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Synthesis and Biological Evaluation of SERMs with Potent Nongenomic Estrogenic Activity

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*We have synthesized novel SERMs that activate a rapid response in CNS neurons, but which lack the ability to bind to the nuclear estrogen receptors (ERα and ERβ). These compounds are analogues of 4-hydroxytamoxifen, but unlike 4-hydroxytamoxifen, they do not exist as a mixture of E/Z isomers. They contain a carboxamide insertion between the olefin and basic phenyl side chain, which results in more stable geometric isomers. The amide insertion also eliminates their ability to bind to the nuclear estrogen receptors, and hence, they are unable to modulate ER-mediated gene transcription as do classical estrogens and SERMs. We show that one of these analogues, **ST-X**, elicits a potent nongenomic estrogen response in the CNS by rapidly inhibiting GIRK activation in hypothalamic γ-aminobutyric acid (GABA) and proopiomelanocortin (POMC) neurons. To our knowledge, **ST-X** is the only SERM that modulates rapid estrogen responses, but which lacks nuclear ER activity.*

Introduction

Estrogen is an important regulatory hormone, with 17β-estradiol (E2) being the primary estrogen present before menopause. E2 exerts its tissue-specific effects by interacting with cells in a variety of target tissues. The main tissues targeted by E2 are breast and uterus; however, E2 also acts on the liver, bone, and brain. Traditionally, E2 action in peripheral tissues has been attributed to E2 binding to nuclear estrogen receptors (ERα or ERβ). The activated ERs bind to estrogen response elements in the promoter region of target genes, thereby regulating gene transcription. Such transactivation is a slow genomic process, which can take hours to days to alter cellular physiology.[1] However, there has been increasing evidence for rapid effects of E2 in various cell types that is attributed to nongenomic signaling.[2–4] In fact, several effects of estrogen in the central nervous system (CNS) are attributed to nongenomic actions since the responses to hormone are rapid, taking from seconds to minutes, and are insensitive to transcription and translation inhibitors.[5]

E2 has been shown to rapidly alter Ca^{2+} levels in mid-brain neurons,[6] transduce signals through MAPK pathways in neuronal cells,[7] elevate cAMP levels in hippocampal CA1 neurons,[8] and stimulate inositol triphosphate generation in rat brain cells.[9] In the hypothalamus, E2 regulates the activity of gonadotropin releasing hormone (GnRH) neurons directly and indirectly. Indirectly, it modulates neurons that form synaptic connections on GnRH neurons such as POMC and GABAergic neurons.[10] These presynaptic neurons contain G-protein-coupled inwardly rectifying potassium (GIRK) channels that are coupled to the μ-opioid and $GABA_B$ G-protein-coupled receptors (GPCRs). GPCR activation leads to GIRK activation, which elicits a K^+ current that hyperpolarizes POMC and GABA neurons.[11] Recently, we have shown that E2 rapidly inhibits the coupling of μ-opioid and $GABA_B$ receptors to GIRKs in POMC and GABA neurons.[12] The same inhibition is observed with a cell-impermeable E2–BSA conjugate; this suggests that the receptor associated with this response is membrane bound. Several selective estrogen receptor modulators (SERMs) were also tested and shown to behave like E2. Surprisingly, we found a novel SERM, namely **ST-X**, which strongly inhibits GIRK activation in GABAergic neurons. The estrogenic effect of **ST-X** is unique because **ST-X** has no binding affinity for the nuclear ERα or ERβ and shows no uterotrophic action in guinea pigs or mice. This suggests that **ST-X** action involves a novel receptor that is distinct from the nuclear ERs. Herein, we present the synthesis of **ST-X**, and the design and synthesis of analogues of this compound.

Results

Design of molecules

Our approach for understanding the molecular mechanism of estrogen signaling is to synthesize novel ligands that activate or block nongenomic signaling selectively over genomic signaling. We initially proposed the synthesis of molecules to target the nuclear ERs to explore the chemical structural elements of SERMs such as 4-hydroxytamoxifen and raloxifene that allowed them to be agonists at AP-1 sites.[13–15] 4-Hydroxytamoxifen and raloxifene have similar overall structure with a noteworthy difference of a ketone hinge in raloxifene that

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allows the basic amine-containing side chain more flexibility (Figure 1). Previously, we have shown that an analogue of raloxifene which lacks the ketone functionality behaves more like 4-hydroxytamoxifen than raloxifene at an AP-1 site.[15] Originally, we hoped to expand on this initial observation through the synthesis and evaluation of novel triphenylethylene analogues such as the ketone-linked molecule shown in Figure 1. The synthesis of this compound proved to be difficult, and the *E* and *Z* isomers were inseparable. Compounds containing an amide group between the vinylic and aryl system were then proposed based on their synthetic feasibility.

