DNA Structures

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A Highly DNA-Duplex-Stabilizing Metal–Salen Base Pair**

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The incorporation of one or multiple metal ions into nucleic acids in a defined fashion is currently of great interest for the development of alternative base pairs and the generation of DNA and RNA with novel catalytic and electronic properties.[1] The general idea regarding catalysis is that the metal ion provides a unique catalytic property, while the nucleic acids surrounding it serve as a chiral ligand amenable to evolutionary optimization.[2,3] Recently, the groups led by Leumann,[4] Meggers,[5] Schultz,[6] Shionoya,[7] Switzer,[8] and Tor[9] presented metal-coordinating nucleic acids, so-called ligandosides, which are able to hold a metal ion inside the double helix.

Regarding electronic application, Shionoya et al. achieved the incorporation of five consecutive metal–base pairs, thereby creating a double helix in which five copper ions are presumably stacked on top of each other.[1] On the way to the goal of creating metal-based catalytic nucleic acids, it would be desirable to create new metal–base pairs in DNA or RNA from chemical entities known to be "privileged ligands" for the construction of powerful catalysts.[10] We therefore decided to develop a DNA metal–base pair based on the *N*,*N*′-bis(salicylidene)ethylenediamine (salen) ligand,[11] which is today one of the most versatile ligands used for the generation of metal catalysts. Metal–salen complexes are able to accelerate a wide range of reactions such as cyclopropanations, epoxidations,[12] and oxidations[13] and are applicable for kinetic resolutions.[14]

The development of our metal–base pair is based on earlier studies of Sheppard and Gothelf et al., who assembled intrastrand salen complexes from phosphate-bound salicylic aldehydes.[15] To create a salen-based metal–base pair that fits optimally into the double-helix structure, we decided to connect the salicylic aldehyde at the C4 atom to the C1′ position of 2′-deoxyribose. Based on molecular modeling we

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envisioned that two such ligands facing each other in a duplex structure may assemble in the presence of a suitable metal ion and ethylenediamine to give a metal–salen complex (Figure 1). This complex was envisioned to stack inside the duplex structure, presenting the metal in the minor groove. In contrast to the metal–base pairs investigated so far, the salen–base pair requires both the metal and ethylenediamine for assembly. The inclusion of ethylenediamine gives the resulting complex cross-linking character, although in water imine formation is generally highly reversible.[16]

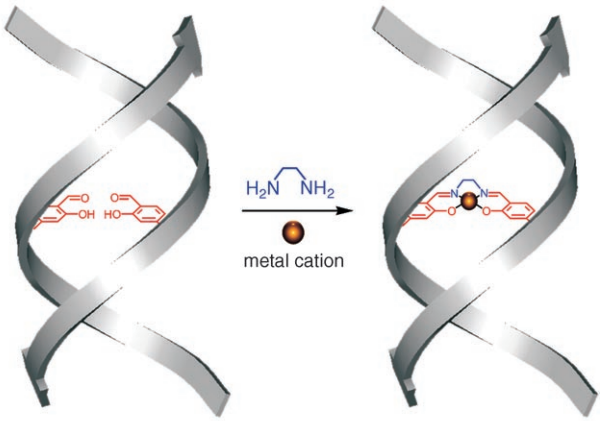
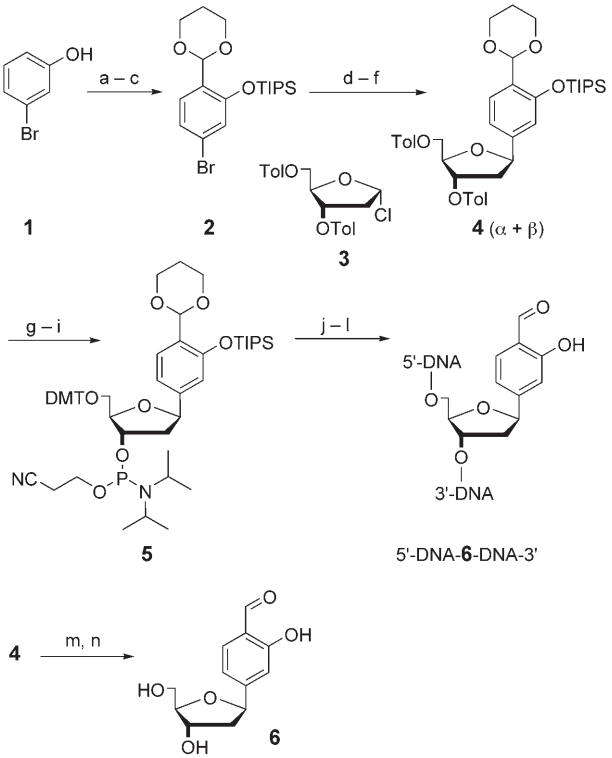
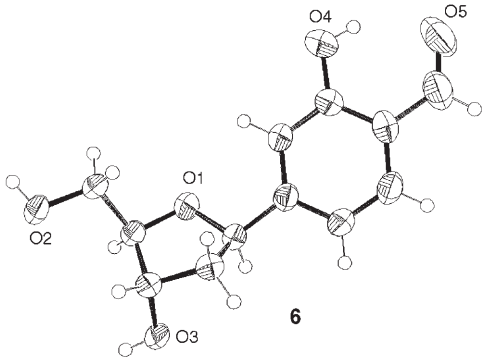


