CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200902986

# A HTS Assay for the Detection of Organophosphorus Nerve Agent Scavengers

**Ludivine Louise-Leriche,[a, b] Emilia Păunescu,[a, b] Géraldine Saint-André,[c] Rachid Baati,*[c] Anthony Romieu,[a, b] Alain Wagner,[c] and Pierre-Yves Renard*[a, b, d]**

*This paper is dedicated to the memory of the late Dr. Charles Mioskowski*

**Abstract:** A new pro-fluorescent probe aimed at a HTS assay of scavengers is able to selectively and efficiently cleave the P–S bond of organophosphorus nerve agents and by this provides non-toxic phosphonic acid has been designed and synthesised. The previously described pro-fluorescent probes were based on a conventional activated P–Oaryl bond cleavage, whereas our approach uses a self-immolative linker strategy that allows the detection of phosphonothioase activity with respect to a non-activated P–Salkyl bond. Further, we have also developed and optimised a high-throughput screening assay for the selection of decontaminants (chemical or biochemical scavengers) that could efficiently hydrolyse highly toxic **V**-type nerve agents. A preliminary screening, realised on a small α-nucleophile library, allowed us to identify some preliminary "hits", among which pyridinealdoximes, α-oxo oximes, hydroxamic acids and, less active but more original, amidoximes were the most promising. Their selective phosphonothioase activity has been further confirmed by using **PhX** as the substrate, and thus they offer new perspectives for the synthesis of more potent **V** nerve agent scavengers.

**Keywords:** fluorescent probes · hydrolysis · nerve agent scavengers · organophosphonothioase activity · phosphorus

## Introduction

Organophosphorus nerve agents (OPNAs) such as chemical warfare agents sarin (**1a**), soman (**1b**), tabun (**2**) or the exceedingly toxic **VX** (**3a**) act as acetylcholine esterase (AChE) irreversible inhibitors.[1] Despite international treaties aimed at preventing their proliferation, the ease of their synthesis and the similarity between their synthetic precursors and those of widely used pest-control agents have prevented any efficient control of the use of these weapons of mass destruction.[2] Moreover, the threat of their use became reality as illustrated by both the 1995 Tokyo subway strike with sarin and the spread of anthrax through the US postal system in the fall 2001. The decontamination and demilitarisation of OPNAs as well as the treatment of organophosphorus (OP)-based pest agent poisonings (a yearly average of 200000 lethal poisoning cases has been estimated recently[3]) are therefore imperative. Most of the methods of decontamination involve neutralising the nerve agents (NAs) by using strong alkaline and/or oxidative media and are thus incompatible with medical use and the treatment of skin or other sensitive material.[4] In the last few years, considerable work has been undertaken to develop new means of chemical and/or biotechnological decontamination.[5]

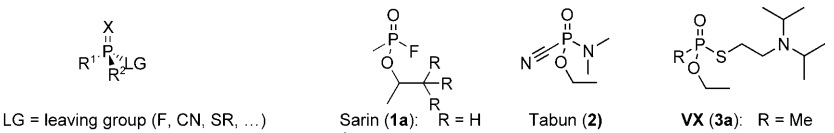
Exceedingly toxic phosphonothioate **VX** ($LD_{50} = 8\ \mu g\,kg^{-1}$ (i.v.) and $28\ \mu g\,kg^{-1}$ (per-coetaneous, rabbit))[1] is by far the most difficult target to deal with[4b] and so far no satisfactory mild means of decontamination have been proposed because the hydrolytic efficiencies of the reported decontami-

[a] L. Louise-Leriche, E. Păunescu, A. Romieu, P.-Y. Renard
Equipe de Chimie Bio-Organique
COBRA—CNRS UMR 6014 & FR 3038
rue Tesnière, 76130 Mont-Saint-Aignan (France)
Fax: (+33) 235-52-29-59
E-mail: pierre-yves.renard@univ-rouen.fr

[b] L. Louise-Leriche, E. Păunescu, A. Romieu, P.-Y. Renard
Université de Rouen, IRCOF
rue Tesnière, 76130 Mont Saint Aignan (France)

[c] G. Saint-André, R. Baati, A. Wagner
Laboratoire de Chimie des Systèmes Fonctionnels CNRS/UMR7199
Faculté de Pharmacie, Université de Strasbourg
BP 24, 67401 Illkirch (France)
Fax: (+33) 3368-854-301
E-mail: baati@bioorga.u-strasbg.fr

[d] P.-Y. Renard
Institut Universitaire de France, 103
boulevard Saint Michel, 75005 Paris (France)




nation agents towards **V**-type NAs like **VX** remain significantly lower than for the less toxic **G**-type nerve and pest-control agents.[6] This is mainly due to the relative stability of the P–S bond compared with the activated P–F or P–CN bond. Because there are different pathways to **VX** hydrolysis,[4b] to avoid the formation of even more toxic species, the reactions involved have to avoid the formation of a penta-coordinated phosphorane intermediate. Scavengers that could meet this requirement should act preferentially by nucleophilic substitution on the phosphorus atom, similar to a $S_N2P$ mechanism. Such scavengers could thus also act on **G** agents and pest control agents. Interestingly, **PhX** (**3b**)[7] displays the same hydrolysis profile as **VX** (**3a**), whereas its toxicity has been estimated to be two orders of magnitude lower and in vitro AChE inhibition appears reversible.[5c] For safety and regulatory[8] reasons, we have chosen to study the hydrolysis of this less toxic NA to validate our strategy.

Reactive decontaminants should be characterised by the ability to neutralise chemical warfare agents in a rapid and safe manner, their ease of handling, stability on long-term storage, availability and easy disposal. They should also be environmentally benign and benefit from low cost and a lack of corrosiveness. The scavengers so far discovered range from metal-centred oxidation catalysts to engineered catalytic antibodies.[5]

For now the most promising agents are still strong oxidants that act as α nucleophiles,[9–12] for example, hydrogen peroxide, peracids or oxidative chlorides or peroxides. The phosphorolytic reactivity of *o*-iodosylcarboxylates and related nucleophiles has also received a lot of interest,[13] yet such very efficient **G** nerve agent scavengers are poorly active towards **V** nerve agents.[4b] As far as inorganic salts are concerned, solvent-free hydrolysis-based chemical destruction of various warfare agents has been described using alumina-supported fluoride reagents[14] or nanosize inorganic oxide particles.[15] Among the enzyme-mediated bioremediation methods recently reported, most promising are the exogenous administration of acetyl cholinesterase or human butyryl cholinesterase used as a stoichiometric trap.[16] Some enzymatic catalytic hydrolysis[17] or oxidation[18] approaches have been proposed, yet, in all these cases, the enzymatic activity is particularly substrate-dependent, and even though some activity against V-type nerve agents has been reported, they are still insufficient.[4]

The activities of such chemical or biochemical scavengers can be easily tuned and improved through parallel or combinatorial synthesis and site-directed or randomised mutagenesis (for example, this has recently been reported for phosphotriesterases (PTE)[19] and cholinesterases[20]) as well as by the use of catalytic antibodies.[5] Consequently, it became of great importance to develop HTS assays aimed at the identification of new scavengers able to selectively and efficiently cleave the P–F, P–CN or P–S bond under mild conditions. As far as the **V** agents are concerned, their hydrolysis releases a thiol. Although this thiol rapidly dimerises under slightly basic conditions, a popular thiol-sensitive chromogenic reagent such as Ellman's reagent[21] could be used in the HTS assay. Yet, in our hands, Ellman's reagent suffers many limitations, namely 1) it does not solve the issue of **G** agents, 2) it is not compatible with most of the thiol-containing culture media used for enzyme production and protein evolution, but most of all, 3) it has been shown not to be compatible with some of the chemistry-based scavengers we have used (see below), such as other thiols, oxidants or even some α nucleophiles at basic pH (for example, by aromatic nucleophilic substitution).

Reported assays for the evaluation of OP nerve agent hydrolysis are based either on the detection of the OP themselves,[22] of their degradation agents[23] or of the remaining activity of the inhibited AChE.[24] Nevertheless, most of the assays developed to detect enzymes displaying phosphatase activity are based on fluorogenic, chromogenic or chemiluminescent substrates.[25] Some of them have been directly applied to OP nerve agents, the leaving group being replaced with a phenol-based chromogenic or fluorescent unit. The most widely used assay exploits the properties of paraoxon, which releases yellow-coloured *p*-nitrophenol after hydrolysis (Figure 1). Soukharev and Hammond synthesised and evaluated a stable fluorogenic substrate, the 7-diethoxyphosphinoyloxy-6,8-difluoro-4-methylcoumarin (DEPFMC), for the specific detection of organophosphorus hydrolases (OPH) enzymes that can hydrolyse NAs such as sarin or soman (Figure 1).[26]

More recently, another 12 fluorogenic analogues of various OPNAs were synthesised by Briseño-Roa et al.[27] using 3-chloro-7-oxy-4-methylcoumarin (Figure 1) to develop an assay that would allow the screening and selection of enzymes that are able to efficiently hydrolyse OP pest control

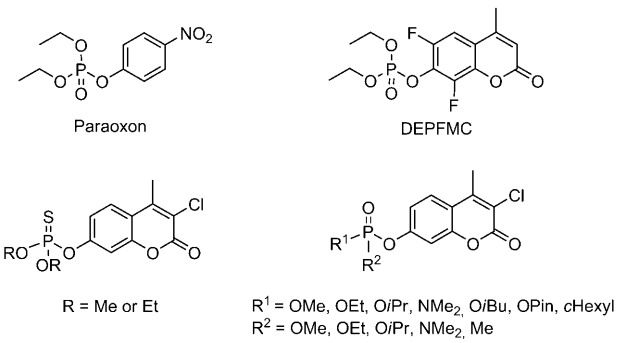



Figure 1. Reported structures of chromogenic or fluorogenic substrates.

agents (paraoxon, parathion and dimefox) and OP warfare agents (DFP, tabun, cyclosarin, soman and **VX**). However, the fact that the phenol-based fluorophore or chromophore (coumarin, fluorescein or *p*-nitrophenol) is directly linked to the organophosphoric unit dramatically slants the result for the most stable OP nerve agents. Under these conditions, the detectable moiety (an activated phenol) is a good leaving group, which activates the P–O bond rendering it more sensitive to hydrolysis. Furthermore, modification of the substrate hampers the detection of enzymes, the recognition sites of which should enclose bulky hydrophobic aromatic leaving groups. Considering the increased stability of the non-activated P–Salkyl bond in **V**-type nerve agents and the toxicity of the S-alkyl phosphonothioic acids resulting from the alternative P–O bond cleavage, this type of phenol-based probe is not suitable for the detection of enzymes or chemical agents susceptible to act as decontaminants or scavengers in this case.

In this article we describe a HTS assay that allows the identification of either chemical or biochemical scavengers able to efficiently cleave not only activated P–F or P–O bonds, but also the non-activated P–Salkyl bond of alkyl phosphonothioates. This assay is based on the use of an original pro-fluorescent probe, provided with a self-immolative linker, which permits the quantification of P–Salkyl bond cleavage through a two-step release of a hydrosoluble 7-hydroxycoumarin derivative. The test has been further validated by the identification of the most active compounds among a small focused library of nucleophiles. New oxime, amidoxime and hydroxamic acid based scavengers able to decontaminate **V**-type OP nerve agents in an aqueous environment close to physiological pH have been identified, and their activities and selectivities have been additionally confirmed by using **PhX** as the substrate.

## Results and Discussion

Inspired by the pioneering research of Reymond and coworkers for the detection of esterase activity[28] and because the targeted scavengers have to act preferentially by nucleophilic substitution on the phosphorus, similar to an $S_N2P$ mechanism,[4b] we have proposed a strategy for the rational design of a new self-immolative fluorogenic probe. Another interesting source of inspiration was the example of Zhu and co-workers who successfully designed and synthesised new fluorogenic probes that specifically target different classes of protein phosphatases.[29]

As illustrated in Figure 2, the designed probe contains a P–Salkyl moiety attached to a fluorescent unit through a reactive linker. This rational approach implies a succession of chain reactions: once the P–S bond is cleaved through an $S_N2P$ nucleophilic addition reaction, the spontaneous decomposition of the self-immolative spacer should lead to the release of the fluorescent specie.

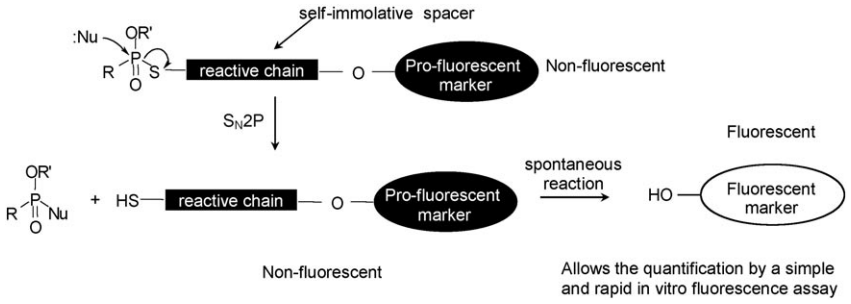
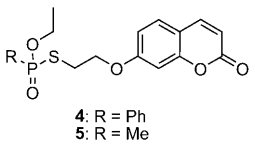



Figure 2. Self-immolative spacer strategy applied to pro-fluorescent probes suitable for organophosphonothioase activity detection assays.

