Hapten Synthesis for (+)-6-(2-Chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*]triazolo[4,3-*a*][1,4]diazepine (E6123)

Shuhei MIYAZAWA, Kazuo OKANO,* Tetsuya KAWAHARA, Yoshimasa MACHIDA, and Isao YAMATSU

Tsukuba Research Laboratories, Eisai Co., Ltd., 1-3 Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-26, Japan. *Received July 26, 1991*

(+)-6-(2-Chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno-[3,2-*f*]triazolo[4,3-*a*][1,4]diazepine (E6123) is a very potent platelet-activating factor (PAF) receptor antagonist and shows potent anti-PAF activities at the microgram level in a variety of animal models. In order to examine the pharmacokinetics of E6123 at low doses, establishment of a radioimmunoassay is required. On the basis of the metabolic pattern of E6123, we synthesized 6-{2-chloro-4-(3-carboxypropyl)phenyl}-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine 22 as a potential hapten. In the synthesis of 22, we developed butynyl carbamate as a piperidine ring *N*-protecting group to prevent possible side reaction, namely oxidation of the methylene at position 2. This protecting group is stable under usual basic and acidic conditions.

Keywords platelet-activating factor (PAF); platelet-activating factor antagonist; E6123; hapten; radioimmunoassay

Platelet-activating factor (PAF) is a phospholipid with a wide variety of potent biological actions; *e.g.*, stimulation of platelets and leukocytes, induction of hypotension, smooth muscle constriction, increase in vascular permeability, and negative ionotropic cardiac effects.[1] Thus, PAF is involved in a wide variety of pathophysiological processes, including endotoxin shock, allergic diseases, and inflammation, and anti-PAF agents should be applicable to the treatment of these diseases. One of these, E6123[2] (Fig. 1) is a very potent PAF receptor antagonist and showed potent anti-PAF activities at the microgram level in animal models in which PAF is considered to play an important role. Therefore, establishment of a radioimmunoassay[3] is very important in order to examine the pharmacokinetics of E6123 at these low doses. Hapten synthesis is very important to developed a specific and sensitive radioimmunoassay. Hapten itself does not elicit the formation of a detectable amount of antibody. When coupled with a carrier protein, however, it does elicit an immune response. In humoral immunity, the antibody specificity is directed primarily to the hapten. On the basis of studies on the metabolic pattern of E6123, we considered that introduction of a carboxylic acid side chain into the chlorobenzene moiety of E6123 is the best way of obtaining a good antibody.[4] Thus, synthesis of 6-{2-chloro-4-(3-carboxypropyl)phenyl}-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido-[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine **22** was attempted for use as a hapten. Our strategy for the synthesis of 2-amino-3-{2-chloro-4-(3-hydroxypropyl)-benzoyl}-6-(3-butynyloxy)-4,5,6,7-tetrahydrothieno[2,3-*c*]-pyridine **10** and its transformation to **22** involved the condensation of the α-cyanoacetophenone derivative **5** with the appropriately protected piperidone **9** as a key step. Selection of suitable protecting groups for the overall reaction is the most significant point.

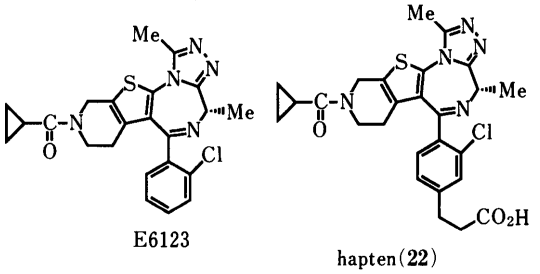
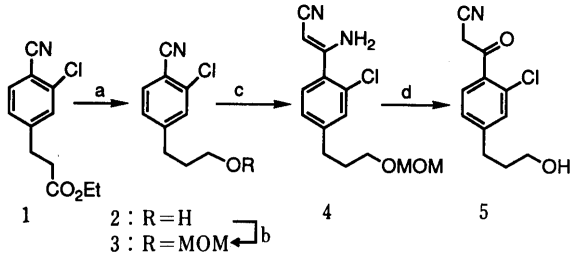


