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Application of novel metallocomplexes for mimicking of cytochrome P450 Analysis of product distribution for 1-hexene as a substrate

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Abstract

While the most catalysts applying for mimicking of oxygenation function of cytochrome P450 rely on the metalloporphyrins, we present here novel approach based on M^{n+} metallocomplexes of novel ligands: arylmethyl substituted bis-β-aminoketones. The efficacy of catalyst for 1-hexene oxygenation was tested as function of M^{n+}, ligand type and reaction conditions. Our results revealed that the best performance is for Co(II) metallocomplexes of ligand *N*,*N*′-ethylenebis[4-amino-1,5-di-(2-naphthyl)]pent-3-en-2-one are the most efficient for conversion of 1-hexene to oxygenation products. Analysis of product distribution was performed with GC–MS analysis, the mild conditions of analysis allowed to detect primary products of oxygenation reaction.

Keywords: Cytochrome P450; Enzyme mimick; Oxygenation; Metallocomplexes; Product distribution

1. Introduction

Mimicking of the catalytic activity of cytochrome P450 represents one of the major challenges in organic and supramolecular chemistry, as specific transformation under very mild conditions are focus of many synthetic methodologies. Oxygenation of non-activated organic compounds, specifically of C–H bond has been unmet challenge for quite long time [1,2]. Mimic of oxygenation function of enzyme cytochrome P450 has faced major obstacle, as easy formation of μ-oxo dimers from variety of porphyrin-metallocomplexes has been major problem in mimicking of such transformation with synthetic receptors.

Excellent examples of c-P450 mimicking have been presented and the major problem of catalyst inactivation has been solved by introduction of electronegative substitution (electron-withdrawing groups on meso-phenyl, or β-pyrrole positions) which prevents undesired reduction of catalytic activity. For leading examples see reviews [3–5]. Another approach has been to attach metalloporphyrin on the solid surface and prevent in this way the μ-oxo dimer formation, which is sterically restricted in this case [6,7]. Examples of such, sometimes very successful catalyst—cytochrome P450 mimicking synthetic metallo-ligands are given in references (above), where the success is based on combination of the catalytically active part of molecule (metalloporphyrin) with a binding part of molecule for substrate (usually cyclodextrin, or cyclophanes), which allows an efficient and selective conversion. The leading examples are given by Breslow and coworkers with elegant way for regioselective transformation e.g. of steroid olefins with very high catalytic turnover [8] and references therein [9,10]. Meunier and coworkers studied cytochrome P450 mimick for DNA damage. It was shown that oxidation of saccharide (deoxyribose) results from hydroxylation reaction by Mn-metallopoprhyrin with $KHSO_5$ as oxygen source [11].

In this project we adopted a completely different strategy for cytochrome P450 mimick.

Instead of classical metalloporphyrins we used novel metallocomplexes. The rational behind our work was to test

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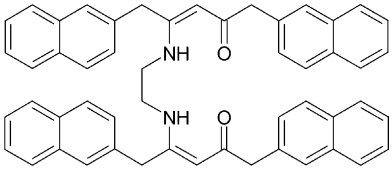
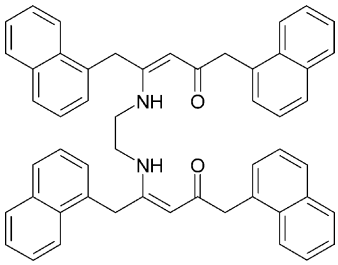
application possibility of novel metallo-ligand receptors, which can work as cytochrome P450 mimick without the problem of the formation of μ-oxo dimers. We tested here novel, recently reported [12] metallocomplexes based on tetradentate open-chain ligand-podands (non-macrocyclic) with two tertiary amines and two oxygens (ketones) forming the binding site for a cation.

