Supplementary Information

Development of on-DNA vinyl sulfone synthesis for DNA-encoded chemical library

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1. Abbreviations

ACN: acetonitrile
DIPEA: N, N-diisopropylethylamine
DMA: N, N-dimethylacetamide
MeOH: methanol
DMT-MM: 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride
HATU: O-(7-aza-1-benzotriazolyl)-N, N, N', N'-tetramethyluroniumhexafluorophosphate
HFIP: 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol
HP: headpiece
HP-P: headpiece primer
HPLC: high performance liquid chromatography
MW: molecular weight
NMR: nuclear magnetic resonance
PAGE: polyacrylamide gel electrophoresis
TBE: tris-borate-EDTA
TEAA: triethylammonium acetate
TEA: trimethylamine
TLC: thin layer chromatography
TIC: total ion chromatogram
UPLC-MS: ultra performance liquid chromatography-mass spectrum
UV: ultraviolet
2. Materials and general methods

2.1. Materials

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Headpiece (HP, 5’/5Phos/GAGTCA/iSp9/iUniAmM/iSp9 /TGACT CCC-3’), Headpiece-primer (HP-P, 5’-/5Phos/ACCTTCGGTCGGGAGTCA /iSp9/iUniAmM/iSp9/TGACTCCCGACCGAAGGTTG-3’) and code sequences were received from HitGen Inc. (Shuangliu District, Chengdu, China). All the DNA sequences were written in 5’- to 3’- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in MeOH, DMA or other solvents, depending on solubility and optimized reaction conditions. T4 DNA ligase and 10× ligation buffer (500 mM Tris pH 7.5, 500 mM NaCl, 100 mM MgCl2, 100 mM DTT and 25 mM ATP) were purchased from HitGen Inc.. All the buffer and aqueous solutions, NaCl (5 M), basic borate buffer (250 mM sodium borate/boric acid, pH 9.4), acetate buffer (3 M sodium acetate/acetic acid, pH 5.2) were prepared in-house. Cestbon water was used in all the reactions unless otherwise stated. All the gel images were captured by a Bio-Rad Chemidoc™ image system.

Figure S1. Structure of HP (5’/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3’), MW = 4937.
2.2. General methods for DNA analysis (UPLC-MS method)

**Analysis of on-DNA reactions by UPLC-MS.** The detection was performed by a high-resolution mass spectrometry-Agilent 6230 Time-of-Flight (TOF) mass spectrometer connected to an Agilent 1290 UPLC. After reaction, an aliquot of the reaction mixture was diluted with water to make the sample approximately 1 µM, and then 10~20 µL of the sample was injected into a reversed-phase UPLC column (Agilent, AdvanceBio Oligonucleotide, C18, 2.1×50 mm, 2.7 µm, maintained at 60 °C) at flow rate of 0.3 mL/min. The effluent was detected by UV absorbance at 260 nm and analysed on Agilent TOF (6230 B) in negative ion mode.

**LCMS method of DNA reaction analysis:**

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<th>Time (min)</th>
<th>Flow (mL/min)</th>
<th>%B</th>
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Solvent A: 200 mM HFIP and 8 mM TEA in H₂O; Solvent B: MeOH

**LCMS method of DNA ligation analysis:**

<table>
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<th>Time (min)</th>
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Conversion calculation. The conversion of on-DNA products was determined by UV absorbance (260 nm) peak area integration using the following equation: Conversion% = UV (desired products)/UV (total products), ignoring UV extinction coefficient difference for DNA species and assuming 100% DNA recovery. Any non-oligo material with UV absorbance at 260 nm was subtracted from the conversion calculation.¹

Analysis of molecular mass. Observed m/z was calculated as m/z = [M – z]/z for the negative ion mode. Data visualization and integration were performed on BioConfirm 10.0 software (Agilent, v10.0).
2.3. General methods for DNA conjugates purification

**General procedure for ethanol precipitation.** To an on-DNA reaction mixture was added 10% (V/V) 5 M NaCl solution and 3 times volume of absolute cold ethanol. Alternatively, to a DNA ligation mixture was added 10% (V/V) 3 M acetate buffer (pH 5.2) and 3 times volume of absolute cold ethanol. After swirling and centrifuging, the solution was maintained at -80 °C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 °C by using Eppendorf 5424R centrifuge. The supernatant was discarded and the pellet was rinsed with 200 μL cold 75% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 °C, the supernatant was discarded again and the DNA pellet was dried by Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in the appropriate solvent for subsequent experiments.

