SUPPORTING INFORMATION for

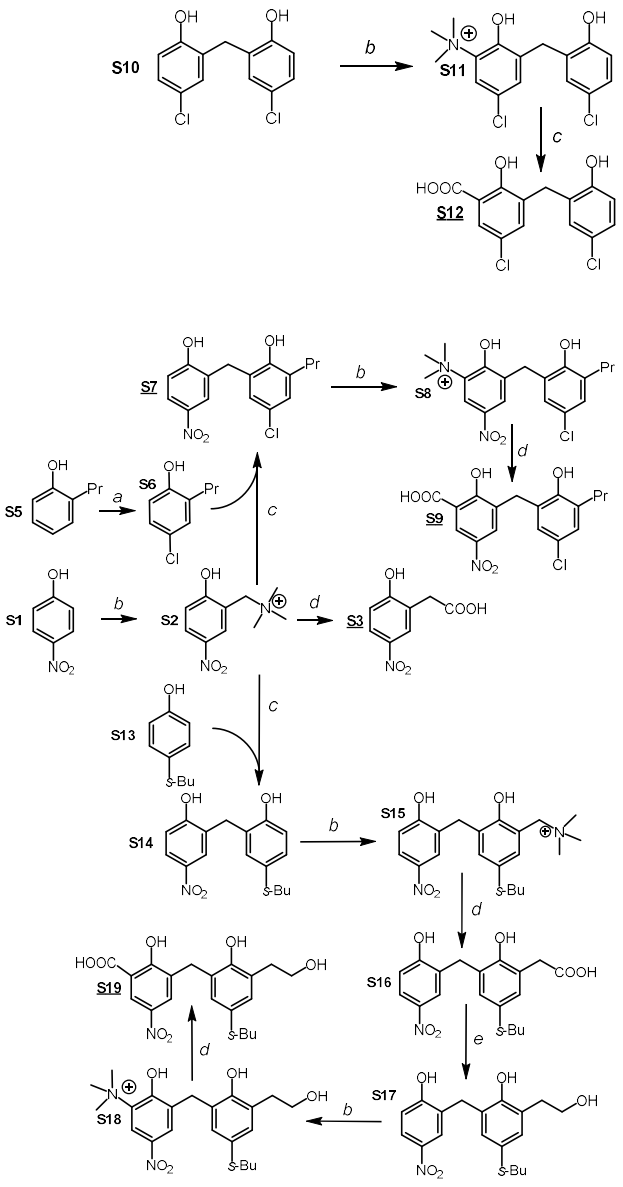
Combined NMR and UV-vis Spectroscopic Studies of Models for the Hydrogen Bond System in the Active Site of Photoactive Yellow Protein: H-Bond Cooperativity and Medium Effects

Benjamin Koeppe*, Peter M. Tolstoy, Jing Guo, Gleb S. Denisov, Hans-Heinrich Limbach

1 Syntheses

1.1 Overview

Scheme S 1. Schematic overview of synthetic routes to neutral precursors of mono-anions **4** through **8** from simple phenolic compounds employing five types of methods labeled *a*-*e* (see main text).



As depicted in Scheme S 1, neutral precursors of species **1b**, **2b**, **3b**, **3c**, **4b** (**S3**, **S7**, **S9**, **S12**, and **S19**, respectively, numbers underlined in the scheme) were obtained from commercially available phenols by a combination of five types of conversions *a*, *b*, *c*, *d* and *e*. Conversion *a* is single step chlorination. Conversion *b* is the Mannich reaction of a phenol with formalin and dimethylamine to form the corresponding Mannich

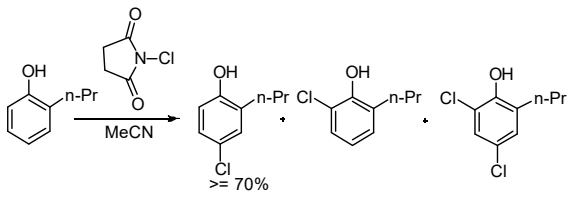
bases and subsequent quaternization with methly iodide.[1] The iodides thus obtained were either coupled to another phenol resulting in bis-(2-hydroxyphenyl)-methane derivatives (bisphenols, conversion *c*)[2,3] or reacted with cyanide and converted to (2-hydroxyphenyl)-acetic acids through hydrolysis of the resulting nitrile (conversion *d*).[1] Conversion *e* is a selective reduction of carboxylic groups to hydroxyl groups by borane.[4]

The Freonic solvent mixture CDF_2Cl/CDF_3 was prepared from $CDCl_3$ according to Ref. 5.

1.2 Methods

1.2.1 Method a: chlorination

4-chloro-2-n-propylphenol was obtained by chlorination of 2-n-propylphenol with N-chlorosuccinimide (NCS):



This procedure, given in Section 2.2.1.1, was chosen in analogy to a procedure for bromination by *N*-bromosuccinimide (NBS) described in Ref. 6. It has conveniently been used in several other conversions (Ref. 7). The reaction of phenols with NCS in acetonitrile solution at room or slightly elevated temperatures seems to be generally slower but more selective than that with NBS. However, one should be aware that in the halogenation of phenols by any *N*-halogenosuccinimide, problems in the separation of succinimide from halogenation products can be an issue that should not be underestimated, especially on large scale reactions.

1.2.2 Method b: Mannich reaction and quaternization

Compounds listed in Table S 1 were obtained according to the scheme in Figure S 1; specific procedure can be found in the given subsection to 2.2.2

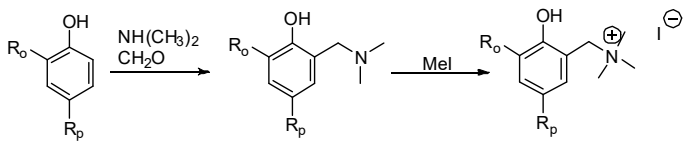
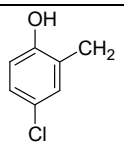
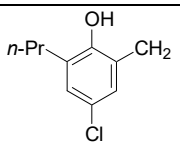
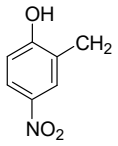
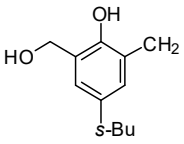


Figure S 1. Mannich reaction of phenolic species to Mannich bases and methylation of Mannich bases to the corresponding tetraalkylammonium iodides. Specific compounds with substituents R_p and R_o are listed in Table S 1.

Table S 1. Nomenclature of compounds with substituents R_p and R_o in Figure S 1 and references to experimental procedures.

R_p	R_o	reactant	product	procedure
NO_2	H	**S1**	**S2**	2.2.2.1
Cl		**S10**	**S11**	2.2.2.2
NO_2		**S7**	**S8**	2.2.2.3
s-Bu		**S14**	**S15**	2.2.2.4
NO_2		**S17**	**S18**	2.2.2.5

Procedures for aminomethylation as well as the subsequent *N*-methylation slightly varied with the reactants. In the cases of reactants bearing only a single hydrogen *ortho* to the hydroxyl group such as bis-(2-hydroxyphenyl)methane derivatives **S7**, a considerable excess of aminomethylation reagent could be used, whereas in all other cases rather stoichiometric amounts and monitoring of reaction progress is necessary to avoid double substitution. Fractions of substance not transformed during the aminomethylation step may in many cases be recovered after *N*-methylation: the tetraalkylammonium salts tend to precipitate leaving the unreacted phenol in solution which may be subjected to the Mannich protocol again. In case of the symmetric bis-(2-hydroxy-5-chloro-phenyl)-methane, products of single and double aminomethylation and unreacted reactant have to be separated by chromatography.[8] An interesting case is the Mannich reaction of asymmetrically substituted bis-(2-hydroxyphenyl)-methane derivative **S14** in which two substitution products are conceivable (see Figure S 2).

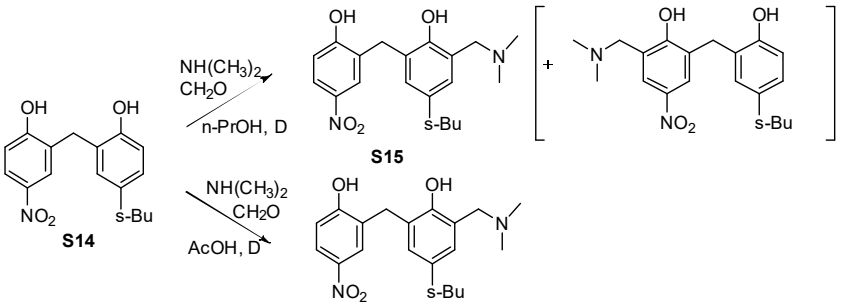
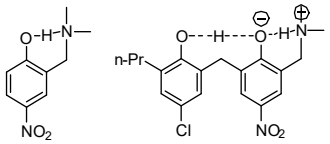


Figure S 2. Considerations concerning regioselectivity in the Mannich reaction of bis-(2-hydroxyphenyl)methane derivative **S14**.

On the one hand, one may expect preferential aminomethylation of the more electron rich butyl substituted ring. On the other hand, this regioselectivity could be compromised – or even inverted – by a higher tendency of the nitrophenol moiety to be deprotonated by the amine component and thus be converted to a more nucleophilic phenolate motif. Therefore, initial experiments were conducted in which the Mannich reaction was performed in alcoholic solution in the presence of one equivalent of KOH per hydroxyl group and, alternatively, in glacial acetic acid solution. Indeed, while in the first case a slow conversion to multiple products was observed, under acidic conditions substitution was found to take place rather rapidly and exclusively on the butyl substituted ring.

N-methylation of the Mannich bases was found to be a slow reaction in many cases. Reactivity of the amino groups is probably reduced due to intramolecular OHN hydrogen bonding. This effect can be expected to be strong in Mannich bases with relatively high OH acidity, and even more so in bis-(2-hydroxyphenyl)-methane derivatives where hydrogen bond cooperativity seems likely:



It was found that addition of KOH or NaOH was accelerating *N* alkylation, and in cases such as the generation of **S8** presence of a stoichiometric amount of base turned out to be necessary to obtain quantitative alkylation at room temperature within reasonable time. Competition from *O* alkylation was not observed under any of the conditions employed.

