**Supporting Information**

# Discovery of an Orally Bioavailable Small-Molecule Inhibitor for the *β*-Catenin/B-Cell Lymphoma 9 Protein−Protein Interaction

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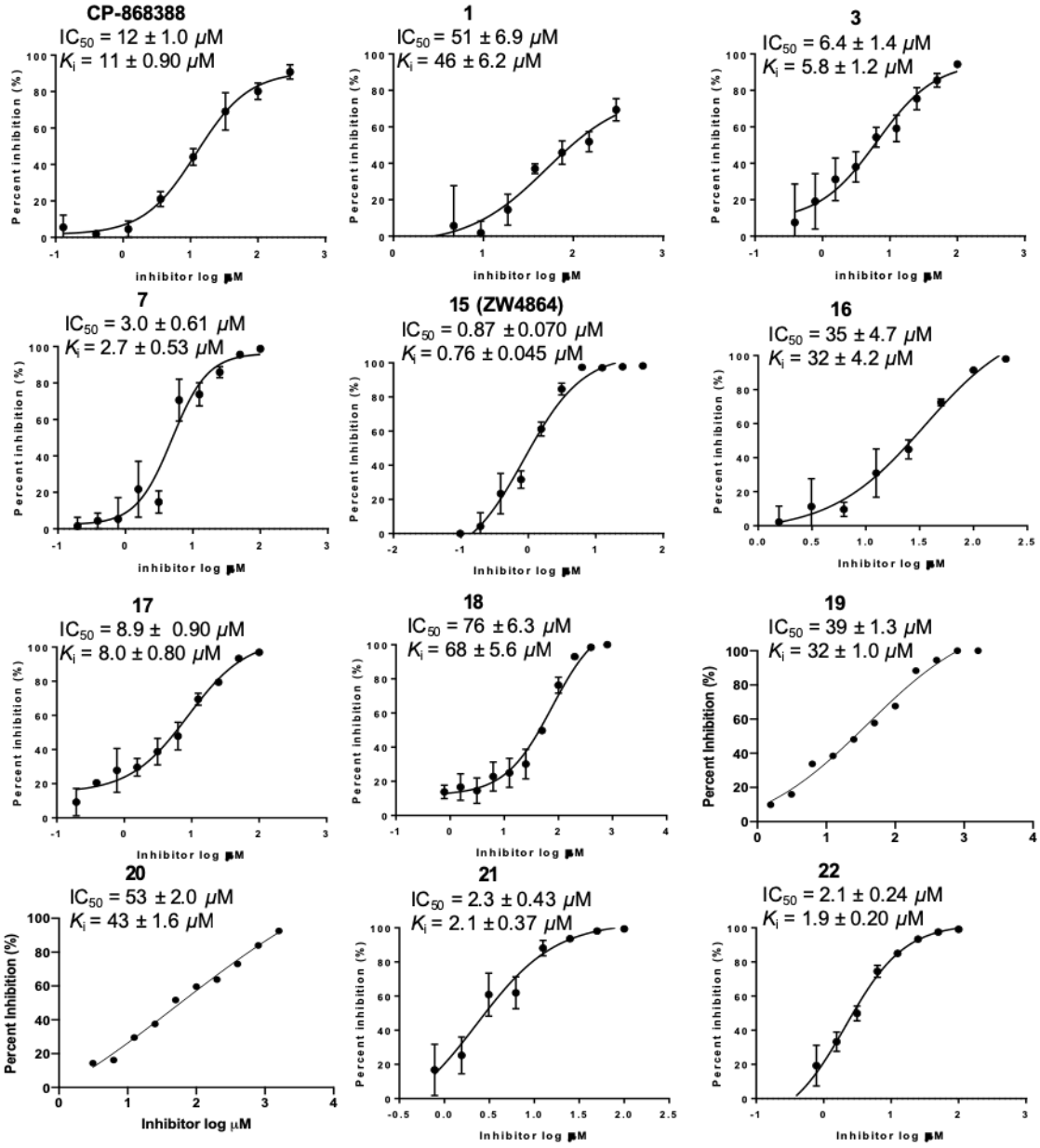
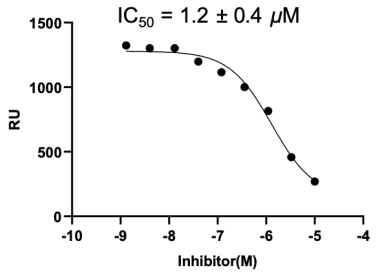
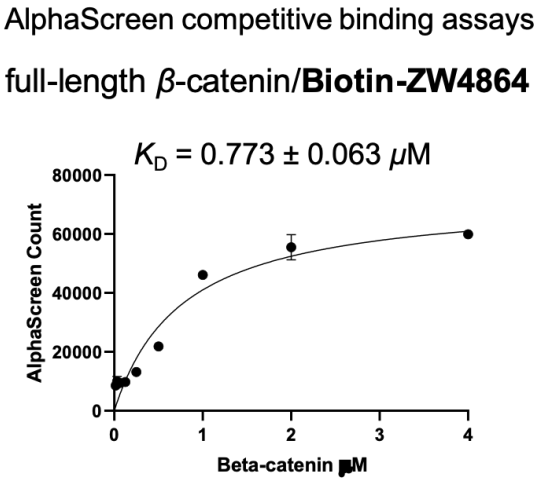
**Table of Contents**



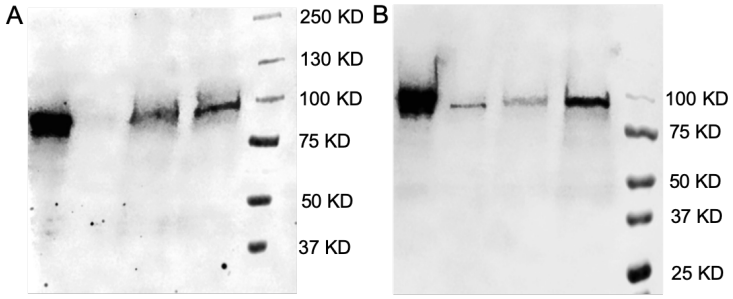

**Supplementary Figure S1**. The dose-response curves of AlphaScreen assays of **CP-868388, 1, 3, 7, 15 (ZW4864), and 16**–**22** for disruption of the full-length *β*-catenin/BCL9 PPI. Each set of data is expressed as mean ± standard deviation (n = 3).




**Supplementary Figure S2**. The fitting curve that derives the $IC_{50}$ of SPR competitive inhibition assay for disruption of $\beta$-catenin/BCL9 PPI. The concentrations of **ZW4864** for curve fitting are 30, 10, 1.1, 0.36, 0.12, 0.04, 0.013, 0.004, and 0.0013 $\mu$M, respectively.



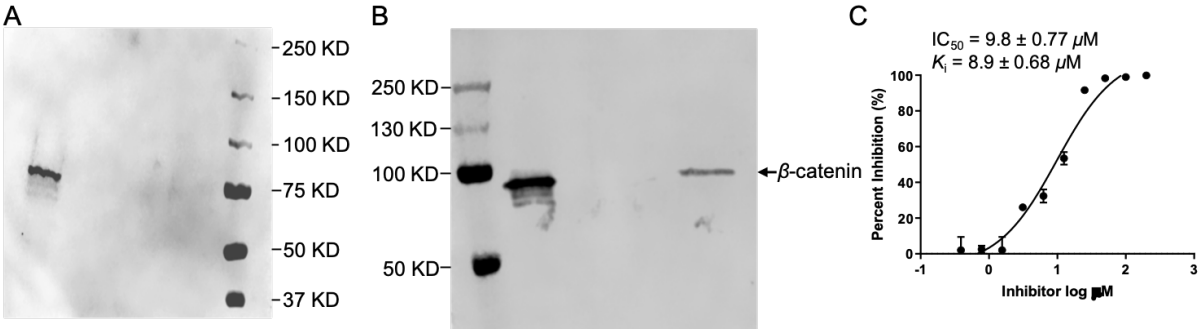

**Supplementary Figure S3.** The $K_D$ of **Biotin-ZW4864** with full-length $\beta$-catenin in AlphaScreen saturation binding assays. Increasing concentrations of $His_6$-tagged full-length $\beta$-catenin were incubated with 10 nM **Biotin-ZW4864** for 1 h. Donor and acceptor beads were then added into each well to a final concentration of 20 $\mu$g/mL in a 25 $\mu$L assay volume. The plate was read after 2 h incubation. Each experiment was performed in triplicate. Data are expressed as mean ± standard deviation (n = 3).




The normalized densities of the images of the protein pull-down experiment.

| **ZW4864,** $\mu$M | full-length $\beta$-catenin | SW480 cell lysates |
|---|---|---|
| Input | 1.00 | 1.00 |
| 0 | 0.01 ± 0.05 | 0.07 ± 0.04 |
| 1 | 0.10 ± 0.03 | 0.05 ± 0.03 |
| 10 | 0.28 ± 0.07 | 0.29 ± 0.05 |

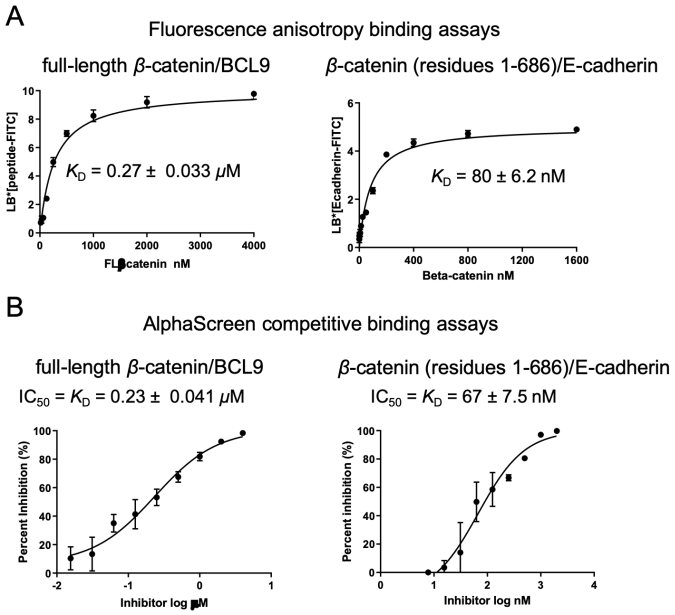
**Supplementary Figure S4**. Uncropped Western blot gels for the pull-down experiments of **Biotin-ZW4864** using (*A*) purified full-length human $\beta$-catenin. and (*B*) SW480 cell lysates.



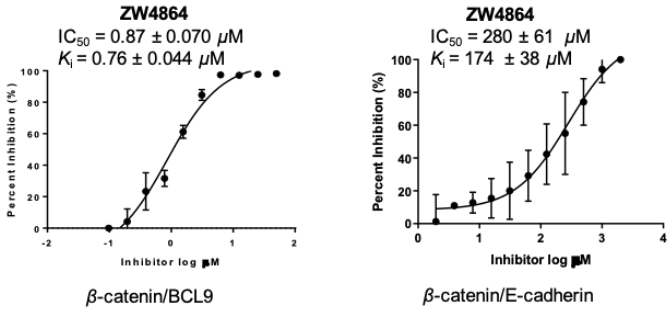

The normalized densities of the images of the protein pull-down experiment.

| **ZW4864**, $\mu$M | RIC | **ZW4864**, $\mu$M | R1C |
|---|---|---|---|
| Input | 1.00 | Input | 1.00 |
| 0 | 0.01 ± 0.01 | 0 | 0.03 ± 0.01 |
| 1 | 0.03 ± 0.02 | 5 | 0.07 ± 0.01 |
| 10 | 0.04 ± 0.02 | 50 | 0.27 ± 0.06 |

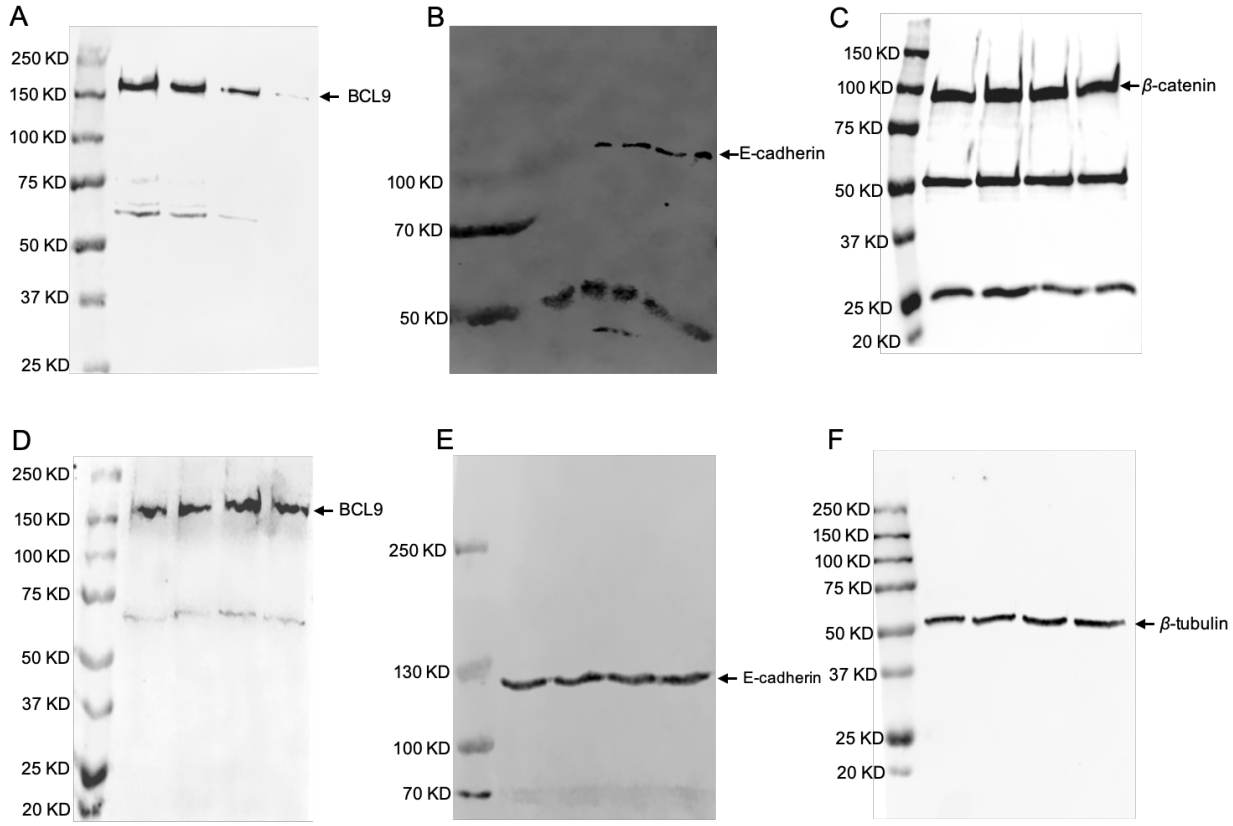
**Supplementary Figure S5**. Uncropped Western blot gels for the pull-down experiments of **Biotin-ZW4864** at (*A*) 1 and 10 $\mu$M, (*B*) 5 and 50 $\mu$M using $\beta$-catenin RIC (residue 138–781). (*C*) AlphaScreen assay results of **ZW4864** for disruption of $\beta$-catenin RIC/BCL9 PPI. Each set of data is expressed as mean ± standard deviation (n = 3).




**Supplementary Figure S6.** The $K_D$s of full-length $\beta$-catenin with BCL9 HD2 peptide (residues 350–375) and the $K_D$s of $\beta$-catenin (residues 1-686) with E-cadherin peptide (residues 824–877) using (*A*) fluorescence anisotropy binding assays and (*B*) AlphaScreen competitive binding assays. Each set of data is expressed as mean ± standard deviation (n = 3).



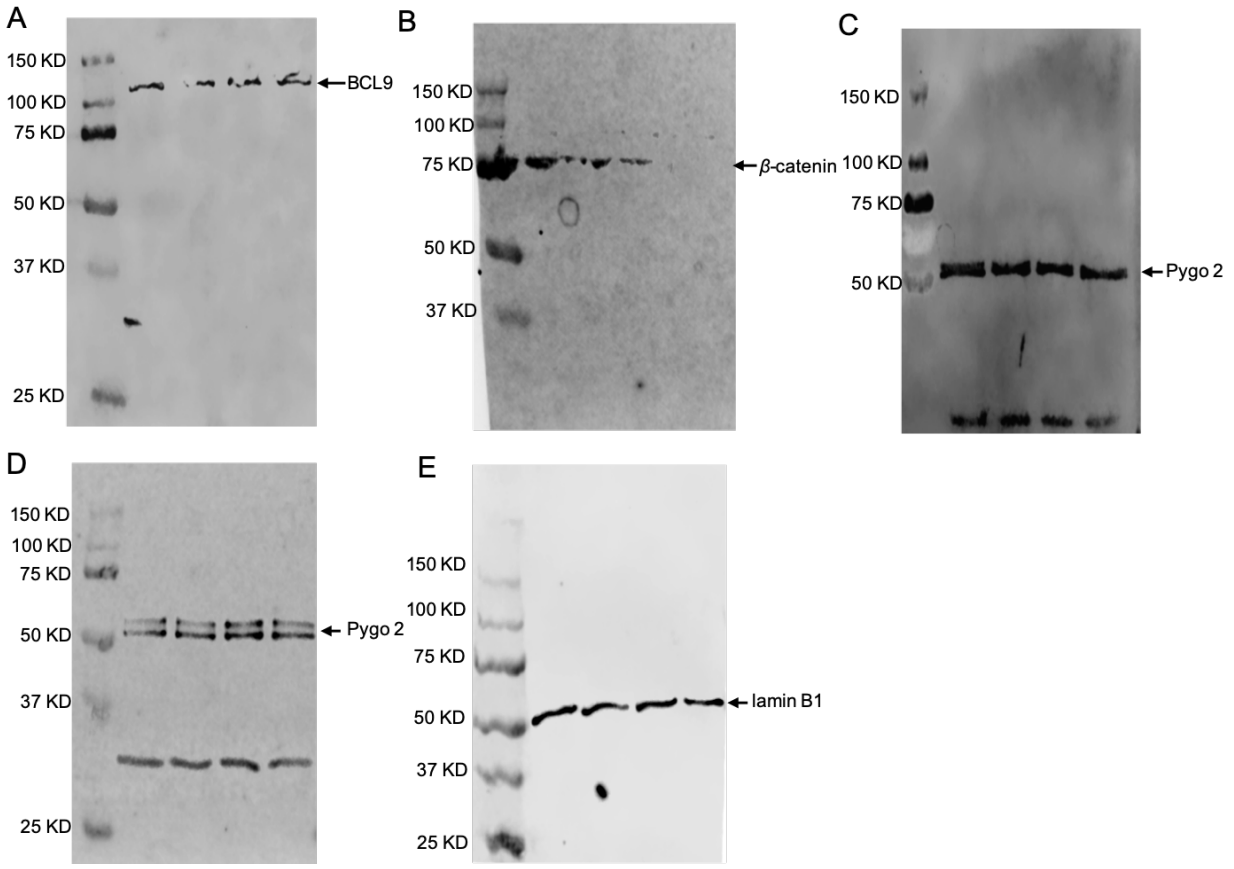

**Supplementary Figure S7**. AlphaScreen selectivity assay results of **ZW4864** for disruption of full-length $\beta$-catenin/BCL9 and full-length $\beta$-catenin/E-cadherin PPIs. Each set of data is expressed as mean ± standard deviation (n = 3).




The normalized densities of the images of the co-IP assays.

| **ZW4864**, $\mu$M | IP: $\beta$-catenin IB: BCL9 | IP: $\beta$-catenin IB: E-cadherin | IP: $\beta$-catenin IB: $\beta$-catenin | Input: E-cadherin | Input: BCL9 | Input: $\beta$-tubulin |
|---|---|---|---|---|---|---|
| 0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 0.73 ± 0.06 | 1.10 ± 0.05 | 1.05 ± 0.08 | 1.10 ± 0.04 | 1.03 ± 0.05 | 1.05 ± 0.08 |
| 20 | 0.42 ± 0.05 | 1.05 ± 0.08 | 0.99 ± 0.08 | 1.05 ± 0.06 | 1.11 ± 0.09 | 1.10 ± 0.06 |
| 40 | 0.20 ± 0.03 | 1.15 ± 0.10 | 0.95 ± 0.06 | 1.10 ± 0.09 | 1.02 ± 0.07 | 1.06 ± 0.05 |

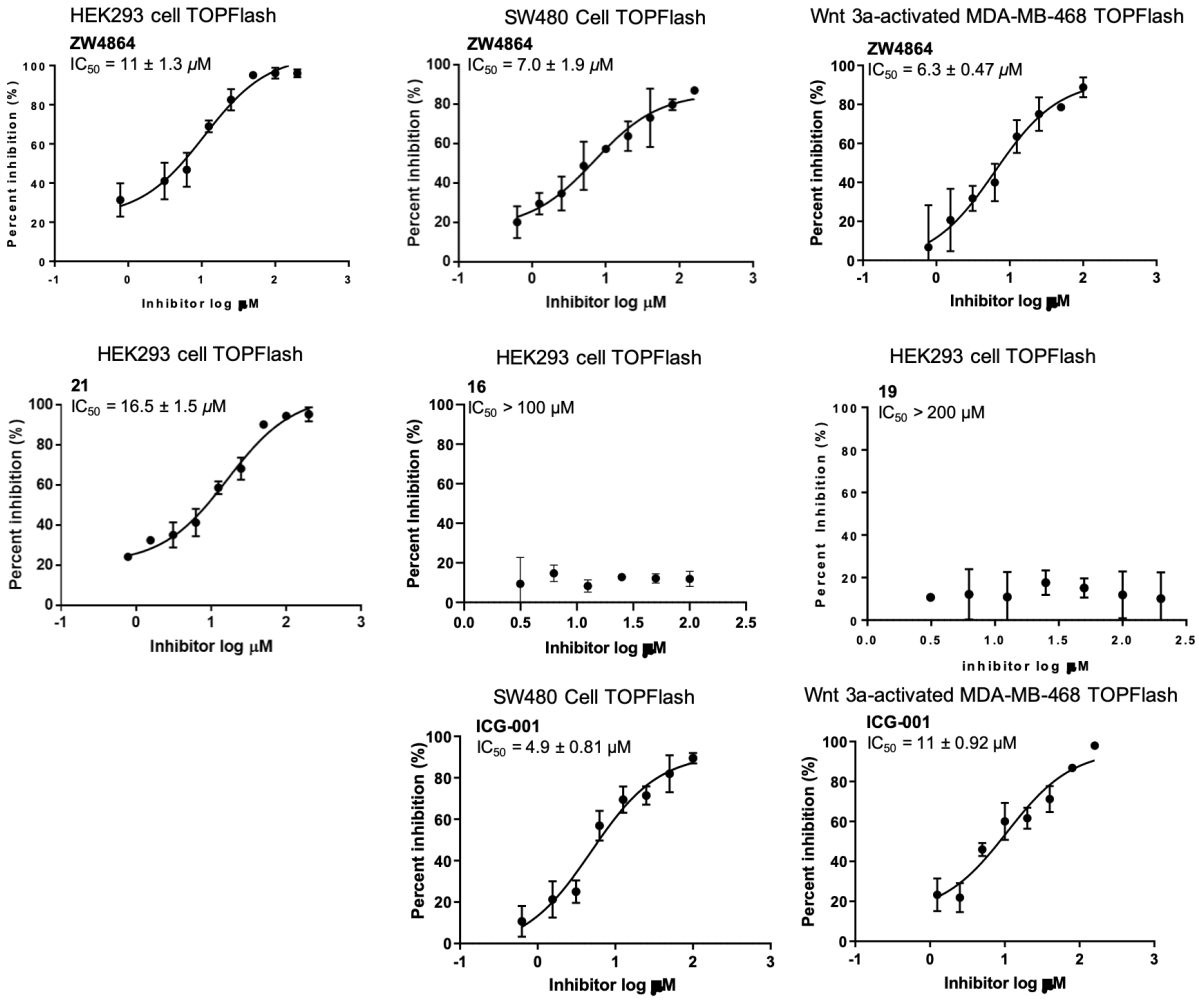
**Supplementary Figure S8**. Uncropped Western blot gels for the co-IP experiments to evaluate effects of **ZW4864** for disruption of the $\beta$-catenin/ BCL9 PPI and selectivity for $\beta$-catenin/BCL9 over $\beta$-catenin/E-cadherin PPIs. (*A*) $\beta$-Catenin immunoprecipitation (IP) and then BCL9 immunoblotting (IB); (*B*) $\beta$-catenin IP and then E-cadherin IB; (*C*) $\beta$-catenin IP and then $\beta$-catenin IB; (*D*) BCL9 IB as the input; (*E*) E-cadherin IB as the input; and (*F*) $\beta$-tubulin IB as the input.



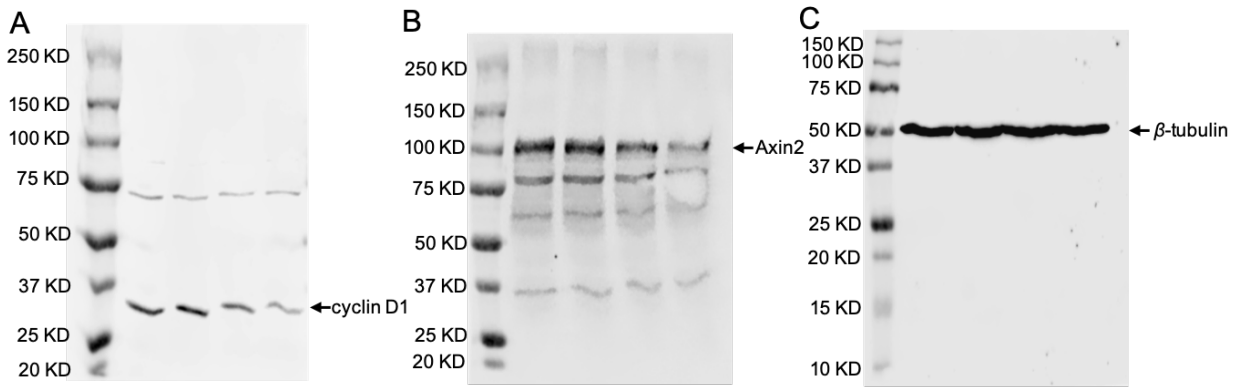

The normalized densities of the images of co-IP experiments to evaluate effects of **ZW4864** for disruption of BCL9/Pygo and *β*-catenin/Pygo interactions.

| **ZW4864**, $\mu$M | IP: Pygo2<br>IB: BCL9 | IP: Pygo2<br>IB: *β*-catenin | IP: Pygo2<br>IB: Pygo2 | Input:<br>Pygo2 | Input:<br>lamin B1 |
|---|---|---|---|---|---|
| 0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 0.91 ± 0.06 | 0.69 ± 0.05 | 1.05 ± 0.05 | 1.06 ± 0.06 | 0.92 ± 0.05 |
| 20 | 1.01 ± 0.07 | 0.36 ± 0.04 | 1.05 ± 0.06 | 1.19 ± 0.08 | 0.96 ± 0.07 |
| 40 | 1.07 ± 0.08 | 0.05 ± 0.01 | 0.99 ± 0.07 | 1.07 ± 0.06 | 0.93 ± 0.04 |

**Supplementary Figure S9**. Uncropped gels for co-IP experiments to evaluate effects of **ZW4864** for disruption of BCL9/Pygo and *β*-catenin/Pygo interactions. (*A*) Pygo 2 immunoprecipitation (IP) and then BCL9 immunoblotting (IB); (*B*) pygo 2 IP and then *β*-catenin IB; (*C*) pygo 2 IP and then pygo 2 IB; (*D*) pygo 2 as the input; and (*E*) lamin B1 as the input.



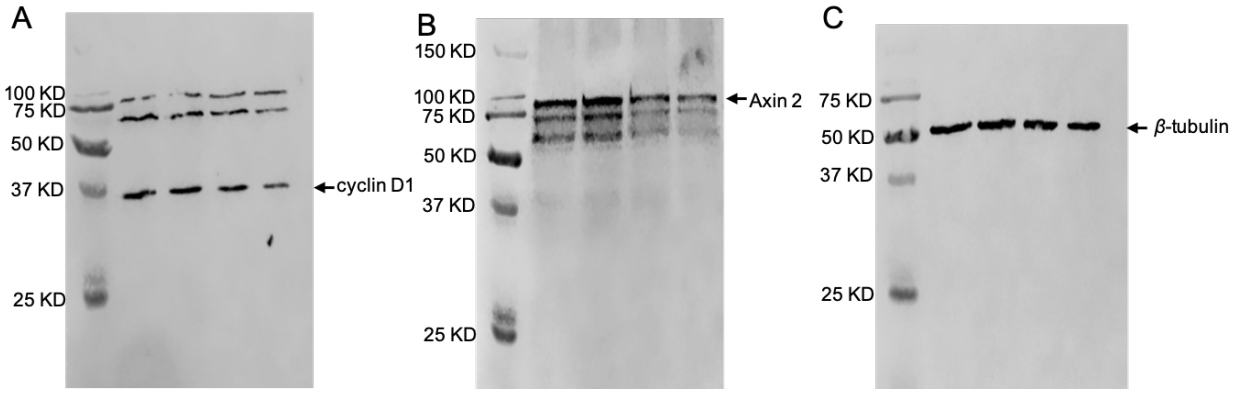

**Supplementary Figure S10**. The dose-response curves of Wnt-responsive TOPFlash luciferase reporter assay results of compounds **ZW4864 (15)**, **21**, **16**, **19** and **ICG-001**.




