

^1H and ^{13}C nuclear magnetic resonance assignments and stereochemistry of *N-n*-butyl-*N*-methyl-11-(16' α -chloro-3',17' β - and 17' α -dihydroxyestra-1',3',5'(10')-trien-7' α -yl) undecanamide

Patricia Dionne, Shankar M. Singh, and Fernand Labrie

Medicinal Chemistry Division, Laboratory of Molecular Endocrinology, CHUL Research Center and Laval University, Québec G1V 4G2, Canada

The stereochemistry of *N-n*-butyl-*N*-methyl-11-(16' α -chloro-3',17' β -dihydroxyestra-1',3',5'(10')-trien-7' α -yl) undecanamide (**4**) and *N-n*-butyl-*N*-methyl-11-(16' α -chloro-3',17' α -dihydroxyestra-1',3',5'(10')-trien-7' α -yl) undecanamide (**5**) at the 17'-position was unambiguously established by one dimensional nuclear Overhauser enhancement (NOE difference spectroscopy). Irradiation of H-18' led to the increase in the signal of H-11' β , H-12' β , H-8' β , H-15' β , and H-16' β for compound **4** and a very small increase in the signal of H-17' indicating the β -orientation of the 17'-OH. In contrast, for compound **5**, the increase in the signal of H-17' indicated the α -orientation of the 17'-hydroxy group. Complete assignment of the ^1H and ^{13}C resonances is facilitated by the following one- and two-dimensional NMR experiments: ^1H homonuclear correlated spectroscopy (COSY), ^1H - ^{13}C heteronuclear shift correlation (HSC), ^1H - ^{13}C heteronuclear shift correlation via long range couplings (COLOC), and distortionless enhancement by polarisation transfer (DEPT). Comparison of the ^1H and ^{13}C NMR chemical shifts indicates that the stereochemistry at the 17' position is more easy to determine by analysing the chemical shifts of C-17', C-12', and C-18'. (*Steroids* **59**:493-497, 1994)

Keywords: ^1H nuclear magnetic resonance; ^{13}C nuclear magnetic resonance; estradiol; antiestrogen; stereochemistry

Introduction

We recently reported the synthesis of a series of 7 α -undecanamide-substituted 17 β -estradiol with different halogens (Cl, Br, I) at C-16.¹ These compounds have shown pure antiestrogenic activity in the highly sensitive mouse uterine in vivo assay.² In order to support biological applications, knowledge of the stereochemistry of each new chiral center has primary importance. Stereochemistry at the C-7 position of the steroids is well established.³ However, the stereochemistry at C-16 and C-17 is incompletely understood. During the preparation of these compounds, the last step was the reduction of the C-17' ketone which could provide a mixture (α and β) of alcohols. Normally, the β -methyl at C-18 provides

an excellent selectivity for the reduction of 17-ketosteroids with the 16-unsubstituted D ring. For example, the reduction of compound **1** with sodium borohydride gives 96% of the β -alcohol **2** (Figure 1).⁴ For compound **3** however, the same reduction with lithium aluminum hydride is not selective and yields 55% of compound **4** and 29% of compound **5**.¹ This low selectivity is caused by the presence of a halogen at the 16 α -position. Similar results were already reported for 16 α -halogeno-17-keto steroids.⁵⁻⁷

In previous studies, the stereochemistry of compounds **4** and **5** at carbons C-16' and C-17' was determined by comparison of the chemical shifts and the coupling constants of numerous derivatives studied on the basis of the molecular models and the dihedral angles.^{5,8-10} Compound **4** is a potent anti-estrogen and it is known that most often the opposite isomer of the potent compound has reduced biological activity. To further pursue our studies of these potent molecules, we needed

Address reprint requests to Dr. Fernand Labrie, CHUL Research Center, 2705 Laurier Boulevard, Québec, QC, G1V 4G2, Canada.
Received December 2, 1993; accepted March 7, 1994.