Fig. 1

Preparation of 2-Chloro-4-(3-hydroxypropyl)cyanoacetophenone 5 Compound **5** possesses the propanol moiety, which is converted to the propanoic acid side-chain. Chart 1 shows the synthetic pathway to **5** from ethyl 3-chloro-4-cyanophenylpropionate **1**.[5]

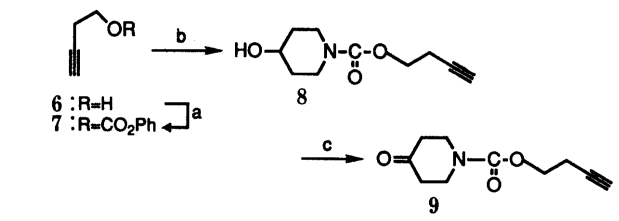
Our first attempt to introduce the α-cyanoketone moiety into the ester **1** was unsuccessful under the usual conditions (lithium diisopropylamide (LDA)–CH_3CN/tetrahydrofuran (THF), −70 °C). Under the conditions employed, α-lithiation of the ester and subsequent self-condensation occurred. To avoid this undesirable reaction, introduction of the α-cyanoketone moiety into the corresponding protected alcohol **3** was examined. Conversion of **1** to **3** was easily achieved by reduction of the ester **1** with sodium borohydride ($NaBH_4$/EtOH, reflux) followed by protection of the alcohol **2** by methoxymethyl (MOM) (MOMCl–diisopropylethylamine/CH_2Cl_2, room temperature). Treatment of **3** with acetonitrile anion (CH_3CN–LDA/THF, −70 °C) followed by hydrolysis of the ketoimine **4** thus obtained under two-phase conditions (concentrated HCl/MeOH, 50 °C) gave the α-cyanoacetophenone **5**.

Preparation of *N*-(3-Butynyloxycarbonyl)-4-piperidone 9 The choice of protecting group is very important, as it should be stable under the usual basic, acidic and oxidative



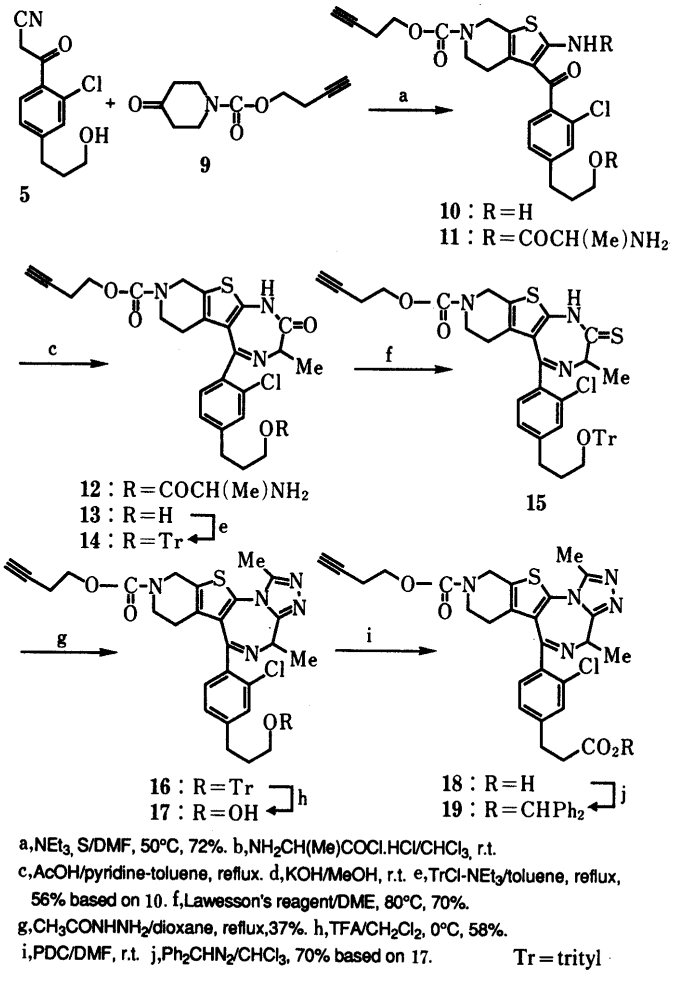
a, $NaBH_4$/EtOH, reflux, 98%.
b, MOMCl-diisopropylethylamine/CH_2Cl_2, r.t., 73%.
c, CH_3CN-LDA/THF, −70°C. d, conc.HCl/MeOH, 50°C, 78%.

