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The use of metallocenic esters of *N*-hydroxysuccinimide for metallohapten synthesis

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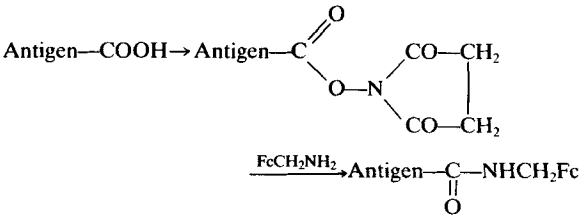
Different organometallic markers have been described in a new technique for the labelling of many drugs. Thus metallocenic esters of

[M = $(CO)_3CrC_6H_5$—; $(CO)_3CrC_6H_5$—$(CH_2)_3$—; η-C_5H_5—FeC_5H_4—; $(CO)_3Mn\eta C_5H_4$—; $(CO)_3Mn\eta C_5H_4COCH_2CH_2$—; $\eta C_5H_4(\eta C_5H_5)Co^+PF_6^-$] react with primary or secondary amine drugs [DRUG—NHR] for a psychostimulant drug: amphetamine; tricyclic antidepressants—desipramine and nortriptyline; a vasodilator—histamine; an adrenergic substance—norfenefrine; and for a central stimulant—methamphetamine, to give the metallohaptens MCON(R)—DRUG. All these compounds have been fully characterized by different analytical methods and have potentialities for biological assays. This synthetic route was found better than one presented previously which utilized the metallocenic acid chloride MCOCl as intermediate, and could be proposed as a general synthetic route for labelling biological compounds which possess an amino group.

Keywords: Organometallic labels, drugs, benchrotrene, cymantrene, cobaltocenium salt, ferrocene, metallohaptens, immunoassays

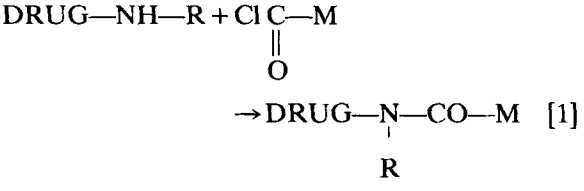
INTRODUCTION

As recently described,[1] the aim of our work is to propose different possible routes to produce metallohaptens which would be included in new immunoassays. In this way a general strategy for a metal-labelling hapten was envisaged;[2] this approach concerned the synthesis of an 'active ester' of a carboxyantigen which is reacted with a suitable functionalized metal-containing reagent, e.g. $FcCH_2NH_2$ (i.e. η-C_5H_5Fe-η-$C_5H_4CH_2NH_2$) according to Scheme 1.



Scheme 1

Another approach was recently reported,[1] in which we developed the use of several different markers for the synthesis of metallohaptens (metallocenic-labelled drugs) as outlined in Eqn. [1].



[1]

A new strategy can also be envisaged. One would plan the synthesis of an organometallic 'active ester' which could react with the amino-drug to produce the desired metallohapten (Scheme 2).

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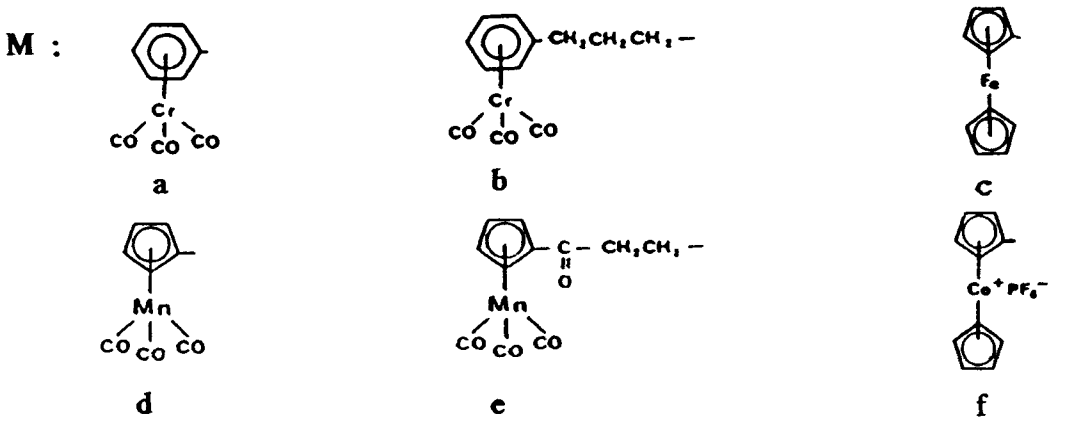
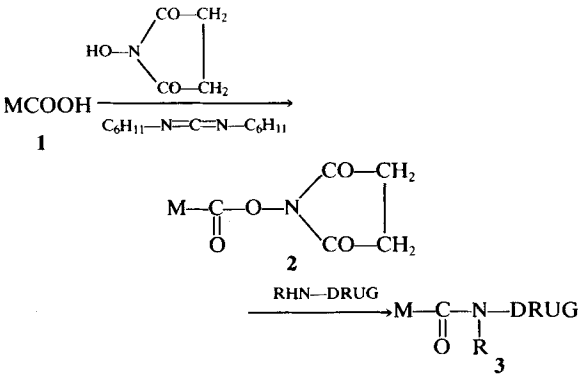


