Metagenome guided medicinal chemistry yields improved Gram-negative active albicidin and cystobactamid type antibiotics

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Experimental Procedures

1.1 Construction of PABA phylogenetic tree

The construction and screening of soil environmental DNA (eDNA) libraries have been described in detail previously.[1, 2] In this study, 15 previously achieved eDNA cosmid libraries were used to screen biosynthetic gene clusters (BGCs) that encode PABA based metabolites. Briefly, each cosmid library containing ~20 million eDNA cosmid clones was constructed into EC100 cells and arrayed into 2x384 well plates for PCR screening and clone recovery. To identify wells containing PABA specific BGCs, all libraries were screened by PCR using barcoded A domain degenerate primers (A3F: 5′-GCSTACSYSATSTACACSTCSGG-3' and A7R: (5′-SASGTCVCCSGTSCGGTA-3').[3] Methods for primer barcoding, PCR conditions, MiSeq sequencing, and sequence data processing have been described previously in detail.[4] The resulting amplicon sequence data were analyzed using eSNaPD (Environmental Surveyor of Natural Product Diversity) software[5] to identify hits related to PABA selective A domain sequences from albicidin and cystobactamid BGCs. Sequences that matched known PABA selective A domain sequences with E-values less than 10^{25} were considered hits. These sequences were aligned together with known PABA selective A domain sequences using MUSCLE.[6] The resulting phylogenetic tree was visualized using iTOLv5 software.[7] Hits found in new clades that are phylogenetically close to those containing known PABA selective A domain sequences were considered to be associated with potentially novel PABA-encoding BGCs.

1.2 Recovery of PABA-containing BGCs

The well locations of cosmids containing BGCs that encode PABA-containing metabolites were identified using well-specific barcode sequences incorporated in the A domain degenerate primers. Specific primers targeting each unique sequence of interest were designed manually. To recover single cosmid clones of interest, a serial dilution PCR method described previously was used.[2, 4] Recovered single clones containing PABA encoding sequences were mini-prepped and sequenced by Illumina MiSeq technology. To identify cosmids with inserts overlapping the initially recovered primary cosmid, primers pairs were designed to amplify ~500 bp from each edge of primary cosmid. These primers were utilized to screen cosmid subpools. Identified overlapping cosmids were recovered as described above. The overlapping cosmids were then sequenced and assembled *in silico* with primary cosmid sequences into a single continuous sequence using Geneious software (Version 11.0.3).

1.3 Bioinformatic prediction of PABA-containing BGCs

Fully assembled PABA-containing BGCs were analyzed using an annotation pipeline consisting of open reading frame (ORF) predictions by MetaGeneMark[8] and BLAST searches[9]. The annotation script was developed using the Python Programming language and is available in the open source repository: https://github.com/brady-lab-rockefeller/geneannotation. Each NRPS A domain was analyzed using antiSMASH 5.0 (bacterial version) to identify its 10-amino acid A domain signature code including amino acids at positions 235, 236, 239, 278, 299, 301, 322, 330, 331, and 517.[10] These 10 amino acid signatures were then compared to known A domain signatures. This information combined with the predicted functions of any an encoded tailor enzyme were used to predict the building blocks of the PABA-containing congeners encoded by each BGC.

1.4 Synthesis of PABA-containing congeners

Instruments, reagents, and consumables: Unless stated otherwise, all reactions conducted in organic solvents were performed in oven-dried glassware under an atmosphere of argon or nitrogen. Dry THF, DMF, and CH_2Cl_2 were purchased and stored in Sure/Seal bottles obtained from Sigma-Aldrich. Coupling reagent 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxid hexafluorophosphate (HATU) and standard amino acid building blocks were purchased from P3 Biosystems (Louisville, KY). All other reagents and consumables were purchased from Sigma-Aldrich (St. Louis, MO), Thermo Fisher Scientific (Waltham, MA), or VWR (Radnor, PA). Purchased reagents and chemicals were used as received, unless stated otherwise. Reactions were monitored by thin-layer chromatography (TLC) on glass silica gel 60 F_{254} plates by EMD Millipore and visualized by UV irradiation or development with p-anisaldehyde or ninhydrin stain. Volatile solvents were removed under reduced pressure using a rotary evaporator. Flash column chromatography was conducted using a CombiFlash R_f 200 system by Teledyne ISCO using normal-phase RediSep Rf silica gel columns or completed manually using Sorbtech standard grade silica gel (particle size: 40-63 μm). Target compounds were purified using an Agilent 1200 series HPLC (Santa Clara, CA) equipped with a Waters XBridge C18 5μm column (10 mm x 250 mm). Samples were eluted using a linear gradient of solvent B (ACN with 0.1% formic acid) over solvent A (H_2O with 0.1% formic acid). Prep-HPLC purifications were completed using a Shimadzu LC-20AB HPLC equipped with a Phenomenex C18 15 μm column (150 mm x 40 mm) or Phenomenex C18 3 μm column (75 mm x 30 mm) eluting with a linear gradient of solvent B (ACN with 0.1% TFA) over solvent A (H_2O with 0.1% TFA). High-resolution mass spectra (HRMS) were obtained using a Sciex X500R Q-TOF system (Framingham, MA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker AV600 or Bruker Avance NEO operating at

600 or 400 MHz for ^{1}H (151 MHz for ^{13}C) in $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in ppm using the residual solvent peak as an internal reference ($CDCl_3$ or DMSO-d_6). Multiplicity (brs = broad singlet, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet) and coupling constants (J = Hz) are quoted where possible.

1.5 Microbial susceptibility assay

Syn-BNPs were tested against a panel of ESKAPE pathogens as well as the ciprofloxacin-resistant bacteria shown in Supplementary Table S1. MIC assays were conducted following the protocol recommended by the Clinical and Laboratory Standards Institute.[11] Assay were performed in duplicate in 96-well microliter plates. Syn-BNPs were dissolved into DMSO to give 1.6 mg/mL stock solutions. Ciprofloxacin (Sigma) was used as positive control. Stock solutions were diluted across 96-well plates using a 2-fold serial dilution to give a concentration range of 16 to 0.015 µg/mL in 50 µL of LB broth. The top and bottom rows of each plate were filled with 100 µL of LB broth without compound to avoid edge effects. The last well in each row contained bacteria but did not contain compounds. A single colony of each bacterial assay strain was inoculated into 5 mL of LB broth medium and grown overnight at 37 °C. The saturated overnight culture was diluted 5,000-fold in fresh LB, and 50 µL were transferred into each well of the assay plates. Each well contained a total volume of 100 µL. MIC values were determined by visual inspection of the minimum concentration that prevented bacteria growth after 18 h of static incubation at 37°C.

1.6 Resistant mutation generation

Autoclaved LB agar was cooled to 55 °C and mixed with ciprofloxacin at a concentration of 4x its MIC against *E. coli* ATCC25922. The mixture was poured into a 150 x 15 mm petri dish and allowed to solidify. A single colony of *E. coli* ATCC25922 was used to inoculate 5 mL of LB medium and was grown overnight at 37 °C. 500 µL of the overnight culture was used to inoculate 50 mL of fresh LB. Upon reaching log phase, $1x10^8$ cells from the culture were spread on the ciprofloxacin-containing agar plate. The plate was incubated overnight at 37 °C and the number of resistant colonies were counted after a 16 h incubation. Resistant colonies were struck on ciprofloxacin-containing plates to confirm the resistant phenotype. To identify point mutations in the quinolone-resistance-determining region (QNDR), resistant colonies were subjected to colony PCR using the following primers: GyrA-FW: TGCCAGATGTCCGAGAT, GyrB-RV: GTATAACGCATTGCCGC. The PCR products were Sanger sequenced and the amplicon sequences were aligned with the wild-type of GyrA QNDR sequence using SnapGene (Version 5.2.1) to reveal point mutations.

1.7 DNA gyrase supercoiling assay

An *E. coli* gyrase supercoiling assay kit from TopoGEN was used for *in vitro* DNA gyrase supercoiling assays. Serial dilutions of PABA syn-BNPs as well as a ciprofloxacin positive control were prepared [concentration range from 0.04 to 10.24 µM] in DMSO. Assays were performed according to the manufacture's protocol. Briefly, fresh diluted *E. coli* gyrase (1U) was mixed with each inhibitor and 250 ng relaxed plasmid pHOT1. The final volume of each reaction was adjusted to 20 µL with H_2O. A reaction with all materials except enzyme was used as a control. Reactions were quenched after 60 min at 37°C by the addition of 2 µL of 10% (w/v) SDS and then treatment for 30 min with 50 µg/mL proteinase K. Relaxed (REL) and supercoiled (SC) plasmids were extracted with chloroform/isoamyl alcohol (24:1 mixture), separated on 1% (w/v) agarose gels, and visualized by 0.5 mg/mL ethidium bromide staining. Image analysis (intensity of SC plasmid bands) was performed using Analytik Jena image software. The intensity of supercoiled bands was used to determine the IC_{50} of each syn-BNP.

1.8 AlbD expression and purification

The gene sequence of AlbD from *Pantoea dispersa* was retrieved from NCBI (AAB71813). It was synthesized by Genewize (USA) to include NdeI and EcoRI restriction sites, which were used to clone the gene into the pET28c vector. pET28c-AlbD was introduced into *E. coli* BL21(DE3) cells (NEB) by electroporation. For recombinant expression of AlbD, a single colony was used to inoculate 50 mL of fresh LB broth containing 50 µg/mL kanamycin. The culture was grown overnight at 37°C with shaking at 220 rpm. The next day, 500 µL of the overnight culture was used to inoculate 1 L of fresh LB containing 50 µg/mL kanamycin. The culture was grown with shaking at 220 rpm to $OD_{600\ nm}$ 0.8-1.0. For protein expression, the culture was cooled down to 16 °C and allowed to grow for additional 8-12 h in the presence of 0.5 mM of isopropyl-ß-D-thiogalactoside (IPTG). The culture was then harvested by centrifugation at 3,724 g for 10 min. The cell pellet was lysed in B-PER bacterial protein extraction solution (ThermoFisher) following the manufacturer's protocol. The lysate was centrifuged at 21,1300 g for 10 min and the insoluble cellular material was removed. The supernatant was injected into an AKTA system (GE-Healthcare) equipped with a 1 mL His-trap-FF column. Recombinant proteins were eluted using a 5 to 100% gradient of buffer A to buffer B over 20 column volume. (Buffer A: 20 mM Tris-HCl pH 7.5, 10% glycerol, 150 mM NaCl. Buffer B: 20 mM Tris-HCl pH 7.5, 10% glycerol, 150 mM NaCl, 1 M Imidazole). Eluted fractions were collected and checked by SDS-PAGE to identify fractions containing AlbD. Fractions containing AlbD were pooled and concentrated by centrifugation using a 10 kDa concentrator (Millipore Corp., USA). The concentration of purified protein was determined using a Bradford assay (Sigma) and BSA as a control.

1.9 AlbD cleavage assay

The AlbD cleavage assay was performed using a previously reported protocol with slight modifications.[12] 120 µM of tested compounds and 50 µM of purified AlbD protein were added to 100 µL of assay buffer - 20mM Tris-HCl pH 7.5, 10% glycerol, 150 mM NaCl. An AlbD-free assay mixture containing only test compound was used as negative control. Reactions were incubated for 20 min at 28°C and stopped by adding 350 µL of methanol to precipitated protein and salts. The precipitated pellet was removed by centrifugation at 211,300 g for 15 min. An aliquot of supernatant (5 µL) was analyzed via LCMS (Waters Acquity UPLC). Runs were completed using a gradient of 5-95% H_2O/acetonitrile (0.1% formic acid) over 6 min. All reactions were performed in duplicate and repeated three times. The efficiency of cleavage for each albicidin congener is calculated by a relative cleavage ratio (RCR) compared to the cleavage of albicidin. The remaining peak area of a digested antibiotic (AUC congener) was divided by the peak area for a reaction with same antibiotic but no AlbD (AUC control). Each normalized cleavage ratio (1-(AUCcongener/AUCcontrol)) was divided by the normalized cleavage ratio an AlbD-catalyzed albicidin cleavage (1-(AUCALBI/AUCALBIcontrol)) to obtain a relative cleavage ratio (RCR) compared to albicidin.

2.0 Time-dependent killing assay

An overnight culture of *E. coli* ATCC25922 were diluted 1:10,000 in MHB medium and incubated at 37°C for 2 h. Exponential phase bacteria (3 mL) were then challenged with syn-BNPs and ciprofloxacin at 10 x MICs in culture tubes at 37°C and 200 r.p.m. An untreated sample was used as negative control. After 1, 4, 9 and 24 hours of incubation, 20 µL aliquots were removed, centrifuged and resuspended in PBS buffer. Serial diluted suspensions were then plated on MHB agar plates and incubated at 37°C overnight. Colonies were counted. Experiments were performed in triplicate.

Supplementary Table S1. Strain information

Group	Name of Bacteria	Strain
Firmicutes	*Escherichia coli*	ATCC25922
	Escherichia coli_gyrA(D87G)	ATCC25922
	Escherichia coli_gyrA(S83L)	ATCC25922
	Escherichia coli	BL21(DE3)
ESKAPE pathogens	*Enterococcus faecium*	Com15
	Staphylococcus aureus	SH1000
	Klebsiella pneumonia	ATCC10031
	Acinetobacter baumannii	ATCC17978
	Pseudomonas aeruginosa	PAO1
	Enterobacter cloacae	ATCC13047
	Staphylococcus aureus	USA300
	Staphylococcus aureus	ATCC BAA-42
	Staphylococcus aureus	ATCC BAA-1721
	Staphylococcus aureus	NRS146
	Staphylococcus epidermidis	RP62A
	Enterococcus faecalis	781
	Enterococcus faecium	EF16
	Enterococcus casseliflavus	788

Supplementary Table S2. MIC values of syn-BNPs against engineered *E. coli*.

Bacteria	Albicidin	PABA48	PABA70	PABA57	PABA34	PABA157	PABA95	PABA95-2	Ciprofloxacin
E.coli-BL21 (pET28c)	0.0019	0.06	0.5	0.5	0.0075	0.0075	0.015	0.06	0.0009
E.coli-BL21 (pET28c-AlbD)	0.25	>8.0	>64	>64	0.03	1.0	2.0	0.5	0.0019
Fold increase	131	>133	>128	>128	4	133	133	8	2

Supplementary Table S3. PABA48 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	1932	P48A	Iterative methylation of PABA	Cobalamin B12-binding domain-containing protein [*Cystobacter ferrugineus*], WP_071903907.1	86%
2	639	P48B	O-methyltransferase of hydroxy-Asn	SAM-dependent methyltransferase [*Cystobacter ferrugineus*], WP_120590329.1	77%
3	696	P48C	Hydroxylation of PABA	Glyoxalase/bleomycin resistance protein/dioxygenase [*Cystobacter sp. Cbv34*], AKP45388.1	73%
4	612	P48D	Self-resistance protein	Pentapeptide repeat-containing protein [*Cystobacter ferrugineus*], WP_071903904.1	76%
5	426	P48E	Unknown	IS5 family transposase [*Methylorubrum extorquens*], OHV16442.1	63%
6	1656	P48F	Unknown	ISL3 family transposase [*Corallococcus macrosporus*], WP_095960599.1	87%
7	1188	P48G	Unknown	Cytochrome P450 [*Dictyobacter kobayashii*], WP_126554500.1	74%
8	198	P48H	A domain activation	MbtH family protein [*Cystobacter ferrugineus*], WP_071903903.1	68%
9	927	P48I	Aminoacyltransfersae	Alpha/beta hydrolase [*Corallococcus sp. CA054B*], WP_120590331.1	69%
10	1377	P48J	Dioxygenation of PABA	Phenylacetate-CoA oxygenase [*Corallococcus sp. CA054B*], WP_120590332.1	75%
11	2193	P48K	PABA biosynthesis	Aminodeoxychorismate synthase component I [*Cystobacter ferrugineus*], WP_071903900.1	79%
12	819	P48M	Thioesterase	Thioesterase [*Cystobacter ferrugineus*], WP_071903899.1	75%
13	1038	P48N	O-methyltransferase of PABA	Methyltransferase domain-containing protein [*Cystobacter ferrugineus*], WP_071903898.1	88%
14	5973	P48O	NRPS	Non-ribosomal peptide synthetase [*Cystobacter ferrugineus*], WP_071903897.1	74%
15	2934	P48P	NRPS	Non-ribosomal peptide synthetase [*Cystobacter ferrugineus*], WP_187345315.1	84%
16	819	P48Q	PABA biosynthesis	aminodeoxychorismate lyase [*Corallococcus sp. CA054B*], WP_158616735.1	86%
17	984	P48R	Hydroxylation of Asn	TauD/TfdA family dioxygenase [*Cystobacter ferrugineus*], WP_071903894.1	85%
18	6135	P48S	NRPS	Non-ribosomal peptide synthetase [*Cystobacter* sp. Cbv34], AKP45399.2	75%
19	4713	P48T	NRPS	Non-ribosomal peptide synthetase [*Cystobacter* sp. Cbv34], AKP45399.2	66%
20	2106	P48U	NRPS	Non-ribosomal peptide synthetase [*Cystobacter* sp. Cbv34], AKP45399.2	82%
21	2721	P48V	Unknown	Benzoate-CoA ligase [*Cystobacter sp. Cbv34*], AKP45400.1	70%
22	1500	P48W	Transporter	MFS transporter [*Corallococcus sp. CA054B*], WP_158616558.1	78%
23	1074	P48R	PABA biosynthesis	3-deoxy-7-phosphoheptulonate synthase [*Cystobacter ferrugineus*], WP_071901016.1	91%

Supplementary Table S4. PABA70 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	639	P70A	O-methyltransferase of hydroxy-Asn	(SAM)-dependent O-methyltransferase [*Cystobacter sp. Cbv34*], AKP45386.1	78%
2	684	P70B	Hydroxylation of PABA	Glyoxalase/bleomycin resistance protein/dioxygenase [*Cystobacter sp. Cbv34*], AKP45388.1	74%
3	657	P70C	Self-resistance protein	Pentapeptide repeat-containing protein [*Cystobacter ferrugineus*], WP_071903904.1	77%
4	216	P70D	A domain activation	MbtH family NRPS accessory protein [*Corallococcus sp. CA054B*],	74%
5	960	P70E	Hydroxylation of PABA	Alpha/beta hydrolase [*Corallococcus sp. CA054B*], WP_120590331.1	71%
6	1407	P70F	Dioxygenation of PABA	Phenylacetate-CoA oxygenase subunit PaaI [*Corallococcus sp. CA054B*], WP_120590332.1	74%
7	2196	P70G	PABA biosynthesis	Para-aminobenzoate synthase [*Cystobacter sp. Cbv34*], AKP45392.1	78%
8	699	P70H	Thioesterase	Thioesterase [*Cystobacter ferrugineus*], WP_071903899.1	78%
9	1038	P70I	O-methyltransferase of PABA	Methyltransferase domain-containing protein [*Cystobacter ferrugineus*], WP_071903898.1	85%
10	5991	P70J	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45395	75%
11	2973	P70K	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45396.1	80%
12	819	P70M	PABA biosynthesis	Aminodeoxychorismate lyase [*Cystobacter ferrugineus*], WP_071903895.1	82%
13	1029	P70N	Hydroxylation of Asn	TauD/TfdA family dioxygenase [*Cystobacter ferrugineus*], WP_071903894.1	82%
14	12948	P70O	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45399.2	70%
15	2154	P70P	Unknown	Benzoate-CoA ligase [*Cystobacter sp. Cbv34*], OJH37549.1	70%
16	1536	P70Q	Transporter	MFS transporter [*Corallococcus sp. CA054B*], RKG70114.1	74%
17	789	P70R	Unknown	Ester cyclase [*Deltaproteobacteria bacterium*], MBA3820360.1	40%
18	893 partial	P70S	Iterative methylation of PABA	Radical SAM protein [*Corallococcus sp. CA054B*], RKG65320.1	85%

Supplementary Table S5. PABA57 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	660 partial	P57A	Dioxygenation of PABA	Phenylacetate-CoA oxygenase [*Corallococcus sp. CA054B*], WP_120590332.1	59%
2	2097	P57B	PABA biosynthesis	Para-aminobenzoate synthase [*Cystobacter sp. Cbv34*], AKP45392.1	67%
3	1797	P57C	Iterative methylation of PABA	Methyltransferase domain-containing protein [*Cystobacter ferrugineus*], WP_071903898.1	74%
4	5895	P57D	NRPS	Non-ribosomal peptide synthetase [*Cystobacter ferrugineus*], WP_071903897.1	64%
5	2760	P57E	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45396.1	71%
6	969	P57F	Hydroxylation of Asn	TauD/TfdA family dioxygenase [*Cystobacter ferrugineus*], WP_071903894.1	72%
7	1455	P57G	NRPS	Non-ribosomal peptide [*Corallococcus sp. CA054B*], WP_147447375.1	71%
8	4113	P57H	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45399.2	64%
9	7095	P57I	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45399.2	66%
10	2745	P57J	Unknown	benzoate-CoA ligase [*Cystobacter sp. Cbv34*], WP_147447000.1	61%

Supplementary Table S6. PABA34 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	603	P34A	Self-resistance protein	Pentapeptide repeat-containing protein [*Raoultella sp. 18102*], WP_159896252.1	59%
2	843	P34B	Unknown	Hypothetical protein [*Pandoraea cepalis*], WP_174975302.1	42%
3	561	P34C	PABA biosynthesis	Chorismate lyase [*Chryseobacterium nakagawai*], ROS09722.1	43%
4	1374	P34D	Dioxygenation of PABA	Phenylacetate-CoA oxygenase [*Corallococcus sp. CA054B*], WP_120590332.1	58%
5	924	P34E	Hydroxylation of PABA	Alpha/beta hydrolase [*Xanthomonas sp. MUS 060*], WP_045739213.1	48%
6	216	P34F	A domain activation	MbtH family protein [*Nonomuraea solani*], WP_103963622.1	52%
7	2236	P34G	PABA biosynthesis	Aminodeoxychorismate synthase component I [*Corallococcus sp. CA054B*], WP_120590333.1	59%
8	804	P34H	NRPS	Thioesterase [*Cystobacter ferrugineus*], WP_071903899.1	52%
9	1023	P34I	O-methyltransferase of PABA	Methyltransferase domain-containing protein [*Corallococcus sp. CA054B*], WP_120590334.1	75%
10	3828	P34J	NRPS	Non-ribosomal peptide synthetase [*Cystobacter ferrugineus*], WP_071903897.1	55%
11	2055	P34K	NRPS	Non-ribosomal peptide synthetase [*Corallococcus sp. CA054B*], WP_147447276.1	46%
12	1416	P34M	NRPS	Non-ribosomal peptide synthetase [*Corallococcus sp. CA054B*], WP_120591726.1	55%
13	18693	P34N	PKS/NRPS	Polyketide synthase [*Bacillus sp. BSC154*], KFI01609.1	39%
14	1602	P34O	Unknown	Benzoate-CoA ligase [*Cystobacter sp. Cbv34*], AKP45400.1	47%
15	1455	P34P	Transporter	MFS transporter [*Corallococcus sp. CA054B],* WP_158616558.1	48%

Supplementary Table S7. PABA157 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	783	P157A	PABA biosynthesis	4-amino-4-deoxychorismate lyase [*Caulobacterales bacterium 32-69-10*], OYX29862.1	55%
2	1170	P157B	PABA biosynthesis	3-deoxy-7-phosphoheptulonate synthase class II [*Caulobacter sp. 17J65-9*], WP_163259901.1	49%
3	2163	P157C	PABA biosynthesis	Aminodeoxychorismate synthase component I [*Xanthomonas sp. MUS 060*], WP_052688199.1	63%
4	801	P157D	NRPS	Thioesterase [*Cystobacter ferrugineus*], WP_071903899.1	48%
5	792	P157E	O-methyltransferase of hydroxy-Asn	SAM-dependent methyltransferase [*Xanthomonas sp. MUS 060*], WP_161795442.1	61%
6	1932	P157F	Unknown	Putative benzoate--coa ligase protein [*Xanthomonas albilineans GPE PC73*], CBA16029.1	54%
7	993	P157G	Hydroxylation of Asn	TauD/TfdA family dioxygenase [*Xanthomonas sp. MUS 060*], WP_082065178.1	67%
8	5076	P157H	NRPS	Non-ribosomal peptide synthetase [*Xanthomonas albilineans*], WP_080928093.1	56%
9	483	P157I	PABA biosynthesis	Chorismate lyase [*Trinickia caryophylli*], WP_085230263.1	56%
10	690	P157J	PABA biosynthesis	4-phosphopantetheinyl transferase superfamily protein [*Luteibacter sp. Sphag1AF*], WP_183423732.1	46%
11	17934	P157K	PKS/NRPS	Polyketide non-ribosomal peptide synthase [*Xanthomonas albilineans*], CAE52339.1	57%
12	1032	P157M	O-methyltransferase of PABA	Methyltransferase domain-containing protein [*Xanthomonas sp. MUS 060*], WP_045739310.1	70%
13	567	P157N	Thioesterase	Thioesterase [*Xanthomonas*], WP_012916037.1	60%
14	2871	P157O	NRPS	Non-ribosomal peptide synthase [*Xanthomonas albilineans*], CAE52342.1	64%
15	225	P157P	A domain activation	MbtH family NRPS accessory protein [*Actinophytocola oryzae*], WP_133900845.1	73%
16	927	P157Q	Hydroxylation of PABA	Alpha/beta hydrolase [*Xanthomonas albilineans*], WP_045767479.1	56%
17	1368	P157R	Dioxygenation of PABA	Phenylacetate-CoA oxygenase subunit PaaI [*Corallococcus sp. CA054B*], WP_120590332.1	57%
18	951	P157S	Hydroxylation of PABA	Alpha/beta fold hydrolase [*Xanthomonas sp. MUS 060*], WP_045739211.1	59%
19	1626	P157T	Transporter	MFS transporter [*Xanthomonas albilineans*], WP_080928090.1	53%
20	759	P157U	Self-resistance protein	Pentapeptide repeat-containing protein [*Bradyrhizobium sp. BTAi1*], WP_011942600.1	41%

Supplementary Table S8. PABA95 biosynthetic gene cluster analysis

Orf	Gene Size (bp)	Gene Name	Proposed Function	Protein [Organism], Accession Number	Protein ID%
1	678	P95A	PABA biosynthesis	Aminodeoxychorismate synthase component I [*Xanthomonas sp. MUS 060*], WP_052688199.1	74%
2	777	P95B	Thioesterase	Thioesterase [*Cystobacter ferrugineus*], WP_071903899.1	59%
3	1035	P95C	O-methyltransferase of PABA	Methyltransferase domain-containing protein [*Cystobacter ferrugineus*], WP_071903898.1	70%
4	5979	P95D	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45395.1	54%
5	2781	P95E	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45396.1	65%
6	819	P95F	PABA biosynthesis	Aminodeoxychorismate lyase [*Cystobacter ferrugineus*], WP_071903895.1	53%
7	1392	P95G	PKS	Acyltransferase [*Ketobacter sp.*], RLU01354.1	31%
8	978	P95H	Hydroxylation of Asn	TauD/TfdA family dioxygenase [*Cystobacter ferrugineus*], WP_071903894.1	68%
9	2238	P95I	PKS/NRPS	Polyketide non-ribosomal peptide synthase [*Xanthomonas albilineans GPE PC73*], CBA16032.1	42%
10	3441	P95J	PKS	SDR family NAD(P)-dependent oxidoreductase [*Sporocytophaga myxococcoides*], WP_051313368.1	54%
11	3519	P95K	PKS	SDR family NAD(P)-dependent oxidoreductase [*Bacillus subtilis*], WP_148343064.1	42%
12	10890	P95M	NRPS	Non-ribosomal peptide synthase [*Cystobacter sp. Cbv34*], AKP45399.2	53%
13	756	P95N	Unknown	Benzoate-CoA ligase [*Cystobacter sp. Cbv34*], AKP45400.1	32%

Supplementary Table S9. Albicidin 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	PKS										MCA
B	A	V	K	Y	V	A	N	D	A	K	PABA
*	E	L	T	Y	V	H	V	A	A	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	Y	V	A	N	D	A	K	PABA
E	A	I	K	Y	F	S	I	D	M	K	AHIBA
F	A	I	K	Y	F	S	I	D	M	K	AHIBA