**General method for HPLC purification.** Preparative reversed-phase high-pressure liquid chromatography (RP-HPLC) for the DNA conjugate was performed on Waters 1575EF Series with the column (Eclipse-XDB C18, 5 µM, 9.4 × 250 mm) using eluent A (100 mM TEAA in H₂O) and eluent B (100 mM TEAA in 80% ACN) with gradient: 10% B (0 - 1 min), 10% to 30% B (1 - 11min), 30% to 100% B (11 - 11.1 min), 100% B (11.1 - 12 min), 100% to 10% B (12 - 12.1 min), 10% B (12.1 - 16 min). Fractions containing the product were combined and lyophilized overnight.

2.4. General procedure for DNA ligation

This reaction contained variably-derivatized **HP-P** starting material (10 nmol in H₂O, 1 equiv), code (12 nmol in H₂O, 1.2 equiv), 10× ligation buffer (4 µL), T4 DNA ligase (1 µL, 1000 units/µL) and nuclease-free water (to the total volume of 40 µL). The reaction mixture was incubated at 20 °C for overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step of synthesis without further purification.
2.5. General procedure for polyacrylamide gel

Ligation reaction was monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). First, the DNA samples were denatured at 95 °C for 10 min and mixed with loading buffer. Then, 10 pmol of treated DNA samples was loaded on the gel, and the gel was run at 200 V for 50 - 60 min. DNA fragments were visualized and analyzed by Bio-Rad Chemidoc™ Image System (Bio-Rad, CA, USA).

2.6. General methods for monitoring reaction and characterizing small molecules

Reactions were monitored by TLC and general staining reagents were used to analyze TLC intuitively. There were no unknown compounds, and known compounds were characterized by $^1$H-NMR or identified by comparison of their physical and spectroscopic data with those reported in the literature. NMR spectrum was recorded on Agilent 400 MHz spectrometer using residual non-deuterated solvent (CDCl$_3$) as the internal standard. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet. Unless otherwise noted, all deuterated solvents were purchased from Adamas.
3. General procedure for on-DNA vinyl sulfone formation

3.1. Preparation of DNA-conjugated alkenes by amide coupling

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. 4-Vinylbenzoic acid (20 µL, 200 mM in DMA, 200 equiv), HATU (10 µL, 400 mM in DMA, 200 equiv), and DIPEA (10 µL, 400 mM in DMA, 200 equiv) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to HP solution (40 µL, 20 nmol). The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 h. After ethanol precipitation, the reaction was analyzed by UPLC-MS. The separated and collected conjugates were vacuum-dried overnight, redissolved in H₂O for subsequent experiments. Deconvoluted molecular mass: calculated: 5067 Da; observed: 5067 Da.

3.2. Preparation of sodium sulfinates

Benzenesulfonyl chloride (1 equiv) was added to a mixture of Na₂SO₃ (2 equiv) and NaHCO₃ (2 equiv) in water (1 M). The result mixture was stirred at 80 °C for 4 h. After cooling down to room temperature, the water was removed in vacuum, then ethanol was added. Then the precipitate was filtered off and the filtrate was concentrated in vacuum to obtain the final product. All the sulfinates are known compounds. They were identified by comparison of their physical and spectroscopic data with those reported in the literature. Unless otherwise noted, sodium sulfinates described in the supplementary information were
synthesized under this standard condition.
3.3. On-DNA vinyl sulfone synthesis

**Standard Condition:** To the solution of DNA-conjugated **aa** (2 μL, 100 μM in H₂O, 0.2 nmol, 1 equiv) was added H₂O (8 μL), **1** (2 μL, 200 mM in H₂O, 400 nmol, 2000 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 2000 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5207 Da; observed: 5207 Da. Unless otherwise noted, on-DNA vinyl sulfones described in the supplementary information were synthesized under this standard condition.

