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Supporting Information

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for

Tunable DNA Cleavage by Intercalating Peptidoconjugates

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Materials

Solvents were purchased from Fisher and reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Acros Organics (Morris Plains, NJ). Amino acids and Fmoc-Lys(Boc)-Wang resin (75-100 mesh) with a substitution level of 0.70 mmol/g were purchased from Advanced ChemTech (Louisville, KY). Fmoc-protected Rink amide resin (100-200 mesh) was purchased from NovaBiochem (San Diego, CA) with a substitution level of 0.64 mmol/g. Fmoc-Lys(Boc)-OH-Wang resin (75-100 mesh) was purchased from Advanced ChemTech (Louisville, KY) with a substitution level of 0.70 mmol/g. All solvents and reagents were used without further purification. HPLC grade acetonitrile and Millipore water were used for HPLC analysis. Molar extinction coefficient (e = 63 000 M^{-1} cm^{-1} in H_2O at 500 nm for TO-conjugates.[1]

Analysis

Reversed-phase HPLC (RP-HPLC) was performed with an Agilent 1100 series HPLC using either an analytical column (Varian C18, 5 micron, 250 x 4.6 mm) at a flow rate of 2 mL/min or a preparative column (Hamilton PRP-1, 10 micron, 250 x 10 mm) at

a flow rate of 3.5 mL/min. For analytical HPLC a linear solvent gradient from 5 to 100% B over 60 min was used (solvent A = 0.1% TFA in H_2O; solvent B = 0.1% TFA in CH_3CN). Mass spectral analysis was performed by the Boston College Mass Spectrometry Facility. Samples were analyzed by MALDI-TOF MS on a Micromass TofSpec 2E using α-cyano-4-hydroxycinnamic acid matrix. UV analysis was performed on an Agilent 8453 Diode Array Spectrophotometer.

General procedure for the synthesis of TO-peptide conjugates

A representative procedure for solid-phase synthesis of TO-conjugate **7** is given below. The term "wash" refers to the following series of rinses: DMF (5 x 1 mL), MeOH (5 x 1 mL) and DCM (5 x 1 mL).

Example synthesis: TO-(d)WK-$CONH_2$ (7)

Fmoc-Rink amide resin (31.3 mg, 0.0200 mmol, 1.0 equiv) and THF (1 mL) was added to a 1.5 mL Bio-Rad chromatography column. The slurry was agitated for 1 h then the resin was washed.

A 20% piperidine in DMF solution (1 mL) was added to the reaction vessel and agitated for 0.5 h. The resin was then washed.

HBTU (30.3 mg, 0.0800 mmol, 4.0 equiv), Fmoc-D-Lys(Boc)-OH (37.5 mg, 4.0 equiv), dry DMF (1 mL) and *i*-Pr_2NEt (27.9 µL, 0.160 mmol, 8.0 equiv) was added to the reaction vessel. The reaction was agitated for 3 h. The resin was then washed.

A 20% piperidine in DMF solution (1 mL) was added to the reaction vessel and agitated for 0.5 h. The resin was then washed.

HBTU (30.3 mg, 4.0 equiv), Fmoc-D-Trp(Boc)-OH (42.1 mg, 4.0 equiv), dry DMF (1 mL) and *i*-Pr_2NEt (27.9 µL, 8.0 equiv) was added to the reaction vessel. The reaction was agitated for 3 h. The resin was then washed.

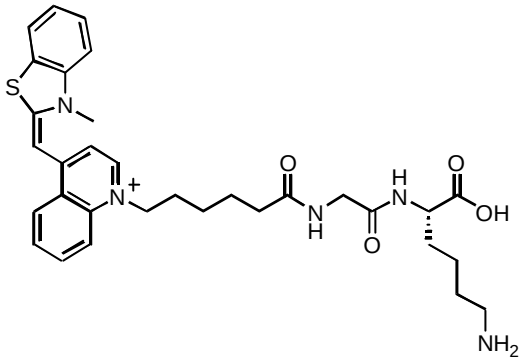
A 50% piperidine in DMF solution (1 mL) was added to the reaction vessel and agitated for 5 min. The resin was then washed.

HBTU (30.3 mg, 4.0 equiv), TO-COOH (38.8 mg, 4.0 equiv), dry DMF (1 mL) and *i*-Pr_2NEt (27.9 µL, 8.0 equiv) was added to the reaction vessel, which was then covered with foil. The reaction was agitated for 3 h. The resin was then washed.

