A novel and practical synthesis of CAT3: a phenanthroindolizidine alkaloid with potential in treating glioblastoma†

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CAT3, one of the (+)-deoxytylophorinine-based phenanthroindolizidine alkaloids, is a promising therapeutic agent for the treatment of hedgehog (Hh)-driven glioblastoma and is currently being evaluated in preclinical studies. In this paper, a novel and practical synthetic route for CAT3 was firstly demonstrated with 10% overall yield in 11 steps and has been successfully validated for pilot-plant scale preparation. Investigation of the substitution at the 3-position of phenanthrene revealed that the electron-donating functionality can well preserve the S configuration. In particular, the excellent enantiomeric excess of CAT3 (≥99% ee) was achieved by introducing the strongly electron-donating tert-butyldimethylsilyl (TBS) group.

Introduction

Glioblastoma (GBM) is one of the most common and malignant brain tumors in the human central nervous system along with high invasion, high mortality and poor prognoses,1,2 which ends with an average survival of 15–17 months even with the best available treatment.3 Temozolomide (TMZ), the principal first-line chemotherapeutic agent, is widely used for the treatment of recurrent and high-grade glioma and is proven to be an efficient inhibitor of GBM proliferation.4,5 However, due to the emergence of intrinsic and acquired TMZ resistance in GBM cells, its use is seriously limited.6,7 Therefore, there is an urgent need to develop new therapeutic agents for GBM disease.

(+)-Deoxytylophorinine (CAT) was isolated from the roots of Tylophora atrofoliculata and Tylophora ovata and showed significant anticancer properties in vivo with its ability to well penetrate the blood brain barrier,8–11 while serious damage on gastrointestinal was also observed. In the study of its metabolic products in vivo, (13αS)-3-hydroxyl-6,7-dimethoxyphenanthro[9,10-b]indolizidine (PF403, Fig. 1) was found to possess excellent antiproliferative activity with the IC50 value less than 0.01 nM in multiple cancer cell lines in vitro.10–12 However, the excellent data in vitro cannot be reflected to its data in vivo probably due to its poor pharmacokinetic properties. To address this issue, the prodrug strategy was employed herein and (13αS)-3-pivaloyloxyl-6,7-dimethoxyphenanthro[9,10-b]indolizidine (CAT3) was designed and synthesized as the prodrug of PF403 (Fig. 1). A series of preclinical studies has proven that CAT3 is a promising therapeutic agent against hedgehog (Hh)-driven GBM.13,14 Although the structure of CAT3 only differs slightly from that of CAT, the synthesis of which has been accomplished in several total syntheses,13–15 the mutigram synthesis of CAT3 is still challenging. A main problem lies in the completely different starting materials due to the methoxy and pivaloxy group for CAT and CAT3, respectively, used in the synthesis. When the same synthetic strategy of CAT was used to synthesize CAT3,13–15 it did not work after attempting multiple times in the past. This challenge, together with its excellent potency for GBM, stimulated us to investigate a novel and efficient synthetic route for CAT3.

Results and discussion

Strategically, CAT3 can be easily synthesized from acylation of PF403. Therefore, the efficient synthesis of PF403 is a key question. Takashi and coworkers disclosed the synthesis of PF403 starting from 4-benzyloxybenzaldehyde and 2-(3,4-dimethoxyphenyl)acetic acid with 10.8% yield in 13 steps.16,17 which

Fig. 1  The development process of the preclinical drug CAT3.
was the first meaningful synthetic route. However, photo-
induced electron cyclization reaction was used to construct
the phenanthrene ring, and it was not efficient for large-scale
preparation.\footnote{pf403} PF403 was also successfully prepared in 16
steps in our previous work, but the route was very tedious.\footnote{pf403}
Moreover, it has been demonstrated that it is only applicable for
small-amount synthesis. When we tried multigram synthesis,
the yield decreased to as low as 2%, making it unacceptable for
the large-scale preparation of CAT3.

Thus it is necessary to develop a novel synthesis of PF403.
The retrosynthetic analysis was illustrated in Scheme 1. We
envisioned that PF403 could be accessible from the inter-
mediate A, which could be readily made by reduction and protec-
tion from B. It was envisaged that B could be assembled from
compound C and D by a substitution reaction.

