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A novel separation technique of diastereomeric esters of pyridylethanols by extraction: formal total synthesis of PNU-142721, HIV-1 reverse transcriptase inhibitor

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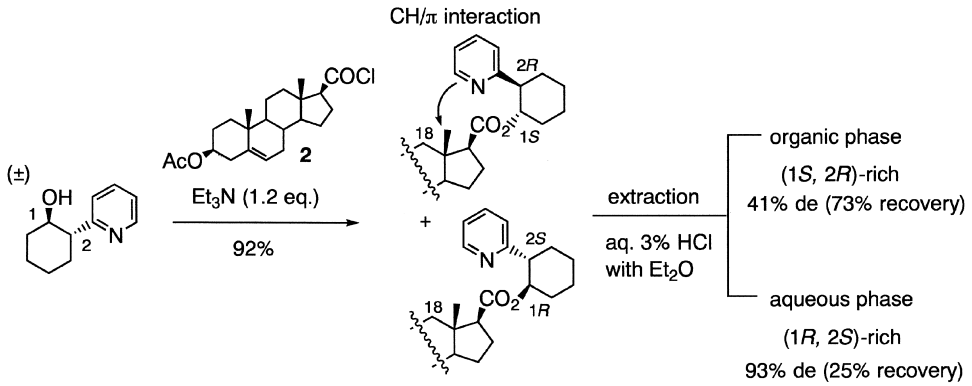
Abstract—The separation of diastereomeric esters derived from (±)-pyridylethanols and 3β-acetoxyetienic acid were achieved by an extraction technique using diethyl ether and aqueous hydrochloric acid. A formal total synthesis of PNU-142721 was effectively carried out to prepare the chiral, non-racemic synthon 1-furo[2,3-*c*]pyridin-5-yl-ethanol (**1**) by means of this technique. The structure optimized using MOPAC calculations on each diastereomer suggested the presence of intramolecular CH/π interaction in only the (*S*)-isomer of the diastereomers.

1. Introduction

We recently disclosed that a diastereomeric mixture derived from (±)-*trans*-2-pyridylcyclohexanols and 3β-acetoxyetienic acid[1] could be separated by a simple extraction procedure using achiral organic media and aqueous acid (Scheme 1).[2] This phenomenon could be assumed by the difference in the pK_a values caused by an intramolecular CH/π interaction[3] in only one of the two diastereomers. This interaction possibly reduces the electron density on the nitrogen atom of the pyridine ring due to the charge transfer character to CH(σ*) from HOMO of the π moiety,[4] or the possibility of the difference in the steric bulkiness around the nitrogen atom in the diastereomers.

Incidentally, (−)-6-chloro-2-[(1-furo[2,3-*c*]pyridin-5-yl-ethyl)thio]-4-pyrimidinamine (PNU-142721) has been announced as an HIV-1 specific, non-nucleoside reverse transcriptase inhibitor[5] and evaluated for its inhibitory activity to the various reverse transcriptases and a panel of mutant RT enzymes etcetera (Fig. 1).[6] The reported synthetic routes for the optically pure PNU-142721 involves



Scheme 1. Diastereomer separation by extraction.

Keywords: Chiral resolution; CH/π interaction; Diastereomers; Extraction; PNU-142721.
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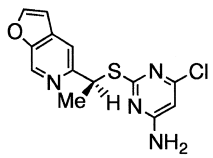


Figure 1.

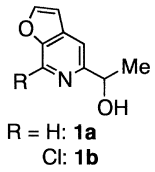
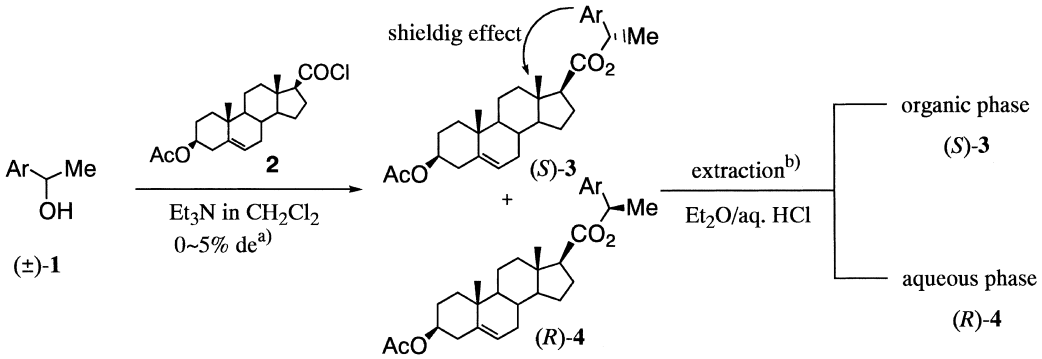


Figure 2.



a) The kinetic resolution was observed in acylation process.
b) Diastereomeric mixture (**3a** and **4a**) / Et_2O / aq. HCl = 300 mg / 20 ml / 50 ml

Scheme 2. The separation of diastereomers derived from pyridylethanols.

an optical resolution of (±)-1-furo[2,3-*c*]pyridin-5-yl-ethanol (**1a**)[5] or (±)-(7-chlorofuro[2,3-*c*]pyridin-5-yl)-ethanol (**1b**)[7] using enzymatic acylation[8] or asymmetric reduction of the corresponding acetylpyridyne[9] as a key step to introduce the chirality to the molecule (Fig. 2). Although these synthetic routes are excellent, the kinetic resolution of (±)-**1a** has somewhat disadvantages such as the use of the expensive acyl-reagent and a long reaction time (9 days).[5] We wish to report here that the concise preparation of both enantiomerically pure (*S*)-**1a** and (*R*)-**1a** using the convenient optical resolution technique of (±)-**1a** via a simple extraction of the corresponding diastereomeric derivatives.

