Bimetallic Cu^{2+} complexes of bis-terpyridine ligands as catalysts of the cleavage of mRNA 5′-cap models. The effect of linker length and base moiety

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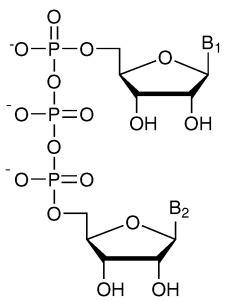
Received (in Montpellier, France) 3rd December 2008, Accepted 7th April 2009
First published as an Advance Article on the web 12th May 2009
DOI: 10.1039/b821724f

Ligands, where two terpyridine units are linked *via* an alkyl chain of three to five methylene units, have been synthesized. Their Cu^{2+} complexes have been studied as catalysts for the hydrolysis of the triphosphate bridge of three different dinucleoside triphosphates. The results show that the bimetallic complexes are up to 600 times more efficient catalysts than monomeric Cu^{2+}-TerPy, and up to 5×10^5-fold rate enhancement in comparison to the uncatalysed reaction, is achieved. However, the catalytic activity strongly depends on the length of the linker and the base composition of the substrate. The differences can be attributed to interactions between the Cu^{2+}-TerPy and nucleic acid base moieties as well as steric factors that may hinder the productive interaction between the substrate and the catalyst.

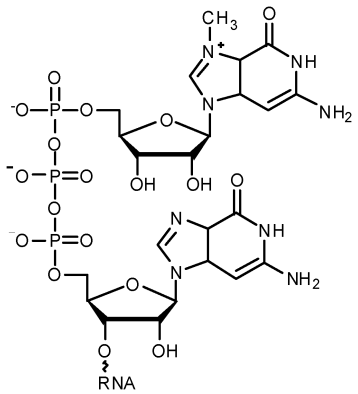
Introduction

Hydrolysis of dinucleoside triphosphates has been studied[1] to understand the reactions of the 5′-terminal *cap* structure (Scheme 1) found in RNA polymerase I synthesized mRNA molecules. While 5′-*cap* containing the more labile N^7-methyl guanine base undergoes three different hydrolytic reactions,[1] the only reaction of substrates such as diadenosine triphosphate (**1a**) under neutral conditions, is the hydrolysis of the triphosphate bridge.[2] This reaction is very slow, as it involves a nucleophilic attack of water molecule on a negatively charged phosphate,[2] and in the case of 5′-*cap* the rate of the hydrolysis of the triphosphate bridge is negligible in comparison to the hydrolysis of *N*-glycosidic bond[1,3] or the cleavage of N^7-methyl guanine base.[1,4] Presumably the spontaneous hydrolysis of the triphosphate bridge is independent of the bases present in the substrate, since nucleoside moieties are not involved in the hydrolysis of the triphosphate bridge.[2] Information on the hydrolysis and reactivity of the triphosphate of 5′-*cap* structure can, hence, be obtained with substrates such as **1a–c** containing more stable base moieties.

Metal ion catalysts, such as complexes of Cu^{2+} and trivalent lanthanide ions efficiently enhance the triphosphate hydrolysis, and the most efficient catalysts reduce the half-life of the reaction from hundreds of years to few hours at 37 °C.[1,5] While the mechanism of the metal ion promoted hydrolysis of internucleosidic phosphodiester bonds of RNA has been extensively studied, and mechanisms are well established,[6] less is known about the hydrolysis of the triphosphate linkage of dinucleoside triphosphates. Intramolecular transesterification of phosphodiester bonds of RNA[7] and the hydrolysis of the phosphoanhydride linkage involving an external nucleophile[2] are mechanistically different reactions, and hence the mechanisms of the metal ion promoted reactions are also likely to be different. In the case of dinucleoside triphosphates, a clear second-order dependence on the concentration of metal ion catalysts has been observed.[5c,e] This suggests that two metal ions are involved, and it has been proposed that a water ligand of one metal ion acts as a nucleophile while coordinated metal ions facilitate the reaction by electrostatic interactions with the triphosphate bridge.[5] The second-order dependence observed



