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Investigation of two-photon absorption properties in new A–D–A compounds emitting blue and yellow fluorescence



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HIGHLIGHTS

• Three new A-D-A structure compounds based on pyridine were synthesized.

• The comprehensive photophysical properties of three compounds were investigated.

• The structure-properties relationship was discussed in various solvents.

• **BYQ** has a good solubility in water.

• BYQ shows promising applications in pharmaceutical and biological fields.

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ABSTRACT

Three new acceptor–donor–acceptor compounds (**LBQ**, **DBQ**, **BYQ**) were synthesized and characterized by infrared, hydrogen nuclear magnetic resonance, mass spectrometry and elemental analysis. Their photophysical properties were investigated including linear absorption, single-photon excited fluorescence, fluorescence quantum yield and two-photon absorption. These compounds in CH_2Cl_2 exhibit good fluorescence quantum yield which are 0.36, 0.26, and 0.25 and the largest two-photon absorption cross-section which are 48, 36, and 181 GM respectively. Under the excitation of Ti: sapphire laser with a pulse width of 140 fs, LBQ and DBQ emit blue two-photon excited fluorescence (TPEF), while **BYQ** emits bright yellow TPEF. **BYQ** has a good solubility in water and the σ can be as large as 130 GM, so it shows promising applications in many pharmaceutical and biological fields.

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Introduction

Two-photon absorption (TPA) can be defined as simultaneous absorption of two photons through virtual states in a medium, and it belongs to the category of the third-order nonlinear optical effect. In 1931, Maria Goeppert-Mayer [1] theoretically predicted the existence of it in her doctoral thesis. However, the laser had not been invented at that time, it was difficult to get the necessary of two-photon absorption's light intensity. Until the 1860s, Kaiser [2] proved this absorption process existed through the actual experiment. TPA cross-section (σ) is the most important parameter of TPA properties, TPA materials with large σ have many potential applications, including two-photon fluorescence imaging [3–5], optical limiting [6–8], three-dimensional (3D) data storage [9,10], 3D microfabrication [11–13], and photodynamic therapy [14–16].

In order to improve σ values of materials, a variety of structural models were designed, including donor- π -bridge-acceptor (D- π -A) type [17,18], donor- π -bridge-donor (D- π -D) type [19,20], acceptor- π -bridge-acceptor (A- π -A) type [21], donor-acceptor-donor (D-A-D)/acceptor-donor-acceptor (A-D-A) type [22-24], and arborization type [25,26]. Among them, the (D-A-D)/(A-D-A) type have some characteristics such as large intramolecular charge transfer (ICT) and small energy band gap which lead to enhanced σ , Chen [27] and Ray et al. [28] designed the molecules of (D-A-D)/(A-D-A) type whose σ were up to an order of magnitude of 10^{-47} .

In this work, three new A–D–A molecules (**LBQ**, **DBQ**, **BYQ**) were focused on their synthesis and TPA properties. Their structures were characterized by IR, ¹H NMR, MS, and elemental analysis. **LBQ** and **DBQ** are composed of pyridine and a formyl group as terminal acceptors (A) and two methoxy groups as central donors (D), while **BYQ** has pyridinium as a stronger electron acceptor. The π -conjugated electron systems of these three molecules are relatively small. In general, molecules possessing small conjugated



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Scheme 1. Synthesis of the target compounds (LBQ, DBQ, BYQ).

systems exhibit short wavelength emission. Emission wavelength exhibits red shifts by increasing the conjugated systems. **LBQ** and **DBQ** emit blue fluorescence accordingly, however, **BYQ** realizes a leap of emission wavelength through the salitying of pyridine acceptor rather than increasing the conjugated system, which emits bright yellow fluorescence. What is more, **BYQ** has a good solubility in water, and its σ can be as large as 130 GM in H₂O, so it shows promising applications in selective, recognition, and analysis in medical diagnostics, pathogen recognition or fundamental research, biological probes and other pharmaceutical and biological fields [29].