2. Results and discussion

First, we examined whether the separation technique of the diastereomeric isomers can be applied to the diastereomers derived from simple (±)-pyridylethanols (**1c**–**1g**)[10] and 3β-acetoxyetienic acid or not (Scheme 2). We used diethyl ether as the organic phase since other solvents previously tested did not work.[2]

Using the acidic extraction, the diastereomers could be separated with moderate distribution if there is no electron-withdrawing group on the pyridine ring (Table 1, entries 3–13). Thus, we found that this separation technique could be applicable not only to the reported cyclic-type *trans*-(±)-2-(2-pyridyl)cyclohexanols[1] but also to the acyclic-type pyridylethanols such as **1c**–**1g**. However, the separation was low when 1-(4-pyridyl)ethanol (**1e**) was used as the (±)-substrate (Table 1, entries 9 and 10). We assume that this is due to the acid catalyzed racemization of (*R*)-**4e** via a cross-conjugated intermediate during the extraction (Fig. 3).

The absolute configuration of the diastereomer which mainly existed in the organic phase, was the (*S*)-isomer.[11] The shielding effect of the C18–CH_3 on steroid ring was observed by ^{1}H NMR. These spectra corresponded to the free form of **3** and not its HCl salt. The chemical shifts of protons on C18–CH_3 in **3** [δ (ppm) in $CDCl_3$] were **3c**: 0.59; **3d**: 0.51; **3e**: 0.56; **3f**: 0.60; **3g**: 0.60; respectively. On the other hand, the chemical shifts of protons in **4** on C18–CH_3 were δ 0.73 in all cases.[12] These results strongly suggest that our target diastereomers derived from

Table 1. Separation of the diastereomers (**3** and **4**) by extraction

Entry	Ar	aq. HCl (%)	Organic phase		Aqueous phase	
			(*S*)-**3** (%)	de (%)	(*R*)-**4** (%)	de (%)
1	2-Pyridyl: **1c**	3.0	99	3	0	—
2		5.0	97	2	1	43
3		6.0	80	15	17	70
4		7.0	63	34	33	67
5	3-Pyridyl: **1d**	2.0	48	47	42	72
6		3.0	40	73	56	58
7		5.0	18	75	79	20
8	4-Pyridyl: **1e**	1.0	72	19	18	45
9		2.0	39	27	54	3
10		3.0	15	39	75	2
11	2-(6-Methypyridyl): **1f**	5.0	60	54	30	71
12		6.0	63	48	30	85
13		7.0	38	61	58	31
14	2-(6-Brompyridyl): **1g**	7.0	97	6	0	—
15		15.0	95	2	0	—
16		30.0	99	3	0	—
17	2-Furo[2,3-*c*]pyridyl: **1a**	3.0	77	34	18	87
18		5.0	39	91	57	49
19		7.0	37	95	54	53

The de were determined by ^{1}H NMR (270 MHz).

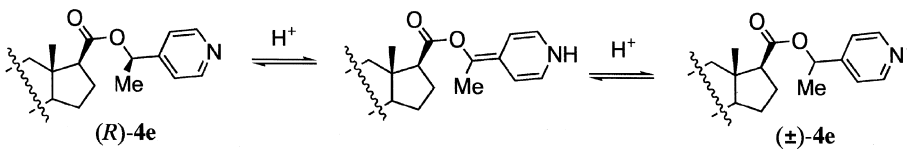


Figure 3.

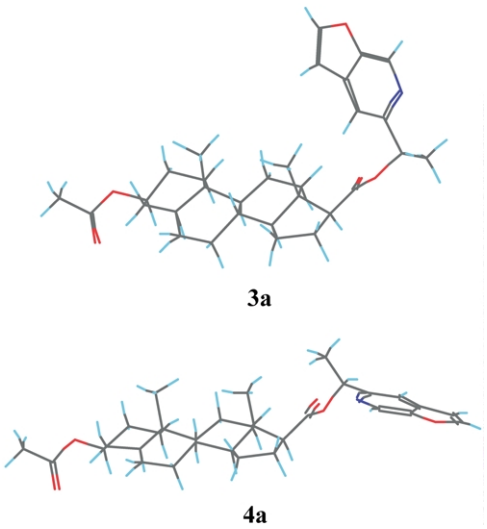


Figure 4.

(±)-furo[2,3-*c*]pyridin-5-yl-ethanol, which has no electron-withdrawing group on the pyridine ring, could also be separated in a similar manner. In fact, we separated the corresponding diastereomers with higher efficiency than we expected (Table 1, entries 17–19).

