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Synthesis and NMR Spectrum of [$^{13}C_{18}$]-*meso*-Hexestrol, a Fully Carbon-13 Substituted Ligand for NMR Studies of the Estrogen Receptor

Monica J. Kochanny
Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, Illinois 61801, USA

Torleif Härd
Center for Structural Biochemistry, Karolinska Institute, NOVUM, S-14157 Huddinge, Sweden

John A. Katzenellenbogen*
Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, Illinois 61801, USA

The estrogen receptor ligand *meso*-hexestrol has been synthesized with ^{13}C enrichment (98 at.%) at every position. ^{13}C NMR spectra of the intermediates were obtained and ^{13}C–^{13}C coupling patterns analyzed. The complex ^{13}C NMR spectrum of [$^{13}C_{18}$]-*meso*-hexestrol was simplified through the use of selective ^{13}C decoupling. Several ^{13}C–^{13}C coupling constants were estimated from the decoupled spectra and refined via iterative simulation. Some additional coupling constants were measured in selective one-dimensional ^{13}C COSY spectra. Coupling constants are reported to an accuracy of ±0.4–1 Hz. This study demonstrates the feasibility of determining ^{13}C–^{13}C coupling constants in highly ^{13}C-substituted compounds; such compounds are expected to be used with increased frequency in studying receptor–ligand interactions by polarization transfer methods.

KEY WORDS ^{13}C–^{13}C coupling ^{13}C enrichment Simulation 1D ^{13}C COSY

INTRODUCTION

Carbon-13 labeling has long been recognized as a powerful tool in the study of biological systems. The synthesis of highly enriched, multiply ^{13}C-substituted molecules has gained increased attention recently owing to the advent of isotope-edited nuclear magnetic resonance spectroscopy.[1] This technique utilizes an isotopically labeled (e.g. ^{13}C or ^{15}N) small molecule (ligand or inhibitor) to simplify the ^{1}H NMR spectrum of a small molecule–protein complex, allowing selective observation of small molecule–protein interactions.[2] The use of an isotopically labeled receptor has also been reported recently.[3] In this manner, conformations and active site interactions have been analyzed for a number of small molecule–receptor complexes.[4]

The use of isotope-edited spectroscopy to observe a steroid–receptor complex would, in principle, provide a multiple-point analysis of ligand–binding site interactions. Such interactions are most commonly studied through affinity labeling techniques, which in most cases provide binding information for only a single point of attachment. As part of a collaborative effort to investigate estrogen receptor binding, we have prepared the non-steroidal estrogen receptor ligand *meso*-hexestrol (**1**) (Fig. 1) uniformly substituted with carbon-13 (98 at.% enrichment). Hexestrol was chosen for its high affinity for the receptor (relative binding affinity = 300 *vs.* estradiol = 100)[5] and its synthetic accessibility.

The literature on carbon–carbon coupling constants is abundant, and both one-bond[6] and long-range[7] carbon–carbon coupling constants have been comprehensively reviewed recently. Coupling constants are not generally determined using highly ^{13}C-substituted molecules, owing to the complexity of spectra caused by the presence of numerous couplings, although partial assignments have been reported in some simple molecules[8] and in some amino acids and peptides.[9] However, as more highly ^{13}C-substituted ligands are synthesized in order to perform polarization transfer experiments of ligand receptor binding,[2–4] it will become important to measure carbon–carbon coupling constants in such highly substituted systems. Particularly with highly flexible molecules, it will be necessary to determine these coupling constants in the uncomplexed state in order to compare them with values for the ligand in the complexed state.

In order to demonstrate the feasibility, that is, the scope and limitations, of determining carbon–carbon coupling constants in highly substituted molecules, we have analyzed the ^{13}C coupling patterns in the non-steroidal estrogen hexestrol (**1**) through the use of selective decoupling experiments and iterative simulation of

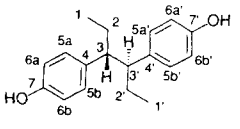

Figure 1. Structure and carbon numbering in *meso*-hexestrol.

* Author to whom correspondence should be addressed.

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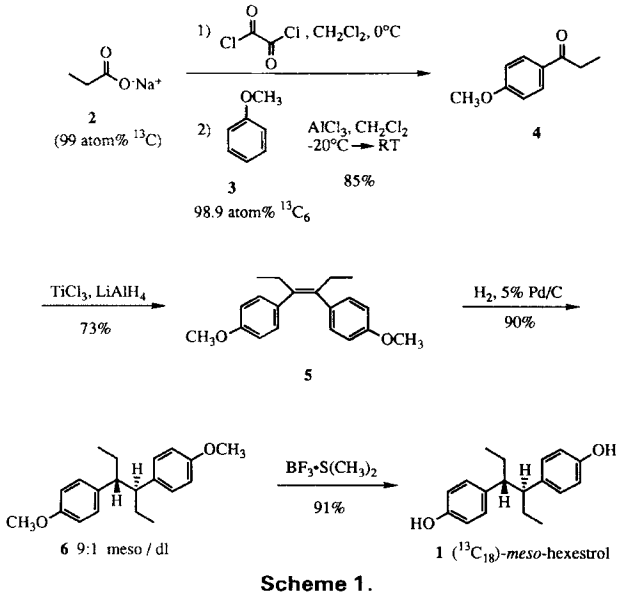
the resulting spectra. In this manner, many of the carbon–carbon coupling constants have been determined to an accuracy of *ca.* ±0.4–1 Hz. Some additional coupling constants which could not be deduced from the decoupling experiments were determined from selective one-dimensional COSY spectra.

RESULTS AND DISCUSSION

Synthesis of [$^{13}C_{18}$]-*meso*-hexestrol

The stereoselective synthesis of [$^{13}C_{18}$]-*meso*-hexestrol (**1**) was carried out in five steps, as outlined in Scheme 1. The synthetic strategy was adapted from the reported synthesis of [$^{2}H_{6}$]-*meso*-hexestrol from [$^{2}H_{3}$]-4′-methoxypropiophenone, but includes several procedural refinements and stoichiometric adjustments to ensure the most efficient utilization of both of the very costly, highly enriched ^{13}C-labeled precursors.[10] The starting materials were sodium [$^{13}C_{3}$]propionate (**2**) and [*ring*-$^{13}C_{6}$]-anisole (**3**), purchased from Isotec, with isotopic purities of ≥99 and 98.9 at.% ^{13}C, respectively.

[$^{13}C_{9}$]-4′-Methoxypropiophenone (**4**) was prepared by conversion of sodium propionate to the acid chloride, followed by Friedel–Crafts acylation of anisole.



Scheme 1.