Supplementary Table S10. Cystobactamid 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	A	V	K	H	I	A	N	D	V	K	PABA
B	A	V	K	H	I	A	N	D	V	K	PABA
*	D	L	A	Y	F	G	V	I	G	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	H	I	A	N	D	V	K	PABA
E	A	I	K	Y	Y	S	I	D	V	K	AHMBA
F	A	I	K	Y	I	A	N	D	I	K	PABA

Supplementary Table S11. PABA48 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	D	A	W	T	I	A	A	V	C	K	Phe
B	A	V	K	H	I	A	N	D	V	K	PABA
*	D	L	A	Y	F	G	V	I	G	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	H	I	A	N	D	V	K	PABA
E	A	I	K	Y	F	S	I	D	V	K	AHIBA
F	A	I	K	Y	I	A	N	D	I	K	AHIBA

Supplementary Table S12. PABA70 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	D	A	A	T	I	A	A	V	C	K	Tyr
B	A	V	K	Y	V	A	N	D	V	K	PABA
*	E	L	A	Y	F	G	I	I	G	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	Y	V	A	N	D	V	K	PABA
E	A	I	K	Y	F	S	I	D	V	K	AHIBA
F	A	I	K	C	F	S	I	D	I	K	AHIBA

Supplementary Table S13. PABA57 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	D	A	A	T	I	A	A	V	C	K	Tyr
B	A	V	K	H	I	A	N	D	I	K	PABA
*	D	L	A	H	F	G	T	I	G	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	H	I	A	N	D	I	K	PABA
E	A	I	K	Y	F	S	I	D	I	K	AHIBA
F	A	V	K	H	I	A	N	D	I	K	PABA

Supplementary Table S14. PABA34 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	PKS										MCA
B	A	V	K	H	V	A	N	D	V	K	PABA
*	D	I	I	I	L	A	I	E	I	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	I	K	Y	F	S	I	D	M	K	AHMBA
E	A	I	K	Y	F	S	I	D	M	K	AHMBA
F	A	V	K	H	V	A	N	D	V	K	PABA

Supplementary Table S15. PABA157 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
A	PKS										CA
B	A	V	K	F	V	A	N	D	V	K	PABA
*	G	L	L	S	E	H	V	H	F	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	F	V	A	N	D	V	K	AHMBA
E	A	I	K	Y	F	S	I	D	I	K	AHMBA
F	A	V	K	F	V	A	N	D	V	K	PABA

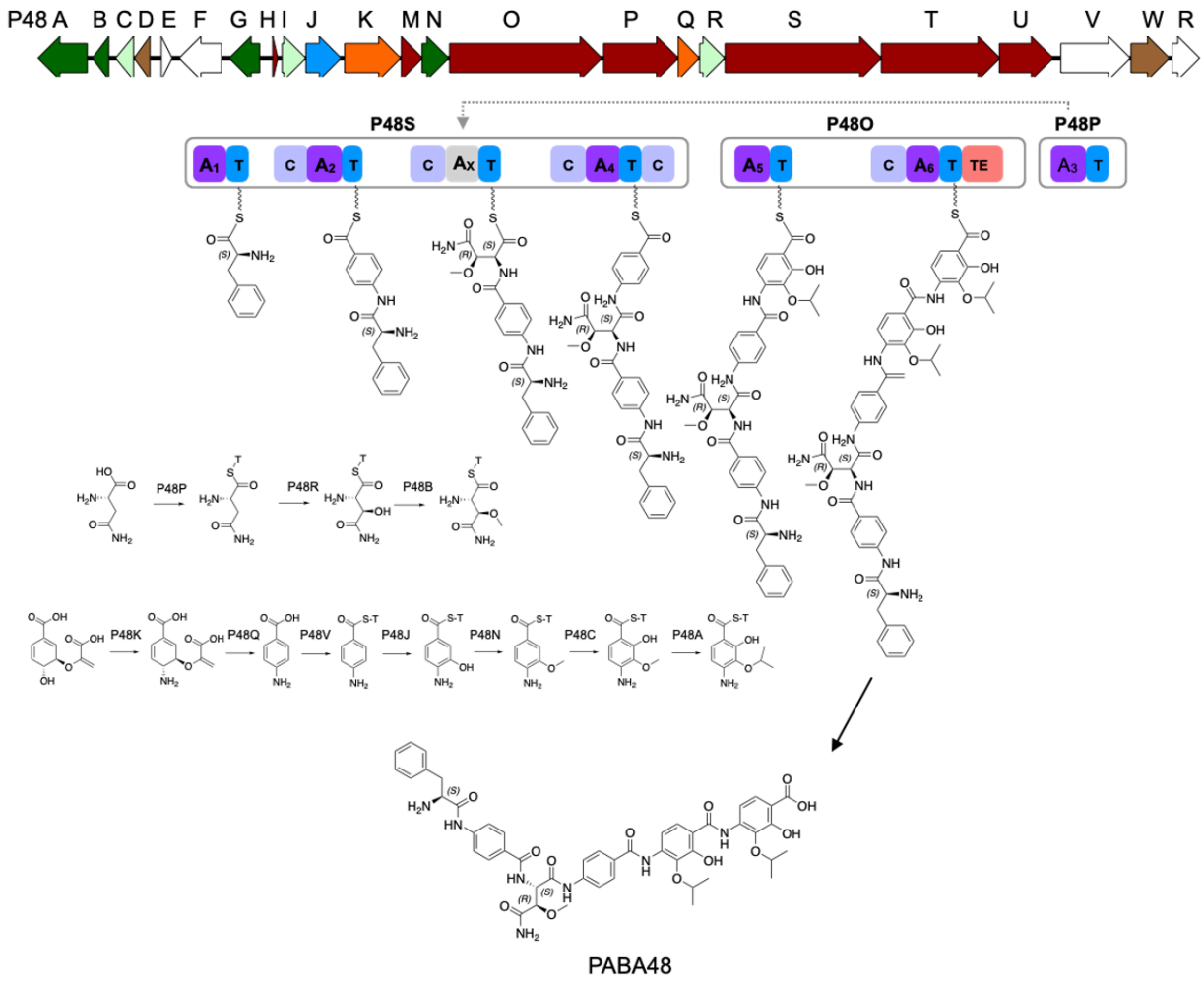
Supplementary Table S16. PABA95 10 signature code analysis

Module	A domain signature										Predicted substrate
	235	236	239	278	299	301	322	330	331	517	
PKS											MCA
B	A	I	K	Y	F	S	I	D	I	K	AHMBA
*	D	V	I	Y	L	G	A	L	G	K	inactive
C	D	L	T	K	I	G	E	V	G	K	Asn
D	A	V	K	H	I	A	N	D	V	K	PABA
E	A	I	K	Y	F	S	I	D	V	K	AHMBA
F	A	V	K	F	V	A	N	D	V	K	PABA

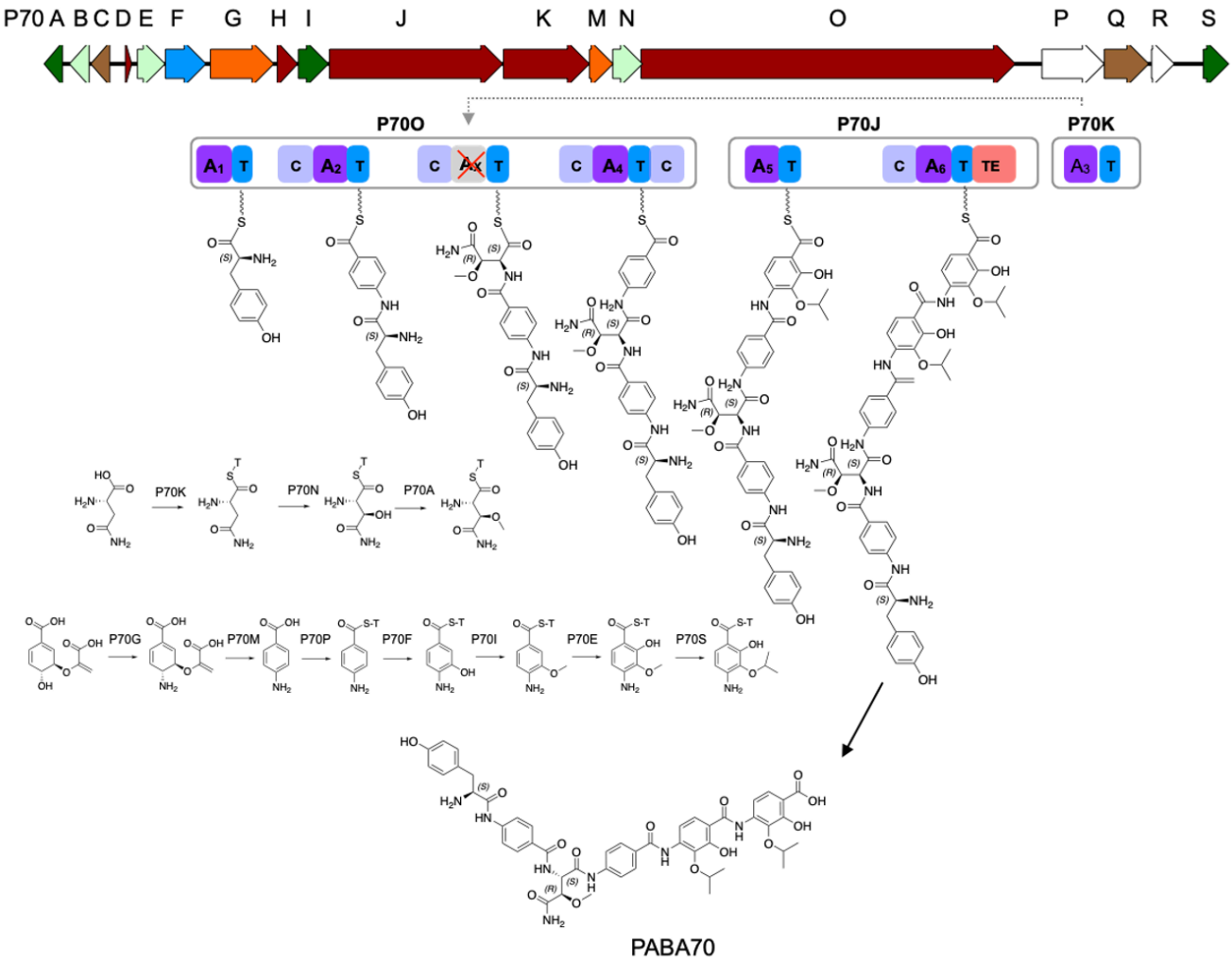
Supplementary Table S17. Resistance frequency of syn-BNPs and ciprofloxacin against *E. coli* 25922 in the presence of 2 x MIC antibiotic.

Compounds	Frequency
Albicidin	7.80×10^{-7}
PABA48	3.50×10^{-7}
PABA70	3.25×10^{-7}
PABA57	4.25×10^{-7}
PABA34	6.67×10^{-7}
PABA157	4.50×10^{-7}
PABA95	3.62×10^{-7}
Ciprofloxacin	4.50×10^{-8}

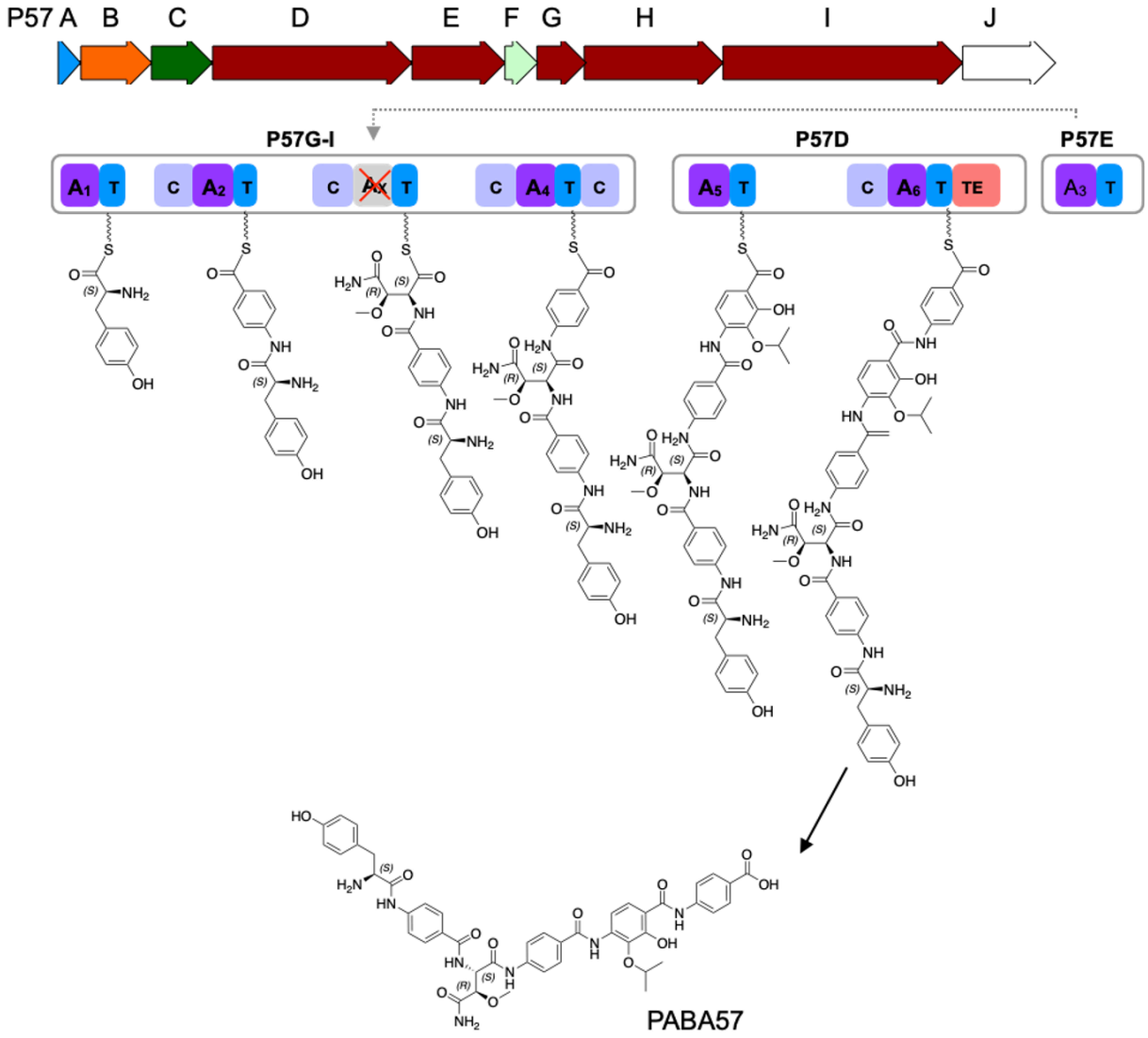
Supplementary Figure S1. Proposed PABA48 biosynthetic pathway



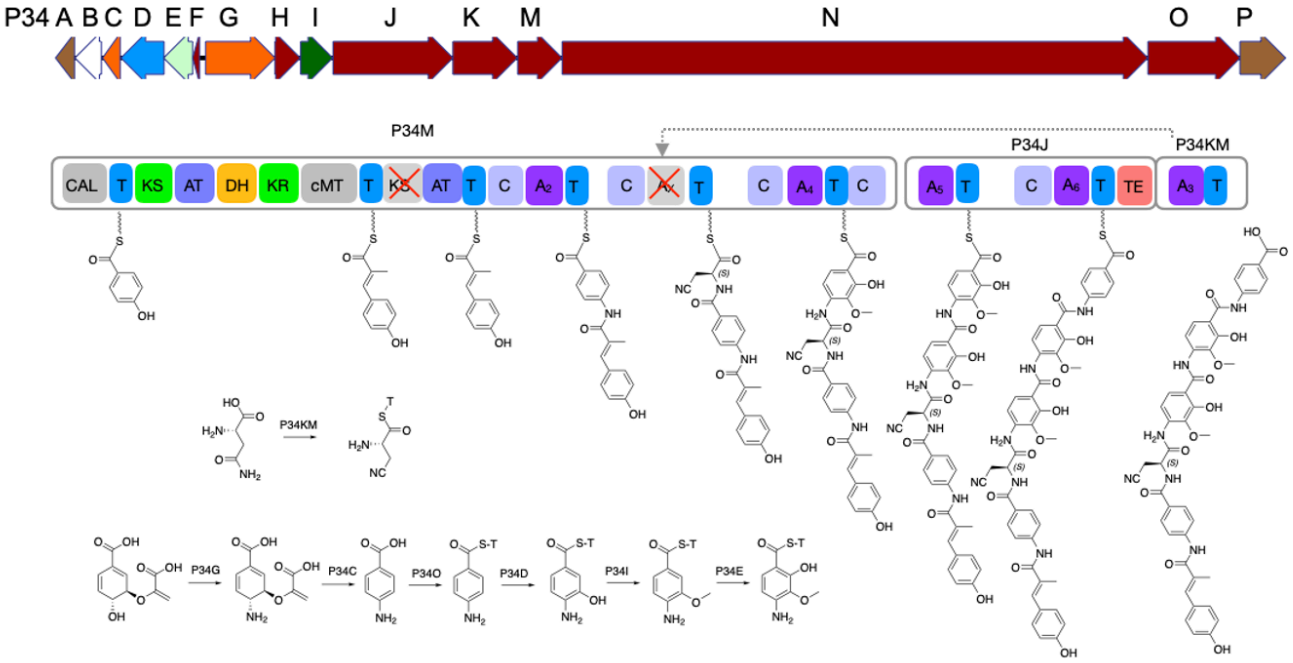
Supplementary Figure S2. Proposed PABA70 biosynthetic pathway



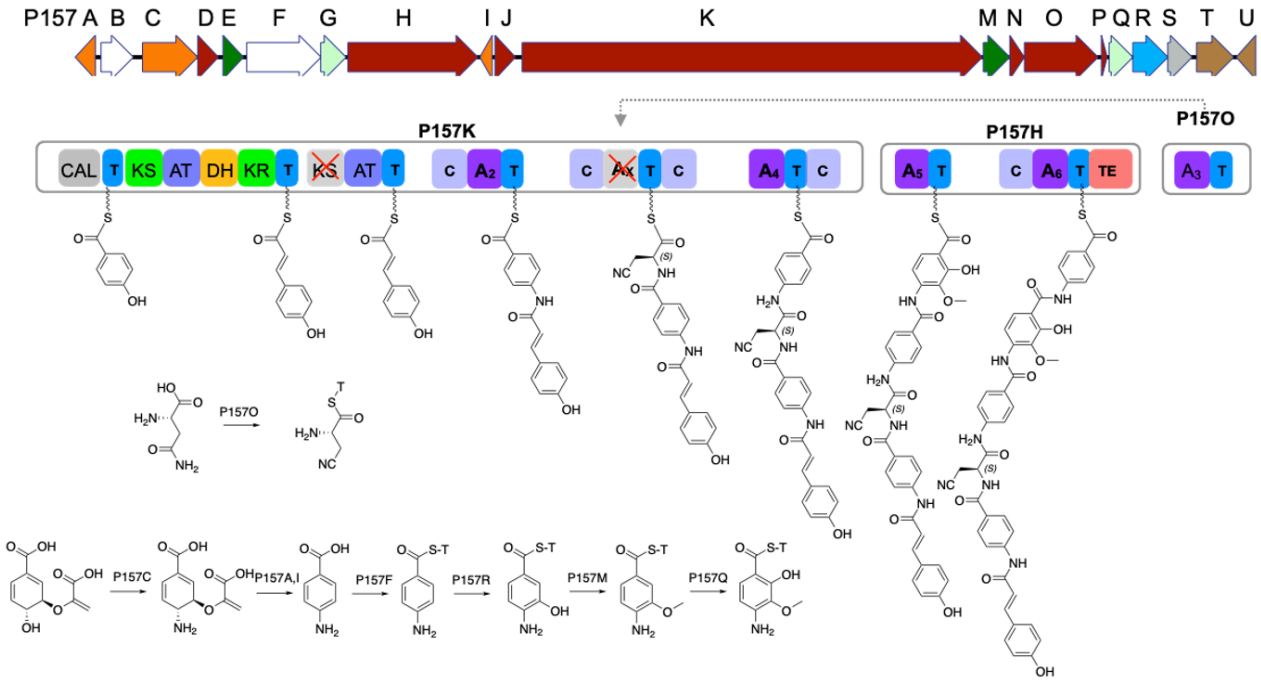
Supplementary Figure S3. Proposed PABA57 biosynthetic pathway



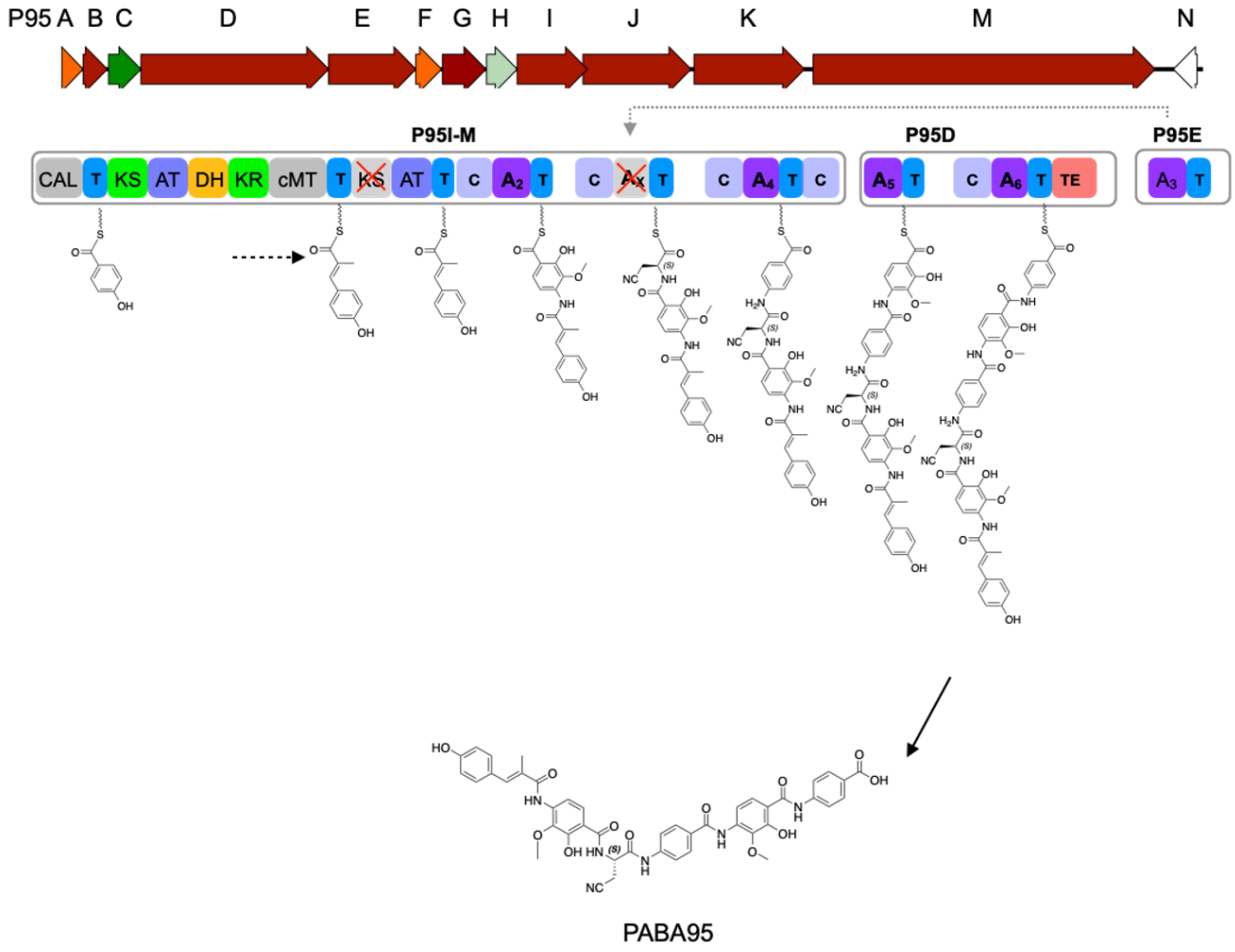
Supplementary Figure S4. Proposed PABA34 biosynthetic pathway

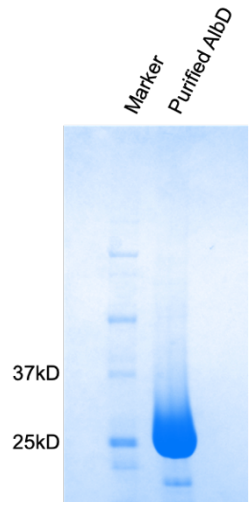


Supplementary Figure S5. Proposed PABA157 biosynthetic pathway



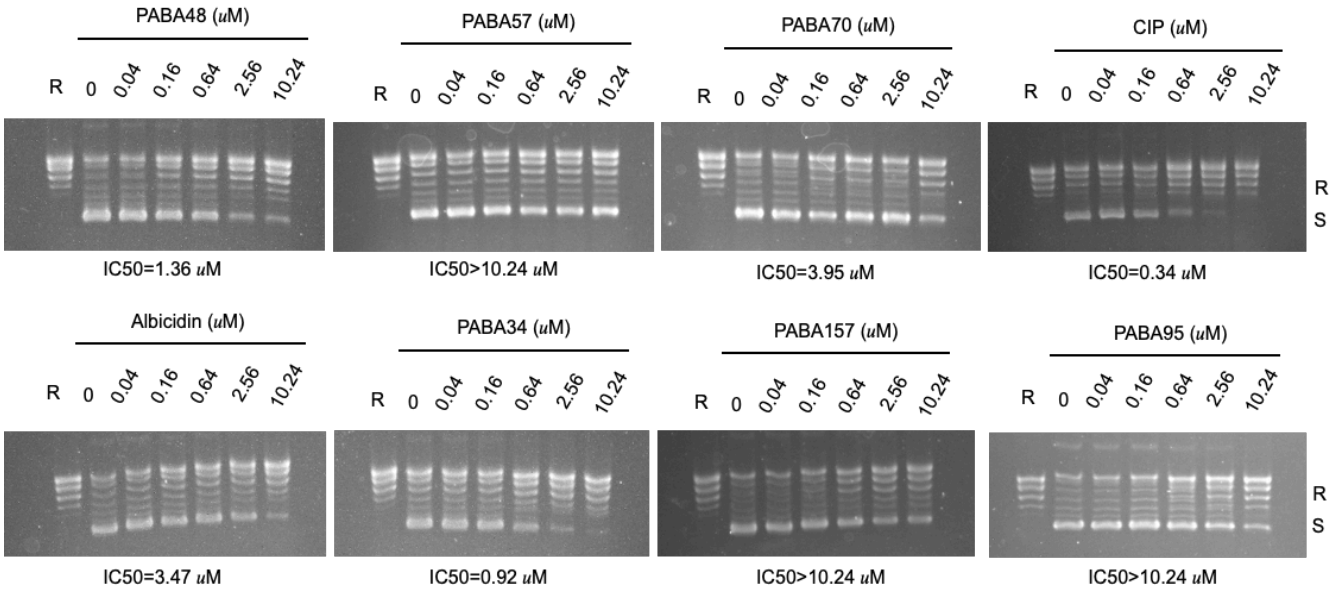
Supplementary Figure S6. Proposed PABA95 biosynthetic pathway



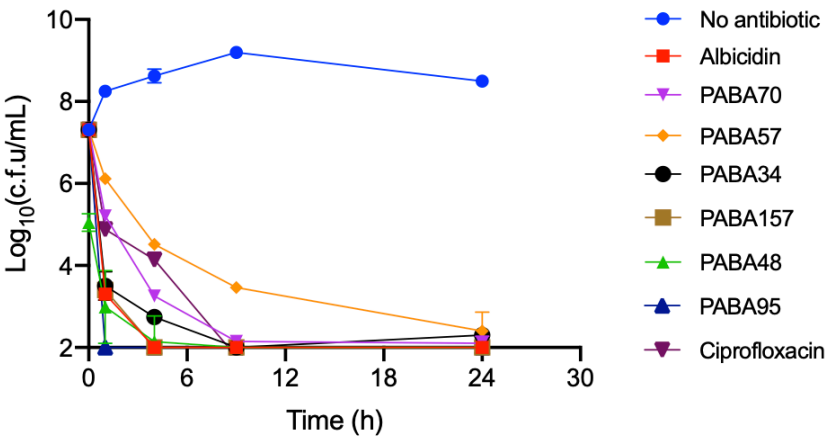


Supplementary Figure S7: SDS-PAGE analysis of purified AlbD protein.