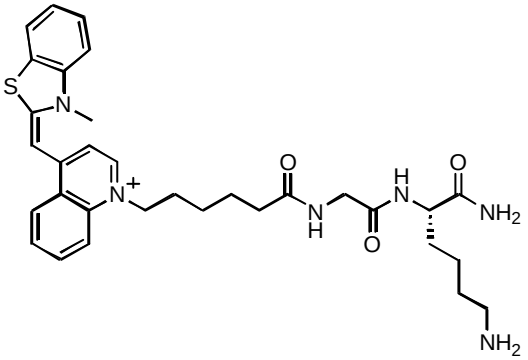
10% TFA in DCM (2 x 1 mL) was added to the reaction vessel and the slurry was then transferred to a glass funnel with a fine sinter. The solvent was allowed to drip slowly through the resin bed and was washed with 5% TFA in DCM (2 x 1 mL) and concentrated in vacuo.

Deprotection was achieved by stirring the residue in a 95:5 TFA:TIS solution at room temperature for 0.5 h and concentrating the solution in vacuo. The residue was dissolved in dry MeOH (150 ?L) and transferred to a 15 mL plastic conical tube. Cold Et_2O (10 mL) was added and the resulting crude mixture was centrifuged and the Et_2O was decanted. This procedure was repeated. The red solid was dissolved in 0.1% TFA/H_2O and purified by RP-HPLC.

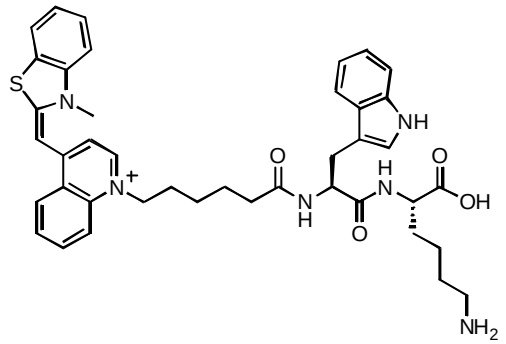
Characterization of TO-conjugates



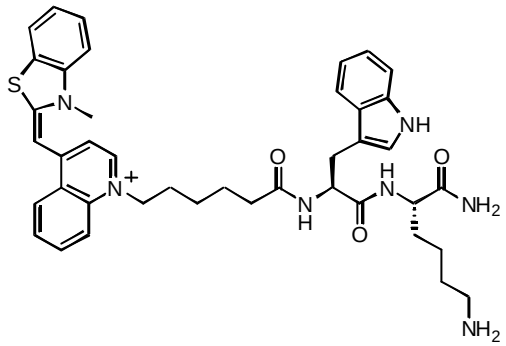
TO-GK-COOH (**1**). MALDI-TOF calculated for [$C_{32}H_{40}N_5O_4S^+$] requires *m/z* 590.8 Found 590.8 HPLC retention time 19.6 min



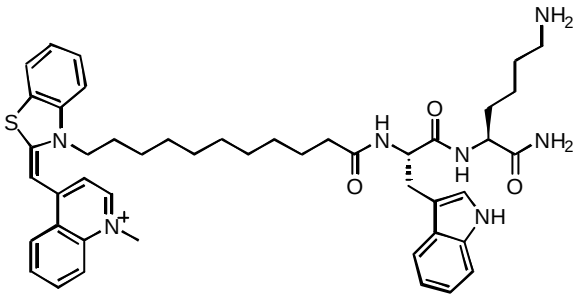
TO-GK-$CONH_2$ (**2**). MALDI-TOF calculated for [$C_{32}H_{41}N_6O_3S^+$] requires *m/z* 589.8 Found 589.7 HPLC retention time 19.3 min



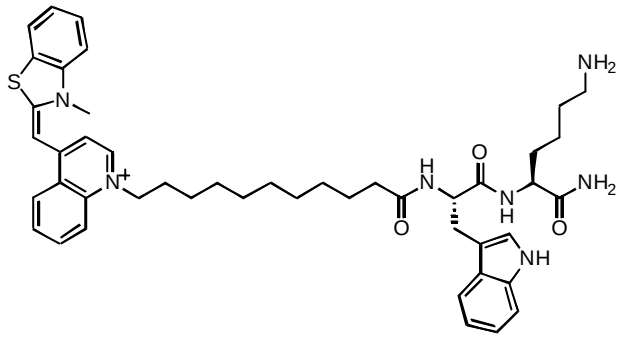
TO-WK-COOH (**3**). MALDI-TOF calculated for [$C_{41}H_{47}N_6O_4S^+$] requires *m/z* 719.9 Found 720.8 HPLC retention time 23.6 min



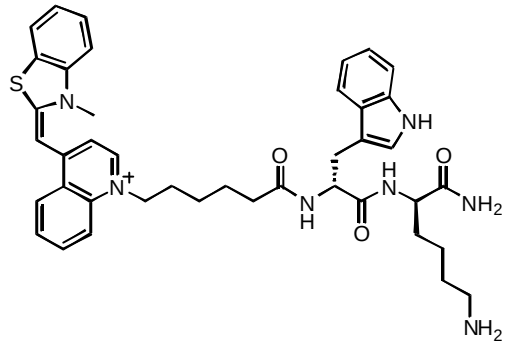
TO-WK-$CONH_2$ (**4**). MALDI-TOF calculated for [$C_{41}H_{48}N_7O_3S^+$] requires *m/z* 718.9 Found 719.2 HPLC retention time 22.8 min



