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Amplification of DNA-binding affinities of protoberberine alkaloids by appended polyamines

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Abstract—This communication describes a synthetic approach toward the amplification of the moderate DNA-binding affinities of protoberberine alkaloids. Specifically, three protoberberine derivatives bearing two to six primary amino groups at the 3- and 9-positions of protoberberine were synthesized and characterized by NMR (^{1}H and ^{13}C) and HRMS. Studies on their affinities toward calf thymus (CT) DNA by ethidium bromide (EB) displacement and spectrophotometric titration experiments indicate that these polyamino protoberberines show more than 10^{3}-fold enhanced DNA-binding affinities relative to palmatine and thus are exploitable as strong DNA-binders.

Multivalent interactions are prevalent in nature and play a crucial role in the function of biological systems. For example, biology uses polyamines, such as spermine, to form strong interaction with DNA and RNA.[1] Therefore, an increasing interest has been currently attracted to the synthesis and biomedical applications of multivalent molecules.[2] Such molecules usually feature a large number of recognition groups that are organized in a well-defined multivalent way so that they can provide a convenient means for generating a high local concentration of binding sites—a feature that enhances the formation of multiple non-covalent bonding. Multivalent binding discloses the molecular basis for the high DNA-binding affinities and sequence selectivities of many naturally occurring antitumor antibiotics that exert their biological activities through specific and non-covalent interaction with DNA.[2]

We have become interested in the design and synthesis of multivalent DNA-binding agents,[3] by using readily available protoberberine alkaloids as building motifs. Protoberberine alkaloids have been demonstrated to be capable of forming complexes with DNA.[4] The main drawback is that they show moderate DNA-binding affinities, in part because they have only one recognition site (i.e., quaternary ammonium cation). In previous study, we have shown that attaching one primary amino group onto berberine can enhance the DNA-binding strength.[5] This result, together with the high DNA-binding affinities displayed by polyamines, such as dendritic spermines and poly(L-lysine),[6] makes us reason that conjugation of protoberberine alkaloids with polyamines may lead to significantly strong DNA binders in which protoberberine subunit serves as an anchor for DNA binding, whereas the polyamino groups are expected to impart enhanced binding affinities through multivalent bonding (e.g., hydrogen bonding and electrostatic interactions) with DNA.[7] With this rationale in mind, we describe herein the synthesis and the remarkably enhanced DNA-binding affinities of three protoberberine derivatives **2**–**4** bearing two, four, and six primary amino groups at the 3- and 9-positions, respectively (Chart 1).

The synthetic approach that was used for the preparation of compounds **2**–**4** is outlined in Scheme 1. Selective demethylation of jatrorrhizine **5** at 190 °C under vacuum for 15 min gave jatrorubine **6** in 87% yield. Alkylation of **6** with 1,3-dibromopropane in DMF afforded **7** in 90% yield. Ammonolysis of **7** with 28% ammonia solution-NH_4Cl gave **2** in 67% yield. Acylation (23%)

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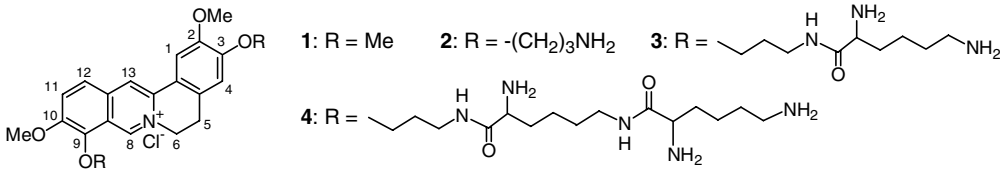
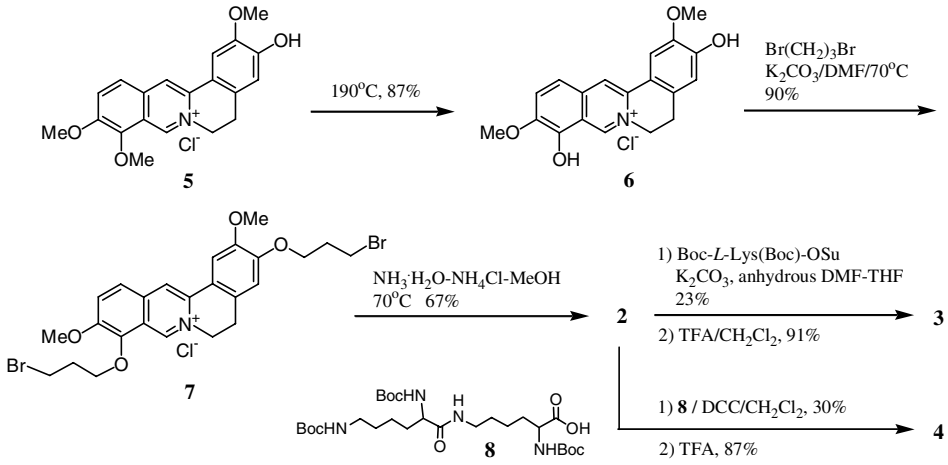


Chart 1.



