

Poplar Milled Wood Lignin Models (Wild type, and COMT-deficient)

This document and the associated [web page](#) (from which 3D and other files may be obtained) accompany a review article for [Annual Reviews in Plant Biology](#) on “Lignin Biosynthesis” by Wout Boerjan, John Ralph, and Marie Baucher, 2003, 54:519-546. The structures were constructed based loosely on the following partial data from NMR spectra (the [HMQC experiment](#) of which is shown) described in the following references.

- Marita JM, Ralph J, Lapierre C, Jouanin L, Boerjan W. 2001. [NMR characterization of lignins from transgenic poplars with suppressed caffeic acid O-methyltransferase activity](#). J. Chem. Soc., Perkin Trans. 1: 2939-45.
- Ralph J, Lapierre C, Marita J, Kim H, Lu F, Hatfield RD, Ralph SA, Chapple C, Franke R, Hemm MR, Van Doorselaere J, Sederoff RR, O'Malley DM, Scott JT, MacKay JJ, Yahiaoui N, Boudet A-M, Pean M, Pilate G, Jouanin L, Boerjan W. 2001. [Elucidation of new structures in lignins of CAD- and COMT-deficient plants by NMR](#). Phytochem. 57: 993-1003.

Lignin	A	B	C	D	J	X	4-O-5	S/G
Wild-Type	88	3	7	0	0	2	?	0.725
COMT-sense	53	13	5	6	18	5	?	0.125

(see the NMR Figure for the designations of the units, **A-D** etc – same as in Fig 2 of the review manuscript).

We chose to use 20 phenylpropanoid units (i.e. constructed from 20 monomers), and included a single 4-O-5 branchpoint in each. No β -1 structures are included (since these are not seen at any significant level in the milled wood lignins).

The unit breakdown is as follows (note the units do not add up to 20 because of the way linkages may involve more than one unit, but the total number of **S+G+5H** units is 20).

WT	linkage	S	G	5H	S-S	S-G	G-G	5H-G	5H-S	5H-5H
A	β -O-4				3	9	4			
B	β -5				-	-	1			
C	β - β				1	-	-			
D2	DBDOX				-	-	-			
J	Benzodioxane				-	-	-			
X1	CA-endgroup	-	-	1						
E	4-O-5					1	-			
Total No		10	10	0						

