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Synthetic Strategies to Derivatizable Triphenylamines Displaying High Two-Photon Absorption

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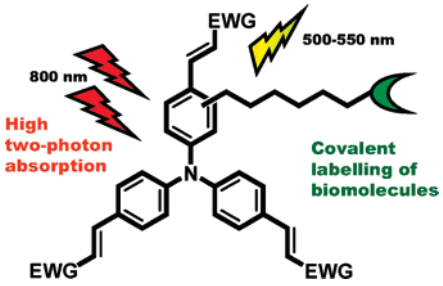

A versatile synthetic strategy to access a set of highly fluorescent π-conjugated triphenylamines bearing a functional linker at various positions on one phenyl ring is described. These compounds were designed for large two-photon absorption (2PA) and in particular for labeling of biomolecules. The monoderivatized trisformylated or trisiodinated intermediates described herein allow introduction of a large variety of electron-withdrawing groups required for large 2PA as well as a panel of chemical functions suitable for coupling to biomolecules. The monoderivatized three-branched compounds and in particular the benzothiazole (TP-3Bz) series show remarkable linear (high extinction coefficients and high quantum yield) and nonlinear (high 2-photon cross sections) optical properties. Interestingly the presence of functional side chains does not disturb the two-photon absorption. Finally, monoderivatized two-branched derivatives also appear to be valuable candidates. Altogether the good optical properties of the new derivatizable π-conjugated TPA combined with their small size and their compatibility with bioconjugation protocols suggest that they represent a new chemical class of labels potentially applicable for the tracking of biomolecules using two-photon scanning microscopy.

Introduction

Optical imaging of living cells is a common method in molecular biology and hence many fluorescent dyes are routinely used as molecular probes. Fluorescence is characterized by emission of a photon from a molecular excited state. However, at least two main processes are known to populate such a state: one-photon absorption (1PA) results from absorption of a single photon of energy $h\nu$ and two-photon absorption (2PA) is based upon simultaneous absorption of two photons of half the excitation energy ($h\nu$/2 each) by a single molecule. In view of biological applications and in vivo imaging the two-photon process offers many advantages:[1,2] better in-depth penetration, reduced cell damage, and higher spatial resolution. As a consequence, two-photon microscopy experiences an increasing popularity. Unfortunately, the two-photon absorption process is statistically disfavored and hence performances of classical one-photon fluorophores are usually low due to their two-photon absorption cross section (δ).[3,4] As a consequence, many efforts have been devoted to design fluorophores displaying higher δ.

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Since intramolecular charge transfer (ICT) contributes strongly to increase the 2PA capacity, many two-photon fluorophores are built up from electron-donating and/or withdrawing groups bridged by π-conjugated systems.[5−8] Therefore, classical approaches toward optimization of 2PA cross section consist of extending the π-electron conjugation throughout additional double bonds and aromatic moieties.[9−11] Following these guidelines, many dendrimeric, highly conjugated large-sized compounds have been designed.[12,13] However, most of them lack suitable functionalities for conjugation to biomolecules.[14−16] In addition, their large size and strong aromatic characteristics are likely to perturb the properties of the biomolecule they might be linked with. Also, a key issue is solubilization in water of these large aromatic systems, albeit several strategies based on the attachment of hydrophilic substituents such as charged groups or polyethyleneglycol (PEG) chains can be applied to improve this parameter.[17−20] A challenging task is thus to design efficient two-photon fluorophores compatible both with the constraints of biolabeling and with the synthetic protocols for conjugation to biomolecules. In addition to exhibiting solubility and stability in aqueous media, the ideal 2PA biolabel should meet the following specific requirements: (i) advantageous balance between as small as possible size and high two-photon absorption cross section and (ii) the presence of a functional linker for labeling with a negligible effect on the optical properties.

For many years, triphenylamine (TPA) derivatives have been shown to be promising materials for two-photon absorption. TPA is an electron-rich, propeller-shaped molecule exhibiting a C_3 symmetry thus displaying an octupolar feature.[21] By introduction of electron-withdrawing groups on the three positions para to the central nitrogen, very efficient 2PA fluorophores were recently obtained.[22,23] Hence TPA represents an excellent scaffold for the design of new 2PA dyes for biolabeling. However, most of the derivatives currently available are large-sized π-extended systems displaying the previously mentioned drawbacks. In addition, the chemistry of derivatizable TPA remains largely unexplored.[24−29] More importantly, no studies were reported on the influence of lateral chains on 2PA properties of TPA that are known to be highly sensitive to symmetry and electron density.

In our ongoing research aimed on nucleic acids/small molecules interactions, we recently described vinylpyridinium triphenylamine derivatives (TP-py) displaying enhanced two-photon excited fluorescence upon binding to double-stranded DNA.[30] DNA-bound TP-py compounds exhibit a strong fluorescence in the far-red range (680 nm) upon two-photon excitation in the NIR (700−800 nm) and are highly efficient for visualization of nuclear DNA in cells via laser scanning two-photon microscopy. This study demonstrated for the first time that TPA derivatives are suitable as labels for biological purposes and hence it was extremely stimulating to continue to develop 2PA biolabels based on the TPA core.

The present paper describes the building of a versatile derivatizable set of TPA synthons and of their development toward two-photon fluorophores suitable for biolabeling. Additionally a structure−property correlation based on the systematic comparison of the photophysical properties of the derivatized and nonderivatized TPA series bearing various functional groups is proposed.

Results and Discussion

The synthetic strategies to construct π-conjugated triphenylamines are essentially based on metal-catalyzed cross-coupling reactions with halogenated derivatives or on Wittig−Horner condensations via formylated precursors. Therefore we focused on the preparation of trishalogeno and trisformyl TPA bearing a functional group on one of the three phenyl rings (Figure 1). The choice of the additional functional group (OH or COOH) was dictated by chemical reactivity consideration, since OH or carboxylate groups can be easily engaged in chain elongation strategies, and by the availability of starting materials as detailed below.

Synthesis of Derivatizable Halogenated TPAs. 4,4′,4″-TrishaloTPA are convenient precursors of various two-photon

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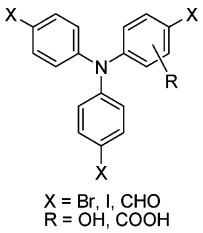
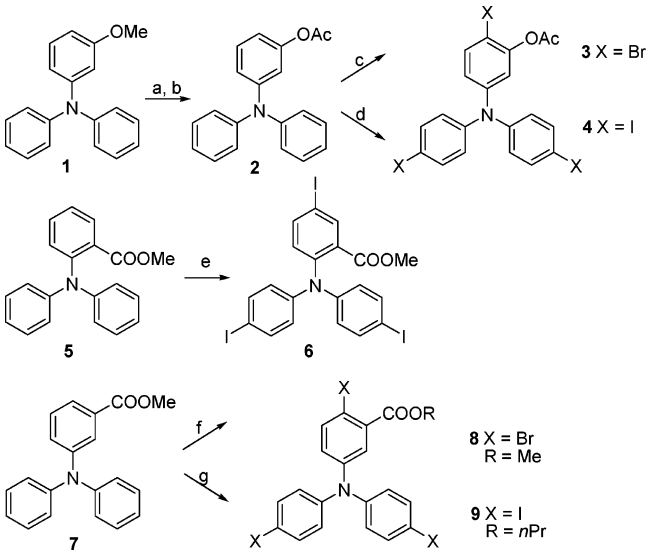


FIGURE 1. General structure of functionalized TPA precursors.

SCHEME 1. Synthesis of Trishalogenated TPA[a]



[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 °C→rt, 2 h; (b) Ac_2O, pyridine, reflux, 1 h; (c) Br_2, $CHCl_3$, 0 °C→rt, 3 h; (d) HgO, I_2, CH_2Cl_2, rt, 3 days; (e) HgO, I_2, CH_2Cl_2, rt, 10 days; (f) NBS, $CHCl_3$, reflux, 3 days; (g) HgO, I_2, 1-propanol, 90 °C, 10 days.

fluorescent TPA dyes since they can be functionalized by numerous palladium-catalyzed reactions. However, little research was published on such compounds bearing a fourth derivatizable reactive group (R) in position 2 or 3 of one phenyl ring, in addition to the three reactive functions in position 4. To the best of our knowledge there are no methods of trisbromination/iodination of monofunctionalized TPA described to date. This can be explained by difficulties that rely mainly on three points: first, introduction of a side chain increases or decreases the electron density of the corresponding phenyl ring leading potentially to over- or underhalogenation, respectively; second, iodination is commonly performed using strong oxidants (to oxidize I_2 into the more electrophilic I^+) whereas the central nitrogen atom of the TPA core is oxidation sensitive,[31,32] and last, putative regioisomers are virtually inseparable from the desired products.

We investigated several halogenation reagents/conditions and developed an efficient set of reactions to obtain regioselectively 4,4′,4″-trishalo, 2-(or 3-)functionalized TPA (Scheme 1). Among the starting materials, **1** is commercially available whereas compound **5** was obtained by Ullmann coupling from 2-aminobenzoic acid followed by esterification and compound **7** by Buchwald−Hartwig coupling from methyl 3-aminobenzoate.

Bromination of **1** following known procedures (Br_2 in $CHCl_3$ at 0 °C[33] or NBS in DMF[34]) led to mixtures of under- and overhalogenated compounds. Because the high electron-donating ability of the methoxy group should strongly perturb the electrophilic bromination, conversion of **1** to the moderately activated arene **2** was performed, which enabled easy and regioselective access to **3** by trisbromination.[33,35] Iodination was revealed to be more problematic: TPA **2** in the presence of the widely used reagents Ag_2SO_4/I_2,[36] I_2/In(OTf),[37] AgOTf/I_2,[38] or R_4N^+, ICl_2^- salt[39] either reacts extremely slowly or leads to intractable mixtures. Finally a HgO/iodine mixture[40] leads to clean conversion of **2** into **4** within 3 days. By using this reagent, the trisiodination, albeit slow, was revealed to be particularly efficient since the fairly deactivated TPA **5** was converted into **6** within 10 days at room temperature, while the strongly deactivated **7** was quantitatively and regioselectively trisiodinated into **9** in hot *n*-propanol accompanied by transesterification[41] with the same reaction time. Its parent brominated compound **8** was also obtained under forced conditions (3 days in refluxing $CHCl_3$)[42] as compared to the unsubstituted TPA. In summary, this set of reactions enables us to develop a pool of regiospecifically halogenated TPA bearing one electron-donating or electron-withdrawing group in position 2 or 3 of the phenyl ring. Hence, the synthesized key intermediates (**3**, **4**, **6**, **8**, and **9**) open the way for the application of palladium-catalyzed reactions.

