Crystal Structure of a Hydrated Molecular 1:2 Complex of Nicotinamide Adenine Dinucleotide (NAD^+) and Gallic Acid: Polar Alignment of the Phenolic Partner Molecules

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Dedicated to the memory of *Emanuel Vogel* (1927–2011)

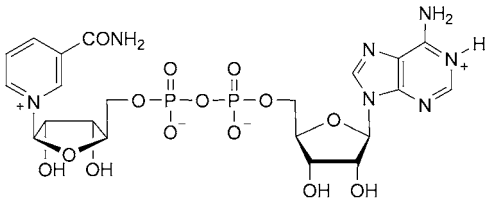
Nicotinamide adenine dinucleotide (oxidized form, free acid, NAD^+) was utilized as an auxiliary for polar alignment of gallic acid (= 3,4,5-trihydroxybenzoic acid, GA) in the crystalline molecular complex $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$. In this adduct, NAD^+ takes up a novel U-shaped conformation accepting a GA molecule to give rise to a pincer-type π-complex. With the assistance of further GA molecules, these pincer assemblies build up infinite donor–acceptor π-stacks in the crystal. An extended network of H-bonds among the multitude of pertinent active functional groups and the water molecules supports the crystal structure. These findings may imply noteworthy material properties, in particular nonlinear optical effects, and might also serve to trigger inspiring biochemical connections to be made. The crystal structure of anhydrous GA is also reported, which is centrosymmetric and non-polar.

An ambitious and rewarding goal of crystal chemistry of ongoing urgency is the predictive design of polar crystals in order to create interesting, 'smart' material properties, for example, nonlinear optical (NLO) effects. No straightforward strategies towards this end are at hand, and unearthing new useful polar crystalline materials continues to represent in significant measure an educated trial-and-error effort at best. One fairly obvious recipe may, however, be cited in order to at least increase the likeliness of polar molecular alignment in crystals, that is the imposition of chirality upon their structure, and thus precluding crystallographic centrosymmetry. Among other options, this may be brought about in the course of a supramolecular approach, with the help of chiral auxiliaries, which are capable of engaging the polar partner molecules to be aligned in molecular complex formation[1]). Clearly, as is well-known, of

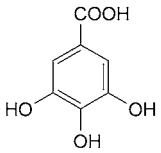
[1]) We refer here to polar crystals in a narrower sense as to those whose symmetries give rise to a permanent dipole moment in the solid state, not to those in a wider sense just lacking a center of symmetry. Of course, the former represent a subset of the latter. It is to be conceded that the effectiveness of the present approach of inciting polar molecular alignment is impaired by the fact that polar crystal symmetries involving mirror planes or glide planes obviously are also excluded. A pertinent example would be the orthorhombic space group $Pna2_1$, which indeed is not particularly rare. Moreover, probably supported by simple electrostatic factors, antiparallel pairing of polar molecules across *local approximate* centers of symmetry is not infrequently met with, irrespective of the crystal's non-centrosymmetric structure as a whole [1]. This leads to extensive dipolar

particular interest is the polar alignment of usually achiral organic substances with extended planar, polarizable push–pull π-electron frameworks.

Apparently and (literally!) quite naturally, an attractive and convenient source of chiral molecular auxiliaries is the environmentally benign ('green') chiral pool of substances provided by the realm of living organisms. In the following, we report the crystal structure of a rather intriguing (hydrated) molecular 1:2 complex of nicotinamide adenine dinucleotide (oxidized form, free acid, NAD^+) and gallic acid (= 3,4,5-trihydroxybenzoic acid, GA), in which the chiral auxiliary NAD^+ induces polar alignment of the GA companion molecules. The formation of the NAD^+–GA–H_2O complex was uncovered in the course of experiments aimed at the study of the interaction of NAD^+ with unsaturated organic molecules involving polar(izable) π-electron systems.



Nicotinamide adenine dinucleotide (NAD^+)



Gallic acid (GA)

Initially, relatively small crystals of the present complex could be grown at room temperature simply by covering the combined NAD^+ and GA components with H_2O in a beaker and sealing the container. Crystals of the sparingly soluble complex appeared after *ca.* 3 days. With some effort, sufficiently large, barely colored crystals were eventually obtained, again from benign aqueous medium, by suitable diffusion techniques. They appear as bundles of faintly pale yellow, flattened, tapering needles, and are stable for at least about two months, if not longer, under normal ambient conditions, but this was not followed up over a still more extended span of time. Thus, it appears that GA chemically stabilizes NAD^+, which otherwise decomposes slowly even at 4°. The good quality and appreciable size of the crystals

compensation frustrating polar molecular alignment. Likewise, the same consequence results if the polar axis of the molecules to be aligned happens to run more or less perpendicular with respect to crystallographic rotation axes, screw axes, mirror planes, or glide planes. According to our experience, occurrence of this sort of dipolar compensation is again not a negligibly rare phenomenon. These structural imponderables may serve to underscore challenge and complexity of the problem of polar molecular alignment in crystals. On the other hand, it is noted that antiparallel pairing of polar molecules does not, of course, *in general* preclude the emergence of *any* useful material properties; consider, for example, the intriguing optoelectronic properties of a class of novel polar push–pull π-systems with antiparallel pairing in the crystal [2].

(needle length up to *ca.* 10 mm, width 0.5 mm) allowed rather precise X-ray measurements[2]).

The crystal lances thus grown are monoclinic with space group $P2_1$. The molecular composition of the present complex corresponds to $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$, and the unit cell holds two of these formula units. In the course of the structural refinements, a residual electron density maximum of 1.65 e Å^{-3} emerged, which could reasonably be treated as a 6th, partially occupied (15%) H_2O position[2]), but is neglected in the following exposition for simplicity reasons.

