Supporting Information

On-DNA Palladium-catalyzed hydrogenation like reaction suitable for DNA-Encoded library synthesis

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I. Materials and Analytical methods

All the reagents were purchased through vendors. They were dissolved in an appropriate solvent before use. On-DNA reactions conducted during validation were analyzed by HPLC/ESI-MS. After reaction, an aliquot of the reaction mixture solution was diluted (typically 1 μL aliquot was diluted in 10 μL of water). Samples were then injected onto a reverse-phase chromatography column (XBridge C18, 3.5 μm, 2.1x50 mm) and eluted with a mixture of Solvent A (10 mM TEAA in H_2O at pH=7) and Solvent B (CH_3CN). Yields were calculated by examination of the UV (260 nm) and TIC traces of the LCMS chromatograms. Samples with MW>12000 D were analyzed by Quadrupole Time-of-flight Mass Spectrometry (QTOF). After reaction, an aliquot of the reaction mixture solution was diluted (typically 1 μL aliquot was diluted in 10 μL of a saturated solution of EDTA in water). Samples were then injected onto a reverse-phase chromatography column (DNAPac RP Column, 4 mm, 3.0x50 mm) and eluted with a mixture of Solvent A (15 mM DBA/25 mM HFIP in H_2O at pH=9.8) and Solvent B (CH_3CN). Mass was obtained after deconvolution with Agilent Mass Hunter Qualitative Analysis.

II. Structure of DNA headpieces

AOP-HP-Primer-NH2: DNA headpiece-NH2 (HP-NH2) was obtained from Biosearch Technologies. The amine of this HP was further extended by an additional 16 atoms by reacting it with Fmoc-15-amino-4,7,10,13-tetraoxapentadecanoic acid (**AOP**) followed by treatment with piperidine to remove the Fmoc protecting group. Following ethanol precipitation and isolation, this material was then reconstituted in water to continue the synthesis. The structure of the HP and detailed experimental procedure followed to get **AOP-HP-NH2** (Figure S1) was described previously by Clark et al.[1] **AOP-HP-NH2** was elongated via ligation with a primer to get **AOP-HP-Primer-NH2** (Figure S2). This AOP-Headpiece-Primer-NH2 (**AOP-HP-Primer-NH2**) served as the starting material for scaffolds **SC1** to **SC36** described in the paper.

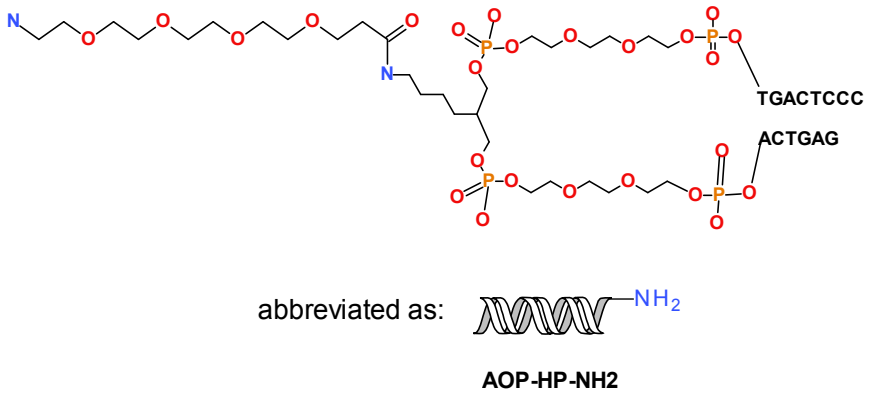

Figure S1. Sequence and structure of the "**AOP-HP-NH2**". MW= 5184 D

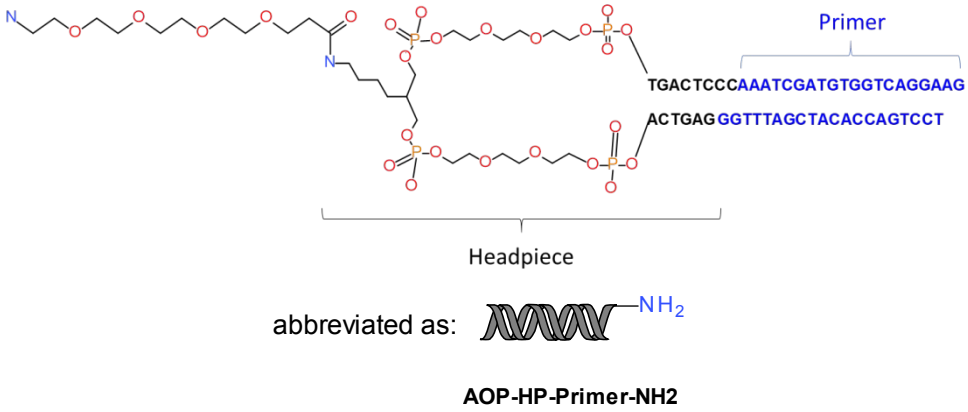

Figure S2. Sequence and structure of the "**AOP-HP-Primer-NH2**". MW= 17606 D

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[1] Clark, M. A.; Acharya, R. A.; Arico-Muendel, C. C.; Belyanskaya, S. L.; Benjamin, D. R.; Carlson, N. R.; Centrella, P. A.; Chiu, C. H.; Creaser, S. P.; Cuozzo, J. W.; Davie, c. F.; Ding, Y.; Franklin, G. J.; Franzen, K. D.; Gefter, M. L.; Hale, S. P.; Hansen, N. J. V.; Israel, D. I.; Jiang, J.; Kavarana, M. J.; Kelley, M. S.; Kollmann, C. DS.; Li, F.; Lind, K.; Mataruse, S.; Medeiros, P. F.; Messer, J. A.; Myers, P.; O'Keefe, H.; Oliff, M. C.; Rise, C. E.; Satz, A. L.; Skinner, S. R.; Svendsen, J. L.; Tang, L.; van Vloten, K.; Wagner, R. W.; Yao, G.; Morgan, B. A. Design, synthesis and selection of DNA-encoded small-molecule libraries. *Nat. Chem. Biol.* **2009**, *5*, 647-654.

III. Synthesis and LCMS of DNA SM SC1 to SC36

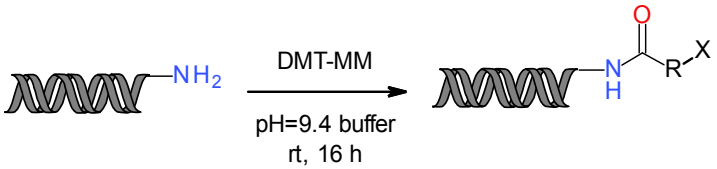


Figure S3: AOP-HP-Primer-NH-CO-X (X = *N*-Cbz, *N*-Alloc, *N*-Allyl, O-Bn, O-Allyl groups, alkene, alkyne, azide and nitro groups) preparation by amide chemistry promoted by DMT-MM.

General preparation of SC1 to SC36: Acylation with DMT-MM

To a solution of **AOP-HP-Primer-NH$_2$** (for **SC1** to **SC36**) in borate buffer 0.5 M pH 9.4 (1000 nmol, 1mL) were added 40 equivalents of the corresponding carboxylic acid (stock solution, 400 mM in DMA, 40000 nmol), followed by 40 equivalents of DMT-MM (stock solution, 400 mM in water, 40000 nmol). The reaction mixture was allowed to proceed at room temperature with stirring overnight. The reaction was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. The pellet was re-dissolved (14 mL water) and charged in one YM3K centrifugal tube (Amicon) and centrifuged at 4000 rpm for 30 min. The sample was collected and its concentration was measured by Nanodrop.

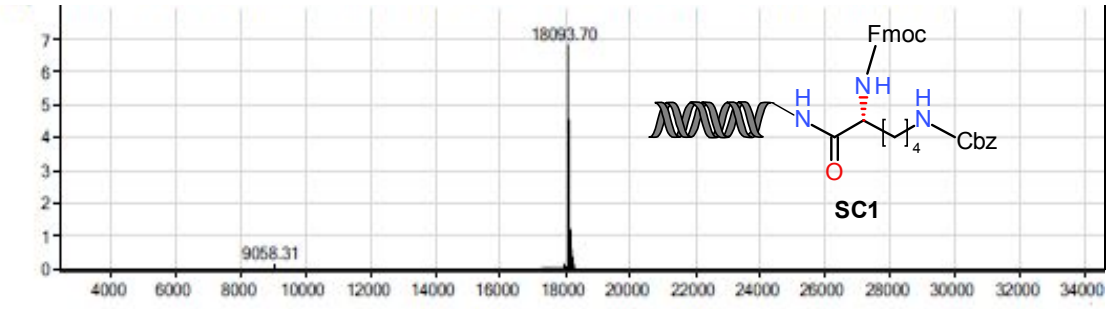


Figure S4: Deconvoluted mass spectrum of compound **SC1**, expected: 18090; observed: 18093

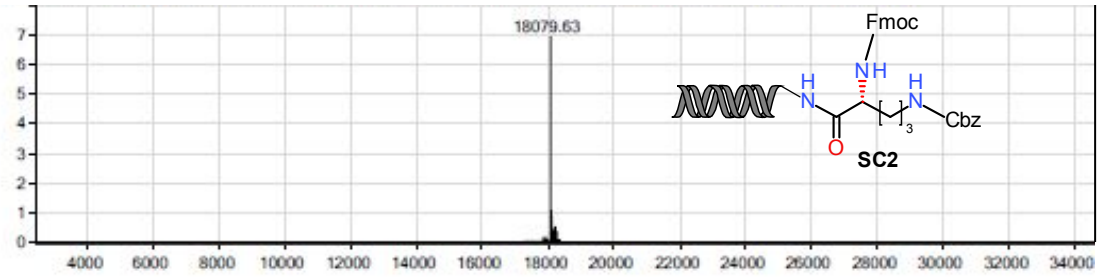


Figure S5: Deconvoluted mass spectrum of compound **SC2**, expected: 18076; observed: 18079

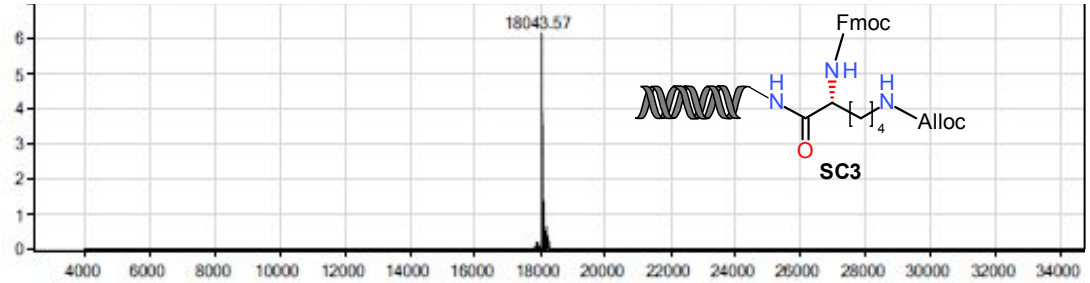


Figure S6: Deconvoluted mass spectrum of compound **SC3**, expected: 18040; observed: 18043

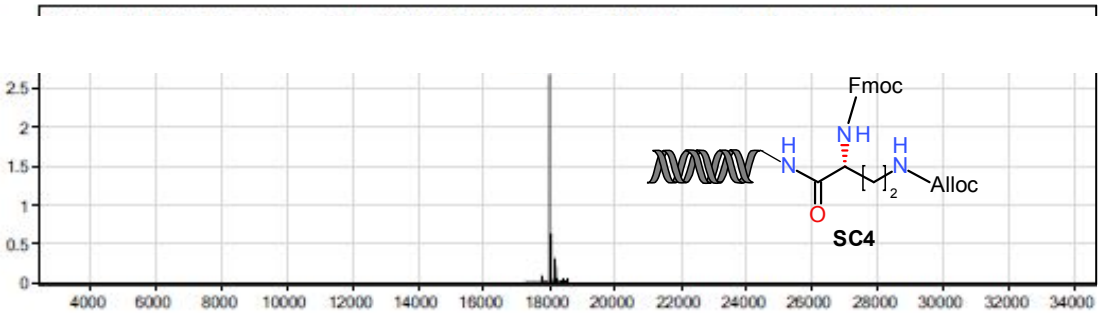


Figure S7: Deconvoluted mass spectrum of compound **SC4**, expected: 18012; observed: 18015

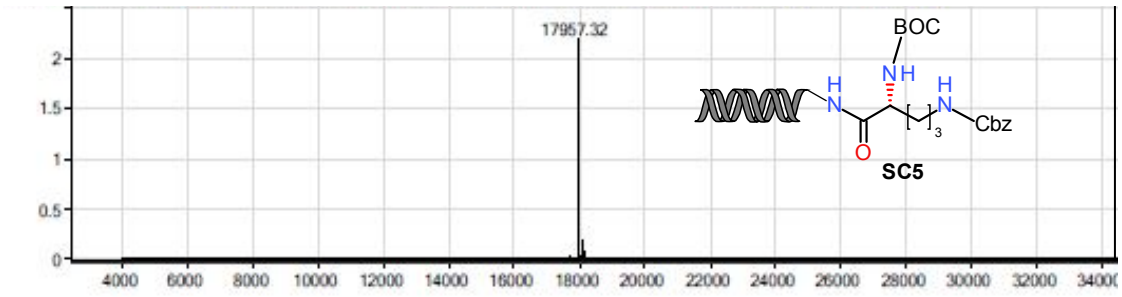


Figure S8: Deconvoluted mass spectrum of compound **SC5**, expected: 17954; observed: 17957

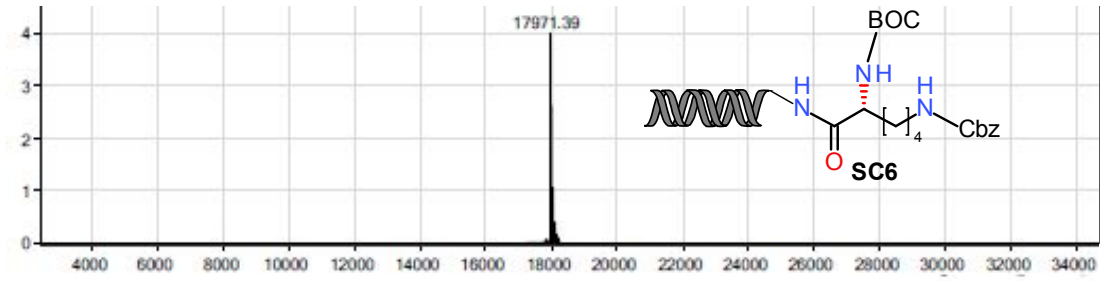


Figure S9: Deconvoluted mass spectrum of compound **SC6**, expected: 17968; observed: 17971

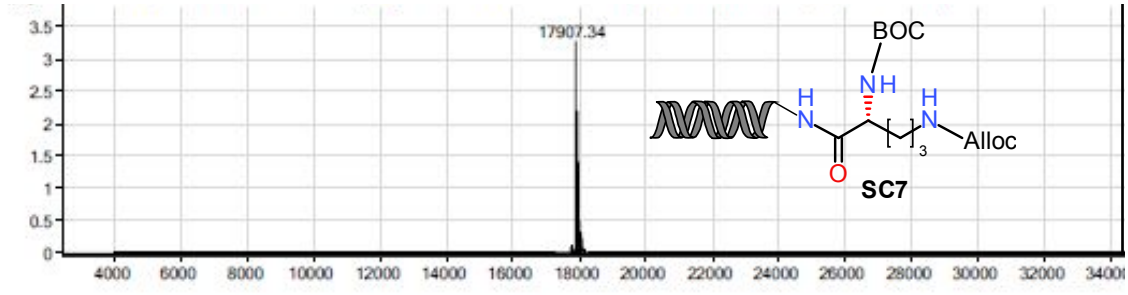
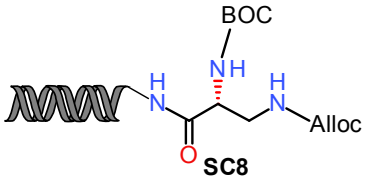


Figure S10: Deconvoluted mass spectrum of compound **SC7**, expected: 17904; observed: 17907



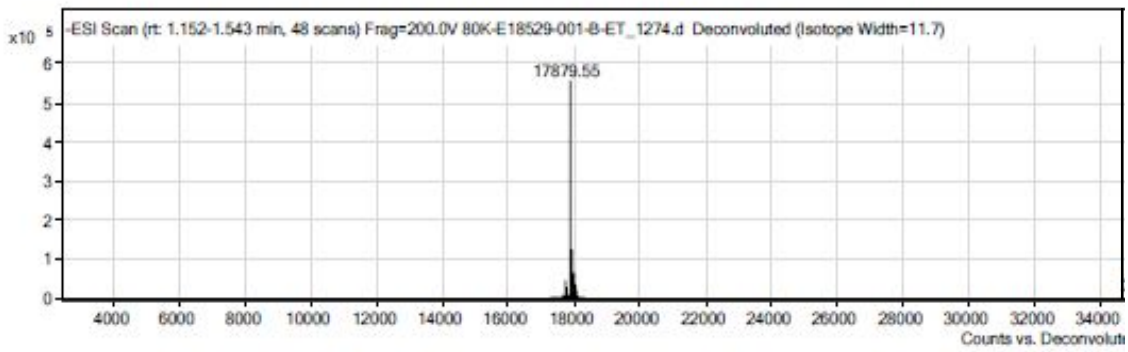


Figure S11: Deconvoluted mass spectrum of compound **SC8**, expected: 17876; observed: 17879

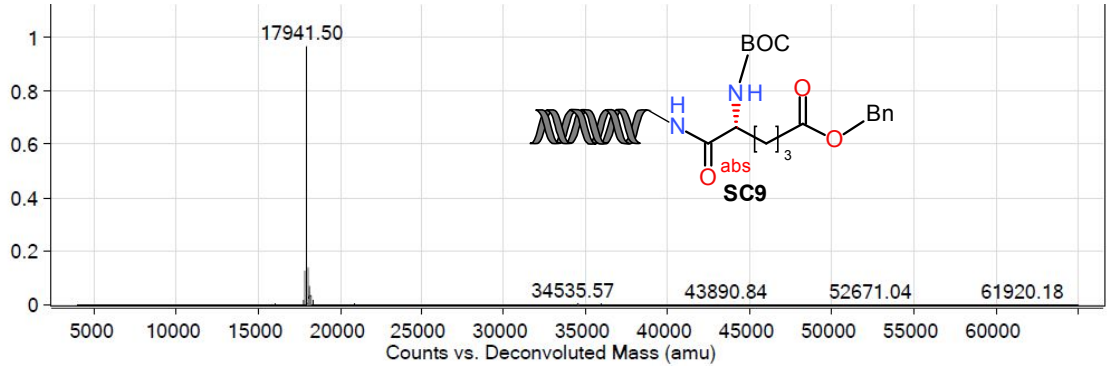


Figure S12: Deconvoluted mass spectrum of compound **SC9**, expected: 17939; observed: 17941