Chart 1



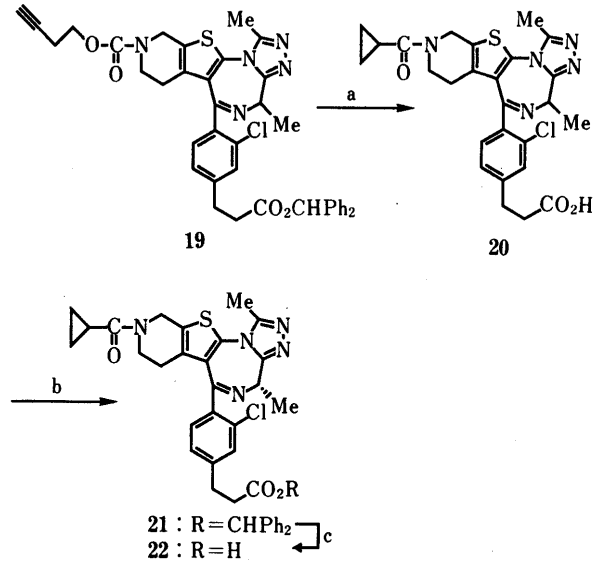
a, $ClCO_2Ph$-pyridine/CH_2Cl_2, r.t. b, 4-hydroxypiperidine, 110°C, 98% based on 6. c, DMSO-$(COCl)_2$-NEt_3/CH_2Cl_2, -50°C—r.t., 91%.

Chart 2



a, NEt_3, S/DMF, 50°C, 72%. b, $NH_2CH(Me)COCl.HCl/CHCl_3$, r.t. c, AcOH/pyridine-toluene, reflux. d, KOH/MeOH, r.t. e, TrCl-NEt_3/toluene, reflux, 56% based on 10. f, Lawesson's reagent/DME, 80°C, 70%. g, $CH_3CONHNH_2$/dioxane, reflux, 37%. h, TFA/CH_2Cl_2, 0°C, 58%. i, PDC/DMF, r.t. j, Ph_2CHN_2/$CHCl_3$, 70% based on 17. Tr = trityl

Chart 3



a, (1) 8 N NaOH/MeOH, reflux. (2) cyclopropanecarbonyl choride-pyridine/$CHCl_3$, 0°C, 44%. b, (1) Ph_2CHN_2/$CHCl_3$. (2) optical resolution by ChiraSpher, 19%. c, TFA/CH_2Cl_2, 0°C, quant.

Chart 4

conditions. We developed butynyl carbamate as a group satisfying these conditions.[6] The *N*-protected α-unsubstituted ketone was easily synthesized by condensation of 4-hydroxypiperidine with 3-butyne phenyl carbonate **7** followed by Swern oxidation (dimethylsulfoxide (DMSO)–$(COCl)_2$–NEt_3/CH_2Cl_2, −50 °C—room temperature) (Chart 2).