In the hydrated complex with GA, the NAD^+ molecular partner is protonated at the heterocyclic pyrimidine-type N-atom nearer to the amino group of the adenine (AD) terminus, creating a favorable amidinium-type moiety. Both aromatic termini of NAD^+ thus carry a formal positive charge qualifying them as electron-deficient π-acceptors. The two bridging phosphate groups are each deprotonated and negatively charged, such that an overall neutral, doubly zwitterionic NAD^+ molecule results (see *Formulae*). The same protonation pattern and distribution of charges have previously been observed for NAD^+ in its crystalline tetrahydrate, $NAD^+ \cdot (H_2O)_4$, of which precise X-ray [3] and neutron [4] diffraction measurements are at hand. The crystal structure of a lithium salt of NAD^+ (dihydrate) has also been reported earlier [5], but is obviously less well comparable to our present case than that of $NAD^+ \cdot (H_2O)_4$.

The conformation of NAD^+ in the present adduct $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$ is hairpin-like or U-shaped, respectively, and the electron-deficient nicotinamide (NA) and (protonated) adenine (AD) termini are positioned right on top of each other with virtually parallel molecular planes *ca.* 6.44 Å apart, a separation apparently corresponding to twice the usual distance of face-to-face stacking of planar π-systems (*Figs. 1* and *3*,*a*). As a cofactor of redox enzymes, *i.e.*, complexed with a protein (the apoenzyme) and H_2O molecules, NAD^+ prefers to take up extended conformations with the aromatic termini widely separated [6]. (It should be noted, though, that, under the usually prevailing more or less neutral physiological conditions, the adenine terminus of NAD^+ is not protonated, and the latter carries a net negative charge.) The

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[2]) The X-ray intensities were measured on a four-circle diffractometer equipped with a CCD area detector applying Mo radiation (λ 0.71073 Å). Structure solution was accomplished by direct methods (SHELXS97) and subsequent least-squares refinement (SHELXL97). The heavy atoms were refined anisotropically, H-atoms isotropically. Crystal data at 100 K: *a*) $NAD^+ \cdot (GA)_2 \cdot (H_2O)_{5.15}$: composition $C_{21}H_{27}N_7O_{14}P_2 \cdot (C_7H_6O_5)_2 \cdot (H_2O)_{5.15}$, pale-yellow needles from H_2O, crystal size $0.4 \times 0.4 \times 0.2$ mm^3, monoclinic, space group $P2_1$, $Z = 2$, $a = 12.3619(3)$, $b = 15.2319(3)$, $c = 13.2574(3)$ Å, $\beta = 116.442(1)^\circ$, $V = 2235.16$ Å^3, $d_x = 1.625$ g cm^{-3}, $\mu = 0.21$ mm^{-1}, $\Theta_{max} = 33^\circ$; 34628 reflections measured, 16819 independent, 15848 significant ($F > 4\sigma(F)$), $R_{int} = 0.018$, R_1 (sign. data) = 0.026, wR_2 (all data) = 0.067, maximum residual electron density $\Delta\varrho_{max} = 0.36$ e Å^{-3}, absolute structure (*Flack*) parameter = −0.04(3); presumptive 6th H_2O molecule with 15% occupancy considered in the refinements, its H atoms neglected, however. *b*) Anhydrous GA ($C_7H_6O_5$): colorless prisms from butan-2-one by removing the solvent *via* the gas phase with P_2O_5 in a desiccator, crystal size $0.2 \times 0.2 \times 0.1$ mm^3, monoclinic, space group $C2/c$, $Z = 8$, $a = 25.675(4)$, $b = 4.9047(4)$, $c = 11.115(2)$ Å, $\beta = 105.770(5)^\circ$, $V = 1347.00$ Å^3, $d_x = 1.678$ g cm^{-3}, $\mu = 0.15$ mm^{-1}, $\Theta_{max} = 27^\circ$; 3204 reflections measured, 1457 independent, 880 significant, $R_{int} = 0.038$, $R_1 = 0.040$, $wR_2 = 0.094$, $\Delta\varrho_{max} = 0.21$ e Å^{-3}. CCDC-857531 (*a*) and CCDC-857530 (*b*) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

conformation of NAD^+ in crystalline $NAD^+ \cdot (H_2O)_4$ [3][4] may be characterized as intermediate: the terminal aromatic planes are approximately parallel, yet the two π-systems do not sit on top of each other, but are substantially displaced laterally.

The present U-shaped conformation of NAD^+ represents an effective molecular pair of pincers (or tweezers) ready to accommodate a GA molecule, conforming to a π-complex. Basically, in simplified terms (see below), the electron-rich polyphenolic region of GA is sandwiched between the electron-deficient aromatic (quaternized) NA and (protonated) AD termini of NAD^+, while the electron-withdrawing carboxy group protrudes towards the interior of the dinucleotide, being anchored by donating a H-bond to an O-atom of a phosphate bridge (*Figs. 1* and *3*,*a*). In the crystal of $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$, the second, independent GA molecule is similarly π-sandwiched, but the flanking nicotinamide and (protonated) adenine termini now belong to different NAD^+ molecules. Thus, we may distinguish *endo* (pincer-type) from *exo* complexation of GA by NAD^+. Expectedly, the more or less planar molecular structures of *endo*-GA and *exo*-GA are largely the same, except for the conformational pattern of the phenolic OH groups (*Figs. 1–3*; see below). Thus, it becomes fairly clear from the above structural outline that, altogether, in the present adduct the NAD^+ and GA molecules self-assemble to build up π-donor–acceptor stacks composed of the aromatic NAD^+ termini, and interspersed *endo*- and *exo*-GA molecules (*Fig. 2*; average stacking distance 3.27 Å; unsurprisingly, stacking of *endo*-GA somewhat narrower than of *exo*-GA, by *ca.* 0.1 Å). The 1:2 stoichiometry of NAD^+ and GA follows from this intriguing supramolecular fabric.