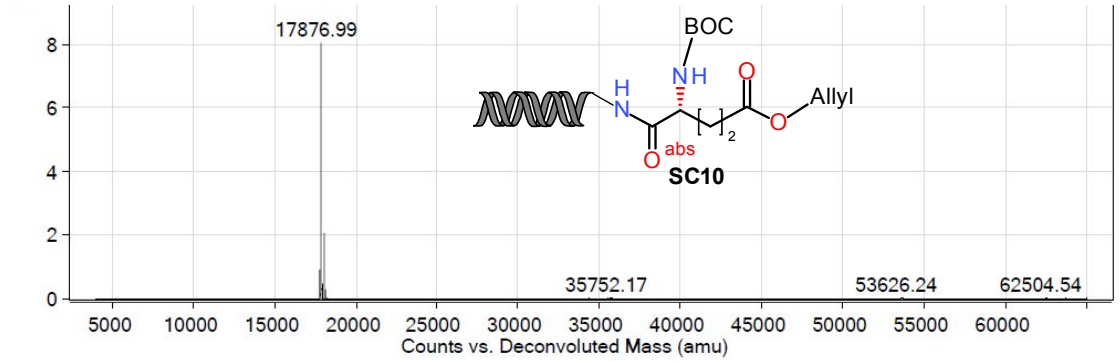
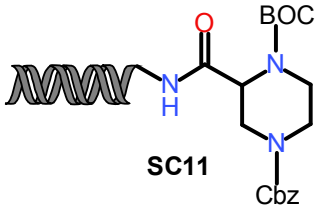


Figure S13: Deconvoluted mass spectrum of compound **SC10**, expected: 17875; observed: 17876



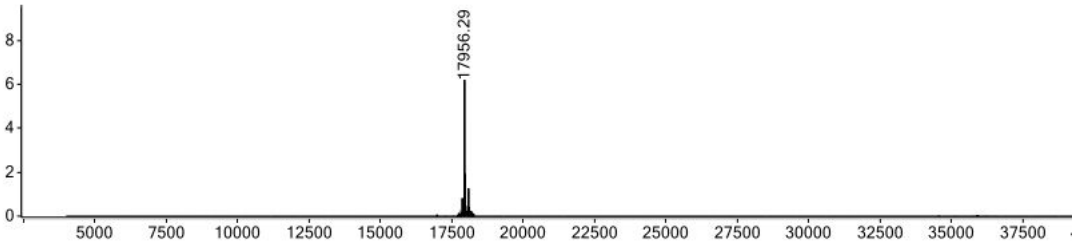


Figure S14: Deconvoluted mass spectrum of compound **SC11**, expected: 17953; observed: 17956

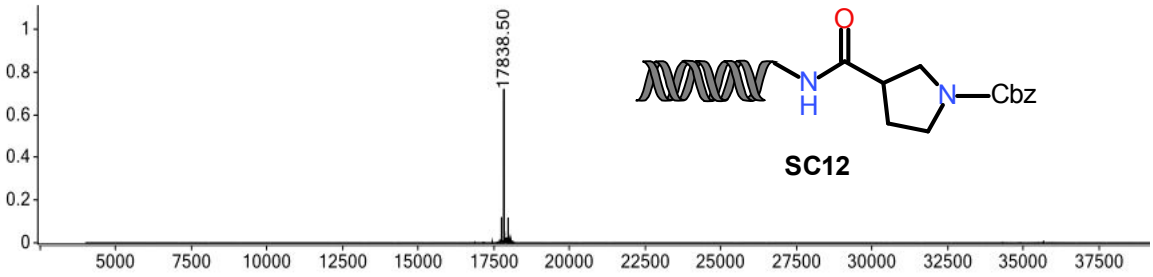


Figure S15: Deconvoluted mass spectrum of compound **SC12**, expected: 17837; observed: 17838

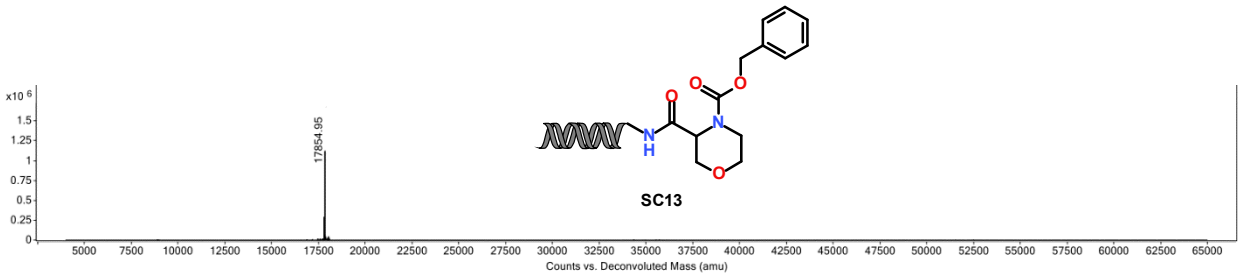


Figure S16: Deconvoluted mass spectrum of compound **SC13**, expected: 17853; observed: 17854

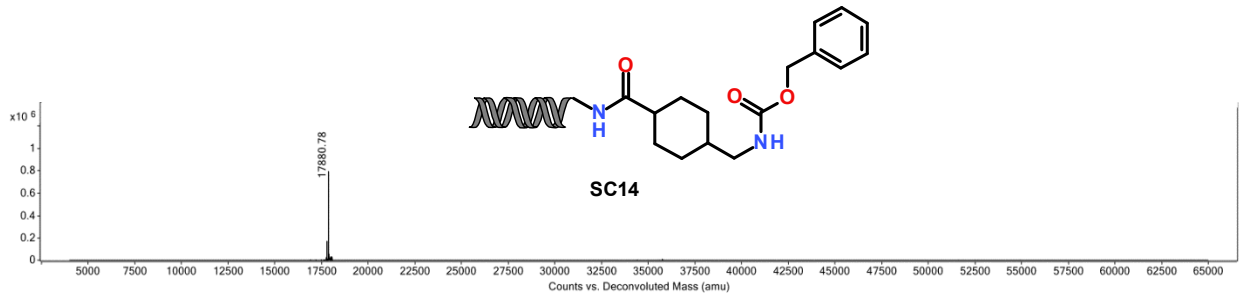


Figure S17: Deconvoluted mass spectrum of compound **SC14**, expected: 17879; observed: 17880

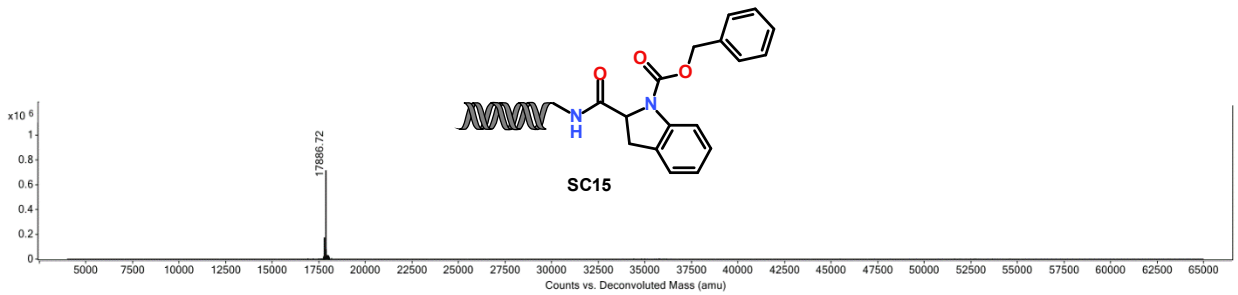


Figure S18: Deconvoluted mass spectrum of compound **SC15**, expected: 17885; observed: 17886

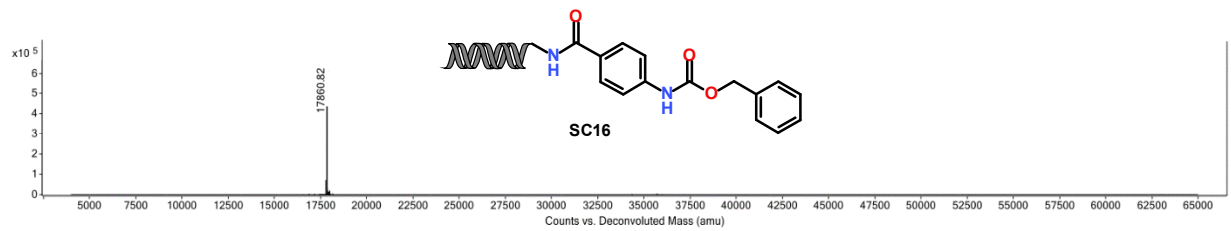


Figure S19: Deconvoluted mass spectrum of compound **SC16**, expected: 17859; observed: 17860

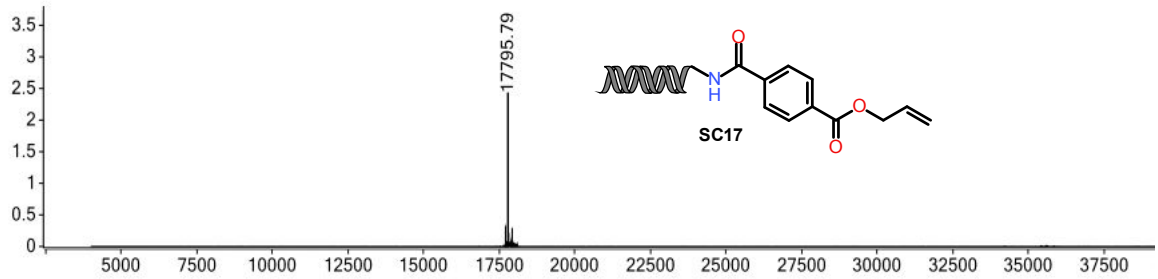


Figure S20: Deconvoluted mass spectrum of compound **SC17**, expected: 17794; observed: 17795

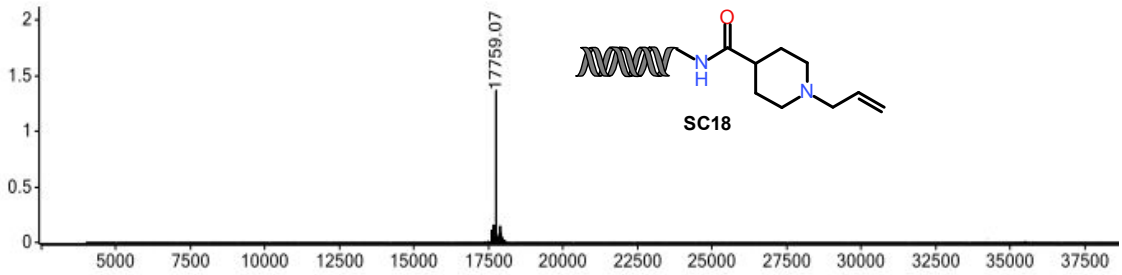


Figure S21: Deconvoluted mass spectrum of compound **SC18**, expected: 17757; observed: 17759

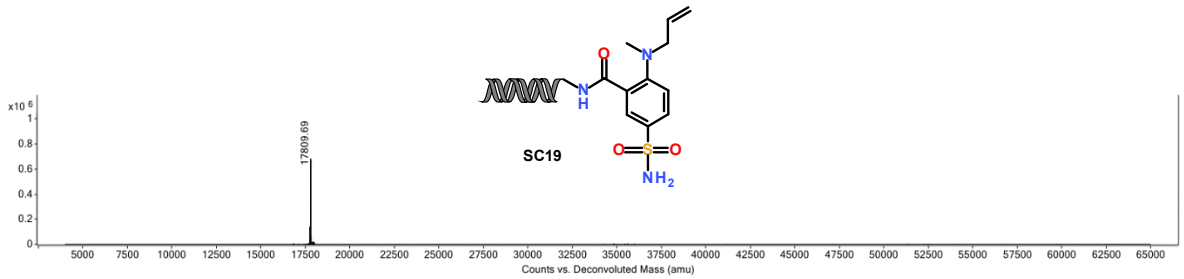


Figure S22: Deconvoluted mass spectrum of compound **SC19**, expected: 17808; observed: 17809

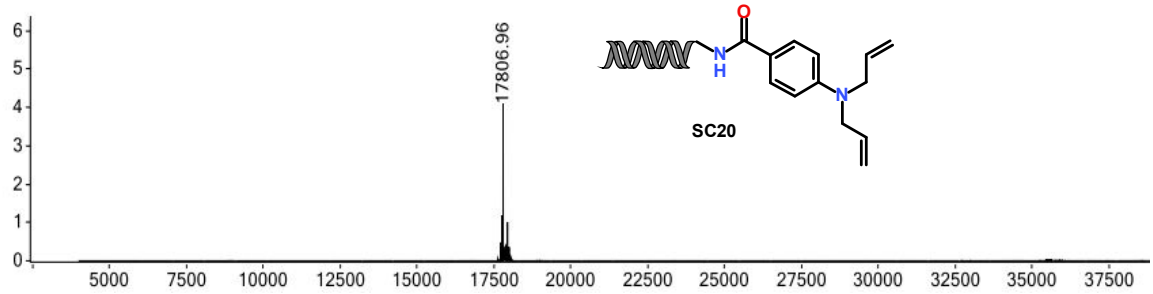


Figure S23: Deconvoluted mass spectrum of compound **SC20**, expected: 17805; observed: 17806

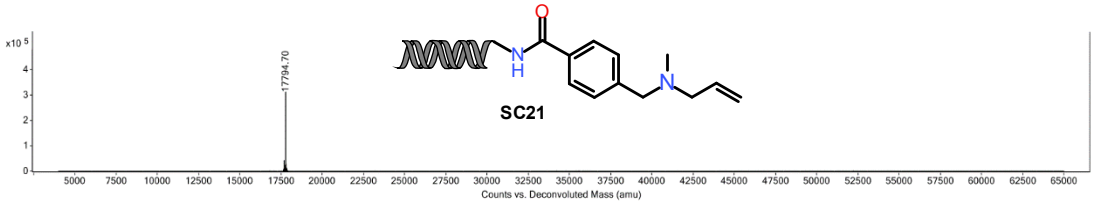


Figure S24: Deconvoluted mass spectrum of compound **SC21**, expected: 17793; observed: 17794

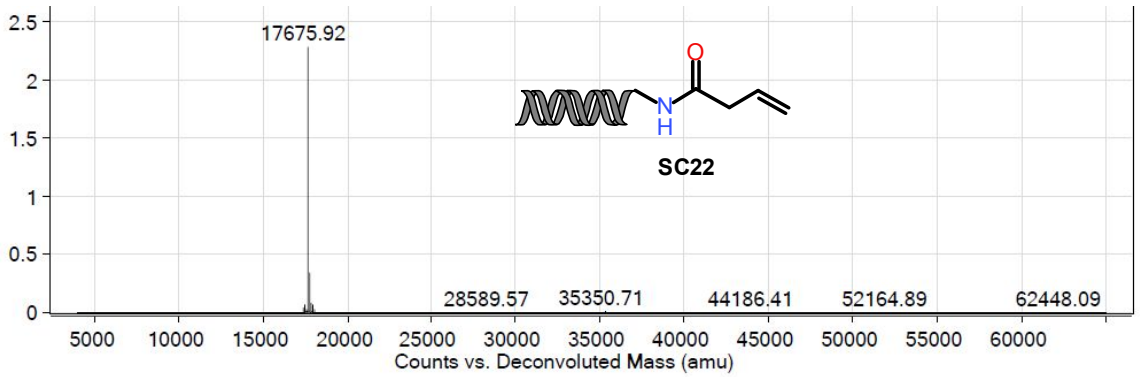


Figure S25: Deconvoluted mass spectrum of compound **SC22**, expected: 17674; observed: 17675

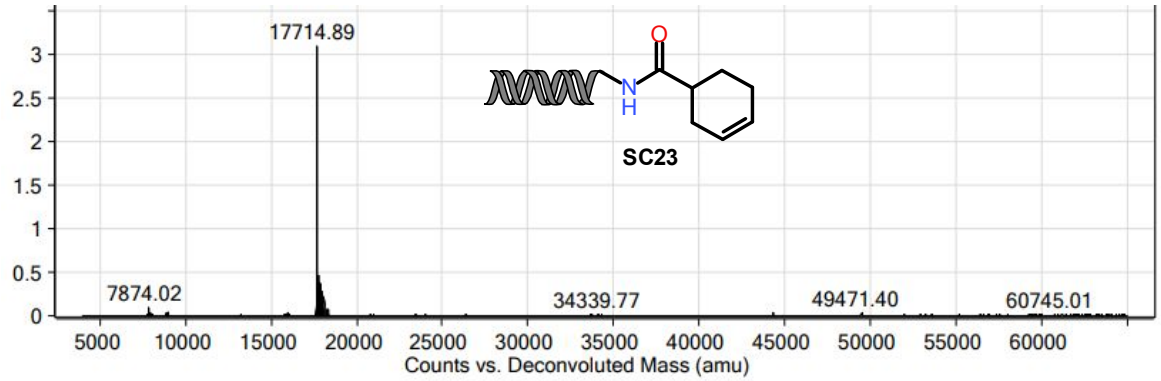


Figure S26: Deconvoluted mass spectrum of compound **SC23**, expected: 17714; observed: 17714

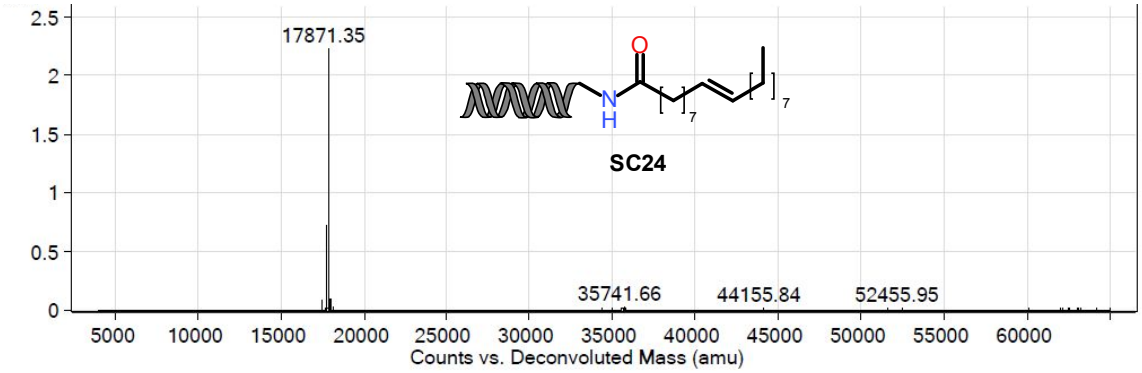


Figure S27: Deconvoluted mass spectrum of compound **SC24**, expected: 17870; observed: 17871

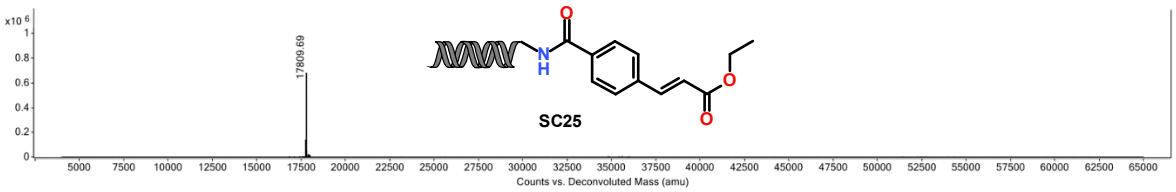


Figure S28: Deconvoluted mass spectrum of compound **SC25**, expected: 17808; observed: 17809

