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The synthesis of metallocene-labelled drugs for biological assays

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Several drugs (amphetamine, desipramine, nortriptyline, phenobarbital) have been labelled with metallocenic fragments in order to develop a new immunoassay method. The metallocenic fragments are cymantrenic or benchrotrenic derivatives: the linkage between the organic and organometallic moieties has been achieved by reactions between amino and acidic functional groups. All the products (metallohaptens), purified by different chromatography techniques, have been fully characterized by IR and ^{1}H NMR spectroscopy and their mass spectra.

Keywords: metallocenic labels, cymantrene, benchrotrene, metallohaptens, drugs, immunoassay

INTRODUCTION

The very important development of organometallic chemistry and the research into new analytical methods have instigated the use of organometallic complexes in the biological field. These metallic moieties can appear under different forms: as organometallic coordination complexes, as chelates or as clusters. In parallel, a great number of sensitive analytical techniques have been investigated including electrochemistry,[1] fluorescence,[2] atomic absorption (AA) spectrometry,[3] infrared spectroscopy[4] and to a lesser extent electronic paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) spectroscopy.

Molecules labelled with metal chelates could be applied to medical imaging,[5] biodistribution,[6] scanning studies,[7] etc., but the best probable utilization will be provided by their potential as labelling agents for biological assays.

During the last years, many workers have developed this approach in particular as a probe for immunoassays. Cais *et al.*[8] first described the use of organometallic moieties as labels for immunoassays of steroids and coined this new immunological technique, 'metalloimmunoassay'. Weber and Purdy[9] have developed a continuous-flow immunoassay for the determination of morphine labelled with ferrocene based upon electrochemical detection; Alam and Christian have reported the immunoassay of human serum albumin after labelling with lead,[10], cobalt[11] or nickel[12] electrochemically detected; Doyle *et al*[13] described a heterogeneous immunoassay for proteins labelled with indium chelate using differential pulse voltammetry; Willmot and co-workers[14] used a terbium–transferrin complex as a fluorescent label for gentamicin. Chelates of lanthanides have been proposed by Hemmilä *et al.* as labelling agents for antibodies[15] and for thyroxin,[16] by Dechaud *et al.* for the assay of prolactin[17] and progesterone,[18] by Bador *et al.* for follitropin[19] and by Reichstein *et al.* for the assay of cortisol.[20]

More recently Butler *et al.*[21] have proposed organotransition metal carbonyl complexes as infrared labels for hormonal steroids.

It seemed interesting to us to apply the metalloimmunoassay concept described by Cais.[3] Immunoassays are vital analytical tools in the biological sciences, particularly in clinical chemistry for the diagnostic information to the clinician and as well as for the monitoring of therapy.

One form of immunoassay is the competition between excess metallo-labelled (Ag.M) and unlabelled antigen (Ag) for a limited amount of specific antibodies (Ab) as outlined in Eqn [1].

$$\begin{array}{l} \text{Ag.M} + \text{Ag} + \text{Ab} \quad \rightarrow \\ \quad \text{Ab—Ag.M} + \text{Ab—Ag} \end{array} \qquad [1]$$

Labelled antigen (drug) provides a considerable improvement in sensitivity of the assay of a drug, in particular using radioisotope labels as first proposed by Yalow and Berson.[22] But drawbacks inherent to

the use of radioactivity have stimulated immunoassay systems based on the use of another label.

The metalloimmunoassay concept can provide an alternative response to the radioimmunoassay, firstly because the field of inorganic chemistry is now considerable and secondly because many analytical methods are available for detecting and quantitating metals. Moreover, the use of metal-carbonyl structures offers the possibility of detection of these carbonyl ligands by Fourier transform infrared spectroscopy (FTIR) as previously described.[23]

The synthesis of the labelled antigen is one of the major steps in the development of every competitive immunoassay.

One of the different approaches to labelling requires the synthesis of a metallo compound with a functional group suitable for linking drug molecules. The first work concerning the labelling of therapeutic drugs with a ferrocenic moiety was described by Brossier and Moise,[24] as well as its use for metalloimmunoassay of tricyclic antidepressants.[25] In this first synthetic method the organometallic complexes bear a primary amino group able to react with a carboxylic derivative of drugs.

In the second method proposed in this paper the organometallic complexes bear an acid or acid chloride functional group which reacts with an amino drug as described in Eqn [2]. Drugs incorporating metallic fragments are designated 'metallohaptens'.

$$\text{Drug—NHR} + \text{M—COOH or MCOCl} \rightarrow \text{Drug—NR—CO—M} + \text{H}_2\text{O or HCl} \quad [2]$$

We report here the synthesis of different organometallic complexes derived from cyclopentadienylmanganese tricarbonyl[26] (cymantrene, Cy—H) and from benzenechromium tricarbonyl[27] (benchrotrene, Bct—H) and their utilization as labels for drugs. The drugs studied are: (a) amphetamine (AMPHE), a psychostimulant; (b) phenobarbital (PHENO), a sedative-hypnotic drug; (c) desipramine (DESI) and (d) nortriptyline (NORTRI), two tricyclic antidepressants.

