In the previous chapter, the first examples of direct double strand DNA cleavage with a synthetic non-heme iron complex were presented. In this chapter, the attempts to increase this direct double strand DNA cleavage are summarized. Two possible strategies are explored: i) the covalent linking of three active single strand DNA cleavage agents together and ii) the covalent attachment of an additional DNA intercalator to the earlier discussed dinuclear complexes. It was envisioned that both approaches would result in a higher activity in terms of direct double strand DNA cleavage. The newly developed trinuclear complexes led only to small but significant additional double strand cleavage activity when compared to the corresponding dinuclear iron complexes. Quite surprisingly, the covalent attachment of a DNA intercalator (i.e. an acridine moiety) to the dinuclear complexes led only to lower or similar kinetics for DNA cleavage compared to analogous complexes without the acridine functionality. However, dramatic differences in the DNA cleavage activity were observed between two dinuclear acridine complexes, which only differ in the relative positioning of this functionality with respect to the active iron centers in the complex.*

* Parts of this chapter will be published: R.P. Megens, T.A. van den Berg, A.D. de Bruijn, B.L. Feringa, G. Roelfes, Chem Eur. J., accepted.
5.1 Introduction

The dinuclear iron complexes discussed in Chapter 4 can be used as catalysts for the aerobic oxidation of DNA and are capable of inducing direct double strand cuts in DNA. It was envisioned that a further derivatization of these dinuclear complexes could result in new complexes, which display an even higher degree of direct double strand DNA cleavage. This chapter summarizes the attempts on increasing the number of direct double strand cut events in DNA without losing activity by derivatization of the dinuclear complexes. Two possible strategies were examined: i) the covalent linking of an additional active complex to the dinuclear complexes and ii) the covalent attachment of an additional DNA intercalator to these dinuclear complexes.

A large increase in direct double strand DNA cleavage was observed when dinuclear (iron) complexes were used instead of a mononuclear analogue. Therefore, an increase of two to three covalently bound active centers was anticipated to even further increase the DNA cleavage activity. The easiest way to examine this approach would be to synthesize and evaluate the DNA cleavage activity of trinuclear Fe(N4Py) complexes. By attaching three of these active Fe(N4Py) complexes to each other, more active centers will be present in the proximity of the DNA at the same time. In turn, this could lead to a higher probability of two successful strand cleavage events in opposite DNA strands and thus a net direct double strand cleavage event.

The intercalator approach combines the knowledge gathered in both Chapter 3 and Chapter 4. It has been demonstrated that the covalent attachment of acridine to Fe(N4Py) increases the reaction rate without changing the overall reaction pathway (Chapter 3). For this reason, it was envisioned that the covalent attachment of this acridine to the dinuclear complexes (Chapter 4) would result in a higher DNA cleavage rate. Moreover, it was thought that this approach could result in higher numbers of double strand cleavage events to be reached, as the intercalating properties of the acridine would result in a higher probability of two active centers to be in close proximity of each other and in close proximity of the DNA at the same time.

These approaches for achieving a higher activity are not known for iron complexes in aerobic DNA oxidation. Related approaches using copper complexes or application of related iron complexes in DNA hydrolysis are briefly addressed in the first part of this chapter. This is followed by the synthesis and characterization of the new target ligands, based on derivatization of the N4Py ligand. The DNA oxidation behavior of the corresponding iron complexes will be evaluated and discussed in the subsequent section. Finally, some general conclusions will be drawn from these results and these will be placed into context with the results obtained in the preceding chapters.

5.1.1 Trinuclear complexes

Literature precedents for this approach are limited and are restricted to the use of (trinuclear) copper complexes in DNA oxidation. In addition to their work on dinuclear copper complexes, Karlin and Rokita have found that trinuclear polypyridyl copper complexes display selective DNA cleavage at junctions between single and double stranded DNA (Figure 5.1, See Chapter 1, Section 1.4.3.2).
Towards a higher degree of double strand DNA cleavage

Figure 5.1 Polypyridyl trinuclear complex \( \text{Cu}_3(\text{Lx})(\text{NO}_3)_2(\text{H}_2\text{O})_3 \) (left) and proposed mechanism for the selective DNA oxidation with the trinuclear copper complexes (right). One of the three copper centers is involved in the binding to guanine and/or adenine. The other two copper centers are involved in the activation of dioxygen, resulting in a DNA strand cut in the opposite strand.

In these complexes, the individual copper centers were demonstrated to have different functions, resulting in a unique selectivity. One copper center is proposed to be engaged in a direct interaction with one of the DNA strands, whereas the other two copper centers are involved in the activation of oxygen (Figure 5.1, right).

Other examples of polypyridyl trinuclear complexes have been reported by Guo and co-workers (Figure 5.2). Trinuclear copper complexes (Figure 5.2, left and center) display significantly higher DNA cleavage activity, compared to the corresponding mononuclear complex. However, a related dinuclear complex (see Chapter 4) displays an even higher DNA cleavage activity. This is remarkable, since fluorescence and circular dichroism (CD) spectroscopic studies show that the binding ability of the trinuclear copper complex is twice as high as the more active dinuclear copper complex. Recently, a trinuclear copper complex based on a polypyridyl triazine ligand was published, which was used in oxidation of DNA, albeit with high copper : base pair ratios (Figure 5.2, right). The observed DNA strand breaks can solely be attributed to a single strand cleavage pathway with these copper complexes.

Figure 5.2 Trinuclear copper complexes reported by Guo and co-workers for the oxidation of DNA.

5.1.2 Dinuclear complexes with an intercalator

The other approach to influence the direct double strand DNA cleavage activity is via the covalent attachment of a DNA intercalator to dinuclear complexes. Examples where DNA intercalators are attached to dinuclear complexes are rare, whereas many examples are reported where DNA intercalators have been attached to mononuclear (iron) complexes. Only recently, a dinuclear iron complex with a DNA intercalator attached covalently has...
been published (Figure 5.3).\textsuperscript{16} Peng and co-workers attached an acridine functionality to the BBPMP ligand,\textsuperscript{17,18} and found a significant increase in the bis-iron mediated hydrolytic DNA cleavage activity.

![Figure 5.3](image)

\textbf{Figure 5.3} The BBPMP ligand\textsuperscript{17} and the BBPMP ligand with the covalently attached acridine functionality.\textsuperscript{16} The corresponding bis-iron complexes are used in hydrolytic DNA cleavage.

## 5.2 Synthesis of the target ligands

### 5.2.1 Tritopic ligands

The goal here is the development of trinuclear complexes, capable of inducing DNA strand cuts. The approach taken here is to connect three active single strand cleavage agents, similar to the approach in Chapter 4 for dinuclear complexes. The active single strand DNA cleavage agent of choice is the Fe(N4Py) complex.

The first approach towards a tritopic N4Py ligand was via a route, which is common in dendrimer synthesis. The use of cyanuric chloride (or 2,4,6-trichloro-1,3,5-triazine) enables a stepwise introduction of (different) amine functionalities via a nucleophilic aromatic substitution by changing the reaction temperature (Scheme 5.1).\textsuperscript{19,20} Reaction between cyanuric chloride and an amine (either primary or secondary) at 0°C results in one chloride to be substituted by an amine. At room temperature, a second substitution reaction can take place and at elevated temperatures the third chloride group can be substituted with an amine.\textsuperscript{19} Since all three chloride functionalities on the cyanuric chloride can be replaced independently, this allows the construction of large functionalized dendrimers,\textsuperscript{21,22} in principle in a one-pot procedure.

![Scheme 5.1](image)

\textbf{Scheme 5.1} Via a stepwise introduction of amine moieties to cyanuric chloride by varying the reaction temperature a trifunctionalized melamine derivative can be obtained.

