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Synthesis and DNA cleaving activity of water-soluble non-conjugated thienyl tetraynes

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Abstract—Water-soluble non-conjugated thienyl tetraynes (**3**–**6**) were synthesized and their DNA cleaving activity was evaluated using electrophoresis, atomic force microscopy (AFM) and *Escherichia coli* (*E. coli*) transformation techniques. The amino-functionalized compound **4** was shown to possess an activity to cleave plasmid DNA by both electrophoresis and *E. coli* transformation techniques. AFM also showed a cleavage of the circular DNA into a linear form with a formation of burst-star-shaped architectures, which were envisaged to be cross-linked DNA oligomers.

1. Introduction

Cyclization reactions of conjugated polyyne systems such as ene–diyne, enyne–allene, and enyne–ketene, forming biradical species as intermediates, have been reported in the 1960s–1970s in the field of structural organic chemistry.[1] On the other hand, from the 1980s to date, many antitumor natural products possessing ene–diyne structures, as shown in Figure 1, have been isolated,[2] and their molecular target was identified as DNA.[2,3] These natural products share some structural and functional similarities. One fragment of a structure is responsible for the recognition and transport, another part acts as a molecular trigger while the third, the reactive ene–diyne unit, undergoes a cycloaromatization (CA) to cleave the backbone of DNA. Although members of the ene–diyne family are already in clinical use to treat various cancers, more general use is limited by their complex structures. Therefore, in order to develop more simple, selective, and potent anticancer agents, there have been many reports on the design and synthesis of artificial ene–diyne compounds,[2c,4] but to date, there have been few studies to change the conjugated ene–diyne core structure. As a new entry to DNA cleaving molecules, we focused on non-conjugated ene–yne systems with an interest to investigate the reactivity of ene–polyyne systems,[5] and as expected, non-conjugated aryl (phenyl and pyridyl) tetraynes (TYs) were found to undergo CA by the way of biradical species with the breakage of a single chain of duplex DNA.[6]

Despite their potential importance in therapeutic application, the TYs we developed have suffered from obstacles in their use under physiological conditions due to their hydrophobicity and lack of a triggering system to generate active biradical species. Thus, to solve these problems, we planned to introduce hydrophilic functionalities and to set a trigger on aryl TYs. Fortunately, during the course of our study to investigate the reactivity of aryl TYs, we recently have found that the CA reaction of thienyl TY **A** does not proceed even at 37 °C, but that of the corresponding ketone derivative **B** proceeds smoothly to form **E** (Scheme 1).[7,8] Since this property was envisaged to be useful as a triggering device, thienyl TY was selected as a core structure. According to the proposed mechanism of the CA of non-conjugated thienyl TYs[8] (Scheme 1), two active species capable of cleaving DNA can be proposed. One is biradical **C1**, which breaks the DNA backbone as a

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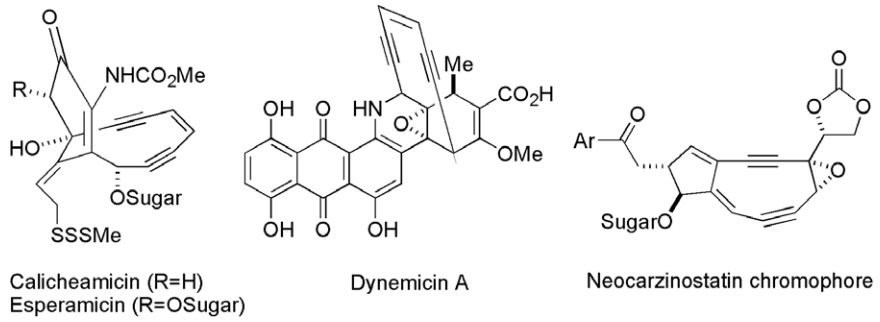
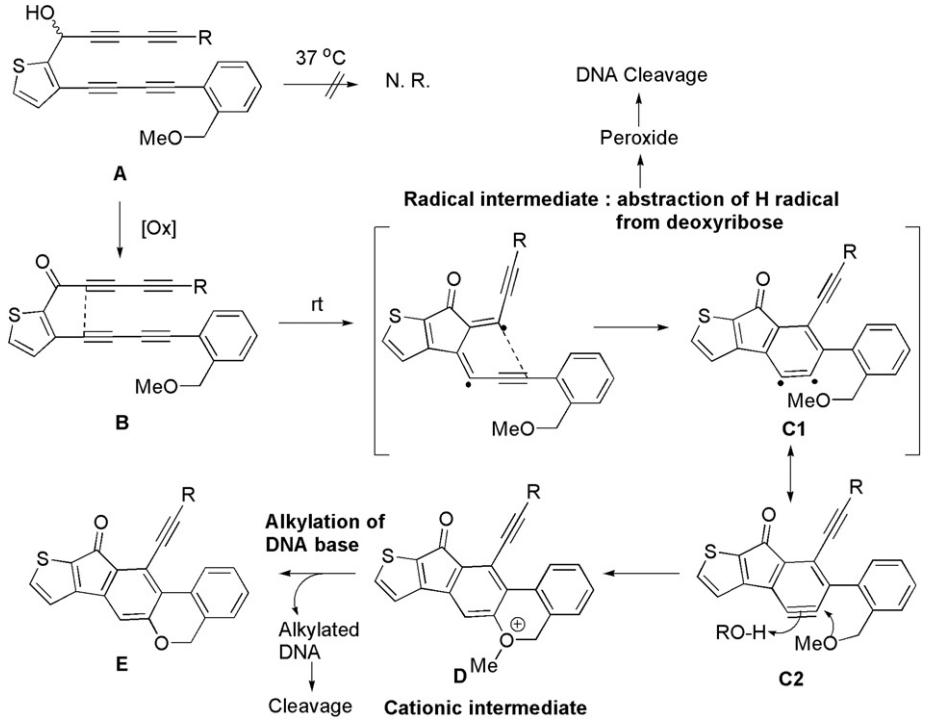


Figure 1. Ene–yne antitumor agents from natural resources.



Scheme 1. Proposed mechanism for thermal cycloaromatization of thienyl tetraynes and hypothesis of dual DNA cleaving pathway.