[$^{13}C_{3}$]propionyl chloride was formed by treatment of the sodium salt with a slight excess of oxalyl chloride.[11] Formation of the acid chloride in CH_2Cl_2 allowed the acylation to be carried out without isolation of the volatile propionyl chloride. Treatment of the crude acid chloride with $AlCl_3$ and one equivalent of [$^{13}C_{6}$]anisole afforded an 85% yield of ketone **4** as the sole product. Coupling of **4** induced by low-valent titanium ($LiAlH_4$–$TiCl_3$)[12] gave predominantly the (*Z*)-alkene **5** [73% yield, *ca.* 95:5 (*Z*)- to (*E*)-alkene ratio by GC and ^{1}H NMR]. In the absence of steric crowding, aryl ketones have been shown to give predominantly the (*Z*)-alkene in the McMurry coupling, a result which has been attributed to complexation of the aromatic rings by titanium.[13] In the present case, alkene geometry was established by the chemical shifts of the methyl protons (*Z*, 0.96; *E*, 0.80) and aromatic protons (*Z*, 6.63, 6.87; *E*, 6.91, 7.15).[13a] Palladium-catalyzed hydrogenation of **5** in ethanol gave a 90% yield of [$^{13}C_{18}$]hexestrol dimethyl ether (**6**) as a 9:1 mixture of *meso*- and *dl*-isomers (GC), from which the pure *meso*-isomer was partially separated by chromatography. Demethylation of *meso*-**6** with boron trifluoride–dimethyl sulfide complex afforded the desired [$^{13}C_{18}$]-*meso*-hexestrol (**1**), which crystallized from ethanol–water as colorless needles. Similar deprotection of the *meso*/*dl*-ether mixture gave a sample of [$^{13}C_{18}$]-*dl*-hexestrol. Verification of the structure of the major product as the desired *meso*-isomer was by comparison of the ^{1}H and ^{13}C NMR chemical shifts of the two [$^{13}C_{18}$]hexestrol isomers with authentic natural abundance *meso*-hexestrol.

Spectral properties

Mass spectrum. The electron impact mass spectrum (70 eV, off-scale) of **1** ([$^{13}C_{18}$]M^+ = 288) indicates the high isotopic purity of the product (Fig. 2). The base peak in the on-scale spectrum is at m/z = 144, which corresponds to cleavage between the two benzylic carbons. The isotope content analysis of **1** *vs.* natural abundance *meso*-hexestrol was performed using field ionization mass spectrometry. The data are inset in Fig. 2.

Infrared spectrum. The carbon–carbon and carbon–oxygen stretching regions of the infrared (IR) spectra of **1** (bold trace) and natural abundance *meso*-hexestrol are

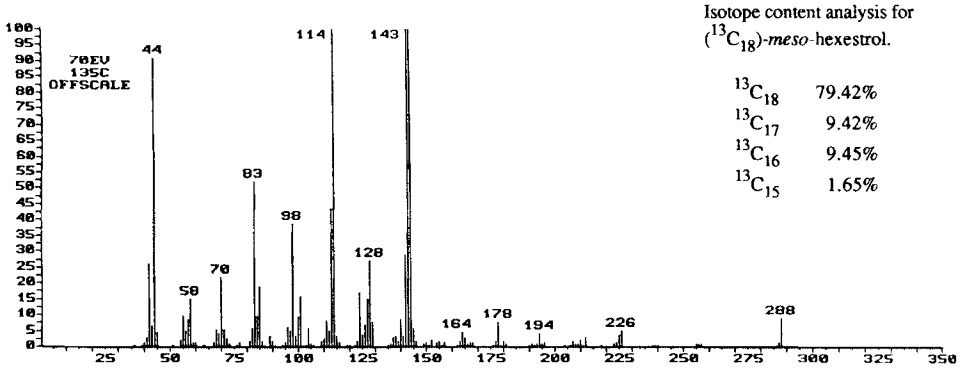


Figure 2. Low-resolution electron impact mass spectrum of [$^{13}C_{18}$]-*meso*-hexestrol (**1**, M^+ = 288). Inset: isotope content analysis of **1**.

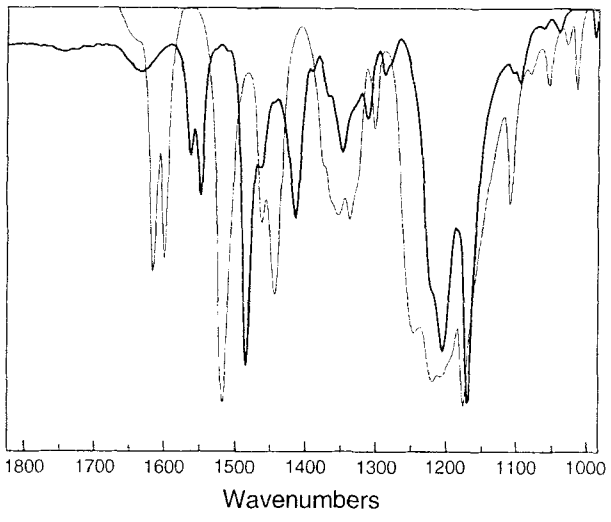


Figure 3. Infrared spectra (KBr) of **1** (bold trace) and natural abundance *meso*-hexestrol superimposed to show isotope-induced shifts (only the portion containing C–C and C–O stretches is shown).

shown superimposed in Fig. 3. Isotopic substitution causes the aromatic carbon–carbon stretches (1559, 1544 and 1478 cm^{-1}) and carbon–oxygen stretches (1200 and 1164 cm^{-1}) of **1** to shift to lower frequency relative to the natural abundance compound. The magnitudes of these shifts (C–C, 37–55 cm^{-1}; C–O, 9–17 cm^{-1}) are similar to the theoretical shifts (C–C, 61–65 cm^{-1}; C–O, 26–27 cm^{-1}) predicted using the oscillator equation.

^{1}H NMR spectrum. The 300 MHz ^{1}H NMR spectra of **1** and of natural abundance *meso*-hexestrol are shown in Fig. 4. For all resonances, the natural abundance signals align with the center of the ^1J(C,H) doublets of **1**. The absence of signals at the center of each doublet indicates the isotopic purity of each position. Coupling constants other than ^1J(C,H) could not readily be extracted, as the presence of vicinal proton–proton and numerous scalar carbon–proton couplings causes all signals to appear as unresolved multiplets. J(HH) values are presumed to be identical with those of the natural abundance compound.

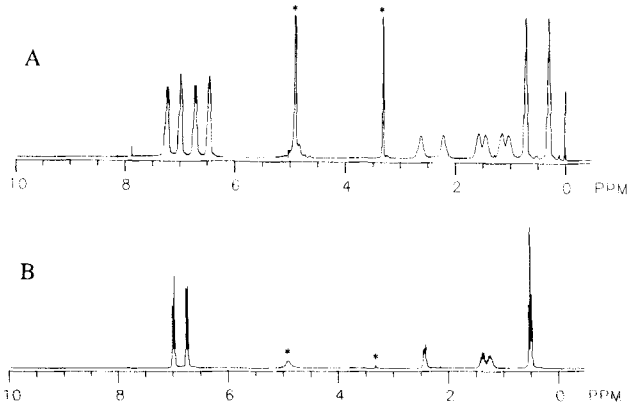


Figure 4. 300 MHz ^{1}H NMR spectra of (A) [$^{13}C_{18}$]-*meso*-hexestrol (**1**) and (B) natural abundance *meso*-hexestrol in CD_3OD at 20 °C. Solvent peaks are indicated with asterisks.

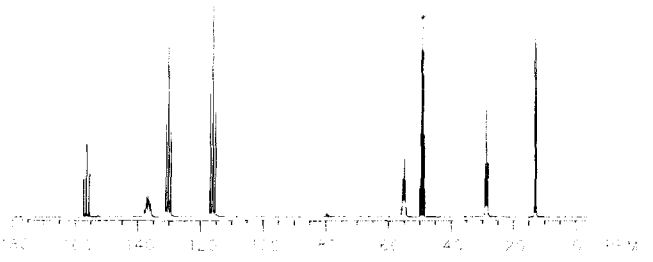


Figure 5. 75.5 MHz $^{13}C\{^1H\}$ spectrum of **1** in CD_3OD at 20 °C. Solvent peak is indicated with an asterisk.