Preparation of Subgoal 19 α-Cyano acetophenone **5** and the protected piperidone **9** thus obtained were converted to subgoal **19** as shown in Chart 3.[7] The 2-amino-3-benzoyl thiophene **10** was prepared by reaction of the two with sulfur in the presence of triethylamine. Introduction of the 2-aminoketone moiety into **10** was easily performed by reacting it with alanine acid chloride. The ring closure of **11** to **12** was carried out by refluxing an equimolar mixture of **11** and AcOH in pyridine–toluene solution with azeotropic removal of water. The diazepine esterified with alanine **12** was unstable under the usual conditions for thioamide preparation (P_2S_5/1,2-dimethoxyethane (DME), Lawesson's reagent[8]/dichloroethane, *etc.*). Thus, the protection of the alcohol moiety of **13** was altered to a trityl group. The alanine moiety of **12** was cleaved under mild basic conditions (KOH/MeOH, room temperature). Tritylation of the alcohol was carried out under the usual conditions (tritylchloride–triethylamine/toluene, reflux) and the amide group of the trityl protected compound **14** was converted to the thioamide without decomposition (Lawesson's reagent/DME, 80 °C). The reaction of the thioamide **15** with acetyl hydrazine in refluxing dioxane gave triazolodiazepine **16** in 37% yield. The trityl group was removed by acid treatment trifluoroacetic acid (TFA)/CH_2Cl_2, 0 °C) to give **17**. Our first attempt at oxidation of the alcohol moiety in **17** using manganese dioxide was a failure, because the methylene at position 2 is easily oxidized under the conditions employed[6] (10 eq MnO_2/CH_3CN, room temperature). This oxidative process leading to **18** was achieved by employment of pyridinium dichromate (PDC) as the oxidant without accompanying side reactions. The crude residue was used for the next step after purification on a short column to remove the chromium residue. The carboxylic acid **18** was esterified with diphenyldiazomethane and was purified by column chromatography to give **19** in 70% yield.

Preparation of Hapten 22 The protecting group of **19** was removed under basic conditions (8 N NaOH/MeOH, reflux). Deprotection of the diphenyl methyl ester occurred concurrently under these conditions. The cyclopropane carbonyl group was introduced into the above compound (cyclopropanecarbonyl chloride–pyridine/$CHCl_3$, 0 °C) and the racemic compound was resolved to obtain optically pure **21** on a column of ChiraSpher, after esterification by diphenyldiazomethane treatment. The optically active **21** was treated with TFA in cold dichloromethane to give **22** (Chart 4).

Experimental

General Methods Reagents and solvents were purchased from usual commercial sources. Silica gel (Kiesel gel 60, Merck) was used for column chromatography and silica gel (Kiesel gel 60 F_{254}, Merck) for analytical thin layer chromatography (TLC). Melting points were measured on a Yanagimoto micro melting apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-100 (100 MHz) or Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Infrared (IR) spectra were obtained on a Hitachi 260-30 IR spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer.

2-Chloro-4-(3-hydroxypropyl)benzonitrile (2) A solution of the ester **1** (89.2 g, 0.38 mol) in EtOH (1 l) was treated with $NaBH_4$ (71.0 g, 1.88 mol) and this mixture was heated under reflux for 30 min, then poured into saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (30% AcOEt/hexane) to give the alcohol **2** as a yellow oil (70 g, 98%). **2**: MS (Pos, field desorption (FD)) *m/z* 195 (M^+). IR (neat): 3400, 3440, 3360, 2220, 1590 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.5—2.2 (m, 3H), 2.4—2.9 (m, 2H), 3.76 (t, J=8 Hz, 2H), 7.16 (dd, J=2, 8 Hz, 1H), 7.32 (d, J=2 Hz, 1H), 7.52 (d, J=8 Hz, 1H).

2-Chloro-4-(3-methoxymethoxypropyl)benzonitrile (3) MOMCl (172.0 g, 1.7 mol) was added dropwise to a mixture of **2** (210 g, 1.08 mol) and diisopropylethylamine (258.5 g, 2 mol) in dichloromethane (1.5 l). The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (9 : 1→1 : 4 hexane/AcOEt) to give **3** (189 g, 73%) as a yellow oil. **3**: MS (Pos, FD) *m/z* 239 (M^+). IR (neat): 3430, 2220, 1595 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.7—2.1 (m, 2H), 2.5—2.9 (m, 2H), 3.36 (s, 3H), 3.52 (t, J=8 Hz, 2H), 4.6 (s, 2H), 7.12 (dd, J=2, 8 Hz, 1H), 7.3 (d, J=2 Hz, 1H), 7.52 (d, J=8 Hz, 1H).