The π-overlap of the NA and AD termini of NAD^+ with *endo*- and *exo*-GA, *i.e.*, the π-sandwiching of the former by the latter, is illustrated in *Fig. 3*. To begin with, and simplifying matters, it may be seen that the overlap behavior of both types of GA molecule is roughly comparable. The π-overlap of GA with terminal AD (more to the point ADH^+) is qualitatively in accord with expectation, in so far as the electron-rich phenolic portion of GA essentially faces the electron-deficient amidinium region of the protonated AD terminus (*Fig. 3*,*b*). Less obvious appears the π-interaction of GA with terminal NA, which is of particular concern, considering the crucial role of the NA terminus as reaction center of cofactorial NAD^+ in enzymatic redox processes. Rather than the formally positively charged pyridinium nucleus of NA, its carboxamide side group emerges to be π-topped by the phenolic arch of GA (*Fig. 3*,*c*). Also, the appreciable π-overlap of the carboxy group of GA with the pyridinium core of the NA terminus does not necessarily conform to expectation (*Fig. 3*,*c*). For deeper insight, experimental and theoretical studies of charge-density distributions would be helpful, also as regards the influence of H-bonding: in the crystals of $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$, *e.g.*, the carboxamide group of NA is doubly H-bonded rather tightly to the carboxy group of an *exo*-GA molecule as evidenced by a comparatively short O–H···O bond (O···O distance 2.578(1) Å; see below).

As outlined initially, the main target of the present supramolecular engineering approach was the polar alignment of GA with the help of the chiral auxiliary NAD^+. Inspection of *Fig. 2* clearly shows that this task was indeed accomplished: the *endo*- and *exo*-GA molecules are roughly translationally equivalent (except for three of the four acid H-atoms) and their molecular polar axes thus not very far from parallel. In turn, these axes run approximately parallel to the crystallographic twofold screw axes (2_1)

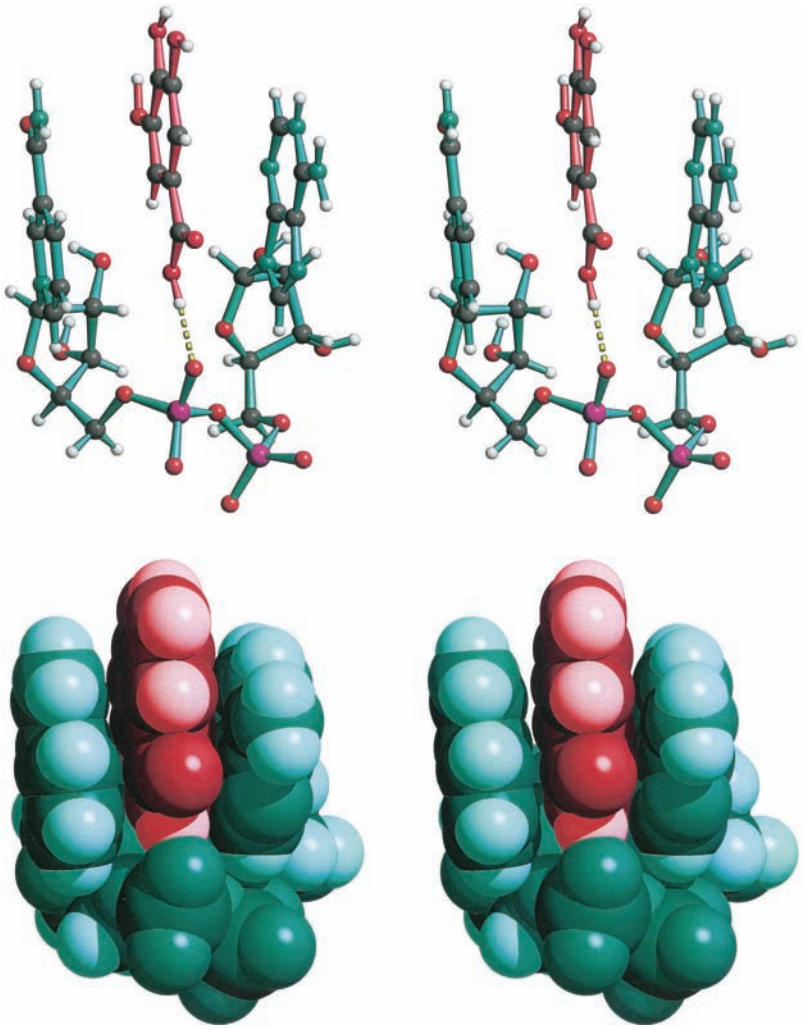

Fig. 1. *Stereoviews of the NAD*$^+$*–gallic acid* (GA) *pincer complex in ball-and-stick* (*top*) *and space-filling* (*bottom*) *representation*. Color coding of *Figs. 1–3* and *5*: C, black; H, white; N, green; O, red; P, magenta; H-bonds (dashed), yellow; bonds of NAD$^+$, green; *endo*-GA, red; *exo*-GA, orange; H_2O, blue; in the space-filling views: NAD$^+$, green; *endo*-GA, red; *exo*-GA, orange.

with the consequence of remarkably good polar alignment of GA in the crystals of $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$, as grown without special precautions from benign aqueous medium. It should be noted that the crystal structures of the two forms of GA monohydrate reported in the literature [7] are centrosymmetric and thus nonpolar (described in space groups $P2_1/c$ and $P2/n$, respectively; different phenolic OH conformations in both structures). According to a crystal-structure search, anhydrous GA has apparently hitherto not been characterized crystallographically. We have filled in this gap of structural knowledge and could grow X-ray-quality crystals relatively

