Tetrahedron Letters,Vol.28,No.37,pp 4339-4342,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

SYNTHESIS OF A CATECHOL COVALENTLY BOUND TO A TRIS-BIPYRIDYL COMPLEX OF RUTHENIUM: A FLUORESCENT POTENTIAL HAPTEN.

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Abstract : The synthesis of a fluorescent potential skin sensitizer (or hapten or allergen), **Ru(bpy)bpycat(BF_4)$_2$** **6** is described here. It should permit to visualize by fluorescence and electron microscopy processing of a skin hapten by epidermal Langerhans cell, both in vivo and in vitro.

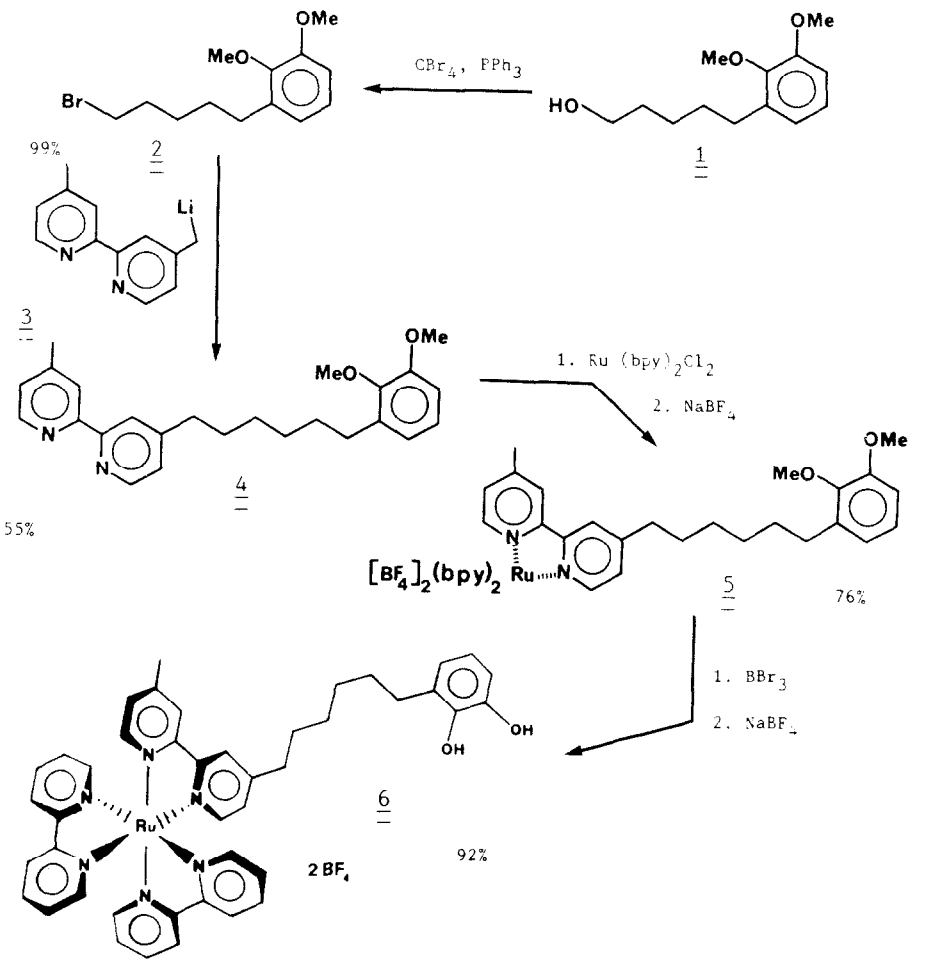
In the course of our investigations on the molecular and cellular mechanism of allergic contact dermatitis (ACD) [1], we have been interested in ACD to alkylcatechols [2], the allergenic principle present in the famous Anacardiaceae plants, Poison Ivy and Poison Oak . These plants are responsible for ACD in over 80% of the Americans. While there seems to be a general agreement concerning molecular aspects of ACD to pyrocatechols (oxidation into an o -quinone followed by conjugation to a cutaneous protein), processing of this "adduct" by Langerhans cells (LC), the skin macrophages, is the center of much debate [3].

Some understanding of these processes could be gained if the hapten (or its protein conjugate) could be visualized on skin sections. We describe here the synthesis of a potential hapten, a pyrocatechol containing a fluorescent probe, a bipyridyl-ruthenium based complex.

