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Process Improvements in the Production of a Novel Non-Xanthine Adenosine A_1 Receptor Antagonist. A "One-Pot" Horner-Emmons Isomerization Reaction

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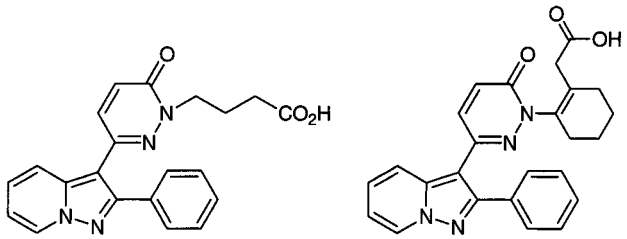
Abstract:

Pilot plant scale synthesis of 2-[3-(2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl-1(6*H*)-pyridazin-6-one)-1-cyclohexen-1-yl] acetic acid (FR166124) is described. The process involved efficient isomerization of regioisomers produced in a Horner-Emmons reaction and employed ester exchange and hydrolysis with NaOH in MeOH. Challenges encountered in the final purification stage to afford high quality drug substance in pure crystalline form are also described. Process improvements and optimization of each step permitted elimination of column chromatography, resulting in a straightforward, practical, and cost-effective synthesis of FR166124. These methods were successfully scaled up in a pilot plant to give bulk drug suitable for pharmacological and toxicological evaluation.

Introduction

In previous papers,[1] we described a pilot scale synthesis of the selective adenosine A_1 receptor antagonist, FK838 (**1**) (3-[2-phenylpyrazolo[1,5-*a*]pyridin-3-yl]-1(6*H*)-pyridazine-6-one butyric acid, Figure 1). This drug has characteristic features related to both diuretic and anti-hypertensive effects.[2] Whilst the development project of **1** was progressing, intense efforts centered on searching for new types of pyrazolo[1,5-*a*]pyridine adenosine A_1 receptor antagonist, led to the discovery of FR166124 (**2d**) as a more potent and versatile second-generation candidate.[3] To support later-stage preclinical development we were charged with efficient preparation of **2d** on a large scale.

Recently, Kuroda et al. reported a novel synthesis of **2d** using a sequential Horner-Emmons/isomerization reaction (Scheme 1).[4] Whilst efficient enough to afford the material in sufficient quantities for early preclinical evaluation, the reported methods had a number of limitations that became apparent only when conducting the process on a large scale. Significant scale-up issues were as follows: (1) low-temperature conditions in a Swern oxidation step, (2) using excess amounts of NaH in a Horner-Emmons reaction, (3) column chromatographic separation of **6d** from the isomer **6b**, (4) selective preparation of **2d** in the desired crystalline form (B-form). This paper describes findings during endeavours that resulted in a reliable and rugged process suitable for a large scale synthesis.



FK838, 1 **FR166124, 2d**

Figure 1. **Structure of adenosine A_1 receptor antagonists.**

Results and Discussion

Pyridazone (**3**) was prepared from 4-phenyl-3-butyn-2-one and pyridine *N*-imine by the methods described in our previous paper.[1b] Ketone (**5a**) was obtained on a laboratory scale by alkylation of **3** with cyclohexene oxide in a mixture of toluene and water under reflux for 16 h, followed by oxidation. However, the long reaction for the alkylation time was an important issue for a large-scale synthesis. During further investigations it was found that the reaction rate was significantly affected by solvent volume, regardless of the presence of a phase-transfer catalyst. Thus, the reaction was complete in 6 h when solvent volumes were reduced by half. At the end of the reaction, water was added to a reaction mixture to dissolve a precipitated trace amount of sodium salt of unreacted pyridazone (**3**) in the aqueous layer, and the mixture cooled to 0 °C. The major by-product **4b** remained dissolved in the organic layer (Scheme 2). Thus, these impurities were cleanly removed in mother liquor (**3** in the aqueous layer and **4b** in the organic layer) by filtration, leading to alcohol (**4a**) in excellent quality. For the subsequent oxidation step, ketone (**5a**) was effectively prepared by Swern oxidation on a laboratory scale, but very low-temperature conditions (∼ −60 °C) were not acceptable for a pilot plant synthesis. As an alternative and improved oxidation system, we examined the Parikh−Doering reac-