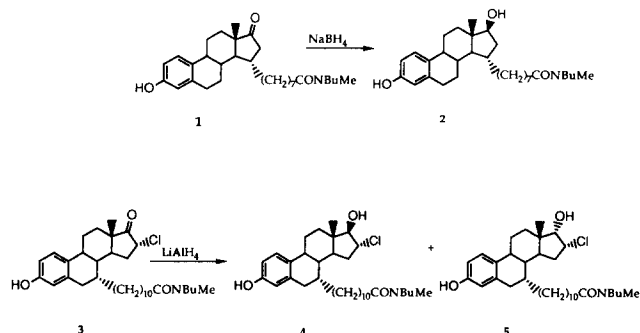


Figure 1 Structures of compounds 1 to 5.

to establish the stereochemistry of compounds 4 and 5 unequivocally and to create the data base of ^1H and ^{13}C NMR chemical shifts to help identify and elucidate the structure of new steroid analogues.

Consequently, in the present study we used the following two-dimensional NMR experiments: ^1H homonuclear correlated spectroscopy (COSY),¹¹ ^1H - ^{13}C heteronuclear shift correlation (HSC),^{12,13} and the ^1H - ^{13}C correlation spectroscopy via long range couplings (COLOC).¹⁴ In addition to the standard ^1H and ^{13}C spectra, the one-dimensional NMR experiments performed were the nuclear overhauser enhancement difference (NOE difference)¹⁵ and the distortionless enhancement by polarization transfer (DEPT).¹⁶ These experiments led to the determination of the exact stereochemistry at positions 16 and 17 and the ^1H and ^{13}C chemical shifts data of compounds 4 and 5.

Experimental

The synthesis of compounds 4 and 5 has been previously reported.¹ For NMR experiments, the sample (5–10 mg for ^1H NMR or 38–100 mg for ^{13}C NMR) was dissolved in 99.8% atom chloroform-*d* (Omega, Québec, Canada) in a 5 mm NMR tube. The spectra were recorded on a Bruker AC-F 300 spectrometer using a QNP probe head (Quattro Nucleus Probe) tuned to 300.13 MHz (^1H) and 75.47 MHz (^{13}C). The chemical shifts are referenced to chloroform (7.26 ppm (^1H) and 77.00 ppm (^{13}C)).

For the 2D COSY experiment, the following pulse sequence was used: RD-90°- t_1 -90°-acq.¹¹ Relaxation delay: 1 s; spectral width in both dimensions: 1213 Hz; number of experiments: 128. The spectrum was acquired with 512 × 512 data points.

The 2D HSC spectrum was obtained using the standard pulse sequence.^{12,13} Relaxation delay: 2 s; $1/2J = 3.45$ ms ($J_{\text{CH}} = 145$ Hz); $1/4J = 1.72$ ms; number of experiments: 128. Spectral windows: 9803 Hz (^{13}C) and 1205 Hz (^1H). The spectrum was acquired with 512 × 2K data points (1K = 1024 points).

The DEPT-135 and -45 experiments¹⁶ were done with the following parameters: relaxation delay = 2 s; spectral width = 18518 Hz; data size = 16K; variable pulse width = 135° or 45°.

The 2D COLOC experiment was performed using the pulse sequence described in reference 14. Relaxation delay: 2 s; $\Delta_1 = 41$ ms; $\Delta_2 = 33$ ms; number of experiments: 128. Spectral windows: 13889 Hz (^{13}C) and 2348 Hz (^1H). The spectrum was acquired with 512 × 4K data points.

The NOE difference experiments resulting from irradiation of H-18' were obtained using a recycle time of 2 s, a delay of 700 ms and a pulse width of 90°. An irradiation at -2 ppm

was done for the control spectrum. For each irradiation, 30 experiments of 32 transients were done.

Results and discussion

Figure 2 illustrates the ^1H NMR spectrum of compound 4 in CDCl_3 . For the aromatic protons, the splitting as well as the coupling constants were used to assign the signals at $\delta = 7.11$, 6.66 and 6.60 ppm to H-1', H-2', and H-4', respectively. However, the aliphatic region which involves more than 50 protons is complex, especially between $\delta = 1.0$ and 2.4 ppm. Starting with the most deshielded signals and considering the multiplicity of the signals and the electronegativity of the substituents at positions 16' and 17', it is reasonable to propose that the multiplet at $\delta = 4.11$ ppm arises from H-16' whereas the doublet of doublets at $\delta = 3.84$ ppm comes from H-17'.¹⁷ For the amide group, it is well known that the rotation around the C-N bond is slow on the NMR time scale and this led to two separated signals which could be assigned for two conformers.¹⁸ For instance, the ^1H NMR spectrum shows two distinct methyl peaks (NCH₃) at $\delta = 2.93$ and 2.98 ppm and two sets of multiplets for H-1'' at $\delta = 3.37$ and 3.26 ppm. We also observed two sets of triplets at $\delta = 0.92$ and 0.95 ppm that come from H-4''. There are two deshielded signals at $\delta = 2.84$ and 2.70 ppm that should be assigned for the diastereotopic protons at C-6' because they are next to the aromatic ring. Finally, the last signal that can be identified in this spectrum is the CH₃-18' which is a singlet at $\delta = 0.81$ ppm.