Via this route, it was proposed that tritopic N4Py ligands could be accessed in one step. Moreover, due to the stepwise introduction of different substituents, it was thought that this
Towards a higher degree of double strand DNA cleavage

route could be also appropriate for the construction of ditopic N4Py ligands with a third functionality to which an acridine could be attached (vide infra).

It was envisioned that reaction between 3 equivalents of the substituted N4Py ligand 1 with the aminopropyl functionality and one equivalent of cyanuric chloride at an elevated temperature would result in the formation of the target tritopic ligand 2 (Scheme 5.2). Initially, the solution of starting materials in a mixture of MeOH and DCM (1:1 v/v) was heated at reflux together with N,N-diisopropylethylamine (DIEA). However, the major product after workup was identified as the monotopic N4Py ligand 3, which has one chloride substituted by a methoxy group, based on electron mass spectral analysis. When methanol was omitted as solvent, only the formation of 4 was observed. Changing the solvent to 1,2-dichloroethane instead of DCM allowed higher reaction temperatures. However, only the ditopic ligand 4 was formed and accompanied by only trace amounts of the desired tritopic ligand 2. The reactions were performed in an autoclave at elevated pressures also, which are reported to be beneficial for the introduction of the third substituent.19 However, this only resulted in degradation of the starting material 1. Finally, attempts of using microwave conditions resulted in degradation of the starting material also. Due to the problems encountered in the attempts to introduce a third functionality, this route was abandoned.

Scheme 5.2 One-pot reaction between 1 and cyanuric acid in the presence of DIEA in different solvents results in the formation of different ligands.

An alternative route was based on the methodology used for the synthesis of the dinuclear ligands (Chapter 4). This involved a coupling of the N4Py synthon 1 to a trifunctionalized central building block (Scheme 5.3). A straightforward (peptide) coupling between 1 and an activated triacid was thought to be most suited for this purpose (See Chapter 4). Two tritopic ligands were prepared via this route; one ligand 5, based on 1,3,5-benzenetricarboxylic acid and a ligand 6, based on the more flexible 4-(carboxymethyl)heptanedioic acid (7) (Scheme 5.3).
Scheme 5.3 Retrosynthetic analysis of the target tritopic ligands 5 and 6.

Coupling in DCM at room temperature of 1 to the succinimide-activated tribenzoic acid 8 (synthesized from a DCC coupling between 1,3,5-tribenzoic acid and N-hydroxysuccinimide (NHS)) yielded the tritopic ligand 5 in 75% yield (Scheme 5.4). This ligand was purified by size exclusion chromatography (Sephadex® LH-20/MeOH) and characterized with NMR, MS-ESI$^+$ and RP-HPLC.

Scheme 5.4 Synthesis of target tritopic ligand 5. Reagents and conditions: (a) NHS, DCC, THF, overnight; (b) 1, DCM, overnight.

The synthesis of the flexible analogue, i.e. linker molecule 7, was found to be more laborious (Scheme 5.5). A Horner-Wadsworth-Emmons reaction between benzyl protected 4-ketopimelic acid (9) and commercially available triethyl phosphonoacetate resulted in compound 10, which was purified by column chromatography (54% yield). Reduction of this compound over Pd/C with $\text{H}_2$ resulted in the removal of the double bond accompanied by the removal of the two benzyl protecting groups in a quantitative one-pot procedure. Hydrolysis of the remaining ester moiety of 11 yielded the target molecule 7 in 56% yield.
Scheme 5.5 Synthesis route of the saturated central building block 7. Reagents and conditions: (a) triethyl phosphonoacetate, NaH, THF, rt, 1 h, then reflux overnight; (b) H₂, Pd/C (10%), MeOH, overnight; (c) LiOH, MeOH/H₂O (1:1 v/v), overnight.

A coupling of triacid 7 and N4Py synthon 1 using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBT) and DIEA in DCM was found to be most efficient; the target ligand 6 was obtained in an excellent yield of 90%. Only traces of impurities were observed by NMR spectroscopy, which were removed by size exclusion chromatography (Sephadex® LH-20/MeOH). The ligands were characterized with NMR spectroscopy, electron spray mass spectral analysis and RP-HPLC. If needed, ligands 5 and 6 were further purified by preparative RP-HPLC.

Scheme 5.6 Synthesis of target tritopic ligand 6. Reagents and conditions: (a) 1, EDCI, HOBT, DIEA, DCM, overnight.

5.2.2 Ditopic ligands with an intercalator

It was demonstrated in Chapter 3 that an acridine functionality causes a fourfold increase in DNA cleavage rate when attached to Fe(N4Py). Therefore, it was envisioned that by covalent attachment of an acridine moiety to a dinuclear complex (whose increase in direct double strand cleavage has been demonstrated in Chapter 4), a similar rate enhancement could be achieved.

The target ligands 12 and 13 are based on a ditopic ligand, to which an acridine can be attached via an additional spacer. A retrosynthetic analysis shows that the N4Py synthon 1 can be used together with an activated diacid (Scheme 5.7). This central moiety should
have a functionality to which a spacer can be attached. Isophthalic acid derivatives are useful starting materials, as an alkylation of the alcohol functionality can result in the introduction of this spacer to which the acridine can be attached in the last step of the synthesis. Two different substitution patterns were tested to investigate a possible substitution effect on the DNA cleavage activity.

Scheme 5.7 Retrosynthetic analysis of target acridine ligands 12 and 13.

The first step towards the target ligands 12 and 13 involves the synthesis of both alkylated isophthalic acid derivatives 14 (Scheme 5.8) and 15 (Scheme 5.9). The synthesis of 14 is straightforward, but requires dimethyl 5-hydroxyisophthalate as starting material for the alkylation step of the alcohol, since the free diacid (5-hydroxyisophthalate) was found to be unreactive towards alkylation. An alkylation with Boc-protected aminopropyl bromide 16 in acetone with K₂CO₃ furnished the desired product 17 in 58% after column chromatography (Scheme 5.8). Hydrolysis of 17 to the diacid 14 proceeded in 83%.

Scheme 5.8 Synthesis of alkylated isophthalic acid 14. Reagents and conditions: (a) K₂CO₃, acetone, reflux overnight; (b) LiOH, THF/H₂O (1:1 v/v), 48 h.

The synthesis of the other regio isomer 15 was somewhat more laborious, as dimethyl 2-hydroxyisophthalate (18) is not commercially available. Therefore, 2-methoxyisophthalic acid was converted into the corresponding dimethyl ester 19 (Scheme 5.9). Deprotection of the phenol moiety by reaction of 19 with BBr₃ in dry DCM at -80°C yielded 18 (50% over two steps). Alkylation of the alcohol with Boc-protected bromopropylamine 16 in N,N-dimethylformamide (DMF) with K₂CO₃ yielded 20, which was hydrolyzed to the desired diacid 15.
Towards a higher degree of double strand DNA cleavage

Scheme 5.9 Synthesis of alkylated isophthalic acid 15. Reagents and conditions: (a) Conc. H$_2$SO$_4$, MeOH, overnight; (b) BBr$_3$, DCM, -78°C, 45 min; (c) 16, K$_2$CO$_3$, DMF, reflux overnight; (d) LiOH, THF/H$_2$O (1:1 v/v), 24 h.

A peptide coupling (EDCI/HOBt/DIEA) between the dialkylated isophthalic acids 14 and 15 and aminopropyl N4Py 1 furnished both 21 and 22 in moderate to high yields (quantitative and 70%, respectively (Scheme 5.10)). Purification was performed over a Sephadex$^8$ LH-20 size exclusion column.

The Boc-protecting group was removed by treatment of 21 and 22 with a 10% TFA solution in DCM overnight (Scheme 5.10). After workup and size exclusion chromatography 23 and 24 were obtained in moderate yields. The high solubility of 23 and 24 in water caused the isolation to be quite difficult. The final step was the coupling between the free amines 23 and 24 and acridine to obtain the target ligands 12 and 13.