The ideal concentration of aq. HCl was ca. 5.0–7.0 wt% for separation of **1a** (Table 1, entries 18 and 19). The structure optimized with MOPAC[13] of **3a** suggested the presence of an intramolecular CH/π interaction (Fig. 4). The shortest distance between the C18–CH_3 and the π moiety was 2.82 Å which is shorter than the sum of the each van der Waals radius.[14] We assume that this interaction may be fixing the conformation to the *ap* plane[15] of the chiral esters, thus suggesting that it maybe superior to the steric effect between the C18–CH_3 and the π system. In particular, the spread π plane of furopyridyl ring acts as a factor of stabilizing the interaction.

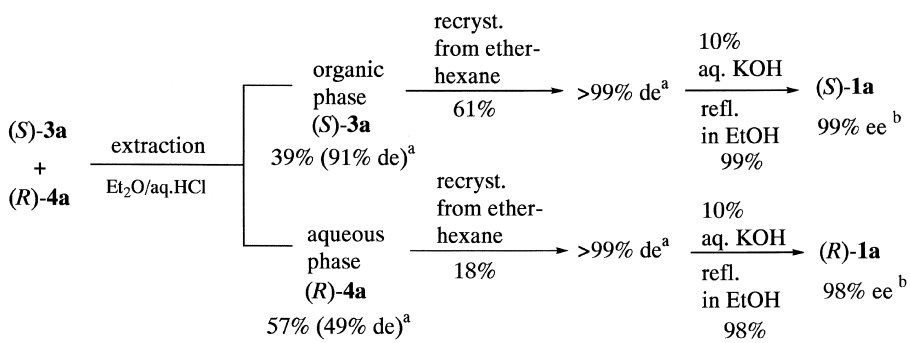
As shown in Table 2, the ^{1}H NMR spectrum of **3a** and **4a** in diethyl ether-D10 showed an enhanced shielding effect on the C18–CH_3 than when using $CDCl_3$, hence the presumed major conformation of **3a** in diethyl ether appears to be close to MO calculation as in Figure 4.

After the extraction, the diastereomeric excess of both (*S*)-**3a** and (*R*)-**4a** can be further enhanced by recrystallization from diethyl ether/*n*-hexane to >99% de (Scheme 3). Cleavage of the 3β-acetoxyetienic acid with aqueous potassium hydroxide, then provides both of **1a** for use in the synthesis of optically pure PNU-142721.

The synthesis of PNU-142721, however, was attempted without recrystallization at the diastereomer-resolution stage on the basis of information that it could be obtained optically pure by recrystallization at the end of the synthesis.[5] The route is shown in Scheme 4. In this case, the distribution of the crude diastereomers was somewhat different from the former case. This maybe due to the change of the acidity of aq. HCl by the presence of an excess of acyl reagent 3β-acetoxyetienic acid, which remained in the reaction mixtures. The (*S*)-furo[2,3-*c*]pyridin-5-yl-ethanol (70% ee) obtained from the organic phase after

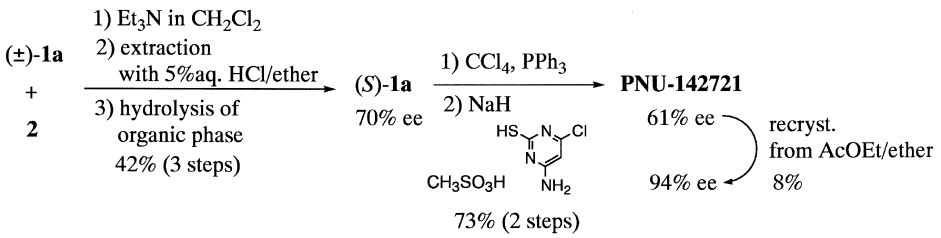
Table 2. Chemical shift values of C18–CH_3 in ^{1}H NMR at room temperature

Solvent	Chemical shift of protons on C18–CH_3 in **3a** [δ (ppm)]	Chemical shift of protons on C18–CH_3 in **4a** [δ (ppm)]	Difference of chemical shift between **3a** and **4a** [δ (ppm)]
CDCl3	0.54	0.73	0.19
$(C_2D_5)_2O$	0.53	0.76	0.23



a) The de was determined by ^{1}H NMR; b) The ee was determined by HPLC (CHIRAL CEL OD®).

Scheme 3. Optical resolution of (±)-**1a** by extraction.



Scheme 4. An alternative route for PNU-142721.

alkaline hydrolysis was converted to PNU-142721 (61% ee) according to the reported method.[5] A single recrystallizing of the crude product from ethyl acetate/ether gave PNU-142721 with 94% ee (Scheme 4). This synthetic route has the benefit that there is no troublesome purifications such as recrystallization or column chromatography throughout the derivation from (±)-**1a** to the crude PNU-142721.

To conclude, we have shown a novel optical resolution technique of (±)-pyridylethanols by a simple extraction method using an achiral-organic solvent and acidic solution. Some features of this optical resolution technique by extraction are summarized as follows. (1) The absolute configurations of the resolved pyridylethanols can be easily determined because of the fact that the (*S*)-isomer showing the shielding effect of the C18–CH_3 of the π system exists in the organic phase upon extraction. (2) The diastereomeric purity of pyridylethanol derivatives following the acidic extraction can be further enriched by a single recrystallization. (3) It does not require a long reaction time to obtain the chiral alcohols. (4) The target compound PNU-142721 can be prepared with only one recrystallization of the final product.

