A Practical Synthesis of Optically Active Platelet-Activating Factor Antagonist, (+)-6-(2-Chlorophenyl)-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 (E6123), and Its Absolute Configuration

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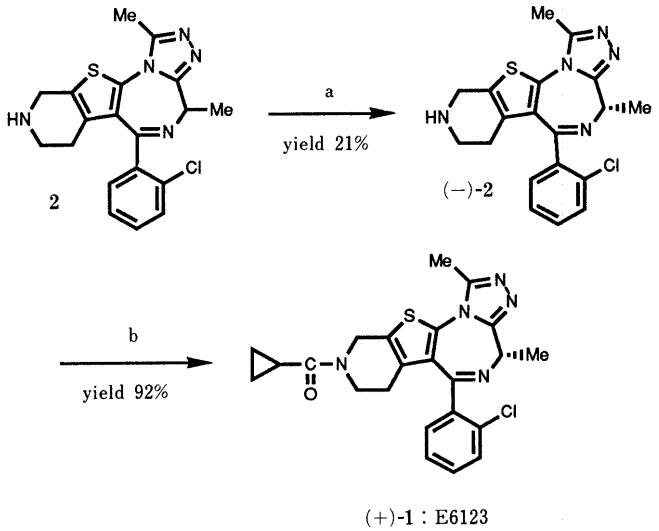
Optically active platelet-activating factor (PAF) receptor antagonist, (+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (E6123), was synthesized on a large-scale by optical resolution using (+)-dibenzoyl-D-tartaric acid. An X-ray crystallographic analysis clearly indicated that the absolute configuration of the synthesized E6123 was *S*.

Keywords platelet activating factor; platelet activating factor antagonist; triazolothienodiazepine; E6123; optical resoltion

Platelet-activating (PAF)[1] is a phospholipid, which has a wide variety of potent biological actions, *e.g.*, stimulation of platelets and leukocytes, induction of hypotension, and increase in vascular permeability. It is considered to be involved in the pathophysiological processes of various diseases, including endotoxin shock, allergic diseases and inflammation. Recently, we have reported (+)-6-(2-chlorophenyl)-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 ((+)-**1**), E6123,[2] as a potent PAF receptor antagonist, having an asymmetric center in its triazolothienodiazepine skeleton[3] (Fig. 1).

Initially, we resolved both enantiomers by using an optical resolving column, ChiraSpher, to define which isomer contributed more to the anti-PAF activity. As we had expected, there were significant differences in biological activity between the two enantiomers of **1**. For example, in the [^{3}H]PAF receptor binding assay system, IC_{50} values were evaluated to be 0.42 μM for (−)-**1** and 2.8 nM for (+)-**1**, so there is a 150-fold difference between the two. Thus, we considered that a large amount of optically pure compound would be required for further evaluation of (+)-**1**. The above synthetic method was unsuitable for large-scale preparation of (+)-**1** because of the troublesome procedure of using an optical resolving column. In this paper, we wish to describe a convenient and practical method for synthesizing (+)-**1** on a large-scale, and the determination of its absolute configuration.

There are several methods to obtain optically active compounds, for example, asymmetric synthesis, optical resolution and chirality transfer from natural products.[4] We succeeded in preparing optically active (+)-**1** by optical resolution. Compound (±)-**2** was prepared by the previously reported method,[2,5] (Fig. 1). We investigated optical resolution of this racemic amine (±)-**2** using a variety of acidic optical resolving agents, such as (+)-dibenzoyl-D-tartaric acid, (+)-camphorcarboxylic acid, (1*S*)-(+)-10-



a, (+)-dibenzoyl-D-tartaric acid. H_2O/aq. EtOH b, cyclopropanecarbonyl chloride–pyridine/CH_2Cl_2 r.t.

