

Supporting Information for:

Ring Conformational Energetics for Rhamnogalacturonan-II Monosaccharides: Implications for Structural Dynamics of Complex Carbohydrates

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Section 1: Free Energy Landscapes for All Sugars

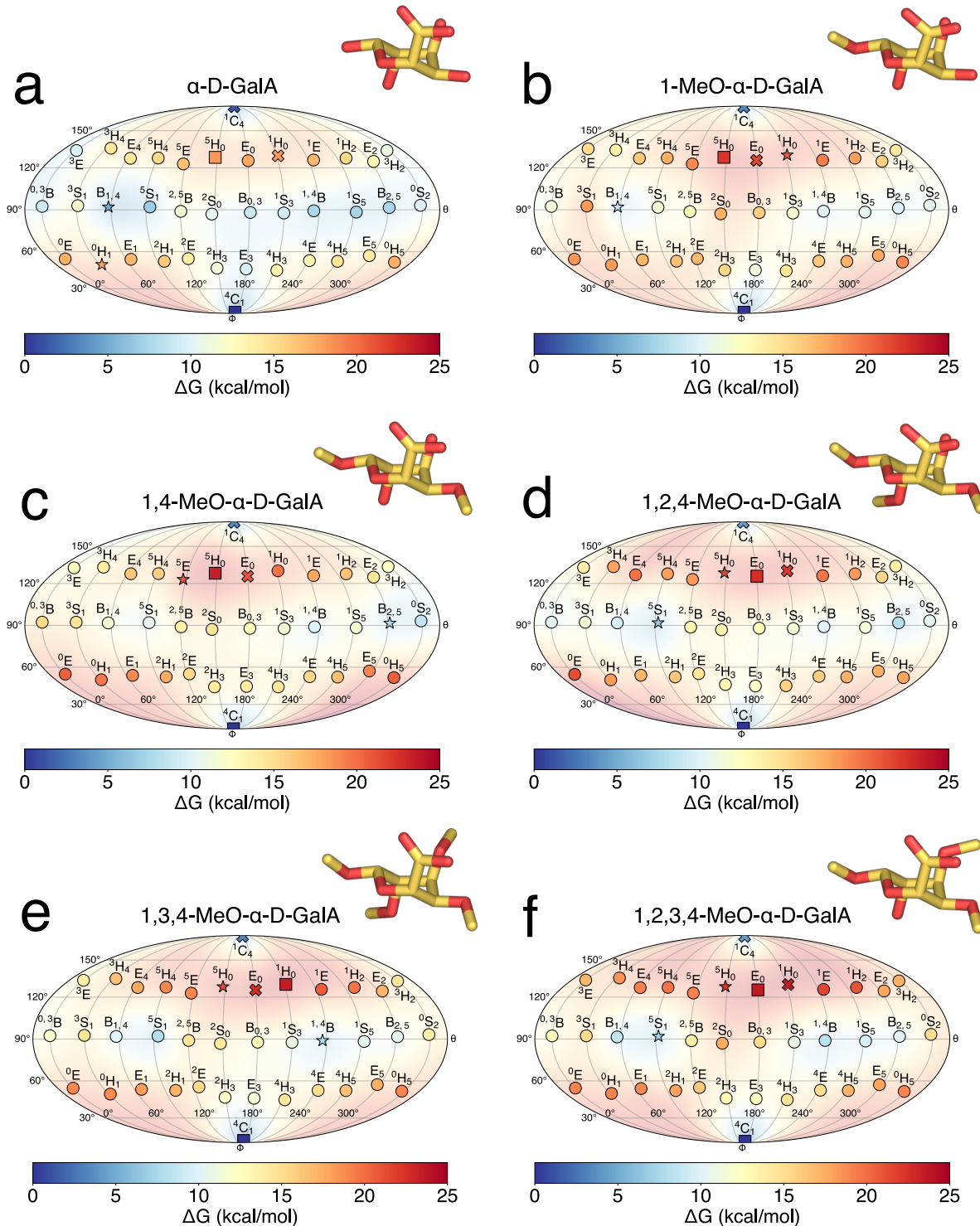


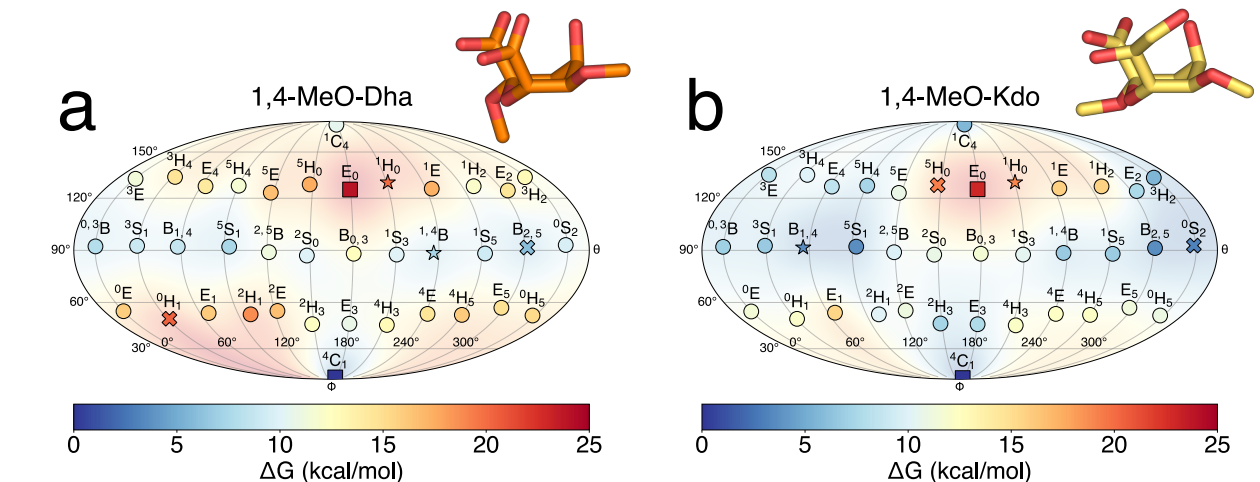
Figure 1.1. (a-f) Group 1 free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents

a 1-MeO- α -L-Arap

b 1,2,3-MeO- α -L-Arap

0 5 10 15 20 25 ΔG (kcal/mol)

0 5 10 15 20 25 ΔG (kcal/mol)



3

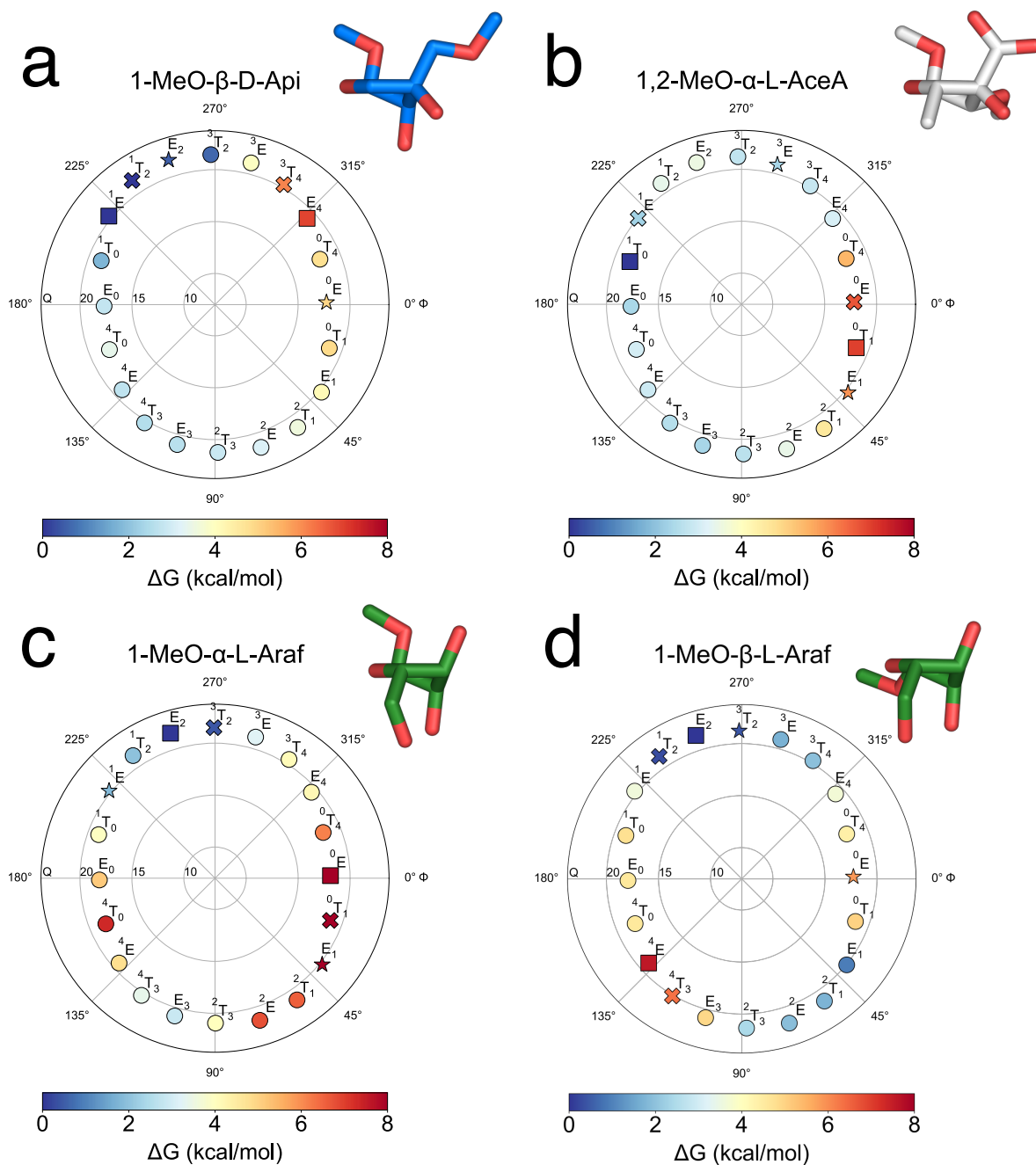
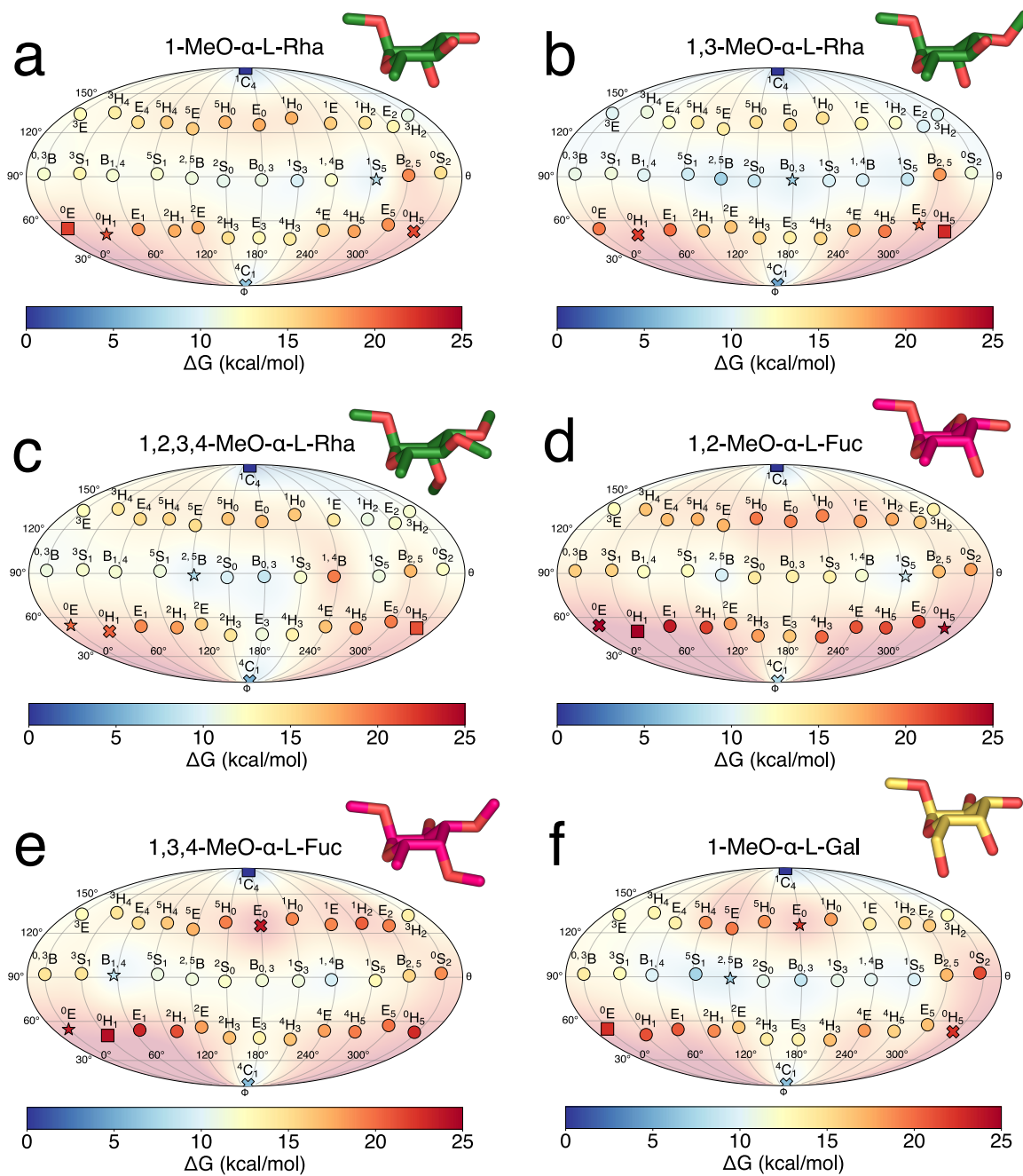


Figure 1.4. (a-d) Group 4 free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.