In order to use this probe with cell culture and realize double labeling experiments, we needed a water soluble fluorophore with excitation and emission wavelengths as far away from each other as possible and which emission spectra would be as different as possible from

fluorescent compounds generally used in histology (fluorescein or rhodamin)[4]. Tris-bipyridyl Ruthenium complexes [5] ($Ru(bpy)_3$) seemed to be ideal candidates (**scheme I**).

SCHEME I



Scheme I summarizes the synthesis of such a fluorescent potential hapten. Sonication of 5-Benzyloxybromobutane with magnesium in THF and subsequent trapping with o -veratraldehyde produced a benzylic alcohol which was hydrogenolyzed over palladium [6]. Alcohol 1 thus obtained in 77% yield was converted into bromide 2 by treatment with triphenylphosphine and carbon tetrabromide (99% yield). Trapping of the anion obtained from 4,4'-dimethyl-2,2'-bipyridine 3 [7] by treatment with LDA yielded bipyridine 4 (55% yield).

Complexation of 4 with $Ru(bpy)_2Cl_2.2H_2O$ in 2-Propanol/water mixture (1/1) under reflux [5], followed by precipitation with an excess of $NaBF_4$, afforded 5 [8] as a red solid (72% yield). Deprotection of the catechol part with BBr_3 in CH_2Cl_2 at -78°C, controlled hydrolysis of BBr_3 in excess and elimination of the resulting boron salts yielded 6 [9] which was precipited from an aqueous solution by subsequent addition of $NaBF_4$ (92% yield).

The complex 6 exhibits two excitation maxima at 284 and 470 nm which both lead to a single emission at 604 nm. Quantum yield for the lower excitation maximum was 0.74 (with respect to $Ru(bpy)_3(BF_4)_2$) . It is interesting to notice the large difference between the excitation and emission wavelength. Use of this compound on skin sections is in progress.

Acknowledgements: The authors are indebted to Dr.J.P.Sauvage and Dr. J.-C. Chambron for helpful discussions.

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[8] **$Ru(bpy)_2bpycatOMe\ (BF_4)_2$ 5**

NMR : 8;50 (4H, d, J=8.21, H_4bpy); 8.39 (1H, bs, H_4bpycat); 8.34 (1H, d, J=2.4, H_4bpycat); 8.04 (4H, m, H_3bpy); 7.73 (4H, d, J=5.61, H_1bpy); 7.54 (2H, dd, J=2.4 J=5.7, H_1bpycat); 7.39 (4H, m, H_2bpy); 7.27 (2H, d, J=5.7, H_2bpycat); 6.97 (1H, dd,t like, J=7.8, Ar-H); 6.85 (1H, dd, J=7.8 J=1.7, Ar-H); 6.77 (1H, dd, J=7.8 J=1.7, Ar-H); 3.82 (3H, s, ArOMe); 3.73 (3H, s ArOMe); 2.80 (2H, t, J=7.6, bpy -CH_2); 2.61 (2H, t, J=7.0, Ar-CH_2); 2.16 (3H, s, bpyMe); 1.25-1.80 (8H, m, CH_2).

UV/VIS : 458 (15800), 284 (136000).

Fluorescence: excitation :308, 470 nm. emission (308;470) : 608 nm

anal. : ($C_{45}H_{46}N_6O_2RuB_2F_8$ + 4 $NaBF_4$) C, H

[9] **$Ru(bpy)bpycat(BF_4)_2$ 6**

NMR : 8;50 (4H, d, J=8.29, H_4bpy); 8.38 (1H, bs, H_4bpycat); 8.34 (1H, d, J=2.4, H_4bpycat); 8.05 (4H, m, H_3bpy); 7.73 (4H, d, J=5.6, H_1bpy); 7.54 (2H, dd, J=2.4 J=5.7, H_1bpycat); 7.39 (4H, m, H_2bpy); 7.27 (2H, d, J=5.7, H_2bpycat); 6.63 (1H, dd,t like, J=5.7, Ar-H); 6.34 (2H, m, Ar-H); 2.80 (2H, t, J=7.6, bpy -CH_2); 2.54 (2H, t, J=7.0, Ar-CH_2); 2.54 (3H, s, bpyMe); 1.20-1.80 (8H, m, CH_2).

UV/VIS : 460 (14300), 284 (81500).

Fluorescence: excitation :284, 470 nm. emission (280;470): 604 nm

Anal. : ($C_{43}H_{42}N_6O_2RuB_2F_8$ + 4 $NaBF_4$) C, H

(Received in UK 12 June 1987)