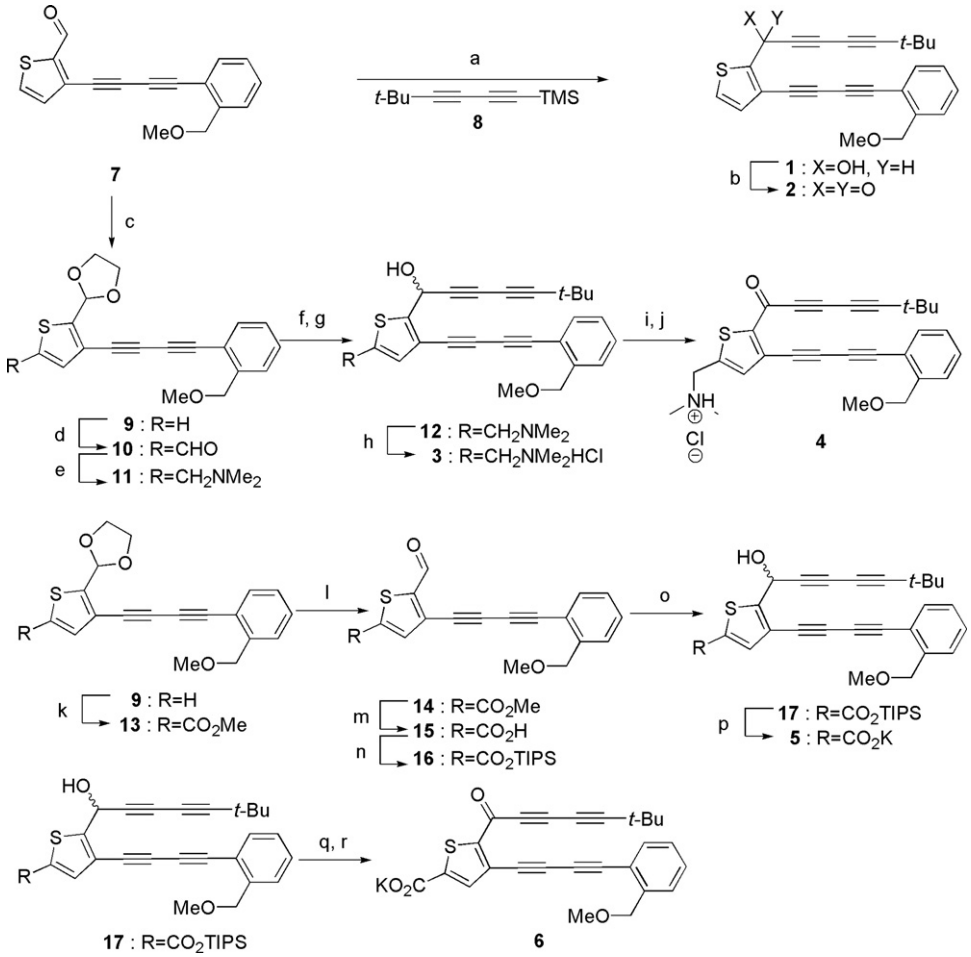
result of abstraction of a hydrogen atom from deoxyribose-phosphate chains, and the other is cation **D** which alkylates DNA bases to cleave. There have been few reports of ene–yne compounds which generate both radical and cationic active species simultaneously,[9] hence, if the reactivity, recognition, and transport could be well controlled, thienyl TY might be a good candidate as a new ene–yne antitumor agent, for instance, to cure the multi-drug resistant cancers. Herein, we report the synthesis of water-soluble thienyl TYs and the evaluation of their DNA damaging abilities via electrophoresis, atomic force microscopy (AFM), and *Escherichia coli* (*E. coli*) transformation techniques (Scheme 2).

2. Results and discussion

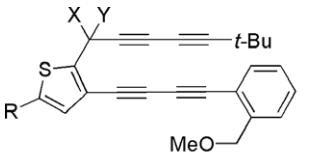
2.1. Design and synthesis of thienyl tetraynes

We designed water-soluble thienyl TYs (**2**, **4**, and **6**) taking into account the following points (Fig. 2): (i) diethynyl terminus was changed from the originally reported trimethylsilyl group[7,8] into a *tert*-butyl group in order to improve the stability; (ii) as hydrophilic functionalities, dimethylamino and carboxy groups, which are positively and negatively charged, respectively, in physiological conditions, were selected for a facile structure–activity relationship (SAR) study; (iii) the above functionalities were introduced onto the thiophene ring at locations where these were believed to least likely affect the reactivity of the thermal cyclization of the TY moiety as well as to be more readily synthesized. In addition, alcohols (**1**, **3**, and **5**), whose thermal CA does not occur at 37 °C, were synthesized to demonstrate whether DNA damage is definitely induced via thermal CA reactions.

First of all, the synthesis of hydrophobic thienyl TY **2** commenced with the previously reported aldehyde **7**.[8] Aldehyde **7** was treated with diethynyllithium, which was generated in situ from butadiyne **8**[10] and MeLi–LiBr in refluxing methyl *tert* -butyl ether, to give racemic



Scheme 2. Reagents and conditions: (a) **8**, MeLi, MeO-*t*-Bu, reflux, 3.5 h, then **7**, −70 to −55 °C, 1.5 h, 88%; (b) MnO_2, Na_2SO_4, CH_2Cl_2, 0–7 °C, 15.5 h, quant.; (c) ethyleneglycol, PPTS, benzene, Dean-Stark trap, reflux, 21 h, 99%; (d) *n*-BuLi, THF, −78 °C, 5 min, then DMF, −78 °C–rt, 2.5 h, 84%; (e) $HNMe_2HCl$, Et_3N, $Ti(O\text{-}i\text{-}Pr)_4$, EtOH, rt, 19 h, then $NaBH_4$, rt, 3.5 h; (f) PPTS, acetone, H_2O, reflux, 2 days, 91% (two steps); (g) *t*-Bu–C≡C–C≡C–Li, MeO-*t*-Bu, Et_2O, THF, −70 to −55 °C, 1.5 h, 66%; (h) HCl/MeOH, $CHCl_3$, 0 °C, 5 min, quant.; (i) MnO_2, Na_2SO_4, 0–7 °C, CH_2Cl_2, 16 h; (j) HCl/MeOH, CH_2Cl_2, $CHCl_3$, 0 °C, 56% (two steps); (k) *n*-BuLi, THF, −78 °C, 5 min, then $ClCO_2Me$, −78 °C, 30 min, 80%; (l) PPTS, acetone, H_2O, reflux, 29 h, 92%; (m) 0.1 M aqueous NaOH, THF, rt, 70 min, quant.; (n) TIPSCl, imidazole, CH_2Cl_2, rt, 1 h, 92%; (o) *t*-Bu–C≡C–C≡C–Li, MeO-*t*-Bu, Et_2O, −72 to 0 °C, 1.5 h, 40%; (p) K_2CO_3 (0.5 equiv), MeOH, 0–4 °C, 41 h, 75%; (q) MnO_2, Na_2SO_4, CH_2Cl_2, 0–7 °C, 27 h, 98%; (r) K_2CO_3 (0.5 equiv), MeOH, ether, $CHCl_3$, 0 °C, 20 min, 77%.



1 : R=H, X=OH, Y=H
2 : R=H, X=Y=O
3 : R=CH_2NMe_2HCl, X=OH, Y=H
4 : R=CH_2NMe_2HCl, X=Y=O
5 : R=CO_2K, X=OH, Y=H
6 : R=CO_2K, X=Y=O

Figure 2. Structures of functionalized thienyl tetraynes.

alcohol **1** in 88%. The final oxidation required to be conducted at around 0 °C, without chromatographic purification if possible, as thermal cyclization of ketone **2** was expected to proceed even at room temperature. Considerable experimentations revealed that MnO_2 in the presence of Na_2SO_4 was the best oxidant to furnish ketone **2** in excellent yield (quantitative). Since we successfully constructed a common core structure, we moved on to the synthesis of dimethylamino-functionalized thienyl TY **4**, starting with the protection of common intermediate **7**, as acetal **9** (99%). Due to the fact that previous attempts at the one-step introduction of a dimethylaminomethyl group to the thiophene ring of **9** via a Mannich-type reaction had failed,[11] a sequential method was examined. Lithiation of **9** with *n*-butyllithium, followed by treatment with DMF furnished formylthiophene **10** (84%), which was converted to dimethylaminomethylthiophene **11** via titanium tetraisopropoxide mediated reductive amination.[12] Removal of the acetal from **11** with pyridinium *p*-toluenesulfonate (PPTS), followed by the addition of diethynyllithium to the resulting aldehyde gave TY **12** in 60% for three steps. Although the final oxidation of **12** using MnO_2 proceeded smoothly to give the corresponding ketone, the resulting amino-free ketone after drying, was found to be unstable, even at −20 °C in an argon atmosphere. Therefore, amino-free alcohol **12** was oxidized with

MnO_2 to the ketone, and converted immediately to the corresponding hydrochloride **4** using 10% HCl–MeOH, followed by evaporation at 0 °C. As a result, synthesis of the desired ketone **4**, which was clarified to be isolable, was achieved.