Based on our retrosynthetic analysis, the synthesis of PF403
commenced with the known compound 1, which was obtained
using our reported method in 6 steps.\footnote{pf403} The ester 2 was easily
prepared from the condensation of 1 with commercially avail-
able methyl L-proline hydrochloride. Compound 3, which was
expected to produce phenanthroindolizidine by eliminating
one molecule of H2O, was easily obtained by reducing 2 with
diisobutylaluminum hydride.\footnote{pf403} However, multiple attempts to
construct the indolizidine ring from 3 in one step failed
according to literature,\footnote{pf403} even if we tried to convert the
hydroxy group to a better leaving group such as MsO– or
TsO–. Except compound 1, other different substitutions on 3-
position was tried, such as TBSO– and HO–, but both result were
disappointed. Thus the preparation of PF403 via this route
proved probably impracticable (Scheme 2).

We deduce that there are two probable pathways starting from
3 under the acid condition to construct phenanthroindolizidine.
Theoretically, the route should work well if the aromatic electron
at 10-position of phenanthrene ring attacks carbocation to
generate phenanthroindolizidine via pathway 1. However, iden-
tification of the chlorinated compound 5 suggests that the car-
bocation, which probably arised from transition state 4 with the
aid of the nitrogen lone-pair electrons, appears to favor the
formation of the nitrogen-containing three-membered ring
structure as a transition state via pathway 2 (Scheme 3).

After multiple attempts, we eventually found a novel and practical
synthetic route to produce CAT3 in 11 steps (Scheme 4). The condensation between 2-(3,4-dimethoxyphenyl)acetic
acid and 4-hydroxybenzaldehyde gave compound 6 in almost
quantitative yield, which was confirmed by difference NOE
spectrum as cis-configuration (see ESI†). Compound 9 was
prepared in 50% overall yield in three steps, including the scroll
coupling in the presence of FeCl3, esterification in methanol
with con. H2SO4, substitution of 3-OH with tert-butyldime-
thylsilyl chloride (TBSCI). The key intermediate 10 was achieved
in 95% yield by the reduction of 9 with diisobutylaluminum
hydride, which was transformed to 11 in 50% yield through
a reaction sequence including iodine substitution, nucleophilic
substitution with L-glutamic acid dimethyl ester hydrochloride,
and subsequent hydrolysis. After formation of 12 in 80% yield
by Friedel–Crafts reaction from 11, its 3-position hydroxyl group
was again protected by TBS to give 13 in 85% yield. The 14-
position carbonyl of 13 was then reduced to compound 14 in
80% yield, in which the lactam carbonyl group was further
reduced to methylene in 95% yield, and PF403 was successfully
synthesized till now. After PF403 in hand, we firstly tried to

Scheme 1 The retrosynthetic analysis of CAT3.

Scheme 2 A simple synthetic route firstly designed for PF403.
Reagents and conditions: (a) PPh3, imidazole, I2, DCM, r.t., then methyl
L-proline hydrochloride, imidazole, r.t.; (b) DIBAL–H, DCM, 0 °C, 40% for two steps.

Scheme 3 A plausible mechanism explaining the failure in con-
structing the phenanthroindolizidine.
prepare CAT3 by acylation of PF403 using pivaloyl chloride. However, a main by-product chlorinated substitution on 4-position of CAT3 was produced, which was difficult to be separated because of its polarity almost the same as CAT3. At last, pivaloyl chloride was replaced by pivalic anhydride and CAT3 was smoothly achieved with a yield as high as 70% avoiding the by-product. In general, there are several aspects of advantages for this route as follows: (1) the conventional design for the synthesis of 6 is broken so that no protection step is required for 4-hydroxybenzaldehyde, thus leading to a shorter synthesis; (2) the operation and post treatment of the reactions in this route are easy and convenient, which also result in a high overall yield (10%), and no traditional column chromatography is used; (3) pilot-scale preparation has been achieved for this route with cheap reagents and materials used, indicating that it is probably an economical route for large-scale synthesis; (4) all the chiral intermediates and products are prepared in high optical purity (∼99% ee), which is vital for a drug candidate.

