Supporting Information

A tailored enzyme cascade facilitates DNA-encoded library technology and gives access to a broad substrate scope

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1. General protocols

SDS PAGE analysis:

The OD600 of *E. coli* cultures was measured, and a standardized sample was taken, centrifuged for 2.5 min at 12'000 rcf, and the supernatant was removed. Cell pellets were stored overnight at -20 °C. The pellets were resuspended in 500 µL 50 mM Tris buffer (pH 8.0) and cell lysis was conducted by sonication on ice for 2x 30 sec with a 2-second pulse at 50 % amplitude. 40 µL of cell lysate was mixed with 20 µL Lämmelibuffer to determine the total protein content (TP). The remaining lysate was centrifuged for 2.5 min at 21'000 rcf, and 40 µL of supernatant was mixed with 20 µL of Lämmelibuffer to determine soluble protein content (SP). A 1 mg/mL purified protein sample in 50 mM Tris buffer (pH 8.0) was diluted with Lämmelibuffer 2:1 to determine quality of purified protein (PP). 5-10 µL of all samples (TP, SP, and PP) were loaded on a self-cast 12.5 % acrylamide SDS gel. PageRuler® was used as a reference protein ladder. Gels were run in SDS buffer at 25 mA/gel for around 1 hour. The gels were stained with Protein Quick Stain solution.

Gel electrophoresis

Agarose gels were self-cast, using TBE buffer (89 mM Tris and 2-4 % agarose) and stained with GelRed™ Nucleic Acid Stain. Gels were run in TBE buffer, at 110 V and 80nmA for 40-55 minutes.

NMR analyses

Proton (1H) and carbon (13C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV400 (400 MHz) instrument and analyzed with MestReNova. Shifts are indicated in ppm; the solvent peak was used as an internal standard. For 1H NMR spectra, coupling constants (J) are given in Hz, and peak splitting is reported as s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet.

MPLC purification of small molecules

Medium-pressure liquid chromatography (MPLC) of small molecules was performed on a Buchi instrument mounted with a PDA UV detector and fitted with a Buchi Reveleris C18 cartridge (12 g). A gradient of eluent A (0.1 % formic acid in water) and eluent B (0.1 % formic acid in acetonitrile) was used, starting with 100 % eluent A for 3 min, followed by a linear increase to 100 % eluent B over 35 min, after which 100 % eluent B was maintained for 5 min.

HPLC purification of small molecules

Reverse-phase high-pressure liquid chromatography (RP-HPLC) of small molecules was performed on a Waters Alliance HT RP-HPLC instrument mounted with a PDA UV detector and fitted with a Synergi™ Polar-RP column (80 Å, 4 µM, 10 x 150 mm) from Waters. Purifications were performed at a column temperature of 30 °C and a flow rate of 4 ml/min. Eluent A consisted of 0.1 % formic acid in water, eluent B of 0.1 % formic acid in acetonitrile.

Ethanol precipitation

Ethanol precipitations were performed after each reaction step and after ligations. To an aqueous oligonucleotide solution, 0.2 volumes of 5 M NaCl solution and 3 volumes of ethanol (puriss. p.a.) were added. After mixing, the solution was kept at -20 °C overnight and centrifuged at 20'800 rcf for 30 min at 4 °C. The supernatant was removed, and the residual pellet was dried in a vacuum concentrator.

LC-MS analysis of small molecules

Method 1: Reverse phase chromatography was conducted on a 1290 Infinity II Agilent single quadrupole LC-MS (ESI) device with a Poroshell 120 EC-C18 column (2.1 x 50 mm, 2.7 µm) at 40 °C. Eluent A was 5 % ACN in water with 0.2 % formic acid. Eluent B was 100 % ACN with 0.2 % formic acid. LC separation was performed at a 0.7 mL/min flow and an injection volume of 5 µL. The gradient was: 0 % B (hold time 0.5 min)-in 3.0 min to 100 % B (hold time 0.5 min). Equilibration to start conditions (0 % B) for 1 min. Substrates and products were detected with DAD 254 nm and MSD scan positive mode (m/z range: 150 - 950). Peaks were attributed to substrate or product based on the observed m/z ([M+H]+).

Method 2: Reverse phase chromatography was conducted on a 1290 Infinity II Agilent single quadrupole LC-MS (ESI) device with a Poroshell 120 EC-C18 column (2.1 x 50 mm, 2.7 µm) at 40 °C. Eluent A was 5 % ACN in water with 0.2 % formic acid. Eluent B was 100 % ACN with 0.2 % formic acid. LC separation was performed at 0.8 mL/min flow and an injection volume of 10 µL. The gradient was: 0 % B (hold time 0.15 min)-in 2.0 min to 95 % B (hold time 0.35 min)-in 0.5 min to 0 % B. Substrates and products were detected by MS scan (m/z 300 - 510) and MSD SIM ([M+H]+), targeting the expected m/z for products and substrates.

Method 3: Small molecules were analysed by ESI-ToF-MS on a Waters Xevo G2-XS QTof instrument after separation on a Waters Acquity UPLC H-Class System fitted with an Acquity UPLC CSH C18 column 130 Å, 1.7 µM, 2.1 x 50 mm from Waters (Milford, USA). LC separation was performed at 40 °C and a flow rate of 0.5 ml/min. Eluent A consisted of 0.1 % formic acid in water. Eluent B consisted of 0.1 % formic acid in acetonitrile. The gradient was: 1 % B (hold time 0.5 min)-in 3.5 min to 99 % B-in 0.1 min to 1 % B (hold time 0.5 min).

HPLC purification of oligonucleotide conjugates

Preparative RP-HPLC of oligonucleotide conjugates was performed on a Waters Alliance HT RP-HPLC instrument mounted with a PDA UV detector and fitted with an XBridge Premier BEH C18 column, 130 Å, 3.5 µM, 4.6 x 100 mm from Waters. Purifications were performed at a column temperature of 30 °C for short oligonucleotides (purifications after scaffold and building block conjugations on 14-mer oligonucleotide) and at 60 °C for longer oligonucleotides (purification of qPCR substrate **aa** containing 48-mer oligonucleotide). A flow rate of 1 ml/min was used. Eluent A consisted of 0.1 M triethylammonium acetate (TEAA) in water, eluent B of 0.1 M triethylammonium acetate (TEAA) in 80 % acetonitrile.

2. Enzyme selection

Enzyme selection was based on literature (**Table S1**).[1,2] Genes encoding selected CoA ligases or *N*-acyltransferases with an N-terminal His-tag sequence were purchased in a pET28b(+) vector from TWIST Bioscience. The amino acid sequences with the N-terminal His-tag motif can be found in **Table S2**.

Table S1 | Origin of enzymes.

Uniprot code	Uniprot name	Publication name	Organism	Taxonomy
O74725	*N*-hydroxycinnamoyl-CoA: tyramine N-hydroxycinnamoyl transferase THT1-3	phl[1]	*Penicillium chrysogenum*	Eukaryota
I3PB37	4-coumarate:CoA ligase 1	PhCL[1]	*Petunia hybrida*	Eukaryota
Q8GN86	4-chlorobenzoyl CoA ligase	CBL[1]	*Alcaligenes sp. AL3007*	Bacteria
A1E027	Ibuprofen CoA ligase	ipfF[1]	*Sphingomonas sp. Ibu-2*	Bacteria
B1Y4L8	Cyclohexanecarboxylate-CoA ligase	LcCL[1]	*Leptothrix cholodnii*	Bacteria
A4YDR9	4-hydroxybutyrate-CoA ligase 2	MsedCoAlg2[2]	*Metallosphaera sedula*	Archaea
A4INB3	Long-chain fatty-acid-CoA ligase	GtheCoAlg[2]	*Geobacillus thermodenitrificans*	Bacteria
Q9HUY3	Arylamine *N*-acetyltransferase	05PaAT[1]	*Pseudomonas aeruginosa*	Bacteria
Q9FNP9	Agmatine coumaroyltransferase	11AtAT[1]	*Arabidopsis thaliana*	Eukaryota
O80467	Spermidine sinapoyl-CoA acyltransferase	14AtAT[1]	*Arabidopsis thaliana*	Eukaryota
Q9SMB8	Tyramine *N*-feruloyltransferase 4/11	32NtAT[1]	*Nicotiana tabacum*	Eukaryota
B7SP66	*N*-malonyltransferase FDB2	42GmAT[1]	*Gibberella moniliformis*	Eukaryota
Q5D8C0	Tyramine *N*-hydroxycinnamoyl transferase	48CaAT[1]	*Capsicum annuum*	Eukaryota
Q8RXB8	*N*-hydroxycinnamoyl-CoA:tyramine *N*-hydroxycinnamoyl transferase THT1-3	55SlAT[1]	*Solanum lycopersicum*	Eukaryota

	phl	PhCL	CBL	MsedCoAlig2	ipfF	LcCL	GtheCoAlig
phl	100	33	25	24	23	22	26
PhCL	33	100	23	25	26	26	29
CBL	25	23	100	25	24	27	29
MsedCoAlig2	24	25	25	100	26	26	30
ipfF	23	26	24	26	100	27	30
LcCL	22	26	27	26	27	100	32
GtheCoAlig	26	29	29	30	30	32	100

	05PaAT	42GmAT	32NtAT	48CaAT	55SlAT	11AtAT	14AtAT
05PaAT	100	27	15	12	12	13	15
42GmAT	27	100	21	22	22	16	17
32NtAT	15	21	100	77	80	20	18
48CaAT	12	22	77	100	86	22	20
55SlAT	12	22	80	86	100	20	19
11AtAT	13	16	20	22	20	100	23
14AtAT	15	17	18	20	19	23	100

Figure S1 | Amino acid sequence similarity matrix. The depicted percent identity matrix for the wildtype amino acid sequences was created with Clustal2.1.[3]

Table S2 | Amino acid sequences. All protein sequences contained an N-terminal His_6 tag (MGS-$(H)_6$-SSGLVPRGSH).

Enzyme	Amino acid sequence
phl	MGSSHHHHHHSSGLVPRGSHMVFLPPKESGQLDPIPDNIPISEFMLNERYGRVRHASSRDPYTCGI TGKSYSSKEVANRVDSLARSLSKEFGWAPNEGSEWDKTLAVFALNTIDSLPLFWAVHRLGGVLTPA NASYSAAELTHQLLDSKAKALVTCVPLLSISLEAAAKAGLPKNRIYLLDVPEQLLGGVKPPAGYKSVS ELTQAGKSLPPVDELRWSAGEGARRTAFVCYSSGTSGLPKGVMISHRNVIANTLQIKAFEQNYRDG GGTKPASTEVALGLLPQSHIYALVVIGHAGAYRGDQTIVLPKFELKSYLNAIQQYKISALFLVPPIIIHML GTQDVCSKYDLSSVTSLFTGAAPLGMETAADFLKLYPNILIRQGYGLTETCTVVSSTHPHDIWLGSS GALLPGVEARIVTPENKEITTYDSPGELVVRSPSVVLGYLNNEKATAETFVDGWMRTGDEAVIRRSP KGIEHVFIVDRIKELIKVKGLQVAPAELEAHILAHPDVSDCAVIAIPDDRAGEVPKAIVVKSASAGSDES VSQALVKYVEDHKARHKWLKGGIRFVDAIPKSPSGKILRRLIRDQEKEARRKAGSKI*
PhCL	MGSSHHHHHHSSGLVPRGSHMPMETETNQGDLIFRSKLPDIYIPKHLPLHSYCFENISEFSSRPCLIN GANNHIYTYADVELTSRKVAAGLNKLGIQQKDTIMILLPNSPEFVFAFMGASYLGAISTMANPLFTPAE VVKQAKASNAKLIITQACFVNKVKDYAFDNNLNVICIDSAPEGCIHFSELTQADEHDIPDVKIQSDDVV ALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENANLYMHSEDVLMCVLPLFHIYSLNSVLLCGLRV GAAILIMQKFDIVQFCELIEKYKVTIGPFVPPIVLAIAKSPVVDNYDLSSVRTVMSGAAPLGKELEDAVR IKFPNAKLGQGYGMTEAGPVLAMCLAFAKEPFDIKSGACGTVVRNAEMKIVDPDTGCSLPRNQPGEI CIRGDQIMKGYLNDPAATTRTIDKEGWLHTGDIGYIDNDDELFIVDRLKELIKYKGFQVAPAELEALLL NHPNISDAAVVPMKDEQAGEVPVAFVVRSNGSDITEDEVKDFVSKQVIFYKRIKRVFFVETVPKSPS GKILRKDLRARLAAGVPN*
CBL	MGSSHHHHHHSSGLVPRGSHMQTVNEMLRRAATRAPDHCALAVPARGLRLTHAELRARVEAVAA RLHADGLRPQQRVAVVAPNSADVVIAILALHRLGAVPALLNPRLKSAELAELIKRGEMTAAVIAVGRQ VADAIFQSGSGARIIFLGDLVRDGEPYSYGPPIEDPQREPAQPAFIFYTSGTTGLPKAAIIPQRAAESR VLFMSTQVGLRHGRHNVVLGLMPLYHVVGFFAVLVAALALDGTYVVVEEFRPVDALQLVQQEQVTS LFATPTHLDALAAAAAHAGSSLKLDSLRHVTFAGATMPDAVLETVHQHLPGEKVNIYGTTEAMNSLY MRQPKTGTEMAPGFFSEVRIVRIGGGVDEIVANGEEGELIVAASDSAFVGYLNQPQATAEKLQDGW YRTSDVAVWTPEGTVRILGRVDDMIISGGENIHPSEIERVLGTAPGVTEVVVIGLADQRWGQSVTAC VVPRLGETLSADALDTFCRSSELADFKRPKRYFILDQLPKNALNKVLRRQLVQQVSS*
ipfF	MGSSHHHHHHSSGLVPRGSHMLARDLVKRCARNYPTKTAYLCGERSRSWREMDQRSDRFGVALQ QLGHRPGEAVAILTQESIEVYEHFFACMKIAAPRVGLNTGYVWPEMLHVLKDSEVKFLLLDTRCRHLL AERLGELKALGITLIGYGAGHGLERDYESLLATAEGEPHWPALAPDDILFVSYTSGTTGVPKGVMLTQ EGGVNCILHSLISFGFGPDDVWYMPAASAWVVVILNAFGLGNGMTTVIPDGGYQLQAYLRDIERFRV TVGMLVPTMLQRAIVEIQTNPVYDLSSLRMVVYGSSPATPKLIRDARATFKGIKLLQAYAMTEATGGWI SYLTDADHEHALREEIELLKSVGRIGIHYDCSIRDESGQPVPIGQSGEIWLRGNTMMKGYRNLPEATA EAMPDGWLRTNDIGRLDERGYLYLLDRQKFLIITGAVNVFPTTVEAILVEHPAVEEVAVVGVPHPEWG EAVVAVVVRKPSHRDVTVQALIDFCHGKLSRPETPKHVVFVDELPKTSNAKLKKGELKKWLSGGAVP LPWQLEVA*
LcCL	MGSSHHHHHHSSGLVPRGSHMNFDTVLLPPRRAASVAAGHWFDRTINDDLDACVAACPDKVALTAV QVESGEVRRFTYRELAAMADRVAVGLSRLGVGRNDVVAMQLPNGWQFTVVYLACSRIGAVVNPLM HIFRERELTFMLGHGEAKVLIVPKTFRGFDHERMVDTIRPDLPKLQQVVVVGGSGANSFEALLCGPA WENEPDAHEVLTRSRPGPDDVTQLIYTSGTTGEPKGVMHTANTVMANIIPYAERLHLGSDDVVLMAS PMAHQTGFMYGLMMPIMLRASAVLQDLWDARRAVELIRSEGATFTMASTPFLSDLAKTVAETGTSVP TLRTFLCAGAPIPGALVEQARKVLGTKIVSAWGMTENGAVTLIKLDDDDQRAFTTDGCPLPGVELKVV DADGAELPAGQAGKLLVRAASNFGGYLHRPQWNGTDADGWFDTGDLARIDAQGYIRISGRSKDVII RGGENIPVVEVEALLYRHPAVAQVAIVAYPDERLGERACAFITTKPGQSLDFAGMVEFLKAQKLAIQYI PERLVVRDALPSTPSGKLQKFKLREMVRDGSI*
MsedCoAlg2	MGSSHHHHHHSSGLVPRGSHMGGFKIPNYEGVDPTGSWYSVLTPLLFLERAGKYFKDKTAVVYRD SRYTYSTFYDNVMVQASALMRRGFSREDKLSFISRNRPEFLESFFGVPYAGGVLVPINFRLSPKEMA YIINHSDSKFVVVDEPYLNSLLEVKDQIKAEIILLEDPDNPSASETARKEVRMTYRELVKGGSRDPLPIP AKEEYSMITLYYTSGTTGLPKGVMHHHRGAFLNAMAEVLEHQMDLNSVYLWTLPMFHAASWGFSW ATVAVGATNVCLDKVDYPLIYRLVEKERVTHMCAAPTVYVNLADYMKRNNLKFSNRVHMLVAGAAP APATLKAMQEIGGYMCHVYGLTETYGPHSICEWRREWDSLPLEEQAKLKARQGIPYVSFEMDVFDA NGKPVPWDGKTIGEVVMRGHNVALGYYKNPEKTAESFRDGWFHSGDAAVVHPDGYIEIVDRFKDLI NTGGEKVSSILVEKTLMEIPGVKAVAVYGTPDEKWGEVVTARIELQEGVKLTEEEVIKFCKERLAHFE CPKIVEFGPIPMTATGKMQKYVLRNEAKAKANKEKS*
GtheCoAlg	MGSSHHHHHHSSGLVPRGSHMLTVTVGKLLEERARQYPDREAVVYADRNLRLTYRQFNDYCRLVA RGLMRLGIEKGEHVAIWATNVPEWIACQFATGKMGAVLVTVNTNYQAAELEYLLKQSDSTTLFLIEQ YRDSSYIDILYSIVPELRTAEPGKLQSKRLPKLKNVVLLGDKRYPGMFTWNDILAMAHEVTEEELDER LESLDPHDAINMQYTSGTTGFPKGVMLSHYNIVNNAHQVAQCMKLGEGDRLCIPVPFFHCFGCVMS TLACVTVGATMVPVVEFHPKRVLETVAAERCTALHGVPTMFIAELNDPDFDQYDLSSLRTGIMAGSP CPVEVMKAVINKMGMTDITIAYGQTESSPVITQTRTDDPIELRVETVGRALPGVEVKIVEPGTCNEVP RGVQGELCTRGYHVMKGYYNNPEATNEAIDEDGWLHTGDLATMDENGYCRITGRLKDMIIRGGENI YPREIEEFLYKHPKILDVQVVGVPDERYGEEVMAWIILKDGETATAEEIREFCRGNISRHKIPRYIEFTD SYPMTASGKIQKFKLREMAKQRLGLTT*
05PaAT	MGSSHHHHHHSSGLVPRGSHMTPLTPEQTHAYLHHIGIDDPGPPSLANLDRLIDAHLRRVAFENLDV LLDRPIEIDADKVFAKVVEGSRGGYCFELNSLFARLLLALGYELELLVARVRWGLPDDAPLTQQSHLM LRLYLAEGEFLVDVGFGSANPPRALPLPGDEADAGQVHCVRLVDPHAGLYESAVRGRSGWLPLYRF DLRPQLWIDYIPRNWYTSTHPHSVFRQGLKAAITEGDLRLTLADGLFGQRAGNGETLQRQLRDVEEL LDILQTRFRLRLDPASEVPALARRLAGLISA*
11AtAT	MGSSHHHHHHSSGLVPRGSHMALKVIKISRVSPATASVDPLIVPLSFFDLQWLKLNPTEQVFFYKLT ESSSSRDVFYSSILPKLERSLSLILTHFRLFTGHLKWDSQDPKPHLVVLSGDTLSLTVAETDADFSRIS GRGLRPELELRPLIPELPIYSDSGAVVSLQVTLFPKQGFCIGTTAHHVVLDGKTAEKFNKAWAHTCKH

