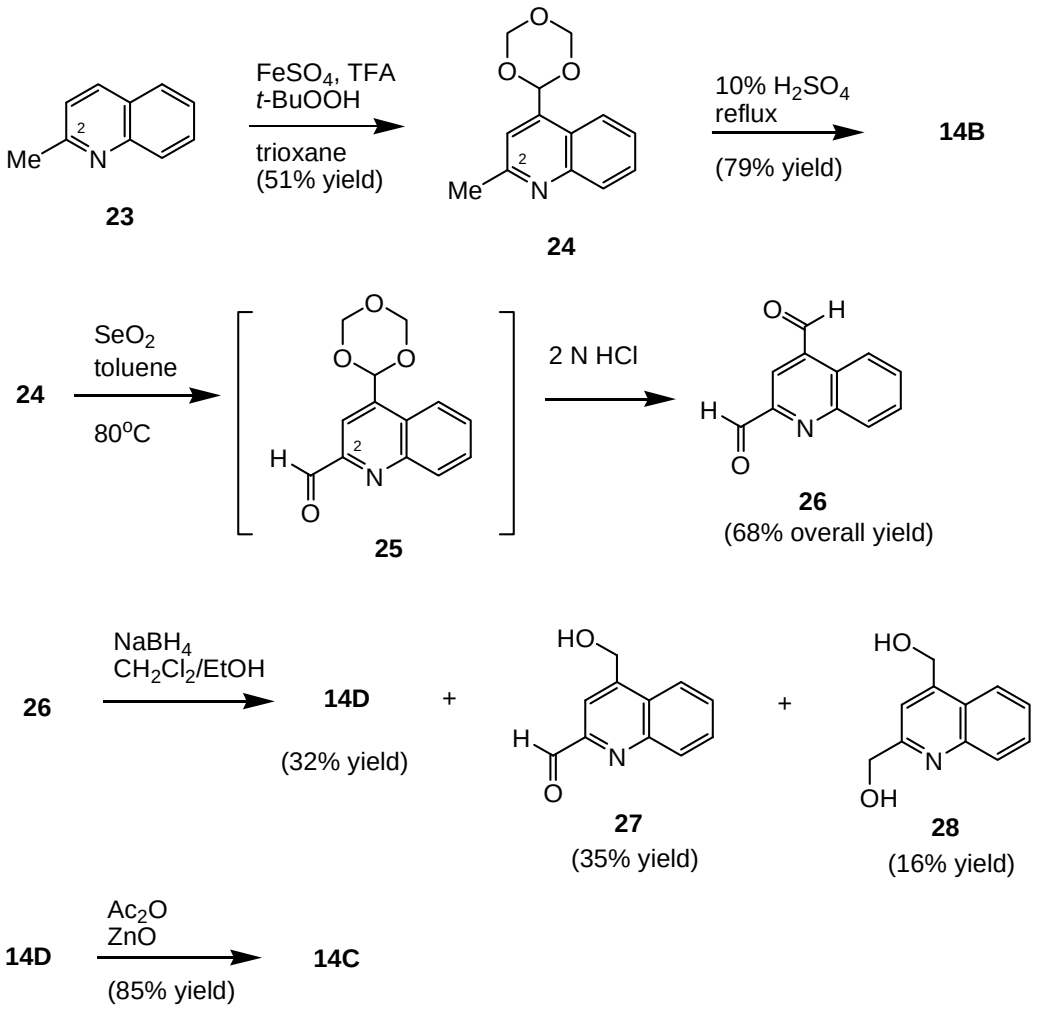
obtained from commercial sources. Aldehydes **14A**, **15**, and **16** were prepared by benzylic oxidation with selenium dioxide[13] or bromination followed by hydrolysis/oxidation[14] of methylquinolines, and aldehydes **13B**, **13C**, **14B - 14F**, and **17** were achieved from free-radical heteroaromatic trioxanylation[15,16] with trioxane-*t*-BuOOH-ferrous sulfate (Schemes 2 – 4). Hence, oxidation of 4-methylquinoline (**19**) with SeO_2 under refluxing toluene gave quinoline-4-carboxaldehyde (**14A**) in 73% yield along with a small amount of over oxidized carboxylic acid **18** (Scheme 2). Similarly, quinoline-6-carboxaldehyde (**15**) was obtained from 6-methylquinoline (**20**) in 54% yield after the treatment with SeO_2 in refluxing xylene. To our surprise, oxidation of 8-methylquinoline (**21**) under similar reaction conditions provided only a trace amount of 8-quinoline-carboxaldehyde (**16**). Apparently, methyl group appended on ring A of quinoline is activated toward oxidation, but methyl group on ring B is not, and a sluggish oxidation resulted. To overcome the problem, benzylic bromination of **21** was carried out. Treatment of **21** with *N*-bromosuccinimide (NBS) and a catalytic amount of azobisisobutyronitrile (AIBN) followed by aqueous hydrolysis accompanying air oxidation gave aldehyde **16** (37% yield)[14] along with the hydrolyzed product, 6-hydroxymethylquinoline (**22**) (53% yield). Oxidation of alcohol **22** with *o*-iodoxybenzoic acid (IBX) and DMSO[17] furnished **16** in a 79% yield.

Scheme 2. Syntheses of Compound 8 and quinolinecarboxaldehydes 14A, 15, and 16.



Substituted quinolinecarboxaldehydes **13B**, **13C**, **14B** - **14F**, and 7,8-benzoquinolinecarboxaldehyde (**17**) were obtained from heteroaromatic trioxanylation reactions (Schemes 3 and 4).[15,16] Treatment of 2-methylquinoline (**23**) with trioxane, *t*-butyl hydroperoxide and trifluoroacetic acid (TFA) in the presence of a catalytic amount of ferrous sulfate afforded trioxanylquinoline **24**, which underwent acidic hydrolysis to give aldehyde **14B**. 2-Hydroxymethyl-4-quinolinecarboxaldehyde (**14D**) was obtained from the benzylic oxidation of **24** with selenium dioxide followed by the hydrolysis of the resulting trioxanyl aldehyde **25** with 2 N HCl to give dialdehyde **26**. Subsequent reduction of **26** with sodium borohydride afforded **14D**. In the reduction process, regioisomer, 4-hydroxymethyl-2-quinolinecarboxaldehyde (**27**) and 2,4-di(hydroxymethyl)quinoline (**28**) were also isolated. Acetylation of the hydroxyl function of **14D** with acetic anhydride and zinc oxide[18] produced acetate **14C** (Scheme 3).

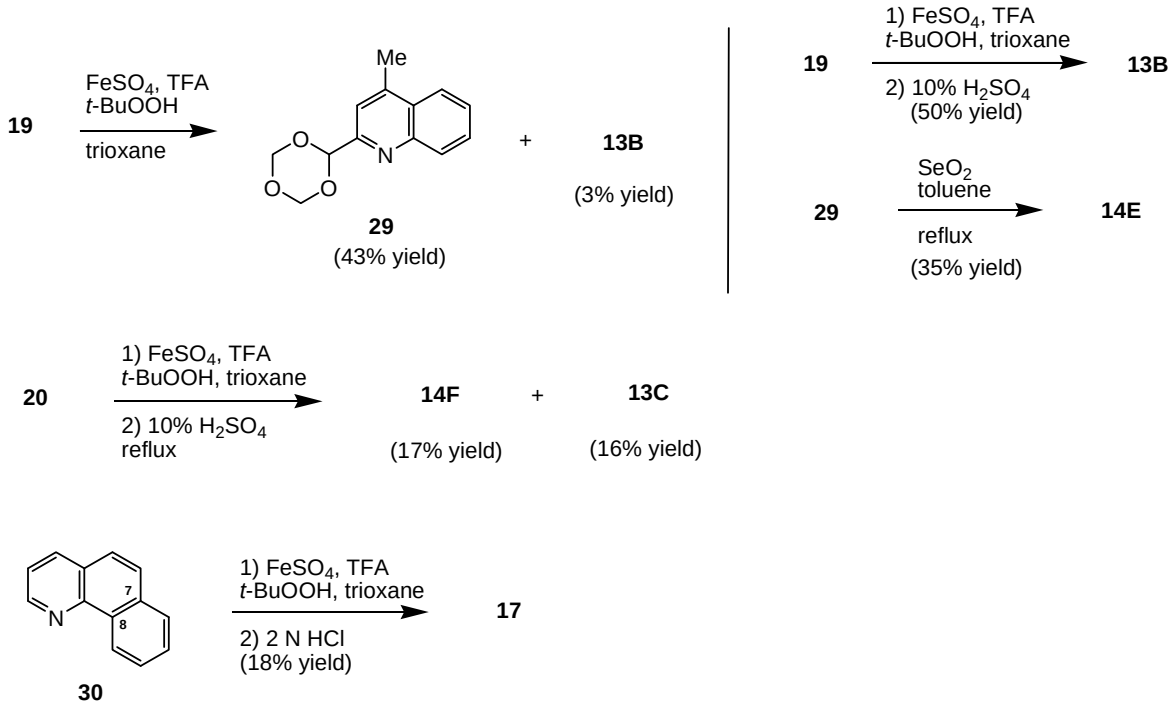
Scheme 3. Syntheses of quinolinecarboxaldehydes 14B - 14D.



Similar trioxanylation of 4-methylquinoline (**19**) furnished 2-trioxanequinoline **29** in 43% yield along with a small amount of 4-methyl-2-quinolinecarboxaldehyde (**13B**) (3% yield), which derived from the hydrolysis of the trioxane moiety of **29** with TFA and water in the reaction mixture (Scheme 4). Alternatively, aldehyde **13B** can be achieved in 50% yield from a two-step trioxanylation of **19** followed by acidic hydrolysis with 10% aqueous sulfuric acid of the resulting trioxane without purification. 2-Trioxanyl-4-carboxaldehyde (**14E**) was obtained from the benzylic oxidation of **29** with selenium dioxide. Similar to the selenylation reaction of 8-methylquinoline (**21**), trioxanylation of 6-methylquinoline (**20**) appeared to be sluggish.

Hence, the reaction of **20** with trioxane, *t*-butyl hydroperoxide, TFA and ferrous sulfate followed by acidic hydrolysis furnished a mixture of regioisomers, 6-methyl-4-carboxaldehyde (**14F**) (17% yield) and 6-methyl-2-carboxaldehyde (**13C**) (16% yield), which were separated by silica gel column chromatography. The spectral data of compound **13C** are similar to that reported,[19] and the assignment of regiochemistry of **14F** is based on its ^{1}H NMR spectral data. The chemical shift of C2-H of **14F** appears at δ 9.14 ppm as a doublet with coupling constant J value of 4.3 Hz, and that of C3-H at δ 7.77 ppm as a doublet with J value of 4.3 Hz, which are similar to that of 4-quinolinecarboxaldehyde (**14A**). Formylation of 4-azaphenanthrene (**30**) under similar reaction conditions followed by hydrolysis with 2 N HCl afforded 7,8-benzoquinoline-4-carboxaldehyde (**17**). Hence, methylquinolines can either be oxidized to the corresponding quinolinecarboxaldehydes or formylated to methylquinolinecarboxaldehydes.

Scheme 4. Syntheses of quinolinecarboxaldehydes 13B, 13C, 14E, 14F and 7,8-benzoquinolinecarboxaldehyde (17).



From our initial screening of the effects of pyranobenzopyrone compounds on the reduction of NV replicon-harboring cells (HG23 cells), 3-pyridyl analog **1** showed promising results with ED_{50} value (effective dosage at reducing NV genome levels by 50% at 24 h post-treatment) and TD_{50} value (cytotoxic dosage in killing 50% HG23 cells at 48 h post-treatment determined by cytotoxicity assay at 48 h of treatment)[7,9,20] of 4 and >200 μM, respectively. Hence, a small library of pyridylmethyl, quinolylmethyl, quinolylcarbonyl, and 4-azaphenanthrenylmethyl derivatives along with their synthetic precursor, amine **10**, was evaluated for their anti-norovirus activities. Results of the inhibition of NV RNA replication are summarized in Table 1. To our surprise, amine **10**, 4-pyridylmethyl **2**, 8-quinolylmethyl **6**, and 2-acetoxymethyl- and 2-hydroxymethyl-4-quinolylmethyls **4C** and **4D** have ED_{50} values >10 μM. Due to high ED_{50} values, TD_{50} values of these compounds were not determined except compound **10**. Other quinolylmethyl, substituted quinolylmethyl, and 4-azaphenanthrenylmethyl derivatives along with 4-quinolyl amide **8** possess ED_{50} values ranging from 2 – 8 μM and TD_{50} values of 61 – >200 μM. In particular, 2-quinolylmethyl **3A**, 4-quinolylmethyl **4A**, and 6-methyl-4-quinolylmethyl **4F** possess the strongest anti-norovirus activities with respective ED_{50} values of 3.4, 2.4, and 3.4 μM and TI (therapeutic index; derived from TD_{50}/ED_{50}) values of 58.8, 40.2, and 18.0, respectively. 6-Methyl-2-quinolylmethyl **3C** on the other hand showed a lower ED_{50} value of 8.1 μM. Hence, addition of substituent at C6 or ring B of 4-quinolylmethyl analog appears to retain its antiviral activity and substituent on ring A diminishes its activity.

Table 1. Effects of pyranobenzopyrone compounds on the reduction of NV RNA replication (ED_{50}) and toxicity (TD_{50}) in HG23 cells (NV replicon-haboring cells) and their therapeutic indexes (TI).

Compound	ED_{50} value in μM	TD_{50} value in μM	Therapeutic index (TI)
1	**4.1**	**>200**	**>50**
2	**9.6**	**ND***	**-**
3A	**3.4**	**>200**	**>58.8**
4A	**2.4**	**96.4**	**40.2**
5	**8.1**	**ND**	**-**
6	**>10**	**>160**	**>16**
4B	**8.2**	**ND**	**-**
4C	**9.7**	**ND**	**-**
4D	**9.5**	**ND**	**-**
4E	**8.4**	**ND**	**-**
3B	**5.3**	**>200**	**>40**
4F	**3.4**	**61.2**	**18.0**
3C	**8.1**	**83.7**	**10.3**
7	**5.3**	**>200**	**>40**
8	**5.5**	**103.5**	**18.8**
10	**>10**	**>200**	**-**

* ND: not determined due to high ED_{50} values. Each value is the average of at least 2 independent tests.