1a: B^1=B^2= Adenine
1b: B^1=Adenine, B^2=Uracil
1c: B^1=B^2= Uracil



Scheme 1

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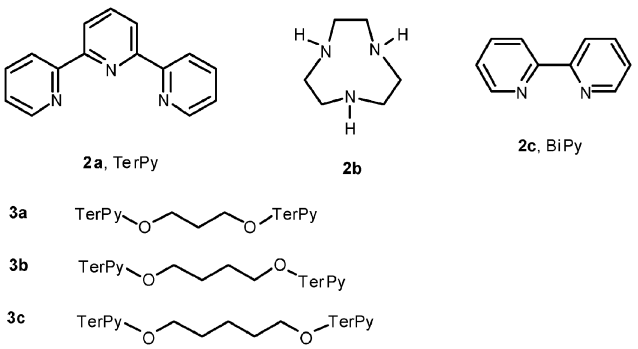
suggests also that efficient catalysts can be obtained by joining two metal ion complexes. Consistent with this, it has been previously shown that bimetallic Cu^{2+} complexes of ligands containing 1,4,7-triazacyclononane (**2b**) as the chelating units, are approximately a hundred times as efficient catalysts as a single Cu^{2+} 1,4,7-triazacyclononane complex.[5c]

In our work we set out to study in more detail the catalysis by bimetallic complexes, and the effect of the structure of the substrate and of the catalyst has been investigated. Cu^{2+}-terpyridine (Cu^{2+}-**2a**) was chosen as the catalytic unit, since Cu^{2+}-TerPy (TerPy = 2,2′:6′,2″-terpyridine) is a robust complex that has been extensively studied as a catalyst of the cleavage of phosphoesters.[8] Our previous studies[5e] with Cu^{2+}-terpyridine (Cu^{2+}-**2a**) have shown that while the complex enhances the hydrolysis of **1a** only modestly, the reaction shows a clear second-order dependence on the complex concentration. Dimers of appropriate structure could, hence, be expected to show clearly enhanced catalytic activity in comparison to monomeric Cu^{2+}-TerPy. Alkyl linked bis-terpyridine ligands were a logical choice, since the flexible alkyl linker could be expected to disturb the interaction between the metal ion chelates and the triphosphate as little as possible. Furthermore, alkyl linked bis-terpyridine ligands are well-known compounds and several reports on their metal ion complexes have been published.[9] A series of terpyridine based catalysts (**3a–c**) was, therefore, synthesized and the catalytic activity of their Cu^{2+} complexes in hydrolysis of three different dinucleoside triphosphate substrates (**1a–c**) was studied.



Results and discussion

The cleavage of the dinucleoside triphosphate substrates **1a–c** was studied at pH 7.0 in the presence of 1 mM Cu^{2+} complexes. The reaction solutions were prepared by mixing appropriate volumes of buffer and metal complex stock solutions. Reaction solutions containing $2Cu^{2+}$-**3c** were slightly cloudy, particularly after the addition of the substrate. Solubility was improved upon addition of acetonitrile (50% in reaction solution), but this resulted in clear decrease of the catalytic activity of $2Cu^{2+}$-**3b** and $2Cu^{2+}$-**3c**. The concentration of the substrate as detected by CZE (capillary zone electrophoresis) analysis was, however, same in cloudy and clear solutions, and therefore the kinetic experiments were carried out without acetonitrile.

The cleavage of dinucleoside triphosphates to a nucleoside monophosphate and a nucleoside diphosphate was followed by capillary zone electrophoresis (CZE). First-order rate constants were calculated on the basis of the decrease of the normalized signal area as a function of reaction time using the integrated first-order rate law. Eight to twelve consecutive aliquots were withdrawn at appropriate time intervals and analyzed as described in the Experimental section. Aliquots were kept on an ice-bath until the analysis. No other quenching method was utilized due to practical problems encountered. Adding EDTA or phosphate buffer to deactivate the metal ions complexes resulted in formation of a precipitate that prevented the analysis. Treatment with chelating agents, such as Chelex, was not possible because of the small sample volumes.