We believe that this technique will play an important role in a large-scale synthesis of similar optically active pyridylethanols.

3. Experimental

3.1. General information

Melting points are taken with a micro hot-stage apparatus (Yanagimoto) and are uncorrected. Infrared (IR) absorption spectra were recorded with a SHIMADZU FTIR-8400 spectrometer as a KBr or a NaCl pellet. ^{1}H NMR spectra were measured on a JEOL JNM-EX270 or a JEOL JNM-AL300 spectrometers with $SiMe_4$ as the internal standard in $CDCl_3$. Mass spectra (MS) were determined on a JEOL JMS-AMII50, a JEOL JMS-700 or a JEOL JMS-600H mass spectrometer. Chiral HPLC analyses were performed with a JASCO Gulliver series PU-986, MD-910 and CO-1560 or 2060 using a Daicel chiral column (Daicel Chiralcel OD, OD-H or OJ). Specific rotations were measured by JASCO P-1020 polarimeter. Kanto Chemical Silica Gel 60 N (spherical, neutral) and Fuji Silysia Chemical silica gel BW-300 were used for flash column chromatography, respectively. (±)-1-(2-Furo[2,3-*c*]pyridyl)ethanol (**1a**),[5] (±)-1-pyridylethanols (**1c**–**g**),[10] 3β-acetoxy-5-etienic acid chloride (**2**)[1] and 4-amino-6-chloro-2-thiopyrimidine mesylate salt[5] were essentially prepared by the reported method.

3.1.1. (±)-1-(2-Furo[2,3-*c*]pyridyl)ethanol (1a).[5] Yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.48 (3H, d, *J*=6.5 Hz, C*H*$_3$), 4.06 (1H, br, O*H*), 4.93 (1H, q, *J*=6.5 Hz, C*H*), 6.73 (1H, d, *J*=1.9 Hz, furan-*H*), 7.46 (1H, s, pyridine-*H*), 7.70 (1H, d, *J*=1.9 Hz, furan-*H*), 8.72 (1H, s, pyridine-*H*); IR (NaCl): ν_{max}/cm^{-1} 3360 (OH).

3.1.2. (±)-1-(2-Pyridyl)ethanol (1c).[10a–c] Yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.51 (3H, d, *J*=6.6 Hz, C*H*$_3$), 4.24 (1H, br, O*H*), 4.90 (1H, q, *J*=6.6 Hz, C*H*), 7.20 (1H, ddd, *J*=7.5, 5.0, 0.6 Hz, pyridine-*H*), 7.28 (1H, ddd, *J*=7.9, 0.6, 0.2 Hz, pyridine-*H*), 7.69 (1H, td, *J*=7.7, 1.8 Hz, pyridine-*H*), 8.54 (1H, dd, *J*=5.0, 1.8 Hz, pyridine-*H*); IR (KBr): ν_{max}/cm^{-1} 3250 (OH).

3.1.3. (±)-1-(3-Pyridyl)ethanol (1d).[10a,b] Yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.54 (3H, d, *J*=6.2 Hz, C*H*$_3$), 2.21 (1H, br, O*H*), 4.97 (1H, q, *J*=6.2 Hz, C*H*), 7.29 (1H, dd, *J*=7.8, 4.7 Hz, pyridine-*H*), 7.74 (1H, d, *J*=7.8 Hz, pyridine-*H*), 8.51 (1H, dd, *J*=4.7, 1.3 Hz, pyridine-*H*), 8.59 (1H, d, *J*=1.7 Hz, pyridine-*H*); IR (KBr): ν_{max}/cm^{-1} 3202 (OH); MS (EI$^+$): *m*/*z* (%) 123 (M$^+$, 17), 108 (100).

3.1.4. (±)-1-(4-Pyridyl)ethanol (1e).[10a] Colourless oil; ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.48 (3H, d, *J*=6.5 Hz, C*H*$_3$), 3.01 (1H, br, O*H*), 4.88 (1H, q, *J*=6.5 Hz, C*H*), 7.29 (2H, d, *J*=4.6 Hz, pyridine-*H*), 8.41 (2H, d, *J*=4.6 Hz, pyridine-*H*); HRMS (EI$^+$) calcd for C_7H_9NO (M$^+$): 123.0684, found: 123.0694.

3.1.5. (±)-1-[2-(6-Methylpyridyl)]ethanol (1f).[10d] Yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.48 (3H, d, *J*=6.2 Hz, C*H*$_3$), 2.55 (3H, s, pyridine-6′-C*H*$_3$), 4.65 (1H, br, O*H*), 4.86 (1H, q, *J*=6.2 Hz, C*H*), 7.03 (1H, d, *J*=2.2 Hz, pyridine-*H*), 7.07 (1H, d, *J*=2.2 Hz, pyridine-*H*), 7.56 (1H, t, *J*=7.6 Hz, pyridine-*H*); IR (NaCl): ν_{max}/cm^{-1} 3376 (OH).