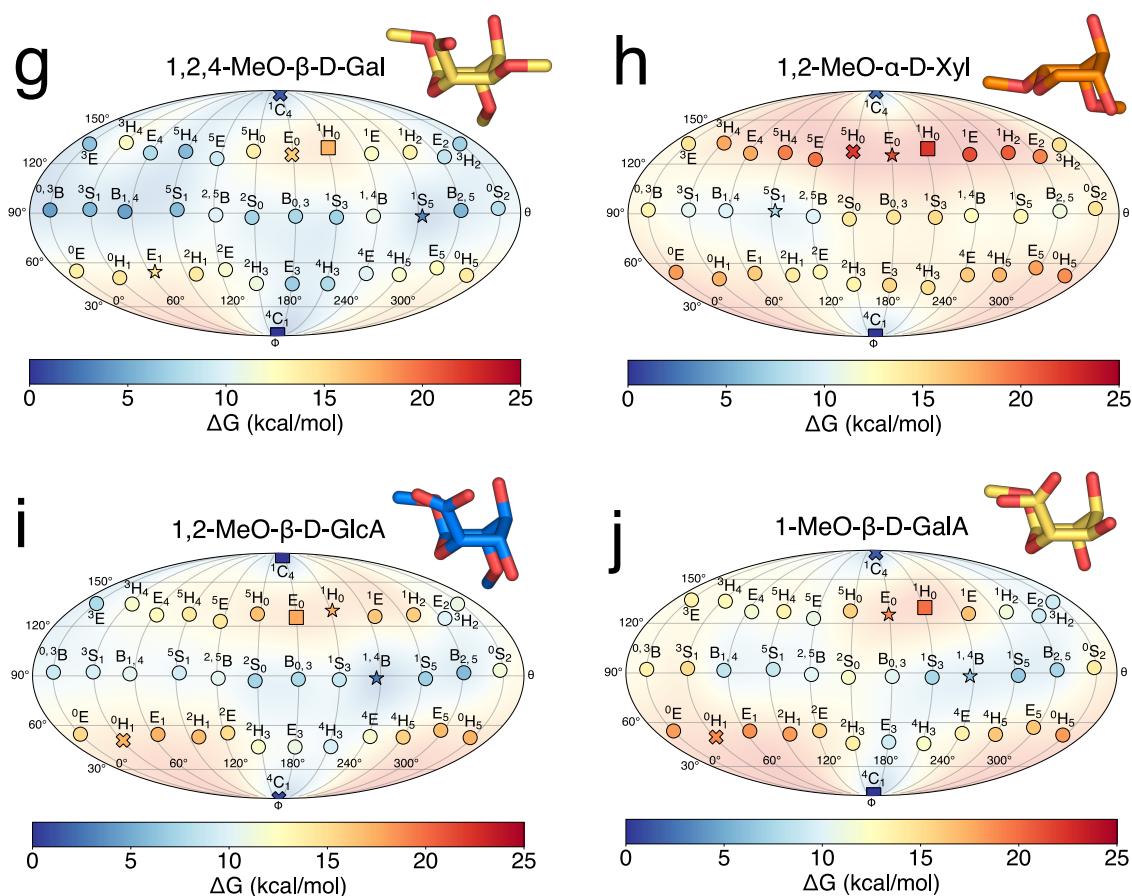


Figure 1.5. (a-j) Group 5 free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

Section 2: Enthalpy Landscapes for All Sugars

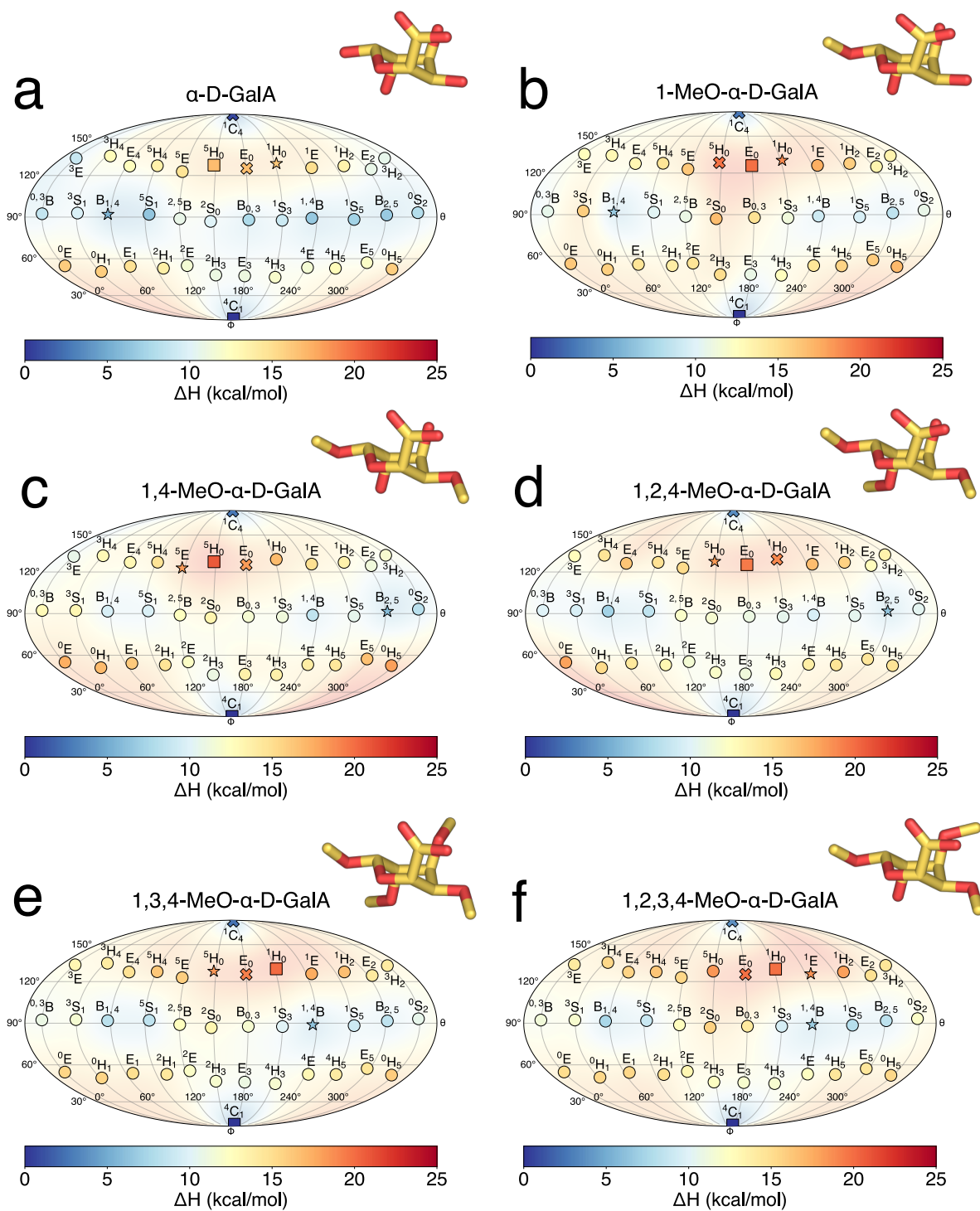


Figure 2.1. (a-f) Group 1 enthalpy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents

the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

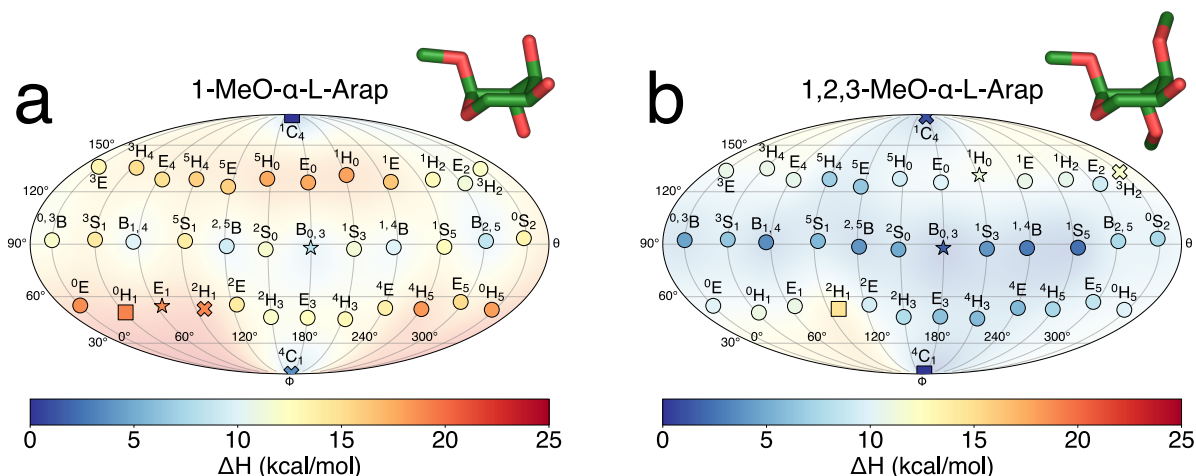


Figure 2.2. (a-b) Group 2 enthalpy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

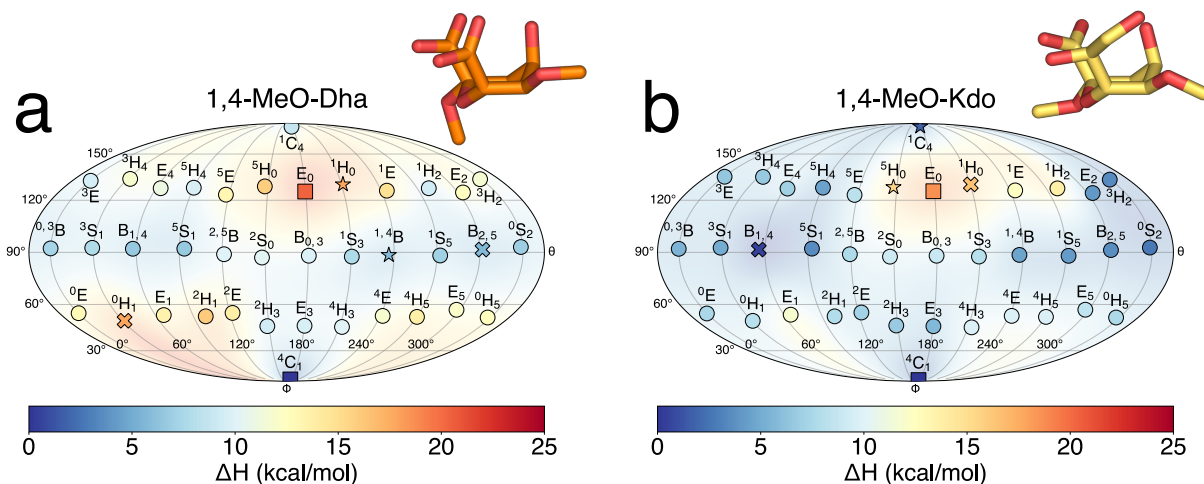


Figure 2.3. (a-b) Group 3 enthalpy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

