Supporting Information

Novel Triazole Nucleoside Analogues Promotes Anticancer Activity via Both Apoptosis and Autophagy

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Table of Contents

Figure S1. Inhibitor of necroptosis, necrostatin-1, did not show notable effect on the antiproliferative activity of **If**. Panc-1 cells were treated with If at various concentrations in the presence and absence of necrostatin-1 (50 μM).

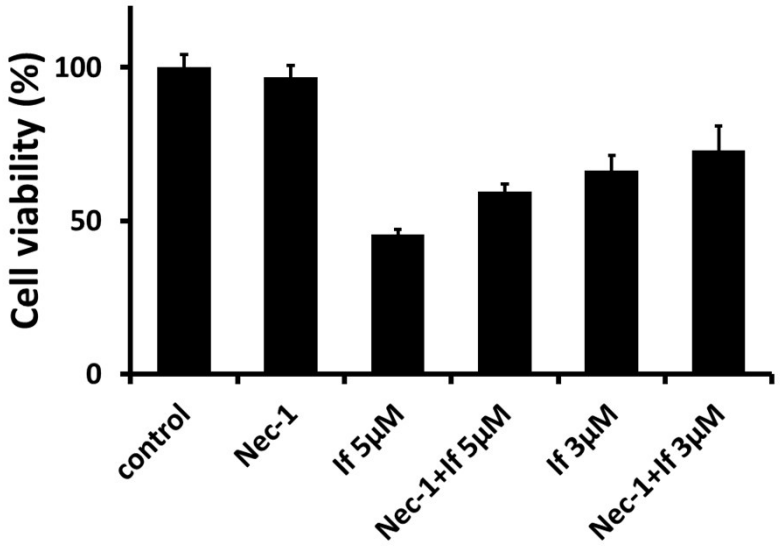
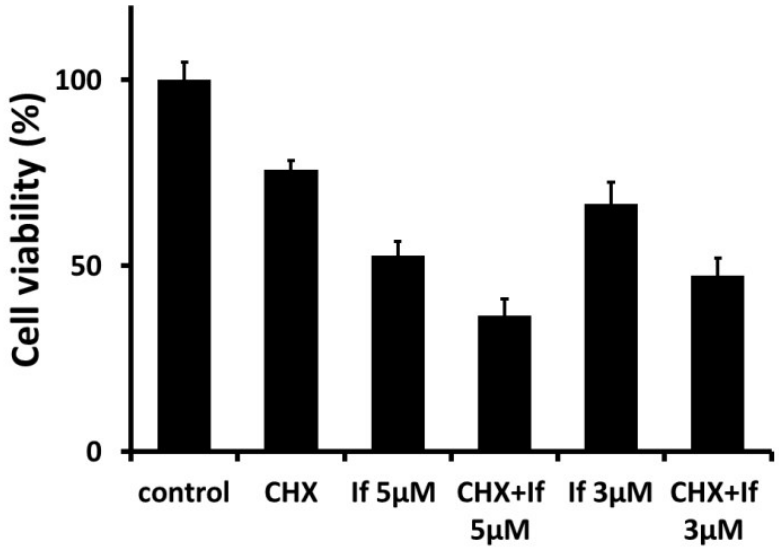


Figure S2. A) The co-treatment of paraptosis inhibitor, cycloheximide, with **If** enhanced the antiproliferative activity of **If** instead of rescuing the cells. Panc-1 cells were treated with **If** at various concentrations in the presence and absence of cycloheximide (10 μM). B) Phase-contrast images show the morphological change of Panc-1 cells treated with **If** at indicated time points from 0−48 h. All images were recorded at the same magnification.

A



B

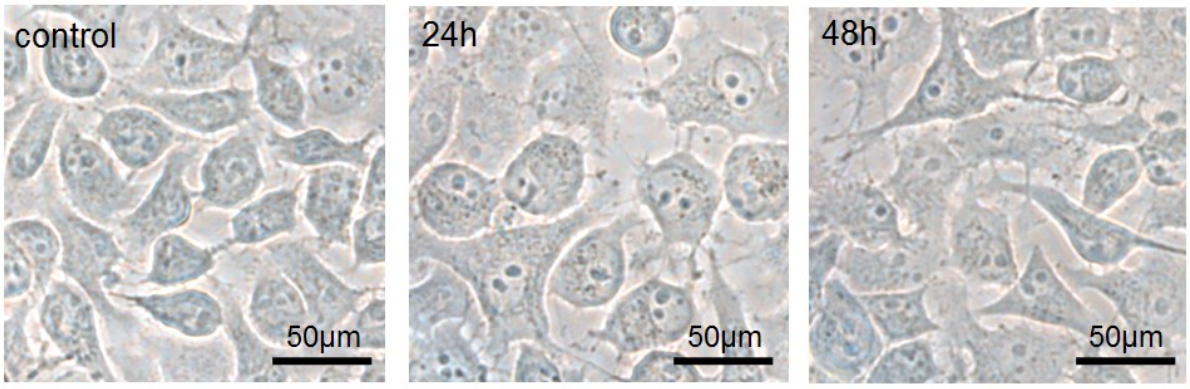
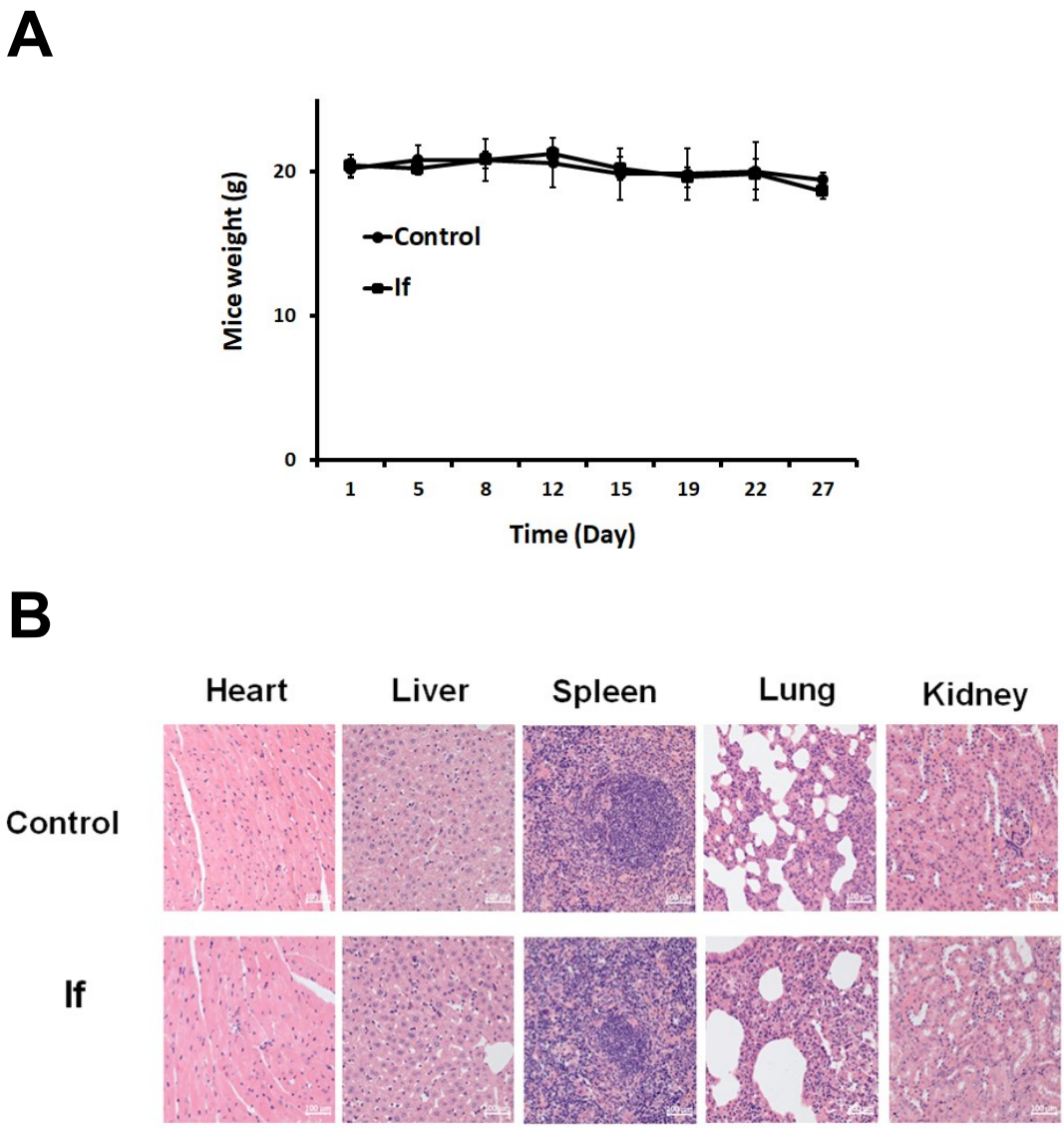


Figure S3. No adverse effect was observed on mice weight with the treatment of **If**. (A) Nude mice bearing Panc-1 tumors of 100 mm^3 were randomly selected for various treatments. **If** was injected intraperitoneally (20 mg/kg) for 4 weeks. The tumor volumes were measured twice a week. All of the results were expressed as the mean ± S.E (n = 5). Points, means of analysis; bars, standard errors. (B) Mouse organs including the heart, liver, spleen, lung, and kidneys were sectioned and stained by H&E to evaluate tissue inflammation and structural damage.



General

All the terminal alkynes and chemical reagents were purchased from Adamas-beta, Energy Chemical, woka and TCI. Solvents were purchased from local supplier and used without further purification. All compounds were purified by performing flash chromatography on silica gel (200-300 mesh) or preparative thin-layer chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent DD2 400-MR. The chemical shifts were recorded in parts per million (ppm) with tetramethylsilane as the internal reference. The microwave assisted reactions were performed on an Initiator EXP EU produced by Biotage. The ESI-MS was recorded on a Waters Acquity SQ Detecter mass spectrometer or Finigan LCQ mass spectrometer. The high resolution of ESIMS was recorded on Bruker SolariX 7.0T mass spectrometer or IonSpec 4.7 Tesla Fourier Transform mass spectrometer. All MS analysis samples were prepared as solutions in methanol. Analytical HPLC runs were performed on gilent Technologies 1260 Infinity connected with Inertsil® ODS-3 (Method 1) and Inertsil hypersil C8 (Method 2) by GL sciences Inc, with 1260 VWD VL, wavelength 282 nm. All the samples were dissolved in MeOH. The mobile phase was consisted of a gradient elution of MeOH and H_2O (contain 0.1% triethylamine). Elution system was the mixture of MeOH/H_2O from 10/90 to 90/10 in 20 min and then kept 90/10 in 20 min. The flow rate was 1.0 mL/min. Temperature was 30 °C. Compound **WMH-116** were synthesized following the reference of previous report.[1]

Synthesis of compound I

*Synthesis of compound **I-2***

A mixture of compound **WMH-116** (384 mg，1.0 mmol), p-toluenesulfonyl chloride (763 mg, 4.0 mmol), 4-dimethylaminopyridine (12.2 mg, 0.1 mmol) and triethylamine (505 mg, 5 mmol) were dissolved in 15 mL dichloromethane. After stirred at 25 °C for 6 hours, the reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 2:1) to afford the corresponding product **I-2**.

I-2: 458 mg, 0.85 mmol, 85%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, *J* = 8.4 Hz, phenyl-H), 7.54 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.32 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.25 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.03 (br s, 1H, -C(O)N*H*), 5.76 (br s, 1H, -C(O)N*H*), 5.64 (s, 2H, -OCH_2N-), 4.15 (t, 2H, *J* = 4.4 Hz, -OCH_2CH_2O-), 3.88 (t, 2H, *J* = 4.4 Hz, -OCH_2CH_2O-), 2.66 (t, 2H, *J* = 7.6 Hz, phenyl-CH_2-), 2.42 (s, 3H, -CH_3), 1.65-1.61 (m, 2H, -CH_2-), 1.33-1.28 (m, 8H, -$(CH_2)_4$-), 0.88 (t, 3H, *J* = 7.6 Hz, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.27, 156.20, 146.36, 145.03, 141.26, 132.59, 132.23, 129.88, 128.88, 127.91, 116.75, 99.12, 78.09, 73.53, 68.32, 67.90, 36.06, 31.75, 31.14, 29.18, 29.10, 22.63, 21.63, 14.08. IR: 2223 cm^{-1} (-C≡C-). MS (ESI, m/z): 561.5 $[M + Na]^+$, 1099.6 $[2M + Na]^+$.

*Synthesis of compound **I-1***

To a solution of compound **I-2** (431 mg, 0.8 mmol) in 10 mL anhydrous acetone was added LiBr (695 mg, 8.0 mmol). Then the mixture was stirred and refluxed for 4 hours under nitrogen. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 2:1) to afford the corresponding product **I-1**.

I-1: 343 mg, 0.77 mmol, 96%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (d, 2H, *J* = 5.2 Hz, phenyl-H), 7.24 (d, 2H, *J* = 5.2 Hz, phenyl-H), 7.04 (br s, 1H, -C(O)N*H*), 5.76 (br s, 1H, -C(O)N*H*), 5.74 (s, 2H, -OCH_2N-), 4.03 (t, 2H, *J* = 4.0 Hz, -O*C*$H_2$$CH_2$Br), 3.46 (t, 2H, *J* = 4.0 Hz, -OCH_2*C*H_2Br), 2.65 (t, 2H, *J* = 5.2 Hz, phenyl-CH_2-), 1.64-1.61 (m, 2H, -CH_2-), 1.32-1.27 (m, 8H, -$(CH_2)_4$-), 0.88 (t, 3H, *J* = 4.4 Hz，-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.32, 156.15, 146.32, 141.32, 132.23, 128.85, 116.80, 99.13, 78.11, 73.58, 70.17, 36.05, 31.75, 31.11, 29.60, 29.17, 29.10, 22.63, 14.08. IR: 2228 cm^{-1} (-C≡C-).

*General procedure for preparing **I**.*

Compound **I-1** (44.7 mg, 0.1 mmol) and various amines (1 mmol) were dissolved in 3 mL anhydrous DMF. After stirred at 25 °C for 12 hours, the reaction mixture was concentrated under reduced pressure and the crude residue was purified by preparative thin-layer chromatography to afford the corresponding product **I**.

Ia: 39.5 mg, 0.096 mmol, 96%, white solid. HPLC: t_R = 27.223 min (Method 1, purity = 99.58%), t_R = 25.212 min (Method 2, purity = 99.19%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (br s, 1H, -C(O)N*H*), 7.72 (br s, 1H, -C(O)N*H*), 7.63 (d, 2H, *J* = 7.6 Hz, phenyl-H), 7.35 (d, 2H, *J* = 7.6 Hz, phenyl-H), 5.71 (s, 2H, -OCH_2N-), 3.67 (t, 2H, *J* = 5.2 Hz, -O*C*$H_2$$CH_2$N-), 2.63 (t, 2H, *J* = 7.6 Hz, phenyl-CH_2-), 2.41 (t, 2H, *J* = 5.2 Hz, -OCH_2*C*H_2N-), 2.10 (s, 6H, -$N(CH_3)_2$), 1.59-1.57 (m, 2H, -CH_2-), 1.27-1.24 (m, 8H, -$(CH_2)_4$-), 0.85 (t, 3H, *J* = 7.2 Hz, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.16, 157.25, 146.21, 140.51, 132.44, 129.46, 117.00, 97.75, 78.33, 74.95, 67.99, 58.20, 45.77, 35.54, 31.64, 31.01, 28.96, 28.90, 22.49, 14.37. IR: 2227 cm^{-1} (-C≡C-). MS (ESI, m/z): 412.5 $[M + H]^+$, 823.8 $[2M + H]^+$.

HRMS: calcd for $C_{23}H_{34}N_5O_2$, $[M + H]^+$, 412.2707, found 412.2713.

Ib: 36.9 mg, 0.084 mmol, 84%, white solid. HPLC: t_R = 30.306 min (Method 1, purity = 99.77%), t_R = 26.612 min (Method 2, purity = 99.25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (br s, 1H, -C(O)N*H*), 7.74 (br s, 1H, -C(O)N*H*), 7.63 (d, 2H, *J* = 8.4 Hz, phenyl-H), 7.35 (d, 2H, *J* = 8.4 Hz, phenyl-H), 5.72 (s, 2H, -OCH_2N-), 3.66 (t, 2H, *J* = 6.0 Hz, -OCH_2CH_2N-), 2.64 (t, 2H, *J* = 7.6 Hz, phenyl-CH_2-), 2.57 (t, 2H, *J* = 5.6 Hz, -OCH_2CH_2N-), 2.44 (q, 4H, *J* = 6.8 Hz, -$(CH_2)_2$), 1.59-1.56 (m, 2H, -CH_2-), 1.27-1.24 (m, 8H, -$(CH_2)_4$), 0.89-0.83 (m, 9H, -CH_3 + -$(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.16, 157.22, 146.20, 140.49, 132.43, 129.45, 117.01, 97.77, 78.51, 74.98, 68.49, 51.93, 47.23, 35.53, 31.66, 31.03, 28.95, 28.92, 22.50, 14.38, 12.00. IR: 2227 cm^{-1} (-C≡C-). MS (ESI, m/z): 440.42 $[M + H]^+$. HRMS: calcd for $C_{25}H_{36}N_5O_2$, $[M + H]^+$, 440.3020, found 440.3016 .

Ic: 38.9 mg, 0.089 mmol, 89%, white solid. HPLC: t_R = 29.924 min (Method 1, purity = 98.84%), t_R = 26.178 min (Method 2, purity = 97.75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (br s, 1H, -C(O)N*H*), 7.74 (br s, 1H, -C(O)N*H*), 7.64 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.35 (d, 2H, *J* = 8.0 Hz, phenyl-H), 5.72 (s, 2H, -OCH_2N-), 3.69 (t, 2H, *J* = 6.4 Hz, -OCH_2CH_2N-), 2.64 (t, 2H, *J* = 7.6 Hz, phenyl-CH_2-), 2.58 (t, 2H, *J* = 6.4 Hz, -OCH_2CH_2N-), 2.51-2.41 (m, 4H, pyrrolidinyl-H), 1.64-1.59 (m, 6H, pyrrolidinyl-H + -CH_2-), 1.27-1.24 (m, 8H, -$(CH_2)_4$-), 0.85 (t, 3H, *J* = 7.2 Hz, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.17, 157.22, 146.20, 140.51, 132.45, 129.45, 117.00, 97.77, 78.37, 74.97, 68.91, 54.81, 54.25, 35.53, 31.66, 31.04, 28.95, 28.93, 23.47, 22.50, 14.38. IR: 2227 cm^{-1} (-C≡C-). MS (ESI, m/z): 438.5 $[M + H]^+$, 875.8 $[2M + H]^+$. HRMS: calcd for $C_{25}H_{36}N_5O_2$, $[M + H]^+$, 438.2863, found 438.2869.

