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# Oligo(phenylene ethynylene) Glucosides: Modulation of Cellular Uptake Capacity Preserving Light ON

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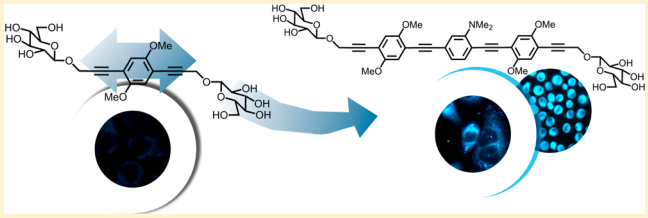
Supporting Information

**ABSTRACT:** A new family of oligo(phenylene ethynylene) (OPE) glucosides has been prepared and characterized. Our results demonstrate that fine-tuning of their photophysical properties can be obtained by acting on the electronics of the core and molecular skeleton. Modulation of the hydrophobic chain length and substituents on the central moieties influences the bioaffinity too. In particular, introducing a $NMe_2$ group on the aromatic central core affords a highly efficient biocompatible fluorescent probe that can be taken up in cytoplasmic vesicles of HEp-2 cells (cells from epidermoid carcinoma larynx tissue). The photophysical behavior, high quantum yield, and stability open the way to the use of the OPE family as stains for cellular imaging analysis by fluorescence microscopy.



## INTRODUCTION

Oligo- and poly(phenylene ethynylene)s (OPEs and PPEs, respectively) represent peculiar classes of luminescent dyes with stable, $\pi$-conjugated, rigid rodlike skeletons.[1] Their photophysical properties are directly connected to their extensive conjugation. In particular, Yamaguchi and Che[2] reported modulation of the photophysical properties of OPEs in dependence on the structure and substitution of the aromatic conjugated system. In the last decades, thanks to their tunable functional properties, OPEs and PPEs have found a great variety of applications, ranging from sensing[3] and electronics[4] to the biological field.[5] OPE-type molecular rods attached to gold electrodes have been created for the development of nanoscale-based molecular circuits with the finding of a direct correlation between molecular structural features and conductance.[4a] Coordination of cationic systems to some PPE polyethers caused their self-aggregation, with a resulting variation or quenching of their optical response.[3b] As a result of the production of singlet $O_2$ upon irradiation with light, some kinds of end-only cationic OPEs[5a,b] were shown to kill specific kinds of bacteria. Moreover, sugar-functionalized oligomers act as inhibitors of *Pseudomonas aeruginosa* lectin LecA[6] and are used as luminescent labeling probes for proteins.[2b] Finally, PPEs can specifically bind and consequently detect *Escherichia coli*[7] and represent efficient probes for lectin concanavaline A.[8]

Imaging represents one of the most efficient techniques for medical diagnosis and therapy. Imaging based on the use of fluorescent probes allows the interpretation of biological processes at the molecular and cellular levels.[9] There are several characteristics that a fluorescent molecule must have to be used as probe in medical imaging, such as optimal excitation and emission wavelengths, strong brightness, bio- and photostability, and the capacity of maintaining the pharmacokinetics without alteration. Usually, when small fluorophores are conjugated with a sugar or an amino acid, this last characteristic is satisfied.[9b]

Herein we describe the synthesis of end-only glucose-functionalized OPEs where the different substitution of the central core and the length modulation allow the tuning of their photophysical properties and the carbohydrate decoration guarantees their biocompatibility.[10] Furthermore, the balanced contribution of the hydrophilic (sugar) and hydrophobic (aryl-conjugated system) moieties gives rise to the permeation of some of these OPEs to the cellular membrane, as shown by preliminary biological experiments, disclosing their potential use as dyes in fluorescent-imaging microscopy.

## RESULTS AND DISCUSSION

**Synthesis.** A Pd(0)-mediated coupling was chosen as the key step in the syntheses of all the new OPE glucosides described in this work. In particular, the copper-free Sonogashira reaction of **1** and **2**[11] in a suitable molecular ratio under optimized reaction conditions led to compound **3**, which was ready for another derivatization on the residual

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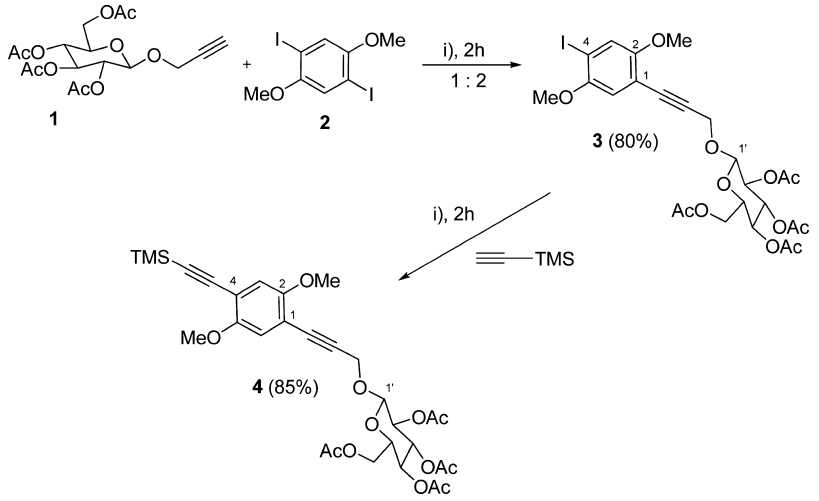
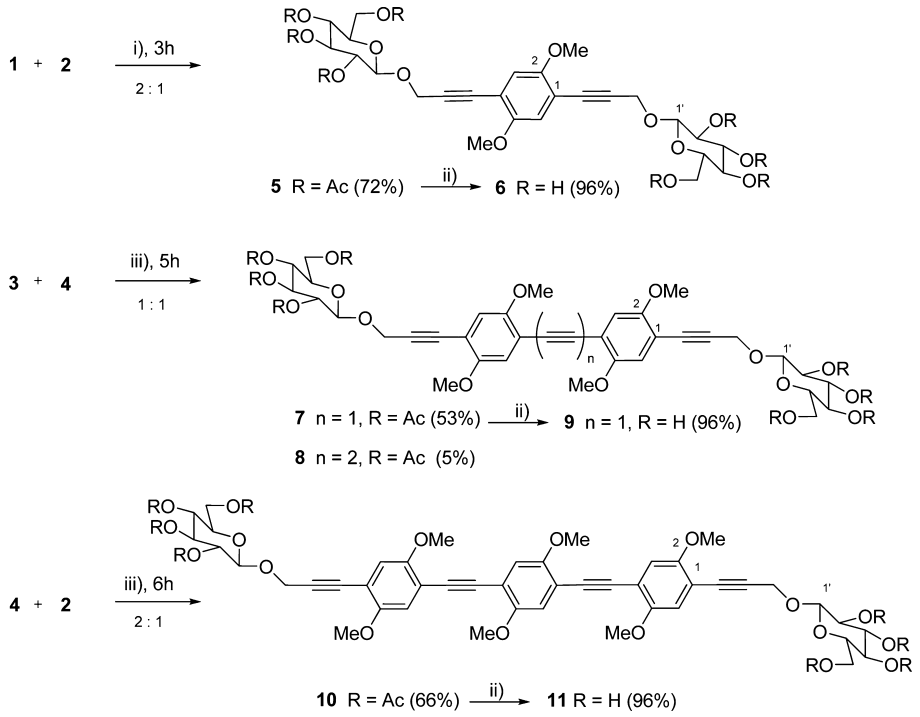
**Scheme 1. Synthetic Route to General Precursors 3 and 4**[a]




[a]Reagents and conditions: (i) $[Pd(PPh_3)_4]$, $NEt_3$, DMF, 60 °C.

**Scheme 2. Synthetic Route to Differently Elongated Oligomers 5−11**[a]




[a]Reagents and conditions: (i) $[Pd(PPh_3)_4]$, $NEt_3$, DMF, 60 °C; (ii) $NH_4OH$, MeOH/THF, rt; (iii) $[Pd(PPh_3)_4]$, $Ag_2O$, DMF/THF, 60 °C.

unreacted arm (Scheme 1). An additional coupling of **3** with an excess of trimethylsilylacetylene quantitatively furnished compound **4**. Compounds **3** and **4** were used as building blocks for subsequent synthetic developments.

Coupling of compounds **1** and **2** with an inverted molecular ratio with respect to the one used for the synthesis of **3** provided a high yield of the shortest phenylene ethynylene D-glucopyranoside, **5** (Scheme 2), together with a small amount of **3**, which was easily separable by column chromatography. A modified cross-coupling reaction, adopting $Ag_2O$ to generate in situ an ethynyl reactive arm,[12] was employed to extend the conjugated chain. Reactions of **4** with **3** and **2** led to the differently elongated oligomers **7** and **10** with two and three conjugated ethynylarene systems, respectively (Scheme 2). In every case, compounds **5**, **7**, and **10** were the major reaction products. In the coupling of **3** with **4**, a small amount of compound **8** was obtained too. Even though **8** can be considered as a side product of the coupling, it was easily separated from **7** and isolated, and its photophysical properties were determined (see the Supporting Information).