Although the literature precedent indicates a quantitative reaction between aminopropyl-N4Py 1 and 9-chloroacridine, coupling between the amines 23 and 24 was found to be quite troublesome. A thorough purification of the amines (size exclusion chromatography) was required together with a lowering of the reaction temperature (< 80°C) to obtain a clean reaction. The resulting ditopic ligands 12 and 13 were isolated as the HCl salts and were characterized after basic workup with $^1$H NMR and high resolution MS-ESI$^+$ analysis. The purity was confirmed by RP-HPLC analysis (Figure 5.4). The stability of the ligands 12 and 13 as HCl salts was high; after prolonged storage no signs of degradation were observed. In contrast, the free ligands (prepared via a basic workup) displayed signs of degradation already after several days.

Figure 5.4 RP-HPLC traces (λ = 254 nm) of 12 (left; Waters XTerra MS C18 column) and 13 (right; Waters Xbridge C18 column). In both cases 10 mM ammonium acetate (pH = 5.5) and MeOH were used as mobile phase. A linear gradient of 40% to 70% MeOH was employed.
5.3 DNA oxidation experiments

5.3.1 DNA oxidation with trinuclear complexes

Prior to DNA oxidation, the ligands 5 and 6 were coordinated to iron(II). To ascertain the uptake of three iron(II) ions per ligand, titrations experiments were performed analogous to the titration studies discussed in Chapter 4 for the dinuclear complexes. Indeed, the titration of [(NH$_4$)$_2$Fe(II)(SO$_4$)$_2$] to 5 resulted in the uptake of three iron ions per ligand as was determined by UV/Vis spectroscopy and $^1$H NMR spectroscopy.$^{26}$

The new trinuclear complexes ([Fe$^{III}_{3}(5)$] (25) and [Fe$^{III}_{3}(6)$] (26)) were tested for their DNA cleavage activity. The oxidation of supercoiled pUC18 plasmid DNA (0.1 μg/μL, 150 μM in bp) in Tris buffer (10 mM, pH = 8.0) at 37°C in the presence of DTT (1.0 mM) was studied. The conversion of the supercoiled DNA into nicked and linear DNA was followed in time for both 25 and 26 (1.0 μM based on Fe) (Figure 5.5).

A similar cleavage pattern is observed for both complexes (Figure 5.5). Initially, only nicked DNA is formed and linear DNA is formed only after a short lag period. It seems that the phenyl based trimer 25 is somewhat more effective in producing linear DNA than the more flexible trimer 26. Already after 30 min, the phenyl based trimer 25 is responsible for the production of nearly 30% linear DNA (Figure 5.5a), which is the maximum quantifiable
Towards a higher degree of double strand DNA cleavage

amount (see Chapter 2). At the same time, the flexible trimer 26 has produced around 20% linear DNA (Figure 5.5b) and the 30% limit is only reached after 60 min (data not shown). Taken together, it seems that both complexes display double strand cleavage, but the more rigid complex 25 is somewhat more effective.

Figure 5.5 Aerobic oxidation of supercoiled plasmid DNA (■) into nicked DNA (●) and linear DNA (▲) followed in time catalyzed by a) [Fe^{III}L_{5}] (25) and b) [Fe^{III}L_{6}] (26). Errors bars represent the root mean square (rms) error based on three runs. A correction factor of 1.31 was used to compensate for the reduced ethidium bromide uptake capacity of supercoiled DNA (see Chapter 2).

To substantiate that both complexes display double strand cleavage, the number of single and double strand cleavage cuts per DNA molecule were calculated using Equation 5.1 and Equation 5.2 (Figure 5.6).

\[
f_{III} = m \times e^{-m}
\]

**Equation 5.1** Average number of double strand cuts in one DNA molecule, calculated from the fraction of linear DNA \( f_{III} \).

\[
f_{I} = e^{-(m+n)}
\]

**Equation 5.2** Total average number of strand cuts (both single \( n \) and double \( m \)) in one DNA molecule, calculated from the fraction of supercoiled DNA \( f_{I} \).

Initially, for both complexes only single strand cuts are observed (Figure 5.6). In course of the reaction, double strand cuts are observed also. This indicates that the double strand cleavage pathway becomes more and more prominent when the reaction proceeds. Although trimeric complex 25 is somewhat faster in producing double strand cuts than trimeric complex 26, this is not reflected in the \( m/n \) ratio. This points to a similar cleavage pathway, albeit with different reaction rates. When the results are contrasted with the results of a dinuclear complex (namely the 1,3-substituted phenyl based dinuclear complex (27), Chapter 4), a similar trend was observed for both 25 and 26. However, the introduction of double strand breaks in the DNA was already observed in an earlier stage of the reaction with the trinuclear complexes compared to the dinuclear complex. Since the double strand cuts are not the result of extensive single strand cleavage (data differs significantly from the Freifelder-Trumbo relationship, dotted line), it seems that the trinuclear complexes are somewhat more effective in producing double strand cuts in the DNA than the
corresponding dinuclear complexes (data from Chapter 4 shows that all dinuclear complexes are quite similar in terms of DNA cleavage behavior).

**Figure 5.6** Number of double strand cuts per DNA molecule (dsc) as a function of the number single strand cuts per DNA molecule (ssc) for 25 (■) and 26 (□). Data of a dinuclear complex is shown for comparison (▲, 1,3-disubstituted phenyl based dinuclear Fe(N4Py) complex (27), see Chapter 4). The dashed line is the Freifelder-Trumbo relation. Error bars represent the uncertainty of the data, based on a Monte Carlo simulation, taking into account a standard deviation $\sigma$ of 0.03.

The increase in double strand cleavage activity is not as large as was observed when going from mononuclear complexes to dinuclear complexes. This indicates that an increase in electrostatic interaction between the negatively charged DNA and the more positively charged trimuclear complexes (compared to the corresponding dinuclear complexes) does not alter the reaction outcome drastically. It seems that the double strand cleavage rate is not dominated by this parameter, but rather by other factors, e.g. accessibility of the target hydrogens of the DNA to the (two or more) active complexes in close proximity.

### 5.3.2 DNA oxidation with dinuclear complexes with an acridine

Prior to the DNA oxidation experiments, the acridine ligands 12 and 13 were coordinated to $[(\text{NH}_4)_2\text{Fe}^{II}(\text{SO}_4)_2]$. The obtained dinuclear complexes $[\text{Fe}^{III}_2(12)]^{4+}$ (28) and $[\text{Fe}^{III}_2(13)]^{4+}$ (29) were tested by following the oxidation of supercoiled pUC18 plasmid DNA into nicked and linear DNA in time (Figure 5.7) under similar conditions as mentioned earlier (vide supra).

Large differences in the DNA cleavage behavior between both complexes 28 and 29 (both at a 1.0 $\mu$M concentration based on Fe) were observed. First of all, a much slower conversion of supercoiled DNA is observed for 28 ($t_{1/2} \sim 25$ min) than for 29 ($t_{1/2} \sim 10$ min). The rate of conversion of supercoiled DNA by the latter complex is comparable to that of the dinuclear complexes discussed in Chapter 4. Another distinct difference is that hardly any linear DNA is produced within 1 h when 28 is employed as catalyst (Figure 5.7a), whereas more than 30% of linear DNA is formed within the same period with 29 (Figure 5.7b). From these results it can be concluded that the substitution pattern on the central phenyl moiety is of crucial importance for the activity of the complexes as catalysts in the DNA oxidation reaction.
Towards a higher degree of double strand DNA cleavage

Figure 5.7 Aerobic oxidation of supercoiled plasmid DNA (■) into nicked DNA (●) and linear DNA (▲) followed in time, catalyzed by a) [[Fe\(^{II}\)\(_2\)(12)]\(^{4+}\) (28) and b) [Fe\(^{II}\)\(_2\)(13)]\(^{4+}\) (29). Errors bars represent the root mean square (rms) error based on three runs. A correction factor of 1.31 was used to compensate for the reduced ethidium bromide uptake capacity of supercoiled DNA (see Chapter 2).

