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# TX-2152: A conformationally rigid and electron-rich diyne analogue of FTY720 with in vivo antiangiogenic activity

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## ABSTRACT

We designed FTY720 analogues with conformationally rigid and electron-rich acetylenic chains as antiangiogenic agents (the monoyne **1**: TX-2148, the diyne **2**: TX-2152, the triyne **3**: TX-2256). Molecular orbital (MO) calculations of our designed acetylenic analogues and FTY720 showed that the localization of the lowest unoccupied MO and the highest occupied MO increased from phenyl ring to acetylenic chain compared with that of FTY720. These acetylenic analogues were synthesized from *p*-hydroxyphenylethanol as a starting material. The construction of the acetylenic chain was carried out by an iterative strategy using a Sonogashira cross-coupling reaction and desilylative bromination in two steps. The corresponding overall yields of the monoyne **1**, the diyne **2**, and the triyne **3** were 27% (11 steps), 13% (13 steps), and 10% (15 steps). The in vivo antiangiogenic activities of these acetylenic analogues and FTY720 were evaluated by the chick embryo chorioallantoic membrane (CAM) assay and compared to the activities of the known antiangiogenic agent TNP-470. The diyne **2** showed more potent antiangiogenic activity (90% inhibition) than FTY720 (77% inhibition) and other acetylenic analogues (the monoyne **1**: 42% inhibition, the triyne **3**: 60% inhibition), and TNP-470 (82% inhibition) at a dose of 10 μg/CAM, without showing toxicity. The diyne **2** also had potent inhibitory activity at a dose of 5 and 2.5 μg/CAM. These results indicate that the flexibility of C8 alkyl chain of FTY720 is not required for its antiangiogenic activity. We suggest that the diyne **2** (TX-2152) may be a promising candidate as an antiangiogenic agent for antineoplastic drug discovery.

## 1. Introduction

FTY720 (Fig. 1),[1–3] an immunosuppressant, is currently in clinical phase III trials for the treatment of relapsing-remitting multiple sclerosis in oral administration.[4] FTY720 exerts its immunosuppressive activity as the phosphate ester formed by phosphorylation in vivo.[3,5] The phosphate interacts with sphingosine-1-phosphate (S1P) receptors.[6,7] Recently, it was reported that FTY720 was able to abrogate the stimulating effect of vascular endothelial growth factor (VEGF: 20 ng/mL) even at subnanomolar concentrations in a human umbilical vein endothelial cell spheroid model.[8] It was also reported that FTY720 potently inhibited VEGF and S1P induced angiogenesis in vivo in growth factor implant and corneal models in mice at well-tolerated and clinically relevant doses.[9]

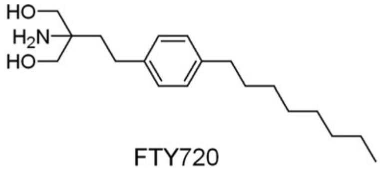

**Figure 1.** The structure of FTY720.

Angiogenesis, the formation of a new capillary network from pre-existing blood vessels, plays a crucial role in the growth of a neoplasm.[10,11] Since the neoplasm actively induces angiogenesis for its own growth,[12] an antiangiogenic agent is considered to be an antineoplastic agent. In the past, many antiangiogenic agents have been developed and some of these are currently undergoing

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clinical trials.[10,13] Bevacizumab, which is a monoclonal antibody against VEGF, was the first antiangiogenic agent approved for colon cancer by the FDA.[14,15] About 60% of human neoplasms express VEGF but many neoplasms produce other angiogenic proteins as well.[16] Furthermore, some neoplasms normally produce only VEGF, but mutation allows them to produce new angiogenic proteins. If more patients respond well to bevacizumab therapy and bevacizumab receives FDA approval for other tumors, we think that co-administration of other antiangiogenic inhibitors should be explored.[13,17] There is a continuing need for the development of new antiangiogenic agents.

Previously, we have developed a series of antiangiogenic agents and have evaluated their in vitro and in vivo antiangiogenic activities.[18–25] We have used the chick embryo chorioallantoic membrane (CAM) assay which is widely employed in this area for evaluation of in vivo antiangiogenic activity.[26] It is well known that the angiogenic endothelium of the CAM expresses VEGF and its recepter-2.[27–29] From this and our previous studies, we were confident that FTY720 would also show antiangiogenic activity in the CAM assay. For these reasons, we decided to design FTY720 analogues as antiangiogenic agents.

From previous structure–activity relationship (SAR) studies of FTY720 analogues, the 2-amino-1,3-propane diol moiety is known to be critical for its immunosuppressive activity.[2] We assume that this moiety has also important role for its antiangiogenic activity. To develop FTY720 analogues, we planned to perform conformational rigidification of the flexible C8 alkyl chain of FTY720. As shown in Figure 2A, the flexible $sp^3$ carbon chain of FTY720 fills a large space. The structural flexibility generates a vast number of conformations.[30,31] Conformational flexibility can be a complicating factor in drug development, and conformational rigidification can be a very important strategy in drug design. To achieve effective rigidification, we selected acetylenic structures, a structural motif widely distributed in nature.[32,33] Acetylenic compounds including polyynes have unique structural properties that include rigidity and high electron-densities,[34] and show diverse biological activities such as antiangiogenesis,[35] antitumor,[36] antiinflammatory,[37] antibiotic,[38] and anti-HIV activities.[39] As can be seen in Figure 2B, such acetylenic units contribute to substantial rigidification of conformations. There are no examples of the use of polyynes for drug rigidification beyond the use of diynes in medicinal chemistry.

In this paper, we describe the design and synthesis of FTY720 analogues that have conformationally rigid and electron-rich acetylenic chains. We also show the in vivo antiangiogenic activities of our designed acetylenic FTY720 analogues evaluated by the CAM assay.

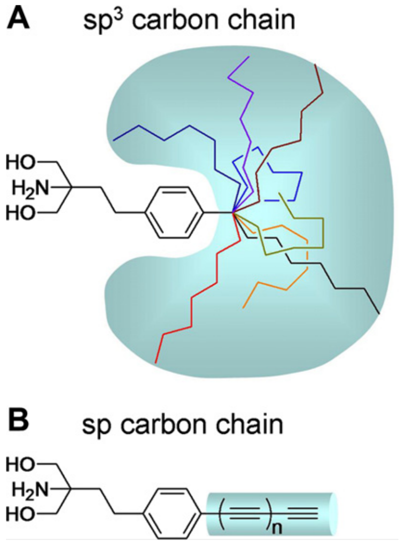


**Figure 2.** Filled space images of (A) $sp^3$ carbon chain and (B) sp carbon chain.

## 2. Results

### 2.1. Design

As shown in Figure 3, we designed acetylenic FTY720 analogues such as the monoyne **1** (TX-2148), the diyne **2** (TX-2152), and the triyne **3** (TX-2256) wherein the flexible C8 alkyl chain of FTY720 has been replaced with a conformationally rigid and electron-rich acetylenic chain. We expected that this molecular design could change the molecular properties and electronic states of the molecules in a manner that would improve the pharmacodynamic properties. We felt that such changes could also affect and possibly increase antiangiogenic activity.

We carried out molecular orbital (MO) calculations to aid in the design of the acetylenic FTY720 analogues. In particular, the MO calculations enabled us to examine the extent to which the acetylenic units influenced the overall electronic states of the molecules. The MO calculations were done using both MOPAC 2000 with PM3 method[40] and Gaussian 03 with B3LYP/6-31G(d).[41] As shown in Figure 4, the lowest unoccupied MO (LUMO) and the highest occupied MO (HOMO) of FTY720 were localized at the phenyl moiety. In contrast, localizations of LUMO and HOMO of the acetylenic FTY720 analogues extended from the phenyl moiety to the acetylenic chain. The energy levels of the LUMOs ($E_{\mathrm{LUMO}}$) of the acetylenic FTY720 analogues were at lower energy than those of FTY720, while the energy levels of their HOMOs ($E_{\mathrm{HOMO}}$) were higher than those of FTY720. The calculated $\log P$ ($\mathrm{c}\log P$) values of these acetylenic FTY720 analogues were obtained using OSIRIS Property Explorer.[42] As shown in Table 1, the corresponding $\mathrm{c}\log P$ values of the monoyne **1** (TX-2148), the diyne **2** (TX-2152), and the triyne **3** (TX-2256) were 0.58, 1.06, and 1.54.