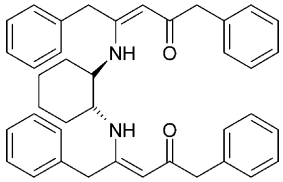
The prepared 15 metallocomplexes are shown below:

- Group A

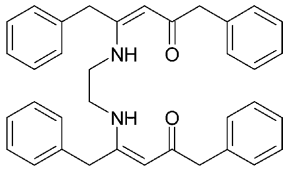




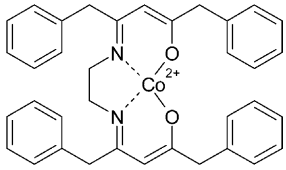
- Group A′



- Group B



- Group C



The complexes were tested in homogeneous catalytic mode for mimicking of oxygenation activity of c-P450 with 1-hexene as the substrate. The optimum conditions were found by variation of several input values.

Firstly, our question was what type of metal cation would be the best in terms of catalytic activity mimicking for given ligand. Secondly, we performed variation of oxygen source and also ligand base. The final testing was made with *tert*-BuOOH as the oxygen donor and pyridine as the metallo-axial ligand, where pyridine was also used as internal standard for conversion evaluation and product distribution measurement. A further question was whether the aromatic rings connected to the catalytic redox center with the rotationally very flexible bonds would have some effect on the reactions: the rotational barriers of the benzylic CH2 linkages are very low [12,13].

For the analytical method we selected GC–MS, which gave as information about product distribution and also about the compounds formed during the oxygenation process. Critically important were conditions for injection of the sample, for all our measurements we kept the temperature of injection cell at 150 °C (at higher temperature we could not observe primary product of the reaction, but only secondary stable products formed from reactive intermediates X).

2. Results and discussion

Typical experiments were performed as follows: 1 mg of catalyst; 100 μl of 1-hexene (substrate); 100 μl of tBuOOH (oxidant); 100 μl of dichlormethane (solvent); 10 μl of pyridine (axial ligand, internal standard).

Time course was followed within 2 h to 7 days period, with usual product maximum from 24 h to 2 days. The mixture reacted for 1 week at a laboratory temperature in a closed vial. After a given period of time, the reaction mixture was injected into the gas chromatograph with the mass spectrometric quadrupole detector and electron ionization (Hewlett Packard 5890-5971A). Quantitative evaluation was made on the GC–MS Hewlett Packard and on the GC Varian 3600 with flame ionization detector under similar conditions. The results obtained from both the instruments are comparable.

It was found that the GC injection temperature had a fundamental influence on the resulting composition of the analyzed mixture. The amount of compounds (peak dimension) called X_1 and X_{2a+b} (there is partial dividing X_{2a} and X_{2b} in some chromatograms, in the others there is one undivided peak X_{2a+b}) decreased with increasing temperature of injection area (Fig. 1a and b).

These processes occur in dependence of temperature of injecting area (in the range of 150–250 °C), but they are not affected by presence or absence of rests of oxidant (tBuOOH).

We made also a parallel experiment, in which the sample with high content of X_1 and X_{2a+b} compounds was heated during 8 h at 180 °C. There was no presence of X_1 and X_{2a+b} compounds after GC–MS analysis of this mixture at low (150 °C) injection temperature. On the other hand, the amounts of compounds of short retention times increased.

We can conclude that the compounds X_1 and X_{2a+b} are in fact stable intermediate products, which decompose at high temperature to compounds with short retention time. The compounds are identified in Fig. 2. Some polymerizing reactions may also occur. The results are summarized in Figs. 3–5.

Fig. 3 shows the structures of compounds X_1 and X_{2a+b}. They were determined after interpretation of mass spectra (Figs. 4 and 5), further study of fragmentation mechanisms using MS–MS method (triple quadrupole Varian was used)

