Thioacylation Achieved by Activation of a Monothiocarboxylic Acid with Phosphorus Reagents

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A selection of phosphorus-based coupling reagents have been tested for their ability to activate the ambident nucleophilic monothio acid group as a thiocarbonyl functionality, suitable for use in thioacylations for the formation of thioamides. For these studies the reaction between thioacetic acid and cyclohexylamine to give the corresponding thioamide was chosen as a model. The obtained O/S-selectivities were monitored by the use of ^{31}P NMR and were found to be dependent on the nature of the phosphorus functionality and on the kind of leaving group involved. Several new analogues of the widely used peptide coupling reagent (benzotriazol-1-yloxy)tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP, **1a**) were prepared and investigated. Best results were obtained with two new analogues of PyBOP: [(6-nitrobenzotriazol-1-yl)oxy]tris(pyrrolidino)-phosphonium hexafluorophosphate (PyNOP, **1b**) and [[6-(trifluoromethyl)benzotriazol-1-yl]oxy]-tris(pyrrolidino)phosphonium hexafluorophosphate (PyFOP, **1c**). Both reagents, containing electron-withdrawing substituents at the benzotriazole ring, secured fast activation of the monothio acid. Other phosphorus reagents, such as bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBrOP, **1d**), gave an undesired O/S-selectivity, leading to the formation of oxoamides and phosphorus sulfides.

Introduction

Apart from their wide use as intermediates in organic synthesis,[1] thioamides (thiocarboxamides) have also attracted attention in the field of peptide chemistry.[2] The thiopeptide bond is isosteric with the natural peptide bond,[3] and several examples of peptides containing one or more thioamide substitutions in the peptide backbone (so-called endothiopeptides) have been reported.[4] These modified peptides have often shown more or less retained biological activity, sometimes associated with significant selectivity between different cellular receptors. Also, partial resistance toward proteases has been observed.[5] Thioamide substitution in a peptide has also been used as a tool for determining the possible involvement of an individual peptide bond in receptor interaction (structure–activity relationships).[6]

The formation of thioamides, as well as of other thiocarbonyl functions, is often achieved by treatment of the corresponding carbonyl compound with an O/S-exchange reagent. Phosphorus pentasulfide and some of its derivatives are most often used; the best known is the Lawesson reagent.[7] An alternative route, thioacylation, offers the potential advantage of regioselectivity in the introduction of thiocarbonyl functions in multifunctional molecules such as peptides. Unfortunately, thio analogues of the classical acylation reagents are not easily available. Thioacyl halides are unstable even at low temperature, and only a few are known.[8] Similarly, thioacyl anhydrides are difficultly accessible and quite unstable.[9] Compounds of this type have, to the best of our knowledge, never been used for thioacylations involving amino acids or peptides. Most works with thioacylations have employed thio esters, mainly the relatively easily available dithio or thiono alkyl esters. Although these esters are often described as efficiently thioacylating reagents,[10] several investigations have demonstrated their inefficiency as thioacylating agents for amino acids and peptides. Their slow reactions lead to racemization problems,[11] and often they actually

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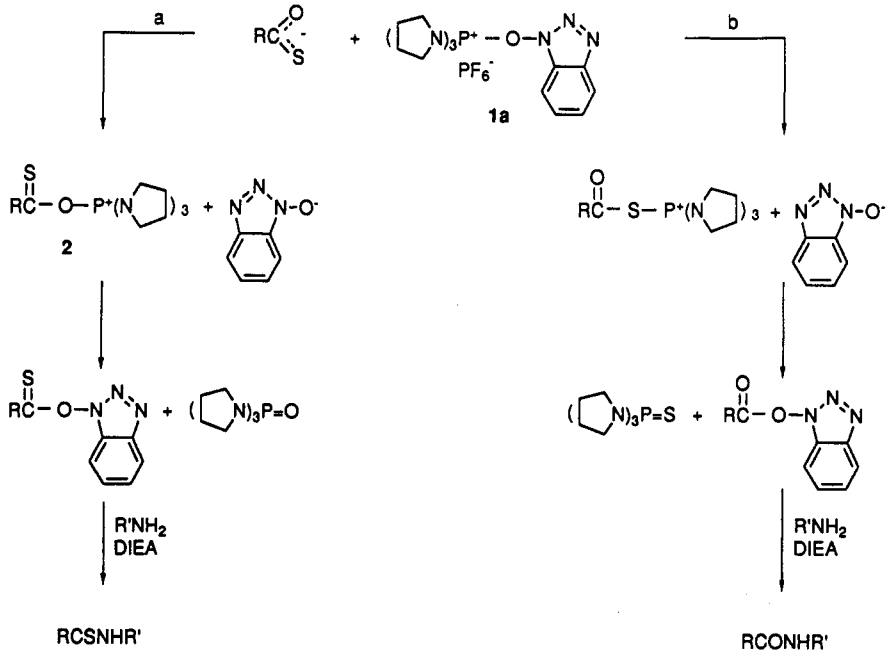
0022-3263/94/1959-1257$04.50/0