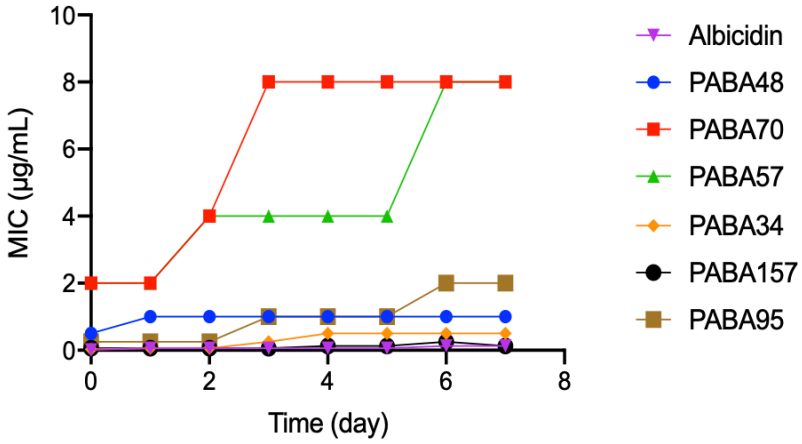
Supplementary Figure S8: DNA gyrase supercoiling assay with PABA syn-BNPs or ciprofloxacin (CIP) at the concentrations indicated.



Supplementary Figure S9: Time-dependent killing curves. An exponential culture of E. coli 25922 was challenged with 10 x MIC of a syn-BNP antibiotic or ciprofloxacin (n=3). Data are mean ± s.d. c.f.u. colony-forming units.

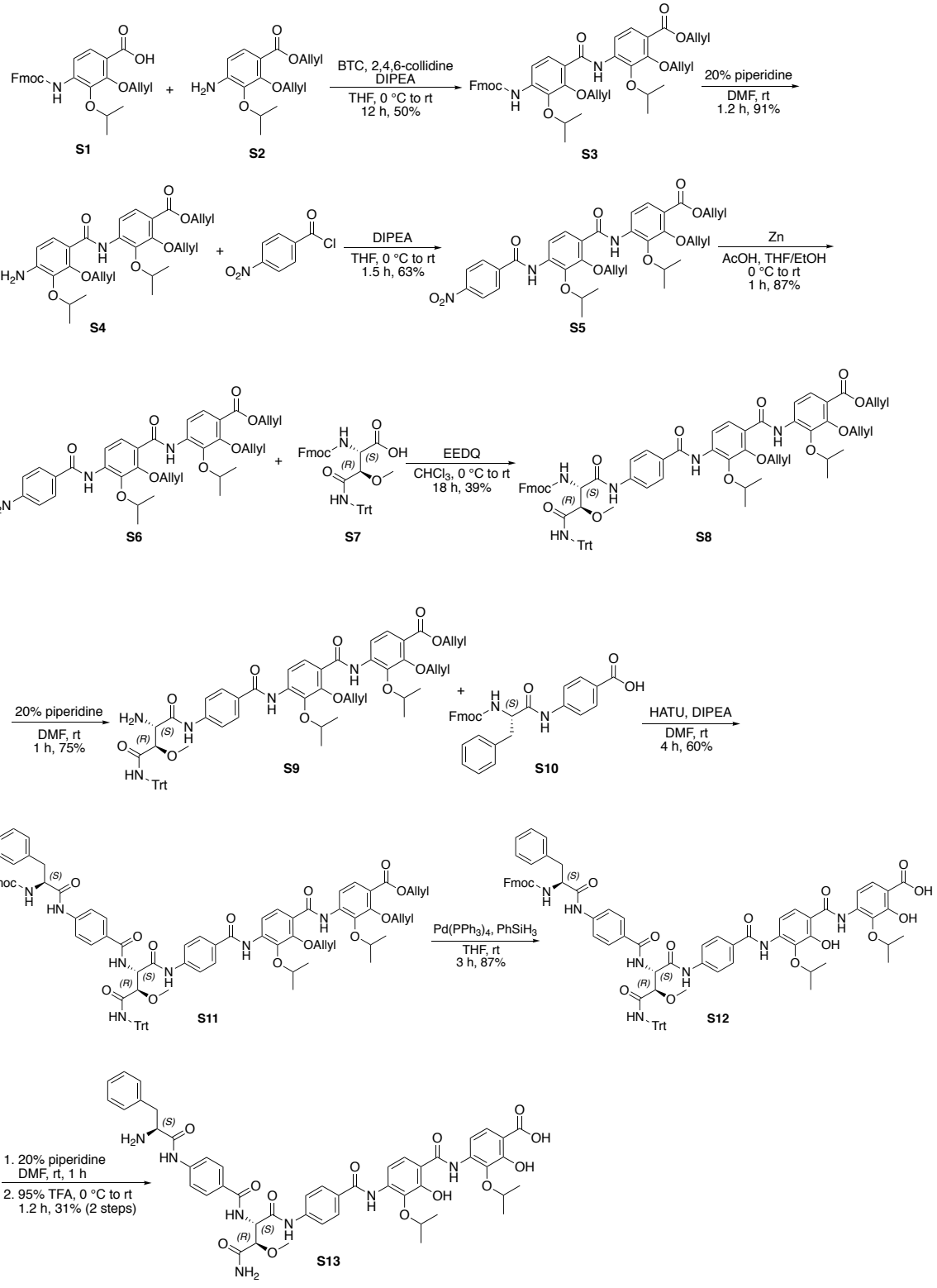


Supplementary Figure S10: Resistance after serial passage. Cultures of E. coli were passaged in the presence of sub-MIC levels of each syn-BNP antibiotic. The MIC of single colony after each day is shown.



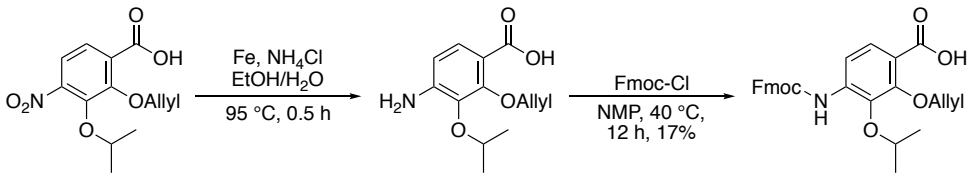
Synthetic procedures:

Total synthesis of PABA48



Scheme S1. Synthetic pathway for the preparation of PABA48 (S13).

4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(allyloxy)-3-isopropoxybenzoic acid (**S1**)



S1a **S1b** **S1**

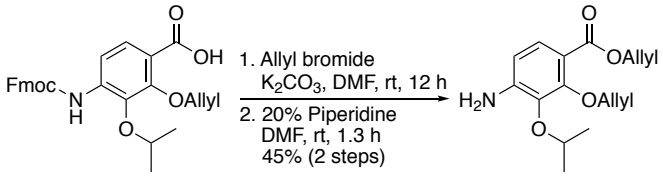
Building block 2-(allyloxy)-3-isopropoxy-4-nitrobenzoic acid (**S1a**) was prepared according to previously reported protocols.[13]

Compound **S1a** (1.10 g, 3.91 mmol) was dissolved in EtOH (20.0 mL) followed by the addition of Fe (655 mg, 11.7 mmol). A solution of NH_4Cl (1.26 g, 23.5 mmol) dissolved in H_2O (6.00 mL) was added and the reaction was warmed to 95 °C. After stirring at 95 °C for 0.5 h, the solution was cooled to 70 °C, filtered and the residue washed with EtOH (100 mL). The EtOH was removed *in vacuo* resulting in a brown solid. The crude product (1.80 g) was used in the next step without further purification.

To a solution of compound **S1b** (1.80 g, 3.58 mmol) in NMP (35.0 mL), Fmoc-Cl (926 mg, 3.58 mmol) dissolved in NMP (15.0 mL) was added. The mixture was warmed to 40 °C and stirred for 12 h. After quenching with H_2O, the pH was adjusted to 5 with 0.5 M HCl. The aqueous layer was extracted with EtOAc (3x) followed by washing of the organic layers with brine (1x) and drying over Na_2SO_4. After concentrating *in vacuo,* the crude material was purified via RP-HPLC (45%-85%, ACN/H_2O). Compound **S1** (300 mg, 17%) was obtained as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 7.91 (d, *J* = 7.50 Hz, 2H), 7.77 (d, *J* = 7.26 Hz, 2H), 7.53 (d, *J* = 9.04Hz, 1H), 7.44 (q, *J* = 7.20 Hz, 3H), 7.34 (t, *J* = 7.20 Hz, 2H), 6.01–6.12 (m, 1H), 5.37 (d, *J* = 17.4 Hz, 1H), 5.23 (d, *J* = 9.71Hz, 1H), 4.50 (d, *J* = 5.29 Hz, 2H), 4.38-4.45 (m, 3H), 4.33 (t, *J* = 6.80 Hz, 1H), 2.58 (s, 3H), 1.23 (d, *J* = 6.17 Hz, 6H); ESI-MS [M - H]$^-$: *m/z* 472.17

Allyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate (**S2**)



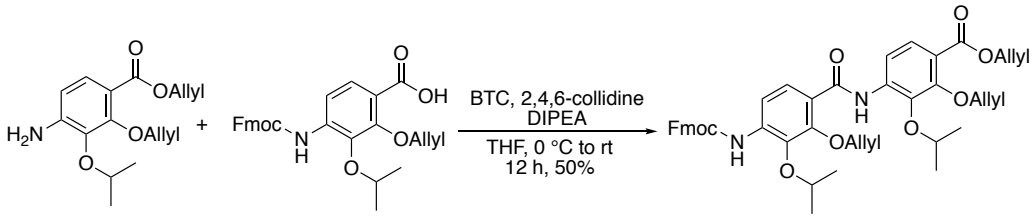
S1 **S2**

S1 (110 mg, 0.232 mmol) was dissolved in dry DMF (1.10 mL) followed by the addition of allyl bromide (21.1 μL, 0.303 mmol) and K_2CO_3 (19.2 mg, 0.139 mmol). The reaction was stirred at room temperature for 18 h under Ar(g). Et_2O (10.0 mL) and H_2O (10.0 mL) were added, and the organic layer was then washed with saturated $NaHCO_3$ (2x), brine (1x), and dried over Na_2SO_4. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford a mixture of deprotected intermediates as a clear oil.

The mixture (59.9 mg, 0.117 mmol) was dissolved in dry DMF (1.48 mL) followed by the addition of piperidine (0.370 mL). The reaction stirred at room temperature under Ar(g) for 1 h and quenched with a saturated solution of NH_4Cl. The mixture was extracted with DCM (3x) followed by washing of the organic layers with saturated $NaHCO_3$ (1x) before drying over Na_2SO_4 and concentrating *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford deprotected dimer **S2** as a clear oil (30.4 mg, 0.104 mmol, 45% over 2 steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.6 Hz, 1H), 6.47 (d, *J* = 8.6 Hz, 1H), 6.14 (ddt, *J* = 16.5, 11.0, 5.9 Hz, 1H), 6.03 (ddt, *J* = 16.4, 10.9, 5.7 Hz, 1H), 5.37 (ddd, *J* = 17.4, 13.3, 2.0 Hz, 2H), 5.23 (dd, *J* = 15.5, 10.4 Hz, 2H), 4.76 (d, *J* = 5.8 Hz, 2H), 4.66 (hept, *J* = 6.3 Hz, 1H), 4.53 (d, *J* = 6.0 Hz, 2H), 4.27 (s, 2H), 1.28 (d, *J* = 6.2 Hz, 6H); ESI-MS [M + H]$^+$: *m/z* 292.14

Allyl 4-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(allyloxy)-3-isopropoxybenzamido)-2-(allyloxy)-3-isopropoxybenzoate (**S3**)



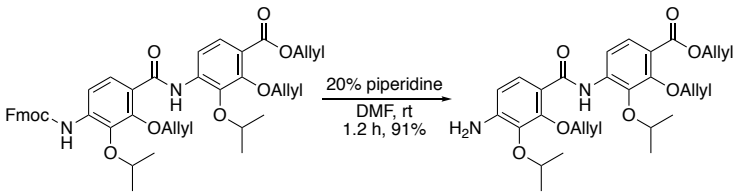
S2 **S1** **S3**

BTC (28.2 mg, 95.0 μmol) and **S1** (135.1 mg, 0.285 mmol) were dissolved in dry THF (0.900 mL) and cooled to 0 °C under Ar(g). To this solution 2,4,6-collidine (252 μL, 1.90 mmol) was added dropwise and the resulting suspension was stirred for 5 min at room temperature. A solution of **S2** (69.3 mg, 0.238 mmol) and DIPEA (414 μL, 1.90 mmol) was prepared under Ar(g) and then added dropwise to the suspension. The reaction was stirred at room temperature overnight. The reaction was diluted with Et_2O followed by washing with 10 % $KHSO_4$ (2x). The organic layer was then washed with saturated $NaHCO_3$ (1x) followed by brine (1x) before drying

over Na_2SO_4 and concentrating *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford dimer **S3** as a clear oil (89.8 mg, 0.120 mmol, 50%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.72 (s, 1H), 8.46 (d, *J* = 8.8 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 2H), 7.68 (d, *J* = 8.9 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.42 (q, *J* = 7.2 Hz, 3H), 7.34 (t, *J* = 7.5 Hz, 2H), 6.20 – 6.00 (m, 3H), 5.40 (dd, *J* = 22.7, 17.2 Hz, 2H), 5.31 – 5.23 (m, 3H), 5.22 (d, *J* = 10.2 Hz, 1H), 4.81 (d, *J* = 5.7 Hz, 2H), 4.76 (h, *J* = 6.2 Hz, 1H), 4.68 (d, *J* = 6.6 Hz, 2H), 4.62 (q, *J* = 6.1 Hz, 1H), 4.58 (d, *J* = 6.3 Hz, 3H), 4.56 (s, 1H), 4.33 (t, *J* = 6.8 Hz, 1H), 1.37 (d, *J* = 6.1 Hz, 6H), 1.28 (d, *J* = 6.2 Hz, 6H); ESI-MS [M + H]$^+$: *m/z* 747.26

Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)-3-isopropoxybenzoate (**S4**)

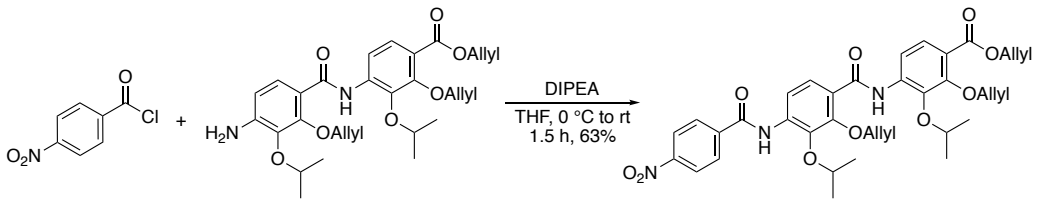


S3 **S4**

The Fmoc-protected dimer **S3** (88.2 mg, 0.118 mmol) was dissolved in dry DMF (1.60 mL) followed by the addition of piperidine (0.4 mL). The reaction stirred at room temperature under Ar(g) for 1 h and quenched with a saturated solution of NH_4Cl. The mixture was extracted with DCM (3x) followed by washing of the organic layers with saturated $NaHCO_3$ (1x) before drying over Na_2SO_4 and concentrating *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford deprotected dimer **S4** as a pale yellow oil (56.3 mg, 0.107 mmol, 91%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.79 (s, 1H), 8.46 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 6.59 (d, *J* = 8.6 Hz, 1H), 6.19 – 6.07 (m, 2H), 6.04 (ddt, *J* = 16.5, 11.0, 5.7 Hz, 1H), 5.39 (dd, *J* = 20.7, 17.2 Hz, 2H), 5.31 – 5.21 (m, 3H), 5.18 (d, *J* = 10.2 Hz, 1H), 4.79 (d, *J* = 5.8 Hz, 2H), 4.73 (p, *J* = 6.2 Hz, 1H), 4.68 (d, *J* = 6.7 Hz, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 4.59 – 4.51 (m, 1H), 4.25 – 4.19 (brs, 2H), 1.35 (d, *J* = 6.3 Hz, 6H), 1.27 (d, *J* = 6.3 Hz, 6H); ESI-MS [M + H]$^+$: *m/z* 525.29

Allyl 2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-(4-nitrobenzamido)benzamido)-3-isopropoxybenzoate (**S5**)

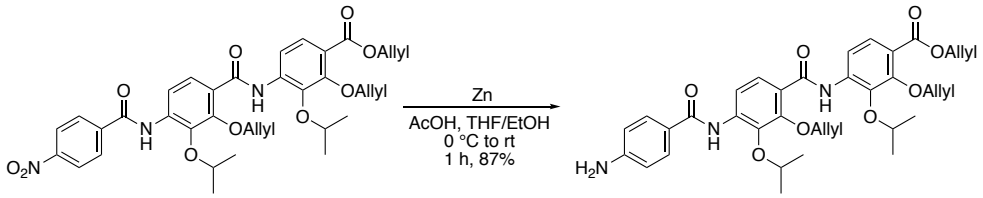


S4 **S5**

Dimer **S4** (56.3 mg, 0.107 mmol) was dissolved in dry THF (250 μL) followed by the addition of DIPEA (56.0 μL, 0.322 mmol) and cooled to 0 °C under Ar(g). To this solution, 4-nitrobenzoyl chloride (29.9 mg, 0.161 mmol) was added portionwise over 30 min keeping the solution at 0 °C. Once the starting material was consumed, the reaction was allowed to warm to room temperature and stirred for 10 min. Following formation of a precipitate, the reaction was diluted with Et_2O and stirred at room temperature for 1 h. The precipitate was filtered and washed with Et_2O. The filtrate and all Et_2O washes were combined and concentrated *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford trimer **S5** as a pale yellow oil (45.2 mg, 67.0 μmol, 63%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.70 (s, 1H), 8.79 (s, 1H), 8.46 (dd, *J* = 8.8, 6.5 Hz, 2H), 8.40 (d, *J* = 8.3 Hz, 2H), 8.08 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 6.19 – 6.00 (m, 3H), 5.40 (dd, *J* = 22.4, 17.2 Hz, 2H), 5.32 – 5.21 (m, 4H), 4.80 (d, *J* = 5.7 Hz, 2H), 4.80 – 4.75 (m, 2H), 4.70 (d, *J* = 6.7 Hz, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 1.41 (d, *J* = 6.2 Hz, 6H), 1.29 (d, *J* = 6.2 Hz, 6H); ESI-MS [M + H]$^+$: *m/z* 674.27

Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropoxybenzamido)-3-isopropoxybenzoate (**S6**)

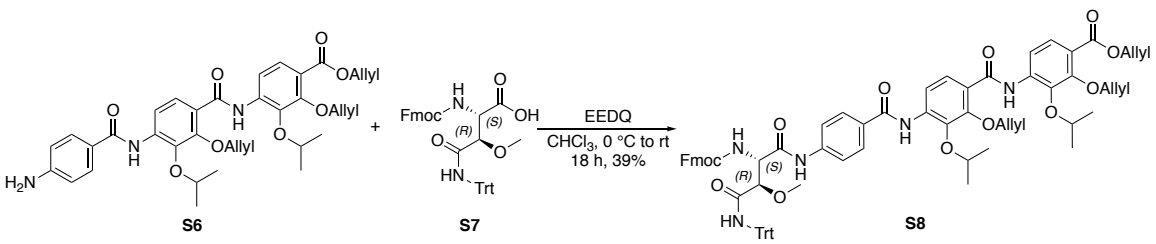


S5 **S6**

Nitrobenzoate **S5** (18.0 mg, 0.027 mmol) was dissolved in a solution of 10% AcOH in THF/EtOH (4:1, 275 μL) and cooled to 0 °C. Zinc powder (33.2 mg, 0.508 mmol) was added portionwise and monitored via TLC. The reaction was allowed to warm to room temperature and stirred for 1 h before filtering through Celite™ and washing with excess DCM as well as a small amount of MeOH. After concentrating *in vacuo*, the crude material was purified via flash column chromatography with a gradient of 0-40% EtOAc in hexanes to afford **S6** as a yellow oil (15.0 mg, 23.0 μmol, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.73 (s, 1H), 8.65 (s, 1H), 8.47 (dd, *J* = 8.9, 3.2 Hz, 2H), 7.99 (d, *J* = 8.9 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 2H), 6.19 – 6.00 (m, 3H), 5.40 (dd, *J* = 21.8, 17.2 Hz, 2H), 5.31 – 5.26 (m, 2H), 5.23 (dd, *J* = 17.2, 10.2 Hz, 2H), 4.80 (d, *J* = 5.8 Hz, 2H), 4.76 (p, *J* = 6.1 Hz, 1H), 4.73 – 4.69 (m, 1H), 4.69 (d, *J* = 6.9 Hz, 2H), 4.58 (d, *J* = 5.9 Hz, 2H), 4.13 (brs, 2H), 1.40 (d, *J* = 6.2 Hz, 6H), 1.28 (d, *J* = 6.2 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 644.30

Allyl 4-(4-(4-((2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)-2-(allyloxy)-3-isopropoxybenzoate (**S8**)

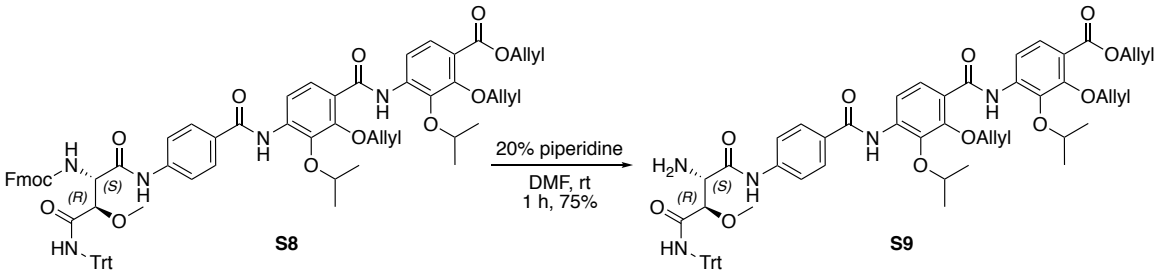


S6 **S7** **S8**

Tripeptide **S6** (28.4 mg, 44.0 μmol) and Fmoc-(*S*,*R*)-β-OMe-Asn **S7** (13.3 mg, 66.0 μmol) were dissolved in $CHCl_3$ (200 μL) and cooled to 0 °C. EEDQ (41.5 mg, 21.0 μmol) was added and the resulting mixture was stirred at 0 °C for 20 min before warming to room temperature and stirring for 18 h under Ar(g). The solution was diluted with EtOAc and washed with 10 % $KHSO_4$ (2x) followed by brine (1x). The organic layer was then dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via manual flash column chromatography (hexanes/EtOAc 5:1 to 4:1 to 2:1) to afford **S8** as a clear oil (21.7 mg, 17.0 μmol, 39%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.73 (s, 1H), 8.73 (s, 1H), 8.48 (d, *J* = 8.9 Hz, 2H), 8.24 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.90 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.70 (dd, *J* = 13.3, 8.2 Hz, 2H), 7.60 (t, *J* = 6.6 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.34 – 7.25 (m, 2H), 7.21 (d, *J* = 5.2 Hz, 8H), 7.15 (d, *J* = 4.0 Hz, 4H), 6.21 – 5.96 (m, 3H), 5.54 (d, *J* = 8.2 Hz, 1H), 5.40 (dd, *J* = 22.0, 17.2 Hz, 2H), 5.32 – 5.19 (m, 4H), 4.83 (s, 1H), 4.81 (d, *J* = 5.9 Hz, 2H), 4.79 – 4.75 (m, 1H), 4.75 – 4.71 (m, 1H), 4.71 (d, *J* = 6.6 Hz, 2H) 4.63 – 4.56 (d, *J* = 6.6 Hz, 2H), 4.59 (d, *J* = 6.2 Hz, 2H), 4.25 (t, *J* = 6.4 Hz, 1H), 3.60 (s, 3H), 1.40 (d, *J* = 3.6, 2.7 Hz, 6H), 1.29 (d, *J* = 6.2 Hz, 6H); HRMS (ESI): *m/z* calculated for $C_{75}H_{74}N_5O_{13}^+$ $[M + H]^+$: 1252.5283; observed 1252.5207

Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-(4-((2*S*,3*R*)-2-amino-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzamido)-3-isopropoxybenzoate (**S9**)



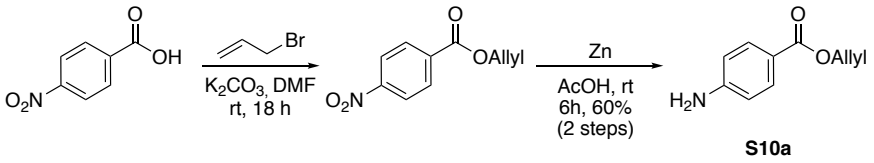
S8 **S9**

Fmoc-protected tripeptide **S8** (21.7 mg, 17.0 μmol) was dissolved in dry DMF (205 μL) followed by the addition of piperidine (51.0 μL). The resulting mixture stirred at room temperature for 1 h under Ar(g). After quenching with saturated NH_4Cl, the solution was extracted with DCM (3x) following by washing of the organic layer with saturated $NaHCO_3$ (1x). The organic layer was then dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via manual flash column chromatography (hexanes/EtOAc 1:1 to 1:2) to afford **S9** as a clear/faint yellow oil (13.3 mg, 13.0 μmol, 75%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.73 (s, 1H), 9.45 (s, 1H), 8.72 (s, 1H), 8.47 (dd, *J* = 8.8, 3.7 Hz, 2H), 8.03 – 7.98 (m, 2H), 7.87 – 7.82 (m, 3H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.30 – 7.23 (m, 8H), 7.25 – 7.20 (m, 7H), 6.19 – 6.00 (m, 3H), 5.40 (dd, *J* = 22.0, 17.2 Hz, 2H), 5.32 – 5.20 (m, 5H), 4.81 (d, *J* = 5.8 Hz, 2H), 4.77 (q, *J* = 6.1 Hz, 1H), 4.72 (m, 1H), 4.70 (d, *J* = 6.9 Hz, 2H), 4.47

(d, *J* = 3.3 Hz, 1H), 3.83 (d, *J* = 3.3 Hz, 1H), 3.57 (s, 3H), 1.61 – 1.55 (m, 1H), 1.39 (dd, *J* = 6.3, 3.4 Hz, 6H), 1.31 – 1.27 (m, 6H); ESI-MS $[M + H]^+$: *m/z* 1030.72

Allyl 4-aminobenzoate (**S10a**)



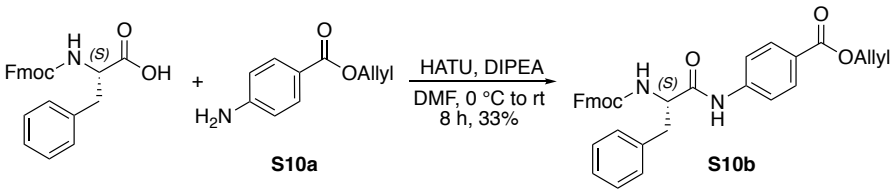
S10a

4-nitrobenzoic acid (1.00 g, 5.98 mmol) was dissolved in dry DMF (12.0 mL) followed by the addition of allyl bromide (0.57 mL, 6.58 mmol) and K_2CO_3 (496 mg, 3.59 mmol). The reaction stirred at room temperature for 18 h under Ar(g). Et_2O (60.0 mL) and H_2O (60.0 mL) were added and the organic layer was then washed with saturated $NaHCO_3$ (2x), brine (1x), and dried over Na_2SO_4. The solvent was removed *in vacuo* resulting in a yellow oil that was used in the next step without further purification.

