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Synthesis and biological properties of tensyuic acids B, C, and E, and investigation of the optical purity of natural tensyuic acid B

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ABSTRACT

The first, concise total synthesis of (±)-tensyuic acids B, C, and E, using chemoselective formal S_N2' type Grignard reactions and selective esterification, is described. In addition, the optical purity of natural (±)-tensyuic acid B was determined using Chirabite-AR. Synthetic tensyuic acids, together with their intermediate compounds, were found to possess useful bioactive properties, with some of them showing potent activity against *Trypanosoma brucei brucei* strain *GUTat* 3.1.

1. Introduction

During the course of screening for new antibiotics, six new compounds, designated tensyuic acids A–F (Fig. 1), were isolated from a culture broth of *Aspergillus niger* FKI-2342.[1] The structure of tensyuic acids A–F is known to belong to the itaconic acid family.[2–4] In addition, tensyuic acids are novel itaconic acid derivatives having the ester carboxyl moieties at the bottom of the alkyl side chain. Although tensyuic acids and structurally related hexylitaconic acid[3] have low $[\alpha]_D$ values (tensyuic acid A: +1.8, B: −1.7, C: −6.0, D: +3.0, E: +4.9, F: −3.1; hexylitaconic acid: −8) in methanol,[1,3] the absolute configuration of related itaconic acid derivatives (i.e., hexylitaconic acid[3] and ceriporic acid A[4]) has never been reported. The property of biological activities for antimicrobial and anticancer actions has been traced to the novel tensyuic acids, and tensyuic acid C (**2**) has been identified as being antibiotic agent against *Bacillus* subtilis with moderate concentration for MIC (inhibition zone: 10 mm at 50 μg/disk).[1] Although other itaconic acid derivatives have been reported to exhibit various activity, such as inhibitor of the glyoxylate cycle,[2] the production of a cellulolytic active oxygen species, the iron redox reaction[3] and p53-HDM2 interaction,[4] studies of the biological properties of tensyuic acids have been hampered by insufficient quantities of products from the natural source. Consequently, the supply of these natural products produced synthetically will allow detailed investigation of their biological properties. Here, we describe the first total synthesis of (±)-tensyuic acids B (**1**), C (**2**), and E (**3**), the optical purity of naturally occurring **1**, and the biological activities of them.

2. Results and discussion

2.1. Retrosynthetic analysis of tensyuic acids B, C, and E

Tensyuic acids containing *exo*-type olefin are generally synthesized using a Wittig reaction,[5] coupling reactions involving reduction of carbon–carbon triple bond,[6] and S_N2' coupling reactions of appropriate substrates and nucleophiles.[7] In our synthetic plan, we envisaged construction of the basic structure of the tensyuic acids to permit a shorter synthetic route via chemoselective formal S_N2' coupling between Grignard reagents and dimethyl bromomethylfumarate (**8**) as shown in Scheme 1.

The C–C linkage for this formal S_N2' reaction will allow access to all forms of tensyuic acids from **8** with suitable nucleophiles. Our approach also has the feature of efficient selective esterification from hydrazide, with appropriate side chains.

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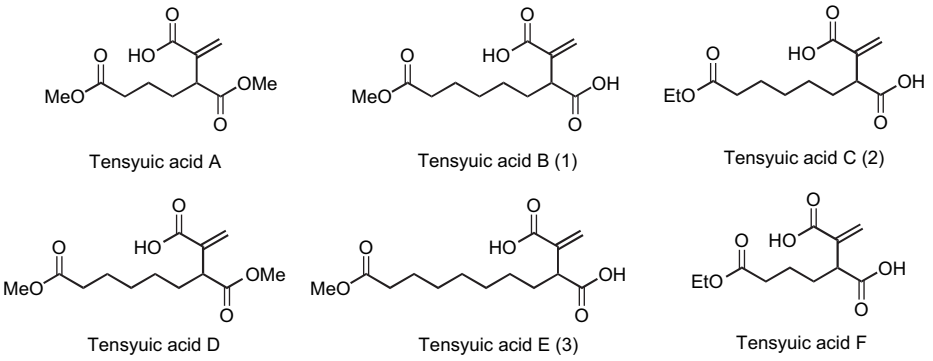
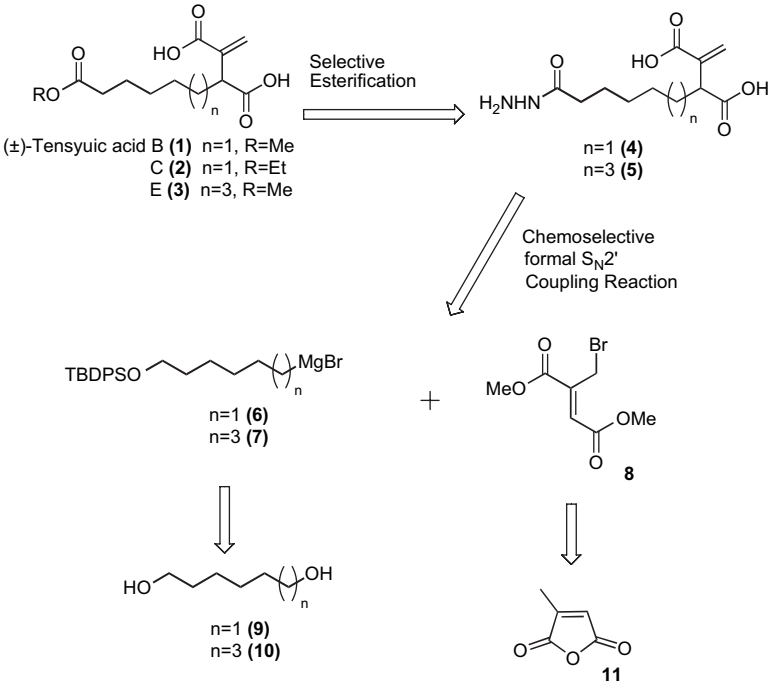


Figure 1. Structure of tensyuic acids A–F.



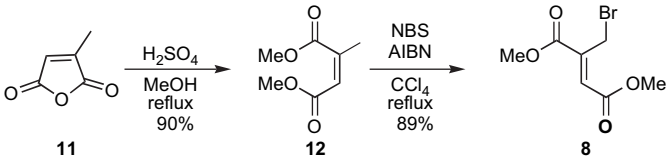
Scheme 1. Retrosynthetic analysis of (±)-tensyuic acids B, C, and E.

