Pure Appl. Chem.; doi:10.1351/PAC-CON-09-10-02

# The Effect of Molecular Clips and Tweezers on Enzymatic Reactions by Binding Coenzymes and Basic Amino acids*

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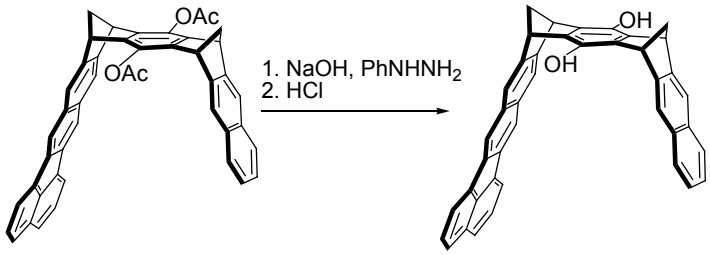
# Supporting Information

1. Synthesis of the methanephosphonate-substituted molecular clip **6b** having one benzo[*k*]fluoranthene and one naphthalene sidewall.
2. Determination of the thermodynamic parameter, free enthalpy $\Delta G$, enthalpy $\Delta H$, and entropy $\Delta S$, of the self-assembled dimerization of clip **6b** to $(\mathbf{6b})_2$

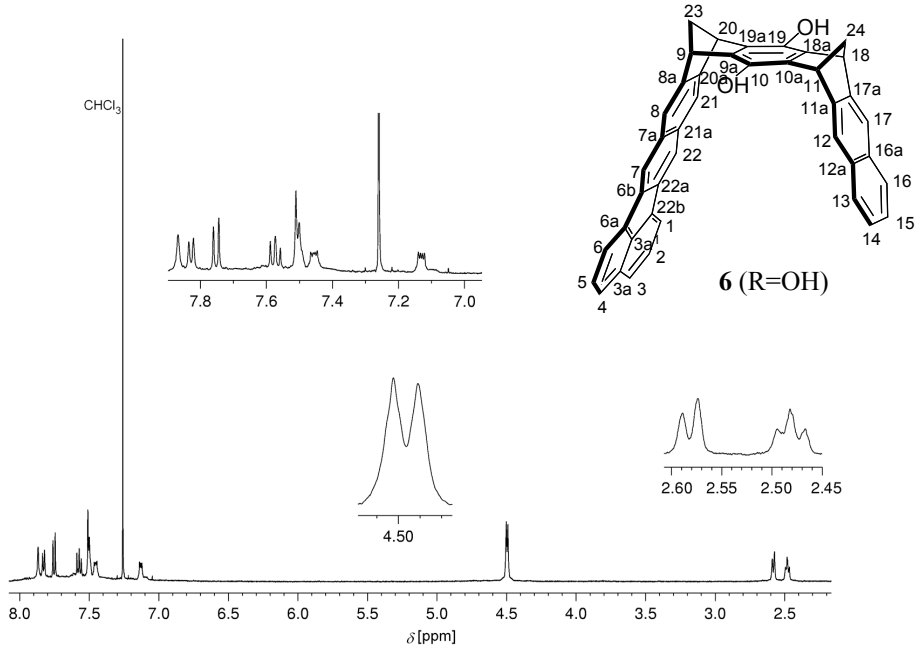
## 1. Synthesis of the methanephosphonate-substituted molecular clip 6b having one benzo[*k*]fluoranthene and one naphthalene sidewall.

**Hydroquinone clip 6 (R=OH):**




**6a** **6** (R=OH):

Aqueous solution of NaOH (2.4 mL, 15%) is added to a stirred suspension of **6a** (180 mg, 0.28 mmol) and Ph-NH-$NH_2$ (34 µL) in EtOH (17 mL). The mixture is stirred for 24 h at room temperature. Aqueous HCl (5 mL, 15%) is added. After addition of ice-water (50 mL) hydroquinone clip **6** (R=OH) precipitates as light-yellow solid which is filtered off, washed with water (10 mL), and dried in an exsiccator over $P_2O_5$. Yield: 154 mg (0.27 mmol, 98%) of **6** (R=OH), m.p. > 300°C.




$^{1}$H-NMR($CDCl_3$, 500 MHz): $\delta$[ppm] = 2.48 (m, 2H, H-23a, H-24a), 2.58 (m, 2H, H-23i, H-24i), 4.49, 4.50 (2 br t, 4H, H-9, H-11, H-18, H-20), 7.13 (m, 2H, H-14, H-15), 7.46 (m, 2H, H-13, H-16), 7.50 (s, 2H, H-8, H-21), 7.51 (s, 2H, H-12, H-17), 7.57 (dd, 2H, $^{3}J$(H-2, H-3) = 8.15 Hz, $^{3}J$(H-2, H-1) = 6.95 Hz, H-2, H-5), 7.75 (d, 2H, H-3, H-4), 7.83 (d, 2H, H-1, H-6), 7.87 (s, 2H, H-7, H-22).

