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Isopyrazole-Masked Tetraketone: Tautomerism and Functionalization for Fluorescent Metal Ligands

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Abstract: An isopyrazole (4*H*-pyrazole)-embedded nonane-2,4,6,8-tetraone derivative was synthesized as a masked-tetraketone for stepwise and multiple functionalization. The masked-tetraketone has six possible tautomers, but exists predominantly as a 12 π -electron conjugated keto-enamine-imine-enol form, which was also observed in the solid state by single crystal X-ray analysis. Stepwise functionalization to introduce metal-coordination and fluorescence-enhancement sites into the masked tetraketone furnished a fluorescent chromophore that indicates metal coordination on a microgram scale by considerably changing the solid-state emission.

Aliphatic polyketones bearing 1,3-diketone subunits are common intermediates in biological synthesis^[1] and in functional organic materials because of their ability to form metal complexes and π -conjugated chromophores.^[2] Their synthetic utility is widely recognized, but site-selective functionalization of polyketones at each carbonyl site is still problematic. Harris *et al.* reported terminal-selective α -acylation of an octane-1,3,5,7-tetraone derivative, which is a natural type β -polyketone, via generation of a quadruple lithium enolate species.^[3] However, further functionalization of internal carbonyl groups has not been reported. A major obstacle to site-selective functionalization of β -polyketones is tautomerization of 1,3-diketone units, which leads to a complicated mixture of possible tautomers with different reactivities.^[3b,4] It is quite important to control such tautomerism for implementation of biosynthesis-like multiple functionalization of polyketones.

Our recent studies of aliphatic polyketones^[5] have shown that isopyrazole (4*H*-pyrazole) can be used to mask 1,3-diketone units. This can prevent complicated tautomerism of β -polyketones (Figure 1). Isopyrazole-masked units can be readily generated from 2,2-dialkylated-1,3-diketones in quantitative yields by treatment with hydrazine, e.g., conversion of **1** into **2** (Figure 2a).^[6] The five-membered ring stabilizes ketimines against hydrolysis, therefore isopyrazole ligands can form various metal complexes. Despite the presence of a quaternary carbon atom, an isopyrazole can be embedded into π -conjugated systems as a 4 π -diimine unit, analogously to enone-forms of 1,3-diketones.



Figure 1. Structures of natural-type polyketone and isopyrazole-masked analogue for multiple functionalization.

Given these isopyrazole characteristics, we explored the possibility of multiple functionalization of isopyrazole-masked polyketones. Here, we describe the synthesis and site-selective, full-functionalization of isopyrazole-masked β -tetraketone **3**. Crystallographic and theoretical analysis showed that masked tetraketone **3** predominantly exists in an enol-enamine form among six possible tautomers. Masked tetraketone **3** underwent stepwise functionalization at each carbonyl group to incorporate a 12 π -conjugated chromophore, a BF_3 -complex unit that can amplify emission, and a metal-ion-coordinating site. The obtained tetraketone-based fluorophore **5** was used as an indicator of metal coordination via solid-state emission changes.

Isopyrazole-masked tetraketone **3** was synthesized from 3,3-dimethylpentane-2,4-dione (**1**) in three steps (Figure 2a). Treatment of **1** with hydrazine furnished 3,4,4,5-tetramethyl-4*H*-pyrazole (**2**) quantitatively.^[6] Such imination reactions are known to be reversible.^[7] Double lithiation of the methyl groups at the 3- and 5-positions of **2** was achieved by addition of 3 equiv of lithium diisopropylamide (LDA) in THF. Subsequent addition of 2.3 equiv of ethyl trifluoroacetate gave masked tetraketone **3** in 64% yield. For double acylation of **2**, use of a trifluoroacetate ester was necessary; use of less-electrophilic ethyl benzoate led to formation of singly acylated products in low yields.

Masked-tetraketone **3** has six possible forms, which are interconvertible via keto-enol and imine-enamine

tautomerizations (Figure 2b), despite the presence of only two enolizable methylene units. Such tautomerism in natural-type β -polyketones has hampered full NMR spectroscopic characterization of related compounds.^[8] However, the ^1H NMR spectrum of **3** in CDCl_3 is simple, with a broad peak at 11.27 ppm, and two singlet peaks at 5.67 and 1.39 ppm, assignable to OH (or NH), olefinic, and methyl protons, respectively. The absence of methylene($-\text{CH}_2-$) proton signals indicates that **3** exists as 12 π -conjugated tautomer **iv**, **v**, or **vi**. Theoretical calculations^[9] performed at the M06-2X/6-311+G(d,p)//B3LYP/6-31+G(d,p) level in chloroform using the polarizable continuum model showed that the enamine–enol form **iv** is the most stable among the six tautomers. The second-most stable tautomer **iii** was 16.0 kJ/mol less stable than **iv**, which is large enough for **3** to exist exclusively as **iv** in solution. These results enable the simple NMR spectrum of **3** to be interpreted as rapid exchange of NH and OH protons in tautomer **iv** on the NMR time scale.

