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Synthesis and acid–base properties of (1*H*-benzimidazol-2-yl-methyl)phosphonate ($Bimp^{2-}$). Evidence for intramolecular hydrogen-bond formation in aqueous solution between (N-1)H and the phosphonate group †

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The synthesis of (1*H*-benzimidazol-2-yl-methyl)phosphonic acid, $H_2(Bimp)^{\pm}$, is described: 2-chloromethylbenzimidazole was reacted with ethylchloroformate to give 1-carboethoxy-2-chloromethylbenzimidazole which was treated with trimethyl phosphite and after hydrolysis with aqueous HBr $H_2(Bimp)^{\pm}$ was obtained. In $H_2(Bimp)^{\pm}$ one proton is at the N-3 site and the other at the phosphonate group; both acidity constants were determined in aqueous solution by potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$) and this furnished the pK_a values of 5.37 ± 0.02 and 7.41 ± 0.02, respectively. The acidity constant for the release of the primary proton from the $P(O)(OH)_2$ group of $H_3(Bimp)^+$ was estimated: $pK_a = 1.5 \pm 0.2$. Moreover, $Bimp^{2-}$ can be further deprotonated at its neutral (N-1/N-3)H site to give the benzimidazolate residue, but this reaction occurs only in strongly alkaline solution (KOH); application of the H_- scale developed by G. Yagil (*J. Phys. Chem.*, 1967, **71**, 1034) together with UV spectrophotometric measurements gave $pK_a = 14.65 \pm 0.12$. Comparisons with acidity constants taken from the literature show that this latter pK_a value is far too large and this allows the conclusion that an intramolecular hydrogen bond is formed between the (N-1/N-3)H site and the phosphonate group of $Bimp^{2-}$; the formation degree of this hydrogen-bonded isomer is estimated to be 98 ± 2%. The general relevance of this and the other results are shortly discussed and the species distribution for the Bimp system in dependence on pH is provided.

1 Introduction

Purines and benzimidazoles are very similar in shape, as demonstrated, *e.g.*, for 4-methyl-1*H*-benzimidazole and adenine derivatives,[1] and consequently, benzimidazole is also sometimes addressed as 1,3-dideazapurine. Hence, it is no surprise that the biological properties of benzimidazole and its derivatives were[2] and still are studied intensively[3–5] and are also used in drug design.[6–8] For example, several 2-[(4-chlorophenoxy)methyl]-benzimidazoles act as selective neuropeptide receptor antagonists,[4] 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole is a powerful inhibitor of casein kinase-2 (CK-2)[9] and also of mRNA production in eukaryotic cells,[10] and the 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl) derivative of 4-methyl-1*H*-benzimidazole is an excellent inhibitor of HIV-1 reverse transcriptase in an *in vitro* assay.[11]

Similarly, simple phosphonate derivatives[12,13] also show many astonishing biological properties: *e.g.*, acetonylphosphonate (AnP^{2-}) is an inhibitor of acetoacetate decarboxylase[14] and it also acts on several other enzyme systems.[15] AnP[16] is further a substrate for the P–C bond-cleaving enzyme phosphonoacetaldehyde hydrolase, where it undergoes enzyme-catalyzed hydrolysis to hydrogen phosphate and acetone.[17] Phosphonoformate (PFA^{3-}; Foscarnet; $^-OOC–PO_3^{2-}$) is another bioactive molecule of this kind, which, *e.g.*, reduces the Na^+-phosphate uptake in osteoclasts, the primary cells responsible for bone resorption,[18] and which also has pronounced antiviral effects,[19] *e.g.*, against human herpes virus 6 (*cf.* ref. 20) and 7 (*cf.* ref. 21) and also against the human immunodeficiency virus 1.[22]

Observations of the indicated kind gave rise to the synthesis of compounds which combine heterocycles, like nucleobase derivatives, with a phosphonate residue,[23] and among these, *e.g.*, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA),[24] also known as Adefovir, is active against the hepatitis B virus and the human immunodeficiency viruses.[19,25] Indeed, its bis-(pivaloyloxymethyl)ester (Adefovir dipivoxyl) was very recently approved by the US Food and Drug Administration (FDA) for use in hepatitis B therapy.[26]

The mentioned biological relevance of benzimidazole derivatives and of phosphonates prompted us to continue our previous work on phosphonate derivatives with a heterocyclic ring system[27,28] and to prepare (1*H*-benzimidazol-2-yl-methyl)-phosphonate ($Bimp^{2-}$; see Fig. 1 in Section 2.1).[16] We report now the synthesis of this compound as well as its detailed acid–base properties; the availability of this information is a precondition for meaningful biochemical and pharmacological studies. The results for the acid–base equilibria turned out to be very interesting because they provide evidence for the formation of an intramolecular hydrogen bond in aqueous solution.

2 Results and discussion

2.1 Synthesis of $H_2(Bimp)^{\pm}$

The route leading to $H_2(Bimp)^{\pm}$ (**4**) is depicted in Fig. 1. The reaction of 2-chloromethylbenzimidazole (**1**) with ethylchloroformate led to 1-carboethoxy-2-chloromethylbenzimidazole (**2**).[29] It was necessary to block the 1-position of **1** in order to prevent self-condensation.[30] The phosphonate derivative **3** was

† Electronic supplementary information (ESI) available: figure showing absorption spectra of Bimp in alkaline aqueous solution. See http://www.rsc.org/suppdata/ob/b3/b301281f/

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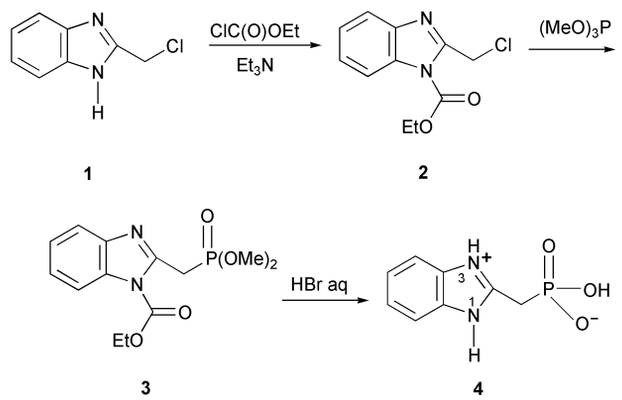

Fig. 1 Synthetic route to (1*H*-benzimidazol-2-yl-methyl)phosphonic acid (**4**), which exists in aqueous solution in the zwitterionic form, $H_2(Bimp)^{\pm}$, shown above.

obtained in an Arbusov reaction of **2** with trimethyl phosphite; to improve the yield the phosphite was used in excess.