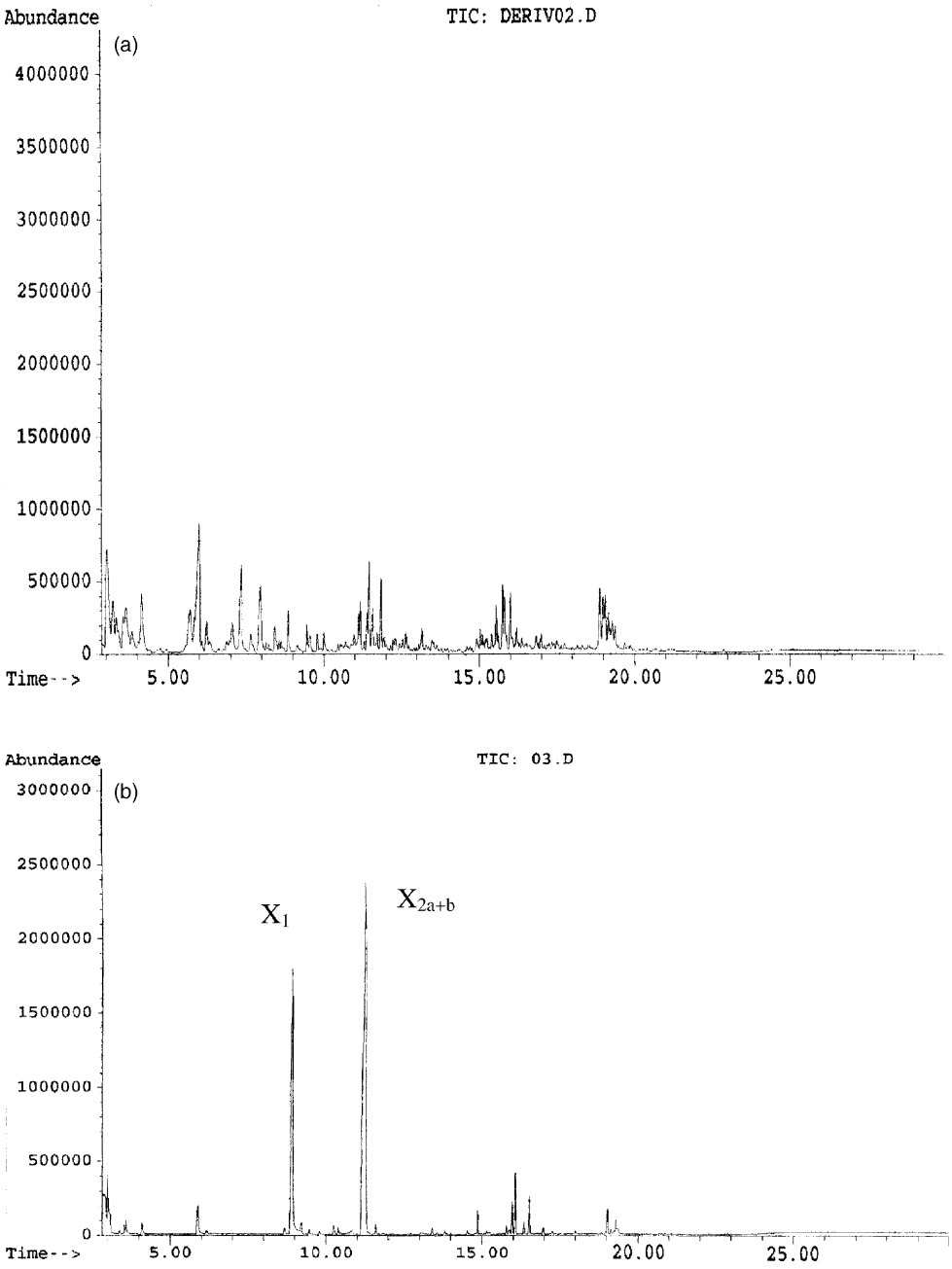


Fig. 1. Product distribution for oxygenation of 1-hexene with catalyst **9**, GC–MS analysis: (a) injection at 250 °C; (b) injection at 150 °C.

and also according to facts mentioned above. Isomers X_{2a} and X_{2b} are partially divided or merged, according to quality of column and chromatographic conditions.

3. Conclusions

Novel catalysts were synthesized and tested for mimicking of cytochrome P450. Catalytic turnover and activity proved that these novel ligands could serve as successful example of metallocomplexes that function as cytochrome P450 mimick for conversion of model substrate 1-hexene.