It should be noted that compound 14 can be synthesized in one step from 12 with high yield (Scheme 5). However, the optical purity of CAT3 from this approach was not satisfactory. We studied all the chiral intermediates and noticed that the optical purity obviously decreased when the 14-position ketone of compound 12 was reduced to the hydroxyl group. In order to figure it out, a batch of experiments were performed. As a common sense, we first focused our efforts on the solvent and temperature used in this step. Different kinds of solvents or/and their combination were tested, such as diethylene glycol dimethyl, tetrahydrofuran, methanol, ethanol, dichloromethane and so on. Meanwhile, we tried the reaction at different temperatures, including −20 °C, 0 °C and 20 °C. Unfortunately, the results were unsatisfactory (27–93% ee, Scheme 5) in all these cases. Then we turned to modify the 3-position of compound 12. Three different substitutes were selected and compared, including the free hydroxyl group, the hydroxyl protected with the electron-donating benzyl and electron-withdrawing acetyl group. The best enantiomeric excess was obtained when 3-position hydroxyl was protected as benzyl ether (95–98% ee, Scheme 6) while the worst result obtained when protected as acetyl ester (10–50% ee, Scheme 7). Thus it can be preliminarily inferred that substitution on 3-position is related with the enantiomeric excess of the final product, and stronger electron-donating ability may further increase the optical purity of the product. As we expect, excellent enantiomeric excess was achieved when the 3-position hydroxyl...
was protected by the strong electron-donating TBS group (≥99% ee, Scheme 8). We deduce that the electron-donating group at C-14 in the plane structure, has a remote control on the stereochemistry of the phenanthroindolizidine moiety in favor of the formation of the S-configured center. The yields and ee values generated from different substitutions were illustrated in Schemes 5–8 and Table 1.

### Experimental

#### General methods

All the reactions sensitive to air or moisture were carried out under argon atmosphere in anhydrous solvents. The purchased extra dry Tetrahydrofuran (THF) with the resealable package was used directly. Thin layer chromatography (TLC) was performed using glass precoated silica gel GF254 plates. Spots were visualized under 254 nm or 365 nm UV light or colored by 1% phosphomolybdic acid in Et2O, r.t.; (c) NaOH, CH3OH, r.t., then HCl; (d) Red-Al, THF, r.t.

(S)-([1-((3-(Benzyloxy)-6,7-dimethoxyphenanthren-9-yl)methyl)pyrrolidin-2-yl]methanol (3)

To a stirring solution of imidazole (90 mg, 1.32 mmol) and triphenylphosphine (267 mg, 1.02 mmol) in DCM (15 ml) was added methyl L-prolinate hydrochloride (198 mg, 1.2 mmol) and iodine (214 mg, 0.84 mmol). A solution of the crude product in dry CH2Cl2 (30 ml), dried with anhydrous Na2SO4, and concentrated under vacuum to furnish the residue as crude product (224 mg, 0.6 mmol) was added to the stirring reaction in sequences. A solution of the crude product in dry CH2Cl2 (30 ml) was added DIBALH (0.8 ml, 1.5 M in toluene) dropwise under argon, and the reaction mixture was allowed to stir at room temperature for 12 h. Then the reaction mixture was quenched with the solution of seignette salt along with stirring for additional 12 h. The reaction mixture was separated as two layers and the water layer was extracted with dichloromethane CH2Cl2 (3 × 20 ml). The combined organic layer was washed with brine (2 × 30 ml), dried with anhydrous Na2SO4, and concentrated under vacuum to furnish the residue as crude product (2). To the cooled (−10 °C) solution of the crude product in dry CH2Cl2 (30 ml) was added DIBALH (0.8 ml, 1.2 mmol, 1.5 M in toluene) dropwise under argon, and the reaction mixture was allowed to stir at room temperature for 12 h. Then the reaction mixture was quenched with the solution of seignette salt along with stirring for additional 12 h. The reaction mixture was separated as two layers and the water layer was extracted with dichloromethane CH2Cl2 (3 × 20 ml). The combined organic layer was washed with brine (2 × 30 ml), dried with anhydrous Na2SO4, and concentrated under vacuum to furnish the crude product, which was purified by PTLC (DCM/MeOH = 10/1) to provide 3 (109 mg, 0.24 mmol) as yellow oil with 40% yield in two steps.