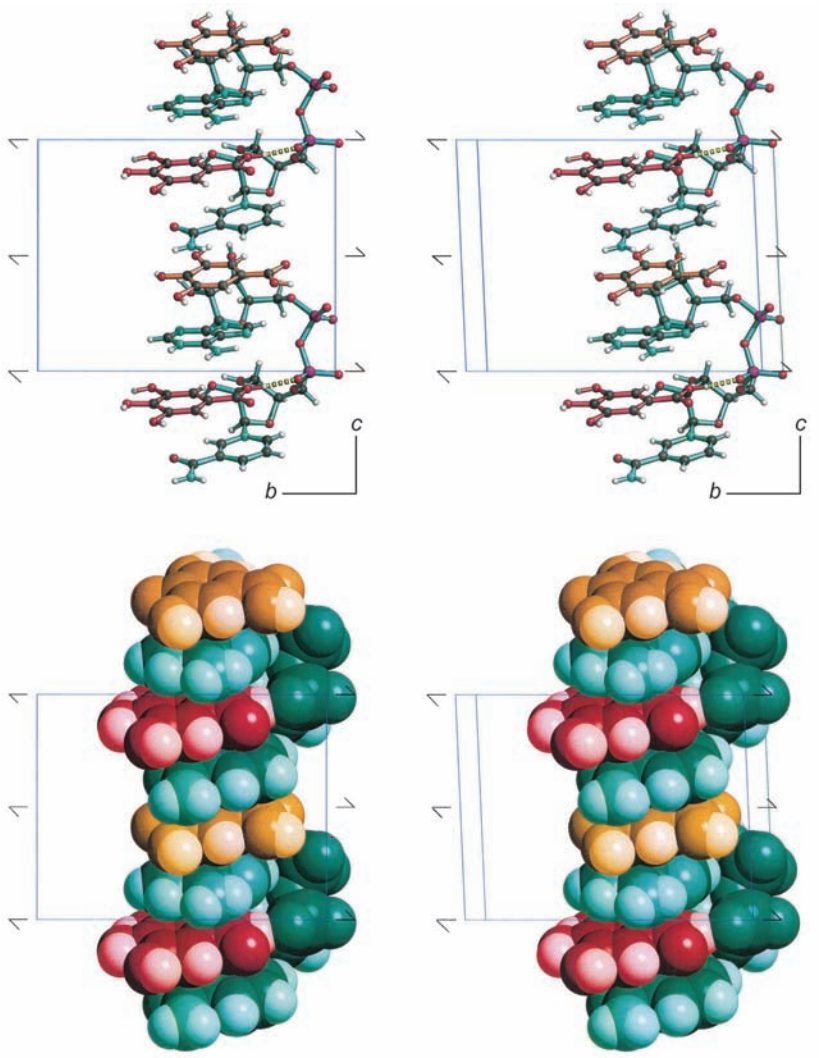

Fig. 2. *π-Stacking of NAD⁺ and GA*. Shown are ball-and-stick (*top*) and space-filling (*bottom*) stereoviews of two NAD⁺–*endo*-GA pincer aggregates with interspersed *exo*-GA molecules. Unit cell edges and twofold screw axes outlined; crystallographic *a* axis vertical in left members of stereopairs.

easily from dry butan-2-one. These crystals are not particularly hygroscopic and surprisingly stable over months under normal ambient conditions. Their crystal structure is again centrosymmetric, space group $C2/c$[2]), and a stereoview is provided in *Fig. 4*. The essentially (yet not entirely) planar GA molecules occur in centrosymmetric dimers doubly H-bonded across the carboxy groups (O···O distance 2.650(2) Å). These dimers set up sheets of oblique π-stacks held together by H-bonds among the phenolic OH groups. In turn, the sheets are connected by additional H-bonds between