The structure of **3** was confirmed by elemental analysis and spectroscopy. The analysis of the ^{1}H NMR spectra reveals the two methyl ester groups as being chemically equivalent; that is, the signals of the six protons from the two methyl groups result in a doublet with a chemical shift of δ = 3.79 ppm and a coupling constant $^3J_{HP}$ = 11 Hz. The two protons of the CH_2-P methylene group at the 2-position of the benzimidazole ring give rise to a doublet with δ = 4.05 ppm and $^2J_{HP}$ = 22 Hz. The three multiplets above 7 ppm are attributed to the aromatic ring system, *i.e.*, δ = 7.33 ppm to the C-5 and C-6 protons, δ = 7.75 ppm to the C-4 proton, and δ = 7.92 ppm to the C-7 proton. The observed proton triplet (δ = 1.54 ppm, $^3J_{HH}$ = 7.1 Hz) and the quartet (δ = 4.59 ppm, $^3J_{HH}$ = 7.1 Hz) are characteristic for an ethyl ester group. The singlet at δ = 24.48 ppm in the ^{31}P NMR spectrum is characteristic for phosphonic compounds. The IR spectrum displays an intensive band at 1259 cm^{-1}, characteristic of the phosphonate group (P=O). The bands at 1056 and 1028 cm^{-1} correspond to a P–O–C group, and the band at 1749 cm^{-1} to a C=O unit.

The phosphonic acid **4**, *i.e.* $H_2(Bimp)^{\pm}$, was synthesized in a single step by hydrolysis of the ester **3** with 40% aqueous hydrobromic acid. In this reaction also the carboethoxy group was removed. The structure of **4** was confirmed by elemental analysis and spectroscopy. The ^{1}H NMR spectrum taken in a solution of trifluoroacetic acid shows two groups of signals: One group appears as a doublet at δ = 3.99 ppm with the coupling constant $^2J_{HP}$ = 22 Hz and this is assigned to the CH_2-P protons. The other group with a multiplet at δ = 7.40–7.70 ppm is attributed to the aromatic ring protons. The singlet at δ = 16.68 ppm in the ^{31}P NMR spectrum confirms further the structure of **4**. The IR spectrum shows a broad band at 2500 cm^{-1} and a sharp one at 1248 cm^{-1} and these are characteristic for a P–O–H and a P=O group, respectively.

2.2 Definition of the acidity constants of $H_3(Bimp)^+$

A phosphonate group may accept two protons and the benzimidazole residue one. Hence, if protonated, for the release of these protons from $H_3(Bimp)^+$ the following equilibria need to be defined:

$$H_3(Bimp)^+ \rightleftharpoons H_2(Bimp)^{\pm} + H^+ \quad (1a)$$

$$K^H_{H_3(Bimp)} = [H_2(Bimp)^{\pm}][H^+]/[H_3(Bimp)^+] \quad (1b)$$

$$H_2(Bimp)^{\pm} \rightleftharpoons H(Bimp)^- + H^+ \quad (2a)$$

$$K^H_{H_2(Bimp)} = [H(Bimp)^-][H^+]/[H_2(Bimp)^{\pm}] \quad (2b)$$

$$H(Bimp)^- \rightleftharpoons Bimp^{2-} + H^+ \quad (3a)$$

$$K^H_{H(Bimp)} = [Bimp^{2-}][H^+]/[H(Bimp)^-] \quad (3b)$$

The first proton according to equilibrium (1a) is released from the $-P(O)(OH)_2$ group leading to $-P(O)_2(OH)^-$. The resulting zwitterionic $H_2(Bimp)^{\pm}$ species is next deprotonated at its (N-3)H$^+$ site [eqn. (2)] which leads to $H(Bimp)^-$ where the proton is at the phosphonate group. The release of this final proton occurs according to equilibrium (3) giving $Bimp^{2-}$.

However, from benzimidazole[31] and related species[32] it is known that the remaining proton at the N-1/N-3 site may also be removed in strongly alkaline solution giving a negatively charged benzimidazolate residue. This is expressed in equilibrium (4a):

$$Bimp^{2-} \rightleftharpoons (Bimp–H)^{3-} + H^+ \quad (4a)$$

$$K^H_{Bimp} = [(Bimp–H)^{3-}][H^+]/[Bimp^{2-}] \quad (4b)$$

The determination of the various acidity constants is described below.

2.3 Acidity constants for the deprotonation reactions of $H_3(Bimp)^+$ to $Bimp^{2-}$

The acidity constants according to equilibria (2a) and (3a) could be determined by potentiometric pH titrations (Section 4.3). The corresponding results are listed in Table 1 together with some related data.[31,33–46] Comparison of the constants provided in entries 1–4 with our results for $H_3(Bimp)^+$, which are summarized in entry 5, demonstrates that the site attributions for the various deprotonation reactions agree with those given in Section 2.2.

For the determination of a low pK_a value by potentiometric pH titration high ligand concentrations are needed[38] and such quantities of Bimp were not available. However, an estimation for the acidity constant, $K^H_{H_3(Bimp)}$, due to equilibrium (1a) is possible in the following way:

Entries 4 and 6–9 of Table 1 list the acidity constants for the release of the two protons from the $-P(O)(OH)_2$ group for systems where the remaining moieties of the ligands are not affected themselves by an acid–base reaction. In accord herewith are the differences defined in equation (5),

$$\Delta\, pK_a = pK^H_{P(O)_2(OH)} - pK^H_{P(O)(OH)_2} \quad (5)$$

within the error limits identical for the phosphonates of entries 4 and 6–8 giving on average ΔpK_a = 5.45 ± 0.07. Moreover, the difference for the phosphate ligand UMP [entry 9; (6.15 ± 0.01) − (0.7 ± 0.3) = 5.45 ± 0.3] demonstrates that this property is the same for phosphonates and phosphates, even though the latter are somewhat less basic.