In conclusion, various pyranobenzopyrone molecules containing pyridine, quinoline, or 4-azaphenanthrene nucleus were synthesized and evaluated. 4-Quinolylmethyl analogs **4A** and **4F** and 2-quinolylmethyl analog **3A** possess the strongest anti-norovirus activities having ED_{50}

values of 2.4 – 3.4 μM and TI values of 18.0 – >58.8. The identified hits are suitable for further optimization via medicinal chemistry and molecular modeling.

Acknowledgement.

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Supplementary data

Synthetic procedure, analytical data, and protocols for antiviral evaluation are included. Supplementary data associate with this article can be found in the online version, at …

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Supplemental Materials.

Experimental Section.

I. General Methods. NMR spectra were obtained from a 400-MHz spectrometer (Varian Inc.), in $CDCl_3$, unless otherwise indicated, and reported in ppm. Infrared spectra were taken from a Nicolet 380 FT-IR instrument (Thermo Scientific) in solid forms and are reported in wave numbers (cm^{-1}). Low-resolution mass spectra were taken from an API 2000-triple quadrupole ESI-MS/MS mass spectrometer (from Applied Biosystems). High-resolution Mass spectra were obtained from a LCT Premier (Waters Corp., Milford MA) time of flight mass spectrometer. The instrument was operated at 10,000 resolution (W mode) with dynamic range enhancement that attenuates large intensity signals. The cone voltage was 60 eV. Spectra were acquired at 16666 Hz pusher frequency covering the mass range 100 to 1200 μ and accumulating data for 2 seconds per cycle. Mass correction for exact mass determinations was made automatically with the lock mass feature in the MassLynx data system. A reference compound in an auxiliary sprayer is sampled every third cycle by toggling a "shutter" between the analysis and reference needles. The reference mass is used for a linear mass correction of the analytical cycles.

Chemicals such as sodium cyanoborohydride, 4-hydroxy-6-methyl-2-pyrone, (*S*)-perillaldehyde, hydroxylamine-*O*-sulfonic acid, pyridine-3-carboxaldehyde (**11**), pyridine-4-carboxaldehyde (**12**), 2-quinolinecarboxaldehyde (**13A**) and 4-quinolinecarboxylic acid (**18**) were purchased from Fisher Scientific and Aldrich Chemical Co. (5a*S*,7*S*)-{7-Isopropenyl-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (**9**) was prepared as described.[21]

II.1. (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Amino-1-methylethyl)-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (10). To a cold (0^oC) solution of 5.0 g (19.4 mmol) of compound **9** in 100 mL of THF under argon was added 10 mL (9.7 mmol) of BH_3•THF complex (1.0 M in THF) dropwise. After stirring the solution at 0^oC for 2 h and 25^oC for 12 h, the borane solution was added to 3.3 g (29.2 mmol) of hydroxylamine-*O*-sulfonic acid under argon via a cannula followed by the addition of 20 mL of chloroform. The reaction mixture was heated to reflux for 7 h, cooled to 25^oC, diluted with 300 mL of ethyl acetate, and extracted with 2 N HCl (100 mL each) twice. The combined HCl layer was washed three times with ethyl acetate (100 mL each), basified carefully with 5% sodium carbonate until pH = 9 ~ 10, and extracted three times with dichloromethane. The combined dichloromethane layer was washed with brine, dried (anhydrous Na_2SO_4), and concentrated to give 2.7 g (50% yield) of compound **10**, whose ^1H and ^{13}C NMR spectra are identical to that reported.[10]

General procedure for the synthesis of compounds 1 – 7 via the reductive amination reaction.

II.2. (5a*S*,7*S*)-3-Methyl-7-(1-(pyridin-3-ylmethylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (1). A solution of 85 mg (0.31 mmol) of amine **10** and 33 mg (0.31 mmol) of 3-pyridinecarboxaldehyde (**11**) in 5 mL of dry MeOH was stirred under argon at 25^oC for 12 h, added acetic acid (5 drops) and a solution of 68 mg (1.1 mmol) of

$NaBH_3CN$ in methanol. After stirring for 1 h, the reaction solution was diluted with 40 mL of 5% aqueous ammonium hydroxide and extracted three times with dichloromethane, and the combined organic layer was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of CH_2Cl_2 and MeOH as eluant to give 68 mg (60% yield) of **1** as a solid. ^{1}H NMR δ 8.57 (s, 1 H), 8.51 (d, *J* = 4 Hz, 1 H), 7.70 (d, *J* = 7.5 Hz, 1 H), 7.28 (dd, *J* = 7.5, 4 Hz, 1 H), 6.05 (s, 1 H), 5.71 (s, 1 H), 5.11 – 4.98 (m, 1 H), 3.81 (s, 2 H), 2.67 – 2.57 (m, 1 H), 2.53 – 2.38 (m, 2 H), 2.19 (s, 3 H), 2.08 – 1.91 (m, 2 H), 1.74 – 1.46 (m, 4 H), 1.29 – 1.06 (m, 1 H), 0.91 (d, *J* = 5.0 Hz, 3 H); ^{13}C NMR δ 163.3, 162.6, 161.6, 149.7, 148.5, 136.0, 135.6, 132.8, 123.5, 109.1, 99.9, 97.4, 79.7, 79.5, 53.1, 53.05, 51.5, 39.2, 38.7, 38.5, 37.7, 37.6, 36.7, 32.4, 32.3, 31.0, 28.4, 20.2, 14.7, 14.6; MS (electrospray ionization) m/z 367.2 ($M+H^+$); HRMS calcd for $C_{22}H_{27}N_2O_3^+$ ($M+H^+$) 367.2022, found 367.2017 (100%).

II.3. (5aS,7S)-3-Methyl-7-(1-(pyridin-4-ylmethylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2). From 85 mg (0.31 mmol) of amine **10** and 33 mg (0.31 mmol) of 4-pyridinecarboxaldehyde (**12**), 72 mg (64% yield) of compound **2** was obtained as a solid. ^{1}H NMR δ 8.54 (d, *J* = 4.5 Hz, 2 H), 7.26 (d, *J* = 4.7 Hz, 2 H), 6.06 (s, 1 H), 5.70 (s, 1 H), 5.11 – 4.98 (m, 1 H), 3.80 (s, 2 H), 2.63 – 2.55 (m, 1 H), 2.50 – 2.39 (m, 2 H), 2.18 (s, 3 H), 2.09 – 1.91 (m, 2 H), 1.75 – 1.47 (m, 4 H), 1.35 – 1.05 (m, 1 H), 0.92 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR δ 163.3, 163.3, 162.6, 161.6, 149.8, 132.8, 123.1, 109.3, 99.9, 97.4, 79.7, 79.6, 53.4, 53.3, 53.0, 39.3, 38.8, 38.7, 38.0, 37.9, 36.9, 32.5, 32.4, 31.1, 28.6, 20.2, 14.8, 14.7; MS (electrospray ionization) m/z 367.2 ($M+H^+$), 139.0, 121.2; HRMS calcd for $C_{22}H_{26}N_2O_3Na^+$ ($M+Na^+$) 389.1841, found 389.1844 (100%).

II.4. (5aS,7S)-3-Methyl-7-(1-(quinolin-2-ylmethylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (3A). From 60 mg (0.22 mmol) of amine **10**

and 35 mg (0.22 mmol) of 2-quinolinecarboxaldehyde (**13A**), 54 mg (60% yield) of **3A** was obtained as a solid. ^{1}H NMR δ 8.11 (d, *J* = 8.6 Hz, 1 H), 8.04 (d, *J* = 8.2 Hz, 1 H), 7.79 (d, *J* = 8.2 Hz, 1 H), 7.69 (t, *J* = 7.4 Hz, 1 H), 7.50 (t, *J* = 7.4 Hz, 1 H), 7.43 (d, *J* = 8.6 Hz, 1 H), 6.04 (s, 1 H), 5.67 (s, 1 H), 5.08 – 5.00 (m, 1 H), 4.09 (s, 2 H), 2.69 (dd, *J* = 11.7, 5.8 Hz, 1 H), 2.59 – 2.50 (m, 1 H), 2.41 (d, *J* = 14 Hz, 1 H), 2.16 (s, 3 H), 2.10 – 1.91 (m, 2 H), 1.78 – 1.45 (m, 4 H), 1.27 – 1.04 (m, 1 H), 0.93 (d, *J* = 6.2 Hz, 1.5 H, CH_3), 0.92, (d, *J* = 6.2 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.4, 163.4, 162.7, 161.6, 160.1, 147.8, 136.6, 133.0, 132.9, 129.7, 129.0, 127.7, 127.5, 126.3, 120.7, 109.2, 99.9, 97.5, 79.8, 79.7, 55.9, 53.7, 53.6, 39.3, 38.7, 38.6, 37.9, 37.8, 36.8, 32.5, 32.4, 31.1, 28.5, 20.2, 14.8, 14.7; MS (electrospray ionization) m/z 417.3 ($M+H^+$); HRMS calcd for $C_{26}H_{29}N_2O_3^+$ ($M+H^+$) 417.2178, found 417.2164 (100%).

II.5. (5aS,7S)-3-Methyl-7-(1-(quinolin-4-ylmethylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (4A). From 1.28 g (4.65 mmol) of amine **10** and 0.73 g (4.65 mmol) of 4-quinolinecarboxaldehyde (**14A**), 1.25 g (65% yield) of **4A** was obtained as a solid. ^{1}H NMR δ 8.88 (d, *J* = 4.3 Hz, 1 H), 8.14 (d, *J* = 7.4 Hz, 1 H), 8.12 (d, *J* = 7.0 Hz, 1 H), 7.73 (t, *J* = 7.6 Hz, 1 H), 7.58 (t, *J* = 7.8 Hz, 1 H), 7.45 (d, *J* = 4.3 Hz, 1 H), 6.07 (s, 1 H), 5.70 (s, 1 H), 5.06 – 4.95 (m, 1 H), 4.27 (s, 2 H), 2.75 – 2.67 (m, 1 H), 2.63 – 2.55 (m, 1 H), 2.44 (d, *J* = 14.4 Hz, 1 H), 2.19 (s, 3 H), 2.07 – 1.88 (m, 2 H), 1.75 – 1.47 (m, 4 H), 1.30 – 1.08 (m, 1 H), 0.94 (d, *J* = 6.6 Hz, 1.5 H, CH_3), 0.93 (d, *J* = 6.6 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.4, 163.37, 162.7, 161.7, 150.4, 148.4, 146.0, 132.8, 130.3, 129.3, 127.3, 126.6, 123.6, 120.1, 109.3, 99.9, 97.5, 79.8, 79.7, 53.9, 53.8, 50.7, 50.7, 39.3, 38.8, 38.6, 38.0, 37.9, 36.9, 32.5, 32.4, 31.1, 28.6, 20.3, 14.9, 14.8; MS (electrospray ionization) m/z 417.5 ($M+H^+$),139.1; HRMS calcd for $C_{26}H_{29}N_2O_3^+$ ($M+H^+$) 417.2178, found 417.2162 (100%).

II.6. (5aS,7S)-3-Methyl-7-(1-(quinolin-6-ylmethylamino)propan-2-yl)-6,7,8,9-tetrahydropyrano[4,3-b]chromen-1(5aH)-one (5). From 0.15 g (0.53 mmol) of amine **10** and 83 mg (0.53 mmol) of 6-quinolinecarboxaldehyde (**15**), 104 mg (47% yield) of **5** was obtained as a solid. ^{1}H NMR 8.87 δ (d, *J* = 3.9 Hz, 1 H), 8.12 (d, *J* = 8.2 Hz, 1 H), 8.06 (d, *J* = 8.6 Hz, 1 H), 7.76 (s, 1 H), 7.71 (d, *J* = 9.0 Hz, 1 H), 7.38 (dd, *J* = 8.4, 4.1 Hz, 1 H), 6.02 (s, 1 H), 5.66 (s, 1 H), 5.01 (dd, *J* = 10.9, 4.7 Hz, 1 H), 3.99 (s, 2 H), 2.70 – 2.62 (m, 1 H), 2.55 – 2.46 (m, 1 H), 2.40 (d, *J* = 14.1 Hz, 1 H), 2.15 (s, 3 H), 2.05 – 1.88 (m, 2 H), 1.74 – 1.42 (m, 4 H), 1.26 – 1.01 (m, 1 H), 0.90 (d, *J* = 6.3 Hz, 1.5 H, CH_3), 0.89 (d, *J* = 6.3 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.4, 163.36, 162.7, 161.6, 150.3, 147.7, 137.4, 136.2, 132.7, 132.7, 130.4, 129.6, 128.3, 126.9, 121.5, 109.2, 99.9, 97.4, 97.4, 79.7, 79.5, 53.5, 52.7, 52.7, 39.2, 38.7, 38.5, 37.4, 37.3, 36.7, 32.4, 32.3, 31.0, 28.4, 20.2, 14.7, 14.6; MS (electrospray ionization) m/z 417.5 ($M+H^+$), 142.0; HRMS calcd for $C_{26}H_{29}N_2O_3^+$ ($M+H^+$) 417.2178, found 417.2174 (100%).

II.7. (5aS,7S)-3-Methyl-7-(1-(quinolin-8-ylmethylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (6). From 43 mg (0.15 mmol) of amine **10** and 24 mg (0.15 mmol) of 8-quinolinecarboxaldehyde (**16**), 34 mg (53% yield) of **6** was obtained as a solid. A mixture of ethanol and THF (2:1) was used as solvent in the reaction. In the column chromatographic separation, the column was deactivated with 2% triethylamine in hexane prior to the loading of the crude product. ^{1}H NMR δ 8.87 – 8.83 (m, 1 H), 8.12 (d, *J* = 8.2 Hz, 1 H), 7.69 (d, *J* = 8.2 Hz, 1 H), 7.61 (d, *J* = 7.0 Hz, 1 H), 7.44 (t, *J* = 7.4 Hz, 1 H), 7.37 (dd, *J* = 8.2, 3.9 Hz, 1 H), 5.99 (s, 1 H), 5.65 (s, 1 H), 5.01 – 4.91 (m, 1 H), 4.30 (s, 2 H), 2.58 (dd, *J* = 11.7, 5.8 Hz, 1 H), 2.50 – 2.42 (m, 1 H), 2.35 (d, *J* = 12.9 Hz, 1 H), 2.13 (s, 3 H), 2.02 – 1.82 (m, 2 H), 1.69 – 1.38 (m, 4 H), 1.30 – 0.99 (m, 1 H), 0.82 (d, *J* = 6.2 Hz, 3 H); ^{13}C NMR δ 163.4, 163.35, 162.7, 161.6, 149.5, 147.0, 136.8, 133.0, 132.9, 129.6, 128.6, 127.5, 126.5, 121.3, 109.2, 109.14,

99.9, 97.5, 79.8, 79.7, 53.1, 53.0, 51.7, 51.67, 39.4, 38.5, 38.45, 37.5, 37.4, 36.7, 32.5, 32.4, 31.1, 28.2, 20.3, 14.7, 14.63; MS (electrospray ionization) 439.5 ($M+Na^+$), 417.3 ($M+H^+$), 142.1; HRMS calcd for $C_{26}H_{29}N_2O_3^+$ ($M+H^+$) 417.2178, found 417.2173 (100%).