Figure 1. Depiction of the salicylic aldehyde base pair (LL) and assembly of the metal–salen complex inside the duplex.

The protecting groups on the ligand were chosen to comply with ligandoside synthesis and DNA chemistry. The triisopropylsilyl (TIPS) protecting group for the phenol is cleavable under the conditions used to remove the synthesized DNA from the solid support. The aldehyde functionality was protected as an acetal, cleavable with 2% dichloroacetic acid in wet dichloromethane. This reagent is also used for the cleavage of the 5′-DMT protecting groups during DNA synthesis.

The preparation of the protected salicylic aldehyde nucleobase was achieved as depicted in Scheme 1 by ortho-formylation of 3-bromophenol (**1**) with paraformaldehyde in the presence of $MgCl_2$ and triethylamine.[17] The formylation was followed by first an acetalization according to a special procedure for salicylic aldehydes[18] and second by TIPS protection to yield the protected ligand **2**. The C-glycosidation[19] was achieved after lithium–bromine exchange with *tert*-butyllithium, transmetalation, and reaction of the resulting aryl copper species with α-2′-deoxyribosylchloride **3**, which was prepared in two steps from 2′-deoxyribose.[20] After separation of the α and β anomers of nucleoside **4** by column chromatography (the configuration at C1′ of both anomers was assigned by evaluation of the NOESY contacts between the hydrogen atoms C1′-H, C2′-H, and C3′-H), we depro-





8-L-a = 5′-CACATTA**L**TGTTGTA-3′
8-L-b = 3′-GTGTAAT**L**ACAACAT-5′
7-L = 5′-GTA**L**AGTTTTCT**L**TAC-3′
L = ligand **6**

Scheme 1. Synthesis of the salen ligandoside **6** and its phosphoramidite together with the crystal structure of **6** and the sequences of the DNA strands prepared for this study. a) $(CH_2O)_n$, NEt_3, $MgCl_2$, toluene, 100 °C, 10 h, 49%; b) 1,3-propanediol, $HC(OEt)_3$, $N(nBu)_4Br_3$, RT, 24 h, 86%; c) TIPS-OTf, $NEt(iPr)_2$, CH_2Cl_2, RT, 12 h, 87%; d) 2 equiv *t*BuLi, Et_2O, −78 °C, 2 h; e) CuBr·SMe_2, −78 → −30 °C, 20 min; f) **3**, CH_2Cl_2, 12 h, −78 °C → RT, 78% (α/β = 3:2); g) K_2CO_3, MeOH, RT, 2 h, 72%; h) DMT-Cl, pyridine, 3 h, 67%; i) CED-Cl, $NEt(iPr)_2$, THF, RT, 2 h, 78%; j) automated DNA synthesis; k) deprotection of aldehydes with wet 2% $CHCl_2COOH$ in CH_2Cl_2; l) cleavage from solid support and cleavage of TIPS with $NH_{3(aq)}$/EtOH = 3:1; m) $N(nBu)_4F$, THF; n) 2% $CHCl_2COOH$, CH_2Cl_2. CED-Cl = (2-cyanoethyl)-*N*,*N*-diisopropylphosphorimidochloridite, DMT = 4,4′-dimethoxytrityl, Tf = trifluoromethanesulfonyl, TIPS = triisopropylsilyl, Tol = tolyl. X-ray structure: Ligandoside **6** crystallized from EtOAc.[24]

