

Supporting Information

Dynamic changes in DNA populations revealed by split-combine selection

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Methods

Chemicals and Materials. All chemicals were purchased from Sigma-Aldrich (Munich, Germany) if not otherwise stated. 5-Ethynyl-dUTP and tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) were obtained from BaseClick (Neuried, Germany). Magnetic beads (Dynabeads® His-Tag Isolation and Pulldown and Dynabeads™ M-280 Streptavidin), salmon sperm DNA, lambda exonuclease and C3-GFP were purchased from Thermo Fisher Scientific (Darmstadt, Germany). mE-GFP was kindly provided by Volkmar Fieberg (LIMES, Bonn). The preparation of azides, if not commercially available, were done following the protocol from Suzuki *et al.*[1].

Oligonucleotides. All oligonucleotides were purchased HPLC purified from Ella Biotech (Planegg, Germany). DNA purification was done according to the manufacturer's recommendation using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Düren, Germany).

Click Chemistry. The click reaction was performed as described in F. Pfeiffer *et al.* [2]. Briefly, Cu(I)-catalyst solution was freshly prepared by dissolving 100 mM sodium ascorbate, 1 mM CuSO₄, and 4 mM THPTA solution in a total volume of 100 µL ddH₂O. For the click reaction, 10 µL of a 10 mM azide solution in DMSO, 10 µL of 10x PBS (pH 7), 10 µL of catalyst solution, and 70 µL DNA in ddH₂O were mixed and incubated for 60 min at 37°C and 650 rpm. Afterwards, the samples were purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Düren, Germany).

HPLC/MS analysis. Separation and analysis of DNA strands were performed with reversed-phase ion-pairing chromatography. 100 pmol of test oligo (TO1) were functionalized as described previously [2]. For the separation of DNA (10 pmol), an analytical 1100 series HPLC system with an Agilent Zorbax 2.1x50mm, 5µm (SB-C18) 2.1x100 mm reverse phase column was used. An aqueous solution of 10 mM triethylammonium (TEA) and 100 mM hexafluoroisopropanol (HFIP) was used as mobile phase. A gradient from 0% to 30% acetonitrile over 20 min, 0.5 ml/min flow was employed. Mass spectrometry was performed with an HTC Esquire (Brucker). Settings for measuring the samples with the ultrascan in the negative mode were used as follows: 50 psi, dry gas: 10 l/min, dry temperature: 365°C, SPS: 1000 m/z, ICC: 70000, scan: 500-1500 m/z.

Bead preparation. The beads were prepared described previously [2]. Briefly, 25 µL Dynabeads® His-Tag Isolation and Pulldown (40 mg/mL) were washed three times with 500 µL washing buffer (1xPBS, 1 mg/mL BSA, 0.1 % TWEEN 20). The beads were resuspended in 500

μL of a 3.5 μM protein (C3-GFP) solution in PBS and incubated for 30 min at 25°C and shaken gently (800 rpm). Afterwards, the protein-loaded beads were washed three times with 500 μL washing buffer and finally resuspended in 500 μL washing buffer. The C3-GFP beads were stored at 4°C for a maximum period of two weeks. 500 μL Dynabeads™ M-280 Streptavidin (10mg/mL) were washed three times with 500 μL washing buffer (1xPBS, 1 mg/mL BSA) and directly used.

PCR amplification. PCR was done in a Mastercycler® Personal from Eppendorf (Hamburg, Germany) or in a Personal Cycler from Biometra®. PWO DNA polymerase (Genaxxon, Ulm, Germany) and the supplied buffer containing 2 mM Mg²⁺ were used for all PCR-reactions. For 100μL PCRreaction Mix, 1μL of the PWO DNA polymerase was used. In addition, the PCR-reaction Mix contained in final concentration 250 μM of dN*TPs (1:1:1:1 dATP, dGTP, dCTP and EdUTP), 1 μM reverse and forward primers (**Supporting Tab. 5**). The samples were used with the following cycling program (2 min 95°C; 30 sec 95°C, 30 sec 62°C, 1 min 72°C; hold 10°C). Afterwards, the samples were purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Düren, Germany).

Split-combine click-SELEX targeting C3-GFP. The selection was done as described in previous work [3] with slight changes. Briefly, the SELEX buffer (SB1) contained D-PBS (Sigma D1283: NaCl (137.9 mM); Na₂HPO₄ (8.1 mM); KCl (2.7 mM); KH₂PO₄ (1.5 mM); CaCl₂ (0.9 mM); MgCl₂ (0.5 mM); pH 5.3), 1 mg/mL bovine serum albumin (BSA), 0.1% TWEEN 20, and 0.1 mg/mL salmon sperm DNA. The target C3-GFP was immobilized on magnetic beads, as described above. To increase the selection pressure a 42-mer sequence with randomized nucleotide distribution was chosen to be used as the click-competitor [2]. This click-competitor was functionalized in the same way as the library, i.e. five aliquots of the click-competitor were functionalized with five different azides and purified separately. 100 pmol of each functionalized click-competitor were added to the incubation mixture with the target (total amount 500 pmol, equals ca. 3 x 10¹⁴ different molecules). For the modification, the OW-1 ssDNA library was split into five aliquots and each aliquot was functionalized with a different azide. Subsequently, the purified functionalized DNA was mixed and incubated with the C3-GFP bearing beads (**Supporting Tab. 1**) for 30 min at 37°C under gentle agitation (800 rpm). After incubation, the beads were washed three times with 200 μL of SB1 and the bound sequences were recovered from the beads by the addition of 100 μL of a 300 mM imidazole solution (in ddH₂O). The recovered DNA was amplified by PCR, without any further purification, in a total volume of 1600 μL. The PCR products were visualized on a 4% agarose gel and ethidium bromide staining. Subsequently, lambda exonuclease digestion was performed to generate single-stranded DNA (ssDNA). For this, 20 μL of the supplied 10x λ-exonuclease reaction buffer was added to 180 μL of the purified PCR product. After addition of 3 μL λ-exonuclease (5000 U/mL), the samples were incubated for 20 min at 37°C and under agitation (1000 rpm). Subsequently, the samples were purified with the NucleoSpin® Clean-Up kit using the NTC buffer for the DNA loading on the column. Finally, the obtained ssDNA was divided into five samples and modified with one of the five azides, recombined and this library was used for the next selection cycle. To increase the selection pressure, the following measures were applied during the selection process: i) reducing the number of magnetic beads per selection cycle; ii) increasing the number of washes, the incubation times during washing steps and the wash buffer volume; iii) introducing a click-

competitor modified with the same azides as the library but lacking the primer region for no amplification during PCR, and iv) introducing a *pre*-selection step with non-modified magnetic beads. The selection details are listed in **Supporting Tab. 1**.

Split-combine click-SELEX targeting streptavidin (SA). The SELEX buffer (SB2) contained D-PBS (Sigma D1283: NaCl (137.9 mM); Na₂HPO₄ (8.1 mM); KCl (2.7 mM); KH₂PO₄ (1.5 mM); CaCl₂ (0.9 mM); MgCl₂ (0.5 mM); adjusted to pH 7.4), and 1 mg/mL bovine serum albumin (BSA). The functionalization of the FT2 DNA library and incubation with the target (Dynabeads™ M-280 Streptavidin) were performed as described above, with the following exception: The recovery of the bound sequences was performed by addition of 90 µL ddH₂O and by heating to 80°C for 3 min. The selection details are listed in **Supporting Tab. 3**.

Flow Cytometry-based interaction analysis. The interaction of Cy5-labeled DNA with protein immobilized on magnetic beads were investigated on a FACSCanto II from BD Biosciences (Heidelberg, Germany). For this, 5 µL of the respective C3-GFP-modified beads or 1 µL of Dynabeads™ M-280 Streptavidin were mixed with 10 pmol or 5 pmol, respectively, of Cy5-labeled DNA in 20 µL SB1 or 10 µL SB2 and incubated for 30 min at 37°C and gentle agitation 800rpm. The beads were washed two times and resuspended in 200 µL SB1 or SB2, respectively. 50.000 events were measured in the flow cytometer and the mean Cy5-fluorescence intensity in the APC-A channel was analyzed. Raw binding data were corrected for unspecific background binding.

Next-generation sequencing (NGS) sample preparation. All NGS samples were prepared according to published procedures [4] and measured on Illumina HiSeq1500 platform. Briefly, the PCR to introduce the indices was performed using the canonical set of nucleotides. Those indices allow multiplexing of 12 different samples in one lane. The purification of the PCR product was done with NucleoSpin® Clean-Up Kit from Macherey-Nagel (Düren, Germany). Equal amounts of DNA with different indices (0.125 µg) were then pooled. Finally, an adapter sequence was added by enzymatic ligation according to the manufacturer's instructions using TruSeq DNA PCR-Free Sample Preparation Kit LT (Illumina). The final purification was done by agarose gel extraction and NucleoSpin® Clean-Up kit from Macherey-Nagel. The DNA was eluted in resuspension buffer (TruSeq DNA PCR-Free Sample Preparation Kit LT, Illumina). Quantitative PCR was performed for library validation and quantification using the KAPA library quantification kit (Sigma-Aldrich) prior to sequencing. The sequencing was carried out with seventy-five bp single-end sequencing. Analysis of demultiplexed raw data for the selections was performed using an in-house developed software.

Surface Plasmon Resonance using clickmers targeting C3-GFP. The interactions of C3-GFP as an analyte to clickmers (I10, F8, F20, and B33) as immobilized ligands were investigated using a Biacore 3000 instrument. 5'-biotinylated clickmers were immobilized according to the manufacturer's recommendation on streptavidin-coated sensor chip from Xantec. C3-GFP (1000 nM, 360 nM, 100 nM, 36 nM, 10 nM, 3.6 nM, 1 nM) was injected for 120-225 s at a flow rate of 40µL/min. The dissociation time was 300 s. Afterwards, 5mM EDTA solution was injected to ensure a total dissociation of the protein. The analysis of these

experiments was done using the software BIAevaluation 4.1 (Biacore). The data were fitted by using the 1:1 Langmuir model.

Surface Plasmon Resonance using clickmers targeting streptavidin – Interaction analysis. The interactions of streptavidin as an analyte to clickmers (B1, G1) as immobilized ligands were investigated in the Biacore 3000. For this, the clickmers were immobilized according to the manufacturer's recommendation on a CMDP sensor chip from Xantec. Streptavidin (360 nM, 100 nM, 36 nM, 10 nM, 3.6 nM, 1 nM) was injected for 120 s at a flow rate of 40 μ L/min. The dissociation time was 300 s. Afterwards, the regeneration solution (5 mM EDTA, 0.1% SDS, 0.5 M NaCl, 25 mM NaOH) was injected to ensure a total dissociation of the protein. The analysis of these experiments was done using the software BIAevaluation 4.1 (Biacore). The data were fitted by using the 1:1 Langmuir model.

Supporting Table 1: Overview of the split-combine SELEX conditions using library OW1 targeting C3-GFP. The deconvolution cycle 9 was performed for each azide separately. †In the split-combine cycles 1-8 (shaded in light grey), 100 pmol of each competitor modified with one of the 5 azides was used, thus in total 500 pmole. In the deconvolution cycle 9 (shaded in light blue), 500 pmole of the competitor modified with the indicated azide was applied.

SELEX cycle	blank beads [μL]	C3-GFP beads [μL]	Wash at 37°C, 800 rpm	Wash vol. [μL]	Competitor [pmol]†	PCR cycles
1	-	50	3x 30 sec	200	-	18
2	-	50	3x 30 sec	200	-	14
3	50	50	1x 30 sec 1x 5 min	200	100	20
4	50	35	1x 30 sec 1x 5 min	200	100	14
5	50	35	1x 30 sec 1x 5 min	200	100	14
6	50	25	3x 5 min	200	100	12
7	50	15	3x 5 min	500	100	10
8	50	5	3x 5 min	750	100	8
9 In-dU	50	5	3x 5 min	750	500	8
9 BF-dU	50	5	3x 5 min	750	500	10
9 Bn-dU	50	5	3x 5 min	750	500	24
9 ClBn-dU	50	5	3x 5 min	750	500	24
9 Ea-dU	50	5	3x 5 min	750	500	24

Supporting Table 2 – Please refer to attached Excel file

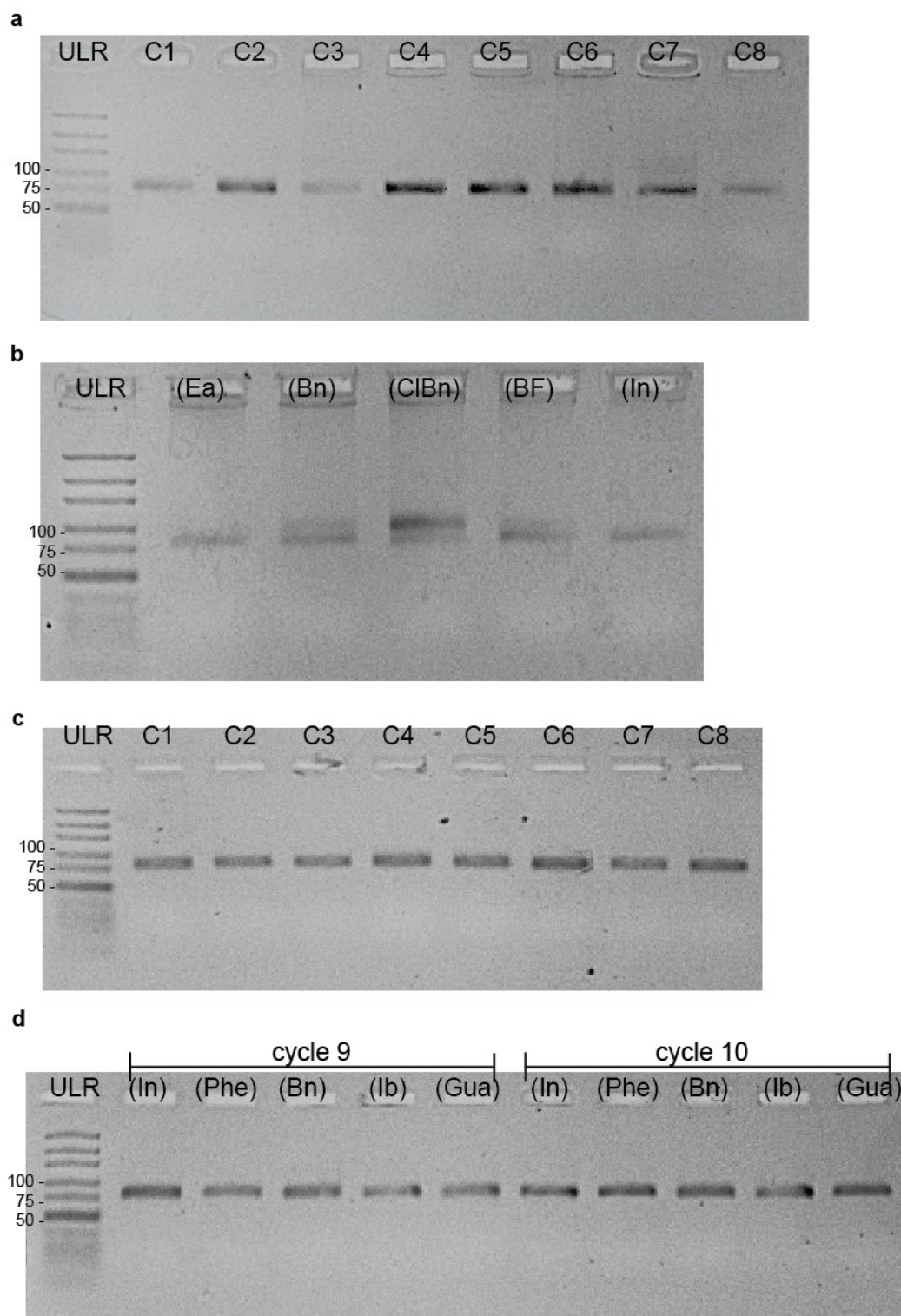
Supporting Table 3: Overview of the split-combine SELEX conditions using library FT2 targeting streptavidin. The deconvolution cycles 9 and 10 were performed for each azide separately. †In the split-combine cycles 1-8 (shaded in light grey), 100 pmol of each competitor modified with one of the 5 azides was used, thus in total 500 pmole. In the deconvolution cycles 9 and 10 (shaded in light blue), 500 pmole of the competitor modified with the indicated azide was applied.