2-Chloro-4-(3-hydroxypropyl)cyanoacetophenone (5) CH_3CN (21 ml, 0.40 mol) was added to a solution of LDA prepared from 1.6 M *n*-BuLi (254 ml, 0.41 mol) and diisopropylamine (57 ml, 0.41 mol) in THF (300 ml) at −70 °C, and the reaction mixture was stirred at this temperature for 30 min. Then **3** (65.1 g, 0.27 mol) in THF (150 ml) was added dropwise to this mixture at −70 °C and stirring was continued at −70 °C for 30 min. The reaction mixture was poured into saturated NH_4Cl and extracted with AcOEt. The extract was dried ($MgSO_4$), filtered and concentrated. The green-gray-colored oil obtained (76.6 g) was used in the next step without further purification. A mixture of **4** (76.6 g) and concentrated HCl (80 ml) in MeOH (450 ml) was stirred at 50 °C for 15 min. The reaction mixture was poured into saturated NaCl and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (1 : 1 hexane/AcOEt) to give **5** (51 g, 78%) as a yellow oil. **5**: IR (neat): 3400, 2920, 2180, 1690, 1590 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.7—2.1 (m, 3H), 2.6—2.9 (m, 2H), 3.66 (t, J=8 Hz, 2H), 4.16 (s, 2H), 7.2 (dd, J=2, 8 Hz, 1H), 7.3 (d, J=2 Hz, 1H), 7.6 (d, J=8 Hz, 1H).

***N*-(3-Butynyloxycarbonyl)-4-hydroxypiperidine (8)** Phenyl chloroformate (45 ml, 0.36 mol) was added to a mixture of 3-butyn-1-ol (25 g, 0.36 mol) and pyridine (30 ml, 0.37 mol) in CH_2Cl_2 at room temperature. The reaction mixture was diluted with AcOEt and washed with H_2O. The extract was dried ($MgSO_4$), filtered and concentrated, and the residue was used in the next step without further purification. A mixture of this residue and 4-hydroxypiperidine (36.0 g, 0.36 mol) was heated at 110 °C for 30 min. After the reaction, the residue was purified by column chromatography (2 : 1→1 : 2 hexane/AcOEt) to give **8** (63.3 g, 98% based on 3-butyn-1-ol). **8**: MS (DI-EI) *m/z* 197 (M^+), 179. IR (neat) 3400, 3280, 2920, 1670, 1430 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.1—1.9 (m, 4H), 2.02 (t, J=2 Hz, 1H), 2.4 (br s, 1H), 2.54 (dt, J=2, 8 Hz, 2H), 2.9—3.3 (m, 4H), 3.6—4.04 (m, 5H), 4.16 (t, J=8 Hz, 2H).

***N*-(3-Butynyloxycarbonyl)-4-piperidone (9)** DMSO (76 ml, 1.07 mol) was slowly added to a solution of oxalyl chloride (65 ml, 0.75 mol) in CH_2Cl_2 (1.5 l) at −70 °C. The mixture was stirred at −50 °C for 30 min and re-cooled to −70 °C. Then the alcohol **8** (63.3 g, 0.35 mol) in CH_2Cl_2 (500 ml) was slowly added. The reaction mixture was allowed to warm to −50 °C, after which triethylamine (250 ml, 1.79 mol) was slowly added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (4 : 1→1 : 1 hexane/AcOEt) to give **9** (57.3 g, 91%) as a pale yellow oil. **9**: MS (DI-EI) *m/z* 196 (M^+). IR (neat): 1690, 1470, 1430 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.02 (t, J=2 Hz, 1H), 2.3—2.7 (m, 6H), 3.78 (t, J=8 Hz, 4H), 4.25 (t, J=8 Hz, 2H).

2-Amino-3-{2-chloro-4-(3-hydroxypropyl)benzoyl}-6-(3-butynyloxy)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (10) Triethylamine (61 g, 0.60 mol) was added to a mixture of the ketone **9** (111.5 g, 0.59 mol), sulfur (18.9 g, 0.59 mol) and **5** (140 g, 0.59 mol) in dimethylformamide (DMF) (400 ml). The reaction mixture was stirred at 50 °C for 1 h and then concentrated with aid of a vacuum pump. The residue was purified by column chromatography (1 : 1 hexane/AcOEt) to give **10** (189 g, 72%) as a yellow solid. **10**: MS (Pos, FD) *m/z* 447 (M^+). IR (neat): 3300, 3000, 1680, 1570, 1430 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.6—2.1 (m, 2H), 1.9 (t, J=2 Hz, 1H), 2.3—2.8 (m, 4H), 3.4 (t, J=8 Hz, 2H), 3.64 (t, J=8 Hz, 2H), 4.16 (t, J=8 Hz, 2H), 4.34 (br s, 2H), 7.1 (br s, 1H), 7.2 (br s, 1H), 7.39 (br s, 1H).