Scheme 1.

of **2** with $N_\alpha N_\varepsilon$-di-Boc-L-lysine hydroxylsuccinimide ester (Boc-L-Lys(Boc)-OSu), followed by the deprotection (91%) by TFA, afforded **3**. Compound **4** was synthesized from the condensation (30%) of **2** with Boc-protected N^6-L-lysyl-L-lysine **8**[8] and subsequent deprotection (87%) by TFA. Compounds **2**–**4** were fully characterized by HRMS and NMR (^{1}H and ^{13}C).[9] They afforded HRMS spectra with the *m*/*z* values corresponding to $[M-Cl]^+$. Their NMR spectra were in full agreement with the given structures. The purity was judged from MS, ^{1}H NMR, and one spot on TLC developed by different eluting solvents.

The binding affinities of **2**–**4** toward calf thymus (CT) DNA were evaluated initially by means of ethidium bromide (EB) displacement experiments. EB strongly fluoresces upon intercalation into DNA duplexes and displacement from its DNA complex leads to a decrease in the fluorescence intensity. This fluorescence-based competition technique is commonly used to study the binding of polyaminonium compounds to DNA,[10] and can lead to the indirect determination of apparent binding constants (K_{app}). That is, K_{app} values can be estimated from the equation $K_{app} = K_{EB} \times [EB]_0/[B]_{50}$ in which K_{EB} is the association constant of EB with CT DNA and $[B]_{50}$ is defined as the compound concentration that generates a 50% decrease in the initial fluorescence intensity of the EB-CT DNA complex.[11] Figure 1 shows the plots of the relative fluorescence intensity (FI, I/I_0) of EB against the concentrations of added **2**–**4**, together with those of palmatine **1** and N^6-L-lysyl-L-lysine methyl amide (LysLys) for comparison. The obtained binding constants are listed in Table 1.

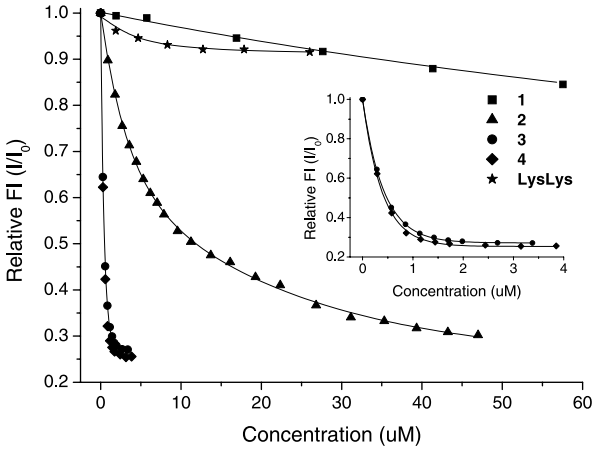


Figure 1. Fluorescence decrease of EB (5.04×10^{-6} M) induced by the competitive binding of **1**–**4** and LysLys to CT DNA (3.98×10^{-6} M) in 50 mM Tris–HCl buffer (pH 6.35, 0.05 mM EDTA) at room temperature, excitation 490 nm, emission 593 nm. Inset shows the data for **3** and **4** with an expanded *x*-axis.

In the measuring conditions, slight quenching was observed upon the addition of palmatine **1** or LysLys, indicating that these two compounds have low affinities toward CT DNA such that their $[B]_{50}$ values were not available. Therefore, the association constant of **1** with CT DNA was measured from spectrofluorimetric titration. LysLys was estimated to have comparable binding ability with **1** from their similar EB displacement patterns. However, **2**–**4** can efficiently substitute EB bound to CT DNA, indicating that they have significantly enhanced DNA-binding affinities. The binding ability of

Table 1. Apparent association constants (K_{app}'s, M^{-1}) and photophysical properties of **1**–**4** bound to CT DNA[a]

Compound	K_{app}[b]	Red shift[c] (nm)	Hypochromicity[c] (%)	Isosbestic point (nm)
1	8.72×10^3	3.0	14.6	351, 382, 437
2	2.81×10^5	5.5	43.3	368, 424, 480
3	1.08×10^7	2.5	44.4	379, 420, 502
4	1.15×10^7	2.5	63.5	376, 424, 499

[a] Measured in 50 mM Tris–HCl buffer (pH 6.35, 0.05 mM EDTA) at room temperature.

