Kinetics of Electrophilic Fluorination of Steroids and Epimerisation of Fluorosteroids

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Abstract: Fluorinated steroids, which are synthesised by electrophilic fluorination, form a significant proportion of marketed pharmaceuticals. To gain quantitative information on fluorination at the 6-position of steroids, kinetics studies were conducted on enol ester derivatives of progesterone, testosterone, cholestenone and hydrocortisonew ith a series of electrophilic N–F reagents. The stereoselectivities of fluorination reactions of progesterone enol acetate and the kinetic effects of additives, including methanol and water, were investigated. The kinetics of epimerisation of 6β-fluoroprogesterone to the more pharmacologically active 6α-fluoroprogesterone isomer in HCl/acetic acid solutions are detailed.

Introduction

In the history of the development of fluorine-containing drug substances, fluorosteroids made significant early contributions when Fried and Sabo discovered that the introduction of a single fluorine atom into a corticosteroid 1 increased its potency tenfold.[1] Since this observation, numerous fluorinated steroids have been marketed for the treatment of various disease classes, including cancers and inflammation.[2] In particular, fluorosteroids bearing a fluorine atom at the 6-position, such as flurandrenolide 2 and fluticasone 3, continue to be commercially significant (Figure 1). Fluticasone therapeutics were ranked in the top 200 drugs prescribed in the USA in 2017.[3] The introduction of a fluorine atom at the 6-position is normally carried out by reaction of a steroid enolate derivative with an electrophilic fluorinating agent. Early examples of this transformation included the use of perchloryl fluoride (ClO$_3$F)[4] and trifluoroacetyl hypofluorite (CF$_3$COOF),[5] but due to the hazardous natures of these reagents, they were not suitable for large scale use. In recent years, several electrophilic fluorinating reagents of the N–F class, such as N-fluoropyridinium salts,[6] NFSI,[7] Selectfluor™,[8] and Accufluor™,[9] have been used for the fluorination of steroid enolate derivatives. Within the N–F class of reagents, Selectfluor™ has been used in larger scale applications, such as in the manufacture of fluticasone 3.[10] Indeed, an estimated 80% of commercially available fluorosteroids are synthesised using Selectfluor™.[11–14]

The introduction of a fluorine atom using an N–F reagent has also been achieved at other positions within the steroid; for example, Lectka et al. recently described the photocatalytic fluorination of steroids at the 15-position using Selectfluor™.[15] 6-Fluorosteroids are generally formed as mixtures of 6α- and 6β-isomers, where the former is usually pharmacologically active.[16, 17] The ratio depends upon the fluorinating reagent employed, steroid structure, temperature and timescale of the reaction. These factors were explored by Herrinton et al.[18] using several N–F reagents, where Selectfluor™ was determined to be the most efficient fluorinating agent.

To our knowledge, there have been no kinetics studies on the electrophilic fluorination of steroidal enolate systems, despite their therapeutic and commercial importance. However,
recent studies by Nelson et al.\(^{19}\) reported the kinetics of fluorination of a related tetralone system using Selectfluor\(^{TM}\). These studies established the mechanistic pathway of fluorination using Hammett correlations, concluding that an S\(_{N}2\) reaction occurred rather than SET. Furthermore, the kinetics and mechanisms of acid-mediated epimerisations of fluorosteroid mixtures to their more pharmacologically active \(\alpha\)-isomers remain underexplored.

General studies on the mechanisms and reactivities of N—F reagents towards carbon nucleophile systems have also recently been performed. Mayr et al.\(^{20}\) used a heterogeneous range of C-nucleophile systems to provide evidence for an S\(_{N}2\) mechanism and quantitative electrophilicity values, based on the Mayr–Patz scale, for five N—F fluorinating reagents. We used a homologous series of enolic para-substituted 1,3-diaryl-1,3-dicarbonyl derivatives to report a quantitative reactivity scale for ten electrophilic N—F fluorinating reagents (Figure 2) and deliver Hammett correlations that support S\(_{N}2\) fluorine transfer.\(^{21}\)

We recently extended our studies to explore the factors affecting fluorination of our 1,3-dicarbonyl compound series, concluding that solvent effects dramatically enhance enolization rates, thus promoting fluorination.\(^{22}\)

