Supporting Information

A new architecture for DNA-templated synthesis in which abasic sites protect reactants from degradation

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Materials, Methods, and Instruments

General

Unless otherwise stated, reagents were purchased from SIGMA with the highest available purity. Gels were cast with a BIORAD Mini-PROTEAN Gel Kit, run with a BIORAD PowerPac Basic Power Supply and observed with an GE Amersham Typhoon FLA9500 gel scanner. Samples were annealed or incubated at specified temperatures using an Eppendorf Mastercycler Nexus. H_2O was purified with a Milli-Q Integral Water Purification System with a 0.22 µm filter. Reactants were stored in 1.5 mL Eppendorf tubes at -20 °C and reactions performed in either 1.5 mL Eppendorf tubes or 0.2 mL PCR tubes (Axygen). Centrifugation was performed with either an Eppendorf 5810R benchtop ultracentrifuge or Eppendorf Minispin benchtop centrifuge. TAE buffer (pH 8) comprised 200 mM Tris, 100 mM acetic acid and 5 mM ethylenediaminetetraacetic acid (EDTA).

DNA sequences

DNA and RNA was purchased from Integrated DNA Technologies, Inc. Unmodified strands were synthesized at 250 nM scale with standard desalting. Modified strands were synthesized at 1 µM scale with HPLC purification.

Key:

	iUniAmM	internal Uni-Link™ Amino Modifier
	iSpC3	internal C3 Spacer (unreactive abasic site)
	idSp	1'2'-dideoxyribose (unreactive abasic site)
	X	iUniAmM or iSpC3

Name	**Sequence (5'->3')**
donor (0)	GCCGAGCCAGCAGTCAGCGC/iUniAmM/GTCCTAATCTACCTG
donor (0, toehold)	GCCGAGCCAGCAGTCAGCGC/iUniAmM/GTCCTAATCTACCTGTTTTTTTTTT
acceptor (fc)	CAGGTAGATTAGGACAGCGCTGACTGCTGGCTCGGC
acceptor (s)	CAGGTAGATTAGGACAGCGCTGACTGCTGG
acceptor (-17)	CAGGTAGATTAGGACAGCGCTGACTGCTGGC/iUniAmM/CGGC
acceptor (-5)	CAGGTAGATTAGGACAGCGC/iUniAmM/GACTGCTGGCTCGGC
acceptor (-4)	CAGGTAGATTAGGACAGCG/**X**/TGACTGCTGGCTCGGC
acceptor (-3)	CAGGTAGATTAGGACAGC/**X**/CTGACTGCTGGCTCGGC
acceptor (-2)	CAGGTAGATTAGGACAG/**X**/GCTGACTGCTGGCTCGGC
acceptor (-1)	CAGGTAGATTAGGACA/**X**/CGCTGACTGCTGGCTCGGC
acceptor (0)	CAGGTAGATTAGGAC/**X**/GCGCTGACTGCTGGCTCGGC
acceptor (1)	CAGGTAGATTAGGA/**X**/AGCGCTGACTGCTGGCTCGGC
acceptor (2)	CAGGTAGATTAGG/**X**/CAGCGCTGACTGCTGGCTCGGC
acceptor (3)	CAGGTAGATTAG/**X**/ACAGCGCTGACTGCTGGCTCGGC
acceptor (4)	CAGGTAGATTA/**X**/GACAGCGCTGACTGCTGGCTCGGC
acceptor (5)	CAGGTAGATT/iUniAmM/GGACAGCGCTGACTGCTGGCTCGGC
1'2'-dideoxyribose (-3)	CAGGTAGATTAGGACAGC/idSp/CTGACTGCTGGCTCGGC
1'2'-dideoxyribose (0)	CAGGTAGATTAGGAC/idSp/GCGCTGACTGCTGGCTCGGC
1'2'-dideoxyribose (2)	CAGGTAGATTAGG/idSp/CAGCGCTGACTGCTGGCTCGGC
acceptor (1, toehold)	AAAAAAAAAACAGGTAGATTAGGA/iUniAmM/AGCGCTGACTGCTGGCTCGGC

Native PAGE

A typical 10% gel was prepared by mixing 1.8 mL 30% 29:1 acrylamide:bisacrylamide, 2.5 mL H_2O, 1.1 mL 5×TAE, 40 µL 10% (w/v) ammonium persulfate (APS), and 4 µL tetramethylethylenediamine (TEMED). Different percentage gels were prepared by varying the proportions of acrylamide and water. After removing well combs, wells were rinsed with running buffer (1×TAE) using a pipette before loading 2 µL samples typically diluted to 200 nM in loading

buffer (1×TAE, 50% glycerol). Electrophoresis was performed at 150 V for 20-30 minutes at RT. Gels were observed both pre- and post-staining. Gels were stained in a mix of 2 µL 10,000× SYBR Gold Nucleic acid stain (Thermofisher) and 50mL H_2O for 5-15 minutes while gently shaking, then washed with H_2O.

Denaturing PAGE

Denaturing gels were made with resolving and stacking layers. A typical 20% resolving gel was prepared by mixing 2.5 mL 40% 19:1 acrylamide:bisacrylamide, 0.7 mL 2.8 M Tris-HCl (pH 8.8), 2.1 g urea (Alfa Aesar) and 1.25 mL formamide. Urea was dissolved by heating in a hot water bath (5 minutes at ~60 °C) before adding 33 µL 10% (w/v) APS and 3 µL TEMED to set the gel. Different percentage gels were prepared by varying the proportions of acrylamide and water. A thin layer of 100% ethanol was added on top of the resolving gel while setting and removed with a tissue before casting the stacking gel above the resolving gel. A typical 5% stacking gel was prepared by mixing 0.2 mL 40% 19:1 acrylamide:bisacrylamide, 0.16 mL H_2O, 0.17 mL 1.25 M Tris-HCl (pH 6.8), 0.7 g urea and 0.42 mL formamide. Urea was dissolved as above before adding 11 µL 10% (w/v) APS and 1 µL TEMED to set the gel. After removing well combs, wells were rinsed in running buffer (25 mM Tris-HCl, 200 mM glycine, no pH adjustment) using a pipette before loading 2 µL samples typically diluted to 200 nM in loading buffer (2.5 mM Tris-HCl, 5 mM glycine and 90% formamide, no pH adjustment). Samples were heated to 95 °C for 2 minutes and loaded while hot before electrophoresis at 300 V for 60 minutes at RT. Gels were observed both pre- and post-staining. Gels were stained as described above.

PAGE densitometry

Scanned gel images were analyzed using GE ImageQuant TL software. Lanes and bands corresponding to free TAMRA, amide product and unhydrolysed donor (as indicated in Fig. 1c) were identified manually and the integrated TAMRA fluorescence intensity corresponding to each

band calculated. A background signal measured from an empty reference lane was subtracted. The yield of a species was calculated as integrated intensity of the corresponding band divided by the total from all fluorescent bands in the lane. Note that this calculation does not take account of variation in TAMRA quantum yield between fluorescent species: the results are useful for comparing relative yields between architectures but not for quantifying DTS yields.

LC-MS

Liquid Chromatography-Mass Spectrometry (LC-MS) was performed with a Xevo G2-XS QToF instrument (Waters). Purified TAMRA-thioester modified DNA samples were adjusted to 10 µM in water in a 50 µL volume before LC-MS analysis in negative ionisation mode. An ACQUITY UPLC H-Class plus system was used for RP-LC with an ACQUITY UPLC BEH 130 Å 1.7 µm C18 column (2.1×50 mm). Buffer A: 75 mM triethylammonium acetate (TEAA) pH 7.0 in H_2O; Buffer B: 75 mM TEAA pH 7.0 in MeCN. Flow rate: 0.2 $mL.min^{-1}$. Column temperature: 60 °C. Leucine enkephalin was used as the reference for the LockSpray correction. The raw continuum data was deconvoluted to produce zero charge mass spectra using ProMass HR for MassLynx (Novatia) software.

RP-HPLC

Reversed Phase High Performance Liquid Chromatography (RP-HPLC) analysis and purification of oligonucleotides was performed on a modular Shimadzu instrument or an instrument from the Agilent 1200 Series. The Shimadzu instrument was equipped with the following modules: CBM-20A system controller, LC-20AD solvent deliver module, SIL-20AC HT autosampler, CTO-20AC column oven, SPD-M20A photodiode array UV-Vis detector, RF-20A spectrofluorometric detector and a FRC-10 fraction collector. Chromatography was performed on a reversed phase Waters Xbridge™ Oligonucleotide BEH C18 column, 130 Å, 2.5 µm, 4.6×50 mm. Column temperature: 35 °C. Flow rate: 0.8 $mL.min^{-1}$. Buffer A: 5% acetonitrile, 0.1 M TEAA, pH 7 in H_2O; buffer B: 70%

acetonitrile, 0.1 M TEAA, pH 7 in H_2O. The buffer gradient for analysis and purification was 1% buffer B for 3 minutes, 1% to 15% B over 17 minutes, 15% to 95% B over 5 min, 95% to 1% B over 2 min and finally 1% B for 4 min. Samples were observed by absorbance at 262 nm (DNA) and fluorescence with λ_{ex}:550 nm and λ_{em}:580 nm fluorescence.