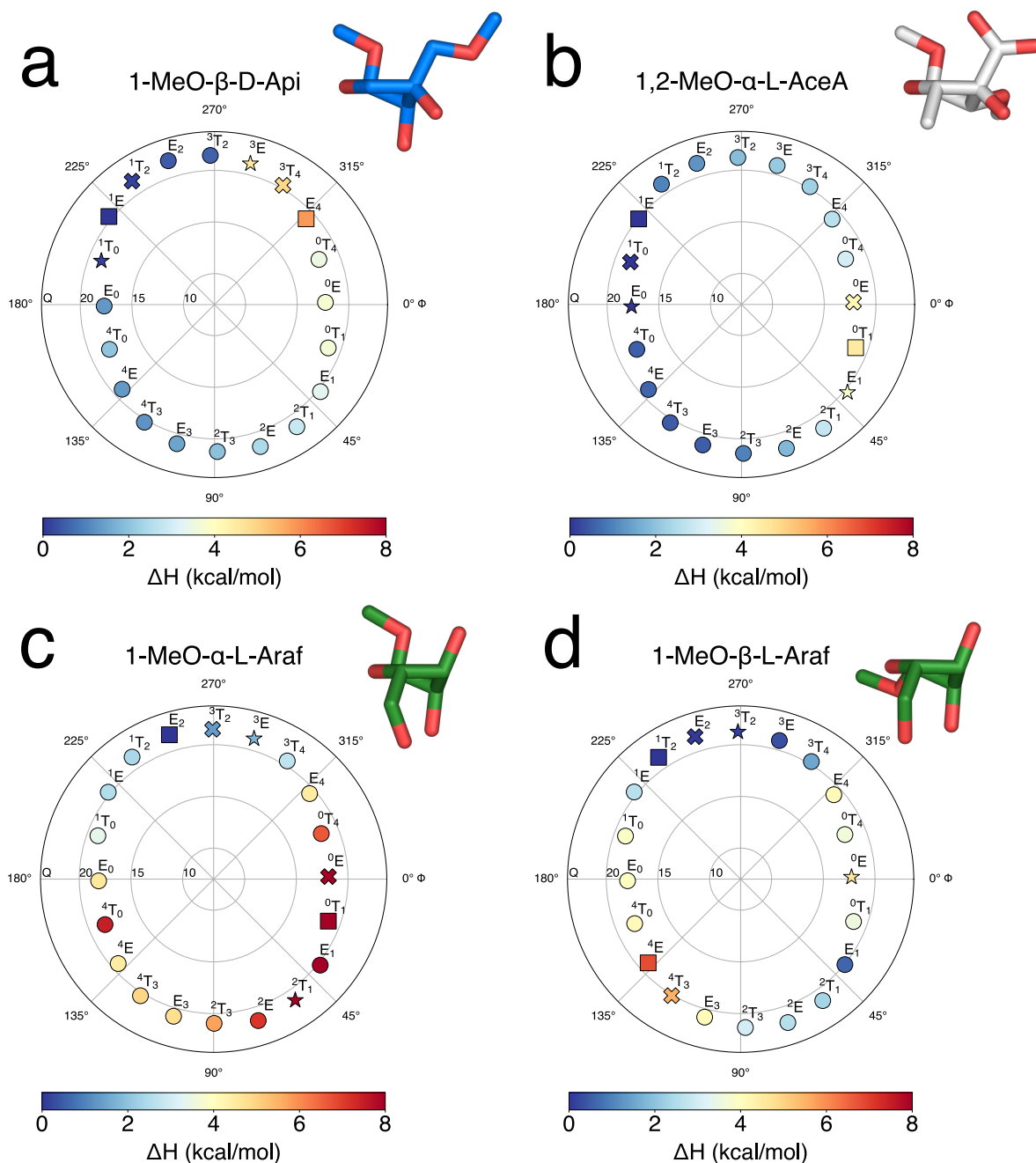
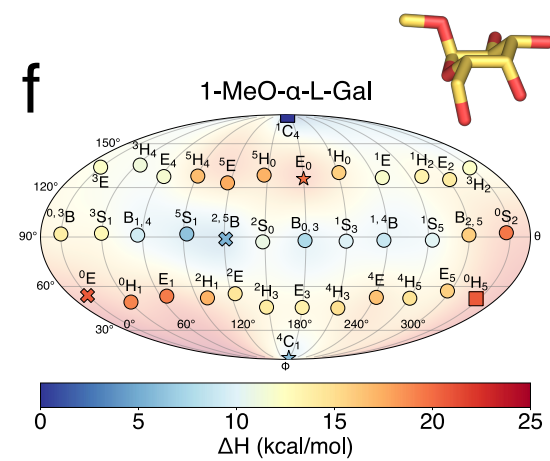
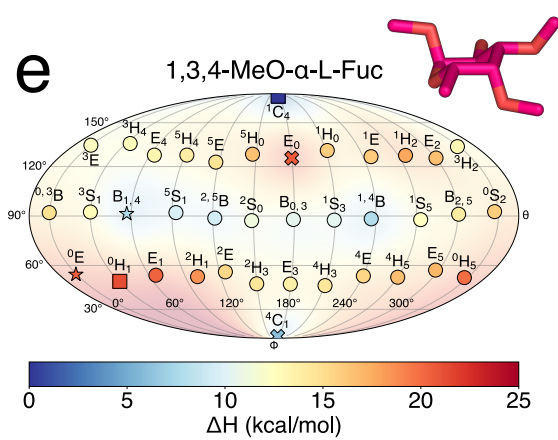
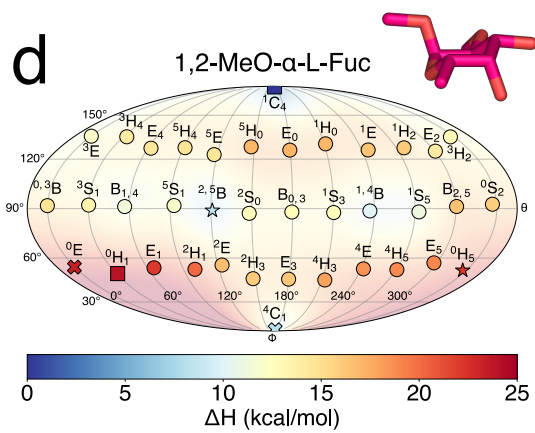
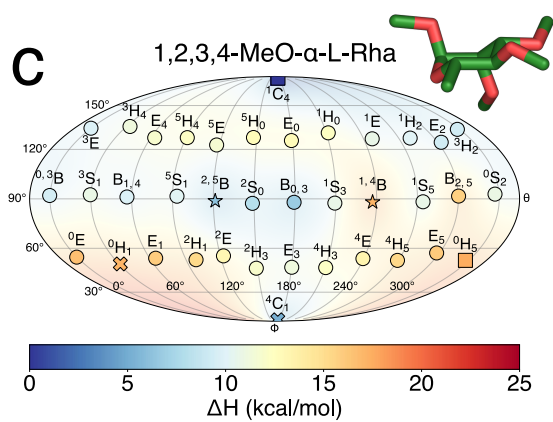
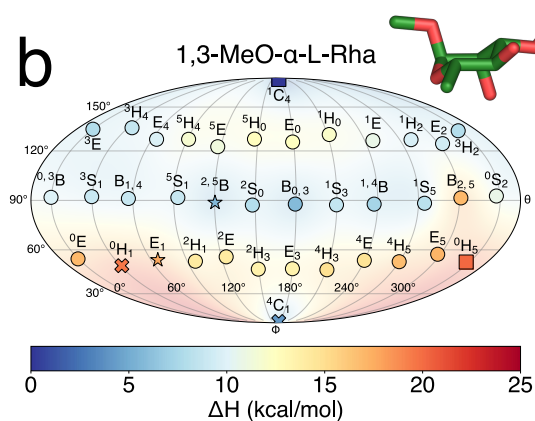
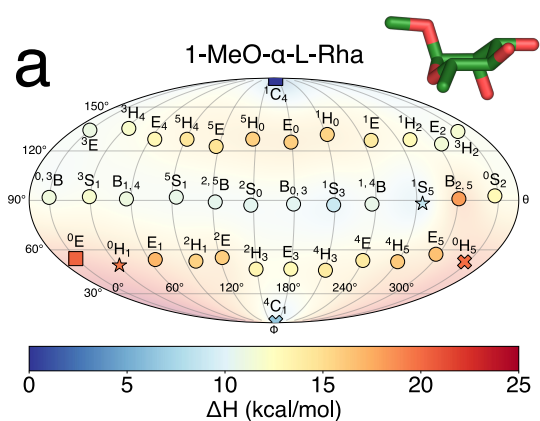


Figure 2.4. (a-d) Group 4 enthalpy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.



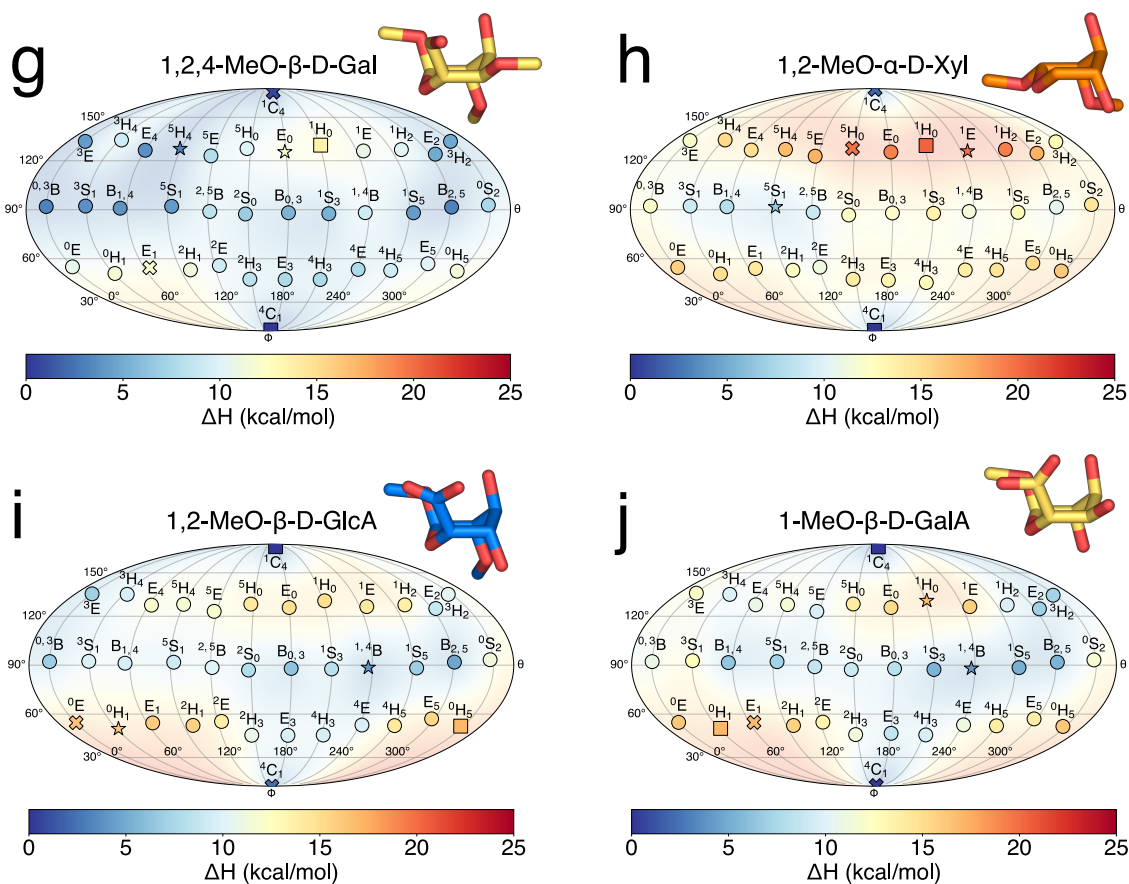


Figure 2.5. (a-j) Group 5 enthalpy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

Section 3: Zero-Point Energy (ZPE) Landscapes for All Sugars

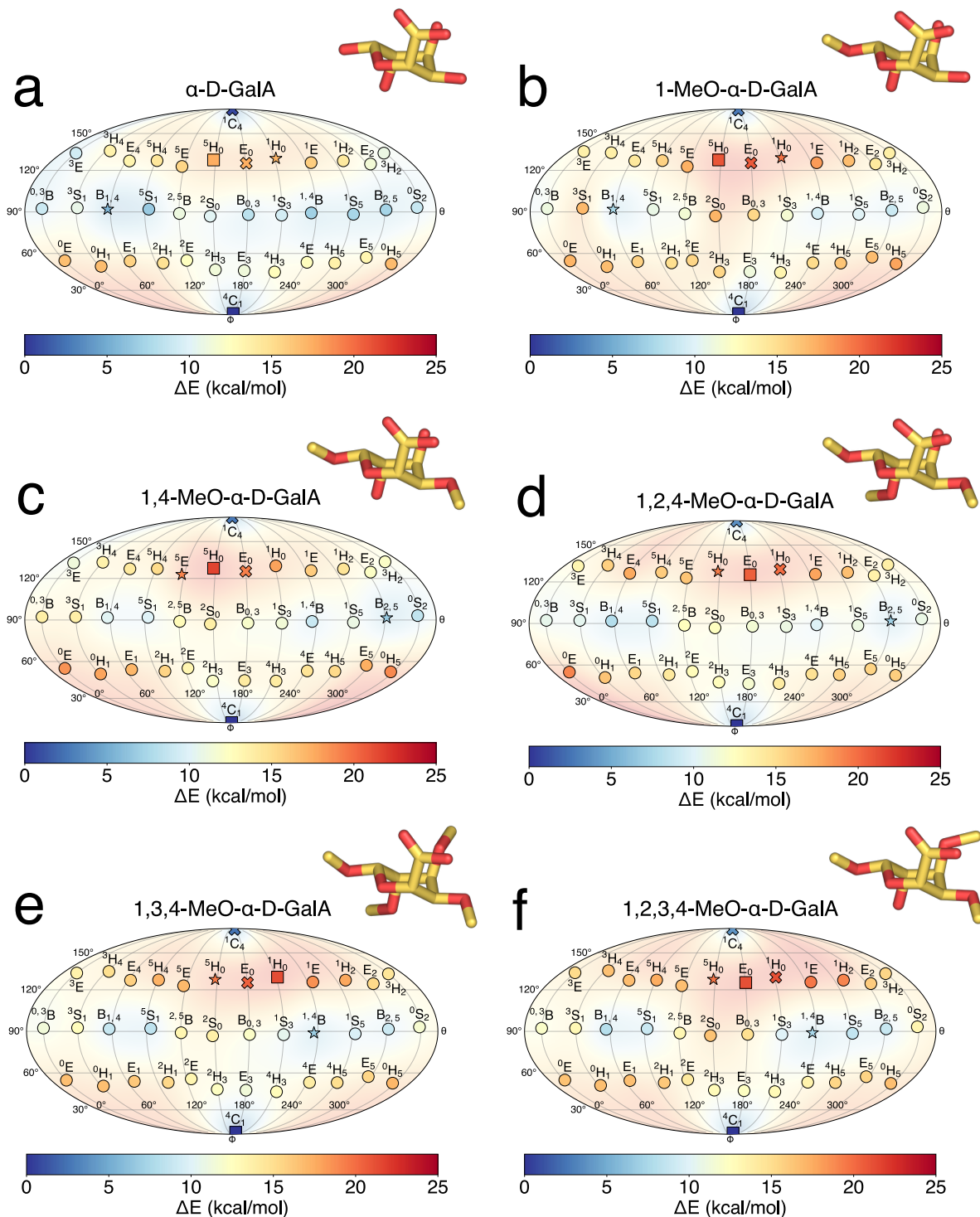


Figure 3.1. (a-f) Group 1 ZPE landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents

the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

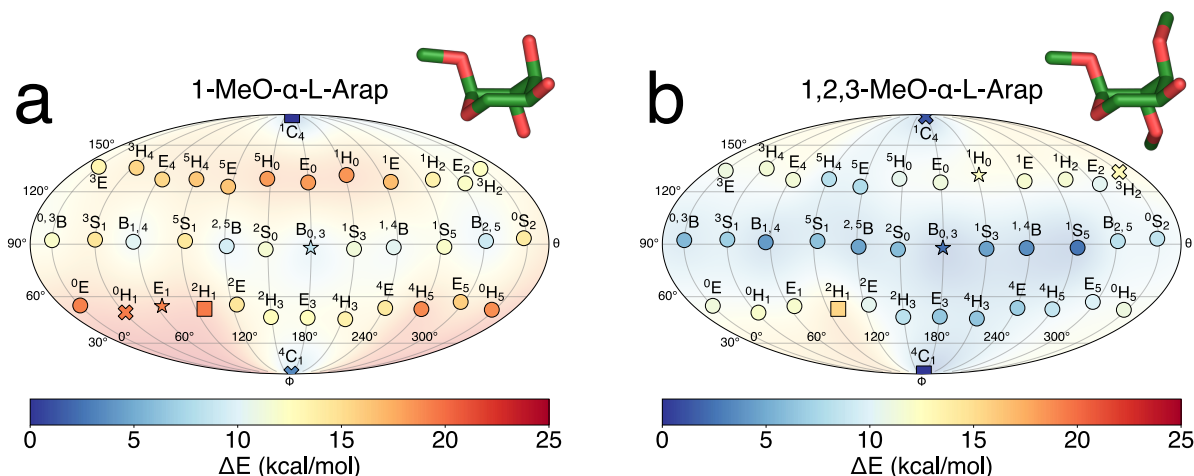


Figure 3.2. (a-b) Group 2 ZPE landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