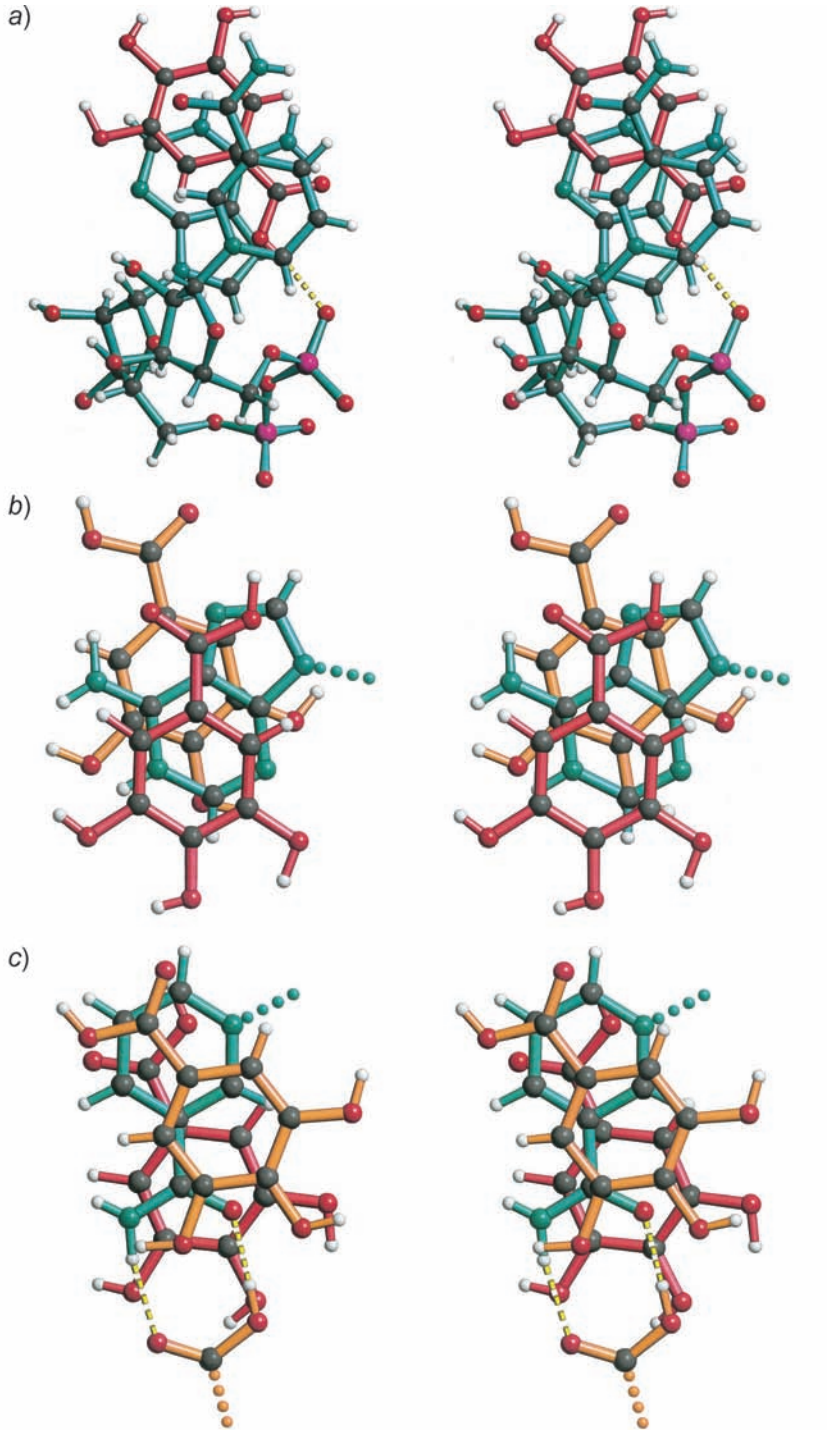

Fig. 3. *π-Overlap of NAD⁺ and GA*. Stereoviews perpendicular to aromatic planes: *a*) NA–*endo*-GA–AD sandwich with complete NAD⁺ pincer host skeleton, *b*) *endo*-GA–AD–*exo*-GA sandwich, *c*) *exo*-GA–NA–*endo*-GA sandwich. Dotted bonds indicate truncation of the NAD⁺ structure.

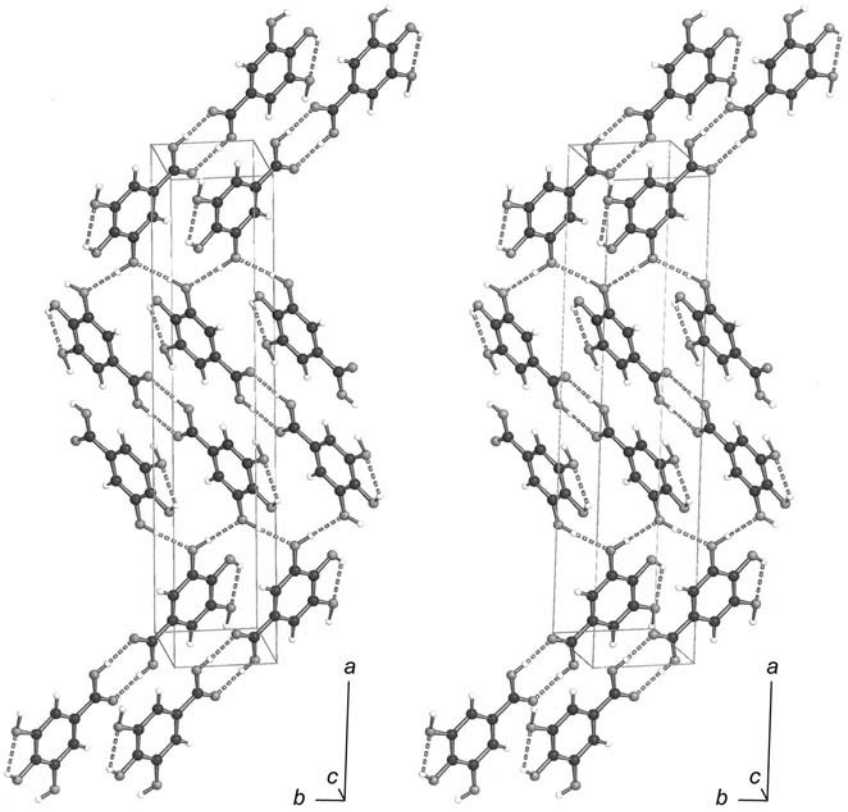

Fig. 4. *Stereoview of the crystal structure of anhydrous GA with unit-cell edges outlined.* Layer section of H-bonded (dashed) molecules shown.

phenolic and carboxylic O-atoms to ultimately give rise to a three-dimensional H-bonded network[3]).