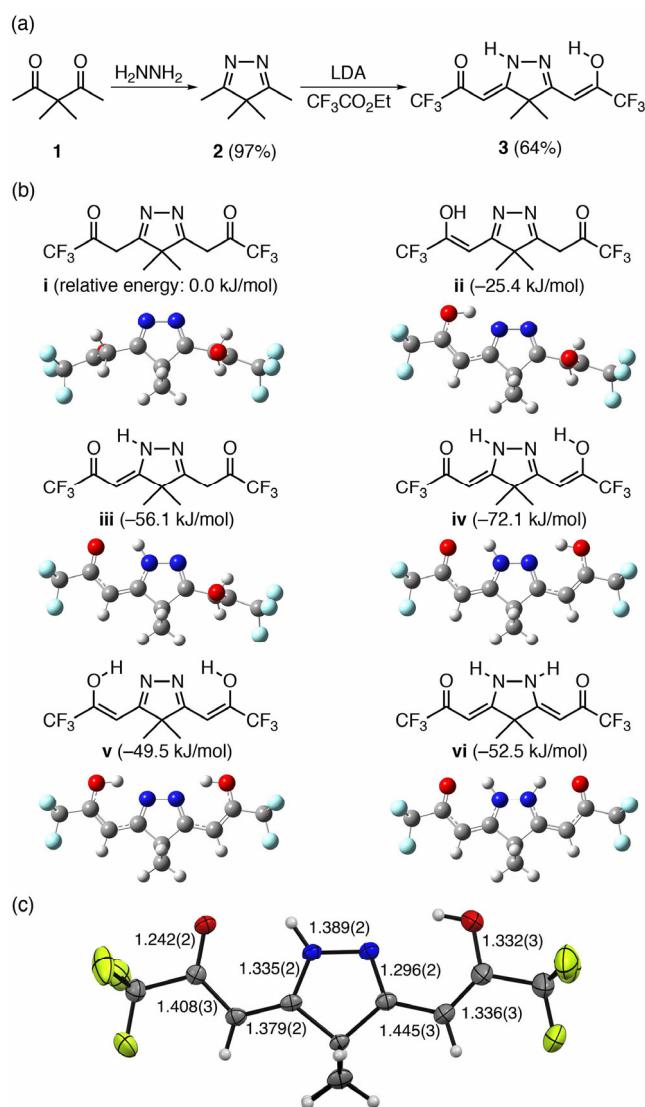


Figure 2. (a) Synthesis of isopyrazole-masked tetraketone **3**. (b) Six possible tautomers **i–vi** of **3** and their relative energies calculated at the M06-2X/6-311+G(d,p)//B3LYP/6-31+G(d,p) level. (c) Crystal structure of **3** drawn at the 50% probability level of thermal ellipsoids (H: white, C: gray, N: blue, O: red, and F: yellow green) and selected bond lengths in angstroms.

Single-crystal X-ray analysis of **3** clearly showed the solid-state structure of tautomer **iv** without disorder from other tautomers. The bond-length alternation shown in Figure 2c indicates 12 π -electronic conjugation consisting of keto–enamine–imine–enol units (from left to right). The observed tautomer **iv** implies that substitution at a ketone site will affect the reactivity of another carbonyl group through π -conjugation.

For functionalization of masked tetraketone **3**, we first introduced a metal-coordination site to harness the π -conjugated system as a probe for interactions with metal ions. When a toluene solution of **3** and 1.5 equiv of 2-picolyamine was refluxed in the presence of *p*-TsOH, the mono-substituted product **4** was obtained in 58% yield (Figure 3a). Only a trace amount of the disubstituted byproduct was formed. For acetylacetone, double substitution with amines requires harsh conditions because of the decreased electrophilicity of the remaining carbonyl group after ketimine formation.^[10] Similarly, the remaining carbonyl group in **4** is less electrophilic than that in **3**, which prevents further substitution. The crystal structure of **4** showed a tautomer with enamine–imine–enamine–keto conjugation in which a tridentate coordination site was formed around the 2-picoly group (Figure 3b).