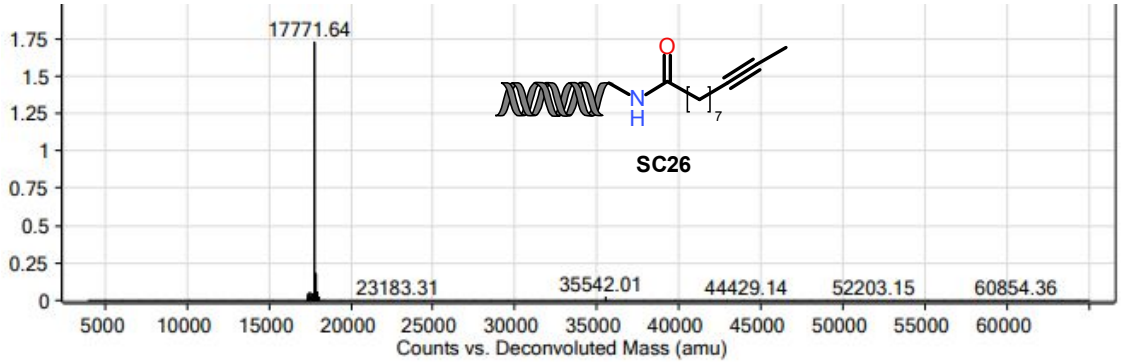


Figure S29: Deconvoluted mass spectrum of compound **SC26**, expected: 17770; observed: 17771

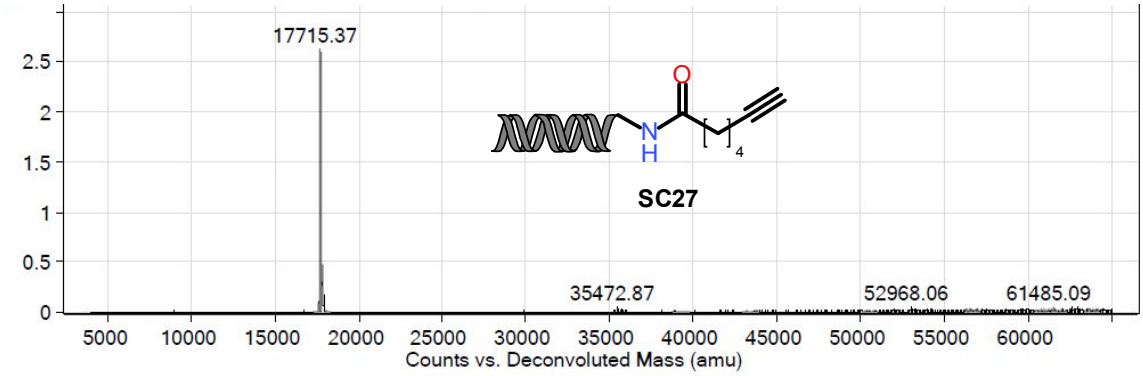


Figure S30: Deconvoluted mass spectrum of compound **SC27**, expected: 17714; observed: 17715

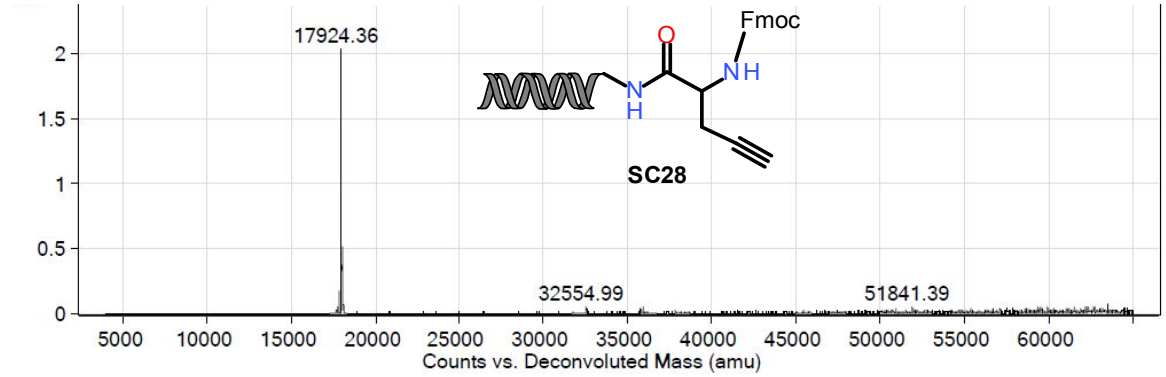


Figure S31: Deconvoluted mass spectrum of compound **SC28**, expected: 17923; observed: 17924

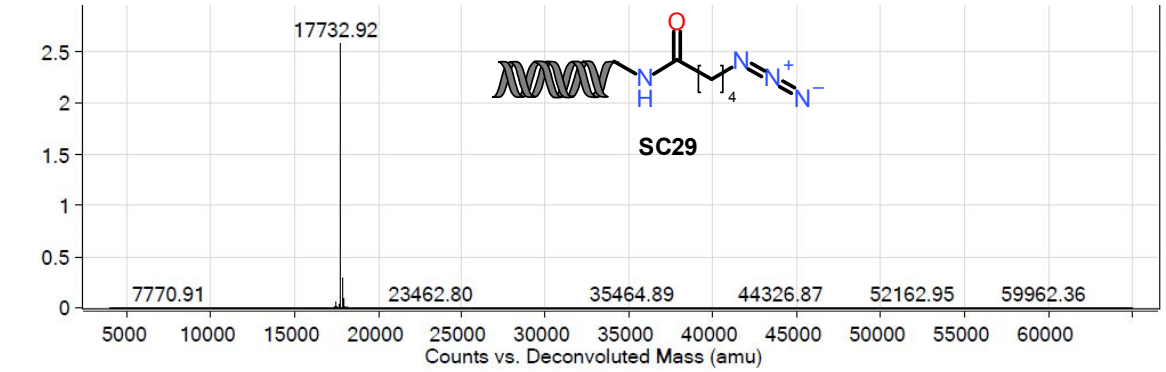


Figure S32: Deconvoluted mass spectrum of compound **SC29**, expected: 17731; observed: 17732

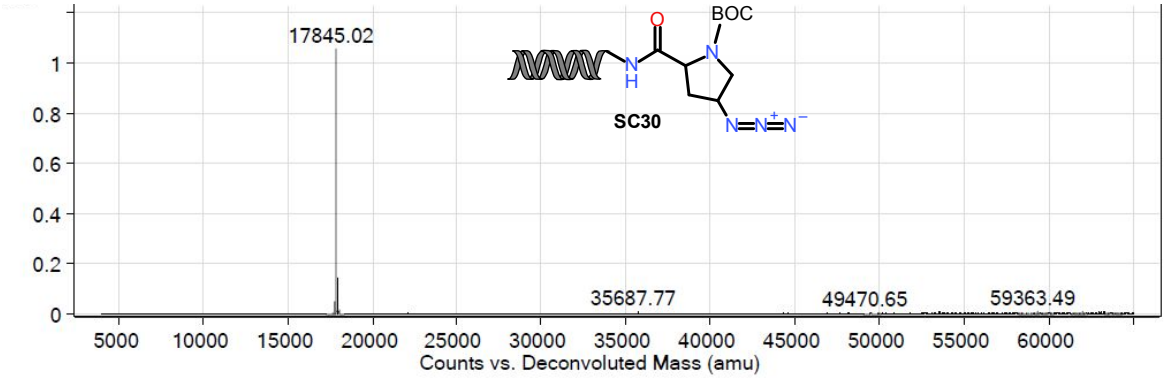


Figure S33: Deconvoluted mass spectrum of compound **SC30**, expected: 17844; observed: 17845

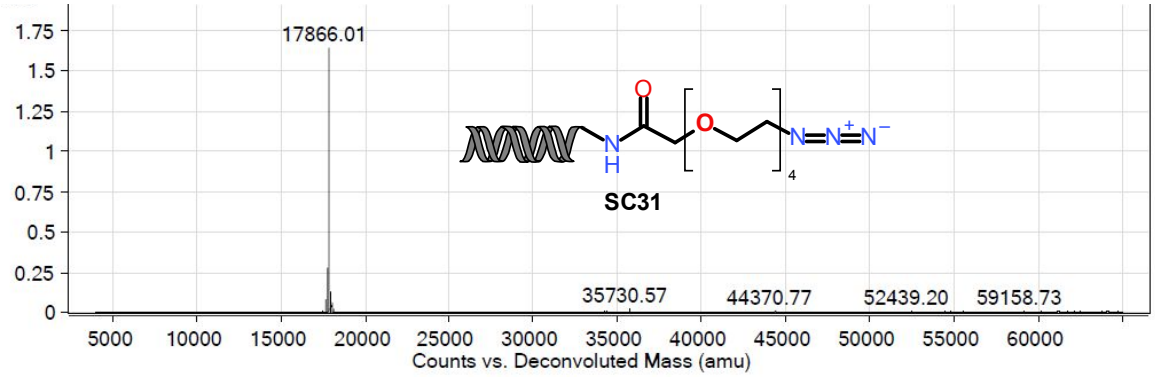


Figure S34: Deconvoluted mass spectrum of compound **SC31**, expected: 17865; observed: 17866

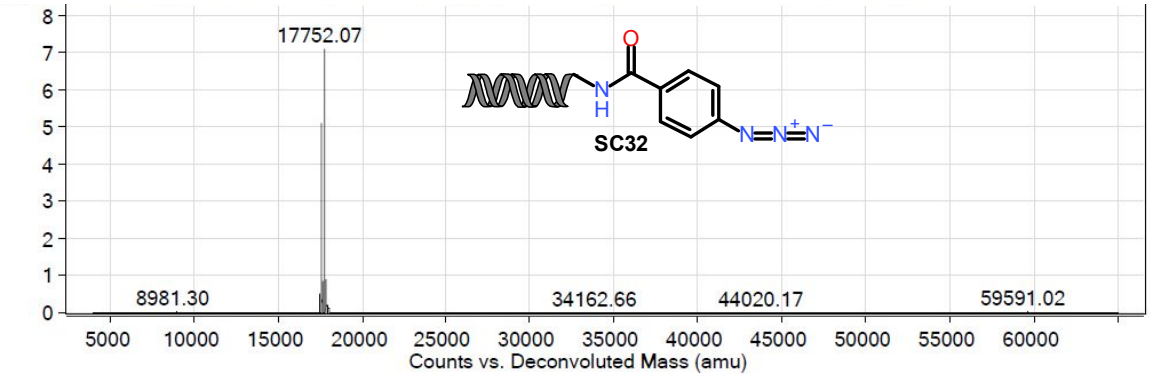


Figure S35: Deconvoluted mass spectrum of compound **SC32**, expected: 17751; observed: 17752

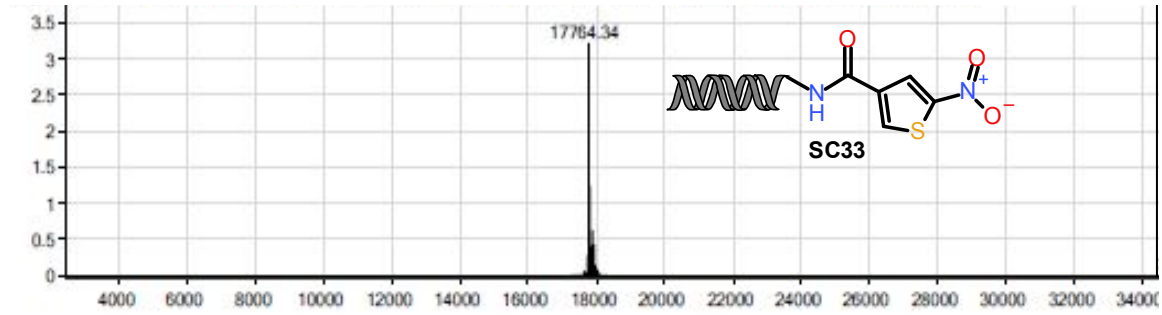


Figure S36: Deconvoluted mass spectrum of compound **SC33**, expected: 17761; observed: 17764

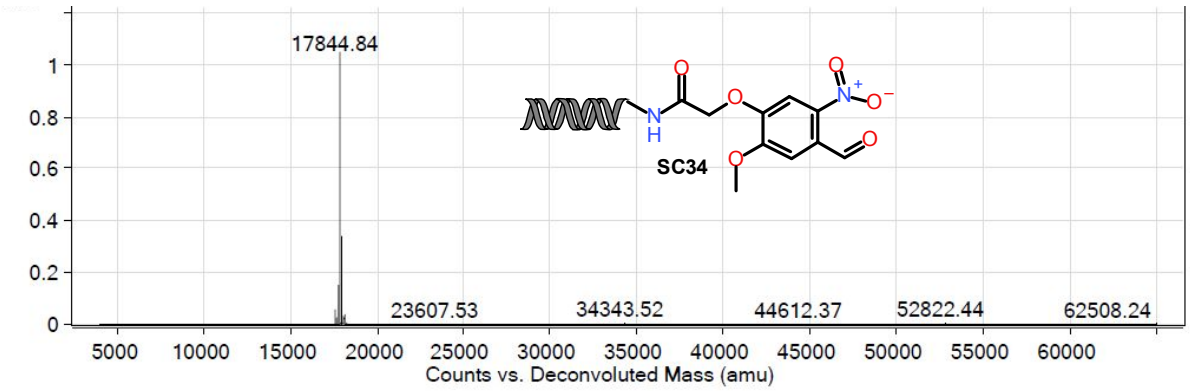


Figure S37: Deconvoluted mass spectrum of compound **SC34**, expected: 17843; observed: 17844

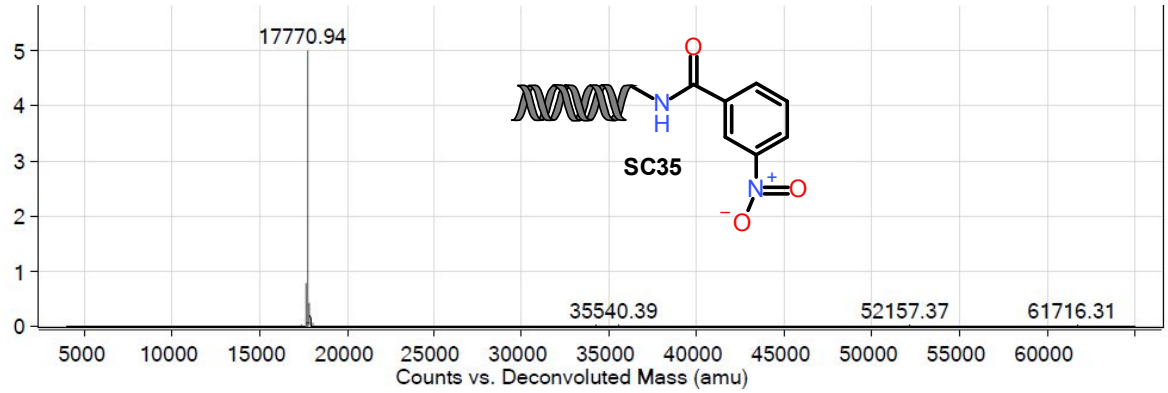


Figure S38: Deconvoluted mass spectrum of compound **SC35**, expected: 17769; observed: 17770

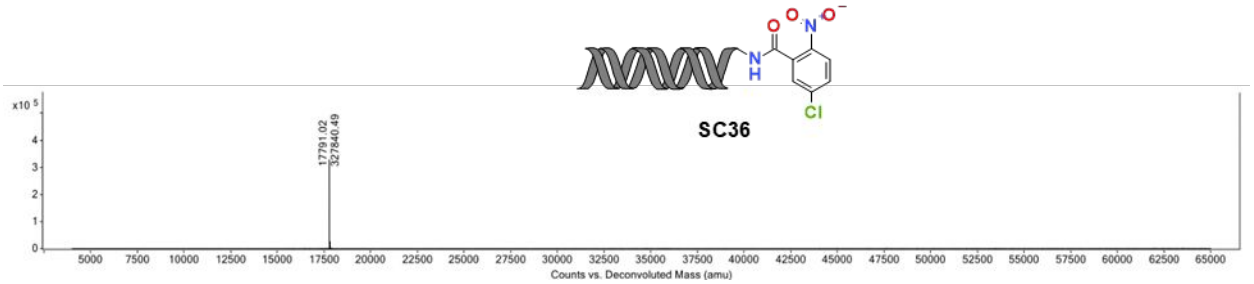


Figure S39: Deconvoluted mass spectrum of compound **SC36**, expected: 17790; observed: 17791

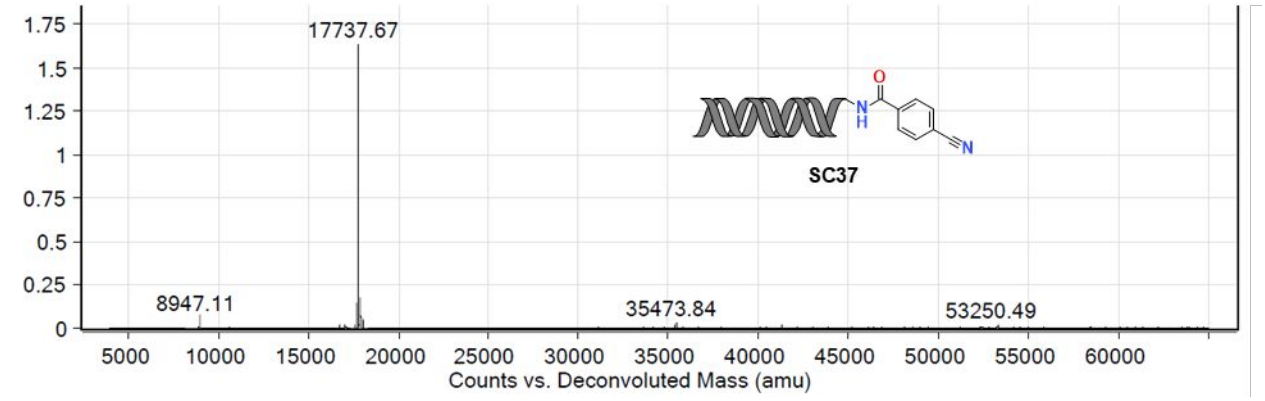


Figure S40: Deconvoluted mass spectrum of compound **SC37**, expected: 17735; observed: 17737

IV. General procedure for deprotection and reduction of DNA-*N*-Cbz, *N*-Alloc, *N*-Allyl, O-Bn and O-Allyl and representative LCMS

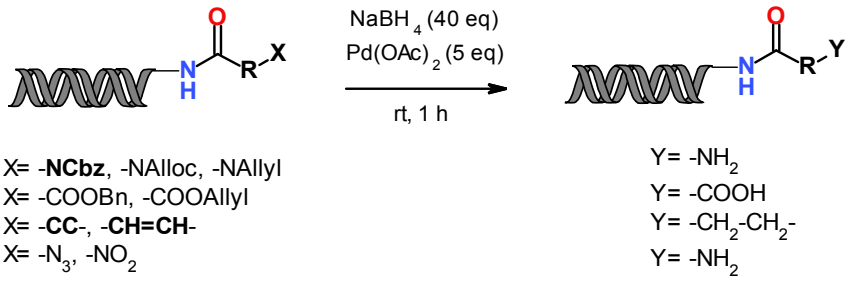


Figure S41: AOP-HP-Primer-NH-CO-R-X (X = *N*-Cbz, *N*-Alloc, *N*-Allyl, O-Bn, O-Allyl groups, alkene, alkyne, azide and nitro groups) deprotection/reduction promoted by $Pd(OAc)_2/NaBH_4$.