SELEX cycle	streptavidin beads [μL]	Wash at 37°C, 800 rpm	Wash vol. [μL]	Competitor [pmol]†	PCR cycles
1	50	3x 30 sec	200	100	18
2	50	3x 30 sec	200	100	16
3	35	1x 30 sec 1x 5 min	200	100	16
4	25	1x 30 sec 1x 5 min 1x 30 sec	200	100	14
5	20	1x 30 sec 2x 5 min	500	100	10
6	5	3x 5 min	750	100	12
7	5	3x 5 min	750	100	10
8	5	3x 5 min	750	100	12
9 In-dU	5	3x 5 min	750	500	10
9 Phe-dU	5	3x 5 min	750	500	10
9 Bn-dU	5	3x 5 min	750	500	10
9 Ib-dU	5	3x 5 min	750	500	10
9 Gua-dU	5	3x 5 min	750	500	14
10 In-dU	5	3x 5 min	750	500	10
10 Phe-dU	5	3x 5 min	750	500	10
10 Bn-dU	5	3x 5 min	750	500	12
10 Ib-dU	5	3x 5 min	750	500	12
10 Gua-dU	5	3x 5 min	750	500	14

Supporting Table 4 – Please refer to attached Excel file

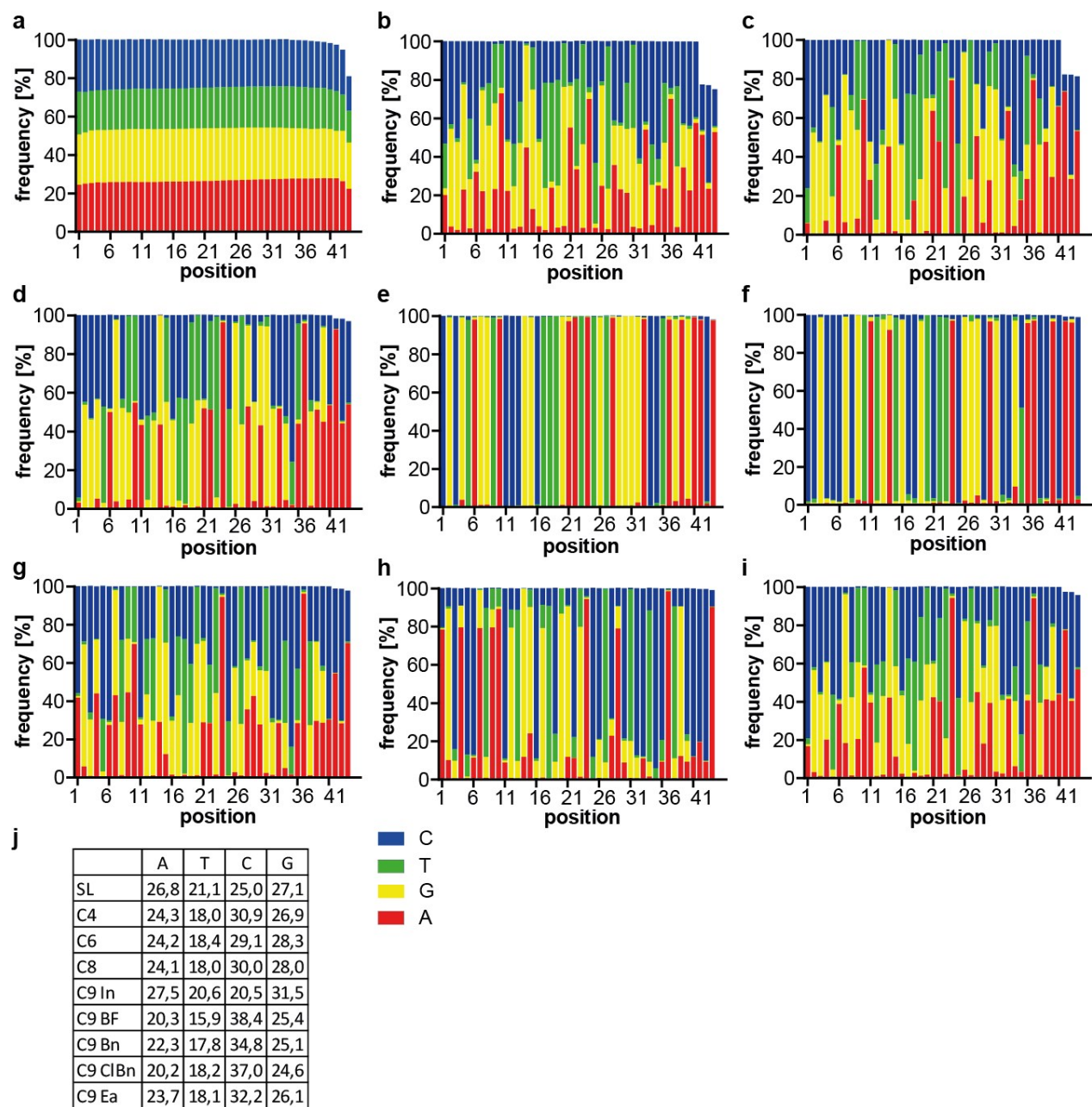
Supporting Table 5: Oligonucleotides used in this study (X=EdU). Sequences related to the library OW1 (used for the selection targeting C3-GFP) are shaded in green, those related to FT2 (used for the selection targeting SA) in orange.

Oligo	sequence
OW1-library	5'- AGCCACGGAAGAACCAGA -N44- GCAGAAGCGACAGCAACA -3' (N= dA:dC:dG:EdU = 1:1:1:1)
OW1_forward primer	5'- AGCCACGGAAGAACCAGA -3'
OW1_forward primer_Cy5	5'- Cy5 - AGCCACGGAAGAACCAGA -3'
OW1_reverse primer	5'Phosphate - TGTTGCTGTCGCTTCTGC -3'
FT2-library	5'- CACGACGCAAGGGACCACAGG -N42- CACGACGACACCGCAGAGGCA -3' (N= dA:dC:dG:EdU = 1:1:1:1)
FT2-forward primer_Cy5	5'- Cy5 - CACGACGCAAGGGACCACAGG -3'
FT2-forward primer	5' - CACGACGCAAGGGACCACAGG -3'
FT2-reverse primer	5'Phosphate – TGCCTCTGCGGTGTCGTGCTG -3'
I10	5'- AGCCACGGAAGAACCAGACGCGXAGGXACCCGGCXXXGAAXAXGX AGGGGACCXAGAGAACAGCAGAAGCGACAGCAACA -3'
B33	5'- AGCCACGGAAGAACCAGAGGGCGXACXXXGXCCACACAXXC XAACCACCACXACGCCAGCAGAAGCGACAGCAACA -3'
F20	5'- AGCCACGGAAGAACCAGACCGCCCGCGXAXGAXGCCGXCCXACGG GCAGCCGXAAACCACAACGCAGAAGCGACAGCAACA -3'
C1	5'- AGCCACGGAAGAACCAGAAAGCACCAXAACGXGGCGXCGGCGACCC CACXCCXCCAXGCCCGAGCAGAAGCGACAGCAACA -3'
F20sc	5'- AGCCACGGAAGAACCAGAGXCGCGCXCCAGACGCXAACCGCCAXA CACAGCXGCCGXGXCGAGCAGAAGCGACAGCAACA -3'
B1	5'- CACGACGCAAGGGACCACAGGCCAGGXCCGGXAXAXXCGGXCGCA GAXACGAACCAGGGXAXACAGCACGACACCGCAGAGGCA -3'
P2	5'- CACGACGCAAGGGACCACAGGGGGXCAAGCCGGGXGGAGXXXA AGGGGXGGGGCAGGACCCAGCACGACACCGCAGAGGCA -3'
G1	5'- CACGACGCAAGGGACCACAGGTTTTGCGAACGCGGGTAGGCGCAC CCTTCGCACAAACGTCCCCAGCACGACACCGCAGAGGCA -3'
G1sc	5'- CACGACGCAAGGGACCACAGGGCGGCTCGTTAGCTGGCCACACCA GGCGACGCATTACCTTACAGCACGACACCGCAGAGGCA -3'
I2	5'- CACGACGCAAGGGACCACAGGGCGGXACGGXXXGACCGXCCGAA GAGCXXXAAGCGAXXGAGGCAGCACGACACCGCAGAGGCA -3'
TO1	5'- GCACTGTXCATTGCGC -3'

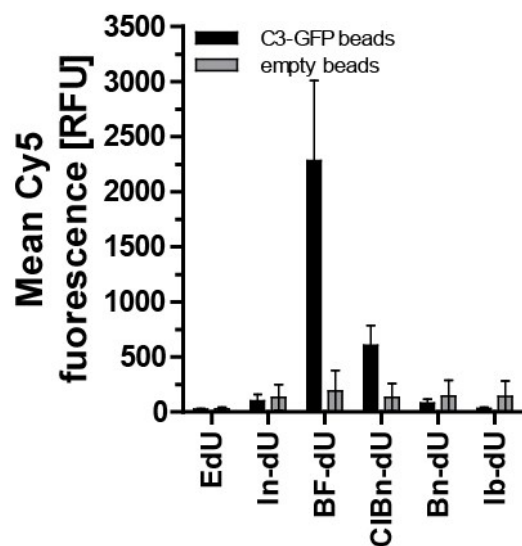


Supporting Figure 1: Agarose gel electrophoresis analysis of the PCR products from split-combine SELEX. In (a) the first eight selections cycles targeting C3-GFP and in (b) the ninth cycle with the different azides ((In) 3-(2-azidoethyl)-1*H*-indole, (BF) 3-(2-azidoethyl)-benzofuran, (ClBn) 1-(azidomethyl)-4-chlorobenzene, (Bn) 1-(azidomethyl)-benzene, and (Ea) 2-azidoethanamine) are depicted. For this SELEX the OW1 library was used (dsDNA: 80 bp). In (c) the first eight selections cycles targeting SA and in (d)

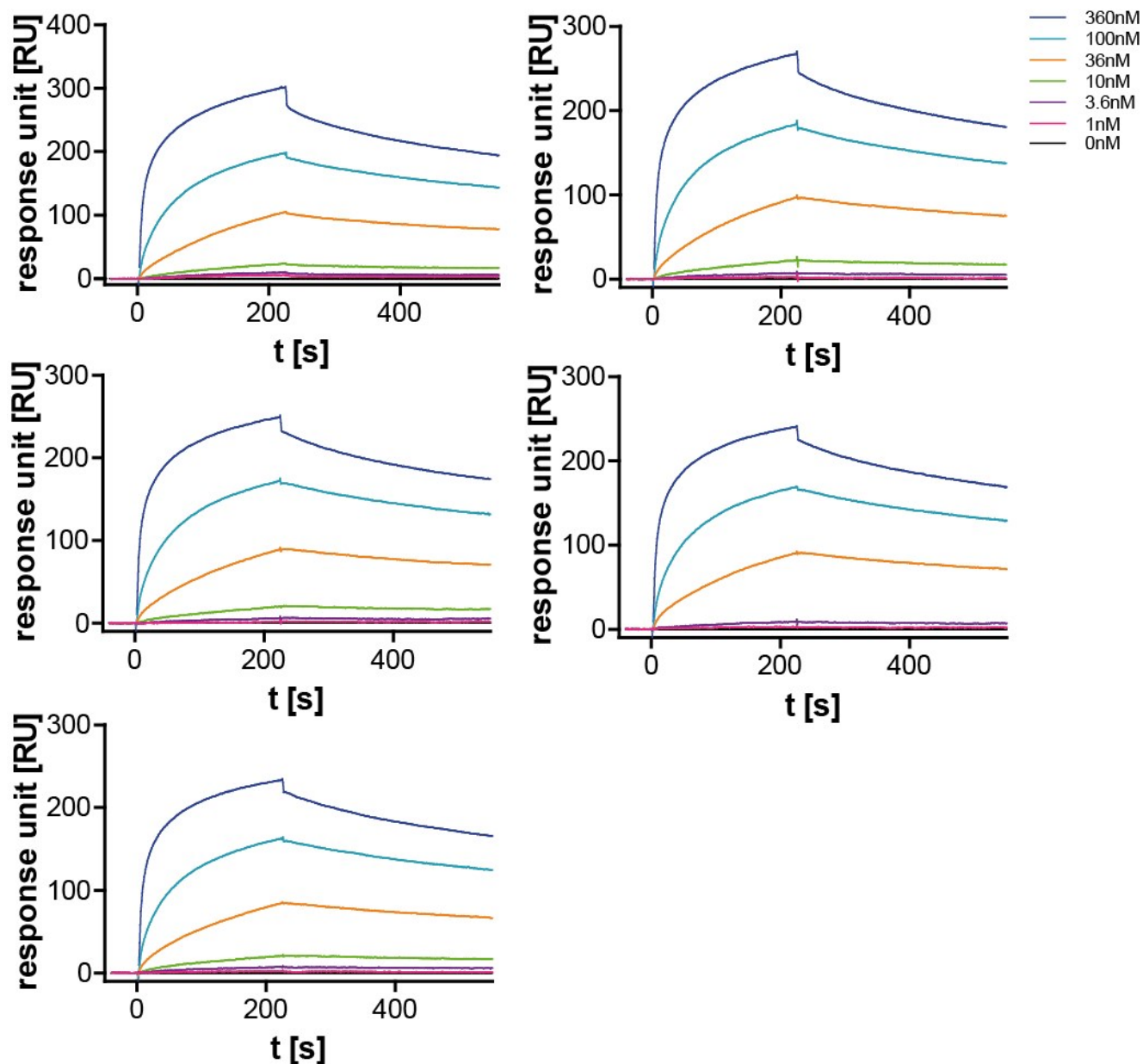
the ninth and tenth cycle with the different azides ((In) 3-(2-azidoethyl)-1*H*-indole, (Phe) 3 4-(2-Azido-ethyl)-phenol, (Bn) 1-(azidomethyl)-benzene, (Ib) 1-azido-2-methylpropane, and (Gua) *N*-(2-Azido-ethyl)-guanidine) are depicted. For this SELEX the FT2 library was used (dsDNA: 82 bp).