Furthermore, we describe several nortriptyline derivatives labelled with cymantrenic fragments differing in the length of the carbon chain bound to the cyclopentadienyl ring. These various metallohaptens present different reaction capabilities towards antibodies.

EXPERIMENTAL

Equipment

All manipulations of air-sensitive products were performed under a purified argon atmosphere using Schlenk techniques. The solvents were distilled under argon from sodium benzophenone immediately before use.

Preparative thin layer chromatography silica gel 7732 G Merck 0.5 mm and silica gel 9285 Merck for column chromatographic separation were used.

Spectra were recorded with the following instruments: infrared, Perkin—Elmer 580 B; ^{1}H NMR, JEOL FX 100 (δ ppm/TMS); mass spectra, Finnigan 3002.

Starting materials

The starting material chromium hexacarbonyl [$Cr(CO)_6$] was purchased from Strem Chemicals; cyclopentadienylmanganese tricarbonyl (cymantrene, Cy—H) was obtained from Aldrich; amphetamine and phenobarbital were purchased from Cooper de Melun (France); desipramine was supplied to be by Ciba—Geigy Laboratories and nortriptyline by Lilly Research Laboratories.

Cy—$COCH_3$ (1)

This was synthesized from Cy—H according to the well-established procedure of Fischer and Pleszke.[28] Yield 93%; m.p. 42°C (lit. 40—41°C).

Cy—COOH (2)

The procedure of Riemschneider and Petzoldt[29] was followed. Yield 60%; brown needles decomposed at *ca* 204°C (recrystallization from ethanol/water).

Cy—COCl (3)

To a stirred suspension of 1.7 g (6.85 mmol) of Cy—COOH in 50 cm^3 of dry benzene was added 1.5 g (7.2 mmol) of PCl_5 in three portions during a 2 h period. The mixture was stirred under argon for 1 h at room temperature. On removal of the solvent, a brown solid resulted. This solid was redissolved in 50 cm^3 of dry benzene and filtered under argon on thoroughly degassed (deoxygenated) silica gel. After removal of solvent under reduced pressure the Cy—COCl was obtained as orange—yellow crystals, m.p.

61°C (1.4 g; 5.22 mmol; yield 76%). IR $(KBr)_I$ cm^{-1}: C≡O 2031, 1965–1948; C=O 1758. ^1H NMR (C_6D_6), ppm: 4.70 (t) and 3.60 (t, Cy). Mass spectrum: *m/e* = 266–268 $(P)^+$, 232 $(P-Cl)^+$, 182–184 $(P-3\ CO)^+$.

Cy—CO—CH_2—CH_2—COOH (4)

Treatment of cymantrene with succinic anhydride under Friedel–Crafts conditions according to the procedure of Dabard and Le Plouzennec[30] yielded 90% of the expected keto-acid, m.p. 114°C (lit. 112–114°C).

Cy—CH_2—CH_2—CH_2—COOH (5)

This γ-butyric acid was prepared following the procedure of Dabard and Le Plouzennec[30] by Clemmensen reduction of the corresponding keto-acid Cy—CO—CH_2—CH_2—COOH (yield 70%).

Cy—CH_2—CH_2—CH_2—CO—Cl (6)

To a lemon-yellow suspension of cymantrenyl γ-butyric acid (710 mg, 2.44 mmol) in 15 cm^3 of benzene was added oxalyl dichloride (1.5 cm^3, 17 mmol) via syringe at room temperature under argon. After vigorous gas evolution had ceased, a homogeneous orange–red solution was obtained. Evaporation of the solvent under reduced pressure gave a brown–yellow oil of the expected cymantrene acid chloride (685 mg, 2.2 mmol; yield 90%). This was used without further purification for the next step. IR (NaCl), cm^{-1}: C≡O 2016, 1927; C=O 1794.

Ph—CO—CH_2—CH_2—COOH (7)

The literature procedure[31] was modified as follows. To a stirred solution of 6.75 g (67.5 mmol) of succinic anhydride in 140 cm^3 of benzene (122 g, 1.568 mol) under argon was added all at once 20 g (150 mmol) of powdered anhydrous aluminium chloride. The mixture was refluxed with continuous stirring for 1 h. The flask was cooled to 0°C and the mixture slowly poured into a suspension of 75 cm^3 of concentrated hydrochloric acid while being vigorously stirred. The resulting material was extracted with ether and ether extracts treated with 2 mol dm^{-3} sodium hydroxide solution. The aqueous layer was then separated and acidified by the slow addition of concentrated hydrochloric acid. A white solid was precipitated and filtered under vacuum. The solid was washed repeatedly with cold water, then thoroughly dried in a vacuum desiccator. Yield 98%, m.p. 116°C (lit. 114–115°C).