tected the sugar hydroxy groups under Zémplen conditions[21] and protected the 5′-OH group with DMT-Cl followed by the synthesis of phosphoramidite **5**. In order to establish the configuration at C1′ unambiguously, **4** was treated with tetrabutylammonium fluoride and dichloroacetic acid to yield the fully unprotected C-nucleoside **6** for crystallization. The crystal structure of the salicylic aldehyde **6** finally confirmed the desired β configuration (Scheme 1).

DNA synthesis was performed using a standard phosphoramidite protocol on an Expedite DNA synthesizer with prolonged coupling times for the incorporation of the salicylic aldehyde phosphoramidite. After additional treatment of the assembled DNA with wet dichloroacetic acid in CH_2Cl_2 and final cleavage from the solid support with saturated ammonia in water/ethanol, the oligonucleotides **7**, **8-a**, and **8-b** containing the salicylic aldehyde nucleobase were purified by reversed-phase high-performance liquid chromatography (RP-HPLC) and analyzed by MALDI-TOF mass spectrometry. The presence of the aldehyde was additionally proven by reaction of the obtained DNA with an amine; the resulting yellow color was indicative for the formation of salicylic aldimines. In addition, reaction of the DNA with *O*-benzylhydroxylamine and reductive amination using different primary amines and sodium cyanoborohydride gave quantitatively the corresponding *O*-benzyloximes and amines, respectively, as shown by mass spectrometric analysis.

All the prepared DNA duplexes and DNA hairpins together with their measured melting temperatures are listed in Table 1. In one sequence, replacement of an AT base pair (entry 1) with the salicylic aldehyde base pair (LL, entry 7) was found to decrease the melting temperature by 9.0 K. Addition of an excess of ethylenediamine (en) to a solution containing the DNA duplex caused an increase of the melting temperature by 4.8 K (Table 1, entries 4 and 5) accompanied by changes in the fluorescence and emission spectra indicative for the formation of salicylic aldimines. More important, however, is our observation that the careful titration of a solution of divalent metal ions to the ethylenediamine-containing DNA solution resulted in systematically increasing duplex stabilization (Figure 2a,b) accompanied by changes of the CD spectra (Figure 3a,b). One equivalent of Cu^{2+} induced a shift of the melting temperature to 82.4 °C (Table 1, entry 3), a shift of more than 30 K with respect to a normal AT base pair (+42.5 K with respect to the duplex containing the LL "base pair", Table 1, entry 2 and Figure 2a). Additional Cu^{2+} had no further effect. To the best of our knowledge, this is the most dramatic increase in duplex stabilization ever observed with a metal–base pair. Addition of one equivalent of Mn^{2+} (which is known to be oxidized to Mn^{3+} upon complexation by salen ligands)[22] increased the T_M by 28.1 K to 68.8 °C (Table 1, entries 4 and 6; Figure 2b).

Table 1: Melting-temperature experiments with oligonucleotides **7** and **8**.