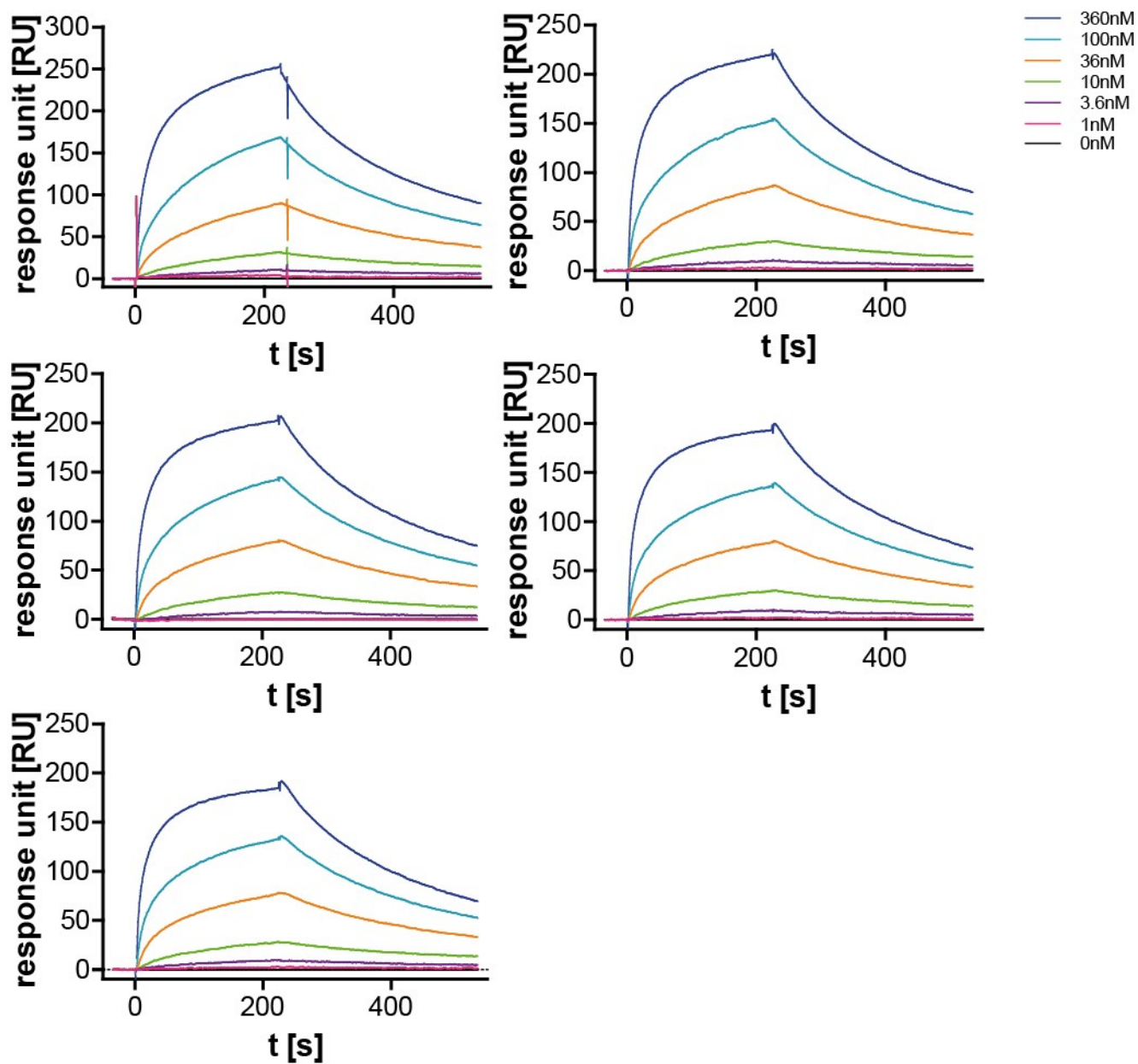
Supporting Figure 2: NGS analysis of all selection cycles of the split-combine SELEX targeting C3-GFP. Statistical base distribution over the 44 nucleobases random region for the starting library (a), selection cycle 4 (b), cycle 6 (c), cycle 8 (d), and cycle 9 for indole azide (In-dU) (e), cycle 9 for benzofuran azide (BF-dU) (f), and cycle 9 for benzyl azide (Bn-dU) (g), cycle 9 for chlorobenzyl azide (ClBn-dU) (h), and cycle 9 for amine azide (Ea-dU) (i). (j) Shown is the distribution of the nt in the random region [%].



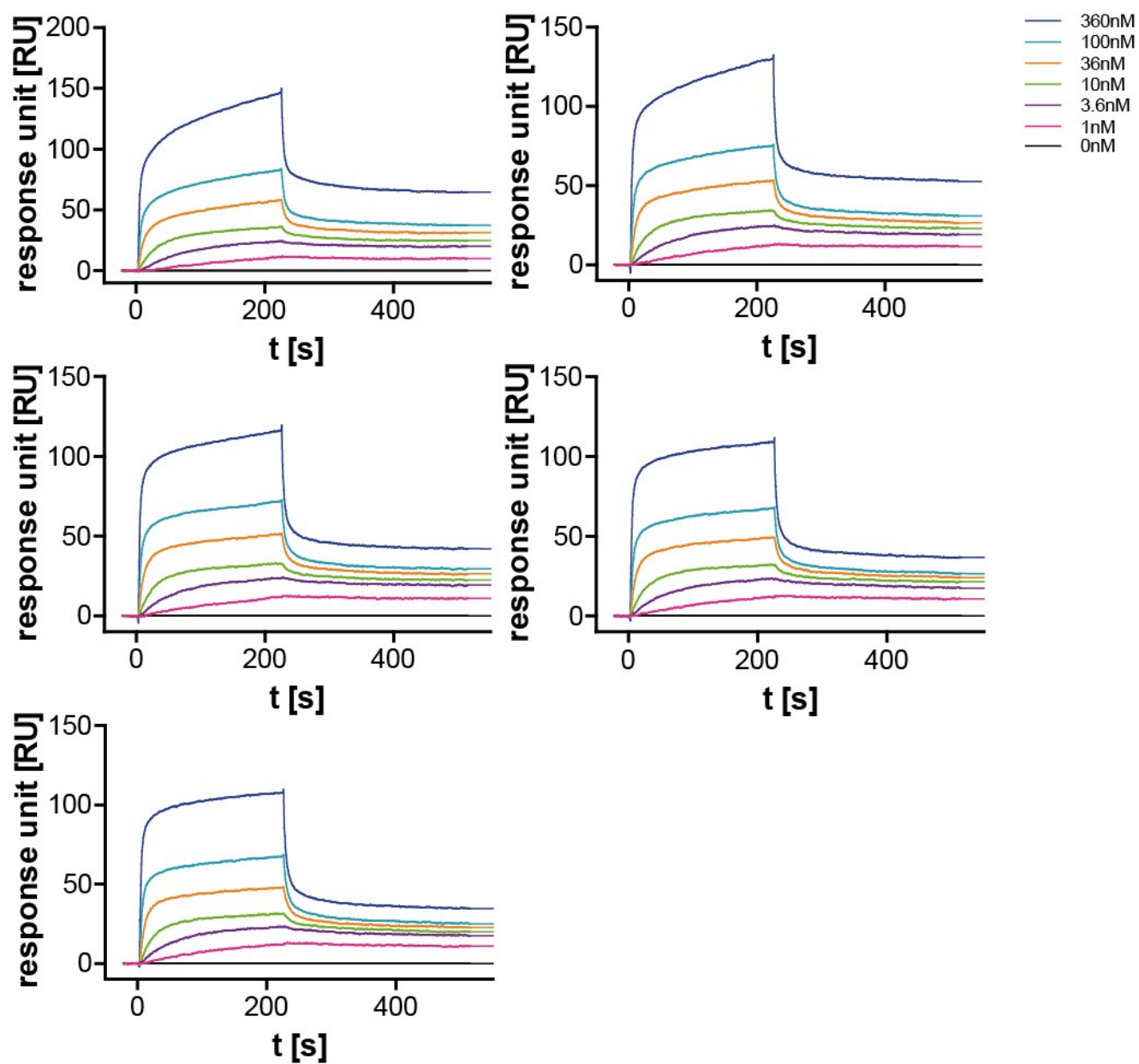
Supporting Figure 3: Flow cytometer binding assay in which 500 nM Cy-5 labelled DNA from the clickmer C1 were incubated with C3-GFP immobilized on magnetic beads (C3-GFP beads). DNA was unmodified (Edu) or modified with indole (In-dU), benzofuran (BF-dU), benzyl (Bn-dU), chlorobenzyl (ClBn-dU) or isobutyl (Ib-dU) (experiments were done at least twice in duplicates, mean $\pm$ SD).



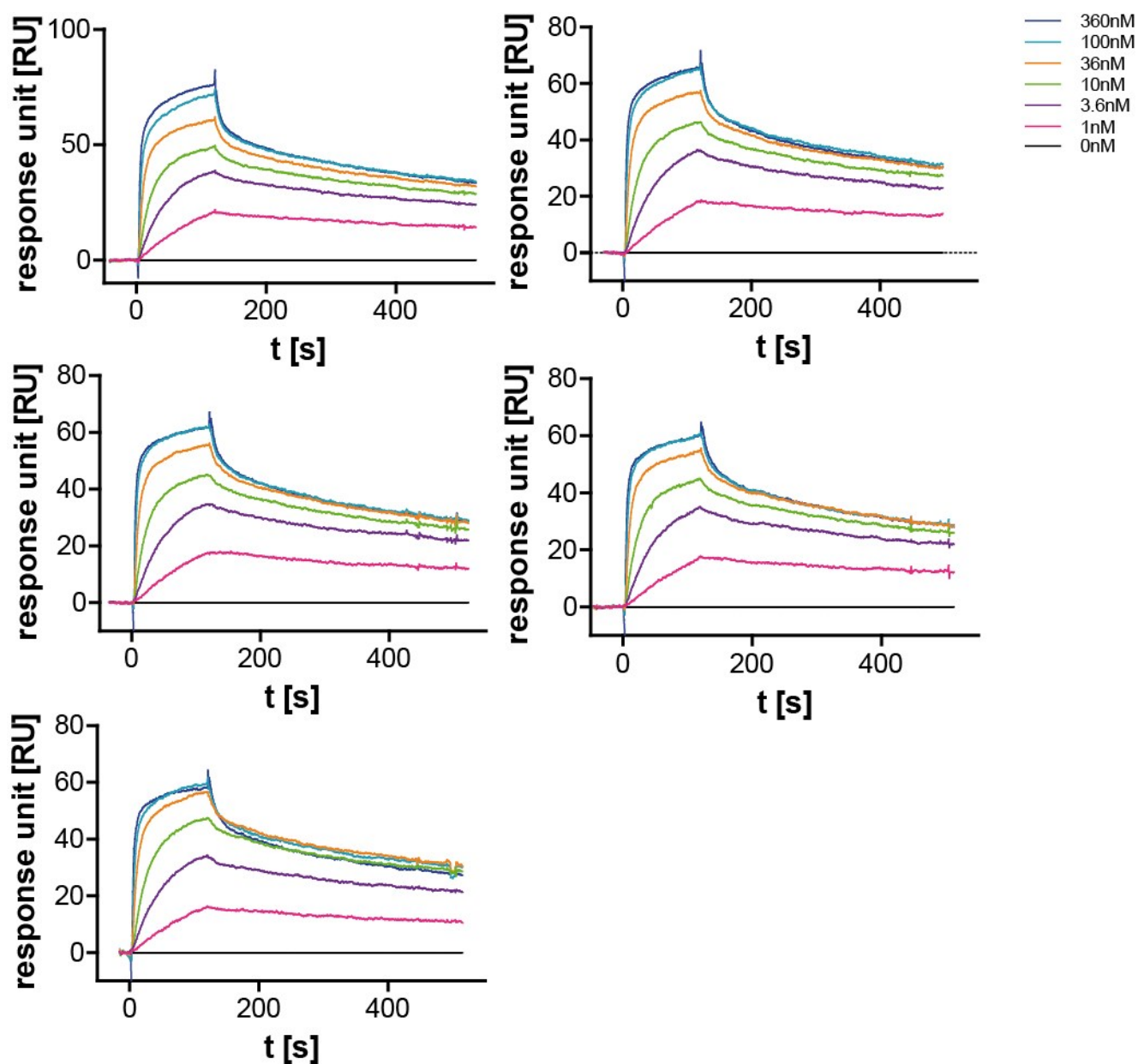
Supporting Figure 4: Surface Plasmon Resonance (SPR) measurement of clickmer I10 modified with In-dU at 25°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand (n=5).



