Electronic Supplementary Information (ESI)

Higher-Order Human Telomeric G-Quadruplex DNA Metalloenzyme Catalyzed Diels-Alder Reaction: An Unexpected Inversion of Enantioselectivity Modulated by K^+ and NH_4^+ ions†

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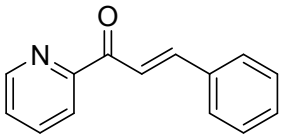
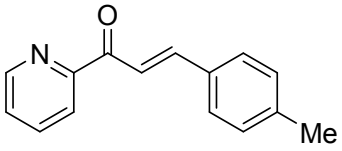
Materials

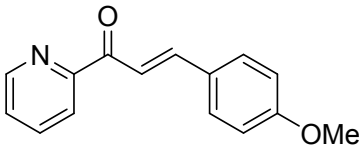
All DNA oligodeoxynucleotides were purchased from Sangon (Shanghai, China). 3-(N-morpholino)propanesulfonic acid (MOPS) was purchased from Sangon (Shanghai, China). $Cu(NO_3)_2 \cdot 3H_2O$ (>99.5%), KCl (>99.5%) and NH_4Cl (>99.5%) were purchased from the Shanghai Chemical Reagent Company of the Chinese Medicine Group. Water was distilled and deionized (specific resistance of 18.2 MΩ at 25 ºC) using a Milli-Q A10 water purification system. All experiments were carried out in 20 mM MOPS buffer (pH 6.5) unless otherwise stated. Other reagents and solvents were obtained from commercial sources and used without further purification. Dienophiles **1a-f** were prepared according to the literature.[1] All of the racemic products were prepared by the Diels-Alder reaction using $Cu(NO_3)_2$ as catalyst in methanol.



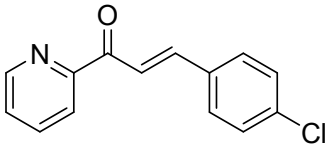
1a



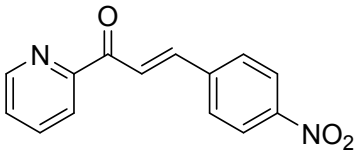
1b



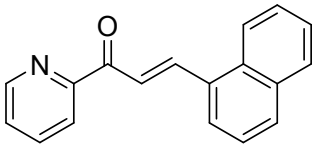
1c



1d



1e



1f

Methods

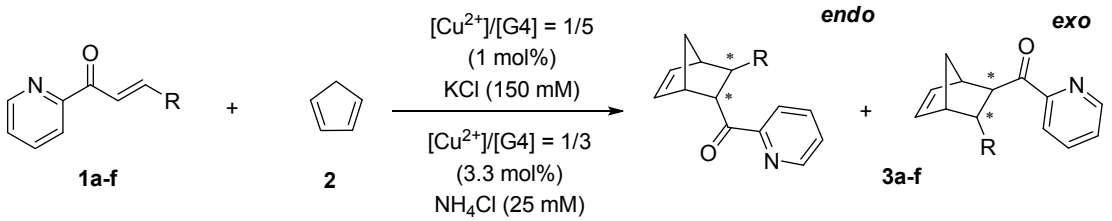
Circular dichroism (CD) spectra were recorded on a dual beam DSM 1000 CD spectrophotometer (Olis, Bogart, GA) with a 10 mm path-length quartz cell. Each measurement was recorded from 220 to 320 nm at 4 ºC under N_2 purge. The scan rate was 0.5 nm per second. The average scan for each sample was subtracted by a background CD spectrum of corresponding buffer solution. The molarities are related to G-quadruplex unit. CD samples of all G-quadruplexes were prepared at a concentration (G4 unit) of 5 μM by using a MOPS buffer solution (20 mM, pH = 6.5) consisting of different concentration of KCl or NH_4Cl as required.

The precise DNA strand concentrations were determined by measuring the UV absorbance of sample at 260 nm using the molar extinction coefficient values provided by the manufacturer. UV-Vis experiments were carried out on a Shimadzu 2450 spectrophotometer (Shimadzu, Japan) equipped with a Peltier temperature control accessory. All UV-Vis spectra were measured using a sealed quartz cell with a path length of 1.0 cm.

^1H NMR spectra were recorded on 400 MHz in $CDCl_3$ and ^{13}C NMR spectra were recorded on 100 MHz in $CDCl_3$ using TMS or residual protic solvent signals as internal standard. Data for ^1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved, coupling constant(s) in Hz, integration).

The enantioselectivity was determined by chiral HPLC analysis using Daicel chiralcel ODH, OJ-H column or chiralpak ADH, AD column with a UV-detector by using isopropanol and n-hexane as eluents at 25 °C.

General procedure



To a MOPS buffer (0.5 mL, 20 mM, pH 6.5) containing KCl (150 mM) or NH_4Cl (25 mM), an aqueous solution of HT45 (final G4 unit conc. of 50 μM) was added. After stirred for a half hour at 4 °C, a solution of $Cu(NO_3)_2$ (final conc. of 10 μM for K^+ case and 16.7 μM for NH_4^+ case) was added. Then, aza-chalcone **1** in CH_3CN (final conc. of 1 mM for K^+ case and 0.5 mM for NH_4^+ case, 1 equiv.) was added. The reaction was initiated by the addition of freshly distilled cyclopentadiene **2** (100 equiv.). The mixture was stirred for 12 hours (**1a**), 48 hours (**1b-e**) and 72 hours (**1f**) at 4 °C, respectively. Followed by the extraction with diethyl ether (3 × 5 mL), the solvent was removed under reduced pressure. The residue was directly analyzed by ^{1}H-NMR and HPLC. The conversions were determined by ^{1}H-NMR and HPLC (only for **3a**) of the crude product.[2] The diastereoselectivity (*endo*/*exo*) and enantiomeric excess (*ee*) were determined by chiral HPLC.

HPLC analysis condition

Product **3a**: Daicel Chiralcel-ODH, *n*-hexane/*i*-PrOH 98:2, flow rate 0.5 mL/min, λ = 212 nm).

Product **3b**: Daicel Chiralcel-ODH, *n*-hexane/*i*-PrOH 98:2, flow rate 0.5 mL/min, λ = 212 nm).

Product **3c**: Daicel Chiralpak-ADH, *n*-hexane/*i*-PrOH 95:5, flow rate 1.0 mL/min, λ = 212 nm).

Product **3d**: Daicel Chiralcel-OJH, *n*-hexane/*i*-PrOH 90:10, flow rate 0.5 mL/min, λ = 212 nm).

Product **3e**: Daicel Chiralpak-AD, *n*-hexane/*i*-PrOH 90:10, flow rate 1.0 mL/min, λ = 254 nm).

Product **3f**: Daicel Chiralpak-ADH, *n*-hexane/*i*-PrOH 90:10, flow rate 1.0 mL/min, λ =254 nm).

Calculation the conversion of 1a

The procedure to determine the conversion of **1a** by HPLC was according to the literature.[2]

Conversions of **1a** were calculated using the formula:

Conversion (%) = $A_{3a} / (A_{3a} + A_{1a} / f)$

Where A_{1a} and A_{3a} are the HPLC peak areas of **1a** and **3a**, respectively. And *f* is the correction factor determined to be 0.73 from a calibration curve.

Kinetic measurements

All kinetic measurements were performed using UV-Vis spectroscopy (Shimadzu 2450) at 298 K by monitoring the disappearance of the absorption of **1a** at 326 nm.[2b] Typical procedure is described as follows: HT45 (final G4 unit conc. 50 μM) was added to MOPS (20 mM, pH 6.5) containing KCl (150 mM) or NH_4Cl (25 mM) in a quartz cuvette. After stirring for 15 min, Cu^{2+} ion (final conc. 10 μM in K^+ media or 16.7 μM in NH_4^+ media) was added. After stirring for another 15 min, 4 μL of a fresh solution of **1a** in CH_3CN was added, resulting in a final concentration of 20 μM. The determination was made after **2** (final conc. 4 mM) was added with the cuvette sealed tightly.

