Syntheses of Potential Metabolites of a Potent κ-Opioid Receptor Agonist, TRK-820

Kuniaki KAWAMURA,* Hiromasa HORIKIRI, Jun HAYAKAWA, Chie SEKI, Ken-ichi YOSHIZAWA, Hideo UMEUCHI, and Hiroshi NAGASE

Pharmaceutical Research Laboratories, Toray Industries, Inc.; 1111 Tebiro, Kamakura, Kanagawa 248–8555, Japan.
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Chemical syntheses of three kinds of potential metabolites of TRK-820, a potent κ-opioid receptor agonist, were described. One of the potential metabolites 2, 17-*N*-dealkylated TRK-820, was synthesized starting from noroxycodone through 8 steps in 21% total yield. Glucuronidation of intermediate 10 and compound 1, the free base of TRK-820, was carried out stereoselectively to give 3-*O*-β-D-glucuronides 15 and 16 in good yields, respectively. Syntheses of potential conjugated metabolites 3 and 4 were accomplished through 10 steps and 2 steps in 11% and 43% total yields, respectively. Among the potential metabolites of TRK-820, compounds 2 and 4 were identified as metabolites in human hepatocytes. The results of pharmacological studies of compounds 2, 3, and 4 are described.

Key words κ-opioid receptor agonist; TRK-820; metabolite; glucuronide

We have described the design and synthesis of (*E*)-*N*-[17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide monohydrochloride, TRK-820 (Fig. 1), and its pharmacological activity.[1)] TRK-820 exhibited a high κ-opioid agonistic activity and effective antipruritic actions in histamine resistant pruritus models using mice.[2,3)] Now clinical trials of TRK-820 are in progress (Phase III in Europe and Phase IIb in Japan) as an antipruritic agent for uraemic pruritus.

In order to investigate pharmacokinetics of TRK-820, it is necessary to synthesize potential metabolites of TRK-820 as standard materials. In general, 17-*N*-dealkylation and conjugation (3-*O*-β-D-glucuronide formation) are well known as major metabolic pathways for detoxification in 4,5-epoxymorphinans.[4)] Therefore, we supposed three kinds of metabolites **2**, **3**, and **4** of TRK-820 would occur in metabolism (Fig. 2). It is also important to investigate whether these compounds are active or inactive metabolites in pharmaceutical research. Herein we wish to report the syntheses of compounds **2**, **3**, and **4**, and the results of their pharmacological studies and *in vitro* metabolism on TRK-820.

Results and Discussion

Noroxycodone **5**, which is a 17-*N*-dealkylated derivative with a 14β-hydroxy-4,5α-epoxymorphinan skeleton, was used as the starting material to synthesize compound **2** (Chart 1). Protection of the 17-amino group in noroxycodone **5** as a *tert*-butoxycarbonyl group (Boc), followed by reductive amination of the resulting compound **6** with *N*-benzylmethylamine using $NaBH_3CN$[5)] gave compound **7** in 72% yield (2 steps). Stereochemistry of the newly introduced amino group was elucidated to be 6β-configuration by ^{1}H-NMR spectral analysis of the 5-proton, which was observed at $\delta = 4.75$ with an axial–axial coupling of 7.1 Hz between the 5- and 6-protons. Compound **7** was converted into amide **8** by debenzylation under hydrogenolysis conditions, followed by acylation with (*E*)-3-(3-furyl)acryloyl chloride in 61% yield (2 steps). The next step was deprotection of the 3-*O*-methyl and Boc groups of compound **8** to obtain the first target compound **2**. For this purpose, the 17-*N*-Boc group of compound **8** was first converted to a 9*H*-fluorenylmethoxycarbonyl group (Fmoc) in 96% (2 steps). When compound **6** was directly treated with 1.0 M BBr_3 for removal of the 3-*O*-methyl and 17-*N*-Boc groups, the desired compound was obtained in *ca.* 50% yield concomitant with a significant amount of a 17-*N*-methylated compound due to side reaction with the resulting methyl bromide under the reaction conditions. Thus, in the case of compound **8**, we had to replace the 17-*N*-Boc group with a 17-*N*-Fmoc group which is stable in Lewis acidic conditions. The reason why an Fmoc group was not used in the first protection step was due to instability of the Fmoc group in the presence of intramolecular basic amine at the 6-position (6-N(CH_3)Bn or 6-NHCH_3) in the

