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Bridgehead-Substituted Triptycenes for Discovery of Nucleic Acid Junction Binders

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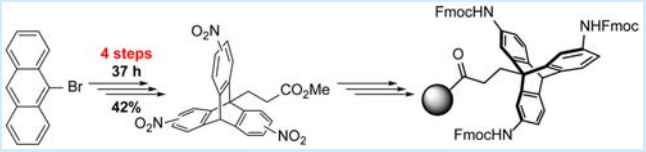
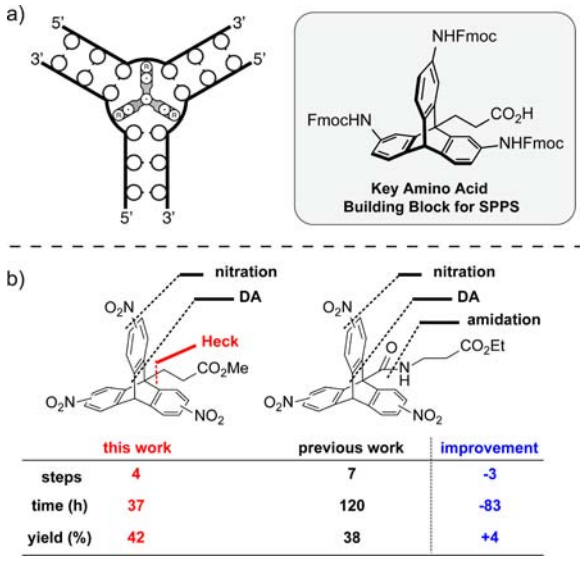
Supporting Information

ABSTRACT: Recently, the utility of triptycene as a scaffold for targeting nucleic acid three-way junctions was demonstrated. A rapid, efficient route for the synthesis of bridgehead-substituted triptycenes is reported, in addition to solid-phase diversification to a new class of triptycene peptides. The triptycene peptides were evaluated for binding to a d(CAG)·(CTG) repeat DNA junction exhibiting potent affinities. The bridgehead-substituted triptycenes provide new building blocks for rapid access to diverse triptycene ligands with novel architectures.



Nucleic acid junctions are important structural intermediates in biology.[1−3] Junctions are present in important biological processes including replication.[4,5] These junctions also occur in viral genomes in addition to trinucleotide repeat expansions associated with numerous neurodegenerative diseases.[6−12] These structures are also present in nanostructures and aptamer-based sensors.[13−20] The ability to selectively modulate a subset of nucleic acid structures using small molecules would allow for the chemical control of cellular processes as well as the reprogramming of cellular events.[21−37] The ability to differentially stabilize predefined nucleic acid structures or to reprogram and bias the equilibrium distribution of an ensemble of structures in a precise manner could have a profound impact not only in biology but also in nucleic acid nanotechnology and materials applications.

We previously demonstrated that triptycene-based molecules can bind to three-way junctions (3WJs).[38] Additionally, we have shown that these molecules bind to d(CAG)·(CTG) repeats implicated in triplet repeat expansion diseases.[39] The ability to synthesize libraries of triptycene derivatives on solid supports will accelerate efforts to identify biologically relevant nucleic acid junction binders and provide further insight into the molecular recognition properties of triptycenes toward diverse junction sequences and topologies. To facilitate solid-phase immobilization, a point of attachment on triptycene is required. The bridgehead position provided a strategic location, as it is equidistant from the three amino groups that serve as sites of diversification (Figure 1a). We recently described a synthesis for bridgehead-substituted triptycene building blocks.[40] Here, we report a modified, more efficient synthesis by utilizing a combined Heck coupling/benzyne Diels−Alder strategy. The new triptycene building block was further diversified on solid phase with short di- and tripeptides, and the final compounds were evaluated for binding to a d(CAG)·(CTG) repeat junction. We discovered new high-affinity lead compounds for this junction motif that will form the basis of further investigations.



	this work	previous work	improvement
steps	4	7	-3
time (h)	37	120	-83
yield (%)	42	38	+4

Figure 1. (a) Schematic of triptycene bound to a three-way junction and a key triptycene building block for diversification by solid-phase synthesis. (b) Improvement of the synthesis of triptycene intermediates in this work.