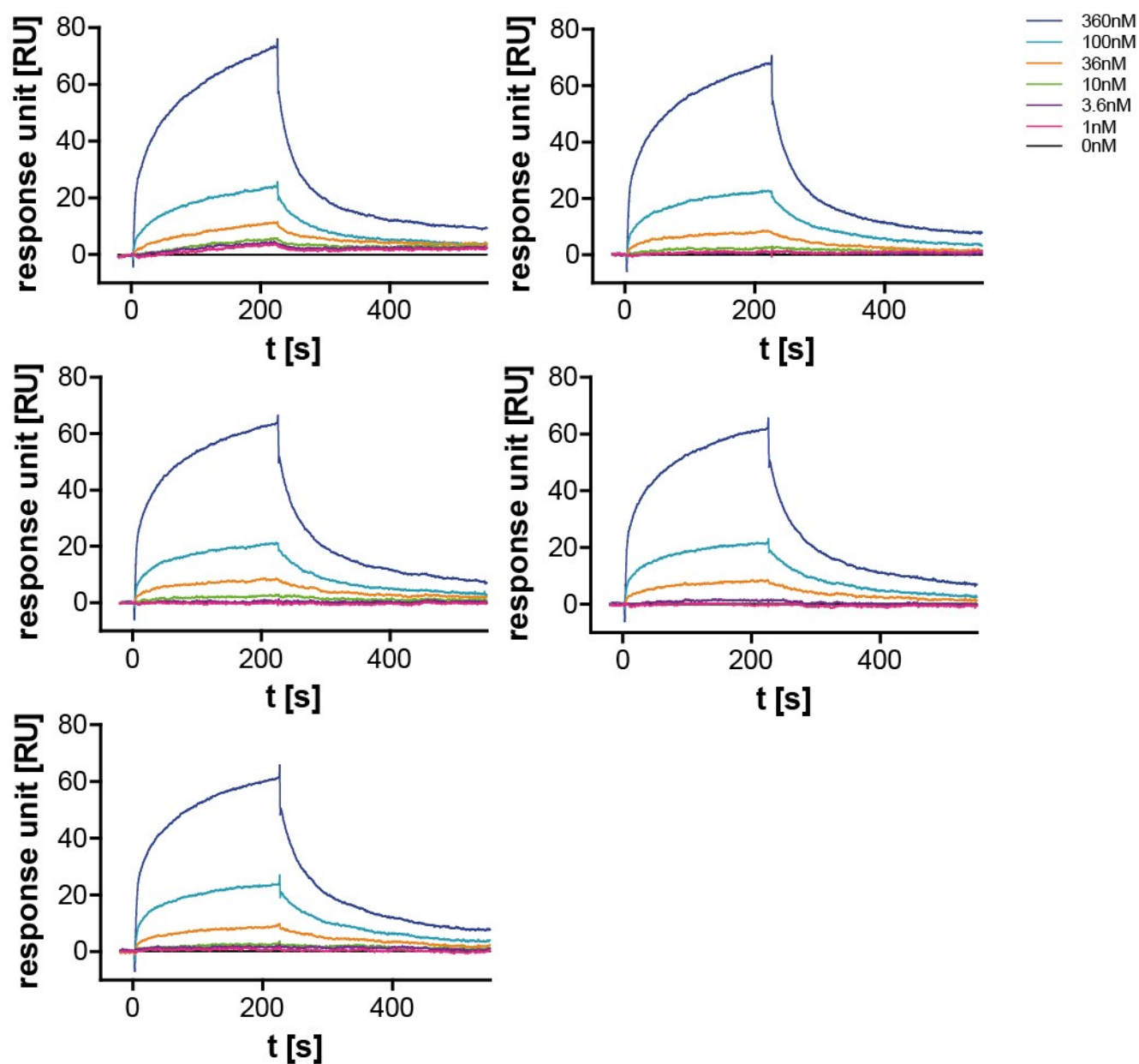
Supporting Figure 5: Surface Plasmon Resonance (SPR) measurement of clickmer I10 modified with In-dU at 37°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand ($n=5$).



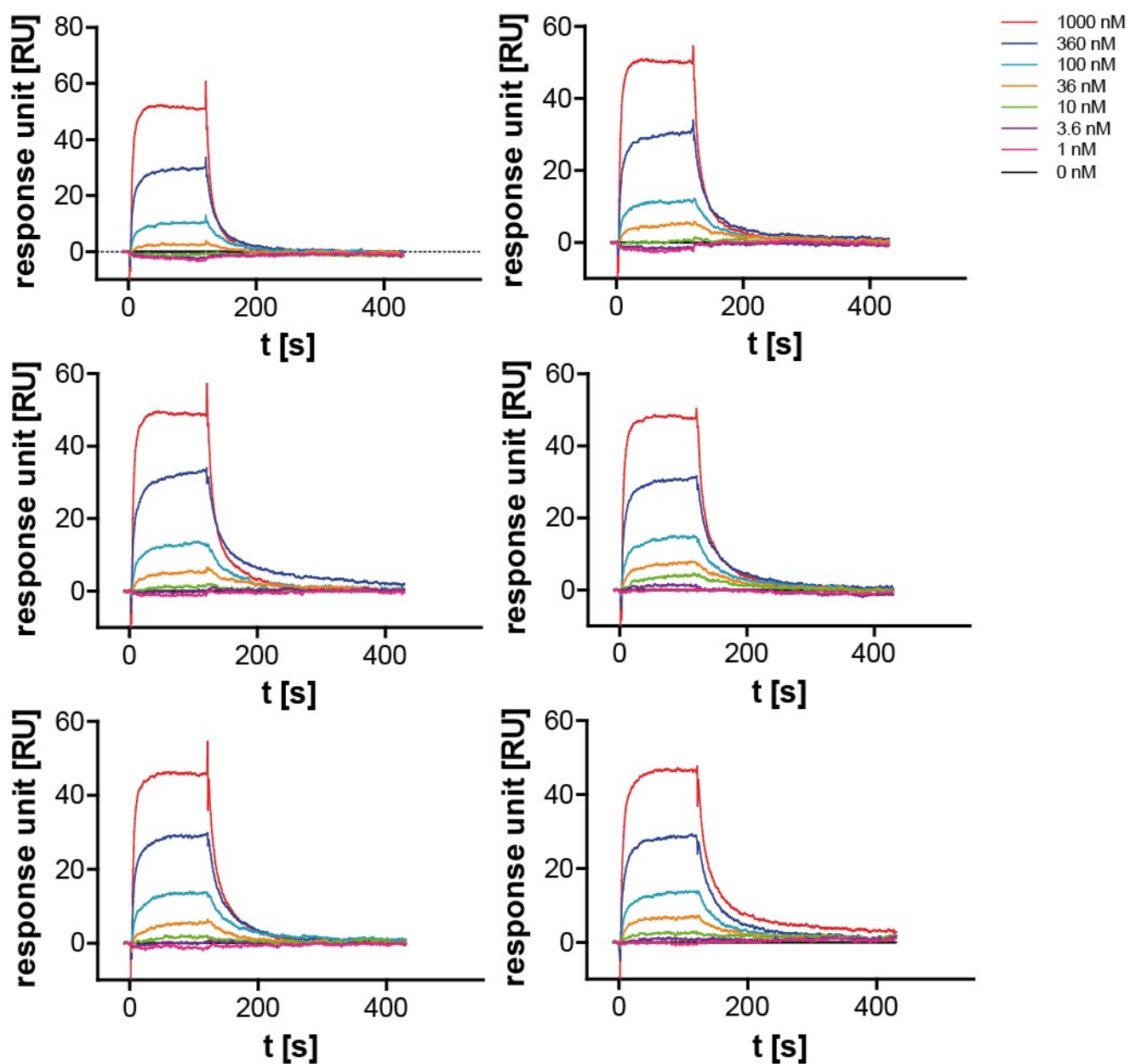
Supporting Figure 6: Surface Plasmon Resonance (SPR) measurement of clickmer F20 modified with BF-dU at 25°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand (n=5).



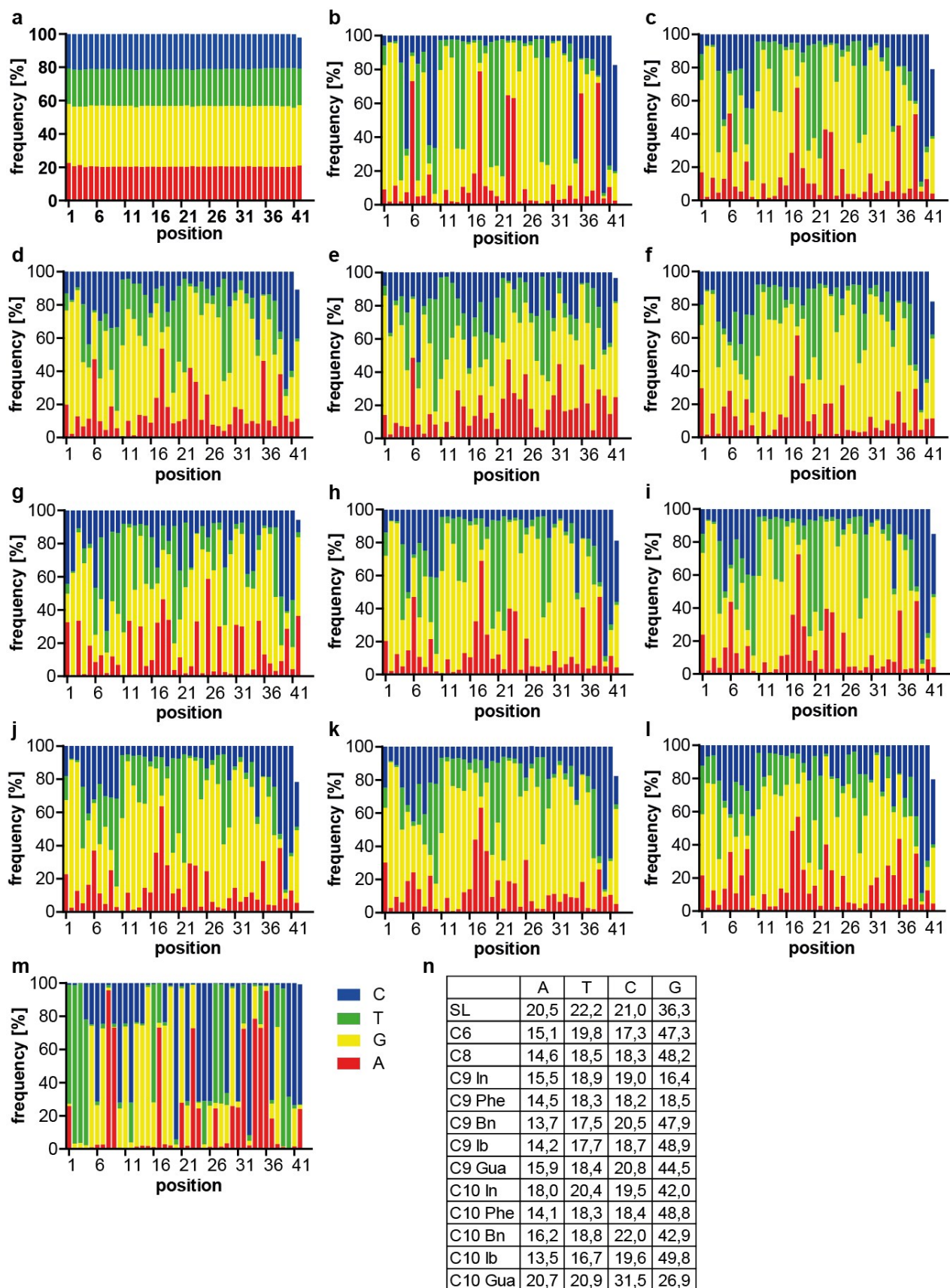
Supporting Figure 7: Surface Plasmon Resonance (SPR) measurement of clickmer F20 modified with BF-dU at 37°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand (n=5).



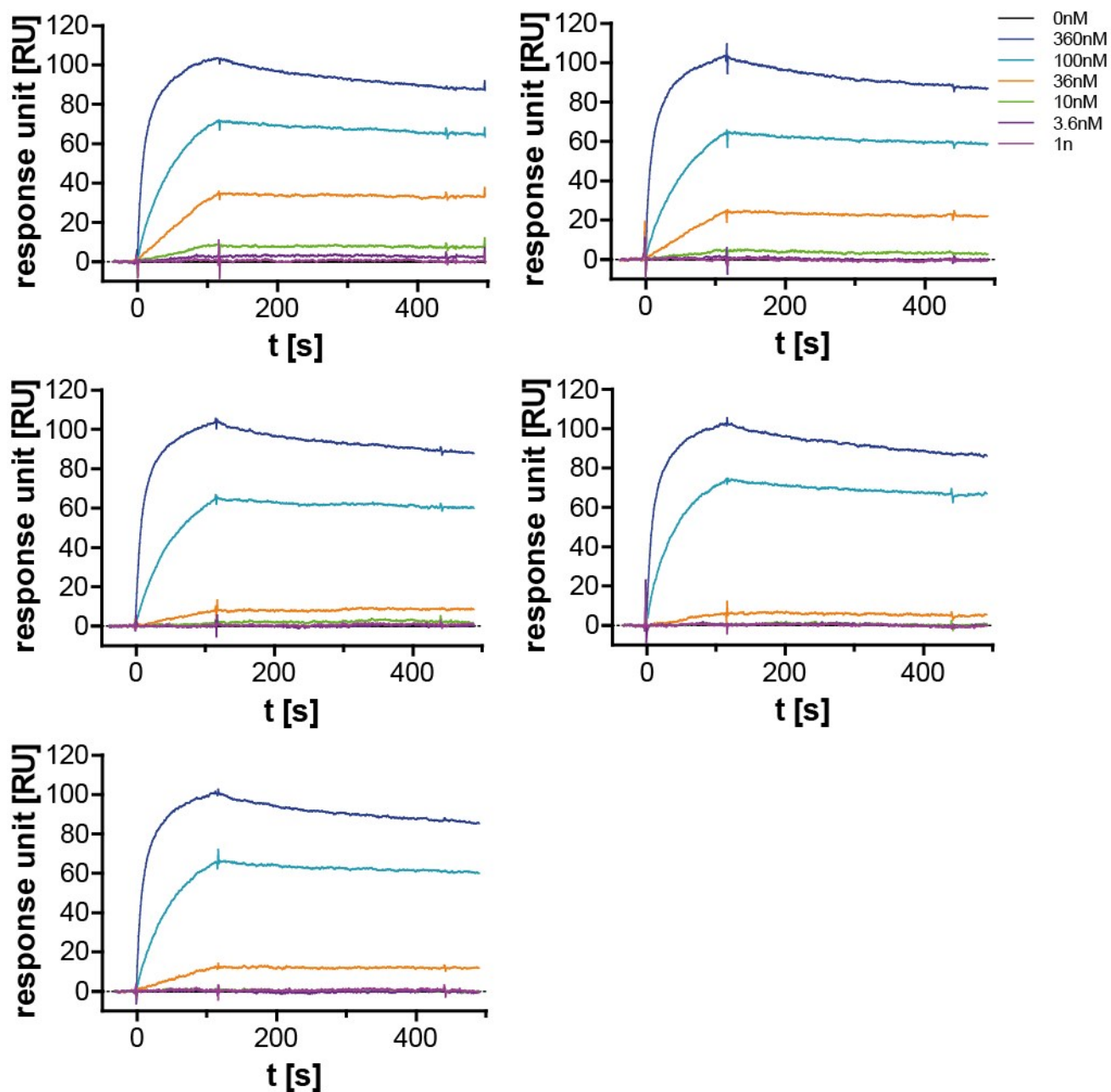
Supporting Figure 8: Surface Plasmon Resonance (SPR) measurement of clickmer B33 modified with Bn-dU at 25°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand ($n=5$).