1.2.3 Method c: quaternary ammonium salts to bisphenols

The key intermediate in C-C bond formations to the benzylic carbon atoms are believed to be *ortho*-quinone methides (Figure S 3) which can be generated in various ways.[9] Here they were formed either thermally by loss of trimethylamine from the quaternary ammonium salts under basic conditions or by dehydration of salicylic alcohols under acidic conditions. Quinone methides can be intercepted by diverse nucleophiles such as phenols, H_2O/OH^- or cyanide (see section 1.2.4).

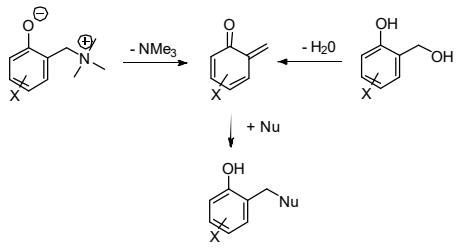


Figure S 3. Generation and nucleophilic interception of *ortho*-quinone methides.

The reaction with water and alcohols is quite slow and thus reactions of quaternary ammonium salts with stronger nucleophiles such as electron rich phenols may be done in aqueous or alcoholic solution.

The bis-(2-hydroxyphenyl)-methane derivative **S14** was obtained by coupling of 4-*s*-butylphenol **S13** with tetraalkylammonium iodide according to the scheme in Figure S 4(procedure in .Section 2.2.3.1).

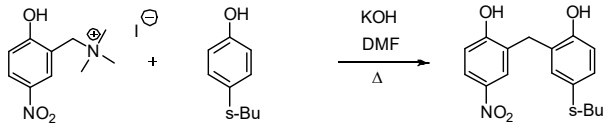
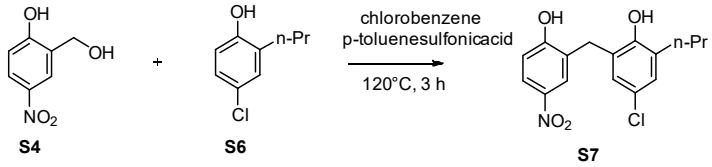
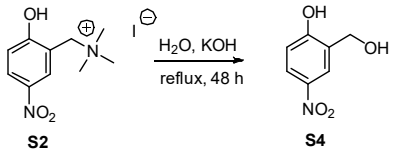


Figure S 4. Coupling of phenols and tetraalkylammonium iodides to bis-(2-hydroxyphenyl)-methanes.

The bis-(2-hydroxyphenyl)-methane derivative **S7** was obtained by heating the salicylic alcohol **S4** and phenol **S6** in chlorobenzene in the presence of catalytic amounts of a very strong acid (experimental procedure is given in section 2.2.3.3).



The salicylalcohol 2-hydroxy-5-nitro-phenylmethanol **S4** was obtained by hydrolysis of (2-hydroxy-5-nitrobenzyl)-trimethylammonium iodide **S2** (see 2.2.3.2), but is also commercially available:



1.2.4 Method d: quaternary ammonium salts to (2-hydroxyphenyl)-acetic acids

Title compounds listed in Table S 2 were obtained according to the scheme in Figure S 5. The synthetic route was adapted from Ref. 1, the use of an aprotic solvent in connection with 18-crown-6 as a catalyst in the substitution step was inspired by Ref. 10. Specific procedures can be found in Section 2.2.

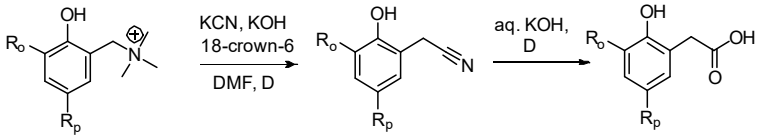
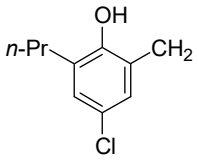
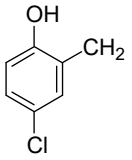
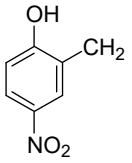
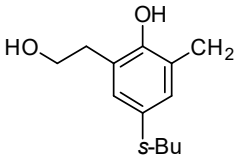


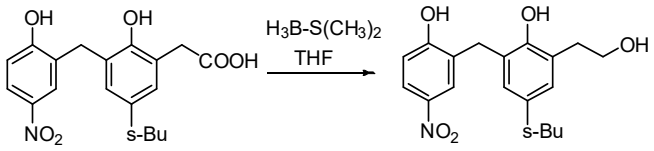
Figure S 5. Conversion of (2-hydroxyphenyl)-trimethylammonium iodides to (2-hydroxyphenyl)-acetic acids via nitriles. Substituents are given in Table S 2.

Table S 2. Definitions of substituents R_p and R_o and nomenclature of the compounds in Figure S6.

R_p	R_o	reactant	product	procedure
NO_2	H	**S2**	**S3**	2.2.4.1
NO_2		**S8**	**S9**	2.2.4.2
Cl		**S11**	**S12**	2.2.4.3
s-Bu		**S15**	**S16**	2.2.4.4
NO_2		**S18**	**S19**	2.2.4.5

1.2.5 Method e: reduction to 2-(2-hydroxyphenyl)-ethanol derivatives

(2-hydroxyphenyl)-acetic acids can be converted to 2-(2-hydroxyphenyl)-ethanols by selective reduction of the carboxylic group with borane.[11] The reagent is employed conveniently as the dimethylsulfide complex $H_3B\text{-}S(CH_3)_2$ in THF solution (procedure in Section 2.2.5.1):



S16 **S17**

1.3 Discussion

It can be summarized that bis-(2-hydroxyphenyl)-methane and (2-hydroxyphenyl)-acetic acid derivatives synthesized according to the given routes are assembled from the corresponding single ring phenolic moieties. Carbon atoms of methylene groups in derivatives of bis-(2-hydroxyphenyl)-methane, (2-hydroxyphenyl)-acetic acid and 2-(2-hydroxyphenyl)-ethanol stem from formalin and C-1 atoms of the latter two are introduced as cyanide. In respect to the synthesis of ^{13}C enriched species, this means that labeling within any aromatic ring requires the corresponding phenol. Labeling in those carbon atoms introduced as formalin or cyanide is readily done in the course of the steps discussed here using those common precursors for isotope enrichment.

Yields of bis-(2-hydroxyphenyl)-methanes depended on the substitution pattern of the phenolic nucleophile: in the presence of electron withdrawing groups and a substituent in one of the *ortho* positions strongly decreased yields were observed. Even with considerable excess of the nucleophile in many cases yields did not exceed 10-20% in respect to the quinone methide precursor; best yield was 60% with 4-*s*-butylphenol similar to yields of 60-90% with 4-methylphenol reported in Ref. 1. In the former cases, isolation of the product from the large variety of side products was not always straightforward. Bis-(2-hydroxyphenyl)-methane derivative **S7** was first obtained through the salicyl alcohol (in 17% yield), as described (see section 2.1.3), after a previous attempts of syntheses from the ammonium salt **S2** had failed. However, **S7** was also obtained analog to **S10** (from **S2** in aqueous solution) in approx. 20% yield. The two alternative coupling protocols with either ammonium salts or salicyl alcohols as quinone methide precursors seem to give similar yields as far as the coupling step itself is concerned. However, the inefficient conversion of the ammonium salts to the salicyl alcohols makes the latter protocol very unattractive. Nonetheless, salicyl alcohols could be key intermediates in alternative syntheses of bis-(2-hydroxyphenyl)-methane derivatives not involving Mannich bases. Salicyl alcohols could be obtained directly from phenols[12] or by formylation[13] and subsequent reduction[14] of the intermediate salicyl aldehyde.

2 Experimental Section

2.1 DFT calculations

We used the B3LYP/6-311++G** basis set for structure optimization as was employed for similar systems in Ref. [15]. Structures of **1** and **2** were optimized first. An initial guess for the structure of **3** was deduced from these results, however, hydrogen bonding protons were placed in covalent bond distance from phenolic oxygen atoms.

2.2 Synthetic procedures

2.2.1 Chlorophenols

*2.2.1.1 2-n-propyl-4-chlorophenol **S6***

15.0 g 2-propylphenol (**S5**, 136.2 g/mol; 0.11 mol) was dissolved in 50 mL acetonitrile and 18.3 g N-chlorosuccinimide (133.53 g/mol; 0.14 mol) was added. The solution was stirred overnight at 50°C. After the quantitative reaction[16] of 2-propylphenol had been verified by ^{1}H NMR, the solution was decanted from precipitated succinimide and the solvent evaporated. The product (R_f = 0.6) was separated from *ortho* chlorination products (R_f = 0.7) and other byproducts by column chromatography (silica gel; dichloromethane). 14.1 g of pure **S6** (170.6 g/mol; 75%) was obtained. ^{1}H NMR (400 MHz, acetone-d6): 6.82 (d, J = 9 Hz, 1H), 7.00 (dd, J_1 = 3 Hz, J_2 = 9 Hz, 1H), 7.08 (d, J = 3 Hz, 1H), 8.47 (s).

2.2.2 (2-hydroxybenzyl)-trimethylammonium iodides

*2.2.2.1 (2-hydroxy-5-nitro-benzyl)-trimethylammonium iodide **S2**.*

8.0 g 4-nitrophenol (**S1**, 139 g/mol, 57.6 mmol) was dissolved in 20 mL 1-propanol. 7.3 mL of a 40% aqueous solution of dimethylamine (45.0 g/mol, 7.9 M, 92 mmol) and 4.3 mL formalin 38% (1.1 g/mol, 30.03 g/mol, 60 mmol) were added. The mixture was refluxed for 14 hours and then left to cool to r. t. over night. Precipitated product was filtered off and dried in vacuum. 8.0 g (71%) of the Mannich base was obtained. ^{1}H NMR (270 MHz, acetone-d6): 8.07 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, H-4), 7.98 (d, J = 3 Hz, 1H, H-6), 6.84 (d, J = 9 Hz, 1H, H-3), 3.87 (s, 2H, ArC$\underline{H}_2$N), 2.40 (s, 6H, N(CH_3)$_2$).

