Supporting Information for

Considerations for achieving maximized DNA recovery in solid-phase DNA-encoded library synthesis

Alexander K. Price*† and Brian M. Paegel‡

Department of Chemistry
Scripps Research
130 Scripps Way
Jupiter, Florida 33458, United States

† Current address:
Plexium
11494 Sorrento Valley Rd., Suite A
San Diego, California 92121, United States

‡ Current address:
Department of Pharmaceutical Sciences
Departments of Chemistry and Biomedical Engineering
101 Theory, Suite 100
University of California
Irvine, California 92617, United States

* Correspondence: aprice@plexium.com and bpaegel@uci.edu
Supporting Information Experimental Procedures

All reagents were from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. 1-[(1-(Cyano-2-ethoxy-2-oxoethyldieneaminoxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU, Acros Organics, Geel, Belgium), N,N-dimethylacetamide (DMA, Acros Organics), Borane-dimethylamine complex (DMAB, Acros Organics), Cyclopentyl methyl ether (CPME, Acros Organics), 1-Hydroxy-7-azabenzotriazole (HOAt, Accela ChemBio Inc., San Diego, CA), N,N-diisopropylethylamine (DIEA, TCI America, Portland, OR), 4-Dimethylaminoazobenzene-4'-carboxylic acid (DABCYL acid, TCI America), Tetrabutylammonium fluoride (TBAF, TCI America), Benzoyl chloride (TCI America), Tetrahydrofuran (THF, TCI America), N,N-diisopropylcarbodiimide (DIC, Thermo Fisher Scientific), Dichloromethane (DCM, Thermo Fisher Scientific), Acetonitrile (ACN, Thermo Fisher Scientific), Dimethyl sulfoxide (DMSO, AMRESCO Inc., Solon, OH), N-α-Fmoc-N-ε-7-methoxycoumarin-4-acetyl-lysine (Fmoc-Lys(Mca)-OH, EMD Millipore, Billerica, MA), Fmoc-12-aminododecanoic acid (Fmoc-12-Ado-OH, Creosalus, Louisville, KY), Fmoc-9-amino-4,7-dioxanonanoic acid (Fmoc-AEEP-OH, Chem-Impex International, Inc., Wood Dale, IL), N-α-Fmoc-N-γ-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-arginine (Fmoc-Arg(Pbf)-OH, Chem-Impex International, Inc.), N-α-Fmoc-glycine (Fmoc-Gly-OH, AnaSpec, Inc., Fremont, CA), N-α-Fmoc-O-t-butyl-tyrosine (Fmoc-Tyr(tBu)-OH, AnaSpec, Inc.), N-α-Fmoc-phenylalanine (Fmoc-Phe-OH, AnaSpec, Inc.), N-α-Fmoc-alanine (Fmoc-Ala-OH, AnaSpec, Inc.), N-α-Fmoc-proline (Fmoc-Pro-OH, AnaSpec, Inc.), N-α-Fmoc-N-ε-4-methyltrityl-lysine (Fmoc-Lys(Mtt)-OH, Combi-Blocks Inc., San Diego, CA), N-α-Fmoc-glutamic acid γ-allyl ester (Fmoc-Glu(OAll)-OH, Combi-Blocks Inc.), N-α-Fmoc-O-t-butyl-dimethylsilyl-serine (Fmoc-Ser(TBDMS)-OH, Combi-Blocks Inc.), 3-cyclohexylpropionic acid (Combi-Blocks Inc.), 4-(2-hydroxyethyl)benzoic acid (Combi-Blocks Inc.), tetrakis(triphenylphosphine)palladium(0) (Pd(PPh₃)₄, Combi-Blocks Inc.), Fmoc-8-amino-3,6-dioxoaoctanoic acid (Fmoc-AEEA-OH, Oakwood Products, Inc., Estill, SC), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Oakwood Products, Inc.), 4-Fmoc-aminobenzoic acid (Fmoc-4-Abz-OH, Oakwood Products, Inc.), decanoyl chloride (Oakwood Products, Inc.), 4-pyrroolidinopyridine (PPY, Oakwood Products, Inc.), and 4-(bromomethyl)benzoic acid (Matrix Scientific, Columbia, SC) were used as provided. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) was recrystallized as previously described.¹ Solvents used in standard solid-phase synthesis were dried over molecular sieves (3 Å, 3.2 mm pellets). The DCM and DMA used in all analysis reactions were
dried over anhydrous MgSO_4_. All solid-phase synthesis was conducted in a UV-free environment with rotation (10 rpm).

