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Practical Synthetic Method of (2*Z*,3*E*)-1,4-Diphenylbutadiene T-2639, an Inhibitor of Plasminogen Activator Inhibitor-1 (PAI-1) Production

Hiroshi Miyazaki, Hiroshi Ohmizu, and Tsuyoshi Ogiku*

Medicinal Chemistry Laboratory, Mitsubishi Tanabe Pharma Co., Ltd., 3-16-89, Kashima, Yodogawa, Osaka 532-8505, Japan

Abstract:

A practical synthetic method of (2*Z*,3*E*)-1,4-diphenylbutadiene derivative T-2369 (1), a potent inhibitor of plasminogen activator inhibitor-1 (PAI-1) production, is described. Conditions of Stobbe condensation in the conventional synthesis of T-2369 were examined, and a new method to efficiently synthesize the key intermediate, (2*Z*,3*E*)-2,was derived. (2*Z*,3*E*)-2 was selectively obtained in 82% (with 91:9 selectivity) by predominant precipitation of (2*Z*,3*E*)-2 Na salt and *in situ* isomerization of (2*E*,3*E*)-2 to (2*Z*,3*E*)-2.

Introduction

It is well-known that plasminogen activator inhibitor-1 (PAI-1), a specific inhibitor of both tissue-type plasminogen activator and urokinase-type plasminogen activator, plays an important role in regulation of the fibrinolytic system.[1] As inhibition of PAI-1 activity or reduction of its production is believed to result in antithrombotic effect, a number of small molecules with the potential to inhibit PAI-1 or suppress PAI-1 production have recently been studied.[2,3]

We have previously reported T-2639 (**1**, Figure 1)[4] as a potent orally active inhibitor of PAI-1 with good DMPK and strong antithrombotic activity. However, the synthetic route of T-2639, which mainly consists of two Stobbe condensations (Scheme 1), has some drawbacks. Notably, the second Stobbe condensation of succinate **4**[5] with 3′,5′-dimethoxyacetophenone **5** is unselective, leading to a mixture of two isomers (2*Z*,3*E*)- and (2*E*,3*E*)-**2.** Moreover, due to the difficult separation of the two isomers as carboxylic acids, the mixture of isomers has to be converted into the corresponding methoxymethyl (MOM) esters. After separation by silica gel column chromatography, (2*Z*,3*E*)-**6** is deprotected to (2*Z*,3*E*)-**2**. For scale-up synthesis of T-2639, it is necessary to overcome these drawbacks. In this paper, we report a practical synthetic method of T-2639.

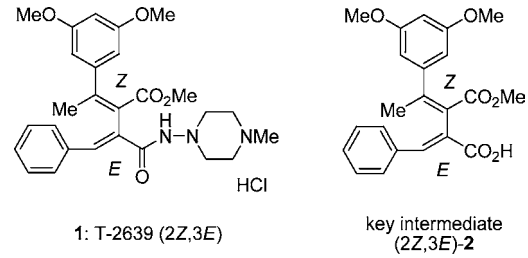

Figure 1. **Structures of T-2639 (1) and the key intermediate (2*Z*,3*E*)-2.**

Results and Discussion

In order to improve selectivity in the second Stobbe condensation, we first examined the reaction conditions. The results are shown in Table 1. Stobbe condensation proceeded according to the amount of *t*-BuOK used, but the ratio of (2*Z*,3*E)* to (2*E*,3*E*) was less than 2 to 1 (entries 1−4).[6] Heating in *t*-BuOH at 60 °C did not improve the selectivity (entry 5). Next, the effect of the solvent on selectivity was examined using 24% w/w NaOMe solution in MeOH as a base. In the case of *t*-BuOH as a solvent, the selectivity was slightly improved (entry 6). However, in the case of other solvents including MeOH, THF, or toluene, no beneficial effect was observed (entries 7−9). Although we could not find ideal conditions to improve selectivity for (2*Z*,3*E*)-**2**, the use of toluene as a solvent allowed a small amount of (2*Z*,3*E*)-**2** Na salt to precipitate as crystals in the reaction vessel after cooling, indicating that crystallinity of (2*Z*,3*E*)-**2** Na salt in toluene is higher than that of (2*E*,3*E*)-**2** Na salt. Thus, we anticipated that (2*Z*,3*E*)-**2** Na salt could predominantly be obtained if isomerization of (2*E*,3*E*)-**2** to (2*Z*,3*E*)-**2** takes place and the generated (2*Z*,3*E*)-**2** is separated

* Author to whom correspondence may be sent. Fax +81-6-6300-2564. E-mail: ogiku.tsuyoshi@me.mt-pharma.co.jp.