Synthesis of Derivatizable Formylated TPA. Beside halogenated TPA, formylated TPA are convenient precursors for conjugated TPA since they can be converted into vinylated analogues by Knoevenagel or Wittig reactions which have been exemplified in many studies.[22,43,44] However, few reports are known on the synthesis of their 2- or 3-monofunctionalized derivatives,[28] hence hampering the design of derivatizable fluorescent TPA (Figure 1).

Several attempts to directly formylate the monofunctionalized TPA core failed: Repeated halogen−metal exchange reactions from compound **10**, followed by quenching by DMF or ethyl formate lead to the expected compound in low yield and poor reproducibility. In a second attempt, we choose to introduce formyl groups directly by palladium-catalyzed coupling between two properly functionalized moieties. The corresponding precursors **11** and **12** were synthesized and their condensation on the corresponding diphenylamines **13** and **14** attempted (Scheme

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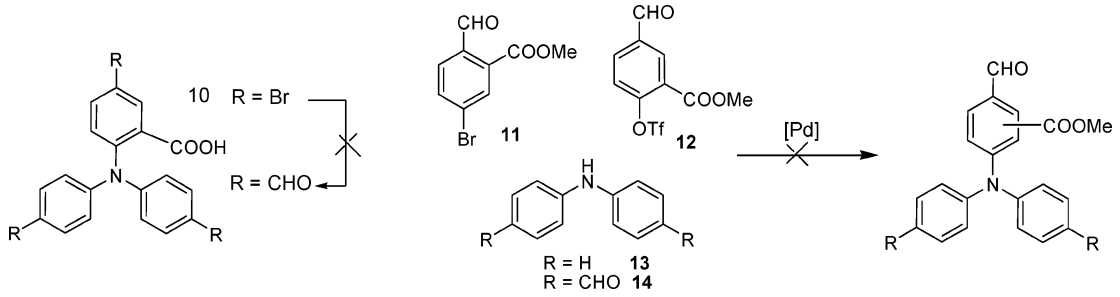
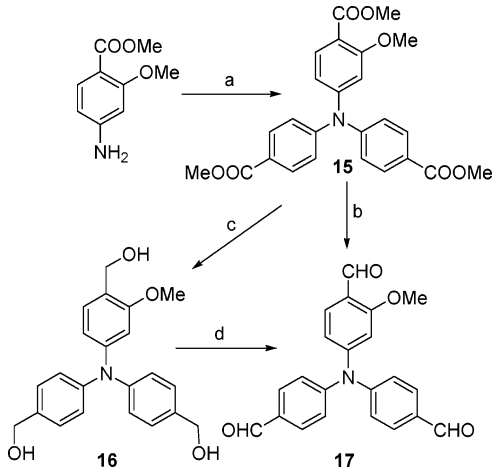
SCHEME 2. First Attempts of Synthesis of Trisformylated TPA

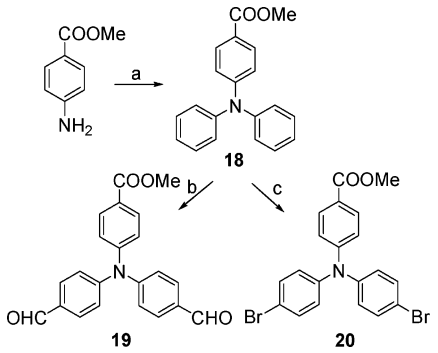


SCHEME 3. Synthesis of Trisformylated TPA[a]



[a] Reagents and conditions: (a) 4-BrC_6H_4COOMe, Cs_2CO_3, $Pd(OAc)_2$, P(*t*Bu)$_3$, toluene, reflux, o/n; (b) Red-Al, NMP, THF, 7 h, 0 °C; (c) $LiAlH_4$, THF, reflux, 1 h; (d) MnO_2, CH_2Cl_2, rt, 48 h.

SCHEME 4. Synthesis of Functionalized Precursors for Two-Branched TPA[a]



[a] Reagents and conditions: (a) PhBr, $Pd(OAc)_2$, P(*t*Bu)$_3$, Cs_2CO_3, toluene, reflux, o/n; (b) $POCl_3$, DMF, 95 °C, 4 h; (c) NBS, $CHCl_3$, reflux, 2 h.

2). Unfortunately, the application of the palladium-catalyzed procedure led in all cases to recovery of starting materials.

Then a new route was devised to access trisformylated key intermediates and we focused our effort on the preparation of the methoxy, trisformylated key compound **17** (Scheme 3). This goal was achieved by construction of the corresponding triester **15** followed by a controlled reduction of all three esters. Although direct reduction of aliphatic esters into aldehydes is well documented,[45] the parent reaction on aromatic esters was revealed to be more difficult. Recently, a report on 2,6-naphthalene-biscarbaldehyde synthesis, using modified Red-Al reagent,[46] prompted us to apply this reagent to our target system. Unfortunately, despite repeated trials, the best yield was only a poor overall yield of 38%. Unexpectedly, the unsymmetrical monoester was isolated as the major byproduct and increasing reaction time or hydrid concentration led only to complex mixtures of over-reduced TPA. Finally, the classical two-step sequence based on reduction into alcohol and smooth reoxydation into aldehyde afforded **17** in far better yield (88%) (Scheme 3).

As previously observed by us[30] and others[43,47] two-branched TPA represent an alternative design of highly fluorescent materials, which were also used for comparative purposes in the photophysical studies. Therefore, in parallel with our work on the trisbranched TPA the preparation of derivatizable precursors leading to two-branched TPA was explored. As depicted in Scheme 4, bisbromo and bisformyl precursors bearing a methoxycarbonyl function on the third phenyl ring (**19** and **20**) were easily obtained following classical synthetic procedures. Comparatively, this straightforward access emphazises the difficulties to be solved in the synthesis of the monofunctionalized trisbranched series.

Synthesis of π-Conjugated Derivatizable Triphenylamines. The versatile set of synthons was further used as starting materials for the introduction of various electron-withdrawing groups via vinylation. The biscyanomethyl, 4-pyridinyl, and 2-benzothiazolyl moieties were chosen since they are relatively small, have been proved to be efficient electron-withdrawing groups, and have been successfully incorporated into various push−pull nonlinear optics materials[23,42,48] and water-soluble fluorophores.[49,50]

Thus, the highly fluorescent TPA **22**, **23**, and **26** were obtained by Heck coupling,[51] whereas **24** was synthesized by Wittig reaction[52] and **21** and **25** by Knoevenagel condensation.[44] It should be noticed that, in spite of the anhydrous conditions used, reaction of **4** in Heck conditions resulted in the concomitant ester hydrolysis to yield the phenolic derivative **23**. The derivatizable TPA **21**−**26** (Scheme 5) display a large structural

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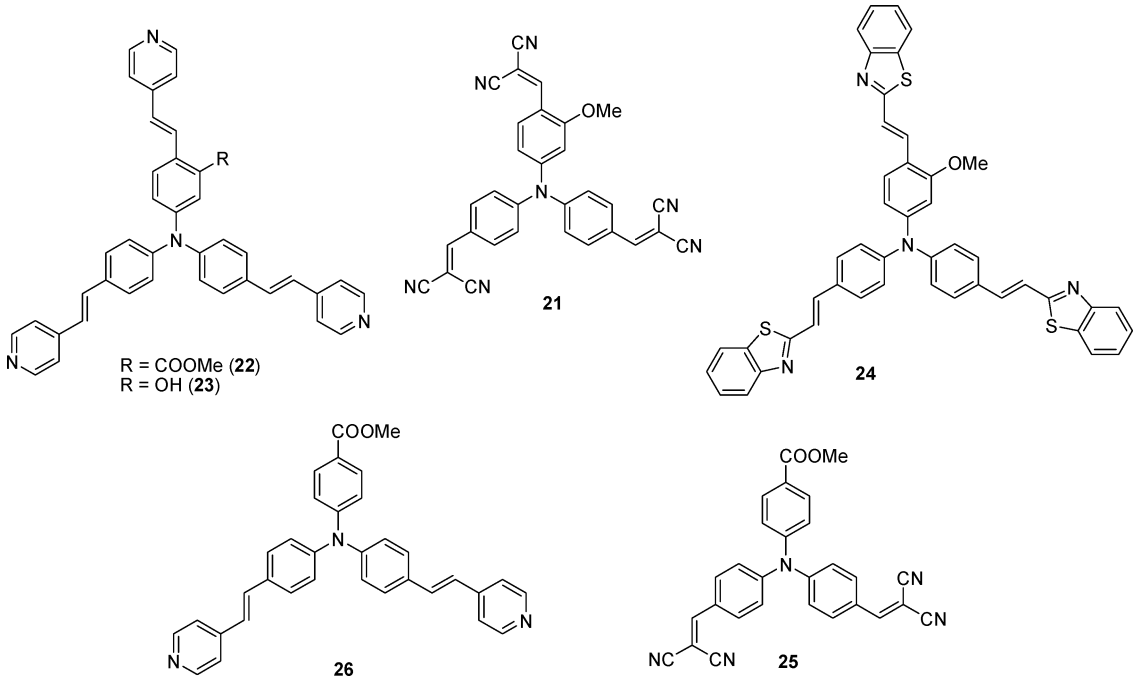
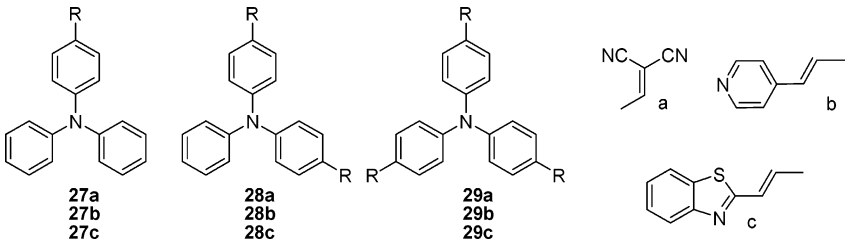
SCHEME 5. Fluorescent Derivatizable TPA



SCHEME 6. Fluorescent Naked TPA



pattern diversity differing by the number of branches (from trisbranched **21**−**24** to bisbranched **25** and **26**) and the electronic character of the functional group (**21**, **23**, and **24** bear a donor group whereas **22**, **25**, and **26** bear the acceptor COOMe). The presence of the functional group induces distortion from the C_3 symmetry and should also influence the electron density repartition of the resulting TPA. Given that 2PA fluorescence is expected to be sensitive to these two parameters, this molecular set of derivatizable TPA will enable evaluation of the structural determinants and optimization of the optical properties aimed at selection of the best candidates for biolabeling.

