Supporting Information

Berberine Molecular Recognition of the Parallel MYC G-Quadruplex in Solution

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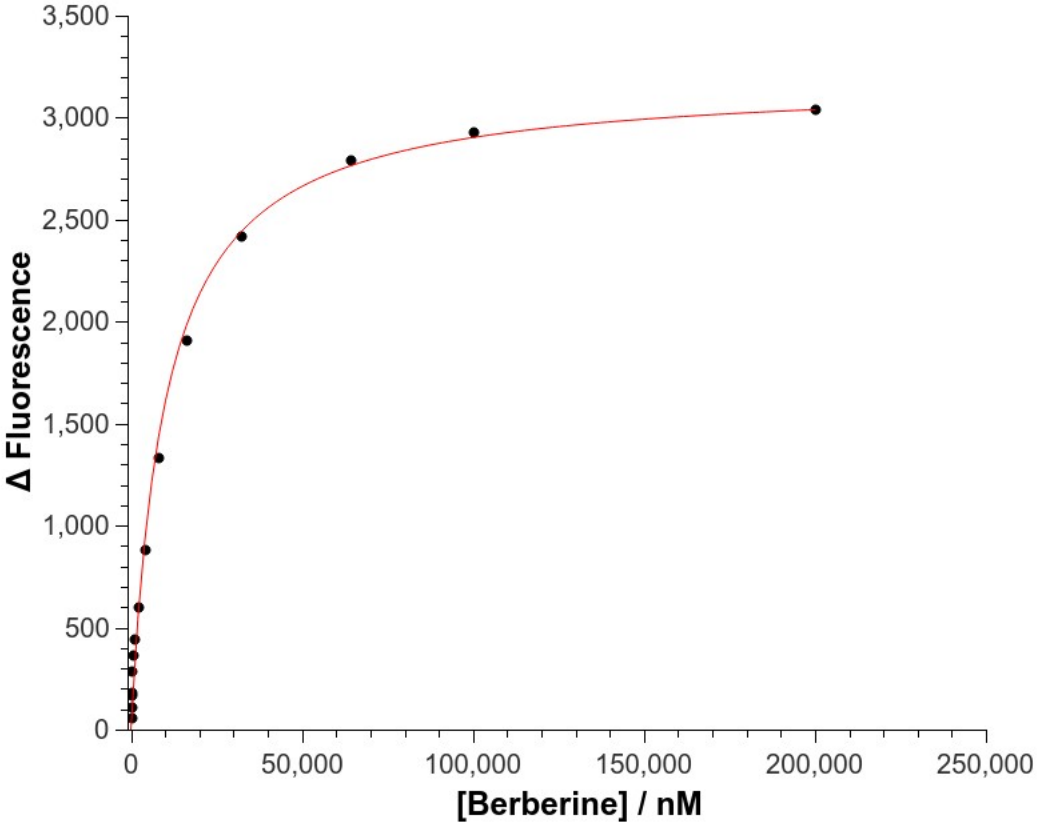

Figure S1. Determination of the apparent dissociation constant $K_{d,app}$ describing the binding affinity of berberine to the MycG4 using a fluorescence assay. Fluorescence intensity change of a 3'-Cy5-labeled MycG4 upon berberine addition was measured and plotted over the berberine concentration. The concentration of the labeled MycG4 was 20 nM in 100 mM K^+ buffer, pH 7, 20 °C. λ_{ex}= 640 nm, λ_{em}= 659 nm