To enhance the fluorescence efficiency, a BF_2 -unit was complexed with the remaining ketone site of **4**. Treatment of **4** with $\text{BF}_3\text{-OEt}_2$ and triethylamine led to the formation of BF_2 -complex **5** in 83% yield. Complexation occurred only at the less-hindered ketone–enamine site. The crystal structure of **5** confirmed the formation of a boron-containing six-membered ring at the ketone site, whereas the tridentate coordination site had not reacted (Figure 3c). The 14 π -electronic conjugation was still preserved after the stepwise, full substitution at ketone sites.

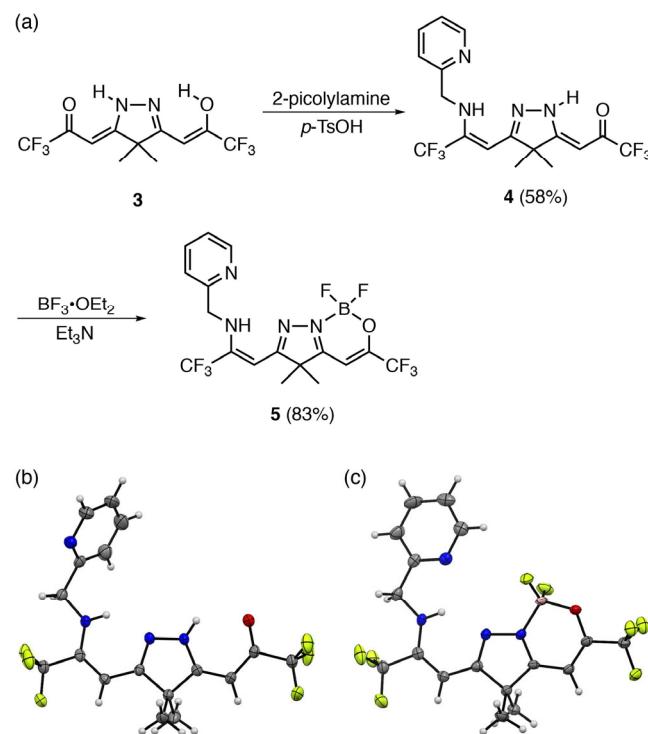


Figure 3. (a) Synthesis of fluorescent ion sensor **5**. ORTEP drawings of crystal structures of (b) **4** and (c) **5** at the 50% probability level.

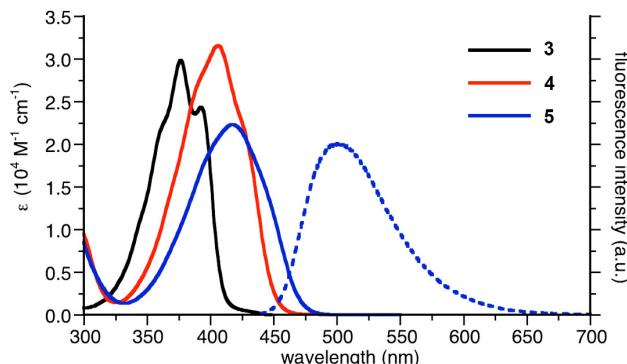


Figure 4. UV-vis absorption spectra (solid lines) of compounds **3**, **4**, and **5**, and fluorescence emission spectrum (dotted lines; excitation wavelength: 418 nm) of **5** in CH_2Cl_2 .

The UV-vis absorption spectra of **3–5** showed intense visible region absorption bands (Figure 4). Masked tetraketone **3** in dichloromethane gave an absorption band at 377 nm with an absorption coefficient of $3.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Similar absorption bands were observed for **4** and **5** at 406 and 418 nm, respectively; their solutions were yellow. Fluorescence emissions of **3** and **4** were not observable at standard concentrations (optical densities of 0.05–0.10), but **5** showed a green emission at 503 nm, which mirrored the absorption band at 418 nm. The fluorescence quantum yield and lifetime were 8% and 0.8 ns, respectively, in dichloromethane. The fluorescence quantum yield of **5** in the solid-state increased to 56%, but solid-state emission of precursor **4** was too weak to obtain emission spectrum. This clearly shows the importance of the BF_2 -unit in enhancing the fluorescence emission of the masked tetraketone chromophore.