For the assignment of other protons, we conducted the following NMR experiments. Figure 3 shows the 2D COSY contour spectrum of compound 4. Analysis of the cross peaks in the COSY spectrum together with the ^1H NMR spectrum led to the assignment of protons H-2'', H-3'', H-7' β , H-8' β , H-9' α , H-14' α , and H-15' (Table 1). Furthermore, the presence of a correlation with CH₃-18' from a long range coupling led to the identification of H-12' α .¹⁹

It is possible to determine the stereochemistry of the alcohol at the 17'-position with a one-dimensional NOE

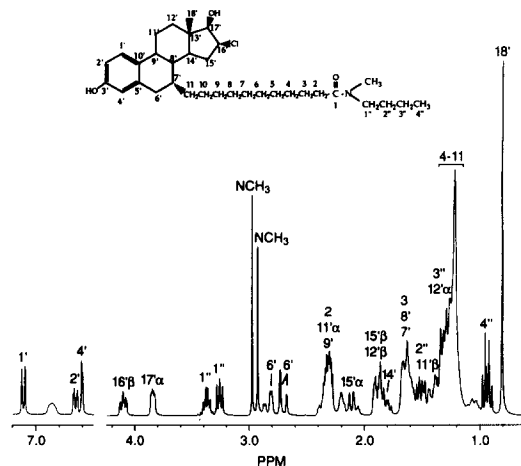


Figure 2 300.13 MHz ^1H NMR spectrum of compound 4 in CDCl_3 .

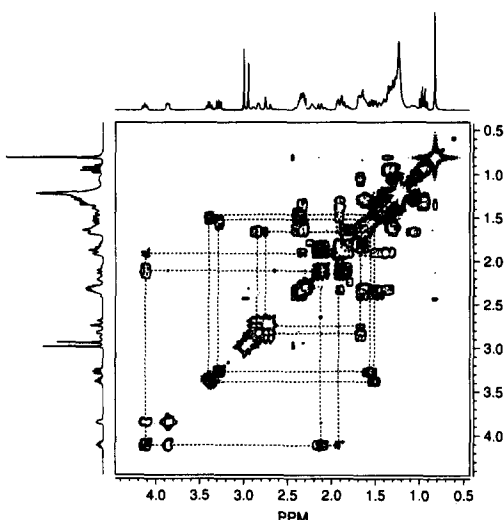


Figure 3 Two-dimensional homonuclear correlated spectroscopy (COSY) spectrum of compound 4 in CDCl₃.

difference spectrum.¹⁵ Figure 4A shows the NOE difference experiment of compound 4 for which irradiation of H-18' gives a complete map of the top right-hand side of the molecule (by the increase in the signals H-11' β , H-12' β , H-8' β , H-15' β and H-16' β). The very small increase in the H-17' signal indicates that this proton is in α -orientation and the 17'-OH is in β -orientation. For comparison, the same experiment was conducted with the isomer 5 (Figure 4B). In this case, the increase in the H-17' signal indicated the β -orientation of this proton and the α -orientation of the 17'-OH group. Although the H-12' β , H-8' β , and H-11' β signals are overlapped, it is important to note the chemical shift difference between the H-12' β and the H-12' α protons of compounds 4 and 5 (Table 1). Thus, when the 17'-alcohol is in the β -orientation, H-12' β resonates at $\delta = 1.90$ ppm and is thus more deshielded than H-12' α that resonates at $\delta = 1.33$ ppm. However, when the 17'-alcohol is in the α -orientation, H-12' β ($\delta = 1.65$ ppm) is less deshielded than H-12' α ($\delta = 1.97$ ppm).

