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Supporting Information

Solid-Phase Synthesis of DNA-Encoded Libraries Via An "Aldehyde Explosion" Strategy

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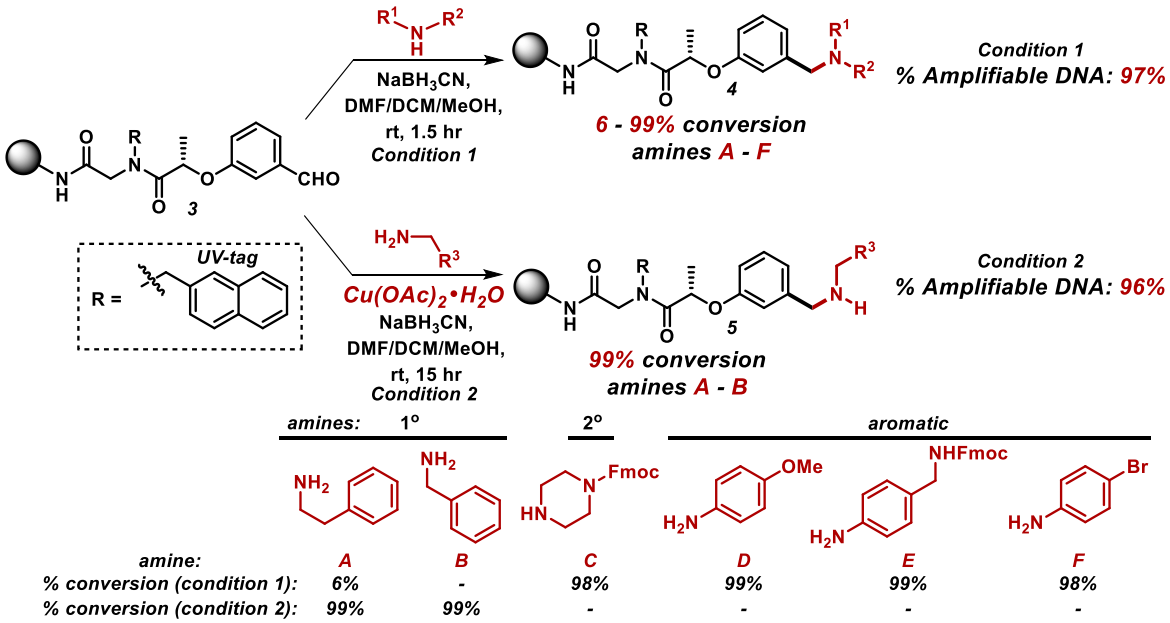
I. Supplementary figure 1: complementary DNA-compatible reductive aminations



Supplementary Figure 1. Complementary DNA-compatible reductive aminations. Percent conversions were determined using LC-MS by comparing peak area integrations of the product and the starting material of the crude solution after release of the compounds from the beads. Gray circles represent TentaGel resin. "-" indicates the reaction was not performed. Amount of amplifiable DNA was quantified via TaqMan qPCR by comparing the amount of amplifiable DNA tags from the reaction chemistry to resins that received no chemistry.

II. General information

All chemical (Sigma, Fisher Scientific) and biological (Integrated DNA Technologies, Thermo Fisher Scientific) reagents were purchased from commercial sources and were used without further purification. A Barnstead Nanopure filtration system was used for any steps that required the use of ultrapure deionized water (ddiH_2O). Analytical thin layer chromatography (TLC) was performed using 250 µm Silica Gel 60 F254 pre-coated plates (EMD Chemicals Inc.). Flash column chromatography was performed using 230-400 Mesh 60Å Silica Gel (Sorbent Technologies). NMR experiments were recorded on a Bruker AM 400 MHz spectrometer (400 MHz for ^{1}H NMR; 100 MHz for ^{13}C NMR). All spectra are presented using Bruker TopSpin 4.0.6 software. Spectra were obtained in the following solvents (reference peaks also included for ^{1}H and ^{13}C NMRs): $CDCl_3$ (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.23 ppm), and d_6-DMSO (^{1}H NMR: 2.50 ppm; ^{13}C NMR: 39.52 ppm). Chemical shift values (δ) are reported in parts per million (ppm) for all ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR multiplicities are reported as: s = singlet, d = doublet, q = quartet, br = broad, m = multiplet. High resolution mass spectra for all small molecules were collected on a Q Exactive HR mass spectrometer. Samples (~1 mg) were dissolved in a 1:1 water:acetonitrile with 0.1% formic acid solution and were analyzed at a flow rate of 5 µL/min. Ions were identified by electrospray ionization (ESI) scanning 50 – 300 m/z range. Optical rotation data were obtained on a Rudolph Research Analytical Autopol IV automatic polarimeter. LC-MS analysis was carried out on an Agilent 1200 Series equipped with a ZORBAX SB-C18 Rapid Res 4.6 x 100 mm x 3.5 µm column, PDA detector, and a 6120 quadrupole mass spectrometer using a 15 minute linear gradient of 95% water/5% acetonitrile/0.1% formic acid to 95% acetonitrile/5% water/0.1% formic acid at 1.0 mL/min flow rate. Real-time quantitative polymerase chain reaction (qPCR) was performed on an Applied Biosystems StepOnePlus Real-Time PCR System. Native-PAGE gels were imaged on a BIORAD Gel Doc XR+ Imaging System with Image Lab software.

Chemical abbreviations

- DBU: 1,8-diazabicyclo[5.4. 0]undec-7-ene
- DIAD: diisopropyl azodicarboxylate
- DIC: *N*,*N'*-diisopropylcarbodiimide
- DIPEA: *N*,*N*-diisopropylethylamine
- HOAt: 1-hydroxy-7-azabenzotriazole
- NMP: *N*-methyl-2-pyrrolidone
- PyAOP: (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

- TBTA: tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine
- TEAA: triethylammonium acetate
- TIPS: triisopropylsilane

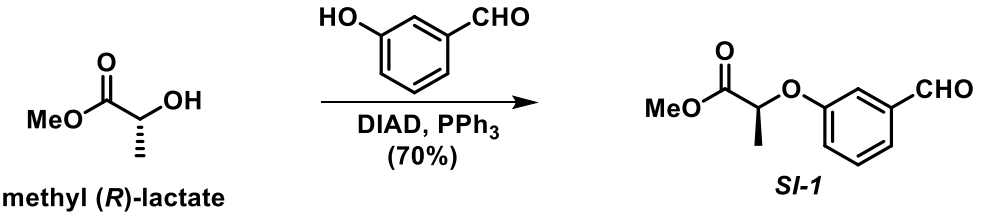
Buffers

- Bis-tris propane ligation buffer (BTPLB): 50 mM NaCl, 10 mM $MgCl_2$, 1 mM ATP, 0.02% Tween 20, 10 mM bis-tris, pH 7.6
- Bis-tris propane wash buffer (BTPWB): 50 mM NaCl, 0.04% Tween 20, 10 mM bis-tris, pH 7.6
- Bis-tris propane breaking buffer (BTPBB): 100 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 1% SDS, 1% Tween 20, 10 mM bis-tris, pH 7.6
- Click reaction buffer (CRB): 50% DMSO, 30 mM triethylammonium acetate, 0.04% Tween 20, pH 7.5
    - **Note:** CRB was made fresh prior to every N_3-hDNA click reaction
- qPCR master mix (10X MM): 100 mM Tris, 500 mM KCl, 15 mM $MgCl_2$, 2 mM dNTPs (N0447L)

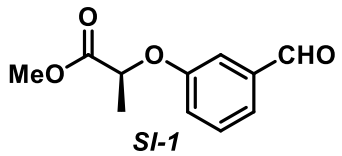
Oligonucleotide sequences

- MLM003: `5′-/5phos/GCCGCCCAGTCCTGCTCGCTTCGCTACATGGACAAAGAGCCGACGACGACTTCCCCGCGGTCTAAACCTCAA-3′`
- MLM004: `5′-/5phos/AGGCTTGAGGTTTAGACCGCGGGGAAGTCGTCGGCTCTTTGTCCATGTAGCGAAGCGAGCAGGACTGGGCGGCGG-3′`
- MLM005: `5′-/5phos/CACTGAGCATATCCCTGATACCG-3′`
- MLM006: `5′-/5phos/GCCTCGGTATCAGGGATATGCTCAGTG-3′`
- Full-length soluble DNA damage standard: `5'-/5phos/GCCGCCCAGTCCTGCTCGCTTCGCTACATGGACAAAGAGCCGACGACGACTTCCCCGCGGTCTAAACCTCAAGCCTCGGTATCAGGGATATGCTCAGTG-3'`
- MLM014: `5′-/5phos/CCTGCTCGCTTCGCTACATGGACAAAG-3′`
- ROX: `5′-6-carboxyl-X-rhodamine-[26-6430-XX]-3′`
- TaqMan FAM probe (BMP259): `5'-/56-FAM/CCGACGACG/ZEN/ACTTCCCCGCG/3IABkFQ/-3′`
- ABM063: `5′-/5phos/GCCGCCCAGTCCTGCTCGCTTCGCTAC-3′`
- ABM062: `/5AmMC6/GTGGCACAACAACTGGCGGGCAAAC-3′`
- ABM096: `5′-/5phos/CGCCAGGGTTTTCCCAGTCACGACCAACCACCCAAACCACAAACCCAAACCCCAAACCCAACACACAACAACAGCCGCCCAGTCCTGCTCGCTTC-3′`
- ABM099: `5′-/5phos/GTGGCAAACAACTG-3′`
- CES001: `5′-/5phos/GTTTTCCCAGTCACGAC-3′`

III. Synthesis procedures and characterization data



Procedure for the synthesis of SI-1 via Mitsunobu reactions: methyl (*R*)-lactate (9.1 g, 31.4 mmol, 1.0 equiv.), triphenylphosphine (9.1 g, 34.7 mmol, 1.11 equiv.) and 3-hydroxybenzaldehyde (5.4 g, 34.4 mmol, 1.10 equiv.) were added to a round bottom flask, dissolved in anhydrous tetrahydrofuran (180 mL) and cooled to 0 °C. Diisopropylazodicarboxylate (6.8 mL, 34.5 mmol, 1.10 equiv.) was dissolved in tetrahydrofuran (30 mL) and added dropwise via addition funnel at 0 °C. The cold bath was removed and the reaction was stirred at room temperature for 30 minutes. The reaction was heated to 80 °C and stirred for 13 hours (overnight). The reaction was cooled to room temperature and the solvent was removed *in vacuo*. The residue was then added a solution of hexanes:ethyl acetate (4:1, 50 mL) and the mixture was filtered and washed with additional hexanes:ethyl acetate (4:1, 50 mL). The filtrate was concentrated and the crude material was purified via silica gel column chromatography using hexanes:ethyl acetate (4:1) to afford **SI-1** (4.6 g, 70%) as a colorless liquid.



SI-1

SI-1, methyl (*S*)-2-(3-formylphenoxy)propanoate, yield: 70%, colorless liquid.

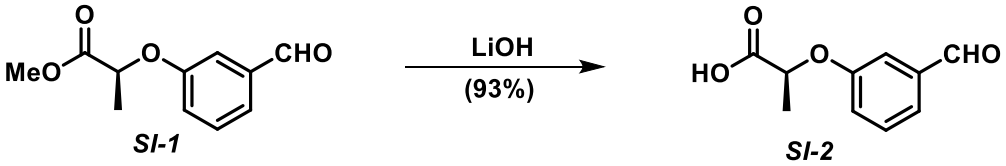
^{1}H NMR: (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.51 – 7.41 (m, 2H), 7.32 (dd, *J* = 2.7, 1.3 Hz, 1H), 7.17 (ddd, *J* = 7.8, 2.8, 1.3 Hz, 1H), 4.85 (q, *J* = 6.8 Hz, 1H), 3.76 (s, 3H), 1.65 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ 192.0, 172.3, 158.3, 138.0, 130.5, 124.3, 122.4, 113.9, 72.8, 52.7, 18.7.

HRMS (ESI): calc. $C_{11}H_{13}O_4$ for $[M+H]^+$: 209.0808, found: 209.0804.

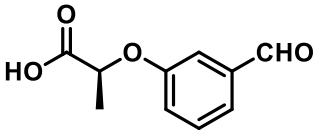
$[\alpha]_D^{23}$: +72.83 (c = 1.06, EtOH)

Note: SI-1 is a known compound (CAS: 289054-20-2) and matches previously published ^{1}H and ^{13}C NMR data.[1]



SI-1 ***SI-2***

Procedure for the synthesis of acid SI-2 via basic hydrolysis: SI-1 (4.2 g, 17.3 mmol, 1.0 equiv.) was added to a round bottom flask, dissolved in tetrahydrofuran (120 mL) and cooled to 0 °C. A solution of lithium hydroxide (0.6 g, 26.0 mmol, 1.50 equiv.) dissolved in water (40 mL) was added to the reaction dropwise via addition funnel at 0 °C. The cold bath was removed, and the solution was stirred at room temperature for 30 minutes. The solvent was concentrated *in vacuo* and the aqueous phase was extracted with ethyl acetate (2 x 30 mL). The aqueous phase was cooled to 0 °C and acidified to pH = 2 via concentrated HCl. The aqueous phase was warmed to room temperature and extracted with ethyl acetate (3 x 50 mL). The organics were collected, dried with sodium sulfate, filtered and concentrated to afford pure **SI-2** (3.8 g, 93%) as a pale-yellow solid.



SI-2

SI-2, (*S*)-2-(3-formylphenoxy)propanoic acid, yield: 93%, pale-yellow solid.

^{1}H NMR: (400 MHz, d_6-DMSO) δ 13.12 (br. s, 1H), 9.96 (s, 1H), 7.55 – 7.50 (m, 2H), 7.33 (m, 1H), 7.24 (m, 1H), 4.96 (q, *J* = 6.8 Hz, 1H), 1.53 (d, *J* = 6.8 Hz, 3H).

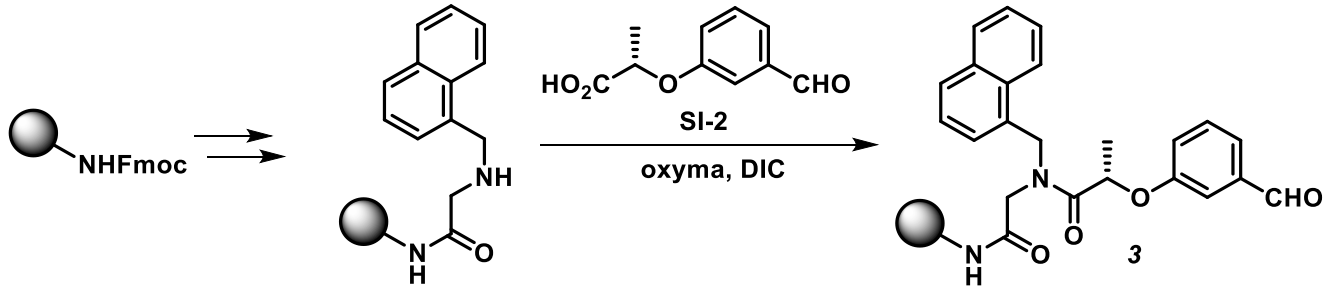
^{13}C NMR: (100 MHz, d_6-DMSO) δ 192.9, 172.8, 158.0, 137.6, 130.5, 123.1, 121.7, 113.7, 71.7, 18.2.

HRMS (ESI): calc. $C_{10}H_{11}O_4$ for $[M+H]^+$: 195.0652, found: 195.0647.

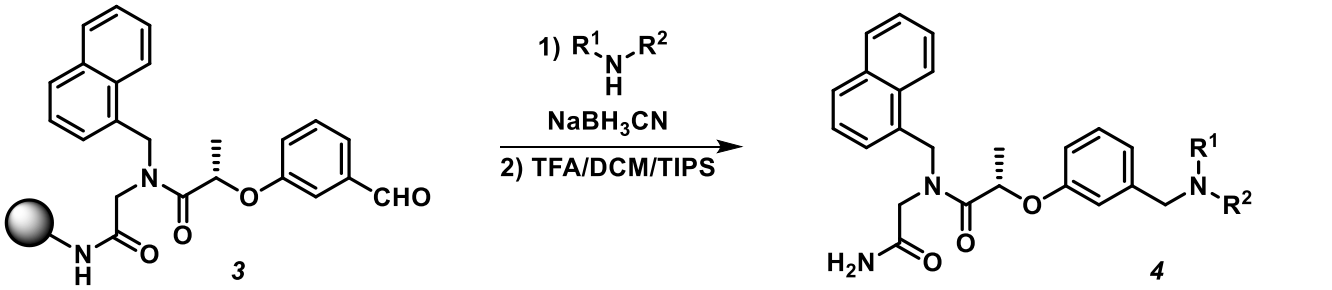
$[\alpha]_D^{23}$: +53.80 (c = 1.08, EtOH)

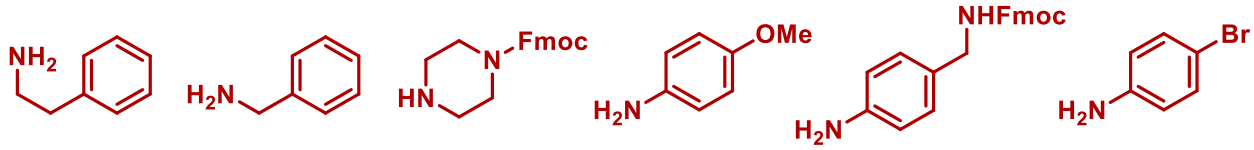
Note: SI-2 is a known compound (CAS: 1704942-49-3); however, no sources detailing the synthesis or characterization were found.

IV. Solid-phase DNA-compatible synthesis procedures



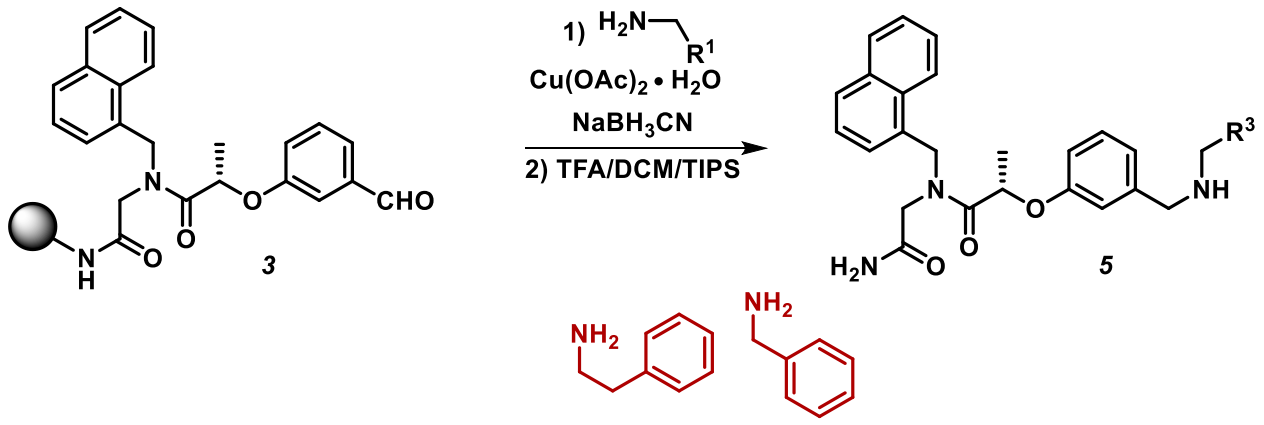
General procedure for the preparation of immobilized aldehydes: TentaGel HL RAM resin (160 µm, 0.41 mmol/g) or TentaGel R RAM resin was used for synthesis. TentaGel HL RAM resin (300 mg, 160 µm, 0.41 mmol/g, 0.123 mmol) was added to a filtered syringe and swelled in DMF (2 mL) at room temperature for 1 hr. The solvent was drained and 2 mL of a 20% piperidine solution in DMF was added and stirred for 2 x 30 minutes at room temperature. The solution was drained, and the resin was washed with DMF 3 x 1.5 mL. The resin was then added 1 mL of a 2 M bromoacetic acid (BAA) solution in DMF, followed by 1 mL of a 50% DIC/DMF solution and the reaction was stirred for 10 minutes at 37 °C. The BAA/DIC solution was drained and the resin was washed with DMF 5 x 1.5 mL. A 1 M solution of 1-naphthylmethylamine, which served as a UV tag during LC-MS analysis, in DMF was added and the reaction was stirred for 30 minutes at 37 °C. The solution was drained, and the resin was washed with DMF 3 x 1.5 mL. The resin was added a pre-mixed solution of the **SI-2** (0.369 mmol, 3 equiv.), oxyma (52.4 mg, 0.369 mmol, 3 equiv.), collidine (48.6 µL, 0.369 mmol, 3 equiv.) and DIC (86.3 µL, 0.554 mmol, 4.5 equiv.) in 2 mL of DMF and stirred at 37 °C for 1 hr. The solution was drained and the resin was washed with DMF 3 x 1.5 mL to afford immobilized aldehyde ***3***. **Note:** this acylation was done on a large scale without DNA conjugation.



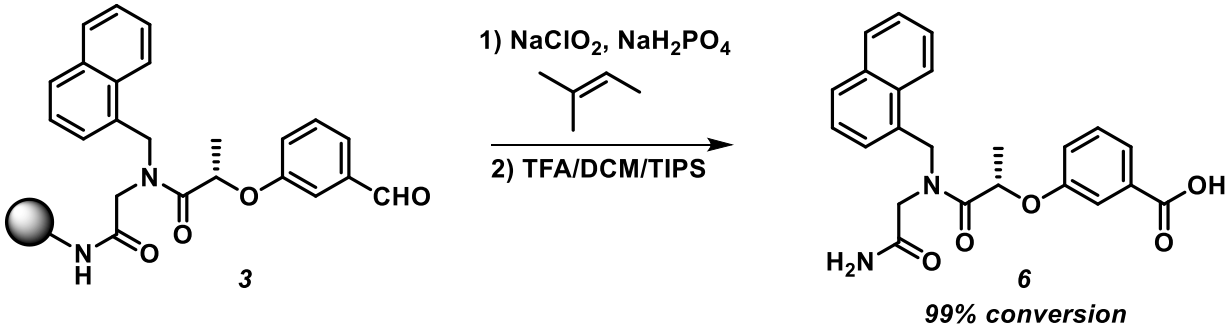


amine:	***A***	***B***	***C***	***D***	***E***	***F***
% conversion:	***6%***	***-***	***98%***	***99%***	***99%***	***98%***

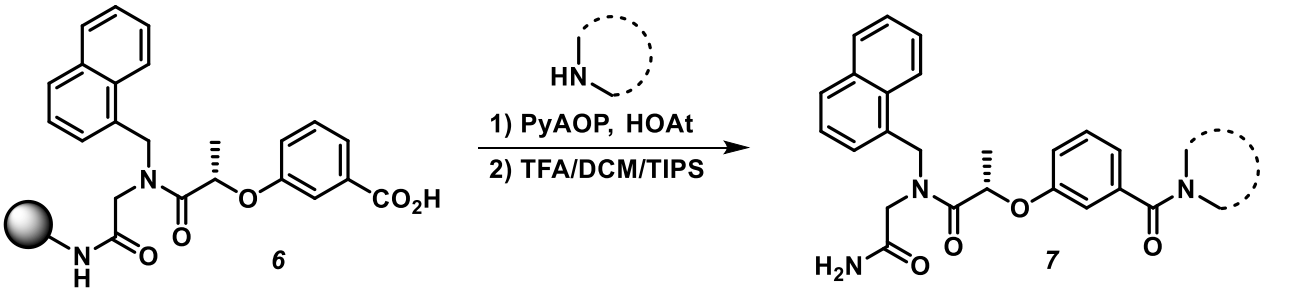
General procedure for condition 1 reductive amination of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with DMF, then added 75 µL of a 1 M amine solution (A, C – F) in DMF containing 1% AcOH. The reaction was stirred at room temperature for 30 minutes and 75 µL of a 2 M $NaBH_3CN$ solution in DCM:MeOH (1:1) with 1% AcOH was added and the reaction was stirred for an additional 1.5 hours. The solution was drained, and the resin was washed with DMF, MeOH, then DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, reductive amination with amines A – F proceeded with 6 – 99% conversions to products ***4***.

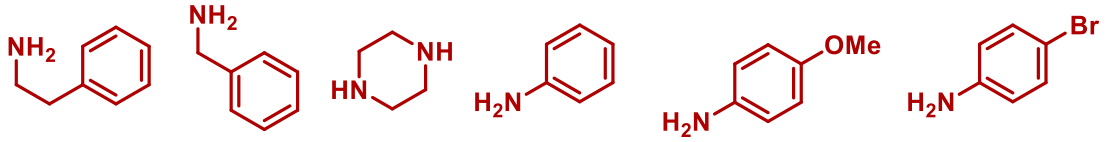


General procedure for condition 2 reductive amination of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, washed with DMF, and the resin was added 75 µL of a solution containing 1 M amine (A or B) and 50 mM $Cu(OAc)_2 \cdot H_2O$ in DMF with 2% AcOH. The reaction was stirred at room temperature for 30 minutes and 75 µL of a 2 M $NaBH_3CN$ solution in DCM:MeOH (1:1) with 2% AcOH was added and the reaction was stirred at room temperature for an additional 15 hours. The solution was drained, and the resin was washed with DMF, MeOH, then DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, reductive amination with amines A – B proceeded with 99% conversions to products ***5***.



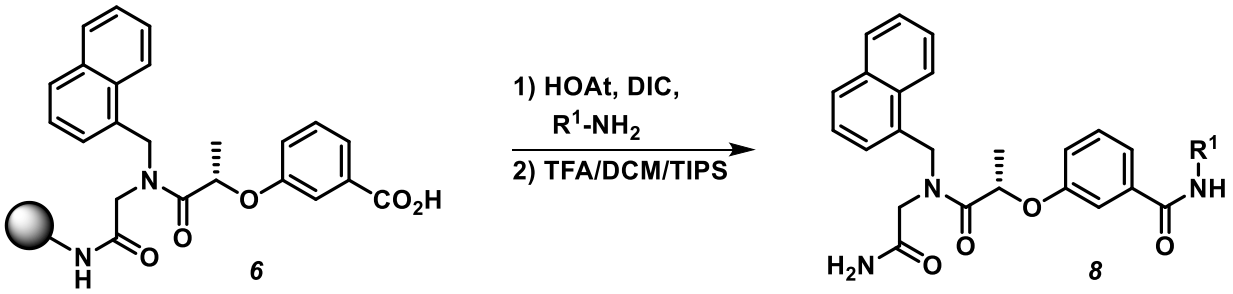
Procedure for Pinnick oxidation of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with THF, and then added 100 µL *t*BuOH, 10 µL of a 1 M NaH_2PO_4 solution in H_2O, 30 µL of a 1 M solution of 2-methyl-2-butene in THF and 10 µL of a 1 M $NaClO_2$ solution in H_2O and the reaction was stirred at room temperature for 1 hour. The solution was drained, and the resin was washed with THF, H_2O, DMF then DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, Pinnick oxidation proceeded with 99% conversion to product ***6***.

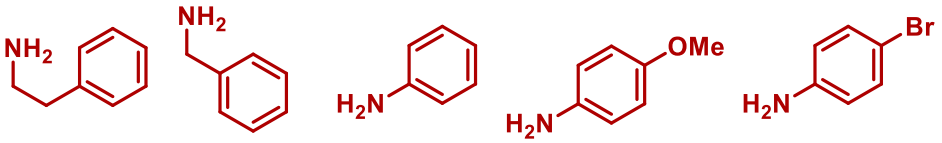




amine:	A	B	C	D	E	F
% conversion:	33%	42%	92%	49%	55%	13%

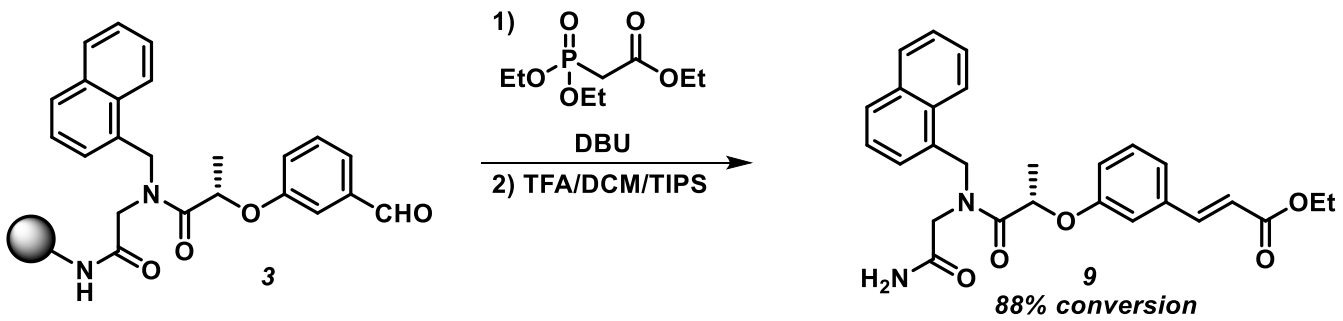
General procedure for condition 1 amidation of immobilized acids: A 96-well filter plate was added 100 µL DCM, followed by the immobilized acid ***6*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with DMF, then added 75 µL of a solution containing 90 mM PyAOP/180 mM HOAt/270 mM DIPEA in DMF, followed by 75 µL of a 500 mM solution of the corresponding amine (A – F) in DMF and the reaction was stirred at 37 °C for three hours. The solution was drained and the resin was washed with DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, amidation with amines A – F proceeded with 13 – 92% conversion to products ***7***.



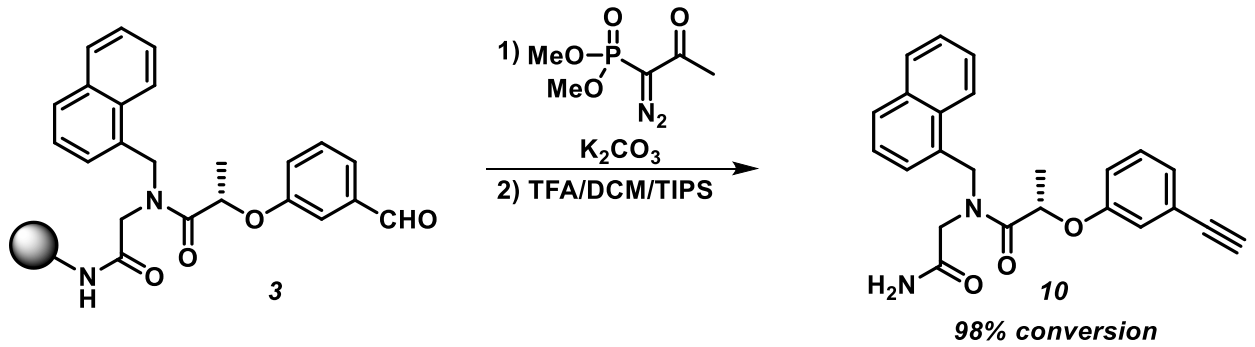


amine:	A	B	D	E	F
% conversion:	95%	99%	98%	97%	98%

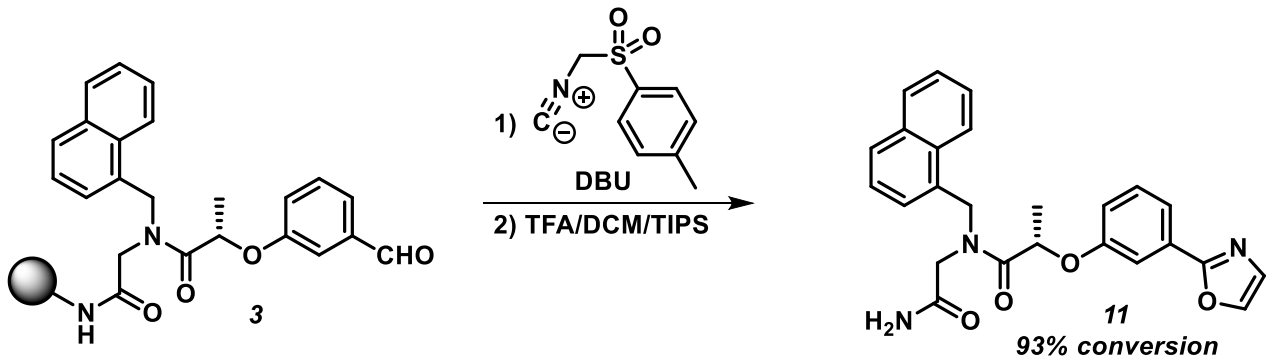
General procedure for condition 2 amidation of immobilized acids: A 96-well filter plate was added 100 µL DCM, followed by the immobilized acid ***6*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with DMF, then added 75 µL of a solution containing 200 mM HOAt/300 mM DIC/200 mM DIPEA in DMF, followed by 75 µL of a 200 mM solution of the requisite amine in DMF and the reaction was stirred at 37 °C for three hours.[3] The solution was drained and the resin was washed with DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, amidation with amines A, B and D – F proceeded with 95 – 99% conversion to products ***8***.



Procedure for the Horner-Wadsworth-Emmons olefination of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with NMP, then added 75 µL of a solution containing 200 mM triethylphosphonoacetate in NMP, followed by 75 µL of a 200 mM solution of DBU in NMP and the reaction was stirred at 37 °C for 16 hours. The solution was drained and the resin was washed with NMP, DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, Horner-Wadsworth-Emmons olefination proceeded with 88% conversion to product ***9***.

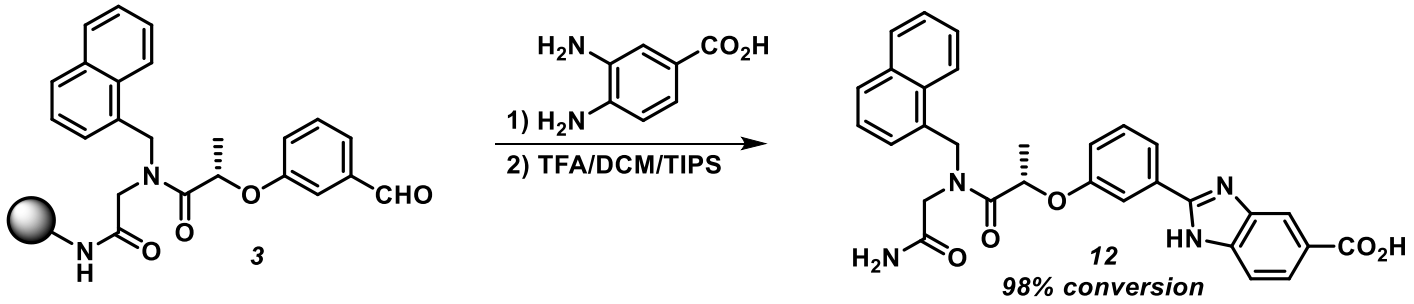


Procedure for the Seyferth-Gilbert homologation of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with MeOH, then added 150 µL of a solution containing 80 mM dimethyl-(1-diazo-2-oxopropyl)phosphonate (Ohira-Bestmann reagent) and 125 mM potassium carbonate in MeOH and the reaction was stirred at room temperature for one hour. The solution was drained, and the resin was washed with MeOH, DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, Seyferth-Gilbert homologation proceeded with 98% conversion to product ***10***.

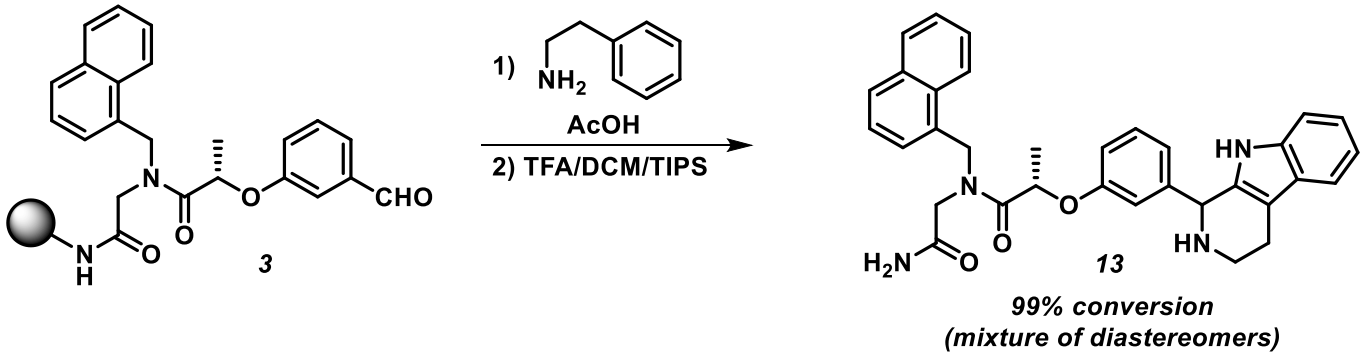


Procedure for the van Leusen cyclization of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with NMP, then added 75 µL of a solution containing 100 mM toluenesulfonylmethyl isocyanide (TosMIC) in NMP and 75 µL of a 200 mM DBU solution and the reaction was stirred at 37 °C for 15 hours. The solution was drained, and the resin

was washed with NMP, DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, van Leusen oxazole synthesis proceeded in 93% conversion to product ***11***.

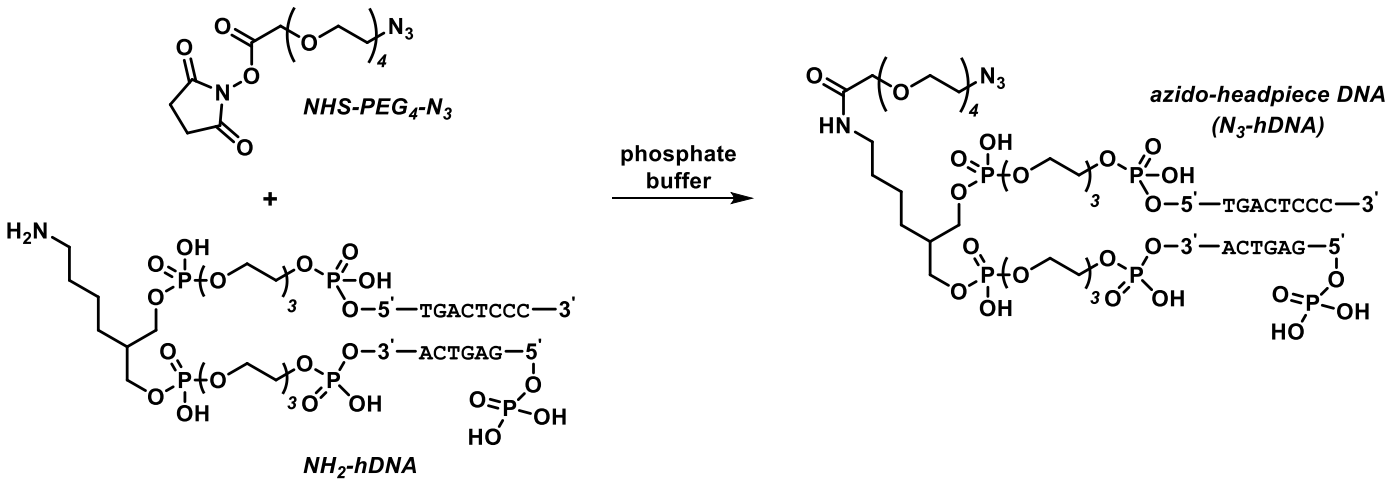


Procedure for benzimidazole synthesis from immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, and the resin was added 150 µL of a solution containing 200 mM 3,4-diaminobenzoic acid in NMP and the reaction was stirred at 37 °C for 15 hours. The solution was drained, and the resin was washed with NMP, DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, benzimidazole synthesis proceeded in 98% conversion to product ***12***.