Similarly, if the corresponding difference according to equation (5) is formed for phosphonate derivatives, which carry an aromatic-ring residue that also can accept a proton (in entries 10–12 this is an adenine moiety protonated at N-1) the ΔpK_a values [eqn. (5)] are again identical within their error limits giving on average $\Delta pK_a'$ = 5.69 ± 0.06, a value which again agrees within the error limits with that of the phosphate ligand AMP [entry 13; (6.21 ± 0.01) − (0.4 ± 0.2) = 5.81 ± 0.2]. This latter average is by 0.24 ± 0.09 [= (5.69 ± 0.06) − (5.45 ± 0.07)] larger because of the repulsion which the first proton released from the $-P(O)(OH)_2$ group experiences due to the positive charge located at the protonated aromatic moiety. For the release of the second proton from the $-P(O)_2(OH)^-$ group no such effect exists since at the corresponding pH values the aromatic residue is also deprotonated.

If one considers molecular models of PMEA[16] and Bimp, one sees that the distance between the (N-3)H$^+$ site and the phosphonate group in Bimp is considerably shorter (about one half) than the corresponding one involving (N-1)H$^+$ in PMEA and its derivatives. We double therefore the repulsion effect[47]

Table 1 Negative logarithms of the acidity constants [eqns. (1)–(4)] for aqueous solutions (25 °C) of $H_3(Bimp)^+$ as well as of some related species[a,b]

		pK_a for the site[c]				
No.	Acid[16]	$-P(O)(OH)_2$	$(N\text{-}3)H^+$ or $(N\text{-}1)H^+$	$-P(O)_2^-(OH)$	(N-1)H	Ref.
1	$H(Bi)^+$		5.63 ± 0.01		12.86 ± 0.05[d]	31, 33
2	$H(1MBi)^+$		5.67 ± 0.01			33
3	$H(2MBi)^+$		5.9		13.18 ± 0.15[e]	31, 34
4	$CH_3P(O)(OH)_2$	2.10 ± 0.03		7.51 ± 0.01		12
5	$H_3(Bimp)^+$	1.5 ± 0.2[f]	5.37 ± 0.02	7.41 ± 0.02	14.65 ± 0.12	—
6	$CH_3CH_2–O–CH_2–P(O)(OH)_2$	1.57 ± 0.15		7.02 ± 0.01		35, 36
7	$(CH_3)_3N^+–CH_2CH_2–O–CH_2–P(O)(OH)_2$	1.11 ± 0.12		6.57 ± 0.01		35, 37
8	$H_2(PMEDAPy)^+$	1.14 ± 0.15		6.62 ± 0.01		35
9	$H_2(UMP)$	0.7 ± 0.3		6.15 ± 0.01		38
10	$H_3(PMEA)^+$	1.22 ± 0.13[g]	4.16 ± 0.02	6.90 ± 0.01		36, 39[g]
11	$H_3(dPMEA)^+$	1.98 ± 0.13	4.17 ± 0.02	7.69 ± 0.01		40
12	$H_3(dPEEA)^+$	2.06 ± 0.08	4.17 ± 0.01	7.75 ± 0.01		41, 42
13	$H_3(AMP)^+$	0.4 ± 0.2[g]	3.84 ± 0.02	6.21 ± 0.01		43,[g] 44

[a] The error limits given, if nothing else is mentioned, are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] So-called practical, mixed or Brønsted constants are listed (see also Section 4.3); the $pK^H_{(N\text{-}1)H}$ values (column 6) are based on the H_- scale as defined by Yagil[31] and these constants were measured by UV spectrophotometry. If nothing else is mentioned the acidity constants were determined by potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$). In those instances where $pK_a < 1$ or $pK_a > 13$ I was higher (see legend for Fig. 2 in Section 2.4). [c] $(N\text{-}3)H^+$ refers to benzimidazole derivatives and $(N\text{-}1)H^+$ to adenine derivatives. [d] This value is from Yagil;[31] it agrees well with those of Walba and Isensee (12.78)[45] and Walba and Ruiz-Velasco (12.80).[46] [e] Yagil's result[31] of 13.18 ± 0.05 is relatively close to the value (13.4) given by Krishnamurthy *et al.*[34] We prefer Yagil's result since it was measured and defined in the same way as our result for Bimp (see Section 4.4). However, we increased the error limit to ±0.15 to include the average (13.3) of the two values[31,34] into the limit and also to be on the safe side with our interpretations. [f] Estimated value; see text in Section 2.3. [g] Determined by ^{1}H-NMR shift measurements.[39,43]

and estimate for $pK^H_{H_3(Bimp)} = pK^H_{H(Bimp)} - \Delta pK_a - [2 \times (0.24 \pm 0.09)] = (7.41 \pm 0.02) - (5.45 \pm 0.07) - (0.48 \pm 0.13) = 1.48 \pm 0.15$ and use the value 1.5 ± 0.2; this estimate is listed in entry 5 of Table 1.

2.4 Acidity constant for the deprotonation of $Bimp^{2-}$

Since $Bimp^{2-}$ is a symmetrical species, the proton can be located either at N-1 or at N-3, written below as (N-1/N-3)H, which also means that in the deprotonated state the negative charge at the benzimidazole ring is distributed between these sites. This means further that the protonation reaction at the ring of $(Bimp–H)^{3-}$ is favored by a factor of two [*cf.* with eqn. (4)] and therefore the intrinsic acidity of (N-1)H is actually described by the microconstant $pk = pK^H_{Bimp} - 0.3$. Similarly, if one considers the deprotonation of a monoprotonated benzimidazole residue or of benzimidazole itself, the (N-1)H and (N-3)H sites, carrying in total a charge of +1 and written below as $(N\text{-}1/N\text{-}3)H_2^+$, are again equal and consequently, the deprotonation reaction is favored by a factor of two, making the compound more acidic; here the pk of the intrinsic micro acidity constant is obtained[33] by adding 0.3. However, since in the present context the comparisons are made with unsubstituted benzimidazole or its 2-substituted derivatives, the symmetrical properties of the compounds, which appear in entries 1, 3 and 5 of Table 1, are identical, the statistical factors thus do not need to be considered because they cancel. In other words, for the comparisons to be made, the macro acidity constants as defined for equilibria (2a) and (4a) may be used directly.