Table 1 ¹H and ¹³C NMR chemical shifts (ppm) of compound 4 and 5 in CDCl₃

Position	Compound 4		Compound 5	
	¹ H	¹³ C	¹ H	¹³ C
1'	7.11 d $J = 8.5$ Hz	126.57	7.13 d $J = 8.5$ Hz	126.78
2'	6.66 dd $J = 8.5, 2.7$ Hz	113.12	6.66 dd $J = 8.5, 2.8$ Hz	113.12
3'		154.46		154.34
4'	6.60 d $J = 2.7$ Hz	116.24	6.59 d $J = 2.8$ Hz	116.24
5'		136.43		136.61
6'	2.70 d $J = 16.8$ Hz	34.46	2.70 d $J = 16.8$ Hz	34.71
7'	2.84 dd $J = 16.8, 4.7$ Hz		2.84 dd $J = 16.8, 5.4$ Hz	
8'	(β) 1.65	33.21	(β) 1.67	34.31
9'	(β) 1.60	41.24	(β) 1.53	41.89
10'	(α) 2.35	37.97	(α) 2.35	37.82
11'		130.29		130.83
	(α) 2.30	26.73	(α) 2.32	26.71
	(β) 1.40		(β) 1.47	
12'	(α) 1.33	36.56	(α) 1.97	31.62
	(β) 1.90		(β) 1.65	
13'		44.14		45.84
14'	(α) 1.80	44.38	(α) 2.22	43.33
15'	(α) 1.89	34.95	(α) 1.89	35.42
	(β) 2.12		(β) 2.14	
16'	(β) 4.11 m	64.21	(β) 4.61 m	62.83
17'	(α) 3.84 dd $J = 6.5, 3.8$ Hz	90.48	(β) 3.70 d $J = 4.6$ Hz	79.21
CH ₃ -18'	0.81 s	11.58	0.79 s	17.42
1''	3.37 m and 3.26 t $J = 7.6$ Hz	49.92	3.37 m and 3.26 t $J = 7.6$ Hz	49.95
		47.62		47.65
2''	1.50 and 1.55	29.31	1.51 and 1.57	29.40
		30.51		30.63
3''	1.35	19.96	1.32	19.97
		19.85		20.06
4''	0.92 t $J = 7.3$ Hz and 0.95 t $J = 7.3$ Hz	13.80	0.92 t $J = 7.4$ Hz and 0.95 t $J = 7.4$ Hz	13.86
NCH ₃	2.93 s and 2.98 s	35.45	2.93 s and 2.98 s	33.60
		33.60		35.42
1		173.61		173.51
		173.72		173.63
2	2.30	32.97	2.30	33.71
		33.66		33.03
3	1.63	25.12	1.61	25.06
		24.48		25.48
4-9	1.2-1.4	29.0-29.7	1.2-1.4	28.7-29.7
10	1.20 and 1.41	27.66	1.19 and 1.40	27.51
11	1.05 and 1.30	25.18	1.05 and 1.30	25.06

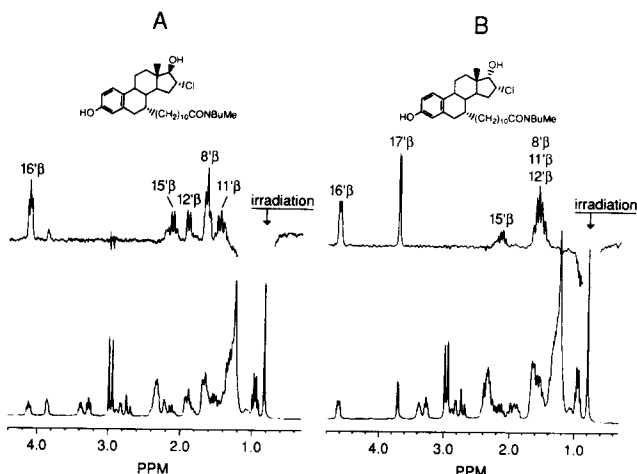


Figure 4 Nuclear Overhauser enhancement (NOE) difference experiments with irradiation of H-18'. A) Compound 4. B) Compound 5. The NOE spectra are at the top and the ordinary spectra are at the bottom.