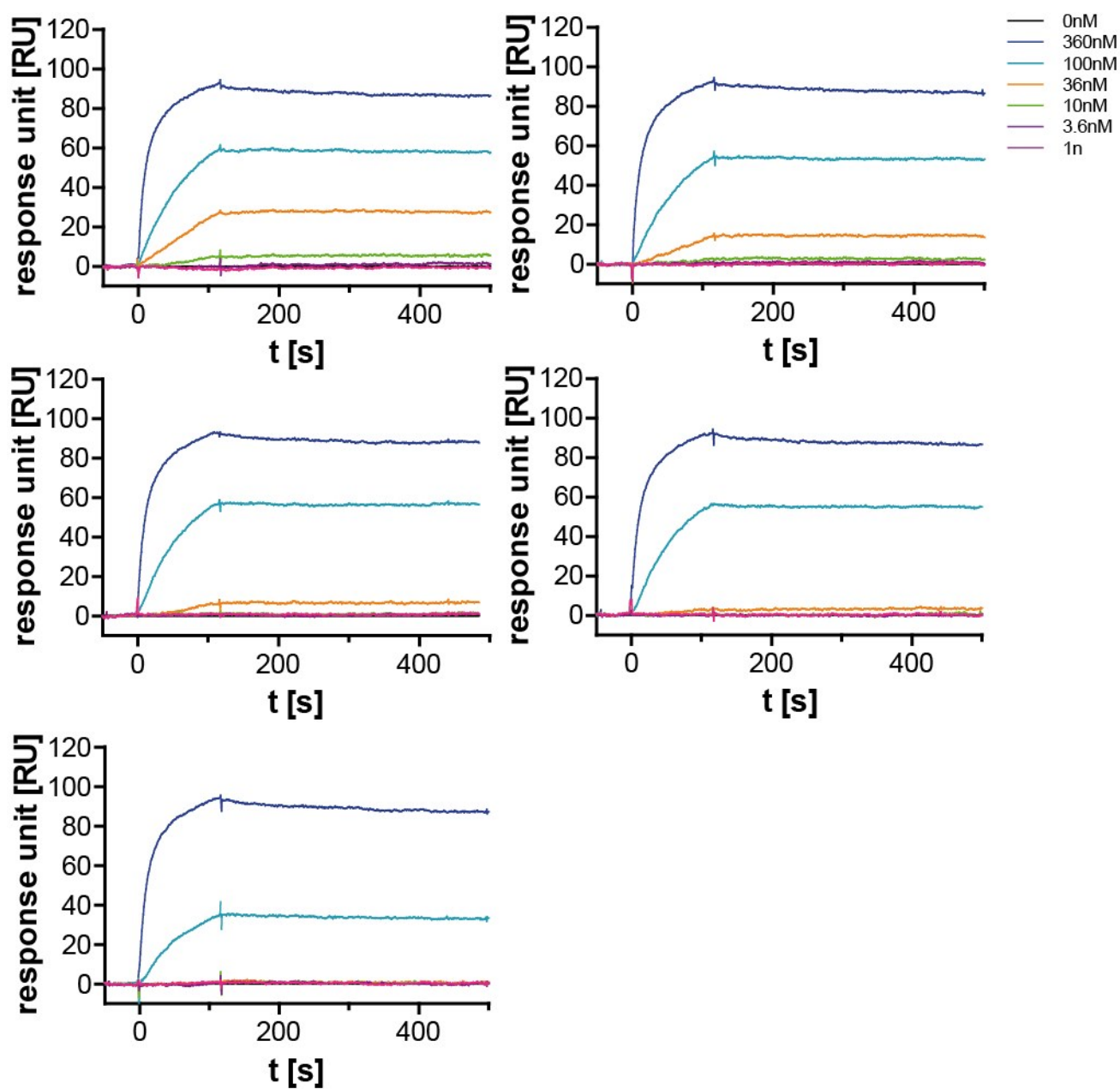
Supporting Figure 9: Surface Plasmon Resonance (SPR) measurement of clickmer B33 modified with Bn-dU at 37°C. C3-GFP in different concentration was used as analyte and clickmer was used as an immobilized ligand ($n=6$).



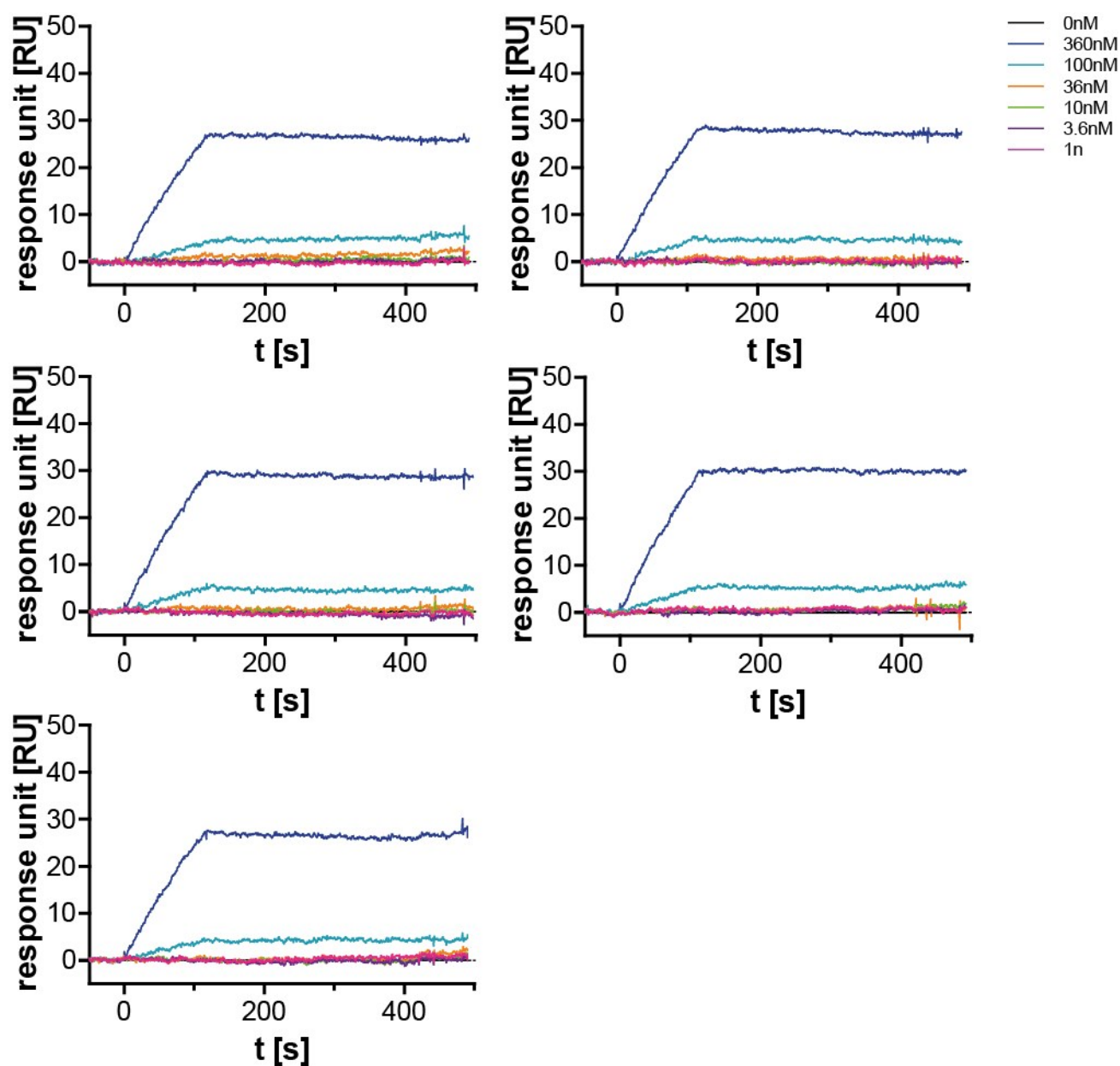
Supporting Figure 10: NGS analysis of all selection cycles of the split-combine SELEX targeting SA. Statistical base distribution over the 42 nucleobases random region for the starting library (a), selection cycle 6 (b), cycle 8 (c), cycle 9 and 10 for indole azide (In-dU) (d, e), cycle 9 and 10 for benzyl azide (Bn-dU) (f, g), cycle 9 and 10 for phenol azide (Phe-dU) (h, i), cycle 9 and 10 for isobutyl azide (Ib-dU) (j, k), as well as cycle 9 and 10 for guanidine azide (Gua-dU) (l, m). (n) Shown is the percentage distribution of the nt in the random regions [%].



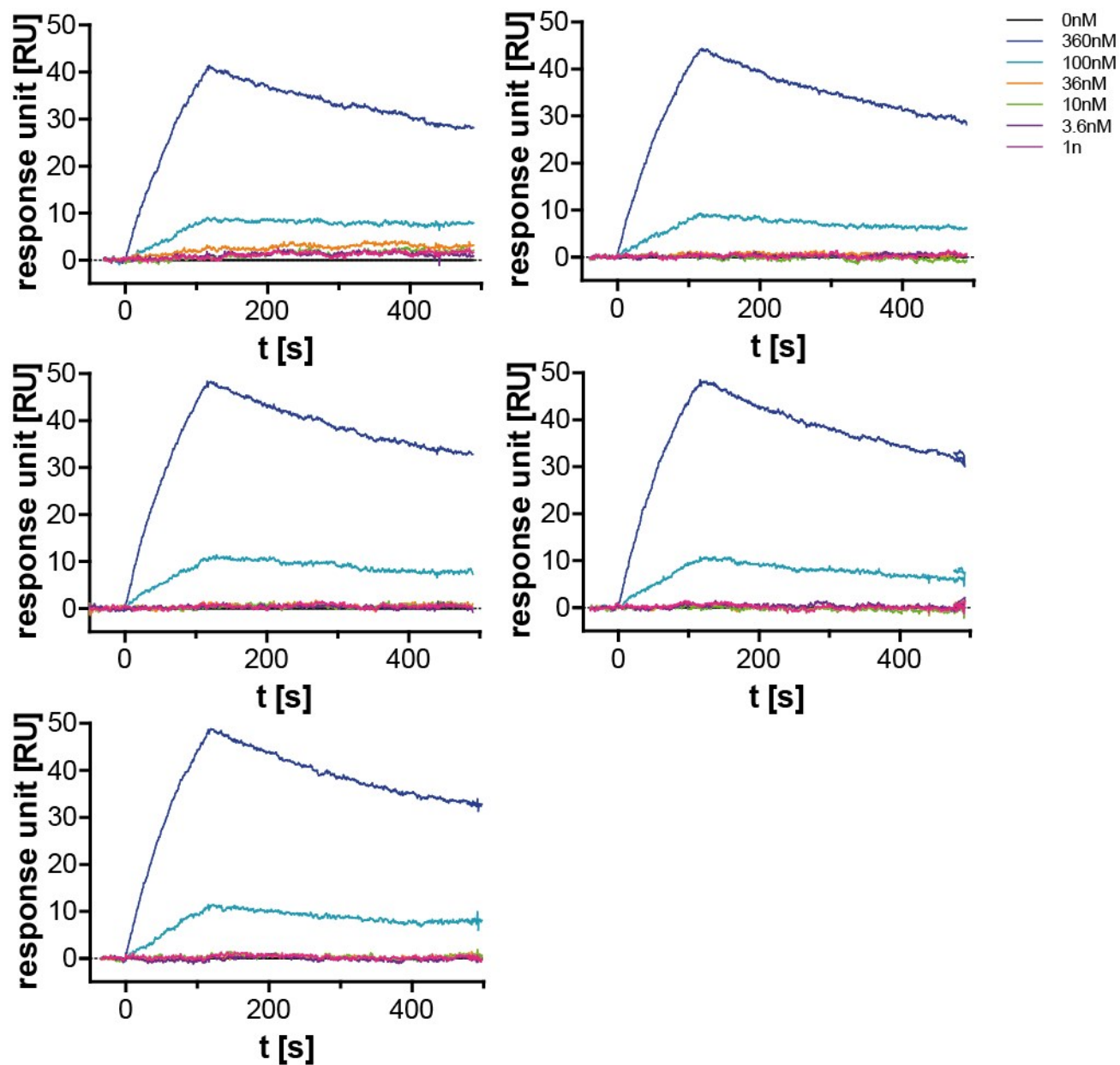
Supporting Figure 11: Surface Plasmon Resonance (SPR) measurement of clickmer B1 modified with Bn-dU in D-PBS buffer at 37°C. Streptavidin in different concentration was used as analyte and clickmer was used as an immobilized ligand ($n=5$).