Id: 41.1 mg, 0.091 mmol, 91%, white solid. HPLC: t_R = 31.320 min (Method 1, purity = 99.82%); t_R = 26.679 min (Method 2, purity = 99.20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (br s, 1H, -C(O)N*H*), 7.74 (br s, 1H, -C(O)N*H*), 7.64 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.35 (d, 2H, *J* = 8.0 Hz, phenyl-H), 5.71 (s, 2H, -OCH_2N-), 3.68 (t, 2H, *J* = 5.6 Hz, -OC*H*$_2$CH$_2$N-), 2.64 (t, 2H, *J* = 7.6 Hz, phenyl-CH$_2$-), 2.42 (t, 2H, *J* = 5.6 Hz, -OCH$_2$C*H*$_2$N-), 2.33-2.25 (m, 4H, piperidyl-H), 1.61-1.56 (m, 2H, -CH$_2$-), 1.41-1.37 (m, 4H, piperidyl-H), 1.28-1.24 (m, 10H, piperidyl-H + -(CH$_2$)$_4$-), 0.85 (t, 3H, *J* = 7.2 Hz, -CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.18, 157.18, 146.20, 140.49, 132.44, 129.46, 117.01, 97.76, 78.42, 74.99, 67.77, 57.86, 54.66, 35.52, 31.66, 31.04, 28.93, 25.90, 24.28, 22.50, 14.38. IR: 2227 cm^{-1} (-C≡C-). MS (ESI, m/z): 452.5 [M + H]$^+$, 903.8 [2M + H]$^+$. HRMS: calcd for $C_{26}H_{38}N_5O_2$, [M + H]$^+$, 452.3020, found 452.3026.

Ie: 42.3 mg, 0.088 mmol, 88%, white solid. HPLC: t_R = 27.928 min (Method 1, purity = 99.30%), t_R = 25.226 min (Method 2, purity = 98.18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (br s, 1H, -C(O)N*H*), 7.74 (br s, 1H, -C(O)N*H*), 7.64 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.35 (d, 2H, *J* = 8.0 Hz, phenyl-H), 5.71 (s, 2H, -OCH_2N-), 3.68 (t, 2H, *J* = 5.6 Hz, -OC*H*$_2$CH$_2$N-), 2.64 (t, 2H, *J* = 7.6 Hz, phenyl-CH$_2$-), 2.45 (t, 2H, *J* = 5.6 Hz, -OCH$_2$C*H*$_2$N-), 2.34-2.20 (m, 10H, piperazinyl-H + -CH$_2$-), 1.61-1.56 (m, 2H, -CH$_2$-), 1.27-1.25 (m, 8H, -(CH$_2$)$_4$-), 0.93 (t, 3H, *J* = 7.2 Hz, -CH$_3$), 0.85 (t, 3H, *J* = 7.2 Hz, -CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.18, 157.18, 146.20, 140.48, 132.46, 129.45, 117.01, 97.78, 78.44, 74.99, 67.73, 57.18, 53.46, 52.72, 52.00, 35.54, 31.65, 31.05, 28.95, 28.93, 22.51, 14.38, 12.40. IR: 2224 cm^{-1} (-C≡C-). MS (ESI, m/z): 481.5 [M + H]$^+$, 961.9 [2M + H]$^+$. HRMS: calcd for $C_{27}H_{41}N_6O_2$, [M + H]$^+$, 481.3285, found 481.3292.

If: 44.5 mg, 0.085 mmol, 85%, white solid. HPLC: t_R = 28.686 min (Method 1, purity = 97.82%); t_R = 24.935 min (Method 2, purity = 95.97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (br s, 1H, -C(O)N*H*), 7.75 (br s, 1H, -C(O)N*H*), 7.64 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.35 (d, 2H, *J* = 8.0 Hz, phenyl-H), 5.71 (s, 2H, -OCH_2N-), 3.68 (t, 2H, *J* = 5.6 Hz, -OC*H*$_2$CH_2N-), 2.64 (t, 2H, *J* = 7.6 Hz, phenyl-CH_2-), 2.44 (t, 2H, *J* = 5.6 Hz, -OCH_2C*H*$_2$N-), 2.38-2.23 (m, 12H, piperazinyl-H + -CH_2CH_2-), 2.09 (s, 6H, -N(CH_3)$_2$), 1.61-1.56 (m, 2H, -CH_2-), 1.27-1.23 (m, 8H, -(CH_2)$_4$-), 0.85 (t, 3H, *J* = 7.2 Hz, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.17, 157.17, 146.19, 140.47, 132.47, 129.45, 117.01, 97.77, 78.43, 75.00, 67.69, 57.17, 57.01, 56.36, 53.47, 45.98, 35.55, 31.66, 31.07, 28.98, 28.93, 22.52, 14.40. IR: 2225 cm^{-1} (-C≡C-). MS (ESI, m/z): 524.6 [M + H]$^+$. HRMS: calcd for $C_{29}H_{46}N_7O_2$, [M + H]$^+$, 524.3707, found 524.3714.

Cell culture

Human liver cancer HepG2 cell, human prostate cancer PC-3 and ovarian cancer SKOV3 cells were purchased from Cell Resource Centre, IBMS, CAMS/PUMC. Human pancreatic cancer Panc-1 cells were the gifts from Prof. Huaizhi Wang (Institute of Hepatopancreatobiliary Surgery, Southwest Hospital, Chongqing). HepG2 cells were grown in Dulbecco's modified eagle's medium (DMEM) (HyClone) supplemented with 10% fetal bovine serum (FBS). Panc-1 and BxPC-3 cells were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) (GIBCO) supplemented with 10% FBS. PC-3 cells were grown in Ham's F12 (Kaighn's modification) (GIBCO) supplemented with 10% FBS. SKOV3 cells were grown in McCoy's 5A (GIBCO) supplemented with 10% FBS. All cells were

incubated at a humidified atmosphere containing 5% CO_2 at 37 °C.

Reagents and Antibodies

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Adamas. The annexin V-FITC/ Propidium Iodide PI apoptosis detection kit was purchased from Sangon Biotech (Shanghai, China). The primary antibodies including PARP antibody, Bcl-2 antibody, HSF1 rabbit mAb, HSP70 rat mAb, HSP27 rabbit mAb, eIF4E rabbit mAb and LC3B rabbit mAb were all obtained from Cell Signaling Technology (Danvers, MA, USA). The β-actin rabbit antibody and goat anti-rat (HRP) secondary antibody were purchased from Bioss (Beijing, China). The goat anti-rabbit (HRP) secondary antibody was purchased from Sino Biological Inc. (Beijing, China). The cell lysis buffer, primary antibody dilution buffer, ECL kit and cycloheximide were purchased from Beyotime (Shanghai, China). Necrostatin-1 was purchased from Selleckchem.

Cell growth inhibition assay

HepG2, PC-3 and SKOV3 cells were seeded into a 96-well plate at 10000 cells per well, while BxPC-3 and Panc-1 at 5000 cells per well, and allowed to adhere overnight. Then the culture medium was removed and replaced with fresh media alone as control or containing various concentrations of the compounds. After indicated treatment, the number of viable cells remained was determined by MTT colorimetric assay. The absorbance was measured in a BioTek® SynergyH4 hybrid reader at a wavelength of 490 nm. All experiments were done in triplicate and repeated three independent times. The IC_{50} values were calculated with the software program SPSS 22.0.

Visualization of cell morphology via microscopy

Panc-1 cells were seeded in 60 mm culture dishes at a density of 2.5×10^5 cells and allowed to adhere overnight. Then the culture medium was removed and replaced with fresh media alone as control or containing 5 μM of **If**. After indicated treatment, the cells were washed with PBS and observed under the microscope (Olympus IX51).

In vivo study in mice model

BALB/c female nude mice (5 weeks) were purchased from the Shanghai Xipur-Bikai Experimental Animal Ltd (Shanghai, China). All animal experimental procedures were done in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of China Pharmaceutical University. Experimental Animals housed under sterile conditions as per standard protocols. The nude mice were injected with 1×10^7 Panc-1 cells suspensions in the right flank region, Cells suspensions were prepared in 0.1 mL of PBS and combined with 50% Matrigel (BD Biosciences, San Diego, CA, USA). When tumors reached approximately 100 mm^3, tumor-bearing mice were randomly divided into 3 groups (n = 5/group).

Animals in the treated group received an intraperitoneal (i.p.) injection of **If** dissolved in sterile PBS containing 1% DMSO at 20 mg/kg body weight twice a week. And the blank control group received vehicle. The tumor volume and body weight were monitored before every administration, and the tumor volume was calculated using the following standard formula: $width^2 \times length \times 0.5$, where the length and the width represent the maximum and

the minimum diameters, respectively. After about 4 weeks, mice were euthanized by cervical dislocation. Tumor samples and organs were excised and fixed in 4% polyformaldehyde for histopathology analyses.

HE Staining

Briefly, after deparaffinization and rehydration with xylene and an ethanol gradient, 4 μm longitudinal sections were stained with hematoxylin solution for 5 min. Then the sections were stained with eosin solution for 5 min and followed by dehydration with graded alcohol and clearing in xylene. The mounted slides were then examined and photographed using a fluorescence microscope (Zeiss Axio Vert A1).

Ki67 staining

The 5 μm longitudinal sections were deparaffinised and rehydrated using xylene and an ethanol gradient. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 30 min. After sealing with serum, the sections were incubated with the primary antibody Ki67 (Wuhan servicebio technology CO., Ltd, Wuhan, China) overnight at 4 °C. The next day, the slides were rinsed and incubated with the corresponding secondary antibody (Wuhan servicebio technology CO., Ltd, Wuhan, China) for 50 min. After washed with PBS, the sections were stained by diaminobenzidine (DAB) and counterstained with hematoxylin, respectively. Finally, the specimens were dehydrated and photographed under the microscope (Zeiss Axio Vert A1).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Paraffin blocks were cut into slices with 4 μm thickness. The TUNEL assay was conducted using a TUNEL apoptosis detection kit (Wuhan servicebio technology CO., Ltd, Wuhan, China). Paraffin sections were incubated with proteinase K at 37 °C for 30 min. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 30 min. The sections were incubated with TUNEL reaction mixture at 37 °C for 2 h. Finally, the sections were counterstained with DAPI for 10 min, and the images were captured under the microscope (Zeiss Axio Vert A1).

Apoptosis and necrosis assay on flow cytometry

The cancer cells were seeded in 6-well plates (2.5×10^5 cells/well) and allowed to adhere and proliferate overnight. Culture medium was then removed and fresh media containing the compound was added. After 48 hours treatment, the cells were harvested and washed with cold PBS. The samples were pelleted again through centrifugation and resuspended in binding buffer. Annexin-V/FITC and propidium iodide (PI) was then added to the cells and the samples were incubated for 15 minutes at room temperature. After staining, flow cytometry was performed on Fluorescence Activated Cell Sorting (Beckman Coulter, CytoFLEX model). Each sample was performed in triplicate.

Western Blotting

The cancer cells after being treated by the compounds for 48 h were lysed in lysis buffer. A total of 10-50 μg protein were quantified and loaded into the 10% SDS-PAGE gel.

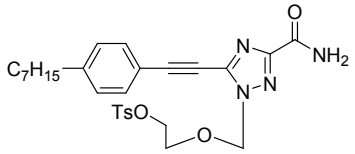
After electrophoresis (BIO-RAD), the proteins in the gel were transferred to a PVDF membrane. Then the membrane was blocked in 5% (w/v) skim milk in TBS-T for 1 h and incubated with the primary antibodies at 4 °C overnight. The membrane was washed with TBS-T and incubated with HRP-conjugated secondary antibody for 1 h. Specific proteins were detected using an ECL kit.

Immunofluorescence assay

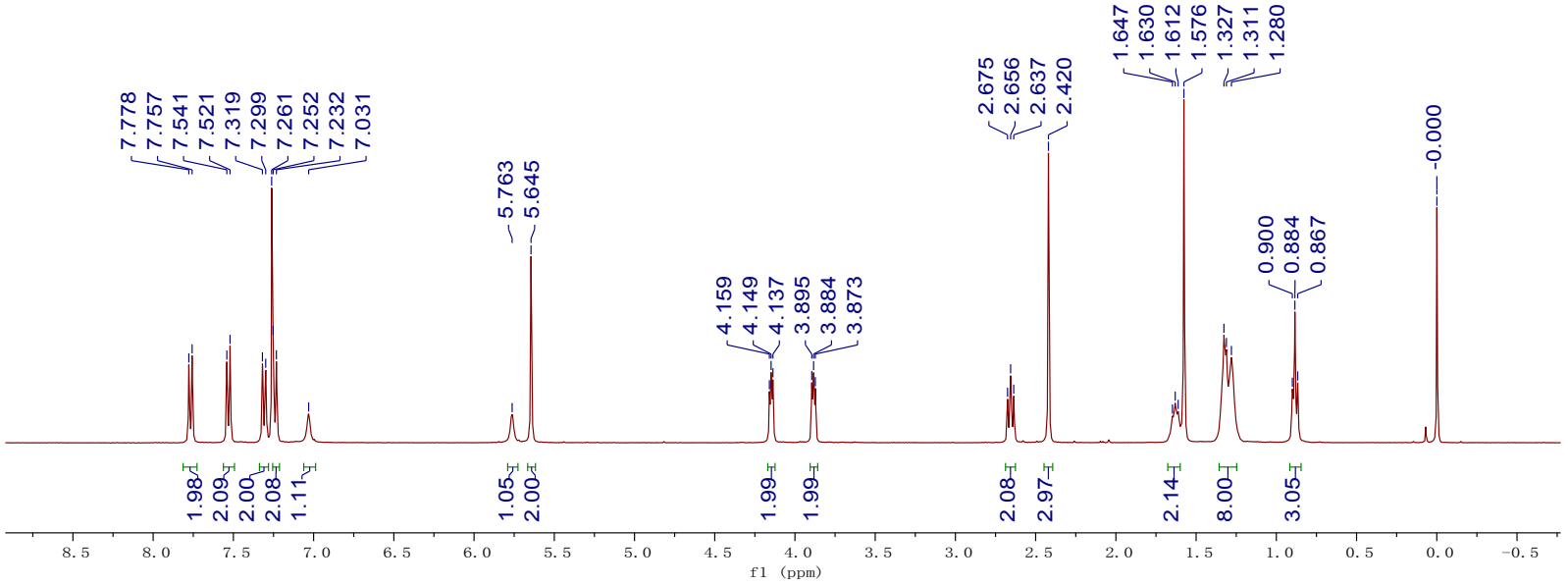
Panc-1 cells were seeded at 100000 cells per well in RPMI 1640 with 10% FBS on cover glass in 6-well plates. After 24 h, cells were treated with the compounds for 12 h. Cells were washed with 1× PBS and fixed in a solution (4% paraformaldehyde in PBS) at room temperature for 15 minutes. After that, cells were blocked with permeable buffer (0.5% Triton-100 in PBS) for 20 minutes and added with goat serum at room temperature for 1 hour, then incubated with LC3B (1:150) antibody at 4 ℃ overnight. Next, cells were washed with PBS and incubated with Alexa Fluor 488 secondary antibodies (1:250) in the dark for 1 hour and the nuclei were stained with Hoechst 33342 (10 μg/mL) for 10 minutes. Each group of Panc-1 cells were observed using inverted fluorescence microscope (Leica DMi8).

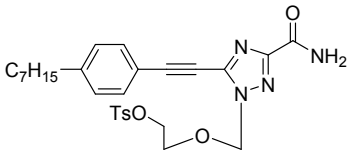
Reference

1. Zhu, R.; Wang, M.; Xia, Y.; Qu, F.; Neyts, J.; Peng, L. Arylethynyltriazole acyclonucleosides inhibit hepatitis C virus replication. *Bioorg. Med. Chem. Lett.* **2008**, 18, 3321-3327.