The normalized densities of the images of the Western blot analyses

| **ZW4864**, $\mu$M | Axin2 | cyclin D1 | $\beta$-tubulin |
|---|---|---|---|
| 0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.06 ± 0.08 | 1.03 ± 0.06 | 1.04 ± 0.06 |
| 20 | 0.62 ± 0.05 | 0.56 ± 0.06 | 1.02 ± 0.08 |
| 40 | 0.35 ± 0.04 | 0.31 ± 0.05 | 0.95 ± 0.07 |

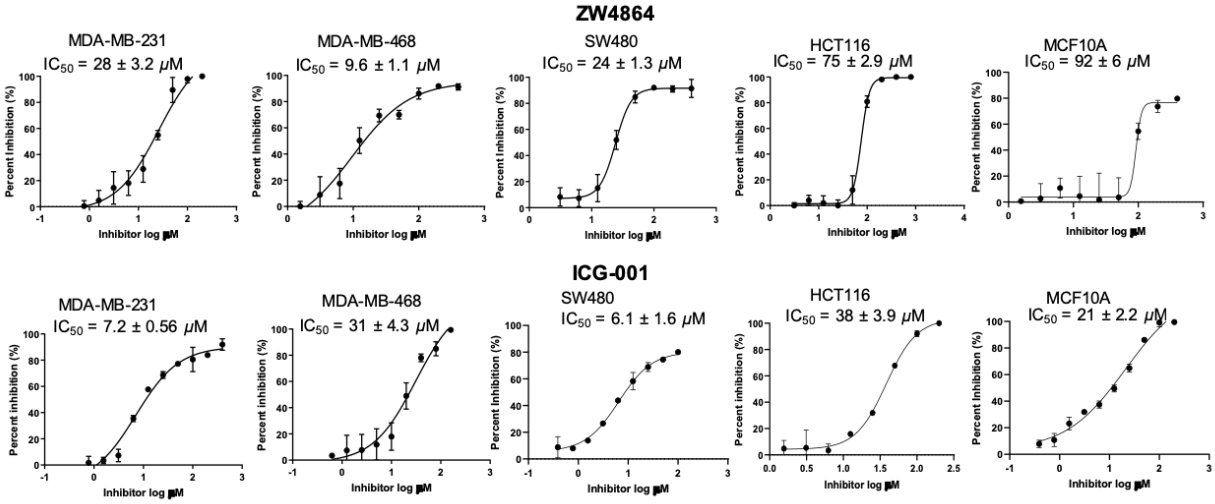
**Supplementary Figure S11.** Uncropped gels of Western blot experiments of **ZW4864** in SW480 cells, as described in Figure 2G. (*A*) Cyclin D1; (*B*) Axin2; and (*C*) $\beta$-tubulin.



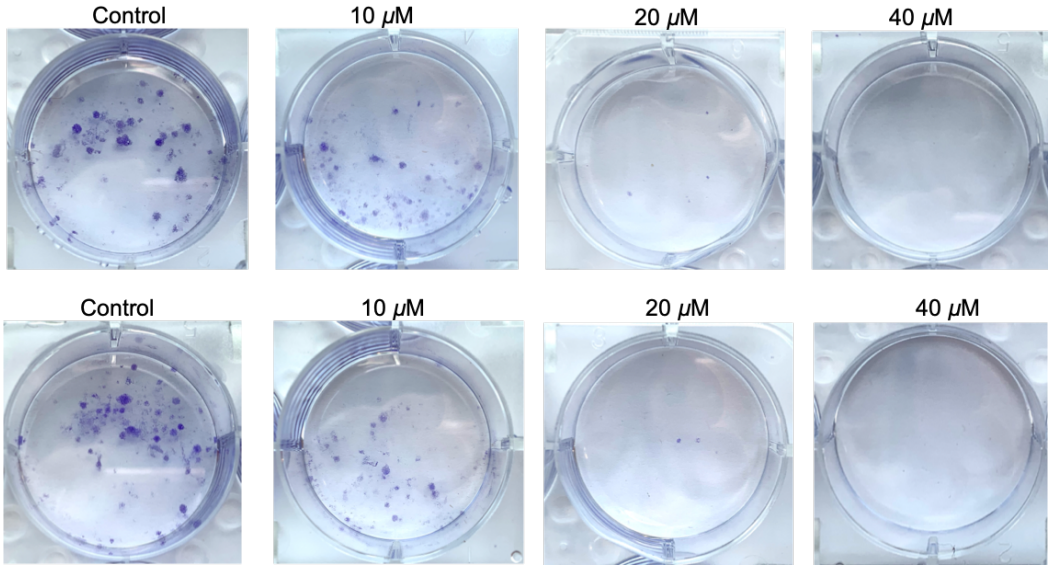

The normalized densities of the images of the Western blot analyses

| **ZW4864**, $\mu$M | Axin2 | cyclin D1 | $\beta$-tubulin |
|---|---|---|---|
| 0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.08 ± 0.09 | 0.98 ± 0.08 | 0.95 ± 0.09 |
| 20 | 0.57 ± 0.06 | 0.67 ± 0.07 | 0.92 ± 0.08 |
| 40 | 0.29 ± 0.04 | 0.32 ± 0.05 | 0.93 ± 0.08 |

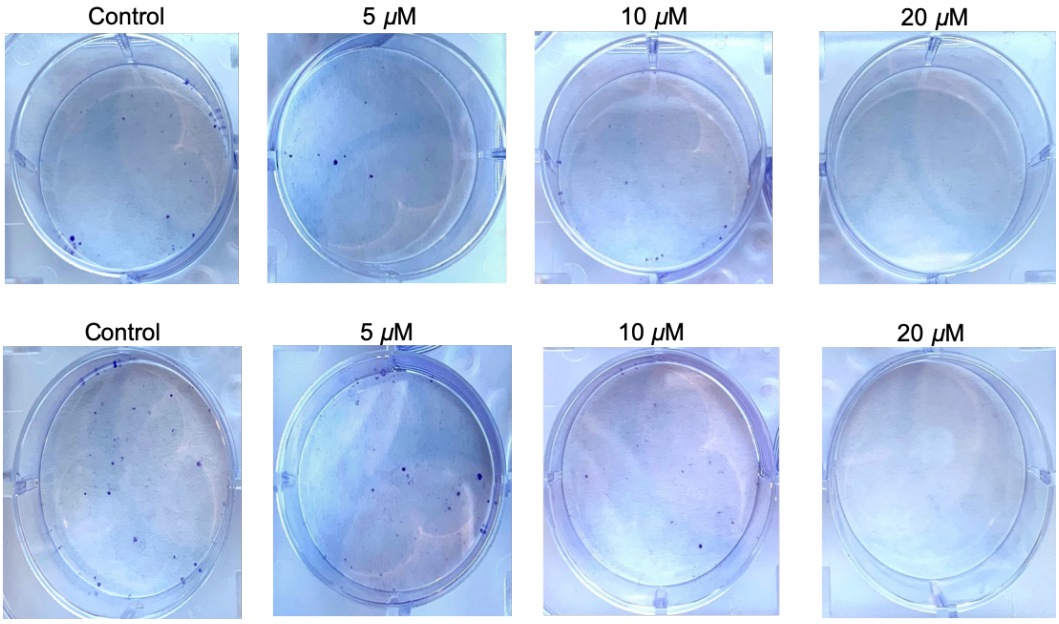
**Supplementary Figure S12**. Uncropped gels of Western blot experiments of **ZW4864** in Wnt 3a-activated TNBC MDA-MB-231 cells, as described in Figure 2G. (*A*) Cyclin D1; (*B*) Axin 2; and (*C*) $\beta$-tubulin.



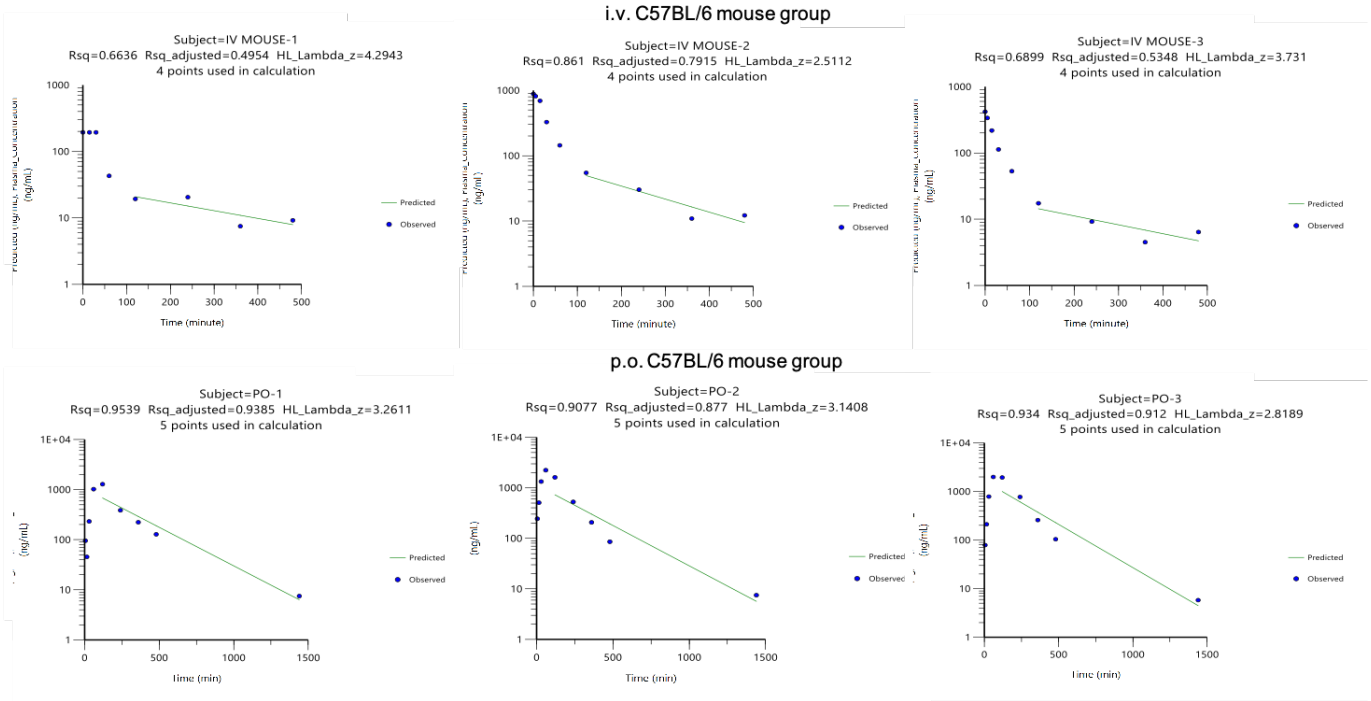

**Supplementary Figure S13.** MTS growth inhibition assay results of **ZW4864** and **ICG-001** against various TNBC cell lines (MDA-MB-231 and MDA-MB-468), colorectal cancer lines (SW480 and HCT116), and normal mammary epithelial MCF10A cell line after 72-h treatment.



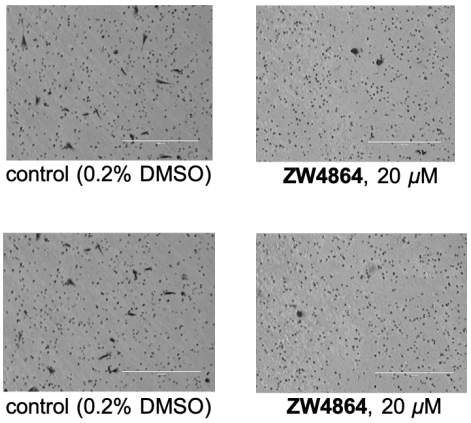

**Supplementary Figure S14.** The clonogenic assay results of **ZW4864** using MDA-MB-231 cells.



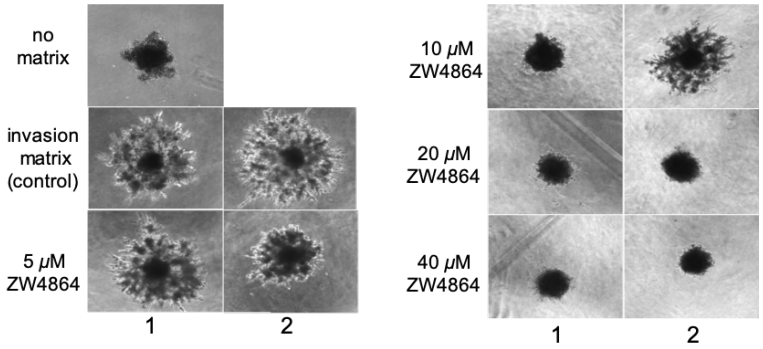

**Supplementary Figure S15.** Clonogenic assay results of **ZW4864** using MDA-MB-468 cells.



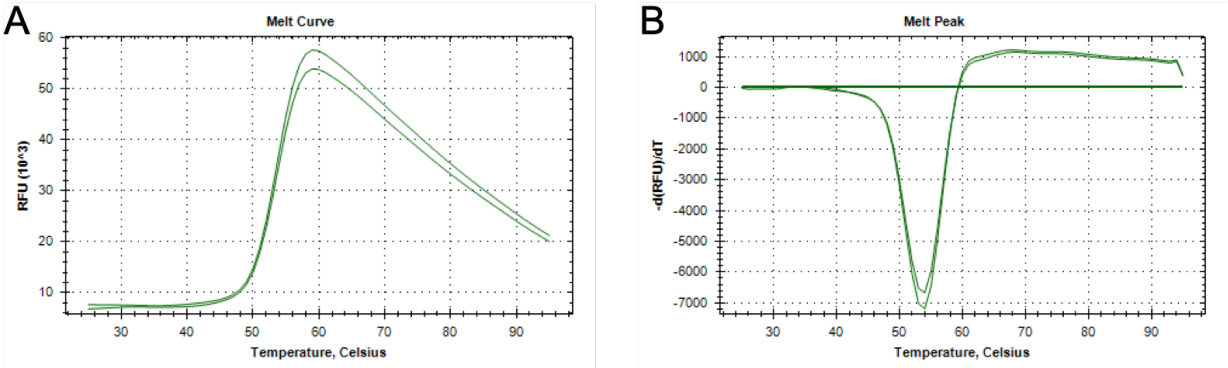

**Supplementary Figure S16.** Original data plots of C57BL/6 mouse PK studies of **ZW4864**.




**Supplementary Figure S17.** Original images of the Matrigel invasion assay results of **ZW4864**.




**Supplementary Figure S18.** Original images of 3D spheroid BME cell invasion assays of **ZW4864.**




**Supplementary Figure S19.** The quality of full-length *β*-catenin proteins after purification was examined by thermal shift assays. Here is one example. A temperature increment of 1°/min was applied.

**HPLC Condition and Traces**

The purity of final compounds was determined by HPLC analysis. The instrument was an Agilent 1260 Infinity II HPLC system with a quaternary pump, a vial sampler, and a DAD detector. A Kromasil 300–5–C18 column (4.6 × 250 mm) was used. The DAD detector was set to 254 nm. The purity of all tested compounds was >95%. Some HPLC traces are shown below.

**Condition**: Elute with gradient starting with 0.1% TFA in water and ending with 0.1% TFA in water and acetonitrile mixture (water with 0.1% TFA : acetonitrile = 1 : 1) for 10 min, and then change to a 10 min-gradient starting with 0.1% TFA in water and acetonitrile 1 : 1 mixture and ending with 100% acetonitrile.

| Compound ID | Purity (%) | Retention time (min) |
|---|---|---|
| **1** | 98.6 | 12.48 |
| **2** | 98.9 | 12.51 |
| **3** | 97.6 | 10.96 |
| **4** | 100 | 11.37 |
| **5** | 98.0 | 11.55 |
| **6** | 100 | 12.33 |
| **7** | 100 | 12.15 |
| **8** | 100 | 12.49 |
| **9** | 99.4 | 13.42 |
| **10** | 100 | 12.29 |
| **11** | 99.4 | 11.90 |
| **12** | 97.1 | 8.05 |
| **13** | 99.2 | 8.21 |
| **14** | 98.8 | 13.07 |
| **15** | 96.8 | 9.35 |
| **16** | 98.8 | 9.34 |
| **17** | 99.1 | 9.41 |
| **18** | 98.0 | 9.86 |
| **19** | 98.5 | 9.23 |
| **20** | 99.7 | 11.52 |
| **21** | 99.9 | 8.99 |
| **22** | 99.7 | 9.34 |
| **Biotin-ZW4864** | 96.0 | 8.83 |

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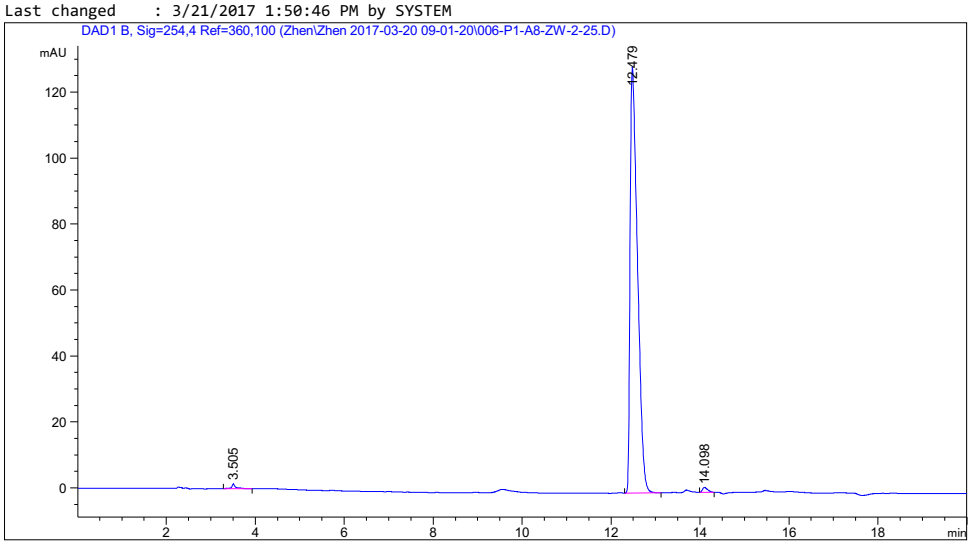


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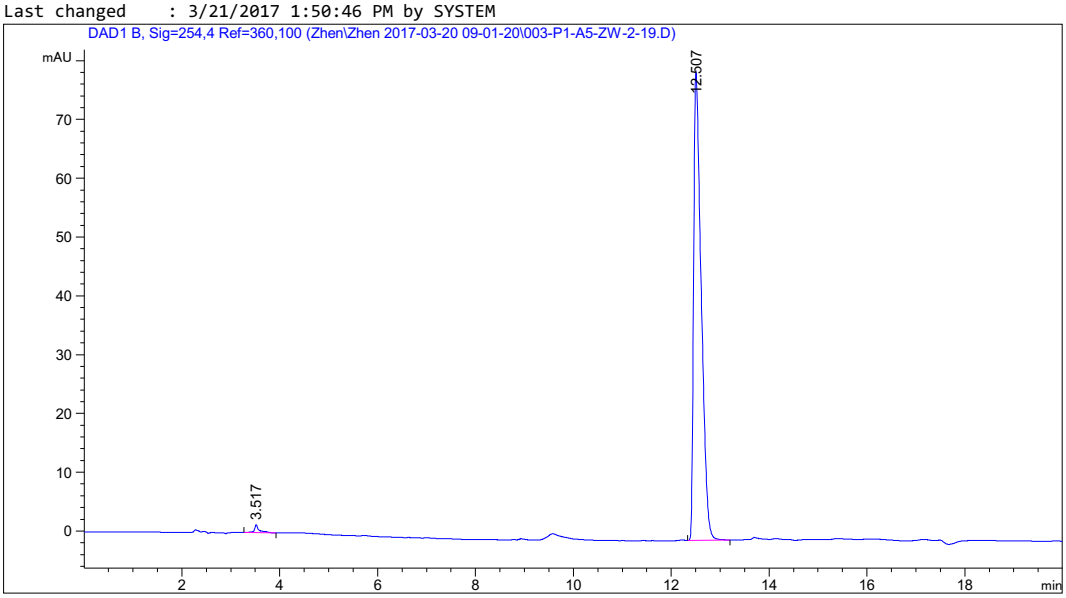


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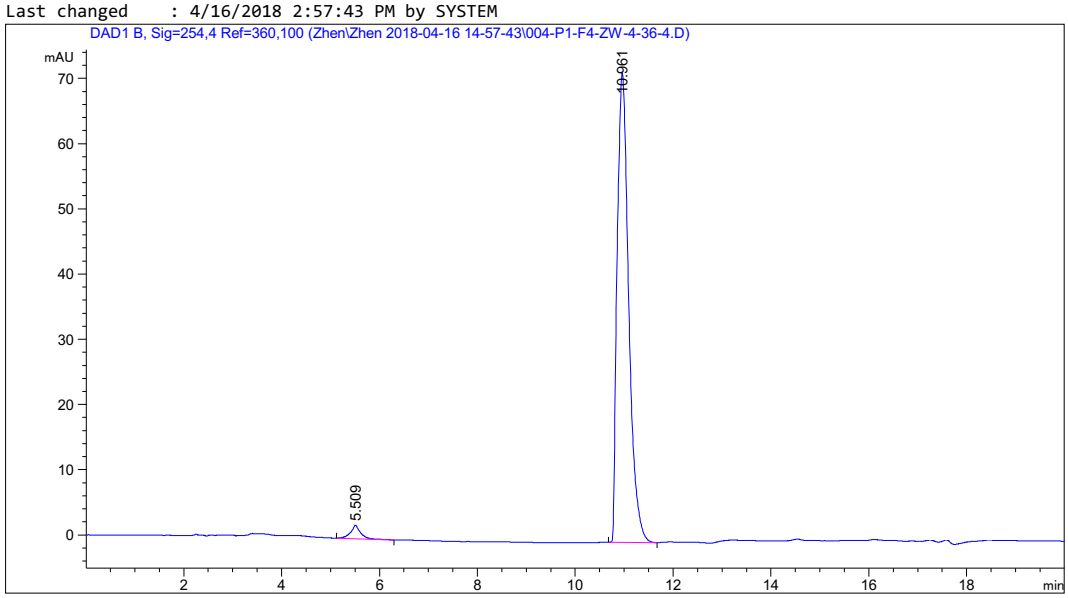


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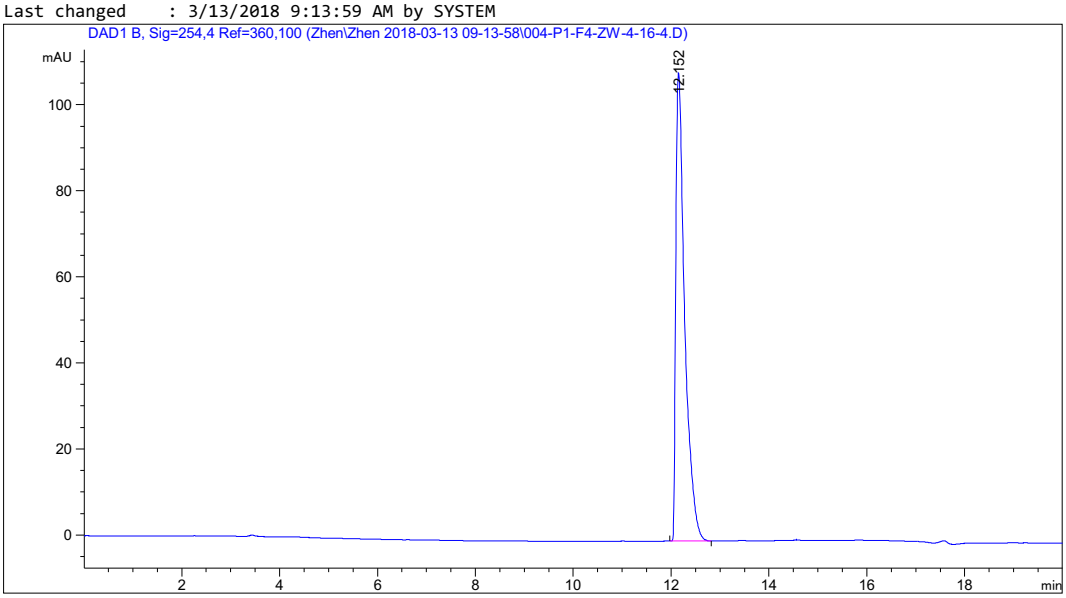


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    Acq. Operator   : SYSTEM                       Seq. Line :   1
    Acq. Instrument : HPLC                         Location :   P1-F1
    Injection Date  : 6/18/2019 1:23:31 PM              Inj :   1
                                                  Inj Volume : 20.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 3.000 µl
    Method          : C:\Chem32\1\Data\Zhen\Zhen 2019-06-18 13-17-06\Zhen.M (Sequence Method)
    Last changed    : 6/18/2019 1:17:07 PM by SYSTEM
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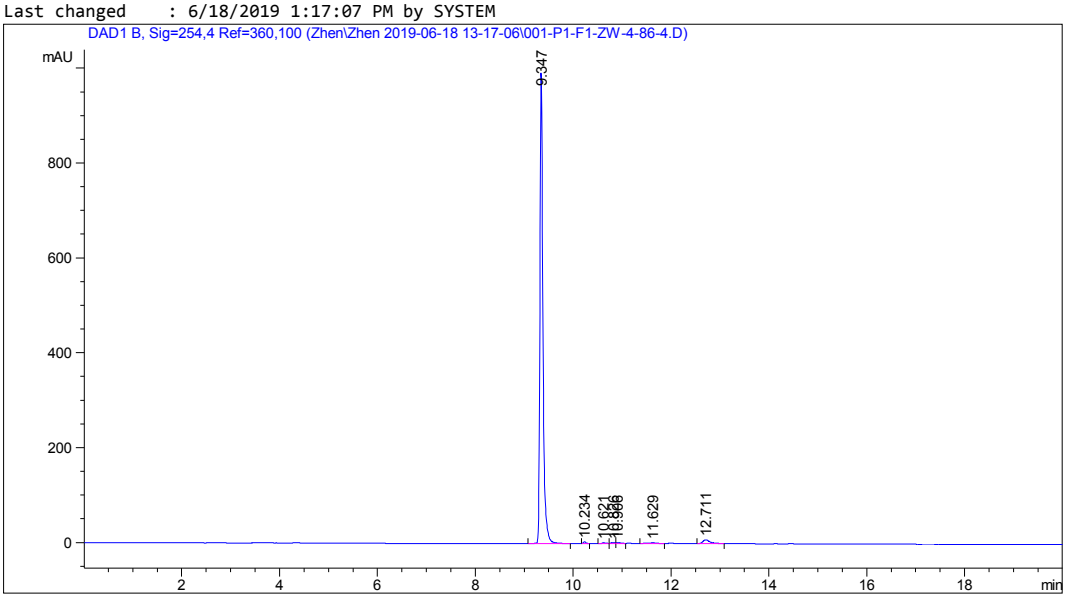


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    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 B, Sig=254,4 Ref=360,100

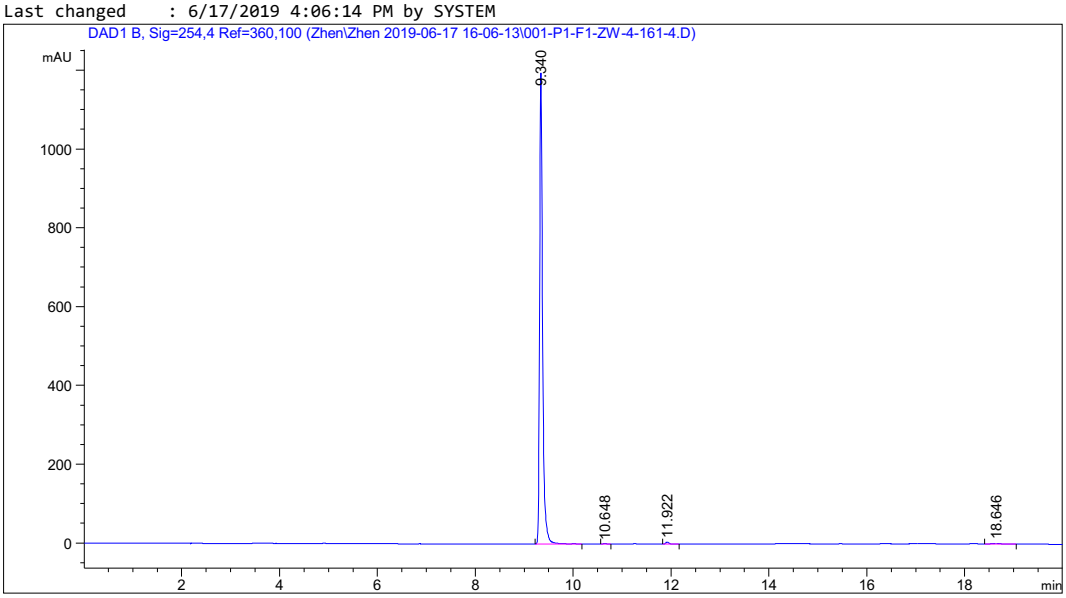
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.347 BB    0.0641 4278.50879  996.16571  96.7588
       2  10.234 BB    0.0544   12.00380    3.46852   0.2715
       3  10.621 BV    0.0808   10.25903    1.79158   0.2320
       4  10.826 VV    0.0862   11.37997    2.00437   0.2574
       5  10.906 VB    0.0915   11.55812    1.83584   0.2614
       6  11.629 BB    0.1676   20.89855    1.65176   0.4726
       7  12.711 BB    0.1467   77.22118    7.85193   1.7464

