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Computational Insights into Functionalized Calcein Derivatives (D1–D5) for Enhanced Nonlinear Optical Response: A DFT-Based Study

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ABSTRACT

Optimization of organic chromophores with higher nonlinear optical (NLO) properties is at the heart of recent optoelectronics, photonics, and data storage. Calcein, being a xanthene-derived fluorescent dye, presents a robust platform for structural tuning towards increased Nonlinear optical response. In the present research, we computationally investigate a series of hypothetical Calcein functional derivatives (D1–D5), with electron-donating and electron-withdrawing substituents. The Density Functional Theory (DFT) and Time-Dependent DFT (TD-DFT) models were used to geometrical optimize, determine frontier orbital distributions, calculate dipole moments, predict UV-Vis absorption spectra, and estimate first-order hyperpolarizability (β). Results show that the functionalization has substantial impacts on intramolecular charge transfer (ICT), resulting in increased NLO responses over parent Calcein. Amongst the derivatives, Amino-Calcein (D1) and Nitro-Calcein (D2) registered the maximum β values, indicating their strong potential as future-generation NLO chromophores. In this work, Calcein derivatives have been brought into focus as lead contenders for experimental synthesis and future use in cutting-edge photonic materials.

Keywords: Calcein, Nonlinear Optics, Second Harmonic Generation, DFT, Hyperpolarizability, Molecular Engineering

Introduction

The area of nonlinear optics (NLO) has seen tremendous development because of its importance in optical communication, photonic switching, and data processing technology. Organic materials, especially, have emerged as vital components for second-order nonlinear processes because of their structural tunability, low cost, and high value of optical nonlinearity over the corresponding inorganic materials. Second-harmonic generation (SHG), perhaps one of the most researched NLO effects, is

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critically dependent on molecular symmetry, electronic delocalization, and intramolecular charge transfer (ICT) processes [1,2].

Molecular design is usually in the form of electron-donating (D) and electron-withdrawing (A) substituents bridged by a π-conjugated bridge (D–π–A systems). These configurations favor charge asymmetry and induce large first-order hyperpolarizabilities (β), a direct indicator of molecular nonlinear response [3,4]. In organic scaffolds, xanthene derivatives fluorescein and rhodamine have been extensively investigated for applications in optics because they exhibit high fluorescence and π-conjugation [5].

Calcein, a hydrophilic xanthene derivative, provides a versatile platform for functionalizing due to the presence of its reactive amine and hydroxyl groups. Although widely used in fluorescence assays, its capability as a nonlinear optical chromophore is yet to be fully explored. The addition of various substituents like amino, nitro, fluoro, thio, and alkyl groups can significantly modify its electronic distribution and ICT nature, thereby potentially enhancing NLO performance [6–8]. Theoretical assessment through Density Functional Theory (DFT) offers a cost-effective pathway to forecast these effects prior to experimental synthesis [9].

The current research seeks to explore the structure–property relationships of Calcein and five functionalized derivatives (D1–D5) based on DFT and time-dependent DFT (TD-DFT) approaches. Computational analysis involves molecular geometries, frontier molecular orbitals, dipole moments, absorption spectra, and hyperpolarizability values. Such a thorough evaluation offers predictive information on how donor–acceptor substitution affects NLO activity and facilitates the identification of the most promising candidates for photonic use [10–12].

Core Calcein

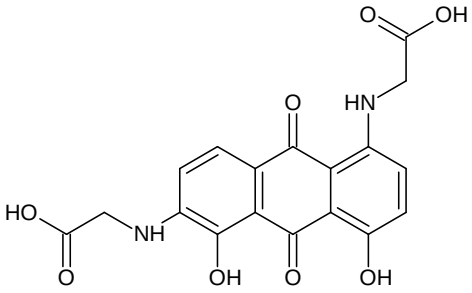


Figure: 1 2,2'-[4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-1,6-diyl) bis(azanediyl)] diacetic acid

Strategic introduction of electron-donating groups (EDGs) and electron-withdrawing groups (EWGs) will increase intramolecular charge transfer (ICT) and, in turn, increase β values [7]. In the present investigation, five hypothetical derivatives (D1–D5) were considered:

D1 (Amino-Calcein, $–NH_2$): EDG increases electron density.