The final task, synthesis of the carboxy-functionalized thienyl TY **6**, commenced with acetal **9**. Lithiation of **9** followed by treatment with methyl chloroformate gave methoxycarbonyl thiophene **13** in 80%. Removal of the acetal from **13** with PPTS (**14**, 92%), followed by saponification, gave carboxylic acid **15** in quantitative yield. Carboxylic acid **15** was protected as triisopropylsilyl (TIPS) ester (**16**, 92%), and subjected to the addition of diethynyllithium to give TY **17** (40%).[13] Finally, MnO_2-oxidation of **17**, followed by the removal of the TIPS group using 0.5 equivalent of potassium carbonate afforded potassium salt **6** in 75% for two steps. The corresponding alcohol **5** was also prepared (75%) as a negative control in experiments.[14]

2.2. Evaluation of DNA cleaving activities of thienyl tetraynes

2.2.1. Electrophoresis. With functionalized thienyl TYs in hand, we turned our attention to the evaluation of their DNA cleaving abilities. First of all, after treatment with thienyl TYs[15] in the presence of DMSO (3%), pBluescript II KS^+ (PB) plasmid DNA was analyzed by agarose gel electrophoresis (Fig. 3a). As shown in lane 1, intact PB exists mostly as a supercoiled form (form I). When PB was treated with cyclophosphamide (CPA, 10 mM), a clinically used anticancer DNA cleaving agent by alkylation, a smear at the lower molecular weight (ca. 700 bp) region was observed, indicative of the cleavage of DNA to small pieces (lane 2). On the other hand, although only a slight or almost no change was observed by treating with alcohol **3**, **5**, and carboxy-functionalized ketone **6** (1.0 mM, lane 3, 5, and 6, respectively), a significant decrease in form I with a concomitant increase in form II (nicked) and form III (linear) was observed, when treated with amino-functionalized ketone **4** (lane 4). Since carboxy-functionalized ketone **6** was supposed to be less accessible to negatively charged DNA due to its anionic properties, it was reasonable to find that ketone **6** was inactive, even though it undergoes CA under the conditions examined. Contrary to **6**, amino-functionalized ketone **4**, which is considered to interact more potently with DNA owing to Coulomb force, exhibited DNA cleaving activity, as evidenced by a significant conversion of the circularized form I to the nicked form II and/or the linearized form III. Furthermore, electrophoretic analysis of the above DNA after treatment with **4** at the lower concentrations (0.1, 0.3, 0.6, and 1 mM, respectively, Fig. 3b) revealed that the DNA cleaving activity of **4** is dose-dependent, and ca. 100 μM (lane 2) would be a minimum concentration to elicit the activity. Meanwhile, a rough comparison of the half-lives of ketones **2**, **4**, and **6** by measuring the time-dependent changes of ^1H NMR indicated that the rate of the CA reaction was **4** > **2** > **6**, an order, which corresponds to the electron-poverty of the thienyl moiety of each ketone. Therefore, the dimethylaminomethyl group may increase the DNA cleaving activity of **4**, not only by enhancing the electrostatic interaction with DNA, but also by providing an electron-withdrawing effect. On the other hand, treatment with **4** also gave an intriguing smear at the higher molecular weight region, concomitant with form II and III DNA (lane 4). For the rational explanation of this unexpected observation, we next examined directly the molecular shape of DNA after treatment with **4** using an atomic force microscope.

2.2.2. Atomic force microscopy. Recently, AFM has attracted much attention in the field of nano-biotechnology.[16] Because AFM is a powerful tool in that it enables the direct observation of a single biomolecule without special treatments, we envisaged that AFM is the most suitable method to accurately visualize the state of DNA,[17] after treatment with **4**, which gave an aberrant electrophoretic pattern. Figure 4a and b are AFM images of intact PB plasmid DNA showing that almost all the PB molecules exist in a supercoiled form I. Contrary to these, images after treatment of **4** (1.0 mM, Fig. 4c–e) indicated both the cleavage of circular DNA into a linear form (form III, Fig. 4d) and the formation of burst-star-shaped architectures (Fig. 4e), that are consistent with the results obtained by electrophoresis. Although the precise structure of the burst-star-shaped architectures was unclear, these can be considered as DNA oligomers resulting from intermolecular conjunction between linear DNA radicals. This unique topological change of DNA by ketone **4**, which differs from that by CPA (treatment with CPA resulted in only the scission of PB, which was confirmed by both electrophoresis and AFM, data not shown), inspired us to evaluate the functional change of PB using a biological method.

2.2.3. Evaluation of functional damage of PB via transformation of *E. coli*. Since PB is a designed plasmid DNA routinely used in cloning application, transformation by PB renders *E. coli* resistant to ampicillin, an antibiotic agent. We postulated that if the function of PB was destroyed by ketone **4**, *E. coli* transformed by damaged PB would not survive in ampicillin-containing media.

The results are depicted in Table 1. When intact PB was introduced into *E. coli* (DH5α or JM109 competent cells), *E. coli* became resistant to ampicillin, yielding many (more than 1000) colonies on Luria–Bertani (LB) agar plates-containing ampicillin. On the other hand, when PB was pretreated with CPA (10 mM), an alkylative DNA cleaving agent, no colonies were observed. As for TYs (1.0 mM), contrary to the observation of many colonies using PB pretreated with **3**, **5**, and **6**, treatment with amino-functionalized ketone **4** yielded only eight colonies indicating the functional disruption of PB. In addition, due to the fact that treatment with both amino-functionalized alcohol **3** and trimethylamine hydrochloride did not affect ampicillin resistance, the functional change is not a result of aggregation of DNA due to an ionic interaction between the dimethylamino group and phosphate (of DNA) neutralizing the