Scheme 1



fail to react at all.[12] For these reasons, a method for the preparation of thio analogues of the so-called active esters is desirable.

We recently reported[13] that the use of readily available monothio acids in combination with the phosphorus-containing PyBOP (**1a**, Scheme 6)[14] leads to the formation of new thioacylating species, most likely thiono OBt esters (HOBt; 1-hydroxy-1,2,3-benzotriazole). Such use of monothio acids for formation of thiocarbonyl functionalities has, to the best of our knowledge, not been described earlier. In another recent method,[15] employing acylation of a phosphoramidothionate followed by rearrangement to the thioamide (intramolecular O/S-exchange), the oxygenophilicity of phosphorus is similarly utilized, but in a completely different way. Whereas that method employs strongly basic conditions (NaH), the monothio acid method proceeds under mild conditions, suitable for the preparation of peptides, also by solid-phase methods.[16] We report here on further investigations of phosphorus reagents for monothio acid activation using a simple model system consisting of monothioacetic acid and cyclohexylamine to give the corresponding thioamide. Some of the investigated reagents afforded thioamide in improved yields relative to what we previously obtained,[13] while other reagents mainly gave the undesired oxoamide.

Results and Discussion

The idea of using phosphorus-containing reagents for the synthesis of thioamides from monothio acids is based on the fact that phosphorus forms a stronger bond to oxygen than to sulfur.[17] In our previous study of PyBOP (**1a**)-promoted activation we observed that, although the ratio between the resulting phosphine oxide to phosphine sulfide was approximately 20:1 (Scheme 1), the yield of thioamide was at best 55%.[13] There may be several explanations for this apparent discrepancy, and we have considered the following possibilities:

1. Monothio acids are weakly acylating agents reacting with amines to give amides and hydrogen sulfide.[18] As we reported previously,[13] PyBOP activation of monothio acids proceeds relatively slow under the prevailing conditions, taking several hours to go to completion. Formation of amides may take place because PyBOP activation is usually performed with the amine component present from the reaction start; this secures immediate consumation of the quite labile OBt esters.

It is of interest to note that the relatively strong carboxylic acid TFA (trifluoroacetic acid) is not activated by PyBOP.[19] The slow activation of monothio acids compared to carboxylic acids, which are activated almost instantaneously, may therefore be a result of their higher acidity.

In order to circumvent the above-described problems, we decided to investigate two strategies: (A) late addition of the amine, waiting until the activation of the monothio acid was complete, and (B) use of a modified PyBOP reagent, giving faster activation of the monothio acid. For this purpose the already known PyBrOP[20] (**1d**) seemed an attractive possibility.

2. The initially formed (thioacyloxy)phosphonium ion **2** may be attacked by the sulfur in the monothiocarboxylate giving a dithio anhydride (acyl thioacyl sulfide, Scheme

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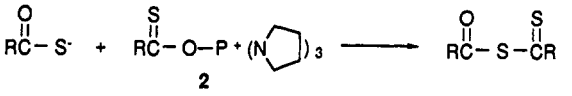
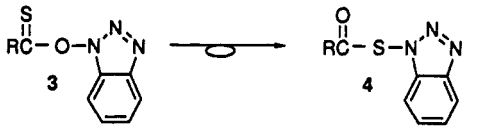
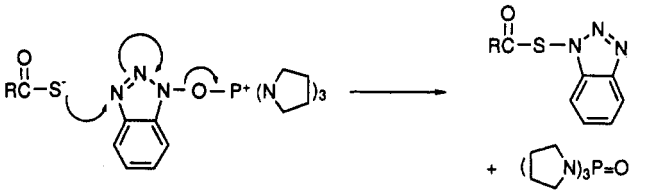
Scheme 2



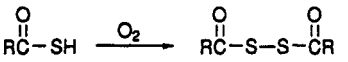
Scheme 3



Scheme 4



Scheme 5



2), which will react further to give amide and dithio acid.[9] Anhydrides do to some extent form as intermediates in normal PyBOP couplings.[19]

3. An intermediary thiono OBt ester **3** may be considered to rearrange to a thiolo ester **4** (Scheme 3), resulting in the formation of amide. Though thiono/thiolo rearrangements have been seen in, for instance, thiono phenyl esters,[21] the usually very weak N–S bond[17] makes this possibility seem very unlikely.