Ph—CH_2—CH_2—CH_2—COOH (8)

This was synthesized according to the literature procedure.[32] Yield 85%; m.p. 46°C (lit. 46–48°C).

Ph—CH_2—CH_2—CH_2—COO—CH_3 (9)

This ester was similarly prepared according to the literature procedure[33] for ethyl γ-phenylbutyrate except that methanol was used and, in the extracting phase, the combined ester and ether layers were washed with *ca* 20% Na_2CO_3, then with water, and dried over anhydrous $CaCl_2$. Removal of the solvent gave a residue which was distilled under diminished pressure. The portion boiling at 160–164°C/30 mm Hg was collected. Yield 50%. IR (NaCl), cm^{-1}: C=O 1739.

Bct—CH_2—CH_2—CH_2—COO—CH_3 (10)

This was prepared according to the procedure of Top and Jaouen[34] starting from **9** (2.5 g, 25.6 mmol) and $Cr(CO)_6$ (6.8 g, 31 mmol) in a dibutyl oxide/THF mixture (125:25). Yield 60%.

Bct—CH_2—CH_2—CH_2—COOH (11)

Compound **10** was treated with 4.24 g of KOH dissolved in 25 cm^3 of water and 73 cm^3 of methanol. The reaction was allowed to stand for 24 h at room temperature under argon in the dark and was then poured into 150 cm^3 of water acidified with 6 mol dm^{-3} hydrochloric acid. The yellow precipitate which formed immediately was collected and washed repeatedly with water. The crude yield (43%) after drying was 3.27 g (11 mmol); m.p. 128°C. IR (toluene), cm^{-1}: C≡O 1970, 1896; C=O 1710.

Bct—CH_2—CH_2—CH_2—CO—Cl (12)

Compound **12** was prepared as for compound **6**. A thick brown oil was obtained (yield 90%). IR (toluene), cm^{-1}: C≡O 1969, 1895; C=O 1800.

Synthesis

Cy—CO—AMPHE (3a)

In a typical experiment, a solution of cymantrenoyl chloride (134 mg, 0.5 mmol) in 1.6 cm^3 of dry THF was slowly added under argon to a solution of amphetamine free base (67 mg, 0.5 mmol) extracted

from its HCl salt, in 6 cm^3 of dry THF and 0.5 cm^3 of pyridine. After 24 h of continuous stirring at room temperature a precipitate was formed. After filtration, the filtrate was evaporated under reduced pressure. The residue was then dissolved in methylene chloride, washed successively with dilute hydrochloric acid, water, dilute sodium hydroxide and water, and dried over anhydrous $CaCl_2$. On removal of the solvent, a yellow oil resulted. A thin-layer chromatography purification (eluent: toluene/acetone, 20/1) followed by crystallization from hexane afforded amide **3a** (90 mg; yield 50%) as yellow crystals, m.p. 122°C. IR (KBr) cm^{-1}: C≡O 2025, 1937; C=O 1638, 1556; N—H 3259. ^1H NMR (CD_3COCD_3), ppm: 7.25 (s, C_6H_5—), 4.27 (pseudo q, CH_3C—H), 2.8 (dd, C_6H_5—CH_2—), 2.8 (s, N—H), 1.15 (d, CH_3), 5.58 (t), 5.03 (t) and 4.95 (t, Cy—).

Cy—CO—PHENO (3b)

In the presence of 0.9 cm^3 of pyridine, the reaction between *p*-aminophenobarbital (230 mg, 0.93 mmol) synthesized following the procedure of Brossier and Moise[24] in 12 cm^3 of dry THF and cymantrenoyl chloride (250 mg, 0.95 mmol) in 5 cm^3 of dry THF was conducted similarly to that described for crude Cy—CO—AMPHE. A yellow solid was obtained, which was washed copiously with water, then ethyl ether, and dried in vacuum; yield 82% (363 mg); m.p. indefinite. IR (KBr), cm^{-1}: C≡O 2024, 1939; —CO—NH 1708, 1751 (sh), 1731 (sh); C_6H_4, 1598. ^1H NMR: this compound was found to be insoluble. Mass spectrum: *m/e* = 477 $(P)^+$.