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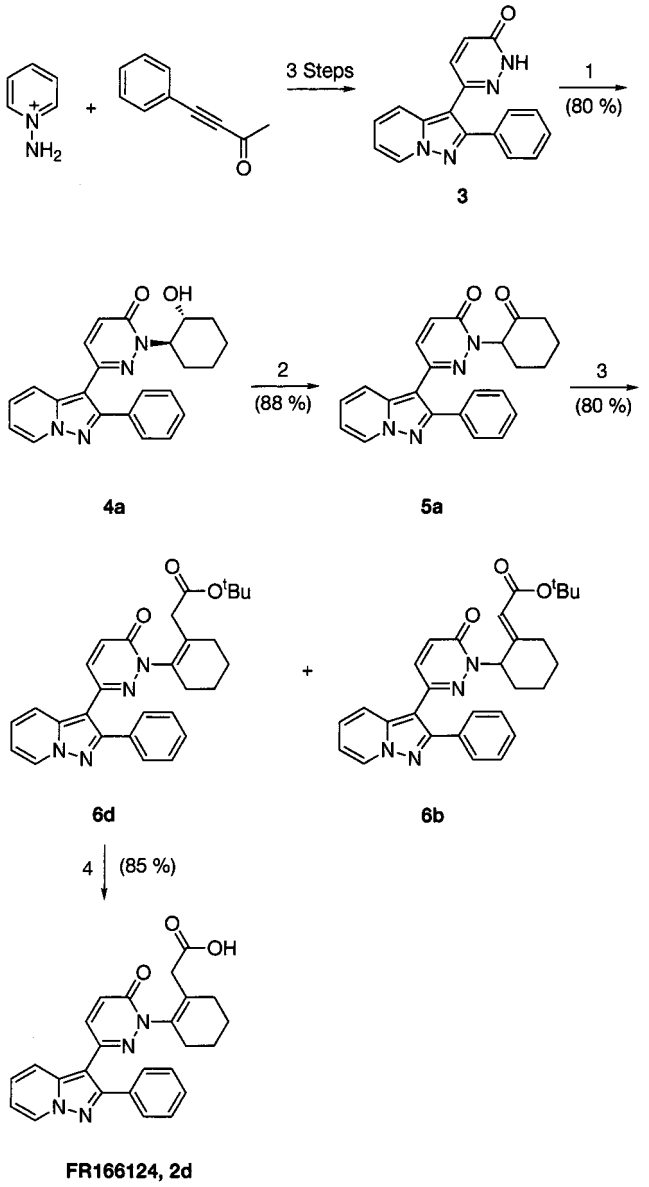
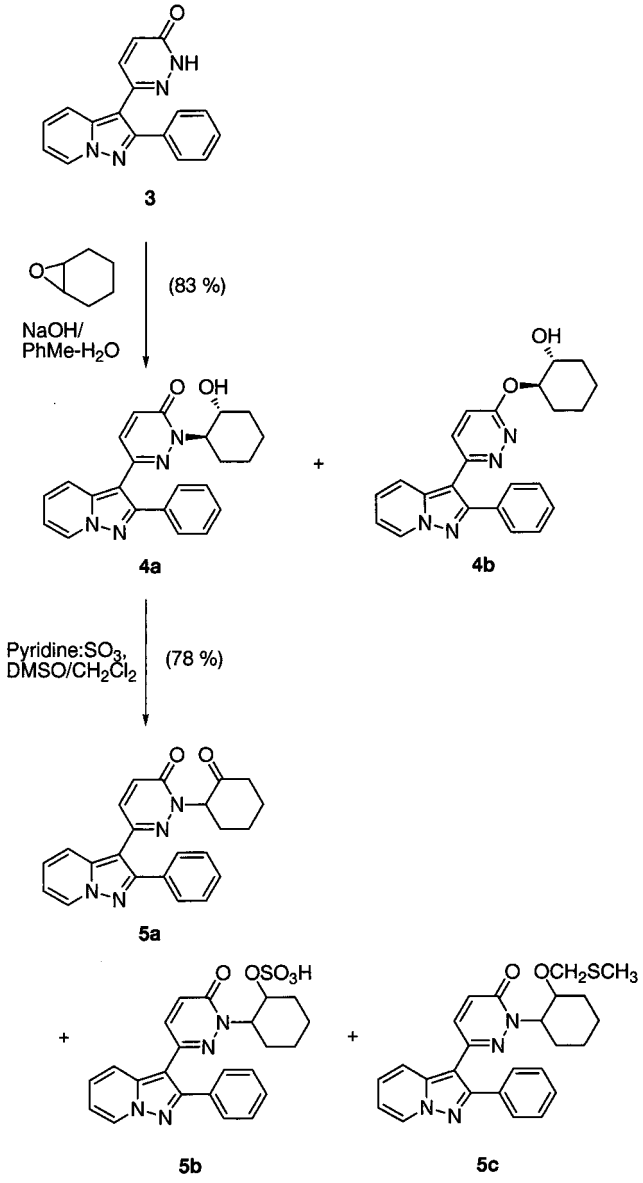
Scheme 1. **Laboratory scale route to 2d**[a]