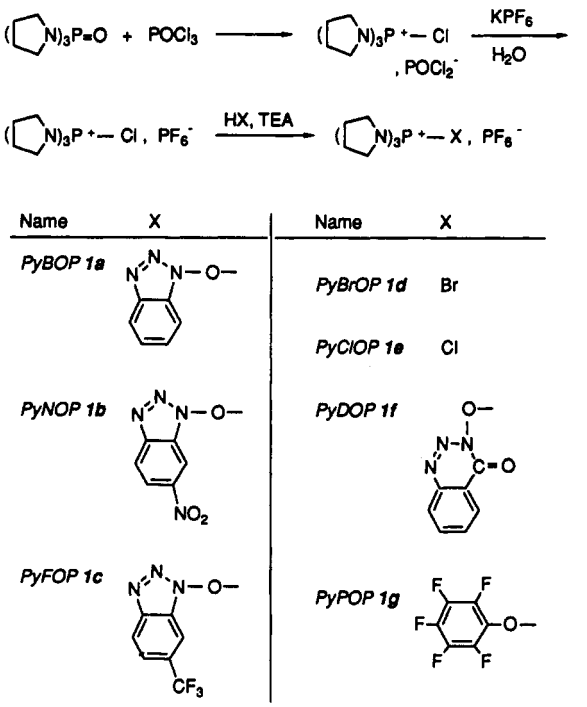
4. The sulfur of the monothiocarboxylate could theoretically attack the 3-nitrogen in PyBOP, yielding phosphine oxide and thiolo ester **4** (Scheme 4). The possibility of attack at the nitrogen has been studied, and eliminated, in a normal BOP coupling by using an ^{18}O-labeled carboxylate.[22] Additionally, considering the weak N–S bond, this possibility seems very unlikely.

5. Although careful precautions are taken to avoid moisture and air, hydrolysis and oxidation of small amounts of the thio compounds may result in the formation of minor amounts of amide. Dithio diacyl compounds (monothio acid disulfides), which are easily formed by oxidation of monothio acids (Scheme 5), are known as excellent acylating agents,[23] and thiono esters have been described as being sensitive toward oxidative O/S-exchange.[24] Furthermore, monothio acids are very difficult to obtain in an absolutely pure state.[25] Purities of 98–99% must usually be accepted.

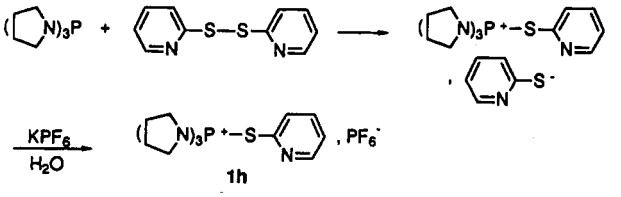
Model Studies

In addition to the already mentioned PyBrOP (**1d**) and the closely related PyClOP (**1e**) we have prepared new analogues of PyBOP and BOP (**5a**).[26] PyBOP was recently introduced as a substitute for BOP, for the purpose of avoiding the use and generation of metabolically alkylating HMPA (hexamethylphosphoramide).

Scheme 6



Scheme 7



By incorporating electron-withdrawing substituents at the benzotriazole ring, we expected to acquire faster working activation reagents.[27] The resulting compounds are PyNOP (**1b**), PyFOP (**1c**), and NOP (**5b**) (Scheme 6). In addition to these, we also prepared new analogues that incorporate structures which are often used for active esters in peptide synthesis. These structures are DhbtOH (3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine), PfpOH (pentafluorophenol), and 2-MP (2-mercaptopyridine), so that the new reagents are PyDOP (**1f**), PyPOP (**1g**) (Scheme 6), and PyTOP (**1h**) (Scheme 7).