**For DNA-conjugated aw-az:** To the solution of DNA-conjugated (2 μL, 100 μM in H₂O, 0.2 nmol, 1 equiv) was added H₂O (8 μL), **1** (2 μL, 200 mM in H₂O, 400 nmol, 2000 equiv), and iodine (2 μL, 50 mM in MeOH, 100 nmol, 500 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above.
4. General procedure for subsequent diversification of vinyl sulfone

4.1. Amide coupling

Figure S2. UPLC chromatogram and deconvoluted MS of D1.

To the solution of aa21 (0.2 nmol, 1 μL, 200 μM in H₂O, 1 equiv) was added benzylamine (1500 nmol, 5 μL, 300 mM in ACN/H₂O, 1:1, 750 equiv), Borate buffer pH 9.4 (2250 nmol, 9 μL, 250 mM in H₂O, 11250 equiv), DMT-MM (4500 nmol, 5 μL, 900 mM in H₂O, 22500 equiv). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 10 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 88%). Deconvoluted molecular mass: calculated: 5340 Da; observed: 5340 Da.

4.2. Buchwald–Hartwig amination
**Figure S3.** UPLC chromatogram and deconvoluted MS of D2.

To the solution of aa7 (0.2 nmol, 2 μL, 100 μM in H₂O, 1 equiv) was added aniline (1600 nmol, 4 μL, 400 mM in DMA, 8000 equiv), Borate buffer pH 9.4 (375 nmol, 1.5 μL, 250 mM in H₂O, 1875 equiv), t-BuXPhos Pd G3 (150 nmol, 1.5 μL, 100 mM in DMA, 750 equiv), and H₂O (5 μL). The reaction mixture was vortexed, centrifuged, and incubated at 30 °C for 2 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 70%). Deconvoluted molecular mass: calculated: 5298 Da; observed: 5298 Da.

**4.3. Nitro reduction**

**Figure S4.** UPLC chromatogram and deconvoluted MS of D3.

To the solution of aa6 (0.2 nmol, 1 equiv) in Borate buffer pH 9.4 (2500 nmol, 10 μL, 250 mM in H₂O, 12500 equiv), was added FeSO₄ (1000 nmol, 5 μL, 200 mM in H₂O, 5000 equiv), NaOH (3000 nmol, 3 μL, 1000 mM in H₂O, 15000 equiv), FeSO₄, NaOH, Borate buffer pH 9.4, 80 °C, 2 h.
equiv). The reaction mixture was vortexed, centrifuged, and incubated at 80 °C for 2 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5222 Da; observed: 5222 Da.

### 4.4. Tetrazole formation

![Reaction Scheme](image)

**Figure S5.** UPLC chromatogram and deconvoluted MS of D4.

To the solution of aa12 (0.2 nmol, 10 μL, 20 μM in H₂O, 1 equiv) was added MES buffer pH 5.8 (2500 nmol, 10 μL, 250 mM in H₂O, 12500 equiv), 1,4-dioxane (18 μL), NaN₃ (2000 nmol, 5 μL, 400 mM in H₂O, 10000 equiv), ZnBr₂ (500 nmol, 2 μL, 250 mM in H₂O, 2500 equiv). The reaction mixture was vortexed, centrifuged, and incubated at 80 °C for 16 h. After the reaction mixture was cooled down to room temperature, sodium cysteinate (1000 nmol, 5 μL, 200 mM in H₂O, 5000 equiv) was added and then heated at 80 °C for 15 min before being quenched by EtOH precipitation. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 75%). Deconvoluted molecular mass: calculated: 5275 Da; observed: 5275 Da.

### 4.5. On-DNA thiol-Michael addition reaction of vinyl sulfone and subsequent sulfoxide formation
Scheme S1. Synthesis of aa35.

To the solution of DNA-conjugated aa1 (2 μL, 100 μM in H2O, 0.2 nmol, 1 equiv) was added H2O (16 μL), NaOH (4 μL, 250 mM in H2O, 1000 nmol, 5000 equiv), and 35 (8 μL, 200 mM in DMA, 1600 nmol, 8000 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (Conversion: 80%). Deconvoluted molecular mass: calculated: 5331 Da; observed: 5331 Da.

Scheme S2. Synthesis of aa36

To the solution of DNA-conjugated aa1 (2 μL, 100 μM in H2O, 0.2 nmol, 1 equiv) was added DMA (16 μL), H2O (6 μL), NaOH (2 μL, 250 mM in H2O, 500 nmol, 2500 equiv), and 36 (4 μL, 200 mM in DMA, 800 nmol, 4000 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5331 Da; observed: 5331 Da.