General Procedures

Synthesis of TAMRA-thioester adapters

Reactions were prepared by mixing 2 µL 1 mM internal amine-modified DNA in H_2O with 2 µL 1 M *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP, Cambridge Bioscience) or *N*-succinimidyl 6-(2-pyridyldithio)hexanoate (SPDH, synthesized see Scheme S1 and Figure S16) in DMF and 8 µL 100 mM borate buffer (pH 9.8). The reaction mixture was incubated overnight (~15 hours) at 21 °C. To remove SPDP the reaction was precipitated using 300 vol% ethanol at –20 °C. After centrifugation at 15000 rpm the resulting internal disulfide-modified DNA pellet was washed twice with ice-cold 70% ethanol. After the DNA pellet was dried, it was dissolved in 2 µL in H_2O and 4 µL 60 mM TCEP solution in H_2O (freshly prepared) was added. The resulting solution was incubated at 21 °C for 1 h, before 7 µL 100 mM borate buffer (pH 9.8) and 1 µL 0.1 M NHS-TAMRA in DMF were added. The reaction mixture was incubated overnight (~15 hours) at 21 °C. DMF and excess TAMRA were removed by ethanol precipitation as described above. The dried TAMRA-thioester DNA containing pellet was dissolved in 500 µL H_2O and remaining TAMRA was removed with an Amicon-Ultra 3 kDa 0.5 mL centrifugal filter. The resulting 80-100 µL sample was then analyzed and purified by RP-HPLC. Fractions were collected and washed with an AmiconUltra 3 kDa 0.5 mL centrifugal filter to remove HPLC buffers. The concentration of DNA was checked by absorbance at 260 nm and 550 nm, while the purity was checked by RP-HPLC and LC-MS (Figure S1). The same protocol was followed substituting NHS-biotin for NHS-TAMRA to produce thioester-biotin modified DTS donor adapters.

DNA templated synthesis (DTS)

Figure 1. DTS reactions were prepared in 20 µL volumes by mixing: 2 µL 100 mM $MgCl_2$, 2 µL 20 µM NH_2-modified acceptor DNA, 2 µL 10 µM donor TAMRA-thioester DNA (with toehold). After duplex formation, 10 µL DTS buffer (pH 11) was added and the remaining volume H_2O. Samples were incubated overnight (~15 hours) at 21 °C before analysis by denaturing PAGE (29:1, 25%, 250 V, RT, 60 minutes).

Figure 3b, c. DTS reactions were performed in two stages. For the incubation stage samples were prepared in 30 µL volumes by mixing 4.4 µL H_2O, 0.6 µL 500 mM $MgCl_2$, 3.33 µL 9 µM donor TAMRA-thioester DNA (with toehold) and 6.67 µL 9 µM keeper oligo incorporating an abasic site (*n* = -3) or a fully complementary keeper. In both cases the keeper was shorter than the donor adapter, leaving unhybridized the 10-bp toehold domain of the donor adapter. After duplex formation, 15 µL 0.25 M DTS buffer (pH 11) was added and samples were incubated for at 5 °C for up to 48 h. The DTS reaction stage was triggered by adding 6 µL 20 µM NH_2-modified acceptor DNA (*n* = 1), which incorporated a complementary toehold domain, to replace the keeper by toehold-mediated strand displacement (Figure S8). Samples were incubated at 5 °C for 30 min and then at 15 °C. Reaction progress was monitored via RP-HPLC (2 µL injection) using the TAMRA fluorescence channel (λ_{ex}:550 nm, λ_{em}:580 nm) (Figure S10 and S11). Product formation, thioester starting material and hydrolyzed TAMRA were quantified using calibration curves (Figure S9). All reactions were performed in triplicate.

Figure S3. DTS reactions were prepared in 20 µL volumes by mixing: 2 µL 100 mM $MgCl_2$, 5 µL 20 µM acceptor-NH_2 DNA or keeper incorporating abasic site (*n* = -3), 2 µL 25 µM donor biotin-thioester DNA. After duplex formation, 10 µL DTS buffer (pH 11) was added and the remaining volume H_2O. Samples were incubated overnight (~15 hours) at 21 °C. Samples were split and one half hydrolyzed by incubating at 95 °C for 15 minutes. 5 µL

samples were diluted in 5 µL 20 µM streptavidin and incubated at 4 °C for 20 minutes, then diluted again in 5 µL native PAGE loading buffer (1×TAE, 50% glycerol) before native PAGE (29:1, 10%, 150 V, 4 °C, 45 minutes).

RP-HPLC time course

RP-HPLC time courses were used to monitor protection assays and DTS reactions with a preceding protection stage, described above. 2 µL samples were withdrawn and injected for analysis. Components of DTS reactions were identified using standards that contained a single species (Figure S15).

Figure 2a. The TAMRA fluorescence intensity under each peak was integrated using a standard window size (e.g., 30 seconds) centered around the signal. The yield of a species was calculated as the integrated intensity of the corresponding peak divided by the total from all fluorescent peaks. For each sample the initial fluorescence intensity of free TAMRA was subtracted to correct for a background of pre-existing TAMRA impurity.

Figure 2c. The integrated intensity of the peak corresponding to hydrolyzed TAMRA was used as a measure of hydrolysis. The intensity under each fluorescence signal was integrated using a standard window size (e.g., 30 seconds) centered around the signal. Data were linearly rescaled to the interval [0,1].

Figure 3b and c. Integrated fluorescence signals corresponding to the thioester starting material, amide product and hydrolyzed TAMRA were used to monitor reaction progression. Dilution series from all three species were used to calibrate the measurements (Figure S9).

