Luminescent rhenium *fac*-tricarbonyl-containing complexes of androgenic oxo-steroids

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Abstract

A new route to luminescent derivatives of androgenic steroids containing a ketone group in the 3- or 17-position has been developed. Reaction with the *fac*-$Re(CO)_3Cl$ complex of 3,3'-diamino-2,2'-bipyridine (complex **1**) afforded a cyclic aminal product with different steroids. The rate of reaction and yield varies according to the conjugation or steric hindrance around the ketone group.

Introduction

The use of complexes containing luminescent transition metal ions to detect molecules of biological interest has gained much attention in recent years. This work has focused mainly upon lanthanide luminescence[1] and more recently the use of d-block metal ions.[2] For example, Yam and co-workers have prepared Pt(II), Ru(II) and Re(I) complexes for detection of biomolecules such as esterase[3], serum albumin[4] and heparin.[5] Lo *et al*. have reported a range of Re(I)- and Ru(II)-containing complexes which act as biomolecular probes, including biotin-transition metal conjugates as non-covalent probes for avidin[6] and indole-appended complexes which interact with indole-binding proteins such as BSA and tryptophanase.[7]

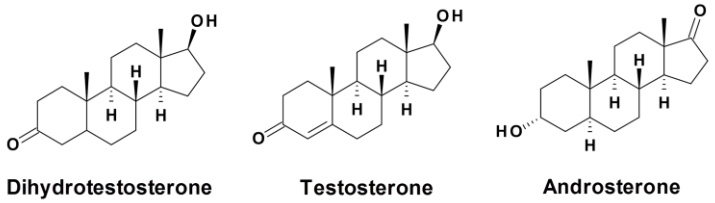
There are many applications for which fluorescent labelling of steroids is required, including immunoassays, receptor binding studies and fluorescence detection (*e.g.* in HPLC). Many fluorescent labelling reagents are in use for derivatisation of steroids; these include 1- and 9-anthroyl nitrile[8,9], nitrobenzoxadiazole[10], dansyl hydrazine[11], 7-alkylcoumarin derivatives[12] and various fluorescein-containing reagents[13], among others. All of these reagents utilise organic fluorescence which tends to possess a short lifetime, typically 0.1 – 20 ns, although this can rise to around 90 ns (*e.g.* for pyrene-containing compounds).[14] In contrast to the estradiols, very few reactions of androgenic steroids with complexes containing luminescent metals have been reported; this approach offers potential advantages since metal-based luminescence often gives a longer emission lifetime, leading to the possibility of time-gated luminescence measurements to remove competing signals from background autofluorescence in biological systems. However, it is frequently the case in such systems that multi-step synthetic modification of the steroid backbone is required prior to coordination of the metal ion.

We report herein a new route to simple, one-step functionalisation of dihydrotestosterone, a biologically important androgenic steroid, with a rhenium *fac*-tricarbonyl bipyridyl complex containing a diamino unit remote from the metal centre. The attempted reactions of testosterone and androsterone are also presented. Some examples of rhenium-containing androgen derivatives

have been reported; Wüst *et al* prepared such complexes by funtionalising testosterone at the 7α-position with thiol-containing ligands capable of coordinating an oxorhenium centre.[15] Jaouen and coworkers have synthesised a range of $CpRe(CO)_3$-substituted complexes of cholest-4-en-3-one[16], and latterly of testosterone and DHT.[17]

Transition metal complexes containing ligands with a 3,3'-diamino-2,2'-bipyridyl core have been shown previously to react readily with ketones[18] and aldehydes[19] to form seven-membered cyclic aminal products and therefore this chemistry was utilised in this study.

The three steroids used in this work (Fig. 1a) are all members of the androstane sub-class whose general structure is shown in Fig. 1b; the androstanes are characterised by the methyl substituents at positions 10 and 13 of the tetracyclic steroid backbone.[20] These three steroids vary in the degree of conjugation or steric hindrance around the ketone moieties; their reactivities may be expected to differ significantly for this reason.



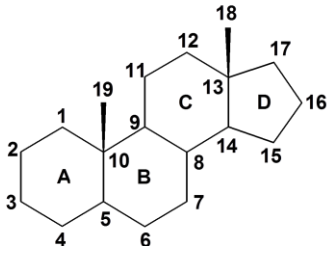
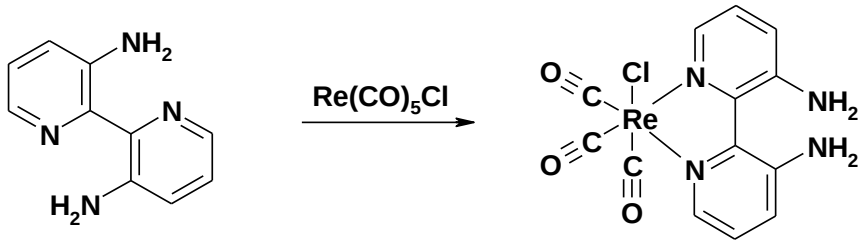