Unless otherwise noted, on-DNA thiol-Michael addition reaction described in the supplementary information were conducted under this standard condition.
Scheme S3. Synthesis of sulfoxide aa44.

To the solution of aa36 (2 µL, 100 µM in H₂O, 0.2 nmol, 1 equiv) was added H₂O (43 µL) and NaIO₄ (5 µL, 100 mM in H₂O, 500 nmol, 2500 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 5 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5347 Da; observed: 5347 Da.
5. Structural confirmation

5.1. Off-DNA synthesis of authentic A1

Scheme S4. Synthesis of authentic A1

Methyl (E)-4-(2-(phenylsulfonyl)vinyl)benzoate (A1). To a suspension of benzenesulfinic acid sodium salt (147.6 mg, 0.9 mmol, 3 equiv) and NaOAc (36.9 mg, 0.45 mmol, 1.50 equiv) in MeCN (1.2 mL) was added styrene (50 mg, 0.3 mmol, 1 equiv) followed by iodine (114.3 mg, 0.45 mmol, 1.50 equiv). The mixture was heated to reflux for 3 h before being allowed to cool and the excess iodine was quenched with 10% aq. sodium thiosulfate. Sat. aq. NaHCO₃ was added and the product was extracted into EtOAc (3 x 20 mL). The organic phase was combined and dried with Na₂SO₄. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate (5:1) as eluent, affording A1 as a white amorphous solid (66 mg, 72%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 7.4 Hz, 2H), 7.70 (d, J = 15.4 Hz, 1H), 7.65 (t, J = 7.4 Hz, 1H), 7.56 (dd, J = 12.2, 8.0 Hz, 4H), 6.95 (d, J = 15.4 Hz, 1H), 3.93 (s, 3H).
$^1$H-NMR of A1
5.2. Co-injection experiment

**Route 1**

\[
\text{aa} + \text{SO}_2\text{Na} \rightarrow \text{aa1}
\]

**Route 2**

\[
\text{A1} \rightarrow \text{A2}
\]

1) NaOH, THF
2) HATU, DIPEA

HPLC chromatography showed that the peak from the co-injection (curve in blue) had the same retention time as the other two peaks (aa1 from route 1, curve in green; A2 from route 2, curve in red)

**Figure S6.** Co-injection experiment of aa1 and A2 from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (curve in blue) had the same retention time as the other two peaks (aa1 from route 1, curve in green; A2 from route 2, curve in red)
6. Scale-up reaction
6.1. Scale-up reaction of aa1

![Chemical diagram](image)

Figure S7. UPLC chromatogram of aa1 at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated aa (2 μL, 5 mM in H2O, 10 nmol, 1 equiv) was added H2O (8 μL), 1 (2 μL, 200 mM in H2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 88%). Deconvoluted molecular mass: calculated: 5207 Da; observed: 5207 Da.
6.2. Scale-up reaction of aa6

![Reaction Scheme]

**Figure S8.** UPLC chromatogram of aa6 at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated aa (2 μL, 5 mM in H2O, 10 nmol, 1 equiv) was added H2O (8 μL), 6 (2 μL, 200 mM in H2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 86%). Deconvoluted molecular mass: calculated: 5252 Da; observed: 5252 Da.
6.3. Scale-up Reaction of aa7

![Chemical Reaction Diagram](image)

Figure S9. UPLC chromatogram of aa7 at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated aa (2 μL, 5 mM in H2O, 10 nmol, 1 equiv) was added H2O (8 μL), 7 (2 μL, 200 mM in H2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 81%). Deconvoluted molecular mass: calculated: 5286 Da; observed: 5286 Da.
6.4. Scale-up reaction of aa12

![Chemical Reaction Diagram]

**Figure S10.** UPLC chromatogram of aa12 at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated aa (2 μL, 5 mM in H₂O, 10 nmol, 1 equiv) was added H₂O (8 μL), 12 (2 μL, 200 mM in H₂O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 77%). Deconvoluted molecular mass: calculated: 5232 Da; observed: 5232 Da.
6.5. Scale-up reaction of aa21

![Chemical reaction diagram]