**Scheme 8** Reagents and conditions for the synthesis of PF403 starting from 12 with TBS substitution at 3-position: (a) TBSCI, imidazole, DMF, r.t.; (b) NaBHa, then Et3SiH, BF3–Et2O, r.t.; (c) Red-Al, THF, r.t.

**Table 1** The ee and yield of the final product from the different synthetic route

| Substitution at 3-position of compound 12 | HO | BnO | TBSO | AcO |
|-----------------------------------------|----|-----|------|-----|
| ee                                      | 27–91% | 95–98% | ≥99% | 10–50% |
| Yield                                  | 13.2% | 2.9% | 10.0% | 10.1% |
solution of 7 (800 g) in methanol (2500 ml) was added concentrated sulfuric acid (125 ml), and the reaction was refluxed for 12 h. After completion of the reaction, it was quenched by the addition of imidazole (306 g) and was stirred for another 30 min. Then the mixture was concentrated under vacuum to furnish the residue as crude product (8). To the stirring solution of the crude product and imidazole (238 g, 3.5 mol) in dichloromethane (2000 ml) was added TBSCI (530 g, 3.5 mol). After stirring for 12 h, the produced solid was filtered and the filtrate was washed by brine (2 × 2000 ml), dried with anhydrous Na2SO4, and concentrated under vacuum to furnish the residue, which was purified by fast silica column chromatography (PE/EtOAc, 5 : 1) to give 9 (213 g, 0.5 mol) as yellow solid with 50% yield in four steps. Mp 144–146 °C. 1H NMR (500 MHz, CDCl3) δ 8.65 (s, 1H), 8.45 (s, 1H), 7.86 (s, 1H). 7.86 (d, J = 2.2 Hz, 1H), 7.82 (d, J = 8.6 Hz, 1H). 7.13 (dd, J = 8.6, 2.2 Hz, 1H), 4.12 (s, 3H), 4.02 (s, 3H), 1.06 (s, 9H), 0.32 (s, 6H), 0.07 (s, 4H). 13C NMR (125 MHz, CDCl3) δ 168.3, 156.6, 149.9, 149.8, 133.6, 131.9, 131.4, 125.0, 124.9, 124.7, 121.9, 120.8, 111.3, 106.9, 103.2, 55.95, 55.90, 52.1, 25.9, 18.5, −4.0. IR (KBr) r\text{max} = 3120, 3004, 2951, 2857, 1708, 1617, 1520, 1469, 1421, 1258, 1208, 1113, 1033, 992, 938, 910, 832, 778 cm⁻¹. HRESIMS, m/z calculated for C23H30O3Si [M + H⁺]: 427.1938. Found: 427.1946.

(E)-3-(4-Acetoxyphenyl)-2-(3,4-dimethoxyphenyl)acrylic acid (6)
To a stirring solution of 3, 4-dimethoxyphenyl acetic acid (196 g, 1 mol) and 4-hydroxybenzaldehyde (123 g, 1 mol) in acetic anhydride (400 ml) was added triethylamine (200 ml) dropwise and the mixture was refluxed overnight under argon. After completion of the reaction, it was cooled to room temperature and 800 ml water was added. The reaction was stirred for 1 h and furnished 6 (341 g, 1 mol) as yellow solid after a sequence of filtration, washing and drying. 1H NMR (500 MHz, CD3OD) δ 7.78 (s, 1H), 7.08 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.2 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 6.73 (s, 1H), 6.72 (dd, J = 8.2, 2.0 Hz, 1H), 3.82 (s, 3H), 3.69 (s, 3H), 2.20 (s, 3H). 13C NMR (125 MHz, CD3OD) δ 171.1, 170.8, 152.6, 150.6, 150.3, 140.1, 134.2, 133.9, 132.6, 129.9, 123.6, 122.6, 114.7, 112.97, 56.4, 56.3, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 20.9. IR (KBr) r\text{max} = 3072, 2992, 2838, 2630, 1768, 1680, 1612, 1514, 1419, 1456, 1369, 1021, 912, 848 cm⁻¹. HRESIMS, m/z calculated for C19H18O6Na [M + Na⁺]: 365.0996. Found: 365.0986.