Supplementary Figures

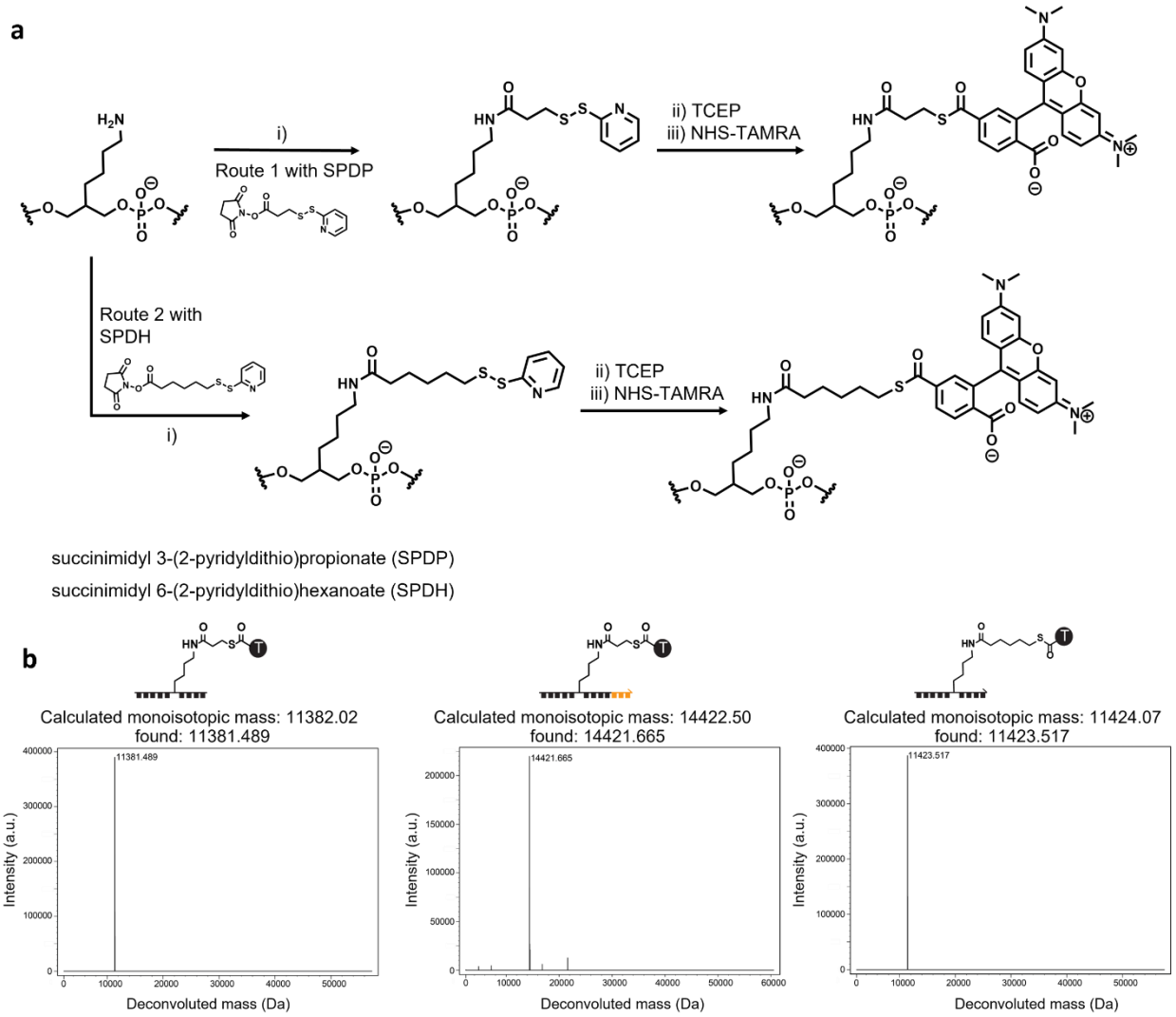


Figure S1. *a) Synthesis of TAMRA-thioester modified DNA donors. i) 0.1 mM NH2-modified DNA, 0.1 M N-succinimidyl 3-(2-pyridyldithio)propionate or N-succinimidyl 6-(2-pyridyldithio)hexanoate (DMF), 0.1 M borate buffer pH 9.8, 21 °C, 18 h; ii) 0.33 mM SPDP-modified DNA, 0.06 M TCEP (water), 19 °C, 1 h; iii) 0.1 mM thiol-modified DNA, 0.01 M TAMRA-NHS ester (DMF), 0.1 M borate buffer pH 9.5, 19 °C, 18 h. b) Deconvoluted mass spectra for TAMRA-thioester modified donor adapters.*

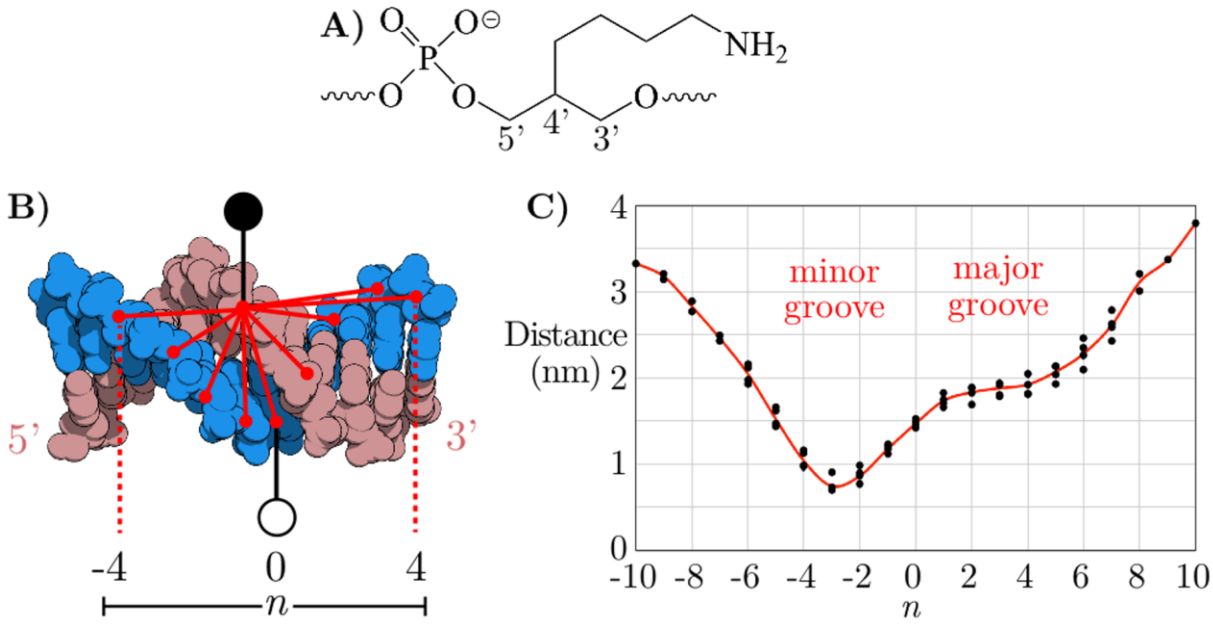


Figure S2. *Proximity of DTS reactants tethered at abasic sites. A) Structure of abasic-site amine modification. Carbon atoms are numbered by analogy to the deoxyribose linkage that is replaced by this minimal linker. This amine modification was used as supplied as acceptor in the acyl transfer reaction and to tether the donor thioester through an amide linkage. B) Model of an unmodified DNA duplex with positions of 4' carbons (taken as the reference position for potential internal amine modifications) indicated. C) Distances between attachment points measured from DNA crystal structures for various positions of the second modification (see main text Figure 1 for definition of offset n) (PDB:5f9i, 2m2c, 2v3l, 5zld, 4j2i).*

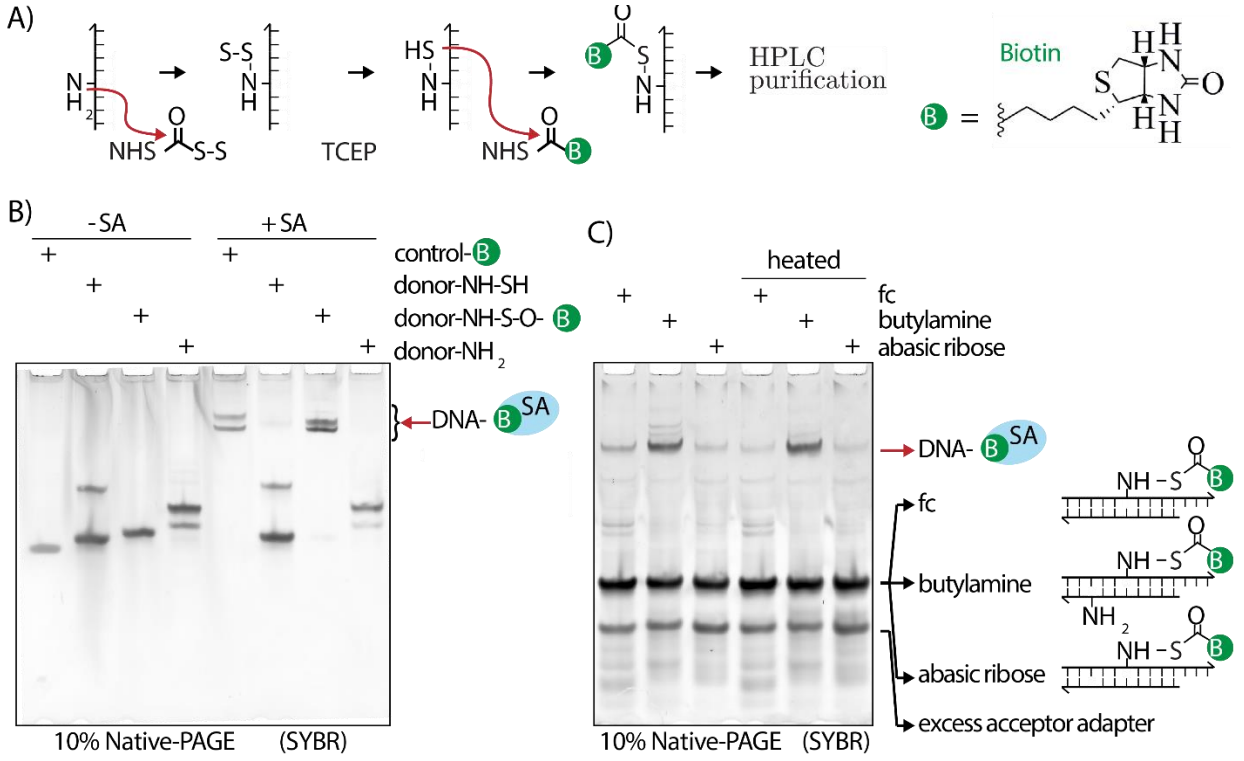


Figure S3. *Exploring protection from hydrolysis of a biotin thioester using a streptavidin (SA) gel-shift assay to indicate the presence of the biotin moiety. A) Biotin-thioester donor adapters were built upon an internal abasic butylamine modification, as described for the synthesis of the TAMRA-thioester adapters. Briefly, a disulfide was added using SPDP (NHS-SS), reduced to a thiol, and incubated with NHS-biotin before RP-HPLC purification. B) Native PAGE analysis of the synthesized biotin donor adapter to confirm purity and streptavidin binding. After RP-HPLC purification, the synthesized biotin-thioester-modified DNA ("donor-NH-S-O-B") was split into two aliquots, one of which was heated to 95 °C for 20 min to hydrolyze the thioester ("donor-NH-SH"). A non-labile covalent biotin modification added during solid-phase synthesis ("control-B", purchased from Integrated DNA Technologies, Inc.) and the butylamine-modified DNA starting material ("donor-NH_2"), both as supplied, served as controls. Lanes 1-8 contain the modified oligonucleotides without (lanes 1-4) and with (lanes 5-8) addition of streptavidin (10-fold excess). The presence of a single band in lane 3 indicates the purity of the synthesized biotin thioester. The appearance of slow bands in lanes 5 and 7 (cf. lanes 1 and 3) indicate that both biotin-modified oligos bind streptavidin stoichiometrically (two bands are observed in each lane consistent with the tetravalency of streptavidin). No interaction between streptavidin and the non-biotinylated controls is observed. We attribute the*

