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Development of an Efficient Synthesis for a Nipecotate-Containing Immunopotentiator

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Abstract:

The preparation of Elanco Animal Health immunopotentiator (*S*)-ethyl-1-(2-thiopheneacetyl)-3-piperidinecarboxylate (1) is described. The synthesis includes a new resolution of racemic ethyl nipecotate with dibenzoyl-L-tartaric acid. The resolved salt is found to couple directly with commercially available 2-thiopheneacetyl chloride under environmentally friendly Schotten−Baumann conditions to afford the amide in high yield. The final product is an oil which is purified by wiped film evaporative distillation.

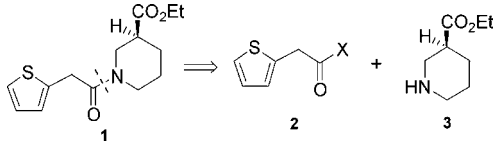
Introduction

The Elanco Animal Health[1] compound (*S*)-ethyl-1-(2-thiopheneacetyl)-3-piperidinecarboxylate (**1**) is an investigational immunopotentiating agent targeting direct stimulation of immune cells, useful for the prevention of infectious diseases in livestock.[2] Promising in vivo studies using 1-day-old broiler chicks as a model species warranted process research involvement to meet increasing material demand and enable further field and toxicological study. Strategically, the preparation of **1** is straightforward where the key bond disconnection involves the amide linkage connecting the 2-thiopheneacetic acid unit **2** with (*S*)-ethyl nipecotate (**3**) (Scheme 1). Key requirements for successfully meeting large material requests were the availability of the necessary raw materials, a classical resolution of racemic **3**, efficiency of the amide bond formation, and since **1** is an oil (mp −0.5 °C by DSC), an economical and simple purification method to give high-quality product.

Results and Discussion

Classical Resolution of Ethyl Nipecotate. The first-generation synthesis of **1**[2] utilized commercially available **3**. However, the only supplier at the time of this project was the Italian company, Chemie S.p.A, who were able to supply **3** on an as-needed basis at a price of $7500 per 500 grams.[3] The projected pricing for ton quantities was significantly reduced at $1200/kg, yet remained prohibitive, particularly for use in the preparation of an animal health product. We thus turned our attention toward the classical resolution of racemic ethyl nipecotate [(±)-**3**], available for <$100/kg in metric ton quantities.[4] Initially, a literature procedure was used which involved three recrystallizations of the diastereomeric salts derived from L-tartaric acid and [(±)-**3**] to effect (*R*)-isomer removal.[5] The combined mother liquors were then treated with base to provide (*S*)-enriched **3** which was reacted with D-tartaric acid to afford, after two crystallizations, **3**·D-tartaric acid as a 1:1 complex in 99% de and 32% overall yield.[6,7] Attempts to obtain the *S*-isomer more directly from the racemate upon fractional crystallization of the diastereomeric salts with D-tartaric acid were met with moderate success. Best results were obtained using 95% aqueous ethanol as solvent and 1 equiv of D-tartaric acid which gave, after seeding and one recrystallization, **3**·D-tartaric acid in 94% de and 32% yield.[6,7] Concurrent with these efforts, a screen of resolving agents was undertaken in an attempt to find a more expedient means of accessing high enantiopurity **3** (≥98% ee). An examination of several standard resolving agents at 100-mg scale revealed dibenzoyl-tartaric acid and mandelic acid to be the most promising acids with respect to crystal production in reasonable yield (ca. 30−40%).[7] Upon scale-up of these hits to 5 g of (±)-**3**, it was found that dibenzoyl-L-tartaric acid and (*S*)-mandelic acid provided diastereomeric salts enriched in the desired (*S*)-isomer of ethyl nipecotate (**3**). Using 10 volumes of refluxing 95% aqueous ethanol and 0.5 equiv of dibenzoyl-L-tartaric acid followed by slow cooling to room temperature resulted in precipitation of the corresponding salt in 38% yield.[7] The salt was shown by 500 MHz ^{1}H NMR spectroscopy to be the 2:1 complex **4**. Treatment of **4** with base followed by Mosher amide formation and analysis (GC, HPLC) indicated the diastereomeric excess to be ≥98%. Similarly, (*S*)-mandelate salt **5** was produced in 32% yield with a de of 94% using 8 volumes of ethyl acetate as solvent