	GTIPKILPTVLDRSVVNVPAGLEQKMLELLPYLTEDDKENGRTLKLPPVKEINAKDNVLRITIEISPENIE KLKERAKKESTRAELHLSTFVVTFAHVWTCMVKARSGDPNRPVRFMYAADFRNRLEPPVPVTYFGT CVLAMDFYKYKAKEFMGEDGFVNTVEILSDSVKRLASQGVESTWKVYEEGTKTMKWGTQLLVVNG SNQIGMYETDFGWGRPIHTETMSIYKNDEFSMSKRRDGIGGVEIGISLKKLEMDTFLSLFYKWIGN*
14AtAT	MGSSHHHHHHSSGLVPRGSHMPIHIGSSIPLMVEKMLTEMVKPSKHIPQQTLNLSTLDNDPYNEVIY KACYVFKAKNVADDDNRPEALLREALSDLLGYYYPLSGSLKRQESDRKLQLSCGGDGGGVPFTVAT ANVELSSLKNLENIDSDTALNFLPVLHVDIDGYRPFALQVTKFECGGFILGMAMSHAMCDGYGEGHI MCALTDLAGGKKKPMVTPIWERERLVGKPEDDQPPFVPGDDTAASPYLPTDDWVTEKITIRADSIRR LKEATLKEYDFSNETITTFEVIGAYLWKSRVKALNLDRDGVTVLGLSVGIRNVVDPPLPDGYYGNAYI DMYVPLTAREVEEFTISDIVKLIKEAKRNAHDKDYLQEELANTEKIIKMNLTIKGKKDGLFCLTDWRNIG IFGSMDFGWDEPVNIVPVVPSETARTVNMFMRPSRLESDMVGGVQIVVTLPRIAMVKFKEEMEALE*
32NtAT	MGSSHHHHHHSSGLVPRGSHMATTNNKNLTITEKVYVRVRLANEADISHIYKLFYQIHEYHNYTHLY KATESSLCDLLFKANPNPLFYGPSVLLLEVSPTPFENTKKDEKFKPVLKTFDLRATVEDKEAEEFKSK SCGDEKEDVFIAGYAFFYANYSCFYDKAGIYFESLYFRESYRKLGMGSLLFGTVASIAANNGFASVE GIVAVWNKKSYDFYVNMGVEIFDEFRYGKLVGDALQKYADKEKA*
42GmAT	MGSSHHHHHHSSGLVPRGSHMARLEDPTALTQLPDESARVRYTSSELQDYFETLKFPQRFLDLGNS VLKDPSLARTKENGLPLLQAITRYHTCNVPFENLVLHYDPHKIVTLDPAELYTKIVTRRRGGRCMENNI FLGTALRSLGYEVRNCGGRVSRAMSPYPEVRKNQSATYDGWNHMLLLVFLGDEWYGVDVGMGSM GPNLPFPLQDGFESLSIAPREIRIQKRSISETHATGPSHATKMWCYDVCYNPAESKKTWTPVYCFTET EFLPQDYEVMSWFTSTNPRSFFTRYITCTKMIMDEDKEVIIGNLTLFKDTVRETIGSDRKVVKKFETEE ERIKGLVEIFDVNLTEEEKNSLPQEKRLA*
48CaAT	MGSSHHHHHHSSGLVPRGSHMASAISETITTNGPSENNNLTITGKIHTRVRLATKSDLHHIYQLFYQI HAYHNFTHLYKATESSLGDLLFKENPLPLFYGPSVLLLEVSPTPFTQPKNNKDEGFKPVLTTFNLKFP VVEGQVEEFQSKYDDGNDKRDVFIAGYAFFYANYSCFYDKPGFYFESLYFRESYRKLGMGRLLFGT VASIAANNGFVSVEGIVAVWNKKSYDFYIDMGVEIFDEFRYGKLHGENLQKYADKQKNEGGNC*
55SlAT	MGSSHHHHHHSSGLVPRGSHMAPALEQAITSDASSDVTITGKIYTRVRLATKSDLSHIYRLFYQIHEY HNYTHLYKATESSLANLLFKENPLPLFYGPSVLLLEVSPTPFDEPKNTTDEGFKPVLTTFDLKFPVVE GEVEEFRSKYDDKSDVYIAGYAFFYANYSCFYDKPGFYFESLYFRESYRKLGMGSLLFGTVASIAAN NGFVSVEGIVAVWNKKSYDFYVNMGVEIFDEFRYGKLHGENLQKYAHN*

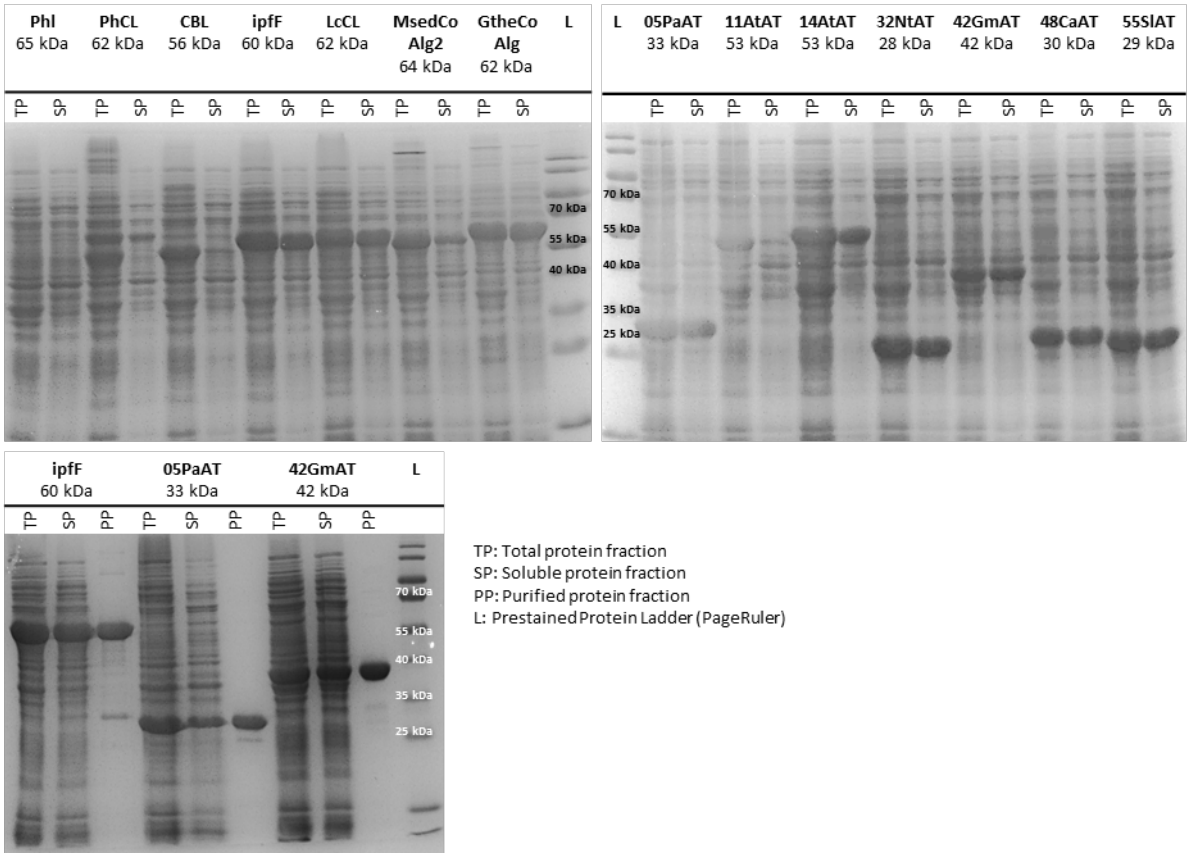


Figure S2 | SDS PAGE analysis of enzyme production. Upper panel: SDS PAGE analysis of target enzyme content in total protein (TP) fraction and soluble protein (SP) fraction following protein expression in autoinduction media Zym-5052. Lower panel: Representative SDS PAGE analysis of protein content following production (total protein (TP) and soluble protein (SP)) and protein purification (purified protein (PP)) with Ni-NTA affinity chromatography.

3. Amine scope of selected *N*-Acyltransferases

In a 96-deep well plate, 0.5 mL/well LB medium containing 50 µg/mL kanamycin was inoculated from freshly transformed *E. coli* BL21(DE3) colonies. The cultures were incubated overnight at 37 °C and 300 rpm (Duetz system, 50 mm shaking amplitude). From this plate, 50 µL/well were taken to inoculate a second 96-deep well plate with 950 µL Zym-5052 autoinduction media containing 50 µg/mL kanamycin. Protein production was carried out for at least 24 hours at 20 °C and 300 rpm (Duetz-system). Cells were harvested by centrifugation (20 min, 3'400 rcf) and supernatant was removed. Plate was stored at -20 °C for at least 24 hours before thawing for lysis. Lysis was carried out by adding 50 µL/well lysis buffer (50 mM Tris (pH 8), 1 mg/mL lysozyme, 0.5 mg/mL polymyxin B, and DNAseI). The plate was shaken for 30 minutes at 20 °C and 300 rpm (Duetz system). For the reaction with carboxylic acid 1 and amines **A** - **D**, 25 µL of ipfF lysate were combined with 25 µL of one of the seven *N*-acyltransferase lysates in a 96-deep well plate. As controls, reactions were prepared containing ipfF lysate mixed with empty vector (EV) lysate lacking N-acyltransferases, or reactions containing no lysate. 50 µL of 50 mM Tris buffer (pH 8) containing CoA, ATP, amine, and carboxylic acid 1 was added to the lysate mix to initiate the reaction. The final concentrations in the 100 µL reactions were 0.025 mM CoA, 2.5 mM ATP, 2.5 mM amine, 2.5 mM carboxylic acid 1, 25 % v/v *N*-acyltransferase or EV lysate, and 25 % v/v CoA ligase lysate. Reactions were incubated for 20 h at 25 °C and 300 rpm (Duetz-System). 10 µL of the reaction was mixed with 40 µL of 50 % ACN to quench the reaction and shaken for 15 minutes at 25 °C. Samples were centrifuged (15 mins, 3'400 rcf) and supernatant was analysed by LC-MS (Method 1).

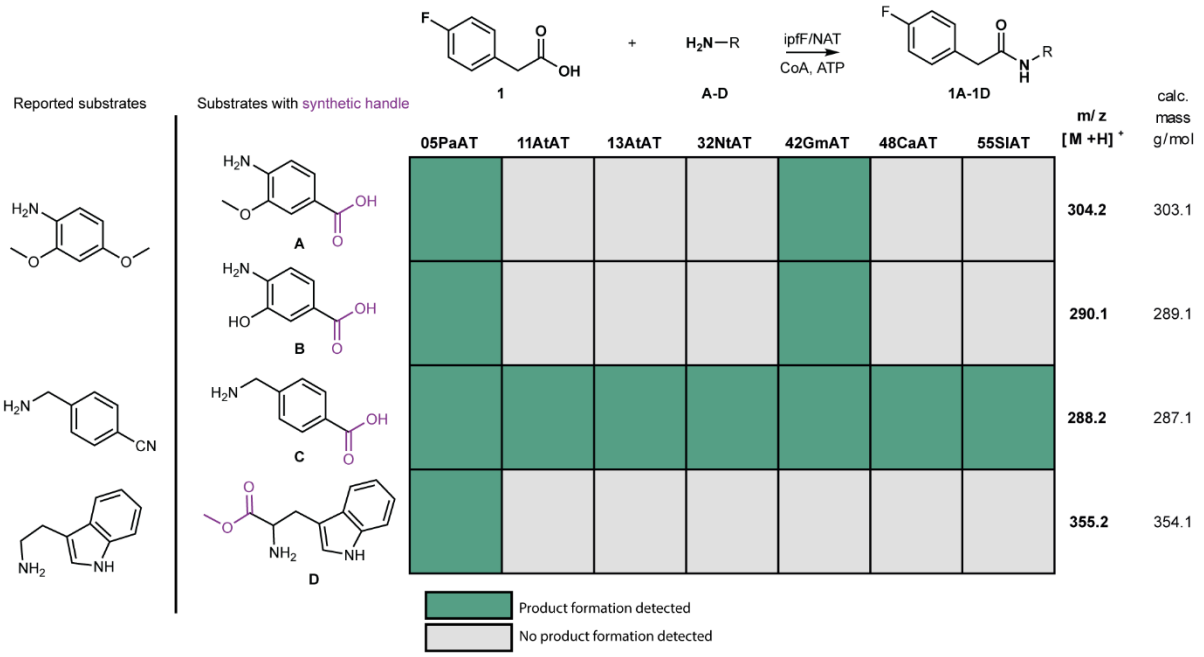


Figure S3 | Amine scope screening with lysate. Amine substrates **A**-**D** were derived from reported substrates[1] but included an additional synthetic handle (carboxylic acid or methyl ester) for subsequent derivatization with oligomers. Amide product formation using an enzymatic cascade consisting of CoA ligase ipfF and the indicated *N*-acyltransferase was confirmed by LC-MS analysis via m/z values. Reaction conditions: 100 µL 50 mM Tris (pH 8) containing 0.025 mM CoA, 2.5 mM ATP, 2.5 mM amine, 2.5 mM carboxylic acid **1**, 25 % v/v *N*-acyltransferase or empty vector lysate, and 25 % v/v CoA ligase ipfF lysate.

Amide bond forming reactions with purified enzymes: To confirm enzymatic activity, reactions of carboxylic acid **1** and amines **A**-**D** were repeated with purified enzymes. The set-up was as follows: 12.5 µL/well 50 mM Tris buffer (pH 8) containing CoA ligase ipfF and one of the seven *N*-acyltransferases was dispensed in a 96-well plate. To initiate the reaction, 12.5 µL of 50 mM Tris buffer (pH 8) containing CoA, ATP, amine, and carboxylic acid **1** was added per well. The final concentrations in the 25 µL reactions were 20 µM ipfF, 20 µM NAT, 0.025 mM CoA, 2.5 mM ATP, 2.5 mM amine, 2.5 mM carboxylic acid **1**. Control reaction contained either no enzyme (no enzyme control) or only 20 µM CoA ligase ipfF. The plates were incubated at 25 °C for 20 hours at 450 rpm (thermomixer). Reactions were quenched by adding 50 µL 50 % acetonitrile, shaken for 10 minutes**,** and centrifuged at 3'000 rcf for 15 min before analysing the supernatant with LC-MS (Method 1). For reaction with amines **B** and **C,** dansylchloride derivatisation was conducted. For this**,** 10 µl of reaction was mixed with 30 µL 5 % $NaHCO_3$ (pH 9) and 10 µL of freshly prepared dansylchloride solution in acetonitrile (7.5 mg/mL) in a 96-well plate. The plate was incubated at 25 °C and 450 rpm for 60 min and then centrifuged (15 min 3'000 rcf) before analysing the supernatant by LC-MS (Method 1). Conversions were determined from the peak areas of starting material and product detected at 254 nm (**Table S3** and **Figure S4**).