Analysis of the DEPT-135, the 2D HSC, and the 2D COLOC NMR spectra led to the assignment of the carbons. For the DEPT-135 (Figure 5A) spectrum,¹⁶ five quaternary carbons are indicated, not accounted for. There are also three methylenes (one of them has split into two lines because of the slow rotation of the C-N bond), nine methines, and methylenes which appear as the negative signals. Analysis of this spectrum together with the ¹³C NMR spectrum (Figure 5B) and the 2D HSC^{12,13} in Figure 6 led to the assignment of the following carbons.

Quaternary carbons

The two downfield signals at $\delta = 173.61$ and 173.72 ppm are assigned to the carbonyl of the amide group C-1. In the aromatic region, the downfield resonance at $\delta = 154.46$ ppm is assigned to C-3' which is deshielded by the hydroxy group. The high field quaternary carbon at $\delta = 44.14$ ppm must be assigned to C-13' because there

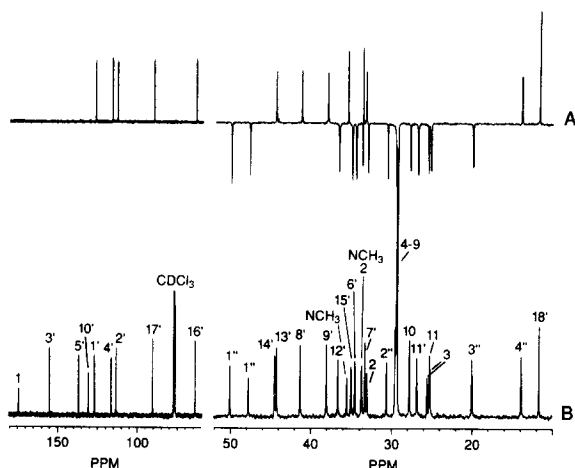


Figure 5 A) Distortionless enhancement by polarization transfer (DEPT; 135°). The CH₃ and CH carbons appear as positive signals, the CH₂ carbons appear as negative signals and the quaternary carbons are absent. (DEPT-135) and B) ¹³C NMR spectrum at 75.47 MHz of compound 4 in CDCl₃.

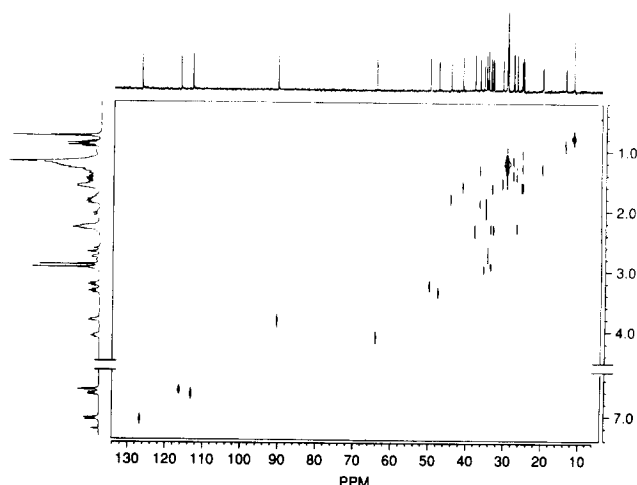


Figure 6 Two-dimensional heteronuclear shift correlation (HSC) spectrum of compound 4 in CDCl₃. The one-dimensional ¹³C spectrum is a DEPT-45.

is only one carbon of this type in the aliphatic region. Finally, the carbons C-5' and C-10' can not be assigned with these present experiments.

Methine carbons

All methines (except C-7' and C-8' for which the corresponding protons are overlapped in the ¹H NMR spectrum (Figure 2)) were easily assigned by their correlations with the proton signals in the 2D HSC experiment (Figure 6).

Methyl and methylene carbons

As for the methine carbons, the methyl and some methylene signals for which the proton chemical shifts are known were assigned by their correlations with the proton signals in the 2D HSC experiment (Figure 6).