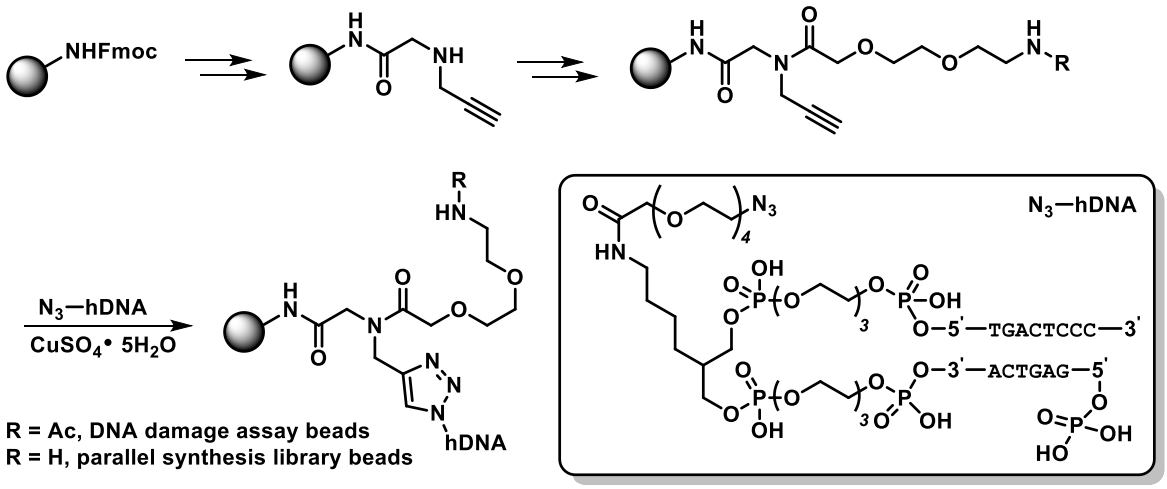


Procedure for the Pictet-Spengler cyclization of immobilized aldehydes: A 96-well filter plate was added 100 µL DCM, followed by the immobilized aldehyde ***3*** (4 mg, 0.41 mmol/g, 0.0016 mmol). The solvent was drained, the resin was washed with NMP, then added 150 µL of a solution containing 0.5 M tryptamine in NMP (containing 2% AcOH) and the reaction was stirred at 37 °C for two hours. The solution was drained, and the resin was washed with NMP, DMF and DCM. The resin was transferred to a 96-well round bottom plate via DCM and the solvent was evaporated. A solution of TFA/DCM/TIPS 49/49/2 (150 µL/well) was added to the resin and stirred at room temperature for 45 minutes. The solvent was evaporated, and the resultant crude residue was utilized for LC-MS. Following LC-MS analysis, Pictet-Spengler cyclization with tryptamine proceeded with 99% conversion to product ***13***.

V. Procedures for DNA-conjugated resin synthesis



Procedure for the large-scale synthesis of azido-headpiece DNA (N_3-hDNA): NHS-PEG_4-N_3 (200 mg, 0.5342 mmol) was dissolved in 860 µL DMF (0.6 M), and the amino-headpiece DNA (NH_2-hDNA) was dissolved in ddiH_2O to afford a 2.8 mM stock solution. A 15 mL conical tube was sequentially added 720 µL of a 1 M K_2HPO_4 buffer (pH = 8, final concentration of 0.4 M), 685 µL ddiH_2O, 328 µL of 2.8 mM NH_2-hDNA (0.9184 µmol) and 66 µL of 0.6 M solution of NHS-PEG_4-N_3 (39.6 µmol, 43 equiv.). The tube was vortexed and tumbled at room temperature for one hour. The tube was centrifuged and added another 43 equivalents of 0.6 M NHS-PEG_4-N_3 and tumbled at room temperature for an additional hour. The reaction was quenched by addition of 100 µL of a 1 M solution of Tris (pH = 7.6) and the reaction was incubated at 60 °C for five minutes. The reaction was acidified by addition of a 3 M solution of sodium acetate (pH = 5, 900 µL), followed by 9.0 mL of EtOH. The reaction was cooled to -20 °C for one hour to induce DNA precipitation. The tube was then centrifuged, and the supernatant was decanted. The pellet was re-dissolved in 1.0 mL ddiH_2O, added 9.0 mL EtOH and chilled at -20 °C overnight to fully precipitate the DNA out of solution. The tube was centrifuged, and the supernatant was decanted again. The resulting pellet was dried under a stream of argon, frozen in liquid nitrogen and lyophilized to afford a colorless solid. The concentration of the azido-headpiece DNA (N_3-hDNA) was measured via NanoDrop.



Procedure for the synthesis of 0.8% hDNA-loaded TentaGel resin: TentaGel M NH_2 resin (50 mg, 0.23 mmol/g, 10 µm, 0.012 mmol) was added to an Eppendorf tube and swelled in DMF (1 mL) at room temperature for 1 hr. The resin was centrifuged, and the solvent was decanted. Then, 1 mL of a 20% piperidine in DMF solution was added and stirred at room temperature for 2 x 30 minutes. The resin was then added 1 mL of a 2 M bromoacetic acid (BAA) solution in DMF, followed by 1 mL of a 50% DIC/DMF solution and the reaction was stirred for 10 minutes at 37 °C.

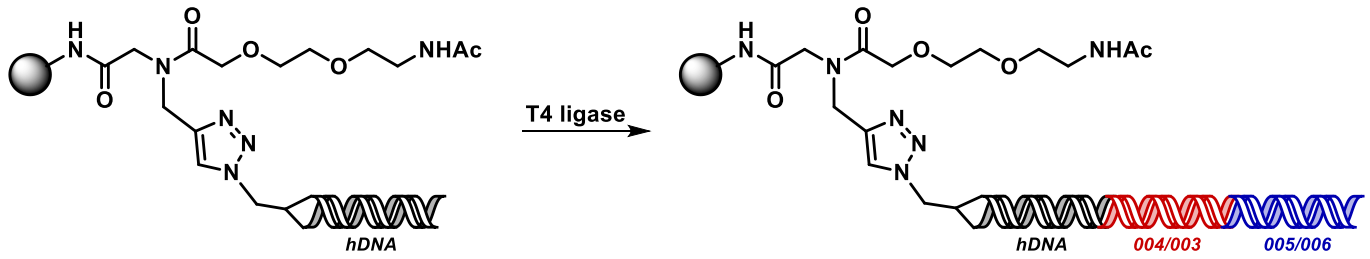
The BAA/DIC solution was decanted and the resin was washed with DMF 5 x 1.5 mL. A 1 M solution of propargylamine in DMF was added and the reaction was stirred for 30 minutes at 37 °C. Immobilized propargylamine resin was added a pre-mixed solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (142.2 mg, 0.369 mmol, 3 equiv.), oxyma (52.4 mg, 0.369 mmol, 3 equiv.), collidine (48.6 µL, 0.369 mmol, 3 equiv.) and DIC (86.3 µL, 0.554 mmol, 4.5 equiv.) in 1 mL of DMF and

stirred at 37 °C for 1 hr. The solution was drained, and the resin was washed three times with DMF (1 mL x 3). The resin was then added 1 mL of a 20% piperidine in DMF solution and stirred at room temperature for 2 x 30 minutes. For beads to be used in the DNA damage assay, the terminal nitrogen was acylated by addition of a solution of 20% Ac_2O and 10% DIPEA in DMF and stirred at room temperature for 3 hours.

DNA-conjugated resin was synthesized based on a previously published procedure.[2] The resin-bound alkyne handle was subjected to a copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC) with azido-headpiece DNA (N_3-hDNA). The resin was split into 5 mg portions (1.20 µmol) and added to Eppendorf tubes and equilibrated in CRB for 1 hour. The beads were centrifuged, the CRB was decanted and the beads were added 166 µL of fresh CRB, followed by 21 µL of copper sulfate solution (**Supplementary Table 1**) and the reaction was stirred at 37 °C for five minutes. Next, 32 µL of N_3-hDNA solution was added and the reaction was stirred at 37 °C for four hours. The resin was centrifuged, drained of the copper/N_3-hDNA solution, washed three times with BTPBB and stirred overnight in BTPBB at room temperature. The beads were drained and washed with BTPWB and stored at 4 °C.

		Amount (µmol)	Equivalents	Volume (µL)	Stock concentration (mM)
Copper solution	$CuSO_4$•$5H_2O$	1.3225	1.1	2.6	500
	TBTA	0.0023	0.2%	0.5	5
	Sodium ascorbate	6.6125	5.5	4.4	1500
	DMSO	-	-	13.3	-
	Total	-	-	21	-
N_3-hDNA solution	TEAA	6.4	5.3	6.4	1000
	DMSO	-	-	12.6	-
	N_3-hDNA	0.0092	0.8%	12.9	0.715
	Sodium ascorbate	0.064	5.3%	0.04	1500
	Total	-	-	32	-

Supplementary Table 1. Calculations for the copper and N_3-hDNA solutions for the subsequent click reaction.



Procedure for the installation of the full-length DNA damage oligonucleotide sequence (headpiece-004/003-005/006) to resin: hDNA-conjugated beads were then ligated on a full-length DNA damage tag for use in DNA damage assays. An aliquot of 0.8%-conjugated DNA-beads was removed (0.4 mg, 45.6 µL, 0.736 nmol) and added to a well in a filter plate (performed in triplicate) and a separate aliquot was removed (0.04 mg, 4.56 µL, 0.0736 nmol) and added to a negative control well (negative control was omitted the 005/006 oligonucleotides). The solution was drained, and resin was equilibrated and washed with BTPLB. The BTPLB was drained and a T4 ligase solution (**Supplementary Table 2**, 1.02 equivalents of oligos relative to [DNA]) was added to each well and the reaction was mixed at room temperature overnight. **Note**: Each T4 ligase solution (positive and negative control) was made by first mixing all components in an Eppendorf tube and stirring at room temperature for 20 minutes. Then, a second aliquot of T4 ligase solution was introduced to each tube and the whole mixture was added to the hDNA-conjugated resin.

	Positive control beads			Negative control beads		
	Volume (µL)	Stock concentration	[oligo]/well (nmol)	Volume (µL)	Stock concentration	[oligo]/well (nmol)
BTPLB	15	10X	-	15	10X	-
MLM004/003	12.5	60 µM	0.750	12.5	60 µM	0.750
MLM005/006	12.5	60 µM	0.750	0	60 µM	0
T4 ligase	3	-	-	3	-	-
ddiH_2O	107	-	-	119.5	-	-
Total	150	-	-	150	-	-

Supplementary Table 2. Calculations for T4 ligase-catalyzed ligation tests for positive and negative control beads.

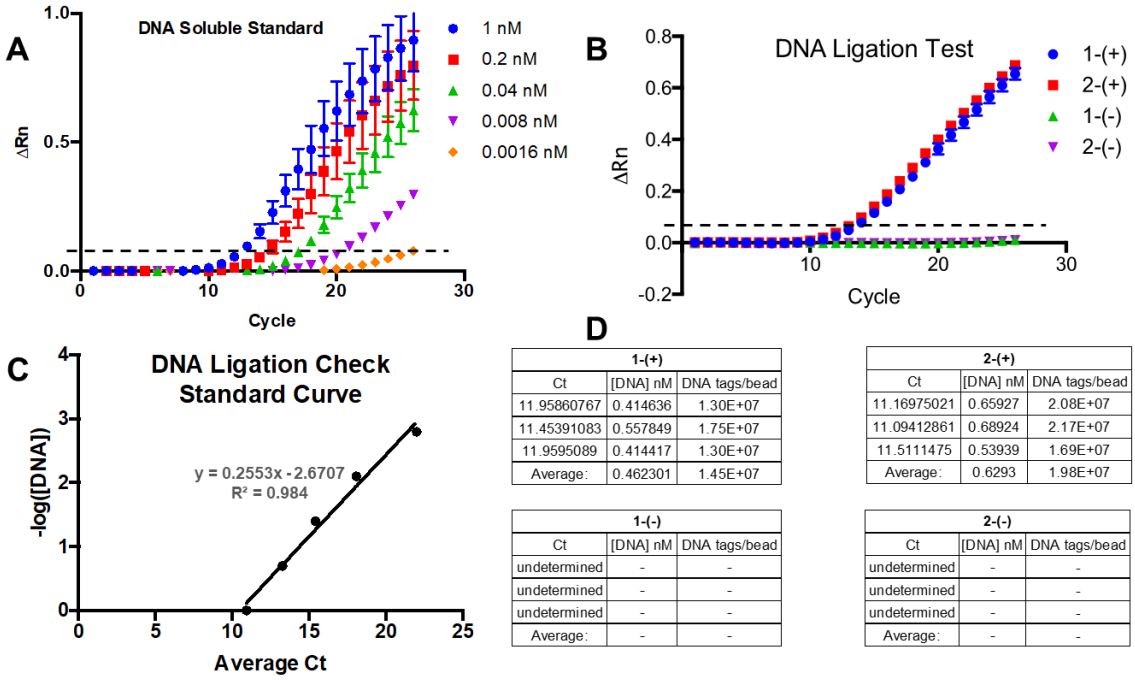
The full-length DNA damage tag ligation was assessed via a TaqMan polymerase real-time qPCR assay with FAM reporter (BMP259) and ROX as a passive reference dye. DNA-conjugated beads were drained and washed with BTPWB, and then added 75 µL BTPWB. Beads were suspended in the solution and transferred to an Eppendorf tube. This process was repeated two more times to afford a solution of DNA-conjugated beads in 225 µL BTPWB. Beads were sonicated and the concentration (beads/µL) was checked via hemocytometer. The beads were centrifuged and additional BTPWB was added to afford a final concentration of 100 beads/µL.

A TaqMan polymerase solution was prepared (**Supplementary Table 3**) along with a soluble DNA standard stock solution. The TaqMan master mix solution (19 µL) was added to wells of a PCR plate. The wells were then added 1 µL of either the diluted bead sample, water, full-length DNA standard serially diluted, or negative bead control (naïve beads) and the plate was placed in a PCR thermal cycler: holding stage (96 °C, 30 seconds), cycle stage (95 °C, 20 seconds; 65.8 °C, 20 seconds; 68 °C, 15 seconds; 26 cycles), then melt curve stage (72 °C, two minutes; 72.3 °C, two minutes).

Reagent function in PCR	Reagents	Volume in PCR reaction (µL)	Stock Concentration
dNTPs	1X MM	2	10X
Reverse primer	0.5 µM MLM005	0.5	20 µM
Forward primer	0.5 µM MLM014	0.5	20 µM
-	8% DMSO	1.6	-
-	1 M betaine	4	5 M
-	$MgCl_2$	0.2	100 mM
Polymerase	*Taq*	0.2	5 U/µL
TaqMan probe	FAM (BMP259)	0.7	10 µM
Passive reference dye	1X ROX	0.4	50X
-	$ddiH_2O$	9.9	-
-	Total	20	-

Supplementary Table 3. Calculations for preparing the TaqMan polymerase master mix for quantitative PCR assay.

Ligation of the full-length DNA tag (and concentration of conjugated DNA on resin) was assessed by first analyzing the threshold cycles (C_t, dashed line) for each serially diluted DNA standard (**Supplementary Figure 2A**), as well as positive and negative control beads (**2B**). ΔRn (FAM reporter signal normalized to the fluorescence signal of ROX dye minus baseline) was plotted against the cycle number and the C_t value was deduced from the intersection of the ΔRn fluorescence with the threshold value (0.024).



1-(+)		
Ct	[DNA] nM	DNA tags/bead
11.95860767	0.414636	1.30E+07
11.45391083	0.557849	1.75E+07
11.9595089	0.414417	1.30E+07
Average:	0.462301	1.45E+07

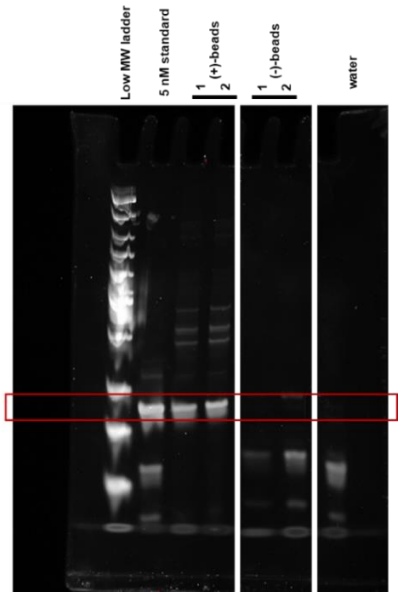
2-(+)		
Ct	[DNA] nM	DNA tags/bead
11.16975021	0.65927	2.08E+07
11.09412861	0.68924	2.17E+07
11.5111475	0.53939	1.69E+07
Average:	0.6293	1.98E+07

1-(-)		
Ct	[DNA] nM	DNA tags/bead
undetermined	-	-
undetermined	-	-
undetermined	-	-
Average:	-	-

2-(-)		
Ct	[DNA] nM	DNA tags/bead
undetermined	-	-
undetermined	-	-
undetermined	-	-
Average:	-	-

Supplementary Figure 2. A) qPCR of serially diluted full-length DNA damage standard. **B)** TaqMan polymerase qPCR of DNA ligation test for positive and negative control beads. **C)** Standard curve resulting from qPCR of serially diluted DNA standard. **D)** Quantification of DNA concentration and DNA tags/bead for each bead control.

By plotting the C_t values from the DNA standard versus the -log([DNA]), a standard curve can be constructed (**2C**), from which overall concentration of DNA on resin can be calculated (**2D**). The two positive control bead populations contained an average [DNA] = 0.546 nM, which corresponds to 1.72 x 10^7 DNA tags per bead. The C_t values for amplifiable DNA for the negative control beads were below the threshold value, so no concentration of DNA was measured. To ensure the appropriate full-length DNA was ligated on the positive beads, a native-PAGE gel was run from the amplified DNA of the full-length ligation (**Supplementary Figure 3**). From the native-PAGE gel, both positive control beads contained a DNA band that corresponds to the appropriate molecular weight of the full-length DNA damage soluble standard. Additionally, the negative and water control lanes did not contain any DNA at the molecular weight area of interest.



Supplementary Figure 3. 6% native-PAGE gel from qPCR of full-length DNA ligation. Lanes (from left to right) correspond to low molecular weight DNA ladder, 5 nM soluble full-length DNA standard, positive control beads, negative control beads and water. The red bar highlights the desired molecular weight band of interest corresponding to full-length DNA (headpiece-004/003-005/006). The gel was pre-run for 50 minutes at 110 V prior to sample addition. DNA samples (5µL) were premixed with 6X gel loading dye (1 µL) then ran on the gel for 60 minutes at 110 V. Nucleic acids were visualized via SYBR gold nucleic acid gel stain (10,000X diluted in 1X TAE buffer).

VI. Procedures and data for DNA-damage assay

Procedure for the DNA-damage assay: A 96-well filter plate was added 100 µL DCM, followed by DNA-conjugated beads: ~20,000 beads per reaction condition. For every DNA damage assay, an aliquot of beads was used for the damaging reaction, along with a solvent control, where beads were incubated in the reaction solvent, but did not receive any reactive chemical reagents and a control, known damaging reaction: chloroacylation (chloroacetic acid/HOAt/DIC 40/40/57 mM). The solvent was drained from all filtered wells and the resins were washed three times with DMF. The resin was subjected to the respective reaction conditions, including solvent controls, and following the reaction the solvents were drained and the resins were washed with DMF, 50% BTPWB/DMF and three times with BTPWB. The beads were shaken in BTPWB at room temperature overnight.

Reagent concentration in PCR	Volume in PCR reaction (µL)	Stock Concentration
1X MM	2	10X
0.5 µM MLM005	0.5	20 µM
0.5 µM MLM014	0.5	20 µM
8% DMSO	1.6	-
1 M betaine	4	5 M
1 mM $MgCl_2$	0.2	100 mM
Taq	0.2	5 U/µL
0.35 µM BMP259	0.7	10 µM
1X ROX	0.4	50X
$ddiH_2O$	9.9	-
Total	20	-

Supplementary Table 4. Calculations for TaqMan polymerase quantitative PCR.

In a similar fashion for installing the full-length DNA damage tag, a TaqMan polymerase solution was utilized for assessing DNA-damage via qPCR (**Supplementary Table 4**). In the 96-well filter plate, the BTPWB was filtered and DNA-conjugated beads (for each reaction condition and solvent controls) were added 75 µL of BTPWB. Beads were suspended in the solution and transferred to an Eppendorf tube. This process was repeated two more times for each condition to afford solution of beads in 225 µL of BTPWB. Beads were sonicated and the concentrations were checked via hemocytometer. The bead solutions were centrifuged and additional BTPWB was added to afford a final concentration of 100 beads/µL.

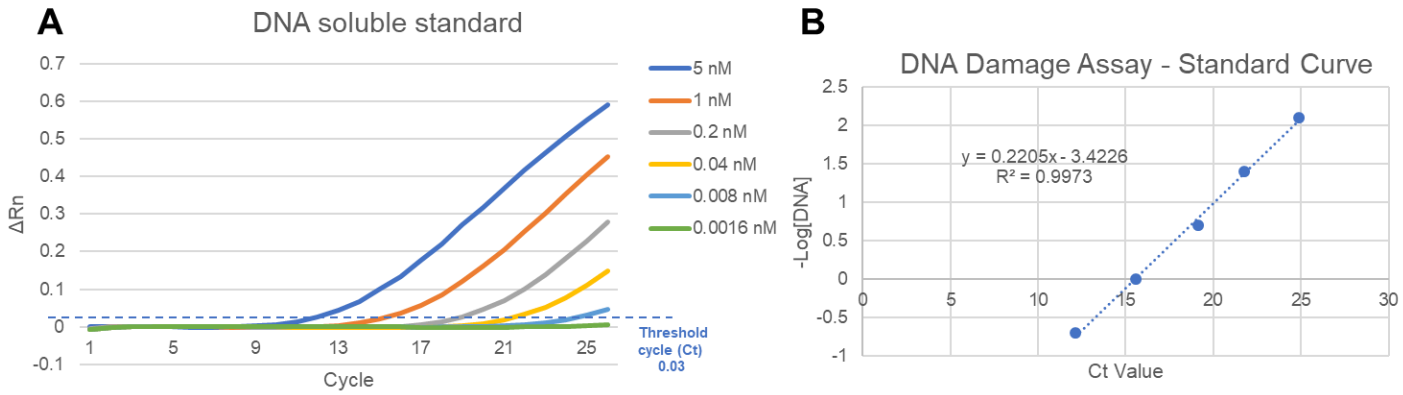
	1	2	3	4	5	6	7	8	9	10	11	12
A	5 nM	1 nM	0.5 nM	0.1 nM	0.02 nM	0.004 nM	0.0008 nM	0.00016 nM	Water	Water	Water	
B	5 nM	1 nM	0.5 nM	0.1 nM	0.02 nM	0.004 nM	0.0008 nM	0.00016 nM				
C	Naïve beads	Naïve Beads	Naïve Beads	DMF	DMF	DMF	CAA	CAA	CAA	NMP	NMP	NMP
D	PyAOP	PyAOP	PyAOP	DMF/DCM/MeOH	DMF/DCM/MeOH	DMF/DCM/MeOH	Cu(II) red. amin.	Cu(II) red. amin.	Cu(II) red. amin.			
E												
F												
G												
H												

Supplementary Figure 4. Representative example of PCR plate layout for subsequent TaqMan polymerase qPCR DNA damage assay.

A TaqMan master mix solution was made (**Supplementary Table 4**) and 19 µL was added to all wells of a PCR plate (**Supplementary Figure 4**). The PCR plate was then added 1 µL of serially diluted full-length DNA damage standard (run in duplicate, wells A1 – A8, B1 – B8), 1 µL ddiH_2O (run in triplicate, wells A9 – A10), 1 µL of naïve beads that did not receive any chemistry (run in triplicate C1 – C3) and 1 µL of the corresponding bead sample for each damaging reaction and solvent control (each run in triplicate C4 – D9). The plate was sealed, centrifuged and subjected to PCR conditions: holding stage (96 °C, 30 seconds), cycle stage (95 °C, 20 seconds; 65.8 °C, 20 seconds; 68 °C, 15 seconds; 26 cycles), then melt curve stage (72 °C, two minutes; 72.3 °C, two minutes). DNA damage was assessed by comparing the remaining amplifiable DNA from the damaging reaction beads, compared to naïve beads that received no reaction chemistry. Additionally, a known DNA-damaging reaction was used for each assay: 40 mM chloroacetic acid (CAA)/40 mM HOAt/ 57 mM DIC, 37°C for one hour.[3]

First, the serially diluted full-length DNA soluble standard was subjected to qPCR and the corresponding Ct values were obtained (**Supplementary Figures 5A – 12A**). The Ct values were plotted against -log([DNA]) in order to produce a standard curve (**Supplementary Figures 5B – 12B**). The standard curve was used to quantify the amount of amplifiable DNA for each reaction condition based on the Ct value for each condition. Finally, the % DNA remaining was calculated by dividing the average [DNA] for the reaction condition to the average [DNA] for naïve beads that did not receive any damaging chemistry (**Supplementary Figures 5C – 12C**).

Quantitative PCR raw data, standard curves and calculations for percent amplifiable DNA remaining for DNA damage assays.

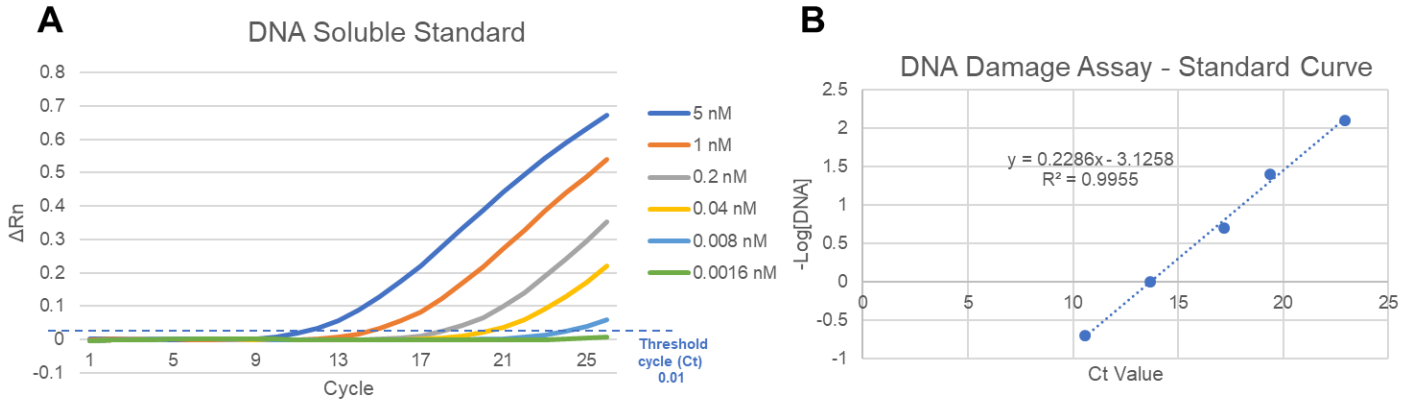


C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	14.49	14.39	13.91	1.69	1.78	2.27	1.91	0.31	-
DMF	13.93	14.30	14.39	2.24	1.86	1.77	1.96	0.25	**102.51**
CAA	15.75	16.07	15.52	0.89	0.76	1.00	0.88	0.12	**46.21**
Reductive amination	14.23	14.44	14.23	1.92	1.73	1.93	1.86	0.11	**97.28**
tBuOH/H2O/THF	14.13	14.39	13.93	2.03	1.78	2.25	2.02	0.23	**105.64**
Pinnick	14.49	14.30	14.29	1.69	1.86	1.87	1.81	0.10	**94.50**

Supplementary Figure 5. Assessment of DNA damage resulting from CAA acylations, reductive aminations and Pinnick oxidations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values,

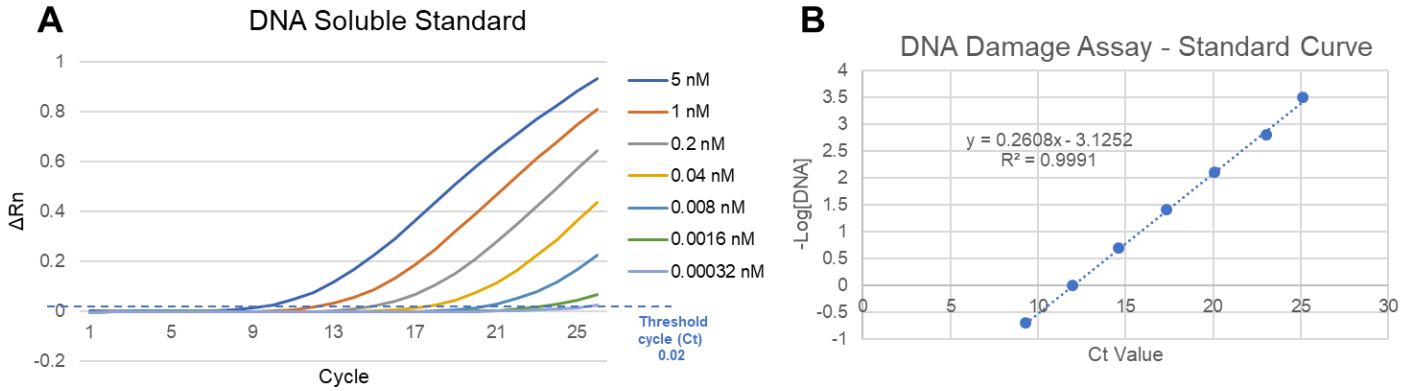
[DNA] for each reaction condition and % DNA remaining after damaging reactions. Conditions that resulted in >100% DNA remaining are attributed to error in bead normalization prior to addition to qPCR. Solvent controls that showed >100% DNA remaining were repeated several times and did not indicate any significant DNA damage.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	12.44	12.52	12.54	1.92	1.83	1.81	1.85	0.06	-
DMF - 1 hr	12.51	12.71	12.63	1.85	1.66	1.74	1.75	0.09	**94.30**
CAA	14.14	14.38	14.37	0.78	0.69	0.69	0.72	0.05	**38.92**
NMP	12.54	12.65	12.63	1.82	1.71	1.73	1.75	0.06	**94.62**
PyAOP	15.34	15.34	15.39	0.42	0.42	0.41	0.41	0.01	**22.23**
Cu(II) reductive amination	12.60	12.60	12.55	1.76	1.76	1.81	1.78	0.03	**95.78**

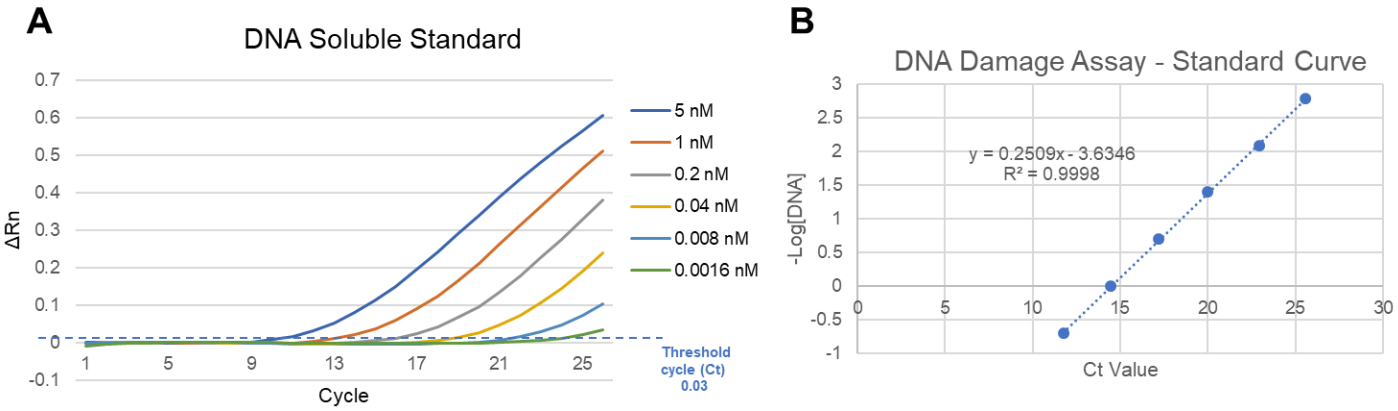
Supplementary Figure 6. Assessment of DNA damage resulting from CAA acylations, PyAOP-mediated amidations and Cu^{II}-catalyzed reductive aminations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	12.94	12.82	12.90	0.56	0.61	0.58	0.58	0.02	-
DMF - 1 h	12.80	12.96	13.26	0.61	0.55	0.46	0.54	0.07	**93.42**
CAA	12.94	13.31	13.31	0.56	0.45	0.45	0.49	0.06	**83.82**
PyAOP/HOAt	12.95	12.73	13.11	0.56	0.64	0.51	0.57	0.07	**97.71**

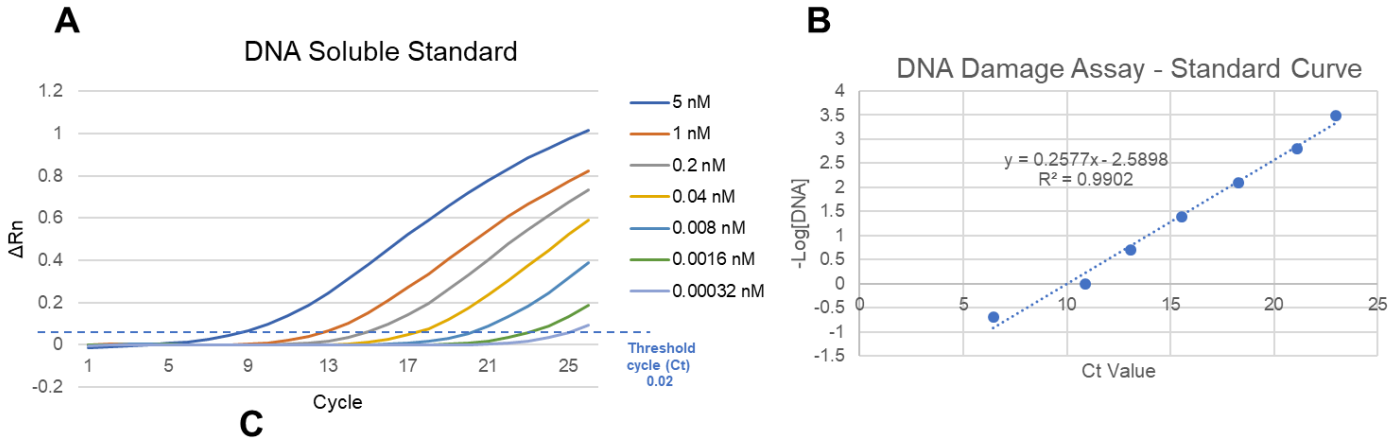
Supplementary Figure 7. Assessment of DNA damage resulting from CAA acylations and PyAOP/HOAt-mediated amidations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	13.62	13.69	13.99	1.65	1.58	1.33	1.52	0.17	**-**
DMF - 1 h	14.14	13.76	13.77	1.22	1.52	1.51	1.42	0.17	**93.22**
CAA	15.22	15.14	15.23	0.66	0.69	0.65	0.66	0.02	**43.62**
HOAt/DIC	13.94	13.85	14.23	1.37	1.45	1.16	1.33	0.15	**87.19**

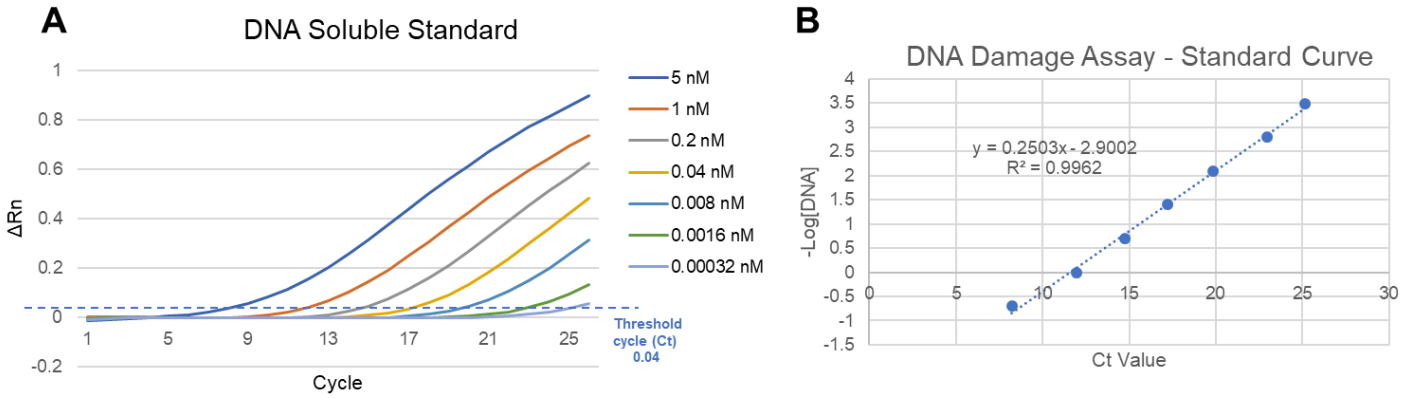
Supplementary Figure 8. Assessment of DNA damage resulting from CAA acylations and HOAt/DIC-mediated amidations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	10.71	10.39	10.80	0.67	0.82	0.64	0.71	0.09	**-**
CAA	10.88	10.83	10.83	0.61	0.63	0.63	0.62	0.01	**87.43**
Horner-Wadsworth-Emmons	10.93	10.68	10.50	0.59	0.69	0.77	0.68	0.09	**96.00**

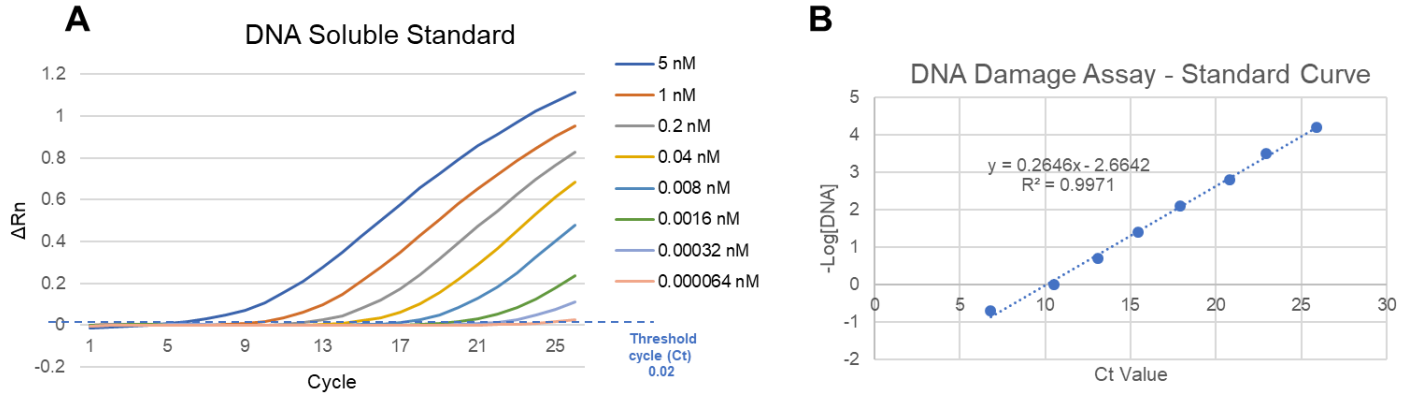
Supplementary Figure 9. Assessment of DNA damage resulting from CAA acylations, Pictet-Spengler cyclizations and Seyferth-Gilbert homologations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	11.95	11.86	11.97	0.37	0.39	0.37	0.38	0.01	**-**
CAA	12.84	12.78	13.01	0.23	0.24	0.21	0.22	0.01	**59.80**
Pictet-Spengler	12.02	11.99	11.94	0.36	0.36	0.37	0.36	0.01	**97.03**
Seyferth-Gilbert	12.01	11.92	12.05	0.36	0.38	0.35	0.36	0.01	**96.43**

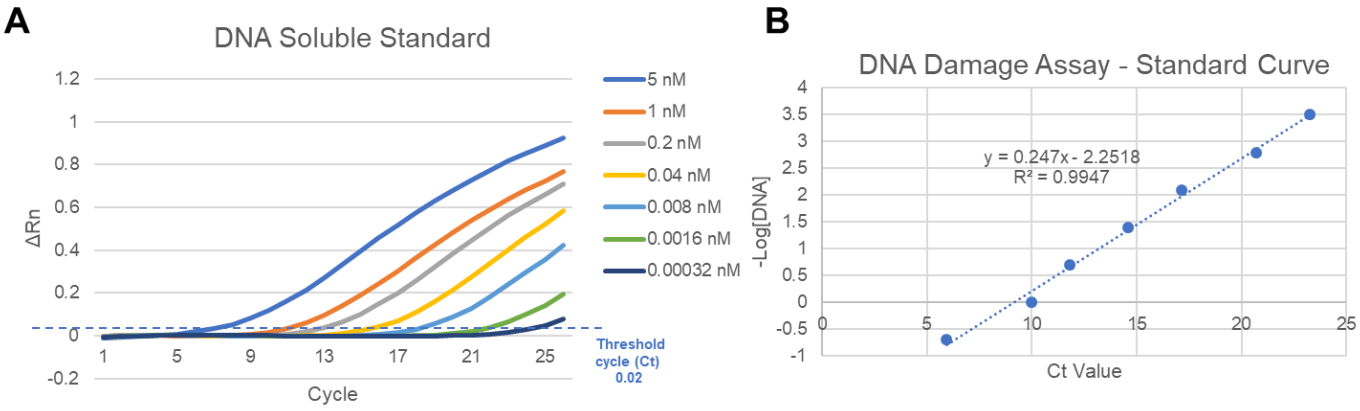
Supplementary Figure 10. Assessment of DNA damage resulting from CAA acylations, Pictet-Spengler cyclizations and Seyferth-Gilbert homologations. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.