Methyl 3-{[(tert-butyl(dimethyl)silyl)oxy]-6,7-dimethoxyphanthrene-9-carboxylate (9)
To a solution of 6 (341 g, 1 mol) and ferric chloride in trifluoroacetic acid (1400 ml) was added dropwise a solution of 3-chloroperbenzoic acid (189.8 g, 1.1 mol) in trifluoroacetic acid (1700 ml) below 0 °C and then stirred for 12 h at room temperature. After completion of the reaction, a solution of sodium thiosulfate pentahydrate (20 g in 100 ml water) was added and then stirred for 30 min. The reaction was evaporated under reduced pressure to afford crude product 7 (800 g), which was used for the next step without further purification. To the
evaporated under vacuum to furnish the residue. The obtained residue was dissolved in methanol (600 ml) and dichloromethane (100 ml), and 4 M NaOH (240 ml) was added to the solution dropwise until the pH was stable around 11–12. After completion of the hydrolysis, most methanol was evaporated under reduced pressure. The obtained crude residue was mixed with 500 ml water and then extracted with dichloromethane (3 × 500 ml). The aqueous phase was further acidified with 4 M HCl to pH 2 at 0–5 °C and off-white solid precipitated out of the reaction mixture. Simple filtration and baking of the solid afforded 11 (53.5 g, 0.135 mol) with 55% yield. Mp 190–192 °C; 1H NMR (500 MHz, CD3OD) δ 7.92 (s, 1H), 9.83 (d, J = 2.1 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.48 (s, 1H), 7.44 (s, 1H), 7.08 (dd, J = 8.6, 2.1 Hz, 1H), 5.49 (d, J = 14.6 Hz, 1H), 4.30 (d, J = 14.6 Hz, 1H), 4.03 (s, 3H), 3.95 (s, 3H), 3.78 (dd, J = 9.4, 3.3 Hz, 1H), 2.62–2.55 (m, 1H), 2.46–2.40 (m, 1H), 2.19–2.13 (m, 1H), 2.04–1.98 (m, 1H). 13C NMR (125 MHz, CD3OD) δ 177.5, 175.0, 157.8, 150.9, 150.2, 133.2, 131.2, 129.0, 127.0, 126.5, 126.1, 125.9, 117.5, 106.7, 106.1, 105.3, 60.0, 56.5, 56.3, 45.6, 30.95, 23.8. IR (KBr) vmax = 3240, 2994, 2966, 2838, 1742, 1644, 1634, 1612, 1581, 1532, 1511, 1443, 1391, 1359, 1320, 1159, 1129, 1109, 1164, 1030, 984, 880, 859, 841, 796 cm⁻¹. HRESIMS, m/z calculated for C23H28O6N6 [M – H]⁺: 394.1296. Found: 394.1308.

(S)-3-Hydroxy-6,7-dimethoxy-13,13α-dihydrodibenzo[j,h]pyrrolo[1,2-b]isoquinoline-11,14(9H,12H)-dione (12)