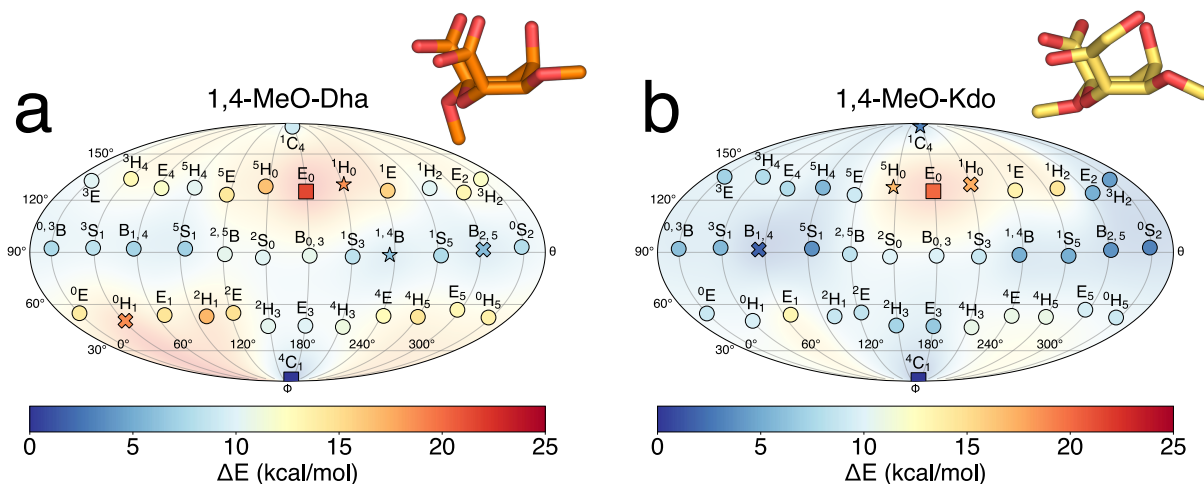


Figure 3.3. (a-b) Group 3 ZPE landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

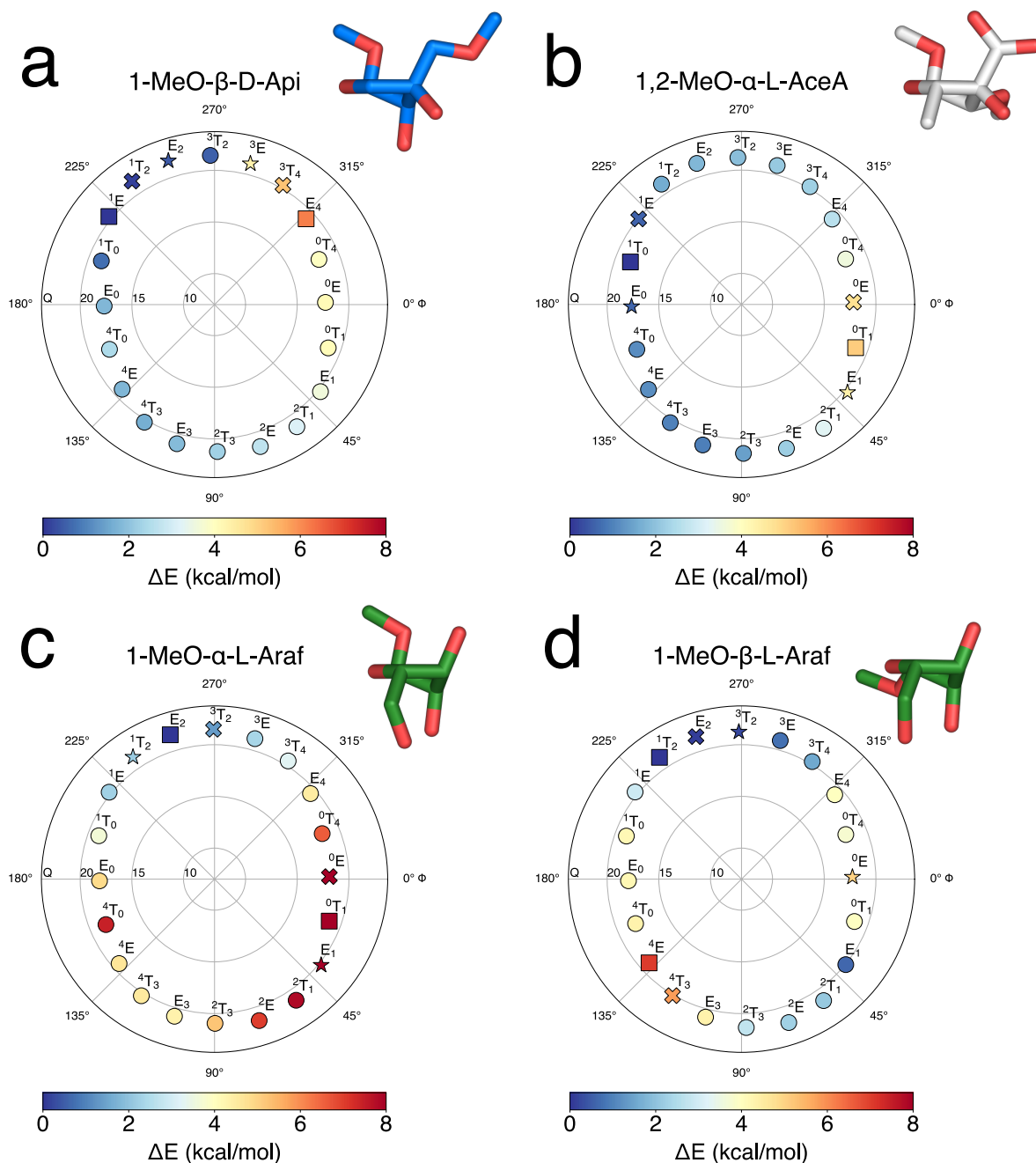
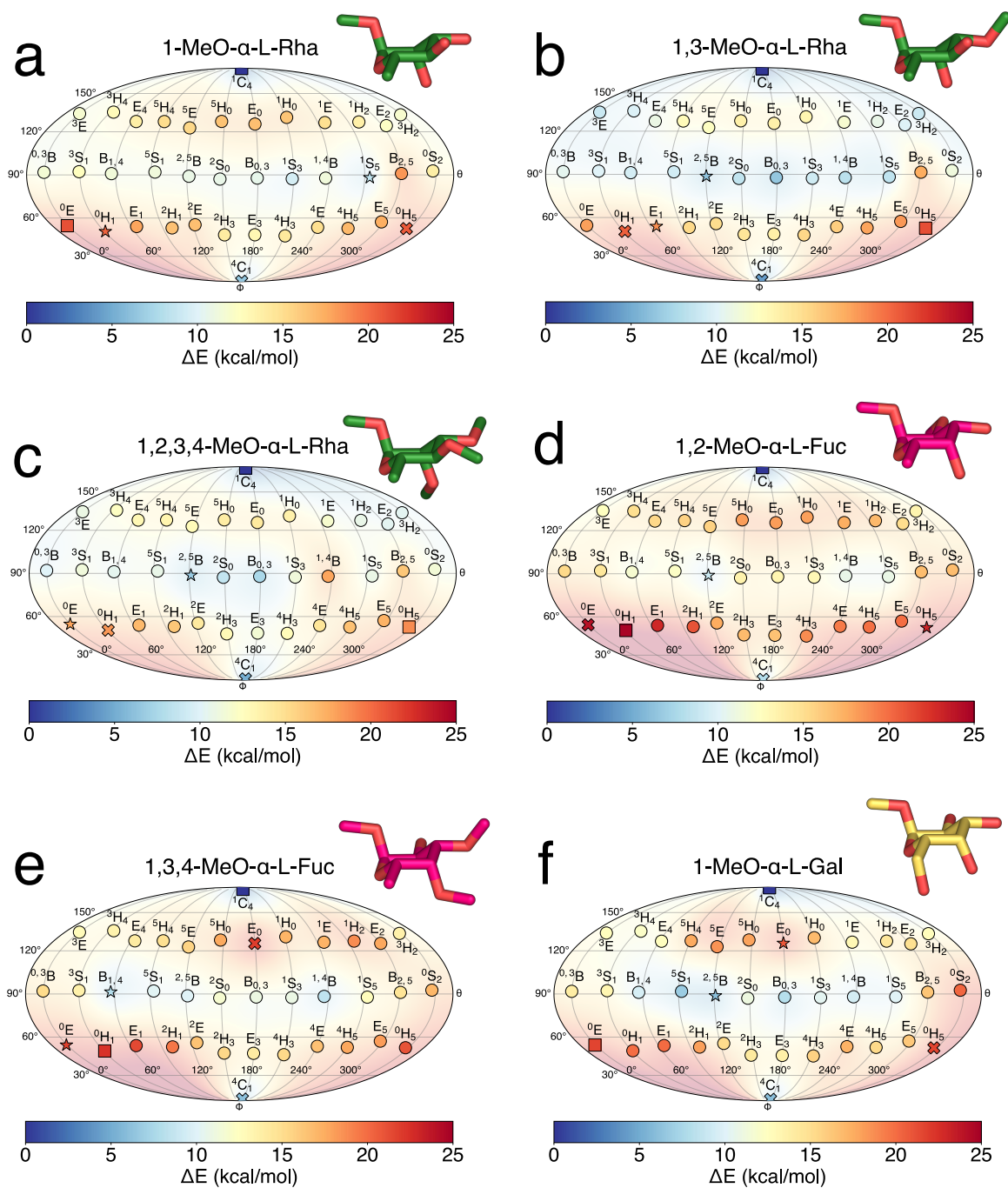


Figure 3.4. (a-d) Group 4 ZPE landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.



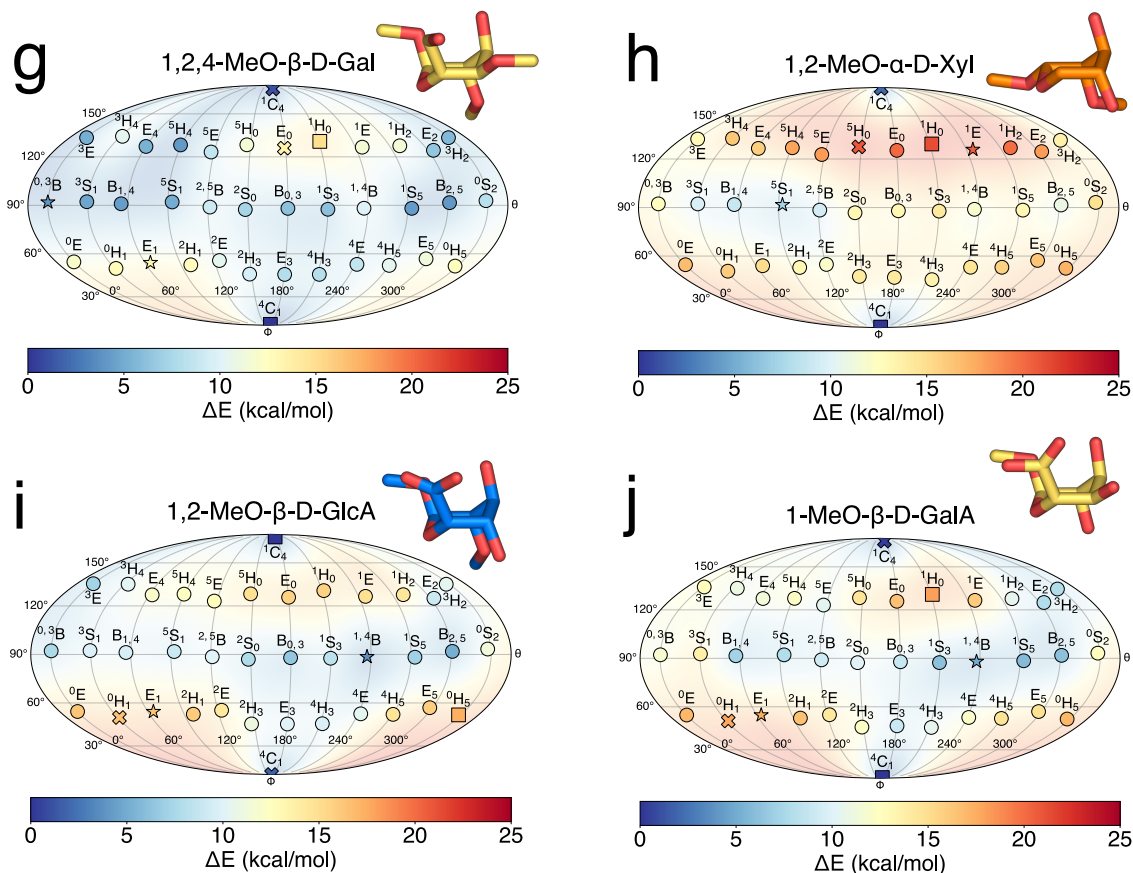


Figure 3.5. (a-j) Group 5 ZPE landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