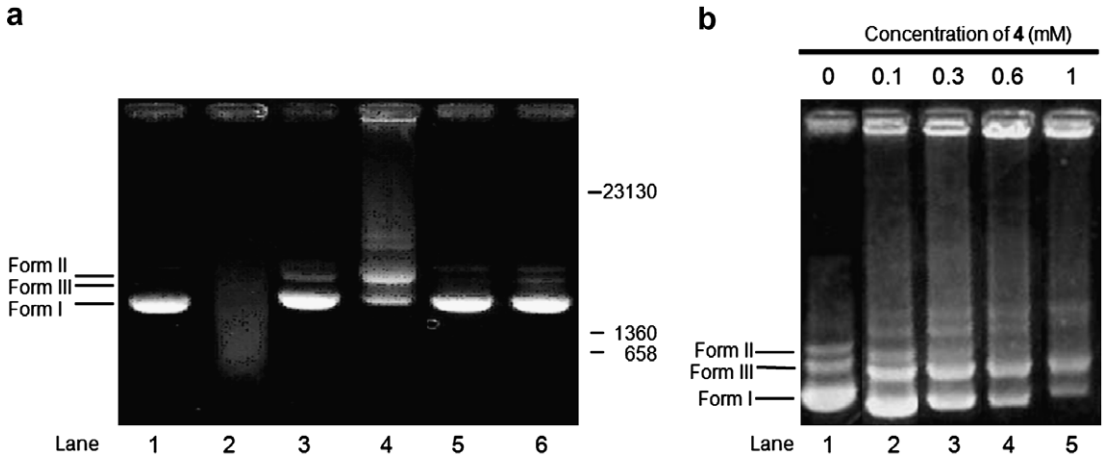


Figure 3. Agarose gel electrophoresis of pBluescript II KS$^+$ plasmid DNA in the presence of DNA cleaving agents. (a) pBluescript II KS$^+$ (2 μg) alone (lane 1); in the presence of cyclophosphamide (CPA, 10 mM, lane 2); in the presence of alcohol **3** (1.0 mM, lane 3); in the presence of ketone **4** (1.0 mM, lane 4); in the presence of alcohol **5** (1.0 mM, lane 5); in the presence of ketone **6** (1.0 mM, lane 6); (b) pBluescript II KS$^+$ (2 μg) alone (lane 1); in the presence of **4** (0.1 mM, lane 2; 0.3 mM, lane 3; 0.6 mM, lane 4; 1 mM, lane 5, respectively). DNA was visualized via treatment of ethydium bromide, followed by the irradiation of UV.

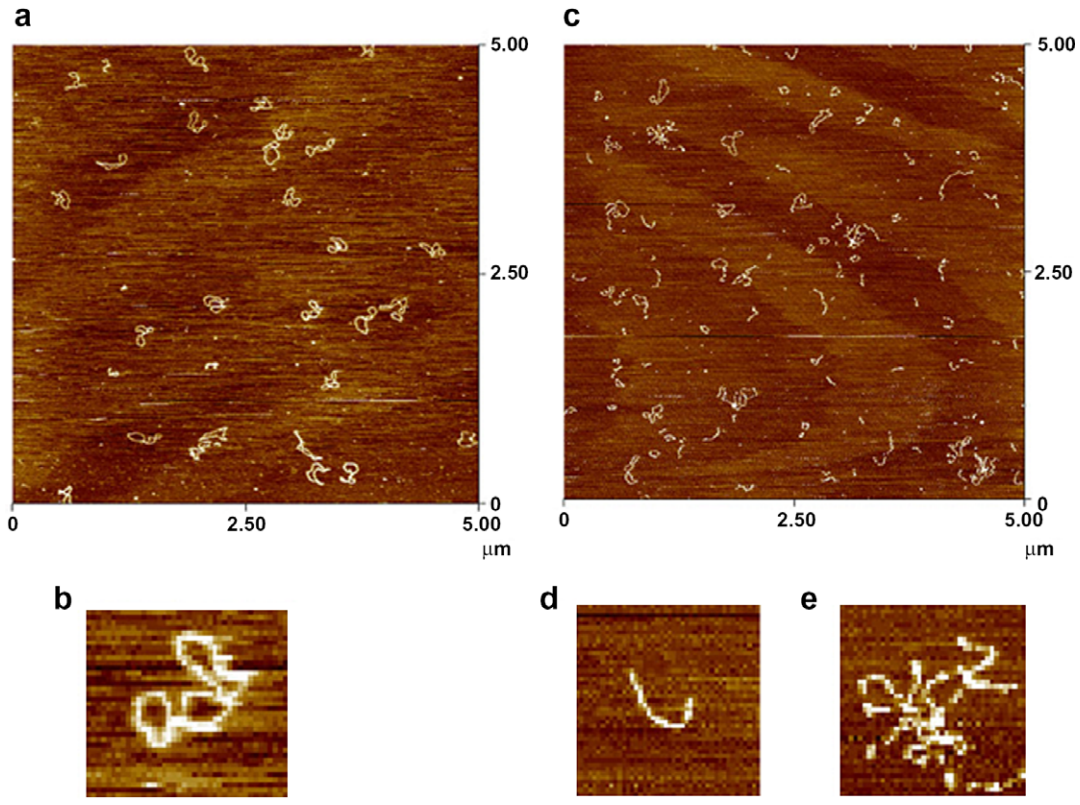


Figure 4. Direct observation of pBluescript II KS$^+$ plasmid DNA by atomic force microscopy. (a) Intact pBluescript II KS$^+$ on a flat mica plate; (b) a representative image of a PB plasmid DNA molecule in supercoiled form from (a); (c) PB after treatment of amino-functionalized ketone **4** (1.0 mM, 37 °C, 6 h); (d) a representative image of cleaved pieces of PB molecule from (c); (e) a representative image of the burst-star-shaped architecture from (c).

Table 1. Numbers of *E. coli* colonies transformed by PBs after treatment with compounds (1.0 mM for tetraynes and trimethylamine hydrochloride, 10 mM for CPA) in ampicillin-containing LB media

Compound	None	CPA	**3**	**4**	**5**	**6**	Me_3N HCl
Colonies/plate[a]	>1000	0	>1000	8	>1000	>1000	>1000

[a] Incubated at 37 °C for 15 h on LB plates.

charge, but from the active species via CA reaction of an enediyne core. Furthermore, other radical species, for example, hydroxyl radical, derived from hydrogen abstraction from water by biradical intermediates, would not be involved in damaging DNA, because the CA reaction of ketone **6** did not cause DNA damage. These findings implied that TYs bound to DNA to exert their activities. A facile SAR study of thienyl TYs in this work should provide important information for improving affinity to DNA and setting a better triggering device, which are expected to be achieved in the near future, to lead TYs to a new class of ene–yne antibiotics.

3. Conclusion

We synthesized water-soluble thienyl tetraynes and evaluated their DNA cleaving activities via electrophoresis, atomic force microscopy and *E. coli* transformation techniques. As a result, it was found that positively charged dimethylamino-fuctionalized thienyl tetrayne **4** cleaves pBluescript II KS^+ plasmid DNA whereas carboxylate **6** is ineffective. Furthermore, concomitant with topological change, the biological function of DNA, as observed in an *E. coli* transformation assay, was also abrogated. The burst-star-shaped architectures of plasmid DNA, which were visualized by atomic force microscopy (AFM), are believed to be covalently cross-linked DNA oligomers, and are a unique feature of **4**, suggesting the involvement of biradical species, additionally to that of previously reported cationic species[8] to cleave DNA. Because of the dual DNA cleaving species, thienyl tetrayne **4** is potentially a lead compound for a novel family of DNA cleaving agents. Further investigations on DNA cleaving cycloaromatization of non-conjugated ene–tetrayne system are now in progress.

4. Experimental

4.1. General methods and instruments for organic synthesis

Anhydrous diethyl ether, methyl *tert*-butyl ether, tetrahydrofuran (THF), and *N*,*N*-dimethylformamide (DMF) were purchased from Kanto Chemical Co. Inc. or Wako Pure Chemical Industries Ltd and used without further drying. Other solvents were dried over activated molecular sieves 4A (3A for methanol). All the other chemicals were obtained from local venders, and used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} pre-coated plates (0.25-mm thickness). For column chromatography, silica gel 60 (70–270 mesh ASTM, Merck), aluminum oxide 90 (Merck), and florisil® (75–150 μm, Wako) were used. For high performance liquid chromatography (HPLC), JEOL RI-2031 UV-2075 PU-2086 system was used. IR spectra were recorded on a Shimadzu FT-IR-8300. NMR spectra were recorded on a JEOL JNM-LA400 (400 MHz). Chemical shifts were reported in ppm from tetramethylsilane with reference to internal residual solvent [^{1}H NMR, $CHCl_3$ (7.26), CHD_2OD (4.78), $CHDCl_2$ (5.32); ^{13}C NMR, $CDCl_3$ (77.0), CD_3OD (49.0), CD_2Cl_2 (53.1). The following abbreviations are used to designate the multiplicities: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet. Mass spectra were recorded on a JEOL JMS600 under FAB conditions using *m*-nitrobenzyl alcohol (NBA) as a matrix. Combustion elemental analyses were performed using Perkin-Elmer 240C. Melting point of solid samples was measured on a device from Gallenkamp.