^{13}C NMR spectrum. The broadband proton-decoupled ^{13}C NMR spectrum of **1** is shown in Fig. 5. The signals are expanded in Fig. 6 to show the fine structure of each multiplet. Chemical shifts and coupling patterns are given in Table 1. Relative to the natural abundance compound, $\Delta\delta$ values of -0.01 to -0.08 ppm are observed for the fully ^{13}C-substituted compound. In comparison, the secondary isotope effect caused by a single adjacent ^{13}C atom has been reported to be -0.00242 ± 0.00006 ppm.[14] As can be seen from Fig. 6, the majority of the J(CC) values could not be directly measured from the spectral data. Factors contributing to this problem were the overlap of many long range couplings (^2J–^4J) giving rise to multiplet splitting beyond the limits of resolution of the spectrum, broad line widths [e.g. C-7 line width = 1.68 Hz (FWHM)] incompatible with the determination of small couplings (e.g. ^2J,^4J) and the existence of complex non-binomial coupling patterns for some resonances.

Two methods were used to assist in the determination of J values. Selective ^{13}C decoupling was performed at each ^{13}C resonance to simplify coupling patterns. In this experiment, C-1 and C-4 could not be independently decoupled owing to a harmonic in the decoupler frequency which resulted in the mutual saturation of both signals. J values estimated from the resulting spectra were then refined using the ITRCAL program, which is a seven-spin LAOCOON-type[15] iterative simulation program. Some additional coupling constants were measured in selective one-dimensional ^{13}C COSY spectra. The coupling constants are given in Table 2. All of the one-bond coupling constants are consistent with

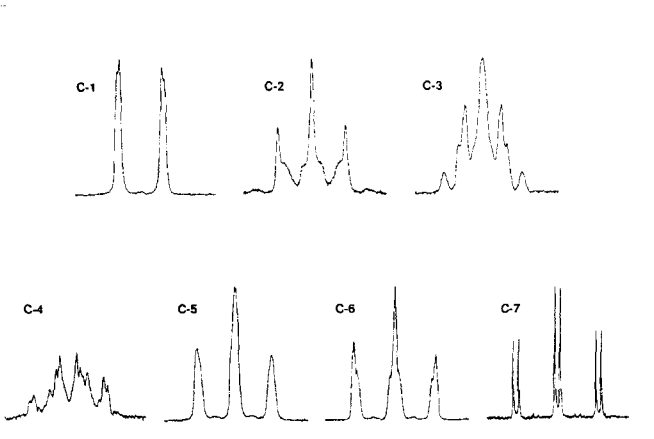
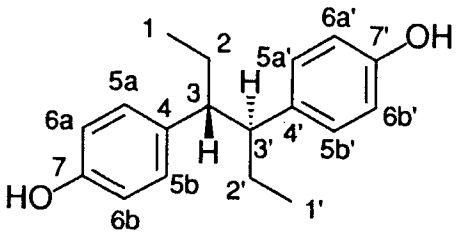


Figure 6. Expansion of ^{13}C resonances taken from Fig. 5 showing ^{13}C coupling patterns.

Table 1. ^{13}C chemical shifts and multiplicities in [$^{13}C_{18}$]-*meso*-hexestrol



Carbon	δ (ppm) Natural abundance	δ (ppm) $^{13}C_{18}$-labelled	Multiplicity	Coupled carbons[a]
1	12.60	12.59	'dd'[b]	1, 2, (3' or 4)
2	28.43	28.42	m[c]	1, 2, 2', 3, 3'
3	54.89	54.87	m[d]	2, 2', 3, 3', 4, 4'
4	136.70	136.68	m[e]	3, 3', 4, 4', 5a, 5b, 7
5	130.17	130.10	't'[b]	3, 3', 4, 5a, 5b, 6a, 6b
6	115.92	115.84	't'[b]	5a, 5b, 6a, 6b, 7
7	156.31	156.30	td	4, 6a, 6b, 7

[a] Carbons involved in the apparent coupling pattern indicated in multiplicity column.
[b] Apparent multiplicity of signals for which not all significant couplings are resolved.
[c] Doublet of XX' portion of an AA'XX' spin system (C-2,C-3).
[d] MM' portion of an AA'MM'XX' spin system (C-2,C-3,C-4).
[e] AA' portion of an AA'XX' spin system (C-3,C-4) split into a binomial triplet of doublets.

the well established effects of substitution and hybridization on the magnitude of ^1J(CC).

Analysis of ^{13}C coupling patterns

Aromatic carbons. Consideration of the $^{13}C\{^1H\}\{C\text{-}3\}$ NMR spectrum allowed simulation of the patterns for C-7, C-6 and C-4 to give the coupling constants listed in Table 2. The C-7 resonance consists of a binomial triplet of doublets (Fig. 6) due to ^1J(C-6,C-7) and ^3J(C-4,C-7). Geminal coupling to C-5 would be expected to split each line further into a triplet. The absence of this coupling indicates a ^2J(C-5,C-7) value near zero. Simulation indicates that a ^2J(C-5,C-7) value of up to 0.5 Hz can exist without significant distortion of the line shape.

The C-6{C-3} resonance shows a poorly resolved complex multiplet. The pattern is predicted to be that of the DD' portion of an ABCC'DD' spin system com-

Table 2. ^{13}C–^{13}C coupling constants in [$^{13}C_{18}$]-*meso*-hexestrol (1)[a,b,c,d]

^1J(C-*i*,C-*j*)	(Hz)	^2J(C-*i*,C-*j*)	(Hz)	^3J(C-*i*,C-*j*)	(Hz)
(C-1,C-2)	36.0	(C-1,C-3)	~0	(C-1,C-3')	3
(C-2,C-3)	37.0	(C-2,C-3')	(−)0.5	(C-1,C-4)	1
(C-3,C-3')	30.0	(C-3,C-4')	(−)1.0	(C-2,C-2')	3.0
(C-3,C-4)	42.0	(C-4,C-6)	(−)0.5	(C-2,C-5)	2.3
(C-4,C-5)	58.0	(C-5a,C-5b)	3.0	(C-3,C-5')	6[e]
(C-5,C-6)	57.5	(C-5,C-7)	0.3	(C-3,C-6)	<5
(C-6,C-7)	65.2	(C-6a,C-6b)	3.0	(C-4,C-4')	4.0
				(C-4,C-7)	8.4
				(C-5a,C-6b)	6.2

[a] See text for estimated accuracies (±0.5–2 Hz).
[b] ^1J and ^3J are assumed to be positive. For ^2J, negative signs in parentheses were predicted by simulation but not verified.
[c] Equivalent couplings [e.g. J(C-5a,C-6b) = J(C-5b,C-6a) = J(C-5a',C-6b') = J(C-5b',C-6a')] are listed under only one combination.
[d] Couplings not listed were not observed or could not be determined.
[e] This coupling may be for ^2J(C-3,C-5).

prised of the aromatic ring carbons. Most of the couplings to C-6a and C-6b could be estimated from the other aromatic resonances (see below). ^2J(C-6a, C-6b) and ^3J(C-5a,C-6b) [$=^3J$(C-5b,C-6a)] were initially assigned values of 2.5 and 10 Hz (^2J and ^3J values in benzene),[16,17] respectively, and refined by simulation, using fragment A (Fig. 7).