Allyl 4-nitrobenzoate (1.01 g, 4.88 mmol) was dissolved in a mixture of AcOH (6.00 mL) and EtOH (10.0 mL) and cooled to 0 °C. Zinc powder (6.38 g, 97.6 mmol) was added portionwise over 30 min. Reaction allowed to warm to room temperature and stirred for 6 h before quenching with saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc (2x) and the combined organic layers were washed with saturated $NaHCO_3$ (1x) followed by brine (1x). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford **S10a** as a white solid (640 mg, 3.61 mmol, 60% over 2 steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 8.4 Hz, 2H), 6.06 – 5.99 (m, 1H), 5.38 (dd, *J* = 17.1, 1.9 Hz, 1H), 5.25 (dd, *J* = 10.3, 1.7 Hz, 1H), 4.77 (d, *J* = 5.6 Hz, 2H), 4.07 (s, 2H); ESI-MS $[M + H]^+$: *m/z* 178.12

Preparation of allyl (*S*)-4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)benzoate (**S10b**)

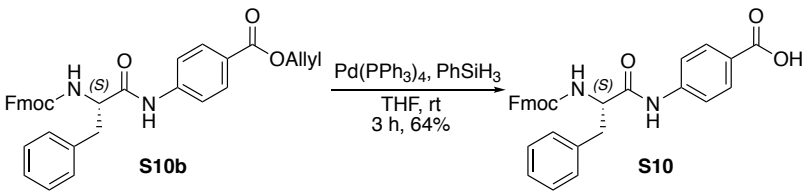


S10a **S10b**

Fmoc-Phe-OH (45.7 mg, 0.118 mmol) was dissolved in 300 μL of dry DMF and cooled to 0 °C before HATU (65.0 mg, 0.171 mmol) and DIPEA (61.6 μL, 0.354 mmol) were added. A solution of **S10a** (20.9 mg, 0.118 mmol) dissolved in 30 μL of dry DMF was added dropwise to the reaction mixture. After stirring at 0 °C for 1 h, the solution was allowed to stir at room temperature for 7 h. The reaction mixture was diluted with EtOAc and washed with 10 % $KHSO_4$ (2x) followed by brine (1x). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via manual flash column chromatography (0.25% MeOH in DCM) to afford dimer **S10b** as a white solid (19.4 mg, 35.0 μmol, 33%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H), 7.96 (d, *J* = 8.3 Hz, 2H), 7.90 – 7.85 (m, 3H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.66 (dd, *J* = 11.4, 7.6 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 7.7 Hz, 2H), 7.29 (dt, *J* = 14.6, 7.4 Hz, 4H), 7.20 (t, *J* = 7.4 Hz, 1H), 6.04 (ddt, *J* = 16.2, 10.6, 5.4 Hz, 1H), 5.40 (d, *J* = 17.3 Hz, 1H), 5.27 (d, *J* = 10.5 Hz, 1H), 4.78 (d, *J* = 5.5 Hz, 2H), 4.47 – 4.43 (m, 1H), 4.22 – 4.14 (m, 3H), 3.06 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.91 (dd, *J* = 13.7, 10.2 Hz, 1H); ESI-MS $[M + H]^+$: *m/z* 569.12

(*S*)-4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)benzoic acid (**S10**)

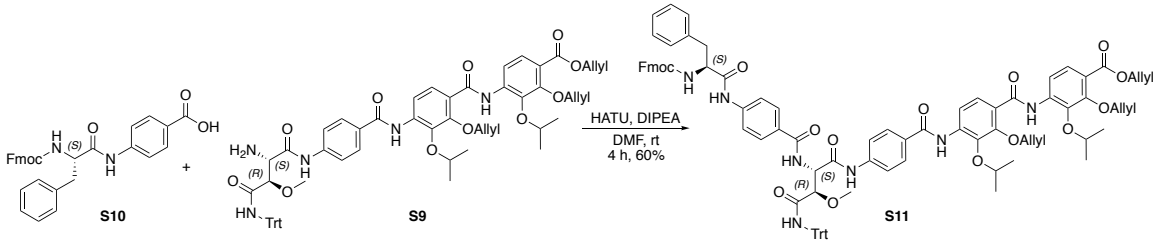


S10b **S10**

Dimer **S10b** (61.3 mg, 0.112 mmol) was dissolved in dry THF (2.58 mL) followed by the addition of $Pd(PPh_3)_4$ (12.6 mg, 11.2 μmol) and phenylsilane (69.2 μL, 0.561 mmol). The reaction mixture was stirred for 3 h at room temperature under the exclusion of light. After removal of all volatile components, the residue was dissolved in DCM and filtered through a short silica plug rinsing with several portions of DCM. The solvent was concentrated *in vacuo*. Crude material was purified via manual flash column chromatography (2-3% MeOH in DCM) to afford **S10** as a white solid (36.4 mg, 0.072 mmol, 64%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 3H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.66 (dd, *J* = 11.7, 7.5 Hz, 2H), 7.39 (dt, *J* = 14.7, 7.7 Hz, 4H), 7.28 (t, *J* = 6.6 Hz, 4H), 7.20 (t, *J* = 7.4 Hz, 1H), 4.47 – 4.43 (m, 1H), 4.17 (dt, *J* = 14.1, 7.3 Hz, 3H), 3.06 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.92 (dd, *J* = 13.7, 10.3 Hz, 1H); ESI-MS $[M + H]^+$: *m/z* 507.10

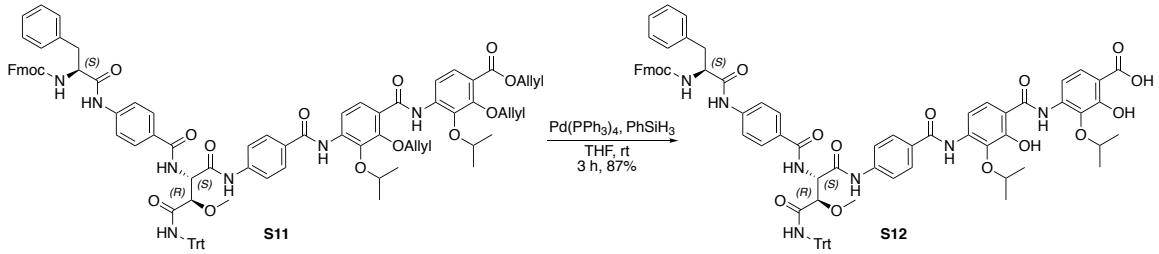
Allyl 4-(4-(4-((2*S*,3*R*)-2-(4-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)benzamido)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)-2-(allyloxy)-3-isopropoxybenzoate (**S11**)



Dipeptide **S10** (4.70 mg, 9.00 μmol) and HATU (3.40 mg, 9.00 μmol) were dissolved in dry DMF (150 μL). DIPEA (5.40 μL, 31.0 μmol) was added followed by addition of **S9** (6.4 mg, 6.20 μmol) dissolved in dry DMF (75.0 μL). The reaction stirred under Ar(g) for 4 h at room temperature. The reaction mixture was then diluted with EtOAc and washed with 10% $KHSO_4$ (2x) and brine (1x). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via manual flash column chromatography (hexanes/EtOAc 2:1 to 1:1) to afford **S11** as a clear oil (5.70 mg, 3.60 μmol, 60%).

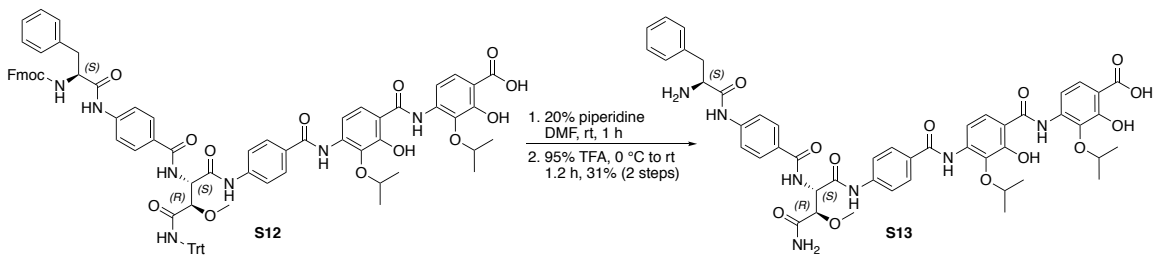
^{1}H NMR (600 MHz, $CDCl_3$) δ 10.73 (s, 1H), 9.33 (s, 1H), 8.73 (s, 1H), 8.47 (d, *J* = 8.9 Hz, 2H), 8.07 (s, 1H), 8.01 (d, *J* = 8.9 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.53 (t, *J* = 7.5 Hz, 3H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 6.9 Hz, 5H), 7.20 (s, 3H), 7.17 (h, *J* = 7.3, 6.4 Hz, 14H), 6.20 – 6.00 (m, 3H), 5.40 (dd, *J* = 22.3, 17.2 Hz, 3H), 5.32 – 5.20 (m, 5H), 4.88 (t, *J* = 6.5 Hz, 1H), 4.81 (d, *J* = 5.9 Hz, 2H), 4.77 (q, *J* = 6.1 Hz, 1H), 4.73 (d, *J* = 6.2 Hz, 1H), 4.70 (d, *J* = 6.7 Hz, 2H), 4.59 (d, *J* = 6.3 Hz, 3H), 4.56 – 4.37 (m, 4H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.65 (s, 3H), 3.16 (d, *J* = 37.1 Hz, 3H), 1.39 (d, *J* = 6.2 Hz, 6H), 1.28 (d, *J* = 6.2 Hz, 6H); HRMS (ESI): *m*/*z* calculated for $C_{91}H_{88}N_7O_{15}^+$ $[M + H]^+$: 1518.6338; observed 1518.6293

4-(4-(4-((2*S*,3*R*)-2-(4-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)benzamido)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (**S12**)



Linear species **S11** (5.70 mg, 3.80 μmol) was dissolved in dry THF (100 μL) followed by the addition of $Pd(PPh_3)_4$ (0.420 mg, 0.362 μmol) and phenylsilane (1.4 μL, 0.012 mmol). The reaction mixture was stirred for 3 h at room temperature under the exclusion of light. After removal of all volatile components, the residue was dissolved in MeOH and filtered through a syringe filter (PTFE, 0.2 μm) and the filter was rinsed with several portions of MeOH. The solvent was concentrated *in vacuo*. Crude material was filtered through a short silica plug (DCM/MeOH 10:1) to afford **S12** as a white solid (4.60 mg, 3.30 μmol, 87%). Presence of product confirmed via MS before proceeding to next step. HRMS (ESI): *m*/*z* calculated for $C_{82}H_{75}N_7O_{15}$ [M + H]: 1397.5321; observed 1397.5380

4-(4-(4-((2*S*,3*R*)-4-amino-2-(4-((*S*)-2-amino-3-phenylpropanamido)benzamido)-3-methoxy-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (**S13**)



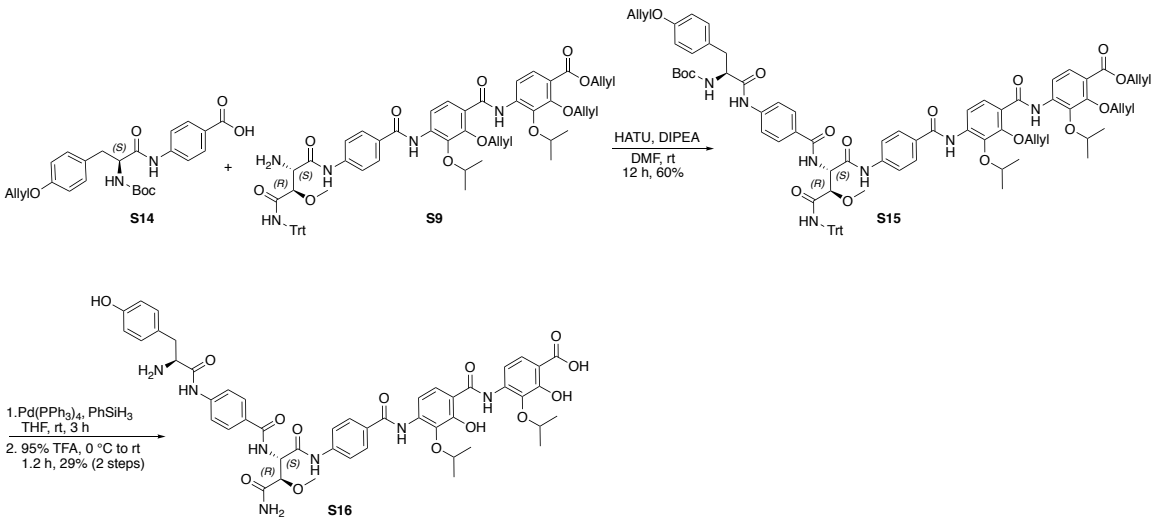
Compound **S12** (4.60 mg, 3.30 μmol) was dissolved in dry DMF (60.0 μL) followed by the addition of piperidine (15.0 μL). The resulting mixture stirred at room temperature for 1 h under Ar(g). After quenching with saturated NH_4Cl, the solution was extracted with EtOAc

(3x) followed by washing of the organic layer with saturated $NaHCO_3$ (1x). The organic layer was then dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was used in the next step without further purification.

Crude residue was dissolved in a pre-cooled solution of 95% TFA (2.5% TIPS, 2.5% H_2O, 175 μL) at 0 °C. After stirring at 0 °C for 5 min, the reaction mixture was allowed to warm to room temperature and kept under Ar(g) for 1.2 h. All volatiles were removed *in vacuo* and the crude residue was dissolved in DCM and evaporated twice. Consumption of starting material monitored via LCMS analysis. Crude material was purified via RP-HPLC (20-80% ACN/H_2O) to afford (**S13**) as a white solid (1.10 mg, 1.20 μmol, 31% over 2 steps).

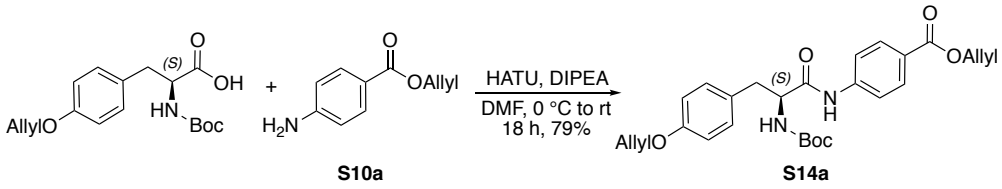
^{1}H NMR (600 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.51 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.16 (s, 1H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.83 (dd, *J* = 8.7, 3.9 Hz, 3H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.48 (d, *J* = 15.7 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.27 (dd, *J* = 17.6, 7.3 Hz, 3H), 7.22 (d, *J* = 7.2 Hz, 1H), 6.90 (s, 1H), 4.91 (t, *J* = 8.1 Hz, 1H), 4.72 (dq, *J* = 13.3, 6.5 Hz, 1H), 4.39 (s, 1H), 4.08 (d, *J* = 7.9 Hz, 1H), 3.73 (s, 1H), 3.31 (s, 3H), 3.05 (dd, *J* = 13.6, 5.5 Hz, 1H), 2.82 (dd, *J* = 13.7, 7.6 Hz, 1H), 2.55 (s, 1H), 1.29 – 1.22 (m, 12H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.94, 168.73, 165.46, 164.23, 163.27, 142.10, 141.58, 138.27, 138.14, 129.37, 128.74, 128.41, 128.27, 126.46, 124.46, 123.95, 118.89, 118.54, 116.32, 79.95, 79.23, 79.00, 78.78, 72.55, 57.75, 56.59, 55.74, 40.43, 35.13, 31.32, 30.75, 29.05, 28.72, 28.61, 25.13, 22.12, 22.08, 14.00; HRMS (ESI): *m*/*z* calculated for $C_{48}H_{51}N_7O_{13}^+$ [M + H]$^+$: 934.3623; observed 934.3613

Total synthesis of PABA70



Scheme S2. Synthetic pathway for the preparation of PABA70 (**S16**).

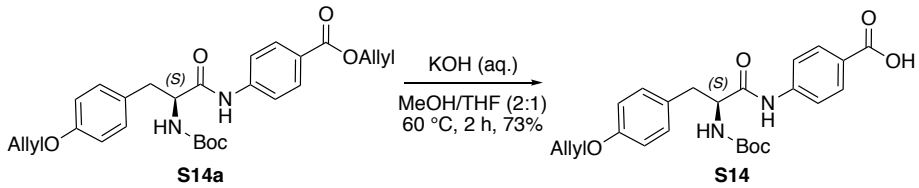
Allyl (*S*)-4-(3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanamido)benzoate (**S14a**)



Boc-Tyr(Allyl)-OH (313 mg, 0.974 mmol) was dissolved in 1.2 mL of dry DMF and cooled to 0 °C before HATU (481 mg, 1.27 mmol) and DIPEA (0.848 mL, 4.87 mmol) were added. After stirring under Ar(g) for 30 min in an ice bath, a solution of **S10a** dissolved in 0.300 mL of dry DMF was added dropwise to the reaction mixture. The solution was allowed to stir at room temperature overnight. After removal of DMF *in vacuo*, the crude material was dissolved in EtOAc and washed with 10% $KHSO_4$ (2x) followed by brine (1x). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified via flash column chromatography with a gradient of 0-20% EtOAc in hexanes to afford dimer **S14a** as a pale yellow oil (371 mg, 0.770 mmol, 79%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.83 (d, *J* = 8.2 Hz, 2H), 6.07 – 5.98 (m, 2H), 5.39 (dd, *J* = 17.3, 4.3 Hz, 2H), 5.27 (dd, *J* = 10.6, 6.2 Hz, 2H), 5.24 (d, *J* = 6.8 Hz, 1H), 4.79 (d, *J* = 5.8 Hz, 2H), 4.48 (d, *J* = 5.5 Hz, 3H), 3.07 (qd, *J* = 14.9, 14.3, 7.1 Hz, 2H), 1.41 (s, 9H); ESI-MS [M + H]$^+$: *m/z* 479.23

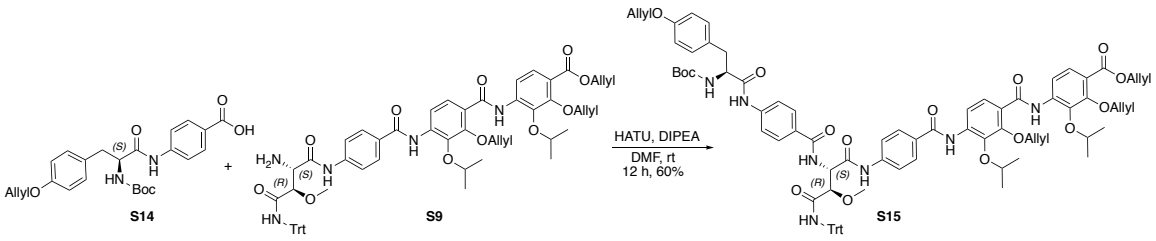
(*S*)-4-(3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanamido)benzoic acid (**S14**)



Dimer **S14a** (28.7 mg, 59.0 μmol) was dissolved in MeOH/THF (2:1, 400 μL). An aqueous solution of KOH (5.0 M, 59.7 μL, 5.0 eq) was added and the reaction mixture stirred for 2 h at 60 °C. The resulting reaction mixture was concentrated *in vacuo* and diluted with H_2O (0.380 mL). The product was precipitated with the addition of 6M HCl (153 μL), filtered, and dried *in vacuo*. An off-white solid was obtained (19.2 mg, 44.0 μmol, 73%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 7.13 (dd, *J* = 11.3, 8.2 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.01 (ddt, *J* = 16.1, 10.4, 5.1 Hz, 1H), 5.39 – 5.33 (m, 1H), 5.22 (d, *J* = 10.6 Hz, 1H), 4.51 (d, *J* = 5.2 Hz, 2H), 4.30 (td, *J* = 9.2, 4.7 Hz, 1H), 2.94 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.78 (dd, *J* = 13.8, 10.2 Hz, 1H), 1.32 (s, 9H); ESI-MS [M + H]$^+$: *m/z* 463.22

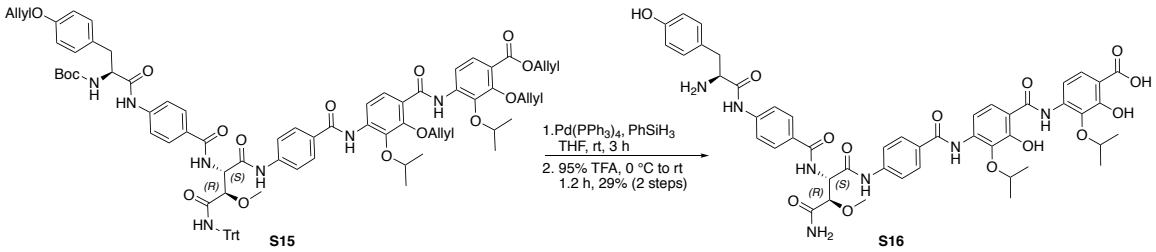
Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-(4-((2*S*,3*R*)-2-(4-((*S*)-3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanamido)benzamido)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzamido)-3-isopropoxybenzoate (**S15**)



The reaction procedure was the same as described for compound **S11**. The product was obtained as a clear oil (5.10 mg, 3.51 μmol, 60%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.72 (s, 1H), 9.33 (s, 1H), 8.73 (s, 1H), 8.47 (d, *J* = 8.9 Hz, 2H), 8.06 (s, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.70 – 7.58 (m, 4H), 7.46 (s, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.17 (p, *J* = 8.9, 8.3 Hz, 15H), 6.86 (d, *J* = 8.1 Hz, 2H), 6.20 – 6.09 (m, 2H), 6.04 (dtt, *J* = 15.8, 10.3, 5.7 Hz, 2H), 5.44 – 5.35 (m, 3H), 5.30 – 5.20 (m, 4H), 5.09 (s, 1H), 4.88 (t, *J* = 6.5 Hz, 1H), 4.80 (d, *J* = 5.8 Hz, 2H), 4.79 – 4.70 (m, 2H), 4.70 (d, *J* = 6.6 Hz, 2H), 4.60 – 4.56 (m, 3H), (d, *J* = 5.0 Hz, 2H), 4.50 (d, *J* = 5.3 Hz, 2H), 4.43 (s, 1H), 3.65 (s, 3H), 3.10 (d, *J* = 7.0 Hz, 2H), 1.44 (s, 9H), 1.39 (d, *J* = 6.1 Hz, 6H), 1.28 (d, *J* = 6.2 Hz, 6H); HRMS (ESI): *m*/*z* calculated for $C_{84}H_{90}N_7O_{16}^+$[M + H]$^+$: 1452.6440; observed 1452.6329

4-(4-(4-((2*S*,3*R*)-4-amino-2-(4-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)benzamido)-3-methoxy-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (**S16**)

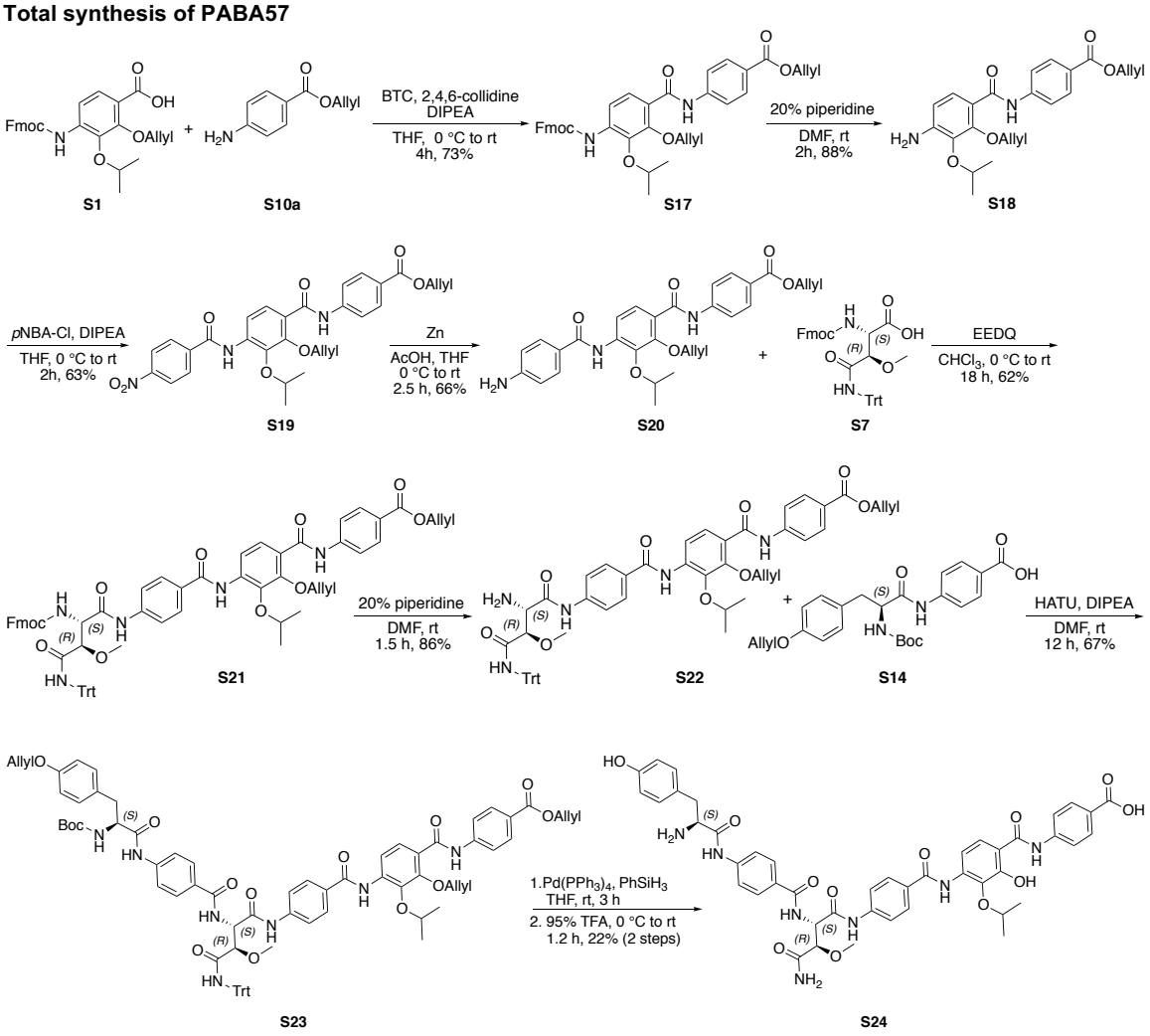


Linear species **S15** (5.20 mg, 3.60 μmol) was dissolved in dry THF (100 μL) followed by the addition of $Pd(PPh_3)_4$ (0.410 mg, 0.358 μmol) and phenylsilane (1.40 μL, 11.0 μmol). The reaction mixture was stirred for 3 h at room temperature under the exclusion of light. After removal of all volatile components, the residue was dissolved in MeOH and filtered through a syringe filter (PTFE, 0.2 μm) and the filter was rinsed with several portions of MeOH. The solvent was concentrated *in vacuo*. Crude material was filtered through a short silica plug (DCM/MeOH 10:1 to 5:1) to afford **S16** as a white solid (3.4 mg, crude). Filtered material was used in the next step without additional purification.

The partially deprotected **S15** was dissolved in a pre-cooled solution of 95% TFA (2.5% TIPS, 2.5% H_2O, 175 μL) at 0 °C. After stirring at 0 °C for 5 min, the reaction mixture was allowed to warm to room temperature and kept under Ar(g) for 1.2 h. All volatiles were removed *in vacuo* and the crude residue was dissolved in DCM and evaporated twice. Consumption of starting material monitored via LCMS analysis. Crude material was purified via RP-HPLC (10-90% ACN/H_2O) to afford **S16** as a white solid (1.00 mg, 1.10 μmol, 29% over 2 steps).