Nonfunctionalized TPA. For comparative purposes a series of "naked" (i.e., nonderivatizable) TPA were synthesized following procedures similar to those described precedently. The mono-, bis-, and trisbranched TPA series **27**−**29** were thus obtained from their corresponding iodinated[30] or formylated[53] counterparts which bear three various vinyl groups: (biscyano)-vinyl (a), vinylpyridine (b) and vinylbenzothiazole (c) (Scheme 6). It should be noted that yields of Wittig−Horner reaction increased from 67% to 95% along with increasing number of introduced benzothiazole moieties reflecting presumably the electron-withdrawing character of this group and, hence, the increased sensitivity of the remaining formyl group toward the nucleophilic phosphonate anion. It is worth noting that the "naked" triphenylamines prepared herein belong to the so-called star-shaped chromophores and that some derivatives (**29a** and **29b**) already have been described.[23,44,54]

Synthesis of TPA Suitable for Biolabeling. To achieve biolabeling subsequent elongation of the initially grafted functional group was then examined in the pyridine and benzothiazole series. In the pyridine series compound **22** was submitted to amidation with a monoprotected diamino PEG linker after saponification, which afforded the derivative **31** in a satisfactory yield (Scheme 7).

In the benzothiazole series, cleavage of the methoxy group of derivative **24** with BBr_3 failed likely due to complexation of the borate reagent with the nitrogens of the benzothiazole moieties as seen from MS analysis. Thus elongation was attempted on the key trisformylated intermediate **17** (Scheme 8). Treatment of **17** with BBr_3 led quantitatively to a tetrabrominated intermediate.[55] Although the latter could be converted into the desired compound **32** by $AgNO_3$, yields and purity were revealed to be poorly reproducible and use of other cleavage agents (BI_3 Me_3SiCl/NaI, *p*-MeC_6H_4SNa) failed. Finally, $AlCl_3$ in dichloromethane under gentle reflux afforded quantitatively pure trisformylated phenol **32**. Subsequent alkylation with 1,8-

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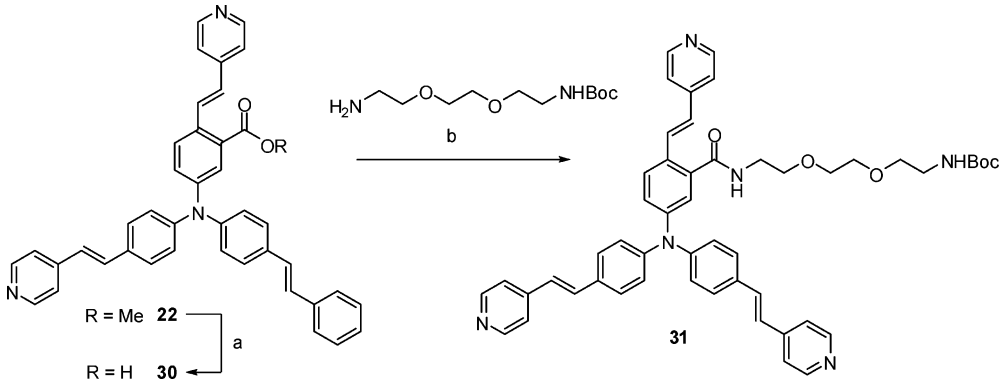
SCHEME 7. Synthesis of Trispyridine TPA Suitable for Bioconjugation[a]



[a] Reagents and conditions: (a) LiOH, THF, H_2O, rt, o/we; (b) EDCI, HOAt, DMF, rt, 7 days.

dibromooctane was easily performed under Williamson conditions to afford **33**. In parallel a carboxylic acid terminated linker was introduced by use of ethyl ω-bromobutyrate, which afforded **34**. Intermediates **33** and **34** were conveniently converted to the highly fluorescent trisbenzothiazole derivatives **35** and **37** with phosphonate **40**[56] under Wittig−Horner conditions. Compound **37** was converted to acid **38** and activated ester **39**. On the other hand, bromide **35** could be efficiently converted into *N*-alkylmaleimide **36** by the method described by Turnbull.[57] These reactional sequences provide us with a pool of highly fluorescent TPA displaying a large set of reactivity for coupling to biomolecules. For example, a haloalkylated terminated compound, such as **35**, might be linked on the 5′ end of oligonucleotides by thiophosphate chemistry under nonaqueous conditions.[58] Equally, acid **38** might be coupled to oligonucleotides as well as to protein amino residues by in situ activation, whereas **39** is expected to react similarly without the need of prior activation. Last, maleimide derivatives (such as **36**) may serve for selective coupling with cysteine residues, which is routinely applied for labeling proteins.[59] Finally introduction of an amino PEG linker should provide water solubility[60] while also allowing amidation reactions.

Optical Measurements. (a) Linear Spectroscopy. The photophysical properties of fluorophores **21**−**29** were studied in dichloromethane and are summarized in Table 1. All the compounds show an intense $\pi-\pi^*$ absorption band ($\epsilon \geq 40\,000$ L·mol^{-1}·cm^{-1}) with absorption maxima between 385 and 476 nm. The two- and three-branched compounds are significantly red-shifted (∼28−30 nm) as compared to the monoderivatives indicating some degree of conjugation between the vinyl conjugated arms. The strongest absorption is obtained in the benzothiazole (Bz) series with ϵ up to 94 700 L·mol^{-1}·cm^{-1} for compound **24**. As expected the molar extinction coefficient ϵ increases with the number of π-conjugated branches. For example, in the biscyano (BCN) series ϵ is increasing from 39 400 L·mol^{-1}·cm^{-1} for the monosubstituted derivative to 49 000 L·mol^{-1}·cm^{-1} for the di- and 82 600 L·mol^{-1}·cm^{-1} for the trisubstituted compound. Importantly, the presence of a lateral functionalized linker does not influence the UV/vis absorption, neither in the two-branched nor in the three-branched series, indicating that the electronic structure is not affected by the linker.

In polar solvents, all fluorophores exhibit a broad structureless emission characteristic of intramolecular charge transfer at excited states[61,62] (Figure 2, and the Supporting Information). All compounds display high quantum yields in dichloromethane ($0.13 < \eta < 0.6$) and large Stokes shifts (50 to 150 nm) (see Table 1). In the benzothiazole series the quantum yield increases with the dimensionality of the molecule since the two- and three-branched compounds exhibit significantly higher quantum yields than their mono counterpart. The same is observed for the two other series but given the low one-photon fluorescence of the monoderivatives the quantum yield was not determined. Most importantly, in the three series introduction of the lateral chain does not have a detrimental influence on the fluorescence emission albeit a decrease in quantum yield of 20−30% is systematically observed (see, for example, compounds **29c** and **24** or compounds **29b** and **22**).

All the fluorophores (mono, bis, and tris) display a strong emission solvatochromism that is reflected by a large bathochromic shift of their fluorescence emission maxima with increasing solvent polarity. In contrast the absorption is not shifted. This solvatochromic behavior, which results from the stabilization of the highly polar emitting state by polar solvents, is typical for compounds presenting an internal charge transfer upon excitation and has been fully documented for numerous fluorophores containing donor−acceptor units including triphenylamine derivatives.[43,61,62] To examine in detail the solvent effect, the emission spectra of compound **29c** were recorded in various media from nonpolar to polar solvents: as can be seen in Figure 2 the maximum emission wavelength of **29c** is red-shifted by 100 nm from cyclohexane (455 nm) to methanol (560 nm). Interestingly in apolar media (toluene, cyclohexane) the vibronic structure is restored likely due to the decrease of polarity in the solvation shell and hence a better vibronic coupling. The solvatochromic behavior is accompanied by a decrease of the fluorescence quantum yield when the polarity of the solvent increases. The origin of this quenching phenomenon is attributed

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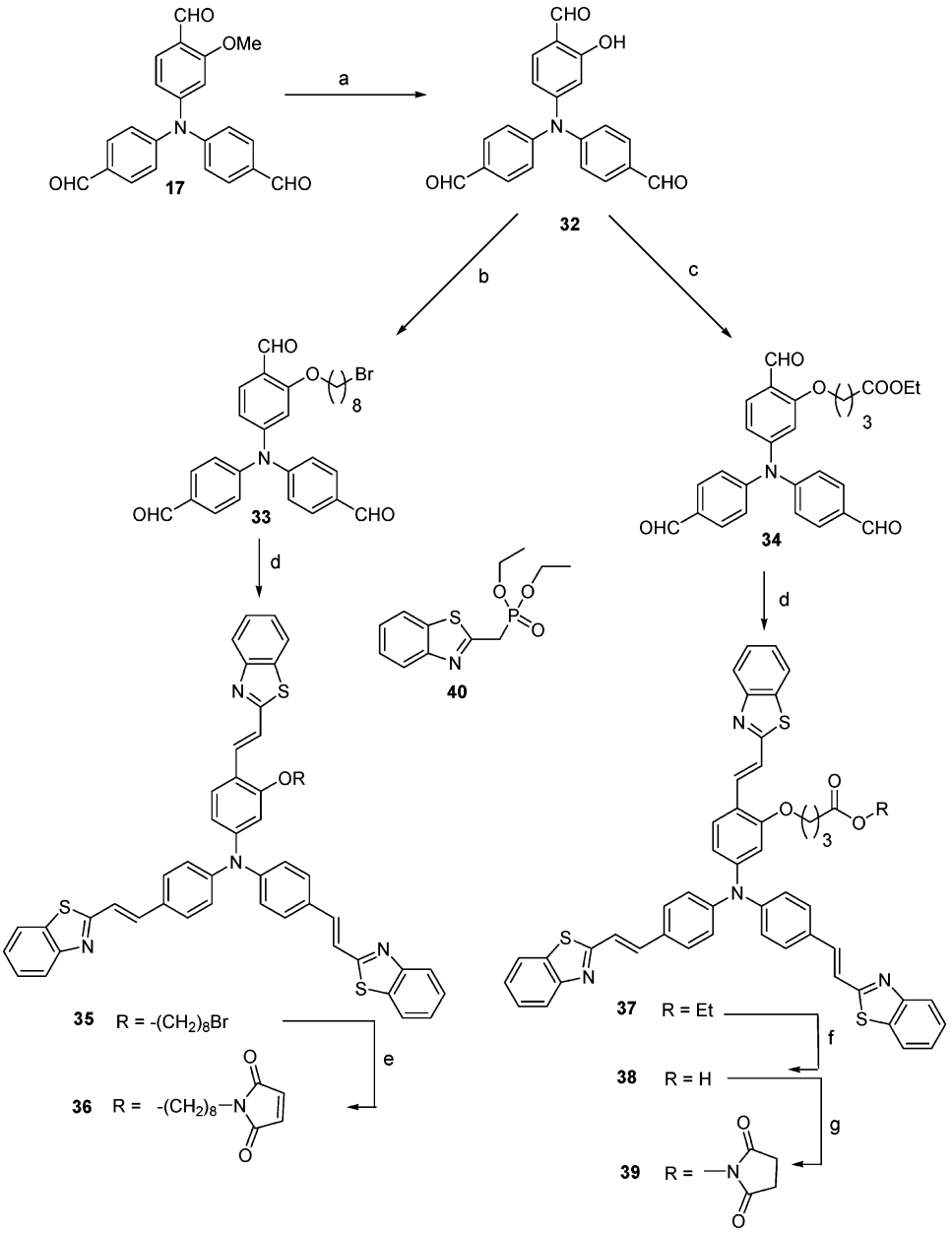
SCHEME 8. Synthesis of Trisbenzothiazole TPA Suitable for Bioconjugation[a]