Compounds **18**, **19**, and **21**, analogues of **10**, were synthesized following similar strategies (Scheme 3), with the aim of inserting new functions on the aromatic core that could increase the biocompatibility of our OPEs. The central aromatic "bricks" **16**, bearing an electron-donating $NMe_2$ group, and **17**, with both $NMe_2$ and electron-withdrawing $NO_2$ groups, were efficiently prepared starting from commercially available 2,4-dibromoaniline (**12**) and its *p*-nitro derivative (**13**).[13a]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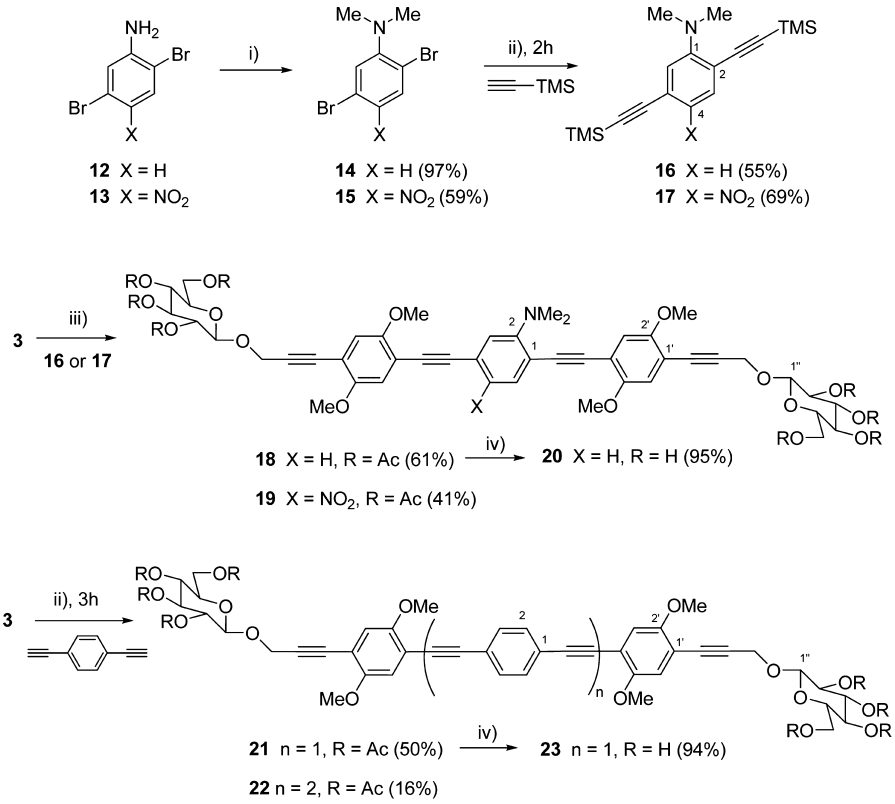
**Scheme 3. Synthetic Route to Differently Substituted Central Cores 16 and 17 and Oligomers 18–23**[a]




[a]Reagents and conditions: (i) MeI, $K_2CO_3$, DMF, 100°C; (ii) $[Pd(PPh_3)_4]$, $NEt_3$, DMF, 60°C; (iii) $[Pd(PPh_3)_4]$, $Ag_2O$, DMF/THF, 60°C; (iv) $NH_4OH$, MeOH/THF, rt.

Methylation of the aromatic nitrogens of **12** and **13** with MeI in the presence of $K_2CO_3$ gave **14**[13b] and **15**, respectively, whose copper-free Sonogashira coupling with an excess of ethynyltrimethylsilane furnished **16** and **17**, respectively, in excellent quantities. The syntheses of compound **18** and **19** were finally reached by the use of the above cited $Ag_2O$-modified Pd(0)-mediated reaction. The cross-coupling of **4** with **14** or **15** was also attempted as an alternative synthetic pathway to **18** or **19**, but poor yields of the target compounds (<20%) confirmed the low reactivity of the dibromoaromatic system involved in cross-coupling reactions.

Glucoside **21** with an unsubstituted aromatic core was prepared in good yield (50%) by the reaction of **3** with commercially available 1,4-diethynylbenzene in a copper-free Sonogashira fashion. In this last reaction, coupling of **3** with two queued central 1,4-diethynylbenzene moieties furnished **22** as minor product (**21**/**22** = 4:1) that was easily separated for photophysical studies (see the Supporting Information).

The protected glucosides were then subjected to deacetylation. Deprotection of compounds **5**, **7**, **10**, **18**, and **21** easily happened in the presence of aqueous ammonia, after one night of stirring in THF/MeOH at rt, giving quantitatively **6**, **9**, **11**, **20**, and **23**, respectively. The same reaction was not attempted on **19** because of its easy decomposition even under refrigerated and degassed conditions. Compounds **18** and **20** bearing an $NMe_2$ substituent are very stable and easy to handle, in spite of what has been reported elsewhere.[2c]

**Photophysical Properties.** When dissolved in solvent/nonsolvent mixtures,[14] most OPEs form aggregates and excimers, which are usually detected by absorption and fluorescence spectroscopies. In our case, however, the concentration used was low enough to avoid these features: concentration dependence was observed only when the concentration was higher than $10^{-4}$ M and in mixed solvents.[15]

The absorption spectra of all the investigated species in DCM solution (for the protected compounds **5**, **7**, **8**, **10**, **18**, **19**, **21**, and **22**) as well as in aqueous solution (buffer phosphate for the deprotected compounds **6**, **9**, **11**, **20**, and **23**), are characterized by intense absorption in the UV region ($\varepsilon$ in the $10^4$–$10^5$ $M^{-1}$ $cm^{-1}$ range) due to spin-allowed $\pi$–$\pi^*$ transitions. Increasing the chain length registered a red shift of the absorption maxima (see Table 1, Figure 1, and the Supporting Information); this behavior is consistent with greater $\pi$ conjugation in passing from **5** to **10**. The molar extinction coefficient ($\varepsilon$) of the lowest-energy transition also increases with the same trend. In the absorption spectrum of **5**

**Table 1. Spectroscopic and Photophysical Data**[a]

| | absorption[b] | luminescence[c] | | |
|---|---|---|---|---|
| compd | $\lambda_{max}$/nm ($\varepsilon$/$M^{-1}$ $cm^{-1}$) | $\lambda_{max}$/nm | Φ | τ/ns |
| **5**/**6** | 344 (11000) | 383 | 0.45 | 0.72 |
| **7**/**9** | 378 (35000) | 404 | 0.87 | 1.54 |
| **10**/**11** | 390 (49500) | 433 | 0.85 | 0.98 |
| **18**/**20** | 388 (38900) | 472 | 0.57 | 2.47 |
| **19** | 381 (38000) | – | – | – |
| **21**/**23** | 377 (39500) | 437 | 0.82 | 0.76 |

[a]The absorption and the room-temperature emission data were obtained in DCM; no significant changes were observed in the DMSO/water mixture. [b]Maxima (or shoulders) of the lower-energy bands are given. [c]At 298 K.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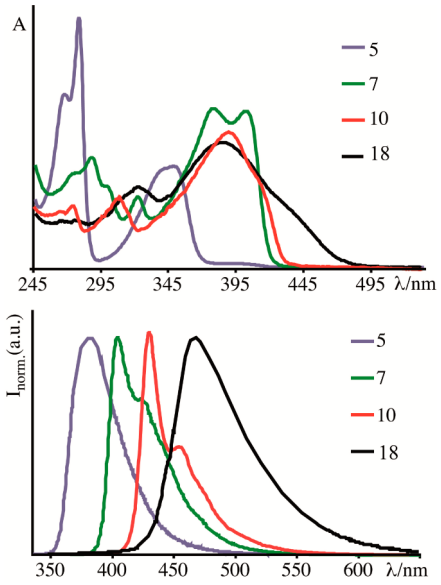


**Figure 1.** (top) Absorption and (bottom) emission spectra of representative species in DCM at rt.

in DCM, additional vibronically resolved absorption bands at $\lambda_{max}$ = 275 and 290 nm are observed. These high-energy absorption features become broad and less resolved as the conjugation increases.

Excitation of compounds **5**−**11**, **18**, and **20**−**23** in the range 280−420 nm at room temperature produces a blue or blue-green emission. The excited-state lifetimes ($\tau$) for all of the investigated species are on the nanosecond time scale. Relevant data are collected in Table 1, whereas representative emission spectra are shown in Figure 1.

As the chain length is increased in moving from **5** to **7**, the emission energy decreases, whereas the emission quantum yield ($\Phi$) doubles (see Figure 1 and Table 1). This is reminiscent of the bathochromic shift in the corresponding absorption spectra. The augmented $\pi$ conjugation between the peripheral subunits in **10** and **21** causes a further decrease in both the luminescence excited-state energy and the quantum yield.

