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O-Alkylation of 3-hydroxyisoxazoles predominates under Mitsunobu conditions

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ABSTRACT

Regiochemical control in the functionalization of ambident nucleophiles is of particular interest in organic chemistry. Herein, we demonstrate that O-alkylation of ambident 3-hydroxyisoxazoles, which are heterocyclic bioisosteres of carboxylic acids, predominates under Mitsunobu conditions. In several cases, excellent *O*-regioselectivity ($\geqslant$95%) was observed. It is noteworthy that reactions were complete within 15 min at room temperature. Furthermore, the conditions are compatible with a range of alcohols that cover all of the typical protecting groups for the 3-hydroxyisoxazole motif, providing milder, simpler and less hazardous protocols to those commonly followed in the literature.

3-Hydroxyisoxazoles (3-isoxazolols or isoxazole-3-ones) feature in naturally occurring compounds and are useful building blocks in the construction of pharmaceuticals.[1] For example, muscimol (**1**) and ibotenic acid (**2**) are psychoactive natural products,[2] and a variety of related 3-hydroxyisoxazoles have been developed that exhibit excellent central nervous system (CNS) activity.[3,4] Bearing a similar pK_a, the 3-hydroxyisoxazole motif is bioisosteric with the carboxylic acid function and may evade some of the metabolic fates suffered by carboxylic acids, further adding to its utility in drug design.[5] Moreover, whilst a carboxylic acid represents the *terminus* to a drug molecule, a 3-hydroxyisoxazole motif can be incorporated *within* a drug molecule (Fig. 1B), allowing for functionality to be added either side of the acidic function analogous to the acylsulfonamide in the Bcl-2/Bcl-x_L inhibitor ABT-737 (**3**).[6a] Indeed, as part of our research program to develop inhibitors of the oncoprotein Mcl-1,[6b] we have enlisted the 3-hydroxyisoxazole heterocycle as an acidic scaffold designed to bind Arg263, whilst substitutions at both the 4- and 5-positions are intended to target hydrophobic domains flanking Arg263. In our hands, however, protection of the acidic hydroxyl was non-trivial and afforded the desired products in a variety of yields, prompting the present research.

Owing to its ambident nature, synthetic modification of the 3-hydroxyisoxazole motif under classical alkylation conditions (RHal, K_2CO_3, room temperature (rt)) frequently results in mixtures of *O*- and *N*-alkylated products.[7] 3-Hydroxyisoxazoles have a pK_a of approximately 5 and are, therefore, suitable pronucleophiles for the Mitsunobu reaction with the triphenylphosphine (PPh_3)/di-isopropyl azodicarboxylate (DIAD) reagent system, wherein the pK_a of the pronucleophile must be around 12 or below for a successful reaction to occur.[8] Encouraged by our recent findings that the regioselective N-alkylation of the benzodiazepine-2,5-dione anilide function can be effectively controlled by the Mitsunobu reaction,[9] coupled with a similar study on pyridone,[10] we wondered if selective O/N-alkylation of 3-hydroxyisoxazoles might likewise be mastered. To the best of our knowledge, although there is precedent for Mitsunobu chemistry with 3-hydroxyisoxazoles,[11] such a study has not been reported. By analysing the pronucleophile, alcohol and reaction solvent, we herein demonstrate the utility of the Mitsunobu reaction in the regioselective O-alkylation of 3-hydroxyisoxazoles. Mild reaction conditions, swift reaction times and yields of up to 91% render this chemistry an attractive means towards the protection/functionalization of this acidic heterocycle.

Employing a slight excess (1.3 equiv) of PPh_3 and DIAD in THF, which represent the most popular Mitsunobu reagents and reaction solvent, we found that the coupling reaction between 5-phenyl-3-hydroxyisoxazole (**4** (R^1 = Ph)) and isopropanol was complete within 15 min at rt. Analysis of the ^{1}H NMR of the crude reaction mixture revealed a ratio of 6.7:1 for the *O*-isopropyl product **5** to the regioisomeric *N*-isopropyl product **6** (Table 1, entry 1). The O:N ratio was determined by comparison of the integrations of

