

Article

Cocrystals versus Salts of Fluorescein

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Abstract: A series of nitrogen-containing organic molecules (4,4'-bipyridyl; *trans*-1,2-bis(4-pyridyl) ethylene; 1,2-bis(4-pyridyl)ethane; 4-aminopyridine and *trans*-1,4-diaminocyclohexane) was envisaged for cocrystallization experiments together with fluorescein. These compounds, containing pyridyl or/and amino nitrogen atoms, can act either as hydrogen bond acceptors for the phenol groups of fluorescein-generating cocrystals or as proton acceptors forming organic salts. Five cocrystals were obtained with the partners containing only pyridyl groups: $\{(H_2Fl)_2(bipy)\}$ (1); $\{(H_2Fl)_2(bipy)(MeOH)_2\}$ (2); $\{(H_2Fl)_2(bpete)(EtOH)_2\}$ (3); $\{(H_2Fl)(bpete)\}$ (4); $\{(H_2Fl)(bpeta)\}$ (5). The compounds bearing amino groups deprotonate fluorescein producing salts: $[(HFl)(Hampy)] \cdot 2H_2O$ (6); $[(HFl)(Hampy)]$ (7); $[(Fl)(H_2diach)] \cdot 3H_2O$ (8); $[(HFl)_2(H_2diach)] \cdot 2H_2O \cdot EtOH$ (9); and $[(HFl)_2(Fl)_2(H_2diach)_3] \cdot 4H_2O$ (10). Optical properties of the cocrystals and salts were investigated.

Keywords: fluorescein; cocrystals; organic salts; luminescence



Citation: Răducă, M.; Mădălan, A.M. Cocrystals versus Salts of Fluorescein. *Crystals* **2021**, *11*, 1217. <https://doi.org/10.3390/cryst11101217>

Academic Editors:
Younes Hanifehpour and
Neil Champness

Received: 22 September 2021
Accepted: 6 October 2021
Published: 9 October 2021

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1. Introduction

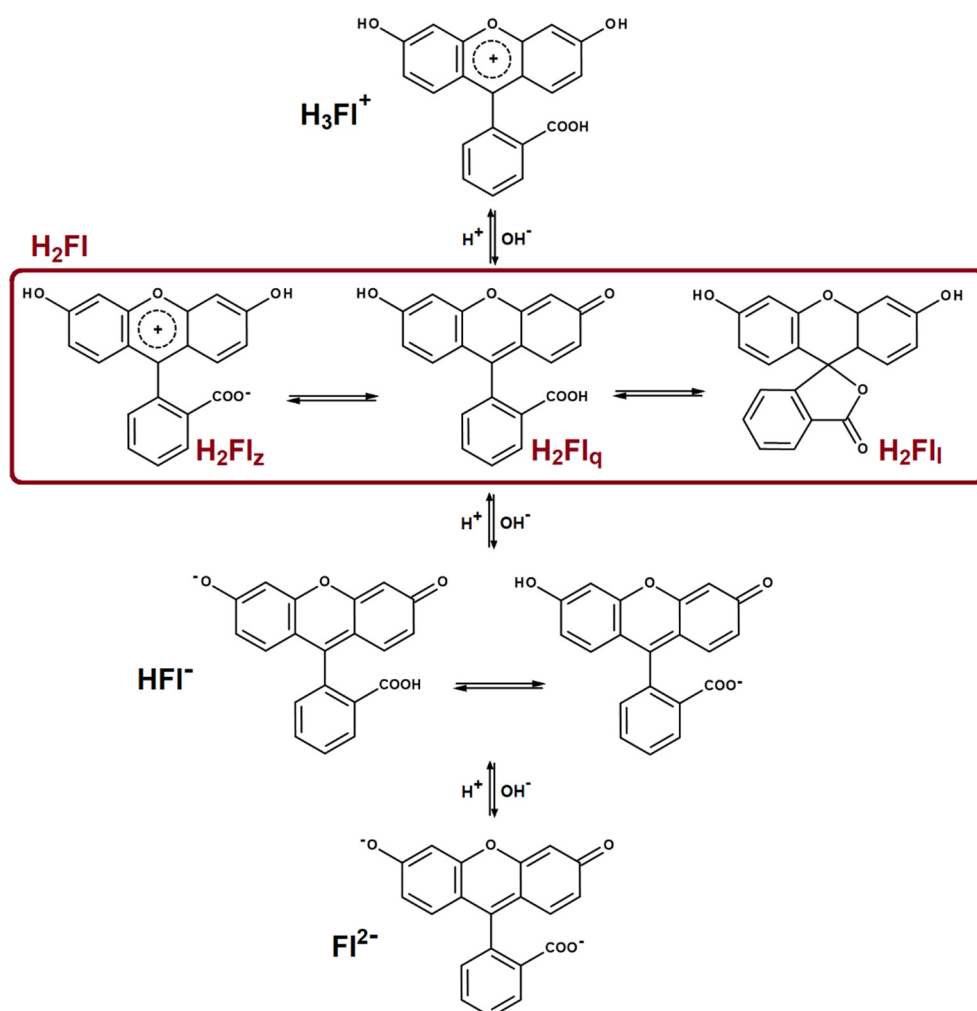
Fluorescein is a xanthene derivative widely used as platform for designing various chromogenic or fluorogenic probes [1]. Fluorescein derivatives can serve as fluorescent chelators for Zn(II) [2,3], Cu(II) [4], Co(II), Ni(II) [5], Hg(II) or Ag(I) [6] metal ions. As biological applications of the fluorescein derivatives we can also mention detection of nitric oxide production in some tissues [7], detection of homocysteine and cysteine [8], detection of hypochlorite [9], study of serotonin receptors [10], enzymes [11] and live bacteria detection [12].

The chromogenic mechanism of fluorescein is based on protonation–deprotonation reactions. Due to the biological applications of the fluorescein dyes, solution studies have attracted particular attention. The ionization equilibria of fluorescein are presented in Scheme 1. Depending on pH, in solution can be identified cationic (H_3Fl^+), neutral (H_2Fl) or anionic species (HFl^- and Fl^{2-}). The neutral form (H_2Fl) presents in solution three tautomers: zwitterion (H_2Flz), quinonoid (H_2Flq) and lactone (H_2FlI). For the monoanionic form (HFl^-), the phenolate tautomer appears in small quantities only in pure solvents such as DMSO, acetonitrile or acetone [13].

In solid state, the three tautomers of the neutral form are characterized by different colors: the zwitterionic form is yellow, the quinonoid form is red and the lactonoid form of fluorescein is colorless. The crystal structures of H_2Flq and H_2Flz have been determined by powder X-ray diffraction [14,15]. The crystal structure of the pure lactonoid form of fluorescein has not been reported. The lactonoid form crystallizes with solvent molecules, and the methanol [16], acetone [15,17] and 1,4-dioxane [15,18] solvates were structurally characterized by X-ray diffraction on a single crystal.

The lactone (H_2FlI) can also cocrystallize with nitrogen-containing heteroaromatic molecules: acridine, phenanthridine and pyrazine [19]. The use of basic nitrogen-containing molecules as partners for cocrystallization offers the theoretical possibility of proton transfer with the formation of organic salts. Such salts were reported for eosin (2',4',5',7'-tetrabromofluorescein) with 4-aza-1-azoniabicyclo[2.2.2]octane [20] and guanidium

cations [21]. In Mannich derivatives of fluorescein, the presence of basic nitrogen atoms attached to the xanthene core allows the intramolecular transfer of the protons with the formation of zwitterions [22].



Scheme 1. The protonation–deprotonation equilibria of fluorescein.

In this paper we report the synthesis and structural characterization of organic cocrystals and salts of fluorescein with different nitrogen-containing partners: 4,4'-bipyridyl (bipy); *trans*-1,2-bis(4-pyridyl)ethylene (bpete); 1,2-bis(4-pyridyl)ethane (bpeta); 4-aminopyridine (ampy) or *trans*-1,4-diaminocyclohexane (diach). Determination of the specific solid form (cocrystal or salt) is of particular interest especially for active pharmaceutical ingredients [23].

2. Materials and Methods

2.1. Synthesis

The chemicals used as well as all the solvents were of reagent grade and were purchased from commercial sources.

2.1.1. Synthesis of $\{(H_2Fl)_2(bipy)\}$ (1)

Fluorescein (0.0664 g, 0.2 mmol) and 4,4'-bipyridyl (0.0156 g, 0.1 mmol) were dissolved in 20 mL ethanol and 20 mL acetonitrile. The mixture was stirred for 15 min and then filtered. The light-yellow crystals formed after several days. FT-IR (cm^{-1}): 3373s, 3053w, 2891w, 2800w, 2166–2700br, 1758vs, 1602vs, 1501s, 1449s, 1338s, 1257s, 1175vs, 1098s, 816s, 680s.

