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Original article

Synthesis and biological evaluation of novel symmetry bis-enediynes

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Abstract

A series of acyclic symmetry bis-enediynes have been synthesized successfully and their bioactivities were evaluated. Among them, 1,6-bis(4-((2-(pyridin-2-ylethynyl)phenyl)ethynyl)phenoxy)hexane **8g** showed good inhibition activity against the CCRF-CEM ($GI_{50} = 0.04$ μM) and HL-60 ($GI_{50} = 0.09$ μM) cell lines of human leukemia.. The cell cycle analysis shows that compound **8g** arrests cell cycle *via* inhibiting Cyclin A and Cyclin B expressions in low concentration and induces a significant apoptosis progress in high concentration.

Keywords: Bis-enediynes; Cell cycle; Anticancer; Apotosis

1. Introduction

Small molecules can arrest cancer cell cycle machinery in G2/M phase by the inhibition of Cdk family that led to the antiproliferation of cancer cell have been reported by many research groups [1–4]. Cyclin A and Cyclin B are critical proteins in G2 and mitosis of cell cycle. Cyclin A can construct complexes that play critical roles in replication and passage through G2 with both Cdk1 and Cdk2 during S and G2 phases. Cyclin B constructs complexes that required for mitosis with Cdk1 during G2 [5]. The report of controlling cell cycle *via* Cdk family is interested in many small molecules. Recently, we have disclosed novel acyclic enediyne derivatives [6] that showed remarkable broad spectrum inhibition of the growth of cancer cell lines and the average GI_{50} values were from 10^{-8} to 10^{-6} M. Particularly, 2-(6-(2-thienyl)-3(*Z*)-hexen-1,5-diynyl)aniline against the MDA-MB-231/ACTT cell line of human breast cancer was found to be less than 10^{-8} M [6c]. Furthermore, the series of acyclic enediynes performs induced apoptosis phenomenon. It has been known that linking of two small molecules as a dimer could enhance the efficiency and selectivity of the biological activities. For instance, the trioxane dimer [7] and bis-acridine [8] have been shown to enhance the efficiency and selectivity of anticancer activity. In continuing search for more potent biologically active compounds, bis-enediynes **8a–8g** have been designed and synthesized (Fig. 1). The main focus of this study is to modify the aryl group at the terminal alkyne for the structure and to study the structure–activity relationship.

2. Chemistry

The synthesis of compounds **8a–8g** is summarized in Scheme 1. Compound **1** [9] was treated with various aryl iodides **2a–2f** using $Pd(PPh_3)_4$ as the catalyst to obtain compounds

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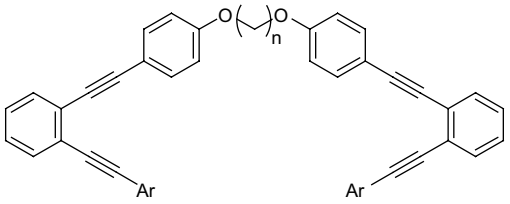
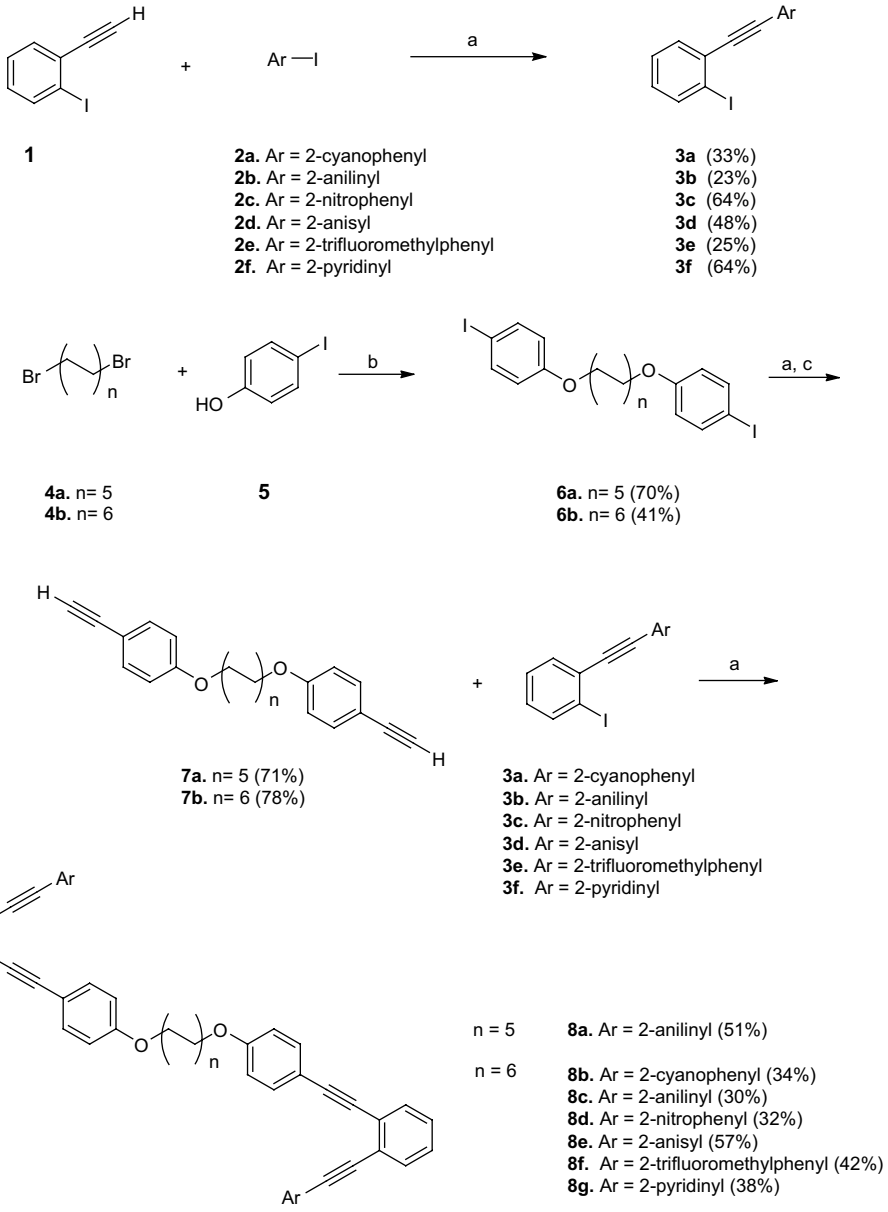