Scheme 1



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(1) Elanco Animal Health is a division of Eli Lilly and Company.
(2) Creemer, L. C.; Herring, J. R.; McGruder, E. D. U.S. Patent 6,664,271, 2003.
(3) Aldrich currently offers both enantiomers of ethyl nipecotate for approximately $90/g.
(4) At the time of this work, the French company Interor offered racemic ethyl nipecotate through Interchem Corporation, U.S.A.
(5) (a) Zheng, X.; Day, C.; Gollamudi, R. *Chirality* **1995**, *7*, 90. (b) Akkerman, A. M.; De Jongh, D. K.; Veldstra, H. *Recl. Trav. Chim. Pays-Bas.* **1951**, *70*, 899.
(6) The enantiomeric purity of the free base was assessed by GC and/or HPLC analysis of the derived Mosher amides (see Experimental Section).
(7) The resolution yields disclosed herein are calculated on the basis of 100% theoretical yield.

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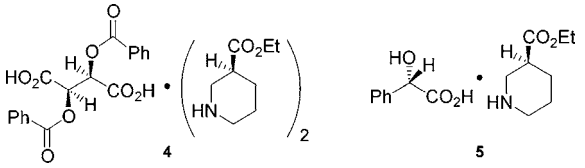
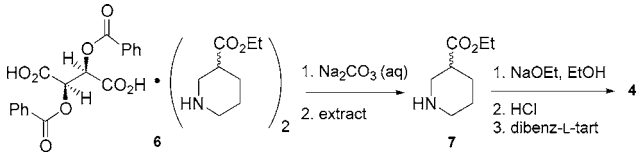
and 1 equiv of (*S*)-mandelic acid. A single recrystallization from 8 volumes of ethyl acetate provided **5** in 27% overall yield and ≥99% de.[6,7] Due to the relative efficiency, simplicity, and high diastereomeric purity with which **4** was produced, we chose the dibenzoyl-L-tartaric acid resolution method for further scale-up and use in the preparation of **1**.[8]



4 **5**

Enantiomer/Mother Liquor Recycle. With a suitable method in hand for attaining **3** via a classical resolution we examined the feasibility of recycling the (*R*)-enantiomer-enriched mother liquor waste stream of the resolution process. Accordingly, the mother liquor and filtrates were concentrated in vacuo to generate (*R*)-enriched salt **6** (Scheme 2). Treatment with aqueous sodium carbonate

Scheme 2



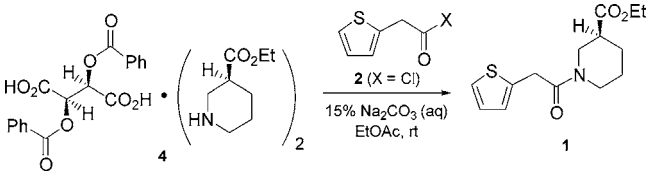
6 **7** **4**

followed by extractions with methyl *tert*-butyl ether and concentration provided (*R*)-enriched ethyl nipecotate **7**. Dissolution of **7** in ethanol and treatment with catalytic sodium ethoxide at reflux for 1 h effected complete racemization. Neutralization with concentrated hydrochloric acid, removal of sodium chloride by filtration, and treatment of the filtrate with dibenzoyl-L-tartaric acid as conducted previously provided **4** in 35% yield and ≥97% de.[6,7] It was observed that neutralization of the sodium ethoxide used for the epimerization with hydrochloric acid was necessary since neutralization with excess dibenzoyl-L-tartaric acid led to inferior quality (tacky) **3** in low yields due to interference from sodium dibenzoyl-L-tartrate. While not optimized for yield and processing efficiency, the recycle of enantiomeric **3** is indeed possible, should it prove necessary, and cost-effective relative to discarding the resolution filtrates and purchasing fresh racemate.