II.8. (5aS,7S)-3-Methyl-7-(1-((2-methylquinolin-4-yl)methylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (4B). From 43 mg (0.15 mmol) of amine **10** and 26 mg (0.15 mmol) of 2-methyl-4-quinolinecarboxaldehyde (**14B**), 40 mg (60% yield) of **4B** was obtained as a solid. ^{1}H NMR δ 8.01 (d, *J* = 8.6 Hz, 2 H), 7.65 (t, *J* = 7.6 Hz, 1 H), 7.47 (t, *J* = 7.6 Hz, 1 H), 7.31 (s, 1 H), 6.03 (s, 1 H), 5.68 (s, 1 H), 5.04 – 4.94 (m, 1 H), 4.18 (s, 2 H), 2.73 – 2.63 (m, 1 H), 2.71 (s, 3 H), 2.59 – 2.52 (m, 1 H), 2.41 (d, *J* = 14.5 Hz, 1 H), 2.16 (s, 3 H), 2.05 – 1.86 (m, 2 H), 1.75 – 1.44 (m, 4 H), 1.26 – 1.05 (m, 1 H), 0.91 (d, *J* = 6.6 Hz, 1.5 H, CH_3), 0.90 (d, *J* = 6.6 Hz, 1.5 H, CH_3); ^{13}C NMR δ 163.4, 163.3, 162.7, 161.6, 158.9, 148.0, 145.8, 132.81, 132.80, 129.3, 129.3, 125.7, 125.4, 123.3, 120.9, 109.2, 99.9, 97.4, 79.7, 79.6, 53.8, 53.79, 50.6, 50.61, 39.3, 38.7, 38.5, 37.9, 37.8, 36.8, 32.5, 32.3, 31.1, 28.5, 25.5, 20.2, 14.9, 14.7; MS (electrospray) 431.4 ($M+H^+$), 144.2; HRMS calcd for $C_{27}H_{31}N_2O_3^+$ ($M+H^+$) 431.2335, found 431.2320 (100%).

II.9. 4-((2-((5aS,7S)-Methyl-1-oxo-1,5a,6,7,8,9-hexahydropyrano[4,3-b]chromen-7-yl)propylamino)methyl)quinolin-2-yl)methyl acetate (4C). From 43 mg (0.15 mmol) of amine **10** and 34 mg (0.15 mmol) of quinolinecarboxaldehyde **14C**, 50 mg (66% yield) of **4C** was obtained as a solid. A mixture of ethanol and THF (2:1) was used as a solvent for the reaction. ^{1}H NMR δ 8.09 (dd, *J* = 8.2, 3.1 Hz, 2 H), 7.71 (t, *J* = 7.6 Hz, 1 H), 7.56 (t, *J* = 7.6 Hz, 1 H), 7.52 (s, 1 H), 6.05 (s, 1 H), 5.70 (s, 1 H), 5.37 (s, 2 H), 5.06 – 4.96 (m, 1 H), 4.25 (s, 2 H), 2.75 – 2.67 (m, 1 H), 2.61 – 2.54 (m, 1 H), 2.43 (d, *J* = 14.0 Hz, 1 H), 2.19 (s, 3 H), 2.18 (s, 3 H), 2.07 – 1.88 (m, 2 H), 1.73 – 1.48 (m, 4 H), 1.31 – 1.06 (m, 1 H), 0.94 (d, *J* = 6.6 Hz, 1.5 H,

CH_3), 0.93 (d, J = 6.6 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 170.9, 163.4, 163.4, 162.7, 161.7, 156.0, 147.9, 147.0, 132.8, 130.1, 129.7, 126.8, 126.5, 123.5, 118.5, 109.3, 99.9, 97.5, 79.8, 79.6, 67.8, 53.9, 53.9, 50.8, 50.8, 39.3, 38.8, 38.7, 38.0, 37.9, 36.9, 32.5, 32.4, 31.1, 28.6, 21.2, 20.3, 15.0, 14.8; MS (electrospray ionization) 511.2 ($M+Na^+$), 489.2 ($M+H^+$); HRMS calcd for $C_{29}H_{32}N_2O_5Na^+$ ($M+Na^+$) 511.2209, found 511.2184 (100%).

II.10. (5aS,7S)-7-(1-((2-(Hydroxymethyl)quinolin-4-yl)methylamino)propan-2-yl)-3-methyl-6,7,8,9-hexahydropyrano[4,3-b]chromen-1(5aH)-one (4D). From 43 mg (0.15 mmol) of amine **10** and 29 mg (0.15 mmol) of (2-hydroxymethyl)quinoline-4-carboxaldehyde (**14D**), 42 mg (61% yield) of **4D** was obtained as a solid. Ethanol was used as a solvent in the reaction. ^1H NMR δ 8.09 (d, J = 8.2 Hz, 1 H), 8.07 (d, J = 7.8 Hz, 1 H), 7.72 (t, J = 7.6 Hz, 1 H), 7.56 (t, J = 7.6 Hz, 1 H), 7.35 (s, 1 H), 6.06 (s, 1 H), 5.70 (s, 1 H), 5.06 – 4.97 (m, 1 H), 4.91 (s, 2 H), 4.25 (s, 2 H), 2.76 – 2.66 (m, 1 H), 2.63 – 2.54 (m, 1 H), 2.43 (d, J = 14.0 Hz, 1 H), 2.19 (s, 3 H), 2.08 – 1.88 (m, 2 H), 1.77 – 1.47 (m, 4 H), 1.33 – 1.06 (m, 1 H), 0.94 (d, J = 6.6 Hz, 1.5 H, CH_3), 0.93 (d, J = 6.6 Hz, 1.5 H, CH_3); ^{13}C NMR δ 163.4, 163.37, 162.7, 161.7, 159.0, 146.9, 146.7, 132.8, 132.79, 129.6, 129.5, 126.5, 126.4, 123.5, 117.1, 109.3, 99.9, 97.5, 79.8, 79.6, 64.3, 53.9, 53.86, 50.7, 50.65, 39.3, 38.8, 38.6, 38.0, 37.9, 36.8, 32.5, 32.4, 31.1, 29.9, 28.6, 20.3, 14.9, 14.8; MS (electrospray ionization) 469.3 ($M+Na^+$), 447.3 (M+1), 142; HRMS calcd for $C_{27}H_{31}N_2O_4^+$ ($M+H^+$) 447.2284, found 447.2284.

II.11. (5aS,7S)-7-(1-((2-(1,3,5-Trioxan-2-yl)quinolin-4-yl)methylamino)propan-2-yl)-3-methyl-6,7,8,9-tetrahydropyrano[4,3-b]chromen-1(5aH)-one (4E). From 102 mg (0.37 mmol) of amine **10** and 91 mg (0.37 mmol) of aldehyde **14E**, 88 mg (47% yield) of compound **4E** was obtained as a solid. Ethanol was used as solvent in the reaction. ^1H NMR δ 8.16 (d, J = 8.2 Hz, 1 H), 8.14 (d, J = 7.6 Hz, 1 H), 7.84 (d, J = 1.6 Hz, 1 H), 7.72 (t, J = 7.6 Hz, 1 H), 7.59 (t, J = 8.1

Hz, 1 H), 6.11 (s, 1 H), 6.05 (s, 1 H), 5.69 (s, 1 H), 5.46 – 5.39 (m, 4 H), 5.07 – 4.98 (m, 1 H), 4.27 (s, 2 H), 2.75 – 2.66 (m, 1 H), 2.63 - 2.54 (m, 1 H), 2.43 (d, *J* = 14.1 Hz, 1 H), 2.18 (s, 3 H), 2.07 – 1.87 (m, 2 H), 1.78 – 1.46 (m, 4 H), 1.33 – 1.06 (m, 1 H), 0.92 (d, *J* = 6.8 Hz, 1.5 H, CH_3), 0.92 (d, *J* = 6.8 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.4, 163.37, 162.7, 161.7, 155.2, 147.7, 147.5, 133.0, 130.5, 129.7, 127.5, 127.3, 123.7, 117.3, 117.2, 109.3, 102.3, 99.9, 97.5, 93.9, 79.8, 79.7, 54.0, 53.9, 51.0, 51.0, 39.4, 38.6, 38.5, 38.0, 37.9, 36.8, 32.6, 32.4, 31.2, 28.5, 20.3, 14.9, 14.8; MS (electrospray ionization) 527.4 ($M+Na^+$), 505.6 ($M+H^+$), 445.1, 199.3, 139.0**;** HRMS calcd for $C_{29}H_{33}N_2O_6^+$ ($M+H^+$) 505.2339, found 505.2340.

II.12. (5aS,7S)-3-Methyl-7-(1-((4-methylquinolin-2-yl)methylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (**3B**). From 43 mg (0.15 mmol) of amine **10** and 26 mg (0.15 mmol) of 4-methyl-2-quinolinecarboxaldehyde (**13B**), 35 mg (53% yield) of compound **3B** was obtained as a solid. Ethanol was used as solvent in the reaction. ^{1}H NMR δ 8.03 (d, *J* = 8.1 Hz, 1 H), 7.96 (d, *J* = 8.2 Hz, 1 H), 7.67 (t, *J* = 7.4 Hz, 1 H), 7.51 (t, *J* = 7.4 Hz, 1 H), 7.27 (s, 1 H), 6.03 (s, 1 H), 5.67 (s, 1 H), 5.12 – 4.97 (m, 1 H), 4.02 (s, 2 H), 2.75 - 2.60 (m, 1 H), 2.68 (s, 3 H), 2.59 – 2.231 (m, 2 H), 2.16 (s, 3 H), 2.11 – 1.86 (m, 2 H), 1.78 – 1.39 (m, 4 H), 1.31 – 1.04 (m, 1 H), 0.92 (d, *J* = 6.4, Hz, 1.5 H), 0.91 (d, *J* = 6.4, Hz, 1.5 H); ^{13}C NMR δ 163.4, 162.7, 161.6, 156.0, 147.7, 144.7, 133.0, 129.6, 129.3, 127.5, 126.0, 123.9, 121.4, 109.2, 99.9, 97.5, 79.9, 79.7, 56.0, 53.8, 53.7, 39.4, 38.7, 38.5, 38.0, 37.8, 36.8, 32.6, 32.4, 31.2, 28.5, 20.2, 18.9, 14.9, 14.7. MS (electrospray ionization) 429.1 (M-1). HRMS calcd for $C_{27}H_{31}N_2O_3^+$ ($M+H^+$) 431.2335, found 431.2308 (100%).

II.13. (5aS,7S)-3-Methyl-7-(1-((6-methylquinolin-4-yl)methylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (4F). From 85 mg (0.31 mmol) of amine **10** and 53 mg (0.31 mmol) of 6-methyl-4-quinolinecarboxaldehyde (**14F**), 82 mg (62%

yield) of compound **4F** was obtained as a solid. A mixture of ethanol and THF (2:1) was used as a solvent in the reaction. ^{1}H NMR δ 8.78 (d, *J* = 4.3 Hz, 1 H), 8.01 (d, *J* = 8.6 Hz, 1 H), 7.85 (s, 1 H), 7.53 (d, *J* = 8.6 Hz, 1 H), 7.39 (d, *J* = 4.3 Hz, 1 H), 6.05 (s, 1 H), 5.69 (s, 1 H), 5.05 – 4.94 (m, 1 H), 4.21 (s, 2 H), 2.75 – 2.66 (m, 1 H), 2.62 – 2.54 (m, 1 H), 2.56 (s, 3 H), 2.43 (d, *J* = 14.0 Hz, 1 H), 2.17 (s, 3 H), 2.07 – 1.88 (m, 2 H), 1.74 – 1.47 (m, 4 H), 1.31 – 1.07 (m, 1 H), 0.94 (d, *J* = 7.0 Hz, 1.5 H, CH_3), 0.93 (d, *J* = 7.0 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.4, 163.35, 162.7, 161.6, 149.5, 147.0, 145.2, 136.5, 132.8, 131.5, 130.0, 127.2, 122.4, 120.0, 109.3, 99.9, 97.5, 79.8, 79.7, 53.9, 53.8, 50.7, 39.3, 38.8, 38.7, 38.0, 37.9, 36.9, 32.5, 32.4, 31.2, 28.6, 22.1, 20.2, 14.9, 14.8; MS (electrospray ionization) 431.4 (M+1), 156.2, 144.3; HRMS calcd for $C_{27}H_{30}N_2O_3Na^+$ ($M+Na^+$) 453.2154, found 453.2151.

II.14. (5aS,7S)-3-Methyl-7-(1-((6-methylquinolin-2-yl)methylamino)propan-2-yl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (3C). From 43 mg (0.15 mmol) of amine **10** and 26 mg (0.15 mmol) of 6-methyl-2-quinolinecarboxaldehyde (**13C**), 37 mg of compound **3C** (56% yield) was obtained as a solid. Ethanol was used as a solvent in the reaction. ^{1}H NMR δ 8.04 (d, *J* = 8.2 Hz, 1 H), 7.94 (d, *J* = 8.6 Hz, 1 H), 7.57 (s, 1 H), 7.53 (dd, *J* = 8.6, 1.6 Hz, 1 H), 7.40 (d, *J* = 8.6 Hz, 1 H), 6.05 (s, 1 H), 5.69 (s, 1 H), 5.10 – 5.02 (m, 1 H), 4.08 (s, 2 H), 2.73 – 2.65 (m, 1 H), 2.59 – 2.49 (m, 1 H), 2.53 (s, 3 H), 2.43 (d, *J* = 14.4 Hz, 1 H), 2.18 (s, 3 H), 2.10 – 1.93 (m, 2 H), 1.77 – 1.46 (m, 4 H), 1.29 – 1.07 (m, 1 H), 0.94 (d, *J* = 6.6 Hz, 1.5 H, CH_3), 0.93 (d, *J* = 6.6 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 163.5, 163.4, 162.8, 161.6, 159.1, 146.4, 136.2, 136.1, 133.0, 132.99, 132.0, 128.7, 127.5, 126.7, 120.7, 109.2, 100.0, 97.5, 79.9, 79.7, 55.9, 53.7, 53.6, 39.4, 38.7, 38.5, 37.9, 37.8, 36.8, 32.6, 32.4, 31.2, 28.4, 21.7, 20.3, 14.9, 14.7; MS (electrosprary ionization) 431.4 (M+1), 156.1, 129.2; HRMS calcd for $C_{27}H_{31}N_2O_3^+$ ($M+H^+$) 431.2335, found 431.2309 (100%).