Again, the average number of single and double strand DNA cuts can be calculated using Equation 5.1 and Equation 5.2 (vide supra) for both complexes 28 and 29. The results are displayed in Figure 5.8.

Figure 5.8 Number of double strand cuts per DNA molecule (dcs) as a function of the number of single strand cuts per DNA molecule (ssc) for 28 (■) and 29 (□). The dashed line is the Freifelder-Trumbo relation. Error bars represent the uncertainty of the data, based on a Monte Carlo simulation, taking into account a standard deviation \(\sigma\) of 0.03.

Both complexes initially follow the Freifelder-Trumbo relationship (dashed line), implying a single strand cleavage pathway (Figure 5.8), which is observed also for other polynuclear complexes (Figure 5.6 and see Chapter 4). However, after this initial single strand cleavage activity, both complexes display double strand cleavage also, evident from the strong deviation from the Freifelder-Trumbo relationship. Interestingly, a higher degree of double strand DNA cleavage is observed at an earlier stage \((n > 0.5)\) with complex 28 compared than with complex 29, although this complex 28 is a much less efficient catalyst compared to complex 29 in terms of DNA conversion (Figure 5.7).
The only difference between the acridine complexes is the relative positioning of the acridine with respect to the two Fe(N4Py) moieties, which could explain the results. The acridine of complex 28 (with a 1,3,5-substitution pattern on the central phenyl moiety) intercalates in the DNA, but due to the relative positioning the two active iron moieties are pointing away from the DNA. In contrast, the active centers of complex 29 are positioned towards the DNA (due to the 1,2,3-substitution pattern of the central phenyl moiety) and this might result in a higher activity. However, to further substantiate these findings more research is required.

### 5.4 Summary and conclusions

This chapter summarizes the research on polynuclear iron complexes that were designed to achieve direct double strand DNA cleavage. These investigations focused on the use of trinuclear iron complexes and dinuclear iron complexes with an additional DNA intercalator bound to the complex.

New trinuclear ligands based on the N4Py ligand were synthesized successfully following standard peptide coupling strategies. The trinuclear ligand 5 with a central phenyl ring could be obtained via direct coupling between the aminopropyl N4Py 1 and an activated tribenzoic acid. An alternative ligand 6 with a more flexible central moiety required a somewhat longer synthesis, but the desired ligand was obtained in 5 steps.

Two ditopic ligands with a covalent attached acridine moiety were synthesized successfully also. The last step was the attachment of the acridine moiety to the ligand, but it was found that this step was not as straightforward as literature precedents would suggest. The ditopic ligands with the acridine bound covalently were found to be quite sensitive to higher temperatures and low pH.

The trinuclear complexes 25 and 26 were tested for their DNA cleavage activity (Figure 5.5, Figure 5.7). A similar cleavage pattern was observed for both trinuclear complexes, as initially only nicked DNA was formed followed by a significant increase in the amount of linear DNA. However, a distinct difference is that the phenyl-based trinuclear complex 25 has a much higher activity than the more flexible trinuclear analogue 26. When the number of single and double strand DNA cuts is calculated for both complexes, it seems that these trinuclear complexes are identical in terms of cleavage behavior (Figure 5.6). Comparison of the DNA cleavage results of the trinuclear complexes and the corresponding dinuclear complexes indicates that only a small but significant increase in the double strand DNA cleavage activity is achieved (Figure 5.6). The increase in double strand cleavage activity is larger between the mono- and dinuclear complexes than between di- and trinuclear complexes. It seems that increased electrostatic interactions between the trinuclear complexes and the DNA hardly affect the cleavage activity.

In contrast to the two trinuclear complexes, the two acridine complexes (28 and 29) differ quite significantly from each other in terms of DNA cleavage activity. Complex 28 displayed quite slow oxidation activity of supercoiled DNA into nicked and linear DNA (Figure 5.7a), whereas the activity of complex 29 is comparable to that of corresponding dinuclear complexes (Figure 5.7b). It is quite surprising that the DNA cleavage activity of these complexes differ so much from each other as the complexes only differ in the relative placement of the acridine moiety in the ligand (Scheme 5.7). After intercalation of the acridine into the DNA, it can be speculated that in case of complex 28 (with a central 1,3,5-substitution pattern) both active Fe(N4Py) moieties are pointed away from the DNA. In the case of complex 29 (with a central 1,2,3-substitution pattern) the positioning of both
Towards a higher degree of double strand DNA cleavage

Fe(N4Py) centers is pointed towards the DNA strand. More research is required to substantiate this rationale for the difference in DNA cleavage activity.

In conclusion, new polynuclear complexes based on the N4Py complex have been synthesized and tested for their DNA cleavage activity. There is a small increase in direct double strand cleavage when the trinuclear complexes are compared to the dinuclear complexes. The covalent attachment of an acridine to the dinuclear complexes did not lead to an expected rate increase of DNA cleavage (as was observed with the mononuclear complexes). In contrast, either a smaller or a lower reaction rate was observed with these acridine complexes. From these results it seems that increasing the DNA affinity of the cleaving agent (by increased electrostatics or via a DNA intercalator) hardly influences the DNA cleavage activity. A parameter which is not changed in this research is the relative positioning of the two active centers with respect to each other, as the spacer length is left unchanged. This factor could be of great importance for the accessibility of the (two) active complexes to the DNA backbone, since both DNA strands should be addressed efficiently at the same time for direct double DNA strand cleavage.

5.5 Experimental section

Instrumentation

For general remarks on used instrumentation, see Chapter 4. High resolution electron spray mass spectral analysis (HRMS (ESI+)) was performed on an Applied Biosystems Q-STAR mass spectrometer. $^{13}$C NMR of 6 was performed on a Varian Unity Plus 500 spectrometer at 126 MHz.

Chemicals and methods

For general remarks, see Chapters 3 and 4. All reactions were performed under an inert atmosphere (N₂ or Ar). Aminopropyl N4Py, benzyl protected 4-ketopimelic acid and Boc-protected bromopropylamine were synthesized according to literature procedures and all data was in agreement with the available data. For general remarks on biochemical studies, see Chapter 2 and 3. All DNA cleavage experiments, including photos of agarose gels slabs and subsequent data analysis, were performed using the method, which is described in Chapter 3. The uncertainty limits of the values of n and m were calculated with a Monte Carlo simulation using the software program Mathematica version 5.2.0.0. as described in Chapter 3.

Purification of compounds with preparative RP-HPLC

Preparative RP-HPLC was performed on a Shimadzu LC-10AD vp at 35°C, which was equipped with a photodiode array detector (SPD M10A) and a fraction collector (Shimadzu FRC 10A). General preparative separation method: A Waters Xbridge C18 preparative column (3.5 μm, 4.6 x 150 mm) was used at a flow rate of 10 mL/min. A linear gradient of 10 mM ammonium acetate (pH = 5.5) and MeOH was used, going from 40% to 60% MeOH over a period of 45 min. The fractions were analyzed using analytical RP-HPLC (for details, see Chapter 4). After pooling the fractions containing pure material, the resulting solution was concentrated, extracted with DCM and dried over Na₂SO₄. Filtration and evaporation of the solvent yielded the pure material.
1-(3,5-Bis[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl]benzoyl]-2,5-pyrrolinedione (8)

A mixture of 1,3,5-benzene tricarboxylic acid (1.01 g; 4.79 mmol), NHS (1.82 g; 15.8 mmol) and DCC (3.30 g; 16.01 mmol) in THF (250 ml) was stirred overnight. The white suspension was filtrated and the supernatant was concentrated. The resulting white solid was recrystallized from IPA. The product 8 was obtained as a white solid (1.82 g; 3.64 mmol; 76%). Mp. 255°C (dec.).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 9.14 (s, 3H), 2.94 (s, 12H).