Figure 1 Carboxylic acids derived from benchrotrene, ferrocene, cymantrene and cobaltocenium hexafluorophosphate (**1a–1f**; MCOOH).



Scheme 2

In fact, a single example has been reported by Cais involving the reaction of cobaltocenium hexafluorophosphate-activated *N*-hydroxysuccinimide for the labelling of oestrogen in this way.[3] More recently, taking a similar view, Tanaka *et al.*[4] have mentioned that *N*-succinimidyl 3-ferrocenylpropionate can be used for the selective and sensitive analysis of amino compounds in a complex matrix, particularly in biological fluids. But no systematic study has been described of the use of metallocenic esters of *N*-hydroxysuccinimide for metallohapten synthesis.

We present here results concerning this pathway using the carboxylic acids **1a–1f** (Fig. 1) derived from benzenechromium tricarbonyl (benchrotrene, Bct-H), dicyclopentadienyl iron (ferrocene, Fc-H), cyclopentadienylmanganese tricarbonyl (cymantrene, Cy-H), dicyclopentadienylcobaltocenium hexafluorophosphate (cobaltocenium hexafluorophosphate, $PF_6^-Cob^+$-H). The amino-drugs studied are: (α) amphetamine (AMPHE), (β) desipramine (DESI), (γ) nortriptyline (NORTRI), (δ) histamine (HIST), (ε) norfenefrine (NORFE) and (ζ) methamphetamine (MET).

The carboxyorganometallic complexes **1a–1f** react with *N*-hydroxysuccinimide to form in good yields the stable 'activated esters' **2a–2f** whose main characteristics are given in Table 1. All these complexes have been obained after purification in the solid state and were fully characterized by IR and ^{1}H NMR spectroscopy. Their infrared spectra exhibit three very strong carbonyl stretching vibrations in the 1825–1734 cm^{-1} range assigned to ester and amide carbonyl groups. In NMR spectroscopy, chemical shifts of succinimidyl protons occurred at about 1.60 ppm. In addition, characteristic absorptions of the metallocenic moiety were evident at 2028–1860 cm^{-1} indicating the $Mn(CO)_3$ or $Cr(CO)_3$ group and in the NMR spectra the well-established signals of the cyclopentadienyl or benchrotrenic protons were observed.

All these metallohaptens (**3**) have been synthetized starting from 'activated ester' **2** as outlined in Scheme 2. In a typical reaction, the amino-drug (free base) was reacted in the appropriate solvent (THF, $CHCl_3$ or acetone) with the 'activated ester' metallocenic complex to form the corresponding metallohapten which was purified generally by chromatography and conveniently characterized.

Thus, the pure metallohaptens Bct-CO-AMPHE (**3aα**) Bct-CO-DESI (**3aβ**), Fc-CO-AMPHE (**3cα**) and Fc-CO-MET (**3cζ**) were obtained easily in about 50% yield after

purification. Spectroscopic details (IR and ^1H NMR) given in the Experimental section indicated unambiguously that the products have a good amide structure.