The other carbons that could not be assigned from the above experiments were assigned by a 2D COLOC experiment that is a 2D HSC experiment via small coupling constants (Figure 7).¹⁴ For example, in the aromatic region, the quaternary carbons C-5' and C-10' were assigned as follows: H-2' can correlate with C-10' via ³J but not with C-5'. Thus, the correlation at $\delta = 130.29$ ppm led to the assignment of the C-10' carbon. Now, all the aromatic signals have been assigned except the resonance at $\delta = 136.43$ ppm that must be C-5' carbon. Furthermore, the correlation study of H-6' in the aliphatic region led to the assignment of C-11. Indeed, H-6' correlates with the methine carbons (C-7' and C-8') and with only one methylene C-11 that resonates at $\delta = 25.18$ ppm. Using this approach, the majority of unknown signals were assigned.

The chemical shifts of carbons C-7' and C-8' of the compounds 4 and 5 were determined by comparing with steroids made in our laboratory* and with some other steroids.²⁰ For most of the steroids, the chemical shift of C-8' is about 39 ppm and that of C-7' is about 27 ppm. For compound 4, there are only two chemical shifts left

* Lévesque, C.; Mérand, Y. and Dufour, J. M. The results are not published.

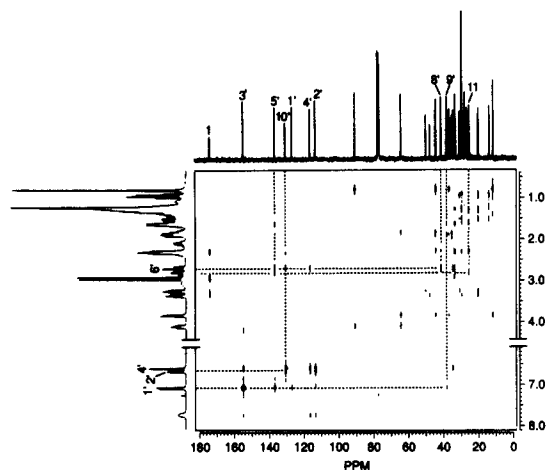


Figure 7 Two-dimensional ^1H - ^{13}C correlation via long range couplings (COLOC) spectrum of compound **4** in CDCl_3 .

unaccountable for that region ($\delta = 41.24$ and 33.21 ppm). They thus correspond to C-8' and C-7', respectively.

Results presented herein indicate that the method of choice to determine the stereochemistry at the 17' position is the NOE difference. However, for similar compounds, it could be ideal to determine the stereochemistry only with the ^1H and ^{13}C chemical shifts. Indeed, comparison of the ^1H and ^{13}C chemical shifts of compounds **4** and **5** led to the observation that the ^{13}C NMR spectrum could be a better probe to differentiate readily between the α - and β -isomers at the 17'-position. There is more than 10 ppm difference between the ^{13}C NMR chemical shift of the α - and β -isomers (Table 1), whereas there is only 0.14 ppm difference between the ^1H NMR chemical shift of H-17 α and H-17 β (Table 1). Moreover, in both cases, the chemical shifts for C-17' of compound **4** are more deshielded than those of compound **5**. In contrast, the ^1H NMR spectrum is a better probe for the identification of the isomers at the 16'-position with a chemical shift difference of 0.50 ppm for H-16 β . However, the ^{13}C NMR spectra show a difference of about 6 ppm between the two isomers (**4** and **5**) at the C-18' position, whereas no difference was noted in the ^1H NMR spectra for the H-18' chemical shifts. As mentioned earlier with the NOE difference experiment, it is possible to locate the position of H-12 β that is also influenced by the orientation of the alcohol (Table 1). In the ^{13}C NMR spectra, there is a difference of about 5 ppm between the chemical shifts of compounds **4** ($\delta = 36.56$ ppm) and **5** ($\delta = 31.62$ ppm) at C-12'. Finally, the above described observations lead to the conclusion that the stereochemistry of the substituents at the C-17' position could be easily determined by analyzing the chemical shifts of C-12', C-17', and C-18' with the ^{13}C NMR experiments. In the future, similar types of compounds which are under preparation in our laboratory will be compared with their spectral values to generalize this method of analysis.

Acknowledgments

We are grateful to Dr Donald Poirier for his help in the redaction of this manuscript. A post doctoral fellowship

from the Fonds de la Recherche en Santé du Québec (FRSQ) is also gratefully acknowledged. This work was supported by Endorecherche and FRSQ.