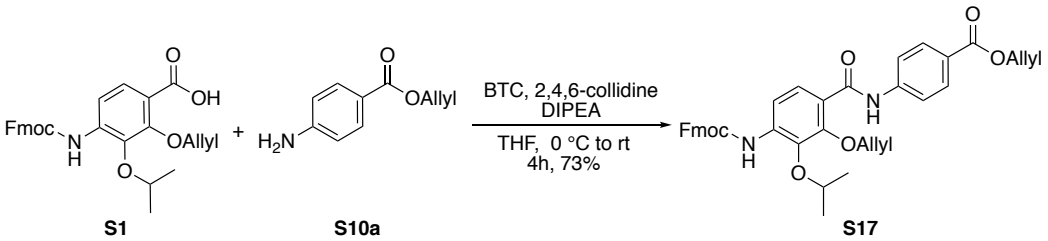
^{1}H NMR (600 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.22 (s, 1H), 8.84 (s, 1H), 8.52 (s, 6H), 8.44 (d, *J* = 8.3 Hz, 1H), 7.88 – 7.80 (m, 3H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.57 (s, 1H), 7.44 (s, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.25 (dd, *J* = 8.6, 4.0 Hz, 1H), 7.02 (t, *J* = 7.9 Hz, 2H), 6.83 (x), 6.65 (d, *J* = 8.0 Hz, 2H), 6.28 (s, 1H), 5.03 (p, *J* = 6.1 Hz, 1H), 4.90 (t, *J* = 8.1 Hz, 1H), 4.66 (p, *J* = 6.1 Hz, 1H), 4.09 (d, *J* = 8.0 Hz, 1H), 3.51 (m, *J* = 7.1 Hz, 1H), 3.31 (s, 3H), 2.89 (dd, *J* = 13.6, 5.5 Hz, 1H), 1.27 (d, *J* = 6.0 Hz, 6H), 1.23 (d, *J* = 5.9 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.20, 171.92, 170.95, 168.68, 165.62, 165.46, 156.59, 155.78, 141.84, 138.21, 138.08, 130.19, 128.35, 128.08, 127.58, 123.83, 119.12, 118.40, 116.49, 114.97, 107.77, 79.98, 72.30, 57.71, 57.43, 29.07, 28.74, 22.93, 22.13, 21.72, 14.01; HRMS (ESI): *m*/*z* calculated for $C_{48}H_{52}N_7O_{14}^+$ [M + H]$^+$: 950.3572; observed 950.3451

Total synthesis of PABA57



Scheme S3. Synthetic pathway for the preparation of PABA57 (**S24**).

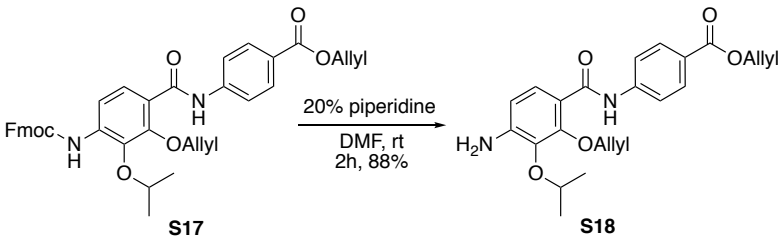
Allyl 4-(4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(allyloxy)-3-isopropoxybenzamido)benzoate (**S17**)



The reaction procedure was the same as described for compound **S3**. The product **S17** was obtained as a clear oil (126 mg, 0.200 mmol, 73%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.06 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 3H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.13 (ddt, *J* = 16.5, 11.1, 5.8 Hz, 1H), 6.05 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.49 (d, *J* = 17.1 Hz, 1H), 5.45 – 5.38 (m, 2H), 5.29 (d, *J* = 10.4 Hz, 1H), 4.82 (d, *J* = 5.7 Hz, 2H), 4.68 (d, *J* = 5.9 Hz, 2H), 4.64 (p, *J* = 6.1 Hz, 1H), 4.56 (p, *J* = 6.4 Hz, 2H), 4.33 (t, *J* = 6.8 Hz, 1H), 1.36 (d, *J* = 6.2 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 633.24

Allyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate (**S18**)

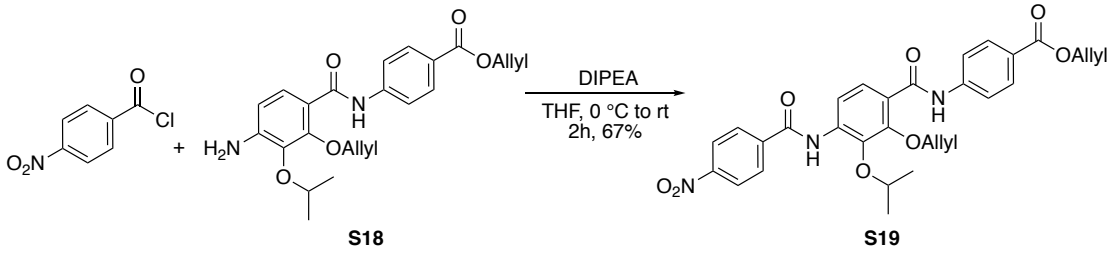


S17 **S18**

The reaction procedure was the same as described for compound **S4**. The product **S18** was obtained as a pale yellow oil (111 mg, 0.271 mmol, 88%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 6.60 (d, *J* = 8.6 Hz, 1H), 6.13 (ddt, *J* = 16.5, 10.9, 5.9 Hz, 1H), 6.04 (ddt, *J* = 16.4, 10.9, 5.6 Hz, 1H), 5.48 (d, *J* = 17.1 Hz, 1H), 5.40 (d, *J* = 17.1 Hz, 1H), 5.37 (d, *J* = 10.5 Hz, 1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.80 (d, *J* = 5.7 Hz, 2H), 4.68 (d, *J* = 5.9 Hz, 2H), 4.56 (h, *J* = 6.1 Hz, 1H), 4.25 (s, *2H*), 1.33 (d, *J* = 6.3 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 411.21

Allyl 4-(2-(allyloxy)-3-isopropoxy-4-(4-nitrobenzamido)benzamido)benzoate (**S19**)

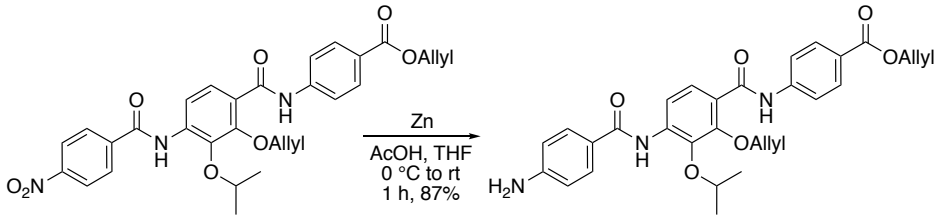


S18 **S19**

The reaction procedure was the same as described for compound **S5**. Crude material was purified via flash column chromatography with a gradient of gradient of 0-30% EtOAc in hexanes to afford **S19** as a pale yellow oil (90.0 mg, 0.160 mmol, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.78 (s, 1H), 8.46 (d, *J* = 8.9 Hz, 1H), 8.39 (d, *J* = 8.4 Hz, 2H), 8.06 (dt, *J* = 8.3, 5.8 Hz, 5H), 7.75 (d, *J* = 8.4 Hz, 2H), 6.14 (ddt, *J* = 16.5, 11.0, 5.9 Hz, 1H), 6.04 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.50 (d, *J* = 17.1 Hz, 1H), 5.44 – 5.38 (m, 2H), 5.29 (d, *J* = 10.4 Hz, 1H), 4.81 (d, *J* = 5.9 Hz, 2H), 4.78 (dd, *J* = 12.3, 6.1 Hz, 1H), 4.70 (d, *J* = 6.0 Hz, 2H), 1.39 (d, *J* = 6.2 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 560.16

Allyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropoxybenzamido)benzoate (**S20**)

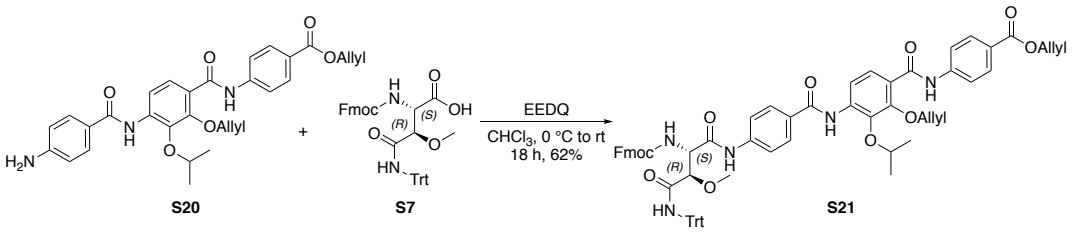


S19 **S20**

The reaction procedure was the same as described for compound **S6**. The product was obtained a pale yellow oil (74.0 mg, 0.140 mmol, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H), 8.65 (s, 1H), 8.49 (d, *J* = 8.9 Hz, 1H), 8.05 (t, *J* = 8.3 Hz, 3H), 7.75 (dd, *J* = 13.1, 8.3 Hz, 4H), 6.75 (d, *J* = 8.1 Hz, 2H), 6.14 (ddt, *J* = 16.5, 11.0, 5.9 Hz, 1H), 6.05 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.50 (d, *J* = 17.1 Hz, 1H), 5.44 – 5.38 (m, 2H), 5.29 (d, *J* = 10.3 Hz, 1H), 4.82 (d, *J* = 5.7 Hz, 2H), 4.73 (q, *J* = 6.2 Hz, 1H), 4.69 (d, *J* = 5.9 Hz, 2H), 4.23 (s, 2H), 1.38 (d, *J* = 6.2 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 530.20

Allyl 4-(4-(4-((2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)benzoate (**S21**)

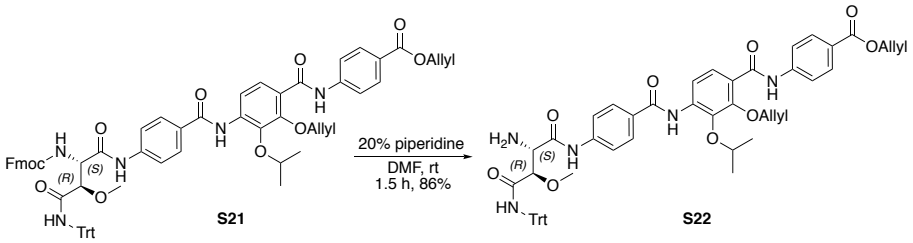


S20 S7 S21

The reaction procedure was the same as described for compound **S8**. The product **S21** was obtained as a clear oil (10.0 mg, 88.0 μmol, 62%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.73 (s, 1H), 8.50 (d, *J* = 8.9 Hz, 1H), 8.24 (s, 1H), 8.07 (dd, *J* = 8.7, 4.7 Hz, 3H), 7.90 (s, 1H), 7.85 (d, *J* = 8.3 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 3H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 6.5 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 9H), 7.15 (dd, *J* = 6.6, 2.8 Hz, 6H), 6.15 (ddt, *J* = 16.6, 11.1, 5.9 Hz, 1H), 6.05 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.52 (dd, *J* = 20.3, 12.4 Hz, 2H), 5.45 – 5.39 (m, 2H), 5.29 (d, *J* = 7.1 Hz, 1H), 4.82 (d, *J* = 5.7 Hz, 3H), 4.75 (p, *J* = 6.1 Hz, 1H), 4.71 (d, *J* = 5.9 Hz, 2H), 4.60 (qd, *J* = 10.8, 6.3 Hz, 3H), 4.25 (t, *J* = 6.3 Hz, 1H), 3.60 (s, 3H), 1.38 (dd, *J* = 6.2, 3.0 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 1139.12

Allyl 4-(2-(allyloxy)-4-(4-((2*S*,3*R*)-2-amino-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzamido)benzoate (**S22**)

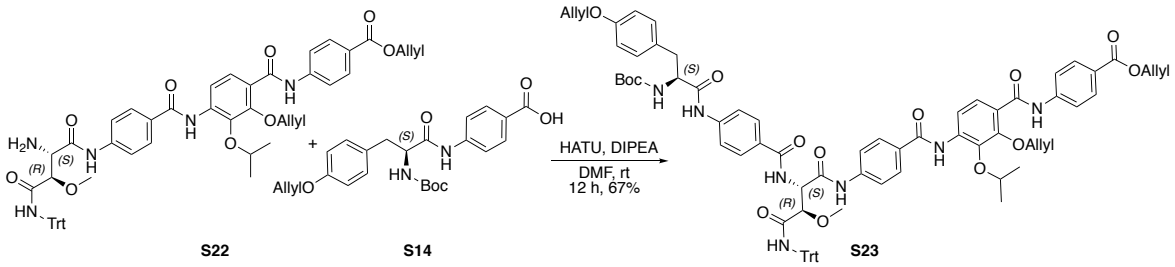


S21 S22

The reaction procedure was the same as described for compound **S9**. The product **S22** was obtained as a clear oil (10.3 mg, 11.0 μmol, 86%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H), 9.44 (s, 1H), 8.72 (s, 1H), 8.50 (d, *J* = 8.9 Hz, 1H), 8.07 (d, *J* = 8.6 Hz, 3H), 7.86 – 7.82 (m, 3H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.34 – 7.25 (m, 3H), 7.27 – 7.22 (m, 3H), 7.22 (d, *J* = 7.6 Hz, 6H), 6.15 (ddt, *J* = 16.6, 11.1, 5.9 Hz, 1H), 6.05 (ddt, *J* = 16.4, 10.9, 5.6 Hz, 1H), 5.50 (d, *J* = 17.1 Hz, 1H), 5.45 – 5.39 (m, 2H), 5.29 (d, *J* = 7.9 Hz, 2H), 4.82 (d, *J* = 5.6 Hz, 2H), 4.72 (dd, *J* = 17.3, 6.0 Hz, 3H), 4.47 (d, *J* = 3.2 Hz, 1H), 3.83 (d, *J* = 3.2 Hz, 1H), 3.58 (s, 3H), 1.65 (s, 2H), 1.57 (d, *J* = 5.6 Hz, 1H), 1.38 (dd, *J* = 6.2, 3.7 Hz, 6H); ESI-MS $[M + H]^+$: *m/z* 916.65

Allyl 4-(2-(allyloxy)-4-(4-((2*S*,3*R*)-2-(4-((*S*)-3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanamido)benzamido)-3-methoxy-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzamido)benzoate (**S23**)

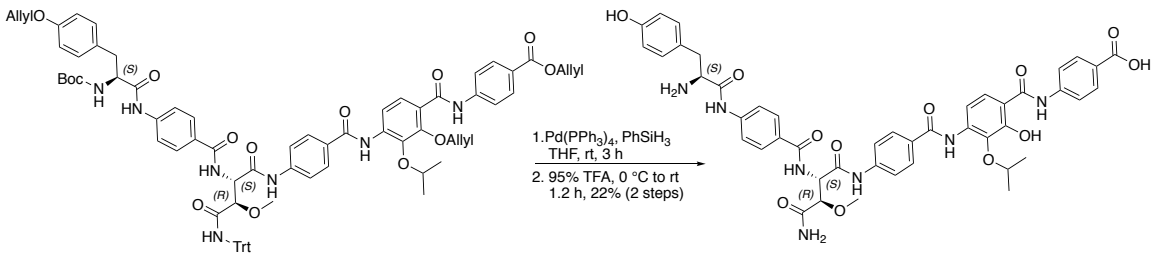


S22 S14 S23

The reaction procedure was the same as described for compound **S15**. The product **S23** was obtained as a clear oil (5.00 mg, 3.74 μmol, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H), 9.34 (s, 1H), 8.73 (s, 1H), 8.50 (d, *J* = 8.9 Hz, 1H), 8.10 (s, 1H), 8.07 (d, *J* = 4.6 Hz, 3H), 7.84 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.62 (dd, *J* = 22.3, 8.1 Hz, 4H), 7.47 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.17 (p, *J* = 9.1, 8.4 Hz, 17H), 6.86 (d, *J* = 8.2 Hz, 2H), 6.14 (ddt, *J* = 16.5, 11.0, 5.9 Hz, 1H), 6.04 (dddd, *J* = 22.9, 12.6, 10.7, 5.4 Hz, 2H), 5.50 (d, *J* = 17.1 Hz, 1H), 5.45 – 5.36 (m, 3H), 5.28 (t, *J* = 11.4 Hz, 2H), 5.10 (s, 1H), 4.88 (t, *J* = 6.5 Hz, 1H), 4.82 (d, *J* = 5.7 Hz, 2H), 4.74 (p, *J* = 6.1 Hz, 1H), 4.70 (d, *J* = 5.9 Hz, 2H), 4.58 (d, *J* = 6.7 Hz, 1H), 4.50 (d, *J* = 5.3 Hz, 2H), 4.44 (s, 1H), 3.65 (d, *J* = 3.5 Hz, 3H), 3.10 (d, *J* = 7.0 Hz, 2H), 2.80 (s, 1H), 2.61 (s, 1H), 1.44 (s, 9H), 1.38 (d, *J* = 6.1 Hz, 6H); HRMS (ESI): *m/z* calculated for $C_{78}H_{80}N_7O_{14}^+$ $[M + H]^+$: 1338.5763; observed 1338.5660

4-(4-(4-((2*S*,3*R*)-4-amino-2-(4-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)benzamido)-3-methoxy-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**S24**)

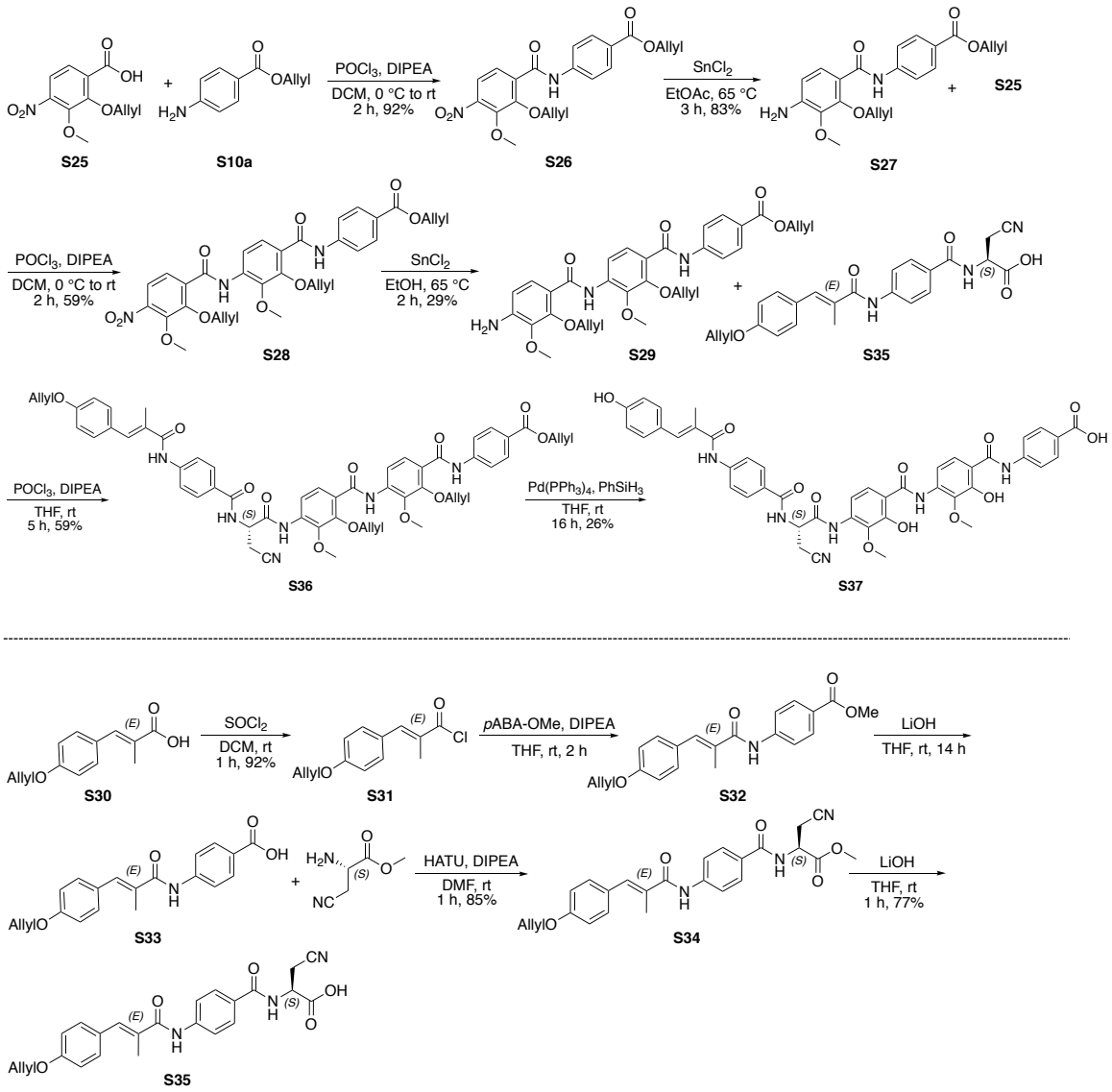


S23 **S24**

The reaction procedure was the same as described for compound **S16**. The product **S24** was obtained as a white solid (0.700 mg, 0.80 μmol, 22% over 2 steps).

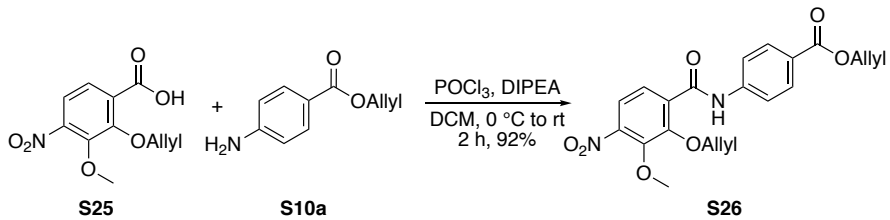
^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.89 (s, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.88 – 7.80 (m, 3H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.73 – 7.69 (m, 1H), 7.59 – 7.54 (m, 2H), 7.47 – 7.44 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.10 (dd, *J* = 11.2, 8.4 Hz, 2H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 5.00 (p, *J* = 6.1 Hz, 1H), 4.92 (dt, *J* = 23.1, 8.1 Hz, 1H), 4.09 (t, *J* = 7.5 Hz, 1H), 4.03 (q, *J* = 7.1 Hz, 1H), 3.31 (s, 3H), 2.89 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.76 (dd, *J* = 14.2, 8.7 Hz, 1H), 1.20 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.40, 166.85, 165.36, 163.15, 162.82, 130.21, 129.68, 128.38, 127.64, 119.12, 117.52, 116.09, 115.00, 79.98, 78.01, 72.30, 70.38, 59.80, 57.77, 57.43, 57.18, 34.22, 31.00, 28.52, 28.08, 26.70, 24.81, 22.72, 22.11, 20.81, 20.65, 14.12, 14.02, 11.30; HRMS (ESI): *m*/*z* calculated for $C_{45}H_{46}N_7O_{12}^+$ [M + H]$^+$: 876.3204; observed 876.3159

Total synthesis of PABA34



Scheme S4. Synthetic pathway for the preparation of PABA34 (**S37**).

Allyl 4-(2-(allyloxy)-3-methoxy-4-nitrobenzamido)benzoate (**S26**)

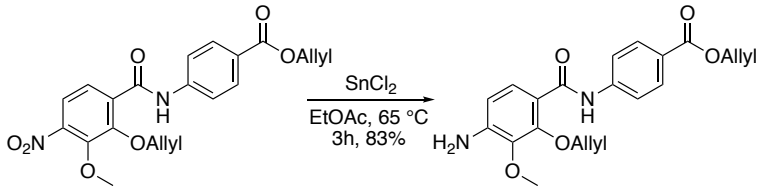


Building block 2-(allyloxy)-3-methoxy-4-nitrobenzoic acid (**S25**) was prepared according to previously reported protocols.[14]

Compound **S25** (2.00 g, 3.01 mmol) was dissolved in DCM (20.0 mL) followed by the addition of $POCl_3$ (1.45 g, 9.48 mmol) and DIPEA (2.75 mL, 15.8 mmol) at 0 °C. Compound **S10a** (1.40 g, 7.90 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with DCM (3x). The combined organic phases were washed with brine (1x), dried over Na_2SO_4, and concentrated. Crude material was purified via manual flash column chromatography (Pet. Ether/EtOAc 15:1 to 10:1) to afford (**S26**) as a yellow solid (3.00 g, 7.27 mmol, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.04 – 8.01 (m, 3H), 7.69 – 7.67 (d, *J* = 8 Hz, 2H), 7.60 – 7.58 (m, 1H), 6.12 – 5.99 (m, 2H), 5.45 – 5.22 (m, 4H), 4.77 – 4.75 (d, *J* = 8 Hz, 2H), 4.71 – 4.69 (d, *J* = 8 Hz, 2H), 4.07 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 413.30

Allyl 4-(2-(allyloxy)-4-amino-3-methoxybenzamido)benzoate (**S27**)

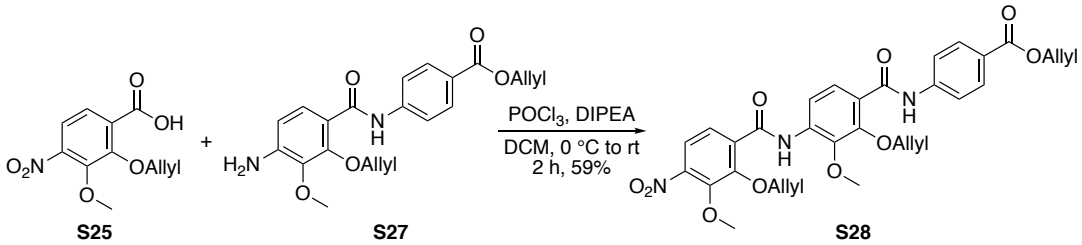


S26 **S27**

Compound **S26** (1.50 g, 3.64 mmol) was dissolved in EtOAc (15.0 mL) followed by the addition of $SnCl_2 \cdot 2H_2O$ (4.10 g, 18.2 mmol) and the mixture was stirred at 60-65 °C for 3 h. After quenching with saturated $NaHCO_3$, the aqueous layer was extracted with EtOAc (3x) and the combined organic phases were washed with brine (1x) and dried over Na_2SO_4. After concentrating *in vacuo*, the crude material was purified via manual flash column chromatography (Pet. ether/EtOAc 15:1 to 6:1) to afford **S27** as a yellow solid (1.15 g, 3.01 mmol, 83%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.97 – 7.95 (d, *J* = 8 Hz, 2H), 7.83 – 7.81 (d, *J* = 8 Hz, 2H), 7.40 – 7.38 (d, *J* = 8 Hz, 1H), 6.58 – 6.54 (d, *J* = 16 Hz, 1H), 6.09 – 6.05 (m, 2H), 5.74 (s, 2H), 5.48 – 5.39 (m, 2H), 5.29 – 5.27 (d, *J* = 8 Hz, 2H), 4.80 – 4.78 (d, *J* = 8 Hz, 2H), 4.66 – 4.64 (d, *J* = 8 Hz, 2H), 3.76 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 383.30

Allyl 4-(2-(allyloxy)-4-(2-(allyloxy)-3-methoxy-4-nitrobenzamido)-3-methoxybenzamido)benzoate (**S28**)

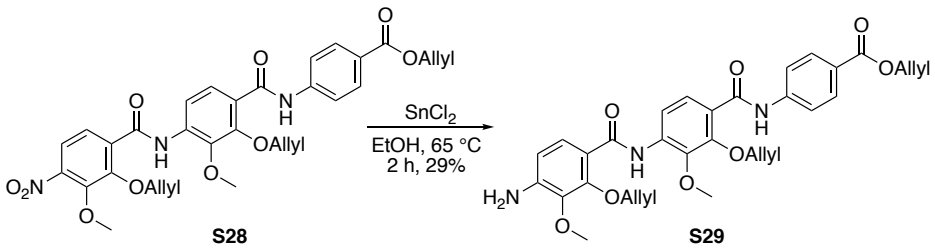


S25 **S27** **S28**

Compound **S25** (761 mg, 3.01 mmol) was dissolved in DCM (10.0 mL) followed by the addition of $POCl_3$ (0.336 mL, 3.61 mmol) and DIPEA (1.05 mL, 6.01 mmol) at 0 °C. Dimer **S27** (1.15 g, 3.01 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into a solution of saturated $NaHCO_3$ and extracted with DCM (3x). The combined organic phases were washed with brine (1x), dried over Na_2SO_4, and concentrated. Crude material was purified via manual flash column chromatography (Pet. Ether/EtOAc 10:1 to 3:1) to afford **S28** as a yellow solid (1.10 g, 1.78 mmol, 59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.56 (s 1H), 10.41 (s, 1H), 8.18 – 8.16 (d, *J* = 8 Hz, 1H), 8.01 – 7.91 (m, 2H), 7.89 – 7.87 (d, *J* = 8 Hz, 2H), 7.80 – 7.78 (d, *J* = 8 Hz, 1H), 7.76 – 7.75 (m, 1H), 7.46 – 7.44 (d, *J* = 8 Hz, 1H), 6.07 – 6.03 (m, 3H), 5.44 – 5.38 (m, 3H), 5.30 – 5.27 (m, 3H), 4.81 – 4.76 (m, 4H), 4.62 – 4.61 (d, *J* = 4 Hz, 2H), 4.00 (s, 3H), 3.93 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 618.00

allyl 4-(2-(allyloxy)-4-(2-(allyloxy)-4-amino-3-methoxybenzamido)-3-methoxybenzamido)benzoate (**S29**)

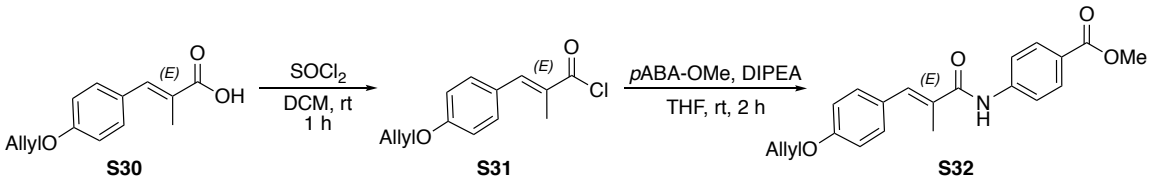


S28 **S29**

Compound **S28** (1.10 g, 1.78 mmol) was dissolved in EtOH (30.0 mL) followed by the addition of $SnCl_2 \cdot 2H_2O$ (2.01 g, 8.91 mmol) and the mixture was stirred at 60-65 °C for 2 h. The solvent was evaporated *in vacuo* and the crude residue was dissolved in EtOAc (400 mL). The organic layer was washed with saturated $NaHCO_3$ (3x) followed by brine (1x) and dried over Na_2SO_4. After concentrating *in vacuo*, the crude material was purified via manual flash column chromatography (Pet. ether/EtOAc 5:1 to 2:1) to afford tripeptide **S29** as a yellow solid (300 mg, 0.510 mmol, 29%).