2-(2-Aminopropionylamino)-3-{2-chloro-4-(3-alanyloxypropyl)benzoyl}-6-(3-butynyloxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (11) Ala(OCl)·HCl (335 g, 2.5 mol) was added portionwise to a solution of **10** (189 g, 0.42 mol) in $CHCl_3$ (2 l) at room temperature. The reaction mixture was concentrated, neutralized with saturated $NaHCO_3$, and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered and concentrated to give **11** (247 g) as a brown oil. **11**: MS (Pos, FD) *m/z* 589 (M^+). IR (neat): 3000, 1670, 1590 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.0—1.7 (m, 6H), 1.7—2.35 (m, 5H), 2.35—3.0 (m, 4H), 4.2—4.6 (m, 2H), 4.6—4.9 (m, 2H), 4.9—5.3 (m, 4H), 5.3—5.6 (m, 2H), 7.13 (s, 3H).

3-Methyl-5-{2-chloro-4-(3-alanyloxypropyl)phenyl}-8-(3-butynyloxycarbonyl)-6,7,8,9-tetrahydro-1*H*,3*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,4]diazepin-2-one (12) A solution of crude **11** (247 g) in a mixture of pyridine (500 ml), AcOH (150 ml), and toluene (3 l) was heated under reflux for 1.5 h in a flask fitted with a Dean–Stark head. The mixture was then concentrated under reduced pressure to give **12** (290 g) as a brown oil. **12**: MS (Pos, FD) *m/z* 571 (M^+). IR (neat): 3000, 1680, 1580 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.0—1.6 (m, 5H), 1.64 (d, J=7 Hz, 3H), 1.96 (t, J=2 Hz, 1H), 1.7—2.2 (m, 4H), 2.3—2.8 (m, 4H), 2.9—3.3 (m, 1H), 3.3—4.0 (m, 3H), 4.0—4.3 (m, 4H), 4.3—4.9 (m, 2H), 7.0—7.4 (m, 3H).

3-Methyl-5-{2-chloro-4-(3-hydroxypropylphenyl}-8-(3-butynyloxycarbonyl)-6,7,8,9-tetrahydro-1*H*,3*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,4]diazepin-2-one (13) A mixture of crude **12** (290 g) and NaOH (57 g, 1.43 mol) in EtOH (1 l) was stirred at room temperature for 1.5 h, then filtered through Celite. The filtrate was acidified with dilute HCl, neutralized with saturated $NaHCO_3$ and brine-extracted with $CHCl_3$. The extract was dried ($MgSO_4$), filtered and concentrated. The brown oil obtained (199 g) was used in the next step without further purification.

3-Methyl-5-{2-chloro-4-(3-trityloxypropyl)phenyl}-8-(3-butynyloxycarbonyl)-6,7,8,9-tetrahydro-1*H*,3*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,4]diazepin-2-one (14) A mixture of crude **13** (199 g), trityl chloride (167 g, 0.6 mol), triethylamine (81 g, 0.8 mol), and toluene (1.3 l) was heated under reflux for 1 h. Further portions of trityl chloride (139 g, 0.5 mol) and triethylamine (50.5 g, 0.5 mol) were then added to drive the reaction to completion. The reaction mixture was poured into H_2O and extracted with $CHCl_3$. The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (1 : 1 hexane/AcOEt) to give **14** (176 g, 56% based on **10**) as a brown oil. **14**: MS (Pos, FD) *m/z* 742 (M^+). IR (neat): 3000, 1675 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.74 (d, J=7 Hz, 3H), 1.4—2.2 (m, 5H), 2.3—2.9 (m, 4H), 3.07 (t, J=7 Hz, 2H), 3.0—3.3 (m, 1H), 3.5—3.9 (m, 1H), 3.86 (q, J=7 Hz, 1H), 4.16 (t, J=7 Hz, 2H), 4.5 (ABq, J=18 Hz, 2H), 6.8—7.6 (m, 18H), 8.4—8.8 (br s, 1H).