Changes in fluorescence emission of **5** upon metal-coordination were investigated using various metal acetate salts in a dichloromethane/methanol (v/v, 1:1) mixed solution [Figure S2 in the Supporting Information(SI)]. Chromophore **5** gave two different responses, which are typified by the responses to Zn^{2+} and Ni^{2+} ions. On addition of excess Zn^{2+} ions, the green fluorescence emission of **5** turned yellow, but addition of Ni^{2+} ions led to the fluorescence quenching. Similar fluorescence quenching was observed for Co^{2+} and Cu^{2+} salts, while Fe^{2+} ions did not show complexation behavior with **5**.

To gain further insights into the response of **5** to Zn^{2+} ions, titration experiments with ^1H NMR spectroscopic analysis were performed. When 1 equiv of zinc acetate was added to a 9.0 mM solution of **5** in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1), a new set of ligand proton signals from a zinc complex **6** appeared and the intensity of the original signal from **5** decreased. On addition of 3 equiv of the zinc salt, ligand **5** was completely converted to complex **6** (Figure 5 and S4 in the SI). High-resolution APCI mass spectrometry indicated the formation of dinuclear zinc complex **6**, the parent ion peak at $m/z = 592.9745$ corresponds to $[\mathbf{6}-\text{OAc}]^+$ (calculated for $[\text{C}_{19}\text{H}_{17}\text{F}_6\text{N}_4\text{O}_3\text{Zn}_2]^+$, 592.9757). Formation of **6** was associated with deboronation of **5**, presumably due to Lewis acidic zinc ions. Complex **6** was also quantitatively obtained by treating **4** with 2 equiv of zinc acetate. Single-crystal X-ray analysis also confirmed the formation of zinc complex **6**, although **6** reversibly formed a dimer on crystallization (Figure S10).

Quantitative formation of **6** from **5** was also observed by titration with UV-vis absorption and fluorescence spectroscopies. On addition of zinc acetate (0~4 equiv) to a 35 μM solution of **5** in dichloromethane/methanol (v/v, 1:1), the absorption band at 412 nm gradually attenuated, with the concomitant appearance of new bands at 309, 443, and 466 nm; these absorption bands are assignable to **6**. Fluorescence titration showed that on addition of Zn^{2+} ions, the intensity of the original emission from **5** at 505 nm progressively decreased, with a concomitant increase in the intensity of a new emission band at 486 nm. Addition of approximately 100 equiv of zinc acetate was needed to reach saturation at a 1.0 μM concentration; the final spectrum was almost identical to that of **6** (fluorescence quantum yield: 18%).

Titration experiments with nickel(II) acetate showed UV-vis spectral changes similar to those with zinc acetate (Figure S3). However, the fluorescence emission was almost completely quenched on addition of 100 equiv of nickel(II) acetate (Figure S6). ESI-TOF mass spectrometric analysis indicated the formation of a dinuclear nickel complex with loss of the BF_2 unit, similarly to the case for **6**. The fluorescence quenching on nickel complexation is attributable to energy transfer by d-d transitions of the nickel center. While fluorescence titration with Co^{2+} ion showed similar quenching to that of Ni^{2+} in the range of 10 to 100 equiv, complete fluorescence quenching was observed only with 10 equiv of Cu^{2+} ions, showing rather high sensitivity of **5** for Cu^{2+} ion over other metal ions. The competition experiment using fluorescence spectroscopy revealed that ligand **5** can detect Cu^{2+} ion at 0.5–10 μM concentrations even in the presence of 30 equiv of Zn^{2+} ions (Figure S7).