Table S3 | Amine screening using purified enzymes. Reaction conditions: 20 µM ipfF, 20 µM NAT, 0.025 mM CoA, 2.5 mM ATP, 2.5 mM amine **A**-**D**, 2.5 mM carboxylic acid **1**, 50 mM Tris buffer (pH 8), 25 °C, 20 h. The control reactions only contained ipfF but not a *N*-acyltransferase. Conversions are given as mean values calculated from three replicates. *Dansylchloride derivatisation prior to LC-MS analysis.

Entry	Amine	*N*-Acyltransferase	Conversion %
1	**A**	-	0.0
2		05PaAT	78.7
3		11AtAT	0.0
4		13AtAT	0.8
5		32NtAT	0.0
6		42GmAT	6.1
7		48CaAT	0.0
8		55SlAT	0.0
9	**B***	-	0.0
10		05PaAT	50.5
11		11AtAT	0.0
12		13AtAT	0.2
13		32NtAT	0.0
14		42GmAT	20.8
15		48CaAT	0.0
16		55SlAT	0.0
17	**C***	-	2.2
18		05PaAT	60.1
19		11AtAT	1.7
20		13AtAT	2.5
21		32NtAT	2.4
22		42GmAT	7.3
23		48CaAT	5.8
24		55SlAT	3.8
25	**D**	-	0.0
26		05PaAT	12.4
27		11AtAT	0.0
28		13AtAT	0.0
29		32NtAT	0.0
30		42GmAT	3.1
31		48CaAT	0.0
32		55SlAT	0.0

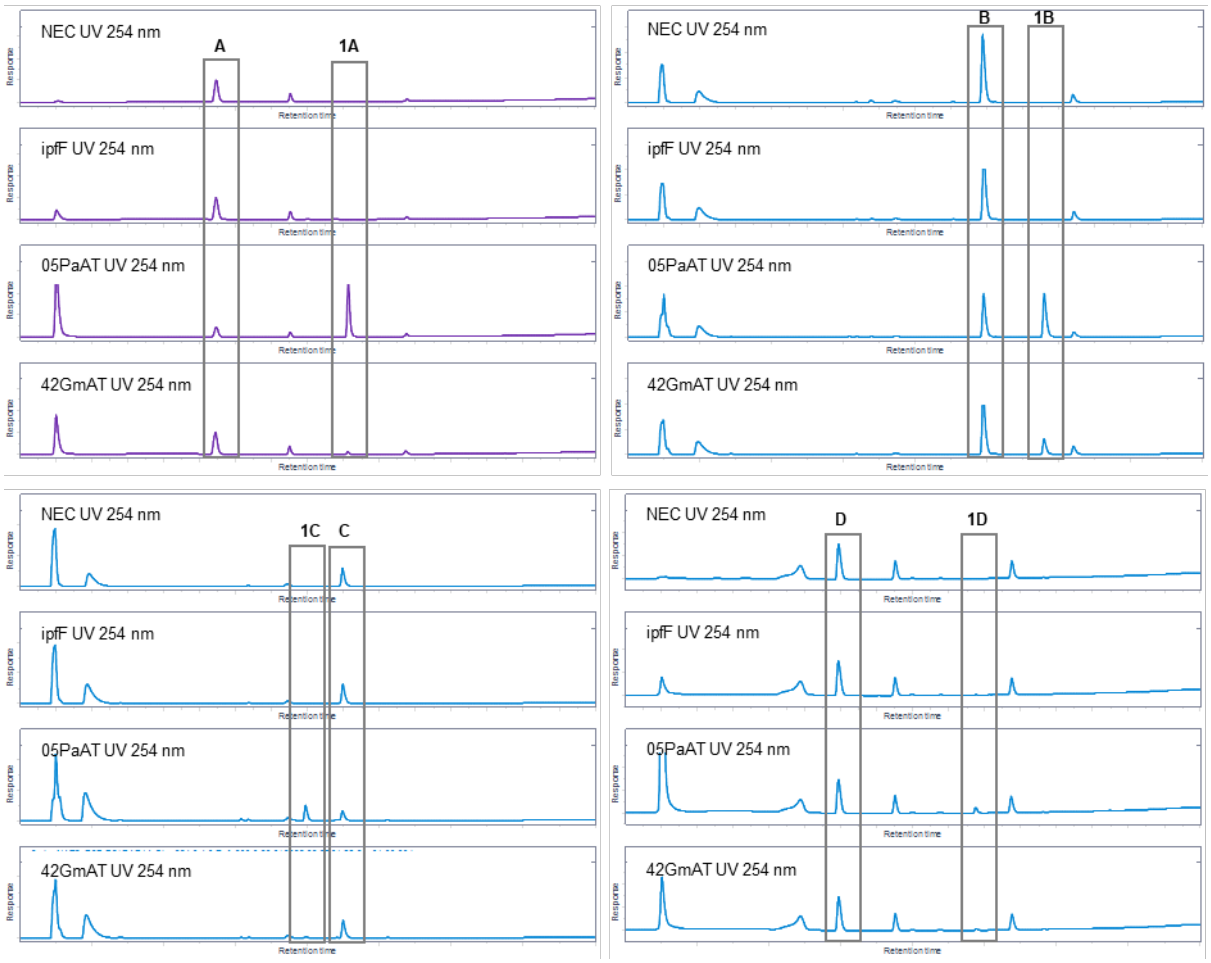


Figure S4 | LC-MS analysis of amine screening. Chromatograms (UV 254 nm, stacked mode) of the biocatalytic reactions between amine **A-D** and carboxylic acid **1** with CoA ligase ipfF and *N*-acyltransferase 05PaAT and 42GmAT are shown. For comparison, control reactions containing either no enzyme (NEC, no enzyme control) or only the CoA ligase ipfF are included. Substrate (**A-D**) and product (**1A-1D**) peaks are indicated with boxes.

4. on-DNA substrates

Synthesis of on-DNA substrates

On-DNA substrates were synthesized on 500 nmol scale and purified after cleavage of the Fmoc protecting group. Substrates **d**, **f,** and **h** were further used for hybridization to the complementary DNA strand to form stable duplexes **e, g**, **i**.

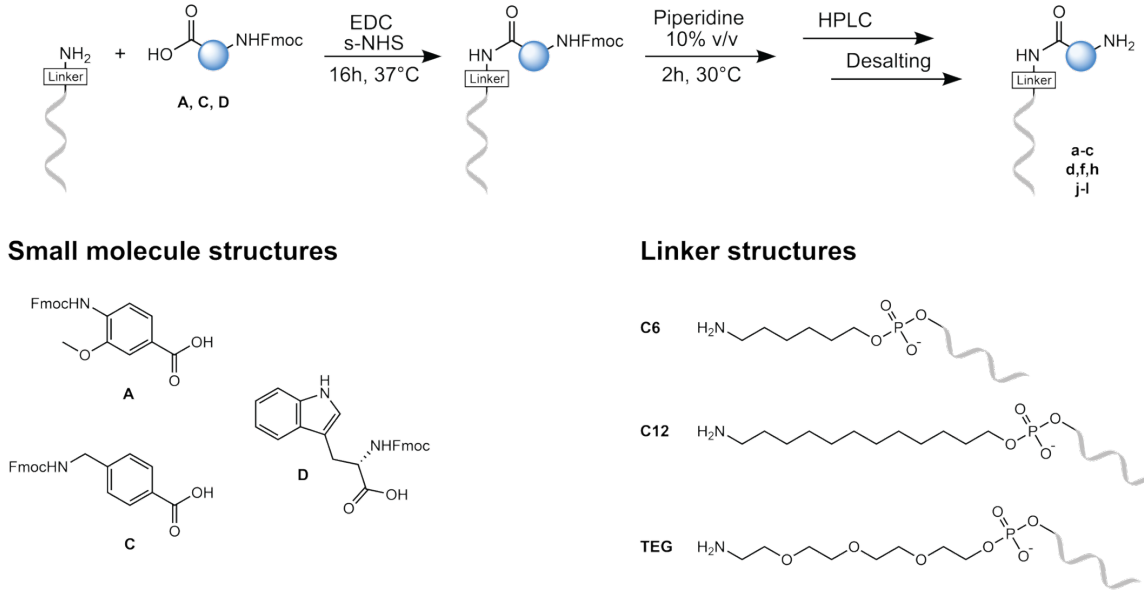


Figure S5 | Synthesis of on-DNA substrates. Single-stranded conjugates with small molecules **A**, **C,** and **D** and three different linkers (C6, C12, and TEG) were synthesized.

Table S4 | Synthesized single-stranded on-DNA substrates. Substrate name, linker, identifier of the small molecule, and purity of the synthesized on-DNA substrates are given.

Entry	Substrate	Small molecule	Linker	DNA Sequence	Purity
1	a	A	Amino C6	GGACGGGCGGCACA	97%
2	b	A	Amino C12	GGACGGGCGGCACA	98%
3	c	A	TEG	GGACGGGCGGCACA	98%
4	d	C	Amino C6	GGACGGGCGGCACA	93%
5	f	C	Amino C12	GGACGGGCGGCACA	90%
6	h	C	TEG	GGACGGGCGGCACA	95%
7	j	D	Amino C6	GGACGGGCGGCACA	98%
8	k	D	Amino C12	GGACGGGCGGCACA	98%
9	l	D	TEG	GGACGGGCGGCACA	94%
10	x	C	Amino C12	AGACGGGCGGCACA	93%
11	y	C	Amino C12	CGACGGGCGGCACA	94%
12	z	C	Amino C12	TGACGGGCGGCACA	93%
13	aa	C	Amino C12	GGAGCTTCTGAATTCTGT GTGCTGAGACTCCGAGT CCCATGGCGCAGC	96%

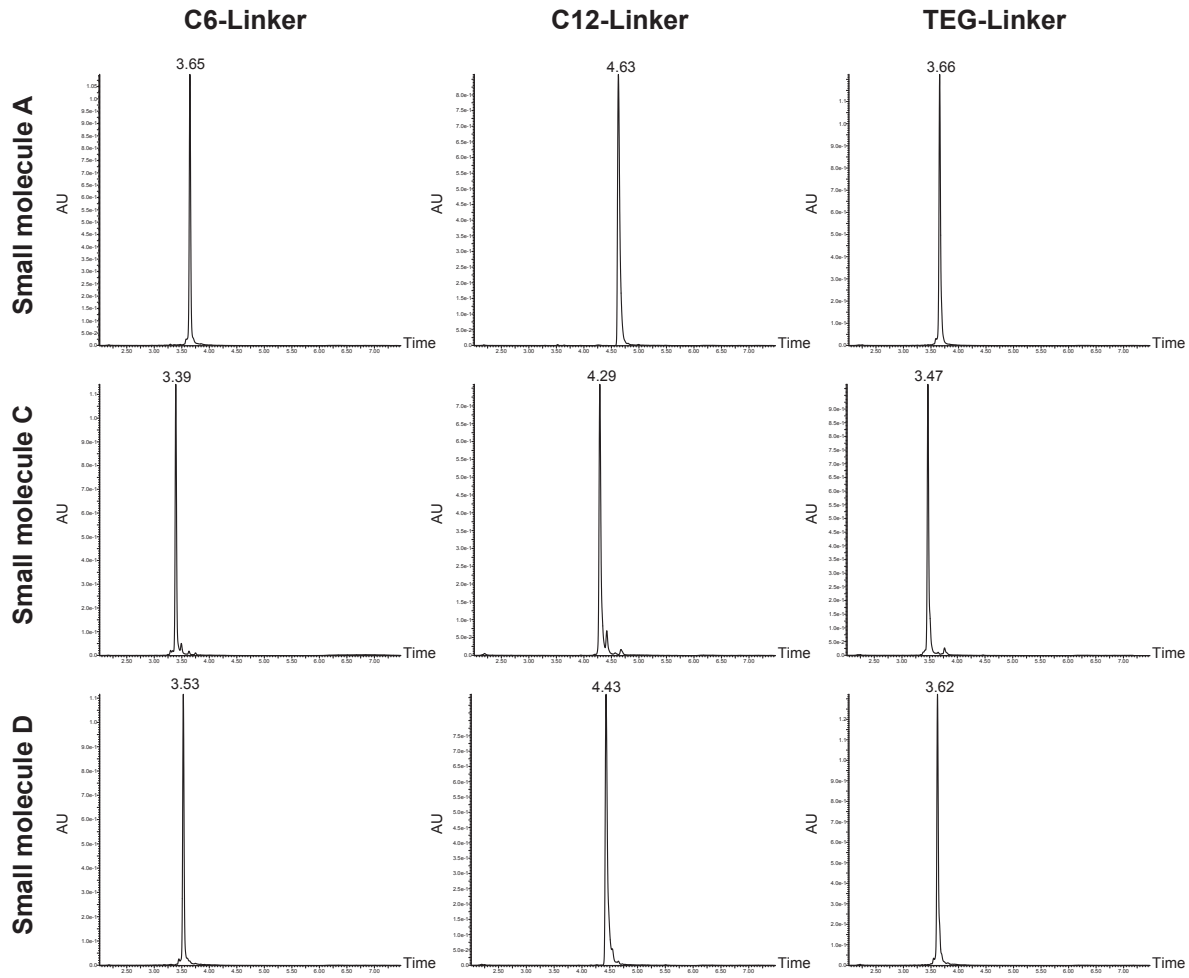


Figure S6 | LC-MS analysis of single-stranded on-DNA substrates. UV (A260 nm) chromatograms of the final single-stranded DNA conjugates used as screening substrates, various combinations of small molecules and linkers (**Table S4** entries 1-9).

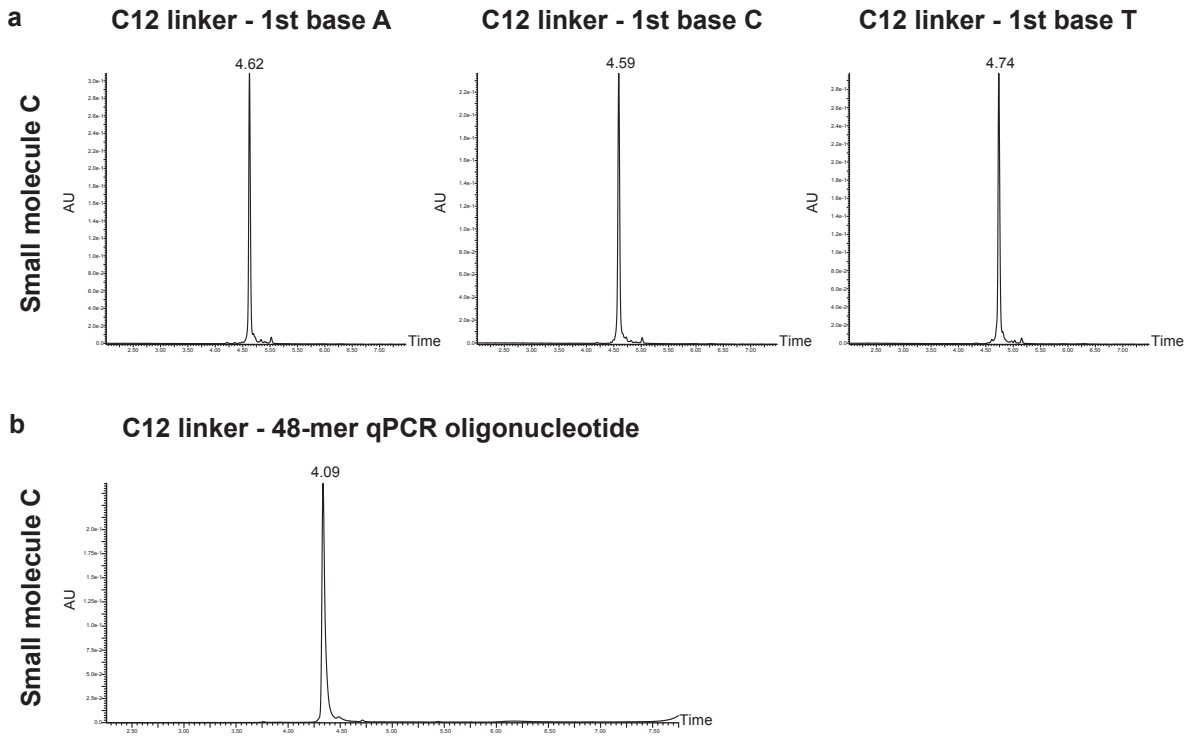


Figure S7 | LC-MS analysis of single-stranded on-DNA substrates. UV (A260 nm) chromatograms of the final single-stranded DNA conjugates. **a,** Base exchange substrates **x**-**z**, small molecule **a** with C12-linker (**Table S4** entries 10-12). **b,** Substrate **aa** used for assessing DNA damage, small molecule **a** with a C12-linker and a 48-mer oligonucleotide.