3-Methyl-5-{2-chloro-4-(3-trityloxypropyl)phenyl}-8-(3-butynyloxycarbonyl)-6,7,8,9-tetrahydro-1*H*,3*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,4]diazepine-2-thione (15) A solution of **14** (176 g, 0.24 mol) and Lawesson's reagent (56.35 g, 0.14 mol) in DME (800 ml) was heated at 80 °C for 1.5 h. The reaction mixture was concentrated, and the residue was dissolved in a small amount of $CHCl_3$. Silica gel was added to this solution and the solvent was pumped off. This silica gel-absorbed reaction mixture was purified by column chromatography (1 : 5→1 : 1 AcOEt/hexane) to give **15** (126 g, 70%) as a brown oil. **15**: ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.9 (d, J=7 Hz, 3H), 1.6—2.3 (m, 5H), 2.3—3.2 (m, 4H), 3.6 (t, J=7 Hz, 2H), 3.4—3.9 (m, 3H), 4.16 (t, J=7 Hz, 2H), 4.5 (ABq, J=18 Hz, 2H), 6.8—7.6 (m, 18H).

3-(3-Butynyloxycarbonyl)-6-{2-chloro-4-(3-trityloxypropyl)phenyl}-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (16) A mixture of the thioamide **15** (126 g,

0.17 mol) and acetyl hydrazine (148 g, 0.20 mol) in dioxane was heated at 130 °C for 2 h, then the dioxane was evaporated off. The residue was purified by column chromatography ($CHCl_3$→3%→10%MeOH/$CHCl_3$) to give **16** (48 g, 37%). **16**: MS (Pos, FD) *m/z* 780 (M^+). IR (neat): 3020, 1690, 1590, 1210 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.5—2.3 (m, 8H), 2.3—2.9 (m, 4H), 2.68 (s, 3H), 2.9—3.2 (m, 2H), 3.6—3.9 (m, 3H), 4.2 (t, J=7 Hz, 2H), 4.64 (ABq, J=18 Hz, 2H), 6.9—7.7 (m, 18H).

3-(3-Butynyloxycarbonyl)-6-{2-chloro-4-(3-hydroxypropyl)phenyl}-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (17) TFA (7.5 ml) was added to an ice-cooled solution of **16** (7.4 g, 9.5 mmol) in $CHCl_3$ (150 ml). The reaction mixture was poured into saturated $NaHCO_3$ and extracted with dichloromethane. The extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (5%MeOH/$CHCl_3$) to give **17** (3.0 g, 58%). **17**: MS (Pos, FD) *m/z* 538 $(M+H)^+$. IR (neat): 3300, 2930, 1690, 1595, 1420 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.5—2.3 (m, 5H), 2.1 (d, J=7 Hz, 3H), 2.3—2.9 (m, 4H), 2.66 (s, 3H), 2.9—3.5 (m, 1H), 3.6—4.06 (m, 1H), 3.62 (t, J=7 Hz, 2H), 4.2 (t, J=7 Hz, 2H), 4.64 (ABq, J=18 Hz, 2H), 7.0—7.4 (m, 3H).

3-(3-Butynyloxycarbonyl)-6-[2-chloro-4-{3-(diphenylmethoxycarbonyl)-propyl}phenyl]-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (19) PDC (3.95 g, 10.5 mmol) was added to a solution of **17** (1.13 g, 2.1 mmol) in DMF (20 ml) at room temperature. The reaction mixture was stirred at this temperature for 15 h, then diluted with dichloromethane and filtered through a Celite-silica gel column. The filtrate was concentrated and the residue was purified by column chromatography (CH_2Cl_2→5%→10%MeOH/CH_2Cl_2) to give the carboxylic acid **18**. To a solution of **18** in $CHCl_3$ (5 ml) was added diphenyldiazomethane (1.89 g, 9.7 mmol). The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was concentrated and the residue was purified by column chromatography (CH_2Cl_2→3%MeOH/CH_2Cl_2) to give **19** (1.05 g, 70%) as a white foam. **19**: MS (Pos, FD) *m/z* 718 (M^+). IR (neat): 1700, 1590 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.5—2.3 (m, 8H), 2.63 (dt, J=2, 8 Hz, 2H), 2.7—2.8 (m, 5H), 2.9—3.2 (m, 2H), 3.7—3.8 (m, 1H), 4.1—4.25 (m, 2H), 4.25—4.9 (m, 2H), 6.85 (s, 1H), 7.1—7.6 (m, 13H).

