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Synthesis of biotinylated diazinon: Lessons learned for biotinylation of thiophosphate esters

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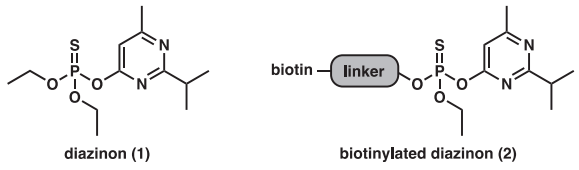
ABSTRACT

Biotinylation permits recovery of a molecule from a complex mixture, with commercially available streptavidin containing products (such as streptavidin-coated beads). As part of a larger effort to evaluate reagents capable of degrading diazinon, a thiophosphate insecticide, we pursued biotinylation of this molecule. Our strategy focused on replacing a single thiophosphate ethyl ester with an ester linkage that contains biotin. Multiple approaches—using published methods—were unsuccessful and resulted in no reactivity, or degradation of starting material. Here, we report a successful strategy for the synthesis of biotinylated diazinon, which is likely applicable to alternative thiophosphate esters and other biotinylated molecules.

Biotinylation is commonly employed to enable recovery or sequestration of a molecule or biopolymer with commercially-available streptavidin-containing reagents, such as streptavidin-coated beads.[1–4] As part of a larger research program that relies on the reactivity and processing of the thiophosphate insecticide diazinon (**1**), we initiated the synthesis of a biotinylated form. Given the reactivity of diazinon, wherein a nucleophile attacks the thiophosphate and the hydroxy pyrimidine moiety is released, we envisaged a molecule in which one of the thiophosphate ethyl esters is replaced by an ester containing biotin (exemplified by **2**). We reasoned that such a molecule could be used in a screen to identify reagents that attack the thiophosphate, since they would become biotinylated as a result of such reactivity, and could therefore be isolated and characterized following enrichment with streptavidin-coated reagents.

By virtue of the utility of biotinylation, and its broad use, the literature is replete with protocols for chemical conjugation of biotin to functionally diverse molecules.[4] However, we quickly found out that many of these synthetic approaches do not work in the context of diazinon. We began by using a previously described method to prepare the chlorothiophosphate **3**, which was achieved at 45% isolated yield.[5,6] We envisaged that **3** could react with biotin alcohol **4** in the presence of mild bases, leading to desired conjugate **5**. However, to our surprise, under various conditions, we observed no desired product (**5**), and decomposition of chlorothiophosphate **3** (Table 1). Under every reaction condition, we observed limited solubility of **4**, and therefore pursued a substrate with improved solubility in common organic solvents.



Toward the goal of improving biotin solubility we prepared biotin alcohol **3**, which features a polyethylene glycol (PEG) linker. However, Table 2 shows that attempted coupling reactions between **3** and **6**, resulted in no desired thiophosphate ester **7** (entries 1–5). We again observed decomposition of the starting alcohol, in line with observations from Table 1. We reasoned that addition of 3 equivalents of AgOTf, a known halide abstractor[7,8] would increase the electrophilicity of **3**. Again, no desired product formation was observed, and **3** decomposed into unknown byproducts (Table 2, entries 6–9). Interestingly, in all of these cases we did not observe protons at the fusion of the urea and tetrahydrothiophene rings in biotin (shown in Table two as wedged hydrogens in **7**), indicating decomposition of the biotin moiety through an unknown mechanism.

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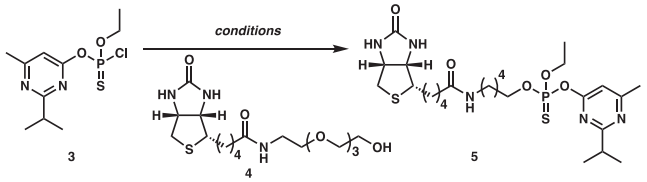
0040-4039/