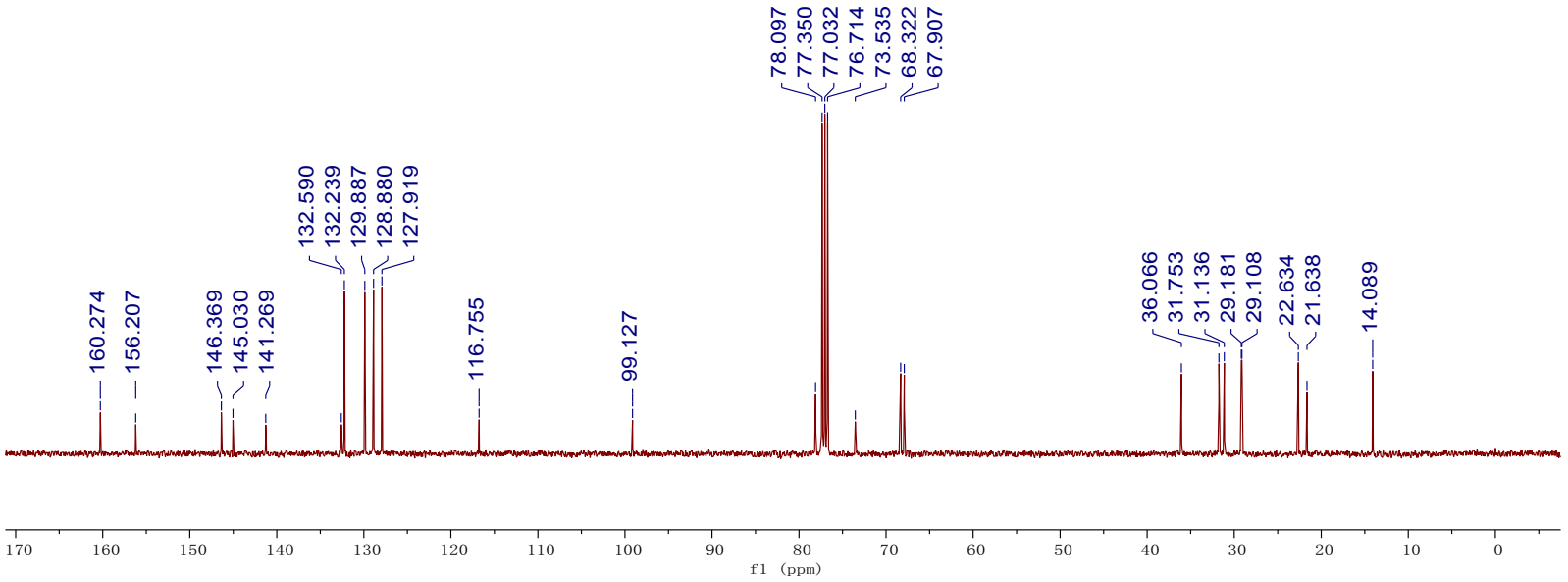

O
NH2
C7H15
N
N
N
TsO
O

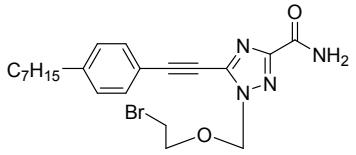
I-2


7.778
7.757
7.541
7.521
7.319
7.299
7.261
7.252
7.232
7.031
5.763
5.645
4.159
4.149
4.137
3.895
3.884
3.873
2.675
2.656
2.637
2.420
1.647
1.630
1.612
1.576
1.327
1.311
1.280
0.900
0.884
0.867
-0.000
1.98
2.09
2.00
2.08
1.11
1.05
2.00
1.99
1.99
2.08
2.97
2.14
8.00
3.05
f1 (ppm)

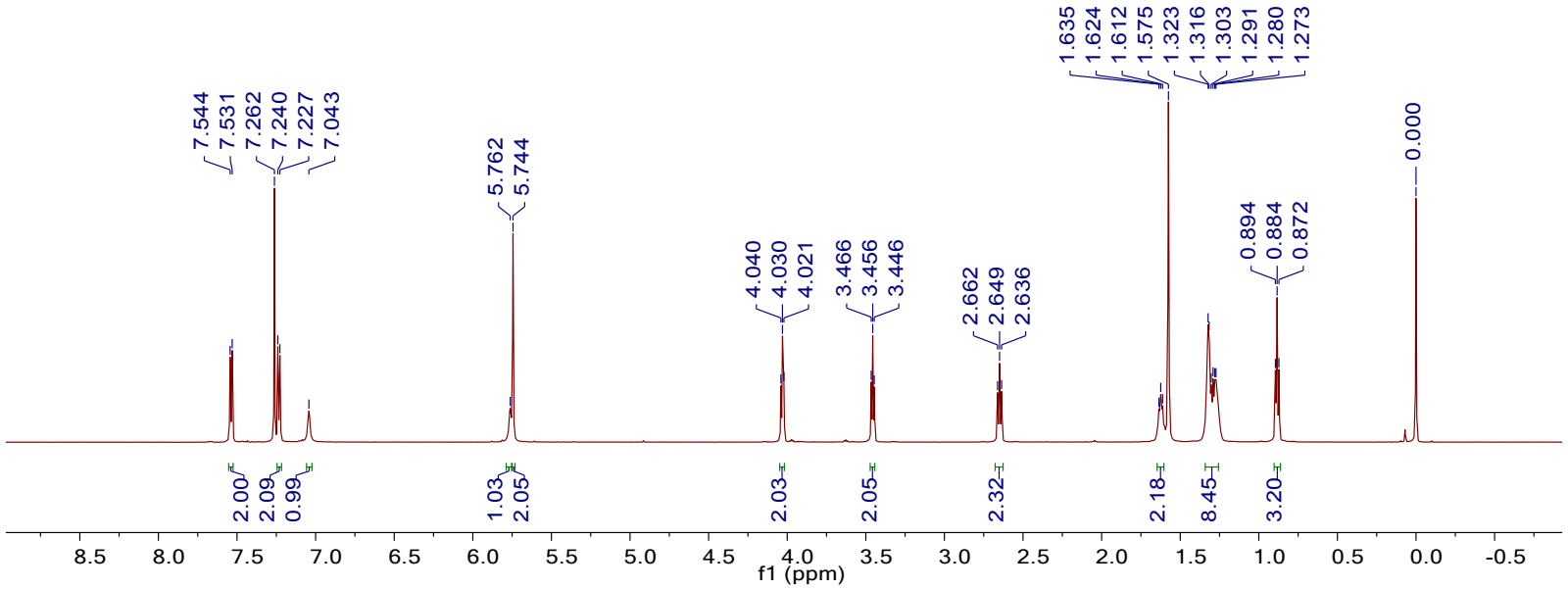

C7H15
TsO
O
N
N
N
O
NH2

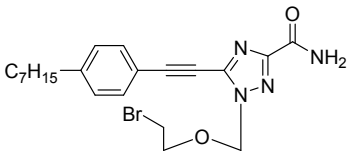
I-2


160.274
156.207
146.369
145.030
141.269
132.590
132.239
129.887
128.880
127.919
116.755
99.127
78.097
77.350
77.032
76.714
73.535
68.322
67.907
36.066
31.753
31.136
29.181
29.108
22.634
21.638
14.089
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

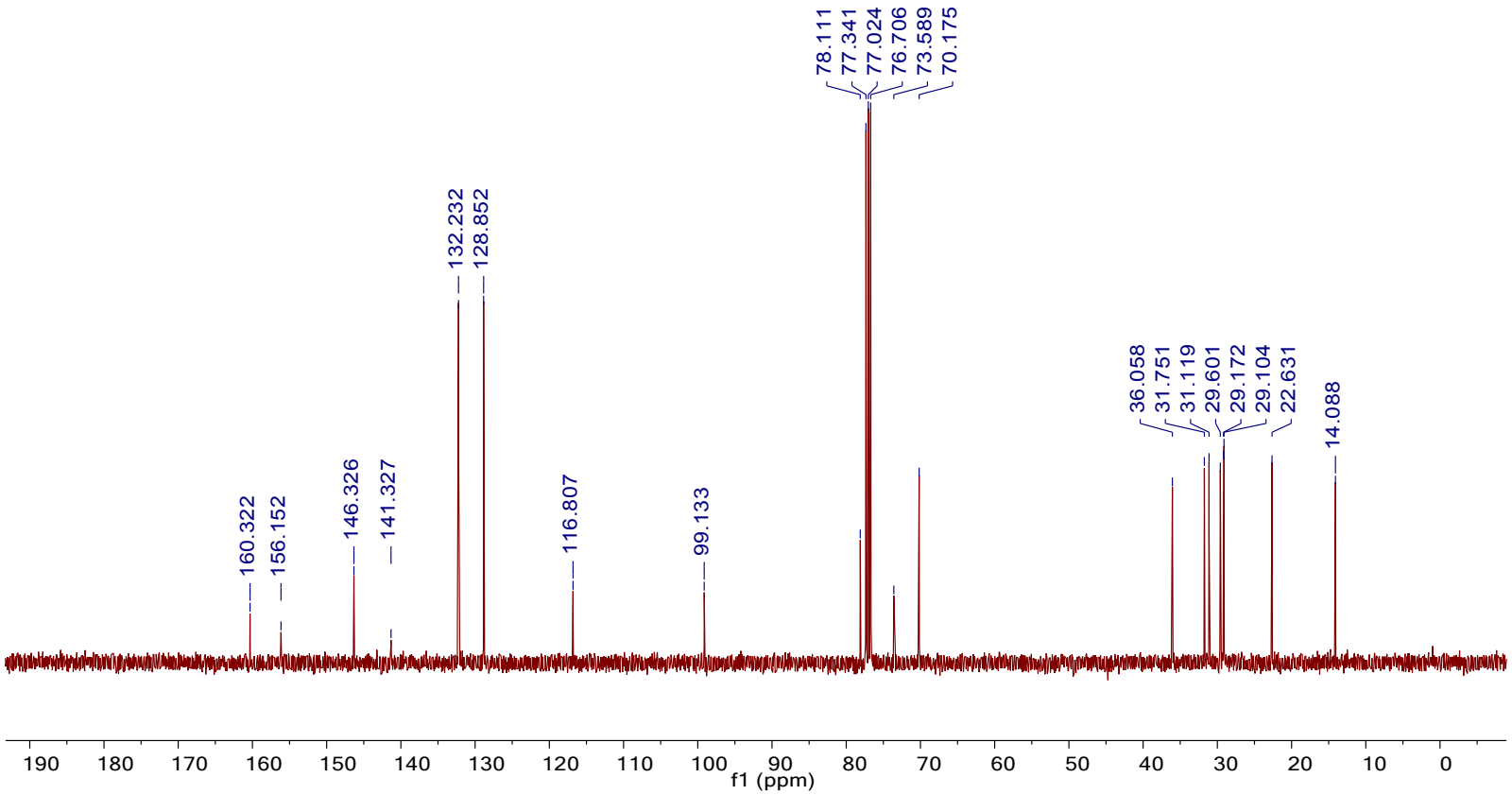

O
NH2
N
N
N
C7H15
Br
O

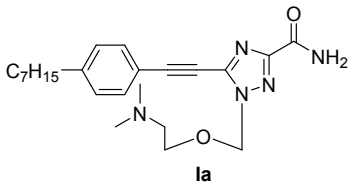
I-1


7.544
7.531
7.262
7.240
7.227
7.043
5.762
5.744
4.040
4.030
4.021
3.466
3.456
3.446
2.662
2.649
2.636
1.635
1.624
1.612
1.575
1.323
1.316
1.303
1.291
1.280
1.273
0.894
0.884
0.872
0.000
2.00
2.09
0.99
1.03
2.05
2.03
2.05
2.32
2.18
8.45
3.20
f1 (ppm)

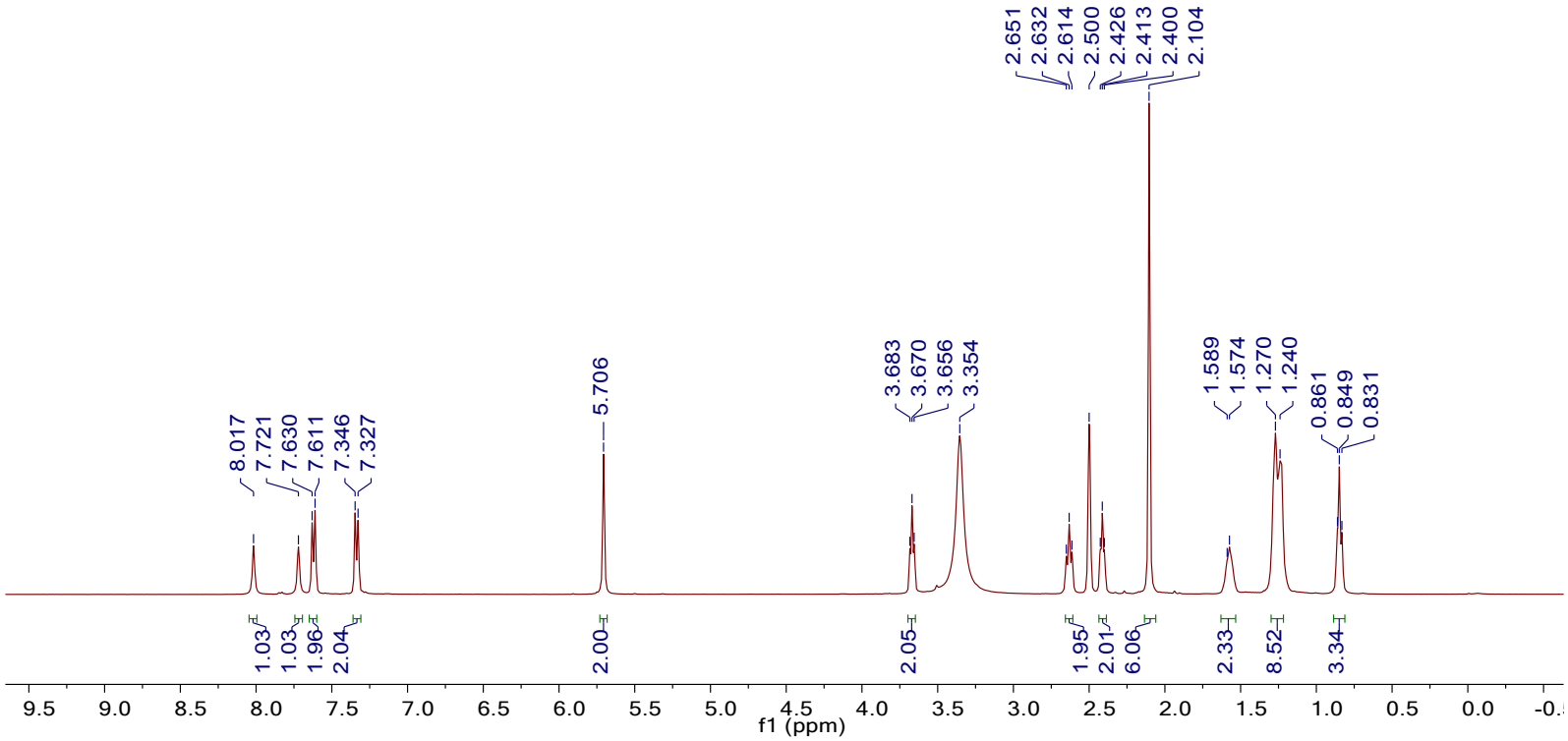

O
NH2
C7H15
N
N
N
Br
O

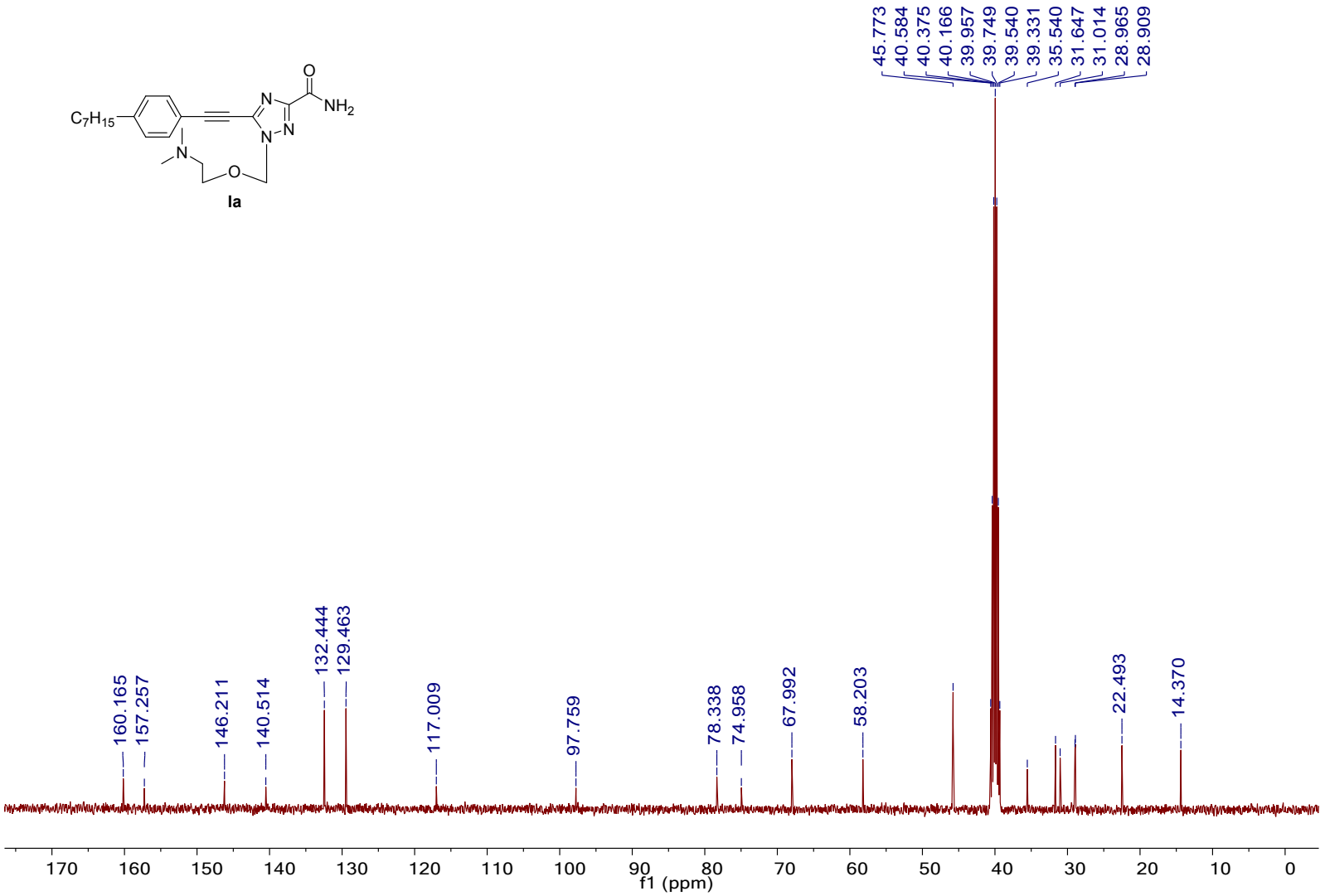
I-1

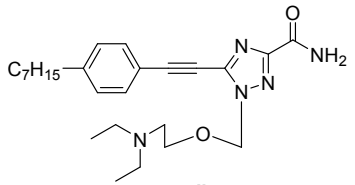

78.111
77.341
77.024
76.706
73.589
70.175
132.232
128.852
160.322
156.152
146.326
141.327
116.807
99.133
36.058
31.751
31.119
29.601
29.172
29.104
22.631
14.088
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