$^{13}$C NMR (50.3 MHz, CDCl$_3$): $\delta$ 168.5, 159.5, 137.4, 127.7, 25.6.

MS-CI$^+$: $m/z$ 388 [M – NHS + H$^+$]$^+$. Anal. calcd (%) for C$_{21}$H$_{15}$N$_3$O$_{12}$: C 50.3; H 3.02, N 8.38; found: C 50.1; H 3.12; N 8.28.

$N_1,N_3,N_5$-Tris(3-[(6-[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl]-3-pyridinyl)carbonyl]amino)propyl)-1,3,5-benzenetricarboxamide (5)

A solution of 1 (93 mg, 0.20 mmol) and 8 (30 mg, 67 μmol) in DCM (20 mL) was stirred overnight. Aq. NaHCO$_3$ (10 mL) was added and the layers were separated. The aqueous layer was extracted with dichloromethane (3 x 25 mL) and the combined organic fractions were washed with aq. NaHCO$_3$ (2 x 10 mL) and dried over Na$_2$SO$_4$. Filtration and evaporation of the solvent yielded a light brown solid, which was further purified on a size exclusion column (Sephadex$^\text{®}$ LH-20 with MeOH). Ligand 5 was obtained as a yellow oil (78 mg; 50 μmol; 75%).

$^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 8.81 (d, $^3$J = 1.8 Hz, 3H), 8.45 (d, $^3$J = 4.6 Hz, 6H), 8.35 (d, $^3$J = 4.7 Hz, 3H), 8.32 (m, 3H), 8.09 (m, 3H), 7.96 (m, 3H), 7.80-7.63 (m, 15H), 7.52 (m, 3H), 7.26 (m, 6H), 7.19 (m, 3H), 7.19 (m, 3H), 7.01 (s, 3H), 3.96 (s, 6H), 3.40 (m, 12H), 1.97-1.83 (m, 6H).

$^{13}$C NMR (50.3 MHz, CD$_3$OD): $\delta$ 168.7, 167.9, 163.8, 160.8, 160.2, 145.0, 149.5, 148.6, 138.4, 136.9, 136.7, 129.9, 125.6, 124.8, 124.2, 124.0, 123.6, 74.4, 58.8, 58.4, 38.5, 38.3, 30.2.

MS-ESI$: m/z$: 1558.3 [M + H$^+$]$^+$, 780.1 [M + 2H$^+$]$^{2+}$, 520.5 [M + 3H$^+$]$^{3+}$.

RP-HPLC (Waters X Terra MS C18 analytical column (3.5 μm, 3.0 x 150 mm), flow rate 0.5 mL/min of water (0.05% acetic acid) and MeOH (0.05% acetic acid) as mobile phase. A linear gradient of 10% to 70% MeOH was employed.): $R_f = 42.6$ min.
6-Benzyl 1-ethyl 3-[3([benzyloxy]-3-oxopropyl]-2-hexenedioate (10)

Triethyl phosphonoacetate (3.1 g; 13.8 mmol) in THF (10 mL) was added slowly to a solution of NaH (332 mg; 13.8 mmol) in THF (20 mL) at 0°C. The resulting reaction mixture was stirred for an additional 30 min at 0°C, before benzyl protected 4-ketopimelic acid (9) (4.46 g; 12.58 mmol) was added. The resulting mixture was stirred for 1 h at room temperature and heated at reflux overnight. Water (20 mL) was added and the mixture was extracted with EtOAc (3x 20 mL). The organic layers were dried (Na2SO4), filtrated and the solvents evaporated. The product was further purified by column chromatography (SiO2; n-pentane/EtOAc 10:1) to afford 2.89 g (6.82 mmol; 54%) of product 10 as a white solid.

1H NMR (400 MHz, CHCl3): δ 7.36 (m, 10H), 5.63 (s, 1H), 5.09 (s, 4H), 4.11 (q, J = 7.0 Hz, 2H), 2.87 (t, J = 6.2 Hz, 4H), 2.50 (t, J = 6.2 Hz, 4H), 1.24 (t, J = 7.0 Hz, 3H).

13C NMR (50.4 MHz, CDCl3): δ 206.8, 206.6, 172.5, 172.3, 135.7, 128.4, 128.1, 128.0, 66.3, 60.5, 36.9, 36.9, 31.8, 29.6, 29.2, 27.8, 27.6, 22.5, 14.0.


4-(2-Ethoxy-2-oxoethyl)heptanedioic acid (11)

To a solution of 10 (1.00 g, 2.36 mmol) in MeOH (25 mL) was added Pd/C (10%, 0.1 g) and the suspension was stirred under a H2 atmosphere overnight. The mixture was filtered over Celite®. After washing the filtercake with MeOH, the solvent was evaporated to afford the reduced diacid 11 (0.54 g; 2.34 mmol; 99%) as a solid.

1H NMR (400 MHz, CDCl3): δ 4.12 (q, J = 7.1 Hz, 2H), 2.23-2.47 (m, 6H), 1.90 (m, 1H), 1.66 (m, 4H), 1.23 (t, J = 7.0 Hz, 3H).

13C NMR (50.3 MHz, CDCl3): δ 177.2, 170.5, 59.5, 38.4, 34.0, 31.2, 28.6.

HRMS (EI-) calcd. for C11H17O5 (M - OH-): m/z 229.107; found: 229.106.

4-(Carboxymethyl)heptanedioic acid (7).

A mixture of 11 (0.21 g, 0.85 mmol) and LiOH (61.33 mg, 2.56 mmol) in MeOH/H2O (5 mL, 1:1 v/v) was stirred overnight. The mixture was washed with DCM (10 mL) and conc. H2SO4 (96 %) was added until pH ~ 2. The resulting suspension was extracted with EtOAc (12 x 10 mL). After drying (Na2SO4), filtration and evaporation of the solvents, 7 was isolated as a white solid (105 mg; 0.48 mmol; 56%).

1H NMR (300 MHz, CD3OD): δ 2.33 (t, J = 7.8 Hz, 4H), 2.27 (d, J = 6.6 Hz, 5H), 1.89 (m, 1H), 1.65 (m, 4H).

13C NMR (50.3 MHz, CDCl3): δ 205.4, 204.6, 67.1, 63.3, 60.1, 57.7.

HRMS (EI) calcd. for C9H13O5 (M - OH-): m/z 201.076; found: 201.076.
A solution of 1 (0.10 g, 0.214 mmol) and HOBt (28.9 mg, 0.214 mmol) in DCM (7.5 mL) was cooled to 0°C. EDCI (59.8 mg, 0.312 mmol) and DIEA (27.8 mg, 0.215 mmol) were added and the reaction mixture was stirred for 1 h at 0°C and overnight at room temperature. The mixture was washed with aq. NaHCO3 (2 x 10 mL) and water (10 mL). The organic phase was dried over Na2SO4, filtrated and concentrated to afford a light yellow solid, which was further purified on size exclusion column (Sephadex® LH-20, MeOH) to afford 6 as a yellow oil (97.1 mg; 62 μmol; 90%).

1H NMR (300 MHz, CD3OD): δ 8.88 (dd, 4J = 4.6 Hz, 4J = 1.5 Hz, 3H), 8.48 (d, 3J = 4.1 Hz, 6H), 8.38 (d, 3J = 3.2 Hz, 3H), 8.27-8.15 (m, 6H), 8.07 (t, 3J = 7.7 Hz, 6H), 7.70-7.35 (m, 27H), 7.17-6.95 (m, 6H), 5.33 (s, 3H), 3.97 (s, 6H), 3.92 (s, 6H), 3.48-3.31 (m, 12H), 1.74-1.47 (m, 6H).