The new PyBOP/BOP analogues were prepared in analogy with literature methods[28] (Scheme 6). Satisfactory

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Table 1. PyBOP Activation of CH_3COSH in Various Solvents (250 mM, unless Otherwise Noted)

solvent	$t_{1/2}$[a] (min)	P=S[b] (%)	CSNH[c] (%)	yield[d] (%)	remarks
$CDCl_3$	41	8	71	68	turns yellow
CH_2Cl_2	25	10	69	88	yellow
CH_3CN	49	12	54	89	dark red
DMF	14	5	39	75	black
DME	9	6	36	77	50 mM, yellow
THF[e]	16	8	52	70	50 mM, yellow
DMF[e]	11	5	35	35	50 mM, black

[a] Half-life for the activation step. [b] Final amount of phosphine sulfide relative to phosphine oxide (18 h). [c] Final amount of thioamide relative to amide (18 h). [d] Total yield of amides (thioamide plus amide). [e] $CH_3COS^-K^+$ was used in place of CH_3COSH and DIEA.

elemental analysis could not be obtained for the two nitro compounds, PyNOP and NOP. Both are rather sensitive toward hydrolysis. Spectroscopic data were, however, consistent with the assigned structures. PyTOP (**1h**) could not be prepared by the usual route. The substitution of 2-MP for chlorine proceeded very slowly. PyTOP was instead prepared by treating tris(pyrrolidino)phosphine (**11**) with 2,2′-dipyridyl disulfide (Scheme 7), followed by precipitation of the phosphonium ion with hexafluorophosphate from an aqueous solution. Tris(pyrrolidino)phosphine (**11**) and the reference compound for ^{31}P NMR, tris(pyrrolidino)phosphine sulfide (**12**), were prepared in analogy with known methods describing the preparation of tris(dimethylamino)phosphine and its sulfide.[29,30]

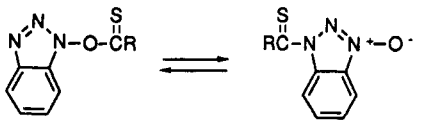
A few other phosphorus compounds, which have been used as coupling reagents in peptide synthesis, were also tested for their monothio acid activation. These compounds are *N,N*-bis(2-oxo-3-oxazolidinyl)phosphorodiamide chloride (BOP-Cl, **6**),[31] the Mukaiyama reagent (Ph_3P/2,2′-dipyridyl disulfide, **7**),[32] and a phosphorus ester, 1,4-epoxy-5-norbornene-2,3-dicarboximido diphenyl phosphate (ENDPP, **8**).[33]

As a model for the activation reaction we chose to study couplings between a simple monothio acid (thioacetic acid) and an aliphatic amine (cyclohexylamine) by the use of ^1H, ^{13}C, and ^{31}P NMR. The potential products *N*-cyclohexylacetamide (**9**), and *N*-cyclohexylthioacetamide (**10**) are both known compounds. For use as references, amide **9** was prepared by acylation of cyclohexylamine with acetic acid anhydride and thioamide **10** was prepared by thionation of amide **9** with $Na_2(P_4S_{10}O)$.[34]

Generally, the ^{31}P spectra of the activation mixtures showed neither (acyloxy)phosphonium ions nor five-coordinated phosphorus compounds (phosphanes). This is in accordance with earlier studies of PyBOP activation; (acyloxy)phosphonium ions have only been observed for a sterically hindered carboxylic acid activated at low temperature.[35]

General Optimization of PyBOP-Promoted Monothio Acid Activation. As can be seen from Table 1, the choice of solvent had a large effect on the thioamide yield; lipophilic solvents gave the best results. The fractions of thioamide obtained with $CHCl_3$ and CH_2Cl_2 (71 and 69%) constitute an improvement from what was obtained with THF (tetrahydrofuran) in our previous study,[13] at best 55%. In this connection it is notable that the solubility of PyBOP is much higher in $CHCl_3$, CH_2Cl_2, CH_3CN, and DMF (*N,N*-dimethylformamide) than in THF and DME (1,2-dimethoxyethane). Therefore, experiments could be carried out with 250 mM solutions or higher in the former solvents but not higher than 50 mM in the latter.

Scheme 8



Alkali metal salts of monothio acids can be used in place of monothio acid plus tertiary amine, as demonstrated by the use of potassium thioacetate in THF and DMF. THF gave the best results. It should be noted that alkali metal monothiocarboxylates often show surprisingly good solubility in organic solvents and that these salts, in contrary to the acids, can be stored without risk of oxidation to the disulfides.[36]

When activation of a monothio acid was done in a lipophilic solvent, a yellow color appeared slowly. This color gradually intensified during the next couple of hours, but the next day the solution was colorless. The transient color may be due to an intermediary thiono OBt ester. In the more polar CH_3CN, the solution soon assumed a dark red color, and in DMF the solution turned completely black. These colors did not disappear later. As the dark colors are accompanied by lower thioamide yields (Table 1), we interpret them as originating from degradation products of the thiono OBt ester.