Fig. 1: a) Structures of dihydrotestosterone, testosterone and androsterone; b) the general structure of androstanes, showing the IUPAC steroid numbering convention[20]

Results & Discussion

Synthesis of complex 1

3,3'-Diamino-2,2'-bipyridine was prepared from 3-amino-2-chloropyridine according to literature methods.[21] The rhenium complex of 3,3'-diamino-2,2'-bipyridine (**1**) was prepared by reaction of the ligand with rhenium pentacarbonyl chloride in dichloromethane under reflux (Scheme 1). As expected the ^1H and ^{13}C NMR analysis was consistent with the formation of the rhenium complex; in addition the ESI-MS gave an ion at m/z 457.0 (corresponding to $[M\text{-}Cl]^+$) whose composition was confirmed by accurate mass measurements.



[1]

Scheme 1: Synthesis of the rhenium complex of 3,3'-diamino-2,2'-bipyridine, **1**

Reaction of 1 with dihydrotestosterone (DHT)

The reaction of **1** with DHT was monitored *via* thin layer chromatography until the rhenium-containing starting material had been consumed. A yellow solid (**1**-DHT) was produced following initial purification of the crude product by passage through a plug of alumina (2% MeOH in DCM). The ^{1}H NMR spectrum of the resultant solid (CD_3CN) showed a complex series of highly coupled signals (Fig 2).

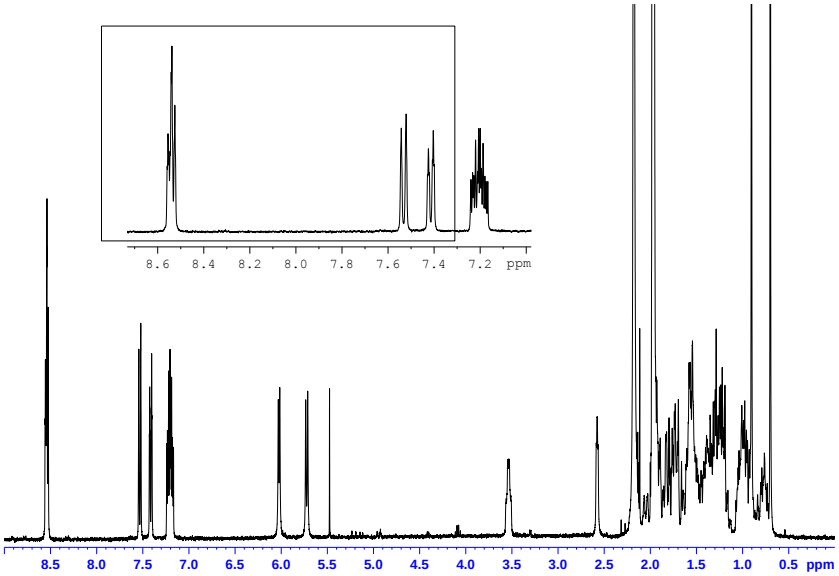


Fig. 2: ^{1}H NMR spectrum of the crude **1**-DHT product showing an expansion of the aromatic region (inset)

Close examination of the aromatic region reveals the presence of four sets of signals from the bipyridyl moiety indicating that there are isomeric products present in solution.

The ESI-mass spectrum of the product (MeCN) gave a predominant ion at *m/z* 787.2 corresponding to ([(**1**-DHT)+Na]$^{+}$); this composition was confirmed by accurate mass measurements. In addition, the measured isotope pattern of the ion at *m/z* value 787.2 closely matched the calculated pattern for $C_{32}H_{38}N_4O_4Re_1Cl_1Na_1$ (Fig. 3).