3.1.6. (±)-1-[2-(6-Bromopyridyl)]ethanol (1g).[10d] Colourless oil; ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.50 (3H, d, *J*=6.5 Hz, C*H*$_3$), 3.45 (1H, br, O*H*), 4.88 (1H, q, *J*=5.5 Hz, C*H*), 7.33 (1H, d, *J*=7.4 Hz, pyridine-*H*), 7.38 (1H, d, *J*=7.4 Hz, pyridine-*H*), 7.55 (1H, t, *J*=7.7 Hz, pyridine-*H*); IR (NaCl): ν_{max}/cm^{-1} 3391 (OH).

3.1.7. 3β-Acetoxyetienic acid chloride[8] **(2).** Colourless solid; mp 193–195 °C (dec.); ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.82 (3H, s, 18β-C*H*$_3$), 0.83–2.59 (19H, m, steroid ring), 1.06 (3H, s, 19β-C*H*$_3$), 1.98 (3H, s, 3β-OCOC*H*$_3$), 2.88 (1H, m, 17α-*H*), 4.81 (1H, m, 3α-*H*), 5.43 (1H, m, 6-olefin-*H*); IR (KBr): ν_{max}/cm^{-1} 1784 (C=O).

3.1.8. 4-Amino-6-chloro-2-thiopyrimidine mesylate salt.[5,6b] Colourless crystal; mp 166.0–167.0 °C (Et_2O); ^1H NMR (270 MHz, $CDCl_3$): δ_H 2.40 (3H, s, CH_3SO_3H), 3.55 (1H, br, 2-S*H*), 4.89 (2H, br, 4-NH_2), 6.23 (1H, s, 5-*H*), 1H of methanesulfonic acid was not observed; HRMS (EI^+) calcd for $C_4H_4N_3SCl$ (M^+): 160.9814, found: 160.9813.

3.1.9. Typical procedure for esterification of 1a, c–g. To a stirred solution of 3β-acetoxyetienic acid (2.56 g, 7.1 mmol) in C_6H_6 (20 ml) under nitrogen atmosphere was added oxalyl chloride (3.60 ml, 40.8 mmol) and the solution was stirred for 2 h at room temperature. The acid chloride **2** was obtained as a white crystal after removal C_6H_6 in vacuo. To a solution of **2** in CH_2Cl_2 (10 ml) under nitrogen atmosphere and shielded from light, were added Et_3N (1.00 ml, 7.2 mmol) and **1a** (1.00 g, 6.1 mmol) in CH_2Cl_2 (10 ml) at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was filtered. The filtrate was washed with saturated $NaHCO_3$ aq. and water, dried over Na_2CO_3, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluent: hexane/AcOEt=3:1) to give **3a** and **4a** (2.41 g, 78%) as a diastereomeric mixture.

3.1.10. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-(2-furo[2,3-*c*]pyridyl)ethyl ester (3a and 4a). Colourless solid; mp 126.5–127.0 °C; IR (KBr): ν_{max}/cm^{-1} 1727 (C=O); HRMS (EI^+) calcd for $C_{31}H_{39}NO_5$ (M^+): 505.2828, found: 505.2827.

Compound (*S*)-**3a**. Mp 127.0–127.5 °C (Et_2O); ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.47 (3H, s, 18β-CH_3), 0.91 (3H, s, 19β-CH_3), 1.05–2.39 (20H, m, steroid ring), 1.57 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.02 (3H, s, 3β-OCOCH_3), 4.53 (1H, m, 3α-*H*), 5.30 (1H, m, 6-olefine-*H*), 5.98 (1H, m, 17β-CO_2C*H*CH_3), 6.73 (1H, d, *J*=7.6 Hz, furan-*H*), 7.55 (1H, s, pyridine-*H*), 7.69 (1H, d, *J*=7.6 Hz, furan-*H*), 8.77 (1H, s, pyridine-*H*).

Compound (*R*)-**4b**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.70 (3H, s, 18β-CH_3), 0.96 (3H, s, 19β-CH_3), 1.05–2.39 (20H, m, steroid ring), 1.58 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.02 (3H, s, 3β-OCOCH_3), 4.53 (1H, m, 3α-*H*), 5.30 (1H, m, 6-olefine-*H*), 5.98 (1H, m, 17β-CO_2C*H*CH_3), 6.73 (1H, d, *J*=7.6 Hz, furan-*H*), 7.55 (1H, s, pyridine-*H*), 7.69 (1H, d, *J*=7.6 Hz, furan-*H*), 8.77 (1H, s, pyridine-*H*).

3.1.11. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-(2-pyridyl)ethyl ester (3c and 4c).[2b] Colourless solid; mp 44.5–51.0 °C; IR (KBr): ν_{max}/cm^{-1} 1732 (C=O); MS (EI^+) *m*/*z* (%): 465 (M^+, 1), 405 (100); HRMS (EI^+) calcd for $C_{29}H_{39}NO_4$ (M^+): 465.2879, found: 465.2885.

Compound (1*S*)-**3c**. ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.59 (3H, s, 18β-CH_3), 1.00 (3H, s, 19β-CH_3), 1.18–2.33 (19H, m, steroid ring), 1.60 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.03 (3H, s, 3β-OCOCH_3), 2.43 (1H, dd, *J*=17.8, 9.0 Hz, 17α-*H*), 4.60 (1H, m, 3α-*H*), 5.37 (1H, m, 6-olefin-*H*), 5.93 (1H, q, *J*=6.5 Hz, 17β-CO_2C*H*CH_3), 7.19 (1H, ddd, *J*=7.5, 4.9, 1.2 Hz, pyridine-*H*), 7.38 (1H, dd, *J*=7.9, 1.2 Hz, pyridine-*H*), 7.68 (1H, td, *J*=7.7, 1.7 Hz, pyridine-*H*), 8.58 (1H, ddd, *J*=4.9, 1.7, 0.9 Hz, pyridine-*H*).