[b] Obtained from spectrofluorimetric titration for **1** and EB displacement experiments for **2**–**4**. The association constants of **2**–**4** were calculated from the equation $K_{app} = K_{EB} \times [EB]_0/[B]_{50}$. The association constant (K_{EB}) of EB with CT DNA was 6.26×10^5 M^{-1} in 50 mM Tris–HCl buffer (pH 6.35, 0.05 mM EDTA).

[c] Obtained at 343 nm. The concentrations of **1**–**4** were 4.42×10^{-6} M, 2.74×10^{-5} M, 1.09×10^{-5} M, and 5.52×10^{-6} M, respectively.

2 is at least 30-fold greater than that of **1**. Compounds **3** and **4** exhibit further enhanced DNA binding with the affinities being over 1200- and 1300-fold greater than that of **1**, respectively. These results clearly suggest that protoberberine subunit and the appending polyamino groups cooperatively participate in DNA binding. More significantly, the DNA-binding abilities of **3** and **4** reach a level of 0.1 μM, comparable to those of some natural antibiotics. Thus, it is clear that **2**–**4** act as very effective DNA binders and that the moderate binding abilities of protoberberine alkaloids can be largely amplified by attaching two or more primary amino groups. These results can be rationalized by taking in account the structural characteristics inherent in **2**–**4**. Under the assay conditions (pH 6.35), all the primary amino groups in **2**–**4** should exist in *protonated* forms, thus *multivalent cooperative interactions* (e.g., hydrogen bonding and electrostatic forces) between negatively charged CT DNA and positively charged multiple amino groups are maximized to afford strong binding. It should be noted that **4** bearing six amino groups is only slightly more effective in DNA binding than **3** bearing four amino groups. This may be related to the spatial orientation of multiple amino groups.

The interactions of **2**–**4** with CT DNA were also monitored by spectrophotometric titrations. It is observed that the addition of CT DNA to the solutions of these compounds resulted in large hypochromicities and bathochromic shifts (Fig. 2 and Table 1). These spectroscopic variations provide unambiguous evidences that **2**–**4** form stable complexes with CT DNA. On the other hand, during the titrations with CT DNA, three well-resolved isosbestic points were observed, revealing the existence of one preferential, almost exclusive binding mode. It is noteworthy that during the spectrophotometric titrations of **3** and **4** with CT DNA, some 'visible' particles were observed when these two compounds were set at the same concentrations with **2**. These particles are thought to be due to the formation of assembly with CT DNA.

In summary, three polyamine-appending protoberberine derivatives have been synthesized and characterized by HRMS and NMR. These compounds show quite high DNA-binding affinities as a result of the multivalent interactions of the polyamino groups with DNA, and thus are exploitable as effective DNA-binding agents. Further efforts aiming at their potential applications, for example, in DNA protection and compaction, are continuing in our labs, which will be reported in due course.

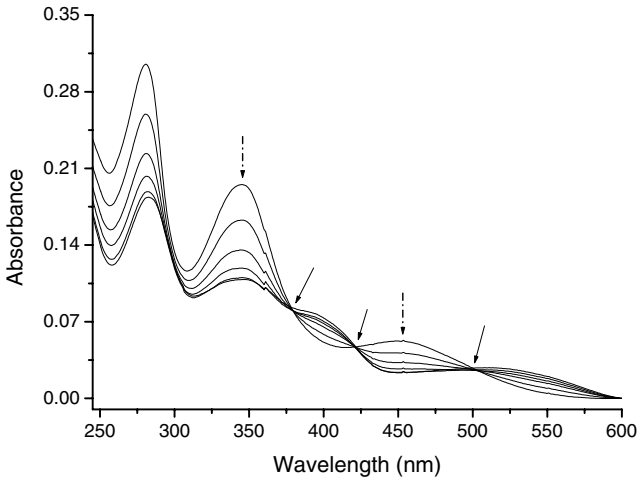


Figure 2. Spectrophotometric titration of **3** (1.09×10^{-5} M) with CT DNA of increasing concentrations (0–3.73×10^{-5} M) in 50 mM Tris–HCl buffer (pH 6.35, 0.05 mM EDTA) at room temperature. The dash–dot arrows indicate the decreasing absorption bands during the course of titration; solid arrows indicate the isosbestic points.