To a 1 mM solution of DNA-derivative (for **SC1** to **SC36**) in water (20 nmol, 20 μL) were added 40 equivalents of sodium borohydride (2 μL, 400 mM in NMP) and 5 equivalents of palladium acetate (2 μL, 50 mM in DMA). The reaction was shaken at rt for 1h and then it was treating with a L-Cysteine (20 μL, 300 mM in water).

Adding L-Cysteine (Pd scavenger) was necessary before running LC/MS; otherwise, the MS signal was messy. The following treatment could be applied for the Pd removal.

L-Cysteine treatment[2]

To the reaction (containing 20 nmol of **SC#**), 300 equivalents of a stock solution of L-cysteine (3000 nmols, 300 mM in water, 20 μL) were added. The suspension was left stirring at room temperature for 4 hours. In some cases, when it was left too much time, L-cysteine can make S-S bonds and precipitate, in those cases centrifugation and collection of the supernatant was carried out. The DNA was then precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C.

[2] Barbaras, D.; Broziu, J.; Johannsen, I.; Allmendinger, T. Removal of Heavy Metals from Organic Reaction Mixtures: Preparation and Application of Functionalized Resins. *Org. Process Res. Dev.* **2009**, *13*, 1068-1079.

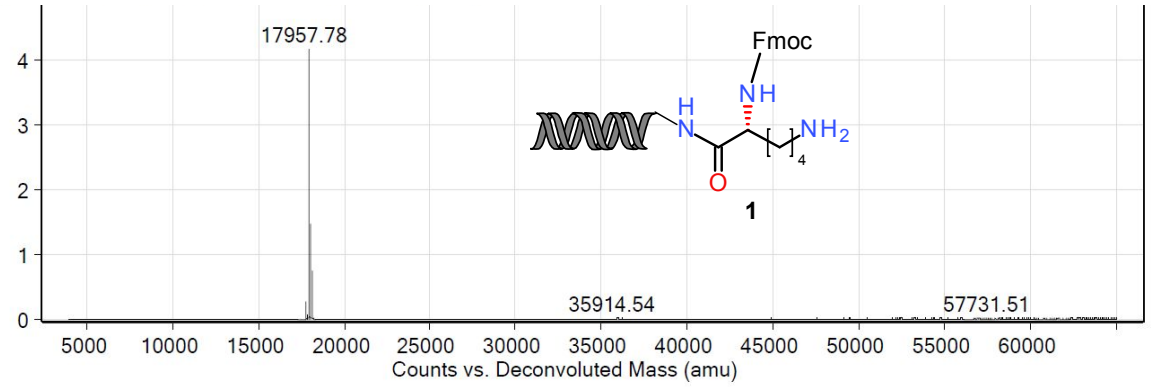


Figure S42: Deconvoluted mass spectrum of compound **1**, expected: 17955; observed: 17957

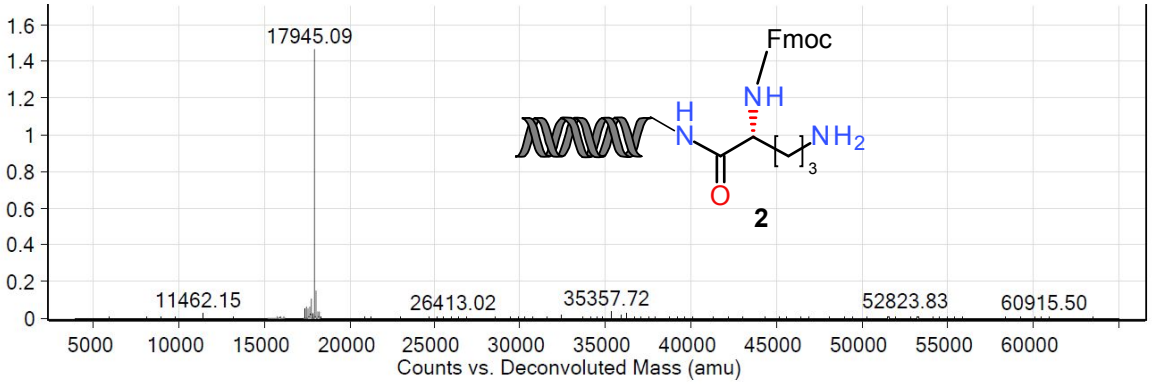


Figure S43: Deconvoluted mass spectrum of compound **2**, expected: 17941; observed: 17945

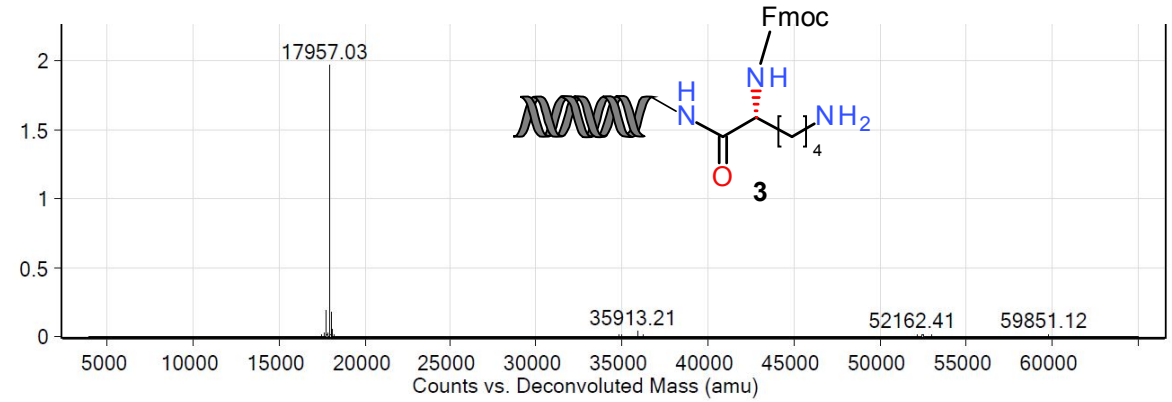


Figure S44: Deconvoluted mass spectrum of compound **3**, expected: 17956; observed: 17957

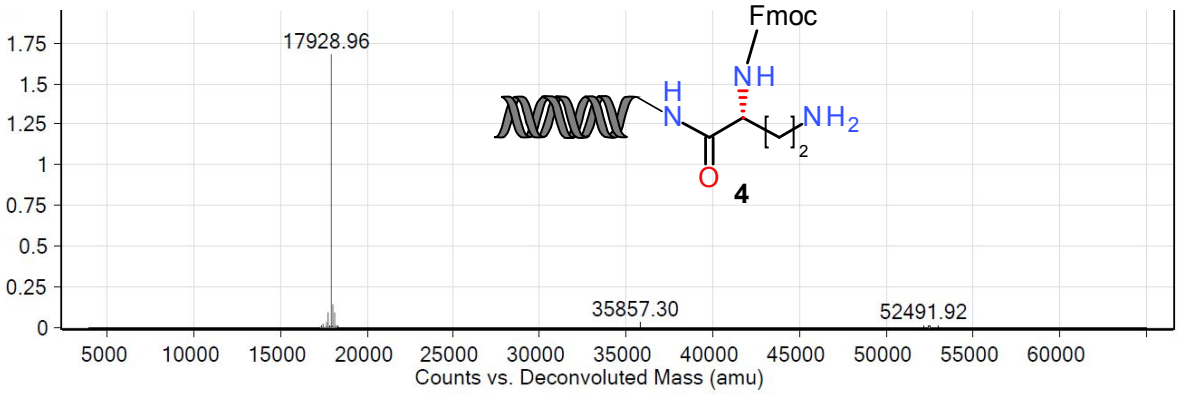


Figure S45: Deconvoluted mass spectrum of compound **4**, expected: 17928; observed: 17928

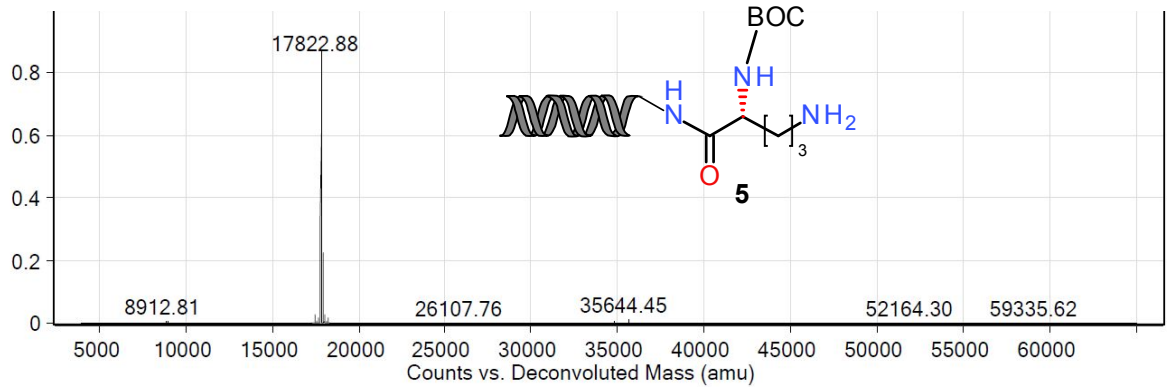


Figure S46: Deconvoluted mass spectrum of compound **5**, expected: 17820; observed: 17822

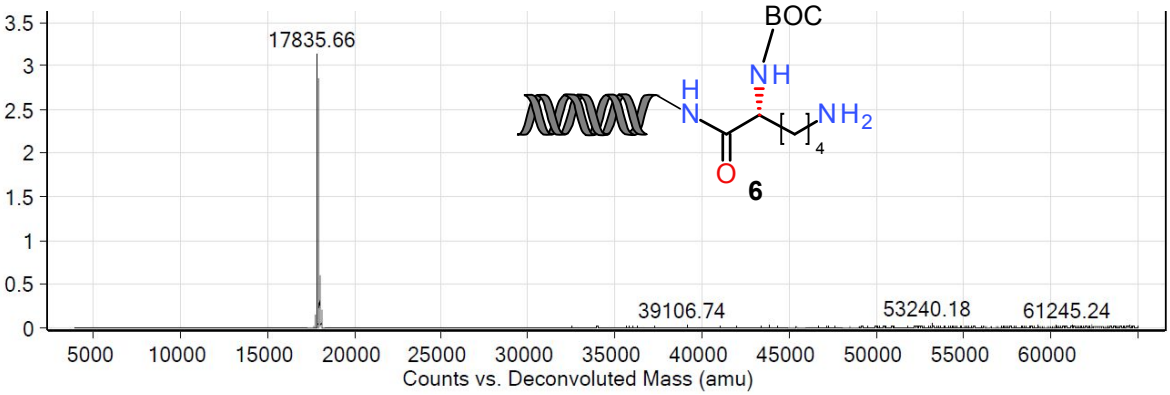


Figure S47: Deconvoluted mass spectrum of compound **6**, expected: 17833; observed: 17835

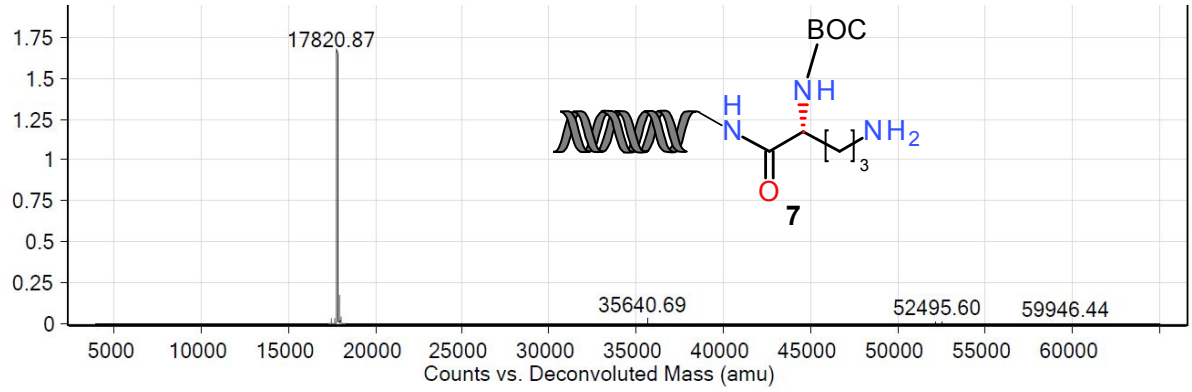


Figure S48: Deconvoluted mass spectrum of compound **7**, expected: 17820; observed: 17820

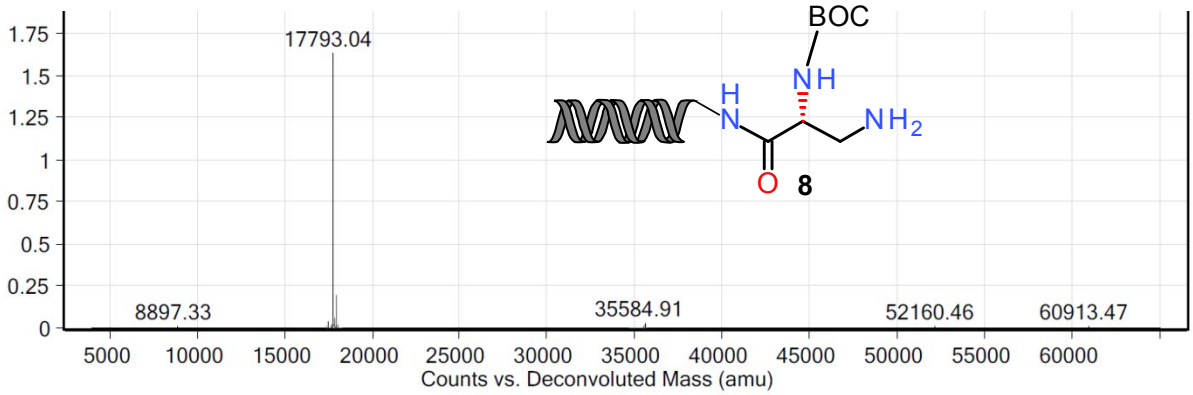


Figure S49: Deconvoluted mass spectrum of compound **8**, expected: 17792; observed: 17793

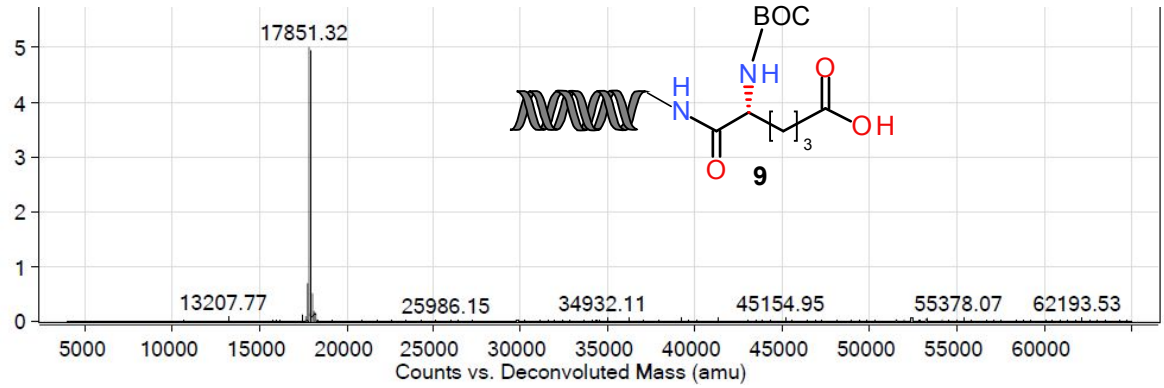


Figure S50: Deconvoluted mass spectrum of compound **9**, expected: 17849; observed: 17851

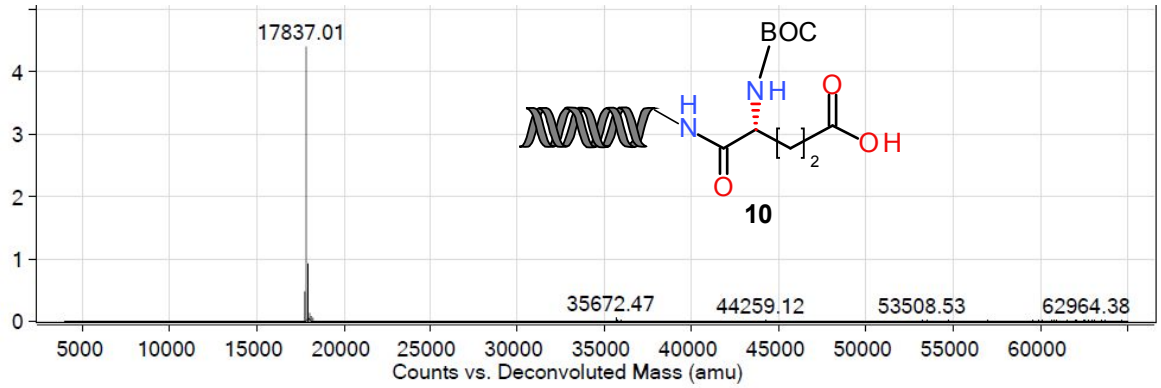


Figure S51: Deconvoluted mass spectrum of compound **10**, expected: 17835; observed: 17837

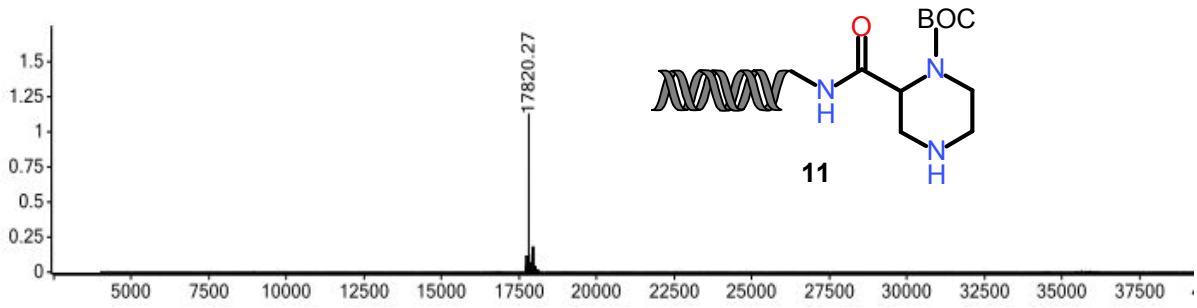


Figure S52: Deconvoluted mass spectrum of compound **11**, expected: 17818; observed: 17820