7.45 g (38 mmol) 2-((dimethylamino)-methyl)-4-nitrophenol was dissolved in 20 mL dry THF. 4.75 mL iodomethane (2.28 g/mL, 141.9 g/mol, 76 mmol) was added and the mixture was stirred for three days at r. t. Solvent and excess iodomethane were removed under reduced pressure. 11.0 g (32.5 mmol, 85%) of (2-Hydroxy-5-nitrophenyl)-trimethylammonium iodide **S2** was obtained. ^{1}H NMR (270 MHz, DMSO-d6): 8.39 (d, J = 3 Hz, 1H, Ar), 8.26 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, Ar), 7.12 (d, J = 9 Hz, 1H, Ar), 4.54 (s, 2H, ArC$\underline{H}_2$N), 3.07 (s, 9H, N(C$\underline{H}_3$)$_3$).

*2.2.2.2 Tetraalkylammonium iodide **S11***

3.10 g di(2-hydroxy-5-chlorobenzyl)methane (269.13 g/mol; 11.5 mmol) was heated for 24 hours with 1.46 mL 40% aqueous dimethylamine solution (1 eq.) and 0.83 mL Formalin 38% (1 eq.) in 25 mL of n-propanol. After removal of the solvent under reduced pressure, the mixture was suspended in water. The suspension was made acidic (pH 2) and extracted with dichloromethane to remove unreacted starting material. The aqueous phase was brought to neutral pH and extracted with ethyl acetate. The organic phase was dried and the solvent was evaporated, resulting in 2.6 g of a mixture of single (R_f = 0.6 in TLC on silica gel with CH_2Cl_2/methanol 10:1) and double (R_f = 0.5) substitution products and traces of residual starting material (R_f = 0.8). These were separated by column chromatography (same phases as in TLC), yielding 1.60 g product of single aminomethylation (326 g/mol, 4.9 mmol, 39%) and 0.53 g by-product di(2-hydroxy-3-(2-(dimethylamino)-ethyl)-5-chlorobenzyl)methane.

Mannich base (product of single aminomethylation): ^{1}H NMR (270 MHz, methanol-d4): 7.01-6.99 (convoluted, 2H, $\underline{Ar}CH_2N$), 6.98 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC$\underline{H}$CClCH), 6.88 (d, J = 3 Hz, 1H, CHCHCClC$\underline{H}$), 6.72 (d, J = 9 Hz, 1H, C$\underline{H}$CHCClCH), 3.80 (s, 2H, ArC$\underline{H}_2$Ar), 3.70 (s, 2H, ArC$\underline{H}_2$N), 2.39 (s, 6H, $(CH_3)_2$). Di(2-hydroxy-3-(2-(dimetylamino)ethyl)-5-chlorobenzyl)methane: ^{1}H NMR (270 MHz, methanol-d4): 6.99 (d, J = 3 Hz, 2H, Ar), 6.93 (d, J = 3 Hz, 2H, Ar), 3.87 (s, 2H, ArCH$_2$Ar), 3.70 (s, 4H, ArCH2N), 2.39 (s, 12H, $(CH_3)_2$).

The Mannich base was dissolved in THF and stirred with 0.90 mL of iodomethane (3 eq.) until TLC indicated complete conversion (one week). Solvent and excess reagent were evaporated under reduced pressure, leaving the sufficiently pure iodide in almost quantitative yield. ^{1}H NMR (270 MHz, DMSO-d6): 7.37 (d, J = 3 Hz, 1H, CHCClC$\underline{H}$CCH$_2$), 7.11 (d, J = 3 Hz, 1H, C$\underline{H}$CClCHCCH$_2$), 7.11 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC$\underline{H}$CClCH), 7.03 (d, J = 3 Hz, 1H, CHCHCClC$\underline{H}$), 6.84 (d, J = 9 Hz, 1H, C$\underline{H}$CHCClCH), 4.50 (s, 2H, ArCH$_2$N), 3.89 (s, 2H, ArCH$_2$Ar), 3.05 (s, 6H, $(CH_3)_3$).

*2.2.2.3 Tetraalkylammonium iodide **S8***

1.50 g of **S7** (321.8 g/mol, 4.66 mmol) was treated for 12 hours with 1.2 equivalents of each 37% formalin and 40% aqueous dimethylamine solution in 10 mL n-propanol. The Mannich base precipitated from the alcoholic solution; 1.47 g was collected by filtration after the reaction mixture had been stirred over night at r. t. ^{1}H NMR (250 MHz, acetone-d6): 8.19 (d, J = 3 Hz, 1H, CH2CCHC(NO_2)C<u>H</u>), 7.87 (d, J = 3 Hz, 1H, CH_2CCH!C(NO_2)CH), 7.20 (d, J = 3 Hz, 1H, CH_2CCHCClC<u>H</u>), 6.87 (d, J = 3 Hz, 1H, CH_2CC<u>H</u>CClCH), 4.26 (s, 2H, ArC<u>H</u>$_2$Ar), 3.80 (s, 2H, ArC<u>H</u>$_2$N), 2.95 (s, 6H, N(CH_3)$_2$), 2.52 (t, J = 7 Hz, 2H, C<u>H</u>$_2$$CH_2CH_3$), 1.55 (qt, J_1 = 7 Hz, J_2 = 7 Hz, 2H, CH_2C<u>H</u>$_2$$CH_3$), 0.89 (t, J = 7 Hz, 3H, CH_2CH_2C<u>H</u>$_3$).

The Mannich base (0.66 g, 378.9 g/mol, 1.74 mmol) and 0.11 g of KOH (1.2 eq.) were suspended in 10 mL acetonitrile and 1.0 mL iodomethane (ca. 10 eq.). This mixture was stirred at r. t. until TLC (silica gel, ethyl acetate + 5% formic acid) indicated almost complete conversion of the Mannich base (two days). The solvent and excess reagent were removed under reduced pressure, and the residue was washed with ethyl acetate. 0.87 g of a yellow substance quite soluble in acetone was obtained.[17] ^{1}H NMR (250 MHz, acetone-d6): 8.16 (d, J = 3 Hz, 1H, CH_2CCHC(NO_2)C<u>H</u>), 8.09 (d, J = 3 Hz, 1H, CH_2CC<u>H</u>C(NO_2)CH), 7.18 (d, J = 3 Hz, 1H, CH_2CCHCClC<u>H</u>), 6.84 (d, J = 3 Hz, 1H, CH_2CC<u>H</u>CClCH), 4.51 (s, 2H, ArC<u>H</u>$_2$N), 3.80 (s, 2H, ArC<u>H</u>$_2$Ar), 3.25 (s, 9H, N(CH_3)$_3$), 2.51 (t, J = 7 Hz, 2H, C<u>H</u>$_2$$CH_2CH_3$), 1.54 (qt, J_1 = 7 Hz, J_2 = 7 Hz, 2H, CH_2C<u>H</u>$_2$$CH_3$), 0.88 (t, J = 7 Hz, 3H, CH_2CH_2C<u>H</u>$_3$).

*2.2.2.4 Tetraalkylammonium iodide **S15***

4.30 mL formalin 38% (13.9 M, 59.6 mmol) and 8.2 mL 40% aqueous solution of dimethylamine (7.9 M, 65 mmol) were mixed under stirring. A rapid exothermic reaction was observed. After the mixture had cooled to ambient temperature (one hour), it was added to a solution of 15.0 g of bis-(2-hydroxyphenyl)-methane **S14** (301.34 g/mol, 49.8 mmol) in 150 mL glacial acetic acid. This mixture was stirred and heated to 100°C for 10 hours. Solvent, water and possible excess reagent were removed under reduced pressure, leaving a light yellow oil. By column chromatography (silica gel, ethyl acetate/methanol 2:1) the desired Mannich base (R_f = 0.3) was separated from starting material **S14** (R_f = 0.9) and side products (R_f << 0.3). 13.84 g (358.4 g/mol, 38.62 mmol, 78%) of the precursor of **S15** were obtained. ^{1}H NMR (500 MHz, acetone-d6): 8.12 (d, J = 3 Hz, 1H, CHCHC(NO_2)C<u>H</u>), 7.90 (dd, J_1 = 9 Hz, J_2 = 3 Hz, CHC<u>H</u>C(NO_2)CH), 7.25 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 6.92 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 6.71 (d, J = 9 Hz, C<u>H</u>CHC(NO_2)CH), 4.18 (s, 2H,

ArC<u>H</u>$_2$Ar), 3.90 (s, 2H, ArCH_2N), 2.79 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.48 (tq, $J_1 = J_2 = 7$ Hz, 1H, $CH_3C\underline{H}CH_2$), 1.51 (qd, $J_1 = J_2 = 7$ Hz, 2H, CHC<u>H</u>$_2$CH$_3$), 1.15 (d, $J = 7$ Hz, 3H, CHC<u>H</u>$_3$), 0.76 (t, $J = 8$ Hz, 3H, $CH_2C\underline{H}_3$).

13.8 g of the precursor of **S15** (38.5 mmol) was dissolved in 50 mL of THF. 5 mL of iodomethane (>2 eq.) were added and the mixture was stirred at r. t. until TLC indicated complete conversion of the Mannich base (one week). Solvent and excess reagent were removed under reduced pressure, leaving 17.7 g (92%) of the tetraalkylammonium salt **S15** crystallizing as a foam. ^{1}H NMR (270 MHz, DMSO-d6): 7.99 (dd, $J_1 = 9$ Hz, $J_2 = 3$ Hz, CHC<u>H</u>C(NO$_2$)CH), 7.73 (d, $J = 3$ Hz, 1H, CHCHC(NO$_2$)C<u>H</u>), 7.15 (d, $J = 3$ Hz, 1H, CHC(s-Bu)CH), 7.10 (d, $J = 3$ Hz, 1H, CHC(s-Bu)CH), 6.95 (d, $J = 9$ Hz, C<u>H</u>CHC(NO$_2$)CH), 4.53 (s, 2H, ArC<u>H</u>$_2$N), 3.96 (s, 2H, ArC<u>H</u>$_2$Ar), 3.04 (s, 9H, N(C<u>H</u>$_3$)$_3$), 2.51 (tq, $J_1 = J_2 = 7$ Hz, 1H, $CH_3C\underline{H}CH_2$), 1.49 (qd, $J_1 = J_2 = 7$ Hz, 2H, CHC<u>H</u>$_2$CH$_3$), 1.14 (d, $J = 7$ Hz, 3H, CHC<u>H</u>$_3$), 0.74 (t, $J = 8$ Hz, 3H, $CH_2C\underline{H}_3$).