Irradiation of C-3 caused the complex multiplet of C-4 (Fig. 6) to collapse to a triplet of doublets (Fig. 8), from which ^1J(C-4,C-5) and ^3J(C-4,C-7) were determined directly. The lines are broadened relative to the triplet of doublets observed for C-7. A ^2J(C-4,C-6) value of 0.5 Hz was determined from the C-6{C-3} resonance. A coupling constant of this magnitude should not cause significant broadening. The peak width, therefore, is probably due to additional vicinial coupling to C-1 and/or C-2'. A value of 2 Hz for this additional coupling gave the simulated spectrum in Fig. 8, but this coupling could not be optimized. Selective 1D COSY spectra with initial excitation of C-2 revealed a coupling of <5 Hz to C-4 and/or C-4', whereas no coherence transfer to C-4 could be observed in 1D COSY experiments with initial excitation of C-1. The additional coupling required to account for the C-4 line shape can therefore be assigned as the vicinal ^3J(C-2',C-4), although a more precise value of this coupling could not be determined owing to cancellation of antiphase components in the selective 1D COSY experiment.

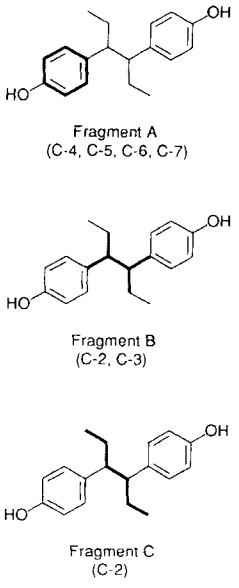


Figure 7. Fragments of *meso*-hexestrol used to generate simulated spectra (see text and Figs 8, 9, 11 and 13).

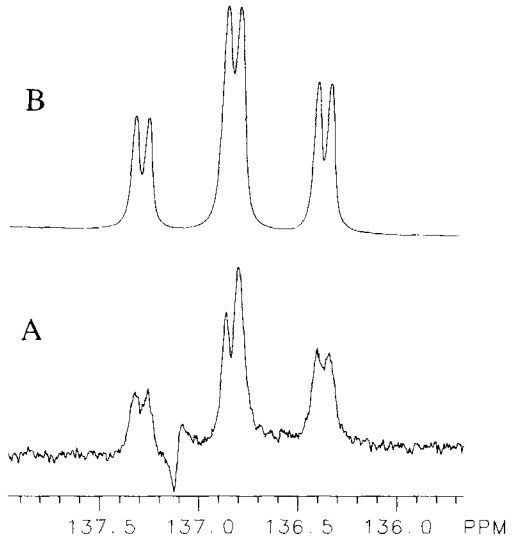


Figure 8. 125.76 MHz $^{13}C\{^1H,C\text{-}3\}$ spectrum of **1** (only the C-4 resonance is shown): (A) observed; (B) computer simulated using fragment A (Fig. 7).

The other selectively decoupled spectra of the C-4 resonance were too poorly resolved to give additional coupling information. Coupling constants to C-3, C-3' and C-4' were obtained from the C-3 resonance (see below). The overall pattern should represent the AA' portion of an AA'XX' spin system (consisting of C-3 and C-4, and excluding couplings to C-2), which is further split into a binomial triplet of doublets by coupling to C-5 and C-7. The fully coupled C-4 resonance could not be simulated owing to the number of spins involved. However, a line spectrum was drawn according to the predicted coupling pattern described above, using the coupling constants indicated in Table 2 (Fig. 9). The line pattern so generated reproduces the main features of the observed C-4 resonance.

Simulation of the aromatic ring failed to reproduce the distorted triplet pattern of the C-5{C-3} resonance. Inclusion of a 2.5 Hz coupling for ^3J(C-2,C-5) more accurately reproduced the observed pattern. A ^3J(C-2, C-5) value of 2.3 Hz could also be measured as the antiphase splitting of the C-2 resonance in 1D COSY experiments with initial excitation of C-5 (Fig. 10).

In general, the aromatic carbons gave good agreement between the observed and simulated spectra. Errors (r.m.s.) of about 0.4 Hz were obtained in the iterative process, and alteration of the long-range (^2J,^3J) coupling constants by more than 0.2–0.5 Hz caused obvious distortion of line shapes. In contrast to the vast literature on carbon–carbon coupling constants in monosubstituted benzens, relatively few data exist for aromatic J(CC) values in disubstituted benzene derivatives. The ^3J(CC) values obtained for **1** are consistent with the observed trend (monosubstituted benzenes) that a substituent internal to a coupling path causes a decrease in ^3J(CC).[7] The ^3J values are comparable in magnitude to those reported for *p*-methylaniline.[18] the

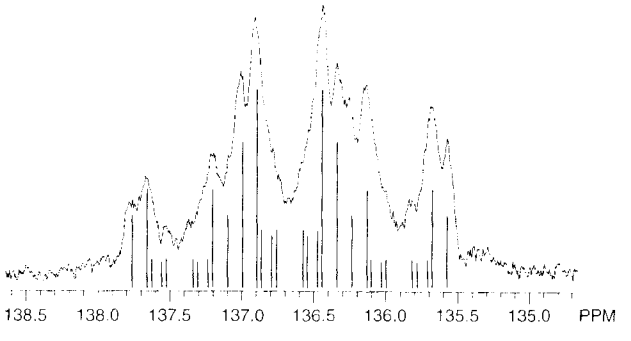


Figure 9. 125.76 MHz $^{13}C\{^1H,C\text{-}3\}$ spectrum of **1** (only the C-4 resonance is shown), inset with the line spectrum predicted for C-4 using the couplig constants in Table 2.

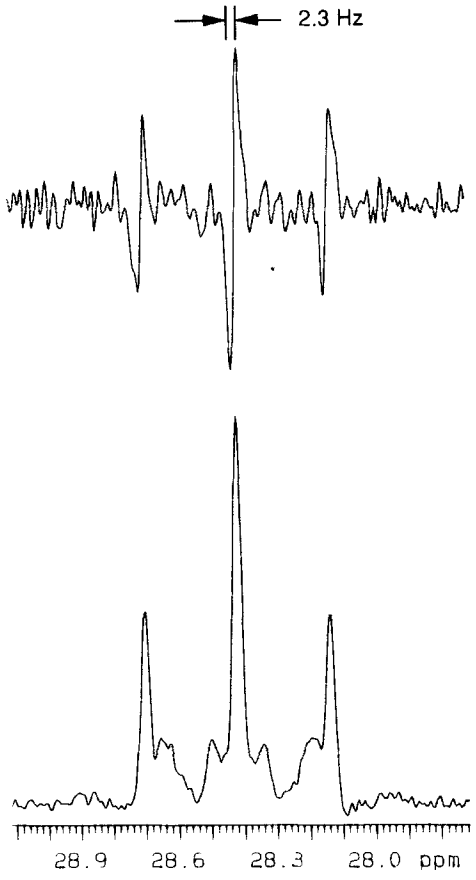


Figure 10. 125.70 MHz ^{13}C spectrum of the C-2 resonance in **1** (bottom) and the corresponding region of a 1D COSY experiment where the C-5 resonance was initially selectively excited (top). The separation of antiphase components in the 1D COSY spectrum was used to determine the ^{3}J(C-2,C-5) coupling constant as indicated in the top spectrum.