Similar to our previous route, our synthetic plan relied on the reduction of nitrated triptycene, a key intermediate, to install the three key amine functional groups that serve as points of future diversification (Figure 1b). The synthetic strategy presented here provides a shorter synthesis with only four steps to the key intermediate compared to seven steps in our previous route. Additionally, this method significantly reduced total reaction times from 120 to 37 h and showed an improvement in overall yield (Figure 1b). Moreover, the solubility of intermediates was improved. After extending the

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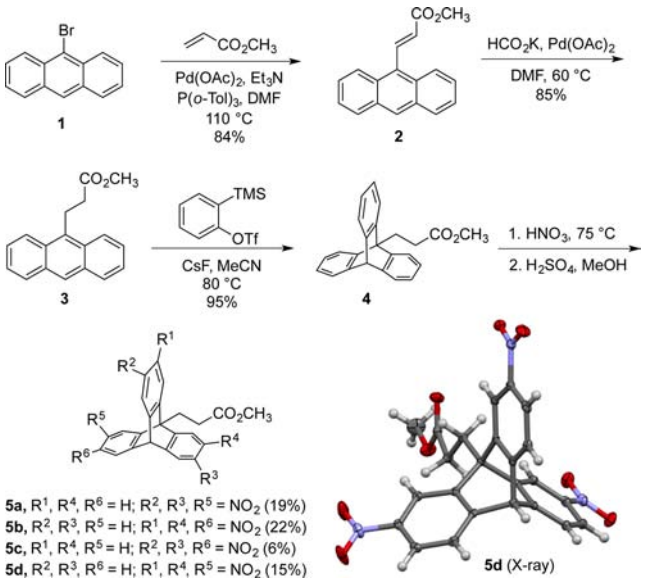
linker at the bridgehead via an amidation reaction in the previous route, the resulting product showed poor to moderate solubility in most organic solvents. However, the intermediates in this synthetic route have good solubility, allowing easier characterization and large-scale reactions. In addition, a new regioisomer **5c** that has all three nitro groups facing away from the linker was isolated in this new synthetic route, whereas this regioisomer was not observed in the previous report.

We initiated our synthesis with a Heck reaction between 9-bromoanthracene **1** and methyl acrylate in the presence of palladium(II) acetate, tri-*o*-tolylphosphine, and triethylamine in a sealed tube. The Heck reaction proceeded cleanly and resulted in the desired product **2** in 84% yield (Scheme 1).

Scheme 1. Synthesis of Bridgehead-Substituted Triptycenes 5a−d



Next, olefin **2** was reduced under mild conditions using palladium(II) acetate as the catalyst and potassium formate as the hydrogen source, producing **3** in 85% yield.[41] The key Diels−Alder reaction with anthracene **3** and benzyne, generated *in situ* from 2-(trimethylsilyl)phenyl trifluoromethanesulfonate and cesium fluoride, proceeded smoothly to yield bridgehead-substituted triptycene **4** in 95% yield. Nitration of triptycene resulted in hydrolysis of the bridgehead ester and four major nitrated regioisomers that proved inseparable by standard chromatographic techniques. Esterification of the crude reaction greatly facilitated the separation of the regioisomeric mixture (**5a−d**) using standard silica gel column chromatography. The nitrated triptycene regioisomers were characterized by HMBC and HSQC (see Supporting Information). A crystal of triptycene **5d** was obtained in chloroform to confirm its structure by X-ray crystallography (Scheme 1).

Next, isomer **5d** was utilized in subsequent transformations that were described in the previous publication.[40] Pd/C-catalyzed hydrogenation, Fmoc protection, and acid-catalyzed hydrolysis of the ester were performed to yield protected triptycene acid **7** in 78% yield over three steps. A key building block **7** was immobilized on 2-chlorotrityl chloride resin in preparation for solid-phase diversification (Scheme 2a). After addition of triptycene and washing of the resin, the Fmoc groups on triptycene were deprotected using piperidine in DMF (20% v/v) for 1 h. A decreased reaction time led to incomplete deprotection of all three Fmoc groups. After deprotection, the first amino acid was coupled onto the immobilized triptycene using HATU and DIEA. Overnight couplings were required for complete reaction with all three hindered aniline nitrogens. Next, subsequent deprotections followed by coupling of the desired amino acids were continued until the final sequence was obtained. The final deprotection of the amino acid side chain protecting groups and cleavage from resin were performed simultaneously using 9:1:1 TFA/TFE/DCM. The resulting triptycene peptides were purified by reversed phase HPLC and characterized prior to evaluation of the junction binding properties. In this manuscript, we focused our efforts on mono-, di-, and tripeptides to maximize diversity while maintaining minimal molecular weight. Longer peptides can certainly be produced although cell permeability will be a consideration as the size increases.