[a] Reagents and conditions: (1) cyclohexene oxide, NaOH/PhMe-H_2O, PTC; (2) Swern oxidation; (3) $(EtO)_2POCH_2CO_2{}^tBu$, NaH/toluene; (4) TFA/CH_2Cl_2.

Scheme 2. **Synthetic route to 5a**



tion.[5] Reaction proceeded smoothly, although two minor by-products were obtained which were presumed to be sulfonated material (**5b**) and methylthiomethyl ether (MTM ether, **5c**). Further studies indicated that formation of **5b** was influenced by reaction temperature and the molar ratio of pyridine:SO_3 and Et_3N. Whilst formation of **5c** was controlled in the presence of excess Et_3N, a large excess led to a reduction in reaction activity. Thus, the optimized reaction conditions were identified as pyridine:SO_3 complex (2 molar equiv) and Et_3N (4 molar equiv) at 3−8 °C in CH_2Cl_2 as solvent. Other methods such as Ac_2O-DMSO[6] produced significant amounts of **5c**.

With an efficient preparation of the ketone (**5a**) in hand and the material available in large quantities, subsequent Horner-Emmons reaction of the ketone (**5a**) with phosphonates, followed by isomerization to **6d** were investigated. First, we examined triethyl phosphonoacetate since this reagent is inexpensive and readily available in large quantities; however, the reaction furnished a mixture of hydrolyzed materials (**2a**−**d,** Scheme 3).

On the other hand, Kuroda et al. reported an efficient synthesis of **6d** applying *tert*-butyl diethylphosphonoacetate. The mechanism of isomerization was clarified in the report, and the authors emphasized that the excess use of both *tert*-butyl diethylphosphonoacetate and NaH as a base in toluene was crucial for efficient isomerization.[4] Despite these discoveries, there still existed obstacles which had to be removed before a fully efficient chemical process was realized. For example, the long reaction time (24 h) was problematic, and furthermore, gummy by-products were present in the reaction mixture, which contained unreacted sodium hydride. After consideration of the pyrophoric hazards associated with sodium hydride, the base employed was changed. First, a mixture of granulated sodium hydroxide and powdered potassium carbonate was examined as an

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Scheme 3. **Horner-Emmons reaction of 5a**

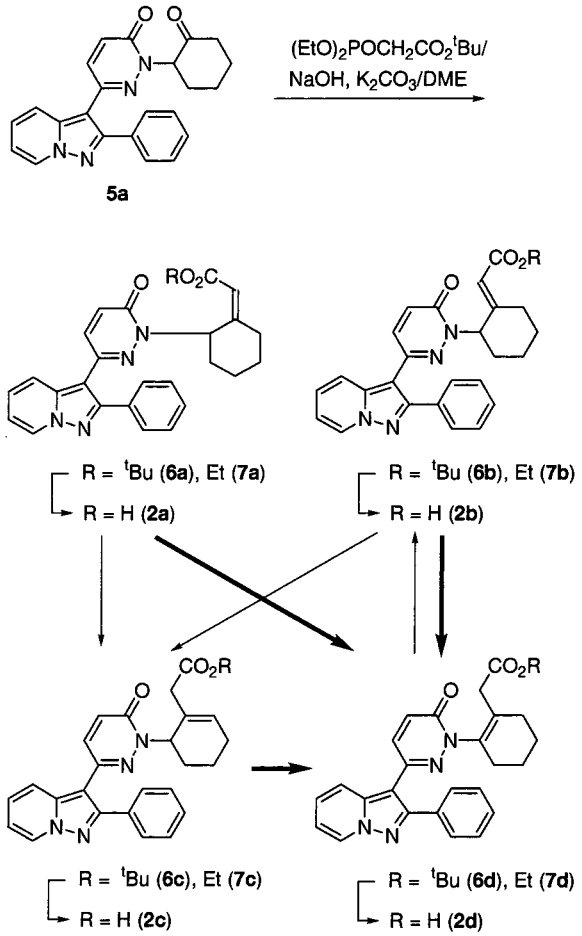


Table 1. **Horner-Emmons reaction of ketone (5a) and *tert*-butyl diethylphosphonoacetate[a]**