**Figure S11.** UPLC chromatogram of aa21 at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated aa (2 μL, 5 mM in H₂O, 10 nmol, 1 equiv) was added H₂O (8 μL), 21 (2 μL, 200 mM in H₂O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 83%). Deconvoluted molecular mass: calculated: 5251 Da; observed: 5251 Da.
7. Enzymatic ligation


Headpiece-primer (HP-P, 20 nmol) was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. 4-Vinylbenzoic acid (10 µL, 200 mM in DMA, 100 equiv), HATU (5 µL, 400 mM in DMA, 100 equiv), and DIPEA (5 µL, 400 mM in DMA, 100 equiv) were mixed by vortex and allowed to pre-activated for 10 minutes at 25 °C, and then the mixture was transferred to HP-P solution in a 0.6 mL tube and mixed. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 hours. After ethanol precipitation, the resulting pellet was vacuum-dried and dissolved in nuclease-free water (200 µL). The reaction was analyzed by UPLC-MS.

To the solution of DNA conjugate b1 (2 µL, 2.5 mM in H2O, 5 nmol, 1 equiv) was added H2O (8 µL), 1 (2 µL, 200 mM in H2O, 400 nmol, 80 equiv), and iodine (2 µL, 200 mM in MeOH, 400 nmol, 80 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 90%). Deconvoluted molecular mass: calculated: 12679 Da; observed: 12679 Da.

DNA conjugate b2 (16 µL, 6 nmol), code (6 µL, 7.2 nmol, 1.2 equiv) and 2× ligation buffer (25 µL) were added into 0.6 mL tube, mixed by vortex, then T4 DNA ligase (3 µL, 350 units/µL) was added and mixed gently. The reaction
mixture was incubated at 20 °C for 16 h. Before ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C for 10 min, and the ligation product was isolated by ethanol precipitation as described above. Deconvoluted molecular mass: calculated: 20719 Da; observed: 20720 Da.

To the solution of DNA conjugate b3 (2 μL, 0.5 mM in H₂O, 1 nmol, 1 equiv) was added H₂O (6 μL) and DMA (16 μL), NaOH (2 μL, 250 mM in H₂O, 500 nmol, 500 equiv), and 4-Chlorobenzylmercaptan (4 μL, 200 mM in H₂O, 800 nmol, 800 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (>90% conversion). Deconvoluted molecular mass: calculated: 20878 Da; observed: 20879 Da. After the reaction, product (10 μL, 50 pmol), code (0.5 μL, 65 pmol, 1.3 equiv) and 2× ligation buffer (10 μL) was added into 0.6 mL tube, mixed by vortex, then T4 DNA ligase (0.2 μL, 350 units/μL) was added and mixed gently. The reaction mixture was incubated at 20 °C for 16 h. Before ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C for 10 min, and the ligation product was isolated by ethanol precipitation as described above. Deconvoluted molecular mass: calculated: 28900 Da; observed: 28983 Da (28900+2Na⁺+K⁺).
Figure S12. 20% denatured PAGE analysis of DEL compatibility of on-DNA vinyl sulfone and subsequent thiol-Michael addition reaction.
8. UPLC chromatogram and deconvoluted MS

8.1. Substrate scope of sodium sulfinates

UPLC chromatogram and deconvoluted MS of aa1

Conversion: >90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da
UPLC chromatogram and deconvoluted MS of aa2

Conversion: 87%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da
UPLC chromatogram and deconvoluted MS of aa3

Conversion: 82%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da
UPLC chromatogram and deconvoluted MS of aa4

Conversion: 86%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da
UPLC chromatogram and deconvoluted MS of aa5

Conversion: >90%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da
UPLC chromatogram and deconvoluted MS of aa6

Conversion: 80%

Calculated Mass: 5252 Da; Observed Mass: 5252 Da
UPLC chromatogram and deconvoluted MS of aa7

Conversion: 80%

Calculated Mass: 5286 Da; Observed Mass: 5286 Da
UPLC chromatogram and deconvoluted MS of aa8

Conversion: >90%

Calculated Mass: 5275 Da; Observed Mass: 5275 Da
UPLC chromatogram and deconvoluted MS of aa9