2.1.2. Synthesis of $\{(H_2Fl)_2(bipy)(MeOH)_2\}$ (2)

Fluorescein (0.1328 g, 0.4 mmol) and 4,4'-bipyridyl (0.0312 g, 0.2 mmol) were dissolved in 80 mL methanol. The mixture was stirred for 15 min and then filtered. The light-yellow crystals formed after several days. FT-IR (cm^{-1}): 3580s, 3478vs, 3035s, 2913s, 2810s, 2692s, 2000–2589br, 1735vs, 1595vs, 1460vs, 1331s, 1279vs, 1176vs, 995s, 833s, 753s, 614m.

2.1.3. Synthesis of $\{(H_2Fl)_2(bpete)(EtOH)_2\}$ (3)

Fluorescein (0.0664 g, 0.2 mmol) and *trans*-1,2-bis(4-pyridyl)ethene (0.0182 g, 0.1 mmol) were dissolved in 20 mL ethanol and 20 mL acetonitrile. The mixture was stirred for 15 min and then filtered. After several days yellow prismatic crystals formed. FT-IR (cm^{-1}): 3400br, 3039m, 28886m, 2801m, 2676s, 2582s, 2113–2468br, 1919m, 1750vs, 1595vs, 1503s, 1420m, 1333m, 1284s, 1246s, 1178s, 1000m, 825s, 543s.

2.1.4. Synthesis of $\{(H_2Fl)(bpete)\}$ (4)

Fluorescein (0.0997 g, 0.3 mmol) and *trans*-1,2-bis(4-pyridyl)ethene (0.0546 g, 0.3 mmol) were dissolved in 40 mL ethanol and 20 mL water. The mixture was stirred for about 15 min and then filtered. After the slow evaporation of the solvent yellow crystals formed. FT-IR (cm^{-1}): 3039w, 2891w, 2803w, 2747w, 2681w, 2580m, 2108–2477br, 1751vs, 1594vs, 1465s, 1417s, 1384s, 1247vs, 1183s, 1070s, 962s, 827s, 587s.

2.1.5. Synthesis of $\{(H_2Fl)(bpeta)\}$ (5)

Fluorescein (0.0664 g, 0.2 mmol) and 1,2-bis(4-pyridyl)ethane (0.0364 g, 0.2 mmol) were dissolved in 40 mL methanol followed by 15 min of stirring and then the mixture was filtered. After one-week yellow crystals were obtained. FT-IR (cm^{-1}): 3496w, 3037m, 2950m, 2895m, 2804m, 2751m, 2680s, 2580s, 2480–2126br, 1834w, 1752vs, 1598vs, 1465s, 1418s, 1332s, 1246vs, 1185vs, 1098vs, 1001s, 895s, 819s, 686s.

2.1.6. Synthesis of $[(HFl)(Hampy)] \cdot 2H_2O$ (6) and $[(HFl)(Hampy)]$ (7)

Compound **6** was obtained by dissolving fluorescein (0.0664 g, 0.2 mmol) and 4-aminopyridine (0.0188 g, 0.2 mmol) in 20 mL ethanol (96%) and 20 mL acetonitrile. The mixture was stirred for 15 min and then filtered, and it was left for slow evaporation of the solvent. After several days prismatic orange-red crystals of **6** appeared on the wall of the beaker. The precipitate obtained on the bottom of the beaker was dissolved in 20 mL ethanol and 20 mL water, then it was stirred for 15 min and filtered. After several days red needle crystals of **7** were obtained. FT-IR (cm^{-1}) (**6**): 3625w, 3166s, 3098s, 2910s, 2669s, 2178–2582br, 2045w, 1873br, 1645s, 1571vs, 1507vs, 1460vs, 1366vs, 1313vs, 1275vs, 1194s, 1112s, 829s, 656m; (**7**): 3640w, 3065m, 2024–2601br, 1764w, 1600vs, 1565vs, 1447vs, 1282vs, 1180vs, 1095vs, 912m, 837s, 752s, 645m.

2.1.7. Synthesis of $[(Fl)(H_2diach)] \cdot 3H_2O$ (8)

Fluorescein (0.0664 g, 0.2 mmol) and *trans*-1,4-diaminocyclohexane (0.0228 g, 0.2 mmol) were dissolved in 40 mL ethanol and 20 mL water. The mixture was stirred for 15 min and then filtered. Red crystals formed after several days. FT-IR (cm^{-1}): 3639w, 3427w, 2861s, 2570s, 1690–2000br, 1627w, 1568vs, 1456vs, 1380vs, 1325vs, 1271vs, 1208vs, 1158s, 1090s, 908m, 845m, 582w.

2.1.8. Synthesis of $[(HFl)_2(H_2diach)] \cdot 2H_2O \cdot EtOH$ (9)

Fluorescein (0.1328 g, 0.4 mmol) and *trans*-1,4-diaminocyclohexane (0.0228 g, 0.2 mmol) were dissolved in 40 mL ethanol and 20 mL water. The mixture was stirred for 15 min and then filtered. After a week red crystals formed. FT-IR (cm^{-1}): 3600–3047br, 2931s, 2861s, 2611s, 2111–2554br, 1907w, 1621vs, 1564vs, 1457vs, 1380vs, 1324vs, 1204s, 1159s, 1051s, 836s, 665m.

2.1.9. Synthesis of $[(\text{HFl})_2(\text{Fl})_2(\text{H}_2\text{diach})_3]\cdot 4\text{H}_2\text{O}$ (**10**)

Fluorescein (0.1328 g, 0.4 mmol) and *trans*-1,4-diaminocyclohexane (0.0342 g, 0.3 mmol) were dissolved in 50 mL ethanol and 20 mL water. The mixture was stirred for 15 min and then filtered. Red crystals formed after a week. FT-IR (cm^{-1}): 3503w, 3431w, 1628s, 1567vs, 1455s, 1377vs, 1321vs, 1203s, 1092s, 833s, 589s.

2.2. Physical Measurements

2.2.1. X-ray Structure Determination

X-ray diffraction measurements for the crystals **1–3**, **5–7**, **9** and **10** were performed on a STOE IPDS II diffractometer, operating with a Mo-K α ($\lambda = 0.71073 \text{ \AA}$) X-ray tube with a graphite monochromator. X-ray diffraction measurements for crystals **4** and **8** were performed on a Rigaku XtaLAB Synergy-S diffractometer operating with a Mo-K α ($\lambda = 0.71073 \text{ \AA}$) micro-focus sealed X-ray tube. The structures were solved by direct methods and refined by full-matrix least-squares techniques based on F^2 . The non-H atoms were refined with anisotropic displacement parameters. Calculations were performed using SHELX-2014 or SHELX-2018 crystallographic software packages. A summary of the crystallographic data and the structure refinement for crystals **1–10** are given in Table S1. CCDC reference numbers: 2111108–2111117.

The X-ray powder diffraction measurements (XRPD) were carried out on a Proto AXRD Benchtop using Cu-K α radiation with a wavelength of $1.54059 \text{ \AA}$ in the 2θ range of $5\text{--}35^\circ$.

2.2.2. Spectroscopy

IR spectra were recorded on an FT-IR Bruker Vertex 70 spectrometer in the $4500\text{--}400 \text{ cm}^{-1}$ range using the ATR technique. The following abbreviations were used: w = weak, m = medium, s = strong, v = very, br = broad. Absorption spectra on powder (diffuse reflectance technique) were measured with a JASCO V-670 spectrophotometer. The fluorescence spectra were collected on powder using a JASCO FP-6500 spectrofluorometer.