presence of two bands in lanes 2 and 6, containing the hydrolyzed thioester, to the formation of a disulfide-bonded dimer. C) Evaluation of the protection effect of an abasic site and DTS yield with a biotin-thioester. The biotin-thioester donor-DNA was hybridized to a complementary oligonucleotide with, at position n = −3, an abasic butylamine modification, an unreactive 1'2' dideoxyribose abasic site or a complementary nucleotide. After duplex formation, DTS buffer was added and the samples were incubated at 21 °C for 15 hours. Each sample was then split into two aliquots of which one was heated as described above to hydrolyze any remaining thioester. All samples were incubated with 10-fold excess streptavidin before analysis by PAGE. The low intensities of the slow-moving bands in lane 1, which are further weakened by heating (lane 4) (SA-biotin conjugated to the donor adapter by an unreacted thioester), indicate that the abasic site at position n = −3 affords little protection from hydrolysis (cf. control lanes 3 and 6). A slow-moving band in lane 2, unaffected by subsequent heating (lane 5) corresponds to successful DTS (SA-bound biotin conjugated to the acceptor adapter by an amide bond): this is in contrast to the behavior of the TAMRA-thioester for which no product formation was detected with the butylamine modification at n = -3 (Figure 1).

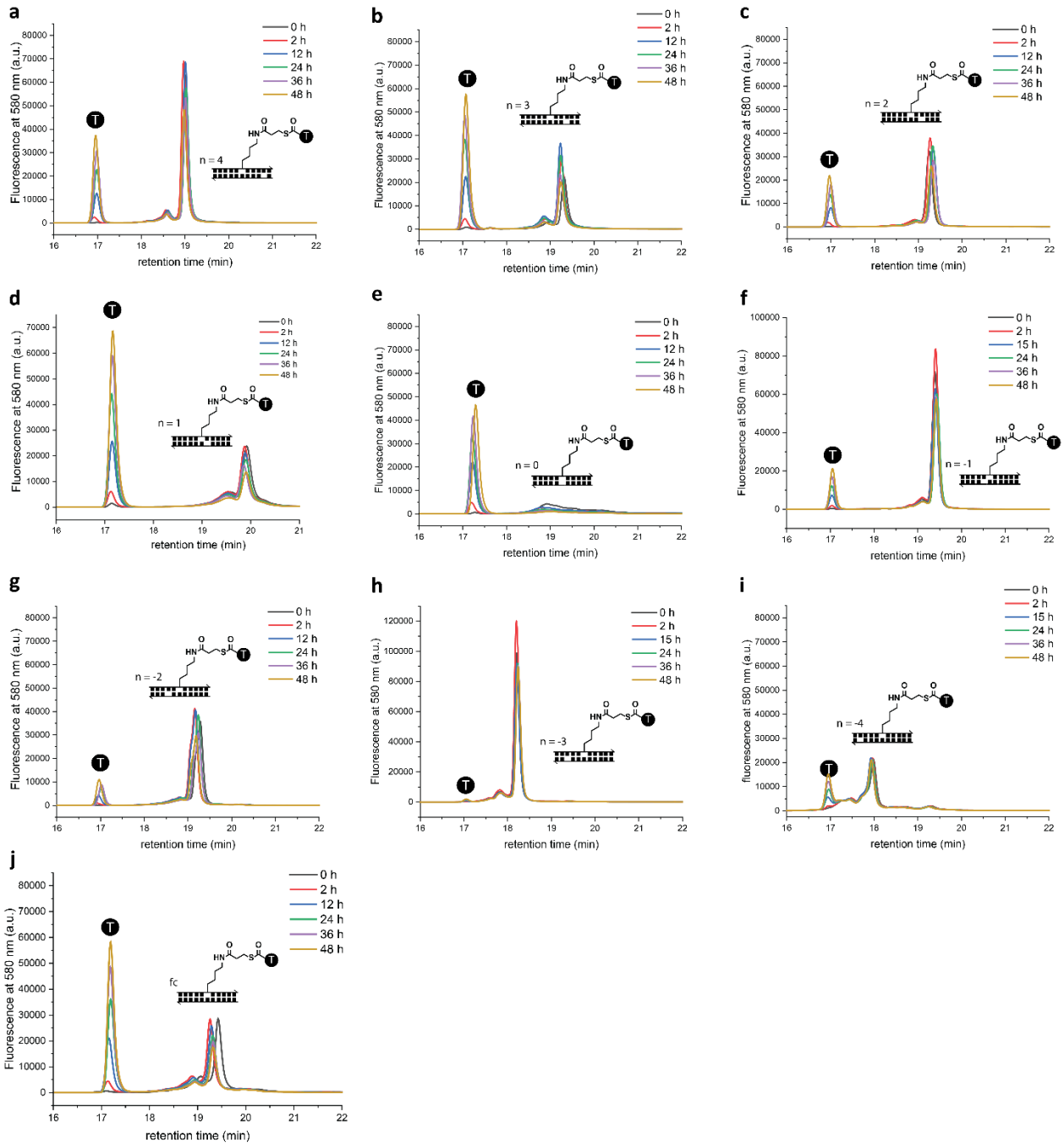


Figure S4. *Hydrolysis of the TAMRA-thioester in the presence and absence of an abasic site used for Figure 2c. The hydrolysis of the TAMRA-thioester was measured by RP-HPLC time-course (5 °C, pH 11, 0-48 h) after the TAMRA-DNA was hybridized with a complementary strand with the abasic site (C_3-spacer) in position 4 (a), 3 (b), 2 (c), 1 (d), 0 (e), –1 (f), –2 (g), –3 (h), –4 (i) or lacking an abasic site as full complementary (fc) strand (j).*

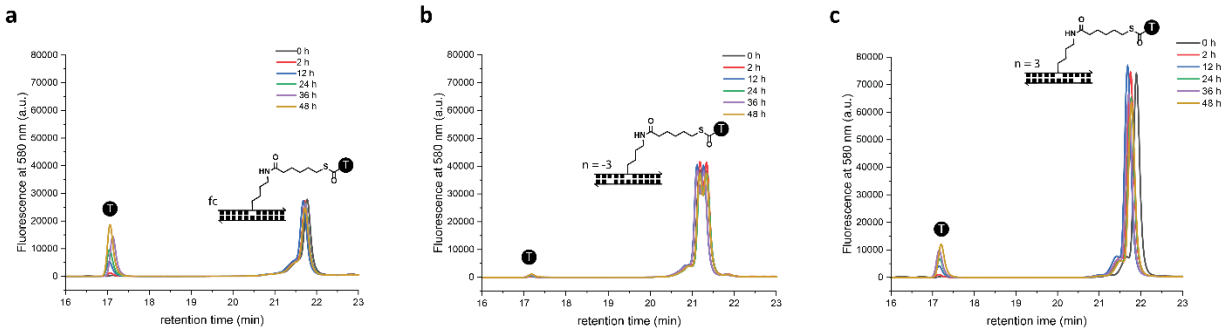


Figure S5. *Hydrolysis of the TAMRA-thioester in presence and absence of an abasic site using an elongated C6-linker. The hydrolysis of the TAMRA-thioester was measured by RP-HPLC time-course (5 °C, pH 11, 0-48 h) after the TAMRA-DNA was hybridized with a complementary strand lacking an abasic site as full match strand (a) and with the abasic site (C3-spacer) in position –3 (b) or 3 (c). Sample concentration was 1 µM, incubation temperature 5 °C and an injection volume of 2 µL.*

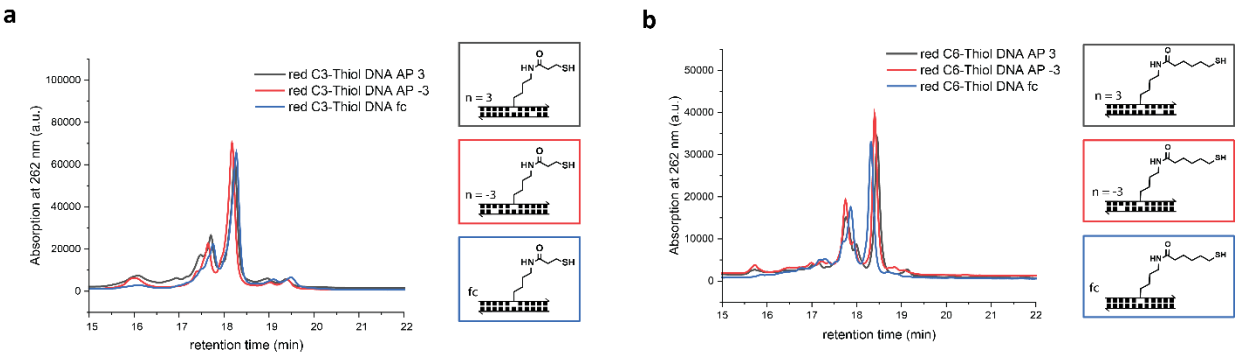


Figure S6. *Comparison of the reduced thiol-linker DNA duplex systems (n = 3, –3 and fc) with a C3- (a) or C6-linker (b) via RP-HPLC (2 µL injection of 1 µM samples) using absorbance channel at 262 nm.*