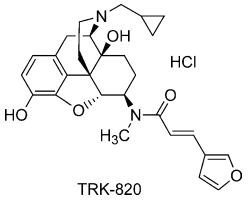


Fig. 1. Structure of TRK-820

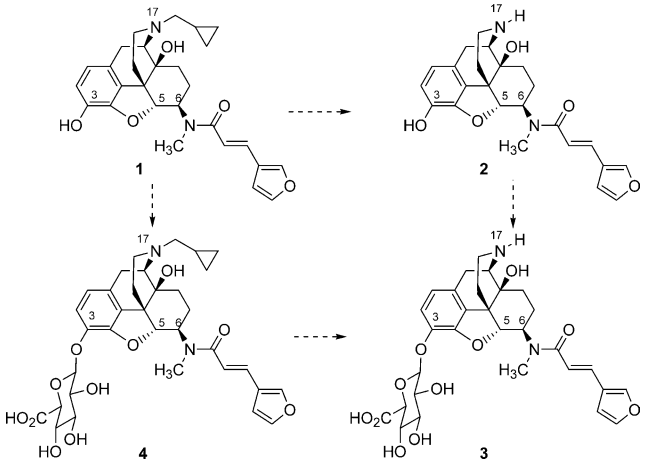
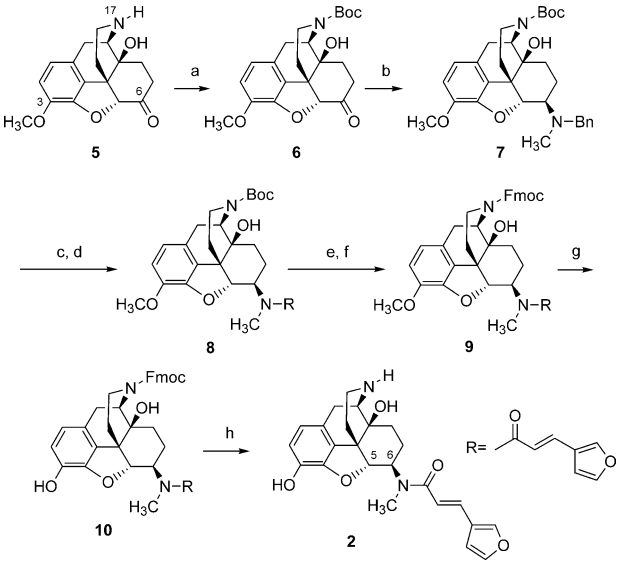


Fig. 2. Potential Metabolites of TRK-820

* To whom correspondence should be addressed. e-mail: Kuniaki_Kawamura@nts.toray.co.jp



Reagents and conditions: (a) $(Boc)_2O$, Na_2CO_3, $CHCl_3$, H_2O, room temperature, 3 h (quant.); (b) $BnNHCH_3$, $PhCO_2H$, PhH reflux, 19 h; $NaBH_3CN$, CH_3OH, 0 °C, 2 h (72%); (c) H_2, 10% Pd–C, *o*-phthalic acid, CH_3OH, room temperature, 25 h; (d) (*E*)-3-(3-furyl)acryloyl chloride, Na_2CO_3, THF, H_2O, 0 °C to room temperature, 14 h (61% 2 steps); (e) CF_3CO_2H, CH_2Cl_2, 0 °C to room temperature, 1 h; (f) FmocCl, Na_2CO_3, 1,4-dioxane, H_2O, room temperature, 2 h (96% 2 steps); (g) BBr_3, CH_2Cl_2, 0 °C to room temperature, 3 h (65%); (h) NaOH, H_2O, THF, room temperature, 16 h (77%).

Chart 1

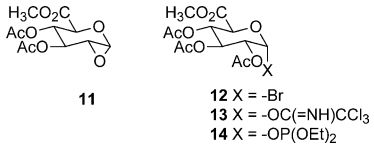
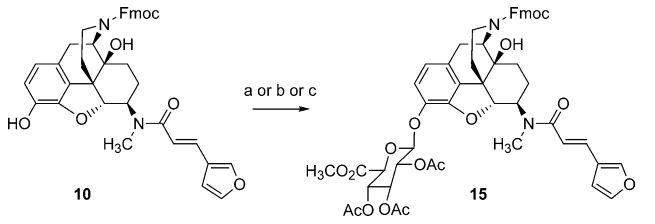


Fig. 3. Glycosyl Donors

compound derived from reductive amination reaction. Then, compound **9** was treated with 1.0 M BBr_3 at 0 °C to room temperature to give phenol **10** in 65% yield. Deprotection of the Fmoc group of compound **10** under basic conditions gave the first target compound **2** in 77% yield. The chemical structure of compound **2** was confirmed by ^{1}H-NMR and electron ionization mass (EI-MS) spectra. ^{1}H-NMR analysis showed that the characteristic 5-proton of 4,5-epoxymorphinan was observed at δ=4.55 (0.7H, d, J=7.8 Hz) and 4.63 (0.3H, d, J=7.8 Hz) with an axial–axial coupling between the 5- and 6-position and the (*E*)-olefinic protons of the amide side chain at δ=6.38 (0.7H, d, J=15.2 Hz), 6.89 (0.3H, d, J=15.2 Hz), 7.20 (0.7H, d, J=15.2 Hz), and 7.36 (0.3H, d, J=15.2 Hz) with *trans* coupling as a mixture of rotation isomers on the amide bond axis. The EI-MS spectrum showed the molecular ion peak at m/z=422 ($[M]^+$).

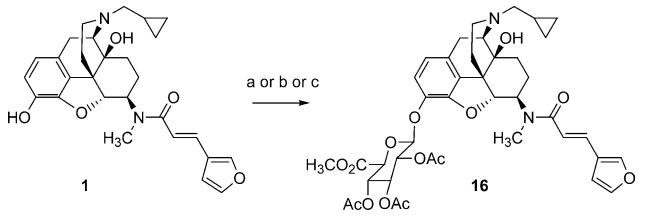
The next step was to establish glucuronidation methods on compounds **10** and **1** to synthesize compounds **3** and **4**. First, we prepared glucuronic acid derivatives **11**,[6] **12**,[7] **13**,[8—11] and **14**[12] in accordance with literature to investigate the reactivity with compounds **10** and **1** (Fig. 3).