From the results presented in Table 1 we can draw some conclusions concerning the ligand function and metal cation. For the evaluation of catalytic activity we used the conditions described in the experimental part: evaluation is based on the amount of formed products X_1 and X_{2a+b} (and final stable products **6**–**10**, see Fig. 2). First, the quantitative evaluation, as compared with pyridine, which function not only as axial ligand for metallocomplex, but also as internal standard, revealed dramatic difference for tested metal cations: while Fe(II) was only slightly active; much better results were obtained with Mn and the best ones with Co as central metal cation in our complexes. This proposes that

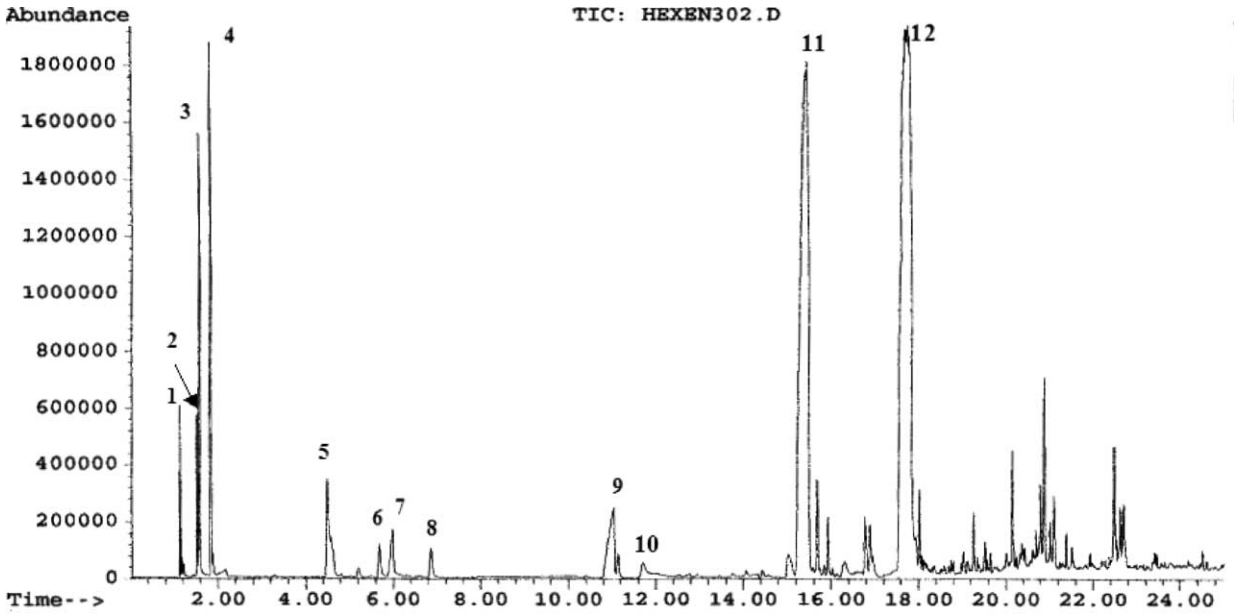


Fig. 2. Product distribution for oxygenation of 1-hexene with catalyst **9**, GC–MS analysis, injection at 150 °C (another chromatographic conditions as in Fig. 1): **1**, air; **2**, *t*-butanol; **3**, dichlormethane; **4**, 1-hexene; **5**, pyridine (IS); **6**, 1-hexen-3-one; **7**, 1-hexen-3-ol; **8**, butyloxirane; **9**, 2-hexenal; **10**, 2-hexen-1-ol; **11**, X_1; **12**, X_{2a+b}.

Table 1
The catalysts tested for oxidation of 1-hexene with *tert*-butylhydroperoxide

Catalyst	tBuOOH (rest) (%IS)	X_1 (%IS)	X_{2a+b} (%IS)	Structure (metal cation)
1	80	71	93	Co-A
2	62	16	25	Mn-A
3	140	4	6	Fe-A
4	90	15	20	Co-A′
5	130	2	2	Mn-A′
6	110	7	11	Fe-A′
7	0	51	69	Co-B + Py
8	0	23	36	Mn-B
9	0	49	69	Mn-B + Py
10	66	12	18	Fe-B
11	0.5	36	46	Co-C
12	0	45	62	Co-C + Py
13	0	28	36	Mn-C + Py
14	95	14	20	Fe-C
15	97	3	4	Fe-C + Py

Characterization of reaction mixture was based on remaining tBuOOH. The obtained primary products X_1 and X_2 are compared in % with the pyridine internal standard.