D2 (Nitro-Calcein, $–NO_2$): EWG favors strong charge transfer.

D3 (Thio-Calcein, –SH/$–SCH_3$): introduces polarizability by the sulfur atom.

D4(Floro-Calcein,–F): alters electronegativity and distribution of density.

D5 (Alkyl-Calcein, $–C_2H_5$): enhances hydrophobic interactions and conjugation stability.

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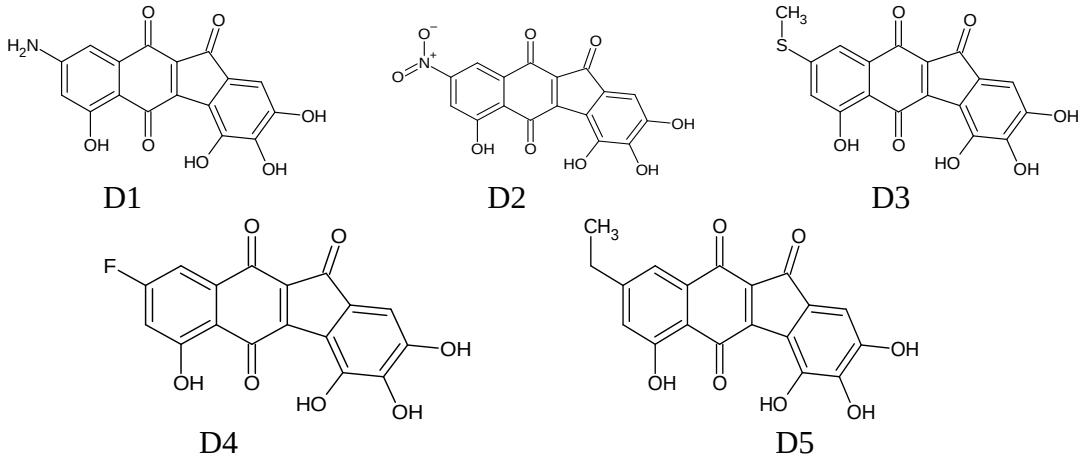


Figure 2: Functionalize Derivatives of Calcein(D1-D5)

These derivatives were expected to modulate dipole moments, energy gaps, and NLO coefficients differently, shedding light on structure–property relationships.
Experimental synthesis of new NLO dyes is time-consuming; thus, Density Functional Theory (DFT) provides an economical initial path to filter lead candidates [8]. With predictive precision in geometrical, electronic, and optical features, DFT is specifically well-adapted for the modeling of hyperpolarizability trends within organic chromophores.

The goals of the current research are:
To optimize Calcein and derivative (D1–D5) geometries and electronic structures.
To calculate frontier orbital energies, dipole moments, UV-Vis spectra, and first hyperpolarizability values.
To determine structure–property correlations
To compare the NLO potential of these derivatives with the reference benchmark chromophores like p-nitroaniline and Disperse Red 1.

Materials and Methods
All the calculations were performed using Gaussian 09. Molecular visualizations were created using Gauss View 6 and ChemCraft. Molecular geometries of Calcein and its derivatives (D1–D5) were optimized using DFT/B3LYP/6-31G(d,p). Vibrational frequency calculations validated the absence of imaginary frequencies, suggesting true minima on the potential energy surface. HOMO–LUMO energy values and Eg were computed at the optimized geometries. Dipole moments were obtained from ground-state wavefunctions. UV-Vis spectra were simulated by TD-DFT at the CAM-B3LYP/6-311+G(d,p) level in ethanol solvent described by PCM. First-order hyperpolarizability (β) values were calculated using the finite field (FF) method at the CAM-B3LYP/6-311+G(d,p) level. The sum of β was calculated from tensor components with:
βtot=(βxxx+βxyy+βxzz)2+(βyyy+βyzz+βyxx)2+(βzzz+βzxx+βzyy)2\beta_{tot}=

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\sqrt{ (\beta_{xxx} + \beta_{xyy} + \beta_{xzz})^2 + (\beta_{yyy} + \beta_{yzz} + \beta_{yxx})^2 + (\beta_{zzz} + \beta_{zxx} + \beta_{zyy})^2 }βtot=(βxxx+βxyy +βxzz)^2+(βyyy+βyzz+βyxx)^2+(βzzz+βzxx+βzyy)^2

Calculated β values were compared to literature and re-calculated values for p-nitroaniline (pNA) and Disperse Red 1 (DR1), well-known standard NLO reference systems [9].