3. Results

A series of nitrogen-containing organic molecules, namely 4,4'-bipyridyl; *trans*-1,2-bis(4-pyridyl)ethylene; 1,2-bis(4-pyridyl)ethane; 4-aminopyridine and *trans*-1,4-diaminocyclohexane, was envisaged for cocrystallization experiments together with fluorescein. These compounds contain pyridyl or /and amino nitrogen atoms and, theoretically, these groups can act either as hydrogen bond acceptors for the phenol groups of fluorescein or as proton acceptors. The following ten compounds were obtained using solution-based crystallization methods (slow evaporation at room temperature) and different stoichiometric ratios between the components: $\{(\text{H}_2\text{Fl})_2(\text{bipy})\}$ (**1**); $\{(\text{H}_2\text{Fl})_2(\text{bipy})(\text{MeOH})_2\}$ (**2**); $\{(\text{H}_2\text{Fl})_2(\text{bpeta})(\text{EtOH})_2\}$ (**3**); $\{(\text{H}_2\text{Fl})(\text{bpeta})\}$ (**4**); $\{(\text{H}_2\text{Fl})(\text{bpeta})\}$ (**5**); $[(\text{HFl})(\text{Hampy})]\cdot 2\text{H}_2\text{O}$ (**6**); $[(\text{HFl})(\text{Hampy})]$ (**7**); $[(\text{Fl})(\text{H}_2\text{diach})]\cdot 3\text{H}_2\text{O}$ (**8**); $[(\text{HFl})_2(\text{H}_2\text{diach})]\cdot 2\text{H}_2\text{O}\cdot \text{EtOH}$ (**9**); and $[(\text{HFl})_2(\text{Fl})_2(\text{H}_2\text{diach})_3]\cdot 4\text{H}_2\text{O}$ (**10**). The cocrystals are indicated by braces (**1–5**) whereas the salts are indicated by square brackets (**6–10**). All ten systems were structurally characterized by X-ray diffraction on a single crystal.

3.1. Description of the Crystal Structures

For a molar ratio between fluorescein and 4,4'-bipyridyl of 2:1, two types of cocrystals were obtained depending on the solvents used in the crystallization experiments: $\{(\text{H}_2\text{Fl})_2(\text{bipy})\}$ (**1**) crystallizes from an ethanol-acetonitrile mixture, while $\{(\text{H}_2\text{Fl})_2(\text{bipy})(\text{MeOH})_2\}$ (**2**) crystallizes from methanol.

Crystal **1** contains only fluorescein and bipy in a 2:1 molar ratio. The asymmetric unit consists of one fluorescein molecule and half a bipy molecule (Figure 1). The fluorescein molecules are in the lactonoid form and the C–O bond lengths for the phenol groups are C3–O1 = $1.350(3)$ and C11–O3 = $1.355(3) \text{ \AA}$. The bipy molecules are hydrogen bonded through the nitrogen atoms to two OH groups belonging to two different fluorescein molecules.

The (O1)-H1...N1 distance for the intermolecular hydrogen interaction is 1.828 Å and the corresponding O1-H1...N1 angle is 165.0°.

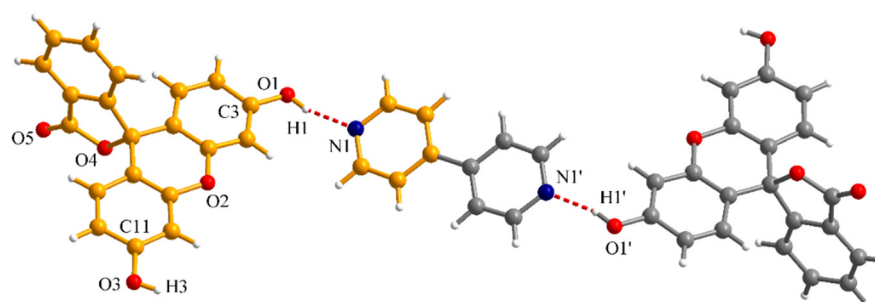


Figure 1. Crystal structure of 1. The asymmetric unit is depicted in orange. Symmetry code: ' = 1 − x, 1 − y, −1 − z.

The O1 oxygen atom also acts as a hydrogen bond acceptor for an O3-H3 phenol group of neighboring fluorescein molecules. The (O3''-H3''...O1 distance for this hydrogen interaction is 1.984 Å and the corresponding O3''-H3''...O1 angle is 144.8° (symmetry code: '' = 0.5 − x, −0.5 + y, −z). The interplay between the two types of hydrogen interactions generates a supramolecular 2D architecture (Figure 2).

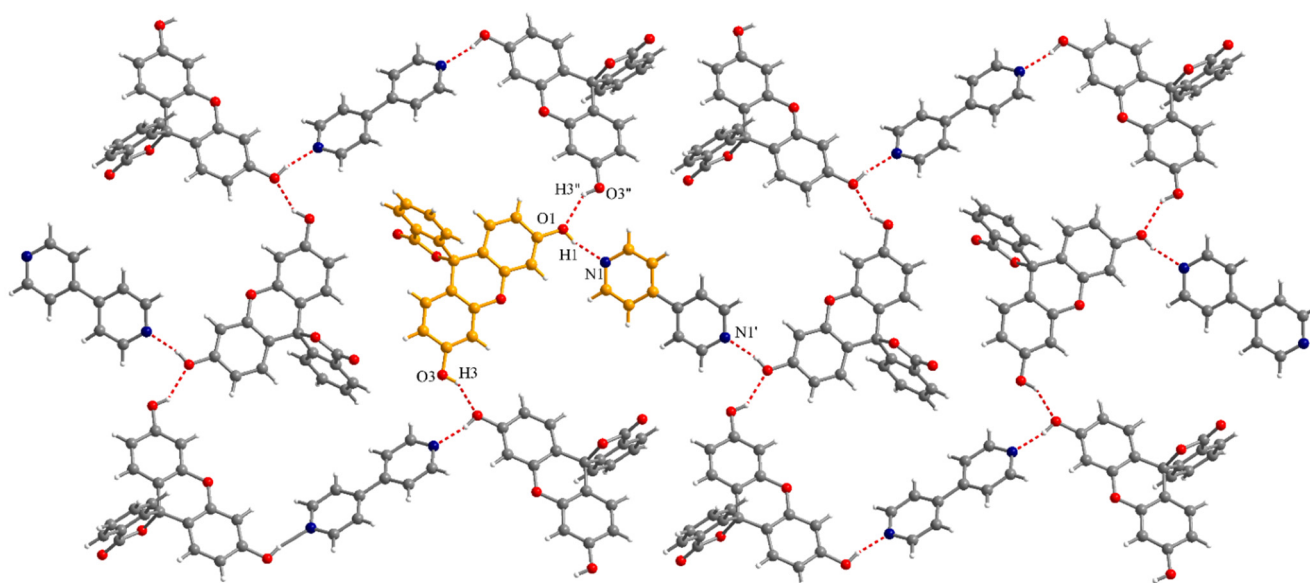


Figure 2. View of the 2D supramolecular array formed through hydrogen interactions in crystal 1. Symmetry codes: ' = 1 − x, 1 − y, −1 − z; '' = 0.5 − x, −0.5 + y, −z.

The supramolecular interactions are further extended to the third dimension through π - π interactions established between the layers. Both organic molecules contain aromatic systems and two patterns of π - π interactions can be described (Figure 3). The first one involves the xanthene cores of the fluorescein molecules with a separation of 3.51–3.62 Å between the fragments. The mean planes of interacting xanthene fragments are parallel. The second type of π - π interaction implicates one pyridine ring of a bipy molecule and one phenol moiety of a fluorescein molecule. The dihedral angle between the mean planes of pyridine and xanthene fragments is 31.1° and the shortest contacts between these fragments range between 3.32 and 3.71 Å.

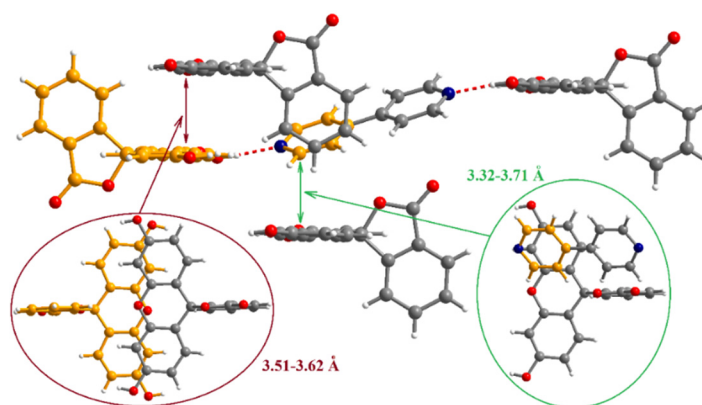


Figure 3. Details of the π - π interactions established in crystal 1.

Cocrystal 2 is a pseudopolymorph of 1 and contains fluorescein, bipy and methanol in a 2:1:2 stoichiometry. It crystallizes in the *triclinic P-1* space group and the asymmetric unit comprises one fluorescein molecule, half a bipy molecule and one methanol molecule (Figure 4).