Table 2
Time course of 1-hexene oxygenation reaction using catalyst **7**

Days	tBuOOH	X_1 (%)	X_{2a+b} (%)
1	Less than 5%	100	100
3	0	140	160
6	0	120	200
8	0	110	190

Product content is expressed in % as related to X_1 (X_2) content at the first day (24 h taken as 100%).

the Co(II)/Co(III)-redox system is correctly balanced for the reaction. Secondly, the comparison of the ligands is not as straightforward: the ligands with naphthyls do not decompose tBuOOH but the 2-naphthyl yielded also the products in a good yield, while the benzylic complexes consumed tBuOOH within a day. Also the difference between the 1- and 2-naphthyl and is remarkable and indicates an essential role of the naphthyl arms in the catalytic events (Table 2).

Thirdly, the product analysis performed with GC–MS analysis showed not only expected products, but also peroxide intermediates, which are not commonly detected (usually only the final stable products of oxygenation, like corresponding alcohol, ketone, aldehyde, or oxirane are reported).

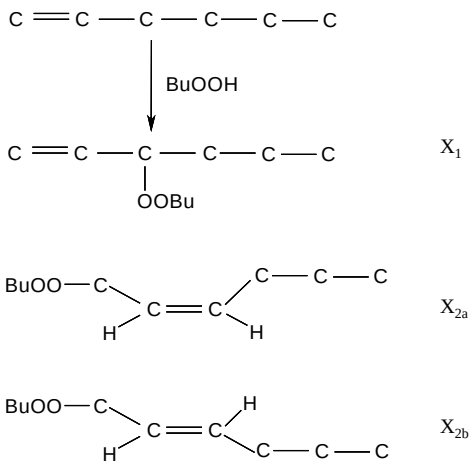


Fig. 3. Conversion scheme indicating the major primary products X. The final conversion led to the products **6**–**10** in Fig. 2.