Section 4: Electronic Energy Landscapes for All Sugars

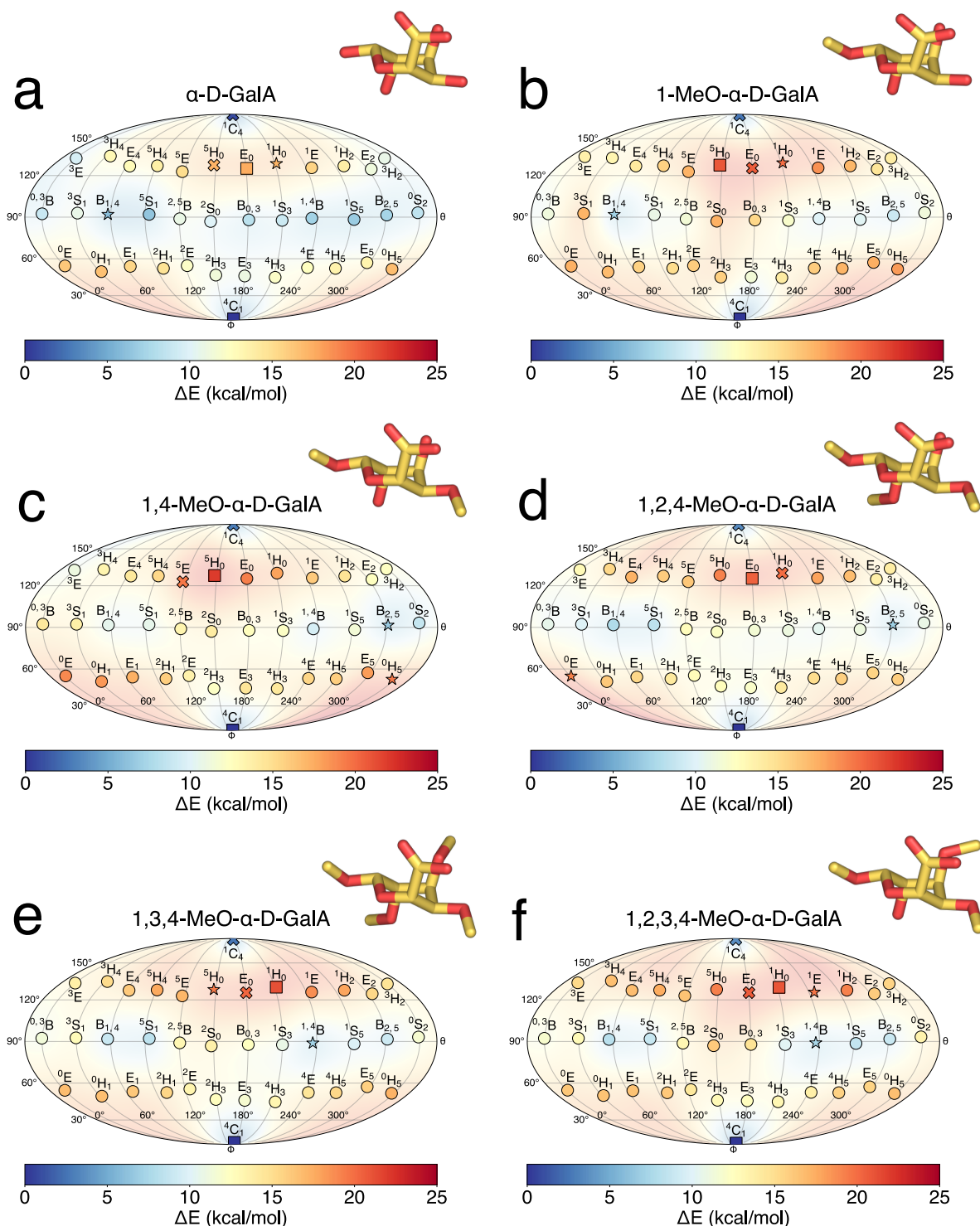


Figure 4.1. (a-f) Group 1 electronic energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents

the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

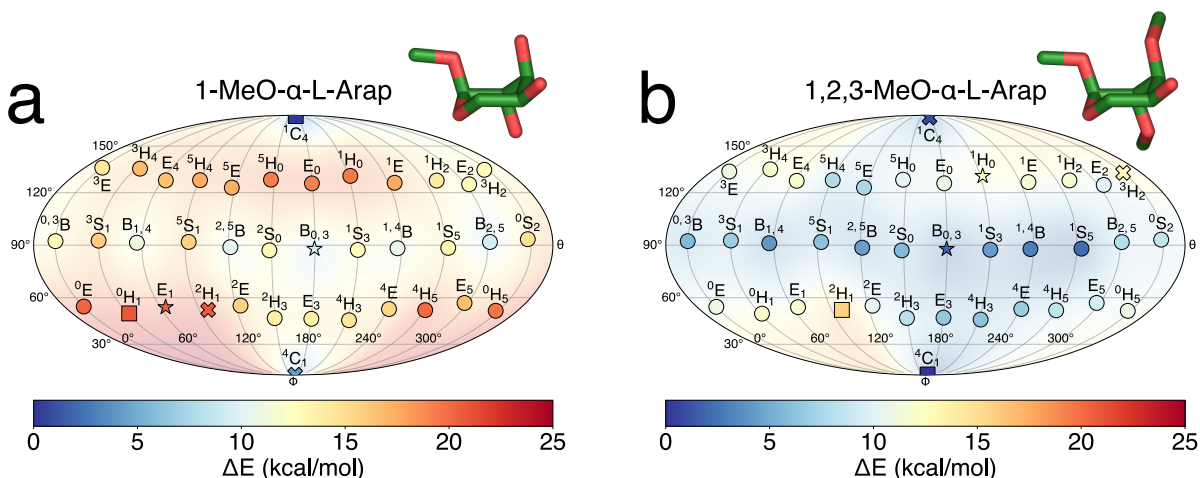


Figure 4.2. (a-b) Group 2 electronic energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

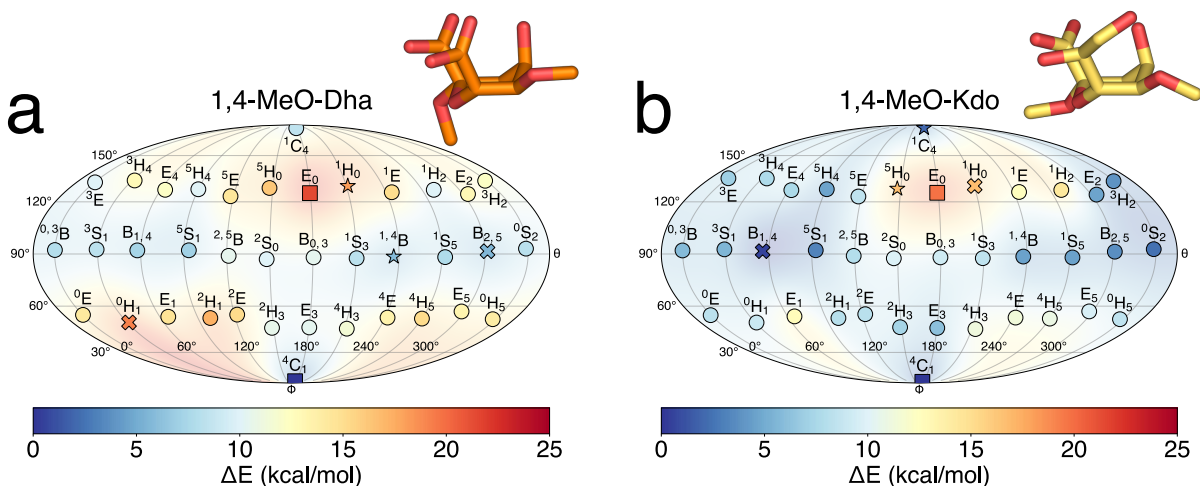


Figure 4.3. (a-b) Group 3 electronic energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

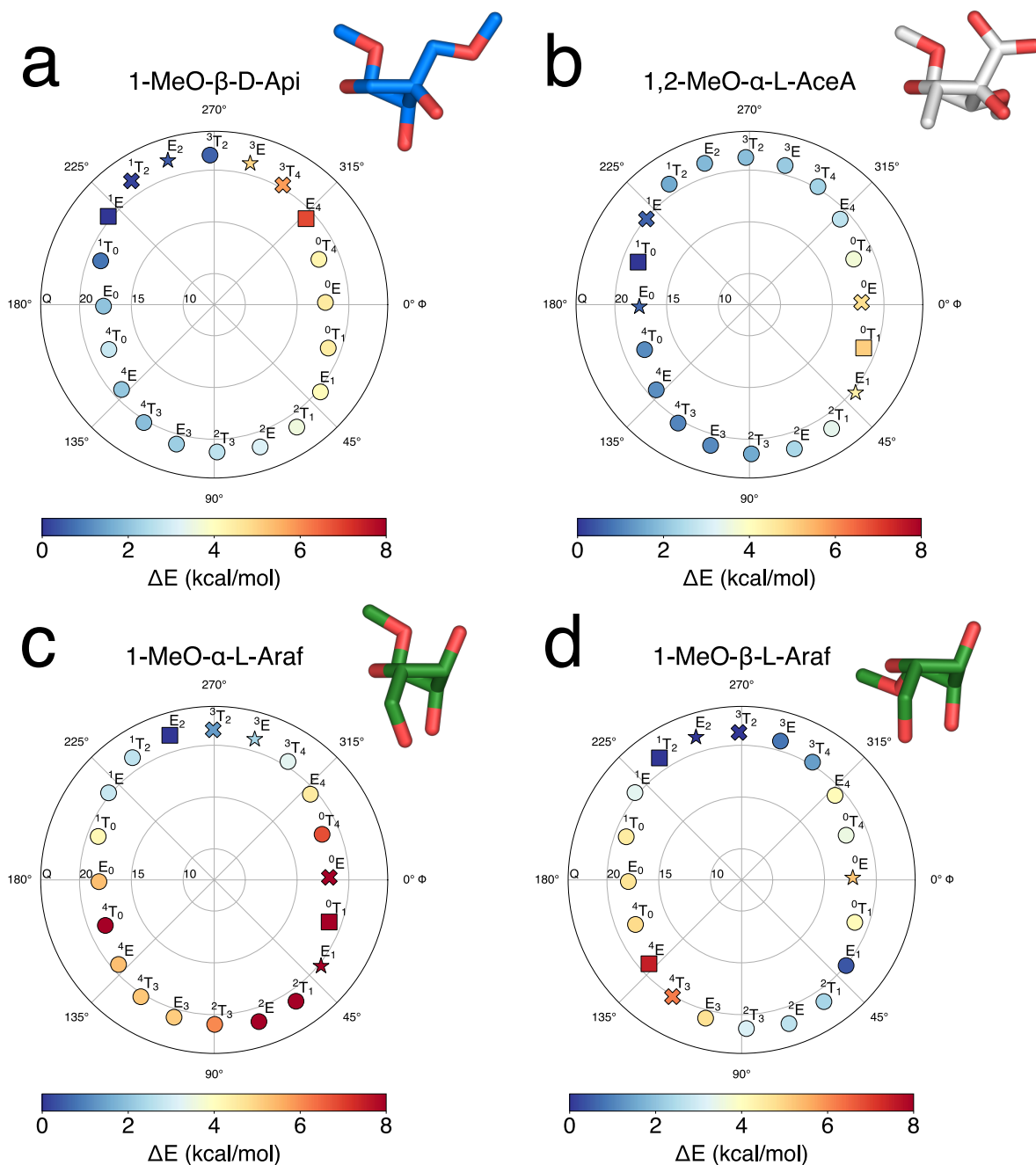
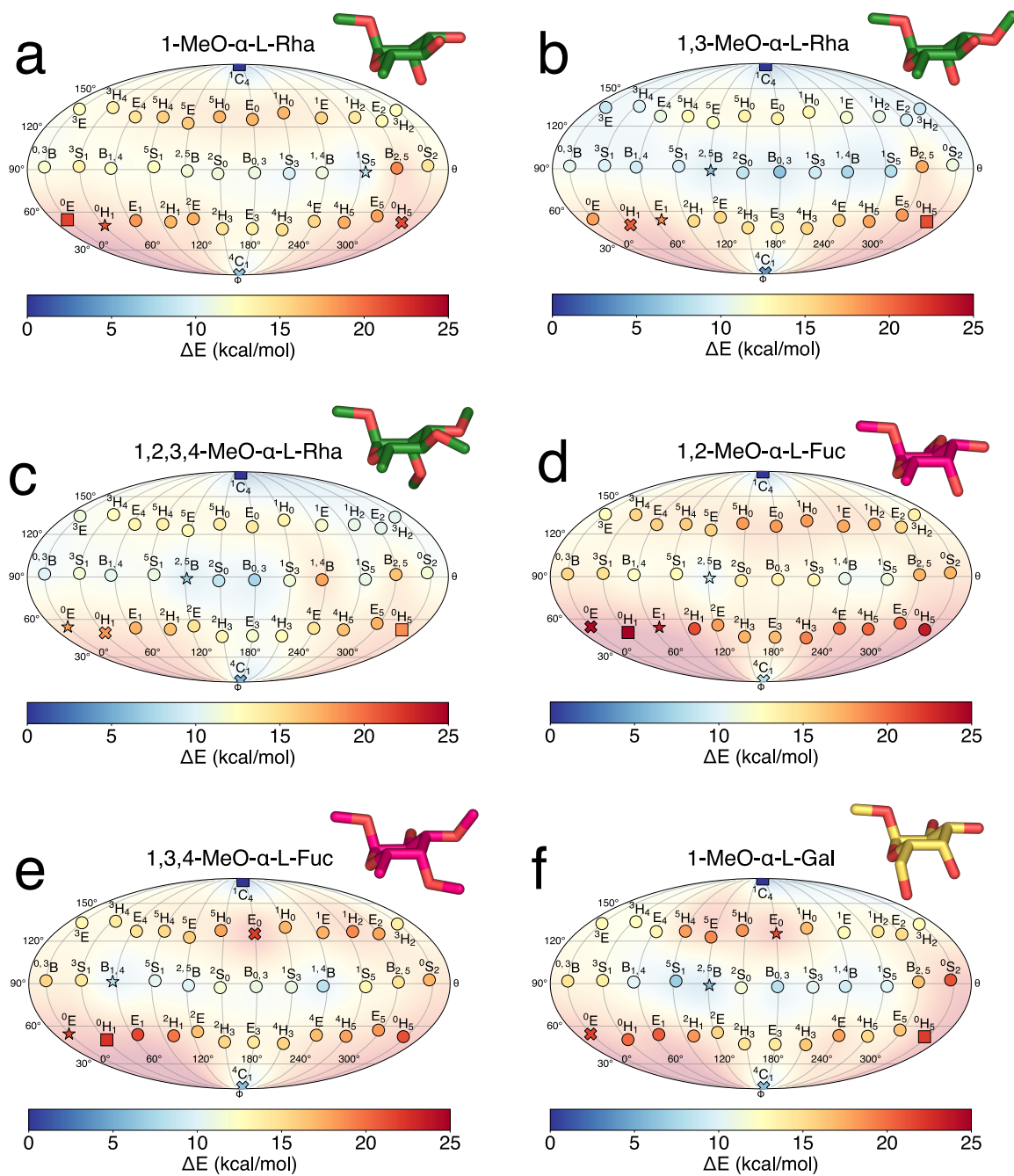


Figure 4.4. (a-d) Group 4 electronic energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.