**Buffers.** All buffers were prepared using distilled, deionized water (18.2 MΩ·cm, Synergy purification system, Millipore, Burlington, MA). All percents are (w/v) unless specified. Bis-tris propane breaking buffer (BTPBB): 10 mM Bis-tris propane, 10 mM NaCl, 1% sodium dodecyl sulfate, 1% Tween-20, adjusted to pH 7.6. Bis-tris propane breaking buffer with EDTA (BTPBB+EDTA): 10 mM Bis-tris propane, 10 mM NaCl, 10 mM EDTA, 1% SDS, 1% Tween-20, adjusted to pH 7.6. Bis-tris propane ligation buffer (BisTL): 10 mM Bis-tris propane, 50 mM NaCl, 10 mM MgCl_2_, 0.02% Tween-20, 1 mM ATP, adjusted to pH 7.6. Bis-tris propane washing buffer (BTPWB): 10 mM Bis-tris propane, 50 mM NaCl, 0.04% Tween-20, adjusted to pH 7.6. PCR master mix: 100 mM Tris, 500 mM KCl, 15 mM MgCl_2_, 2 mM each of dATP, dCTP, dGTP and dTTP, adjusted to pH 8.3. TentaGel resin click reaction buffer (CRB): 30 mM TEAA, 0.04% Tween-20, 50% (v/v) DMSO, adjusted to pH 7.6.

**DNA.** DNA oligomers were purchased from Integrated DNA Technologies (Coralville, IA) and reconstituted in DI water to make 1 mM primary stock solutions. ≈01: 5’-GCCGCCCCAGTCCTGCTCGCTCGCTACATGGACAAAGAGCCGACGACGACTTCCCCGCGGTCTAAACCTCAA-3’. ≈02: 5’-GCCTCGGTATCAGGGATATGCTCAGTG-3’. ≈03: 5’-/FAM/-CCGACGACG/ZEN/ACTTCCCGCG/3IABkFQ/-3’. ≈04: 5’-/5Phos/GCCGCCCCAGTCCTGCTCGCTCGCTACATGGACAAAGAGCCGACGACGACTTCCCCGCGGTCTAAACCTCAA-3’. ≈05: 5’-/5Phos/AGGCTTGAGGTTTAGACCGCCGGGAAGTCGTCGCTCGCTGCTTTTGCTCCATGTAGCGAA GCGAGCAGGACTGGGCAGCGCCGGG-3’. ≈06: 5’-/CACTGAGCATATCCCTGATACCG-3’.

**Base resin synthesis.** Solid-phase synthesis was performed in Mobicol spin columns (Boca Scientific Inc., Westwood, MA) containing 10-µm filters. Base resins S1 and S11 were synthesized in 10-mg batches (TentaGel 160-µm MB RAM resin, Rapp-Polymere GmbH, Tuebingen, Germany) using a standard three-step reaction cycle consisting of 1) acid coupling, 2) protecting group removal, and 3) washing. Reaction and wash volumes were constant (400 µL). Unless stated otherwise, carboxylic acids were coupled onto amine-displaying resin by adding a cocktail composed of acid/COMU/DIEA (50/50/100 mM) and incubating (15 min, RT). This coupling step was repeated once. Fmoc was removed by adding piperidine (20% in DMF) and incubating (10 min, RT). This deprotection step was repeated once. Mtt groups were removed by adding TFA (2% in DCM) to dry resin and incubating (10 min, RT). This
deprotection step was repeated twice. Resin washing consisted of 3X DMF, 3X DCM, and 3X DMF.

Base resin **S1** was synthesized by coupling Fmoc-Lys(Mtt)-OH onto the resin, deprotecting the Mtt group, and coupling DABCYL acid onto the epsilon-amino group of lysine. The Fmoc-group was removed and two Fmoc-AEEA-OH building blocks were coupled to the resin. Base resin **S11** was synthesized by coupling Fmoc-Tyr(tBu)-OH, Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Phe-OH, and Fmoc-Glu(OAll)-OH onto the resin. Base resins were washed (6X DCM), dried under vacuum (10 min) and stored at −20℃.