entry	base (equiv)	solvent	temp (°C)	time (h)	product ratio[b] 5a	2[c]	6a	6b	6c	6d
1	NaH (1.5)	toluene	25	24	0	0	20	18	0	62
2	NaOH (2.0), K_2CO_3 (2.0)	DMSO	50	2	0	0	7	8	9	76
3	NaOH (2.0), K_2CO_3 (2.0)	DME	25	2	0	0	47	15	0	38
4	NaOH (2.5), K_2CO_3 (2.5)	DME	25	2	0	3	33	13	0	50
5	NaOH (2.5), K_2CO_3 (2.5)	DME	25	5	0	6	0	12	1	81
6	NaOH (4.0), K_2CO_3 (4.0)	DME	25	2	0	8	15	15	0	63
7	NaOH (4.0), K_2CO_3 (4.0)	DME	25	5	0	10	1	11	0	78
8[d]	NaOH (2.5), K_2CO_3 (2.5)	DME	25	5	0	1	25	15	1	58
9[d]	NaOH (2.5), K_2CO_3 (2.5)	DME	25	8	0	3	6	12	1	78
10[e]	NaOH (2.5), K_2CO_3 (2.5)	DME	25	8	0	9	3	8	2	78

[a] 1.5 equiv of *tert*-butyl diethylphosphonoacetate. [b] Product ratio was determined by quantitative HPLC using authentic samples.[4] [c] A mixture of **2a**, **2b**, **2c**, and **2d**. [d] 'Scale-down experiment'. [e] Pilot plant scale synthesis.

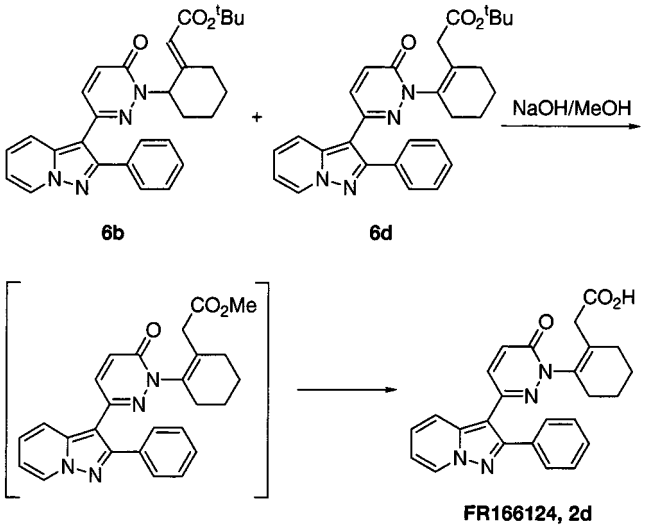
alternative base. Interestingly, whilst the reaction conducted in DMSO as solvent afforded a mixture of four kinds of isomers (**6a**−**d**, Table 1, entry 2), **6c** was not present in DME as solvent (entry 3). Furthermore, after optimization of the amount of base and reaction time, a mixture of **6b** and **6d**

Table 2. **Regioselective isomerization and hydrolysis of 6a−d[a]**

Entry		solvent	product ratio[b] 6a → 2a	6b → 2b	6c → 2c	6d → 2d
	ester		0	9	5	86
1	acid	DME	0	10	1	89
2	acid	MeOH	0	1	1	98
	ester		6	12	1	81
3[c]	acid	MeOH	0	1	1	98
	ester		23	15	1	59
4	acid	MeOH	0	2	1	97
	ester		3	9	2	86
5[d]	acid	MeOH	0	1	1	98

[a] In the presence of 3 equiv of NaOH at 55 °C. [b] Ratio determined by HPLC. [c] 'Scale-down experiment'. [d] Pilot plant scale synthesis.

