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Synthesis of linked berberine dimers and their remarkably enhanced DNA-binding affinities

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Abstract—This communication describes the facile synthesis of five novel berberine dimers and their strong affinities toward double-stranded DNA. These berberine dimers were synthesized in 37–84% yields from the reaction of berberrubine with dihaloalkanes of varying lengths, and fully characterized by HRMS and ^{1}H NMR. Compared with the monomeric parent berberine, these dimers showed greatly enhanced binding affinities up to approximately 100-fold, with two double helical oligodeoxynucleotides, $d(AAGAATTCTT)_2$ and $d(TAAGAATTCTTA)_2$, which was investigated by means of fluorescence spectrometry.

Much interest is currently focused on the development of efficient DNA-binding agents with improved binding affinities and site specificities toward target DNA, because such agents have potentially wide applications, for example, in elucidating the action mechanism of antitumor and antivirus drugs[1] and in developing new chemotherapeutic agents.[2] One sophisticated approach to obtain more effective DNA-binding candidate compounds is the modification of natural products whose binding affinities and modes with DNA have been well established. A typical example of this is the rational structural modification of the antibiotics netropsin and distamycin leading to the development of lexitropsins.[3]

In these aspects, berberine, a typical naturally-occurring protoberberine isoquinoline alkaloid, is attractive as a versatile platform for the development of more efficient DNA-binding agents, because berberine is known as a DNA binder and its binding affinities have been extensively explored by several analytical techniques including absorption, fluorescence, NMR, and electrospray ionization mass (ESI-MS) spectrometries,[4] though its binding modes as a groove binder or an intercalator remain controversial. The binding affinities of berberine, usually in the range of $1.0–2.0 \times 10^4\ M^{-1}$ with duplex DNA, are modest and necessitate the appropriate structural modification as to serve as a novel DNA-binding agents with enhanced binding affinities. In this paper, we describe the remarkably enhanced DNA-binding affinities of berberine dimers **3a–e** (Scheme 1) synthesized in moderate to high yields from the linkage of berberrubine **2**[5] with alkyl chains of varying lengths, aiming at developing more effective protoberberine alkaloid-based DNA-binding agents.

Berberine dimers **3a–e** were synthesized as described in Scheme 1. Pyrolysis of berberine **1** at 190 °C under vacuum, according to the reported protocols,[5] gave berberrubine **2** in 60% yield. Coupling of **2** with dibromo- or diiodoalkanes of varying lengths from two to six carbons in DMF, followed by the exchange of all anions into chloride, afforded corresponding berberine dimers **3a–e** in 37–84% yields. The structures of compounds **3a–e** were confirmed by HR-ESI-MS and NMR.[6] All these compounds afforded the two-charged ESI-MS peaks ($[M-2Cl]^{2+}$) in the mass spectra. The ^{1}H NMR spectra of these compounds, for example, **3a** in Figure 1, showed only one set of berberine subunits, indicating the formation of a symmetric structure. The ratios of the integrated areas for the protons of berberine subunits to those for the alkyl chain protons in the ^{1}H NMR spectra

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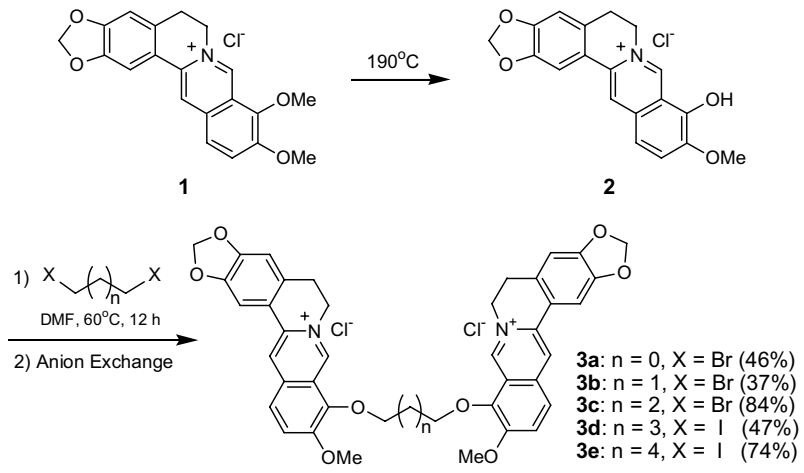

Scheme 1. Synthetic route for berberine dimers **3a**–**e**.