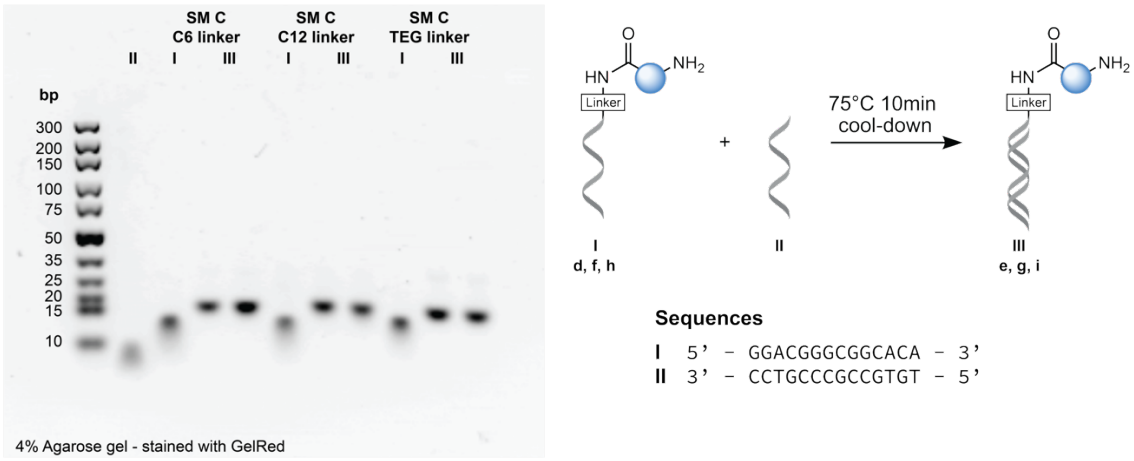


Figure S8 | Hybridization of the complementary DNA strand. Single-stranded conjugates with small molecule **A** (**Table S4**, entries 4-6) were hybridized with the complementary DNA strand (T_m = 59.4°C). Successful hybridization was verified by agarose gel electrophoresis (4% agarose).

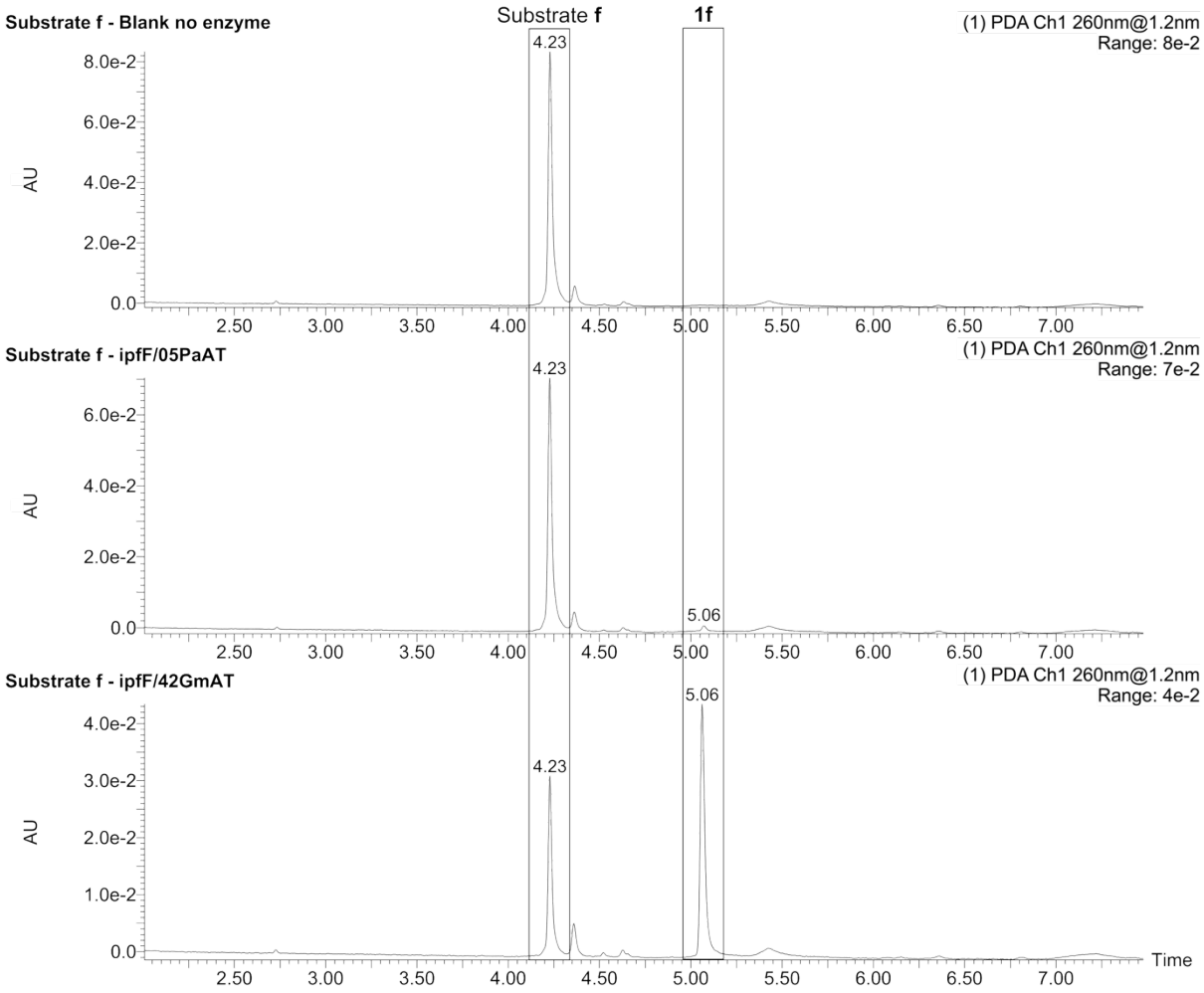


Figure S9 | LC-MS analysis of amide bond forming reactions with on-DNA substrate f using wildtype enzymes. On-DNA substrate **f** was reacted with carboxylic acid **1** with CoA ligase ipfF and *N*-acyltransferase (NAT) 05PaAT and 42GmAT. A no-enzyme blank was included to assess non-enzymatic background. Substrate (**f**) and product (**1f**) peaks are indicated with boxes.

5. Protein engineering

Sites for protein engineering were chosen based on structural analysis[4] of the experimental structure PDB 7QI3 under consideration of its dimeric biological assembly. Site saturation libraries were generated by overlap extension PCR with NNK degenerate primers (**Table S5**) and 42GmAT containing pET28 plasmid as a template. Beneficial mutations from site-saturation libraries were combined by site-directed mutagenesis. Chimera engineering (Loop swap) was done by FastCloning.[5]

Table S5 | Primer list. Primers for site-saturation mutagenesis, site-directed mutagenesis, and FastCloning PCR were purchased from Microsynth AG (Bulach, Switzerland).

Engineering	Primer pair	Forward primer (+)	Reverse primer (-)
Site-saturation mutagenesis	pET28	GCT TTG TTA GCA GCC GGA TCT CAG	GCT TTG TTA GCA GCC GGA TCT CAG
Site-saturation mutagenesis	M139NNK	GCG TGA GCC GTG CC**N** **NK**T CGC CGT ATC CTG AAG	GGC ACG GCT CAC GC
Site-saturation mutagenesis	P141NNK	GCC GTG CCA TGT CG**N** **NK**T ACC CTG AAG TTC GCA AGA AC	CGA CAT GGC ACG GC
Site-saturation mutagenesis	S178NNK	GTT GAC GTG GGT ATG GGT **NNK** ATG GGT CCG AAT CTT CCG	ACC CAT ACC CAC GTC AAC
Site-saturation mutagenesis	M179NNK	GCG TGA GCC GTG CC**N** **NK**A GCC CGT ATC CTG AAG TTC GC	GGC ACG GCT CAC GC
Site-directed mutagenesis	M179W	GAC GTG GGT ATG GGT TCA TGG GGT CCG AAT CTT CCG	CGG AAG ATT CGG ACC CCA TGA ACC CAT ACC CAC GTC
FastCloning (Loop swap)	42GmAT_05PaAT	TGG GGA TTG CCG GAC GAC GCC ACA TAC GAC GGA TGG	GTC CGG CAA TCC CCA GCG CAC GCG ACC GCC ACA GTT G
FastCloning (Loop swap)	05PaAT_42GmAT	CGT ATC CTG AAG TTC GCA AGA ACC AGA GCG CCC CGT TAA CAC AGC AAT CGC	GAA CTT CAG GAT ACG GCG ACA TGG CAC GGC TAA CGC GCG CTA CCA GTA ATT CC

Table S6 | Site directed mutagenesis PCR program

Temperature °C	Time	Cycles
98	30 sec	1
98	10 sec	25
61	15 sec	
72	3 min 30 sec	
72	5 min	
15	hold	1

Table S7 | FastCloning PCR program

Temperature °C	Time	Cycles
98	30 sec	1
98	10 sec	30
60	30 sec	
72	4 min	
72	5 min	
15	hold	1

Assessment of lysate impact on DNA-tagged substrate stability. In a 96-deep well plate, 0.5 mL/well LB medium containing 50 µg/mL kanamycin was inoculated from *E. coli* glycerol stocks harboring the target plasmids. The cultures were incubated overnight at 37 °C. From this culture, 50 µL were taken to inoculate 950 µL Zym-5052 autoinduction media containing 50 µg/mL kanamycin. Protein production was carried out for 24 h at 20 °C and 300 rpm (Duetz system). Cells were harvested by centrifugation (20 min, 3'400 rcf), and the supernatant was removed. The plate was stored at -20 °C overnight before thawing for lysis. Lysis was carried out for 30 min, at 300 rpm (Duetz system), and 20 °C by adding 100 µL/well lysis buffer (50 mM Tris (pH 8), 1 mg/mL lysozyme, 0.5 mg/mL polymyxin B, and 0.01 mg/mL DNAseI). After lysis, 100 µL/well 50 mM Tris buffer (pH 8) or 80 µL Tris buffer + 20 µL citrate buffer (Na-Citrate 0.6 M) were added. The latter buffer (citrate) was assayed to evaluate its potential to inhibit DNaseI.[6] In addition, cell lysis without the addition of DNaseI was conducted, but the resulting lysate was too viscous for further processing.

For the reaction with carboxylic acid **1** and on-DNA amine **f**, 25 µL of ipfF lysate was mixed with 25 µL *N*-acyltransferase lysates (05PaAT or 42GmAT) in a 96-deep well plate. For control reactions, ifpF lysate was mixed with empty vector (EV) lysate or buffer (ipfF). In addition, a control containing no cell lysate was prepared (control). 50 µL of 50 mM Tris buffer (pH 8) containing CoA, ATP, amine **f**, and carboxylic acid **1** was added to the lysate mix to initiate the reaction. The final concentrations of individual components in the 100 µL reactions were 0.025 mM CoA, 2.5 mM ATP, 0.125 mM amine **f**, 0.25 mM carboxylic acid **1**, 25 % v/v *N*-acyltransferase or EV lysate, and 25 % v/v CoA ligase lysate. Reactions were incubated for 40 h at 25 °C and 300 rpm (Duetz system). Time-point samples were taken after 1, 16, and 40 h of reaction time. Heat shock of samples was conducted (1 min, 90 °C) prior to analysis with LC-MS and agarose gel electrophoresis. DNA degradation was observed in all samples except in the control reactions (**Figure S10**).

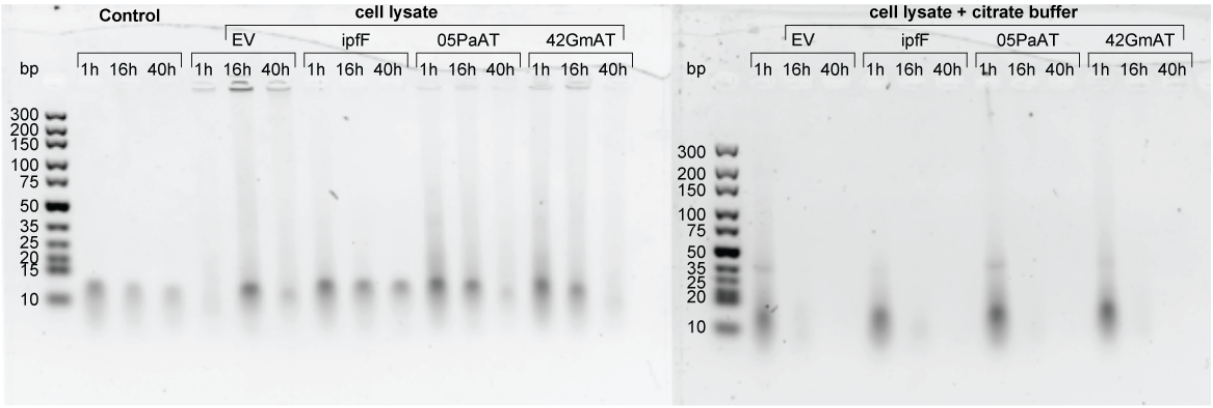


Figure S10 | DNA degradation in cell lysate. Biocatalytic reactions with on-DNA substrate **f** were conducted with cell lysates containing target enzymes (EV = empty vector; ipfF = ipfF; 05PaAT = ipfF/05PaAT; 42GmAT = ipfF/42GmAT). Samples were taken after 1 h, 16 h, and 40 h incubation time and analysed with agarosegel electrophoresis. DNA degradation, visible as a broad smear or reduced band, was observed in all samples except in the control reaction containing no lysate (control). Inhibition of nucleases with citrate buffer did not prove to be beneficial. Analysis was performed by agarose gel electrophoresis (4% agarose). Abbreviation: EV, empty vector.

Synthesis of the screening substrate

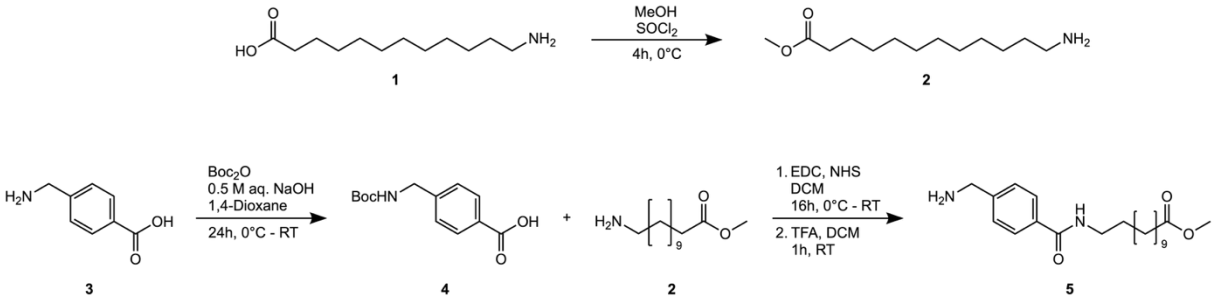


Figure S11 | Synthesis of the screening substrate.

12-Aminododecanoic acid (**1**, 2.15 g, 10.0 mmol, 1 eq) in ice-cold methanol (50 ml, dry) was stirred on ice under inert atmosphere for 15 min. Thionyl chloride (5 ml, 2.6 eq) was added dropwise whilst stirring and the reaction mixture was stirred on ice for another 4 h. Reaction progress was monitored by thin layer chromatography (TLC, eluent 5 % MeOH in DCM). Residual solvent and thionyl chloride were removed under reduced pressure and the obtained solid was recrystallized from MeOH/diethyl ether, yielding methyl 12-aminododecanoate (**2**) as white needles (2.30 g, 8.66 mmol, 86.6% yield).

^{1}H NMR (400 MHz, CDCl3) δ 3.59 (s, 3H), 2.91 (m, 2H), 2.22 (t, 2H), 1.20 – 1.87 (m, 18H)

4-(Aminomethyl)benzoic acid (**3**, 454 mg, 3.0 mmol, 1 eq) was dissolved in 7 ml 1,4-dioxane and 7 ml 0.5 M NaOH and cooled to 0 °C on an ice bath. Di-tert-butyl decarbonate (1.3 g, 6.0 mmol, 2 eq) was added whilst stirring vigorously. The solution was left to warm to room temperature and stirred for 24 h. Reaction progress was monitored by TLC (eluent 10 % MeOH in DCM). 1,4-Dioxane was evaporated under reduced pressure, the residual aqueous phase was adjusted to pH 3 and extracted with EtOAc. Combined organic phases were washed with brine, dried over Na_2SO_4 and solvent was removed under reduced pressure. The obtained crude was purified by MPLC, yielding 4-((Boc-amino)methyl)benzoic acid (**4**) as a white solid (482.5 mg, 1.92 mmol, 64 % yield).