    Totals :                  4421.82944 1014.76970
```

# Compound 16

Data File C:\Chem32\1\Data\Zhen\Zhen 2019-06-17 16-06-13\001-P1-F1-ZW-4-161-4.D
Sample Name: ZW-4-161-4

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   1
    Acq. Instrument : HPLC                         Location :   P1-F1
    Injection Date  : 6/17/2019 4:12:37 PM              Inj :   1
                                                  Inj Volume : 20.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 10.000 µl
    Method          : C:\Chem32\1\Data\Zhen\Zhen 2019-06-17 16-06-13\Zhen.M (Sequence Method)
    Last changed    : 6/17/2019 4:06:14 PM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 B, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.340 BV R  0.0638 5124.51270 1200.06848  98.8473
       2  10.648 BB    0.0667    7.66544    1.76335   0.1479
       3  11.922 BB    0.0694   24.49493    5.35611   0.4725
       4  18.646 BB    0.1797   27.60105    2.27311   0.5324

    Totals :                  5184.27412 1209.46105

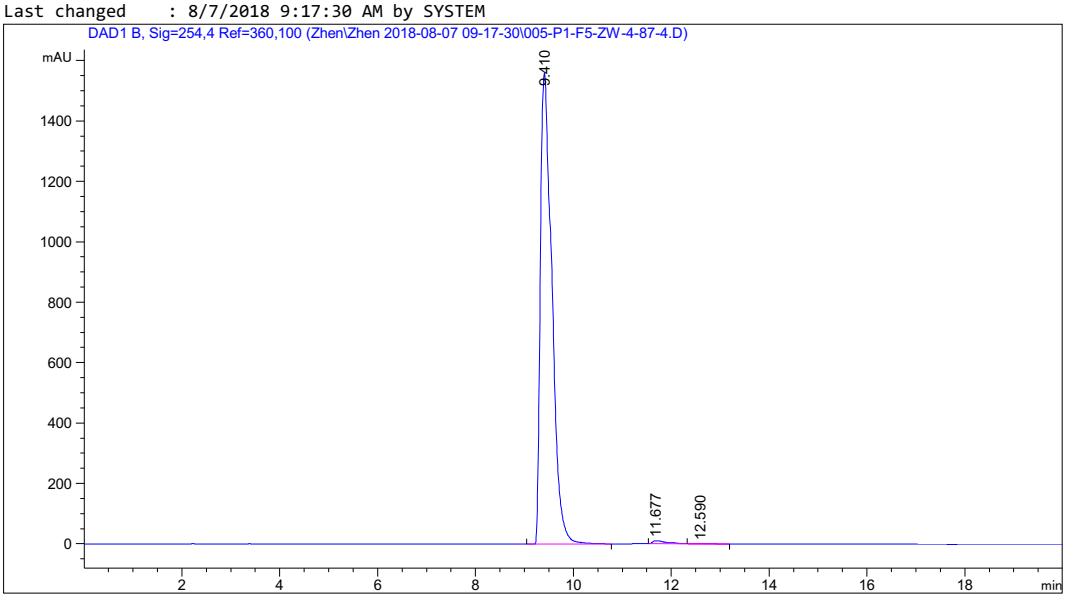

    ======================================================================
                            *** End of Report ***

HPLC 6/17/2019 4:38:26 PM SYSTEM                              Page   1 of 1
```

# Compound 17

Data File C:\Chem32\1\Data\Zhen\Zhen 2018-08-07 09-17-30\005-P1-F5-ZW-4-87-4.D
Sample Name: ZW-4-87-4

```
==================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : HPLC                           Location :   P1-F5
Injection Date  : 8/7/2018 11:07:25 AM                Inj :   1
                                                Inj Volume : 80.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
Method          : C:\Chem32\1\Data\Zhen\Zhen 2018-08-07 09-17-30\Zhen.M (Sequence Method)
Last changed    : 8/7/2018 9:17:30 AM by SYSTEM
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   9.410 BB    0.2323 2.54579e4  1560.33081  99.1045
   2  11.677 BV R  0.2573  197.27127    9.86483   0.7680
   3  12.590 VB E  0.2857   32.75716    1.62133   0.1275

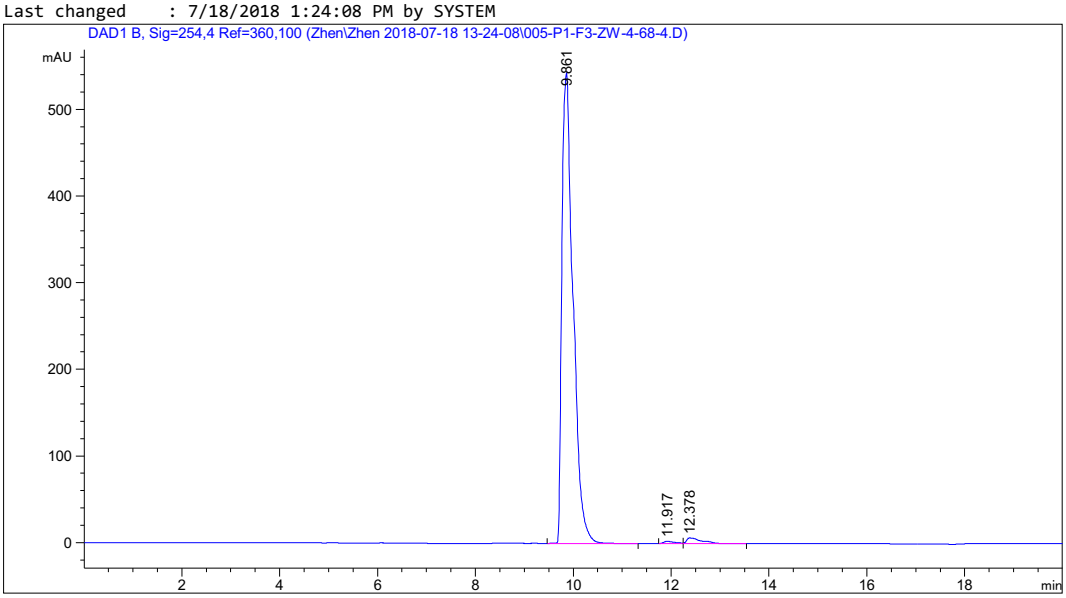
Totals :                  2.56879e4  1571.81698


==================================================================
                        *** End of Report ***
```

# Compound 18

Data File C:\Chem32\1\Data\Zhen\Zhen 2018-07-18 13-24-08\005-P1-F3-ZW-4-68-4.D
Sample Name: ZW-4-68-4

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   5
    Acq. Instrument : HPLC                          Location :   P1-F3
    Injection Date  : 7/18/2018 3:20:13 PM               Inj :   1
                                                  Inj Volume : 80.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
    Method          : C:\Chem32\1\Data\Zhen\Zhen 2018-07-18 13-24-08\Zhen.M (Sequence Method)
    Last changed    : 7/18/2018 1:24:08 PM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 B, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.861 BB    0.2346 8590.25000  543.04675  97.9676
       2  11.917 BV    0.2363   38.83273    2.43210   0.4429
       3  12.378 VB    0.2807  139.37563    6.48695   1.5895

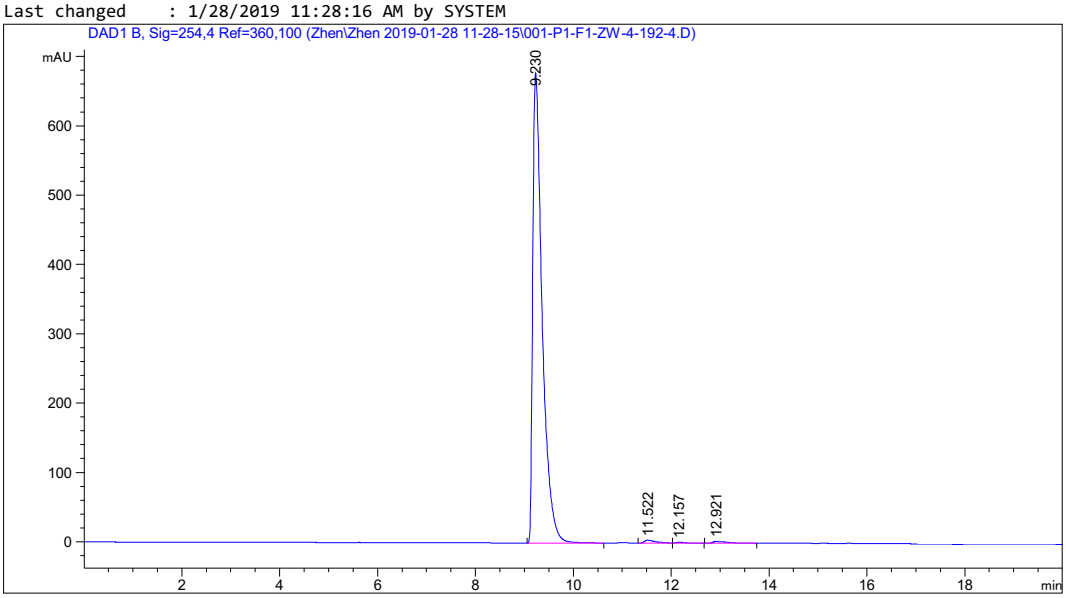
    Totals :                  8768.45835  551.96581


    ======================================================================
                              *** End of Report ***
```

# Compound 19

Data File C:\Chem32\1\Data\Zhen\Zhen 2019-01-28 11-28-15\001-P1-F1-ZW-4-192-4.D
Sample Name: ZW-4-192-4

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   1
    Acq. Instrument : HPLC                         Location :   P1-F1
    Injection Date  : 1/28/2019 11:37:47 AM             Inj :   1
                                                  Inj Volume : 80.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
    Method          : C:\Chem32\1\Data\Zhen\Zhen 2019-01-28 11-28-15\Zhen.M (Sequence Method)
    Last changed    : 1/28/2019 11:28:16 AM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 B, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.230 BB    0.2025 9097.58008  678.28180  98.5312
       2  11.522 BV    0.2205   69.13280    4.32439   0.7487
       3  12.157 VB    0.1917   17.16881    1.33839   0.1859
       4  12.921 BB    0.2458   49.31326    2.64198   0.5341

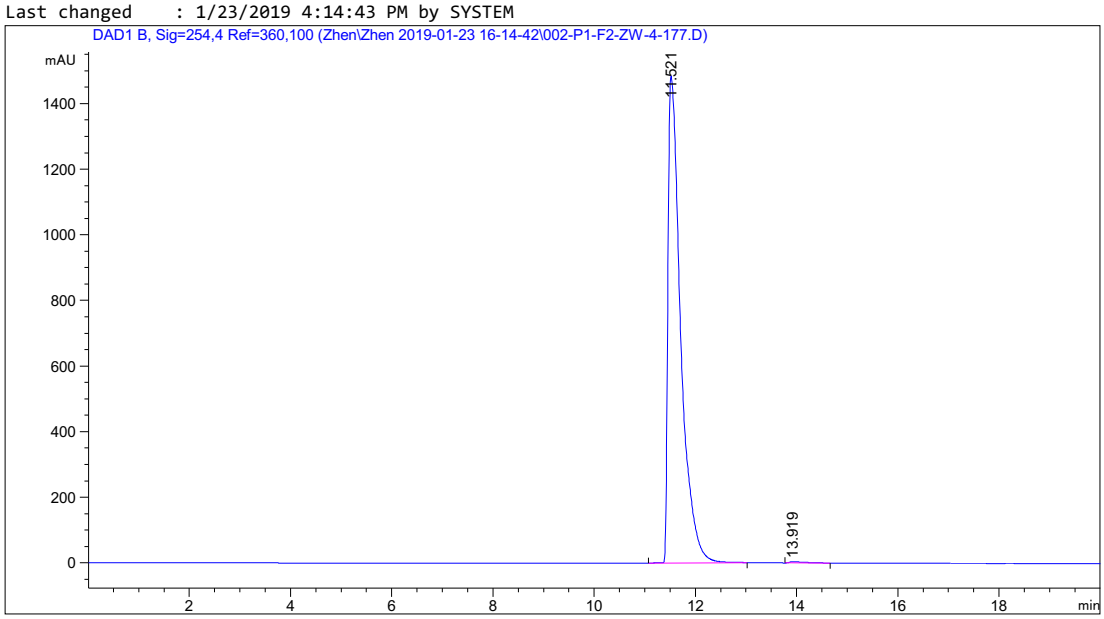
    Totals :                  9233.19495  686.58656


    ======================================================================
                            *** End of Report ***
```

# Compound 20

Data File C:\Chem32\1\Data\Zhen\Zhen 2019-01-23 16-14-42\002-P1-F2-ZW-4-177.D
Sample Name: ZW-4-177

```
======================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   2
Acq. Instrument : HPLC                           Location :   P1-F2
Injection Date  : 1/23/2019 4:47:52 PM                Inj :   1
                                                 Inj Volume : 80.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
Method          : C:\Chem32\1\Data\Zhen\Zhen 2019-01-23 16-14-42\Zhen.M (Sequence Method)
Last changed    : 1/23/2019 4:14:43 PM by SYSTEM
```




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.521 BB    0.2646 2.57907e4  1484.96643  99.6940
   2  13.919 BB    0.2485   79.15220    4.11319   0.3060

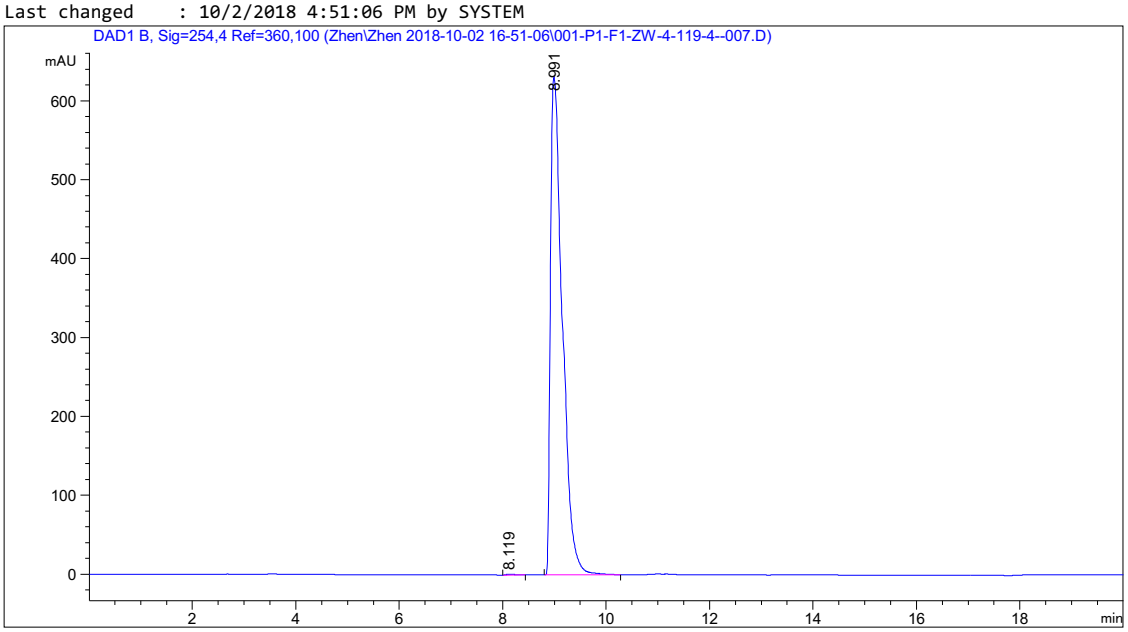
Totals :                  2.58699e4  1489.07962


======================================================================
                          *** End of Report ***
```

# Compound 21

Data File C:\Chem32\1\Data\Zhen\Zhen 2018-10-02 16-51-06\001-P1-F1-ZW-4-119-4--007.D
Sample Name: ZW-4-114-4

```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   7
Acq. Instrument : HPLC                            Location :   P1-F7
Injection Date  : 10/2/2018 7:40:15 PM                 Inj :   1
                                                 Inj Volume : 80.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
Method          : C:\Chem32\1\Data\Zhen\Zhen 2018-10-02 16-51-06\Zhen.M (Sequence Method)
Last changed    : 10/2/2018 4:51:06 PM by SYSTEM
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.119 BB    0.1395   11.79428    1.15122   0.1169
   2   8.991 BB    0.2364 1.00763e4   630.53009  99.8831

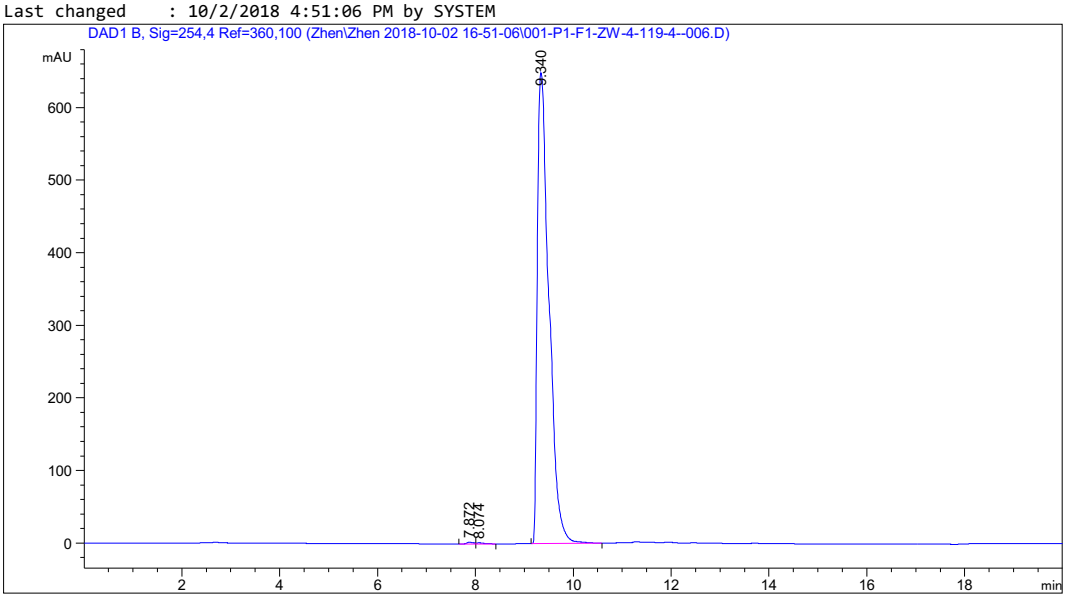
Totals :                  1.00881e4   631.68131


==========================================================================
                        *** End of Report ***
```

# Compound 22

Data File C:\Chem32\1\Data\Zhen\Zhen 2018-10-02 16-51-06\001-P1-F1-ZW-4-119-4--006.D
Sample Name: ZW-4-112-4

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   6
    Acq. Instrument : HPLC                         Location :   P1-F6
    Injection Date  : 10/2/2018 7:13:39 PM              Inj :   1
                                                  Inj Volume : 80.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
    Method          : C:\Chem32\1\Data\Zhen\Zhen 2018-10-02 16-51-06\Zhen.M (Sequence Method)
    Last changed    : 10/2/2018 4:51:06 PM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 B, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   7.872 BV    0.1227   20.46256    2.32472   0.1909
       2   8.074 VB    0.1309   14.41464    1.48991   0.1345
       3   9.340 BB    0.2442 1.06845e4   648.63574  99.6746

    Totals :                   1.07194e4   652.45038


    ======================================================================
                              *** End of Report ***
```

# Biotin-ZW4864

Data File C:\Chem32\1\Data\Zhen\Zhen 2019-01-10 13-51-59\001-P1-F3-ZW-4-181-new.D
Sample Name: ZW-4-181-new

```
=====================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   1
Acq. Instrument : HPLC                         Location :   P1-F3
Injection Date  : 1/10/2019 1:58:38 PM              Inj :   1
                                               Inj Volume : 80.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
Method          : C:\Chem32\1\Data\Zhen\Zhen 2019-01-10 13-51-59\Zhen.M (Sequence Method)
Last changed    : 1/10/2019 1:52:00 PM by SYSTEM
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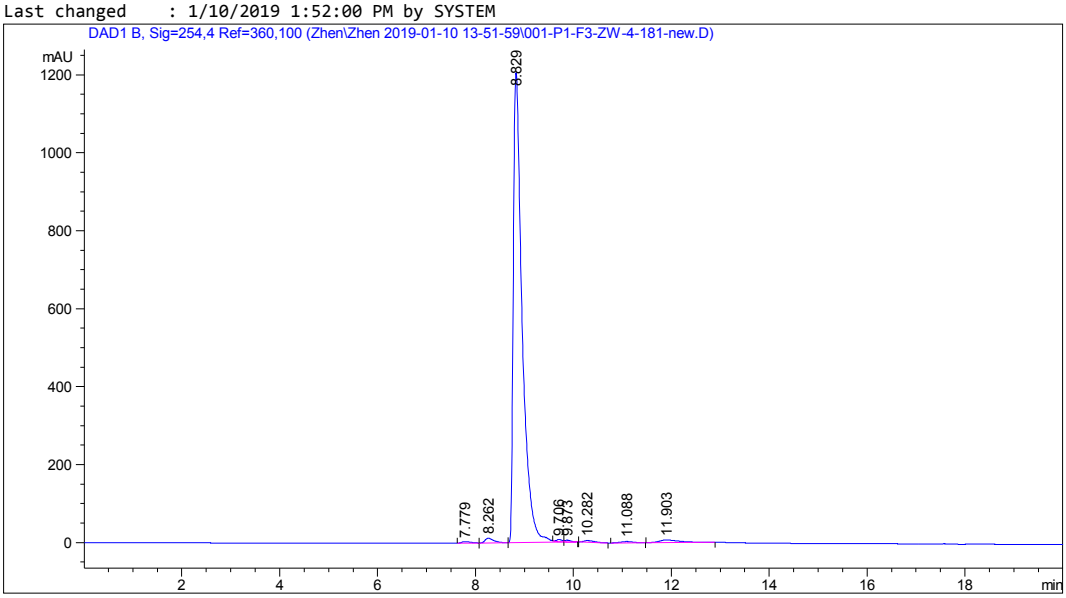
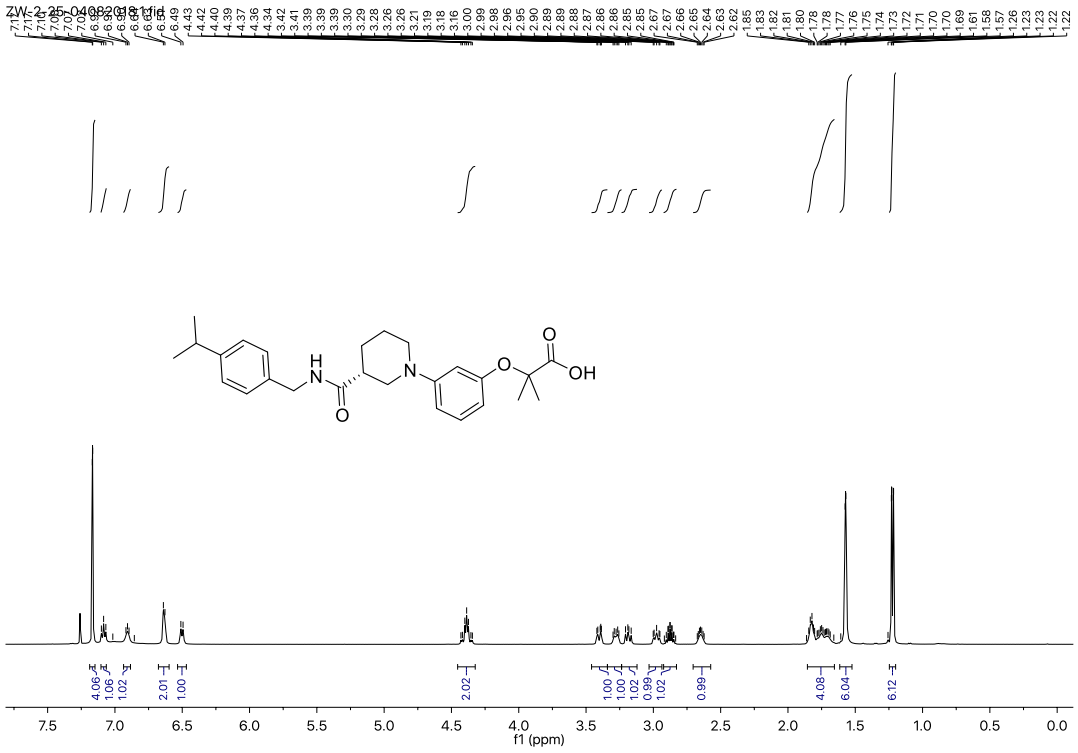



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,4 Ref=360,100
```

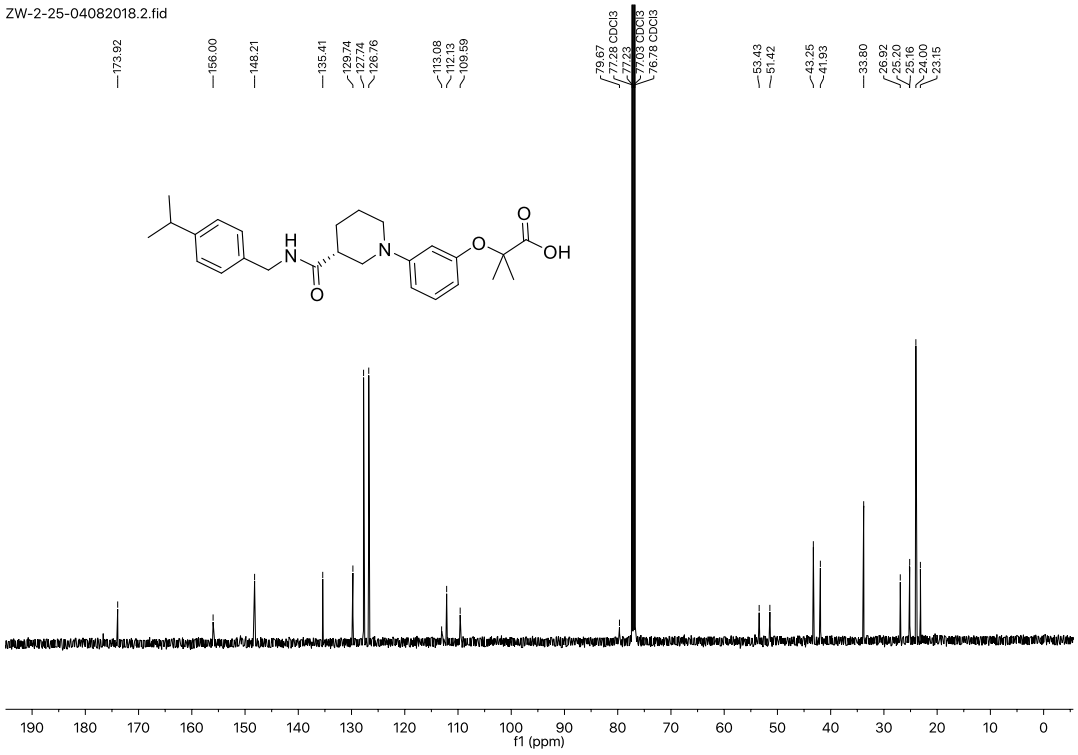
| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 7.779 | BV | 0.1983 | 41.73529 | 3.42166 | 0.2643 |
| 2 | 8.262 | VB | 0.1851 | 150.59569 | 12.10558 | 0.9536 |
| 3 | 8.829 | BV R | 0.1867 | 1.51688e4 | 1205.94653 | 96.0505 |
| 4 | 9.706 | VV E | 0.1159 | 44.89343 | 5.82460 | 0.2843 |
| 5 | 9.873 | VB E | 0.1318 | 42.79675 | 4.81042 | 0.2710 |
| 6 | 10.282 | BB | 0.1951 | 64.29467 | 4.54352 | 0.4071 |
| 7 | 11.088 | BB | 0.2791 | 65.54153 | 3.52163 | 0.4150 |
| 8 | 11.903 | BB | 0.4381 | 213.86261 | 6.96707 | 1.3542 |
| Totals : | | | | 1.57925e4 | 1247.14102 | |