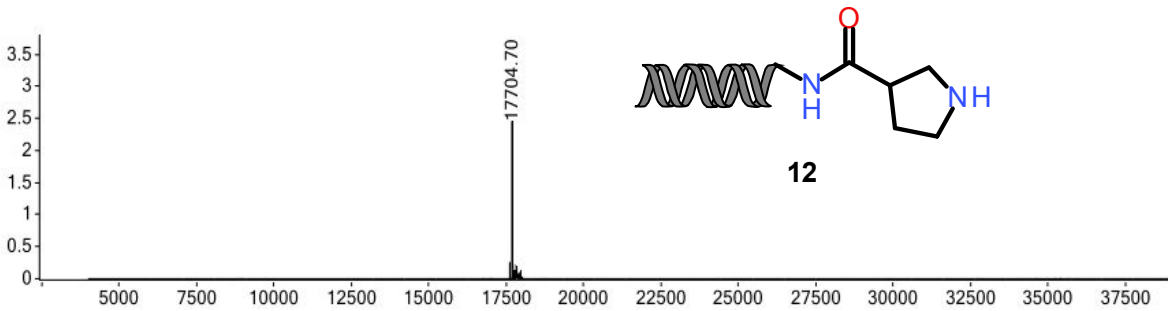


Figure S53: Deconvoluted mass spectrum of compound **12**, expected: 17703; observed: 17704

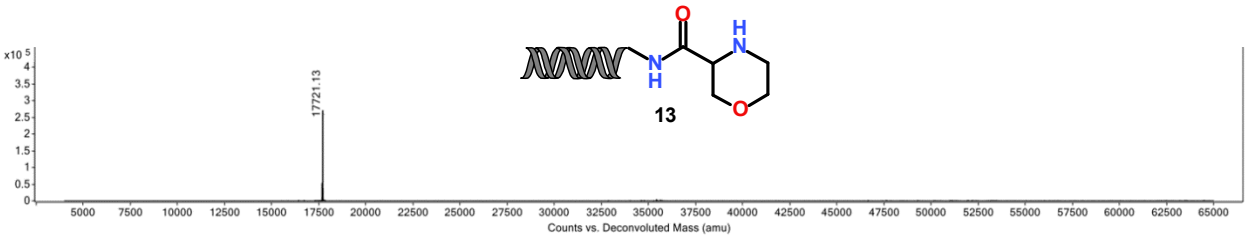


Figure S54: Deconvoluted mass spectrum of compound **13**, expected: 17719; observed: 17721

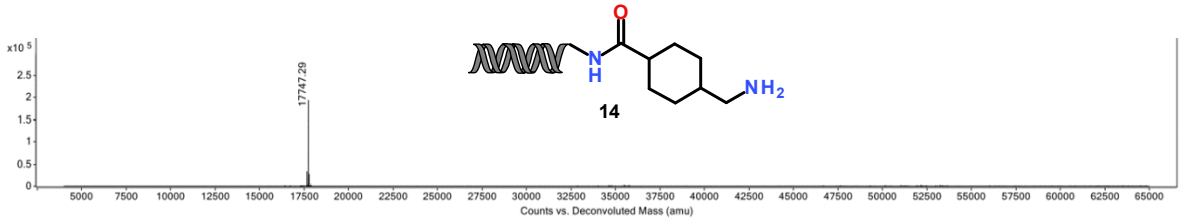


Figure S55: Deconvoluted mass spectrum of compound **14**, expected: 17745; observed: 17747

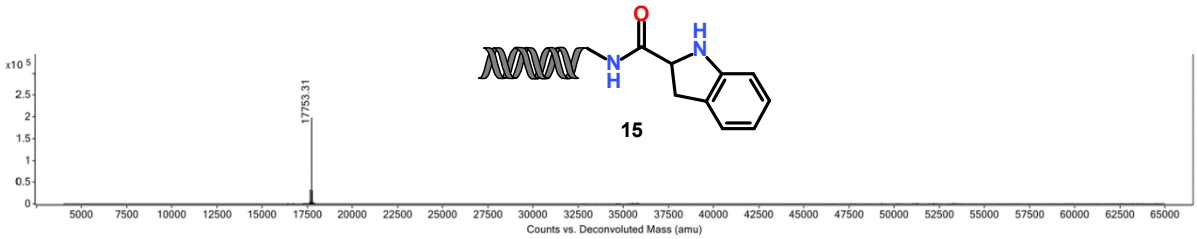


Figure S56: Deconvoluted mass spectrum of compound **15**, expected: 17751; observed: 17753

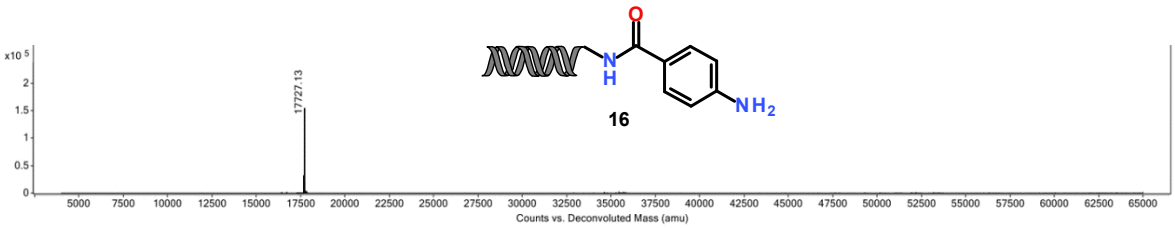


Figure S57: Deconvoluted mass spectrum of compound **16**, expected: 17725; observed: 17727

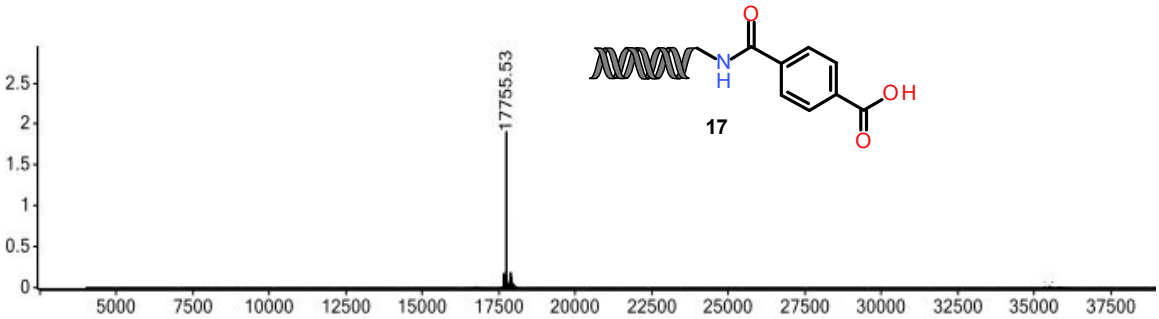


Figure S58: Deconvoluted mass spectrum of compound **17**, expected: 17754; observed: 17755

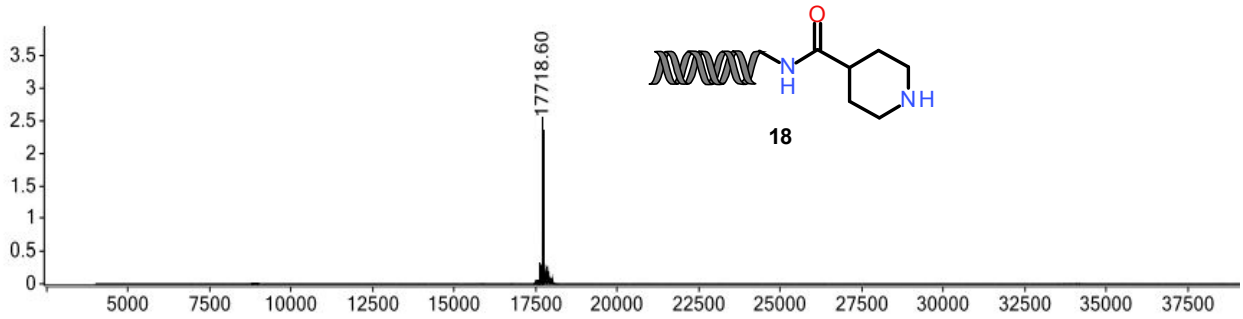


Figure S59: Deconvoluted mass spectrum of compound **18**, expected: 17716; observed: 17718

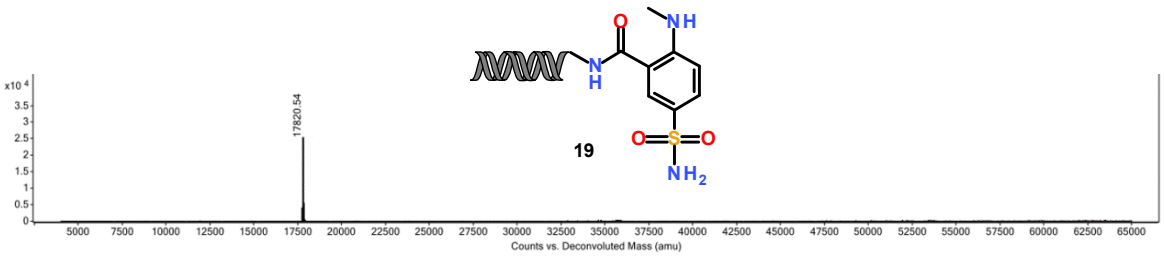


Figure S60: Deconvoluted mass spectrum of compound **19**, expected: 17818; observed: 17820

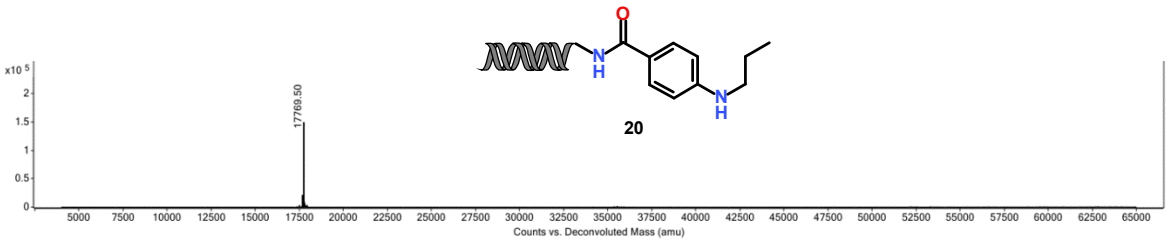


Figure S61: Deconvoluted mass spectrum of compound **20**, expected: 17767; observed: 17769

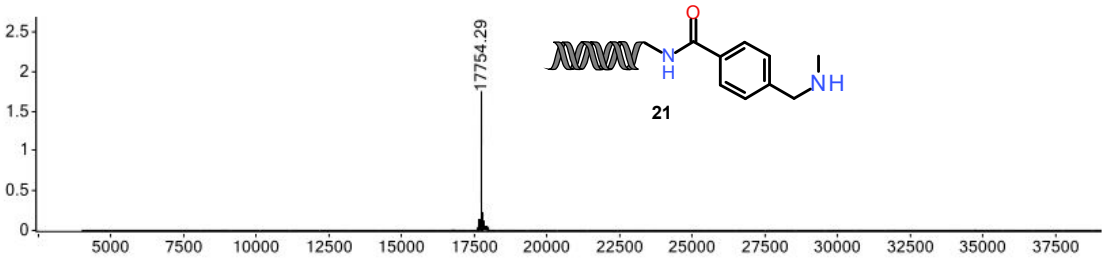


Figure S62: Deconvoluted mass spectrum of compound **21**, expected: 17753; observed: 17754

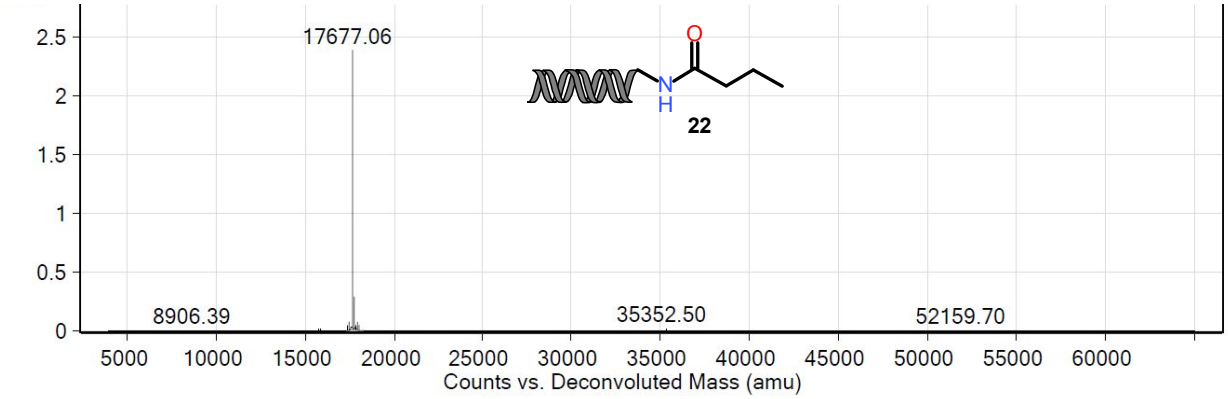


Figure S63: Deconvoluted mass spectrum of compound **22**, expected: 17676; observed: 17677

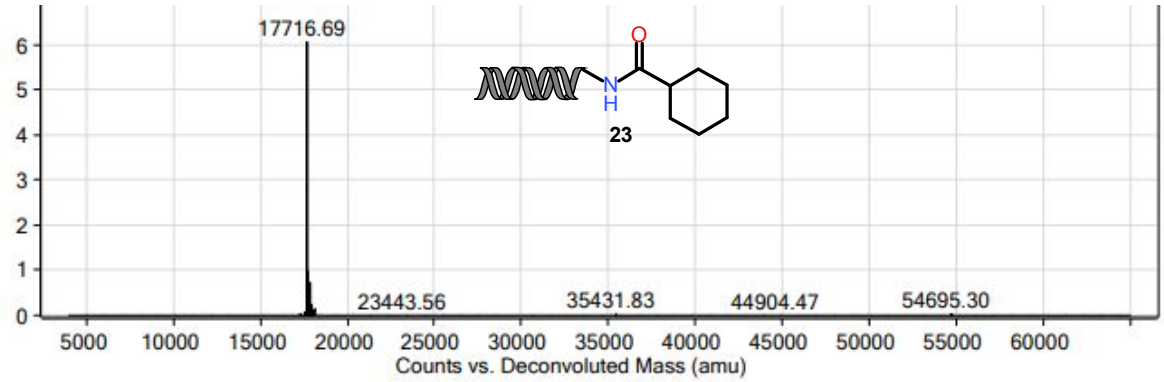


Figure S64: Deconvoluted mass spectrum of compound **23**, expected: 17716; observed: 17716

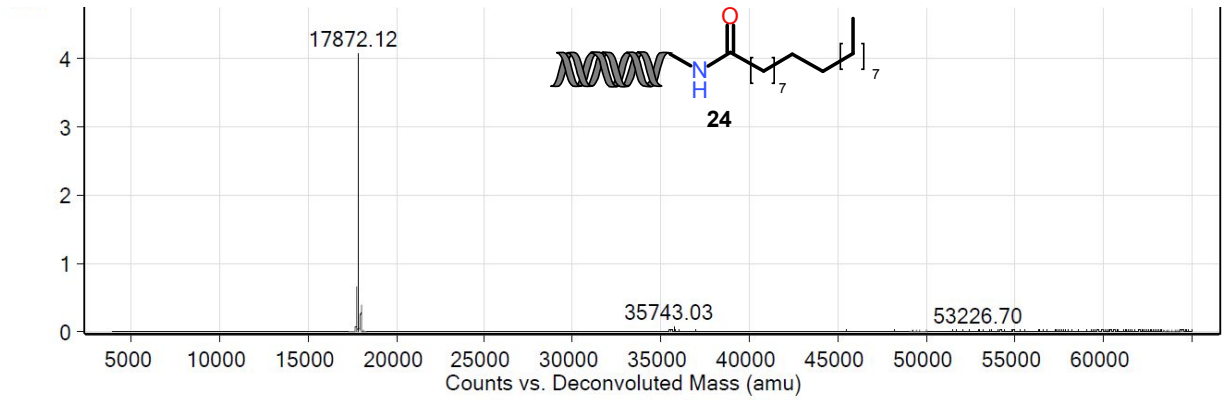


Figure S65: Deconvoluted mass spectrum of compound **24**, expected: 17872; observed: 17872

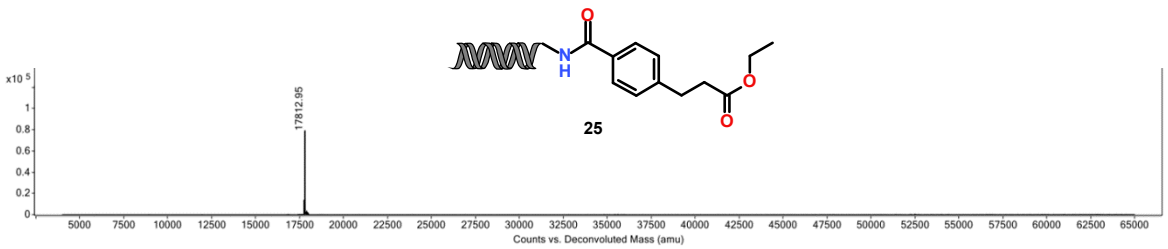


Figure S66: Deconvoluted mass spectrum of compound **25**, expected: 17810; observed: 17812

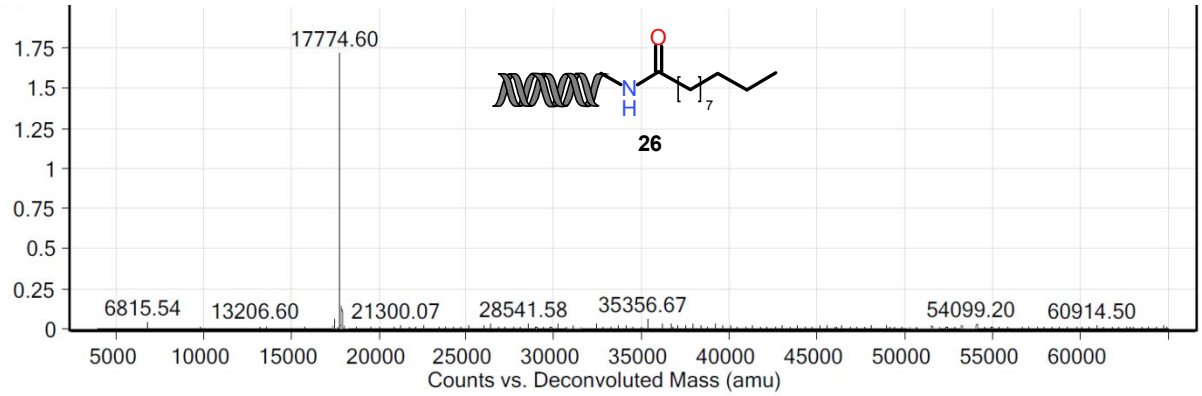


Figure S67: Deconvoluted mass spectrum of compound **26**, expected: 17774; observed: 17774