Analysis of complex samples required long analysis times and extensive flushing of the capillary between each sample. This caused problems with the analysis, since in the absence of an efficient quenching method, the reactions tended to proceed while the aliquots were awaiting the analysis, even when kept on an ice-bath. Rate constants were therefore determined also on the basis of individually prepared samples: the reaction solution was prepared and reaction was allowed to proceed while the capillary was being conditioned for the analysis. Once the capillary was ready, the sample was injected immediately. The procedure was repeated a few times varying the reaction time. The mol fraction of the substrate remaining was determined and the integrated first-order rate law was applied to calculate a rate constant. Values given in Table 1 are mean values of two–five of such individual values. This method was applied also for the reactions involving ligand **3c**. The $2Cu^{2+}$-**3c** complex tended to block the capillary after the analysis of a few samples and therefore sequence analysis of several samples was not possible.

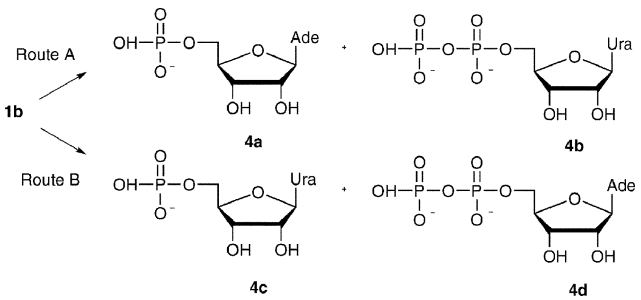
In cases where both methods were applied to calculate the rate constants, values obtained were fully consistent with each other. Calculation of rate constants on the basis of a sequence of samples is, in principle, the most reliable method, but in the present case, suffers from a slight uncertainty because the reaction could not be quenched entirely. Analysis of an individual sample, as described above, is carried out immediately after the sample is withdrawn and therefore shows the extent of the reaction at that precise moment. Since both methods still gave the same rate constants, it can be concluded that the potentially insufficient quenching does not distort the results, but the rate constants based on the analysis of sequences of 10–12 samples also reliably describe the progress of the reactions.

The cleavage of dinucleoside triphosphates yielded a nucleoside monophosphate and diphosphate as products as is shown in Scheme 2. In the case of ApppU (**1b**), two pairs of products were formed, AMP (**4a**) + UDP (**4b**) and UMP (**4c**) + ADP (**4d**) (routes A and B, respectively). Products were identified by spiking with authentic samples. The mole fractions of adenosine and uridine nucleotides were normalized using the molar adsorbtion coefficients reported for AMP and UMP.[10] No other reaction other than the hydrolysis of the triphosphate bridge was observed. Hydrolysis of a nucleoside diphosphate to the corresponding monophosphate was not observed to any significant extent either.

Table 1 Rate constants of the hydrolysis of dinucleoside triphosphates **1a–c** in the presence of Cu^{2+}-TerPy catalysts at pH 7.0 and 60 °C

Catalyst	10^5k(**1a**)/s^{-1}	10^5k(**1b**)[b]/s^{-1}	10^5k(**1c**)/s^{-1}
$2Cu^{2+}$-**3a** (1 mM)	4.7 ± 0.6	2.3 ± 0.1 (55%)	1.2 ± 0.2
$2Cu^{2+}$-**3b** (1 mM)	150 ± 10	420 ± 20 (25%)	460 ± 20
$2Cu^{2+}$-**3c** (1 mM)	60[a]	160[a] (34%)	170[a]
Cu^{2+} -TerPy(**2a**) (2 mM)	1.28 ± 0.03	1.46 ± 0.03 (60%)	0.78 ± 0.03
Cu^{2+}-BiPy(**2c**) (2 mM)[c]	16[d]	10.1 ± 0.2 (63%)	4.5 ± 0.1

[a] Average of two–four individual rate constants calculated on the basis of single sample. [b] Percentage of reaction route resulting in formation of AMP + UDP (route A, Scheme 2). [c] At pH 7.5 and 60 °C. [d] From ref. 5*e*.