*2.2.2.5 Tetraalkylammonium iodide **S18***

0.62 mL formalin 38% (13.9 M, 8.6 mmol) and 1.32 mL 40% aqueous solution of dimethylamine (7.9 M, 10.4 mmol) were mixed. A rapid exothermic reaction was observed. After the mixture had cooled to ambient temperature, it was added to a suspension of 2.0 g of bis-(2-hydroxyphenyl)-methane **S17** (345.4 g/mol, 5.79 mmol) in n-propanol. This mixture was stirred and heated to 90°C. By TLC (silica gel, dichloromethane/methanol 10:1), aminoalkylation of **S17** ($R_f = 0.75$) to the Mannich base ($R_f = 0.25$) was monitored. After 10 hours, approx. half conversion was detected. A second batch of reagent mixture as described above was added. Another 10 hours later the conversion was complete. Solvent, excess reagent and water were removed under reduced pressure leaving the sufficiently pure Mannich base (2.40 g, 402.5 g/mol, 5.96 mmol, ~100%) as a foamy yellow residue. ^{1}H NMR (250 MHz, acetone-d6): 8.12 (d, $J = 3$ Hz, 1H, CHC(NO$_2$)CH), 7.86 (d, $J = 3$ Hz, 1H, CHC(NO$_2$)CH), 7.01 (s, 1H, CHC(s-Bu)CH), 6.77 (s, 1H, CHC(s-Bu)CH), 4.22 (s, 2H, ArC<u>H</u>$_2$Ar), 3.83 (s, 2H, ArC<u>H</u>$_2$N), 3.72 (t, $J = 6$ Hz, 2H, C<u>H</u>$_2$CH$_2$OH), 2.89 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.81 (t, $J = 6$ Hz, 2H, CH$_2$C<u>H</u>$_2$OH), 2.45 (qt, $J_1 = J_2 = 7$ Hz, 1H, $CH_3C\underline{H}CH_2$), 1.52 (dq, $J_1 = J_2 = 7$ Hz, 2H, CHC<u>H</u>$_2$CH$_3$), 1.16 (d, $J = 7$ Hz, 3H, CHC<u>H</u>$_3$), 0.78 (t, $J = 8$ Hz, 3H, $CH_2C\underline{H}_3$).

The Mannich base (2.40 g, 402.5 g/mol, 5.96 mmol) was dissolved in THF, and 0.75.5 mL iodomethane (2.5 eq.) and 0.34 g KOH (1 eq.) were added. This mixture was stirred at r. t. for one week. After evaporation of solvent and excess reagent an orange colored foam was obtained. This was taken up in acetone. A small amount of an insoluble component (potassium iodide?) was filtered off. After evaporation of the solvent, 4.0 g of substance remained. ^{1}H NMR (250 MHz, DMSO-d6): 8.14 (d, $J = 3$ Hz, 1H, CHC(NO$_2$)CH), 8.00 (d, $J = 3$ Hz,

1H, CHC(NO_2)CH), 6.87 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 6.73 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 4.45 (s, 2H, ArC$\underline{H}_2$N), 3.79 (s, 2H, ArC$\underline{H}_2$Ar), 3.52 (t, J = 6 Hz, 2H, C$\underline{H}_2$$CH_2$OH), 3.02 (s, 9H, N(C$\underline{H}_3$)$_3$), 2.67 (t, J = 6 Hz, 2H, CH_2C$\underline{H}_2$OH), 2.40 (qt, J_1 = J_2 = 7 Hz, 1H, CH_3C$\underline{H}$$CH_2$), 1.45 (dq, J_1 = J_2 = 7 Hz, 2H, CHC$\underline{H}_2$$CH_3$), 1.09 (d, J = 7 Hz, 3H, CHC$\underline{H}_3$), 0.73 (t, J = 8 Hz, 3H, CH_2C$\underline{H}_3$).

2.2.3 Bis-(2-hydroxyphenyl)methanes (bisphenols)

*2.2.3.1 (2-hydroxy-5-nitrobenzyl)-(2-hydroxy-5-s-butly)-methane (**S14**)*

40.0 g of (2-hydroxy-5-nitrobenzyl)-trimethylammonium iodide (**S2**, 338 g/mol, 118 mmol) and 35.4 g 4-*s*-butylphenol (150.2 g/mol, 236 mmol) were suspended in 250 mL of water. 18.9 g NaOH (40 g/mol, 472 mmol) were added and the mixture was refluxed (PEG bath 120°C) for 20 hours. Then the solution was made acidic and steam distilled until no more 4-*s*-butylphenol was carried over (3 liters of water). Finally, the residue was concentrated to approx. 200 mL water volume and extracted with ethyl acetate. The organic phase was dried and the solvent evaporated. The dark residue contained almost exclusively the desired product. Recrystallization from toluene readily furnished sufficiently pure **S14** (21.1 g, 301.34 g/mol, 70.0 mmol, 60%). ^{1}H NMR (250 MHz, acetone-d6): 8.00 (d, J = 3 Hz, 1H, CHCHC(NO_2)C$\underline{H}$), 7.98 (dd, J_1 = 8 Hz, J_2 = 3 Hz, CHC$\underline{H}$C(NO_2)CH), 7.08 (d, J = 3 Hz, 1H, CHCHC(*s*-Bu)C$\underline{H}$), 7.03 (d, J = 8 Hz, C$\underline{H}$CHC(NO_2)CH), 6.94 (dd, J_1 = 3 Hz, J_2 = 8 Hz, 1H, CHC$\underline{H}$C(*s*-Bu)CH), 6.83 (d, J = 8 Hz, 1H, CHCHC(*s*-Bu)C$\underline{H}$), 4.01 (s, 2H, ArC$\underline{H}_2$Ar), 2.48 (qt, J_1 = J_2 = 7 Hz, 1H, CH_3C$\underline{H}$$CH_2$), 1.52 (dq, J_1 = J_2 = 7 Hz, 2H, CHC$\underline{H}_2$$CH_3$), 1.16 (d, J = 7 Hz, 3H, CHC$\underline{H}_3$), 0.77 (t, J = 7 Hz, 3H, CH_2C$\underline{H}_3$).

*2.2.3.2 (2-hydroxy-5-nitro-phenyl)-methanol (**S4a**)*

In a flask equipped with inert gas inlet and a reflux condenser attached to a bubble counter, 10 g of (2-hydroxy-5-nitrobenzyl)-trimethylammonium iodide (29.6 mmol) were dissolved in solution prepared from 30 g KOH and 100 mL water and heated at reflux. Progress of the reaction was monitored by checking for trimethylamine in the exiting inert gas stream by a wet pH paper. After gas evolution had ceased (2 days), the solution was acidified and the mixture was extracted with ethyl acetate several times. The combined organic phases were dried with sodium sulfate and the solvent was evaporated. 4.0 g raw product were obtained that were subjected to column chromatography (silica gel; dichloromethane/methanol 10:1). 1.6 g (9.5 mmol; 32%) (2-hydroxy-5-nitro-phenyl)methanol were obtained.

*2.2.3.3 (2-hydroxy-5-nitrobenzyl)-(2-hydroxy-3-propyl-5-chlorobenzyl)-methane (**S7**)*

4.8 g 2-hydroxy-5-nitro-phenylmethanol (28 mmol) and 5.3 g 2-propyl-4-chlorophenol (31 mmol) in 8 mL chlorobenzene were stirred and heated to 120°C. After a homogenous mixture had been obtained, a catalytic amount of 4-toluenesulfonic acid (0.05 g, 1.3 mmol) was added. After three hours, the solvent was distilled off in high vacuum. The residue was dissolved in ethyl acetate. The solution was washed with water and dried. Column chromatography (silica gel; dichloromethane/methanol 20:1) afforded 2.0 g of a brown product. This brown product was treated with as much refluxing dichloromethane as was necessary to completely dissolve the dark byproducts. The solution was separated from the colorless crystalline product (2-hydroxy-5-nitrobenzyl)-(2-hydroxy-3-propyl-5-chlorobenzyl)methane: 1.5 g (4.7 mmol, 17 %). ^{1}H NMR (250 MHz, acetone-d6): 8.05 (d, J = 3 Hz, 1H, CHCHC(NO_2)C<u>H</u>), 8.00 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC<u>H</u>C(NO_2)CH), 7.06 (d, J = 3 Hz, 1H, CHCClCH), 7.04 (d, J = 9 Hz, 1H, C<u>H</u>CHC(NO_2)CH), 7.01 (d, J = 3 Hz, 1H, CHCClCH), 4.03 (s, 2H, ArC<u>H</u>$_2$Ar), 2.62 (t, J = 7 Hz, 2H, C<u>H</u>$_2$$CH_2CH_3$), 1.60 (qt, J_1 = 7 Hz, J_2 = 7 Hz, 2H, CH_2C<u>H</u>$_2$$CH_3$), 0.93 (t, J = 7 Hz, 3H, CH_2CH_2C<u>H</u>$_3$).