The insertion of one electron-donating $NMe_2$ group on the central subunit of the oligomer **18** shifts the luminescence to the red, while the quantum yield decreases but still remains at the high value of 0.57. The unstructured large emission profile suggests that in this case the excited state has a partial charge transfer character that could be tentatively attributed to n → $\pi^*$ transitions. The absence of luminescence in **19** can be ascribed to the presence of the electron-withdrawing $NO_2$ group, which acts as a quencher for these systems.[2c] It is important to stress that no changes in the photophysical properties were observed in going from DCM to aqueous solution.

**Biological Data.** To investigate the ability of these new dyes to achieve cell internalization, we performed uptake experiments for **6**, **9**, **11**, **18**, **20**, and **23** on HEp-2 cells (cells from epidermoid carcinoma larynx tissue). The shortest glucoside, **6**, is slightly internalized in HEp-2 cells (see the Supporting Information), while OPE glucosides **9**, **11**, and **23** were not taken up (data not shown). Figure 2 displays the massive cell internalization and efficient enlightening of glucoside **20**, showing the main localization in vesicles within the cytoplasmic compartment.

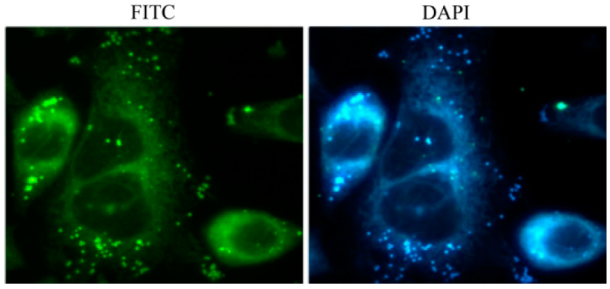


**Figure 2.** Fluorescence microscopy images of Hep-2 cells incubated with **20** (100 $\mu$M) and analyzed using a FITC (green emission) or DAPI (blue emission) filter.

The great luminescence quantum yield and the high degree of endocytosis of **20** permit the detection of fluorescence emission even when cells are incubated at low concentrations of up to 1 $\mu$M (Figure 3). The more lipophilic OPE glucoside **18**, the acetylated analogue of **20**, is slightly internalized by Hep-2 cells (see the Supporting Information).

Finally, the analysis of cell viability, evaluated using the trypan blue assay, showed that these compounds are biocompatible since they had no toxic effects for HEp-2 cells at all concentrations tested for the time of exposure used (48 h).

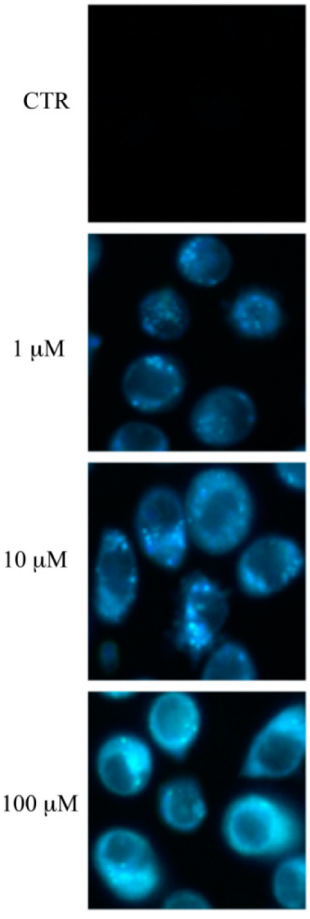


**Figure 3.** Fluorescence microscopy images (DAPI filter) of Hep-2 cells incubated for 24 h with solutions of **20** with final concentrations of 1, 10, and 100 $\mu$M. The image labeled CTR shows the untreated cells.


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## CONCLUSIONS

We have efficiently synthesized a series of new OPE glucosides by exploiting simple synthetic routes involving a Pd(0)-catalyzed cross-coupling as a key step. The biological behavior of such an OPE family is directly related to their structural features, which on the other hand guarantee the observed photophysical properties. The synergic effect of (i) elongation of the hydrophobic chain in going from glucoside **6** to **20**, (ii) substitution of the aromatic central core with a dimethylamino group in **20** instead of the bis(methoxy) moiety present in glucoside **11**, and (iii) deacetylation of the sugar moieties to improve the hydrophilicity of **20** with respect to glucoside **18** creates the right combination for **20** to serve as a highly efficient biocompatible fluorescent cell probe. The broad emission, biocompatibility, high quantum yield, and stability open the way to the application of OPE glucosides as dyes in fluorescence microscopy. Further studies to assess the applicability of OPEs in imaging techniques for sensitive cancer detection are in progress.

## EXPERIMENTAL SECTION

**General Experimental Methods.** Solvents were purified according to standard procedures. All of the reactions were monitored by TLC on commercially available precoated plates (silica gel 60 F254), and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. $H_2SO_4$ (0.6 mL)]. Silica gel 60 was used for column chromatography. $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ solutions, unless differently stated, at 500 and 125 MHz, respectively; coupling constants ($J$) are given in hertz, and the attributions are supported by heteronuclear single-quantum coherence (HSQC) and correlation spectroscopy (COSY) experiments.

**Equipment and Methods for the Absorption Spectroscopy and Photophysical Experiments.** The absorption spectra were recorded in ultrapure spectroscopic solvents. All of the emission spectra were corrected for the photomultiplier response using software purchased with the fluorimeter. For the luminescence lifetimes, a time-correlated single-photon-counting spectrometer was used. As the excitation source, a laser diode (59 ps pulse width at 408 nm) or a nitrogen discharge lamp (2 ns pulse width at 337 nm) was employed. Emission quantum yields for deaerated acetonitrile solutions were determined using the optical dilution method.[16a] As a luminescence quantum yield standard we used an air-equilibrated ethanol solution of anthracene ($\Phi$ = 0.2).[16b]

Experimental uncertainties in the absorption and photophysical data are as follows: absorption maxima, 2 nm; molar absorption, 15%; luminescence maxima, 4 nm; luminescence lifetimes, 10%; luminescence quantum yields, 20%.

**General Procedure A for the Preparation of Compounds 3–5, 16, 17, 21, and 22 by a Coupling Reaction in the Presence of $Pd(PPh_3)_4$ and $Et_3N$.** $Pd(PPh_3)_4$, the halogenoarene, and the alkyne were dissolved in dry DMF. To this mixture was added $Et_3N$ under an Ar atmosphere with stirring. The mixture was heated at 60 °C and maintained under continuous stirring until the disappearance of the limiting reagent as determined by TLC. Solvents were removed under reduced pressure. The reaction crude was subjected to silica gel column chromatography.

**General Procedure B for the Preparation of Compounds 6, 9, 11, 20, and 23.** The starting material (0.2 mmol) was dissolved in 1:1 THF/MeOH (40 mL). To this mixture was added a large excess of aqueous ammonia (12 mL), and the reaction mixture was then maintained under continuous stirring at rt overnight until the disappearance of the starting material as determined by TLC. Solvents were removed under reduced pressure, and the undesired acetamide was eliminated by a series of MeOH washings of the obtained solid.

**General Procedure C for the Preparation of Compounds 7, 8, 10, 18, and 19 by a Coupling Reaction in the Presence of $Pd(PPh_3)_4$ and $Ag_2O$.** $Pd(PPh_3)_4$, $Ag_2O$, the halogenoarene, and the trimethylsilylethynylarene were dissolved in dry DMF and THF. The mixture was heated at 60 °C and maintained under an Ar atmosphere with continuous stirring until the disappearance of the limiting reagent as determined by TLC. After filtration over Celite, the solvents were removed under reduced pressure, and the obtained reaction crude was subjected to silica gel column chromatography.