Experimental

Materials and instruments

1-Ethyl-4-methylpyridinium bromide was synthesized according to the literature procedures [30]. Other materials were commercially available and were used without further purification.

Melting points were measured on an X-4 micromelting point apparatus without correction. ¹H NMR spectra were collected on a Bruker AVANCE III 500 apparatus, with TMS as internal standard and DMSO-d₆ and CDCl₃ as solvent. FT-IR spectra were recorded on a Thermo Nicolet 6700 spectrometer using KBr pellets. Mass spectra were taken on a Therm LCQ TM Deca XP plus ion trap mass spectrometry instrument. Elemental analyses were conducted on a Thermo Finnigan Flash EA 1112 apparatus.

The linear absorption spectra were measured on a Shimadzu UV-2550 UV-visible spectrophotometer. The SPEF spectra measurements were performed using a RF-5301PC fluorescence

spectrophotometer with the maximum absorption wavelengths as the excitation wavelengths. The fluorescence quantum yields were determined using fluorescein in 0.1 mol L^{-1} sodium hydroxide as the standard. The TPEF spectra were measured using a femtosecond laser pulse and Ti: sapphire system (680–1080 nm, 80 MHz, 140 fs) as the light source.

Synthesis

1,4-Bis(bromomethyl)-2,5-dimethoxybenzene (1)

A mixture of 1,4-dimethoxybenzene (8.28 g, 60 mmol), paraformaldehyde (8.10 g, 270 mmol) and acetic acid (150 mL) was heated to 60 °C, then 40% HBr (40 mL) was added to it using a pressure equalizing funnel and the reaction mixture was stirred for 4 h, cooled to room temperature and refrigerated overnight at 0 °C. The solid was filtered and dissolved in CH₂Cl₂, repeatedly washed with saturated sodium bicarbonate, then the solvent was evaporated under reduced pressure and the residue was recrystallized from CHCl₃ to give white needle crystals (13.65 g, 70.2%). m.p. = 197–199 °C (Lit. m.p. = 197–198 °C [31]); ¹H NMR (500 MHz, CDCl₃) δ : 6.89 (s, 2H), 4.55 (s, 4H), 3.89 (s, 6H); FT-IR (KBr) ν /cm⁻¹: 3044, 2962, 2934, 2834, 1509, 1461, 1404, 1319, 1229, 1205, 1040, 874, 718, 664, 551.

2,5-Dimethoxy-1,4-benzenedicarboxaldehyde (2)

A mixture of hexamethylenetetramine (12.60 g, 90 mmol), **1** (9.72 g, 30 mmol) and $CHCl_3$ (200 mL) was heated under reflux for 3.5 h, the solvent was evaporated under reduced pressure and 50% acetic acid (200 mL) was added to the residue, heated under reflux for 4.5 h, then concentrated hydrochloric acid (10 mL) was

Table 1		
Linear and nonlinear optical	properties of the target compounds (LBQ, DBQ, BYQ)).

Compounds	Solvents	λ_{\max}^{abs} a nm	$10^{-4} \epsilon^{\rm b} {\rm mol}^{-1} {\rm L} {\rm cm}^{-1}$	$\lambda_{\max}^{\text{SPEF}c}$ nm	$\Delta v^{\rm d} \ {\rm cm}^{-1}$	ϕ^{e}	$\lambda_{\max}^{\text{TPEFf}}$ nm	$\sigma^{ m g}{ m GM}$
LBQ	Toluene	324	1.03	444	8342	0.16		
	THF	325	1.10	446	8348	0.19	459	26
	CH_2Cl_2	323	1.67	455	9078	0.36	467	48
	DMF	322	2.78	456	9126	0.21		
	CH ₃ CN	321	2.99	458	9316	0.24		
DBQ	Toluene	312	1.46	446	9630	0.09		
	THF	311	1.72	448	9833	0.11	463	19
	CH_2Cl_2	306	2.17	460	10941	0.26	472	36
	DMF	307	3.12	463	10975	0.15		
	CH ₃ CN	307	3.38	465	11068	0.17		
BYQ	Toluene	342	1.52	554	11189	0.08		
	THF	342	2.04	557	11287	0.10	573	97
	CH_2Cl_2	343	2.89	561	11329	0.25	582	181
	DMF	344	3.59	565	11371	0.12		
	CH ₃ CN	343	3.80	566	11487	0.06		
	H ₂ O	335	5.41	558	11930	0.01	572	130