^{2}J(CC) values obtained in this study agree less well with data from monosubstituted benzenes, which are reported to have ^{2}J(CC) on the order of 2–4 Hz, with little apparent substituent effect.[7] In this light, ^{2}J(C-5,C-7) and ^{2}J(C-4,C-6) in **1** appear anomalously low, whereas the ^{2}J(C-5a,C-5b) and ^{2}J(C-6a,C-6b) values fall within the expected range. The former two coupling constants are of similar magnitude to the ^{2}J(C-2,C-3) value (0.78 Hz) reported for [1-^{13}C]toluene,[19] although studies performed using natural abundance toluene have indicated larger values for this coupling (2.042 Hz[14] or 2.05 Hz[16]). The ^{2}J(CC) values obtained in this study are, however, similar in magnitude to results obtained in polyaromatic systems,[20] where a terminal hydroxyl or methyl substituent has been reported to cause a reduction in the magnitude of ^{2}J(CC).

Aliphatic carbons. The observed resonances for C-3 and C-2 (Fig. 6) were less well reproduced by simulation owing to the restriction of the simulation program to seven spins *vs.* the highly coupled nature of these carbons. Coupling constants reported for diphenylethane[21] suggest that C-3 and C-3′ in **1** will have >1 Hz coupling to all carbons except C-7 and C-7′. The errors determined by simulation for these coupling constants are thus inherently larger (r.m.s. = 1–2 Hz).

The one-bonded coupling pattern for C-3 is that of the MM′ portion of an AA′MM′XX′ spin system. Irradiation at C-2 caused the resonance to collapse to a broad AA′XX′ pattern, from which the four coupling constants involving C-3 and C-4 were estimated. The couplings involving C-2 and C-3 were more readily estimated from the C-2 resonance of the {C-1,C-4} spectrum, as fewer significant couplings remained resulting in a sharper pattern. The coupling constants for each AA′XX′ system were refined separately, before combining all six carbons to give the simulated spectrum shown in Fig. 11. The observed pattern for C-3 was insensitive to the coupling constants ^{2}J(C-2,C-4) [$=^{2}J$(C-2′,C-4′)] and ^{3}J(C-2,C-4′) [$=^{3}J$(C-2′,C-4)], which gave 'acceptable' simulations over a range of 0–5 Hz. Values of 0.5 Hz (^{2}J) and 2 Hz (^{3}J) were used in the spectrum shown in Fig. 11.

The vicinal ^{3}J(C-3,C-5′) and ^{3}J(C-3,C-6) couplings could be estimated from selective 1D COSY spectra with initial excitation of C-5 and C-6, respectively. Cancellation of antiphase intensities in the C-3 resonance made a precise determination of ^{3}J(C-3,C-6) difficult, and only allowed an estimate of the upper limit, i.e. ^{3}J(C-3,C-6) < 5 Hz. The ^{3}J(C-3,C-5′) coupling was estimated to be *ca.* 6 Hz (Fig. 12); this coupling could also be due to ^{2}J(C-3,C-5). The measured J(C-3,C-5) is the largest of the two possible couplings.

Other couplings to C-3 could not be resolved in the experimental spectra. However, coupling constants between the aromatic and benzylic carbons have been reported for a number of diphenylethylene and diphenylethane derivatives enriched with ^{13}C at the benzylic carbons.[21,22] Those studies demonstrated only small variations in the magnitudes of individual coupling constants over a variety of benzylic substituents. With the exception of ^{3}J(C-3,C-5′) [or ^{2}J(C-3,C-5], estimated to be *ca.* 6 Hz in **1** *vs.* literature values of 1.8–3.8 Hz,[21,22] all the couplings in **1** fall within the ranges reported for similar compounds.

The C-2 resonance represents the XX′ portion of an AA′MM′XX′ spin system (consisting of C-2, -3 and -4), which is further split into a doublet due to ^{1}J(C-1,C-2). As this system involves eight spins, the calculated spectrum could not be generated. The simulated spectrum

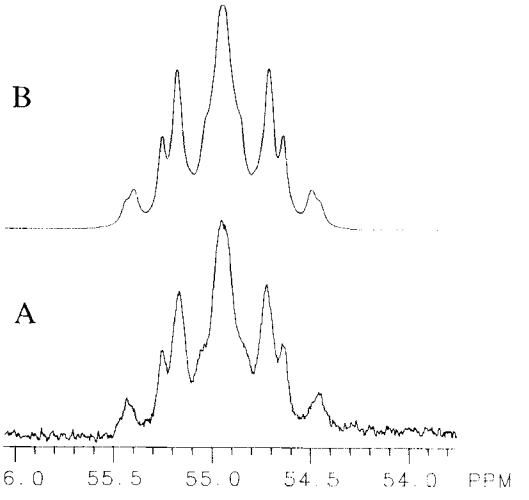


Figure 11. 125.76 MHz $^{13}C\{^{1}H,C\text{-}5\}$ spectrum of **1** (only the C-3 resonance is shown): (A) observed; (B) computer simulated using fragment B (Fig. 7).

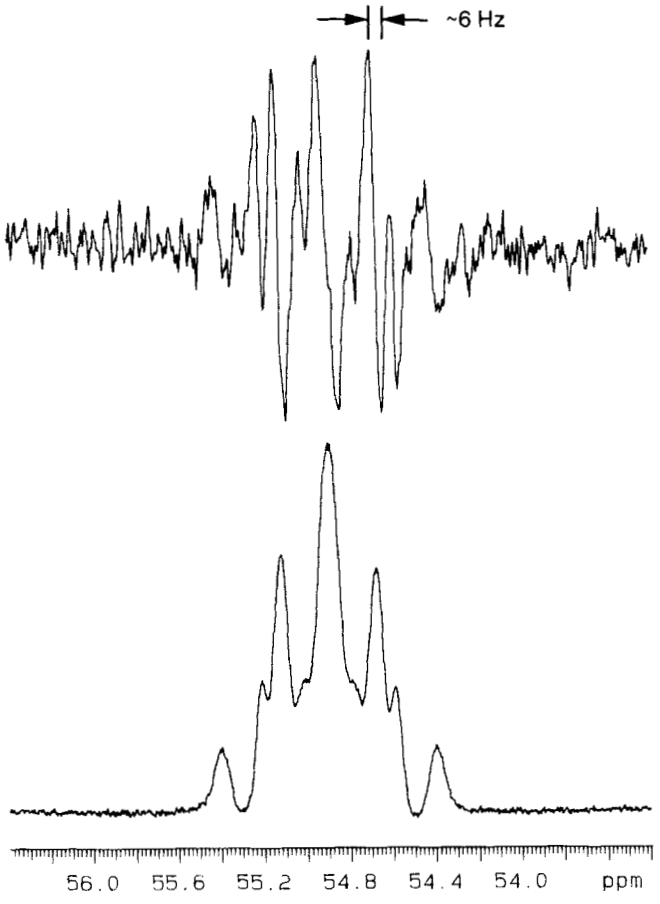


Figure 12. 125.70 MHz ^{13}C spectrum of the C-3 resonance in **1** (bottom) and the corresponding region of a 1D COSY experiment where the C-5 resonance was initially selectively excited (top). The separation of antiphase components in the 1D COSY spectrum was used to estimate the ^3J(C-3,C-5′) coupling constant as indicated in the top spectrum.

