Sustainable synthesis of enantiopure fluorolactam derivatives by a selective direct fluorination – amidase strategy†

Nicky J. Willis,a Craig A. Fisher,b Catherine M. Alder,a Antal Harsanyi,b Lena Shukla,*a Joseph P. Adamsa and Graham Sandford*b

Pharmaceutically important chiral fluorolactam derivatives bearing a fluorine atom at a stereogenic centre were synthesized by a route involving copper catalyzed selective direct fluorination using fluorine gas for the construction of the key C–F bond and a biochemical amidase process for the crucial asymmetric cyclisation stage. A comparison of process green metrics with reported palladium catalyzed enantioselective fluorination methodology shows the fluorination-amidase route to be very efficient and more suitable for scale-up.

Introduction

Enzyme catalysed reaction of functional fluoromalonate building blocks, prepared using fluorine gas, has been used for the first time for the enantioselective synthesis of a pharmaceutically important chiral fluorolactam derivative. An inexpensive, highly economically competitive, lower waste stream process that does not rely on precious metal catalysis and has been quantified by green metric analysis is described.

The synthesis of chemical intermediates bearing a fluorine atom at a stereogenic centre is becoming increasingly important for applications across the materials and life-science sectors.1 While fluoroaromatic derivatives appear as sub-units in many commercially valuable pharmaceutical products,2 there are far fewer fluorinated drugs on the market where a single fluorine atom is attached to an sp³ carbon, apart from several anti-inflammatory fluoroesteroid derivatives.3 One reason for the relative lack of commercial products that bear fluorine at a stereogenic centre is the often very difficult synthesis, but much progress in the field of enantioselective chemical fluorination has been made in recent years.4 Fluorination of positions α to a carbonyl group by an electrophilic fluorination process is a common approach to the synthesis of enantiopure fluorinated systems and various Selectfluor™, cinchona alkaloid combinations,5 palladium or zinc catalysed processes using N-fluorobenzenesulphonamide (NFSI),6 organocatalyst-fluorinating agent combinations7 and chiral fluorinating agents based upon Selectfluor™-type derivatives8 have been devised and successfully implemented to give a range of enantiopure fluorinated building blocks (Scheme 1). Whilst these chemical approaches can be very valuable at the discov-

Scheme 1. Examples of reagent combinations used for the synthesis of enantiopure systems with fluorine located at a stereogenic centre.
ery stage of a medicinal chemistry process, the application of chemical enantioselective fluorination strategies at larger scale is severely hampered by the usually prohibitive expense of the reagent-ligand combinations and the large waste streams generated.

Pharmaceutical companies are increasingly concerned about the environmental impact of their commercial products and, for example, GSK recently announced an environmental strategy with the objective that the company’s operations become carbon neutral by 2050. Additionally, the European Federation of Pharmaceutical Industries and Associations (EFPIA) continues to develop the Eco-Pharmaco-Stewardship (EPS) proposal to develop methods to minimise the effect of pharmaceuticals within the environment including in the development and manufacturing stages.

Consequently, highly efficient low-waste synthetic processes for pharmaceutical manufacture are required to meet the industry’s ambitious environmental goals. Therefore, methods for assessing the efficiency and amount of waste generated by a synthetic strategy are used, in part, to identify a suitable final process for pharmaceutical manufacture. Green metrics packages allow a holistic comparison between potential synthetic reaction pathways using a mixture of quantitative and qualitative assessment criteria. Calculations of total process mass intensity (PMI) enables the synthetic chemist to simply compare the environmental effect of competing synthetic strategies from common starting materials, thus aiding the selection of the final preparative route.

A series of pre-clinical candidate spleen tyrosine kinase (Syk) inhibitors have been synthesised from chiral fluoro-lactam building blocks (Scheme 2). General synthetic procedures for the preparation of enantiopure 2-fluoro-1,3-amidoesters are relatively rare and are limited to enantioselective fluorination of malonate esters using NFSI with Zn(OAc)$_2$/DBFOX-Ph catalyst followed by amide formation, ligand catalysed chiral alkylation of fluoromalonate derivatives followed by amide formation or fluorination using NFSI with chiral palladium catalysis. The NFSI–palladium catalysis protocol reported by Sodeoka was adopted for scale up and 2a was synthesised on 100 g scale (Scheme 3). However, the route requires a structurally complex palladium catalyst prepared by multi-step procedures and purification of the desired enantiomer by time-consuming chiral HPLC due to the relatively low 44% ee obtained for the fluorination stage when performed on the large scale.