$^{13}$C-NMR($CDCl_3$, 125.7 MHz): $\delta$[ppm] = 46.93, 46.99 (C-9, C-11, C-18, C-20), 64.80, 64.90 (C-23, C-24), 119.02 (C-1, C-6), 119.71 (C-12, C-17), 120.22 (C-7, C-22), 120.67 (C-8, C-21), 125.26 (C-14, C-15), 125.94 (C-3, C-4), 127.60 (C-13, C-16), 128.16 (C-2, C-5), 130.45 (C-3a), 132.08 (C-12a, C-16a), 132.10 (C-7a, C-21a), 134.65, 134.69, 134.82 (C-6b, C-22a and C-3a[1] and C-9a, C-19a or C-10a, C-18a) 137.21, 137.27 (C-6a, C-22b and C-9a, C-19a or C-10a, C-18a), 139.16 (C-10, C-19), 146.62 (C-11a, C-17a), 146.97 (C-8a, C-20a).

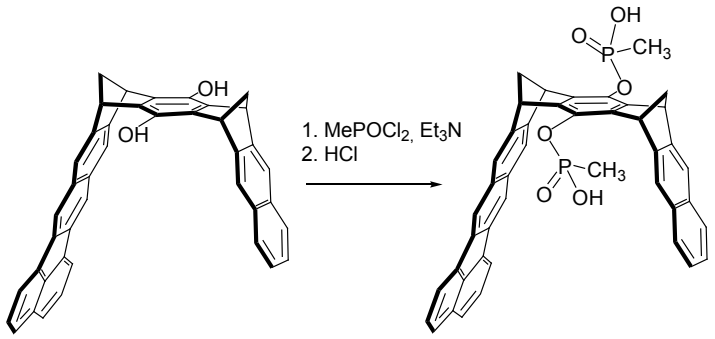
IR(KBr): $\tilde{\nu}$[$cm^{-1}$] = 3402 (O-H), 3047 (C-H), 2991 (C-H), 2964 (C-H), 2932 (C-H), 2859 (C-H), 1485 (C=C), 1279 (O-H).

UV/Vis ($CHCl_3$): $\lambda_{max}$ [nm] (lg $\varepsilon$) = 245 (4.74), 313 (4.68), 359 (3.91), 384 (3.99), 404 (3.98).

MS (70 eV): m/z (%) = 562 (100) [$M^+$].

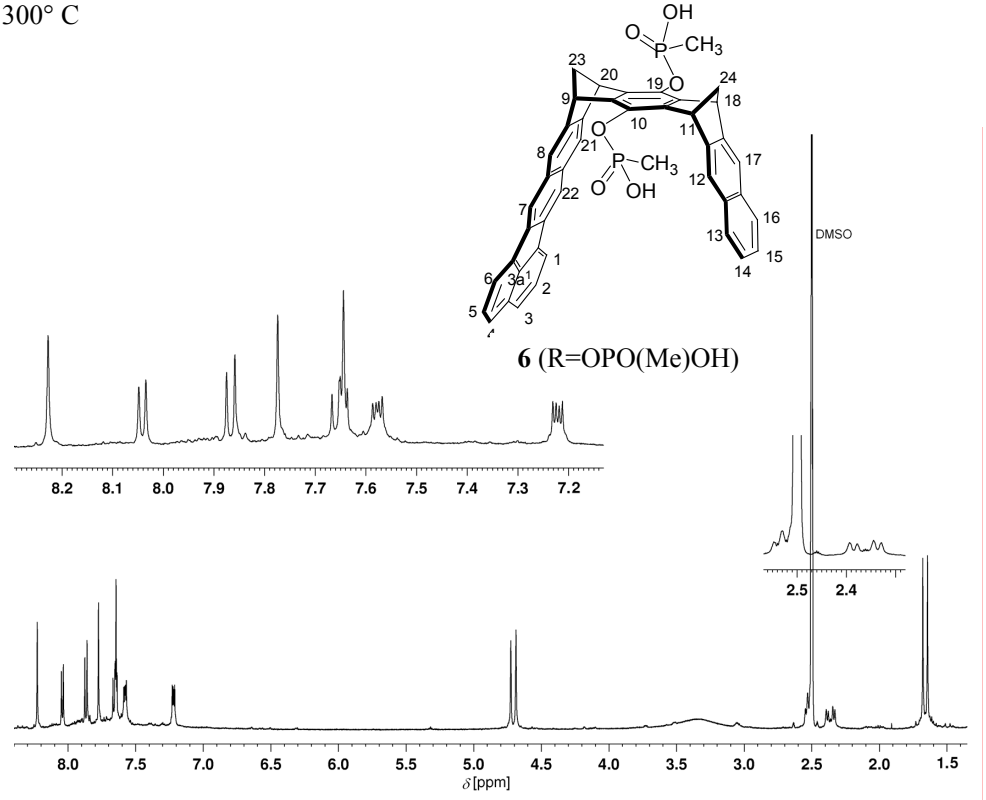
HR-MS (70 eV): m/z = 562.1872 found; 562.1933 calculated for $C_{42}H_{26}O_2$.