**Compound 3.** This compound was obtained in 2 h following general procedure A starting from prop-2-yn-1-yl β-D-glucopyranoside-2,3,4,6-tetraacetate (**1**) (1.42 g, 3.67 mmol, 1 equiv), 1,4-diiodo-2,5-dimethoxybenzene (**2**) (3.00 g, 7.69 mmol, 2.1 equiv), and $Pd(PPh_3)_4$ (0.50 g, 0.43 mmol, 0.12 equiv) in dry DMF (30 mL) and $Et_3N$ (30 mL). Column chromatography was performed with 70:30 hexane/EtOAc as the eluent, and compound **3** was obtained as a white solid (1.90 g, 2.93 mmol, 80%). TLC: $R_f$ 0.64 (40:60 hexane/EtOAc). Mp: 65–67 °C. $^1H$ NMR: $\delta$ 7.29 (s, 1H, H-3), 6.84 (s, 1H, H-6), 5.25 (t, $J_{2',3'} = J_{3',4'}$ = 9.3, 1H, H-3′), 5.11 (t, $J_{3',4'} = J_{4',5'}$ = 9.3, 1H, H-4′), 5.03 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 9.3, 1H, H-2′), 4.88 (d, $J_{1',2'}$ = 7.7, 1H, H-1′), 4.61 (s, 2H, $CH_2C{\equiv}$), 4.27 and 4.15 (split AB system, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.5, $J_{6'A,6'B}$ = 12.2, 2H, $H_2$-6′), 3.84 and 3.83 (two s, 6H, 2 × $OCH_3$), 3.75 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.5, 1H, H-5′), 2.07, 2.03, 2.02, and 2.00 (four s, 12H, 4 × $CH_3CO$). $^{13}C$ NMR: $\delta$ 170.7, 170.3, and 169.4 (4 × CO), 154.8 (C-2), 152.3 (C-5), 122.3 (C-3), 115.1 (C-6), 111.9 (C-1), 98.3 (C-1′), 88.4 (C-4), 87.3 and 85.4 (C≡C), 72.8 (C-3′), 71.9 (C-5′), 71.1 (C-2′), 68.4 (C-4′), 61.8 (C-6′), 57.2, 57.0, and 56.5 (2 × $OCH_3$ and $CH_2C{\equiv}$), 20.7 and 20.6 (4 × $CH_3CO$). Anal. Calcd for $C_{25}H_{29}IO_{12}$ (648.40): C, 46.31; H, 4.51. Found: C, 46.44; H, 4.50.

**Compound 4.** This compound was obtained in 2 h following general procedure A starting from **3** (1.00 g, 1.54 mmol, 1 equiv), commercial ethynyltrimethylsilane (0.65 mL, 4.62 mmol, 3 equiv), and $Pd(PPh_3)_4$ (0.18 g, 0.16 mmol, 0.1 equiv) in dry DMF (7 mL) and $Et_3N$ (7 mL). Column chromatography was performed with 80:20 hexane/EtOAc as the eluent, and compound **4** was obtained as a white solid (0.81 g, 1.31 mmol, 85%). TLC: $R_f$ 0.78 (40:60 hexane/EtOAc). Mp: 59–61 °C. $^1H$ NMR: $\delta$ 6.95 and 6.89 (two s, 2H, H-3,6), 5.25 (t, $J_{2',3'} = J_{3',4'}$ = 9.4, 1H, H-3′), 5.10 (t, $J_{3',4'} = J_{4',5'}$ = 9.4, 1H, H-4′), 5.03 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 9.4, 1H, H-2′), 4.88 (d, $J_{1',2'}$ = 7.7, 1H, H-1′), 4.62 (s, 2H, $CH_2C{\equiv}$), 4.28 and 4.15 (split AB system, $J_{5',6'A}$ = 4.7, $J_{5',6'B}$ = 2.4, $J_{6'A,6'B}$ = 12.2, 2H, $H_2$-6′), 3.85 and 3.84 (two s, 6H, 2 × $OCH_3$), 3.74 (ddd, $J_{4',5'}$ = 10.0, $J_{5',6'A}$ = 4.7, $J_{5',6'B}$ = 2.5, 1H, H-5′), 2.07, 2.04, 2.03, and 2.01 (four s, 12H, 4 × $CH_3CO$), 0.27 [s, 9H, $Si(CH_3)_3$]. $^{13}C$ NMR: $\delta$ 170.7, 170.3, 169.4, and 169.3 (4 × CO), 154.2 and 153.9 (C-2,5), 116.1 and 115.8 (C-3,6), 113.7 and 112.4 (C-1,4), 100.7 and 100.6 (C≡CSi), 98.2 (C-1′), 89.0 and 83.4 ($CH_2C{\equiv}C$), 72.8 (C-3′), 71.9 (C-5′), 71.1 (C-2′), 68.3 (C-4′), 61.8 (C-6′), 57.0 ($CH_2C{\equiv}$), 56.5 and 56.3 (2 × $OCH_3$), 20.7, 20.6, and 20.5 (4 × $CH_3CO$), −0.05 [$Si(CH_3)_3$]. Anal. Calcd for $C_{30}H_{38}O_{12}Si$ (618.70): C, 58.24; H, 6.19. Found: C, 58.30; H, 6.20.

**Compound 5.** This compound was obtained in 3 h following general procedure A starting from prop-2-yn-1-yl β-D-glucopyranoside-2,3,4,6-tetraacetate (**1**) (1.00 g, 2.59 mmol, 2.1 equiv), 1,4-diiodo-2,5-dimethoxybenzene (**2**) (0.48 g, 1.23 mmol, 1 equiv), and $Pd(PPh_3)_4$ (0.17 g, 0.15 mmol, 0.12 equiv) in dry DMF (10 mL) and $Et_3N$ (10 mL). Column chromatography was performed with 65:35 hexane/EtOAc as the eluent, and compound **5** was obtained as a white solid (0.80 g, 0.88 mmol, 72%). TLC: $R_f$ 0.55 (40:60 hexane/EtOAc). Mp: 178–180 °C. $^1H$ NMR: $\delta$ 6.92 (s, 2H, H-3,6), 5.27 (t, $J_{2',3'} = J_{3',4'}$ = 9.3, 2H, 2 × H-3′), 5.12 (t, $J_{3',4'} = J_{4',5'}$ = 9.3, 2H, 2 × H-4′), 5.05 (dd, $J_{1',2'}$ = 8.3, $J_{2',3'}$ = 9.3, 2H, 2 × H-2′), 4.90 (d, $J_{1',2'}$ = 8.3, 2H, 2 × H-1′), 4.64 (s, 4H, 2 × $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A}$ = 5.6, $J_{5',6'B}$ = 2.4, $J_{6'A,6'B}$ = 12.2, 4H, 2 × $H_2$-6′), 3.85 (s, 6H, 2 × $OCH_3$), 3.76 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 5.6, $J_{5',6'B}$ = 2.4, 2H, 2 × H-5′), 2.07, 2.04, 2.02, and 2.00 (four s, 24H, 8 × $CH_3CO$). $^{13}C$ NMR: $\delta$ 170.7, 170.3, 169.5, and 169.4 (8 × CO), 154.0 (C-2,5), 115.7 (C-3,6), 112.8 (C-1,4), 98.3 (2 × C-1′), 89.2 and 83.2 (2 × C≡C), 72.8 (2 × C-3′), 71.9 (2 × C-5′), 711 (2 × C-2′), 68.3 (2 × C-4′), 61.8 (2 × C-6′), 57.0 (2 × $CH_2C{\equiv}$), 56.4 (2 × $OCH_3$), 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{42}H_{50}O_{22}$ (906.83): C, 55.63; H, 5.56. Found: C, 55.49; H, 5.58.

**Compound 6.** This compound was obtained as a white solid (0.13 g, 0.19 mmol, 96%) following general procedure B starting from **5**


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(0.18 g). TLC: $R_f$ 0.15 (80:20 $CHCl_3$/MeOH). Mp: 230−233 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.04 (s, 2H, H-3,6), 5.12 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.96 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.91 (d, $J_{vic}$ = 5.4, 2H, 2 × OH), 4.65 and 4.52 (AB system, $J_{gem}$ = 15.7, 4H, 2 × $CH_2C{\equiv}$), 4.53 (t, $J_{OH,6}$ = 4.9, 2H, 2 × 6′-OH), 4.32 (d, $J_{1',2'}$ = 7.9, 2H, 2 × H-1′), 3.77 (s, 6H, 2 × $OCH_3$), 3.65 and 3.43 (split AB m, 4H, 2 × $H_2$-6′), 3.16−2.93 (m, 8H, 2 × H-2′−5′). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 153.6 (C-2,5), 115.8 (C-3,6), 112.2 (C-1,4), 101.1 (2 × C-1′), 91.1 and 81.9 (2 × C≡C), 77.0 and 76.7 (2 × C-3′,5′), 73.3 (2 × C-2′), 70.0 (2 × C-4′), 61.2 (2 × C-6′), 56.1 (2 × $OCH_3$), 55.8 (2 × $CH_2C{\equiv}$). Anal. Calcd for $C_{26}H_{34}O_{14}$ (570.54): C, 54.73; H, 6.01. Found: C, 54.79; H, 6.00.

**Compounds 7 and 8.** These compounds were obtained in 5 h following general procedure C starting from **3** (0.50 g, 0.77 mmol, 1 equiv), **4** (0.48 g, 0.78 mmol, 1 equiv), $Pd(PPh_3)_4$ (0.13 g, 0.11 mmol, 0.14 equiv), and $Ag_2O$ (0.18 g, 0.78 mmol, 1 equiv) in dry DMF (10 mL) and THF (5 mL). Column chromatography was performed with 50:50 hexane/EtOAc as the eluent, giving first compound **8** as a yellow low-melting solid (42 mg, 0.04 mmol, 5%) and then compound **7** as a yellow solid (0.44 g, 0.41 mmol, 53%).