n = 5 **8a**. Ar = 2-anilinyl

n = 6 **8b.** Ar = 2-cyanophenyl
8c. Ar = 2-anilinyl
8d. Ar = 2-nitrophenyl
8e. Ar = 2-anisyl
8f. Ar = 2-trifluoromethylphenyl
8g. Ar = 2-pyridinyl

Fig. 1. Chemical structures of bis-enediynes **8a**–**8g**.

3a–**3f**. On the other hand, 1,5-dibromopentane (**4a**) and 1,6-dibromohexane (**4b**) were reacted with 4-iodophenol (**5**) in DMF using K_2CO_3 as the base to give compounds **6a** and **6b** in 70 and 41% yields, respectively. Compounds **6a** and **6b** were then coupled with trimethylsilylacetylene under Sonogashira coupling reaction conditions [10], followed by desilyation gave compounds **7a** and **7b** in 71 and 78% yields, respectively. Finally, palladium-catalyzed coupling reaction of **7a** and **7b** with various aryl iodides **3a**–**3f** gave **8a**–**8g** in the yields from 30 to 57%.



Scheme 1. Reagents and condition: (a) $Pd(PPh_3)_4$, CuI, *n*-$BuNH_2$, ether, rt; (b) K_2CO_3, DMF, rt; (c) K_2CO_3, methanol, rt.

3. Biological assay result

3.1. Cytotoxicity activities

Compounds **8a–8g** were submitted to the National Cancer Institute for testing against a panel of nine cancer cell lines. Details of this test system have been published by others [11]. Compounds **8a–8f** were found to be inactive in the pre-screening test. However, compound **8g** is found to be active against 50 tumor cell lines at low concentration, especially against the CCRF-CEM ($GI_{50} = 0.04$ μM) and HL-60 ($GI_{50} = 0.09$ μM) cell lines of human leukemia. The GI_{50} and LC_{50} values of compound **8g** are summarized in Table 1. It was also noted that most of the LC_{50} values of compound **8g** for these cancer cell lines were 10–1000 times higher than the GI_{50} values. This result indicates that compound **8g** exhibits very high growth inhibition activity but not cytotoxic toward human cancer cell lines. This phenomenon is meaningful to the advancement of medical therapies of human cancer diseases, whereas drugs with highly cytotoxic activities always caused the damage to normal cells.

3.2. Cell cycle assay

To confirm the mechanism of the biological activity of bisenediyne **8g**, we examined cell cycle phase distribution by treating K-562 cell line of leukemia for 24 h by flow cytometry. The pattern of cell cycle is shown in Fig. 2. The result of cell cycle percentage is summarized in Fig. 3(a). On the other hand, Fig. 3(b) summarized the result of apoptosis percentage. According to Fig. 3(a), the cell cycle was blocked in G2/M phase compared with control (17.3%) by compound **8g** of 10 μM (35.8%). Furthermore, treatment with **8g** in 20 μM with K-562 cell showed diphasic arrest in S phase (59.7%) and G2/M (26.6%) that compared with S phase (51.2%) and G2/M (17.3%) of control. Comparing the percentage of sub-G1 area (3.13%) of control, compound **8g** showed significant apoptotic activity against K-562 cell line in 50 μM (15.54%). This experiment indicated that compound **8g** can cease cell cycle machinery in G2/M phase in low concentration (10 μM), diphasic arrest in S phase and G2/M phase (20 μM), and induce significant apoptosis phenomenon in high concentration (50 μM).