**Methanephosphonic acid-substituted clip 6 (R=OPO(Me)OH):**




**6** (R=OH) **6** (R=OPO(Me)OH)

$Et_3N$ (65 µL, 0.49 mmol) is dropped to the stirred solution of hydroquinone **6** (R=OH) (101 mg, 0.18 mmol) and $MePOCl_2$ (63 mg, 0.49 mmol) cooled to 0°C under argon. A colorless solid precipitates. After 1 h the cooling bath is removed and the mixture is stirred for 1h at room temperature. The colorless precipitate is filtered off under argon. Aqueous HCl (2.9 mL, 15%) is dropped to the stirred filtrate. After 15 min hexane (5.7 mL) is added to the solution. The methanephosphonic acid-substituted clip **6** (R=OPO(Me)OH) precipitates as a viscous oil. The suspension is stirred overnight and solvent is decanted off. Aqueous HCl (2.5%, 5 mL) is added to the oil and the mixture is placed in an ultrasound bath. The precipitated solid is filtered off, washed several times with small portions of HCl (2.5%), and dried over $P_2O_5$ in an exsiccator. Yield: 76 mg (0.106 mol, 59%) of **6** (R=OPO(Me)OH) as brownish solid, m.p. > 300° C




**6** (R=OPO(Me)OH)

$^{1}$H-NMR(DMSO-$d_6$, 500 MHz): $\delta$[ppm] = 1.66 (s, 6H, $^{2}J$(P, H-25) = 17.25 Hz, -C**H**$_3$), 2.34, 2.39 (2d, 2H, H-23a, H-24a), 2.53 (m, 2H, H-), 3.34 (s, 2H, -OH), 4.69 (s, 2H, H-11, H-18 or H-9, H-20), 4.73 (s, 2H, H-11, H-18 oder H-9, H-20), 7.22 (m, 2H, H-14, H-15), 7.58 (m, 2H, H-13, H-16), 7.64 (s, 2H, H-12, H-17), 7.65 (dd, 2H, $^{3}J$(H-2, H-3) = 8.15 Hz, $^{3}J$(H-2, H-1) = 6.95 Hz, H-2, H-5), 7.77 (s, 2H, H-8, H-21), 7.87 (d, 2H, H-3, H-4), 8.04 (d, 2H, H-1, H6), 8.23 (s, 2H, H-7, H-22).

$^{13}$C-NMR(DMSO-$d_6$, 125.7 MHz): $\delta$[ppm] = 12.78 (d, $^{1}J$(C-P) = 140.36 Hz, -**C**H$_3$), 47.71, 47.82 (C-9, C-11, C-18, C-20), 64.95 (C-23, C-24), 119.57 (C-1, C-6), 120.06 (C-12, C-17), 120.51 (C-7, C-22), 121.16 (C-8, C-21), 125.10 (C-14, C-15), 126.02 (C-3, C-4), 127.48 (C-13, C-16), 128.34 (C-2, C-5), 129.90 (C-3a). 131.43 (C-12a, C-16a), 131.49 (C-7a, C-21a), 133.74 (C-3a$^{1}$), 136.03 (C-6a, C-22b), 136.09 (C-6b, C-22a), 136.28 (C-10, C-19), 140.66 (C-9a, C-10a, C-18a, C-19a), 146.42 (C-11a, C-17a), 147.06 (C-8a, C-20a).

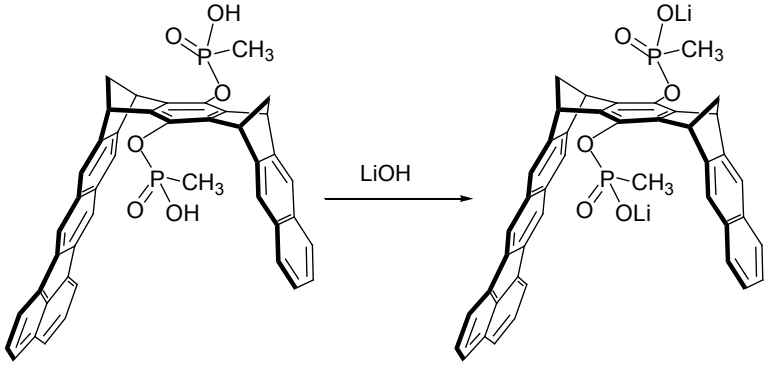
$^{31}$P-NMR(DMSO-$d_6$, 202.4 MHz):$\delta$[ppm] = 24.42.

IR (KBr): $\tilde{\nu}$ [cm$^{-1}$] = 3437 (O-H), 3048 (C-H), 2962 (C-H), 2934 (C-H), 2867 (C-H), 1466 (C=C), 1312 (P=O), 1179 (P-O).

UV/Vis (MeOH):$\lambda_{max}$ [nm] (lg $\varepsilon$) = 225 (4.90), 310 (4.71), 360 (3.95), 380 (4.04), 400 (4.03).