To the solution of 11 (52.0 g, 0.132 mol) in trifluoroacetic anhydride (300 ml) was added boron fluoride ether ethyl (420 ml) and the reaction was stirred for 30 min at 45 °C. After completion of the reaction, it was poured to ice water (1500 ml) and stirred for 1 h with light green precipitating out. Simple filtration and baking of the solid afforded 12 (43.7 g, 0.116 mol) with 85% yield. Mp 300 °C (decomposed); 1H NMR (500 MHz, DMSO-d6) δ 9.92 (s, 1H), 9.13 (d, J = 9.2 Hz, 1H), 8.02 (d, J = 2.5 Hz, 1H), 8.00 (s, 1H), 7.48 (s, 1H), 7.16 (dd, J = 9.2, 2.5 Hz, 1H), 5.53 (d, J = 18.1 Hz, 1H), 4.80 (d, J = 18.1 Hz, 1H), 4.57 (t, J = 6.7 Hz, 1H), 4.07 (s, 3H), 4.03 (s, 3H), 2.48–2.29 (m, 4H). 13C NMR (125 MHz, DMSO-d6) δ 196.2, 173.1, 156.2, 151.5, 149.7, 137.4, 131.1, 128.6, 126.9, 122.8, 122.3, 120.8, 117.5, 106.5, 105.2, 104.0, 60.8, 55.84, 55.79, 29.5, 20.4. IR (KBr) vmax = 3167, 3000, 2925, 2835, 1708, 1663, 1613, 1519, 1457, 1401, 1353, 1245, 1207, 1178, 1117, 1029, 940, 924, 844, 806, 760 cm⁻¹. HRESIMS, m/z calculated for C22H20O8N6 [M + H]+: 378.1336. Found: 378.1335.

(S)-3-(tert-Butyldimethylsilyloxy)-6,7-dimethoxy-13α-dihydrodibenzo[j,h]pyrrolo[1,2-b]isoquinoline-11,14(9H,12H)-dione (13)

To the stirring solution of 12 (43.7 g, 0.116 mol) and N,N-diisopropylethylamine (45.0 g, 0.348 mol) in DMF (300 ml) was added TBDSCI (34.9 g, 0.232 mol). After completion of the reaction, water (800 ml) was carefully added to the reaction dropwise. After it was stirred for additional 1 h at ice water bath, yellow solid precipitated. Simple filtration, washing by methanol and baking of the solid afforded 13 (48.2 g, 0.098 mol) with 85% yield. Mp 212–214 °C; 1H NMR (500 MHz, CDCl3) δ 9.27 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 2.5 Hz, 1H), 7.85 (s, 1H), 7.29 (s, 1H), 7.20 (dd, J = 9.2, 2.5 Hz, 1H), 5.70 (d, J = 17.8 Hz, 1H), 4.65 (d, J = 17.8 Hz, 1H), 4.44–4.41 (m, 1H), 4.15 (s, 3H), 4.09 (s, 3H), 2.63–2.51 (m, 4H), 1.05 (s, 9H), 0.30 (s, 6H). 13C NMR (125 MHz, CDCl3) δ 195.5, 174.2, 154.6, 151.7, 149.9, 137.4, 131.4, 129.1, 127.7, 123.1, 122.8, 122.6, 121.6, 111.4, 104.3, 103.6, 61.2, 56.3, 56.1, 40.7, 30.2, 25.9, 21.0, 18.5, –4.01, –4.02. IR (KBr) vmax = 3106, 2953, 2934, 2856, 1691, 1612, 1582, 1521, 1462, 1419, 1391, 1260, 1209, 1177, 1120, 1025, 953, 886, 825, 785 cm⁻¹. HRESIMS, m/z calculated for C25H34N4O6 [M + H]+: 492.2228. Found: 492.2241. HPLC (Daicel Chiralpak OD-H, 4.6 mm × 250 mm, 10 µm, hexane/iPrOH = 50 : 50, flow rate = 1 ml min⁻¹, λ = 264, 355 nm): tR = 25.9 min; 99.5% ee.
3.64–3.57 (m, 1H), 3.50 (d, J = 15.0 Hz, 1H), 2.75 (dd, J = 15.6, 10.6 Hz, 1H), 2.36–2.31 (m, 2H), 2.22–2.09 (m, 1H), 1.91–1.79 (m, 2H), 1.78–1.73 (m, 1H), 1.68–1.56 (m, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 155.6, 149.1, 148.0, 130.3, 126.6, 125.0, 124.8, 124.5, 123.8, 122.6, 116.3, 106.4, 104.0, 103.4, 59.9, 55.5, 55.4, 54.6, 54.3, 33.1, 30.9, 21.3. IR (KBr) $v_{\text{max}}$ = 3565, 3092, 2965, 2825, 1626, 1585, 1535, 1511, 1481, 1461, 1437, 1414, 1386, 1359, 1323, 1256, 1237, 1150, 1033, 1009, 844, 788 cm$^{-1}$. HRESIMS, m/z calculated for C$_{32}$H$_{41}$NO$_3$ [M + H$^+$]: 434.2332. HPLC [Daicel Chiralpak OZ-H, 4.6 mm X 250 mm, 5 µm, hexane (0.2% TEA)/iPrOH = 90 : 10, flow rate = 1 ml min$^{-1}$, $\lambda$ = 258, 287 nm]: $t_R$ = 11.6 min; 99.6% ee.