Treatment of compound **10** with glycosyl donor **12** or **14** gave the desired glucuronated derivative **15** in low yields (27—32%). Unfortunately, our efforts to improve the yield by using **12** or **14** were in vain. We finally found that trichloroacetimidate **13** was the best glycosyl donor for the glucuronidation on compound **10** (Chart 2).



Reagents and conditions: (a) **13**, $BF_3\cdot OEt_2$, MS4A, CH_2Cl_2, room temperature, 3 h (92%); (b) **12**, $CdCO_3$, $PhCH_3$, reflux, 16 h (27%); (c) **14**, $BF_3\cdot OEt_2$, MS4A, CH_2Cl_2, −78 °C, 1 h (32%).

Chart 2



Reagents and conditions: (a) **13**, $BF_3\cdot OEt_2$, MS4A, CH_2Cl_2, room temperature, 19 h (82%); (b) **11**, NaH, 15-crown-5-ether, DMF, room temperature, 2 h (0%); (c) **12**, $CdCO_3$, $PhCH_3$, 110 °C, 20 h (0%).

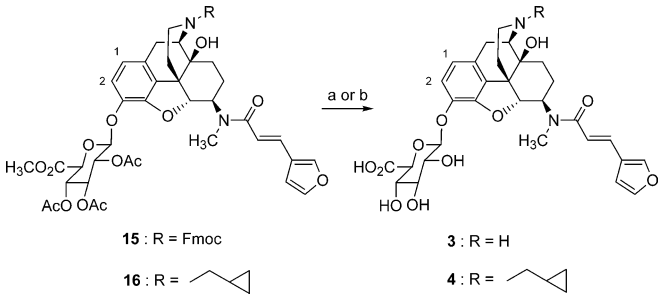
Chart 3

Treatment of compound **10** with 2 equivalents of trichloroacetimidate **13** in the presence of 2.3 equivalents of $BF_3\cdot OEt_2$ in CH_2Cl_2 at room temperature for 75 min afforded the glucuronated derivative **15** in 92% yield after chromatographic purification. The ^{1}H-NMR spectrum of compound **15** showed signals indicative of a methyl 2,3,4-tri-*O*-acetylglucuronate function at δ=1.96 (3H, s), 1.99 (3H, s), 2.00 (3H, s), and 3.72 (3H, s) and also showed signals indicative of an *N*-methyl amide function at δ=3.14 (3H, s). The electrospray ionization mass (ESI-MS) spectrum showed the molecular ion peak at m/z=961 ($[M+H]^+$).

In the case of glucuronidation on compound **1**, the free base of TRK-820, the use of epoxide **11** and bromide **12** was unsuccessful and the desired glucuronated derivative **16** was not produced. The reason why compound **16** was not obtained is unclear. The only point of difference between **1** and **10** is that the substrate **1** has a basic nitrogen. It has been reported that reproducibility of the Koenig-Knorr reaction using bromide **12** was poor.[13,14]

Similarly, trichloroacetimidate **13** was an appropriate glycosyl donor in this case (Chart 3). Treatment of compound **1** with 1.5 equivalents of trichloroacetimidate **13** in the presence of 1.8 equivalents of $BF_3\cdot OEt_2$ in CH_2Cl_2 at room temperature afforded the glucuronated derivative **16** in 82% yield after chromatographic purification. The ^{1}H-NMR spectrum showed signals indicative of a methyl 2,3,4-tri-*O*-acetylglucuronate function at δ=1.95 (3H, s), 1.98 (3H, s), 2.00 (3H, s), and 3.71 (3H, s) and also showed signals indicative of an *N*-methyl amide function at δ=3.16 (3H, s). The ESI-MS spectrum showed the molecular ion peak at m/z=793 ($[M+H]^+$).

Deprotection of both compounds **15** and **16** was accomplished by treatment with lithium hydroxide followed by neutralization using 1 M HCl to produce the target compounds **3** and **4**, respectively (Chart 4). Because both compounds **3** and **4** have high polarity, they were distributed into water layer on



Reagents and conditions: (a) $LiOH \cdot H_2O$, THF, CH_3OH, H_2O, room temperature, 18 h for **15** (43%); (b) $LiOH \cdot H_2O$, THF, H_2O, room temperature, 25 h for **16** (54%).

Chart 4

work-up. Therefore, their purification was carried out as follows. The water layer containing compound was washed with an organic solvent, and the resulting water layer was directly concentrated *in vacuo*. To the residue which contained the desired compound and inorganic salts was added an alcoholic solvent to precipitate the desired compound and inorganic salts, and the resulting precipitation was purified through Sep-Pak Vac® filled with ODS using water and CH_3OH as eluent. As the results of the above purification methods, pure compounds **3** and **4** were obtained in 43% and 54% yields, respectively. The chemical structure of compound **3** was confirmed by ^{1}H-NMR and ESI-MS spectra. The anomeric α-proton was observed as a doublet signal at δ=5.09 with an axial–axial coupling of 7.4 Hz. One- and two-position protons were observed at δ=7.13 (1H, d, J=8.2 Hz) and 6.79 (1H, d, J=8.2 Hz), and the (*E*)-olefinic protons of the amide side chain at δ=6.87 (1H, d, J=15.0 Hz) and 7.64 (1H, d, J=15.0 Hz) with a *trans* coupling. The ESI-MS spectrum showed the molecular ion peak at m/z=599 ($[M+H]^+$).