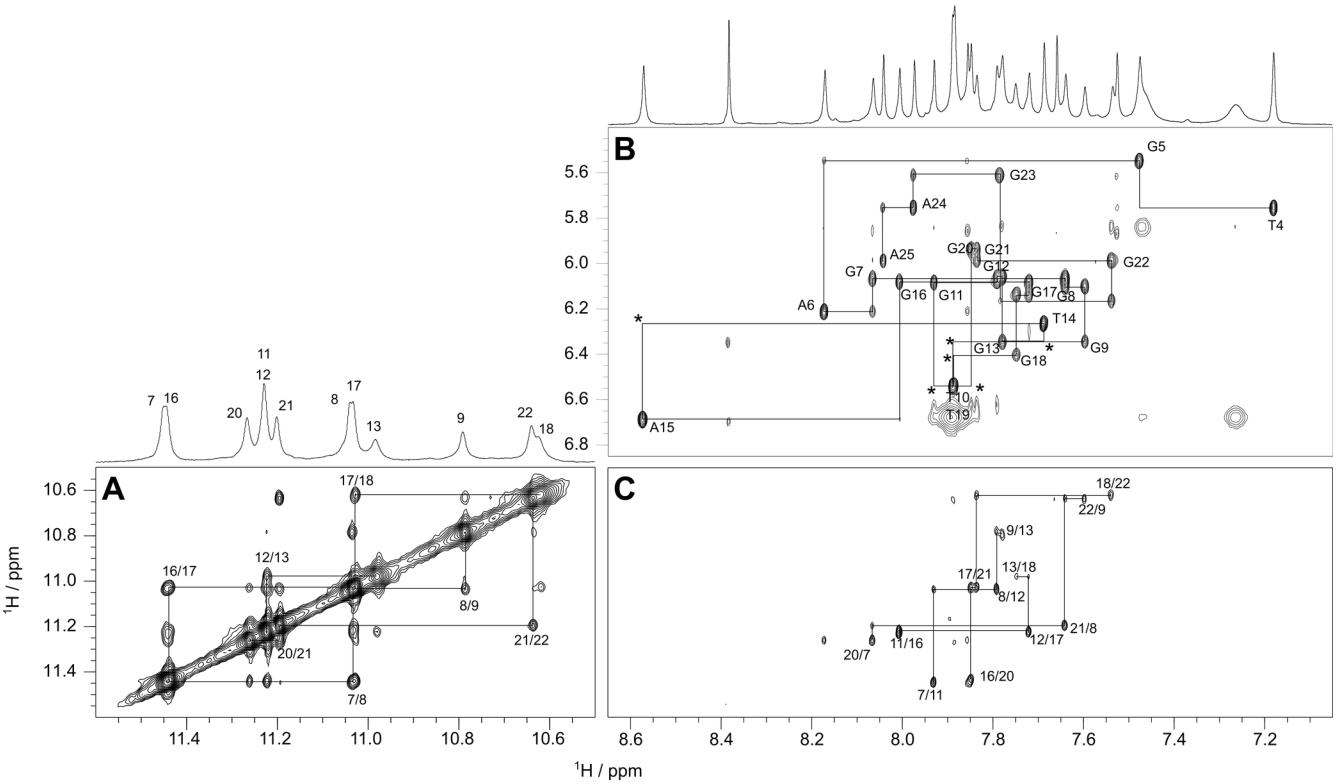

Figure S2. 2D NOE spectral regions of the 2:1 berberine-MycG4 complex acquired at 25 °C with a mixing time of 300 ms showing (**A**) sequential imino-imino cross peaks along the parallel G-stretches, (**B**) sequential H8-H1' contacts traced by solid lines (missing NOE cross peaks are marked with an asterisk), and (**C**) guanine H8/H6–imino cross peaks showing the intra-tetrad and inter-tetrad guanine connectivities indicated by solid lines. Spectrum acquired on an 800 MHz spectrometer in 10 mM K^+ buffer, pH 7, with 1.2 mM DNA and a 3:1 ligand-DNA ratio.

