A lanthanum macrocycle catalysed hydrolysis of 2,4-dinitrophenyl diethyl phosphate and O-isopropyl methylfluorophosphonate (Sarin)

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Abstract—The lanthanum complex of a hexa–aza macrocycle is shown to be an effective catalyst for the hydrolysis of the water soluble phosphate triester 2,4-dinitrophenyl diethyl phosphate. At pH 9, using a catalyst concentration of 2.5×10^{-3} mol dm^{-3} at 25°C, the rate enhancement is *ca* 10^3 fold. The reaction is shown to be catalytic rather than stoichiometric and possible mechanisms involving a metal-bound hydroxide nucleophile are considered to account for the catalysis. The macrocyclic complex is also shown to be an effective catalyst for the hydrolysis of the anticholinesterase agent O-isopropyl methylfluorophosphonate (Sarin). © 1997 Elsevier Science Ltd

Keywords: Lanthanum macrocycle; catalysis; phosphotriester hydrolysis; sarin hydrolysis; kinetics.

Recently there has been growing interest in the metal–ion-promoted hydrolysis of phosphate esters as model systems for metallo–phosphatase enzymes and as potential catalysts for the detoxification of anticholinesterase agents used in chemical warfare [1–7]. In addition, reagents that cleave RNA by promoting transesterification of the phosphate diester linkage of RNA have been the subject of considerable attention [8].

Early studies by Butcher and Westheimer [9] established that lanthanide hydroxide gels promoted the hydrolysis of esters such as β-methoxyethyl and β-aminoethyl phosphates at pH 8.5 and 78°C. Bamann [10] also noted that the hydroxides of La^{3+}, Ce^{3+} and Th^{3+} promoted the hydrolysis of α-glycerophosphate in the pH range 7–10 and suggested that the reaction could be regarded as a model for the metal-containing alkaline phosphatases which cleave phosphate esters around pH 9. Blewett and Watts [11] first observed that in the presence of yttrium(III) the rate of hydrolysis of 4-nitrophenyl methylphosphonate is accelerated by a factor of 10^5–10^6 fold. The catalytically active

yttrium(III) species was considered to be a polymeric hydroxo-complex which acted bifunctionally. Lanthanide salts are also effective in promoting the transesterification of RNA [12] and the decarboxylation of β oxo-acids [13].

Angelici and coworkers [14] carried out a number of elegant investigations on lanthanide ion catalysis of carboxylic ester hydrolysis and established that there was a direct relationship between the catalytic activity of the ion and its ionic potential (charge/ionic radius). In the phosphate ester area, because of the heterogeneous nature of the reactions, it has been difficult to come to firm mechanistic conclusions. However, it seems probable that metal-bound hydroxide nucleophiles are involved.

Lanthanide complexes of macrocyclic ligands have become the subject of considerable interest and the topic has been reviewed [15]. Lanthanide complexes of the macrocycle (1) are fairly robust [16]. Thus [LaL]$^{3+}$ and [EuL]$^{3+}$ undergo little decomposition over 3 days at 37°C and pH 7.0 (8 and less than 5%, respectively) [8]. The present paper discusses the catalytic activity of [LaL]$^{3+}$ towards the phosphate triester 2,4-dinitrophenyl diethyl phosphate (2) and the anticholinesterase agent O-isopropyl methylfluoro phosphonate (Sarin = 3). Since our preliminary report [17] on catalysis by this macrocyclic complex, Morrow and coworkers [8] have shown that the Eu^{III} complex is an

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This paper is dedicated to Professor Ralph G. Wilkins on the occasion of his 70th birthday.

(1)

(2)

(Sarin)

(3)

(4)

(5)
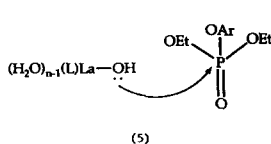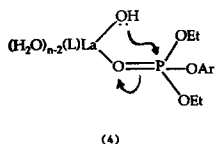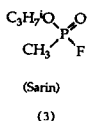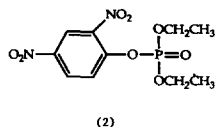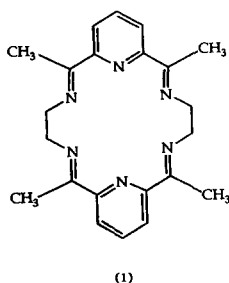
effective catalyst for the hydrolysis of RNA. Rare earth ions have also been shown to rapidly hydrolyse adenylyl(3'-5')adenosine and uridyl(3'-5')uridine at pH 8 and 30°C. The half life of the former substrate is 10 min. with 0.01 mol dm^{-3} Tm^{III} [18].