2.2. Preparation of fragments and chemoselective formal S_N2' coupling

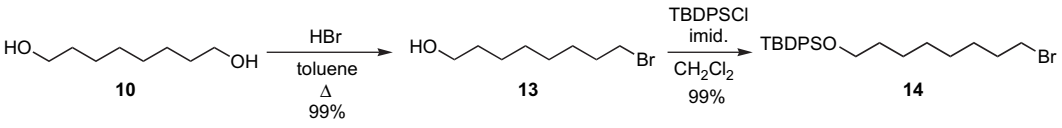
The total synthesis of (±)-tensyuic acid E (**3**), which has the longest alkyl chain among the tensyuic acids, began with the preparation of the 8*C*-Gignard reagent (**7**) and dimethyl bromomethylfumarate (**8**).[8] The fumarate derivative (**8**), conveniently available from citraconic anhydride (**11**), is a convenient starting material via the Argade protocol[8] (Scheme 2). The reaction of **11** with methanol/H_2SO_4 under reflux gave the desired diester (**12**) in 90% yield. Treatment of **12** with NBS/AIBN in refluxing carbon tetrachloride underwent allylic bromination and isomerization of the carbon–carbon double bond to yield **8** in 89% yield.

The precursor of Grignard reagent (**14**)[9] was prepared from 1,8-octanediol (**10**) as a starting material by mono-bromination with HBr under reflux in 99% yield and protection of the remaining hydroxyl group with TBDPS in 99% yield (Scheme 3).

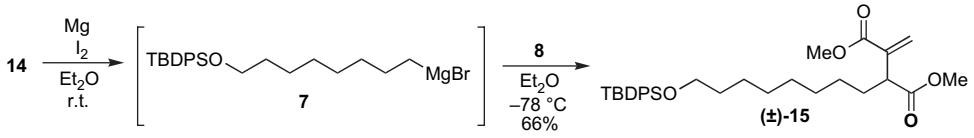
Turning to the formal S_N2' coupling tactic to construct the *C*3-alkyl itaconic acid structure, the Grignard reagent (**7**) was derived from **14** with activated magnesium metal to react with **8** (Scheme 4). However, it is difficult to prepare the high-molecular weight Grignard reagent from unreactive halides by direct reaction of the halides and magnesium. This difficultly was conquered by the use of I_2 to activate magnesium metal in freshly distilled Et_2O, and the long chain Grignard reagent (**7**) was obtained as a dark gray solution in quantitative conversion. The Grignard reagent reacted in a highly chemo- and regioselective fashion with **8** and the exclusive Michael addition, followed by elimination of the allylic bromine atom, gave the formal S_N2' product in 66% yield. In a previous report,[8] Argade and Kar reported that HMPA as an additive is very effective for this formal S_N2' coupling reaction, but we obtained a better yield in the absence of HMPA for our substrate.



Scheme 2. Preparation of dimethyl bromomethylfumarate (**8**).



Scheme 3. Preparation of 1-bromo-8-silyloxy octane (**14**).



Scheme 4. Synthesis of (±)-3-alkyl itaconic acid derivative (**15**).

2.3. Completion of total synthesis of (±)-tensyuic acid E

Having the basic carbon frame of **3**, we turned our attention to complete the synthesis of (±)-**3** via a selective esterification strategy (Scheme 5). Deprotection of TBDPS from (±)-**15** with TBAF in THF gave the desired *exo*-olefin product (±)-**16** in 47% yield with the more thermodynamically stable *endo*-olefin as a by-product in 53% yield. To avoid this isomerization, we examined several deprotection conditions, with 47% HF aq/pyridine (1/2.4, v/v) being the most effective for suppression of the undesired isomerization, affording 88% yield of *exo*-product (±)-**16** and none of the *endo*-product. Subsequently, primary alcohol (±)-**16** was subjected to Parikh–Doering oxidation and Pinnick oxidation to furnish the desired carboxylic acid (±)-**17** in 81% yield in two steps. Then, (±)-**17** was converted to acid chloride with $(COCl)_2$, followed by condensation with Boc-hydrazine to give the Boc-hydrazide (±)-**18** in 99% yield in two steps. Because hydrazide (±)-**18** is a key intermediate in this total synthesis, two benefits are bestowed. Firstly, hydrazide can be easily converted to the desired ester under simple oxidation conditions in the solution of suitable alcohol. Secondly, hydrazide can be maintained under saponification conditions. Consequently, dicarboxylic acid with Boc-hydrazide (±)-**19** was easily prepared under basic condition, from (±)-**18**, with (±)-**19** then being subjected to the next reaction without silica gel purification, due to its high polarity.

After deprotection of the Boc group with TFA from the crude (±)-**19**, azide (±)-**5** was prepared using sodium nitrate and aqueous HCl.[10] The reaction was quenched with suitable alcohol (in this case, methanol) to complete full construction of (±)-tensyuic acid E (**3**) in 30% yield from (±)-**19** in three steps (Scheme 6).

Utilization of HONO for this selective esterification, under several conditions, was examined but (±)-tensyuic acid E (**3**) could not be obtained in satisfactory yields. Esterification with another oxidizing agents eventually proved successful, the deprotected hydrazide being treated with CAN[11] as oxidant in methanol to directly furnish (±)-**3** in 58% yield in three steps from (±)-**19** (Scheme 7).

The mechanism of the reaction of hydrazide with CAN and esterification with alcohol is unclear.[11,12] Although the reaction process is not fully understood, a proposed mechanism for the formation of the corresponding ester is depicted in Scheme 8.

Regarding the evolution of nitrogen, an acyldiazonium salt (**c**) would be generated by the oxidation of hydrazide. Alcohol would then attack **c** to give the ester (**d**).

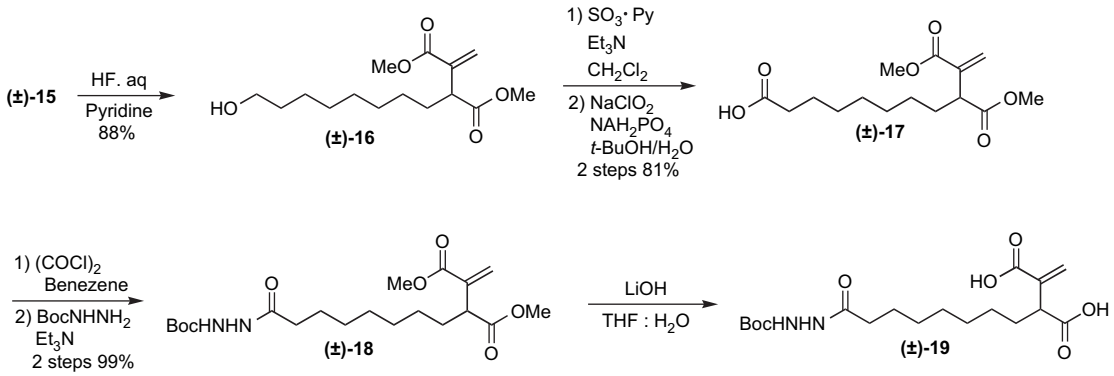
2.4. Total synthesis of (±)-tensyuic acids B and C

After completion of the total synthesis of (±)-**3**, our focus turned to the total synthesis of tensyuic acids B (**1**) and C (**2**). Accordingly, the Grignard reagent (**6**) was prepared by the same reaction sequences as for the preparation of **7**, using commercially available 1,6-hexanediol (**9**). Subsequently, **6** and **8** underwent the same reaction sequence as (±)-**15** for the synthesis of (±)-tensyuic acid E (**3**) to give (±)-tensyuic acid B (**1**) (Scheme 9). Additionally, (±)-tensyuic acid C (**2**) was prepared via the use of ethanol instead of methanol as a reaction solvent for final reaction step from (±)-**4** to selectively give the ethyl ester (Scheme 9).