Quantitative approaches offer the possibility of matching the reactivities of nucleophilic and electrophilic partners, allowing reaction rates to be estimated and rapid, but controlled, processes to be designed.\(^{23}\) Given the pharmaceutical importance of 6-fluorosteroidal systems, we sought to quantify the nucleophilicities of enol equivalents of four main classes of steroids, namely; progesterone 13 (a progestogen), testosterone 14 (an androgen), cholestenone 15 (a cholesterol precursor) and hydrocortisone 16 (a corticosteroid). Each of these steroids contains an enolisable \(\alpha,\beta\)-unsaturated ketone system that can direct fluorination to their 6-positions via the corresponding enol ester systems 17–20 (Scheme 1), which are readily synthesised in one step.\(^{24,25}\)

We explore a range of fluorinating reagents, including N—F reagents and fluorine gas, and the factors that affect stereo-

chemical outcome. We also perform kinetic studies upon epimerisation processes that are used to redress the balance between the kinetically favoured \(\beta\)-isomers that arise from fluorination processes and the pharmaceutically desired \(\alpha\)-isomers.

Most electrophilic fluorinating reagents are synthesised from fluorine gas (F\(_2\)), however, there have been no reports of the corresponding fluorinations of steroidal enol ester systems at the 6-position using fluorine gas itself, although there are several reports of fluorination of tertiary C—H positions in steroid substrates using F\(_2\).\(^{26,27}\) Since selective direct fluorination of steroids by fluorine gas could provide a more cost-effective, greener route to commercially important 6-fluorosteroid derivatives, we used progesterone enol acetate 17 to study direct fluorination using fluorine gas.\(^{28}\)

**Results and Discussion**

**Preparation of materials and stereochemical characterisation**

In order to assess kinetics of enol acetate fluorination and subsequent epimerisation of fluorosteroids, we prepared a series of enol acetate substrates and isolated \(\alpha\)- and \(\beta\)-fluorosteroid isomers. The facial selectivities of a range of reagents were determined by NMR spectroscopy. The absolute configurations of the fluorosteroid products were, in some cases, confirmed crystallographically.

Progesterone enol acetate 17 was synthesised in 65% yield following a modified literature procedure (Scheme 1).\(^{24}\) Spectroscopic analyses were in agreement with previous reports\(^{25}\) and the structure was further confirmed by X-ray crystallography (see Supporting Information Section 2.1). Fluorination of 17 was conducted using Selectfluor\(^{TM}\) (Scheme 1) to obtain a mixture of \(\alpha\) and \(\beta\) isomers of 6-fluoroprogesterone 21.\(^{26}\) The

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**Figure 2.** Fluorinating reagents of the N—F class investigated in this study (NFPy = N-fluoropyridinium).
fluorination proceeded cleanly, with 100% conversion as determined by $^1$H and $^{19}$F NMR spectroscopy, and 96% yield of the $\alpha/\beta$ isomer mixture, where the $\alpha$ and $\beta$ isomers were present in a 34:66 ratio. The isomer mixture was resolved using column chromatography to afford isolated yields of 19% $\alpha$-isomer and 46% $\beta$-isomer. The structures of the isomers were assigned by X-ray crystallography (Scheme 2).

![Scheme 2. Epimerisation of 6-fluoroprogesterone $21-\beta$ to $21-\alpha$ in HCl/AcOH and X-ray crystallographic structures for each isomer.](image)

Table 1. When Selectfluor™ was used as the fluorinating reagent, testosterone enol diacetate 18 resulted in the highest levels of the desired $\alpha$ isomer compared with the other steroidal nucleophiles. The crude $\alpha/\beta$ isomer ratios for fluorinations of progesterone enol acetate 17 by seven N–F reagents were determined, and the reaction with Selectfluor™ gave the highest proportion of the $\alpha$ isomer. The stereoselectivity does not follow the trend in reactivities of the N–F reagents. As we have previously reported,[21] diCl-NFPy TFO−/BF₄⁻ 11a/b are 4-fold less reactive than Selectfluor™ and show the lowest selectivities for the $\alpha$ isomer. NFSI 8, NFPy TFO−/BF₄⁻ 9 and triMe-NFPy TFO−/BF₄⁻ 10 are 4–6 orders of magnitude less reactive than Selectfluor™, and pentaCl-NFPy TFO−/BF₄⁻ 12 is 1 order of magnitude more reactive, yet, all lead to similar stereoselectivities.