The following equations were used to calculate k_{app} and k_{rel}:

$$k_{app} = d[A_{\mathbf{1a}}]/dt \cdot (d \cdot (\varepsilon_{\mathbf{1a}} - \varepsilon_{\mathbf{3a}}) \cdot [\mathbf{1a}]^0 \cdot [\mathbf{2}]^0)^{-1}$$

$$k_{rel} = k_{app\text{-}catalyst} / k_{app\text{-}no\ catalyst}$$

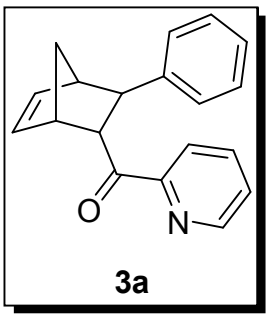
Where $d[A_{\mathbf{1a}}]/dt$ is the slope of the absorption of **1a** *vs.* time during the first 15% of the reaction, and d is the path length of the cuvette. $\varepsilon_{\mathbf{1a}}$ (22173 $M^{-1}cm^{-1}$) and $\varepsilon_{\mathbf{3a}}$ (177 $M^{-1}cm^{-1}$) are the molar extinction coefficient of **1a** and **3a** at 326 nm, respectively.

References

1. (a) S. Otto, F. Bertoncin and J. B. F. N. Engberts, *J. Am. Chem. Soc.* **1996,** 118, 7702; (b) L. Wang, J. Zhang, N. Wang, X. Yang, Q. Wang and X. Yu, *Lett. Org. Chem.* **2009,** 6, 392.
2. (a) N. S. Oltra and G. Roelfes, *Chem. Commun.* **2008**, 6039; (b) C. H. Wang, G. Q. Jia, J. Zhou, Y. H. Li, Y. Liu, S. M. Lu and C. Li, *Angew. Chem. Int. Ed.* **2012,** 51, 9352.

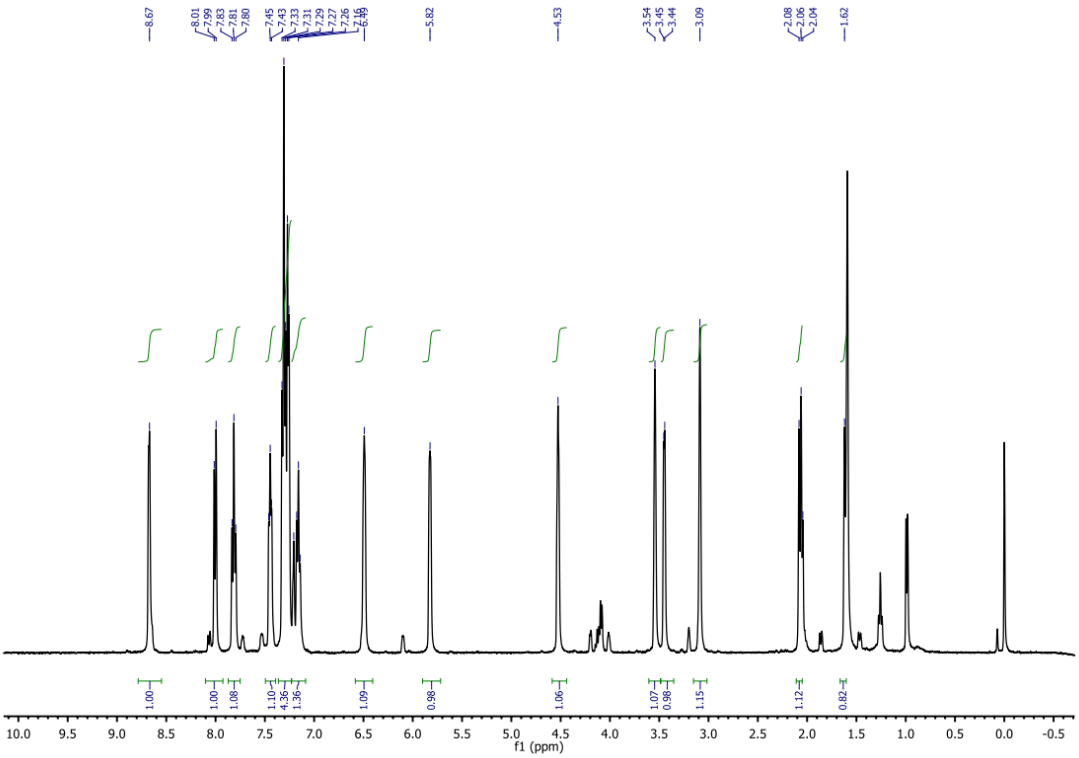
1H NMR spectra

(3-phenylbicyclo[2.2.1]hept-5-en-2-yl)(pyridin-2-yl)methanone (**3a**)

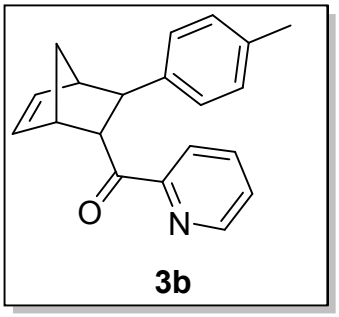


3a

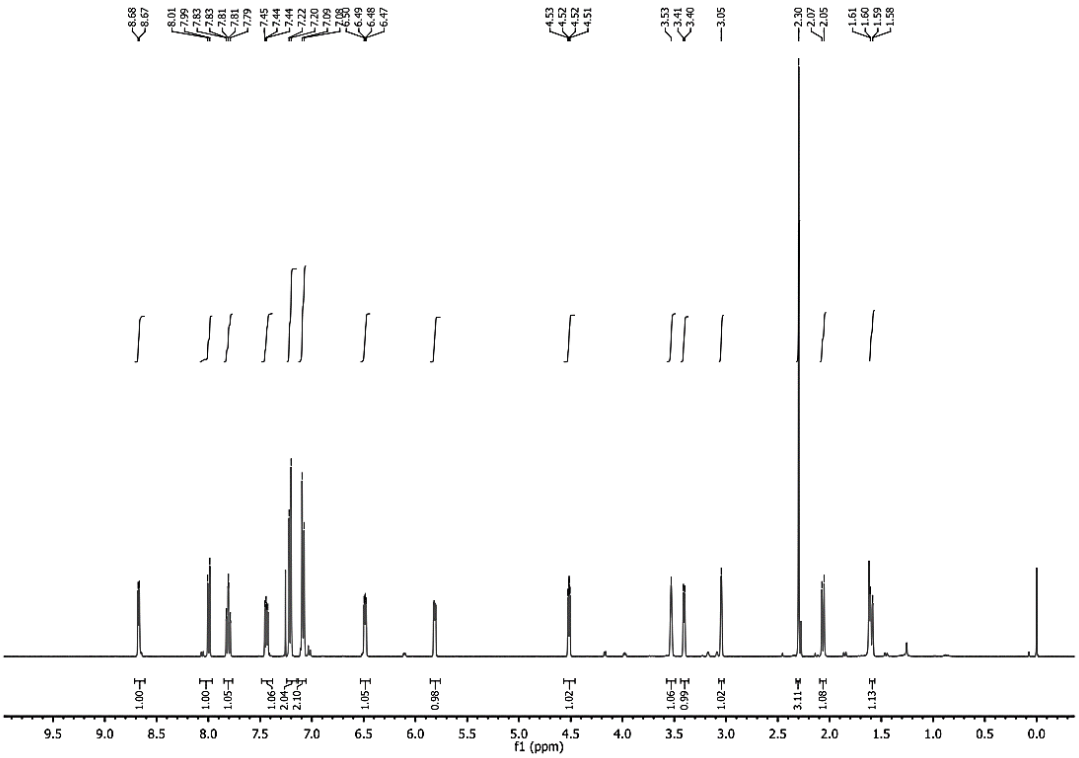
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (m, 1H), 8.00 (m, 1H), 7.81 (m, 1H), 7.49 – 7.39 (m, 1H), 7.36 – 7.23 (m, 4H), 7.17 (m, 1H), 6.49 (m, 1H), 5.82 (m, 1H), 4.53 (m, 1H), 3.54 (s, 1H), 3.45 (d, J = 3.9 Hz, 1H), 3.09 (s, 1H), 2.07 (d, J = 8.4 Hz, 1H), 1.62 (m, 1H).