(13αS)-3-Pivaloyloxyl-6,7-dimethoxyphenanthro[9,10-b]-indolizidine (CAT3)

To the cooled solution of $\text{PF}_4\text{O}$ (25.8 g, 0.077 mol), triethylamine (34.5 ml, 0.23 mol) and DMAP (0.94 g, 7.7 mmol) in dichloromethane (300 ml) was added trimethylacetic anhydride (28.1 g, 0.15 mol). After completion of the reaction, it was quenched by water. The reaction mixture was extracted into dichloromethane CH$_2$Cl$_2$ (3 x 200 ml). The combined organic layer was washed with brine (3 x 200 ml), dried with anhydrous Na$_2$SO$_4$, and concentrated under vacuum to furnish the residue, which was recrystallized by anhydrous ethanol (400 ml) to afford CAT3 (22.4 g, 0.052 mol) as off-white crystal with 70% yield. Mp 200.4–200.8 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.41 (s, 1H), 8.07 (s, 1H), 8.01 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.22 (s, 1H), 4.58 (d, J = 15.3 Hz, 1H), 4.02 (s, 3H), 3.94 (s, 3H), 3.53 (d, J = 15.3 Hz, 1H), 3.38–3.32 (m, 2H), 2.87–2.73 (m, 1H), 2.37–2.31 (m, 2H), 2.16–2.13 (m, 1H), 1.85–1.81 (m, 2H), 1.68–1.59 (m, 1H), 1.40 (s, 9H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 176.9, 149.5, 148.8, 148.6, 129.6, 128.0, 127.8, 126.4, 124.9, 124.8, 122.9, 120.5, 114.9, 104.6, 103.4, 59.8, 55.9, 55.5, 54.5, 53.4, 38.7, 33.1, 30.8, 27.0, 21.3. IR (KBr) $v_{\text{max}}$ = 3097, 2965, 2830, 1740, 1619, 1530, 1510, 1468, 1427, 1415, 1367, 1339, 1256, 1205, 1164, 1135, 1034, 899, 841, 821, 786 cm$^{-1}$. HRE- SIMS, m/z calculated for C$_{32}$H$_{41}$NO$_3$ [M + H$^+$]: 434.2331. Found: 434.2332. HPLC [Daicel Chiralpak OZ-H, 4.0 mm X 250 mm, 5 µm, hexane (with 0.2% TEA)/iPrOH = 82 : 18, flow rate = 1 ml min$^{-1}$, $\lambda$ = 258, 287 nm]: $t_R$ = 14.6 min; 99.6% ee.

Conclusions

In summary, a novel and facile synthesis for CAT3 was achieved in 11 steps with 10% overall yield after multiple attempts. This route represents a very economical and practical synthesis, in which all the reactions and post treatments are easy to handle. Importantly, pilot-scale preparation has been achieved following this route, and it would be suitable for large-scale preparation. Based on preliminary studies of different substitutions at 3-position of phenanthrene, the relationship between the electronic effect of the 3-position substituent group and the enantiomeric excess of chiral intermediates and the final product has been preliminarily elucidated. The introduction of electron-donating group significantly retains the configuration of phenanthroindolizidine as S at its 13a position. By virtue of

the strong electron-donating TBS group, the excellent enantio-meric excess was achieved for CAT3 ($\geq$99% ee) which is critical for its further preclinical investigations. This interesting finding not only represents a big breakthrough in the synthesis of CAT3, but also greatly inspires the construction and studies of chiral phenanthroindolizidine or other similar structures which is highly important for researchers in diverse fields, including natural product synthesis, organic chemistry, and medicinal chemistry.

Conflicts of interest

There are no conflicts to declare.

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