3.3. Inhibiting Cyclin A and Cyclin B expressions

To obtain the understanding of mechanism of cell cycle in these enediyne analogues, we examined the expression of G2/M associated proteins, including Cyclin A, Cyclin B, Cdk1, Cdk2 and Cdk4 expression analyses which are summarized in Fig. 4. According to the electrophoresis, we found that compound **8g** showed significant inhibition of Cyclin A and Cyclin B expressions in 20 μM. It is indicted that compound **8g** affects cancer cell cycle *via* controlling the expressions of Cyclin A and Cyclin B.

Table 1
The in vitro testing results of full panel screen of 50 human tumor cell lines[a]

Panel/cell line	**8g**
	GI_{50}[b]/LC_{50}[c]
Leukemia	
CCRF-CEM	0.04/>50.0
HL-60	0.09/2.7
K-562	1.38/22.8
MOLT-4	0.37/28.0
RPMI-8226	0.62/>50.0
Non-Small Cell Lung cancer	
A549/ATCC	6.41/43.2
EKVX	4.33/22.9
HOP-62	6.31/38.8
NCI-H226	6.53/30.3
NCI-H23	7.97/>50.0
NCI-H460	1.69/18.7
NCI-H522	2.87/38.4
Colon cancer	
COLO 205	8.18/30.9
HCC-2998	7.21/26.9
HCT-116	8.69/>50.0
HCT-15	2.78/22.0
HT29	7.89/27.0
KM12	1.38/20.8
SW-620	4.00/22.7
CNS cancer	
SF-268	3.63/22.7
SF-295	1.24/15.8
SF-539	2.38/21.3
SNB-19	6.00/26.2
SNB-75	5.13/31.3
U251	5.63/24.1
Melanoma	
LOX IMVI	0.27/18.4
M14	7.47/41.6
SK-MEL-28	5.73/24.3
SK-MEL-5	2.30/20.5
UACC-257	7.11/31.4
UACC-62	0.47/16.4
Ovarian cancer	
OVCAR-3	2.14/21.3
OVCAR-4	1.91/23.4
OVCAR-5	3.56/23.4
SK-OV-3	7.05/42.0
Renal cancer	
786-0	7.45/>50.0
ACHN	1.34/19.6
CAKI-1	5.42/23.8
RXF 393	7.65/27.9
SN12C	6.55/32.7
UO-31	8.51/>50.0
Prostate cancer	
PC-3	4.46/29.2
DU-145	6.14/24.9
Breast cancer	
MCF7	1.91/21.8
NCI/ADR-RES	2.30/>50.0
MDA-MB-231/ATCC	2.67/23.5
HS 578T	5.41/34.6
MDA-MB-435	1.54/21.7
BT-549	3.45/22.7
T-47D	2.35/>50.0

[a] Data obtained from the NCI's in vitro human tumor cell screen.
[b] The concentration produces 50% reduction in cell growth.
[c] The concentration produces 50% cells death.

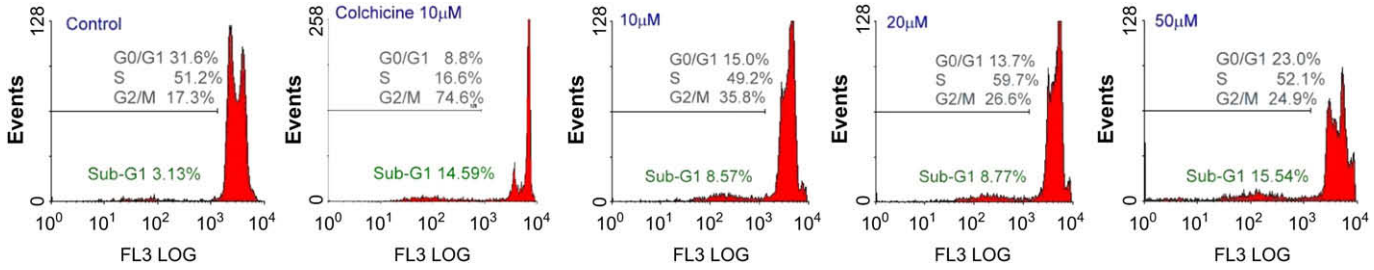


Fig. 2. Cell cycle distribution of K-562 cells treated with colchicine (10 μM) and compound **8g** for 24 h used at the concentration of 10, 20 and 50 μM by flow cytometry analysis. Sub G1 = apoptotic area.

