Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 10

TRITIUM LABELLING OF ANTITUMOR DNA BIS-INTERCALATORS : SYNTHESIS OF [^{3}H] DITERCALINIUM.

P. Léon*, C. Garbay-Jaureguiberry*, S. Le Greneur†, R. Besselièvre† and B.P. Roques *.

* Département de Chimie Organique, UA 498 CNRS, U 266 INSERM, UER des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, 75270 PARIS, Cedex 06, France.
† Present adress : Institut de Chimie des Substances Naturelles, CNRS, 91190 GIF SUR YVETTE, France.

SUMMARY

Ditercalinium : 2,2'-[4,4'-bipiperidine-1,1'-bis (ethane-1,2-diyl)] bis (10-methoxy-7H-pyrido[4,3-c] carbazolium) tetramethanesulfonate (NSC 366241) is a potent antitumor DNA bis-intercalator characterized by a new mechanism of action. In order to elucidate its biological action at the cellular level and its pharmacokinetic and metabolic properties, radiolabelled ditercalinium was prepared. In the present paper, we report the synthesis of the 11-bromo-10-methoxy-7H-pyrido [4,3-c] carbazole presursor and its reductive tritiation by exchange with [^{3}H]$_2$. The pyrido[4,3-c]carbazole ring labelled in position 11 is then condensed either with the 1,1' bis (2-chloroethyl)- 4,4'-bipiperidine chain to provide tritiated ditercalinium or with the 1-(2-chloroethyl) piperidine chain to yield its monomeric analog.

Key words : tritiated ditercalinium, DNA bis-intercalator, antitumor drug.

INTRODUCTION

Ditercalinium (NSC 366241) is a highly potent antitumor DNA bis-intercalator developped in our laboratory (1-2). Several biochemical studies have evidenced that ditercalinium elicits its cytotoxicity through a new mechanism of action largely different from that of other DNA intercalating antitumor agents (3-6). Ditercalinium induces a delayed cytotoxicity in sensitive L 1210 cells and arrests the cell growth almost randomly at any phase of the cell cycle (3). On the other hand, this bifunctional intercalator does not produce any protein-associated DNA breaks such as those obtained with most of the other antitumor intercalating agents such as mAMSA (7). However ditercalinium inhibits the formation of linear DNA's in presence of mAMSA (4). Moreover ditercalinium provokes the formation of high molecular weight DNA constituted by catenanes or aggregates (4). Electronic microscopy studies of L1210 cells treated with

0362–4803/88/101143–05$05.00

Received February 2, 1988
Revised March 8, 1988

ditercalinium showed a strong effect on mitochondria whereas the nucleus and chromatin were altered (6). Further investigations by means of structure-activity analyses (8,9) and NMR studies on its DNA-complex structure (10) have been performed in order to determine the mechanism of action of ditercalinium. In addition pharmacokinetic and metabolic studies are in progress (11), in order to determine information on the plasma profile and tissue distribution of ditercalinium as well as its biotransformation *in vivo*. For this purpose radiolabelled ditercalinium was required. Therefore, the dimer was labelled with tritium at the C_{11} position of each 7H pyrido [4,3-c] carbazole ring. The synthetic pathway is presented in figure 1. The inactive monomeric analog 5 was prepared as a standard.