^a Maximum linear absorption wavelength, $c = 1 \times 10^{-5} \text{ mol L}^{-1}$.

^b Molar absorption coefficients.

^c Maximum single-photon excited fluorescence wavelength, $c = 1 \times 10^{-7}$ mol L⁻¹.

d Stokes shift.

^e Fluorescence quantum yield were measured using fluoresceine as the standard in 0.1 mol L⁻¹ sodium hydroxide. ($\phi_{\text{fluorescein}} = 0.90$) [36].

^f Maximum two-photon excited fluorescence wavelength, $c = 1 \times 10^{-3} \text{ mol L}^{-1}$.

^g Two-photon absorption cross-section, 1 GM = 1×10^{-50} cm⁴ s photon⁻¹.



Fig. 1. UV-visible absorption spectra of the target compounds (LBQ, DBQ, BYQ) in CH₂Cl₂ (c = 1 \times 10⁻⁵ mol L⁻¹).

added to the mixture, cooled to room temperature, extracted with CHCl₃ and dried with anhydrous sodium sulfate. after the solvent was evaporated under reduced pressure and the residue was recrystallized from ethyl alcohol to give bright yellow needle crystals (1.98 g, 34.0%). m.p. = 202–203 °C (Lit. m.p. = 201 °C [32]); ¹H NMR (500 MHz, CDCl₃) δ : 10.52 (s, 2H), 7.47 (s, 2H), 3.96 (s, 6H); FT-IR (KBr) v/cm⁻¹: 3047, 2985, 2953, 2870, 1679, 1483, 1411, 1396, 1301, 1214, 1130, 1027, 878, 660.

2,5-Dimethoxy-4-[(1E)-2-(2-pyridinyl)ethenyl]benzaldehyde (LBQ)

A flask fitted with a magnetic stirrer and condenser was charged with 2-methyl-py-ridine (0.93 g, 10 mmol), **2** (1.94 g, 10 mmol), acetic anhydride (3 mL), and acetic acid (1.5 mL). The mixture was heated under reflux for 6 h, then cooled to room temperature. After concentrated hydrochloric acid (15 mL) was added, the mixture was filtered. The filtrate was neutralized with 30% aqueous sodium hydroxide solution (30 mL) and gave a precipitate. The precipitate was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (10:1) as eluent to give bright

yellow needle crystals (1.69 g, 62.8%). m.p = $110-112 \circ C^{-1}H$ NMR (500 MHz, DMSO-d₆): δ 10.34 (s, 1H), 8.62 (d, *J* = 3.9 Hz, 1H), 7.99 (d, *J* = 16.2 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 16.2 Hz, 1H), 7.60 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.31 (dd, *J*_1 = 7.5 Hz, *J*_2 = 4.8 Hz, 1H), 7.28 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H); FT-IR (KBr) ν/cm^{-1} : 3051, 2921, 2872, 1670, 1603, 1466, 1406, 1214, 1123, 1041, 970, 870, 770, 685 cm⁻¹; ESI-MS: *m/z* (%): 270.1 (100) [M + H]⁺; elemental analysis calcd (%) for C₁₆H₁₅NO₃: C 71.36, H 5.61, N 5.20; found: C 71.48, H 5.69, N 5.41.