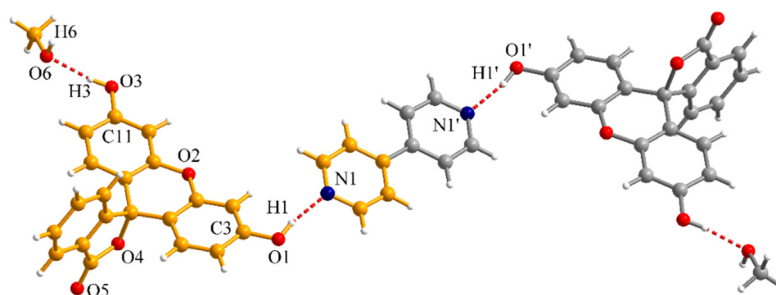


Figure 4. Crystal structure of 2. The asymmetric unit is depicted in orange. Symmetry code: ' = $3 - x$, $3 - y$, $2 - z$.

The fluorescein is also in the lactonoid form with the bond lengths for the phenol groups of 1.357(4) (C3-O1) and 1.358(4) Å (C11-O3). The two phenol groups of fluorescein act as hydrogen bond donors towards one bipy molecule and one methanol molecule, with the (O1)-H1...N1 and (O3)-H3...O6 distances of 1.890 and 1.858 Å, respectively. The corresponding O1-H1...N1 and O3-H3...O6 angles are 169.4 and 171.3°.

The methanol molecule is also a hydrogen donor for another phenol group of a different fluorescein molecule generating supramolecular units formed by two fluorescein molecules and two methanol molecules (Figure 5). The (O6)-H6...O1'' distance is 2.264 Å, while the O6-H6...O1 angle is 135.5°. These supramolecular units are connected by the bipy molecules in a 1D array. In crystal 2, the presence of the solvent molecules prevents the extension of the hydrogen networking to a 2D system. Within the supramolecular dimers the xanthene fragments also establish π - π interactions (3.38–3.55 Å).

For the cocrystallization experiments of fluorescein with *trans*-1,2-bis(4-pyridyl)ethylene two different molar ratios were used: 2:1 and 1:1. The corresponding cocrystals obtained are $\{(H_2Fl)_2(bpete)(EtOH)_2\}$ (3) and $\{(H_2Fl)(bpete)\}$ (4). Cocrystal 3 contains, similarly with crystal 2, fluorescein, bpete and solvent in a 2:1:2 stoichiometry (Figure 6). In this case, the solvent is ethanol and the CH₃-CH₂- fragment is disordered on two independent crystallographic positions with site occupancy factors of 0.5 each. Similar to compound 2, the two phenol groups of fluorescein act as hydrogen bond donors towards one bpete molecule and one ethanol molecule. The (O1)-H1...N1 distance is 1.823 Å and the corresponding O1-H1...N1 angle is 161.0°.

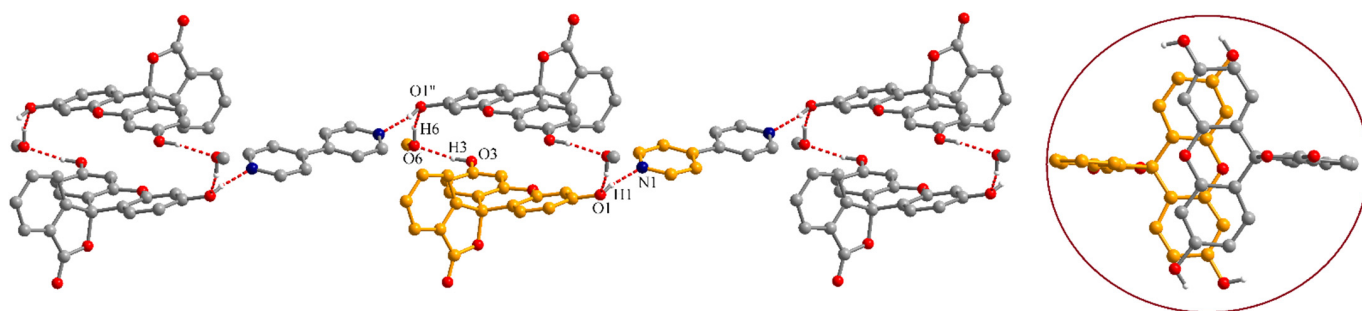


Figure 5. Perspective view of the 1D supramolecular array formed through hydrogen interactions in crystal 2. The inset shows a detail of the π - π interactions established within the supramolecular dimers. Symmetry code: $" = 1 - x, 2 - y, 1 - z$.

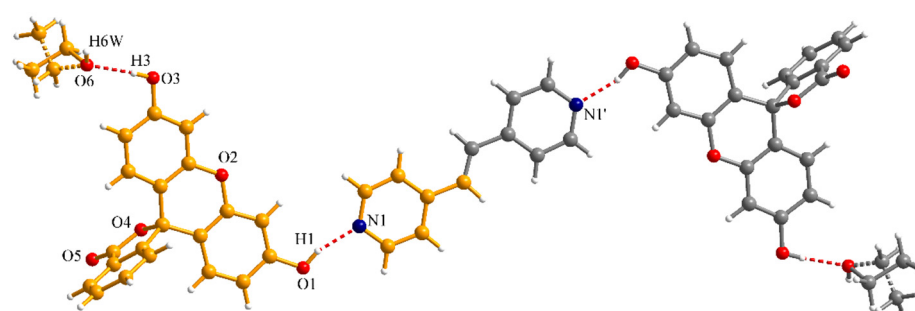


Figure 6. Crystal structure of 3. The asymmetric unit is depicted in orange. Symmetry code: $' = 2 - x, 3 - y, 2 - z$.

In crystal 3, extension of the hydrogen bonding follows a different pattern with formation of a railroad-like arrangement. The alternating fluorescein and ethanol molecules connected by hydrogen interactions, with the (O3)-H3 $\cdots$ O6 and (O6)-H6W $\cdots$ O1'' distances of 1.896 and 1.957 Å respectively, are generating the rails while the sleepers are formed by the bppe molecules (Figure 7).

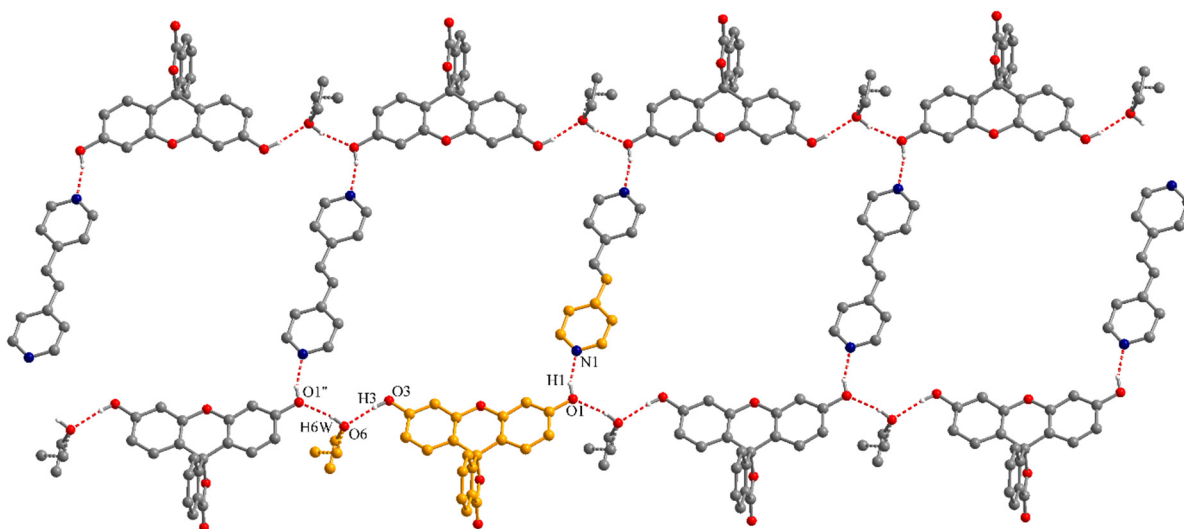


Figure 7. Perspective view of the 1D supramolecular array formed through hydrogen interactions in crystal 3 (the hydrogen atoms not involved in hydrogen interactions were omitted for clarity). Symmetry code: $" = -1 + x, y, -1 + z$.