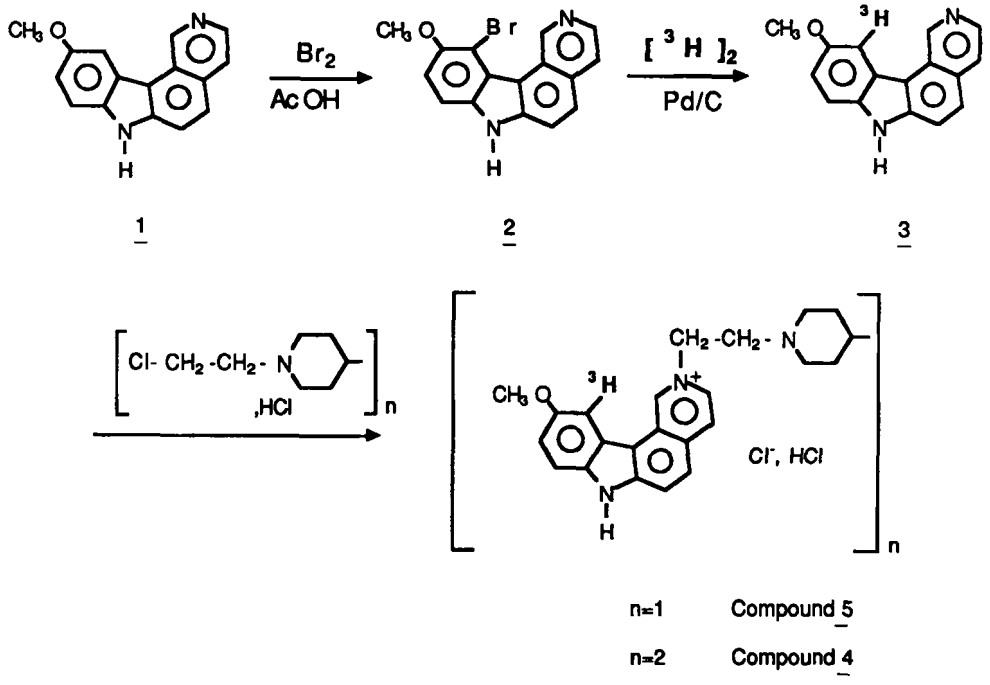

Figure 1 - Synthetic pathway of [^{3}H] ditercalinium and related monomer.

EXPERIMENTAL PART

Materials :

The structure of the brominated precursor 2 was confirmed by 1H NMR spectroscopy (Brüker WH 270 MHz) using HMDS as an internal reference and mass spectroscopy (Ribermag R-10-10-C). The purity was checked by thin layer chromatography on silica gel plates (Merck) in the following

solvent systems (v/v) : A, chloroform-methanol (9:1) ; B, chloroform-methanol-triethylamine (95:5:0.5) ; C, ethyl formate-isopropanol-ammonia (45:35:20). The purity of ditercalinium 4 was checked by HPLC at 262 nm on a Waters apparatus equipped with a μ Bondapak C18 column with acetonitrile-water-heptanesulfonic acid-NH_4Cl (64 ml : 36 ml : 24 ml : 400 mg) as eluent and the purity of compound 5 by t.l.c. with elution system C. The catalyst Pd/C was supplied by Engelhardt (France). Tritium gas was from Lumina (France). [^3H] scannings of TLC plates were performed with a Dunnsicht scanner II Berthold LB 2722.

Radioactivity was determined with a LKB rack 1211 B scintillator counter. Specific activity was measured or by the U.V./scintillation method (Beckmann 5230 spectrometer) or by mass spectroscopy (Varian CH 7A, Impact electronic)

Methods

I-Precursor synthesis 2

Bromine (5.3 μl, 1 mmol) was added to a solution of 10-methoxy-7H-pyrido [4,3-c] carbazole 1 (0.25g, 1 mmol) in a mixture AcOH-$CHCl_3$ (15 ml : 8 ml) with stirring. A yellow precipitate appeared immediately and the suspension was stirred at room temperature in the dark for 48 h. The precipitate was collected by filtration, thoroughly washed with n-hexane, and dried in vacuo. Chromatography over a silica gel column ($CHCl_3$-MeOH, 20:1) afforded 2 : 0.22 g (53% yield), mp >260°C, 1H NMR [$(C^2H_3)_2$ SO] : δ (ppm) 12.89 (s, 1H, H_7) ; 11.16 (s, 1H, H_1) ; 8.67 (d, 1H, H3) 8.59 (d, 1H, H_4) ; 8.40 (d, 1H, H_6) 8.29 (d, 1H, H_5) ; 7.81 (d, 1H, H_8) ; 7.49 (D, 1H, H_9) 3.93 (s,3H, OCH_3). MS : m/e 326.

II-Tritiation of the 10-Methoxy-7H-pyrido [4,3-c] carbazole ring : 3.

The Pd/C (10%) catalyst (41 mg) and 40 μl of ethyldiisopropylamine were added to 11-bromo-10-methoxy-7H-pyrido [4,3-c] carbazole 2 (41 mg, 0.125 mmol) in methanol (1.5 ml). The suspension was frozen, purged from air under vacuum (10^{-4} Torr with Toepler pump) and then stirred at room temperature in the presence of tritium gas (25 Ci, 425 GBq) at atmospheric pressure for 1 h. The system was again frozen, the excess of tritium gas removed and the mixture allowed to return to room temperature under constant stirring for 3 h. After millipore filtration of the catalyst, the labile tritium atoms were eliminated by successive flash rotative evaporations with a large volume of methanol. Then, the residue was purified by silicagel chromatography under pressure (Miniprep system-10 bar) with chloroform-methanol-triethylamine (95-5-0.5) as eluent system (Rf : 0.74). Chemically and radiochemically pure [^3H]-10-methoxy-7H-pyrido [4,3-c] carbazole 3

(490 mCi) was collected and the specific activity : 8.6 Ci/mmole (318 GBq/mmol) determined by mass spectroscopy and by the combination U.V/scintillation counting.