Data for **7**. TLC: $R_f$ 0.35 (30:70 hexane/EtOAc). Mp: 80−81 °C. $^1$H NMR: $\delta$ 7.04 and 6.94 (two s, 4H, 2 × H-3,6), 5.27 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.3, 2H, 2 × H-3′), 5.12 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.3, 2H, 2 × H-4′), 5.05 (dd, $J_{1',2'}$ = 8.3, $J_{2',3'}$ = 9.3, 2H, 2 × H-2′), 4.92 (d, $J_{1',2'}$ = 8.3, 2H, 2 × H-1′), 4.65 (s, 4H, 2 × $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.4, $J_{6'A,6'B}$ = 12.3, 4H, 2 × $H_2$-6′), 3.90 and 3.88 (two s, 12H, 4 × $OCH_3$), 3.77 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.4, 2H, 2 × H-5′), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.7, 170.3, 169.4, and 169.3 (8 × CO), 154.1 and 153.9 (2 × C-2,5), 115.7 and 115.5 (2 × C-3,6), 113.8 and 112.4 (2 × C-1,4), 98.3 (2 × C-1′), 91.2, 89.1, and 83.4 (3 × C≡C), 72.8 (2 × C-3′), 71.9 (2 × C-5′), 71.1 (2 × C-2′), 68.4 (2 × C-4′), 61.8 (2 × C-6′), 57.0 (2 × $CH_2C{\equiv}$), 56.5 and 56.3 (4 × $OCH_3$), 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{52}H_{58}O_{24}$ (1067.00): C, 58.53; H, 5.48. Found: C, 58.65; H, 5.47.

Data for **8**. TLC: $R_f$ 0.40 (30:70 hexane/EtOAc). $^1$H NMR: $\delta$ 6.98 and 6.92 (two s, 4H, 2 × H-3,6), 5.26 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.3, 2H, 2 × H-3′), 5.12 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.3, 2H, 2 × H-4′), 5.04 (t, $J_{1',2'}$ = $J_{2',3'}$ = 9.3, 2H, 2 × H-2′), 4.90 (d, $J_{1',2'}$ = 9.3, 2H, 2 × H-1′), 4.64 (s, 4H, 2 × $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 1.9, $J_{6'A,6'B}$ = 12.2, 4H, 2 × $H_2$-6′), 3.86 and 3.84 (two s, 12H, 4 × $OCH_3$), 3.76 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 1.9, 2H, 2 × H-5′), 2.08, 2.04, 2.03, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.9, 170.6, 169.7, and 169.6 (8 × CO), 155.6 and 154.2 (2 × C-2,5), 116.3 and 115.9 (2 × C-3,6), 113.6 and 112.6 (2 × C-1,4), 98.5 (2 × C-1′), 90.0, 83.5, 79.7, and 79.6 (4 × C≡C), 73.1 (2 × C-3′), 72.2 (2 × C-5′), 71.4 (2 × C-2′), 68.6 (2 × C-4′), 62.1 (2 × C-6′), 57.3 (2 × $CH_2C{\equiv}$), 56.7 and 56.6 (4 × $OCH_3$), 20.9 and 20.8 (8 × $CH_3CO$). Anal. Calcd for $C_{54}H_{58}O_{24}$ (1091.02): C, 59.45; H, 5.36. Found: C, 59.38; H, 5.34.

**Compound 9.** This compound was obtained as a white solid (0.14 g, 0.19 mmol, 96%) following general procedure B starting from **7** (0.23 g). TLC: $R_f$ 0.10 (80:20 $CHCl_3$/MeOH). Mp: 206−207 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.10 and 7.08 (two s, 4H, 2 × H-3,6), 5.14 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.97 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.91 (d, $J_{vic}$ = 5.4, 2H, 2 × OH), 4.67 and 4.55 (AB system, $J_{gem}$ = 15.6, 4H, 2 × $CH_2C{\equiv}$), 4.54 (t, $J_{OH,6}$ = 4.9, 2H, 2 × 6′-OH), 4.34 (d, $J_{1',2'}$ = 7.8, 2H, 2 × H-1′), 3.81 and 3.80 (two s, 12H, 4 × $OCH_3$), 3.67 and 3.46 (split AB m, 4H, 2 × $H_2$-6′), 3.19−2.98 (m, 8H, 2 × H-2′−5′). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 153.7 and 153.2 (2 × C-2,5), 115.9 and 115.3 (2 × C-3,6), 112.8 and 112.2 (2 × C-1,4), 101.0 (2 × C-1′), 91.3, 91.1, and 81.9 (3 × C≡C), 77.0 and 76.7 (2 × C-3′,5′), 73.3 (2 × C-2′), 70.1 (2 × C-4′), 61.2 (2 × C-6′), 56.3 and 56.1 (4 × $OCH_3$), 55.8 (2 × $CH_2C{\equiv}$). Anal. Calcd for $C_{36}H_{42}O_{16}$ (730.71): C, 59.17; H, 5.79. Found: C, 59.14; H, 5.80.

**Compound 10.** This compound was obtained in 6 h following general procedure C starting from 1,4-diiodo-2,5-dimethoxybenzene **2** (0.50 g, 1.28 mmol, 1 equiv), **4** (1.58 g, 2.55 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.22 g, 0.19 mmol, 0.15 equiv), and $Ag_2O$ (0.59 g, 2.55 mmol, 2 equiv) in dry DMF (10 mL) and THF (5 mL). Column chromatography was performed with 50:50 hexane/EtOAc as the eluent, and compound **10** was obtained as a yellow solid (1.04 g, 0.85 mmol, 66%). TLC: $R_f$ 0.47 (40:60 hexane/EtOAc). Mp: 169−171 °C. $^1$H NMR: $\delta$ 7.06, 7.05, and 6.94 (three s, 6H, 3 × H-3,6), 5.27 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.3, 2H, 2 × H-3′), 5.12 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.3, 2H, 2 × H-4′), 5.05 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 9.3, 2H, 2 × H-2′), 4.91 (d, $J_{1',2'}$ = 7.8, 2H, 2 × H-1′), 4.65 (s, 4H, 2 × $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A}$ = 4.9, $J_{5',6'B}$ = 2.4, $J_{6'A,6'B}$ = 12.3, 4H, 2 × $H_2$-6′), 3.92, 3.90, and 3.88 (three s, 18H, 6 × $OCH_3$), 3.77 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 4.9, $J_{5',6'B}$ = 2.4, 2H, 2 × H-5′), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.7, 170.3, 169.5, and 169.4 (8 × CO), 154.1, 153.9, and 153.8 (3 × C-2,5), 115.7 and 115.5 (3 × C-3,6), 113.8, 113.4, and 112.3 (3 × C-1,4), 98.3 (2 × C-1′), 91.5, 91.2, 89.1, and 83.5 (4 × C≡C), 72.8 (2 × C-3′), 71.9 (2 × C-5′), 71.1 (2 × C-2′), 68.3 (2 × C-4′), 61.8 (2 × C-6′), 57.1 (2 × $CH_2C{\equiv}$), 56.6, 56.5 and 56.3 (6 × $OCH_3$), 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{62}H_{66}O_{26}$ (1227.17): C, 60.68; H, 5.42. Found: C, 60.80; H, 5.43.

**Compound 11.** This compound was obtained as a yellow solid (0.17 g, 0.19 mmol, 96%) following general procedure B starting from **10** (0.25 g). TLC: $R_f$ 0.05 (80:20 $CHCl_3$/MeOH). Mp: 248−250 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.13, 7.12, and 7.09 (three s, 6H, 3 × H-3,6), 5.15 (d, $J_{vic}$ = 5.4, 2H, 2 × OH), 4.98 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.93 (d, $J_{vic}$ = 5.4, 2H, 2 × OH), 4.68 and 4.55 (AB system, $J_{gem}$ = 15.6, 4H, 2 × $CH_2C{\equiv}$), 4.55 (t, $J_{OH,6}$ = 5.5, 2H, 2 × 6′-OH), 4.34 (d, $J_{1',2'}$ = 7.9, 2H, 2 × H-1′), 3.84, 3.82, and 3.80 (three s, 18H, 6 × $OCH_3$), 3.69 and 3.45 (split AB m, 4H, 2 × $H_2$-6′), 3.19−2.98 (m, 8H, 2 × H-2′−5′). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 153.7, 152.4, and 153.3 (3 × C-2,5), 115.9, 115.5, and 115.4 (3 × C-3,6), 112.8, 112.7, and 112.3 (3 × C-1,4), 101.1 (2 × C-1′), 91.3, 91.2, and 82.0 (4 × C≡C), 77.0 and 76.7 (2 × C-3′,5′), 73.3 (2 × C-2′), 70.0 (2 × C-4′), 61.2 (2 × C-6′), 56.3, 56.2, and 56.1 (6 × $OCH_3$), 55.8 (2 × $CH_2C{\equiv}$). Anal. Calcd for $C_{46}H_{50}O_{18}$ (890.88): C, 62.02; H, 5.66. Found: C, 62.05; H, 5.67.