**Primary amine acylation.** Base resin **S1** (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) to produce reaction resin **S2**. After washing, the resin was combined with a reaction cocktail and incubated (50/50/50 mM Fmoc-Ala-OH/Oxyma/DIC, 9:1 dry DCM/dry DMA, 20 min, RT). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Fmoc deprotection.** Base resin **S1** (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and two Fmoc-Phe-OH building blocks were successively coupled onto the resin using standard conditions (50/50/100 mM acid/COMU/DIEA, 15 min, RT, 2X) to produce reaction resin **S3**. The resin was incubated with piperidine (20% in dry DCM, 5 min, RT). This deprotection step was repeated twice. Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Steglich esterification.** Base resin **S1** (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH was coupled onto the resin using standard conditions. After deprotection, the resin was washed, combined with 4-(2-hydroxyethyl)benzoic acid reaction cocktail (50/50/50/100 mM acid/Oxyma/2,4,6-trimethylpyridine/DIC, 9:1 dry DCM/dry DMA), and incubated (1 h, 37 ℃) to produce reaction resin **S4**. The resin was combined with reaction cocktail (80/100 mM Fmoc-Pro-OH/DIC, 3.2 mol% PYY, 9:1 dry DCM/dry DMA) and incubated (2 h, 4 ℃). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Nucleophilic substitution of an alkyl bromide.** Base resin **S1** (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH was coupled onto the resin using standard conditions. After deprotection, the resin was washed, combined with 4-(bromomethyl)benzoic acid reaction cocktail (50/50/50/100 mM acid/Oxyma/2,4,6-trimethylpyridine/DIC, 9:1 dry DCM/dry DMA), and incubated (1 h, RT) to produce reaction resin **S5**. The resin was combined with benzylamine (1 M in dry DCM) and incubated (1 h, RT).
Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Acetylation of a primary amine.** Base resin S1 (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH was coupled onto the resin using standard conditions. After deprotection (reaction resin S6), the resin was washed, combined with acetic anhydride (5% v/v) and Oxyma/DIEA (15/125 mM, 9:1 dry DCM/dry DMA), and incubated (10 min, RT). Following a washing step (6X DCM), the resin was combined with Fmoc-Ala-OH reaction cocktail (50/50/50 mM Fmoc-Ala-OH/Oxyma/DIC, 9:1 dry DCM/dry DMA) and incubated (20 min, RT). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Silyl ether deprotection.** Base resin S1 (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT). Fmoc-Phe-OH and Fmoc-Ser(TBDM)-OH were successively coupled onto the resin using standard conditions. After deprotection, the resin was washed, combined with 3-cyclohexylpropionic acid reaction cocktail (50/50/50 mM acid/Oxyma/DIC in 9:1 dry DCM/dry DMA), and incubated (30 min, RT) to produce reaction resin S7. The resin was combined with TBAF (2.5 mM in 100:1 dry THF/MeOH) and incubated (1 h, RT). Following a washing step (6X DCM), the resin was combined with dry DCM/benzoyl chloride/pyridine (90:5:5) and incubated (1 h, RT). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Secondary amine acylation.** Base resin S1 (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH was coupled onto the resin using standard conditions. After deprotection, the resin was combined with chloroacetic acid reaction cocktail (50/50/50 mM acid/Oxyma/DIC, 9:1 dry DCM/dry DMA) and incubated (1 h, RT). Following a washing step (6X DCM), the resin was combined with benzylamine (1 M in DMF) and incubated (3 h, 37 °C) to produce reaction resin S8. Following a washing step (6X DCM), the resin was combined with Fmoc-Pro-OH reaction cocktail (60/60/60 mM Fmoc-Pro-OH/HOAt/DIC, 9:1 dry DCM/DMA) and incubated (3 h, RT). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Arylamine acylation.** Base resin S1 (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH was coupled onto the resin using standard conditions. After deprotection, the resin was combined with Fmoc-4-Abz-OH reaction cocktail (50/50/50/100 mM acid/Oxyma/2,4,6-trimethylpyridine/DIC in 9:1 dry DCM/dry DMA) and incubated (1 h, 50 °C). After deprotection (reaction resin S9), the resin was combined with decanoyl
chloride/pyridine (50/100 mM) in dry DCM, and incubated (15 min, RT). Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Mtt deprotection.** Base resin S1 (0.5 mg) was deprotected (20% piperidine, 2X, 10 min, RT) and Fmoc-Phe-OH and Fmoc-Lys(Mtt)-OH were coupled onto the resin using standard conditions. After Fmoc deprotection, the resin was washed, combined with DMF/hexanoic anhydride/DIEA (90:5:5), and incubated (10 min, RT) to produce reaction resin S10. The resin was combined with HFIP/DCM/triisopropylsilane (75:22:3), incubated (2 x 8 min, RT) and neutralized (95:5 DCM/DIEA, 5 min, RT). Following a washing step (6X DCM), Fmoc-12-Ado-OH was coupled onto the deprotected epsilon-amino group of lysine using standard conditions at 37 °C. Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**Allyl group deprotection.** Base resin S11 (0.5 mg) was combined with borane-dimethylamine (267 mM in 300 µL dry DCM) and incubated (15 min, RT). Pd(PPh₃)₄ (10 mM in 100 µL dry DCM) was added to the resin and incubated (10 min, RT). The resin was combined with sodium diethyldithiocarbamate (100 mM in water) and incubated (10 min, 37 °C). This step was repeated once. Afterwards, the resin was washed (3X DCM, 3X DMF, 3X DCM) and dried under vacuum (10 min).