C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	11.14	11.16	10.95	0.52	0.51	0.58	0.54	0.04	**-**
CAA	11.34	11.29	11.46	0.46	0.47	0.43	0.45	0.02	**84.30**
Oxazole	11.43	11.38	11.37	0.44	0.45	0.45	0.45	0.01	**82.88**

Supplementary Figure 11. Assessment of DNA damage resulting from CAA acylations and oxazole synthesis. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.

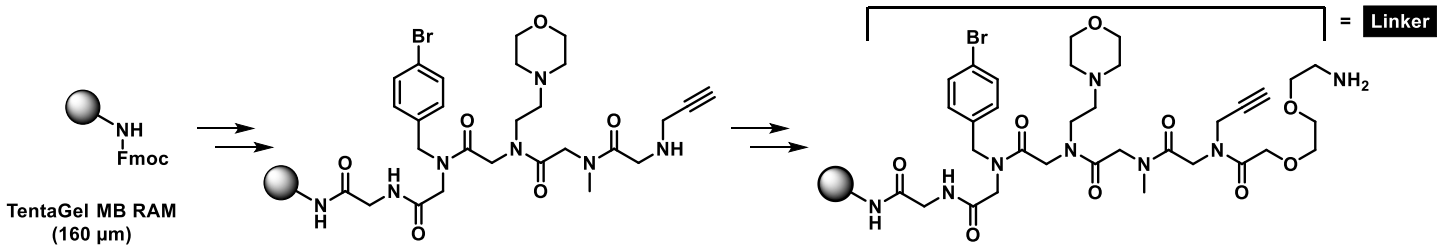


C

	Ct values			[DNA] nM			Average	Std. Dev.	% DNA Remaining
No chemistry	12.51	12.70	12.57	0.15	0.13	0.14	0.14	0.01	-
DMF	12.97	12.98	12.94	0.11	0.11	0.11	0.11	0.00	**80.89**
CAA	14.82	14.53	14.73	0.04	0.05	0.04	0.04	0.00	**30.34**
NMP	12.80	12.80	12.77	0.12	0.12	0.13	0.12	0.00	**89.37**
Benzimidazole	12.85	12.82	13.03	0.12	0.12	0.11	0.12	0.01	**83.99**

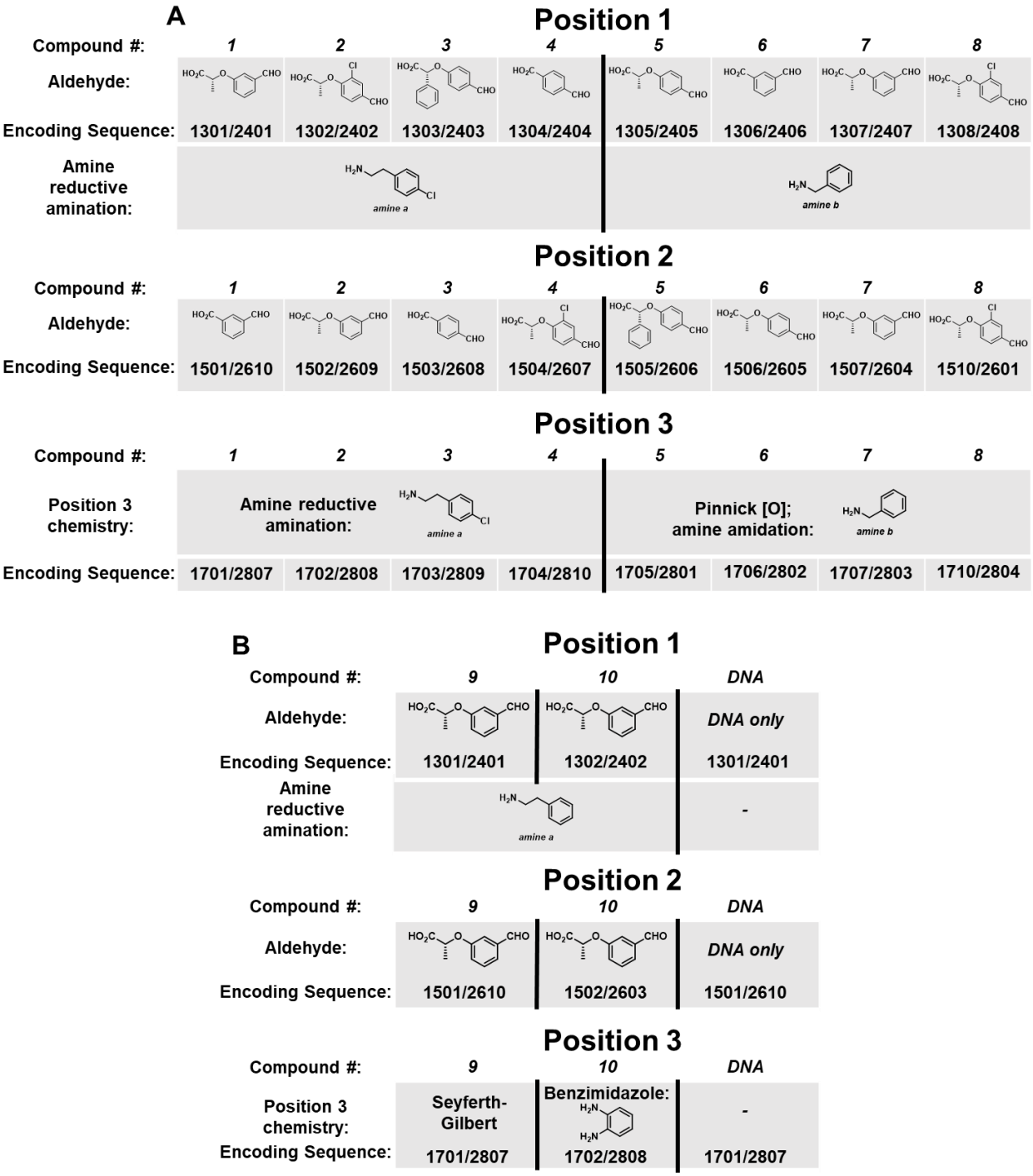
Supplementary Figure 12. Assessment of DNA damage resulting from CAA acylations and benzimidazole synthesis. **A)** qPCR of serially diluted full-length DNA standard. **B)** Standard curve resulting from qPCR of serially diluted DNA standard. **C)** Ct values, [DNA] for each reaction condition and % DNA remaining after damaging reactions.

VII. Procedures for parallel synthesis of DNA-encoded small molecules



Procedure for the synthesis of the linker for 160 µm beads: The linker was installed on 160 µm TentaGel MB RAM resin (5 mg, 0.41 mmol/g, 0.0021 mmol). The resin was added to a filtered syringe and swelled in DMF (2 mL) at room temperature for 1 hr. The solvent was drained and 2 mL of a 20% piperidine solution in DMF was added and stirred for 2 x 30 minutes at room temperature. The solution was drained, and the resin was washed with DMF 3 x 1.5 mL. The resin was then added 0.5 mL of a premixed solution containing Fmoc-Gly-OH (3 eq.), oxyma (3 eq.), collidine (3 eq.) and DIC (4.5 eq.) and the reaction was stirred at 37 °C for one hour. The solution was drained, the resin was washed with DMF 3 x 1.5 mL, then stirred in a 20% piperidine solution in DMF for 2 x 30 minutes at room temperature. The resin was then added 0.3 mL of a 2 M bromoacetic acid solution in DMF, followed by 0.3 mL of a 50% DIC solution in DMF and was stirred at 37 °C for 10 minutes. The resin was washed with DMF 3 x 1.5 mL and subsequently added a 1 M 4-bromobenzylamine solution in DMF and was stirred at 37 °C for 30 minutes. The solution was drained, washed with DMF 3 x 1.5 mL and this process of bromoacylation and amine displacement was repeated for installation of the subsequent 2-morpholinoethan-1-amine peptoid unit.

Next, the resin was added 0.5 mL of a premixed solution containing Fmoc-Sar-OH (3 eq.), oxyma (3 eq.), collidine (3 eq.) and DIC (4.5 eq.) and the reaction was stirred at 37 °C for one hour. The solution was drained, and the resin was washed with DMF 3 x 1.5 mL then subjected to bromoacylation conditions and propargylamine displacement to generate the secondary amine intermediate. A final acylation was performed by adding 0.5 mL of a premixed solution containing Fmoc-Amino-3,6 dioxaoctanoic acid (3 eq.), oxyma (3 eq.), collidine (3 eq.) and DIC (4.5 eq.) and the reaction was stirred at 37 °C for one hour. The solution was drained, the resin was washed with DMF 3 x 1.5 mL, then stirred in a 20% piperidine solution in DMF for 2 x 30 minutes at room temperature to afford the linker-conjugated 160 µm quality control beads.



Supplementary Figure 13. Synthesis and encoding schemes for the parallel encoded synthesis of **A)** compounds **1** – **8** and **B)** compounds **9** – **10**, including a DNA control lane that did not receive any chemistry.

A

Overhangs		Encoding Sequences			
Overhang #	Sequence	Identifier #	Sequence	Identifier #	Sequence
X1XX	/5Phos/ATGG	1X01	AAGAGGCA	2X01	CCTCCTAA
X2XX	/5Phos/TCA	1X02	ACGGAGCA	2X02	AACCTCAA
X3XX	/5Phos/GTT	1X03	ACGAGATT	2X03	ATTCTCGG
X4XX	/5Phos/CTA	1X04	AAGGAGGT	2X04	AACCCTAC
X5XX	/5Phos/TTC	1X05	AGAAAGCA	2X05	GACTCCGC
X6XX	/5Phos/CGC	1X06	ATAGAGCC	2X06	CATTTCAA
X7XX	/5Phos/GTA	1X07	CAGAAGGA	2X07	CCCTCCGG
X8XX	/5Phos/TGG	1X08	GAGGAACA	2X08	CGTTCCTG
X9XX	/5Phos/TCT	1X09	TGAAGGAA	2X09	TTCTTCAT
XAXX	/5Phos/AAG	1X10	TTGAGGAT	2X10	TCTCCTCC

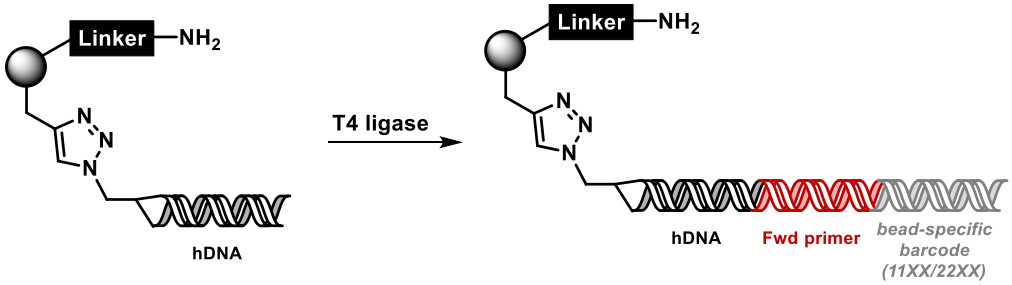
B

Forward Primer (FwdPr)		Reverse Primer (RevPr)	
Primer #	Sequence	Primer #	Sequence
ABM063	/5Phos/GCCGCCCAGTCCTGCTCGCTTCGCTAC	-	/5Phos/AAGCGTTCCTG

C

		Bead Specific Barcode		Position 1		Position 2		Position 3		Library ID	
Compound #	Full Encoding Sequence	11XX	22XX	13XX	24XX	15XX	26XX	17XX	28XX	19XX	2AXX
1	hDNA-FwdPr-110222011301240115012610170128071902 2A06-RevPr	1102	2201	1301	2401	1501	2610	1701	2807	1902	2A06
2	hDNA-FwdPr-110222021302240215 02/26091702280819022A06-RevPr	1102	2202	1302	2402	1502	2609	1702	2808	1902	2A06
3	hDNA-FwdPr-110522011303240315032608170328091902 2A06-RevPr	1105	2201	1303	2403	1503	2608	1703	2809	1902	2A06
4	hDNA-FwdPr-110622021304240415042607170428101902 2A06-RevPr	1106	2202	1304	2404	1504	2607	1704	2810	1902	2A06
5	hDNA-FwdPr-110722071305240515052606170528011902 2A06-RevPr	1107	2207	1305	2405	1505	2606	1705	2801	1902	2A06
6	hDNA-FwdPr-110822081306240615062605170628019022A06-RevPr	1108	2208	1306	2406	1506	2605	1706	2802	1902	2A06
7	hDNA-FwdPr-110922031307240715072604170728031902 2A06-RevPr	1109	2203	1307	2407	1507	2604	1707	2803	1902	2A06
8	hDNA-FwdPr-111022091308240815102601171028041902 2A06-RevPr	1110	2209	1308	2408	1510	2601	1710	2804	1902	2A06
9	hDNA-FwdPr-110222091301240115012610170828071902 2A06-RevPr	1102	2209	1301	2401	1501	2610	1708	2807	1902	2A06
10	hDNA-FwdPr-110222021302240215022609170228081902 2A06-RevPr	1102	2202	1302	2402	1502	2609	1702	2808	1902	2A06
DNA only	hDNA-FwdPr-110222091301240115012610170828071902 2A06-RevPr	1102	2209	1301	2401	1501	2610	1708	2807	1902	2A06

Supplementary Figure 14. Oligonucleotide sequence lookup table corresponding to the [+] strands of **A)** the encoding DNA sequences and **B)** the forward and reverse primers. **C)** Full encoding sequences for DNA-encoded small molecules **1** – **10**, along with a DNA only control.

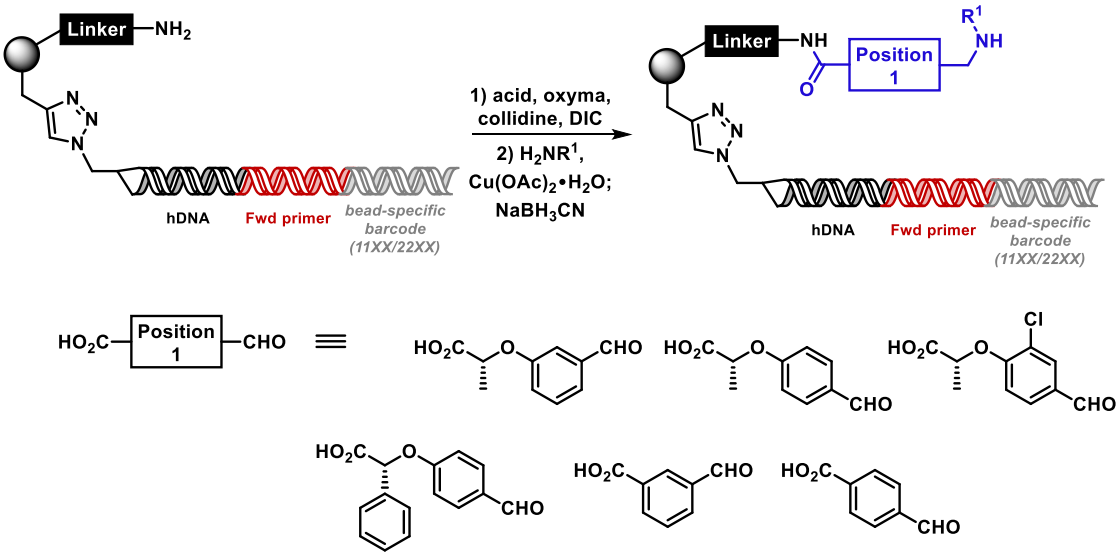


Procedure for the ligation of the forward primer and bead-specific barcode: ~1 mg of TentaGel M NH_2 resin (10 µm) was added to each corresponding well of a filter plate with TentaGel MB RAM resin (160 µm) spiked in at a ~30,000:1 ratio. The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained then 110 µL of a premixed reaction solution was added (**Supplementary Table 5**) followed by 40 µL of mixed barcodes (1:1 mixture of 60 µM 11XX and 60 µM 22XX to afford a final concentration of 30 µM. These premixed codes were heated to 50 °C for five minutes prior to ligation reaction). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature.

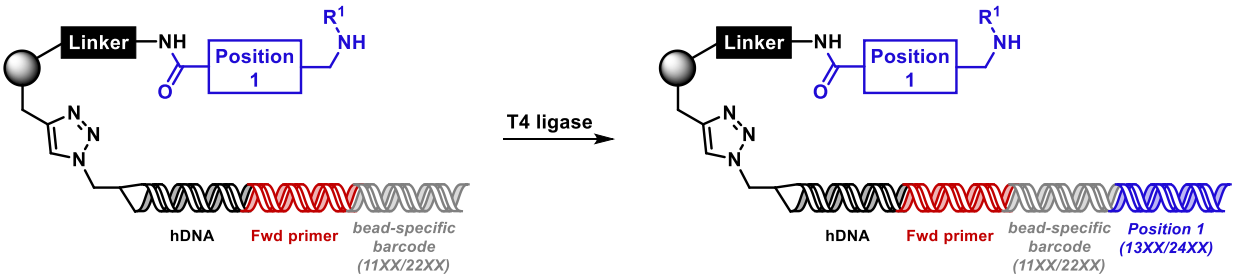
Reagent (Final conc.)	Stock Concentration	Volume in PCR reaction (µL)
BTPLB (1X)	10X	15
T4 ligase	-	0.45
FwdPr (8 µM)	60 µM	20
$ddiH_2O$	-	74.55
Total	-	110

Supplementary Table 5. Calculations for preparing the T4 ligase-catalyzed primer ligation.

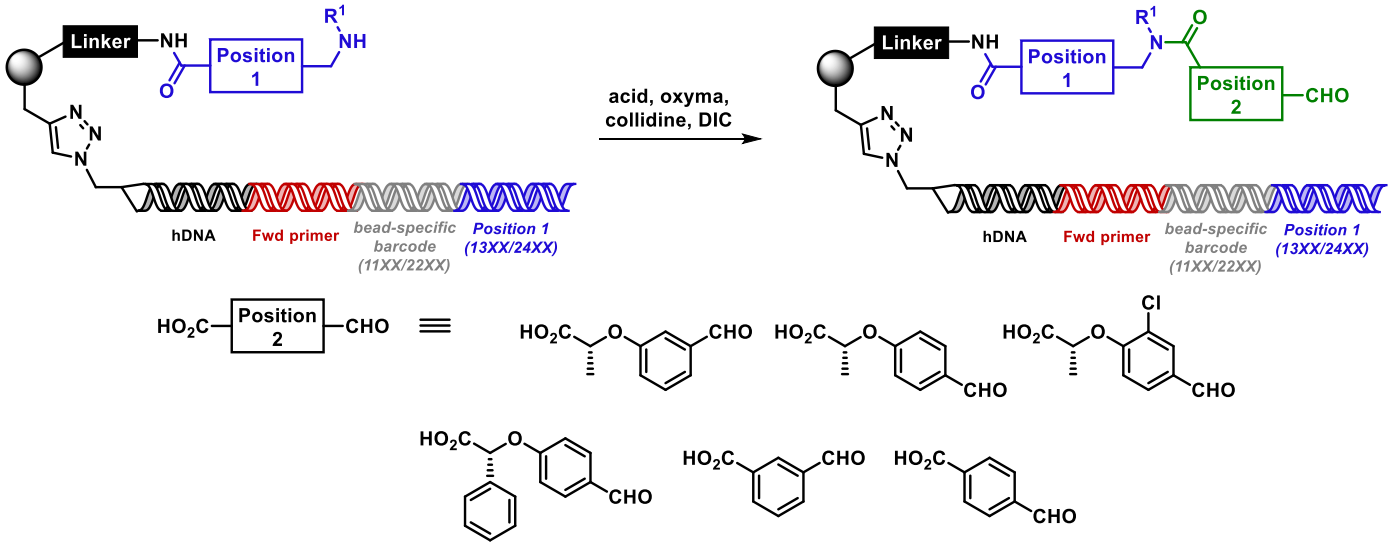
Following the ligation the solution was drained, the wells were washed with BTPWB 4 x 150 µL, then 50% BTPWB/DMF 3 x 150 µL, then 100% DMF 5 x 150 µL and the resin was equilibrated in DMF overnight at room temperature.



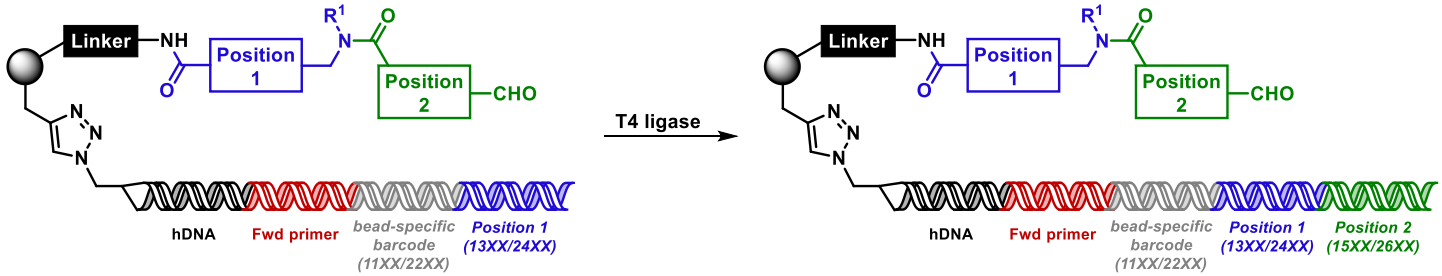
Procedure for the synthesis of position one: The filter plate was drained of DMF and to each well was added 150 µL of a premixed solution containing 80 mM carboxylic acid, 80 mM oxyma, 80 mM collidine and 125 mM DIC in DMF. The reaction was stirred at 37 °C for three hours. Following the incubation, the solutions were drained and washed 4 x 150 µL with DMF. The resin was then added 75 µL of a 1 M amine solution in DMF (containing 2% AcOH), along with 50 mM copper(II) acetate monohydrate and the resin was stirred at room temperature for 20 minutes. The resin was then added 75 µL of a 2 M sodium cyanoborohydride solution in 1:1 DCM:MeOH (with 2% AcOH) and the reaction was stirred at room temperature overnight. The solution was drained, the resin was washed with DMF, THF, H_2O, DMF, 50% BTPWB/DMF, BTPWB and equilibrated in BTPWB at room temperature for three hours.



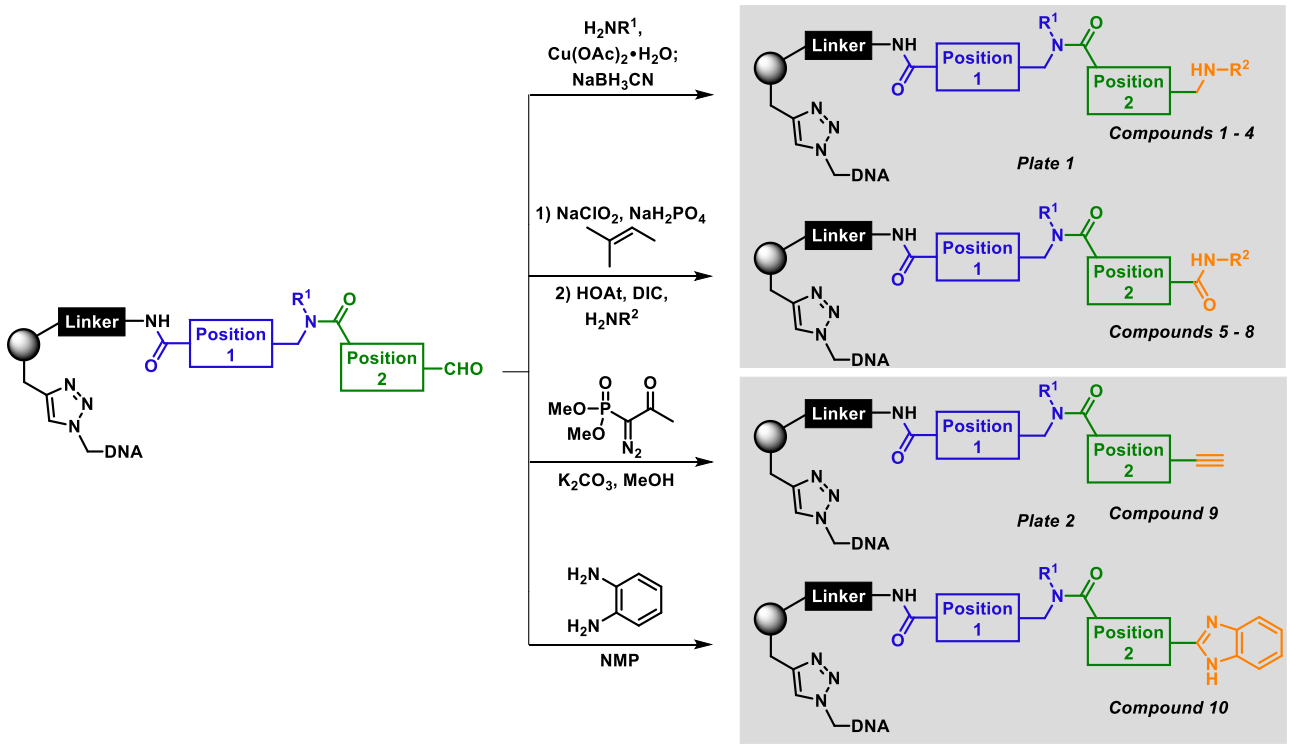
Procedure for the ligation of position one codes (13XX/24XX): The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained, then 110 µL of a premixed master mix solution was added (94.55 µL H_2O, 15 µL 10X BTPLB, 0.45 µL T4 ligase) to each well, followed by 40 µL of mixed barcodes. (**Note:** the 13XX and 24XX barcodes were added as a 1:1 mixture of 60 µM 13XX and 60 µM 24XX to afford a final concentration of 30 µM. These premixed codes were heated to 50 °C for five minutes prior to ligation reaction). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature. Following the ligation, the solution was drained, the wells were washed with BTPWB 4 x 150 µL, then 50% BTPWB/DMF 3 x 150 µL, then 100% DMF 5 x 150 µL and the resin was equilibrated in DMF overnight at room temperature.



Procedure for the synthesis of position two: The filter plate was drained of DMF and to each well was added 150 µL of a premixed solution containing 80 mM carboxylic acid, 80 mM oxyma, 80 mM collidine and 125 mM DIC in DMF. The reaction was stirred at 37 °C for three hours. Following the incubation, the solutions were drained and washed with DMF, THF, H_2O, DMF, 50% BTPWB/DMF, BTPWB and equilibrated in BTPWB at room temperature overnight.



Procedure for the ligation of position two codes (15XX/26XX): The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained, then 110 µL of a premixed master mix solution was added (94.55 µL H_2O, 15 µL 10X BTPLB, 0.45 µL T4 ligase) to each well, followed by 40 µL of mixed barcodes. (**Note:** the 15XX and 26XX barcodes were added as a 1:1 mixture of 60 µM 15XX and 60 µM 26XX to afford a final concentration of 30 µM. These premixed codes were heated to 50 °C for five minutes prior to ligation reaction). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature. Following the ligation, the solution was drained, the wells were washed with BTPWB 4 x 150 µL, then 50% BTPWB/DMF 3 x 150 µL, then 100% DMF 5 x 150 µL and the resin was equilibrated in DMF overnight at room temperature.



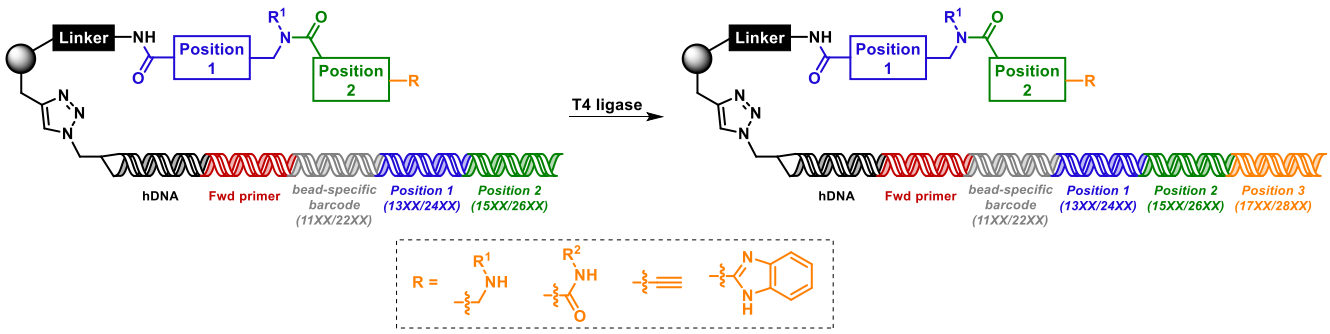
Procedure for the synthesis of position three: On two separate filter plates (plate 1 and plate 2), DMF was drained and the wells were split for each reaction. Plate 1: four wells received reductive amination conditions and four wells received Pinnick oxidations, followed by amidation reactions. Plate 2: one well received Seyferth-Gilbert homologation reaction conditions, one well received benzimidazole synthesis conditions and one well received no chemistry (DNA only). **Note:** Each reaction condition was performed in duplicate.

Reductive amination: Each well was added 75 µL of a 1 M amine solution in DMF (containing 2% AcOH), along with 50 mM copper(II) acetate monohydrate and the resin was stirred at room temperature for 20 minutes. The resin was then added 75 µL of a 2 M sodium cyanoborohydride solution in 1:1 DCM:MeOH (with 2% AcOH) and the reaction was stirred at room temperature overnight. The solution was drained, the resin was washed with DMF, THF, H_2O, DMF, 50% BTPWB/DMF, then BTPWB and equilibrated in BTPWB at room temperature for three hours.

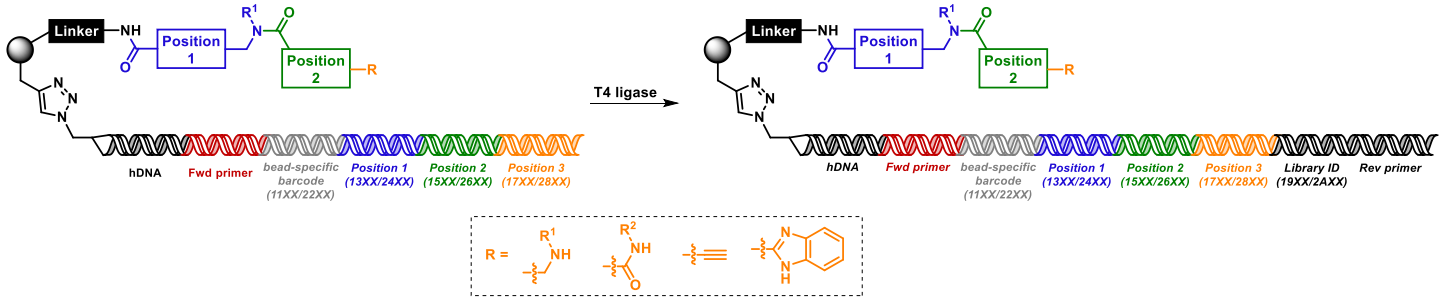
Pinnick oxidation/amidation: The resin was added 100 µL *t*BuOH, 10 µL of a 1 M NaH_2PO_4 solution in H_2O, 30 µL of a 1 M solution of 2-methyl-2-butene in THF and 10 µL of a 1 M $NaClO_2$ solution in H_2O and the reaction was stirred at room temperature for 1 hour. The solution was drained, and the resin was washed with THF, H_2O, and DMF. The solvent was drained, and the resin was added 75 µL of a solution containing 200 mM HOAt/300 mM DIC/200 mM DIPEA in DMF, followed by 75 µL of a 200 mM solution of the requisite amine in DMF and the reaction was stirred at 37 °C for three hours. The solution was drained, and the resin was washed with DMF, 50% BTPWB/DMF, then BTPWB and equilibrated in BTPWB at room temperature for three hours.

Seyferth-Gilbert homologation: The resin was drained of DMF and washed with MeOH. The resin was then added 150 µL of a solution containing 80 mM dimethyl-(1-diazo-2-oxopropyl)phosphonate (Ohira-Bestmann reagent) and 125 mM potassium carbonate in MeOH and the reaction was stirred at room temperature for one hour. The solution was drained, and the resin was washed with MeOH, DMF, 50% BTPWB/DMF, then BTPWB and equilibrated in BTPWB at room temperature for three hours.

Benzimidazole synthesis: The resin drained of DMF, washed 3 x 150 µL with NMP and then added 150 µL of a solution containing 200 mM 3,4-diaminobenzoic acid in NMP and the reaction was stirred at 37 °C for 15 hours. The solution was drained, and the resin was washed with NMP, DMF, 50% BTPWB/DMF, then BTPWB and equilibrated in BTPWB at room temperature for three hours.



Procedure for the ligation of position three codes (17XX/28XX): The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained, then 110 µL of a premixed master mix solution was added (94.55 µL H_2O, 15 µL 10X BTPLB, 0.45 µL T4 ligase) to each well, followed by 40 µL of mixed barcodes. (**Note:** the 17XX and 28XX barcodes were added as a 1:1 mixture of 60 µM 17XX and 60 µM 28XX to afford a final concentration of 30 µM. These premixed codes were heated to 50 °C for five minutes prior to ligation reaction). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature. Following the ligation, the solution was drained, the wells were washed with BTPWB 4 x 150 µL, then 50% BTPWB/DMF 3 x 150 µL, then 100% DMF 5 x 150 µL and the resin was equilibrated in DMF overnight at room temperature.



Procedure for the ligation of the library ID tag (19XX/2AXX) and reverse primer: The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained, then 150 µL of a premixed reaction solution was added (**Supplementary Table 6**). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature.

Reagent (Final conc.)	Stock Concentration	Volume in PCR reaction (µL)
BTPLB (1X)	10X	15
T4 ligase	-	0.45
Library ID (1902/2A06, 8 µM)	30 µM	40
$ddiH_2O$	-	94.55
Total	-	150

Supplementary Table 6. Calculations for preparing the T4 ligase-catalyzed library ID oligonucleotide ligation.

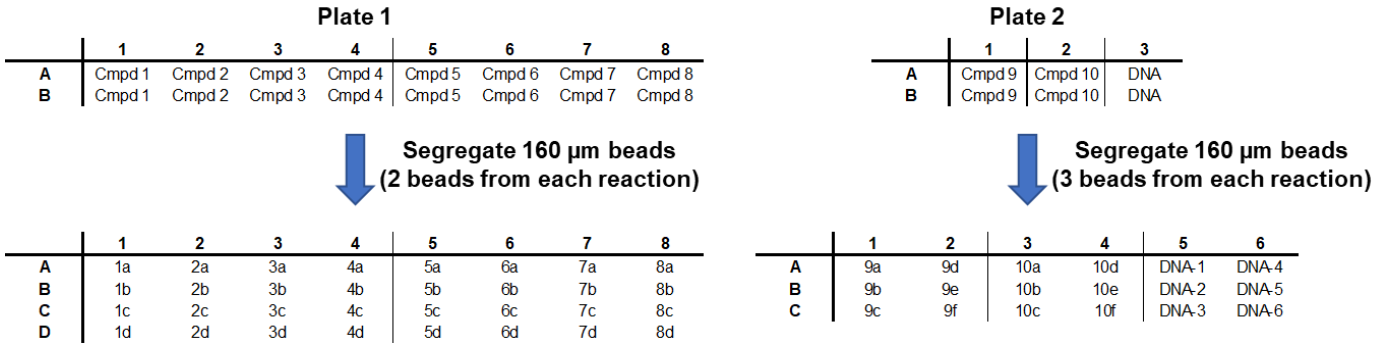
Following the ligation, the solution was drained, the wells were washed with BTPWB 4 x 150 µL and equilibrated in BTPWB at room temperature overnight. The resin was washed with BTPWB then equilibrated in 1X BTPLB for 20 minutes at room temperature. The BTPLB was drained, then 150 µL of a premixed reaction solution was added (**Supplementary Table 7**). The filter plate was sealed with adhesive foil and shaken at 750 rpm for four hours at room temperature.

Reagent (Final conc.)	Stock Concentration	Volume in PCR reaction (µL)
BTPLB (1X)	10X	15
T4 ligase	-	0.45
Reverse primer (8 µM)	30 µM	40
$ddiH_2O$	-	94.55
Total	-	150

Supplementary Table 7. Calculations for preparing the T4 ligase-catalyzed reverse primer ligation.

Following the ligation, the solution was drained, the wells were washed with BTPWB 4 x 150 µL and equilibrated in BTPWB at room temperature overnight.

VIII. Procedures for quality control of the DNA-encoded parallel synthesis library



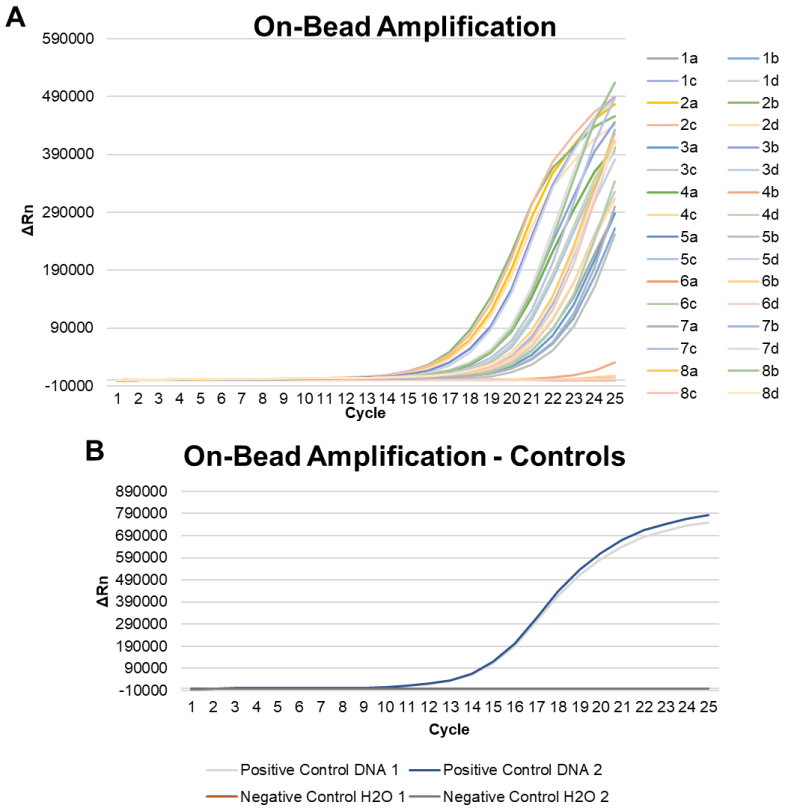
Supplementary Figure 15. Plate layouts for parallel synthesis of encoded small molecules. Plate 1: compounds **1** – **8** performed in duplicate. Following synthesis, two 160 µm beads from each well were segregated for subsequent quality control analysis. Plate 2: compounds **9** – **10** and DNA only performed in duplicate. Following synthesis, three 160 µm beads from each well were segregated for subsequent quality control analysis.