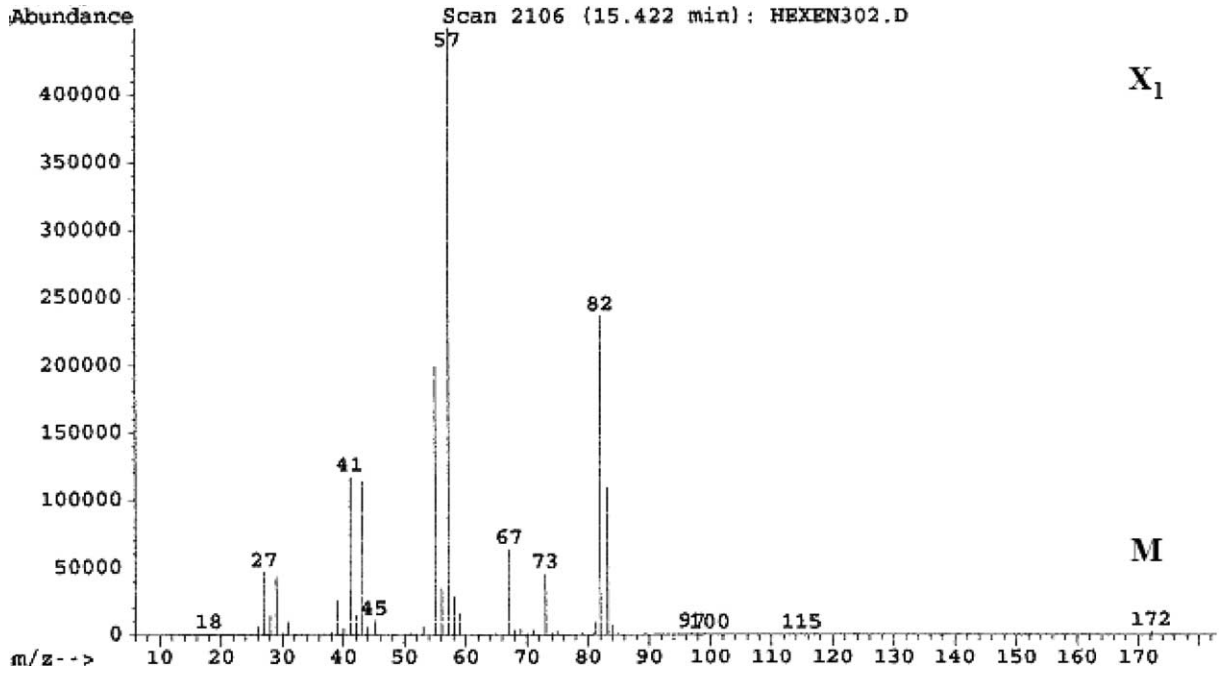


Fig. 4. Mass spectrum of X_1.

In the products analysis the critical point was temperature of the GC injection system. When temperature was kept around 150 °C, the primary products of oxygenation reaction were observed. The conversion yields were from 10 to 50% of starting substrate (based on internal standard).

4. Experimental

4.1. General

All chemicals and solvents were reagent grade and used as received. The precursor diketones were prepared according to our previous general procedure [14]. Melting points were measured with Stuart Scientific SMP2 apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra (TMS/$CDCl_3$) were recorded on a Bruker AM 400 WB spectrometer operating at 400 and 101 MHz, respectively. All the coupling constants are given in Hz. The mass spectra were obtained on a Varian VG 70-250SE spectrometer. Elemental analyses were performed with Carlo Erba 1106 elemental analyzer.

4.2. General procedure for the synthesis of the free ligands A, A′ and C

A solution of the appropriate diketone (2 mmol), ethylenediamine (1 mmol) and *p*-TsOH (0.1 mmol) in toluene (8 ml) was refluxed in a Dien-Stark apparatus for 5 h. After cooling to room temperature, the solution was dried in vacuo, dissolved in CH_2Cl_2 and washed with water. The organic phase was dried in vacuo giving brown viscous oil to which 50:50 ethylacetate-*n*-hexane was added. The solid product, which separated as white powder, was filtered off and evaporated to dryness.

4.3. N,N′-ethylenebis[4-amino-1,5-di-(2-naphthyl)]pent-3-en-2-one (A)

Yield 82%; mp 154–156 °C; δ_H 10.92 (2H, br t, *J* 6.1, N*H*), 7.79–7.70 (8H, m, H_{Ar}), 7.67–7.61 (6H, m, H_{Ar}), 7.45–7.40 (8H, m, H_{Ar}), 7.36 (2H, d, *J* 8.1, H_{Ar}), 7.07 (2H, d, *J* 8.0, H_{Ar}), 5.01 (2H, s, C*H*), 3.71 (4H, s, CH_2), 3.43 (4H, s, CH_2), 3.15 (4H, m, CH_2); δ_C 195.9, 164.7, 134.7, 133.6, 133.4, 133.1, 132.3, 132.2 (8s), 128.5, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 126.9, 126.5, 126.3, 125.9, 125.9, 125.4, 96.8 (15d), 49.3, 43.5, 38.3 (3t); *m/z* (Found: M^+–C_8H_6 626.26952. $C_{44}H_{38}N_2O_2$ requires *M* 626.26813) 626 (M^+–C_8H_6, 23%), 560 (10), 419 (61), 392 (5), 210 (32), 141 (100).