**Compound 14.** A DMF solution (12 mL) of 2,5-dibromoaniline (**12**) (1.00 g, 3.98 mmol, 1 equiv) was added to anhydrous $K_2CO_3$ (5.00 g, 36.18 mmol) at rt under an Ar atmosphere. To the obtained suspension was added 1.2 mL of iodomethane (20 mmol, 5 equiv), and the mixture was heated to 100 °C and maintained under these conditions with continuous stirring until completion of the reaction as determined by TLC. After 48 h, the reaction was quenched by the addition of water (7 mL), and the resulting mixture was extracted with dichloromethane (3 × 10 mL). The organic phases were dried over anhydrous $Na_2SO_4$, and the solvent was evaporated under reduced pressure to give pure 2,5-dibromo-*N*,*N*-dimethylaniline[13b] (**14**) as a transparent oil (1.08 g, 3.87 mmol, 97%). TLC: $R_f$ 0.85 (95:5 hexane/EtOAc). $^1$H NMR: $\delta$ 7.35 (d, 1H, $J_{3,4}$ = 8.8, H-3), 7.14 (d, 1H, $J_{4,6}$ = 2.4, H-6), 6.96 (dd, 1H, $J_{3,4}$ = 8.8, $J_{4,6}$ = 2.4, H-4), 2.76 [s, 6H, $N(CH_3)_2$]. $^{13}$C NMR: $\delta$ 152.8 (C-1), 134.7 (C-3), 126.2 (C-4), 123.5 (C-6), 119.9 and 117.2 (C-4,5), 43.7 [$N(CH_3)_2$]. Anal. Calcd for $C_8H_9Br_2N$ (278.97): C, 34.44; H, 3.25; N, 5.02. Found: C, 34.50; H, 3.26; N, 5.03.

**Compound 15.** A DMF solution (5 mL) of 2,5-dibromo-4-nitroaniline (**13**)[13a] (0.40 g, 1.35 mmol, 1 equiv) was added to anhydrous $K_2CO_3$ (2.38 g, 17.22 mmol) at rt under an Ar atmosphere. To the obtained suspension was added 0.41 mL of iodomethane (6.75 mmol, 5 equiv), and the mixture was heated to 100 °C and maintained under these conditions with continuous stirring until completion of the reaction as determined by TLC. After 70 h, the reaction was quenched by the addition of water (5 mL), and the resulting mixture was extracted with dichloromethane (3 × 10 mL). The organic phases were dried over anhydrous $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The reaction crude was subjected to silica gel column chromatography (90:10 hexane/EtOAc as the eluent), and **15** was isolated as a yellow solid (0.26 g, 0.80 mmol, 59%). TLC: $R_f$ 0.80 (80:20 hexane/EtOAc). Mp: 81−83 °C. $^1$H NMR: $\delta$ 8.23 (s, 1H, H-3), 7.18 (s, 1H, H-6), 2.97 [s, 6H, $N(CH_3)_2$]. $^{13}$C NMR: $\delta$ 155.7 (C-1), 141.8 (C-4), 132.3 (C-3), 124.6 (C-6), 115.3 (C-5), 113.4 (C-2), 43.3 [$N(CH_3)_2$]. Anal. Calcd for $C_8H_8Br_2N_2O_2$ (323.97): C, 29.66; H, 2.49; N, 8.65. Found: C, 29.71; H, 2.50; N, 8.68.

**Compound 16.** This compound was prepared in 2 h following general procedure A starting from **14** (1.00 g, 3.58 mmol, 1 equiv), commercial ethynyltrimethylsilane (3.05 mL, 21.60 mmol, 6 equiv),


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and $Pd(PPh_3)_4$ (0.41 g, 0.35 mmol, 0.1 equiv) in dry DMF (15 mL) and $Et_3N$ (15 mL). Column chromatography was performed with hexane as the eluent, and compound **16** was obtained as a transparent oil (0.62 g, 1.98 mmol, 55%). TLC: $R_f$ 0.67 (90:10 hexane/EtOAc). $^1$H NMR: $\delta$ 7.33 (d, 1H, $J_{3,4}$ = 7.9, H-3), 6.94 (m, 2H, H-4,6), 2.93 [s, 6H, $N(CH_3)_2$], 0.25 [s, 18H, 2 × $Si(CH_3)_3$]. $^{13}$C NMR: $\delta$ 154.7 (C-1), 134.6 (C-3), 123.8 and 120.1 (C-4,6), 115.0 and 105.1 (C-2,5), 104.3, 104.2, 101.4, and 95.3 (2 × C≡C), 43.1 [$N(CH_3)_2$], −0.02 and −0.17 [2 × $Si(CH_3)_3$]. Anal. Calcd for $C_{18}H_{27}NSi_2$ (313.58): C, 68.94; H, 8.68; N, 4.47. Found: C, 68.88; H, 8.70; N, 4.48.

**Compound 17.** This compound was prepared in 2 h following general procedure A starting from **15** (0.25 g, 0.77 mmol, 1 equiv), commercial ethynyltrimethylsilane (0.65 mL, 4.62 mmol, 6 equiv), and $Pd(PPh_3)_4$ (0.09 g, 0.08 mmol, 0.1 equiv) in dry DMF (6 mL) and $Et_3N$ (6 mL). Column chromatography was performed with hexane as the eluent, and compound **17** was obtained as a yellow oil (0.19 g, 0.53 mmol, 69%). TLC: $R_f$ 0.48 (80:20 hexane/EtOAc). $^1$H NMR: $\delta$ 8.19 (s, 1H, H-3), 6.85 (s, 1H, H-6), 3.16 [s, 6H, $N(CH_3)_2$], 0.28 and 0.24 [two s, 18H, 2 × $Si(CH_3)_3$]. $^{13}$C NMR: $\delta$ 156.1 (C-1), 139.6 (C-4), 133.1 (C-3), 120.7 (C-6), 119.7 and 110.7 (C-2,5), 103.7, 102.6, 102.5, and 100.8 (2 × C≡C), 42.5 [$N(CH_3)_2$], −0.27 and −0.42 [2 × $Si(CH_3)_3$]. Anal. Calcd for $C_{18}H_{26}N_2O_2Si_2$ (358.58): C, 60.29; H, 7.31; N, 7.81. Found: C, 60.40; H, 7.29; N, 7.78.

**Compound 18.** This compound was obtained in 7 h following general procedure C starting from **16** (0.18 g, 0.57 mmol, 1 equiv), **3** (0.75 g, 1.16 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol, 0.16 equiv), and $Ag_2O$ (0.27 g, 1.16 mmol, 2 equiv) in dry DMF (4 mL) and THF (2 mL). Column chromatography was performed with 50:50 hexane/EtOAc as the eluent, and compound **18** was obtained as a brilliant-yellow solid (0.42 g, 0.35 mmol, 61%). TLC: $R_f$ 0.56 (40:60 hexane/EtOAc). Mp: 91−93 °C. $^1$H NMR: $\delta$ 7.46 (d, $J_{5,6}$ = 8.4, 1H, H-6), 7.08 (m, 2H, H-3,5), 7.00, 6.99, 6.93, and 6.91 (four s, 4H, 2 × H-3′,6′), 5.25 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.5, 2H, 2 × H-3″), 5.10 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.5, 2H, 2 × H-4″), 5.03 (dd, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 9.5, 2H, 2 × H-2″), 4.90 (d, $J_{1'',2''}$ = 8.0, 2H, 2 × H-1″), 4.64 (s, 4H, 2 × $CH_2C$≡), 4.27 and 4.16 (split AB system, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, $J_{6''A,6''B}$ = 12.7, 4H, 2 × $H_2$-6″), 3.87, 3.86, and 3.85 (three s, 12H, 4 × $OCH_3$), 3.76 (ddd, $J_{4'',5''}$ = 9.5, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, 2H, 2 × H-5″), 3.02 [br s, 6H, $N(CH_3)_2$], 2.06, 2.03, 2.02, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.7, 170.3, 169.5, and 169.4 (8 × CO), 154.5, 154.1, 153.9, and 153.8 (C-2, 2 × C-2′,5′), 134.3 (C-6), 128.2 (C-4), 123.7 and 120.0 (C-3,5), 115.7, 115.6, 115.5, and 115.1 (2 × C-3′,6′), 115.0, 114.4, 113.8, 112.3, and 111.9 (C-1, 2 × C-1′,4′), 98.3 (2 × C-1″), 95.5, 94.6, 92.6, 89.1, 88.9, 86.6, 83.5, and 83.4 (4 × C≡C), 72.8 (2 × C-3″), 71.9 (2 × C-5″), 71.1 (2 × C-2″), 68.3 (2 × C-4″), 61.8 (2 × C-6″), 57.1 (2 × $CH_2C$≡), 56.5, 56.4, and 56.3 (4 × $OCH_3$), 43.5 [$N(CH_3)_2$], 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{62}H_{67}NO_{24}$ (1210.19): C, 61.53; H, 5.58; N, 1.16. Found: C, 61.66; H, 5.57; N, 1.16.