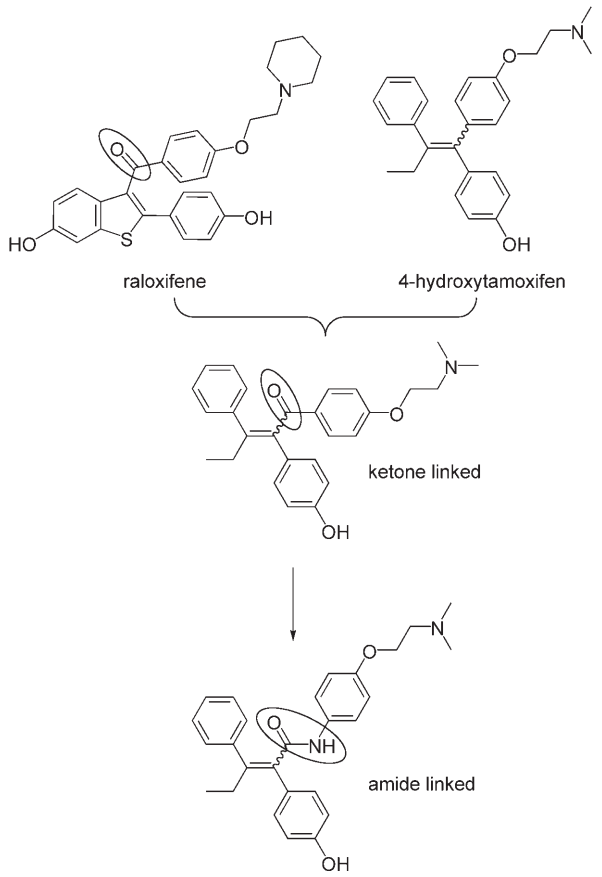
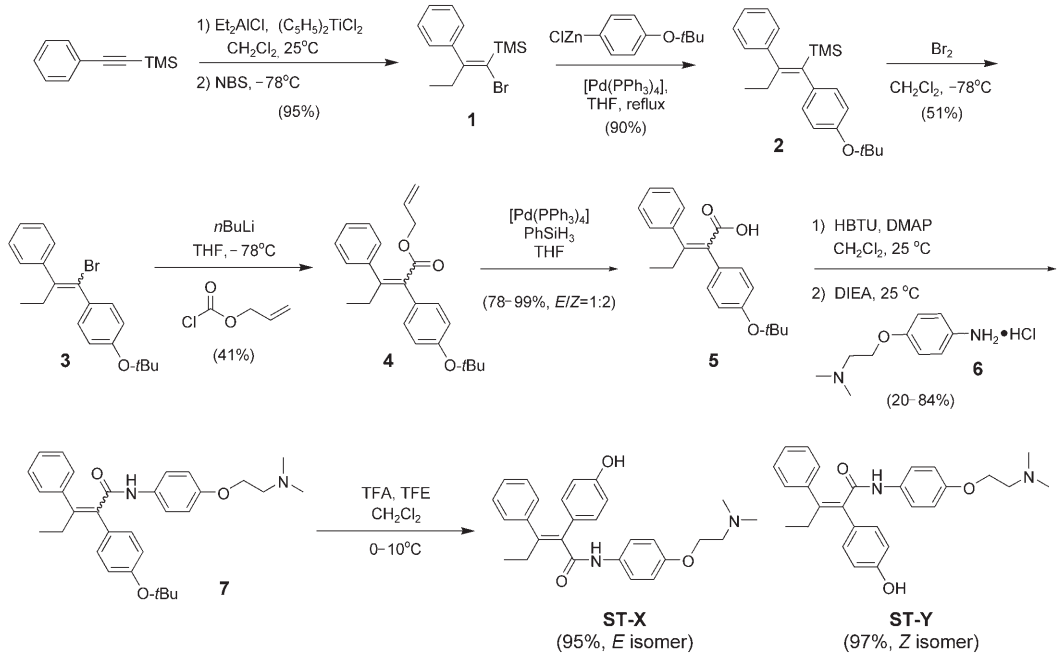


Figure 1. Structures of raloxifene, 4-hydroxytamoxifen, and the proposed raloxifene–hydroxytamoxifen hybrid molecules.

Synthesis of ST-X and ST-Y

As shown in Scheme 1, the synthesis began by reacting commercially available 1-phenyl-2-(trimethylsilyl)acetylene with diethylaluminum chloride followed by treatment with *N*-bromosuccinimide (NBS) to obtain the tetrasubstituted olefin **1**.[16] This vinyl bromide was cross coupled with *tert*-butoxybenzene by palladium-catalyzed coupling to give vinylsilane **2**.[14] Replacement of the trimethylsilyl group was accomplished by treatment with bromine to yield vinyl bromide **3**;[14] at this stage in the synthesis, two inseparable isomers formed in a 2:1 ratio in favor of the *Z* isomer. The vinyl bromide was transmetalated with *n*-butyl lithium and quenched with allyl chloroformate to give **4**, followed by removal of the allyl group to yield the carboxylic acid **5**. At this point, the two isomers were separated by using silica gel chromatography. Each isomer was coupled with HBTU and DMAP to the *para*-substituted aniline **6**, followed by removal of the protecting group to give **ST-X** (*E* isomer) and **ST-Y** (*Z* isomer).



Scheme 1. Synthesis of amide-linked compounds **ST-X** and **ST-Y**. DIEA = *N*,*N*-diisopropylethylamine; DMAP = (4-dimethylaminopyridine); HBTU = (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); NBS = *N*-bromosuccinimide; TFA = trifluoroacetic acid; TFE = trifluoroethanol.

Binding assay to nuclear ERs

These initial compounds were tested for binding affinity to ERα and ERβ in a competitive binding assay by using fluorescence polarization (data for **ST-X** were reported previously[12]). Relative binding affinities (RBA) are expressed as a percentage of E2 binding (Table 1). These data demonstrate that the affinity of **ST-X** and **ST-Y** for ERα and ERβ is more than 10000-fold lower than that of E2. It is also worth mentioning that the binding affinity of **ST-X** toward these receptors is weaker than that of **ST-Y**; the same trend is observed for 4-hydroxytamoxifen, in which the *Z* isomer has higher ER affinity.

Assay for rapid response effects

ST-X and **ST-Y** were subsequently tested in a GIRK activation assay in hypothalamic arcuate neurons. The rapid effects of ligands on the activation of the GIRK conductance by baclofen was measured by using the whole-cell recording paradigm illustrated in Figure 2. Cells were perfused for 5 min with tetrodotoxin (TTX) alone before they were treated with baclofen for 5 min. Baclofen generated the first $GABA_B$ receptor-mediated outward current response (R1). After washout, it returned to its resting level, and cells were treated for 15 min with vehicle or ligand. Baclofen was perfused again and a second response (R2) was measured. The ligand effect on the baclofen response is expressed as a percentage of R2/R1.[12] As shown in Figure 3, 0.1 μM E2 decreased the potency of the $GABA_B$ receptor agonist baclofen to activate GIRK by 41%. The membrane-impermeable estrogen conjugate (E2–BSA, 0.1 μM) and raloxifene (1.0 μM) had a similar effect on the outward current. 4-Hydroxytamoxifen (1.0 μM) and **ST-Y** (1.0 μM) blocked the baclofen response by 25%. Most fascinating, we found that 0.01 μM **ST-X** is as effective as 0.1 μM E2. These results allowed us to determine that the *E* isomer is important for this rapid response. In addition, we determined that insertion of the amide linker abolished binding to the nuclear ERs and prevented isomerization. Our next step was to try to look at the chemical determinants of **ST-X** activation by synthesizing analogues.