[a] Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, reflux, o/n; (b) excess $Br(CH_2)_8Br$, K_2CO_3, acetone, 45 °C, 24 h; (c) $Br(CH_2)_3COOEt$, K_2CO_3, DMF, rt, 24 h; (d) **40**, NaH, 15-crown-5, THF, rt, o/we; (e) (i) *rac*-7-oxabicyclo-[2.2.1]-heptene-2,3-dicarboxylic imide, K_2CO_3, DMF, 55 °C, o/n, (ii) anisole, reflux, 2 h; (f) LiOH, THF/H_2O, rt, o/n; (g) NHS, DCC, CH_2Cl_2, rt, 24 h.

to the lowered energies of the ICT states[62] but still remains unclear and its amplitude rather unpredictable.[63−65]

(b) Two-Photon Absorption (2PA) Properties. 2PA spectra of the TP dyes were obtained after two-photon-induced fluorescence (TPIF) measurements, with a femtosecond Ti:sapphire laser source delivering 90 fs pulses at 76 MHz repetition rate over the spectral range from 740 to 840 nm. The TPIF intensities of the samples were measured relative to a solution of fluorescein, the ratio of the fluorescent signals enabling further determination of the 2PA cross section, if equal one- and two-photon fluorescence quantum yields are assumed.[3,4]

2PA cross section of the monosubstituted compounds was revealed to be low, on the order of magnitude of that of standard fluorophores (1−10 GM, 1 GM = 10^{-50} $cm^4 \cdot s \cdot photon^{-1}$), and for this reason was not systematically determined. In the Bz and BCN series, 2PA excitation spectra of two- and three-branched TPA strongly differ in shape (Figure 3A,B). While two-branched compounds have their maximum 2PA wavelength between 760 and 820 nm, three-branched compounds exhibit an absorption maximum below 740 nm, which is the lowest excitation wavelength allowed by the titanium−sapphire laser used in this study. Thus, the maximum 2PA wavelength appears blue-shifted relative to twice the maximum 1PA wavelength,

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TABLE 1. Linear Optical Data and Two-Photon Absorption Properties

compd	λ_{abs} (nm)[a]	ϵ (L·mol^{-1}·cm^{-1})	λ_{em} (nm)[b]	η[c]	δ_{max} (GM)[d]	δ_{max}/M (GM·g^{-1}·mol)
			Bz series			
27c (TP-1Bz)	403	19 700	531	0.24	16 (740 nm)	0.04
28c (TP-2Bz)	431	59 400	530	0.59	158 (760 nm)	0.28
29c (TP-3Bz)	433	89 200	538	0.54	345 (740 nm)	0.48
24 (TP-3Bz-OMe)	432	94 700	542	0.45	341 (740 nm)	0.45
37 (TP-3Bz-Oalk-ester)	428	90 000	540	0.38	365 (745 nm)	0.43
			BCN series			
27a (TP-1BCN)	446	39 400	609	nd[f]	nd[f]	nd[f]
28a (TP-2BCN)	476	49 000	535	0.34[e]	205 (780 nm)	0.52
25 (TP-2BCN-COOMe)	467	42 300	515	0.20[e]	136 (780 nm)	0.30
29a (TP-3BCN)	461	82 600	509	0.22[e]	204 (745 nm)	0.43
21 (TP-3BCN-OMe)	464	40 300	539	0.14	140 (745 nm)	0.28
			Py series			
27b (TP-1Py)	384	32 000	494	nd[f]	nd[f]	nd[f]
28b (TP-2Py)	402	50 400	497	0.64	102 (820 nm)	0.23
26 (TP-2Py-COOMe)	385	47 200	488	0.81	34 (820 nm)	0.07
29b (TP-3Py)	406	63 900	519	0.53	90 (820 nm)	0.16
23 (TP-3Py-OH)	408	71 000	521	0.36	73 (820 nm)	0.13
22 (TP-3Py-COOMe)	402	66 100	550	0.37	97 (820 nm)	0.16

[a] One-photon maximum absorption wavelength in dichloromethane. [b] Maximum fluorescence emission wavelength in dichloromethane. [c] Fluorescence emission quantum yield, measured using quinine bisulfate in 1 N H_2SO_4 as a reference. [d] Maximum two-photon absorption cross section. [e] Measurements were performed in chloroform. [f] nd: not determined.

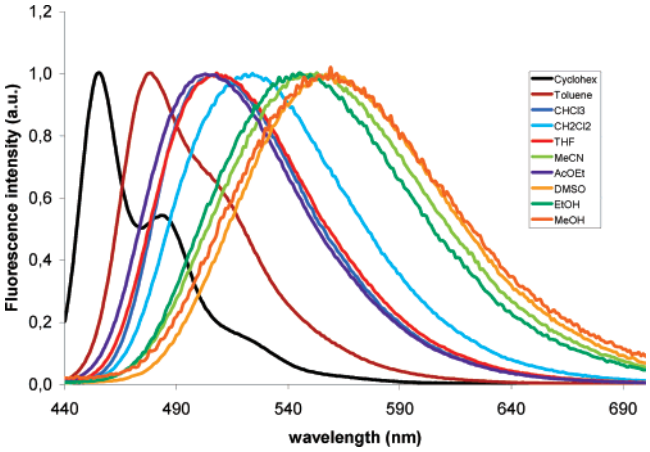

FIGURE 2. Normalized emission spectra of compound **29c** recorded in various solvents.

indicating that higher order excited states may also contribute to the signal.[4,43] Additionally this indicates that the maximum 2PA wavelength is not reached in the scanned optical window (740−840 nm), and suggests that it would be of interest to test the three-branched compounds at lower excitation wavelengths.[66] This trend is not observed in the Py series where the 2PA maximum is situated around 820 nm for all compounds (Figure 3C). The pyridine group being much less electron-withdrawing than the Bz and BCN functions, this indicates that the EW character of the end group and thus the magnitude of the ICT is a determinant of the 2PA spectral shape. In addition variations of charge transfer occurring on each single branch are likely to induce differences in the coupling between branches.

Determination of the 2PA cross section of the compounds at their respective absorption maxima (δ_{max}) was achieved and the obtained values are listed in Table 1. As can be seen triphenylamines substituted by 3 benzothiazole units (**29c**, **24**, and **37**) and three biscyanovinyl groups (**29a**) are the most efficient compounds, with two-photon absorption cross sections δ_{max} reaching respectively 365 and 204 GM. Triphenylamines substituted with pyridine groups have smaller two-photon absorption cross sections, which remain slightly under 100 GM. On the whole, in the Bz series the cross section increases strongly with the number of branches and three-branched compounds exhibit significantly increased two-photon absorption properties compared to their two-branched and monobranched counterparts. This increase is not linear with respect to the number of branches, which is consistent with a cooperative enhancement effect already observed for other vinyl triphenylamines[43,47] and for multimeric molecular systems.[67] The δ_{max} enhancement is dramatic when the number of branches is increased from one to two (approximately 10-fold from compound **27c** to **28c**) and then more modest but still significant when increasing from two to three (approximately 2−2.5-fold from **28c** to **29c**). This is consistent with a strong electronic coupling between at least two/several units and implies that bisbranched systems could display both dipolar and quadrupolar characteristics. However, the mechanism of this enhancement still remains to be fully established.[43,47]

This trend is not observed in the BCN series and in the Py series showing that the 2PA performance of the designed triphenylamine dyes is dependent on several structural parameters (at least on the electron-withdrawing group and the number of branches) thus confirming the difficulty to predict optimized molecular structures.

However, in the three families (BCN, Bz, and Py) the two-branched derivatives still reach a satisfying 2PA level, which falls in the 100−200 GM range. This activity combined with the easy synthetic access of these two-branched structures and with their high quantum yields make these compounds worth considering for biolabeling applications.

Finally and very interestingly no significant difference was observed between the two-photon absorption cross section of compounds bearing a derivatizable linker and that of their unsubstituted counterparts. This can be exemplified in the Bz series where a systematic comparison can be conducted, i.e., compare **29c**, **24**, and **37**. This important feature was observed in the case of the two- and three-branched series irrespective

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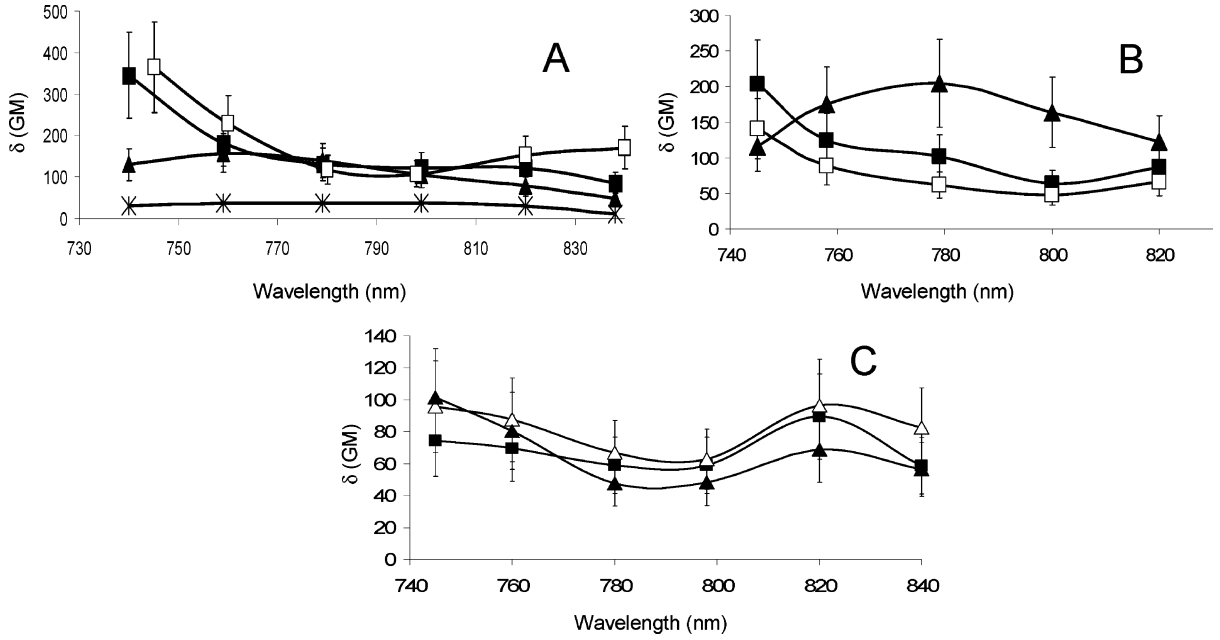

FIGURE 3. Two-photon absorption cross sections of compounds in dichloromethane and fluorescein (∗) in aqueous KOH (pH 10−11): (A) benzothiazole series **28c** (TP-2Bz, ▲), **29c** (TP-3Bz, ■), **37** (TP-3Bz O-alkyl-ester, □); (B) biscyano series **28a** (TP-2BCN, ▲), **29a** (TP-3BCN, ■), **21** (TP-3BCN-OMe, □); and (C) pyridine series **28b** (TP-2Py, ▲), **29b** (TP-3Py, ■), **22** (TP-3Py -COOMe, △).

of the electron-withdrawing group present on the TPA and of the linker length (Figure 3 and Table 1). This result indicates that the distortion from the C_3-symmetry induced by the linker does not influence significantly the two-photon absorption ability. This observation, made for the first time, fully validates our initial design (Figure 1), which is especially important for future applications.