Procedure for on-bead amplification via qPCR: For each well of an encoded small molecule, individual 160 µm beads were physically segregated from the 10 µm resin. The 160 µm beads were added to individual wells of a PCR plate via transferring 1 µL of the bead in BTPWB. To first amplify the corresponding DNA on the 160 µm beads, a qPCR master mix was prepared (**Supplementary Table 8**).

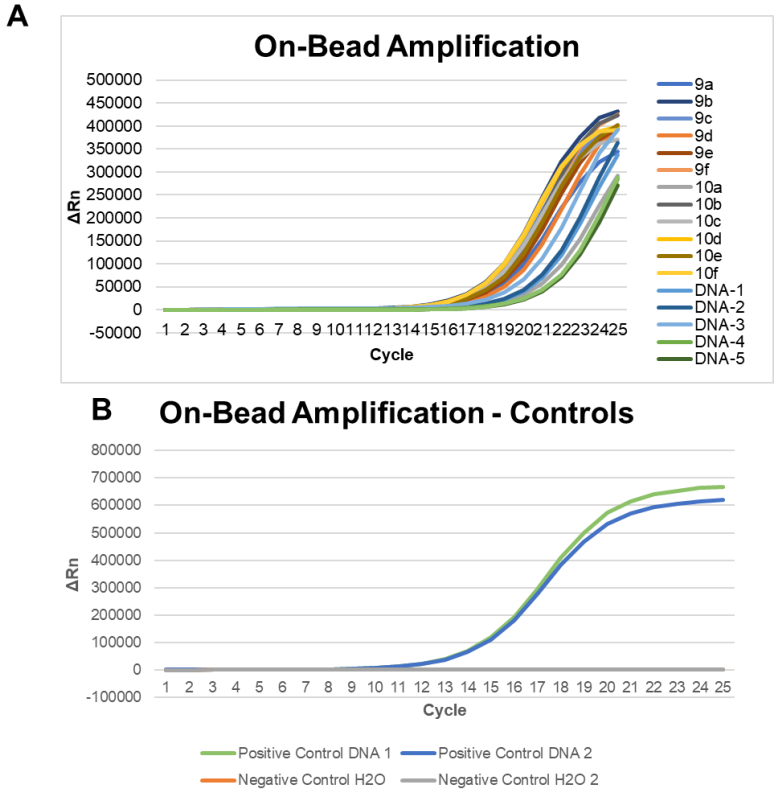
Reagent function in PCR	Reagents	Volume in PCR reaction (µL)	Stock Concentration
dNTPs	MM (1X)	2	10X
Reverse primer	ABM063 (0.3 µM)	0.3	20 µM
Forward primer	ABM062 (0.3 µM)	0.3	20 µM
-	8% DMSO	1.6	-
-	Betaine (1 M)	4	5 M
-	$MgCl_2$	0.2	100 mM
Polymerase	*Taq*	0.2	5 U/µL
Nucleic acid stain	SYBR Green (2X)	0.4	100X
-	$ddiH_2O$	11	-
-	Total	20	-

Supplementary Table 8. Calculations for preparing the TaqMan polymerase master mix for on-bead amplification via qPCR.

The master mix solution (20 µL) was added to each well of the PCR plate that contains individual 160 µm beads. The plate was placed in a qPCR thermal cycler: holding stage (96 °C, 10 seconds), cycle stage (95 °C, 8 seconds; 72 °C, 24 seconds; 25 cycles), then melt curve stage (72 °C, two minutes). On-bead amplification was assessed by first analyzing the overall amplification of DNA fluorescence via nucleic acid staining by SYBR green. ΔRn was plotted against the cycle number and DNA amplification for each bead was identified along with positive controls (full length encoding DNA standard) and negative controls (water).

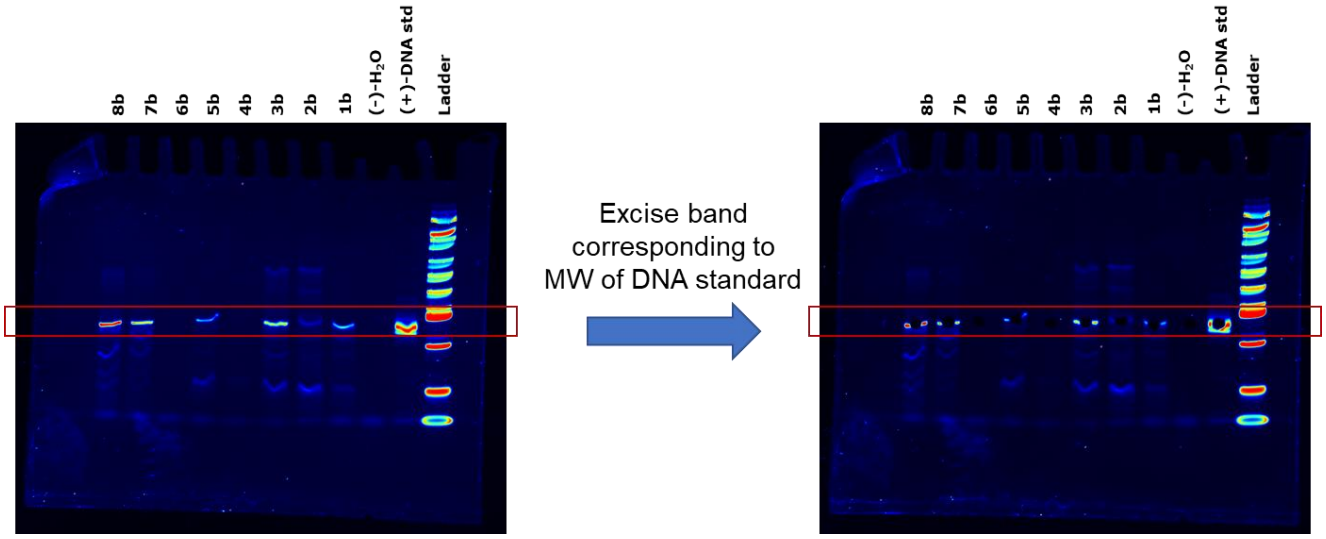


Supplementary Figure 16. Assessment of on-bead DNA amplification. **A)** qPCR of each individual 160 µm bead corresponding to compounds **1** – **8**. **B)** qPCR of positive control DNA standards and negative control (H_2O).



Supplementary Figure 17. Assessment of on-bead DNA amplification. **A)** qPCR of each individual 160 µm bead corresponding to compounds **9** – **10**. **B)** qPCR of positive control DNA standards and negative control (H_2O).

A second PCR amplification is needed in order to fully sequence the DNA off of the 160 µm beads via Sanger. To do so, each sample was ran on a native-PAGE gel alongside a DNA standard in order to confirm the full-length encoded DNA is intact (**Supplementary Figure 18**).



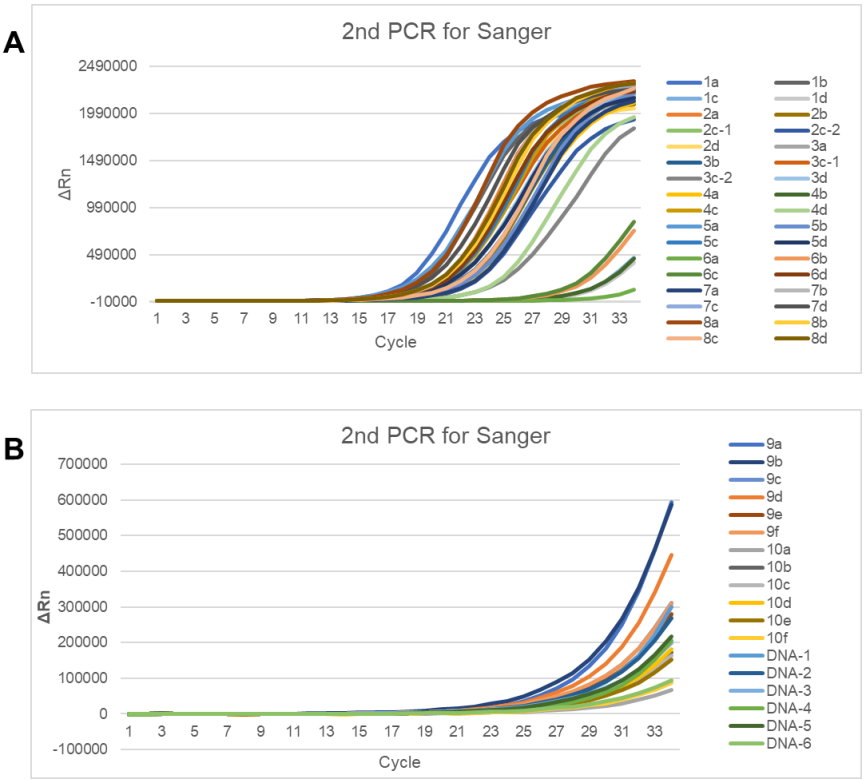
Supplementary Figure 18. A representative 6% native-PAGE gel from qPCR of the on-bead amplification. The gel was first pre-run for 60 minutes at 110 V. Lanes (from right to left) correspond to low molecular weight DNA ladder, positive control full-length DNA standard, negative control water lane, and representative samples from plate 1 (**1b** – **8b**). The red bar highlights the desired molecular weight band of interest corresponding to full-length DNA. The DNA band within the highlighted portion was excised and transferred to a 1.5 mL Eppendorf tube prefilled with 100 µL of a 100 mM Tris solution, which was tumbled at room temperature for 24 hours in order to elute the DNA out of the gel for subsequent PCR amplification for Sanger sequencing. DNA samples (5µL) were premixed with 6X gel loading dye (1 µL), added to the gel and ran for 60 minutes at 110 V. Nucleic acids were visualized via SYBR gold nucleic acid stain (10,000X diluted in 1X TAE buffer).

Procedure for the second qPCR for Sanger sequencing: Each excised DNA band from the on-bead amplification was subjected to a second qPCR in order to amplify the DNA tags for Sanger sequencing. Prior to the qPCR, a master mix solution was prepared (**Supplementary Table 9**).

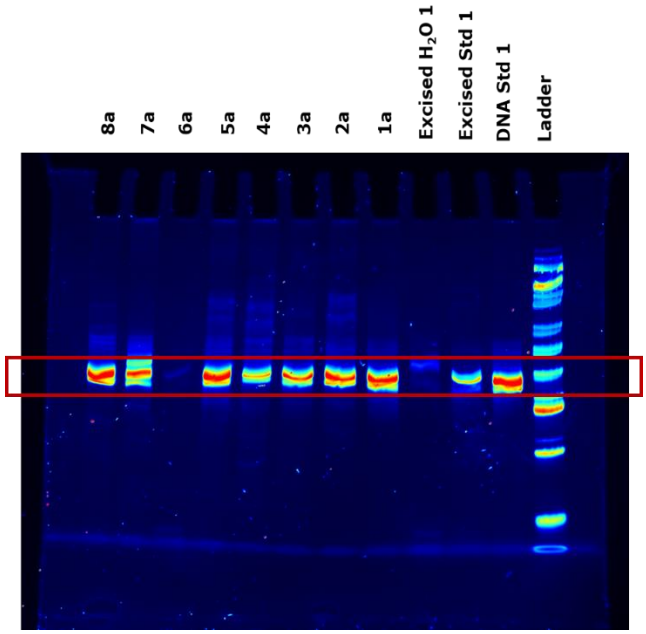
Reagent function in PCR	Reagents	Volume in PCR reaction (µL)	Stock Concentration
dNTPs	MM (1X)	5	10X
Reverse primer	ABM099 (0.3 µM)	0.75	20 µM
Forward primer	ABM096 (0.02 µM)	0.5	2 µM
Bridge primer	CES001 (0.28 µM)	0.7	20 µM
-	8% DMSO	4	-
-	Betaine (1 M)	10	5 M
Polymerase	*Taq*	0.5	5 U/µL
Nucleic acid stain	SYBR Green (2X)	1	100X
-	$ddiH_2O$	27.55	-
-	Total	50	-

Supplementary Table 9. Calculations for preparing the TaqMan polymerase master mix for the second qPCR for Sanger sequencing.

Each well of a PCR plate was added 48 µL of the master mix obtained above. The Eppendorf tubes containing the eluted DNA resulting from the gel excision were centrifuged and 2 µL of each sample were added to individual wells of the PCR plate containing the master mix. The plate was sealed and placed in a qPCR thermal cycler: holding stage (95 °C, 2 minutes), cycle stage (95 °C, 20 seconds, 52 °C, 15 seconds, 72 °C, 20 seconds; 34 cycles), then melt curve stage (72 °C, 2 minutes). The second PCR amplification was assessed by first analyzing the overall amplification of DNA fluorescence via nucleic acid staining by SYBR green. ΔRn was plotted against the cycle number and DNA amplification for each bead was identified.



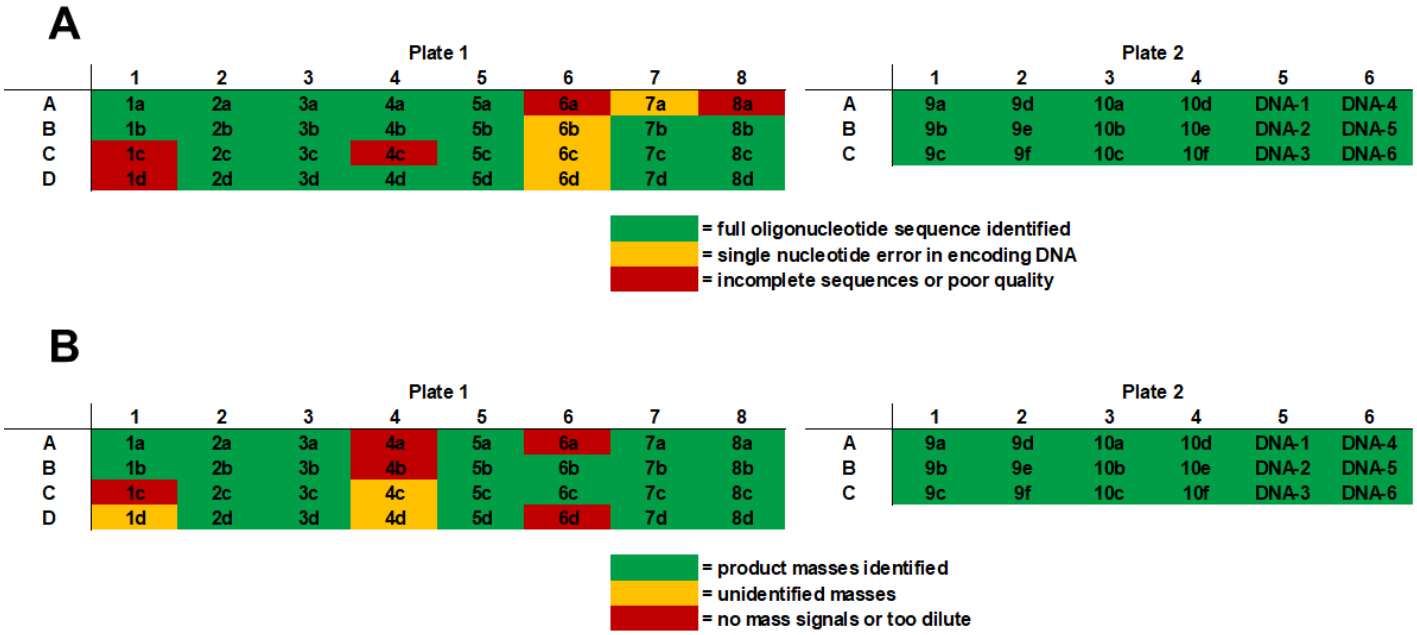
Supplementary Figure 19. Assessment of second DNA amplification. **A)** qPCR of each excised DNA band corresponding to compounds **1** – **8**. **B)** qPCR of each excised DNA band corresponding to compounds **9** – **10**.



Supplementary Figure 20. A representative 6% native-PAGE gel from the qPCR of DNA amplification. The gel was pre-run for 60 minutes at 110 V. Lanes (from right to left) correspond to low molecular weight DNA ladder, positive control full-length DNA standard, excised DNA standard from on-bead amplification, excised water band from on-bead amplification and representative samples from plate 1: **1a** – **8a**. The red bar highlights the desired molecular weight band of interest corresponding to the full-length encoding DNA. DNA samples (5µL) were premixed with 6X gel loading dye (1 µL) and added to the gel, which was run for 60 minutes at 110 V. Nucleic acids were visualized via SYBR gold nucleic acid stain (10,000X diluted in 1X TAE buffer).The entire solution of amplified DNA product from the second PCR (45 µL) was purified via Qiagen MinElute PCR purification kit (250) prior to Sanger sequencing.

Procedure for the acidic cleavage of encoded molecules from the TentaGel resin: Following PCR amplification for Sanger sequencing, each individual 160 µm bead was transferred to a 96-well round bottom plate containing 150 µL of

ddiH_2O. The wells were agitated, then 125 µL of ddiH_2O was removed. Fresh ddiH_2O was then added (125 µL), the well was mixed, then 125 µL was removed. Next, 150 µL of EtOH was added, the well was mixed, and the entire solution was removed (while leaving the 160 µm bead in the well). An additional wash with 150 µL of EtOH was performed, the solvent was carefully removed, and the plate solvent was evaporated. To cleave the molecule from the resin, 50 µL of a 49:49:2 TFA:DCM:TIPS solution was added, and the plate was shaken at room temperature for 40 minutes. The solvents were evaporated and to each well was added 5 µL of α-cyano-4-hydroxycinnamic acid (CHCA) MALDI matrix. The solution was mixed and 0.75 µL was removed and spotted on a corresponding MALDI plate for analysis.

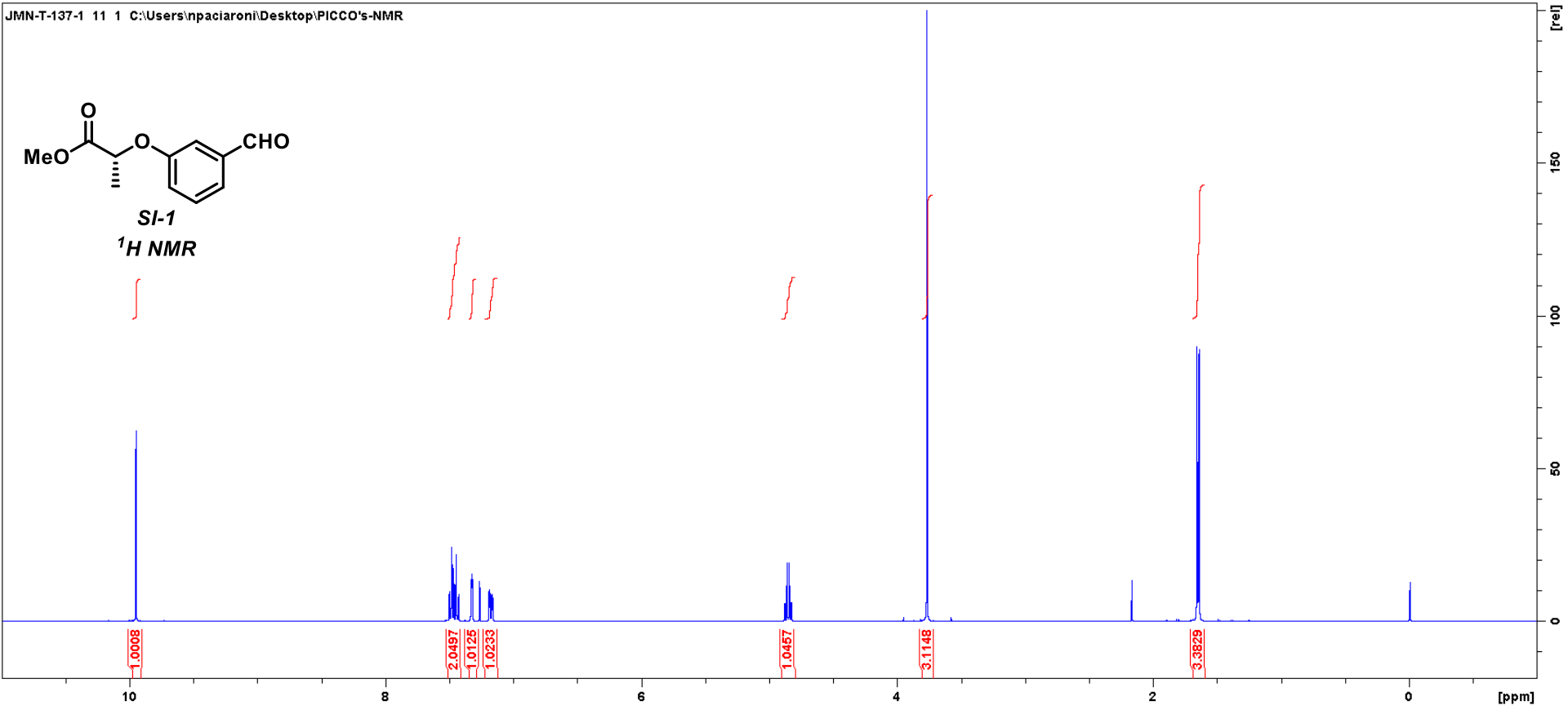


Supplementary Figure 21. Overview of quality control analysis of the parallel synthesis of DNA-encoded small molecules. **A)** Results of Sanger sequencing of each 160 µm bead. Of the 50 beads analyzed, 45 beads (90%) were fully sequenced and contained the full-length encoding DNA (including beads with single nucleotide errors). **B)** Results of MALDI analysis from compounds cleaved from the TentaGel resin. Of the 50 beads that were cleaved for MALDI analysis, 42 beads (84%) contained the appropriate molecular weight of the desired encoded product.

IX. References

1) Minguet M, Amabilino DB, Cirujeda J, Wurst K, Mata II, Molins E, Novoa JJ, Veciana J. Stereochemistry of phenyl alpha-nitronyl nitroxide radicals. *Chemistry* **2000**, *6*, 2350.
2) Mendes KR, Malone ML, Ndungu JM, Suponitsky-Kroyter I, Cavett VJ, McEnaney PJ, MacConnell AB, Doran TM, Ronacher K, Stanley K, Utset O, Walzl G, Paegel BM, Kodadek T. High-throughput identification of DNA-encoded IgG ligands that distinguish active and latent Mycobacterium tuberculosis infections. *ACS Chem. Biol.* **2017**, *12*, 234.
3) Malone ML, Paegel BM. What is a “DNA-compatible” reaction? *ACS Comb. Sci.* **2016**, *18*, 182.

X. NMR Spectra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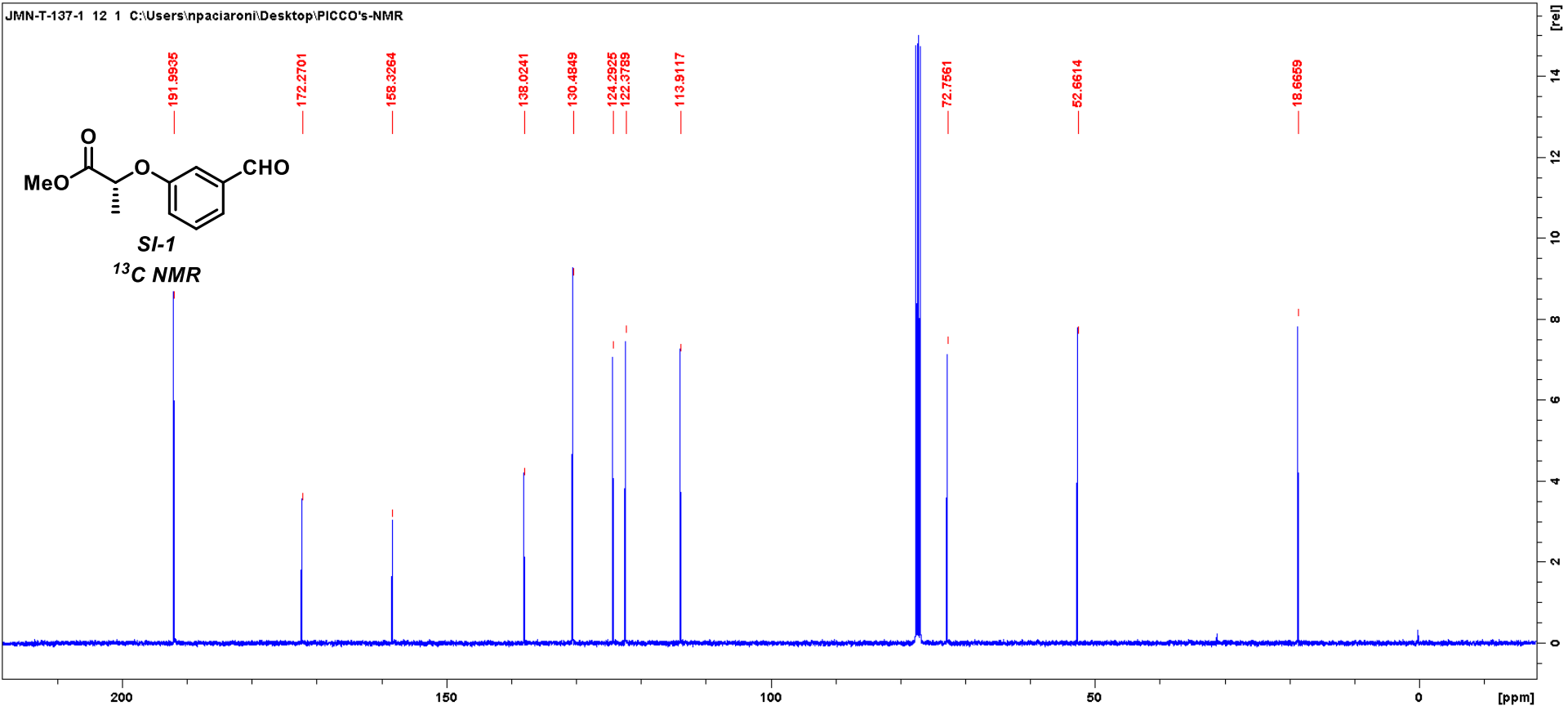
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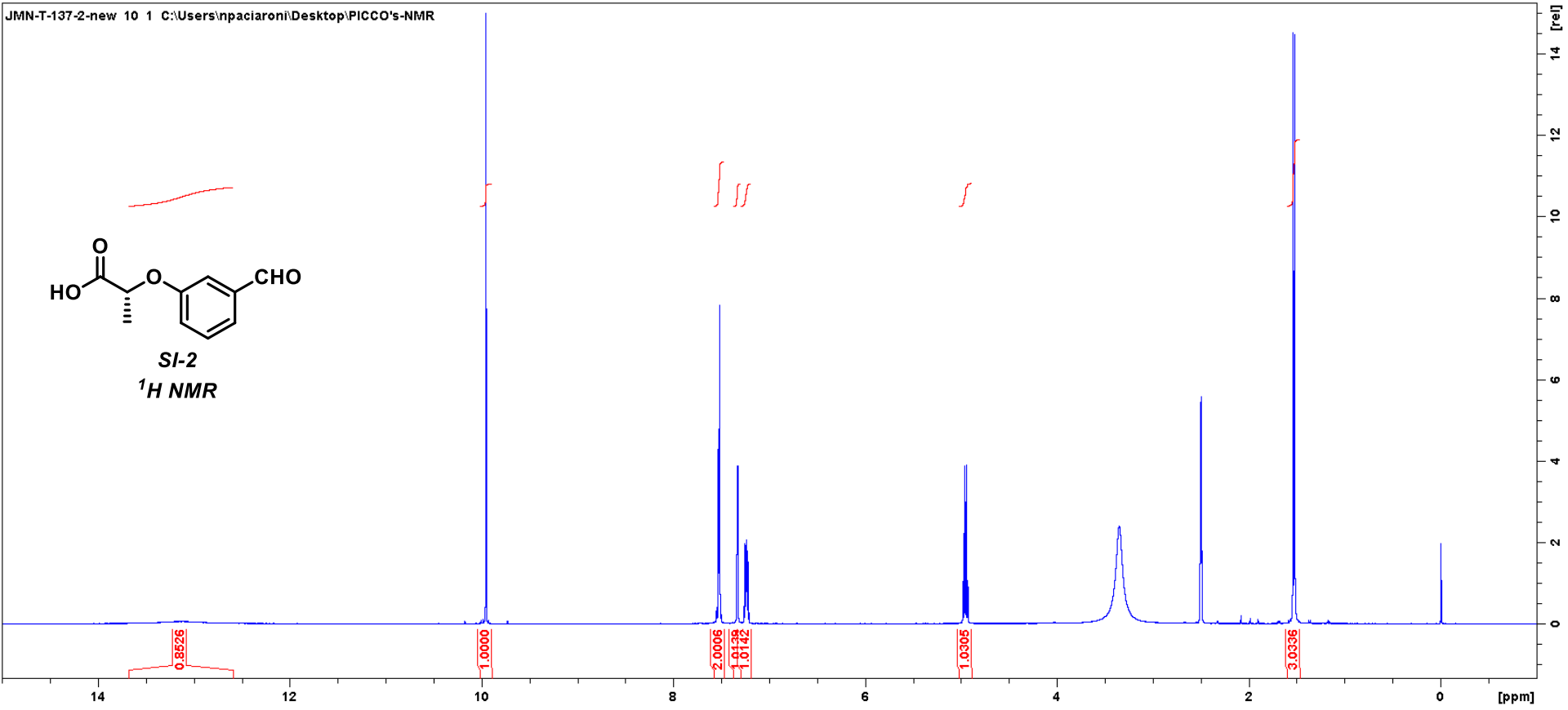
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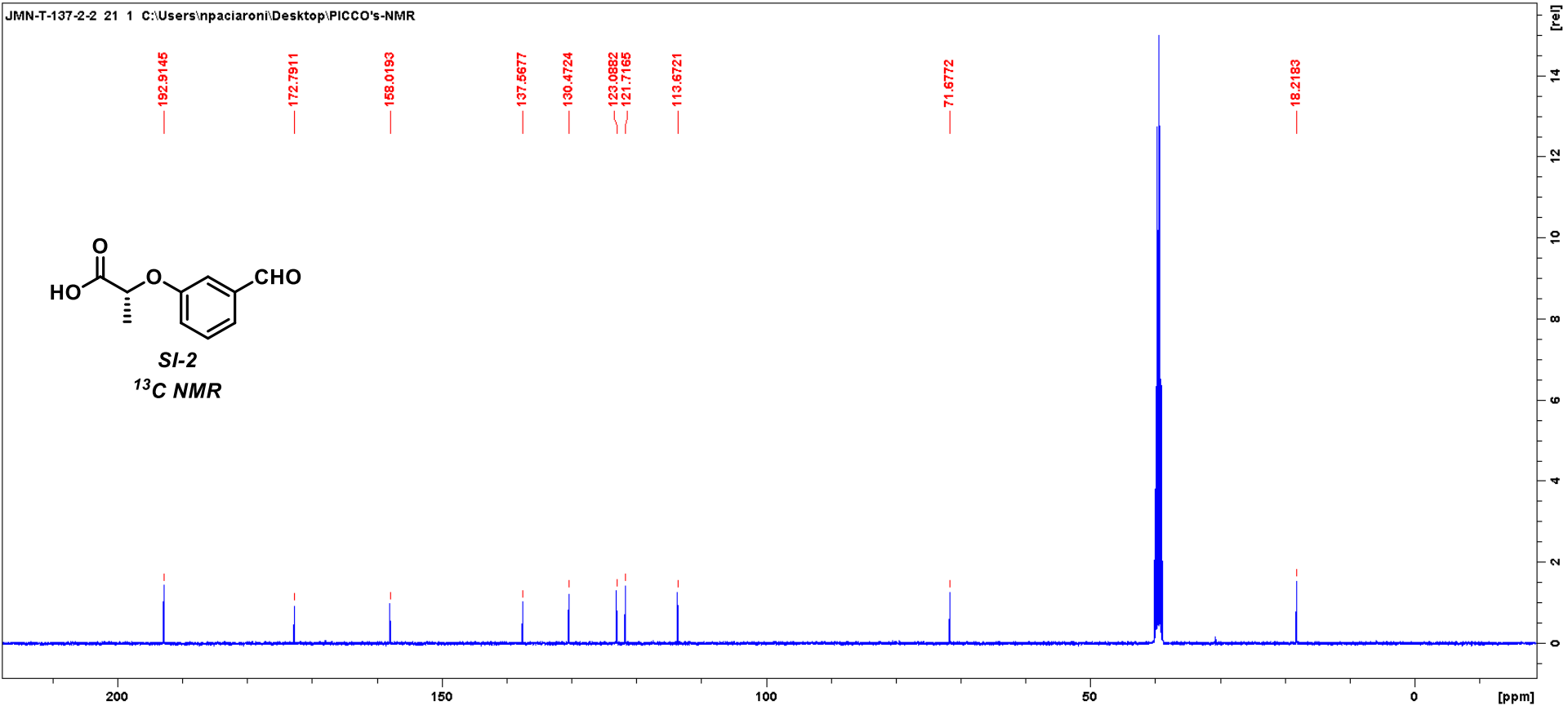
NMR3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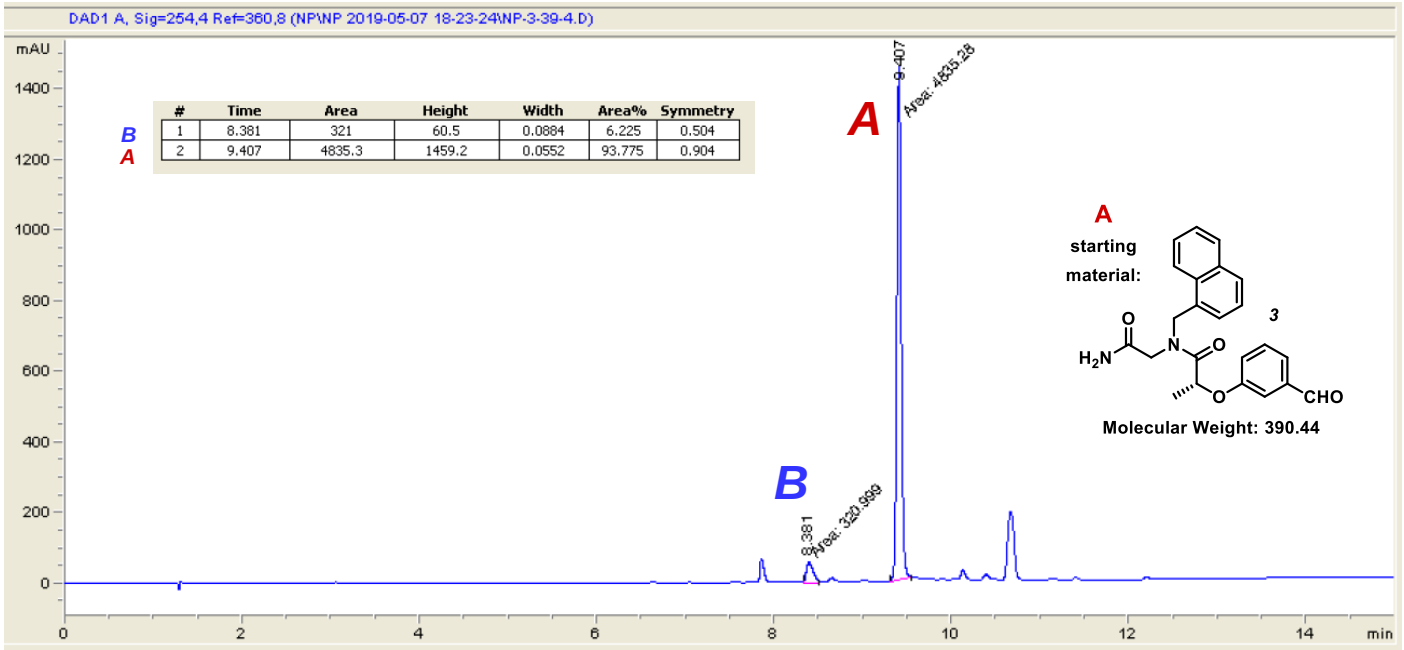
Crude LC spectrum of ***4A***.



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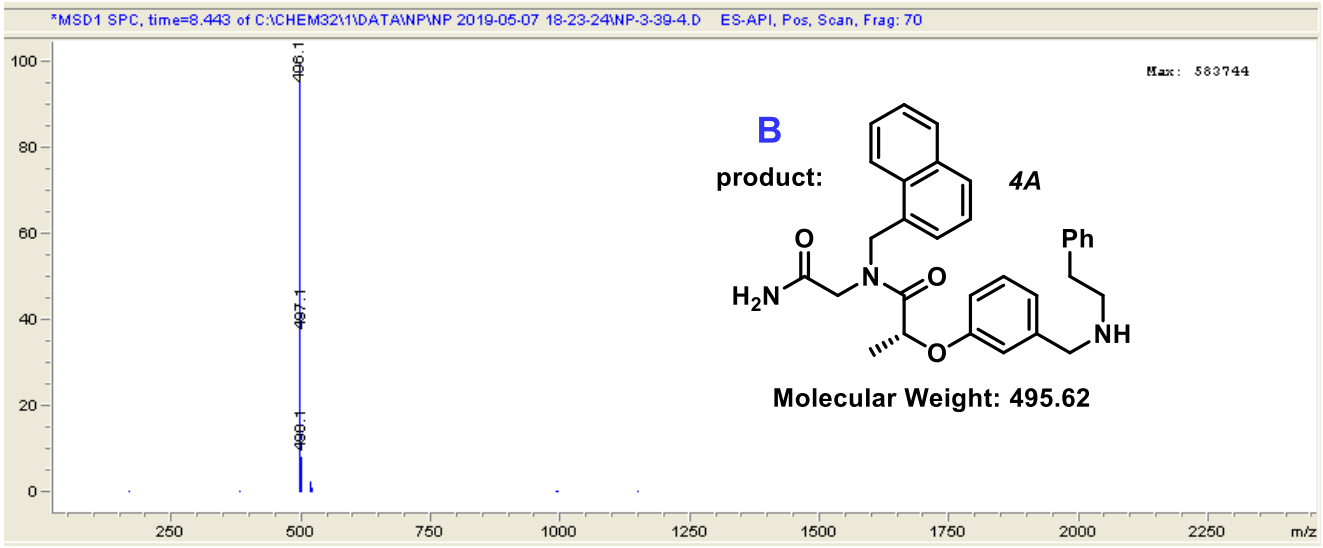
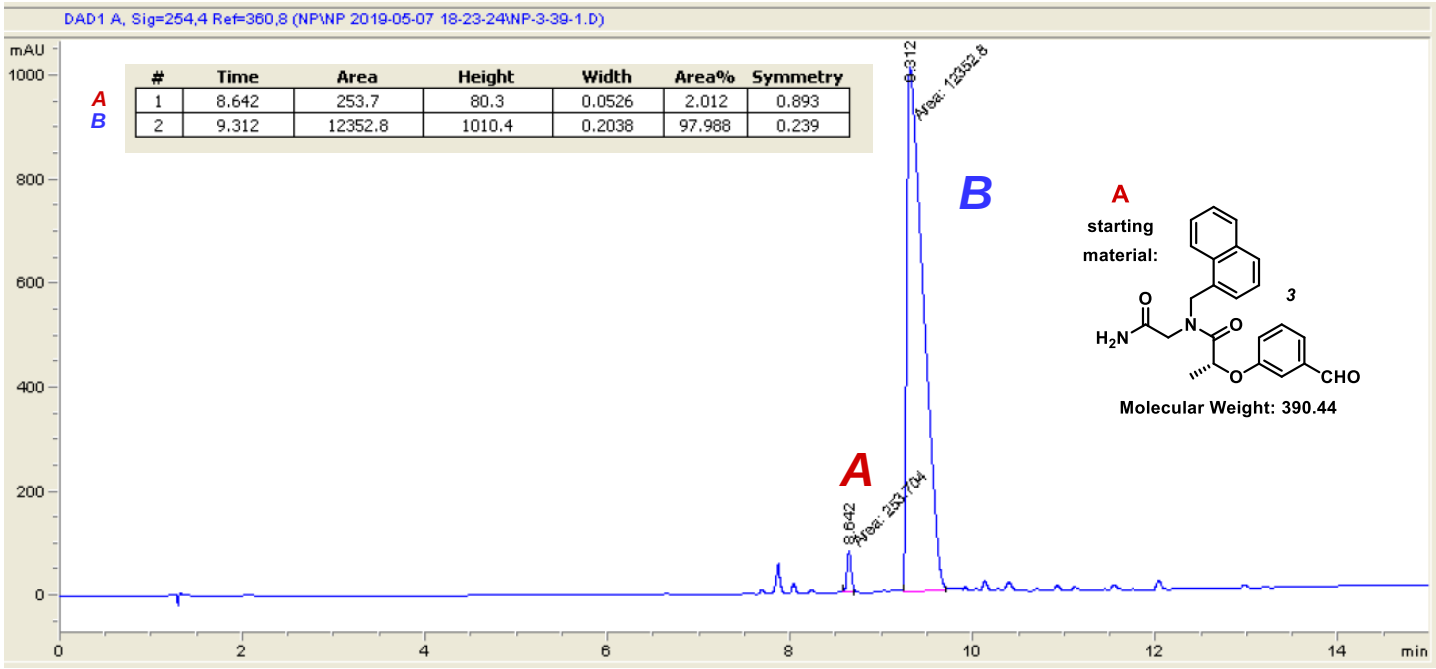
Corresponding MS spectrum for product peak (***B***) at 8.44 minutes.