These results are surprising considering the vast range of reactivities and differing steric requirements of the fluorinating reagents. The near-identical $\alpha/\beta$ isomer ratios suggested that epimerisation at the newly-formed C–F centre could be in operation, with the isomer ratio being determined by solvent-product interactions. Closer inspection of $^1$H NMR kinetic data for the fluorinations of progesterone enol acetate 17 with NFPy TFO−/BF₄⁻ 9 and NFSI 8 showed constant $\alpha/\beta$ isomer ratios over the courses of the reactions. In order to further investigate the potential for in situ epimerisation we explored whether ‘spent’ Selectfluor™ (CICH$_2$-DABCO$^+$/BF$_4^-$) could play an acid/base catalysis role in this process. When $21-\beta$ was incubated with CICH$_2$-DABCO$^+$/BF$_4^-$ in MeCN-$d_3$, no formation of $21-\alpha$ was observed by $^{19}$F NMR spectroscopy over the course of 1 week. To probe the possibility of protonated ‘spent’ Selectfluor™ catalysing in situ epimerisation, we attempted to prepare a sample of this protonated species, although our efforts were unsuccessful (see Supporting Information Section 2.1.12).

To explore the effects of other enol derivatives upon fluorination kinetics and stereoselectivity, the ethylated form of the progesterone enol, 3-ethoxy-pregna-3,5-dien-20-one 25, was prepared using a previously described method.[50] This compound is the starting material for the synthesis of birth control drug quingestrone and several related compounds.[51] The electrophilic chlorination of 25 with N-chlorosuccinimide (NCS) was
conducted by Ringold et al.\textsuperscript{[32]} although, to the best of our knowledge, the fluorination of 25 has not been reported. On mixing 25 and Selectfluor\textsuperscript{TM} (0.95 equiv) in CDCl$_3$, an immediate colour change from yellow to red was observed (Scheme 3). CDCl$_3$ was used due to the lower solubility of 25 in MeCN and acetone. Analysis of the reaction mixture by $^{19}$F NMR spectroscopy showed the disappearance of the N–F signal, however, new signals were not observed. Analysis by high resolution mass spectrometry (HR-MS) showed the formation of a product consistent with oxidation of 25 by introduction of a single oxygen atom. The fragment ions showed loss of Ac- and AcO- groups, which indicate that the oxidised product is progesterone enol acetate 17. Selectfluor\textsuperscript{TM} is a known oxidant, for example, the copper-mediated oxidation of amides to imides by Selectfluor\textsuperscript{TM} has been reported.\textsuperscript{[33]} The oxidation of an ethoxy group to an acetyl group was previously reported for the conversion of 6-ethoxybenzothiazole-2-sulphonamide to the corresponding acetate with trichloroisocyanuric acid,\textsuperscript{[34]} a reagent that is known to carry out both chlorination and oxidation.\textsuperscript{[35]} Oxidation may be specific at this position due to the non-bonding electrons on the ethereal oxygen atom that could activate the neighbouring C–H bonds by hyperconjugation effects.\textsuperscript{[36]} We believe oxidation processes are likely to result in the formation of HF, which will be lost from the reaction mixture, thus leading to the loss of detectable $^{19}$F NMR signals over the course of the reaction.

When the reaction was conducted in MeCN-d$_3$, with 1 equiv of Selectfluor\textsuperscript{TM} (with sonication to fully solubilise 25), small amounts of 21-$\alpha$ and 21-$\beta$ were detected by $^1$H and $^{19}$F NMR spectroscopy, as well as traces of unidentified fluorinated products (NMR spectra are included in the Supporting Information Section 2.1.11). Progesterone enol acetate 17 was not detected; however, the major product of the reaction was progesterone 13, which could have formed by oxidation of 25 to 17, followed by hydrolysis of the ester group to form 13. The formation of 13 was confirmed by comparison of the spectra with an authentic sample of 13.