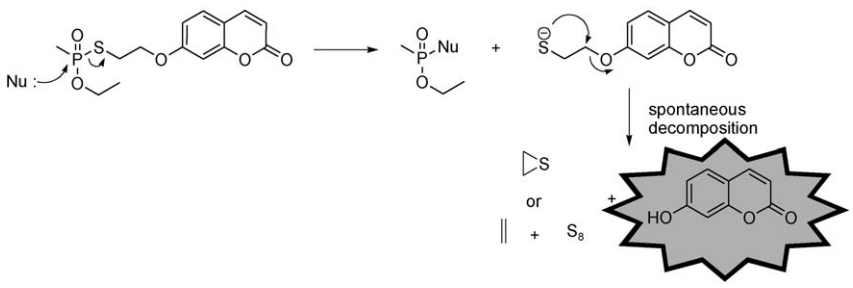
As a consequence, the design of the self-immolative linker-containing probes **4** and **5** was based on the observation that thiolates are susceptible to forming the corresponding thiiranes when they are substituted with a labile oxygenated group at the β position.[30] Therefore the cleavage of the probe P–S bond (mediated by an enzyme or by a nucleophile) should generate an unstable 7-(2-mercaptoethoxy)coumarin,




**4**: R = Ph
**5**: R = Me

which might spontaneously decompose further into the corresponding highly fluorescent 7-hydroxycoumarin ($\lambda_{em}$ = 452 nm, $\Phi$ = 0.76 in phosphate buffer at pH 7.4)[31] and thiirane (Scheme 1).

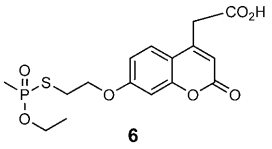
We have to be aware that the decomposition of the self-immolative linker could lead to the formation of two prod-




Scheme 1. Mechanism proposed for the decomposition of the pro-fluorogenic probe.

ucts, either the thiirane resulting from nucleophilic attack of the thiolate on the β-carbon atom or the ethylene corresponding to the β-elimination product. An alternative to these mechanisms is a Smiles-type rearrangement,[32] but this would involve nucleophilic attack of the thiolate upon the carbon atom of the aromatic phenol, which is less probable. The two first generation probes (**4** and **5**) were not fluorescent and our strategy was validated by the preliminary results obtained in a base-induced (8:2 borate buffer (pH 9.0)/acetonitrile) hydrolysis of probe **4**,[33] which proved that the product obtained from the hydrolysis of the P–S bond cleavage is indeed 7-hydroxycoumarin. However, because both probes **4** and **5** exhibit poor hydrosolubility, we designed a second-generation water-soluble pro-fluorogenic probe **6** containing an additional hydrophilic carboxylic acid function at the 4-position of the coumarin unit.



**6**

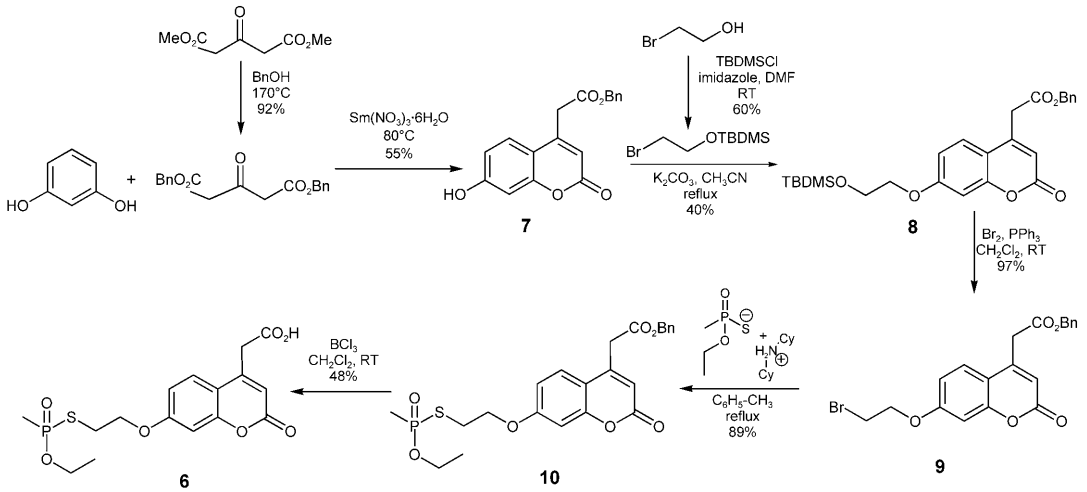
The first challenge was the synthesis of a suitable fluorophore. Because the selective protection of the carboxylic acid function in the presence of a phenol group (for example, 7-hydroxycoumarin-4-acetic acid) proved problematic, we preferred to synthesise directly the 7-hydroxycoumarin unit bearing a suitable carboxylic ester at the 4-position. The classic methods for the synthesis of 4-substituted coumarins use Pechmann,[34] Perkin,[35] Knoevenagel,[36] Reformatsky[37] or Wittig[38] reactions, which involve the condensation of a phenol with a β-keto ester in the presence of different Brønsted[39] or Lewis acids.[40] Recently, solventless synthetic methods have been developed and applied to Pechmann and Knoevenagel reactions, the condensation reactions taking place in the presence of an appropriate catalyst (*p*-toluenesulfonic acid, piperidine and samarium(III) salts).[41] In our case the best results were obtained by using the latter conditions, as presented in Scheme 2.

Dibenzyl 3-oxopentanedioate, isolated (92% yield) after transesterification of the corresponding commercially available dimethyl ester, was treated with resorcinol at 80°C in the presence of samarium(III) nitrate under solventless conditions,[41b] which allowed the isolation of the desired coumarin **7** in a moderate, yet optimised yield (55%). Phenol **7** was alkylated with *O*-silyl-protected 2-bromoethanol to give ether **8** in a yield of 40%, which was further brominated in the presence of dibromophosphorane (quantitative yield). Finally, the brominated derivative **9** was treated with a dicyclohexylammonium thiophosphonate salt to give the protected probe **10** in good yield (89%).

Unfortunately, none of the attempts to remove the benzyl protecting group by hydrogenolysis was very successful. Only a 12% conversion was obtained under pressure (20 bar) in the presence of the Pd/C catalyst in acetic acid at room temperature, whereas heating led to a complete degradation of the reaction mixture (due to the fragile P–S bond), and palladium-, titanium- and platinum-based catalysts were poisoned by the sulfur. Other methods for the soft deprotection of carboxylic acid esters, such as the use of bis(tributyl)tin oxide in an aprotic solvent,[42] also failed. Finally, the use of $BCl_3$ in anhydrous dichloromethane at room temperature gave satisfactory results. Purification by reversed-phase flash column chromatography ($C_{18}$ silica gel) allowed the isolation of the fluorogenic probe **6** in a satisfactory yield of 48% (overall yield of 8% for the six steps).

In contrast to the first-generation fluorogenic probes and as expected, the introduction of the carboxylic acid function led to a good hydrosolubility for **6**. As depicted in Figure 3, compared with 7-hydroxycoumarin-4-acetic acid, fluorogenic probe **6** displays a very low fluorescence in water (200 μM in borate buffer at pH 8.75), which designates it as a valid pro-fluorescent probe candidate because it fulfils perfectly the imposed requirements (Figure 3).

Under simulated physiological conditions, **6** proved to have good stability even after prolonged storage. For this




Scheme 2. Preparation of fluorogenic probe **6**.

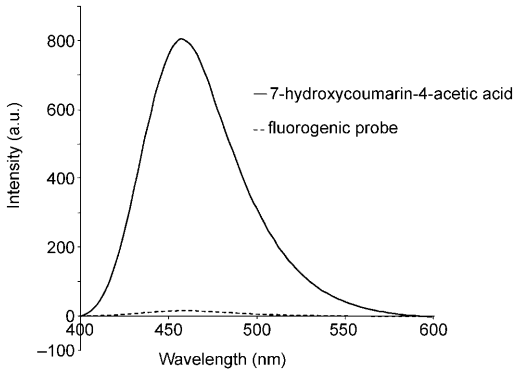



Figure 3. Comparison of the fluorescence emissions of the fluorogenic probe **6** and 7-hydroxy coumarin-4-acetic acid (200 μM in borate buffer at pH 8.75, excitation wavelength 370 nm).

type of thiophosphonate, it is important to avoid the use of phosphate buffers because, as has already been reported,[5c,43] phosphate ions can also act as weak nucleophiles leading to a significant increase in the rate of hydrolysis of phosphonothioates and thus distort the results. That is why, contrary to the commonly used procedures, the experiments were performed in Tris·HCl buffer (pH 7.4). Under the conditions used, after one week of storage at room temperature only traces of P–S bond hydrolysis were detected by HPLC analysis and fluorescence measurements at $\lambda_{em}=455$ nm ($\lambda_{ex}=370$ nm). Considering this particular and unexpected stability, we were concerned by the potential toxicity of phosphonothioate **6**, but inhibition experiments performed on human recombinant AChE (at the French Ministry of Defence Procurement Agency - DGA, Centre d'Etude du Bouchet) revealed only a moderate inhibition activity.

Subsequently, the kinetics for the hydrolysis of the fluorogenic probe **6** were evaluated in 0.1 M borate and Tris·HCl buffers (pH range from 7.0 to 10.0). The presence and quantification of the expected hydrolysis products of **6** (7-hydroxycoumarin-4-acetic acid, *O*-ethyl methylphosphonic acid, 7-(2-mercaptoethoxy)coumarin-4-acetic acid and the corresponding disulfide dimer) were analysed both by LC–MS and fluorescence assays. Similar HPLC experiments performed in parallel on **PhX** (**3b**) at pH > 9.0 revealed significant amounts of the P–O bond cleavage product, which is consistent with literature reports.[9–12] In the case of the fluorogenic probe **6**, no traces of phosphonothioic acid were detected. As expected, the rate of hydrolysis of **6** is significantly accelerated under basic pH conditions because hydroxide ions are the active species during the spontaneous hydrolysis of the phosphonothioates. A slow spontaneous hydrolysis of the fluorogenic probe was detected at pH 8.0, and the coumarin unit showed satisfactory stability at pH < 9.0 but proved troublesome above this pH (Figure 4). The LC–MS experiments showed that the optimal pH for the fluorescence experiments was between 8.5 and 8.75. Under these slightly basic pH conditions, the only hydrolysis product of **6** detected was the expected fluorescent 7-hydroxycoumarin-4-acetic acid; only a weak and apparently constant amount

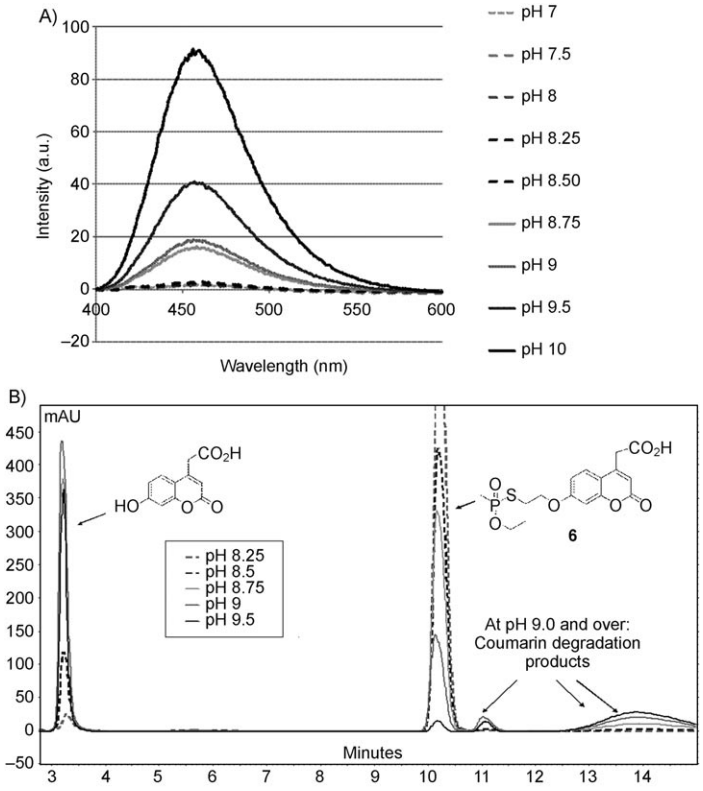


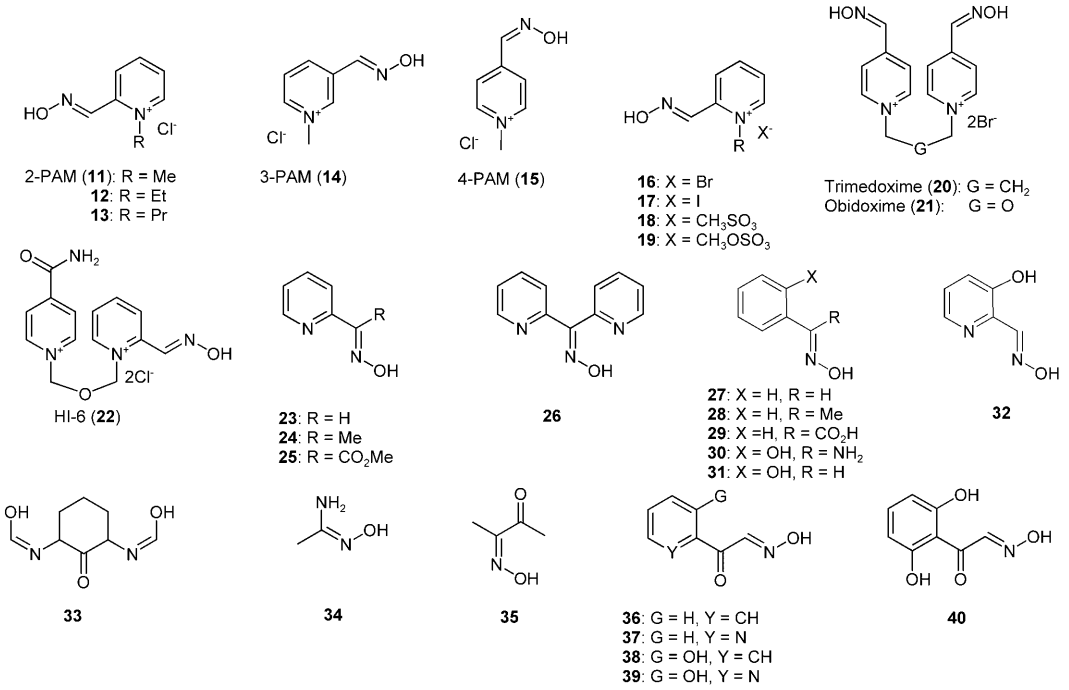

Figure 4. A) Fluorescence emission ($\lambda_{em}=370$ nm) and B) RP-HPLC elution profile (detection at $\lambda_{ex}=320$ nm) for the fluorogenic probe **6** (concentration: 200 μM) after a) 1 and b) 10 days at 37 °C in 0.1 M borate buffer at the appropriate pH.

of 7-(2-mercaptoethoxy)coumarin-4-acetic acid was observed (below 0.5% as estimated by HPLC). Because the pH range used in these experiments corresponds to the $pK_a$ of the free thiol, and although we were unable to detect either episulfur or ethylene, we concluded that these experiments confirm the expected mechanism for the decomposition of the self-immolative linker: nucleophilic attack of the thiolate anion on the carbon atom at the β position (Scheme 1).