EXPERIMENTAL

Materials

The macrocyclic complex [LaL(NO$_3$)$_3$] was prepared by template condensation of 2,6-diacetyl-pyridine (Aldrich) with 1,2-diaminoethane. Hydrated lanthanum nitrate (0.325 g, 0.75 mmol), 1,2-diamino-ethane (0.09 g, 1.50 mmol) and 2,6-diacetylpyridine (0.25 g, 1.5 mmol) were refluxed in methanol (30 cm^3) for ca 4 h giving pale pink–brown crystals of the complex (0.38 g, 73%). The crystals were collected, washed with ethanol, then diethyl ether and dried in vacuo. Found: C, 37.6; H, 3.7; N, 18.1. Calc. for C$_{22}$H$_{26}$N$_9$O$_9$La: C, 37.8; H, 3.75; N, 18.0%. ^{1}H NMR (D$_6$-DMSO); TMS), 8.3 (6H), 3.9 (8H), 2.5 (12H). The IR spectrum (KBr disc) had bands at 1633 (C=N); 1588 (pyridine), 1445, 1421, 1374 and 1304 cm^{-1} (NO$_3$). Bands due to NH and C=O were absent from the spectrum. The complex is a 3:1 electrolyte in aqueous solution with $\Lambda_M = 354$ S cm^2 mol^{-1} at 25°C.

2,4-Dinitrophenyl diethyl phosphate (DNPDEP)

Diethyl chlorophosphate (63.0 g, 0.366 mol) was added dropwise to a stirred suspension of dry sodium

2,4-dinitrophenoxide (75.4 g, 0.366 mol) in dry toluene (450 cm^3). On completion of the addition, the mixture was refluxed for 5 h, cooled, washed with ice water (150 cm^3), cold 5% sodium carbonate (150 cm^3) and ice water (150 cm^3). The toluene layer was dried over MgSO$_4$ and concentrated in vacuo to give crude DNPDEP in 60% yield. The crude product was kept in the deep freeze and aliquots purified immediately before use as described below.

CAUTION: The ester displays potent anticholinesterase activity. Great care should be exercised in its handling and use.

Decontamination of glassware and other equipment was carried out using solutions of hypochlorite (bleach). Found: C, 36.9; H, 4.1; N, 8.2 Calc. for C$_{10}$H$_{13}$N$_2$O$_8$P: C, 37.5; H, 4.1; N, 7.7%. ^{13}C NMR (CDCl$_3$, TMS): 16.3, 65.8, 121.6 (CH), 123.6 (CH), 129.1 (CH), 140.7 (C—X), 143.8 (C—X), 148.4 (C—X). IR, 1540 (NO$_2$), 1350 (P=O), 1032 (P—O—alkyl), 1270 (P—O—aryl) cm^{-1}.

Immediately prior to use the ester (1.0 g) was dissolved in ice-cold chloroform (20 cm^3) and the solution shaken in a separating funnel with ice-cold NaHCO$_3$ (25 cm^3, 5%) then ice-cold water (25 cm^3) followed by ice-cold HCl (25 cm^3, 0.1 mol dm^{-3}) then two portions of ice-cold water (25 cm^3). The organic layer was dried over Na$_2$SO$_4$ for several hours and the solvent removed on a rotary evaporator at 40°C.

Kinetic measurements

The hydrolysis of DNPDEP was monitored both by pH-stat and stopped-flow methods. The stopped-flow measurements were unsuitable for the determination of the k_O rate constants, due to the small contribution made by the solvolytic reaction at high base concentrations. pH-Stat measurements were made using a Radiometer Titralab system in the pH-stat mode interfaced with a PC. Hydroxide ion concentrations were derived from the pH using the appropriate values of pK_w [19] and activity coefficients calculated from the Davies' equation [20]. Stopped-flow measurements were made with a Hi-Tech Sample Handling Unit fitted with a grating monochromator, tungsten–halogen light source and EMI 9798 QB 11 stage end window photomultiplier to monitor absorbance changes. The output from the PM tube was fed into an Infotek AD 2000 A to D converter (high speed) and then via a Hewlett–Packard 98622 GP10 16-bit parallel interface to an HP-310 PC, where curve-fitting routines based on the algorithm of Gamp [21] were used to calculate the rate constants. A total of 400 data points were collected during each run, irrespective of the time base used.