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(6) Even if the reaction time was extended to more than 5 h, further Stobbe condensation in *t*-BuOH didn't proceed.

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Scheme 1. **Conventional synthesis of T-2639 (1)**

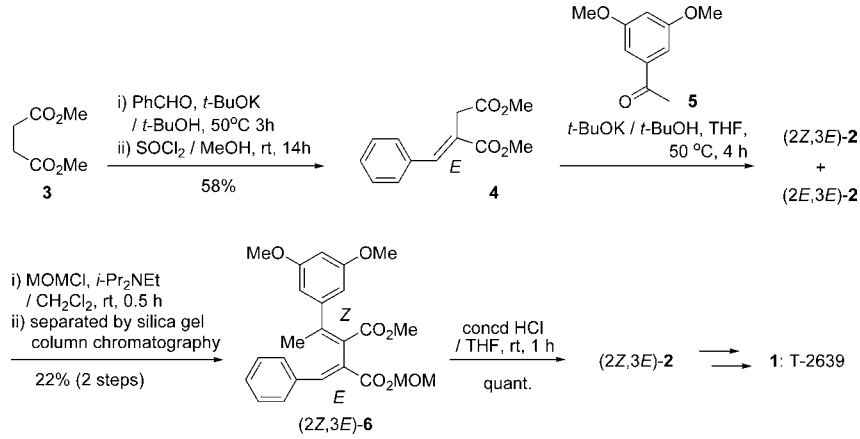


Table 1. **Stobbe condensation of succinate 4 and 3′,5′-dimethoxyacetophenone 5 with various bases and solvents**[a]

4 + **5** → (base, 5h) → (2*Z*,3*E*)-**2** + (2*E*,3*E*)-**2**

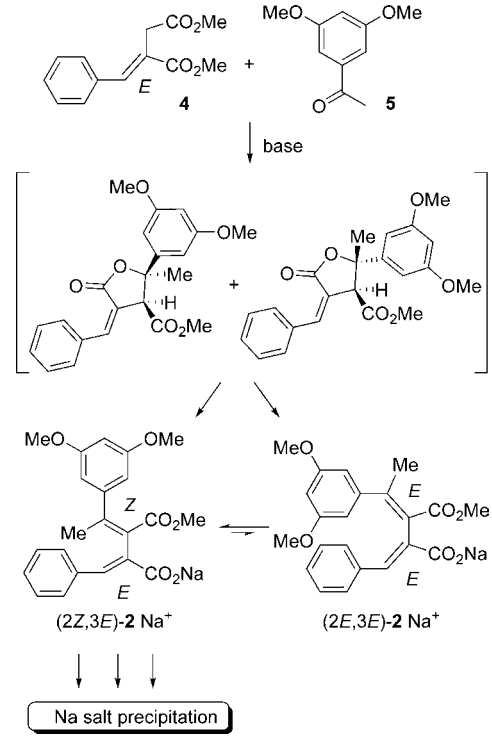
entry	base (equiv)	solvent (3 v/w)	temp.	ratio[b] (2*Z*,3*E*)-**2**:(2*E*,3*E*)-**2**	yield (%) (2*Z*,3*E*)-**2** + (2*E*,3*E*)-**2**
1	*t*-BuOK (1.0)	*t*-BuOH	rt	0.8:1	45[b]
2	*t*-BuOK (1.2)	*t*-BuOH	rt	1.3:1	55[b]
3	*t*-BuOK (1.5)	*t*-BuOH	rt	1.9:1	70[b]
4	*t*-BuOK (2.0)	*t*-BuOH	rt	2.0:1	75[b]
5	*t*-BuOK (1.5)	*t*-BuOH	60 °C	1.9:1	79[b]
6	NaOMe[c] (1.5)	*t*-BuOH	reflux	2.9:1	65[d]
7	NaOMe[c] (1.5)	MeOH	reflux	1.3:1	67[d]
8	NaOMe[c] (1.5)	THF	reflux	1.5:1	59[d]
9	NaOMe[c] (1.5)	toluene	reflux	1.8:1	57[d]
10[e]	NaOMe[c] (1.5)	toluene	reflux	1.8:1	65[d]
11[f]	NaOMe[c] (1.5)	toluene	reflux	3.4:1	78[d]
12[e,f]	NaOMe[c] (1.5)	toluene	reflux	11.5:1	82[d]