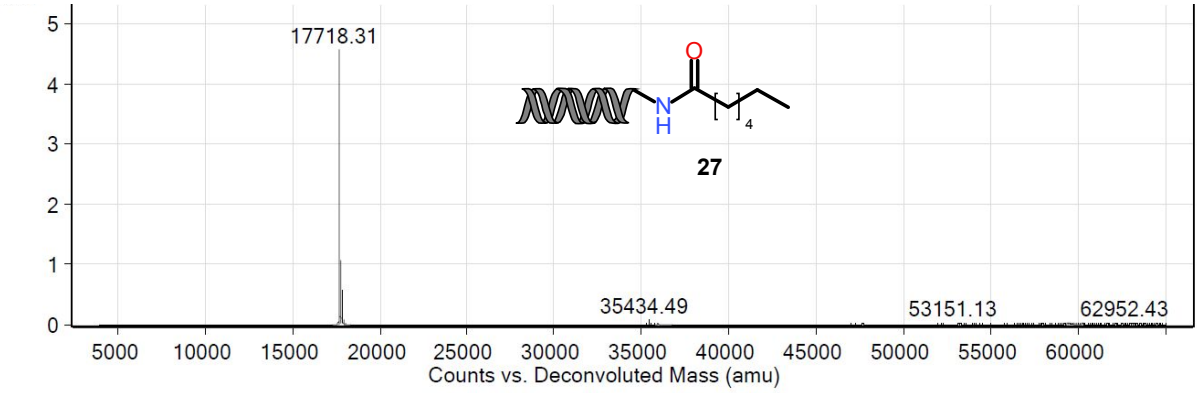


Figure S68: Deconvoluted mass spectrum of compound **27**, expected: 17718; observed: 17718

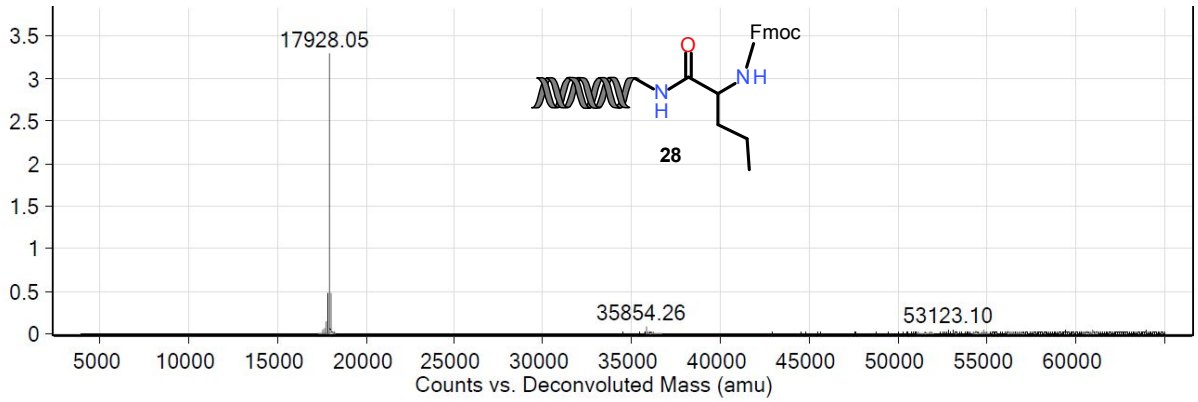


Figure S69: Deconvoluted mass spectrum of compound **28**, expected: 17927; observed: 17928

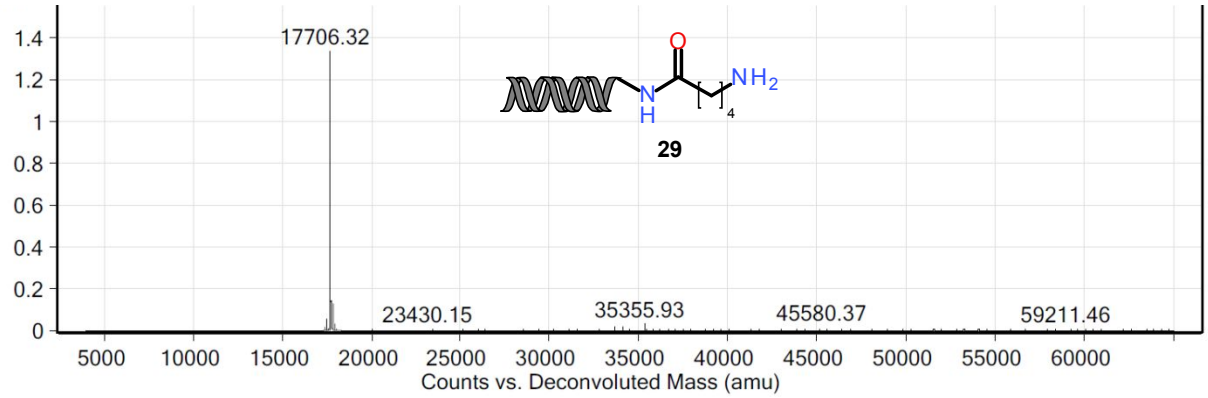


Figure S70: Deconvoluted mass spectrum of compound **29**, expected: 17705; observed: 17706

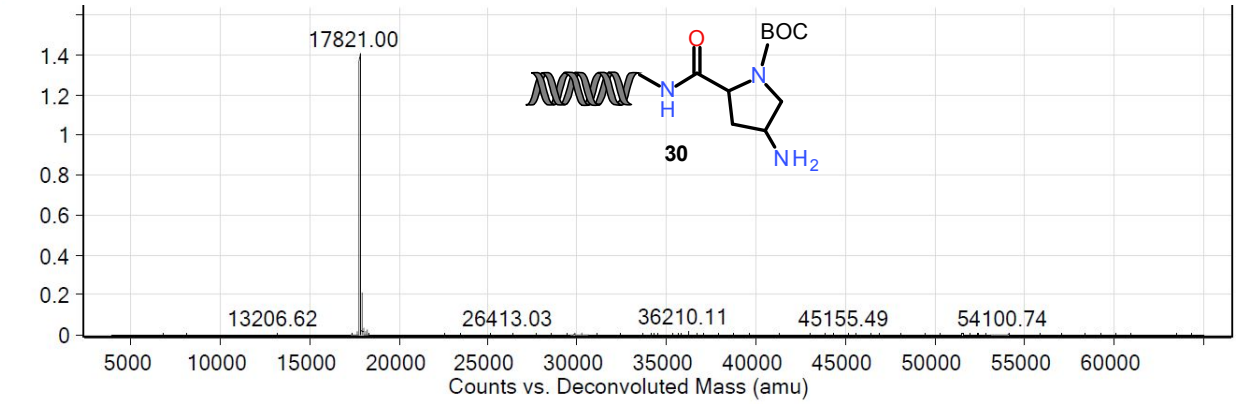


Figure S71: Deconvoluted mass spectrum of compound **30**, expected: 17818; observed: 17821

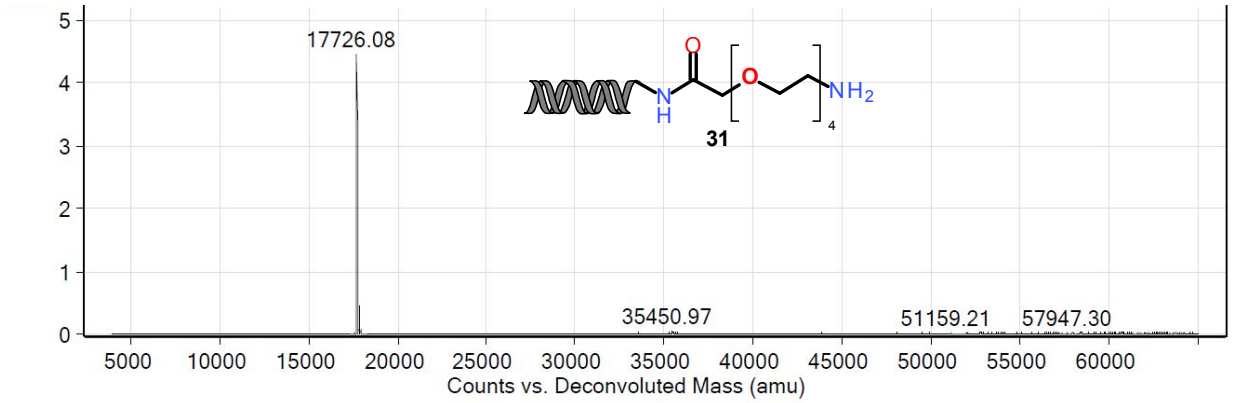


Figure S72: Deconvoluted mass spectrum of compound **31**, expected: 17725; observed: 17726

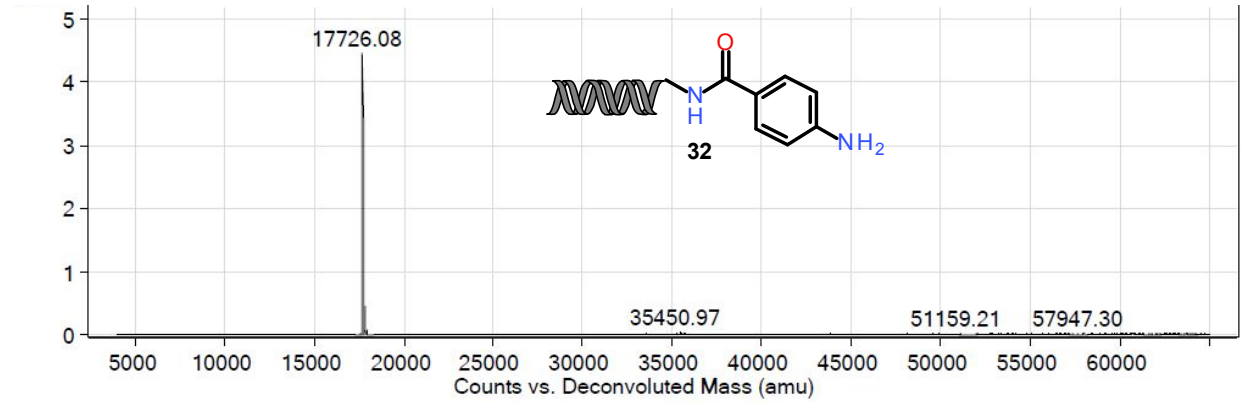


Figure S73: Deconvoluted mass spectrum of compound **32**, expected: 17725; observed: 17726

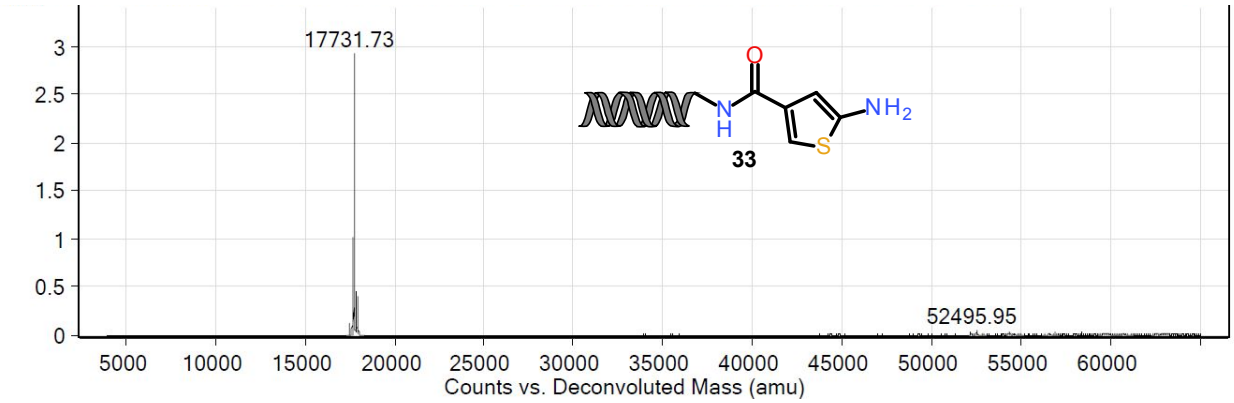


Figure S74: Deconvoluted mass spectrum of compound **33**, expected: 17731; observed: 17731

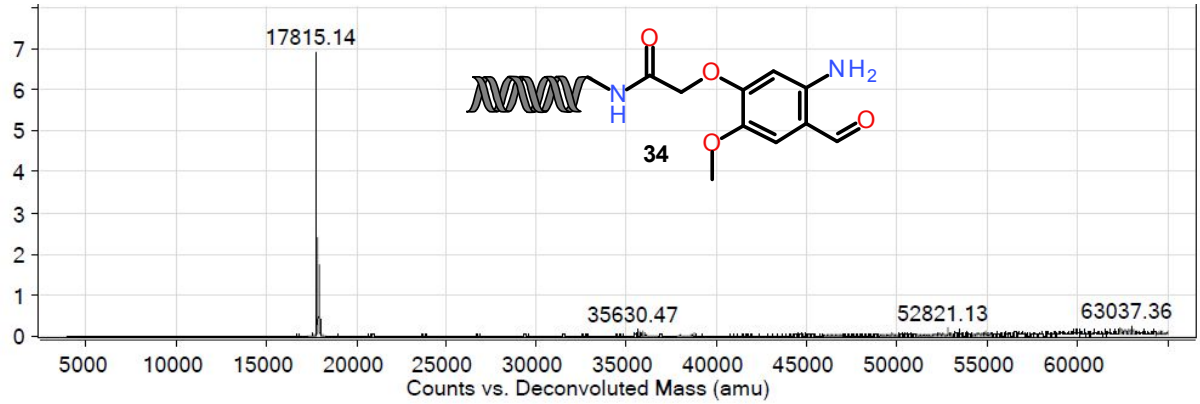


Figure S75: Deconvoluted mass spectrum of compound **34**, expected: 17813; observed: 17815

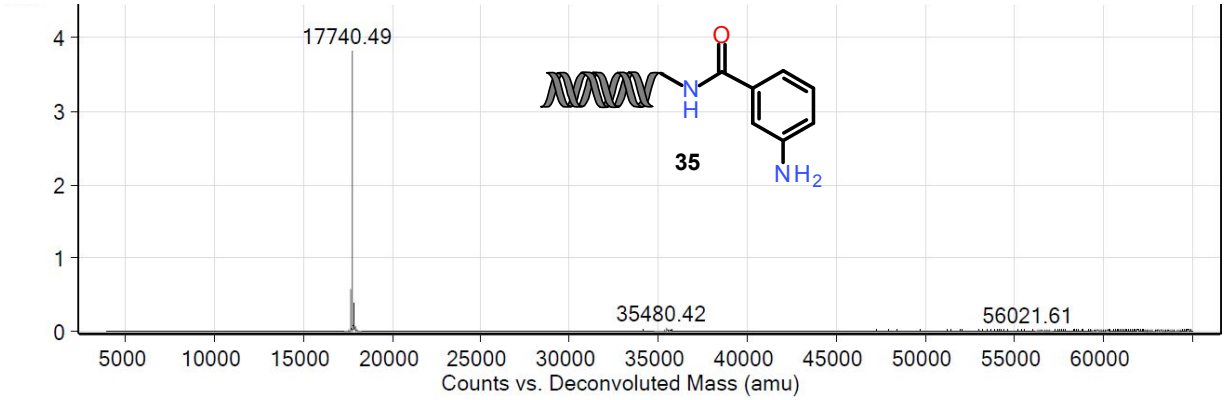


Figure S76: Deconvoluted mass spectrum of compound **35**, expected: 17739; observed: 17740

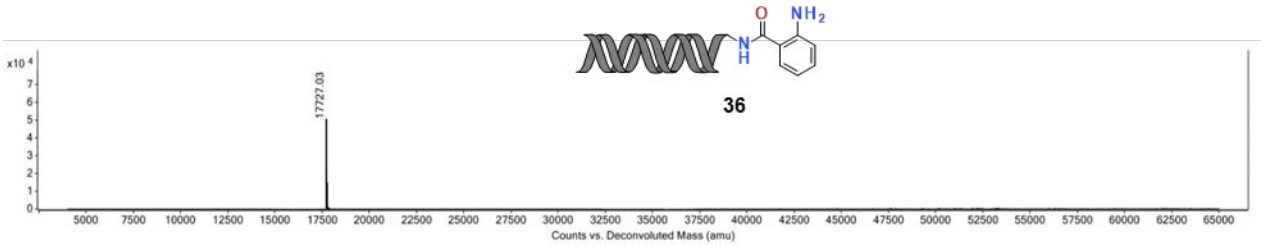


Figure S77: Deconvoluted mass spectrum of compound **36**, expected: 17760; observed: 17727.

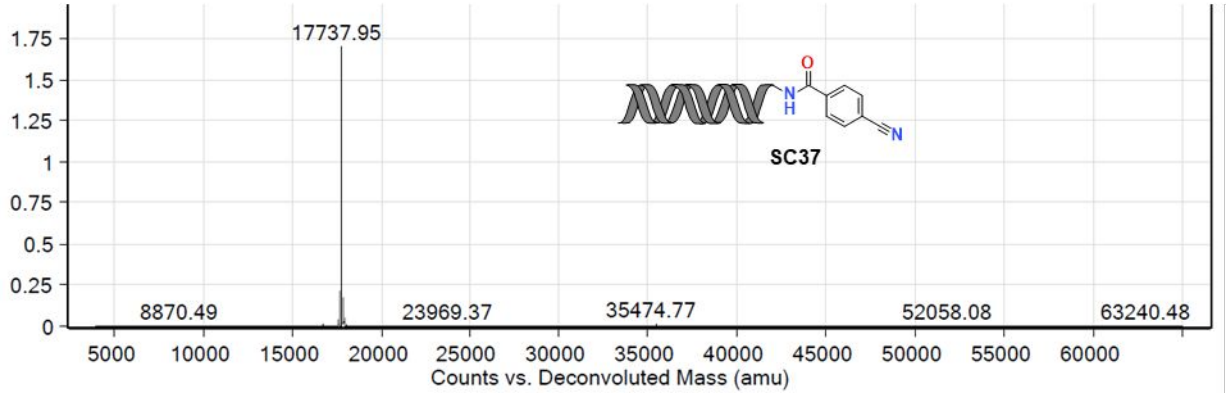


Figure S78: Deconvoluted mass spectrum of compound **37**, expected: 17739; observed: 17737 (SM, **SC37**). No nitrile reduction observed.