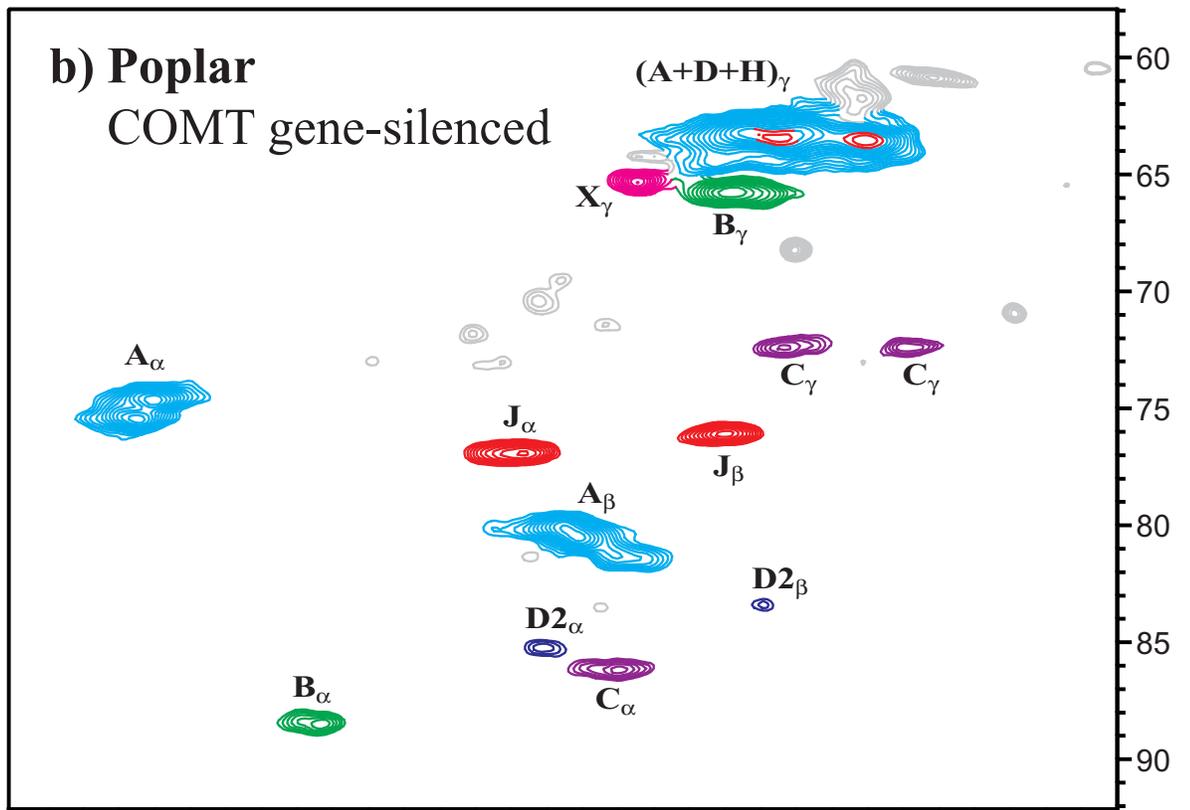
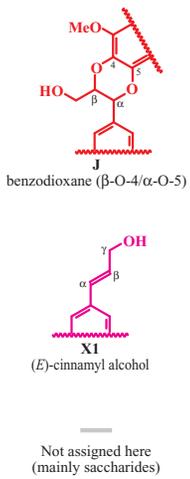
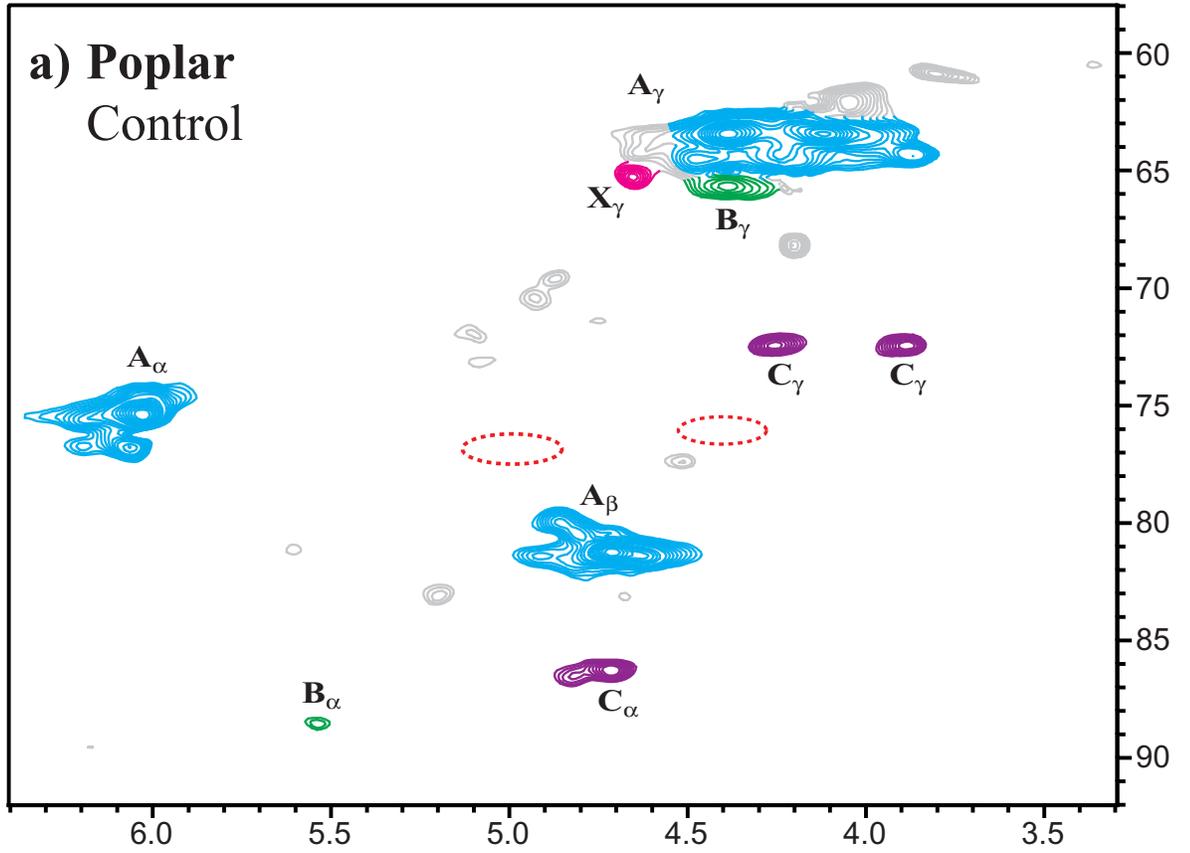
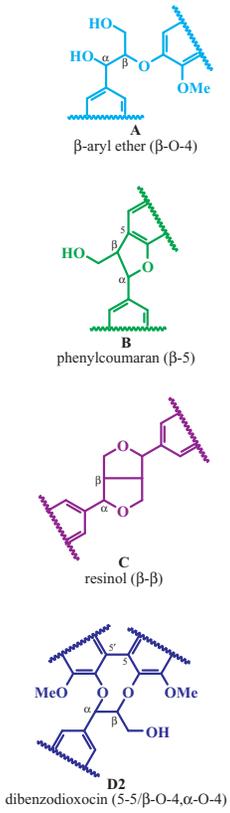
COMT-	linkage	S	G	5H	S-S	S-G	G-G	5H-G	5H-S	5H-5H
A	β -O-4					1	5	1	1	
B	β -5				-	-	3			
C	β - β					1	-			
D2	DBDOX				-	-	1			
J	Benzodioxane				-	-	-	3		1
X1	CA-endgroup	-	1	-						
X7	glycerol endgroup	-	-	1						
E	4-O-5					1	-			
Total No		2	14	4						