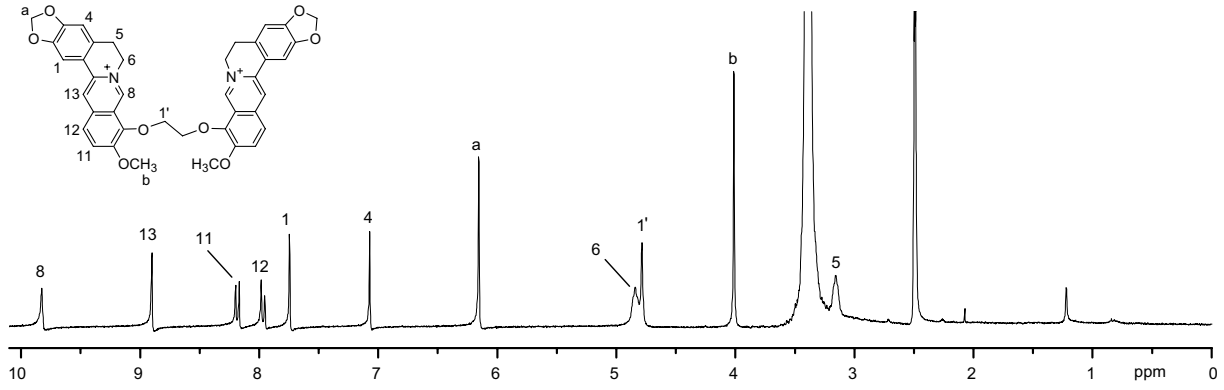

Figure 1. ^{1}H NMR (300 MHz) spectrum of **3a** in DMSO-d_6.

were in full accord with the linked berberine dimeric structures.

The binding affinities of **3a**–**e** toward two self-complementary double-stranded oligodeoxynucleotides, d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$, were evaluated by means of fluorescence spectrometry. Figure 2 shows the fluorescence titration spectra of **3b** with d(TAAGAATTCTTA)$_2$. It is observed that the weak fluorescence of **3b** was largely enhanced upon the addition of DNA, suggesting the interaction of **3b** with DNA added. The 1:1 binding stoichiometry between **3b** and d(TAAGAATTCTTA)$_2$ was determined by molar ratio methods (inset in Fig. 2). Analyses of the relationship between the fluorescence intensities and the DNA concentrations by nonlinear curve fitting methods[7] afforded the association constants (K_a's) of **3a**–**e** with d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$ (Fig. 3 and Table 1). For comparison, the binding constants of **1** with the two oligodeoxynucleotide duplexes were measured in a similar way. The binding affinities of **3a**–**e** relative to **1** with d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$ are illustrated in Figure 4.

Some interesting observations can be extracted from Table 1 and Figure 4. The first observation is that linked