**Compound 19.** This compound was obtained in 30 h following general procedure C starting from **17** (0.22 g, 0.61 mmol, 1 equiv), **3** (0.75 g, 1.16 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol, 0.15 equiv), and $Ag_2O$ (0.27 g, 1.16 mmol, 2 equiv) in dry DMF (4 mL) and THF (2 mL). Column chromatography was performed with 50:50 hexane/EtOAc as the eluent, and compound **19** was obtained as a pale-green oil (0.31 g, 0.25 mmol, 41%). TLC: $R_f$ 0.36 (40:60 hexane/EtOAc). $^1$H NMR: $\delta$ 8.38 (s, 1H, H-6), 7.10 (s, 1H, H-3), 6.98, 6.96, and 6.93 (three s, 4H, 2 × H-3′,6′), 5.27 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.4, 2H, 2 × H-3″), 5.12 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.4, 2H, 2 × H-4″), 5.05 (dd, $J_{1'',2''}$ = 8.2, $J_{2'',3''}$ = 9.4, 2H, 2 × H-2″), 4.90 (d, $J_{1'',2''}$ = 8.2, 2H, 2 × H-1″), 4.65 (s, 4H, 2 × $CH_2C$≡), 4.27 and 4.16 (split AB system, $J_{5'',6''A}$ = 4.1, $J_{5'',6''B}$ = 2.3, $J_{6''A,6''B}$ = 12.3, 4H, 2 × $H_2$-6″), 3.91, 3.89, and 3.86 (three s, 12H, 4 × $OCH_3$), 3.78 (ddd, $J_{4'',5''}$ = 9.4, $J_{5'',6''A}$ = 4.1, $J_{5'',6''B}$ = 2.3, 2H, 2 × H-5″), 3.28 [s, 6H, $N(CH_3)_2$], 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.2, 169.4, and 169.3 (8 × CO), 155.6, 154.3, 154.1, and 154.0 (C-2 and 2 × C-2′,5′), 139.0 (C-5), 133.0 (C-6), 120.1, 119.9, 115.8, 115.4, and 114.9 (C-3 and 2 × C-3′,6′), 113.4, 113.2, 112.5, and 110.5 (C-1 and 2 × C-1′,4′), 98.2 (2 × C-1″), 93.3, 93.1, 93.0, 92.1, 89.5, 89.3, and 83.3 (4 × C≡C), 72.8 (2 × C-3″), 71.9 (2 × C-5″), 71.1 (2 × C-2″), 68.3 (2 × C-4″), 61.8 (2 × C-6″), 57.0 (2 × $CH_2C$≡), 56.5, 56.4, and 56.2 (4 × $OCH_3$), 42.8 [$N(CH_3)_2$], 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{62}H_{66}N_2O_{26}$ (1255.19): C, 59.33; H, 5.30; N, 2.23. Found: C, 59.26; H, 5.29; N, 2.23.

**Compound 20.** This compound was obtained as a pale-yellow solid (0.17 g, 0.19 mmol, 95%) following general procedure B starting from **18** (0.24 g). TLC: $R_f$ 0.05 (80:20 $CHCl_3$/MeOH). Mp: 202−203 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.44 (d, $J_{5,6}$ = 8.3, 1H, H-6), 7.16, 7.10, 7.07, and 7.06 (four s, 4H, 2 × H-3′,6′), 7.01 (m, 2H, H-3,5), 5.14 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.97 (d, $J_{vic}$ = 4.8, 2H, 2 × OH), 4.92 (d, $J_{vic}$ = 5.4, 2H, 2 × OH), 4.67 and 4.54 (AB system, $J_{gem}$ = 16.1, 4H, 2 × $CH_2C$≡), 4.56 (t, $J_{OH,6}$ = 5.9, 2H, 2 × 6′-OH), 4.33 (d, $J_{1'',2''}$ = 7.8, 2H, 2 × H-1″), 3.81 and 3.80 (two s, 12H, 4 × $OCH_3$), 3.66 and 3.44 (split AB m, 4H, 2 × $H_2$-6″), 3.18−2.97 (m, 8H, 2 × H-2″−5″), 2.97 [s, 6H, $N(CH_3)_2$]. $^{13}$C NMR (DMSO-$d_6$): $\delta$ 154.1, 153.8, 153.7, and 153.5 (C-2, 2 × C-2′,5′), 134.5 (C-6), 123.3 (C-4), 122.7 and 119.2 (C-3,5), 115.8, 115.6, and 115.0 (2 × C-3′,6′), 113.7, 113.2, 112.5, 112.4, and 112.0 (C-1, 2 × C-1′,4′), 101.1 (2 × C-1″), 94.8, 94.3, 92.8, 91.3, 91.2, 87.3, 82.1, and 82.0 (4 × C≡C), 77.1 and 76.7 (2 × C-3″,5″), 73.3 (2 × C-2″), 70.1 (2 × C-4″), 61.2 (2 × C-6″), 56.3 and 56.2 (4 × $OCH_3$), 55.9 (2 × $CH_2C$≡), 42.7 [$N(CH_3)_2$]. Anal. Calcd for $C_{46}H_{51}NO_{16}$ (873.89): C, 63.22; H, 5.88; N, 1.60. Found: C, 63.25; H, 5.87; N, 1.60.

**Compounds 21 and 22.** These compounds were obtained in 3 h following general procedure A starting from **3** (1.98 g, 3.05 mmol, 2.1 equiv), 1,4-diethynylbenzene (0.18 g, 1.43 mmol, 1 equiv), and $Pd(PPh_3)_4$ (0.20 g, 0.17 mmol, 0.12 equiv) in dry DMF (12 mL) and $Et_3N$ (12 mL). Column chromatography was performed with 60:40 hexane/EtOAc as the eluent, and a mixture of **21** and **22** was obtained. A second column chromatography run (90:10 toluene/acetonitrile as the eluent) was needed to obtain first **22** as a yellow solid (0.30 g, 0.23 mmol, 16%) and then **21** as a yellow solid (0.83 g, 0.71 mmol, 50%).

Data for **21**. TLC: $R_f$ 0.59 (40:60 hexane/EtOAc). Mp: 182−184 °C. $^1$H NMR: $\delta$ 7.54 (s, 4H, H-2,3,5,6), 7.01 and 6.94 (two s, 4H, 2 × H-3′,6′), 5.27 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.3, 2H, 2 × H-3″), 5.12 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.3, 2H, 2 × H-4″), 5.05 (dd, $J_{1'',2''}$ = 8.3, $J_{2'',3''}$ =9.3, 2H, 2 × H-2″), 4.91 (d, $J_{1'',2''}$ = 8.3, 2H, 2 × H-1″), 4.65 (s, 4H, 2 × $CH_2C$≡), 4.28 and 4.17 (split AB system, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, $J_{6''A,6''B}$ = 12.2, 4H, 2 × $H_2$-6″), 3.92 and 3.89 (two s, 12H, 4 × $OCH_3$), 3.76 (ddd, $J_{4'',5''}$ = 9.3, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, 2H, 2 × H-5″), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.6, 170.3, 169.4, and 169.3 (8 × CO), 154.1 and 153.9 (2 × C-2′,5′), 131.6 (C-2,3,5,6), 123.1 (C-1,4), 115.8 and 115.5 (2 × C-3′,6′), 113.7 and 112.4 (2 × C-1′,4′), 98.2 (2 × C-1″), 94.9, 89.1, 87.4, and 83.4 (4 × C≡C), 72.8 (2 × C-3″), 71.9 (2 × C-5″), 71.1 (2 × C-2″), 68.3 (2 × C-4″), 61.8 (2 × C-6″), 57.0 (2 × $CH_2C$≡), 56.5 and 56.3 (4 × $OCH_3$), 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{60}H_{62}O_{24}$ (1167.12): C, 61.75; H, 5.35. Found: C, 61.62; H, 5.36.