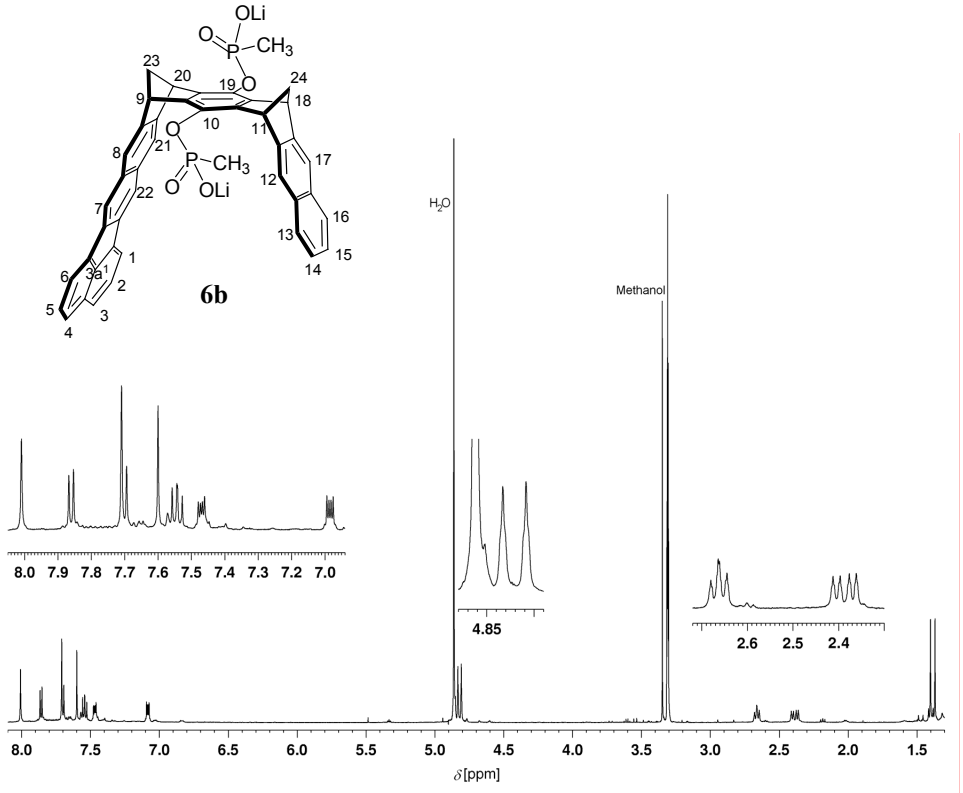
HR-MS (ESI, negative ionic modus): 358.0803 found; 358.0764 calculated for ($C_{44}H_{30}O_6P_2$)$^{2}$.

**Lithium methanephosphonate-substituted clip 6b**




**6** (R=OPO(Me)OH) **6b**

The solution of LiOH•$H_2O$ (5.9 mg) in 0.7 mL of methanol is added to the intensively stirred solution of **6** (R=OPO(Me)OH) (50.5 mg, 0.07 mmol) in 50 mL of dichloromethane. The mixture is stirred for 2 h and the precipitated salt is filtered off. Yield: 47 mg of **6b** (92%) as brownish solid m. p. > 300°C.




$^{1}$H-NMR(MeOH-$d_4$, 500 MHz): $\delta$[ppm] = 1.38 (d, 6H, $^{2}J$(P, H-25) = 16.44 Hz, C**H**$_3$), 2.37, 2.40 (2d, $^{2}J$(H-23i, H-23a) = 7.82 Hz, H-23a, H-24a), 2.66 (m, H-23i, H-24i), 4.81 (br t, 2H, H-11, H-18), 4.84 (br t, 2H, H-9, H-20), 7.11 (m, 2H, H-14, H-15), 7.49 (m, 2H, H-13, H-16), 7.57 (dd, 2H, $^{3}J$(H-2, H-3)=8.07 Hz, $^{3}J$(H-2, H-1) = 7.00 Hz, H-2, H-5), 7.61 (s, 2H, H-12, H-17), 7.73 (d, 2H, H-3, H-4), 7.74 (s, 2H, H-8, H-21), 7.89 (d, 2H, H-1, H-6), 8.06 (s, 2H, H-7, H-22).

$^{13}$C-NMR(MeOH-$d_4$, 125.7 MHz): $\delta$[ppm] = 12.28 (d, $^{1}J$(C-P) = 137.64 Hz, -**C**H$_3$), 48.33, 48.42 (C-9, C-11, C-18, C-20), 64.51, 64.55 (C-23, C-24), 118.49 (C-1, C-6), 119.56 (C-12, C-17), 119.89 (C-7, C-22), 120.62 (C-8, C-21), 124.48(C-14, C-15), 125.39 (C-3, C-4), 127.21 (C-13, C-16), 127.82 (C-2, C-5), 130.49 (C-6a, C-22b), 132.20 (C-7a, C-12a, C-16a, C-21a), 134.54 (C-3a$^1$), 136.80 (C-6b, C-22a), 137.14 (C-3a), 138.06 (C-10, C-19), 140.80, 140.84 (C-9a, C-10a, C-18a, C-19a), 147.44 (C-11a, C-17a), 148.04 (C-8a, C-20a).