2.2.4 (2-hydroxyphenyl)-acetic acids

*2.2.4.1 2-(2-hydroxy-5-nitrophenyl)-acetic acid(**S3**)*

In a flask equipped with inert gas inlet, a reflux condenser attached to a bubble counter, 3.0 g (8.9 mmol) (2-hydroxy-5-chloro-benzyl)-trimethylammonium iodide was stirred and heated to 100°C with 0.5 g KOH (9 mmol) and 0.87 g (13 mmol) KCN in the presence of 0.35 g 18-crown-6 (15%) in DMF solution (10 mL). Progress of the reaction was monitored by checking for trimethylamine in the exiting inert gas stream by a wet pH paper. After evolution of trimethylamine had ceased (5 hours), DMF was distilled off in high vacuum. The residue was taken up in water, the pH was adjusted to slightly acidic, and the mixture was extracted with ethyl acetate several times. The combined organic phases were dried with sodium sulfate and the solvent was evaporated. Column chromatography (silica gel; ethyl acetate/hexane 3:1) afforded 1.05 g (5.9 mmol; 70%) 2-(2-hydroxy-5-nitrophenyl)acetonitrile. ^{1}H-NMR (270 MHz, acetone-d6): 8.28 (d, J=2.5Hz, 1H), 8.11 (dd, J_1=9.2Hz, J_2=2.1Hz, 1H), 7.15 (d, J=9.2Hz, 1H), 3.93 (s, 2H).

In a flask equipped with inert gas inlet and a reflux condenser attached to a bubble counter, 1.75 g (9.8 mmol) 2-(2-hydroxy-5-nitrophenyl)acetonitrile was suspended in an aqueous sodium hydroxide solution

(10% w/w) and refluxed (120°C bath temperature) until evolution of ammonia ceased (10 hours). The solution was acidified and the mixture was extracted with ethyl acetate several times. The combined organic phases were dried with sodium sulfate and the solvent was evaporated. Column chromatography (silica gel; dichloromethane/methanol 14:1 +3 vol.-% HCOOH) afforded 1.73 g (8.8 mmol, 90%) 2-(2-hydroxy-5-nitrophenyl)acetic acid. Mp.: 158-159°C (Lit. 160 - 162°C)[18]. ^{1}H NMR (270 MHz, acetone-d6): 8.18 (d, J = 3 Hz, 1H), 8.06 (dd, J_1 = 9 Hz, J_2=3 Hz, 1H), 7.04 (d, J = 9 Hz, 1H), 3.76 (s, 2H).

*2.2.4.2 Carboxylic acid **S9***

Analog to the procedure described in section 2.2.4.1 0.46 g of the quaternary ammonium salt **S8** obtained according to section 2.2.2.3 (maximum amount[19] of the organic component is 0.92 mmol) was treated with 0.06 g K^{13}CN (0.90 mmol) and 0.10 g 18-crown-6 (0.38 mmol) for three hours at 120°C. After acidic work-up and extraction with ethyl acetate, 0.40 g raw product formally corresponding to 1.1 mmol of the nitrile (369.7 g/mol, 120%) was obtained. ^{1}H NMR (250 MHz, acetone-d6): 8.14 (d, J = 3 Hz, 1H, $CH_2CCHC(NO_2)C\underline{H}$), 8.08 (d, J = 3 Hz, 1H, $CH_2CC\underline{H}C(NO_2)CH$), 7.15 (d, J = 3 Hz, 1H, $CH_2CCHCClC\underline{H}$), 6.98 (d, J = 3 Hz, 1H, $CH_2CC\underline{H}CClCH$), 4.00 (s, 2H, $ArC\underline{H}_2Ar$), 3.85 (d, J = 8 Hz, 2H, $ArC\underline{H}_2{}^{13}CN$), 2.60 (t, J = 7 Hz, 2H, $C\underline{H}_2CH_2CH_3$), 1.59 (qt, J_1 = 7 Hz, J_2 = 7 Hz, 2H, $CH_2C\underline{H}_2CH_3$), 0.93 (t, J = 7 Hz, 3H, $CH_2CH_2C\underline{H}_3$).

The nitrile was hydrolyzed by refluxing for three hours in 10 mL of 12.5% aqueous NaOH. After acidification and extraction with ethyl acetate, as described in section 2.2.4.1, 0.40 g of raw product was obtained. The raw product was dissolved in 10 mL dichloromethane and 3 mL hexane was added (avoiding immediate precipitation). The solution was left over night at -15°C. 0.11 g of crystals of the acid **S9** were collected by filtration. ^{1}H NMR (250 MHz, acetone-d6): 8.05-8.03 (2H, $C\underline{H}C(NO_2)C\underline{H}$), 7.14 (d, J = 3 Hz, 1H, $CH_2CCHCClC\underline{H}$), 7.03 (d, J = 3 Hz, 1H, $CH_2CC\underline{H}CClCH$), 4.06 (s, 2H, $ArC\underline{H}_2Ar$), 3.80 (d, J = 8 Hz, 2H, $ArC\underline{H}_2{}^{13}COOH$), 2.63 (t, J = 7 Hz, 2H, $C\underline{H}_2CH_2CH_3$), 1.61 (qt, J_1 = 7 Hz, J_2 = 7 Hz, 2H, $CH_2C\underline{H}_2CH_3$), 0.94 (t, J = 7 Hz, 3H, $CH_2CH_2C\underline{H}_3$).

*2.2.4.3 Carboxylic acid **S12***

Analog to the procedure described in section 2.2.4.1, 1.10 g (2.35 mmol) of iodide **S11** was treated with 0.14 g KOH (1 eq.), 0.17 g KCN (1 eq.) and 0.09 g 18-crown-6 (0.15 eq.) for 6 hours at gradually increasing temperatures between 70-110°C. The raw product was 0.95 g of brown oil. After purification by column chromatography (silica gel, ethyl acetate, R_f = 0.8) 0.50 g (308 g/mol, 1.6 mmol, 68%) of the nitrile intermediate was obtained. ^{1}H NMR (270 MHz, $CDCl_3$): 7.64 (s, 1H, $ArO\underline{H}$), 7.22 (d, J = 3 Hz, 1H, Ar), 7.20 (d,

J = 3 Hz, 1H, Ar), 7.15 (d, J = 3 Hz, 1H, Ar), 7.05 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC<u>H</u>CClCH), 6.70 (d, J = 9 Hz, 1H, C<u>H</u>CHCClCH), 6.20 (s, 1H, ArO<u>H</u>), 3.81 (s, 2H, ArC<u>H</u>$_2$Ar), 3.64 (s, 2H, ArC<u>H</u>$_2$CN).

The nitrile was hydrolyzed analog to the description in 2.2.4.1. Chromatographic isolation of the carboxylic acid was done with silica gel and ethyl acetate/hexane 2:1 + 4% formic acid as eluent.

0.34 g (327 g/mol, 1.0 mmol, 63%) of the carboxylic acid **S12** was obtained. ^{1}H NMR (270 MHz, acetone-d6): 9.52 (s (broad), 2H, O<u>H</u>), 7.23 (d, J = 3 Hz, 1H, Ar), 7.15 (d, J = 3 Hz, 1H, Ar), 7.13 (d, J = 3 Hz, 1H, Ar), 7.09 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC<u>H</u>CClCH), 6.91 (d, J = 9 Hz, 1H, C<u>H</u>CHCClCH), 3.95 (s, 2H, ArC<u>H</u>$_2$Ar), 3.69 (s, 2H, ArC<u>H</u>$_2$COOH).

*2.2.4.4 Carboxylic acid **S16***

21.5 g tetramethylammonium iodide **S15** (500.38 g/mol, 43 mmol), 5.0 g KCN (65 g/mol, 75 mmol), 2.0 g KOH (56 g/mol, 36 mmol) and 2.0 g 18-crown-6 (ca. 9 mmol) were heated to 90°C in 40 mL DMF for nine hours. After removal of the solvent in high vacuum, water was added and pH was adjusted to slightly acidic. The product was extracted with ethyl acetate. The raw product was filtered through silica gel with ethyl acetate/hexane 1:1 and 2% formic acid as solvent. 10.0 g of the nitrile (340.4 g/mol, 29.4 mmol, 68%) was obtained. ^{1}H NMR (270 MHz, acetone-d$_6$): 8.03 (s, 1H, CHCHC(NO$_2$)C<u>H</u>), 8.01 (d, J = 9 Hz, CHC<u>H</u>C(NO$_2$)CH), 7.10 (s, 2H, C<u>H</u>C(*s*-Bu)C<u>H</u>), 7.08 (d, J = 9 Hz, C<u>H</u>CHC(NO$_2$)CH), 4.07 (s, 2H, ArC<u>H</u>$_2$Ar), 3.82 (s, 2H, ArC<u>H</u>$_2$CN), 2.52 (qt, J_1 = J_2 = 7 Hz, 1H, CH$_3$C<u>H</u>CH$_2$), 1.53 (dq, J_1 = J_2 = 7 Hz, 2H, CHC<u>H</u>$_2$CH$_3$), 1.17 (d, J = 7 Hz, 3H, CHC<u>H</u>$_3$), 0.77 (t, J = 7 Hz, 3H, CH$_2$C<u>H</u>$_3$).

The nitrile was suspended in 35 mL 10% KOH and refluxed for 24 hours. The solution was made acidic and extracted with ethyl acetate. The organic phase was dried, and the solvent was evaporated. The raw product was filtered through silica gel (solvent ethyl acetate and 2 % formic acid). The filtrate contained 8.5 g of substance. The product was isolated by column chromatography (silica gel, dichloromethane/methanol 10:1 to 5:1). 7.5 g carboxylic acid **S16** (359.4 g/mol, 20.9 mmol, 71 %) was obtained. ^{1}H NMR (500 MHz, acetone-d6): 8.03 (d, J = 3 Hz, 1H, CHCHC(NO$_2$)C<u>H</u>), 7.99 (dd, J_1 = 9 Hz, J_2 = 3 Hz, CHC<u>H</u>C(NO$_2$)CH), 7.03 (d, J = 9 Hz, C<u>H</u>CHC(NO$_2$)CH), 7.03 (s, 2H, CHC(*s*-Bu)CH), 6.95 (s, 2H, CHC(*s*-Bu)CH), 4.05 (s, 2H, ArC<u>H</u>$_2$Ar), 3.71 (s, 2H, ArC<u>H</u>$_2$COOH), 2.49 (tq, J_1 = J_2 = 7 Hz, 1H, CH$_3$C<u>H</u>CH$_2$), 1.52 (qd, J_1 = J_2 = 7 Hz, 2H, CHC<u>H</u>$_2$CH$_3$), 1.16 (d, J = 7 Hz, 3H, CHC<u>H</u>$_3$), 0.78 (t, J = 7 Hz, 3H, CH$_2$C<u>H</u>$_3$).