Compounds **3dα**, **3dβ** and **3dγ** were identified by comparison with each of the authentic samples obtained previously when the corresponding organometallic acid chloride reacted with the appropriate amino-drug.[1]

The metallohaptens Fc-CO-HIST (**3cδ**) and Cy-CO-HIST (**3dδ**) were obtained as above except that reaction was performed at room temperature instead of at 40°C and in a shorter reactional period to avoid the decomposition of histamine.

With these conditions the yield was about 25%. The absorption frequencies of the amide group were characteristic in the IR spectrum—CO—N stretch: 1628 cm^{-1} and 1541 cm^{-1} (**3cδ**); 1638 cm^{-1} and 1568 cm^{-1} (**3dδ**). Furthermore the infrared spectrum of **3dδ** showed absorption bands at 2030, 1952 and 1935 cm^{-1} assigned to the terminal carbonyl groups. The NMR spectra clearly showed the signals of a metallocenic moiety and of the drugs as indicated in the Experimental section. For the cymantrenic derivatives (**3d**), it was worth noting that the signals of the cyclopentadienyl protons at 3.69 and 4.28 ppm were well resolved, whereas those of the drug moiety were not.

Fc-CO-NORFE (**3cε**) was prepared starting from the norfenefrine hydrochloride salt according to the literature procedure via peptide synthesis.[5] In fact it was not easy to complete the preparation of norfenefrine free base in good yield due to the simultaneous presence of hydroxyl and amino groups. In this case a selective protection of the functional groups by the silylation method was not successful.[6] Spectroscopic details for **3cε** presented in the Experimental section indicate unambiguously that the product possesses the amide metallocenic structure. In addition, the ^1H NMR spectrum shows resonances due to phenolic and alcoholic hydroxyl groups at 8.36 and 5.03 ppm respectively. The label, ester cobaltocenium hexafluorophosphate (**2f**), was reacted with amphetamine free base in acetone to form a yellow compound which was purified directly by recrystallization. IR and ^1H NMR indicates clearly that the product has the structure **3fα**.

This general new synthetic approach with metallohaptens involving the 'active ester' intermediate presents many advantages compared with the synthetic route which utilizes a metallocenic acid chloride as reported previously:[1]

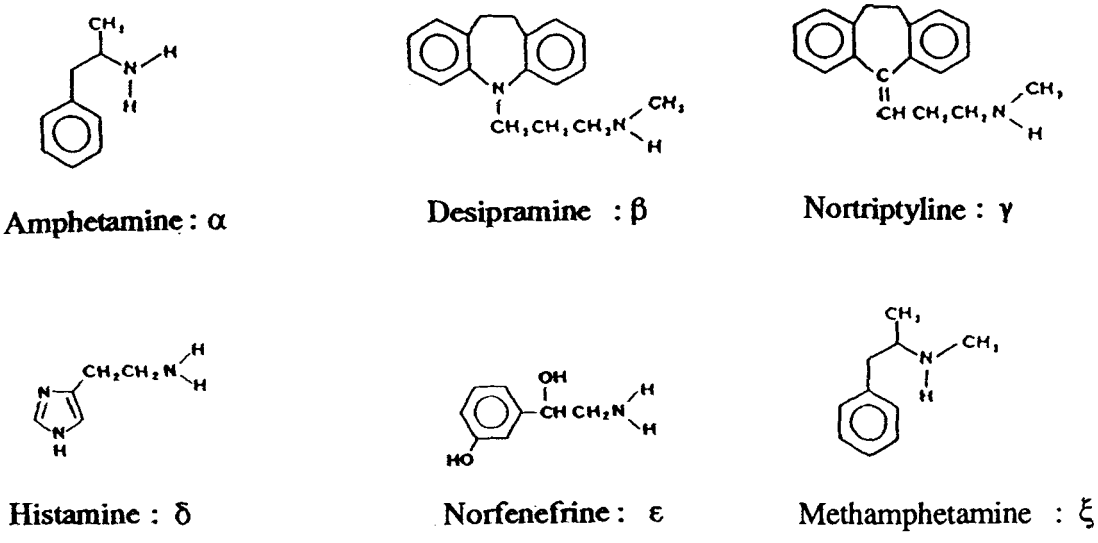


Figure 2 Amino-drugs.