### 2.2. Synthesis

The synthesis of a key intermediate **11** began with commercially available *p*-hydroxyphenylethanol as shown in Scheme 1. Selective protection of the primary alcohol gave the phenol **4**.[43] Silylation of the phenol **4** followed by selective deprotection of the acetyl group produced the alcohol **5**. Iodination of the alcohol **5** gave the ethyl iodide **6**, which was condensed with diethyl acetamidomalonate to give the diester **7**. The reduction of the diester **7** with $LiAlH_4$ followed by acetylation gave the triacetate **8**. Desily-

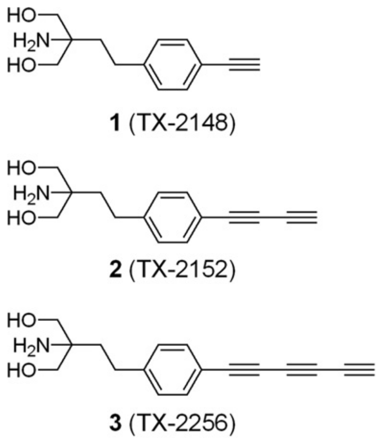


**Figure 3.** The structures of our designed acetylenic FTY720 analogues, the monoyne 1 (TX-2148), the diyne 2 (TX-2152), and the triyne 3 (TX-2256).

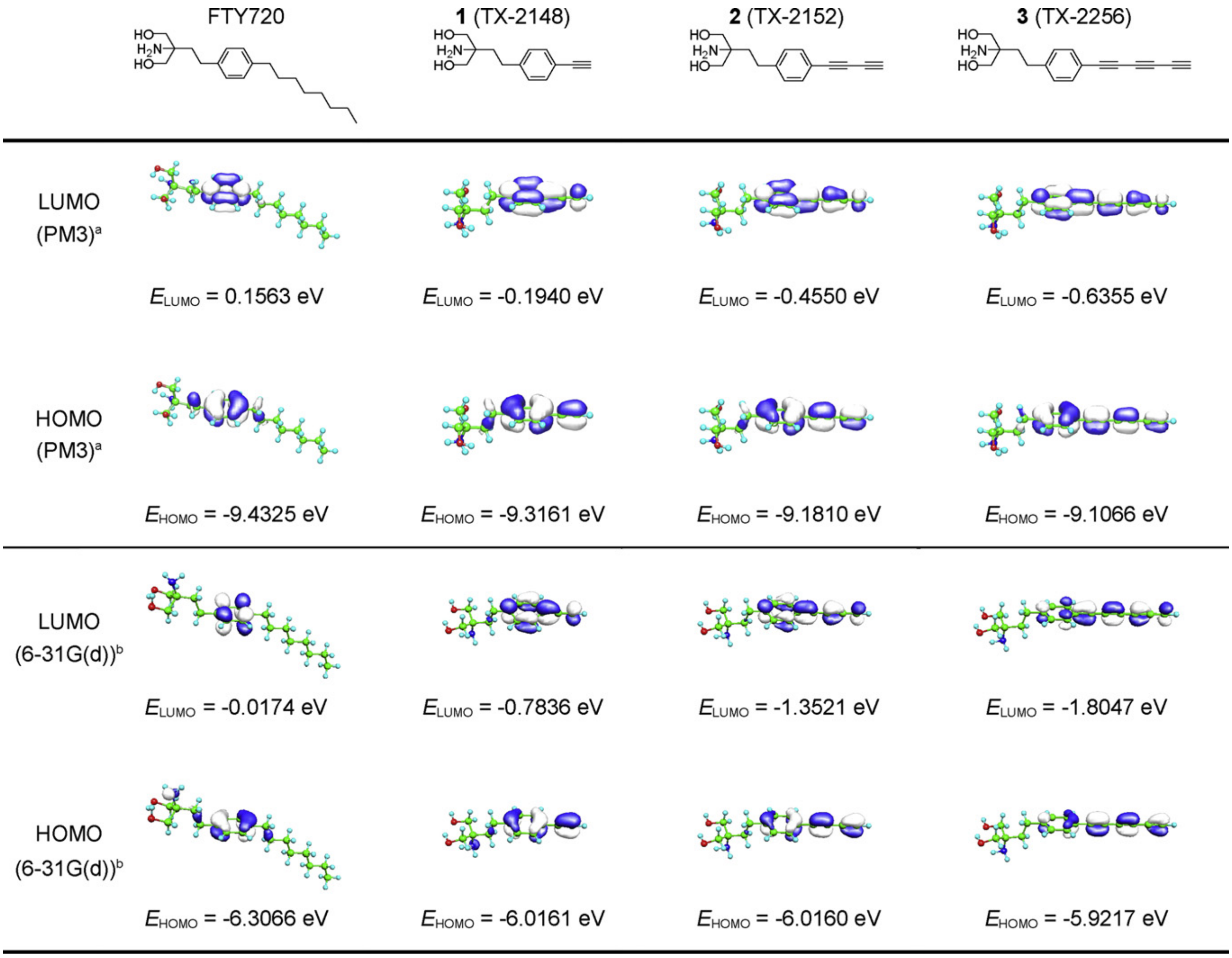


**Figure 4.** Molecular orbitals and their energy levels of FTY720 and acetylenic FTY720 analogues. [a]MOPAC 2000 with PM3. [b]Gaussian 03 with B3LYP/6-31G(d).

lation of the triacetate **8** afforded the phenol **9**. The phenol **9** was converted to the triflate **10**, which was subjected to a modified palladium-catalyzed Sonogashira cross-coupling[44] with trimethylsilylacetylene to provide the TMS-monoyne **11**.

The homologation of the acetylenic chain was carried out using the two-step acetylene homologation sequence as shown in Scheme 2.[45] An in situ desilylation–bromination reaction of the TMS-monoyne **11** gave the bromo-monoyne **12**.[46] The bromo-monoyne **12** was treated with trimethylsilylacetylene under Sonogashira cross-coupling conditions to give the TMS-diyne **13**. The TMS-triyne **15** was similarly obtained from the TMS-diyne **13**. As shown in Scheme 3, the TMS-triyne **15** was also synthesized via one-step reaction from the bromo-monoyne **12** using bis-trimethylsilylbutadiyne but the yield of this transformation was relatively low.[47] All the acetylenic FTY720 analogues, including the monoyne **1** (TX-2148), the diyne **2** (TX-2152), and the triyne **3** (TX-2256) were obtained by hydrolysis of the corresponding trimethylsilyl alkynes. The corresponding overall yields of the monoyne **1** (TX-2148), the diyne **2** (TX-2152), and the triyne **3** (TX-2256) were 27% (11 steps), 13% (13 steps), and 10% (15 steps) from *p*-hydroxyphenylethanol.

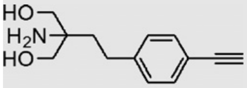
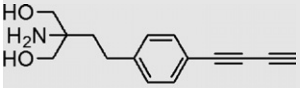
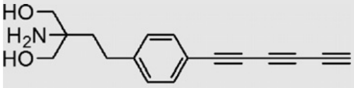
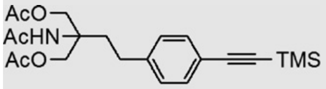
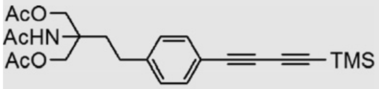
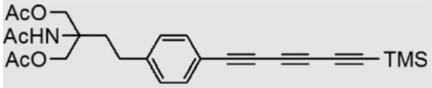
## 2.3. Determination of calculated and observed hydrophobicity of ethynyl group

We measured the observed log*P* (log*P*ow) values of **11**, **13**, and **15** by an HPLC method to determine the gain in an observed substituent hydrophobic constant ($\pi$) per acetylenic bond. To estimate log*P* and $\pi$, we calculated the clog*P* values of **11**, **13**, and **15**. As shown in Table 1, the corresponding clog*P* values of **11**, **13**, and **15** were 4.32, 4.80, and 5.27. The estimated $\pi$ of the ethynyl group obtained from these clog*P* values of the acetylenic FTY720 analogues was approximately 0.48. The corresponding log*P*ow values of **11**, **13**, and **15** were 4.07, 4.97, and 5.90. The $\pi$ of ethynyl group obtained from these log*P*ow values was approximately 0.92.