On the whole, the cross-section values obtained for the most efficient compounds fall in the range obtained for recently optimized 2PA dyes dedicated to biolabeling.[60,68] Nevertheless, δ values remain 1 order of magnitude below that of the extended π-conjugated TPA reported so far in the literature.[9,23] But the large size of the latter represents a major issue for the labeling of biomolecules. In this respect and in order to compare the 2PA performances of different chromophoric series, the two-photon absorption/molecular weight ratio (δ/MW) is a relevant figure of merit. In this regard, our compounds fall in the range of the best chromophores reported so far with a δ/MW ratio around 0.5 GM·g^{-1}·mol for compounds **29c** (TP-3Bz) and **28a** (see Table 1) compared to values of 0.5−1.0 GM·g^{-1}·mol described for large-sized systems optimized for 2-photon absorption.[9,66,67]

In the aggregate, our data fully validate the initial design of derivatizable vinyl triphenylamines for covalent labeling of biomolecules and subsequent tracking using multiphoton microscopy. They also demonstrate that 2-branched compounds might be good candidates to consider given their good performance and the short and easy synthetic access of the derivatizable derivatives. As a result, we are currently focusing our efforts in the development of functionalized TPA based on both designs.

Conclusions

In conclusion, new synthetic methodologies were developed opening access to three series of novel fluorescent π-conjugated triphenylamines. The versatility of the synthetic routes described allows introduction of side chains of various nature and length and terminated with chemically reactive groups suitable for conjugation to biomolecules. The one-photon and two-photon characterizations in organic solvents show high extinction coefficients, high quantum yields, and high 2PA cross sections. Comparison of the derivatized series with their nonderivatized counterparts shows that the former retain all of the desirable nonlinear optical properties. This indicates that the presence of the linker although affecting the C_3-symmetry does not have significant consequences on the 2PA absorption, which fully validates the initial three-branched monosubstituted design. Interestingly the good 2PA performances of the two-branched series highlight this design as a valuable alternative for fluorescent labeling. Finally the relatively small size of our compounds allows us to anticipate that water solubility could be provided either by quaternerization of heterocyclic ring nitrogens[30] or by the use of highly hydrophilic linkers (PEG or polyammonium and peptides chains)[69] which will be the next step in exploring fully the biolabeling potential of the newly synthesized triphenylamines.

Experimental Section

2-Bromo-5-(*N*,*N*-Bis(4-bromophenyl)amino)phenyl Acetate (3). Compound **2** (153 mg, 525 μmol) was dissolved in chloroform (3 mL) and cooled to 0 °C and bromine (79 μL, 1.54 mmol) in solution in chloroform (5 mL) was added dropwise during 1 h. The reaction mixture was stirred at rt for 2 h. Then it was diluted in CH_2Cl_2 and washed with a $Na_2S_2O_3$ solution (0.1 M), a $NaHCO_3$ solution (10%), and water. The organic layers were collected, dried (Na_2SO_4), and evaporated to afford 225 mg (426 μmol) of a light green powder in 81% yield (product is spoiled by a few isomers).

Alternatively, **3** could be prepared from **2** (450 mg, 1.48 mmol, 1 equiv) and benzyltrimethylammonium tribromide (1.70 g, 4.45

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mmol, 3 equiv) dissolved in a $CHCl_3$/MeOH mixture (10 mL, 1:1, v/v) containing excess $CaCO_3$. Once the solution became colorless, the crude mixture was filtered, washed with water, dried (Na_2SO_4), concentrated, and purified by column chromatography (*n*-hexane/CH_2Cl_2, 3:1, v/v) affording the title compound in lower yield (67%) but isomer free.

Mp 68−70 °C; IR (KBr pellet) 1774 ($\nu_{C=O}$, ester), 1579 (Ar), 1486 (Ar) cm^{-1}; ^1H NMR (acetone-d_6) δ 2.27 (s, 3H), 6.89 (dd, J = 2.7 Hz, 8.4 Hz, 1H), 7.07 (d, J = 9.0 Hz, 4H), 7.51 (d, J = 9.0 Hz, 4H), 7.56 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 20.8, 109.1, 116.9, 118.1, 121.8, 126.2, 132.7, 134.6, 145.6, 147.2, 148.9, 167.4. HRMS (FAB+) calcd for $C_{20}H_{14}{}^{79}Br_2{}^{81}BrNO_2$ 538.8555, found 538.8556. HRMS (FAB+) calcd for $C_{20}H_{14}{}^{79}Br^{81}Br_2NO_2$ 540.8536, found 540.8541.

2-Iodo-5-(*N*,*N*-bis(4-iodophenyl)amino)phenyl Acetate (4). To a well-stirred solution of **2** (77 mg, 254 μmol) in CH_2Cl_2 (2 mL) was added an excess of iodine (449 mg, 1.77 mmol, 7 equiv). After complete dissolution, red mercury oxide (384 mg, 1.77 mmol, 7 equiv) was added and the solution was stirred for 3 d at rt. The suspension was filtered through a pad of Celite and washed with $Na_2S_2O_3$ (0.1 M) and water. The organic layers were collected, dried (Na_2SO_4), and concentrated to dryness to afford dark brown residue. This residue was purified by chromatography on a short column (eluant: *n*-hexane/CH_2Cl_2 (2/1, v/v)) to afford the title compound as a white foam (153 mg, 223 μmol) in 88% yield.

Mp 74 °C; IR (KBr pellet) 1771 ($\nu_{C=O}$, acetate), 1575 (Ar), 1483 (Ar) cm^{-1}. ^1H NMR (acetone-d_6) δ 2.28 (s, 3H), 6.77 (dd, J = 8.7 Hz, 2.7 Hz, 1H), 6.89 (d, J = 2.7 Hz, 1H), 6.94 (d, J = 9.0 Hz, 4H), 7.69 (d, J = 9.0 Hz, 4H), 7.75 (d, J = 8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 21.2, 81.9, 87.4, 117.7, 122.3, 126.6, 138.6, 139.5, 146.2, 148.2, 152.0, 168.4. HRMS (ESI−) calcd for $C_{18}H_{11}I_3NO$ [M − Ac] 637.7975, found 637.7977.

Alternatively, compound **4** was prepared by stirring **2** (100 mg, 330 mol) and silver(I) trifluoroacetate (219 mg, 991 μmol, 3 equiv) and iodine (251 mg, 989 mol, 3 equiv) in chloroform (5 mL) for 3 d at rt. Yield: 80%.

Methyl 5-[Bis(4-bromophenyl)amino]-2-bromobenzoate (8). A solution of 3-(diphenylamino)benzoic acid methyl ester **7** (5.20 g, 17.1 mmol, 1 equiv) and NBS (6.71 g, 37.7 mmol, 2.2 equiv) in $CHCl_3$ was heated at reflux. The reaction course was followed by ^1H NMR. After 2 h, a second portion of NBS (6.10 g, 34.3 mmol, 2.0 equiv) was added. After 2 d of reflux, the chloroform was removed in vacuo and the residue was filtered through a short silica pad and washed with CH_2Cl_2/*n*-pentane (1:1, v/v) to give **8** (8.78 g, 95%) as a light brown solid. The compound purity was estimated by ^1H NMR to be >95%, and it was used without further purification.

Mp 109−111 °C; IR (KBr pellet) 1486 (Ar), 1580 (Ar), 1732 ($\nu_{C=O}$) cm^{-1}. ^1H NMR (CD_2Cl_2) δ 3.87 (s, 3H), 6.99 (d, J = 9.0 Hz, 4H), 7.05 (dd, J = 8.7 Hz, 2.7 Hz, 1H), 7.40−7.46 (m, 5H), 7.54 (d, J = 8.7 Hz, 1H). ^{13}C ($CDCl_3$) δ 52.4, 114.0, 116.5, 125.5, 126.0, 127.2, 132.6, 133.5, 135.1, 145.7, 146.3, 166.1. HRMS (DCI+) calcd for $C_{20}H_{15}Br_3NO_2$ 537.8653 ($^{79}Br)_3$, 539.8633 ($^{79}Br)_2$ + ^{81}Br, 541.8614 ($^{81}Br)_2$ + ^{79}Br, 543.8598 ($^{81}Br)_3$, found 537.8661, 539.8623, 541.8602, 543.8574.

Propyl 2-Iodo-5-(*N*,*N*-bis(4-iodophenyl)aminobenzoate (9). To a solution of **7** (134 mg, 441 μmol, 1 equiv) dissolved in technical *n*-propanol (5 mL) were added iodine (1.12 g, 4.41 mmol, 10 equiv) and red mercury oxide (956 mg, 4.41 mmol, 10 equiv). The resulting suspension was heated at 85 °C for 2 weeks. Regularly after ca. 72 h, I_2 (2 equiv) and HgO (2 equiv) were added portionwise again. Then the crude mixture was filtered through a Celite pad and abundantly washed with hot toluene. Mother liquors were concentrated to dryness, redissolved in a few milliliters, and filtered again. This procedure was repeated until removal of excess HgO. The crude mixture (215 mg) contained the expected product along with the methyl ester (in a 5.6:1 ratio) as impurity and was purified by preparative TLC (*n*-hexane/Et_2O, 95:5, v/v) to afford the title compound as a yellow oil (160 mg) in 51% yield.

Mp 242−244 °C. ^1H NMR ($CDCl_3$) δ 1.00 (d, J = 7.2 Hz, 3H), 1.80 (hex, J = 7.2 Hz, 2H), 4.27 (t, J = 7.2 Hz, 2H), 6.93−6.90 (m, 5H), 7.44 (d, J = 2.7 Hz, 1H), 7.59 (d, J = 8.7 Hz, 4H), 7.80 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 10.6, 21.9, 67.5, 85.3, 87.4, 125.3, 126.4, 127.3, 137.2, 138.7, 141.9, 146.1, 146.9, 166.5. HRMS (MALDI+) calcd for $C_{22}H_{18}NO_2I_3$ 708.8466, found 708.8442.