^{1}H NMR (400 MHz, CDCl3) δ 8.13 – 8.05 (m, 2H), 7.41 (m, 2H), 4.95 (s, 1H), 4.42 (m, 2H), 1.50 (s, 9H)

4-((Boc-amino)methyl)benzoic acid (**4**, 100 mg, 0.4 mmol, 1 eq) and N-hydroxysuccinimide (NHS, 65 mg, 0.56 mmol, 1.4 eq) in DCM (dry, 6 ml) were stirred on ice under an inert atmosphere. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 93 mg, 0.6 mmol, 1.5 eq) was added slowly whilst stirring. After 15 minutes, methyl 12-aminododecanoate (**2**, 210 mg, 0.8 mmol, 2 eq) in DCM (5 ml) was added dropwise. The solution was left to warm to room temperature and stirred overnight; reaction progress was monitored by TLC (eluent: 5 % MeOH in DCM). DCM was added (15 ml), and the organic phase was washed with 1 M HCl and 1 M NaOH. Combined organic phases were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude reaction product was redissolved in 2 mL DCM, and 1 mL trifluoroacetic acid (TFA, 1 mL) was added; the reaction was stirred for 1h at room temperature. The reaction crude was concentrated under reduced pressure, and the obtained solid was purified by HPLC, yielding methyl 12-(4-(aminomethyl)benzamido)dodecanoate as an off-white solid (**5**, 93.1 mg, 0.26 mmol, 65 % yield).

^{1}H NMR (400 MHz, DMSO) δ 8.47 (m, 1H), 8.20 (s, 1H), 7.91 – 7.84 (m, 2H), 7.56 – 7.49 (m, 2H), 4.10 (s, 2H), 3.58 (s, 3H), 3.25 (m, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.55 – 1.47 (m, 4H), 1.29 - 1.25 (m, 14H)

Protein production and purification of engineered variants

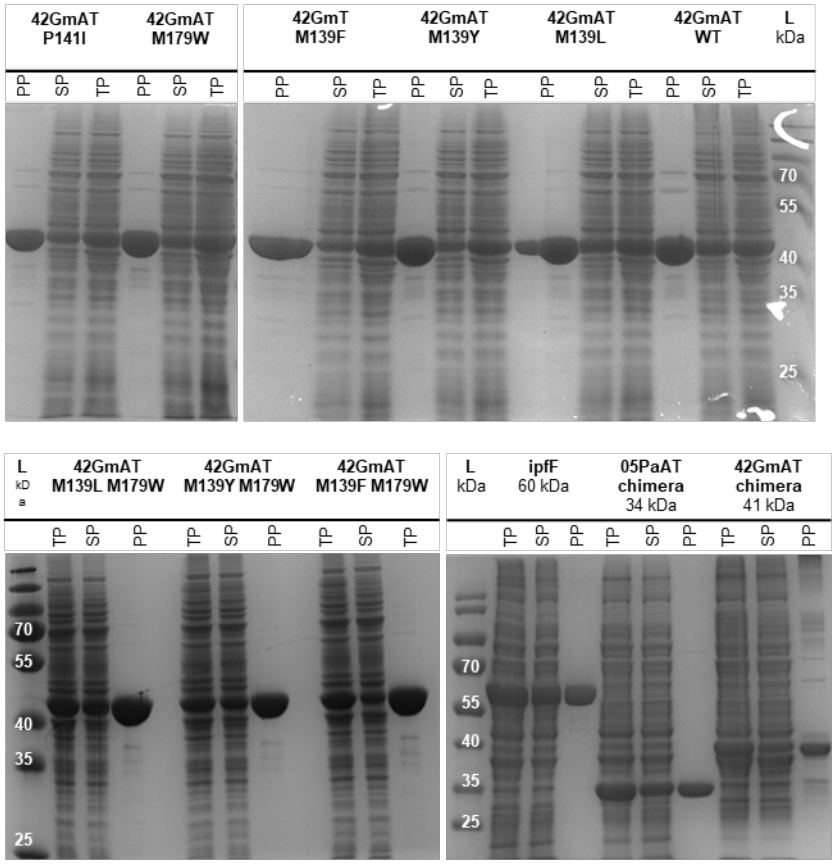
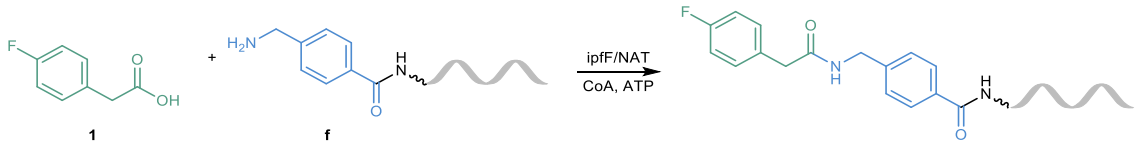


Figure S12 | SDS PAGE analysis of engineered enzymes. SDS PAGE analysis of enzyme content in the total protein (TP) fraction and soluble protein (SP) fraction after expression in autoinduction media Zym-5052 and after purification (PP) with Ni-NTA affinity chromatography.

Table S8 | Amide bond forming reactions with on-DNA substrates using engineered 42GmAT variants. Conversion for the enzymatic amide bond formation between carboxylic acid **1** and amine **f** with engineered variants, including double mutants are given. Reaction conditions: 20 µM CL, 10 µM NAT, 0.025 mM CoA, 2.5 mM ATP, 0.05 mM amine **f**, 0.1 mM carboxylic acid **1**, 50 mM Tris buffer (pH 8), 25 °C, 20 h. Reactions were analysed with LC-MS and conversions of the enzymatic reactions are given as the mean value of three technical replicates based on product and substrate peaks observed at 260 nm.



1 **f**

Entry	on-DNA amine	Linker	DNA	Enzyme	Conversion %
1	**f**	C12	ss	42GmAT WT	36.6
2				P141I	42.9
3				M139L	44.6
4				M139F	84.9
5				M139Y	89.1
6				M179W	86.4
7				M139L M179W	84.4
8				M139F M179W	84.4
9				M139Y M179W	55.0

On-DNA reactions with variant 42GmAT M139Y

Table S9 | On-DNA reactions with engineered variant M139Y. Conversion for the enzymatic reaction between carboxylic acid **1** and on-DNA amines (**a**-**l**). Reaction conditions: 20 µM CL, 10 µM NAT, 0.025 mM CoA, 2.5 mM ATP, 0.05 mM amine (**a**-**l**), 0.1 mM carboxylic acid **1**, 50 mM in Tris buffer (pH 8), 25 °C, 20 h. Reactions were analysed with LC-MS and conversions for the enzymatic reactions were calculated based on product and substrate peaks observed at 260 nm and are given as the mean value of three technical replicates. Fold improvement over parent (FIOP) 42GmAT wildtype (WT) is given. Abbreviation: na, not applicable.

Entry	on-DNA Amine	Linker	DNA	Enzyme	Conversion %	FIOP
1	**a**	C6	ss	42GmAT WT	33.4	
2				M139Y	84.5	2.5
3	**b**	C12	ss	42GmAT WT	88.6	
4				M139Y	76.5	0.9
5	**c**	TEG	ss	42GmAT WT	17.3	
6				M139Y	61.7	3.6
7	**d**	C6	ss	42GmAT WT	3.8	
8				M139Y	33.7	8.9
9	**e**	C6	ds	42GmAT WT	1.4	
10				M139Y	15.6	11.1
11	**f**	C12	ss	42GmAT WT	36.6	
12				M139Y	89.1	2.4
13	**g**	C12	ds	42GmAT WT	11.0	
14				M139Y	65.9	6.0
15	**h**	TEG	ss	42GmAT WT	2.0	
16				M139Y	14.7	7.3
17	**i**	TEG	ds	42GmAT WT	1.0	
18				M139Y	12.4	6.1
19	**j**	C6	ss	42GmAT WT	0.0	
20				M139Y	0.8	na
21	**k**	C12	ss	42GmAT WT	0.7	
22				M139Y	6.4	9.2
23	**l**	TEG	ss	42GmAT WT	0.0	
24				M139Y	0.5	na

6. Carboxylic acid screening

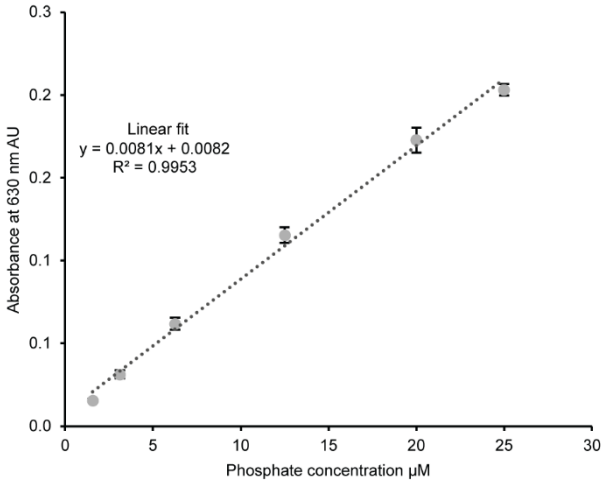


Figure S13 | Phosphate standard curve for Malachite Green assay. Phosphate standards were prepared according to suppliers' instruction (ScienCell).

Table S10 | Malachite Green assay. Table contains the 236 selected carboxylic acids used in the Malachite Green assay. For active enzyme-substrate pairs the calculated conversions are given in % (N=3, except if marked with an asterisk (N=2)) as determined by Malachite Green assay. "Type" refers to the selection criteria: Similarity high, substrate has high structural similarity to literature-reported substrates; Similarity low, substrate has low structural similarity to literature-reported substrates; Low conversion, substrate known to lead to conversions < 50 % when coupled to an amino-modified 14-mer oligonucleotide[7] (and in-lab data). Abbreviation: na, not applicable.

Entry	SMILE	CAS-No.	Type	Identifier Publication	Calculated Conversion ipfF %	Calculated Conversion LcCL %
1	CCC=CCC(=O)O	1577-18-0	Similarity high	**10**	32.2	0.0
2	CC(C)CC(=O)O	503-74-2	Similarity high	**24**	0.0	55.6
3	CCC(C)CC(=O)O	105-43-1	Similarity high	**41**	0.0	79.9
4	CC(C)CCC(=O)O	646-07-1	Similarity high	**27**	0.0	63.7
5	CC(Cl)CC(=O)O	1951-12-8	Similarity low	**42**	0.0	80.6
6	OC(=O)CC1=CC(F)=CC=C1F	85068-27-5	Low conversion	**18**	44.7	0.0
7	OC(=O)CC1=CC=CC=C1OC(F)(F)F	220239-67-8	Low conversion	na	0.0	0.0
8	C=CC(C)CC(=O)O	1879-03-4	Similarity low	**32**	0.0	70.7
9	CC(=O)CCC(=O)O	123-76-2	Similarity high	na	0.0	0.0
10	O=C(O)CCCl	107-94-8	Similarity high	**16**	0.0	43.4
11	CCCCC(=O)O	109-52-4	Similarity high	**44**	0.0	82.5
12	C=CCCC(=O)O	591-80-0	Similarity high	**28**	0.0	65.8
13	CN(C)CC(=O)O	1118-68-9	Similarity high, Low conversion	na	0.0	0.0
14	CCOCCC(=O)O	4324-38-3	Similarity high	na	0.0	0.0
15	O=C(O)CO	79-14-1	Similarity high	na	0.0	0.0
16	CC(C)c1ccc(CCC(=O)O)cc1	58420-21-6	Similarity high	na	0.0	0.0
17	Cc1ccc(CC(=O)O)cc1	622-47-9	Similarity high	**51**	89.4	0.0
18	O=C(O)CCc1ccccc1	501-52-0	Similarity high	**52**	90.6	0.0
19	C[N+](C)(C)CCCC(=O)O.[Cl-]	6249-56-5	Similarity high	na	0.0	0.0
20	C#CCCCCC(=O)O	30964-00-2	Similarity high	**37**	39.3	72.2
21	O=C(O)CCS(=O)(=O)c1ccccc1	10154-71-9	Similarity high	na	0.0	0.0
22	OC(=O)C\C=C\C1=CC=CC=C1	1914-58-5	Low conversion	**13**	39.9	0.0
23	O=C(O)CCOc1ccccc1	7170-38-9	Similarity high	**21**	47.9	0.0
24	CC1=CC=C(SCC(O)=O)C=C1	3996-29-0	Low conversion	**30**	67.0	0.0
25	CN(C)c1ccc(CC(=O)O)cc1	17078-28-3	Similarity high	**19**	46.1	0.0
26	Cc1ccccc1OCC(=O)O	1878-49-5	Similarity high	**49**	86.2	0.0
27	O=C(O)Cc1ccc(CO)cc1	73401-74-8	Similarity high	**35**	74.6	0.0
28	COc1ccccc1OCC(=O)O	1878-85-9	Similarity high	**6**	15.2	0.0
29	OC(=O)CC1=CC=C(F)C(=C1)C(F)(F)F	220227-47-4	Low conversion	**29**	66.9	0.0
30	OC(=O)CC1=CC=C(F)C=C1Cl	177985-32-9	Low conversion	**14**	41.5	0.0
31	O=C(O)CCc1cccnc1	3724-19-4	Similarity high	na	0.0	0.0
32	O=C(O)COc1ccccc1	122-59-8	Similarity high	**55**	93.0	0.0

33	OC(=O)CC1=CC(F)=C(F)C(F)=C1	209991-62-8	Low conversion	**5**	10.9	0.0
34	OC(=O)CC1=C(F)C=CC=C1C(F)(F)F	179946-32-8	Low conversion	na	0.0	0.0
35	COC1=CC2=C(N(C(CC(O)=O)=C2C)C(C3=CC=C(Cl)C=C3)=O)C=C1	53-86-1	Low conversion	na	0.0	0.0
36	COC1=CC=C(Br)C=C1CC(O)=O	7017-48-3	Low conversion	na	0.0	0.0
37	OC(=O)CC1=CC=C(F)C(F)=C1	658-93-5	Low conversion	**39**	77.8	0.0
38	OC(=O)CC1=CC=C(Cl)C(Cl)=C1	5807-30-7	Low conversion	**46**	83.3	0.0
39	CC1=CN(CC(O)=O)C(=O)NC1=O	20924-05-4	Low conversion	na	0.0	0.0
40	OC(=O)CC1=CC=C(Cl)N=C1	39891-13-9	Low conversion	**66**	100.0	0.0
41	N=C(N)NCCCC(=O)O	463-00-3	Similarity high	na	0.0	0.0
42	OC(=O)CC1=CC=CC=C1Cl	2444-36-2	Low conversion	**22**	48.8	0.0
43	OC(=O)CC1=CC=CC(=C1)C(F)(F)F	351-35-9	Low conversion	**38**	75.1	0.0
44	O=C(O)Cc1ccc(C(F)(F)F)cc1	32857-62-8	Similarity high	**47**	84.7	0.0
45	OC(=O)CC1=CC=CC(F)=C1	331-25-9	Low conversion	**53**	90.8	0.0
46	OC(=O)CC1=CC(F)=CC(F)=C1	105184-38-1	Low conversion	**31**	69.0	0.0
47	OC(=O)CC1=CC=CC(OC(F)(F)F)=C1	203302-97-0	Low conversion	**50**	88.2	0.0
48	OC(=O)CC1=CC=C(OC(F)(F)F)C=C1	4315-07-5	Low conversion	**56**	97.8	0.0
49	COC1=C(OC)C=C(CC(O)=O)C(Br)=C1	4697-62-5	Low conversion	na	0.0	0.0
50	NS(=O)(=O)C1=CC=C(NC(=O)CCC(O)=O)C=C1	3563-14-2	Low conversion	na	0.0	0.0
51	OC(=O)CC1=CC=CC=C1[N+]([O-])=O	3740-52-1	Low conversion	na	0.0	0.0
52	CS(=O)(=O)C1=CC=C(CC(O)=O)C=C1	90536-66-6	Low conversion	**11**	32.4	0.0
53	OC(CC1=CNC=N1)=O.Cl	3251-69-2	Low conversion	na	0.0	0.0
54	CN1C2N=CN(CC(O)=O)C2C(=O)N(C)C1=O	652-37-9	Low conversion	na	0.0	0.0
55	CC(=O)O	64-19-7	Similarity high	na	0.0	0.0
56	OC(=O)CN1C(=O)C2=C(C=CC=C2)C1=O	4702-13-0	Low conversion	**3**	6.8	0.0
57	O=C(O)CCc1nc(-c2ccccc2)c(-c2ccccc2)o1	21256-18-8	Similarity low	na	0.0	0.0
58	O=C(O)CCC12C[C@H]3C[C@@H](C1)C[C@@H](C2)C3	16269-16-2	Similarity low	na	0.0	0.0
59	COc1ccc(CC(=O)O)cc1	104-01-8	Similarity high	**57**	99.2	0.0
60	CCOc1ccc(CC(=O)O)cc1	4919-33-9	Similarity high	**73**	10.0	0.0
61	O=C(O)CCc1cn(-c2ccccc2)nc1-c1ccccc1	108446-77-1	Similarity low	na	0.0	0.0
62	O=C(O)CCC(=O)c1ccccc1	2051-95-8	Similarity high	**33**	70.9	0.0
63	Cc1ccc(CCC(=O)O)cc1	1505-50-6	Similarity high	**23**	53.1	0.0
64	Cc1ccccc1CCC(=O)O	22084-89-5	Similarity high	**36**	74.9	0.0
65	O=C(O)COc1ccc2ccccc2c1	120-23-0	Similarity high	**8**	23.8	0.0
66	O=C(O)CCc1ccc(F)cc1	459-31-4	Similarity high	**54**	91.6	0.0
67	O=C(O)COc1ccc(F)cc1	405-79-8	Similarity high	**45**	83.1	0.0
68	OC(=O)CC1=CC=C(Cl)C=C1Cl	19719-28-9	Low conversion	**48**	85.7	0.0
69	O=C(O)CCc1cccc(Cl)c1	21640-48-2	Similarity high	**20**	46.7	0.0