Table 1 Physical data of the 'activated esters' (compounds **2**), M—C(=O)—O—N(CO CH_2)(CO CH_2)

Complex	M.P. (°C)	Yield (%)	IR (cm^{-1})(KBr) $\nu_{C\equiv O}$	IR (cm^{-1})(KBr) $\nu_{C=O}$	^1H NMR (C_6D_6, TMS as internal reference) —CH_2—CH_2— (succinimidyl)	Ring	$(CH_2)_n$
2a	184	65	1975 1901	1800 1778 1739	1.59(s)	3.98(t)(2) 4.46(tt)(1) 5.62(dd)(2)	
2b	134	30	1956 1886 1860	1812 1784 1739	1.62(s) 1.58(s)	(4.26–4.55) (m)(5)	$n=3$ 1.75–2.06(m)(4) 1.29–1.53(m)(2)
2c	172	67		1798 1765 1734	1.67(s) 1.61(s)	C_5H_4: 4.88(t)(2) 4.01(t)(2) C_5H_5: 4.23(s)(5)	
2d	80	80	2028 1946	1800 1767 1738	1.56(s)	3.67(t)(2) 4.98(t)(2)	
2e	140	40	2021 1937	1825 1784 1739	1.57(s)	3.77(t)(2) 4.67(t)(2)	$n=2$ 2.54(t)(2) 2.27(t)(2)
2f	260_{dec}	98		1810 1781 1741	[a]3.03(s)	[a]C_5H_4: 6.25(t)(2) 6.61(t)(2) C_5H_5: 6.15(s)(5)	

[a]Solvent CD_3COCD_3.

(1) The main advantage of such 'activated ester' intermediates is their easy preparation from the acid precursors in good yields and their great stability compared with metallocenic and chloride analogues.

(2) As shown in Table 2, we note a difference in the overall yield (two steps) for the synthesis of the metallohaptens **3** from **1** in both synthetic approaches. For example, **3dα** was obtained in 63% yield via the acid chloride route and in 75% yield via the 'active ester' route.

(3) It is noteworthy that the labelling of drugs which bear different functional groups (e.g. norfenefrine) able to react with the acid chloride function of the organometallic complex, cannot be prepared by the acylation reaction but only by the 'activated ester' method. Furthermore, cobaltocenium hexafluorophosphate (**3fα**) was not able to be prepared by the metallocenic

Table 2 Comparative yields of the two synthetic routes for metallohaptens **3xα** (x: **a, c, d, f**) (via MCOCl or 'activated ester' **2** intermediates)

MCOOH	Yield (%)		Metallohapten	Yield (%)[a]	Yield (%)[b]
1a	MCOCl	48	**3aα**	90	69
1a	**2a**	65	**3aα**	52	58.5[c]
1c	MCOCl	64	**3cα**	21	34.5
1c	**2c**	67	**3cα**	31	49
1d	MCOCl	76	**3dα**	50	63
1d	**2d**	80	**3dα**	73	76.5
1f	MCOCl	73	**3fα**	64[d]	68.5[d]
1f	**2f**	98	**3fα**	57	77.5

[a]Transformation MCOCl or **2a**→**3aα**. [b]Successive transformations **1a**→MCOCl (or **2a**)→**3aα**. [c]Decomplexation was observed. [d]Tetraphenylborate cobaltocenium salt **3f′α**.

acid chloride route because partial dissolution of the product during the usual purification reaction was observed. In these conditions it was necessary to precipitate the metallohapten by treatment with $Na^+BPh_4^-$; thus the water-insoluble tetraphenylborate cobaltocenium salt **3f'α** was obtained in 64% yield. This was fully characterized by IR and ^{1}NMR spectroscopy as indicated in the Experimental section.