Binding of the amino acid substituted triptycenes was evaluated against a slipped-out d(CAG)·(CTG) repeat nucleic acid junction. Lysine and histidine containing triptycenes were synthesized due to their large presence in nucleic acid−protein interfacial interactions. Among the molecules previously tested, TripNL-$(Lys)_3$ and TripNL-$(His)_3$ exhibited the highest affinity toward the junction. Several dimeric and trimeric amino acid substituents were synthesized for comparison (Scheme 2b). A high-throughput assay in which the 3WJ was labeled with a fluorophore and a quencher was used to determine binding. The addition of a 10 bp oligonucleotide strand that was complementary to the 5′ end of the junction (**I10**) opened the structure, resulting in a highly fluorescent state (**TNR*-I10**), as shown in Scheme 2c. Titration of junction-stabilizing molecules resulted in quenching of fluorescence due to displacement of the inhibitor strand and reformation of the junction (**TNR*-Trip**). To determine if increased flexibility of the amino acid may play an important role in binding, glycine was coupled directly to the triptycene core followed by lysine or histidine. Trip-$(Gly\text{-}Lys)_3$ (**8**) exhibited increased potency compared to that of Trip-$(Lys)_3$, with a K_d of 90 nM, indicating that the increased flexibility may allow for better binding. This triptycene derivative demonstrates the highest binding affinity toward the TNR junction thus far. Interestingly, Trip-$(Gly\text{-}His)_3$ (**9**) did not exhibit improved binding compared to that of Trip-$(His)_3$. Triptycenes substituted with three amino acids were also synthesized using lysine, histidine, and asparagine. Trip-$(His\text{-}Lys\text{-}His)_3$ (**10**), Trip-$(His\text{-}Lys\text{-}Lys)_3$ (**11**), and Trip-$(His\text{-}Lys\text{-}Asn)_3$ (**12**), which only differ in their final amino acid, exhibited K_d values of 0.20, 0.17, and 0.39 μM, respectively. It should be noted that most triptycene derivatives synthesized in this work showed improved binding affinity compared to the most potent triptycene derivative from the previous work, which exhibited a K_d value of 0.27 μM. We also compared the binding affinity of Trip-$(His\text{-}Lys\text{-}Asn)_3$ (**12**) to that of TripAM-$(His\text{-}Lys\text{-}Asn)_3$, which have the same peptide sequence but an amide linker at the bridgehead. They exhibited similar binding affinities toward the junction. Triptycenes **8−12** were also characterized using a gel shift assay, where the inhibitor strand was incubated with unlabeled 3WJ (see Supporting Information). This change resulted in an electrophoretic shift that is consistent with a larger complex. Titration of triptycene with this complex resulted in reformation of the nucleic acid junction (Figure S2).

In summary, we have developed a shorter, more efficient synthetic strategy toward a bridgehead-substituted triptycene