*2.2.4.5 Carboxylic acid **S19***

4.0 g of the quaternary ammonium salt **S18** obtained according to section 2.2.2.5 (maximum amount[17] of the organic component was 6.0 mmol) was dissolved in 40 mL DMF. 1.0 g KCN (2.5 eq.) and 1.0 g 18-crown-6 (ca. 0.6 eq.) were added. The mixture was stirred and heated to 90°C- 110°C for 18 hours until the evolution of ammonia had ceased. DMF was removed by distillation in high vacuum followed by an acidic aqueous work-up and extraction with ethyl acetate. 4.4 g of a brown oil was obtained. This was dissolved in ethyl acetate and filtered through silica gel. After evaporation of the solvent, 1.6 g of a golden colored foam remained.

The nitrile (1.6 g) was hydrolyzed by refluxing in 20% NaOH until evolution of ammonia had ceased (12 hours). The solution was made acidic and extracted with acetyl acetate. The raw product was subjected to column chromatography (silica gel, ethyl acetate /hexane 1:1 with 2% formic acid). 0.80 g of the carboxylic acid **S19** was isolated. ^{1}H NMR (500 MHz, acetone-d6): 8.15 (d, J = 3 Hz, 1H, CHC(NO_2)CH), 8.04 (d, J = 3 Hz, 1H, CHC(NO_2)CH), 7.15 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 6.87 (d, J = 3 Hz, 1H, CHC(s-Bu)CH), 4.05 (s, 2H, ArC$\underline{H}_2$Ar), 3.94 (t, J = 6 Hz, 2H, C$\underline{H}_2$$CH_2$OH), 3.76 (s, 2H, ArC$\underline{H}_2$COOH), 2.90 (t, J = 6 Hz, 2H, CH_2C$\underline{H}_2$OH), 2.49 (qt, J_1 = J_2 = 7 Hz, 1H, CH_3C$\underline{H}$$CH_2$), 1.53 (dq, J_1 = J_2 = 7 Hz, 2H, CHC$\underline{H}_2$$CH_3$), 1.15 (d, J = 7 Hz, 3H, CHC$\underline{H}_3$), 0.77 (t, J = 7 Hz, 3H, CH_2C$\underline{H}_3$).

2.2.5 2-(2-hydroxyphenyl)-ethanol derivatives

*2.2.5.1 Reduction of the carboxylic acid **S16** to alcohol*

5.50 g of the carboxylic acid **S16** (359.4 g/mol; 15.3 mmol) was dissolved in 15 mL of dry THF. 14 mL of a 2 M solution of $BH_3S(CH_3)_2$ (28 mmol) was added dropwise. Gas evolution was observed. Conversion of the acid (R_f = 0.5) to the alcohol (R_f = 0.8) and a side product (R_f = 0.9) was monitored by TLC (silica gel; dichloromethane/methanol 10:1). The solution was stirred over night at room temperature. Water was added carefully. The solution was acidified (pH 1-4) and extracted with ethyl acetate. The organic phase was dried with sodium sulfate and the solvent was evaporated. The product was separated from the less polar side product by column chromatography (silica gel; ethyl acetate /hexane 1:1): 4.58 g (345.4 g/mol; 13.3 mmol; 86%). ^{1}H NMR (250 MHz, acetone-d6): 8.05 (d, J = 3 Hz, 1H, CHCHC(NO_2)C$\underline{H}$), 7.96 (dd, J_1 = 9 Hz, J_2 = 3 Hz, 1H, CHC$\underline{H}$C(NO_2)CH), 7.01 (s, 1H, CHC(*s*-Bu)CHCCl), 6.98 (d, J = 9 Hz, 1H, C$\underline{H}$CHC(NO_2)CH), 6.84 (s, 1H, CHC(*s*-Bu)CHCCl), 4.01 (s, 2H, ArC$\underline{H}_2$Ar), 3.90 (t, J = 6 Hz, 2H, C$\underline{H}_2$$CH_2$OH), 2.86 (t, J = 7 Hz, 2H,

CH2C<u>H</u>$_2$OH), 2.45 (qt, $J_1 = J_2 = 7$ Hz, 1H, $CH_3C\underline{H}CH_2$), 1.51 (dq, $J_1 = J_2 = 7$ Hz, 2H, $CHC\underline{H}_2CH_3$), 1.14 (d, $J = 7$ Hz, 3H, $CHC\underline{H}_3$), 0.76 (t, $J = 7$ Hz, 3H, $CH_2C\underline{H}_3$).

2.3 Spectroscopy

2.3.1 Instrumentation, sample preparation

The equipment and general procedures for combined NMR and UV-vis spectroscopy were previously described in Refs. 20.

Solutions of monoanions were prepared from the neutral compounds by addition of a slight excess of tetraethylammonium hydroxide solution in methanol. Solvent and water were removed in vacuum (10 mL flask, rotary evaporator, < 10 mbar residual pressure). Further drying was achieved by repeated addition and evaporation of dry CH_2Cl_2.under reduced pressure (rotavap < 10 mbar). The residue was taken up in CD_2Cl_2 and transferred to a flat bottomed quartz tube ("UVNMR cuvette", see Ref. 30) with PTFE insert for combined NMR and UV-vis spectroscopy. In cases of evidence for the presence of di-anionic species (according to any part of the combined spectroscopy), split equivalent amounts of the corresponding neutral compound were added as a solution in CD_2Cl_2 until the monoanion was present exclusively (a titration guided by NMR or the combined spectroscopy, see Refs. 20).

For subsequent experiments in CDF_2Cl/CDF_3 as solvent, sample solutions were transferred to medium wall sized NMR tubes equipped with *J*. Young valves and PTFE inserts. After evaporation of CD_2Cl_2 in vacuum, CDF_2Cl/CDF_3 was added by vacuum transfer.

Partial deuteration in mobile proton sites was generally done in NMR tubes equipped with *J*. Young valves. From a sample solution prepared as described above, the solvent was removed and MeOD (0.1 mL) was added to the residual sample substance. Methanol was removed again under reduced pressure and the solvent desired for spectroscopic studies was reintroduced.

Solute concentrations typically were 1 mM for the combined spectroscopy and up to 3 mM if only NMR spectra were to be obtained. For the former, concentrations were practically limited by optical density. For the latter, increase in concentration was principally motivated by sensitivity concerns but limitations arose from issues of solubility (solute-solute interactions).

2.3.2 Evaluation

^{1}H and ^{13}C NMR chemical shifts were determined using solvent signals as internal standard, and converted to the conventional TMS scale: $\delta(C\underline{H}DCl_2)$ = 5.32 ppm, $\delta(^{13}\underline{C}D_2Cl_2)$ = 53.5 ppm, $\delta(C\underline{H}F_2Cl)$ = 7.18 ppm, $\delta(^{13}\underline{C}DF_2Cl)$ = 117.37 ppm. Proton decoupled ^{13}C NMR spectra were obtained using the inverse-gated decoupling scheme allowing us to employ signal integration in the attribution to different isotopologues and their isotopomers (if any).

In systems with coupled H-bonds, integral signal intensities of the isotopologues/isotopomers were analyzed in order to assess isotope fractionation effects. ^{1}H NMR, by nature, can give information only on species with at least one hydrogen atom in the H-bond chain (if only the H-bonding protons are considered). While ^{13}C NMR, e.g. of the carboxylic carbon, can provide that information for all species whose individual signal contributions are resolved, spectra tend to suffer from low signal to noise ratios - or are not available at all, namely for species with natural carbon isotope abundance.

The information gap on the fully deuterated species that ^{1}H NMR leaves can be filled if one assumes, in an approximation, that the nature of the light particle in one binding site does not affect the potential of the particles in the other sites of the H-bond chain (negligence of the vicinal isotope effect). This simplification seems justified because in all the systems studied in this work, the vicinal isotope effects (in terms of chemical shifts) are at least an order of magnitude smaller than the shifts between signals of the protons in the individual binding sites. Neglecting the vicinal isotope means that the equilibrium constants of the following two exchange reactions (in which e.g. “HH” identifies a system of two coupled H-bonds containing only protons as light particles) are identical:

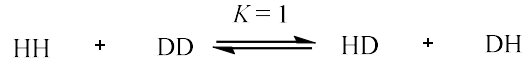
$$HH + D^{+} \underset{}{\overset{K_H}{\rightleftharpoons}} HD + H^{+}$$

$$DH + D^{+} \underset{}{\overset{K_D}{\rightleftharpoons}} DD + H^{+}$$

For this situation, it can readily be deduced from the chemical equilibrium law that the concentration of the fully deuterated species is (within the limitations of the above approximation):

$c(DD) \approx c(HD)\, c(DH) / c(HH)$

which corresponds to:



Analogue relationships can be established also for systems with three coupled H-bonds. Thus, (relative) concentrations of isotopologues/isotopomers (Table S 8) were established from integral intensities of proton as well as carbon spectra (Table S 7). Whenever redundant information was present in the spectra (e.g. the intensity of the two proton signals in a system of the type HH), averaged values were used for evaluation. Furthermore, deuterium fractions in individual light particle sites for isotopologues/isotopomers and isotope fractionation factors were calculated wherever possible (Table S 9). The site specific deuterium fractions are defined as follows, for the example of the bond to the carboxylic group in **4b**:

$x_{\underline{D}LL} = c_{\underline{D}LL} / (c_{\underline{H}LL} + c_{\underline{D}LL})$

where the concentrations containing the light particle wildcard L = H or D expand to, e.g.:

$c_{\underline{D}LL} = c_{\underline{D}HH} + c_{\underline{D}HD} + c_{\underline{D}DH} + c_{\underline{D}DD}$

and (relative) values for the concentrations *c* are derived from integral signal intensities.