The chemical structure of compound **4** was also confirmed by ^{1}H-NMR and ESI-MS spectra. Protons of the cyclopropylmethyl part were observed at δ=0.40—0.50 (2H, m), 0.65—0.90 (2H, m), and 1.00—1.20 (1H, m), and the anomeric α-proton was observed as a doublet signal at δ=5.06 with an axial–axial coupling of 7.4 Hz. One-position proton was observed at δ=7.20 (1H, d, J=8.2 Hz), and the (*E*)-olefinic proton of the amide side chain at δ=7.66 (1H, d, J=15.6 Hz) with a *trans* coupling. The ESI-MS spectrum showed the molecular ion peak at m/z=653 ($[M+H]^+$).

To confirm the presence of compounds **2**, **3** and **4** as the metabolites of TRK-820, radio-chromatograms of an incubation mixture of [^{3}H]TRK-820 with cryopreserved human hepatocytes were compared with UV-chromatograms of compounds **2**, **3** and **4**.[15] Metabolite peaks in the incubation mixture were detected at retention times of 9.4 and 8.0 min on radio-chromatograms, corresponding to those of compound **2** and **4** simultaneously analyzed. These results indicated that compounds **2** and **4** were the metabolites derived from TRK-820 in human hepatocytes.

Pharmacological studies of compounds **2**, **3**, and **4** were carried out to investigate their antipruritic activity.[16] Scratching behavior was induced by the intradermal injection of Substance P, a pruritogen, in 5-weeks-old male ddY mice.[3] Subcutaneous administration of compounds **2**, **3**, and **4** (1, 10, 100, 1000 μg/kg each) did not inhibit the scratching behavior induced by Substance P, though TRK-820 inhibited the scratching behavior at low dose.[3] These results indicated that compounds **2**, **3**, and **4** were substantially inactive on the above experimental conditions.

Conclusion

Chemical syntheses of three kinds of potential metabolites of TRK-820 were accomplished. Compound **2** was prepared from noroxycodone **5** in 21% total yield (8 steps). Stereoselective glucuronidation of compounds **1** and **10** succeeded by using trichloroacetimidate methods. Compound **3** was prepared from noroxycodone **5** in 11% total yield (10 steps), and compound **4** was prepared from compound **1** in 43% yield (2 steps). Among the potential metabolites of TRK-820, compounds **2** and **4** were indeed confirmed to occur in human hepatocytes, and the synthesized compounds **2**, **3**, and **4** are unlikely to have antipruritic activity like TRK-820. The availability of these metabolites by chemical synthesis should facilitate further clinical studies on TRK-820.

Experimental

General Methods All reactions were proceeded under protection from light and the reactions requiring anhydrous conditions were conducted under atmosphere of dry argon. The progress of reactions and purity of intermediates were monitored on Merck analytical silica gel TLC plates 60 F_{254}. For column chromatography Merck silica gel 60 (0.063—0.200 mm) was used. Melting points were measured on Yanaco MP-500D melting point apparatus without correction. ^{1}H-NMR spectra were measured (TMS internal standard) on a Varian Gemini 2000 (300 MHz) spectrometer or on a JEOL JNM-AL 400 (400 MHz) spectrometer. IR spectra were measured on JASCO FT/IR-410. Electron ionization mass spectra (EI-MS) and high-resolution electron ionization mass spectra (HR-EI-MS) were measured on JEOL DX-303. Electrospray ionization mass spectra (ESI-MS) and high-resolution electrospray ionization mass spectra (HR-ESI-MS) were measured on micromass LCT.

17-*N*-(*tert*-Butoxycarbonyl)-4,5α-epoxy-14β-hydroxy-3-methoxymorphinan-6-one (6) To a stirred suspension of noroxycodone hydrochloride **5** (102.45 g, 303.29 mmol) and $NaHCO_3$ (55.35 g, 658.8 mmol) in 900 ml of water and 1050 ml of $CHCl_3$ was added dropwise $(Boc)_2O$ (73.41 g, 336.3 mmol) at room temperature, and the suspension became a clear solution soon. The solution was stirred at room temperature for 3 h, and the organic layer was separated. The water layer was extracted with $CHCl_3$ and the combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to give 149.42 g of compound **6** as an amorphous solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 (9H, s), 1.59—1.72 (3H, m), 1.89—1.94 (1H, m), 2.30 (1H, dt, J=14.6, 3.0 Hz), 2.33—2.45 (1H, m), 2.60—2.80 (2H, m), 2.90 (1H, d, J=18.3 Hz), 2.98—3.08 (2H, m), 3.90 (3H, s), 4.30—4.60 (1H, m), 4.67 (1H, s), 6.64 (1H, d, J=8.3 Hz), 6.73 (1H, d, J=8.3 Hz). IR (KBr) cm^{-1}: 3437, 1724, 1681. HR-EI-MS m/z M^+ Calcd for $C_{22}H_{27}NO_6$: 401.1838. Found: 401.1855.