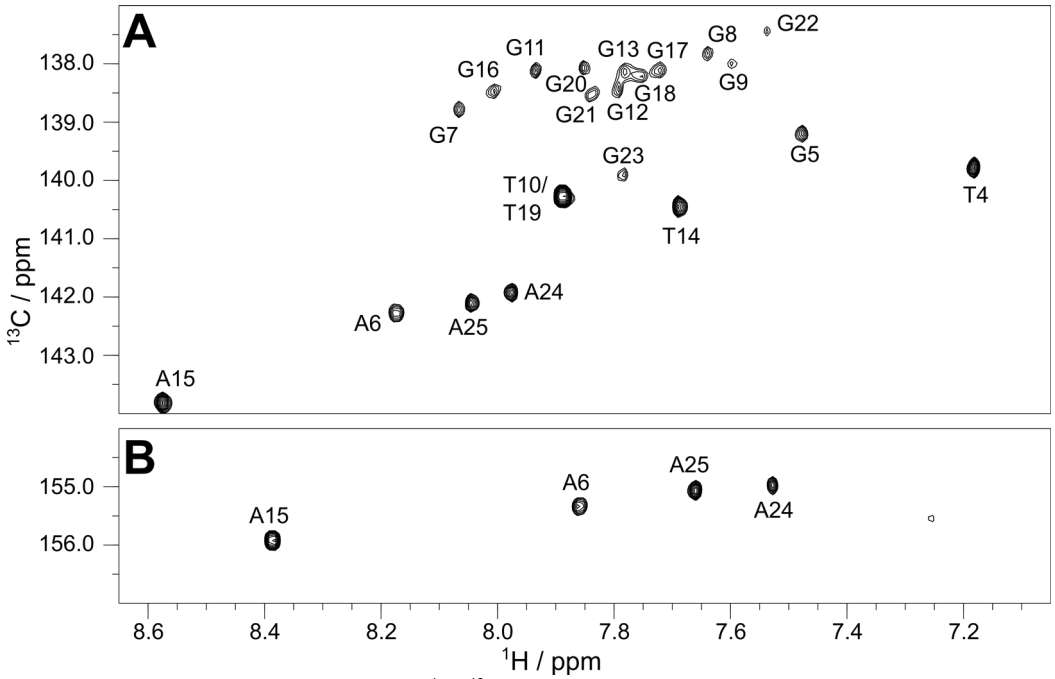

Figure S3. Spectral regions of the 2D ^{1}H−^{13}C HSQC of the 2:1 berberine-MycG4 complex at 25 °C showing the (**A**) H6−C6/H8−C8 peaks for all bases and the (**B**) adenine H2−C2 contacts. Spectrum acquired on an 800 MHz spectrometer in 10 mM K^{+} buffer, pH 7, with a 1.2 mM DNA and a 3:1 ligand-DNA ratio.