Detection of traces of the 7-(2-mercaptoethoxy)coumarin intermediate also clearly shows that the limiting-step of the whole process is this latter nucleophilic attack, and that the stability of the intermediate thiol is the key feature in the design of the probe. We and others[44] have already observed that such a thiol is stable up to pH 7.5, and that its spontaneous decomposition is pH-dependant, the decomposition being spontaneous above pH 8.0, which is consistent with our results described herein.

With the pro-fluorescent probe in hand, we decided to evaluate a library of known and potential chemical scavengers. Because most of the previously described efficient methods for the decontamination of **V**-type agents are based on strong nucleophiles, and more particularly on α nucleophiles, we chose to focus our library on this type of compound. Beyond the classic α nucleophiles, recent computational results regarding the mechanism of the nucleophilic

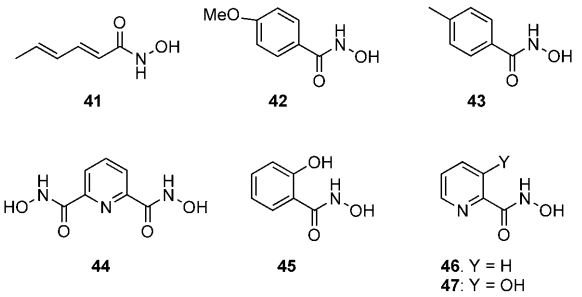



substitution of oximes on phosphonates[45] have attracted our attention. From these studies it was concluded that, even if they do not act through the $S_N2P$ mechanism, no phosphorane intermediate can be expected, and thus this type of structure represents a basis for the construction of our chemical library.

The reactions of various oximes with different OP analogues have already been studied[46] and the reactivity of oximes towards OPs (such as diisopropyl fluorophosphates; DFP) was the basis of a strategy for the design of chemically reactive colorimetric or fluorescent sensors.[47] Bromberg and Hatton[48] used recyclable catalytic magnetic nanoparticles (magnetite ($Fe_3O_4$) nanoparticles modified with a common antidote, 2-pralidoxime (2-PAM) or its polymeric analogue, poly(4-vinylpyridine-*N*-phenacyloxime-*co*-acrylic acid)) for nerve agent destruction. Oximes are well known to act as reactivators for organophosphorus-inhibited AChE.[49] Thus, pralidoxime or 2-PAM (an oxime reactivator) has been approved for clinical use in poisonings with insecticidal organophosphates, facilitating the reactivation of phosphorylated AChE back to normal activity. However, 2-PAM is not an effective reactivator of AChE inhibited with nerve agents.[50]

We extended the library with oxime-related compounds also known to act as phosphorylated cholinesterase reactivators such as hydroxamic acids.[51] In this context it is interesting to note the early report of Sloan et al.[52] who found a marked acceleration of paraoxon hydrolysis with various *N*-alkylhydroxamic acids in micellar conditions.

To evaluate the newly synthesised fluorogenic probe **6** we first selected 60 potential α nucleophiles, the large majority being commercially available products. Our library contained oximes (such as pralidoxime (2-PAM, **11**) and its derivatives (**12**–**19**), trimedoxime (TMB-4, **20**), obidoxime (**21**) and HI-6 (**22**)) already known for their ability to reactivate OPNA-inhibited AChE. Their efficiencies were compared with those of other oximes and strong nucleophiles such as hydroxy- or mercaptopyridines, hydroxypyridinones, hydroxylamines, pyrimidines, hydroxamic acids, quinazolines and peracids.




The screening test performed with the fluorogenic substrate **6** was conducted in 96 well-plates, the fluorescence measurements being realised daily for each sample. As evaluated in the HTS fluorescent assay summarised in Figure 5, most of the tested nucleophiles exhibit a weak phosphonothioate activity. Thus, as shown by the colour-coded pattern (Figure 5), HOBt (**65**), peracids **68** and **69**, iodosobenzoic acid (IBA, **71**), which according to the literature survey and to our previous experiments give satisfactory results with **G**-type agents and the HTS assay using fluorogenic probes bearing the coumarin unit directly linked to the phosphorus,[13] in our case provided very disappointing results (which were further confirmed for the hydrolysis of **PhX** (**3b**)). As

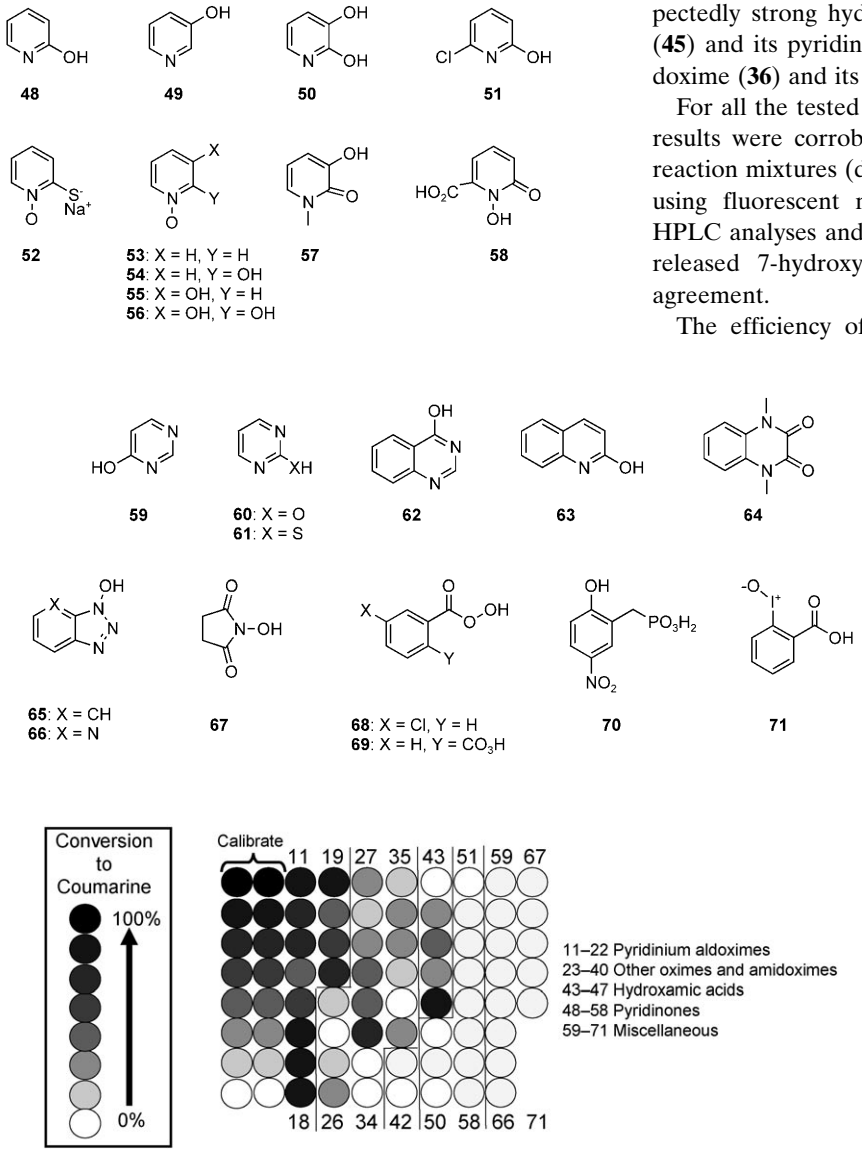


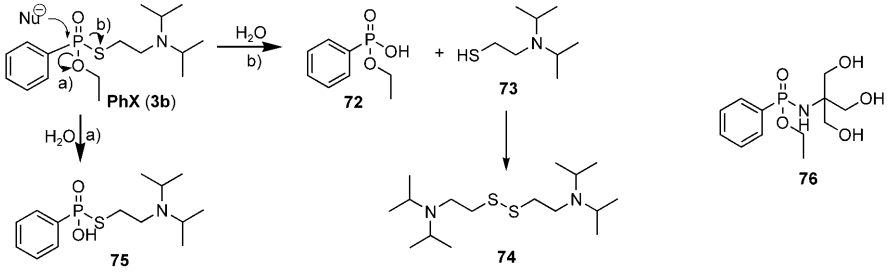

Figure 5. Representative HTS results after 36 h of reaction of 100 μM nucleophile and 50 μM fluorogenic probe **6** in 0.1 M borate buffer at pH 8.75 and 37 °C.

we expected, the quaternised pyridinealdoximes, which are described as being the best OP-poisoned AChE reactivators, display the highest phosphonothioate activity, 2-PAM (**11**) being slightly better. Their nucleophilicity is high because at pH 8.75 the oxime is totally deprotonated. The four uncharged oximes **25**, **26**, **31** and **32**[42] also gave satisfactory rates of hydrolysis; the difference in their reactivity compared with the corresponding pyridinium analogues remained high, but is lessened at a slightly basic pH at which they are also deprotonated. Interestingly, other α nucleophiles exhibited unexpectedly strong hydrolytic activity: salicylohydroxamic acid (**45**) and its pyridine-derived analogue (**47**), benzoylformaldoxime (**36**) and its derivatives (**37**–**40**) and amidoxime (**30**).

For all the tested compounds, the fluorescence HTS assay results were corroborated by HLPC analyses of the crude reaction mixtures (data not shown). Except for the reactions using fluorescent nucleophiles (such as **32** and **58**), the HPLC analyses and fluorescence yield measurements of the released 7-hydroxycoumarin-4-acetic acid are in perfect agreement.

The efficiency of some representative nucleophiles (**11**, **23**, **25**, **26**, **30**, **32**, **36** and **47**) was further tested directly in the hydrolysis of **PhX** (**3b**) at pH 8.75 in either 0.1 M borate or Tris·HCl buffer at 37 °C. As we have previously observed,[5c] temperature has a very strong effect on these phenylphosphonic esters because, under our conditions, the initial rate of spontaneous hydrolysis of **PhX** is significantly higher at 37 °C than at 20 °C. The two main hydrolytic pathways are shown in Scheme 3. The reaction was monitored by HPLC as previously described.[5c] Further LC–MS experiments allowed us to identify the main hydrolysis products: phosphonic acid **72** (which is the targeted product), thiol **73** and its corresponding oxidised form **74** (the formation of which is favoured by the slightly basic conditions), and the undesired P–O bond cleavage product **75**. In a control experiment performed only with Tris·HCl buffer, traces of the Tris addition product (**76**) were also detected, with Tris adding to the phosphorus atom.

To study the hydrolysis of **PhX** (**3b**) in the presence of the nucleophiles, two possibilities were considered: monitor-

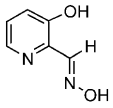
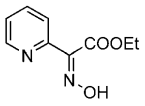
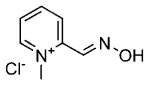
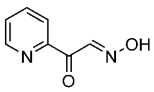
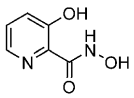
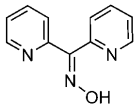
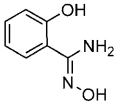
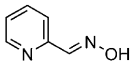



Scheme 3. Pathways for the hydrolysis reaction of **PhX** (**3b**) in the presence of a nucleophile.

ing of the decrease of **PhX** (**3b**) or of the increase of the newly formed hydrolysis product **72**. The first approach allows the reduction of toxicity to be studied directly, whereas the second allows the confirmation of the selectivity of the hydrolysis with respect to P–S bond cleavage in **PhX**. However, monitoring the formation of **72** could be problematic with some of the nucleophiles, because in the chosen elution system their HPLC retention times are close to the retention time of **72**. Because the undesired P–O bond-cleavage product (phosphonothioic acid **75**) is easier to detect, we decided to evaluate the consumption of **PhX**. For the majority of the evaluated nucleophiles (the reaction conditions were as follows: 1.0 μM PhX and 5.0 μM nucleophile in 0.1 M Tris·HCl buffer at pH 8.75 and 37 °C, see Table 1 and the Supporting Information for further details), the HPLC results were consistent with those of the HTS assay. The two main exceptions were crowded nucleophiles such as dipyridine oxime **26**, for which access to the phosphorus atom seems hampered by the pyridine group, and, to a lesser extent hydroxamic acid **47**, which seems slightly less reactive with phenylphosphonic ester **3b** than with methylphosphonic ester **6**. The two most active compounds, pralidoxime (**11**) and 3-hydroxy-2-pyridinealdoxime (**32**) (which could not be evaluated through the fluorescent assay because it is also fluorescent),[53] presented a very similar initial rate. Interestingly, the activity of two slightly less efficient compounds with structures that are more original, hydroxamic acid **47** and amidoxime **30**, was also confirmed. Note that in these four cases (**11**, **30**, **32** and **47**), even after completion of the reaction, no traces of undesired phosphonothioic acid **75** were detected by HPLC.