Entry	Strands [a]	Additive(s)	T_M [°C]
1	**8-A-a/-T-b**	[d]	50.1
2	**8-L-a/b**	[b]	39.9
3	**8-L-a/b**	100 μM en, 3 μM Cu^{2+} [b]	82.4
4	**8-L-a/b**	[c]	40.7
5	**8-L-a/b**	100 μM en [c]	45.5
6	**8-L-a/b**	100 μM en, 3 μM Mn^{2+} [c]	68.8[e]
7	**8-L-a/b**	[d]	41.1
8	**8-L-a/b**	100 μM en, 400 μM Zn^{2+} [d]	48.8[e]
9	**8-L-a/b**	100 μM en, 400 μM Ni^{2+} [d]	36.5
10	**8-L-a/b**	200 μM $MeNH_2$, 4 μM Cu^{2+} [b]	52.3
11	**8-L-a/b**	4 μM Cu^{2+} [b]	54.9
12	**8-L-a/b**	6 μM Mn^{2+} [c]	40.7
13	**7-L**	[b]	19.9
14	**7-L**	100 μM en, 6 μM Cu^{2+} [b]	65.2
15	**7-L**	[c]	22.1
16	**7-L**	100 μM en, 4 μM Mn^{2+} [c]	[f]
17	**7-T/A**		46.5

[a] For sequences see Scheme 1. In **8-A-a** and **8-T-b** the salicylic aldehyde is replaced by A and T, respectively. All samples contained 3 μM DNA (duplex or hairpin) and 150 mM NaCl. Melting profiles were measured from 0 °C to 85 °C (for Cu^{2+}: 95 °C) with a rate of 0.5 K min^{-1}. Further details are given in the Supporting Information. [b] All experiments using Cu^{2+} and corresponding controls were carried out in 10 mM Ches (*N*-cyclohexyl-2-aminoethanesulfonic acid) buffer at pH 9.0. [c] All experiments using Mn^{2+} and corresponding controls were carried out in 10 mM Hepes (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)) buffer at pH 9.0. [d] Measured in 10 mM Tris(2-amino-2-(hydroxymethyl)propane-1,3-diol) buffer at pH 7.4. [e] Reproducible differences in de- and renaturing profiles due to thermal instability of the complex (see the Supporting Information). [f] No T_M determined (see the Supporting Information).

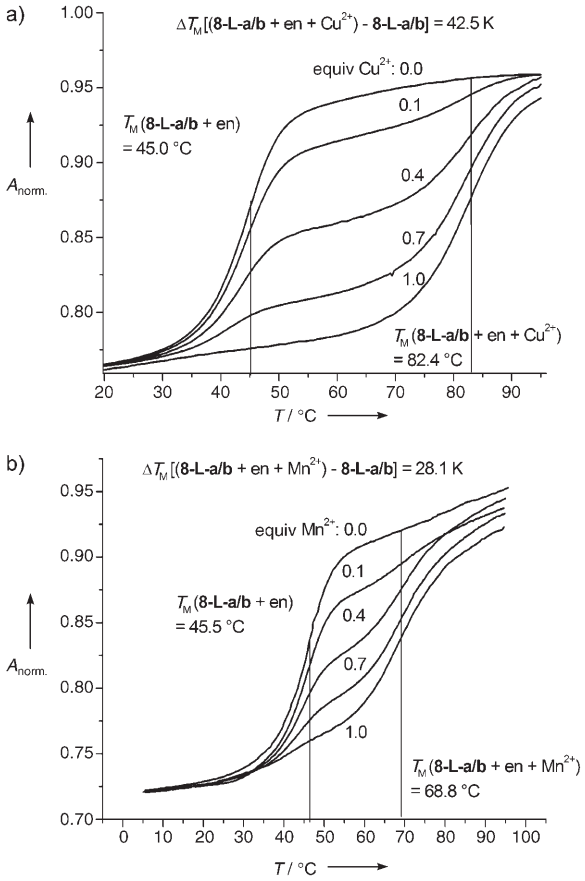


Figure 2. Melting profiles of 3 μM **8-L-a/b** with 100 μM ethylenediamine (en) in the presence of various amounts of a) Cu^{2+} (0–1 equiv) and b) Mn^{2+} (0–1 equiv). The samples contained 150 mM NaCl and 10 mM buffer (Ches pH 9 for Cu^{2+}, Hepes pH 9 for Mn^{2+}).