2,5-Dimethoxy-4-[(1E)-2-(4-pyridinyl)ethenyl]benzaldehyde (DBQ)

The Synthesis of this compound was similar to **LBQ**. Light yellow needle crystals. Yield 71.3%. m.p. = 144–148 °C ¹H NMR (500 MHz, DMSO-d₆): δ 10.34 (s, 1H), 8.59 (d, *J* = 5.9 Hz, 2H), 7.70 (d, *J* = 16.6 Hz, 1H), 7.59 (s, 1H), 7.58 (d, *J* = 5.9 Hz, 2H), 7.54 (d, *J* = 16.6 Hz, 1H), 7.29 (s, 1H), 3.98 (s, 3H), 3.89 (s, 3H); FT-IR (KBr) *v*/cm⁻¹: 3064, 2947 2873, 1681, 1600, 1485, 1408, 1215, 1124 1035, 971, 879, 771, 691 cm⁻¹; ESI-MS: *m*/*z* (%): 269.8 (100) [M + H]⁺; elemental analysis calcd (%) for C₁₆H₁₅NO₃: C 71.36, H 5.61, N 5.20; found: C 71.59, H 5.78, N 5.47.

1-Ethyl-4-[(1E)-2-(2,5-dimethoxy-4-formylphenyl)ethenyl] pyridinium bro- mide (BYQ)

A flask fitted with a magnetic stirrer and condenser was charged with 1-ethyl-4-methylpyridinium bromide (2.01 g, 10 mmol), 2 (1.94 g, 10 mmol), piperidine (0.85 g, 10 mmol) and methyl alcohol (50 mL). The mixture was heated under reflux for 7 h, then cooled to room temperature. The mixture was filtered and the filtrate was evaporated by rotary evaporators. The solid was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (1:5) as eluent to give a orange-yellow crystalline powder (2.11 g, 55.7%). m.p. = 255–257 °C ¹H NMR (500 MHz, DMSO-d6): δ 10.37 (s, 1H), 9.00 (d, J = 6.9 Hz, 2H), 8.31 (d, J = 6.8 Hz, 2H), 8.09 (d, J = 16.5 Hz, 1H), 7.77 (d, J = 16.5 Hz, 1H), 7.63 (s, 1H), 7.35 (s, 1H), 4.57 (q, 2H), 3.99 (s, 3H), 3.93 (s, 3H), 1.55 (t, J = 7.3 Hz, 3H); FT-IR (KBr) v/cm⁻¹: 3084, 2941, 2897, 1685, 1618, 1490, 1410, 1211, 1123, 1041, 978, 873, 692 cm⁻¹; ESI-MS: m/z (%): 298.3 (100) [M]⁺; elemental analysis calcd (%) for C₁₈H₂₀BrNO₃: C 57.15, H 5.33, N 3.70; found: C 57.43, H 5.52, N 3.96.



Fig. 2. SPEF spectra of the target compounds (LBQ, DBQ, BYQ) in various solvents ($c = 1 \times 10^{-7}$ mol L⁻¹).

Results and discussion

Synthesis and characterization

The new target compounds **LBQ**, **DBQ**, and **BYQ** were synthesized according to Scheme 1. 2,5-Dimethoxy-1,4-benzenedicarboxaldehyde (2) was obtained according to Sommelet's procedure by reaction of 1,4-bis(bromomethyl)-2,5-dimethoxybenzne (1) with hexamethylenetetramine in chloroform, followed by hydrolysis in acetic acid. **LBQ** and **DBQ** were synthesized by the acid condensation of 2-methyl-pyridine and 4-methyl-pyridine with 2, while **BYQ** was synthesized by the alkaline condensation reaction of 1-ethyl-4-methylpyridinium bromide with 2. In ¹H NMR spectra, compared with these three compounds, one can see chemical shift (δ) of **BYQ** is higher than these of **LBQ** and **DBQ**, it indicates that pyridinium's electron-accepting ability is stronger than that of pyridine. The (*E*)-configurations of the C–C double bonds were certified by the coupling constants ³*J*(H, H) = 16.2–16.6 Hz for the ole-finic AB spin systems.