Table 1. Initial rates of reaction for the hydrolysis of PhX.[a]

| Nuc.[b] | Structure | Initial rate [μM min$^{-1}$] | Nuc.[b] | Structure | Initial rate [μM min$^{-1}$] |
|---|---|---|---|---|---|
| None | | 1.27 | | | |
| **32** |  | 41.5 | **25** |  | 16.8 |
| **11** |  | 37.1 | **36** |  | 15.5 |
| **47** |  | 32.3 | **26** |  | 6.1 |
| **30** |  | 25.2 | **23** |  | 4.5 |

[a] Reagents and conditions: 1.0 M PhX and 5.0 M nucleophile in 0.1 M Tris·HCl buffer at pH 8.75 and 37 °C. [b] Nucleophile.

## Conclusion

We have developed and optimised a new HTS fluorogenic assay that has allowed us to identify efficient and selective α nucleophiles displaying phosphatase activity on non-activated phosphonothioate. The method used is simple and inexpensive to implement. This assay was further used to elaborate potential scavengers of the highly toxic OP nerve agents used as chemical warfare agents such as **VX** and **PhX**. In this preliminary study, we have been able to identify four very simple structures: pralidoxime (**11**), 3-hydroxy-2-pyridinealdoxime (**32**), 3-hydroxy-2-pyridinecarbohydroxamic acid (**47**) and to a lesser extent 3-hydroxy-2-amidoxime (**30**), which exhibit significant scavenging activity towards **PhX**. With these interesting "hits" in hand, supplementary and complementary tests are presently being undertaken to further elaborate new α nucleophiles structures and to apply these scavengers to more toxic methylphosphonates such as **VX** (**3a**). Further tests are necessary to analyse the influence of relative concentrations and to identify scavengers that could be used in a wider pH range. To put the results of this work into perspective, this assay could also be applied to bioscavanger libraries elaborated for use at a physiological pH. Interestingly, among the new scavengers we have highlighted, 2-hydroxyarylaldoximes similar to **31** and **32** have very recently been used as pro-fluorescent probes for the detection of organophosphorus nerve agents by Dale and Rebek.[47c] These hydroxy oximes have been shown to efficiently react with OP nerve agent surrogates such as diisopropylfluorophosphate at a physiological pH to yield the corresponding isoxazole and non-toxic phosphonic acid, which further supports the results presented herein on their role as potential nerve agent scavengers. In another recent article, the mechanism of hydroxamic acids (such as the ones we have highlighted in this article) in the dephosphorylation of bis(2,4-dinitrophenyl) phosphates has also been investigated.[51d]

## Experimental Section

**General**: The chemicals used in the syntheses were from Aldrich, Acros or Alfa-Aesar and were used without further purification. The solution of **PhX** in $CH_3CN$ (20 mg/mL) was stored at −20 °C. **PhX** and phosphonic acids **72** and **75** were prepared as previously described.[5c,7] All solvents were dried following standard procedures ($CH_3CN$: distillation over $CaH_2$; MeOH: distillation over $Mg(OMe)_2$ and storage over 4 Å molecu-

lar sieves; DMF: distillation over BaO and storage over 4 Å molecular sieves; DIEA and triethylamine: distillation over $CaH_2$ and storage over BaO; $CH_2Cl_2$: distillation over $P_2O_5$; THF: distillation over Na/benzophenone; toluene: distillation over Na). Purifications by column flash chromatography were performed on silica gel SDS Si 60 (40–63 μm). TLC was carried out on Merck DC Kieselgel 60 F-254 aluminium sheets and visualised by employing a short wavelength UV lamp ($\lambda = 254$ nm) or by staining with a 3.5% (w/v) phosphomolybdic acid solution in absolute ethanol.

Reversed-phase column flash chromatography was performed on octadecyl-functionalised silica gel (200–400 mesh, loading 20–22%, surface area ~550 $m^2 g^{-1}$, and mean pore size 60 Å) from Aldrich.

All aqueous buffers were prepared by using water purified with a Milli-Q system (purified to 18.2 MW $cm^{-1}$).

Borate buffers 0.1 N, pH ranging from 7.0 to 10.0: [boric acid] = 0.1 N adjusted with 1 N NaOH solution.

Tris·HCl buffer 0.1 N, pH 8.75: [Tris] = 0.1 N adjusted to pH 8.75 with 1 N HCl solution.

Tris·HCl buffer 0.1 N, pH 7.4: [Tris] = 0.1 N adjusted to pH 7.4 with 1 N HCl solution.

Tris·HCl buffer 0.1 N, 0.15 N NaCl, pH 7.4: [Tris] = 0.1 N, [NaCl] = 0.15 N, adjusted to pH 8.75 with 1 N HCl solution.

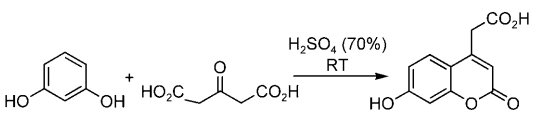
Tris·HCl buffer 0.01 N, 0.15 N NaCl, pH 7.3: [Tris] = 0.01 N, [NaCl] = 0.15 N, adjusted to pH 7.3 with 1 N HCl solution.

Phosphate buffer 0.01 N, 0.015 N NaCl, pH 7.4: $[KH_2PO_4] = 0.002$ N, $[Na_2HPO_4] = 0.008$ N, [NaCl] = 0.015 N.

Phosphate buffer 0.01 N, 0.15 N NaCl, pH 7.3: $[KH_2PO_4] = 0.002$ N, $[Na_2HPO_4] = 0.008$ N, [NaCl] = 0.15 N.

Melting points were recorded on a LEICA VMHB Kofler system at atmospheric pressure and are uncorrected. Infrared spectra were recorded using a Perkin–Elmer FT-IR Paragon 500 spectrometer in KBr pellets with frequencies given in $cm^{-1}$. NMR spectra were recorded on a Bruker AM300 spectrometer (300.15 MHz for $^1H$, 75.4 MHz for $^{13}C$ and 121.5 for $^{31}P$). Chemical shifts ($\delta$) are expressed in parts per million (ppm) with [D$_6$]acetone ($\delta_H = 2.05$, $\delta_C = 29.84$, 206.26 ppm) or $CDCl_3$ ($\delta_H = 7.26$, $\delta_C = 77.16$ ppm) as internal references and coupling constants ($J$) values are expressed in Hz. The multiplicities are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), quint. (quintuplet) and m (multiplet). Analytical HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector. High-performance liquid chromatography separation was performed on a Thermo Hypersil GOLD $C_{18}$ column (5 μm, 10×250 mm) with $CH_3CN$ and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as eluents (100% aq. TFA (5 min), linear gradient from 0 to 40% of $CH_3CN$ (40 min), then 40 to 60% of $CH_3CN$ (10 min)) at a flow rate of 1.0 mL $min^{-1}$. Dual UV detection was achieved at 290 and 350 nm. GC–MS spectra were recorded on a Thermoquest Finnigan Trace GC spectrometer coupled to an Auto Mass Multi III analyser using electron impact ionisation. ESI-MS spectra and LC–MS experiments were performed with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source. UV/Vis spectra were recorded on a Varian Cary 50 scan spectrophotometer. Fluorescence spectroscopic studies were performed in a semi-micro fluorescence cell (Hellma, 104FQS, 10×4 mm, 1400 μL) or in 96 well-plates (Corning or Nunc) using a Varian Cary Eclipse spectrophotometer.

**7-Hydroxycoumarin-4-acetic acid**: 7-Hydroxycoumarin-4-acetic acid used as a reference for the HTS assay was synthesised following an improved literature procedure (Scheme 4).[54] 1,3-Acetonedicarboxylic acid (7.3 g, 50 mmol) was added portionwise to a solution of resorcinol (5.5 g, 50 mmol) in a 70% sulfuric acid aqueous solution (50 mL) at 0°C. The mixture was allowed to warm to room temperature and stirred overnight (18 h). The resulting yellow solution was poured onto crushed ice and the white precipitate formed was collected by filtration, washed with water, EtOAc and diethyl ether and then dried overnight under reduced pressure to afford the desired product (8.12 g, 70%) as a white solid.




Scheme 4. Synthesis of 7-hydroxycoumarin-4-acetic acid.

$^1H$ NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.55$ (d, $^3J$(H-H) = 9 Hz, 1H), 6.78 (dd, $^3J$(H-H) = 9 Hz, $^4J$(H-H) = 2 Hz, 1H), 6.70 (d, $^3J$(H-H) = 2 Hz, 1H), 6.19 (s, 1H), 3.81 ppm (s, 2H); $^{13}C$ NMR (75 MHz, [D$_6$]DMSO): $\delta =$ 172.0, 162.8, 162.3, 156.1, 151.3, 126.9, 113.7, 112.5, 112.4, 102.9, 37.8 ppm; IR (KBr): $\tilde{\nu} = 3267$, 1702, 1618, 1560, 1373, 1328, 1251, 1214, 1141, 1063, 860, 684, 617 $cm^{-1}$.

**Dibenzyl 1,3-acetonedicarboxylate** This intermediate was synthesised following an adapted literature procedure.[55] Dimethyl 1,3-acetonedicarboxylate (30 mL, 204 mmol) and benzyl alcohol (43 mL, 408 mmol) were heated at 170–180°C and the methanol formed during the reaction was removed from the reaction mixture by distillation. When no more methanol was formed, the reaction mixture was cooled to room temperature and the residual methanol was removed under reduced pressure. The residual benzyl alcohol was then distilled from the mixture under a pressure of 0.4 mmHg to give the benzyl ester (61.6 g, 92%) as a yellow oil.

$^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 7.35$ (s, 10H), 5.15 (s, 4H), 3.64 ppm (s, 4H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 195.6$, 166.9, 135.5, 129.0 (2C), 128.8 (2C), 128.6, 67.7, 49.3 ppm; IR (KBr): $\tilde{\nu} = 1738$, 1652, 1455, 1327, 1261, 1223, 1177, 1145, 1000, 748, 697 $cm^{-1}$; MS (ESI): $m/z$: calcd for [$M$]: 326.16; found: 327; elemental analysis calcd (%) for $C_{19}H_{18}O_5$: C 69.93, H 5.56; found: C 69.37, H 5.86.

**Benzyl 7-hydroxycoumarin-4-acetate (7)**: This intermediate was synthesised using an adapted literature protocol.[56] A neat mixture of resorcinol (7.6 g, 68.9 mmol), dibenzyl 1,3-acetonedicarboxylate (22.5 g, 68.9 mmol) and samarium(III) nitrate hexahydrate (6.1 g,13.73 mmol) was heated at 80°C for 18 h. The dark-red oil obtained was dissolved in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The crude solid was then triturated in diethyl ether and filtered to give coumarin derivative (**7**; 6.6 g, 55%) as a beige solid.

$^1H$ NMR (300 MHz, [D$_6$]acetone): $\delta = 9.44$ (s, 1H), 7.55 (d, $^3J$(H-H) = 9 Hz, 1H), 7.34 (m, 5H), 6.81 (dd, $^3J$(H-H) = 9 Hz, $^4J$(H-H) = 3 Hz, 1H), 6.77 (d, $^4J$(H-H) = 3 Hz, 1H), 6.24 (s, 1H), 5.18 (s, 2H), 3.97 ppm (s, 2H); $^{13}C$ NMR (75 MHz, [D$_6$]acetone): $\delta = 170.1$, 162.4, 161.2, 156.9, 150.3, 137.3, 129.7, 129.4, 129.3, 127.9, 114.1, 114.0, 113.1, 103.9, 67.8, 38.5 ppm; IR (KBr): $\tilde{\nu} = 3259$, 1728, 1693, 1614, 1562, 1328, 1193, 1144, 832, 740, 700 $cm^{-1}$; MS (ESI): $m/z$: calcd for [$M$]: 310.08; found: 311 $[M+H]^+$, 643 $[2M+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{14}O_5$: C 69.67, H 4.55; found: C 69.68, H 4.69.

**(2-Bromoethoxy)(*tert*-butyl)dimethylsilane**: This intermediate was synthesised using a literature procedure.[57] 2-Bromoethanol (4.28 mL, 60.3 mmol) was added to a solution of *tert*-butyldimethylsilyl chloride (10 g, 66.3 mmol) and imidazole (5.34 g, 78.4 mmol) in anhydrous DMF. The reaction mixture was stirred for 18 h at room temperature. Water (50 mL) was added and the reaction mixture was extracted twice with DCM (50 mL). The organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The crude product was then purified by distillation under vacuum to give the desired product (8.02 g, 60%) as a colourless oil.