**Kinetics of fluorination of steroid enol acetates**

The kinetics of fluorination of progesterone enol acetate 17 by NFSI 8, NFpy TfO\textsuperscript{•} 9 and triMe-NFpy TfO\textsuperscript{•} 10 were monitored by quantitative $^1$H NMR spectroscopy in MeCN-d$_3$ (Figure 3). These kinetics experiments were carried out with excess N–F reagent to achieve pseudo-first order conditions. Due to the wide range of reactivity of the N–F reagents, reactions involving Selectfluor\textsuperscript{TM} 7, diCl-NFpy TfO\textsuperscript{•} 11a, diCl-NFpy BF\textsubscript{3} 11b and pentaCl-NFpy TfO\textsuperscript{•} 12 were too rapid to be monitored by NMR spectroscopy. Hence, we used UV/Vis spectrophotometry, where the use of lower concentrations of the reaction partners was expected to afford lower observed rates. Although the fluorinations of steroid enol acetates discussed in the previous section were carried out in MeCN-acetone mixtures to maximise the solubilities of the reaction partners, solubility was not an issue at the lower concentrations used in UV/Vis spectrophotometry and NMR spectroscopy studies. Hence, kinetics studies were conducted in MeCN only. Extinction coefficients were determined for 17, 21-$\alpha$ and 21-$\beta$ (see Supporting Information Section 2.4.4) with the aim of enabling us to determine 21-$\alpha$:21-$\beta$ ratios in our reaction mixtures spectrophotometrically. Although there was a difference between the extinction coefficients for 21-$\alpha$ and 21-$\beta$, we were unable to reliably extract ratios. Since Selectfluor\textsuperscript{TM} 7 is not chromophoric, we were able to monitor the decays in absorbance of progesterone enol acetate 17 at 236 nm with an excess of Selectfluor\textsuperscript{TM} 7. However, the UV/Vis spectra of 11a, 11b and 12 contain absorbance bands between 200–350 nm and it was not possible to selectively monitor the steroid band at 236 nm. Therefore, the kinetics experiments involving reagents 11a, 11b and 12 were conducted with excess 17 by monitoring the N–F reagent absorbance at $\lambda_{\text{max}}$ = 288 nm (for 11a and 11b) and $\lambda_{\text{max}}$ = 320 nm (for 12).

A representative example is shown in Figure 4 for the fluorination of progesterone enol acetate 17 by Selectfluor\textsuperscript{TM} 7.

![Scheme 3. Reaction of 3-ethoxy-pregna-3,5-dien-20-one 25 with Selectfluor\textsuperscript{TM} (0.95 equiv) in CDCl$_3$ gave the oxidised product 17, as identified by HR-MS, and fluorinated products were not detected. The HR-MS fragment ions consistent with 17 were: [M+H]+ = 357.243, [M+H-CH$_3$COO]$^-$ = 315.228, [M-CH$_3$COO]$^-$ = 297.215. However, in the reaction of 25 with Selectfluor\textsuperscript{TM} (1.0 equiv) in MeCN-d$_3$, progesterone 13 was the major product, and traces of fluorinated products were present.](image-url)

![Figure 3. Fluorination of progesterone enol acetate 17 by NFSI in MeCN-d$_3$ at 25 °C, monitored by $^1$H NMR spectroscopy. Starting concentrations of reagents: [17] = 17.5 mM, [NFSI] = 526.0 mM. Signals corresponding to 17 are labelled as C6H and C4H. Peaks associated with fluoroprogesterone isomers 21-$\alpha$ and 21-$\beta$ are also labelled.](image-url)

![Chem. Eur. J. 2020, 26, 12027 – 12035 www.chemeurj.org 12030 © 2020 The Authors. Published by Wiley-VCH GmbH](image-url)
were obtained from the fitting of plots of absorbance values are reported in Table 2, as well as those of $k$ in MeCN or MeCN-$d_3$. A linear (i.e., first order) correlation was observed (Figure 4b). The direct dependence upon N–F reagent concentration demonstrates rate-limiting fluorination and thus the slope of this graph gave the second-order rate constant, $k_2$ [M$^{-1}$ s$^{-1}$], according to the second-order rate Equation (1):

$$\text{Rate} = -\frac{d[N\text{uc}]}{dt} = k_2[N\text{uc}][\text{NF reagent}]$$ (1)