Conversion: 78%

Calculated Mass: 5285 Da; Observed Mass: 5285 Da

Unknown by-product
UPLC chromatogram and deconvoluted MS of **aa10**

**Conversion:** 83%

**Calculated Mass:** 5225 Da; **Observed Mass:** 5225 Da
UPLC chromatogram and deconvoluted MS of **aa11**

**Conversion:** 86%

**Calculated Mass:** 5248 Da; **Observed Mass:** 5248 Da
UPLC chromatogram and deconvoluted MS of \textbf{aa12}

\textbf{Conversion: 75\%}

\textbf{Calculated Mass: 5232 Da; Observed Mass: 5232 Da}
UPLC chromatogram and deconvoluted MS of aa13

Conversion: 57%

Calculated Mass: 5249 Da; Observed Mass: 5249 Da
UPLC chromatogram and deconvoluted MS of aa14

Conversion: >90%

Calculated Mass: 5243 Da; Observed Mass: 5243 Da
UPLC chromatogram and deconvoluted MS of **aa15**

**Conversion:** 76%

**Calculated Mass:** 5277 Da; **Observed Mass:** 5277 Da
UPLC chromatogram and deconvoluted MS of **aa16**

**Conversion:** >90%

**Calculated Mass:** 5259 Da; **Observed Mass:** 5259 Da
UPLC chromatogram and deconvoluted MS of **aa17**

**Conversion:** 82%

**Calculated Mass:** 5316 Da; **Observed Mass:** 5316 Da
UPLC chromatogram and deconvoluted MS of **aa18**

**Conversion:** >90%

**Calculated Mass:** 5233 Da; **Observed Mass:** 5233 Da
UPLC chromatogram and deconvoluted MS of aa19

Conversion: 82%

Calculated Mass: 5264 Da; Observed Mass: 5264 Da
UPLC chromatogram and deconvoluted MS of aa20

Conversion: >90%

Calculated Mass: 5291 Da; Observed Mass: 5291 Da
UPLC chromatogram and deconvoluted MS of aa21

**Conversion:** >90%

**Calculated Mass:** 5251 Da; **Observed Mass:** 5251 Da
UPLC chromatogram and deconvoluted MS of **aa22**

**Conversion:** >90%

**Calculated Mass:** 5251 Da; **Observed Mass:** 5251 Da
UPLC chromatogram and deconvoluted MS of \texttt{aa23}

Conversion: $>90\%$

Calculated Mass: 5257 Da; Observed Mass: 5257 Da
UPLC chromatogram and deconvoluted MS of aa24

Conversion: 80%

Calculated Mass: 5213 Da; Observed Mass: 5213 Da
UPLC chromatogram and deconvoluted MS of **aa25**

**Conversion:** 85%

**Calculated Mass:** 5248 Da; **Observed Mass:** 5248 Da
UPLC chromatogram and deconvoluted MS of aa26

Conversion: 61%

Calculated Mass: 5271 Da; Observed Mass: 5271 Da
UPLC chromatogram and deconvoluted MS of aa27

Conversion: 90%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da
UPLC chromatogram and deconvoluted MS of aa28

Conversion: 38%

Calculated Mass: 5226 Da; Observed Mass: 5226 Da
UPLC chromatogram and deconvoluted MS of **aa29**

**Conversion:** 28%

**Calculated Mass:** 5258 Da; **Observed Mass:** 5258 Da
UPLC chromatogram and deconvoluted MS of aa30

Conversion: 75%

Calculated Mass: 5280 Da; Observed Mass: 5281 Da

Unknown by-product
UPLC chromatogram and deconvoluted MS of aa31

Conversion: 85%

Calculated Mass: 5145 Da; Observed Mass: 5145 Da
UPLC chromatogram and deconvoluted MS of aa32

Conversion: 70%

Calculated Mass: 5159 Da; Observed Mass: 5159 Da
UPLC chromatogram and deconvoluted MS of aa33

**Conversion: 80%**

**Calculated Mass: 5187 Da; Observed Mass: 5187 Da**
UPLC chromatogram and deconvoluted MS of aa34

Conversion: 72%

Calculated Mass: 5171 Da; Observed Mass: 5171 Da
8.2. Substrate Scope of DNA Conjugated Alkenes

UPLC chromatogram and deconvoluted MS of 1ab

Conversion: >90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da
UPLC chromatogram and deconvoluted MS of 1ac

Conversion: 90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da
UPLC chromatogram and deconvoluted MS of 1ad