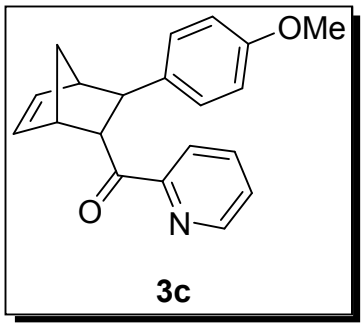
pyridin-2-yl(3-p-tolylbicyclo[2.2.1]hept-5-en-2-yl)methanone (**3b**)



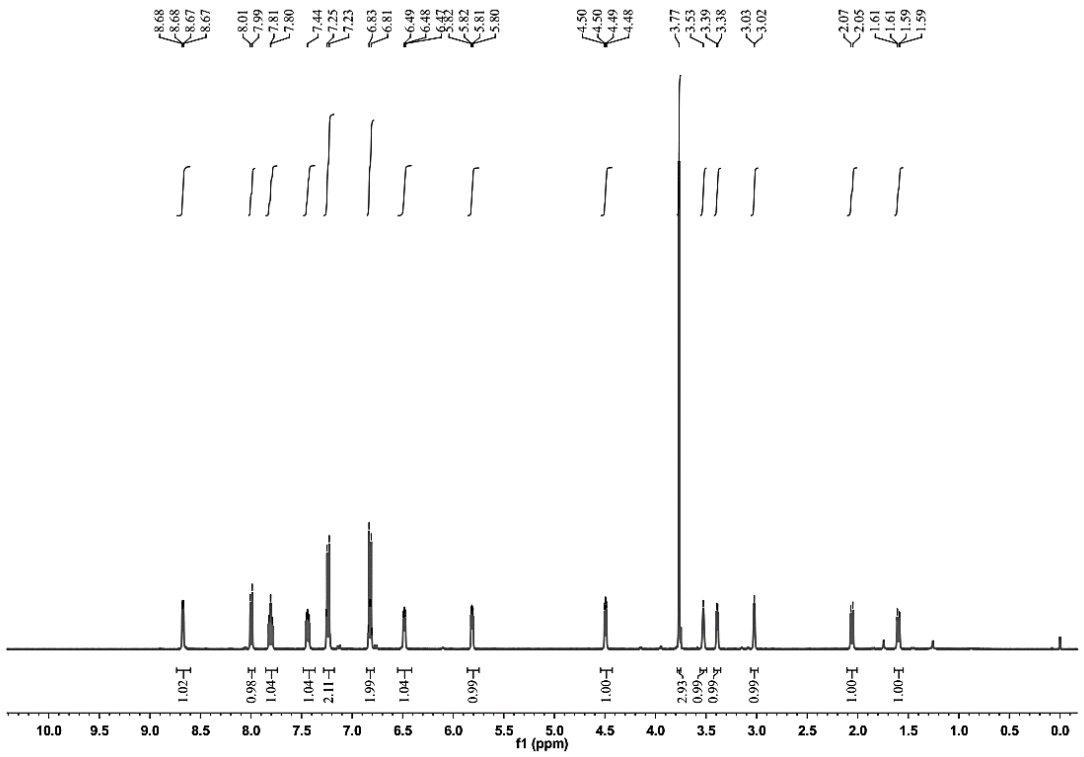
^{1}H NMR (400 MHz, $CDCl_3$, *endo* isomer): δ 8.67 (d, *J* = 4.6 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.81 (td, *J* = 7.7, 1.3 Hz, 1H), 7.44 (dd, *J* = 7.0, 5.3 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.49 (dd, *J* = 5.4, 3.3 Hz, 1H), 5.81 (dd, *J* = 5.6, 2.7 Hz, 1H), 4.52 (dd, *J* = 4.9, 3.7 Hz, 1H), 3.53 (s, 1H), 3.41 (d, *J* = 5.0 Hz, 1H), 3.05 (s, 1H), 2.30 (s, 3H), 2.06 (d, *J* = 8.4 Hz, 1H), 1.59 (dd, *J* = 8.5, 1.1 Hz, 1H).



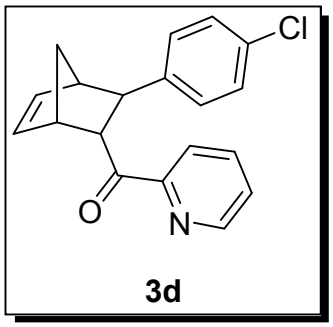
(3-(4-methoxyphenyl)bicyclo[2.2.1]hept-5-en-2-yl)(pyridin-2-yl)methanone (**3c**)



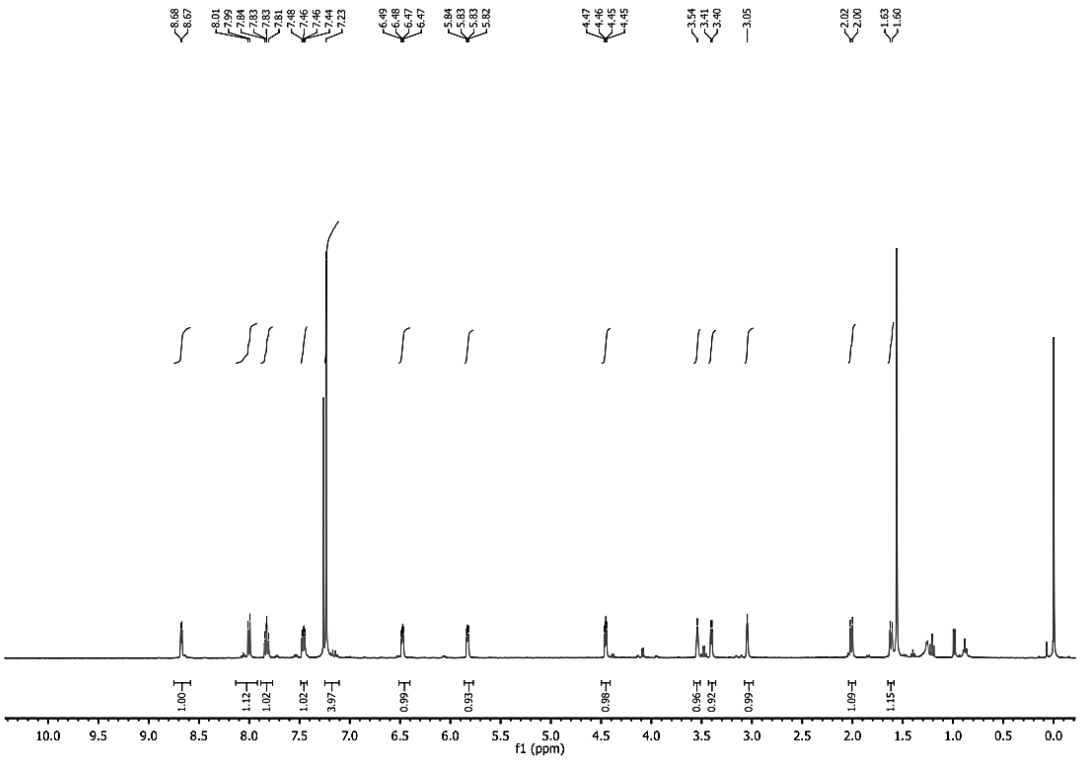
^{1}H NMR (400 MHz, $CDCl_3$, *endo* isomer): δ 8.74 – 8.60 (m, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.81 (td, *J* = 7.7, 1.7 Hz, 1H), 7.44 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.28 – 7.18 (m, 2H), 6.86 – 6.78 (m, 2H), 6.48 (dd, *J* = 5.5, 3.2 Hz, 1H), 5.81 (dd, *J* = 5.6, 2.7 Hz, 1H), 4.49 (dd, *J* = 5.1, 3.5 Hz, 1H), 3.77 (s, 3H), 3.53 (s, 1H), 3.39 (d, *J* = 4.5 Hz, 1H), 3.02 (d, *J* = 1.2 Hz, 1H), 2.06 (d, *J* = 8.4 Hz, 1H), 1.60 (dd, *J* = 8.5, 1.6 Hz, 1H).