II.15. 5aS,7S)-7-(1-(Benzo[h]quinolin-4-ylmethylamino)propan-2-yl)-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (7). From 51 mg (0.18 mmol) of amine **10** and 38 mg (0.18 mmol) of 7,8-benzoquinoline-4-carboxaldehyde (**17**), 54 mg (62% yield) of compound **7** was obtained as a solid. Ethanol was used as a solvent in the reaction. ^{1}H NMR δ 9.32 (d, *J* = 7.4 Hz, 1 H), 8.96 (d, *J* = 4.3 Hz, 1 H), 8.03 (d, *J* = 9.0 Hz, 1 H), 7.92 (d, *J* = 7.4 Hz, 1 H), 7.85 (d, *J* = 9.4 Hz, 1 H), 7.78 – 7.68 (m, 2 H), 7.56 (d, *J* = 4.7 Hz, 1 H), 6.05 (s, 1 H), 5.64 (s, 1 H), 5.05 – 4.94 (m, 1 H), 4.30 (s, 2 H), 2.76 – 2.66 (m, 1 H), 2.64 – 2.56 (m, 1 H), 2.43 (d, *J* = 14.4 Hz, 1 H), 2.18 (s, 3 H), 2.04 – 1.89 (m, 2 H), 1.76 – 1.46 (m, 4 H), 1.43 – 1.06 (m, 1 H), 0.95 (d, *J* = 7.0 Hz, 1.5 H, CH_3), 0.93 (d, *J* = 7.0 Hz, 1.5 H, CH_3); ^{13}C NMR δ 163.4, 162.8, 161.6, 148.8, 146.7, 145.7, 133.4, 132.9, 132.0, 128.3, 127.9, 127.8, 127.3, 125.1, 124.9, 121.3, 121.1, 109.2, 99.9, 97.5, 79.8, 79.6, 53.8, 53.7, 51.1, 39.2, 38.8, 38.6, 38.0, 37.8, 36.8, 32.5, 32.4, 31.1, 28.7, 20.3, 15.0, 14.8; MS (electrospray ionization) m/z 467.6 ($M+H^+$), 192.3; HRMS calcd for $C_{30}H_{31}N_2O_3^+$ ($M+H^+$) 467.2335, found 467.2335 (100%).

II.16. N-(2-((5aS,7S)-3-Methyl-1-oxo-1,5a,6,7,8,9-hexahydropyrano[4,3-b]chromen-7-yl)propyl)quinolie-4-carboxamide (8). A mixture of 43 mg (0.15 mmol) of amine **10**, 42 mg (0.24 mmol) of quinoline-4-carboxylic acid (**18**), 56 mg (0.36 mmol) of EDC, and 2 mg (0.02 mmol) of DMAP in 5 mL of dichloromethane was stirred under argon at 25^{o}C for 12 h. The reaction mixture was diluted with 100 mL of dichloromethane, washed with water, and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluant to give 43 mg (65% yield) of compound **8**, as a solid. ^{1}H NMR δ 8.82 (d, *J* = 4.3 Hz, 1 H), 8.16 (d, *J* = 8.6 Hz, 1 H), 8.08 (d, *J* = 8.6 Hz, 1 H), 7.73 (t, *J* = 7.6 Hz, 1 H), 7.57 (t, *J* = 7.6 Hz, 1 H), 7.34 (d, *J* = 4.3 Hz, 1 H), 6.72 (br. s, 1 H, NH), 5.97 (s, 1 H), 5.68 (s, 1 H), 5.09 – 4.98 (m, 1 H), 3.63 – 3.52 (m, 1 H), 3.42 – 3.29 (m, 1

H), 2.43 (d, *J* = 13.3 Hz, 1 H), 2.14 (s, 4 H), 2.06 – 1.90 (m, 1 H), 1.87 – 1.71 (m, 2 H), 1.71 – 1.51 (m, 2 H), 1.43 – 1.11 (m, 1 H), 0.99 (d, *J* = 6.6 Hz, 1.5 H, CH_3) 0.98 (d, *J* = 6.6 Hz, 1.5 H, CH_3) (2 diastereomers at C12); ^{13}C NMR δ 167.7, 163.5, 162.7, 161.8, 149.9, 148.7, 142.4, 132.4, 130.2, 129.9, 127.8, 125.4, 124.6, 118.5, 109.4, 99.9, 97.4, 79.6, 79.4, 43.9, 39.2, 38.8, 38.7, 38.0, 36.9, 32.4, 32.3, 31.1, 28.6, 20.2, 14.5, 14.4; MS (electrospray ionization) m/z 453.3 ($M + Na^+$), 431.1 ($M+H^+$); HRMS calcd for $C_{26}H_{27}N_2O_4^+$ ($M+H^+$) 431.1971, found 431.1957 (100%).

General procedure for the syntheses of compounds 14A, 15, 14E, and 25.[13]

II.17. Quinoline-4-carboxaldehyde (14A). A solution of 5.0 g (35 mmol) of 4-methylquinoline (**19**) and 5.0 g (45 mmol) of SeO_2 in toluene under argon was heated to reflux for 24 h. The reaction mixture was diluted with dichloromethane, washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a mixture of hexanes and ethyl acetate (4:1) as eluant to give 4.0 g (73% yield) of compound **14A**,[13] as a solid. ^{1}H NMR δ 10.54 (s, 1 H), 9.22 (d, *J* = 4.3 Hz, 1 H), 9.04 (d, *J* = 8.6 Hz, 1 H), 8.24 (d, *J* = 8.2 Hz, 1 H), 7.84 (t, *J* = 7.6 Hz, 1 H), 7.81 (d, *J* = 4.3 Hz, 1 H), 7.76 (t, *J* = 8.0 Hz, 1 H); ^{13}C NMR δ 193.1, 150.7, 149.5, 137.0, 130.4, 130.3, 129.6, 126.0, 124.7, 124.1. MS (electrospray ionization) m/z 158.0 (100%) ($M+H^+$), 130.2.

II.18. Quinoline-6-carboxaldehyde (15). From 0.50 g (3.5 mmol) of 6-methylquinoline (**20**), 0.12 g (54% yield based on recovered compound **20**) of **15**[13] as a solid and 0.30 g of **20** was recovered. ^{1}H NMR δ 10.20 (s, 1 H), 9.05 (dd, *J* = 4.3, 1.6 Hz, 1 H), 8.35 (s, 1 H), 8.32 (dd, *J* = 8.2, 1.9 Hz, 1 H), 8.23 – 8.17 (m, 2 H), 7.52 (dd, *J* = 8.2, 4.3 Hz, 1 H); ^{13}C NMR δ 191.6, 153.3, 151.1, 137.6, 134.5, 133.8, 131.0, 127.9, 126.9, 122.4. MS (electrospray ionization) m/z 157.9 (100%) ($M+H^+$).

II.19. 2-(1,3,5-Trioxan-2-yl)quinoline-4-carboxaldehyde (14E). From 0.30 g (1.3 mmol) of **29**, 96 mg (35% yield based on recovered **29**) of compound **14E** was obtained as a solid and 40 mg of **29** was recovered. ^{1}H NMR δ 10.53 (s, 1 H), 9.08 (dd, *J* = 8.0, 1.2 Hz, 1 H), 8.26 (dd, *J* = 7.8, 0.8 Hz, 1 H), 8.25 (s, 1 H), 7.86 (ddd, *J* = 7.8, 7.0, 1.6 Hz, 1 H), 7.78 (ddd, *J* = 7.8, 7.0, 1.6 Hz, 1 H), 6.21 (s, 1 H), 5.49 – 5.45 (m, 4 H); ^{13}C NMR δ 193.0, 155.8, 148.3, 138.0, 130.7, 130.2, 130.1, 124.7, 124.3, 124.0, 101.3, 93.8. MS (electrospray ionization) m/z 268.3 (100%) ($M+Na^+$).

II.20. 4-(1,3,5-Trioxan-2-yl)quinoline-2-carboxaldehyde (25). From 0.70 g (3.0 mmol) of **24**, 0.60 g (81% yield) of **25** as a solid. The reaction was conducted at 80 °C for 15 h. ^{1}H NMR δ 10.24 (s, 1 H), 8.32 (d, *J* = 9.0 Hz, 2 H), 8.31 (s, 1 H), 7.85 (ddd, *J* = 8.5, 7.1, 1.2 Hz, 1 H), 7.75 (ddd, *J* = 8.4, 6.6, 1.2 Hz, 1 H), 6.47 (s, 1 H), 5.51 – 5.46 (m, 4 H); ^{13}C NMR δ 193.5, 152.5, 148.6, 142.1, 131.3, 130.6, 129.8, 127.0, 124.6, 115.5, 99.1, 93.9. MS (electrospray ionization) m/z 267.9 (100%) ($M+Na^+$), 245.9 ($M+H^+$). See Experiment II.26. for a direct conversion of **24** to dialdehyde **26**.

General procedure for the syntheses of compounds 13B, 14F, 13C, 17, 24, and 29.

II.21. 4-Methylquinoline-2-carboxaldehyde (13B). To a solution of 0.95 g (8.4 mmol) of trifluoroacetic acid and 1.0 g (7.0 mmol) of 4-methylquinoline (**19**), were added 3.6 mL (28 mmol) of 70% *tert*-butyl hydroperoxide, 78 mg (0.28 mmol) of $FeSO_4 \cdot 7H_2O$, 20 mL of acetonitrile, and 20 g of trioxane. The reaction mixture was heated to reflux for 12 h, cooled to 25 °C, basified with 5% NaOH, and extracted three times with diethyl ether. The combined organic layer was washed with brine, and concentrated. The residue was diluted with 100 mL of 10% H_2SO_4, heated to reflux for 5 h, cooled to 25 °C, basified with 10% NaOH, and extracted three times with diethyl ether. The combined organic layer was washed with brine, dried

($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluant to give 0.38 g (50% yield based on recovered **19**) of **13B**[15,16] as a solid and 0.37 g of **19** was recovered. ^{1}H NMR δ 10.19 (s, 1 H), 8.24 (d, *J* = 8.2 Hz, 1 H), 8.06 (d, *J* = 8.2 Hz, 1 H), 7.86 (s, 1 H), 7.81 (ddd, *J* = 8.4, 6.8, 1.2 Hz, 1 H), 7.70 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1 H), 2.78 (s, 3 H); ^{13}C NMR δ 194.3,152.4, 147.9, 146.2, 131.2, 130.3, 130.25, 129.1, 124.2, 118.1, 19.1. MS (electrospray ionization) m/z 172.0 (100%) ($M+H^+$).

II.22. 6-Methylquinoline-4-carboxaldehyde (14F) and 6-methylquinoline-2-carboxaldehyde (13C). From 1.5 g (10.5 mmol) of 6-methylquinoline (**20**), 0.24 g (17% yield based on recovered **20**) of **14F** and 0.23 g (16% yield) of **13C**[20] as solids, and 0.31 g of **20** was recovered. Compound **14F**: ^{1}H NMR δ 10.51 (s, 1 H), 9.14 (d, *J* = 4.3 Hz, 1 H), 8.83 (s, 1 H), 8.12 (d, *J* = 8.6 Hz, 1 H), 7.77 (d, *J* = 4.3 Hz, 1 H), 7.67 (dd, *J* = 8.6, 1.9 Hz, 1 H), 2.63 (s, 3 H); ^{13}C NMR δ 192.8, 149.2, 147.7, 139.6, 135.8, 132.2, 129.4, 125.8, 123.7, 123.1, 22.0; MS (electrospray ionization) m/z 172.0 (100%) ($M+H^+$), 144.1.

Compound **13C**: ^{1}H NMR δ 10.21 (s, 1 H), 8.21 (d, *J* = 8.2 Hz, 1 H), 8.14 (d, *J* = 9.4 Hz, 1 H), 8.00 (d, *J* = 8.6 Hz, 1 H), 7.67 (s, 1 H), 7.66 (dd, *J* = 8.0, 2.0 Hz, 1 H), 2.59 (s, 3 H); ^{13}C NMR δ 194.0, 152.2, 146.8, 139.9, 136.8, 133.1, 130.4, 130.3, 126.9, 117.7, 22.1. MS (electrospray ionization) m/z 172.0 (100%) ($M+H^+$).

II.23. Benzo[*h*]quinoline-4-carboxaldehyde (17). From 0.50 g (2.79 mmol) of benzo[*h*]quinoline (**30**), 90 mg (18% yield based on recovered **30**) of **17**[22] as a solid and 85 mg of **30** was recovered. ^{1}H NMR δ 10.50 (s, 1 H), 9.26 (d, *J* = 7.8 Hz, 1 H), 9.16 (d, *J* = 4.3 Hz, 1 H), 8.74 (d, *J* = 9.4 Hz, 1 H), 7.94 – 7.84 (m, 2 H), 7.80 – 7.68 (m, 3 H); ^{13}C NMR δ 192.9, 148.9, 147.8, 136.5, 133.3, 131.2, 130.9, 129.0, 127.9, 127.7, 125.0, 124.9, 122.9, 120.8. MS (electrospray ionization) m/z 208 ($M+H^+$), 186.2.

II.24. 2-Methyl-4-(1,3,5-trioxan-2-yl)quinoline (24).[15,16] From 10.0 g (70 mmol) of 2-methylquinoline (**23**), 5.3 g (50% yield based on recovered **23**) of **24** and 3.5 g of **23** was recovered. ^{1}H NMR δ 8.05 (d, *J* = 8.6 Hz, 2 H), 7.66 (t, *J* = 7.8 Hz, 1 H), 7.55 (s, 1 H), 7.49 (t, *J* = 7.8 Hz, 1 H), 6.33 (s, 1 H), 5.44 – 5.36 (m, 4 H), 2.73 (s, 3 H); ^{13}C NMR δ 159.0, 148.4, 140.4, 129.4, 129.39, 126.1, 123.6, 123.3, 119.5, 98.8, 93.8, 25.5. MS (electrospray ionization) m/z 232.2 ($M+H^+$), 172.0, 144.1.