Compound (1*R*)-**4c**. ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.73 (3H, s, 18β-CH_3), 1.03 (3H, s, 19β-CH_3), 1.18–2.33 (19H, m, steroid ring), 1.60 (3H, d, *J*=6.7 Hz, 17β-CO_2CHCH_3), 2.03 (3H, s, 3β-OCOCH_3), 2.43 (1H, dd, *J*=17.8, 9.0 Hz, 17α-*H*), 4.60 (1H, m, 3αH), 5.38 (1H, d, *J*=4.2 Hz, 6-olefine-*H*), 5.95 (1H, q, *J*=6.7 Hz, 17β-CO_2C*H*CH_3), 7.19 (1H, ddd, *J*=7.5, 4.9, 1.3 Hz, pyridine-*H*), 7.36 (1H, d, *J*=7.9 Hz, pyridine-*H*), 7.67 (1H, td, *J*=7.7, 1.7 Hz, pyridine-*H*), 8.57 (1H, ddd, *J*=4.9, 1.7, 0.9 Hz, pyridine-*H*).

3.1.12. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-(3-pyridyl)ethyl ester (3d and 4d). Colorless amorphous; IR (KBr): ν_{max}/cm^{-1} 1732 (C=O); MS (FAB^+) *m*/*z* (%): 466 (MH^+), 106 (100); HRMS (FAB^+) calcd for $C_{29}H_{40}NO_4$ (MH^+): 466.2958, found: 466.2943.

Compound (*S*)-**3d**. ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.51 (3H, s, 18β-CH_3), 0.96–2.19 (18H, m, steroid ring), 0.98 (3H, s, 19β-CH_3), 1.57 (3H, d, *J*=6.8 Hz, 17β-CO_2-CHCH_3), 2.03 (3H, s, 3β-OCOCH_3), 2.32–2.41 (3H, m, 17α-*H* and steroid ring), 4.61 (1H, m, 3α-*H*), 5.36 (1H, d, *J*=5.1 Hz, 6-olefine-*H*), 5.91 (1H, q, *J*=6.8 Hz, 17β-CO_2-C*H*CH_3), 7.27 (1H, m, pyridine-*H*), 7.69 (1H, ddd, *J*=8.1, 2.1, 1.6 Hz, pyridine-*H*), 8.54 (1H, dd, *J*=4.8, 1.6 Hz, pyridine-*H*), 8.66 (1H, d, *J*=2.1 Hz, pyridine-*H*).

Compound (*R*)-**4d**. ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.71 (3H, s, 18β-CH_3), 0.96–2.19 (18H, m, steroid ring), 1.03 (3H, s, 19β-CH_3), 1.57 (3H, d, *J*=6.7 Hz, 17β-CO_2-CHCH_3), 2.04 (3H, s, 3β-OCOCH_3), 2.32–2.41 (3H, m, 17α-*H* and steroid ring), 4.61 (1H, m, 3α*H*), 5.38 (1H, d, *J*=5.0 Hz, 6-olefine-*H*), 5.94 (1H, q, *J*=6.7 Hz, 17β-CO_2-C*H*CH_3), 7.27 (1H, m, pyridine-*H*), 7.67 (1H, ddd, *J*=7.9, 2.0, 1.7 Hz, pyridine-*H*), 8.54 (1H, dd, *J*=4.7, 1.7 Hz, pyridine-*H*), 8.63 (1H, d, *J*=2.0 Hz, pyridine-*H*).

3.1.13. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-(4-pyridyl)ethyl ester (3e and 4e). Colorless amorphous; IR (KBr): ν_{max}/cm^{-1} 1732 (C=O); HRMS (CI^+) calcd for $C_{29}H_{39}NO_4$: 465.2879, found: 465.2876.

Compound (*S*)-**3e**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.56 (3H, s, 18β-CH_3), 1.10–2.44 (20H, m, steroid ring), 1.00 (3H, s, 19β-CH_3), 1.57 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.03 (3H, s, 3β-OCOCH_3), 4.62 (1H, m, 3α-*H*), 5.37 (1H, d, *J*=5.0 Hz, 6-olefine-*H*), 5.84 (1H, m, 17β-CO_2C*H*CH_3), 7.26 (2H, m, pyridine-*H*), 8.58 (2H, m, pyridine-*H*).

Compound (*R*)-**4e**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.72 (3H, s, 18β-CH_3), 1.10–2.44 (20H, m, steroid ring), 1.03 (3H, s, 19β-CH_3), 1.54 (3H, d, *J*=6.5 Hz, 17β-CO_2-CHCH_3), 2.04 (3H, s, 3β-OCOCH_3), 4.62 (1H, m, 3α-*H*), 5.37 (1H, d, *J*=5.0 Hz, 6-olefine-*H*), 5.84 (1H, m, 17β-CO_2C*H*CH_3), 7.26 (2H, m, pyridine-*H*), 8.58 (2H, m, pyridine-*H*).