6β-(*N*-Benzyl)methylamino-17-(*tert*-butoxycarbonyl)-4,5α-epoxy-3-methoxymorphinan-14β-ol (7) To a stirred solution of compound **6** (21.50 g, 53.55 mmol) in 500 ml of benzene were added *N*-benzylmethylamine (14.2 ml, 110 mmol) and benzoic acid (6.68 g, 54.7 mmol). This mixture was stirred under reflux conditions for 19 h, using a Dean-Stark apparatus. After removal of benzene, the crude products were dissolved in 100 ml of CH_3OH, and $NaBH_3CN$ (4.11 g, 65.4 mmol) was added to the resulting solution, and the mixture was stirred at 0 °C. After stirring for 2 h, the solution was concentrated and the crude products were partitioned in EtOAc and saturated aq. $NaHCO_3$ solution. The organic layer was separated and the water layer was extracted with EtOAc. The combined organic layer was washed successively with saturated aq. $NaHCO_3$ solution and saturated aq. NaCl solution, dried over Na_2SO_4 and concentrated *in vacuo* to give 33.70 g of crude products. This was chromatographed on silica gel. Elution with *n*-hexane/EtOAc (10/1—1/1) gave 19.65 g (72%) of compound **7** as an amorphous solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35—1.54 (3H, m), 1.48 (9H, s), 1.62—1.65 (3H, m), 1.90—2.01 (1H, m), 2.17—2.28 (1H, m), 2.32 (3H, s), 2.57—2.64 (1H, m), 2.65—2.80 (1H, m), 2.86 (1H, d, J=17.8 Hz), 3.01—3.08 (1H, m), 3.71 (1H, d, J=13.6 Hz), 3.76 (1H, d, J=13.6 Hz), 3.89 (3H, s), 4.75 (1H, d, J=7.1 Hz), 6.59 (1H, d, J=8.3 Hz), 6.72 (1H, d, J=8.3 Hz),

7.18—7.44 (5H, m). IR (KBr) cm^{-1}: 3432, 1669, 1606. HR-EI-MS *m/z* M^+ Calcd for $C_{30}H_{38}N_2O_5$: 506.2781. Found: 506.2790.

(*E*)-*N*-[17-(*tert*-Butoxycarbonyl)-4,5α-epoxy-14β-hydroxy-3-methoxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide (8) *o*-Phthalic acid (2.97 g, 17.87 mmol) and 10% Pd–C (2.23 g, 2.09 mmol) were added to a solution of compound **7** (17.07 g, 33.69 mmol) in 70 ml of CH_3OH. The mixture was stirred under hydrogen atomosphere (1 atm) at room temperature for 25 h. The Pd–C catalyst was then removed by filtration with hyflo super-cell® and washed with CH_3OH. The filtrate was concentrated *in vacuo* to give 16.64 g of crude debenzylated compound. To a stirred solution of the crude products in 100 ml of THF and 50 ml of water was added Na_2CO_3 (2.83 g, 26.7 mmol) followed by (*E*)-3-(3-furyl)acryloyl chloride (7.96 g, 50.8 mmol) at 0 °C, and the temperature was gradually raised to room temperature. After stirring for 14 h, the organic layer was separated and the water layer was extracted with EtOAc. The combined organic layer was concentrated and dissolved in EtOAc again, and washed with a saturated aq. $NaHCO_3$ solution and saturated aq. NaCl solution, dried over Na_2SO_4 and concentrated *in vacuo* to give 13.72 g of crude products. This was chromatographed on silica gel (250 g) with $CHCl_3/CH_3OH$ (50/1) as eluent to give 11.03 g (61% 2 steps) of compound **8** as an amorphous solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.40—1.70 (6H, m), 1.49 (9H, s), 2.17—2.30 (2H, m), 2.35—2.80 (2H, m), 2.85—3.11 (2H, m), 3.00 (2H, s), 3.14 (1H, s), 3.82 (2H, s), 3.85 (1H, s), 4.20—4.50 (1H, m), 4.61 (0.7H, d, J=8.0 Hz), 4.76 (0.3H, d, J=8.0 Hz), 6.45 (0.7H, d, J=15.6 Hz), 6.47 (0.7H, s), 6.56—6.66 (0.9H, m), 6.70 (0.7H, d, J=8.3 Hz), 6.76 (0.3H, d, J=8.3 Hz), 6.83 (0.7H, d, J=8.3 Hz), 7.38 (0.7H, s), 7.42 (0.3H, s), 7.48 (1H, d, J=15.6 Hz), 7.57 (0.7H, s), 7.61 (0.3H, s). IR (KBr) cm^{-1}: 3383, 1652, 1591. HR-EI-MS *m/z* M^+ Calcd for $C_{30}H_{36}N_2O_7$: 536.2523. Found: 536.2541.