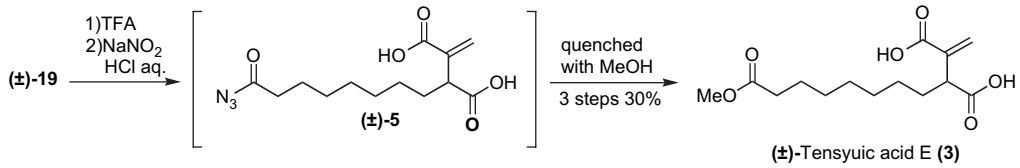
The three specific synthetic natural products, (±)-tensyuic acids B, C, and E, were found to be identical to the naturally occurring tensyuic acid products in all respect.

2.5. Application of Chirabite-AR to determine ee% of natural-(1)

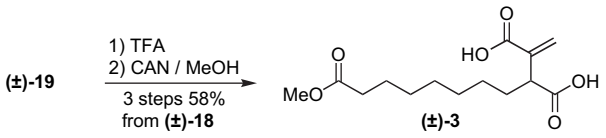
In possession of the racemic tensyuic acids, we next investigated the optical purity of naturally occurring tensyuic acids using Chirabite-AR, a commercially available chiral shift reagent, newly developed by Ema et al.[13] (Fig. 2).



Scheme 5. Synthesis of (±)-**19**.



Scheme 6. Completion of the synthesis of (±)-tensyuic acid E (**3**).



Scheme 7. Advanced condition for the selective esterification.

So far, various types of chiral shift reagents such as lanthanide complexes,[14] cyclodextrins,[15] crown ethers,[16] calixarenes,[17] porphyrins,[18] and others have been developed. However, few have been commercialized except for lanthanide complexes, which often cause signal broadening, particularly in a high magnetic field because of the paramagnetic metal. To demonstrate the practical utility of Chirabite-AR, at first, synthetic (±) and naturally occurring tensyuic acid B (**1**) was tested. Before comparison between synthetic and natural tensyuic acid B with Chirabite-AR, we examined the effect of differing amounts of Chirabite-AR regarding (±)-**1**, to determine sufficient signal separations between (+)- and (−)-**1**. As shown in Figure 3, two signals of *exo*-olefin of (±)-**1** were strongly influenced by Chirabite-AR in $CDCl_3$ at room temperature, the extent depending on increasing amounts of Chirabite-AR, together with the signal broadening. Fortunately, the problem of signal broadening was resolved by heating the mixture of (±)-**1** and Chirabite-AR in $CDCl_3$ to 50 °C.

A mixture of (±)-tensyuic acid B with 70 mol % of Chirabite-AR was measured sequentially by 400 MHz ^{1}H NMR at 50 °C in $CDCl_3$ (Fig. 4-1), marked signal separations were observed for *exo*-olefin, without notable line broadening, and good enantiomeric discrimination was achieved for racemic **1**.

NMR analysis of natural **1** under the same conditions as used to obtain the results displayed in Figure 4-1 indicated that separated signals exhibited 5/3 ratio in numerical integration value (Fig. 4-2). Therefore, the optical purity of natural **1** was determined as 25% ee.

2.6. Biological activity

The bioactivity of synthetic tensyuic acids, including all synthetic intermediates, was examined, with respect to their anti-infectious properties, notably those focused on Type III secretion systems,[19] influenza,[20] methicillin-resistant *Staphylococcus aureus* (MRSA),[21] *Trypanosoma*,[22] vancomycin-resistant *Enterococcus* (VRE),[21] and toll-like receptor (TLR).[23]

Both (±)-tensyuic acid C (**2**) and a synthetic intermediate (**16**) show promising anti-trypanosomal properties in vitro, with IC_{50} values of 2.23 μg/mL and 1.95 μg/mL, respectively (Table 1). The values of these IC_{50} are close to the IC_{50} value of suramin (1.58 μg/mL)[22a] (Fig. 5), which is still widely used as therapeutic agent in humans. Currently, only four drugs are registered for the treatment of Human African trypanosomiasis (HAT): pentamidine, suramin, melarsoprol, and eflornithine.[22a] All four drugs used to treat HAT are unsatisfactory, since they cannot be given orally and are all hampered by severe toxicity and increasing resistance of the parasites. Consequently, there is an urgent need for new anti-trypanosomal drugs, which have novel structures and mechanisms of action and which are both safe and effective. Tensyuic acid C and a synthetic intermediate (**16**) may develop to be novel anti-trypanosomal drugs.

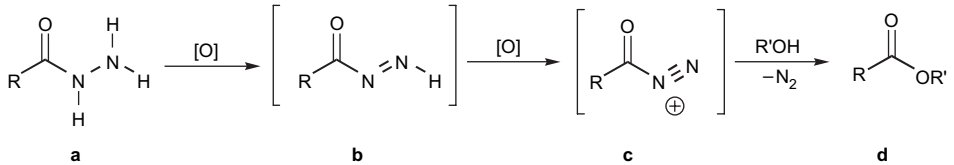
3. Conclusion

In conclusion, we have achieved the first, a concise, total synthesis of (±)-tensyuic acids B (**1**), C (**2**), and E (**3**) using chemoselective formal S_N2' type Grignard reactions and selective esterification. In addition, using Chirabite-AR we found that the optical purity of natural **1** is 25% ee. We have also identified some important bioactive properties for (±)-tensyuic acids and their synthetic intermediates, and found potent activity against *Trypanosoma brucei brucei* for compounds (±)-**2** and **16**. Further in vitro and in vivo studies on tensyuic acid C and analogues are in progress.