Cy—CO—DESI (3c)

Desipramine free base (500 mg, 1.8 mmol) extracted from its HCl salt in 38 cm^3 of dry THF with 2 cm^3 of pyridine was reacted with cymantrenoyl chloride (482 mg, 1.8 mmol) in 20 cm^3 of dry THF by the procedure described above. A yellow oil was obtained. This was chromatographed. Elution with benzene/acetone (20:1) gave immediately a lemon-yellow zone from which metallohapten **3c** was isolated as oil of clear yellow consistency (500 mg, yield 55%). IR (NaCl), cm^{-1}: C≡O 2024, 1935; CO—N 1625. ^1H NMR (C_6D_6), ppm: 6.9 (m, aromatic H); 3.37 (m, N—CH_2—); 3.07 (m, —CH_2—N); 1.52 (m, —CH_2—CH_2—CH_2—); 2.94 (s, —CH_2—CH_2—); 4.63 (t) and 3.81 (t, Cy). Mass spectrum: *m/e* = 497 $(P + H)^+$; 413 $(P + H - 3CO)^+$.

Cy—CO—NORTRI (3d)

Nortriptyline labelled with cymantrene was synthesized as previously for metallohapten **3c**. Starting from nortriptyline free base (600 mg, 2.5 mmol) in 30 cm^3 of dry THF, pyridine (2.2 cm^3) and cymantrenoyl chloride (670 mg, 2.5 mmol) in 8 cm^3 of dry THF, a yellow oil was obtained. Chromatographic purification (eluent: toluene/acetone, 50:3) gave the metallohapten **3d**) as a thick yellow oil (820 mg, yield 66%). IR (NaCl), cm^{-1}: C≡O 2017, 1938; —CO—N 1628. ^1H NMR (C_6D_6) ppm: 7.04 (m, aromatic H); 5.62 (t, C—H); 4.65 (t) and 3.83 (t, Cy); 2.4 (s, —CH_2—CH_2); 3.1 (sb, N—CH_3); 2.2 (m, —CH_2—CH_2—). Mass spectrum: *m/e* = 493 $(P)^+$.

Cy—CO—CH_2—CH_2—CO—NORTRI (4d)

In 50 cm^3 of dry THF was dissolved 604 mg (2 mmol) of cymantrene keto-acid (**4**); the solution was cooled to 4°C and *N*-hydroxysuccinimide (250 mg, 2.2 mmol) then dicyclohexylcarbodi-imide (600 mg, 2.8 mmol) were added with stirring. After a period of 3 h at 4°C, the urea was separated by filtration. To the resulting filtrate was added nortriptyline free base (595 mg, 2.26 mmol) in THF (10 cm^3) and triethylamine (1.5 cm^2). The stirring was continued for another 24 h at room temperature. On removal of the solvent, a brown solid resulted which was purified by flash column chromatography (eluent: methylene chloride/toluene/acetone, 3:1:1). Finally an orange oil of pure metallohapten **4d** (525 mg, 0.95 mmol; yield 42%) was obtained. IR (CH_2Cl_2) cm^{-1}: C≡O 2018, 1950; —CO—N 1628. ^1H NMR (C_6D_6), ppm: 7.3–6.85 (m, aromatic H); 5,78 (t) and 5.58 (t, = CH); 2.10 (s, N—CH_3); 2.55 (s, —CH_2—CH_2—); 3.25 (t, —CO—CH_2—$\underline{CH_2}$— CO—Cy); 2.85–2.25 (m, —CO—$\underline{CH_2}$—CH_2—CO—Cy and —CH_2—CH_2—N); 4.88 (t) and 3.78 (t, Cy). Mass spectrum: *m/e* = 466 $(P + H - 3CO)^+$.

Cy—CH_2—CH_2—CH_2—CO—NORTRI (6d)

Cymantrene acid chloride, **6** (685 mg, 2.2 mmol) in 8 cm^3 of benzene was added dropwise to a benzene solution (8 cm^3) of nortriptyline free base (637 mg, 2.4 mmol) and pyridine (1 cm^3). The mixture was stirred at room temperature for 4 h. Some precipitate formation was observed (pyridinium salt). After filtration and removal of the solvent under reduced pressure, a yellow oily residue was left. This was

dissolved in methylene chloride, washed with dilute HCl then water until neutral pH, and dried over anhydrous $CaCl_2$. Evaporation of the volatiles followed by column chromatography on silica gel (eluent: CH_2Cl_2/hexane, 5:2) gave pure metallohapten (**6d**) as a yellow oil (320 mg, yield 25%). IR (toluene), cm^{-1}: C≡O 2018, 1938; —CO—N 1653. ^1H NMR (CD_3COCD_3), ppm: 7.3–7.0 (m, aromatic H); 5.9 (m, =CH); 4.0 (t, —CO—CH_2—); 2.5–2.0 (m, —CH_2—CH_2—Cy); 2.9 (s, —CH_2—CH_2—); 2.73 (s, N—CH_3); 4.84 (s) and 4.79 (s, Cy). Mass spectrum: m/e = 452 $(P + H - 3CO)^+$.