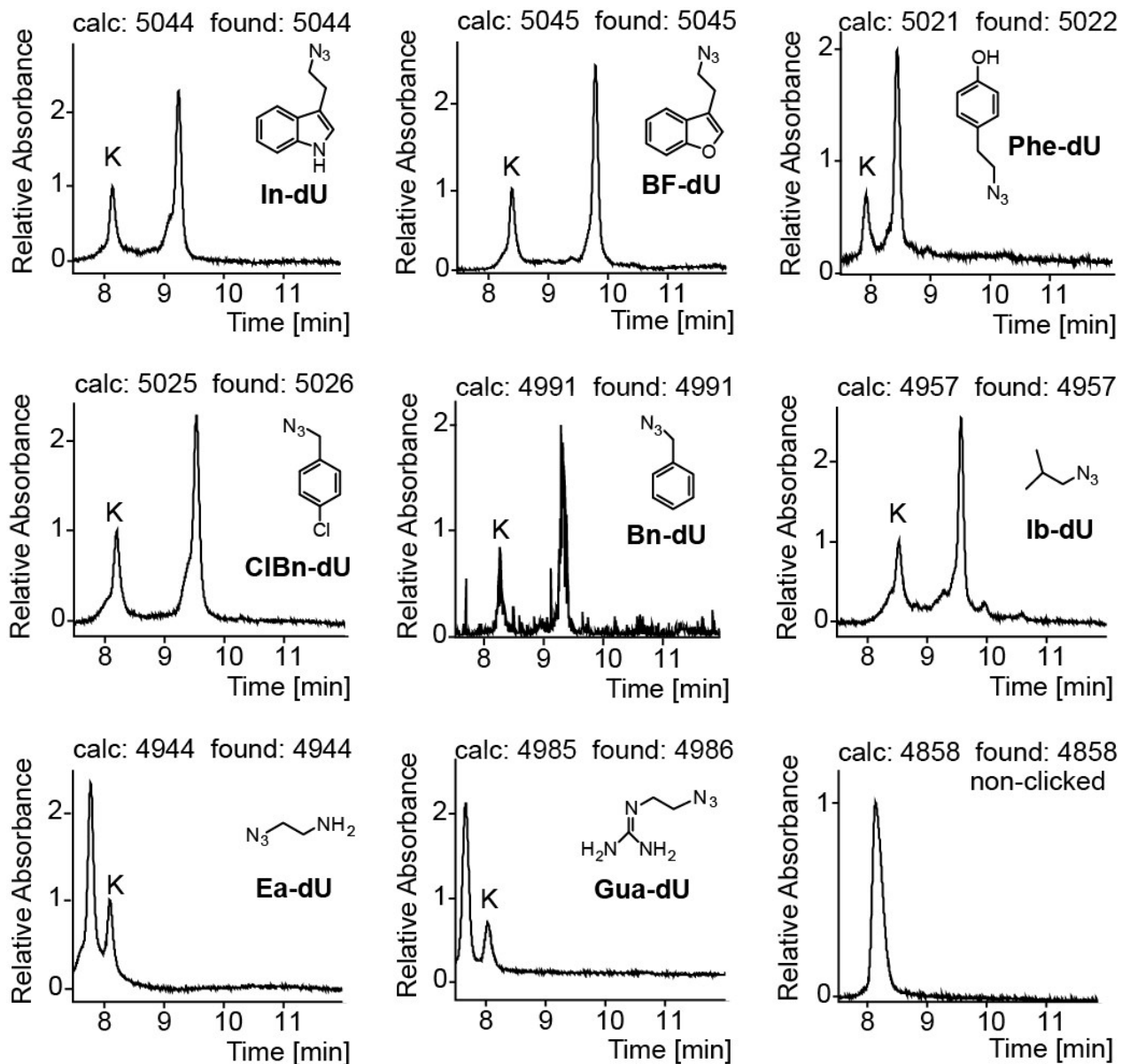
Supporting Figure 12: Surface Plasmon Resonance (SPR) measurement of clickmer B1 modified with Bn-dU in D-PBS buffer at 21°C. Streptavidin in different concentration was used as analyte and clickmer was used as an immobilized ligand (n=5).



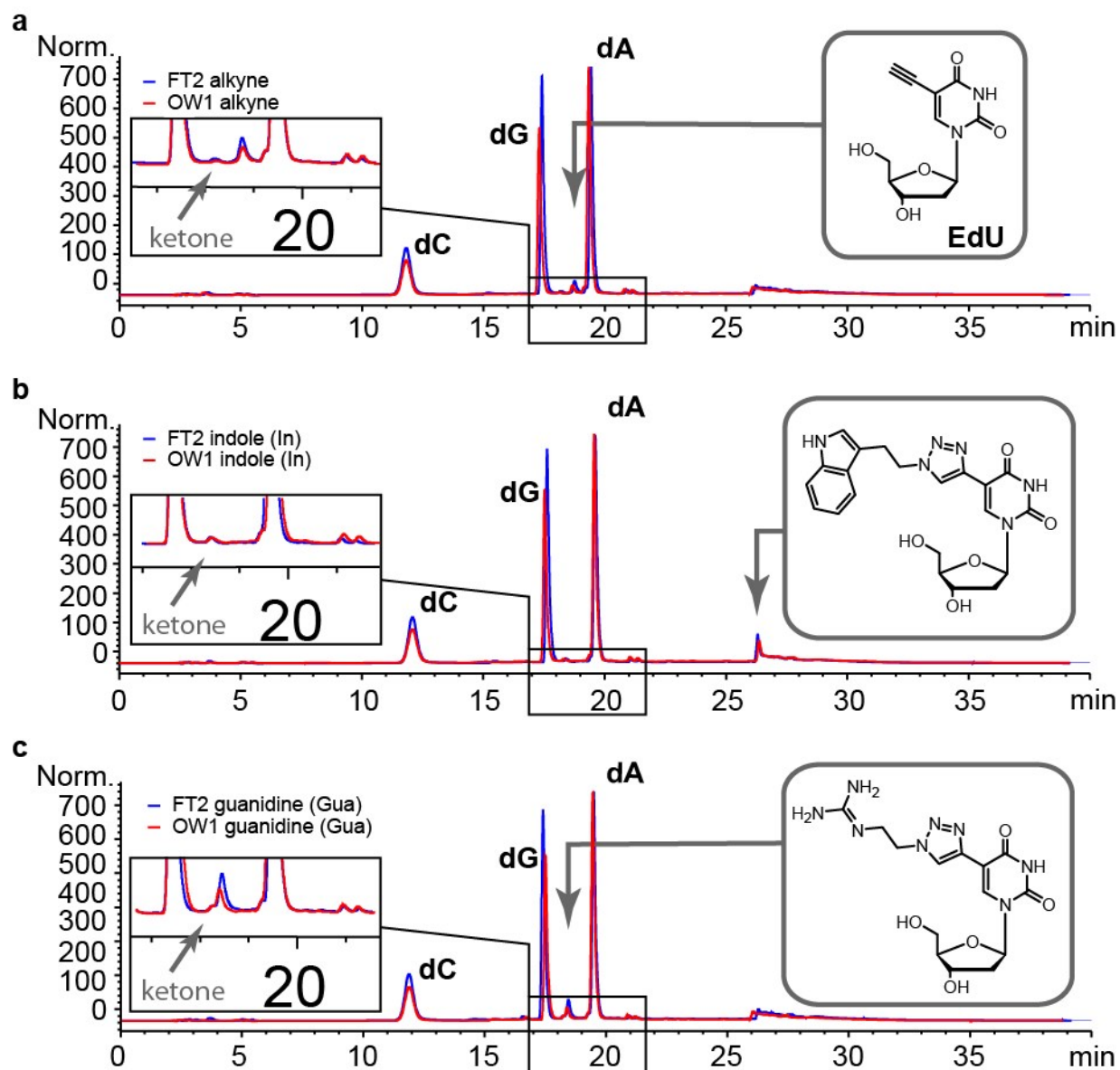
Supporting Figure 13: Surface Plasmon Resonance (SPR) measurement of aptamer G1 (naïve, unmodified DNA) in D-PBS buffer at 21°C. Streptavidin in different concentration was used as analyte and clickmer was used as an immobilized ligand ($n=5$).



Supporting Figure 14: Surface Plasmon Resonance (SPR) measurement of aptamer G1 (naïve, unmodified DNA) in D-PBS buffer at 37°C. Streptavidin in different concentration was used as analyte and clickmer was used as an immobilized ligand (n=5).

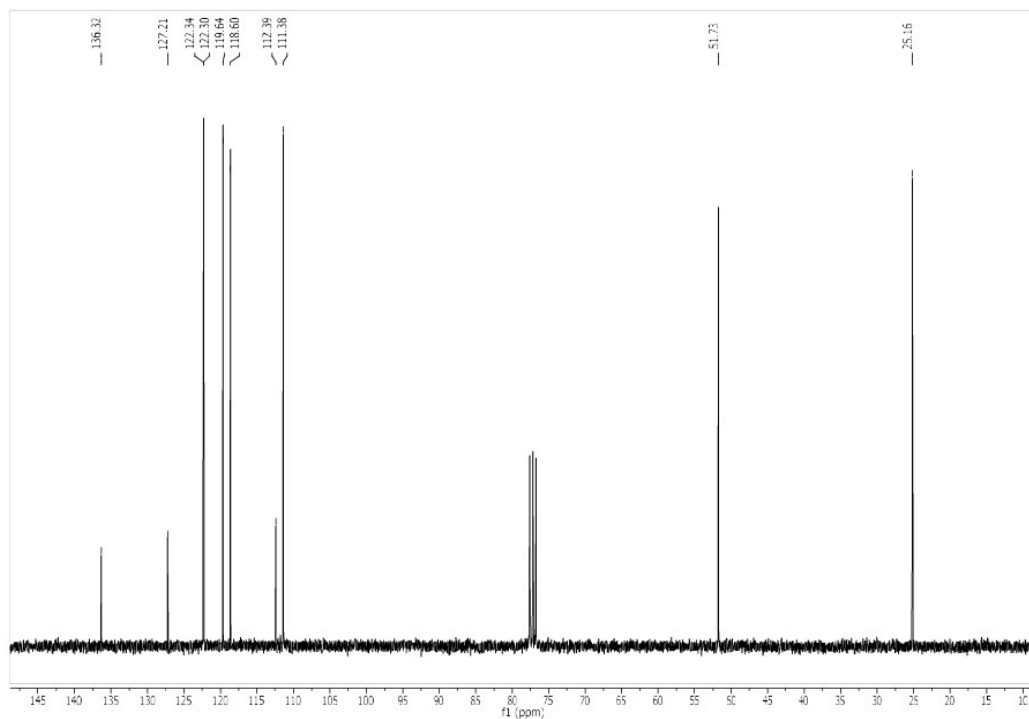
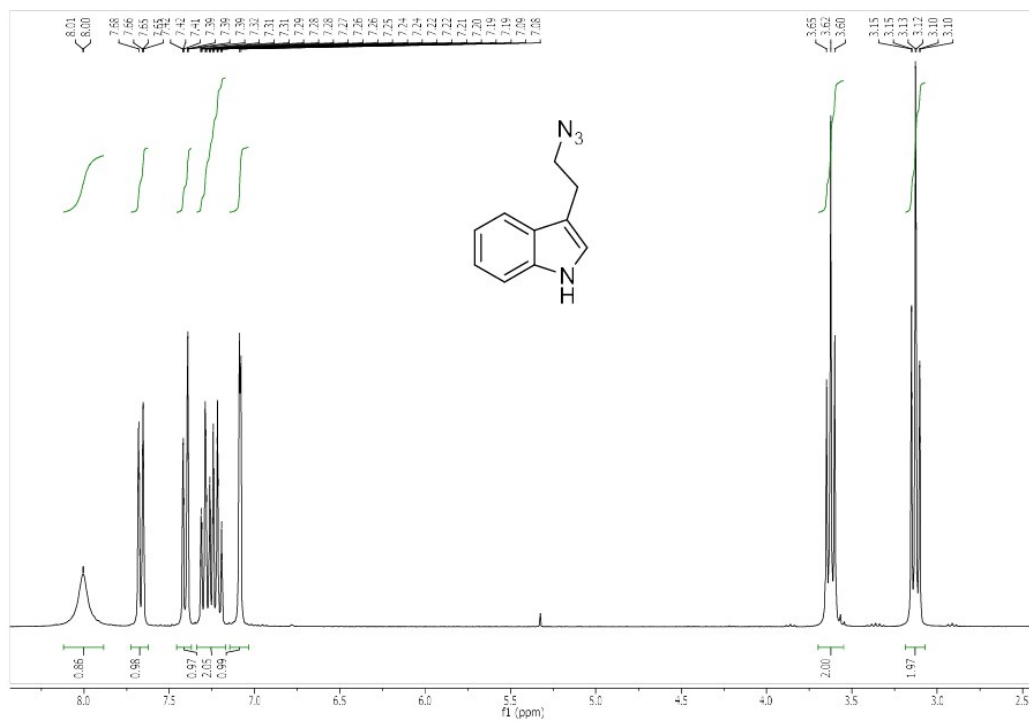


HPLC/MS analysis of clicked test ODN and unclicked test ODN, K refers to KdU which is not clickable[6]. 10 pmol of functionalized test ODN are analyzed with HPLC-MS. tested Azides: (**In-dU**) 3-(2-azidoethyl)-1H-indole, (**BF-dU**) 3-(2-azidoethyl)benzofuran, (**Phe-dU**) 4-(2-Azido-ethyl)-phenol, (**ClBn-dU**) 1-(azidomethyl)-4-chlorobenzene, (**Bn-dU**) 1-(azidomethyl) benzene, (**Ib-dU**) 1-azido-2-methylpropane, (**Ea-dU**) 2-azidoethanamine, (**Gua-dU**) N-(2-Azido-ethyl)-guanidine. Relative Absorbance was normalized to KdU Absorbance.



