Formyloxyacetoxyphenylmethane as an N-Formylating Reagent for Amines, Amino Acids and Peptides

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ABSTRACT: Formyloxyacetoxyphenylmethane is a stable, water-tolerant, N-formylating reagent for primary and secondary amines that can be used under solvent-free conditions at room temperature to prepare a range of N-formamides, N-formyl-anilines, N-formyl-α-amino-acids, N-formyl-peptides and an isocyanide.

N-Formylation reactions of primary and secondary amines are important transformations in organic chemistry, because they provide direct access to the highly versatile formamide group.1 Highlighting their synthetic utility, N-formyl derivatives can be used as precursors for the synthesis of formamidines,3 ureas,3 carbamates,3 aryl amides,4 isocyanates,5 nitriles6 and isocyanides.7 Consequently, they have often been used as intermediates for the synthesis of pharmaceuticals.8 Alternatively, the N-formyl group may be used as a protecting group for peptide synthesis,9 whilst many naturally occurring formamides exhibit important medicinal and biological activities.10 The formamide moiety is also present in pharmaceuticals, including Leucovorin (chemotherapeutic),11 Formoterol (asthma)12 and Orlistat (anti-obesity).13

Due to their importance, a number of N-formylating reagents for primary and secondary amines have been developed,1 including the use of formic acid,14 formic acid in the presence of coupling reagents (e.g. DCC),15 acetic formic anhydride,16 formate esters,17 formamides,18 trialkylthiourea-formamides,19 enol formates,20 ammonium formate,21 and N-formyl-benzotriazole.18 However, many of these reagents are only moderately reactive towards sterically hindered or electronically deactivated amines, often requiring elevated temperatures, extended reaction times or the use of excess reagent for their N-formylation reactions to proceed to completion.5 A number of these formylating reagents are also susceptible to hydrolysis, whilst some reagents can decompose to liberate hazardous by-products (e.g. CO).1 Given these limitations, a number of catalytic N-formylation protocols have also been developed that employ N-formyl donors in combination with acid catalysts, Lewis acids, organocatalysts, metal catalysts and biocatalysts.13

Given the limitations of using many N-formylating reagents under catalyst free conditions, we now report herein that formyloxyacetoxyphenylmethane (FAPM) 5 can be used as a bench-stable formylating reagent for primary/secondary amines under solvent-free conditions at room temperature. Importantly, the stability of FAPM 5 towards hydrolysis, enables it to be used as a catalyst free N-formylating reagent for α-amino acids and peptides in aqueous based solvent systems.

As part of investigations into the Baeyer-Villiger oxidation reactions of α,β-unsaturated ketones, we found that treatment of benzylidene acetone 1 with 4 equiv. of meta-chloroperbenzoic acid (mCPBA) in toluene for 24 h gave FAPM 5 in 84% isolated yield (Scheme 1a).14 In this remarkable one-pot 4-step reaction, benzylidene acetone 1 first undergoes an mCPBA mediated Baeyer-Villiger reaction to afford enol acetate 2, whose electron-rich alkene bond is then epoxidised by a second equivalent of mCPBA to afford epoxy-acetate 3. This unstable epoxy-acetate 3 then undergoes intramolecular rearrangement to afford aldehyde 4, which then undergoes a further mCPBA mediated Baeyer-Villiger reaction to afford FAPM 5. The formation of intermediates 2 and 4 in this oxidation reaction were confirmed by 1H NMR spectroscopic analysis of a series of control reactions that were carried out using fewer equivalents of mCPBA over shorter periods of time (see SI for details).15

A second route to FAPM 5 was also developed using a variant of a protocol previously developed by Raphaël and coworkers for the synthesis of non-symmetric aclyoxyalkyl esters.26 This involved treatment of benzaldehyde with 1.5 equiv. of acetyl chloride in the presence of a catalytic amount of para-toluene sulfonyl acid to afford α-chlorobenzyl acetate 6,27 that was subsequently reacted with formic acid andEt3N in acetone to afford FAPM 5 in 76% yield (Scheme 1b).28

FAPM 5 proved to be a bench-stable liquid that could be stored at room temperature under nitrogen for up to 6 months without decomposition. We reasoned that FAPM 5 might act as a selective N-formyl donor for amines, which was confirmed by reacting 1.5 equiv. of FAPM 5 with 1 equiv. of benzylamine 7a in THF at rt for 4.5 h to give N-formyl-benzylamine 8a and imine 9 with 90% and 10% conversion respectively (Table 1, entry 1). Importantly, 1H NMR spectroscopic analysis revealed no evidence of any N-acetyl-benzylamide having been formed via competing N-acyl transfer from FAPM 5. Mechanistically, we propose that
benzylamine \(10a\) attacks the reactive formyl group of FAPM to irreversibly afford formamide \(8a\),