[a] 3′,5′-Dimethoxyacetophenone **5** was reacted with succinate **4** (1.2 equiv) for 5 h. [b] Ratio and yield were determined by ^{1}H NMR of the reaction mixture after acid quenching [relative integration ratio between **5** (δ = 2.58 ppm, 3H), (2*Z*,3*E*)-**2** (δ = 1.78 ppm, 3H) and (2*E*,3*E*)-**2** (δ = 2.44 ppm, 3H)]. [c] 24% w/w NaOMe solution in MeOH was used. [d] Isolated yield (%). The ratio of (2*Z*,3*E*)-**2**:(2*E*,3*E*)-**2** was almost the same as that of the reaction mixture. [e] MeOH originating from NaOMe solution was removed. [f] Powder of (2*Z*,3*E*)-**2** Na salt (0.01 equiv) was added to the reaction mixture, and the new mixture was heated under refluxing for 2 h.

as a solid from the reaction mixture under the reaction conditions (Scheme 2).[7,8]

In order to accelerate isomerization of (2*E*,3*E*)-**2** to (2*Z*,3*E*)-**2** and precipitation of (2*Z*,3*E*)-**2** Na salt, MeOH originating from NaOMe solution was removed during the reaction (entry 10). However, the expected precipitation of the salt was not observed, and the yield and selectivity for (2*Z*,3*E*)-**2** were not improved. Next, to precipitate the desired salt, powder of (2*Z*,3*E*)-**2** Na salt (0.01 equiv) was added as seeds to the reaction mixture (entry 11). In this case, generation of a substantial amount of the salt was

Scheme 2. **Selective preparation of (2*Z*,3*E*)-2 by predominant precipitation of (2*Z*,3*E*)-2 Na salt and *in situ* isomerization of (2*E*,3*E*)-2 to (2*Z*,3*E*)-2**



observed, and the selectivity of the product was improved ((2*Z*,3*E*)-**2**:(2*E*,3*E*)-**2** = 3.4:1), indicating that predominant precipitation of (2*Z*,3*E*)-**2** Na salt and *in situ* isomerization of (2*E*,3*E*)-**2** to (2*Z*,3*E*)-**2** occurred. The best result was obtained by a combination of seeding and removal of MeOH from the reaction system (entry 12).[9,10] In this case, selectivity was greatly improved by up to 11.5:1.

As described above, we could selectively prepare (2*Z*,3*E*)-**2**.[11] Actually, a scale-up synthesis of T-2639 using this optimized method, in which the undesired isomer was

(7) In fact, the isomerization from (2*E*,3*E*)-**2** to (2*Z*,3*E*)-**2** had been observed under the basic conditions. When (2*E*,3*E*)-**2** had been treated with *t*-BuOK (1.1−1.2 equiv) in *t*-BuOH at 50−60 °C for 10 min, 1:1 mixture of (2*E*,3*E*)-**2** and (2*Z*,3*E*)-**2** had been obtained, which had been monitored by TLC.

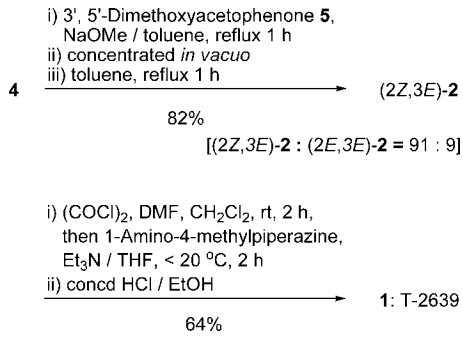
(8) Because such salt precipitation was not observed with *t*-BuOK, the effect of Na may be related to precipitation.

(9) See Experimental Section.

(10) NaOMe solution was used instead of powder NaOMe because it was easy to handle in consideration of application to process chemistry. The reaction will take place similarly even if powder NaOMe is used.