Crude LC spectrum of ***4C***.



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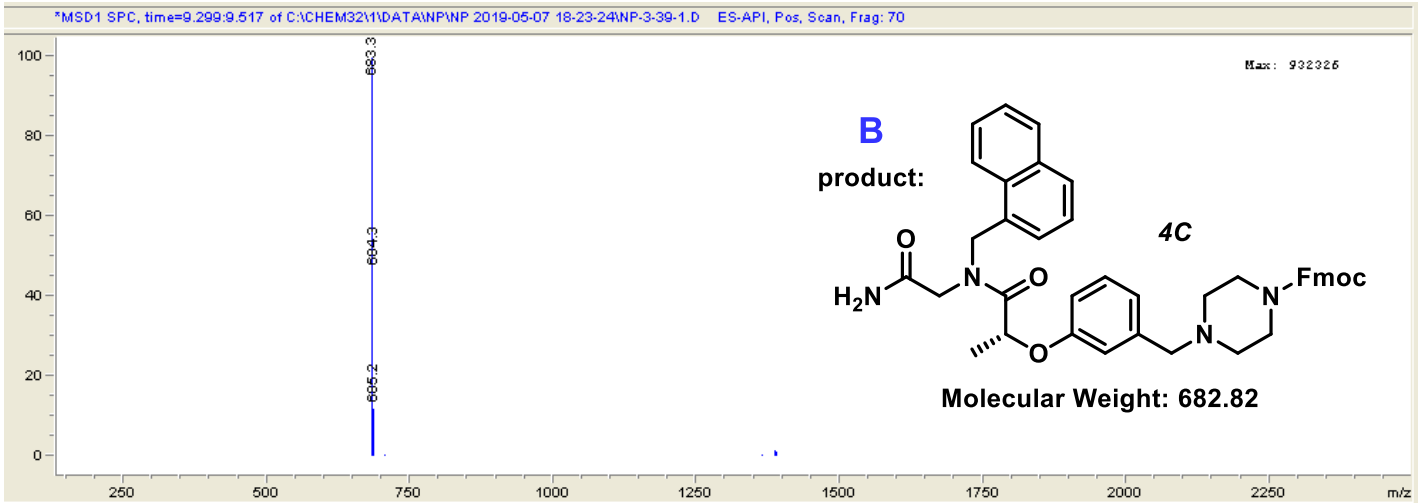
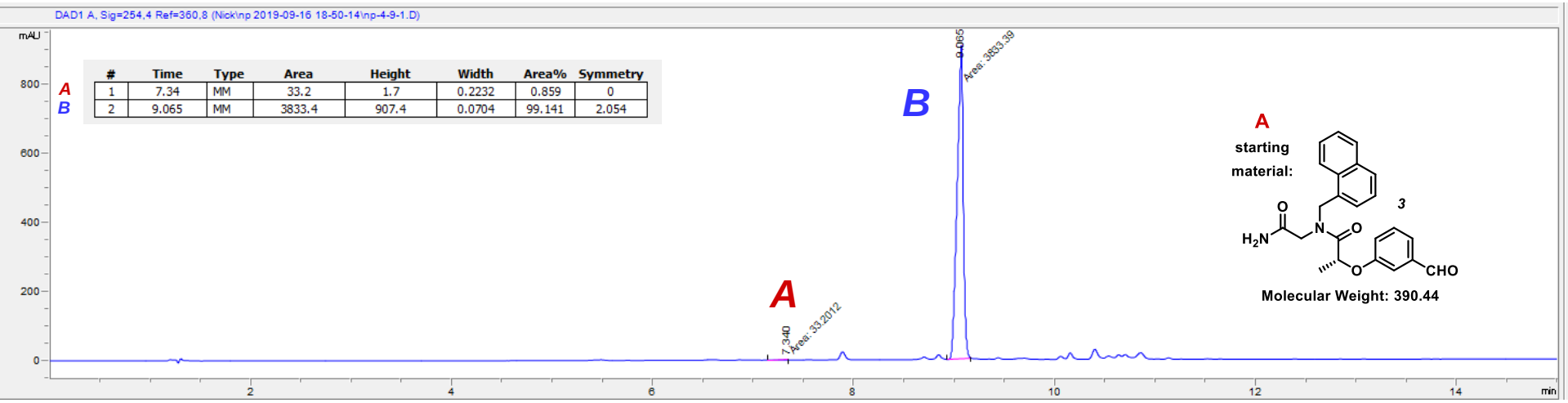
Corresponding MS spectrum for product peak (***B***) at 9.30 minutes.



Crude LC spectrum of ***4D***.



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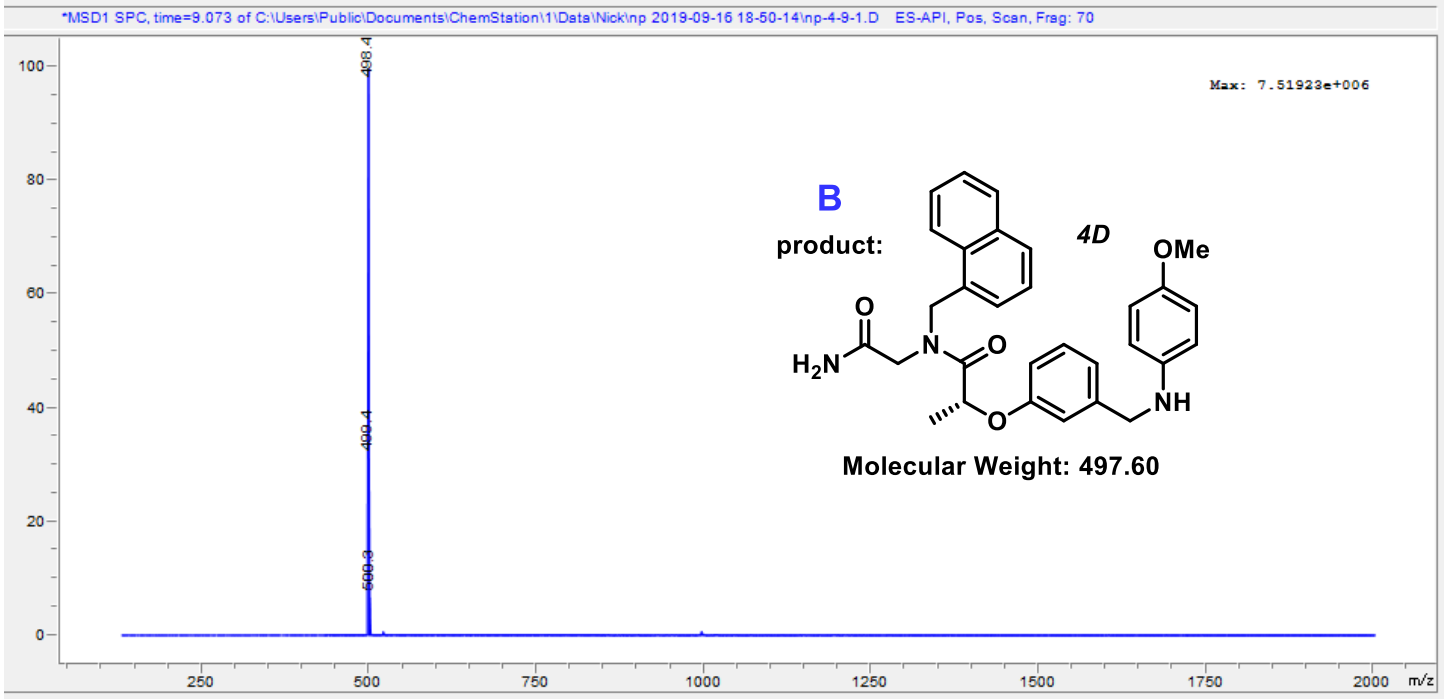
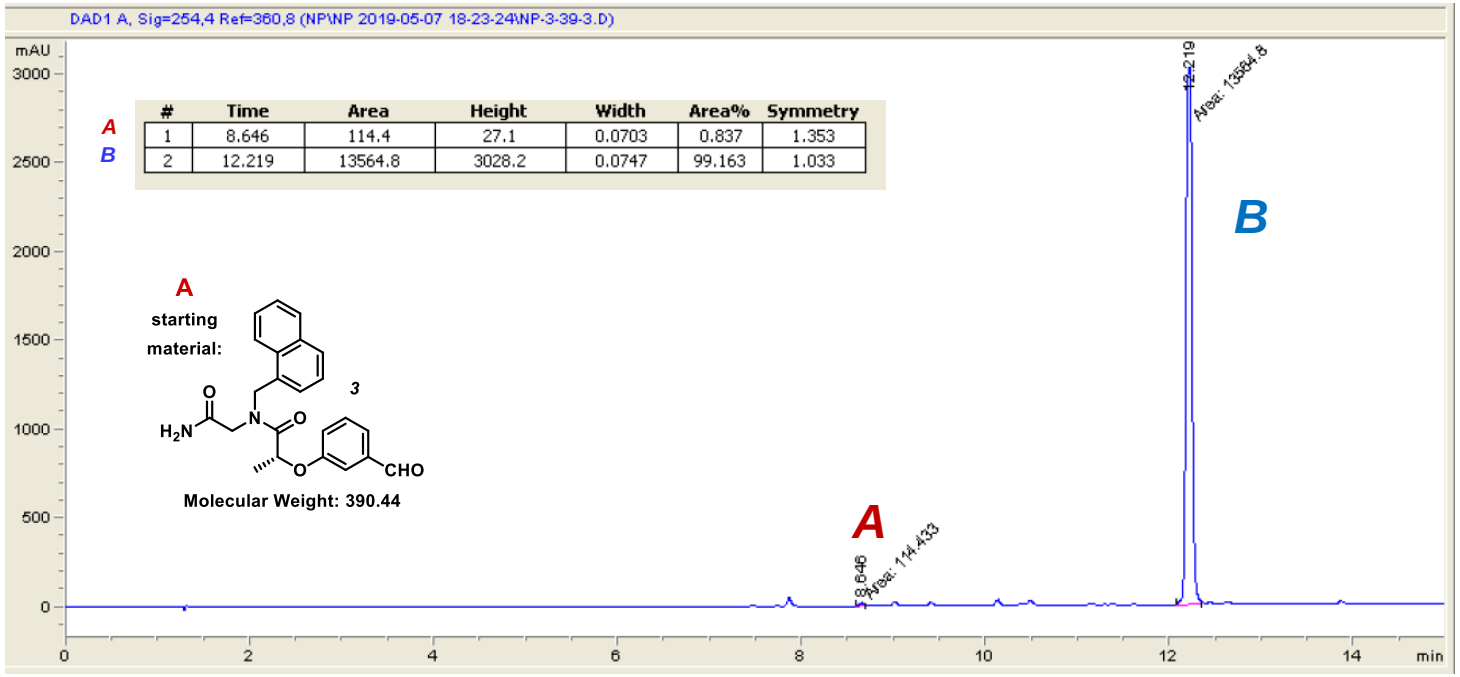
1	7.34	MM	33.2	1.7	0.2232	0.859	0
2	9.065	MM	3833.4	907.4	0.0704	99.141	2.054

Corresponding MS spectrum for product peak (***B***) at 9.07 minutes.



Crude LC spectrum of ***4E***.



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1	8.646	114.4	27.1	0.0703	0.837	1.353
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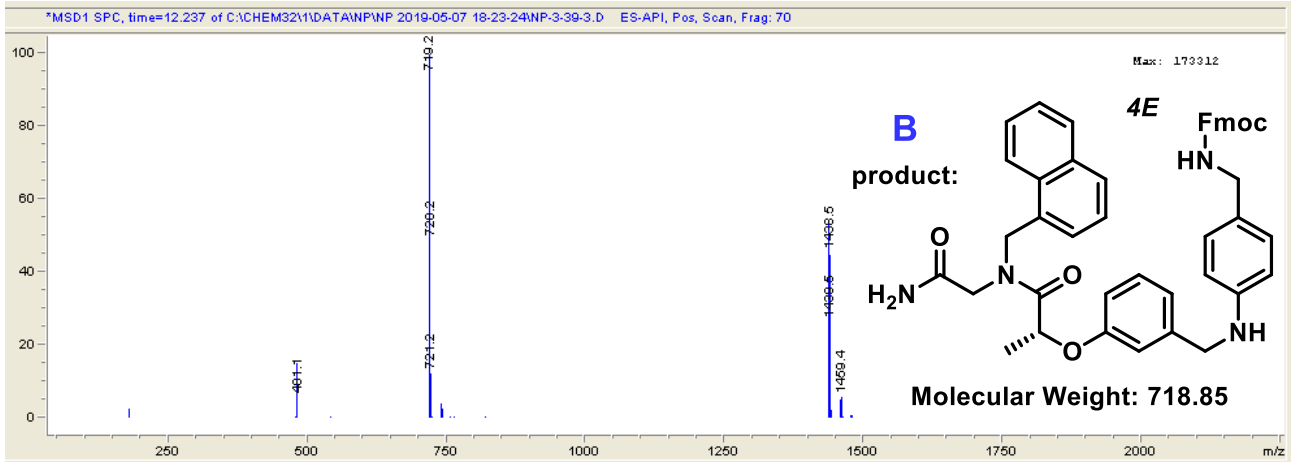
Corresponding MS spectrum for product peak (***B***) at 12.24 minutes.



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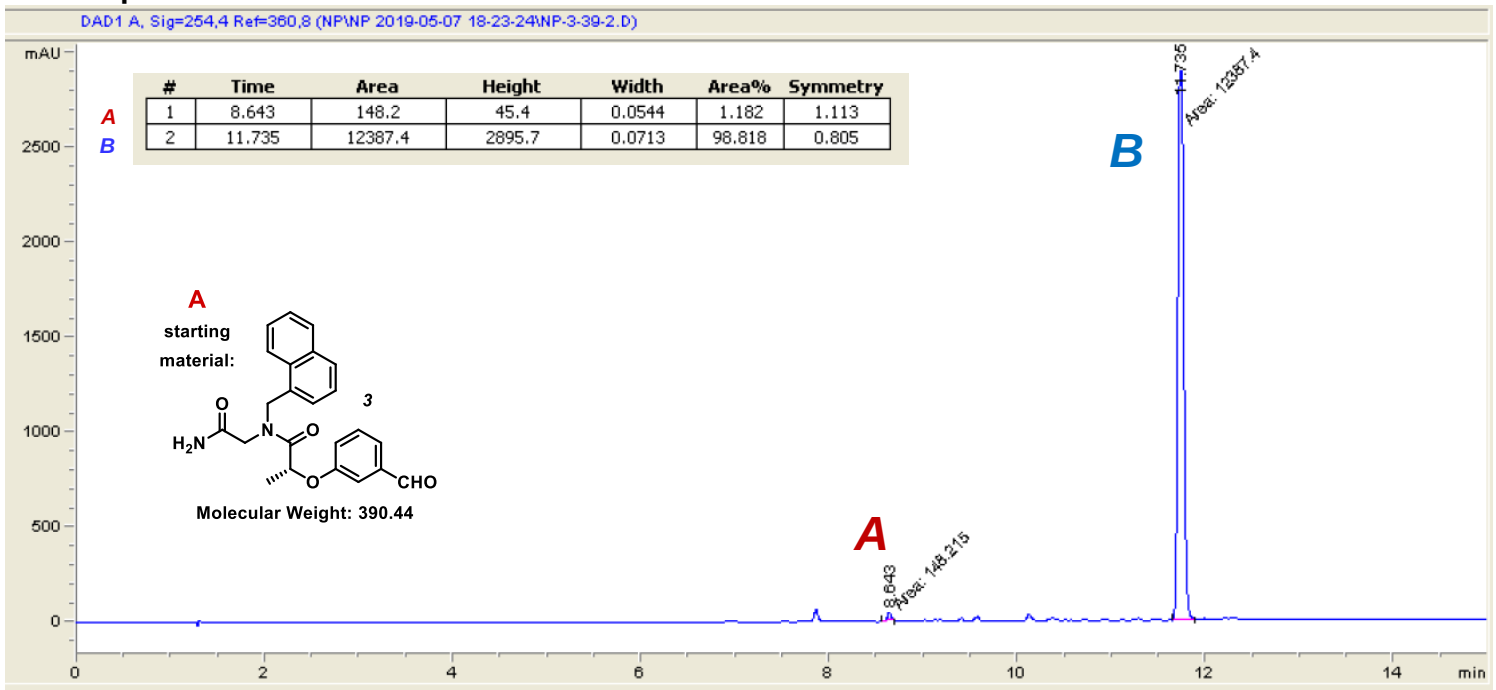

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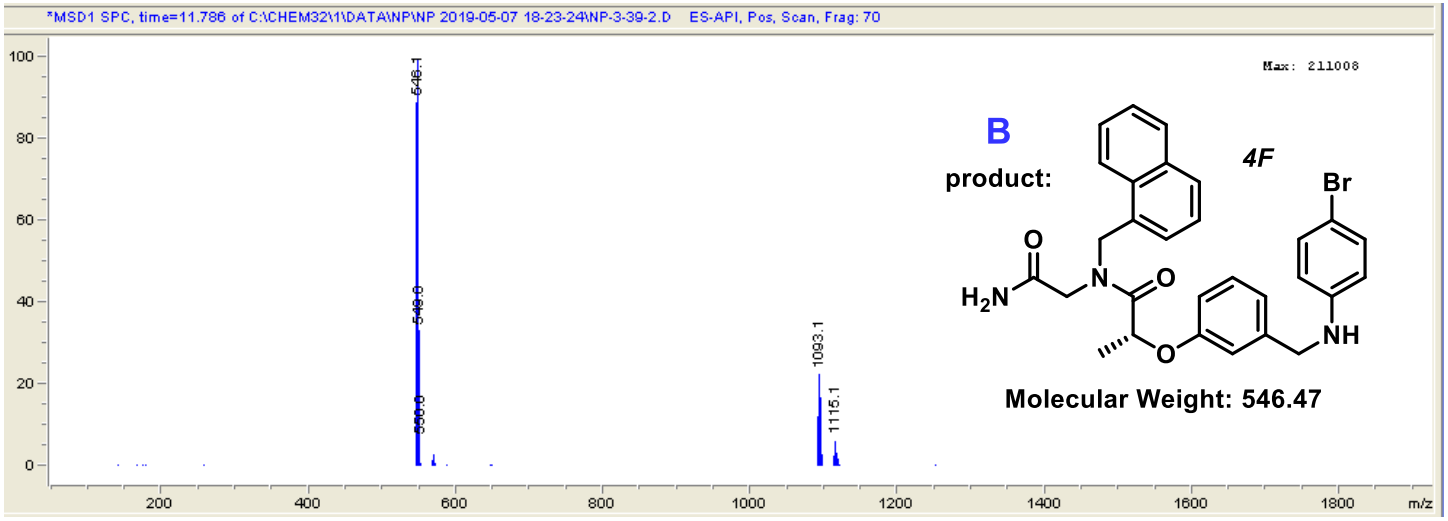
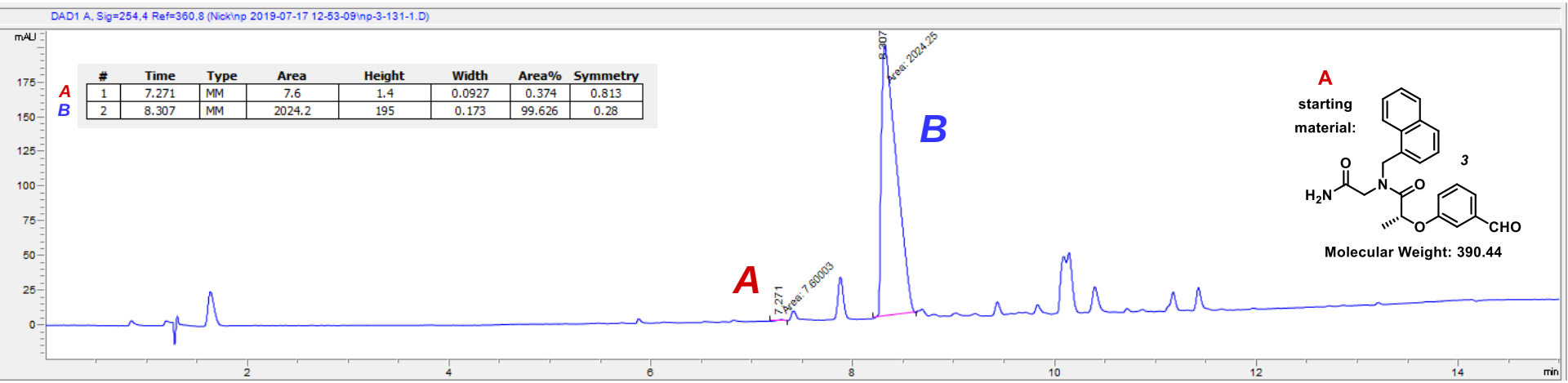
1	8.643	148.2	45.4	0.0544	1.182	1.113
2	11.735	12387.4	2895.7	0.0713	98.818	0.805

Corresponding MS spectrum for product peak (***B***) at 11.79 minutes.



Crude LC spectrum of ***5A***.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.271	MM	7.6	1.4	0.0927	0.374	0.813
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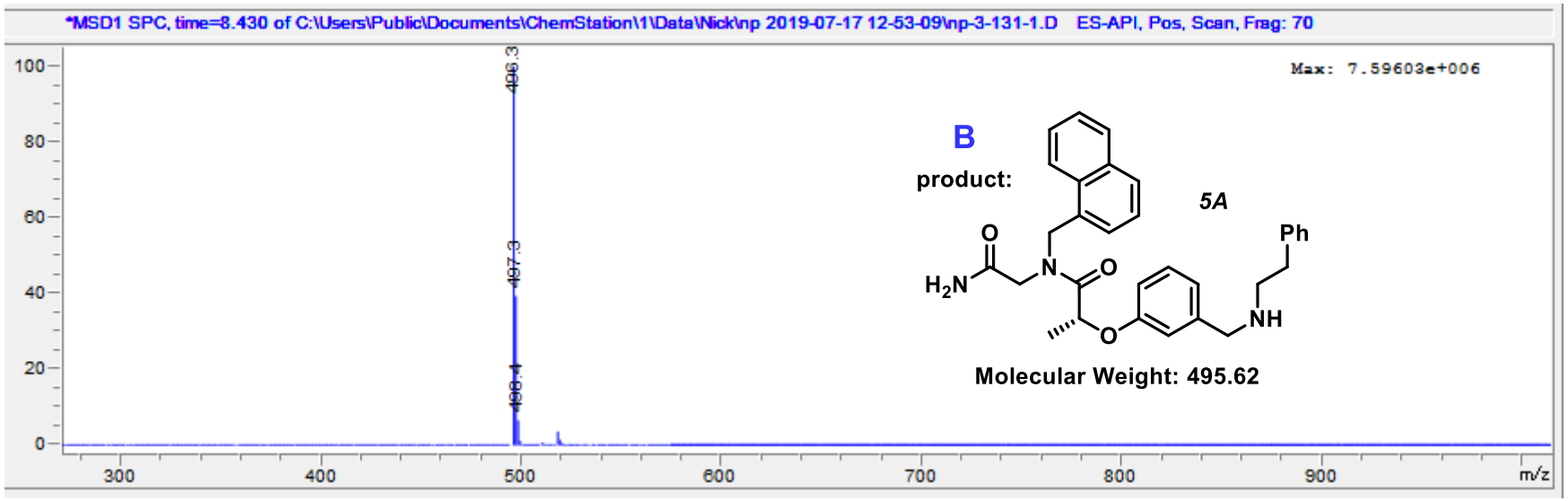
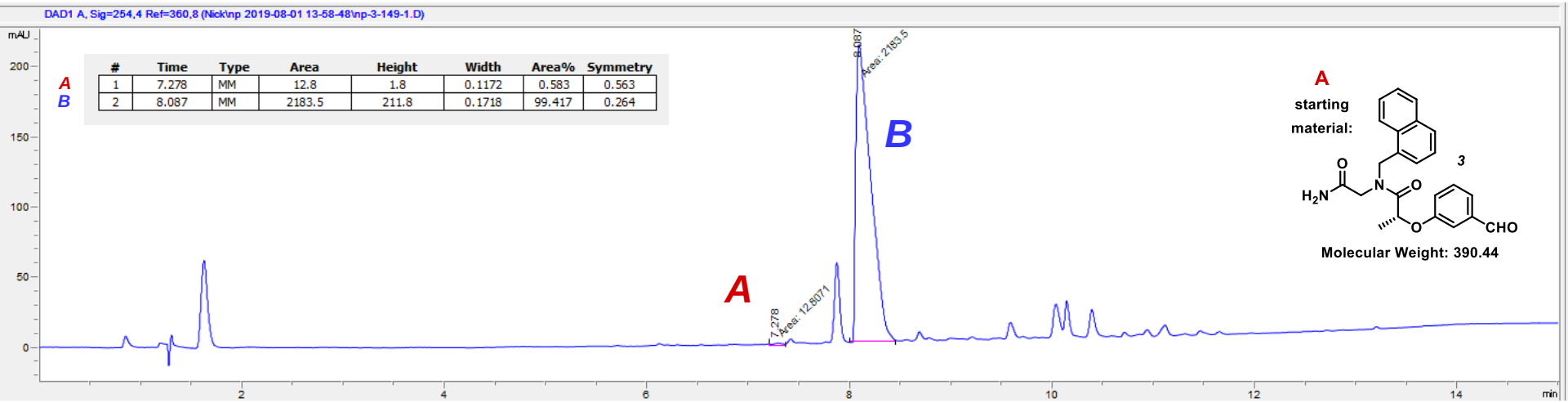
Corresponding MS spectrum for product peak (***B***) at 8.43 minutes.



Crude LC spectrum of ***5B***.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.278	MM	12.8	1.8	0.1172	0.583	0.563
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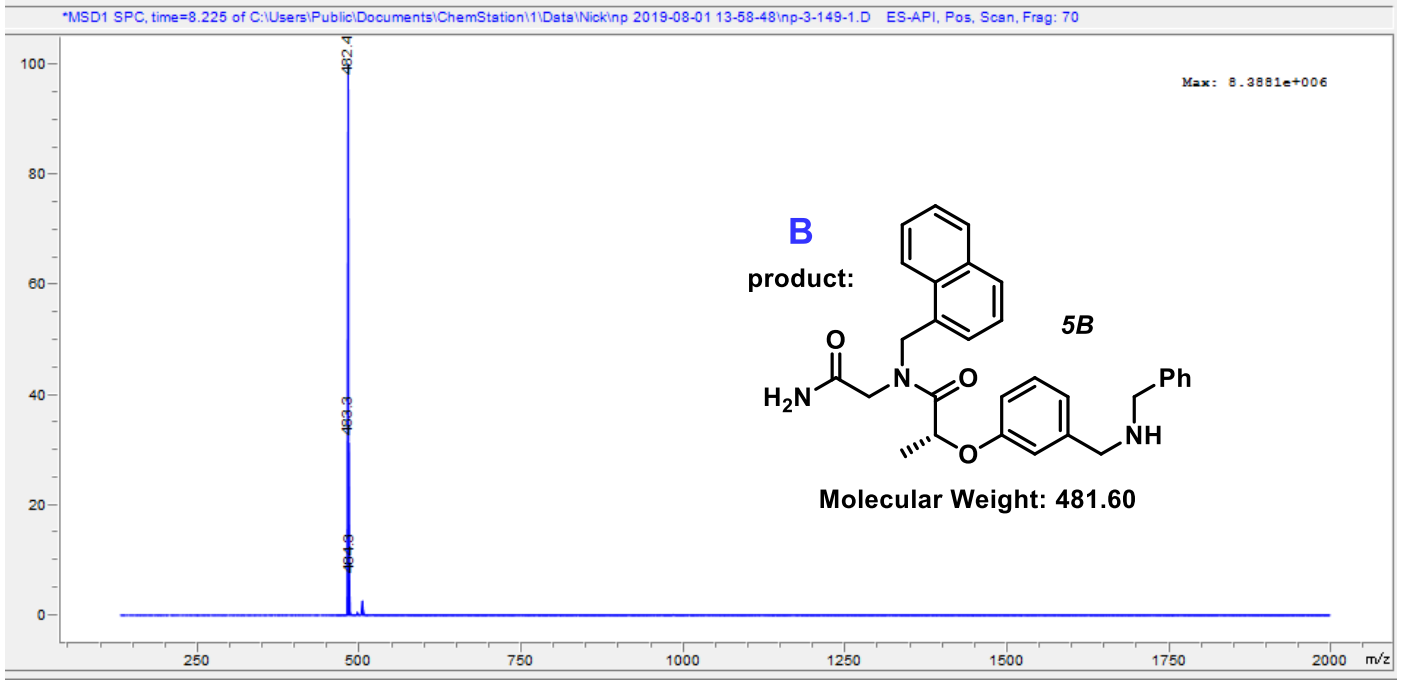
Corresponding MS spectrum for product peak (***B***) at 8.23 minutes.



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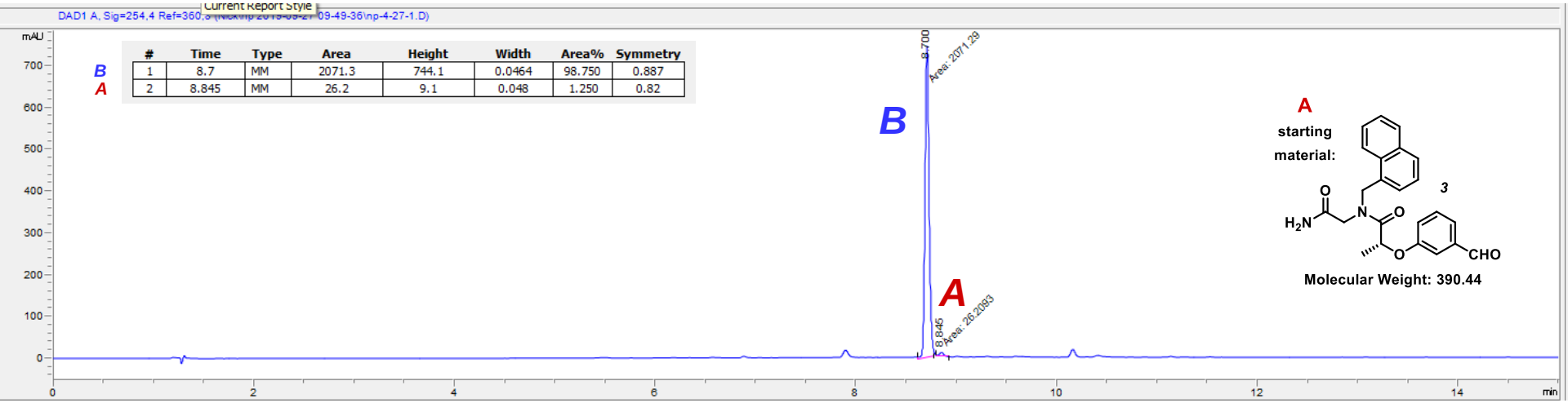
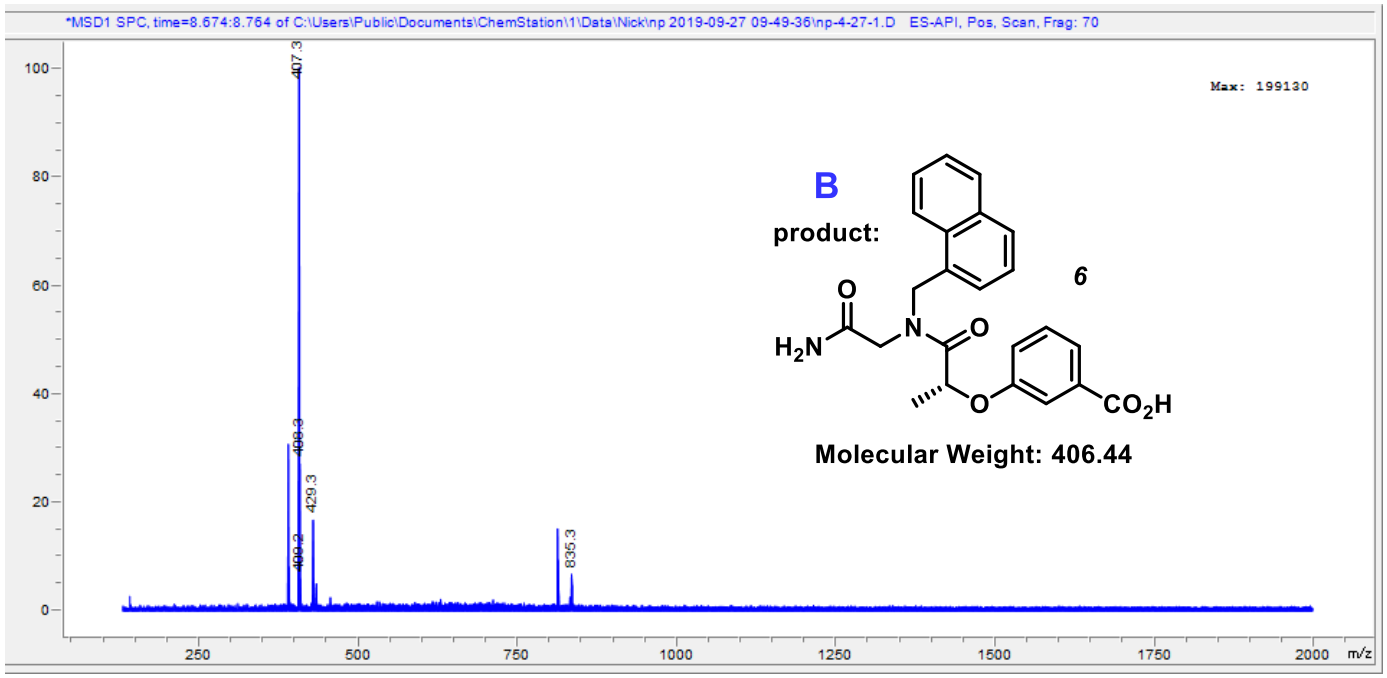
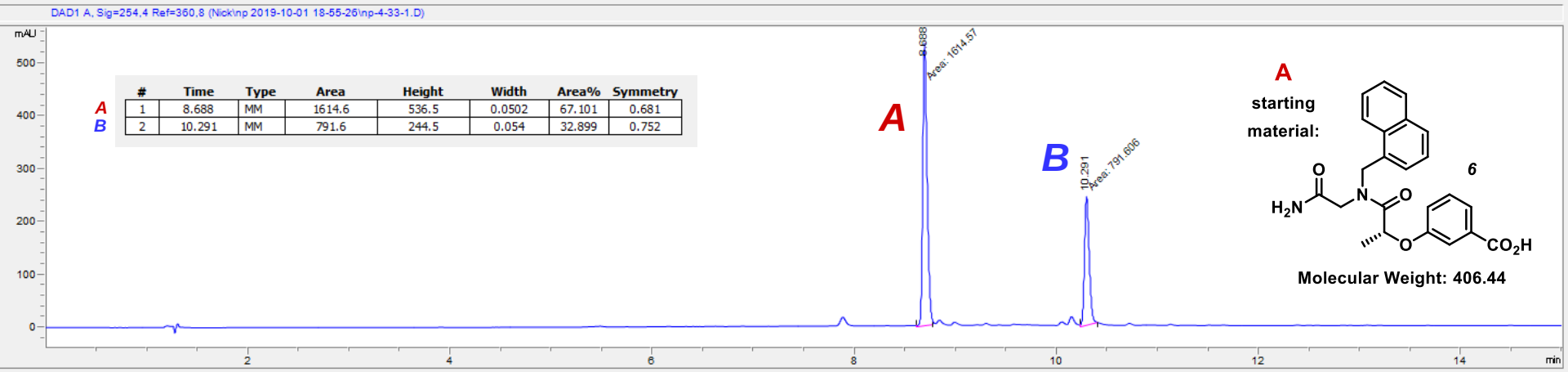


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.7	MM	2071.3	744.1	0.0464	98.750	0.887
2	8.845	MM	26.2	9.1	0.048	1.250	0.82

Corresponding MS spectrum for product peak (***B***) at 8.76 minutes.

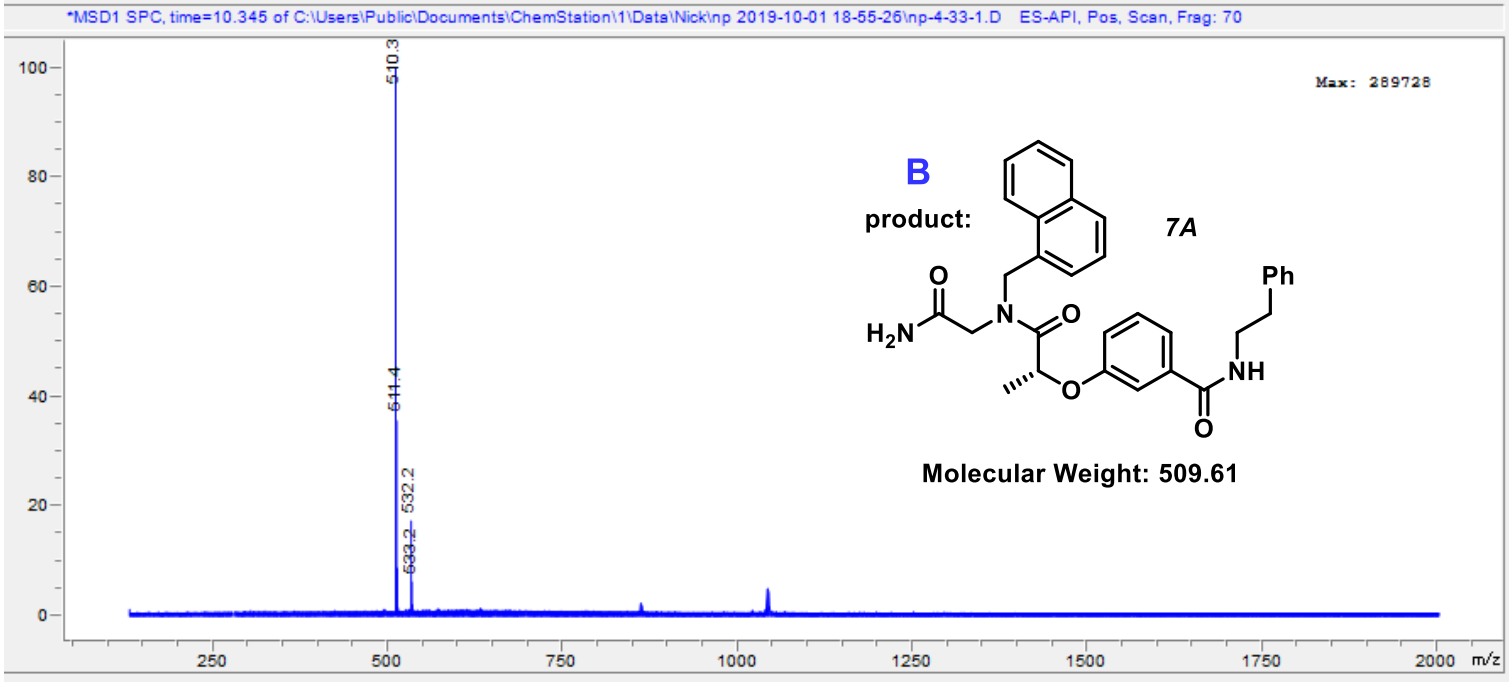


Crude LC spectrum of ***7A***.