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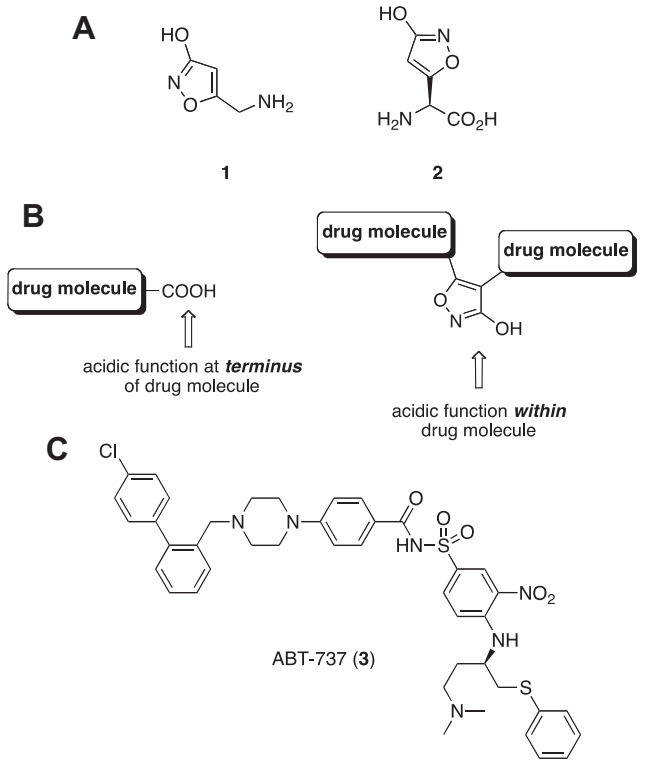
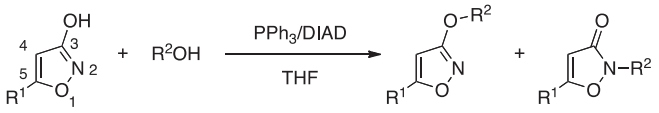
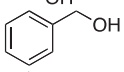
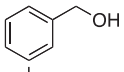
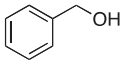
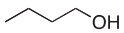
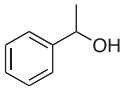


Figure 1. (A) The structures of muscimol (**1**) and ibotenic acid (**2**). (**B**) The 3-hydroxyisoxazole motif allows the incorporation of an acidic function *within* a drug molecule, in contrast to its bioisosteric carboxylic acid motif. (**C**) The structure of ABT-737.

Table 1
Mitsunobu reactions of 3-hydroxyisoxazoles with alcohols R^2OH[a]



4 **5** **6**

Entry	R^1	R^2OH	**5**[b] (%)	**5**:**6**[c]
1	—Ph		82	6.7:1
2	—Ph		45	1:1
3	—H		ND	7.3:1
4	—H		33	1.7:1
5			84	13:1
6			65	2.3:1
7		MeOH	70	2.3:1
8			84	19:1
9			60	13:1
10			18[d]	3:1

ND, not determined.
[a] Reaction conditions:[12] 3-hydroxyisoxazole (1 mmol), R^2OH (1.25 mmol) and PPh_3 (1.3 mmol) were dissolved in anhydrous THF (14 mL) at rt. After 2 min, DIAD (1.3 mmol) was added dropwise. The reaction mixture was stirred at rt under an inert atmosphere until complete by TLC (typically within 15 min).
[b] Isolated yield.
[c] ^1H NMR-determined ratio of O:N products in crude reaction mixture.
[d] Reaction time = 48 h.

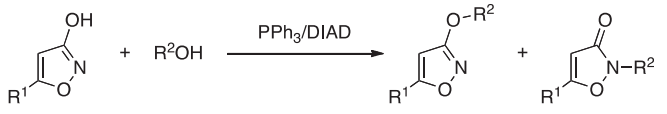
the C4-H protons of **5** and **6** (δ_H (DMSO-d_6) 6.73 (O), 6.43 (N)). In addition, after purification of the reaction mixture by silica gel flash column chromatography, the identity of the faster-running product (R_f 0.68 (Hex/EtOAc, 1:1)) was confirmed to be *O*-isopropyl-**5** through comparison of the NMR spectral data with an authentic sample that was prepared unambiguously.[13] The more polar *N*-regioisomer **6** (R_f 0.15 (Hex/EtOAc, 1:1)) was readily separated from **5** owing to the large difference in R_f.