Kinetics studies were conducted on the fluorination of steroid enol acetates 18, 19 and 20 using similar procedures and the $k$ values are reported in Table 2, as well as those of 17. All spectra relating to kinetics studies on fluorination of 17–20 are included in the Supporting Information Section 2.4. Relative rate constants ($k_{rel}$) were determined using Equation (2), with Selectfluor$^\text{TM}$ as the reference electrophile, thus enabling a comparison of reactivities (Table 2). The reactivity trends of the N–F fluorinating agents match those we previously reported for fluorinations of enolic 1,3-dicarbonyl systems, which reinforces the predictive nature of our reactivity scale. More detailed comparisons of reactivities between the two nucleophile systems are included in the Supporting Information Section 2.5.

$$k_{rel} = \frac{k_2(\text{NF reagent})}{k_2(\text{Selectfluor})}$$ (2)

Selectfluor$^\text{TM}$ 7 shows excellent solubility and good stability in water, additionally, the use of benign solvents such as water is attractive due to the potential for reducing the environmental impact of the process. Furthermore, we recently showed that the addition of water significantly increased fluorination rate constants of the enol forms of 1,3-dicarbonyl species. We also wondered whether the trapping of the cationic intermediate generated upon fluorination of 17, with a weak nucleophilic species, could improve product yield by sequestering this reactive intermediate (Scheme 4, Pathway B). Thus, taking these factors together, we performed kinetics studies using water and MeOH as co-solvents for fluorination of 17.

Clean exponential decays of absorbance of the nucleophile were observed in all runs, and the first-order rate constants $k_{obs}$ were obtained from the fitting of plots of absorbance versus time (Figure 4a). The $k_{obs}$ values were plotted against N–F reagent concentration and a linear (i.e., first order) correlation was observed.

![Figure 4](image_url)

**Figure 4.** (a) Exponential decays of absorbance of progesterone enol acetate 17 (50 $\mu$M) with different concentrations of Selectfluor$^\text{TM}$ (0.5–2.5 mM) in MeCN at 25 °C. (b) Correlation of $k_{obs}$ with [Selectfluor$^\text{TM}$].

| Nucleophile                  | Electrophile                  | $k_2$ [M$^{-1}$ s$^{-1}$] | $k_{rel}$ |
|-----------------------------|-------------------------------|---------------------------|----------|
| progesterone enol acetate 17| Selectfluor$^\text{TM}$ 7     | 2.38                      | 1.0      |
|                            | NFSI 8                        | 3.33×10$^{-4}$            | 1.4×10$^{-4}$ |
|                            | NFPy TFO$^\text{TM}$ 9        | 2.08×10$^{-5}$            | 8.7×10$^{-6}$ |
|                            | triMe-NFPy TFO$^\text{TM}$ 10 | 7.19×10$^{-6}$            | 3.0×10$^{-6}$ |
|                            | diCl-NFPy TFO$^\text{TM}$ 11a | 4.72×10$^{-6}$            | 2.0×10$^{-6}$ |
|                            | diCl-NFPy BF$_3^\text{TM}$ 11b| 5.03×10$^{-6}$            | 2.1×10$^{-6}$ |
|                            | pentaCl-NFPy TFO$^\text{TM}$ 12| 1.31×10$^{-5}$            | 5.5×10$^{-6}$ |
| testosterone enol diacetate 18 | Selectfluor$^\text{TM}$ 7     | 2.11                      | 1.0      |
|                            | diCl-NFPy TFO$^\text{TM}$ 11a | 4.41×10$^{-1}$            | 2.1×10$^{-1}$ |
|                            | pentaCl-NFPy TFO$^\text{TM}$ 12| 1.42×10$^{3}$             | 6.7×10$^{3}$ |
| cholestenone enol acetate 19 | Selectfluor$^\text{TM}$ 7     | 3.18                      | 1.0      |
|                            | pentaCl-NFPy TFO$^\text{TM}$ 12| 1.94×10$^{2}$             | 6.1×10$^{2}$ |
| hydrocortisone enol tetraacetate 20 | Selectfluor$^\text{TM}$ 7 | 1.06                      | 1.0      |
|                            | pentaCl-NFPy TFO$^\text{TM}$ 12| 5.54×10$^{1}$             | 5.2×10$^{1}$ |
Reactions were conducted in the presence of different quantities of water and MeOH, and rate constants were determined using UV/Vis spectrophotometry. All rate constants ($k_2$) are summarised in the Supporting Information Section 2.4.3. There was little variation in second-order rate constants, $k_2$, upon addition of 10–50% MeOH (v/v) in MeCN. For example, with 30% MeOH, there was only a 1.2-fold rate enhancement compared to without MeOH. With water, on the other hand, fluorination rate constants, $k_2$, decreased as the amount of water was increased (Figure 5). For example, with 30% water in MeCN (v/v), the rate of fluorination decreased 4-fold compared with the analogous reaction in MeCN. Thus, water is not a suitable co-solvent for increasing the rate of fluorination of 17, and we tentatively attribute its inhibitory effects to the differing solvation requirements of fluoroenol species in our earlier study and enol ester 17 along with their fluorination reaction coordinates. Product analyses of crude reaction mixtures by LC-MS were also unchanged by the addition of MeOH or water, and thus we conclude that the use of these co-solvents offers no advantage to the fluorination process.