13C NMR (126 MHz, CD3OD): δ 176.2, 175.3, 167.8, 163.8, 160.8, 160.2, 150.0, 149.5, 148.6, 138.6, 138.5, 137.0, 130.0, 128.3, 126.6, 125.7, 124.9, 124.3, 124.1, 123.8, 74.6, 58.5, 58.5, 41.6, 38.4, 37.9, 36.1, 34.3, 30.7, 30.2

MS-ESI+: m/z 523.2 [M + 3H+]3+, 392.7 [M + 4H+]4+. RP-HPLC (Waters XTerra MS C18 analytical column (3.5 μm, 3.0 x 150 mm), flow rate 0.5 mL/min of water (0.05% acetic acid) and MeOH (0.05% acetic acid) as mobile phase. A linear gradient of 10% to 70% methanol was employed): Rf = 33.0 min.

**Dimethyl 5-{3-[(tert-butoxycarbonyl)amino]propoxy}isophthalate (17)**

Dimethyl 5-hydroxyisophthalate (1.00 g; 4.73 mmol) was dissolved in acetone (50 mL). To this solution the Boc-protected bromopropylamine 16 (0.563 g; 2.36 mmol) and K2CO3 (0.980 g; 7.09 mmol) were added and the white suspension was heated at reflux overnight. After cooling to room temperature the suspension was filtrated and the solvent of the supernatant was removed. DCM (50 mL) was added and after an additional filtration, the solution was washed with aq. NaHCO3 (2 x 50 mL) and water (50 mL). Drying (Na2SO4), filtration and evaporation of the solvents yielded a colorless oil, which was purified by column chromatography (SiO2; n-pentane/EtOAc 4:1 to 1:1). Rf = 0.25. Yield 0.505 g (1.37 mmol; 58%) of 17 as a white solid. M.p. 76.8-77.0°C.
Towards a higher degree of double strand DNA cleavage

$^1$H NMR (200 MHz, CDCl$_3$): δ 8.26 (t, $^3J = 1.4$ Hz, 1H), 7.72 (d, $^4J = 1.4$ Hz, 2H), 4.88-4.58 (br. s, 1H), 4.09 (t, $^3J = 6.0$ Hz, 2H), 3.91 (s, 6H), 3.33 (m, 2H), 2.01 (m, 2H), 1.43 (s, 9H).

$^{13}$C NMR (50.3 MHz, CDCl$_3$): δ 166.0, 158.8, 155.9, 131.7, 123.0, 119.7, 79.3, 66.2, 52.3, 37.8, 29.4, 28.3.

HRMS (EI$^+$) calcd. for C$_{18}$H$_{25}$NO$_7$: m/z 367.163; found 367.164.

Anal. calcd (%) for C$_{18}$H$_{25}$NO$_7$: C 58.84, H 6.86, N 3.81; found: C 59.0, H 6.78, N 3.61.

Dimethyl-2-methoxyisophthalate (19)

To a solution of 2-methoxy-isophthalic acid (5.07 g, 25.8 mmol) in MeOH (250 mL) was added conc. H$_2$SO$_4$ (96%, 1 mL) and the solution was heated at reflux overnight. After removal of the MeOH, DCM (50 mL) was added to the residue. The resulting solution was washed with water (2 x 50 mL) and dried over Na$_2$SO$_4$. The product obtained after filtration and evaporation of the solvents was purified through column chromatography (SiO$_2$; n-pentane/EtOAc 9:1 to 3:1). $R_f$ = 0.30. Yield 4.37 g (19.5 mmol, 75%) of 19 as a yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.94 (d, $^3J = 7.7$ Hz, 2H), 7.21 (t, $^3J = 7.7$ Hz, 1H), 3.93 (s, 9H).

$^{13}$C NMR (50.3 MHz, CDCl$_3$): δ 166.0, 159.5, 134.9, 126.5, 123.4, 63.6, 52.3.

HRMS (EI$^+$) calcd. for C$_{11}$H$_{12}$O$_5$: m/z 224.068; found: 224.067.

Dimethyl-2-hydroxyisophthalate (18)

To a solution of 19 (2.60 g, 11.6 mmol) in DCM (50 mL) was added BBr$_3$ (1.0 M in DCM, 12.7 mL, 12.7 mmol) slowly at -78°C. MeOH (15 mL) was added after stirring for an additional 45 min at -78°C. After warming to room temperature, the mixture was washed with aq. NaHCO$_3$ (3 x 30 mL) and dried over Na$_2$SO$_4$. Filtration and evaporation of the solvents yielded 18 as a yellow oil (1.57 g, 64%).

$^1$H NMR (400 MHz, CDCl$_3$): δ 11.81 (s, 1H), 8.03 (d, $^3J = 7.9$ Hz, 2H), 6.92 (t, $^3J = 7.9$ Hz, 3H), 3.93 (s, 6H).

$^{13}$C NMR (50.3 MHz, CDCl$_3$): δ 168.03, 161.49, 136.29, 118.41, 116.40, 52.44.

HRMS (EI$^+$) calcd. for C$_{10}$H$_{10}$O$_5$: m/z 210.053; found: 210.052.

Anal. calcd. (%) for C$_{10}$H$_{10}$O$_5$: C 57.1, H 4.80; found: C 56.9, H 4.79.

5-{3-[[(tert-Butoxycarbonyl)amino]propanoyl]isophthalic acid (14)

To a solution of the diester 17 (0.525 g; 1.43 mmol) in THF/H$_2$O (20 mL; 1:1 v/v) was added LiOH (0.103 g; 4.29 mmol). The mixture was stirred at room temperature for 48 h. Conc. H$_2$SO$_4$ (96%; 0.24 mL; 4.3 mmol) was added, resulting in a white suspension, which became clear after standing (pH < 1). The solution was extracted with DCM (4 x 10 mL). The combined organic fractions were washed with water (3 x 10 mL) and dried over
Na$_2$SO$_4$. Filtration and evaporation of the solvents yielded 14 as a white solid (0.404 g; 1.19 mmol; 83%). M.p. 270.6-272.4°C (dec.).

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.04 (t, $^3J = 1.4$ Hz, 1H), 7.60 (d, $^4J = 1.4$ Hz, 2H), 6.88 (t, $^2J = 5.2$ Hz, 1H), 4.05 (t, $^3J = 6.2$ Hz, 2H), 3.07 (m, 2H), 1.81 (m, 2H), 1.34 (s, 9H).

$^{13}$C NMR (50.3 MHz, DMSO-$d_6$): $\delta$ 166.4, 158.8, 155.6, 132.6, 122.1, 119.0, 77.5, 65.9, 36.7, 29.1, 28.2.

HRMS (EI$^+$) calcld. for C$_{16}$H$_{21}$NO$_7$: m/z 339.132; found: 339.132.

Anal. calcd (%) for C$_{16}$H$_{21}$NO$_7$: C 56.6, H 6.24, N 4.13; found: C 56.7, H 6.25, N 4.15.

**Dimethyl-2-(3-(tert-butoxycarbonylamino)propoxy)isophthalate (20)**

To a solution of 18 (1.1 g, 4.7 mmol) and K$_2$CO$_3$ (3.24 g, 23.5 mmol) in DMF (150 mL) was added a solution of 16 (1.0 g, 4.7 mmol) in DMF (10 mL). After heating at reflux overnight, the suspension was filtered and the supernatant was concentrated. DCM (50 mL) was added to the residue and the resulting solution was washed with water (3 x 20 mL) and brine (20 mL). The product was isolated after drying (Na$_2$SO$_4$), filtration and evaporation of the solvent. The product 20 was purified by column chromatography (SiO$_2$; n-pentane/EtOAc 5:1 to 3:1) and isolated as a yellow oil (1.30 g, 3.5 mmol, 75%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.95 (d, $^3J = 7.8$ Hz, 2H), 7.18 (d, $^3J = 7.8$ Hz, 1H), 5.49 (s, 1H), 4.07 (t, $^3J = 5.6$ Hz, 2H), 3.92 (s, 6H), 3.38 (m, 2H), 1.96 (m, 2H), 1.45 (s, 9H).