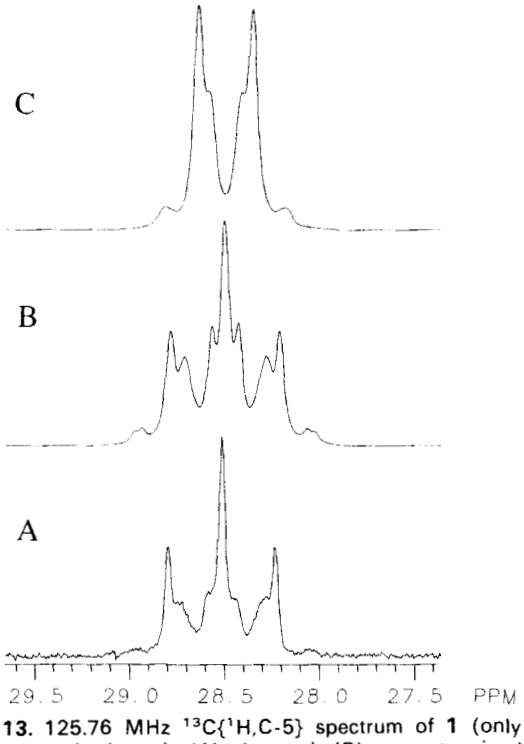


Figure 13. 125.76 MHz $^{13}C\{^1H,C\text{-}5\}$ spectrum of **1** (only the C-2 resonance is shown): (A) observed; (B) computer simulated using fragment A (Fig. 7); (C) computer simulated C-2$\{^1H,C\text{-}1\}$ spectrum generated using fragment B (Fig. 7).

shown in Fig. 13 was generated using the carbons of the hexane backbone to give a doublet of AA′XX′ pattern, with the nearly identical values of ^1J(C-1,C-2) and ^1J(C-2,C-3) giving the overall triplet appearance. Values of 0.5 and 4 Hz were assigned to ^2J(C-1,C-3) and ^3J(C-1,C-3′), respectively, but could not be optimized; ^4J(C-1,C-2′) and ^5J(C-1,C-1′) were assumed to be zero. A better fit with the observed pattern might be imagined from overlapping the AA′MM′*XX*′ pattern obtained for C-2 [Fig. 13(C)] from the simulation described above for C-3.

The resolution-enhanced selective 1D spectrum of C-1 (Fig. 14) showed a doublet due to ^1J(C-1,C-2) with two additional doublet splittings of *ca.* 3 and *ca.* 1 Hz,

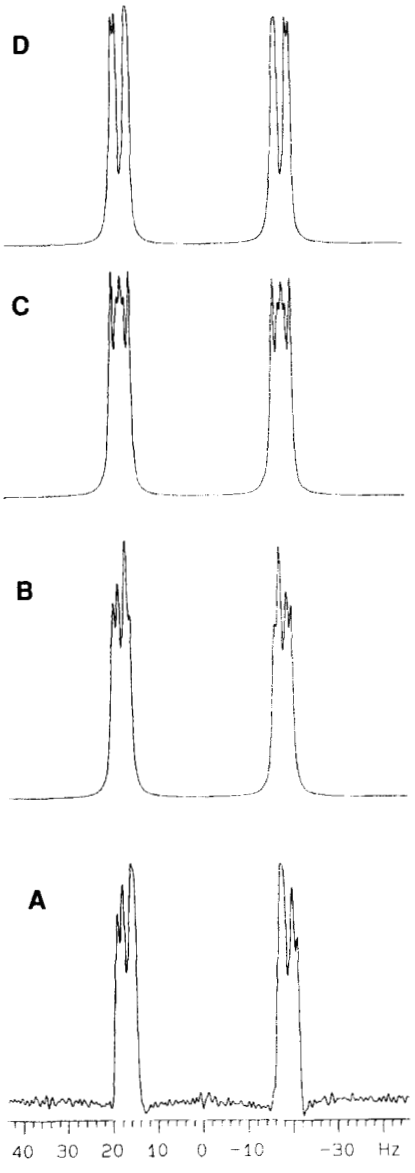


Figure 14. (A) Resolution-enhanced selective 125.70 MHz ^{13}C 1D spectrum of the C-1 resonance in **1**, showing a doublet due to ^1J(C-1,C-2) with two additional doublet splittings of *ca.* 3 and *ca.* 1 Hz, respectively. (B) Simulation of the C-1 resonance assuming ^1J(C-1,C-2) = 36 Hz, ^2J(C-1,C-3) = 0 Hz, ^{3}(C-1,C-3′) = 3 Hz and ^3J(C-1,C-4) = 1 Hz. (C) Simulation assuming ^1J(C-1,C-2) = 36 Hz, ^2J(C-1, C-3) = 1 Hz, ^3J(C-1,C-3′) = 3 Hz and ^3J(C-1,C-4) = 0 Hz. (D) Simulation assuming ^1J(C-1,C-2) = 36 Hz, ^2J(C-1,C-3) = 0 Hz, ^3J(C-1,C-3′) = 1 Hz and ^3J(C-1,C-4) = 3 Hz.

respectively. The additional couplings might be due to the geminal ^2J(C-1,C-3) coupling or the vicinal ^3J(C-1, C-3′) and ^3J(C-1,C-4) couplings. Simulations (without optimization) of a fragment containing the eight C-1 to C-4 and C-1′ to C-4′ nuclei (using a version of the LAOCOON program provided with the VNMR software from Varian) indicated that the larger (3 Hz) coupling is due to ^3J(C-1,C-3′) and the smaller (1 Hz) coupling is ^3J(C-1,C-4), with ^2J(C-1,C-3) $\approx$ 0 (Fig. 14).

CONCLUSIONS

In order to study ligand interactions with the estrogen receptor by polarization transfer methods, we prepared the non-steroidal ligand *meso*-hexestrol in ^{13}C-substituted form, with an isotopic purity of >98 at.% ^{13}C. To demonstrate the feasibility of determining carbon–carbon coupling constants in such a highly substituted molecule, we analyzed the coupling patterns by homonuclear decoupling and 1D ^{13}C COSY experiments, and determined many of the coupling constants via spectral simulation. The magnitudes of all one-bond and many of the long range coupling constants are consistent with established effects of hybridization and substitution on J(CC) values. The relatively low accuracy (0.4–1 Hz) of the coupling constants is attributed primarily to the broad line widths, which prevent the resolution of long-range couplings.

EXPERIMENTAL

General

Sodium [1,2,3-$^{13}C_3$]propionate (minimum 99 at.% ^{13}C) and [*ring*-$^{13}C_6$]anisole (98.9 at.% ^{13}C) were purchased from Isotec. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl immediately prior to use. CH_2Cl_2 was distilled from CaH_2. Other solvents and reagents were used as purchased. Authentic *meso*-hexestrol was purchased from Sigma. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Flash chromatography was performed according to Still *et al.*,[23] using Merck silica gel (40–63 μm). Low-resolution electron impact mass spectra were obtained on a Finnigan MAT CH-5 spectrometer. Infrared (IR) spectra were recorded on a Mattson Galaxy Series 3000 spectrometer with Win First software.