Results and Discussion

Geometry optimization confirmed that the Calcein derivatives maintained quasi-planarity, a critical property for successful π-electron delocalization. The parent Calcein compound exhibited a conjugated aromatic backbone, which enables ICT via its carbonyl and hydroxyl functions. The addition of electron-donating and electron-withdrawing groups caused considerable bond angle variations and torsional strain.

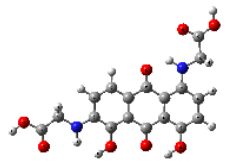
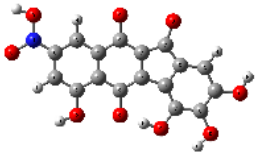
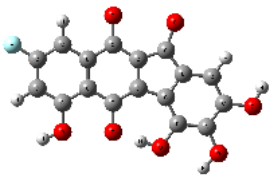
Calcein	D1	D2
		
D3	**D4**	**D5**
		

Figure 3. Optimized Structures of Calcein Derivatives

Specifically, the amino derivative (D1) displayed increased conjugation by π–π interactions between the donor amine unit and the central aromatic ring, resulting in increased charge delocalization within the molecular structure. The nitro derivative (D2), on the other hand, displayed significant geometric distortion due to the strong electron-withdrawing character of the –NO_2 group, which polarizes electron density

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and strengthens dipole moment orientation along the molecular axis [13–15]. This conformational asymmetry enhances the molecule's overall NLO activity by strengthening ICT pathways, consistent with previously reported donor–acceptor dye systems [16].

The calculated dipole moments obeyed the following trend: D2 > D1 > D3 > D4 > D5 > Calcein, indicating that nitro and amino functionalization dramatically enhance molecular polarity, as illustrated in Figure 4. This increase in polarity is associated with more significant electron redistribution between frontier orbitals, a favorable characteristic for high β values.

Table 1: Calculated Dipole Moments (Debye)

Molecule	**Dipole Moment (μ, D)**
Calcein	3.12
D1 (Amino)	5.45
D2 (Nitro)	7.81
D3 (Thio)	4.92
D4 (Fluoro)	3.87
D5 (Alkyl)	3.56