$^{13}$C NMR (50.3 MHz, CDCl$_3$): $\delta$ 166.10, 158.83, 156.39, 135.37, 126.79, 123.76, 78.92, 74.16, 52.75, 37.60, 30.14, 28.65.


**2-(3-(tert-Butoxycarbonylamino)propoxy)isophthalic acid (15)**

To a solution of 20 (0.92 g, 2.37 mmol) in THF/H$_2$O (40 mL, 1:1 v/v) was added LiOH (212 mg, 7.10 mmol). After stirring at room temperature for 24 h, conc. H$_2$SO$_4$ (96%, 3.5 mL, 7.10 mmol) was added. The solution was extracted with DCM (5 x 20 mL), the combined organic phases were washed with H$_2$O (3 x 20 mL) and dried over Na$_2$SO$_4$. Filtration and evaporation of the solvent yielded 15 as a white solid (300 mg, 0.88 mmol, 68%). M.p. 148-149°C.

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.80 (m, 2H), 7.25 (m, 1H), 6.75 (s, 1H), 3.95 (m, 2H), 3.09 (m, 2H), 1.78 (m, 2H), 1.37 (s, 9H).

$^{13}$C NMR (50.3 MHz, DMSO-$d_6$): $\delta$ 167.8, 157.0, 156.2, 134.1, 128.6, 124.3, 78.2, 74.3, 38.9, 31.0, 28.9.


Anal. calcd. (%) for C$_{16}$H$_{21}$NO$_7$: C 56.6, H 6.24, N 4.13; found: C 57.1, H 6.47, N 4.02.
Towards a higher degree of double strand DNA cleavage


To a mixture of aminopropyl N4Py 1 (0.324 g; 0.693 mmol), HOBt (94 mg; 0.696 mmol) and diacid 14 (0.107 g; 0.315 mmol) in DCM (40 mL) was added EDCI (0.187 g; 2.04 mmol) and DIEA (89.9 mg; 115 μL; 0.696 mmol). The reaction mixture was stirred overnight at room temperature. Aq. NaHCO₃ (20 mL) was added and the layers were separated. The organic phase was washed with aq. NaHCO₃ (2 x 20 mL) and water (20 mL). Drying over Na₂SO₄, filtration and evaporation yielded a yellow foam (0.417 g; 0.337 mmol; quant.). Further purification of 21 could be obtained via size exclusion chromatography (Sephadex® LH 20/MeOH) or via preparative RP-HPLC.

**1H NMR (400 MHz, CD₃OD):**

δ 8.81 (dd, 4 J = 2.3 Hz, 5 J = 0.7 Hz, 2H), 8.46 (m, 4H), 8.35 (ddd, 3 J = 4.9 Hz, 4 J = 1.7 Hz, 5 J = 0.9 Hz, 2H), 8.09 (dd, 3 J = 8.2 Hz, 5 J = 2.3 Hz, 2H), 7.88 (m, 1H), 7.82-7.61 (m, 14H), 7.52 (d, 3 J = 1.3 Hz, 2H), 7.26 (ddd, 3 J = 6.3 Hz, 5 J = 4.9 Hz, 4 J = 2.4 Hz, 4H), 7.19 (ddd, 3 J = 7.2 Hz, 5 J = 4.9, 4 J = 1.4 Hz, 2H), 5.34 (s, 2H), 4.09 (s, 4H), 3.96 (s, 4H), 3.47 (m, 10H), 3.23 (t, 3 J = 6.8 Hz, 2H), 1.92 (m, 6H), 1.40 (s, 9H).

**13C NMR (100 MHz, CD₃OD):**

δ 168.1, 166.7, 162.7, 159.6, 159.5, 159.1, 157.3, 149.8, 148.3, 147.4, 137.3, 137.2, 136.2, 135.7, 128.8, 124.5, 123.6, 123.0, 122.8, 122.5, 118.1, 116.2, 78.8, 73.2, 65.9, 57.6, 57.2, 37.3, 37.2, 29.4, 29.0, 27.6.

**MS-ESI+:**


**t-Butyl-3-(2,6-bis[6-(((dipyridin-2-ylmethyl)(pyridine-2-ylmethyl)amino)methyl]nicotinamido)propylcarbamoyl]phenoxy)propylcarbamate (22)**

As for 21, using 15 (127 mg, 0.375 mmol), 1 (350 mg, 0.75 mmol), HOBt (112 mg, 0.825 mmol), EDCI (180 mg, 1.16 mmol) and DIEA (0.14 mL, 0.825 mmol). The product was obtained as a light brown foam (322 mg, 0.261 mmol, 70%).

**1H NMR (300 MHz, CD₃OD):**

δ 8.81 (m, 2H), 8.47 (m, 4H), 8.35 (m, 2H), 7.68 (m, 16H), 7.27 (m, 4H), 7.21 (m, 3H), 5.35 (s, 2H), 4.01 (s, 4H), 3.96 (s, 4H), 3.49 (t, 3 J = 6.7 Hz, 4H), 3.30 (m, 8H), 3.18 (t, 3 J = 6.9 Hz, 2H), 1.92 (m, 6H), 1.33 (s, 9H).
13C NMR (50.3 MHz, CD$_3$OD) δ 167.8, 166.7, 162.7, 159.6, 159.0, 157.2, 154.4, 148.8, 148.4, 147.5, 137.2, 135.8, 132.0, 130.2, 128.8, 124.5, 124.2, 123.7, 123.0, 122.9, 122.6, 78.8, 74.1, 73.2, 57.6, 37.3, 30.3, 29.2, 27.7.

MS-ESI$: m/z 621 [M + 2H$^+$]$^2$, 415 [M + 3H$^+$]$^3$.

**5-(3-Aminopropoxy)-N$_1$N$_3$-bis(3-[[6-[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl]-3-pyridinyl]carbonyl]amino[propyl]isophthalamide (23)**

Ligand 21 (0.417 g; 0.337 mmol) was taken up in DCM (9 mL) and TFA (1 mL) was added carefully to this solution. After stirring overnight at room temperature, 2M aq. NaOH was added to the mixture until pH > 10. The resulting solution was stirred for an additional 10 min, before the solution was extracted with DCM (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL) and dried over Na$_2$SO$_4$. Filtration and evaporation of the solvents yielded a brown/yellow oil, which was purified over a Sephadex® LH-20 column with MeOH as the mobile phase. A light brown solid was obtained (0.166 g, 0.146 mmol; 43%).

1H NMR (400 MHz, CD$_3$OD): δ 8.81 (d, $^3J$ = 2.3 Hz, 2H), 8.45 (m, 4H), 8.35 (ddd, $^3J$ = 4.9 Hz, $^4J$ = 1.6 Hz, $^5J$ = 0.89 Hz, 2H), 8.09 (dd, $^4J$ = 8.2 Hz, $^5J$ = 2.3 Hz, 2H), 7.89 (t, $^3J$ = 1.5 Hz, 1H), 7.82-7.62 (m, 14H), 7.53 (d, $^4J$ = 1.5 Hz, 2H), 7.26 (ddd, $^3J$ = 6.3 Hz, $^4J$ = 4.9 Hz, $^5J$ = 2.4 Hz, 4H), 7.19 (ddd, $^4J$ = 7.2 Hz, $^5J$ = 4.9 Hz, $^3J$ = 1.4 Hz, 1H), 5.34 (s, 2H), 4.15 (t, $^3J$ = 6.1 Hz, 2H), 4.01 (s, 4H), 3.96 (s, 4H), 3.47 (m, 8H), 2.89 (t, $^3J$ = 7.0 Hz, 2H), 1.99 (m, 2H), 1.90 (dd, $^3J$ = 6.4 Hz, $^4J$ = 6.3 Hz, 4H).