Bct—CH_2—CH_2—CH_2—CO—NORTRI (12d)

This was prepared like the metallohapten **6d** by using the benchrotrenic acid chloride (700 mg; 2.1 mmol) instead of the cymantrenic derivative. An orange–yellow oil was obtained which was stored under argon in the freezer. This oil crystallized during storage (383 mg, 0.7 mmol; yield 32%). IR (NaCl), cm^{-1}: C≡O 1958, 1881; —CO—N 1638. ^1H NMR (C_6D_6), ppm: 7.3–6.9 (m, aromatic H); 5.8 (t, =C—H); 3.35 (t, —CO—CH_2—); 2.6 (s, —CH_2—CH_2—); 2.17 (s, N—CH_3); 4.52–4.3 (m) and 4.47 (s, Bct).

RESULTS AND DISCUSSION

Labelled antidepressant drugs (desipramine, nortriptyline), a sedative-hypnotic agent (phenobarbital) and a psychostimulant (amphetamine) are studied in this work. Suitable carboxylic derivatives from cymantrene or benchrotrene have been synthesized. Figure 1 describes the synthesis of metallohaptens derived from cymantrene and bearing a short lateral carbon chain. The synthesis of cymantrenic and benchrotrenic derivatives with a long lateral carbon chain is reported in Fig. 2.

Organometallic complexes synthesis

Cymantrenic derivatives

These were synthesized via slight modifications of literature methods. Details of the synthetic route are outlined in Figs 1 and 2. All these complexes have been obtained starting from cymantrene. The intermediate acetylcymantrene (**1**) was obtained according to the convenient method of Fischer and Pleszke.[28] In this procedure treatment of cymantrene with acetyl chloride under Friedel–Crafts conditions yielded 93% of the expected ketone **1**. This was converted to cymantrene carboxylic acid (**2**) by the haloform reaction following the procedure of Riemschneider and Petzoldt.[29] The carboxylic compound **2** was transformed into the acid chloride **3** by treatment with PCl_5 in benzene and after purification by filtration on thoroughly deoxygenated silica gel. The pure chloroformylcymantrene was obtained in a 76% yield.

The synthesis of the acid chloride **6** present in Fig. 2 required the Clemmensen reduction of the keto-acid **4** obtained by the treatment of cymantrene with succinic anhydride under Friedel–Crafts conditions (90% yield), followed by the halogenation of cymantrenyl γ-butyric acid (**5**) with $(COCl)_2$ in benzene. The yield of **5** and **6** were 70% and 90% respectively.

Benchrotenic derivatives

These organometallic complexes (**11, 12**) have been prepared starting from the intermediate **10**, which was synthesized as described by Top and Jaouen[34] when chromium hexacarbonyl reacted with the ester **9** in a refluxing dibutyl oxide/THF mixture.

The precursor γ-benzoylpropionic acid (**7**) was obtained according to a slightly modified literature procedure[31] starting from benzene, which reacted with succinic anhydride under Friedel–Crafts conditions in a 98% yield. The compound **7** was converted to γ-phenylbutyric acid (**8**) by Clemmensen reduction following the literature procedure[32] in an 85% yield. Compound **8** was transformed to the methyl ester **9** by acid-catalysed esterification[33] and reacted under Top–Jaouen conditions with $Cr(CO)_6$ to yield a yellow oil (**10**) which was converted to the γ-benchrotrenoyl butyric acid (**11**) in 43% yield by saponification with KOH in water/methanol and precipitation from hydrochloric acid. The acid **11** reacted with $(COCl)_2$ to yield over 90% of a thick brown oil of the acid chloride (**12**).

Metallohaptens synthesis

All the metallohaptens **3a, 3b, 3c** and **3d** have been synthesized starting from cymantrenoyl chloride (Cy—CO—Cl). In typical reactions, the amino drugs desipramine, nortriptyline, amphetamine or amino-functionalized therapeutic molecules (phenobarbital) were reacted in THF with cymantrenoyl chloride in the presence of pyridine to form yellow compounds which were generally purified by chromatography.