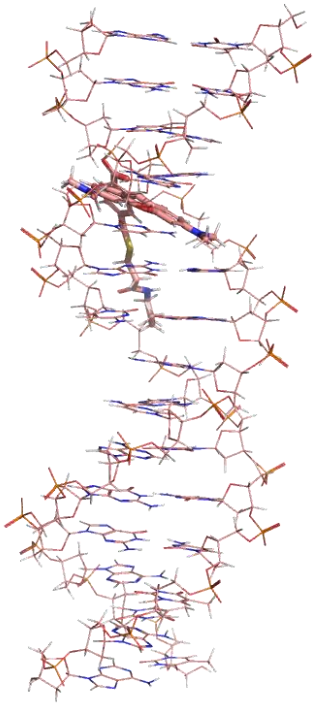


Figure S7. *3D molecular model of the TAMRA-thioester-modified DNA duplex with the acceptor modification in position n = −3. The structure was created in PyMOL using a truncated DNA duplex of 15 base pairs. Insertion of the TAMRA moiety into the hydrophobic core of the duplex at the abasic site was achieved through the manual rotation of bond angles on the linker component. Duplex configuration i.e., base rise, twist (spacing) was automatically configured with built-in forcefield.*

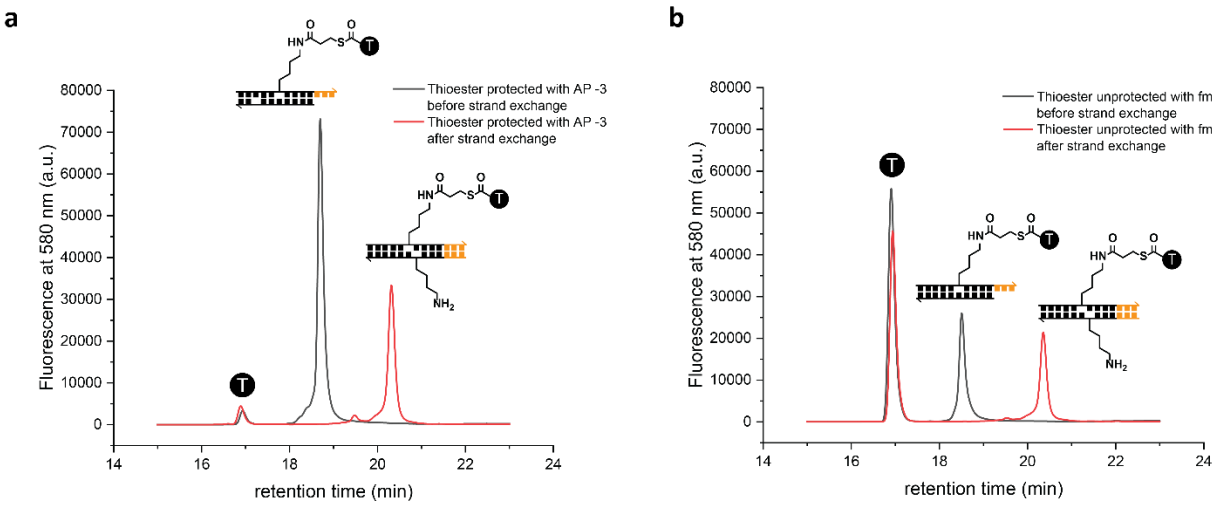


Figure S8. *Triggering DTS reaction by toehold-mediated strand-displacement. Donor adapters bearing the TAMRA-thioester, hybridized to a complementary keeper strand with (a) or without (b) an abasic protective site, were incubated at 5 °C, pH 11 for 48 h before a complementary toehold acceptor adapter bearing the nucleophile (butylamine) at the n = 1 position was added.*

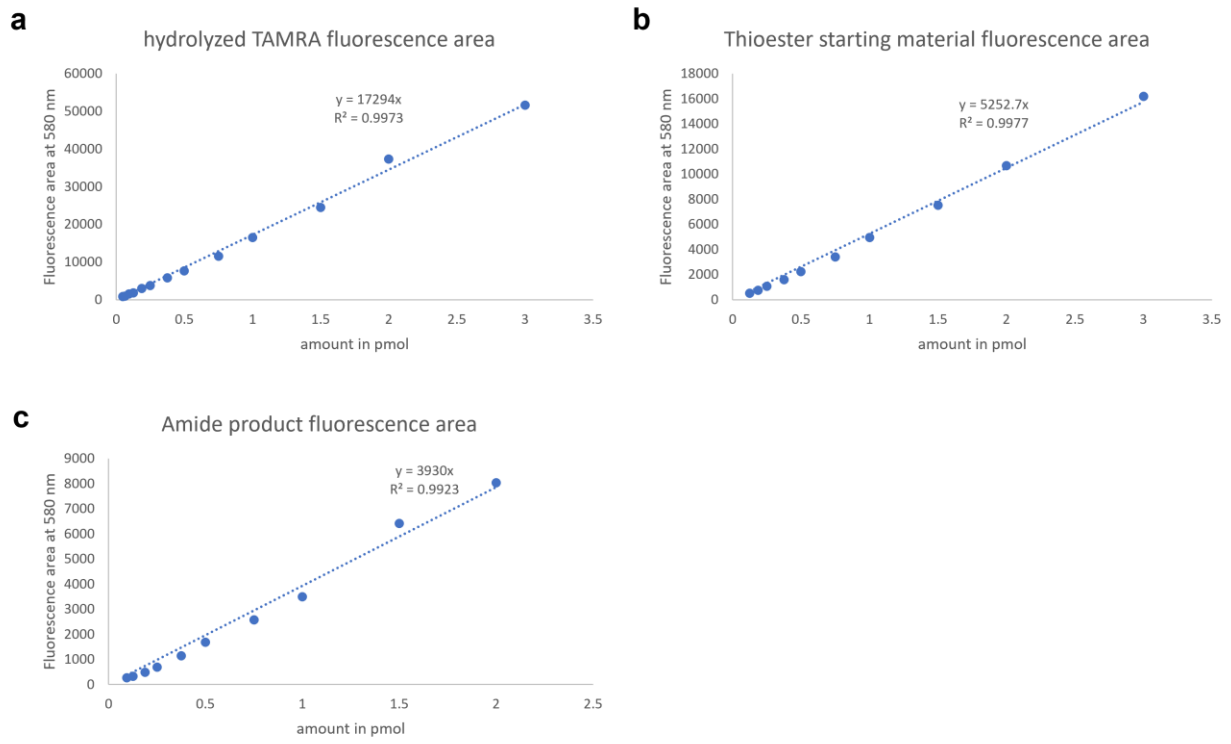


Figure S9. *Calibration curves of the hydrolyzed TAMRA, thioester starting material and amide product. The hydrolyzed TAMRA (a) was obtained by hybridizing the TAMRA-thioester toehold-donor with the toehold-acceptor strand bearing the reactive butylamine in H_2O and $MgCl_2$, as described above, with the addition of the DTS buffer with a final concentration of 1.5 µM. The resulting solution was heated at 95 °C for 20 min, followed by a stepwise cooling procedure to 5 °C over 20 min using the Eppendorf Mastercycler Nexus. The thioester starting material (b) was obtained by hybridizing the TAMRA-thioester toehold-donor with the toehold-acceptor strand bearing the reactive butylamine in H_2O and $MgCl_2$, as described above, without the addition of the DTS buffer with a final concentration of 1.5 µM. The amide product (c) was obtained by hybridizing the reduced thiol-linker toehold-donor with the toehold-acceptor strand bearing the TAMRA-amide in H_2O and $MgCl_2$, as described above, with the addition of the DTS buffer with a final concentration of 1 µM. Calibration curves were obtained by RP-HPLC with an injection volume of 2 µL and stepwise dilution series and signal integration using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm).*