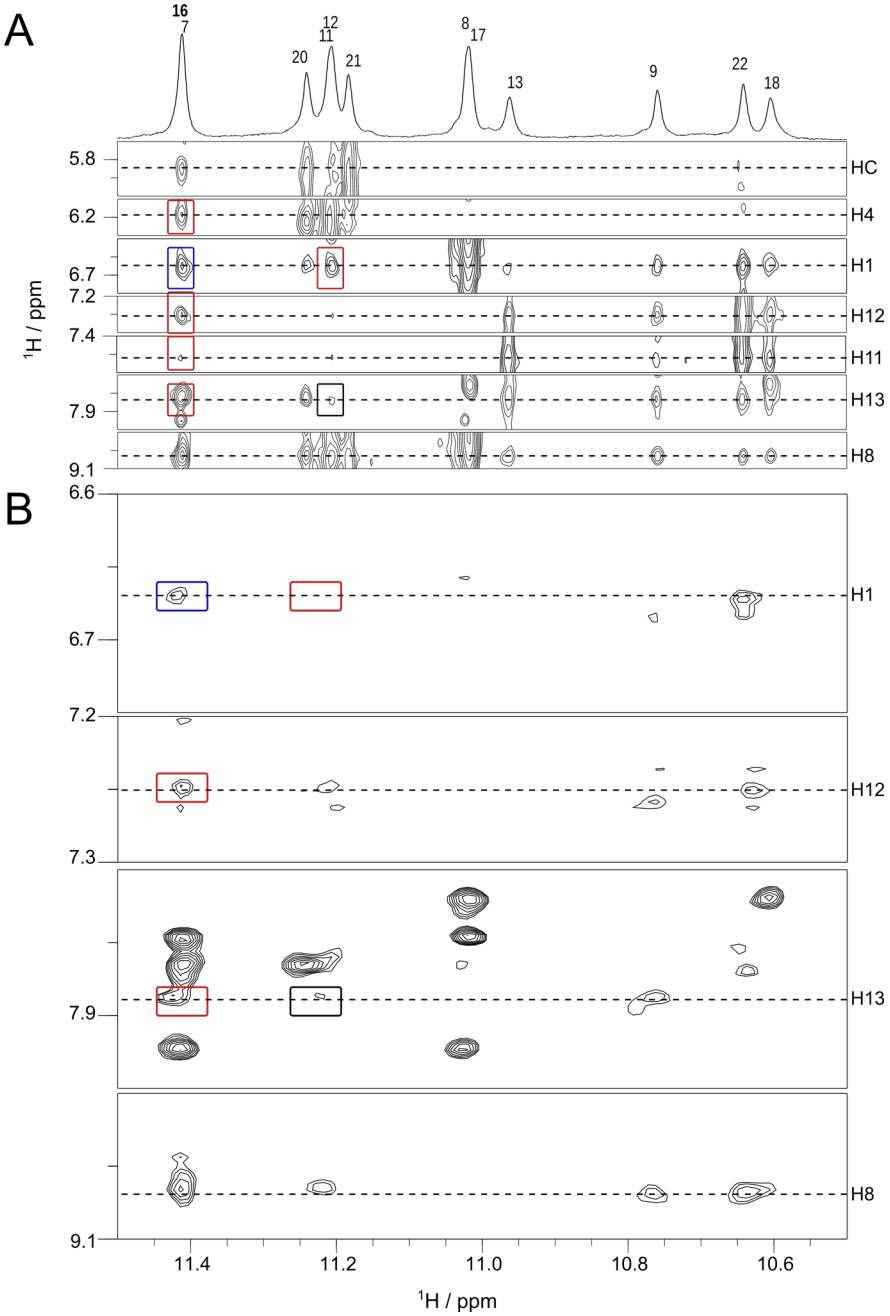

Figure S4. Additional intermolecular NOE contacts between MycG4 and berberine involving the DNA imino protons. Both symmetric spectral regions (A) above and (B) below the diagonal are shown. Measured on an 800 MHz spectrometer at a berberine-MycG4 ratio of 3:1 with 1.2 mM DNA and a mixing time of 300 ms in presence of 10 mM K^+, pH 7, 35 °C. The berberine contacts, which were used for structure determination, are boxed in red, blue, or black for conformation A, B, or both, respectively.

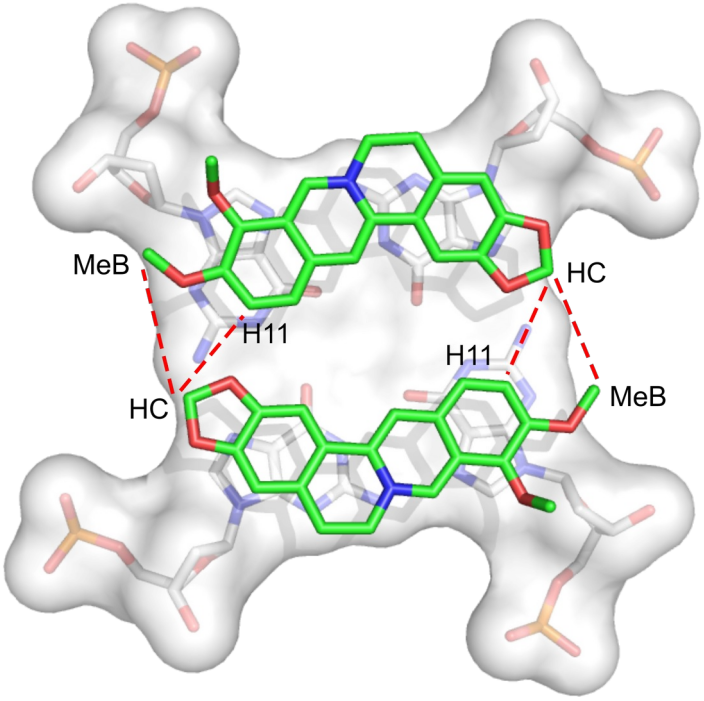

Figure S5. X-ray crystal structure of a berberine-telomeric G4 complex (PDB: 3R6R).[13] Berberine binds as a dimer at both ends of the parallel G4 formed by the human telomeric sequence. The berberine dimer (head-to-tail) at the 3'-end is shown and the observed NOE (red lines) indicate the formation of a similar dimer at the 3'-end of the MycG4.