Synthesis of ST-X analogues

We first examined whether the *para*-hydroxy group or the alkenyl ethyl group were important in inducing this rapid effect. Scheme 2 shows the synthesis of the des-hydroxy **ST-X** derivative. Commercially available diphenyl acetylene was alkylated and subsequently allowed to react with diethylaluminum chloride and bis(cyclopentadienyl)titanium dichloride, followed by iodination with *N*-iodosuccinimide (NIS) to yield the vinyl iodide **8**. Replacement of the iodine atom was carried out by CO insertion in the presence of methanol to yield the methyl ester **9,** which was then subjected to saponification to give **10**. Methyl ester **9** was obtained as a 2:1 mixture of *E* and *Z* isomers, which were separable at this stage or at the acid stage (compound **10**). Compound **10** was finally coupled to the *para*-substituted aniline to yield the desired product **ST-4**.

Scheme 3 shows the preparation of the des-ethyl **ST-X** derivative. The synthesis begins with commercially available 4-hydroxyphenylacetic acid that was treated with allyl bromide to yield protected phenyl acetate **11**. Addition of the lithium eno-

Table 1. RBA of ligands to nuclear ERs.

Ligand	RBA [%][a]	
	ERα	ERβ
17β-estradiol	100	100
4-hydroxytamoxifen	36	43
raloxifene	34	76
ST-X (*E* isomer)	4.3×10^{-6}	9.0×10^{-6}
ST-Y (*Z* isomer)	2.8×10^{-4}	2.1×10^{-5}

[a] RBA = relative binding affinity (with respect to E2).

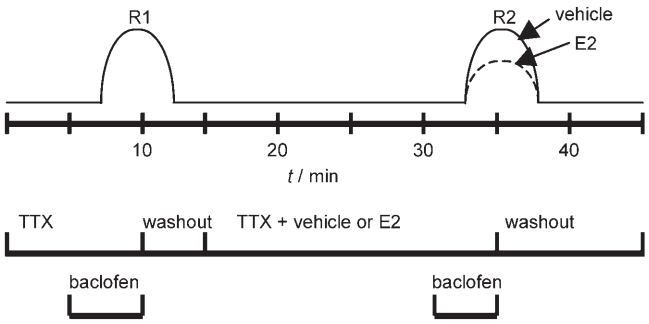


Figure 2. Schematic protocol for drug administration in arcuate neurons. Cells were perfused for 5 min with tetrodotoxin (TTX) to block action potentials before they were perfused with baclofen (at the EC_{50} concentration of 5 μM) for 5 min. Baclofen generated the first $GABA_B$ receptor-mediated outward current response (R1). After washout, the current returned to its resting level, and cells were treated for 15 min with vehicle or ligand. Baclofen was perfused again, and a second outward current response (R2) was measured. The ligand effect on the baclofen response is expressed as a percentage of R2/R1.

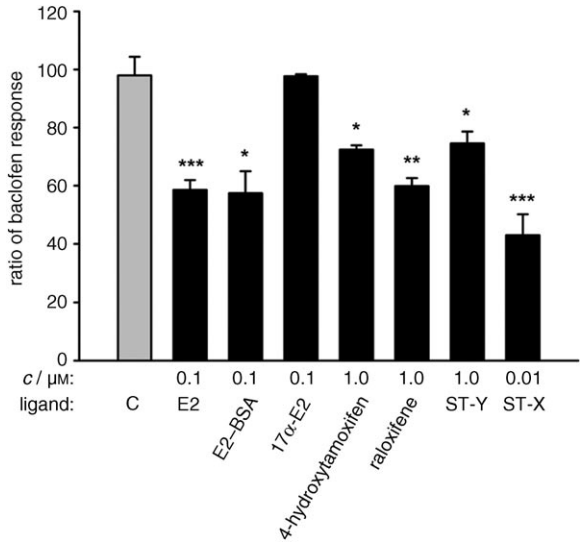
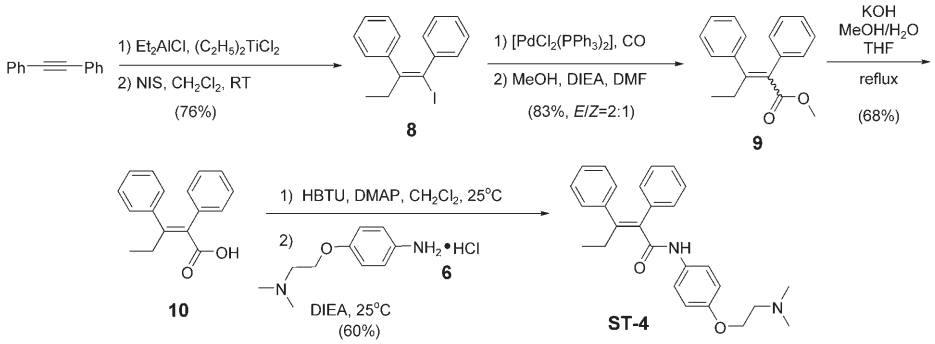


Figure 3. Attenuation of the $GABA_B$ response by SERMs. Data for all SERMs except **ST-Y** have been previously reported.[12] Error bars represent the mean ± SEM of 4–11 cells tested per group; $^*p<0.05$, $^{**}p<0.01$, and $^{***}p<0.005$ versus control group (C).



Scheme 2. Synthesis of **ST-4**: analogue lacking the hydroxy group. DMF = *N*,*N*-dimethylformamide; NIS = *N*-iodosuccinimide.

late of **11** to benzaldehyde followed by mesylation of the alcohol **12** and elimination gave vinyl derivative **13**. Removal of the allyl groups followed by coupling to **6** gave the desired product as a mixture of isomers (*E*/*Z* = 4:1) that were separated by silica gel chromatography to give purified **ST-6**.