## 2.4. In vivo antiangiogenic activity

In vivo antiangiogenic activity of FTY720 and the acetylenic FTY720 analogues were evaluated by the CAM assay. The known antiangiogenic inhibitor TNP-470[48,49] was also evaluated as a positive control. In this assay, we determined that the more potent antiangiogenic agent showed its inhibitory activity of more than 60% with a dose lower than 100 μg/CAM. The antiangiogenic activities of FTY720, the acetylenic FTY720 analogues, and TNP-470 are listed in Table 2. FTY720 showed 100% inhibition at a dose of 100 μg/CAM. FTY720 also showed potent inhibitory activity at 10 μg/CAM (77% inhibition). The monoyne **1** (TX-2148) showed poor activity at all doses of 10, 5, and 1 μg/CAM tested. The diyne **2** (TX-2152) had potent inhibitory activities from 10 to 2.5 μg/CAM. The triyne **3** (TX-2256) also showed potent inhibitory activity at 2.5 μg/CAM (67% inhibition) but its activity did not increase at its higher dose. At 10 μg/CAM, the diyne **2** (TX-2152) showed more potent inhibitory activity than FTY720 and TNP-470. But the

**Table 1**
The c log *P* and the log *P*ow values of acetylenic FTY720 analogues

| Compound | c log *P* | log *P*ow |
|---|---|---|
|  **1** (TX-2148) | 0.58 | – |
|  **2** (TX-2152) | 1.06 | – |
|  **3** (TX-2256) | 1.54 | – |
|  **11** | 4.32 | 4.07 |
|  **13** | 4.80 | 4.97 |
|  **15** | 5.27 | 5.90 |

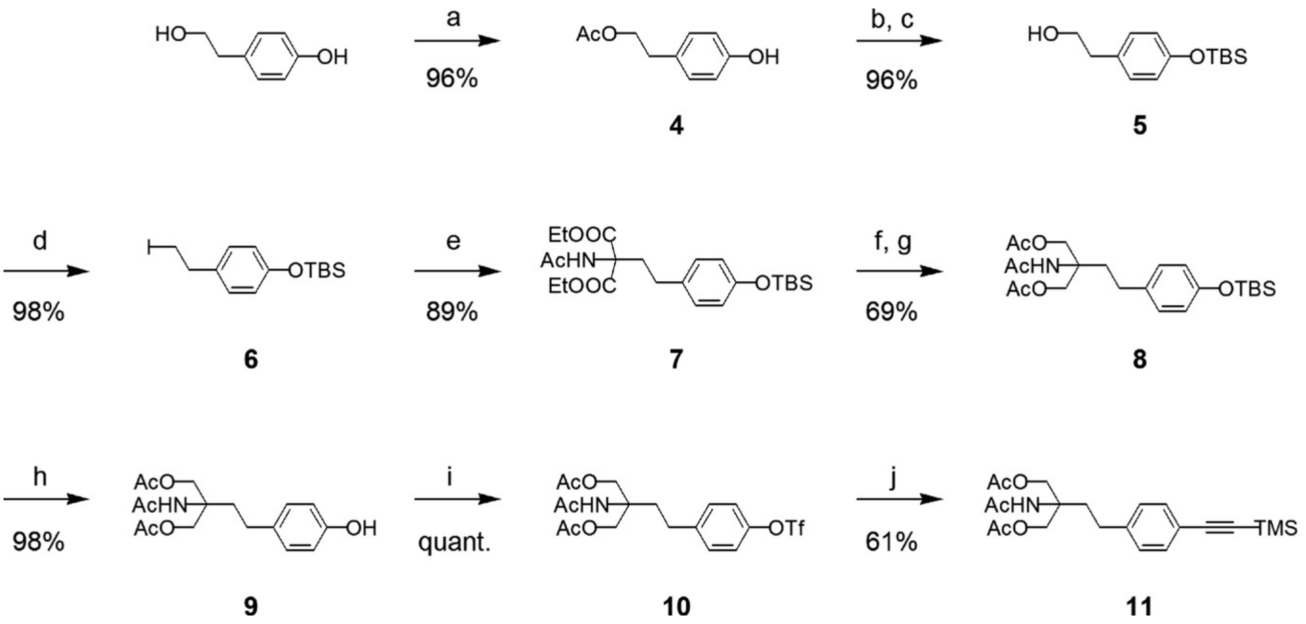
monoyne **1** (TX-2148) and the triyne **3** (TX-2256) were less potent than FTY720 and TNP-470. As shown in Figure 5, the diyne **2** (TX-2152) clearly inhibited antiangiogenesis compared with control. As noted above, the diyne **2** (TX-2152) was the most potent antiangiogenic agent among all the acetylenic FTY720 analogues tested. These compounds were not observed to be toxic to developing chick embryo in the CAM assay.

## 3. Discussion

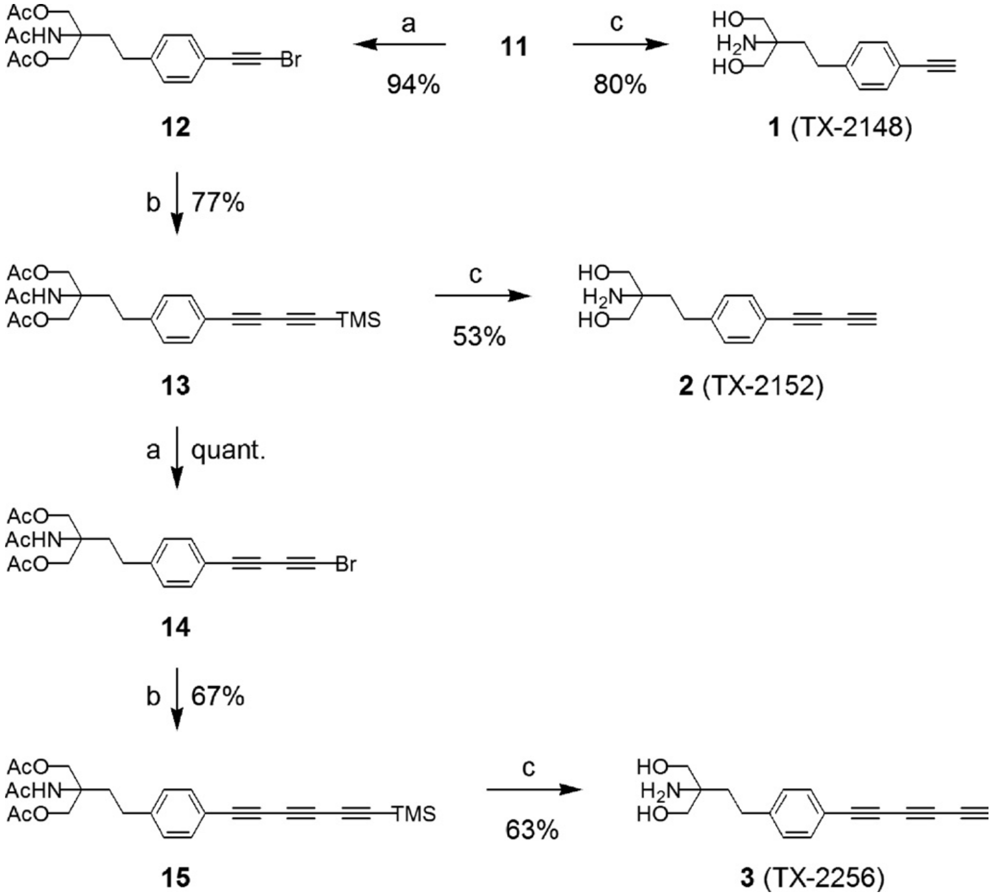
In this study, we have demonstrated that the acetylenic FTY720 analogues have potent antiangiogenic activity comparable to TNP-470 in vivo in the CAM assay. We also showed that the C8 alkyl chain of FTY720 could be replaced by the diyne or the triyne with respect to antiangiogenic activity, and introducing conformational rigidity did not diminish antiangiogenic activity. Since S1P did not show any antiangiogenic activities in the CAM assay from our previous study (data not shown), FTY720 and the acetylenic FTY720 analogues are thought to exert these antiangiogenic activities without involving S1P receptors.