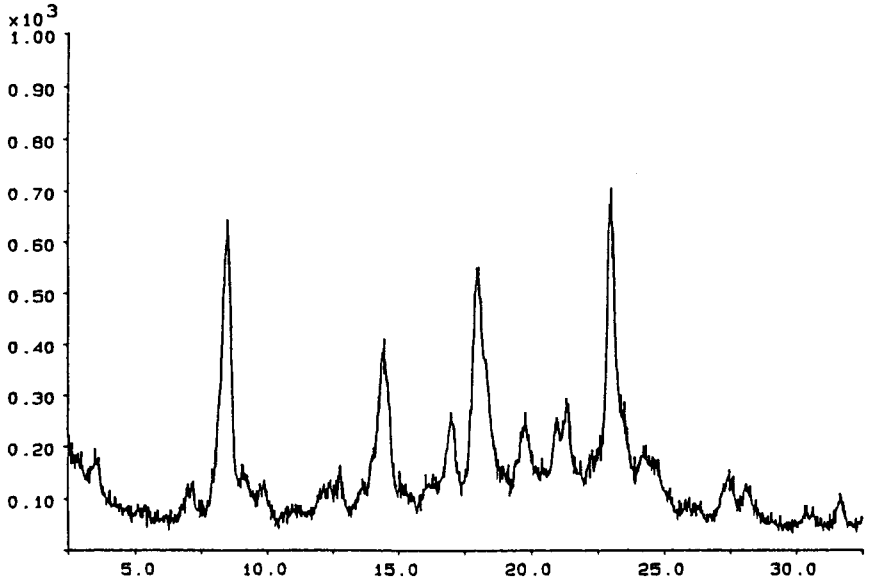
Scheme 4. **Isomerization and hydrolysis of a mixture of 6b and 6d to FR166124, 2d**



was selectively obtained (entry 5). These unexpected results indicated that the presence of excess molar amounts of granulated NaOH and powdered K_2CO_3 are crucial for the isomerization. Whilst using an excess amount of base enhanced the isomerization rate (entry 3, 4, 6), a very large excess increased the saponified materials (**2a**−**d**, entry 6, 7). Thus, optimized reaction conditions were identified as in entry 5. Small amounts of hydrolyzed by-products (**2a**−**d**) were not of serious concern since they were cleanly removed in the extraction.

Next, we investigated a practical purification of **6d**. On a scale of less than 100 g, **6d** was separated from the mixture using column chromatography, however, the required amount of silica gel was large and thus precluded implementation of this approach on a large scale. Despite further efforts with a goal of selective "one-pot" preparation of **6d**, the ratio of **6b** and **6d** was not influenced to any significant degree by changing reaction conditions. Control experiments showed that isolated **6b** afforded an approximately 1:8 mixture of **6b** and **6d** with trace amounts of **6c** when exposed to the reaction conditions. These results indicated that **6b** and **6d** might be in an equilibrium state (Scheme 3).

Chart 1. **X-ray powder diffraction analysis of A-form 2d**



During further studies, it was found that the esters (**6a**−**d**) were completely hydrolyzed to acids (**2a**−**d**) under elevated temperature conditions (55 °C) in the presence of excess granulated NaOH (3 equiv), as shown in Table 2. Furthermore, when the reaction was conducted in MeOH as solvent, **2d** was selectively obtained (entry 2). To understand the mechanism of this unexpected isomerization, **6b** and a mixture of **6b** and **6d** were exposed to MeONa in MeOH to afford a mixture of 25% of **2d** and 75% of the precursor methyl ester of **2d** in both cases. Control experiments indicated that no isomerization occurred when exposing hydrolyzed materials (**2b** or a mixture of **2b** and **2d**) to these conditions. Thus, isomerization presumably occurs after ester moiety exchange from *tert*-butyl to methyl, followed by hydrolysis to give selectively **2d** (Scheme 4).

Although a practical and efficient preparative method of **2d** was established, this process is promising only when the isomerization is much faster than hydrolysis of the precursor esters. Therefore, we had to investigate the reproducibility for a proposed pilot plant scale trial. In effect, reactions can be influenced by impeller shape or agitation speed, and poor stirring efficacy of pilot plant vessels sometimes leads to unexpected results. These results are sometimes effectively foreseen by a "scale-down experiment" which is conducted using the same shape equipment as in the pilot plant under conditions where power consumption per unit volume is constant.[7] As shown in Table 1, the scale-down experiment indicated that small amounts of isomer **6a** may remain unisomerised when the reaction was conducted in a pilot plant (Table 1, entry 8, 9). However, this was not a serious concern, since this reaction system proved to be highly successful for direct and selective preparation of **2d**, even from a mixture of **6a, 6b**, and **6d** (Table 2, entry 3, 4).

Final purification of crude **2d** required significant elaboration to realize suitable quality material. As shown in Chart 1 and Chart 2, two kinds of crystalline forms of **2d** [A-form (metastable form), B-form (stable form)] were possible, and pure and selective preparation of B-form crystals was required.