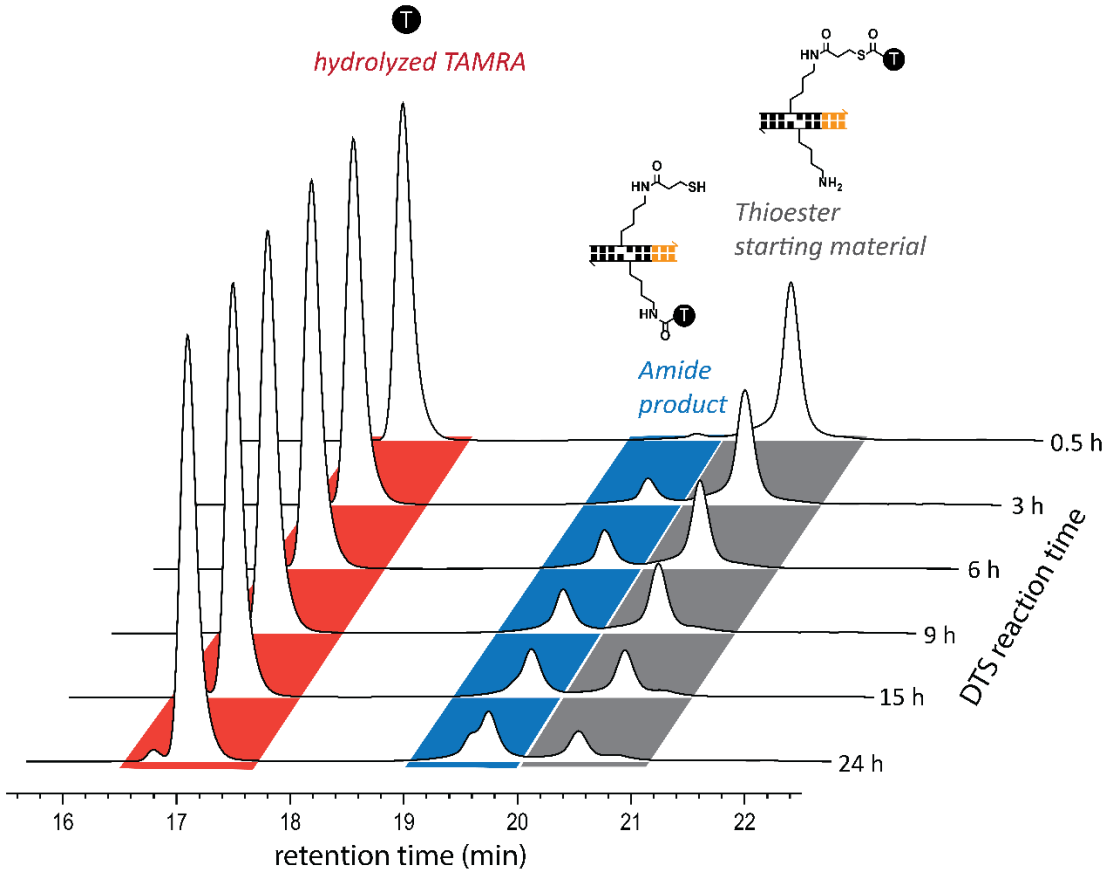


Figure S10. *DTS reaction progress after 48 h of protection stage for the unprotected system. The DTS reaction progress was monitored over 24 h via RP-HPLC using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm).*

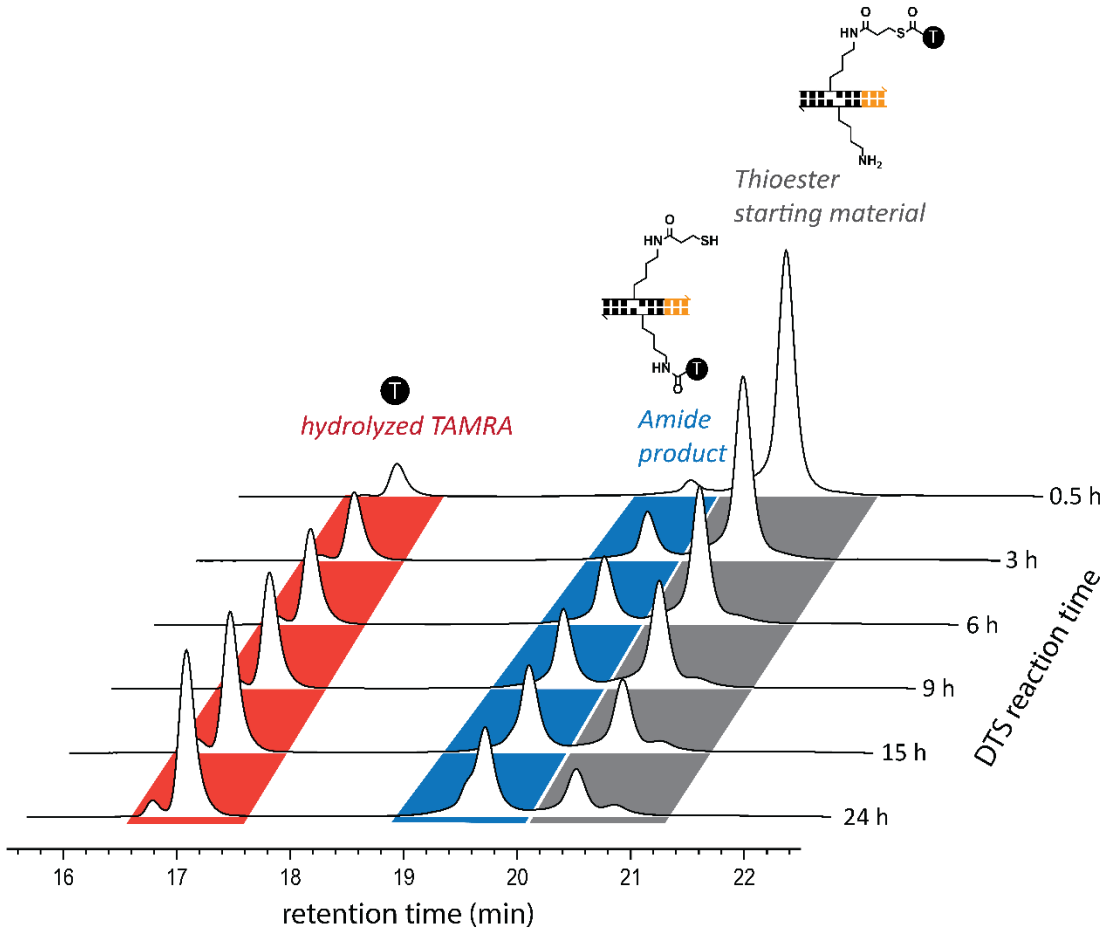


Figure S11. *DTS reaction progress after 48 h of protection stage for the protected system. The DTS reaction progress was monitored over 24 h via RP-HPLC using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm).*

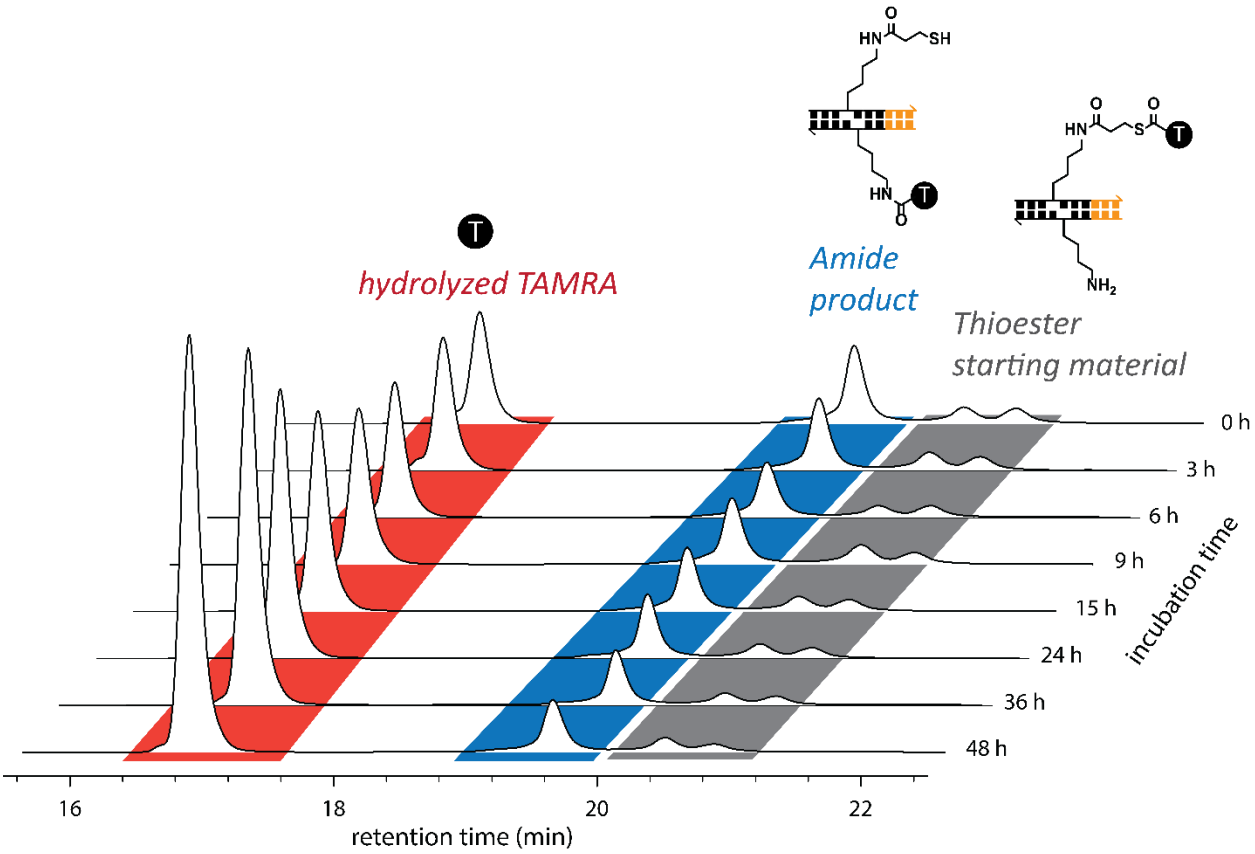


Figure S12. *Impact of DNA-thioester hydrolysis on amide product formation of the unprotected system with varying incubation times during protection stage. DTS reaction progress (15 °C, pH 11, 24 h) was monitored via RP-HPLC using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm) utilizing increasing incubation times during the protection stage from 0 to 48 h. Displayed chromatograms represent one reaction from the performed triplicates.*