4.4. N,N′-ethylenebis[4-amino-1,5-di-(1-naphthyl)]pent-3-en-2-one (A′)

Yield 87%; mp 184.5–185.5 °C; δ_H 10.99 (2H, br t, *J* 6.1, N*H*), 7.93 (2H, d, *J* 8.4, H_{Ar}), 7.79 (2H, d, *J* 7.1, H_{Ar}), 7.77 (2H, d, *J* 7.1, H_{Ar}), 7.68 (2H, d, *J* 8.1, H_{Ar}), 7.67 (2H, d, *J* 8.1, H_{Ar}), 7.53 (2H, d, *J* 8.6, H_{Ar}), 7.45−7.28 (10H, m, H_{Ar}), 7.24 (2H, d, *J* 7.1, H_{Ar}), 7.22 (2H, dd, *J* 8.2, 7.1, H_{Ar}), 7.02 (2 H, d, *J* 7.1, H_{Ar}), 4.87 (2H, s, C*H*), 3.93 (4H, s, CH_2), 3.61 (4H, s, CH_2), 3.10 (4H, m, CH_2); δ_C 196.1, 165.1, 134.0, 133.8, 133.8, 132.6, 131.8, 131.7 (8s), 128.9, 128.8, 128.1, 128.0, 127.5, 126.6, 126.2, 126.1, 126.1, 125.7, 125.7, 125.6, 124.7, 123.3, 96.9 (15d), 47.0, 43.8, 35.3 (3t); *m/z* (Found: M^+–C_8H_6 626.29333. $C_{44}H_{38}N_2O_2$ requires *M*

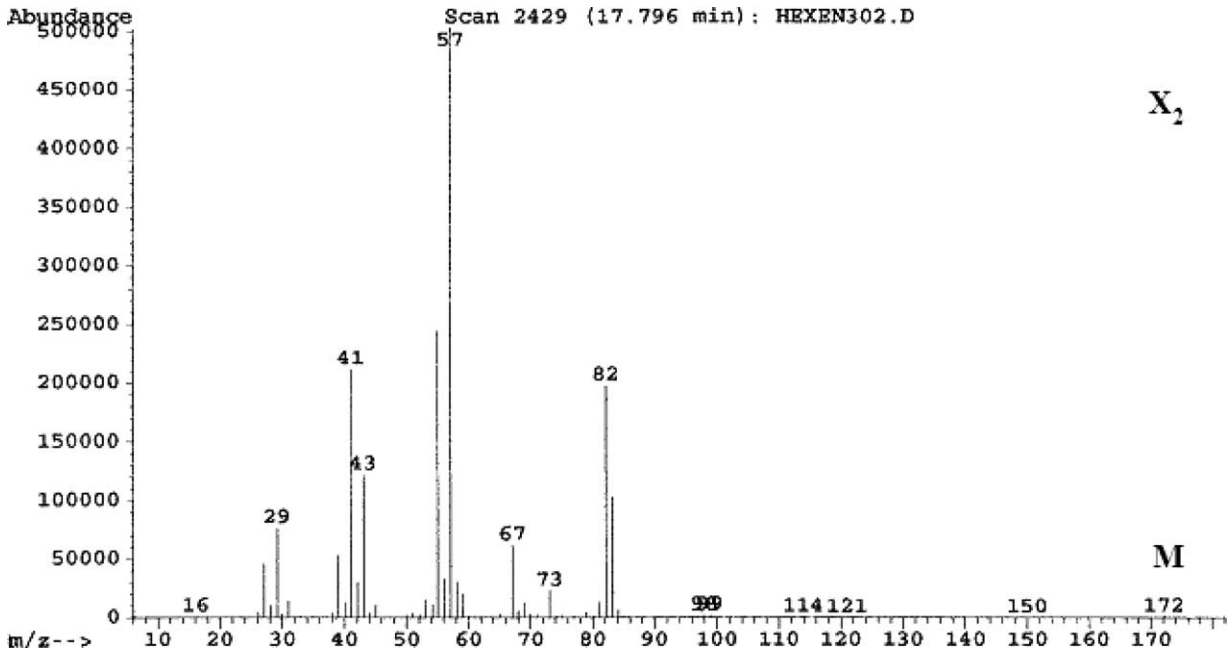


Fig. 5. Mass spectrum of X_2.

626.26813) 626 (M^+–C_8H_6, 11%), 560 (6), 419 (77), 392 (7), 210 (26), 141 (100).

