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Efficient Synthesis of an Indoloquinolizinium Alkaloid Selective DNA-Binder by Ring-Closing Metathesis

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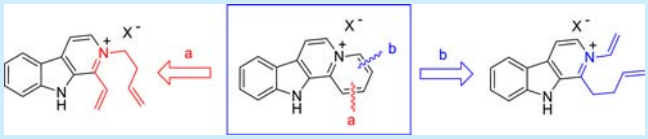
Supporting Information

ABSTRACT: Two total syntheses of the indolo[2,3-*a*]-quinolizinium cation have been accomplished through the application of two ring-closing metathesis reactions to form the pyridinium ring. One of these approaches provides the tetracyclic cation in only five steps from commercially available harmane. Fluorescence-based thermal denaturation experiments, as well as spectrofluorimetric titration, circular dichroism measurements, and theoretical simulations, showed a consistent DNA-binding capacity by intercalation with a marked preference for AT-rich sequences.



The indolo[2,3-*a*]quinolizinium zwitterion is probably the most widespread betainic heterocyclic system in nature, and Flavopereirine, Flavocoryline, Flavocorynanthrine, and Sempervirine are the simplest alkaloids that contain an indoloquinolizinium core[1] (Figure 1). Structurally more

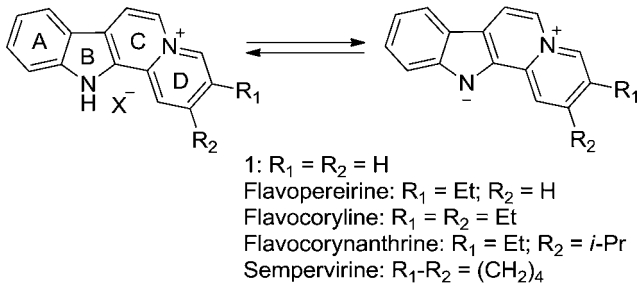


Figure 1. Simple indolo[2,3-*a*]quinolizinium alkaloids.

complex alkaloids such as Mitrasulgynine,[2] Strychnocrysine,[3] and Afrocurarine[4] are also based on this tetracyclic core. Several relevant pharmacological activities have been reported for this family of alkaloids, including anticancer,[5] immunostimulative,[6] anti-HIV,[5d] sedative, and antipsychotic[7] activity. Recently, it has also been reported that Sempervirine and other indole alkaloids are inhibitors of the CYP2D6 enzyme.[8] In addition, DNA intercalative binding[9] and fluorescence properties[10] have been described for some tetracyclic alkaloids.

To date, five total syntheses of the indolo[2,3-*a*]-quinolizinium cation (**1**) or its betainic form have been described in the literature, with overall yields in the range 10–31%.[11] In all of these syntheses the key step is the construction of the pyridinium ring C from a 2-pyridyl-substituted indole using different cyclization reactions on the C3 position of the indole ring. Our approach to this system involves a novel and complementary strategy in which the key step is the construction of pyridinium ring D by two different ring-closing metathesis reactions, which have proven to be very efficient for the construction of the dihydro- and quinolizinium systems from azinium salts.[12]

The strategy, which is depicted retrosynthetically in Figure 2, involves the formation of the quinolizinium moiety by two alternative synthetic routes. Both routes are based on the formation of ring D via the advanced dienic intermediates **3** and **6**, which would undergo ring-closing metathesis (RCM) to provide the corresponding 1,2- and 3,4-dihydroindolo[2,3-*a*]quinolizinium derivatives **2** and **5**, respectively. Both of these compounds would give **1** by oxidation. Retrosynthetically, we envisioned that both dienic substrates could be obtained from the same starting indole derivative **8** (tryptamine).

Our synthesis involving the C3–C4 disconnection from commercially available **8** would allow the preparation of **4** by adapting the literature procedure for the synthesis of 1-alkyl-*β*-carboline derivatives.[13] Unfortunately, the formation of the *β*-carboline system from the indole derivative **9** gave low yields of the desired 1-(3-butenyl)-*β*-carboline (**4**) under the reported conditions (Scheme 1). However, more satisfactory yields of **4** were obtained by an alternative procedure from the commercially available harmane **10**, which could be converted into **4** in a single step by reaction with allyl bromide in the presence of *t*-BuLi in THF as shown in Scheme 1. Cationic diene **3** was obtained from *β*-carboline **4** in a two-step sequence that involved *N*-alkylation with 2-chloroethyl triflate followed by dehydrohalogenation of the pyridinium salt **11** under basic conditions.[12d]