^{1}H NMR (400 MHz, $CDCl_3$) δ10.60 (s 1H), 10.50 (s, 1H), 8.38 – 8.36 (d, *J* = 8 Hz, 1H), 8.00 – 7.98 (d, *J* = 8 Hz, 2H), 7.88 – 7.86 (d, *J* = 8 Hz, 2H), 7.60 – 7.58 (d, *J* = 8 Hz, 1H), 7.44 – 7.42 (d, *J* = 8 Hz, 1H), 7.46 – 7.44 (d, *J* = 8 Hz, 1H), 6.58 – 6.56 (d, *J* = 8 Hz, 1H), 6.17 – 6.04 (m, 3H), 5.86 (s, 2H), 5.43 – 5.38 (m, 3H), 5.30 – 5.27 (m, 3H), 4.80 – 4.79 (d, *J* = 4 Hz, 4H), 4.63 – 4.61 (d, *J* = 8 Hz, 2H), 3.95 (s, 3H), 3.76 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 588.20

Methyl (*E*)-4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)benzoate (**S32**)

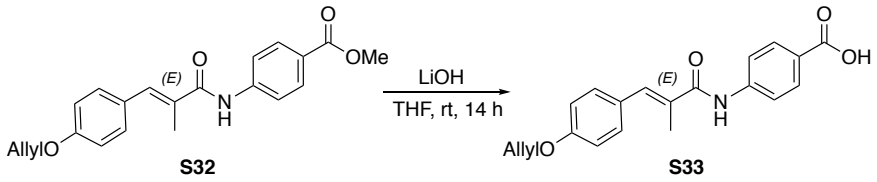


S30 **S31** **S32**

Building block (*E*)-3-(4-(allyloxy)phenyl)-2-methylacrylic acid (**S30**) was prepared according to previously reported protocols.[14, 15]

Compound **S30** (300 mg, 1.37 mmol) was dissolved in DCM (10.0 mL) followed by the dropwise addition of $SOCl_2$ (0.50 mL, 6.87 mmol). The mixture was stirred at room temperature for 1 h before concentrating *in vacuo* and the crude residue dissolved in THF (10.0 mL). Methyl 4-aminobenzoate (180 mg, 1.19 mmol) and DIPEA (1.20 mL, 6.87 mmol) were added to the mixture and stirred for another 2 h at room temperature. The reaction mixture was poured into a solution of saturated NH_4Cl (50.0 mL) and extracted with EtOAc (3x). The combined organic phases were washed with brine (1x), dried over Na_2SO_4, and concentrated. The crude compound **S32** (0.600 g, crude) was obtained as a yellow solid.

(*E*)-4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)benzoic acid (**S33**)

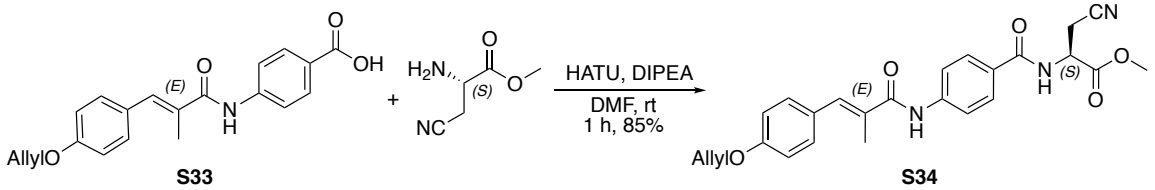


S32 **S33**

Methyl ester **S32** (500 mg, 1.42 mmol) was dissolved in THF (10.0 mL) followed by the addition of LiOH · H_2O (0.50 M, 8.54 mL) and the mixture stirred for 14 h at room temperature. The reaction was acidified to pH 2-3 with 1 M HCl followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. The crude compound **S33** (500 mg, crude) was obtained as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 10.17 (s, 1H), 7.90 (d, *J* = 8.9 Hz, 2H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.29 (s, 1H), 7.03 (d, *J* = 8.7 Hz, 2H), 6.06 (ddt, *J* = 17.4, 10.5, 5.2 Hz, 1H), 5.42 (dq, *J* = 17.3, 1.8 Hz, 1H), 5.28 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.61 (dt, *J* = 5.3, 1.6 Hz, 2H), 2.12 (d, *J* = 1.4 Hz, 3H); ESI-MS [M + H]$^+$: *m/z* 338.00

Methyl (*S*,*E*)-2-(4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)benzamido)-3-cyanopropanoate (**S34**)

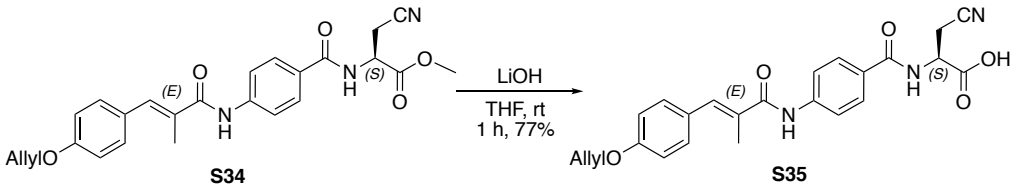


S33 **S34**

Compound **S33** (400 mg, 1.19 mmol) and methyl (2*S*)-2-amino-3-cyano-propanoate (430 mg, 1.78 mmol, 1.50 eq TFA) were dissolved in DMF (10.0 mL) followed by the addition of DIPEA (0.62 mL, 3.56 mmol) and HATU (541 mg, 1.42 mmol). The mixture stirred for 1 h at room temperature before diluting with H_2O (50.0 mL) and extracted with EtOAc (3x). The combined organic phases were washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified via manual flash column chromatography (Pet. ether/EtOAc 3:1 to 1:1) to afford **S34** as a white solid (450 mg, 1.01 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.80 (m, 2H), 7.77 – 7.69 (m, 3H), 7.41 – 7.33 (m, 3H), 7.04 (d, *J* = 6.4 Hz, 1H), 7.00 – 6.92 (m, 2H), 6.07 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 1H), 5.44 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.36 – 5.28 (m, 1H), 4.95 (td, *J* = 5.5, 4.2 Hz, 1H), 4.58 (dt, *J* = 5.3, 1.5 Hz, 2H), 3.91 (s, 3H), 3.23 (dd, *J* = 17.0, 5.4 Hz, 1H), 3.07 (dd, *J* = 17.0, 4.3 Hz, 1H), 2.24 (d, *J* = 1.4 Hz, 3H); ESI-MS [M + H]$^+$: *m/z* 448.20

(*S*,*E*)-2-(4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)benzamido)-3-cyanopropanoic acid (**S35**)

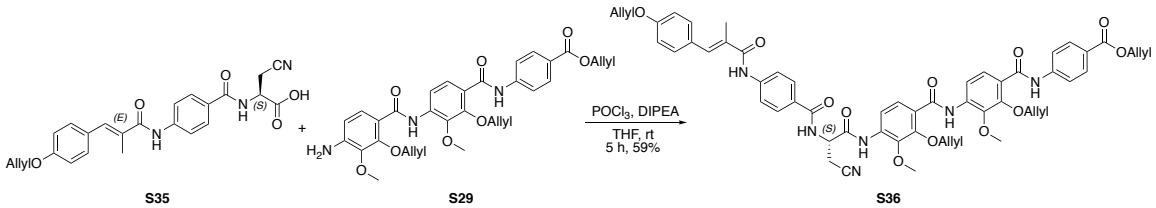


S34 **S35**

Compound **S34** (0.400 g, 0.894 mmol) was dissolved in a mixture of dioxane (10.0 mL) and H_2O (5.0 mL) followed by the addition of $LiOH \cdot H_2O$ (56.2 mg, 1.34 mmol). After stirring at room temperature for 1 h, EtOAc (20.0 mL) was added to the reaction mixture and the pH was adjusted to 3-4 with 1 M HCl. The mixture was extracted with EtOAc (3x), washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by prep RP-HPLC (32-62% ACN/H_2O) to afford **S35** as a white solid (300 mg, 0.692 mmol, 77%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.96 (d, *J* = 7.9 Hz, 1H), 7.90 – 7.80 (m, 4H), 7.49 – 7.41 (m, 2H), 7.30 (s, 1H), 7.07 – 6.99 (m, 2H), 6.06 (ddt, *J* = 17.3, 10.5, 5.2 Hz, 1H), 5.42 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.32 – 5.24 (m, 1H), 4.70 (td, *J* = 8.5, 5.5 Hz, 1H), 4.61 (dt, *J* = 5.3, 1.6 Hz, 2H), 3.15 – 2.97 (m, 2H), 2.13 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 434.00

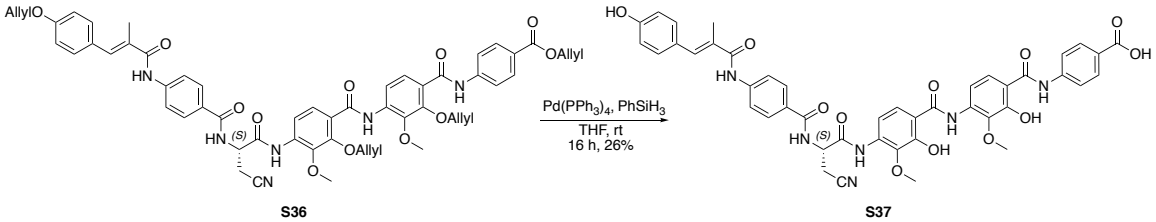
Allyl (*S*,*E*)-4-(2-(allyloxy)-4-(2-(allyloxy)-4-(2-(4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)benzamido)-3-cyanopropanamido)-3-methoxybenzamido)-3-methoxybenzamido)benzoate (**S36**)



S35 **S29** **S36**

Compound **S35** (90.0 mg, 0.208 mmol) and compound **S29** (0.100 g, 0.170 mmol) were dissolved in THF (10.0 mL) followed by the addition of $POCl_3$ (47.60 μL, 0.510 mmol) and DIPEA (88.8 μL, 0.510 mmol). After stirring at room temperature for 5 h the reaction was quenched with a solution of saturated $NaHCO_3$ (15.0 mL) and extracted with DCM (3x). The organic layer was washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified via prep RP-HPLC (72-100% ACN/H_2O) resulting in compound **S36** as a brown solid (0.100 g, 99.7 μmol, 59%). Presence of coupled linear species confirmed via MS and carried forward to the final deprotection step. ESI-MS $[M + H]^+$: *m/z* 1003.00

(*S*,*E*)-4-(4-(4-(3-cyano-2-(4-(3-(4-hydroxyphenyl)-2-methylacrylamido)benzamido)propanamido)-2-hydroxy-3-methoxybenzamido)-2-hydroxy-3-methoxybenzamido)benzoic acid (**S37**)

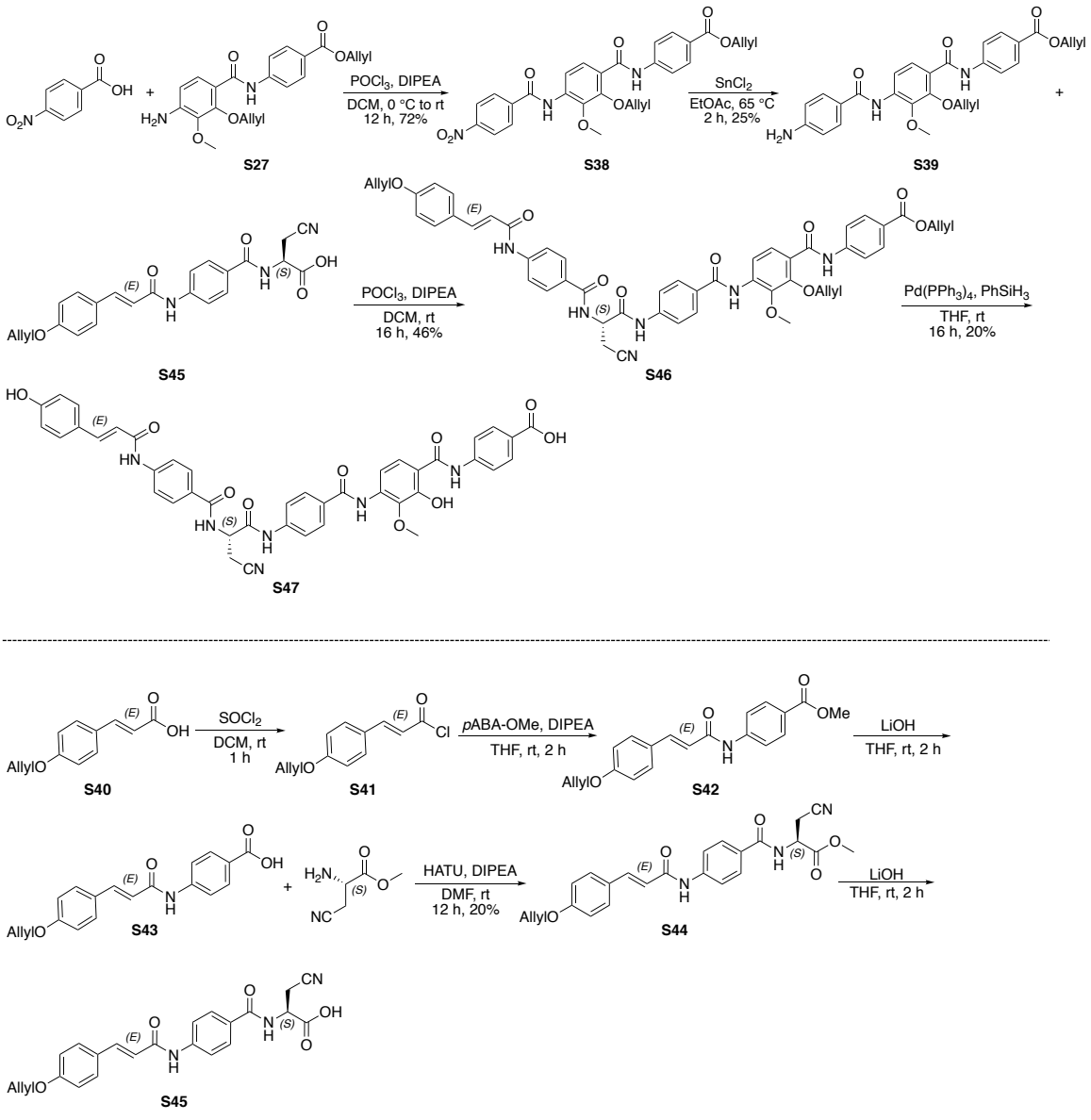


S36 **S37**

Linear species **S36** (90.0 mg, 0.897 mmol) was dissolved in THF (5.00 mL) followed by the addition of $Pd(PPh_3)_4$ (25.9 mg, 0.224 mmol) and phenylsilane (38.8 mg, 0.359 mmol). The reaction mixture was stirred at room temperature for 16 h under an atmosphere of N_2. After removal of all volatile components, the crude residue was purified by RP-HPLC (H_2O/ACN+0.1%TFA) resulting in **S37** as a white solid (20.0 mg, 23.7 μmol, 26%).

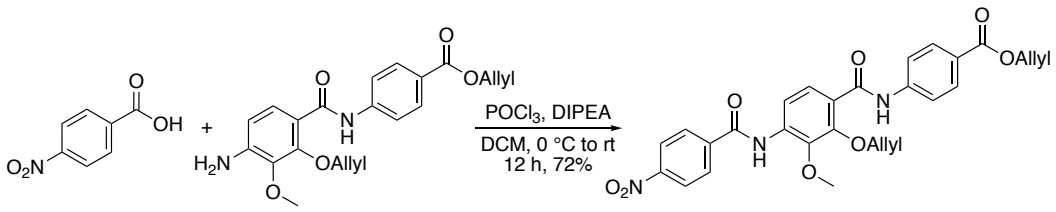
^{1}H NMR (600 MHz, DMSO-d_6) δ 12.79 (s, 1H), 12.26 (s, 1H), 11.61 (s, 1H), 11.12 (s, 1H), 10.58 (s, 1H), 10.13 (s, 1H), 9.76 (d, *J* = 11.5 Hz, 2H), 9.15 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 1H), 7.96 (dd, *J* = 17.3, 8.4 Hz, 4H), 7.87 (t, *J* = 8.0 Hz, 5H), 7.82 (s, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.28 (s, 1H), 6.85 (d, *J* = 8.1 Hz, 2H), 5.20 (q, *J* = 7.9 Hz, 1H), 3.91 (s, 3H), 3.73 (s, 3H), 3.14 (qd, *J* = 17.0, 7.4 Hz, 2H), 2.13 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.38, 168.97, 168.62, 167.34, 167.18, 163.79, 158.02, 153.95, 150.12, 143.41, 142.51, 138.27, 137.32, 137.28, 136.15, 134.43, 131.78, 130.69, 129.97, 128.80, 127.73, 127.01, 126.67, 126.54, 124.32, 121.05, 119.70, 118.81, 116.13, 115.87, 112.56, 112.17, 110.57, 61.20, 60.77, 51.09, 19.84, 15.01; HRMS (ESI): *m/z* calculated for $C_{44}H_{39}N_6O_{12}^+$ $[M + H]^+$: 843.2626; observed 843.2627

Total synthesis of PABA157



Scheme S5. Synthetic pathway for the preparation of PABA157 (**S47**).

Allyl 4-(2-(allyloxy)-3-methoxy-4-(4-nitrobenzamido)benzamido)benzoate (**S38**)

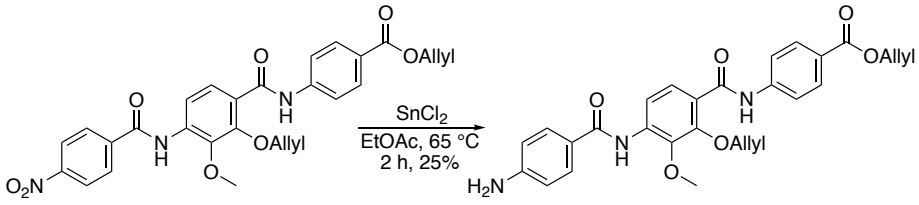


S27 **S38**

Compound **S27** (1.20 g, 3.14 mmol) and 4-nitrobenzoic acid (629 mg, 3.77 mmol) were dissolved in DCM (10.0 mL) followed by the addition of $POCl_3$ (0.438 mL, 4.71 mmol) and DIPEA (1.09 mL, 6.28 mmol) at 0 °C. After stirring at room temperature for 12 h, the reaction mixture was poured into a solution of saturated $NaHCO_3$ and extracted with DCM (3x). The combined organic phases were washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. Crude material was purified via RP-HPLC (H_2O/ACN+0.1%TFA) resulting in compound **S38** as a brown solid (1.20 g, 2.26 mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.61 (s, 1H), 8.40 – 8.38 (d, *J* = 8 Hz, 1H), 8.33 – 8.31 (d, *J* = 8 Hz, 2H), 8.04 – 7.99 (m, 5H), 7.70 – 7.68 (d, *J* = 8 Hz, 2H), 6.11 –5.97 (m, 2H), 5.47 – 5.33 (m, 3H), 5.24 – 5.21 (m, 1H), 4.76 – 4.75 (d, *J* = 4 Hz, 2H), 4.65 – 4.64 (d, *J* = 4 Hz, 2H), 3.99 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 532.20

Allyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-methoxybenzamido)benzoate (**S39**)

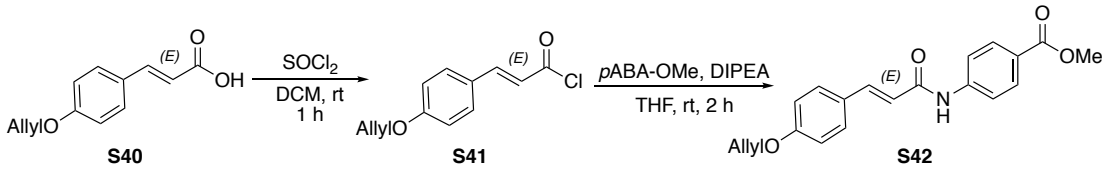


S38 **S39**

Compound **S38** (500 mg, 0.94 mmol) was dissolved in EtOAc (10.0 mL) followed by the addition of $SnCl_2 \cdot 2H_2O$ (849 mg, 3.76 mmol) and the mixture was stirred at 65 °C for 2 h. After quenching with saturated $NaHCO_3$, the aqueous layer was extracted with EtOAc (3x) and the combined organic phases were washed with brine (1x) and dried over Na_2SO_4. After concentrating *in vacuo*, the crude material was purified via prep RP-HPLC (55-75% ACN/H_2O) resulting in tripeptide **S39** as a brown solid (120 mg, 0.239 mmol, 25%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.57 (s, 1H), 8.48 (d, *J* = 9.2 Hz, 1H), 8.12 –8.02 (m, 3H), 7.81 – 7.73 (m, 4H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.21 – 6.15 (m, 1H), 6.11 –6.01 (m, 1H), 5.52 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.47 – 5.39 (m, 2H), 5.35 – 5.27 (m, 1H), 4.89 – 4.82 (m, 2H), 4.75 – 4.69(m, 2H), 4.11 (s, 2H), 4.03 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 502.20

Methyl (*E*)-4-(3-(4-(allyloxy)phenyl)acrylamido)benzoate (**S42**)



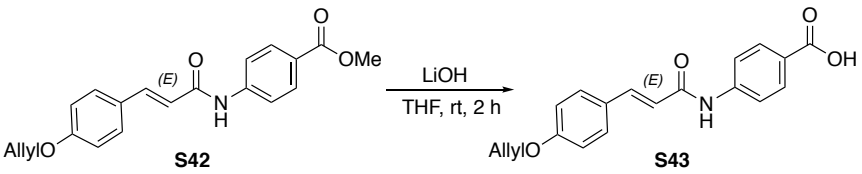
S40 **S41** **S42**

Building block (*E*)-3-(4-(allyloxy)phenyl)acrylic acid (**S40**) was prepared according to previously reported protocols.[15]

The reaction procedure was the same as reported for compound **S32**. The product **S42** was obtained as yellow solid (1.30 g, crude).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 7.98 – 7.90 (m, 2H), 7.87 – 7.79 (m, 2H), 7.62 – 7.54 (m, 3H), 7.07 – 7.00 (m, 2H), 6.70 (d, *J* = 15.7 Hz, 1H), 6.05 (ddt, *J* = 17.2, 10.4, 5.2 Hz, 1H), 5.41 (dq, *J* = 17.3, 1.8 Hz, 1H), 5.27 (ddq, *J* = 9.4, 4.7, 1.6 Hz, 1H), 4.63 (dt, *J* = 5.2, 1.6 Hz, 2H), 3.83 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 338.00

(*E*)-4-(3-(4-(allyloxy)phenyl)acrylamido)benzoic acid (**S43**)

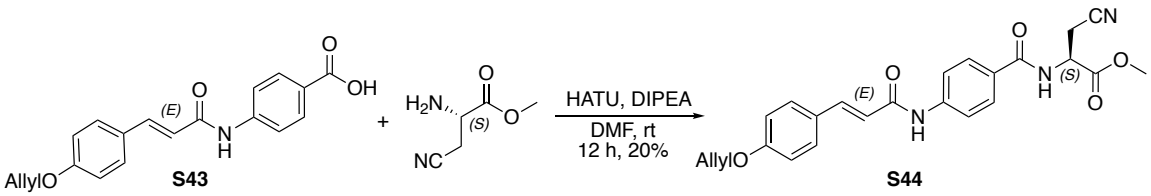


S42 **S43**

The reaction procedure was the same as described for compound **S33**, with the exception that the reaction was run for 2 h. The product **S43** was obtained as yellow solid (800 mg, crude).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 10.43 (s, 1H), 7.96 – 7.88 (m, 2H), 7.84 – 7.78 (m, 2H), 7.63 – 7.54 (m, 3H), 7.04 (d, *J* = 8.8 Hz, 2H), 6.71 (d, *J* = 15.7 Hz, 1H), 6.06 (ddt, *J* = 17.3, 10.4, 5.1 Hz, 1H), 5.41 (ddd, *J* = 17.3, 4.0, 2.3 Hz, 1H), 5.28 (ddd, *J* = 10.6, 4.0, 2.4 Hz, 1H), 4.64 (dt, *J* = 5.3, 1.6 Hz, 2H); ESI-MS [M + H]$^+$: *m/z* 324.00

Methyl (*S*,*E*)-2-(4-(3-(4-(allyloxy)phenyl)acrylamido)benzamido)-3-cyanopropanoate (**S44**)

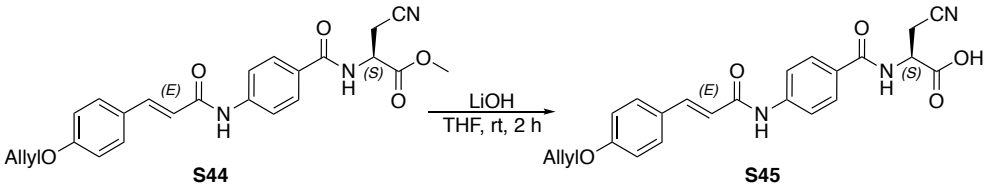


S43 **S44**

Compound **S43** (0.480g, 1.49 mmol) and methyl (2*S*)-2-amino-3-cyano-propanoate (0.300 g, 1.24 mmol) were dissolved in DMF (3.0 mL) followed by the addition of DIPEA (0.43 mL, 2.48 mmol) and HATU (565 mg, 1.49 mmol). The mixture stirred at room temperature for 12 h before concentrating *in vacuo*. The residue was purified via prep RP-HPLC (35-65% ACN/H_2O) resulting in compound **S44** as a white solid (0.110 g, 0.254 mmol, 20%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 9.13 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.80 (d, *J* = 8.9 Hz, 2H), 7.62 – 7.53 (m, 3H), 7.07 – 7.00 (m, 2H), 6.70 (d, *J* = 15.7 Hz, 1H), 6.06 (ddt, *J* = 17.3, 10.5, 5.2 Hz, 1H), 5.41 (dq, *J* = 17.3, 1.8 Hz, 1H), 5.28 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.80 (td, *J* = 8.4, 5.8 Hz, 1H), 4.63 (dt, *J* = 5.3, 1.6 Hz, 2H), 3.67 (s, 3H), 3.17 – 3.00 (m, 2H); ESI-MS [M + H]$^+$: *m/z* 434.00

(*S*,*E*)-2-(4-(3-(4-(allyloxy)phenyl)acrylamido)benzamido)-3-cyanopropanoic acid (**S45**)

