Supplementary data

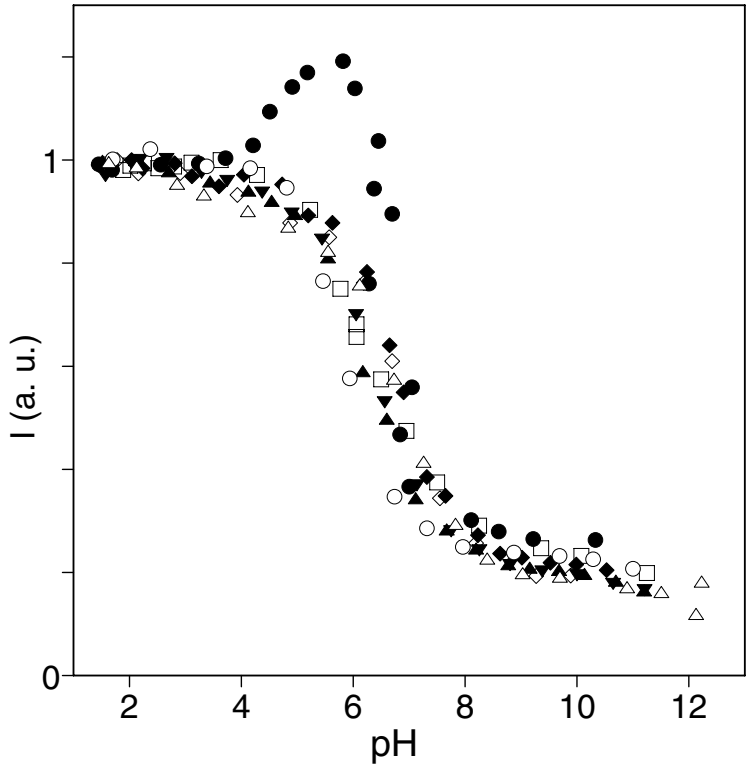

Figure S1. Titration of compound (**1**), $1x10^{-5}$ M, in the presence of 10-fold excess of the Krebs cycle components: free ligand (□), citrate (●), DL-isocitrate (◆), α-ketoglutarate (○), fumarate (▼), succinate (▲), L-malate (△), oxaloacetate (◇). All of them except citrate (●) behave like free compound (**1**).

Table S1. Coefficients of the fitting plotted in Figure 2 of the manuscript: compound (**1**) in the presence of citrate (10-fold excess)

L	LH	LH_2	LH_3	LH_4	LH_5	LH_6	LHA	LH_2A	LH_3A	LH_4A	LH_5A	LH_6A	LH_7A	LH_8A
0.2	0.25	0.28	0.28	0.35	0.35	0.97	0.15	0.3	0.4	0.8	1.1	1.4	1.0	1.0

a) Charges omitted

The coefficients of the fitting of compound (**1**) in the presence of citrate (10 fold excess) show that increase of the emission is mainly due to the species H_6(**1**)A^{2+} (1.85) and H_5(**1**)A^{2+} (1.1).

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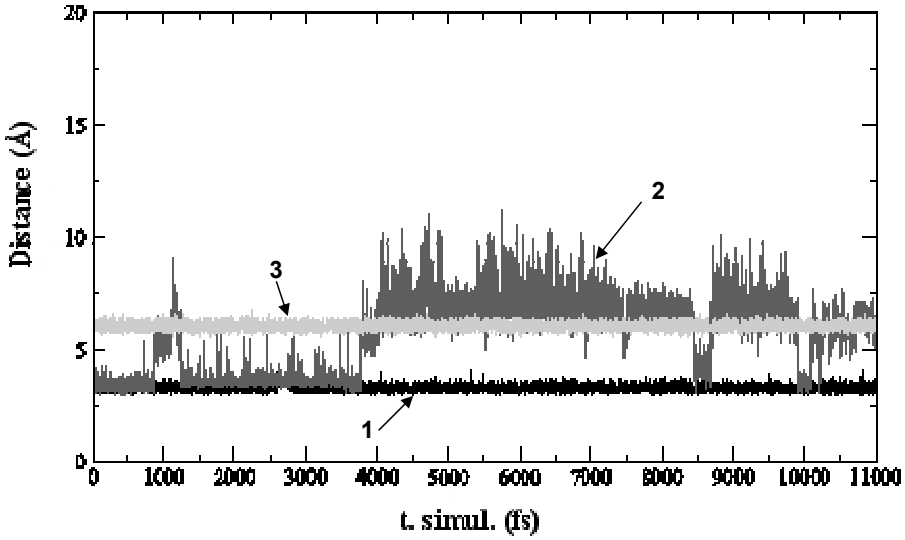

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The stability constants presented in Table S2 have been determined by pH-metric titrations in 0.15 mol dm^{-3} NaCl at 298.1 K with the data analysed by means of the program HYPERQUAD.