V. General procedure for 3-cycle library dummy synthesis and representative LCMS of intermediates 38 to 42

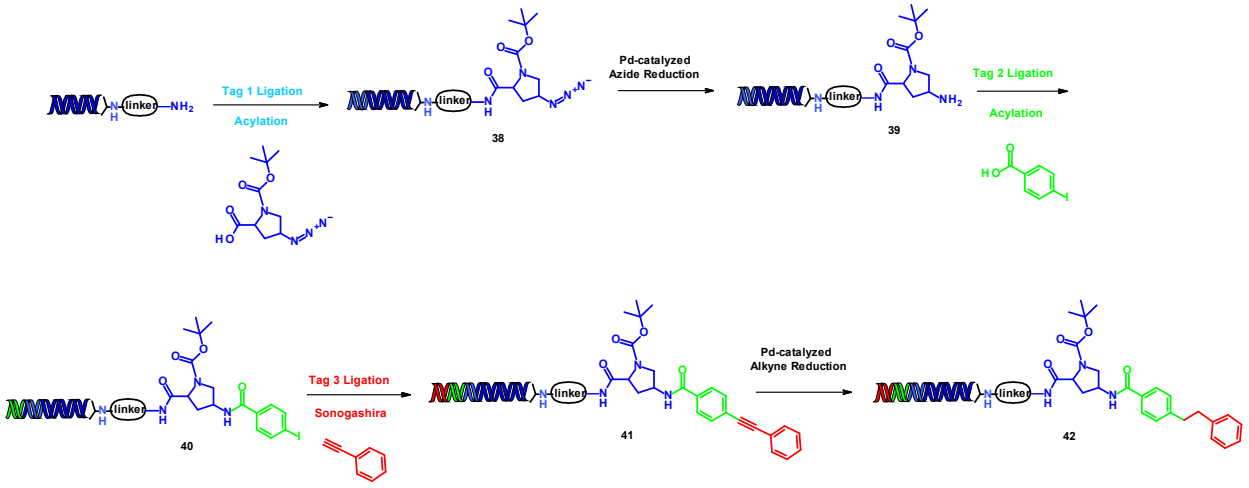


Figure S79: 3-cycle library dummy synthesis

Dummy Cycle 1: Tag 1 ligation, Acylation and Pd hydrogenation steps (39)

Tag 1 ligation: To a solution of AOP-HP-Primer-NH2 (200 nmol, in 514 ul of water) were added Tag 1 (240 nmol in 198 µL of water, 1.2 equiv) and a premixture formed by 10X T4 DNA Ligase Buffer (80 µL) and T4 DNA LIGASE (8 µL). The mixture was slightly vortexed and incubated at 16 ºC, without stirring, for 16 h. The crude material was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, it was used in the next step synthesis without further purification.

Acylation: To a solution of previous Tag 1 ligated AOP-HP-Primer-NH2 in borate buffer 0.5 M pH 9.4 (123 nmol, 1 mM) were added the corresponding carboxylic acid BOC-azido-proline (37 µL, 200 mM in DMA, 60 equiv), followed by DMT-MM (18.5 µL, 400 mM in water, 60 equiv). The reaction mixture was allowed to proceed at room temperature with shaking overnight. The crude material was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, it was used in the next step synthesis without further purification, to afford Tag1-AOP-HP-Primer-NH-Azidoproline derivative (**38**).

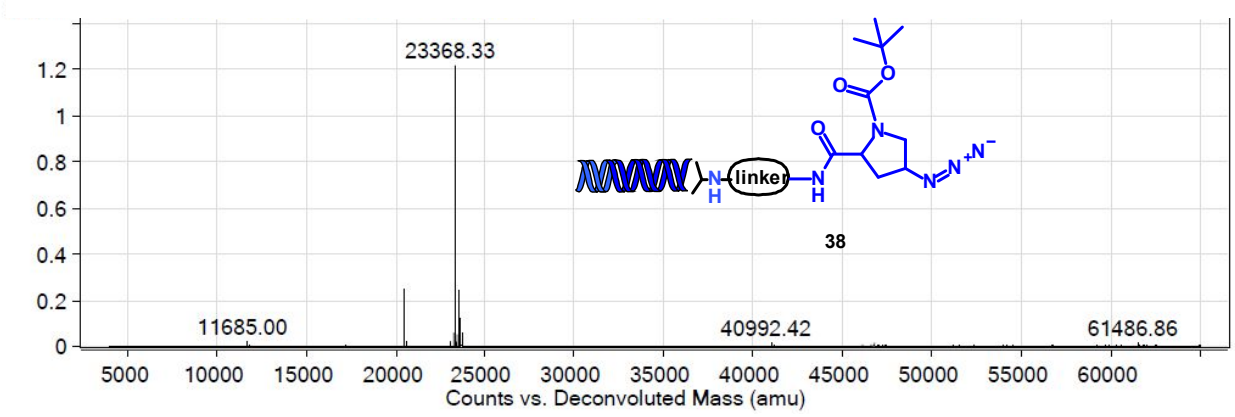


Figure S80: Deconvoluted mass spectrum of compound **38**, expected: 23365; observed: 23368

Pd hydrogenation: After acylation step, Tag1-AOP-HP-Primer-NH-Azidoproline was reduced to amine, following general procedure for hydrogenation (see general procedure, section IV, pag 18 of SI), to afford Tag1-AOP-HP-Primer-NH-amino-pyrrolidine derivative (**39**).

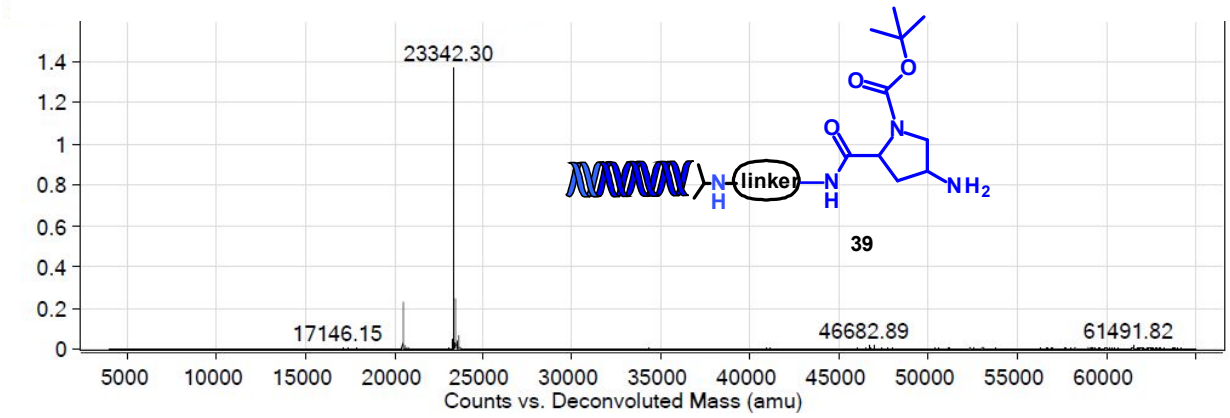


Figure S81: Deconvoluted mass spectrum of compound **39**, expected: 23339; observed: 23342

Dummy Cycle 2: Tag 2 ligation and Acylation step (40)

Tag 2 ligation: To a solution of Tag1-AOP-HP-Primer-NH-amino-pyrrolidine (**39**) (108 nmol, in 344 ul of water) were added Tag 2 (151.2 nmol in 47.6 µL of water, 1.4 equiv) and a premixture formed by 10X T4 DNA Ligase Buffer (44 µL) and T4 DNA LIGASE (4.4 µL). The mixture was slightly vortexed and incubated at 16 ºC, without stirring, for 16 h. The crude material was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, it was used in the next step synthesis without further purification.

Acylation: To a solution of previous Tag 2 ligated Tag1-AOP-HP-Primer-NH-amino-pyrrolidine in borate buffer 0.5 M pH 9.4 (83 nmol, 1 mM) were added the corresponding carboxylic acid iodobenzoic acid (25 µL, 200 mM in DMA, 60 equiv), followed by DMT-MM (12.5 µL, 400 mM in water, 60 equiv). The reaction mixture was allowed to proceed at room temperature with shaking overnight. The reaction was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, the pellet was redissolved in water and centrifuged through a YM10K centrifugal tube (Amicon) at 4000 rpm for 30 min. The sample was collected and its concentration was measured by Nanodrop, to afford Tag1-Tag2-AOP-HP-Primer-NH-iodobenzoyl-amino-pyrrolidine derivative (**40**).

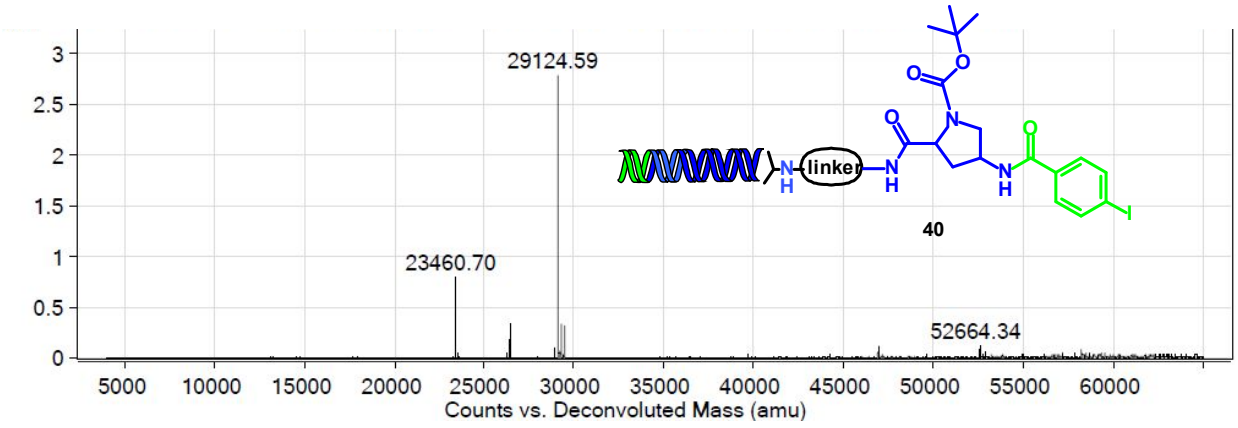


Figure S82: Deconvoluted mass spectrum of compound **40**, expected: 29121; observed: 29124

Dummy Cycle 3: Tag 3 ligation, Sonogashira and Pd hydrogenation steps (42)

Tag 3 ligation: To a solution of Tag1-Tag2-AOP-HP-Primer-NH-iodobenzoyl-amino-pyrrolidine derivative (**40**) (45 nmol, in 90 ul of water) were added Tag 3 (72 nmol in 79.1 µL of water, 1.6 equiv) and a premixture formed by 10X T4 DNA Ligase Buffer (19 µL) and T4 DNA LIGASE (1.9 µL). The mixture was slightly vortexed and incubated at 16 ºC, without stirring, for 16 h. The crude material was precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, it was used in the next step synthesis without further purification.

Sonogashira: To a solution of previous Tag 3 ligated Tag1-Tag2-AOP-HP-Primer-NH-iodobenzoyl-amino-pyrrolidine in water (20 nmol, 1 mM) were added the corresponding phenylacetylene (6 µL, 200 mM in DMA, 60 equiv), followed by pyrrolidine (1 µL, 600 equiv, net) and tetrakis(triphenylphosphine)palladium (0) (10 µL, 10 mM in DMA, 5 equiv). The reaction mixture was allowed to proceed at 65 ºC with shaking for 3 h. Then, to the crude material was added L-Cysteine (Pd scavenger, see L-Cysteine treatment in Section IV, page 18) and precipitated by addition of 10% volume stock solution of 5 M NaCl in water and 3 times volume of cold EtOH. The mixture was kept in the freezer for 1 h and then it was centrifuged for 45 min. The supernatant was discarded and the residue was dried under N_2 at 37 °C. Then, it was used in the next step synthesis without further purification, to afford Tag1-Tag2-Tag3-AOP-HP-Primer-NH-phenylethynyl-benzoyl-amino-pyrrolidine derivative (**41**).

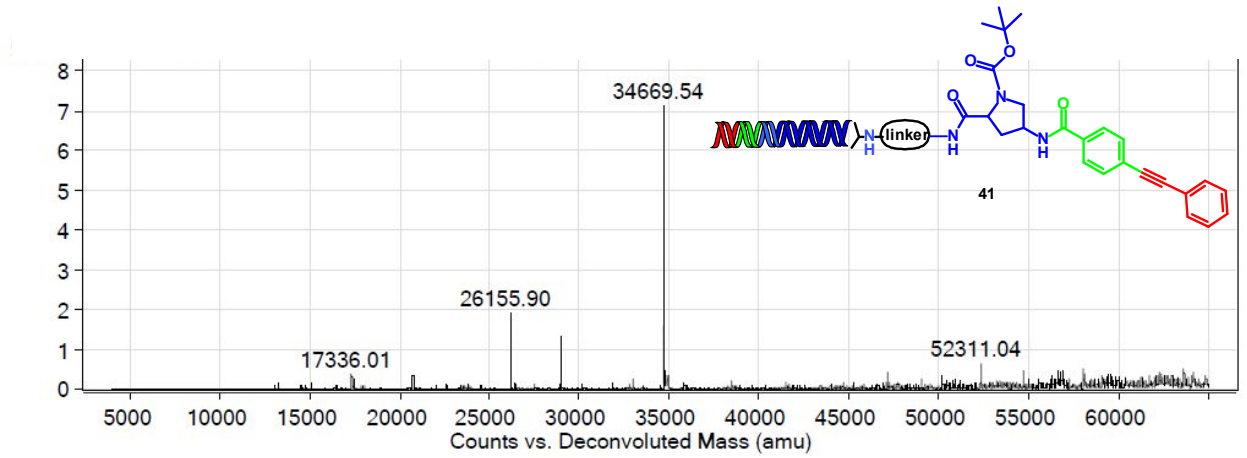


Figure S83: Deconvoluted mass spectrum of compound **41**, expected: 34668; observed: 34669

Pd hydrogenation: After Sonogashira step, Tag1-Tag2-Tag3-AOP-HP-Primer-NH-phenylethynyl-benzoyl-amino-pyrrolidine (**41**) was reduced to alkane, following the general procedure for hydrogenation (Section IV, page 18), to afford Tag1-Tag2-Tag3-AOP-HP-Primer-NH-phenylethyl-benzoyl-amino-pyrrolidine derivative (**42**).

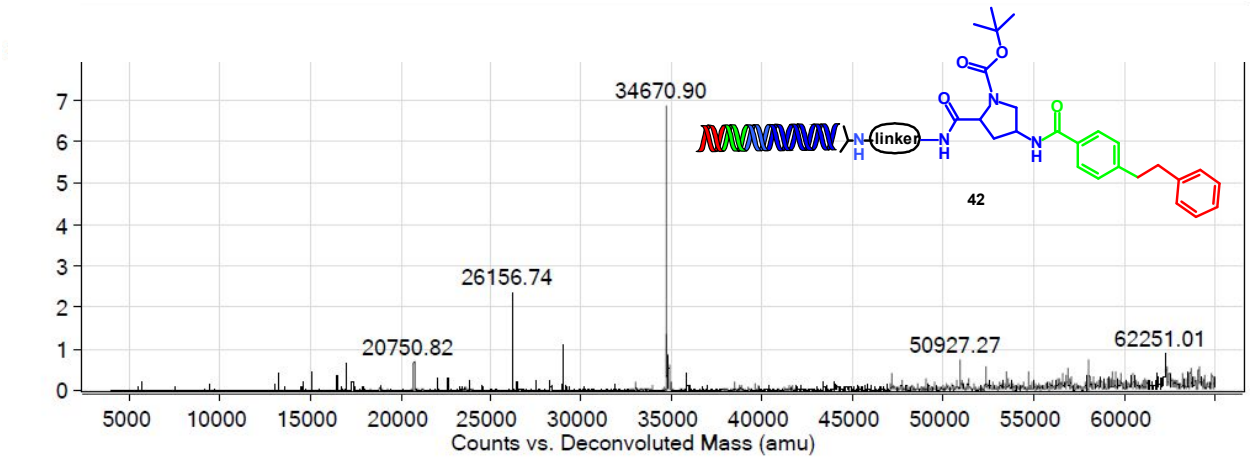


Figure S84: Deconvoluted mass spectrum of compound **42**, expected: 34672; observed: 34671

VI. DNA Damage Evaluation: Assessment of DNA degradation using Quantitative PCR

We generated a DNA sequence containing the Headpiece, Primer, three DNA tags and Closing Primer. This sequence was purified and referenced as Starting Material (SM) to assess the DNA degradation under the different chemistry conditions.

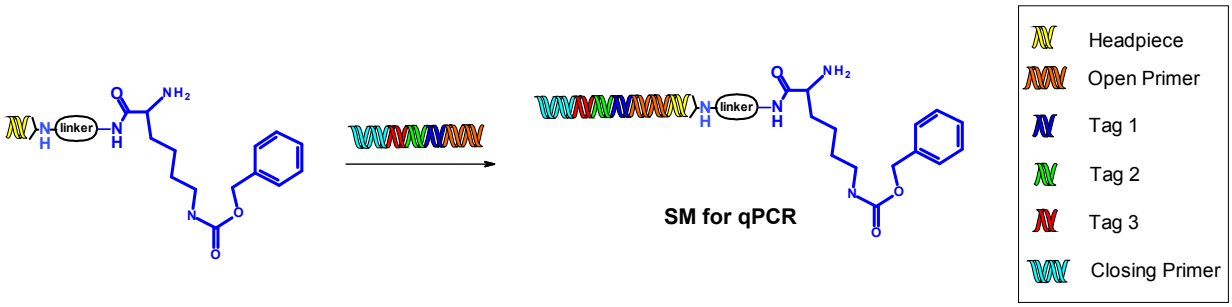


Figure S85: Synthesis of SM for qPCR

The sequence was divided in 5 wells of a 96 well plate with 5 nmol (5 ul at 1 mM concentration) of DNA SM per well. The total volume with the reagents was 14 ul of reaction, the volume of the sample with no chemistry was corrected to a final volume of 14 ul with ddH_2O. The chemistry conditions were as follows:

1. SM only (No chemistry control).
2. SM, 5 equivalents of $Pd(OAc)_2$ and 40 equivalents of $NaBH_4$.
3. SM, 5 equivalents of $Pd(OAc)_2$ and 40 equivalents of $NaBH_4$ and 300 equivalents of L-Cysteine.
4. SM, 20 equivalents of $Pd(OAc)_2$ and 160 equivalents of $NaBH_4$.
5. SM, 20 equivalents of $Pd(OAc)_2$ and 160 equivalents of $NaBH_4$ and 300 equivalents of L-Cysteine.

All the samples were incubated 60 minutes at 30 ºC. After incubation, the samples were purified by ethanol precipitation adding 10% (V/V) NaCl 5M and 2,5 volumes of cold ethanol and centrifugated for 1 hour at 4 ºC. The supernatant was removed, the pellets were dried under nitrogen flow and once the samples were completely dry, the five residues were resuspended in 50 ul of ddH_2O.

The samples were diluted 100.000 times and subjected to Quantitative PCR analysis using triplicates for each condition using a StepOnePlus Real-Time PCR Systems.