II.25. 4-Methyl-2-(1,3,5-trioxan-2-yl)quinoline (29).[15,16] From 5.0 g (35 mmol) of 4-methylquinoline (**19**), 3.0 g (43% yield based on recovered **19**) of **29** and 0.24 g (4% yield) of **13B**, along with 0.70 g of **19**. ^{1}H NMR δ 8.11 (d, *J* = 8.2 Hz, 1 H), 7.92 (d, *J* = 8.5 Hz, 1 H), 7.67 (t, *J* = 7.6 Hz, 1 H), 7.61 (s, 1 H), 7.52 (t, *J* = 7.6 Hz, 1 H), 6.04 (s, 1 H), 5.41 – 5.33 (m, 4 H), 2.66 (s, 3 H); ^{13}C NMR δ 154.9, 147.0, 145.9, 130.1, 129.5, 128.4, 127.0, 123.7, 118.9, 102.2, 93.7, 18.9. MS (electrospray ionization) m/z 270.2 ($M+K^+$), 254.1 ($M+Na^+$), 232.2 ($M+H^+$), 172.0.

II.26. 2-Methylquinoline-4-carboxaldehyde (14B).[15,16] From 0.50 g (2.2 mmol) of **24**, 0.29 g (79% yield) of **14B** was obtained as a solid. ^{1}H NMR δ 10.49 (s, 1 H), 8.97 (dd, *J* = 8.6, 1.2 Hz, 1 H), 8.13 (d, *J* = 8.6 Hz, 1 H), 7.80 (ddd, *J* = 8.3, 7.0, 1.6 Hz, 1 H), 7.70 (s, 1 H), 7.68 (ddd, *J* = 8.5, 6.8, 1.2 Hz, 1 H), 2.88 (s, 3 H); ^{13}C NMR δ 193.0, 159.2, 149.0, 137.0, 130.2, 129.2, 128.3, 127.2, 124.3, 122.2, 25.3. MS (electrospray ionization) m/z 172.0 (100%) ($M+H^+$).

II.27. Quinoline-2,4-dicarboxaldehyde (26). From 0.70 g of **24**, **0.**38 g (68% yield in two steps) of compound **26**[23] was obtained as a solid. Hydrolysis with 2 N HCl was used. ^{1}H NMR δ 10.53 (s, 1 H), 10.28 (s, 1 H), 9.11 (dd, *J* = 8.2, 1.2 Hz, 1 H), 8.40 (s, 1 H), 8.35 (dd, *J* = 8.0, 1.4 Hz, 1 H), 7.92 (ddd, *J* = 8.2, 7.0, 1.6 Hz, 1 H), 7.87 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1 H); ^{13}C NMR δ

192.9, 192.7, 153.0, 149.2, 138.1, 132.3, 131.3, 131.2, 125.6, 125.2, 122.8. MS (electrospray ionization) m/z 186.1 ($M+H^+$), 172.0.

II.28. Quinoline-8-carboxaldehyde (16). A solution of 0.50 g (3.5 mmol) of 8-methylquinoline (**21**), 1.87 g (10.5 mmol) of *N*-bromosuccinamide (NBS), and 50 mg (0.3 mmol) of azobisisobutyronitrile (AIBN) in dichloroethane was refluxed under argon for 30 h. The reaction mixture was diluted with 150 mL dichloromethane, washed three times with 2 N NaOH, and brine, dried ($MgSO_4$), and concentrated. The residue was refluxed in 50 mL of water for 4 h, cooled to 25 °C, diluted with 50 mL of 2 N NaOH, and extracted three times with diethyl ether. The combined organic layer was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 0.20 g (37% yield) of **16**[14] and 0.30 g (53% yield) of **22**[24] as solids. Compound **22** was converted to compound **16** by treating with IBX and DMSO in 79 % yield. Compound **16**: ^{1}H NMR δ 11.45 (s, 1 H), 9.04 (dd, *J* = 4.3, 1.9 Hz, 1 H), 8.32 (dd, *J* = 7.0, 1.6 Hz, 1 H), 8.24 (dd, *J* = 8.4, 1.8 Hz, 1 H), 8.08 (dd, *J* = 8.2, 1.6 Hz, 1 H), 7.67 (t, *J* = 7.6 Hz, 1 H), 7.51 (dd, *J* = 8.4, 4.1 Hz, 1 H); ^{13}C NMR δ 192.8, 151.5, 147.8, 136.5, 134.4, 131.9, 129.5, 128.5, 126.4, 122.0. MS (electrospray ionization) m/z 158.0 ($M+H^+$), 128.1.

Compound **22**: ^{1}H NMR δ 8.77 (dd, *J* = 3.9, 1.2 Hz, 1 H), 8.07 (dd, *J* = 8.2, 1.2 Hz, 1 H), 7.65 (d, *J* = 8.2 Hz, 1 H), 7.55 (d, *J* = 7.0 Hz, 1 H), 7.40 (t, *J* = 7.6 Hz, 1 H), 7.32 (dd, *J* = 8.2, 4.3 Hz, 1 H), 5.20 (s, 2 H); ^{13}C NMR δ 149.0, 146.8, 138.2, 136.7, 128.3, 127.5, 127.3, 126.4, 121.1, 64.2. MS (electrospray ionization) m/z 160.3 ($M+H^+$), 142.0, 141.1.

II.29. 2-(Hydroxymethyl)quinoline-4-carboxaldehyde (14D). To a solution of 0.30 g (1.62 mmol) of **26** in 15 mL of dichloromethane and ethanol (3:1) at 0 °C under argon, was added 15 mg (0.40 mmol) of $NaBH_4$, and the solution was stirred for 1 h. The reaction mixture was

diluted with 50 mL of brine, extracted twice with dichloromethane, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluant to give 73 mg (32% yield) of **14D**, 81 mg (35% yield) of **27**, and 38 mg (16% yield) of **28**[25] along with 72 mg of recovered **26**. Compound **14D**: ^{1}H NMR δ 10.53 (s, 1 H), 9.00 (dd, *J* = 8.4, 1.0 Hz, 1 H), 8.20 (d, *J* = 8.2 Hz, 1 H), 7.84 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1 H), 7.75 (s, 1 H), 7.73 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1 H), 5.06 (s, 2 H); ^{13}C NMR δ 192.8, 159.7, 148.1, 137.8, 130.7, 129.4, 129.1, 124.7, 123.8, 123.5, 64.5. MS (electrospray ionization) m/z 188.3 ($M+H^+$), 128.2, 115.2.

Compound **27**: ^{1}H NMR δ 10.24 (s, 1 H), 8.30 (d, *J* = 8.6 Hz, 1 H), 8.19 (s, 1 H), 8.05 (dd, *J* = 8.2, 0.8 Hz, 1 H), 7.85 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1 H), 7.73 (ddd, *J* = 8.4, 7.0, 1.4 Hz, 1 H), 5.29 (d, *J* = 0.8 Hz, 2 H); ^{13}C NMR δ ($CDCl_3$ and DMSO-d_6) δ 193.1, 151.6, 148.9, 146.7, 130.1, 129.2, 128.3, 126.8, 122.5, 113.4, 59.7. MS (electrospray ionization) m/z 188.3 (100%) ($M+H^+$), 115.1.

Compound **28**: ^{1}H NMR δ 8.12 (d, *J* = 9.0 Hz, 1 H), 7.94 (d, *J* = 8.2 Hz, 1 H), 7.75 (ddd, *J* = 8.4, 6.8, 1.2 Hz, 1 H), 7.58 (ddd, *J* = 8.4, 7.0, 1.2 Hz, 1 H), 7.46 (s, 1 H), 5.25 (d, *J* = 3.5 Hz, 2 H), 4.93 (d, *J* = 3.9 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 162.2, 148.0, 146.5, 129.1, 128.9, 125.8, 124.6, 123.3, 115.7, 65.0, 59.8. MS (electrospray ionization) m/z 190.2 (100%) ($M+H^+$).

II.30. (4-Formylquinolin-2-yl)methyl acetate (14C). To a mixture of 50 mg (0.27 mmol) of **14D**, 5 mg (0.05 mmol) of ZnO in 8 mL of dichloromethane under argon, was added 0.28 g (2.7 mmol) of acetic anhydride. The reaction mixture was stirred at 25°C for 2 h, diluted with 100 mL of dichloromethane, washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel to give 52 mg (85% yield) of compound **14C** as a solid. ^{1}H NMR δ 10.53 (s, 1 H), 8.99 (d, *J* = 8.2 Hz, 1 H), 8.18 (d, *J* = 8.6 Hz, 1 H), 7.87 (s, 1 H), 7.82 (t, *J* = 7.6

Hz, 1 H), 7.73 (t, J = 8.2 Hz, 1 H), 5.48 (s, 2 H), 2.23 (s, 3 H); ^{13}C NMR δ 192.9, 170.8, 156.8, 149.0, 137.8, 130.7, 130.0, 129.6, 124.5, 124.5, 123.5, 67.2, 21.1. MS (electrospray ionization) m/z 230.1 ($M+H^+$), 216.5.

Bioactivity assay of pyranobenzopyrone compounds.

The antiviral effects of each compound were examined in NV replicon-harboring cells (HG23 cells). The detailed procedures for studying the antiviral effects using HG23 cells were reported elsewhere. Briefly, One-day old**,** 80 - 90% confluent HG23 cells were treated with varying concentrations of each compound (0 [mock-DMSO] - 10 μM) to examine their effects on the replication of NV. At 48 hrs of treatment, the NV genome was analyzed with real time qRT-PCR. The ED_{50} of each compound for NV genome levels was determined at 48 hrs post-treatment. HG23 cells were also treated with varying concentrations (0 [mock-DMSO] - 200 μM) of each compound to assess the cytotoxic effects. Cell cytotoxicity assay kit (Promega, Madison, WI) was used to calculate the median toxic dose (TD_{50}) at 48 hrs of treatment.

References:

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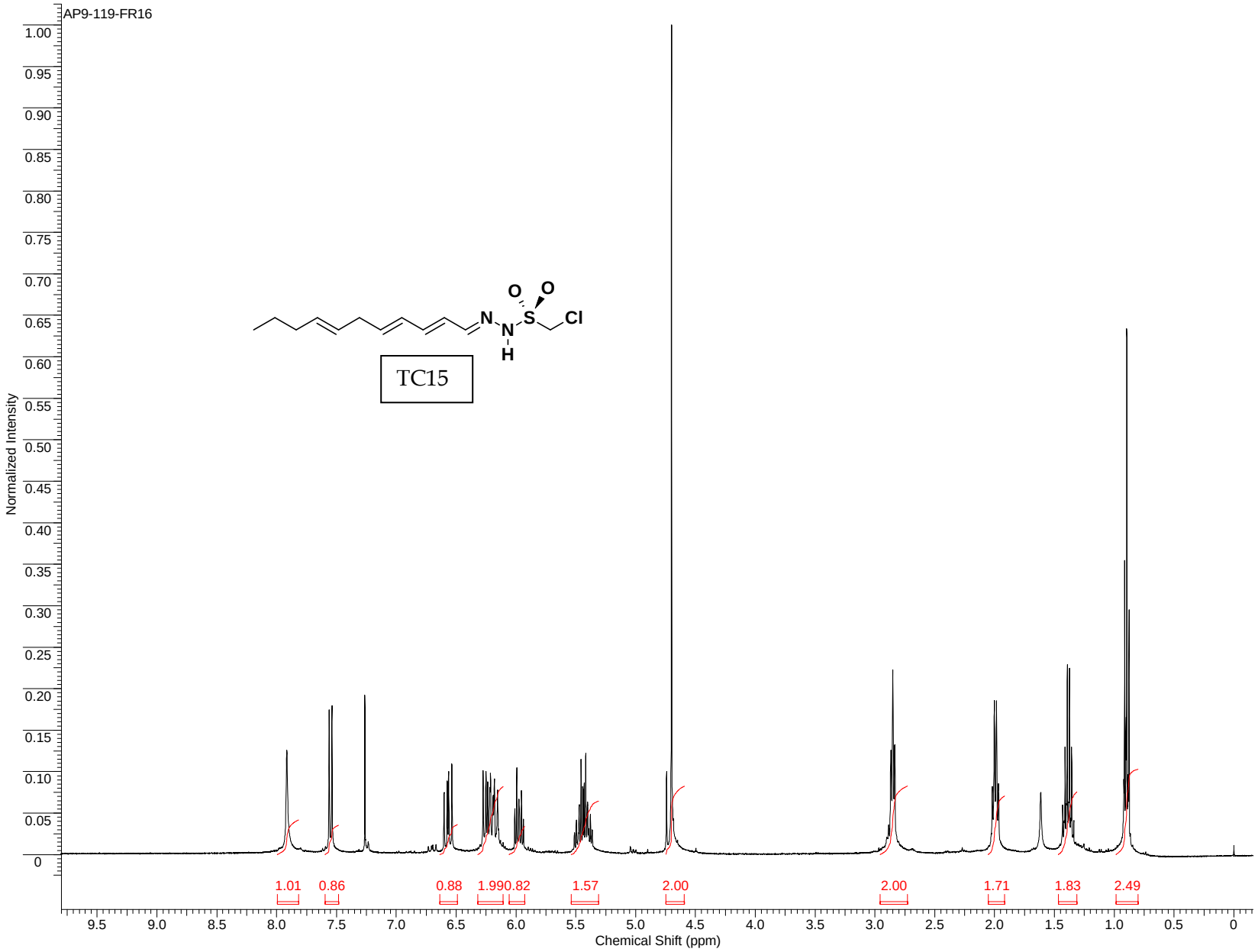
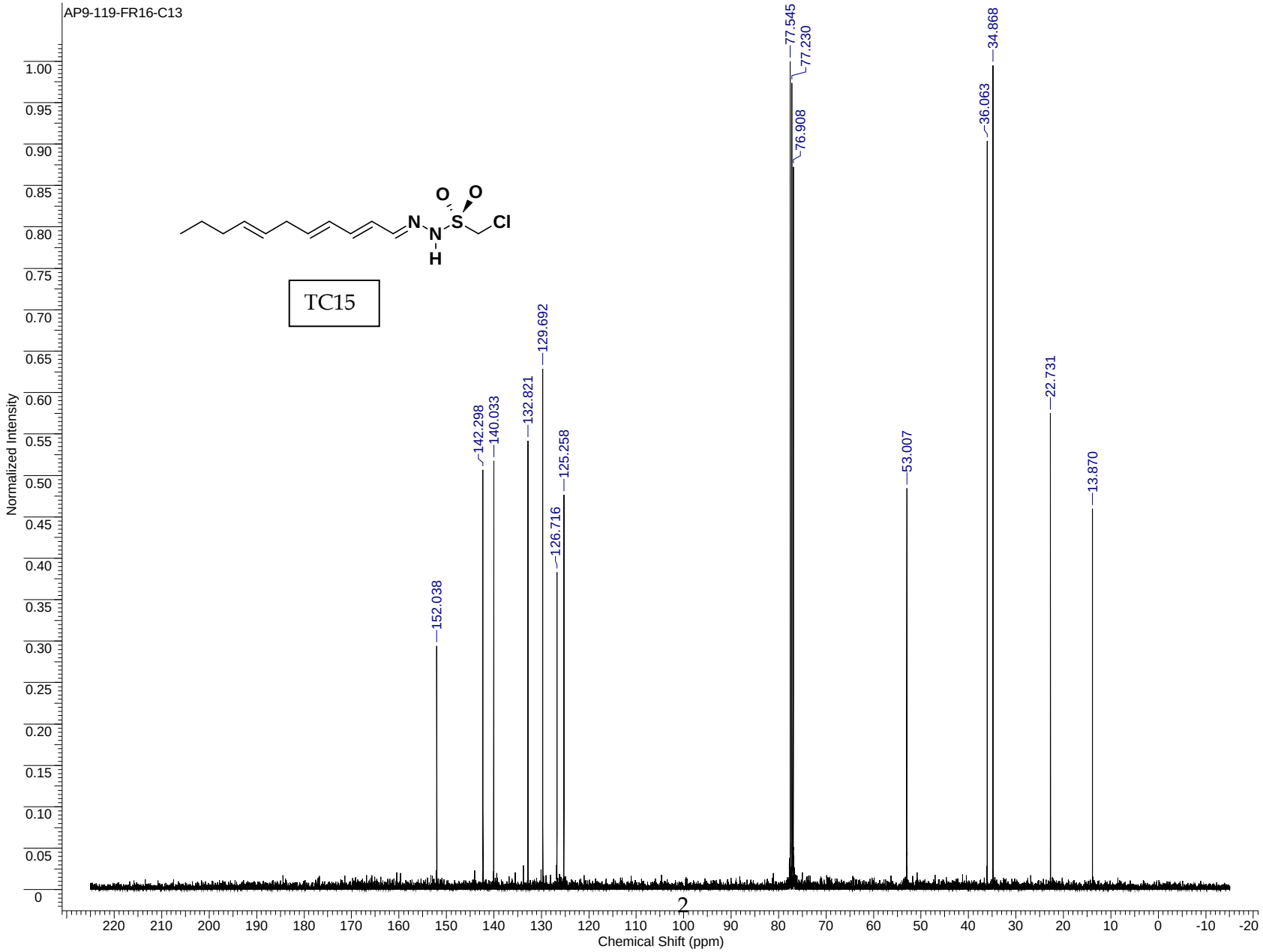