Acknowledgments

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9. Selected structural data for **2**: ^{1}H NMR (CD_3OD, 300 MHz) δ 1.89–1.98 (m, 2H), 2.02–2.10 (m, 2H), 2.97 (t, J = 6.6 Hz, 2H), 3.25 (t, J = 6 Hz, 2H), 3.72 (t, J = 6.6 Hz, 2H), 3.78 (t, J = 6 Hz, 2H), 3.99 (s, 3H), 4.04 (s, 3H), 4.20 (t, J = 6 Hz, 2H), 4.84 (t, J = 6 Hz, 2H), 7.01 (s, 1H), 7.59 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 8.58 (s, 1H), 9.75 (s, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 151.32, 149.75, 148.30, 145.26, 137.77, 136.52, 133.71, 128.42, 123.12, 119.97, 119.55, 117.91, 117.66, 112.26, 108.61, 67.58, 33.22, 56.37, 55.95, 59.24, 45.68, 38.73, 30.74, 29.71, 27.00; HR-MALDI-TOFMS for $C_{25}H_{32}N_3O_4$ ($[M-Cl^-]^+$) Calcd: 438.2392. Found: 438.2412. **3**: ^{1}H NMR (CD_3OD, 300 MHz) δ 1.45–1.53 (m, 4H), 1.65–1.75 (m, 4H), 1.84–1.95 (m, 4H), 2.04–2.12 (m, 4H), 2.86–2.92 (m, 4H), 3.23–3.28 (m, 2H), 3.44–3.52 (m, 2H), 3.70 (t, J = 6.0 Hz, 2H), 3.86 (t, J = 6.0 Hz, 2H), 3.98 (s, 3H), 4.04 (s, 3H), 4.18 (t, J = 6.0 Hz, 2H), 4.37–4.44 (m, 2H), 4.84 (m, 2H, overlapped), 7.01 (s, 1H), 7.63 (s, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 8.62 (s, 1H), 9.70 (s, 1H);^{13}C NMR (CD_3OD, 125 MHz) δ 170.56, 170.07, 152.70, 151.03, 149.95, 147.06, 138.20, 137.89, 135.31, 129.74, 125.37, 121.26, 120.81, 120.05, 119.72, 113.44, 110.02, 67.88, 65.38, 57.52, 57.07, 54.32, 53.76, 46.45, 45.39, 40.24, 37.89, 33.04, 32.05, 31.01, 30.70, 29.93, 28.10, 27.99, 23.12, 22.94, 22.80; HR-MALDI-TOFMS for $C_{37}H_{56}N_7O_6$ ($[M-Cl^-]^+$) Calcd: 694.4292. Found: 694.4332. **4**: ^{1}H NMR (CD_3OD, 300 MHz) δ 1.42–1.49 (m, 4H), 1.54–1.61 (m, 4H), 1.66–1.73 (m, 4H), 1.78–1.98 (m, 12H), 2.06–2.12 (m, 4H), 2.92–2.96 (m, 4H), 3.31 (m, 2H, overlapped), 3.44–3.57 (m, 2H), 3.69–3.80 (m, 8H), 3.84 (t, J = 6.0 Hz, 2H), 3.98 (s, 3H), 4.06 (s, 3H), 4.18 (t, J = 6.0 Hz, 2H), 4.86 (m, 2H, overlapped), 7.02 (s, 1H), 7.64 (s, 1H), 7.65 (d, J = 9.0 Hz, 1H), 7.88 (d, J = 9.0 Hz, 1H), 8.61 (s, 1H), 9.75 (s, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.20, 172.62, 155.16, 153.51, 152.39, 149.62, 140.42, 137.80, 132.30, 127.87, 123.77, 123.33, 122.50, 122.18, 116.00, 112.55, 81.95, 76.36, 70.43, 67.08, 66.93, 60.08, 59.69, 57.02, 56.79, 56.50, 48.94, 47.91, 42.78, 42.63, 40.39, 36.36, 35.52, 34.59, 33.99, 33.62, 33.43, 33.20, 32.91, 32.25, 30.54, 28.57, 26.19, 25.94, 25.75, 25.47; HR-MALDI-TOFMS for $C_{49}H_{80}N_{11}O_8$ ($[M-Cl^-]^+$) Calcd: 950.6191. Found: 950.6177.
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