4.2. Synthesis of thienyl tetraynes

4.2.1. 1-{3-[4-(2-Methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-6,6-dimethyl-hepta-2,4-diyn-1-ol (1). In argon atmosphere, to a solution of asymmetric butadiyne **8** (975 mg, 5.47 mmol) in methyl *tert*-butyl ether (20 mL) was added methyllithium–lithium bromide complex (1.5 M, in ether, 1.9 mL, 2.7 mmol) at room temperature. The mixture was stirred under reflux for 3.5 h, and the resulting alkynyl lithium solution was added to a solution of aldehyde **7** (300 mg, 1.07 mmol) in ether (10 mL) at −78 °C. After being stirred at −78 °C for 1.5 h, the mixture was treated with satd NH_4Cl aq to quench the reaction, and diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford alcohol **1** (363 mg, 88%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.40 (dd, J = 7.6, 7.6 Hz, 1H), 7.25–7.29 (m, 2H), 7.07 (d, J = 5.1 Hz, 1H), 5.99 (d, J = 5.4 Hz, 1H), 4.66 (s, 2H), 3.48 (s, 3H), 2.46 (d, J = 5.4 Hz, 1H), 1.24 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 141.5, 133.1, 130.2, 129.5, 127.5, 127.4, 125.3, 120.1, 118.8, 90.7, 80.3, 77.8, 77.7, 75.4, 74.5, 72.4, 71.4, 62.9, 59.3, 58.6, 30.3, 28.0; IR (ether, $CDCl_3$): 3424, 2401, 2251 cm^{-1}; FAB-MS (NBA) *m*/*z* 369 [$(M-OH)^+$]; Anal. Calcd for $C_{25}H_{22}O_2S$: C, 77.69; H, 5.74; S, 8.30. Found: C, 77.44; H, 5.97; S, 8.67.

4.2.2. 1-{3-[4-(2-Methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-6,6-dimethyl-hepta-2,4-diyn-1-one (2). To a solution of alcohol **1** (200 mg, 0.518 mmol) in CH_2Cl_2 (10 mL) was added Na_2SO_4 (300 mg, 2.11 mmol) and active γ-MnO_2 (4.0 g, 46 mmol) at 0 °C. After being vigorously stirred at 0–7 °C for 15.5 h, the heterogeneous mixture was filtered and the filtrate was concentrated at 0 °C to give ketone **2** (199 mg, quantitative) as a yellow powder: mp 90.7 °C (dec); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.65 (d, J = 5.1 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.42 (ddd, J = 1.2, 7.3, 7.8 Hz, 1H), 7.29 (m, 2H), 4.65 (s, 2H), 3.45 (s, 3H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 166.6, 145.7, 141.8, 133.5, 133.2, 132.8, 129.4, 127.2, 127.0, 125.2, 119.4, 98.6, 81.7, 80.1, 78.2, 77.4, 75.9, 72.0, 71.9, 62.3, 58.1, 29.3, 28.1; IR (CD_2Cl_2): 2304, 2224, 2199, 1602 cm^{-1}; FAB-MS (NBA) *m*/*z* 385 [$(M+H)^+$].

4.2.3. 2-{3-[4-(2-Methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-[1,3]dioxolane (9). To a solution of aldehyde **7** (8.25 g, 29.4 mmol) in benzene (400 mL) was added ethyleneglycol (8.2 mL, 147 mmol) and

pyridinium *p*-toluenesulfonate (PPTS, 740 mg, 2.95 mmol). The mixture was stirred under reflux for 37 h in a glassware equipped with a Dean-Stark trap, and the resulting mixture was treated with satd $NaHCO_3$ aq at room temperature to quench the reaction and diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 7:1) to afford acetal **9** (9.07 g, 95%) as a pale yellow powder: mp. 73.2–73.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.38 (dd, J = 7.6, 7.8 Hz, 1H), 7.27 (d, J = 5.1 Hz, 1H), 7.26 (dd, J = 7.8, 7.9 Hz, 1H), 7.10 (d, J = 5.1 Hz, 1H), 6.31 (s, 1H), 4.66 (s, 2H), 4.17 (m, 2H), 4.06 (m, 2H), 3.47 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.1, 141.6, 133.1, 130.5, 129.4, 127.4, 127.3, 125.7, 120.1, 120.1, 98.9, 80.0, 77.9, 77.0, 75.6, 72.4, 65.5, 58.6; IR ($CDCl_3$): 2401 cm^{-1}; FAB-MS (NBA) *m*/*z* 325 [(M+H)$^+$]; Anal. Calcd for $C_{19}H_{16}O_3S$: C, 70.35; H, 4.97; S, 9.88. Found: C, 70.20; H, 5.19; S, 9.84.

4.2.4. 5-[1,3]Dioxolan-2-yl-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carbaldehyde (10). In argon atmosphere, to a solution of acetal **9** (320 mg, 0.983 mmol) in THF (15 mL) was added *n*-butyllithium (1.5 M in hexane, 0.80 mL, 1.2 mmol) dropwise at −78 °C. The mixture was stirred for 5 min, and to the resulting bright blue solution was added DMF (0.12 mL, 1.6 mmol) and stirred for 2 h at temperature elevated to −50 °C. The reaction mixture was quenched with satd NH_4Cl aq and the resulting mixture was diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to afford aldehyde **10** (306 mg, 88%) as an orange powder: mp 66.8–68.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 7.74 (s, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 7.6 Hz, 1H), 7.40 (dd, J = 7.3, 7.7 Hz, 1H), 7.27 (dd, J = 7.6, 7.3 Hz, 1H), 6.31 (s, 1H), 4.66 (s, 2H), 4.18 (m, 2H), 4.08 (m, 2H), 3.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.6, 156.0, 142.4, 141.7, 138.8, 133.2, 129.7, 127.5, 127.4, 120.8, 119.7, 98.6, 80.8, 77.9, 77.4, 73.8, 72.4, 65.7, 58.6; IR ($CDCl_3$): 2254, 1680 cm^{-1}; FAB-MS (NBA) *m*/*z* 353 [(M+H)$^+$], 321 [(M−OMe)$^+$]; Anal. Calcd for $C_{20}H_{16}O_4S$: C, 68.16; H, 4.58; S, 9.10. Found: C, 68.21; H, 4.93; S, 8.79.