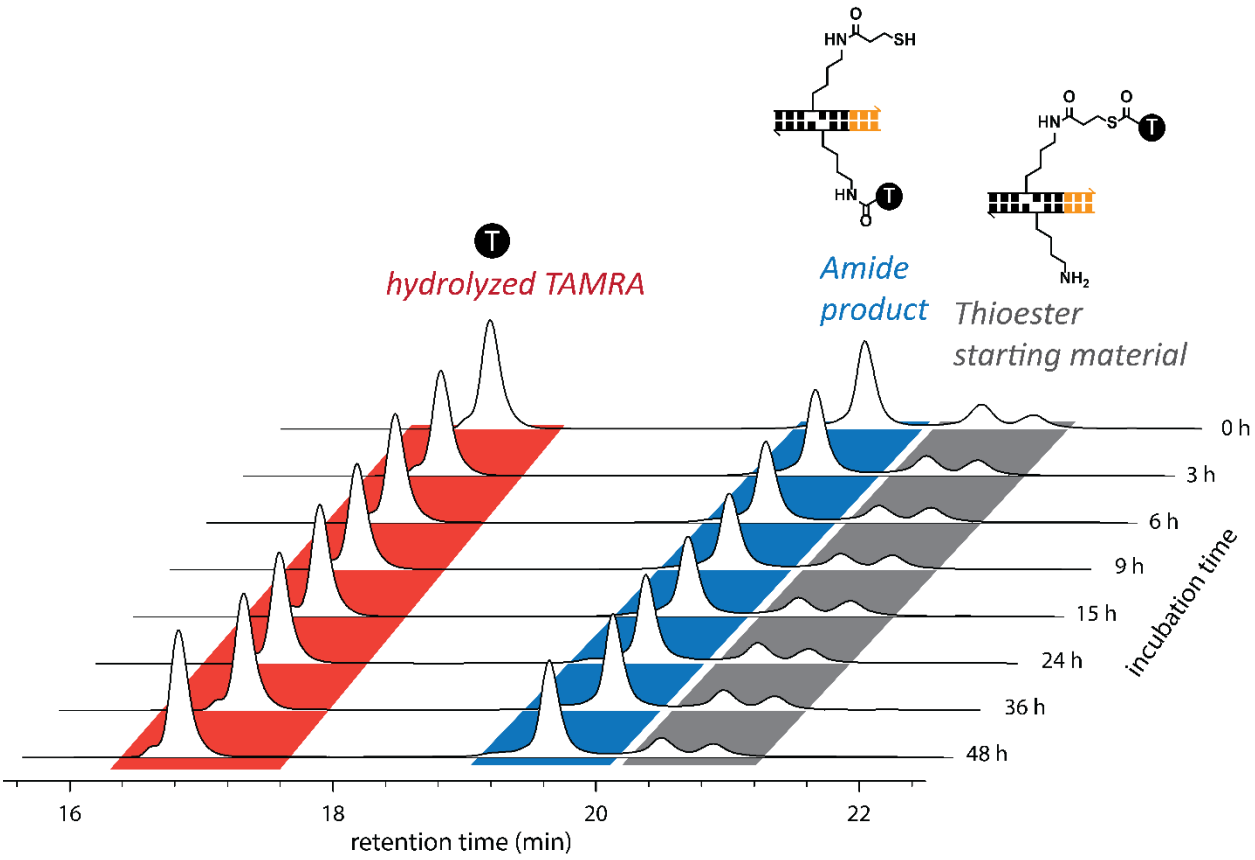


Figure S13. *Impact of DNA-thioester hydrolysis on amide product formation of the protected system with varying incubation times during protection stage. DTS reaction progress (15 °C, pH 11, 24 h) was monitored via RP-HPLC using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm) utilizing increasing incubation times during the protection stage from 0 to 48 h. Displayed chromatograms represent one reaction from the performed triplicates.*

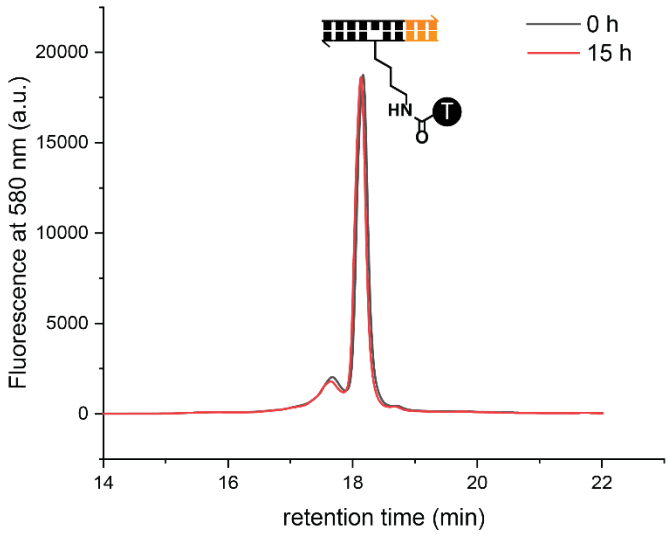


Figure S14. *Stability of amide product at pH 11. The amide product was obtained by hybridizing fully complementary toehold-DNA with the toehold-acceptor strand bearing the TAMRA-amide in H_2O and $MgCl_2$, as described above, with the addition of the DTS buffer with a final concentration of 1 µM. The amide product was incubated at 15 °C for 15 h before analysis via RP-HPLC using the TAMRA fluorescence channel (λ_{ex}:550 nm and λ_{em}:580 nm).*

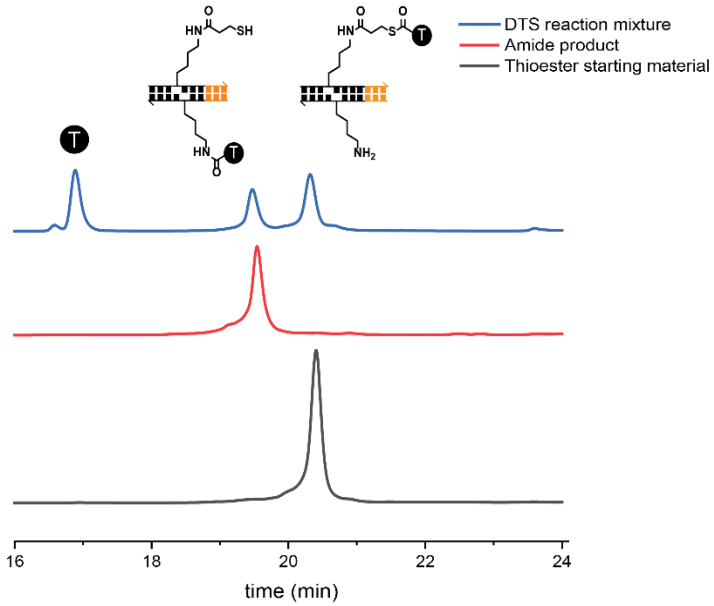
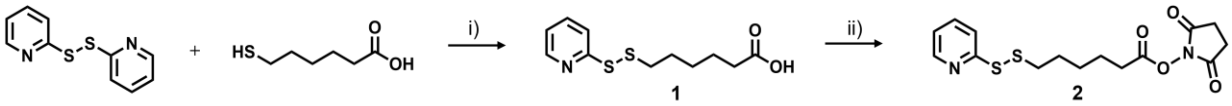


Figure S15. *Identity of the DTS reaction mixture signals. The thioester starting material was obtained by hybridizing the TAMRA-thioester toehold-donor with the toehold-acceptor strand bearing the reactive butylamine in H_2O and $MgCl_2$, as described above, without the addition of the DTS buffer with a final concentration of 1 µM. The amide product was obtained by hybridizing the reduced thiol-linker toehold-donor with the toehold-acceptor strand bearing the TAMRA-amide in H_2O and $MgCl_2$, as described above, with the addition of the DTS buffer with a final concentration of 1 µM.*

Small molecule synthesis



Scheme S1. *Synthesis of succinimidyl 6-(2-pyridyldithio)hexanoate (SPDH). i) 1.5 equiv. 2,2'-dipyridyldisulfide (0.4 g, 1.84 mmol), 1.0 equiv. 6-mercaptohexanoic acid (166 µL, 1.2 mmol), acetic acid (50 µL), ethanol (15 mL), room temperature, 18 h. ii) 1.0 equiv.* ***1*** *(0.3 g, 1.17 mmol), 1.2 equiv. N-hydroxysuccinimide (0.16 g, 1.41 mmol), 1.2 equiv. EDC·HCl (0.27 g, 1.41 mmol), CH_2Cl_2 (3.75 mL), room temperature, 18 h.*

Synthesis of 6-(pyridin-2-yldithio)hexanoic acid (**1**): 2,2'-dipyridyldisulfide (0.4 g, 1.84 mmol) was dissolved in ethanol (10 mL), which was followed by the addition of acetic acid (50 µL). 6-Mercaptohexanoic acid (166 µL, 1.2 mmol) was dissolved in ethanol (5 mL) and the resulting solution was added dropwise over a time-period of 30 min to the 2,2'-dipyridyldisulfide solution containing acetic acid. The resulting reaction mixture was stirred overnight at room temperature. The ethanol solvent was removed under reduced pressure, followed by the isolation of the reaction product **1** by column chromatography using hexane and ethyl acetate as eluent with a gradient of 2:1 hexane/ethyl acetate to 1:1 hexane/ethyl acetate. The product was obtained as a cream white solid with a yield of 63% (0.3 g, 1.17 mmol).