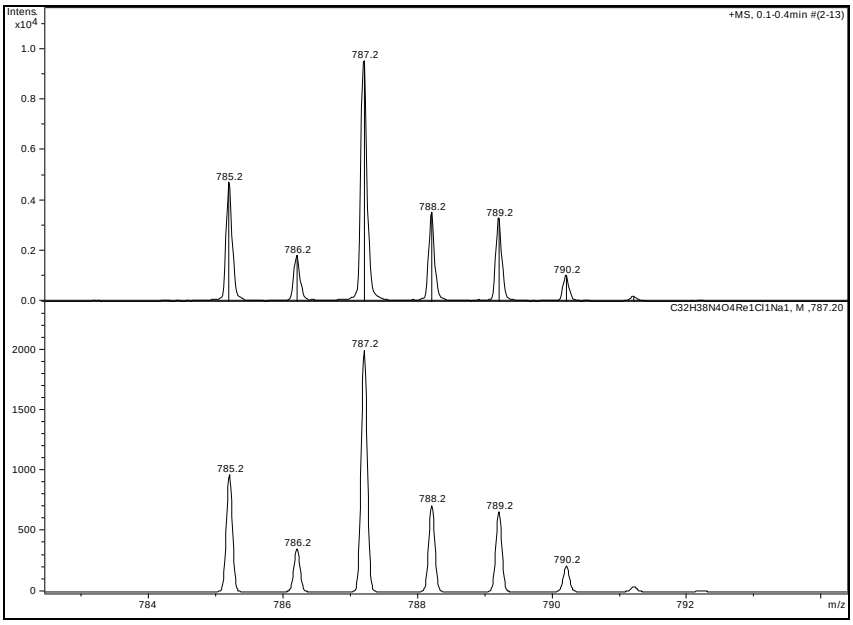


Fig. 3: Measured (top) and calculated (bottom) isotope patterns of $C_{32}H_{38}N_4O_4Re_1Cl_1Na_1$

This evidence clearly shows that DHT has reacted with the rhenium complex; however, the NMR analysis is consistent with the formation of isomeric products. Furthermore, thin layer chromatography shows two products with very similar retention factors. Careful column chromatography produced small amounts of the purified materials which upon analysis gave much simplified NMR spectra (Fig. 4).

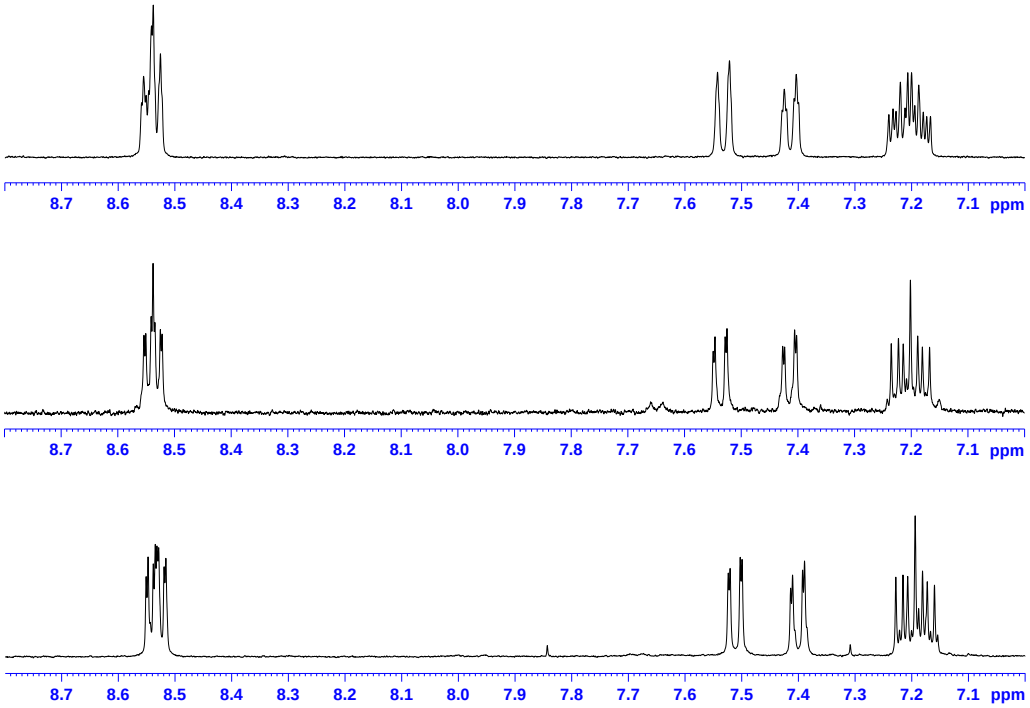


Fig. 4: Selected regions of the ^{1}H NMR spectra of the crude material (top), the early eluting fraction (centre), the late eluting fraction (bottom)

Solid-state analysis

Crystallisation was achieved on one of the fractions by slow diffusion of diisopropyl ether into an acetonitrile solution of the purified product. X-ray analysis showed that in the solid state the rhenium centre adopts an octahedral geometry and is coordinated by two pyridyl nitrogen atoms in its equatorial positions (Fig. 5a). Two carbonyl ligands fill the other two equatorial sites and the axial positions are occupied by another carbonyl ligand and a chloride ion (Re-N distances 2.164(3) and 2.161(3) Å; Re-C distances 1.906(5) – 1.925(5) Å; Re-Cl distance 2.4904(11) Å).
The amine groups of complex **1** have reacted with the ketone group on position 3 of the steroid to form a 7-membered aminal ring; the steroid is positioned in a *trans*-oid geometry relative to the chloride on the rhenium centre. The structure is highly unsymmetrical, with the steroid moiety leaning significantly towards one pyridyl ring (Fig. 5b).