## NMR Spectra

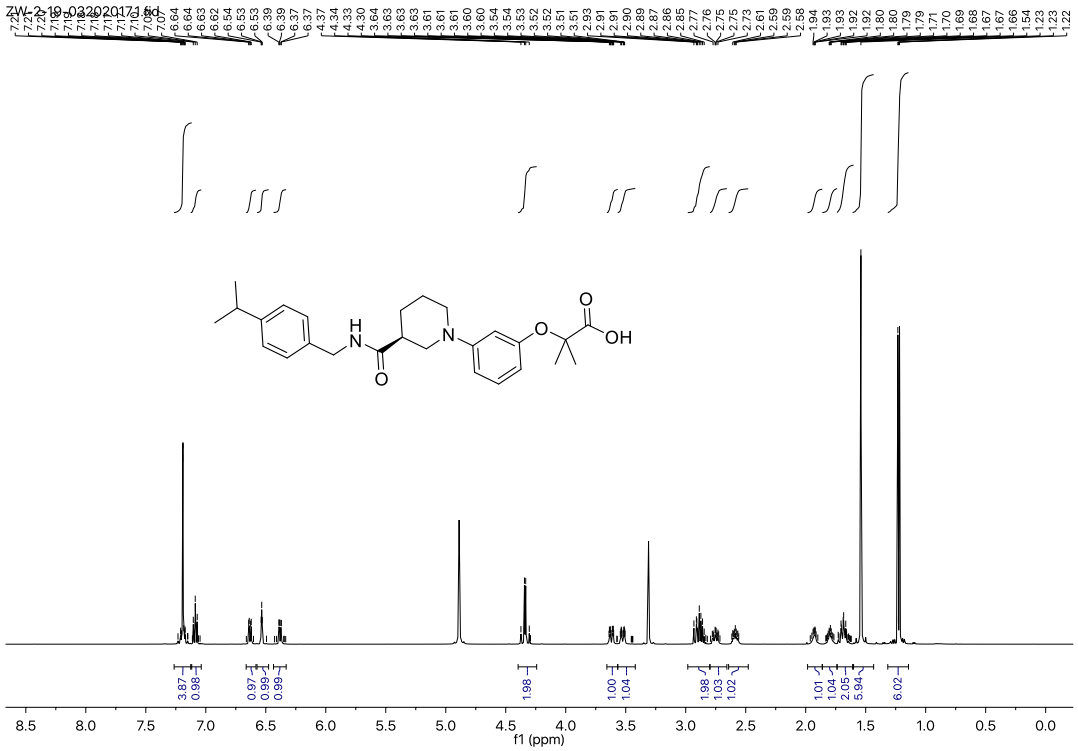
$^{1}$H NMR for compound **1**



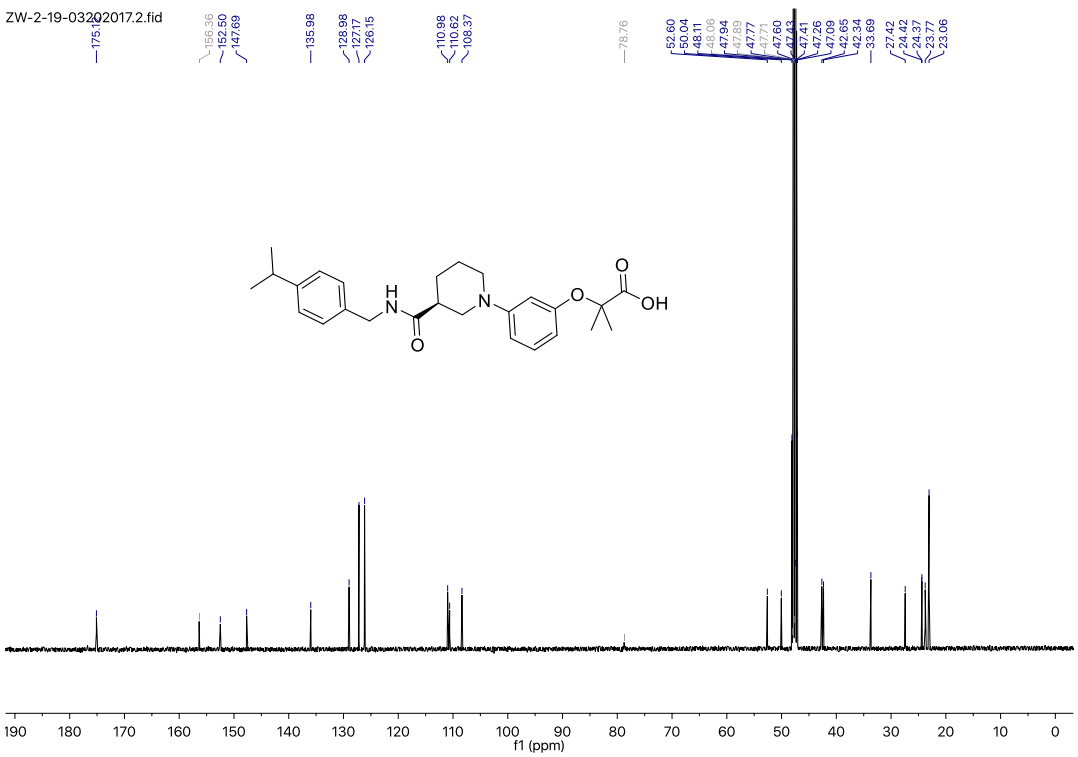

$^{13}$C NMR for compound **1**



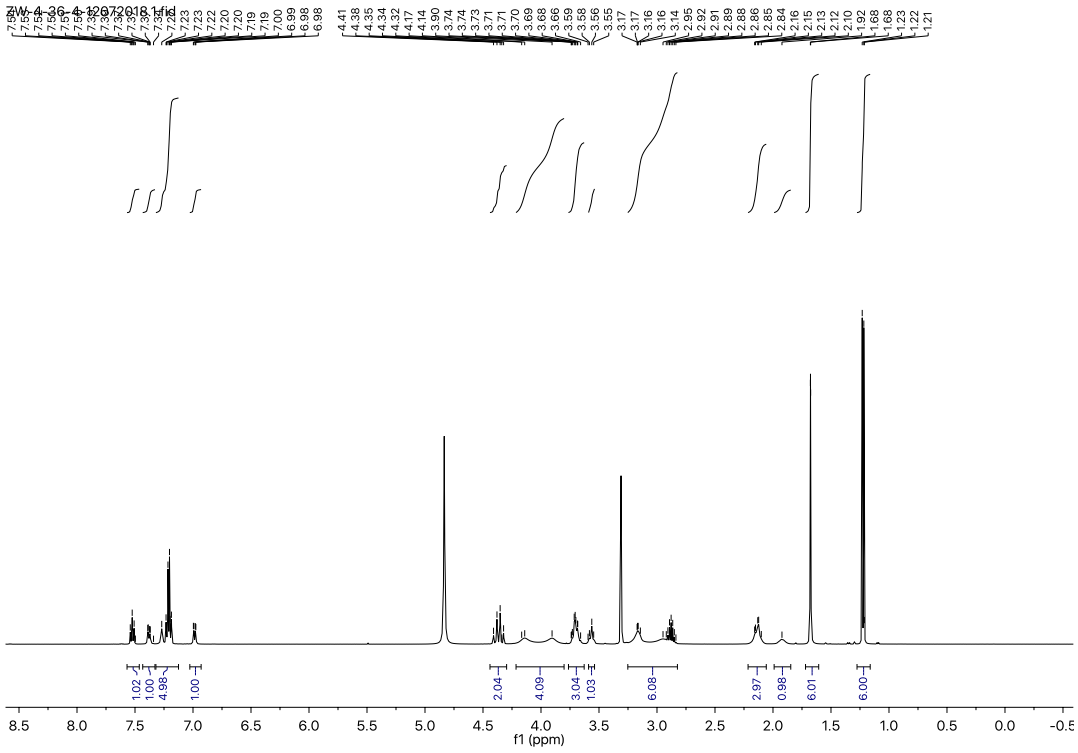
$^{1}$H NMR for compound **2**



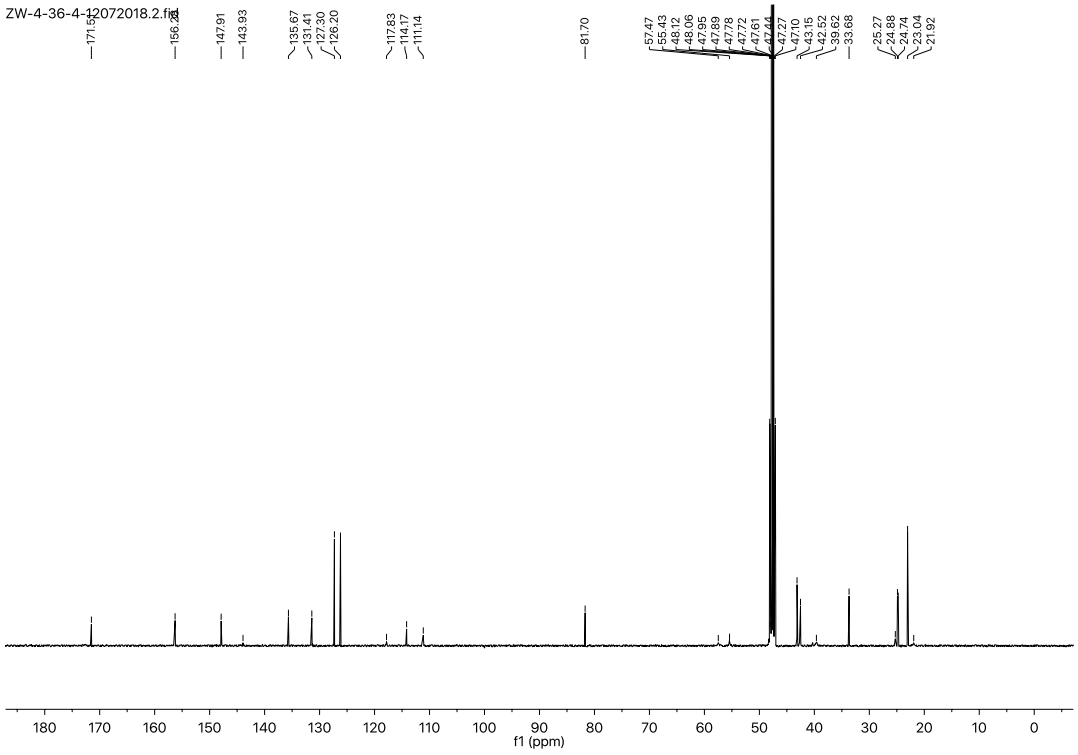

$^{13}$C NMR for compound **2**



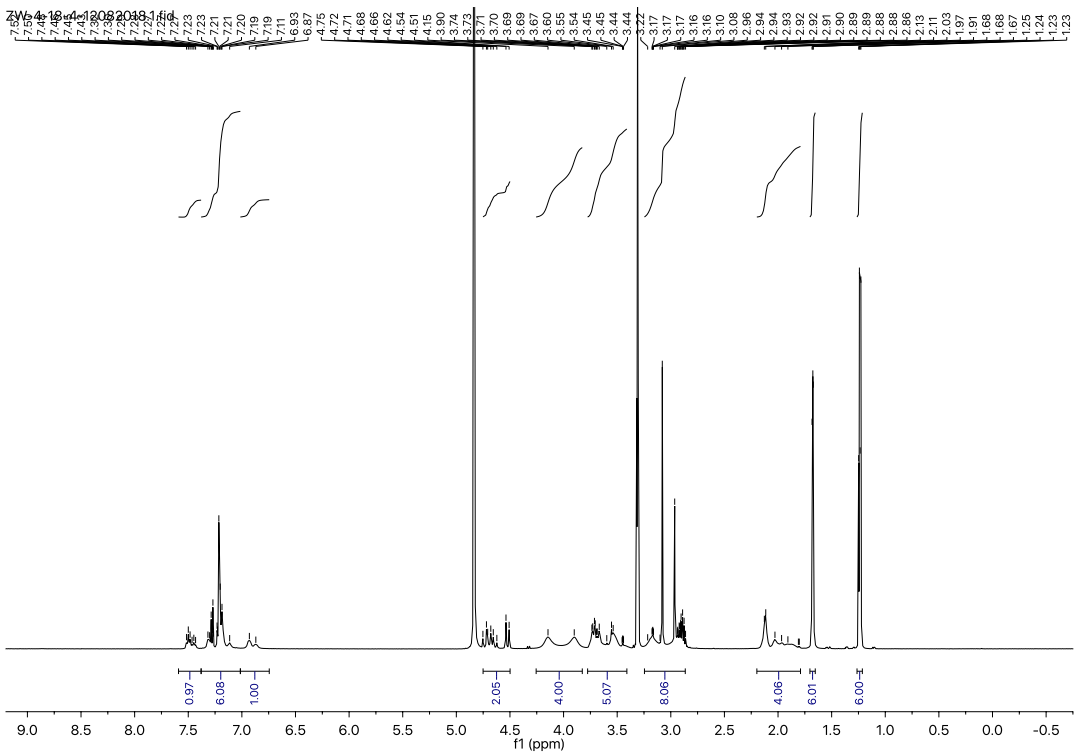
$^{1}$H NMR for compound **3**



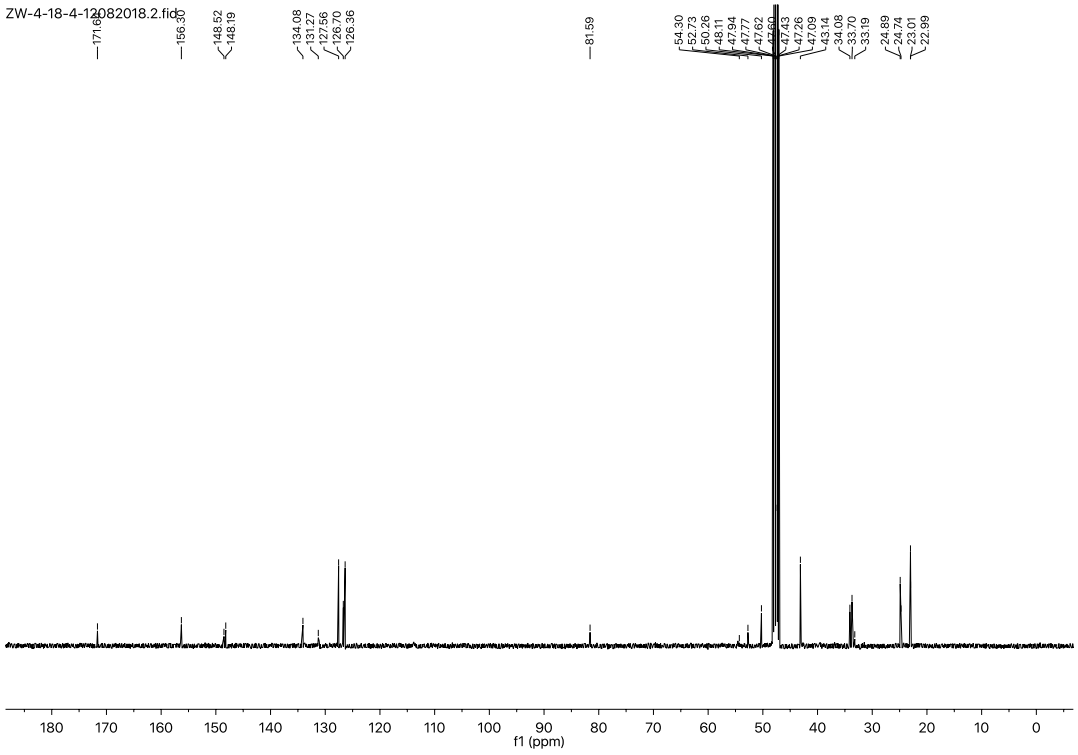

$^{13}$C NMR for compound **3**



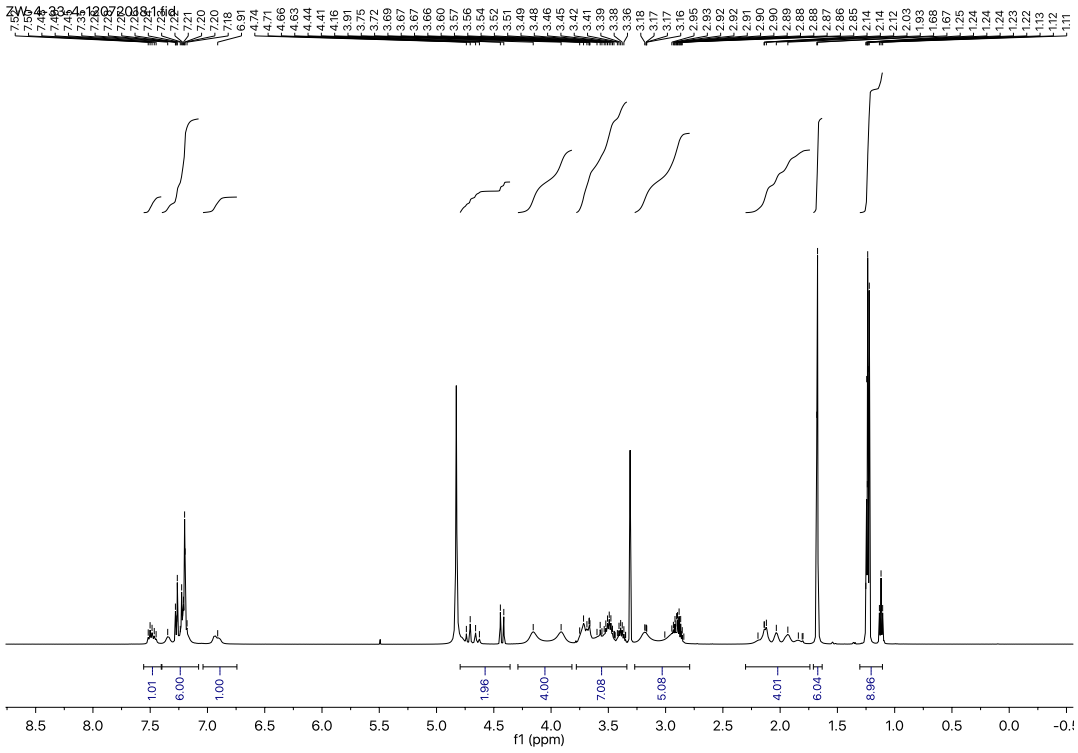
$^{1}$H NMR for compound **4**



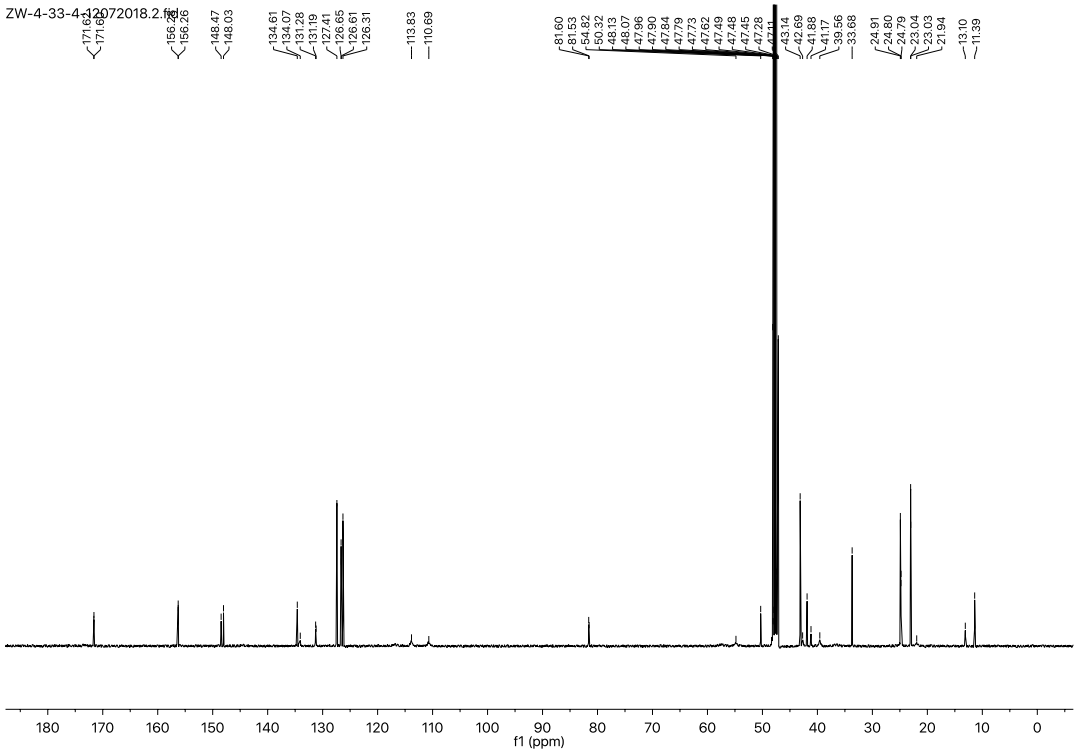

$^{13}$C NMR for compound **4**



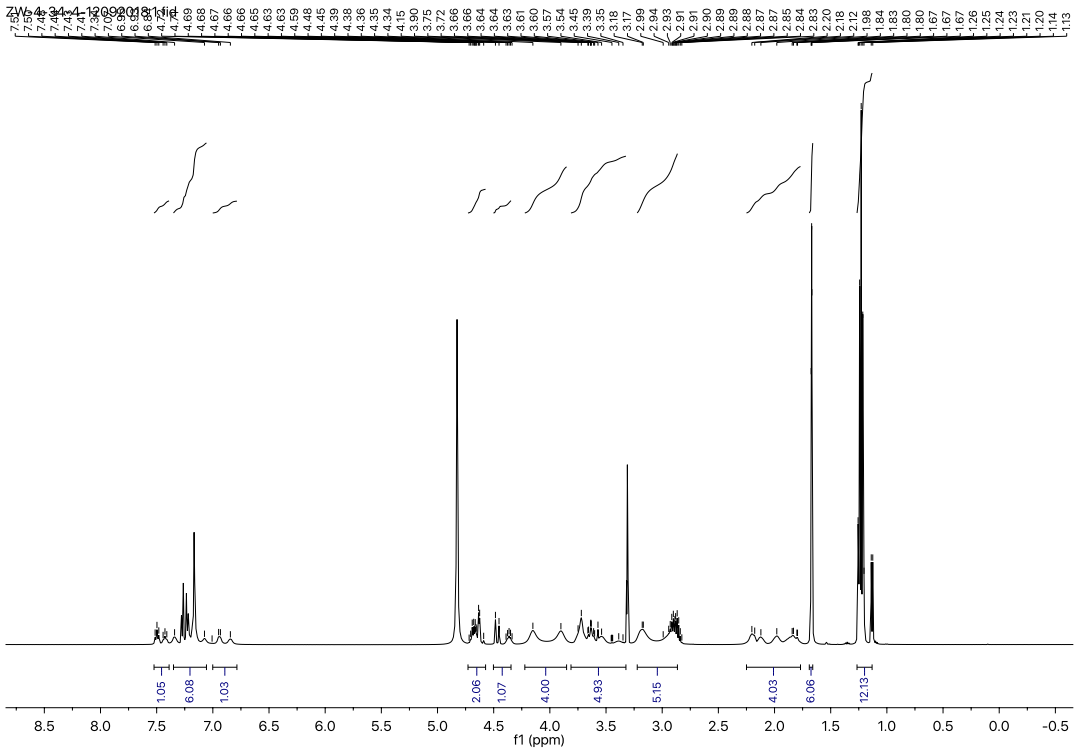
$^{1}$H NMR for compound **5**



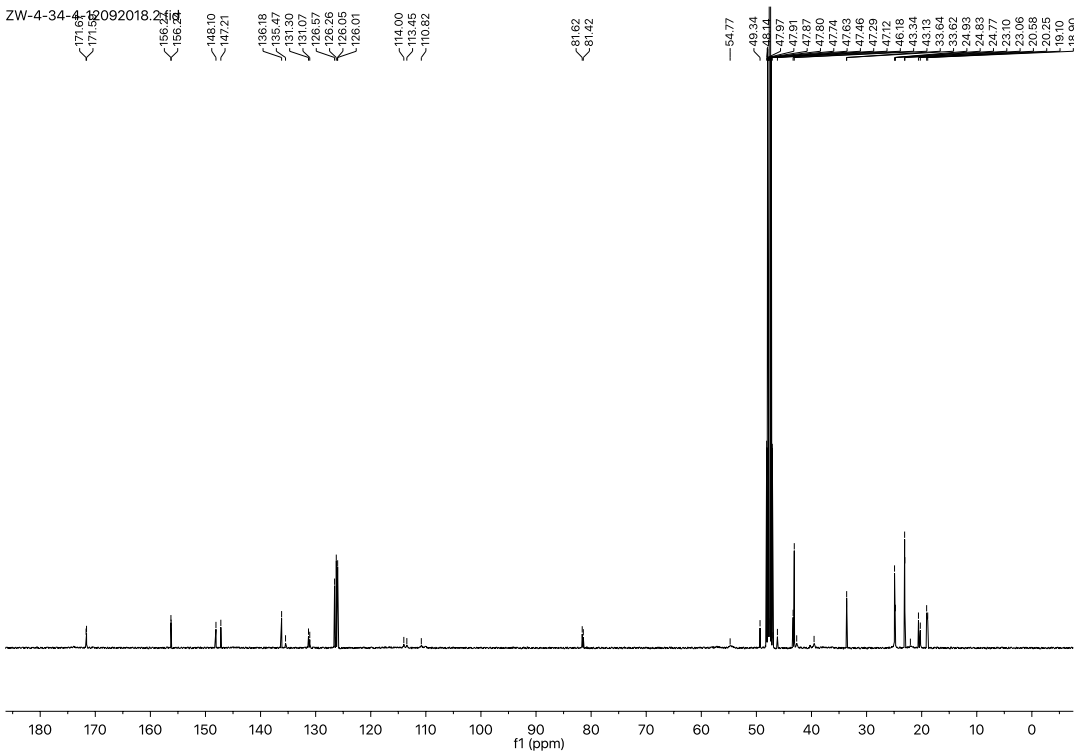
$^{13}$C NMR for compound **5**



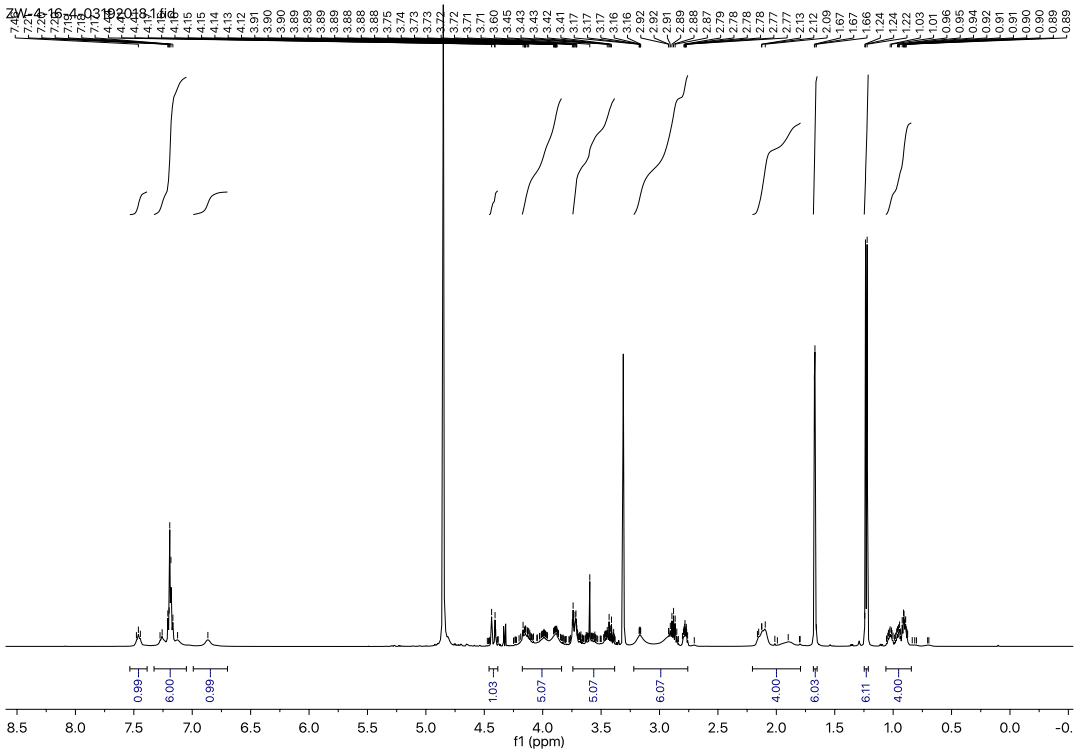
$^{1}$H NMR for compound **6**



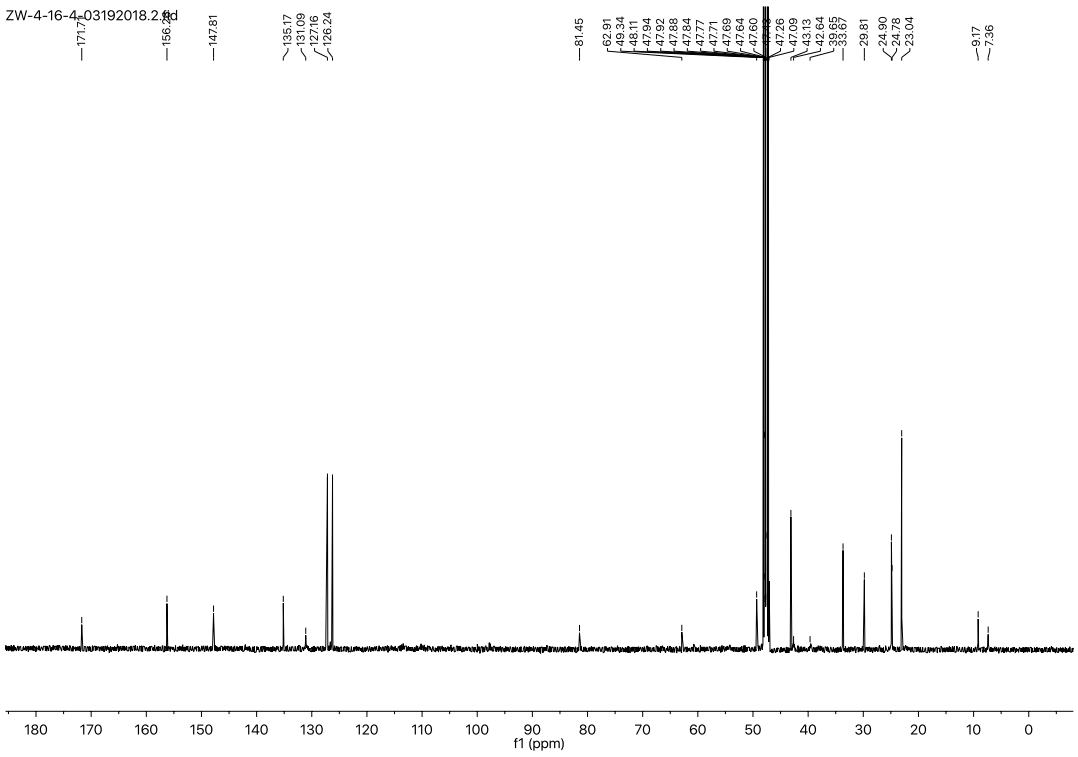

$^{13}$C NMR for compound **6**



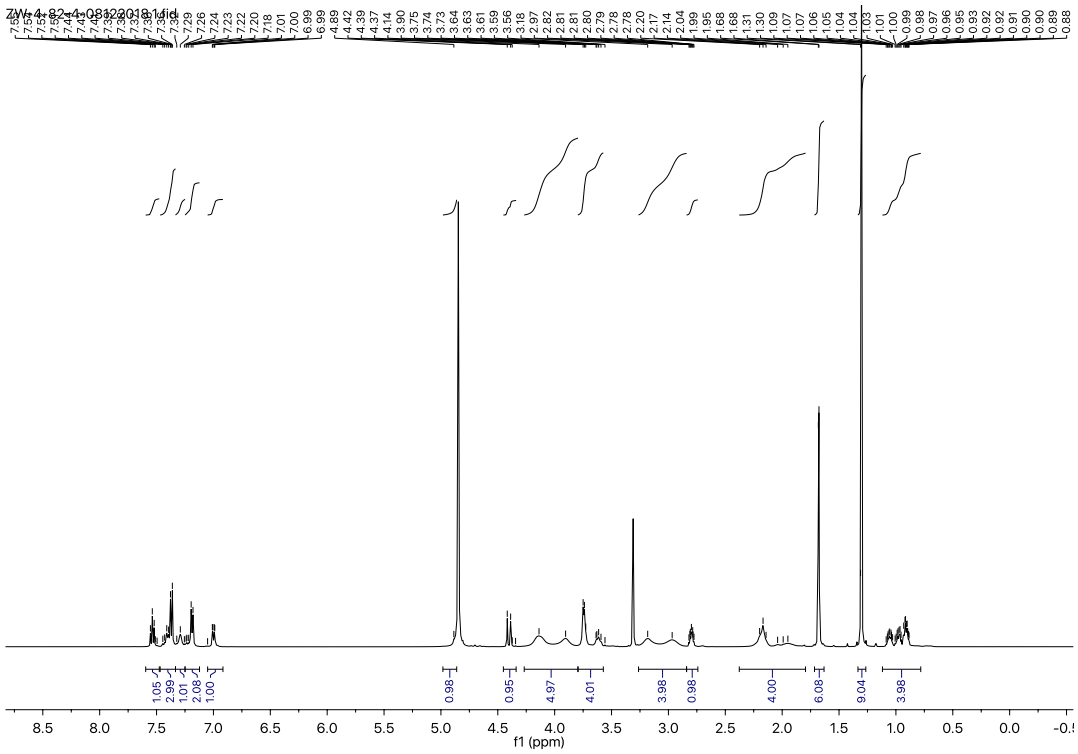
$^{1}$H NMR for compound **7**



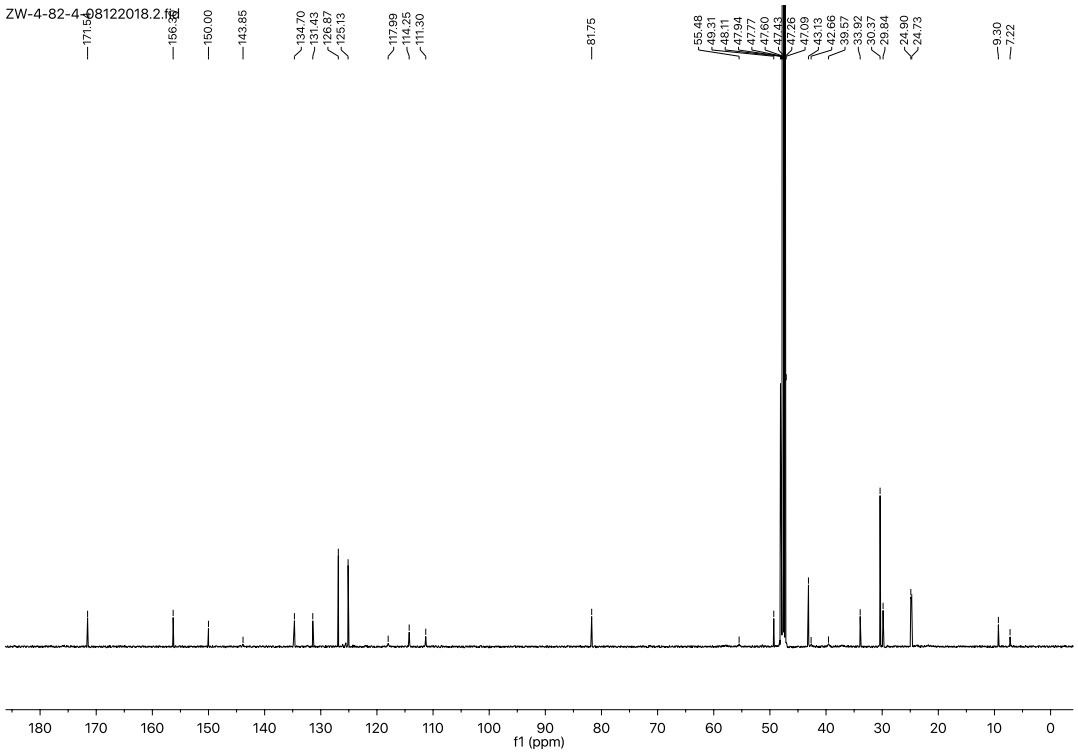

$^{13}$C NMR for compound **7**



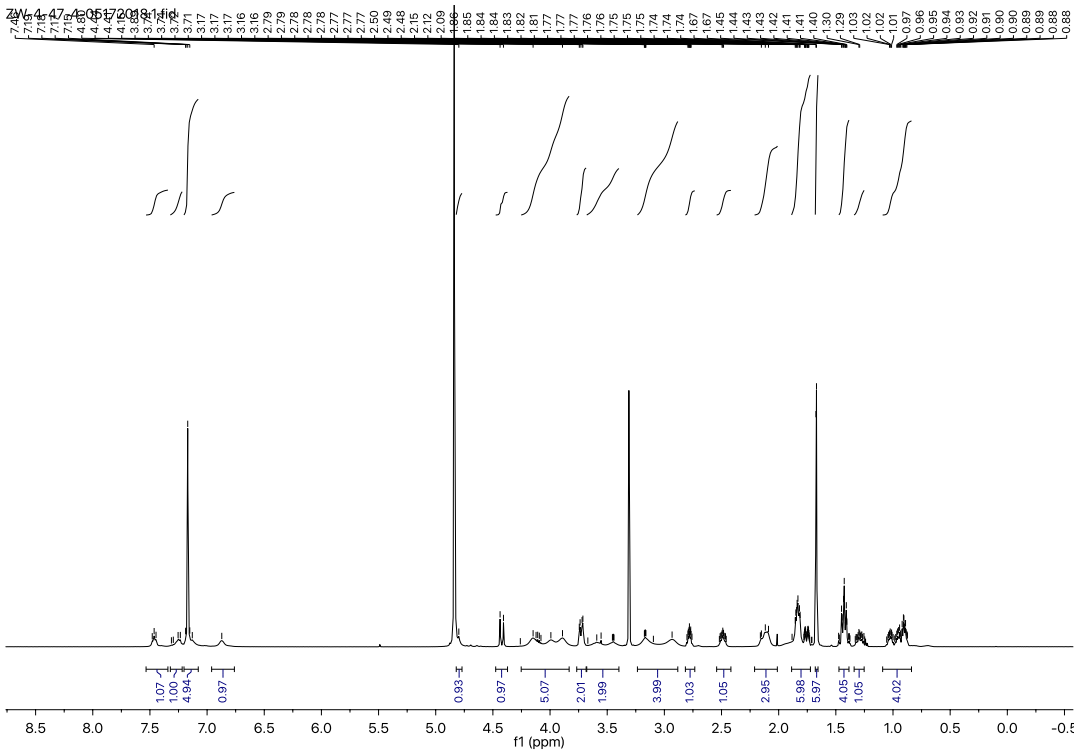
$^{1}$H NMR for compound **8**