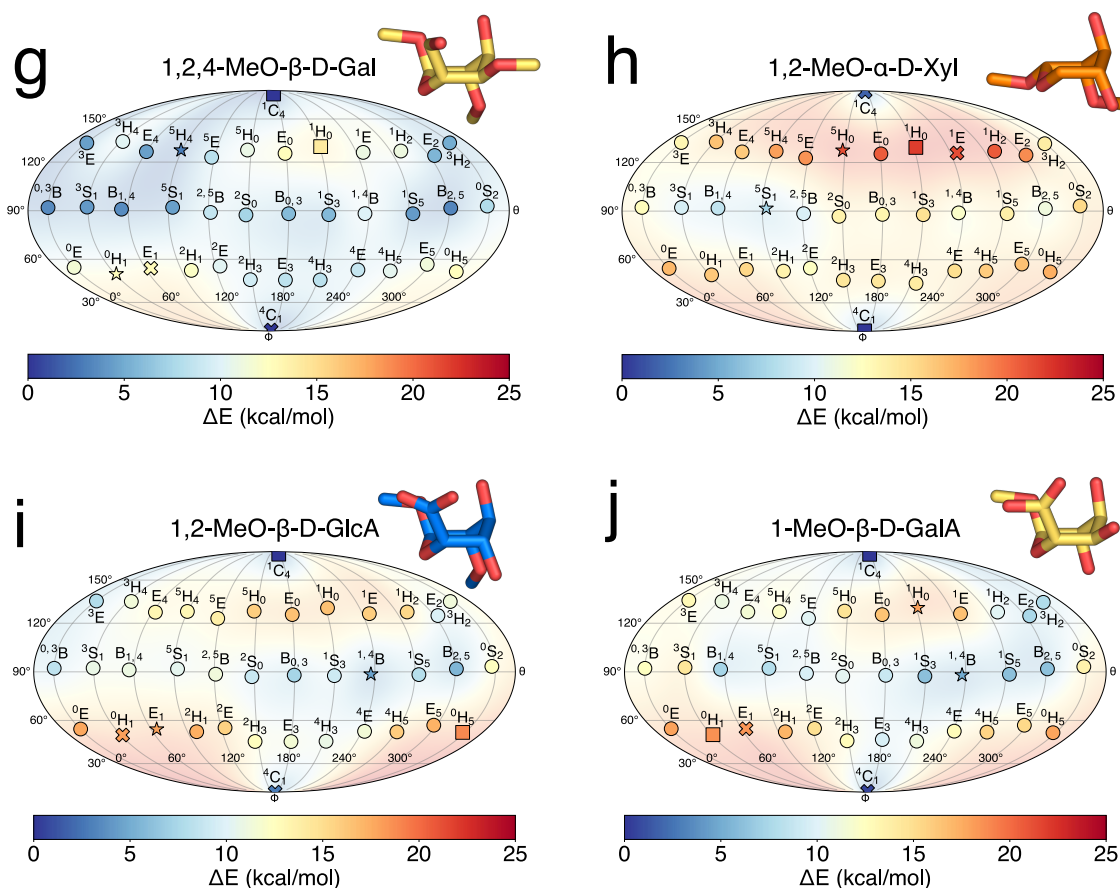


Figure 4.5. (a-j) Group 5 electronic energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

Section 5: Unconstrained Optimizations for All Sugars in RG-II

In addition to the constrained optimizations presented in the main text, fully unconstrained optimizations were conducted for all pucker states across all sugars and their respective variants. These unconstrained optimizations were conducted to verify the accuracy of the constrained optimizations. Dihedral angle constraints that were placed on the ring for all constrained optimizations, which caused the Q value to remain relatively fixed, were lifted to ensure a fully relaxed optimization. Unconstrained optimizations were therefore conducted to ensure that stable and metastable pucker states identified during the constrained energy optimizations are structurally valid. If higher energy pucker states collapse primarily to stable or metastable regions identified in the constrained optimizations, then it can be reasonably concluded that the constrained optimizations are able to reproduce physically valid energy minima across the puckering landscape. Unconstrained calculations were initiated with a fully unconstrained geometry optimization lifting all ring dihedral constraints. The starting structure was chosen as the final structure obtained from the constrained optimization to clearly show the transition. Next, a frequency calculation was conducted to ensure the absence of imaginary frequencies. All unconstrained optimizations were conducted at the MP2/cc-PVDZ level of theory to enable direct comparison with the constrained optimizations. In each of the plots, a black line is drawn to connect the geometry obtained from each constrained optimization (small black dot) to the corresponding geometry obtained from each unconstrained optimization (large green dot). The line does not represent the exact Φ and θ values of the intermediate geometries obtained during the optimization but is rather included to conveniently connect each constrained optimization to its corresponding unconstrained optimization. Geometries that would not converge at the MP2/cc-PVDZ level were optimized at the MP2/6-311g(d) level and are marked with an asterisk next to the starting point (black dot) in the corresponding figures.

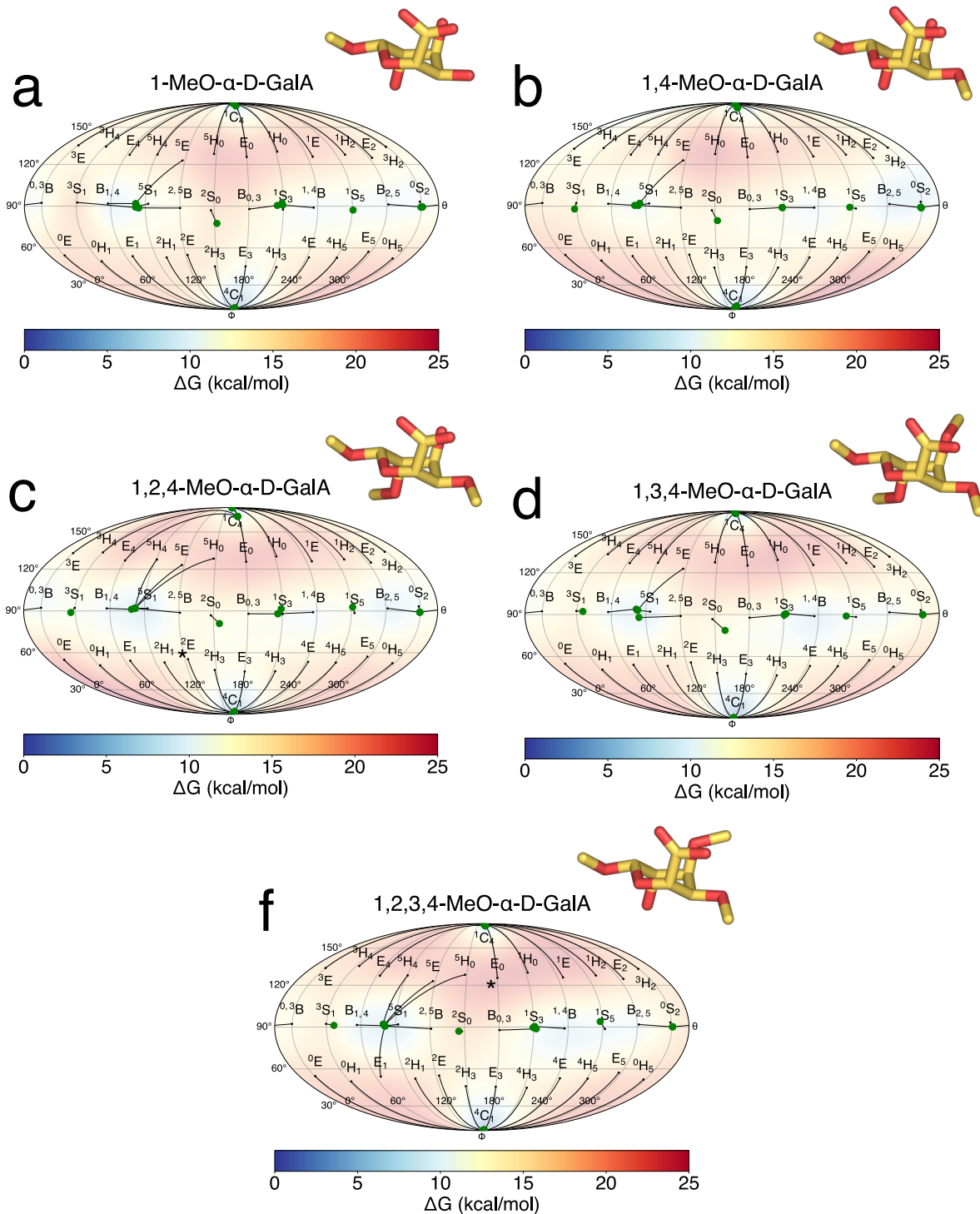


Figure 5.1. (a-f) Group 1 unconstrained free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

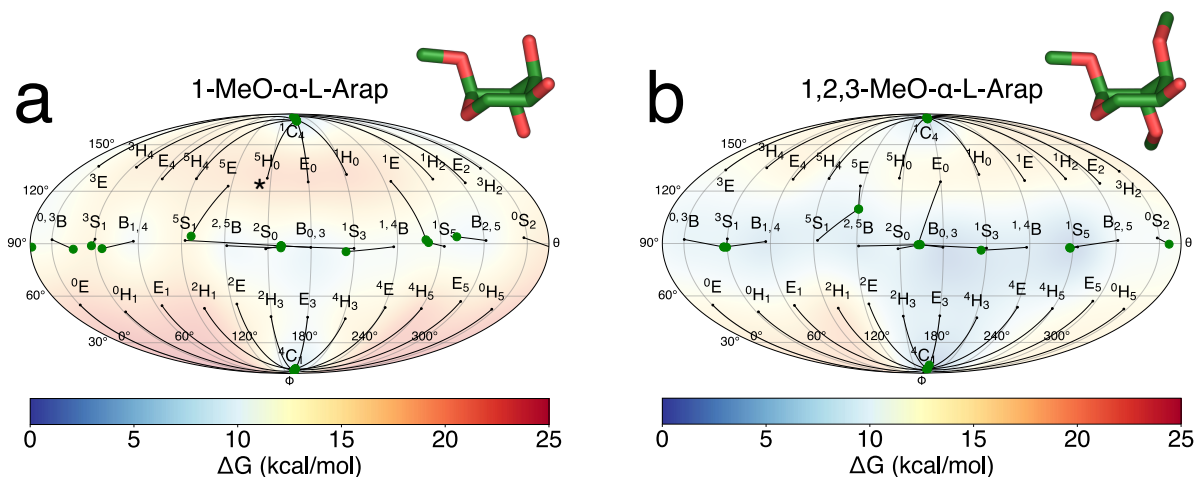


Figure 5.2. (a-b) Group 2 unconstrained free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

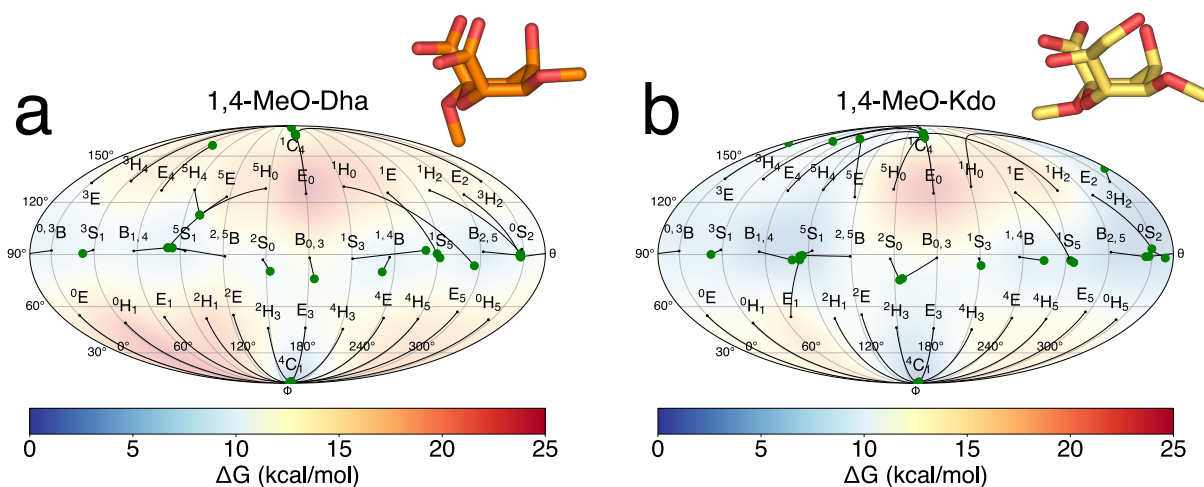


Figure 5.3. (a-b) Group 3 unconstrained free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