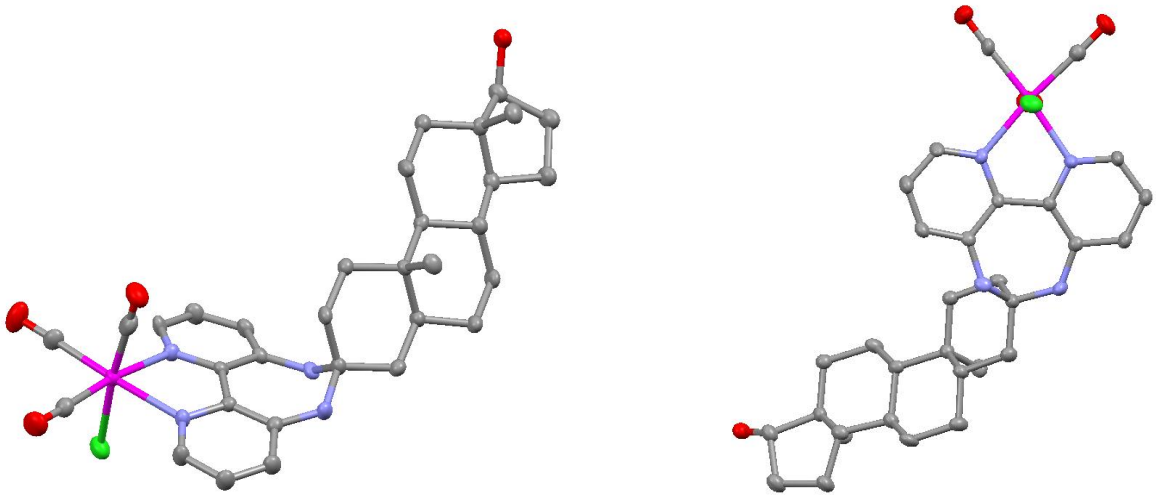


Fig. 5: The single crystal X-ray structure of **1**-DHT (a) as a displacement ellipsoid plot drawn at the 50% probability level; (b) showing the inequivalence of the bipyridyl rings

Examination of the intermolecular packing (Fig. 6) shows that the chloride ion from each molecule forms a hydrogen bond to the hydroxyl group in the 17-position of DHT (Cl-H distance 2.414 Å).

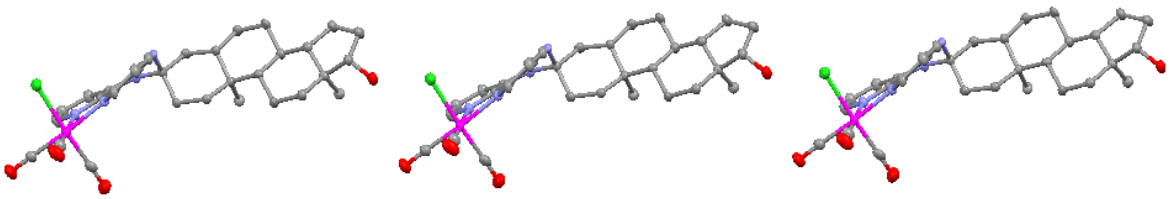


Fig. 6: The single crystal X-ray structure of **1**-DHT showing the intermolecular packing

Solution-state analysis

Close examination of the solid state structure demonstrates why the ^{1}H NMR spectrum is so complex. Firstly, the steroid can react with the complex by approaching it in two different ways, giving the isomers **i** and **ii** (Fig. 7) which can be separated by chromatography and result in simplified ^{1}H NMR spectra. Secondly, due to the non-planarity of the steroid each of the pyridyl rings is not equivalent to the other (Fig 5b) resulting in the doubling-up of the aromatic signals in the ^{1}H and ^{13}C NMR spectra. It is therefore clear that the two geometric isomers can be separated by column chromatography giving a pure complex which contains inequivalent bipyridyl heterocyclic rings.