EXPERIMENTAL

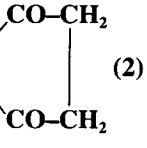
Starting materials

The starting material ferrocene carboxylic acid (Fc-COOH) was purchased from Ventron Chemicals; Cy-COOH was prepared following the procedure of Riemschneider and Petzoldt;[7] Bct-COOH was obtained according to the procedure of Dabard and Meyer;[8] amphetamine, methamphetamine, histamine and norfenefrine were purchased from Cooper de Melun (France); desipramine was supplied by Ciba–Geigy Laboratories and nortriptyline by Eli Lilly Research Laboratories. 1-Carboxycobaltocenium hexafluorophosphate ($PF_6^-Cob^+$-COOH) was obtained by the reaction of anhydrous cobalt(II) chloride with an equimolar mixture of cyclopentadiene and methylcyclopentadiene followed by oxidation using potassium permanganate in an alkali medium.[9]

Equipment

All manipulations 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 was used. Spectra were recorded with the following instruments: infrared, Perkin–Elmer 580 B; ^{1}H NMR, JEOL FX 100 (δ ppm/TMS).

Synthesis

Metallocenic esters of *N*-hydroxysuccinimide MCOO-N  **(2)**

Ferrocenic 'activated acid' **(2c)**
In a typical experiment, ferrocene carboxylic acid (460 mg, 2 mmol), *N*-hydroxysuccinimide (253 mg, 2.2 mmol) and dicyclohexylcarbodiimide (440 mg, 2.2 mmol) in 20 cm^3 of dry THF were stirred for a period of 24 h at room temperature in the dark. The urea by-product was separated by filtration. After removal of solvent a thin-layer chromatography purification (eluent: toluene/acetone, 10:1) followed by crystallization from toluene/hexane afforded 360 mg of orange crystals of **2c** in 67% yield: m.p. 172°C. Calcd for $C_{15}H_{13}NO_4Fe$: C, 55.04; H, 3.97; N, 4.28; Fe, 17.12. Found: C, 55.02; H, 4.23; N, 4.37; Fe, 15.12%. Spectroscopic data are reported in Table 1.

Benchrotrenic 'activated acid' **(2a, 2b)** *and cymantrenic 'activated acid'* **(2d, 2e)**
These were prepared by the same route as for compound **2c** above. Spectroscopic data are summarized in Table 1.

Cobaltocenium hexafluorophosphate 'activated acid' **(2f)**
This was prepared similarly to the above, except that 1-carboxycobaltocenium hexafluorophosphate and *N*-hydroxysuccinimide were reacted on an equimolar basis vigorously in acetone. After removal of solvent a yellow solid was obtained, which was washed with pentane, and dried in vacuum; yield 98%, m.p. 260°C (dec.) (Table 1).

Metallohaptens M—C(=O)—N(R)—DRUG (3)

Cy-CO-AMPHE **(3dα)**
In a typical reaction, a solution of 'activated acid' **2d** (68 mg, 0.2 mmol) in 5 cm^3 of dry tetrahydrofuran (THF) was slowly added under argon to a solution of amphetamine free base (53 mg, 0.4 mmol) in 2 cm^3 of THF. The mixture was stirred during a period of 48 h at 40°C. 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 **3dα** (53 mg; yield 73%) as yellow crystals, m.p. 122°C (lit. 122°C). IR and NMR characteristics were described previously.[1]

Fc-CO-AMPHE **(3cα)**
Amide **3cα** was prepared from ferrocenic ester and amphetamine free base by the method described above. An orange solid resulted, yield 31%, m.p. 160°C. IR (KBr), cm^{-1}: —CO—N 1621, 1531. ^{1}H NMR (CD_3COCD_3), ppm: 7.10–7.09 (m, C_6H_5—); 5.16 (d, N—H); 4.51 (m,

C—H); 4.64 (m, 1H); 4.37 (m, 1H) and 3.96 (t, 2H, C_5H_4); 3.92 (s, C_5H_5); 2.58 (d, CH_2); 1.02 (d, CH_3).