In the catalytic studies the hydrolysis of 2,4-dinitrophenyl diethyl phosphate was monitored using the absorbance increase at 360 nm due to the release of 2,4-dinitrophenolate. To avoid complications due to buffer catalysis, the metal complex was employed as

the buffer and the pH was adjusted by the dropwise addition of hydrochloric acid. Plots of $\ln(A_\infty - A_t)$ versus time were linear for several half lives and the rate constants were evaluated from such plots. The ionic strength of the solution was adjusted to $I = 0.1$ mol dm^{-3} with KNO$_3$. The concentration of [LaL(NO$_3$)$_3$] employed in the measurements was 2.5×10^{-3} mol dm^{-3} and the concentration of 2,4-dinitrophenyl diethyl phosphate 4×10^{-5} mol dm^{-3}.

Measurements with Sarin were carried out at CBDE Porton Down. Hydrolysis of O-isopropyl methyl-fluorophosphonate was monitored in solution using a fluoride specific electrode. All measurements were made using HEPES buffer at pH 7.0.

RESULTS AND DISCUSSION

The phosphotriester 2,4-dinitrophenyl diethyl phosphate provides a useful water soluble substrate whose hydrolysis to diethyl phosphate can readily be monitored spectrophotometrically by following the release of 2,4-dinitrophenolate at 360 nm. The hydrolysis of the ester was monitored both by pH-stat and stopped-flow methods. A typical set of pH-stat measurements at 30°C and $I = 0.1$ mol dm^{-3} are summarised in Table 1. A plot of k_{obs} (the observed first order rate constant at constant pH) versus the hydroxide ion concentration is linear with a positive intercept indicating that $k_{obs} = k_o + k_{OH}[OH^-]$, where k_o relates to the solvolytic reaction (attack by water) and k_{OH} is the second order rate constant for base hydrolysis, Fig. 1. At 30°C, $k_o = 1.66 \times 10^{-6}$ s^{-1} and $k_{OH} = 0.42$ dm^3 mol^{-1} s^{-1}.

The results of pH-stat and stopped-flow studies over the temperature range 10–45°C are summarised in Table 2. For base hydrolysis $\Delta H\ddagger = 50.7 \pm 1.0$ kJ mol^{-1} and $\Delta S^\ddagger_{298} = -79 \pm 2$ JK^{-1} mol^{-1}. At 25°C the second order rate constant for water attack is given by $k_o/55.5 = 1.22 \times 10^{-6}/55.5 = 2.2 \times 10^{-8}$ dm^3 mol^{-1} s^{-1}. The relative nucleophilicities of hydroxide and water towards the ester are given by the ratio $3.05 \times 10^{-1}/2.2 \times 10^{-8} = 1.4 \times 10^7$.

Catalytic studies

The complex [LaL(NO$_3$)$_3$] is readily prepared by a [2+2] template cyclisation of 1,2-diaminoethane and

Table 1. Hydrolysis of 2,4-dinitrophenyl diethyl phosphate at 30°C and $I = 0.1$ mol dm^{-3} monitored by pH-stat

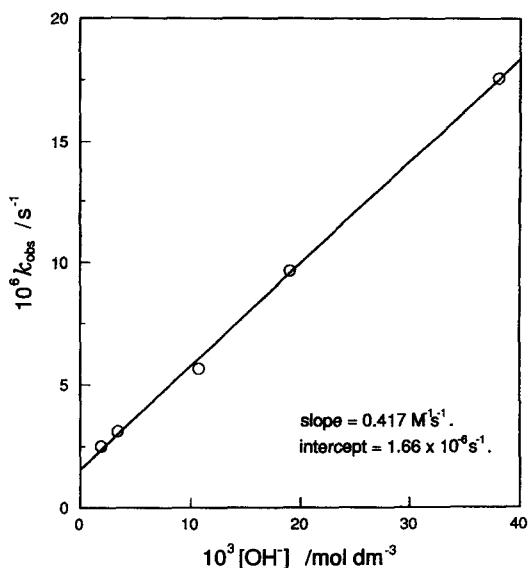| pH	$10^6[OH]^-$ (mol dm^{-3})	$10^6 k_{obs}$ (s^{-1})
8.00	1.91	2.51
8.25	3.39	3.12
8.75	10.73	5.64
9.00	19.07	9.66
9.30	38.06	17.58