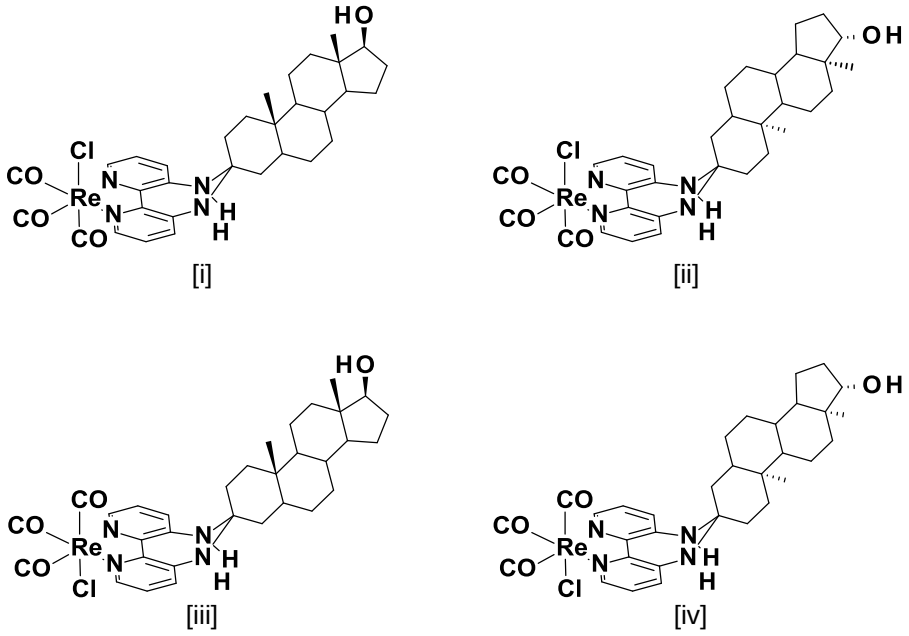


Fig. 7: The possible isomers of **1**-DHT

It is worth noting that it is entirely feasible that there are conformers of **i** and **ii** generated by the inability of the aminal ring to invert (**iv** and **iii** respectively, Fig. 7). However, this would give rise to another set of signals which we do not observe. It is therefore likely that in solution the barrier to inversion is sufficiently low that it is not observed on the NMR timescale at room temperature. To confirm this, cyclohexanone was reacted with **1** to give a similar aminal species, but one which contains a highly symmetrical cyclohexyl unit. If the aminal interconversion were slow, on the NMR timescale, this would give rise to two different species. However, analysis of the crude material demonstrated only one species was formed (Figs S7 and S8, ESI).

Characterisation of the product by NMR

The purified product was characterised fully using 1D and 2D NMR techniques (including COSY, HSQC, HMBC, TOCSY and NOESY); spectra were acquired in DMSO-d_6 to aid solubility and thus facilitate acquisition of ^{13}C-detected experiments.

Carbons 1, 2, 4 and 5 (see Fig. 1) exhibit a marked upfield shift relative to the starting material, which confirms that derivatisation of the steroid has taken place at position 3 (Fig. 8). The signal from C3 itself moves from 210.97 ppm in the underivatised steroid to 67.52 ppm in the product which is typical for a quaternary carbon bonded to nitrogen. In addition, the HMBC spectrum shows $^{2}J_{H,C}$ couplings from the NH groups of the aminal ring to C3 of the steroid (Fig. S4, ESI).

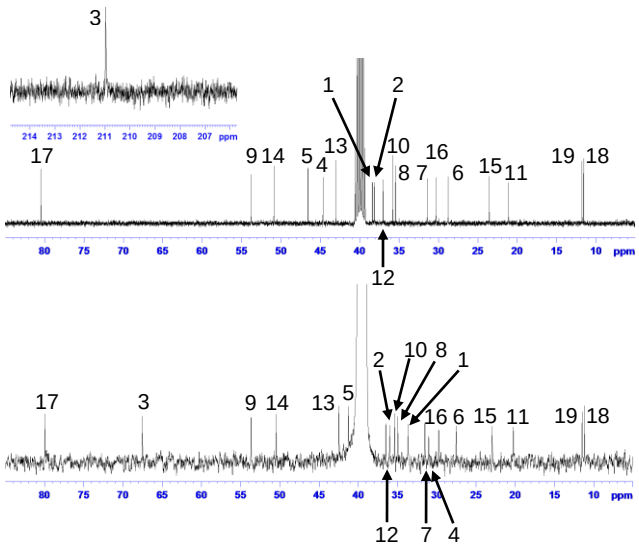


Fig. 8: Selected regions of the $^{13}C\{^{1}H\}$ spectra (DMSO-d_6) of DHT (top) and **1**-DHT (bottom)

As expected due to their inequivalence, two sets of signals are observed for the pyridyl protons as shown in the HSQC spectrum (Fig. 9). The remaining, uncorrelated carbon signals are from the quaternary carbons (2 and 3) in the pyridyl rings.

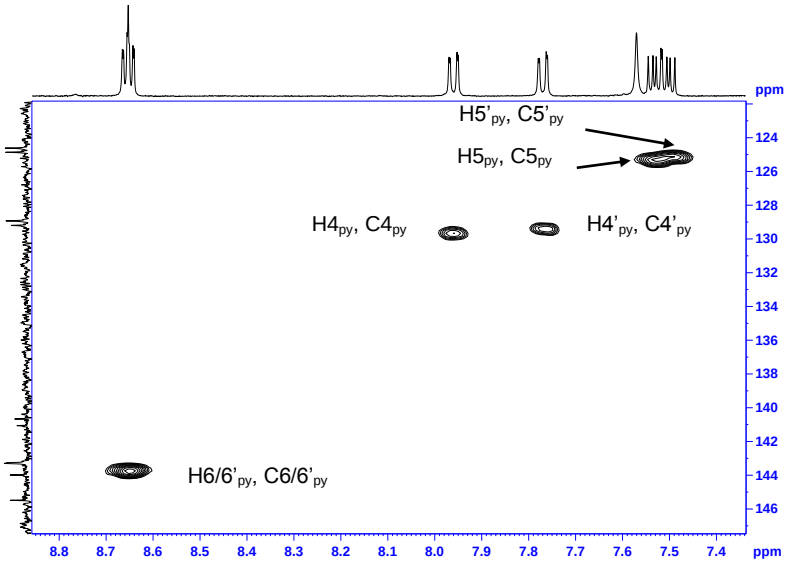


Fig. 9: Selected region of the ^{1}H-^{13}C HSQC spectrum (DMSO-d_6) of **1**-DHT