The dienic salt **3** was subjected to RCM reaction conditions. The yields obtained for 2,3-dihydro-*β*-carboline **2** under the different conditions explored for the optimization of this RCM

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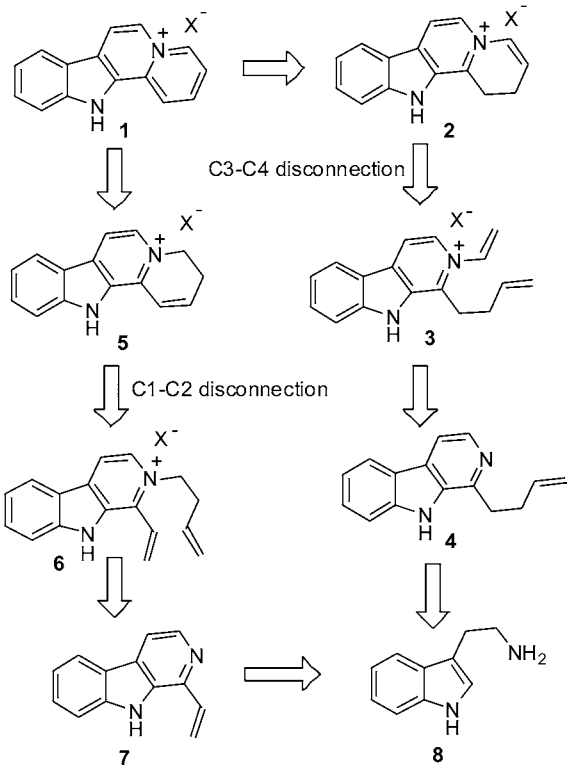
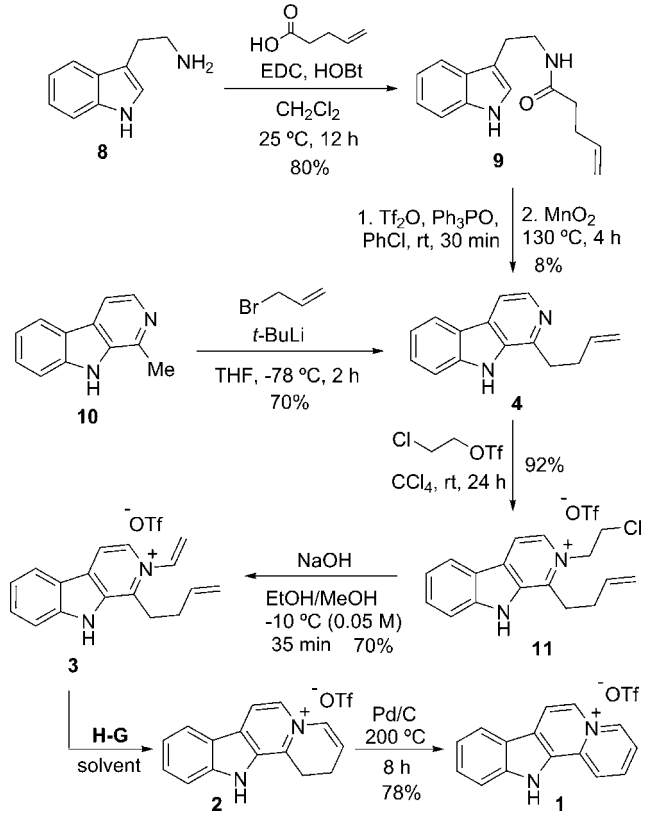


Figure 2. Retrosynthetic analysis of **1**.

Scheme 1. Synthesis of 1



reaction are shown in Table 1. As expected, the best results were obtained on using the Hoveyda–Grubbs catalyst,[14] which has already proven to be more efficient than the Grubbs catalysts for the formation of the dihydro- or pyridinium