Fig. 1. Plot of k_{obs} versus the hydroxide ion concentration for the base hydrolysis of 2,4-dinitrophenyl diethyl phosphate at 30°C monitored by pH-stat at $I = 0.1$ mol dm^{-3} (KNO$_3$).

Table 2. Summary of base hydrolysis (k_{OH}) and solvolytic rate constants $(k_o)^a$ for the hydrolysis of 2,4-dinitrophenyl diethyl phosphate over the temperature range 10 to 45°C at $I = 0.1$ mol dm^{-3} (KNO$_3$)

Temp. (°C)	$10^6 k_o$ (s^{-1})	$10^2 k_{OH}$ (dm^3 mol^{-1} s^{-1})	$10^2 k_{OH}{}^{(a)}$ (dm^3 mol^{-1} s^{-1})
10.0	—	—	11.7
15.0	—	—	16.9
20.0	—	—	22.2
25.0	1.22	30.5	29.2
30.0	1.71	41.7	39.6
35.0	2.26	56.5	57.8
40.0	3.03	75.8	80.2
45.0	4.10	100	101

^{a} Rate constants determined by stopped flow methods.

2,6-diacetylpyridine in the presence of La(NO$_3$)$_3$ in methanol solution. The crystal structure of the complex [20] establishes 12-coordination of the metal ion to the six macrocyclic ring nitrogens and to three bidentate nitrates. Conductivity measurements on aqueous solutions indicate that the complex is a 3 : 1 electrolyte ($\Lambda_M = 354$ S cm^2 mol^{-1}) so that in water the complex exists as [LaL(H$_2$O)$_n$]$^{3+}$. The hexadentate ligand L forms complexes with all lanthanide(III) ions and many of these complexes are resistant to decomposition under a variety of conditions. ^{1}H NMR studies on [EuL]$^{3+}$ and [LaL]$^{3+}$ over a period of 3 days at pH 7.0 and 37°C indicate only some 5 and 8% decomposition, respectively [8].

A comparison of the luminescent lifetimes of [EuL(OH$_2$)$_n$]$^{3+}$ in H$_2$O and D$_2$O indicates that the number of coordinated water molecules is 3 or 4 [8].

As the ionic radii of La^{III} and Eu^{III} are 106.1 and 95 pm, respectively it appears probably that in aqueous solution the La^{III} complex is 9 or 10-coordinate. Most lanthanum salts such as $La(SO_4)_3 \cdot 9H_2O$ are nine coordinate in the solid state [22].

The catalytic studies were carried out with the macrocyclic complex in large excess. The reaction is first order in the phosphate ester and excellent pseudo first order kinetics were observed in the presence of millimolar concentrations of the complex. In order to avoid possible complications due to buffer catalysis the metal complex was employed as the buffer and the pH was adjusted by the dropwise addition of hydrochloric acid. The pH dependence of the catalysis was studied over the pH range 7.0–9.5 (Table 3). The reaction displays a sigmoidal pH-rate profile of the type seen in a number of phosphate ester hydrolyses catalysed by hydroxoaqua complexes of copper(II) and cobalt(III) [23] (Fig. 2). Such pH-rate profiles are indicative of the involvement of metal–hydroxo complexes and suggest that the reaction takes place either by an intramolecular process or an inter-molecular process of the type shown in (4) and (5), respectively.

Potentiometric titration of $[LaL(NO_3)_3]$ in aqueous solution with hydrochloric acid gives the titration curve shown in Fig. 3. A well pronounced end point is observed after addition of one equivalent of hydro-chloric acid consistent with the protonation of a La-OH species. The apparent pK for the equilibrium is ca 7.4.