NMR spectra

Proton magnetic resonance (^{1}H NMR) spectra were obtained on a General Electric QE-300 (300 MHz) spectrometer. Spectra of **4**–**7** were obtained with 6024 Hz sweep widths and 4.8 μs (50°) pulse widths. Data sets were 16K points and were zero-filled to 32K points for a digital resolution of 0.38 Hz per point. The spectrum of **1** was obtained with 32K points zero-filled to 64K points for a digital resolution of 0.19 Hz per point. With the exception of the unlabeled methoxy group, all resonances appeared as unresolved multiplets with doublet splitting due to ^{1}J(C,H). The data are reported in the form chemical shift [^1J(C,H), number of protons, assignment]. Carbon-13 magnetic resonance (^{13}C NMR) spectra were recorded at 75.5 MHz on a General Electric QE-300 spectrometer. Spectra of **4**–**7** were obtained with 20 kHz sweep and 3.1 μs (26°) pulse widths. Data sets were 32K points zero-filled to 64K for a digital resolution of 0.62 Hz per point. The spectrum of **1** was obtained with 64K points zero filled to 128K for a digital resolution of 0.31 Hz per point. For most signals, only one-bond carbon–carbon coupling patterns are reported. A designation such as 't' refers to the appearance of a non-first order unresolved coupling pattern with the indicated apparent coupling constant(s) ('J'). Selective ^{13}C decoupling experiments were performed on a General Electric GN 500 spectrometer at 125.76 MHz for ^{13}C. Spectra were obtained with a 23256 Hz sweep width and an 8 μs (45°) pulse width. Data sets were 64K points zero-filled to 128K for a digital resolution of 0.36 Hz per point. Chemical shifts for both ^{1}H and ^{13}C NMR are reported relative to tetramethylsilane (δ scale). All spectra were obtained at a sample concentration of *ca.* 10 mg of compound per 500 μl of solvent at 20 °C. All ^{13}C NMR spectra were obtained with broadband proton decoupling. Computer simulations of NMR spectra with iterative refinement of coupling constants were performed with the ITRCAL software program from Nicolet using a Nicolet Model 1180E computer. Simulated spectra were plotted using the GENSIM program from General Electric.

Some complementary proton decoupled ^{13}C NMR spectra, including selective 1D and selective 1D COSY experiments, were recorded at 26 °C on a Varian Unity 500 spectrometer operating at 125.70 MHz equipped with a programmable pulse modulator for ^{13}C excitation. The sample used for these additional studies consisted of 9 mg of **1** dissolved in 700 μl of methanol-d_3. Selective 1D spectra were obtained using 'eburp 1' shaped pulses,[24] using spectral widths of 400 Hz and 2048 data points. Zero-filling to 16K yielded a digital resolution of 0.05 Hz per point after Fourier transformation. Selective 1D COSY experiments were performed according to Bossenec *et al.*[25] using 'eburp 1' shaped pulses for excitation and a hard pulse for coherence transfer, following an adjustable evolution delay. The 1D COSY spectra were recorded with 32K data points using a sweep width of 20000 Hz; 2048 transients were averaged for each value of the adjustable evolution delay. The spectra were processed with shifted Gaussian or non-shifted sine-bell apodization functions for resolution enhancement. Coupling constants can be determined in selective 1D COSY spectra by measuring the splitting of the antiphase multiplet (where the active coupling gives rise to the antiphase appearance) and/or from the 'transfer delay' at which optimum coherence transfer is obtained.[25]

Compounds

[$^{13}C_9$]-4′-methoxypropiophenone (4). To a cooled (0 °C) suspension of sodium [$^{13}C_3$]propionate (200 mg, 2.02 mmol) in 1.5 ml of CH_2Cl_2 under N_2 was added oxalyl chloride (210 µl, 2.41 mmol) dropwise via a syringe. Gas evolution began immediately. The mixture was stirred at 0 °C for 1 h, then warmed to room temperature (RT) and stirred for 1 h. After cooling to −20°C, $AlCl_3$ (295 mg, 2.21 mmol) was added in one portion followed by a solution of [$^{13}C_6$]anisole (229 mg, 2.01 mmol) in 500 µl of CH_2Cl_2. The orange solution was stirred at −20 °C for 30 min, then warmed to RT, at which point the color deepened to red. After 15 min the reaction was quenched by dropwise addition to water (30 ml), followed by extraction of the product into Et_2O (3 × 15 ml). The combined organic fractions were dried ($MgSO_4$) and the solvents removed *in vacuo* to yield ketone **4** (296 mg, 1.71 mmol, 85%) as a pale pink liquid. ^{1}H NMR ($CDCl_3$), δ 1.21 [^1J(C,H) = 127.9 Hz, 3 H, —CH_2CH_3], 2.96 [^1J(C,H) = 125.0 Hz, 2 H, —CH_2CH_3], 3.87 [*d*, ^3J(C,H) = 4.0 Hz, 3 H, —OCH_3], 6.93 [^1J(C,H) = 165 Hz, 2 H, H *ortho* to —OCH_3], 7.95 [^1J(C,H) = 167 Hz, 2 H, H *ortho* to —C=O]. ^{13}C NMR (75.5 MHz, $CDCl_3$), δ 8.40 [dd, ^1J(CC) = 35.5 Hz, ^3J(CC) = 2.1 Hz, —CH_2CH_3], 30.8–32.0 (m, —CH_2CH_3), 112.5–114.4 (m, aryl C α to —C—O—CH_3), 129.5–130.8 (m, aryl C bearing C=O and aryl C β to —C—O—CH_3), 162.3–164.2 (m, —C—O—CH_3), 198.8–204.2 (m, —C=O). MS (70 eV), *m/z* (relative intensity, %) 173 ([$^{13}C_9$]M^+, 12), 172 (1.3), 142 (100), 113 (11), 98 (12), 83 (15).

[$^{13}C_{18}$]-(*Z*)-3,4-bis(4-methoxyphenyl)hex-3-ene (5). To a stirred suspension of $TiCl_3$ (1.39 g, 9.01 mmol) in 20 ml of THF under argon was added $LiAlH_4$ (171 mg, 4.51 mmol) in one portion. Once vigorous gas evolution had subsided, the black suspension was heated at reflux for 30 min. To the cooled (RT) suspension was added a solution of ketone **4** (260 mg, 1.50 mmol) in 5 ml of THF, and the mixture was refluxed for 21 h. The cooled dark brown suspension was diluted with light petroleum (25 ml) and filtered through Florisil, rinsing with EtOAc (75 ml). The filtrate was washed with water (3 × 50 ml), dried ($MgSO_4$) and concentrated. Flash chromatography (95:5 hexanes–EtOAc) gave the (*Z*)-alkene **5** as a colorless oil (173 mg, 0.55 mmol, 73%), contaminated with a small amount of the *E*-isomer (estimated <5% by GC and ^{1}H NMR). ^{1}H NMR ($CDCl_3$), δ 0.94 [^1J(C,H) = 126.4 Hz, 6 H, —CH_2CH_3], 2.51 [^1J(CH) = 123 Hz, 4 H, —CH_2CH_3], 3.71 [d, ^3J(C,H) = 4.2 Hz, 6 H, —OCH_3], 6.61 [^1J(C,H) = 157 Hz, 4 H, H *ortho* to —OCH_3], 6.86 [^1J(C,H) = 156 Hz, 4 H, H *ortho* to alkene]. ^{13}C NMR (75.5 MHz, $CDCl_3$), δ 13.31 [d, ^1J(CC) = 33.5 Hz, —CH_2CH_3], 27.34 (doublet of XX′ portion of AA′XX′ spin system, —CH_2CH_3), 112.7 ['t', '^1J(CC)' = 65 Hz, aryl C α to —C—O—CH_3], 130.7 ['t', '^1J(CC)' = 57 Hz, aryl C β to —C—O—CH_3], 134.4–136.8 and 137.4–138.8 (m, alkene C and aryl C attached to alkene), 157.1 [td, ^1J(CC) = 67.4 Hz, ^3J(CC) = 7.9 Hz, —C—O—CH_3]. MS (70 eV), *m/z* (relative intensity, %) 315 ([$^{13}C_{18}$]M^+ + 1, 2.3), 314 ([$^{13}C_{18}$]M^+, 100), 313 (17), 312 (1.9), 298 (14), 283 (38), 267 (11), 252 (12), 184 (15), 169 (48), 143 (13), 128 (44), 84 (10).