During early investigations, it was found that B-form **2d** was selectively precipitated from a high content (>66%) of ethanol in water; however, this method involved considerable loss in the mother liquor (Table 3, entry 4), and with a large amount of **2b** (0.75%) or residual ethanol (0.5%) in the purified **2d** (entry 3, 4). On the other hand, **2d** was more efficiently purified by recrystallization from 50% ethanol in water; however, the obtained material was a mixture of A- and B-forms (entry 1, 2). In general, crystalline form is effectively controlled in many cases by addition of a seed possessing the desired crystalline form;[8] however, the required amount for selective precipitation of the B-form of **2d** was large and prevented implementation on a large scale. Extensive studies provided the following practical solution to these problems. Crude **2d** was dissolved in the presence of NaOH in a mixture of ethanol and purified water. The resulting solution was filtered to remove solid impurities, and the solution was acidified with hydrochloric acid (to give an ethanol concentration of 66%), leading to selective crystallization of **2d** in the desired form (B). The resulting slurry was heated to reflux and then cooled and further diluted with ethanol and water to adjust the conditions to that for entry 2 (50% ethanol in water), allowing efficient and selective removal of isomer **2b** in the mother liquor. At this stage, B-form **2d**, which had precipitated in the previous step, acted as a seed, and avoided crystallization of the A-form **2d**. To improve filtration, the mixture was again refluxed, followed by cooling to 23−27 °C over 1 h and then to 0 °C. The precipitate was collected and dried under reduced pressure to afford pure B-form material in high chemical purity, suitable for pharmacological and toxicological evaluation.

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Chart 2. **X-ray powder diffraction analysis of B-form 2d**

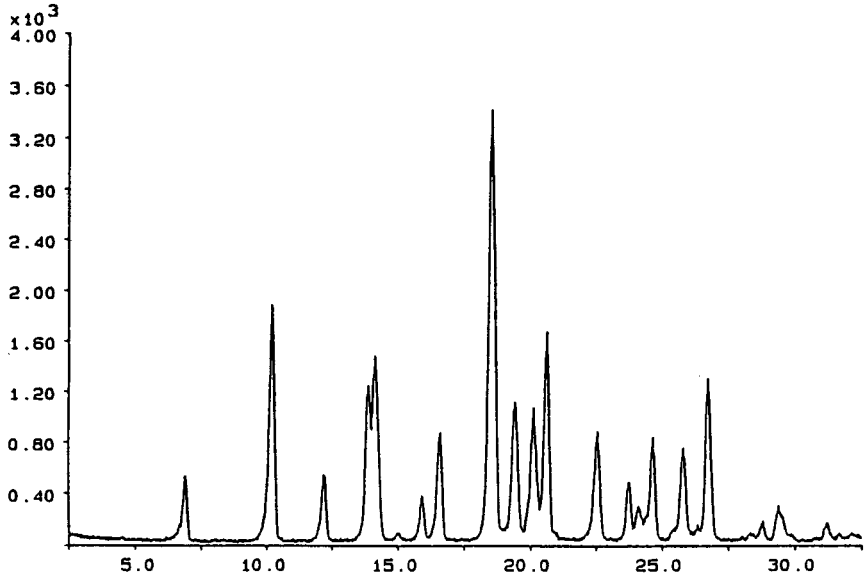

Table 3. **Recrystallization of crude 2d from aqueous ethanol**[a]

entry	solvent (mL)	yield (%)	**2b** (%)[b]	residual EtOH (%)	crystalline form(s)[c]
1	50% EtOH (50)	97.0	0.62	0.1	a mixture of A and B
2	50% EtOH (200)	91.0	0.24	0.1	a mixture of A and B
3	66% EtOH (75)	89.4	0.75	0.2	B
4	80% EtOH (125)	64.4	0.19	0.5	B

[a] Crude **2d** (mixture of A- and B-forms) was contaminated with 1.1% of **2b** recrystallization was conducted on a 5 g scale of crude **2d**. [b] Quantity of **2b** contaminated in purified **2d** was determined by quantitative HPLC. [c] Crystalline form was determined by X-ray analysis.

Conclusions

In this paper we have described process development of the novel non-xanthine adenosine A_1 receptor antagonist FR166124, **2d**. Horner-Emmons reaction using *tert*-butyl diethylphosphonoacetate, followed by ester moiety exchange and hydrolysis in the presence of granulated NaOH in MeOH as solvent, resulted in a successful selective isomerization. The drug substance of pure crystalline form and high chemical purity was obtained in excellent yield by controlling the recrystallization conditions. Process improvement efforts for each step, focusing on safe and optimized reaction conditions resulted in a chromatography-free, inexpensive, and practical synthesis of **2d** applicable to a pilot plant scale.