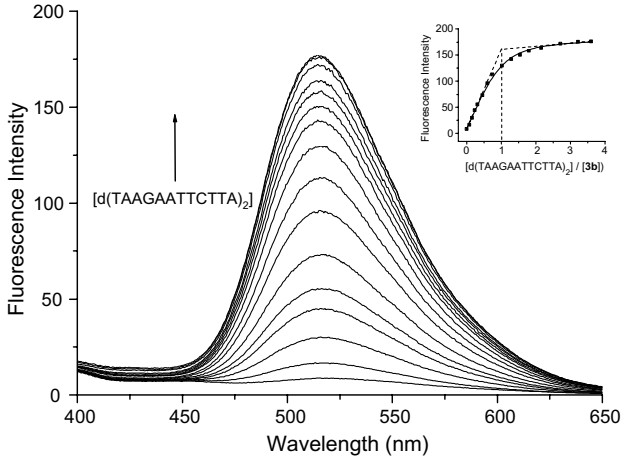

Figure 2. Fluorescence spectra of **3b** (2.47×10^{-6} M) with d(TAAGAATTCTTA)$_2$ of increasing concentrations (0–8.89×10^{-6} M) in 50 mM Tris–HCl (pH 6.35) at room temperature, ex 355 nm. The inset indicates the relationship between the fluorescence intensities (at em 516.7 nm) and the molar ratio of d(TAAGAATTCTTA)$_2$ to **3b**.

berberine dimers **3a**–**e** exhibited much higher affinities than their monomeric parent compound **1**. It can be seen that **3a**–**e** bound to d(AAGAATTCTT)$_2$ at least

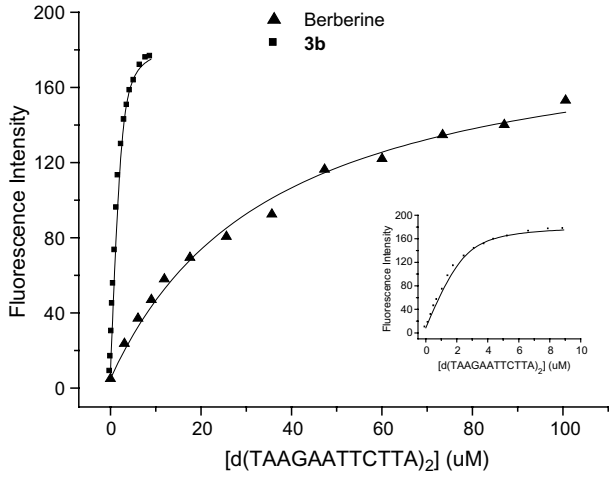

Figure 3. Fluorescence titrations of **1** (2.08×10^{-6} M) and **3b** (2.47×10^{-6} M) with $d(TAAGAATTCTTA)_2$ in 50 mM Tris–HCl (pH 6.35) at room temperature, ex 355 nm, em 516.7 nm. Inset shows data for **3b** with an expanded *x*-axis.

Table 1. Association constants (K_a's, M^{-1}) of **3a–e** with $d(AAGAATTCTT)_2$ and $d(TAAGAATTCTTA)_2$[a]

Compound	$d(AAGAATTCTT)_2$		$d(TA\ AGAATTCTTA)_2$	
	K_a	RA[b]	K_a	RA[b]
1	$(1.24 \pm 0.12) \times 10^4$	1	$(2.93 \pm 0.31) \times 10^4$	1
3a	$(1.18 \pm 0.10) \times 10^5$	9.5	$(1.62 \pm 0.35) \times 10^6$	54.9
3b	$(2.46 \pm 0.07) \times 10^5$	19.8	$(2.76 \pm 0.37) \times 10^6$	94.2
3c	$(8.78 \pm 0.26) \times 10^4$	7.1	$(3.49 \pm 0.60) \times 10^5$	11.9
3d	$(5.77 \pm 0.13) \times 10^4$	4.7	$(3.21 \pm 0.20) \times 10^5$	11.0
3e	$(3.42 \pm 0.07) \times 10^4$	2.8	$(2.48 \pm 0.34) \times 10^5$	8.5

[a] In 50 mM Tris–HCl (pH 6.35) at room temperature.
[b] RA denotes relative affinity.