C7H15
O
NH2
N
N
N
N
O

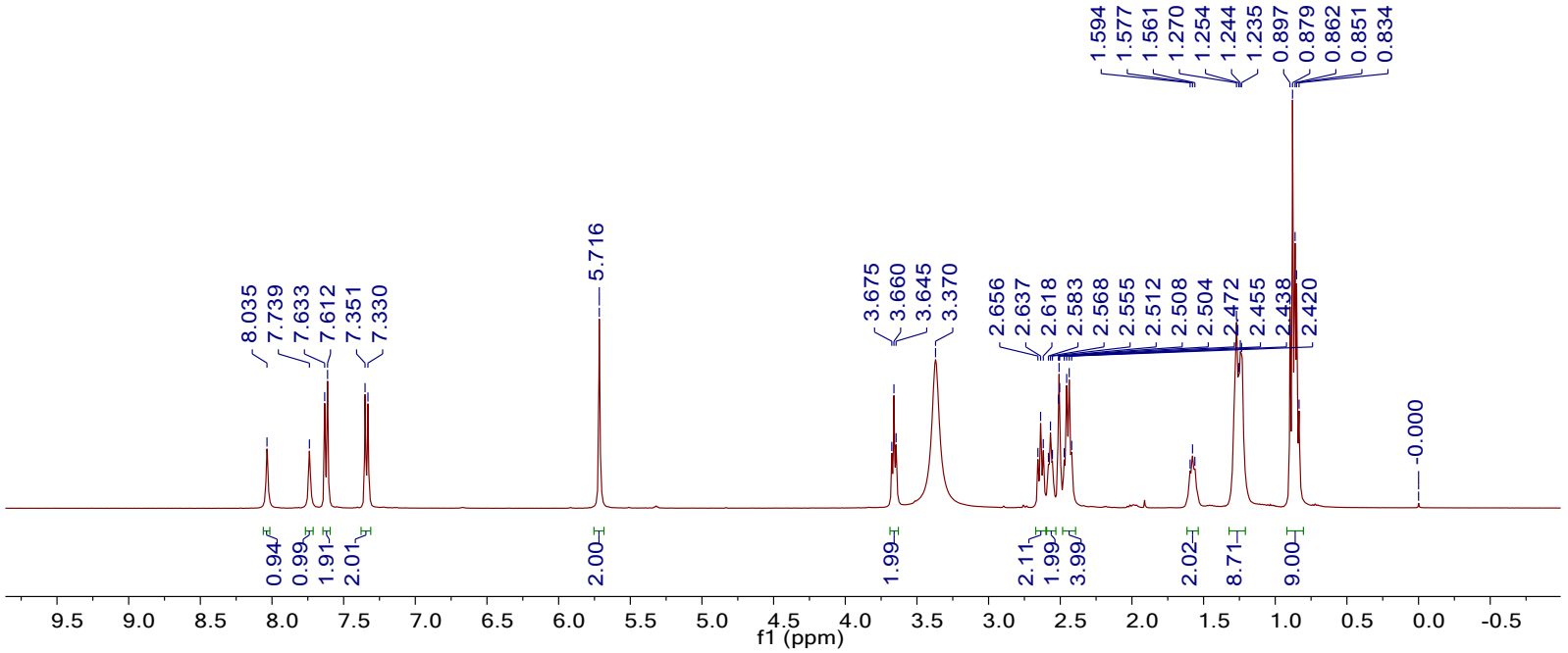
Ia

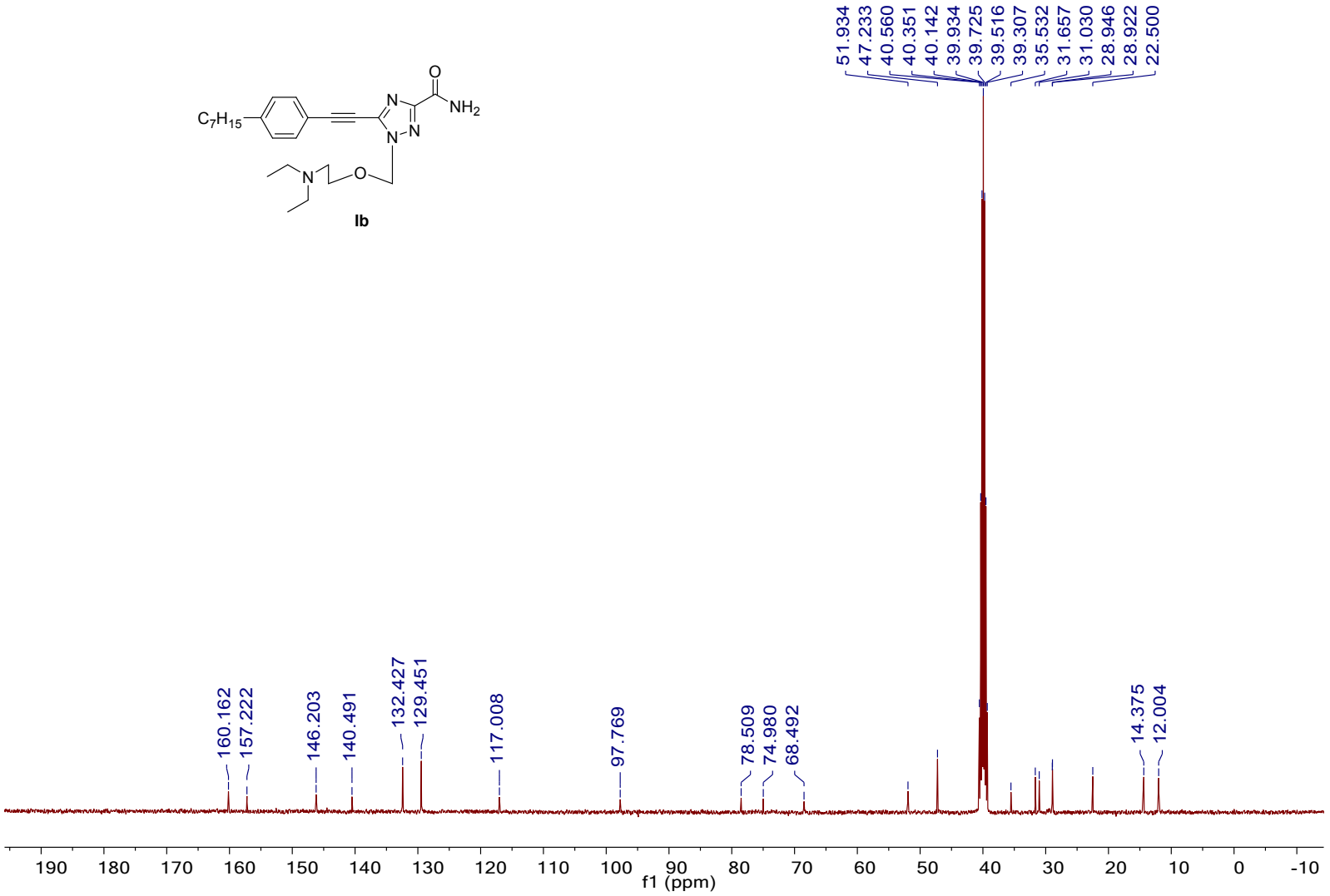

8.017
7.721
7.630
7.611
7.346
7.327
5.706
3.683
3.670
3.656
3.354
2.651
2.632
2.614
2.500
2.426
2.413
2.400
2.104
1.589
1.574
1.270
1.240
0.861
0.849
0.831
1.03
1.03
1.96
2.04
2.00
2.05
1.95
2.01
6.06
2.33
8.52
3.34
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

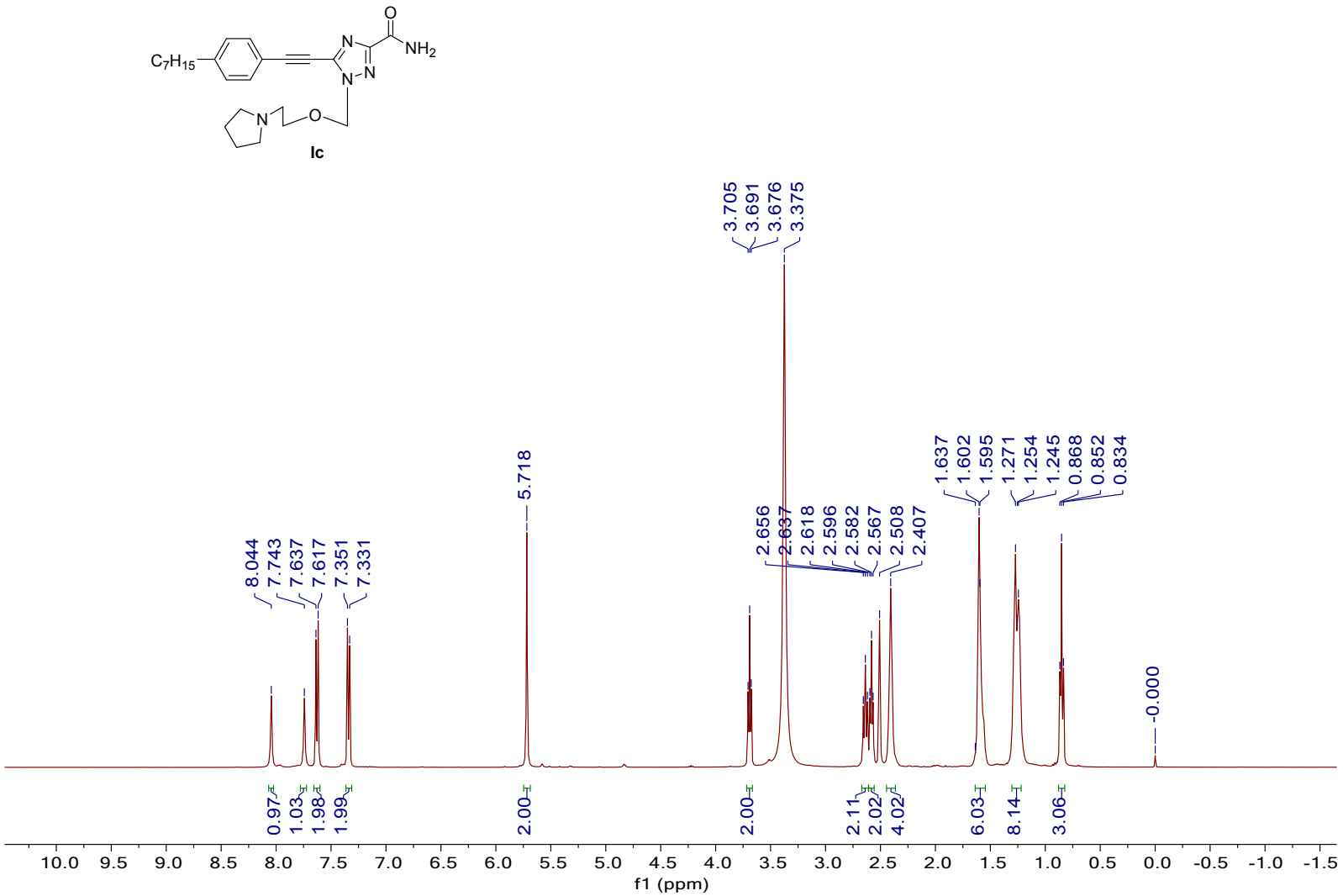

C7H15
O
NH2
N
N
N
N
O
Ia
45.773
40.584
40.375
40.166
39.957
39.749
39.540
39.331
35.540
31.647
31.014
28.965
28.909
160.165
157.257
146.211
140.514
132.444
129.463
117.009
97.759
78.338
74.958
67.992
58.203
22.493
14.370
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

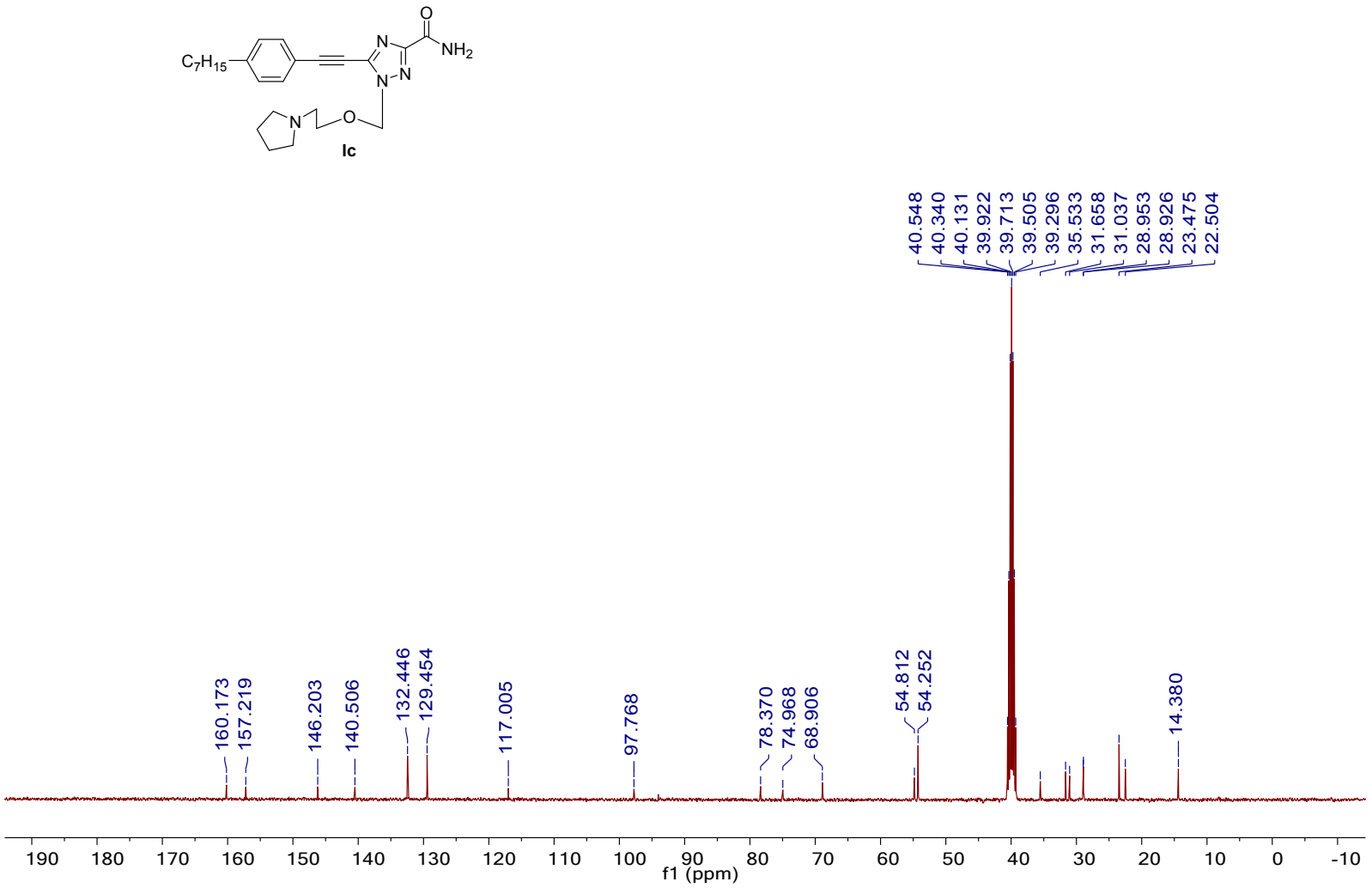

C7H15
N
N
N
O
NH2
N
O

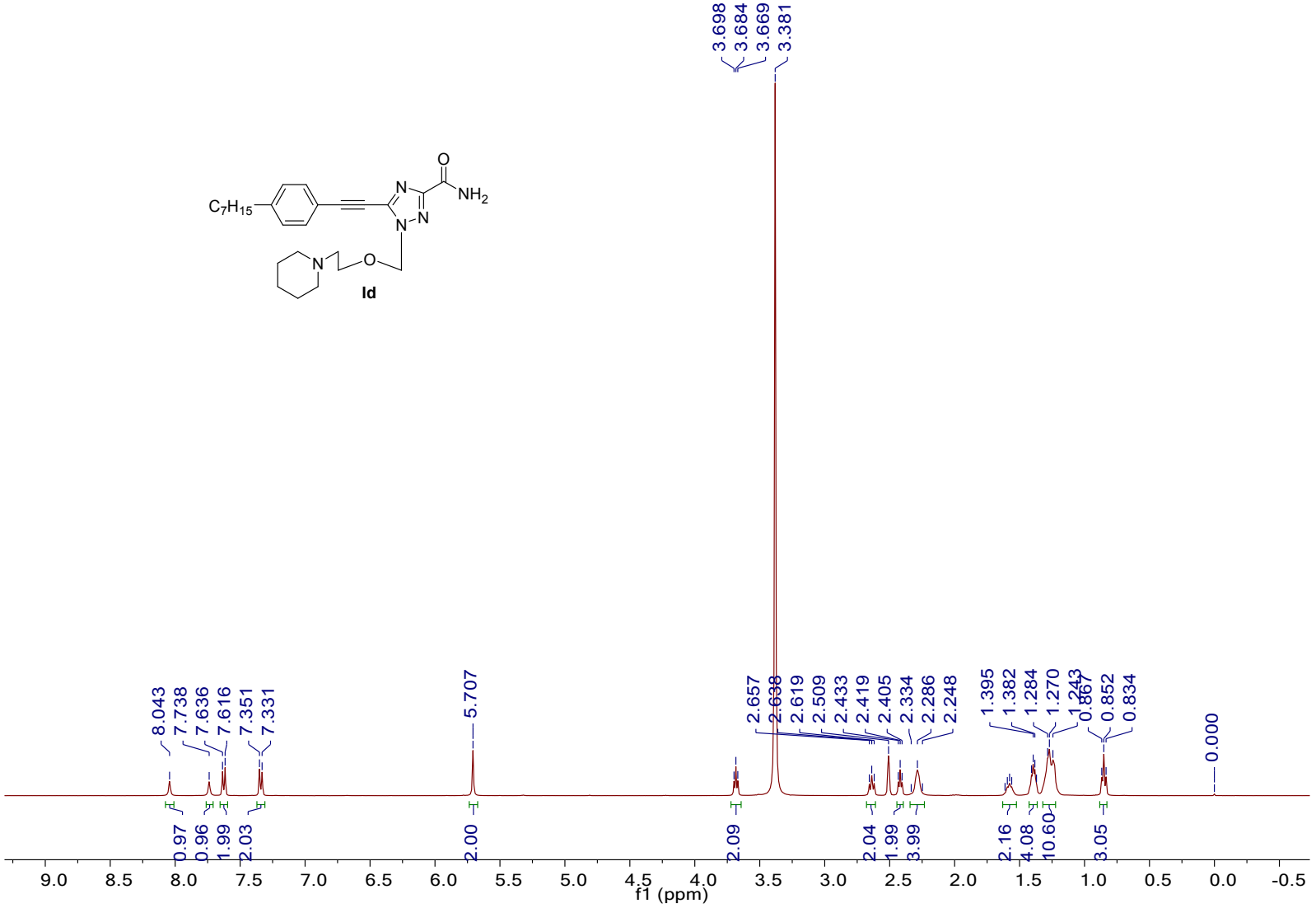
Ib


8.035
7.739
7.633
7.612
7.351
7.330
5.716
3.675
3.660
3.645
3.370
2.656
2.637
2.618
2.583
2.568
2.555
2.512
2.508
2.504
2.472
2.455
2.438
2.420
1.594
1.577
1.561
1.270
1.254
1.244
1.235
0.897
0.879
0.862
0.851
0.834
-0.000
0.94
0.99
1.91
2.01
2.00
1.99
2.11
1.99
3.99
2.02
8.71
9.00
f1 (ppm)