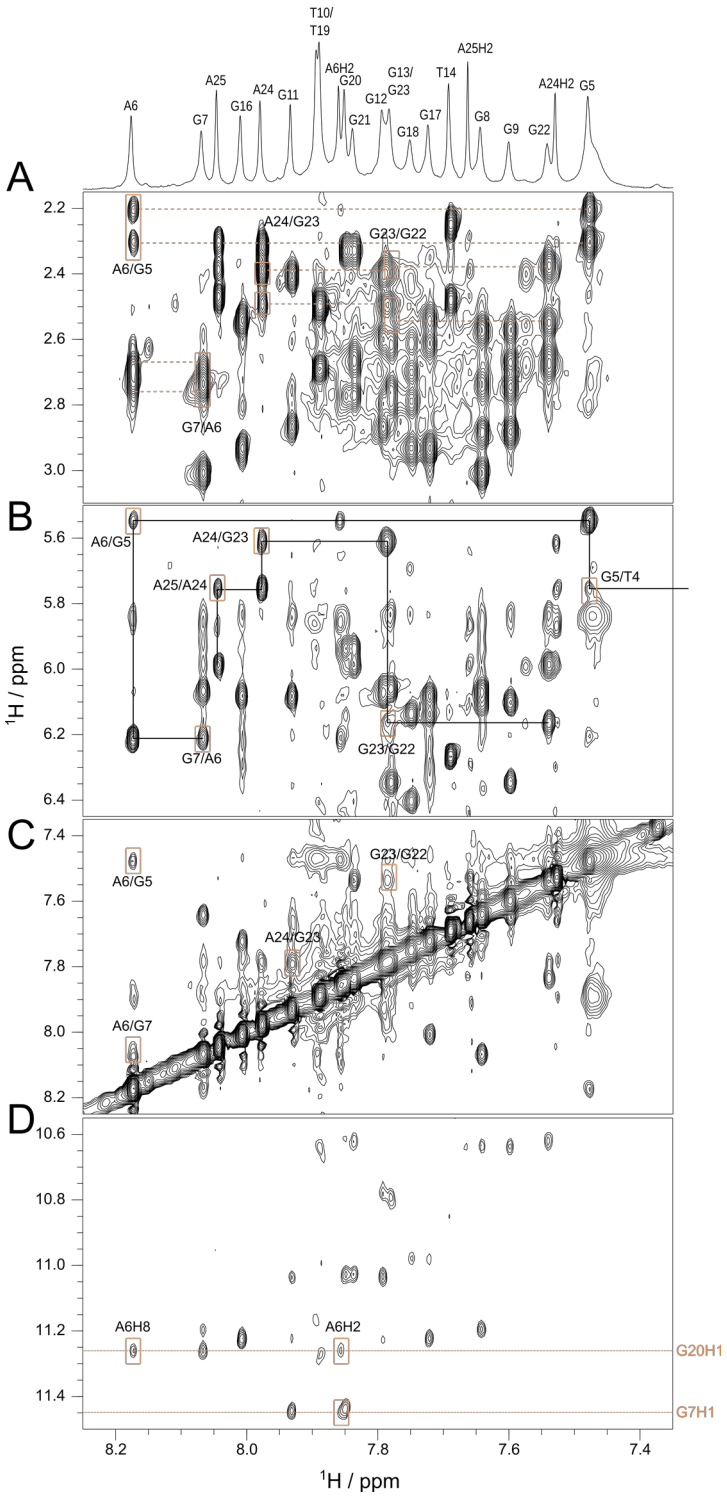

Figure S6. 2D NOE spectral regions of the 2:1 berberine-MycG4 complex defining the position of the recruited residues A6 and G23. (A) Sequential H8/H2' and H8/H2'' contacts. (B) Sequential H8/H1' contacts. (C) Sequential H8-H8 contacts. (D) Interactions of A6-H8 and H2 protons with imino protons from the 5'-tetrad. Acquired on an 800 MHz spectrometer at 25 °C with a mixing time of 300 ms in 10 mM K^+ buffer, pH 7, with 1.2 mM DNA and a 3:1 ligand-DNA ratio.