In the past, various acetylenic coupling reactions have been developed and applied[50] to natural product synthesis.[32,34] To install the acetylenic chains in our new FTY720 analogues, we used an iterative homologation strategy based on the Sonogashira cross-coupling reaction together with desilylative bromination.[45,51] An advantage of this strategy is the ability to add both even- and odd-numbers of acetylenic units by controlling the number of iterations of the chain growth cycles. This allows the establishment of a SAR study of the effect of the length of the acetylenic chain.

Parish et al. isolated the ene-triyne, an amide of the C-9 ene-triyne carboxylic acid with *p*-aminobenzoic acid, from the Basidomycete fungus *Baeospora myosura*.[38] The ene-triyne showed extremely potent antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus*, while the reduced analogue of the ene-triyne and 4-acetimidobenzoic acid were not active. It is not clear that the altered activity results from loss of conformational rigidity, the electronic character of acetylenic structure, and/or other factors. Despite this, introduction of the acetylenic structure greatly influ-



**Scheme 1.** Reagents and conditions: (a) $NaHSO_4 \cdot SiO_2$/*n*-hexane/EtOAc, reflux, 24 h; (b) TBSCl/imidazole/$CH_2Cl_2$, rt, 15 min; (c) $LiAlH_4$/THF, rt, 15 min; (d) imidazole/$PPh_3$/$I_2$/$CH_2Cl_2$, rt, 1.5 h; (e) $AcNH(COOEt)_2$/NaH/DMF, rt, 4 h; (f) $LiAlH_4$/THF, rt, 6 h; (g) $Ac_2O$/$Et_3N$/DMAP/THF, rt, 15 min; (h) $(n\text{-Bu})_4NF$/THF, rt, 20 min; (i) $Tf_2O$/pyridine/$CH_2Cl_2$, 0 °C–rt, 15 min; (j) TMSCCH/$Et_3N$/$PdCl_2(PPh_3)_2$/$CH_3CN$, reflux, 1.5 h.



**Scheme 2.** Reagents and conditions: (a) NBS/$AgNO_3$/acetone, rt, 2 h; (b) TMSCCH/$Et_3N$/$PdCl_2(PPh_3)_2$/CuI/THF, rt, 2 h; (c) LiOH/MeOH, rt, 40 min–23 h.

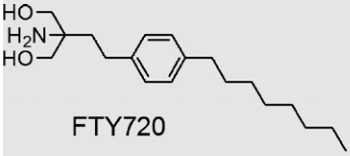
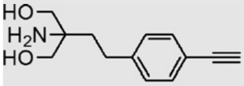
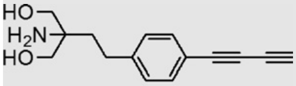
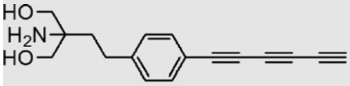
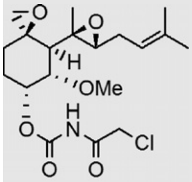
enced the biological activity. This and our present results have shown the utility of our approach. A number of acetylenic moieties have been used as architectures of several compounds.[34] However, there were no examples of drug design in which acetylenic units were extended beyond diyne structures as pharmacophoric descriptors. Our present study may become a new application strategy for primary SAR exploration for drug development.[52]

Good in vitro activity for a target is one important criterion upon which medicinal chemists base drug development. This criterion alone can be misleading. Our previous study indicated that potent antiangiogenic activities shown in vitro did not always correlate with similar tendencies in vivo.[18–25] However, compounds which had potent activity in the CAM assay advanced to the next stage of development with no difficulty. We think that it is mainly because of support from surrounding tissues in the in vivo enviroment.[10,12] In general, it is well documented that many promising candidate compounds with good in vitro activity have been dropped based on poor in vivo results. Given this perspective, we suggest the use of the in vivo CAM assay as an early or even initial screening procedure for antiangiogenic agents. In vitro assays evaluate individual antiangiogenic properties such as effects on cell proliferation, migration, tube formation, as well as toxicity.[26] Their environments differ greatly from that of in vivo. Furthermore, these tests are time consuming, expensive, and technically difficult. In vivo assays using animals such as mice and rats are also expensive, time consuming, and require special facilities. On the other hand, the CAM assay can easily evaluate the total antiangiogenic activity with a small amount of drug at a very low cost in an incubator and is suitable for large-scale screening.[26] In addition, this assay will concurrently provide information on toxicity based on the life or death of CAM. For these reasons, we have taken the position that if we carry out our initial screening of antiangiogenic agents using the CAM assay, this will reduce the risk of failure at a later phase of our research.

Yamaguchi et al. carried out an SAR study of caryonencin analogues including compounds possessing acetylenic moieties.[53] Their study showed that the tetrayne analogue exhibited potent antibacterial and antifungal activities in vitro, while the triyne and diyne analogues were less active. These results indicate that SAR studies of acetylenic compounds related to the number of acetylenic units present can reveal important information on the biological activity of acetylenic compounds that should be useful in drug development. The results of the CAM assay of our acetylenic FTY720 analogues also showed that the length of the acetylenic structure (monoyne, diyne, triyne) influenced the in vivo antiangiogenic activity. Although many unsaturated fatty acids found in nature contain one or more acetylenic structures,[36,54–57] the pharmacological, especially pharmacokinetic and pharmacodynamic, properties related to this functionality are still largely unknown. The SAR study of these acetylenic compounds should add to the knowledge of the effects and potential of acetylenic groups in drug development.

The hydrophobic constant $\pi$ of the ethynyl group as an aromatic substituent has been shown to be 0.4, but the $\pi$ of the extended acetylenic substituents greater than the diyne has not been determined.[58] The differences between the c log*P* values of **11**, **13**, and **15** and the log*P*ow values show the importance of the experimental measurement of log*P*ow values. There are few reported values of log*P*ow of acetylenic compounds in spite of the importance of their substituent hydrophobic constants in their quantitative SAR. Our determination of the substituent hydrophobic constants for these acetylenic compounds based on the experimental measurement of log*P*ow will be very useful for further rational drug design.

**Table 2**
In vivo antiangiogenic activities of FTY720, acetylenic FTY720 analogues, and TNP-470 in the CAM assay

| Compound | Dose[a] (μg/CAM) | Inhibition (%) |
|---|---|---|
|  FTY720 | 100 | 100 |
| | 10 | 77 |
| | 1 | 40 |
|  **1** (TX-2148) | 10 | 42 |
| | 5 | 17 |
| | 1 | 17 |
|  **2** (TX-2152) | 10 | 90 |
| | 5 | 79 |
| | 2.5 | 71 |
| | 1 | 35 |
|  **3** (TX-2256) | 10 | 60 |
| | 5 | 67 |
| | 2.5 | 67 |
| | 1 | 40 |
|  TNP-470 | 10 | 82[c] |
| | 5 | 78[c] |
| | 1 | 50 |
| Control[b] | 0 | 0 |

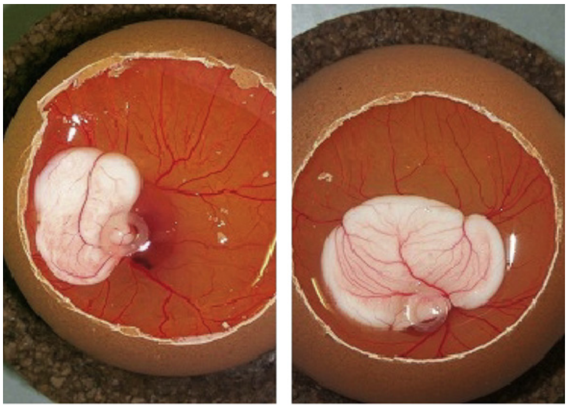
[a] The test compounds were dissolved in DMSO, and then mixed in 1% methyl cellulose/0.9% NaCl (0.05% DMSO).