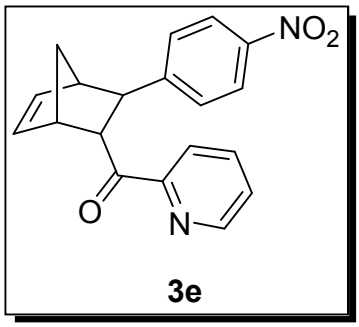
(3-(4-chlorophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(pyridin-2-yl)methanone (**3d**)



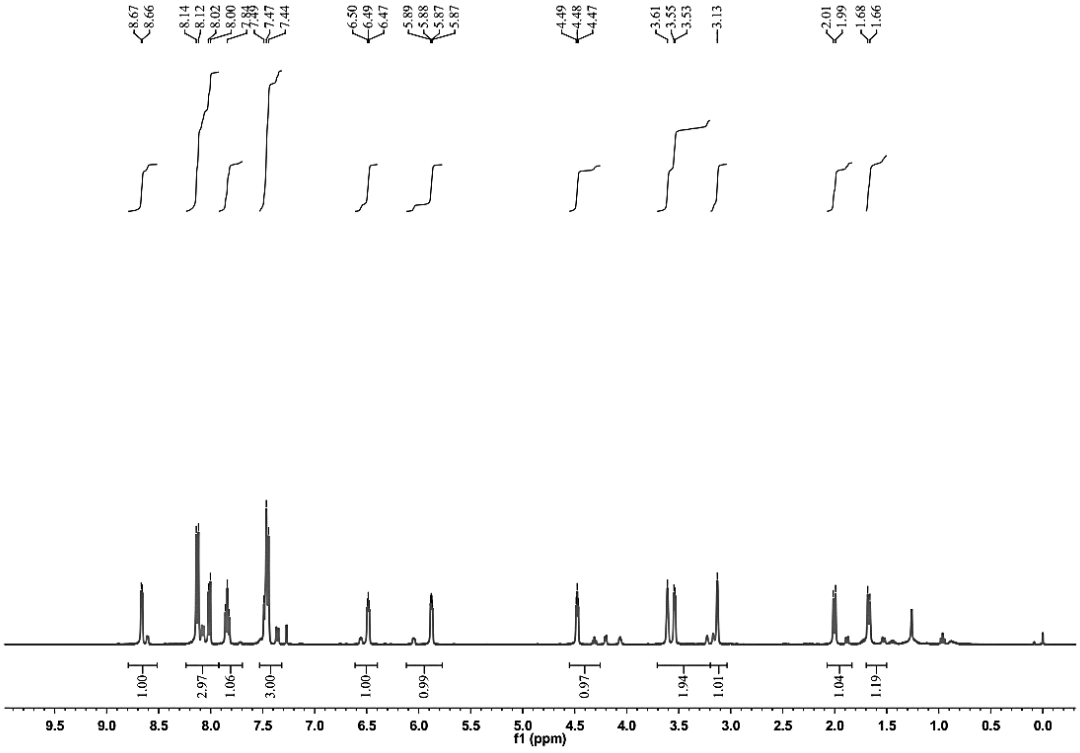
^{1}H NMR (400 MHz, $CDCl_3$, *endo* isomer): δ 8.67 (d, *J* = 4.6 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.83 (dd, *J* = 8.5, 6.9 Hz, 1H), 7.46 (dd, *J* = 7.4, 4.9 Hz, 1H), 7.25 – 7.10 (m, 4H), 6.48 (dd, *J* = 5.4, 3.2 Hz, 1H), 5.83 (dd, *J* = 5.5, 2.7 Hz, 1H), 4.46 (dd, *J* = 5.1, 3.5 Hz, 1H), 3.54 (s, 1H), 3.40 (d, *J* = 5.1 Hz, 1H), 3.05 (s, 1H), 2.01 (d, *J* = 8.5 Hz, 1H), 1.62 (d, *J* = 8.6 Hz, 1H).



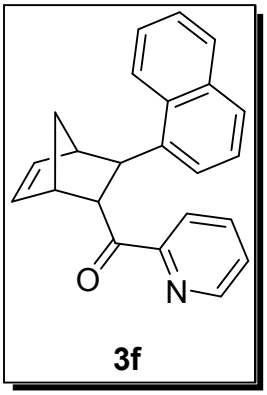
(3-(4-nitrophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(pyridin-2-yl)methanone (**3e**)



^{1}H NMR (400 MHz, *endo* isomer): δ 8.66 (d, *J* = 4.5 Hz, 1H), 8.07 (m, 3H), 7.84 (t, *J* = 7.6 Hz, 1H), 7.47 (m, 3H), 6.52 – 6.44 (m, 1H), 5.88 (dd, *J* = 5.3, 2.5 Hz, 1H), 4.51 – 4.44 (m, 1H), 3.66 – 3.50 (m, 2H), 3.13 (s, 1H), 2.00 (d, *J* = 8.6 Hz, 1H), 1.67 (d, *J* = 8.4 Hz, 1H).

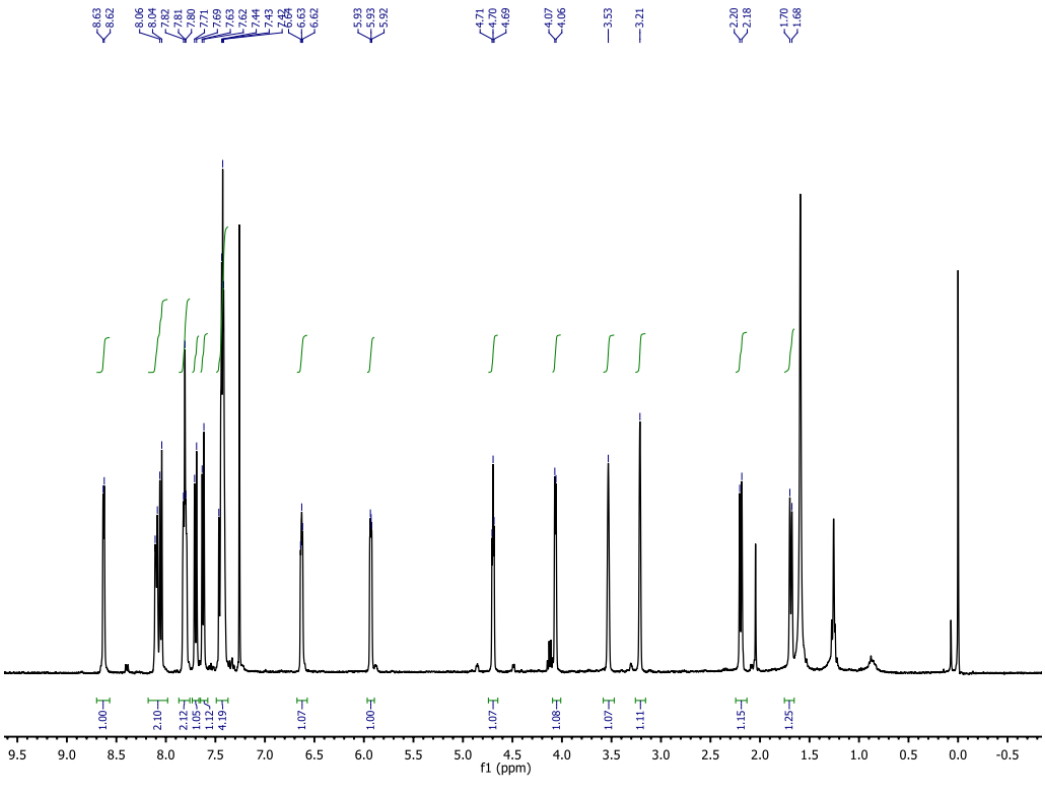


(3-(naphthalen-1-yl)bicyclo[2.2.1]hept-5-en-2-yl)(pyridin-2-yl)methanone (**3f**)