Synthesis of succinimidyl 6-(2-pyridyldithio)hexanoate (**2**): *N*-hydroxysuccinimide (0.16 g, 1.41 mmol) and EDC·HCl (0.27 g, 1.41 mmol) were added sequentially to a solution of 6-(pyridin-2-yldithio)hexanoic acid **1** (0.3 g, 1.17 mmol) in dichloromethane (3.75 mL) at 0 °C. The resulting reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, followed by the isolation of the reaction product **2** by column chromatography using hexane and ethyl acetate as eluent (2:1). The product was obtained as a white powder with a yield of 43% (0.18 g, 0.5 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (ddd, J = 4.8, 1.9, 0.9 Hz, 1H), 7.88 – 7.80 (m, 1H), 7.78 (dt, J = 8.1, 1.1 Hz, 1H), 7.25 (ddd, J = 7.3, 4.8, 1.2 Hz, 1H), 2.86 (t, J = 7.1 Hz, 2H), 2.82 (s, 4H), 2.66 (t, J = 7.2 Hz, 2H), 1.64 (m, 4H), 1.52 – 1.40 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 169.4, 159.9, 150.0, 138.3, 121.6, 119.7, 38.3, 30.5, 28.3, 27.2, 25.9, 24.3.

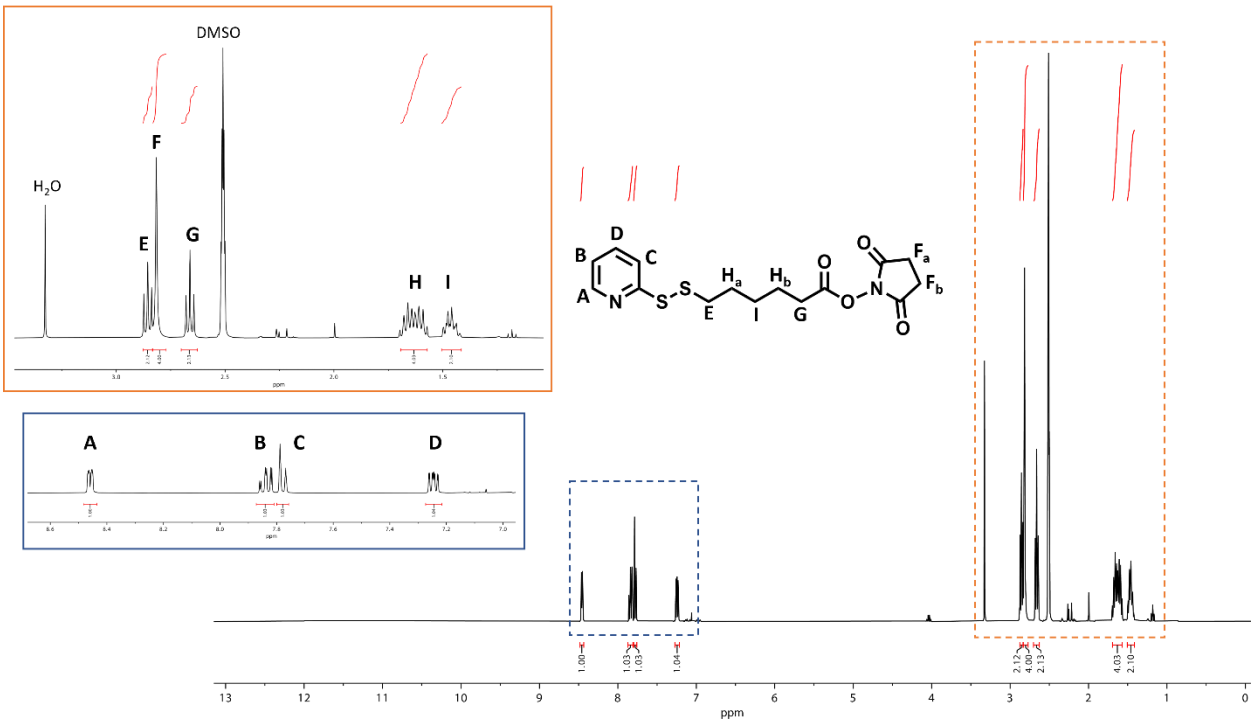


Figure S16. *^{1}H NMR spectrum of succinimidyl 6-(2-pyridyldithio)hexanoate (**2**) (400 MHz, DMSO-d_6).*

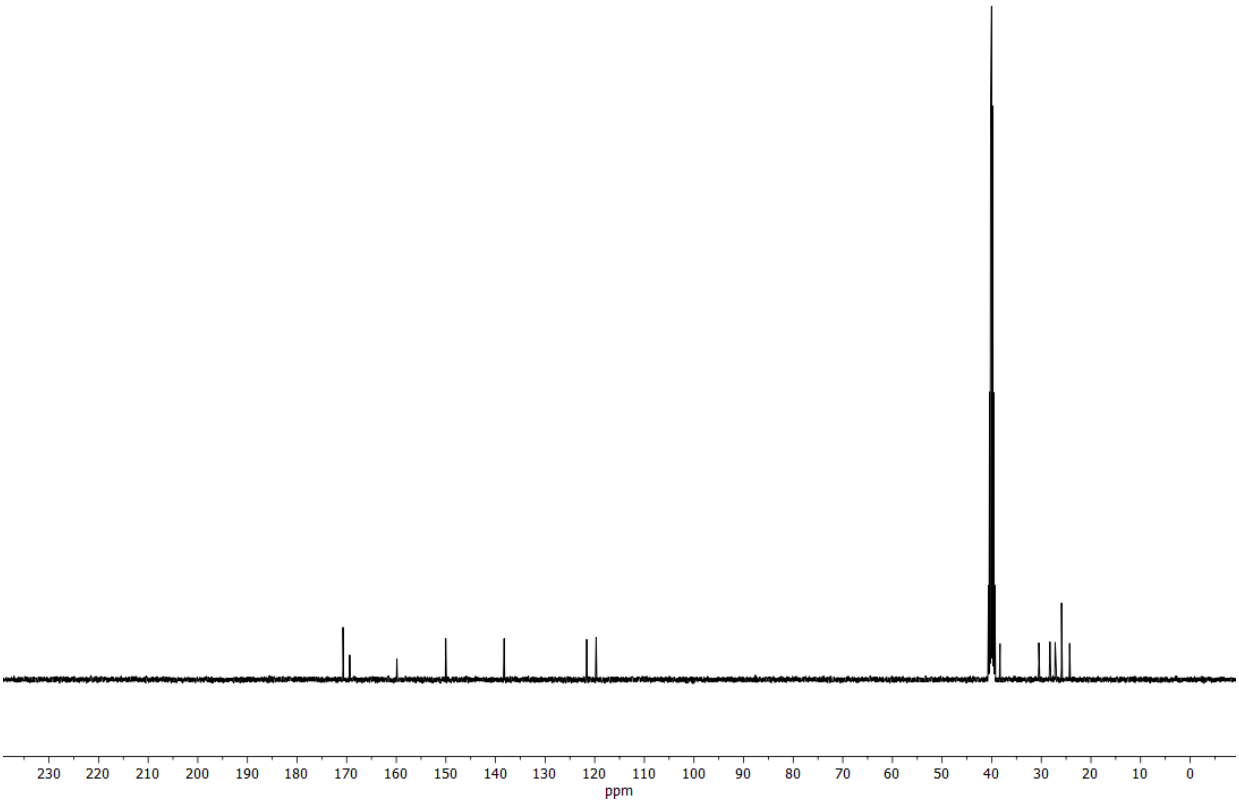


Figure S17. *^{13}C NMR spectrum of succinimidyl 6-(2-pyridyldithio)hexanoate (**2**) (101 MHz, DMSO-d_6).*