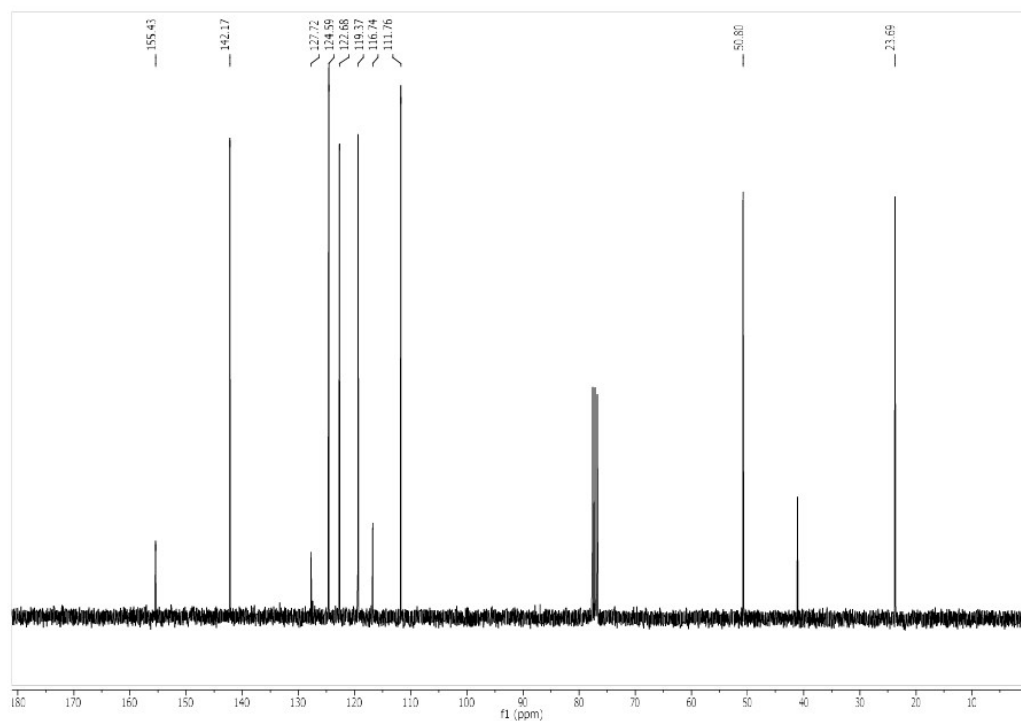
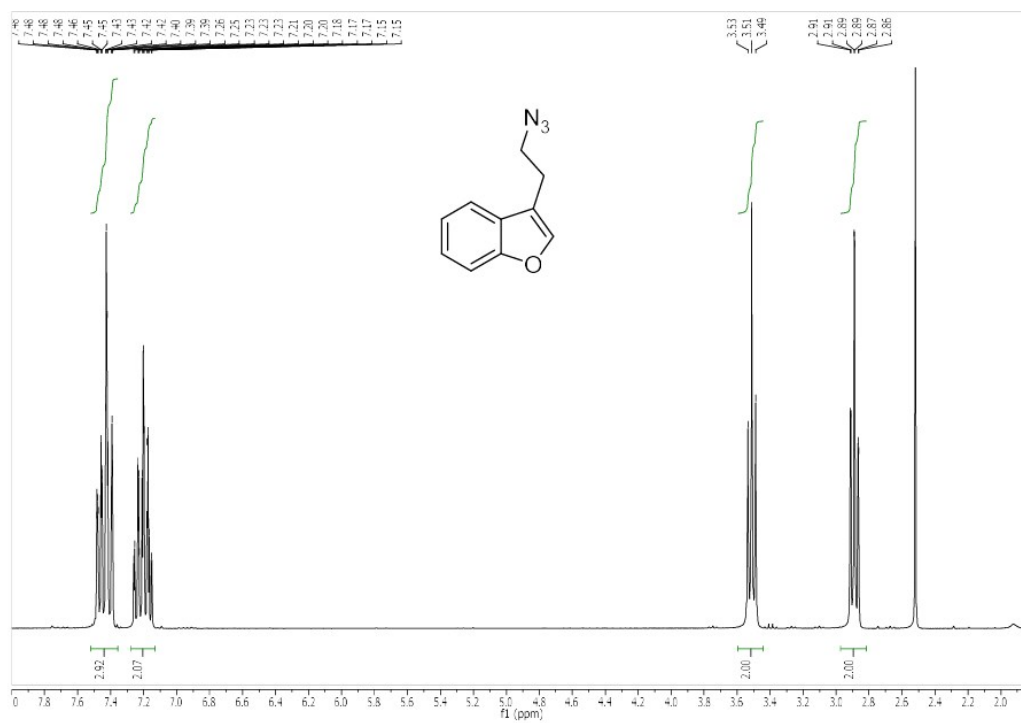
HPLC analysis of the libraries FT2 and OW1. The non-functionalized DNA (alkyne) (a), the indole (In) (b), and guanidine (Gua) (c) modified libraries were digested to the nucleotide level and analysed by HPLC.

NMR Spectra



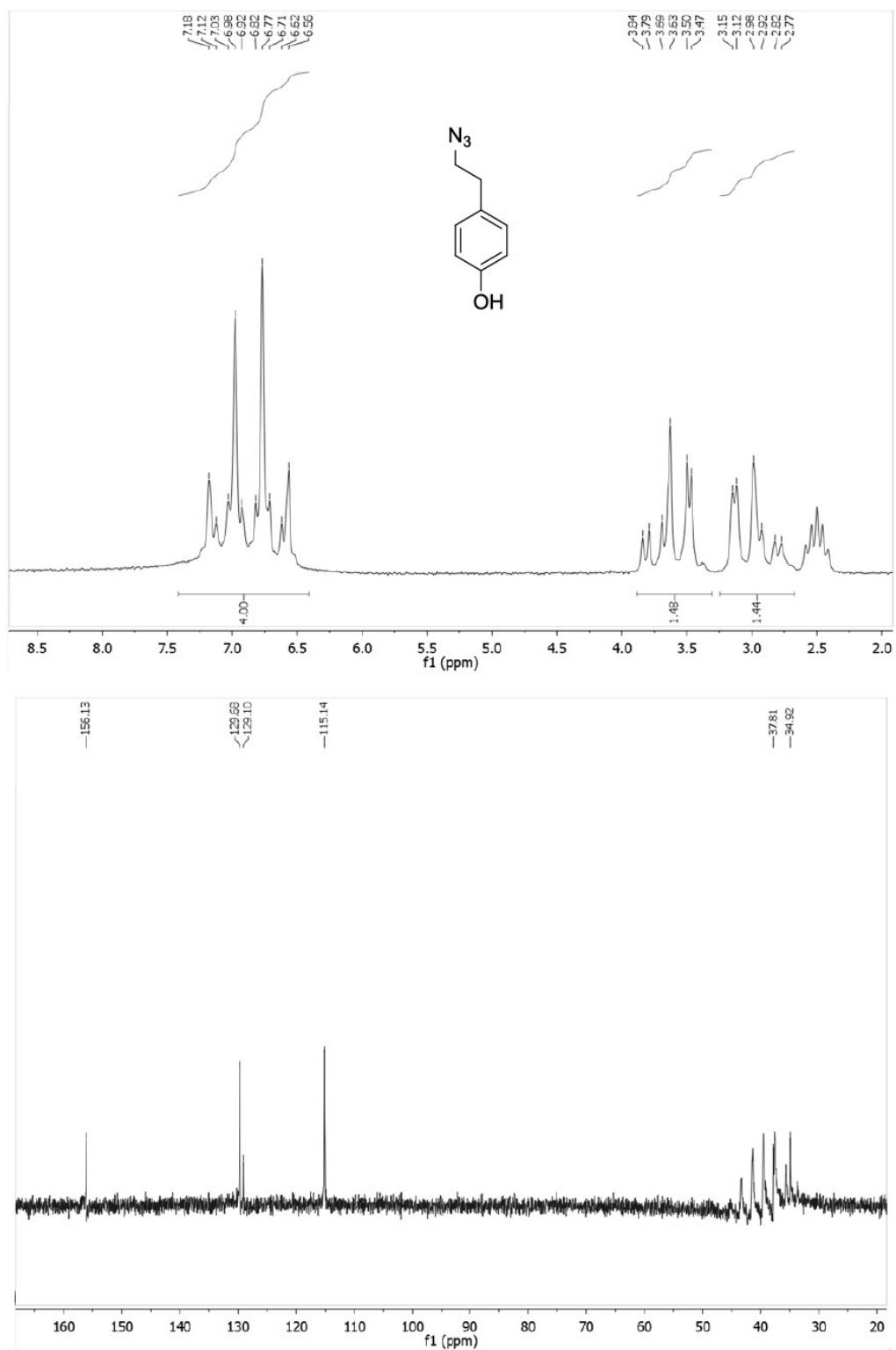
¹H NMR (300 MHz, CDCl₃) δ 8.01 (br s, 1H), 7.67 (dt, J = 7.8, 1.1 Hz, 1H), 7.40 (dt, J = 8.1, 1.0 Hz, 1H), 7.34 – 7.17 (m, 2H), 7.08 (d, J = 2.4 Hz, 1H), 3.62 (t, J = 7.2 Hz, 2H), 3.13 (td, J = 7.2, 0.9 Hz, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 136.32, 127.21, 122.34, 122.30, 119.64, 118.60, 112.39, 111.38, 51.73, 25.16.



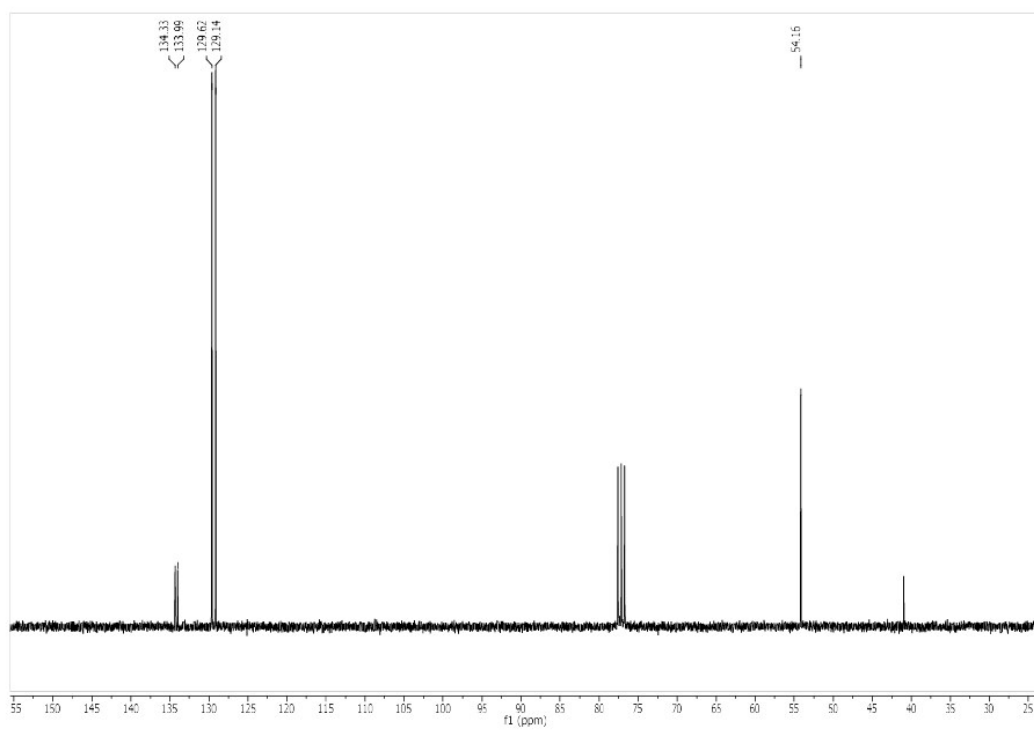
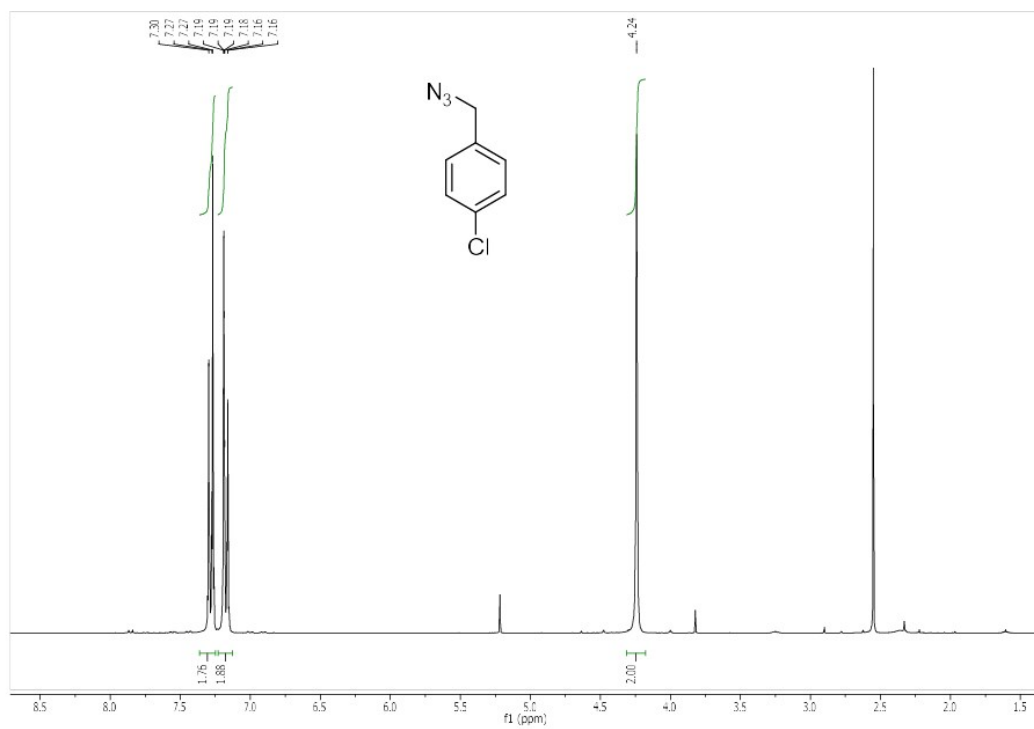
¹H NMR (300 MHz, CDCl₃) δ 7.52 – 7.36 (m, 3H), 7.30 – 7.12 (m, 2H), 3.51 (t, J = 7.1 Hz, 2H), 2.89 (td, J = 7.1, 1.0 Hz, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 155.43, 142.17, 127.72, 124.59, 122.68, 119.37, 116.74, 111.76, 50.80, 23.69.