Fig. 1

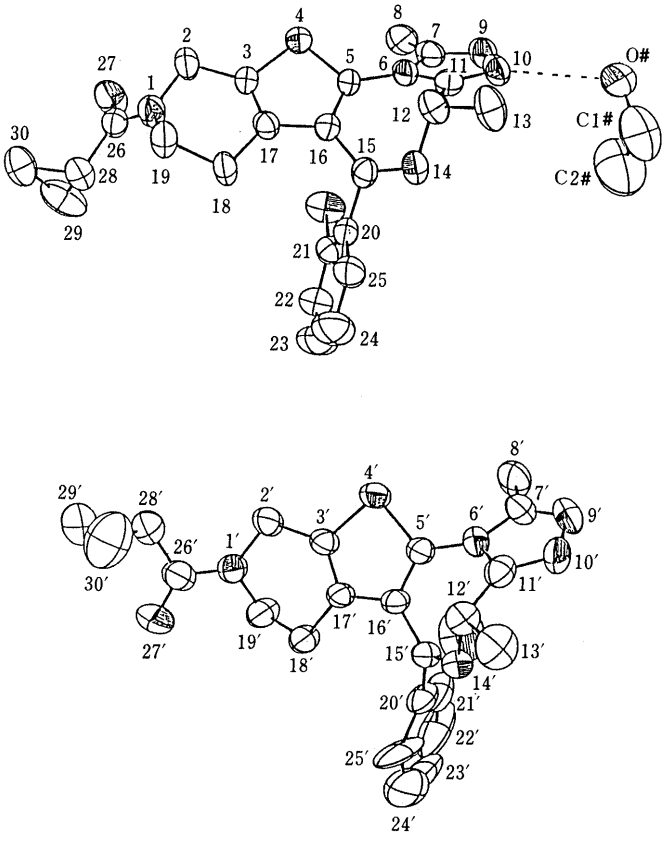


Fig. 2. ORTEP Drawing of (+)-**1**; E6123

TABLE I. Positional Parameters and Their Estimated Standard Deviation

Atom	x	y	z	B(Å²)
Mole-A				
Cl	0.6933 (2)	0.2453 (fix)	0.7142 (2)	5.75 (5)
S4	0.9610 (1)	0.3770 (2)	0.7293 (2)	4.50 (4)
O27	0.8824 (5)	0.6693 (6)	0.9745 (4)	6.4 (2)
N1	0.9239 (5)	0.4657 (7)	0.9883 (5)	4.7 (2)
N6	0.9137 (4)	0.1677 (7)	0.6159 (4)	3.8 (1)
N9	0.8978 (4)	0.1181 (8)	0.4639 (4)	5.0 (2)
N10	0.9198 (4)	0.0099 (8)	0.5182 (5)	4.7 (2)
N14	0.8695 (4)	−0.0472 (7)	0.7315 (4)	4.0 (1)
C2	0.9673 (6)	0.483 (1)	0.9101 (6)	5.9 (2)
C3	0.9387 (5)	0.3752 (9)	0.8407 (5)	4.0 (2)
C5	0.9169 (5)	0.2300 (8)	0.7028 (5)	3.5 (2)
C7	0.8940 (5)	0.215 (1)	0.5209 (5)	4.4 (2)
C8	0.8731 (6)	0.350 (1)	0.4927 (6)	5.4 (2)
C11	0.9239 (5)	0.0392 (9)	0.6089 (6)	4.1 (2)
C12	0.9511 (5)	−0.0374 (9)	0.7009 (6)	4.5 (2)
C13	0.9808 (7)	−0.173 (1)	0.6842 (7)	6.0 (2)
C15	0.8463 (5)	0.0541 (8)	0.7692 (5)	3.7 (2)
C16	0.8875 (5)	0.1787 (8)	0.7770 (5)	3.4 (2)
C17	0.9008 (5)	0.2667 (8)	0.8566 (5)	3.6 (2)
C18	0.8776 (5)	0.2454 (8)	0.9505 (5)	4.1 (2)
C19	0.9343 (6)	0.3361 (9)	1.0286 (6)	4.7 (2)
C20	0.7697 (5)	0.0365 (8)	0.8088 (5)	3.7 (2)
C21	0.6996 (5)	0.1118 (9)	0.7880 (5)	4.1 (2)
C22	0.6272 (6)	0.089 (1)	0.8215 (7)	5.5 (2)
C23	0.6301 (7)	−0.016 (1)	0.8820 (8)	7.2 (3)
C24	0.6992 (7)	−0.093 (1)	0.9054 (8)	7.0 (3)
C25	0.7725 (6)	−0.071 (1)	0.8691 (6)	5.7 (2)
C26	0.8855 (5)	0.5666 (9)	1.0137 (6)	4.2 (2)
C28	0.8403 (6)	0.5452 (9)	1.0942 (6)	4.7 (2)
C29	0.7660 (7)	0.630 (1)	1.0910 (7)	7.6 (3)
C30	0.8509 (8)	0.656 (1)	1.1638 (8)	8.9 (3)
Mole-B				
Cl′	0.4509 (2)	0.1162 (5)	0.1784 (4)	12.0 (1)
S4′	0.6703 (1)	0.1322 (2)	0.4730 (1)	4.37 (4)
O27′	0.6039 (5)	−0.4171 (7)	0.5390 (5)	6.7 (2)
N1′	0.5833 (5)	−0.2079 (7)	0.5314 (5)	4.8 (2)
N6′	0.6946 (4)	0.2212 (6)	0.3044 (4)	3.9 (1)
N9′	0.7223 (5)	0.4006 (8)	0.2436 (6)	5.7 (2)
N10′	0.7658 (5)	0.3051 (8)	0.2089 (5)	5.5 (2)
N14′	0.6825 (4)	0.0094 (7)	0.1643 (5)	4.1 (2)
C2′	0.6098 (6)	−0.0780 (9)	0.5575 (6)	5.2 (2)
C3′	0.6222 (5)	−0.0145 (8)	0.4668 (5)	3.8 (2)
C5′	0.6629 (5)	0.1243 (8)	0.3509 (5)	3.7 (2)
C7′	0.6800 (6)	0.3510 (9)	0.3003 (7)	5.0 (2)
C8′	0.6189 (7)	0.415 (1)	0.3482 (7)	6.7 (3)
C11′	0.7477 (5)	0.1978 (9)	0.2441 (6)	4.2 (2)
C12′	0.7658 (5)	0.0646 (9)	0.2237 (6)	4.1 (2)
C13′	0.8340 (5)	0.058 (1)	0.1635 (7)	6.2 (2)
C15′	0.6229 (5)	−0.0152 (8)	0.2046 (5)	3.7 (2)
C16′	0.6273 (5)	0.0135 (8)	0.3086 (5)	3.5 (2)
C17′	0.6011 (5)	−0.0648 (8)	0.3757 (5)	3.6 (2)
C18′	0.5524 (6)	−0.1890 (9)	0.3535 (6)	4.9 (2)
C19′	0.5120 (6)	−0.218 (1)	0.4388 (6)	5.3 (2)
C20′	0.5455 (6)	−0.076 (1)	0.1415 (6)	5.2 (2)
C21′	0.4660 (7)	−0.026 (1)	0.1244 (8)	7.8 (3)
C22′	0.3869 (8)	−0.080 (2)	0.0629 (9)	13.1 (5)
C23′	0.403 (1)	−0.186 (2)	0.0179 (8)	14.0 (6)
C24′	0.480 (1)	−0.237 (2)	0.0284 (9)	14.5 (5)
C25′	0.5665 (9)	−0.203 (1)	0.0958 (7)	12.5 (3)
C26′	0.6277 (6)	−0.3139 (9)	0.5733 (6)	5.1 (2)
C28′	0.7029 (7)	−0.299 (1)	0.6611 (7)	6.1 (3)
C29′	0.7798 (8)	−0.381 (2)	0.6688 (9)	9.8 (4)
C30′	0.7257 (8)	−0.407 (1)	0.7309 (8)	7.5 (3)
Ethanol				
O#	0.9077 (5)	−0.2012 (9)	0.3938 (5)	8.1 (2)
C1#	0.8652 (9)	−0.313 (2)	0.4245 (9)	9.7 (4)
C2#	0.785 (1)	−0.268 (2)	0.405 (1)	13.3 (6)

Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: (4/3) [a^2B(1,1) + b^2B(2,2) + c^2B(3,3) + ab(cos gamma)B(1,2) + ac(cos beta)B(1,3) + bc(cos alpha)B(2,3)].

camphorsulfonic acid and L-(+)-mandelic acid. Among them, (+)-dibenzoyl-D-tartaric acid gave the most satisfactory result. The racemate (±)-**2** was recrystallized twice and the salt thus obtained was treated with aqueous $NaHCO_3$ to give the free amine (−)-**2** in 99.3% ee ($[\alpha]_D$ −23.5° (EtOH, c=1), 21%). Subsequent amidation of (−)-**2** with cyclopropanecarbonyl chloride in the presence of pyridine in dichloromethane resulted in the formation of (+)-**1**. We have used this methodology for the mass production (killogram scale) of (+)-**1** without any decrease in quality or yield of the product.

The stereochemistry of (+)-**1** was established by X-ray crystallographic analysis (Fig. 2, Table I). The crystal of (+)-**1** recrystallized from an ethanol solution contained two molecules of (+)-**1** and one ethanol molecule in an asymmetric unit. The result clearly indicated that (+)-**1** has *S*-configuration.

Experimental

General Methods Reagents and solvents were purchased from usual commercial sources. Silicagel (Kiesel gel 60, Merck) was used for column chromatography and silicagel (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. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-100 (100 MHz) or a 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 infrared spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter.