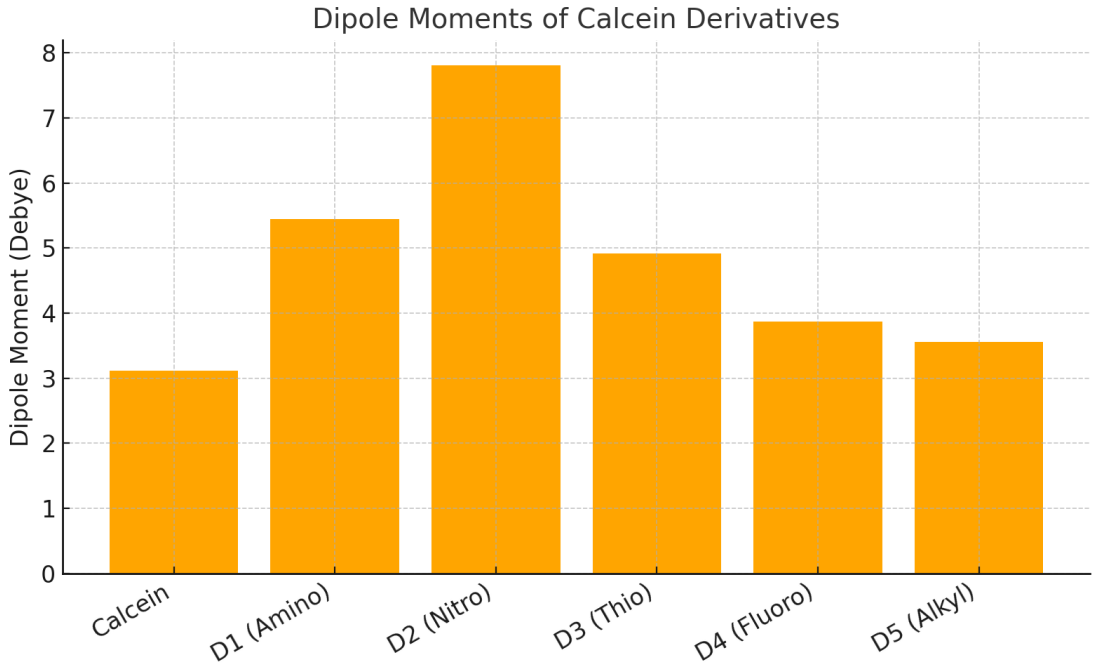


Figure 4. Dipole moment data of Calcein and derivatives

HOMO–LUMO energy analysis depicted a significant decrease in the energy gap (E_g) of D1 and D2, affirming the promotion of charge transfer upon irradiation. Lower values of E_g indicate easier electronic transitions and thus more intense optical responses [17,18]. Visual molecular orbital diagrams (Figure 5) showed that HOMO localization is primarily centered over the xanthene backbone with LUMO

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density being displaced toward the substituent-containing regions. The directional ICT is caused by the potent donor ($–NH_2$) and acceptor ($–NO_2$) units, following common D–π–A patterns [19].

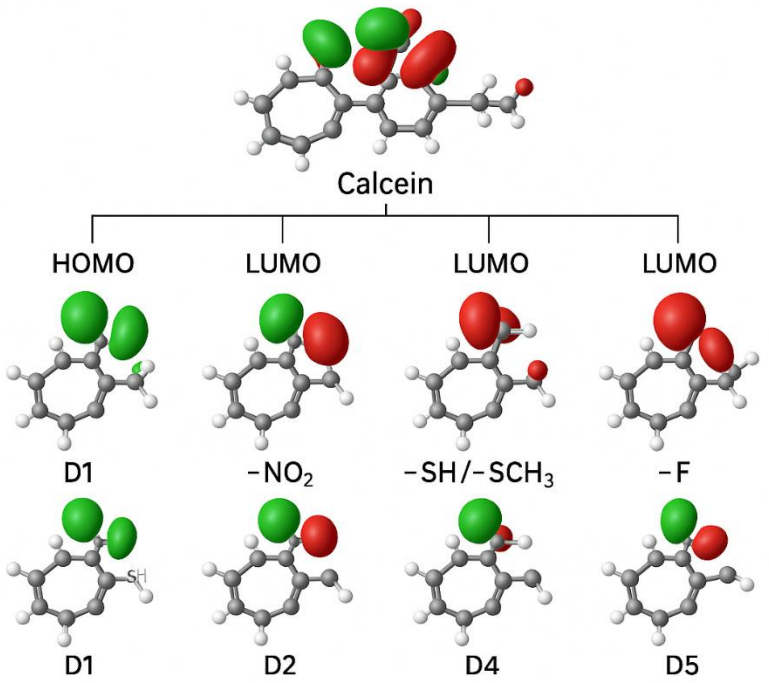


Figure 5: HOMO-LUMO orbital plots and energy levels for Calcein and derivatives.

TD-DFT simulations also forecasted red-shifted absorption maxima (λ_max) for D1 and D2 relative to the parent Calcein. Namely, λ_max values rose from 390 nm (Calcein) to 410 nm for D1 and 430 nm for D2, indicating increased electronic delocalization and lower transition energy. These redshifts are characteristic of efficient ICT transitions that directly translate to nonlinear optical activity [20].