Table 1. Results for the RCM of 3

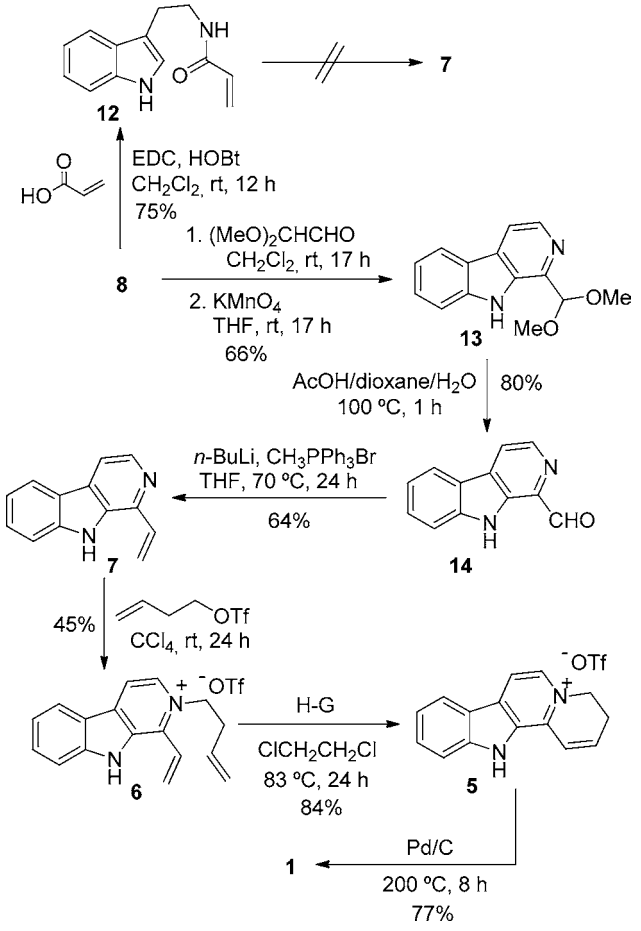
entry	catalyst	conditions	**2** yield (%)[a]
1	**G-I** (5%)	CH_2Cl_2, rt, 24 h	9
2	**G-II** (5%)	CH_2Cl_2, rt, 24 h	19
3	**H-G** (5%)	CH_2Cl_2, rt, 16 h	25
4	**H-G** (5%)	CH_2Cl_2, 40 °C, 10 h	54
5	**H-G** (10%)	CH_2Cl_2, 40 °C, 10 h	63
6	**H-G** (10%)	$ClCH_2CH_2Cl$, 83 °C, 4 h	83
7	**H-G** (5%)	$ClCH_2CH_2Cl$, 83 °C, 6 h	83

[a]Isolated yields from first (G-I) and second (G-II) generation Grubbs' catalysts: H-G: Hoveyda–Grubbs catalyst.

ring.[12a,c] Subsequent oxidation of **2** under previously reported conditions[12d] for dihydroquinolizinium salts, namely Pd/C at 200 °C, led to **1** in 78% yield.

The approach based on the C1–C2 disconnection involves the preparation of the key diene **6** from 1-vinyl-β-carboline (**7**) (Scheme 2). The synthesis of compound **7** is reported in the literature from tryptamine in a five-step sequence with an overall yield of 5%.[15]

Scheme 2. Alternative Synthesis of 1



A shorter route was explored using the same procedure as employed in the synthesis of **4**, but in this case all attempts to cyclize the amide **12**[16] were unsuccessful. Consequently, we focused on the synthesis of the formyl-β-carboline derivative **14**, which was obtained by following the literature procedure[17] shown in Scheme 2. The subsequent Wittig reaction between **14** and methyltriphenylphosphonium bromide gave **7** in a

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better yield. The carbolinium salt **6** was easily obtained by *N*-alkylation of **7** with 3-butenyl triflate. The diene **6** was then subjected to the same optimized RCM reaction conditions found for **2** to give the 3,4-dihydroderivative **5** in good yield (84%). Finally, compound **5** was oxidized by treatment with Pd/C to give **1** in 77% yield.

The photophysical properties of **1** were measured. Absorption and emission bands appeared at 334 and 467 nm, respectively. Values for molar absorptivity at the excitation wavelength(λ_{ex}), fluorescence quantum yield (Φ_f = 0.15), and fluorescence lifetimes at the maximum of the emission band (λ_{em}) show the interesting fluorescence behavior of **1**.

The affinity of **1** for calf thymus DNA (ctDNA) was also investigated by spectrofluorimetric titration. Initial studies showed that this cation is able to bind DNA by intercalation with an affinity constant of $K = (4.8 \pm 1.1) \times 10^4\ M^{-1}$, with the most stable complex **1**:DNA being formed when the number of nucleic base pairs per bound dye was 7.8 ± 0.2.