At pH 9.0, values of k_{obs} for the base hydrolysis of the triester to give diethyl phosphate are given by the expression $k_{obs} = k_o + k_{OH}[OH^-]$ where $k_o = 1.22 \times 10^{-6}$ s^{-1} and $k_{OH} = 0.305$ dm^3 mol^{-1} s^{-1} at 25°C and $I = 0.1$ mol dm^{-3}. The hydroxide ion concentration calculated from the pH using an activity coefficient of 0.774 and $pK_W = 13.997$ is 1.30×10^{-5} mol dm^{-3} giving $k_{obs} = 5.2 \times 10^{-6}$ dm^3 mol^{-1} s^{-1}. At pH 9, k_{obs} for the catalysed reaction is ca 7×10^3 s^{-1} leading to a rate enhancement of at least 10^3 fold using a catalyst concentration of 2.5×10^{-3} mol dm^{-3}.

A true catalytic reaction should exhibit turnover. Turnover experiments were carried out by pH-stat
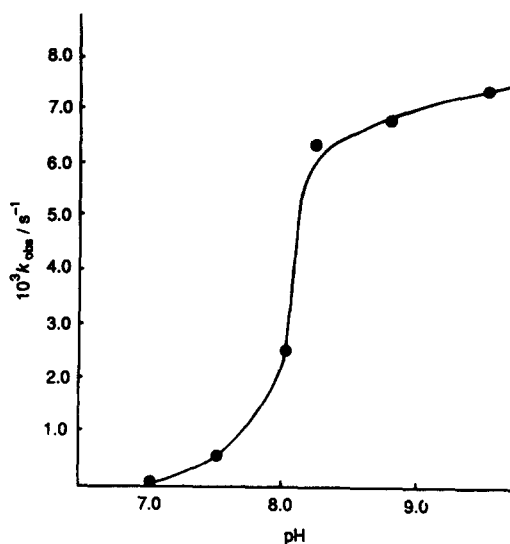

Fig. 2. pH-Rate profile for the lanthanum macrocycle catalysed hydrolysis of 2,4-dinitrophenyl diethyl phosphate at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3). The concentration of $[LaL(NO_3)_3]$ employed was 2.5×10^{-3} mol dm^{-3} and the concentration of 2,4-dinitrophenyl diethyl phosphate was 4×10^{-5} mol dm^{-3}.
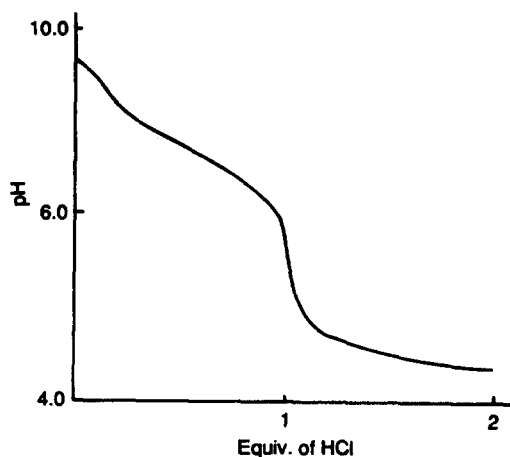

Fig. 3. Potentiometric titration of $[LaL(NO_3)_3]$ in aqueous solution with hydrochloric acid.

Table 3. The lanthanum macrocycle catalysed hydrolysis of 2,4-dinitrophenyl diethyl phosphate at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3)[a]

pH	k_{obs} (s^{-1})	pH	k_{obs} (s^{-1})
7.00	6.25×10^{-5}	8.25	6.30×10^{-3}
7.50	5.22×10^{-4}	8.80	6.70×10^{-3}
8.00	2.45×10^{-3}	9.50	7.28×10^{-3}

[a] Hydrolysis monitored spectrophotometrically at 360 nm. Concentration of $[LaL(NO_3)_3]$ employed $= 2.5 \times 10^{-3}$ mol dm^{-3}. Concentration of 2,4-dinitrophenyl diethyl phosphate $= 4 \times 10^{-5}$ mol dm^{-3}.

methods. A solution (10 cm^3) containing 5×10^{-4} mol dm^{-3} 2,4-dinitrophenyl diethyl phosphate and 5×10^{-5} mol dm^{-3} $[LaL(NO_3)_3]$ was adjusted to pH 8.50 at 25°C and the release of H^+ due to ester hydrolysis monitored by the automatic addition of 2×10^{-3} sodium hydroxide solution. The initial slope of the plot, Fig. 4 gives a release rate of H^+ of 1.9×10^{-4} mol min^{-1} with a turnover of $1.9 \times 10^{-4}/5 \times 10^{-5} = 3.8$ turnovers min^{-1}. At pH 8.50 and 25°C, 10 moles of the ester are hydrolysed by one mole of the catalyst in ca 50 min. At this pH the half life of the free ester is 71.5 h.