(*E*)-*N*-[4,5α-Epoxy-17-(9*H*-fluorenylmethoxycarbonyl)-14β-hydroxy-3-methoxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide (9) Trifluoroacetic acid (16 ml, 207 mmol) was added dropwise to a stirred solution of compound **8** (9.46 g, 17.6 mmol) in 60 ml of CH_2Cl_2 at 0 °C. After stirring at room temperature for 1 h, the solution was concentrated *in vacuo*. The excess trifluoroacetic acid in the mixture was removed azeotropically by using CH_3OH, C_2H_5OH followed by $CHCl_3$. The crude products (9.75 g) were then dissolved in 50 ml of 1,4-dioxane, and a solution of 10% aq. Na_2CO_3 (50 ml, 47.1 mmol) was added with stirring. Then, a solution of FmocCl (5.53 g, 21.37 mmol) in 20 ml of 1,4-dioxane was added dropwise to the solution at room temperature. After stirring for 2 h, the mixture was extracted with EtOAc and the combined organic layer was concentrated *in vacuo* to give 13.69 g of crude products. This was chromatographed on silica gel (360 g) with *n*-hexane/EtOAc (1/1—0/1) as eluent to give 11.02 g (96% 2 steps) of compound **9** as an amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.40—1.50 (3H, m), 1.55—1.80 (3H, m), 2.10—2.40 (2H, m), 2.60—2.75 (1H, m), 2.80—2.90 (1H, m), 3.00 (2H, s), 3.15 (1H, s), 3.75—4.00 (2H, m), 3.81 (2H, s), 3.85 (1H, s), 4.20—4.30 (1H, m), 4.40—4.80 (4H, m), 6.40—6.80 (4H, m), 7.26—7.62 (9H, m), 7.77 (2H, broad s). IR (KBr) cm^{-1}: 3426, 1690, 1652, 1604. HR-ESI-MS *m/z* $[M+H]^+$ Calcd for $C_{40}H_{39}N_2O_7$: 659.2757. Found: 659.2784.

(*E*)-*N*-[4,5α-Epoxy-17-(9*H*-fluorenylmethoxycarbonyl)-3,14β-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide (10) To a stirred solution of compound **9** (10.98 g, 16.66 mmol) in 100 ml of CH_2Cl_2 was added dropwise BBr_3 (1.0 M in CH_2Cl_2, 180 ml, 180 mmol) at 0 °C during 1 h, and the temperature was gradually raised to room temperature. After stirring for 2 h, the flask was cooled to 0 °C again and the mixture was quenched by adding crushed ice and slowly adding 20 ml of water. After that, a solution of 10% aq. Na_2CO_3 was slowly added until the solution showed pH 8, and then the mixture was extracted with $CHCl_3$. The organic layer was washed with water and a saturated aq. NaCl solution, and the organic layer was concentrated *in vacuo* to give 10.50 g of crude products. This was chromatographed on silica gel (360 g) with EtOAc as eluent to give 6.99 g (65%) of compound **10** as an amorphous solid.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.20—1.40 (4H, m), 1.45—1.60 (1H, m), 1.95—2.15 (1H, m), 2.20—2.40 (1H, m), 2.45—2.65 (1H, m), 2.80—3.00 (1H, m), 2.86 (2H, s), 3.10 (1H, s), 3.56 (0.7H, broad s), 3.75—3.85 (1H, m), 4.09 (0.3H, broad s), 4.15—4.50 (4H, m), 4.60—4.65 (1H, m), 4.91—5.08 (1H, m), 6.30—6.40 (0.7H, m), 6.50—6.65 (2.3H, m), 6.75—6.80 (0.7H, m), 6.89 (0.3H, d, J=15.2 Hz), 6.99 (0.3H, s), 7.21 (0.7H, d, J=15.2 Hz), 7.30—7.45 (4H, m), 7.61—7.72 (3H, m), 7.85—7.89 (2H, m), 7.91 (0.7H, s). 8.03 (0.3H, s), 9.09 (0.3H, broad s), 9.50 (0.7H, broad s). IR (KBr) cm^{-1}: 3398, 1678, 1649, 1593. HR-ESI-MS *m/z* $[M+H]^+$ Calcd for $C_{39}H_{37}N_2O_7$: 645.2601. Found: 645.2600.

(*E*)-*N*-[4,5α-Epoxy-3,14β-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide (2) To a stirred solution of compound **10** (655 mg, 1.01 mmol) in 10 ml of THF was added dropwise a solution of 2.5 M aq. NaOH (2.0 ml, 5.0 mmol). The mixture was stirred for 16 h at room temperature, and then a solution of 1 M aq. HCl was added to neutralize (pH 7). This solution was extracted with EtOAc to remove Fmoc residue and the water layer was concentrated *in vacuo* to give crude products. This was purified by Sep-Pak Vac® with water and CH_3OH as eluent to remove inorganic salts to give 338 mg (77%) of compound **2** as a white powder.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.00—1.15 (1H, m), 1.16—1.40 (4H, m), 1.40—1.50 (1H, m), 2.00—2.15 (2H, m), 2.30—2.40 (1H, m), 2.45—2.60 (2H, m), 2.70—3.00 (3H, m), 2.86 (2H, s), 3.09 (1H, s), 3.50—3.60 (0.7H, m), 4.15—4.25 (0.3H, m), 4.55 (0.7H, d, J=7.8 Hz), 4.63 (0.3H, d, J=7.8 Hz), 6.38 (0.7H, d, J=15.2 Hz), 6.51—6.73 (2.7H, m), 6.89 (0.3H, d, J=15.2 Hz), 6.99 (0.3H, s), 7.20 (0.7H, d, J=15.2 Hz), 7.36 (0.3H, d, J=15.2 Hz), 7.66 (0.7H, s), 7.72 (0.3H, s), 7.91 (0.7H, s), 8.02 (0.3H, s). IR (KBr) cm^{-1}: 3399, 1647, 1591. HR-EI-MS *m/z* M^+ Calcd for $C_{24}H_{26}N_2O_5$: 422.1842. Found: 422.1832. mp 286 °C (dec.).