Results and discussion

Our assessment of the reported synthesis of 2 (Scheme 3) using green metric analysis (SI-2†), shows that the single-step enantioselective fluorination reaction has an estimated calculated process mass intensity (PMI) value of 925 (SI-2†). Inspection of each stage of the synthetic route shows that most waste is generated in the key enantioselective fluorination stage because, of course, NFSI is synthesised by reaction of the corresponding sulfonamide with fluorine gas, which must be taken into account when calculating PMI measurements, and loss of material due to the low ee and subsequent resolution. We assumed that all solvent used in the HPLC resolution was recycled and the waste generated in the multi-step synthesis of the palladium catalyst was not included in the PMI calculation. Consequently, the PMI 925 is a low estimate and offers a reasonable benchmark for process development.

As an alternative synthetic strategy, initially we investigated the synthesis of related fluoro-lactam derivative 2b (R = Me) using a combined chemical and biochemical synthetic approach from fluoromalonic acid ester starting materials (Scheme 2). While enzyme catalysed asymmetric hydrolysis of various fluoromalonic derivatives have been developed, no asymmetric amidase reactions of fluoromalonic derivatives have been reported.
Fluoromalonate ester 4a is synthesised in the high yield direct fluorination reaction of dimethyl malonate ester using fluorine gas catalysed by copper nitrate in acetonitrile solution.\(^{18a}\) Recently, we described the optimisation of this process which is routinely carried out on the 50 g scale and assessed to have a mass intensity MI = 9 (Scheme 4).\(^{18b}\)

Initial unoptimised synthesis of a range of racemic monofluorinated functional precursors 4 for subsequent enzymatic transformation reactions were carried out. Michael addition of acrylonitrile\(^{19}\) to fluoromalonate 4a gave the desired nitrile 4b in 90% yield and subsequent reduction of the nitrile group of 4b by hydrogen over palladium enabled the isolation of salt 4c. Base catalysed ring closure gave racemic fluorolactam 4d (Scheme 4, SI-1.2\(^{†}\)). With products 4b–d in hand we began attempts to resolve each fluorinated intermediate by appropriate enzymatic methods to identify the most effective synthetic sequence for the large scale synthesis of the desired enantiopure chiral fluorolactam 2b.

Initially, hydrolase catalysed resolution of 4b was attempted adapting literature protocols.\(^{20}\) However, nitrile 4b was unstable in mildly basic aqueous media (pH 7.0–7.1) and so this approach was discounted as a viable starting material for desymmetrisation (SI-1.3\(^{†}\)).

Attempted hydrolase promoted amidation in anhydrous tertiary amyl alcohol as the solvent\(^{21}\) gave only racemic product 4d from salt 4c using various enzyme catalysts (SI-1.4\(^{†}\)). After determining that 4d does not hydrolyse in aqueous phosphate buffer (pH 7.3) to the corresponding acid at 20–25 °C over 16 hours (SI-1.4\(^{†}\)), enzymatic transformations of 4c were explored in this aqueous buffered medium and, indeed, 4d could be resolved by a variety of hydrolases. Following an initial screening process of 56 enzymes (SI-1.5\(^{†}\)), 25 promising hydrolases that afforded 10–60% hydrolysis of 4d in 8 hours were analysed further (SI-1.5\(^{†}\)). A number of highly enantioselective processes were observed (Table 1) giving both acids 5a,b by hydrolysis (SI-1.6\(^{†}\)) and the corresponding esters 2b,c as reaction products. Both 2b and 2c were isolated by preparative scale HPLC (SI-3\(^{†}\)) and their structures and absolute stereochromies confirmed by X-ray crystallography (Fig. 1, SI-4\(^{†}\)).

CAL-B 10 000 is a recombinant Candida Antartica Lipase B that is commercially available from Fermase and used to catalyse a range of biotransformations on the large scale.\(^{22}\) Since inexpensive CAL-B 10 000 affords the desired fluorolactam (S)-2b (entry 4, Table 1), and is available for purchase on the multi-kilogram and tonne scale, this hydrolase was selected for further reaction optimisation. The possibility of telescoping the formation and resolution of 2b from salt 4c was explored to reduce the work-up process. Initially when 4c was added to buffer solution at room temperature to form a 25 mM solution, we observed that the pH reduced from 7.3 to 6.7 after 15 minutes and that no side-reactions or degradation could be detected. However, when the pH of the solution was readjusted to 7.3 by addition of 2 N NaOH (0.92 equiv.), \(^{19}\)F NMR spectroscopic and chiral HPLC (SI-3\(^{†}\)) analysis of the crude reaction product