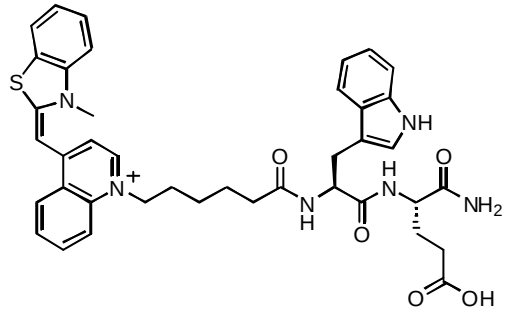
TO_Z-WK-$CONH_2$ (**5**). MALDI-TOF calculated for [$C_{46}H_{58}N_7O_3S^+$] requires *m/z* 789.1 Found 787.9 HPLC retention time 29.1 min



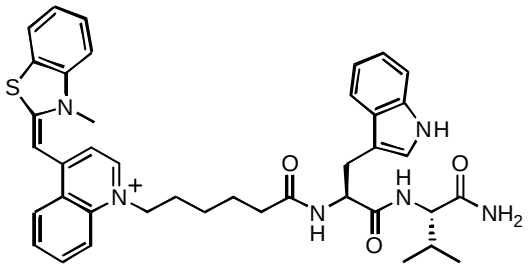
TO_Q-WK-$CONH_2$ (**6**). MALDI-TOF calculated for [$C_{46}H_{58}N_7O_3S^+$] requires *m/z* 789.1 Found 790.1 HPLC retention time 29.7 min



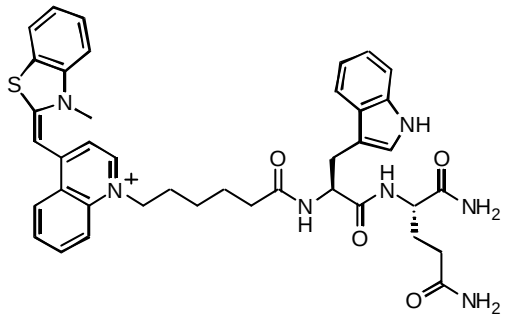
TO-(d)WK-$CONH_2$ (**7**). MALDI-TOF calculated for [$C_{41}H_{48}N_7O_3S^+$] requires *m/z* 718.9 Found 719.2 HPLC retention time 22.8 min



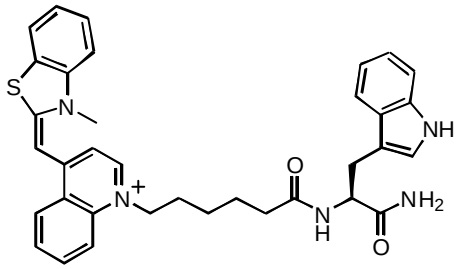
TO-WE-$CONH_2$ (**8**). MALDI-TOF calculated for [$C_{40}H_{43}N_6O_5S^+$] requires *m/z* 719.9 Found 720.2 HPLC retention time 24.5 min



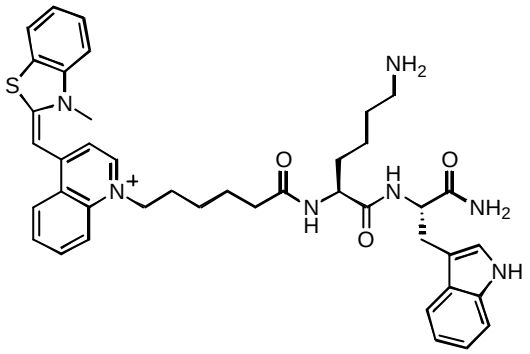
TO-WV-$CONH_2$ (**9**). MALDI-TOF calculated for [$C_{40}H_{45}N_6O_3S^+$] requires *m/z* 689.9 Found 690.1 HPLC retention time 26.6 min



TO-WQ-$CONH_2$ (**10**). MALDI-TOF calculated for [$C_{40}H_{44}N_7O_4S^+$] requires *m/z* 718.9 Found 719.1 HPLC retention time 23.6 min



TO-W-$CONH_2$ (**11**). MALDI-TOF calculated for [$C_{35}H_{36}N_5O_2S^+$] requires *m/z* 590.8 Found 590.8 HPLC retention time 25.7 min



TO-KW-$CONH_2$ (**12**). MALDI-TOF calculated for [$C_{41}H_{48}N_7O_3S^+$] requires *m/z* 718.9 Found 719.2 HPLC retention time 22.9 min

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(1) R. P. Haugland, *Handbook of Fluorescent Probes and Research Products*; 9th ed.; Molecular Probes, Eugene, OR, **2002**.