Scheme 2

Rate constants of the hydrolysis of **1a–c** in the presence of Cu^{2+} complexes of TerPy (**2a**) and *bis*-TerPy ligands (**3a–c**) are collected in Table 1. The results show that the bis-terpy complexes of Cu^{2+} are significantly more efficient catalysts than monomeric Cu^{2+}-TerPy, as expected, but the rate-enhancement achieved depends clearly on the length of the linker joining the two terpyridine moieties. When the terpyridines are joined with a propyl linker, the Cu^{2+} complex is only marginally better a catalyst than a single Cu^{2+}-TerPy complex. Complexes of ligands **3b** and **3c**, with butyl and pentyl linkers respectively, are, in contrast, clearly more efficient catalysts than Cu^{2+}-TerPy. The catalytic advantage achieved varies also with different substrates. The hydrolysis of ApppA with $2Cu^{2+}$-**3b** is 120 times faster than with Cu^{2+}-TerPy. With ApppU and UpppU the differences are 290- and 590-fold, respectively. $2Cu^{2+}$-**3c** appears to be a slightly less active catalyst than $2Cu^{2+}$-**3b** with all substrates studied.

The rate-enhancement obtained with the bis-Cu^{2+} complexes of ligands **3b** and **3c** is even higher than observed with other bimetallic catalysts. Comparison between two systems that have been studied under different conditions is difficult, but in this case an extrapolation can be attempted on the basis of available data. The catalysis by the bis-Cu^{2+} complexes based on **2b**, reaches maximal efficiency at about 1 mM catalyst concentration, and the saturating rate constants at pH 7.3 and 37 °C are 4–7 × 10^{-5} s^{-1}.[5c] At 25 °C and pH 7.0 the rate constants of the hydrolysis of **1a–c** in the presence of 1 mM $2Cu^{2+}$-**3b** vary from 0.9 to 2.0 × 10^{-4} s^{-1}. It is known that below pH 7.5 the Cu^{2+}-TerPy reaction shows a first-order dependence on hydroxide ion concentration.[5e] Furthermore, an approximately three-fold rate enhancement could be expected on going from 25 to 37 °C.[2] Using this information it can be estimated that the rate constants obtained at 1 mM concentration of $2Cu^{2+}$-**3b** are approximately ten times larger than the maximal values obtained with bimetallic catalysts based on **2b**. A more accurate comparison would be obtained if the maximal rate constant referring to saturating concentration of $2Cu^{2+}$-**3b** was known, but due to poor solubility of the complex, results obtained at higher concentrations were difficult to interpret, and hence such experiments were not attempted. Using the same kind of reasoning as above, the catalysis by $2Cu^{2+}$-**3b** can be estimated to be of the same order as that obtained with Eu^{3+} -THED (THED = 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane) complex together with $Zn^{2+}{}_{aq}$, which is the most efficient catalyst for triphosphate hydrolysis reported to date.[5d]

Comparison to the rate of the uncatalysed reaction shows that the rate-enhancement by the bimetallic complexes is remarkable. Previously reported data[2] allows a fairly reliable estimate of 1 × 10^{-8} s^{-1} for the rate constant for the hydrolysis of the triphosphate bridge of dinucleoside triphosphates under neutral conditions at 60 °C. The rate constants collected in Table 1 represent, hence, a 150 000 to 500 000-fold rate enhancement by the complexes.

The proportion of the reaction route resulting in formation of AMP and UDP (route A in Scheme 2) is also included in Table 1, and this data shows that while the cleavage by the less efficient catalysts Cu^{2+}-TerPy and $2Cu^{2+}$-**3a** slightly favor reaction route A, the catalysis by $2Cu^{2+}$-**3b** and $2Cu^{2+}$-**3c** results predominantly in formation of UMP and ADP as products (route B). A 1.5-fold excess of AMP over UMP is observed in the former case, whereas the latter reactions produce UMP in 3- to 4-fold excess.

The results obtained clearly show that two TerPy-moieties can enhance the hydrolysis co-operatively provided that the linker joining the metal complexes is long enough. While this is clearly possible with complexes of ligands with butyl and pentyl linkers, the propyl linker is too short to allow the simultaneous interaction of the two complexes with the phosphate group. Since the rate constants are nearly the same, it seems that $2Cu^{2+}$-**3a** acts in the same way as a single Cu^{2+}-TerPy complex. The cleavage pattern of ApppU is also similar to that of Cu^{2+}-TerPy supporting the suggestion that the catalysis mechanisms are the same.