3 Results

3.1.1 UV-vis absorption spectra of 4-nitrophenols, their anions and homoconjugated anions

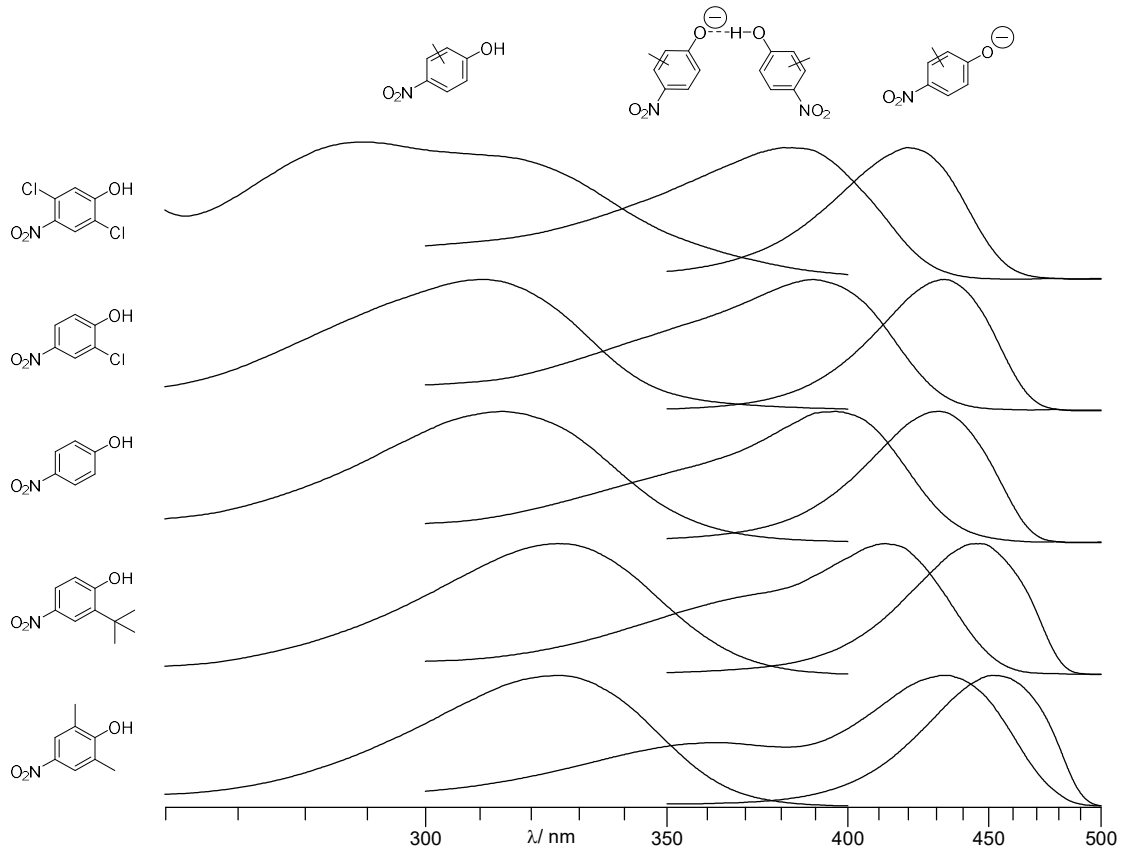


Figure S 6. Absorption spectra of the π-π* transition of 4-nitrophenols, their anions and homoconjugated anions in CD_2Cl_2 solution at 170-180 K. Counter ions are tetraethylammonium. All spectra were normalized to equal maxima. For clarity, traces were reduced to the essential spectral regions. Note how the dual band character of homoconjugated anions seems to become particular obvious in the last two (sterically hindered) species.

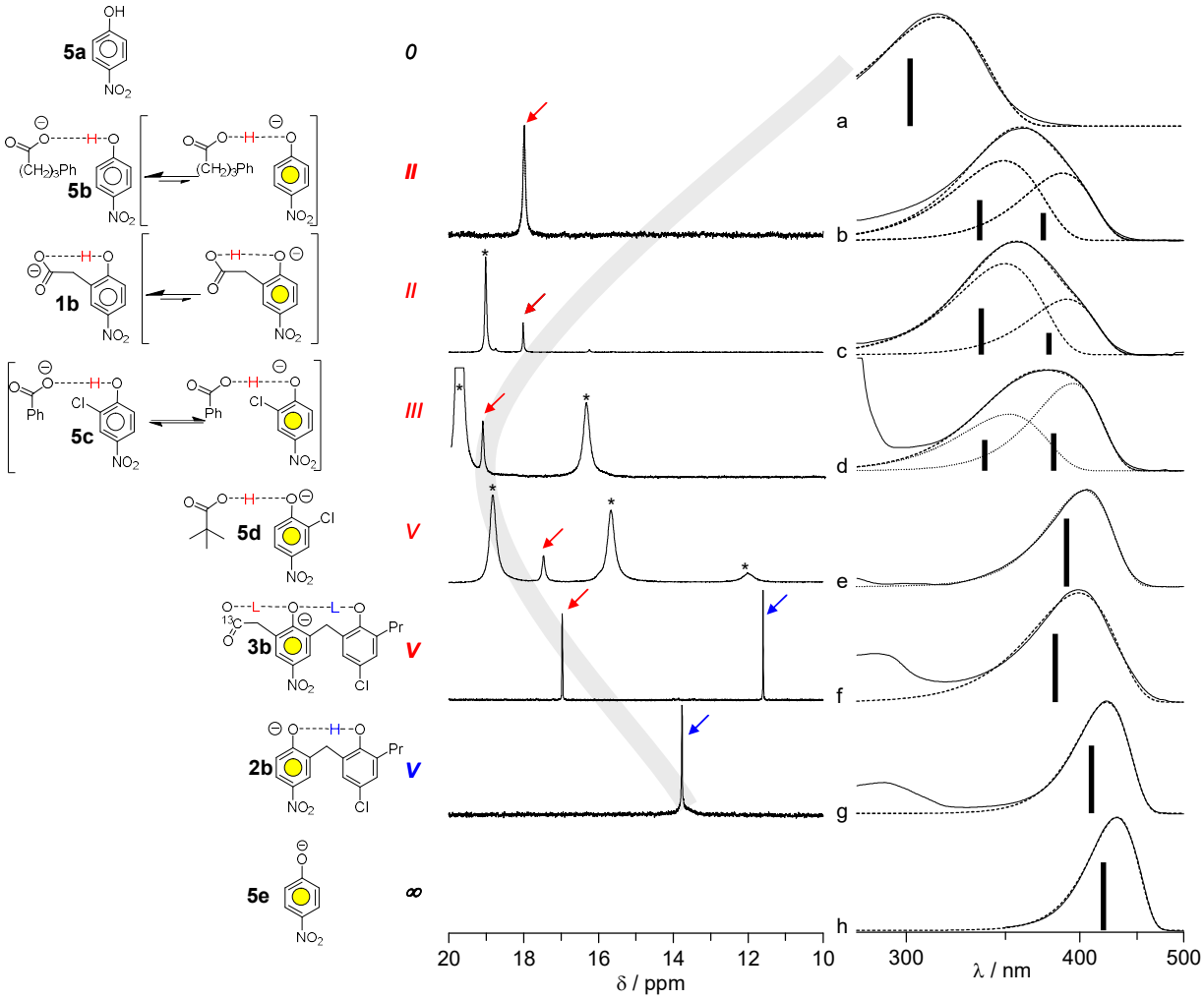


Figure S 7. UVNMR spectra (simultaneously obtained ^{1}H NMR and UV-vis absorption spectra of CD_2Cl_2 solution at 180 K) of anions with intramolecular hydrogen bonds (studied in this work) and reference systems **5** (Refs. 20 and 21). The same conventions apply as in Figure 3 of the main text. Diagonal arrows indicate NMR signals that originate from protons in the respective chem. structures and the roman numbers characterize H-bonds according to Figure 4 (**3b**: left proton → left signal; characterization of H-bond to carboxylic group). The eight systems are given in the sequence of progressive deprotonation (increasing anionic character) of the nitrophenolic moieties in the fashion of Figure 4, as evidenced by the red-shift in light absorption and the strong deshielding of the bridging protons culminating in stage *III* (light gray arc in the background is a rough guide for the eye). The minor discontinuity among the optical spectra of **5d** and **3b** could be a direct spectral effect of the ring substituents of the phenolic moieties (see Figure S 6: the spectra of neutral phenols and "free phenolates" display some spectral variations on ring substitution.).

3.2 Tables of spectroscopic data

Table S 3. UV-vis spectroscopic parameters of monoanions 1b-3b, and 4b at various conditions.

species	solvent	T	λ_{max}	$\tilde{\nu}_{COG}$	$\Delta\tilde{\nu}$	$\tilde{\nu}_{blue}$	$\Delta\tilde{\nu}_{blue}$	$\tilde{\nu}_{red}$	$\Delta\tilde{\nu}_{red}$	x_{blue}
1b	CD_2Cl_2	180	360	28550	6180	29800	5030	26750	4070	0.59
2b	CD_2Cl_2	180	422	24410	3090					0
3b	CD_2Cl_2	180	400	25900	4500					0
3b	CDF_2Cl/CDF_3	130	404	26200	5910					0
4b	CD_2Cl_2	210	391	26900	5710					0

Temperatures T are given in Kelvin, wavenumbers $\tilde{\nu}$ in cm^{-1} and wavelength λ in nm. Parameters of UV-vis absorption bands are center of gravity $\tilde{\nu}_{COG}$, half height full width $\Delta\tilde{\nu}$ and wavelength of maximum in absorbance λ_{max}. Results of deconvolution in terms of dual bands are given as center of gravities $\tilde{\nu}_{blue}$ and $\tilde{\nu}_{red}$ and half height widths $\Delta\tilde{\nu}_{blue}$ and $\Delta\tilde{\nu}_{red}$ where the indices denote the components at higher and lower energies, respectively. Relative intensities of the components are given as fraction of integral absorbance E of the higher energy component $x_{blue} = \frac{E_{blue}}{E_{blue} + E_{red}}$.