In crystal 4 the molar ratio between the fluorescein and bppe is 1:1. The fluorescein is in the neutral lactonoid form and both phenol groups are involved in hydrogen interactions

with bpete molecules generating a crenel-like supramolecular chain (Figure 8). The C–O bond lengths for the phenol groups are C3–O1 = 1.358(5) and C11–O3 = 1.355(5) Å. The distances for the intermolecular hydrogen interactions are (O1–)H1...N1 = 1.894 and (O3–)H3...N2' = 1.905 Å, with the corresponding angles of 171.9 and 174.6°, respectively (symmetry code: ' = $-x, -0.5 + y, -0.5 - z$). The ethene fragment of the bpete molecule is disordered on two crystallographic positions with site occupancy factors of 0.75 and 0.25.

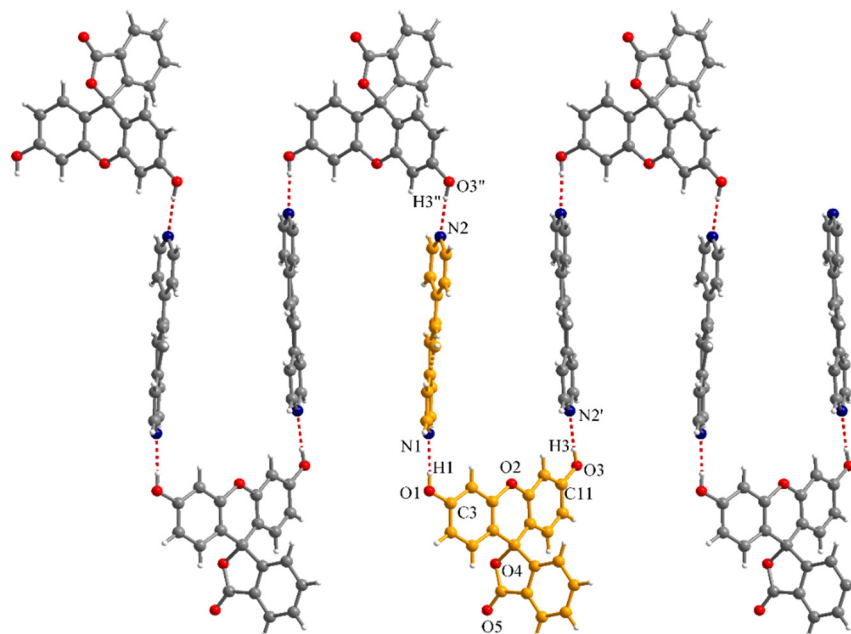


Figure 8. Perspective view of the supramolecular chain formed through hydrogen interactions in crystal 4. The asymmetric unit is depicted in orange. Symmetry codes: ' = $-x, -0.5 + y, -0.5 - z$; '' = $-x, 0.5 + y, -0.5 - z$.

The supramolecular chains formed by hydrogen interactions intercalate and the bpete molecules from neighboring chains establish π – π interactions ranging between 3.27 and 3.64 Å (Figure 9).

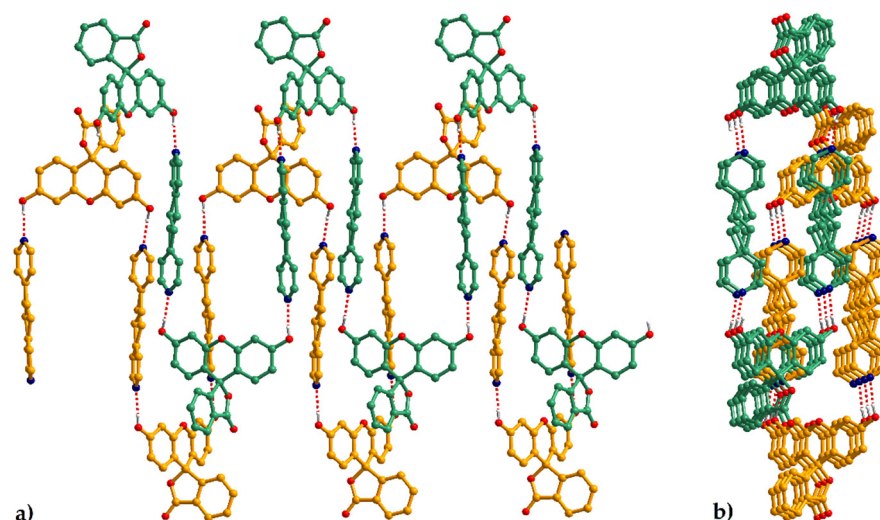


Figure 9. Perspective (a) and side views (b) of two neighboring supramolecular chains in crystal 4.

We have to mention here that compound 4 is not crystallizing as a pure phase and the bulk sample probably contains a small amount of compound 3 (Figures S3–S5).

Cocrystal **5**, $\{(H_2Fl)(bpeta)\}$, was obtained by slow evaporation of a methanolic solution containing fluorescein and 1,2-bis(4-pyridyl)ethane in a 1:1 stoichiometry. In this case, the powder X-ray diffraction shows that the sample contains only one crystalline phase (Figure S6). Structural characterization of compound **5** by X-ray diffraction on a single crystal reveals formation of crenel-like supramolecular chains, similarly to crystal **4**, by hydrogen interactions established between phenol and pyridyl groups (Figure 10). The C–O bond lengths for the phenol groups are: C3–O1 = 1.361(5) and C11–O3 = 1.356(6) Å. The distances for the hydrogen interactions are (O1)–H1...N1 = 1.867 and (O3)–H3...N2' = 1.894 Å, with the corresponding angles of 170.8 and 175.1°, respectively (symmetry code: ' = 1.5 – x, –0.5 + y, 1 – z).

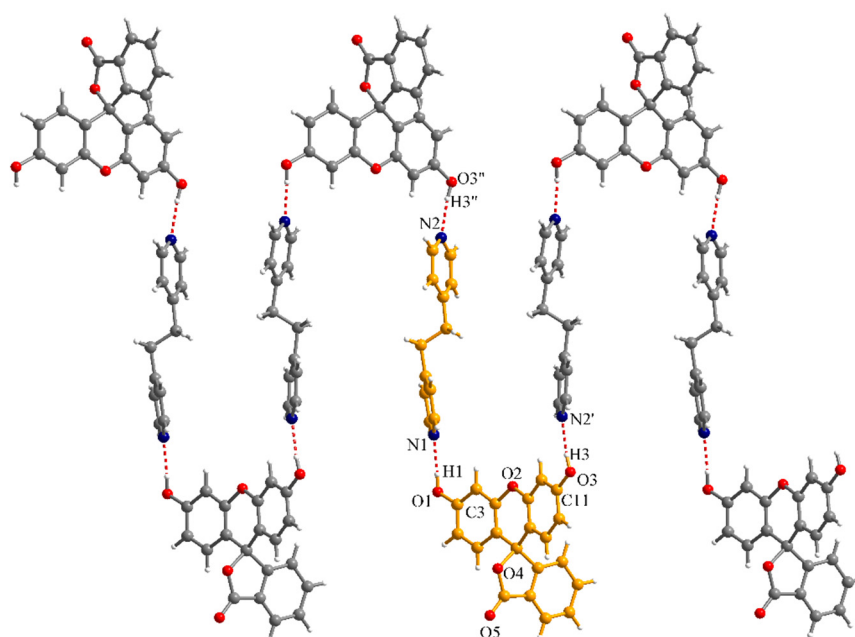


Figure 10. Perspective view of the supramolecular chain formed through hydrogen interactions in crystal **5**. The asymmetric unit is depicted in orange. Symmetry codes: ' = 1.5 – x, –0.5 + y, 1 – z; '' = 1.5 – x, 0.5 + y, 1 – z.

The second type of nitrogen-containing organic molecules used as partners for fluorescein to obtain binary systems were the amino derivatives 4-aminopyridine and *trans*-1,4-diaminocyclohexane. The first notable observation is the color change induced by the amino derivatives. The solid products obtained in the presence of 4-aminopyridine and *trans*-1,4-diaminocyclohexane are red, while the compounds **1–5** are pale yellow or colorless. Since the chromogenic mechanism of fluorescein is based on protonation–deprotonation reactions, the color change is most probably an indication of the proton transfer between the fluorescein and the amino partner.

For a 1:1 stoichiometry between fluorescein and 4-aminopyridine, two types of crystals were obtained depending on the solvents used for crystallization: $[(HFl)(Hampy)] \cdot 2H_2O$ (**6**), obtained in small amounts as orange-red prismatic crystals on the wall of the beaker from a mixture of ethanol (96%)-acetonitrile, and $[(HFl)(Hampy)]$ (**7**) as red needle-like crystals resulted from the evaporation of an ethanol–water solution.