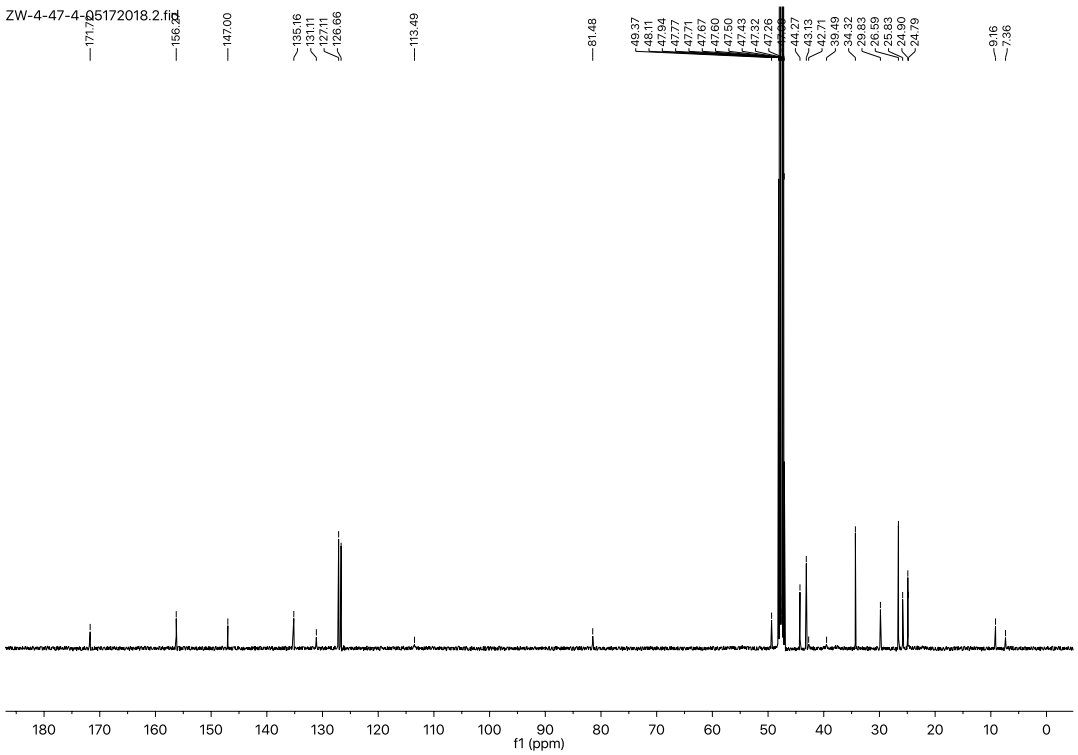

$^{13}$C NMR for compound **8**



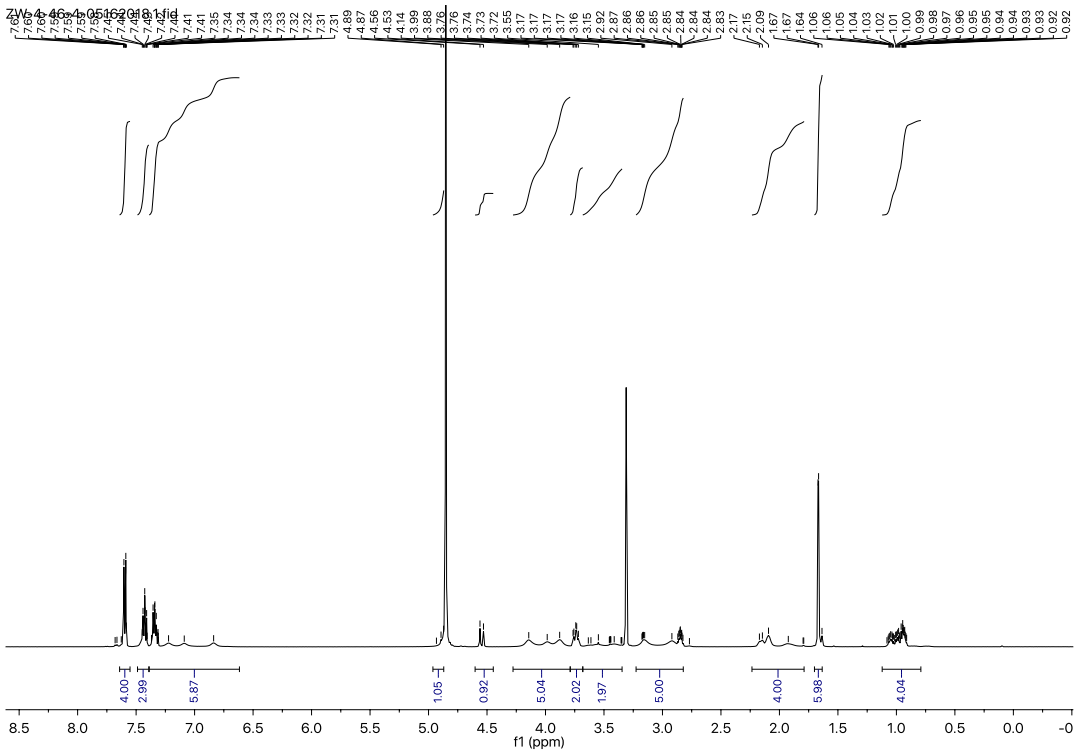
$^{1}$H NMR for compound **9**



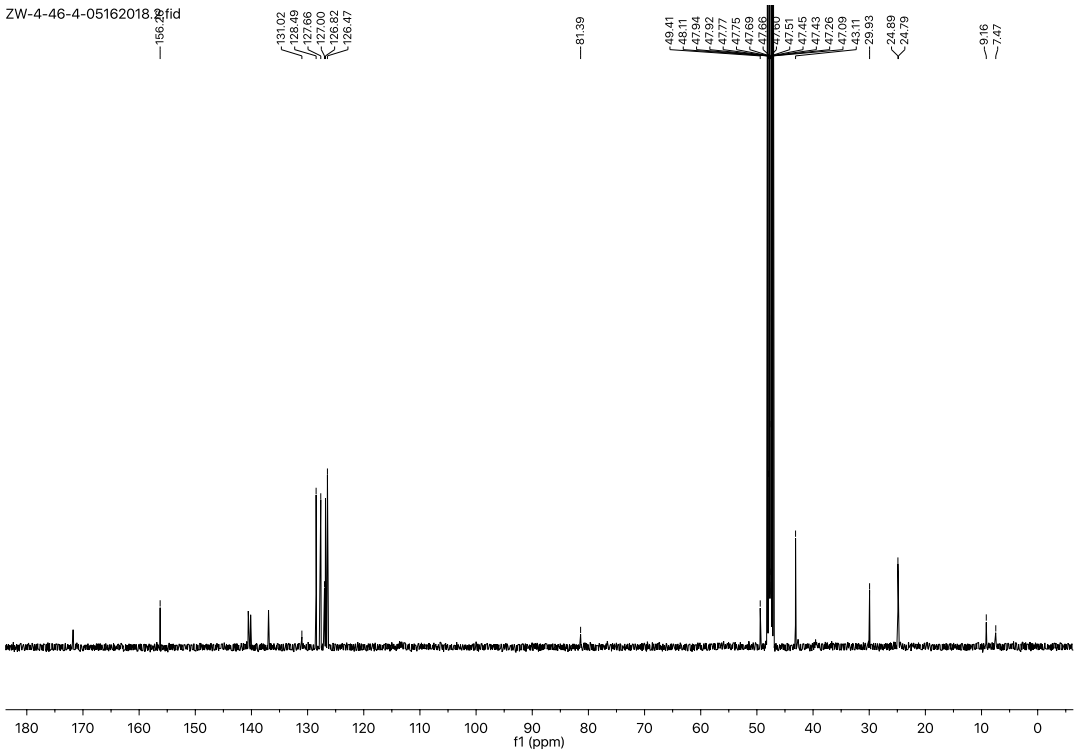

$^{13}$C NMR for compound **9**



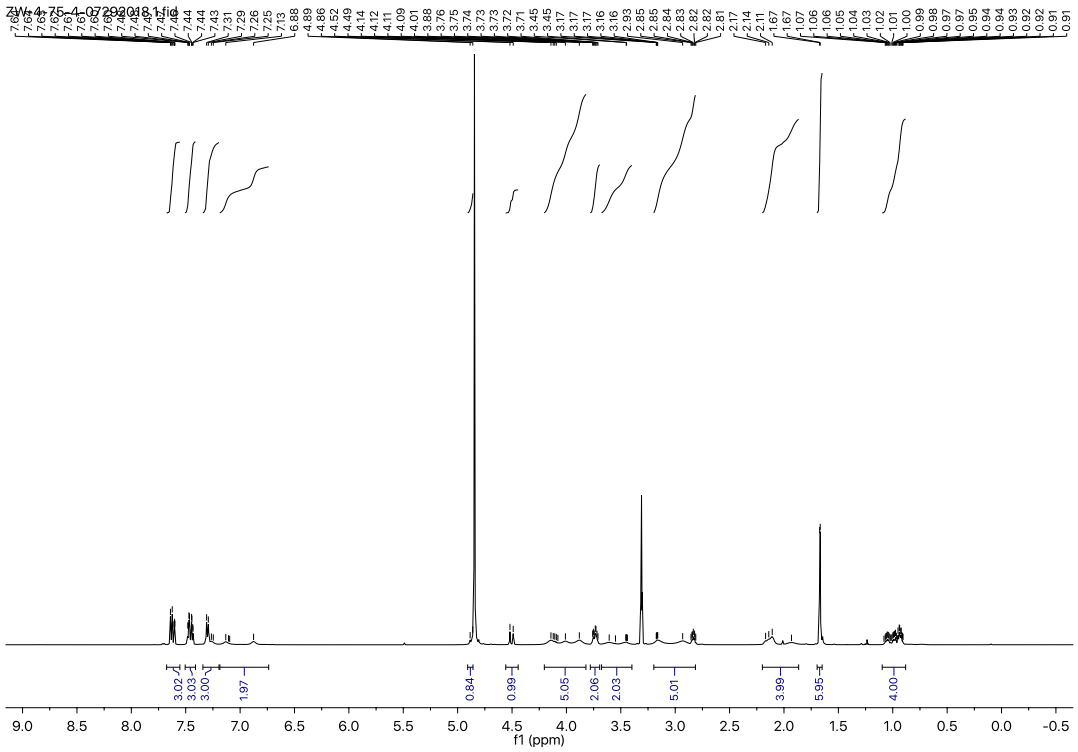
$^{1}$H NMR for compound **10**



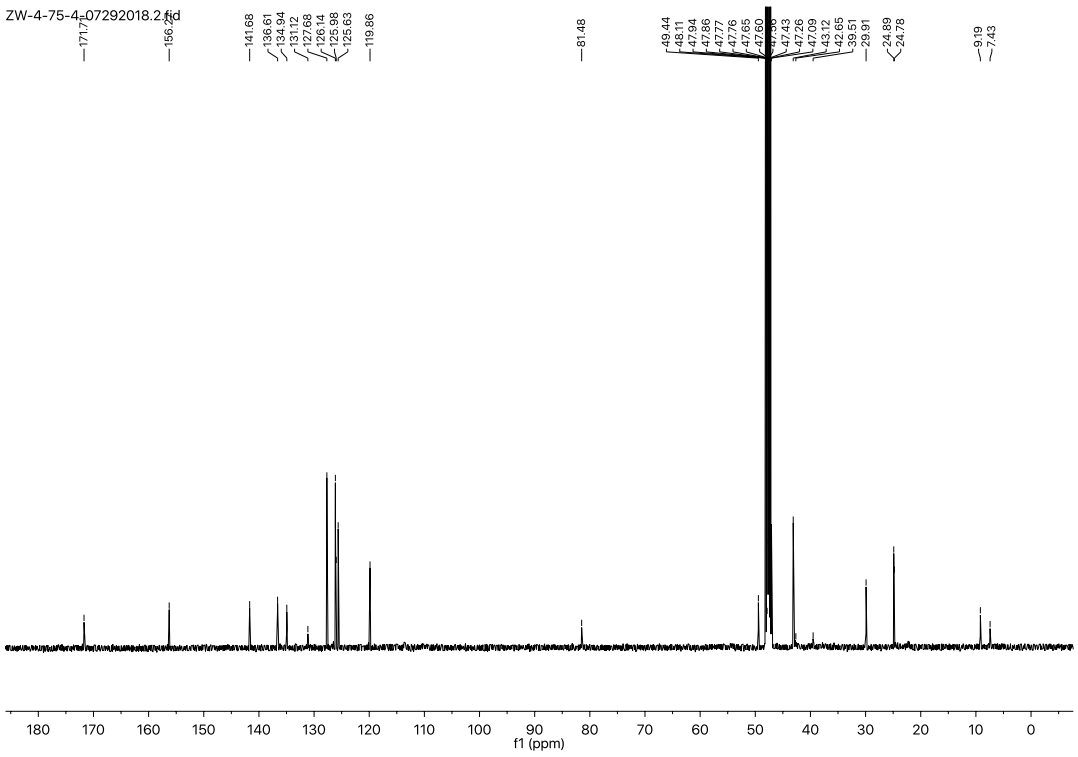
$^{13}$C NMR for compound **10**



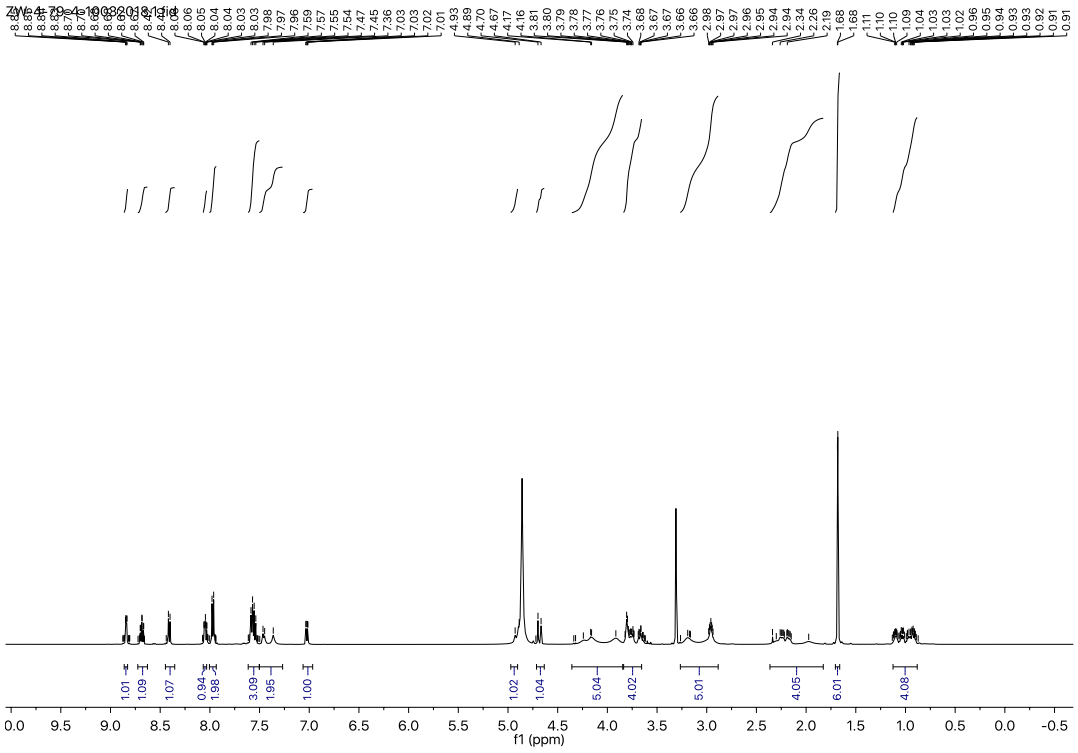
$^{1}$H NMR for compound **11**



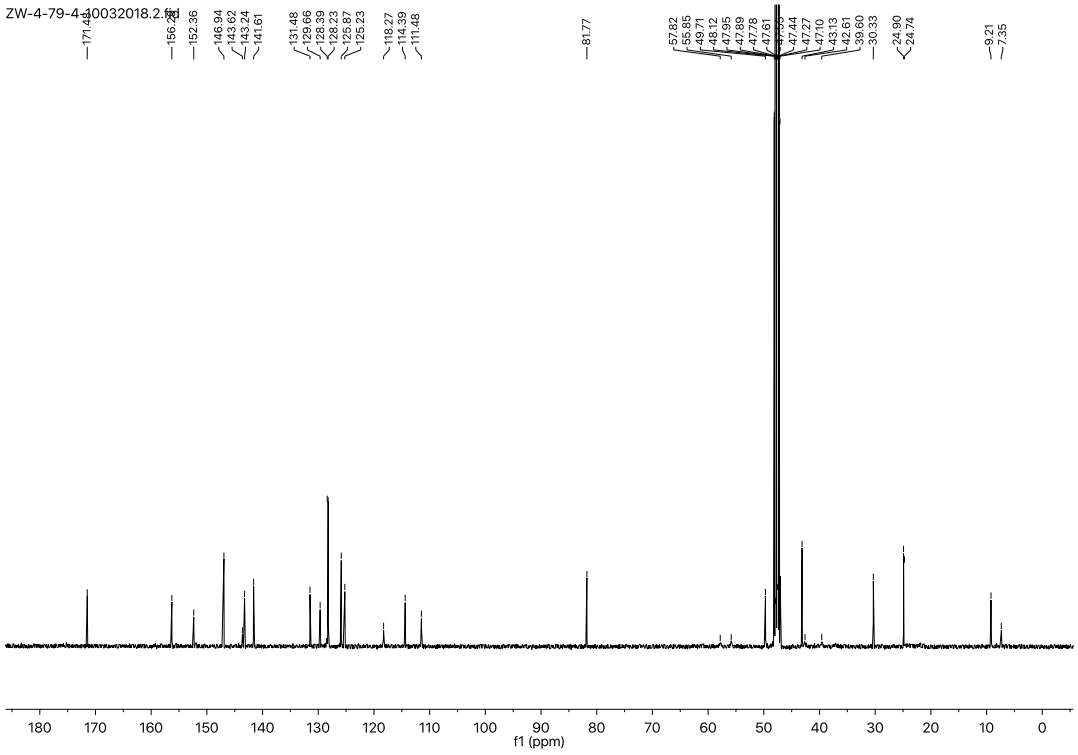

$^{13}$C NMR for compound **11**



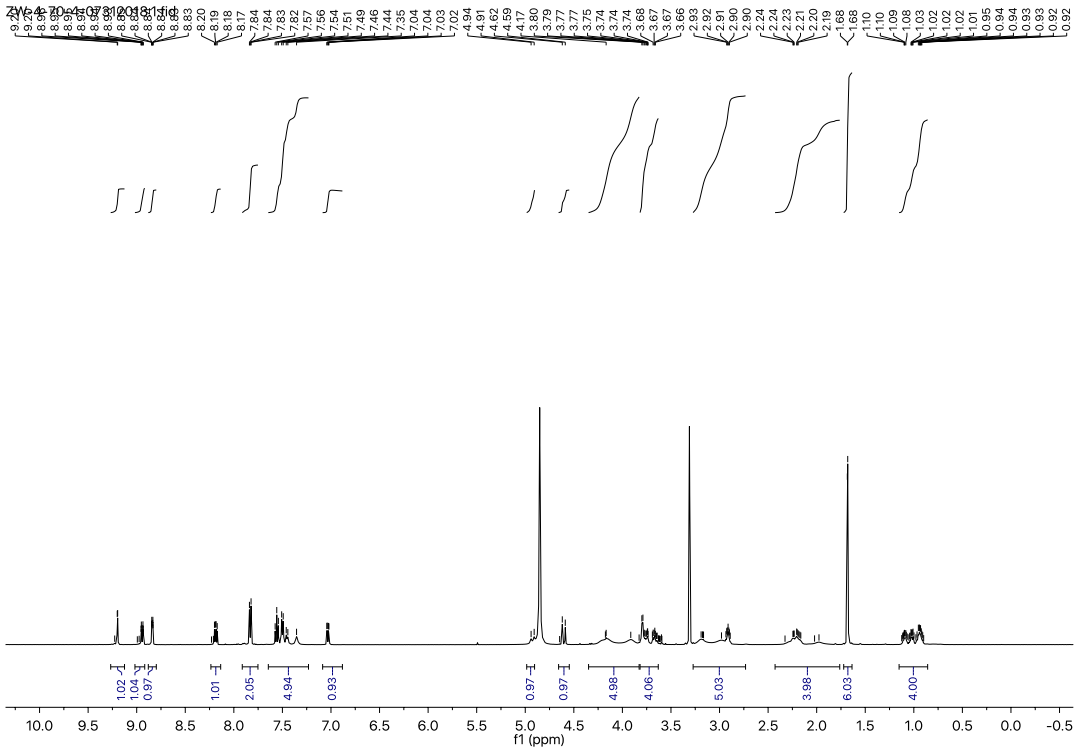
$^{1}$H NMR for compound **12**



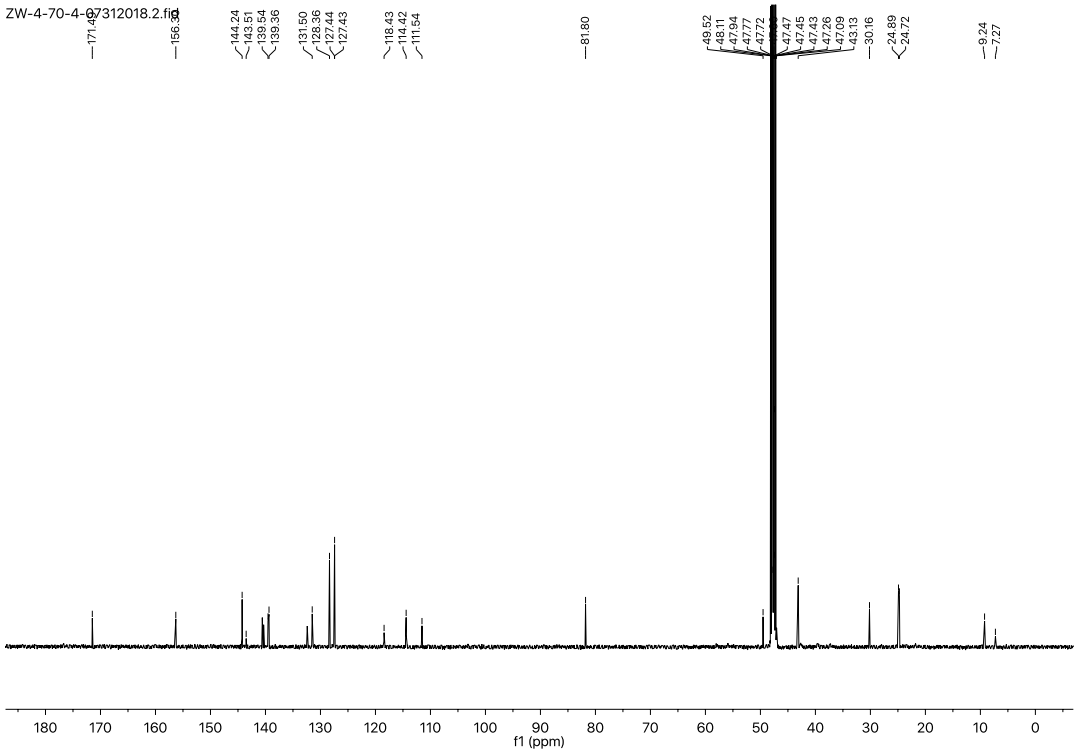
$^{13}$C NMR for compound **12**



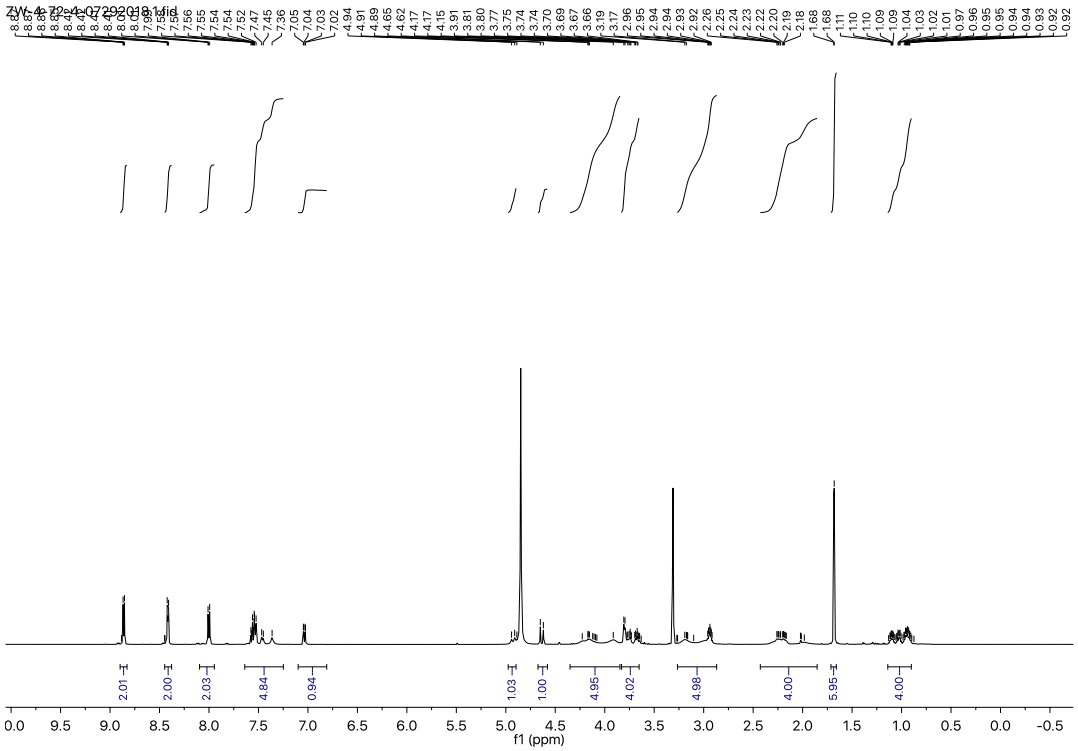
$^{1}$H NMR for compound **13**



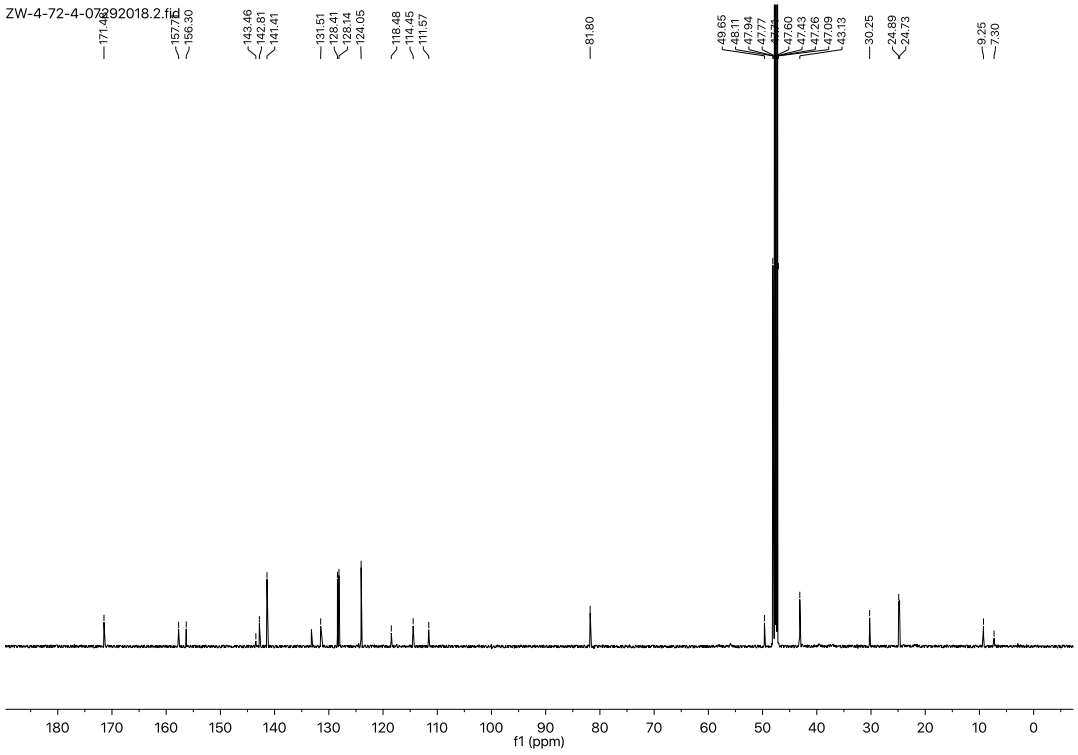

$^{13}$C NMR for compound **13**



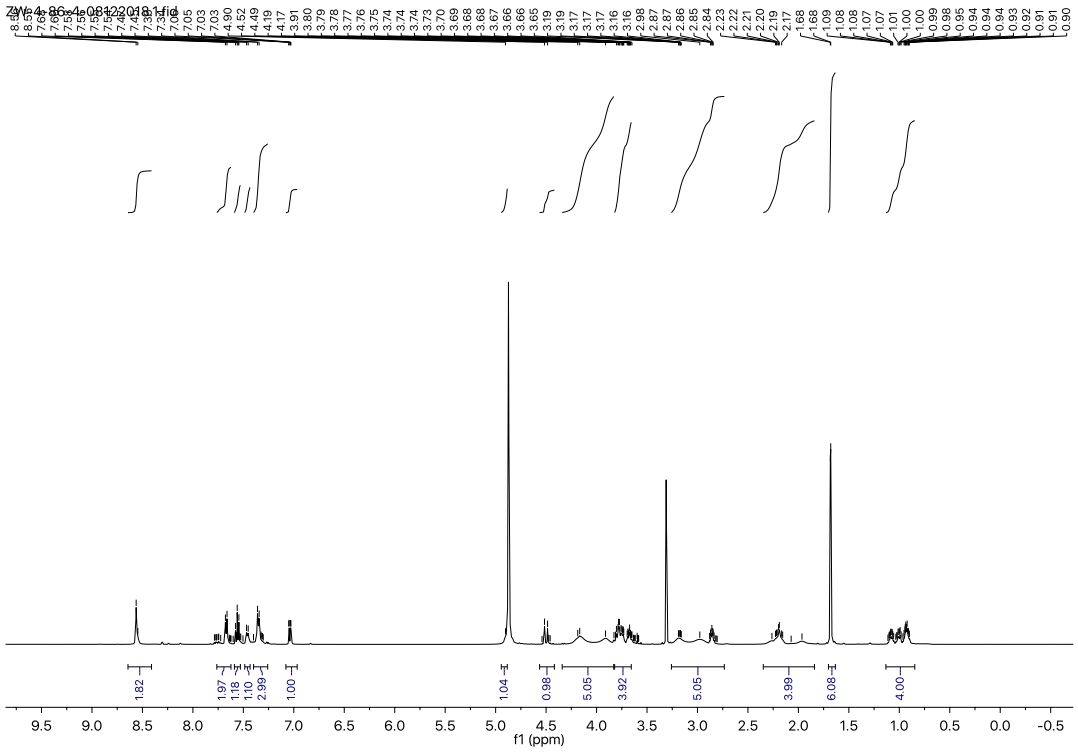
$^{1}$H NMR for compound **14**



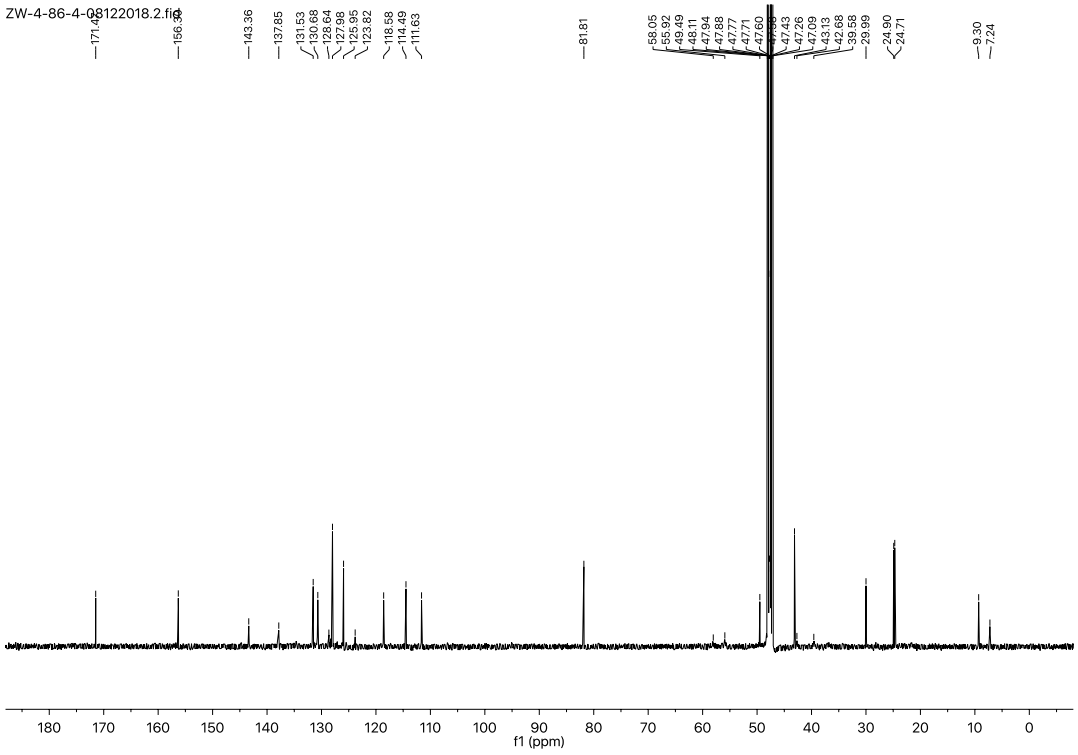

$^{13}$C NMR for compound **14**



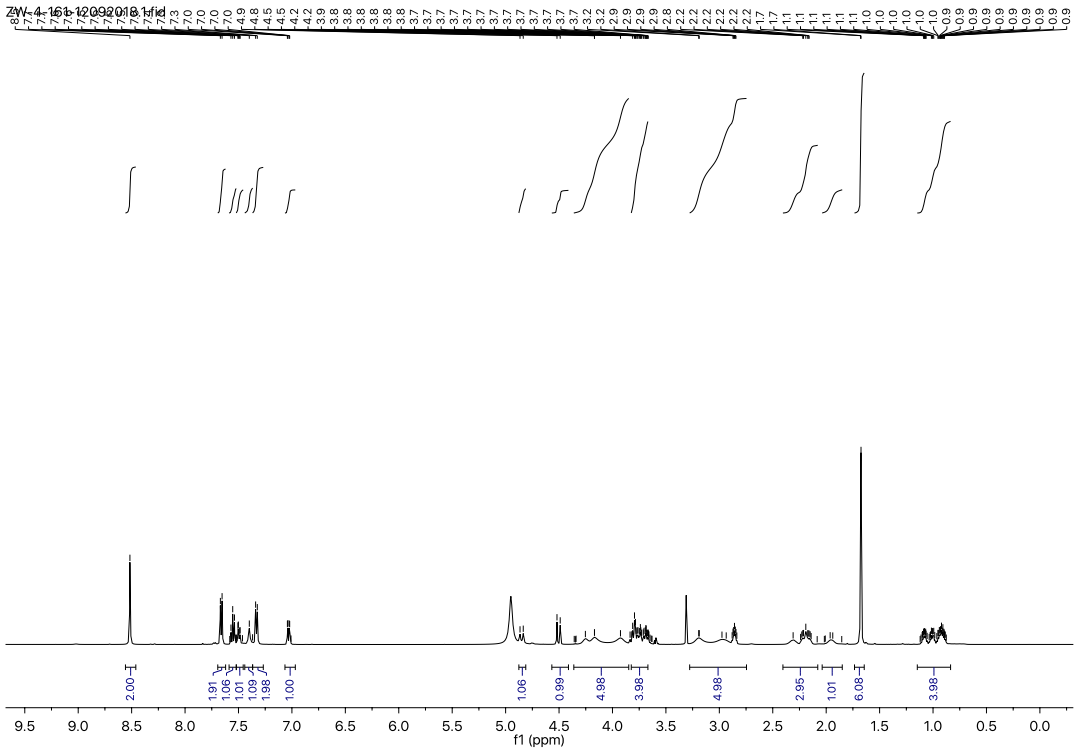
$^{1}$H NMR for compound **15**



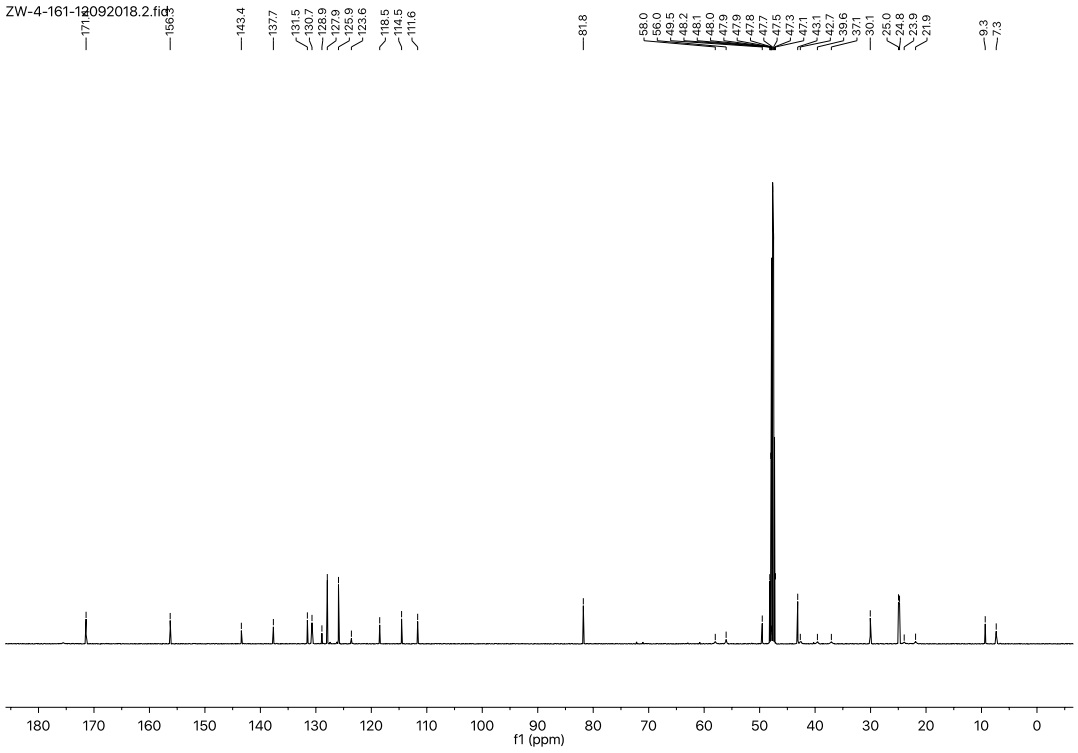
$^{13}$C NMR for compound **15**