[b] In a control experiment, 0.05% DMSO mixed in 1% methyl cellulose/0.9% NaCl without the test compound was used.

[c] The inhibition values were from Ref. 23.

$$\mathrm{TMS{-}C{\equiv}C{-}C{\equiv}C{-}TMS} \xrightarrow[44\%]{a,\ b} \mathbf{15}$$

**Scheme 3.** Reagents and conditions: (a) MeLi·LiBr, THF, 0 °C, 1 h, then CuBr, 0 °C–rt, 30 min; (b) **12**, pyridine, rt, 4 h.



**2** (TX-2152) Control

**Figure 5.** The pictures of CAM treated with the diyne 2 (TX-2152) or a control.

Electron transfers in molecules are important contributing factors to their chemical properties and reactivity. Kovacic and Pozos coined the term 'bioelectronome' to characterize the multiple reactions undergone by electrons in biological systems, including electron transfer and redox processes.[59] Because of the abundance and reactivity of π electrons in acetylenic structures that would be available for diverse biological effects, we considered this moiety to be a bioelectronome. This was a factor in our decision to introduce conformationally rigid and electron-rich acetylenic chains into known drugs or biomolecules as a means to change their electronic structures in order to create new bioelectronomes or new 'electronic drugs'.

Because pharmacodynamic properties are greatly influenced by electrostatic and steric alterations, the alteration of electronic state and the rigidification of conformation in our new analogues should have an effect on biological or pharmacological activities. Our molecular design strategy, the structural transfer from $sp^3$ hybrid to sp hybrid, should be applicable to other known drugs. Although this strategy represents simple modifications, it should lead to substantial changes in biological or pharmacological activities.

In conclusion, we have presented the design and synthesis of conformationally rigid and electron-rich acetylenic analogues of FTY720, based on our new molecular drug design strategy. The length of acetylenic chain affected in vivo antiangiogenic activities in the CAM assay. The diyne **2** (TX-2152) showed more potent antiangiogenic activity than FTY720, the monoyne **1** (TX-2148), the triyne **3** (TX-2256), and TNP-470, without showing toxicity. We suggest that diyne **2** (TX-2152), as an acetylenic FTY720 analogue, shows promise to be an antiangiogenic agent candidate for antineoplastic drug discovery.

## 4. Experimental

### 4.1. General procedures

$^1$H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer (400 MHz) with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm. Coupling constants are reported in Hz. Mass spectra were measured on a Shimazu GC-MS QP-1000 mass spectrometer using an electron ionization (EI) method. HRMS were measured on a JOEL JMS-700 mass spectrometer using a CI method. IR spectra were measured on a Perkin-Elmer spectrum RX 1. Elemental analyses were performed with a Yanako CHN recorder MT-5. Reactions were monitored by analytical TLC with use of Merck silica gel 60 $F_{254}$ glass plates. Column chromatography was performed on Kanto Chemical silica gel 60 N (230–400 mesh). HPLC was performed on C18 column (Inertsil®, 5 μm, 4.6 mm I.D. × 250 mm, GL Sciences Inc.) with a JASCO PU-1586 pump equipped with a UV–vis detector JASCO UV-1570. The c log*P* values were determined by OSIRIS Property Exploler[42] which available free on the Web.[60,61] All chemicals were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), or Sigma–Aldrich Japan (Tokyo, Japan). The fertilized chicken eggs were purchased from Goto hatchery, Inc. (Gifu, Japan).

### 4.2. Molecular modeling methods

The semi-empirical MO calculation was performed with the PM3 methods of Stewart using MOPAC 2000 in a WinMOPAC ver. 3.0 package.[40] The ab initio MO calculation was performed with the B3LYP hybrid density functional in conjugation with the 6-31G(d) basis set using the GAUSSIAN 03 suite of programs.[41] The visualization of the molecular geometries calculated by GAUSSIAN 03 were carried out with MOLKEL 4.3.[62]

## 4.3. Synthesis

### 4.3.1. Preparation of the $NaHSO_4{\cdot}SiO_2$ Catalyst[43]

To a solution of $NaHSO_4{\cdot}SiO_2$ (12 g, 89 mmol) in water (60 mL) was added $SiO_2$ (30 g). The mixture was stirred for 15 min and then gently heated on a hot plate, with intermittent swirling, until a free-flowing white solid was obtained. The catalyst was further dried at 120 °C for at least 48 h prior to use.

### 4.3.2. 4-Hydroxyphenethyl acetate (4)

*p*-Hydroxyphenylethanol (40.4 g, 0.292 mol) was dissolved in a 33% solution by volume of EtOAc in *n*-hexane (900 mL). To this solution was added $NaHSO_4{\cdot}SiO_2$ (35 g). The mixture was heated at reflux for 24 h, then cooled to room temperature and filtered through Celite. The filtrate was evaporated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9:1) to give the phenol **4** (50.7 g, 96%) as white solid. $^1$H NMR ($CDCl_3$): $\delta$ 7.05 (d, 2H, *J* = 8.3 Hz), 6.78 (d, 2H, *J* = 8.3 Hz), 4.24 (t, 2H, *J* = 7.1 Hz), 2.85 (t, 2H, *J* = 7.1 Hz), 2.04 (s, 3H); FT-IR (KBr): 3382, 3043, 2972, 2951, 2890, 2360, 1713, 1615, 1516, 1445, 1268, 1173, 830, 650 cm$^{-1}$; EI-MS *m/z*: 180 (M$^+$).

### 4.3.3. 2-(4-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}phenyl)-1-ethanol (5)

To a solution of the phenol **4** (39.5 g, 0.220 mol) dissolved in $CH_2Cl_2$ (600 mL) and cooled to 0 °C were added *tert*-butyldimethylsilylchloride (40 g, 0.26 mol) and imidazole (22 g, 0.33 mol). After 15 min, $H_2O$ was added, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9:1) to give 4-{[1-(tert-butyl)-1,1-dimethylsilyl]oxy}phenethyl acetate (65.8 g, quant.) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 7.06 (d, 2H, *J* = 8.5 Hz), 6.77 (d, 2 H, *J* = 8.5 Hz), 4.23 (t, 2H, *J* = 7.3 Hz), 2.86 (t, 2H, *J* = 7.3 Hz), 2.03 (s, 3H), 0.98 (s, 9H), 0.18 (s, 6H); FT-IR (KBr): 3470, 3034, 2960, 2934, 2899, 2861, 2361, 1743, 1612, 1513, 1390, 1364, 1258, 1171, 1107, 1035, 919, 841, 810, 782, 689 cm$^{-1}$; EI-MS *m/z*: 294 (M$^+$).

The intermediate (2.00 g, 6.79 mmol) was dissolved in THF (23 mL). $LiAlH_4$ (1.0 M solution in $Et_2O$, 7.5 mL, 7.5 mmol) was added dropwise to the solution at 0 °C. The mixture was warmed to room temperature. After 15 min, the reaction was quenched with saturated aqueous $Na_2SO_3$. The mixture was filtered through Celite and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:1) to give the alcohol **5** (1.65 g, 96%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.07 (d, 2H, *J* = 8.5 Hz), 6.78 (d, 2H, *J* = 8.5 Hz), 3.82 (t, 2H, *J* = 6.6 Hz), 2.80 (t, 2H, *J* = 6.6 Hz), 0.98 (s, 9H), 0.19 (s, 6H); FT-IR (KBr): 3369, 2959, 2934, 2889, 2860, 2364, 1612, 1513, 1473, 1464, 1391, 1363, 1262, 1170, 1104, 1047, 918, 840, 811, 780, 690 cm$^{-1}$; EI-MS *m/z*: 252 (M$^+$).