III -Tritiated ditercalinium : 4

To [^{3}H]-10-methoxy-7H-pyrido [4,3-c] carbazole 3 (490 Ci, 18.13 GBq) in DMF (0.5 ml) , were added 1,1'-bis (2-chloroethyl)-4,4' bipiperidine dihydrochloride (4 mg, 1.1 x 10^{-5} mol) in water (0.2 ml). The mixture was stirred under nitrogen at 80°C for 4 hours until a t.l.c control indicated no more evolution. After evaporation under reduced pressure, two successive additions of water (1ml) followed by evaporation were performed to eliminate the DMF. The residue was purified by elution on two successive LH20 columns with a 0.01 M HCl-methanol (50-50) mixture to yield pure [^{3}H] ditercalinium 4 (26 mCi, 962 MBq). Specific activity : 18 Ci/mmol (666GBq/mmol) determined by the combinationU.V/scintillation counting.

IV -Tritiated monomer : 5

To [^{3}H]-10-methoxy-7H-pyrido [4,3-c] carbazole 3 (420 mCi, 15.44 GBq : 12 Ci/mmol) : (a different batch than that used for ditercalinium tritiation) in DMF (0.5 ml) were added 7.5 mg of 1-(2-chloroethyl)-piperidine, hydrochloride (6.10^{-5} mol) in water (0.1 ml). The solution was stirred under nitrogen at 90°C for 37 hours. After evaporation and similar treatment as for ditercalinium to eliminate DMF, the monomer 5 was purified on a Sephadex G10 column with 0.01M HCl methanol (50-50) as eluent to yield pure 5 (120 MCi, 4.44 GBq) with a specific activity of 12 Ci/mmole (444 GBq/mmol) as determined by the combination U.V/scintillation counting.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. R. Oberlin for technical assistance, I. Bonetti for typing the manuscript, the PIRMED du CNRS and the Service des Molécules Marquées (CEA-CEN Saclay) for allowing them to perform the radiolabelling experiments in their facilities.

This work was supported by grants from the Université René Descartes, the Ligue Nationale contre le Cancer and the Fondation pour la Recherche Médicale.

REFERENCES

1. Roques, B.P., Pelaprat, D., Le Guen, I., Porcher, G., Gosse, C and Le Pecq, J.B-Biochem. Pharmacol. 28, 1811 (1979).
2. Pélaprat, D., Delbarre, A., Le Guen, I., Roques, B.P.and Le Pecq, J.B-J. Med. Chem. 23 : 1336 (1980).
3. Esnault, C., Roques, B.P., Jacquemin-Sablon, A. and Le Pecq, J.B-Canc. Res. 44 : 4355 (1984).
4. Markovits, J., Pommier, J., Matterns, M.R., Esnault, C., Roques, B.P., Le Pecq, J.B. and Kolh, K.W-Canc. Res. 46 : 5821 (1986).
5. Le Pecq, J.B. and Roques, B.P., in "Mechanism of DNA damage and repair" Simic, M.G., Grossman L. and Upton A.C. Eds, Plenum : New York, pp 219 (1986).
6. Esnault, C. Lambert, B., Markovits, J., Segal-Bendirdjian, E., Muzard, G., Garbay-Jaureguiberry, C., Roques, B.P. and Le Pecq, J.B. In "Molecular mechanisms of carcinogenic and antitumor activity", Chagas, C., Pullman, B. Eds. Adenine. Press. N. York, pp 295 (1987).
7. Rowe, T., Wang, J.C. and Liu L.F. Mol-Cell. Biol., 6 : 985 (1986).
8. Garbay-Jaureguiberry, C., Laugâa, P:, Delepierre, M., Laalami, S., Muzard, G., Le Pecq, J.B. and Roques, B.P-Anticancer Drug. Design., 1 : 323, (1987).
9. Léon, P., Garbay-Jaureguiberry, C., Barsi, M.C., Le Pecq, J.B. and Roques, B.P-J. Med. Chem., 30 : 2074 (1987).
10. Delbarre, A., Delepierre, M., Garbay-Jaureguiberry, C., Igolen, J., Le Pecq, J.B. and Roques, B.P. Proc. Natl. Acad. Sci. USA, 84 : 2155 (1987).
11. Gouyette, A. , Fellous, R. , El Abed, I. , Le Chevalier, T. , Léon, P. , Garbay, C. , Besselière, R.,Roques B.P. and Le Pecq J.B. 5th NCI-EORTC Symposium on new drugs in Cancer Therapy. Amsterdam, (October 1986).