Table S2. Stability constants of Krebs cycle compounds with ligand (**1**) in 0.15 mol dm^{-3} NaCl at 298.1 K.

Reaction	A=Kglr	A=Lmal	A=Succ	A=Cit	A=DL-Isocit	A=Fum	A=Oxalac
H(**1**)+A = A(**1**)H[a]		3.90(4)	3.52(2)	3.69(1)		3.85(2)	4.64(1)
H_2(**1**)+ A = A(**1**)H_2	4.21(2)[b]	4.92(2)	4.41(1)	4.09(1)	5.38(1)	4.86(2)	5.41(1)
H_3(**1**)+ A = A(**1**)H_3	4.48(3)	5.44(2)	4.75(1)	4.28(1)	5.51(2)	5.22(2)	5.89(2)
H_4(**1**)+ A = A(**1**)H_4	4.82(2)	5.67(2)	4.85(1)	3.92(2)	5.79(2)	5.43(2)	6.04(2)
H_5(**1**) + A = A(**1**)H_5	4.92(3)	5.89(3)	5.11(2)	4.67(1)	6.01(2)	5.60(3)	6.26(2)
H_6(**1**) + A = A(**1**)H_6	5.37(2)	6.25(2)	5.31(1)	4.71(1)	6.15(2)	5.93(2)	6.53(2)
H_6(**1**) + HA = A(**1**)H_7	5.57(3)	6.60(3)	5.32(1)	4.21(1)	5.79(2)	6.27(3)	6.47(2)
H_6(**1**) + H_2A= A(**1**)H_8			5.57(1)	4.31(1)	6.02(2)	6.44(3)	

b) Charges omitted
c) Values in parentheses are standard deviations in the last significant figure

The protonation constants presented in Table S3 have been determined by pH-metric titrations in 0.15 mol dm^{-3} NaCl at 298.1 K with the data analysed by means of the program HYPERQUAD.

Table S3. Protonation constants of Krebs cycle compounds

Reaction	A=Kglr	A=Lmal	A=Succ	A=Cit	A=DL-Isocit	A=Fum	A=Oxalac
A + H = AH[a]	4.716(8)[b]	4.65(5)	5.25	5.33	5.54(7)	4.163(1)	4.13(1)
A + 2H =AH_2	6.82(6)	7.55(1)	9.24	9.50	9.46(1)	6.983(3)	6.46(2)
AH + H = AH_2	2.10(2)	2.90(1)	3.99	4.17	3.923(5)	2.819(1)	2.33(1)
A + 3H = AH_3				12.27	12.19(7)		
AH_2 + H = AH_3				2.77	2.72(3)		

a) Charges omitted
b) Values in parentheses are standard deviations in the last significant figure

Experimental

The synthetic pathway of chemosensor (**1**) was reported previously.[1]

emf Measurements. The potentiometric titrations were carried out at 298.1±0.1 K using NaCl 0.15 mol dm^{-3} as supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer, etc.) has been fully described elsewhere.[2] The acquisition of the emf data was performed with the computer program PASAT.[3] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen-ion concentration probe by titration of previously standardized amounts of HCl with CO_2-free NaOH solutions and the equivalent point determined by the Gran's method,[4] which gives the standard potential, Eº', and the ionic product of water (pKw=13.73(1)). The protonation constant of (**1**) have been taken from ref. 6 in the main text.

Spectroscopic measurements. Water was twice distilled and passed through a Millipore apparatus. All aqueous solutions were prepared in 0.15 mol dm^{-3} NaCl. The measured pH values were obtained with a MeterLab pHM281 potentiometer from Radiometer-Copenhagen and adjustments of H^+ concentration was made with diluted HCl and NaOH solutions. Absorption and fluorescence spectra were recorded on Shimadzu UV-2100 and Jovin-Yvon Spex Fluorog 3-2.2. spectrometers, respectively. The stock solution of each Krebs cycle components (ca. 3×10^{-3} M) were prepared by dissolving an appropriate amount of the acids in a 10 mL volumetric flask and diluting to the mark with twice distilled water.

Comments on the Detection Limit. The routine determination of citric acid is carried out by reverse phase liquid chromatography with UV or refraction index detection,[5] by capillary electrophoresis[6] or by flow injection techniques [7] In the last years some papers have been reported employing metal complexes as chemosensors[8] but few of them reported in the literature, shows the detection limit in water. The values reported varied from 0.779-25.0 mM [9]or 5mM.[10]

Molecular dynamics simulations. Molecular dynamics simulations were carried out using AMBER8 and GAFF potentials at 325 K. The process comprised a convenient heating stage (in several steps until a final temperature of 325 K is reached) after which the simulation was done. Minimum energy conformers were extracted from the trajectories and are given in Scheme 2 of the manuscript. The calculations were

performed for the interaction of the hexaprotonated macrocycle with citrate, DL-isocitrate and succinate. The protons are located in the secondary amino groups of the receptor. Files with trajectories in AMBER format are available on request

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