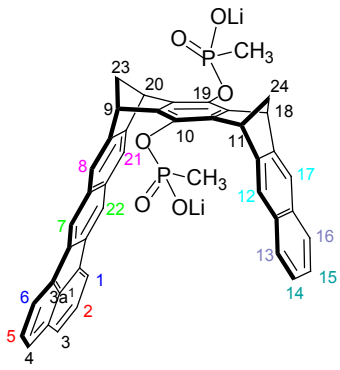
$^{31}$P-NMR(MeOH-$d_4$, 202.4 MHz):$\delta$[ppm] = 21.55 (s).

IR (KBr): $\tilde{\nu}$ [cm$^{-1}$] = 3055 (C-H), 2931 (C-H), 1451 (C=C), 1279 (P=O), 1184 (P-O).

UV/Vis (MeOH):$\lambda_{max}$ [nm] (lg $\varepsilon$) = 223 (4.88), 310 (4.71), 360 (3.91), 383 (4.03), 401 (4.02).

HR-MS (ESI, negative ionic modus): 358.0777 found; 358.0764 calculated for $(C_{44}H_{30}O_6P_2)^{2-}$

## 2. Determination of the thermodynamic parameter, free enthalpy Δ*G*, enthalpy Δ*H*, and entropy Δ*S*, of the self-assembled dimerization of clip 6b to $(6b)_2$




**6b**

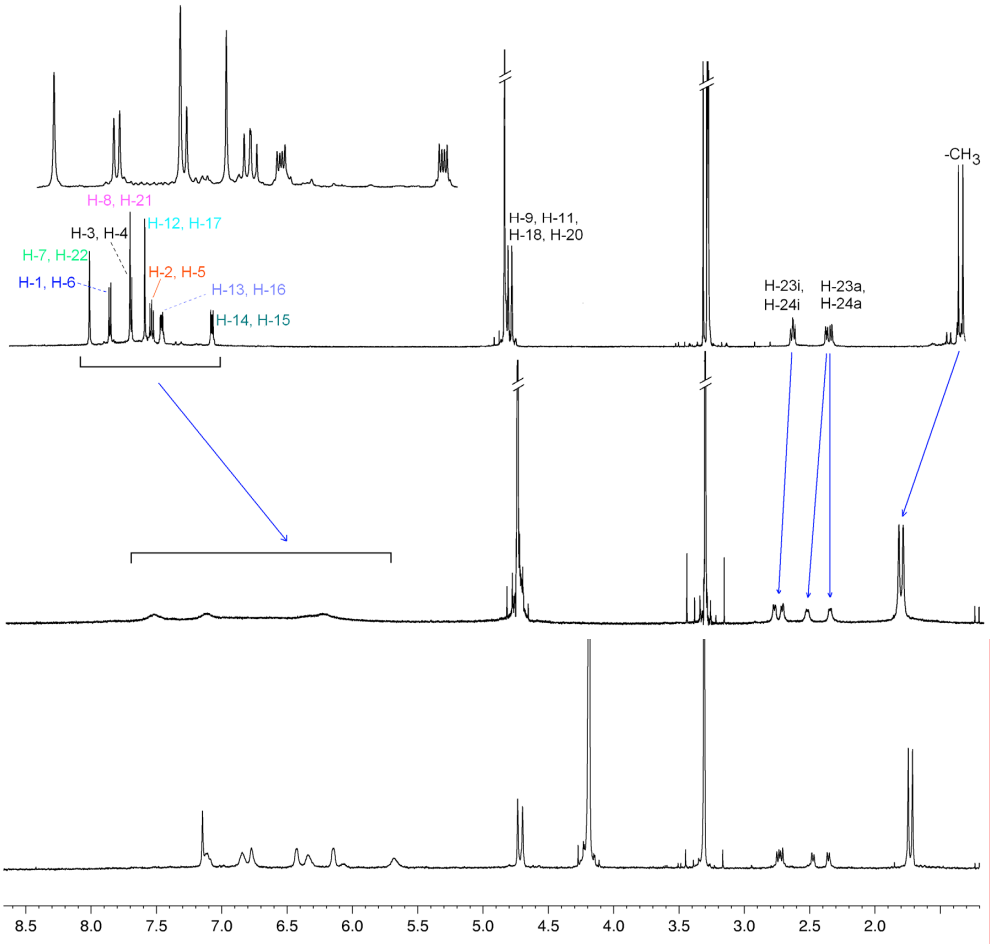
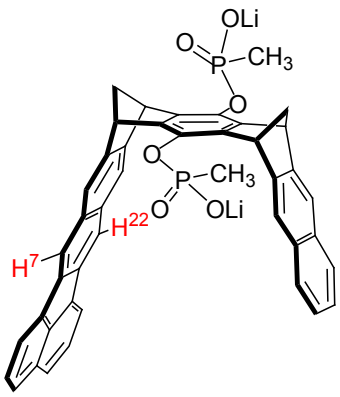



**Figure S1:** $^1$H NMR spectra (500 MHz) of clip **6b** in $CD_3OD$ (top) at 25 °C, in $D_2O$ (middle) at 25 °C and in $D_2O$ (bottom)at 65 °C.

