Supporting Information 1

for

Association of aescin with β- and γ-cyclodextrins studied by DFT calculations and spectroscopic methods

Ana I. Ramos*¹,², Pedro D. Vaz³,⁴, Susana S. Braga⁵ and Artur M. S. Silva⁵

Address: ¹CICECO, Complexo de Laboratórios Tecnológicos, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; ²Current affiliation: INEGI-FEUP Faculty of Engineering of the University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal; ³CQB, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal; ⁴ISIS Neutron & Muon Source, Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, United Kingdom and ⁵QOPNA, Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

Email: Ana I. Ramos - shortinha.sa@gmail.com

* Corresponding author

Supplement to ¹H NMR studies in solution
a) Attemptive Job plot for the H3 proton of γCD in γCD-aescin mixtures

**Figure S1.1:** Job plot for the H3 proton of γ-CD in aqueous solutions containing γ-CD and aescin in a gradient of molar fraction. Note how the inconsistency in the proton shifts affords a negative value for the data point corresponding to the molar fraction of 0.9 (circled with red).
b) Apparent Inclusion Constants for βCD·aescin and γCD·aescin

Figure S1.2: Graphical analysis of $^1$H NMR shifts of the H5 proton of β-CD of one assay comprising a series of aqueous solutions containing 0.25 mM of β-CD and 2.5, 3.0, 3.5, 4.0 and 4.5 mM of aescin. Data is fitted to a line with $r^2$ value of 0.972 and $x_0$ (yy-intercept) of 0.00151, which allows estimating, for this data set, $K_{app} = 1/x_0 = 662$ M$^{-1}$ (the values presented in the manuscript are the average (± RSD) of different calculated $K_{app}$ values). The first data point was not used in the data fitting.
Figure S1.3: Graphical analysis of $^1$H NMR shifts of the H5 proton of γ-CD of one assay comprising a series of aqueous solutions containing 0.25 mM of γ-CD and 2.5, 3.0, 3.5, 4.0 and 4.5 mM of aescin. Data is fitted to a line with $r^2$ value of 0.9834 and $x_0$ (yy-intercept) of -0.00102, which allows estimating $K_{app} = 1/x_0 = 980$ M$^{-1}$. 