Conversion: 88%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da
UPLC chromatogram and deconvoluted MS of 1ae

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da
UPLC chromatogram and deconvoluted MS of 1af

Conversion: >90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da
UPLC chromatogram and deconvoluted MS of 1ag

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da
UPLC chromatogram and deconvoluted MS of 1ah

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da
UPLC chromatogram and deconvoluted MS of 1ai

Conversion: >90%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da
UPLC chromatogram and deconvoluted MS of \textbf{1aj}

\textbf{Conversion:} >90\%

\textbf{Calculated Mass:} 5241 Da; \textbf{Observed Mass:} 5241 Da
UPLC chromatogram and deconvoluted MS of 1ak

Conversion: 72%

Calculated Mass: 5242 Da; Observed Mass: 5242 Da
UPLC chromatogram and deconvoluted MS of 1a1

Conversion: 25%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da
UPLC chromatogram and deconvoluted MS of 1am

Conversion: 90%

Calculated Mass: 5373 Da; Observed Mass: 5373 Da
UPLC chromatogram and deconvoluted MS of 1an

**Conversion:** 81%

**Calculated Mass:** 5221 Da; **Observed Mass:** 5221 Da
UPLC chromatogram and deconvoluted MS of 1ao

Conversion: >90%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da
UPLC chromatogram and deconvoluted MS of 1ap

Conversion: 76%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da
UPLC chromatogram and deconvoluted MS of 1aq

Conversion: 81%

Calculated Mass: 5239 Da; Observed Mass: 5239 Da
UPLC chromatogram and deconvoluted MS of 1ar

Conversion: 87%

Calculated Mass: 5239 Da; Observed Mass: 5239 Da
UPLC chromatogram and deconvoluted MS of 1as

Conversion: 70%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da
UPLC chromatogram and deconvoluted MS of 1at

Conversion: 63%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da
UPLC chromatogram and deconvoluted MS of 1au

Conversion: >90%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da
UPLC chromatogram and deconvoluted MS of 1av

Conversion: >90%

Calculated Mass: 5303 Da; Observed Mass: 5303 Da
UPLC chromatogram and deconvoluted MS of 1aw

Conversion: 82%

Calculated Mass: 5131 Da; Observed Mass: 5131 Da
UPLC chromatogram and deconvoluted MS of 1ax

Conversion: 80%

Calculated Mass: 5145 Da; Observed Mass: 5145 Da
UPLC chromatogram and deconvoluted MS of 1ay

Conversion: 82%

Calculated Mass: 5159 Da; Observed Mass: 5159 Da
UPLC chromatogram and deconvoluted MS of 1az

Conversion: 90%

Calculated Mass: 5173 Da; Observed Mass: 5173 Da
8.3. Substrate Scope of Thiols

UPLC chromatogram and deconvoluted MS of aa35

Conversion: 80%

Calculated Mass: 5331 Da; Observed Mass: 5331 Da
UPLC chromatogram and deconvoluted MS of aa36

**Conversion:** >90%

**Calculated Mass:** 5331 Da; **Observed Mass:** 5331 Da
UPLC chromatogram and deconvoluted MS of aa37

Conversion: >90%

Calculated Mass: 5366 Da; Observed Mass: 5366 Da
UPLC chromatogram and deconvoluted MS of aa38

Conversion: >90%

Calculated Mass: 5361 Da; Observed Mass: 5361 Da
UPLC chromatogram and deconvoluted MS of aa39

Conversion: >90%

Calculated Mass: 5353 Da; Observed Mass: 5353 Da
UPLC chromatogram and deconvoluted MS of **aa40**

**Conversion:** >90%

**Calculated Mass:** 5327 Da; **Observed Mass:** 5327 Da
UPLC chromatogram and deconvoluted MS of \textbf{aa41}

\textbf{Conversion: >90\%}

\textbf{Calculated Mass: 5345 Da; Observed Mass: 5345 Da}
UPLC chromatogram and deconvoluted MS of **aa42**

**Conversion:** >90%

**Calculated Mass:** 5328 Da; **Observed Mass:** 5328 Da
UPLC chromatogram and deconvoluted MS of aa43

Conversion: 80%

Calculated Mass: 5514 Da; Observed Mass: 5514 Da
9. References