DFT and luminescence studies

DFT calculations on a structurally simplified bis-aminal analogue (*i.e.* assuming the condensation of the diamine with acetone) were undertaken to explore the nature of the frontier orbitals. Unexpectedly the optimised lowest energy conformation of the complex predicts an out of equatorial plane distortion (20.04°) for the bpy ligand, which is not as pronounced in the crystal structure of **1**-DHT. The HOMO (E = -5.52 eV) and HOMO-1 (E = -5.61 eV) are close enough in energy ($\Delta E < 0.2$ eV) to be considered isoenergetic and mainly situated on Re and Cl, whilst the LUMO (E = -2.37 eV) is sufficiently different in energy to be considered independent and located primarily on the bpy ligand.

The pictorial representations and relative distributions of the frontier orbitals are shown in Fig. 10 and Table 1, respectively. The mixed metal/halide character is consistent with previous descriptions for complexes of this type[22] and suggests an excited state delocalised positive hole. The results suggest the lowest energy absorption should be reported as a HOMO→π* (bpy), in turn suggesting that significant MLCT and LLCT character be predicted for the lowest energy excited state.

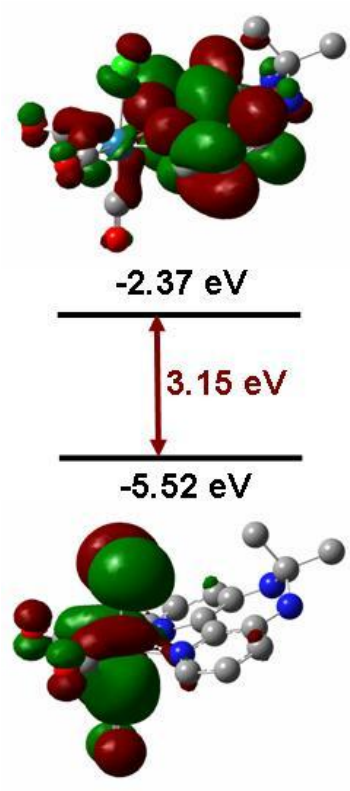


Fig 10: Representations of the calculated HOMO and LUMO

	HOMO-1	HOMO	LUMO
Re	38.2	41.6	3.6
Cl	36.9	38.1	2.1
CO (eq 1)	3.1	3.7	1.0
CO (eq 2)	3.1	3.7	1.0
CO (ax)	8.8	9.5	0.9
bpy	10.9	3.4	91.4

Table 1: Percentage distribution of HOMO-1, HOMO and LUMO over complex

The absorption spectra for the complexes show two main features with ligand-centred transitions dominating < 300 nm and broad visible absorption at 330-450 nm associated with LLCT/MLCT character (*cf*. DFT); the spectrum of **1**-DHT showed a definitive red-shift in the absorption maximum of this band to *ca.* 410 nm.

The room temperature luminescence properties of the free ligand (3,3'-diamino-2,2'-bipyridine), complexes **1** and **1**-DHT were assessed in two aerated solvents (MeOH, and $CHCl_3$). The free ligand gave emission spectra with a fluorescence peak at 436 nm (MeOH, τ = 1.4 ns) or 478 nm ($CHCl_3$, τ = 1.7 ns). In methanol the diamino complex **1** (λ_{ex} = 335 nm) was emissive at 443 nm, whereas in chloroform only one band at 581 nm was evident. The lifetime in MeOH was 2.7 ns, and this was attributed to a metal-perturbed ligand-centred emission. The long wavelength band observed in $CHCl_3$ must be attributed to the MLCT/LLCT character, as discussed in the context of the DFT results, and the lifetime associated with this emission was longer-lived (λ_{ex} = 295 nm) at 119 ns. For comparison, solvent-sensitive photophysical properties are obtained for $[ReCl(CO)_3(bpy)]$ where lifetimes were 18.8 ns (MeOH) and 39.8 ns ($CHCl_3$); a value of 39 ns has also been reported in mthf.[23] Formation of the bis-aminal **1**-DHT species resulted in a bathochromic shift of the lowest energy broad absorption band to *ca*. 440 nm and irradiation of this peak (λ_{ex} = 435 nm) gave emission bands at 533 (MeOH) and 564 nm ($CHCl_3$), each attributed to the MLCT/LLCT character, as described above. However, the lifetimes were very short (3.5 ns in $CHCl_3$; < 1ns in MeOH) for this species, suggesting that the presence of the bis-aminal functionality may provide an effective quenching pathway for the excited state. Interestingly, it was possible to solubilise complexes **1** and **1**-DHT in buffered (MOPS) aqueous solution. The differences in absorption wavelength allowed the selective and differential irradiation (λ_{ex} = 435 nm) of **1**-DHT (Fig. 11), indicating the potential of the complex in a sensing or biomarker capacity.