Co-operative action of two Cu^{2+}-TerPy complexes observed for $2Cu^{2+}$-**3b** and $2Cu^{2+}$-**3c** shows a slight preference for uracil containing substrates **1b** and **1c** over diadenosine triphosphate **1a**. In the presence of these catalysts, the reaction of **1a** is slower than that of **1b** and **1c**, and the cleavage site closer to the uracil base is preferred. This is in contrast to the situation with Cu^{2+}-TerPy, Cu^{2+}-BiPy and $2Cu^{2+}$-**1a**, which cleave adenine containing substrates faster, and which prefer a cleavage site closer to the adenine base. A likely explanation of these differences arises from interactions

between the ligands and nucleic acid bases. Since the smaller catalysts (Cu^{2+}-TerPy, Cu^{2+}-BiPy) show a slight preference for adenine containing substrates, it can be suggested that TerPy and BiPy complexes tend to interact with adenine base, either *via* direct coordination of the Cu^{2+} ion or *via* a hydrophobic interaction between the aromatic rings of adenine base and the ligands. The interaction brings the catalyst closer to the substrate and thus facilitates the cleavage. The bifunctional catalysis by $2Cu^{2+}$-**3b** and $2Cu^{2+}$-**3c** may, however, require a more ordered structure, where two catalytic moieties interact with the phosphate group. The linker will also have to be accommodated. The interaction between the adenine base and the Cu^{2+} complex may, in this case, interfere with the catalysis by steering the catalyst away from the triphosphate bridge, and therefore the adenine containing substrates react more slowly than diuridine triphosphate. The interactions may also dictate the predominant cleavage site in ApppU and steers the catalyst closer to the uridine site of the molecule.

Conclusions

Efficient catalysts of the cleavage of dinucleoside triphosphates can be obtained by joining two terpyridine moieties with a simple alkyl linker. Co-operative catalysis of the two complexes is, however, achieved only when the linker is long enough. Interactions between the ligand and the nucleic acid bases also influence the catalytic activity.

Experimental

Kinetic experiments

Reaction solutions were prepared in doubly-distilled water. The pH was adjusted with a MOPSO buffer (3-morpholino-2-hydroxypropanesulfonic acid; pK_a 6.9 at 25 °C). The complex was added as a 5 mM stock solution. The complex stock was prepared by weighing an appropriate amount of the ligand, suspending it in water and adding $Cu(NO_3)_2$ solution slowly while vigorously mixing. The stock solutions were checked by UV-spectrometry and the concentrations were found to be the same. UV maxima were detected at 280, 315 and 325 nm.

Reactions were carried out in Eppendorf tubes. The temperature was controlled by a water-bath that was adjusted to 60.0 ± 0.1 or 25.0 ± 0.1 °C. Reactions were followed by withdrawing aliquots at appropriate intervals and analyzing them either by capillary electrophoresis of reversed-phase HPLC. Aliquots were kept on an ice-bath until the analysis. Samples from reactions carried out at 25 °C were analysed immediately.

Capillary electrophoresis analysis was carried out with HP ^{3}D instrument utilizing a UV-detection at 260 nm. Fused silica capillary (77 cm length, 50 μm i.d.) and a 50 mM phosphate buffer, pH 6.0, were used for the separation of the starting material and the products. HPLC analysis was carried out with a Perkin Elmer instrument consisting of two micropumps, autosampler and UV detector. The column was a Supelco LC-18-T reversed-phase column (25 × 4.6 cm, 5 μm particle size). 50 mM phosphate buffer, pH 6.0, was used as an eluent. UV-detection at 260 nm was employed.