4. Conclusion

In conclusion, we have found a new acyclic bis-enediyne derivative that is potent in growth inhibition on many kinds of human tumor cancer cells. In this study, several points were achieved. (i) The structure–activity relationship of bis-enediynes showed the distinct cytotoxic activities in 2-pyridinyl of changed aryl group. (ii) The average GI_{50} values of compound **8g** were from 10^{-8} to 10^{-6} M, especially for against the CCRF-CEM (0.04 μM) and HL-60 (0.09 μM) cell lines of human leukemia. (iii) For 24 h treatment of compound **8g**, it can cease cell cycle machinery in G2/M phase (10 μM), diphasic arrest in S phase and G2/M phase (20 μM) and induce significant apoptosis phenomenon (50 μM) (Fig. 3) and (iv) Compound **8g** showed significant inhibition of Cyclin A and Cyclin B expressions that play critical roles during cell cycle in low concentration (Fig. 4). According to the results of this investigation, we found that there is no biological activity correlation between the substituent on the bis-enediyne derivatives and the mono-enediynes.

5. Experimental

5.1. General procedure for coupling compound *3a–3f*, *7a–7b* and *8a–8g*

To a degassed solution of terminal alkyne (12 mmol) containing CuI (3.2 mmol) and *n*-$BuNH_2$ (30 mmol) in MeOH (15 ml) was added a degassed solution of aryl iodides (12 mmol) containing $Pd(PPh_3)_4$ (0.8 mmol) in ether (20 ml). The resulting reaction mixture was stirred for 6 h. Methanol was removed *in vacuo*, and the residue was quenched with saturated aqueous NH_4Cl solution. The aqueous layer was extracted with EtOAc (50 ml) and the combined organic extracts were washed with saturated aqueous Na_2CO_3 solution (40 ml) and dried over anhydrous $MgSO_4$. After filtration and removal of solvent *in vacuo*, the residue was purified by column chromatography on silica gel to yield the desired products.

5.1.1. Cell cycle analysis

Flow cytometry was used to measure cell cycle profile and apoptosis. For cell cycle analysis, K-562 cells treated with compounds **8g** for 24 h were harvested by centrifugation. After being washed with PBS, the cells were fixed with ice-cold 70% ethanol for 30 min, washed with PBS, and then treated with 1 ml of 1 mg/ml of RNase A solution at 37 °C for 30 min. Cells were harvested by centrifugation at 1000 rpm for 5 min and further stained with 250 μl of DNA staining solution (10 mg of propidium iodide [PI], 0.1 mg of trisodium citrate, and 0.03 ml of Triton X-100 were dissolved in 100 ml H_2O) at room temperature for 30 min in the dark. After loading 500 μl of PBS, the DNA contents of 10,000 cells were measured by FACScan (Elite ESP, Beckman Coulter, Brea, CA) and the cell cycle profile was analyzed from the DNA content histograms with WinCycle software. When cells were undergoing apoptosis, the containing DNA was digested

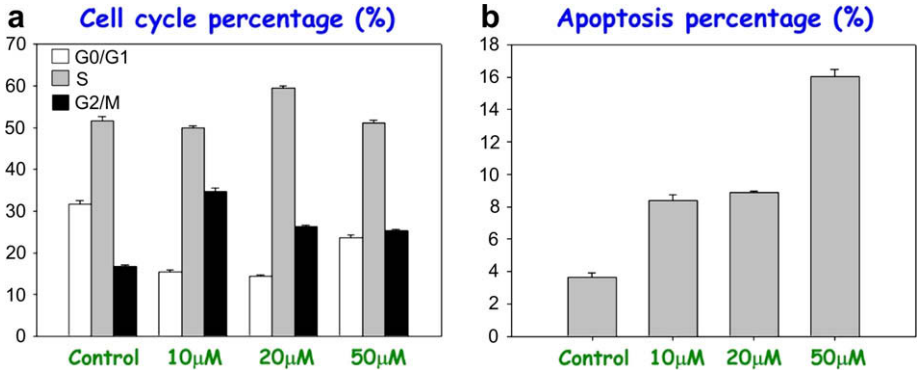


Fig. 3. (a) Cell cycle percentage of K-562 cells treated with compound **8g**. The percentages of accumulation of G2/M phase cells of the control and compound **8g** (10, 20 and 50 μM) were 17.3 ± 0.4, 35.8 ± 0.4, 26.6 ± 1.4 and 24.9 ± 2.3%, respectively ($n = 3$). (b) Apoptosis percentage of K-562 cells treated with compound **8g**. The proportions of apoptotic cells for the control and compound **8g** (10, 20 and 50 μM) were control (3.13 ± 0.5%), 10 μM (8.57 ± 0.5%), 20 μM (8.77 ± 0.2%) and 50 μM (15.54 ± 0.7%).