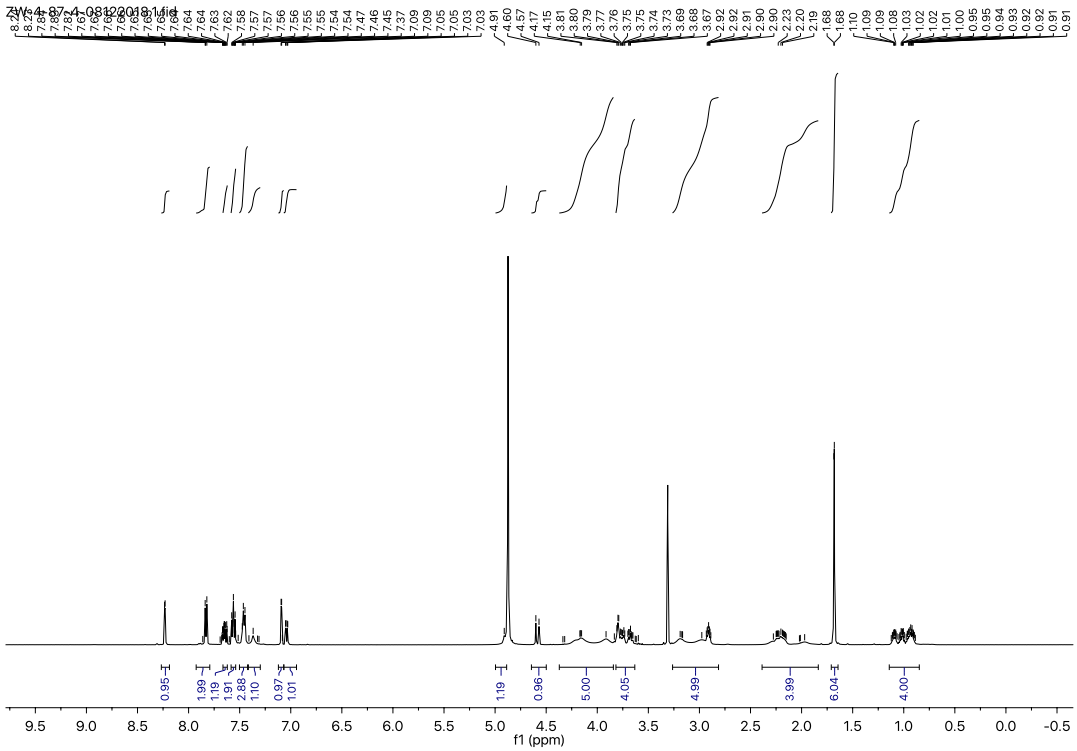
$^{1}$H NMR for compound **16**



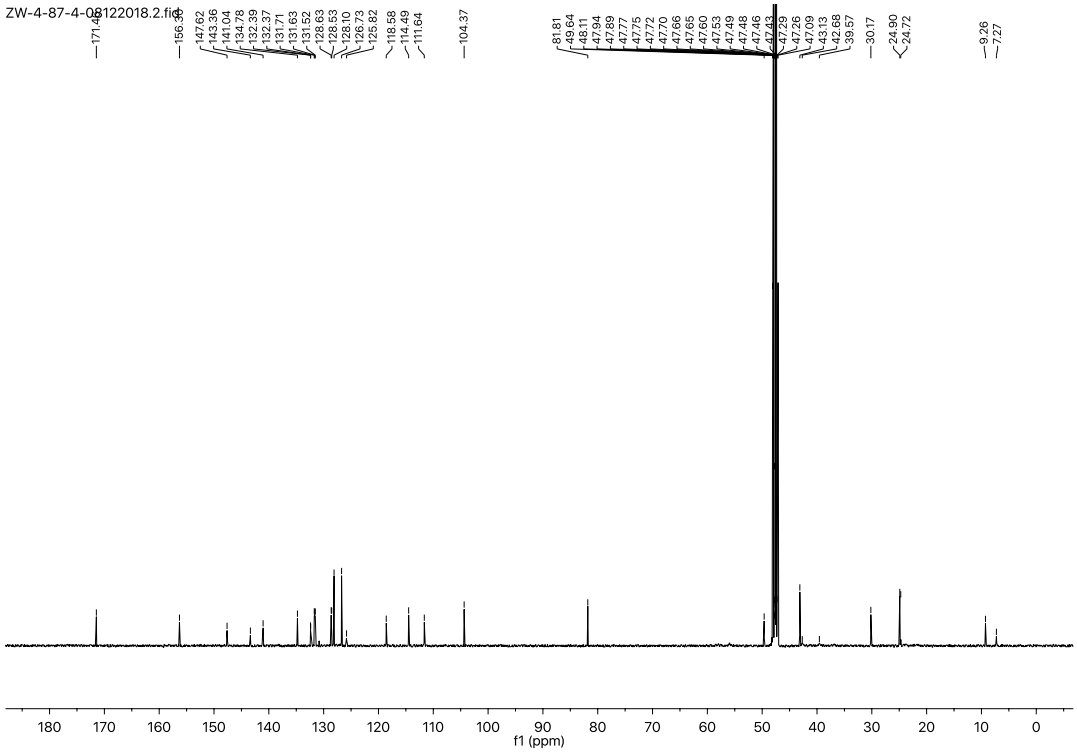

$^{13}$C NMR for compound **16**



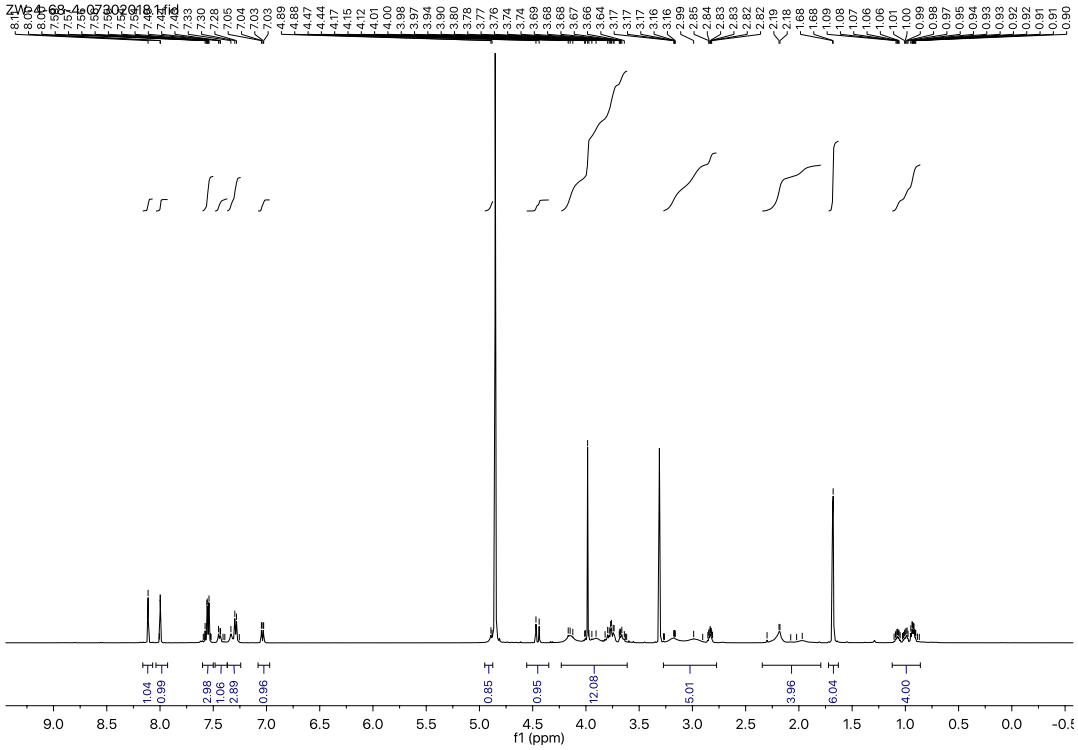
$^{1}$H NMR for compound **17**



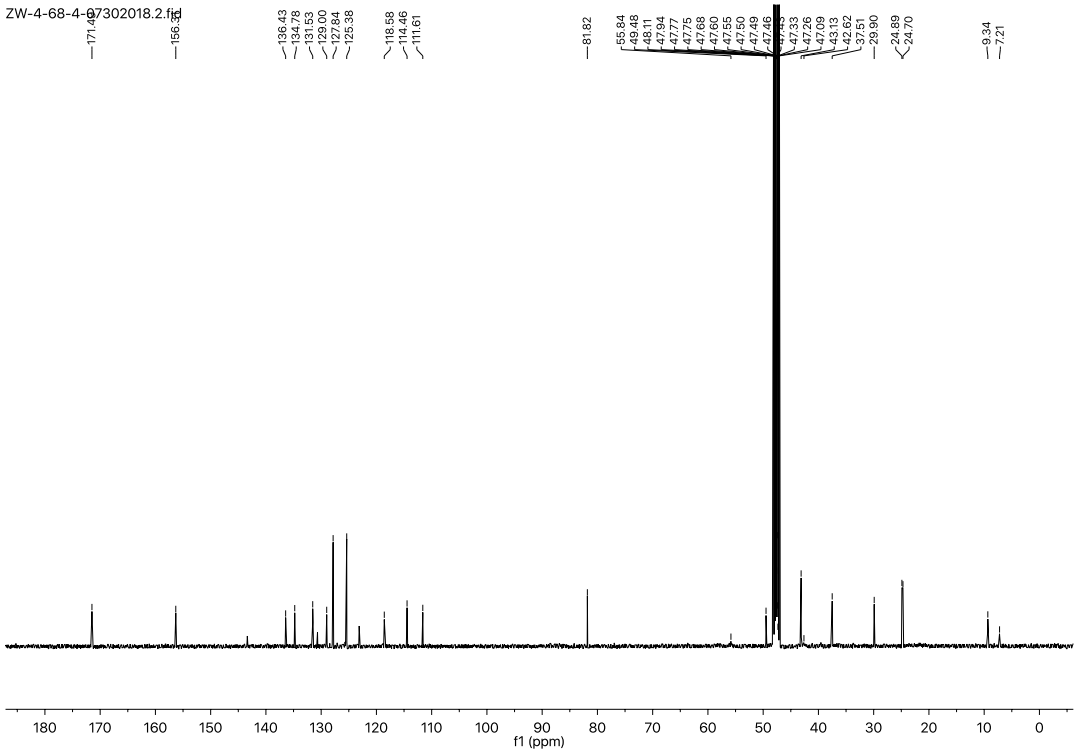

$^{13}$C NMR for compound **17**



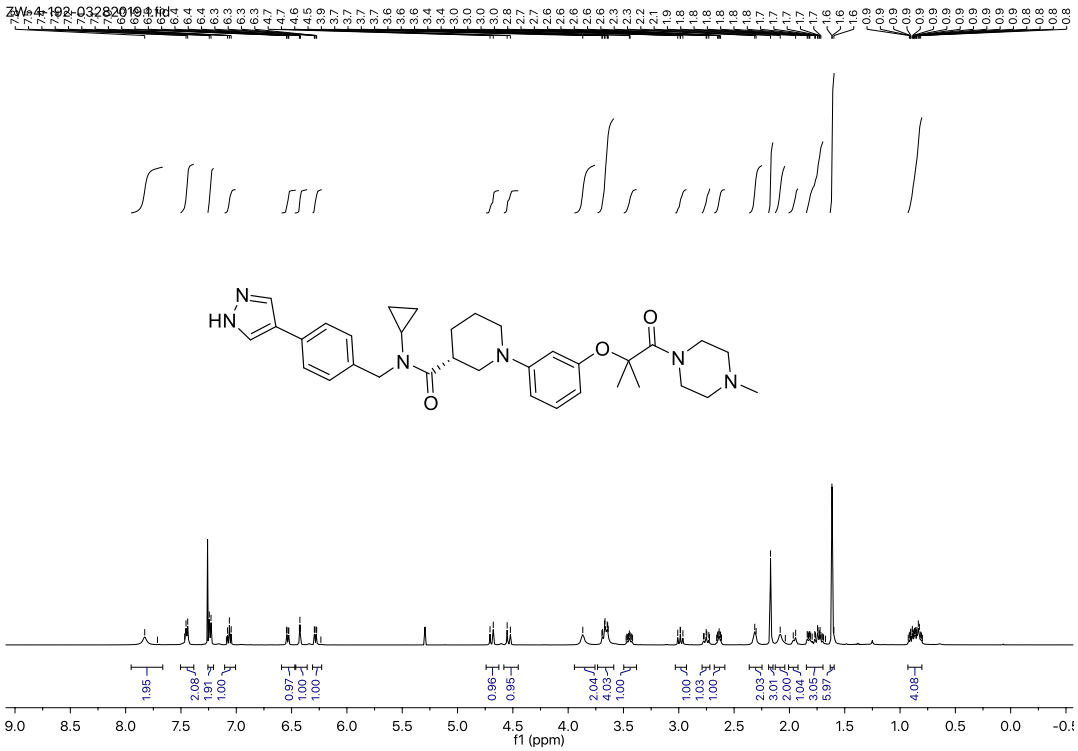
$^{1}$H NMR for compound **18**



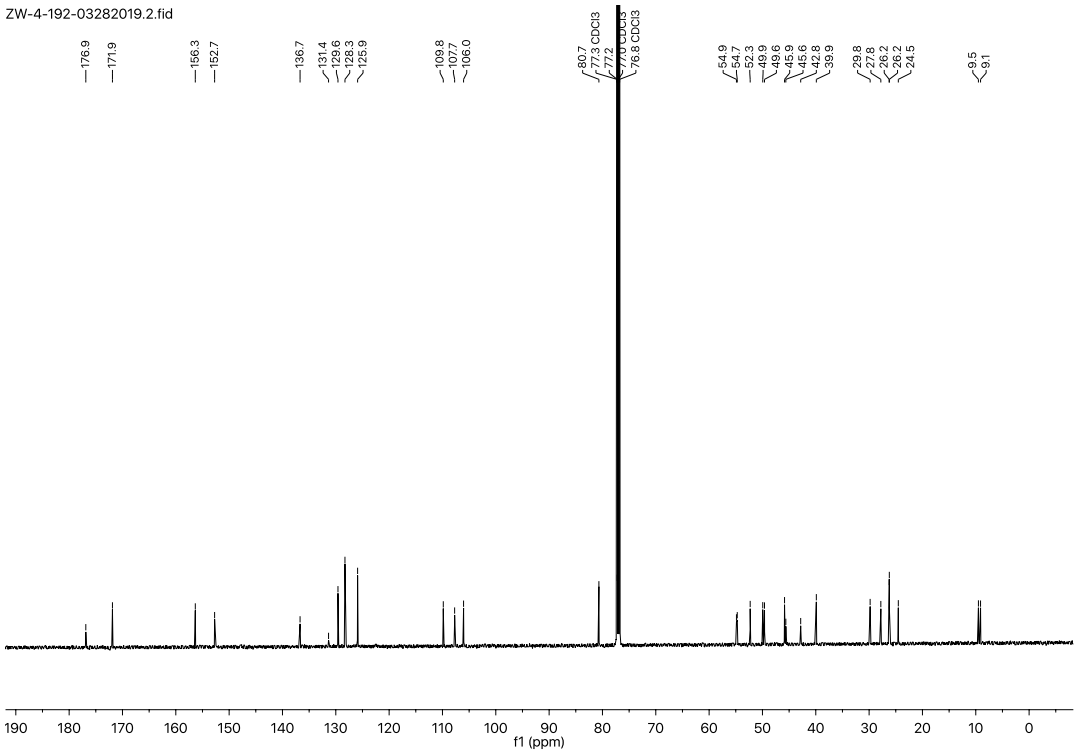

$^{13}$C NMR for compound **18**



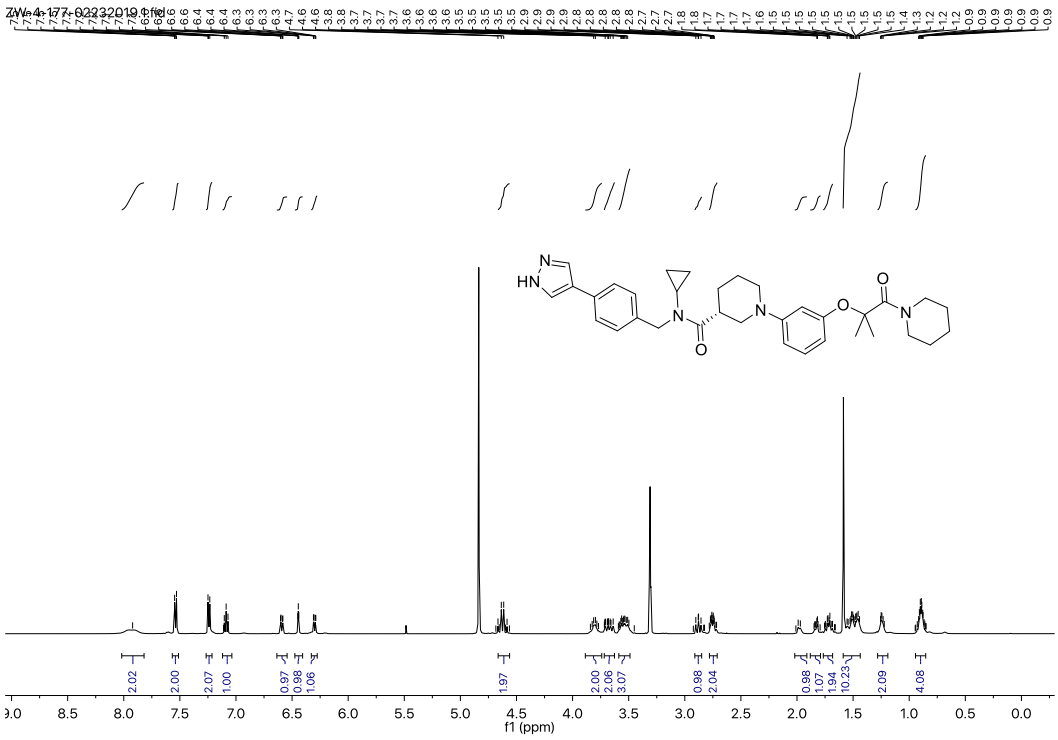
$^{1}$H NMR for compound **19**



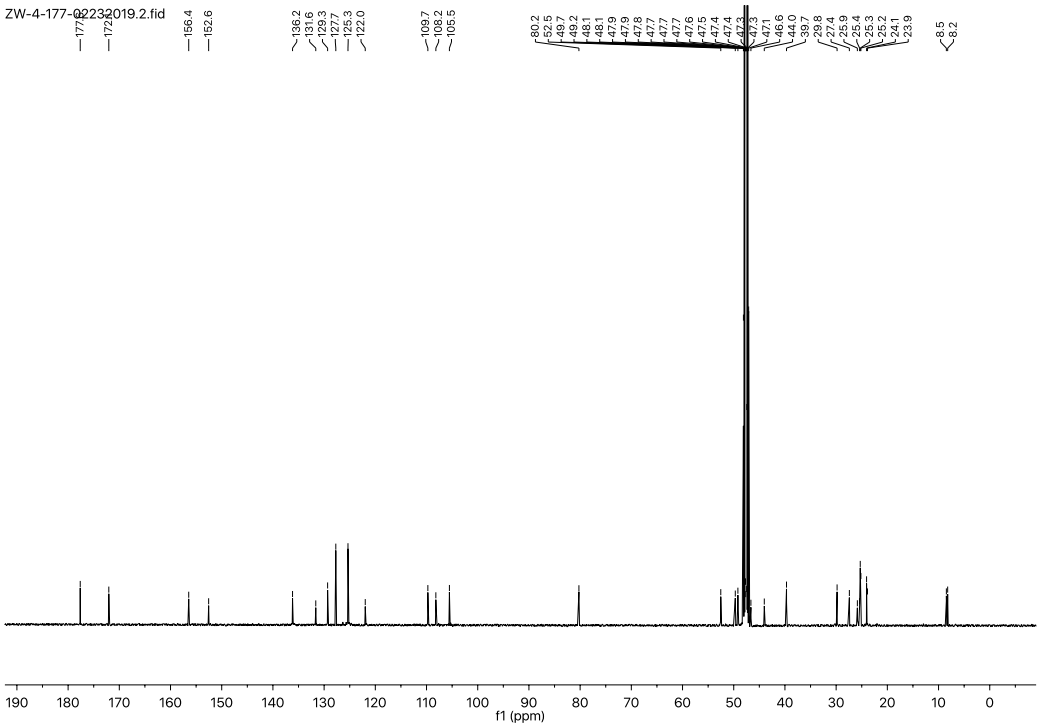

$^{13}$C NMR for compound **19**



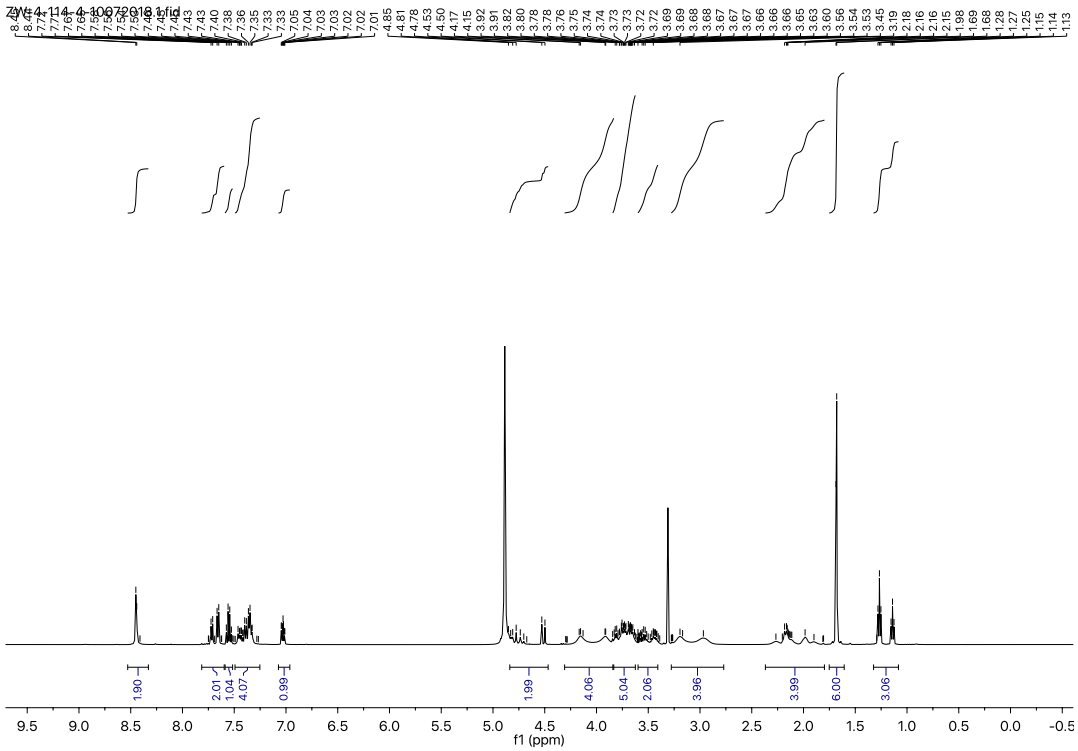
$^{1}$H NMR for compound **20**



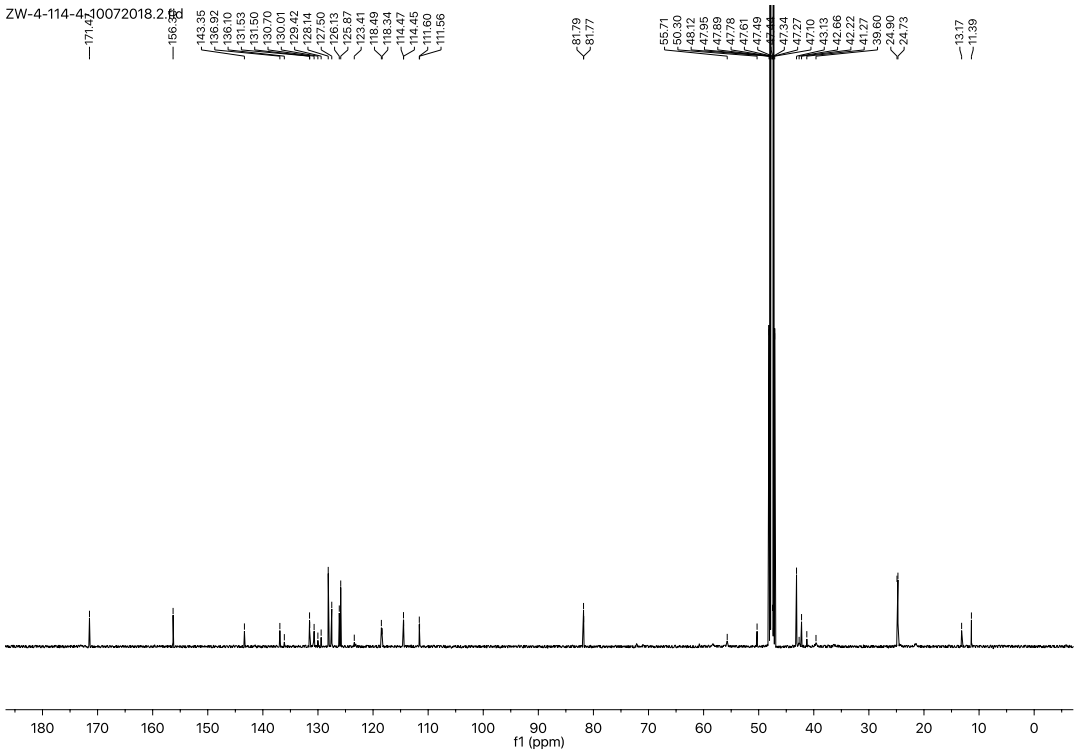

$^{13}$C NMR for compound **20**



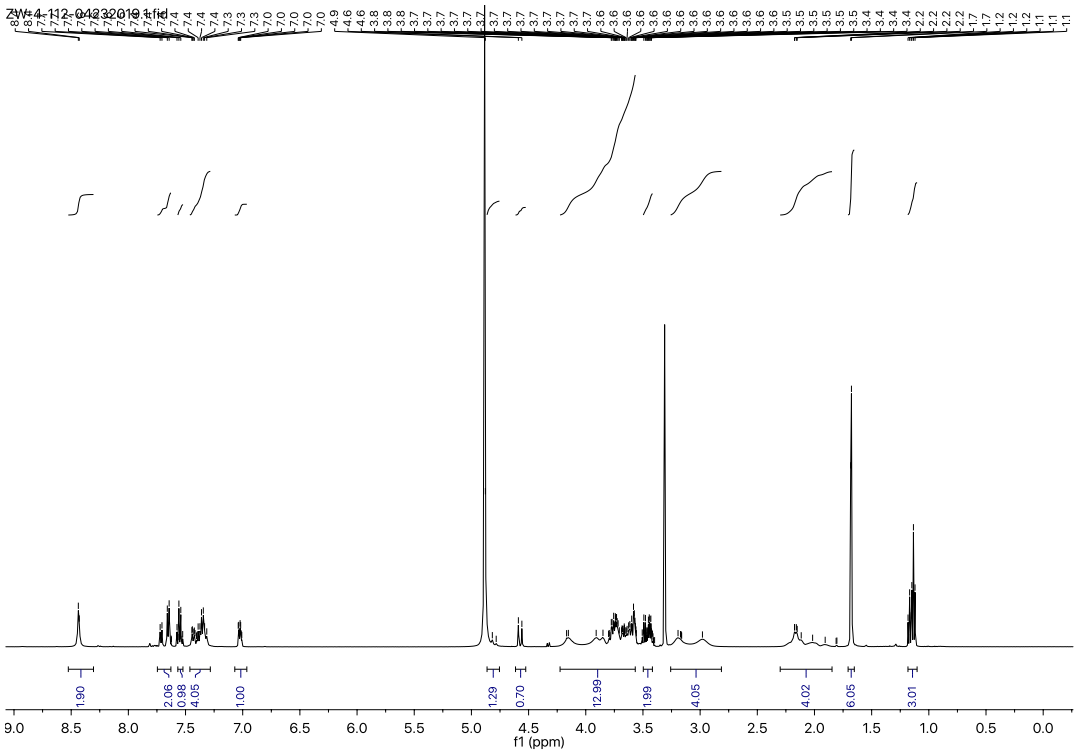
$^{1}$H NMR for compound **21**



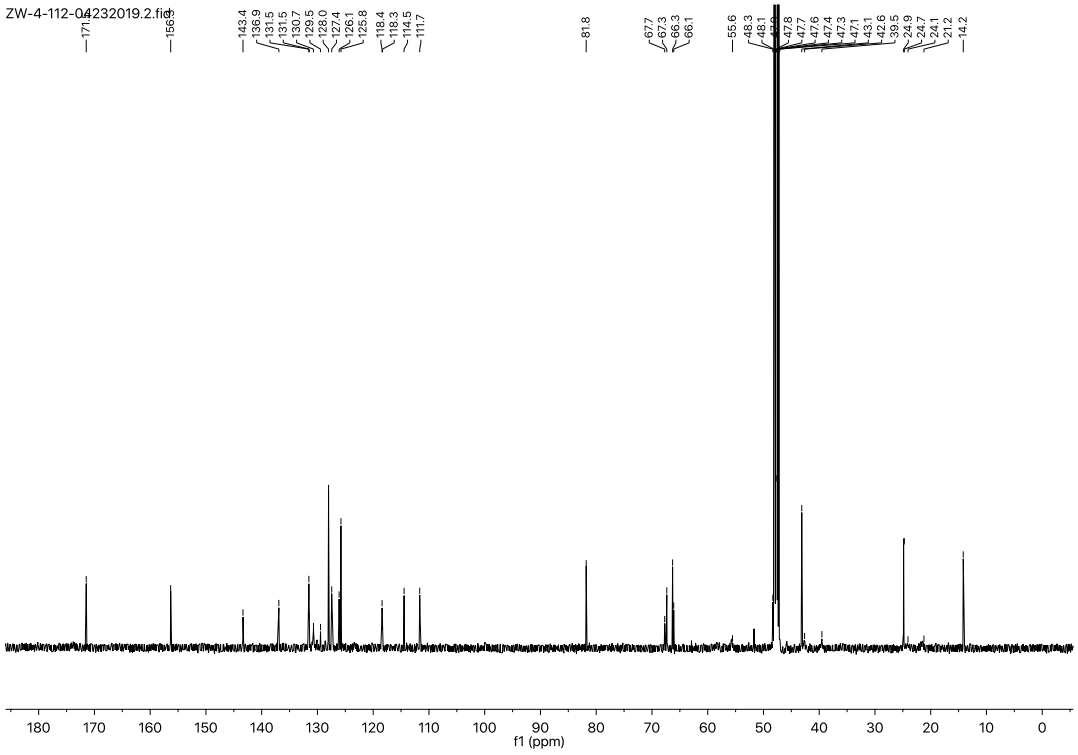

$^{13}$C NMR for compound **21**



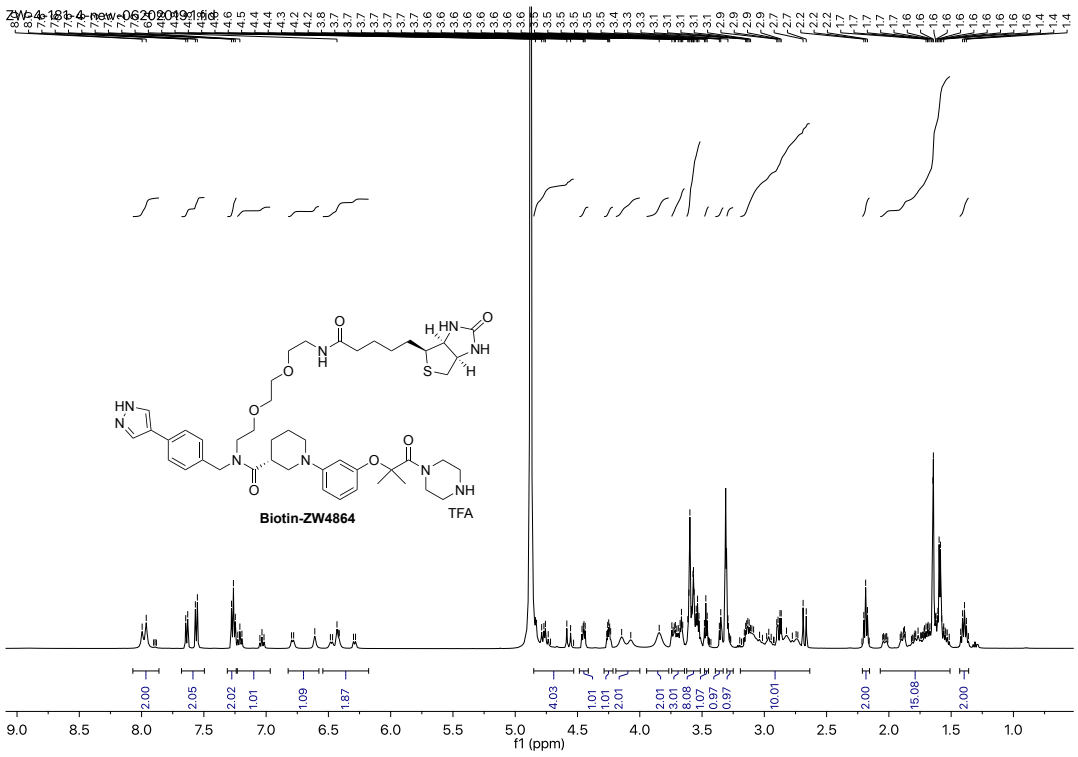
$^{1}$H NMR for compound **22**



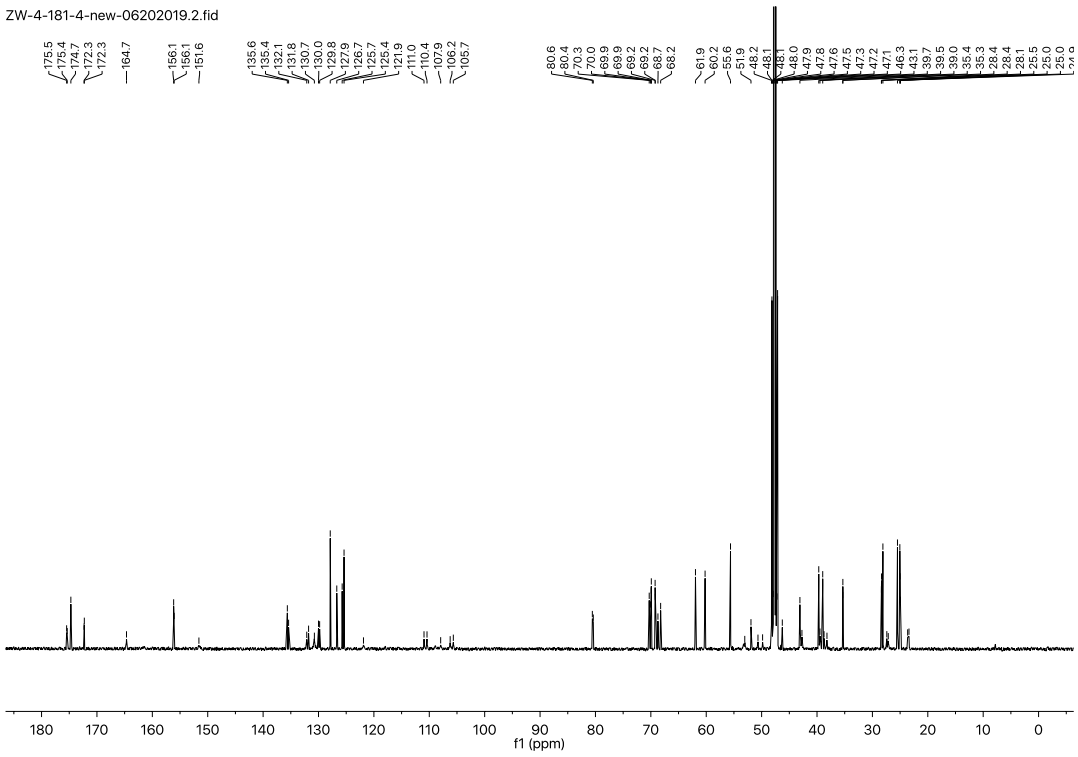

$^{13}$C NMR for compound **22**



$^{1}$H NMR for compound **Biotin-ZW4864**

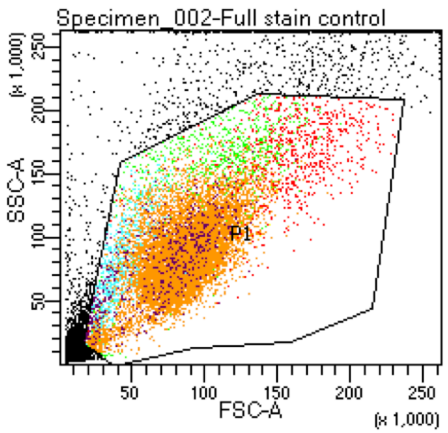