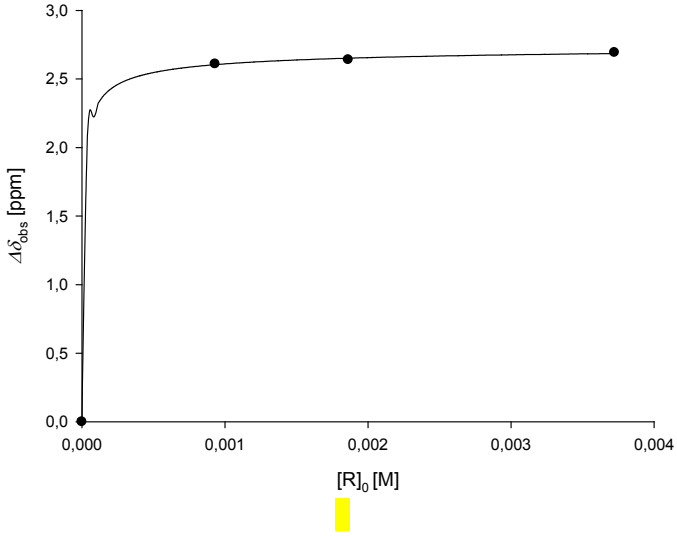
The $^1$H NMR spectrum of **6b** in $D_2O$ only shows broad signals at room temperature which are sharpened at higher temperature (Figure S1) The signals assigned to the protons of the benzo[*k*]fluoranthene and naphthalene sidewalls of clip **6b** show substantial up-field shifts in $D_2O$ solution compared to the corresponding signals in $CD_3OD$, for example the signals assigned to H-7, H-22 of **6b** show an up-field shift of $\Delta\delta_{obs}$ = 2.6 ppm at 50°C. These findings are similar to those found for the phosphonate-substituted clip **5b** having two anthracene sidewalls. They are good evidence for a self-assembled dimerization of clip **6b** in water with a rate of equilibration comparable to the NMR timescale which is indicated by the line-broadening. The equilibrium constants, $K_{Dim}$, were determined for the dimerization 2 **6b** ⇌ (**6b**)$_2$ in aqueous solution from the concentration dependence of the $^1$H NMR signals assigned to the clip protons H-7 and H-22 by dilution titration experiments at different temperatures as described for the naphthalene tweezer **2b** and anthracene clip **5b** by F.-G. Klärner, B. Kahlert, A. Nellesen, J. Zienau, C. Ochsenfeld, T. Schrader, *J. Am. Chem. Soc.* **2006**, *128*, 4831-4841.

| | | | |
|---|---|---|---|
| Receptor, R: | **6b** | $M_R$ [g/mol]: | 730.53 |
| Solvent: | $D_2O$ | $m_R$ [mg]: | 5.44 |
| $T$ [°C]: | 50 | $V_0$ [mL]: | 2 |
| | | $[R]_0$ [mM]: | 3.72 |




$\delta_0$ (H-7) [ppm] = 8.06 ($CD_3OD$)

| $[R]_0$ [M] | $\delta_{obs}$ (H-7) [ppm] | $\Delta\delta_{obs}$ (H-7) [ppm] | $\Delta\delta_{calc}$ (H-7) [ppm] |
|---|---|---|---|
| 0.00372332 | 5.368 | 2.692 | 2.685 |
| 0.00186166 | 5.420 | 2.640 | 2.651 |
| 0.00093083 | 5.451 | 2.609 | 2.604 |

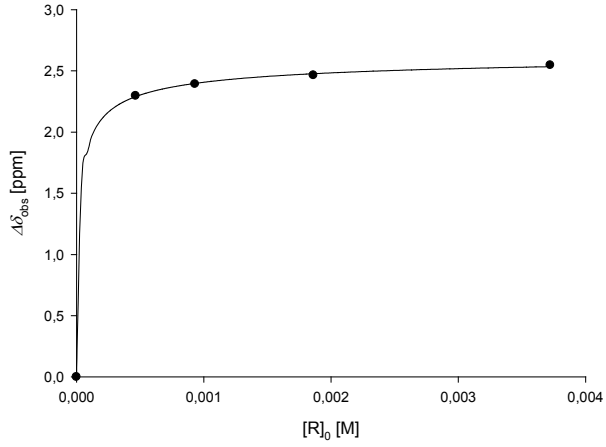



$K_{Dim}$ [$M^{-1}$] = 142300 ± 14200

$\Delta\delta_{max}$(H-7) [ppm] = 2.77

*T* [°C]: 65

| $[R]_0$ [M] | $\delta_{obs}$ (H-7) [ppm] | $\Delta\delta_{obs}$ (H-7) [ppm] | $\Delta\delta_{calc}$ (H-7) [ppm] |
|---|---|---|---|
| 0.00372332 | 5.514 | 2.546 | 2.534 |
| 0.00186166 | 5.596 | 2.464 | 2.477 |
| 0.00093083 | 5.668 | 2.392 | 2.397 |
| 0.00046542 | 5.764 | 2.296 | 2.290 |