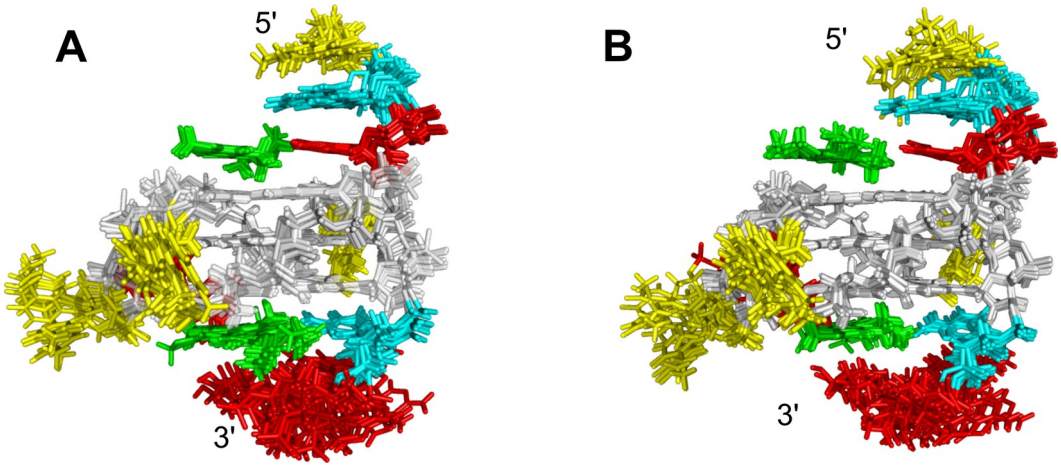

Figure S7. Superposition of the 10 lowest energy structures for (**A**) conformer A (PDB: 7N7D) and (**B**) conformer B (PDB: 7N7E) of berberine in complex with the MycG4.

