

Identification of 4-*cis* hexadecaheptaene by nuclear magnetic resonance spectroscopy

Hexadecaheptaene was purified by HPLC using a YMC C₁₈-A column (5 μm particle size, 12 nm pore size) prior to the NMR measurements. 1D and 2D ¹H spectra, including ¹H-¹H COSY and ¹H-¹H NOESY, were recorded in chloroform-d at room temperature using a 600 MHz FT NMR spectrometer (AVANCE 600, Bruker).

Two doublets ($J_{1,1\text{Me}} = J_{1',1'\text{Me}} = 14 \text{ Hz}$) with small splittings ($J_{2,1\text{Me}} = J_{2',1'\text{Me}} = 2 \text{ Hz}$) in the methyl region (1-3 ppm) were assigned to the 1 and 1' methyl protons (Fig. S1). The 1Me and 1'Me protons exhibited different chemical shifts, indicating an asymmetrical configuration. The possibility of significant contamination by 4,4'-di-*cis* or all-*trans* isomers was eliminated based on the almost identical intensities of the well-separated methyl signals. Starting from signals due to the 1Me protons, the 2D correlations allowed unambiguous assignment of 2H through 8H. The same protocol was applied to the correlations starting from the 1'Me protons to identify the 2'H to 4'H signals. Assignments also were based on the splitting types, coupling constants, and COSY cross peaks between neighboring olefinic protons. The assigned ¹H signals are summarized in Table T1. Coupling constants listed in Table T1 were based on a simple first-order analysis, rather than a simulation of the multi-spin system.¹ The “isomerization shift” is defined as the change in chemical shift upon introduction of a *cis* double bond.¹ In general, the signal of each olefinic ¹H on the concave side of the *cis*-bond exhibits a low-field shift due to steric hindrance and the resultant removal of the shielding electron

from the proton. On the other hand, each olefinic ^1H on the convex side exhibits a high-field shift. In the present context for hexadecaheptaene, the “isomerization shift” is defined as the difference between the chemical shift for a proton on an un-primed carbon (Fig. S1) and that of a corresponding proton on a primed carbon. These shifts are given in parentheses in Table 1. The differences in signs of the isomerization shifts of the 3H and 6H protons and those of the 4H and 5H protons strongly implicate a 4-*cis* configuration for this isomer. The clear NOE correlation between 3H and 6H confirms the presence of the *cis* 4C=5C bond. Other NOE correlations confirmed *trans* configurations for the 2C=3C, 6C=7C, 2'C=3'C and 4'C=5'C double bonds. The NOE cross peaks between 7H and 8'H, 8'H and 6'H, 6'H and 4'H, 8H and 7'H, and 7'H and 5'H could not be detected because of diagonal noise.

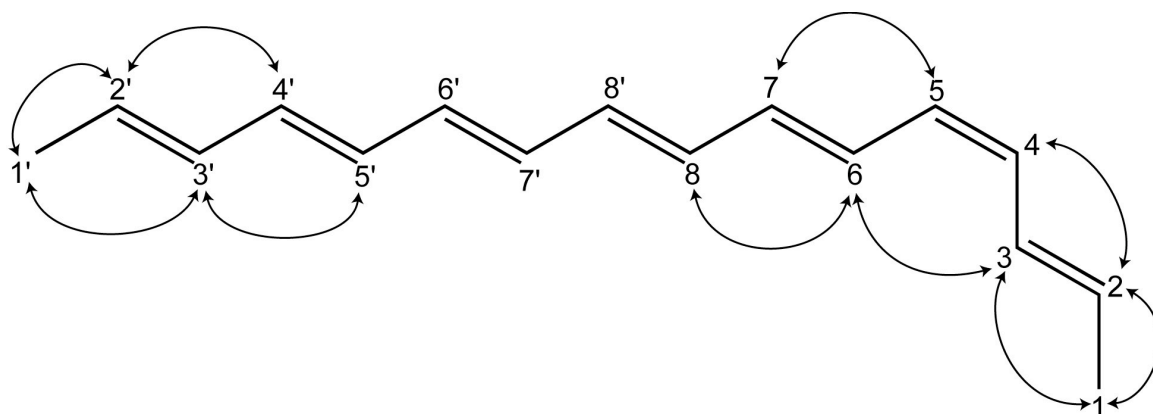


Figure S1 4-*cis*-2,4,6,8,10,12,14-hexadecaheptaene with NOE correlations (arrows).

Proton	1'H ₃	2'H	3'H	4'H	5'H	6'H	7'H	8'H	8'H	7'H	6'H	5'H	4'H	3'H	2'H	1'H ₃
δ (ppm)*	1.79	5.74	6.11	6.22	~6.28	~6.28	~6.28	~6.28	~6.28	~6.26	6.68 (+0.4)	5.92 (-0.4)	5.97 (-0.3)	6.55 (+0.4)	5.76	1.83
splittings**	d	m	dd	dd					dd	dd	dd	dd	dd	dd	m	d
J (Hz)		1'H ₃ -2'H 14	2'H-3'H 15	3'H-4'H 10	4'H-5'H 10	5'H-6'H	6'H-7'H	7'H-8'H 13	8'H-8'H 10	8'H-7'H 13	7'H-6'H 10	6'H-5'H 10	5'H-4'H 10	4'H-3'H 10	3'H-2'H 15	2'H-1'H ₃ 14
COSY		←→←→←→←→	←→←→←→←→	←→←→←→←→						←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	
NOESY		←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→					←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	←→←→←→←→	

* Isomerization shifts are shown in parentheses

** Abbreviations for empirical splitting types: d, doublet; m, multiplet; dd, double doublet

Table T1. ¹H chemical shifts (δ in ppm), splittings, first order coupling constants (*J* in Hz) and correlations of ¹H-¹H COSY and ¹H-¹H NOESY of 4-*cis* hexadecaheptaene in chloroform-*d* at 298 K. Isomerization shifts larger than 0.05 ppm are shown in parentheses.

1. Englert, G. In *Carotenoids*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Eds. Birkhauser Verlag: Basel, Switzerland, 1995; Vol. 1B.