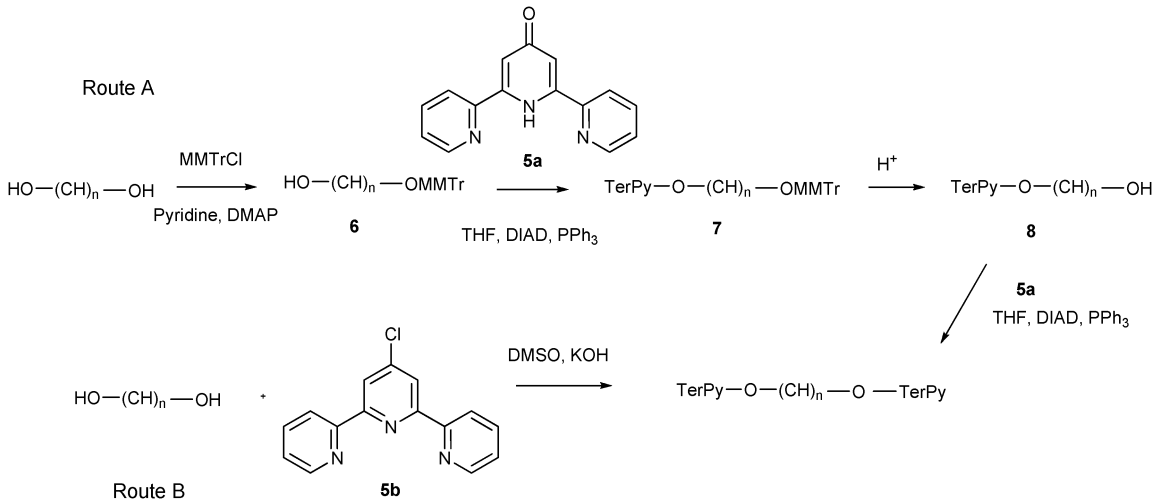
Rate constants were calculated on the basis of the decrease of the mole fraction of the starting material that was calculated on the basis of the normalized signal areas. In case of ApppU, the different molar absorption coefficients[10] of adenine and uracil bases were taken into account when calculating the proportions of the two reaction routes. Signal areas from CE analysis were normalized by dividing them by the migration time. The integrated rate law of a first-order reaction was applied to calculate the rate constants.

Synthetic procedures

Two different methods were used in synthesis of ligands **3a–c**: Sequential addition of two 2,6-bis(2-pyridyl)-4(1*H*)-pyridones (**5a**) by a Mitsunobu reaction with a corresponding diol (route A in Scheme 3) was carried out applying a procedure reported in ref. 11. A more direct method utilized a nucleophilic substitution reaction between 4-chloroterpyridine (**5b**) and a diol (route B in Scheme 3), following a procedure reported[12] for the synthesis of a corresponding ethyl linked bis-terpyridine ligand. Ligand **3b** was synthesized according to route A while **3a** and **3c** were prepared *via* route B, but in principle either method could have been utilized in the synthesis of all bis-terpyridine ligands. Yields obtained with the latter method were clearly better (80% *vs.* 20%). Dinucleoside triphosphate substrates **1b** and **1c** were synthesized according to procedure reported in the literature.[13] Both syntheses and characterization of the products are described below. The diadenosine compound **1a** is commercially available from Sigma. All synthesis products were characterised by ^{1}H NMR spectroscopy. For dinucleoside triphosphates[13] and propyl linked *bis*-TerPy ligand **3a**[9d] this was regarded sufficient as their synthesis and characterization has been reported before. Butyl and pentyl linked *bis*-TerPy ligands **3b** and **3c** were characterized also by HR-MS and ^{13}C NMR. To improve the solubility of the ligands, they were treated with aqueous HCl and then dissolved in D_2O for ^{13}C NMR analysis.

4-((4-Methoxyphenyl)diphenylmethoxy)butan-1-ol (6). Butane-1,4-diol (16.19 mmol, 1.43 mL), 1 eq. of monomethoxytrityl chloride and a catalytic amount of DMAP in 75 ml of pyridine were stirred at room temperature for one day. After evaporation, the crude product was purified on silica gel with dichloromethane as eluent. δ_H (400 MHz, $CDCl_3$): 1.69 (4H, m, $HOCH_2CH_2CH_2CH_2O$–), 3.11 (2H, t, $HOCH_2CH_2CH_2CH_2O$–), 3.64 (2H, t, $HOCH_2CH_2CH_2CH_2O$–), 3.79 (3H, s, CH_3O–), 7.2–7.45 (14H, m, arom. *H*).