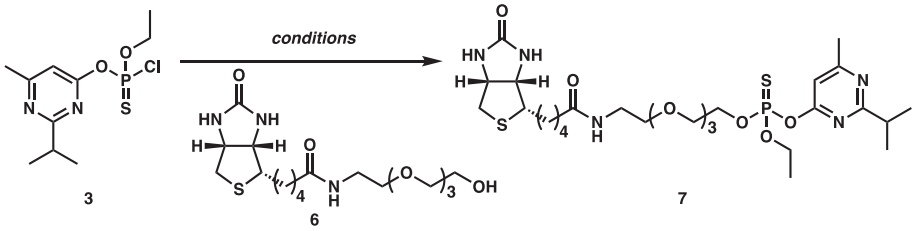
Table 1
Attempted couplings between **3** and biotin alcohol **4**.[a]



Entry	Solvent	Base	Temp (°C)	% Yield **5**[b]
1	DMF	$NaHCO_3$	25	0
2	DMF	NEt_3	25	0
3	DMF	K_2CO_3	25	0
4	MeCN	$NaHCO_3$	25	0
5	MeCN	K_2CO_3	25	0
6	CH_2Cl_2	NEt_3	25	0

[a] Reaction conditions: **3** (0.2 mmol), **4** (1.9 equiv.), base (3.0 equiv.), solvent (2.5 mL), 24 h, nitrogen atmosphere.
[b] Yields obtained by ^{1}H NMR analysis of the crude reaction mixture.

Table 2
Attempted couplings between chlorothiophosphate **3** and biotin alcohol **6**.[a]



Entry	Solvent	Base	Additive	Temp (°C)	% Yield **7**[b]
1	MeCN	$NaHCO_3$	–	25	0
2	MeCN	NEt_3	–	25	0[c,d]
3	CH_2Cl_2	K_2CO_3	–	25	0
4	CH_2Cl_2	$NaHCO_3$	–	25	0[c,d,e]
5	CH_2Cl_2	DIPEA/DMAP	–	25	0[c]
6	CH_2Cl_2	NEt_3	AgOTf	25	0[c,d,e]
7	MeCN	NEt_3	AgOTf	25	0[c,d,e]
8	CH_2Cl_2	K_2CO_3	AgOTf	25	0[c,d,e]
9	MeCN	K_2CO_3	AgOTf	25	0[c,d,e]

[a] Reaction conditions: **3** (0.1 mmol), **6** (1.9 equiv.), base (3.0 equiv.), solvent (1.0 mL), AgOTf (3 equiv. for entries 6–9), 48 h, nitrogen atmosphere.
[b] Yields obtained by ^{1}H NMR analysis of the crude reaction mixture.
[c] Trace amounts of desired mass observed by LC–MS.
[d] Multiple undesired products observed.
[e] Biotin degradation observed by ^{1}H NMR.

On the basis of the above observations, we abandoned direct conjugation between a biotin alcohol (**4** or **6**) and chlorothiophosphate **3**, and pursued conjugation of an alkynyl alcohol to **3**. We envisaged that a subsequent Hüisgen 1,3-dipolar cycloaddition ('click' reaction)[9,10] with an azide-conjugated biotin would result in a desired triazole, containing biotin and diazinon components. We began by reacting propargyl alcohol **8** with chlorothiophosphate **3**. As seen in Table 3, entry 1, reaction in acetonitrile with sodium bicarbonate as a base was unsuccessful. In contrast however, we could isolate 63% of the desired product when potassium carbonate was used as the base (Table 3, entry 2). This is in contrast to our result using biotin alcohol (**4**, Table 1, entry 5), which suggests that the biotin moiety suppresses desired reactivity through unwanted side reactions. Initial attempts to perform a preliminary 'click' reaction, using **9** as an alkynyl reactant, were unsuccessful; none of the anticipated triazole was observed.

As compound **9** was not appreciably miscible in water, and anticipating the need for excellent solubility in aqueous solutions (for subsequent screening) we pursued synthesis of a PEGylated form (**11**, Table 4). However, as shown in Table 4 (entries 1 and 2), initial attempts were unsuccessful. Analogous to previous reactions, we observed consumption of **3**, but no appreciable desired