Data for **22**. TLC: $R_f$ 0.63 (40:60 hexane/EtOAc). Mp: 109−111 °C. $^1$H NMR: $\delta$ 7.52 (m, 8H, 2 × H-2,3,5,6), 7.01 and 6.95 (two s, 4H, 2 × H-3′,6′), 5.27 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.3, 2H, 2 × H-3″), 5.12 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.3, 2H, 2 × H-4″), 5.05 (dd, $J_{1'',2''}$ = 7.8, $J_{2'',3''}$ = 9.3, 2H, 2 × H-2″), 4.91 (d, $J_{1'',2''}$ = 7.8, 2H, 2 × H-1″), 4.65 (s, 4H, 2 × $CH_2C$≡), 4.28 and 4.17 (split AB system, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, $J_{6''A,6''B}$ = 12.2, 4H, 2 × $H_2$-6″), 3.89 and 3.88 (two s, 12H, 4 × $OCH_3$), 3.76 (ddd, $J_{4'',5''}$ = 9.3, $J_{5'',6''A}$ = 4.9, $J_{5'',6''B}$ = 2.4, 2H, 2 × H-5″), 2.08, 2.05, and 2.04 (three s, 24H, 8 × $CH_3CO$). $^{13}$C NMR: $\delta$ 170.7, 170.3, 169.5, and 169.4 (8 × CO), 154.1 and 153.9 (2 × C-2′,5′), 132.4 and 131.6 (2 × C-2,3,5,6), 124.1 and 121.6 (2 × C-1,4), 115.7 and 115.5 (2 × C-3′,6′), 113.5 and 112.6 (2 × C-1′,4′), 98.2 (2 × C-1″), 94.6, 89.2, 88.2, 83.4, 82.1, and 77.2 (6 × C≡C), 72.8 (2 × C-3″), 71.9 (2 × C-5″), 71.1 (2 × C-2″), 68.3 (2 × C-4″), 61.8 (2 × C-6″), 57.0 (2 × $CH_2C$≡), 56.5 and 56.3 (4 × $OCH_3$), 20.7 and 20.6 (8 × $CH_3CO$). Anal. Calcd for $C_{70}H_{66}O_{24}$ (1291.26): C, 65.11; H, 5.15. Found: C, 65.26; H, 5.13.

**Compound 23.** This compound was obtained as a pale-yellow solid (0.15 g, 0.19 mmol, 94%) following general procedure B starting from **21** (0.23 g). TLC: $R_f$ 0.05 (80:20 $CHCl_3$/MeOH). Mp: ≥280 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.58 (s, 4H, H-2,3,5,6), 7.18 and 7.09 (two s, 4H, 2 × H-3′,6′), 5.15 (d, $J_{vic}$ = 4.9, 2H, 2 × OH), 4.98 (d, $J_{vic}$


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= 4.9, 2H, 2 × OH), 4.94 (d, $J_{vic}$ = 5.3, 2H, 2 × OH), 4.68 and 4.55 (AB system, $J_{gem}$ = 15.6, 4H, 2 × $CH_2C\equiv$), 4.54 (t, $J_{OH,6}$ = 5.7, 2H, 2 × 6′-OH), 4.34 (d, $J_{1'',2''}$ = 7.9, 2H, 2 × H-1″), 3.82 and 3.81 (two s, 12H, 4 × $OCH_3$), 3.70 and 3.45 (split AB m, 4H, 2 × $H_2$-6″), 3.19−2.97 (m, 8H, 2 × H-2″−5″). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 153.7 and 153.5 (2 × C-2′,5′), 131.6 (s, C-2,3,5,6), 122.5 (C-1,4), 115.8 and 115.6 (2 × C-3′,6′), 112.5 and 112.2 (2 × C-1′,4′), 101.1 (2 × C-1″), 94.1, 91.4, 88.1.2, and 82.0 (4 × C≡C), 77.0 and 76.7 (2 × C-3″,5″), 73.3 (2 × C-2″), 70.1 (2 × C-4″), 61.2 (2 × C-6″), 56.3 and 56.1 (4 × $OCH_3$), 55.8 (2 × $CH_2C\equiv$). Anal. Calcd for $C_{44}H_{46}O_{16}$ (830.83): C, 63.61; H, 5.58. Found: C, 63.63; H, 5.59.

## ■ ASSOCIATED CONTENT

### Supporting Information

$^1$H NMR and $^{13}$C NMR spectra of compounds **3**−**23**, cellular toxicity data, and absorption and emission spectra of **8** and **21**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605−1644.

(2) (a) Yamaguchi, Y.; Shimoi, Y.; Ochi, T.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-I. *J. Phys. Chem. A* **2008**, *112*, 5074−5084. (b) Zhi, Y.-G.; Lai, S.-W.; Chan, Q. K.-W.; Law, Y.-C.; Tong, G. S.-M.; Che, C.-M. *Eur. J. Org. Chem.* **2006**, 3125−3139. (c) Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. *J. Am. Chem. Soc.* **2005**, *127*, 9332−9333.

(3) (a) Kim, U.-I.; Suk, J.-m.; Naidu, V. R.; Jeong, K.-S. *Chem.—Eur. J.* **2008**, *14*, 11406−11414. (b) Thomas, S. W., III; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339−1386. (c) Kim, I.-K.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. *Chem.—Eur. J.* **2004**, *10*, 6247−6254.

(4) (a) Kaliginedi, V.; Moreno-García, P.; Valkenier, H.; Hong, W.; García Suarez, V. M.; Buiter, P.; Otten, J. L. H.; Hummelen, J. C.; Lambert, C. J.; Wandlowski, T. *J. Am. Chem. Soc.* **2012**, *134*, 5262−5275. (b) Yamaguchi, Y.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*, 7040−7044.

(5) (a) Hill, E. H.; Evans, D. G.; Whitten, D. G. *Langmuir* **2013**, *29*, 9712−9720. (b) Dascier, D.; Ji, E.; Parthasarathy, A.; Schanze, K. S.; Whitten, D. G. *Langmuir* **2012**, *28*, 11286−11290. (c) McRae, R. L.; Phillips, R. L.; Kim, I.-B.; Bunz, U. H. F.; Fahrni, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 7851−7853.

(6) Pertici, F.; Varga, N.; van Duijn, A.; Rey-Carrizo, M.; Bernardi, A.; Pieters, R. J. *Beilstein J. Org. Chem.* **2013**, *9*, 215−222.

(7) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343−13346.

(8) Kelly, T. L.; Lam, M. C. W.; Wolf, M. O. *Bioconjugate Chem.* **2006**, *17*, 575−578.

(9) (a) He, X.; Wang, K.; Cheng, Z. *Nanomed. Nanobiotechnol.* **2010**, *2*, 349−366. (b) Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620−2640. (c) Rao, J.; Dragulescu-Andrasi, A.; Yao, H. *Curr. Opin. Biotechnol.* **2007**, *18*, 17−25.

(10) (a) Bonaccorsi, P.; Di Gioia, M. L.; Leggio, A.; Minuti, L.; Papalia, T.; Siciliano, C.; Temperini, A.; Barattucci, A. *Beilstein J. Org. Chem.* **2013**, *9*, 2410−2416. (b) Bonaccorsi, P.; Aversa, M. C.; Barattucci, A.; Papalia, T.; Puntoriero, F.; Campagna, S. *Chem. Commun.* **2012**, *48*, 10550−10552. (c) Bonaccorsi, P.; Marino-Merlo, F.; Barattucci, A.; Battaglia, G.; Papaianni, E.; Papalia, T.; Aversa, M. C.; Mastino, A. *Bioorg. Med. Chem.* **2012**, *20*, 3186−3195. (d) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Synlett* **2011**, 254−258. (e) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Temperini, A. *Eur. J. Org. Chem.* **2011**, 5668−5673. (f) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Marino-Merlo, F.; Mastino, A.; Sciortino, M. T. *Bioorg. Med. Chem.* **2009**, *17*, 1456−1463. (g) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Eur. J. Org. Chem.* **2009**, 6335−6339. (h) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659−7683. (i) Aversa, M. C.; Barattucci, A.; Bilardo, M. C.; Bonaccorsi, P.; Giannetto, P.; Rollin, P.; Tatibouet, A. *J. Org. Chem.* **2005**, *70*, 7389−7396.

(11) (a) Giovenzana, G. B.; Lay, L.; Monti, D.; Palmisano, G.; Panza, L. *Tetrahedron* **1999**, *55*, 14123−14136. (b) Yi, C.; Blum, C.; Lehmann, M.; Keller, S.; Liu, S.-X.; Frei, G.; Neels, A.; Hauser, J.; Schürch, S.; Decurtins, S. *J. Org. Chem.* **2010**, *75*, 3350−3357.

(12) (a) Babudri, F.; Colangiuli, D.; Di Lorenzo, P. A.; Farinola, G. M.; Hassan Omar, O.; Naso, F. *Chem. Commun.* **2003**, 130−131. (b) Mori, A.; Kondo, T.; Kato, T.; Nishihara, Y. *Chem. Lett.* **2001**, 286−287.

(13) (a) Moroni, M.; Le Moigne, J.; Pham, T. A.; Bigot, J.-Y. *Macromolecules* **1997**, *30*, 1964−1972. (b) Parham, W. E.; Piccirilli, R. M. *J. Org. Chem.* **1977**, *42*, 257−260.

(14) Chu, Q.; Pang, Y. *Macromolecules* **2003**, *36*, 4614−4618.

(15) Babudri, F.; Colangiuli, D.; Di Bari, L.; Farinola, G. M.; Hassan Omar, O.; Naso, F.; Pescitelli, G. *Macromolecules* **2006**, *39*, 5206−5212.

(16) (a) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991−1024. (b) Dempster, D. N.; Morrow, T.; Quinn, M. F. *J. Photochem.* **1974**, *2*, 329−341.