Knowing that the pK_a value for the deprotonation of benzimidazole[31,45,46] is approximately 12.9, one had to expect that for the deprotonation of Bimp $pK^H_{Bimp} > 12.5$ holds. For the determination of an acidity constant of this size potentiometric pH titrations are not applicable. Therefore, we used spectrophotometric measurements[33,48] as we had done previously for the determination of the acidity constant of monoprotonated 5,6-dinitrobenzimidazole which has a rather low pK_a value.[33]

We recorded the UV spectra of Bimp in the pH range above 11.5 in the wavelength range 220 to 320 nm, an example of part of such an experiment is shown in the figure of the ESI,† but used for the evaluation of the data only the absorptions at ≥260 nm, since it is known[31] that OH^- begins to absorb light at 230 nm (and below). It soon became evident from the experiments that the pK_a value of $Bimp^{2-}$ was beyond the pH range accessible with a pH meter (see Section 4.4). It was Schwarzenbach and Sulzberger[49] who first developed a H_- scale based on indicators to quantify the activity of OH^- beyond pH 14, a method in analogy to the H_0 scale for strongly acidic solutions which was originally conceived by Hammett and coworkers.[50] These early attempts[49] were hampered by solubility problems of the indicators, but these were overcome by Yagil[31] who listed H_- data up to 18.2, which corresponds to a 15 M KOH solution at 25 °C, and therefore we used his H_- scale.[51]

In Fig. 2 the absorption of a Bimp solution is plotted as a function of pH; we use here the term "pH" also above 14 because with a pH meter and a glass electrode the "activity" is measured[52] and it is also the OH^- activity which is defined by the H_- scale. This means, the acidity constants given are so-called practical constants,[53] also known as mixed or Brønsted constants.[52–54] In Fig. 2 the plots of absorption *versus* pH are shown for the wavelengths at 260, 277 and 287 nm; the absorption difference is most pronounced at 287 nm and therefore, for this wavelength the most exact results are expected. However, it is evident from Fig. 2 that with the weighted mean of the three results for pK^H_{Bimp} the data points at all three wavelengths can excellently be fitted (see also Section 4.4). The final result $pK^H_{Bimp} = 14.65 \pm 0.12$, listed in Table 1 in column 6 of entry 5, is the average of three independent series of experiments.

A comparison of the acidity constants listed in entries 1, 3 and 5 of Table 1 is revealing:

(i) Substitution of a hydrogen in position 2 of benzimidazole by a methyl group enhances the basicity of the N-1/N-3 site by about 0.3 pK units (= 5.9 − 5.63). This result is corroborated by observations made with pyridines.[55]

(ii) It is important to note that within the error limits the same increase in basicity of about 0.3 pK units (= 13.18 − 12.86) is observed if the release of the proton from (N-1/N-3)H is considered, and in fact, this is expected because the electronic effect of the methyl group should be the same for both mentioned cases.

(iii) The observed acidification of $(N\text{-}1/N\text{-}3)H^+$ by the replacement of a hydrogen in the 2-methyl group by a PO_3H^- unit may at first appear surprising, but it is in accord with

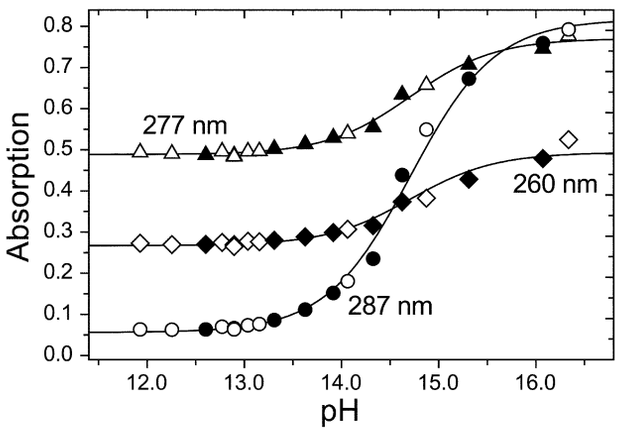

Fig. 2 The UV absorption spectra of (1*H*-benzimidazol-2-ylmethyl)phosphonate (Bimp) in aqueous solution, as shown in part in the figure of the ESI,† were evaluated at 260 (◇,◆), 277 (△,▲) and 287 nm (○,●) as a function of pH. These evaluations furnished the following acidity constants for the (N-1/N-3)H deprotonation of $Bimp^{2-}$, *i.e.* $pK^{H}_{Bimp} = 14.90 \pm 0.06$ (1σ; 260 nm), 14.70 ± 0.04 (1σ; 277 nm) and 14.67 ± 0.03 (1σ; 287 nm), giving as the weighted mean for this experiment $pK^{H}_{Bimp} = 14.71 \pm 0.19$ (3σ) (25 °C; I = 0.1 M, *i.e.* KCl was added to those solutions with [KOH] < 0.1 M) (see also Section 4.4). The solid curves shown are the computer calculated best fits for the various wavelengths through the experimental data points obtained at pH 11.93, 12.26, 12.60, 12.77, 12.90, 12.90, 13.04, 13.16, 13.31, 13.63 ([KOH] = 0.455 M), 13.92 ([KOH] = 0.727 M), 14.06 ([KOH] = 0.909 M), 14.33 ([KOH] = 1.364 M), 14.62 ([KOH] = 2.273 M), 14.87 ([KOH] = 3.182 M), 15.31 ([KOH] = 4.546 M), 16.07 ([KOH] = 7.273 M), and 16.34 ([KOH] = 8.182 M) (from left to right) by using the mentioned average of the acidity constant. The eight solid points in each curve are those that correspond to the spectra shown in the figure of the ESI.† It needs to be noted that the pH values below 13.60 were measured with a pH meter equipped with a glass electrode and where necessary they were corrected for the "alkali error" (see Section 4.4). The pH values above 13.60 were calculated from the KOH concentration employed by using the H_- scale provided by Yagil[31] (see also Sections 2.4 and 4.4).