3f

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 – 8.57 (m, 1H), 8.18 – 7.98 (m, 2H), 7.87 – 7.76 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.49 – 7.37 (m, 4H), 6.68 – 6.57 (m, 1H), 5.97 – 5.89 (m, 1H), 4.74 – 4.65 (m, 1H), 4.07 (d, *J* = 4.8 Hz, 1H), 3.53 (s, 1H), 3.21 (s, 1H), 2.19 (dd, *J* = 8.4 Hz, 1H), 1.69 (dd, *J* = 8.3 Hz, 1H).



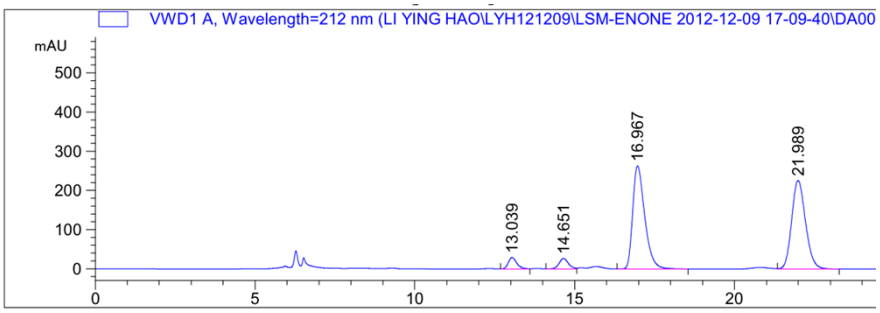
HPLC traces

1. Product **3a**

Ee's were determined by HPLC analysis (Daicel Chiralcel-ODH, *n*-hexane/*i*-PrOH 98:2, flow rate 0.5 mL/min, λ = 212 nm).

(1) Racemic **3a**

Retention times: 13.0, 14.7 (*exo* isomer) and 17.0, 22.0 (*endo* isomer) mins

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                      Area Percent Report
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Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  13.039 VV    0.2821  545.54974   29.38276   3.6834
   2  14.651 VV    0.3169  557.33136   26.79624   3.7630
   3  16.967 VB    0.4002 6895.75879  263.32379  46.5584
   4  21.989 VB    0.4662 6812.34863  226.33138  45.9952

Totals :                  1.48110e4   545.83416

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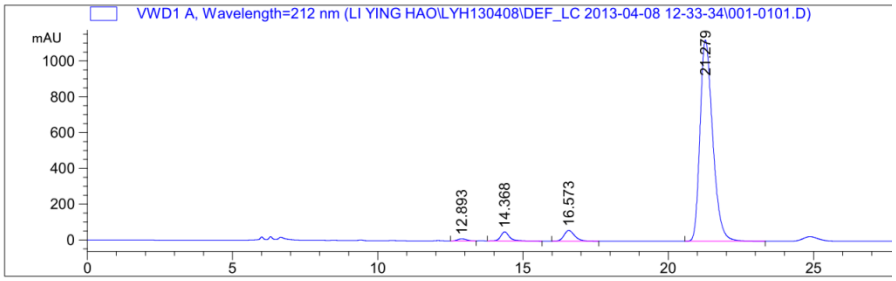
(2) Product **3a** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 12.9, 14.4 (*exo* isomer) and 16.6, 21.3 (*endo* isomer) mins (92% *ee*)

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  12.893 VV    0.3479  261.99924   11.73342   0.7084
   2  14.368 VB    0.3246 1105.62134   50.92263   2.9895
   3  16.573 BB    0.3856 1502.77612   59.65440   4.0634
   4  21.279 BB    0.4676 3.41129e4  1124.07214  92.2387

Totals :                  3.69833e4  1246.38260

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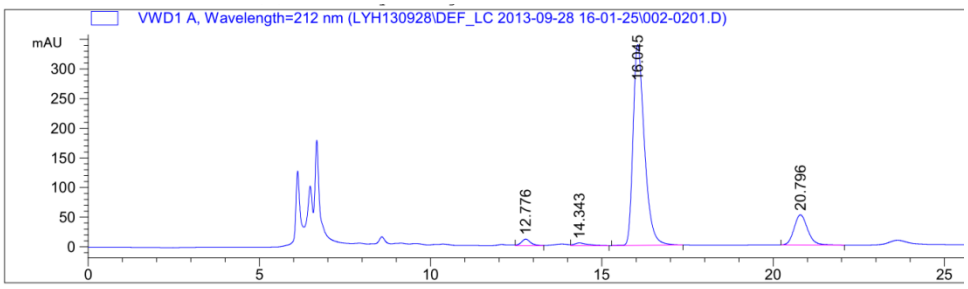
(3) Product **3a** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 12.8, 14.3 (*exo* isomer) and 16.0, 20.8 (*endo* isomer) mins (-73% *ee*)

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  12.776 VV    0.2814  201.28246   10.87555   2.1110
   2  14.343 VB    0.3756  116.61436    4.42889   1.2230
   3  16.045 BB    0.3522 7813.04346  338.53836  81.9418
   4  20.796 BB    0.4272 1403.92444   51.03665  14.7241

Totals :                  9534.86471  404.87945

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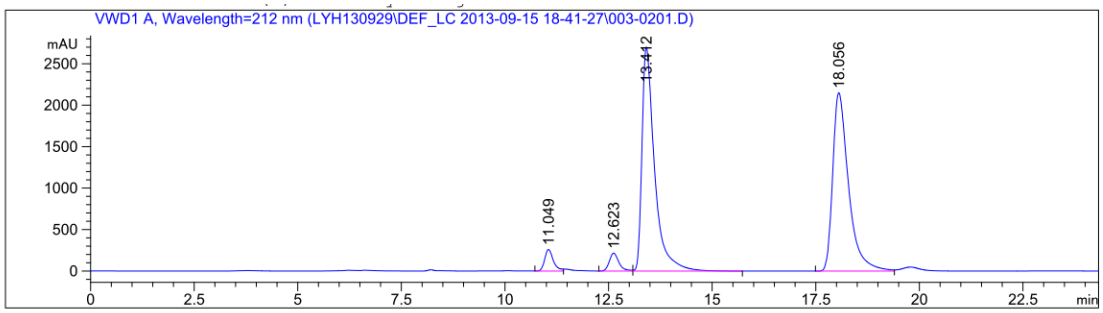
2. Product **3b**

Ee's were determined by HPLC analysis (Daicel Chiralcel-ODH, *n*-hexane/*i*-PrOH 98:2, flow rate 0.5 mL/min, λ = 212 nm).