Methyl 2-Methoxy-4-(*N*,*N*-bis(4-methoxycarbonylphenyl)amino)benzoate (15). To dry and degassed toluene (30 mL) were introduced $Pd(OAc)_2$ (186 mg, 828 μmol, 5%) and P(*t*-Bu)$_3$ (7.7 mL, 2.48 mmol, 15%, 10% in hexanes (w/w)). After 15 min of stirring, methyl 4-bromobenzoate (10.7 g, 49.7 mmol, 3 equiv), methyl 2-methoxy-4-aminobenzoate (3.0 g, 16.6 mmol, 1 equiv), and Cs_2CO_3 (13.5 g, 41.4 mmol, 2.5 equiv) were added. The solution was refluxed overnight, cooled to rt, and diluted with CH_2Cl_2 (100 mL). The crude mixture was filtered through a Celite pad, evaporated to dryness, and purified by silica gel column chromatography (*n*-hexane/CH_2Cl_2 (2:1, v/v) to CH_2Cl_2 gradient) to afford a light yellow powder (6.35 g, 14.8 mmol) in 89% yield.

Mp 77−79 °C. IR (KBr pellet) 2839 (ν_{C-OMe}), 1719 ($\nu_{C=O}$, ester), 1597 (Ar), 1507 (Ar) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.76 (s, 3H), 3.91 (s, 3H), 3.94 (s, 6H), 6.68 (m, 2H), 7.17 (d, J = 8.7 Hz, 4H), 7.80 (d, J = 9.0 Hz, 1H), 7.98 (d, J = 8.7 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 166.3, 165.8, 160.6, 151.0, 150.2, 133.2, 131.1, 125.4, 123.8, 116.0, 115.5, 108.0, 56.0, 52.0, 51.8. HRMS (DCI+) calcd for $C_{25}H_{24}NO_7$ 450.1553, found 450.1548.

(4-{Bis[4-(hydroxymethyl)phenyl]amino}-2-methoxyphenyl)-methanol (16). To a slurry of $LiAlH_4$ (1.7 g, 45 mmol, 15 equiv) in dry THF (30 mL) was added a solution of **15** (1.35 g, 3 mmol) in THF (20 mL) dropwise at −78 °C. The reaction mixture was allowed to warm to rt and then was stirred at reflux for 1 h. The mixture was cooled again to −78 °C then diluted in CH_2Cl_2, and water (10 mL) was added. The solids were filtered and washed thoroughly with CH_2Cl_2 and the filtrate was washed with water and brine, dried (Na_2SO_4), and concentrated to afford a green paste (1.05 g, 2.88 mmol) in 97% yield.

Mp 138 °C. ^1H NMR (acetone-d_6) δ 3.67 (s, 3H), 3.90 (t, J = 5.7 Hz, 1H), 4.17 (t, J = 5.7 Hz, 2H), 4.59 (s, 4H), 4.61 (s, 2H), 6.57 (dd, J = 1.8 Hz, 8.1 Hz, 1H), 6.67 (d, J = 1.8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 4H), 7.29 (d, J = 8.4 Hz, 4H), 7.30 (d, J = 8.1 Hz, 1H). ^{13}C NMR (acetone-d_6) δ 54.8, 59.0, 63.5, 106.4, 115.8, 123.7, 125.2, 127.8, 128.4, 136.9, 146.8, 148.1, 157.5. MS (DCI+) m/z 348 (100%) [M − H_2O + H]$^+$. HRMS (ESI+) calcd for $C_{22}H_{23}NO_4Na$ 388.1525, found 388.1509.

4-[Bis(4-formylphenyl)amino]-2-methoxybenzaldehyde (17). To a solution of **16** (27 mg, 74 μmol) in CH_2Cl_2 (2 mL) was added MnO_2 (40 mg, 444 μmol). The resulting suspension was stirred for 48 h at rt and filtered. Solids were washed well with CH_2Cl_2 and the filtrate was concentrated to afford the product as a yellow solid (25 mg, yield 91%).

Alternatively, **17** could be obtained directly from **15** as follows:

In a dry flask and under an inert atmosphere, Red-Al solution (3.70 mL, 65% in toluene (w/w)) was diluted in dry toluene (5 mL). After cooling to 0 °C, freshly distilled *N*-methylpiperazine (1.48 mL) was added dropwise. The resulting Red-Al-modified colorless solution was stirred 20 min and stored at rt.

To a solution of **15** (300 mg, 667 μmol, 1 equiv) dissolved in dry toluene (10 mL) cooled to 0 °C was added Red-Al-modified solution dropwise (1.90 mL, 2.30 mmol hydride, 3.44 equiv). Light green coloration occurred rapidly. After 8 h of stirring at rt, the appearance of the expected product (R_f 0.29 in *n*-hexane/THF, 7:3, v/v) and residual monoester was noticed. Water was added (2 mL) and the resulting biphasic mixture was filtered through a Celite pad. Mother liquor was decanted, diluted by CH_2Cl_2, and washed several times with HCl (10 mM) and water. After evaporation to dryness, the residue was redissolved in THF/LiOH saturated solution (20 mL, 1:1, v/v) and stirred for 2 h. Once disappearance of residual monoester derivative was noticed by TLC (in *n*-hexane/THF, 7:3, v/v), the mixture was treated using classical procedures and purified

by column chromatography (*n*-hexane/THF, 3:1, v/v) to afford 92 mg (256 μmol) in 38% yield.

Mp 130−131 °C. IR (KBr pellet) 2841 (ν_S, CH_3), 2735 (ν_{C-H}, formyl), 1697 ($\nu_{C=O}$, formyl), 1592 (Ar), 1504 (Ar) cm^{-1}. NMR ^1H ($CDCl_3$) δ 3.81 (s, 3H), 6.71 (s, 1H), 6.77 (dd, J = 8.4 Hz, 0.9 Hz, 1H), 7.29 (d, J = 8.4 Hz, 4H), 7.84 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 4H), 9.89 (s, 2H), 10.39 (s, 1H). ^{13}C NMR (CD_2Cl_2) δ 55.8, 107.5, 116.8, 121.5, 124.6, 129.8, 131.2, 132.6, 151.3, 152.6, 163.1, 187.8, 190.4. HRMS (DCI+) calcd for $C_{22}H_{18}NO_4$ 360,1236, found 360.1229.

Before LiOH treatment, asymmetric monoester could be isolated (12% yield). Despite increased reaction time, its presence was always noticed.

Methyl 4-(*N*,*N*-Bis(4-formylphenyl)amino)benzoate (19). Dry DMF (2.9 mL, 37.5 mmol) was cooled to 0 °C and $POCl_3$ (3.7 mL, 40 mmol) was added dropwise under nitrogen atmosphere. The mixture was stirred for 1 h at 0 °C and in case it became solid, warmed until melted. To this mixture **18** (500 mg, 1.6 mmol) was added with vigorous stirring during the addition. The reaction was carried out at 95 °C for 4 h. After cooling to rt, the reaction mixture was poured onto ice and neutralized by addition of NaOH pellets. The product was extracted with CH_2Cl_2. The organic phase was washed with brine and dried over sodium sulfate. The crude product was purified by column chromatography (*n*-hexane/AcOEt, 3:1, v/v). The expected product was obtained as an orange foam (205 mg, 553 μmol) in 35% yield and the monoformylated byproduct as a yellow oily major byproduct (358 mg, 1.04 mmol) in 65% yield.

Mp 129 °C. ^1H NMR ($CDCl_3$) 3.96 (s, 3H), 7.27 (m, 6H), 7.85 (d, J = 8.7 Hz, 4H), 8.04 (d, J = 8.7 Hz, 2H), 9.96 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 52.2, 124.0, 124.8, 126.6, 131.4 (2C), 132.2, 149.8, 151.4, 166.3, 190.5. HRMS (DCI+) calcd for $C_{22}H_{18}NO_4$ 360.1236, found 360.1223.

4-(2,2-Dicyanovinyl)-3-methoxy-[*N*,*N*-(4-(2,2-dicyanovinyl)-phenyl)]aniline (21). To aldehyde **17** (61 mg, 170 μmol, 1 equiv) in dry pyridine (5 mL) were added malononitrile (40 mg, 606 μmol, 3.6 equiv), $AcONH_4$ (one crystal), AcOH (one drop), and crushed molecular sieve (3 Å). The reaction mixture was stirred 16 h at rt. Pyridine was removed under reduced pressure. The residue was redissolved in CH_2Cl_2 and filtered through a Celite pad. The mother liquor was washed with diluted HCl and water, and then concentrated to dryness, redissolved in CH_2Cl_2, and filtered through a short silica plug. The residue was triturated in *n*-hexane and Et_2O to afford the title compound as a red powder (53 mg, 105 μmol) in 62% yield.

Mp 125 °C. IR (KBr pellet) 1503 (Ar), 1573 (Ar), 2224 (ν_{CN}) cm^{-1}. NMR ^1H (CD_2Cl_2) δ 8.26 (s, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.7 Hz, 4H), 7.77 (s, 2H), 7.29 (d, J = 8.4 Hz, 4H), 6.83 (dd, J = 8.7 Hz, 2.9 Hz, 1H), 6.72 (d, J = 2.9 Hz, 1H), 3.80 (s, 3H). ^{13}C ($CDCl_3$) δ 56.3, 80.3, 81.6, 107.0, 112.8, 113.4, 113.8, 114.6, 117.4, 117.5, 124.8, 127.6, 130.5, 132.7, 150.0, 151.9, 152.1, 157.5, 160.4. HRMS (DCI+) calcd for $C_{31}H_{18}N_7O$ 504.1573, found 504.1574.

Methyl 5-{*N*,*N*-Bis{4-[(*E*)-2-(pyridin-4-yl)vinyl]phenyl}}amino-2-[(*E*)-2-(pyridin-4-yl)vinyl]benzoate (22). To a dry and degassed TEA/DMF (9 mL, 2:1, v/v) mixture were added $Pd(OAc)_2$ (191 mg, 85 μmol, 8%) and P(*o*-tolyl)$_3$ (78 mg, 255 μmol, 24%) and the suspension was stirred 10 min. Then **8** (575 mg, 1.06 mmol, 1 equiv) and 4-vinylpyridine (574 μL, 5.30 mmol, 5 equiv) were added and the resulting mixture was heated to 85 °C for 18 h. The crude mixture was allowed to cool to rt, diluted with CH_2Cl_2, and washed with a $NaHCO_3$ solution (10%) and water. The organic layer were collected, dried, and evaporated to dryness. The resulting red oil was diluted with a few drops of CH_2Cl_2 then redissolved by *n*-pentane and the resulting precipitate was filtered to afford impure title compound (741 mg). The red powder was subjected to column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH (96:4, v/v) to afford 495 mg of the product as an orange powder (495 mg, 806 μmol) in 76% yield.