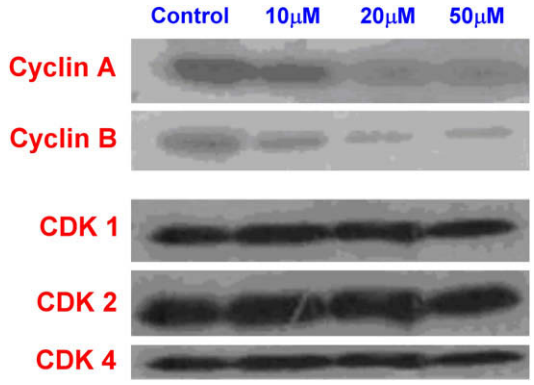


Fig. 4. The western blotting analysis of the G2/M related protein, includes Cyclin A, Cyclin B, CDK1, CDK2 and CDK4 in 10, 20 and 50 μM.

by endonuclease, then the sub G1 pick appeared. The percentage of sub G1 was analyzed by gating on cell cycle dot blots using Windows Multiple Document Interface software (WinMDI).

5.1.2. Western blotting analysis

Cells were washed in PBS, suspended in lysis buffer containing 50 mM Tris (pH 7.5), 1% NP-40, 2 mM EDTA, 10 mM NaCl, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride and placed on ice for 30 min. After centrifugation at 20,000*g* for 30 min at 4 °C, the supernatant was collected. The protein concentration in the supernatant was determined with a BCA protein assay kit (Pierce, Rockford, IL, USA). Whole lysate (50 μg) was resolved by 12% SDS-PAGE, transferred onto PVDF membranes (Roche) by electroblotting, and probed with, anti-Cdk1, -Cdk2, -Cdk4, anti-cyclin A, and anti-cyclinB1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA). The blot was developed by enhanced chemiluminescence.

*5.1.3. 1-Iodo-2-((2-cyanophenyl)ethynyl)benzene (**3a**)*

The compound was purified by column chromatography, eluting with hexane/EA (30:1) to give 33% of yellow oil according to general procedure. Mp: 90–91 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89 (dd, 1H, $J = 8.0$, 1.2 Hz), 7.73–7.57 (m, 4H), 7.44 (td, 1H, $J = 7.6$, 1.2 Hz), 7.36 (td, 1H, $J = 7.6$, 1.2 Hz), 7.07 (td, 1H, $J = 7.6$, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.8, 133.4, 132.7, 132.5, 132.3, 132.1, 130.2, 128.6, 127.9, 126.7, 117.5, 114.9, 100.5, 97.3, 88.6. HRMS calcd for $C_{15}H_8IN$, Mr = 329.1352; found 328.9706.

*5.1.4. 1-Iodo-2-((2-anilinyl)ethynyl)benzene (**3b**)*

The compound was purified by column chromatography, eluting with hexane/EA (15:1) to give 23% of yellow oil according to general procedure. Mp: 67–68 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.87 (dd, 1H, $J = 6.8$, 1.2 Hz), 7.55 (dd, 1H, $J = 6.0$, 2.0 Hz), 7.41 (dd, 1H, $J = 5.6$, 2.0 Hz), 7.34 (td, 1H, $J = 7.2$, 1.2 Hz), 7.17 (td, 1H, $J = 7.6$, 1.2 Hz), 7.01 (td, 1H, $J = 8.0$, 2.0 Hz), 6.74–6.71 (m, 2H), 4.58–4.48 (bs, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.4, 138.5, 132.3, 132.1, 130.1, 129.9, 129.1, 127.9, 117.7, 114.3, 107.2, 100.4, 96.5, 90.2. HRMS calcd for $C_{14}H_{10}IN$, Mr = 319.1404; found 318.9852.

*5.1.5. 1-Iodo-2-((2-nitrophenyl)ethynyl)benzene (**3c**)*

The compound was purified by column chromatography, eluting with hexane/EA (20:1) to give 64% of yellow oil according to general procedure. Mp: 82–83 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (dd, 1H, $J = 7.2$, 1.2 Hz), 7.90 (dd, 1H, $J = 8.4$, 1.2 Hz), 7.85 (dd, 1H, $J = 7.6$, 1.6 Hz), 7.65 (td, 2H, $J = 7.2$, 1.6 Hz), 7.50 (td, 1H, $J = 7.6$, 1.6 Hz), 7.37 (td, 1H, $J = 7.6$, 1.2 Hz), 7.09 (td, 1H, $J = 7.6$, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.8, 134.8, 133.4, 132.9, 130.3 (2C), 129.0, 128.9, 127.9, 124.7, 118.5, 100.7, 98.8, 88.0. HRMS calcd for $C_{14}H_8INO_2$, Mr = 349.1233; found 348.9601.

*5.1.6. 1-Iodo-2-((2-methoxyphenyl)ethynyl)benzene (**3d**)*

The compound was purified by column chromatography, eluting with hexane/EA (20:1) to give 48% of yellow oil according to general procedure. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (dd, 1H, $J = 6.8$, 1.2 Hz), 7.58 (td, 2H, $J = 6.4$, 2.0 Hz), 7.36–7.30 (m, 2H), 7.02–6.91 (m, 3H), 3.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.0, 138.6, 133.6, 132.5, 130.1(2C), 129.1, 127.6, 120.4, 112.1, 110.8, 100.9, 95.3, 89.6, 55.8. HRMS calcd for $C_{15}H_{11}IO$, Mr = 334.1517; found 333.9861.