### Table 1  Initial hydrolase resolution screening of 4d

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hydrolase</th>
<th>Conv. %(^{a})</th>
<th>Acid 5 ee %</th>
<th>Ester 2 ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>JM X14</td>
<td>30</td>
<td>&gt;95</td>
<td>(S)-5a 62</td>
</tr>
<tr>
<td>2</td>
<td>JM X35</td>
<td>19</td>
<td>&gt;95</td>
<td>(S)-5a 19</td>
</tr>
<tr>
<td>3</td>
<td>JM X50</td>
<td>28</td>
<td>&gt;95</td>
<td>(S)-5a 37</td>
</tr>
<tr>
<td>4</td>
<td>CAL-B 10 000</td>
<td>51</td>
<td>&gt;95</td>
<td>(R)-5b &gt;95</td>
</tr>
</tbody>
</table>

\(^{a}\) Calibrated UPLC-MS conversion.
Consequently, reactions in which a short series of environment-
the presence of copper nitrate and HF in the reaction mixture.
alkylation reaction occurred due to problems associated with
the Michael addition reaction between the crude direct fluori-
any work-up after the fluorination stage was explored. Firstly,
possibility of carrying out the subsequent Michael addition
selective fluorination strategy (Scheme 6).

| Scheme 6 | Optimised synthesis of 2b. |

 mixture indicated full conversion to the desired enantiopure lactam 2b in 47% yield and 98% ee (Scheme 5, SI-1.7†).

The most operationally simple experimental protocol for
the transformation of 4c to 2b would be to add 4c in one
portion to the reaction mixture and then adjust the pH to 7.3. Unfortunately, at 250 mM concentrations, the solution became
too acidic (pH 4.9) and hydrolysis by-products were formed.
This issue was, however, resolved by slow addition of the salt
and base, such that the pH was maintained between 6.8 and
7.3. Consequently, the desymmetrization reaction could be
telescoped very successfully at 257 mM concentration and the
desired fluorolactam 2b was separated efficiently by solid
phase extraction. CAL-B enzyme was recovered quantitatively
and recycled three times without any observed loss of reactivity
profile in subsequent cyclisation processes.

With basic operational parameters for the synthesis of
enantiopure 2b using inexpensive reagents and solvents in
place, we carried out studies to optimise the multistep
synthesis in order to assess the green metrics of the chemo-
enzymatic process in comparison to the published enantio-
selective chemical synthesis strategy used previously.15

In order to reduce the solvent use in reaction work-up, the
possibility of carrying out the subsequent Michael addition reaction of 4a with acrylonitrile in a one-pot process without
any work-up after the fluorination stage was explored. Firstly,
the Michael addition reaction between the crude direct fluor-
ination product mixture and acrylonitrile was assessed but no
alkylation reaction occurred due to problems associated with the
presence of copper nitrate and HF in the reaction mixture.
Consequently, reactions in which a short series of environment-
ally benign bases including DBU, potassium phosphate and
2-methyl pyridine, were added to the crude direct fluorination
product mixture were screened. Addition of 0.5 equivalents of
potassium phosphate to the reaction mixture allowed the
Michael reaction to proceed to full conversion at room
temperature. Scale-up of the one-pot process on 100 mmol scale,
where the acrylonitrile was added to the crude direct fluorina-
tion reaction mixture via syringe pump over 30 minutes, gave
4b in 60% yield after 1.5 hours.

Reduction of the nitrile group of 4b was carried out in a
Parr hydrogenator with palladium/carbon in methanol and conc.
hydrochloric acid. Upon completion of the hydrogenation,
a white precipitate formed upon washing the crude reaction mixture with ethanol which allowed simple filtration of the
ammonium hydrochloride salt 4c. After process optimi-
sation, the solvent volume used for the reduction could be
reduced significantly, providing 4c in 84% yield after recrystallisation. The telescoped cyclisation and resolution process was
carried out on 10 g scale to obtain realistic metrics data, gener-
at 2b in 43% isolated yield, 99% ee from 4c (Scheme 6, SI-1.9–11†).

The three stage, enhanced synthesis of (S)-2b from dimethyl
maltone ester gave a calculated PMI = 201, over four times
lower than the corresponding enantioselective chemical syn-
thesis strategy used previously.15

Experimental

Optimised synthesis of 2b (Scheme 6)