3.1.14. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-[2-(6-methyl-pyridyl)]ethyl ester (3f and 4f). Colourless solid; mp 57.0–58.0 °C; IR (KBr): ν_{max}/cm^{-1} 1732 (C=O); HRMS (CI^+) calcd for $C_{29}H_{39}NO_4$ (M^+): 479.3036, found: 479.3036.

Compound (*S*)-**3f**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.60 (3H, s, 18β-CH_3), 0.96 (3H, s, 19β-CH_3), 1.10–2.48 (20H, m, steroid ring), 1.56 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.04 (3H, s, 3β-OCOCH_3), 2.54 (3H, s, pyridine-6′-CH_3), 4.61 (1H, m, 3α-*H*), 5.37 (1H, m, 6-olefine-*H*), 5.90 (1H, m, 17β-CO_2C*H*CH$_3$), 7.04 (1H, d, *J*=7.8 Hz, pyridine-*H*), 7.17 (1H, d, *J*=7.8 Hz, pyridine-*H*), 7.57 (1H, t, *J*=7.8 Hz, pyridine-*H*).

Compound (*R*)-**4f**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.73 (3H, s, 18β-CH_3), 1.00 (3H, s, 19β-CH_3), 1.10–2.48 (20H, m, steroid ring), 1.58 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.05 (3H, s, 3β-OCOCH_3), 2.54 (3H, s, pyridine-6′-CH_3), 4.61 (1H, m, 3α-*H*), 5.37 (1H, m, 6-olefine-*H*), 5.90 (1H, m, 17β-CO_2C*H*CH$_3$), 7.04 (1H, d, *J*=7.8 Hz, pyridine-*H*), 7.17 (1H, d, *J*=7.8 Hz, pyridine-*H*), 7.57 (1H, t, *J*=7.8 Hz, pyridine-*H*).

3.1.15. 3β-Acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthrene-17-carboxylic acid 1-[2-(6-bromo-pyridyl)]ethyl ester (3g and 4g). Colourless solid; mp 56.0–57.0 °C; IR (KBr): ν_{max}/cm^{-1} 1732 (C=O); HRMS (CI^+) calcd for $C_{29}H_{38}BrNO_4$ (M^+): 543.1984, found: 543.1987.

Compound (*S*)-**3g**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.60 (3H, s, 18β-CH_3), 1.02 (3H, s, 19β-CH_3), 1.06–2.46 (20H, m, steroid ring), 1.58 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.04 (3H, s, 3β-OCOCH_3), 4.61 (1H, m, 3α-*H*), 5.38 (1H, m, 6-olefine-*H*), 5.88 (1H, m, 17β-CO_2C*H*CH$_3$), 7.32 (1H, d, *J*=7.6 Hz, pyridine-*H*), 7.39 (1H, d, *J*=7.6 Hz, pyridine-*H*), 7.57 (1H, m, pyridine-*H*).

Compound (*R*)-**4g**. ^1H NMR (270 MHz, $CDCl_3$): δ_H 0.73 (3H, s, 18β-CH_3), 1.04 (3H, s, 19β-CH_3), 1.06–2.46 (20H, m, steroid ring), 1.60 (3H, d, *J*=6.5 Hz, 17β-CO_2CHCH_3), 2.04 (3H, s, 3β-OCOCH_3), 4.61 (1H, m, 3α-*H*), 5.38 (1H, m, 6-olefine-*H*), 5.88 (1H, m, 17β-CO_2C*H*CH$_3$), 7.32 (1H, d, *J*=7.6 Hz, pyridine-*H*), 7.39 (1H, d, *J*=7.6 Hz, pyridine-*H*), 7.57 (1H, m, pyridine-*H*).

3.2. Typical procedure for extraction

To a solution of **3a** and **4a** (300 mg, 1:1 of diastereomeric mixture) in Et_2O (50 ml) was added aq. 5.0 wt% HCl (20 ml, diluted 36% HCl with dist. H_2O). After vigorous shaking, the ethereal solution was separated from the aqueous layer, dried over Na_2CO_3 and filtered. The filtrate was concentrated in vacuo to give **3a** in 39% yield with 91% de. The aqueous layer was made alkaline with $NaHCO_3$ (pH 8) to precipitate a white solid. The precipitation was collected by suction filtration and dried to give **4a** in 57% yield with 49% de.

3.3. General procedure for alkaline hydrolysis of 3a or 4a

3a (>99% de, 135 mg, 0.27 mmol), which was obtained from the ethereal layer of extraction described above and purified by recrystallization from ether, was dissolved in EtOH (25 ml). To this alcohol solution was added 10% KOH aq. (21 ml). After refluxing for 4 h, the reaction mixture was diluted with dist. H_2O (22 ml). The alkaline aqueous mixture was extracted with four portions of Et_2O. The ethereal layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2CO_2, filtered and concentrated to give (*S*)-1-[2-furo[2,3-*c*]pyridyl]ethanol [(*S*)-**1a**, 43 mg, 99%, 99% ee; Chiral HPLC analysis [Daicel Chiralcel OD; 0.5 ml/min; hexane/^{i}PrOH=95:5; 25 °C, ^{t}*R*: 32.4 min]].