Notes

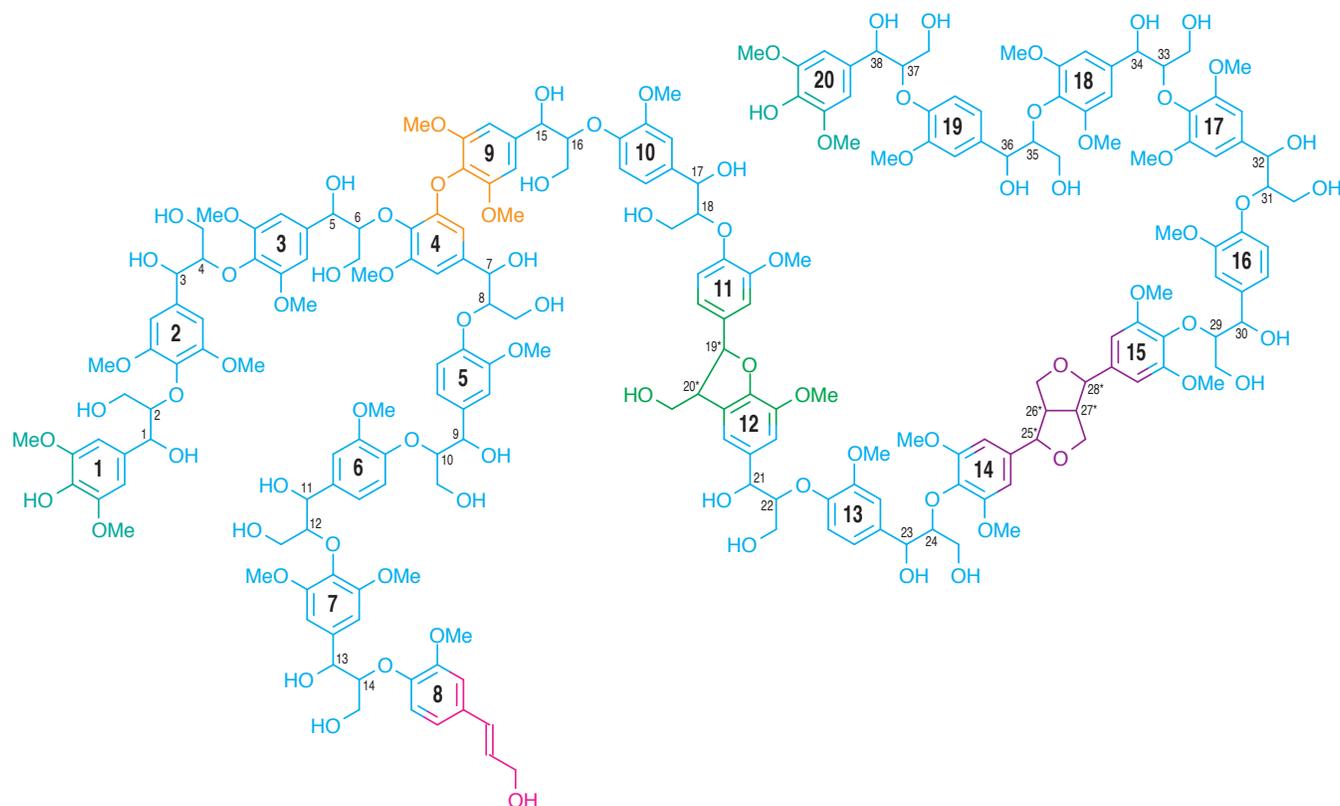
1. The colors of contours in the [NMR spectra](#) and of the groups in the [jpeg stills](#) and the 3D ViewerLite (.msv) files are similar. This way it is readily seen in the [NMR spectra](#) and in the models that, for example, the β -ether units **A** (cyan) are prevalent in the wild-type lignins, but less prevalent in the COMT-deficient lignin. Since it is only these units that cleave by thioacidolysis, DFRC, or in pulping reactions, it is clear why the COMT-deficient lignin is less easily degraded. The new benzodioxane units **J** in the COMT-deficient lignin are colored red (in the NMR and in the models). The branch points (4-O-5 units **E**, orange; dibenzodioxocin units **D2**, dark blue) are also easily identified in the Models.
2. The wild type has only a single branchpoint (4-O-5); the COMT-deficient has two (a 4-O-5 **E** again, and now a dibenzodioxocin **D2**, due to its higher guaiacyl content, as revealed by the NMR). Branchpoints provide the only deviation from “linear” structures.
3. The structures represent only *one* of many millions of isomers as described on the 2D structural pages, [page 3 for the wild type](#), and [page 4 for the COMT-deficient](#) lignins. For the wild type, we calculate that there are 2^{33} (8,589,934,592) real viable isomers for the structure shown. For the COMT-deficient model we deduce 2^{27} (134,217,728) physically distinct isomers. {This note is provided to emphasize the important point that their racemic nature alone confers incredible complexity and variability on polymeric lignins}.
4. The structures have been minimized in place, but it must be emphasized that each unit is probably just a local minimum – full optimization over all conformational space in a molecule of this size is impractical. They are ONLY MODELS!