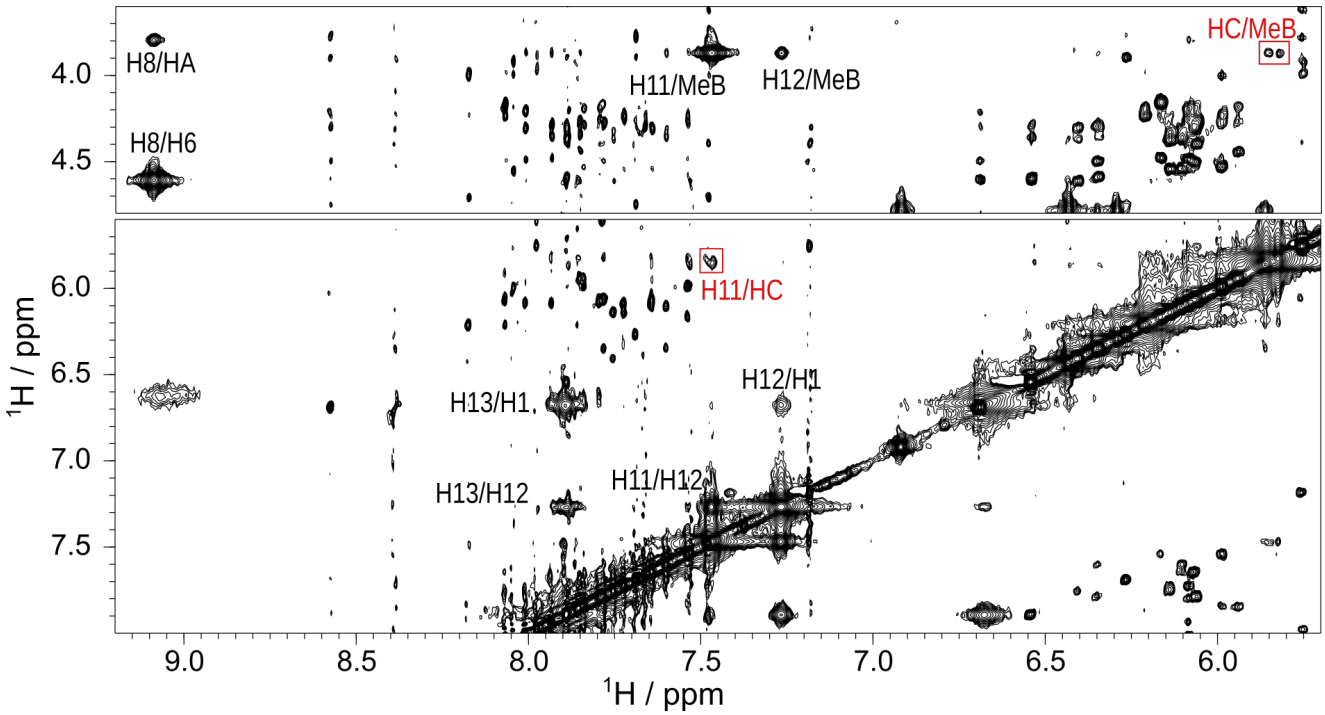

Figure S8. 2D NOE spectral regions of the 2:1 berberine-MycG4 complex acquired at 25 °C with a mixing time of 80 ms showing selected berberine-berberine cross peaks within a monomer (black) and within a dimer (red). Spectrum acquired n an 800 MHz spectrometer in 10 mM K^+ buffer, pH 7, and a 3:1 ligand-DNA ratio with 1.2 mM DNA.

Table S1. ^{1}H and C8/C6 ^{13}C chemical shifts (ppm) of the 2:1 berberine-MycG4 quadruplex complex.[a]

	imino	H8/H6	H2/Me	H1'	H2'/H2''	H3'	H4'	H5'/H5''[b]	C6/C8
T4	---	7.18	1.62	5.75	1.51/1.97	4.39	3.78	3.43/3.43	139.79
G5	---	7.48	---	5.55	2.30/2.21	4.71	4.00	3.62/3.74	139.21
A6	---	8.17	7.86	6.21	2.69/2.74	4.96	4.24	3.97/4.01	142.28
G7	11.44	8.07	---	6.07	2.74/3.01	5.01	4.50	4.18/4.23	138.79
G8	11.04	7.64	---	6.10	2.57/2.88	5.02	4.54	4.30/4.32	137.83
G9	10.79	7.60	---	6.34	2.75/2.67	5.18	4.59	4.315/4.35	138.00
T10	---	7.89	2.02	6.54	2.50/2.69	5.11	4.61	4.30/4.36	140.27
G11	11.22	7.93	---	6.08	2.41/2.87	5.09	4.48	4.29/4.35	138.12
G12	11.22	7.79	–-	6.06	2.60/2.80	5.05	4.39	4.18/4.26	138.43
G13	10.98	7.78	---	6.35	2.76/2.63	5.06	4.50	4.26/4.28	138.13
T14	---	7.69	1.96	6.26	2.25/2.48	4.75	3.90	3.76/3.79	140.45
A15	---	8.57	8.38	6.69	3.10/2.96	5.19	4.61	4.215/4.31	143.81
G16	11.44	8.01	---	6.08	2.54/2.93	5.01	4.49	4.21/4.30	138.47
G17	11.03	7.72	---	6.14	2.62/2.93	5.03	4.54	4.22/4.25	138.10
G18	10.62	7.75	---	6.40	2.79/2.70	5.18	4.61	4.33/4.37	138.21
T19	---	7.89	2.02	6.54	2.50/2.69	5.11	4.59	4.31/4.38	140.27
G20	11.26	7.85	---	5.94	2.33/2.77	5.08	4.44	4.26/4.34	138.08
G21	11.20	7.84	---	5.99	2.66/2.68	5.09	4.53	4.18/4.28	138.52
G22	10.64	7.54	---	6.16	2.38/2.55	5.02	4.48	4.23/4.28	137.45
G23	---	7.79	---	5.61	2.49/2.39	4.90	4.37	4.16/4.18	139.89
A24	---	7.98	7.53	5.75	2.32/2.39	4.79	3.98	3.89/3.96	141.92
A25	---	8.04	7.66	5.99	2.47/2.30	4.55	4.00	3.91/4.00	142.11