At this point, it is intriguing to relate that even standard dinucleotides with comparatively short monophosphate diester 3′,5′-linkers are capable of accommodating planar aromatic molecules in the fashion of pincer-type π-complexes similar to the present NAD^+ aggregate with GA. For example, the crystal structure of the ethidium salt of the self-complementary dinucleotide composed of 5-iodouridine and adenosine (5-iodo-UpA; monophosphate linker p anionic) has been reported quite some time ago [8]: the ethidium molecule, a substituted planar diaminophenanthridinium cation (3,8-

[3]) Within planarity, two conformational patterns of the phenolic functions of GA are possible, which do not involve short nonbonded H ··· H contacts between adjacent OH groups. The two patterns are realized by *endo*-GA and *exo*-GA in the present hydrated complex with NAD^+ (*Figs. 2*, *3*, and *5*). The respective GA conformation of one crystalline monohydrate [7a] corresponds to that of *exo*-GA, of the other monohydrate [7b] to that of *endo*-GA. Finally, the orientation of the phenolic groups in the crystals of anhydrous GA follows that of *endo*-GA.

diamino-5-ethyl-6-phenylphenanthridinium; Et and Ph substituents rotated out of diaminophenanthridinium plane), *i.e.*, a polar push–pull π-system, is embraced by *two* anionic dinucleotide pincers, which are held together by H-bonds corresponding to *Watson–Crick* base pairing. The closed-loop dinucleotide host duplexes with included ethidium are π-stacked *via* another ethidium cation, again immediately comparable to the present NAD^+/GA case. Thus, again *endo*-ethidium may be differentiated from *exo*-ethidium, of course this time with a 1:1 overall molecular ratio of dinucleotide and ethidium. The benzidine-type amino groups of *endo*-ethidium are H-bonded, albeit rather weakly, to opposite phosphoric ester O-atoms, comparable to the H-bond anchoring of *endo*-GA in the present NAD^+ complex. However, essentially no polar alignment of the ethidium ions is observed, since the respective *endo*- and *exo*-species are related by an approximate local center of symmetry (antiparallel relative orientation, except for the *N*-Et groups of ethidium), and the molecular planes of both are more or less perpendicularly oriented with respect to the twofold rotation and screw axes of the crystal (space group *C*2 [8])[1]). Nonetheless, in related dinucleotide salts with planar quaternized *N*-heteroaromatic cations [9], interesting, at least partial polar alignment phenomena may be discerned. It should be noted that these double-pincer complexes were studied in order to model important processes of intercalation of planar π-systems in between the π-stacked base pairs of double-helical DNA [10] and the related mode of action of certain potent cancer-targeting antibiotics, for example, actinomycin D [11] and daunomycin [12]. Indeed, it would appear that, circumstances permitting, NAD^+ might possibly qualify as a nucleic acid (weak) π-intercalator given the cationic electron-deficient pyridinium moiety of its NA terminus bearing some resemblance with, *e.g.*, the ethidium cation and its congeners. Thus, it seems not entirely unrealistic to infer NAD^+ to even conditionally influence tumor growth *via* nucleic acid intercalation. Also, speculatively, such intercalation processes perhaps play a role as to the mode of action of nicotinamide and nicotinic acid (vitamin B_3) in preventing the deficiency disease pellagra. Lastly, these considerations might serve to invite contemplation as to structures and properties of oligo- as well as polynucleotide analogues with pyrophosphate linkers replacing the original monophosphate connectors; *e.g.*, fourfold-helical super-architectures might be fancied with two interpenetrating, self-intercalating base-paired double helices[4]).

Expectedly, the overall pattern of H-bonding set up in the crystal of the present molecular complex $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$ is pretty intricate, given the multitude of H-bond-active functionalities and the rather low crystal symmetry. We confine the discussion to a selection of three characteristic H-bond motifs (*Fig. 5*): *i*) The already mentioned anchoring H-bond donated by the carboxy group of *endo*-GA to a monofunctional side O-atom of a phosphate group of the NAD^+ molecular pincer

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[4]) Alternative linkers of similarly long extension as pyrophosphate, but with strings of other bonding groups, could also be considered. For oligonucleotide analogues mimicking or modifying the comparatively short standard monophosphodiester linkers, in the context of so-called 'antisense' inhibition of nucleic acids, see, *e.g.*, [13]. For more background information regarding oligonucleotide analogues, see a very recent retrospective review [14]. In this context, it would appear that NAD^+ analogues with chemically different linkers modeling pyrophosphate and/or sugar components other than D-ribose should represent intriguing systems as regards their structures, supramolecular behavior, and bioactivities, for example.

(*Figs. 1* and *3*,*a*). The associated observed O···O distance of 2.658(1) Å is not particularly short despite the partially negatively charged acceptor O-atom. *ii*) The likewise charge-assisted pair of H-bonds connecting the amidinium-type function of the AD terminus with a phosphate group of a NAD^+ molecule of a neighboring π-stack. The two side O-atoms of the same phosphate group function as H-bond acceptors, which also receives the H-bond from *endo*-GA just highlighted (*Fig. 5*). The pertinent N···O distances of 2.787(1) and 2.806(1) Å are roughly 0.15 Å shorter than appropriate reference values without charge assistance. *iii*) The particularly relevant pair of H-bonds already referred to above, which unites the carboxamide side group of the NA terminus and the carboxy group of an *exo*-GA molecule engaged in an adjacent π-stack. The measured O···O and N···O distances of 2.578(1) and 2.889(1) Å, respectively, are relatively short (standard values of *ca.* 2.65 and 2.94 Å, as applying to the familiar dimeric carboxy and carboxamide H-bond motifs, resp.). This may be rationalized in terms of simple resonance considerations implying in particular an appreciable negative partial charge located on the carboxamide O-atom and thus enhancing its H-bond acceptor capabilities. Incidentally, these considerations might suggest to lead to an inclination of carboxamides in general to engage in molecular complex formation with carboxylic acids, a conjecture indeed borne out by experiment [15].