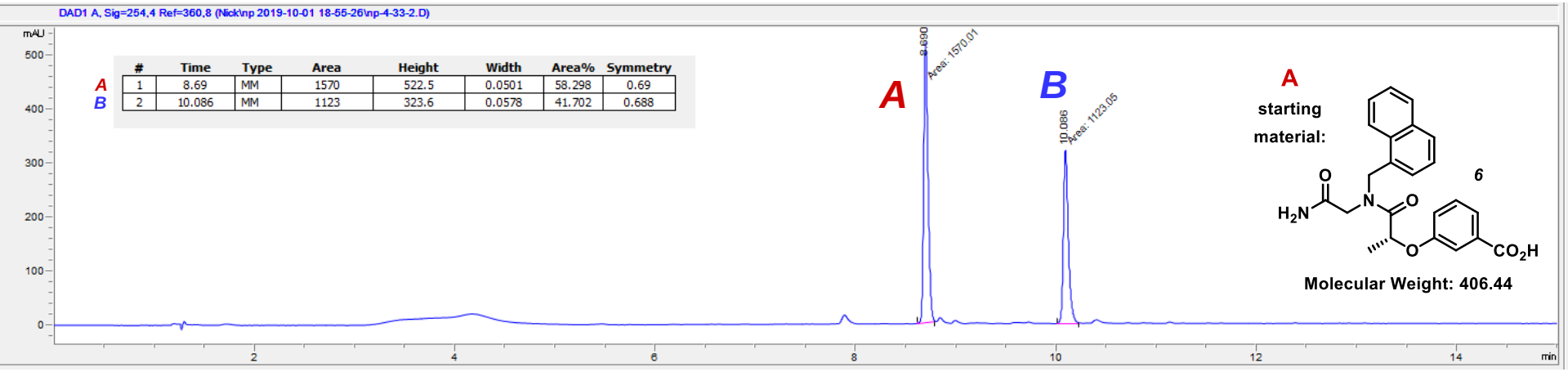


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.688	MM	1614.6	536.5	0.0502	67.101	0.681
2	10.291	MM	791.6	244.5	0.054	32.899	0.752

Corresponding MS spectrum for product peak (***B***) at 10.29 minutes.



Crude LC spectrum of ***7B***.



#	Time	Type	Area	Height	Width	Area%	Symmetry
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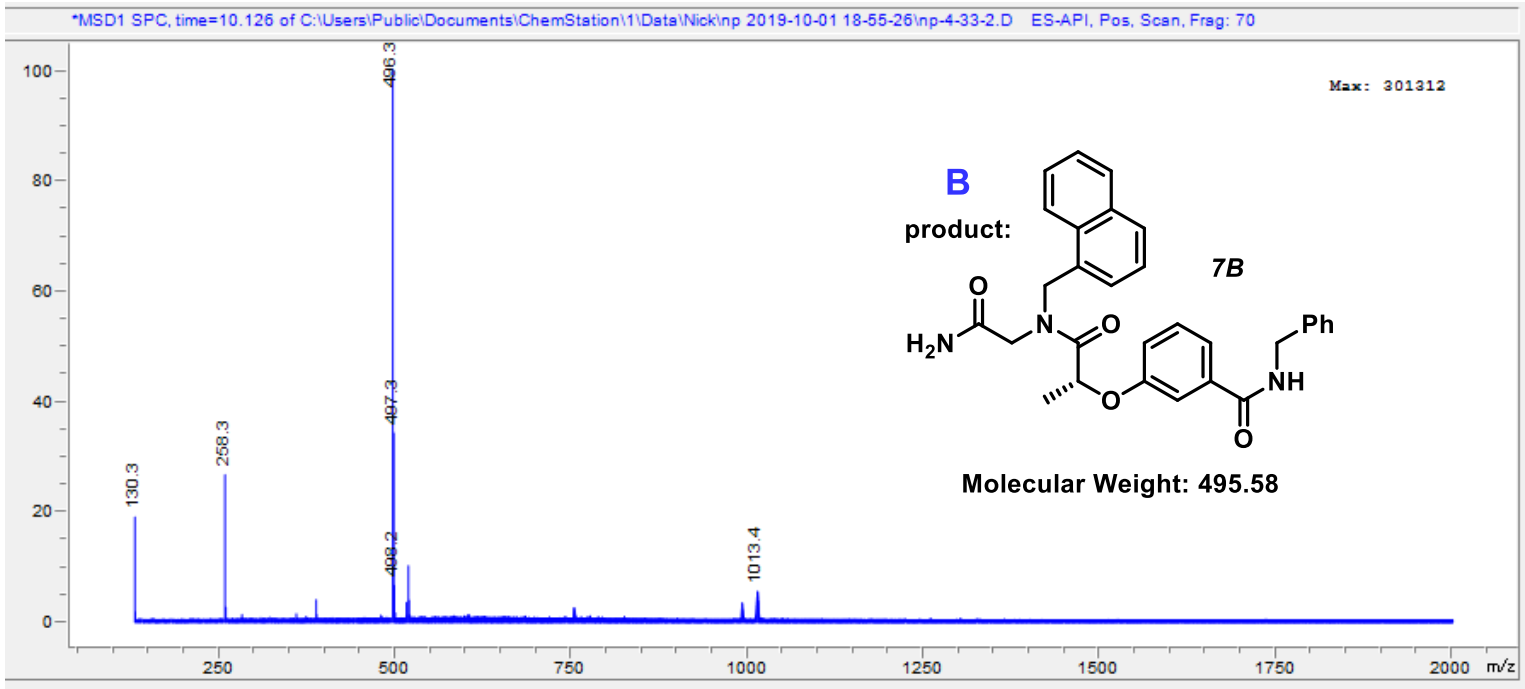
Corresponding MS spectrum for product peak (***B***) at 10.09 minutes.



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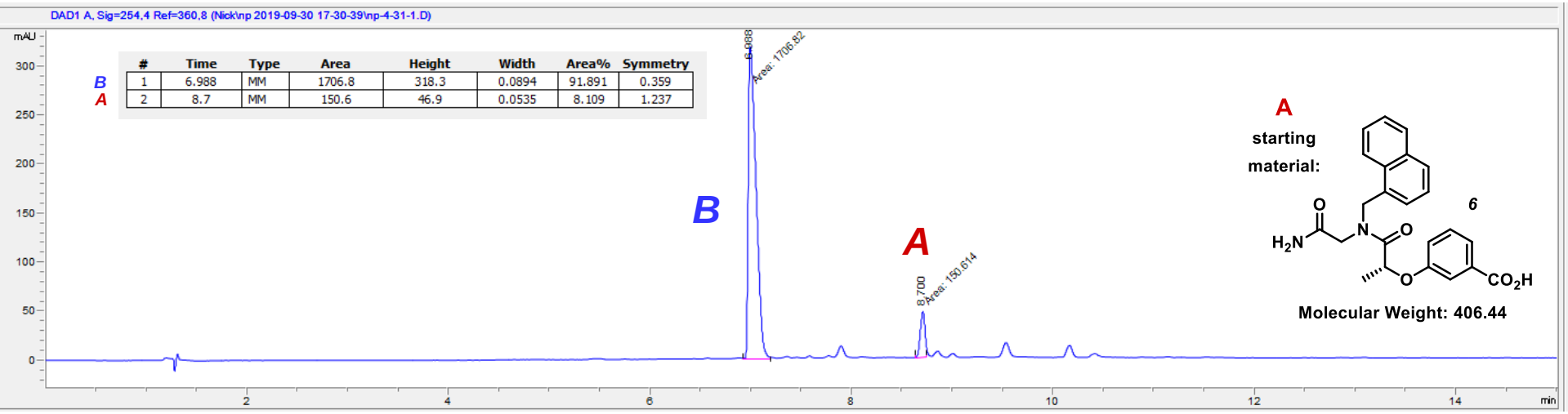

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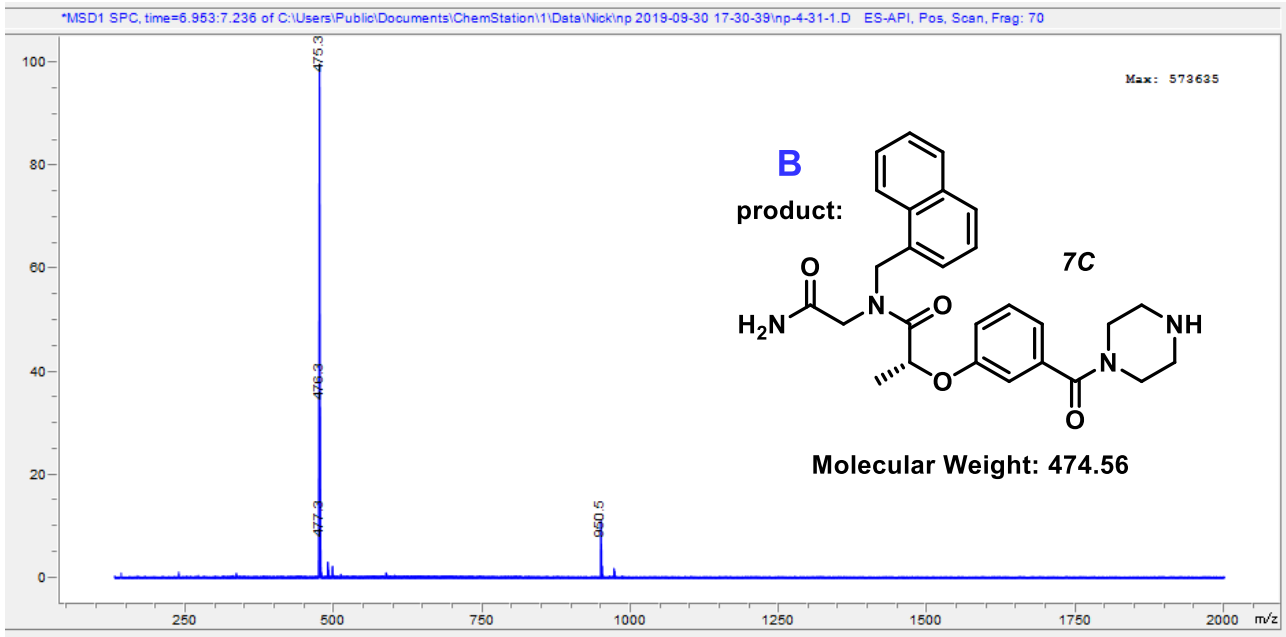
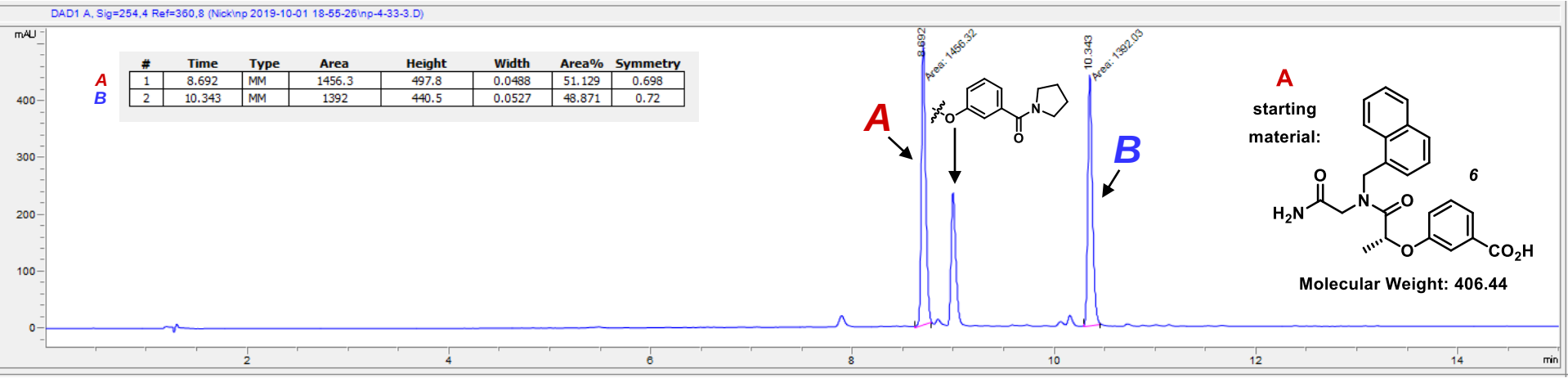
Corresponding MS spectrum for product peak (***B***) at 6.95 minutes.

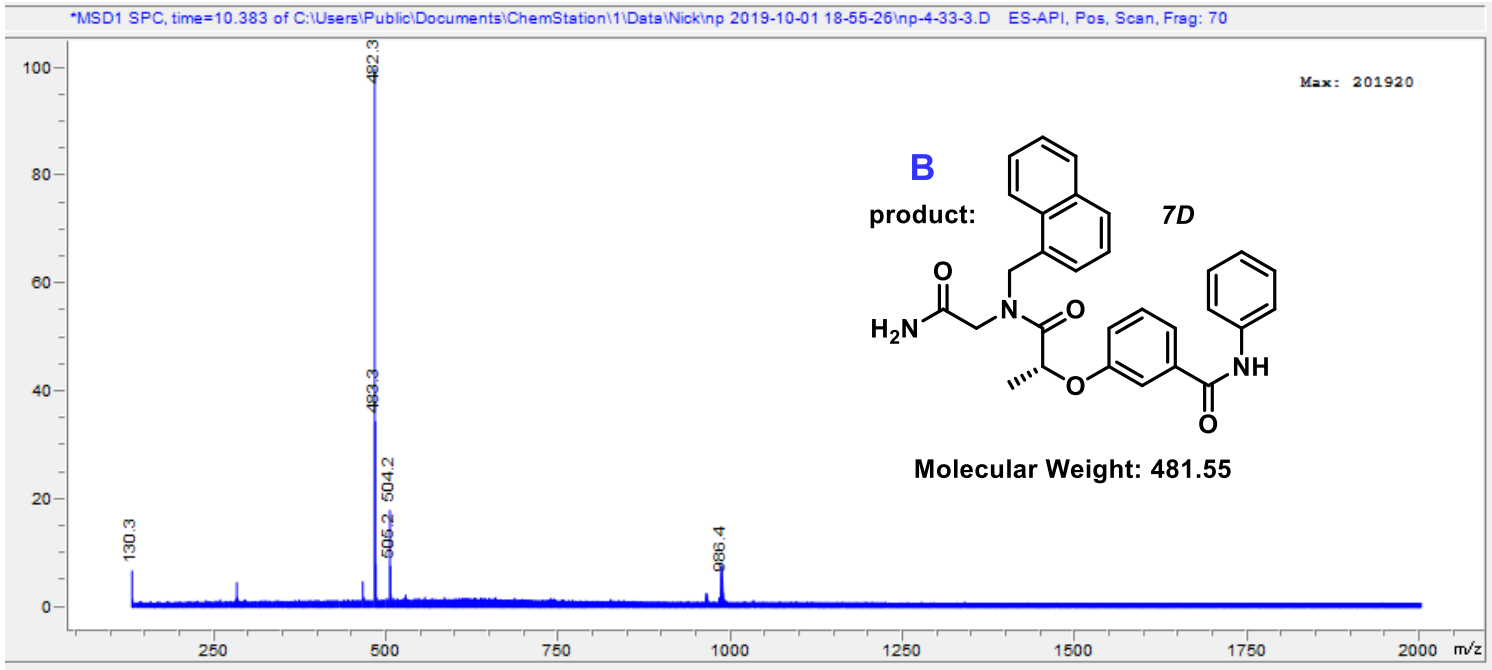


Crude LC spectrum of ***7D***.

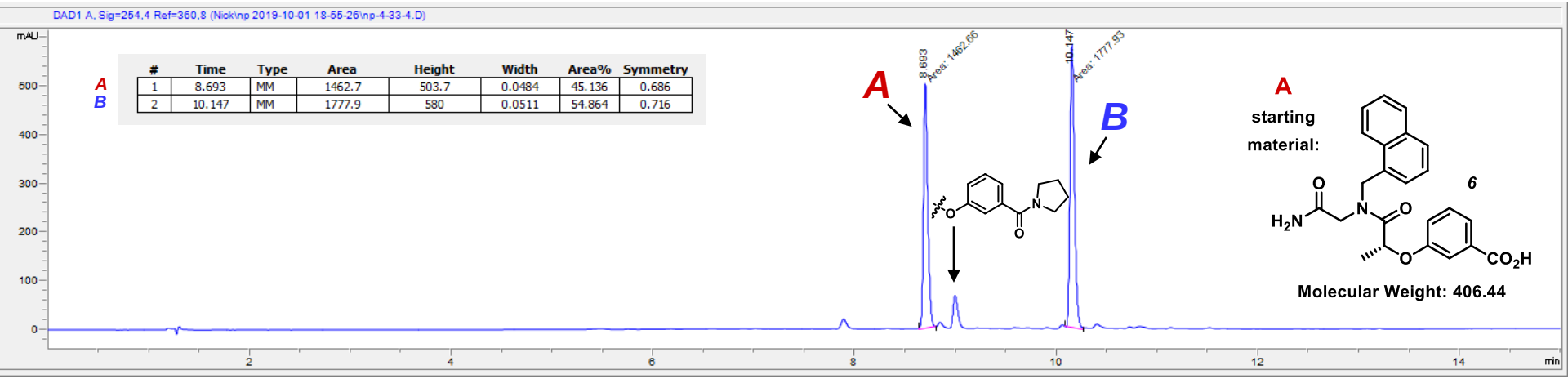


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.692	MM	1456.3	497.8	0.0488	51.129	0.698
2	10.343	MM	1392	440.5	0.0527	48.871	0.72

Corresponding MS spectrum for product peak (***B***) at 10.38 minutes.

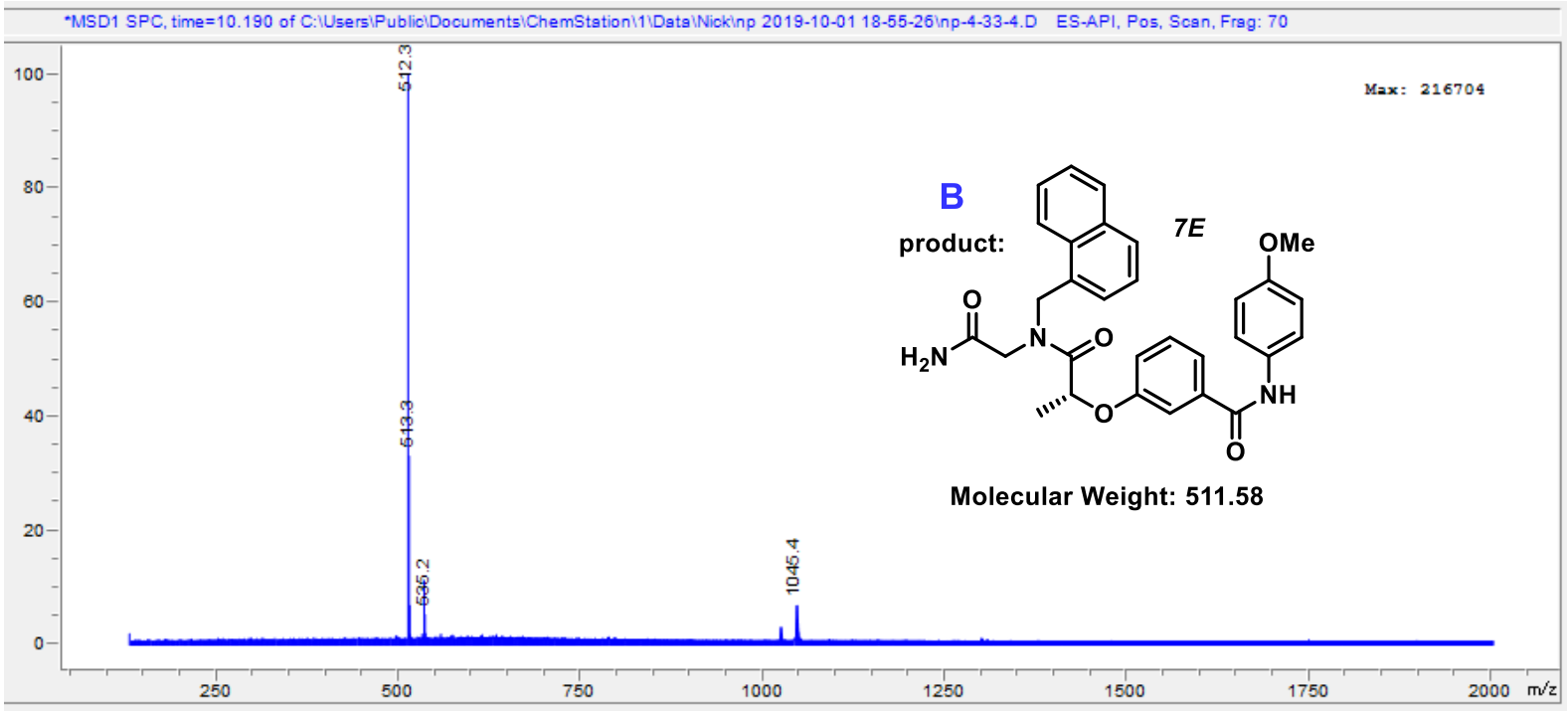


Crude LC spectrum of ***7E***.

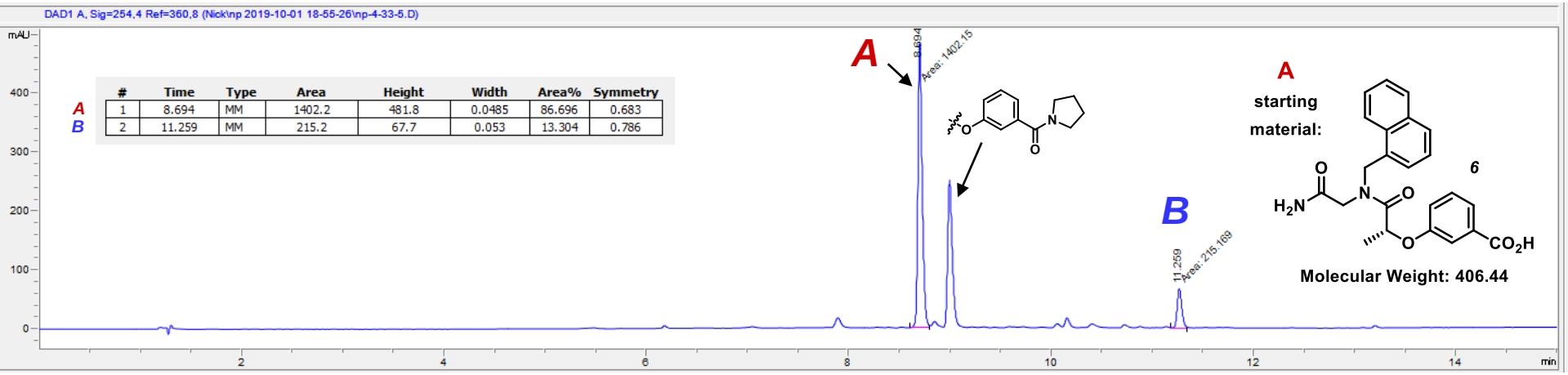


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.693	MM	1462.7	503.7	0.0484	45.136	0.686
2	10.147	MM	1777.9	580	0.0511	54.864	0.716

Corresponding MS spectrum for product peak (***B***) at 10.15 minutes.

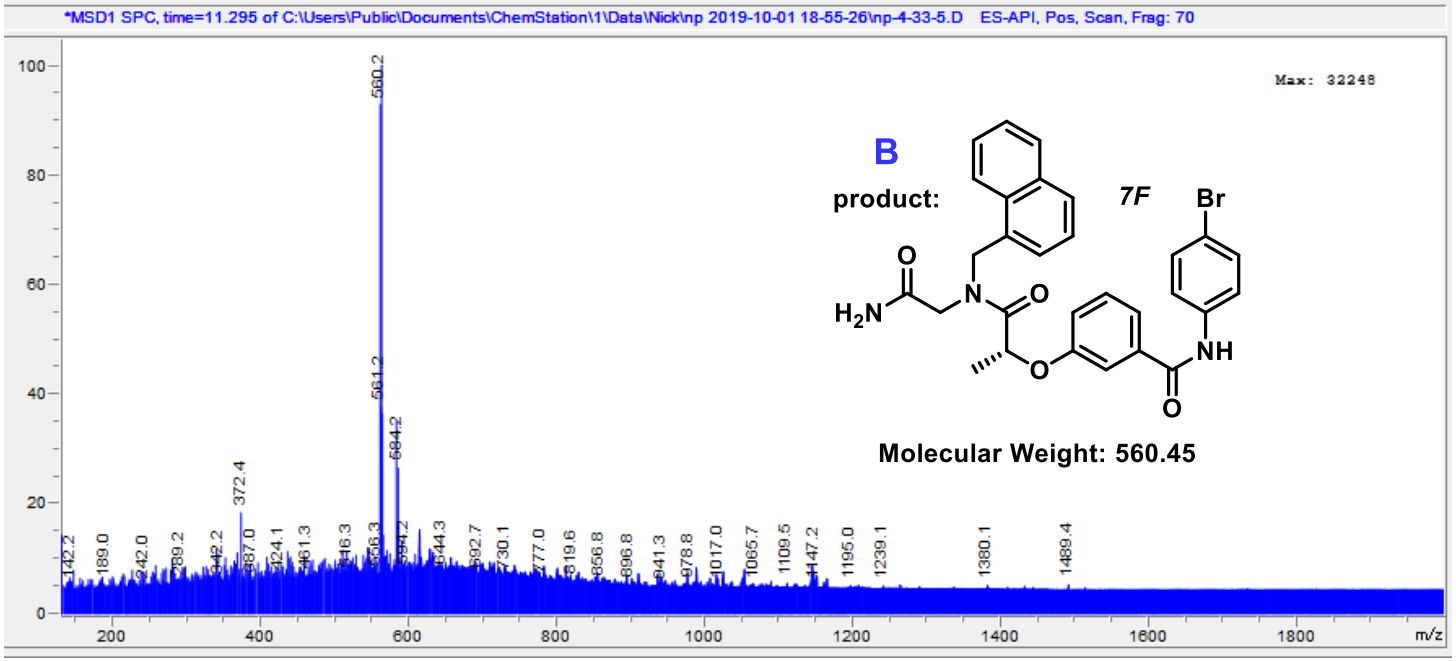


Crude LC spectrum of ***7F***.

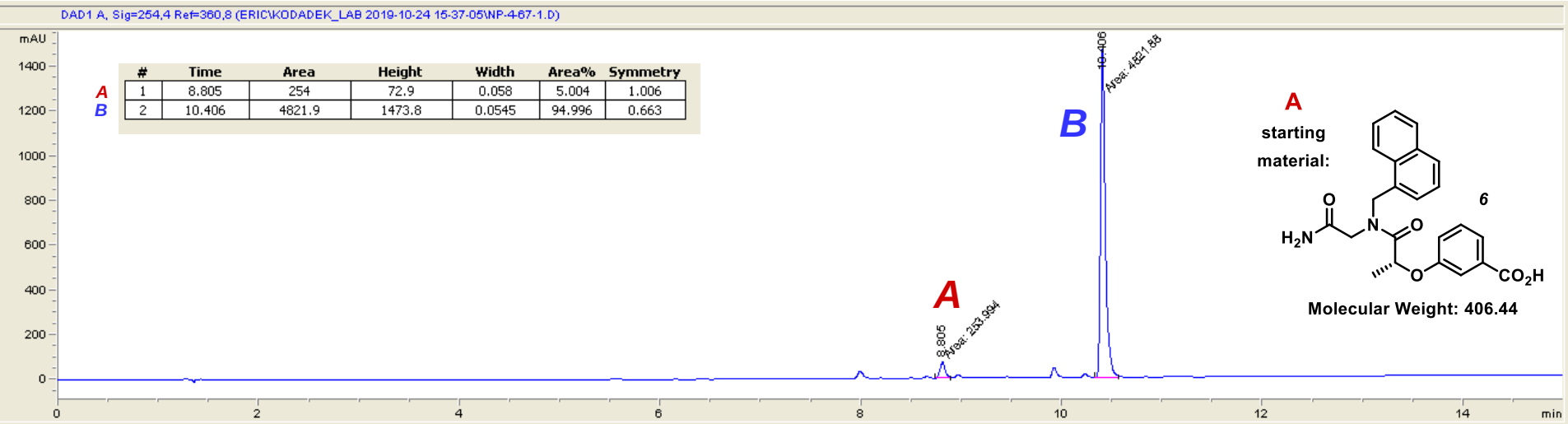


	#	Time	Type	Area	Height	Width	Area%	Symmetry
A	1	8.694	MM	1402.2	481.8	0.0485	86.696	0.683
B	2	11.259	MM	215.2	67.7	0.053	13.304	0.786

Corresponding MS spectrum for product peak (***B***) at 11.30 minutes.

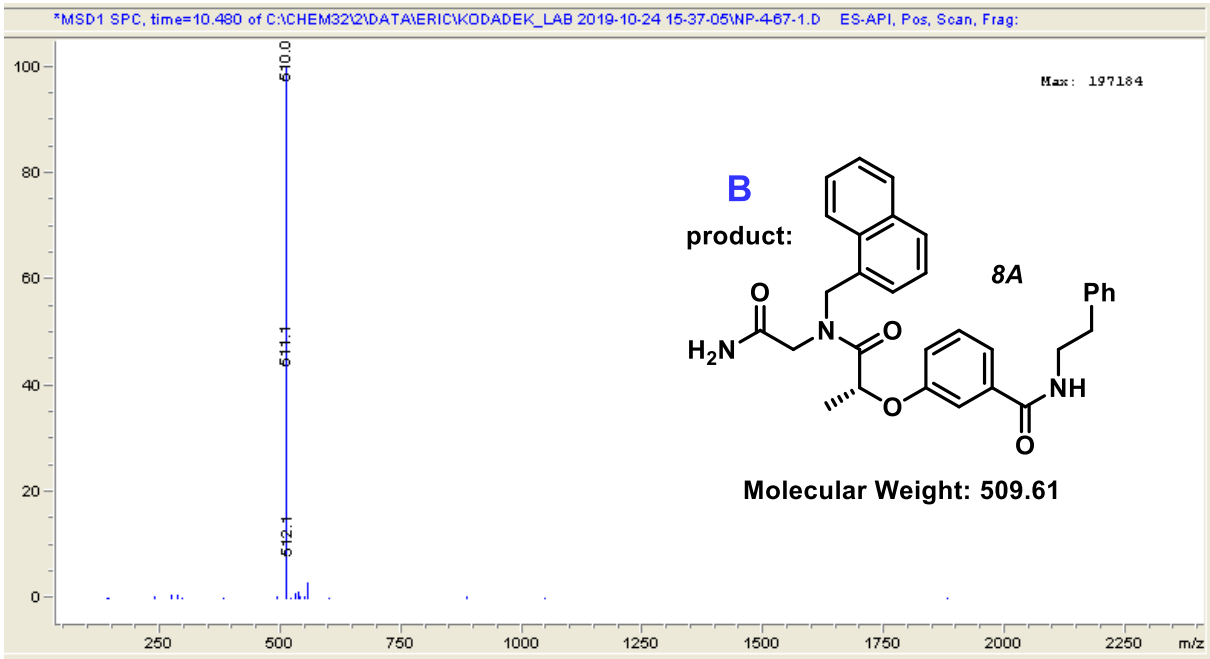


Crude LC spectrum of ***8A***.

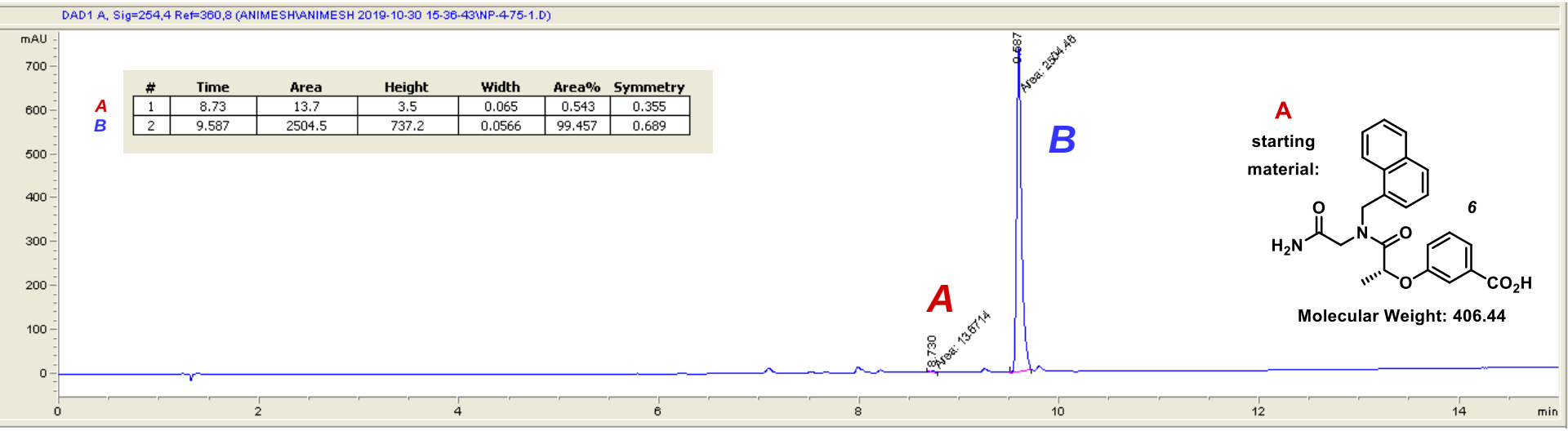


#	Time	Area	Height	Width	Area%	Symmetry
1	8.805	254	72.9	0.058	5.004	1.006
2	10.406	4821.9	1473.8	0.0545	94.996	0.663

Corresponding MS spectrum for product peak (***B***) at 10.01 minutes.

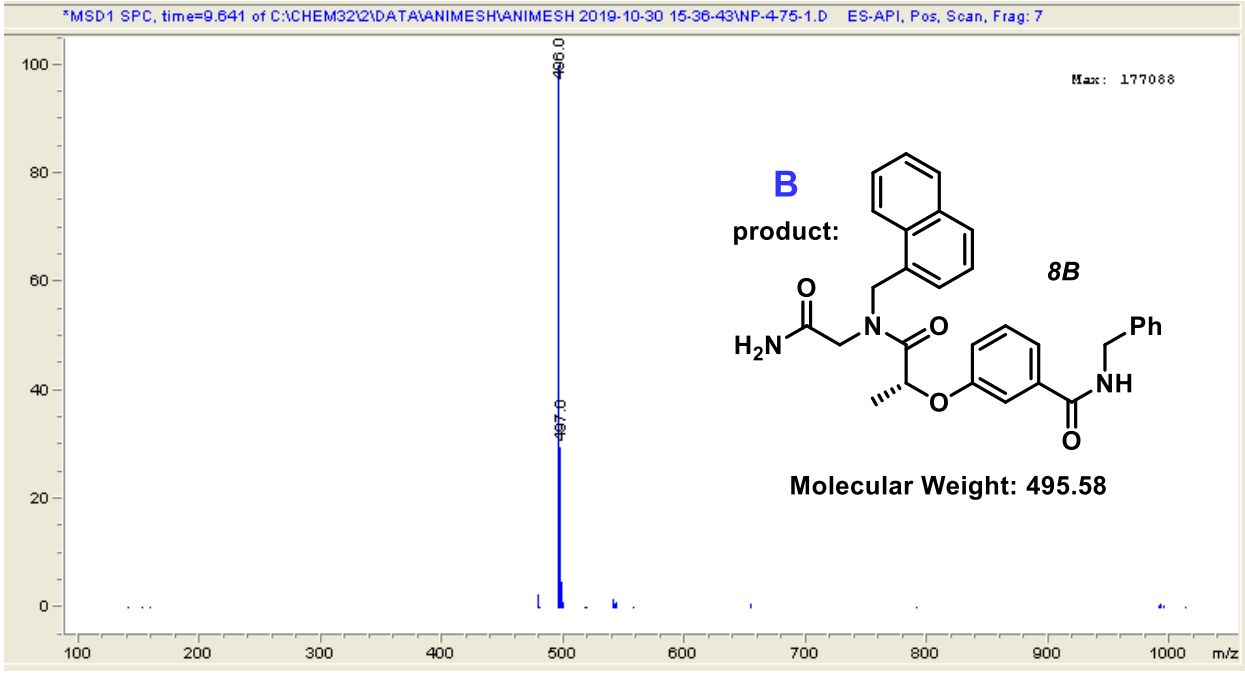


Crude LC spectrum of ***8B***.

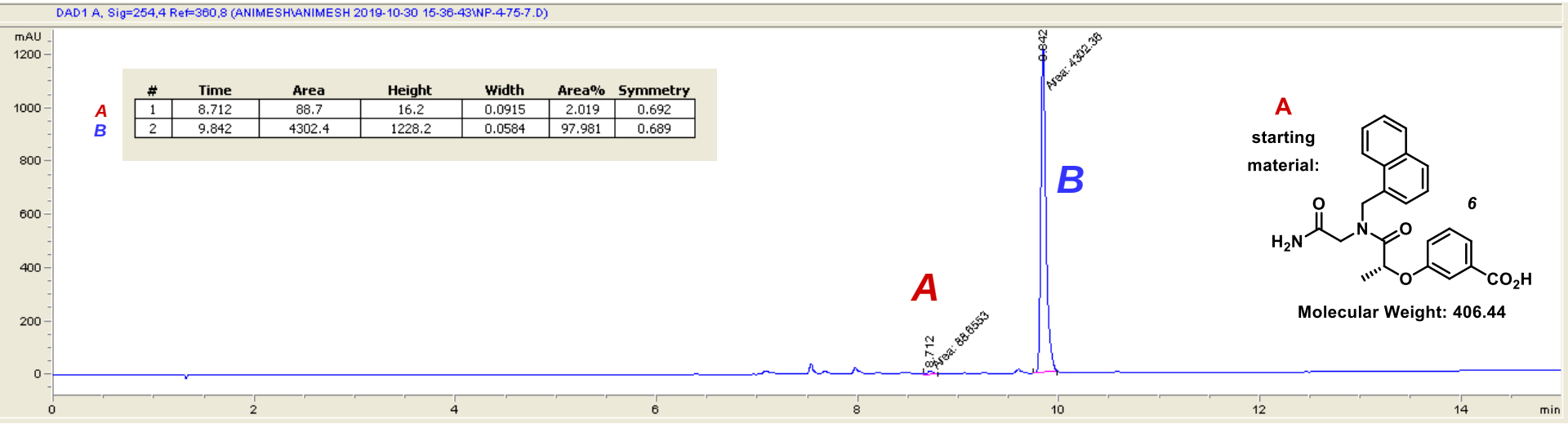


#	Time	Area	Height	Width	Area%	Symmetry
1	8.73	13.7	3.5	0.065	0.543	0.355
2	9.587	2504.5	737.2	0.0566	99.457	0.689

Corresponding MS spectrum for product peak (***B***) at 9.64 minutes.