In contrast to the reaction with isopropanol, no selectivity was observed with benzyl alcohol (Table 1, entry 2). Similar ratios were observed with 3-hydroxyisoxazole (**4** (R^1 = H); Table 1, entries 3 and 4). On the other hand, O-alkylation predominated with methyl 3-hydroxyisoxazole-5-carboxylate (**4** ($R^1 = CO_2Me$); Table 1, entries 5–10), demonstrating the structure of the 3-hydroxyisoxazole, to some degree, dictates the regiochemistry in the Mitsunobu reaction. In the context of methyl 3-hydroxyisoxazole-5-carboxylate, the preference for O-alkylation may result from reduced electron density on the nitrogen atom due to the electron-withdrawing methyl ester at C5. As shown by entry 10 in Table 1, the coupling of methyl 3-hydroxyisoxazole-5-carboxylate with *tert*-butanol generated a notable yield of *O*-*tert*-butyl-**5**. This finding is especially striking since tertiary alcohols do not usually participate in the Mitsunobu reaction due to steric hindrance, although this transformation under Mitsunobu conditions has been reported on occasion.[14] In addition to comparing the integrations of the ^1H NMR chemical shifts of the C4-H protons of regioisomers **5** (for example, δ_H (DMSO-d_6) 7.04–7.11 for $R^1 = CO_2Me$) and **6** (δ_H (DMSO-d_6) 6.58–6.72 for $R^1 = CO_2Me$), O:N ratios were also determined by comparisons of the integrations for *O*- and *N*-methyl, methylene or methine protons originating from the alcohol R^2OH, when not obscured by the isopropyl CH signals from DIAD and DIAD-H_2. These assignments were corroborated by published ^1H NMR spectral data where available.[7,13,15] Due to large differences in R_f (around 0.5 units), *O*-alkylated products **5** were readily separated from their more polar *N*-regioisomers by column chromatography.

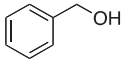
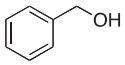
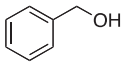
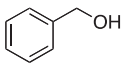
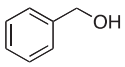
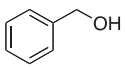
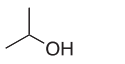
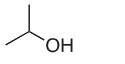
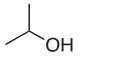
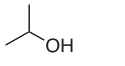
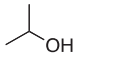
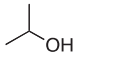
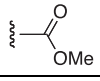
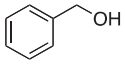
We, and others, have shown that the reaction solvent can influence the regiochemical outcome of the Mitsunobu-mediated alkylation of ambident nucleophiles.[9,10] In the present study, however, we observed little solvent effect on the Mitsunobu reaction with the pronucleophiles 3-hydroxyisoxazole and 5-phenyl-3-hydroxyisoxazole, although THF afforded a slight preference for O-alkylation relative to the chlorinated solvents and toluene (Table 2, entries 1–6). In contrast, the coupling between methyl 3-hydroxyisoxazole-5-carboxylate and isopropanol gave the lowest *O*-selectivity of 13:1, O:N in THF (Table 2, entry 7), whilst toluene delivered the best *O*-selectivity of 32:1 (Table 2, entry 11) for the panel of solvents evaluated. Surprisingly, the Mitsunobu reactions in DMSO and CH_3CN worked well, generating the *O*-alkylated product **5** in high yields (Table 2, entries 10 and 12).

Standard protecting groups for 3-hydroxyisoxazoles include methyl ether, isopropyl ether and benzyl ether. Treatment of 3-hydroxyisoxazoles with MeI often results in N-methylation or, at best, a 1:1 mixture of the *O* and *N* regioisomers, whilst explosive diazomethane promotes O-methylation to afford a 2:1 mixture of O:N products.[7] Regioselective O-isopropylation is accomplished in very good yields with isopropyl halides, although heating may

Table 2
Solvent study of Mitsunobu reactions of 3-hydroxyisoxazoles with alcohols R^2OH[a]



4 5 6

Entry	R^1	R^2OH	Solvent	**5**[b] (%)	**5**:**6**[c]
1			THF	33	1.7:1
2			$CHCl_3$	39	1.3:1
3			THF	45	1:1
4			$CHCl_3$	42	1:1.4
5			CH_2Cl_2	28	1:1.4
6			Toulene	26	1:1.7
7			THF	84	13:1
8			$CHCl_3$	91	13:1
9			CH_2Cl_2	85	19:1
10			DMSO	79	19:1
11			Toulene	85	32:1
12			CH_3CN	82	24:1
13		MeOH	Toulene	66	2.3:1
14			Toulene	72	3:1