The affinity of **1** for DNA was also evaluated by circular dichroism (CD) measurements. This cation exhibited a negative Cotton effect in the ∼305−330 nm region and a positive effect extended between ∼330 and ∼420 nm, both very weak. On the assumption that the transition moments of the bands that appear at longer wavelengths are almost parallel to the long intercalator axis, the results reveal that **1** is aligned with the long-molecular axis perpendicular to the binding bases pocket.[10]

The interaction energies responsible for the stability of the corresponding **1**:DNA complex were studied by Molecular Mechanics (MM) and Molecular Dynamics (MD) calculations. For the Minima Binding Energy (MBE) structure the results indicate that **1** intercalates DNA mostly parallel to both central pairs of bases but that it prefers to locate slightly outside the pocket by the minor groove face (Figure 3).

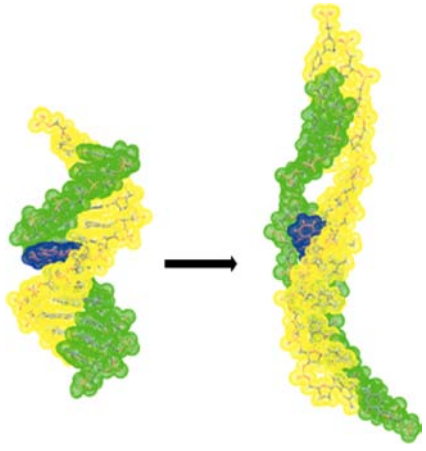

Figure 3. MBE structures for the **1**-DNA complex used as the starting conformation for the 1 ns MD trajectory at 300 K (left) and the one obtained from the analysis of the MD trajectory (right). Helix DNA end-to-end distances for the initial (left) and for the MBE structure from the MD analysis (right) are ∼44 and ∼63 Å, respectively.

Analysis of the MD trajectories revealed that interactions for the intercalation are favorable throughout the whole trajectory, with electrostatics providing the most important contributions to stabilization. The center of the dye intercalator tends to stay around the +1 (Å) *y* coordinate (average 1.0 ± 0.8 Å) most of the time. The average of the angle between the bisector of the base pairs pocket and the long axis of the dye is 62.1 ± 9.8°, which means that it deviates by 30° from the perpendicular (see Supporting Information).

In order to gain a deeper insight into the intercalation of **1** with DNA, binding studies were performed by fluorescence-based thermal denaturation experiments using custom synthetic oligonucleotides with defined AT- or GC-rich sequences.[18] The results showed a consistent double-stranded DNA binding capacity with a clear preference for AT-rich sequences (Figure 4).

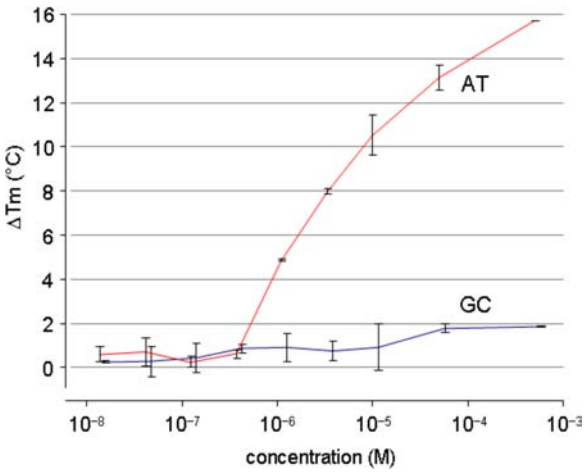

Figure 4. Plot of the experimental ΔT_m versus ligand concentration (logarithmic scale) obtained for reaction mixtures of **1** and double-stranded DNA oligonucleotides of different base composition and sequence. Oligonucleotide AT.-5′-CAATTAAATATAAC-3′ and its complementary. Oligonucleotide GC.-5′-GCGCGGCGTCCGGGCC-3′ and its complementary. Each data point is the average of two separate experiments. GC data points are slightly right-shifted to better visualize the standard deviation error bars in the four cases of lower concentration.

In summary, we report two total syntheses of the indolo[2,3-*a*]quinolizinium salt, both of which involve an RCM reaction for the formation of the pyridinium ring. One of these approaches provided the tetracyclic cation in only five steps from commercially available harmane in 36% overall yield. Photophysical and DNA binding experiments showed that **1** is a fluorescent compound that binds to double-stranded DNA by intercalation, with a significant preference for AT-rich sequences. The findings described above will allow further studies on **1** or its derivatives as DNA probes.

■ ASSOCIATED CONTENT

Supporting Information

Experimental synthetic procedures, photophysical and DNA binding experiments, MM and MD protocols and results, and ^1H and ^{13}C NMR data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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