(1) Racemic **3b**

Retention times: 11.0, 12.6 (*exo* isomer) and 13.4, 18.1 (*endo* isomer) mins

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=====================================================================
                        Area Percent Report
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.049 VV    0.2193 3786.92505  260.22565   3.1164
   2  12.623 VV    0.2440 3466.84985  216.15584   2.8530
   3  13.412 VB    0.3139 5.71527e4  2701.50098  47.0337
   4  18.056 VV    0.3987 5.71079e4  2151.03516  46.9969

Totals :                  1.21514e5  5328.91762


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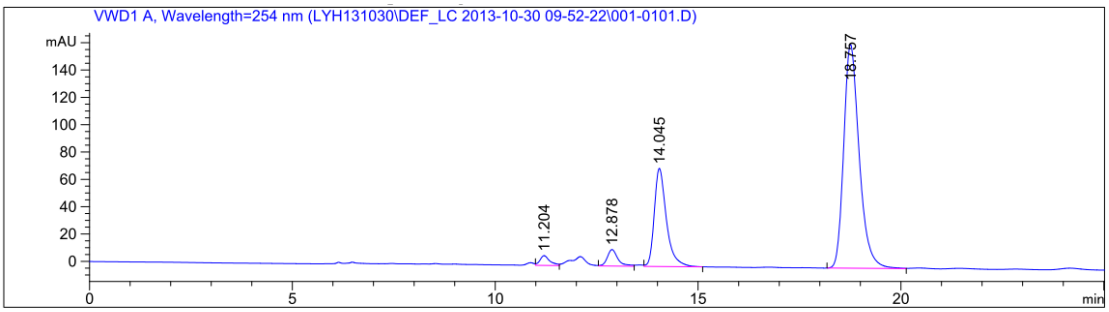
                        *** End of Report ***
```

(2) Product **3b** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 11.2, 12.9 (*exo* isomer) and 14.0, 18.8 (*endo* isomer) mins (50% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.204 VV    0.2469  120.00939    7.14752   1.9482
   2  12.878 VV    0.2698  217.48340   12.08158   3.5305
   3  14.045 VB    0.3117 1488.25195   71.81748  24.1596
   4  18.757 BB    0.4035 4334.34521  163.78825  70.3617

Totals :                  6160.08996  254.83484


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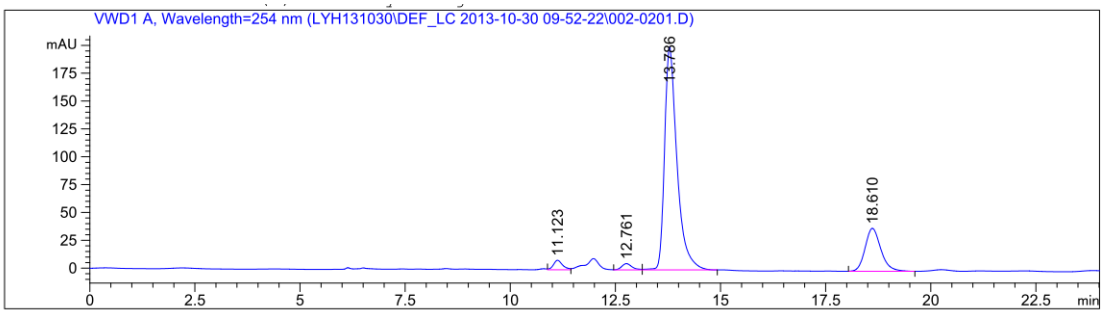

(3) Product **3b** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 11.1, 12.8 (*exo* isomer) and 13.8, 18.6 (*endo* isomer) mins (-61% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.123 VV    0.2276  129.98175    8.51987   2.4360
   2  12.761 VV    0.2549   93.61814    5.59692   1.7545
   3  13.786 VB    0.3082 4108.95508  199.96890  77.0059
   4  18.610 BB    0.3968 1003.33954   38.57032  18.8036

Totals :                  5335.89451  252.65601


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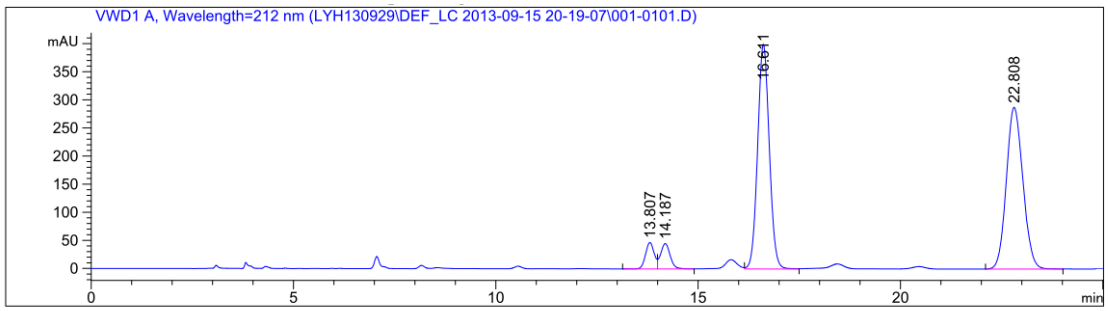
                        *** End of Report ***
```

3. Product **3c**

Ee's were determined by HPLC analysis (Daicel Chiralpak-ADH, *n*-hexane/*i*-PrOH 95:5, flow rate 1.0 mL/min, λ = 212 nm).

(1) Racemic **3c**

Retention times: 13.8, 14.2 (*exo* isomer) and 16.6, 22.8 (*endo* isomer) mins

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  13.807 BV    0.2309  708.48175   47.10786   4.0206
   2  14.187 VB    0.2528  748.52386   45.24762   4.2478
   3  16.611 VB    0.3120 7994.28027  399.87192  45.3670
   4  22.808 BB    0.4420 8170.07568  287.82578  46.3646

Totals :                  1.76214e4   780.05318


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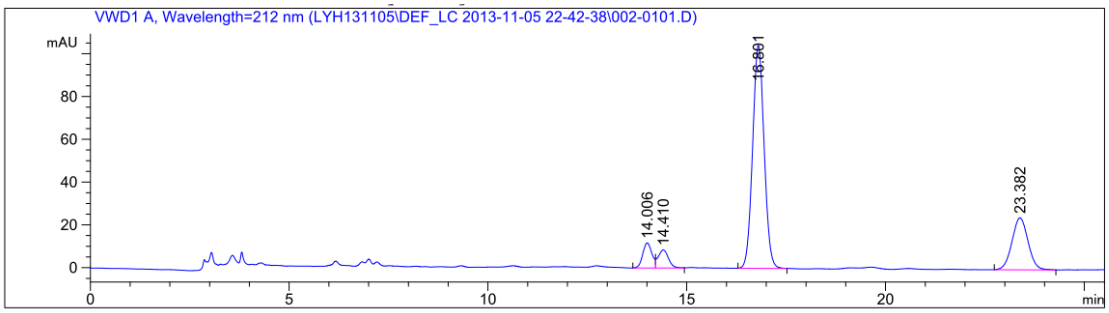

(2) Product **3c** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 14.0, 14.4 (*exo* isomer) and 16.8, 23.4 (*endo* isomer) mins (52% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  14.006 BV    0.2506  187.07259   11.70692   6.0067
   2  14.410 VB    0.2723  150.79552    8.45136   4.8419
   3  16.801 BB    0.3133 2096.36963  104.24008  67.3119
   4  23.382 BB    0.4362  680.17413   24.27889  21.8396