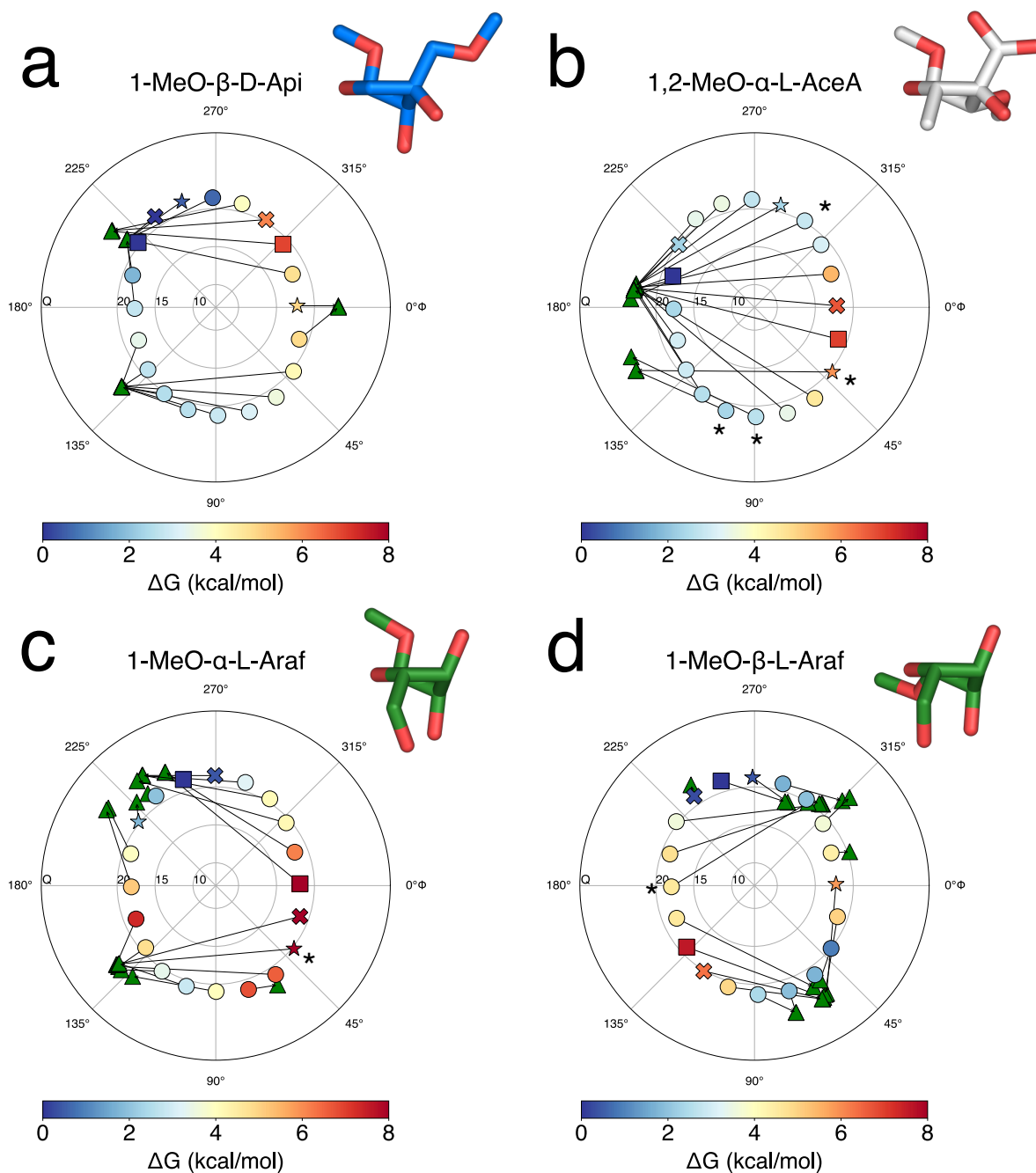
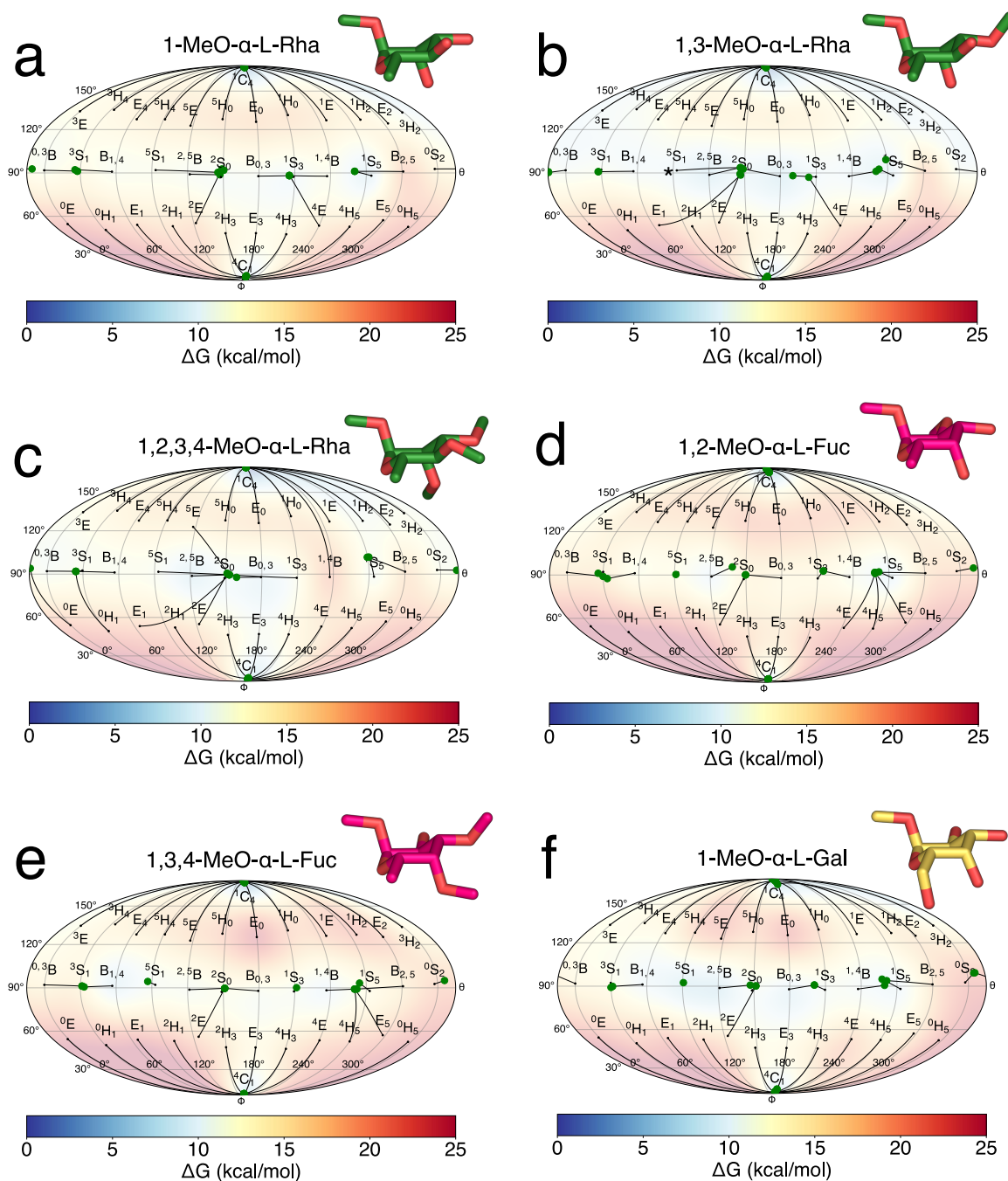


Figure 5.4. (a-d) Group 4 unconstrained free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.



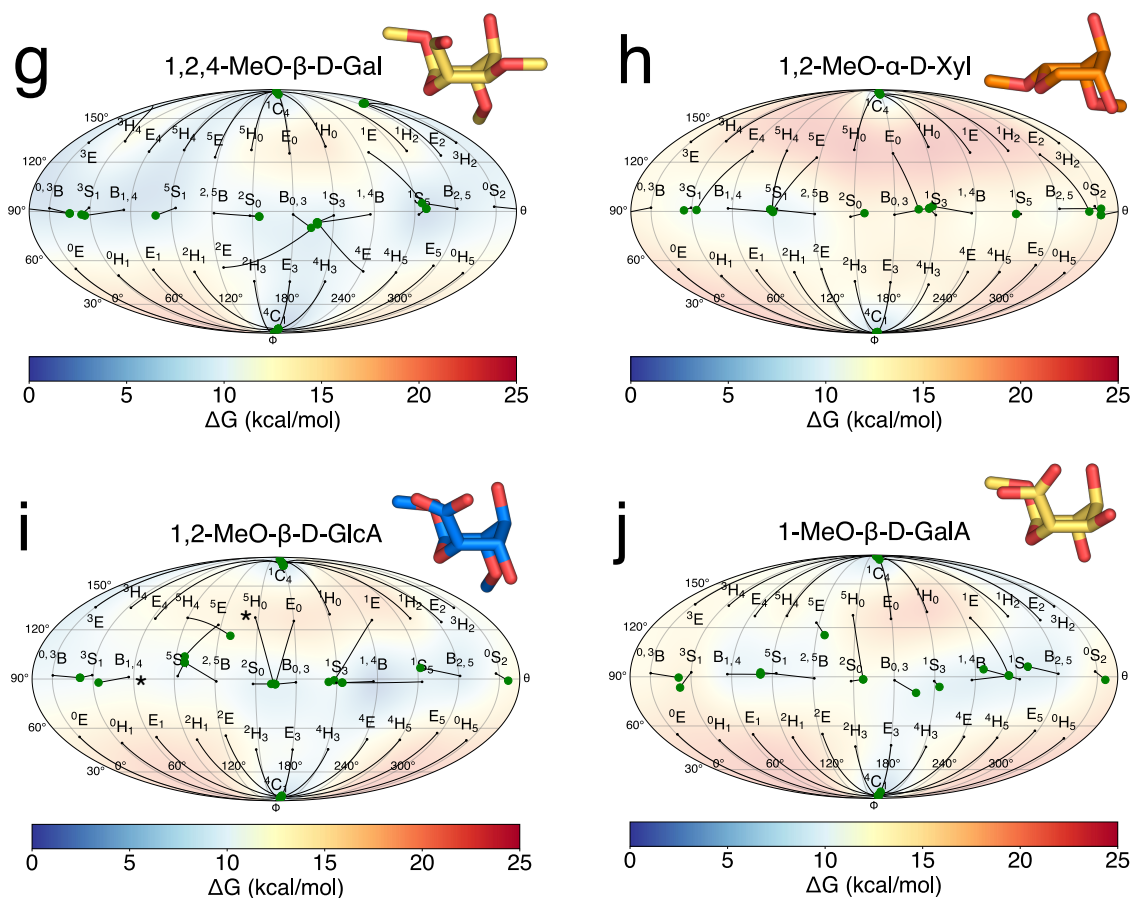


Figure 5.5. (a-j) Group 5 unconstrained free energy landscapes. The three special markers on each plot are as follows: (1) square – represents the lowest or highest energy pucker state, (2) cross – represents the second lowest or second highest energy pucker state, and (3) star – represents the third lowest or third highest energy pucker state. All free energies are reported relative to the lowest energy pucker state for that respective sugar.

Section 6: Minimum energy transition pathways

Lowest energy pucker states in the northern, equatorial, and southern regions are plotted along with the 1C_4 and 4C_1 chair for every pyranose sugar to represent the hypothetical lowest energy ring interconversion pathway from 1C_4 to 4C_1 . From left to right, the points are: (1) 1C_4 chair, (2) CP-northern minimum energy barrier, (3) CP-equatorial minimum energy barrier, (4) CP-southern minimum energy barrier, and (5) 4C_1

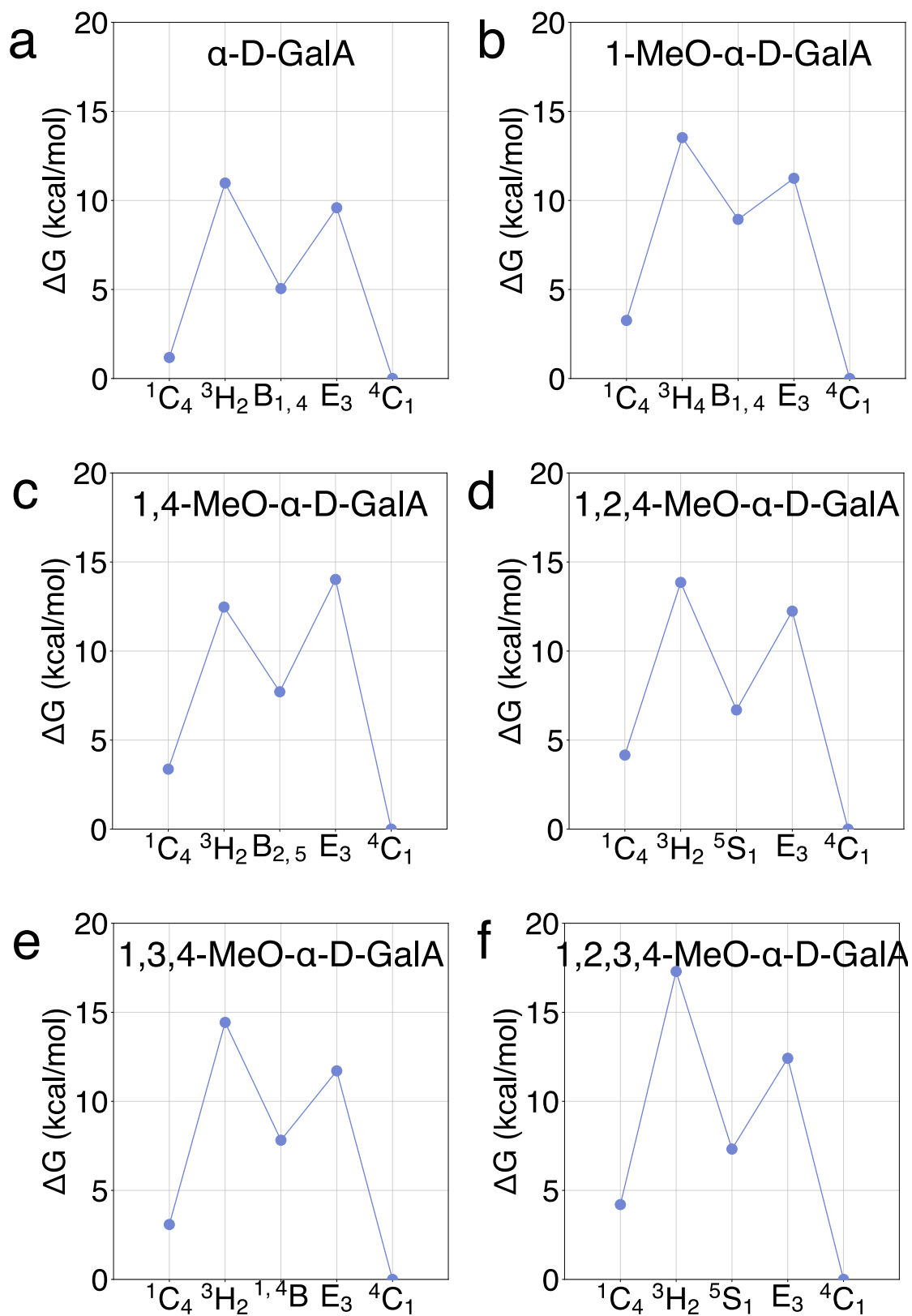


Figure 6.1. (a-f) Group 1 lowest energy ring interconversion pathways.

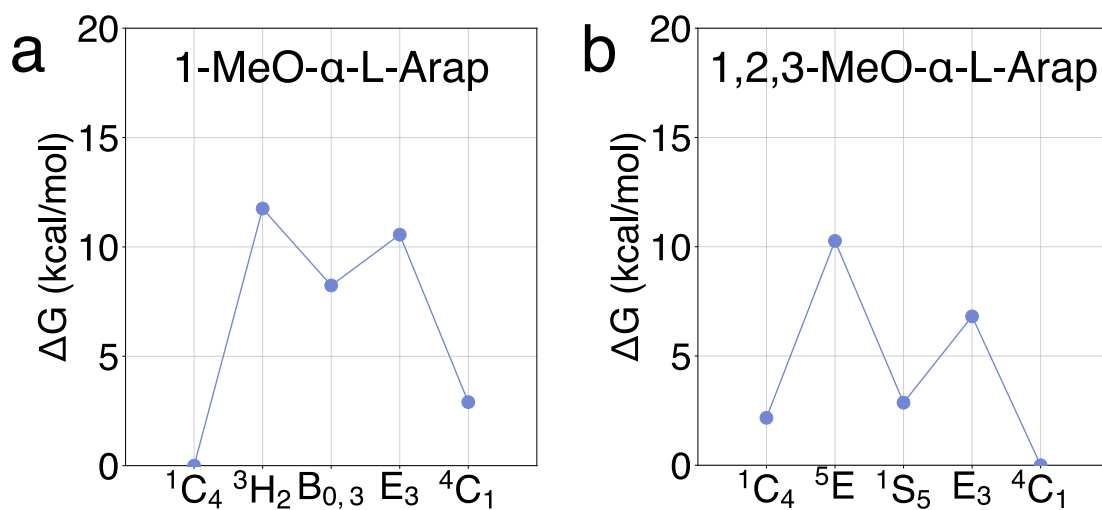


Figure 6.2. (a-b) Group 2 lowest energy ring interconversion pathways.