4.2.5. 1-{5-Dimethylaminomethyl-3-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-6,6-dimethyl-hepta-2,4-diyn-1-ol (12). In argon atmosphere, to a solution of aldehyde **10** (1.317 g, 3.72 mmol) in ethanol (50 mL) were added triethylamine (2.60 mL, 18.6 mmol), dimethylamine hydrochloride (1.50 g, 18.4 mmol), and titanium tetra *i*-propoxide (2.2 mL, 7.5 mmol) at room temperature. After being stirred at room temperature for 20 h, the reaction mixture was cooled to 0 °C, followed by the addition of sodium borohydride (700 mg, 18.5 mmol). The mixture was stirred at room temperature for 5.5 h, and the reaction was quenched with concentrated aqueous ammonia with vigorous stirring for 20 min. The resulting slurry was filtered and the filtrate was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residual oil was roughly purified by alumina column chromatography (hexane/ethyl acetate = 7:1) to give a crude product **11** which was used in the next reaction without further purification. To a solution of the above crude **11** in a mixed solvent of acetone/H_2O = (20 mL:3 mL), was added PPTS (4.00 g, 15.9 mmol) at room temperature. After being stirred under reflux for 46 h, the reaction mixture was cooled to room temperature and the reaction was quenched with satd $NaHCO_3$ aq and the resulting mixture was diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by alumina column chromatography (hexane/ethyl acetate = 5:1) to afford aldehyde (1.15 g, 91% for two steps) as orange oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.10 (s, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 7.6, 7.6 Hz, 1H), 7.29 (dd, J=7.6, 8.1 Hz, 1H), 7.06 (s, 1H), 4.66 (s, 2H), 3.63 (s, 2H), 3.48 (s, 3H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.1, 153.3, 145.3, 141.8, 133.2, 129.8, 129.6, 128.6, 127.6, 127.4, 119.5, 81.8, 79.5, 77.3, 74.5, 72.3, 58.5, 58.3, 45.2; IR ($CDCl_3$): 2252, 2217, 1666 cm^{-1}; FAB-MS (NBA) *m*/*z* 338 [(M+H)$^+$], 307 [(M−OMe)$^+$]; Anal. Calcd for $C_{20}H_{19}NO_2S$: C, 71.19; H, 5.68; N, 4.15; S, 9.50. Found: C, 71.41; H, 5.73; N, 4.04; S, 9.40.

In argon atmosphere, to a solution of asymmetric diyne **8** (1.3 g, 6.7 mmol) in methyl *tert*-butyl ether (35 mL) was added methyllithium–lithium bromide complex (1.4 M in ether, 2.5 mL, 3.5 mmol) at room temperature. The reaction mixture was stirred under reflux for 3.5 h, and the resulting diethynyl lithium reagent was cooled to 0 °C. In argon atmosphere, at −70 °C, to the solution of the aldehyde (300 mg, 0.889 mmol) in THF (15 mL) was added the above diethynyllithium solution. After being stirred at −70 to −55 °C for 1.5 h, the reaction mixture was treated with satd NH_4Cl aq to quench the reaction, and the resulting mixture was diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to afford alcohol **12** (263 mg, 66%) as a pale yellow powder: mp 124.5 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.39 (ddd, J = 1.2, 7.6, 7.6 Hz, 1H), 7.26 (dd, J = 7.6, 7.8 Hz, 1H), 6.86 (s, 1H), 5.93 (s, 1H), 4.66 (s, 2H), 3.57 (s, 2H), 3.48 (s, 3H), 2.86 (broad s, 1H), 2.29 (s, 6H), 1.24 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.9, 141.6, 141.5, 133.1, 129.4, 128.7, 127.4, 127.3, 120.1, 117.9, 90.4, 80.2, 77.9, 77.2, 75.8, 74.9, 72.4, 71.1, 63.1, 59.3, 58.6, 57.9, 44.8, 30.3, 28.0; IR ($CDCl_3$): 3422, 2401, 2254 cm^{-1}; FAB-MS (NBA) *m*/*z* 442 [(M−H)$^+$], 426 [(M−OH)$^+$]; Anal. Calcd for $C_{28}H_{29}NO_2S$: C, 75.81; H, 6.59; N, 3.19; S, 7.23. Found: C, 75.51; H, 6.70; N, 3.12; S, 7.00.

4.2.6. 1-{5-Dimethylaminomethyl-3-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-6,6-dimethyl-hepta-2,4-diyn-1-ol hydrochloride (3). At 0 °C, to a solution of alcohol **12** (108 mg, 0.243 mmol) in $CHCl_3$ (5 mL) was added HCl–methanol solution (10%, 0.17 mL, 0.36 mmol). The reaction mixture was stirred at 0 °C for 5 min, and the solvent was removed in vacuo. To the residual oil was then added ether and the resulting gummy slurry was irradiated by ultrasound to solidify. The solvent was removed to dryness to afford HCl salt **3** (118 mg, quantitative) as a colorless powder: mp 109.3 °C (dec); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 11.93 (broad s, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.39 (m, 2H), 7.27 (dd, J = 7.3, 7.6 Hz, 1H), 5.95 (s, 1H), 5.73 (broad s, 1H), 4.61 (s, 2H), 4.44 (broad s, 2H), 3.43 (s, 3H), 2.84 (s, 6H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 141.5, 135.0, 132.8, 129.2, 128.5, 127.3, 127.1, 119.5, 117.5, 90.3, 80.4, 77.7, 77.1, 74.7, 74.5, 72.0, 70.3, 62.5, 58.7, 58.1, 54.0, 41.5, 29.7, 27.6; IR ($CDCl_3$): 3232, 2304, 2198 cm^{-1}; FAB-MS (NBA) m/z 444 [(M−Cl)$^+$]; Anal. Calcd for $C_{28}H_{30}ClNO_2S$: C, 70.05; H, 6.30; N, 2.92. Found: C, 70.06; H, 6.34; N, 2.88.

4.2.7. 1-{5-Dimethylaminomethyl-3-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophen-2-yl}-6,6-dimethyl-hepta-2,4-diyn-1-one hydrochloride (4). At 0 °C, to a solution of alcohol **12** (150 mg, 0.338 mmol) in a mixed solvent (CH_2Cl_2/$CHCl_3$ = 4.5 mL:3.0 mL) were added Na_2SO_4 (300 mg, 2.11 mmol) and active γ-MnO_2 (2.9 g, 33 mmol). After being vigorously stirred at 0–7 °C for 16 h, the heterogeneous mixture was filtered. To the filtrate was added HCl–methanol solution (10%, 0.24 mL, 0.51 mmol) and the solvent was removed at 0 °C in vacuo. The residue was washed with ether and hexane, followed by drying at 0 °C to afford HCl salt **4** (90 mg, 56%) as a yellow powder: mp 83.5 °C (dec); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 13.32 (broad s, 1H), 7.74 (s, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.42 (dd, J = 7.3, 7.6 Hz, 1H), 7.28 (dd, J = 7.3, 7.6 Hz, 1H), 4.63 (s, 2H), 4.45 (s, 2H), 3.44 (s, 3H), 2.82 (s, 6H), 1.19 (s, 9H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 166.3, 147.4, 141.9, 137.9, 137.0, 132.9, 129.6, 127.2, 127.0, 125.3, 119.1, 99.4, 82.3, 80.9, 79.4, 77.1, 75.0, 72.0, 71.6, 62.2, 58.1, 53.5, 41.6, 29.2, 28.1; IR ($CDCl_3$): 2304, 2223, 2198, 1604 cm^{-1}; FAB-MS (NBA) m/z 442 [(M−Cl)$^+$].