AP9-119-FR16
TC15
Normalized Intensity
Chemical Shift (ppm)
1.01
0.86
0.88
1.99
0.82
1.57
2.00
2.00
1.71
1.83
2.49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Chemical Shift (ppm)
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132.821
129.692
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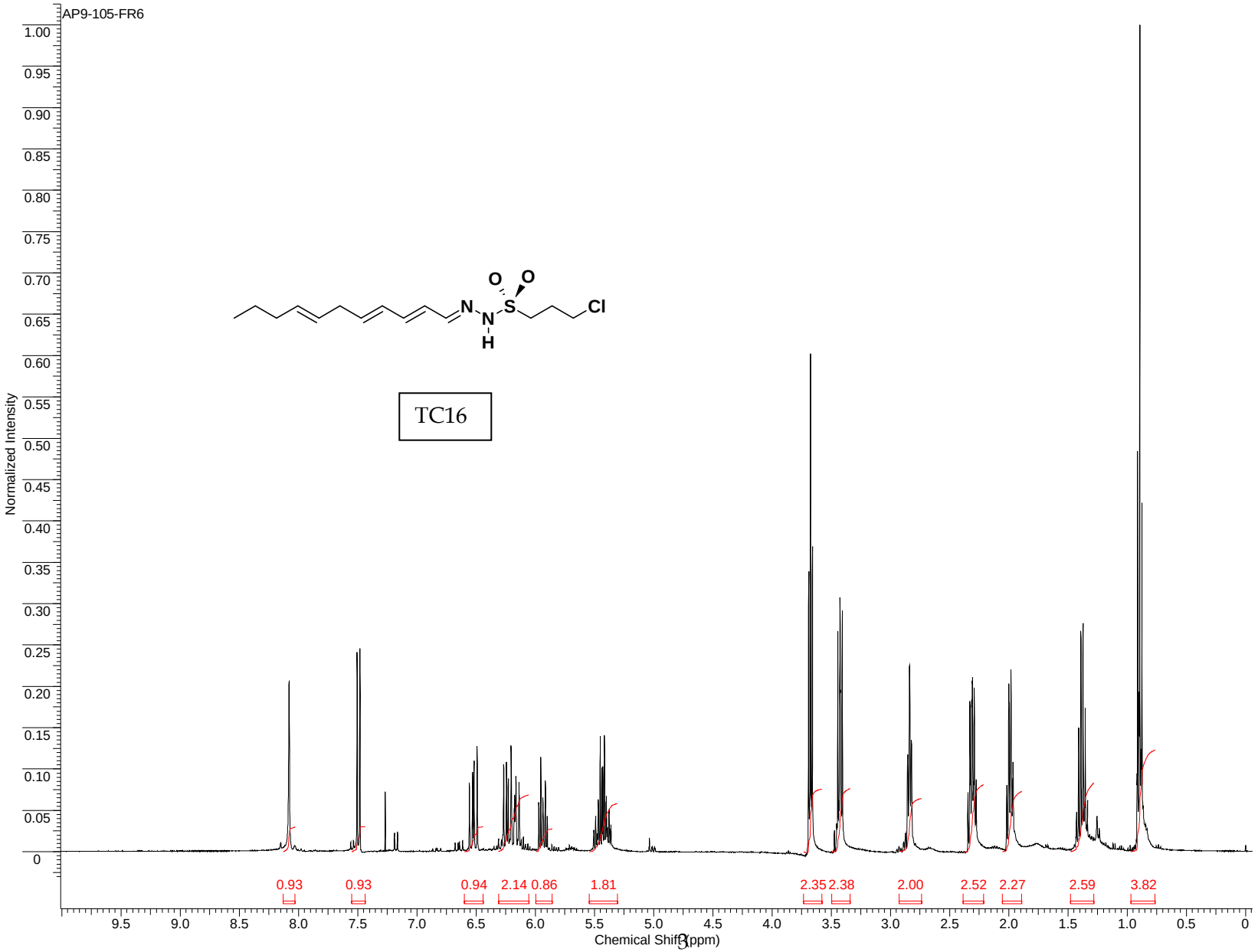
125.258
77.545
77.230
76.908
53.007
36.063
34.868
22.731
13.870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Normalized Intensity
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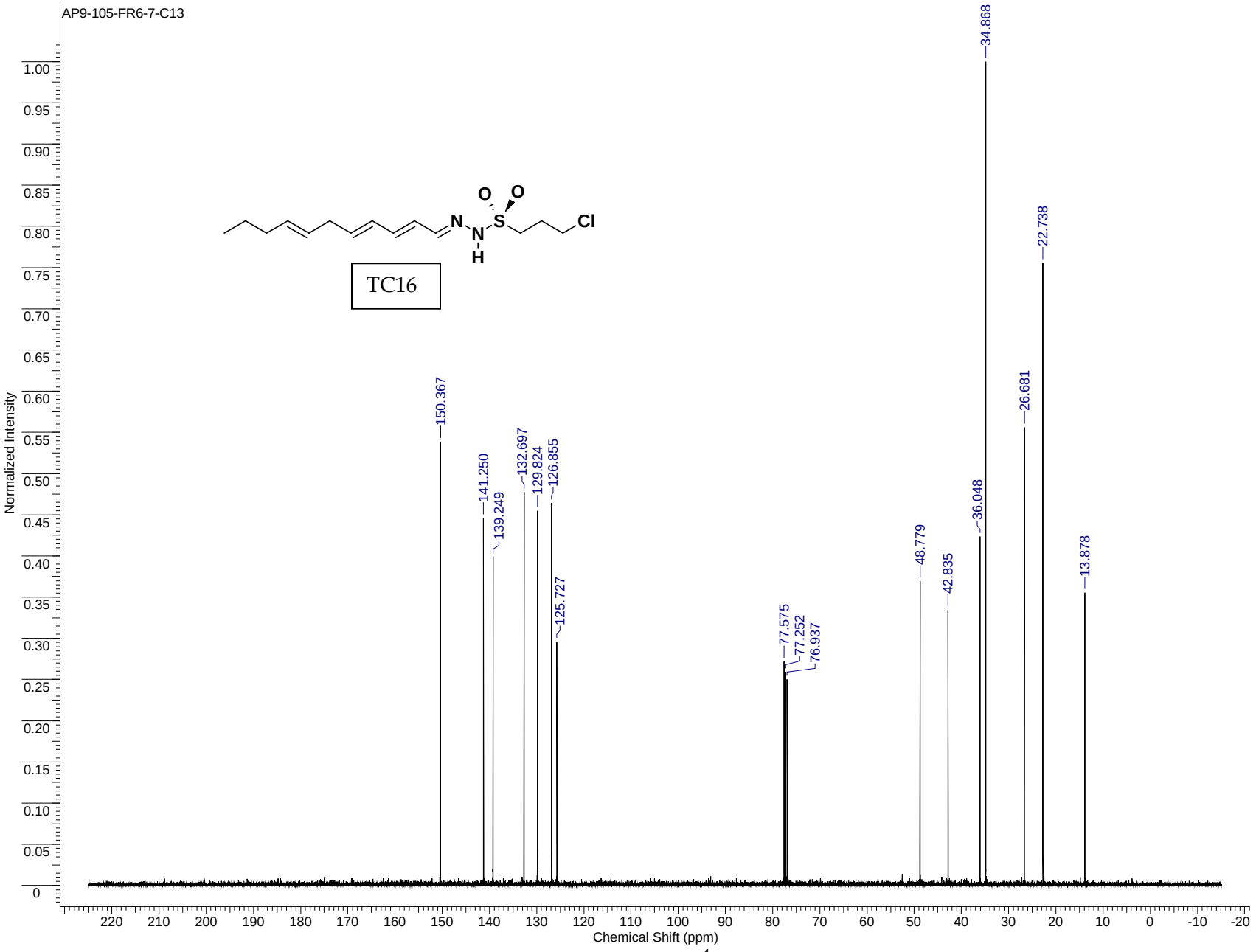
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
O
O
N
S
Cl
N
H
TC16
0.93
0.93
0.94
2.14
0.86
1.81
2.35
2.38
2.00
2.52
2.27
2.59
3.82
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)