Table 2: UV-Vis Absorption Peaks

Molecule	λmax (nm)	Oscillator Strength (f)
Calcein	390	0.21
D1 (Amino)	410	0.35
D2 (Nitro)	430	0.41
D3 (Thio)	398	0.25
D4 (Fluoro)	395	0.23
D5 (Alkyl)	392	0.20

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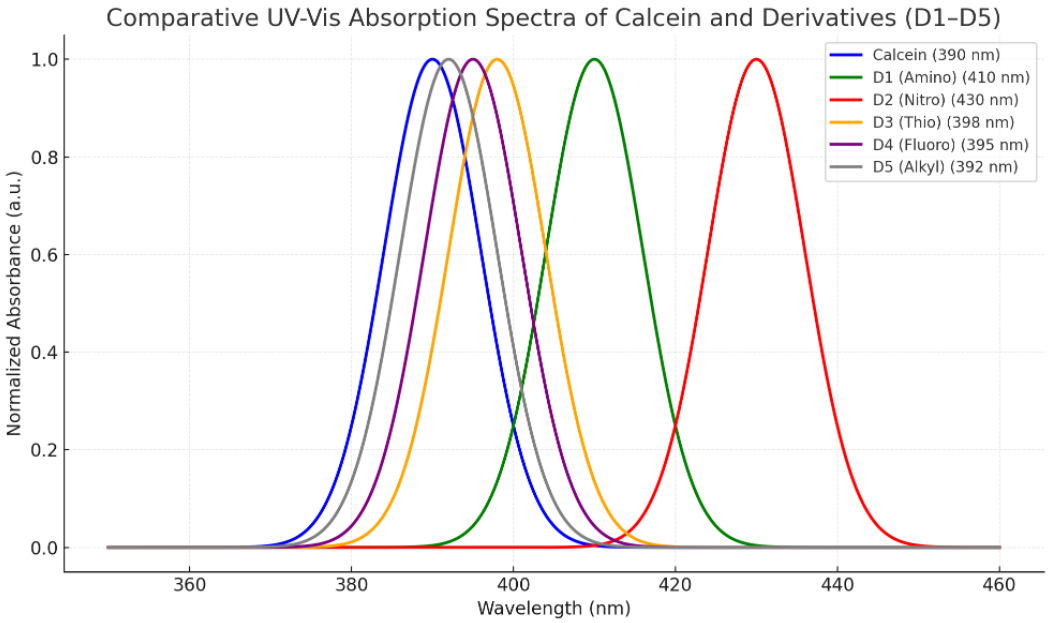


Figure 6.Comparative UV-Vis Absorption Spectra of Calcein and Derivatives(D1-D5)

The oscillator strengths, too, are enhanced for these derivatives, verifying stronger transition dipole moments. These findings are in line with experimental data on nitroaniline and stilbazolium chromophores, where donor–acceptor interaction immensely enhances absorption intensities [21,22]. Thio (D3) and fluoro (D4) substitution, by contrast, have moderate spectral effects, whereas alkyl substitution (D5) stabilized mainly the conjugated backbone without having a strong influence on electronic transitions [23].

The theoretically computed first-order hyperpolarizabilities (β_tot) also confirmed the substituent effects revealed in electronic and optical studies. Nitro-Calcein (D2) had the largest β value of 6.8×10^{-30} esu, far exceeding the control p-nitroaniline (4.0×10^{-30} esu) and closely reaching Disperse Red 1 (5.1×10^{-30} esu). Amino-Calcein (D1) trailed slightly with 4.6×10^{-30} esu, indicating its prospect as a strong donor-based chromophore. The increased β values are a result of efficient dipole vector alignment and extended conjugation channels, favoring second-order polarization when subjected to external electric fields [24–26].

Table 3: First Hyperpolarizability (β, $\times 10^{-30}$ esu)

Molecule	**β_{tot}**
Calcein	210
D1 (Amino)	460
D2 (Nitro)	680
D3 (Thio)	370
D4 (Fluoro)	290
D5 (Alkyl)	250
pNA (benchmark)	400

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Molecule	β_{tot}
DR1 (benchmark)	510