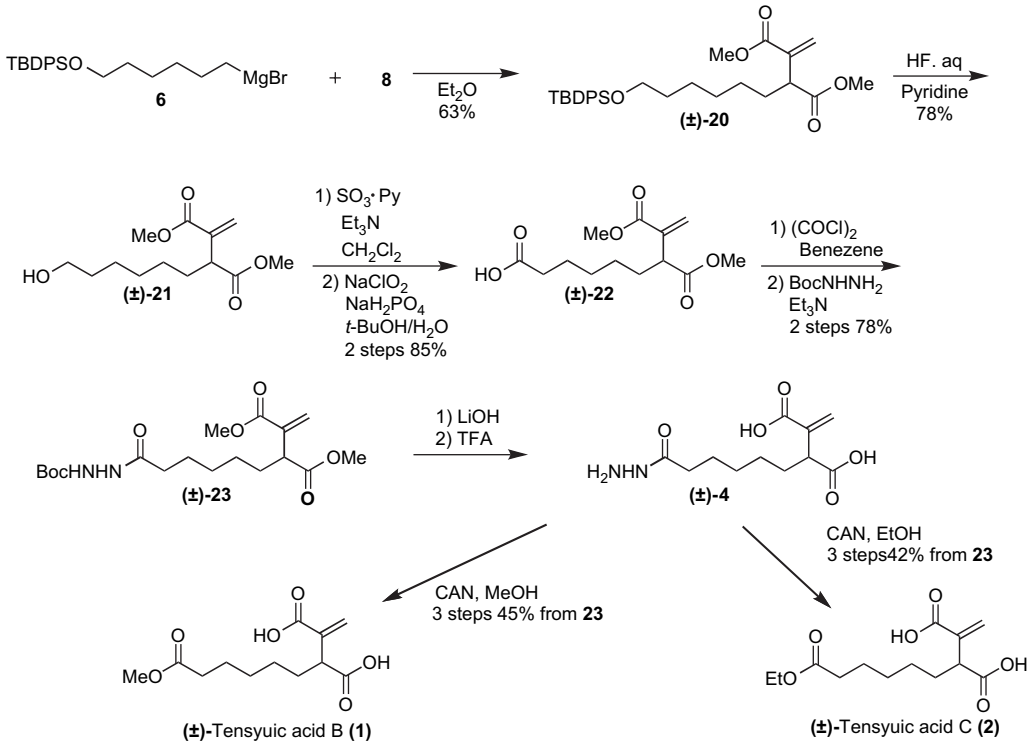
4. Experimental

4.1. General

Dry tetrahydrofuran (THF), toluene, and CH_2Cl_2 were purchased from Kanto Chemical Co., Inc. Dry diethyl ether (Et_2O) was freshly distilled from sodium/benzophenone under argon. Activated magnesium turnings were purchased from Kanto Chemical Co., Inc. Chirabite-AR was purchased from Tokyo Chemical Industry (TCI) Co., Ltd. Pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical and preparative thin layer chromatography. Flash column chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical Co., Inc., Silica gel 60N, spherical neutral, 0.040–0.050 mm, Cat. No. 37563-84). ^{1}H NMR spectra were recorded at 270 MHz or 400 MHz and ^{13}C NMR spectra were recorded at 67.5 MHz or 100 MHz on JEOL JNM-EX270 (270 MHz) or Varian XL-400 (400 MHz) or Varian UNITY-400 (400 MHz). Chemical shifts are expressed in parts per million downfield from internal solvent peaks $CHCl_3$ (7.26 ppm, ^{1}H NMR) and *J* values are given in hertz. The coupling patterns are expressed by s (singlet), d (doublet), t (triplet), and m (multiplet). The all infrared spectra were measured on a Horiba FT-210 spectrometer. High- and low-resolution mass spectra were measured on a JEOL



Scheme 8. Proposed mechanism for esterification from hydrazide by CAN.



Scheme 9. Total synthesis of (±)-tensyuic acids B (**1**) and C (**2**).

JMS-DX300 and JEOL JMS-AX505 HA spectrometers. Liquid chromatographic preparation was conducted on a Jasco PU-980 with Senshu Pak-PEGASIL ODS.

4.2. Total synthesis of (±)-3

*4.2.1. 1-(tert-Butyldiphenylsilyloxy)-8-octylmagnesium bromide (**7**) and methyl 11-(tert-butyldiphenylsilyloxy)-2-methylene-3-methoxycarbonylundecanate ((±)-**15**)*

A solution of Grignard reagent in Et_2O was prepared as follows: a catalytic amount of I_2 (one crystal) was added to activated magnesium turnings (310 mg, 12.9 mmol), which were soaked in dry Et_2O (0.5 mL) under argon at room temperature, and the mixture was stirred for 10 min. To the mixture with gentle stirring was added the solution of **14** (1.9 g, 4.26 mmol) in Et_2O (0.5 mL), and the solution was stirred for further 3 h to afford the desired Grignard reagent in Et_2O. Subsequently, to the solution of dimethyl bromomethylfumarate (**8**) (500 mg, 2.13 mmol) in ether (5 mL) was slowly added the prepared Grignard reagent **7** at −78 °C. The reaction mixture was stirred for further 19 h. The reaction was then quenched by addition of NH_4Cl aq solution (10 mL) and warmed to room temperature. After stirring for 10 min, an additional Et_2O (10 mL) was added to the mixture and two layers were separated. The aqueous layer was extracted with Et_2O (3×30 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate=70:1) afforded (±)-**15** (730 mg, 66%) as a colorless oil. IR ν_{max} (NaCl): 1739, 1724 (C=O), 1199, 1141 (C–O), 1101 (C–O–Si) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 7.68 (4H, m, Ar–H), 7.36 (6H, m, Ar–H), 6.36 (1H, s, 1′-H), 5.75 (1H, s, 1′-H), 3.76 (3H, s, 1-OCH_3), 3.67 (3H, s, 1″-OCH_3), 3.64 (2H, t, J=6.4 Hz, 11-H), 3.50 (1H, t, J=7.3 Hz, 3-H), 1.98–1.44 (4H, complex m, 4, 10-H_2), 1.42–1.17 (10H, complex m, 5, 6, 7, 8, 9-H_2), 1.04 (9H, s, SiC(CH_3)$_3$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 173.7 (C-1″), 166.6 (C-1), 138.3 (C-2), 135.5 (2C, Ar×2), 134.1 (Ar), 129.4 (Ar), 127.5 (2C, Ar×2), 126.6 (C-1′), 63.9 (C-11), 52.0 (COOCH_3), 51.9 (COOCH_3), 46.5 (C-3), 32.5 (C-10), 31.2 (C-4), 29.2 (3C, C-6, 7, 8), 27.4 (C-5), 26.8 (3C, SiC(CH_3)$_3$), 25.7 (C-9), 19.1 (SiC(CH$_3$)$_3$). HRMS (FAB, *m*-NBA) *m*/*z*: 547.2829 $[M+Na]^+$; calcd for $C_{31}H_{44}O_5SiNa$: 547.2856 [M+Na].