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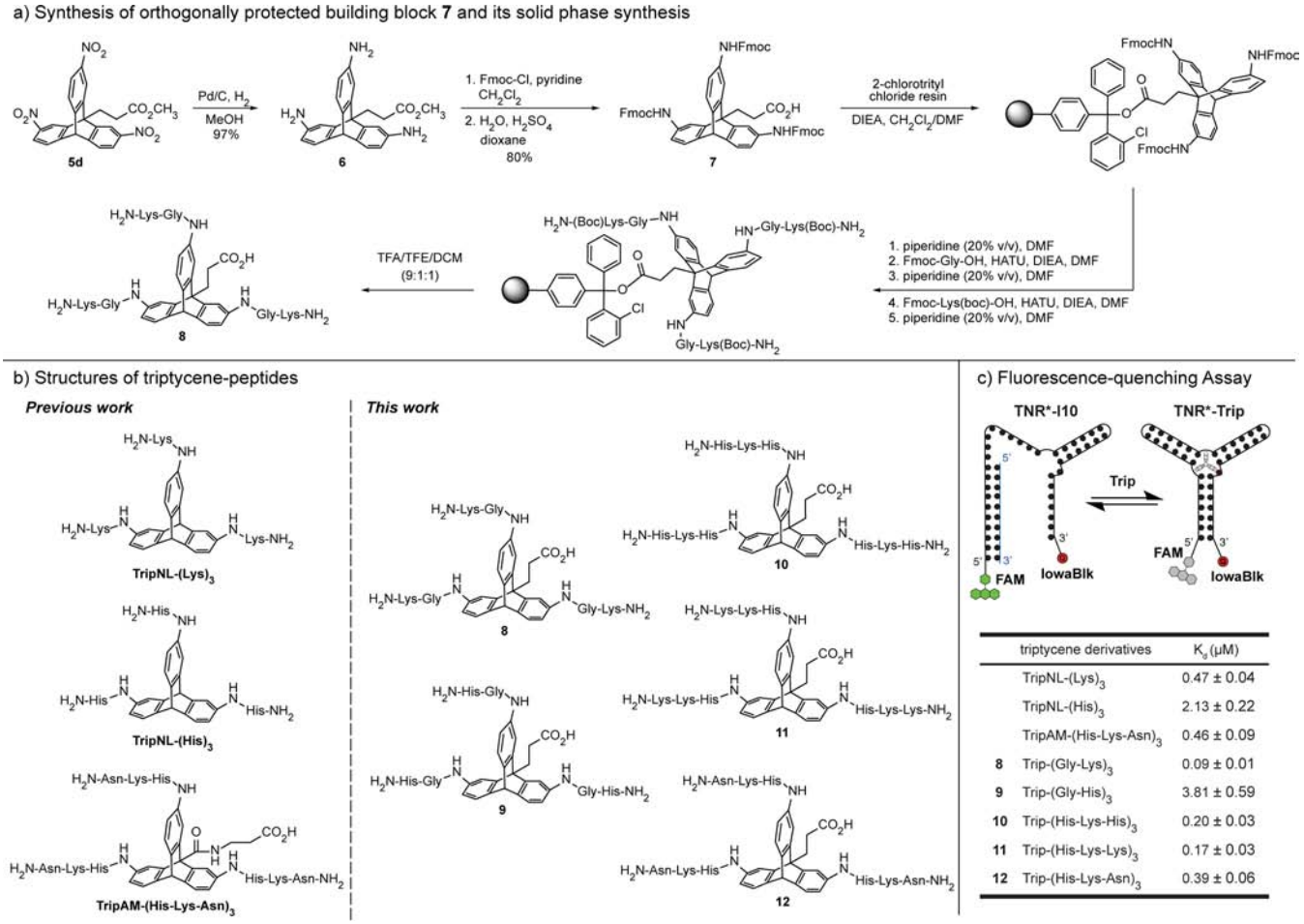
Scheme 2. Solid-Phase Synthesis of Orthogonally Protected Building Block 7 and Fluorescence-Quenching Experiment of Triptycene Peptides



	triptycene derivatives	K_d (μM)
	TripNL-$(Lys)_3$	0.47 ± 0.04
	TripNL-$(His)_3$	2.13 ± 0.22
	TripAM-(His-Lys-Asn)$_3$	0.46 ± 0.09
8	Trip-$(Gly-Lys)_3$	0.09 ± 0.01
9	Trip-$(Gly-His)_3$	3.81 ± 0.59
10	Trip-$(His-Lys-His)_3$	0.20 ± 0.03
11	Trip-$(His-Lys-Lys)_3$	0.17 ± 0.03
12	Trip-$(His-Lys-Asn)_3$	0.39 ± 0.06

building block. This new synthetic route is improved in terms of solubility, enabling large-scale reactions. Moreover, this route provides an interesting new regioisomer that was not observed through the previous route. A building block with an attachment point at the bridgehead provided rapid access to new triptycene peptide derivatives using solid-phase synthesis methods. The triptycene peptides were evaluated for nucleic acid junction binding to a triplet repeat expansion oligonucleotide using a fluorescence-based assay, which revealed the most potent binder to this junction to date. New triptycene building blocks that are amenable to solid-phase diversification provide a path for the discovery of new junction binders with superior properties. This new class of bridgehead-substituted triptycenes may allow for the generation of one-bead−one-compound combinatorial libraries for the rapid discovery of new junction binders using fluorescently labeled junctions.[42−44] Additionally, this new class of bridgehead-substituted triptycenes opens the door for the creation of pull-down probes to identify cellular targets in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00945.

Experimental details and NMR spectra (PDF)

Crystallographic data for **5d** (CIF)

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Notes

The authors declare no competing financial interest.

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