*5.1.7. 1-Iodo-2-((2-(trifluoromethyl)phenyl)ethynyl)-benzene (**3e**)*

The compound was purified by column chromatography, eluting with hexane/EA (3:1) to give 25% of yellow oil according to general procedure. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (dd, 1H, $J = 7.2$, 0.8 Hz), 7.78 (d, 1H, $J = 7.6$ Hz), 7.70 (d, 1H, $J = 8.0$ Hz), 7.57–7.53 (m, 2H), 7.45 (t, 1H, $J = 7.6$ Hz), 7.35 (td, 1H, $J = 7.6$, 1.2 Hz), 7.05 (td, 1H, $J = 7.6$, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.5, 133.9, 132.9, 131.2, 129.6, 129.1, 128.1, 127.6, 125.6, 124.6, 121.9, 120.9, 100.1, 96.3, 88.4. HRMS calcd for $C_{15}H_8F_3I$, Mr = 372.1237; found 371.9630.

*5.1.8. 1-Iodo-2-((2-pyridinyl)ethynyl)benzene (**3f**)*

The compound was purified by column chromatography, eluting with hexane/EA (15:1) to give 64% of yellow oil according to general procedure. . ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.64 (dd, 1H, $J = 4.8$, 0.4 Hz), dd, 7.89 (1H, $J = 6.8$, 1.2 Hz), 7.70 (td, 1H, $J = 7.6$, 1.6 Hz), 7.62 (td, 2H, $J = 7.2$, 1.2 Hz), 7.34 (td, 1H, $J = 7.6$, 1.2 Hz), 7.28–7.25 (m, 1H), 7.05 (td, 1H, $J = 7.2$, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.0, 143.0, 138.7, 136.2, 133.1, 130.0, 128.8, 127.8, 127.5, 123.0, 101.0, 91.7, 91.1. HRMS calcd for $C_{13}H_8IN$, Mr = 304.9701; found 304.9701.

*5.1.9. 1,5-Bis(4-iodophenoxy)pentane (**6a**)*

To a stirred solution of 1,5-dibromopentane (10 mmol) and 4-iodophenol (10 mmol) in DMF (20 ml) was added K_2CO_3 (50 mmol) under nitrogen at room temperature. The resulting solution was stirred for 24 h. The aqueous layer was extracted

with EtOAc (50 ml) and the combined organic extracts were washed with saturated aqueous NH_4Cl solution (40 ml) and dried over anhydrous $MgSO_4$. After filtration and removal of solvent *in vacuo*, the residue was purified by column chromatography on silica gel to yield the desired products. The compound was purified by column chromatography, eluting with hexane/EA (50:1) to give 70% of white solid according to general procedure. Mp: 106–107 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54 (dd, 4H, $J = 4.4$, 2.4 Hz), 6.66 (dd, 4H, $J = 4.4$, 2.4 Hz), 3.94 (t, 4H, $J = 6.4$ Hz), 1.84 (quin, 4H, $J = 6.4$ Hz), 1.64–1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.8, 138.1, 116.8, 82.5, 67.7, 28.81, 22.6. HRMS calcd for $C_{17}H_{18}I_2O_2$, Mr = 508.1326; found 507.9395.

5.1.10. 1,6-Bis(4-iodophenoxy)hexane (**6b**)

To a stirred solution of 1,6-dibromohexane (10 mmol) and 4-iodophenol (10 mmol) in DMF (20 ml) was added K_2CO_3 (50 mmol) under nitrogen at room temperature. The resulting solution was stirred for 24 h. The aqueous layer was extracted with EtOAc (50 ml) and the combined organic extracts were washed with saturated aqueous NH_4Cl solution (40 ml) and dried over anhydrous $MgSO_4$. After filtration and removal of solvent *in vacuo*, the residue was purified by column chromatography on silica gel to yield the desired products. The compound was purified by column chromatography, eluting with hexane/EA (50:1) to give 41% of yellow solid according to general procedure. Mp: 104–108 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.539 (dt, 4H, $J = 8.8$, 3.2 Hz), 6.665 (dt, 4H, $J = 8.8$, 3.2 Hz), 3.923 (t, 4H, $J = 6.4$ Hz), 1.799 (t, 4H, $J = 6.4$ Hz), 1.575–1.499 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.905, 138.150, 116.872, 82.473, 67.858, 29.039, 25.772. HRMS calcd for $C_{18}H_{20}I_2O_2$, Mr = 522.1591; found 521.9548.