1-((4-Methoxyphenyl)diphenylmethoxy))-4-(2,2′:6′,2″-terpyridine-4-yloxy)butane (7). Compound **6** (2 mmol, 0.7 g), 0.8 eq. of 2,6-bis(pyridine-2-yl)pyridine-4(1*H*)-one (**5a**; 1.6 mmol, 0.41 g) and 1 eq. of triphenylphosphine (2 mmol, 0.52 g) were dissolved in dry THF (50 mL). 1 eq. of DIAD (2 mmol, 0.4 mL) was added dropwise and the mixture is stirred during 2 h at room temperature. The pale and clear pink mixture obtained was purified on basic aluminium oxide using dichloromethane as the eluent. Product **7** was obtained as a white solid by precipitation from diethyl ether. δ_H (400 MHz,



Scheme 3

$CDCl_3$); 1.9 (4 H, m, –$OCH_2CH_2CH_2CH_2O$–), 3.17 (2 H, t, TrO$CH_2CH_2CH_2CH_2O$–TerPy), 3.79 (3H, s, CH_3O–), 4.22 (2 H, t, TrO$CH_2CH_2CH_2CH_2O$–TerPy), 7.2–7.45 (24H, m, arom. *H*).

4-(2,2′:6′,2″-Terpyridine-4-yloxy)-butan-1-ol (8). Compound **7** was dissolved in 80% acetic acid in water–acetonitrile (1 : 1) and stirred for 24 h. The product was purified on silica gel CH_2Cl_2–MeOH, 95 : 5 as the eluent. δ_H (400 MHz, $CDCl_3$): 1.69 (4 H, m, –$OCH_2CH_2CH_2CH_2O$–), 3.13 (2 H, t, HO$CH_2CH_2CH_2CH_2O$–), 3.63 (2 H, t, HO–$CH_2CH_2CH_2$-CH_2O–TerPy), 7.2–7.45 (10 H, m, arom. *H*).

1,4-Bis(2,2′:6′,2″-terpyridine-4-yloxy)butane (3b). Compound **8** (2.8 mmol, 0.9 g), 0.8 eq. of 2,6-bis(pyridine-2-yl)-pyridine-4(1*H*)-one (2.2 mmol, 0.55 g) and 1 eq. of triphenylphosphine (2.8 mmol, 0.74 g) were dissolved in (dry) THF (50 mL). 1 eq. of DIAD (2.8 mmol, 0.55 mL) was added dropwise and the mixture was stirred for 2 h at room temperature. The pale and clear pink mixture obtained was purified on basic aluminum oxide using dichloromethane as the eluent followed by precipitation from diethyl ether to yield compound **3b**. δ_H (400 MHz, CD_2Cl_2): 2.16 (4 H, m, –$OCH_2CH_2CH_2CH_2O$–), 4.28 (2 H, t, –$OCH_2CH_2CH_2$-CH_2O–), 7.36 (4H, m, *H5*, *H5″*), 7.86 (4H, m, *H4*, *H4″*), 8.07 (4H, s, *H3′*, *H5′*), 8.63 (4H, d, *H3*, *H3″*), 8.68 (4H, m, *H6*, *H6″*). δ_C (100 MHz, D_2O): 30, 70, 112, 124, 127, 142-148, 168; *m*/*z* (EI) 552.2272 ($C_{34}H_{28}N_6O_2$ requires 552.2274).

1,3-Bis(2,2′:6′,2″-terpyridine-4-yloxy)propane (3a). 1,3-Propanediol (13.8 μL, 0.187 mmol) was mixed in 2 mL of DMSO with 100 mg KOH. Separately 158 mg (0.590 mmol) 4′-chloro-2,2′:6′,2″-terpyridine (**5b**) was added in 6 cm^3 of DMSO. The suspensions were stirred for 1 h at 70 °C after which they were mixed. The mixture was heated for 27 h at 70 °C under nitrogen atmosphere and the reaction was followed with $CHCl_3$ as an eluent. After cooling to room temperature, the orange mixture was poured into water (25 cm^3) and NaCl-saturated water (5 cm^3). The milky suspension was extracted with chloroform (3 × 15 cm^3) and the extracts washed with water (10 + 5 cm^3) until the pH remained neutral. The organic layer was evaporated to dryness. The purification was done in two steps: first on alumina eluting with chloroform and then on silica eluting with $CHCl_3$–CH_2Cl_2 (9.2 : 0.8). The ^1H NMR spectrum obtained is consistent with that reported.[9d] δ_H (500 MHz, $CDCl_3$); 2.45 (2 H, m, –$OCH_2CH_2CH_2O$–), 4.51 (4 H, t, –$OCH_2CH_2CH_2O$–), 7.34 (4 H, m, *H5*, *H5″*), 7.85 (4 H, m, *H4*, *H4″*), 8.09 (4 H, s, *H3′*, *H5′*), 8.64 (4 H, d, *H6*, *H6″*), 8.71 (4 H, d, *H3*, *H3″*).