Totals :                  3114.41187  148.67725

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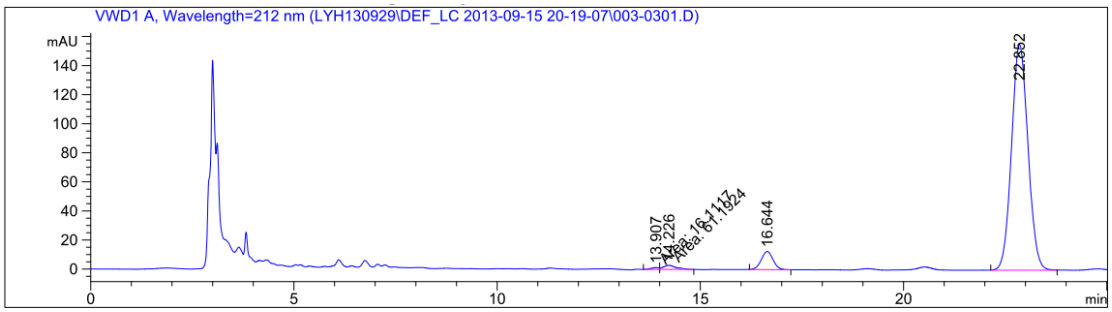
                        *** End of Report ***
```

(3) Product **3c** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 13.9, 14.2 (*exo* isomer) and 16.6, 22.9 (*endo* isomer) mins (-90% *ee*)

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=====================================================================
                        Area Percent Report
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  13.907 MF    0.2181   16.11171    1.23139   0.3443
   2  14.226 FM    0.3617   61.19242    2.81986   1.3076
   3  16.644 BB    0.3134  251.33250   12.49429   5.3706
   4  22.852 BB    0.4347 4351.16846  155.31119  92.9776

Totals :                  4679.80510  171.85673


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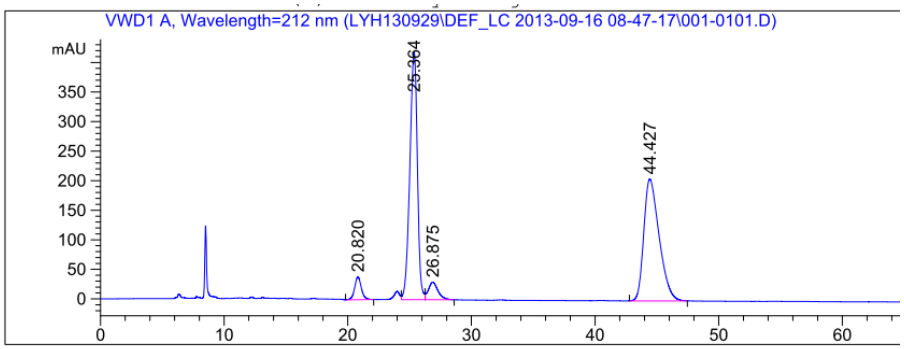

4. Product **3d**

Ee's were determined by HPLC analysis (Daicel Chiralcel-OJH, *n*-hexane/*i*-PrOH 90:10, flow rate 0.5 mL/min, λ = 212 nm).

(1) Racemic **3d**

Retention times: 20.8, 26.9 (*exo* isomer) and 25.4, 44.4 (*endo* isomer) mins

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  20.820 BB    0.5783 1459.81567   38.59794   3.7885
   2  25.364 VV    0.6586 1.77287e4   418.76682  46.0090
   3  26.875 VB    0.8094 1611.82507   29.70839   4.1830
   4  44.427 BB    1.3103 1.77327e4   206.02675  46.0196

Totals :                  3.85330e4   693.09989


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                          *** End of Report ***
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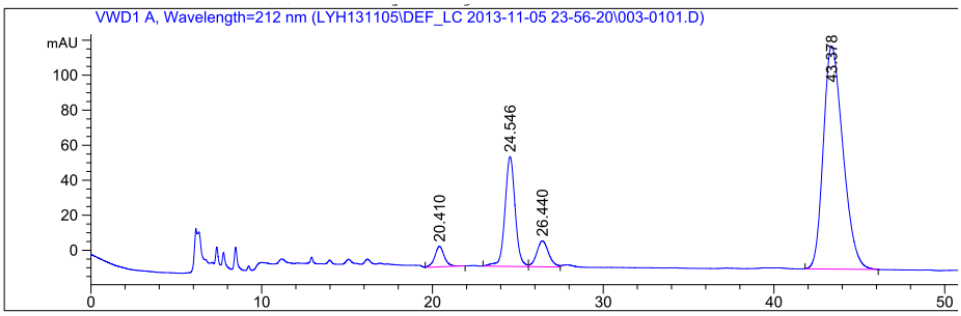
(2) Product **3d** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 20.4, 26.4 (*exo* isomer) and 24.5, 43.4 (*endo* isomer) mins (60% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  20.410 BB    0.5824  456.64355   11.73192   3.2758
   2  24.546 BB    0.6369 2593.16675   62.71894  18.6024
   3  26.440 BV    0.7504  719.75439   14.81583   5.1633
   4  43.378 BB    1.2209 1.01704e4   127.46981  72.9585

Totals :                  1.39399e4   216.73650

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                        *** End of Report ***
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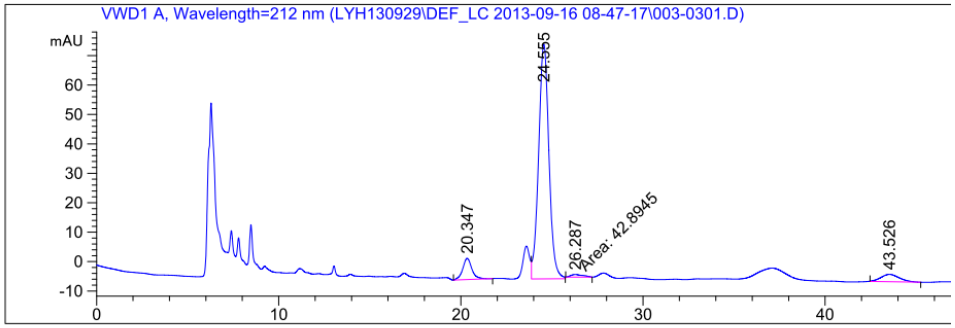
(3) Product **3d** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 20.3, 26.3 (*exo* isomer) and 24.6, 43.5 (*endo* isomer) mins (-90% *ee*)

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==================================================================
                        Area Percent Report
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  20.347 BB    0.5247  253.57475    7.22437   7.0393
   2  24.555 VB    0.6033 3129.88916   79.80411  86.8869
   3  26.287 MM    0.7885   42.89445 9.06635e-1   1.1908
   4  43.526 BB    0.9123  175.89861    2.50564   4.8830

Totals :                  3602.25697   90.44075

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                        *** End of Report ***
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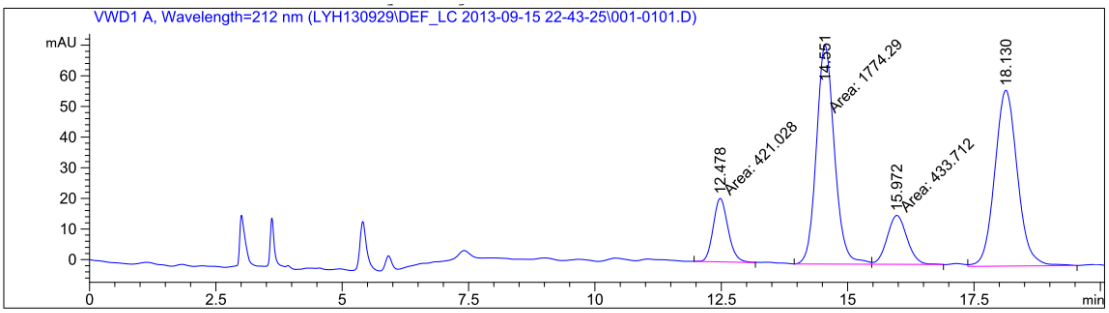
5. Product **3e**

Ee's were determined by HPLC analysis (Daicel Chiralpak-AD, *n*-hexane/*i*-PrOH 90:10, flow rate 1.0 mL/min, λ = 254 nm).

(1) Racemic **3e**

Retention times: 12.5, 16.0 (*exo* isomer) and 14.6, 18.1 (*endo* isomer) mins

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  12.478 MM    0.3388  421.02759   20.71424   9.5208
   2  14.551 MF    0.4143 1774.29187   71.37769  40.1225
   3  15.972 FM    0.4531  433.71246   15.95338   9.8077
   4  18.130 VV    0.4826 1793.14966   57.39640  40.5490

Totals :                  4422.18158  165.44171

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                        *** End of Report ***
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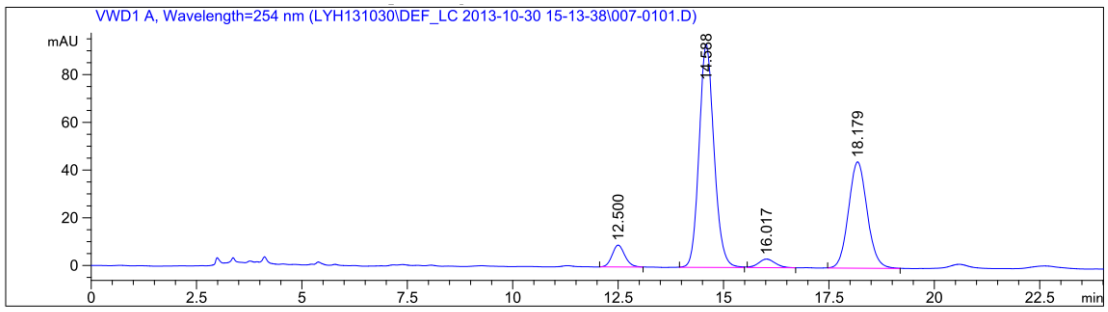
(2) Product **3e** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 12.5, 16.0 (*exo* isomer) and 14.6, 18.2 (*endo* isomer) mins (26% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  12.500 BB    0.3237  192.64001    9.17357   4.8566
   2  14.588 BB    0.3793 2305.09131   93.54002  58.1136
   3  16.017 BB    0.4225   98.47121    3.58528   2.4826
   4  18.179 BB    0.4753 1370.32532   44.57116  34.5472

Totals :                  3966.52785  150.87002


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                        *** End of Report ***
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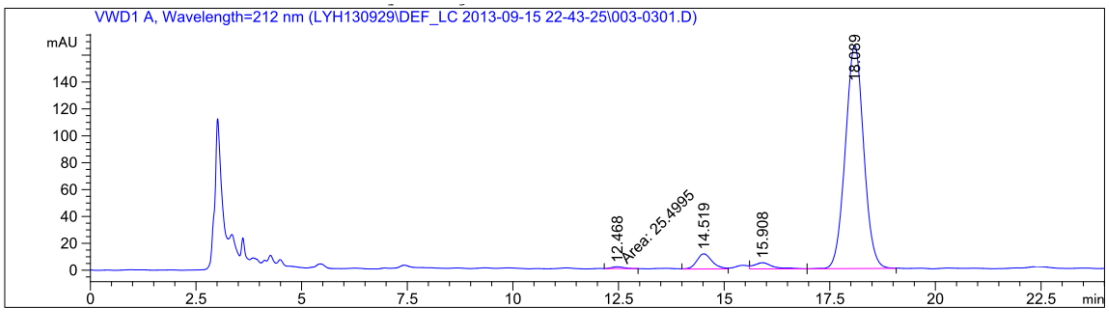
(3) Product **3e** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 12.5, 15.9 (*exo* isomer) and 14.5, 18.1 (*endo* isomer) mins (-90% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=212 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  12.468 MM    0.3222   25.49954    1.31906   0.4667
   2  14.519 VV    0.3839  285.72260   11.29949   5.2294
   3  15.908 VV    0.4406  139.37328    4.58510   2.5509
   4  18.089 VB    0.4658 5013.14209  166.12804  91.7530

Totals :                  5463.73750  183.33169


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                      *** End of Report ***
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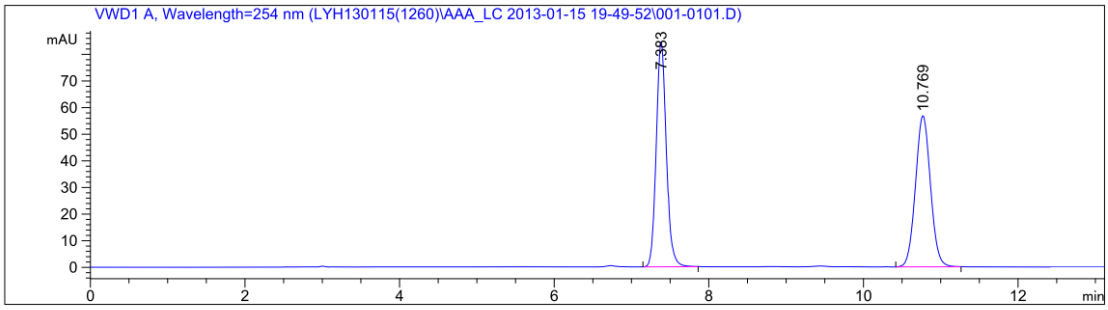
6. Product **3f**

Ee's were determined by HPLC analysis (Daicel Chiralpak-ADH, *n*-hexane/*i*-PrOH 90:10, flow rate 1.0 mL/min, λ = 254 nm).

(1) Racemic **3f**

Retention times: 7.4 and 10.8 mins

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.383 BB    0.1393  764.62390   84.33156  49.9907
   2  10.769 BB    0.2097  764.90961   56.76023  50.0093

Totals :                  1529.53351  141.09179

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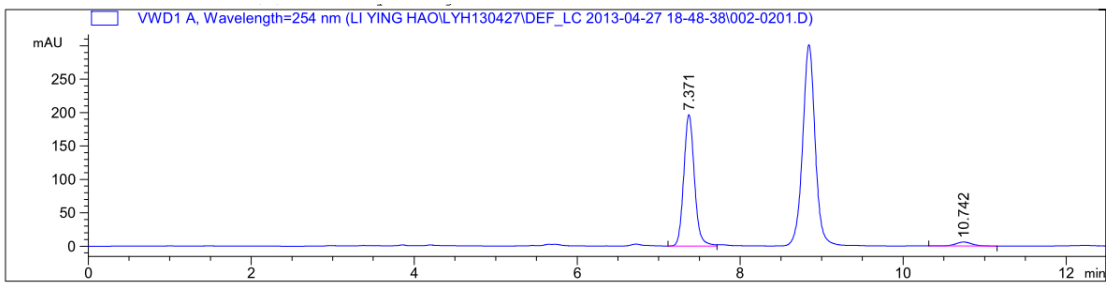
                        *** End of Report ***
```