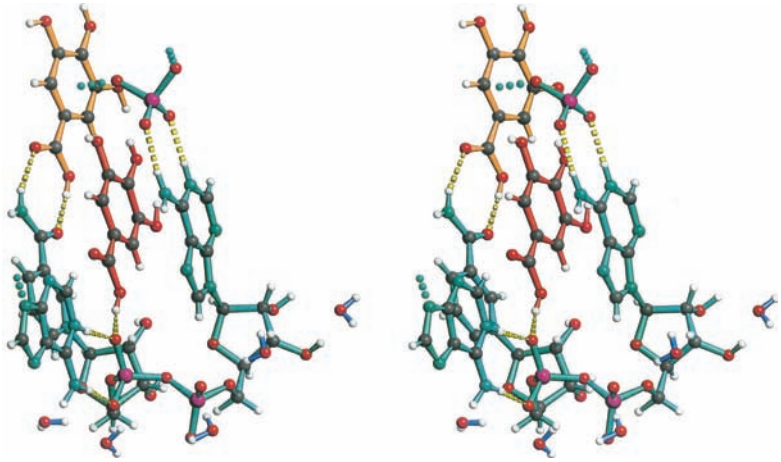

Fig. 5. *Characteristic H-bonds* (dashed) *emanating from* NAD^+. Dotted bonds mean structural truncation. The five independent H_2O molecules present in the crystal are also shown in this stereoview.

Adding to the complexity of the H-bonding web of the present supramolecular system are of course the five H_2O molecules per NAD^+ molecule, which stabilize the crystal structure (*Fig. 5*). However, no details are discussed here; suffice it to remark that the majority of H-bonds involving H_2O are established through contacts with the pertinent active functionalities of NAD^+ and GA. Comparatively few H-bonds bridge H_2O molecules, meaning that no extensive H_2O clustering is observed. An H-bonded chain of four H_2O molecules may be discerned as the largest respective aggregate present. As a matter of fact, we submit that a comprehensive analysis of the vast and

intricate H-bonding grid of the present hydrated NAD^+/GA complex should constitute the subject of a separate study in its own right, deemed beyond intended thrust and scope of this report.

In conclusion, this study shows that it may be rewarding to tap the chiral molecular pool of nature in order to bring about intriguing (supra-) molecular alignment phenomena. A plethora of promising other natural possibilities opens up of further chiral, polarizing auxiliaries to be judiciously picked from this prodigious source, and to be examined amidst benign chemical environment. Summarizing, in the specific present piece of research we have successfully engineered GA to align in polar array in the crystalline solid state by pursuing a bio-organic supramolecular approach and utilizing NAD^+ as a chiral, polarizing supporter molecule. Crystals of the molecular complex $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$ were obtained under mild conditions from aqueous medium. In contrast, GA itself crystallizes centrosymmetrically, both in anhydrous and hydrated forms. In the hydrated complex with GA, NAD^+ takes up a novel U-shaped conformation. Half of the GA molecules (*endo*-GA) are π-sandwiched between the electron-deficient aromatic NA and (protonated) AD termini of one and the same NAD^+ molecule, corresponding to a pincer-type molecular π-complex, which, in addition, is H-bond assisted. The emergence of the U-shaped NAD^+ structure may be regarded as an extreme example of an induced-fit conformational adaptation. The second half of the GA molecules (*exo*-GA) is sandwiched between the aromatic termini of different pincer complexes to ultimately give rise to infinite donor–acceptor π-stacks. These stacks are held together in the crystal by an extensive and rather complex network of H-bonds among the multitude of H-bond-active functionalities available and the H_2O molecules present.

Future measurements will show, whether the polar alignment of the GA molecules in the only lightly colored, transparent, acicular crystals of the presented complex $NAD^+ \cdot (GA)_2 \cdot (H_2O)_5$ might lead to interesting material properties, in particular to significant or even useful NLO (nonlinear optical) effects, *e.g.*, SHG intensities (second-harmonic generation). The presence of the polarizing NAD^+ auxiliary and the hydration may, of course, be expected to exert a certain 'dilution' effect, which is kept in check, however, by the rather favorable stoichiometry of the molecular complex.

It almost goes without saying that our findings are also of some significance as regards the biochemical context, *i.e.*, the study of bioactive substances in general, given the respective relevance of NAD^+ and polyphenolic natural products[5]). The well-known antioxidative properties of the latter may be cited at this point. Moreover, GA itself has been reported to show antitumor activity by inducing apoptosis [18]. In drawing conclusions, it should be kept in mind, however, that unlike in the present case, the AD terminus of NAD^+ is not protonated under usual physiological conditions, reducing its π-acceptor capabilities. This *caveat* would not apply to an AD terminus quaternized by aromatic *N*-alkylation rather than protonation, indeed leading to intriguing NAD^+ derivatives to be envisaged in the present context. Very recently, it

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[5]) For a recent review on polyphenols in plants, see [16]. It is worthy of note that, in a crystalline complex of the enzyme prostaglandin-F-synthase (PGFS) and the inhibitor rutin, a polyphenolic flavonoid, short contacts between the catechol moiety of rutin and the (reduced) NA terminus of NADPH, cofactor of PGFS, have been observed [17].

has been shown that a more rigid synthetic (achiral), polycyclic tweezer molecule roughly about the same overall size as NAD^+, composed of fused norbornadiene moieties and benzene rings (equipped with two phosphonate groups for solubilization), which binds lysine, is capable of disentangling amyloid protein aggregates, and preventing their formation [19]. The latter are supported by intermolecular interactions of lysine residues and notoriously held responsible to trigger neurodegenerative diseases, in particular *Alzheimer*'s and *Parkinson*'s. Conceivably, systems of the type of NAD^+ might possibly bring about similar powers.