the well-known electron-withdrawing effect of this unit.[56] For example, substitution of the C-bound H-atom in HCOOH (pK_a = 3.58)[57] by PO_3H^- gives $HOOC–PO_3H^-$ (phosphonoformic acid) with pK_a = 3.61 for the carboxylic acid ionization.[56] This means, the electron-withdrawing effect of the PO_3H^- unit and the influence of the now also present negative charge cancel each other. To give a further example, let us consider the substitution of a C-bound H-atom in $CH_3NH_3^+$ (pK_a = 10.7)[58] by a PO_3^{2-} group; the resulting $^+H_3NCH_2–PO_3^{2-}$ [(aminomethyl)-phosphonate] has pK_a = 10.08 for the deprotonation of the ammonium group,[56] which means an acidification of 0.6 pK units, and thus the electron-withdrawing properties of the PO_3^{2-} unit clearly dominate over the charge effect which should operate in the opposite direction.

(iv) To conclude, for the release of the proton from (N-1/N-3)H to give the benzimidazolate residue, one also expects an acidification in the order of about 0.5 pK units, yet the observation is to the contrary, *i.e.*, there is a large basicity enhancement of *ca.* 1.5 pK_a units [$(14.65 \pm 0.12) - (13.18 \pm 0.15) = 1.5 \pm 0.2$]. In other words, the pK_a value for the deprotonation of the benzimidazole ring in $Bimp^{2-}$ is far too high.

2.5 Evidence for the formation of an intramolecular hydrogen bond between (N-1/N-3)H and the phosphonate group in $Bimp^{2-}$

The only way to rationalize the described (at the end of Section 2.4) enhanced basicity or better, reduced acidity, is to postulate the occurrence of an intramolecular hydrogen bond.[59] Evidently, if the (N-1/N-3)H site forms a hydrogen bond with the PO_3^{2-} residue, a six-membered "chelate" results and this would clearly inhibit the release of the proton from (N-1/N-3)H and thus the formation of the benzimidazolate residue.

To be able to judge the situation in more detail it is necessary to estimate the expected pK_a value for the release of the proton from (N-1/N-3)H in $Bimp^{2-}$ under conditions where no hydrogen bond with the neighboring phosphonate group is formed. The basic value for such an estimation is clearly the corresponding acidity constant of 2-methylbenzimidazole (2MBi), $pK^{H}_{2MBi} = 13.18 \pm 0.15$ (Table 1, entry 3, column 6). This value needs to be corrected, firstly, for the electron-withdrawing effect of the PO_3^{2-} group which was discussed in point (iii) of the terminating paragraph of Section 2.4, and secondly, for the charge effect of the PO_3^{2-} group:

(1) Based on the discussion in points (ii) and (iii) of the terminating paragraph in Section 2.4, it is evident that the effect of a substituent at position 2 of benzimidazole influences the acid–base properties of N-1 and N-3 to the same extent. This means, the electron-withdrawing effect of the PO_3^{2-} unit, which gives rise to an acidification of the (N-1/N-3)H_2^+ site as follows from the comparison of the acidity constants of $H(2MBi)^+$ and $H_2(Bimp)^{\pm}$ (Table 1, entries 3 and 5) and which amounts to $\Delta pK_{a/electronic} = (5.37 \pm 0.02) - 5.9 = -0.53$, to which we attribute the generous error limit of ±0.15, also holds for the (N-1/N-3)H site.

(2) The charge effect may be estimated by comparing the acidity constants for the release of the proton from monoprotonated ethylamine, $^+H_3NCH_2CH_3$, $pK_a = 10.66 \pm 0.05$ (25 °C; I close to 0.1 M; error limit estimated),[58] and from monoprotonated 2-aminoethylphosphonate, $^+H_3NCH_2CH_2–PO_3^{2-}$, $pK_a = 11.01 \pm 0.05$ (25 °C; I = 0.1 M),[60] which gives $\Delta pK_{a/charge} = (11.01 \pm 0.05) - (10.66 \pm 0.05) = 0.35 \pm 0.07$. There are five atoms between the H-atom and the negative charge in $Bimp^{2-}$ and the same holds for 2-aminoethylphosphonate. One could argue that the charge effect in $Bimp^{2-}$ regarding the release of H^+ is 2−/0 whereas in $^+H_3NCH_2CH_2–PO_3^{2-}$ it is 2−/+; if at all, this would lead to an overestimation of the charge effect, but one could also argue that this would be compensated by the electron-withdrawing effect of PO_3^{2-}, though this is diminished by the presence of another CH_2 group instead of the aromatic ring system. In any case, to be on the safe side, we enlarge the error limit and use $\Delta pK^{*}_{a/charge} = 0.35 \pm 0.15$.

Application of the two correction terms to pK^{H}_{2MBi} leads to the expected acidity constant for $Bimp^{2-}$ in its "open" form in which no hydrogen bonds exist, *i.e.*, $pK^{H}_{Bimp/op} = pK^{H}_{2MBi} + \Delta pK_{a/electronic} + \Delta pK^{*}_{a/charge} = (13.18 \pm 0.15) - (0.53 \pm 0.15) + (0.35 \pm 0.15) = 13.00 \pm 0.26$. Consequently, the difference, $\log \Delta = pK^{H}_{Bimp} - pK^{H}_{Bimp/op} = (14.65 \pm 0.12) - (13.00 \pm 0.26) = 1.65 \pm 0.29$, between the measured acidity constant and the estimated one is real and may thus be attributed to intramolecular hydrogen bonding.