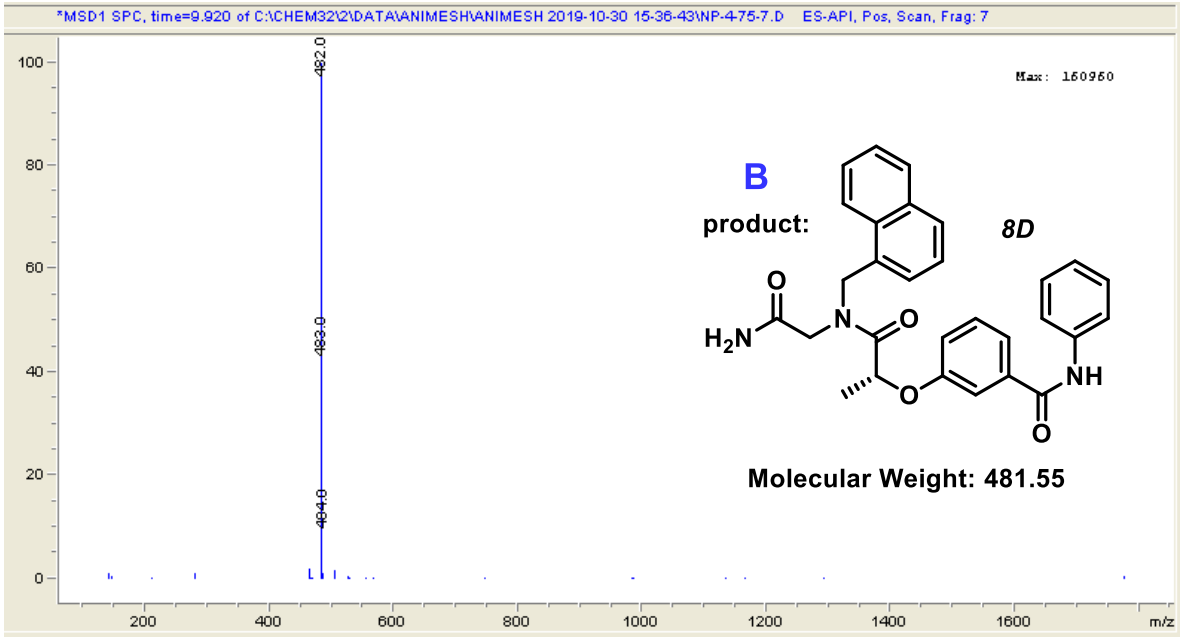


Crude LC spectrum of ***8D***.

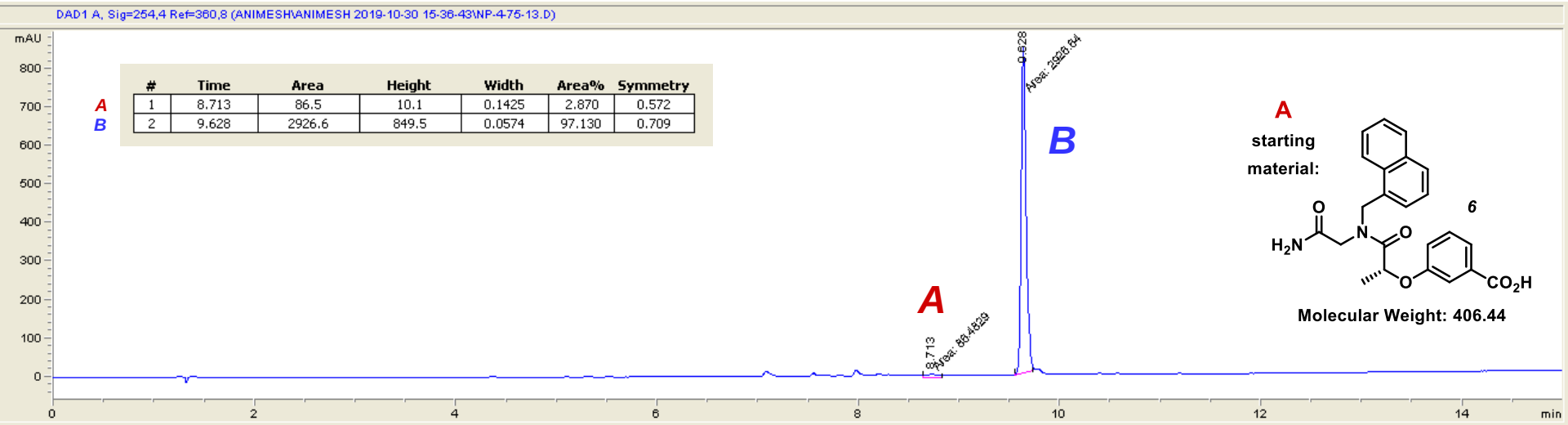


#	Time	Area	Height	Width	Area%	Symmetry
1	8.712	88.7	16.2	0.0915	2.019	0.692
2	9.842	4302.4	1228.2	0.0584	97.981	0.689

Corresponding MS spectrum for product peak (***B***) at 9.92 minutes.

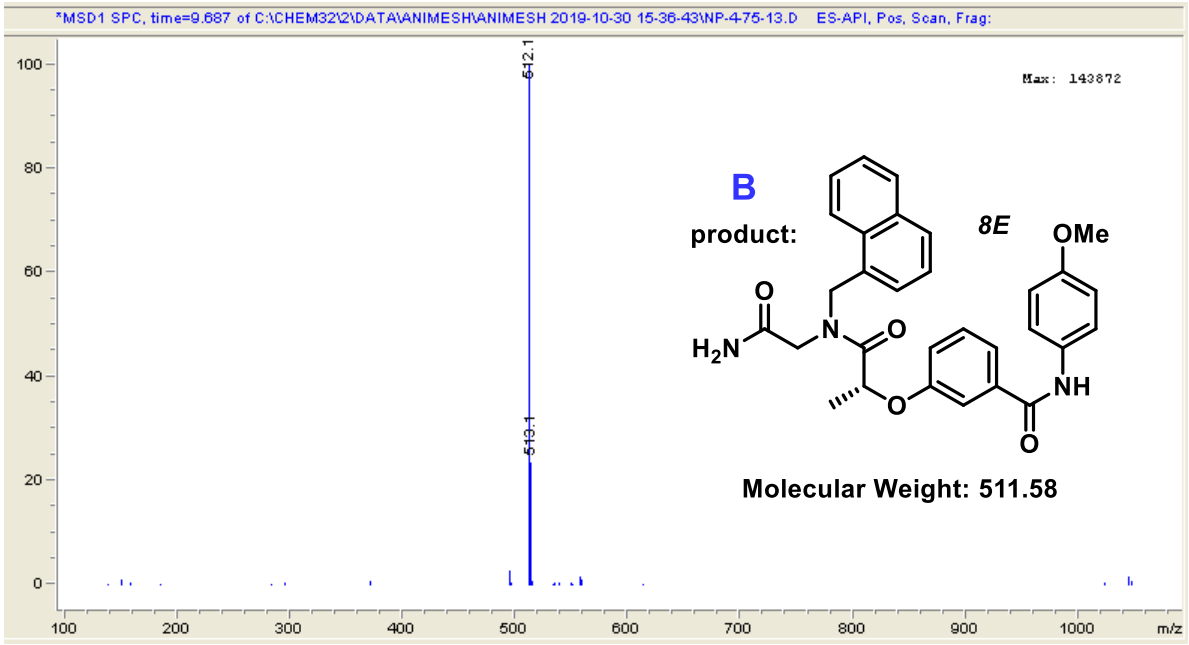


Crude LC spectrum of ***8E***.

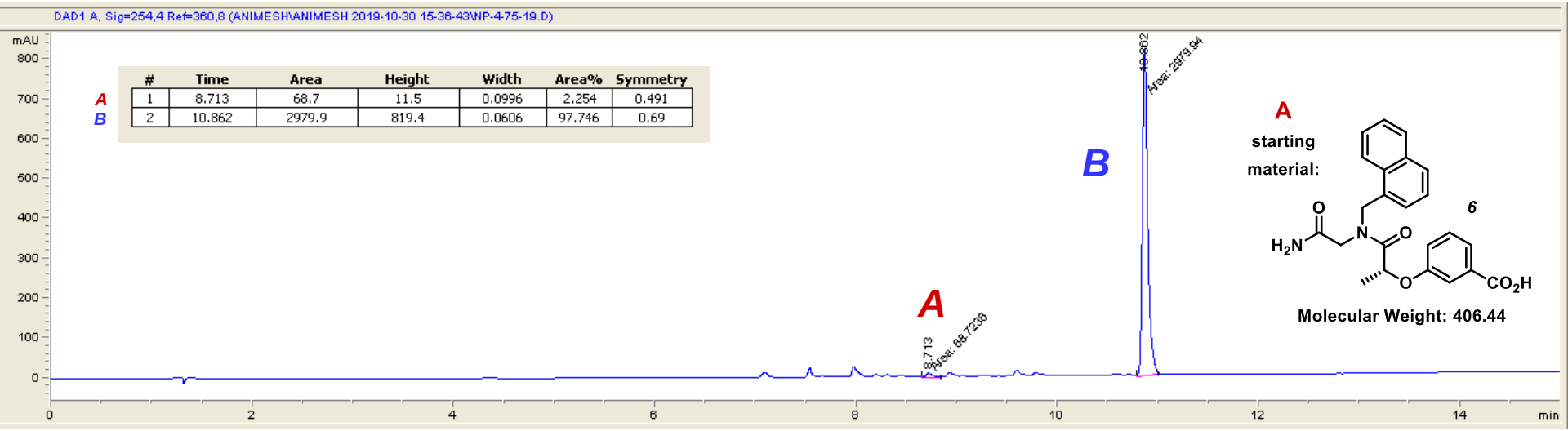


#	Time	Area	Height	Width	Area%	Symmetry
1	8.713	86.5	10.1	0.1425	2.870	0.572
2	9.628	2926.6	849.5	0.0574	97.130	0.709

Corresponding MS spectrum for product peak (***B***) at 9.69 minutes.

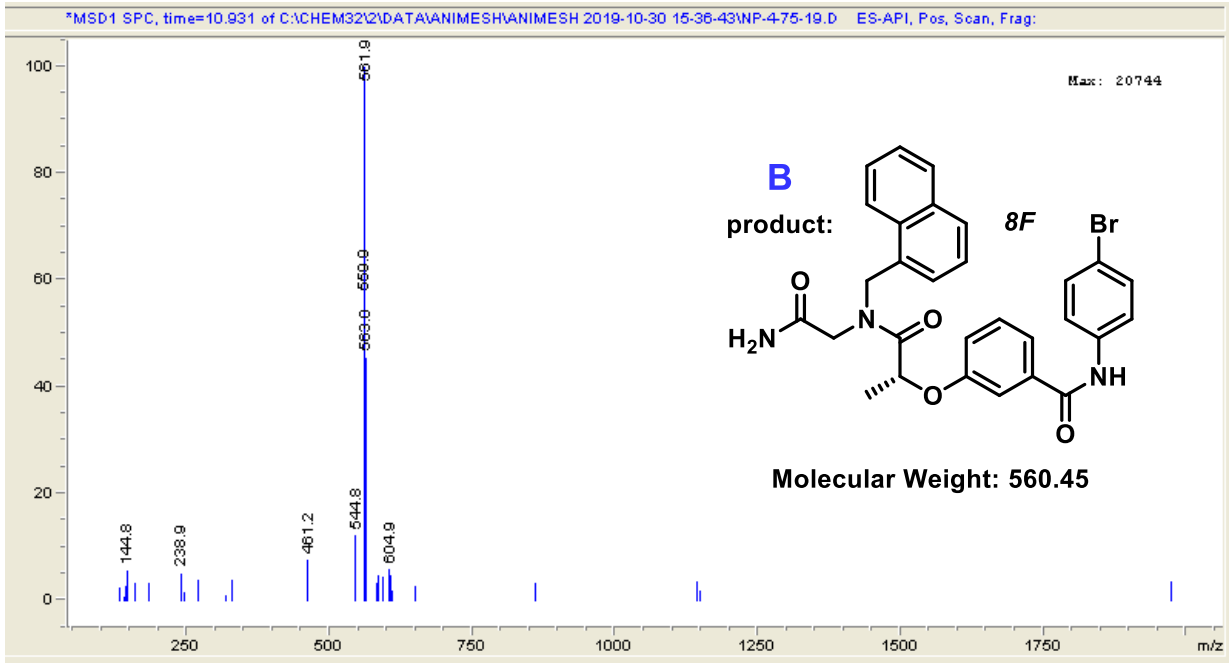


Crude LC spectrum of ***8F***.

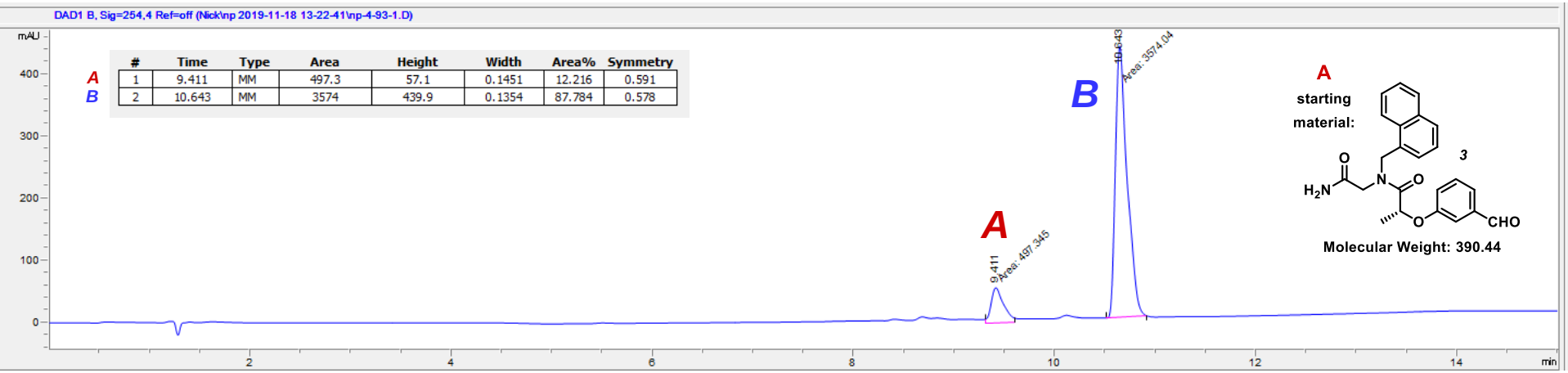


#	Time	Area	Height	Width	Area%	Symmetry
1	8.713	68.7	11.5	0.0996	2.254	0.491
2	10.862	2979.9	819.4	0.0606	97.746	0.69

Corresponding MS spectrum for product peak (***B***) at 10.93 minutes.

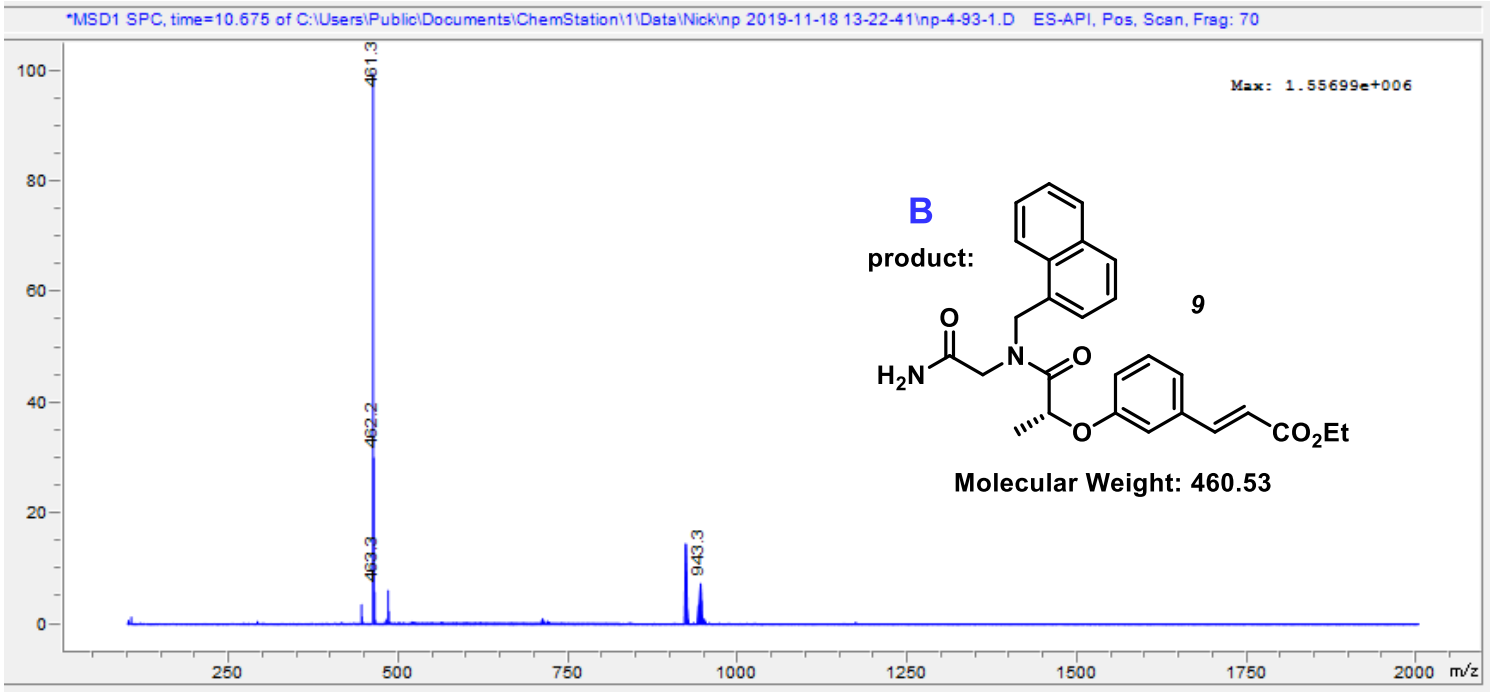


Crude LC spectrum of ***9***.

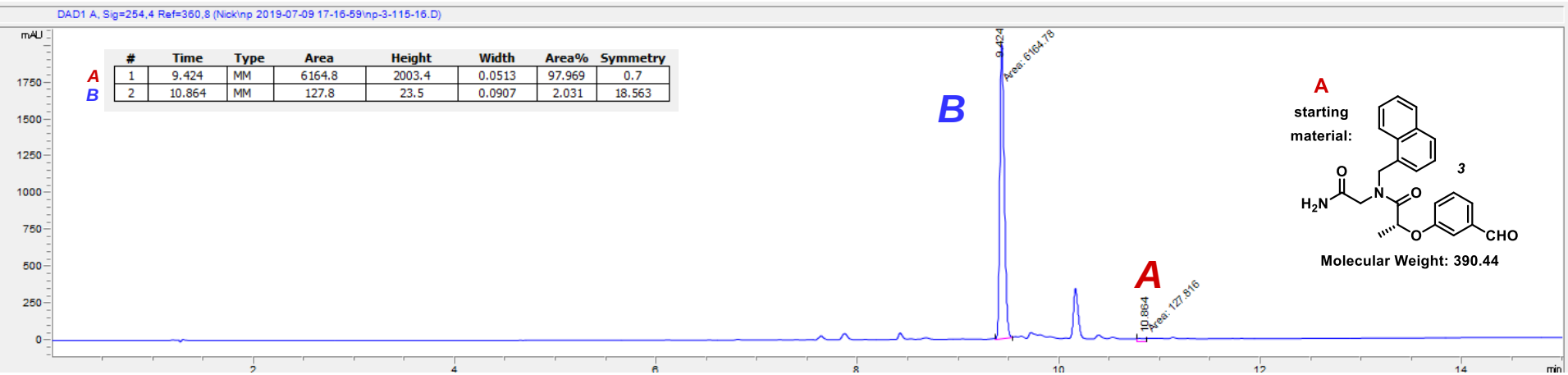


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.411	MM	497.3	57.1	0.1451	12.216	0.591
2	10.643	MM	3574	439.9	0.1354	87.784	0.578

Corresponding MS spectrum for product peak (***B***) at 10.68 minutes.

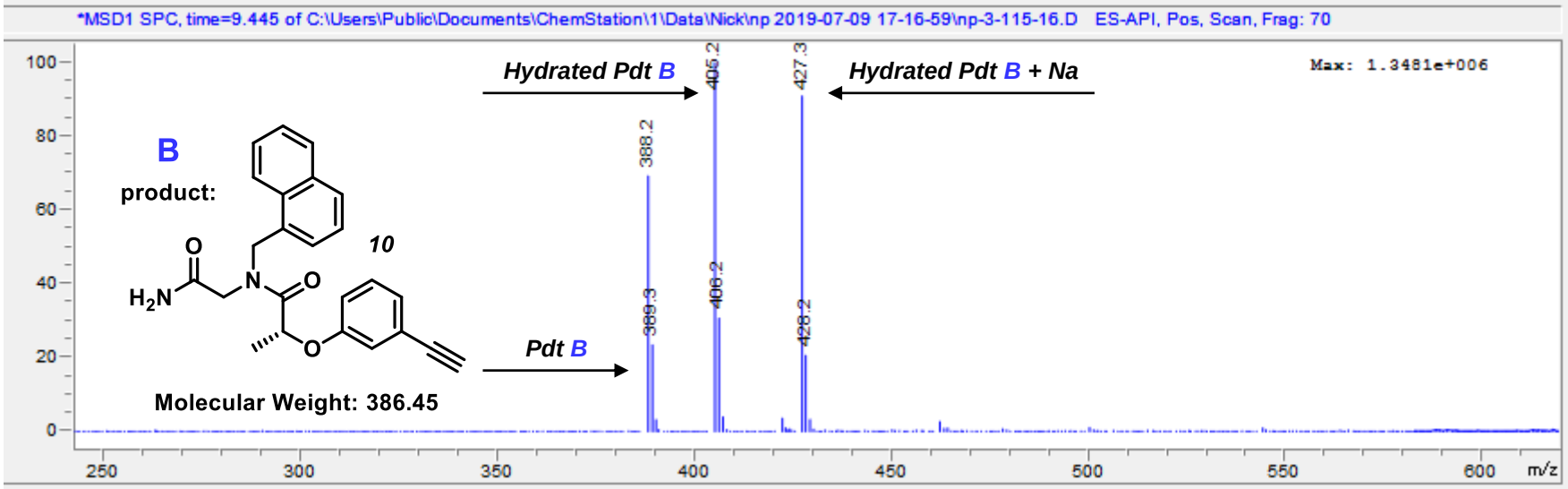


Crude LC spectrum of ***10***.

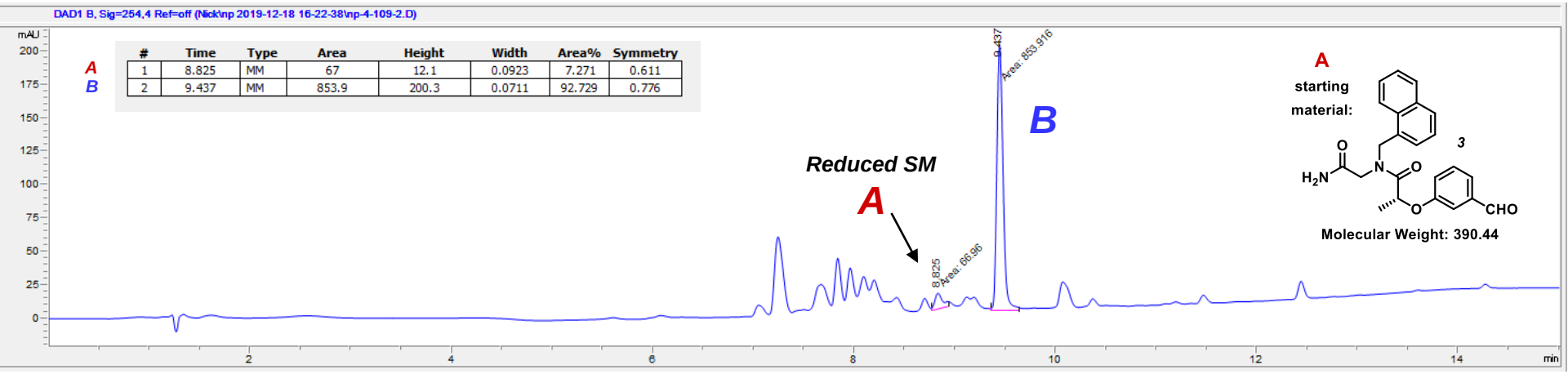


	#	Time	Type	Area	Height	Width	Area%	Symmetry
A	1	9.424	MM	6164.8	2003.4	0.0513	97.969	0.7
B	2	10.864	MM	127.8	23.5	0.0907	2.031	18.563

Corresponding MS spectrum for product peak (***B***) at 9.45 minutes.

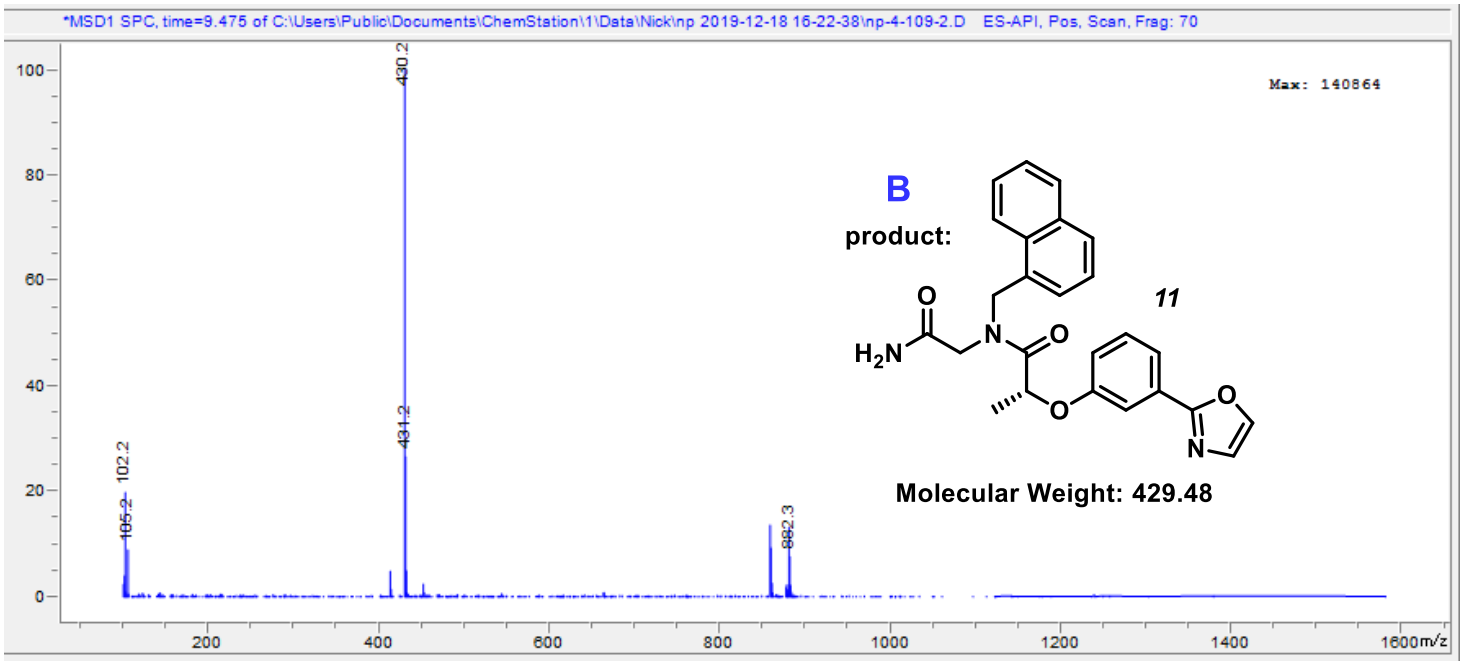


Crude LC spectrum of ***11***.

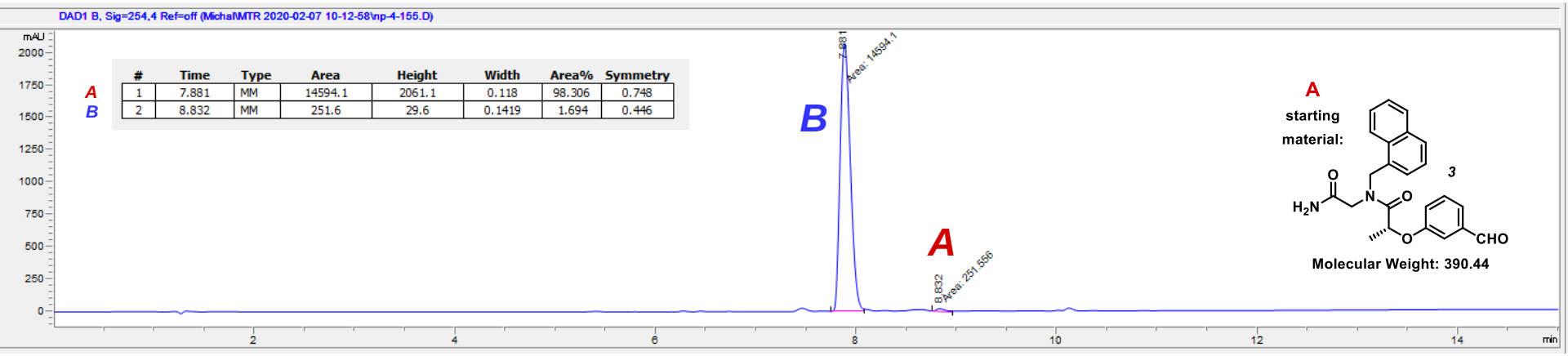


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.825	MM	67	12.1	0.0923	7.271	0.611
2	9.437	MM	853.9	200.3	0.0711	92.729	0.776

Corresponding MS spectrum for product peak (***B***) at 9.48 minutes.

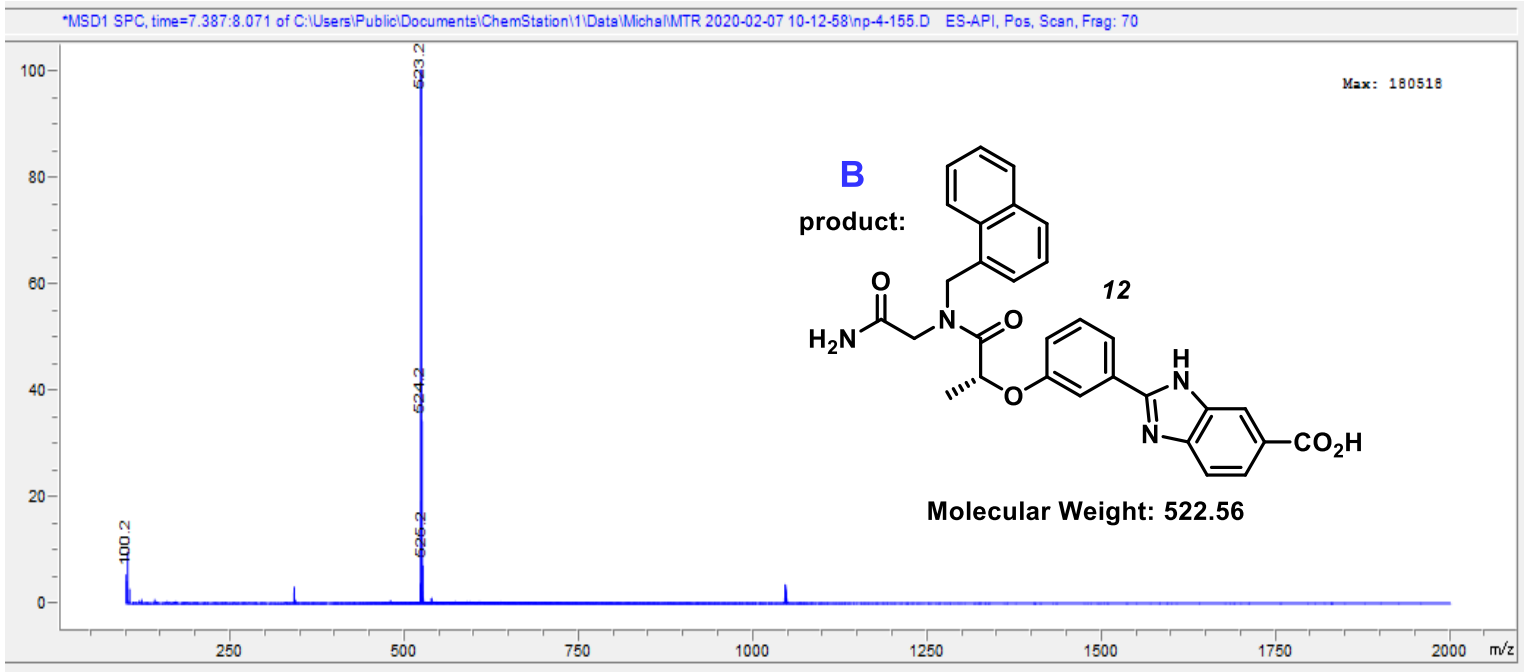


Crude LC spectrum of ***12***.



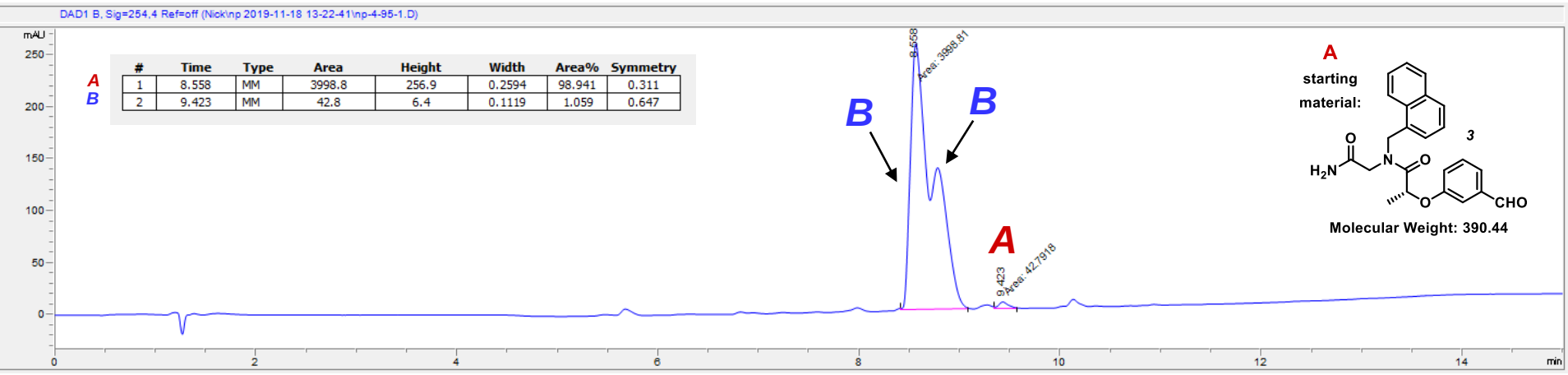
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.881	MM	14594.1	2061.1	0.118	98.306	0.748
2	8.832	MM	251.6	29.6	0.1419	1.694	0.446

Corresponding MS spectrum for product peak (***B***) at 7.92 minutes.



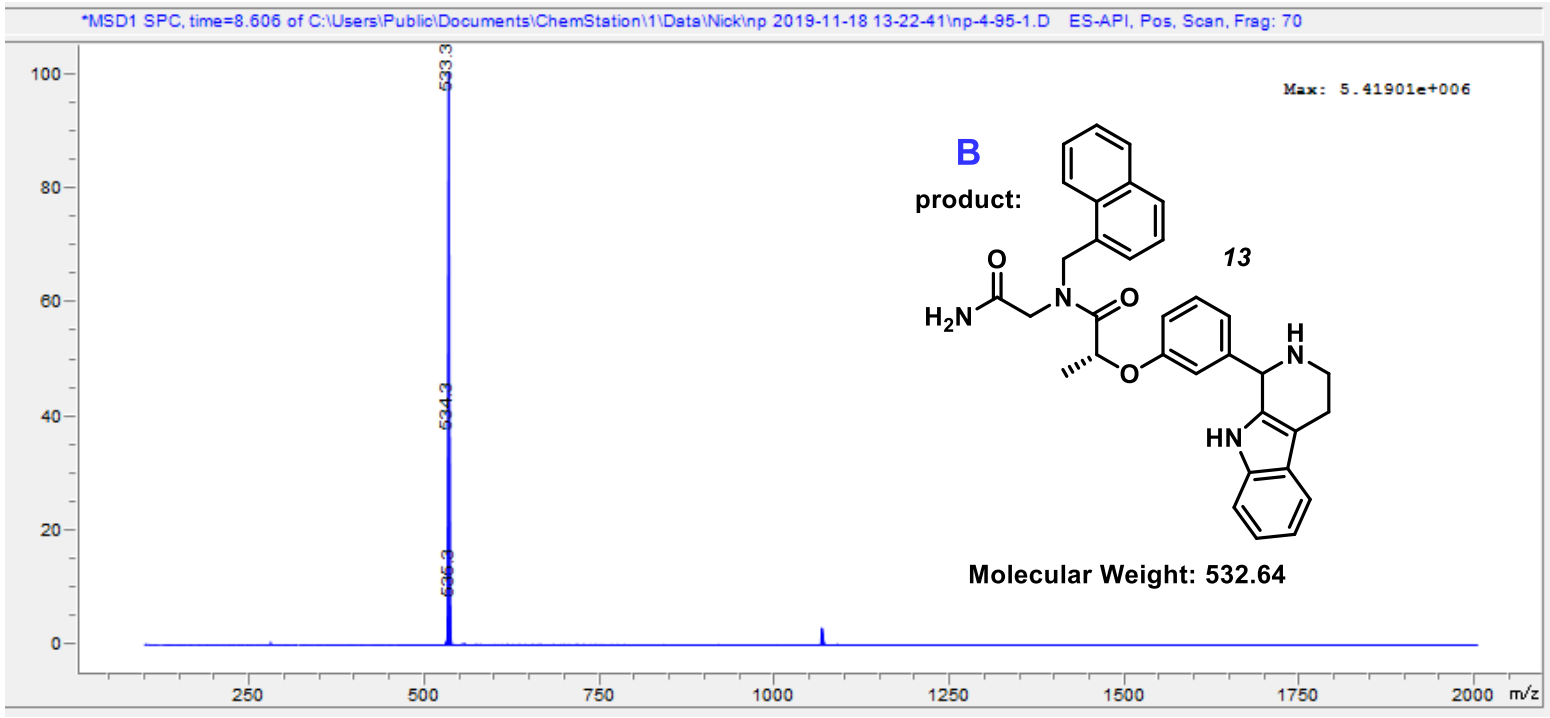
Crude LC spectrum of ***13***.

- Two peaks were integrated for product **B** (***13***) that represents a mixture of diastereomers.



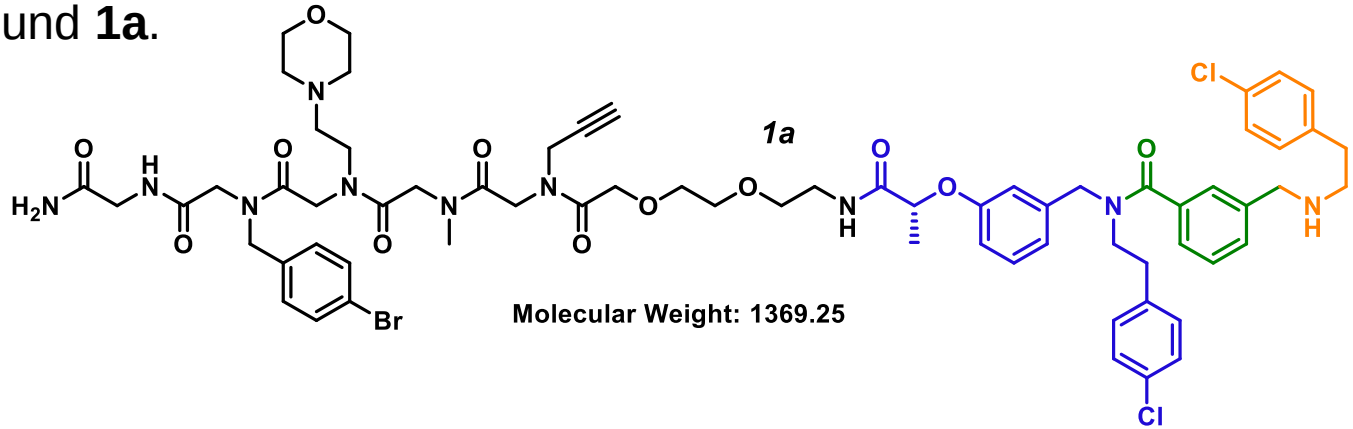
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.558	MM	3998.8	256.9	0.2594	98.941	0.311
2	9.423	MM	42.8	6.4	0.1119	1.059	0.647

Corresponding MS spectrum for product peak (***B***) at 8.61 minutes.