4.5. *N,N′-ethylenebis(4-amino-1,5-diphenylpent-3-en-2-one) (C)*

Yield 91%; mp 162.5−163.5 °C; δ_H 10.82 (2H, br t, N*H*), 7.30−7.19 (16H, m, H_{Ar}), 7.08−7.06 (4H, m, H_{Ar}), 4.96 (2H, s, C*H*), 3.55 (4H, s, CH_2), 3.35 (4H, s, CH_2), 3.07 (4H, m, CH_2); δ_C 195.9, 164.6, 137.1, 135.7 (4s), 129.3, 128.8, 128.4, 128.4, 127.0, 126.3, 96.5 (7d), 49.1, 43.4, 38.3 (3t); *m/z* (Found: M^+ 528.27575. $C_{36}H_{36}N_2O_2$ requires *M* 528.27768) 528 (M^+, 1%), 437 (58), 319 (45), 278 (38), 264 (33), 186 (97), 173 (52), 91 (100).

4.6. *General procedure for the synthesis of the free ligand B*

A solution of the 1,5-diphenylpentane-2,4-dione (4 mmol), (1*S*,2*S*)-(+)-1,2-diaminocyclohexane (2 mmol) and *p*-TsOH (0.2 mmol) in toluene (10 ml) was refluxed in a Dien-Stark apparatus for 20 h. After cooling to room temperature, the solution was dried in vacuo, dissolved in CH_2Cl_2 and washed with water. The organic phase was purified by column chromatography using CH_2Cl_2 as eluent.

4.7. *N,N′-trans-1,2-cyclohexylenebis(4-amino-1,5-diphenylpent-3-en-2-one) (B)*

Yield 87%; brown viscous oil; δ_H 11.01 (2H, br d, *J* 9.4 N*H*), 7.29−7.21 (12H, m, H_{Ar}), 7.22–7.16 (4H, m, H_{Ar}), 7.07−7.04 (4H, m, H_{Ar}), 4.86 (2H, s, C*H*), 3.54 (2H, d, *J* 14.0 CH_2), 3.53 (2H, d, *J* 14.0 CH_2), 3.47 (2H, d, *J* 16.4, CH_2), 3.09 (2H, d, *J* 16.4, CH_2), 3.07 (2H, m, CH_2), 1.60–1.50 (4H, m, CH_2), 1.15 (2H, m, CH_2), 0.91 (2H, m, CH_2); δ_C 195.4, 165.2, 137.2, 136.3 (4s), 129.2, 128.5, 128.4, 128.2, 126.6, 126.2, 95.6, 57.9 (8d), 49.0, 38.1, 32.3, 24.2 (4t); *m/z* (Found: M^+–C_7H_7 491.26985. $C_{33}H_{35}N_2O_2$ requires *M* 491.26901) 491 (M^+, 3%), 308 (3), 253 (6), 186 (97), 161 (48), 91 (100).

4.8. *General procedure for the synthesis of complexes **1**–**6**, **8**, **10**, **11** and **14***

The free ligand A, A′, B or C (0.038 mmol) was dissolved to 2.5 ml of refluxing EtOH. The appropriate metal salt [$Co(AcO)_2{\cdot}4H_2O$, $Mn(AcO)_2{\cdot}4H_2O$ or $Fe(ClO_4)_3{\cdot}9H_2O$, 0.038 mmol] in 2 ml of EtOH was then added and the resulting mixture refluxed for 1 h. After cooling to room temperature, the solvent was removed in vacuo.

4.9. *cis(?)N,N′-ethylenebis[4-amino-1,5-diphenylpent-3-en-2-onecobalt(II)cation] (**11**)*

Orange red plates, mp 144.5−146.0 °C (decomp.) (Found: C, 73.48; H, 5.86; N, 4.66. $C_{36}H_{34}N_2O_2Co$ requires C, 73.84; H, 5.85; N, 4.78%).

4.10. *General procedure for the synthesis of complexes **7**, **9**, **12**, **13** and **15***

The free ligand B or C (0.038 mmol) was dissolved to 2.5 ml of refluxing EtOH. The appropriate metal salt [$Co(AcO)_2{\cdot}4H_2O$, $Mn(AcO)_2{\cdot}4H_2O$ or $FeSO_4{\cdot}7H_2O$, 0.038 mmol] in 2 ml of EtOH was added and the resulting

mixture refluxed for 15 min. Then 0.5 ml of pyridine was added and the reflux continued for 45 min. After cooling to room temperature, the product was evaporated to dryness in vacuo.

Acknowledgements

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