### 4.3.4. *tert*-Butyl[4-(2-iodoethyl)phenoxy]dimethylsilane (6)

To a solution of the alcohol **5** (2.06 g, 8.16 mmol) in $CH_2Cl_2$ (80 mL) cooled to 0 °C were added $PPh_3$ (4.3 g, 16 mmol), imidazole (1.1 g, 17 mmol), and $I_2$ (4.2 g, 16 mmol). After 1.5 h, the reaction was quenched with $Na_2SO_3$. The mixture was extracted with $CH_2Cl_2$. The water layer was back-extracted with $CH_2Cl_2$ and the combined organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane) to give the ethyl iodide **6** (2.91 g, 98%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.03 (d, 2 H, *J* = 7.3 Hz), 6.77 (d, 2H, *J* = 7.3 Hz), 3.30 (t, 2H, *J* = 7.1 Hz), 3.10 (t, 2H, *J* = 7.1 Hz), 0.98 (s, 9H), 0.19 (s, 6H). FT-IR (KBr): 2960, 2932, 2888, 2860, 1610, 1510, 1473, 1464, 1264, 1170, 917, 841, 814, 781, 688, 591 cm$^{-1}$; EI-MS *m/z*: 362 (M$^+$).

### 4.3.5. Diethyl 2-(acetylamino)-2-(4-{[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy}phenethyl)malonate (7)

To a suspension of NaH (60% dispersion in mineral oil, 1.4 g, 33 mmol) in DMF (35 mL) was added a solution of diethyl acetamidomalonate (7.2 g, 33 mmol) in DMF (24 mL). After 1 h, the ethyl iodide **6** (3.0 g, 8.3 mmol) in DMF (4 mL) was added. The suspension was stirred for 4 h, poured into water at 0 °C and extracted with $Et_2O$. The organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1) to give the diester **7** (3.3 g, 89%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 6.99 (d, 2H, *J* = 8.5 Hz), 6.77 (s, 1H), 6.73 (d, 2H, *J* = 8.5 Hz), 4.21 (m, 4H), 2.65 (m, 2H), 2.41 (m, 2H), 2.00 (s, 3H), 1.25 (t, 6H, *J* = 7.1 Hz), 0.97 (s, 9H), 0.17 (s, 6H); FT-IR (KBr): 3372, 2960, 2933, 2896, 2863, 1758, 1741, 1666, 1612, 1513, 1474, 1463, 1454, 1391, 1371, 1308, 1260, 1194, 1171, 1097, 1082, 1031, 1016, 923, 841, 811, 781, 692, 590 cm$^{-1}$; EI-MS *m/z*: 451 (M$^+$).

### 4.3.6. 2-(Acetylamino)-2-[(acetyloxy)methyl]-4-(4-{[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy}phenyl)butyl acetate (8)

To a solution of the diester **7** (5.03 g, 11.1 mmol) in THF (60 mL) cooled to 0 °C was added dropwise a solution of 1.0 M $LiAlH_4$ in $Et_2O$ (32 mL, 32 mmol). The mixture was warmed to room temperature. After 6 h, the reaction was quenched with saturated aqueous $Na_2SO_3$. The mixture was filtered through Celite and evaporated. The residue was purified by silica gel column chromatography (EtOAc) to give 2-amino-2-(4-{[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy}phenethyl)-1,3-propanediol (2.82 g, 69%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 7.04 (d, 2H, *J* = 8.5 Hz), 6.76 (d, 2H, *J* = 8.5 Hz), 5.87 (s, 1H), 3.86 (d, 2 H, *J* = 10.5 Hz), 3.84 (s, 2H), 3.62 (d, 2H, *J* = 10.5 Hz), 2.59 (m, 2H), 1.96 (s, 3H), 1.93 (m, 2H), 0.97 (s, 9H), 0.17 (s, 6H); FT-IR (KBr): 3499, 3311, 3095, 3034, 2955, 2931, 2887, 2881, 1630, 1554, 1510, 1473, 1461, 1434, 1417, 1378, 1322, 1303, 1263, 1200, 1174, 1106, 1062, 997, 978, 912, 840, 782, 646, 616, 588 cm$^{-1}$; EI-MS *m/z*: 367 (M$^+$).

To a solution of the diol (610 mg, 1.66 mmol) in THF (5.5 mL) were added $Ac_2O$ (610 μL, 6.6 mmol), triethylamine (900 μL, 6.6 mmol), and DMAP (10 mg, 80 μmol). After 15 min, the reaction was quenched with $H_2O$ and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (EtOAc) to give the triacetate **8** (751 mg, quant.) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 7.62 (d, 2H, *J* = 8.3 Hz), 6.75 (d, 2H, *J* = 8.3 Hz), 5.64 (s, 1H), 4.34 (s, 4H), 2.54 (m, 2H), 2.17 (m, 2H), 2.09 (s, 6H), 1.96 (s, 3H), 0.97 (s, 9H), 0.18 (s, 6H); FT-IR (KBr): 3485, 3312, 2955, 2861, 2357, 2343, 1738, 1651, 1558, 1513, 1470, 1382, 1259, 1229, 1054, 922, 842, 780 cm$^{-1}$; EI-MS *m/z*: 451 (M$^+$).

### 4.3.7. 2-(Acetylamino)-2-[(acetyloxy)methyl]-4-(4-hydroxyphenyl)butyl acetate (9)

The triacetate **8** (2.73 g, 6.03 mmol) was dissolved in THF (90 mL). Tetrabutylammonium fluoride (1.0 M solution in $Et_2O$, 6.0 mL, 6.0 mmol) was added dropwise to the solution. After 20 min, $H_2O$ was added and the mixture was extracted with $Et_2O$. The organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:4) to give the phenol **9** (1.99 g, 98%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 7.03 (d, 2H, *J* = 8.5 Hz), 6.76 (d, 2H, *J* = 8.5 Hz), 5.77 (s, 1H), 4.34 (s, 4H), 2.53 (m, 2H), 2.20 (m, 2H), 2.10 (s, 6H), 1.99 (s, 3H); FT-IR (KBr): 3352, 3104, 3018, 2962, 2932, 2902, 2859, 1744, 1658, 1616, 1598, 1550, 1518, 1455, 1379, 1260, 1173, 1044, 868, 838, 776, 668, 608 cm$^{-1}$; EI-MS *m/z*: 337 (M$^+$).

### 4.3.8. 2-(Acetylamino)-2-[(acetyloxy)methyl]-4-(4-{[(trifluoromethanesulfonyl)oxy]phenyl}butyl acetate (10)

To a chilled (0 °C) solution of the phenol **9** (555 mg, 1.64 mmol) in $CH_2Cl_2$ (3.3 mL) was added pyridine (637 μL, 7.87 mmol). $Tf_2O$ (331 μL, 1.97 mmol) in $CH_2Cl_2$ (720 μL) was then added dropwise at 0 °C. The reaction mixture was warmed to room temperature. After 15 min, the reaction was quenched with saturated aqueous $NaHCO_3$, extracted with $CH_2Cl_2$, washed with 10% HCl, washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:2) to give the triflate **10** (769 mg, quant.) as a light yellow solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.26 (d, 2H, *J* = 8.5 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 5.71 (s, 1H), 4.33 (s, 4H), 2.64 (m, 2H), 2.23 (m, 2H), 2.10 (s, 6H), 1.98 (s, 3H); FT-IR (KBr): 3339, 3216, 3082, 3012, 2996, 2963, 2944, 2870, 1754, 1657, 1547, 1505, 1427, 1394, 1381, 1328, 1248, 1143, 1045, 1020, 903, 831, 640, 607, 503, 478 $cm^{-1}$; EI-MS *m/z*: 469 ($M^+$).