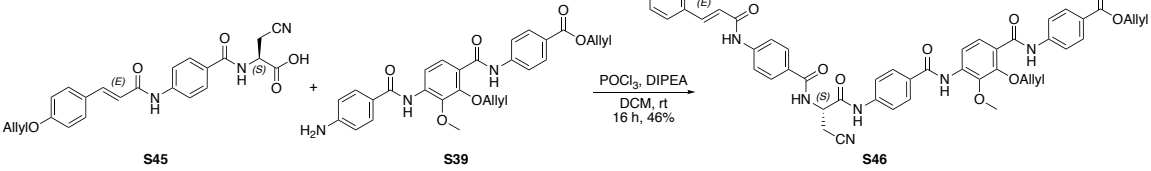


S44 **S45**

The reaction procedure was the same as described for compound **S35**. Following work-up, the deprotected product **S45** was obtained as white solid (0.100 g) and used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.96 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.61 – 7.53 (m, 3H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 15.7 Hz, 1H), 6.05 (ddt, *J* = 17.4, 10.5, 5.2 Hz, 1H), 5.46 – 5.36 (m, 1H), 5.28 (dd, *J* = 10.5, 1.7 Hz, 1H), 4.68 (td, *J* = 8.3, 5.5 Hz, 1H), 4.65 – 4.61 (m, 2H), 3.13 – 2.97 (m, 2H); ESI-MS [M + H]$^+$: *m/z* 420.00

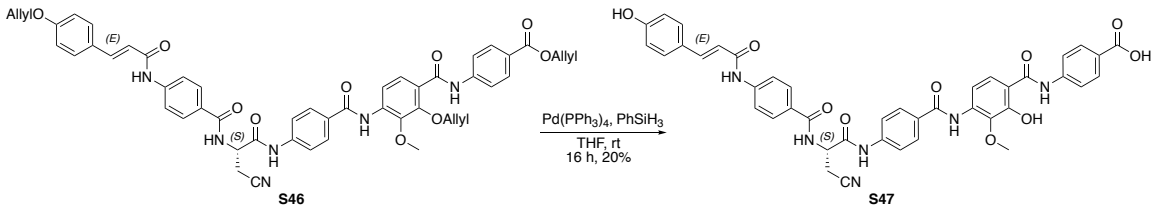
Allyl (*S*,*E*)-4-(2-(allyloxy)-4-(4-(2-(4-(3-(4-(allyloxy)phenyl)acrylamido)benzamido)-3-cyanopropanamido)benzamido)-3-methoxybenzamido)benzoate (**S46**)



S45 **S39** **S46**

Compound **S45** (64.4 mg, 0.153 mmol) and compound **S39** (70.0 mg, 0.139 mmol) were dissolved in DCM (2.0 mL) followed by the addition of DIPEA (48.5 μL, 0.279 mmol) and $POCl_3$ (19.5 μL, 0.209 mmol) at 0 °C. After stirring at room temperature for 16 h, the reaction was quenched with a solution of saturated $NaHCO_3$ (5.0 mL) and extracted with DCM (3x). The organic layer was washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified via prep RP-HPLC (65-95% ACN/H_2O) resulting in compound **S46** as a yellow solid (58 mg, 64.2 μmol, 46%). Presence of coupled linear species confirmed via MS and carried forward to the final deprotection step. ESI-MS [M + H]$^+$: *m/z* 903.00

(*S*,*E*)-4-(4-(4-(3-cyano-2-(4-(3-(4-hydroxyphenyl)acrylamido)benzamido)propanamido)benzamido)-2-hydroxy-3-methoxybenzamido)benzoic acid (**S47**)

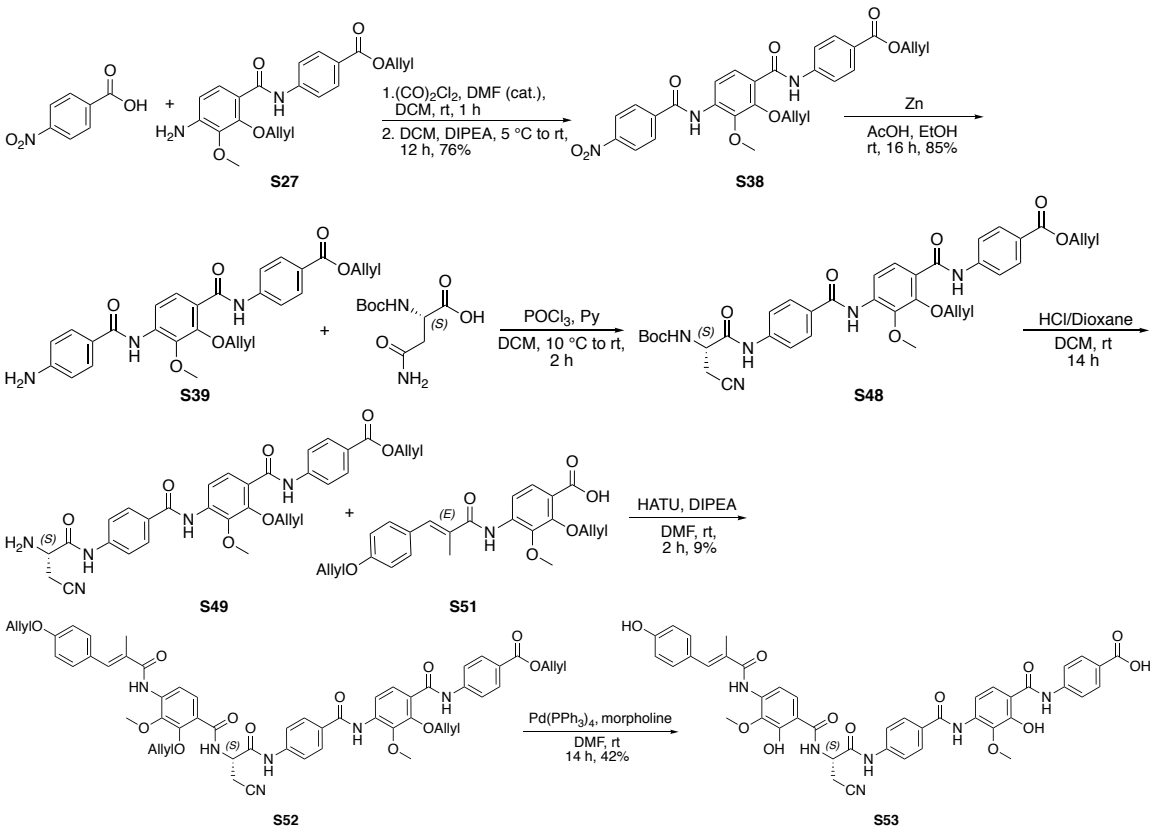


S46 **S47**

The reaction procedure was the same as described for compound **S37**. The product **S47** was obtained as white solid (10.5 mg, 12.8 μmol, 20%).

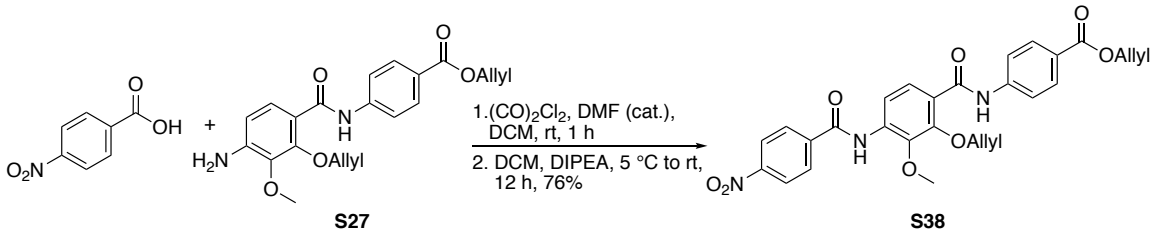
1H NMR (600 MHz, DMSO-d_6) δ 12.52 (s, 2H), 10.56 (s, 1H), 10.37 (s, 1H), 9.97 (s, 1H), 9.36 (s, 1H), 9.01 (d, J = 7.7 Hz, 1H), 7.94 (dd, J = 14.8, 8.4 Hz, 6H), 7.84 – 7.77 (m, 6H), 7.70 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 15.5 Hz, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.43 – 7.39 (m, 1H), 6.83 (d, J = 8.2 Hz, 2H), 6.64 (d, J = 15.6 Hz, 1H), 4.98 (q, J = 8.1 Hz, 1H), 3.86 (s, 3H), 3.15 (dd, J = 16.9, 5.4 Hz, 1H), 3.07 (dd, *J* = 16.9, 8.8 Hz, 1H), 3.01 (q, *J* = 7.4 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.16, 167.70, 166.13, 164.43, 164.36, 159.37, 157.82, 157.62, 142.60, 141.75, 141.03, 139.77, 135.26, 130.26, 129.65, 129.29, 128.62, 128.51, 127.64, 125.59, 123.37, 119.75, 119.01, 118.34, 118.27, 118.19, 116.34, 115.88, 114.09, 59.65, 50.58, 45.69, 39.94, 39.80, 39.66, 39.52, 39.38, 39.24, 39.10, 29.00, 19.98, 13.94; HRMS (ESI): *m*/*z* calculated for $C_{42}H_{35}N_6O_{10}^+$ $[M + H]^+$: 783.2415; observed 783.2384

Total synthesis of PABA95



Scheme S6. Synthetic pathway for the preparation of PABA95 (**S53**).

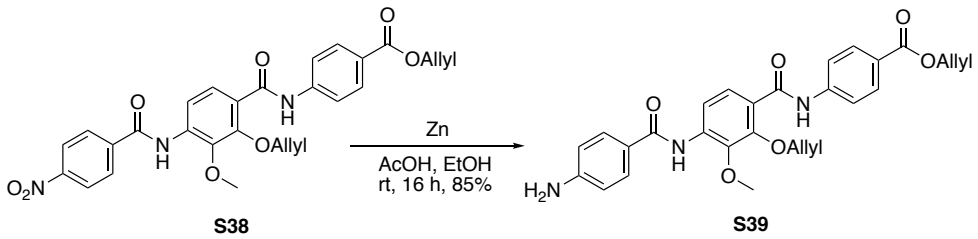
Allyl 4-(2-(allyloxy)-3-methoxy-4-(4-nitrobenzamido)benzamido)benzoate (**S38**)



4-nitrobenzoic acid (0.500 g, 2.99 mmol) was dissolved in DCM (10.0 mL) followed by the addition of one drop of dry DMF and dropwise addition of oxalyl dichloride (524 μL, 5.98 mmol). After stirring at room temperature for 1 h, the reaction was quenched with MeOH and concentrated *in vacuo*. Remaining reagents were co-evaporated with dry DCM and the crude aryl chloride was dissolved in 2.00 mL of dry DCM and added to a mixture of dipeptide **S27** (1.14 g, 2.99 mmol) and DIPEA (1.56 mL, 8.98 mmol) in DCM (10.0 mL) at 5 °C. The reaction mixture was stirred at room temperature for 12 h. After quenching with 0.50 mL of MeOH the solvent was removed *in vacuo,* resulting in crystallized trimer **S38** (1.20 g, 2.26 mmol, 76% yield) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.70 (s, 1H), 8.46 (d, *J* = 8.8 Hz, 1H), 8.42 – 8.35 (m, 2H), 8.12 – 8.05 (m, 5H), 7.84 – 7.71 (m, 2H), 6.24 – 6.13 (m, 1H), 6.12 – 5.98 (m, 1H), 5.53 (dd, *J* = 17.2, 1.2 Hz, 1H), 5.47 – 5.44 (m, 1H), 5.43 – 5.39 (m, 1H), 5.34 – 5.28 (m, 1H), 4.83 (dt, *J* = 5.6, 1.2Hz, 2H), 4.73 (dt, *J* = 5.6, 1.2Hz, 2H), 4.07 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 532.2

Allyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-methoxybenzamido)benzoate (**S39**)

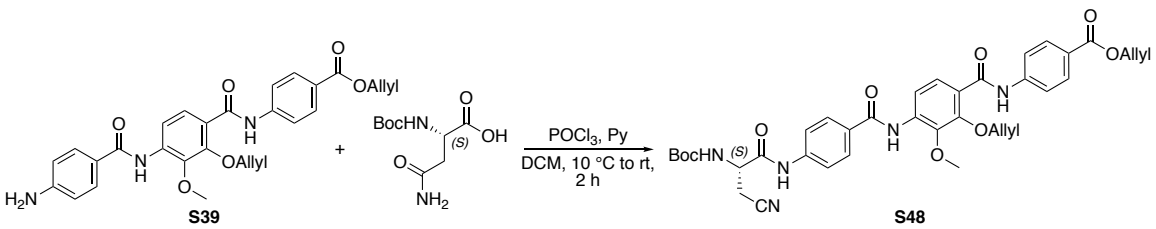


S38 **S39**

Nitrobenzoate **S38** (1.00 g, 1.88 mmol) was dissolved in a solution of 10% AcOH in EtOH (20.0 mL) and cooled to 5 °C. Zinc powder (2.46 g, 37.6 mmol) was added portionwise and the reaction was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was filtered and the filtrate concentrated *in vacuo*. The crude residue was partitioned between saturated $NaHCO_3$ (10.0 mL) and DCM (10.0 mL). The organic phase was separated and concentrated *in vacuo*. Recrystallization from DCM/EtOAc (5 mL/3 mL) by evaporating the DCM afforded trimer **S39** (0.800 g, 1.60 mmol, 85%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.57 (s, 1H), 8.48 (d, *J* = 9.2 Hz, 1H), 8.12 –8.02 (m, 3H), 7.81 – 7.73 (m, 4H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.21 – 6.15 (m, 1H), 6.11 –6.01 (m, 1H), 5.52 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.47 – 5.39 (m, 2H), 5.35 – 5.27 (m, 1H), 4.89 – 4.82 (m, 2H), 4.75 – 4.69(m, 2H), 4.11 (s, 2H), 4.03 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 502.2

Allyl (*S*)-4-(2-(allyloxy)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-cyanopropanamido)benzamido)-3-methoxybenzamido)benzoate (**S48**)

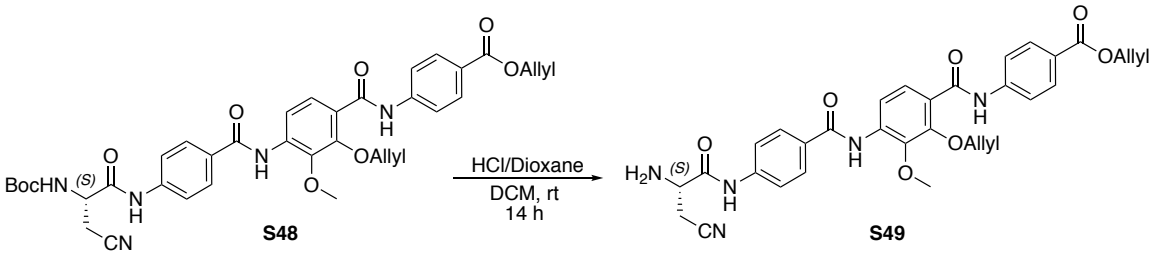


S39 **S48**

Tripeptide **S39** (0.80 g, 1.60 mmol) and Boc-Asn-OH (408 mg, 1.75 mmol) were dissolved in DCM (5.00 mL) followed by the addition of pyridine (773 μL, 9.57 mmol) and $POCl_3$ (341 μL, 3.67 mmol) at 10 °C. The reaction mixture was allowed to slowly warm to room temperature and stirred for 2 h. Following consumption of starting material, the reaction mixture was poured into 0.5 M $NaHCO_3$ (30.0 mL) and extracted with DCM (50.0 mL). The organic phase was separated and concentrated *in vacuo* to give crude product, which was re-crystallized from EtOAc/Pet. Ether (5 mL/10 mL) to afford intermediate **S48** (1.20 g, crude) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (d, *J* = 23.8 Hz, 1H), 9.67 (s, 1H), (7.99 - 7.97 (m, 3H), 7.90 – 7.83 (m, 2H), 7.77 (dd, *J* = 11.9, 8.6 Hz, 2H), 7.72 – 7.65 (m, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 6.09 – 6.02 (m, 2H), 5.43 - 5.38 (m, 2H), 5.28 (dd, *J* = 10.5, 1.5 Hz, 1H), 5.19 (dd, *J* = 10.5, 1.6 Hz, 1H), 4.79 (d, *J* = 5.4 Hz, 2H), 4.60 (d, *J* = 5.7 Hz, 2H), 4.53 – 4.46 (m, 1H), 3.89 (s, 3H), 3.03 (dd, *J* = 16.8, 5.1 Hz, 1H), 2.88 (dd, *J* = 16.8, 9.3 Hz, 1H), 1.42 (s, 9H); ESI-MS $[M + H]^+$: *m/z* 698.30

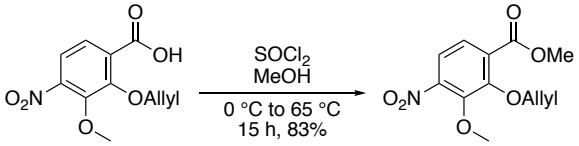
Allyl (*S*)-4-(2-(allyloxy)-4-(4-(2-amino-3-cyanopropanamido)benzamido)-3-methoxybenzamido)benzoate (**S49**)



Boc-protected intermediate **S48** (1.20 g, 1.72 mmol) was dissolved in a mixture of HCl/dioxane (4 M, 4.30 mL) and DCM (50.0 mL) and stirred at room temperature for 14 h. The solvent was removed *in vacuo* to provide a residue which was triturated in DCM/Pet. Ether (10.0 mL/5.0 mL) to afford crude compound **S49** (1.10 g, HCl salt) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 9.66 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 4H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 6.09 – 6.02 (m, 2H), 5.43 – 5.35 (m, 2H), 5.28 (d, *J* = 10.4 Hz, 1H), 5.21 (d, *J* = 9.4 Hz, 1H), 4.80 (d, *J* = 5.2 Hz, 2H), 4.61 (d, *J* = 5.6 Hz, 2H), 3.89 (s, 3H), 3.74 (dd, *J* = 7.2, 5.6 Hz, 1H). 2.88 (dd, *J* = 16.4, 5.2 Hz, 1H), 2.75 (dd, *J* = 24.0, 7.2 Hz, 1H); ESI-MS [M + H]$^+$: *m/z* 598.30

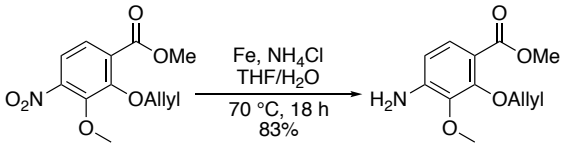
Methyl 2-(allyloxy)-3-methoxy-4-nitrobenzoate (**S25-1**)



Compound **S25** (5.70 g, 7.50 mmol) was dissolved in MeOH (50.0 mL) and cooled to 0 °C followed by the slow addition of $SOCl_2$ (5.44 mL, 75.0 mmol) via syringe. After stirring at 5 °C for 1 h, the mixture was heated to 65 °C for 14 h. After concentrating *in vacuo*, the crude material was dissolved in 1M $NaHCO_3$ (100 mL) and extracted with EtOAc (50.0 mL). The organic phase was separated, dried over Na_2SO_4 and concentrated *in vacuo*. Compound **S25-1** was obtained as a light yellow oil (5.00 g, 18.7 mmol, 83%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 6.14 – 5.96 (m, 1H), 5.38 (dd, *J* = 17.2 Hz, 1.6 Hz, 1H), 5.26 (dd, *J* = 10.4 Hz, 1.6 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 2H), 3.93 (s, 3H), 3.86 (s, 3H); ESI-MS [M + H]$^+$: *m/z* 268.08

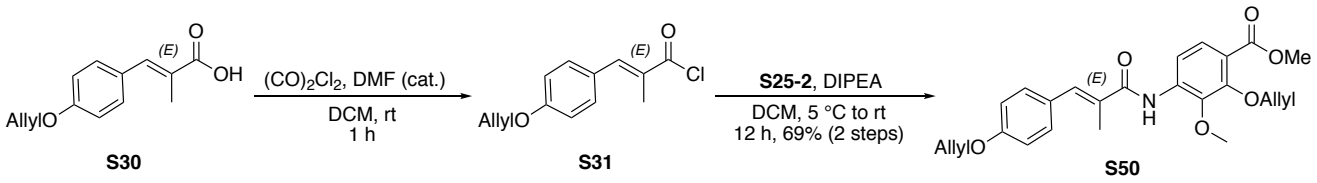
Methyl 2-(allyloxy)-4-amino-3-methoxybenzoate (**S25-2**)



Compound **S25-1** (2.00 g, 7.48 mmol) was dissolved in a mixture of THF (30.0 mL) and H_2O (20.0 mL) followed by the addition of NH_4Cl (2.00 g, 37.4 mmol) and Fe (1.67 g, 29.9 mmol). The reaction was heated at 70 °C for 18 h. After cooling and filtering, the filtrate was washed with EtOAc (0.150 L) and the organic phase separated. The organic phase was concentrated *in vacuo* and the crude material purified via flash column chromatography with a gradient of 0-20% EtOAc in Pet. ether to afford **S25-2** as light oil that appears as a yellow solid when cooled. (1.47 g, 6.21 mmol, 83%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (d, *J* = 8.8 Hz, 1H), 6.44 (d, *J* = 8.8 Hz, 1H), 6.12 – 6.04 (m, 1H), 5.75 (s, 2H), 5.36 (dd, J_1 = 18.0 Hz, J_2 = 2.0 Hz, 1H), 5.20 (dd, J_1 = 10.4 Hz, J_2 = 2.0 Hz, 1H), 4.44 (dd, J_1 = 5.6 Hz, J_2 = 1.6 Hz, 2H), 3.70 (s, 3H), 3.68 (s, 3H); ESI-MS [M + H]$^+$: *m/z* 238.10

Methyl (*E*)-2-(allyloxy)-4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)-3-methoxybenzoate (**S50**)

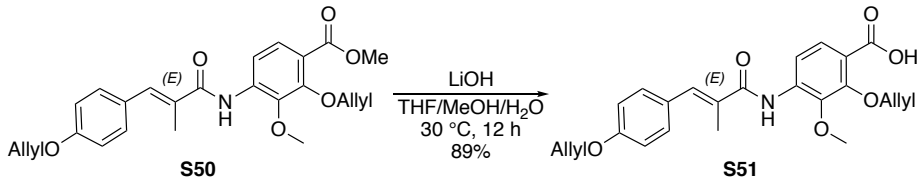


S30 **S31** **S50**

Compound **S30** (3.22 g, 14.75 mmol) was dissolved in DCM (20.0 mL) followed by the addition of one drop of dry DMF and dropwise addition of oxalyl dichloride (3.87 mL, 44.2 mmol). After stirring at room temperature for 1 h, the reaction was quenched with MeOH and concentrated *in vacuo*. Remaining reagents were co-evaporated with DCM and the crude aryl chloride was dissolved in 20.0 mL of dry DCM and added to a mixture of **S25-2** (3.50 g, 14.7 mmol) and DIPEA (12.8 mL, 73.7 mmol) in DCM (10.0 mL). The reaction mixture was stirred at room temperature for 3 h. After quenching with 50.0 mL of H_2O, the solution was diluted with DCM (0.100 L). The organic phase was washed with 1M HCl and separated. After concentrating *in vacuo* the crude material was purified via RP-HPLC resulting in **S50** (4.42 g, 10.1 mmol, 69%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.33 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.45 (s, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.25 – 6.01 (m, 2H), 5.48 – 5.42 (m, 2H), 5.36 – 5.25 (m, 2H), 4.60 – 4.56 (m, 4H), 4.00 (s, 3H), 3.90 (s, 3H), 2.25 (d, *J* = 1.2 Hz, 3H); ESI-MS $[M + H]^+$: *m/z* 437.90

(*E*)-2-(allyloxy)-4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)-3-methoxybenzoic acid (**S51**)

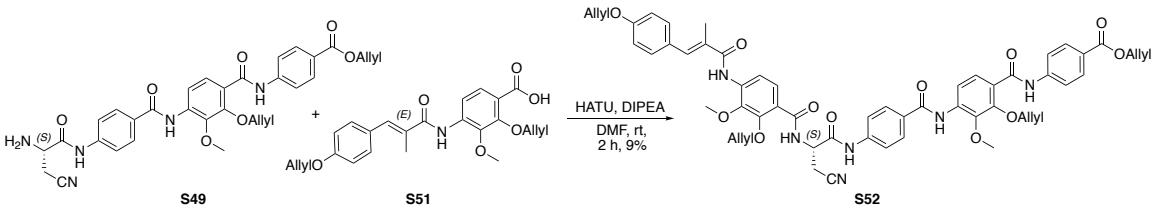


S50 **S51**

Compound **S50** (5.00 g, 11.4 mmol) was dissolved in a mixture of THF (30.0 mL), MeOH (5.00 mL) and H_2O (6.00 mL) followed by the addition of LiOH · H_2O (1.37 g, 57.1 mmol). After stirring at 30 °C for 12 h, the mixture was concentrated *in vacuo*. The crude residue was dissolved in H_2O (30.0 mL) and the pH was adjusted to 6 with 1 M HCl resulting in a precipitate. The white solid was filtered, washed with H_2O (50.0 mL), and concentrated *in vacuo*. The resulting white solid (**S51**) was used without further purification (4.29 g, 10.1 mmol, 89%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.45 (dd, *J* = 8.4, 4.4 Hz, 3H), 7.37 (s, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.18 – 5.95 (m, 2H), 5.43 -5.35 (m, 2H), 5.32 – 5.17 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.53 (d, *J* = 5.6 Hz, 2H), 3.86 (s, 3H), 2.15 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 424.20

Allyl (*S*,*E*)-4-(2-(allyloxy)-4-(4-(2-(2-(allyloxy)-4-(3-(4-(allyloxy)phenyl)-2-methylacrylamido)-3-methoxybenzamido)-3-cyanopropanamido)benzamido)-3-methoxybenzamido)benzoate (**S52**)

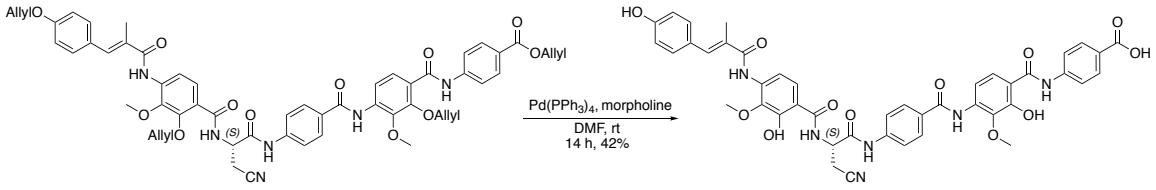


S49 **S51** **S52**

Compound **S49** (1.10 g, 1.73 mmol, HCl salt), and dimer **S51** (661 mg, 1.56 mmol) were dissolved in DMF (10.0 mL) followed by the addition of DIPEA (907 μL, 5.20 mmol) and HATU (792 mg, 2.08 mmol). The reaction mixture was stirred at room temperature for 2 h. Following consumption of starting material, the mixture was then diluted with EtOAc (20.0 mL) and washed with H_2O (1x), and brine (1x) successively. The organic phase was separated and concentrated *in vacuo*. The crude residue was purified via prep RP-HPLC (67-97% H_2O/ACN) to afford **S52** as an off-white solid (0.160 g, 0.160 mmol, 9%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 9.42 (s, 1H), 8.91 (d, *J* = 7.6 Hz, 1H), 8.64 (s, 1H), 8.52 – 8.38 (m, 3H), 8.12 – 8.04 (m, 3H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 2H), 7.80 – 7.71 (m, 4H), 7.46 (s, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.00 – 6.94 (m, 2H), 6.25 - 5.98 (m, 4H), 5.62 – 5.38 (m, 5H), 5.37 – 5.28 (m, 3H), 5.26 – 5.18 (m, 1H), 4.83 (d, *J* = 5.6 Hz, 2H), 4.76 (d, *J* = 6.0 Hz, 2H), 4.72 (d, *J* = 6.0 Hz, 2H), 4.59 (td, *J* = 5.2, 1.2 Hz, 2H), 4.04 (s, 3H), 3.98 (s, 3H), 3.13 – 2.98 (m, 2H), 2.26 (s, 3H); ESI-MS $[M + H]^+$: *m/z* 1003.60

(*S*,*E*)-4-(4-(4-(3-cyano-2-(2-hydroxy-4-(3-(4-hydroxyphenyl)-2-methylacrylamido)-3-methoxybenzamido)propanamido)benzamido)-2-hydroxy-3-methoxybenzamido)benzoic acid (**S53**)