X-ray diffraction on a single crystal shows for compound **6** the presence of fluorescein monoanions (HFl^-), $Hampy^+$ cations and crystallization water molecules (Figure 11). The lactone spirocycle of fluorescein is open and the carboxylate bond lengths are C20–O4 = 1.249(5) and C20–O5 = 1.270(6) Å. For the phenol group, the C3–O1 bond length is 1.340(5) Å, while the quinonic C11–O3 bond length is 1.271(5) Å. The ampy molecule is protonated on a pyridine nitrogen atom (N1). The quinonic O3 atom acts as a hydrogen bond acceptor for a crystallization water molecule, which is also a hydrogen bond acceptor

for the pyridinium fragment (Figure 11). The (O6W-)H1W...O3 and (N1-)H1N...O6W distances are 1.996 and 1.836 Å, respectively, while the corresponding O6W-H1W...O3 and N1-H1N...O6W angles are 163.1 and 167.9°.

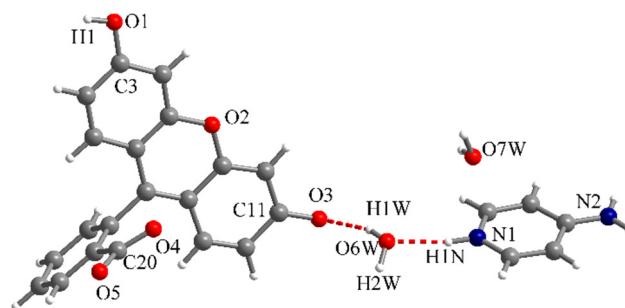


Figure 11. Perspective view of the asymmetric unit in crystal 6.

The xanthene fragments establish π - π interactions (3.57–3.65 Å) forming supramolecular dimers. The fluorescein supramolecular dimers are connected into an extended supramolecular network through hydrogen interactions with crystallization water molecules and 4-aminopyridinium cations (Figure 12). Except the xanthene O2 atom, all the other oxygen atoms of the fluorescein are involved in hydrogen interactions.

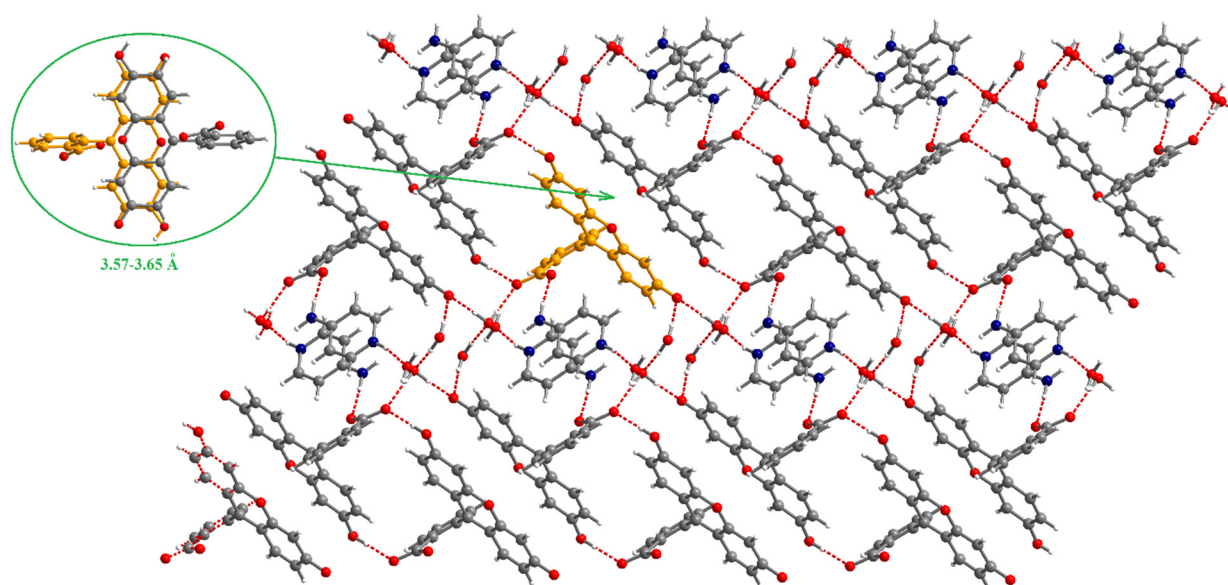


Figure 12. View of a packing diagram in crystal 6. The inset shows a detail of the π - π interactions established within the supramolecular dimers formed by the fluorescein monoanions.

In crystal 7, the lactone spirocycle of fluorescein is also open but only half of the molecules are deprotonated. The proton from one phenol group is transferred on a pyridine nitrogen atom or on a carboxylate O5 atom (Figure 13). The ampy molecules are disordered on two crystallographic positions with site occupancy factors of 0.5. Due to the disorder of ampy molecules we could not refine the percentages of proton transfer on pyridine nitrogen atoms. We estimated that half of the protons are transferred on the carboxylate due to the short O5...O5' distance of 2.506 Å between the carboxylate groups of neighboring fluorescein molecules, which can be explained by hydrogen interactions (the occupancy of the H5A atom was fixed at 0.5). The C3-O1 bond length is 1.336(7) Å, while the C11-O3 bond length is 1.300(6) Å. The carboxylate bond lengths are C20-O4 = 1.218(7) and C20-O5 = 1.275(7) Å.

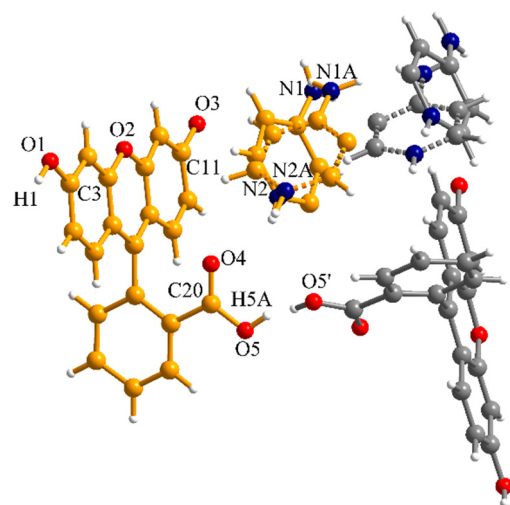


Figure 13. Crystal structure of 7. The asymmetric unit is depicted in orange. Symmetry code: $' = -x, y, 1.5 - z$.

In the reactions of fluorescein with *trans*-1,4-diaminocyclohexane, we initially attempted two stoichiometric ratios, 1:1 and 2:1. In the 1:1 reaction we obtained the compounds $[(\text{Fl})(\text{H}_2\text{diach})] \cdot 3\text{H}_2\text{O}$ (8) and $[(\text{HFl})_2(\text{Fl})_2(\text{H}_2\text{diach})_3] \cdot 4\text{H}_2\text{O}$ (10), while for the 2:1 ratio one type of crystal resulted: $[(\text{HFl})_2(\text{H}_2\text{diach})] \cdot 2\text{H}_2\text{O} \cdot \text{EtOH}$ (9). Compound 10 was obtained subsequently as pure phase using a 4:3 molar ratio between fluorescein and diach.

Compound 8 contains fluorescein dianions (Fl^{2-}), $\text{H}_2\text{diach}^{2+}$ dications and crystallization water molecules. The asymmetric unit is formed by one Fl^{2-} dianion, two halves of $\text{H}_2\text{diach}^{2+}$ dications and three water molecules (Figure 14). One water molecule is disordered on two crystallographic positions with site occupancy factors of 0.6 (O8) and 0.4 (O9). The C3-O1 and C11-O3 bond lengths are 1.288(3) and 1.278(3) Å, respectively. In the carboxylate group the bond lengths are C20-O4 = 1.225(3) and C20-O5 = 1.231(4) Å. The protonated amino groups are hydrogen bonded to the O1 atom with the (N1)-H1A...O1 and (N2)-H2B...O1 distances of 1.831 and 1.881 Å, respectively. The distances for the hydrogen interactions involving the O6 and O7 water molecules are (O6)-H1O...O4 = 2.169, (O7)-H3O...O3 = 1.814 and (O7)-H4O...O5' = 1.918 Å (symmetry code: $' = 2 - x, 2 - y, 2 - z$).