Cy-CO-DESI (**3dβ**)
The same preparative method as for **3dα** was applied to the reaction with desipramine base to afford amide **3dβ**. The yield was 55%; oil (lit. oil).[1]

Cy-CO-NORTRI (**3dγ**)
Treatment of cymantrenic ester **2d** with nortriptyline base according to the above procedure yielded 40% of the expected amide **3dγ**, oil (lit. oil).[1]

Bct-CO-AMPHE (**3aα**)
This was prepared similarly to the other amides above and purified by chromatography using as eluent ether/hexane, 9:2, instead of toluene/acetone, 20:1. A yellow oil was obtained (yield 52%). IR (NaCl), cm^{-1}: C≡O 1975, 1905; —CO—N 1635, 1541. ^1H NMR (CD_3COCD_3), ppm: 7.26 (s, $C_6H_5^-$); 6.23 (dd, 2H) and 5.76–5.60 (m, 3H, Bct); 3.16 (m, C—H); 3.08–2.65 (m, CH_2); 1.18 (d, CH_3).

Bct-CO-DESI (**3aβ**)
This metallohapten **3aβ** was obtained in the same manner as **3aα**. Yellow crystals, m.p. 58°C (yield 44%) were obtained. IR (KBr), cm^{-1}: C≡O 1972, 1885; —CO—N 1632. ^1H NMR (CD_3COCD_3), ppm: 7.14–6.89 (m, aromatic 8H); 5.77–5.37 (m, 5H, Bct); 3.77 (t, $—CH_2—N—CH_3$); 3.55 (t, $—CH_2—CH_2—CH_2NCH_3$); 3.07 (t, $—CH_2—CH_2—$); 2.96 (s, $N—CH_3$); 1.86 (q, $—CH_2—CH_2—N—CH_3$).

Fc-CO-HIST (**3cδ**)
Histamine free base (90 mg, 0.81 mmol) and ferrocenic ester **2c** (132.5 mg, 0.405 mmol) were reacted in chloroform ($CHCl_3$) (25 cm^3) at room temperature during a period of 29 h. A precipitate was formed. After filtration, the filtrate and THF extracts of the precipitate were evaporated under reduced pressure. The crude product was purified by flash column chromatography [eluent: toluene/acetone, 1:1, for elimination of the unreacted starting material; then methanol]. Methanol was evaporated to afford amide **3cδ**, which was recrystallized from ethanol/water. The yield was 20% (ochre crystals); m.p. 190°C. IR (KBr), cm^{-1}: —CO—N 1628, 1541. ^1H NMR (CD_3OD), ppm: 6.35 (s, CH); 5.66 (s, CH); 3.85 (s, C_5H_5); 3.50 (t, 2H) and 3.10 (t, 2H, C_5H_4); 2.31 (t, CH_2); 1.63 (t, CH_2).

Cy-CO-HIST (**3dδ**)
This was prepared like the metallohapten **3cδ** by using the cymantrenic ester **2d** (143 mg; 0.405 mmol) instead of the ferrocenic derivative. The crude product was directly recrystallized from ethanol/water. Amide **3dδ** was obtained as ochre cubic crystals; m.p. 180–181°C (yield 25%). IR (KBr), cm^{-1}: C≡O 2030, 1952, 1935; —CO—N 1638, 1568. ^1H NMR (CD_3OD), ppm: 6.33 (br s, CH); 5.62 (br s, CH); 4.28 (t, 2H) and 3.69 (t, 2H, C_5H_4); 2.24 (br s, CH_2); 1.57 (br s, CH_2).

Fc-CO-NORFE (**3cε**)
A solution of ferrocenic ester (150 mg, 0.458 cm^3) in 10 cm^3 of THF was slowly added to a solution of norfenefrine, HCl salt (86.9 mg, 0.458 mmol) and $NaHCO_3$ (76.81 mg, 0.914 cm^3) in 2 cm^3 of distilled water according to the literature procedure.[5] The mixture was stirred at room temperature for 96 h. After evaporation of the solvent, the resulting material was diluted in water, acidified by 0.25 M-HCl and extracted with CH_2Cl_2. Methylene chloride extracts were dried over anhydrous calcium chloride. On removal of the solvent an orange solid resulted. A thin-layer chromatography purification (eluent: toluene/acetone, 1:1; extraction: methanol) gave pure metallohapten (**3cε**) as orange crystals (60 mg, yield 36%), m.p. 172°C. IR (KBr), cm^{-1}: —CO—N, 1585, 1558. ^1H NMR (CD_3COCD_3), ppm: 8.35 (s, —C—OH); 7.34 (br s, NH); 7.15 (t, 1H); 6.97 (t, 1H); 6.89 (m, 1H) and 6.71 (m, 1H, C_6H_4—); 5.03 (d, —CHOH); 4.82 (q, CH); 4.77 (m, 2H) and 4.32 (t, 2H, C_5H_4); 4.15 (s, C_5H_5); 3.65–3.58 (m, 1H) and 3.36–3.44 (m, 1H, CH_2).