**TFA cleavage and reaction analysis.** Dried reaction resin was combined with TFA/triisopropylsilane/DCM (90:5:5, 350 µL) and incubated (1 h, RT). The supernatant was collected and evaporated to dryness. The residue was resuspended in DMSO (60–200 µL). TFA cleavage products (60 µL) were analyzed via reversed-phase HPLC (XBridge BEH130 C18, 3.5 µm, 4.6 x 100 mm, Waters Corporation, Milford, MA) with gradient elution (mobile phase A: ACN; mobile phase B: 0.1% TFA in water). Analyses used a gradient of 5–55% A over 20 min, except for silyl ether deprotection analysis, which used a gradient of 25–75% A over 20 min. HPLC fractions (1 µL) were spotted on a MALDI target plate, dried, covered in α-cyano-4-hydroxycinnamic acid matrix solution (1.5 mg/mL in 50% v/v ACN/0.1% TFA in water), and subjected to MALDI-TOF MS analysis in positive ionization mode (Microflex LT, Bruker, Billerica, MA).

**dsDNA magnetic sensor bead synthesis.** dsDNA magnetic sensor beads were prepared as described previously.² Briefly, carboxylic acid-functionalized magnetic resin (M-270 Dynabeads, carboxylic acid, 300 µL) were washed (BTPWB, 3 x 600 µL). Resin was combined with propargylamine reaction cocktail (500/500/700 mM propargylamine/HOAt/DIC in DMF, 200 µL total) and incubated (3 h, 50 °C, 10 rpm). The resin was washed (DMF, 6 x 150 µL; DCM, 3 x 150 µL; 1% Tween-20 in DI water, 2 x 150 µL), resuspended (1% Tween-20 in DI
water, 1 mL), incubated with rotation (1 h, 50 °C, 10 rpm), washed again (1% Tween-20 in DI water, 2 x 150 µL), and resuspended (1% Tween-20 in DI water, 300 µL). Propargyl amide-functionalized resin (45 µL) was transferred into a clean 1.5-mL microcentrifuge tube and the supernatant was removed. Resin was combined with Cu (I) catalyst mix (5.6/6.7/27.8 mM CuSO₄/TBTA/ascorbic acid, 9 µL), vortexed, and combined with N₃-HDNA mix (1.1/16.7/300 mM ascorbic acid/N₃-HDNA/TEAA, 60% DMSO, 1.5% Tween-20 in DI water, 18 µL), vortexed, and incubated (1 h, 50 °C, 10 rpm). CuSO₄ and ascorbic acid stock solutions were prepared in HPLC-grade water (Thermo Fisher Scientific) that was sparged with argon. The resin was washed (BTPBB+EDTA, 2 x 1 mL), incubated (ON, 50 °C, 10 rpm), and washed (10X BisTL, 50 µL). Oligo-pair stocks ≈0.4/≈0.5 and ≈0.6/≈0.7 were heated (5 min, 60 °C) and cooled to RT. ≈0.4/≈0.5 (925 pmol each) and ≈0.6/≈0.7 (925 pmol each) were added together in BisTL buffer (185 µL) that contained T4 DNA ligase (1,200 U, New England BioLabs) and incubated (20 min, RT). Another aliquot of T4 DNA ligase (1,200 U) was added and the solution was combined with the resin and incubated (ON, RT, 10 rpm). The beads (Figure S14) were washed (BTPBB, 150 µL), resuspended (BTPBB, 500 µL), incubated (1 h, 50 °C, 10 rpm), washed (BTPBB, 150 µL; BTPWB, 3 x 150 µL), and resuspended in 500 µL of BTPWB.