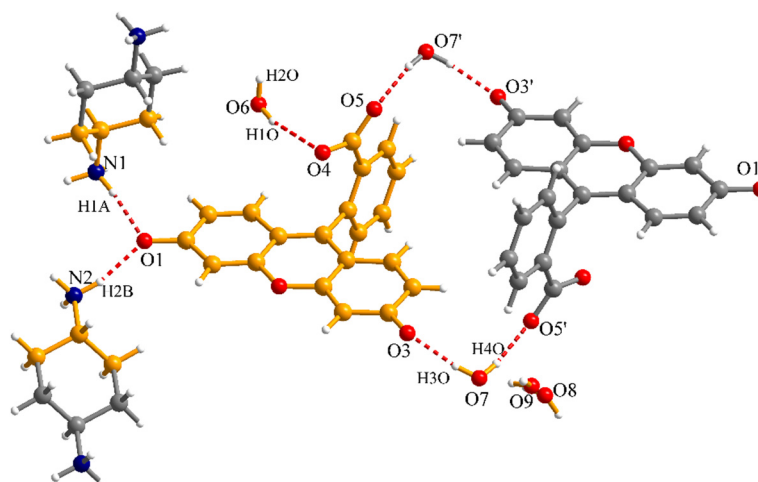


Figure 14. Crystal structure of 8. The asymmetric unit is depicted in orange. Symmetry code: $' = 2 - x, 2 - y, 2 - z$.

In compound **9**, the stoichiometry used in the reaction was recovered in the crystal structure. The asymmetric unit contains two fluorescein monoanions (HFl^-), one $\text{H}_2\text{diach}^{2+}$ dication and crystallization solvent molecules, two water molecules and one ethanol molecule (Figure 15). The ethanol molecule is disordered on two crystallographic positions. The two HFl^- monoanions are in the quinonoid open-ring form. The bond lengths for the phenol and carbonyl groups are $\text{C3-O1} = 1.333(2)$, $\text{C23-O6} = 1.338(2)$ Å, $\text{C11-O3} = 1.270(2)$ and $\text{C31-O8} = 1.261(2)$ Å. The bond lengths in the two carboxylate groups are $\text{C20-O4} = 1.240(2)$, $\text{C20-O5} = 1.254(2)$, $\text{C40-O9} = 1.234(2)$ and $\text{C40-O10} = 1.255(2)$ Å.

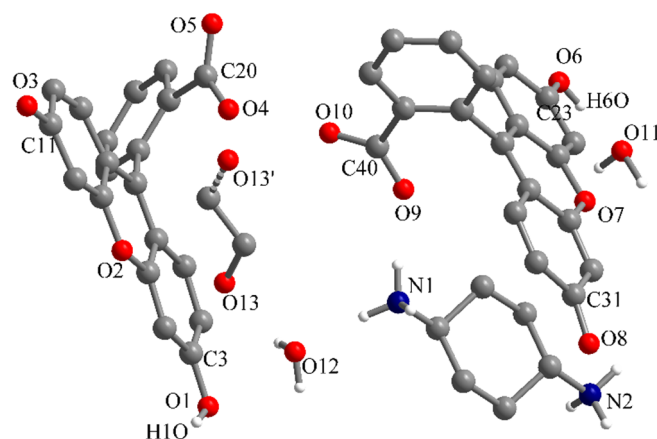


Figure 15. View of the asymmetric unit in crystal **9** (part of the hydrogen atoms was omitted for clarity). Symmetry code: $' = -x, 1 - y, -z$.

The two protonated amino groups of the $\text{H}_2\text{diach}^{2+}$ dication are hydrogen bond donors towards four carboxylate groups, one carbonyl group and one water molecule (Figures 16 and 17). The distances for these hydrogen interactions are $(\text{N1})\text{H1A} \cdots \text{O12} = 1.885$, $(\text{N1})\text{H1B} \cdots \text{O9} = 1.885$, $(\text{N1})\text{H1C} \cdots \text{O3}' = 1.975$, $(\text{N2})\text{H2A} \cdots \text{O10}'' = 1.852$, $(\text{N2})\text{H2B} \cdots \text{O5}''' = 1.861$ and $(\text{N2})\text{H2C} \cdots \text{O4}'' = 2.085$ Å. The corresponding angles are $\text{N1-H1A} \cdots \text{O12} = 172.8$, $\text{N1-H1B} \cdots \text{O9} = 155.0$, $\text{N1-H1C} \cdots \text{O3}' = 169.7$, $\text{N2-H2A} \cdots \text{O10}'' = 174.5$, $\text{N2-H2B} \cdots \text{O5}''' = 166.6$ and $\text{N2-H2C} \cdots \text{O4}'' = 156.2^\circ$.

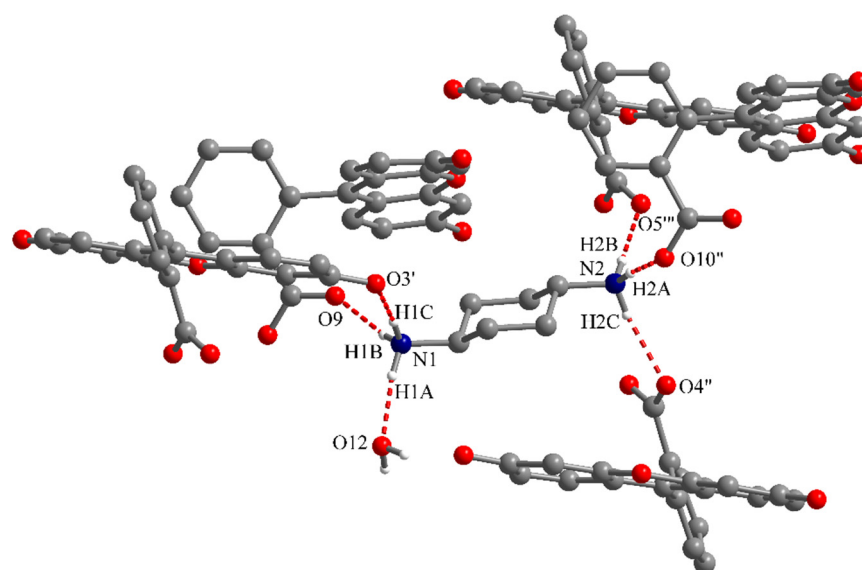


Figure 16. Detail of the hydrogen interactions established by $\text{H}_2\text{diach}^{2+}$ dications in crystal **9** (part of the hydrogen atoms was omitted for clarity). Symmetry codes: $' = -x, 1 - y, -z$; $'' = 1 + x, y, z$; $''' = 1 - x, 1 - y, 1 - z$.

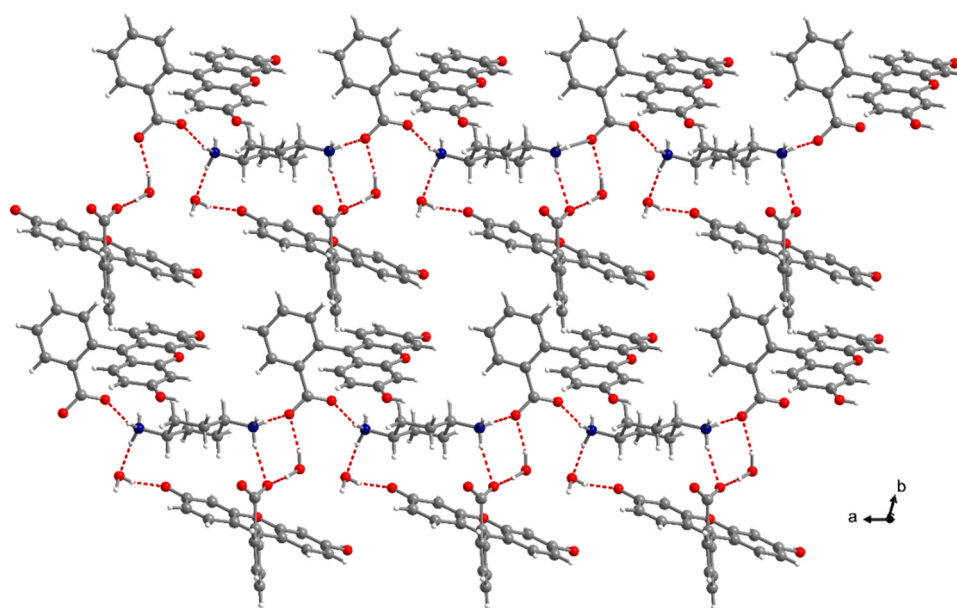


Figure 17. View along the crystallographic c axis of a packing diagram in crystal 9.