Methyl {4,5α-Epoxy-17-(9*H*-fluorenylmethoxycarbonyl)-14β-hydroxy-6β-[(*E*)-*N*-methyl-3-(3-furyl)-2-propenamido]morphinan-3-yl-2′,3′,4′-tri-*O*-acetyl-1′β-D-glucopyranosid}uronate (15) To a stirred solution of compounds **13** (2.61 g, 5.45 mmol) and **10** (1.75 g, 2.72 mmol) in 27 ml of dry CH_2Cl_2 was added 3.42 g of MS4A, and the mixture was stirred at room temperature for 2.5 h. To this suspension was added dropwise $BF_3\cdot OEt_2$ (0.780 ml, 6.15 mmol) at room temperature, and the mixture was stirred for 75 min. Then, a saturated aq. $NaHCO_3$ solution was added to the suspension, and the mixture was filtered through hyflo super-cell® and washed with $CHCl_3$. The organic layer was separated and the water layer was extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give 4.52 g of crude products. This was chromatographed on silica gel (130 g) with *n*-hexane/EtOAc (1/1) as eluent to give 2.41 g (92%) of compound **15** as a white powder.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.30—1.50 (3H, m), 1.96 (3H, s), 1.99 (3H, s), 2.00 (3H, s), 2.03—2.21 (4H, m), 2.50—2.90 (2H, m), 3.14 (3H, s), 3.72 (3H, s), 3.74—3.77 (1H, m), 4.20—4.30 (1H, m), 4.40—4.63 (4H, m), 4.65 (1H, d, J=10.4 Hz), 5.14—5.24 (3H, m), 5.33—5.43 (2H, m), 6.50—6.70 (2H, m), 6.67 (1H, d, J=15.4 Hz), 6.95—7.05 (1H, m), 7.25—7.44 (5H, m), 7.55—7.61 (2H, m), 7.70 (1H, d, J=15.4 Hz), 7.70—7.85 (3H, m). IR (KBr) cm^{-1}: 3443, 1756, 1690, 1653, 1606. HR-ESI-MS *m/z* $[M+H]^+$ Calcd for $C_{52}H_{53}N_2O_{16}$: 961.3395. Found: 961.3401. mp 157 °C.

Methyl {17-Cyclopropylmethyl-4,5α-epoxy-14β-hydroxy-6β-[(*E*)-*N*-methyl-3-(3-furyl)-2-propenamido]morphinan-3-yl-2′,3′,4′-tri-*O*-acetyl-1′β-D-glucopyranosid}uronate (16) To a stirred solution of compounds **13** (2.53 g, 5.28 mmol) and **1** (1.65 g, 3.46 mmol) in 26 ml of dry CH_2Cl_2 was added 7.95 g of MS4A, and the mixture was stirred at room temperature for 2 h. To the suspension was added dropwise $BF_3\cdot OEt_2$ (0.800 ml, 6.31 mmol), and the mixture was stirred at room temperature for 17 h. Then, a saturated aq. $NaHCO_3$ solution was added to the suspension, and the mixture was filtered through hyflo super-cell® and washed with $CHCl_3$. The organic layer was separated and the water layer was extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give 4.48 g of crude products. This was chromatographed on silica gel (158 g) with EtOAC/CH_3OH (10/0—10/1) as eluent to give 2.26 g of a mixture. This was chromatographed on silica gel (113 g) with $CHCl_3/CH_3OH$ (10/1) as eluent to give 2.25 g (82%) of compound **16** as an amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.12—0.14 (2H, m), 0.51—0.55 (2H, m), 0.78—0.90 (1H, m), 1.20—1.70 (3H, m), 1.95 (3H, s), 1.98 (3H, s), 2.00 (3H, s), 2.04—2.15 (3H, m), 2.36 (2H, d, J=6.3 Hz), 2.55—2.70 (2H, m), 3.00—3.10 (3H, m), 3.16 (3H, s), 3.71 (3H, s), 4.53 (1H, d, J=7.6 Hz), 4.60—4.75 (1H, m), 4.67 (1H, d, J=9.6 Hz), 5.14—5.23 (2H, m), 5.35—5.45 (2H, m), 6.59 (1H, d, J=8.2 Hz), 6.66 (1H, s), 6.68 (1H, d, J=15.2 Hz), 6.96 (1H, d, J=8.2 Hz), 7.43 (1H, s), 7.70 (1H, d, J=15.2 Hz), 7.78 (1H, s). IR (KBr) cm^{-1}: 3426, 1756, 1652, 1605. HR-ESI-MS *m/z* $[M+H]^+$ Calcd for $C_{41}H_{49}N_2O_{14}$: 793.3184. Found: 793.3201.