$^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 3.88$ (t, $^3J$(H-H) = 6 Hz, 2H), 3.39 (t, $^3J$(H-H) = 6 Hz, 2H), 0.90 (s, 9H), 0.08 ppm (s, 6H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 63.9$, 33.6, 26.2, 18.7, −4.9 ppm; IR (KBr): $\tilde{\nu} = 2956$, 2930, 2886, 2858, 1472, 1463, 1389, 1362, 1288, 1257, 1189, 1125, 1096, 1022, 947, 892, 838, 778, 674 $cm^{-1}$; elemental analysis calcd (%) for $C_8H_{19}BrOSi$: C 40.17, H 7.95; found: C 40.21, H 7.72.

**Benzyl 7-[2-(*tert*-butyldimethylsilyloxy)ethoxy]coumarin-4-acetate (8)**. This intermediate was synthesised using an adapted literature protocol.[58] A solution of (2-bromoethoxy)-*tert*-butyldimethylsilane (1.7 g, 7.12 mmol) in acetonitrile (30 mL) was added to a suspension of coumarin derivative **7** (1.7 g, 5.48 mmol) and $K_2CO_3$ (2.3 g, 16.44 mmol) in anhydrous acetonitrile (30 mL). The reaction mixture was heated at reflux for

24 h and then concentrated under vacuum. The crude was dissolved in ethyl acetate and the organic layer obtained was washed with water, dried over anhydrous $Na_2SO_4$ and concentrated. The product was purified by flash chromatography (2 to 10% ethyl acetate in cyclohexane) to give the desired compound (1 g, 40%) as a yellow solid.

$^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.63 (d, $^3J$(H-H) = 7 Hz, 1H), 7.35 (m, 5H), 6.92 (s, 1H), 6.89 (d, $^3J$(H-H) = 7 Hz, 1H), 6.29 (s, 1H), 5.18 (s, 2H), 4.21 (t, $^3J$(H-H) = 6 Hz, 2H), 4.04 (t, $^3J$(H-H) = 6 Hz, 2H), 4.00 (s, 2H), 0.90 (s, 9H), 0.11 ppm (s, 6H); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 170.1, 163.6, 161.1, 156.9, 150.2, 137.3, 129.7, 129.4, 129.3, 127.6, 114.7, 113.8, 113.7, 102.6, 71.5, 67.8, 63.0, 38.4, 26.6, 19.3, −4.5 ppm; IR (KBr): $\tilde{\nu}$ = 2952, 2930, 2885, 2851, 1732, 1614, 1560, 1510, 1454, 1392, 1288, 1262, 1126, 1069, 1003, 962, 837, 776 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 468.20; found: 469 [*M*+H]$^+$, 937 [2*M*+H]$^+$, 659 [2*M*+Na]$^+$; elemental analysis calcd (%) for $C_{26}H_{32}O_6Si$: C 66.64, H 6.88; found: C 66.98, H 7.03.

**Benzyl 7-(2-bromoethoxy)coumarin-4-acetate (9)**: A solution of bromine (426 μL, 8.3 mmol) in anhydrous DCM (40 mL) was added dropwise to a solution of triphenylphosphine (2.2 g, 8.3 mmol) in anhydrous DCM (40 mL) until the persistence of a pale-yellow colour. A solution of the coumarin derivative **8** (3 g, 6.4 mmol) in anhydrous DCM (10 mL) was then added and the reaction mixture was stirred for 5 h at room temperature. The mixture was washed twice with water, dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was triturated in cold ethanol and filtered to give the desired product (2.6 g, 97%) as a pale-yellow solid.

$^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.64 (d, $^3J$(H-H) = 7 Hz, 1H), 7.35 (m, 5H), 6.95 (s, 1H), 6.92 (d, $^3J$(H-H) = 7 Hz, 1H), 6.31 (s, 1H), 5.18 (s, 2H), 4.51 (t, $^3J$(H-H) = 5 Hz, 2H), 4.00 (s, 2H), 3.84 ppm (t, $^3J$(H-H) = 5 Hz, 2H); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 170.0, 162.7, 161.0, 156.8, 150.1, 137.3, 129.7, 129.5, 129.4, 127.8, 114.7, 114.2, 113.7, 102.8, 69.8, 67.8, 38.5, 30.5 ppm; IR (KBr): $\tilde{\nu}$ = 3063, 1722, 1618, 1396, 1330, 1296, 1274, 1238, 1190, 1149, 1065, 1024, 963, 853, 742 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 416.03; 417–419 [*M*+H]$^+$, 857–859 [2*M*+Na]$^+$; elemental analysis calcd (%) for $C_{20}H_{17}BrO_5$: C 57.57, H 4.11; found: C 57.54, H 4.14.

**Benzyl 7-{2-[ethoxy(methyl)phosphinoylsulfanyl]ethoxy}coumarin-4-acetate (10)** Coumarin derivative **9** (2.3 g, 5.5 mmol) and *O*-ethyl methylphosphonothioate (2.3 g, 7.2 mmol) were dissolved in toluene (60 mL) and the mixture was heated at reflux for 3 h. Once the reaction evolution monitored by $^{31}$P NMR analysis showed completion, the reaction mixture was filtered, the precipitate was washed with diethyl ether and the filtrate was concentrated under vacuum. After purification by flash chromatography (DCM/acetone/MeOH, 92:2.5:0.5), the desired product (2.35 g, 89%) was obtained as a yellow oil.

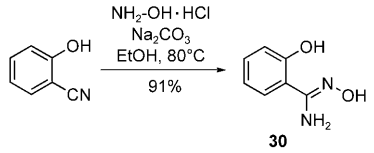
$^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.61 (d, $^3J$(H-H) = 9 Hz, 1H), 7.35 (m, 5H), 6.95 (s, 1H), 6.92 (d, $^3J$(H-H) = 9 Hz, 1H), 6.29 (s, 1H), 5.17 (s, 2H), 4.35 (m, 2H), 4.12 (m, 2H), 3.99 (s, 2H), 3.30 (m, 2H), 1.80 (d, $^2J$(H-P) = 16 Hz, 3H), 1.30 ppm (t, $^3J$(H-H) = 7 Hz, 3H); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 170.1, 162.8, 161.1, 156.8, 150.1, 137.3, 129.8, 129.5, 129.4, 127.7, 114.9, 114.1, 113.6, 102.8, 69.6, 67.9, 62.3, 38.5, 30.1, 21.4, 17.0 ppm; $^{31}$P NMR (121.5 MHz, [D$_6$]acetone): $\delta$ = 53.0 ppm; IR (KBr): $\tilde{\nu}$ = 2985, 2938, 1731, 1715, 1621, 1618, 1393, 1301, 1266, 1219, 1144, 1030, 961, 883, 743 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 476.11; 477 [*M*+H]$^+$, 499 [*M*+Na]$^+$, 975 [2*M*+Na]$^+$; elemental analysis calcd (%) for $C_{23}H_{25}O_7PS$: C 57.98, H 5.29; found: C 58.05, H 5,37.

**7-{2-[Ethoxy(methyl)phosphinoylsulfanyl]ethoxy}coumarin-4-acetic acid (6)**: Boron trichloride (18.4 mL, 1 M in DCM) was added dropwise to a cooled solution (0 °C) of the coumarin derivative **10** (2.2 g, 4.61 mmol) in anhydrous DCM (20 mL) and the reaction mixture was stirred at room temperature for a further 2 h. The reaction was quenched by the addition of water/MeOH (10 mL/10 mL) and concentrated under reduced pressure. The crude product was purified by reversed-phase silica gel chromatography (MeOH/$H_2O$ (0.1% TFA), 10:90 to 35:65) to give the desired product (860 mg, 48%) as a white solid.

$^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.71 (d, $^3J$(H-H) = 9 Hz, 1H), 7.02 (d, $^3J$(H-H) = 9 Hz, 1H), 6.98 (s, 1H), 6.30 (s, 1H), 4.35 (m, 2H), 4.12 (m, 2H), 3.92 (s, 2H), 3.30 (m, 2H), 1.80 (d, $^2J$(H-P) = 16 Hz, 3H), 1.30 ppm (t, $^3J$(H-H) = 7 Hz, 3H); $^{13}$C NMR (75 MHz, [D$_6$]acetone)**:** $\delta$ = 170.6, 162.5, 160.7, 156.5, 150.1, 127.4, 114.5, 113.9, 113.2, 102.4, 69.2 (d), 61.9 (d), 37.8, 29.6, 20.9 (d), 16.6 ppm (d); $^{31}$P NMR (121.5 MHz, [D$_6$]acetone): $\delta$ = 52.9 ppm; IR (KBr): $\tilde{\nu}$ = 2904, 1718, 1614, 1394, 1304, 1267, 1200, 1142, 1029, 971, 896, 749 cm$^{-1}$; MS (ESI): *m/z*: found: 341 [*M*−$CO_2$−H]$^-$; elemental analysis calcd (%) for $C_{16}H_{19}O_7PS$: C 49.74, H 4.96, S 8.30; found: C 49.71, H 4.88, S 8.11.

**Synthesis of the new α nucleophiles**

2-Hydroxybenzamidoxime (30): Amidoxime (**30**) was obtained in one step by the nucleophilic addition of hydroxylamine to the electrophilic cyanide (Scheme 5).

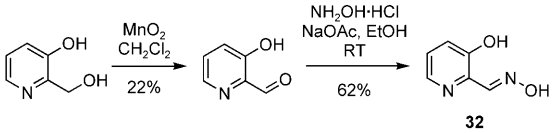



Scheme 5. Synthesis of 2-hydroxybenzamidoxime (**30**).

A solution of hydroxylamine hydrochloride (278 mg, 4 mmol) and $Na_2CO_3$ (212 mg, 2 mmol) in water (3 mL) was added to a solution of 2-hydroxybenzonitrile (119 mg, 1 mmol) in ethanol (0.5 mL) and the mixture obtained was heated at 80 °C for 16 h. The reaction mixture was allowed to cool to room temperature and then was extracted with ethyl acetate. The organic phase was dried over $Na_2SO_4$ and concentrated. The crude was purified by column chromatography using cyclohexane/ethyl acetate (40:60) as eluent to provide the desired compound as a beige solid (138 mg, 91%).

M.p. 97 °C; $^1$H NMR (300 MHz, MeOD): $\delta$ = 7.50 (dd, $^3J$(H-H) = 1.5 Hz, $^3J$(H-H) = 8 Hz, 1H), 7.22 (td, $^3J$(H-H) = 1.5 Hz, $^3J$(H-H) = 8 Hz, 1H), 6.86 ppm (t, $^3J$(H-H) = 8 Hz, 2H); $^{13}$C NMR (50 MHz, MeOD): $\delta$ = 158.7, 155.2, 131.4, 126.5, 119.7, 117.8, 116.2 ppm; IR (KBr): $\tilde{\nu}$ = 3367, 2360, 2342, 1645, 1254 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 152.15; found: 153.

**3-Hydroxy-2-pyridinealdoxime (32)**: This compound was synthesised in two steps starting from commercially available 2-hydroxymethyl-3-hydroxypyridine, which was first oxidised in the presence of $MnO_2$ to give the corresponding aldehyde in a modest yield (22%). This aldehyde was then transformed under classic conditions into the corresponding aldoxime **32** in a yield of 62% (Scheme 6).




Scheme 6. Synthesis of 3-hydroxy-2-pyridinealdoxime (**32**).

A solution of 3-hydroxy-2-(hydroxymethyl)pyridine hydrochloride (2 g, 10.52 mmol, 85%) in MeOH (10 mL) was neutralised with KOH (590 mg, 10.5 mmol) in a salt-ice bath for 1 h and the precipitated KCl was removed by filtration. The filtrate was concentrated to dryness under vacuum. The free 3-hydroxy-2-(hydroxymethyl)pyridine was suspended in DCM (20 mL) and treated with active $MnO_2$ (5 g, 58%). The reaction mixture was heated at reflux for 5 h and was then allowed to cool to room temperature overnight. The crude mixture was filtered and the $MnO_2$ cake was washed with DCM (2×40 mL). The filtrate was concentrated to dryness and the crude product was purified by flash chromatography on silica gel with cyclohexane/ethyl acetate (90:10 to 80:20) as eluent to give the desired product (3-hydroxy-2-pyridinecarbaldehyde) as a pale-green solid (288 mg, 22%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 10.71 (s, 1H), 10.05 (s, 1H), 8.32 (dd, $^3J$(H-H) = 4 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.42 (dd, $^3J$(H-H) = 9 Hz, $^3J$(H-H) =

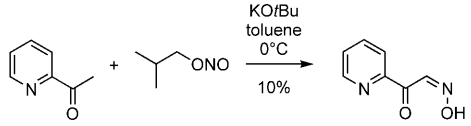
  

4 Hz, 1H), 7.33 ppm (dd, $^3J$(H-H) = 9 Hz, $^4J$(H-H) = 1 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 198.8, 158.7, 142.6, 136.8, 130.1, 126.1 ppm; IR (KBr): $\tilde{\nu}$ = 1681, 1578, 1467, 1353, 1320, 1233, 1149, 1113, 1063, 871, 809, 646 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 123.03; found: 124; elemental analysis calcd (%) for $C_6H_5NO_2$: C 58.54, H 4.09, N 11.38; found: C 58.59, H 4.04, N 11.39.