Experimental Section

General Procedures. Pure grade *tert*-butyl diethylphosphonoacetate is available from Jyohoku Chemical Co. Cyclohexene oxide was from Toray Industries, Inc. Pyridine sulfur trioxide complex was from Saeurefabrik Schweizerhall Co. All other chemicals were obtained from the usual commercial suppliers. IR spectra were recorded on a HORIBA FT-210 spectrometer. NMR spectra were measured on a Brucker AC200P (^{1}H, 200 MHz). Chemical shifts were given in parts per million, and tetramethylsilane was used as the internal standard. Mass spectra were measured on a Hitachi model M-80 mass spectrometer using EI for ionization. Elemental analyses were carried out on a Perkin-Elmer 2400 CHN Elemental Analyzer. HPLC analyses were performed using a YMC GEL ODS 120 Å S-5 column and an acetonitrile/water or methanol/water mobile phase and detection at 254 nm. Purity of each obtained product was determined by comparison with purified authentic samples using quantitative HPLC. Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. X-ray powder diffraction analyses were performed using a Philips MPD 1880 X-ray Powder Diffraction System.

3-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl-1(6*H*)-pyridazin-6-one)-1-cyclohexan-2-ol (4a). To a solution of pyridazone (**3**) (34.0 kg, 118 mol) and NaOH (4.72 kg, 118 mol) in a mixture of water (170 L) and toluene (170 L) was added cyclohexene oxide (34.7 kg, 354 mol). After completion of the addition, the resulting mixture was refluxed for 6 h and then cooled to 76 °C. To this solution was added water (170 L) and cooled to 0 °C. The precipitate was filtered off, washed with water (68 L) and toluene (68 L), and dried under reduced pressure to afford **4a** (37.6 kg, 83% yield) of 95% chemical purity as a yellowish solid: mp 226−228 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.32−1.62 (m, 3H), 1.59 (d, 3H, J = 12.2 Hz), 2.21 (d, 1H, J = 12.1 Hz), 2.67 (d, 1H, J = 6.9 Hz), 3.97 (t, 1H, J = 4.0 Hz), 4.94 (td, 1H, J = 10.8, 4.3 Hz), 6.78 (d, 1H, J = 9.6 Hz), 6.90 (td, 1H, J = 6.9, 1.3 Hz), 7.02 (d, 1H, J = 9.6 Hz), 7.44 (t, 1H, J = 3.7 Hz), 7.56−7.63 (m, 2H), 7.94 (d, 1H, J = 8.9 Hz), 8.53 (d, 1H, J = 6.9 Hz); IR (KBr) 2928, 2854, 1652, 1631, 1586, 1522 cm^{-1}; MS (EI) m/z 387 $(M + H)^+$, 369, 289. Anal. Calcd for $C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N,14.50. Found: C, 71.12; H, 5.75; N,14.37.

3-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl-1(6*H*)-pyridazin-6-one)-1-cyclohexan-2-one (5a). To a solution of the alcohol (**4a**) (37.6 kg, 97.3 mol) and Et_3N (39.5 kg, 390 mol) in a

mixture of CH_2Cl_2 (188L) and DMSO (376 L) was added pyridine sulfur trioxide complex (31.0 kg, 195 mol) at 3−8 °C, and stirring was continued at ambient temperature for 4 h. Methylene chloride (752 L) and water (942 L) were added to an extraction vessel, and to this mixture was added the reaction mixture. The separated organic layer was concentrated to ∼376 L under reduced pressure. To this residual organic solution was added ethanol (376 L) and then reconcentrated to ∼376 L under reduced pressure. To obtain **5a** in high quality and facilitate the filtration, the precipitate was dissolved at 82 °C and the solution cooled to 50 °C followed by addition of purified **5a** (38g). After the precipitation of **5a**, stirring was continued at ambient temperature for 30 min and cooled to 0 °C for 2 h. The precipitated **5a** was filtered off, washed with cooled (<10 °C) ethanol (76 L), and dried under reduced pressure to afford **5a** (29.0 kg, 78% yield) of 99% chemical purity as a yellowish solid: mp 182−183 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.72−2.26 (m, 4H), 2.34−2.70 (m, 4H), 5.79 (dd, 1H, J = 11.7, 7.0 Hz), 6.79 (d, 1H, J = 9.7 Hz), 6.91 (td, 1H, J = 6.2, 1.4 Hz), 7.04 (d, 1H, J = 9.7 Hz), 7.28 (td, 1H, J = 6.3, 1.5 Hz), 7.43−7.47 (m, 3H), 7.61−7.66 (m, 2H), 7.88 (dd, 1H, J = 7.8, 1.1 Hz), 8.52 (dd, 1H, J = 6.0, 0.9 Hz); IR (KBr) 1714, 1661, 1632, 1588, 1527 cm^{-1}; MS (EI) m/z 385 $(M + H)^+$, 355, 289, 236. Anal. Calcd for $C_{23}H_{20}N_4O_3$: C,71.86; H,5.24; N,14.57. Found C,71.90; H, 5.27; N,14.53.