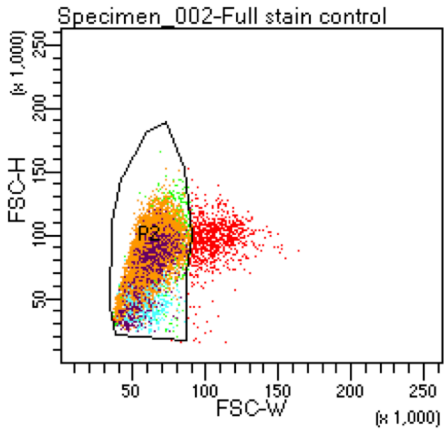

$^{13}$C NMR for compound **Biotin-ZW4864**

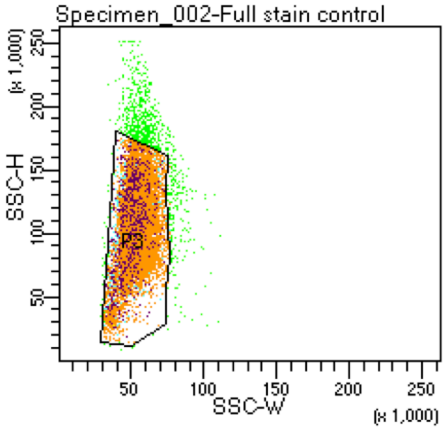


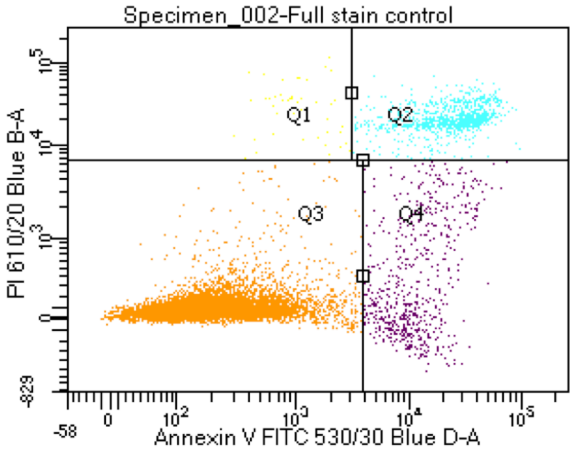
# MDA-MB-231 vehicle control

BD FACSDiva 8.0.1



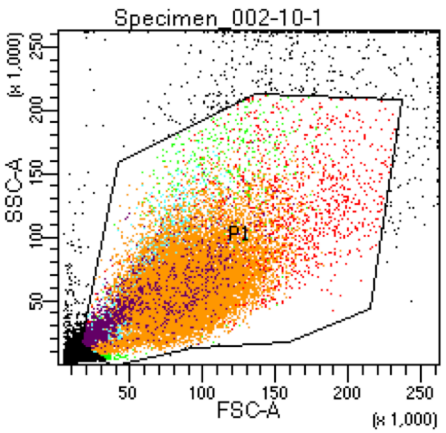



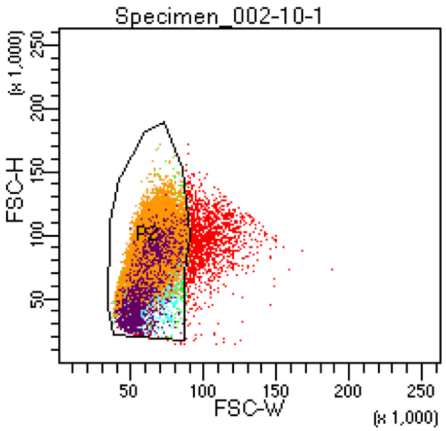

Tube: Full stain control

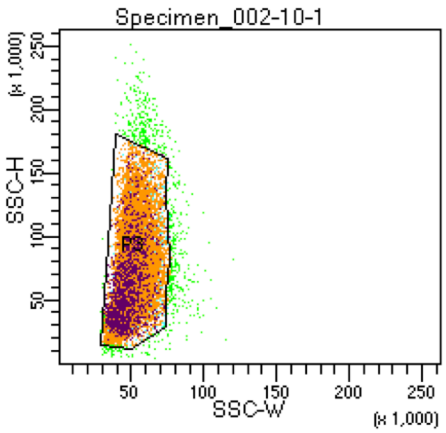
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 19,051 | #### | 100.0 |
| P1 | 13,269 | 69.6 | 69.6 |
| P2 | 12,417 | 93.6 | 65.2 |
| P3 | 11,557 | 93.1 | 60.7 |
| Q1 | 45 | 0.4 | 0.2 |
| Q2 | 1,019 | 8.8 | 5.3 |
| Q3 | 9,883 | 85.5 | 51.9 |
| Q4 | 610 | 5.3 | 3.2 |

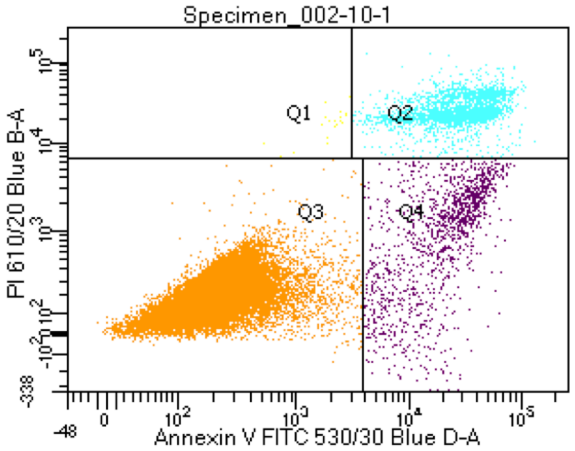
# MDA-MB-231 10 uM ZW4864

BD FACSDiva 8.0.1



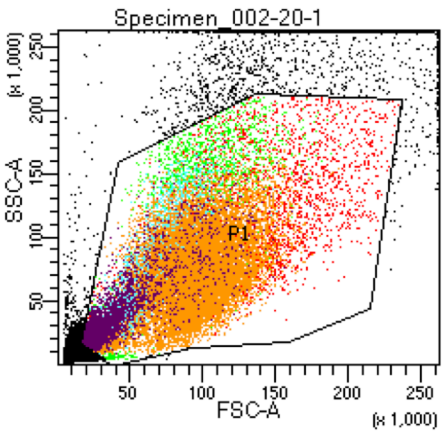



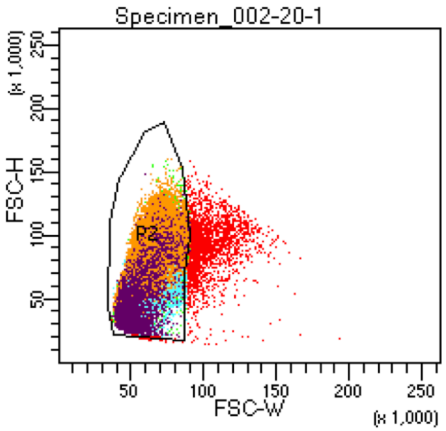

Tube: 10-1

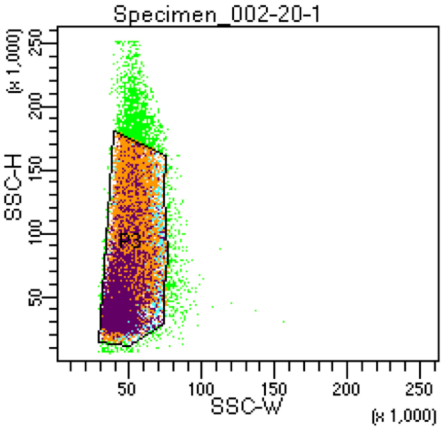
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 35,804 | #### | 100.0 |
| P1 | 26,380 | 73.7 | 73.7 |
| P2 | 24,902 | 94.4 | 69.6 |
| P3 | 24,254 | 97.4 | 67.7 |
| Q1 | 22 | 0.1 | 0.1 |
| Q2 | 2,961 | 12.2 | 8.3 |
| Q3 | 19,965 | 82.3 | 55.8 |
| Q4 | 1,306 | 5.4 | 3.6 |