Scheme 1. Syntheses of formyloxacetophenylmethanes via:
(a) \(m\)CPBA mediated oxidation reaction of benzylidene acetone \(1\).
(b) Nucleophilic attack of formic acid on \(\alpha\)-chlorobenzyl acetate \(6\).

with benzaldehyde and acetic acid being generated as by-products. Unwanted formation of imine \(9\) then occurs through acid catalyzed reaction of benzylamine \(7a\) with the benzaldehyde by-product. A solvent screen was carried out with the aim of identifying conditions that would suppress formation of imine \(9\). Use of ethanol and 1,4-dioxane (Table 1, entries 2-3) also afforded significant amounts of the unwanted imine \(9\) by-product (8-12%). Carrying out the \(N\)-formylation reaction in MeCN (Table 1, entry 4) gave reduced amounts of imine (4%), whilst \(\text{CH}_2\text{Cl}_2\), toluene and EtOAc (Table 1, entries 5-7) gave excellent conversion to \(N\)-formyl-benzylamine \(8a\) with only trace amounts of imine \(9\) (±2%) being formed. Since imine formation is potentially reversible, a series of \(N\)-formylation reactions were carried out in mixed aqueous solvents, in the hope that the presence of bulk water would perturb the reaction equilibrium away from imine formation. Gratifyingly, use of \(1:1\) mixtures of THF : \(\text{H}_2\text{O}\), MeCN : \(\text{H}_2\text{O}\) and 1,4-dioxane : \(\text{H}_2\text{O}\) (Table 1, entries 8-10) gave good conversions to \(N\)-formyl-benzylamine \(8a\), with ≤2% of imine \(9\) being formed in each case. The tolerance of FAPM \(5\) towards hydrolysis in aqueous systems was confirmed by carrying out an \(N\)-formylation reaction in water,\(^{29}\) with 1.5 equiv. of FAPM \(5\) resulting in complete conversion of benzylamine to \(N\)-formyl-benzylamine \(8a\) (Table 1, entry 11). Finally, FAPM \(5\) could also be used under solvent free conditions (Table 1, entry 12), resulting in clean \(N\)-formylation of benzylamine, with only 2% of imine \(9\) being produced.\(^{30}\)

\(^{1}\)H NMR spectroscopic analysis of the \(N\)-formylation reaction of benzylamine in d\(_8\)-toluene revealed that complete consumption of benzylamine \(7a\) occurred after 1 h. Analysis after 15 min revealed the presence of a \(87:13\) mixture of formamide \(8a\) : imine \(9\), which subsequently equilibrated to a \(99:1\) mixture of formamide \(8a\) : imine \(9\) after 1 hr. With this information in hand, we employed 1.5 equiv. of FAPM to \(N\)-formylate a range of sixteen primary and secondary amines \(7a-\) to afford a series of \(N\)-formamides \(8a-p\) in 53-66% yield after chromatographic purification (Scheme 2).

Table 1. Solvent screen to optimize the \(N\)-formylation reaction of benzylamine with FAPM \(5\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Formamide (8a)</th>
<th>Imine (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THF</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>EtOH</td>
<td>88</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Dioxane</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>MeCN</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Toluene</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>EtOAc</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>THF : (\text{H}_2\text{O} : 1)</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>MeCN : (\text{H}_2\text{O} : 1)</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Dioxane : (\text{H}_2\text{O} : 1)</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>(\text{H}_2\text{O})</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Neat</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Product ratios determined from integrals of diagnostic resonances for \(8a\) and \(9\) in \(^1\)H NMR spectra of crude reaction products.

\(8e\) in 95% and 85% yields respectively. FAPM \(5\) reacted exclusively with the primary amine functionality of tryptamine to afford formamide \(8d\) in 90% yield. Whilst the formamide \(8f\) of sterically congested benzhydrylamine was formed in good 83% yield, formylation of tert-butylamine gave \(N\)-formamide \(8g\) in only 53% isolated yield, arising from losses during reaction work-up due to its volatility. (S)-Valine methyl ester afforded \(N\)-formamide \(8h\) in an excellent 93% yield, with no evidence of any racemization having occurred. Acyclic and cyclic secondary amines were also formylated using FAPM \(5\), with moderate to excellent 58-96% yields of \(N\)-formamides \(8i-k\) being isolated. Pleasingly, electron-deficient aniline and its secondary \(N\)-methyl derivative gave their corresponding formamides \(8l-m\) in excellent 90% and 91% yields respectively. However, formylation of anilines containing deactivating halogen or ester groups required extended reaction times of 24 h for their corresponding \(N\)-formamides \(8o-p\) to be formed in 87-95% yields respectively.