Effects of ST-4 and ST-6 on attenuating rapid response

Next, we tested these two compounds to see if they blocked the baclofen-induced activation of GIRKs. The data is summarized in Figure 4 and expressed as the ratio of the baclofen response. Neither compound blocked the GIRK channel activation by baclofen at 0.01 μM (data not shown), however **ST-6** was more efficacious at blocking the baclofen response than **ST-4** at 1.0 μM. There was a decrease of only 15% in the response when **ST-4** was applied, compared with 45% for **ST-6** and 25% for **ST-Y** (all at 1.0 μM), whereas **ST-X** inhibits 57% at 0.01 μM. These results indicate that the *E*-olefin geometry is important and both the hydroxy and ethyl groups are necessary for the rapid response observed in hypothalamic arcuate neurons.

Discussion

It is known that 4-hydroxytamoxifen exists in a dynamic equilibrium and that the active ER ligand is (*Z*)-4-hydroxytamoxifen, and not the *E* isomer.[17] Compounds **ST-X** and **ST-Y** differ from 4-hydroxytamoxifen in that they contain an amide that links the basic amine-containing side chain and the stilbene system. We found that this simple amide linker allows the mixture of isomers to be easily separated and results in geometrical stability that prevents *E*/*Z* isomerization. We were surprised to find that these compounds have essentially no binding affinity for ERα or ERβ. Although **ST-X** has 100-fold less binding affinity for ERα than **ST-Y** and 10-fold less binding affinity for ERβ, the ER binding of these compounds is sufficiently poor that it can be considered negligible in any pharmacology study we have conducted.

Next, we were interested in looking at whether these compounds could induce rapid E2 responses, and found that both **ST-X** and **ST-Y** elicit rapid estrogen responses in hypothalamic neurons. **ST-X** caused greater inhibition of the baclofen-in-

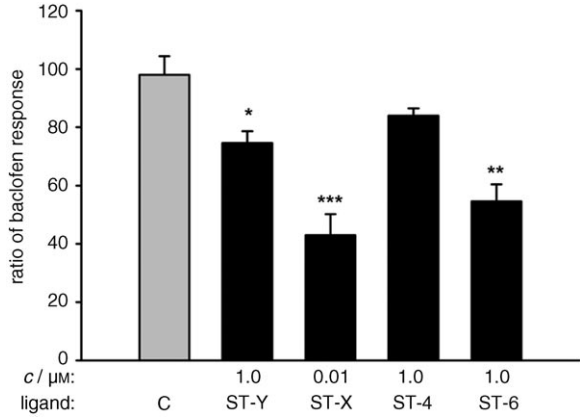
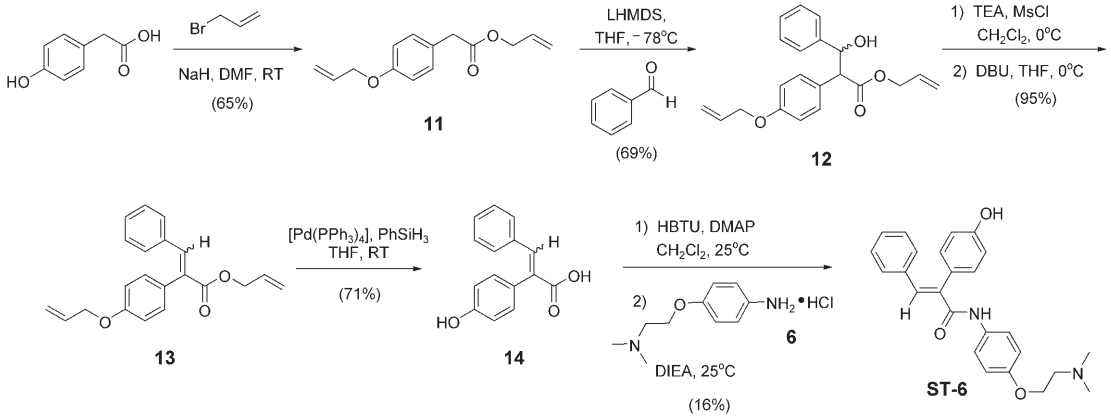


Figure 4. Attenuation of the $GABA_B$ response by **ST-X**, **ST-Y**, **ST-4**, and **ST-6**. Error bars represent the mean ± SEM of 3–4 cells tested per group; $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$ versus control group (C).



Scheme 3. Synthesis of **ST-6**: analogue lacking the alkenyl ethyl group. LHMDS = lithium hexamethyldisilazide.

duced activation of GIRK in GABAergic neurons than **ST-Y**. These results establish that the *E* olefin is critical for this rapid response. More importantly, we found that 0.01 μM **ST-X** induces greater inhibition than 0.1 μM E2. The next step was to examine the structural elements of **ST-X** that endow it with rapid, nongenomic estrogen responses and no slow transcriptional responses. As the *E* geometry proved to be critical, we synthesized and tested **ST-4** and **ST-6**. Both of these compounds, which lack either the phenolic hydroxy group or the alkenyl ethyl group, respectively, were not as effective at eliciting the rapid response as shown with **ST-X**. Thus, **ST-X** is currently the most potent and efficacious ligand observed that elicits rapid estrogenic responses and which does not bind to the nuclear estrogen receptors. Furthermore, the results obtained with these analogues provide evidence that a novel estrogen receptor that is different from ERα and ERβ mediates these nongenomic responses. In fact, we have characterized this novel estrogen receptor as being Gαq-coupled to activation of a phospholipase C–protein kinase C-protein signaling pathway in hypothalamic neurons.[12]

In addition to having beneficial effects on the brain, estrogen has therapeutic effects on the bone and cardiovascular system, but its effects on the uterus and breast might be harmful in post-menopausal women. In the CNS, estrogen modulates thermal regulation, cognitive function, and mood. Decreased estrogen concentrations in post-menopausal women can lead to debilitating symptoms including hot flashes, memory loss, and anxiety. However, hormone-replacement therapy for the treatment of menopausal symptoms is currently controversial due to the deleterious effects of estrogen on reproductive tissues and the cardiovascular system.[18] A ligand that is specific for this nongenomic estrogen signaling is important because it is less likely to have the harmful side effects that are caused by hormone-replacement therapy. As a result, there is a need for a new generation of SERMs to selectively attenuate the CNS estrogen responses for the relief of menopausal symptoms that lack the peripheral estrogenic effects.