Acknowledgements: These models are derived from data in the papers mentioned above. and based on [Brunow's softwood lignin model](#) (in Biopolymers. 2001. vol 1, pp 89-116). In addition, special thanks to Jane Marita for determining this data, to Tom Elder (Auburn University) and Ray Fort (U. Maine) for discussion and for alerting us to the Accelrys ViewLite program – the only one we currently know of that will allow us to color-code the lignin units, and to Bill Lindmeier for arranging the web page.

HSQC NMR Spectra



2D Model Structure: Wild-type Poplar Milled Wood Lignin

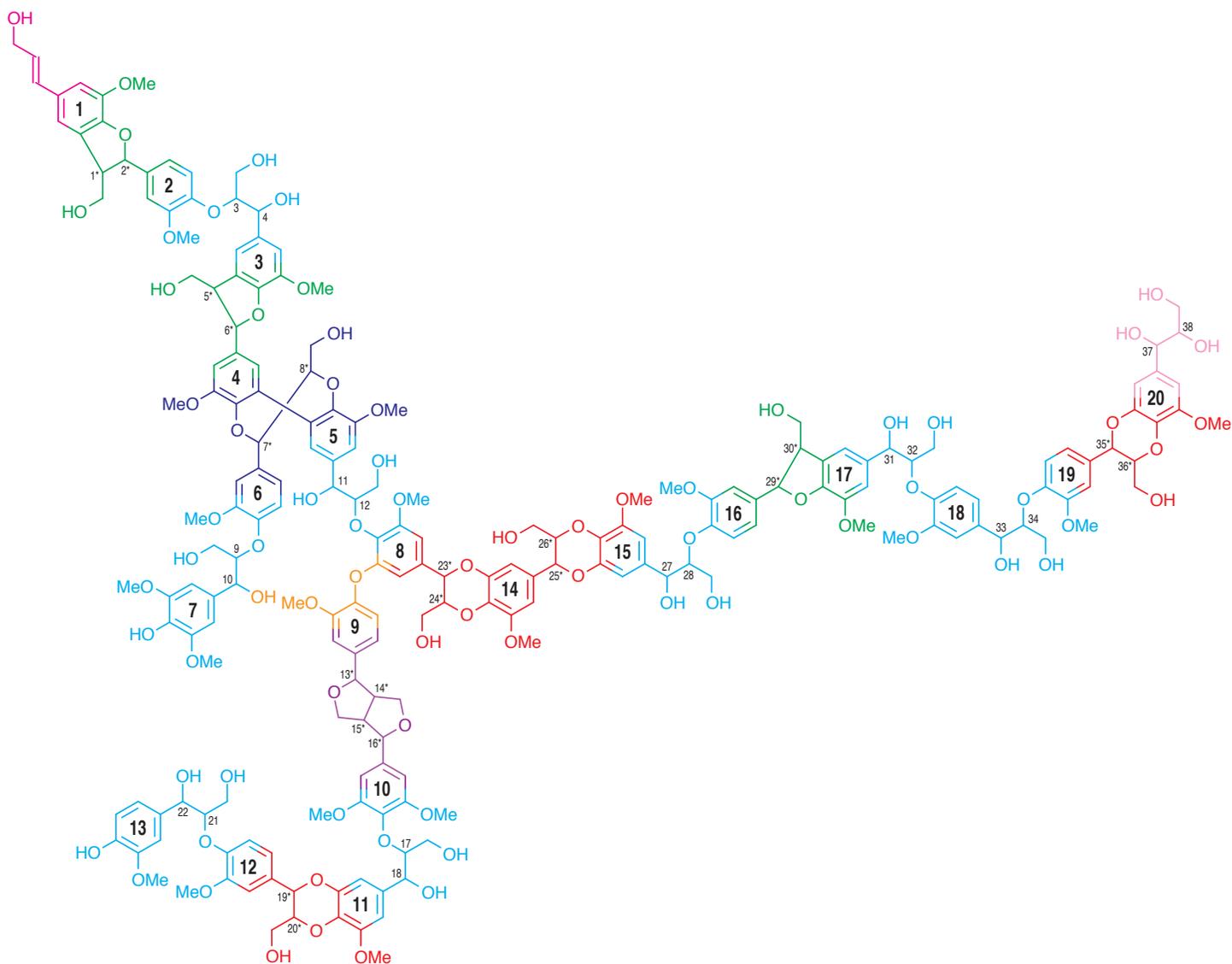


For the model for milled wood lignin from the wild type poplar, containing 20 phenylpropanoid units, there are 38 optical centers leading to 2^{38} potential optical isomers. However, the relative stereochemistries of 3 pairs of centers are fixed (asterisked centers; e.g. trans-ring junctions in phenylcoumarans). We therefore insinuate that there are 2^{35} optical isomers and therefore half that number of physically distinct isomers, i.e. 2^{34} (17,179,869,184) real viable isomers for the structure shown.

Pictures of the 3D Model are on [page 5](#).

The .pdb file for viewing in any molecular display program, e.g. RasMol and the .msv file for using with Accelrys' ViewerLite (with the same color scheme as used here to identify the lignin subunits) are available from our web site at <http://www.dfrc.ars.usda.gov/LigninModels.html>