![Scheme 4. Potential trapping of the reactive intermediate using water or MeOH (Nu = OH, OMe).](image)

Activation parameters ($\Delta G^\ddagger$, $\Delta H^\ddagger$, $\Delta S^\ddagger$) were obtained from kinetic data for the reactions of progesterone enol acetate 17 with two N–F reagents (see Table 3 and Supporting Information Sections 2.4.5 and 2.4.6). With Selectfluor™ 7, these values were $\Delta H^\ddagger = +51 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -66 \text{ JK}^{-1}\text{mol}^{-1}$ and $\Delta G^\ddagger = +71 \text{ kJ mol}^{-1}$. With diCl-NFPy TfO 11a, the values were $\Delta H^\ddagger = +52 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -76 \text{ JK}^{-1}\text{mol}^{-1}$ and $\Delta G^\ddagger = +75 \text{ kJ mol}^{-1}$. We previously obtained activation parameters for the fluorination of para-substituted enolic 1,3-dicarboxyl derivatives 26 (vide infra) by Selectfluor™, which were $\Delta H^\ddagger = +55$ to $+64 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -54$ to $-72 \text{ JK}^{-1}\text{mol}^{-1}$ and $\Delta G^\ddagger = +74$ to $+83 \text{ kJ mol}^{-1}$. Activation parameters determined by Nelson et al. for the fluorination of four tetralone derivatives using Selectfluor™ were $\Delta H^\ddagger = +62$ to $+65 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -84$ to $-100 \text{ JK}^{-1}\text{mol}^{-1}$ and $\Delta G^\ddagger = +90$ to $+93 \text{ kJ mol}^{-1}$. The similarities in these parameters with those of enol ester 17 are consistent with a common $S_n^2$ mechanism for fluorination of these substrates.

| N–F reagent       | $\Delta G^\ddagger$ (kJ mol$^{-1}$) | $\Delta H^\ddagger$ (kJ mol$^{-1}$) | $\Delta S^\ddagger$ (JK$^{-1}$mol$^{-1}$) |
|-------------------|-----------------------------------|-----------------------------------|---------------------------------------|
| Selectfluor™ 7    | +71                               | +51                               | -66                                   |
| diCl-NFPy TfO 11a | +75                               | +52                               | -76                                   |

We also attempted to employ fluorenone gas to fluorinate progesterone enol acetate 17. Exploratory experiments were carried out in formic acid solution, which is a preferred solvent for the direct fluorination of enolic systems. The crude product mixture contained progesterone 13, 6α-fluoroprogesterone (21-α) and 6β-fluoroprogesterone (21-β) along with some minor impurities. However, upon analysis by HPLC, integration of the chromatogram revealed that only half of the crude product mass could be accounted for by these three compounds. Direct fluorination in MeCN yielded mixtures of 21-α, 21-β and unreacted progesterone enol acetate 17 as well as other fluorinated side-products, but progesterone 13 was not detected. The selectivity of the direct fluorinations were α:β, 38:62, which is a similar ratio to that obtained using Selectfluor™ 7. Ultimately, we found batch-based direct fluorinations to be ineffective, however, flow-based systems may offer improved performance. Further details of direct fluorination experiments are contained in Supporting Information Section 2.3.