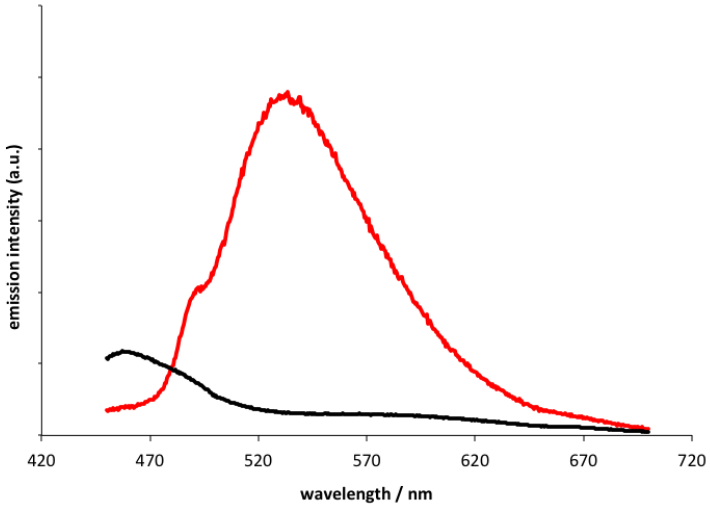


Fig. 11: Emission spectra for complex **1** (black) and **1**-DHT (red) recorded in MOPS buffer (λ_{ex} = 435 nm)

Reaction of 1 with other oxo-steroids

Reaction of equimolar amounts of **1** and testosterone (T) in CD_3CN proceeded significantly more slowly than the analogous DHT reaction; after heating for 8 hours analysis of the reaction mixture by ^1H NMR spectrometry showed that approximately 60% conversion to **1**-T had been achieved. In a similar fashion reaction of equimolar amounts of **1** and androsterone (ADT) in CD_3CN proceeded very slowly; again after heating for 8 hours analysis of the reaction mixture by ^1H NMR spectrometry showed that only approximately 30% conversion to **1**-ADT had been achieved.
The difference in rate and yield of these compounds compared to DHT is due to the conjugated ketone of T and the close proximity of a methyl group in ADT which reduce the rate of reaction due to electronic and steric effects respectively.

Conclusion

A new route to one-step derivatisation of oxo-steroids has been developed. The Re(I)-containing diamine complex reacts with DHT to give two isomers which can be separated and examined in the solution and solid states. Furthermore, reaction with DHT bathochromically shifts the visible absorption profile and results in a luminescent Re-based product emitting in the visible region.

Experimental

General

All photophysical data were obtained on a JobinYvon-Horiba Fluorolog spectrometer fitted with a JY TBX picosecond photodetection module. Lifetimes were obtained using the provided deconvolution software DAS6. Electrospray ionisation mass spectra were recorded from 10^{-3} M solutions on a Bruker MicrOTOF-q instrument.

Synthesis of complex 1

3,3'-Diamino-2,2'-bipyridine was prepared from 3-amino-2-chloropyridine according to literature methods.[21]

To 3,3'-diamino-2,2'-bipyridine (0.10 g, 0.54 mmol) in dichloromethane (25 mL) was added rhenium pentacarbonyl chloride (0.19 g, 0.54 mmol) and the reaction was heated under reflux in the dark for 18 hours. The reaction was cooled and filtered *in vacuo*; the pale yellow product **1** was washed with Et_2O and dried. (128.8 mg, 48%)

^{1}H NMR = [400 MHz, DMSO-d_6] δ 8.37 (dd, *J* = 5.0, 1.3, 2H, py), 7.58 (dd, *J* = 8. 5, 1.3, 2H, py), 7.39 (dd, *J* = 8.5/5.0 Hz, 2H, py), 6.20 (s, broad, 4H, NH_2)

^{13}C NMR = [400 MHz, DMSO-d_6] δ 199.10 (C=O), 191.99 (C=O), 144.00 (py quaternary), 142.34 (py CH), 139.93 (py quaternary), 128.29 (py CH), 125.81 (py CH)

ESI-MS found *m/z* 457.0 $[M-Cl]^+$, HR-ESI-MS found 457.0325, $C_{13}H_{10}N_4O_3Re_1$ requires 457.0305. UV-vis ($CHCl_3$): λ_{max} (ε / dm^3 mol^{-1} cm^{-1}) 274(3900), 380(8200) nm.