AP9-105-FR6-7-C13
TC16
Normalized Intensity
Chemical Shift (ppm)
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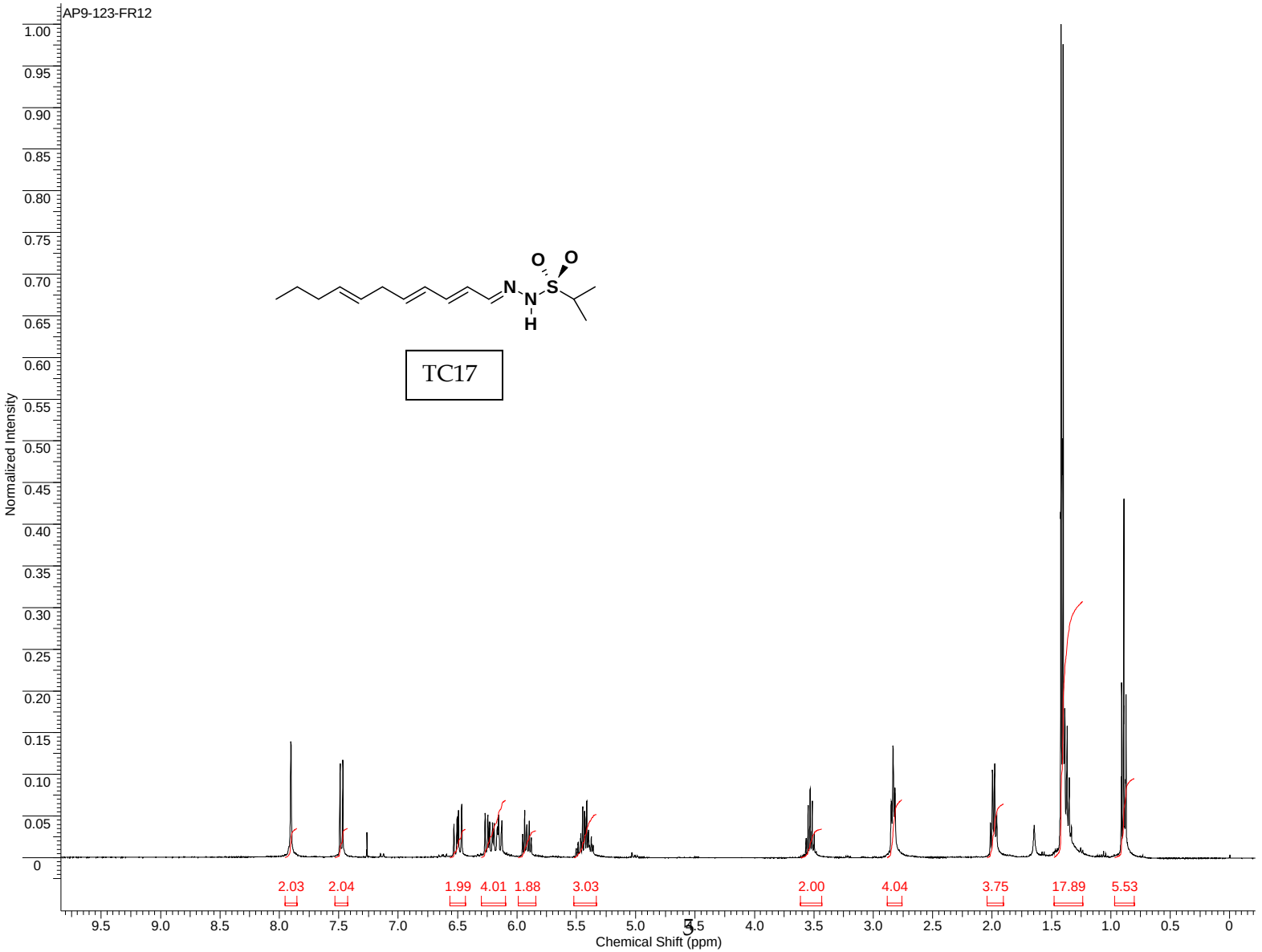
139.249
132.697
129.824
126.855
125.727
77.575
77.252
76.937
48.779
42.835
36.048
34.868
26.681
22.738
13.878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Normalized Intensity
Chemical Shift (ppm)
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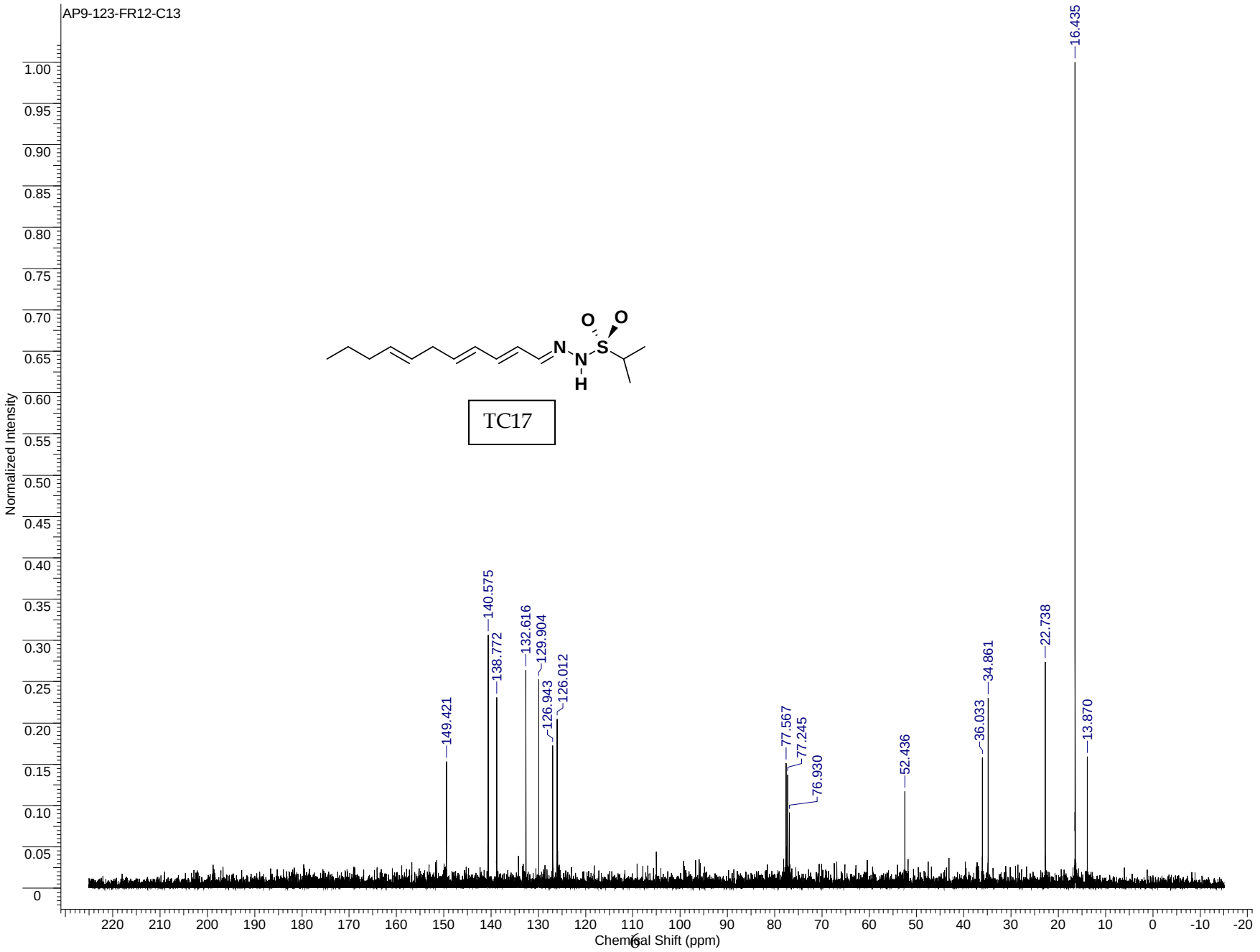
1.99
4.01
1.88
3.03
2.00
4.04
3.75
17.89
5.53
TC17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TC17
Normalized Intensity
Chemical Shift (ppm)
δ
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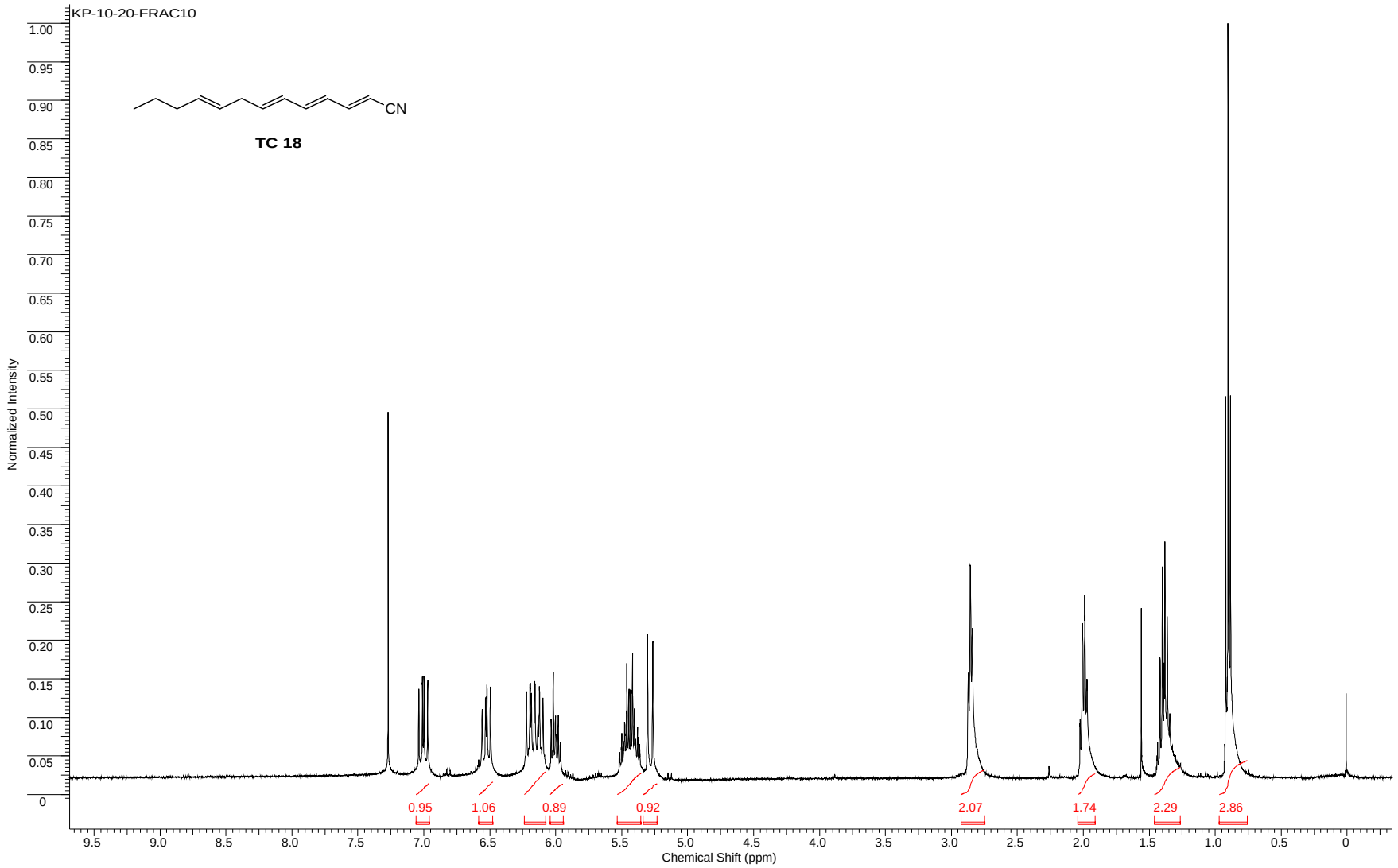
22.738
34.861
36.033
52.436
77.567
77.245
76.930
126.012
126.943
129.904
132.616
138.772
140.575
149.421
13.870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CN
TC 18
Normalized Intensity
Chemical Shift (ppm)
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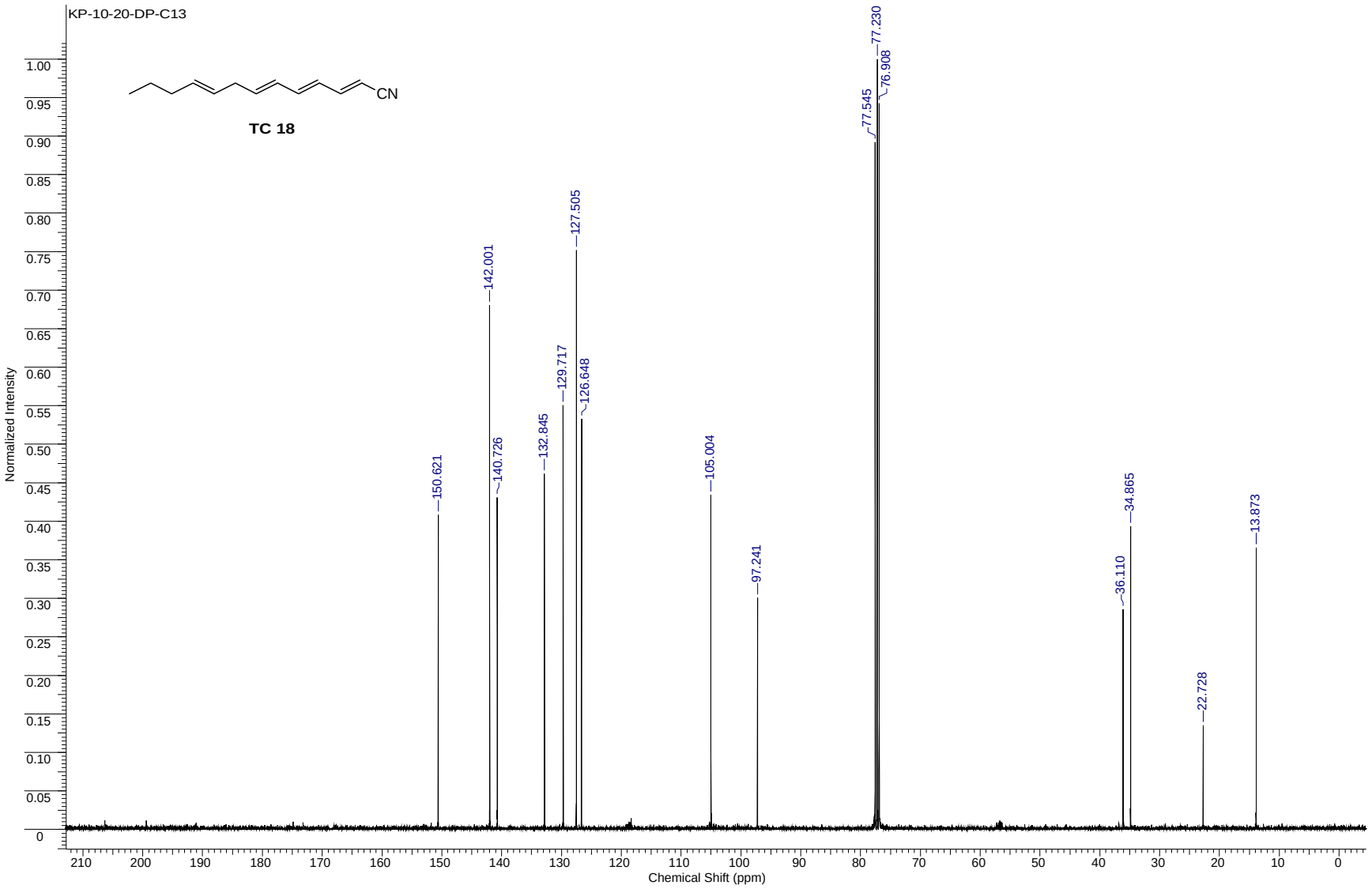
0.89
0.92
2.07
1.74
2.29
2.86