Fc-CO-MET (**3cζ**)
The same preparative method as for **3dα** was applied to the reaction with the methamphetamine free base (reaction period 96 h; thin-layer chromatography purification eluent: ligroïn/acetone, 2:1) to afford amide **3cζ** as orange needles, m.p. 112–114°C (yield 71%, based on the reacted ferrocenic ester; non-reacted was 21 mg). IR (KBr), cm^{-1}: —CO—N 1609. ^1H NMR (CD_3COCD_3), ppm: 7.26 (s, C_6H_5—); 4.91 (t, 2H) and 4.43 (m, 2H, C_5H_4); 4.06 (s, C_5H_5);

4.94 (br s, CH); 2.99 (s, N—CH_3); 2.79–2.89 (m, CH_2); 1.18 (d, CH_3).

$Cob^+PF_6^-$-CO-AMPHE (**3fα**)
A mixture of ester cobaltocenium hexafluorophosphate (63.95 mg, 0.13 mmol) and amphetamine free base (2.02 mg, 0.149 mmol) in 8 cm^3 of acetone was stirred at room temperature for 24 h. After evaporation of the solvent, a microcrystalline product resulted. Crystallization from ethanol afforded 38 mg of fine yellow needles of **3fα** (57%), m.p. 175°C. IR (KBr), cm^{-1}: —CO—N, 1637, 1555. ^1H NMR (CD_3COCD_3), ppm: 7.83 (d, N—N); 7.34 (m, C_6H_5—); 6.84 (t, 2H) and 5.92 (t, 2H, C_5H_4); 5.64 (s, C_5H_5); 4.48 (qd, C—H); 2.91 (d, CH_2); 1.26 (d, CH_3).

$Cob^+BPh_4^-$-CO-AMPHE (**3f'α**)
1-Chlorocarbonylcobaltocenium hexafluorophosphate (73.8 mg, 0.186 mmol) prepared according to the literature procedure[5] and amphetamine free base (50.2 mg, 0.372 mmol) in 10 cm^3 of acetone and 0.5 cm^3 of pyridine were reacted at room temperature for 4 h. After filtration and evaporation of the volatiles, the resulting material was diluted with 20 cm^3 of a mixture of distilled water and acetone (1:1). Then $Na^+BPh_4^-$ (100 mg, 0.29 mmol) in 2 cm^3 of water was added to the yellow solution. A yellow emulsion was obtained and the yellow oil decanted after centrifugation was washed with toluene, then with pentane. The yellow solid which formed immediately was recrystallized from methanol. Yellow needles resulted with a yield of 64% (79 mg), m.p. 74°C. IR (KBr), cm^{-1}: —CO—N, 1669, 1523; N—H, 3392. ^1H NMR (CD_3COCD_3), ppm: 7.82 (br d, N—H); 7.33–6.72 (m, C_6H_5); 6.12 (t, 2H) and 5.64 (m, 2H, C_5H_4); 5.45 (s, C_5H_5); 4.47 (qd, C—H); 2.91 (d, CH_2); 1.25 (d, CH_3).

CONCLUSION

The labelled compounds described in this publication can be regarded as markers for immunoassay as demonstrated in a recent European patent.[10] Moreover, we consider this approach to labelling drugs which posseses primary or secondary amine groups in their structure as a general synthetic route for labelling biological compounds. Furthermore, this reaction can in many cases progress rapidly in a partial aqueous medium because the 'peptidic reaction' is more rapid than the hydrolysis reaction of the activated ester.[11] Labelling of peptides is now under way and will be described in a further publication.

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