Table S 4. NMR spectroscopic parameters of monoanions 1b and 2b under various conditions.

Species	Solvent	T	δ(O<u>H</u>O)	δ(<u>C</u>OOHO)	δ(<u>C</u>OODO)- δ(<u>C</u>OOHO)
1b	CD_2Cl_2	180	18.09	175.31	
		170	17.99		
	CDF_2Cl/CDF_3	170	17.32	175.07	-0.075
		120	17.22	175.40	-0.058
2b	CD_2Cl_2	180	13.77		

Temperatures T are given in Kelvin, chemical shifts δ in ppm. For the attribution of chemical shifts, molecular fragments are given in shorthand notation and the observed nucleus is underlined. L = H or D.

Table S 5. NMR spectroscopic parameters of monoanions 3b and 3c under various conditions.

Species	Solvent	T	δ(COOLOLO)				δ(<u>C</u>OOLOLO)			
			<u>H</u>H	H<u>D</u>	H<u>H</u>	D<u>H</u>	HH	HD	DH	DD
3b	CD_2Cl_2	180	17.03	17.13	11.61	11.86	173.92	173.97	173.58	173.62
	CDF_2Cl/CDF_3	130	18.13	18.25	11.72	12.09	178.09	178.15	177.60	177.66
3c	CD_2Cl_2	180	18.77		11.37	11.45				
	CDF_2Cl/CDF_3	150	18.52		10.93					
		140	18.36	18.27	10.81	10.44				

Temperatures T are given in Kelvin, chemical shifts δ in ppm. For the attribution of chemical shifts, molecular fragments are given in shorthand notation and the observed nucleus is underlined. Light particle wildcard L = H or D. The assignment of isotopologues and their isotopomers follows the syntax introduced in main text Figure 3, i.e. "HD" corresponds to a semi-deuterated species with the proton in the hydrogen bond of the carboxylic group.

Table S 6. Mobile proton chemical shifts of monoanion 4b under various conditions.

Species	Solvent	*T*	δ(COOLOLOLO)							
			HHL	HDL	HHL	HHH	HHD	DHL	DHH	DHD
4b	CD_2Cl_2	210	16.94	17.06	11.87			12.14		
	CDF_2Cl/CDF_3	150	17.85	18.00		12.28[a]	12.24[a]		12.65[a]	12.69[a]

Temperatures *T* are given in Kelvin, chemical shifts δ in ppm. For the attribution of chemical shifts, molecular fragments are given in shorthand notation and the observed nucleus is underlined. Light particle wildcard L = H or D. The assignment of isotopologues and their isotopomers follows the syntax introduced in main text Figure 6, i.e. "DHL" corresponds to the signal of a proton in the phenol-phenol H-bond of a partially deuterated species with a deuteron in the H-bond of the carboxylic group and any light particle in the aliphatic alcohol. [a] Signals are asymmetric doublets due to a non-isotope related 0.035 ppm splitting; in each case the weighted average chemical shift is given.

Table S 7. Integral signal intensities *I* in ^{1}H and ^{13}C spectra of species 3b, 3c and 4b.

a.

species	spectrum	solvent	*T*	*I*(COOLOLO)				*I*(^{13}COOLOLO)			
				HH	HD	HH	DH	HH	HD	DH	DD
3b	Figure 3d	CD_2Cl_2	185	28.1	27.9	27.5	16.5	35.1	30.3	17.2	17.4
3b	Figure 3e	CDF_2Cl/CDF_3	130	27.4	29.0	30.9	12.6	32.9	39.5	12.6	14.9
3b	Figure 3f	CDF_2Cl/CDF_3	130	35.4	21.3	34.6	8.7	47.8	35.0	11.7	5.5
3c	Figure 5	CDF_2Cl/CDF_3	140	39.71	15.5	40.32	4.4	n.d.	n.d.	n.d.	n.d.

b.

species	spectrum	solvent	*T*	*I*(COOLOLOLO)							
				HHL	HDL	HHL	HHH	HHD	DHL	DHH	DHD
4b	Figure 6a	CD_2Cl_2	210	30.3	21.6	34.5	n.r.	n.r.	13.6	n.r.	n.r.
4b	Figure 6b	CDF_2Cl/CDF_3	150	35.6	21.1	33.6	14.9	18.7	9.7	4.0	5.7

Light particle wildcard L = H or D. Temperatures *T* are given in Kelvin, intensities in each spectrum are normalized to a sum of 100. The assignment of isotopologues and their isotopomers follows the syntax introduced in the respective main text Figures. Entries n.d. = not determined; n.r. = not resolved.

Table S 8. Abundance of the HD isotopomers/isotopologues of 3b and 3c.

a.

species	spectrum	solvent	*T*	^{1}H NMR				^{13}C NMR			
				x(HH)	*x*(HD)	*x*(DH)	*x*(DD)	*x*(HH)	*x*(HD)	*x*(DH)	*x*(DD)
3b	Figure 3d	CD_2Cl_2	185	0.31	0.31	0.19	0.19	0.35	0.30	0.17	0.17
3b	Figure 3e	CDF_2Cl/CDF_3	130	0.35	0.35	0.15	0.15	0.33	0.40	0.13	0.15
3b	Figure 3f	CDF_2Cl/CDF_3	130	0.50	0.30	0.12	0.08	0.48	0.35	0.12	0.05
3c	Figure 5	CDF_2Cl/CDF_3	140	0.65	0.25	0.073	0.028	n.d.	n.d.	n.d.	n.d.

b.

species	spectrum	solvent	*T*	^{1}H NMR							
				x(HHH)	*x*(HHD)	*x*(HDH)	*x*(HDD)	*x*(DHH)	*x*(DHD)	*x*(DDH)	*x*(DDD)
4b	Figure 6a	CD_2Cl_2	210	*0.42*		*0.28*		*0.18*		*0.12*	
4b	Figure 6b	CDF_2Cl/CDF_3	150	*0.21*	*0.27*	*0.13*	*0.16*	*0.06*	*0.08*	*0.04*	*0.05*

Light particle wildcard L = H or D. See also Section 2.3.2 of this document. Temperatures *T* are given in Kelvin. The assignment of isotopologues and their isotopomers follows the conventions in the respective main text figures. Entries n.d. = not determined. In sub-table b, joined cell entries correspond to sums of abundances e.g. *x*(HHH)+ *x*(HHD) = *x*(HHL) = 0.42.

Table S 9. Deuterium fractions in mobile proton sites and isotope fractionation in 3b, 3c and 4b.

a.

species	spectrum	solvent	*T*	^{1}H NMR				^{13}C NMR			
				x(D<u>L</u>)	*x*(L<u>D</u>)	*x*(L<u>D</u>)/*x*(D<u>L</u>)	*x*(D)	*x*(D<u>L</u>)	*x*(L<u>D</u>)	*x*(L<u>D</u>)/*x*(D<u>L</u>)	*x*(D)
3b	Figure 3d	CD_2Cl_2	185	0.38	0.50	1.3	0.44	0.35	0.48	1.4	0.41
3b	Figure 3e	CDF_2Cl/CDF_3	130	0.29	0.51	1.8	0.40	0.28	0.54	2.0	0.41
3b	Figure 3f	CDF_2Cl/CDF_3	130	0.20	0.38	1.9	0.29	0.17	0.40	2.4	0.29
3c	Figure 5	CDF_2Cl/CDF_3	140	0.1	0.28	2.8	0.19	n.d.	n.d.	n.d.	n.d.

b.

species	spectrum	solvent	*T*	^{1}H NMR					
				x(D<u>L</u>L)	*x*(L<u>D</u>L)	*x*(LL<u>D</u>)	*x*(D)	*x*(L<u>D</u>L)/*x*(DLL)	*x*(LLD)/*x*(LDL)
4b	Figure 6a	CD_2Cl_2	210	0.28	0.42	n.d.	n.d.	n.d.	1.5
4b	Figure 6b	CDF_2Cl/CDF_3	150	0.22	0.37	0.56	0.39	2.5	1.7

Light particle wildcard L = H or D. See also Section 2.3.2 of this document. Temperatures *T* are given in Kelvin. The assignment of isotopologues and their isotopomers follows the conventions introduced in respective Figures, i.e. *x*(D<u>L</u>) is the deuterium fraction in the H-bond of the carboxylic group; *x*(D) is the all-over deuterium fraction. Entries n.d. = not determined.

Table S 10. Spectroscopic parameters of the π-π* absorption bands of 4-nitrophenols, their tetraethylammonium salts and homoconjugated anions in CD_2Cl_2 solution at 170-180 K.

	neutral form				tetraethylammonium phenolates				tetraethylammonium hydrogenbisphenolates		
	λ_{max}	$\tilde{\nu}_{COG}$	λ_{COG}	$\Delta\tilde{\nu}$	λ_{max}	$\tilde{\nu}_{COG}$	λ_{COG}	$\Delta\tilde{\nu}$	λ_{max}	$\tilde{\nu}_{COG}$	$\Delta\tilde{\nu}$
2,5-dichloro-4-nitrophenol	298	33480	299	7620	420	24460	409	2990	383	28550	5320
2-chloro-4-nitrophenol	310	33220	299	6000	433	23540	425	2660	390	27360	5410
4-nitrophenol	314	32860	304	5990	431	23790	420	2820	395	27270	5129
2-tert-butyl-4-nitrophenol	325	31690	316	5470	445	23040	434	2680	412	26220	5090
2,6-dimethyl-4-nitrophenol	325	31820	314	5490	453	22650	442	2720		25950	

Parameters are center of gravity $\tilde{\nu}_{COG}$, half height full width $\Delta\tilde{\nu}$ and wavelength of maximum in absorbance λ_{max}. Wavenumbers $\tilde{\nu}$ are given in cm^{-1} and wavelengths λ in nm.

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