(11) It was possible to further enrich the isomeric purity of (2*Z*,3*E*)-**2** via further recrystallization from AcOEt, but in case the ratio of (2*Z*,3*E*)-**2**:(2*E*,3*E*)-**2** was 85:15 or more, (2*E*,3*E*)-**2** didn't influence the purity of **1** (T-2639).

Scheme 3. **Scale-up synthesis of T-2639**



removed by recrystallization in the final step, provided up to 420 g of pure T-2639 without chromatography (Scheme 3).

Conclusions

In conclusion, we have succeeded in improving the synthesis of T-2639 using predominant precipitation of (2*Z*,3*E*)-**2** Na salt and *in situ* isomerization of (2*E*,3*E*)-**2** to (2*Z*,3*E*)-**2**.

Experimental Section

General. Melting points were measured using a Büchi 535 capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer or Perkin-Elmer PARAGON1000. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer or Varian UNITY INOVA500 with Me_4Si as an internal standard. Mass spectra data were obtained on a ThermoFisher FINNIGAN LXQ or a Q-TOF Ultima API mass spectrometer. Elemental analyses were obtained on a Perkin-Elmer 2400 II (C, H, N) and Dionex DX-320 (Cl).

HPLC was conducted using an L-column ODS (3 μm, 4.6 mm $\times$ 50 mm). The mobile phase consisted of acetonitrile and 20 mM phosphate buffer, pH 6.5. The analytes were eluted at a flow rate of 1 mL/min with column temperature of 40 °C and observed by UV absorption at 210 nm.

1-Methyl 2-[1-(3′,5′-Dimethoxyphenyl)-(*Z*)-ethylidene]-3-[1-phenyl-(*E*)-methylidene]-4-succinic Acid ((2*Z*,3*E*)-2). Twenty-four percent w/w NaOMe solution in MeOH (20.5 g, 91.5 mmol) was added to a solution of dimethyl (*E*)-2-benzylidenesuccinate[5] (**4**) (17.1 g, 73 mmol) in toluene (50 mL), and the mixture was stirred at 30−40 °C for 30 min. After 3′,5′-dimethoxyacetophenone (**5**) (11.0 g, 61 mmol) was added to the reaction mixture at 30−40 °C, powder of (2*Z*,3*E*)-**2** Na salts (233 mg, 0.61 mmol) was added as seeds to the reaction mixture, and the mixture was stirred at reflux for 1 h. After the salts were precipitated, the mixture was concentrated *in vacuo* until the volume was about 22 mL (2 v/w of 3′,5′-dimethoxyacetophenone (**5**)). The mixture was again stirred at reflux for 1 h. Acetic acid (2.9 g, 49 mmol) was then added to the mixture below 15 °C, and ice−water was poured into the mixture.[12] The organic and aqueous layers were separated, and the organic layer was washed with 5% aqueous $NaHCO_3$ solution (100 mL). AcOEt (250 mL) was added to the previous aqueous layer, and the mixture was acidified with concd HCl below 15 °C (pH 2). The organic and aqueous layers were separated, and the organic layer was washed with 10% brine (100 mL) and saturated brine (100 mL) and dried over $MgSO_4$. After activated charcoal (2 g, nacalai tesque, code: 079-09) was added to the solution, the suspension was filtered, and the filtrate was concentrated *in vacuo*. The residue was diluted with *i*-Pr_2O and filtered to give **2** (19.1 g, 82%, (2*Z*,3*E*)-**2**:(2*E*,3*E*)-**2** = 91:9) as a solid.

HPLC retention time: 2.8 min (elution with 35:65 acetonitrile/20 mM phosphate buffer, pH 6.5), relative retention time (RRT) to **1** (T-2639): 0.47; IR (Nujol) 2924, 2853, 1710, 1685, 1603, 1589, 1451, 1424, 1274, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (s, 1H), 7.60−7.70 (m, 2H), 7.30−7.45 (m, 3H), 6.40 (t, J = 2.3 Hz, 1H), 6.33 (d, J = 2.3 Hz, 2H), 3.80 (s, 6H), 3.53 (s, 3H), 1.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 167.6, 160.5, 150.2, 144.9, 144.6, 134.7, 130.0, 129.8, 128.7, 128.0, 124.8, 104.6, 99.4, 55.3, 51.9, 23.6; MS ESI *m*/*z*: 381 ([M − H]$^-$); HR-MS ESI Calcd for $C_{22}H_{23}O_6$ ([M + H]$^+$): 383.1495. Found: 383.1485.