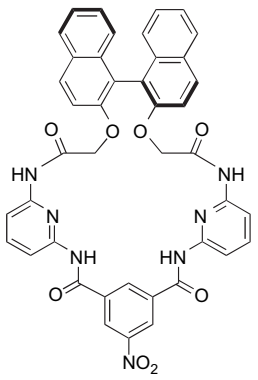


Figure 2. Structure of Chirabite-AR.

*4.2.2. Methyl 11-hydroxy-2-methylene-3-methoxycarbonylundecanate ((±)-**16**)*

Compound (±)-**15** (250 mg, 0.464 mmol) was dissolved into a solution of 47% HF aq (1.4 mL) and pyridine (3.3 mL) at room temperature. The resultant mixture was stirred for 30 min, and then quenched by addition of saturated $NaHCO_3$ aq (20 mL) and extracted with $CHCl_3$ (3×20 mL). The combined organic layers were washed with saturated $NaHCO_3$ aq (20 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate=4:1) afforded (±)-**16** (117 mg, 88%) as a colorless oil. IR ν_{max} (NaCl): 3473 (OH), 1735, 1725 (C=O), 201, 1143 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.36 (1H, s, 1′-H), 5.75 (1H, s, 1′-H), 3.77 (3H, s, 1-OCH_3), 3.67 (3H, s, 1″-OCH_3), 3.63 (2H, t, J=6.4 Hz, 11-H), 3.50 (1H, t, J=7.3 Hz, 3-H), 1.98–1.40 (4H, complex m, 4, 10-H_2), 1.40–1.13 (10H, complex m, 5, 6, 7, 8, 9-H_2); ^{13}C NMR (67.5 MHz,

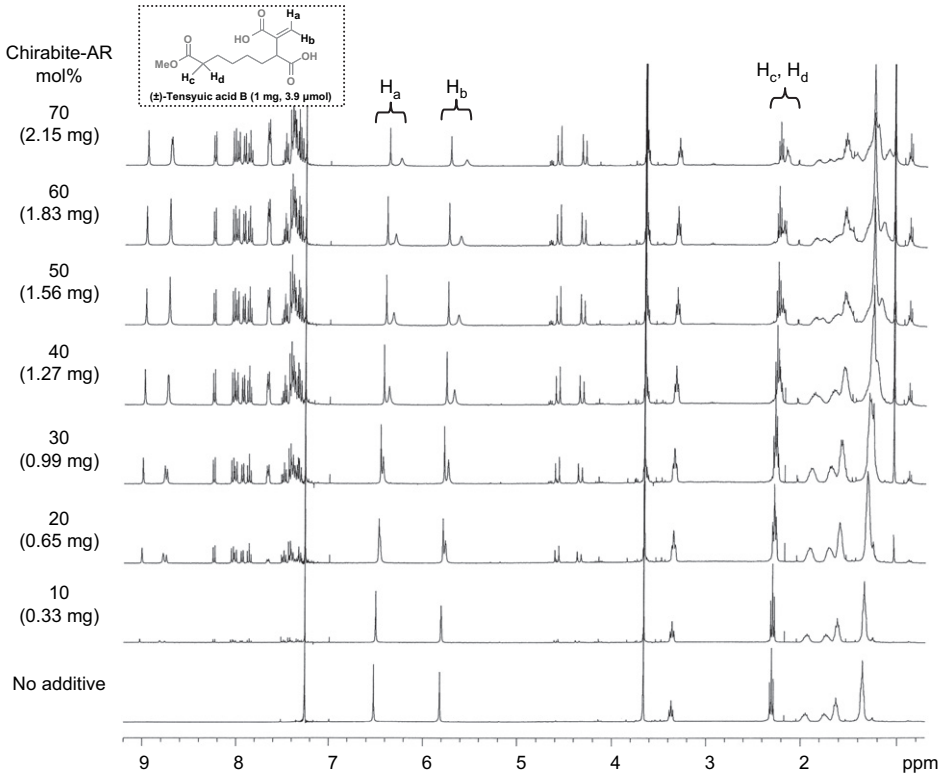


Figure 3. Superposition of ^{1}H NMR (400 MHz) spectra in the presence of increasing amounts of Chirabite-AR (10–70 mol %, from bottom to top) in a $CDCl_3$ solution of synthetic (±)-**1** (1 mg, 3.9 μmol) at room temperature.

$CDCl_3$) δ: 173.8 (C-1″), 166.7 (C-1), 138.3 (C-2), 126.7 (C-1′), 64.0 (C-11), 52.1 ($COOCH_3$), 52.0 ($COOCH_3$), 46.5 (C-3), 32.7 (C-10), 31.2 (C-4), 29.2 (3C, C-6, 7, 8), 27.4 (C-5), 25.6 (C-9). HRMS (FAB, *m*-NBA) *m*/*z*: 309.1682 $[M+Na]^+$; calcd for $C_{15}H_{26}O_5Na$: 309.1687 $[M+Na]^+$.

4.2.3. *Methyl 10-carboxy-2-methylene-3-methoxycarbonyl-decanate ((±)-**17**)*

To a solution of (±)-**16** (450 mg, 1.57 mmol), dimethyl sulfoxide (1.1 mL, 15.7 mmol) and triethyl amine (1.1 mL, 7.86 mmol) in CH_2Cl_2 (3.1 mL) at 0 °C was added SO_3·pyrdine (630 mg, 3.96 mmol). After warming to room temperature, the reaction mixture was stirred for 1 h, and then quenched by addition H_2O (5 mL) and extracted with hexane/AcOEt (1/1) (3×5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give crude aldehyde, which was used in the next step without further purification. To the solution of crude aldehyde in *t*-BuOH (15.7 mL) were added 2-methyl-2-butene (3.3 mL, 31.4 mmol) and a solution of $NaHPO_4$ (3.1 g, 20.4 mmol) and $NaClO_2$ (1.4 g, 15.7 mmol) in H_2O (3.4 mL) at