### 4.3.9. 2-(Acetylamino)-2-[(acetyloxy)methyl]-4-{4-[2-(1,1,1-trimethylsilyl)-1-ethynyl]phenyl}butyl acetate (11)

The triflate **10** (1.0 g, 2.1 mmol) was dissolved in $CH_3CN$ (28 mL), and trimethylsilylacetylene (400 μL, 3.2 mmol), $PdCl_2(PPh_3)_2$ (74 mg, 0.11 mmol), and $Et_3N$ (1.3 mL, 9.3 mmol) were added. The reaction mixture was heated at reflux. After 1.5 h, the mixture was cooled, and then filtered through Celite. The filtrate was evaporated and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:2 to 1:4 gradient) to give the TMS-monoyne **11** (538 mg, 61%) as a white solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.37 (d, 2H, *J* = 8.0 Hz), 7.11 (d, 2H, *J* = 8.0 Hz), 5.70 (s, 1H), 4.33 (s, 4H), 2.59 (m, 2H), 2.19 (m, 2H), 2.09 (s, 6H), 1.97 (s, 3H), 0.24 (s, 9H); $^{13}C$ NMR ($CD_3CN$): $\delta$ 171.4, 171.4, 144.5, 132.7, 129.6, 121.3, 106.1, 94.3, 64.6, 58.6, 33.9, 29.9, 23.9, 21.0, 0.0; FT-IR (KBr): 3450, 3338, 3302, 3081, 2958, 2155, 1747, 1653, 1560, 1376, 1249, 1228, 1041, 866, 844 $cm^{-1}$; EI-MS *m/z*: 417 ($M^+$); Anal. Calcd for $C_{22}H_{31}NO_5Si$: C, 63.28; H, 7.48; N, 3.35. Found: C, 63.21; H, 7.45; N, 3.33.

### 4.3.10. 2-Amino-2-[4-(1-ethynyl)phenethyl]-1,3-propanediol (1: TX-2148)

The TMS-monoyne **11** (100 mg, 0.240 mmol) was dissolved in MeOH (1.0 mL) and 2 N aqueous LiOH (810 μL) was added dropwise at room temperature. After 2 h, the mixture was evaporated. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH = 1:4) to give the monoyne **1** (42.1 mg, 80%) as a white solid. $^1H$ NMR ($CD_3CN$): $\delta$ 7.39 (d, 2H, *J* = 8.3 Hz), 7.21 (d, 2H, *J* = 8.3 Hz), 3.39 (dd, 4H, *J* = 18.5, 10.7 Hz), 3.32 (s, 1H), 2.64 (m, 2 H), 1.61 (m, 2H); FT-IR (KBr): 3318, 3073, 3060, 3024, 3000, 2947, 2923, 2868, 2856, 2646, 2110, 1928, 1814, 1575, 1503, 1476, 1465, 1454, 1438, 1415, 1401, 1355, 1312, 1297, 1281, 1238, 1190, 1160, 1148, 1108, 1083, 1068, 1047, 1023, 966, 923, 853, 827, 810, 793, 763, 649, 641, 629, 556, 514 $cm^{-1}$; CI-HRMS *m/z* calcd for $C_{13}H_{18}NO_2$ [$(M+H)^+$] 220.1337, found 220.1330.

### 4.3.11. 2-Amino-2-[4-(1,3-butadiynyl)phenethyl]-1,3-propanediol (2: TX-2152)

The TMS-monoyne **11** (446 mg, 1.07 mmol) was dissolved in acetone (3.6 mL), and NBS (209 mg, 1.18 mmol) and $AgNO_3$ (182 mg, 1.07 mmol) were added. After 2 h, $H_2O$ was added at 0 °C. The mixture was extracted with $Et_2O$, the extracts were washed with brine, dried with $Na_2SO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to give the bromo-monoyne **12** (428 mg, 94%) as a light yellow solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.36 (d, 2H, *J* = 8.3 Hz), 7.13 (d, 2H, *J* = 8.3 Hz), 5.69 (s, 1H), 4.33 (s, 4H), 2.60 (m, 2H), 2.20 (m, 2H), 2.09 (s, 6H), 1.97 (s, 3H); FT-IR (KBr): 3321, 3300, 3212, 3168, 3082, 3032, 2972, 2203, 1739, 1654, 1558, 1511, 1470, 1430, 1382, 1316, 1259, 1230, 1194, 1058, 1034, 968, 848, 821, 654, 626, 543 $cm^{-1}$.

The bromo-monoyne **12** (165 mg, 0.389 mmol) was dissolved in THF (4.0 mL), and trimethylsilylacetylene (84 μL, 0.59 mmol), $PdCl_2(PPh_3)_2$ (14 mg, 20 μmol), CuI (3.8 mg, 20 μmol), and $Et_3N$ (110 μL, 0.78 mmol) were added. After 2 h, the reaction was quenched with saturated aqueous $NH_4Cl$. The mixture was extracted with $Et_2O$ and the organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:1 to 1:1 gradient) to give the TMS-diyne **13** (132 mg, 77%) as a white solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.39 (d, 2H, *J* = 7.8 Hz), 7.13 (d, 2H, *J* = 7.8 Hz), 5.78 (s, 1H), 4.32 (s, 4H), 2.60 (m, 2H), 2.19 (m, 2H), 2.09 (s, 6H), 1.97 (s, 3H), 0.23 (s, 9H); $^{13}C$ NMR ($CD_3CN$): $\delta$ 171.9, 171.8, 146.2, 134.2, 130.2, 119.6, 92.0, 88.9, 78.3, 74.5, 65.1, 59.1, 34.3, 30.5, 24.3, 21.4, 0.0; FT-IR (KBr): 3316, 3084, 2963, 2364, 2342, 2207, 2106, 1747, 1654, 1558, 1511, 1468, 1379, 1251, 1228, 1049, 1016, 848, 820, 761, 700, 606 $cm^{-1}$; EI-MS *m/z*: 441 ($M^+$); Anal. Calcd for $C_{24}H_{31}NO_5Si$: C, 65.28; H, 7.08; N, 3.17. Found: C, 65.02; H, 7.14; N, 3.22.

The TMS-diyne **13** (33.6 mg, 0.0761 mmol) was dissolved in MeOH (320 μL), and 2 N aqueous LiOH (300 μL) was added dropwise at room temperature. After 40 min, the mixture was evaporated. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH = 3:1 to 2:1 gradient) to give the diyne **2** (9.8 mg, 53%) as a green solid. $^1H$ NMR ($CD_3OD$): $\delta$ 7.42 (d, 2H, *J* = 8.3 Hz), 7.24 (d, 2H, *J* = 8.3 Hz), 3.49 (dd, 4H, *J* = 21.5, 10.7 Hz), 2.67 (m, 2H), 1.68 (m, 2H); FT-IR (KBr): 3295, 3282, 2939, 2896, 2868, 2208, 1914, 1646, 1609, 1556, 1509, 1456, 1436, 1413, 1376, 1343, 1329, 1319, 1298, 1263, 1242, 1206, 1174, 1062, 1019, 986, 966, 853, 820, 703, 666, 614, 607 $cm^{-1}$; CI-HRMS *m/z* calcd for $C_{15}H_{18}NO_2$ [$(M+H)^+$] 244.1338, found 244.1349. 2·HCl: $^1H$ NMR ($CD_3OD$): $\delta$ 7.45 (d, 2H, *J* = 8.3 Hz), 7.26 (d, 2H, *J* = 8.3 Hz), 3.68 (s, 4H), 3.12 (s, 1H), 2.69 (m, 2H), 1.94 (m, 2H).