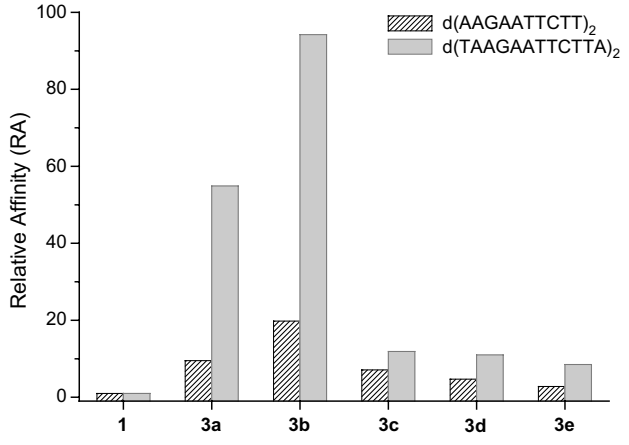

Figure 4. Relative binding affinities of **3a–e** with $d(AAGAATTCTT)_2$ and $d(TAAGAATTCTTA)_2$ in 50 mM Tris–HCl (pH 6.35) at room temperature.

2–20 times and to $d(TAAGAATTCTTA)_2$ at least 10–90 times more strongly than **1**. More significantly, the binding constant of **3b** with $d(TAAGAATTCTTA)_2$ did not only increase by a factor of 94 relative to **1**, but was comparable with those of some natural antibiotics. This was further supported by the ESI-MS results on the comparison of the binding affinities of dimers **3a–e** and berberine **1**. This great increase in the binding affinities may be ascribed to the cooperative interactions of the two berberine subunits in **3a–e**. Secondly, compounds **3a–e** showed a prominent structure–activity correlation and their binding abilities with both duplex DNA could be modulated by the lengths of the alkyl chains. Five dimers **3a–e** showed similar relative binding affinities toward both oligodeoxynucleotide duplexes in the order of **3b > a > c > d > e**. Dimer **3b** linked by a propyl chain exhibited the highest affinity in both cases, indicating that propyl chain may be the most suitable linker to bridge the two berberine units. This result may guide future rational design efforts. Thirdly, compounds **3a–e** showed higher affinities with 12-mer $d(TAAGAATTCTTA)_2$ than with 10-mer $d(AAGAATTCTT)_2$. For example, the binding constants of **3a–b** with $d(TAAGAATTCTTA)_2$ were over 10-fold greater than with $d(AAGAATTCTT)_2$. This result suggested that dimeric berberines would necessarily occupy a greater number of base pairs,[8] with potentially more stringent sequence recognition relative to the monomeric berberine.

In summary, five novel spacer-linked berberine dimers have been successfully synthesized in moderate to high yields. These berberine dimers show much higher DNA-binding affinities than their monomeric parent berberine. Great efforts are being currently made to systematically explore the DNA-binding mechanisms and sequence selectivities of these linked berberine dimers.