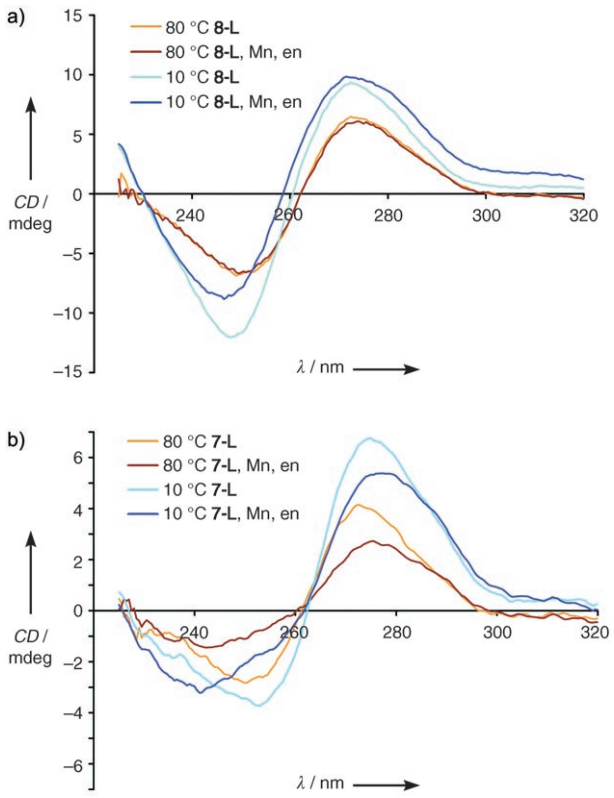


Figure 3. Comparison of CD spectra at 10 °C and 80 °C of a) **8-L-a/b** and b) **7-L** in the absence and presence of ethylenediamine and Mn^{2+}.

Again, additional Mn^{2+} did not further increase the melting temperature. Addition of Zn^{2+} gave an increase of T_M by 7.7 K (Table 1, entries 7 and 8). Interestingly, addition of Ni^{2+} caused reproducibly a decrease of the melting temperature by 4.6 K (Table 1, entries 7 and 9; see the Supporting Information). For Zn and Ni, however, high concentrations of the metal salts were required. When ethylenediamine was replaced by propylenediamine, only Cu^{2+} was able to shift the melting point (addition of butylenediamine had no effect). Addition of Cu^{2+} and not ethylenediamine resulted in an increase of T_M by 15.0 K relative to the melting temperature of the duplex with the pure LL "base pair" (Table 1, entries 2 and 11). In contrast, Mn^{2+} did not cause a shift in melting temperature when ethylenediamine was absent. No changes in the melting temperatures were observed in the absence of either one or both salicylic aldehyde nucleobases, showing that formation of the salen complex inside the duplex is indeed responsible for these dramatic shifts. Formation of the salen complex could be completely reversed by addition of an excess of EDTA to the DNA solution. In order to estimate the effect of the cross-linking, we added to the LL-containing duplex **8-L-a/b** first Cu^{2+} and then methylamine. In this case, a much smaller stabilization of only 12 K was observed (Table 1, entry 10).

Formation of the salen complex inside the duplex was further confirmed by CD spectroscopy of the DNA duplex **8-L-a/b** and the DNA hairpin **7-L** measured with and without metal and ethylenediamine (Figure 3). The CD spectra measured between 80 °C and 10 °C show clearly formation of B-type DNA structures in all cases (see the Supporting Information).[23] Addition of ethylenediamine and either Mn^{2+} or Cu^{2+} resulted in changes of the CD spectra below the individual melting temperatures, indicating formation of the salen complex (only the spectra with Mn^{2+} are shown). Above the melting temperatures the obtained CD spectra of the duplex are undistinguishable from those of DNA strands without the metal. However, the CD spectrum of the hairpin **7-L** at 80 °C in the presence of Mn^{2+} is clearly different from that without the metal. This indicates that the salen complex may stay intact to some extent in the hairpin even at rather high temperatures. (Analogous results were obtained for samples with Cu^{2+}).

In conclusion, we have described the synthesis of a salen-based metal–base pair inside a DNA duplex, in which the metal is located inside the duplex structure. We believe that the extreme increase of the melting temperature by more than 40 KC is induced by the reversible cross-linking of the ligand with ethylenediamine and additionally maybe by axial coordination of the metal with heteroatoms present in the base pairs below and above the metal–base pair.

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[24] CCDC 271234 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