$K_{Dim}$ [$M^{-1}$] = 43500 ± 4400

$\Delta\delta_{max}$(H-7) [ppm] = 2.62

*T* [°C]: 80

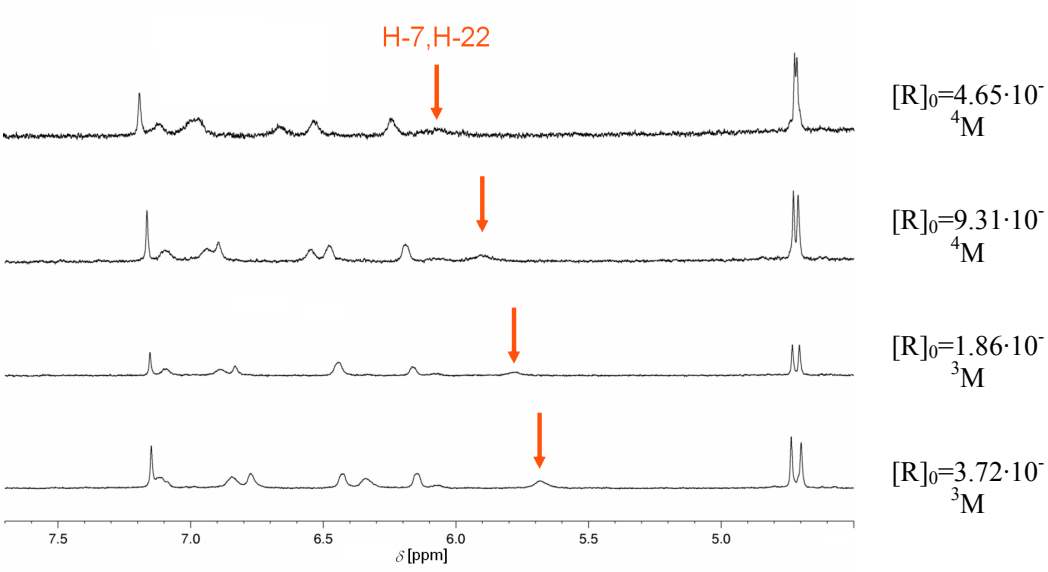
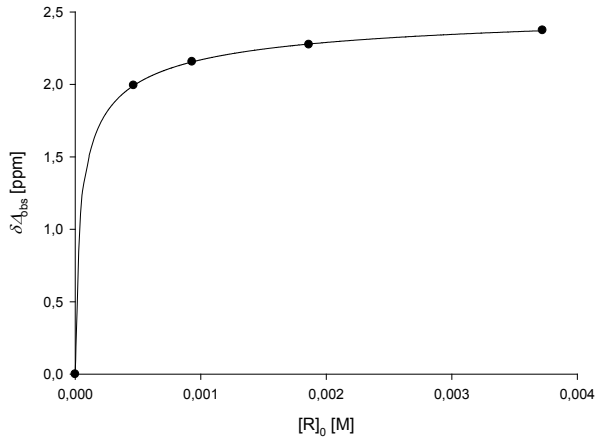



**Figure S2:** $^1$H NMR spectra of **6b** in $D_2O$ at 80 °C and different concentrations.

| $[R]_0$ [M] | $\Delta_{bbs}$ (H-7) [ppm] | $\delta\Delta_{bbs}$ (H-7) [ppm] | $\delta\Delta_{calc}$ (H-7) [ppm] |
|---|---|---|---|
| 0.00372332 | 5.686 | 2.374 | 2.371 |
| 0.00186166 | 5.785 | 2.275 | 2.279 |
| 0.00093083 | 5.903 | 2.157 | 2.156 |
| 0.00046542 | 6.067 | 1.993 | 1.993 |

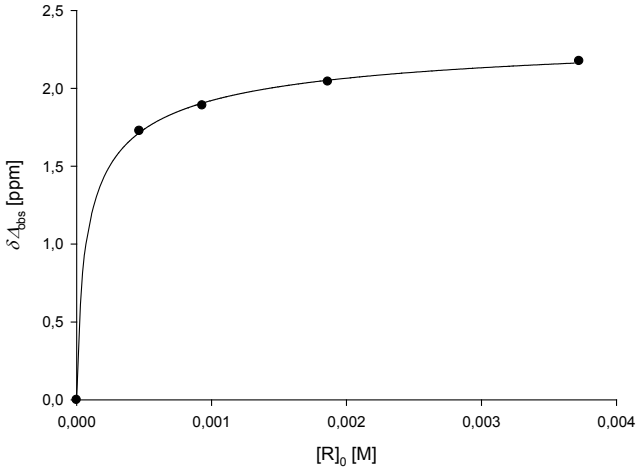



$K_{Dim}$ [$M^{-1}$] = 14700 ± 1041

$\delta\Delta_{max}$(H-7) [ppm] = 2.61

*T* [°C]: 95

| $[R]_0$ [M] | $\Delta_{obs}$ (H-7) [ppm] | $\delta\Delta_{obs}$ (H-7) [ppm] | $\delta\Delta_{calc}$ (H-7) [ppm] |
|---|---|---|---|
| 0.00372332 | 5.884 | 2.176 | 2.164 |
| 0.00186166 | 6.017 | 2.043 | 2.052 |
| 0.00093083 | 6.169 | 1.891 | 1.905 |
| 0.00046542 | 6.333 | 1.727 | 1.716 |