References

- Lévesque C, Mérand Y, Dufour JM, Labrie C, Labrie F (1991). Synthesis and biological activity of new halo-steroidal antiestrogens. *J Med Chem* **34**:1624–1630.
- Labrie C, Martel C, Dufour J-M, Lévesque C, Mérand Y, Labrie F (1992). Novel compounds inhibit estrogen formation and action. *Cancer Res* **52**:610–615.
- Bowler J, Lilley TJ, Pittam JD, Wakeling AE (1989). Novel steroidal pure antiestrogens. *Steroids* **54**:71–99.
- Poirier D, Mérand Y, Labrie F (1991). Synthesis of 17 β -estradiol derivatives with *N*-butyl, *N*-methyl alkylamide side chain at position 15. *Tetrahedron* **47**:7751–7766.
- Heiman DF, Senderoff SG, Katzenellenbogen JA, Neeley RJ (1980). Estrogen receptor based imaging agents. 1. Synthesis and receptor binding affinity of some aromatic and D-ring halogenated estrogens. *J Med Chem* **23**:994–1002.
- Kiesewetter DO, Katzenellenbogen JA, Kilbourn MR, Welch MJ (1984). Synthesis of 16-fluoroestrogens by unusually facile fluorine ion displacement reactions: prospects for the preparation of fluorine-18 labeled estrogens. *J Org Chem* **49**:4900–4905.
- Hochberg RB (1979). Iodine-125-labeled estradiol: a gamma-emitting analog of estradiol that binds to the estrogen receptor. *Science* **205**:1138–1140.
- Fevig TL, Mao MK, Katzenellenbogen JA (1988). Estrogen receptor binding tolerance of 16 α -substituted estradiol derivatives. *Steroids* **51**:471–497.
- Longcope C, Arunachalam T, Raffkind I, Capsi E (1981). Biological activity of [^{127}I] and [^{125}I] estradiol analogs in vitro and in vivo. *J Steroid Biochem* **14**:261–268.
- Arunachalam T, Capsi E (1981). Syntheses of seleno estrogens. *J Org Chem* **46**:3415–3420.
- Bax A, Freeman R (1981). Investigation of complex networks of spin-spin coupling by two-dimensional NMR. *J Magn Reson* **44**:542–561.
- Bodenhausen G, Freeman R (1977). Correlation of proton and carbon-13 NMR spectra by heteronuclear two-dimensional spectroscopy. *J Magn Res* **28**:471–476.
- Bax A, Morris GA (1981). An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. *J Magn Res* **42**:501–505.
- Hull WE (1981). Experimental aspects of two-dimensional NMR. In: Croasum WR, Carlson RMK (eds.) *Two Dimensional NMR Spectroscopy, Applications for Chemists and Biochemists*, VCH Publishers, New York, pp. 199–202.
- Sanders JKM, Mersh JD (1982). Nuclear magnetic double resonance; the use of difference spectroscopy. *Prog Nucl Magn Reson Spectrosc* **15**:353–400.
- Sanders JKM, Hunter BK (1988). Connections through chemical exchange. In: *Modern NMR Spectroscopy, a Guide for Chemists*. Oxford University Press, New York, pp. 253–256.
- Pavia DL, Lampman GM, Kriz GS Jr (1979). Nuclear magnetic resonance spectroscopy. Part one: Basic concepts. In: *Introduction to Spectroscopy: A Guide for Students or Organic Chemistry*. Saunders College Publishing, Philadelphia, p. 93.
- Pretsch E, Clerc T, Seibl J, Simon W (1989). Proton resonance spectroscopy. In: Frenesius W, Huber JFK, Pungor E, Rechnitz GA, Simon W, West TS (eds.) *Table of Spectral Data for Structure Determination of Organic Compounds*. Second Edition. Springer-Verlag, Berlin/Heidelberg, pp. H-150–H-160.
- Davis D, Garces FO (1992). H-1, C-13 and F-19 nuclear magnetic resonance assignments of 3,3-difluoro-5 α -androstane-17 β -ol acetate. *Steroids* **57**:563–568.
- Boucheau V, Renaud M, Rolland de Ravel M, Mappus E, Cuilleron CY (1990). Proton and carbon-13 nuclear magnetic resonance spectroscopy of diastereoisomeric 3- and 17 β -tetrahydropyranyl ether derivatives of estrone and estradiol. *Steroids* **55**:209–221.