Amide Formation and Purification. With an efficient procedure for the resolution of ethyl nipecotate in hand our attention was turned toward fashioning the amide linkage in **1**. The first-generation procedure[2] employed commercially available 2-thiopheneacetyl chloride **2** (X = Cl) along with purchased **3** and *i*-Pr_2NEt in methylene chloride followed by extractive workup and Kugelrohr distillation to provide **1** as a light-yellow oil in 69% yield. While overall this direct coupling between an acid chloride and an amine was quite

(8) After the completion of this work, a report was published in this journal describing the efficient resolution of ethyl nipecotate with D-tartaric acid using aqueous 2-propanol as solvent. See: Cohen, J. H.; Bos, M. E.; Cesco-Cancian, S.; Harris, B. D.; Hortenstine, J. T.; Justus, M.; Maryanoff, C. A.; Mills, J.; Muller, S.; Roessler, A.; Scott, L.; Sorgi, K. L.; Villani, F. J., Jr.; Webster, R. R. H.; Weh, C. *Org. Process Res. Dev.* **2003**, *7*, 866.

Scheme 3



4 **1**

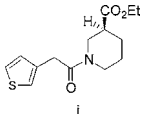
simple, we sought to use salt **4** directly in the coupling reaction and at the same time avoid the use of methylene chloride. Most gratifyingly, environmentally friendly Schotten−Baumann conditions gave excellent results. Specifically (Scheme 3), the dibenzoyl-L-tartrate salt **4** was reacted with 15% aqueous sodium carbonate followed by addition of an ethyl acetate solution of 2-thiopheneacetyl chloride[9] (**2**, X = Cl) which provided **1** as a colorless to off-white oil in near quantitative yield and high purity after silica gel chromatography. The use of sodium hydroxide in place of sodium carbonate as the base in the amidation reaction resulted in yields depressed by as much as 10−12%, primarily due to competitive acid chloride hydrolysis. The carbonate method followed by chromatography routinely produced material of greater than 99% enantiomeric and chemical purity.[10] Chromatography was introduced at this early point primarily to remove color and trace impurities which were evident by TLC.[11] Obviously, it was desirable to dispense with relying on a chromatographic purification of **1** should the project progress further in development. An attractive option was found in wiped film evaporation (WFE) using a Pope Scientific 2-in. wiped film still. Crude material from the amidation reaction was typically distilled at ∼160 °C and 0.06−0.09 Torr to provide **1** as a faint-yellow oil with 90% recovery and 98% HPLC purity.

Conclusions

A simple and efficient two-step preparation of **1** has been developed. The synthesis features a new method for the resolution of ethyl nipecotate [(±)-**3**], an enantiomer recycle,

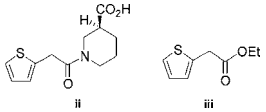
(9) For the present study, 2-thiopheneacetyl chloride was prepared from 2-thiopheneacetic acid and thionyl chloride and purified by distillation according to the following reference: Miller, W. H.; Dessert, A. M.; Anderson, G. W. *J. Am. Chem. Soc*. **1948**, *70*, 500. Subsequently, Clariant was identified as a potential bulk supplier of the acid chloride (ca. $100/kg).

(10) (a) The enantiomeric purity of **1** was determined by chiral capillary electrophoresis. (b) The chemical purity of **1** was determined by HPLC and/or GC analysis (see Experimental Section). The largest impurity, apart from enantiomer, in typical lots of **1** has been identified as 3-thiopheneacetyl isomer **i** due to trace isomer contamination of purchased 2-thiopheneacetic acid. The contamination level is consistently less than 0.5%.



i

(11) These impurities were identified as aqueous carbonate induced hydrolysis product **ii** and ethyl 2-thiopheneacetate (**iii**). The origin of **iii** has not been rigorously determined. It is suspected to arise from reaction of **2** (X = Cl) with residual ethanol present in **4**.



ii **iii**

and a high-yielding Schotten−Baumann coupling. API purification by WFE was shown to be a feasible and attractive option with long-term potential for commercial operations. A 34% yield was achieved for the overall process from readily available starting materials, not including enantiomer recycle.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-500 spectrometer or a Varian MercuryVx 400 MHz spectrometer as noted. Diastereomeric purities (% de) of the diastereomeric salts of ethyl nipecotate were determined by Mosher amide analysis using capillary gas chromatography on a Hewlett-Packard model 5890 instrument equipped with a 25 m × 0.25 mm 30M DB-1 column (initial temperature 60 °C ramped to 300 °C over 13 min, injector temperature 250 °C) with FID detection at 250 °C and by HPLC analysis on a Shimadzu SCL-10A instrument equipped with a 4.6 mm × 250 mm Zorbax SB-Phenyl column with gradient elution (1 mL/min, acetonitrile−water both with 0.5% TFA, 60% acetonitrile ramped to 90% over 25 min) and detection at 220 nm. Absolute configuration of resolved ethyl nipecotate was determined through Mosher amide formation and comparison of retention times (GC and HPLC) with Mosher amides derived from authentic **3** from Chemie S.p.A. and (*R*)-**3** obtained according to ref 5a. Analysis of the chemical purity of **1** was conducted via the above GC method as well as by HPLC using a 4.6 mm × 150 mm Zorbax SB-C18 column with gradient elution (90% water with 0.1% TFA/10% acetonitrile ramped to 80% acetonitrile/20% water with 0.1% TFA over 70 min) at a 1 mL/min flow rate and detection at 250 nm. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses, Fourier transform infrared (FTIR) spectra, and mass spectra were performed at the Structural and Organic Chemistry Research Laboratory, Eli Lilly and Company, Indianapolis, Indiana, U.S.A.