6-[2-Chloro-4-{3-(diphenylmethoxycarbonyl)propyl}phenyl]-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (20) A mixture of **19** (1.05 g, 1.46 mmol) and 8 N NaOH (1.5 ml, 12 mmol) in MeOH 6 ml was heated under reflux. The reaction mixture was concentrated. Cyclopropanecarbonyl chloride (0.7 ml, 7.7 mmol) was added to the above residue in pyridine (5 ml) and dichloromethane (10 ml) at 0 °C. The reaction mixture was diluted with 4:1 $CHCl_3$/MeOH and filtered. The filtrate was then concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$→10%→20%MeOH/$CHCl_3$) to give **20** (0.34 g, 44%) as a yellow-red oil.

6-[2-Chloro-4-{3-(diphenylmethoxycarbonyl)propyl}phenyl]-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (21) Diphenyldiazomethane (0.41 g, 2.1 mmol) was added to a solution of **20** (0.34 g, 0.65 mmol) in chloroform, and the mixture was stirred at room temperature and then concentrated. The residue was purified by column chromatography (3:1 benzene/acetone) to give **21** (0.14 g, 31%) as a yellow oil. The racemate was optically resolved on a ChiraSpher column (5 μm 10 i.d. ×250 mm, ultraviolet (UV) detector 254 nm, flow rate 10 ml/min, eluent 1:1 THF/hexane) to give optically active **21** (80 mg, 93% ee). **21**: MS (Pos, FD) *m/z* 690 (M^+). IR (neat): 1710, 1600 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.65—1.1 (m, 4H), 1.5—2.2 (m, 5H), 2.14 (d, J=7 Hz, 3H), 2.6—2.8 (m, 2H), 2.7 (s, 3H), 2.85—3.05 (m, 2H), 4.25—4.35 (m, 1H), 4.4—5.1 (m, 2H), 6.85 (s, 1H), 7.0—7.6 (m, 13H).

6-{2-Chloro-4-(3-carboxypropyl)phenyl}-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (22) TFA (0.5 ml) was added to a solution of **21** (80 mg, 0.12 mmol) in chloroform (4 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The mixture was concentrated and the residue was purified by column chromatography (10%→25%MeOH/$CHCl_3$) to give **22** (62 mg, yield quant.) as an off-white powder. **22**: MS (Pos, FD) *m/z* 524 (M^+). IR (Nujol) 3300, 1620 cm^{-1}. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 0.5—1.8 (m, 5H), 1.9 (d, J=7 Hz, 3H), 1.9—2.3 (m, 2H), 2.6 (s, 3H), 2.8 (t, J=7 Hz, 2H), 3.9—5.3 (m, 4H), 4.35 (q, J=7 Hz, 1H), 7.2—7.6 (m, 3H).

References and Notes

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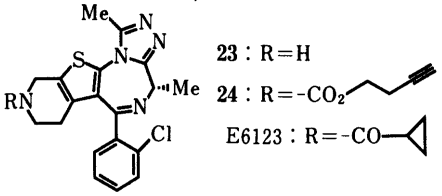
6) We initially investigated manganase dioxide oxidation with the following model substrates. In each case, the methylene at position



23 : R=H
24 : R=-CO_2
E6123 : R=-CO

2 was easily oxidized (-CH_2NR-→-CONR-) under these conditions (10 eq MnO_2/CH_3CN, room temperature). So we investigated PDC oxidation with the above substrates. We found that in this case also the oxidation of the methylene at position 2 easily occurred in **23**, E6123, but compound **24** showed no change under these conditions.
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