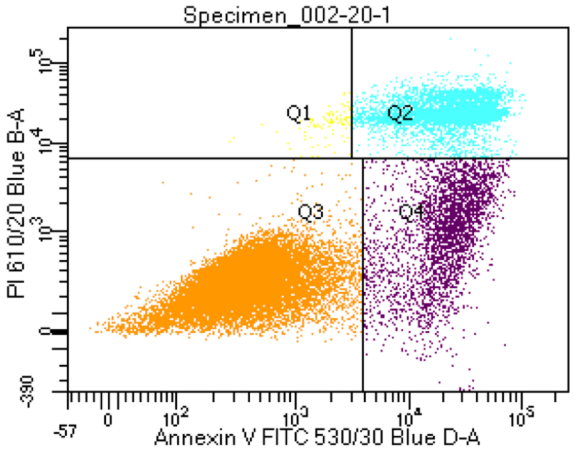
# MDA-MB-231 20 uM ZW4864

BD FACSDiva 8.0.1



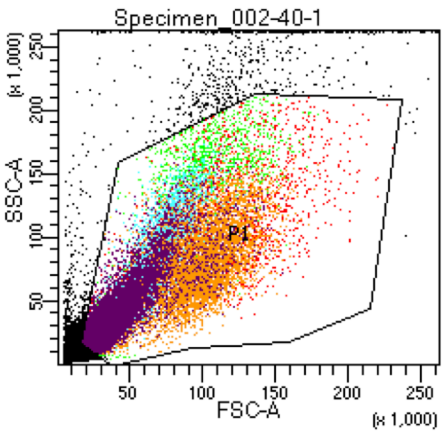



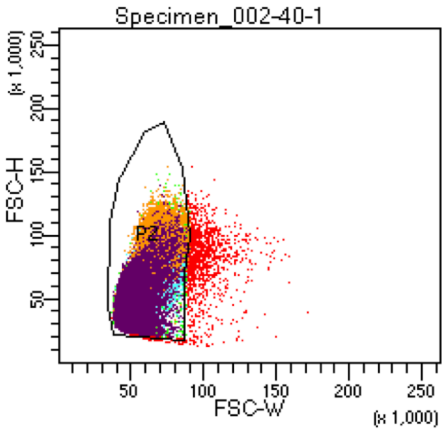

Tube: 20-1

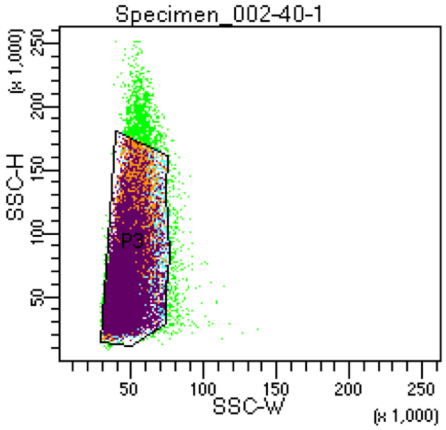
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 43,344 | #### | 100.0 |
| P1 | 26,462 | 61.1 | 61.1 |
| P2 | 24,116 | 91.1 | 55.6 |
| P3 | 22,351 | 92.7 | 51.6 |
| Q1 | 92 | 0.4 | 0.2 |
| Q2 | 5,147 | 23.0 | 11.9 |
| Q3 | 14,116 | 63.2 | 32.6 |
| Q4 | 2,996 | 13.4 | 6.9 |

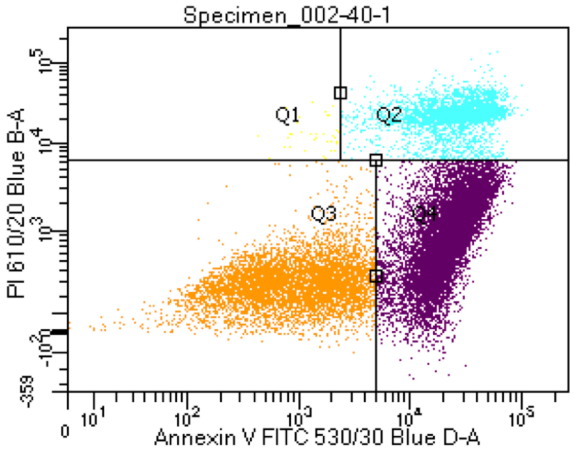
# MDA-MB-231 40 uM ZW4864

BD FACSDiva 8.0.1



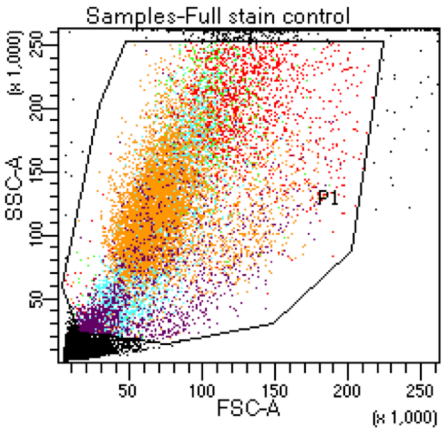



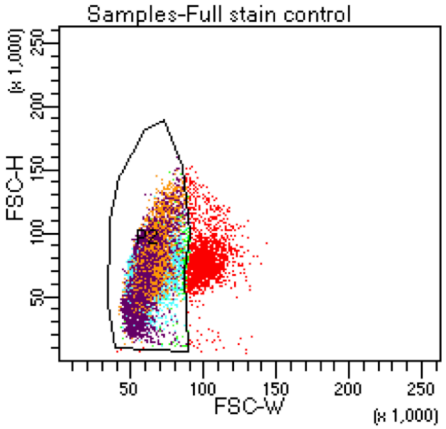

Tube: 40-1

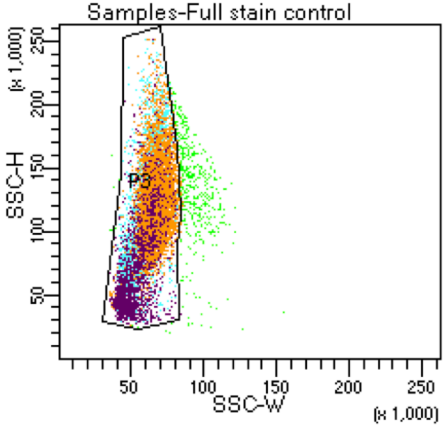
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 76,090 | #### | 100.0 |
| P1 | 25,557 | 33.6 | 33.6 |
| P2 | 24,118 | 94.4 | 31.7 |
| P3 | 22,555 | 93.5 | 29.6 |
| Q1 | 35 | 0.2 | 0.0 |
| Q2 | 4,252 | 18.9 | 5.6 |
| Q3 | 6,281 | 27.8 | 8.3 |
| Q4 | 11,987 | 53.1 | 15.8 |

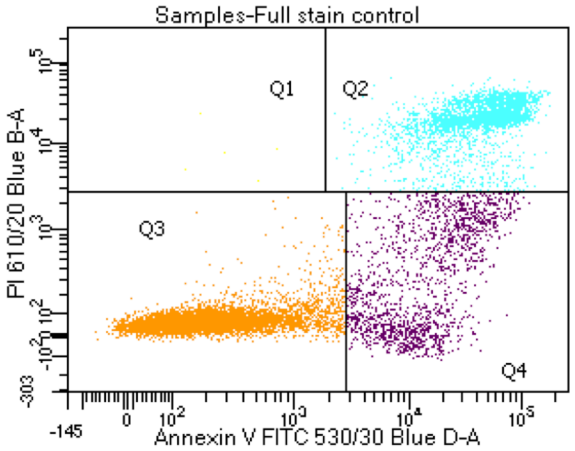
# MDA-MB-468 vehicle control

BD FACSDiva 8.0.1



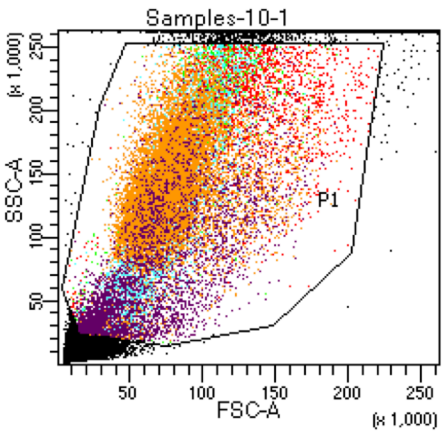



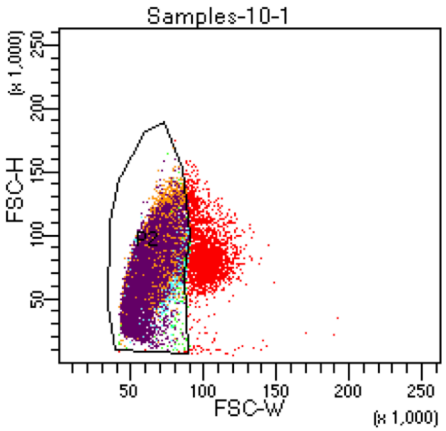

Tube: Full stain control

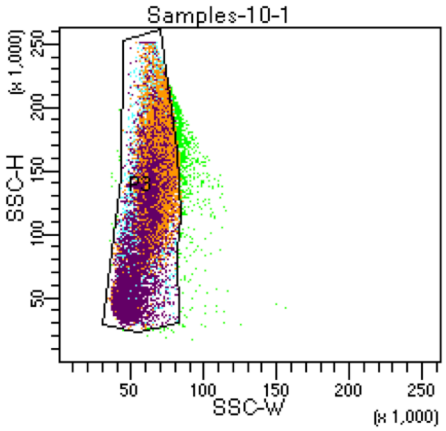
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 22,537 | #### | 100.0 |
| P1 | 13,648 | 60.6 | 60.6 |
| P2 | 11,824 | 86.6 | 52.5 |
| P3 | 11,370 | 96.2 | 50.5 |
| Q1 | 5 | 0.0 | 0.0 |
| Q2 | 3,288 | 28.9 | 14.6 |
| Q3 | 6,492 | 57.1 | 28.8 |
| Q4 | 1,585 | 13.9 | 7.0 |

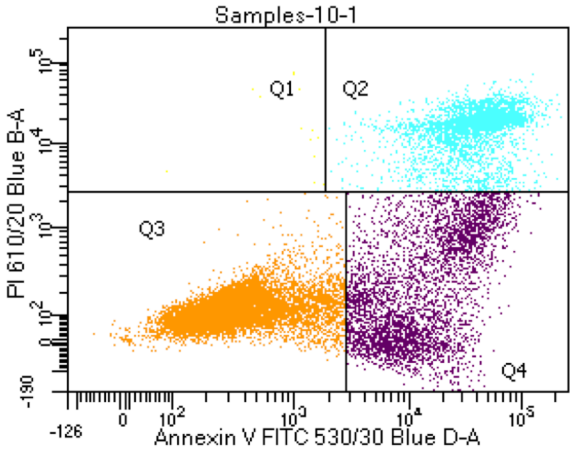
# MDA-MB-468 10 uM ZW4864

BD FACSDiva 8.0.1



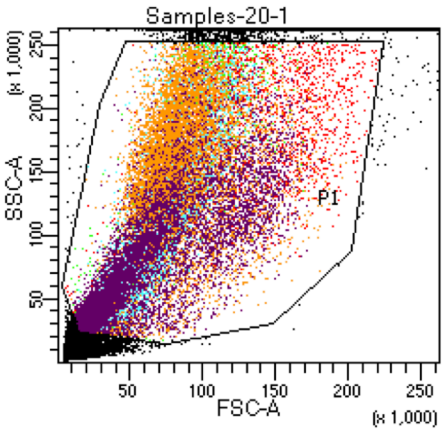



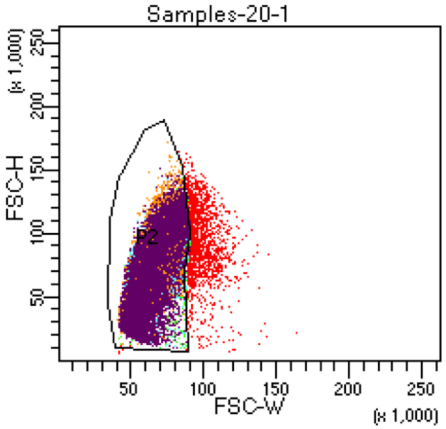

Tube: 10-1

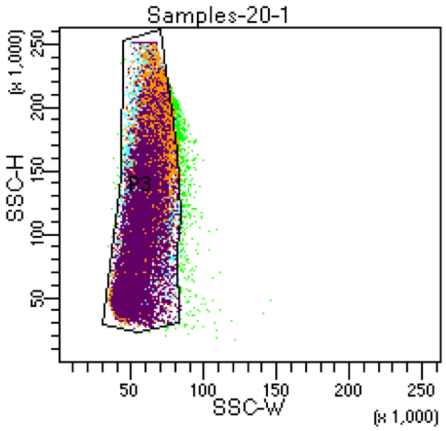
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 44,726 | #### | 100.0 |
| P1 | 25,283 | 56.5 | 56.5 |
| P2 | 22,969 | 90.8 | 51.4 |
| P3 | 22,306 | 97.1 | 49.9 |
| Q1 | 13 | 0.1 | 0.0 |
| Q2 | 3,925 | 17.6 | 8.8 |
| Q3 | 14,460 | 64.8 | 32.3 |
| Q4 | 3,908 | 17.5 | 8.7 |

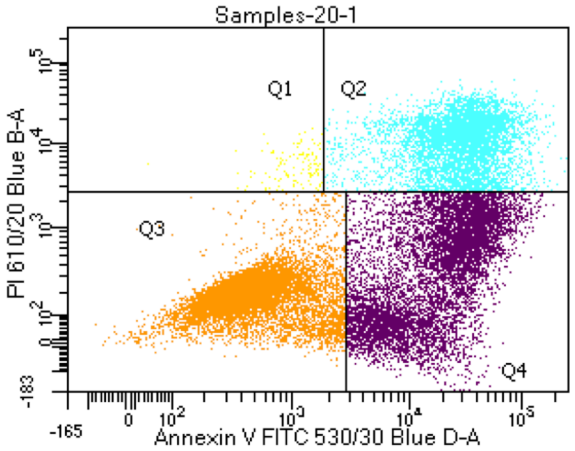
# MDA-MB-468 20 uM ZW4864

BD FACSDiva 8.0.1



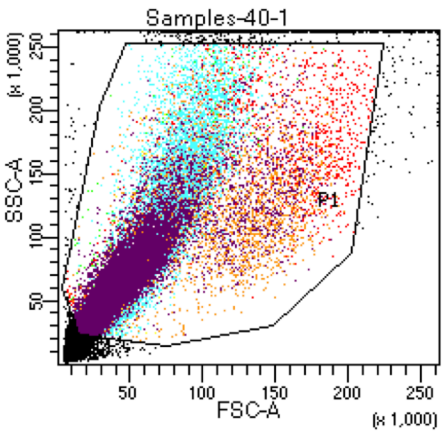



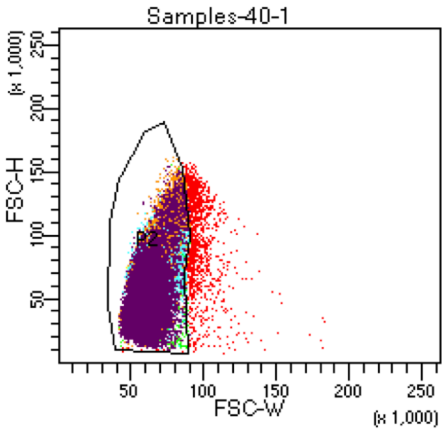

Tube: 20-1

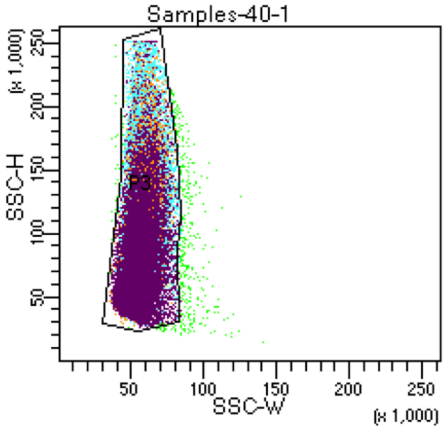
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 46,880 | #### | 100.0 |
| P1 | 25,581 | 54.6 | 54.6 |
| P2 | 23,954 | 93.6 | 51.1 |
| P3 | 23,353 | 97.5 | 49.8 |
| Q1 | 126 | 0.5 | 0.3 |
| Q2 | 5,735 | 24.6 | 12.2 |
| Q3 | 9,406 | 40.3 | 20.1 |
| Q4 | 8,086 | 34.6 | 17.2 |

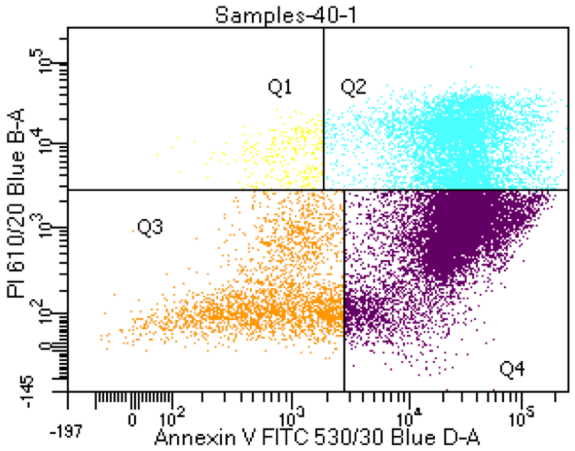
# MDA-MB-468 40 uM ZW4864

BD FACSDiva 8.0.1



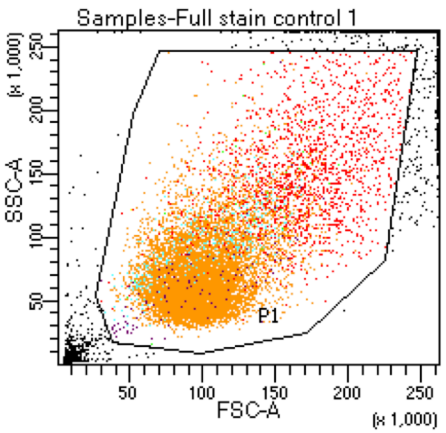



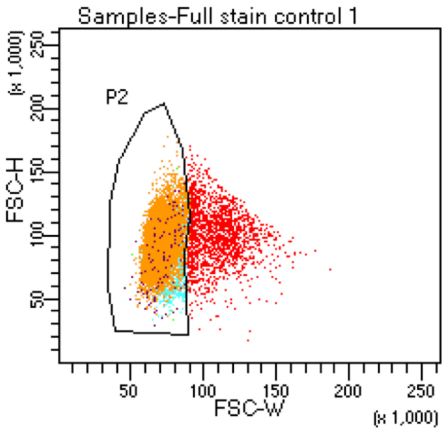

Tube: 40-1

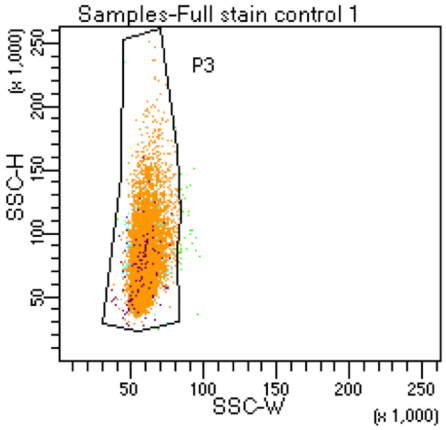
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 40,946 | #### | 100.0 |
| P1 | 24,454 | 59.7 | 59.7 |
| P2 | 23,221 | 95.0 | 56.7 |
| P3 | 22,813 | 98.2 | 55.7 |
| Q1 | 281 | 1.2 | 0.7 |
| Q2 | 7,609 | 33.4 | 18.6 |
| Q3 | 2,712 | 11.9 | 6.6 |
| Q4 | 12,211 | 53.5 | 29.8 |

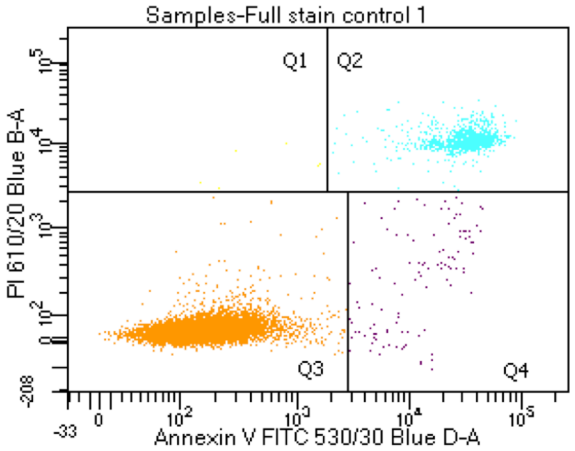
# MCF10A vehicle control

## BD FACSDiva 8.0.1



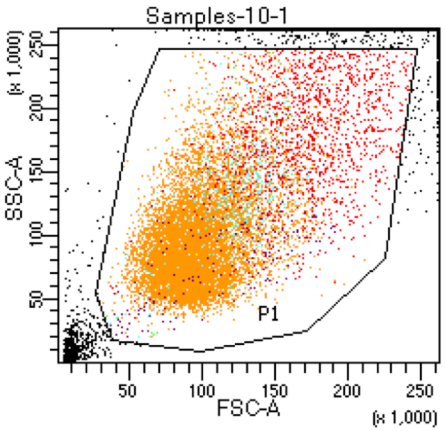



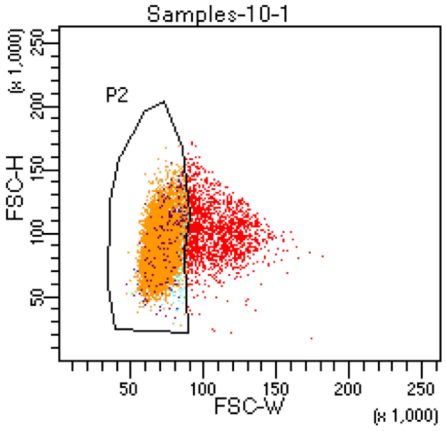

Tube: Full stain control 1

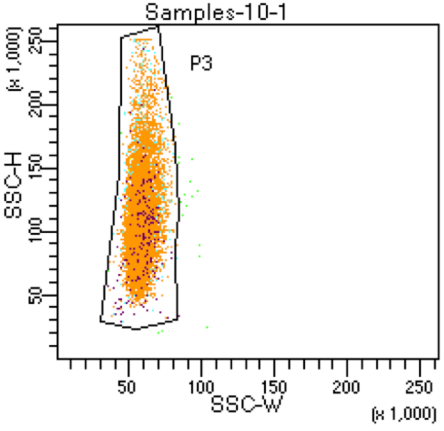
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 13,566 | #### | 100.0 |
| P1 | 11,809 | 87.0 | 87.0 |
| P2 | 10,036 | 85.0 | 74.0 |
| P3 | 10,000 | 99.6 | 73.7 |
| Q1 | 6 | 0.1 | 0.0 |
| Q2 | 1,263 | 12.6 | 9.3 |
| Q3 | 8,620 | 86.2 | 63.5 |
| Q4 | 111 | 1.1 | 0.8 |

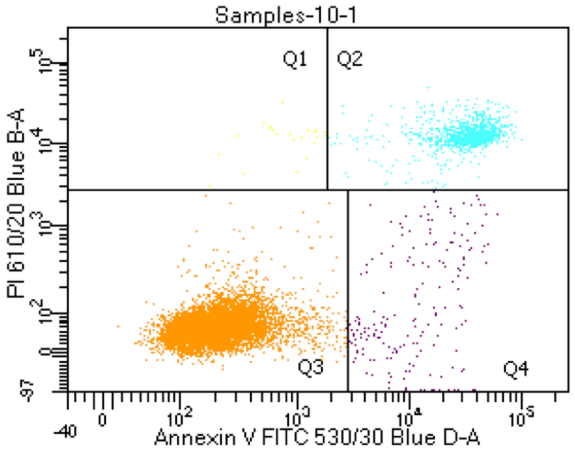
# MCF10A 10 uM ZW4864

BD FACSDiva 8.0.1



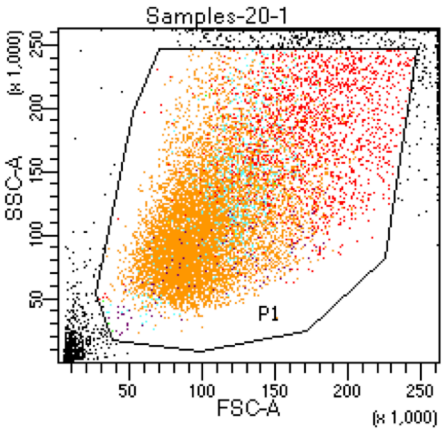



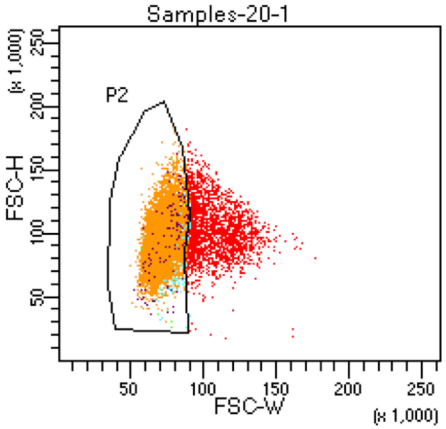

Tube: 10-1

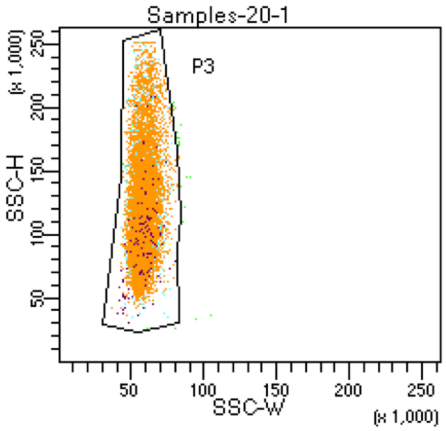
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 14,805 | #### | 100.0 |
| P1 | 11,754 | 79.4 | 79.4 |
| P2 | 10,102 | 85.9 | 68.2 |
| P3 | 10,082 | 99.8 | 68.1 |
| Q1 | 24 | 0.2 | 0.2 |
| Q2 | 1,238 | 12.3 | 8.4 |
| Q3 | 8,628 | 85.6 | 58.3 |
| Q4 | 192 | 1.9 | 1.3 |

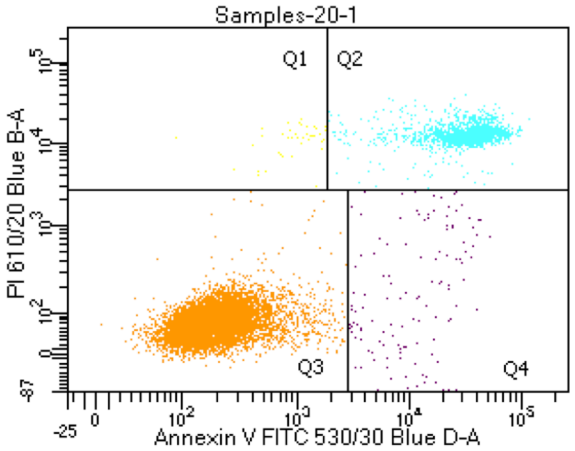
# MCF10A 20 uM ZW4864

BD FACSDiva 8.0.1



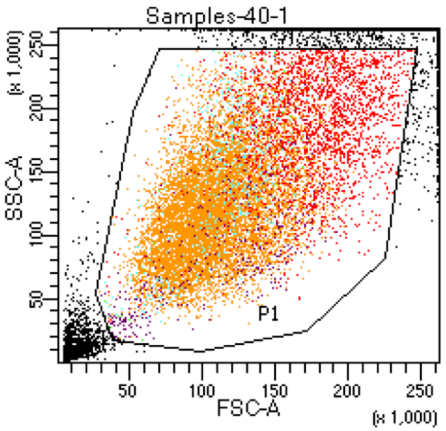



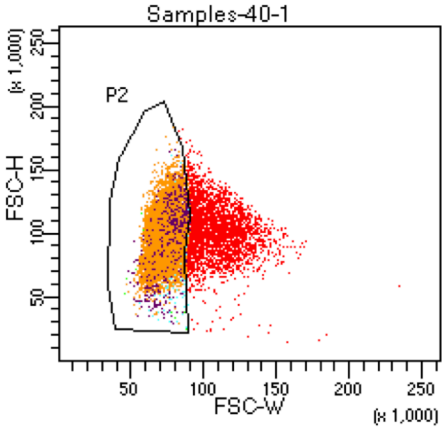

Tube: 20-1

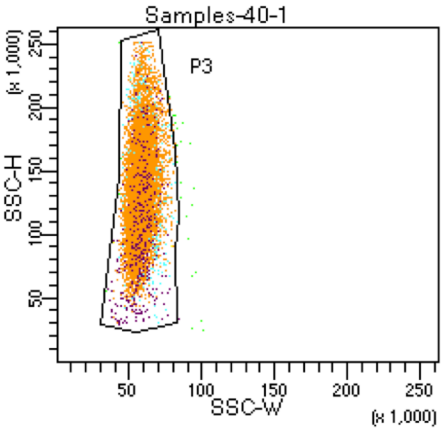
| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 15,847 | #### | 100.0 |
| P1 | 12,108 | 76.4 | 76.4 |
| P2 | 10,022 | 82.8 | 63.2 |
| P3 | 10,000 | 99.8 | 63.1 |
| Q1 | 40 | 0.4 | 0.3 |
| Q2 | 1,726 | 17.3 | 10.9 |
| Q3 | 8,107 | 81.1 | 51.2 |
| Q4 | 127 | 1.3 | 0.8 |

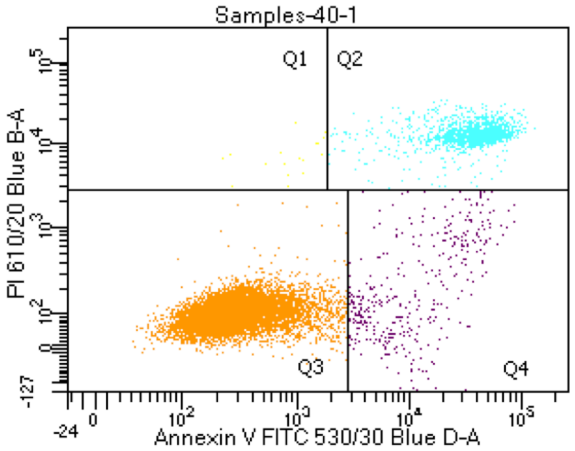
# MCF10A 40 uM ZW4864

BD FACSDiva 8.0.1




Tube: 40-1

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 18,450 | #### | 100.0 |
| P1 | 13,209 | 71.6 | 71.6 |
| P2 | 10,034 | 76.0 | 54.4 |
| P3 | 10,000 | 99.7 | 54.2 |
| Q1 | 17 | 0.2 | 0.1 |
| Q2 | 1,528 | 15.3 | 8.3 |
| Q3 | 8,068 | 80.7 | 43.7 |
| Q4 | 387 | 3.9 | 2.1 |