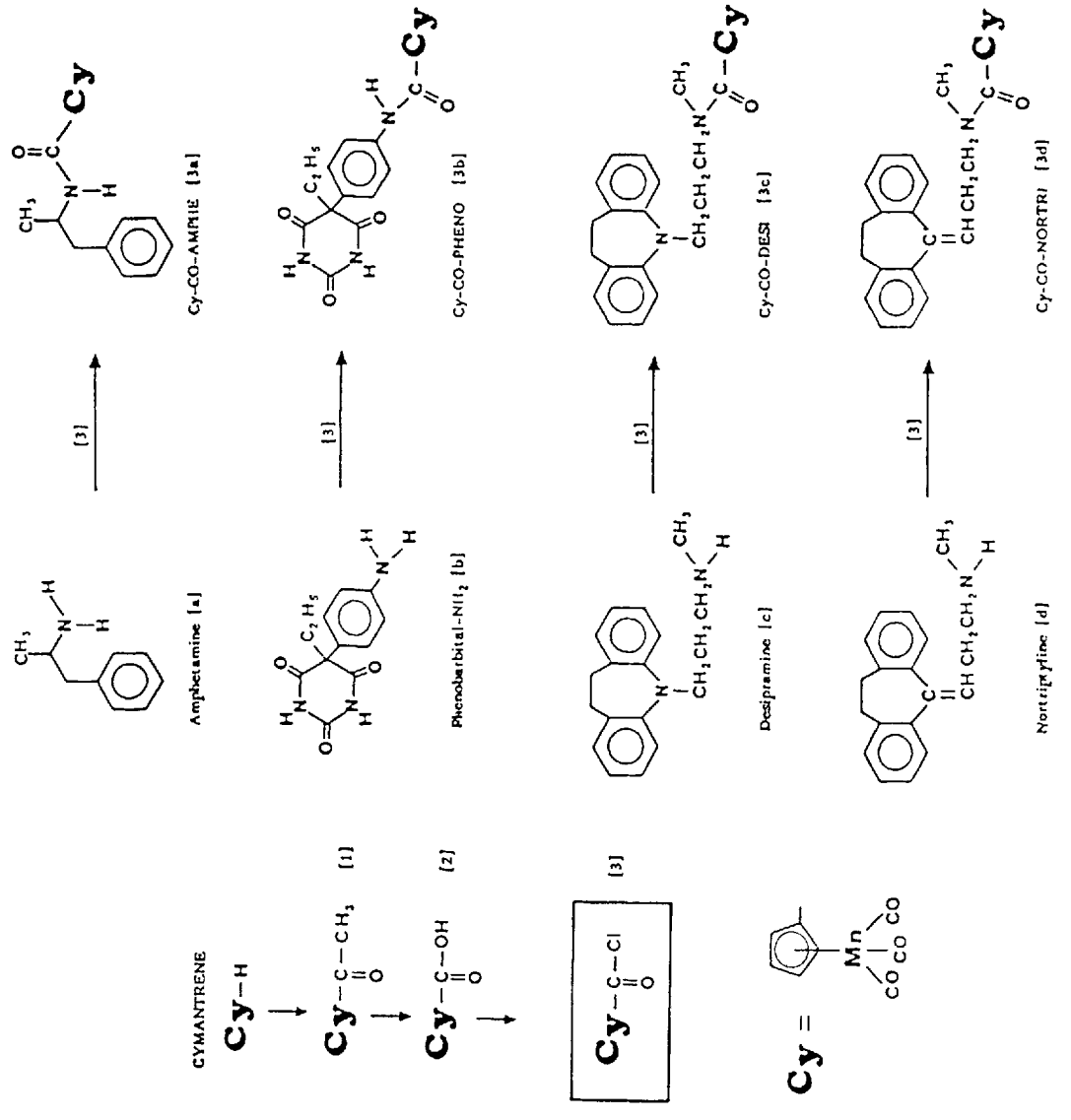

Figure 1 Synthesis of metallohaptens derived from cymantrenic acid chloride.