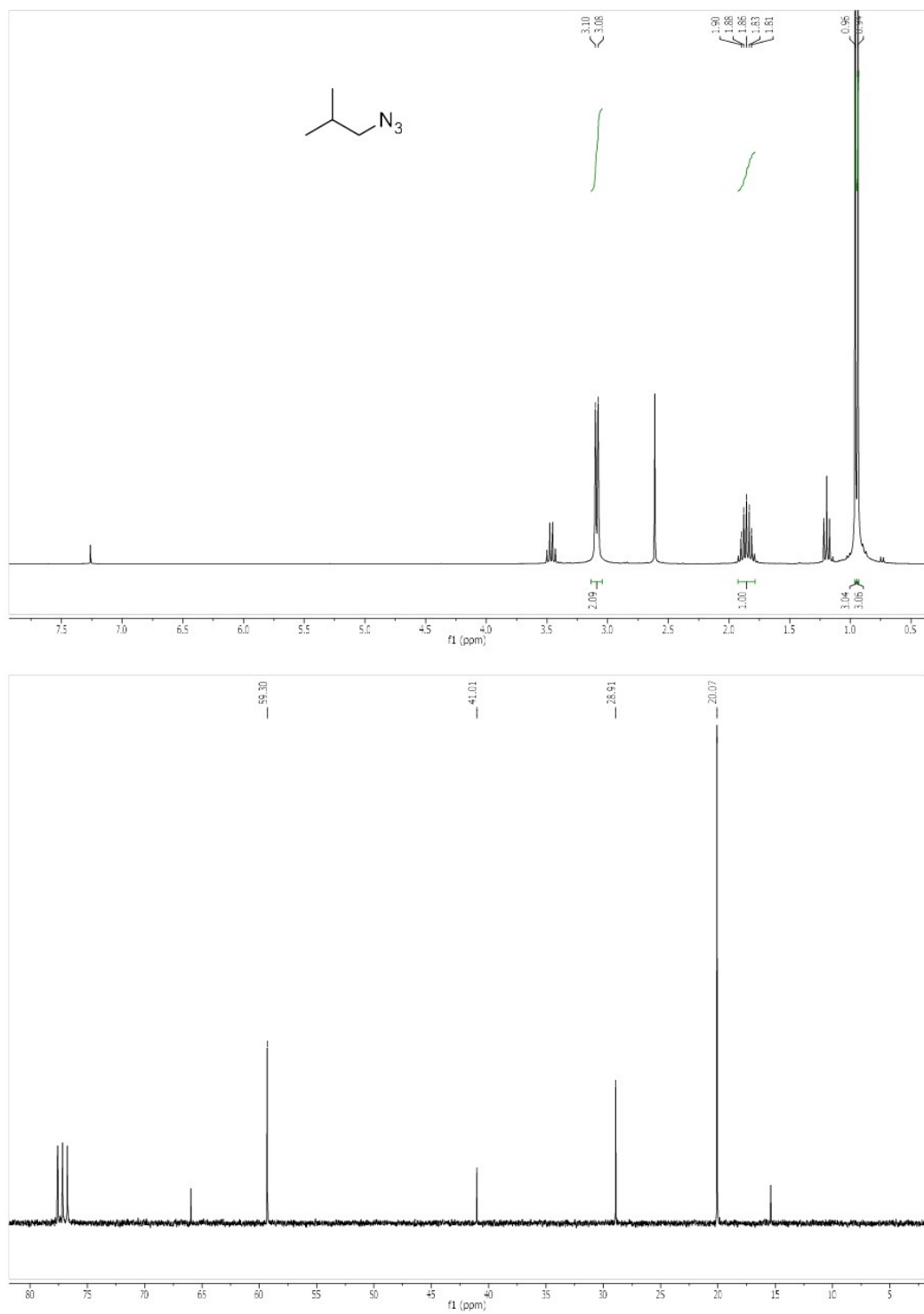
¹H NMR (42.5 MHz, DMSO) δ 3.00 (m, 2H), 3.62 (m, 2H), 6.87 (m, 4H).

¹³C NMR (10.8 MHz, DMSO) δ 34.92, 37.81, 115.14, 129.10, 129.68, 156.13.



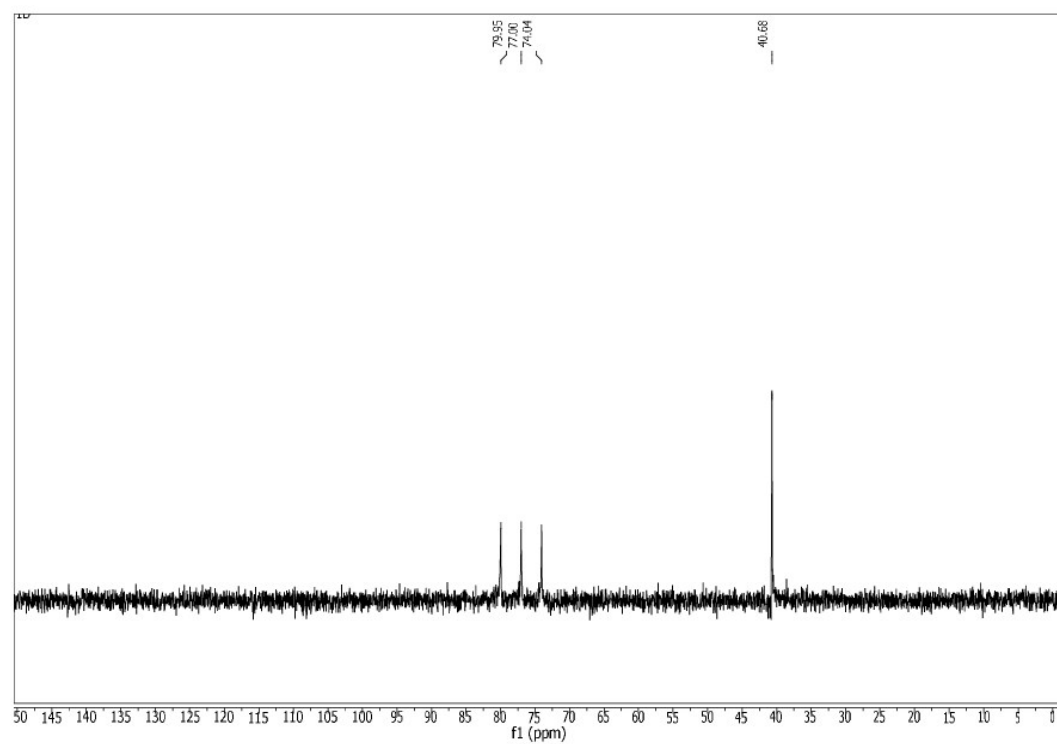
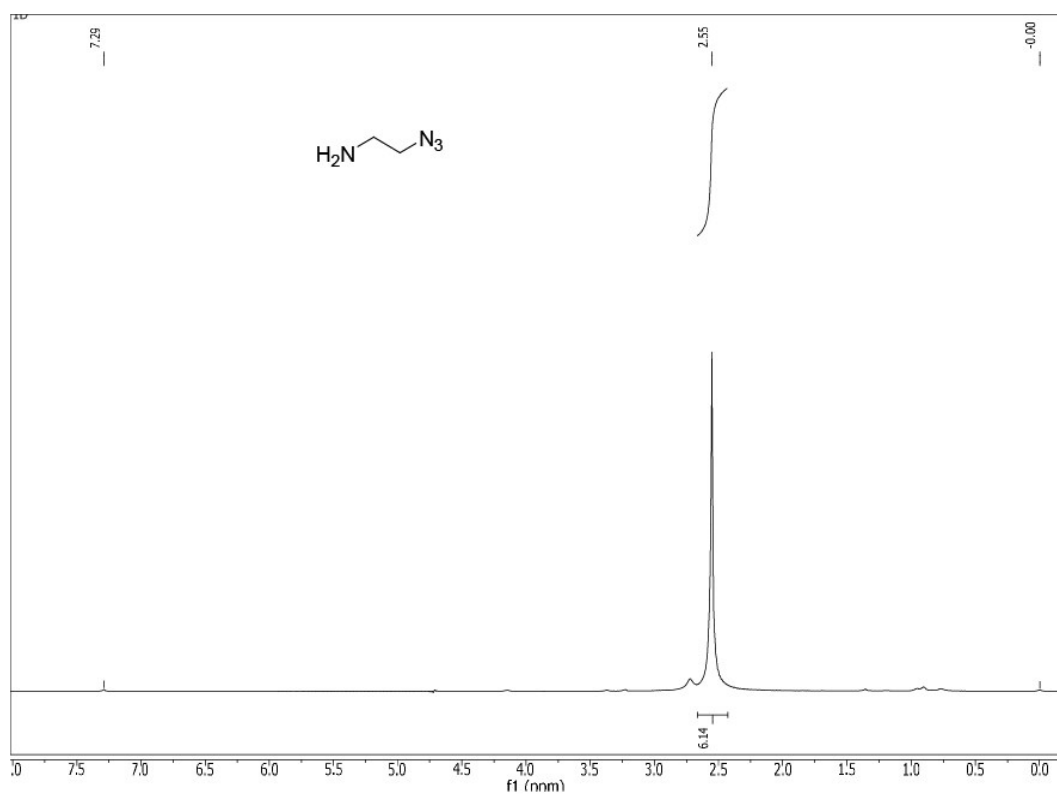
^1H NMR (300 MHz, CDCl_3) δ 7.33 – 7.25 (m, 2H), 7.20 – 7.14 (m, 2H), 4.24 (s, 2H).

^{13}C NMR (75 MHz, CDCl_3) δ 134.33, 133.99, 129.62, 129.14, 54.16.



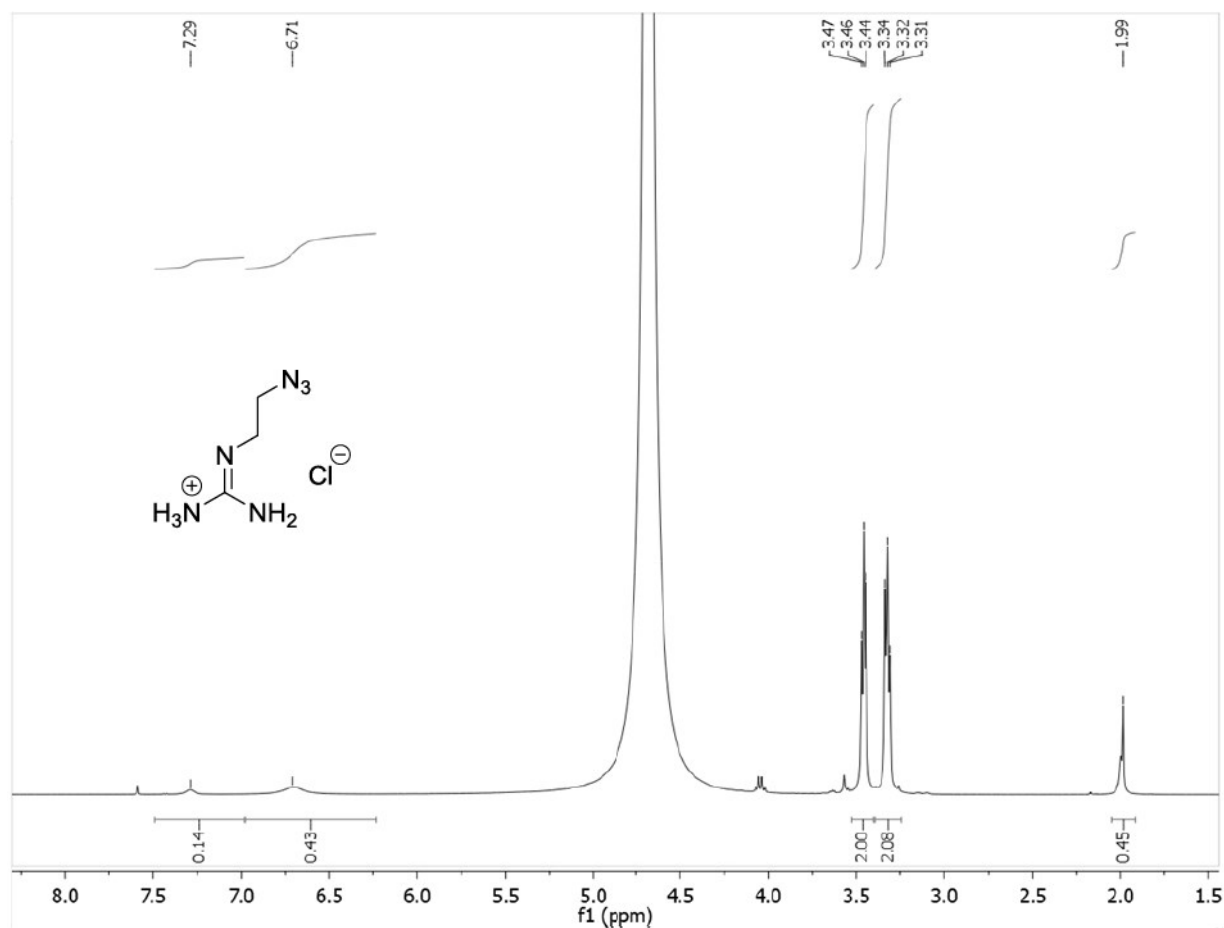
¹H NMR (300 MHz, CDCl₃) δ 3.09 (d, J = 6.7 Hz, 2H), 1.86 (dp, J = 13.3, 6.7 Hz, 1H), 0.96 (s, 3H), 0.94 (s, 3H). (Et₂O impurity)

¹³C NMR (75 MHz, CDCl₃) δ 59.30, 28.91, 20.07. (Et₂O impurity)



^1H NMR (42.5 MHz, CDCl_3) δ 2.55 (br s, 6H)

^{13}C NMR (10.8 MHz, CDCl_3) δ 40.7



¹H NMR (400 MHz, D₂O) δ 2.10 (s, 1H), 3.44 (t, 2H), 3.58 (t, 2H), 6.83 (br s, 2H), 7.41 (br s, 2H).

References

1. Suzuki, T., et al., *Rapid discovery of highly potent and selective inhibitors of histone deacetylase 8 using click chemistry to generate candidate libraries*. J Med Chem, 2012. 55(22): p. 9562-75.
2. Pfeiffer, F., et al., *Identification and characterization of nucleobase-modified aptamers by click-SELEX*. Nat Protoc, 2018. 13(5): p. 1153-1180.
3. Tolle, F., et al., *A Versatile Approach Towards Nucleobase-Modified Aptamers*. Angew Chem Int Ed Engl, 2015. 54(37): p. 10971-4.
4. Tolle, F. and G. Mayer, *Preparation of SELEX Samples for Next-Generation Sequencing*. Methods Mol Biol, 2016. 1380: p. 77-84.
5. Zuker, M., *Mfold web server for nucleic acid folding and hybridization prediction*. Nucleic Acids Res, 2003. 31(13): p. 3406-15.

6. Ingale, S.A., et al., *Ethynyl side chain hydration during synthesis and workup of "clickable" oligonucleotides: bypassing acetyl group formation by triisopropylsilyl protection*. J Org Chem, 2013. 78(22): p. 11271-82.