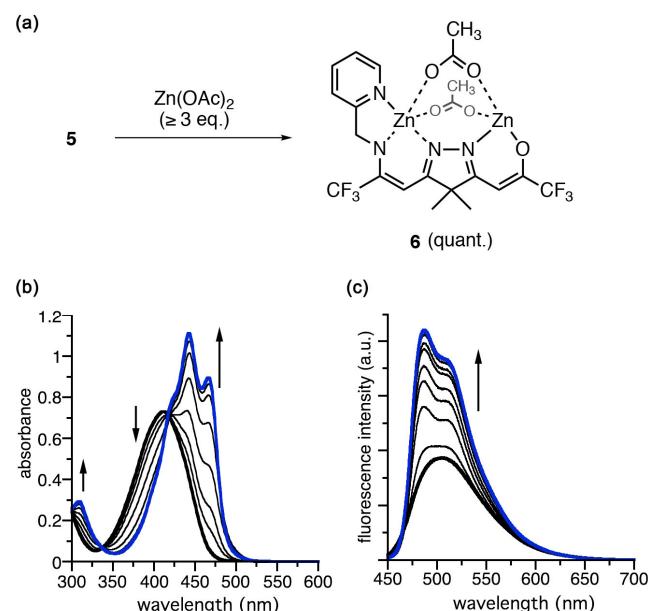


Figure 5. (a) Quantitative formation of zinc complex **6** from **5**. (b) UV-vis absorption spectra of **5** (35 μM) upon addition of 0.0 (bold black line), 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 4.0 equivalents (bold blue line) of zinc acetate in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1). (c) Fluorescence spectra obtained during titration of **5** (1.0 μM) with 0.0 (bold black line), 10, 20, 30, 40, 60, 80, and 100 equivalents (bold blue line) of zinc acetate ($\lambda_{\text{ex}} = 418 \text{ nm}$) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1).

We used the emission and quantitative metal-complexation properties of **5** for microgram-scale metal-ion detection.^[11] A solution drop (20 μL) containing **5** (1.0 μg) was mixed with

various metal acetate salts, and the solution and emission color changes were investigated. The solution color changes were too slight to be distinguished by the naked eyes, and the fluorescence emission behavior varied depending on the metal ion (Figure 6a). The fluorescence from a solution drop containing Zn^{2+} ions turned slightly yellowish green as compared to **5** (blank). Solution drops containing Co^{2+} , Ni^{2+} , and Cu^{2+} ions gave fluorescence quenching, but strong emission from uncomplexed **5** was still observable in each case.

Solvent evaporation led to considerable changes in the solid color and emission intensity (Figure 6b). A solution drop containing Zn^{2+} ions left an orange solid that corresponded to zinc complex **6**. The fluorescence emission of **6** in the solid state was too weak to be observed, in sharp contrast to that of the solution. Solid spots containing Co^{2+} , Ni^{2+} , and Cu^{2+} salts were also non-fluorescent in the solid state. This indicates complexation-induced fluorescence quenching of **5**. Whereas, Fe^{2+} salt did not affect solid-state emission of **5**. Although the detection limit is rather high (approximately 10 μM),^[12] these results clearly show the potential of masked-tetraketone-based chromophores for metal-ion detection.

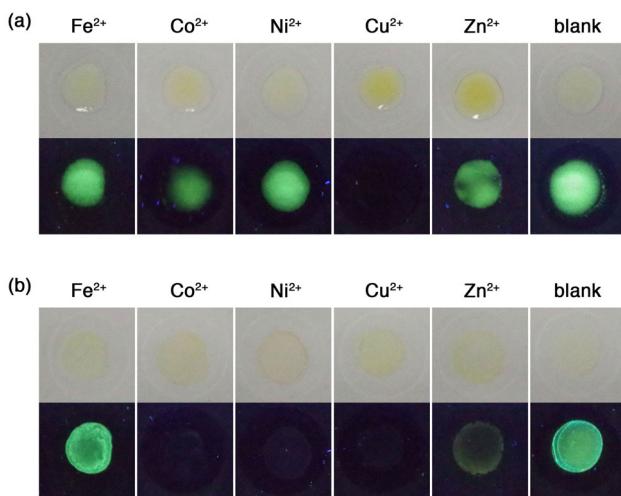


Figure 6. (a) Photographs of solution droplets containing 1.0 μg of ligand **5** in 20 μL of $CH_2Cl_2/MeOH$ (v/v, 1:1) with 3.0 μg of (from left to right) iron(II), cobalt(II), nickel(II), copper(II), and zinc(II) acetate salts. The rightmost column shows control solution of **5** without any salt. (top: under ambient light, bottom: under 365 nm UV light) (b) Photographs after evaporation of the solvent.

In summary, we have shown that isopyrazole-masked tetraketone **3** provides a good scaffold for stepwise, multiple functionalization of β -polyketone analogues. Control of the complex tautomerism of **3** allowed sequential introduction of 12 π -electronic conjugation, tridentate metal-coordination site, and emission enhancement part onto the C9 main chain. The solid-state emission changes of the masked-tetraketone-based fluorophore **5** were used to indicate metal coordination on a microgram-scale. It should be emphasized that these multiple molecular functions were installed into a small molecule, by using the diverse reactivity of polyketones. Such functionalization of polyketone analogues is reminiscent of polyketide biosynthesis. Although enzymes play an important role in biosynthesis by controlling the tautomerism and reactivity

of polyketones, isopyrazole masking provides a useful strategy for artificial functionalization of β -polyketones.