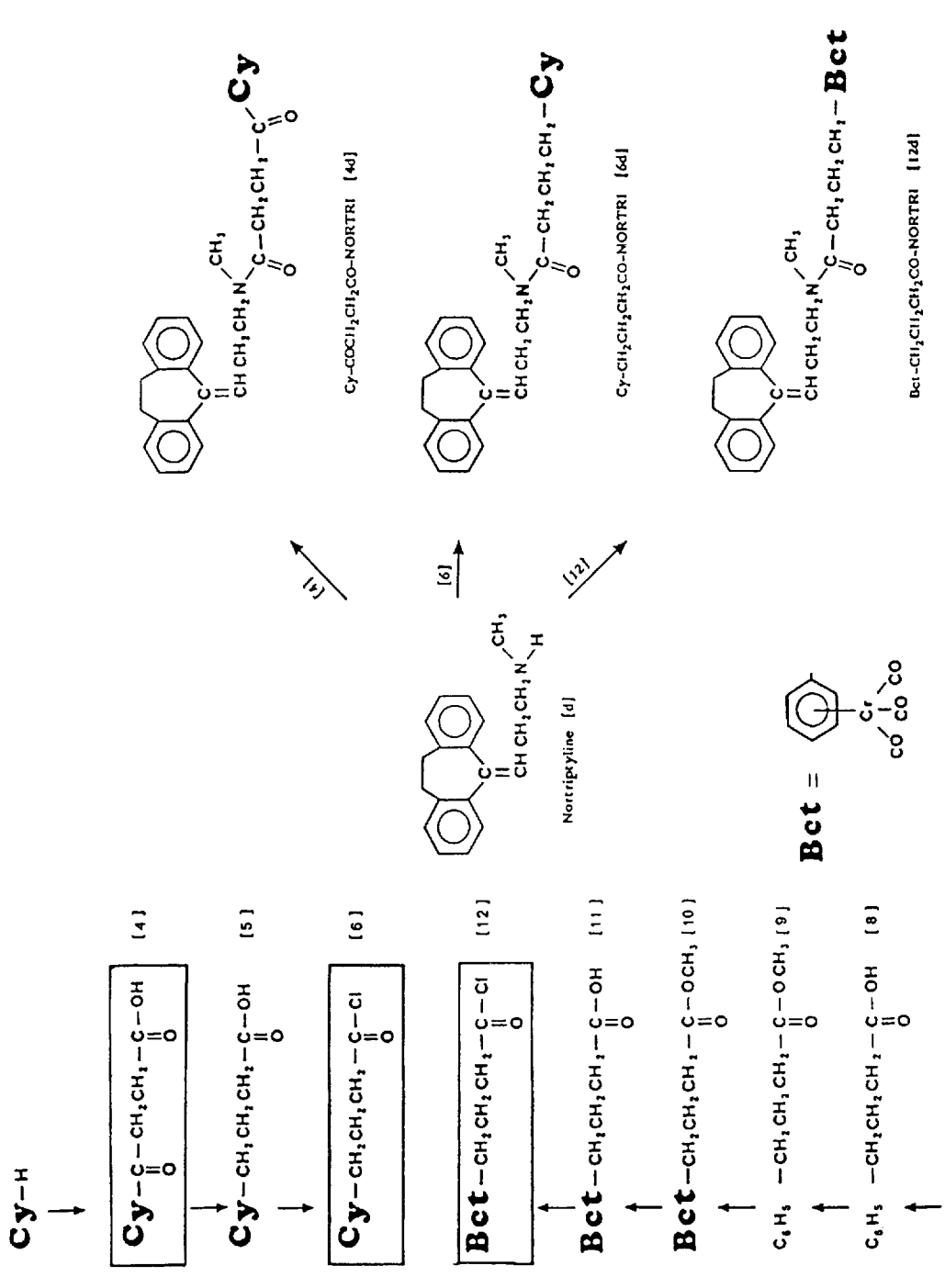

Figure 2 Synthesis of nortriptyline metallohaptens derived from cymantrenic and benchrotrenic complexes.

Thus, the pure metallohapten Cy—CO—AMPHE (**3a**) was obtained in 50% yield after crystallisation as crystals, m.p. 122°C. IR and ^{1}H NMR indicated unambiguously that the product has the structure **3a**. The absorption frequencies of the amide group were characteristic in the IR spectrum: —NH stretch, 3259 cm^{-1}; C=O: 1638 cm^{-1} and 1556 cm^{-1}. In addition, characteristic absorptions of the cymantrenyl group were evident at 2025 cm^{-1} and 1937 cm^{-1} indicating the $Mn(CO)_3$ group. The ^{1}H NMR (100 MHz) spectrum showed the typical cyclopentadienyl ring signals at 4.95, 5.03 and 5.58 ppm together with the resonances of the drug moiety.

The metallohapten Cy—CO—PHENO (**3b**) was obtained as above starting from *p*-aminophenobarbital synthesized following the procedure of Brossier and Moise.[24] A yellow solid was isolated in 82% yield, m.p. indefinite, and found to be insoluble in most solvents, precluding an NMR characterization. The structural problem was resolved by mass spectroscopy [m/e = 477 $(P)^+$] and IR spectroscopy with terminal carbonyl absorptions at 2024 and 1939 cm^{-1} and amide absorptions at 1708, 1751 and 1731 cm^{-1}.