[$^{13}C_{18}$]-(*R,S*)-3,4-bis(4-methoxyphenyl)hexane (6). A solution of (*Z*)-alkene **5** (150 mg, 0.48 mmol) in 3 ml of absolute ethanol was hydrogenated over palladium on carbon (5%, 70 mg) at atmospheric pressure. After 2 h at RT, GC analysis showed conversion of the alkene into a 9:1 mixture of *meso*- and *dl*-alkanes. The mixture was filtered through Celite, rinsing with EtOAc, and concentrated *in vacuo*. Flash chromatography (95:5 hexanes–EtOAc) afforded 104.5 mg (0.33 mmol, 69%) of the pure *meso*-isomer **6** as a white solid. A further 31 mg (0.099 mmol, 21%) were obtained as a mixture of *meso*- and *dl*-isomers. ^{1}H NMR ($CDCl_3$), δ 0.52 [^1J(C,H) = 124.9 Hz, 6 H, —CH_2CH_3], 1.26 [^1J(C,H) = 125 Hz, 2 H, —CH_2CH_3], 1.36 [^1J(C,H) = 125 Hz, 2 H, —CH_2CH_3], 2.47 [^1J(C,H) = 121 Hz, 2 H, benzylic H], 3.82 [d, ^3J(C,H) = 4.2 Hz, 6 H, —OCH_3],6.86 [^1J(C,H) = 162 Hz, 4 H, H *ortho* to —OCH_3],7.07 [^1J(C,H) = 155 Hz, 4 H, H *meta* to —OCH_3]. ^{13}C NMR (75.5 MHz, $CDCl_3$), δ 12.22 [d, ^1J(CC) = 34.5 Hz, —CH_2CH_3], 27.36 (doublet of XX′ portion of AA′XX′ spin system, —CH_2CH_3), 53.48 (MM′ portion of AA′MM′XX′ spin system, benzylic C), 113.42 'dd', '^1J(CC)' = 65, 66 Hz, aryl C α to —C—O—CH_3), 129.08 ['t', '^1J(CC)' = 60 Hz, aryl C β to —C—O—CH_3], 135.5–137.7 (m, aryl C attached to alkane), 157.69 [td, ^1J(CC) = 67.4 Hz, ^3J(CC) = 8.0 Hz, —C—O—CH_3]. MS (70 eV), *m/z* (relative intensity, %) 316 ([$^{13}C_{18}$]M^+, 2.1), 158 (dibenzyl cleavage, 100), 157 (17), 128 (30), 98 (7); (70 eV off-scale, M^+ isotope peaks) 317 (1.8), 316 (47), 315 (8.7), 314 (8.1), 313 (2.0).

[$^{13}C_{18}$]-(*R,S*)-3,4-bis(4-hydroxyphenyl)hexane (1). To a cooled (0 °C) solution of *meso*-hexestrol dimethyl ether (**6**) (90 mg, 0.28 mmol) in 4.5 ml of CH_2Cl_2 was added 1.3 ml of boron trifluoride–dimethyl sulfide complex. The mixture was stirred under N_2 and allowed to warm to RT over 2 h then stirred at RT for 18 h. A white precipitate formed. The mixture was carefully added to water and the solid product extracted into EtOAc (3 × 50 ml), dried ($MgSO_4$) and concentrated. Flash chromatography (80:20 hexanes–EtOAc) gave pure *meso*-hexestrol (75 mg, 0.26 mmol, 91%) as a white solid. Crystallization from EtOH–H_2O gave needles, m.p. 184–186 °C. ^{1}H NMR (CD_3OD), δ 0.50 ^1J(C,H) = 124.7 Hz, 6 H, —CH_2CH_3], 1.23 [^1J(C,H) = 124 Hz, 2 H, —CH_2CH_3], 1.36 [^1J(C,H) = 124 Hz, 2 H, —CH_2CH_3], 2.40 [^1J(C,H) = 131 Hz, 2 H, benzylic H], 6.73 [^1J(C,H) = 155 Hz, 4 H, H *ortho* to —OH), 6.98 [^1J(C,H) = 154 Hz, 4 H, H *meta* to —OH]. IR (KBr) (cm^{-1}), 3028, 2949, 2925 (C–H); 1559, 1544, 1478 (ar C–C); 1409 (C–C); 1200, 1164 (C–O). MS (70 eV) *m/z* (relative intensity, %) 288 ([$^{13}C_{18}$]M^+, 1.03), 144 (dibenzyl cleavage, 100), 143 (21), 114 (48). See Results section for ^{13}C NMR and isotope content analysis.

[$^{13}C_{18}$]-(*R,R*)- and (*S,S*)-3,4-bis(4-hydroxyphenyl)hexane (7). Similar treatment of a mixture of *meso*- and *dl*-dimethyl ethers (25 mg, 0.08 mmol) in CH_2Cl_2 (1.25 ml) with boron trifluoride–dimethyl sulfide complex (360 µl) gave, after flash chromatography, 16 mg (0.056 mmol,

70%) of *meso*-hexestrol (**1**) as a white solid and 6.4 mg (0.022 mmol, 28%) of *dl*-hexestrol (**7**) as a colorless oil. Data for [$^{13}C_{18}$]-*dl*-hexestrol: ^{1}H NMR (CD_3OD), δ 0.70 [^1J(C,H) = 124.8 Hz, 6 H, —CH_2CH_3], 1.47 [^1J(C,H) = 115 Hz, 2 H, —CH_2CH_3], 1.84 [^1J(C,H) = 115 Hz, 2 H, —CH_2CH_3], 2.58 [^1J(C,H) = 123 Hz, 2 H, benzylic H], 6.54 [^1J(C,H) = 159 Hz, 4 H, H *ortho* to —OH], 6.65 [^1J(C,H) = 153 Hz, 4 H, H *meta* to —OH]. ^{13}C NMR (75.5 MHz, $CDCl_3$), δ 12.79 [d, ^1J(CC) = 35.0 Hz, —CH_2CH_3], 27.29 (doublet of XX' portion of AA'XX' spin system, —CH_2CH_3), 53.91 (MM' portion of AA'MM'XX' spin system, benzylic C), 115.13 ['t', '^1J(CC)' = 64.5 Hz, aryl C α to —C—OH], 130.92 ('t', '^1J(CC)' = 57.5 Hz, aryl C β to —C—OH], 134.1–136.2 (m, aryl C attached to alkane), 155.99 [td, ^1J(CC) = 65.7 Hz, ^{3}J(CC) = 8.4 Hz].

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