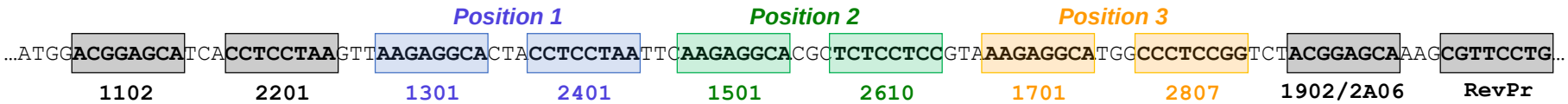


Quality control of the parallel synthesis of DNA-encoded molecules: compound **1**.

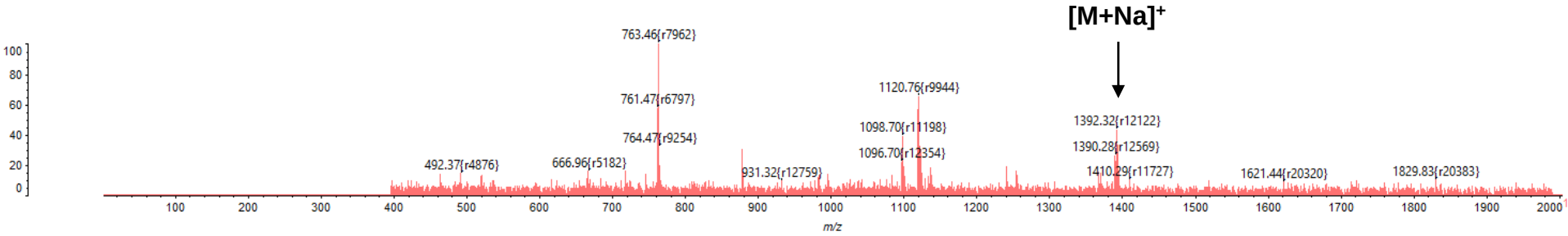
A) Compound **1a**.



B) Sanger sequencing results from amplification of **1a**. Full DNA sequence intact.

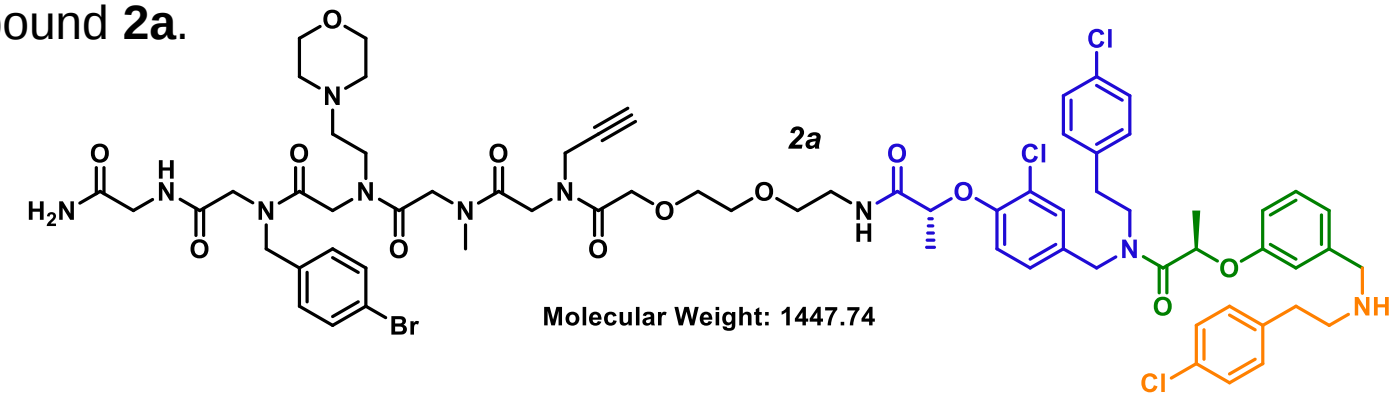


C) MALDI spectrum resulting from acidic cleavage of **1a** from the TentaGel resin.

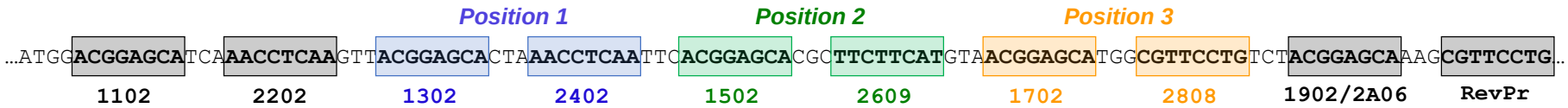


Quality control of the parallel synthesis of DNA-encoded molecules: compound **2**.

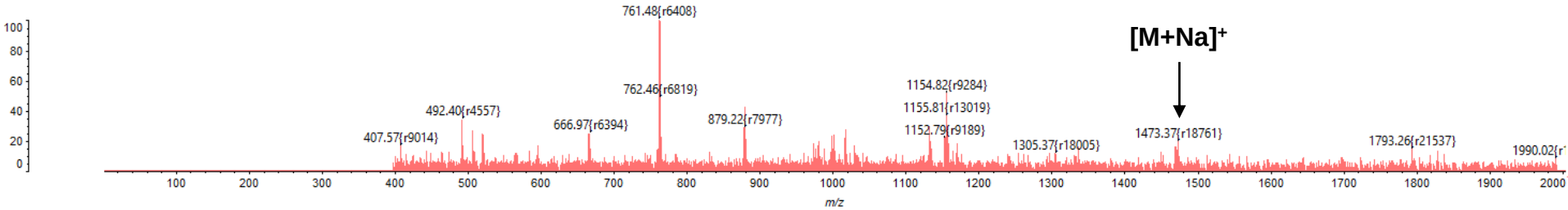
A) Compound **2a**.



B) Sanger sequencing results from amplification of **2a**. Full DNA sequence intact.

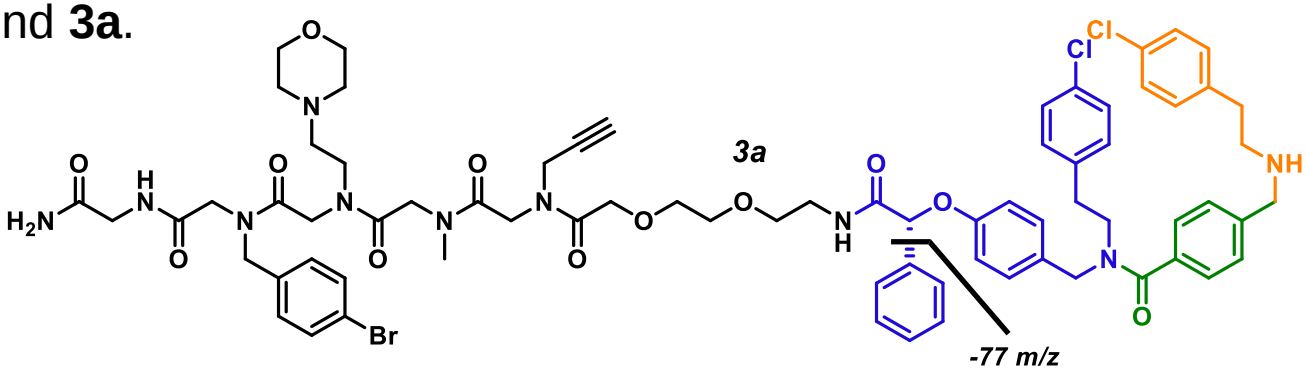


C) MALDI spectrum resulting from acidic cleavage of **2a** from the TentaGel resin.

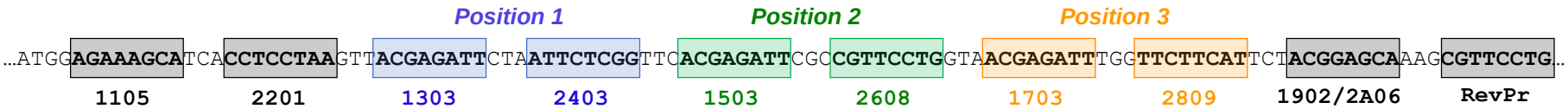


Quality control of the parallel synthesis of DNA-encoded molecules: compound **3**.

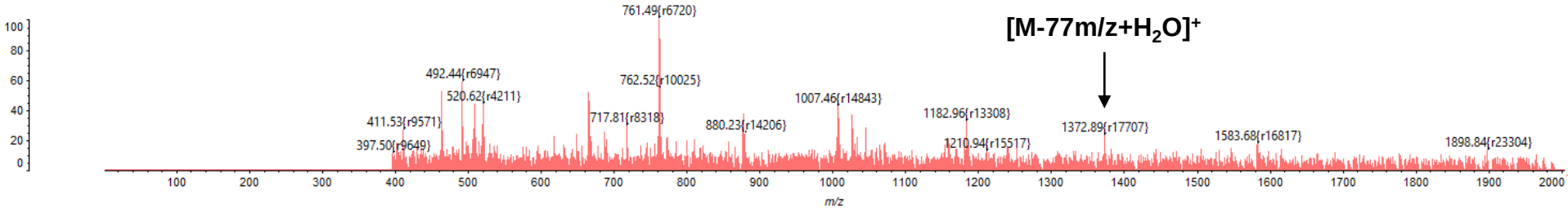
A) Compound **3a**.



B) Sanger sequencing results from amplification of **3a**. Full DNA sequence intact.

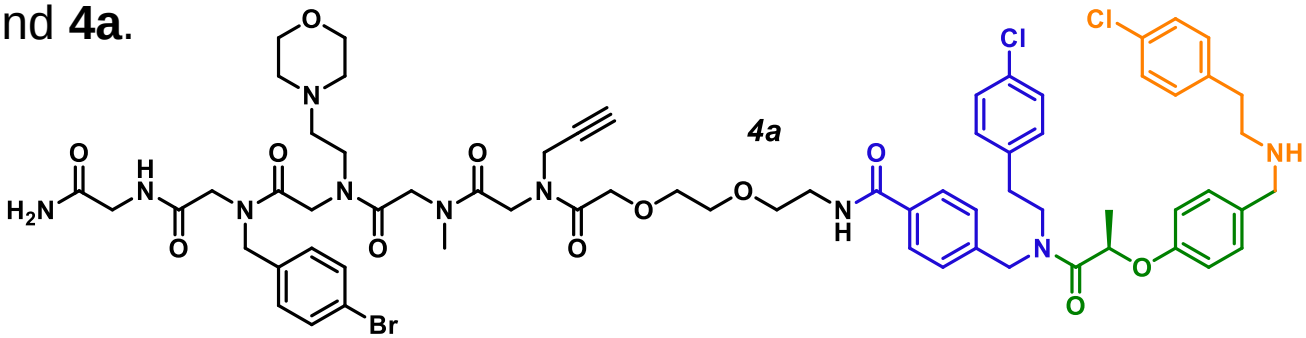


C) MALDI spectrum resulting from acidic cleavage of **3a** from the TentaGel resin.



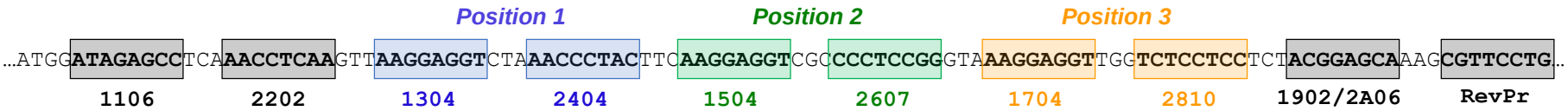
Quality control of the parallel synthesis of DNA-encoded molecules: compound **4**.

A) Compound **4a**.



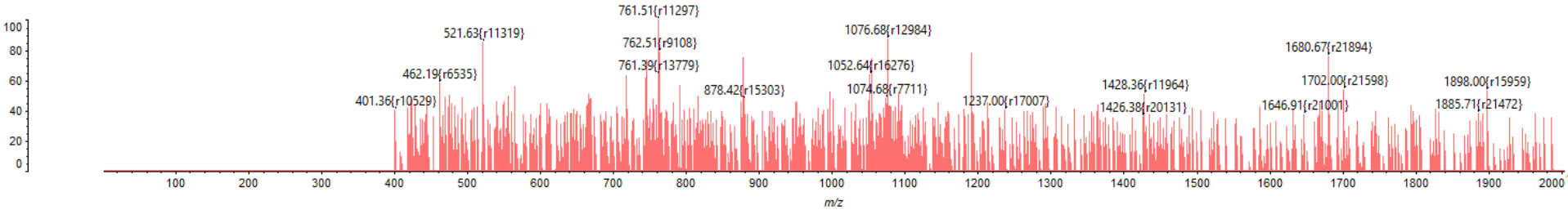
Molecular Weight: 1369.25

B) Sanger sequencing results from amplification of **4a**. Full DNA sequence intact.



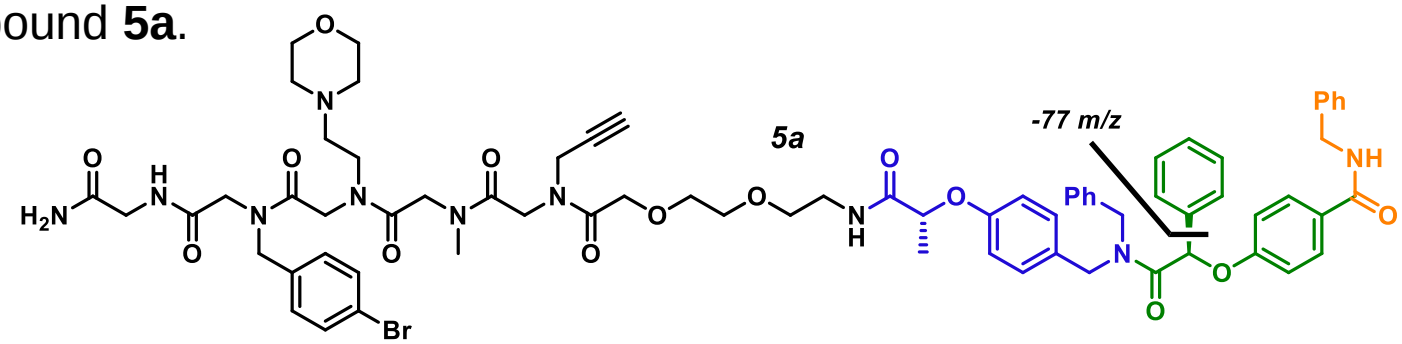
C) MALDI spectrum resulting from acidic cleavage of **4a** from the TentaGel resin.

- *MALDI cleavage inconclusive: too dilute.*



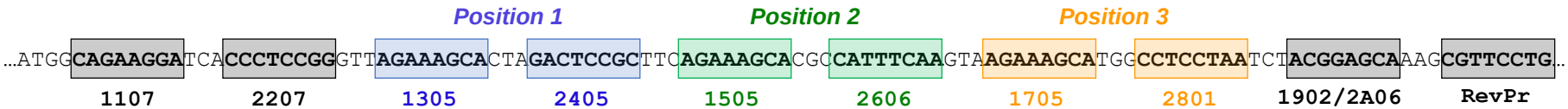
Quality control of the parallel synthesis of DNA-encoded molecules: compound **5**.

A) Compound **5a**.

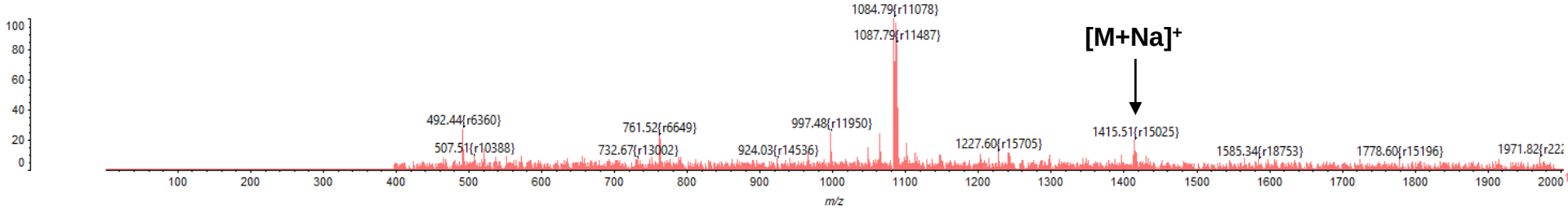


Molecular Weight: 1392.42

B) Sanger sequencing results from amplification of **5a**. Full DNA sequence intact.

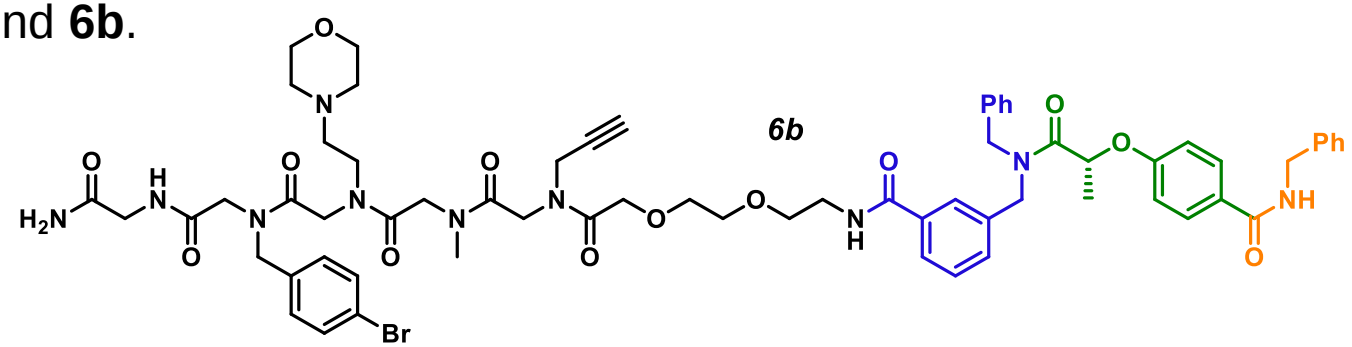


C) MALDI spectrum resulting from acidic cleavage of **5a** from the TentaGel resin.



Quality control of the parallel synthesis of DNA-encoded molecules: compound **6**.

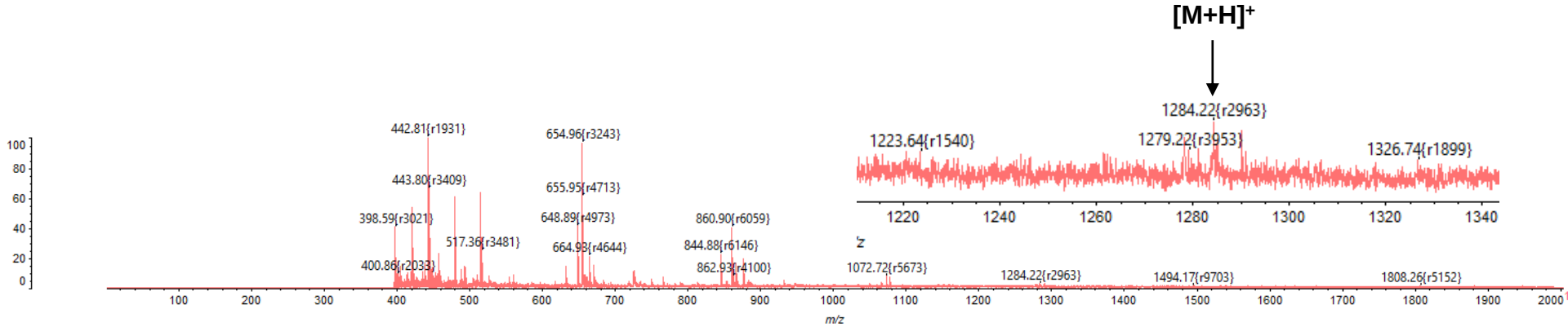
A) Compound **6b**.



Molecular Weight: 1286.29

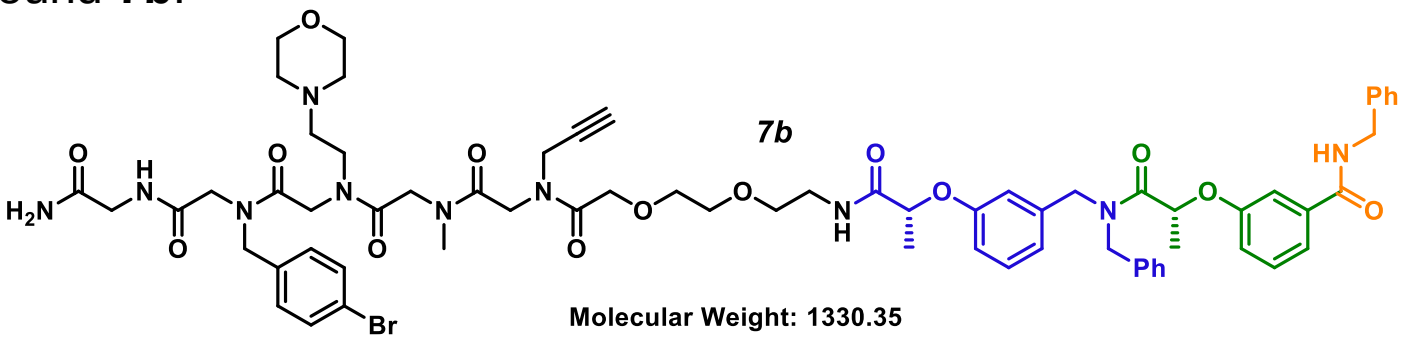
B) Sanger sequencing results from amplification of **6b**. Full DNA sequence intact.

C) MALDI spectrum resulting from acidic cleavage of **6b** from the TentaGel resin.

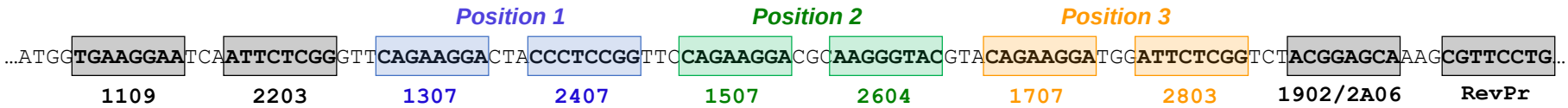


Quality control of the parallel synthesis of DNA-encoded molecules: compound **7**.

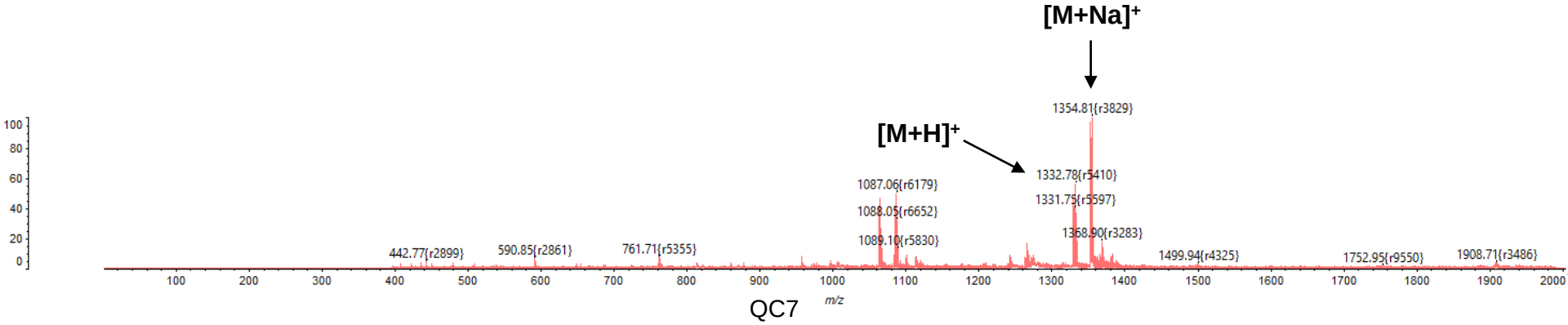
A) Compound **7b**.



B) Sanger sequencing results from amplification of **7b**. Full DNA sequence intact.

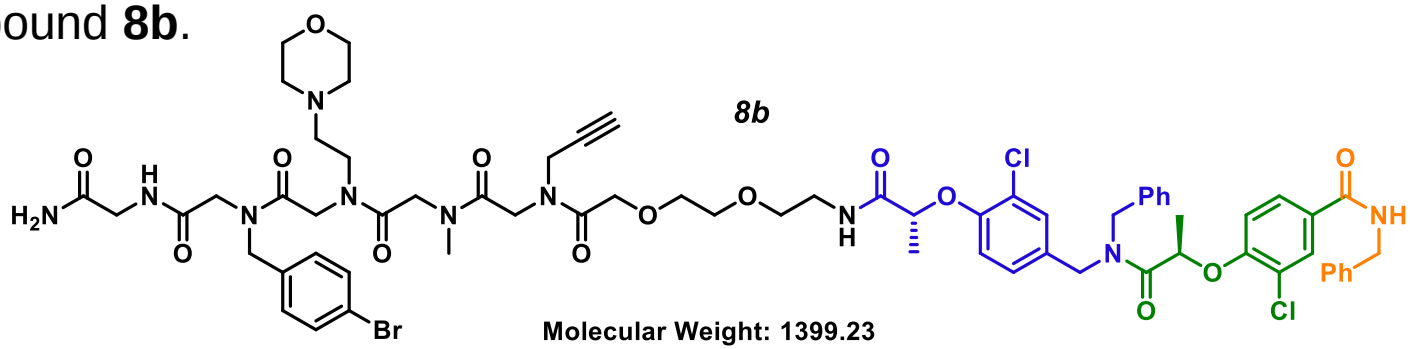


C) MALDI spectrum resulting from acidic cleavage of **7b** from the TentaGel resin.

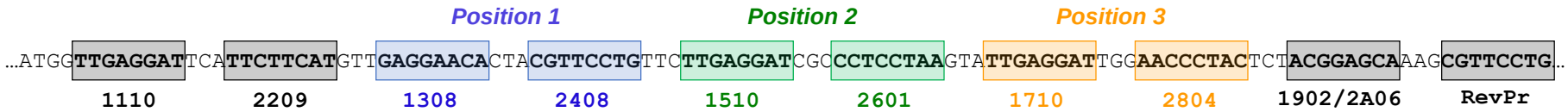


Quality control of the parallel synthesis of DNA-encoded molecules: compound **8**.

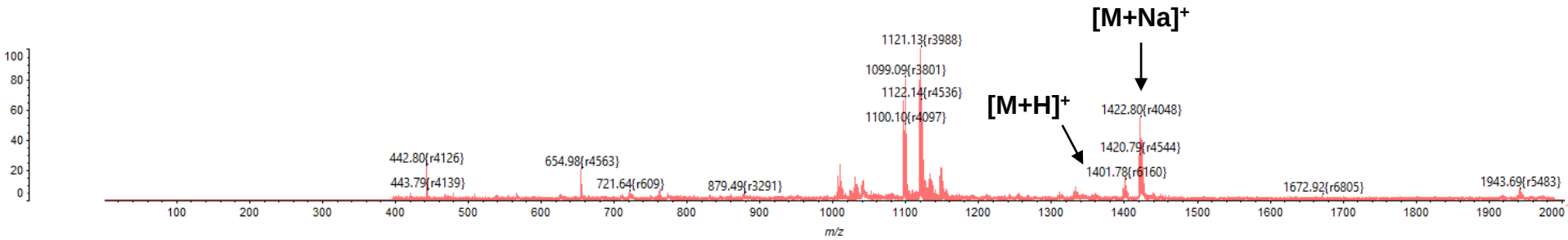
A) Compound **8b**.



B) Sanger sequencing results from amplification of **8b**. Full DNA sequence intact.

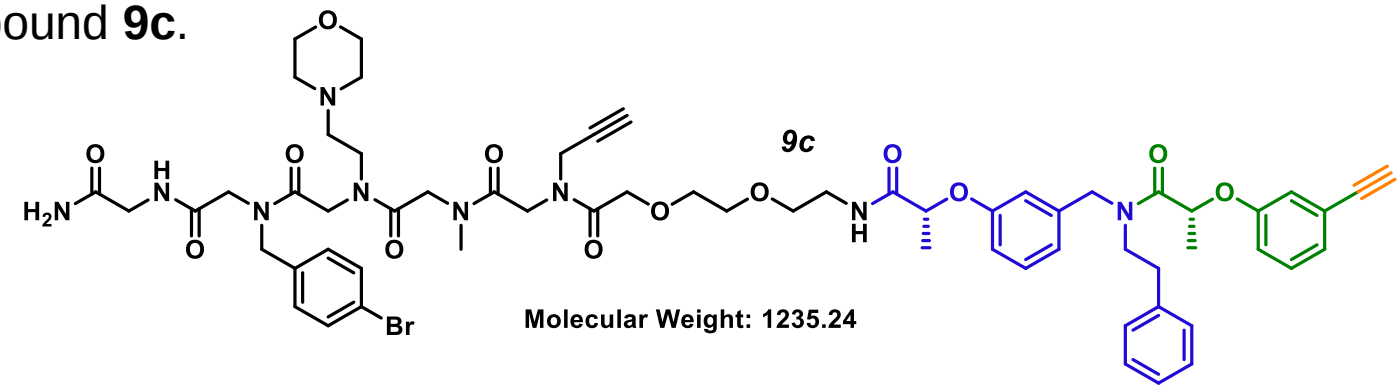


C) MALDI spectrum resulting from acidic cleavage of **8b** from the TentaGel resin.

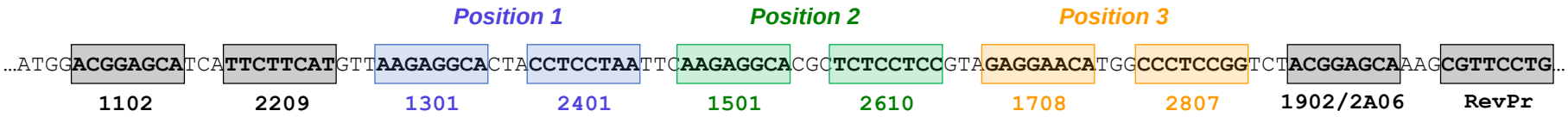


Quality control of the parallel synthesis of DNA-encoded molecules: compound **9**.

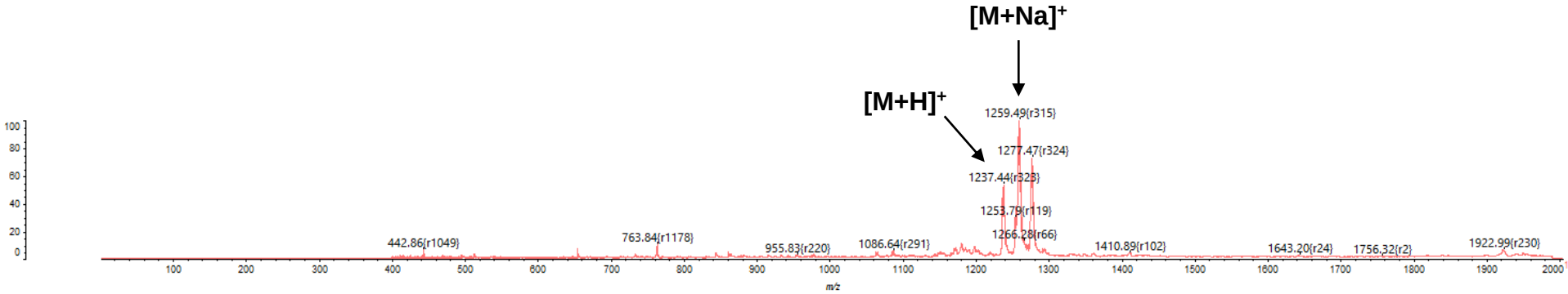
A) Compound **9c**.



B) Sanger sequencing results from amplification of **9c**. Full DNA sequence intact.

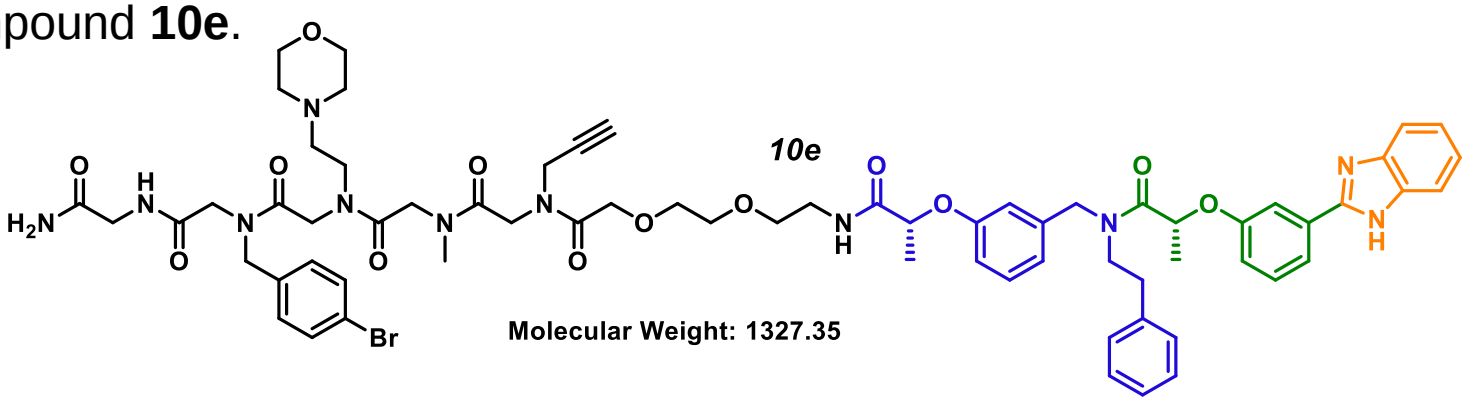


C) MALDI spectrum resulting from acidic cleavage of **9c** from the TentaGel resin.

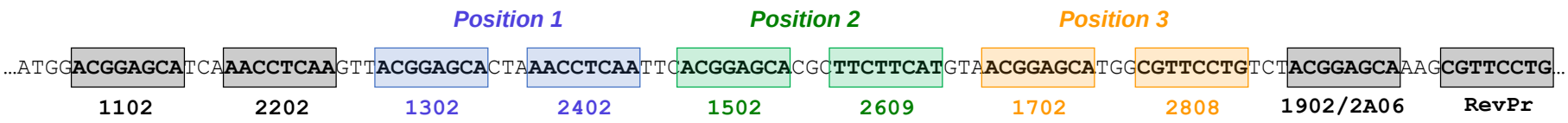


Quality control of the parallel synthesis of DNA-encoded molecules: compound **10**.

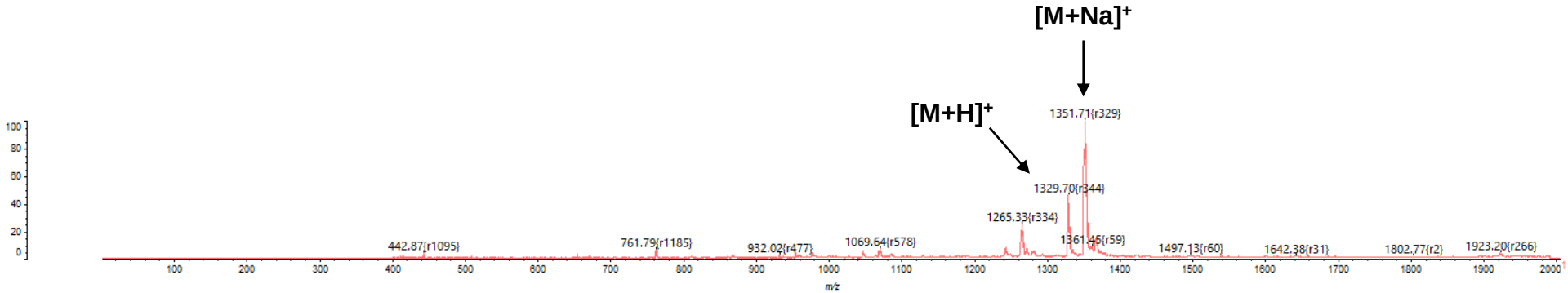
A) Compound **10e**.



B) Sanger sequencing results from amplification of **10e**. Full DNA sequence intact.



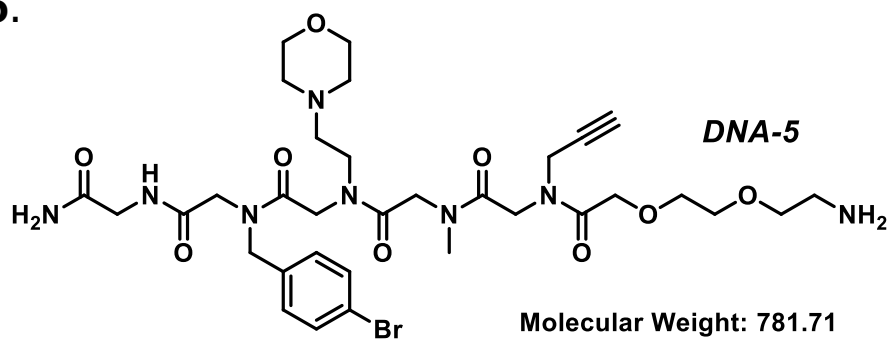
C) MALDI spectrum resulting from acidic cleavage of **10e** from the TentaGel resin.



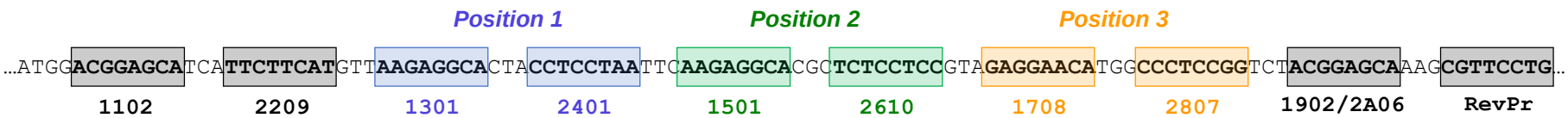
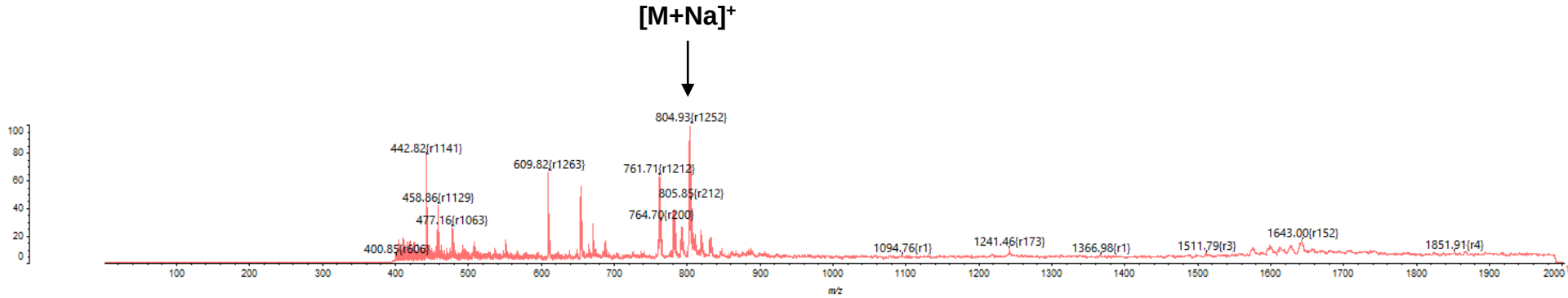
Quality control of the parallel synthesis of DNA-encoded molecules: **DNA-5**.

- *DNA only wells received no chemistry and the corresponding cleaved molecule is the linker component.*

A) Compound **DNA-5**.



B) Sanger sequencing results from amplification of **DNA-5**. Full DNA sequence intact.



C) MALDI spectrum resulting from acidic cleavage of **DNA-5** from the TentaGel resin.