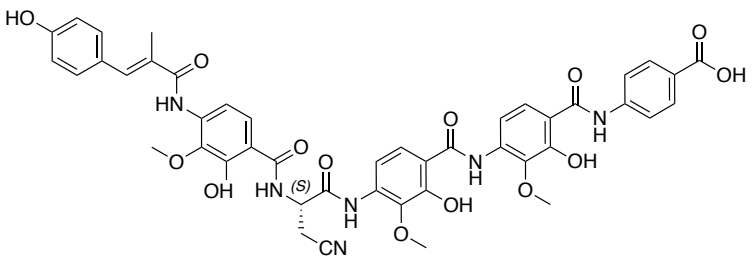


S52 **S53**

Linear compound **S52** (0.100 g, 99.7 μmol) was dissolved in DMF (3.00 mL) followed by the addition of morpholine (70.2 μL, 798 μmol) and $Pd(PPh_3)_4$ (34.6 mg, 29.9 μmol) under N_2(g). The reaction mixture stirred at room temperature for 14 h under the exclusion of light. The reaction mixture was then cooled to 5 °C and acidified to pH 5 with TFA. The mixture was then directly purified via RP-HPLC (H_2O/MeOH+0.1%TFA) to give **S53** (35.0 mg, 41.5 μmol, 42%) as an off-white solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.44 (s, 1H), 12.14 (s, 1H), 10.62 (d, *J* = 18.1 Hz, 2H), 9.89 (s, 1H), 9.58 (s, 1H), 9.39 (d, *J* = 7.3 Hz, 1H), 9.07 (s, 1H), 7.96 (t, *J* = 8.2 Hz, 4H), 7.84 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 9.2 Hz, 3H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 1H), 7.35 (d, *J* = 10.2 Hz, 3H), 6.83 (d, *J* = 7.8 Hz, 2H), 5.03 (t, *J* = 7.0 Hz, 1H), 3.84 (d, *J* = 8.0 Hz, 6H), 2.14 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.31, 168.39, 168.07, 167.77, 167.15, 165.12, 157.80, 153.69, 153.54, 142.21, 141.89, 139.38, 138.33, 136.58, 136.27, 134.89, 131.65, 130.46, 129.35, 129.14, 128.97, 126.65, 126.41, 123.38, 123.23, 120.80, 119.35, 118.15, 115.61, 113.57, 113.11, 111.87, 111.56, 60.49, 60.42, 50.34, 20.35, 14.51; HRMS (ESI): *m/z* calculated for $C_{44}H_{39}N_6O_{12}^+$ $[M + H]^+$: 843.2626; observed 843.2627

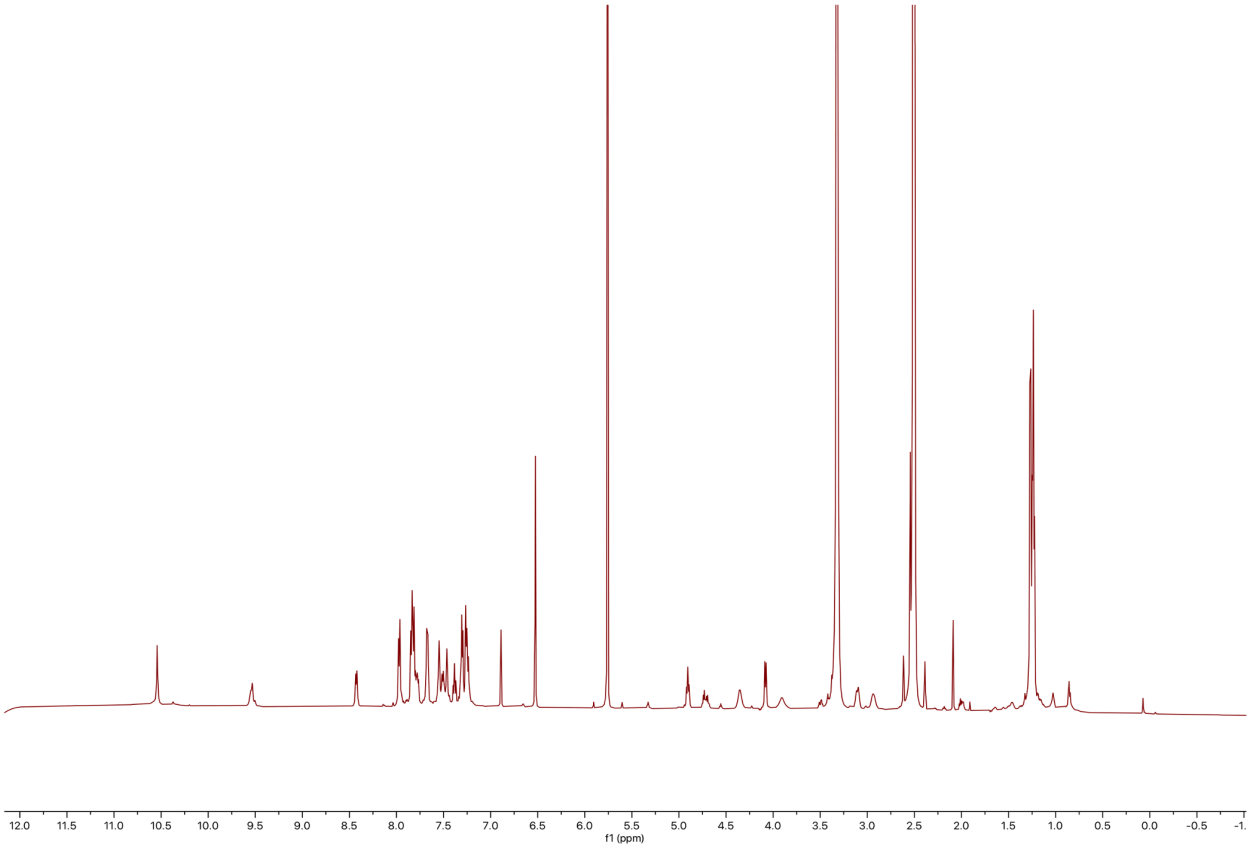
(*S*,*E*)-4-(4-(4-(3-cyano-2-(2-hydroxy-4-(3-(4-hydroxyphenyl)-2-methylacrylamido)-3-methoxybenzamido)propanamido)-2-hydroxy-3-methoxybenzamido)-2-hydroxy-3-methoxybenzamido)benzoic acid (**PABA95-2**)



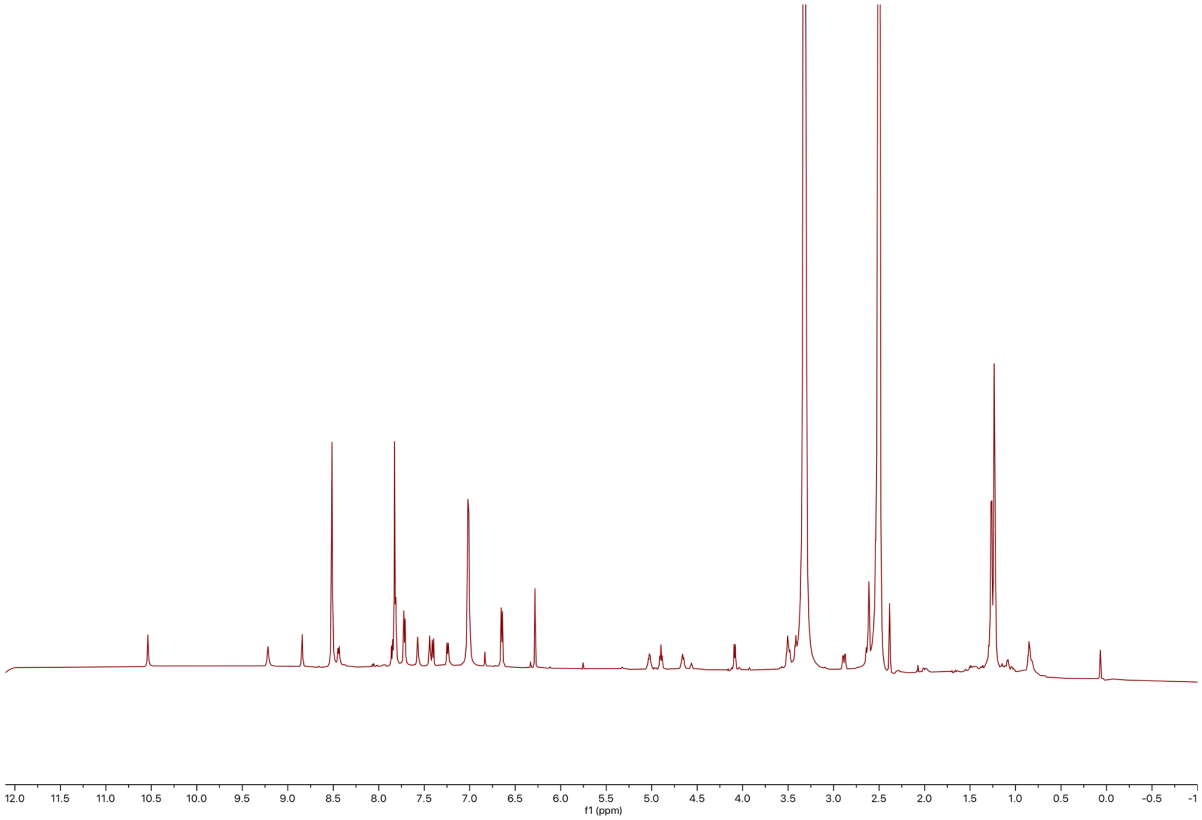
PABA95-2

The synthetic protocols for the preparation of PABA95-2 were the same as described for PABA34 (**S37**) and PABA95 (**S53**). The product **PABA95-2** was obtained as white solid (18.0 mg, 11%).

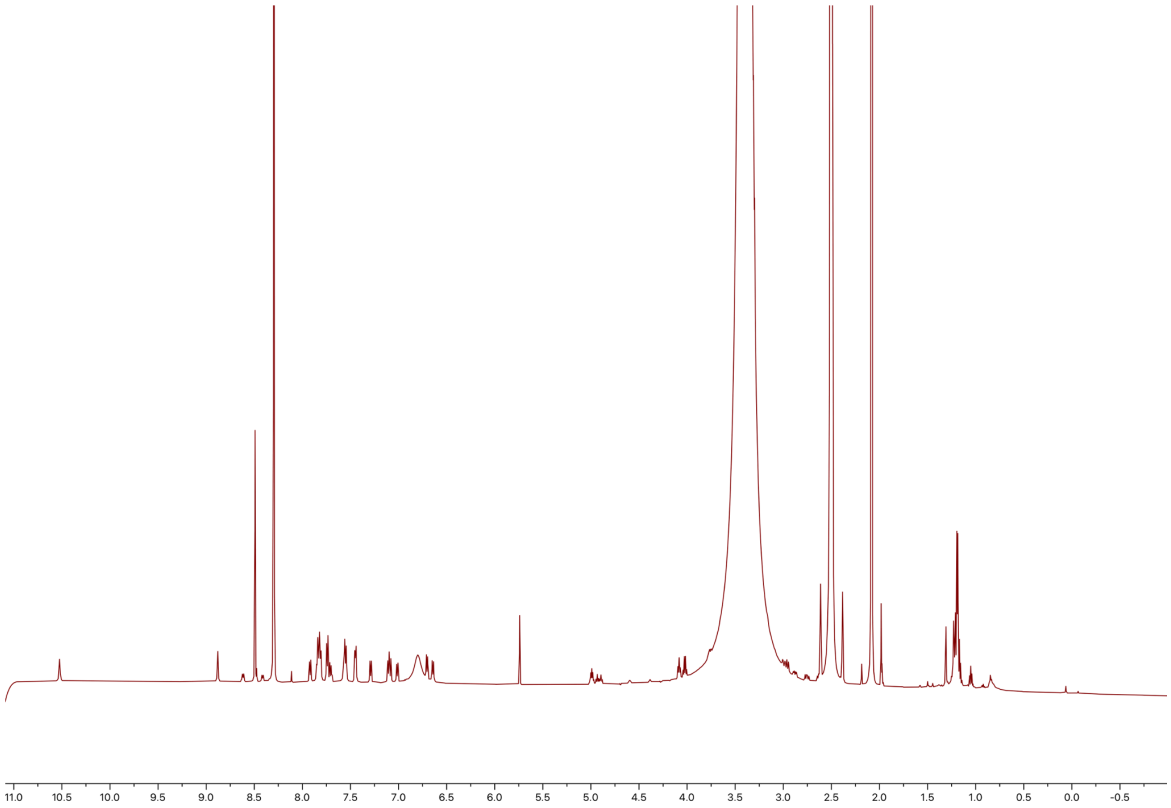
^{1}H NMR (600 MHz, DMSO-d_6) δ 12.72 (s, 1H), 12.44 (s, 1H), 12.25 (s, 1H),11.65 (s, 1H), 11.10 (s, 1H), 10.65 (s, 1H), 9.82 (s, 1H), 9.47 (s, 1H), 9.07 (s, 1H), 8.10 – 7.99 (m, 1H), 7.95 (d, *J* = 8.3 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.81 (s, 1H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 3H), 6.84 (d, *J* = 8.2 Hz, 2H), 5.25 (d, *J* = 7.2 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.73 (s, 3H), 3.20 – 3.11 (m, 2H), 2.15 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.34, 168.08, 158.06, 157.86, 157.66, 157.45, 138.09, 136.50, 134.62, 131.41, 130.20, 128.92, 126.42, 123.84, 122.94, 120.34, 120.29, 118.29, 118.10, 116.30, 115.92, 115.38, 114.31, 111.12, 60.17, 60.05, 50.20, 19.83, 14.32; HRMS (ESI): *m*/*z* calculated for $C_{45}H_{41}N_6O_{14}^+$ $[M + H]^+$: 889.2681; observed 889.2607



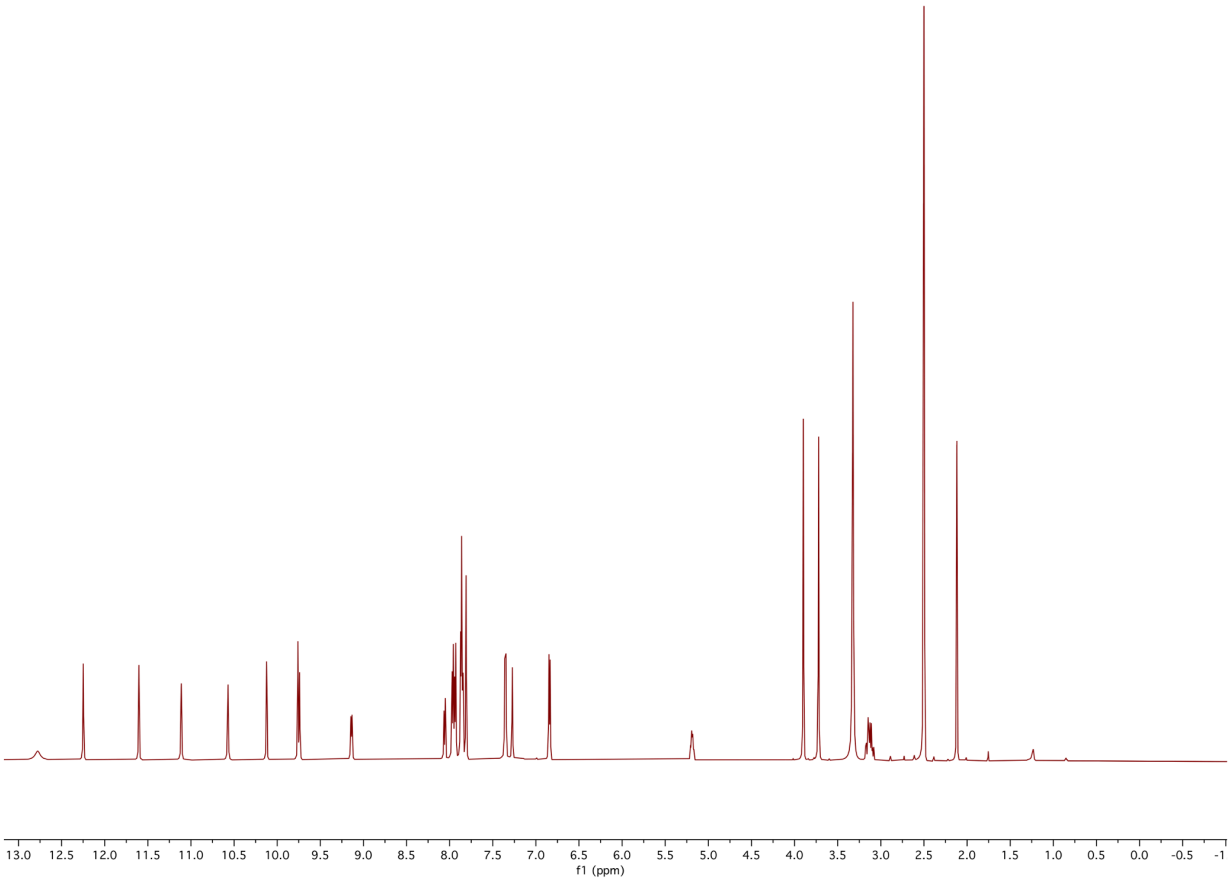
Supplementary Figure S11. ^{1}H-NMR spectrum of PABA48 (S13) in DMSO-d_6.



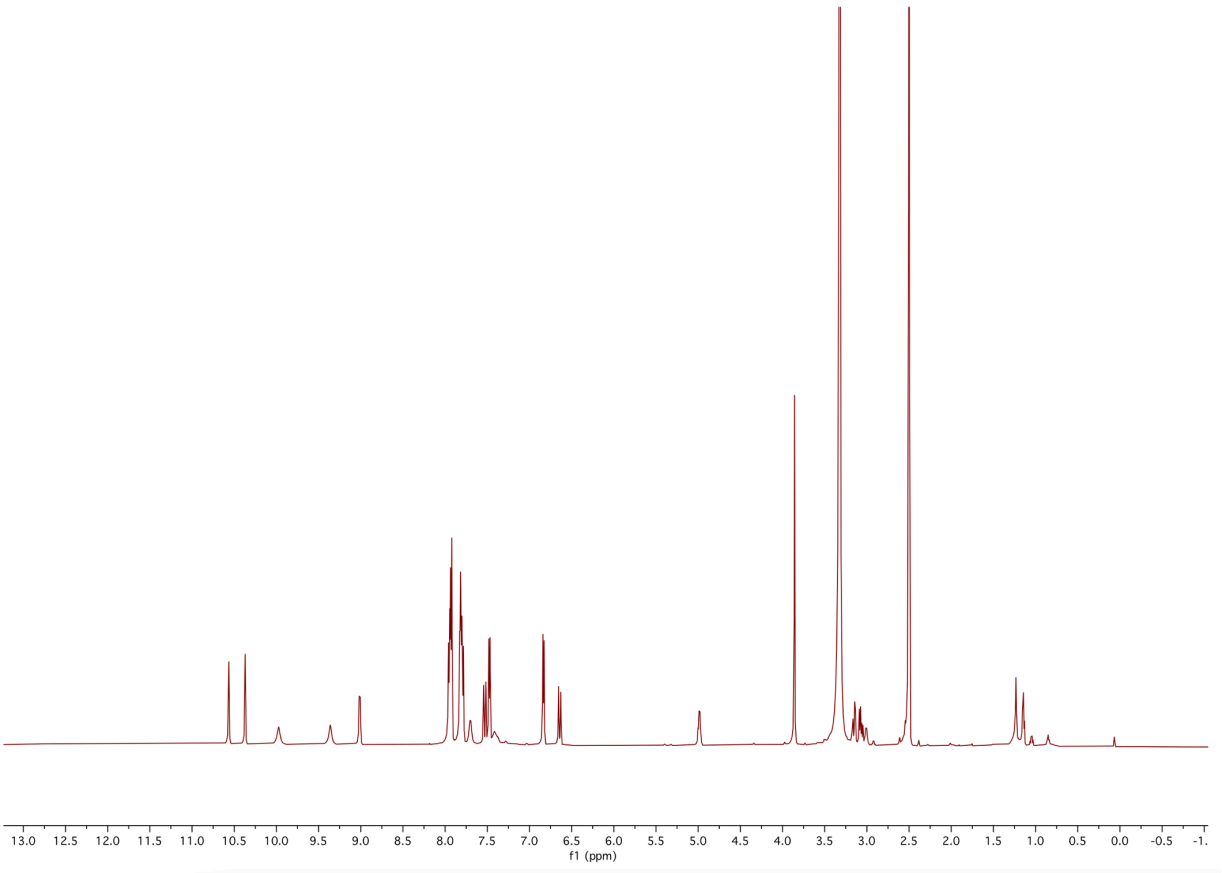
Supplementary Figure S12. ^{1}H-NMR spectrum of PABA70 (S16) in DMSO-d_6.



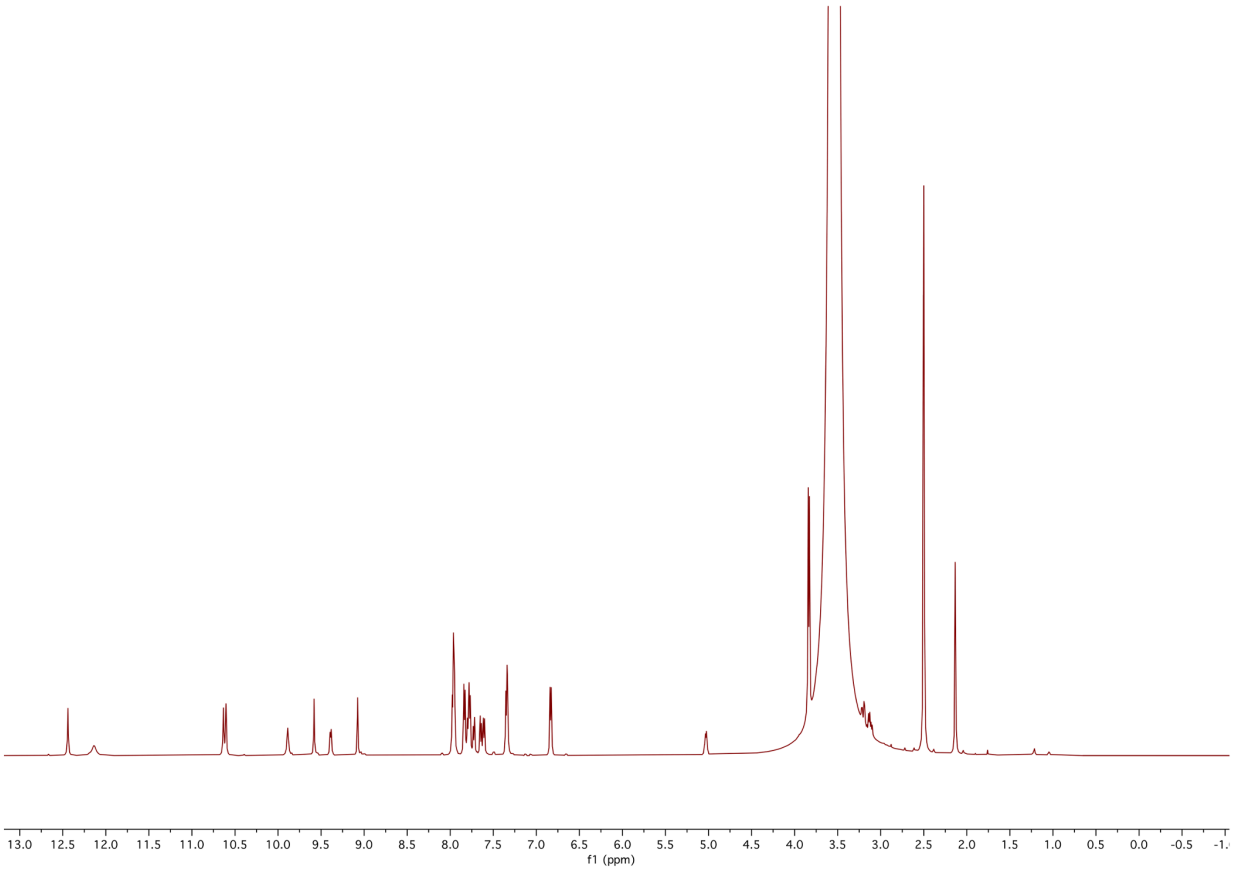
Supplementary Figure S13. ^{1}H-NMR spectrum of PABA57 (S24) in DMSO-d_6.



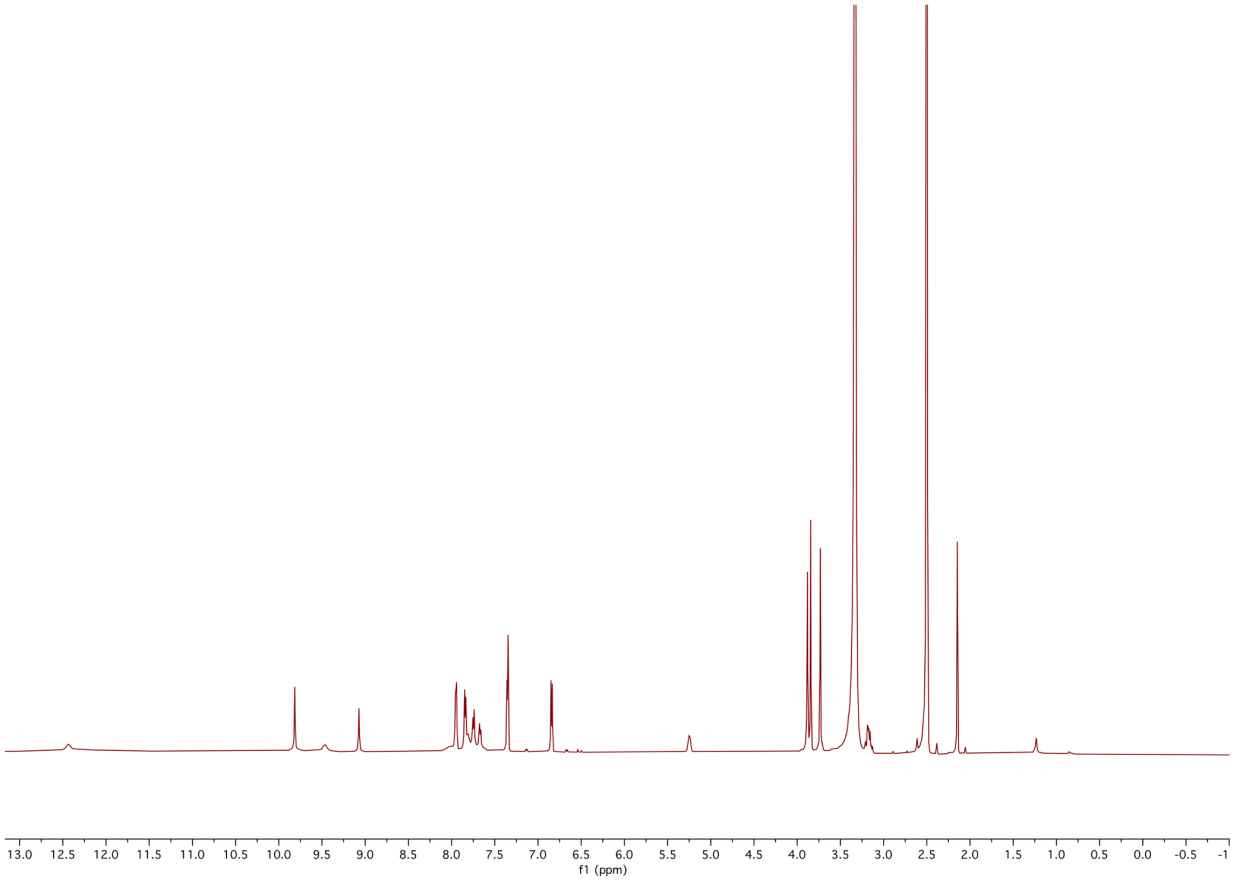
Supplementary Figure S14. ^{1}H-NMR spectrum of PABA34 (S37) in DMSO-d_6.



Supplementary Figure S15. ^{1}H-NMR spectrum of PABA157 (S47) in DMSO-d_6.



Supplementary Figure S16. ^{1}H-NMR spectrum of PABA95 (S53) in DMSO-d_6.



Supplementary Figure S17. ^{1}H-NMR spectrum of PABA95-2 in DMSO-d_6

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