**Multi-reaction sensor bead synthesis.** 5 mg of 160-µm TentaGel MB RAM resin (Rapp-Polymere GmbH) was swelled in DMF and Fmoc-deprotected using standard conditions. After washing, Fmoc-Lys(Mca)-OH was coupled onto the resin (50/50/50/100 mM acid/Oxyma/DIC/DIEA in DMF, 1 h, 50 °C, 10 rpm). Unreacted amines were capped using acetic anhydride (20% in DMF, 30 min, 50 °C, 10 rpm) and the resin was washed and Fmoc-deprotected. Fmoc-Arg(Pbf)-OH and Fmoc-Gly-OH were coupled onto the resin using identical conditions. Bromoacetic acid was coupled onto the resin (50/100 mM acid/DIC, 1 h, 50 °C, 10 rpm). Afterwards, the resin was incubated in 1 M propargylamine in DMF (3 h, 50 °C, 10 rpm) and washed before Fmoc-Gly-OH and Fmoc-AEEP-OH were subsequently coupled onto the resin (50/50/50/100 mM acid/Oxyma/DIC/DIEA in DMF, 1 h, 50 °C, 10 rpm) to produce reaction resin S12.

The resin was washed (CRB, 3 x 600 µL), resuspended (CRB, 900 µL) and then incubated (1 h, RT, 10 rpm) in a microcentrifuge tube. The bead suspension was spun down, the supernatant was removed, and the beads were resuspended in 797.3 µL of CRB and 77.7 µL of Cu+ catalyst mix (63.5/0.2/318 mM CuSO₄/TBTA/ascorbic acid in 2:1 DMSO/DI water) for 5 min at 37 °C (10 rpm). At this time, 25.1 µL of N₃-HDNA mix (1.8/0.7/101.4 mM ascorbic acid/N₃-HDNA/TEAA in 1:1 DMSO/DI water) was added to suspension, which was vortexed and incubated (4 h, 37 °C, 10 rpm). Afterwards, the beads were washed (BTPBB+EDTA, 3 x 600
µL), resuspended (BTPBB+EDTA, 900 µL) and incubated (ON, RT, 10 rpm). The beads were subsequently washed (BTPBB+EDTA, 3 x 600 µL; BTPWB, 3 x 600µL; BisTL, 2 x 1 mL) and resuspended (BisTL, 150 µL). The \( \approx 0.4/\approx 0.5 \) and \( \approx 0.6/\approx 0.7 \) oligo-pair stocks were heated (5 min, 60 °C) and then cooled to RT. \( \approx 0.4/\approx 0.5 \) and \( \approx 0.6/\approx 0.7 \) were ligated together in BisTL buffer that contained 20 U/µL T4 DNA ligase (New England BioLabs) and 24 µM of each oligo (20 min, RT). This solution was then added to the beads and ligated onto the HDNA (ON, RT, 10 rpm). Afterwards, the beads were washed (BTPWB, 3 x 600 µL) and resuspended (BTPWB, 500 µL).

**ssDNA sensor bead synthesis.** Monosized TentaGel M NH2 resin (30-µm, 5 mg, Rapp-Polymere GmbH) was swelled in DMF and Fmoc-Gly-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, and chloroacetic acid were coupled onto the resin using standard conditions. The resin was combined with propargylamine (1 M in DMF), incubated (3 h, 37 °C) and washed (6X DMF) before Fmoc-Gly-OH was coupled onto the resin (60/60/60 mM Fmoc-Gly-OH/HOAt/DIC in 9:1 DCM/DMA, 37 °C). One coupling was performed for 1 h, and a second was performed overnight. Fmoc-AEEA-OH and chloroacetic acid were coupled onto the resin using standard conditions. The resin was combined with benzylamine (1 M in DMF), incubated (3 h, 37 °C), and washed (6X DMF) to produce secondary amine resin S13.

Resin S13 was washed (CRB, 3 x 400 µL), resuspended (CRB, 400 µL) and incubated (1 h, RT, 10 rpm). The supernatant was removed and the beads were resuspended in CRB (259 µL) and Cu (I) catalyst mix (63.5/0.09/318 mM CuSO\(_4\)/TBTA/ascorbic acid in 2:1 DMSO/DI water, 30 µL), incubated (5 min, 37 °C, 10 rpm), combined with N\(_3\)-HDNA mix (0.005/0.61/93.5 mM ascorbic acid/N\(_3\)-HDNA/TEAA in DI water, 10.7 µL), vortexed, and incubated (4 h, 37 °C, 10 rpm). The resin was washed (BTPBB+EDTA, 3 x 400 µL), resuspended (BTPBB+EDTA, 500 µL), and incubated (ON, RT, 10 rpm). The resin was washed (BTPBB+EDTA, 3 x 400 µL; BTPWB, 5 x 1 mL; BisTL, 2 x 1 mL), and resuspended (BisTL, 150 µL). The \( \approx 0.1/\approx 0.5 \) oligo-pair stock was heated (5 min, 60 °C) and then cooled to RT. A ligation mixture containing \( \approx 0.1/\approx 0.5 \) (600 pmol each) and T4 DNA ligase (400 U) in BisTL buffer (120 µL) was added to the resin and incubated (3.5 h, RT, 10 rpm). Afterwards, the resin was washed (BisTL, 2 x 1 mL) and resuspended (BisTL, 150 µL). This ligation process was repeated for the \( \approx 0.2/\approx 0.6 \) oligo pair and the resin was washed (BTPWB, 3 x 1 mL).