4,5α-Epoxy-14β-hydroxy-6β-[(*E*)-*N*-methyl-3-(3-furyl)-2-propenamido]morphinan-3-yl-β-D-glucopyranosiduronic acid (3) To a stirred suspension of compound **15** (2.37 g, 2.46 mmol) in 50 ml of THF and 10 ml of CH_3OH was added a solution of $LiOH\cdot H_2O$ (808 mg, 19.3 mmol) in 20 ml of water, and the mixture was stirred at room temperature. After stirring for 18 h, a solution of 1 M aq. HCl was added to neutralize, and then the mixture was concentrated to remove THF and CH_3OH. The resulting water layer was washed with EtOAc and concentrated *in vacuo*. A mixture of water and 2-propnol (1/4) was added to the residue to precipitate the desired compound and inorganic salts to give 0.80 g of a solid after drying. This

solid was further purified through Sep-Pak Vac® with water and CH_3OH as eluent to give 0.63 g (43%) of compound **3** as a pale yellow powder.

^{1}H-NMR (300 MHz, CD_3OD) δ: 1.21—1.60 (3H, m), 1.65—1.80 (1H, m), 2.10—2.30 (1H, m), 2.40—2.60 (2H, m), 3.00—3.10 (1H, m), 3.15—3.24 (5H, m), 3.38—3.64 (4H, m), 4.00 (1H, d, J=9.6 Hz), 4.30—4.50 (1H, m), 4.81 (1H, d, J=8.8 Hz), 5.09 (1H, d, J=7.4 Hz), 6.79 (1H, d, J=8.2 Hz), 6.87 (1H, d, J=15.0 Hz), 6.88 (1H, s), 7.13 (1H, d, J=8.2 Hz), 7.57 (1H, s), 7.64 (1H, d, J=15.0 Hz), 7.89 (1H, s). IR (KBr) cm^{-1}: 3409, 1707, 1648, 1602. HR-ESI-MS m/z $[M+H]^{+1}$ Calcd for $C_{30}H_{35}N_2O_{11}$: 599.2241. Found: 599.2221. mp 239 °C (dec.).

17-Cyclopropylmethyl-4,5α-epoxy-14β-hydroxy-6β-[(*E*)-*N*-methyl-3-(3-furyl)-2-propenamido]morphinan-3-yl-β-D-glucopyranosiduronic acid (4) To a stirred suspension of compound **16** (1.89 g, 2.39 mmol) in 20 ml of THF was added a solution of $LiOH \cdot H_2O$ (980 mg, 23.3 mmol) in 6 ml of water, and the mixture was stirred at room temperature. After stirring for 25 h, a solution of 1 M aq. HCl was added to neutralize, and then the mixture was concentrated to remove THF. The resulting water layer was washed with $CHCl_3$ and concentrated *in vacuo*. 2-Propanol was added to the residue to precipitate the desired compound and inorganic salts to give 1.03 g of a solid after drying. This solid was further purified with Sep-Pak Vac® with water and CH_3OH as eluent to give 0.85 g (54%) of compound **4** as a yellow powder.

^{1}H-NMR (300 MHz, CD_3OD) δ: 0.40—0.50 (2H, m), 0.65—0.90 (2H, m), 1.00—1.20 (1H, m), 1.40—1.50 (1H, m), 1.50—1.70 (2H, m), 1.70—1.85 (1H, m), 2.20—2.40 (1H, m), 2.45—2.70 (2H, m), 2.70—2.90 (1H, m), 3.00—3.20 (2H, m), 3.25 (3H, s), 3.40—3.60 (5H, m), 3.70—4.00 (2H, m), 4.30—4.50 (1H, m), 4.80—5.00 (1H, m), 5.06 (1H, d, J=7.4 Hz), 6.81—6.90 (3H, m), 7.20 (1H, d, J=8.2 Hz), 7.52—7.78 (1H, m), 7.66 (1H, d, J=15.6 Hz), 7.90 (1H, s). IR (KBr) cm^{-1}: 3405, 1648, 1600. HR-ESI-MS m/z $[M+H]^+$ Calcd for $C_{34}H_{41}N_2O_{11}$: 653.2710. Found: 653.2737. mp 206 °C (dec.).

***In Vitro* Metabolic Experiment of TRK-820** [^{3}H]TRK-820 was incubated with cryopreserved human hepatocytes for 1, 2 and 4 h at 37 °C and the metabolic reaction was terminated by the addition of ice-cold acetonitrile. The incubation mixture was centrifuged at 37 °C, 3000 rpm for 5 min and the entire supernatant obtained was evaporated to dryness with a centrifugal evaporator. The residue was dissolved in the mobile phase and injected to an analytical column (YMC-Pack ODS-AM, S-5 μm, AM-302, 150×4.6 mm; YMC). The radio-HPLC system consisted of a radio detector (Radiomatic FLO-ONE/βeta Series A-505; Packard Co.) and HPLC (LC-10Ai Liquid Chromatograph System; Shimadzu Co.). The HPLC conditions were as follows: mobile phase, CH_3OH (A) and 50 mM NaH_2PO_4 aq. solution (B); flow rate, 0.9 ml/min; column temperature, 40 °C. The gradient elution profile was 30 to 40% B for 0 to 20 min, 40 to 100% B for 20 to 25 min and 100% B for 25 to 30 min.

References and Notes

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15) The use of human hepatocytes was in compliance with the Declaration of Helsinki and approved by the Ethical Committee of Toray Research Laboratories.
16) Pharmacological studies were conducted in accordance with the guidelines for Care and Use of Laboratory Animals in Toray Research Laboratories.