5.1.11. 1,5-Bis(4-ethynylphenoxy)pentane (**7a**)

According to general procedure and followed by desilylation, we can obtain compound **7a**. The compound was purified by column chromatography, eluting with hexane/EA (50:1) to give 71% of yellow solid according to general procedure. Mp: 86–88 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41 (dt, 4H, $J = 8.8$, 2 Hz), 6.823 (dt, 4H, $J = 8.8$, 2 Hz), 3.99 (t, 4H, $J = 6.4$ Hz), 2.991 (s, 2H), 1.89–1.82 (m, 4H), 1.68–1.63 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 133.568, 114.404, 113.979, 83.684, 68.725, 28.881, 22.668. HRMS calcd for $C_{21}H_{20}O_2$, Mr = 304.3823; found 304.1462.

5.1.12. 1,6-Bis(4-ethynylphenoxy)hexane (**7b**)

After general procedure and desilyation, we can obtain compound **7b**. The compound was purified by column chromatography, eluting with hexane/EA (50:1) to give 78% of yellow solid according to general procedure. Mp: 94–96 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41 (dt, 4H, $J = 8.0$, 2.8 Hz), 6.827 (dt, 4H, $J = 7.8$, 2 Hz), 3.968 (t, 2H, $J = 6.4$ Hz), 2.991 (s, 1H), 1.816 (t, 4H, $J = 6.4$ Hz), 1.563–1.515 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 133.564, 114.439, 113.969, 83.716, 67.843, 29.077, 25.803. HRMS calcd for $C_{22}H_{22}O_2$, Mr = 318.4089; found 318.1625.

5.1.13. 1,6-Bis(4-((2-((2-anilinyl)ethynyl)phenyl)ethynyl)-phenoxy)pentane (**8a**)

According to general procedure, we can obtain compound **8a**. The compound was purified by column chromatography, eluting with hexane/EA (5:1) to give 51% of yellow oil according to general procedure. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59–7.49 (m, 8H), 7.41 (dd, 2H, $J = 8.0$, 1.6 Hz), 7.33–7.26 (m, 4H), 7.13 (td, 2H, $J = 8.0$, 1.2 Hz), 6.87 (dt, 4H, $J = 8.8$, 2.8 Hz), 6.73–6.66 (m, 4H), 4.60–6.20 (bs, 2H), 3.99 (t, 4H, $J = 6.4$ Hz), 1.85 (quin, 4H, $J = 8.0$ Hz), 1.65 (quin, 2H, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.2, 148.1, 133.2, 131.9, 131.8, 131.2, 129.8, 127.7, 127.6, 125.5, 125.2, 117.4, 114.7, 114.4, 113.9, 107.4, 93.7, 93.1, 90.2, 87.4, 67.7, 28.8, 22.6. HRMS (FAB$^+$) calcd for $C_{49}H_{39}N_2O_2$ $(M + H)^+$, Mr = 687.3012; found 687.3008.

5.1.14. 1,6-Bis(4-((2-((2-cyanophenyl)ethynyl)phenyl)-ethynyl)phenoxy)hexane (**8b**)

According to general procedure, we can obtain compound **8b**. The compound was purified by column chromatography, eluting with hexane/EA (5:1) to give 34% of yellow solid according to general procedure. Mp: 147–148 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.69–7.63 (m, 6H), 7.57–7.52 (m, 8H), 7.51–7.29 (m, 6H), 6.87 (dt, 4H, $J = 9.2$, 2.8 Hz), 3.99 (t, 4H, $J = 6.8$ Hz), 1.83 (t, 4H, $J = 6.4$ Hz), 1.55 (quin, 4H, $J = 4.0$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.3, 133.2, 132.7, 132.6, 132.4, 132.3, 131.7, 128.9, 128.3, 127.7, 127.2, 126.3, 124.7, 117.4, 115.1, 115.0, 114.5, 94.8, 94.1, 89.1, 86.6, 67.8, 29.6, 25.8. HRMS calcd for $C_{52}H_{36}N_2O_2$, Mr = 720.2777; found 720.2766.

5.1.15. 1,6-Bis(4-((2-((2-anilinyl)ethynyl)phenyl)ethynyl)-phenoxy)hexane (**8c**)

According to general procedure, we can obtain compound **8c**. The compound was purified by column chromatography, eluting with hexane/EA (5:1) to give 30% of yellow solid according to general procedure. Mp: 124–125 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59–7.51 (m, 8H), 7.41 (dd, 2H, $J = 7.6$, 1.6 Hz), 7.32–7.29 (m, 4H), 7.14 (td, 2H, $J = 7.2$, 1.6 Hz), 6.91–6.87 (m, 4H), 6.73–6.67 (m, 4H), 4.60–4.20 (br, 4H), 4.00 (t, 4H, $J = 6.4$ Hz), 1.85 (t, 4H, $J = 6.4$ Hz), 1.56 (quin, 4H, $J = 4.0$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.3, 148.1, 133.3, 131.9, 131.8, 131.2, 129.8, 127.8, 127.6, 125.5, 125.2, 117.4, 114.7, 114.4, 113.9, 107.4, 93.8, 93.1, 90.2, 87.4, 67.8, 29.0, 25.8. HRMS (FAB$^+$) calcd for $C_{50}H_{41}N_2O_2$ $(M + H)^+$, Mr = 701.3168; found 701.3173.