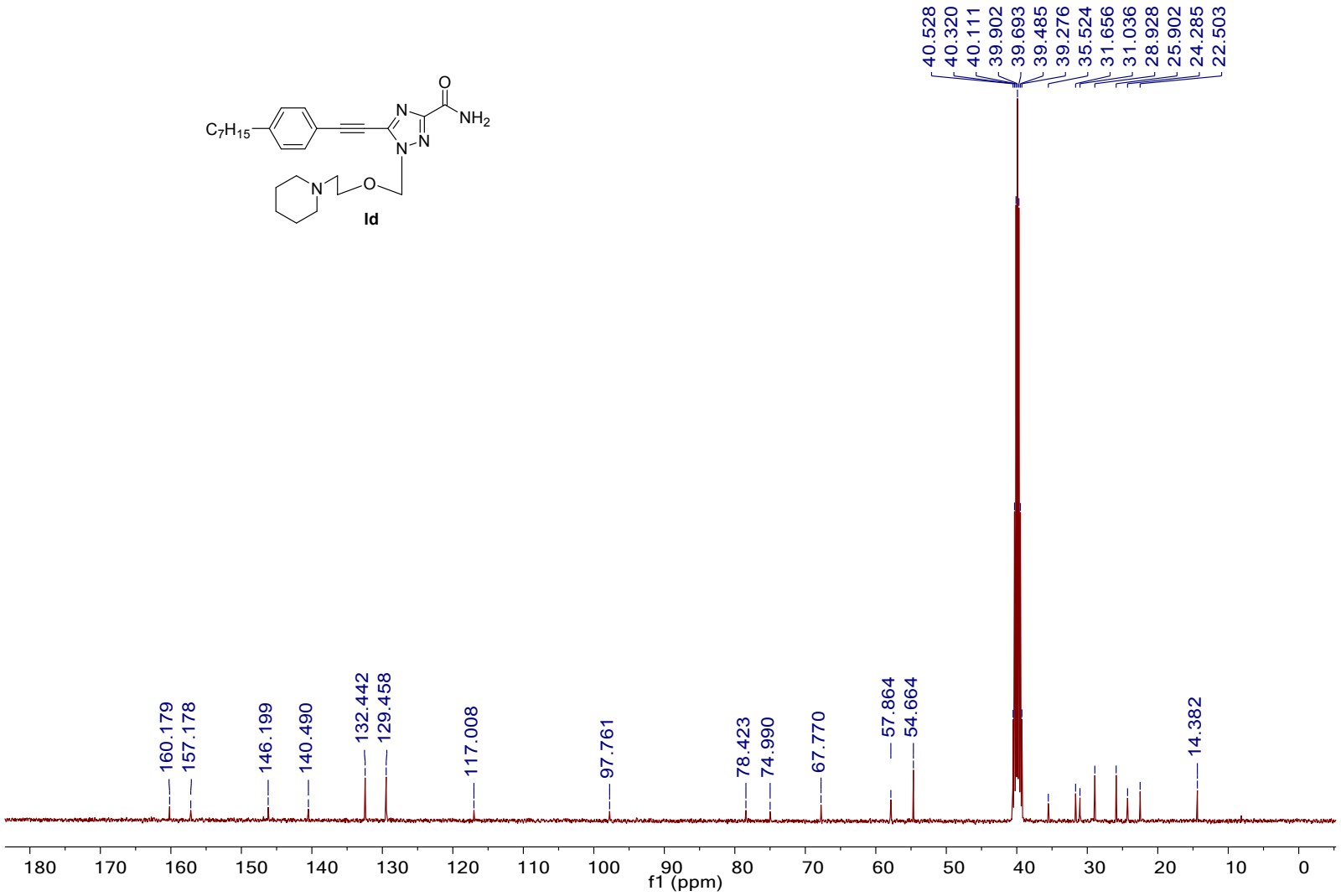

C7H15
N
N
N
O
NH2
N
O
Ib
51.934
47.233
40.560
40.351
40.142
39.934
39.725
39.516
39.307
35.532
31.657
31.030
28.946
28.922
22.500
160.162
157.222
146.203
140.491
132.427
129.451
117.008
97.769
78.509
74.980
68.492
14.375
12.004
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

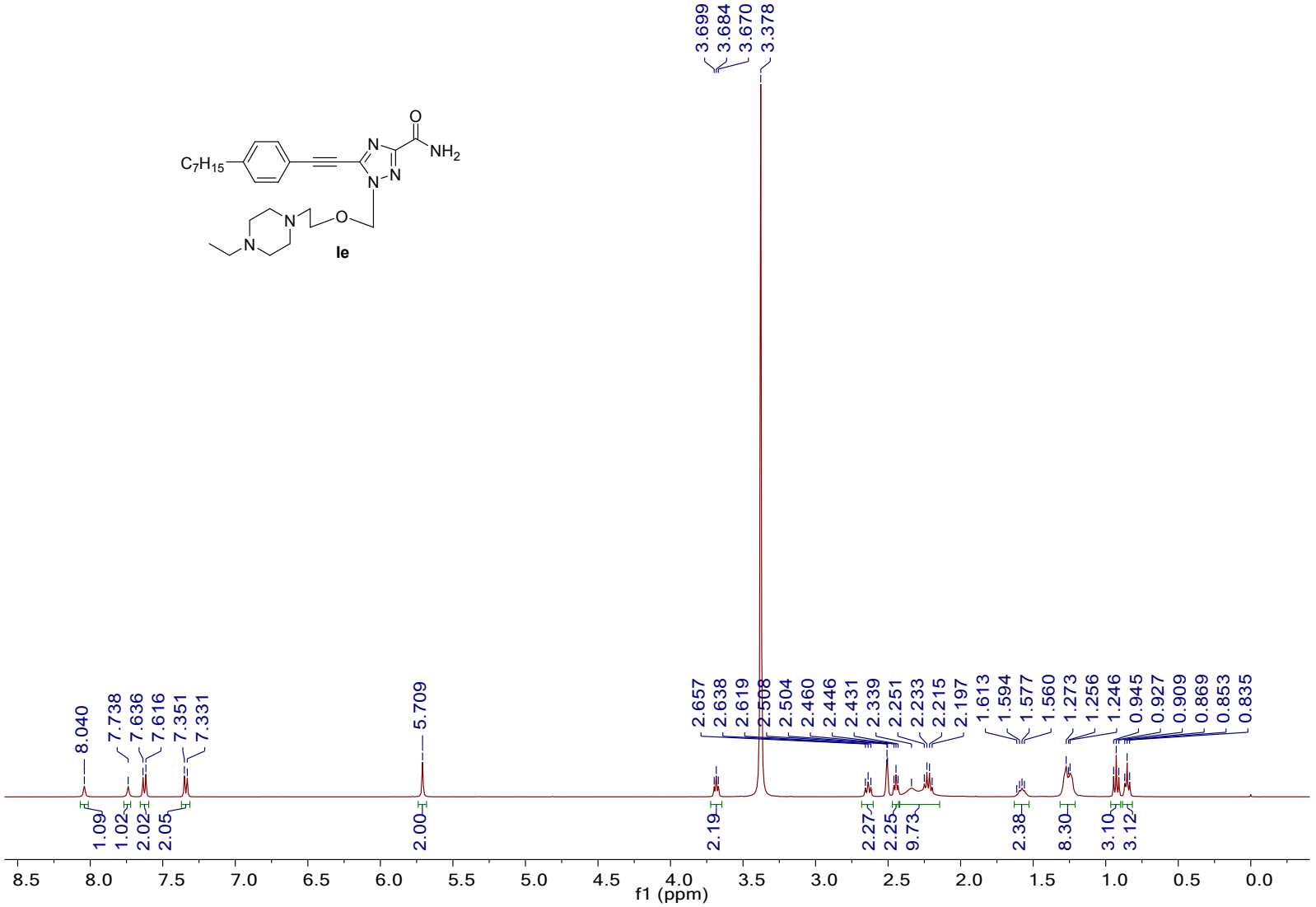

C7H15
N
N
N
O
NH2
N
O
Ic
8.044
7.743
7.637
7.617
7.351
7.331
5.718
3.705
3.691
3.676
3.375
2.656
2.637
2.618
2.596
2.582
2.567
2.508
2.407
1.637
1.602
1.595
1.271
1.254
1.245
0.868
0.852
0.834
-0.000
0.97
1.03
1.98
1.99
2.00
2.00
2.11
2.02
4.02
6.03
8.14
3.06
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
f1 (ppm)

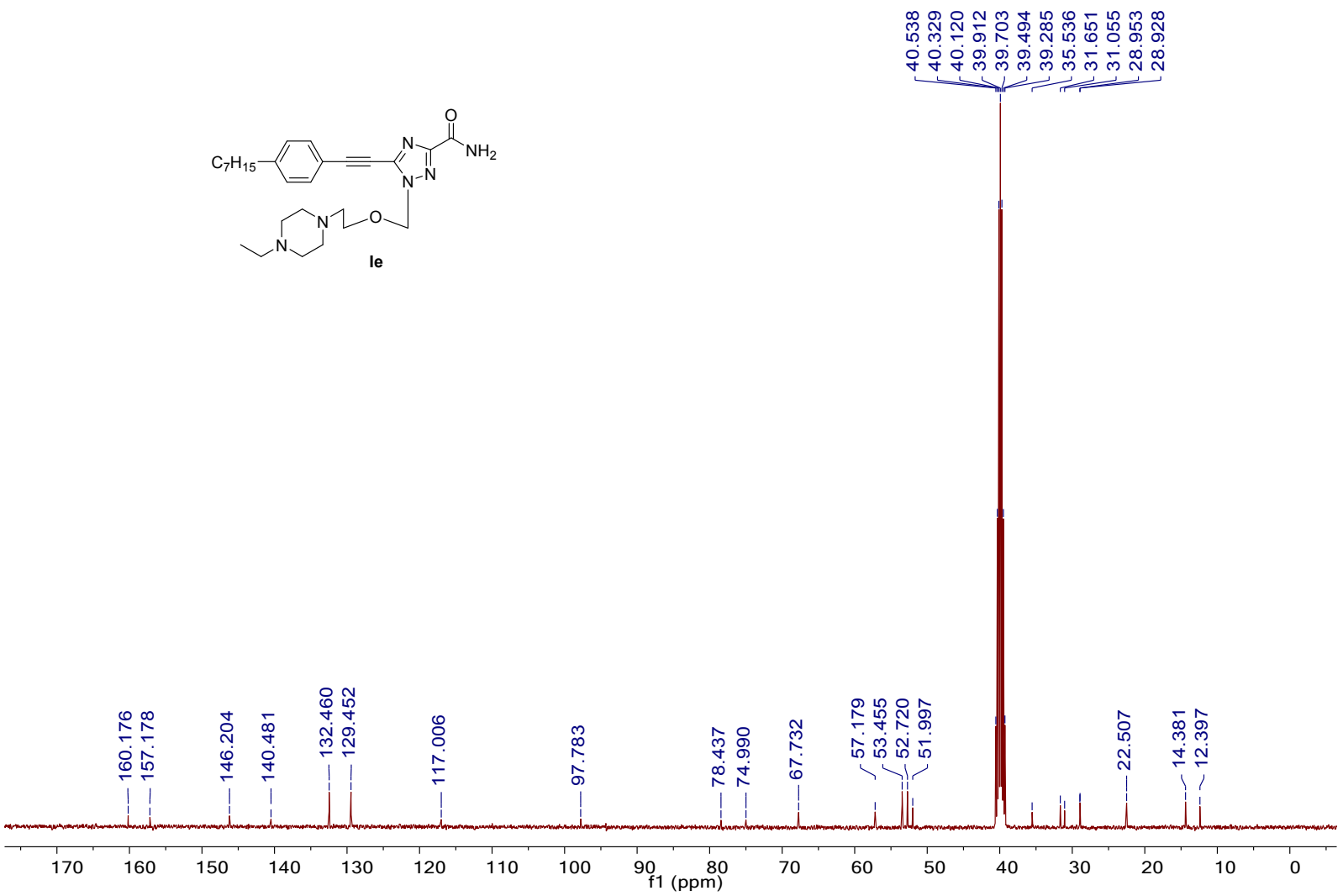

C7H15
O
NH2
N
N
N
N
O
Ic
40.548
40.340
40.131
39.922
39.713
39.505
39.296
35.533
31.658
31.037
28.953
28.926
23.475
22.504
160.173
157.219
146.203
140.506
132.446
129.454
117.005
97.768
78.370
74.968
68.906
54.812
54.252
14.380
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


3.698
3.684
3.669
3.381
8.043
7.738
7.636
7.616
7.351
7.331
5.707
2.657
2.638
2.619
2.509
2.433
2.419
2.405
2.334
2.286
2.248
1.395
1.382
1.284
1.270
1.243
0.867
0.852
0.834
0.000
0.97
0.96
1.99
2.03
2.00
2.09
2.04
1.99
3.99
2.16
4.08
10.60
3.05
f1 (ppm)

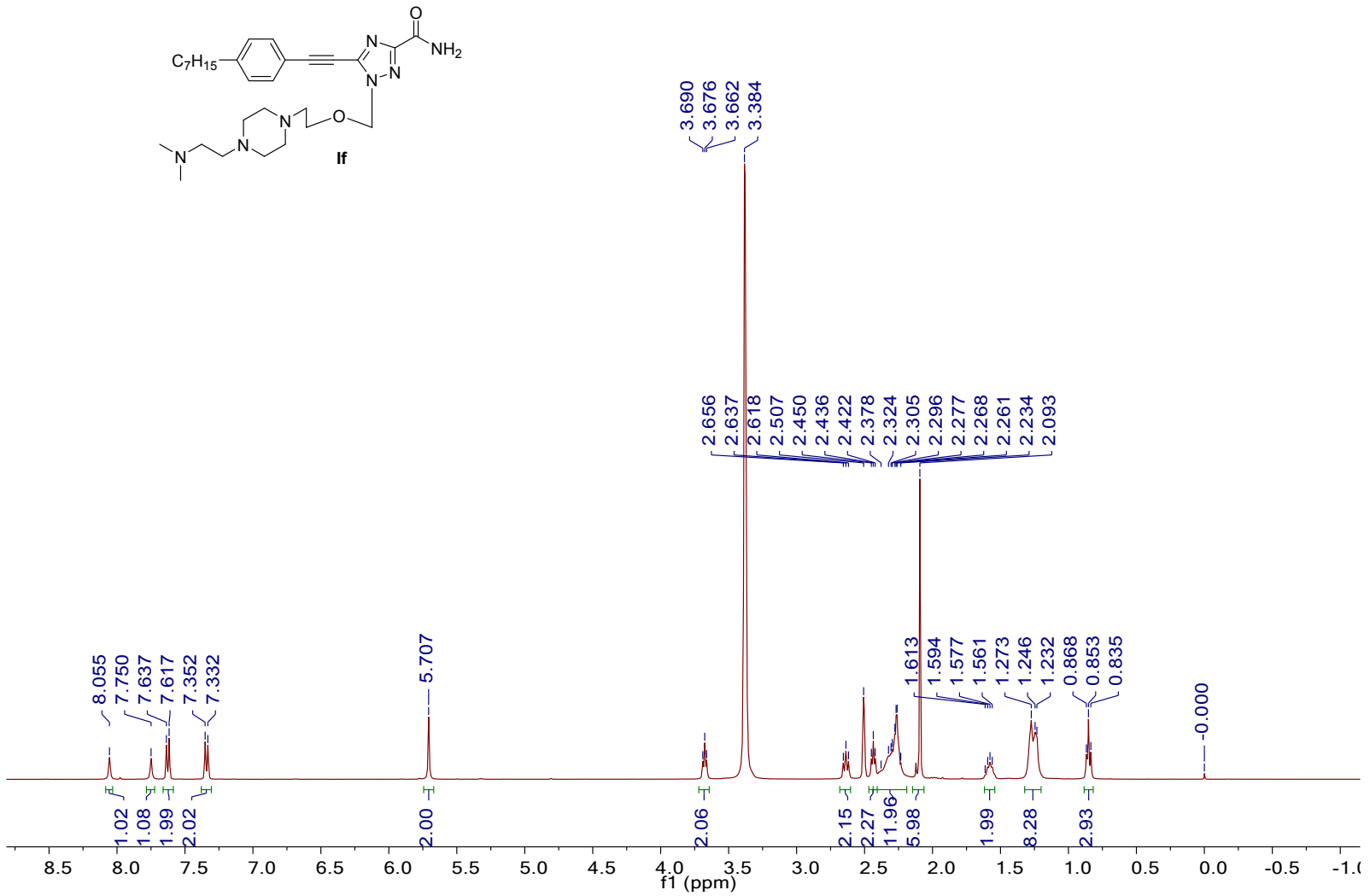
Id

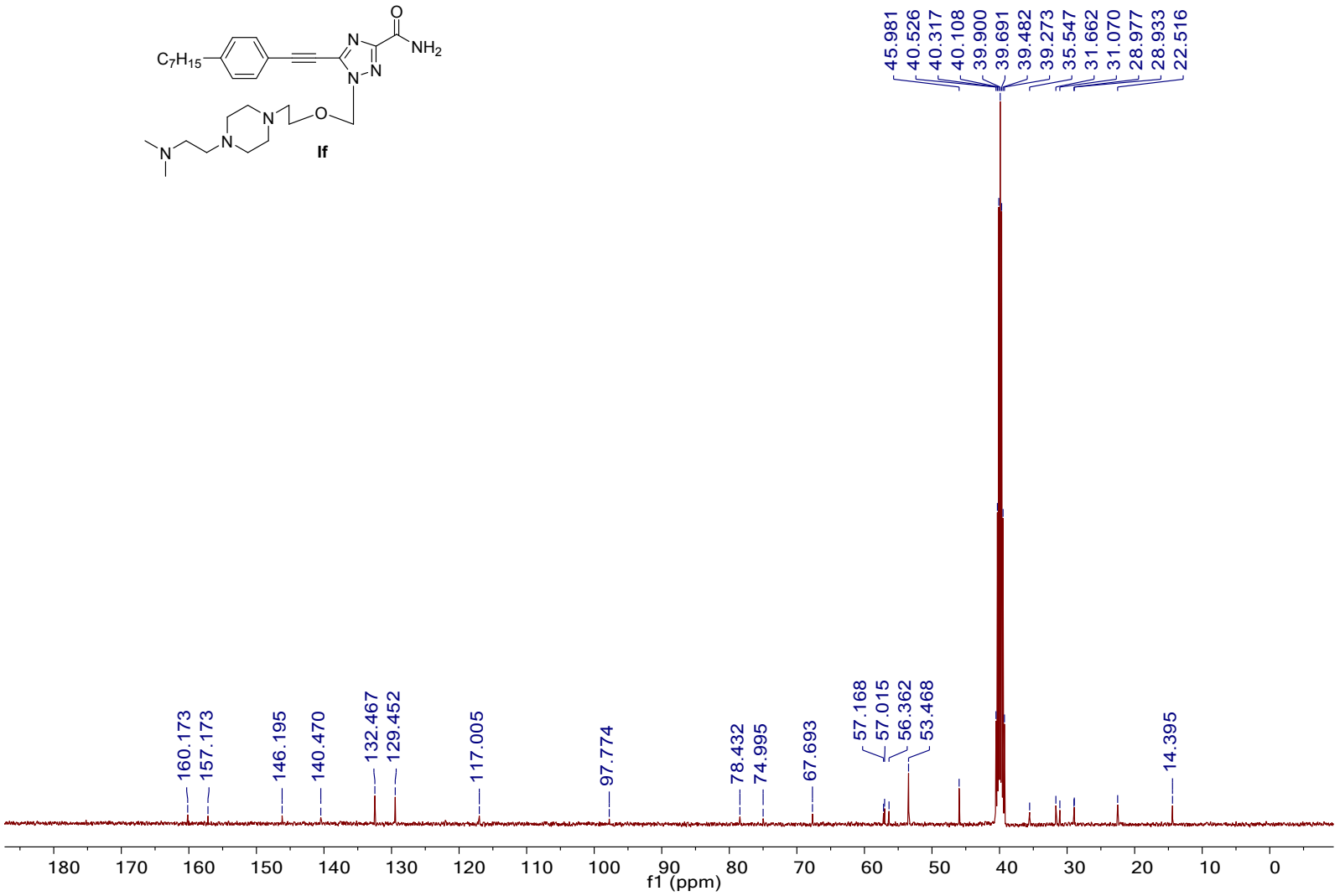

C7H15
N
O
NH2
N
N
N
O
Id
40.528
40.320
40.111
39.902
39.693
39.485
39.276
35.524
31.656
31.036
28.928
25.902
24.285
22.503
160.179
157.178
146.199
140.490
132.442
129.458
117.008
97.761
78.423
74.990
67.770
57.864
54.664
14.382
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