13C NMR (75.5 MHz, CD$_3$OD): δ 168.0, 166.7, 162.7, 159.6, 159.4, 159.1, 148.8, 148.4, 147.4, 137.2, 135.7, 128.8, 124.5, 123.7, 123.0, 122.8, 122.6, 118.2, 116.2, 73.3, 66.3, 57.6, 57.2, 38.2, 37.3, 37.2, 31.1, 29.0.

MS-ESI$: m/z 1160.5 [M + Na$^+$]$^+$, 1138.8 [M + H$^+$]$^+$, 968.6 [M – Py-CH$_2$-Py + H$^+$]$^+$, 570.5 [M + 2H$^+$]$^2$, 380.5 [M + 3H$^+$]$^3$.

As 23, only with 22 (320 mg, 0.26 mmol). Compound 24 was obtained as a yellow foam (200 mg, 0.176 mmol, 68%).

1H NMR (300 MHz, CD$_3$OD): δ 8.81 (m, 2H), 8.47 (m, 4H), 8.35 (m, 2H), 8.10 (m, 2H), 7.68 (m, 16H), 7.27 (m, 4H), 7.21 (m 3H), 5.35 (s, 2H), 4.05 (t, $^3J$=6.1 Hz, 2H), 4.02 (s, 4H), 3.96 (s, 4H), 3.49 (m, 8H), 2.78 (t, $^3J$=6.9 Hz, 2H), 1.93 (m, 6H).

13C NMR (50.3 MHz, CD$_3$OD): δ 167.9, 166.8, 162.7, 159.6, 159.1, 154.3, 148.8, 148.4, 147.4, 137.2, 135.7, 131.9, 130.4, 128.8, 124.5, 123.7, 123.0, 122.9, 122.5, 73.3, 57.6, 57.2, 53.6, 37.7, 29.1.

MS-ESI$: m/z 380 [M + 3H$^+$]$^3$. 

114
Towards a higher degree of double strand DNA cleavage

5-[3-(9-Acridinylamino)propoxy]-N,N'-bis(3-[[6-[[di(2-pyridinyl)methyl]2-pyridinylmethyl]amino][methyl]-3-pyridinyl]carbonyl]amino[propyl]isophthalamide (12)

A mixture of amine 23 (92 mg; 81 μmol), 9-chloroacridine (17.3 mg; 81 μmol) and phenol (1.0 g) was heated at 80°C for 3 h. After cooling to room temperature, Et2O (10 mL) was added, which resulted in the formation of a yellow/brown precipitate. After stirring for 5 min, the Et2O was decanted and fresh Et2O (10 mL) was added. The yellow suspension was stirred overnight. After decanting of the Et2O, again fresh Et2O (10 mL) was added and the suspension was placed in an ultrasonic bath for 10 min. After stirring for an additional hour, the Et2O was removed and the yellow solid was isolated and dried. This HCl salt of 12 was isolated in a quantitative yield. 100 mg of this yellow solid was taken up in 2M NaOH (10 mL) and extracted with DCM (3 x 10 mL). The combined organic phases were washed with 2M NaOH (3 x 10 mL) and dried over Na2SO4. Filtration and evaporation yielded 12 a yellow oil (95 mg; quant.). Pure material was obtained with preparative RP-HPLC.

1H NMR (400 MHz, CD3OD): δ 8.80 (dd, 4 J = 2.3 Hz, 5 J = 0.5 Hz, 2H), 8.44 (m, 4H), 8.33 (dd, 4 J = 5.0 Hz, 5 J = 1.4 Hz, 2H), 8.29 (d, 4 J = 8.7 Hz, 2H), 8.07 (dd, 4 J = 8.2 Hz, 4 J = 2.3 Hz, 2H), 7.83 (t, 4 J = 1.4 Hz, 1H), 7.81-7.55 (m, 20H), 7.30 (ddd, 3 J = 8.7 Hz, 4 J = 6.9 Hz, 5 J = 1.4 Hz, 2H), 7.24 (dd, 4 J = 6.0 Hz, 4 J = 4.9 Hz, 4 J = 2.6 Hz, 4H), 7.16 (ddd, 4 J = 6.9 Hz, 5 J = 5.0 Hz, 4 J = 1.5 Hz, 2H), 5.33 (s, 2H), 4.15 (t, 4 J = 5.6 Hz, 2H), 4.11 (t, 4 J = 6.6 Hz, 2H), 3.99 (s, 4H), 3.94 (s, 4H), 3.46 (m, 8H), 2.28 (m, 2H), 1.89 (m, 4H).

13C NMR (100 MHz, CD3OD): δ 167.9, 166.7, 162.7, 159.6, 159.1, 159.0, 153.7, 148.8, 148.3, 147.4, 137.22, 137.16, 136.2, 135.7, 130.731, 128.7, 126.5-126.0, 124.4, 123.9, 123.6, 123.0, 122.8, 122.5, 122.4, 118.2, 116.02, 115.97, 73.2, 65.9, 57.6, 57.2, 48.7, 37.24, 37.18, 30.3, 29.0.


RP-HPLC (Waters XTerra MS C18 column (3.5 μm, 3.0 x 150 mm), flow rate 0.5 mL/min of 10 mM ammonium acetate (pH = 5.5) and methanol as mobile phase. A linear gradient of 40% to 70% methanol was employed): Rf = 40.6 min (Figure 5.4, left).
Chapter 5

2-(3-(Acridin-9-ylamino)propoxy)-N1,N3-bis(3-(6-(((dipyridin-2-ylmethyl)(pyridine-2-ylmethyl)amino)methyl)nicotinamido)propyl)isophtalamide (13)

As for 12, using 24 (150 mg, 0.13 mmol), 9-chloroacridine (30 mg, 0.13 mmol) and phenol (1.0 g, 10 mmol). After basic workup, 13 was obtained as a yellow foam. Yield 13 (170 mg, 0.13 mmol, 99%). Pure material was obtained with preparative RP-HPLC.

\[ \text{H NMR (300 MHz, CD}_2\text{OD):} \delta 8.73 (m, 2H), 8.44 (s, 4H), 8.32 (m, 2H), 7.98 (m, 2H), 7.74 (m, 16H), 7.67 (m, 8H), 7.25 (m, 4H), 7.16 (m, 3H), 5.31 (s, 2H), 4.20 (t, \frac{J}{2} = 5.8 \text{ Hz}, 2H), 4.08 (t, \frac{J}{2} = 6.5 \text{ Hz}, 2H), 3.95 (s, 2H), 3.91 (s, 2H), 3.31 (m, 8H), 2.25 (m, 2H), 1.80 (m, 4H). \]

\[ \text{C NMR (50.3 MHz, CD}_2\text{OD):} \delta 168.0, 166.6, 162.5, 147.3, 137.2, 135.6, 131.7, 130.6, 128.6, 124.4, 123.6, 122.9, 122.8, 122.5, 74.3, 73.2, 57.6, 57.1, 53.7, 37.1, 31.1, 29.1. \]

\[ \text{MS-ESI+:} m/z 658 [M + 2H]^2+, 439 [M + 3H]^3+. \]

RP-HPLC (Waters Xbridge C18 analytical column (3.5 μm, 4.6 x 150 mm), flow rate 0.8 mL/min of 10 mM ammonium acetate (pH = 5.5) and MeOH as the mobile phase. A linear gradient of 40% to 70% MeOH was employed): Rf = 27.0 min (Figure 5.4, right).

5.6 References and notes

1. See Appendix for abbreviations used.
Towards a higher degree of double strand DNA cleavage

25 RP-HPLC analysis requires a moderate pH of the mobile phase (i.e. pH > 5.0) for a reliable analysis. A lower pH results in decomposition of the material during the chromatography.
31 Signals missing due to peak overlap.