To denature the unligated strand from the ligated strand, the beads were washed (1 mM NaCl, 4 x 1 mL) and resuspended (1 µM \( \approx 0.5/\approx 0.6 \) in 1 mM NaCl, 500 µL). The beads were vortexed, heated (5 min, 60 °C, 10 rpm) and allowed to cool to RT. The beads were centrifuged and the supernatant was removed. This denaturation process was repeated once. Afterwards,
the beads were washed (1 mM NaCl, 4 x 1 mL; BTPWB, 3 x 1 mL) and resuspended (BTPWB, 500 µL). To validate that the sensor beads displayed ssDNA, ssDNA bead stock (9 µL) and dsDNA magnetic bead stock (3 µL) were added to separate microcentrifuge tubes. Each sample was washed (BTPWB, 3 x 200 µL; mung bean nuclease buffer, 150 µL), resuspended in mung bean nuclease (MBN) buffer (100 µL) containing MBN (5 U) and incubated (1 h, 30 °C, 10 rpm). Afterwards, the tubes were centrifuged and the supernatant was removed. The beads were washed (BTPBB, 4 x 200 µL; BTPWB, 4 x 400 µL), and resuspended in 150 µL (dsDNA) or 50 µL (ssDNA) of BTPWB. The resin was then subjected to a standard DNA damage assay (Figure S15).

**DNA damage assay.** A standard DNA damage assay consisted of a set of buffer controls (BUF), a set of reaction samples (RXN), and a set of DNA damage controls (TFA), each present as three biological replicates. dsDNA magnetic sensor bead stock was washed (BTPWB, 3 x 200 µL), and diluted 10-fold in BTPWB before 30-µL aliquots of DNA sensor beads were transferred into individual microcentrifuge tubes. BUF controls were set aside. For the RXN samples, the sensor beads were washed (dry DMF, 3 x 200 µL) before 0.5 mg of reaction resin was added to each tube. As much DMF as possible was removed prior to the addition of reagents. These reactions were performed using the conditions detailed in the reaction sections above. The TFA controls were incubated with 10% TFA in DCM (10 min, RT). Afterwards, both the RXN and TFA beads were washed (dry DMF, 6 x 200 µL) and the reaction resin was removed from the RXN samples. The DNA sensor beads in BUF, RXN, and TFA were washed (BTPWB, 6 x 200 µL) and resuspended in BTPWB (150 µL). DNA sensor bead concentration was calculated using a hemacytometer.

DNA sensor beads (1 µL) were added to qPCR mix (20 µL) in a 96-well PCR plate as a minimum of three technical replicates of each biological replicate. DNA standards (1 µL, full PCR product in BTPWB) as 10-fold dilutions (5 nM – 5 fM) were added to qPCR mix (20 µL) as three technical replicates. The qPCR mix had a final concentration of 500 nM ≈06, 500 nM ≈08, 360 nM ≈03, and 50 U/mL Taq DNA polymerase (New England BioLabs, Ipswich, MA). The plate was centrifuged (1 min, 100 rpm) and thermally-cycled (C1000 Touch, Bio-Rad, Hercules, CA) [(95 °C, 20 s; 65.8 °C, 20 s; 65 °C, 15 s] x 28 cycles, 72 °C, 2 min). PCR amplification data were analyzed using CFX Maestro (Bio-Rad) and the number of amplifiable DNA molecules per bead was determined.

**Solvent panel for secondary amine acylation.** A standard DNA damage assay was performed in triplicate for each solvent investigated, consisting of reaction resin S8 (0.5 mg) and dsDNA magnetic sensor bead stock (3 µL). The resins were combined with 60/60/60 mM...
Fmoc-Pro-OH/HOAt/DIC in 9:1 solvent/DMA and incubated (3 h, RT). The solvents used in the panel were CPME, DCM, THF, DMF, N-methyl-2-pyrrolidone (NMP), and HFIP. Afterward, the magnetic resin was recovered from each reaction and the number of amplifiable DNA molecules per bead was determined via qPCR. The DNA damage assay was also performed in 9:1 solvent/DMA alone for each member of the panel. Reactions performed on reaction resin S8 in the absence of dsDNA sensor beads were used to investigate the performance of the reaction in each solvent via HPLC and MALDI-TOF MS.