1-Methyl 2-[1-(3′,5′-Dimethoxyphenyl)-(*E*)-ethylidene]-3-[1-phenyl-(*E*)-methylidene]-4-succinic Acid ((2*E*,3*E*)-2). HPLC retention time: 2.6 min (elution with 35:65 acetonitrile/20 mM phosphate buffer pH 6.5), relative retention time (RRT) to **1** (T-2639): 0.43; IR (ATR) 2841, 2524, 1711, 1658, 1592, 1426, 1316, 1205, 1157 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50 (s, 1H), 7.25−7.40 (m, 5H), 6.22 (t, J = 2.3 Hz, 1H), 5.85 (d, J = 2.3 Hz, 2H), 3.79 (s, 3H), 3.54 (s, 6H), 2.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6, 167.5, 160.1, 154.7, 144.8, 143.4, 135.0, 129.9, 129.4, 129.2, 128.3, 124.0, 103.9, 100.4, 55.1, 52.0, 23.1; MS ESI *m*/*z*: 381 ([M − H]$^-$); HR-MS ESI Calcd for $C_{22}H_{23}O_6$ ([M + H]$^+$): 383.1495. Found: 383.1485.

Methyl (*Z*)-3-(3′,5′-Dimethoxyphenyl)-2-[(*E*)-1-(4-methylpiperazin-1-ylcarbamoyl)-2-phenylvinyl]-2-butenoate Hydrochloride (1, T-2369). Oxalyl chloride (194 g, 133.3 mL, 1.52 mol) was added dropwise over 20 min to (2*Z*,3*E*)-**2** (487 g, 1.27 mol, containing <10% of (2*E*,3*E*)-**2**) solution in CH_2Cl_2 (1 L) and DMF (1 L). The reaction mixture was stirred for 2 h and then concentrated *in vacuo*. After the residue was dissolved in THF (4 L), a mixture of Et_3N (214 mL, 1.52 mol) and 1-amino-4-methylpiperazine (168 mL, 1.52 mol) was added dropwise to the mixture, maintaining the temperature below 20 °C. After 2 h, the reaction mixture was diluted with AcOEt (4 L) and washed with water (3 L) twice and brine (2 L). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was diluted with CH_2Cl_2 (3 L), and *i*-Pr_2O (1 L) was added. The mixture was concentrated *in vacuo* until the volume was about 1.5 L. After the mixture was stirred for 5 h at room temperature, the crystals were collected by filtration and washed with *i*-Pr_2O. The crystals obtained were next dissolved in EtOH (4.9 L), and concd HCl (86 mL) was added dropwise at room temperature. The reaction mixture was left at room temperature for 1 h

(12) It was confirmed that isomerization between (2*Z*,3*E*)-**2** and (2*E*,3*E*)-**2** didn't occur during aqueous workup.

and cooled in a refrigerator (−25 °C) for 14 h. Crystals were collected by filtration and washed with Et_2O (1 L) to provide 420 g of **1** (64%).

Mp = 235−240 °C; HPLC area: 99.7%; HPLC retention time: 6.0 min (elution with 35:65 acetonitrile/20 mM phosphate buffer pH 6.5); IR (Nujol) 3196, 2924, 2854, 1698, 1662, 1595, 1464, 1262 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 10.11 (br, 1H), 9.48 (s, 1H), 7.54 (d, J = 7.2 Hz, 2H), 7.30−7.50 (m, 4H), 6.43 (s, 1H), 6.29 (d, J = 1.8 Hz, 2H), 3.73 (s, 6H), 3.37 (s, 3H), 3.00−3.50 (m, 8H), 2.79 (s, 3H), 1.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.9, 164.9, 160.8, 149.0, 143.5, 140.9, 134.7, 129.7, 129.5, 128.6, 128.6, 125.1, 104.7, 99.9, 55.5, 53.3, 52.4, 51.3, 43.2, 22.2; MS APCI *m/z*: 480 ($[M + H]^+$). Anal, Calcd for $C_{27}H_{33}N_3O_5 \cdot HCl$: C 62.84; H 6.64; N 8.14; Cl 6.87. Found: C 62.61; H 6.71; N 8.09; Cl 6.79.

Supporting Information Available

NMR chart of (2*Z*,3*E*)-**2**, (2*E*,3*E*)-**2**, and T-2639 (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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