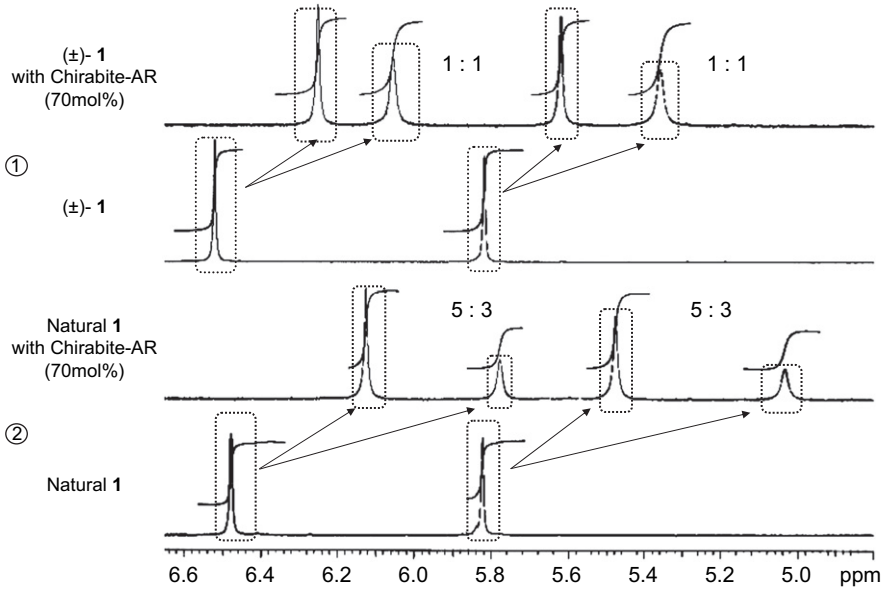
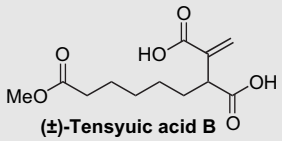
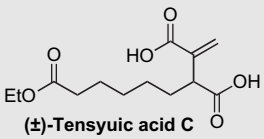
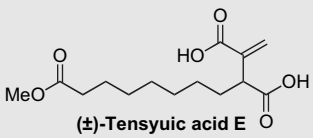
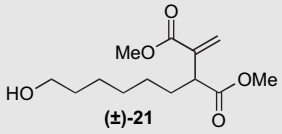
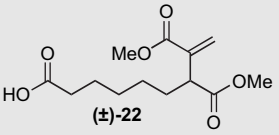
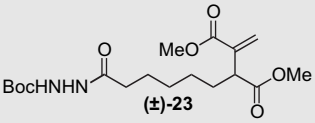
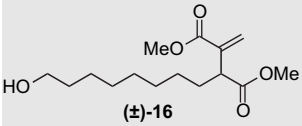
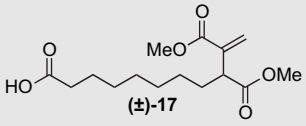
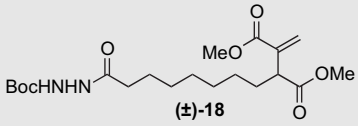


Figure 4. Comparison of ^{1}H NMR (400 MHz) spectra between (±)-**1** and natural **1**, in 70 mol % of Chirabite-AR in $CDCl_3$ at 50 °C.

Table 1
In vitro anti-trypanosomal activity against *Trypanosoma brucei brucei* strain *GUTat* 3.1 for (±)-tensyuic acids and synthetic intermediate

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
 (±)-Tensyuic acid B	>12.5	 (±)-Tensyuic acid C	2.23	 (±)-Tensyuic acid E	>12.5
 (±)-21	4.39	 (±)-22	>12.5	 (±)-23	>12.5
 (±)-16	1.95	 (±)-17	>12.5	 (±)-18	4.93

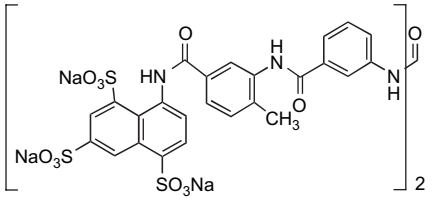


Figure 5. Structure of suramin.

room temperature. The reaction mixture was stirred for 1 h, and then quenched by addition of saturated NH_4Cl solution (20 mL). The resultant mixture was extracted with $CHCl_3$ (30 mL) and the aqueous layer was extracted with $CHCl_3$ (3×60 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate=30/1 to 1/2) afforded (±)-**17** (450 mg, 94%) as a light yellow oil. IR ν_{max} (NaCl): 3151 (COOH), 1739, 1731, 1724 (C=O), 1199, 1141 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.35 (1H, s, 1′-H), 5.75 (1H, s, 1′-H), 3.77 (3H, s, 1-OC*H*$_3$), 3.68 (3H, s, 1″-OC*H*$_3$), 3.50 (1H, t, *J*=7.3 Hz, 3-H), 2.34 (2H, t, *J*=7.2 Hz, 10-H_2), 1.98–1.50 (4H, complex m, 4, 9-H_2), 1.38–1.19 (8H, complex m, 5, 6, 7, 8-H_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 179.5 (C-1‴), 173.8 (C-1″), 166.7 (C-1), 138.3 (C-2), 126.7 (C-1′), 52.1 (COO*C*H$_3$), 52.0 (COO*C*H$_3$), 46.5 (C-3), 32.7 (C-10), 31.2 (C-4), 29.2 (3C, C-6, 7, 8), 27.4 (C-5), 25.6 (C-9). HRMS (FAB, *m*-NBA) *m*/*z*: 323.1457 $[M+Na]^+$; calcd for $C_{15}H_{24}O_6Na$: 323.1471 [M+Na].

4.2.4. *N′-tert-Butyl ester-9,10-dimethoxycarbonyl-undecanohydrazide-10-ene ((±)-**18**)*

To a solution of (±)-**17** (300 mg, 1.0 mmol) in benzene (2.0 mL) was added oxalyl chloride (428 μL, 5.0 mmol) at room temperature. The mixture was then warmed to 80 °C and stirred for 2 h. After stirring, the reaction mixture was cooled to room temperature and concentrated in vacuo to afford crude acid chloride, which was used in the next step without purification. To the solution of crude product in CH_2Cl_2 (15.7 mL) was added the solution of $BocNHNH_2$ (145 mg, 1.1 mmol) and Et_3N (121 μL, 1.1 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C; the resultant mixture being stirred for 30 min. The reaction was then quenched by addition of saturated NH_4Cl solution (5 mL) followed by extraction with $CHCl_3$ (3×10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate=1:1) afforded (±)-**18** (315 mg, 99%) as a yellow oil. IR ν_{max} (NaCl): 3282 (C=O), 2360 (NH), 1704 (C=O), 1205 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.35 (1H, s, 11-H), 5.75 (1H, s, 11-H), 3.77 (3H, s, 1′-OC*H*$_3$), 3.68 (3H, s, 1″-OC*H*$_3$), 3.50 (1H, t, *J*=7.3 Hz, 3-H), 2.21 (2H, t, *J*=7.6 Hz, 2-H_2), 1.95–1.49 (4H, complex m, 3,8-H_2), 1.47 (9H, s, C(*CH*$_3$)$_3$), 1.35–1.15 (8H, complex m, 4, 5, 6, 7-H_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 173.7 (C-1″), 172.6 (C-1), 166.7 (C-1′), 155.7 (–NHNHCOOC(CH$_3$)$_3$), 138.2 (C-10), 126.7 (C-11), 81.5 (–NHNHCOO*C*(CH$_3$)$_3$), 52.1 (2C, COO*C*H$_3$×2), 46.4 (C-9), 33.9 (C-2), 31.0 (C-8), 28.9 (3C, C-4, 5, 6), 28.0 (3C, –NHNHCOOC(*C*H$_3$)$_3$), 27.2 (C-7), 25.1 (C-3); HRMS (FAB, *m*-NBA) *m*/*z*: 415.2430 $[M+H]^+$; calcd for $C_{20}H_{35}N_2O_7$: 415.2444 [M+H].