(−)-6-(2-Chlorophenyl)-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 ((−)-2) Water (0.3 l) was added to a hot solution of the racemate **2** (323 g, 0.84 mol) and (+)-dibenzoyl-D-tartaric acid·H_2O (300 g, 0.80 mol) in EtOH (8.4 l). The precipitate that appeared was filtered off, giving the salt. This diastereoisomer (218 g) was further recrystallized from EtOH (6.0 l), giving 149.5 g of white crystals. The product was taken up in CH_2Cl_2 (4.6 l) and aqueous $NaHCO_3$ (80 g/1.6 l H_2O), and the layers were allowed to separate. Aqueous $NaHCO_3$ (20 g/0.4 l H_2O) was added to the aqueous layer and this was then extracted with CH_2Cl_2 (1 l). The combined organic layers were washed with brine and concentrated to give (−)-**2** (68.3 g, c.y. 21%, 99.3% ee) as a white powder. (−)-**2**: $[\alpha]_D$ −23.5° (c=1, EtOH) The opposite enantiomer was obtained from (−)-dibenzoyl-L-tartaric acid·H_2O. (+)-**2**: $[\alpha]_D$ +17.56° (c=0.02, EtOH). The optical purity was determined by high performance liquid chromatography (HPLC) analysis. Column conditions: Chiracel OJ colum, i.d. 4.6×250 mm, mobile phase *n*-hexane–ethanol (75:25) containing 0.5% NEt_3; flow rate, 1.0 ml/min; column temperature, ambient; detector, UV 254 nm; retention time, (−)-**2** 7.25 min; (+)-**2** 11.16 min.

(+)-6-(2-Chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*]-[1,4]diazepine ((+)-1) Cyclopropionyl chloride (21.5 ml, 0.24 mol) was added dropwise to a cooled (ice bath) solution of (−)-**2** (79.2 g, 0.21 mol) and pyridine (21 ml, 0.26 mol) in CH_2Cl_2 (500 ml), and the resulting solution was stirred at room temperature for 1 h. The mixture was poured into saturated aqueous $NaHCO_3$ (1 l) and extracted with CH_2Cl_2 (1 l×2). The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography. Elution with firstly 40% AcOEt in *n*-hexane and then successively from 1% to 5% MeOH in CH_2Cl_2 gave (+)-**1** (87.6 g, c.y. 92%, 99.3% ee) as a pale yellow-white powder (+)-**1**: $[\alpha]_D$ +16.5—18.5° (c=0.1, $CHCl_3$), mp 150 °C. MS (Pos, Fab) m/z 452 $(M+H)^+$: IR (Nujol): 1635, 1590, 1530 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.55—1.15 (m, 4H), 1.45—2.5 (m, 3H), 2.10 (d, J=6.8 Hz, 3H), 2.66 (s, 3H), 2.8—4.8 (m, 3H), 4.26 (q, J=6.8 Hz, m, 1H), 4.8—5.2 (m, 1H), 7.05—7.65 (m, 4H).

X-Ray Crystallography of (+)-1 The crystals of (+)-**1** recrystallized from an ethanol solution contained two molecules of (+)-**1** and one ethanol molecule in an asymmetric unit. The observed cell parameters for a crystal of (+)-**1** (0.43 × 0.31 × 0.25 mm) were as follows: molecular

formula $2(C_{23}H_{22}ClNOS)\cdot C_2H_5OH$, formula weight 950.02, space group $P2_1$ (monoclinic), $Z=2$, $a=16.098(6)$, $b=10.592(3)$, $c=14.345(4)$ Å, $\beta=106.47(2)°$, $V=2345.5$ Å^3, $D_c=1.35$ g cm^{-3}. Reflection data were collected on a Enraf–Nonius CAD4 diffractometer using graphite-monochromated Cu K_α radiation by the 2θ–ω scan method. The data were corrected for Lorentz and polarization factors, but no absorption correction was applied. A total of 4041 reflections were measured within the 2θ angle of 126°. The crystal structure was determined by the direct method and refined by the full-matrix least-squares procedure. The final R value was 0.067 for 3917 reflections above 3σ (I) including anisotropic thermal factors for nonhydrogen atoms. Hydrogen atoms were not included in the calculations. The final atomic coordinates are listed in Table I. The absolute configuration of (+)-**1** was determined by comparing intensity differences in some Bijvoet pair reflections induced by anomalous dispersion of S and Cl. The result showed that the configuration of the asymmetric carbon of (+)-**1** was *S*.

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