The compound **10**, $[(\text{HFl})_2(\text{Fl})_2(\text{H}_2\text{diach})_3] \cdot 4\text{H}_2\text{O}$, was obtained initially as by-product in the synthesis of compound **8** but subsequently we managed to obtain it as pure phase using a 4:3 molar ratio between fluorescein and diach (Figures S10–S13). Compound **10** is also a salt containing two types of fluorescein anions, mono- (HFl^-) and dianions (Fl^{2-}), $\text{H}_2\text{diach}^{2+}$ dications and crystallization water molecules (Figure 18). Within monoanions the bond lengths for the phenol and carbonyl groups are $\text{C23-O6} = 1.320(5)$ and $\text{C31-O8} = 1.283(5)$ Å, while for the dianions the C3-O1 and C11-O3 bond lengths are $1.285(5)$ and $1.296(5)$ Å, respectively. The phenol group of the monoanion is involved in hydrogen interaction with a carbonyl group of the dianion. The second carbonyl group of the dianion is hydrogen bonded to a crystallization water molecule. The distances for these hydrogen interactions are $(\text{O6})\text{H6} \cdots \text{O3} = 1.700$ and $(\text{O11})\text{H1W} \cdots \text{O1} = 1.739$ Å. The bond lengths in the two carboxylate groups are $\text{C20-O4} = 1.221(5)$, $\text{C20-O5} = 1.263(5)$, $\text{C40-O9} = 1.239(5)$ and $\text{C40-O10} = 1.245(5)$ Å. The simultaneous presence of both mono- and dianions of fluorescein was previously reported for lanthanide coordination polymers [24].

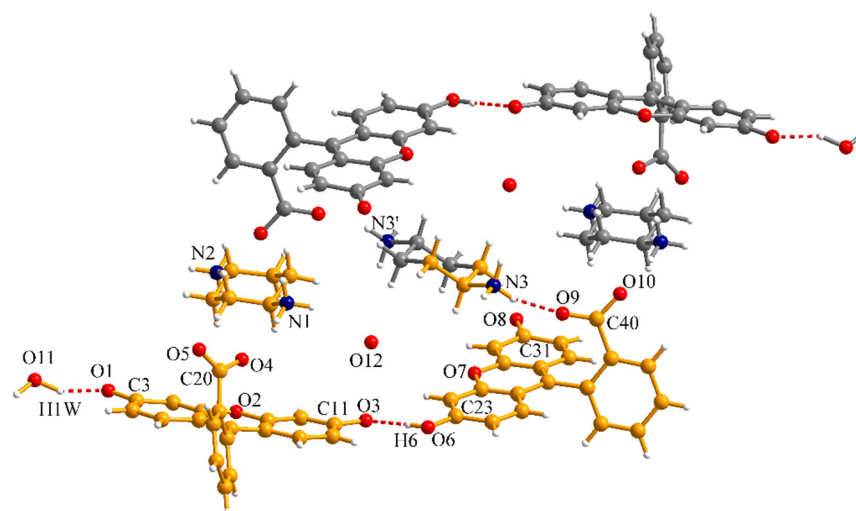


Figure 18. Crystal structure of compound **10**. The asymmetric unit is depicted in orange. Symmetry code: $' = 1 - x, 1 - y, 1 - z$.

3.2. Spectral Properties

The absorption spectra of compounds **1–5** and **7–10** have been acquired over a wavelength range from 200 to 1000 nm on solid samples (using the diffuse reflectance technique). Compound **6** was obtained only in small amounts on the wall of the beaker and we were not able to investigate the optical properties for this compound. As we already mentioned, there is significant color difference between the cocrystals of fluorescein (compounds **1–5**) and the organic salts of fluorescein (compounds **6–10**). The cocrystals are pale yellow or colorless and the salts are red. The cocrystals present clearly separated absorption bands in the UV (with maxima around 300–350 nm) and visible regions (with maxima around 460 and 490 nm). The salts present broad bands covering the UV and visible regions with bathochromic shifts in the absorptions. The solid-state absorption spectra of compounds **1–5** and **7–10** are presented in Figure 19a,b and in Table 1 the wavelengths for the absorption maxima are gathered.

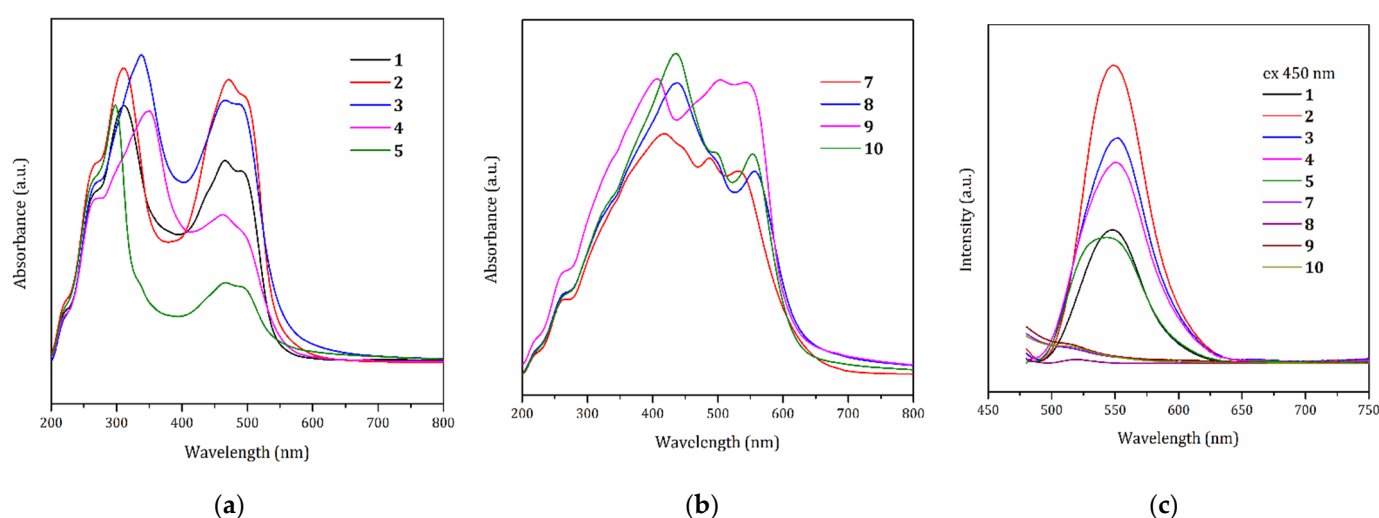


Figure 19. The solid-state absorption spectra of cocrystals **1–5** (a) and salts **7–10** (b). Emission spectra of compounds **1–5** and **7–10** (c).

Table 1. Absorption maxima for compounds **1–5** and **7–10**.

Compound		Wavelength (nm)	
1	311	465	490
2	311	471	494
3	337	465	485
4	350	463	
5	298	466	490
7	418	487	531
8	435	497	553
9	438	497	555
10	406	504	542

The room temperature photoluminescence of compounds **1–5** and **7–10** was investigated using different wavelengths for excitation in the 430–460 nm range. The resulting emission spectra using $\lambda_{\text{ex}} = 450$ nm are presented in Figure 19c. First of all, we have to notice that no emission was observed in solid-state for the salts. The emission spectra of compounds **1–4** display symmetric bands with maxima at 550 nm. Cocrystal **5** presents a broader emission band with a maximum at 542 nm.

4. Conclusions

Fluorescein proved to be a versatile molecule in the binary systems formed with different nitrogen-containing organic molecules. The phenol groups of the fluorescein can act either as hydrogen bond donors generating cocrystals or as proton donors producing organic salts. For molecules containing only pyridine rings such as 4,4'-bipyridyl; *trans*-1,2-bis(4-pyridyl)ethylene; and 1,2-bis(4-pyridyl)ethane, we obtained cocrystals. The presence of more basic amino groups on the nitrogen-containing organic partner conducted syntheses to salts and the deprotonation degree of the fluorescein depended on the molar ratio used. With *trans*-1,4-diaminocyclohexane, by modifying the stoichiometry, we obtained salts containing only monoanions of fluorescein, only dianions of fluorescein or both mono- and dianions. This versatility of fluorescein allows modulation of the optical properties of the binary systems by choosing a suitable partner.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/cryst11101217/s1>, Figures S1–S13: Powder X-ray diffraction patterns, Table S1: Crystallographic data, details of data collection and structure refinement parameters for compounds 1–10.

Author Contributions: Conceptualization, A.M.M.; methodology, A.M.M.; synthesis, M.R.; formal analysis, M.R. and A.M.M.; investigation, M.R. and A.M.M.; writing—original draft preparation, A.M.M.; writing—review and editing, M.R. and A.M.M.; funding acquisition, A.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by UEFISCDI (Project PN-III-P4-ID-PCE-2016-0442 nr. 89/2017).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: M.R. is grateful to Maria Maganu for recording the IR spectra.

Conflicts of Interest: The authors declare no conflict of interest.

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