4.2.5. *3-Carboxy-2-methylene-10-methoxycarbonyldecanic acid [tensyuic acid E, (±)-**3**]*

To a solution of LiOH (14.4 mg, 0.6 mmol) in H_2O (450 μL) was added a solution of (±)-**18** (300 mg, 1.0 mmol) in THF (150 μL) at room temperature and stirred for 5 h. The reaction was quenched by addition of 1 N HCl aq solution (0.3 mL) and extracted with AcOEt (3×5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to furnish crude dicarboxylic acid, which was used in the next step without purification. Crude product was dissolved into TFA (0.2 mL) and the resultant mixture was stirred for 1 h at room temperature. The reaction mixture was then concentrated in vacuo to give crude product, which was subjected to next reaction without further purification. To a solution of crude hydrazide in MeOH (1.0 mL) at room temperature was added CAN (183 mg, 0.334 mmol) and stirred for 30 min. The reaction was quenched by addition of 0.1 N HCl aq solution (0.5 mL). The resultant two layers were separated and the aqueous layer was extracted with AcOEt (3×3 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Preparative HPLC (Senshu Pak-PEGASIL ODS 20∅×250 mm, MeCN/H_2O=35/65, 8.0 mL/min, UV at 210 nm) afforded tensyuic acid E ((±)-**3**) (12 mg, 58%) as a colorless oil. IR ν_{max} (NaCl): 3482 (COOH), 1704 (C=O), 1205 (C–O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.53 (1H, s, 1′-H), 5.82 (1H, s, 1′-H), 3.66 (3H, s, 1‴-OC*H*$_3$), 3.50 (1H, t, *J*=7.2 Hz, 3-H), 2.30 (2H, t, *J*=7.5 Hz, 10-H_2), 2.01–1.67 (2H, m, 4-H), 1.66–1.54 (2H, m, 9-H_2), 1.39–1.21 (8H, complex m, 5, 6, 7, 8-H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 179.1 (C-1″), 174.4 (C-1‴), 171.3 (C-1), 137.2 (C-2), 129.8 (C-1′), 51.4 (C1‴–OC*H*$_3$), 47.3 (C-3), 34.0 (C-10), 29.5 (C-4), 29.0 (C-6, 7, 8), 27.2 (C-5), 24.9 (C-9); HRMS (FAB, *m*-NBA) *m*/*z*: 287.1505 $[M+H]^+$; calcd for $C_{14}H_{23}O_6$: 287.1495 [M+H].

4.3. Total synthesis of tensyuic acids B and C

4.3.1. *Methyl 9-(tert-butyldiphenylsilyloxy)-2-methylene-3-methoxycarbonylnonanate ((±)-**20**)*

The Et_2O solution of Grignard reagent **6**, which was prepared from the corresponding alkyl bromide[24] (2.0 g, 4.78 mmol) by same

procedure for the preparation of **7**, and **8** (561 mg, 2.38 mmol) was converted to (±)-**20** (750 mg, 63%) by same procedure as that described above for the formation of (±)-**15**. IR ν_{max} (NaCl): 1739, 1724 (C=O), 1199, 1141 (C–O), 1110 (C–O–Si) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 7.68 (4H, m, Ar–H), 7.40 (6H, m, Ar–H), 6.36 (1H, s, 1′-H), 5.75 (1H, s, 1′-H), 3.76 (3H, s, 1-OCH_3), 3.68 (3H, s, 1″-OCH_3), 3.64 (2H, t, J=6.4 Hz, 9-H), 3.50 (1H, t, J=7.4 Hz, 3-H), 1.97–1.45 (4H, complex m, 4, 8-H_2), 1.42–1.18 (6H, complex m, 5, 6, 7-H_2), 1.04 (9H, s, SiC(CH_3)$_3$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 173.6 (C-1″), 166.5 (C-1), 138.3 (C-2), 135.4 (2C, Ar×2), 134.0 (Ar), 129.4 (Ar), 127.5 (2C, Ar×2), 126.6 (C-1′), 63.9 (C-9), 52.2 (COOCH_3), 51.8 (COOCH_3), 46.4 (C-3), 32.3 (C-8), 31.1 (C-4), 28.9 (C-6), 27.3 (C-5), 26.8 (3C, SiC(CH_3)$_3$), 25.4 (C-9), 19.1 (SiC(CH$_3$)$_3$); HRMS (FAB, *m*-NBA) *m*/*z*: 519.2557 $[M+Na]^+$; calcd for $C_{29}H_{40}O_5SiNa$: 519.2534 [M+Na].

4.3.2. Methyl 9-hydroxy-2-methylene-3-methoxycarbonylnonanate ((±)-*21*)

TBDPS ether (±)-**20** (700 mg, 1.41 mmol) was converted to (±)-**21** (270 mg, 78%) by the same procedure as that described above for the formation of (±)-**16**. IR ν_{max} (NaCl): 3473 (OH), 1737, 1727 (C=O), 1143 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.34 (1H, s, 1′-H), 5.73 (1H, s, 1′-H), 3.75 (3H, s, 1-OCH_3), 3.66 (3H, s, 1″-OCH_3), 3.60 (2H, t, J=6.6 Hz, 9-H), 3.48 (1H, t, J=7.3 Hz, 3-H), 1.98–1.43 (4H, complex m, 4, 8-H_2), 1.39–1.19 (6H, complex m, 5, 6, 7-H_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 173.8 (C-1″), 166.7 (C-1), 138.3 (C-2), 126.7 (C-1′), 62.8 (C-9), 52.1 (COOCH_3), 52.0 (COOCH_3), 46.5 (C-3), 32.6 (C-8), 31.1 (C-4), 29.0 (C-6), 27.3 (C-5), 25.4 (C-7). HRMS (FAB, *m*-NBA) *m*/*z*: 259.1537$[M+H]^+$; calcd for $C_{13}H_{23}O_5$: 259.1545 [M+H].