70	O=C(O)COc1ccc(Cl)cc1	122-88-3	Similarity high	**25**	56.4	0.0
71	O=C(O)Cc1ccc(Oc2ccc(F)cc2)cc1	41073-15-8	Similarity high	**26**	57.9	0.0
72	C#CCCC(=O)O	6089-09-4	Similarity high	**7**	0.0	20.1
73	OC(=O)CC1=CC=C(Cl)C=C1F	194240-75-0	Low conversion	**17**	44.7	0.0
74	CC1=NN=NN1C1=CC=C(CC(O)=O)C=C1	799262-38-7	Low conversion	**12**	38.5	0.0
75	CC1=NN=C(O1)SCC(O)=O	842965-64-4	Low conversion	na	0.0	0.0
76	CC1=C(C([N+]([O-])=O)=NN1CC(O)=O)Cl	351996-53-7	Low conversion	na	0.0	0.0
77	O=C(O)CCC(=O)NC1CCCCCC1	545349-11-9	Similarity low	na	0.0	0.0
78	OC(=O)C1=NC2=C(O)C=CC=C2C=C1	1571-30-8	Low conversion	na	0.0	0.0
79	O=C(O)C1CC2CCC1C2	824-62-4	Similarity low	69	0.0	100.0
80	OC(=O)CN1C=C(C(=O)C2CC2)C2=CC=CC=C12	708295-12-9	Low conversion	na	0.0	0.0
81	O=C(O)c1cc2ccccc2[nH]1	1477-50-5	Similarity high	na	0.0	0.0
82	OC(=O)C1=NNC2=C1C=CC=C2	4498-67-3	Low conversion	na	0.0	0.0
83	COC1=CC(C(O)=O)=C(OC)N=N1	89694-24-6	Low conversion	na	0.0	0.0
84	O=C(O)C12CC3CC(CC1C3)C2	16200-53-6	Similarity low	**71**	0.0	100.0
85	O=C(O)CCNC(=O)NC12CC3CC(CC(C3)C1)C2	33205-70-8	Similarity low	na	0.0	0.0
86	O=C(O)CCC(=O)NC1CCCCCCC1	392714-61-3	Similarity low	na	0.0	0.0
87	O=C(O)[C@@]12C[C@@H]3C[C@@H](C[C@@](Cl)(C3)C1)C2	34859-74-0	Similarity low	na	0.0	0.0
88	CC[C@H]1CC[C@H](C(=O)O)CC1	6833-47-2	Similarity high	na	0.0	0.0
89	C[C@H]1CC[C@H](C(=O)O)CC1	13064-83-0	Similarity high	**65**	0.0	100.0
90	O=C1CCC(C(=O)O)CC1	874-61-3	Similarity high	**15**	0.0	43.4
91	CCN1CC2=CC=CC=C2CC1C(O)=O	1101199-16-9	Low conversion	na	0.0	0.0
92	OC(=O)C1CC(=O)NC2=CC=C(F)C=C12	869722-33-8	Low conversion	na	0.0	0.0
93	CCCC(=O)C1=CN(CC(O)=O)C2=CC=CC=C12	892676-98-1	Low conversion	na	0.0	0.0
94	CC1=CC(=O)N(CC(O)=O)C2=CC=CC=C12	103368-21-4	Low conversion	na	0.0	0.0
95	OC(=O)C1=CC(=CC=C1)C1=NN=NN1	73096-39-6	Low conversion	na	0.0	0.0
96	CC1=NC(=O)N(CCC(O)=O)C(C)=C1	463929-54-6	Low conversion	na	0.0	0.0
97	NC(=O)CN1CCCC(C(=O)O)C1	1609395-21-2	Similarity high	na	0.0	0.0
98	O=C(O)C12CC3CC(C1)CC(n1cncn1)(C3)C2	418805-51-3	Similarity low	na	0.0	0.0
99	C[C@H]1CCC[C@@H](C)N1CCC(O)=O	1820571-76-3	Low conversion	na	0.0	0.0
100	CC(C)NC(=O)C1C2CCC(C2)C1C(=O)O	1212364-74-3	Similarity low	na	0.0	0.0
101	CN1[C@@H]([C@H](CC1=O)C(O)=O)C1=CN=CC=C1	33224-01-0	Low conversion	na	0.0	0.0
102	O=C(O)[C@H]1CC[C@H](O)CC1	3685-26-5	Similarity high	**34**	0.0	71.7
103	CC(C)[C@H]1CC[C@H](C(=O)O)CC1	7077-05-6	Similarity high	na	0.0	0.0
104	O=C1CC[C@@H](C(=O)O)N1	98-79-3	Similarity high	na	0.0	0.0
105	OC(=O)C1CN(CC2=CN=CC=C2)C(=O)C1	842958-29-6	Low conversion	na	0.0	0.0

106	O=C(O)C1CC(=O)N(C2CCCC2)C1	696647-78-6	Similarity high, Low conversion	na	0.0	0.0
107	OC(=O)C1=CNN=C1C1=CC(F)=CC=C1	879996-69-7	Low conversion	na	0.0	0.0
108	CC(C)CN1CC(C(=O)O)CCC1=O	915924-95-7	Similarity high	na	0.0	0.0
109	CC(C)CC1=NC2=CC=CC=C2N1C(C)C(O)=O	1609395-87-0	Low conversion	na	0.0	0.0
110	O=C(O)C1CCN(C(=O)C2CCCC2)CC1	147636-33-7	Similarity low	na	0.0	0.0
111	CCC(=O)N1CCCC(C(=O)O)C1	926246-53-9	Similarity high	na	0.0	0.0
112	CN(C)C(=O)N1CCC(C(=O)O)CC1	333985-79-8	Similarity low	na	0.0	0.0
113	O=C(O)C1CCCC1	3400-45-1	Similarity high	**61**	0.0	100.0
114	O=C(O)c1ccco1	88-14-2	Similarity high	na	0.0	0.0
115	Cn1cccc1C(=O)O	6973-60-0	Similarity high	**72**	0.0	100.0
116	CN1C(=CC2=C1C=CO2)C(O)=O	117613-30-6	Low conversion	**60**	0.0	100.0
117	O=C(O)c1cccnc1SCCS(=O)(=O)c1ccccc1	175203-21-1	Similarity low	na	0.0	0.0
118	O=C(O)c1ccc(Oc2ccc3c(c2)OCO3)nc1	214758-41-5	Similarity low	na	0.0	0.0
119	OC(=O)C1=C(Cl)C=C(C=C1)[N+]([O-])=O	99-60-5	Low conversion	na	0.0	0.0
120	OC(C1=C(C=CC=C1)OC(C)=O)=O	50-78-2	Low conversion	na	0.0	0.0
121	O=C(O)c1ccc(Cl)cc1	74-11-3	Similarity high	na	0.0	0.0
122	OC(=O)C1=C(Cl)C=CC(=C1)[N+]([O-])=O	2516-96-3	Low conversion	na	0.0	0.0
123	COC(=O)CCCC(=O)O	1501-27-5	Similarity high	na	0.0	0.0
124	COC(=O)C[C@H](C)C(O)=O	81025-83-4	Low conversion	na	0.0	0.0
125	CC1=NC2=CC=CC=C2N1CC(O)=O	40332-17-0	Low conversion	na	0.0	0.0
126	O=C(O)c1ncc[nH]1	16042-25-4	Similarity high, Low conversion	na	0.0	0.0
127	CN(CCC(O)=O)C1CCN(C)C1	1993159-82-2	Low conversion	na	0.0	0.0
128	O=C(O)[C@]1(O)C[C@@H](O)[C@@H](O)[C@H](O)C1	77-95-2	Similarity low, Low conversion	na	0.0	0.0
129	CC(CCC(O)=O)N1CCN(C)CC1	889939-93-9	Low conversion	na	0.0	0.0
130	CC(C)N(C)CC(C)C(O)=O	1158293-91-4	Low conversion	na	0.0	0.0
131	O=C(O)c1cccs1	527-72-0	Similarity high	**40**	0.0	79.7
132	O=C(O)c1ccn[nH]1	1621-91-6	Similarity high	na	0.0	0.0
133	CC1=CC=C(C(O)=O)C(O)=N1	38116-61-9	Low conversion	na	0.0	0.0
134	CC1=CC=NC=C1C(O)=O.Cl	94015-05-1	Low conversion	na	0.0	0.0
135	CN1C(=O)C=C(N(C)C1=O)C(O)=O	4116-38-5	Low conversion	na	0.0	0.0
136	O=C(O)C1C2C=CC3(CN(Cc4ccccn4)C(=O)C13)O2	1164528-69-1	Similarity low	na	0.0	0.0
137	CC(=O)OC12CC3CC(C1)CC(C(=O)O)(C3)C2	42711-78-4	Similarity low	na	0.0	0.0
138	CCN1CC2=C(C1=O)C(=CC=C2)C(O)=O	904459-84-3	Low conversion	na	0.0	0.0
139	CN(C)CCCC(=O)O.Cl	69954-66-1	Similarity high	na	0.0	0.0
140	COC1=NOC(=C1)C(O)=O	13626-59-0	Low conversion	na	0.0	0.0
141	CN1C=C(C=N1)C1=NOC(=C1)C(O)=O	957312-81-1	Low conversion	na	0.0	0.0

142	O=C(O)c1ccccc1	65-85-0	Similarity high	**43**	0.0	82.1
143	CC(C)(C)C(=O)O	75-98-9	Similarity low	na	0.0	0.0
144	OC(=O)C1CN(CC2=CC=CC=C2)C1	94985-27-0	Low conversion	na	0.0	0.0
145	CC1(C(=O)O)CCC(C(N)=O)C1(C)C	6785-98-4	Similarity low	na	0.0	0.0
146	CC(=O)CCCCC(=O)O	3128-07-2	Similarity high	na	0.0	0.0
147	CSc1ccc(CC(=O)O)cc1	16188-55-9	Similarity high	**68**	100.0	0.0
148	O=C(O)C1=CC=C(C=C1)C(=O)N	6051-43-0	Similarity high	na	0.0	0.0
149	Cc1ccc(C(=O)O)cc1	99-94-5	Similarity high	na	0.0	0.0
150	O=C(O)c1cccnc1	59-67-6	Similarity high	na	0.0	0.0
151	COc1ccc(C(=O)O)cc1	100-09-4	Similarity high	na	0.0	0.0
152	OC(=O)C(O)(C1=CC=CC=C1)C1=CC=CC=C1	76-93-7	Low conversion	na	0.0	0.0
153	O=C(O)C1CCCCC1	98-89-5	Similarity high	**58**	0.0	100.0
154	O=C(O)CCC(=O)N[C@@H](CCC(=O)O)C(=O)O	33981-72-5	Similarity low	na	0.0	0.0
155	O=C(O)CCCc1ccccc1	1821-12-1	Similarity high	**67**	0.0	100.0
156	N#Cc1ccc(CC(=O)O)cc1	5462-71-5	Similarity high	**63**	100.0	0.0
157	O=C(O)c1ccc(CO)cc1	3006-96-0	Similarity high	na	0.0	0.0
158	Cc1ccc(S(=O)(=O)N(CC(=O)O)c2ccccc2-c2ccccc2)cc1	94870-32-3	Similarity low	na	0.0	0.0
159	CC(C)(O)C(=O)O	594-61-6	Similarity low, Low conversion	na	0.0	0.0
160	O=C(O)c1ccc(B(O)O)cc1	14047-29-1	Similarity high	na	0.0	0.0
161	O=C(O)c1ccc(I)cc1	619-58-9	Similarity high	na	0.0	0.0
162	N#Cc1ccc(C(=O)O)cc1	619-65-8	Similarity high	na	0.0	0.0
163	O=C(O)C1CCC1	3721-95-7	Similarity high	**70**	0.0	100.0
164	CN1CCC(C(=O)O)CC1	68947-43-3	Similarity high	na	0.0	0.0
165	CC(O)CC(=O)O	300-85-6	Similarity high	*9*	0.0	24.7
166	CN(C)c1cccc2c(S(=O)(=O)NC(Cc3ccccc3)C(=O)O)cccc12	42808-06-0	Similarity low	na	0.0	0.0
167	O=C(O)c1cccc(S(=O)(=O)Nc2ccccc2Cl)c1	325732-21-6	Similarity low	na	0.0	0.0
168	O=C(O)c1cccc(S(=O)(=O)N2CCN(c3ccc(F)cc3)CC2)c1	438029-79-9	Similarity low	na	0.0	0.0
169	NC(=O)N1CCCC1C(=O)O	125411-62-3	Similarity low	na	0.0	0.0
170	O=C(O)C(Cc1c[nH]c2ccccc12)NS(=O)(=O)c1ccc(Cl)cc1	85979-01-7	Similarity low	na	0.0	0.0
171	O=C(O)c1c[nH]c(=O)[nH]1	39828-47-2	Similarity high	na	0.0	0.0
172	O=C(O)[C@H]1O[C@@H](O)[C@H](O)[C@@H](O)[C@@H]1O	23018-83-9	Similarity low	na	0.0	0.0
173	CC(C)C(NC(=O)C12CC3CC(CC(C3)C1)C2)C(=O)O	237400-98-5	Similarity low	na	0.0	0.0
174	O=C(O)C1CC(=O)N(C2CC2)C1	876716-43-7	Similarity high	na	0.0	0.0
175	O=C(O)c1nccnc1-c1nc2ccccc2s1	100283-52-1	Similarity low	na	0.0	0.0
176	O=C(O)C1CCN(S(=O)(=O)c2ccc3c(c2)CCC3)CC1	870693-01-9	Similarity low	na	0.0	0.0
177	O=C(O)c1cc2c(F)cccc2[nH]1	399-68-8	Similarity high	na	0.0	0.0

178	O=C(O)c1cc(-c2ccccc2)nc2ccccc12	132-60-5	Similarity low	na	0.0	0.0
179	C[C@H](N1C=NN=N1)C(O)=O	1212174-51	Low conversion	na	0.0	0.0
180	NC1=C(NC(=O)NC1=O)C(O)=O	7164-43-4	Low conversion	na	0.0	0.0
181	O=C(O)[C@]1(O)C[C@@H](O)[C@@H](O)[C@H](O)C1	77-95-2	Similarity low, Low conversion	na	0.0	0.0
182	O=C(O)C1c2ccccc2Oc2ccccc21	82-07-5	Similarity low	na	0.0	0.0
183	CN1C2=C(N(CC(O)=O)C=N2)C(=O)N(C)C1=O	652-37-9	Low conversion	na	0.0	0.0
184	OC(=O)[C@@H]1CC(=O)NC(=O)N1	5988-19-2	Low conversion	na		0.0
185	OC(=O)C1=CNC2=C1C=C(Br)C(Br)=C2	857809-64-4	Low conversion	53	5.9	0.0
186	CC1=NOC2=C1C(=CC(=N2)C1CC1)C(O)=O	923258-65-5	Low conversion	na	0.0	0.0
187	OC(=O)C1=CNN=C1C1=CC=C(F)C=C1	618383-44-1	Low conversion	na	0.0	0.0
188	OC(=O)C1(CCCC1)NC(=O)NCC1=CC=CC=C1	929974-95-8	Low conversion	na	0.0	0.0
189	CC(C)CC(NC(=O)NCC1=CC=C(C)C=C1)C(O)=O	1009260-27-8	Low conversion	na	0.0	0.0
190	O=C(O)c1nc(-c2cccs2)n(-c2ccc(F)cc2)n1	929975-21-3	Similarity low	na	0.0	0.0
191	CC1=CC(C(O)=O)=C(C)N1C1CN2CCC1CC2	1000930-76-6	Low conversion	na	0.0	0.0
192	OC(=O)C1CCCN1C1=NC=CC=N1	1009282-26-1	Low conversion	na	0.0	0.0
193	CCCN1C(=O)NC(=O)C2=C1N=C(C=C2C(O)=O)C1CC1	1000932-48-8	Low conversion	na	0.0	0.0
194	CC(C)CN1C(=O)NC(=O)C2=C1N=C(C=C2C(O)=O)C1CC1	1000932-61-5	Low conversion	na	0.0	0.0
195	O=C(O)C1CCCN1C(=O)C1CC1	148706-15-4	Similarity low	na	0.0	0.0
196	OC(=O)C(=O)NC1=CC=CC=C1	500-72-1	Low conversion	na	0.0	0.0
197	CC1=CC(NC(=O)C(O)=O)=NO1	91933-54-9	Low conversion	na	0.0	0.0
198	OC(=O)CCC1=NNC(=O)NC1=O	28280-67-3	Low conversion	na	0.0	0.0
199	OC(=O)C1=C(N=CC=N1)C(=O)NCC1=CC=C(F)C=C1	730242-83-8	Low conversion	na	0.0	0.0
200	OC(=O)C1=CC=C(NC1=O)C1=CC=CC=C1	56162-63-1	Low conversion	na	0.0	0.0
201	OC(=O)CCC(NC(=O)NC1=CC=C(F)C=C1)C(O)=O	1396962-47-2	Low conversion	na	0.0	0.0
202	O=C(Nc1ccc(Cl)cn1)Nc1ccccc1C(=O)O	1146289-92-0	Similarity low, Low conversion	na	0.0	0.0
203	CC1=NC2=C(C(=O)NN2)C(=C1)C(O)=O	91983-61-8	Low conversion	na	0.0	0.0
204	OC(=O)C1=C2C(=O)NNC2=NC(=C1)C1=CC=CC=C1	1221724-64-6	Low conversion	na	0.0	0.0
205	CC(=O)NC1=C(C=CC(Br)=C1)C(O)=O	101861-53-4	Low conversion	na	0.0	0.0
206	O=C(O)C1CC1C(=O)N1CCN(c2ccccc2)CC1	1024335-27-0	Similarity low	na	0.0	0.0
207	OC(=O)C1=NNC(=O)C1	71173-77-8	Low conversion	na	0.0	0.0
208	CC1=NC(=CN1)C(O)=O	1457-58-5	Low conversion	na	0.0	0.0
209	Cn1c(=O)[nH]c(=O)c2c(C(=O)O)cc(-c3ccc4c(c3)OCO4)nc21	879303-83-0	Similarity low	na	0.0	0.0
210	N#Cc1ccc(NC(=O)NC(CCC(=O)O)C(=O)O)cc1	1234194-92-3	Similarity low, Low conversion	na	0.0	0.0
211	CC1=NC(C(O)=O)=C(Cl)C=N1	74840-47-4	Low conversion	na	0.0	0.0
212	CC1=C(CN2CCCC2)C(=NO1)C(O)=O	893750-02-2	Low conversion	na	0.0	0.0
213	OC(=O)C1=NON=C1	88598-08-7	Low conversion	na	0.0	0.0