Experimental Section

Materials and methods: NMR spectra were recorded using a Varian Utility 400 MHz spectrometer. Mass spectral analyses were obtained from the Biomedical Mass Spectrometry Resource at UCSF or the Mass Spectrometry Facility at UC Berkeley. Final compounds were analyzed for purity by analytical HPLC, which was performed by using a Waters AllianceHT LC and a Waters AllianceHT LC–MS. Condition A (using HPLC): gradient of 0→100% acetonitrile to water (0.05% TFA) over 10 min. Condition B (using LC–MS): gradient of 0→100% methanol to water (0.05% TFA) over 10 min. All anhydrous reactions were carried out in flame-dried flasks under argon and all organic solvents were anhydrous. Crude products were purified by flash chromatography using silica gel grade 60 (230–400 mesh). Procedures for the estrogen receptor binding assay and the electrophysiology for the rapid-response effects have been published elsewhere.[12]

Synthetic protocols: Syntheses of compounds (*E*)-1-bromo-2-phenyl-1-trimethylsilyl-1-butene (**1**), (*Z*)-2-phenyl-1-(4-*tert*-butoxyphenyl)-1-trimethylsilyl-1-butene (**2**), and 1-bromo-2-phenyl-1-(4-*tert*-butoxyphenyl)-1-butene (**3**) were carried out as previously reported.[14]

2-(4-*tert*-Butoxyphenyl)-3-phenylpent-2-enoic acid allyl ester (4): *n*-Butyl lithium (6.68 mL, 16.7 mmol; 2.5 M) was added dropwise to a solution of vinyl bromide **3** (3.00 g, 8.35 mmol) in anhydrous THF (75 mL) that was cooled to −78 °C. After stirring for 15 min, allyl chloroformate (4.43 mL, 41.8 mmol) was added neat and dropwise. This was stirred from −78 °C to room temperature for 4 h. Saturated sodium bicarbonate was added, and the solution was extracted with ethyl acetate, then dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography (5% EtOAc/hexanes) to give **4** as a light yellow oil (1.80 g, 4.94 mmol) in 59% yield. $R_f = 0.20$ for the *E* isomer and 0.14 for the *Z* isomer (5% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) *E* isomer: $\delta = 1.01$ (t, 3H), 1.25 (s, 9H), 2.68 (q, 2H), 4.72 (d, 2H), 5.25 (m, 2H), 5.94 (m, 1H), 6.69 (d, 2H), 6.87 (d, 2H), 7.00 (m, 2H), 7.11 ppm (m, 3H); *Z* isomer: $\delta = 0.82$ (t, 3H), 1.35 (s, 9H), 2.37 (q, 2H), 4.27 (d, 2H), 4.96 (m, 2H), 5.43 (m, 1H), 7.00 (d, 2H), 7.26 ppm (m, 7H); ^{13}C NMR ($CDCl_3$) *Z* isomer: $\delta = 12.71$, 14.10, 23.32, 25.66, 27.98, 28.87, 38.98, 65.12, 74.35, 78.57, 117.82, 123.73, 127.36, 127.64, 128.06, 129.60, 131.74, 131.80, 131.90, 141.17, 149.25, 154.93, 169.18 ppm; HRMS (EI) mass calcd for $C_{24}H_{28}O_3$: 364.2038, found: 364.2038.

2-(4-*tert*-Butoxyphenyl)-3-phenylpent-2-enoic acid (5): $PhSiH_3$ (0.11 mL, 0.878 mmol) was added to a solution of compound **4** (0.16 g, 0.439 mmol) in anhydrous dichloromethane (10 mL), followed by [$Pd(PPh_3)_4$] (10 mg, 8.78 μmol). The reaction was stirred at room temperature under argon for 30 min. It was then quenched with ddH_2O, and the pH was decreased to 2 with 1 M HCl. The solution was extracted with dichloromethane, then dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude yellow oil was purified by column chromatography (35% EtOAc/hexanes) to give a white solid (0.14 g, 0.432 mmol) in 99% yield. $R_f = 0.33$ for the *E* isomer and 0.49 for the *Z* isomer (10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$) *E* isomer: $\delta = 1.02$ (t, 3H), 2.80 (q, 2H), 6.70 (d, 2H), 6.90 (d, 2H), 6.98 (m, 3H), 7.10 ppm (m, 2H); *Z* isomer: $\delta = 0.84$ (t, 3H), 2.35 (q, 2H), 7.00 (d, 2H), 7.22 (d, 2H), 7.27 ppm (m, 7H); HRMS (EI) mass calcd for $C_{21}H_{24}O_3$: 324.1725, found: 324.1570.

4-(2-(Dimethylamino)ethoxy)aniline (6): 4-Aminophenol (5.00 g, 45.8 mmol) was suspended in chloroform (90 mL). A solution of sodium chloride (8.03 g, 13.7 mmol) in water (70 mL) was added, followed by di-*tert*-butyl dicarbonate (10.0 g, 45.8 mmol) and $NaHCO_3$ (3.85 g, 45.8 mmol). After 1 h at reflux, the reaction was cooled to room temperature. The layers were separated, and the aqueous layer was extracted with chloroform, dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude Boc-protected product was purified through a plug of silica gel using 1:1 EtOAc/hexanes to give a tan solid (8.50 g, 45.4 mmol) in 99% yield (Boc = *tert*-butoxycarbonyl). $R_f = 0.47$ (EtOAc/hexanes 1:1 *v/v*). ^{1}H NMR ($CDCl_3$): $\delta = 6.81$ (d, 2H), 6.55 (d, 2H), 4.52 (s, 2H), 4.25 (m, 2H), 1.25 ppm (t, 3H). The Boc-4-aminophenol (2.00 g, 9.55 mmol) was dissolved in DMF (40 mL), followed by the addition of 2-(dimethylamino)ethyl chloride (1.38 g, 9.55 mmol), $CsCO_3$ (16.86 g, 47.8 mmol) and KI (3.2 mg, 0.019 mmol). The reaction mixture was heated without reflux overnight. After cooling to room temperature, DMF was removed under high vacuum, then re-dissolved in chloroform and washed with water. The water layer was extracted with chloroform, and the organic layers were dried over magnesium sulfate, filtered, and concentrated under decreased pressure to produce a brown oil that was purified in 20% MeOH/$CHCl_3$ to give a tan solid (2.20 g)