Furthermore, the extent of the difference $\log \Delta$ is a quantitative measure[59] for the formation degree of the "chelate" involving the hydrogen bond. Hence, if we define the "open" isomer, *i.e.* the $Bimp^{2-}$ species without a hydrogen bond, as $Bimp_{op}^{2-}$ and the closed species with the intramolecular hydrogen bond between (N-1/N-3)H and the PO_3^{2-} group as $(Bimp)_{NHO}^{2-}$, we may consider the position of the intramolecular equilibrium (6a). The dimensionless equilibrium constant K_I [eqn. (6b)] can be calculated by following known routes.[13,44,61] The interrelation between the mentioned $\log \Delta$ and K_I is given by equation (7), and the percentage of the isomer closed by the hydrogen bond follows from equation (8).

$$(Bimp)_{op}^{2-} \rightleftharpoons (Bimp)_{NHO}^{2-} \quad (6a)$$

$$K_I = [(Bimp)_{NHO}^{2-}]/[(Bimp)_{op}^{2-}] \quad (6b)$$

$$K_I = 10^{\log \Delta} - 1 \quad (7)$$

$$\%(Bimp)_{NHO}^{2-} = 100K_I/(1 + K_I) \quad (8)$$

Table 2 Extent of intramolecular hydrogen bond formation in the $Bimp^{2-}$ and in related systems as calculated from acidity constants. Given is the extent of the reduced acidity log Δ (see text in Section 2.5), the resulting intramolecular and dimensionless equilibrium constant K_I [eqns. (6) and (7)], and the percentage of the "chelated" (= closed) isomer in aqueous solution at 25 °C[a,b]

No.	H-bonded isomer	log Δ	K_I	% closed
1[c]	$(Bimp)^{2-}_{NHO}$	1.65 ± 0.29	43.67 ± 29.83	98 ± 2
2	$(6Umpa)^{2-}_{NHO}$	0.85 ± 0.20	6.08 ± 3.26	86 ± 7
3	$(6Urca)^{-}_{NHO}$	1.27 ± 0.37	17.6 ± 15.9	95 ± 5
4	$H(5Urca)_{OHO}$	1.08 ± 0.51	11.0 ± 14.1	92 ± 10

[a] The data for entry 1 are from the text in Section 2.5; here $I > 0.1$ M (see legend of Fig. 2). The data for entries 2–4 are from ref. 59 ($I = 0.1$ M). [b] For the error limits see footnote *a* of Table 1. The error limits (3σ) of all the above given derived data were calculated according to the error propagation after Gauss. [c] See also note 62.

Hence, one obtains $K_I = 10^{(1.65 \pm 0.29)} - 1 = 43.67 \pm 29.83$ and a formation degree of 97.8 ± 1.5% for $(Bimp)^{2-}_{NHO}$.[62]

Such intramolecular hydrogen-bond formation in aqueous solution has been observed before, *e.g.*, for 6-uracilmethylphosphonate ($6Umpa^{2-}$) which forms a six-membered "chelate" very similar to the one quantified now, as well as for 6-uracilcarboxylate ($6Urca^{-}$) and the monoprotonated form of 5-uracilcarboxylate ($5Urca^{-}$), *i.e.*, 5-uracilcarboxylic acid [H(5Urca)]; the corresponding structures of the anions are shown in Fig. 3. To allow comparisons these previous results[59] are summarized together with the present one in Table 2.

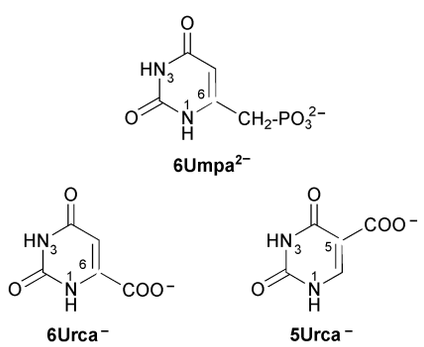

Fig. 3 Chemical structures of 6-uracilmethylphosphonate (= uracil-6-ylmethylphosphonate = $6Umpa^{2-}$) as well as of 6-uracilcarboxylate (= uracil-6-carboxylate = orotate = $6Urca^{-}$) and 5-uracilcarboxylate (= uracil-5-carboxylate = isoorotate = $5Urca^{-}$).

The examples given in entries 1, 2 and 4 of Table 2 allow the formation of six-membered "chelates" involving a H-atom; in the case of entry 3 a five-membered ring is formed. In accord herewith, the formation degree of the hydrogen-bonded isomers in all examples of Table 2 is large, *i.e.*, of the order of 85 to 98%. This contrasts with several examples, where also in aqueous solution, hydrogen-bonded "macrochelates" involving nucleotides were observed, the formation degree being of the order of 40%.[63]

For two of the species given in Table 2 X-ray crystal structure analyses exist for the corresponding protonated compounds, *i.e.*, for the acids. In the case of H_2(6Umpa) intermolecular hydrogen bonding dominates in the solid state,[59] but still (N-1)H and a phosphonic acid oxygen are directed toward each other (intramolecular distance 2.83 Å) thus supporting the observations in solution, where the negatively charged phosphonate group is of course a much better hydrogen acceptor. For H(6Urca), *i.e.*, orotic acid (Fig. 3), the distance between the hydrogen atom of the (N-1)H site and the carbonyl oxygen of the (C-6)COOH group amounts to 2.337 Å,[59] clearly indicating intramolecular hydrogen bonding, which of course will again be further promoted by deprotonation of the COOH group as observed in aqueous solution.

3 Conclusions

The present study shows that the release of the first proton from $H_3(Bimp)^+$ is nearly four pK units separated from the release of the next one (see Table 1, entry 5). Similarly, the acidity constants for the $H(Bimp)^-$ and $Bimp^{2-}$ species are about seven pK units apart and thus, these deprotonation reactions evidently cannot affect each other. Only the deprotonation of $H_2(Bimp)^{\pm}$ and $H(Bimp)^-$ occur with pK_a values of 5.37 and 7.41 in a relatively narrow pH range, but even here the separation amounts to about two pK units and this means that the species $H(Bimp)^-$, which is part of both connected equilibria (2a) and (3a), still can be formed to about 84% as is seen in Fig. 4 which provides the species distribution for the Bimp system as a function of pH in the range 0 to 14. This means that the macro acidity constants $K^H_{H_2(Bimp)}$ and $K^H_{H(Bimp)}$ [eqns. (2) and (3)] quantify relatively well the intrinsic acidities of the $(N\text{-}3)H^+$ and $P(O)_2(OH)^-$ sites, respectively, and that no micro acidity constants need to be derived.[64] This is confirmed by the acidity constant of $CH_3P(O)_2(OH)^-$ (pK_a = 7.51; Table 1) which is close to the one of $H(Bimp)^-$ (pK_a = 7.41); in fact, the first mentioned constant may be considered as a good estimate for the micro acidity constant of the $P(O)_2(OH)^-$ site in $H(Bimp)^-$.