Effect of strandedness on DNA damage. DNA damage assays for secondary amine acylation (60/60/60 mM Fmoc-Pro-OH/HOAt/DIC in 9:1 solvent/DMA, 3 h) were performed using dsDNA magnetic sensor beads/reaction resin S8 and the bifunctional 30-µm ssDNA sensor beads. These reactions were investigated using both DMF and DCM as the primary solvent at RT and 55 °C. The dsDNA and ssDNA sensor beads were also subjected to similar conditions omitting the carboxylic acid building block (60/60 mM Oxyma/DIC in 9:1 solvent/DMA, 3 h) and solvent alone (9:1 solvent/DMA, 3 h) for comparison.

DNA damage for a multi-step synthesis. Prior synthesis conditions: 0.5 mg of 160-µm multi-reaction sensor beads were transferred into a mobicol column and washed with 3 volumes of DMF. The terminal Fmoc was removed (20% piperidine in DMF, 20 min, RT) and the resin was washed (DCM, 3 x 400 µL; DMF, 3 x 400 µL; DCM, 3 x 400 µL). These steps were repeated for every Fmoc deprotection. Subsequently, Fmoc-N-Me-Ala-OH was coupled onto the resin (40/40/57 mM acid/HOAt/DIC in DMF, 1 h, 37 °C) followed by Fmoc-Pro-OH (80/80/100 mM acid/Oxyma/2,4,6-trimethylpyridine/DIC in DMF, 3 h, 37 °C), Fmoc-Ser(TBDMS)-OH and Fmoc-Phe-OH (40/40/57 mM acid/HOAt/DIC in DMF, 1 h, 37 °C) with intermittent Fmoc-deprotection steps. Afterwards, the resin was washed (DMF, 6 x 400 µL; BTPWB, 6 x 400 µL) and resuspended in BTPWB (400 µL).

New (this work) synthesis conditions: 0.5 mg of 160-µm multi-reaction sensor beads were transferred into a mobicol column and washed (DMF, 3 x 400 µL). The terminal Fmoc was deprotected (20% piperidine in DCM, 3 x 5 min, RT) and the resin was washed (DCM, 3 x 400 µL; DMF, 3 x 400 µL; DCM, 3 x 400 µL). These steps were repeated for every Fmoc deprotection. Subsequently, Fmoc-N-Me-Ala-OH was coupled onto the resin (50/50/50 mM acid/Oxyma/DIC in 9:1 DCM/DMA, 20 min, RT) followed by Fmoc-Pro-OH (60/60/60 mM acid/HOAt/DIC in 9:1 DCM/DMA, 3 h, RT), Fmoc-Ser(TBDMS)-OH and Fmoc-Phe-OH (50/50/50 mM acid/Oxyma/DIC in 9:1 DCM/DMA, 20 min, RT) with intermittent Fmoc-deprotection steps. Afterwards, the resin was washed (DCM, 6 x 400 µL; DMF, 6 x 400 µL; BTPWB, 6 x 400 µL), and resuspended in BTPWB (400 µL).
As a damage control, 160-µm multi-reaction sensor beads (0.5 mg) were transferred into a mobicol column and washed (DMF, 3 x 400 µL). The resin was then incubated in 20% TFA in DCM (30 min, RT). The resin was washed (DCM, 6 x 400 µL; DMF, 6 x 400 µL; BTPWB, 6 x 400 µL), and resuspended in BTPWB (400 µL). Another aliquot of multi-reaction sensor beads was kept in BTPWB and served as a buffer control. Six individual beads from each reaction set were isolated and the number of amplifiable DNA molecules per bead was determined via qPCR.