Dibenzoyl-L-tartrate Salt 4. To a three-neck, 5-L flask equipped with a heating mantle, mechanical stirrer, a temperature probe, and a reflux condenser topped with a calcium carbonate drying tube was charged 250.8 g (1.60 mol) of racemic ethyl nipecotate [(±)-**3**] followed by 2100 mL of 95% aqueous 2B-ethanol (denatured with toluene). To the solution was added 286.7 g (0.80 mol) of dibenzoyl-L-tartaric acid as a solid resulting in a temperature rise to 37 °C. Residual resolving agent was rinsed into the reaction mixture with 400 mL of 95% aqueous ethanol. The mixture was heated to 78 °C, effecting complete dissolution. The heat was turned off, and the clear yellow solution was allowed to gradually cool to 68 °C at which time seed was added followed by cooling slowly to room temperature and stirring for a total of 15 h after seeding (crystal formation was observed at 66 °C). The white precipitate was collected and washed with ethanol (1 × 400 mL) followed by vacuum-drying at 45−50 °C to provide 208 g (39%) of the title compound as a white solid: mp 173−175 °C; $[\alpha]_D$ −61 (c 1.22, MeOH), 98% de; FTIR (KBr) 3428 (m), 2996 (m), 2854 (m), 2317 (w), 1721 (s), 1623 (s), 1454 (s), 1383 (s), 1269 (s), 1196 (s), 1121 (s), 715 (s) cm^{-1}; 500 MHz ^{1}H NMR (D_2O) δ 7.96 (d, 4H, J = 8.0 Hz), 7.55 (t, 2H, J = 7.5 Hz), 7.42−7.37 (m, 4H), 5.55 (s, 2H), 4.09−3.97 (m, 4H), 3.27 (dd, 2H, J = 13, 3.5 Hz), 3.11−3.00 (m, 4H), 2.88−2.80 (m, 2H), 2.77−2.63 (m, 2H), 1.94−1.90 (m, 2H), 1.72−1.52 (m, 6H), 1.08 (t, 6H, J = 7.3 Hz); 125 MHz ^{13}C NMR (D_2O) δ 174.63, 173.58, 168.44, 134.66, 130.43, 129.52, 129.36, 75.87, 63.01, 44.71, 44.42, 38.77, 25.03, 21.23, 13.82. Anal. calcd. For $C_{34}H_{44}N_2O_{12}$: C, 60.69; H, 6.59; N, 4.18. Found: C, 60.58; H, 6.66; N, 4.27.

Epimerization/Resolution/Recycle. To ca. 100 g (wet) of concentrated resolution filtrates and mother liquors was added *tert*-butyl methyl ether (500 mL) followed by 500 mL of 15% sodium carbonate. After stirring mechanically for 1 h to effect dissolution, the layers were separated, and the aqueous layer was extracted with *tert*-butyl methyl ether (1 × 500 mL, 2 × 250 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo to give 27.0 g of (*R*)-enriched ethyl nipecotate as a yellow oil. To a solution of the above oil (10 g, 63.5 mmol) in 58 mL of 2B-ethanol (denatured with toluene) was added 5.9 mL (15.9 mmol) of a 21 wt % solution of sodium ethoxide in ethanol. After heating at reflux for 1 h, the mixture was cooled to room temperature and treated with concentrated HCl (1.31 mL, 15.9 mmol) and filtered through Celite washing with 36 mL of 2B-ethanol (denatured with toluene). The combined filtrate and washings were treated with 11.29 g (31.7 mmol) of dibenzoyl-L-tartaric acid, and the mixture was heated to reflux. Addition of 2 mL of water effected complete dissolution, after which time the mixture was allowed to cool slowly. After seeding at 64 °C the mixture was allowed to cool further to room temperature and to stir a total of 16 h. The crystals were collected and dried in vacuo at 45−50 °C to yield 7.30 g (35%) of **4** as a white solid (≥97% de).