Mp 130 °C dec. IR (KBr pellet) 1715 ($\nu_{C=O}$), 1631 ($\nu_{C=C}$), 1588 (Ar), 1504 (Ar) cm^{-1}. ^1H NMR (CD_2Cl_2) δ 3.88 (s, 3H), 6.95 (d, J = 16.2 Hz, 1H), 7.02 (d, J = 16.2 Hz, 2H), 7.17 (d, J = 8.7 Hz, 4H), 7.32 (s, 1H), 7.32 (dd, J = 2.7 Hz, 8.4 Hz, 1H), 7.36−7.46 (m, 7H), 7.55 (d, J = 8.7 Hz, 4H), 7.70 (d, J = 2.7 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 16.2 Hz, 1H), 8.58 (br, 6H). ^{13}C NMR ($CDCl_3$) δ 167.2, 150.2, 147.0, 146.8, 144.8, 144.6, 132.6, 132.2, 131.8, 131.1, 130.3 128.3, 127.7, 127.6, 125.6, 125.2, 124.4, 121.0, 120.7, 52.4. HRMS (DCI+) calcd for $C_{41}H_{33}N_4O_2$ 613.2604, found 613.2599.

Tris[4-(2,2-dicyanovinyl)phenyl]amine (29a). Molecular sieve (1.00 g, 3 Å), malononitrile (101 mg, 1.53 mmol, 3.6 equiv), acetic acid (few drops), and ammonium acetate (1 crystal) were added to a solution of tris(4-formylphenyl)amine (140 mg, 430 μmol, 1 equiv) in dry pyridine (15 mL). After a few minutes of stirring at rt, the solution turned red. After 2.5 h of stirring at rt, pyridine was removed in vacuo, and the residue was dissolved in CH_2Cl_2, filtered through a Celite pad, and washed with water. The organic phase was dried over Na_2SO_4 and its volume was reduced to ca. 1 mL. Then **29a** was obtained as a red powder (164 mg, 344 μmol) in 80% yield by precipitation with hexane.

Mp 180 °C dec. IR (KBr pellet) 1497 (Ar), 1581 (Ar), 2222 (ν_{CN}) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.29 (d, J = 8.7 Hz, 6H), 7.73 (s, 3H), 7.96 (d, J = 8.7 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 81.70, 112.8, 113.8, 124.9, 127.7, 132.8, 150.1, 157.5. HRMS (DCI+) calcd for $C_{30}H_{16}N_7$ 474.1467, found 474.1458.

{4-[(*E*)-2-(Benzothiazol-2-yl)vinyl]}-*N*,*N*-bis{4-[(*E*)-2-(benzothiazol-2-yl)vinyl]phenyl}aniline (29c). Phosphonate **40** (574 mg, 2.01 mmol, 3.3 equiv), NaH (60% dispersion, 54 mg, 2.25 μmol, 3.7 equiv), and one drop of 15-crown-5 were dissolved in dry THF (5 mL). To this reddish solution was added tris(4-formylphenyl)amine (200 mg, 607 μmol, 1 equiv) in dry THF (20 mL) dropwise. After 70 h of stirring, disappearance of tris(4-formylphenyl)amine was noticed by TLC and the solution became yellow greenish fluorescent. The reaction was quenched with water, diluted with diethyl ether, decanted, and washed with water. After evaporation, the resulting yellow powder was triturated in *n*-pentane affording the title compound as a yellow powder (412 mg, 570 μmol) in 94% yield.

Mp 154 °C dec. IR (KBr pellet) 1625 ($\nu_{C=C}$), 1591 (Ar), 1505 (Ar), 1454 (Ar) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.14 (d, J = 8.7 Hz, 6H), 7.43 (dt, J = 1.2 Hz, 8.1 Hz, 3H), 7.51 (dt, J = 1.2 Hz, 8.1 Hz, 3H), 7.53 (d, J = 16.2 Hz, 3H), 7.65 (d, J = 16.2 Hz, 3H), 7.78 (d, J = 8.7 Hz, 6H), 7.96 (d, J = 7.8 Hz, 3H), 8.09 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 167.1, 154.0, 147.7, 136.8, 134.4, 130.1, 128.7, 126.3, 125.3, 124.5, 122.9, 121.5, 121.0. HRMS (DCI+) calcd for $C_{45}H_{31}N_4S_3$ 723.1702, found 723.1721.

5-(Bis{4-[(*E*)-2-(pyridin-4-yl)vinyl]phenyl}amino)-2-[(*E*)-2-(pyridin-4-yl)vinyl]benzoic Acid (30). The methyl ester **22** (50 mg, 82 μmol) was dissolved in 2 mL of THF. Aqueous LiOH (1 mL, 20 mg·mL^{-1}) was added and the mixture was stirred at rt in the dark for 72 h. The solvents were removed under reduced pressure; the residue was dissolved in ethanol and filtered. The filtrate was concentrated under reduced pressure to give **30** as a red powder with quantitative yield.

Mp >260 °C. IR (KBr pellets) 1505 (Ar), 1587 (Ar), 1630 ($\nu_{C=C}$), 1759 ($\nu_{C=O}$) cm^{-1}. ^1H NMR (MeOD) δ 7.05−7.18 (m, 8H), 7.30 (d, J = 2.1 Hz, 1H), 7.50 (d, 2H, J = 16.2 Hz), 7.56−7.62 (m, 10H), 7.77 (d, J = 8.7 Hz, 1H), 8.05 (d, J = 16.2 Hz, 1H), 8.45 (m, 6H). ^{13}C NMR (MeOD + D_2O) δ 121.0, 121.2, 122.7, 123.8, 124.0, 124.1, 124.6, 126.6, 128.1, 128.3, 131.4, 132.2, 133.2, 142.5, 146.2, 146.6, 146.7, 147.3, 148.8, 148.9, 175.6. MS (FAB+) *m*/*z* 605.5 ($[M + Li]^+$, 15%), *m*/*z* 599 ($[M + H]^+$, 10%), *m*/*z* 314.2 ($[M + 2Li]^{2+}$/2, 25%). HRMS (ESI+) calcd for $C_{40}H_{31}N_4O_2$ 599.2447, found 599.2398.

[2-(2-{2-[5-{Bis-[4-(2-pyridin-4-ylvinyl)phenyl]amino}-2-(2-pyridin-4-ylvinyl)benzoylamino]ethoxy}ethoxy)ethyl]-carbamic Acid *tert*-Butyl Ester (31). To a solution of 2-[2-(2-aminoethoxy)ethoxy]ethylcarbamate (19 mg, 76.5 μmol, 1.2 equiv)

in DMF (2 mL) were added acid **30** (39.5 mg, 6.53×10^{-2} mmol, 1.0 equiv), EDCI (13.7 mg, 71.5 μmol, 1.1 equiv), and HOAt (11 mg, 80.8 μmol, 1.2 equiv). The mixture was stirred 7 days at rt in the dark. The mixture was then diluted with CH_2Cl_2 (60 mL) and washed with water (3×15 mL). The aqueous phase was extracted with CH_2Cl_2 (10 mL). The organic phases were combined and dried over Na_2SO_4. The product was dried under reduced pressure to remove DMF and then purified by preparative TLC (SiO_2 2 mm, elution with CH_2Cl_2/MeOH 95/5) to give **31** as an orange solid (16 mg, 30% yield).

^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.22 (br s, 2H), 3.44 (t, J = 5.4 Hz, 4H), 3.49 (d, J = 5.1 Hz, 2H), 3.68 (br s, 4H), 4.91 (br s, 1H, NH), 6.43 (br s, 1H, NH), 6.95 (d, J = 16.2 Hz, 1H), 6.98 (d, J = 16.2 Hz, 2H), 7.17 (d, J = 8.4 Hz, 4H), 7.22 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 16.2 Hz, 2H), 7.41 (br s, 6H), 7.51 (d, J = 8.4 Hz, 4H), 7.67 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 16.2 Hz, 1H), 8.63 (br s, 6H). ^{13}C NMR ($CDCl_3$) δ 28.4, 39.9, 40.3, 69.7, 39.8, 70.1, 70.3, 79.5, 122.2, 124.7, 125.2, 127.1, 127.6, 128.3, 129.0, 129.9, 131.8, 132.3, 144.7, 146.9, 147.1, 150.1, 155.9. MS (FAB+) *m*/*z* 829.5 (75%) $[M + H]^+$, *m*/*z* 729.5 (18%) $[M - Boc + H]^+$, *m*/*z* 581.3 (88%) $[M + H - (NH\text{-}((CH)_2O)_2\text{-}(CH_2)_2\text{-}NH_2)]^+$. HRMS (DCI+) calcd for $C_{51}H_{53}N_6O$ 828.3999, found 829.4048.

4-[Bis(4-formylphenyl)amino]-2-hydroxybenzaldehyde (32). To a suspension of $AlCl_3$ (575 mg, 4.31 mmol, 5 equiv) in dry CH_2Cl_2 (10 mL) at −10 °C was added a solution of **17** (310 mg, 860 μmol) in CH_2Cl_2 (5 mL) dropwise. The mixture was stirred at reflux overnight then poured into an ice/water mixture and vigorously stirred for 10 min. The mixture was extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated to afford a crude solid that was triturated in *n*-hexane to give **32** as a yellow powder (260 mg, 754 μmol) in 87% yield.

Mp 242−244 °C. ^{1}H NMR ($CDCl_3$) δ 6.64 (d, J = 1.8 Hz, 1H), 6.71 (dd, J = 1.8, 8.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 4H), 7.49 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 4H), 9.78 (s, 1H), 9.97 (s, 2H), 11.33 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 110.9, 115.0, 117.1, 125.4, 131.5, 133.1, 135.1, 150.8, 153.3, 163.4, 190.5, 194.3. HRMS (DCI+) calcd for $C_{21}H_{16}NO_4$ 346.1079, found 346.1088.

2-(8-Bromooctyloxy)-4-[*N*,*N*-bis(4-formylphenyl)]aminobenzaldehyde (33). Compound **32** (100 mg, 290 μmol), 1,8-dibromooctane (800 μL, 1.18 g, 4.34 mmol, 15 equiv), and K_2CO_3 (100 mg, 723 μmol, 2.5 equiv) were dissolved in dry acetone (10 mL). The white suspension was stirred at 45 °C for 24 h. Diethyl ether was added and the mixture filtered through a Celite pad. After concentration, the residue was purified by column chromatography (CH_2Cl_2/*n*-hexane 1:1 to 4:1 then CH_2Cl_2 and CH_2Cl_2/MeOH 99.5:0.5). The title compound was isolated as a yellow solid (130 mg, 242 μmol) in 84% yield.