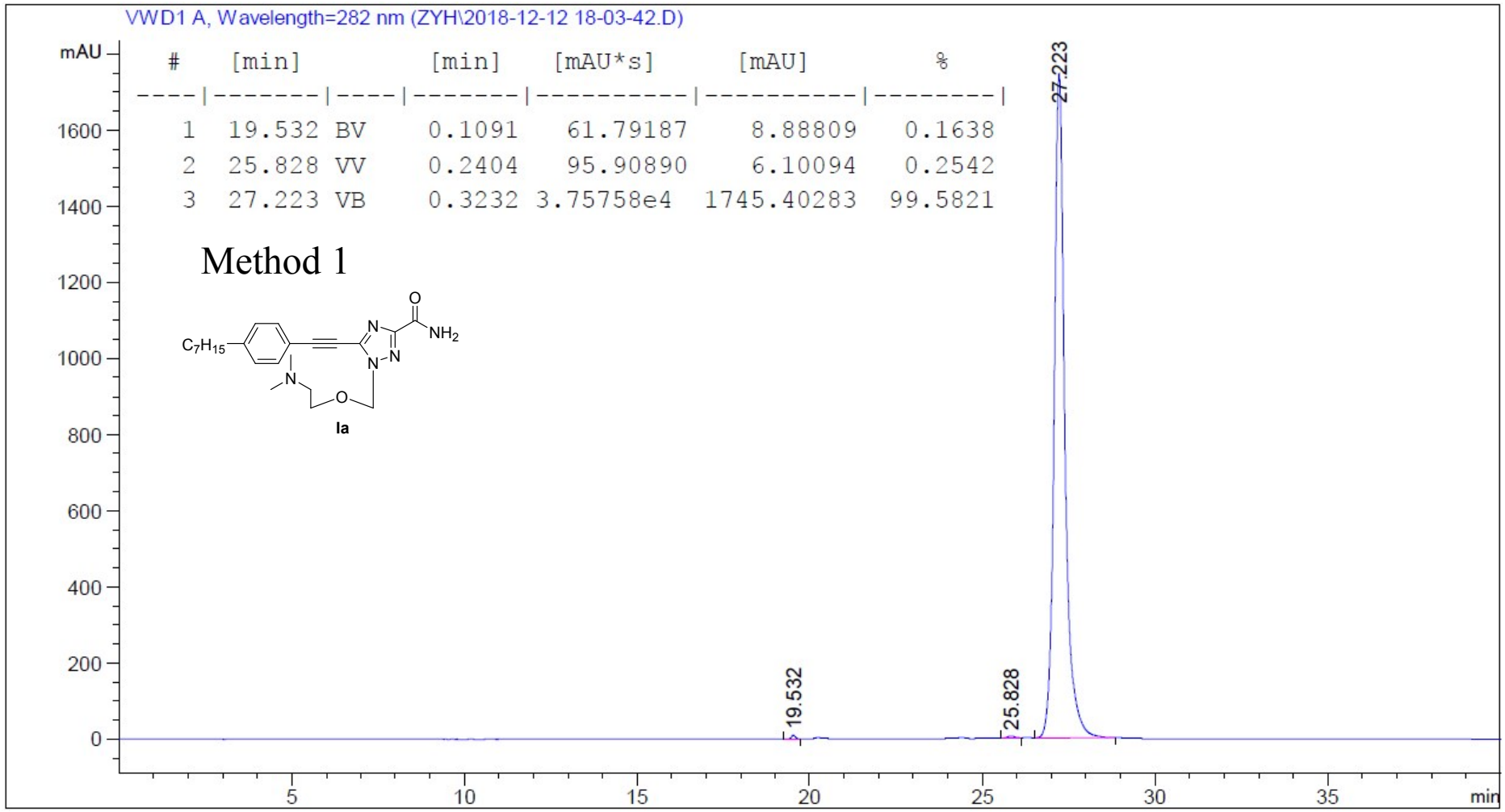

3.699
3.684
3.670
3.378
O
NH2
C7H15
N
N
N
N
O
N
Ie
8.040
7.738
7.636
7.616
7.351
7.331
5.709
2.657
2.638
2.619
2.508
2.504
2.460
2.446
2.431
2.339
2.251
2.233
2.215
2.197
1.613
1.594
1.577
1.560
1.273
1.256
1.246
0.945
0.927
0.909
0.869
0.853
0.835
1.09
1.02
2.02
2.05
2.00
2.19
2.27
2.25
9.73
2.38
8.30
3.10
3.12
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

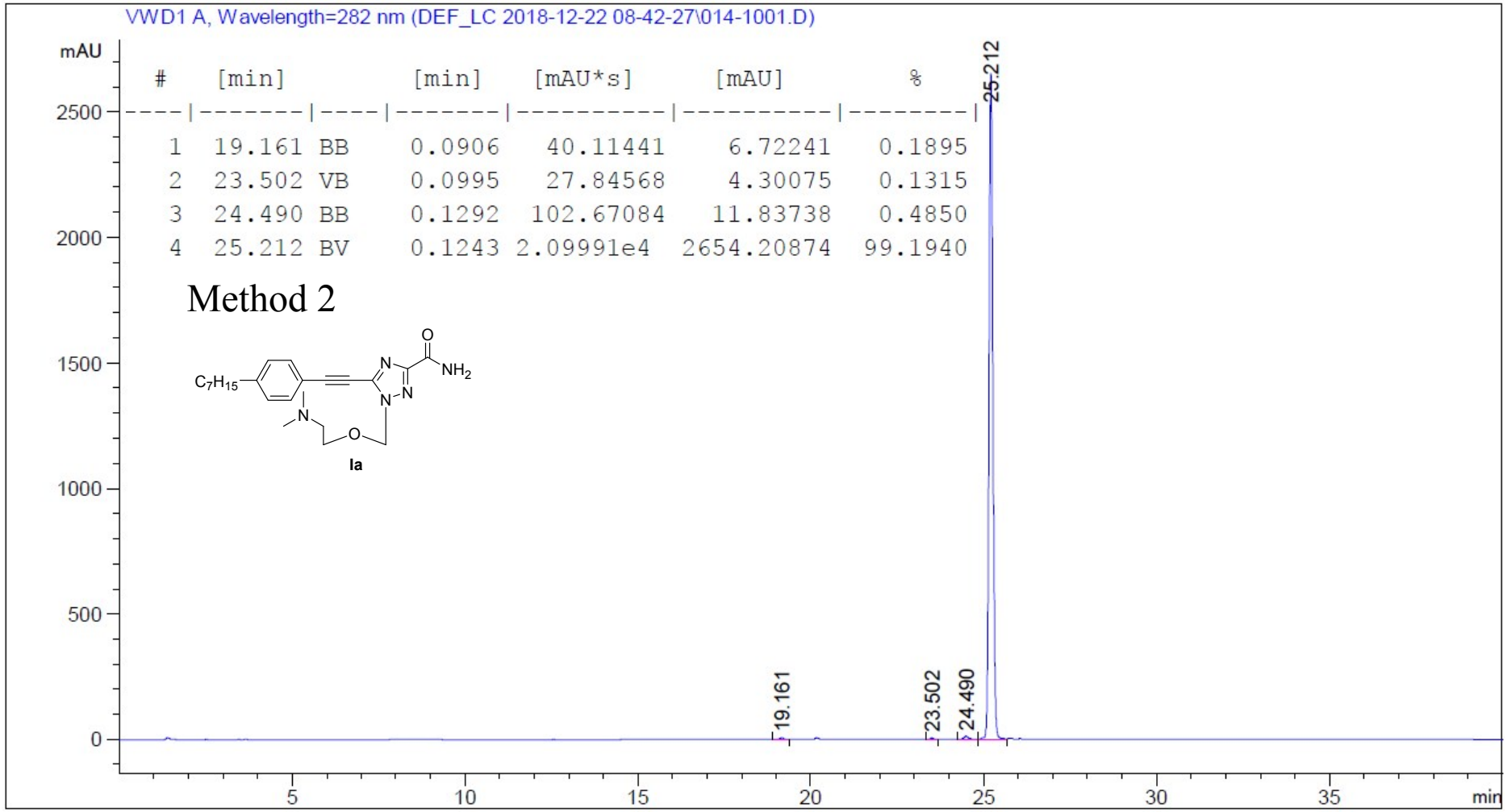

40.538
40.329
40.120
39.912
39.703
39.494
39.285
35.536
31.651
31.055
28.953
28.928
160.176
157.178
146.204
140.481
132.460
129.452
117.006
97.783
78.437
74.990
67.732
57.179
53.455
52.720
51.997
22.507
14.381
12.397
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

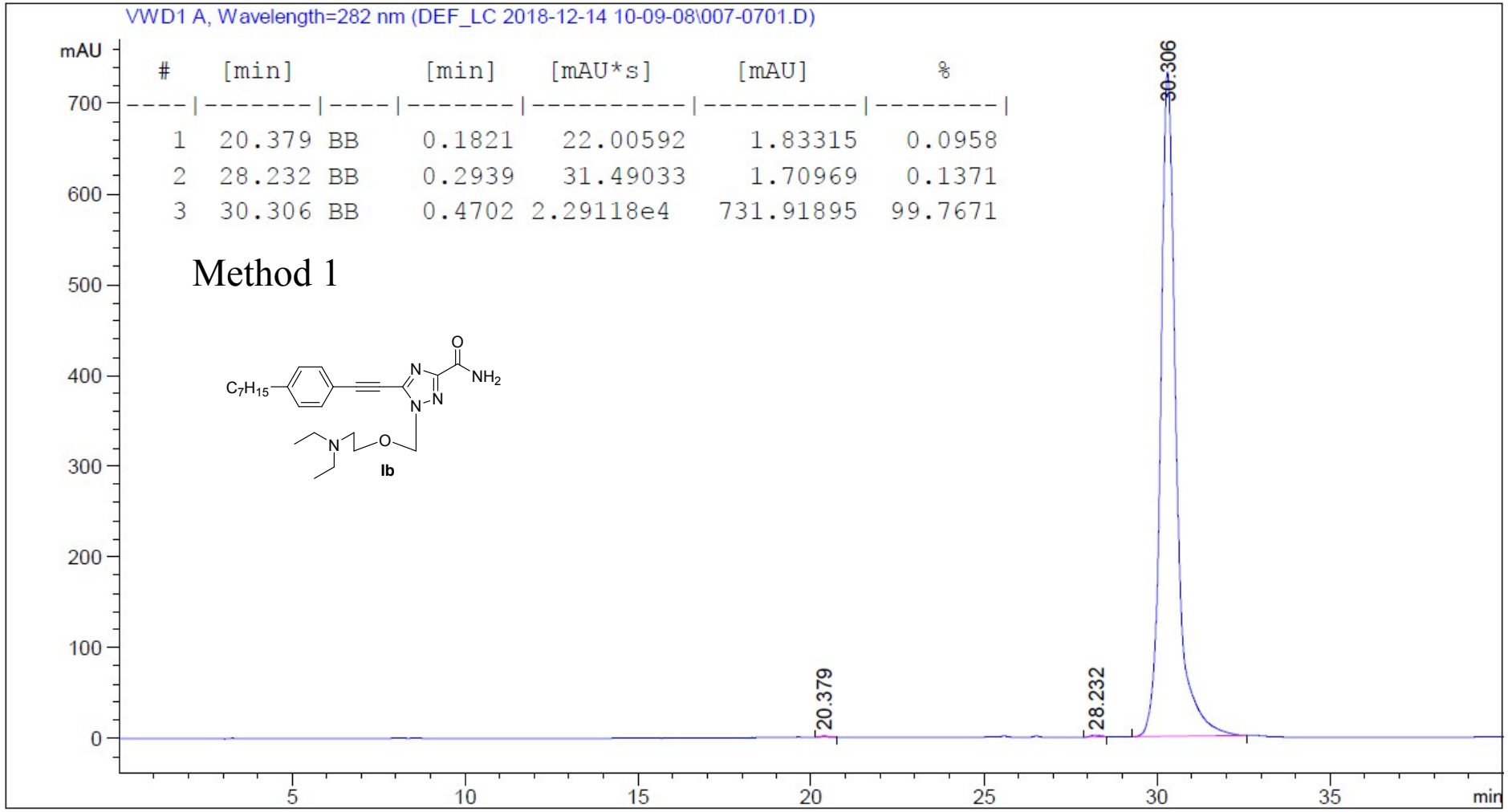
Ie

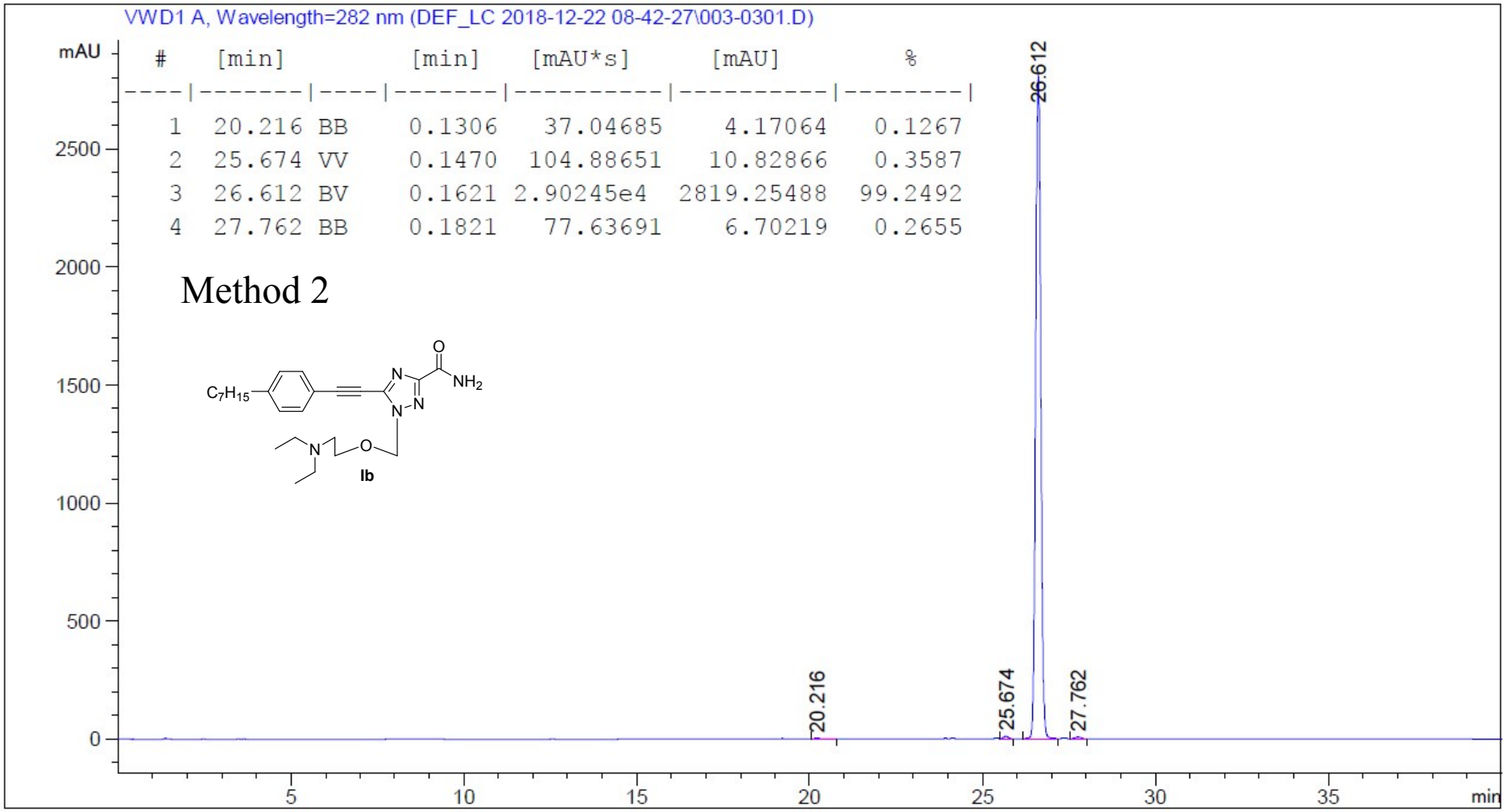

C7H15
O
NH2
N
N
N
N
N
O
N
If
3.690
3.676
3.662
3.384
2.656
2.637
2.618
2.507
2.450
2.436
2.422
2.378
2.324
2.305
2.296
2.277
2.268
2.261
2.234
2.093
8.055
7.750
7.637
7.617
7.352
7.332
5.707
1.613
1.594
1.577
1.561
1.273
1.246
1.232
0.868
0.853
0.835
-0.000
1.02
1.08
1.99
2.02
2.00
2.06
2.15
2.27
11.96
5.98
1.99
8.28
2.93
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