On the basis of the IR, ^{1}H NMR and mass spectra, the metallohaptens Cy—CO—DESI and Cy—CO—NORTRI were assigned respectively the structures **3c** and **3d**. The characteristic absorptions of the $Mn(CO)_3$ and amide groups were evident in the IR spectrum. Furthermore, the NMR spectra clearly showed the signals of the cymantrenoyl moiety and of the drugs as indicated in the Experimental section. Thus, for **3c**, the cyclopentadienyl protons occurred at 4.63 (t) and 3.81 (t) ppm and the two methylene units between the two benzene rings at 2.94 (s) ppm. The metallohapten **3d** showed the corresponding proton signals respectively at 4.65 (t), 3.83 (t) and 2.4 (s) ppm. In the mass spectra, the parent ion P^+ was observed for **3d** while $(P + H)^+$ and $(P + H - 3CO)^+$ peaks were formed for **3c** as described for arene–chromium and molybdenum complexes.[35]

Nortriptyline was also labelled with compounds which contain a lengthened carbon chain between the functional group and the metal moiety (Fig. 2).

The first label, Cy—CH_2—CH_2—CH_2—CO—Cl (**6**), was reacted with nortriptyline free base. After the usual treatment and chromatographic purification, the metallohapten Cy—CH_2—CH_2—CH_2—CO—NORTRI (**6d**) was obtained as a yellow oil in 25% yield. As above, the infrared spectrum of **6d** showed in the $Mn(CO)_3$ stretch at 2018 and 1938 cm^{-1}, the amide at 1653 cm^{-1}, and in the NMR spectra the well-established signals of the cyclopentadienyl protons at 4.84 and 4.79 ppm and the resonance at 2.9 ppm of the two methylene protons of the cycloheptadienyl ring.

The second label, Cy—CO—CH_2—CH_2—COOH was treated according to the dicyclohexylcarbodi-imide method of ester synthesis in the presence of *N*-hydroxysuccinimide.[36] To the resulting compound were added nortriptyline free base and triethylamine in THF. Flash column chromatography pufication gave pure Cy—CO—CH_2—CH_2—CO—NORTRI (**4d**) in 42% yield as an orange oil. The infrared spectra of (**4d**) exhibited broad, intense terminal carbonyl stretching vibrations at 2018 and 1950 cm^{-1} and a very strong carbonyl stretching vibration at 1628 cm^{-1}. In the NMR spectrum were observed the cyclopentadienyl proton signals at 4.88 (t) and 3.78 (t) ppm; the two adjacent methylene protons of the cycloheptadienyl ring resonate at 2.55 (s) ppm. It was interesting to note that the methylene protons adjacent to the carbonyl of the cymantrenyl group were more deshielded than those adjacent to the amido carbonyl group. In mass spectrometry $(P + H - 3CO)^+$ appeared as fragment ions.

The third label, Bct—CH_2—CH_2—CH_2—CO—Cl (**12**) was reacted with nortriptyline base similarly to Cy—CH_2—CH_2—CH_2—CO—Cl and gave the corresponding metallohapten Bct—CH_2—CH_2—CH_2—CO—NORTRI (**12d**) in 32% yield as yellow cystals. The infrared spectrum of **12d** showed absorption bands at 1958 and 1881 cm^{-1} assigned to the terminal carbonyl groups, and at 1638 cm^{-1} assigned to amide carbonyl. In NMR spectroscopy, chemical shifts of benchrotrenic protons at 4.52–4.3 and 4.47 ppm and the resonance at 2.6 (s) ppm of the two methylene protons of the cycloheptadienyl ring were observed.

CONCLUSION

The aim of this work is to produce metallohaptens to be used in new competitive immunoassays.

The disadvantages that we encountered with ferrocene derivatives previously proposed as organometallic labels included the difficulty of the assessment of iron and the danger of contamination from the chemical or biological materials that contain the same metal, and

Table 1 The inhibition of [^{3}H]imipramine binding by different metallohaptens

Metallohapten	Inhibition (%)
Imipramine	100
Cy—CO—DESI (**3c**)	40
Cy—CO—NORTRI (**3d**)	28
Cy—CO—CH_2—CH_2—CO—NORTRI (**4d**)	17
Cy—CH_2—CH_2—CH_2—CO—NORTRI (**6d**)	<8

have stimulated research of labels devoid of these drawbacks.

Metal—carbonyl complexes have been chosen because they offer the possibility of detection both from the metal constituting the organometallic structure and from the carbonyl ligands as recently described.[23]

For the formation of the complex Ab—Ag.M, both the presence and the site of attachment of the label could be inauspicious. Likewise, the 'bridge' between the hapten and the organometallic label severely influence the specificity and the sensitivity of the immunoassay.

As a result, Table 1 presents the inhibition data of different metallohaptens. The reported results show a variation of the percentage inhibition from 8 to 40% and demonstrate the very important role of the length of the 'bridge' in the recognition of the metallohapten by the antibodies produced against hapten as reported in a recent publication.[37]

Further refinements are under way: the trend of our research is to supply each hapten with a specific label.

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