Comparison of nucleophilicity

The relative nucleophilicities, $k_{rel}$, of enol acetates 17–20, expressed, defined by Equation (3) (see Table 4) were determined using the second-order rate constants, $k_2$ (from Table 2). Unsurprisingly, the reactivity differences are small across the four compounds. Progesterone enol acetate 17 and testosterone enol diacetate 18 have, on average, very similar reactivities. Cholestenone enol acetate 19 is, on average, 1.4-fold more reactive than 17, and hydrocortisone enol tetracetate 20 is 2.3-
fold less reactive than 17. The major structural change across this range of steroids is the substituent at the remote C-17 position which, unsurprisingly, appears to have limited effects on their respective nucleophilicities. The electron-withdrawing acetate groups in 20 result in reduced nucleophilicity of this compound, whereas the inductive electron-donating alkyl chain at the C-17 position of 19 increases its nucleophilicity. 

$$k_{\text{rel}} = \frac{k_2(\text{Steroid enol acetate})}{k_2(\text{Progesterone enol acetate 17})}$$

(3)

The nucleophilic reactivity of progesterone enol acetate 17 was compared with those of the 1,3-dicarbonyl derivatives 26a–f using the second-order rate constants, $k_2$, for fluorination of these substrates by Selectfluor™ and NFSI (from ref. [21]). These two N–F reagents were selected for this comparison since they show markedly different reactivities, as well as having the most extensive datasets for fluorination kinetics. Equation (4) defines $k_{\text{rel}}$. The reactivities of the nucleophiles span 5 orders of magnitude (Figure 6) and enol ester 17 is one order of magnitude more reactive than enol 26a. More detailed comparisons of reactivities are included in the Supporting Information Section 2.5.

$$k_{\text{rel}} = \frac{k_2(\text{Nucleophile})}{k_2(26a \text{ enol})}$$

(4)

### Kinetics of epimerisation

The α-isomers of 6-fluorosteroids are generally desired because they display higher levels of biological activity.16,17 However, the low stereoselectivities of the fluorination reactions discussed earlier result in the formation of the 6β-isomer as the major product (Table 1). We carried out studies on the rates of epimerisation of 6β-fluoroprogesterone (21-β) to 6α-fluoroprogesterone (21-α) by HCl in AcOH, using appropriately diluted solutions of a commercially available reagent. Reactions were monitored directly by quantitative time-arrayed "in-magnet" $^{19}\text{F}$ NMR spectroscopy with four different concentrations of HCl in acetic acid (0.25–1.00 M). A representative example is shown in Figure 7 for an epimerisation reaction conducted with 0.50 M HCl in acetic acid. The triplet of doublets at δ = −165.60 to −165.90 ppm, corresponding to 21-β, decreased in intensity over time, whereas the doublet of doublet of doublets at δ = −183.00 to −183.16 ppm associated with 21-α increased in intensity. Additional peaks were present at δ = −165.56 ppm, which overlapped with part of the adjacent 21-β signals. Similarly, small peaks appeared over time adjacent to the 21-α signals.

The reaction profiles for each species in the epimerisation mixture are shown in Figure 8. Due to the overlap between the peaks, partial signal integration was employed. The integrals corresponding to the growth of 21-α (black data points) were fitted to an exponential rise function for all concentrations of HCl. A plot of $k_{\text{obs}}$ versus HCl concentration showed a linear relationship (Figure 9). The integrals of the small signals

### Table 4. Comparison of reactivities of steroid enol acetates 17–20 using $k_{\text{rel}}$ values defined by Equation 3, determined using the $k_2$ values summarised in Table 2.

| Nucleophile                                | Selectfluor™ 7 | Selectfluor™ diCl-NFPy TfO 11a | pentaCl-NFPy TfO 12 |
|-------------------------------------------|----------------|-------------------------------|-------------------|
| progesterone enol acetate 17              | 1.0            | 1.0                           | 1.0               |
| testosterone enol diacetate 18            | 0.89           | 0.93                          | 1.08              |
| cholestenone enol acetate 19              | 1.34           |                               | 1.48              |
| hydrocortisone enol tetraacetate 20       | 0.45           | –                             | 0.42              |

Figure 6. Reactivity scale for nucleophilic enols 26a–f and enol ester 17, with enol 26a as the reference nucleophile.