2-[3-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl-1(6*H*)-pyridazin-6-one)-1-cyclohexen-1-yl] Acetic Acid (FR166124, 2d). To a mixture of *tert*-butyl diethylphosphonoacetate (14.8 kg, 58.7 mol), fine granulated NaOH (3.90 kg, 97.5 mol), potassium carbonate (13.5 kg, 97.5 mol) in DME (113 L) was added **5a** (15.0 kg, 39.0 mol) at 15 °C. The reaction was continued for 5 h at 15−20 °C. The reaction mixture cooled to 10 °C, and to the reaction mixture was added methylene chloride (113 L) and purified water (150 L) at <20 °C. The organic layers were separated and washed with purified water (75 L) and brine (75 L) and concentrated to ∼30 L. To this solution was added MeOH (60 L) and then concentrated to ∼30L under reduced pressure. In another vessel, NaOH (4.68 kg, 117 mol) was dissolved in MeOH (45 L), and to this solution was added dropwise the previous solution maintaining the temperature below 45 °C. After stirring at 55 °C for 8 h, the reaction mixture was cooled to 25 °C, followed by addition of purified water (300 L) and *n*-heptane (75 L). To the separated aqueous layer was added methylene chloride (300 L), followed by adjusting to pH = 1.8−2.0 with 18% hydrochloric acid (∼21 L). The organic layer was separated and washed with saturated sodium bicarbonate in purified water (135 L). The separated organic layer was concentrated to ∼150 L under reduced pressure, followed by addition of ethyl acetate (180 L) and then concentrated again to ∼150 L. To obtain **2d** in high quality, the precipitate was dissolved by refluxing and then cooled to 0 °C. After the precipitation of **2d**, stirring was continued at ambient temperature for over 1 h. The precipitated **2d** was filtered off, washed with cooled (<10 °C) ethyl acetate (30 L), and dried under reduced pressure to afford crude **2d** (12.7 kg, 79% yield) of 98% chemical purity as a white solid. The crude **2d** (12.7 kg, 29.8 mol) was added to a solution of NaOH (1.25 kg, 31.3 mol) in a mixture of purified water (58 L) and ethanol (130 L) and purified by passing through a 0.6 μm filter. The filtrate was adjusted to pH = 3.9−4.2 by 30% hydrochloric acid (∼6.5 L) at 25 °C. This mixture was then heated to reflux for 30 min. During this procedure the precipitate was dissolved at 65 °C and again precipitated at ∼80 °C. The mixture was then cooled to 65 °C, followed by addition of purified water (195 L) and ethanol (130 L) at ambient temperature, and then refluxed for 30 min. The slurry was stirred at 25 °C for 1 h and cooled to 0 °C. Stirring was continued at ambient temperature overnight, and the precipitate was filtered off, washed with cooled (<10 °C) ethanol (13 L) and purified water (13 L), and dried under reduced pressure to afford purified **2d** (12.2 kg, 96% yield) of 99% chemical purity as a white solid: mp 147−148 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85−1.92 (m, 4H), 2.32−2.52 (m, 4H), 2.92 (d, 1H, J = 14.4 Hz), 3.14 (d, 1H, J = 14.1 Hz), 6.90 (d, 1H, J = 9.7 Hz), 6.92 (t, 1H, J = 7.0 Hz), 7.13 (d, 1H, J = 9.7 Hz), 7.35 (td, 1H, J = 6.8, 1.0 Hz), 7.47 (t, 3H, J = 2.9 Hz), 7.55−7.60 (m, 2H), 7.93 (dd, 1H, J = 7.8, 1.1 Hz), 8.26 (dd, 1H, J = 6.9, 1.0 Hz); IR (KBr) 2938, 1725, 1634, 1580, 1532, 1495 cm^{-1}; MS (EI) m/z 427 $(M + H)^+$, 409, 361, 289. Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H,5.20; N, 13.14. Found: C, 70.57; H, 5.27; N, 13.11.

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