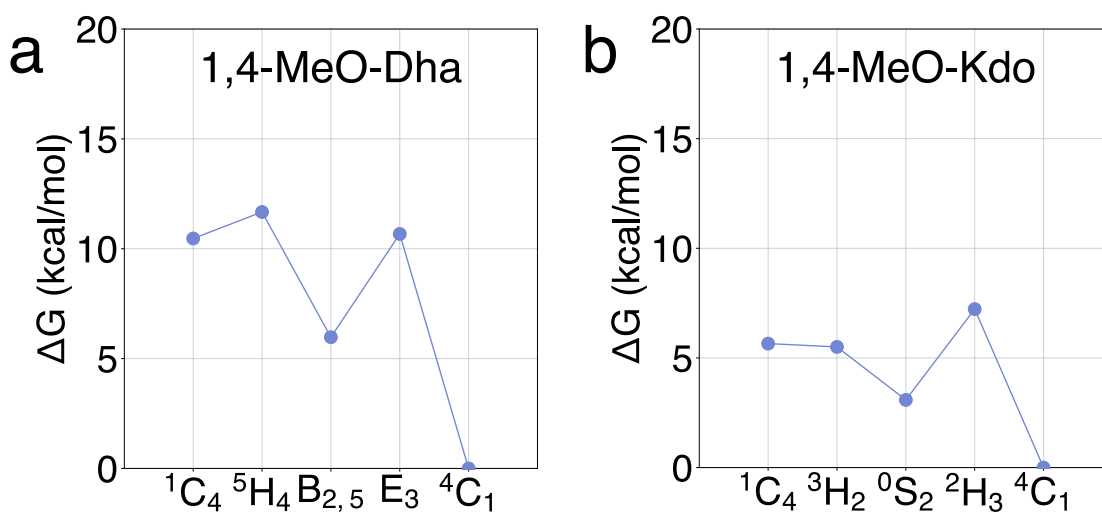
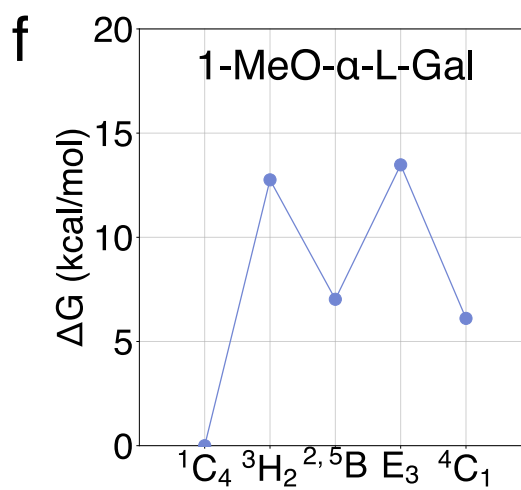
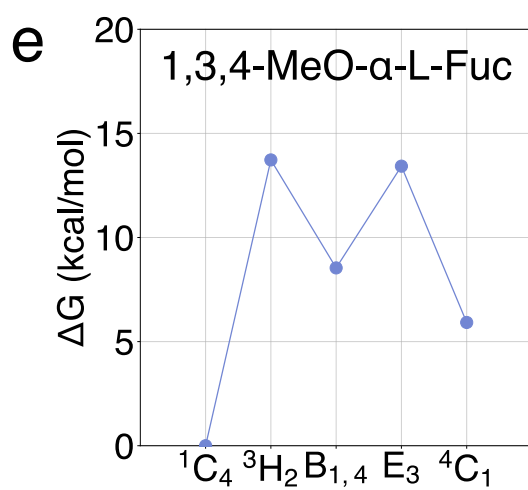
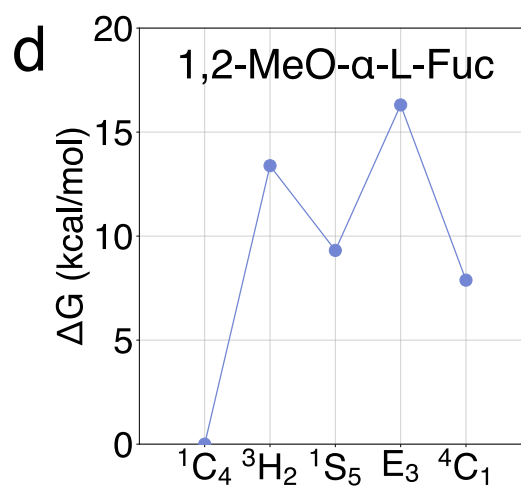
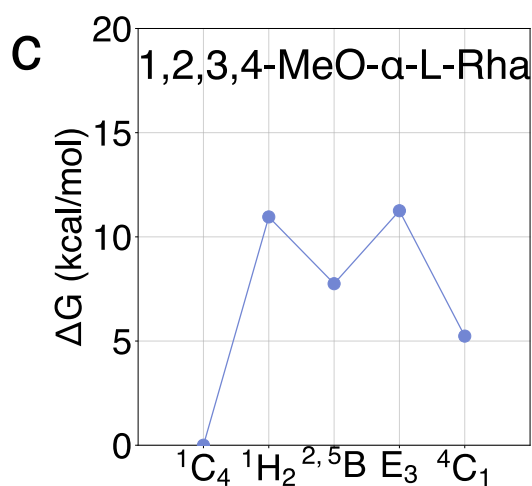
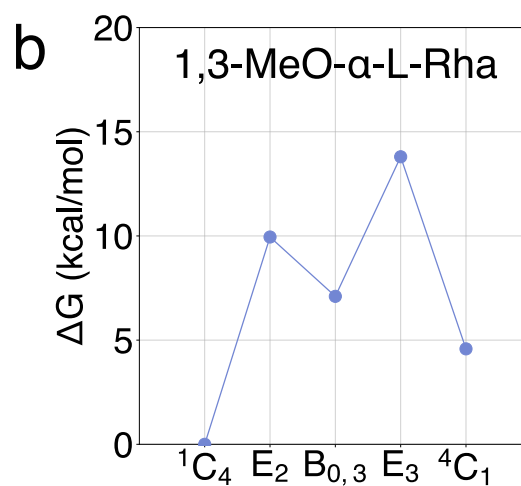
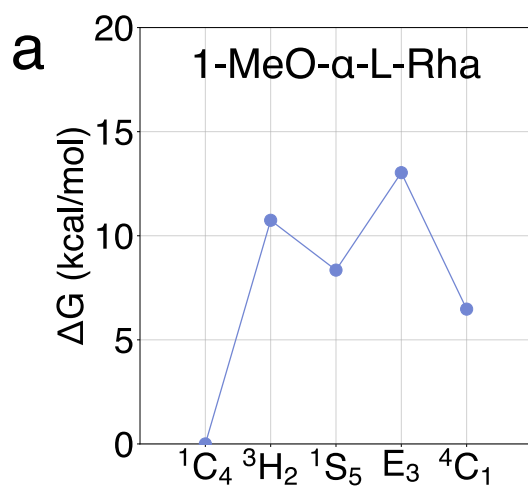


Figure 6.3. (a-b) Group 3 lowest energy ring interconversion pathways.



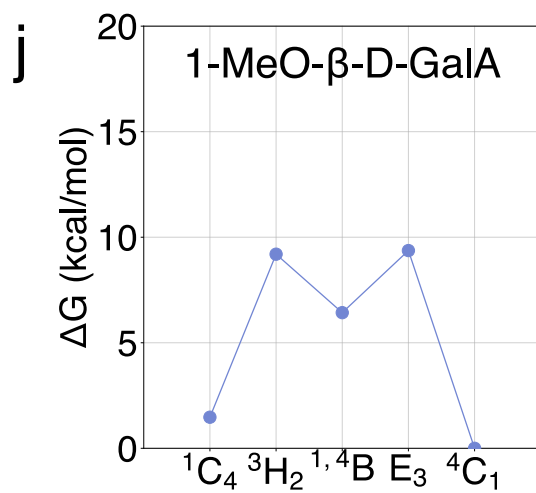
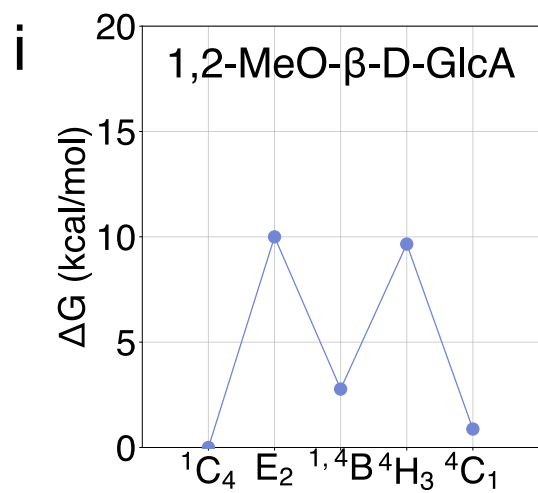
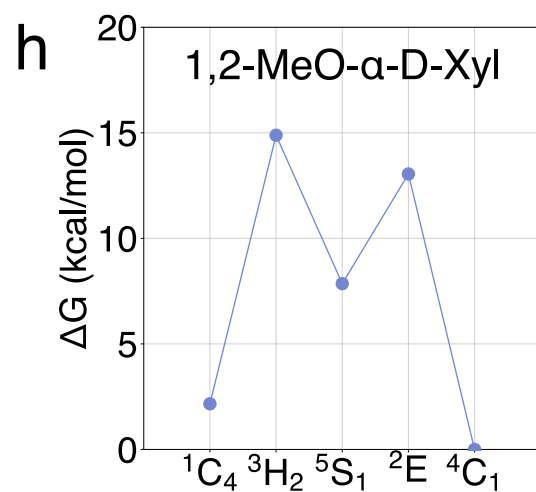
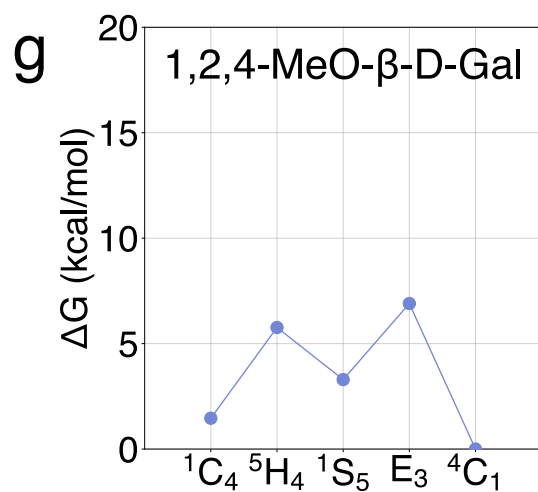


Figure 6.4. (a-j) Group 5 lowest energy ring interconversion pathways.

Section 7: NMR GIAO Calculations

Gauge-Including Atomic Orbitals (GIAO) calculations were conducted for $^1\text{C}_4$ and $^4\text{C}_1$ chair conformations of both 1-MeO-Arap and 1,2,3-MeO-Arap to determine expected NMR peak shifts. These calculations were used to verify the location of peaks corresponding to the $^1\text{C}_4$ and $^4\text{C}_1$ chair conformations in experimental NMR spectra. The full, detailed procedure is described in prior work by Bharadwaj, Westawker, and Crowley¹. In short, the optimized geometry from the final dihedral scan (B3LYP/6-31+(g,d)) was used as the input geometry. A second optimization was performed with the same level of theory and basis set using D₂O implicit solvation (IEFPCM, included via $\epsilon=78.06$ in the route section) to correspond with experimental conditions. Trimethylsilane (TMS) was used as the reference molecule. Peak shifts for the C5 ring carbon are calculated using the equation¹:

$$\delta_{\text{comp}} = \sigma_{\text{ref}} + \delta_{\text{ref}} - \sigma_{\text{comp}} \quad (1)$$

The symbols are as follows: δ_{comp} is the Gaussian-computed NMR peak shift for the C5 carbon of the sugar (referenced to TMS), σ_{ref} is the peak shift for the C5 carbon in TMS determined from an experimental NMR study², δ_{ref} is the Gaussian-computed NMR peak shift for the C5 carbon in TMS, and σ_{comp} is the Gaussian-computed NMR peak shift for the C5 carbon of the sugar. Experimental and computationally derived NMR peak shifts are shown in Table 5.1.

Sugar	Pucker	Experimental Shift (ppm)	Gaussian Shift (ppm)
1-MeO-Arap	$^1\text{C}_4$	Not Observed	65.5
1-MeO-Arap	$^4\text{C}_1$	71	71.3
1,2,3-MeO-Arap	$^1\text{C}_4$	68	67.1
1,2,3-MeO-Arap	$^4\text{C}_1$	69	67.3

Table 5.1. Experimental and Gaussian (computationally determined) NMR peak shifts for both chair conformations of each Arap variant.

The experimental value for the $^4\text{C}_1$ chair of 1-MeO-Arap agrees closely with the computational result. For 1,2,3-MeO-Arap, the $^1\text{C}_4$ chair exhibits a lower peak shift value than for $^4\text{C}_1$ in both the experimental and computational results, which indicates that the same shielding trend is observed in both cases. Thus, the experimental results align with the computationally predicted results.

References

- ¹ V.S. Bharadwaj, L.P. Westawker, and M.F. Crowley, “Towards Elucidating Structure–Spectra Relationships in Rhamnogalacturonan II: Computational Protocols for Accurate ¹³C and ¹H Shifts for Apiose and Its Borate Esters,” *Front. Mol. Biosci.* **8**, 756219 (2022).
- ² G.R. Fulmer, A.J.M. Miller, N.H. Sherden, H.E. Gottlieb, A. Nudelman, B.M. Stoltz, J.E. Bercaw, and K.I. Goldberg, “NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist,” *Organometallics* **29**(9), 2176–2179 (2010).