in 60% yield. $R_f = 0.54$ (20% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 7.22$ (d, 2H), 6.85 (d, 2H), 4.05 (t, 2H), 2.72 (t, 2H), 2.58 (s, 6H), 1.45 ppm (s, 9H). The desired product was obtained by treating 4-(2-(dimethylamino)ethoxy)-Boc-aniline (1.15 g, 5.30 mmol) with 1 M HCl in ethyl acetate (40 mL, anhydrous) overnight. The next day, the ethyl acetate was filtered off to give a tan solid (1.03 g, 4.75 mmol) in 90% yield. ^1H NMR (MeOD): $\delta = 7.33$ (d, 2H), 7.15 (d, 2H), 4.35 (t, 2H), 3.58 (t, 2H), 2.95 ppm (s, 6H); HRMS (EI) mass calcd for $C_{10}H_{16}N_2O$: 180.1263, found: 180.1268.

2-(4-*tert*-Butoxyphenyl)-3-phenylpent-2-enoic acid [4-(2-dimethylaminoethoxy)phenyl]amide (7): HBTU (25.7 mg, 0.0617 mmol) and DMAP (0.4 mg, 3.09 μmol) were added to a solution of compound **5** (20.0 mg, 0.0617 mmol) in dichloromethane (1 mL) that was pre-cooled to 0 °C. The reaction was stirred at 0 °C for 30 min and at room temperature for an additional 30 min. It was then re-cooled to 0 °C, and the amine hydrochloride salt **6** (14.7 mg, 0.0679 mmol) was added, followed by the dropwise addition of DIEA (0.04 mL, 0.247 mmol). The reaction was stirred at room temperature for 30 min, then quenched with saturated ammonium chloride, and extracted with dichloromethane. The combined organic layers were washed with saturated sodium bicarbonate, then dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography (10% MeOH/$CHCl_3$) to give a white foam (25.4 mg, 0.519 mmol) in 84% yield. $R_f = 0.15$ for the *E* isomer and 0.33 for the *Z* isomer (10% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$) *E* isomer: $\delta = 1.02$ (t, 3H), 1.25 (s, 9H), 2.35 (s, 6H), 2.82 (t, 2H), 2.79 (q, 2H), 4.02 (t, 2H), 6.72 (d, 2H), 6.85 (d, 2H), 6.92 (d, 2H), 7.01 (m, 3H), 7.10 (m, 3H), 7.38 ppm (d, 2H); *Z* isomer: $\delta = 0.91$ (t, 3H), 1.38 (s, 9H), 2.40 (s, 6H), 2.45 (q, 2H), 2.82 (t, 2H), 3.96 (t, 2H), 6.63 (d, 2H), 6.82 (m, 3H), 7.01 (d, 2H), 7.30 ppm (m, 6H).

2-(4-Hydroxyphenyl)-3-phenylpent-2-enoic acid [4-(2-dimethylaminoethoxy)phenyl]amide (ST-X, ST-Y): Compound **7** (25.3 mg, 0.0514 mmol) was dissolved in anhydrous dichloromethane (0.47 mL) and cooled to 0 °C. 2,2,2-Trifluoroethanol (0.37 mL) and trifluoroacetic acid (0.74 mL) were added, and the reaction mixture was stirred from 0 to 10 °C over 1.5 h. After this time, the solution was poured into 1 M HCl, extracted with CH_2Cl_2, then dried over magnesium sulfate, filtered, and the solvent was removed under decreased pressure. The crude product was purified by column chromatography (15% MeOH/$CHCl_3$) to give a white foam (21.5 mg, 0.499 mmol) in 97% yield. $R_f = 0.18$ for the *E* isomer and 0.22 for the *Z* isomer (15% MeOH/$CHCl_3$). ^1H NMR (MeOD) *E* isomer: $\delta = 0.90$ (t, 3H), 2.49 (s, 6H), 2.58 (q, 2H), 2.95 (t, 2H), 4.10 (t, 2H), 6.43 (d, 2H), 6.86 (m, 4H), 7.10 (m, 5H), 7.47 ppm (d, 2H). ^{13}C NMR (MeOD): $\delta = 13.20$, 30.40, 45.18, 58.66, 65.68, 115.69, 115.82, 123.41, 127.93, 129.11, 130.42, 171.77, 133.29, 136.06, 141.48, 143.52, 156.74, 157.57, 171.96 ppm. ^1H NMR (MeOD) *Z* isomer: $\delta = 0.90$ (t, 3H), 2.49 (s, 6H), 2.50 (q, 2H), 2.92 (t, 2H), 4.05 (t, 2H), 6.75 (d, 2H), 6.83 (d, 2H), 7.01 (d, 2H), 7.27 (m, 6H), 7.39 ppm (d, 2H). ^{13}C NMR (MeOD): $\delta = 13.21$, 28.22, 45.35, 58.75, 65.78, 117.2, 116.5, 123.8, 128.5, 129.2, 129.5, 131.2, 132.5, 137.2, 142.2, 147.8, 156.5, 158.4, 171.8 ppm; HRMS (EI) mass calcd for $C_{27}H_{30}N_2O_3$: 430.2256, found: 430.2263.