Hydroxylamine hydrochloride (104 mg, 1.5 mmol) and sodium acetate (204 mg, 1.5 mmol) were added to a solution of 3-hydroxy-2-pyridinecarbaldehyde (123 mg, 1 mmol) in dry ethanol (2.5 mL).The mixture was stirred at room temperature overnight and then concentrated. The crude product was diluted in EtOAc (10 mL), washed with water (2×10 mL), dried over $Na_2SO_4$ and purified by flash column chromatography on silica gel with cyclohexane/EtOAc (80:20 to 70:30) as eluent to give the desired product as a white solid (86 mg, 62%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 9.84 (s, 1H), 8.48 (s, 1H), 8.45 (s, 1H), 8.22 (dd, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.34 (dd, $^3J$(H-H) = 9 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.25 ppm (dd, $^3J$(H-H) = 9 Hz, $^3J$(H-H) = 5 Hz, 1H); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 155.1, 154.1, 142.0, 137.0, 125.6, 124.4 ppm; IR (KBr): $\tilde{\nu}$ = 3075, 2994, 2874, 2737, 1447, 1319, 1278, 1183, 1020, 910, 812, 703, 656 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 138.04; found: 139.

**2-Oxo-2-(pyridin-2-yl)acetaldehyde oxime (37)**: Oximes at the α position of a ketone were obtained by the reaction of the carbonyl compound with an alkyl nitrite in the presence of a base.[59] Thus, the expected compound **37** could be isolated by treating isoamyl nitrite with 2-acetylpyridine in the presence of potassium *tert*-butoxide in toluene, but a poor yield of only 10% was obtained (Scheme 7). As the quantity of the product obtained (~135 mg) was sufficient to realise the hydrolysis tests, this reaction was not further optimised.



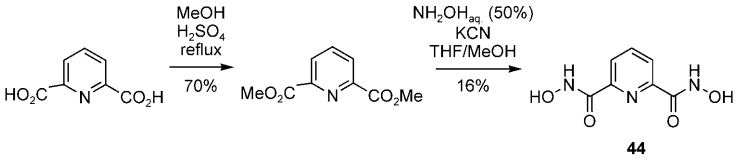

**37**

Scheme 7. Nitrosation of 2-acetylpyridine.

2-Acetylpyridine (1 mL, 8.9 mmol) and potassium *tert*-butoxide (2.5 g, 22.3 mmol) were dissolved in anhydrous toluene (44 mL) at −10 °C. After 15 min of stirring, isoamyl nitrite (1.42 mL, 10.7 mmol) was added dropwise and stirring was maintained for 30 min. Iced water (40 mL) and acetic acid (4.5 mL) were added to the reaction mixture. The solution was then extracted with EtOAc (2×40 mL) and the combined organic layers were washed with brine, dried over $Na_2SO_4$, concentrated and purified by silica gel chromatography with cyclohexane/EtOAc (80:20) as eluent to give the desired product as a white solid (135 mg, 10%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 11.64 (s, 1H), 8.70 (d, $^3J$(H-H) = 5 Hz, 1H), 8.50 (s, 1H), 8.12 (d, $^3J$(H-H) = 8 Hz, 1H), 7.91 (td, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.52 ppm (ddd, $^3J$(H-H) = 8 Hz, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 186.7, 153.0, 148.9, 146.6, 137.7, 127.6, 124.3 ppm; IR (KBr): $\tilde{\nu}$ = 3306, 1693, 1593, 1582, 1454, 1438, 1314, 1286, 1226, 1070, 982, 877, 808, 745, 695, 617 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 150.04; found: 151; elemental analysis calcd (%) for $C_7H_6N_2O_2$: C 56.00, H 4.03, N 18.66; found: C 55.72, H 4.11, N 18.63.

**2,6-Pyridinedicarbohydroxamic acid (44)**: Dihydroxamic acid **44** was synthesised in two steps (Scheme 8) from the corresponding commercially available 2,6-pyridinedicarboxylic acid. Thus, the intermediate methyl diester could be obtained as previously described in a yield of 70%. The reaction of this diester in the presence of hydroxylamine led to a complete conversion of the starting compound, but due to the high polarity of the obtained diacid the purification was tricky, and product **44** was finally isolated after recrystallisation in water with a poor yield of only 16%.




**44**

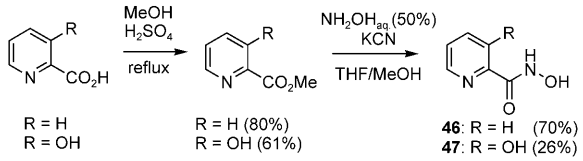
Scheme 8. Synthesis of the 2,6-pyridinedicarbohydroxamic acid (**44**).

Pyridine-2,6-dicarboxylic acid (1 g, 6 mmol) was dissolved in MeOH (12 mL). Concentrated $H_2SO_4$ (2 mL) was added and the mixture was heated at reflux for 48 h. After cooling to room temperature, the mixture was concentrated under vacuum, diluted in $H_2O$ (50 mL) and neutralised by the addition of solid $K_2CO_3$. The solution was extracted with EtOAc (2×50 mL) and the organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated to give the desired product (dimethyl 2,6-pyridinedicarboxylate) as a yellow solid (820 mg, 70%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 8.29 (d, $^3J$(H-H) = 8 Hz, 2H), 8.01 (t, $^3J$(H-H) = 8 Hz, 1H), 4.00 ppm (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 165.14, 148.2, 138.5, 128.1, 53.3 ppm; IR (KBr): $\tilde{\nu}$ = 1741, 1572, 1449, 1291, 1246, 1197, 1164, 1144, 1080, 995, 952, 853, 812, 757, 722, 607, 645 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 195.05; found: 196 [*M*+H]$^+$, 218 [*M*+Na]$^+$; elemental analysis calcd (%) for $C_9H_9NO_4$: C 55.39, H 4.65, N 7.18; found: C 55.53, H 4.59, N 7.05.

Dimethyl 2,6-pyridinecarboxylate (200 mg, 1.02 mmol) was dissolved in THF/MeOH (1.5 mL/1.5 mL). An aqueous hydroxylamine solution (50%, 0.63 mL, 10.2 mmol) and KCN (13 mg, 0.2 mmol) were added to the solution and the mixture was stirred at room temperature for 36 h. The crude mixture was concentrated under vacuum and recrystallisation from water gave the desired product as a white solid (32 mg, 16%). $^1$H NMR (300 MHz, [D$_6$]DMSO): $\delta$ = 11.83 (s, 2H), 9.32 (s, 2H), 8.13 ppm (s, 3H); $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 160.9, 148.3, 139.6, 123.8 ppm; IR (KBr): $\tilde{\nu}$ = 3283, 3138, 2886, 1674, 1644, 1514, 1436, 1250, 1189, 1022, 1002, 887, 841, 743, 630 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 197.04; found: 198 [*M*+H]$^+$, 220 [*M*+Na]$^+$; elemental analysis calcd (%) for $C_7H_7N_3O_4$: C 42.65, H 3.58, N 21.31; found: C 42.82, H 3.53, N 21.49.

**Synthesis of hydroxamic acids 46 and 47**: Hydroxamic acids **46** and **47** were obtained by a two-step procedure, the first step consisting of the synthesis of the methyl esters of the starting acids by heating them in methanol at reflux in acidic media ($H_2SO_4$),[60] followed by hydroxyamination. In this manner, the corresponding two esters could be isolated in yields of 80 and 61%, respectively (Scheme 9). The hydroxyamination reaction was achieved by using aqueous hydroxylamine in MeOH/THF in the presence of a catalytic amount of KCN.[61] After purification, we were able to isolate the two hydroxamic acids **46** and **47** in yields of 56 and 16%, respectively, for the two steps.




Scheme 9. Synthesis of the hydroxamic acids **46** and **47**.

***N*-Hydroxypicolinamide (46)**: Picolinic acid (1 g, 8.12 mmol) was dissolved in MeOH (16 mL). Concentrated $H_2SO_4$ (1 mL) was added and the mixture was heated at reflux for 24 h. After cooling to room temperature, the mixture was concentrated under vacuum, diluted in $H_2O$ (10 mL) and neutralised by the addition of solid $K_2CO_3$. The solution was extracted with EtOAc (2×20 mL) and the organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated to give methyl picolinate as a yellow oil (890 mg, 80%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta = 8.75$ (dd, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H), 8.12 (dd, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.85 (td, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.48 (ddd, $^3J$(H-H) = 9 Hz, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H), 4.00 ppm (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 165.79$, 149.9, 147.9, 137.1, 127.0, 125.2, 53.01 ppm; IR (KBr): $\tilde{\nu} = 3418$, 2954, 1731, 1586, 1445, 1434, 1312, 1249, 1196, 1134, 1090, 751, 706 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 137.05; found: 138.

Methyl picolinate (50 mg, 0.36 mmol) was dissolved in THF/MeOH (0.5 mL/0.5 mL). An aqueous hydroxylamine solution (50%, 0.24 mL, 3.6 mmol) and KCN (5 mg, 0.07 mmol) were added to the solution and the mixture was stirred at room temperature overnight. After the addition of 10% citric acid (15 mL), the mixture was extracted with EtOAc (2×15 mL). The organic layers were dried over $Na_2SO_4$ and concentrated to give the desired product as a pale-rose solid (35 mg, 70%).

$^1$H NMR (300 MHz, $CD_3CN$): $\delta = 8.53$ (dd, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H), 8.01 (dd, $^3J$(H-H)) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.88 (td, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.50 ppm (ddd, $^3J$(H-H) = 8 Hz, $^3J$(H-H) = 5 Hz, $^4J$(H-H) = 1 Hz, 1H); $^{13}$C NMR (75 MHz, $CD_3CN$): $\delta = 162.9$, 149.7, 149.4, 138.6, 127.6, 122.8 ppm; IR (KBr): $\tilde{\nu} = 3449$, 3266, 2804, 1672, 1518, 1474, 1177, 1029, 815, 751, 632 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 138.04; found: 139.

***N*,3-Dihydroxypicolinamide (47)**: 3-Hydroxypicolinic acid (500 mg, 3.6 mmol) was dissolved in MeOH (7 mL). Concentrated $H_2SO_4$ (0.5 mL) was added and the mixture was heated at reflux for 24 h. After cooling to room temperature, the mixture was concentrated under vacuum, diluted in $H_2O$ (10 mL) and neutralised by the addition of solid $K_2CO_3$. The solution was extracted with EtOAc (2×20 mL) and the organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated to give methyl 3-hydroxypicolinate as a white solid (335 mg, 61%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta = 10.57$ (s, 1H), 8.22 (dd, $^3J$(H-H) = 4 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.37 (dd, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 4 Hz, 1H), 7.29 (dd, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H), 3.99 ppm (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 169.9$, 158.8, 141.5, 129.9, 129.6, 126.2 ppm; IR (KBr): $\tilde{\nu} = 1676$, 1449, 1369, 1304, 1209, 1100, 862, 807, 709, 665 cm$^{-1}$; MS (ESI): *m/z*: calcd for [*M*]: 153.04; found: 154; elemental analysis calcd (%) for $C_7H_7NO_3$: C 54.90, H 4.61, N 9.15; found: C 55,43, H 4.76, N 9.12.

Methyl 3-hydroxypicolinate (150 mg, 0.98 mmol) was dissolved in THF/MeOH (1.5 mL/1.5 mL). An aqueous hydroxylamine solution (50%, 0.6 mL, 9.8 mmol) and KCN (13 mg, 0.19 mmol) was added to the solution and the mixture was stirred at room temperature overnight. The mixture was concentrated and 10% citric acid (20 mL) was added to the crude product. The mixture was extracted with EtOAc (2×20 mL) and the organic layers were dried over $Na_2SO_4$, concentrated and purified by flash column chromatography on silica gel using cyclohexane/EtOAc (50:50) as eluent to give the desired product as a beige solid (40 mg, 26%).

$^1$H NMR (300 MHz, MeOD): $\delta = 8.11$ (dd, $^3J$(H-H) = 4 Hz, $^4J$(H-H) = 1 Hz, 1H), 7.46 (dd, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 4 Hz, 1H), 7.38 ppm (dd, $^3J$(H-H) = 8 Hz, $^4J$(H-H) = 1 Hz, 1H); $^{13}$C NMR (75 MHz, MeOD): $\delta =$ 166.5, 158.3, 149.5, 131.9, 129.8, 127.6 ppm; MS (ESI): *m/z*: calcd for [*M*]: 154.04; found: 153.

**Biochemical assays**

High-performance liquid chromatography separations: Several chromatographic systems were used for the analytical experiments. System A: RP-HPLC (Thermo Hypersil GOLD $C_{18}$ column, 5 μm, 4.6×150 mm) with $CH_3CN$ and 0.1% aq. trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as eluents (20% $CH_3CN$ (2 min), linear gradient from 20 to 25% (5 min), 25% (10 min), linear gradient from 25 to 20% (2 min) and 20% (2 min) at a flow rate of 1 mL min$^{-1}$) and UV detection at 320 nm. System B: System A with isocratic elution at 25% $CH_3CN$ (15 min). The tray, column and oven were heated at 37 °C. System C: System B with isocratic elution at 20% $CH_3CN$ (15 min) and UV detection at 264 nm.

**Study of the influence of pH on the hydrolysis of fluorogenic substrate 6**
The samples were prepared in 0.1 N borate buffer with pH values ranging from 7.0 to 10.0 (7, 7.5, 8, 8.25, 8.5, 8.75, 9, 9.5 and 10) with a substrate concentration of 200 μM. The hydrolysis was followed by RP-HPLC (System A, injected volume = 20 μL) and by fluorescence measurement (in a fluorescence cell of 1400 μL, $\lambda_{ex} = 370$ nm, $\lambda_{em} = 400$–600 nm, voltage = 600 W).