Acknowledgements

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6. Structural data for compound **3a**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.17 (s, 4H, 5-H), 4.02 (s, 6H, OCH_3), 4.79 (s, 4H, OCH_2), 4.85 (s, 4H, 6-H), 6.16 (s, 4H, OCH_2O), 7.08 (s, 2H, 4-H), 7.75 (s, 2H, 1-H), 7.98 (d, 2H, J = 9.6 Hz, 12-H), 8.19 (d, 2H, J = 9.6 Hz, 11-H), 8.91 (s, 2H, 13-H), 9.83 (s, 2H, 8-H); HRMS for $C_{40}H_{34}N_2O_8$ ($[M-2Cl]^{2+}$) calcd: 335.1152. Found: 335.1129. Compound **3b**: ^{1}H NMR (CD_3OD–$CDCl_3$, 300 MHz,) δ 2.65 (m, 2H, CH_2CH_2O), 3.23 (t, 4H, J = 6.3 Hz, 5-H), 4.09 (s, 6H, OCH_3), 4.76 (t, 4H, OCH_2CH_2), 4.92 (t, 4H, 6-H), 6.09 (s, 4H, OCH_2O), 6.91 (s, 2H, 4-H), 7.59 (s, 2H, 1-H), 7.98 (d, 2H, J = 9.0 Hz, 12-H), 8.07 (d, 2H, J = 9.0 Hz, 11-H), 8.65 (s, 2H, 13-H), 9.87 (s, 2H, 8-H); HRMS for $C_{41}H_{36}N_2O_8$ ($[M-2Cl]^{2+}$) calcd: 342.1231. Found: 342.1213. Compound **3c**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.14 (s, 4H, CH_2CH_2O), 3.15–3.24 (m, 4H, 5-H), 4.04 (s, 6H, OCH_3), 4.40 (t, 4H, J = 5.6 Hz, OCH_2CH_2), 4.93 (s, 4H, 6-H), 6.17 (s, 4H, OCH_2O), 7.08 (s, 2H, 4-H), 7.78 (s, 2H, 1-H), 7.98 (d, 2H, J = 9.0 Hz, 12-H), 8.19 (d, 2H, J = 9.0 Hz, 11-H), 8.92 (s, 2H, 13-H), 9.78 (s, 2H, 8-H); HRMS for $C_{42}H_{38}N_2O_8$ ($[M-2Cl]^{2+}$) calcd: 349.1309. Found: 349.1318. Compound **3d**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.65–1.80 (m, 2H, $CH_2CH_2CH_2O$), 1.99 (m, 4H, $CH_2CH_2CH_2O$), 3.15–3.23 (m, 4H, 5-H), 4.04 (s, 6H, OCH_3), 4.32 (t, 4H, J = 6.3 Hz, OCH_2CH_2), 4.93 (t, 4H, J = 6.4 Hz, 6-H), 6.16 (s, 4H, OCH_2O), 7.07 (s, 2H, 4-H), 7.75 (s, 2H, 1-H), 7.97 (d, 2H, J = 8.7 Hz, 12-H), 8.18 (d, 2H, J = 8.7 Hz, 11-H), 8.90 (s, 2H, 13-H), 9.75 (s, 2H, 8-H); HRMS for $C_{43}H_{40}N_2O_8$ ($[M-2Cl]^{2+}$) calcd: 356.1387. Found: 356.1385. Compound **3e**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.60 (s, 4H, $CH_2CH_2CH_2O$), 1.90–2.00 (m, 4H, $CH_2CH_2CH_2O$), 3.15–3.23 (m, 4H, 5-H), 4.03 (s, 6H, OCH_3), 4.29 (t, 4H,J = 5.8 Hz, OCH_2CH_2), 4.92 (t, 4H, J = 6.9 Hz, 6-H), 6.16 (s, 4H, OCH_2O), 7.07 (s, 2H, 4-H), 7.75 (s, 2H, 1-H), 7.97 (d, 2H, J = 9.3 Hz, 12-H), 8.18 (d, 2H, J = 9.3 Hz, 11-H), 8.90 (s, 2H, 13-H), 9.73 (s, 2H, 8-H); HRMS for $C_{44}H_{42}N_2O_8$ ($[M-2Cl]^{2+}$) calcd: 363.1471. Found: 363.1504.
7. Association constants are derived from nonlinear curve fitting, using the equation $I = I_0 + ((I_\infty - I_0)/2[B]_0)\ \{([DNA]_0 + [B]_0 + 1/K_a) - (([DNA]_0 + [B]_0 + 1/K_a)^2 - 4[DNA]_0[B]_0)^{1/2}\}$, wherein $[DNA]_0$ and $[B]_0$ are the initial concentrations of DNA and **3a**–**e**, respectively. I, I_0, and I_∞ represent the fluorescence intensities of the sample, **3a**–**e** alone and the intensity when **3a**–**e** are totally bound, respectively. For reference, see: (a) Schneider, H.-J.; Yatsimirski, A. K. *In Principles and Methods in Supramolecular Chemistry*; J Wiley: New York, 2000, pp 137–143; (b) Xi, Z.; Jones, G. B.; Qabaja, G.; Wright, J.; Johnson, F.; Goldberg, I. H. *Org. Lett.* **1999**, *1*, 1375–1377.
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