1-Iodo-1,2-diphenylbut-1-ene (8): Diethylaluminum chloride (9.78 g, 0.0393 mol) was added to a solution of bis(cyclopentadienyl)titanium dichloride (21.8 mL, 0.0393 mol; 1.8 M) in anhydrous dichloromethane (200 mL), and the mixture was stirred at room temperature for 10 min under an atmosphere of argon. Diphenylacetylene (5.0 g, 0.0281 mol) was added slowly and stirred for 5 h. The reaction mixture was cooled to −78 °C and diluted with dichloromethane (70 mL). NIS (14.5 g, 0.0645 mol) was added slowly so that the temperature remained at −78 °C; after addition, the reaction was stirred at room temperature overnight. The next day, it was poured into hexanes (100 mL); 5% Na_2SO_3 in 3 N NaOH (200 mL) was added, and the reaction mixture was filtered. After filtration, the two layers were separated, and the organic layer was washed with 5% Na_2SO_3 (200 mL), followed by 3 N HCl (200 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography in hexanes to give a light brown oil (7.15 g, 0.0214 mol) in 76% yield. ^1H NMR ($CDCl_3$): $\delta = 1.03$ (t, 3H), 2.82 (q, 2H), 7.05 ppm (m, 9H). ^{13}C NMR ($CDCl_3$): $\delta = 11.77$, 38.58, 98.89, 126.58, 126.92, 127.50, 127.78, 128.22, 128.32, 129.05, 129.88, 131.59, 139.60, 144.51, 150.24 ppm; HRMS (EI) mass calcd for $C_{16}H_{15}I$: 334.0218, found: 334.0216.

2,3-Diphenylpent-2-enoic acid methyl ester (9): Methanol (5 mL), DIEA (0.17 mL, 0.987 mmol), and [$PdCl_2(PPh_3)_2$] (107 mg, 0.153 mmol) were added to vinyl iodide **8** (300 mg, 0.898 mmol), which was dissolved in anhydrous DMF. Carbon monoxide was bubbled into the reaction for 5 min, and the reaction mixture was stirred at 80 °C under an atmosphere of CO for 3 days. The cooled reaction mixture was diluted with ethyl acetate and washed with ddH_2O; the layers were separated and the organic layer was dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography in chloroform to give the product (198 mg, 0.743 mmol; *E*/*Z* = 2:1) in 83% yield. $R_f = 0.47$ for the *E* isomer and 0.53 for the *Z* isomer ($CHCl_3$). ^1H NMR ($CDCl_3$) *E* isomer: $\delta = 1.01$ (t, 3H), 2.69 (q, 2H), 3.78 (s, 3H), 7.07 (m, 7H), 7.36 ppm (m, 4H); *Z* isomer: $\delta = 0.86$ (t, 2H), 2.38 (q, 1H), 3.38 (s, 1H), 7.07 (m, 7H), 7.36 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 12.72$, 12.80, 27.99, 29.96, 51.57, 51.94, 126.75, 126.97, 127.45, 127.57, 127.73, 127.84, 128.08, 128.37, 128.93, 128.99, 129.75, 130.19, 132.20, 137.05, 140.07, 149.91 ppm.

2,3-Diphenylpent-2-enoic acid (10): A solution of KOH (9.4 mL, 1.88 mmol; 0.2 M) was added dropwise to a solution of compound **9** (50 mg, 0.188 mmol) in methanol (4 mL) and THF (9 mL). The reaction was then heated at reflux for 2 days. The next day, it was poured into 10 mL of 1 N HCl and stirred for 10 min, then extracted with $CHCl_3$, and the organic layer was dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give a white solid (32 mg, 0.127 mmol) in 68% yield. $R_f = 0.33$ for the *E* isomer and 0.47 for the *Z* isomer (5% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$) *E* isomer: $\delta = 1.02$ (t, 3H), 2.80 (q, 2H), 7.02 (m, 4H), 7.11 ppm (m, 6H); *Z* isomer: $\delta = 0.84$ (t, 3H), 2.33 (q, 2H), 7.04 (d, 2H), 7.33 ppm (m, 8H); HRMS (EI) mass calculated for $C_{17}H_{15}O_2$: 252.1150, found: 252.1150.

2,3-Diphenylpent-2-enoic acid [4-(2-dimethylaminoethoxy)phenyl]amide (ST-3, ST-4): Compounds **ST-3** (the *Z* isomer of **ST-4**) and **ST-4** were synthesized as described for compounds **ST-X** and **ST-Y** using compound **10** (29 mg, 0.115 mmol), HBTU (48 mg, 0.126 mmol), DMAP (0.7 mg, 5.7 μmol), amine hydrochloride **6** (27 mg, 0.126 mmol), and DIEA (0.08 mL, 0.459 mmol) to give the desired product **ST-3** (19 mg, 0.0488 mmol) in 32% yield and **ST-4** (29 mg, 0.070 mmol) in 60% yield. $R_f = 0.15$ for the *E* isomer and 0.10 for the *Z* isomer (5% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$) *E* isomer: $\delta = 1.05$ (t, 3H), 2.32 (s, 6H), 2.70 (t, 2H), 2.82 (q, 2H), 4.05 (t, 2H), 6.85 (d, 2H), 7.10 (m, 8H), 7.42 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta =$ 13.09, 18.01, 29.69, 45.77, 58.17, 66.16, 114.86, 121.59, 126.93, 127.14, 127.92, 128.17, 128.99, 129.97, 131.07, 137.23, 140.50, 148.77, 155.66, 167.72 ppm; *Z* isomer: $\delta = 0.90$ (t, 3H), 2.39 (s, 6H),

2.41 (q, 2H), 2.81 (t, 2H), 4.00 (t, 2H), 6.62 (d, 2H), 6.82 (d, 2H), 7.39 ppm (m, 10H); HRMS (EI) mass calcd for $C_{27}H_{30}N_2O_2$: 414.23073, found: 414.23017 (**ST-3**), 414.23079 (**ST-4**).