For each well, 5 ul of Fast SYBR Green Master MIX (2X) (Thermo-Fisher), 1 ul of the Forward Primer (5 uM), 1 ul of the Reverse Primer (5 uM), 2 ul of ddH_20 and 1 ul of diluted sample was used.

qPCR Cycling Conditions (fast mode):

Step	Temperature	Time	Cycles
UDG activación	50ºC	2min	1
Polymerase	95ºC	2min	1
Denaturation	95ºC	1sec	40
Annealing/extension	60ºC	30sec	40

Figure S86: qPCR Cycling Conditions

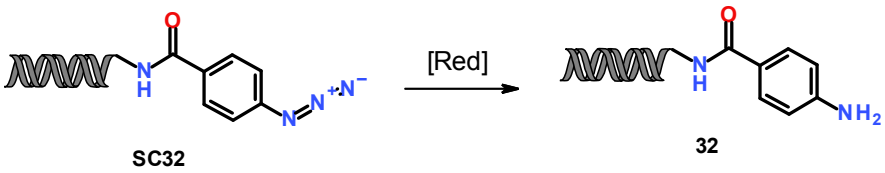
The results were analyzed using the StepOne Software v2.3 and the Cycle Threshold (Ct) of each sample, the Ct Mean and Standard Deviation (Ct SD) for the triplicates were calculated by the software. To calculate the percentage of degradation, the sample set as no chemistry control was used as reference. The difference between the Ct of the sample of interest and the reference (sample 1, No Chemistry control) was calculated and transformed to the power of 2 of the -dCT to set the percentage of sample remaining.

Well	Target Name	C_T Mean	C_T SD	dCT (Ct Target - Ct Reference)	2^-dCT	Percentage
1	**SM only**	11.87	0.056	0	1	100
2	**SM + Pd 5 equiv + 40 equiv BoroH**	11.91	0.018	0.038122	0.973922	97.4
3	**SM + Pd 20 equiv + 160 equiv BoroH**	11.9	0.082	0.028113	0.980702	98.1
4	**SM + Pd 5 equiv + 40 equiv BoroH + 300 equiv Cys**	11.93	0.014	0.062666	0.957493	95.7
5	**SM + Pd 20 equiv + 160 equiv BoroH + 300 equiv Cys**	11.92	0.033	0.049963	0.965961	96.6

Figure S87: Results of qPCR

As assessed by qPCR there is very low (<5%) degradation of the samples under the experimental conditions used in comparison with the sample set as reference.

VII. Azide reduction methods comparison



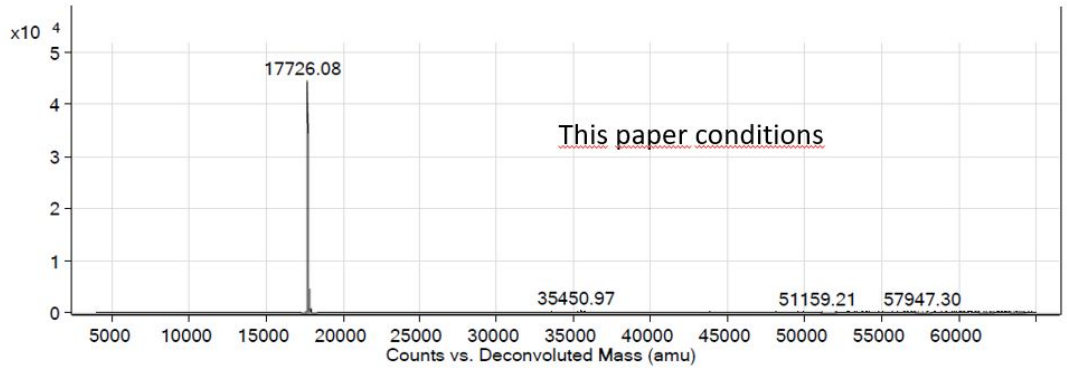


Figure S88: Deconvoluted mass spectrum of compound **32**, expected: 17725; observed: 17726. Azide reduction under the described conditions in this paper.

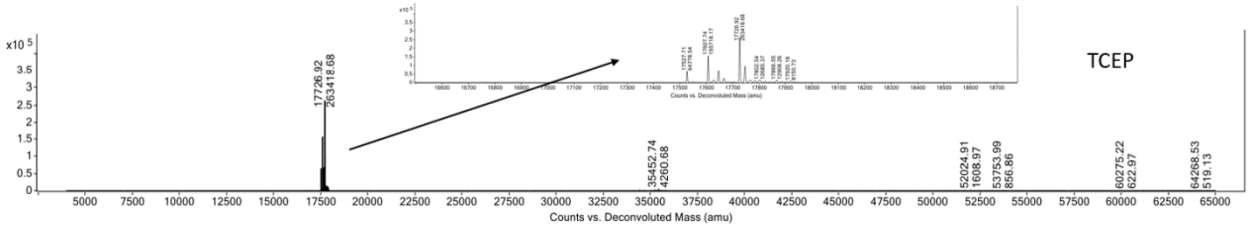
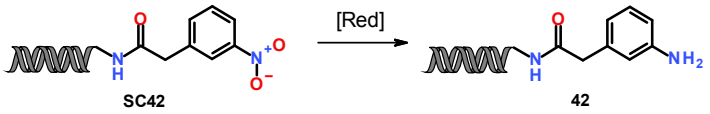


Figure S89: Deconvoluted mass spectrum of compound **32**, expected: 17725; observed: 17726. Azide reduction under TCEP conditions.

VIII. Nitro reduction methods comparison



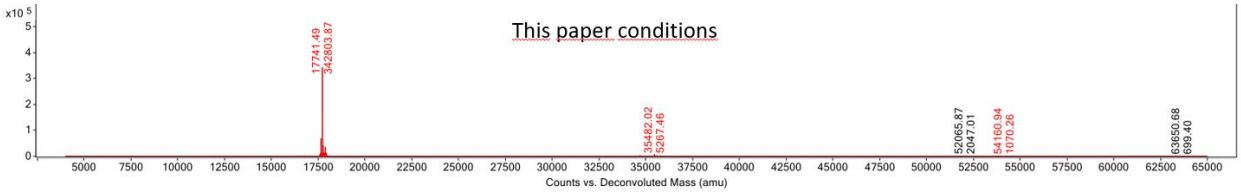


Figure S90: Deconvoluted mass spectrum of compound **42**, expected: 17739; observed: 17741. Nitro reduction under the conditions described in this paper: .

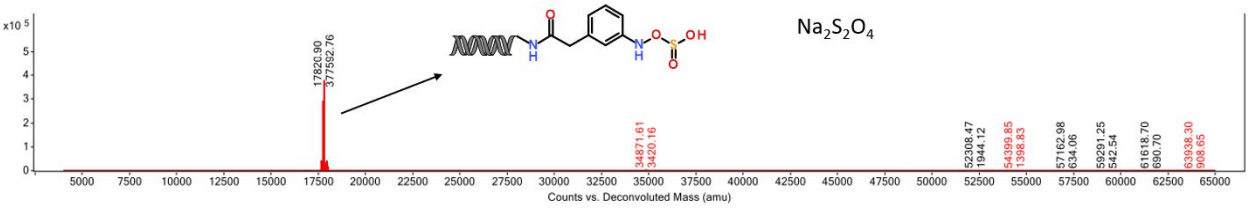


Figure S91: Deconvoluted mass spectrum of compound **42**, expected: 17739; observed: 17820 (hydrogen sulfite intermediate). Nitro reduction with $Na_2S_2O_4$.

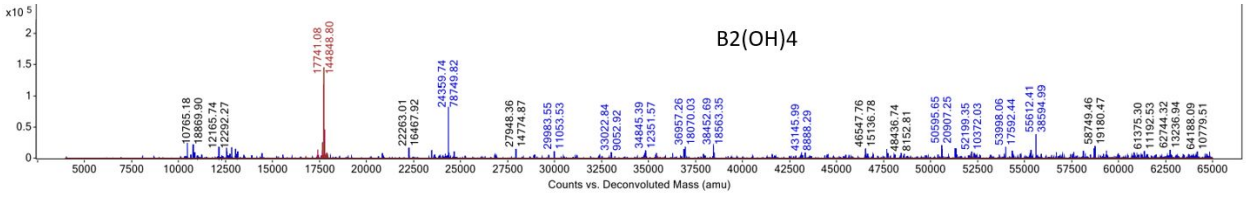


Figure S92: Deconvoluted mass spectrum of compound **42**, expected: 17739; observed: 17741. Nitro reduction with $B_2(OH)_4$.

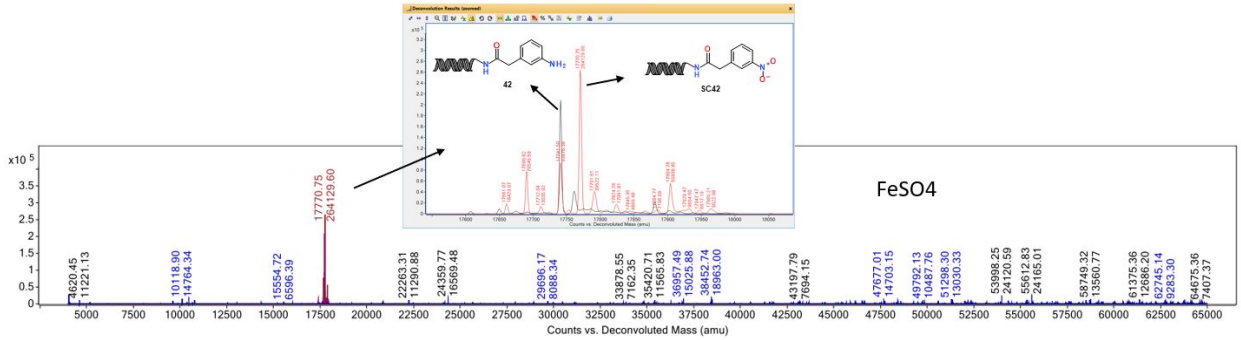
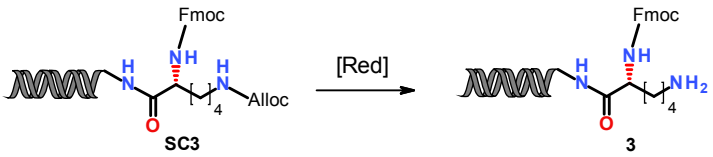


Figure S93: Deconvoluted mass spectrum of compound **42**, expected: 17739; observed: 17741 and 17770 (SM, **SC42**). Nitro reduction with $FeSO_4$.

IX. *N*-Alloc deprotection methods comparison



- **<u>This paper conditions</u>:** 1 equiv. of **SC3** (1.0 mM in H2O), 40 equiv. of $NaBH_4$ (400 mM in NMP), 5 equiv. of $Pd(OAc)_2$ (50 mM in DMA), rt, 1h. Desired deprotected product **3** obtained.

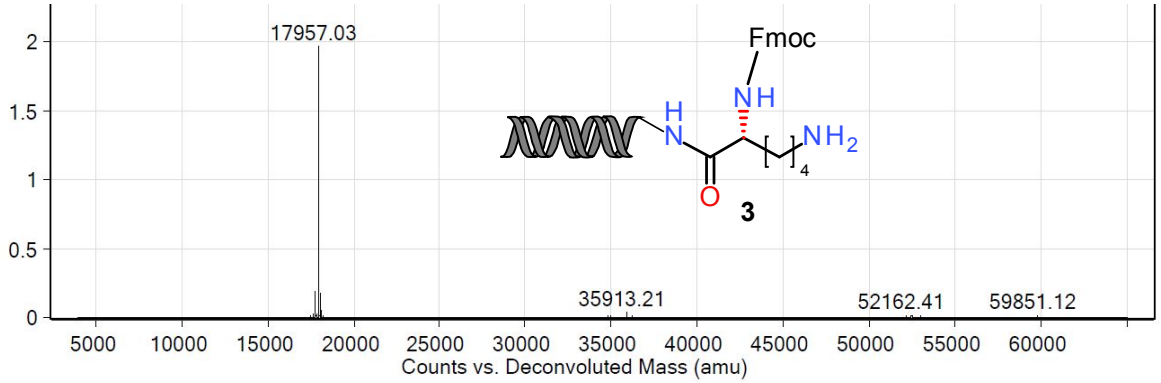


Figure S96: Deconvoluted mass spectrum of compound **3**, expected: 17956; observed: 17957

- **<u>Satz conditions</u>**: 1 equiv. of **SC3** (1.0 mM in H_2O), 10 equiv. of $NaBH_4$ (200 mM in ACN), 10 equiv. of $Pd(PPh_3)_4$ (10 mM in DMA), rt, 2h. Desired deprotected product **3** + unknown cpd obtained.

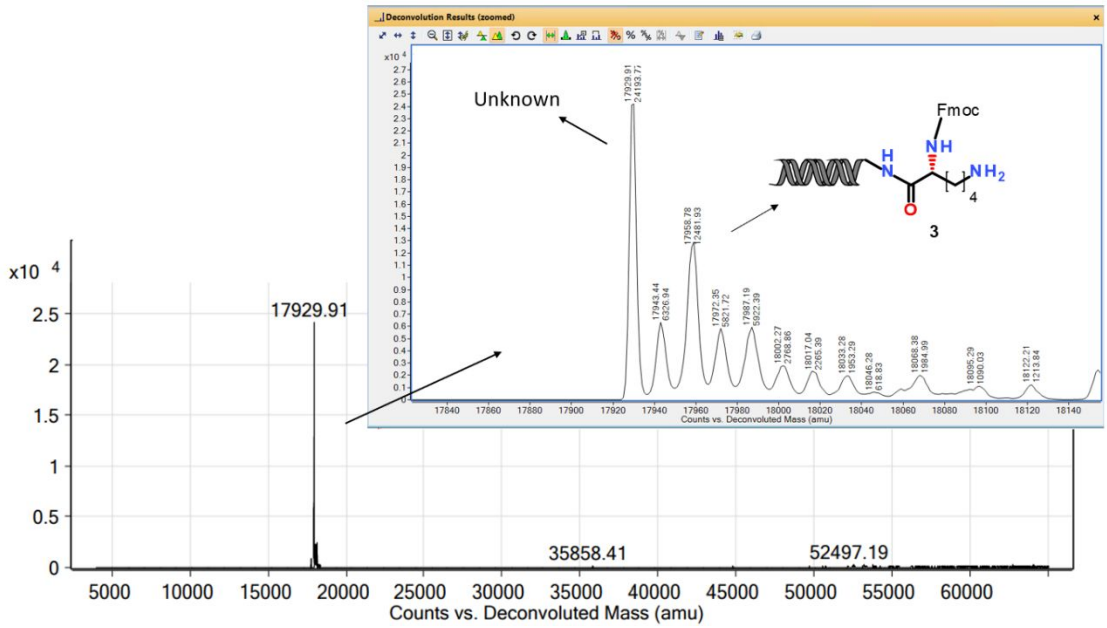
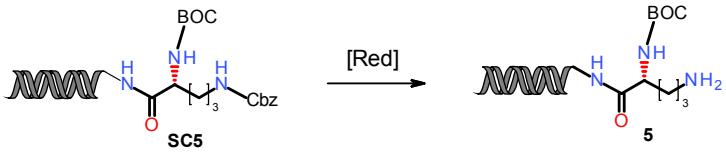


Figure S97: Deconvoluted mass spectrum of compound **3**, expected: 17956; observed: 17958 + 17929 (unknown compound).

X. *N*-Cbz deprotection methods comparison



- **<u>This paper conditions</u>:** 1 equiv. of **SC5** (1.0 mM in H_2O), 40 equiv. of $NaBH_4$ (400 mM in NMP), 5 equiv. of $Pd(OAc)_2$ (50 mM in DMA), rt, 1h. Desired deprotected product obtained.

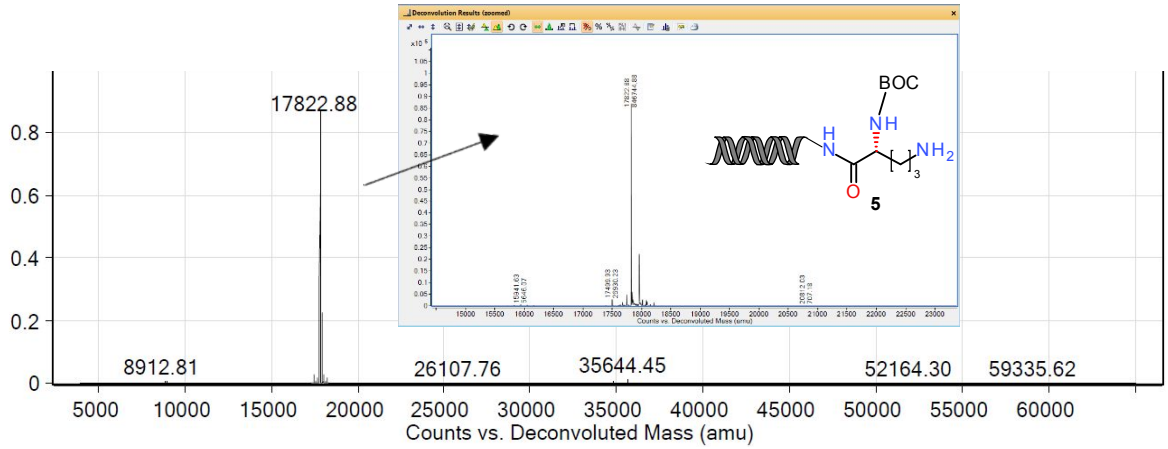


Figure S94: Deconvoluted mass spectrum of compound **5**, expected: 17820; observed: 17822.

- **<u>Satz conditions</u>**: 1 equiv. of **SC5** (1.0 mM in H_2O), 10 equiv. of $NaBH_4$ (200 mM in ACN), 10 equiv. of $Pd(PPh_3)_4$ (10 mM in DMA), rt, 2h. Mainly SM **SC5** obtained.

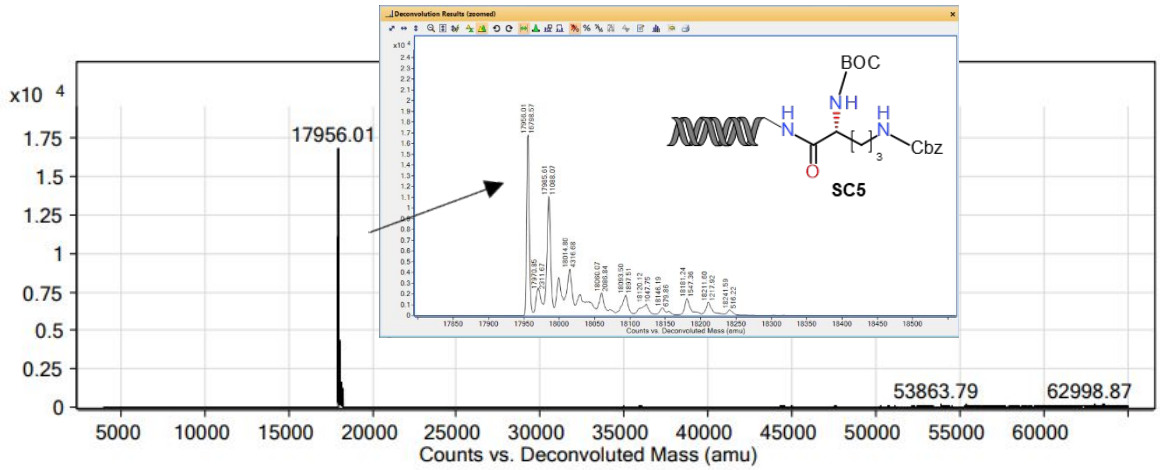


Figure S95: Deconvoluted mass spectrum. Expected product: 17820; observed: 17956 (SM, **SC5**).