[a] Reaction conditions:[12] 3-hydroxyisoxazole (1 mmol), R^2OH (1.25 mmol) and PPh_3 (1.3 mmol) were dissolved in anhydrous solvent (14 mL) at rt. After 2 min, DIAD (1.3 mmol) was added dropwise. The reaction mixture was stirred at rt under an inert atmosphere until complete by TLC (typically within 15 min).
[b] Isolated yield.
[c] ^1H NMR-determined ratio of O:N products in crude reaction mixture.

be required for up to 2 days.[16] Meanwhile, O-benzylation is achieved under classical alkylation conditions with benzyl bromide and K_2CO_3 in yields of 50% and upwards.[4] Our Mitsunobu conditions afforded the desired *O*-methyl (Table 1 entry 7), *O*-isopropyl (Table 1, entry 5) and *O*-benzyl (Table 1, entry 6) protected 3-hydroxyisoxazoles in good to very good yields and in excellent selectivities that are comparable with those in the literature for similar reactions that employ alternative, non-Mitsunobu conditions. For example, O-methylation of ethyl 2-(3-hydroxyisoxazol-5-yl)acetate with diazomethane proceeded in 63% yield in a 2:1 ratio of O:N products,[7] whilst treatment of methyl 4-(3-hydroxyisoxazol-5-yl)piperidine-1-carboxylate with isopropyl bromide and benzyl bromide delivered the corresponding O-alkylated products in 82% and 50% yields, respectively.[16,4] Significantly, though, our reaction conditions are more appealing, with transformations typically being complete within 15 min at rt under essentially neutral conditions, and, in the case of methylation, avoid the use of hazardous diazomethane.

In summary, the Mitsunobu reaction of ambident 3-hydroxyisoxazole pronucleophiles with a panel of alcohols resulted in O-alkylation as the predominant product in most cases with yields of up to 91%. Notably, the structure of the 3-hydroxyisoxazole played a role in the regiochemical control of the reaction with an electron-withdrawing carboxylic ester at C5 particularly favouring O-alkylation. THF proved a suitable reaction solvent to deliver high *O*-selectivities (up to 13:1, O:N), whilst toluene may furnish even greater *O*-selectivities with electron-withdrawing groups at position 5 of the isoxazole ring (up to 32:1, O:N). Transformations were typically complete within 15 min at rt, demonstrating the mildness of the reaction conditions. Collectively, our findings provide compelling reasons to adopt the Mitsunobu reaction as the preferred chemistry to achieve the regioselective O-functionalization of 3-hydroxyisoxazoles.

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12. *Typical Mitsunobu procedure:* 5-phenyl-3-hydroxyisoxazole (161 mg, 1.0 mmol, 1 equiv) was dissolved in anhydrous THF (14 mL), followed by isopropanol (96 μL, 1.25 mmol, 1.25 equiv) and PPh_3 (341 mg, 1.3 mmol, 1.3 equiv). The reaction mixture was stirred for 2 min at rt. DIAD (256 μL, 1.3 mmol, 1.3 equiv) was added dropwise. After stirring for a further 15 min at rt under an inert (N_2) atmosphere, TLC analysis confirmed the reaction was complete. The solvent was concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography (eluent gradient: 100% Hexane to 9:1 Hexane/EtOAc to 1:1 Hexane/EtOAc) to give *O*-regioisomer (**5**) 3-isopropoxy-5-phenyl-isoxazole as a colourless oil (169 mg, 82%): R_f 0.68 (Hex/EtOAc, 1:1); δ_H ($CDCl_3$, 400 MHz) 1.41 (d, J = 6.0 Hz, 6H, $2CH_3$), 4.94 (sep, J = 6.0 Hz, 1H, $CH(CH_3)_2$), 6.08 (s, 1H, C4-H), 7.41–7.71 (m, 5H, Ph) and *N*-regioisomer (**6**) 2-isopropoxy-5-phenyl-isoxazolone as a colourless film (21 mg, 10.3%): R_f 0.15 (Hex/EtOAc, 1:1); δ_H ($CDCl_3$, 400 MHz) 1.41

(d, J = 6.7 Hz, 6H, $2CH_3$), 4.69 (sep, J = 6.7 Hz, 1H, $CH(CH_3)_2$), 6.02 (s, 1H, C4-H), 7.44–7.67 (m, 5H, Ph).

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