4.2.8. 5-[1,3]Dioxolan-2-yl-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid methyl ester (13). In argon atmosphere, to a solution of acetal **9** (2.50 g, 7.71 mmol) in THF (80 mL) at −78 °C was added n-butyllithium (1.4 M in hexane, 6.5 mL, 9.4 mmol). The mixture was stirred at −78 °C for 10 min, to the resulting bright blue solution was added methyl chloroformate (1.8 mL, 23 mmol) and the reaction mixture was stirred at −78 °C for 30 min. The reaction mixture was quenched with satd NH_4Cl aq and diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford methyl ester **13** (2.355 g, 80%) as a yellow powder: mp 69.5–71.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.31 (dd, J = 7.6, 7.6 Hz, 1H), 7.18 (dd, J = 7.6, 7.8 Hz, 1H), 6.20 (s, 1H), 4.57 (s, 2H), 4.03 (m, 4H), 3.81 (s, 3H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 153.3, 141.6, 136.2, 133.1, 132.8, 129.6, 127.4, 127.3, 120.1, 119.8, 98.6, 80.4, 77.5, 77.4, 74.3, 72.3, 65.6, 58.6, 52.5; IR ($CDCl_3$): 2254, 1717 cm^{-1}; FAB-MS (NBA) m/z 382 [M$^+$]; Anal. Calcd for $C_{21}H_{18}O_5S$: C, 65.95; H, 4.74; S, 8.38. Found: C, 65.93; H, 4.77; S, 8.55.

4.2.9. 5-Formyl-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid methyl ester (14). To a solution of acetal **13** (3.00 g, 7.84 mmol) in a mixed solvent of acetone/H_2O = (130 mL:13 mL) was added PPTS (6.50 g, 25.9 mmol) at room temperature. After being stirred under reflux for 41 h, the reaction mixture was cooled to room temperature and the reaction mixture was quenched with satd $NaHCO_3$ aq and the resulting mixture was diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford aldehyde **14** (2.532 g, 95%) as a yellow powder: mp 69.7–71.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.85 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.43 (dd, J = 7.6, 7.8 Hz, 1H), 7.29 (dd, J = 7.6, 7.8 Hz, 1H), 4.66 (s, 2H), 3.95 (s, 3H), 3.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.8, 161.2, 149.3, 141.9, 139.9, 136.7, 133.4, 130.1, 128.3, 127.7, 127.5, 119.3, 82.4, 80.4, 76.9, 73.2, 72.4, 58.7, 53.0; IR ($CDCl_3$): 2255, 1723, 1675 cm^{-1}; FAB-MS (NBA) m/z 339 [(M+H)$^+$]; Anal. Calcd for $C_{19}H_{14}O_4S$: C, 67.44; H, 4.17; S, 9.48. Found: C, 67.42; H, 4.21; S, 9.43.

4.2.10. 5-Formyl-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid (15). In argon atmosphere, to a solution of methyl ester **14** (2.55 g, 7.54 mmol) in THF (100 mL) was added 1 M NaOH aq (11 mL, 11 mmol). The mixture was stirred at room temperature for 70 min, and the reaction mixture was quenched with 1 M HCl aq (20 mL, 20 mmol) at 0 °C. The resulting mixture was diluted with ethyl acetate and the organic layer was separated, washed with brine, dried over Na_2SO_4, decolorized with charcoal, filtered and the filtrate was concentrated under reduced pressure to give carboxylic acid **15** (2.44 g, quantitative) as an orange powder: mp 128.4–134.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 1H), 7.89 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.43 (dd, J = 7.6, 7.6 Hz, 1H), 7.31 (dd, J = 7.6, 7.8 Hz, 1H), 4.69 (s, 2H), 3.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.8, 164.5, 150.0, 141.5, 139.6, 137.6, 133.5, 130.1, 128.4, 128.1, 127.7, 119.6, 80.6, 77, 73.1, 72.4, 60.7, 58.4; IR ($CDCl_3$): 3422, 2256, 1679 cm^{-1}; FAB-MS (NBA) m/z 325 [(M+H)$^+$].

4.2.11. 5-Formyl-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid triisopropylsilyl ester (16). To a solution of carboxylic acid **15** (2.4 g,

7.4 mmol) in CH_2Cl_2 (40 mL) were added imidazole (600 mg, 8.81 mmol) and triisopropylsilyl chloride (TIPSCl, 1.9 mL, 9.0 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. 4 wt% of the residual crude oil (150 mg) was purified by florisil (deactivated with 20% of H_2O) column chromatography (hexane/ether = 8:1) to afford TIPS ester **16** (98 mg, ca. 70%) as an organge wet powder: mp 55.5–62.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.81 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.43 (dd, J = 7.5, 7.8 Hz, 1H), 7.29 (dd, J = 7.5, 7.8 Hz, 1H), 4.66 (s, 2H), 3.48 (s, 3H), 1.40 (m, 3H), 1.14 (d, J = 7.6 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9, 159.8, 149.3, 142.3, 142.0, 136.8, 133.5, 130.1, 128.5, 127.8, 127.6, 119.4, 82.5, 80.3, 73.5, 72.5, 58.6, 31.7, 13.7, 12.0; IR ($CDCl_3$): 2254, 2219, 1700, 1675 cm^{-1}; FAB-MS (NBA) *m/z* 481 [(M+H)$^+$]; Anal. Calcd for $C_{27}H_{32}O_4SSi$: C, 67.46; H, 6.71; S, 6.67. Found: C, 67.55; H, 6.64; S, 6.70.

4.2.12. 5-(1-Hydroxy-6,6-dimethyl-hepta-2,4-diynyl)-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid triisopropylsilyl ester (17). In argon atmosphere, to a solution of asymmetric butadiyne **8** (1.48 g, 8.30 mmol) in methyl *tert*-butyl ether (35 mL) was added methyllithium–lithium bromide complex (1.4 M, in ether, 3.0 mL, 4.2 mmol) at room temperature. The mixture was stirred under reflux for 3 h, and the resulting alkynyl lithium solution was cooled to 0 °C and added to a solution of aldehyde **16** (500 mg, 1.04 mmol) in ether (14 mL) at −72 °C. After being stirred at −72 to −48 °C for 2 h, the reaction mixture was treated with satd NH_4Cl aq to quench the reaction and diluted with ethyl acetate. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. The residue was roughly purified by deactivated silica gel (by adding 25wt% of H_2O) column chromatography (hexane/ether = 5:1) to give crude alcohol **17**. The above crude (40 mg) was purified by HPLC ($CHCl_3$) to give pure **17** (23 mg, ca. 40%) as yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.40 (dd, J = 7.3, 7.8 Hz, 1H), 7.27 (dd, J = 7.3, 7.6 Hz, 1H), 5.95 (s, 1H), 4.66 (s, 2H), 3.48 (s, 3H), 2.74 (broad s, 1H), 1.38 (m, 3H), 1.24 (s, 9H), 1.13 (d, J = 7.6 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.5, 155.5, 141.7, 136.1, 134.9, 133.2, 129.7, 127.6, 127.4, 119.9, 119.2, 91.3, 80.8, 78.2, 77.5, 74.4, 73.6, 72.4, 72.1, 62.8, 59.8, 58.6, 30.3, 28.1, 17.8, 12.0; IR ($CDCl_3$): 3360, 1689 cm^{-1}; FAB-MS (NBA) *m/z* 586 [M$^+$]; Anal. Calcd for $C_{35}H_{42}O_4SSi$: C, 71.63; H, 7.21; S, 5.46. Found: C, 71.37; H, 7.16; S, 5.62.