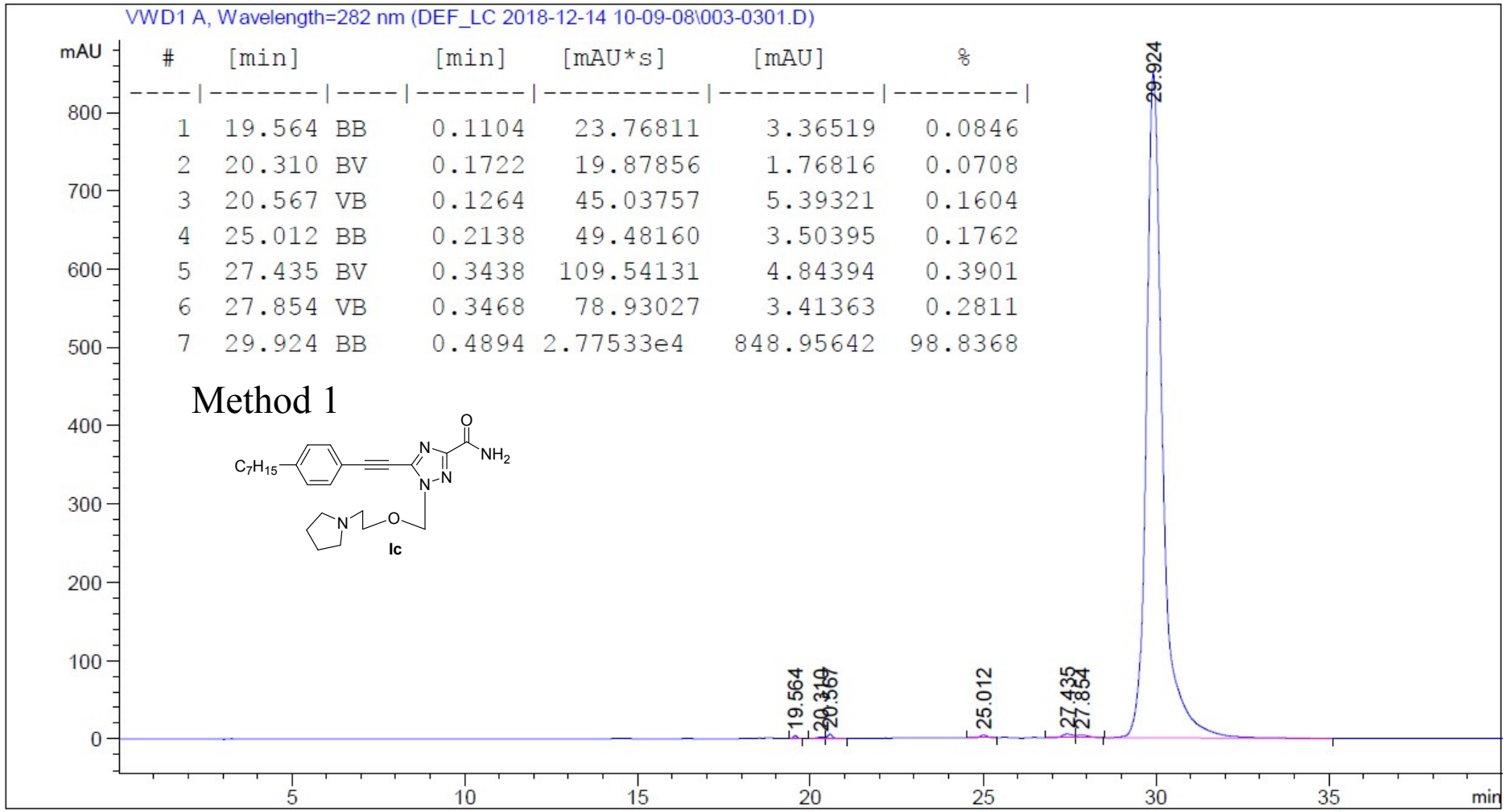

C7H15
O
NH2
N
N
N
N
N
O
N
If
45.981
40.526
40.317
40.108
39.900
39.691
39.482
39.273
35.547
31.662
31.070
28.977
28.933
22.516
160.173
157.173
146.195
140.470
132.467
129.452
117.005
97.774
78.432
74.995
67.693
57.168
57.015
56.362
53.468
14.395
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

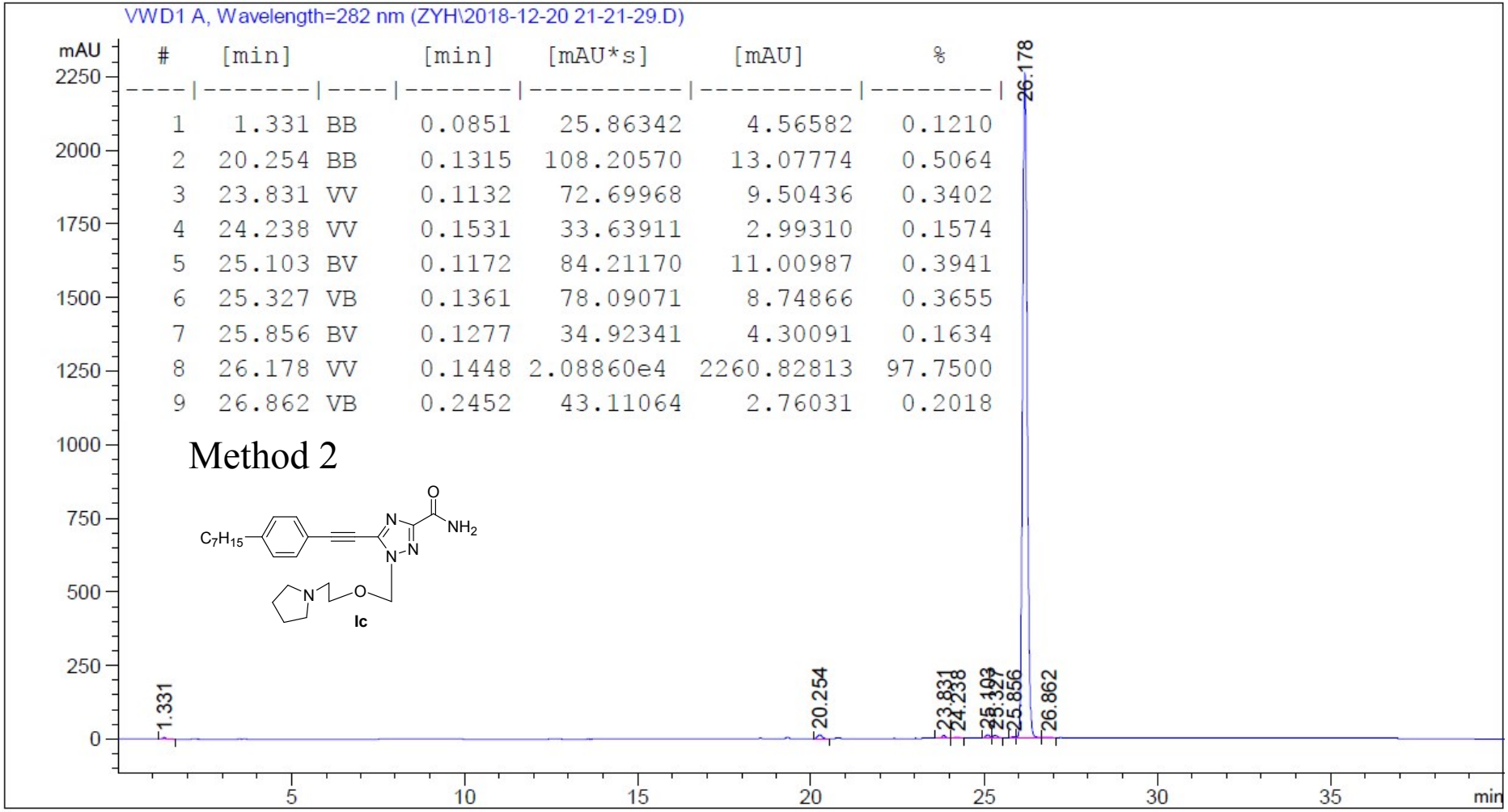

VWD1 A, Wavelength=282 nm (ZYH\2018-12-12 18-03-42.D)
mAU
[min] [min] [mAU*s] [mAU] %
1 19.532 BV 0.1091 61.79187 8.88809 0.1638
2 25.828 VV 0.2404 95.90890 6.10094 0.2542
3 27.223 VB 0.3232 3.75758e4 1745.40283 99.5821
Method 1
Ia
19.532
25.828
27.223
min


VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-22 08-42-27\014-1001.D)
mAU
2500
2000
1500
1000
500
0
[min] [min] [mAU*s] [mAU] %
1 19.161 BB 0.0906 40.11441 6.72241 0.1895
2 23.502 VB 0.0995 27.84568 4.30075 0.1315
3 24.490 BB 0.1292 102.67084 11.83738 0.4850
4 25.212 BV 0.1243 2.09991e4 2654.20874 99.1940
Method 2
Ia
19.161
23.502
24.490
25.212
5
10
15
20
25
30
35
min


VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-14 10-09-08\007-0701.D)
mAU
700
600
500
400
300
200
100
0
[min] [min] [mAU*s] [mAU] %
1 20.379 BB 0.1821 22.00592 1.83315 0.0958
2 28.232 BB 0.2939 31.49033 1.70969 0.1371
3 30.306 BB 0.4702 2.29118e4 731.91895 99.7671
Method 1
C7H15
N
N
N
O
NH2
N
O
Ib
20.379
28.232
30.306
5
10
15
20
25
30
35
min


VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-22 08-42-27\003-0301.D)
mAU
[min] [min] [mAU*s] [mAU] %
1 20.216 BB 0.1306 37.04685 4.17064 0.1267
2 25.674 VV 0.1470 104.88651 10.82866 0.3587
3 26.612 BV 0.1621 2.90245e4 2819.25488 99.2492
4 27.762 BB 0.1821 77.63691 6.70219 0.2655
Method 2
Ib
20.216
25.674
26.612
27.762
min

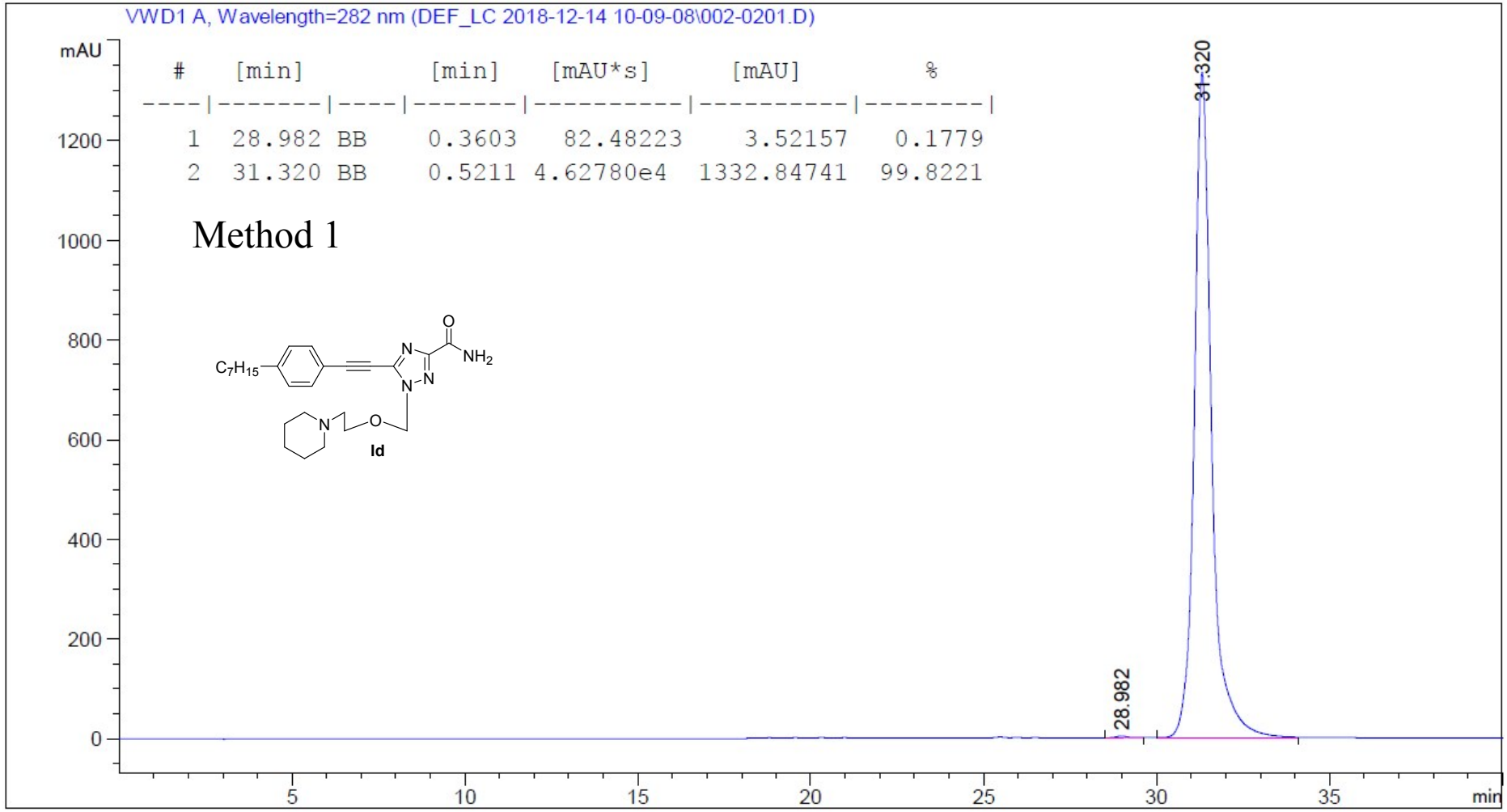

VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-14 10-09-08\003-0301.D)
mAU
[min] [min] [mAU*s] [mAU] %
1 19.564 BB 0.1104 23.76811 3.36519 0.0846
2 20.310 BV 0.1722 19.87856 1.76816 0.0708
3 20.567 VB 0.1264 45.03757 5.39321 0.1604
4 25.012 BB 0.2138 49.48160 3.50395 0.1762
5 27.435 BV 0.3438 109.54131 4.84394 0.3901
6 27.854 VB 0.3468 78.93027 3.41363 0.2811
7 29.924 BB 0.4894 2.77533e4 848.95642 98.8368
Method 1
Ic
19.564
20.310
20.567
25.012
27.435
27.854
29.924
min