2D Model Structure: COMT-deficient Poplar Milled Wood Lignin

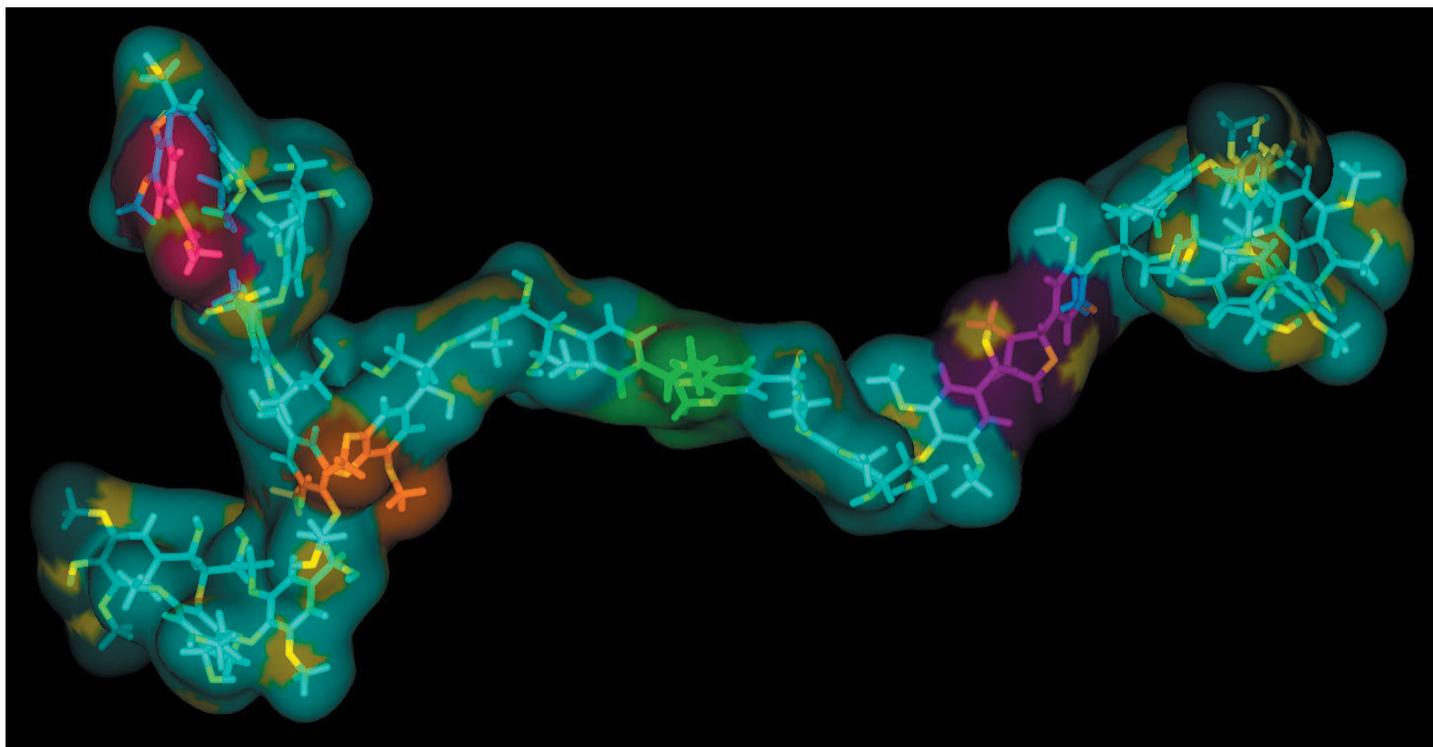
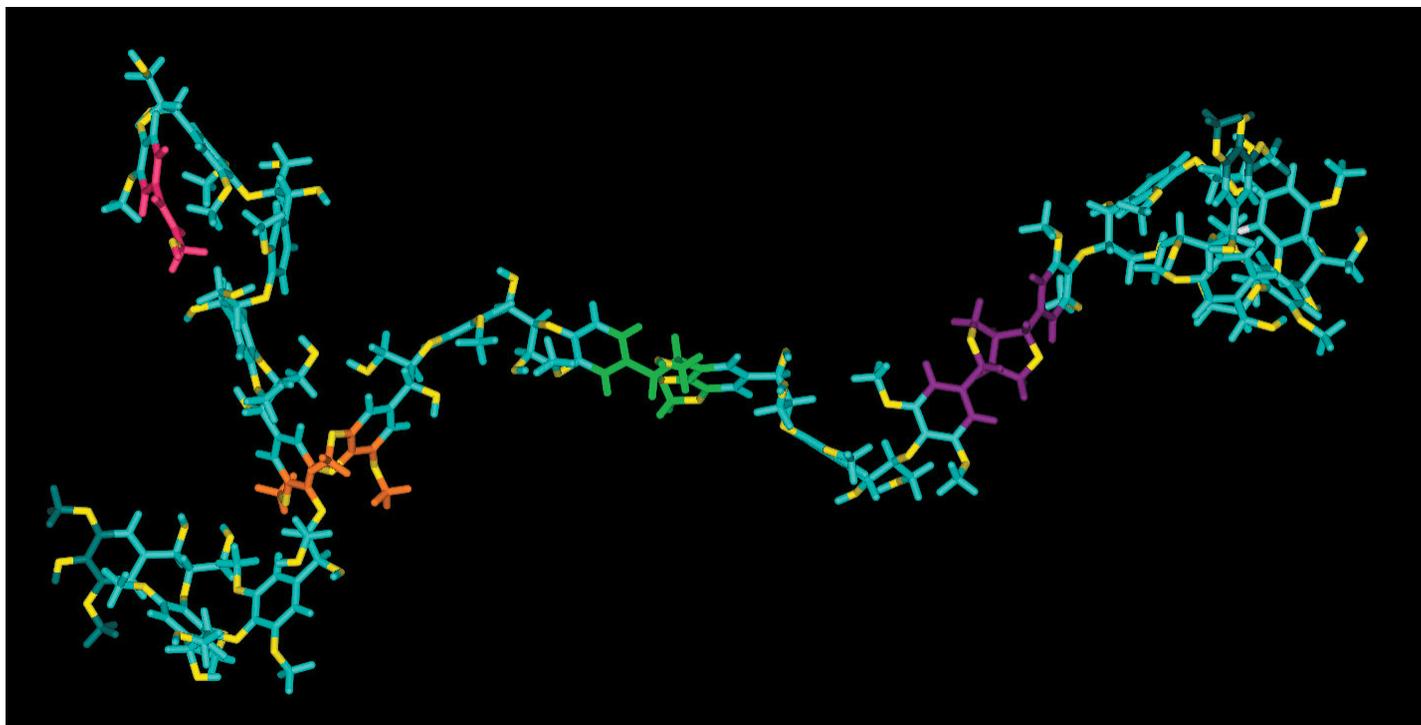