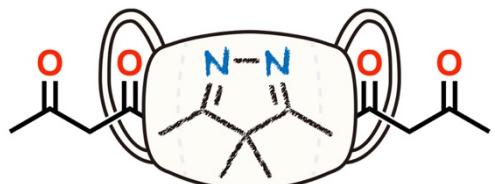
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Keywords: Fluorescence • Metal-coordination • Tautomerism • Polyketone

- [1] a) C. Hertweck, *Angew. Chem. Int. Ed.* **2009**, *48*, 4688–4716; b) V. C. Fäseke, R. C. Raps, C. Sparr, *Angew. Chem. Int. Ed.* **2020**, *59*, 6975–6983. c) J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, *18*, 380; d) T. P. Stockdale, N. Y. S. Lam, M. J. Anketell, I. Paterson, *Bull. Chem. Soc. Jpn.* **2012**, *94*, 713.
- [2] a) G. Aromí, P. Gamez, J. Reedijk, *Coord. Chem. Rev.* **2008**, *252*, 964–989; b) Y. Inokuma, T. Yoneda, Y. Ide, S. Yoshioka, *Chem. Commun.* **2020**, *56*, 9079–9093.
- [3] a) T. A. Oster, T. M. Harris, *Tetrahedron Lett.* **1983**, *24*, 1851–1854; b) P. J. Wittek, T. M. Harris, *J. Am. Chem. Soc.* **1973**, *95*, 6865–6867.
- [4] a) A. J. Birch, P. Fitton, D. C. C. Smith, D. Steere, A. R. Stelfox, *J. Chem. Soc.* **1963**, 2209–2216; b) T. M. Harris, C. M. Harris, *Pure Appl. Chem.* **1986**, *58*, 283–294.
- [5] a) M. Uesaka, Y. Saito, S. Yoshioka, Y. Domoto, M. Fujita, Y. Inokuma, *Commun. Chem.* **2018**, *1*, 23; b) Y. Manabe, M. Uesaka, T. Yoneda, Y. Inokuma, *J. Org. Chem.* **2019**, *84*, 9957–9964; c) Y. Ashida, Y. Manabe, S. Yoshioka, T. Yoneda, Y. Inokuma, *Dalton Trans.* **2019**, *48*, 818–822.
- [6] A. L. Baumstark, A. Choudhary, P. C. Vasquez, M. Dotrong, *J. Heterocycl. Chem.* **1990**, *27*, 291–294.
- [7] P. Paredes, H. Bastos, R. Montoya, A. L. Chavez, *Tetrahedron* **1988**, *44*, 6821–6830.
- [8] R. M. Sandifer, A. K. Bhattacharya, T. M. Harris, *J. Org. Chem.* **1981**, *46*, 2260–2267.
- [9] a) A. R. Hajipour, M. Karimzadeh, S. Jalilvand, H. Farrokhpour, A. N. Chermahini, *Comput. Theor. Chem.* **2014**, *1045*, 10–21; b) K. Jana, B. Ganguly, *ACS Omega* **2018**, *3*, 8429–8439; c) D. K. Dhaked, P. V. Bharatam, *J. Inorg. Biochem.* **2015**, *142*, 84–91.
- [10] a) H. Bredereck, F. Effenberger, D. Zeyfang, *Angew. Chem. Int. Ed. Engl.* **1965**, *4*, 242; b) S. G. McGeachin, *Can. J. Chem.* **1968**, *46*, 1903–1912.
- [11] a) Z. Liu, W. He, Z. Guo, *Chem. Soc. Rev.* **2013**, *42*, 1568–1600; b) Z. Xu, J. Yoon, D. R. Spring, *Chem. Soc. Rev.* **2010**, *39*, 1996–2006; c) J. F. Zhang, Y. Zhou, J. Yoon, J. S. Kim, *Chem. Soc. Rev.* **2011**, *40*, 3416–3429; d) D. T. Quang, J. S. Kim, *Chem. Rev.* **2010**, *110*, 6280–6301.
- [12] Several BF_3 -complex-based fluorescent detectors (quantum yield >80%) for ion detection at submicro molar are known: N. Boens, V. Leen, W. Dehaen, *Chem. Rev.* **2012**, *41*, 1130–1172.

Entry for the Table of Contents



Isopyrazole-masking strategy allowed to control complicated tautomerism of a nonane-2,4,6,8-tetraone derivative having a natural type polyketone sequence. Stepwise, multiple functionalization of isopyrazole-masked tetraketone furnished a fluorescent metal ligand that considerably changes solid-state emission upon metal coordination.