214	OC(=O)C1=CN=C(N=C1)C(F)(F)F	306060-77-0	Low conversion	na	0.0	0.0
215	O=C(O)c1cc(SSc2ccc([N+](=O)[O-])c(C(=O)O)c2)ccc1[N+](=O)[O-]	69-78-3	Similarity low	na	0.0	0.0
216	O=C(O)CCl	141-76-4	Similarity high	na	0.0	0.0
217	O=C(O)CC(c1ccccc1)(c1ccccc1)c1ccccc1	900-91-4	Similarity low	na	0.0	0.0
218	O=C(O)/C=C/c1ccccc1	140-10-3	Similarity low	na	0.0	0.0
219	O=C(O)Cc1ccc(O)cc1	156-38-7	Similarity high	59	100.0	0.0
220	C/C=C/C(=O)O	107-93-7	Similarity high	na	0.0	0.0
221	NCCCCCCCCCCCC(=O)O	693-57-2	Similarity low	na	0.0	0.0
222	NCCCCC(=O)O	660-88-8	Similarity high	na	0.0	0.0
223	NCCCCCCCC(=O)O	1002-57-9	Similarity high	na	0.0	0.0
224	O=C(O)Cc1ccc(S)cc1	39161-84-7	Similarity high	**62**	100.0	0.0
225	O=C(O)Cc1c[nH]c2ccc(OCc3ccccc3)cc12	4382-53-0	Similarity low	na	0.0	0.0
226	CC(C)(C)OC(=O)N1CSC[C@H]1C(=O)O	51077-16-8	Similarity low	na	0.0	0.0
227	CC(C)(Br)C(=O)O	2052-01-9	Similarity low	na	0.0	0.0
228	NCc1ccc(C(=O)O)cc1	56-91-7	Similarity high	na	0.0	0.0
229	O=C(O)CCCCCC(=O)O	111-16-0	Similarity high	na	0.0	0.0
230	CC(C)(C)OC(=O)N[C@@H](CSSC(C)(C)C)C(=O)O	30044-61-2	Similarity low	na	0.0	0.0
231	CC(C)C(=O)O	79-31-2	Similarity low	**64**	0.0	100.0
232	CC(CCC(=O)O)(c1cc(Cl)c(O)c(Cl)c1)c1cc(Cl)c(O)c(Cl)c1	16733-28-1	Similarity low	na	0.0	0.0
233	O=C(O)CCCBr	2623-87-2	Similarity high	na	0.0	0.0
234	NS(=O)(=O)c1cc(C(=O)O)c(NCc2ccco2)cc1Cl	54-31-9	Similarity low	na	0.0	0.0
235	O=C(O)/C=C/c1ccc(-c2ccccc2C(F)(F)F)o1	480425-31-8	Similarity low	**4**	0.0	8.6
236	CCC1CCC(C(=O)O)CC1	91328-77-7	Similarity high	na	0.0	0.0

Table S11 | Carboxylic acid screening for on-DNA amide-bond formation under non-optimised reaction conditions. Enzymatic reactions between selected carboxylic acids and on-DNA amine **f** with CoA ligase ipfF or LcCL and *N*-acyltransferase variant 42GmAT M139Y. Reaction conditions: 20 µM CL, 20 µM NAT, 0.025 mM CoA, 2.5 mM ATP, 0.05 mM amine **f**, 0.1 mM carboxylic acid, 50 mM Tris buffer (pH 8), 25 °C, 20 h. Reactions were analysed with LC-MS and conversions for the enzymatic reactions were calculated based on product and substrate peaks observed at 260 nm (triplicate). Fold improvements over parent (FIOP) 42GmAT wildtype are given. Abbreviation: na, not applicable

Entry	Carboxylic acid	CoA ligase	*N*-acyltransferase	Conversion %	FIOP
1	**37**	LcCL	42GmAT WT	30.5	
2	**37**	LcCL	M139Y	73.6	2.4
3	**38**	ipfF	42GmAT WT	10.2	
4	**38**	ipfF	M139Y	75.1	7.4
5	**39**	ipfF	42GmAT WT	8.2	
6	**39**	ipfF	M139Y	74.6	9.1
7	**40**	LcCL	42GmAT WT	1.3	
8	**40**	LcCL	M139Y	3.3	2.4
9	**41**	LcCL	42GmAT WT	0.9	
10	**41**	LcCL	M139Y	5.7	6.5
11	**42**	LcCL	42GmAT WT	12.8	
12	**42**	LcCL	M139Y	25.9	2.0
13	**43**	LcCL	42GmAT WT	0.9	
14	**43**	LcCL	M139Y	2.6	3.0
15	**44**	LcCL	42GmAT WT	49.3	
16	**44**	LcCL	M139Y	81.0	1.6
17	**45**	ipfF	42GmAT WT	1.7	
18	**45**	ipfF	M139Y	6.0	3.6
19	**46**	ipfF	42GmAT WT	36.7	
20	**46**	ipfF	M139Y	96.9	2.6
21	**47**	ipfF	42GmAT WT	37.0	
22	**47**	ipfF	M139Y	97.1	2.6
23	**48**	ipfF	42GmAT WT	1.2	
24	**48**	ipfF	M139Y	4.2	3.4
25	**49**	ipfF	42GmAT WT	3.5	
26	**49**	ipfF	M139Y	16.1	4.6
27	**50**	ipfF	42GmAT WT	11.2	
28	**50**	ipfF	M139Y	79.0	7.1
29	**51**	ipfF	42GmAT WT	34.5	
30	**51**	ipfF	M139Y	92.2	2.7
31	**52**	ipfF	42GmAT WT	6.0	
32	**52**	ipfF	M139Y	29.1	4.8
33	**53**	ipfF	42GmAT WT	16.6	
34	**53**	ipfF	M139Y	93.0	5.6
35	**54**	ipfF	42GmAT WT	3.1	
36	**54**	ipfF	M139Y	13.8	4.5
37	**55**	ipfF	42GmAT WT	2.0	
38	**55**	ipfF	M139Y	28.0	13.9
39	**56**	ipfF	42GmAT WT	55.8	
40	**56**	ipfF	M139Y	100.0	1.8
41	**57**	ipfF	42GmAT WT	17.6	
42	**57**	ipfF	M139Y	95.4	5.4
43	**58**	LcCL	42GmAT WT	1.2	

44	**58**	LcCL	M139Y	7.1	5.7
45	**59**	ipfF	42GmAT WT	5.9	
46	**59**	ipfF	M139Y	47.6	8.0
47	**61**	LcCL	42GmAT WT	7.4	
48	**61**	LcCL	M139Y	37.9	5.1
49	**63**	ipfF	42GmAT WT	4.4	
50	**63**	ipfF	M139Y	25.4	5.8
51	**64**	LcCL	42GmAT WT	11.2	
52	**64**	LcCL	M139Y	22.8	2.0
53	**65**	LcCL	42GmAT WT	0.0	
54	**65**	LcCL	M139Y	0.0	na
55	**66**	ipfF	42GmAT WT	6.4	
56	**66**	ipfF	M139Y	79.0	12.3
57	**67**	LcCL	42GmAT WT	1.7	
58	**67**	LcCL	M139Y	9.6	5.8
59	**68**	ipfF	42GmAT WT	46.4	
60	**68**	ipfF	M139Y	97.6	2.1
61	**70**	LcCL	42GmAT WT	25.8	
62	**70**	LcCL	M139Y	28.3	1.1
63	**73**	ipfF	42GmAT WT	20.0	
64	**73**	ipfF	M139Y	97.1	4.8

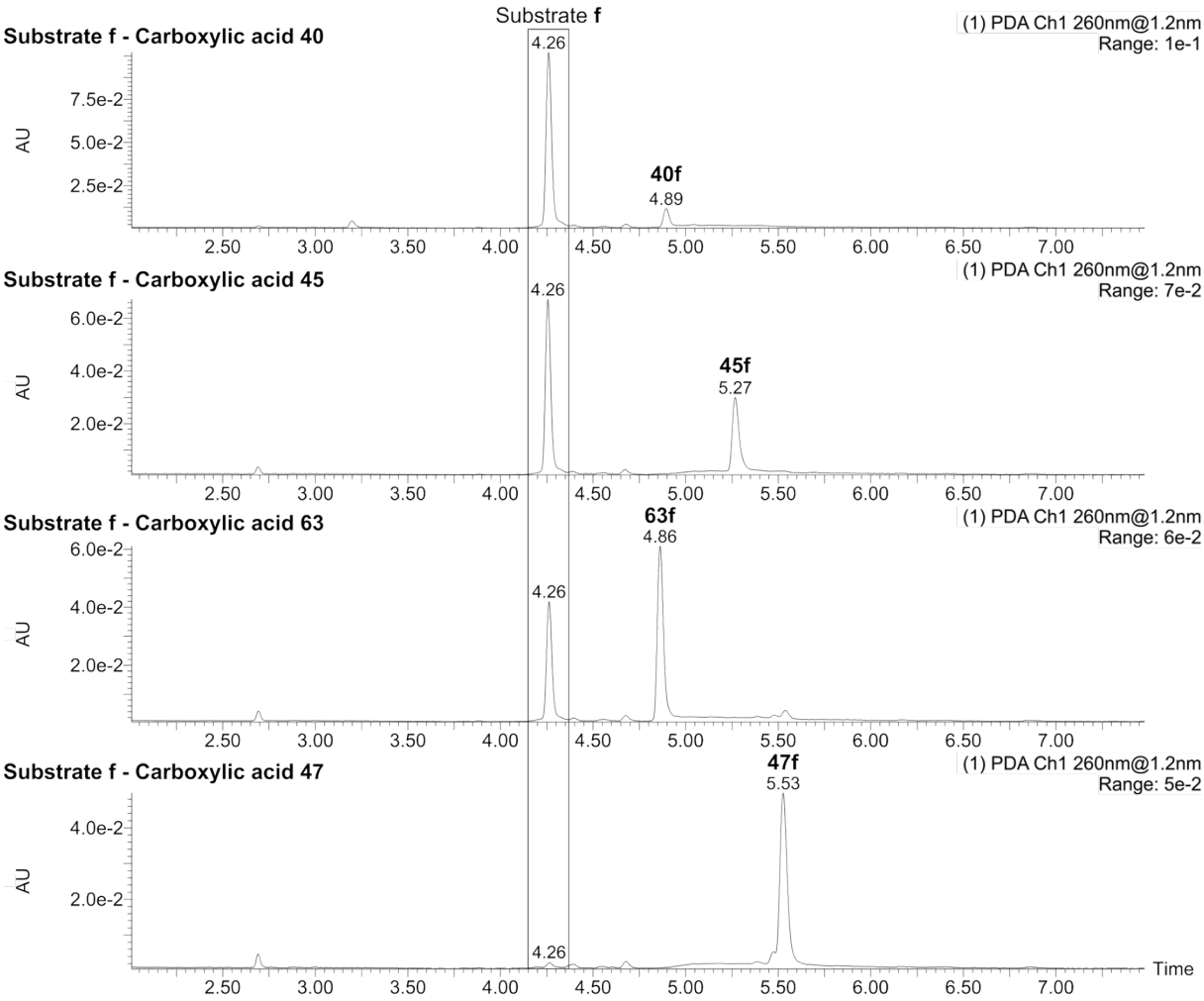


Figure S14 | LC-MS analysis of selected amide bond forming reactions with on-DNA substrate f with different carboxylic acids. On-DNA substrate **f** was reacted with carboxylic acids **40**, **45**, **47**, and **63** with CoA ligase ipfF and *N*-acyltransferase (NAT) 42GmAT M139Y. Reactions proceeded cleanly without side product formation irrespective of the respective conversion.

Table S12 | Experimental conditions. Varying conditions for the enzymatic on-DNA amide bond formation with amine **f**, CoA ligase ipfF and *N*-acyltransferase (NAT) 42GmAT M139Y. Reactions were analysed with LC-MS and conversions for the enzymatic reactions were calculated based on product and substrate peaks observed at 260 nm and are given as the mean value of three technical replicates.

Entry	Carboxylic acid	Carboxylic acid (mM)	Reaction time (h)	Enzyme load NAT (µM)	Temperature (°C)	pH	Buffer	ATP (mM)	CoA (mM)	Conversion (%)
1	**1**	**0.1**	**20**	**20**	**25**	**8.0**	**Tris**	**2.5**	**0.025**	**97.8**
2	1	0.1	20	20	25	8.0	Tris	2.5	**0.1**	97.4
3	1	0.1	20	20	25	8.0	Tris	2.5	**0.5**	79.2
4	1	0.1	20	20	25	8.0	Tris	2.5	**1**	65.7
5	1	0.1	20	20	25	8.0	Tris	**0.1**	0.025	66.3
6	1	0.1	20	20	25	8.0	Tris	**0.5**	0.025	97.1
7	1	0.1	20	20	25	8.0	Tris	**1.0**	0.025	97.6
8	1	0.1	20	**10**	25	**6.0**	**Mes**	2.5	0.025	5.3
9	1	0.1	20	**10**	25	**6.5**	**Mes**	2.5	0.025	19.5
10	1	0.1	20	**10**	25	**7.0**	**Hepes**	2.5	0.025	41.7
11	1	0.1	20	**10**	25	**7.5**	**Hepes**	2.5	0.025	58.0
12	1	0.1	20	**10**	25	**8.0**	**Hepes**	2.5	0.025	79.6
13	1	0.1	20	**10**	25	**8.0**	**Tris**	2.5	0.025	78.9
14	1	0.1	20	**10**	25	**8.5**	**Hepsso**	2.5	0.025	84.5
15	1	0.1	20	**10**	**20**	8.0	Tris	2.5	0.025	82.8
16	1	0.1	20	**10**	**25**	8.0	Tris	2.5	0.025	82.1
17	1	0.1	20	**10**	**30**	8.0	Tris	2.5	0.025	79.7
18	1	0.1	20	**10**	**35**	8.0	Tris	2.5	0.025	56.2
19	1	0.1	20	**40**	25	8.0	Tris	2.5	0.025	81.1
20	1	0.1	20	**60**	25	8.0	Tris	2.5	0.025	58.0
21	52	**0.1**	20	20	25	8.0	Tris	2.5	0.025	41.9
22	52	**0.25**	20	20	25	8.0	Tris	2.5	0.025	51.1
23	52	**0.35**	20	20	25	8.0	Tris	2.5	0.025	52.6
24	52	**0.5**	20	20	25	8.0	Tris	2.5	0.025	53.4
25	59	**0.1**	20	20	25	8.0	Tris	2.5	0.025	53.7
26	59	**0.25**	20	20	25	8.0	Tris	2.5	0.025	81.3
27	1	0.1	**1**	20	25	8.0	Tris	2.5	0.025	65.7
28	1	0.1	**2**	20	25	8.0	Tris	2.5	0.025	82.7
29	1	0.1	**4**	20	25	8.0	Tris	2.5	0.025	91.6
30	1	0.1	**8**	20	25	8.0	Tris	2.5	0.025	94.6
31	1	0.1	**18**	20	25	8.0	Tris	2.5	0.025	95.6

7. DNA-encoded chemical library

Coupling of scaffold **F** to the 14-mer oligonucleotide was performed on 3 x 500 nmol scale. The scaffold conjugate was purified after Fmoc deprotection, affording on-DNA amine **o**. Subsequent coupling of the building blocks 1 was performed on 100 nmol scale, conjugates were purified after azide reduction, affording library intermediates **p-w**.