For the model for milled wood lignin from the COMT-deficient poplar, also containing 20 phenylpropanoid units, there are again 38 optical centers leading to 2^{38} potential optical isomers. However, the relative stereochemistries of 10 pairs of centers are fixed (asterisked centers; e.g. *trans*-ring junctions in phenylcoumarans, *trans*-ring junctures in the benzodioxanes) in this case; the significant incorporation of 5-hydroxyconiferyl alcohol leads to a high proportion of benzodioxane structures which are mostly in the *trans*-ring form. We therefore calculate that there are 2^{28} optical isomers and therefore half that number of physically distinct isomers, i.e. 2^{27} (134,217,728) real viable isomers for the structure shown.

Pictures of the 3D Model are on [page 6](#).

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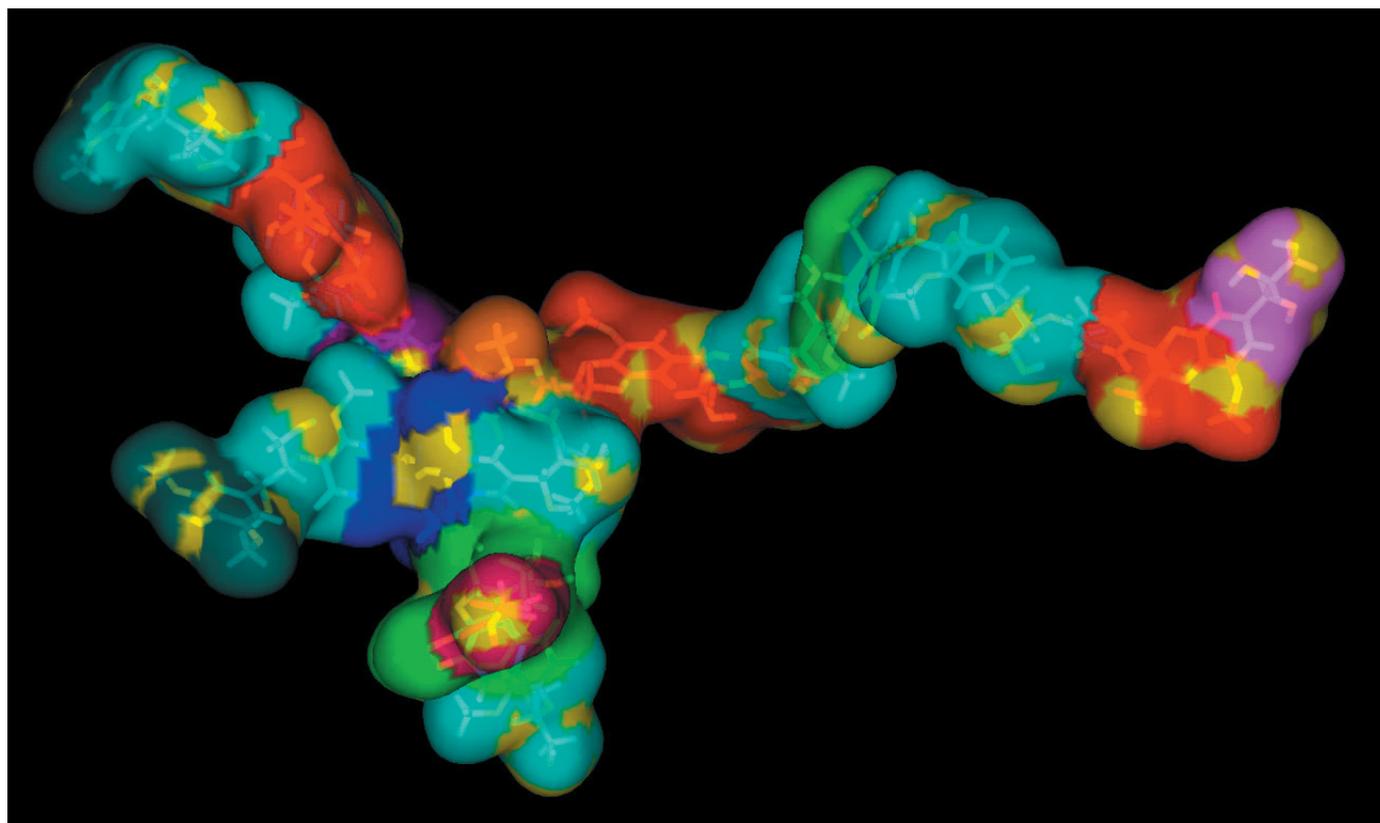
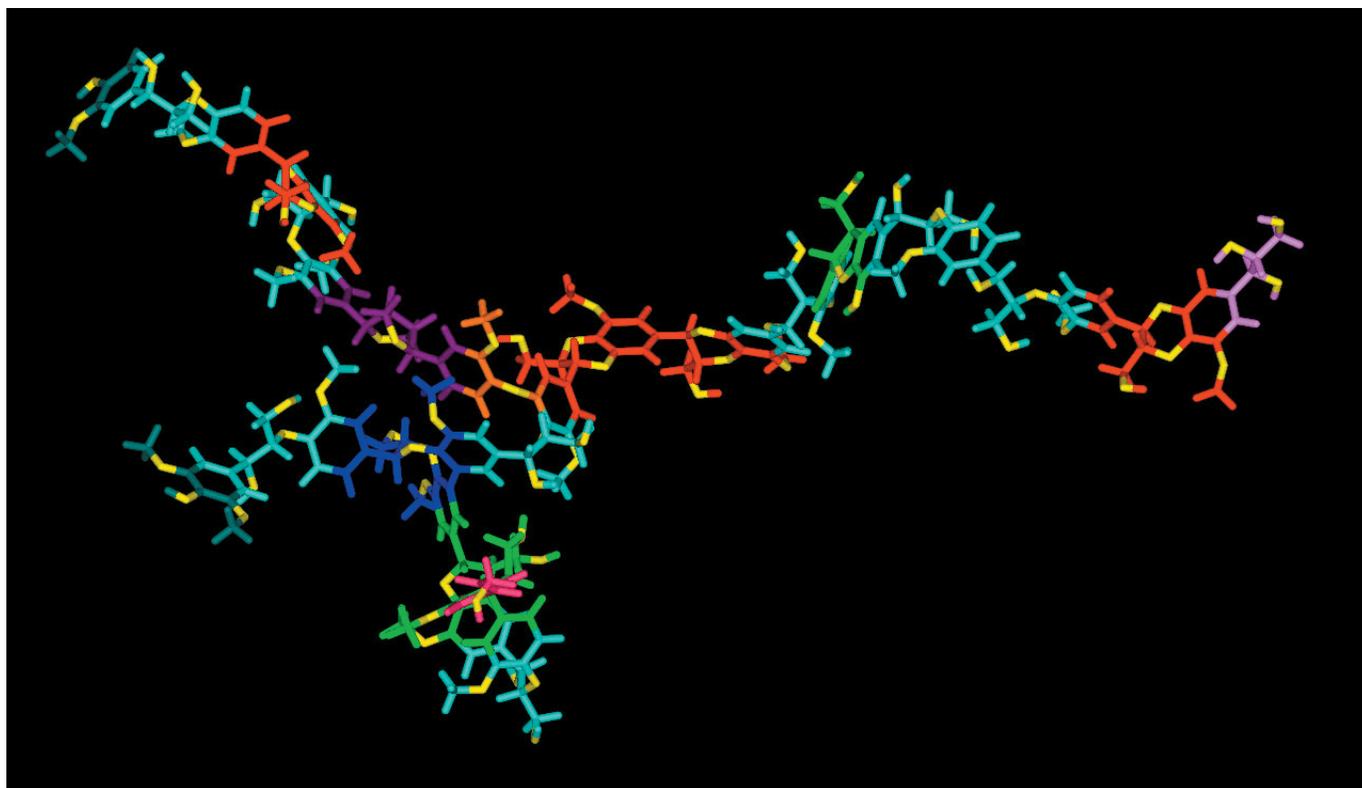
3D Model Structure: Wild-type Poplar Milled Wood Lignin



Stills are from the ViewerLite program.

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3D Model Structure: COMT-deficient Poplar Milled Wood Lignin



Stills are from the ViewerLite program.

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