(2) Product **3f** from the Diels-Alder reaction catalyzed by HT45-K-Cu

Retention times: 7.4 (*major*) and 10.7 (*minor*) mins (91% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   7.371 VV    0.1391 1778.98389  196.59781  95.5101
   2  10.742 BB    0.2167   83.62959    5.93761   4.4899

Totals :                  1862.61347  202.53542

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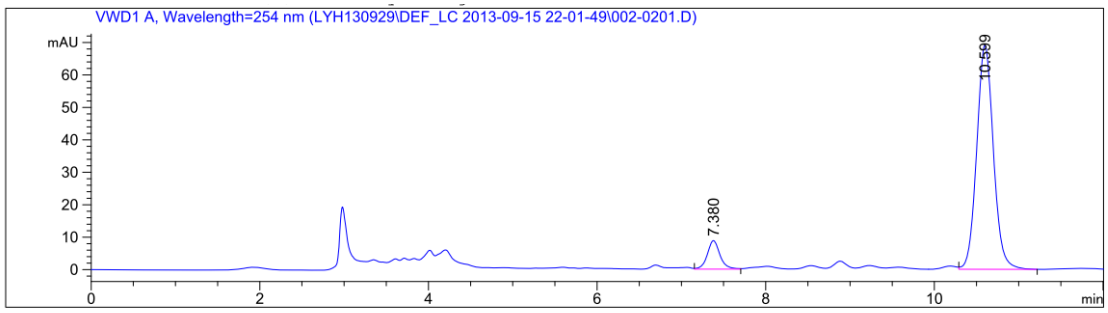

(3) Product **3f** from the Diels-Alder reaction catalyzed by HT45-NH_4-Cu

Retention times: 7.4 (*minor*) and 10.6 (*major*) mins (-82% *ee*)

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

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


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.380 VV    0.1565   91.58588    8.79649   8.8002
   2  10.599 VB    0.2128  949.13885   69.06776  91.1998

Totals :                  1040.72473   77.86425


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                        *** End of Report ***
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