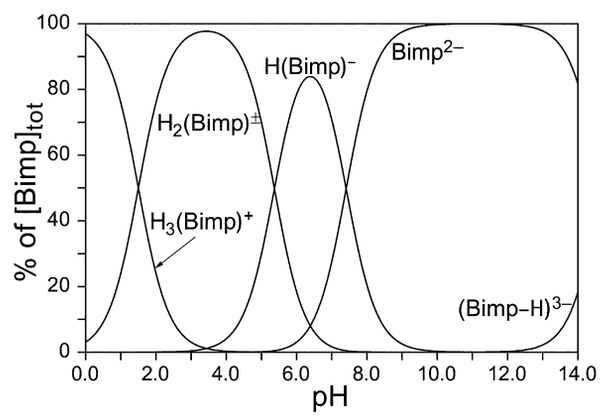

Fig. 4 Effect of pH on the concentration of the species present in an aqueous solution of Bimp at 25 °C ($I = 0.1$ M, $NaNO_3$, in the pH range 1–13). The results are plotted as the percentage of the total Bimp present. The calculations are based on the acidity constants listed in entry 5 of Table 1.

Another very interesting result of the present study is the proof that intramolecular hydrogen-bond formation occurs between the (N-1/N-3)H site and the PO_3^{2-} residue of $Bimp^{2-}$ and that in fact the formation degree of the "chelated" species is quite large showing further that the effect of hydrogen-bond formation can have a profound influence on the acid–base behavior of a compound in aqueous solution. To what extent, if any, this strong internal hydrogen bond might affect possible biological properties of Bimp remains to be seen. However, this study proves that the PO_3^{2-} group is an excellent H-bond acceptor and the (N-1/N-3)H site of a benzimidazole residue a very suitable donor.

Finally, one may also expect that a metal ion can take the place of the H-atom, thus giving rise to chelate formation and indeed, this is the case as preliminary results of studies of several M^{2+}/Bimp systems show.[65] For example, Zn(Bimp) exists to nearly 100% in the form of a six-membered chelate, the "open" species occurring only in trace amounts.

4 Experimental

4.1 Materials

2-Chloromethylbenzimidazole was obtained from Sigma-Aldrich, Poznań, Poland. Potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), KOH (solid and Titrisol), KCl and

$NaNO_3$ (all *pro analysi*) as well as the pH 9.97 (for 25 °C) buffer solution were purchased from Merck KGaA, Darmstadt, FRG. The other buffer solutions (pH 4.00, 7.00, 9.00), all based on the NBS scale (now US National Institute of Science and Technology, NIST) were from Metrohm AG, Herisau, Switzerland.

For all solutions deionized, ultrapure (Milli-Q185 Plus; from Millipore S.A., 67120 Molsheim, France) CO_2-free water was used. The stock solutions of Bimp, used for the physicochemical measurements, were freshly prepared daily and the titre of the NaOH solution was established with potassium hydrogen phthalate.

4.2 Synthesis of (1*H*-benzimidazol-2-yl-methyl)phosphonic acid, $H_2(Bimp)^{\pm}$

4.2.1 Equipment. Melting points were taken on a Boetius apparatus and are uncorrected. The ^1H and ^{31}P NMR spectra were recorded on a Varian EM 360 spectrometer. Chemical shifts δ for ^1H and ^{31}P are reported in parts per million (ppm) and are referenced to tetramethylsilane (TMS) and the ^{31}P spectra to 80% H_3PO_4 as external standard. Coupling constants J are given in Hz. The IR spectra were performed on a Perkin-Elmer PE 380 spectrophotometer.

4.2.2 Preparation of 1-carboethoxy-2-chloromethylbenzimidazole (2).[29] To a suspension of 2-chloromethylbenzimidazole (**1**; see Fig. 1) (3.3 g, 20 mmol) in dry dioxane (50 mL) ethyl chloroformate (1.9 mL, 20 mmol) was added,[30] followed gradually by triethylamine (2.8 mL, 20 mmol); the mixture was stirred at room temperature for 1 h, and then refluxed for 3 h. The mixture was filtered while hot and the filtrate was evaporated to oil. Dry diethyl ether (50 mL) was added portionwise. The evaporation of the ether caused the oil to solidify. The crude product was recrystallized from hexane; yield 3.4 g (71%), mp 80–82 °C. ^1H-NMR ($CDCl_3$): δ 1.57 (t, 3H, $^3J_{HH}$ = 7.1, CH_3), 4.63 (q, 2H, $^3J_{HH}$ = 7.1, CH_2O), 5.11 (s, 2H, CH_2Cl), 7.40 (m, 2H, aryl H-C_5, H-C_6), 7.80 (m, 1H, aryl H-C_4), 8.00 (m, 1H, aryl H-C_7).

4.2.3 Preparation of (1-carboethoxy-benzimidazol-2-yl-methyl)phosphonic acid dimethyl ester (3). A mixture of **2** (0.6 g; 2.5 mmol) and trimethylphosphite (5 mL) was stirred under reflux for 10 h in dry argon atmosphere and then the excess of phosphite was evaporated. The product was separated from impurities by means of column chromatography (silica gel, chloroform–acetone 4 : 1, R_F = 0.28); yield 0.6 g of thick yellow oil (76%). ^1H-NMR ($CDCl_3$): δ 1.54 (t, 3H, $^3J_{HH}$ = 7.1, CH_3CH_2), 3.79 (d, 6H, $^3J_{HP}$ = 11, two OCH_3), 4.05 (d, 2H, $^2J_{HP}$ = 22, CH_2P), 4.59 (q, 2H, $^3J_{HH}$ = 7.1, CH_3CH_2), 7.33 (m, 2H, aryl H-C_5, H-C_6), 7.75 (m, 1H, aryl H-C_4), 7.92 (m, 1H, aryl H-C_7). ^{31}P-NMR ($CDCl_3$): δ 24.48. IR (film), ν(cm^{-1}): 1749 (C=O), 1259 (P=O), 1056, 1028 (P–O–C).