References


Figure S1. Base resin S1 and modifications. Base resin S1 was modified as described to investigate primary amine acylation (S2), Fmoc deprotection (S3), esterification (S4), nucleophilic substitution (S5), acetylation (S6), silyl ether deprotection (S7), secondary amine acylation (S8), and arylamine acylation (S9), and Mtt deprotection (S10).
Figure S2. Base resin S11, multi-reaction base resin, and ssDNA base resin. Base resin S11 was used to investigate allyl ester deprotection. Multi-reaction resin S12 was used to investigate the cumulative effects of DNA damage over three primary amine acylations, a secondary amine acylation, and four Fmoc deprotections. ssDNA sensor base resin S13 was used to investigate the effect of DNA strandedness on reaction compatibility.
Figure S3. Acylation of a primary amine. HPLC and MALDI-TOF MS analysis of the reaction resin S2 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) acylation with N-Fmoc-Ala-OH support conversion of starting material (peak i) into the expected amide product (peak ii). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]^+. 
Figure S4. Fmoc deprotection. HPLC and MALDI-TOF MS analysis of the reaction resin S3. TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) treatment with piperidine support conversion of starting material (peak ii) into the expected free amine (peak i). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]^+. 
Figure S5. Steglich esterification. HPLC and MALDI-TOF MS analysis of the reaction resin S4 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) esterification with Fmoc-Pro-OH support conversion of starting material (peak i) into the expected ester (peak ii). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+Na]⁺.
Figure S6. Nucleophilic substitution of an alkyl bromide. HPLC and MALDI-TOF MS analysis of the reaction resin $S_5$ TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) substitution with benzylamine support conversion of starting material (peak $i$) into the expected secondary amine (peak $ii$). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]$^+$ and [M-Br]$^+$. 
Figure S7. Acetylation of an amine. HPLC and MALDI-TOF MS analysis of the reaction resin S6 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) acetylation followed by attempted primary amine acylation with Fmoc-Ala-OH support conversion of starting material into the expected acetylated product (peak i) instead of the further-modified amide (peak ii). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]^+. 
Figure S8. Silyl ether deprotection. HPLC and MALDI-TOF MS analysis of the reaction resin S7 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) deprotection with TBAF followed by esterification with benzoyl chloride support conversion of starting material into the expected ester product (peak ii) instead of the hydroxyl product produced by TFA cleavage (peak i). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]^+.
**Figure S9.** Secondary amine acylation. HPLC and MALDI-TOF MS analysis of the reaction resin S8 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) acylation with Fmoc-Pro-OH support conversion of starting material (peak i) into the expected amide product (peak ii). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+H]+ and [M+Na]+.
**Figure S10.** Arylamine acylation. HPLC and MALDI-TOF MS analysis of the reaction resin S9 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) acylation with decanoyl chloride support conversion of starting material (peak i) into the expected amide product (peak ii). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+Na]^+.
Figure S11. Mtt group deprotection. HPLC and MALDI-TOF MS analysis of the reaction resin TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) deprotection with HFIP/DCM/TIPS cocktail support conversion of starting material into the expected amine product that is further elaborated with Fmoc-Ado-OH (peak ii) while the starting material is deprotected to the amine product after TFA cleavage (peak i). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+Na]^+.
Figure S12. Allyl group deprotection. HPLC and MALDI-TOF MS analysis of the reaction resin S11 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) deprotection with Pd(PPh₃)₄ and borane-dimethylamine complex support conversion of starting material (peak ii) into the expected acid product (peak i). Mass spectra display both theoretical exact masses (blue) and observed masses (black) for [M+Na]⁺.
Figure S13. Solvent panel reaction yield of secondary amine acylation. HPLC analysis of the reaction resin S8 TFA cleavage products before (starting material, gray trace) and after (reaction products, magenta trace) acylation of the secondary amine starting material with Fmoc-Pro-OH in 9:1 CPME/DMA (top left), 9:1 DMF/DMA (top right), 9:1 HFIP/DMA (middle left), 9:1 NMP/DMA (middle right), and 9:1 THF/DMA (bottom left).
Figure S14. Magnetic dsDNA sensor resin. (A) Azido-modified headpiece DNA (N$_3$-HDNA) is clicked onto 2.8-µm magnetic beads displaying an alkyl group. (B) DNA oligo pairs $\approx04/\approx05$ and $\approx06/\approx07$ are ligated onto the resin to create the fully-elaborated dsDNA sensor resin.
Figure S15. ssDNA-bead analysis. Upon denaturation of the unligated DNA strand off the bead, the most stable conformation (A) for the remaining ssDNA sequence was modeled (mFold web server, http://unafold.rna.albany.edu/?q=mfold). In this conformation, 61 of the 99 nucleobases are unpaired, while 38 adopt paired secondary structure. (B) Incubation of ssDNA beads with MBN resulted in a ~96% loss of DNA amplifiability compared to ssDNA beads incubated without MBN (green bars, n = 8), confirming the single-stranded nature of these sensor beads. Incubation of beads displaying dsDNA with MBN actually produced a ~67% increase in DNA amplifiability (purple bars, n = 6), which we cannot currently explain.