Synthesis of derivatives of complex 1

Dihydrotestosterone

Dihydrotestosterone (3.04 mg, 0.01 mmol) and **1** (5.04 mg, 0.01 mmol) were dissolved in acetonitrile (1 mL). A few grains of camphorsulfonic acid were added and the solution was heated at 60°C for two hours. The product was purified *via* column chromatography (Al_2O_3, 2% MeOH in DCM). The yield of the two purified fractions was 4.97 mg, 62%.

NMR – see the text for discussion of the NMR spectra. Characterisation of the purified product was carried out in DMSO-d_6 on a Bruker Avance 500 MHz spectrometer using Bruker's standard pulse programs, with increased numbers of scans where necessary.

ESI-MS found *m/z* 787.2 $[M+Na]^+$, HR-ESI-MS found 787.2011, $C_{32}H_{38}N_4O_4Re_1Cl_1Na_1$ requires 787.2031. IR (ATR) ν/cm^{-1}: 3308 (w, OH), 2931 (m, CH), 2847 (m, CH), 2014 (s, C≡O), 1900 (s, C≡O), 1879 (s, C≡O). UV-vis (MeCN): λ_{max} (ε / dm^3 mol^{-1} cm^{-1}) 290(12600), 411(19000) nm.

Testosterone and androsterone

1-T and **1**-ADT were prepared by dissolving *ca*. 2 mg of complex **1** with 1 eq. of steroid and a few grains of camphorsulfonic acid in 0.65 mL CD_3CN and heating to 60°C for 8 hours. The reactions were monitored using ^{1}H NMR spectrometry.

Cyclohexanone

Complex **1** (20 mg, 0.04 mmol) was dissolved in dichloromethane (10 mL). Cyclohexanone (10 μL, 2.5 eq.) and a few grains of camphorsulfonic acid were added and the solution was stirred at room temperature for 2 hours. The resulting precipitate was filtered under vacuum and washed with dichloromethane (2 x 2mL), giving a bright yellow solid (15 mg, 65%).

^{1}H NMR = [500 MHz, DMSO-d_6] δ 8.42 (dd, *J* = 5.02/1.32, 2H, py), 7.66 (dd, *J* = 8.50/1.32, 2H, py), 7.30 (dd, *J* = 8.48/5.06 Hz, 2H, py), 7.21 (s, broad, 2H, NH), 1.73 (m, broad, 4H, CH_2), 1.62 (m,

broad, 2H, CH_2), 1.42 (m, broad, 4H, CH_2)

^{13}C NMR = [500 MHz, DMSO-d_6] δ 199.15 (C=O), 192.06(C=O), 145.12 (py quaternary), 143.92 (py CH), 141.41 (py quaternary), 129.76 (py CH), 125.40 (py CH), 67.50 (cyclohexyl quaternary), 35.71, 34.35, 25.30, 21.70 (cyclohexyl CH_2s)

ESI-MS found *m/z* 537.1 $[M\text{-}Cl]^+$, HR-ESI-MS found 537.0926, $C_{19}H_{18}N_4O_3Re_1$ requires 537.0931.

Crystallographic data

Single crystal X-ray diffraction data were collected on a Bruker Apex Duo diffractometer equipped with a graphite monochromated Mo(Kα) radiation source and a cold stream of N_2 gas.

Crystal data for {**1**-DHT·DIPE} ($C_{19}H_{26}Cl_{0.5}N_2O_{2.5}Re_{0.5}$): M = 866.49; Monoclinic, $P2_1$, a = 12.8902(7), b = 10.9298(5), c = 13.7766(7) Å, β = 109.1860(10)°; V = 1833.14(16) $Å^3$, Z = 2; ρ_{calc} = 1.570 Mg m^{-3}, F(000) = 880; dimensions 0.18×0.10×0.03 mm; $\mu(Mo_{K\alpha})$ = 0.71073 mm^{-1}, *T* = 150 (2) K. A total of 28751 reflections were measured in the range 1.57 ≤ θ ≤ 33.25 ° (*hkl* range indices: -19≤*h*≤17, -13≤*k*≤16, -21≤*l*≤21), 13094 unique reflections (R_{int} = 0.0472). The structure was refined on F^2 to R_w = 0.0630, R = 0.0356 (10555 reflections with $I > 2\sigma(I)$) and GOF = 0.918 on F^2 for 449 refined parameters, 16 restraints. The diisopropyl ether molecule within the unit cell showed some disorder and was constrained using SIMU and DELU commands in the refinement. Largest peak and hole 1.271 and -0.973 e$Å^{-3}$. CCDC 887418.

DFT calculations

DFT calculations were performed on the Gaussian 03 program.[24] Geometry optimisations were carried out without constraints using the B3PW91 functional. The LANL2DZ basis set was used for the Re centre, and was invoked with pseudo-potentials for the core electrons, a 6-31G(d,p) basis set for all coordinating (and oxygen of CO) atoms with a 6-31G basis set for all remaining atoms. All optimisations were followed by frequency calculations to ascertain the nature of the stationary point (minimum or saddle point).

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