KP-10-20-DP-C13
CN
TC 18
Normalized Intensity
Chemical Shift (ppm)
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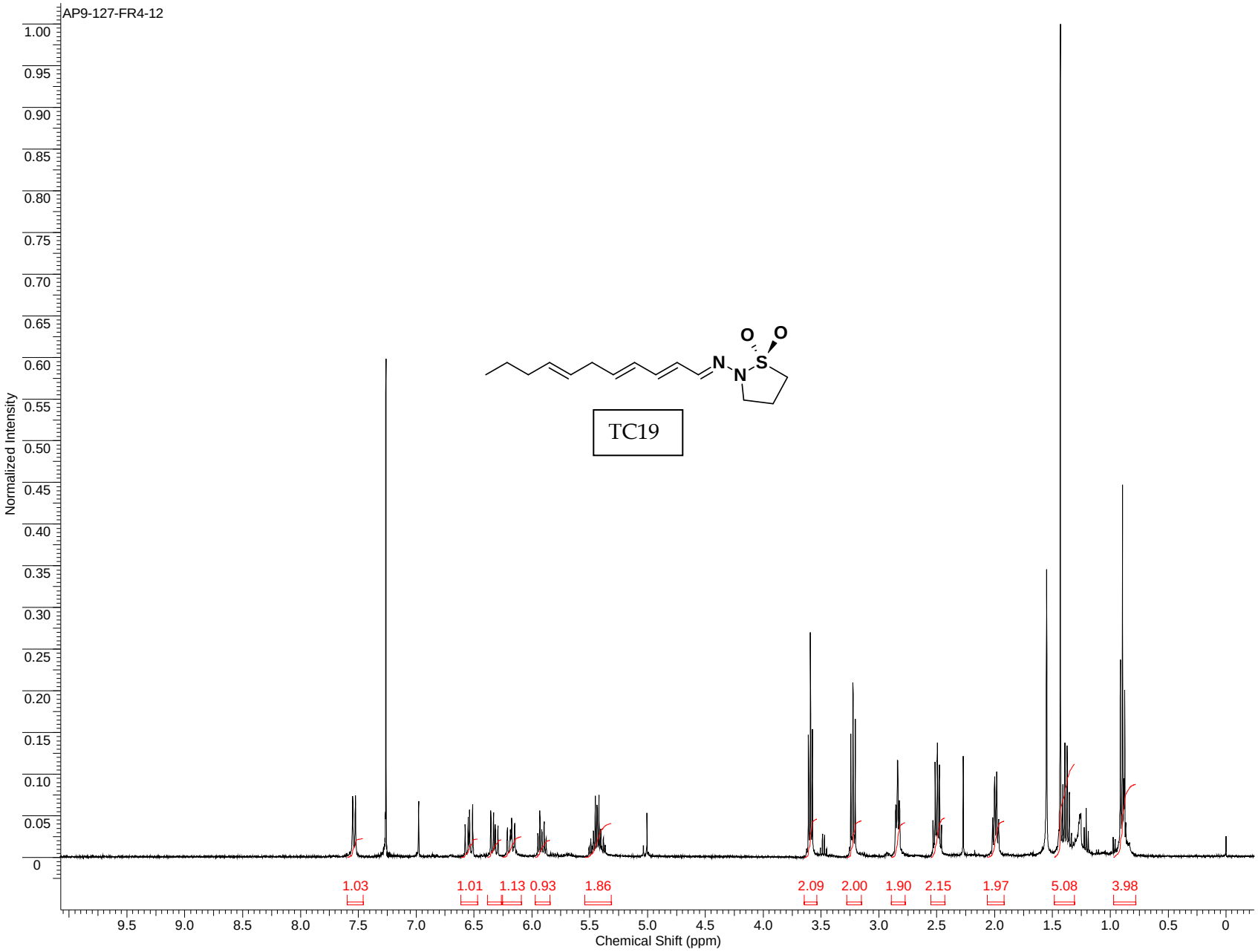
105.004
97.241
77.545
77.230
76.908
36.110
34.865
22.728
13.873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Normalized Intensity
Chemical Shift (ppm)
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1.13
0.93
1.86
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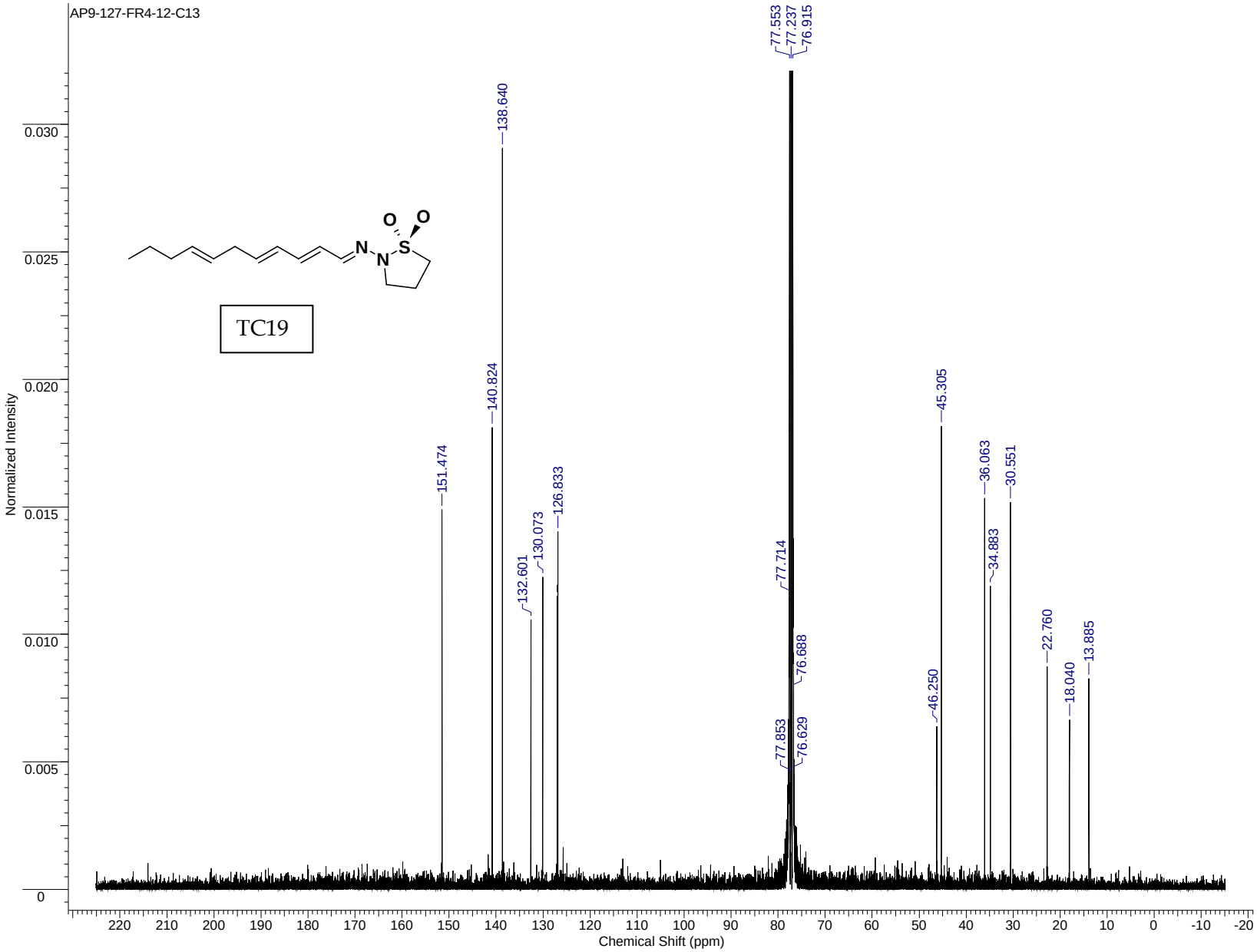
2.00
1.90
2.15
1.97
5.08
3.98
O
O
N
N
S
TC19


AP9-127-FR4-12-C13
TC19
Normalized Intensity
Chemical Shift (ppm)
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0.025
0.020
0.015
0.010
0.005
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
151.474
140.824
138.640
132.601
130.073
126.833
77.714
77.553
77.237
76.915
77.853
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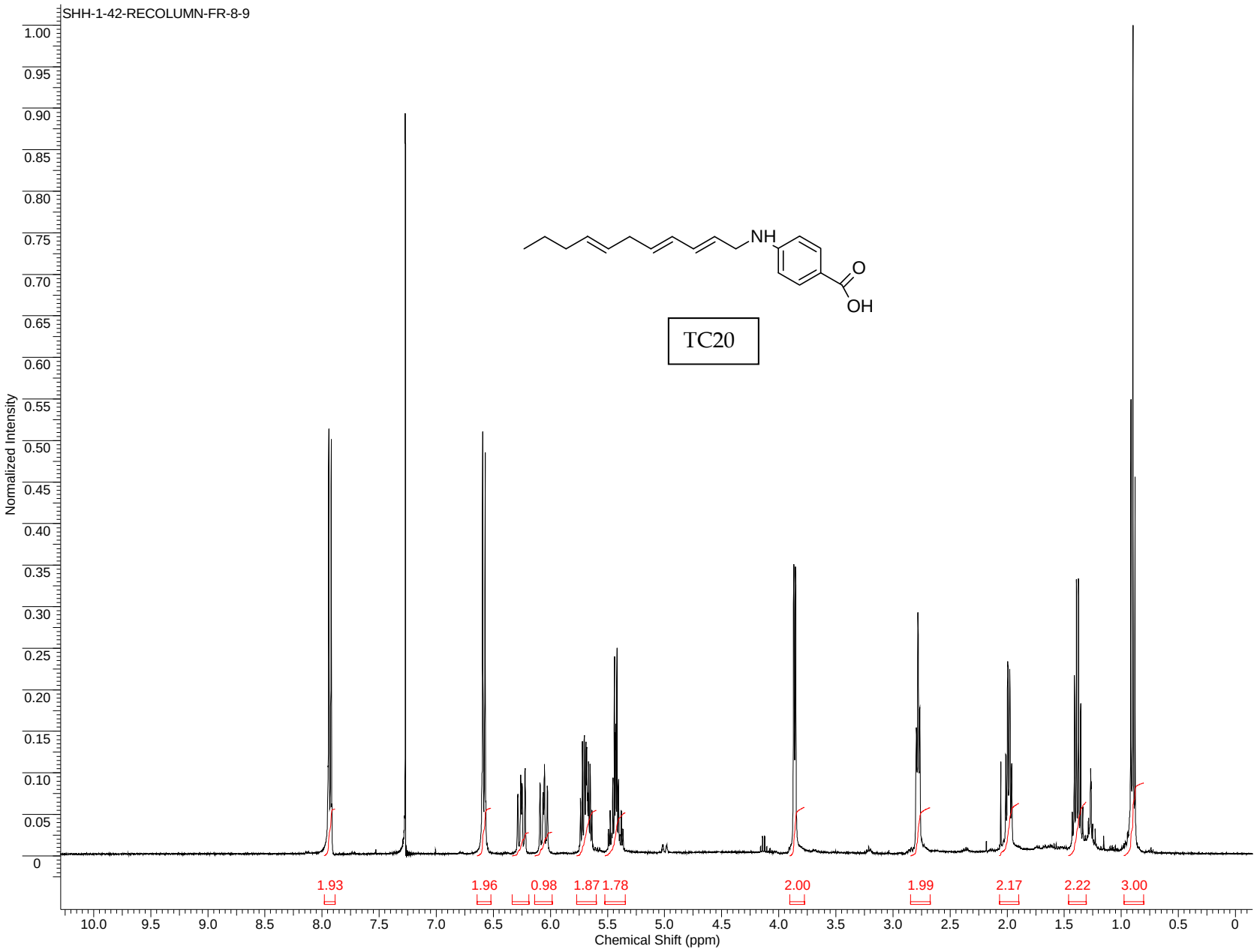
36.063
34.883
30.551
22.760
18.040
13.885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Normalized Intensity
Chemical Shift (ppm)
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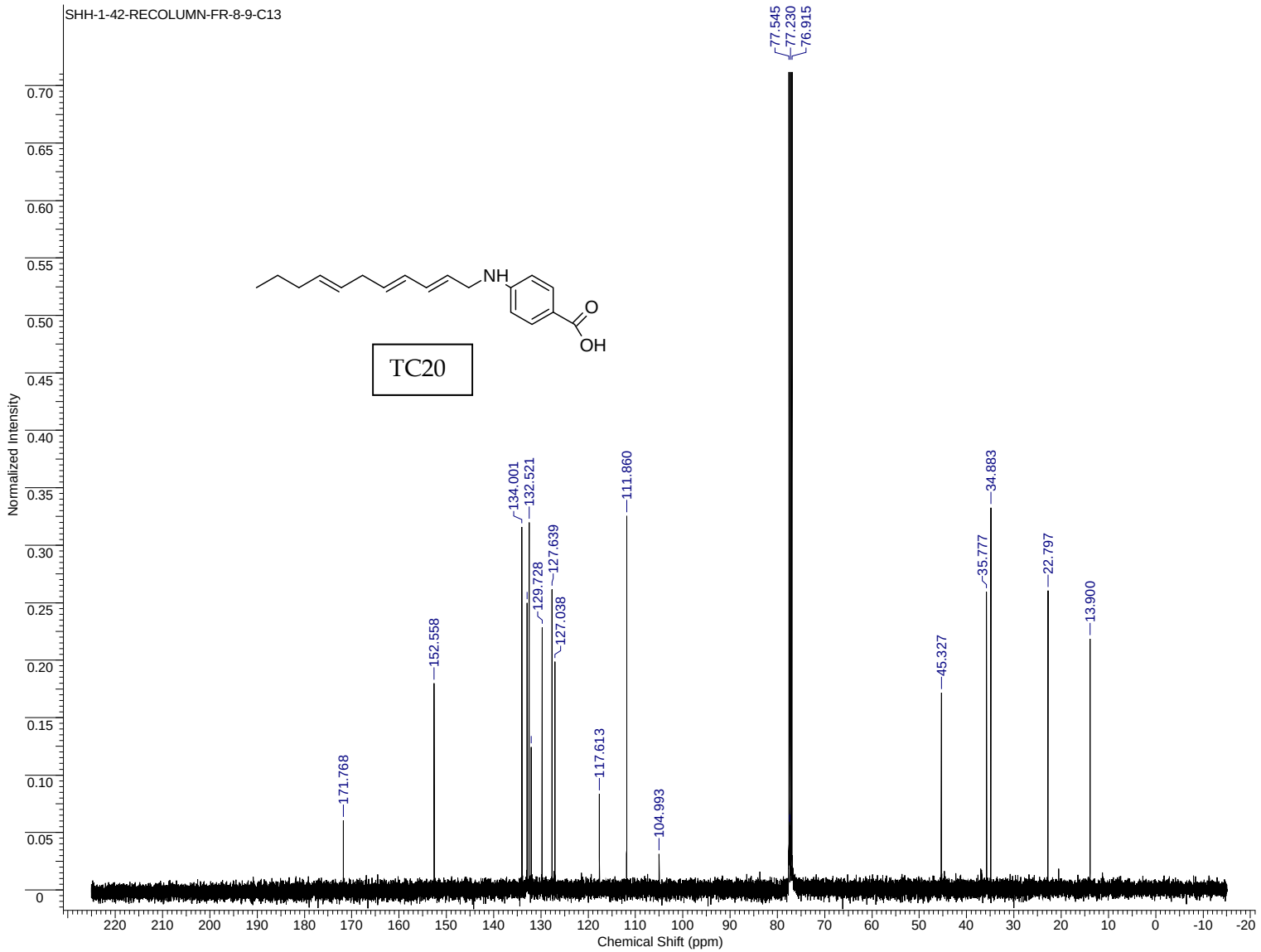
1.87
1.78
2.00
1.99
2.17
2.22
3.00
NH
O
OH
TC20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77.230
76.915
NH
O
OH
TC20
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132.521
129.728
127.639
127.038
111.860
152.558
171.768
117.613
104.993
45.327
35.777
34.883
22.797
13.900
Normalized Intensity
Chemical Shift (ppm)