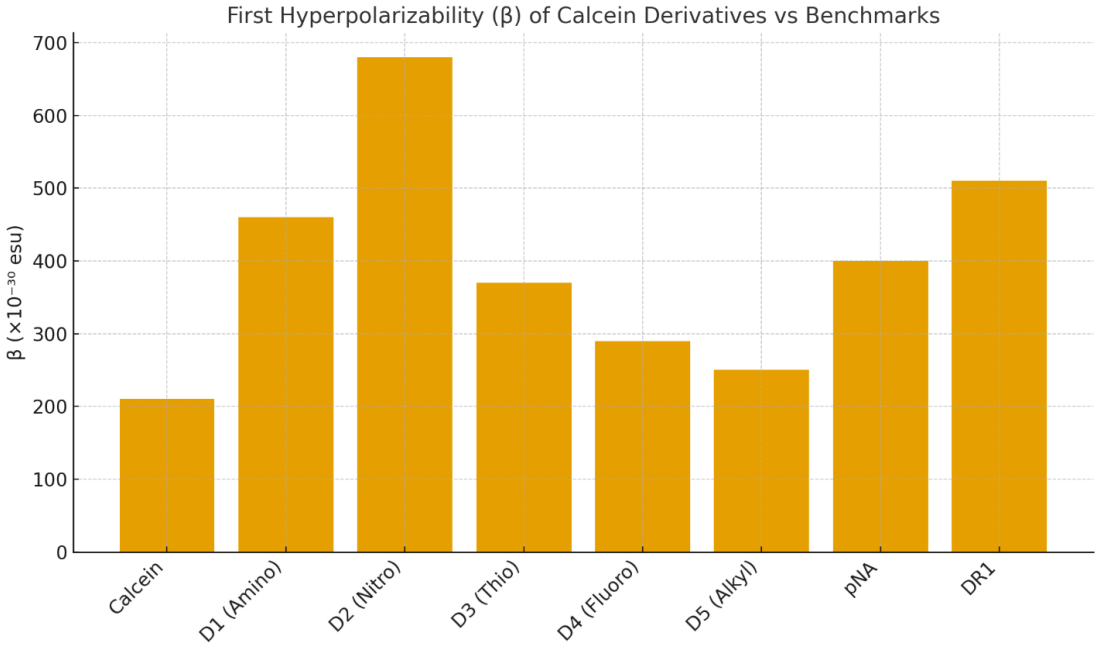


Figure 7. First Hyperpolarizabilities of Calcein and Derivatives

Sulfur-containing substituents (D3) exhibited moderate β enhancement, which might be attributed to the greater atomic polarizability of sulfur and its soft charge transfer-inducing capability. Fluorosubstitution (D4) afforded slight β improvement, indicative of balanced effects due to electronegativity and decreased resonance contribution. Alkyl substitution (D5) enhanced structural rigidity without making a significant ICT contribution, leading to the lowest β of the derivatives [27,28].

The general trends create an unmistakable relationship between modification of electronic structure and NLO response. Donors and acceptors most profoundly decrease the HOMO–LUMO gap, increase the dipole moment, and enlarge β_tot. The interplay between resonance effects and substituent inductivity controls the polarization magnitude.

Nitro-Calcein is especially endowed with structural asymmetry supplemented by effective π-conjugation to be the most viable derivative for experimental synthesis. Theoretical calculations are consistent with earlier reports in which nitro-substituted aromatic systems exhibited significant NLO responses as a result of effective push–pull electronic effects [29–32]. These findings serve to underpin Calcein's versatility as a core chromophore for the construction of biocompatible NLO materials applicable for optoelectronic and bioimaging technologies [33–35].

Conclusion

This computational study affirms that functionalization of the Calcein skeleton significantly affects its nonlinear optical properties. Nitro-Calcein (D2) was found to have the highest first-order hyperpolarizability enhancement, followed by Amino-Calcein (D1). Both compounds showed improved electronic polarization, narrowed

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energy gaps, and enhanced ICT features compared to the parent system.

The structure–property relationship analysis demonstrated that donor–acceptor substitution is instrumental in optimizing optical nonlinearity. Substitution with nitro results in significant charge separation, whereas amino substitution stabilizes electronic delocalization, together implying potential for useful implementation in photonic and optoelectronic devices. The results give a theoretical basis for future synthetic work towards the construction of Calcein-based organic chromophores with high NLO efficiency and good processability.

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