4.2.13. 5-(1-Hydroxy-6,6-dimethyl-hepta-2,4-diynyl)-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid potassium salt (5). To a solution of TIPS ester **17** (80 mg, 0.14 mmol) in MeOH (3.5 mL) was at 0 °C added K_2CO_3 (10 mg, 0.072 mmol). After being stirred at room temperature for 5 h, the reaction mixture was diluted with H_2O, acidified with 1 M HCl aq (0.15 mL, 0.15 mmol), and extracted with ether. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. The residual solid was washed with hexane and then, with a mixed solvent of hexane/$CHCl_3$ = 10:1 for several times to afford carboxylic acid (50 mg, 85%) as an orange powder: mp 158.8–165.0 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (s, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.40 (dd, J = 7.6, 7.8 Hz, 1H), 7.30 (dd, J = 7.6, 7.6 Hz, 1H), 5.84 (s, 1H), 4.62 (s, 2H), 3.45 (s, 3H), 1.22 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.1, 158.7, 142.7, 136.8, 134.6, 134.2, 130.8, 129.2, 128.9, 121.5, 119.4, 91.1, 81.5, 78.6, 78.3, 75.6, 75.5, 73.6, 71.6, 64.1, 60.2, 58.9, 30.7, 29.1; IR (CD_2Cl_2): 3500–2300 (broad), 2254, 1685 cm^{-1}; FAB-MS (NBA) *m/z* 413 [(M−OH)$^+$], 399 [(M−OMe)$^+$].

To a solution of the above carboxylic acid (133 mg, 0.309 mmol) in MeOH (5 mL) was added K_2CO_3 (21.5 mg, 0.156 mmol) at 0 °C. The mixture was stirred at 0–4 °C for 41 h, and the solvent was removed in vacuo. To the residual viscous oil was added ether to solidify to afford potassium salt **5** (127 mg, 88%) as a greenish yellow powder: ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.3 Hz, 1H), 7.44 (s, 1H), 7.41 (dd, J = 7.3, 7.6 Hz, 1H), 7.31 (dd, J = 7.6, 7.6 Hz, 1H), 5.79 (s, 1H), 4.63 (s, 2H), 3.46 (s, 3H), 1.23 (s, 9H); IR ($CDCl_3$): 3362 (broad), 2254, 1588, 1365 cm^{-1}; FAB-MS (NBA) *m/z* 429 [(M−K)$^+$].

4.2.14. 5-(6,6-Dimethyl-hepta-2,4-diynoyl)-4-[4-(2-methoxymethyl-phenyl)-buta-1,3-diynyl]-thiophene-2-carboxylic acid potassium salt (6). To a solution of alcohol **17** (100 mg, 0.170 mmol) in CH_2Cl_2 (10 mL) was added Na_2SO_4 (142 mg, 1.00 mmol) and active γ-MnO_2 (1.5 g, 17 mmol) at 0 °C. After being vigorously stirred at 0–7 °C for 27 h, the heterogeneous mixture was filtered and the filtrate was concentrated at 0 °C to give ketone (98 mg, 98%) as an orange oil: ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.81 (s, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.42 (dd, J = 7.6, 7.8 Hz, 1H), 7.29 (dd, J = 7.6, 7.6 Hz, 1H), 4.64 (s, 2H), 3.45 (s, 3H), 1.41 (m, 3H), 1.21 (s, 9H), 1.14 (d, J = 7.6 Hz, 18H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 167.6, 160.1, 149.8, 142.6, 141.8, 138.5, 133.6, 130.3, 128.0, 127.8, 125.5, 120.0, 100.3, 82.8, 81.0, 80.3, 77.9, 75.9, 72.7, 72.5, 63.0, 58.8, 30.0, 28.9, 17.9, 12.4; IR (CD_2Cl_2): 2220, 1701, 1609 cm^{-1}; FAB-MS (NBA) *m/z* 585 [(M+H)$^+$].

To a solution of the above ketone (95 mg, 0.16 mmol) in a mixed solvent (MeOH/CH_2Cl_2/ether = 10:1:1, 8.4 mL) was at 0 °C added K_2CO_3 (12 mg, 0.087 mmol). The mixture was stirred at 0 °C for 20 min, and the solvent was removed at 0 °C. The residual amorphous was washed with cold ether for several times to afford potassium salt **6** (58 mg, 77%) as a yellow powder: mp 73.0 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (s, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.43 (dd, J = 7.6, 7.8 Hz, 1H), 7.32 (dd, J = 7.3, 7.6 Hz, 1H), 4.64 (s, 2H), 3.45 (s, 3H), 1.18 (s, 9H); IR (CD_2Cl_2): 2304, 2224, 2198, 1604, 1359 cm^{-1}; FAB-MS (NBA) *m/z* 467 [(M+H)$^+$].

4.3. Agarose gel electrophoresis

Stock solutions of thienyl tetraynes were prepared as follows: thienyl tetraynes were dissolved in DMSO followed by dilution with distilled deionized water (DDW) till the final concentration of DMSO reached to 5.0%. Before adding to DNA, the above stock solution was diluted with 5.0% DMSO. To a solution of pBluescript II KS^+ DNA (2.0 μg/7.5 μL) was added the diluted tetrayne stock solution to set the final incubation conditions: total volume 20 μL; final DMSO concentration 3.0%. The mixture was incubated at 37 °C over night, DNA was precipitated by treatment with 3 M sodium acetate (2 μL) and ethanol (60 μL). After being incubated for 20 min at −80 °C, the suspension was centrifuged (15000 rpm, 4 °C, 10 min). The supernatant was removed and the residue was washed with 70% ethanol, followed by centrifugation (15,000 rpm, 4 °C, 10 min) and the removal of supernatant again. The residue was dried in vacuo, dissolved in H_2O (8.0 μL), and the resulting solution was incubated at 60 °C for 10 min, at 37 °C for 30 min. To the above DNA solution was added loading buffer (2.0 μL), the resulting solution was developed on 0.7% agarose gel. After electrophoresis, DNA was visualized by treating the gel with ethydium bromide solution for 10 min followed by the irradiation of UV.

4.4. Atomic force microscopy

pBluescript II KS^+ DNA was treated with tetraynes (1.0 mM, 37 °C, 6 h) and isolated by ethanol precipitation as described in Section 4.3. After ethanol precipitation, pellet was dissolved in 800 μL of H_2O to give a sample solution. DNA was settled on a mica plate as follows: 10 μL of the above sample solution was dropped on a flat mica plate, incubated at room temperature for 1 min and unnecessary water was flushed away with air. The surface of the mica plate possessing DNA was visualized using an atomic force microscope (Multimode atomic force microscope (Nanoscope IV) from Veeco Instruments, Inc., USA).

4.5. *Escherichia coli* transformation assay

pBluescript II KS^+ DNA was treated with tetraynes (1.0 mM) or other reagents, isolated by ethanol precipitation as described in Section 4.3. The resulting DNA was dissolved in 8.0 μL of H_2O, and the solution was incubated at 60 °C for 10 min. To a solution of competent cell of *E. coli* (DH5α or JM109) at 0 °C was added the above DNA solution (8.0 μL) and the mixture was incubated at 42 °C for 1 min (heat shock). The resulting solution was immediately cooled to 0 °C and incubated for 3 min at 0 °C, then Luria–Bertani (LB) media (900 μL) was added. The mixture was incubated at 37 °C for 1 h (transformation), the resulting *E. coli* solution (100 μL) was spread to an LB agar plate-containing ampicillin (100 mg/L). The plate was incubated at 37 °C for 15 h, and the number of colonies was counted.

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