**Screening tests on the fluorogenic substrate 6**

*Calibration curves*: A 5 mM solution of 7-hydroxycoumarine-4-acetic acid was prepared in 0.1 N borate buffer at pH 8.75. Dilutions of this stock solution were performed to obtain concentrations ranging from 1.56 to 50.0 μM. Each solution was analysed by fluorescence measurement (300 μL in six different wells of a 96 well-plate, $\lambda_{ex} = 370$ nm, $\lambda_{em} =$ 470 nm, voltage = 570 W, five measures per well) and by RP-HPLC (System B, injected volume = 20 μL).

*Sample without nucleophile*: A sample containing a solution of **6** (50 μM) was prepared in 0.1 N borate at buffer pH 8.75 ($V_{tot} = 12$ mL). The sample was thermostatted at 37 °C.

*Samples with nucleophiles*: Samples containing a solution of **6** (50 μM) and the nucleophile (100 μM) were prepared in 0.1 N borate buffer at pH 8.75 ($V_{tot} = 12$ mL). The samples were thermostatted at 37 °C.

*Fluorescence measurements*: Each sample was analysed every 24 h in a 96 well-plate (300 μL in two different wells), $\lambda_{ex} = 370$ nm, $\lambda_{em} = 470$ nm, voltage = 570 W, five measures per well).

*HPLC analysis*: Each sample was analysed by HPLC (System B, injected volume = 20 μL) at the end of the experiment.

**Hydrolysis of PhX in the presence of nucleophiles**

*Calibration curve of **PhX***: A 1.0 mM solution of **PhX** was prepared in Tris·HCl buffer (0.1 N, pH 8.75). Dilutions of this solution were performed to obtain concentrations ranging from 0.062 to 1.0 mM. Each solution was analysed by RP-HPLC (System C, injected volume = 20 μL).

*Calibration curve of **72***: A 1.0 mM solution of **72** was prepared in Tris·HCl buffer (0.1 N, pH 8.75). Dilutions of this solution were performed to obtain concentrations ranging from 0.062 to 1.0 mM. Each solution was analysed by RP-HPLC (System C, injected volume = 20 μL)).

*Sample preparation*: Samples containing a solution of **PhX** (1.0 mM) and the nucleophile (5.0 mM) were prepared in Tris·HCl buffer (0.1 N, pH 8.75, $V_{tot} = 1$ mL) for HPLC analysis. The samples were placed in the HPLC autosampler thermostatted at 37 °C.

*HPLC analysis*: Each sample was analysed by RP-HPLC every 30 min (for the first 3 h) and further injections were made after longer periods of time if necessary (20 μL eluted with System C).

## Acknowledgements

This work was supported by the Région Haute-Normandie, the Délegation Générale de l'Armement (French Ministry of Defence Procurement Agency) for Ph. D. grants to L.L.-L. and G.S.A., and the Agence Nationale pour la Recherche (ANR 06 BLAN 0163 DETOXNEURO) for a postdoctoral grant to E.P. We thank Elisabeth Roger, Laëtitia Bailly and Annick Le Boisselier (UMR 6014 CNRS) for IR measurements, mass analyses and elemental analyses, and the company QUIDD for open access to their HPLC instruments. Dr. Laurent Verdier (centre d'Etude du Bouchet) is gratefully acknowledge for the pro-fluorescent probe toxicity evaluation. Dr. Florian Nachon and Prof. Patrick Masson (CRSSA - Grenoble) are gratefully acknowledged for the gift of commercially available oximes, as well as Dr. Frédéric Taran (CEA Saclay) for the gift of hydroxypyridines, pyridine *N*-oxides and pyridinones.

[1] a) P. Taylor in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed. (Eds.: J. G. Hardman, L. E. Limbird, A. G. Goodman), McGraw-Hill, New York, **2001**, pp. 175–192; b) R. E. Langford in *Introduction to Weapons of Mass Destruction, Radiological, Chemical and Biological*, Wiley, New York, **2004**.

[2] Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction;

The Technical Secretariat of the Organization for the Prohibition of Chemical Weapons, Den Hague, Netherlands, **1993**.

[3] M. Eddleston, N. A. Bucklay, P. Eyer, A. H. Dawson, *Lancet* **2008**, *371*, 597–607.

[4] a) Y. C. Yang, J. A. Baker, J. R. Ward, *Chem. Rev.* **1992**, *92*, 1729–1743; b) Y. C. Yang, *Acc. Chem. Res.* **1999**, *32*, 109–115.

[5] a) D. Crabb, J. DeFrank, C. Penet, *Chem. Ind.* **2004**, *14*, 15; b) L. M. Eubanks, T. J. Dickerson, K. D. Janda, *Chem. Soc. Rev.* **2007**, *36*, 458–470; c) P. Vayron, P.-Y. Renard, F. Taran, C. Créminon, Y. Frobert, J. Grassi, C. Mioskowski, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7058–7063; d) B. M. Smith, *Chem. Soc. Rev.* **2008**, *37*, 470–478; e) S. S. Talmage, A. P. Watson, V. Hauschild, N. B. Munro, J. King, *Curr. Org. Chem.* **2007**, *11*, 285–298; f) A. W. Reshetnyak, M. F. Armentano, N. A. Pomarenko, D. Vizzuso, O. M. Durova, R. Ziganshin, M. Serebryakova, V. Govorun, G. Gololobov, H. C. Morse III, A. Friboulet, S. P. Makker, A. G. Gabibov, A. Tramontano, *J. Am. Chem. Soc.* **2007**, *129*, 16175–16182.

[6] a) D. P. Dumas, H. D. Durst, W. G. Landis, F. M. Raushel, J. R. Wild, *Arch. Biochem. Biophys.* **1990**, *277*, 155–159; b) J. E. Kolakowski, J. J. DeFrank, S. P. Harvey, L. L. Szafraniec, W. Beaudry, K. Lai, J. R. Wild, *Biocatal. Biotransform.* **1997**, *15*, 297–312.

[7] P.-Y. Renard, H. Schwebel, P. Vayron, L. Josien, A. Valleix, C. Mioskowski, *Chem. Eur. J.* **2002**, *8*, 2910–2916.

[8] C.o. Disarmament, The Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, **1992**, CD/1170.

[9] Activated hydrogen peroxides: a) Y. C. Yang, L. L. Szafraniec, W. T. Beaudry, C. A. Bunton, *J. Org. Chem.* **1993**, *58*, 6964–6965; b) G. W. Wagner, Y.-C. Yang, *Ind. Eng. Chem. Res.* **2002**, *41*, 1925–1928; c) W. Wagner, L. R. Procell, Y.-C. Yang, C. A. Bunton US 6723891B1, **2004**; d) G. W. Wagner, D. C. Sorrick, L. R. Procell, M. D. Brickhouse, I. F. Mcvey, L. I. Schwartz, *Langmuir* **2007**, *23*, 1178–1186.

[10] Activated chlorides: a) Y. C. Yang, J. A. Baker, J. R. Ward, *Chem. Rev.* **1992**, *92*, 1729–1743; b) "Decontamination": C. G. Hurst in *Medical Aspects of Chemical and Biological Warfare* (Eds.: F. R. Sidell, E. T. Takafuji, D. R. Franz), The Borden Institute, Chapter 15, **2008**.

[11] FOxone: Y. C. Yang, F. J. Berg, L. L. Szafraniec, W. T. Beaudry, C. A. Bunton, A. Kumar, *J. Chem. Soc. Perkin Trans. 2* **1997**, 607–613.

[12] Peracids : a) Y. C. Yang, L. L. Szafraniec, W. T. Beaudry, D. K. Rohrbaugh, *J. Am. Chem. Soc.* **1990**, *112*, 6621–6627; b) T. Cassagne, H.-J. Cristau, G. Delmas, M. Desgranges, C. Lion, G. Magnaud, E. Torreilles, D. Virieux, *Heteroat. Chem.* **2001**, *12*, 485–490.

[13] a) P. S. Hammond, J. S. Forster, C. N. Lieske, H. Dupont Durst, *J. Am. Chem. Soc.* **1989**, *111*, 7860–7866; b) R. A. Moss, H. Morales-Rojas, H. Zhang, B.-D. Park, *Langmuir* **1999**, *15*, 2738–2744; c) F. J. Berg, R. A. Moss, Y.-C. Yang, H. Zhang, *Langmuir* **1995**, *11*, 411–413; d) H. Morales-Rojas, R. A. Moss, *Chem. Rev.* **2002**, *102*, 2497–2522; e) N. Masurier, F. Estour, M.-T. Froment, B. Lefevre, J.-C. Debouzy, B. Brasme, P. Masson, O. Lafont, *Eur. J. Med. Chem.* **2005**, *40*, 615–623; f) N. Masurier, O. Lafont, R. Le Provost, D. Lesur, P. Masson, F. Djedaini-Pilard, F. Estour, *Chem. Commun.* **2009**, 589–591.

[14] E. Gershonov, I. Columbus, Y. Zafrani, *J. Org. Chem.* **2009**, *74*, 329–338.

[15] a) G. W. Wagner, O. B. Koper, E. Lucas, S. Decker, K. J. Klabunde, *J. Phys. Chem. B* **2000**, *104*, 5118; b) G. W. Wagner, P. W. Bartram, O. B. Koper, K. J. Klabunde, *J. Phys. Chem. B* **1999**, *103*, 3225; c) G. W. Wagner, P. W. Bartram, *Langmuir* **1999**, *15*, 8113; d) G. W. Wagner, L. R. Procell, R. J. O'Connor, S. Munavalli, C. L. Carnes, P. N. Kapoor, K. J. Klabunde, *J. Am. Chem. Soc.* **2001**, *123*, 1636; e) G. W. Wagner, L. R. Procell, S. J. Munavalli, *J. Phys. Chem. C* **2007**, *111*, 17564; f) G. W. Wagner, Q. Chen, Y. J. Wu, *J. Phys. Chem. C* **2008**, *112*, 11901; g) I. Columbus, D. Waysbort, L. Shamueli, I. Nir, D. Kaplan, *Environ. Sci. Technol.* **2006**, *40*, 3952–3958.

[16] a) L. Raveh, E. Grauer, J. Grunwald, E. Cohen, Y. Ashani, *Toxicol. Appl. Pharmacol.* **1997**, *145*, 43–53; b) A. Saxena, D. M. Maxwell, D. M. Quinn, Z. Radic, P. Taylor, B. P. Doctor, *Biochem. Pharmacol.* **1997**, *54*, 269- 274; c) B. P. Doctor, A. Saxena, *Chem.-Biol. Interact.* **2005**, *157–158*, 167–171.

[17] For OPH (organophosphorous hydrolases), see: a) F. C. G. Hoskin, J. E. Walker, W.-D. Dettbarn, J. R. Wild, *Biochem. Pharmacol.* **1995**, *49*, 711–715; b) V. K. Rastogi, J. J. Defrank, T.-C. Cheng, J. R. Wild, *Biochem. Biophys. Res. Commun.* **1997**, *241*, 294–296; c) A. A. Wang, W. Chen, A. Mulchandani, *Biotech. Bioeng.* **2005**, *91*, 379–386; d) A. Singh, Y. Lee, W. J. Dressick, *Adv. Mater.* **2004**, *16*, 2112–2115; for PTE–phosphotriesterase see : e) E. Ghanem, F. M. Raushel, *Toxicol. Appl. Pharmacol.* **2005**, *207*, 459–470.

[18] a) Using laccase: G. Amitai, R. Adani, G. Sod-Moriah, I. Rabinovitz, A. Vincze, H. Leader, B. Chefetz, L. Leibovitz-Persky, D. Friesem, Y. Hadar, *FEBS Lett.* **1998**, *438*, 195–200; b) using chloroperoxidase (CPO) together with urea hydrogen peroxide: G. Amitai, R. Adani, M. Hershkovitz, P. Bel, I. Rabinovitz, H. Meshulam, *J. Appl. Toxicol.* **2003**, *23*, 225–233.

[19] a) E. Ghanem, F. M. Raushel, *Toxicol. Appl. Pharmacol.* **2005**, *207*, 459–470; b) E. Carletti, L. Jacquamet, M. Loiodice, D. Rochu, P. Masson, F. Nachon, *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1045–1055.

[20] a) C. B. Millard, O. Lockridge, C. A. Broomfield, *Biochemistry* **1998**, *37*, 237–247; b) F. Nachon, Y. Nicolet, N. Viguié, P. Masson, C. Ju, O. Fontecilla-Camps, Lockridge, *Eur. J. Biochem.* **2002**, *269*, 630–637; c) P. Masson, E. Carletti, F. Nachon, *Protein Pept. Lett.* **2009**, *16*, 1215–1224.

[21] G. L. Ellman, *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.

[22] a) T. J. Dale, J. Rebek, Jr., *J. Am. Chem. Soc.* **2006**, *128*, 4500–4501; b) S. Royo, R. Martínez-Mánez, F. Sancenon, A. M. Costero, M. Para, S. Gil, *Chem. Commun.* **2007**, 4839–4847; c) M. Burnworth, S. J. Rowan, C. Weder, *Chem. Eur. J.* **2007**, *13*, 7828–7836.

[23] a) Y. R. Shu, A. K. Su, J. T. Liu, C. H. Lin, *Anal. Chem.* **2006**, *78*, 4697–4701; b) J. Wang, G. Chen, A. Muck, M. P. Chatrathi, A. Mulchandani, W. Chen, *Anal. Chim. Acta* **2004**, *505*, 183–187; c) A. L. Jenkins, O. M. Uy, G. M. Murray, *Anal. Comm.* **1997**, *34*, 221–224.

[24] a) S. Jin, Z. Xu, J. Chen, X. Liang, Y. Wu, X. Qian, *Anal. Chim. Acta* **2004**, *523*, 117–123; b) F. Arduini, A. Amine, D. Moscone, F. Ricci, G. Palleschi, *Anal. Bioanal. Chem.* **2007**, *388*, 1049–1057.