1,5-Bis(2,2′:6′,2″-terpyridine-4-yloxy)pentane (3c). 1,5-Pentanediol (20.3 μL, 0.187 mmol) was mixed in 2 mL of DMSO with 100 mg KOH. Separately 200 mg (0.740 mmol) 4′-chloro-2,2′:6′,2″-terpyridine was added in 6 cm^3 of DMSO. The details of the reaction and work-up are similar to those described above. The solid residue was washed with cyclo-hexane to remove an unknown by-product. The product was purified by column chromatography on silica gel eluting with CH_2Cl_2–MeOH (9.2 : 0.8). NMR analysis shows that there is some pentanediol left in the product, which could not be fully removed. This was taken into account and the concentration of the complexes was checked by UV-spectrometry. δ_H (500 MHz, $CDCl_3$): 1.6–1.7 (6 H, m, –$OCH_2CH_2CH_2CH_2$-CH_2O–), 4.26 (4 H, m, –$OCH_2CH_2CH_2CH_2CH_2O$–), 7.35 (4 H, t, *H5*, *H5″*), 7.86 (4 H, t, *H4*, *H4″*), 8.03 (4 H, s, *H3′*, *H5′*), 8.64 (4 H, d, *H6*, *H6″*), 8.71 (4 H, d, *H3*, *H3″*); δ_C (100 MHz, D_2O): 27, 39, 70, 110, 125, 128, 142–149, 169; *m*/*z* (ESI) 567.2563 ($C_{35}H_{31}N_6O_2^+$ requires 567.2508).

Synthesis of dinucleoside triphosphates

A nucleoside monophosphate triethylammonium salt was first converted to the corresponding imidazolidate in a mixture of anhydrous DMF and triethylamine in the presence of 2,2′-dipyridyl sulfide and triphenylphosphine. The product was crystallized by adding anhydrous $NaClO_4$ and acetone. The nucleotide imidazolidate was coupled to appropriate nucleoside 5′-diphosphate triethylammonium salt in anhydrous dimethylformamide using $ZnCl_2$ as the catalyst. The product was purified on DEAE–Sephadex in HCO_3^- form using a gradient elution from 0 to 0.1 M ammonium bicarbonate

buffer, pH 7.4. In the case of the asymmetric substrate **1b**, AMP-imidazolidate was coupled with UDP. The products were analysed by ^{1}H and ^{31}P NMR spectroscopy. Spectra are fully consistent with those reported previously.[14] **1b**: δ_H (400 MHz, D_2O): 4.06 (4H, m, *H5′*, *H5″*), 4.15 (2H, m, *H4′*), 4.26 (2H, m, *H3′*), 4.40 (2H, m, *H2′*), 5.61 (1H, d, J = 8.0 Hz, *H5*; Ura), 5.70 (2H, d, J = 5.6 Hz, *H1′*), 7.63 (1H, d, J = 8.4 Hz, *H6*; ura), 8.10 (1H, s, *H2*; ade), 8.42 (1H, s, *H8*; ade); δ_P (162 MHz, D_2O): –11,28, –22,59. **1c**: δ_H (400 MHz, D_2O): 4,12 (2H, m, *H5′*,*H5″*), 4,17 (1H, m, *H4′*), 4,25 (1H, m, *H3′*), 4,28 (1H, m, *H2′*), 5,87 (1H, s, *H1′*), 5,87 (1H, d, J = 7,2 Hz, *H5*), 7,86 (1H, d, J = 8,4 Hz, *H6*). δ_P (162 MHz, D_2O): −11,45, −22,86.

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