Linear absorption and single-photon excited fluorescence (SPEF)

The comprehensive photophysical properties of the compounds (**LBQ**, **DBQ**, and **BYQ**) in various solvents with different polarity are listed in Table 1. One can see that absorption properties of three compounds show little dependence on the polarity of the solvents. With increasing polarity of the solvents from toluene to CH₃CN, the absorption peak position of **LBQ**, **DBQ**, and **BYQ** almost have no variation, but the absorption peak position of **BYQ** in water exhibits blue shift. This blue shift can be attributed to hydrogen bonding formed by n electron and water in the ground state S₀, which results in the increase of the energy gap between S₀ and the excited state S₁. As shown in Fig. 1 and listed in Table 1, compared the maximum absorption wavelength $\left(\lambda_{max}^{abs}\right)$ of three compounds, we

find the λ_{max}^{abax} exhibits obvious red shift from **DBQ** to **BYQ** (**BYQ** > **LBQ** > **DBQ**). The reason for this characteristic is that the electron-accepting ability of pyridinium is stronger than that of 2-pyridine, which is stronger than that of 4-pyridine. Among them, the molar absorption coefficients (ε) of **BYQ** is higher than the other two, it shows that the ability of absorbing light of **BYQ** is the strongest.

As shown in Fig. 2, all these compounds emit SPEF between 400 and 600 nm under the excitation at their maximum absorption wavelengths. The SPEF maxima (λ_{max}^{SPEF}) and the stokes shifts (Δv) of **LBQ**, **DBQ**, and **BYQ** increase with increasing polarity of the solvents from toluene to CH₃CN. We can explain the slight red shifts are that strong solvent–solute dipole–dipole interactions, a manifestation of the large dipole moment and orientational polarizability. An increased dipole–dipole interaction between the solute and solvent leads to a lower energy level. H₂O is a solvent whose



Fig. 3. SPEF spectra and fluorescence photos of the target compounds (**LBQ**, **DBQ**, **BYQ**) in CH_2CI_2 ($c = 1 \times 10^{-7}$ mol L⁻¹).



Fig. 4. Two-photon absorption cross sections of the target compounds (LBQ, DBQ, BYQ) in CH_2CI_2 ($c = 1 \times 10^{-3}$ mol L^{-1}).

polarity is stronger than CH₃CN, however, the SPEF of **BYQ** in H₂O exhibits slight blue shift. The reason is the influence of water via hydrogen bonding, too. In Table 1, we find the fluorescence quantum yields (ϕ) of three compounds are maximum in CH₂Cl₂, it is possible that CH₂Cl₂ has special solvation effect which increases molecular structure rigidity and coplanarity. As shown in Fig. 3, compared these three compounds, one can see the λ_{max}^{SPEF} increases from **LBQ** to **BYQ** (**BYQ** > **DBQ** > **LBQ**). For example, in CH₂Cl₂ solution, the λ_{max}^{SPEF} of **BYQ** presenting bright yellow fluorescence at 561 nm is red-shifted by about 100 nm relative to that of **LBQ** and **DBQ** presenting blue fluorescence at about 460 nm. This can be attributed to the stronger electron-accepting ability of pyridinium which reduces the energy gap between S₀ and S₁.