4.2.4 Preparation of (1*H*-benzimidazol-2-yl-methyl)-phosphonic acid (4). The ester **3** (0.6 g, 1.9 mmol) in 40% aq. HBr solution (5 mL) was refluxed for 10 h. The solvent was removed under reduced pressure and the residue dissolved in saturated aq. $NaHCO_3$ solution which was filtered of any impurities and acidified with 6 M HCl. The resulting precipitate was filtered off, washed with water and dried *in vacuo*. Yield 0.33 g (82%); mp 330–333 °C. Anal. calc. for $C_8H_9N_2O_3P$: C 45.28, H 4.25, N 13.21. Found: C 45.21, H 4.38, N 13.01%. ^1H-NMR (CF_3COOH): δ 3.99 (d, 2H, $^2J_{HP}$ = 22, CH_2P), 7.40–7.70 (m, 4H, aryl). ^{31}P-NMR (CF_3COOH): δ 16.68. IR (film), ν(cm^{-1}): 2500 (OH), 1248 (P=O), 1161, 1079, 745 (1,2-disubstituted benzene).

4.3 Potentiometric pH titrations

The acidity constants $K^H_{H_2(Bimp)}$ and $K^H_{H(Bimp)}$ of $H_2(Bimp)^{\pm}$ and $H(Bimp)^-$, respectively [eqns. (2) and (3)], where one proton is at the benzimidazole ring and the other at the phosphonate group were determined by potentiometry. The experimental conditions for the aqueous Bimp solutions were such (see below) that self-stacking, as is well-known for purine derivatives[66] and may be correspondingly anticipated for benzimidazoles, was certainly negligible.[67]

The pH titrations of aqueous solutions were carried out with a Metrohm E536 potentiograph connected with a Metrohm E765 dosimat and a Metrohm 6.0222.100 combined single-junction glass electrode. The instrument was calibrated with the buffer solutions mentioned in Section 4.1.

The direct pH meter readings were used in the calculations for the acidity constants,[52,68] *i.e.*, the constants determined at I = 0.1 M ($NaNO_3$) and 25 °C are so-called practical, mixed or Brønsted constants.[52] They may be converted into the corresponding concentration constants by subtracting 0.02 from the measured pK_a values.[52] This conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[52,54] It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do not enter into our calculation procedures because we always evaluate the differences in NaOH consumption between a pair of solutions, *i.e.*, with and without ligand.[52,68]

The two acidity constants mentioned above were determined by titrating 50 mL of aqueous 0.59 mM HNO_3 (25 °C; I = 0.1 M, $NaNO_3$) in the presence and absence of 0.26 mM $Bimp^{2-}$ under N_2 with 1 mL of 0.03 M NaOH. The differences in NaOH consumption between such a pair of titrations were used for the calculations. The evaluation of the data was carried out by a curve-fitting procedure using a Newton-Gauss nonlinear least-squares program. All calculations were performed with the equipment described recently.[69] The pH range evaluated was 4.0–8.8, which corresponds to an initial formation degree of nearly 96% for $H_2(Bimp)^{\pm}$; at pH 8.8 a neutralization degree of about 96% is reached for the $H(Bimp)^-/Bimp^{2-}$ system, meaning that only about 4% of $H(Bimp)^-$ remain untitrated. The final results for these two acidity constants are the averages of 26 independent titrations.

4.4 Spectrophotometric measurements

The acidity constant that describes the release of the proton from the (N-1/N-3)H site of $Bimp^{2-}$, K^H_{Bimp} [eqn. (4)], to give $(Bimp–H)^{3-}$ was determined by spectrophotometric measurements. The UV-spectra of Bimp (0.08 mM) were recorded in aqueous solution (25 °C) in 1 cm quartz cells with a Varian Cary 3C spectrophotometer connected to an IBM-compatible desk computer (Windows 95 system) and a Hewlett-Packard 1600 printer.

The solutions were prepared with certain aliquots of concentrated KOH (I = 0.1 M was adjusted with KCl for those solutions in which [KOH] < 0.1 M) and their pH was measured with a Metrohm 713 pH meter connected with a Metrohm 6.204.100 glass electrode (calibrated with buffers of pH 4.00, 7.00, 9.00 and 9.97; see Section 4.1) in those instances where the pH was below 13.60. The measured pH values in the range 13 to 13.6 were corrected based on the "correction curve for the alkali error" provided by Metrohm (see also legend to Fig. 2). The pH values above 13.60 were calculated from the KOH concentration employed by using the H_- scale provided by Yagil[31] (see also Section 2.4 and legend to Fig. 2).

The spectrophotometric measurements (for an example see the figure of the ESI) were evaluated at 260, 277 and 287 nm (see Fig. 2) in the way described previously for ^1H-NMR shift measurements.[39,43] The spectra of some solutions with the highest KOH concentrations were recorded again after 20 minutes; there were no significant differences compared to those measured immediately after preparation. In total three independent experimental series were carried out; the final results described in Section 2.4 and listed in Table 1 (entry 5, column 6) is the weighted mean of the individual results.

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16 Abbreviations and definitions: see also Figs. 1 and 3. AMP^{2-}, adenosine 5′-monophosphate; Bi, benzimidazole; $dPEEA^{2-}$, dianion of 9-(5-phosphonopentyl)adenine (= 3′-deoxa-PEEA, where PEEA = 9-[2-(2-phosphonoethoxy)ethyl]adenine); $dPMEA^{2-}$, dianion of 9-(4-phosphonobutyl)adenine (= 3′-deoxa-PMEA); *I*, ionic strength; K_a, general acidity constant; M^{2+}, divalent metal ion; 1MBi, 1-methylbenzimidazole; 2MBi, 2-methylbenzimidazole; $PMEA^{2-}$, dianion of 9-[2-(phosphonomethoxy)ethyl]adenine; $PMEDAPy^{-}$, quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine; UMP^{2-}, uridine 5′-monophosphate. Species written without a charge either do not carry one or represent the species in general (*i.e.*, independent of their protonation degree); which of the two possibilities applies is always clear from the context. A formula like $(Bimp–H)^{3-}$ means that the compound has lost a further proton and is to be read as Bimp *minus* H^+.

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