Telescoped fluorination-Michael addition: synthesis of
dimethyl (2-cyanoethyl)-2-fluoromalonate 4b. Dimethyl malo-
none 4a (26.40 g, 200 mmol) and copper(ii) nitrate hemi(penta-
hydrate) (4.65 g, 20 mmol) were dissolved in acetonitrile
(100 mL) and the mixture was cooled to 0–5 °C and stirred at
650 rpm using an overhead stirrer. After purging the system
with N2 for 5 minutes, fluorine gas (20% v/v in N2, 100 mL
min⁻¹, 220 mmol) was introduced into the reaction mixture
for 4 h 25 min. After purging with nitrogen for 5 min, potassium
phosphate tribasic (anhydrous) (42.45 g, 200 mmol) was
added to the reaction mixture and stirred. After 1 h the potas-
sium phosphate was removed by filtration and washed with
acetonitrile (2 × 20 mL) before a further portion of potassium
phosphate (42.45 g, 200 mmol) was added to the solution
which was heated to 55 °C. Acrylonitrile (12.73 g, 240 mmol)
in acetonitrile (10 mL) was added over 30 min and the solution
stirred. After a further 3.25 h the potassium phosphate was
removed by filtration and washed with acetonitrile (3 × 20 mL)
and the filtrate was concentrated in vacuo. Vacuum distillation
(140–141 °C, 6 mbar) of the crude product yielded dimethyl(2-
cyanoethyl)-2-fluoromalonate 4b (24.45 g, 60%) as a clear oil;
([MH]+, 204.0652. C9H12FNO4 requires: [MH]+, 204.0672); IR
(CF 201.0, CH2); 1H NMR (400 MHz,
CDCl3) δ 3.85 (6H, s, OCH3), 2.60–2.49 (4H, m, CH2);19F NMR
(376 MHz, CDCl3) δ −167.83−168.04 (m);13C NMR (101 MHz,
CDCl3) δ 165.42 (d, J_{CF} 25.3, C=O), 117.88 (s, CN), 92.73 (d,
J_{CF} 201.0, C-F), 53.86 (s, CH3O), 30.16 (d, J_{CF} 21.5, CH2),
11.48 (d, J_{CF} 5.5, CH3); m/z (ASAP) 204.1 (100%, [MH]+),
162.1 (25).
Reduction: synthesis of dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt 4c. 10% Pd/C (2.62 g, 5 mol%) and conc. HCl (4.85 mL) were added into a Hastelloy autoclave. A solution of dimethyl[2-cyanoethyl]-2-fluoromalonate 4b (10 g, 49.2 mmol) in methanol (43.3 mL) was added and the vessel sealed. The vessel was pressurized with H₂ (4 bar) and the contents were stirred at 600 rpm. After 16 h the solution was filtered through celite (2 g) with methanol (20 mL) and evaporated to give crude 4c. The solid was washed with methanol (2 × 20 mL) and acetone (2 × 15 mL) to give dimethyl[2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt 4c (10.43 g, 84%) as white crystals; mp 147–148 °C; [[M − Cl]⁺, 208.0978. C₉H₆F₂N,O₄ requires [M + Cl]⁺ 208.0985]; IR (neat, cm⁻¹) 3016, 2942, 1748, 1437, 1249, 1033; ¹H NMR (400 MHz, methanol-d₄) δ 3.87 (6H, s, OCH₃), 3.08–2.96 (2H, m, CH₂), 2.32 (2H, ddd, J₉HF 23.1, J₉HH 9.2, J₇HH 6.9, CH₃), 1.89–1.77 (2H, m, CH₂); ¹³C NMR (376 MHz, methanol-d₄) δ −167.20 (t, J₉HF 23.1); ¹³C NMR (101 MHz, methanol-d₄) δ 167.60 (d, J₇CF 25.8, C=O), 95.57 (d, J₇CF 197.4, C-F), 54.10 (s, s, CH₂O), 40.21 (s, CH₂NH₂), 32.18 (d, J₇CF 21.6, CH₂CF), 22.38 (d, J₇CF 3.2, CH₂), m/z (ASAP) 208.1 [100%, [M − Cl]⁺], 191 (14), 176 (8).

Cyclisation: synthesis of (S)-methyl 3-fluoro-2-oxopiperidine-3-carboxylate 2b. To a 500 mL round bottomed flask was added 0.06 M Na₂HPO₄: 0.06 M KH₂PO₄ buffer (164 mL, 3 : 1, pH 7.3) followed by 4c (10.00 g, 41.0 mmol) in small portions using 0.5 M NaOH to buffer the solution to pH 7.3. The solution was filled to 328 mL total volume with further 0.06 M Na₂HPO₄: 0.06 M KH₂PO₄ buffer (3 : 1, pH 7.3) to give a 257 mM solution Fermase immobilised CAL-B 10 000 (7.2 g) of which are composed of financial contribution from the Innovative Medicines Initiative Joint Undertaking project CHEM21 under grant agreement no 115360, resources of which are composed of financial contribution from the European Union’s Seventh Framework Programme (FP7/2007–2013) and EFPIA companies’ in kind contribution. We thank Dr D.S. Yufit for X-ray crystallography.

**Notes and references**


2. For reviews on fluorinated pharmaceuticals, see: (a) K. Muller, C. Faeh and F. Diether, Science, 2007, 317, 1881–1886; (b) Fluorine in Medicinal Chemistry and Chemical