$K_{Dim}$ [$M^{-1}$] = 8200 ± 721

$\delta\Delta_{max}$ (H-7) [ppm] = 2.46

**Table S1**: Constants, $K_{Dim}$, Gibbs enthalpies, $\delta G$, and the maximum dimerization-induced 1H NMR shifts, $\delta\Delta_{max}$, of the dimerization of **6b** at the given temperatures, T [°C]in $D_2O$.

| T | $K_{Dim}$ | $\delta G$ | $\delta\Delta_{max}$ (H-7, H-22) |
|---|---|---|---|
| 50 | 142300 | -7.58 | 2.78 |
| 65 | 43500 | -7.14 | 2.62 |
| 80 | 14700 | -6.70 | 2.61 |
| 95 | 8200 | -6.56 | 2.46 |

$\ln K_{Dim} =$

$$\ln K_a = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R}$$

$R = 1.978$ cal·K$^{-1}$·mol$^{-1}$

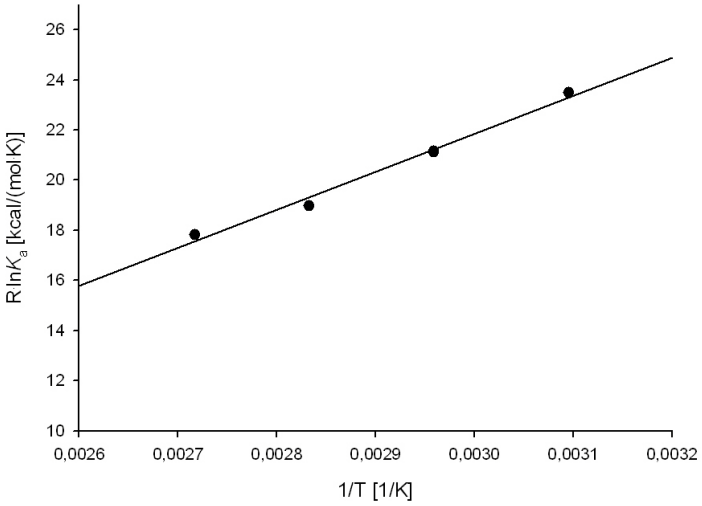



**Figure S3**: van't Hoff plot of the temperature dependence of the $K_{Dim}$ values listed in Table S1.

**Table S2:** The thermodynamic parameters of the dimerization of **6b** resutling from the van't Hoff plot shown in Figure S6**.**.

| T [°C] | Δ***H*** [kcal·mol$^{-1}$] | Δ***S*** [cal·mol$^{-1}$·K$^{-1}$] | Δ***G*** [kcal·mol$^{-1}$] | $K_{dim}$ [M$^{-1}$] |
|---|---|---|---|---|
| 25 | -15.2 | -23.7 | -8.1 | 961700 |
| 50 | -15.2 | -23.7 | -7.5 | 131100 |

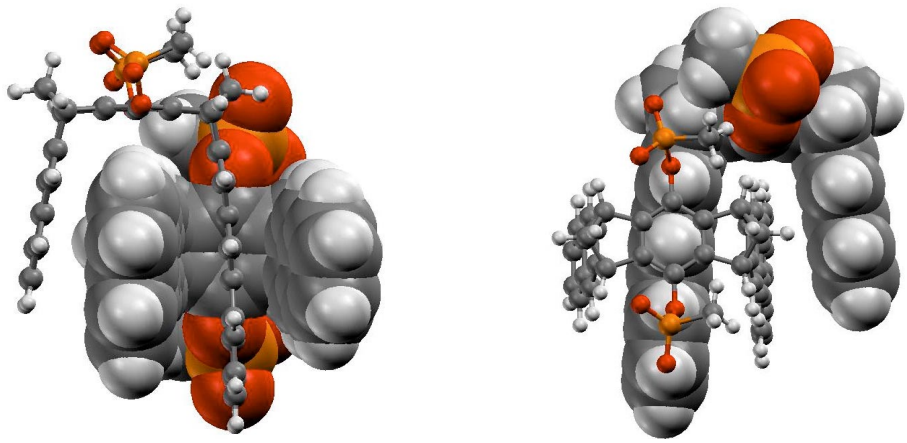


**Figure S4:** Structure of the dimer $(\mathbf{6b})_2$ calculated by a Monte-Carlo conformer search (MacroModel 6.5, Amber*, 5000 structures).