5.1.16. 1,6-Bis(4-((2-((2-nitrophenyl)ethynyl)phenyl)-ethynyl)phenoxy)hexane (**8d**)

According to general procedure, we can obtain compound **8d**. The compound was purified by column chromatography, eluting with hexane/EA (4:1) to give 32% of yellow solid according to general procedure. Mp: 150–151 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (dd, 2H, $J = 8.0$, 1.2 Hz), 7.75

(dd, 2H, $J = 7.6$, 1.2 Hz), 7.62 (dt, 2H, $J = 7.2$, 1.2 Hz), 7.57–7.43 (m, 10H), 7.37–7.29 (m, 4H), 6.87 (dt, 4H, $J = 9.6$, 2.8 Hz), 3.98 (t, 4H, $J = 6.4$ Hz), 1.83 (t, 4H, $J = 6.4$ Hz), 1.55 (quin, 4H, $J = 4.0$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.3, 149.3, 134.9, 133.2, 132.7, 132.6, 131.8, 128.9, 128.6, 127.7, 126.5, 124.7, 124.4, 118.9, 115.0, 114.5, 96.2, 94.0, 88.4, 86.6, 67.8, 29.0, 25.8. HRMS (FAB^+) calcd for $C_{50}H_{37}N_2O_6$ $(M + H)^+$, Mr = 761.2652; found 761.2657.

5.1.17. 1,6-Bis(4-((2-((2-methoxyphenyl)ethynyl)phenyl)-ethynyl)phenoxy)hexane (**8e**)

According to general procedure, we can obtain compound **8e**. The compound was purified by column chromatography, eluting with hexane/EA (5:1) to give 57% of yellow solid according to general procedure. Mp: 105–106 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55–7.45 (m, 10H), 7.28–7.21 (m, 6H), 6.90–6.78 (m, 8H), 3.92 (t, 4H, $J = 6.4$ Hz), 1.76 (t, 4H, $J = 6.4$ Hz), 1.48 (quin, 4H, $J = 3.6$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 159.0, 133.6, 133.0, 131.7, 131.3, 129.7, 127.3, 125.8, 125.7, 120.2, 115.2, 114.2, 112.4, 110.5, 93.5, 92.2, 89.7, 86.9, 67.6, 55.5, 28.9, 25.6. HRMS calcd for $C_{52}H_{42}O_4$, Mr = 730.3083; found 730.3088.

5.1.18. 1,6-Bis(4-((2-((2-trifluoromethylphenyl)ethynyl)-phenyl)ethynyl)phenoxy)hexane (**8f**)

According to general procedure, we can obtain compound **8f**. The compound was purified by column chromatography, eluting with hexane/EA (3:1) to give 42% of yellow solid according to general procedure. Mp: 147–148 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (t, 4H, $J = 8.0$ Hz), 7.59–7.43 (m, 10H), 7.41 (t, 2H, $J = 8.0$ Hz), 7.35–7.28 (m, 4H), 6.87 (dd, 4H, $J = 6.8$, 2.4 Hz), 3.9 (t, 4H, $J = 6.4$ Hz), 1.84 (t, 4H, $J = 6.4$ Hz), 1.56 (quin, 4H, $J = 3.6$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.2, 134.0, 133.1, 132.3, 131.8, 131.3, 128.5, 128.0, 127.6, 126.0, 125.8, 124.9, 124.8, 122.1, 124.6, 115.1, 114.4, 93.8, 89.0, 86.6, 67.8, 29.1, 25.8. HRMS calcd for $C_{52}H_{36}F_6O_2$, Mr = 806.2619; found 806.2623.

5.1.19. 1,6-Bis(4-((2-(pyridin-2-ylethynyl)phenyl)ethynyl)-phenoxy)hexane (**8g**)

According to general procedure, we can obtain compound **8g**. The compound was purified by column chromatography, eluting with hexane/EA (3:1) to give 38% of yellow solid according to general procedure. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63 (d, 2H, $J = 4.4$ Hz), 7.65–7.60 (m, 4H), 7.55–7.52 (m, 8H), 7.44–7.20 (m, 6H), 6.86–6.80 (m, 4H), 3.97 (td, 4H, $J = 6.4$, 3.6 Hz), 1.82 (t, 4H, $J = 6.4$ Hz), 1.52 (quin, 4H, $J = 3.2$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.2, 149.9, 143.4, 136.0, 133.9, 133.1, 132.1, 131.4, 128.6, 127.5, 127.2, 126.6, 124.4, 122.7, 115.0, 114.5, 114.4, 94.1, 92.3, 88.1, 86.7, 67.8, 29.0, 25.7. HRMS (FAB^+) calcd for $C_{48}H_{37}N_2O_2$ $(M + H)^+$, Mr = 672.2777; found 673.2857.

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