(*S*)-Ethyl-1-(2-thiopheneacetyl)-3-piperidinecarboxylate (1). To a three-neck, 5-L flask equipped with a mechanical stirrer and a dropping funnel was charged 182 g (271 mmol) of tartrate salt **4** followed by 590 mL of ethyl acetate. To the rapidly stirring slurry was added 715 mL of water followed by 482 mL (ca. 2.5 equiv) of 15% sodium carbonate over 15 min via dropping funnel. After stirring briefly, acid chloride **2** (X = Cl)[9] (91.3 g, 568 mmol) was added as a solution in 111 mL of ethyl acetate over 10 min via a dropping funnel (mild gas evolution observed). Upon completion of the addition, the mixture was allowed to stir for 30 min at which time the layers were separated, and the aqueous layer was washed with ethyl acetate (2 × 350 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo to a yellow oil. Chromatography (1200 g of flash SiO_2, 1:1 then 1:3 hexanes/ethyl acetate) provided 150 g (99%) of the title compound as a faintly off-white oil: $[\alpha]^{22}_D$ +63 (*c* 10.8, MeOH); 400 MHz ^{1}H NMR (CD_3-OD; mixture of two rotamers) δ 1.24 (dt, J = 5.37, 7.33 Hz, 3H), 1.28−1.40 (m, 0.5H), 1.40−1.51 (m, 0.5H), 1.61−1.73 (m, 1.5H), 1.73−1.84 (m, 0.5H), 1.93−2.03 (m, 1H), 2.31−2.39 (m, 0.5H), 2.39−2.47 (m, 0.5H), 3.12 (dd, J = 13.19, 9.77 Hz, 0.5H), 3.15−3.26 (m, 1.5H), 3.52 (dd, J = 13.68, 8.3 Hz, 0.5H), 3.80−4.02 (m, 3H), 4.09−4.18 (m,

2H), 4.34 (dm, $J = 12.7$ Hz, 0.5H), 6.91−6.97 (m, 2H), 7.28 (dt, $J = 5.37$, 1.46 Hz, 1H); 100 MHz ^{13}C NMR ($CDCl_3$; mixture of two rotamers) δ 172.98, 168.70, 136.82, 127.03, 126.30, 124.97, 61.03, 60.91, 48.19, 46.91, 44.11, 42.60, 41.68, 41.35, 35.51, 35.36, 27.35, 25.01, 24.01, 14.42; FTIR ($CHCl_3$) cm^{-1} 3019 (br, m), 1725 (s), 1636 (s), 1455 (m), 1179 (m); ES-MS (Positive) 585 (dimer + Na^+), 304 (M + Na^+), 282 (MH^+); UV (EtOH) λ_{max} nm 234; Anal. calcd. For $C_{14}H_{19}NO_3S$: C, 59.76; H, 6.81; N, 4.98; S, 11.39. Found: C, 59.95; H 6.82; N, 5.10; S, 11.26.

Mosher Amide Formation for Enantiopurity Analysis. *General Procedure*. To a slurry of 55 mg (0.082 mmol) of salt **4** in 1 mL of *tert*-butyl methyl ether was added 200 μL of 15% sodium carbonate. After stirring to dissolve, the layers were separated, and the aqueous layer was washed with *tert*-butyl methyl ether (2 × 1 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated to give 22 mg (0.14 mmol, 86%) of **3** as a colorless oil. The free base was dissolved in 220 μL of CH_2Cl_2 and treated sequentially with 66 mg (0.28 mmol) of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-Mosher acid], 2 mg (0.02 mmol) of 4-dimethylamino pyridine, and 58 mg (0.28 mmol) of 1,3-dicyclohexylcarbodiimide as a solution in 60 μL of CH_2Cl_2. After stirring for 30 min the cloudy, white reaction was filtered through Celite, and the filtrate was analyzed by GC and HPLC as indicated above in the General Experimental section.

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