[25] a) M. Burnworth, S. J. Rowan, C. Weder, *Chem. Eur. J.* **2007**, *13*, 7828–7836; b) S. Royo, R. Martínez-Máñez, F. Sancenón, A. M. Costero, M. Parra, S. Gil, *Chem. Commun.* **2007**, 4839–4847.

[26] S. Soukharev, D. J. Hammond, *Anal. Biochem.* **2004**, *327*, 140–148. S. Soukharev, D. J. Hammond, WO 2004/094658, **2004**.

[27] L. Briseno-Roa, J. Hill, S. Notman, D. Sellers, A. P. Smith, C. M. Timperley, J. Wetherell, N. H. Williams, G. R. Williams, A. R. Fersht, A. D. Griffiths, *J. Med. Chem.* **2006**, *49*, 246–255.

[28] a) J.-P. Goddard, J.-L. Reymond, *Trends Biotechnol.* **2004**, *22*, 363–370; b) J.-P. Goddard, J.-L. Reymond, *Curr. Opin. Biotechnol.* **2004**, *15*, 314–322; c) J.-L. Reymond, V. S. Fluxà, N. Maillard, *Chem. Commun.* **2009**, 34–49.

[29] a) Q. Zhu, X. Huang, G. Y. J. Chen, S. Yao, *Tetrahedron Lett.* **2004**, *45*, 707–710; b) M. S. T. Gonçalves, *Chem. Rev.* **2009**, *109*, 190–212; c) D. Wahler, F. Badalassi, J. L. Reymond, *Chem. Eur. J.* **2002**, *8*, 3211–3228; d) Q. Zhu, M. Uttamchandani, D. B. Li, M. L. Lesaicherre, S. Q. Yao, *Org. Lett.* **2003**, *5*, 1257–1260; e) D. Wahler, F. Badalassi, P. Crotti, J. L. Reymond, *Angew. Chem.* **2001**, *113*, 4589–4592; *Angew. Chem. Int. Ed.* **2001**, *40*, 4457–4460.

[30] a) M. Lapeyre, J. Leprince, M. Massonneau, H. Oulyadi, P.-Y. Renard, A. Romieu, G. Turcatti, H. Vaudry, *Chem. Eur. J.* **2006**, *12*, 3655–3671; b) M. Michalska, E. Brzezinska, P. Lipka, *J. Am. Chem. Soc.* **1991**, *113*, 7945–7951; c) C. Schultz, *Bioorg. Med. Chem.* **2003**, *11*, 885–898; d) H. Kawa, N. Ishikawa, *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2097–2098; e) R. A. Boigegrain, B. Gross, *Carbohydr. Res.* **1975**, *41*, 135–142.

[31] K.-I. Setsukinai, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, *J. Chem. Soc. Perkin Trans. 2* **2000**, 2453–2457.

[32] P. C. H. Eichinger, J. H. Bowie, R. N. Hayes, *J. Am. Chem. Soc.* **1989**, *111*, 4224–4227.

[33] G. Roger, University of Toulouse and P. Badol, University Paris VI, MS internship, results not shown.
[34] H. Von Pechmann, C. Duisberg, *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 2119.
[35] J. R. Jonson, *Org. React.* **1942**, *1*, 210.
[36] a) G. Brufola, F. Fringuelli, O. Piermatti, F. Pizzo, *Heterocycles* **1996**, *43*, 1257–1266; b) G. Jones, *Org. React.* **1967**, *15*, 204–599.
[37] R. L. Shriner, *Org. React.* **1942**, *1*, 1–37.
[38] a) I. Yavari, R. Hekmat-Shoar, A. Zonouzi, *Tetrahedron Lett.* **1998**, *39*, 2391–2392; b) N. S. Narasimhan, R. S. Mali, M. V. Barve, *Synthesis* **1979**, 906–909.
[39] W.-C. Sun, K. R. Gee, R. P. Haugland, *Bioorg. Med. Chem.* **1998**, *6*, 3107–3110.
[40] a) S. P. Chavan, K. Shivasankar, R. Sivappa, R. Kale, *Tetrahedron Lett.* **2002**, *43*, 8583–8586; b) D. Subhas Bose, A. P. Rudradas, M. Hari Babu, *Tetrahedron Lett.* **2002**, *43*, 9195–9197; c) Y. Ma, W. Luo, P. J. Quinn, Z. Liu, R. C. Hider, *J. Med. Chem.* **2004**, *47*, 6349–6362.
[41] a) T. Sugino, K. Tanaka, *Chem. Lett.* **2001**, 110–111; b) S. S. Bahekar, D. B. Shinde, *Tetrahedron Lett.* **2004**, *45*, 7999–8001.
[42] a) E. G. Mata, O. A. Mascaretti, *Tetrahedron Lett.* **1988**, *29*, 6893–6896; b) C. J. Salomon, E. G. Mata, O. A. Mascaretti, *Tetrahedron Lett.* **1991**, *32*, 4239–4242; c) C. J. Salomon, E. G. Mata, O. A. Mascaretti, *J. Org. Chem.* **1994**, *59*, 7259–7266.
[43] Y. C. Yang, L. L. Szafraniec, W. T. Beaudry, D. K. Rohrbaugh, L. R. Procell, J. B. Samuel, *J. Org. Chem.* **1996**, *61*, 8407–8413.
[44] a) A. Satyam, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3196–3199; b) S. Far, O. Melnyk, *Tetrahedron Lett.* **2004**, *45*, 7163–7165; c) M. Lapeyre, J. Leprince, M. Massonneau, H. Oulyadi, P.-Y. Renard, A. Romieu, G. Turcatti, H. Vaudry, *Chem. Eur. J.* **2006**, *12*, 3655–3671; d) O. P. Varghese, W. Sun, J. Hilborn, D. A. Ossipov, *J. Am. Chem. Soc.* **2009**, *131*, 8781–8783.
[45] a) I. B. Wilson, *J. Biol. Chem.* **1951**, *190*, 111–117; b) K. Kuca, J. Cabal, J. Patocka, J. Kassa, *Lett. Org. Chem.* **2004**, *1*, 84–86; c) J. Wang, J. Gu, J. Leszczynski, M. Feliks, A. Sokalski, *J. Phys. Chem. B* **2007**, *111*, 2404–2408; d) A. S. Khan, M. K. Kesharwani, T. Bandyopadhyay, B. Ganguly, *J. Mol. Graphics Modell.* **2009**, *28*, 177–182.
[46] a) G. Becker, A. Kawan, D. Gutzeit, F. Worek, L. Szinicz, *Arch. Toxicol.* **2007**, *81*, 415–420; b) V. P. Kumar, B. Ganguly, S. Bhattacharya, *J. Org. Chem.* **2004**, *69*, 8634–8642; c) J. Epstein, J. J. Kaminski, N. Bodor, R. Enever, J. Sowa, T. Higuchi, *J. Org. Chem.* **1978**, *43*, 2816–2821; d) R. E. Plapinger, O. O. Owens, *J. Org. Chem.* **1956**, *21*, 1186–1187; e) J. C. Lamb, G. M. Steinberg, S. Solomon, B. E. Hackley, Jr., *Biochemistry* **1965**, *4*, 2475–2484.
[47] a) K. J. Wallace, R. I. Fagbemi, F. J. Folmer-Andersen, J. Morey, V. M. Lynch, E. V. Anslyn, *Chem. Commun.* **2006**, 3886–3888; b) K. J. Wallace, J. Morey, V. M. Lynch, E. V. Anslyn, *New J. Chem.* **2005**, *29*, 1469–1474; c) T. J. Dale, J. Rebek, Jr., *Angew. Chem.* **2009**, *121*, 7990–7992; *Angew. Chem. Int. Ed.* **2009**, *48*, 7850–7852; d) Q. Zhu, M. Uttamchandani, D. Li, M. L. Lesaicherre, S. Q. Yao, *Org. Lett.* **2003**, *5*, 1257–1260.
[48] L. Bromberg, T. A. Hatton, *Ind. Eng. Chem. Res.* **2005**, *44*, 7991–7998.
[49] a) G. Y. Yang, K.-A. Oh, N.-J. Park, Y.-S. Jung, *Bioorg. Med. Chem.* **2007**, *15*, 7704–7710; b) K. Musilek, J. Kucera, D. Jun, V. Dohnal, V. Opletalova, K. Kuca, *Bioorg. Med. Chem.* **2008**, *16*, 8218–8223; c) T.-H. Kim, K. Kuca, D. Jun, Y.-S. Jung, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2914–2917; d) F. Terrier, P. Rodriguez-Dafonte, E. Le Guével, G. Moutiers, *Org. Biomol. Chem.* **2006**, *4*, 4352–4362; e) S. Eckert, P. Eyer, N. Herkert, R. Bumm, G. Weber, H. Thiermann, F. Worek, *Biochem. Pharmacol.* **2008**, *75*, 698–703; f) F. Worek, H. Thiermann, L. Szinicz, P. Eyer, *Biochem. Pharmacol.* **2004**, *68*, 2237–2248; g) M. Jokanović, M. P. Stojiljković, *Eur. J. Pharmacol.* **2006**, *553*, 10–17; h) C. M. Timperley, M. Bird, S. C. Heard, S. Notman, R. W. Read, J. E. H. Tattersall, S. R. Turner, *J. Fluorine Chem.* **2005**, *126*, 1160–1165; i) K. Musilek, K. Kuca, D. Jun, M. Dolezal, *Curr. Org. Chem.* **2007**, *11*, 229–238; j) J. Kassa, K. Kuca, L. Bartosova, G. Kunesova, *Curr. Org. Chem.* **2007**, *11*, 267–283; k) J. Bajgar, J. Fusek, K. Kuca, L. Bartosova, D. Jun, *Mini-Rev. Med. Chem.* **2007**, *7*, 461–466; l) K. Kuca, D. Jun, K. Musilek, *Mini-Rev. Med. Chem.* **2006**, *6*, 269–277; m) J. Wang, J. Gu, J. Leszczynski, M. Feliks, W. A. Sokalski, *J. Phys. Chem. B* **2007**, *111*, 2404–2408; n) F. Worek, P. Eyer, N. Aurbek, L. Szinicz, H. Thiermann, *Toxicol. Appl. Pharmacol.* **2007**, *219*, 226–234; o) D. M. Maxwell, I. Koplovitz, F. Worek, R. E. Sweeney, *Toxicol. Appl. Pharmacol.* **2008**, *231*, 157–164.
[50] J. E. Chambers, S. F. Oppenheimer, *Toxicol. Sci.* **2004**, *77*, 185–187.
[51] a) B. E. Hackley, R. Plapinger, M. Stolberg, T. Wagner-Jauregg, *J. Am. Chem. Soc.* **1955**, *77*, 3651–3653; b) M. A. Stolberg, W. A. Mosher, *J. Am. Chem. Soc.* **1957**, *79*, 2618–2620; c) R. Swidler, R. E. Plapinger, G. M. Steinberg, *J. Am. Chem. Soc.* **1959**, *81*, 3271–3274; d) M. Silva, R. S. Mello, M. A. Farrukh, J. Venturini, C. A. Bunton, H. M. S. Milagre, M. N. Eberlin, H. D. Fiedler, F. Nome, *J. Org. Chem.* **2009**, *74*, 8254–8260.
[52] K. B. Sloan, N. Bodor, T. Higuchi, R. Little, M.-S. Wu, *J. Chem. Res.* **1977**, 290–291.
[53] The strong fluorescence of 3-hydroxy-2-pyridinealdoxime (**32**; $\lambda_{ex}=$ 380 nm, $\lambda_{em}=435$ nm, $\Phi=0.93$, ethanol/water 1:4, see: J. V. Ruiz, A. G. de Torres, J. M. Cano-Pavon, *Talanta* **1984**, *31*, 29–32) and *N*-hydroxy-2-oxo-6-pyridinecarboxylic acid (**58**) prevented any use of the fluorescence-based HTS assay with these nucleophiles. The results given here are those of the HPLC assays.
[54] P. Heinz, P. Polczynski, *J. Prakt. Chem.* **1970**, *312*, 240–244.
[55] S. S. Bahekar, D. B. Shinde, *Tetrahedron Lett.* **2004**, *45*, 7999–8001.
[56] J. Vader, H. Sengers, A. De Groot, *Tetrahedron* **1989**, *45*, 2131–2142.
[57] a) Q. Zhu, X. Huang, G. Y. J. Chen, S. Q. Yao, *Tetrahedron Lett.* **2004**, *45*, 707–710; b) B. Wang, X. Guan, Y. Hu, Z. Su, *Adv. Polym. Technol.* **2007**, *18*, 529–534.
[58] a) M. G. Bock, R. M. DiPardo, B. E. Evans, K. E. Rittle, D. F. Veber, R. M. Freidinger, J. Hirshfield, J. P. Springer, *J. Org. Chem.* **1987**, *52*, 3232–3239; b) F. M. Hauser, W. P. Ellenberger, *J. Org. Chem.* **1988**, *53*, 1118–1121.
[59] J. Swiatek-Kozlowska, E. Gumienna-Kontecka, A. Dobosz, I. A. Golenya, I. O. Fritsky, *J. Chem. Soc. Dalton Trans.* **2002**, *4639*–4643.
[60] H. L. Yale, *Chem. Rev.* **1943**, *33*, 209–256.
[61] C. Y. Ho, E. Strobel, J. Ralbovsky, R. A. Galemmo, *J. Org. Chem.* **2005**, *70*, 4873–4875.

Received: October 28, 2009
Published online: February 8, 2010