### 4.3.12. 2-Amino-2-[4-(1,3,5-hexatriynyl)phenethyl]-1,3-propanediol (3: TX-2256)

The TMS-diyne **13** (117 mg, 0.265 mmol) was dissolved in acetone (3.5 mL), and NBS (87 mg, 0.49 mmol) and $AgNO_3$ (42 mg, 0.25 mmol) were added. After 2 h, $H_2O$ was added at 0 °C. The mixture was extracted with $Et_2O$, the extracts were washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:2) to give the bromo-diyne **14** (123 mg, quant.) as a yellow solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.42 (d, 2H, *J* = 8.3 Hz), 7.14 (d, 2H, *J* = 8.3 Hz), 5.68 (s, 1H), 4.32 (s, 4H), 2.61 (m, 2H), 2.20 (m, 2H), 2.09 (s, 6H), 1.97 (s, 3H); FT-IR (KBr): 3380, 3072, 2927, 2857, 2219, 2130, 1745, 1711, 1695, 1662, 1551, 1509, 1457, 1420, 1375, 1247, 1185, 1143, 1047, 891, 850, 822, 638, 605, 546 $cm^{-1}$.

The bromo-diyne **14** (50.6 mg, 0.113 mmol) was dissolved in THF (1.1 mL), and trimethylsilylacetylene (19 μL, 0.13 mmol), $PdCl_2(PPh_3)_2$ (0.8 mg, 1 μmol), CuI (0.2 mg, 1 μmol), and $Et_3N$ (31 μL, 0.22 mmol) were added. After 2 h, the reaction was quenched with saturated aqueous $NH_4Cl$. The mixture was extracted with $Et_2O$ and the organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to give the TMS-triyne **15** (34.5 mg, 67%) as a white solid. $^1H$ NMR ($CDCl_3$): $\delta$ 7.43 (d, 2H, *J* = 8.5 Hz), 7.15 (d, 2H, *J* = 8.5 Hz), 5.68 (s, 1H), 4.32 (s, 4H), 2.61 (m, 2H), 2.20 (m, 2H), 2.09 (s, 6H), 1.97 (s, 3H), 0.22 (s, 9H); $^{13}C$ NMR ($CD_3CN$): $\delta$ 172.0, 172.0, 147.1, 134.8, 130.6, 118.9, 91.2, 88.8, 79.1, 74.5, 67.8, 65.2, 62.8, 59.2, 34.4, 30.8, 30.4, 24.5, 21.6, 0.0; FT-IR (KBr): 3315, 3208, 3081, 2962, 2931, 2858, 2175, 2077, 1742, 1654, 1551, 1511, 1469, 1437, 1376, 1248, 1141, 1049, 845, 761, 642, 603 $\text{cm}^{-1}$; EI-MS *m/z*: 465 ($M^+$); Anal. Calcd for $C_{26}H_{31}NO_5Si$: C, 67.07; H, 6.71; N, 3.01. Found: C, 66.82; H, 6.80; N, 2.97.

The TMS-triyne **15** (29.7 mg, 0.064 mmol) was dissolved in MeOH (270 μL), and 2 N aqueous LiOH (144 μL) was added to the solution dropwise at room temperature. After 23 h, the mixture was evaporated. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH = 2:1) to give the triyne **3** (10.8 mg, 63%) as a light brown solid. $^1H$ NMR ($CD_3CN$): $\delta$ 7.42 (d, 2H, $J$ = 8.5 Hz), 7.22 (d, 2H, $J$ = 8.5 Hz), 3.37 (dd, 4H, $J$ = 21.0, 10.7 Hz), 2.65 (m, 2H), 1.59 (m, 2H); FT-IR (KBr): 3394, 3355, 3336, 3306, 3057, 2932, 2864, 2855, 2362, 2195, 2134, 1624, 1576, 1510, 1466, 1455, 1400, 1146, 1129, 1085, 1022, 1067, 1049, 829, 807, 723, 639, 545 $\text{cm}^{-1}$; CI-HRMS *m/z* calcd for $C_{17}H_{18}NO_2$ [$(M+H)^+$] 268.1338, found 268.1342.

#### 4.3.13. 2-(Acetylamino)-2-[(acetyloxy)methyl]-4-{4-[6-(1,1,1-trimethylsilyl)-1,3,5-hexatriynyl]phenyl}butyl acetate (15)

Bistrimethylsilylbutadiyne (229 mg, 1.2 mmol) was dissolved in THF (2.3 mL), and MeLi·LiBr (1.5 M solution in THF, 790 μl, 1.2 mmol) was added at 0 °C. After 1 h, CuBr (169 mg, 1.2 mmol) was added, and then the solution was warmed to room temperature. After 30 min, the solution was evaporated. The bromo-monoyne **12** (500 mg, 1.2 mmol) in pyridine (3.0 mL) was added to the residue at room temperature. After 4 h, the reaction was quenched with saturated aqueous $NH_4Cl$. The mixture was extracted with $Et_2O$, and the organic layer was washed with brine, dried with $Na_2SO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:1 to 1:1 gradient) to give the TMS-triyne **15** (244 mg, 44%) as a brown solid.

### 4.4. Determination of log*P*ow

The measurement of log*P*ow values was carried out using an HPLC method. UV detection was performed at 254 nm. Methanol and water in a ratio 3:1 (v/v) were employed as the mobile phase at a flow rate of 1.0 mL/min. The test compounds and the reference compounds were dissolved in the mobile phase for their injection. The log*P*ow is deduced from the logarithm of the capacity factor *k* (log*k*), calculated according to the following equation.

$$k = (t_r - t_0)/t_0$$

where $t_r$ is the retention time and $t_0$ is the dead time. Thiourea dissolved in the mobile phase was used to determine the $t_0$. In order to correlate the log*k* of a compound with its log*P*ow value, a calibration graph using 11 reference compounds, ranging in log*P*ow values from 0.3 to 5.7, was established. Since the calibration graph showed strong correlation ($R^2$ = 0.9806) between log *k* and log*P*ow, the log*P*ow value of any compound exceeding 5.7 was determined by extrapolation.

### 4.5. Chick embryo CAM assay

Antiangiogenic activity was assayed using the CAM assay according to the method described by Oikawa.[63] All compounds tested were dissolved in DMSO. In a control experiment, a test compound solution was replaced with DMSO. Fertilized chicken eggs were incubated in a humidified incubator at 37.6 °C for 4 days and then two small holes were bored in the eggshell at the lateral and the blunt end. After albumin (3 mL) was extracted from the lateral hole with a syringe, mild suction was applied to the blunt end hole to displace the air sac and drop the CAM away from the shell membrane. The lateral hole was sealed with adherent tape. The blunt end of the shell was picked away with a tweezer to expose the shell membrane. A part of this was removed so that a window could be made and the CAM identified. The diameter of the window was about 1 cm. The windows were closed with stainless-steel caps. The eggs were incubated in a humidified incubator at 39.0 °C. After 1 day, the cap was removed to check if the size of the CAM had become 2–3 mm. A silicon ring (3.0 mm external diameter, 5.0 mm internal diameter, 1.5 mm thickness) was placed on the CAM, and then 10 μL of test compound solution or control solution mixed in 1% methyl cellulose/0.9% NaCl (0.05% DMSO) was applied into the silicon ring. The windows were closed with stainless-steel caps. The eggs were incubated in a humidified incubator at 39.0 °C. After 1 day, the position of silicon ring was checked, and temperature of incubator was raised to 39.5 °C. The eggs were incubated for 1day and then the shell was cut out with scissors to make the CAM easy to observe. Inhibition of angiogenesis was evaluated after visualization by injection of a fat emulsion Intralipos® (1 mL) into the CAM. The angiogenic inhibition was indicated by an avascular zone around a ring of 3 mm diameter. It was evaluated in five stages. The egg whose vessels around a ring showed no inhibition of angiogenesis was designated minus. The egg whose vessels were not clear as to whether these were positive or negative was designated plus-minus. The egg whose capillaries were partially inhibited was designated plus, while the egg whose capillaries were completely inhibited was double plus. The egg whose large vessels showed inhibition was designated triple plus. The percent inhibition of angiogenesis was calculated by the following equation.

$$\text{Inhibition }(\%) = N_i/N_s \times 100$$

where $N_i$ is number of eggs evaluated greater than or equal to plus, $N_s$ is number of total survived eggs.

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