3.3.1. (*S*)-1-(2-Furo[2,3-*c*]pyridyl)ethanol [(*S*)-1a].[5] Yellow oil; $[\alpha]_D^{26}$ −29.2 (*c* 0.94, $CHCl_3$); 99% ee [chiral HPLC analysis (Daicel Chiralcel OD, hexane/^{i}PrOH=95:5, flow rate: 0.5 ml/min, 25 °C, ^{t}*R*: 32.4 min)].

3.3.2. (*R*)-1-(2-Furo[2,3-*c*]pyridyl)ethanol [(*R*)-1a].[5] Yellow oil; $[\alpha]_D^{25}$ +20.1 (*c* 0.86, $CHCl_3$); 98% ee [chiral HPLC analysis (Daicel Chiralcel OD, hexane/^{i}PrOH=99:1, flow rate: 0.5 ml/min, 25 °C, ^{t}*R*: 49.3 min)].

3.4. Optical resolution of (±)-1a by extraction technique and synthesis of PNU-142721

The diastereomeric mixture of **3a** and **4a** (3.21 g) was separated by the extraction technique descried above [Et_2O: 568 ml, aq. 5.0 wt% HCl: 247 ml (diluted 36% HCl with dist. H_2O)] and 80% de of **3a** (1.49 g) was obtained from the ethereal layer. The steroid moiety of **3a** was removed by the alkaline hydrolysis (EtOH: 278 ml, 10% KOH aq.: 223 ml) to form (*S*)-**1a** (0.43 g, 39% for 3 steps, 70% ee).

To a solution of (*S*)-**1a** (70% ee, 0.41 g, 2.5 mmol) in $CHCl_3$ (1.60 ml) under nitrogen atmosphere was added the solution of triphenylphosphine (1.34 g, 5.0 mmol) in carbon tetrachloride (4.8 ml) and the resulting mixture was stirred for 26 h. Hexane (1.6 ml) was added and the reaction mixture was continued to stir for 1 h. The generated precipitate was removed by succession filtration and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (eluent: hexane/AcOEt=4:1) to give (+)-(*R*)-5-(1-chloroethyl)furo[2,3-*c*]pyridine [0.19 g, 42% from (*S*)-**1a**] as a colourless oil.

To a suspension of sodium hydride (90 mg, 3.8 mmol), which was washed with hexane, in DMF (3 ml) was added 4-amino-6-chloro-2-mercaptopyrimidine mesylate salt (280 mg, 1.1 mmol) at 0 °C under nitrogen atmosphere. The DMF solution was then stirred at room temperature for 1 h. To this solution was added a solution of (+)-(*R*)-5-(1-chloroethyl)furo[2,3-*c*]pyridine (190 mg, 1.1 mmol) in DMF (5 ml). After stirred for 5 days, the resulting mixture was diluted with AcOEt (25 ml), washed with 50% NaCl aq., dried over $K_2CO_3/MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (eluent: hexane/AcOEt=1:1) to give (*S*)-6-chloro-2-[[1-(furo[2,3-*c*]pyridin-5-yl)ethyl]thio]-4-pyrimidinamine

(PNU-142721) [240 mg, 73% from (+)-(*R*)-5-(1-chloroethyl)furo[2,3-*c*]pyridine, 61% ee] as a white crystal. This crystal was purified by recrystallization from AcOEt/Et_2O to give optically pure PNU-142721 (18 mg, 8% from low optical purity of PNU-142721, 94% ee).

3.4.1. (*R*)-5-(1-Chloroethyl)furo[2,3-*c*]pyridine.[5] Colourless oil; ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.94 (3H, d, J=6.8 Hz, CH_3), 5.30 (1H, q, J=6.8 Hz, C*H*), 6.80 (1H, s, pyridyl-*H*), 7.72 (d, J=1.9 Hz, furan-*H*), 7.76 (1H, d, J=1.9 Hz, furan-*H*), 8.83 (1H, s, pyridyl-*H*).

3.4.2. (*S*)-6-Chloro-2-[[1-(furo[2,3-*c*]pyridin-5-yl)ethyl]thio]-4-pyrimidinamine (PNU-142721).[5] Colourless crystal; 146.0–147.0 °C (AcOEt/Et_2O); ^{1}H NMR (270 MHz, DMSO-d_6): δ_H 1.72 (3H, d, J=6.8 Hz, CH_3), 5.12 (1H, q, J=6.8 Hz, C*H*), 6.16 (1H, s, pyridyl-*H*), 7.04 (1H, d, J=2.2 Hz, furan-*H*), 7.34 (2H, br, NH_2), 7.81 (1H, s, pyrimidyl-*H*), 8.23 (1H, d, J=2.2 Hz, furan-*H*), 8.91 (1H, s, pyridyl-*H*); IR (KBr): ν_{max}/cm^{-1} 3308 and 3152 (NH); $[\alpha]_D^{26}$ −280.6 (*c* 0.34, $CHCl_3$); 94% ee [chiral HPLC analysis (Daicel Chiralcel OD, hexane/^{i}PrOH=75:25, flow rate: 0.5 ml/min, 25 °C, ^{t}*R*: 33.6 min)].

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