4.3.3. Methyl 8-carboxy-2-methylene-3-methoxycarbonyloctanate ((±)-*22*)

Alcohol (±)-**21** (270 mg, 1.05 mmol) was converted to (±)-**22** (245 mg, 85%) by the same procedure as that described above for the formation of (±)-**17**. IR ν_{max} (NaCl): 3151 (COOH), 1737, 1731, 1712 (C=O), 1174, 1143 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.35 (1H, s, 1′-H), 5.74 (1H, s, 1′-H), 3.73 (3H, s, 1-OCH_3), 3.66 (3H, s, 1″-OCH_3), 3.48 (1H, t, J=7.4 Hz, 3-H), 2.32 (2H, t, J=7.3 Hz, 8-H_2), 1.99–1.53 (4H, complex m, 4, 8-H_2), 1.43–1.20 (4H, complex m, 5, 6-H_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 179.2 (C-1″), 173.7 (C-1‴), 166.6 (C-1), 138.2 (C-2), 126.8 (C-1′), 52.2 (COOCH_3), 52.1 (COOCH_3), 46.5 (C-3), 33.8 (C-8), 31.0 (C-4), 28.6 (C-6), 27.1 (C-5), 24.4 (C-7). HRMS (FAB, *m*-NBA) *m*/*z*: 273.1343 $[M+H]^+$; calcd for $C_{13}H_{21}O_6$: 273.1338 [M+H].

4.3.4. N′-tert-Butyl ester-7,8-dimethoxycarbonylnonanohydrazide-8-ene ((±)-*23*)

Carboxylic acid (±)-**22** (245 mg, 0.90 mmol) was converted to (±)-**23** (270 mg, 78%) by the same procedure as that described above for the formation of (±)-**18**. IR ν_{max} (NaCl): 3288 (C=O), 1737, 1731, 1727 (C=O), 1438 (CH), 1201 (C–O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.33 (1H, s, 9-H), 5.72 (1H, s, 9-H), 3.74 (3H, s, 1′-OCH_3), 3.65 (3H, s, 1″-OCH_3), 3.47 (1H, t, J=7.4 Hz, 7-H), 2.18 (2H, t, J=7.5 Hz, 2-H_2), 1.95–1.53 (2H, complex m, 5-H_2), 1.43 (9H, s, C(CH_3)$_3$), 1.40–1.19 (4H, complex m, 3, 4-H_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 173.7 (C-1″), 172.6 (C-1), 166.6 (C-1′), 155.7 (NHNHCOC(CH$_3$)$_3$), 138.2 (C-8), 126.8 (C-9), 81.7 (NHNHCOC(CH$_3$)$_3$), 52.1 (2C, COOCH_3×2), 46.4 (C-7), 33.8 (C-2), 30.9 (C-6), 28.0 (3C, NHNHCOC(CH_3)$_3$), 28.7 (C-4), 27.0 (C-5), 24.9 (C-3). HRMS (FAB, *m*-NBA) *m*/*z*: 409.1936 $[M+Na]^+$; calcd for $C_{18}H_{30}N_2O_7Na$: 409.1951 [M+Na].

4.3.5. 3-Carboxy-2-methylene-8-methoxycarbonyloctanic acid ((±)-tensyuic acid B)

Boc-hydrazide (±)-**23** (245 mg, 0.90 mmol) was converted to (±)-tensyuic acid B (36 mg, 45%) by the same procedure as that described above for the formation of (±)-tensyuic acid E. IR ν_{max} (NaCl): 3482 (COOH), 1737, 1698 (C=O), 1216 (C–O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.53 (1H, s, 1′-H), 5.83 (1H, s, 1′-H), 3.66 (3H, s, 1‴-OCH_3), 3.40 (2H, t, J=8.8 Hz, 3-H_2), 2.30 (2H, t, J=9.2 Hz, 8-H_2), 2.02–1.54 (4H, complex m, 4, 7-H_2), 1.44–1.28 (4H, complex m, 5, 6-H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 179.4 (C-1″), 174.3 (C-1‴), 171.5 (C-1), 137.2 (C-2), 129.9 (C-1″), 51.5 (9-OCH_3), 47.0 (C-3), 33.9 (C-8), 29.5 (C-4), 28.7 (C-6), 27.0 (C-5), 24.6 (C-7). HRMS (FAB, *m*-NBA) *m*/*z*: 259.1192 $[M+H]^+$; calcd for $C_{12}H_{19}O_6$: 259.1182 [M+H].

4.3.6. 3-Carboxy-2-methylene-8-ethoxycarbonyloctanic acid ((±)-tensyuic acid C)

Boc-hydrazide (±)-**23** (245 mg, 0.90 mmol) was converted to (±)-tensyuic acid C (36 mg, 45%) with ethanol by the procedure as that described above for the formation of (±)-tensyuic acid E. IR ν_{max} (NaCl): 3482 (COOH), 1707 (C=O), 1193 (C–O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.51 (1H, s, 1′-H), 5.82 (1H, s, 1′-H), 4.12 (2H, q, J=7.2 Hz, 1‴-OCH_2CH$_3$), 3.38 (2H, t, J=8.2 Hz, 3-H_2), 2.28 (2H, t, J=9.2 Hz, 8-H_2), 2.0–1.67 (2H, m, 4-H), 1.67–1.54 (2H, m, 7-H_2), 1.42–1.29 (4H, complex m, 5, 6-H_2), 1.25 (3H, t, J=10.0 Hz, 9-OCH$_2$CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 179.1 (C-1″), 173.9 (C-1‴), 171.2 (C-1), 137.3 (C-2), 129.6 (C-1″), 60.3 (1″-OOCH_2CH$_3$), 47.2 (C-3), 34.2 (C-8), 29.5 (C-4), 28.7 (C-6), 27.0 (C-5), 24.7 (C-7), 14.2 (1″-OOCH$_2$CH_3). HRMS (FAB, *m*-NBA) *m*/*z*: 273.1339 $[M+H]^+$; calcd for $C_{13}H_{21}O_6$: 273.1338 [M+H].

4.4. In vitro anti-trypanosomal assay

T. brucei brucei strain *GUTat* 3.1 was cultured in IMDM with various supplements containing 10% heat-inactivated FBS at 37 °C, under 5% CO_2/95% air. Ninety five microliters of the trypanosomal suspension (2.0–2.5×10^4 trypanosomes/mL) of the bloodstream form was seeded in a 96-well microplate, and 5 μL of a test compound solution (dissolved in 5% dimethyl sulfoxide, DMSO) was added, followed by incubation for 72 h (long incubation–low inoculation test: LILIT). Ten microliters of the fluorescent dye Alamar Blue was added to each well. After the incubation for 3–6 h, the resulting solution was read at 528/20 nm excitation wavelength and 590/35 nm emission wavelength by an FL×800 fluorescent plate reader (Bio-Tek Instrument, Inc., Vermont, USA). Data were transferred into a spreadsheet program (Excel) and IC_{50} values were determined using fluorescent plate reader software (KC-4, Bio-Tek).

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