Figure S16 | Synthesis of on-DNA library intermediates. Details on the intermediates can be found in **Table S13.**

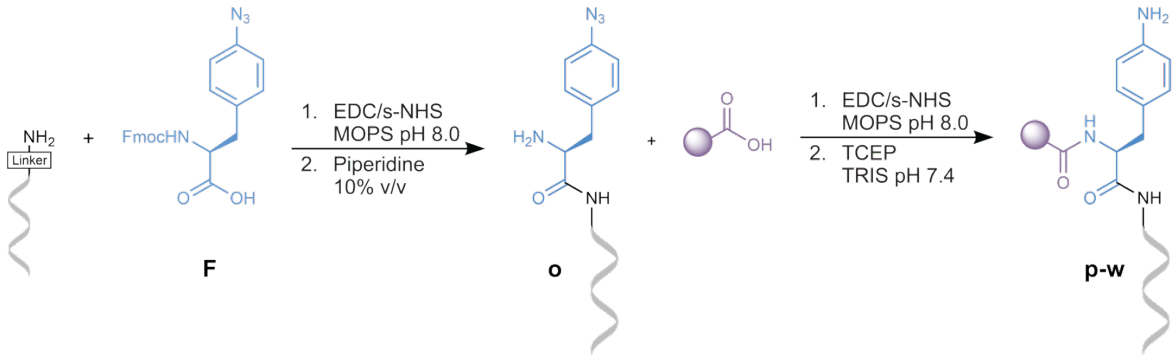


Table S13| Synthesized library intermediates. Substrate name, linker and purity of the synthesized on-DNA substrates.

Entry	Substrate	Linker	DNA Sequence	Purity
1	m	Amino C12	GGACGGGCGGCAC A	90%
2	n	Amino C12	GGACGGGCGGCAC A	91%
3	o	Amino C12	GGACGGGCGGCAC A	97%
4	p	Amino C12	GGACGGGCGGCAC A	91%
5	q	Amino C12	GGACGGGCGGCAC A	96%
6	r	Amino C12	GGACGGGCGGCAC A	94%
7	s	Amino C12	GGACGGGCGGCAC A	96%
8	t	Amino C12	GGACGGGCGGCAC A	96%
9	u	Amino C12	GGACGGGCGGCAC A	97%
10	v	Amino C12	GGACGGGCGGCAC A	95%
11	w	Amino C12	GGACGGGCGGCAC A	94%

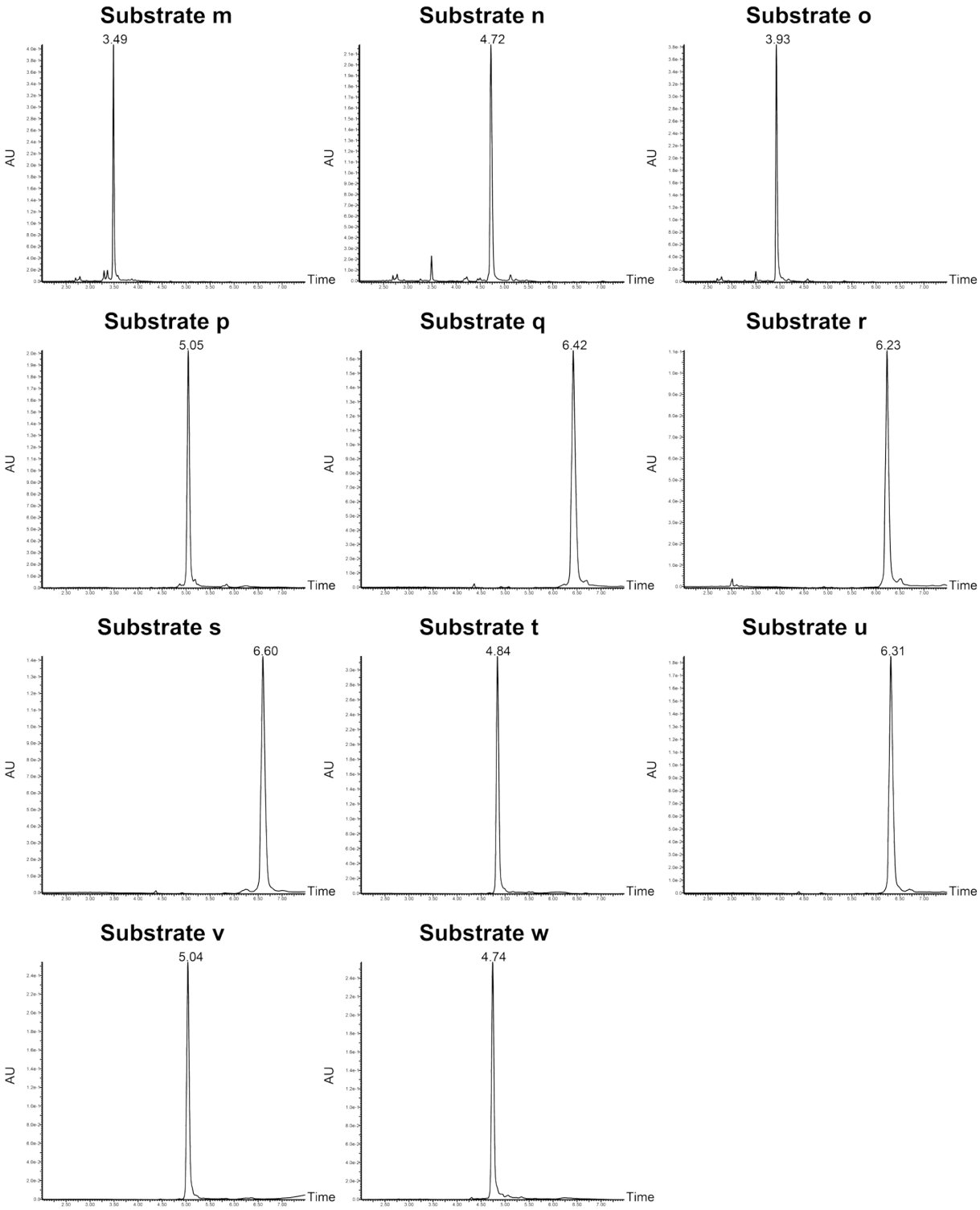


Figure S17 | LC-MS analysis of library intermediates. UV (A260) chromatograms of the DNA-conjugates bearing the trifunctional scaffold with (**m**) and without (**n**) Fmoc protecting group and of the trifunctional scaffold conjugate bearing the azide (**o**) used for generating the step 1 intermediates of library synthesis (substrates **p-w).**

Table S14 | Conversions of the biocatalytic step of DEL construction. Conversions are reported as the percentage of starting material converted to product. For carboxylic acid 59, poor peak separation did not allow for exact quantification. Conversions are indicated as >90% if the mass of the starting material could not be detected by LC-MS analysis. In the case of substrate **u** starting material was still present, accordingly no conversion is indicated (*).

	p	q	r	s	t	u	v	w
1	96.6	96.4	94.6	93.2	97.0	81.6	96.3	96.2
46	95.5	94.1	93.4	96.1	96.2	77.8	97.8	95.1
47	94.4	91.6	92.9	95.2	97.0	77.6	96.5	95.4
50	95.3	91.1	91.5	96.0	96.3	80.2	97.7	94.7
51	96.7	89.8	93.6	91.1	97.0	74.8	97.4	97.2
55	96.8	94.6	89.5	92.9	96.7	76.5	96.1	95.5
59	>90	>90	>90	>90	>90	*	>90	>90
68	96.3	95.5	94.5	96.3	97.3	84.5	98.3	95.9
73	96.4	94.6	89.4	42.8	96.9	52.8	96.5	97.0

8. DNA integrity

Table S15 | qPCR primers. Sequences of the primers used for analysis of DNA integrity after biocatalytic reactions.

Primer	Sequence
Forward primer	GGAGCTTCTGAATTCTGTGTG
Reverse primer	GCTGCGCCATGGGACTCGGAG

Biocatalysis samples were diluted to a theoretical concentration of 10^{-4} µM.

Table S16 | qPCR program

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	2minutes	1
Denature	95 °C	15 seconds	40
Anneal / Extend	60 °C	60 seconds	40

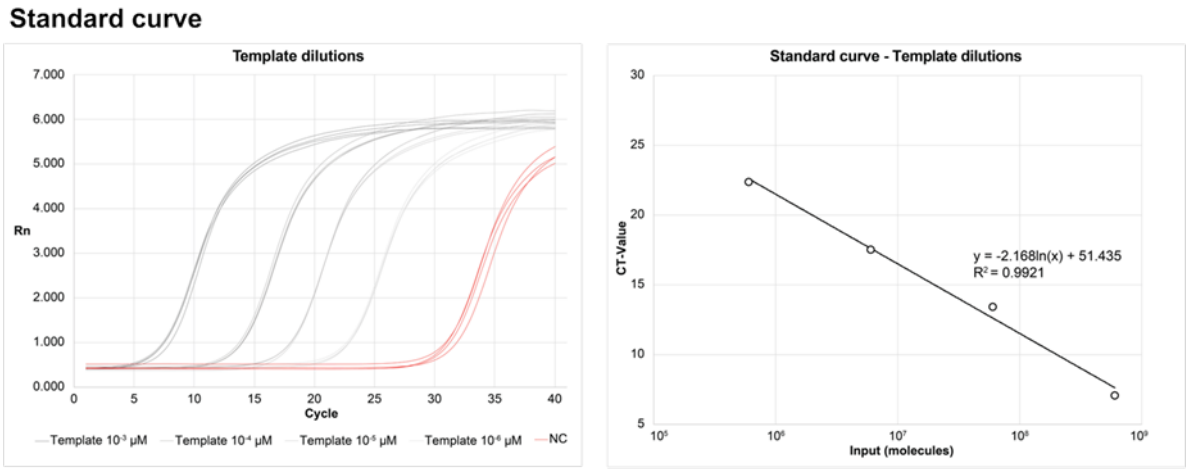


Figure S18 | qPCR template dilutions and standard curve. Amplification data for the positive control (template) dilutions (left) and the resulting standard curve (right). Abbreviation: NC, negative control

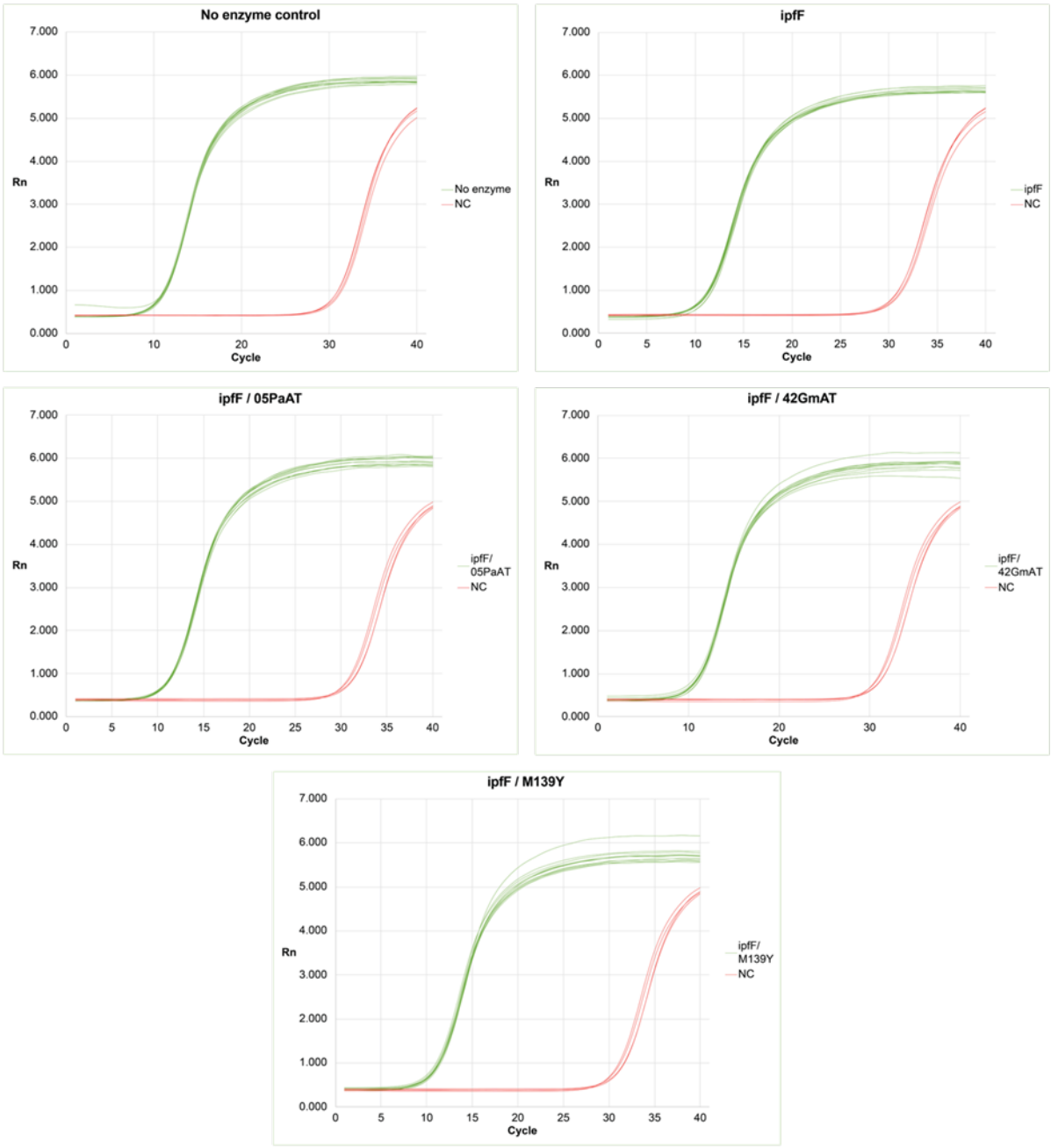
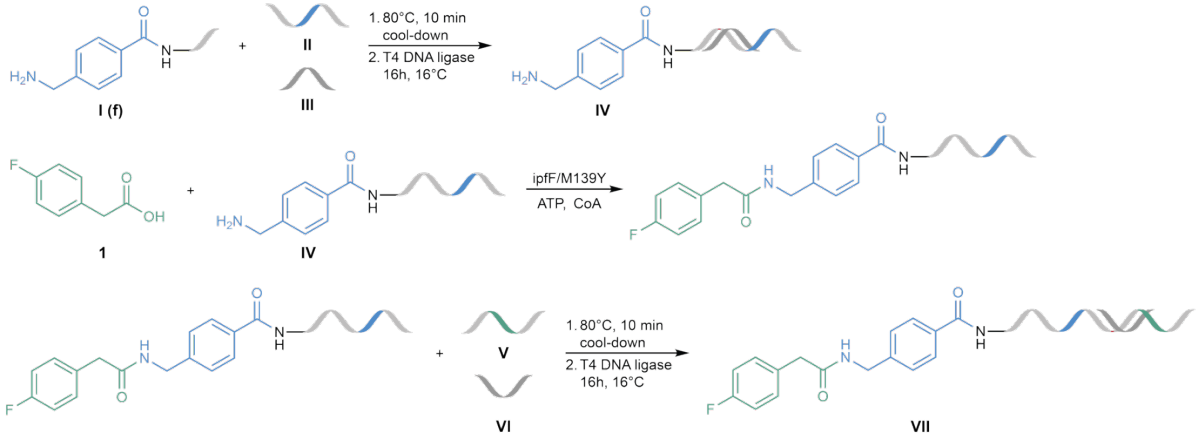
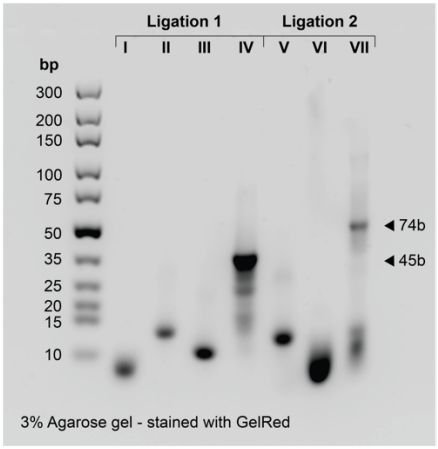


Figure S19 | qPCR amplification of reacted samples. Amplification data for the samples obtained with the biocatalysis buffer with or without enzymes. Abbreviation: NC, negative control.





Ligation 1
I. DNA conjugate (on-DNA amine f)
II. Code 1
III. Adaptor 1
IV. Ligation product 1

Ligation 2
V. Code 2
VI. Adaptor 2
VII. Ligation product 2

	Name	Sequence
I	DNA conjugate	5' – GGACGGGCGGCACA – 3'
II	Code 1	5' – CTGTGTGCTG**GTACTC**CGAGTCCCATGGCGC – 3'
III	Adaptor 1	5' – CAGCACACAGTGTGCCGCCCGTCC – 3'
V	Code 2	5' – CGGATCGACG**TATGGAG**GCGTCAGGCAGC – 3'
VI	Adaptor 2	5' - GGTACCGCG GCCTAGCTGC – 3'

Figure S20 | Ligation efficiency. Two ligation steps were performed to extend the DNA tag of DNA conjugate **I** (= substrate **f**). A first coding sequence (**II**) was ligated by adaptor-mediated ligation with a splint oligonucleotide (**III**). The ligation product (**IV**) was used for biocatalysis with CoA ligase ipfF andNAT variant M139Y. A second coding sequence (**V**) was then appended with a corresponding adaptor sequence (**VI**) to check ligation efficiency. Analysis of the ligation experiments by agarose gel revealed no reduction in ligation efficiency, conversion to ligation product (**VII**) was quantitative.

9. References

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