Alternative work-up procedures were then developed to allow for these \(N\)-formylation reactions to be carried out on-scale, without the need for chromatographic purification. When the desired formamide product was crystalline, or a high boiling oil, then the acetic acid and benzaldehyde by-products could be removed by fractional distillation in vacuo. For more volatile formamides, purification could be achieved by first extracting the crude reaction product with \(\text{NaHCO}_3(aq)\) to remove acetic acid, followed by extraction with \(\text{NaISO}_4(aq)\) to remove benzaldehyde. This affords a crude product containing the formamide that is then purified by recrystallisation or fractional distillation as required (See SI for details).

Although several reagents have been developed for the \(N\)-formylation of amino ester derivatives,\(^{31}\) extension of these
methods to unprotected α-amino acids has not been widely reported. This is due to the instability of many formylating reagents towards hydrolysis in aqueous media and the insolubility of α-amino acids in non-aqueous solvents. Currently, N-formyl-α-amino acids are most commonly prepared via treatment of the parent α-amino acids with a large excess of Ac₂O and formic acid (via formation of formyl acetate in situ), which often requires forcing conditions and/or extended reaction times. N-formylation of α-amino acids using formic acid in water has been reported, however this protocol requires Oxyra as a stoichiometric coupling reagent. Given the observed stability of FAPM in aqueous solvents, a range of six l-amino acids 7a-p were treated with 1.5 equiv. of FAPM in water containing 5 equiv. of NaHCO₃ as a base, which resulted in 80-85% conversion to their corresponding formamides 8a-f after 16 h. However, we subsequently found that modifying these formylation reactions, by incorporating addition of a second portion of FAPM (1.5 equiv.) after 8 h, resulted in complete consumption of each α-amino acid, giving N-formyl-amino acids 11a-f in good to excellent 71-89% isolated yields, with no racemization having occurred (Scheme 3).

**Scheme 2:** FAPM 5 as an N-formylating reagent for the synthesis of N-formamides 8a-p

N-Formylmethionine-leucyl-phenylalanine (F-MLP) 14 is a chemotactic N-formyl-tripeptide that is a potent chemotactant for leukocytes and a macrophage activator that is involved in triggering the innate immune response towards bacterial pathogens, which was chosen as a suitable target to test the utility of FAPM for the N-formylation of peptidic substrates. The parent tripeptide ester 12 was treated with 1.5 equiv. of FAPM in EtOAc for 5 h to afford formamide 13 in 87% yield, which was then hydrolysed using NaOH/MeOH to afford F-MLP 14 in 93% yield (Scheme 4a). Similarly, a decapeptide with the sequence ADGIVNGVKA 15 containing N-acetamide and C-primary amide termini, was reacted with 5 equiv. of FAPM in a mixture of MeCN/H₂O (pH 8.0) containing a few drops of DMSO. This resulted in N-formylation of the free ω-amino group of its lysine residue affording N-formyl-decapeptide 16, as confirmed from HRMS analysis (Scheme 4b).

Formamides are often employed for the synthesis of isocyanides, and so we investigated whether FAPM 5 could be used to develop a stepwise ‘one-pot’ protocol to directly convert an amine into its corresponding isocyanide. 4-Bromoaniline 7n and FAPM 5 (1.5 equiv.) were stirred in CH₂Cl₂ at rt for 24 h to afford its corresponding formamide 8n in situ (confirmed by 1H NMR spectroscopic analysis). Sequential dropwise addition of diisopropylamine (4 equiv.) and POCl₃ (2.2 equiv.) to this solution of formamide 8n in CH₂Cl₂, at 0 °C, followed by stirring at 0 °C for 2 h and rt for 4 h, gave 1-bromo-4-isocyanobenzene 17 in 74% yield over two steps (Scheme 5).

**Scheme 3:** FAPM 5 as an N-formylating reagent for the synthesis of N-formyl-α-amino acids 11a-f.

In conclusion, two practical syntheses of formylglyoxyacetophenylethylmethane 5 have been developed and its use as a water toler-
ant N-formylating reagent for primary and secondary amines under solvent-free conditions demonstrated. Its potential as a reagent for the synthesis of N-formyl-$\alpha$-amino acids and N-formyl-peptides in aqueous systems has been established and it has also been used as an N-formylating reagent in a one pot protocol to convert an aniline into its corresponding isocyanide.

ASSOCIATED CONTENT
Complete experimental procedure and relevant spectra (¹H and ¹³C NMR spectra) for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes
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15. A similar mechanistic rationale was proposed to explain formation of mixed acrylals 19 and 20: See refs 14b-e for details.
18. Formyl-acrylals have been prepared previously in moderate 44-57% yields via reaction of aldehydes with an acid anhydride and formic acid in the presence of phosphorus pentoxide, see: Scheeren, J. W.; Tax, W. J. M.; Schijf, R. Synthesis 1973, 151-153.