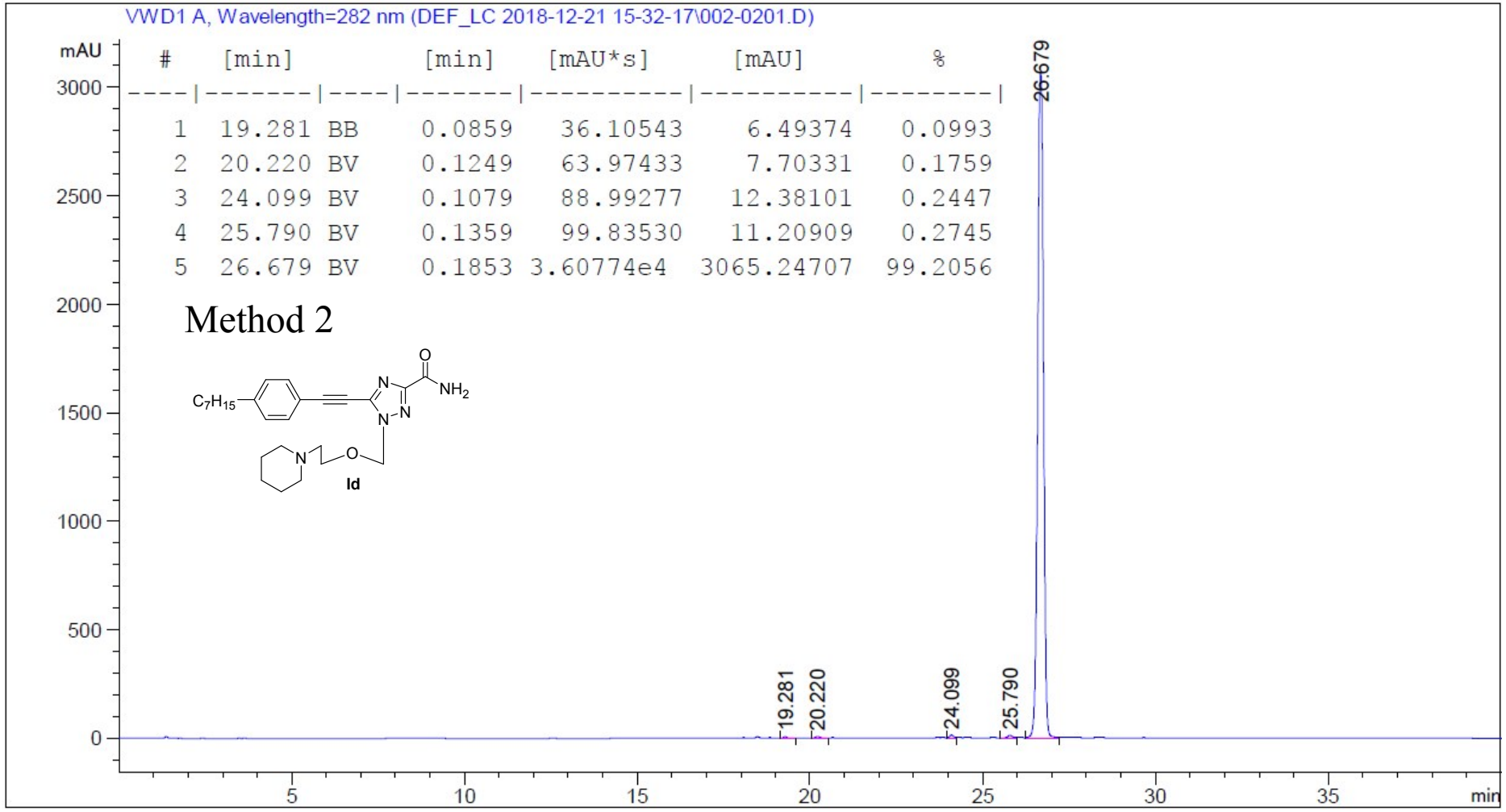


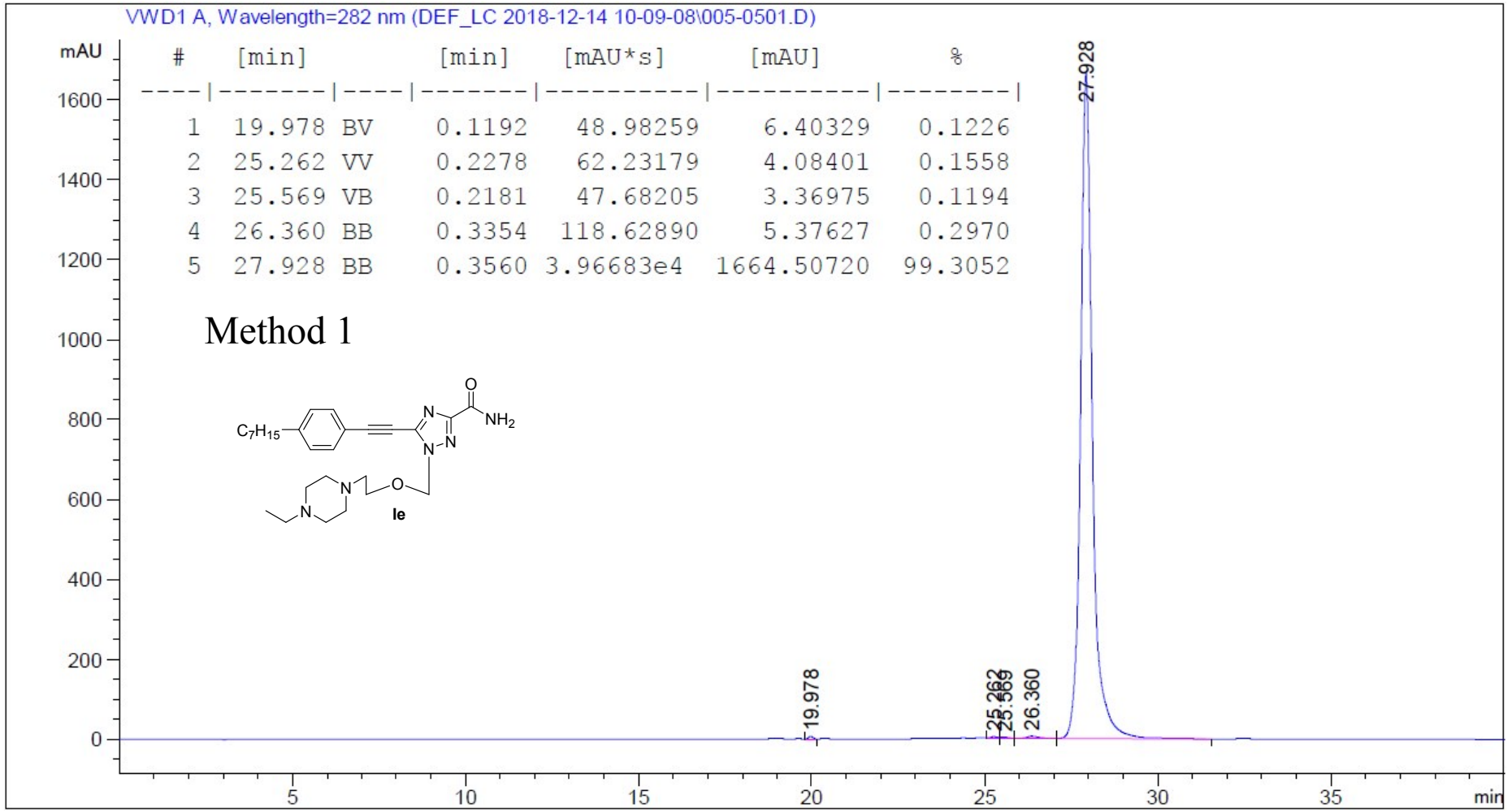
#	[min]		[min]	[mAU*s]	[mAU]	%
1	1.331	BB	0.0851	25.86342	4.56582	0.1210
2	20.254	BB	0.1315	108.20570	13.07774	0.5064
3	23.831	VV	0.1132	72.69968	9.50436	0.3402
4	24.238	VV	0.1531	33.63911	2.99310	0.1574
5	25.103	BV	0.1172	84.21170	11.00987	0.3941
6	25.327	VB	0.1361	78.09071	8.74866	0.3655
7	25.856	BV	0.1277	34.92341	4.30091	0.1634
8	26.178	VV	0.1448	2.08860e4	2260.82813	97.7500
9	26.862	VB	0.2452	43.11064	2.76031	0.2018

Method 2

Ic


VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-14 10-09-08\002-0201.D)
mAU
[min] [min] [mAU*s] [mAU] %
1 28.982 BB 0.3603 82.48223 3.52157 0.1779
2 31.320 BB 0.5211 4.62780e4 1332.84741 99.8221
Method 1
Id
31.320
28.982
min

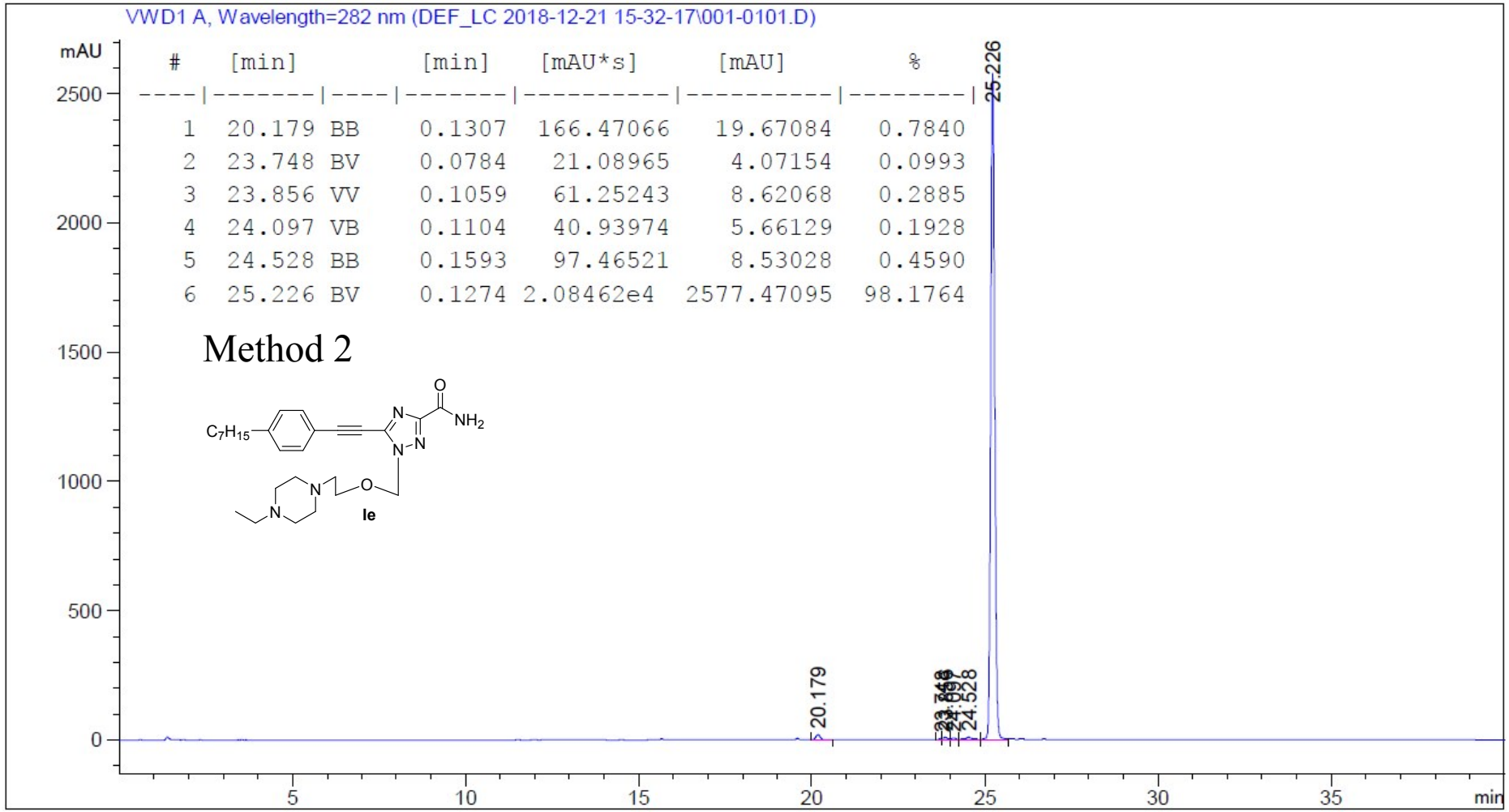

VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-21 15-32-17\002-0201.D)
mAU
3000
2500
2000
1500
1000
500
0
[min] [min] [mAU*s] [mAU] %
1 19.281 BB 0.0859 36.10543 6.49374 0.0993
2 20.220 BV 0.1249 63.97433 7.70331 0.1759
3 24.099 BV 0.1079 88.99277 12.38101 0.2447
4 25.790 BV 0.1359 99.83530 11.20909 0.2745
5 26.679 BV 0.1853 3.60774e4 3065.24707 99.2056
Method 2
C7H15
NH2
Id
19.281
20.220
24.099
25.790
26.679
5
10
15
20
25
30
35
min



#	[min]		[min]	[mAU*s]	[mAU]	%
1	19.978	BV	0.1192	48.98259	6.40329	0.1226
2	25.262	VV	0.2278	62.23179	4.08401	0.1558
3	25.569	VB	0.2181	47.68205	3.36975	0.1194
4	26.360	BB	0.3354	118.62890	5.37627	0.2970
5	27.928	BB	0.3560	3.96683e4	1664.50720	99.3052

Method 1

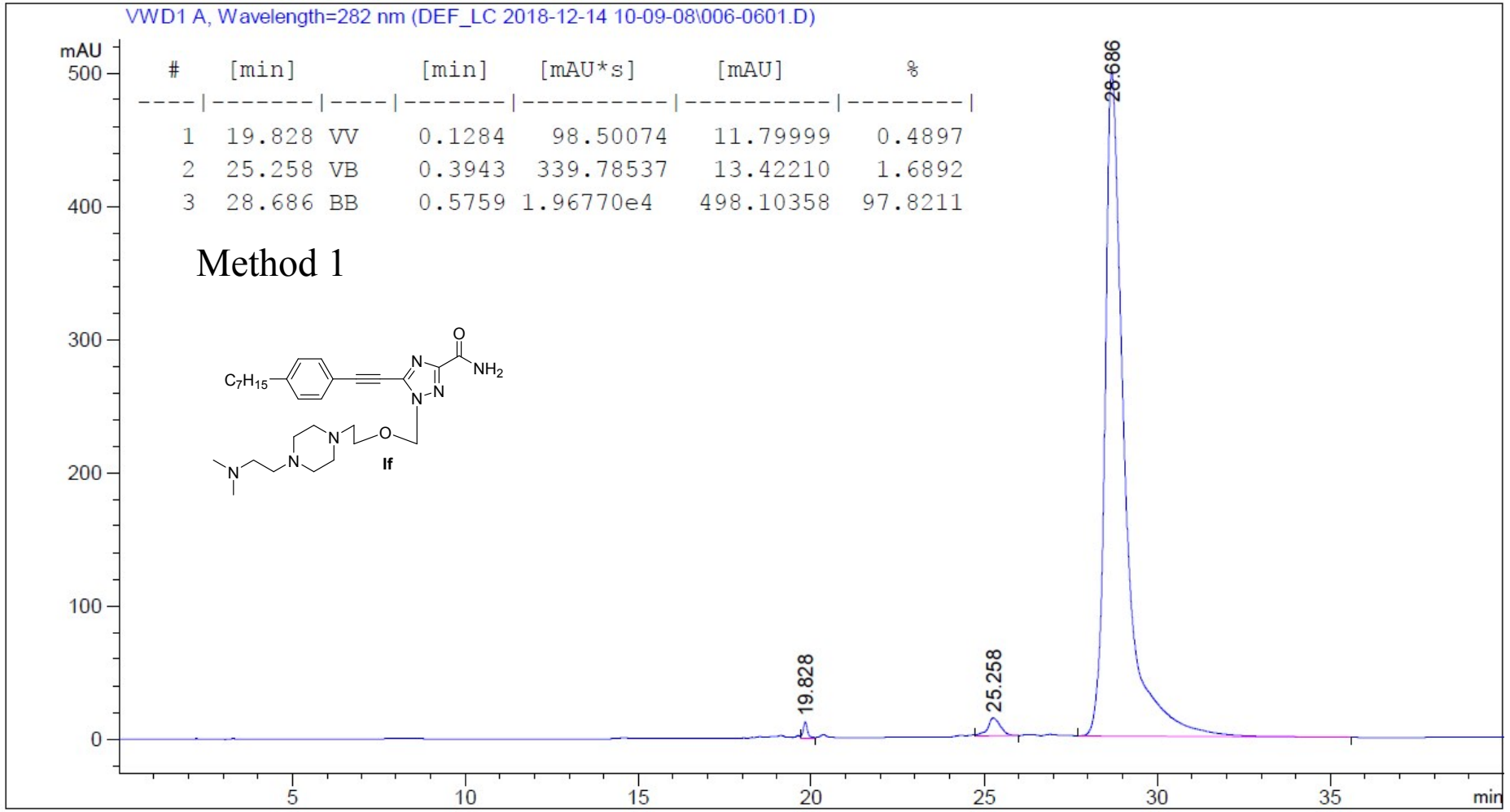
Ie

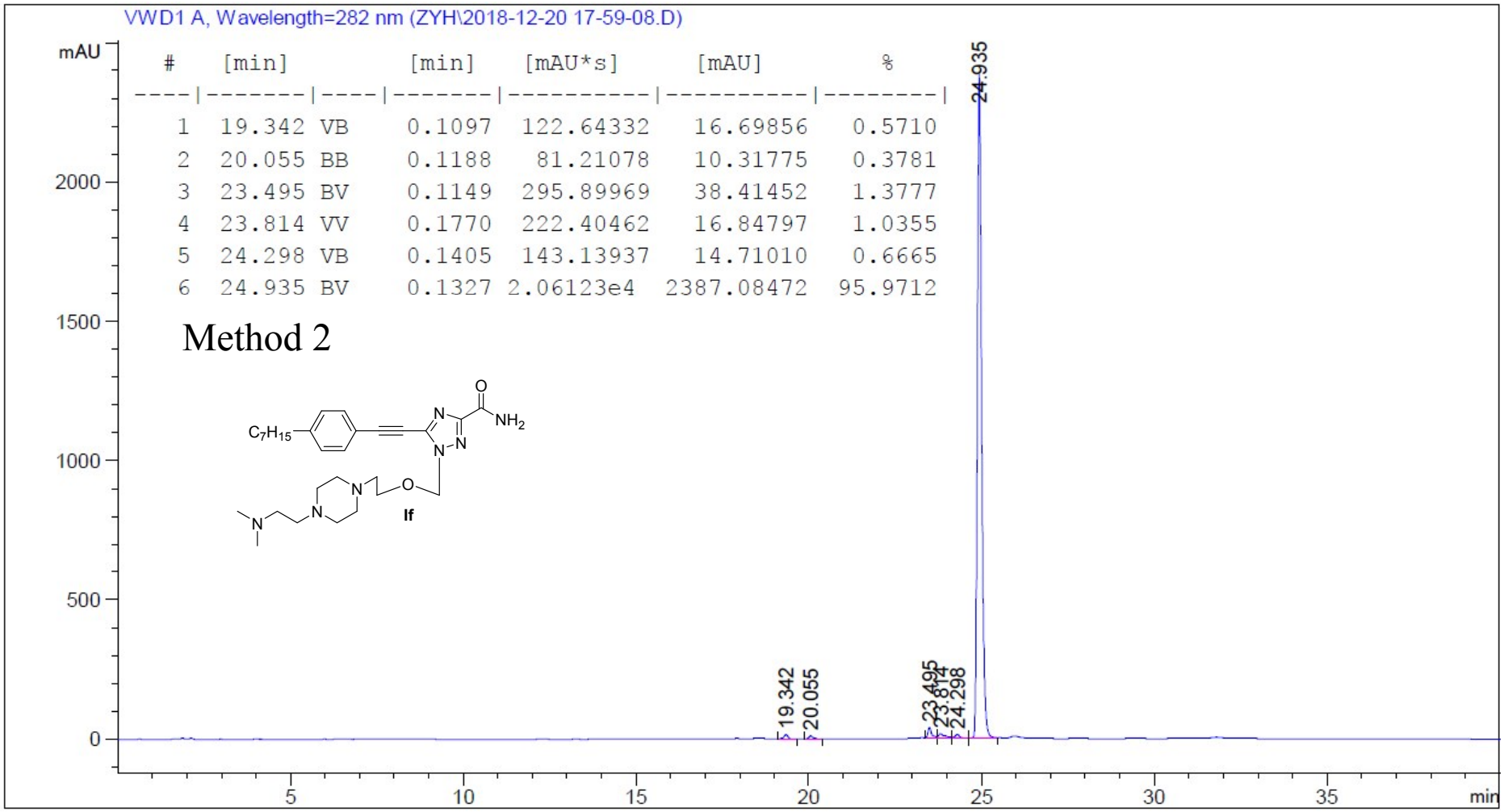


#	[min]		[min]	[mAU*s]	[mAU]	%
1	20.179	BB	0.1307	166.47066	19.67084	0.7840
2	23.748	BV	0.0784	21.08965	4.07154	0.0993
3	23.856	VV	0.1059	61.25243	8.62068	0.2885
4	24.097	VB	0.1104	40.93974	5.66129	0.1928
5	24.528	BB	0.1593	97.46521	8.53028	0.4590
6	25.226	BV	0.1274	2.08462e4	2577.47095	98.1764

Method 2

Ie


VWD1 A, Wavelength=282 nm (DEF_LC 2018-12-14 10-09-08\006-0601.D)
mAU
500
400
300
200
100
0
[min] [min] [mAU*s] [mAU] %
1 19.828 VV 0.1284 98.50074 11.79999 0.4897
2 25.258 VB 0.3943 339.78537 13.42210 1.6892
3 28.686 BB 0.5759 1.96770e4 498.10358 97.8211
Method 1
If
19.828
25.258
28.686
5
10
15
20
25
30
35
min


VWD1 A, Wavelength=282 nm (ZYH\2018-12-20 17-59-08.D)
mAU
[min] [min] [mAU*s] [mAU] %
1 19.342 VB 0.1097 122.64332 16.69856 0.5710
2 20.055 BB 0.1188 81.21078 10.31775 0.3781
3 23.495 BV 0.1149 295.89969 38.41452 1.3777
4 23.814 VV 0.1770 222.40462 16.84797 1.0355
5 24.298 VB 0.1405 143.13937 14.71010 0.6665
6 24.935 BV 0.1327 2.06123e4 2387.08472 95.9712
Method 2
If
19.342
20.055
23.495
23.814
24.298
24.935
2000
1500
1000
500
0
5
10
15
20
25
30
35
min