Mp 80 °C. IR (KBr pellet) 2852 (ν_{C-H}, formyl), 2738 (ν_{C-H}, formyl), 1696 ($\nu_{C=O}$), 1588 (Ar), 1505 (Ar) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.30−1.55 (br, 8H), 1.79 (quint, J = 6.6 Hz, 2H), 1.88 (quint, J = 6.6 Hz, 2H), 3.43 (t, J = 6.6 Hz, 2H), 3.91 (t, J = 6.6 Hz, 2H), 6.68 (d, J = 1.8 Hz, 1H), 6.74 (dd, J = 1.8, 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 4H), 7.82 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.4 Hz, 4H), 9.98 (s, 2H), 10.42 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.9, 28.0, 28.6, 28.9, 29.1, 32.7, 33.9, 68.7, 108.2, 116.9, 121.7, 124.5, 130.0, 131.4, 132.6, 151.2, 152.5, 162.7, 188.1, 190.5. HRMS (DCI+) calcd for $C_{29}H_{31}{}^{79}BrNO_4$ 536.1436, found 536.1437. HRMS (DCI+) calcd for $C_{29}H_{31}{}^{81}BrNO_4$ 538.1421, found 538.1434.

Ethyl 4-{5-[Bis(4-formylphenyl)amino]-2-formylphenoxy}butanoate (34). To a solution of **32** (280 mg, 810 μmol) in dry DMF (12 mL) was added K_2CO_3 (1.1 g, 8.0 mmol, 10 equiv). The reaction was stirred for 15 min and ethyl bromobutanoate (175 μL, 1.22 mmol, 1.5 equiv) was added slowly. The mixture was stirred 24 h at rt and concentrated. The residue was redissolved in CH_2Cl_2 and water and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated to afford a crude solid purified by silica gel column chromatography (elution with a MeOH gradient 0 to 1% in CH_2Cl_2) to afford the product as an orange powder (275 mg, 530 μmol) in 75% yield.

Mp 80 °C. ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 6.6 Hz, 3H), 2.12 (quint, J = 6.6 Hz, 2H), 2.50 (t, J = 6.6 Hz, 2H), 3.96 (t, J = 6.6 Hz, 2H), 4.11 (q, 6.6 Hz, 2H), 6.69 (br, 1H), 6.73 (br, d, J = 8.4 Hz, 1H), 7.25 (br, dd, J = 8.7 Hz, 4H), 7.77 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 8.7 Hz, 4H), 9.93 (s, 2H), 10.35 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 14.2, 24.2, 30.5, 60.6, 67.5, 108.1, 117.0, 121.7, 122.8, 124.6, 130.1, 131.4, 132.6, 151.1, 152.5, 162.3, 172.8, 187.9, 190.5. HRMS (ESI+) calcd for $C_{27}H_{25}NaNO_6$ 482.1580, found 482.1563.

3-[8-(2,5-Dioxo-1-aza)cyclopent-3-enyl]octyloxy- 4-[(*E*)-2-(benzothiazol-2-yl)vinyl]-*N*,*N*-(bis{4-[(*E*)-2-(benzothiazol-2-yl)]-vinyl}phenyl)aniline (36). Compound **35** (24 mg, 25.8 μmol), *rac*-7-oxabicyclo[2.2.1]heptene-2,3-dicarboxylic imide (5 mg, 28.4 μmol, 1.1 equiv), and K_2CO_3 (18 mg, 129 μmol, 5 equiv) were dissolved in dry DMF (500 μL). The resulting suspension was stirred at 55 °C overnight. The reaction course was monitored by TLC. Once starting material disappearance was noticed, the crude mixture was diluted in CH_2Cl_2 and washed with water. The combined organic phases were dried and concentrated. The residue was redissolved in anisole (5 mL) and refluxed 2 h. Anisole was vacuum distilled and the residue was purified by preparative TLC (CH_2Cl_2/MeOH, 400:3, v/v) repeatedly, until separation. The title compound was isolated as an orange powder (11 mg, 12 μmol) in 47% yield.

Mp 80 °C. IR (KBr pellet) 1508 (Ar), 1590 (Ar), 1702 ($\nu_{C=O}$) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.30−1.60 (br, 10H), 1.85 (quint, 2H), 3.52 (t, J = 7.2 Hz, 2H), 3.93 (t, J = 6.3 Hz, 2H), 6.67 (s, 4H), 6.72 (d, J = 1.8 Hz, 1H), 6.77 (dd, J = 1.8 Hz, 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 4H), 7.33−7.60 (m, 16 H), 7.84 (d, J = 16.2 Hz, 1H), 7.90 (m, 3H), 8.01 (d, J = 7.6 Hz, 1H), 8.03 (d, J = 7.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 26.1, 26.6, 28.5, 29.0, 29.1, 29.2, 37.9, 68.6, 108.0, 116.9, 120.4, 121.0, 121.4, 121.5, 122.7, 122.9, 124.4, 125.0, 125.2, 126.3, 126.4, 128.7, 128.9, 130.6, 132.7, 134.0, 134.4 (2C), 136.8, 147.6, 148.9, 153.9, 154.0, 158.1, 167.3, 168.6, 171.0. HRMS (FAB+) calcd for $C_{57}H_{47}N_5O_3S_3$ 946.2919, found 946.2884.

4-{5-[Bis(4-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]phenyl)amino]-2-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]phenoxy}butanoic Acid (38). A mixture of ester **37** (60 mg, 0.070 mmol) in THF (2 mL) and a saturated aqueous LiOH solution (2 mL) was stirred overnight at rt. The reaction was acidified to pH 2 and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated to afford a crude oil. Purification by silica gel column chromatography (CH_2Cl_2/MeOH, 98:2, v/v) afforded the product as an orange powder (52 mg, 63 μmol) in 90% yield.

Mp 146−149 °C. ^{1}H NMR ($CDCl_3$) δ 2.32 (br, 2H), 2.59 (br, 2H), 4.01 (t, J = 6.0 Hz, 2H), 6.67 (d, J = 1.8 Hz, 1H), 6.75 (dd, J = 1.8 Hz, 8.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 4H), 7.30−7.65 (m, 16H), 7.86−7.91 (m, 3H), 8.01−8.04 (m, 3H), 8.14 (d, J = 15.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 25.0, 32.0, 68.6, 107.5, 116.9, 119.4, 120.1, 121.0, 121.5, 122.2, 122.8, 124.6, 125.0, 125.2, 126.5, 128.5, 128.7, 130.9, 132.8, 134.1, 134.3, 137.0, 147.5, 149.0, 153.1, 153.9, 158.1, 167.2, 168.7. HRMS (FAB+) calcd for $C_{49}H_{36}N_4O_3S_3$ 825.1980, found 825.2030.

Succinimidyl 4-{5-[Bis(4-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]-phenyl)amino]-2-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]phenoxy}-butanoate (39). A solution of compound **38** (40 mg, 48.5 μmol, 1 equiv), *N*-hydroxysuccinimide (9 mg, 78 μmol, 1.6 equiv), and DCC (11 mg, 56 μmol, 1.1 equiv) in dry CH_2Cl_2 was stirred for 24 h. The cloudy suspension was filtered, concentrated, and redissolved in CH_2Cl_2. The procedure was repeated five times to afford the title compound as a yellow powder (25 mg, 27 μmol) in 66% yield.

Mp 80 °C. ^{1}H NMR ($CDCl_3$) δ 2.34 (quint, J = 6.3 Hz, 2H), 2.83 (s, 4H), 2.93 (t, J = 6.3 Hz, 2H), 4.06 (t, J = 6.0 Hz, 2H), 6.73 (d, J = 1.8 Hz, 1H), 6.80 (dd, J = 1.8 Hz, 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 4H), 7.35−7.65 (m, 16H), 7.83 (d, J = 16.5 Hz,

1H), 7.90 (m, 3H), 8.00 (d, $J = 8.4$ Hz, 2H), 8.02 (d, $J = 8.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 22.7, 25.6 (3C), 66.8, 108.0, 117.3, 120.2, 121.0, 121.4, 121.5, 122.7, 122.8, 124.6, 125.1, 125.3, 126.2, 126.4, 128.5, 128.7, 130.8, 132.3 134.3, 138.9, 147.6, 148.8, 153.9, 154.0, 157.4, 167.2, 168.2, 168.3, 169.0. HRMS (FAB+) calcd for $C_{53}H_{39}N_5O_5S_3$ 921.2113, found 921.2099.

Optical Measurements. Fluorescence spectra were recorded at a temperature maintained between 19.9 and 20.5 °C with a thermostated cell holder. The solvents were of spectrophotometric grade. Measurements were performed with solutions of OD ≤ 0.1 to avoid reabsorption of the emitted light, and data were corrected with a blank sample and from the variations of the detector with the emitted wavelength.

Measurements of Fluorescence Quantum Yields. Fluorescence quantum yields were measured with use of quinine bisulfate in H_2SO_4 (1 N) as a reference. The quantum yield was calculated with eq 1, where the indices *s* and *u* stand for the standard and the

$$\phi_u = \frac{(1 - 10^{-A_s})F_u n^2}{(1 - 10^{-A_u})F_s n_0^2}\phi_s \quad (1)$$

compound to measure, respectively. *F* represents the area of the fluorescence spectrum, *A* the absorbance of the solution, and *n* and n_0 the refraction indices of the solvents in which the compound and the standard were dissolved. Experimental errors can be estimated to ±10%.

Measurements of Two-Photon Absorption Cross Sections. Two-photon induced fluorescence (TPIF) measurements were performed with a mode-locked Ti−sapphire laser delivering 90 fs pulses with a 76 MHz repetition rate, with fluorescein as reference. The experimental setup used was adapted from the experiment described already.[4] The Ti−Saph excitation beam was focused in a 1 cm long and 2 mm wide cuvette filled with moderately concentrated solutions, with use of a 20 mm focal length lens. The signal was collected at an angle of 90° from the excitation source with use of a high NA lens: a spectrometer was used for detailed study of the emission spectra. The laser beam was linearly polarized and a set of half-wave plates and polarizers were used to vary the fundamental beam intensity. Excitation spectra were determined from measurements of the whole emitted light with use of an amplified photodiode equipped with filters cutting the fundamental beam. For each dye, the quadratic dependence of the signal with the pump beam was checked.

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Supporting Information Available: Experimental details and analytical and spectroscopic data for compounds **2**, **6**, **7**, **11**, **12**, **14**, **20**, **23**−**26**, **27c**, **28c**, **35**, **37**, and **40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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