(4-Allyloxyphenyl) acetic acid allyl ester (11): Sodium hydride (1.90 g, 0.0789 mmol) was suspended in anhydrous DMF (70 mL) and cooled to 0 °C. 4-Hydroxyphenylacetic acid (5.00 g, 0.329 mmol) was dissolved in DMF (30 mL), added to the cooled sodium hydride suspension and allowed to stir at room temperature for 2 h. After this time, the reaction was re-cooled to 0 °C, and allyl bromide (11.3 mL, 0.131 mmol) was added. After stirring for 4.5 h at room temperature, the solution was poured into saturated brine, extracted with ether, and the organic layer was washed with 10% KOH and then brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography ($CHCl_3$) to give an oil (4.97 g, 0.0214 mmol) in 65% yield. $R_f = 0.68$ ($CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 3.58$ (s, 2H), 4.51 (d, 2H), 4.58 (d, 2H), 5.24 (m, 2H), 5.90 (m, 1H), 6.02 (m, 1H), 6.87 (d, 2H), 7.19 ppm (d, 2H); ^{13}C NMR ($CDCl_3$): $\delta = 40.39$, 65.96, 68.80, 114.81, 117.60, 118.15, 126.16, 130.26, 132.05, 133.26, 157.72, 171.50 ppm; HRMS (EI) mass calcd for $C_{14}H_{16}O_3$: 232.1100, found [*M*+H]: 232.1104.

2-(4-Allyloxyphenyl)-3-hydroxy-3-phenyl propionic acid allyl ester (12): LHMDS (9.47 mL, 9.47 mmol) was added to a solution of **11** (2.00 g, 8.61 mmol) in anhydrous THF (120 mL) that was precooled to −78 °C. Benzaldehyde (0.88 mL, 8.61 mmol) was dissolved in THF and added to the cooled mixture. The reaction was warmed to −20 °C and stirred overnight. The next day, it was quenched with saturated ammonium chloride, and the aqueous layer was extracted with EtOAc; the organic layers were combined and dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude product was purified by column chromatography (10% EtOAc/hexanes) to give compound **12** (2.01 g, 5.94 mmol) in 69% yield. $R_f = 0.16$ (10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): $\delta = 3.07$ (d, 1H), 3.84 (d, 1H), 4.44 (d, 2H), 4.64 (m, 2H), 5.24 (m, 4H), 5.85 (m, 1H), 6.01 (m, 1H), 6.71 (d, 2H), 6.99 (d, 2H), 7.09–7.18 ppm (m, 5H).

2-(4-Allyloxyphenyl)-3-phenylacrylic acid allyl ester (13): TEA (1.2 mL, 8.87 mmol) followed by MsCl (0.46 mL, 5.91 mmol) were added to a precooled (0 °C) solution of **12** (1.00 g, 2.96 mmol) in dichloromethane (10 mL). The reaction was stirred for 2 h, then diluted with ether, filtered through a plug of celite, and concentrated under decreased pressure. The crude mesylate was diluted in anhydrous THF (40 mL), cooled to 0 °C, and DBU (1.3 mL, 8.87 mmol) was added. After stirring for 2 h at room temperature, 10% HCl was added. The reaction mixture was extracted with chloroform, and the organic layer was washed with saturated sodium bicarbonate, brine, and magnesium sulfate, then filtered and concentrated under decreased pressure. The crude product was purified by column chromatography (15% EtOAc/hexanes) to produce **10** (0.90 g, 2.81 mmol) in 95% yield. $R_f = 0.59$ (15% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): $\delta = 4.53$ (d, 2H), 4.69 (d, 2H), 5.28 (m, 4H), 5.93 (m, 1H), 5.07 (m, 1H), 6.90 (d, 2H), 7.13 (m, 7H), 7.81 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 65.63$, 68.76, 114.83, 117.72, 117.77, 128.03, 128.17, 128.90, 130.55, 131.01, 132.08, 132.24, 133.17, 134.81, 140.21, 158.22, 167.65 ppm; LRMS (+ESI) mass calcd for $C_{21}H_{20}O_3$: 320.1, found [*M*+H]: 321.2.

2-(4-Hydroxyphenyl)-3-phenyl acrylic acid (14): $PhSiH_3$ (0.78 mL, 6.24 mmol) was added to a solution of **13** in THF (35 mL) followed by the addition of [$Pd(PPh_3)_4$] at room temperature. The reaction was stirred under argon for 15 min. It was quenched by adding ddH_2O and then 1 N HCl to decrease the pH to 2. The reaction was extracted with dichloromethane, dried over magnesium sulfate, filtered, and concentrated under decreased pressure. The crude mixture was purified by column chromatography (1:1 EtOAc/hexanes) to produce **14** (266.9 mg, 1.11 mmol) in 71% yield. $R_f = 0.33$ (5% EtOAc/hexanes). ^{1}H NMR (MeOD): $\delta = 6.71$ (d, 2H), 6.91 (d, 2H), 7.02 (m, 2H), 7.11 (m, 9H), 7.71 ppm (s, 1H); LRMS (+ESI) mass calcd for $C_{15}H_{12}O_3$: 240.1, found [*M*+H]: 241.0.

***N*-[4-(2-Dimethylaminoethoxy)phenyl]-2-(4-hydroxyphenyl)-3-phenylacrylamide (ST-5, ST-6):** Compounds **ST-5** (the *Z* isomer of **ST-6**) and **ST-6** were synthesized as described for compounds **ST-X** and **ST-Y** by using compound **14** (266 mg, 1.11 mmol), HBTU (462 mg, 1.22 mmol), DMAP (6.8 mg, 55.4 μmol), amine hydrochloride (264 mg, 1.22 mmol), and DIEA (0.77 mL, 4.43 mmol) to give the desired product (72 mg, 0.178 mmol, *E*/*Z* = 4:1) in 16% yield. $R_f = 0.27$ (10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 2.36$ (s, 6H), 2.77 (t, 2H), 4.06 (t, 2H), 6.80 (d, 2H), 6.86 (d, 2H), 7.19 (m, 10H), 7.80 (s, 1H), 8.06 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 45.60$, 58.03, 65.76, 114.74, 116.06, 121.82, 123.29, 126.71, 127.94, 128.62, 130.40, 130.82, 131.16, 133.74, 151.71, 155.92, 156.84, 166.63 ppm; HRMS (EI) mass calcd for $C_{25}H_{26}N_2O_3$: 402.19434, found: 402.19382 (**ST-5**), 402.19511 (**ST-6**).

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