[a]In 90% H_2O/10% D_2O, 10 mM potassium phosphate buffer, pH 7.0, 3:1 berberine-MycG4 ratio, at 25 °C. [b]No stereospecific assignments.

Table S2. ^{1}H chemical shifts (ppm) of berberine in the 2:1 Ber-MycG4 complex in 90% H_2O/10% D_2O, 10 mM potassium phosphate buffer, pH 7.0, 3:1 Ber-MycG4 ratio at 25 °C.

	H1	H4	H5[a]	H6[a]	H8	H11	H12	H13	HC[a]	MeA	MeB
Ber	6.68	6.20	2.74/2.80	4.61/4.61	9.09	7.47	7.27	7.89	5.83/5.86	3.80	3.87

[a]No stereospecific assignments.

Table S3. Sequential and long-range intramolecular NOE interactions of MycG4 in the 2:1 complex with berberine involving the 5'-terminal residues T4-A6.[a]

T4		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**	**Me**	**H6**
G5	H5'	m								
	H5''	m								
	H8	w	m	m	m		v	v		w

G5		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**		**H8**
T4	H1'						m	m		w
	H2'									m
	H2''									m
	H3'									m
	H5'									v
	H5''									v
	H6									w
A6	H2	v								
	H8	v	m	m	m					m

A6		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**	**H2**	**H8**
G5	H1'								v	v
	H2'									m
	H2''									m
	H3'									m
	H8									m
G7	H5'	w								
	H5''	w								
	H1								w	
	H8	m	m	m	w		v	v		w
G20	H1	w							w	w

[a]Cross peak intensities are classified as strong (s), medium (m), weak (w), very weak (v), or ambiguous (a) in case of overlapped resonances.

Table S4. Sequential and long-range intramolecular NOE interactions of MycG4 in the 2:1 complex with berberine involving the 3'-terminal residues G23-A25.[a]

G23		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**		**H8**
G22	H1'				w	w	m	m		w
	H2'						m			m
	H2''							m		m
	H3'									w
	H8									w
A24	H2	w								
	H8	m	m	s	w	v				w

A24		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**	**H2**	**H8**
G23	H1'								w	m
	H2'									m
	H2''									s
	H3'									w
	H4'									v
	H8									w
A25	H5'	m								
	H5''	m								
	H8	m	m	m	w					a

A25		**H1'**	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**H5''**	**H2**	**H8**
A24	H1'						m	m		m
	H2'									m
	H2''									m
	H3'									w
	H8									a

[a]Cross peak intensities are classified as strong (s), medium (m), weak (w), very weak (v), or ambiguous (a) in case of overlapped resonances.

Table S5. Intermolecular NOE interactions of MycG4 with berberine in the 2:1 complex that were used for the structure determination. Interactions that were assigned to conformer A, to conformer B, or to both are shown in red, blue, and black, respectively.[a]

5'binding

Ber		HC	H4	H6	H8	MeA	MeB	H11	H12	H13	H1
A6	H2		m		m						
G7	H1		w	m							
G11	H1			m						w	w
	H8	v					m				
G16	H1		w	m				v	w	w	w
	H8	v				m					
G20	H1			m							
	H8	w				m					

3'-binding

Ber		HC	H4	H6	H8	MeA	MeB	H11	H12	H13	H1
G9	H1			m							
	H8	v				w	m				
G13	H1			m							
	H8	w								w	
G18	H1			m							
	H8					w	m				
G22	H1			m							

[a]Cross peak intensities are classified as medium (m), weak (w), or very weak (v).