Two-photon properties

Methods for σ measurements include nonlinear transmission method, Z-scan technology, two-photon induced fluorescence method and two-photon transient absorption spectroscopy method. Three compounds (**LBQ**, **DBQ**, **BYQ**) were measured by the two-photon induced fluorescence method. The σ values of the target compounds in CH₂Cl₂ were recorded under 500 mW



Fig. 5. TPEF spectra of **BYQ** in various solvents ($c = 1 \times 10^{-3} \text{ mol } \text{L}^{-1}$).

from 680 nm to 910 nm in Fig. 4. The σ is obtained by comparing the two-photon excited fluorescence (TPEF) intensity of the sample with that of a reference compound by the following Eq. (1) [33]:

$$\sigma_{\rm s} = \frac{F_{\rm s}}{F_{\rm r}} \frac{\phi_{\rm r}}{\phi_{\rm s}} \frac{n_{\rm r}}{n_{\rm s}} \frac{c_{\rm r}}{c_{\rm s}} \sigma_{\rm r} \tag{1}$$

where the subscripts s and r denote the sample and the reference compound. *F* and ϕ represent the TPEF integral intensity and the SPEF quantum yield. *n* and *c* are the refractive index and the concentration of the solution. In this work, we selected fluorescein in 0.1 mol L⁻¹ sodium hydroxide ($c = 1 \times 10^{-3} \text{ mol L}^{-1}$) as the reference ($\sigma = 36 \text{ GM}$ [33]).

As shown in Fig. 4, the wavelength of **LBQ**, **DBQ**, and **BYQ** at maximum σ values in CH₂Cl₂ are 680 nm, 770 nm and 700 nm, respectively. The peak σ values of **LBQ** and **DBQ** are 48 and 36 GM, which are not the best values due to our wavelength of light source ranges from 680 nm to 1080 nm. This range cannot reach their relative twofold λ_{max}^{abs} . The σ of **BYQ** which is as large as 181 GM in CH₂Cl₂ is higher than the other two obviously, the reason is also electron-accepting ability of pyridinium is stronger than pyridine and it is easy to realize the polarization transition under the light induced. **BYQ** has a good solubility in water and its σ can be as large as 130 GM in H₂O, it shows promising applications in pharmaceutical and biological fields.

As shown in Fig. 5, the two-photon fluorescence intensity of three compounds in CH₂Cl₂ is the strongest among three solvents, the reason is similar to the SPEF. In water, two-photon fluorescence intensity has a bit decrease compared with the other two solvents, the reason is the hydrogen bonds consuming some energy from the excited state molecules [34]. Compared with the TPEF maxima (λ_{max}^{TPEF}) and the λ_{max}^{SPEF} of three compounds, the λ_{max}^{TPEF} exhibits red shifts, this can be explained by the effect of reabsorption for the linear absorption band. It has a slight overlap with the emission band and our two-photon fluorescence experiments were carried out in concentrated solutions that made reabsorption significant.

BYQ in CH_2Cl_2 were pumped by femtosecond laser pulses at different pump intensities with 700 nm excitation wavelength. Fig. 6 shows a log–log plot of the excited fluorescence signal *vs.* excited light power. The slope value of **BYQ** is 2.02. It provides direct evidence for the squared dependence of excited fluorescence intensity and input laser power, suggesting a two-photon excitation mechanism.



Fig. 6. Output fluorescence intensity (I_{out}) vs. the square of input laser power (I_{in}) for **BYQ**, excitation carried out at 700 nm, with $c = 1 \times 10^{-3}$ mol L⁻¹ in CH₂Cl₂.

Conclusions

In this work, three new A–D–A structure compounds containing pyridine and pyridinium were synthesized. The linear absorption. single-photon excited fluorescence, fluorescence quantum yield, two-photon absorption of three compounds were investigated. The structure-properties relationship was discussed in various solvents. Three compounds in CH₂Cl₂ exhibit the largest fluorescence quantum yield which are 0.36, 0.26, and 0.25 and the largest twophoton absorption cross-section which are 48, 36, and 181 GM respectively. LBO and DBO emit blue two-photon excited fluorescence, while BYQ emits bright yellow TPEF. What is more the water-solubility of **BYQ** whose σ is 130 GM provides the possibility for selective, recognition, and analysis in medical diagnostics, pathogen recognition or fundamental research, more importantly, it is anticipated to provide insights into small molecule-protein and protein-protein recognition at the molecular level [35].

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