Figure 7. Quantitative time-arrayed $^{19}\text{F}$ NMR spectroscopic monitoring of the epimerisation of 21-β (60 mm) to 21-α with HCl (0.50 M in AcOH) at 25 °C. Spectra were acquired every 15 min for 17 h with relaxation delays (T1) of 10 s.
at $\delta = -165.56$ ppm (blue data points) clearly showed the formation and decay of an intermediate (Scheme 5).

The small signals at $\delta = -165.56$ and $-183.20$ ppm in Figure 7 are likely to be product-related species, due to their similarity in chemical shift and coupling patterns. When an authentic sample of 21-$\alpha$ was incubated in a solution of 0.50 M HCl in AcOH for 45 min, the proton-coupled $^{19}$F NMR spectrum of the solution showed the presence of signals corresponding to 21-$\alpha$ (at $\delta = -183.03$ to $-183.15$ ppm) as well as smaller adjacent signals due to the intermediate species (at $\delta = -183.10$ to $-183.20$ ppm). The proton-decoupled $^{19}$F NMR spectrum confirmed that two different species were present (see Supporting Information Section 2.6). However, when 21-$\alpha$ was incubated in AcOH alone, no signals corresponding to only one species, 21-$\alpha$, were observed. These results suggest acid-catalysed formation of intermediates such as hemiacetyl aldehydes 29-$\beta$ and 29-$\alpha$ (Scheme 5, blue pathways) or enol esters, however, we have no direct evidence of their structures. The change in epimer preference under the reaction conditions from 21-$\beta$ to 21-$\alpha$ probably derives from differing solvent-product interactions. These interactions are likely to be markedly different in AcOH in comparison to the MeCN solvent that was applied for fluorinations.

Conclusions

The kinetics of fluorination of progesterone enol acetate 17 using seven N-F reagents were studied. The method of analysis was tuned to the reactivity of the system: less powerful electrophiles were studied by $^1$HNMR spectroscopy while more reactive reagents were studied using UV/Vis spectrophotometry. Relative rate constants were determined from absolute rate constants, and they correlate well with our recently reported reactivity scale.$^{[21]}$ These results highlight the successful predictive power of our scale towards a different class of carbon nucleophiles. Activation parameters were determined for the fluorination of progesterone enol acetate 17 by Selectfluor$^{TM}$ 7 and diCl-NFPy TOF 11a. The moderately negative values of $\Delta S^*$ are consistent with those from our previous studies on fluorination of enolic 1,3-dicarbonyl systems$^{[21, 22]}$ and recent studies by Nelson et al.$^{[19]}$ on closely-related tetrahydronorbornene systems. Kinetics studies on the fluorination of testosterone enol diacetate 18, cholesterol enol acetate 19 and hydrocortisone enol tetraacetate 20 were conducted. The substituent at the C-17 position has a small but measurable effect upon the rate of fluorination.

The epimerisation of 6$\beta$-fluoroprogesterone 21-$\beta$ to 6$\alpha$-fluoroprogesterone 21-$\alpha$ with increasing concentrations of HCl in acetic acid proved to be more rapid. Additional signals in the $^{19}$F NMR spectra of the product mixtures also gave evidence for the formation of intermediates in an acid catalyst-dependent manner.

Overall, we have delivered a clearer understanding of the kinetics of fluorination of steroidal systems and the subsequent epimerisation of fluorosteroids. These results highlight the op-
opportunities for achieving more efficient synthetic routes through kinetic understanding.

**Experimental Section**
All experimental details can be found in the Supporting Information, which contains characterisations of compounds, details of kinetics experiments and kinetics data.

Deposition Numbers 1985095, 1985096, 1985097, 1985098 and 1985099 contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

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**Conflict of interest**
The authors declare no conflict of interest.

**Keywords:** epimerisation | fluorination | fluorosteroid | kinetics | stereoselectivity

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