We have attempted to grow useful crystals of possible molecular complexes of NAD^+ with a number of polar aromatic π-systems other than GA, as yet to no avail. Likewise, analogous co-crystallization efforts, in which NAD^+ was replaced by flavin adenine dinucleotide (FAD), did not bear fruit so far. Finally, we take the opportunity to propose for potentially rewarding supramolecular study the (dis-)symmetric congeners of NAD^+ and FAD, that is to say nicotinamide nicotinamide dinucleotide (NND), adenine adenine dinucleotide (AAD), and flavin flavin dinucleotide (FFD). AAD is a well-known, naturally occurring and commercially available compound (diadenosine pyrophosphate). Higher homologues of AAD with more extended oligophosphoric ester linkers are also known (diadenosine oligophosphates) and commercially available as salts. Apparently, NND and FFD have as yet received little attention at all. An obvious candidate for related testing would, of course, also be the oxidized form of nicotineamide adenine dinucleotide phosphate, $NADP^+$. Further, quaternization of adenine termini by aromatic *N*-alkylation could possibly bring about an interesting new structural facet in designing smart dinucleotide-type molecular pincers. Clearly, more general dinucleotide analogues with terminal π-systems modified otherwise, sugar components other than D-ribose, or alternative linker strings mimicking pyrophosphate, would qualify as rewarding targets, too, for future experimenting[4]).

REFERENCES

[1] O. Ermer, C. Röbke, *Angew. Chem.* **1994**, *106*, 1832; *Angew. Chem., Int. Ed.* **1994**, *33*, 1755.

[2] H. Bürckstümmer, E. V. Tulyakova, M. Deppisch, M. R. Lenze, N. M. Kronenberg, M. Gsänger, M. Stolte, K. Meerholz, F. Würthner, *Angew. Chem.* **2011**, *123*, 11832; *Angew. Chem., Int. Ed.* **2011**, *50*, 11628.

[3] B. Guillot, N. Muzet, E. Artacho, C. Lecomte, C. Jelsch, *J. Phys. Chem. B* **2003**, *107*, 9109; R. Parthasarathy, S. M. Fridey, *Science* **1984**, *226*, 969.

[4] B. Guillot, C. Lecomte, A. Cousson, C. Scherf, C. Jelsch, *Acta Crystallogr., Sect. D* **2001**, *57*, 981.

[5] W. Saenger, B. S. Reddy, K. Mühlegger, G. Weimann, *Nature (London)* **1977**, *267*, 225; B. S. Reddy, W. Saenger, K. Mühlegger, G. Weimann, *J. Am. Chem. Soc.* **1981**, *103*, 907.

[6] O. Carugo, P. Argos, *Proteins* **1997**, *28*, 10.

[7] a) R.-W. Jiang, D.-S. Ming, P. P. H. But, T. C. W. Mak, *Acta Crystallogr., Sect. C* **2000**, *56*, 594; b) N. Okabe, H. Kyoyama, M. Suzuki, *Acta Crystallogr., Sect. E* **2001**, *57*, o764.

[8] C.-C. Tsai, S. C. Jain, H. M. Sobell, *J. Mol. Biol.* **1977**, *114*, 301.

[9] S. C. Jain, C.-C. Tsai, H. M. Sobell, *J. Mol. Biol.* **1977**, *114*, 317; T. D. Sakore, B. S. Reddy, H. M. Sobell, *J. Mol. Biol.* **1979**, *135*, 763.

[10] D. S. Johnson, D. L. Boger, in 'Comprehensive Supramolecular Chemistry', Eds. J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, Pergamon, Oxford, 1996, Vol. 4, pp. 73–176 (Sect. 3.3); E. C. Long, J. K. Barton, *Acc. Chem. Res.* **1990**, *23*, 271.

[11] S. Kamitori, F. Takusagawa, *J. Am. Chem. Soc.* **1994**, *116*, 4154.
[12] A. H. J. Wang, G. Ughetto, G. J. Quigley, A. Rich, *Biochemistry* **1987**, *26*, 1152; C. M. Nunn, L. Van Meervelt, S. Zhang, M. H. Moore, O. Kennard, *J. Mol. Biol.* **1991**, *222*, 167.
[13] E. Uhlmann, A. Peyman, *Chem. Rev.* **1990**, *90*, 543; B. Meng, S. H. Kawai, D. Wang, G. Just, P. A. Giannaris, M. J. Damha, *Angew. Chem.* **1993**, *105*, 733; *Angew. Chem., Int. Ed.* **1993**, *32*, 729; A. De Mesmaeker, A. Waldner, J. Lebreton, P. Hoffmann, V. Fritsch, R. M. Wolf, S. M. Freier, *Angew. Chem.* **1994**, *106*, 237; *Angew. Chem., Int. Ed.* **1994**, *33*, 226.
[14] A. Eschenmoser, *Angew. Chem.* **2011**, *123*, 12618; *Angew. Chem., Int. Ed.* **2011**, *50*, 12412.
[15] L. Leiserowitz, F. Nader, *Acta Crystallogr., Sect. B* **1977**, *33*, 2719.
[16] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységu, *Angew. Chem.* **2011**, *123*, 610; *Angew. Chem., Int. Ed.* **2011**, *50*, 586.
[17] J. Komoto, T. Yamada, K. Watanabe, F. Takusagawa, *Biochemistry* **2004**, *43*, 2188.
[18] N. Sakaguchi, M. Inoue, K. Isuzugawa, Y. Ogihara, K. Hosaka, *Biol. Pharm. Bull.* **1999**, *22*, 471; K. Satoh, H. Sakagami, *Anticancer Res.* **1997**, *17*, 2181.
[19] S. Sinha, D. H. J. Lopes, Z. Du, E. S. Pang, A. Shanmugam, A. Lomakin, P. Talbiersky, A. Tennstaedt, K. McDaniel, R. Bakshi, P.-Y. Kuo, M. Ehrmann, G. B. Benedek, J. A. Loo, F.-G. Klärner, T. Schrader, C. Wang, G. Bitan, *J. Am. Chem. Soc.* **2011**, *133*, 16958.

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