The activity of the lanthanum macrocycle as a catalyst for the hydrolysis of the anticholinesterase agent
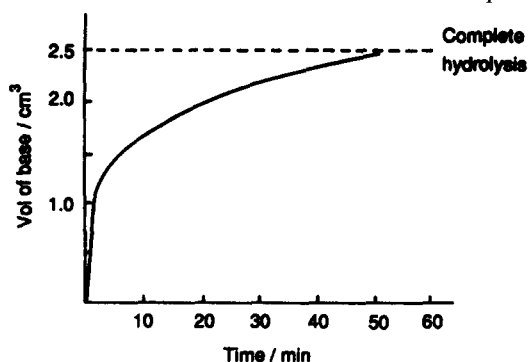
Fig. 4. pH-Stat trace for the hydrolysis of a 10 cm^3 sample of 5×10^{-4} dm^{-3} solution of 2,4-dinitrophenyl diethyl phosphate in the presence of 5×10^{-5} mol dm^{-3} [LaL(NO$_3$)$_3$] at pH 8.50 and 25°C. The titrating alkali was 2×10^{-3} mol dm^{-3} sodium hydroxide.

sarin (O-isopropyl methyl-fluorophosphonate) (3) was also investigated. The reaction was carried out in 1 mol dm^{-3} HEPES buffer pH 7.0 and hydrolysis of fluoride was monitored using a fluoride specific electrode. The measurements were made at 25°C using a [LaL(NO$_3$)$_3$] concentration of 5×10^{-3} mol dm^{-3} and a sarin concentration of 4×10^{-2} mol dm^{-3} (an eightfold excess of the substrate). Fluoride release was monitored as a function of time as shown in Fig. 5. Some three moles of sarin are hydrolysed per mole of the catalyst in 20 min. Although the lanthanum
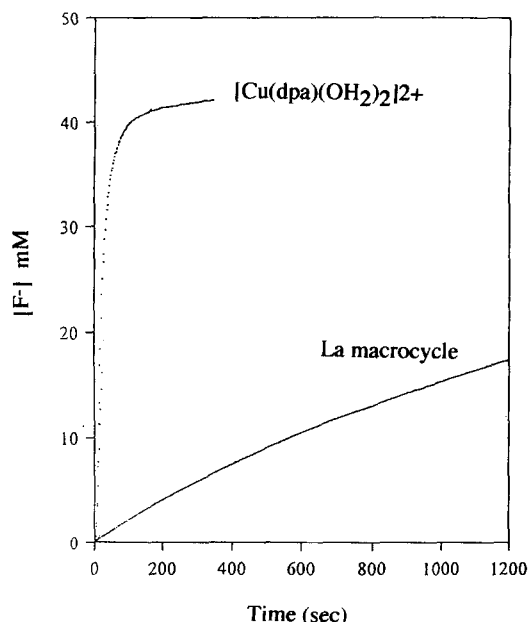

Fig. 5. Hydrolysis of O-isopropyl methylfluorophosphonate (Sarin) at pH 7.0 at 25°C in 1 mol dm^{-3} Hepes buffer in the presence of [Cu(dpa)(OH$_2$)$_2$]$^{2+}$ (dpa = 2,2'-dipyridylamine) and [LaL(NO$_3$)$_3$]. The Sarin concentration was 4×10^{-2} mol dm^{-3} and the concentration of the metal complexes was 5×10^{-3} mol dm^{-3}.

complex is a quite active catalyst it does not show the level of activity at pH 7 observed with the copper(II) complex [Cu(dpa)(OH$_2$)$_2$]$^{2+}$ (dpa 2,2'-dipyridylamine) (Fig. 4) [24].

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