As support for an intermediary thiono OBt ester, we used ^{13}C NMR spectroscopy to search for thiocarbonyl signals. When an activation mixture with no added amine component was recorded overnight, two new peaks were seen at 215.8 and 217.1 ppm. We propose that these signals originate from the thiono OBt ester in its two isomeric forms (Scheme 8). Though three tautomeric OBt esters are actually possible, it has been shown for the oxygen analogues that only the two depicted in Scheme 8 are formed.[37] Attempts to isolate the thiono OBt ester by chromatography were unsuccessful.

In the ^{13}C NMR spectrum, signals from dithio acid derivatives (~230 ppm) could not be observed, but due to their slow relaxations, carbonyl carbons show low signal intensity under normal NMR conditions. Therefore, a signal may exist below the signal-to-noise ratio, and formation of small amounts of anhydride (Scheme 2) cannot be excluded. ^1H NMR spectra of the activation mixture with no added amine component displayed several signals in the thioaceto range (~2.4–2.9 ppm). Major peaks were observed at 2.45, 2.55, and 2.85 ppm. Since reference compounds such as dithio acetic acid anhydride were not available, these signals could not be assigned to

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Table 2. PyBOP Activation under Various Conditions ($CDCl_3$, 250 mM unless Otherwise Noted)[a]

description	$t_{1/2}$	P=S (%)	CSNH (%)	yield (%)
2 equiv of acylation reagents[b]	nd[c]	4	42	98
3 equiv of acylation reagents[b]	nd	5	32	100
500 mM solution	41 min	8	71	92
5 °C	192 min	7	78	66
−18 °C	>6 h	5	71	61
1 equiv of DIEA	57 min	4	57	63
2 h of preactivation[b]	nd	8	50	66
15 h of preactivation at 5 °C[b]	nd	10	55	54

[a] For explanations of columns, see Table 1. [b] Solution turned black. [c] Not determined.

specific structures. However, the peak at 2.85 ppm may well originate from a dithio acid derivative.

The use of auxiliary nucleophiles may inhibit formation of anhydrides. Despite this, addition of HOBt or 2-MP to activation mixtures did not improve the thioamide yield.

Attempts to raise the total yield of amides obtained in $CDCl_3$ (68%) by applying excess of acylation reagents resulted in reduction of the yield of thioamide (Table 2) and in darkening of the reaction mixtures. Consequently, it seems that the use of equivalent amounts of coupling partners is preferable. This is a problem for solid-phase peptide synthesis applications, since here a large excess of reagents is usually applied. Yields could, however, be raised by using a more concentrated solution (500 mM, Table 2).

Lowering of the reaction temperature slowed down the activation and diminished the yield of thioamide slightly (Table 2). Also, use of only 1 equiv of DIEA (diisopropylethylamine) resulted in a slower reaction, along with a slightly depressed thioamide yield. Apparently, the standard 2 equiv of DIEA should optimally be employed: 1 equiv for the formation of the carboxylate and 1 equiv for neutralization of the acidic HOBt released during coupling.[19]

We also investigated whether tris(pyrrolidino)phosphine sulfide could function as a thionating agent, in analogy with the Lawesson-type reagents. This could lead to a serious side reaction if the method was employed with a molecule containing multiple carbonyl groups. However, when a $CDCl_3$ solution of phosphine sulfide **12** and amide **9** was monitored by the use of ^{31}P NMR and ^1H NMR, no reaction was observed within 1 week. This is in accordance with earlier reports of tris(dimethylamino)phosphine sulfide being inert toward amides.[38] Similarly, no reaction could be detected between thioamide **10** and PyBOP.

Late Addition of the Amine Component. To investigate for a direct acylation, thioacetic acid was mixed with cyclohexylamine, DIEA, and acetanilide (standard) in DME at 25 mM concentration each. Indeed after 2 h, HPLC analysis showed a 27% formation of *N*-cyclohexylacetamide. This reaction may thus be the main competitor during PyBOP activation of monothio acids, suggesting that the amine should be added at the end of the activation. In this connection we verified the expected lack of reactivity of PyBOP toward amines.[39] When PyBOP and cyclohexylamine were mixed in $CDCl_3$ (250 mM) and the resulting solution monitored by use of ^{31}P NMR, the only change within 1 week was the appearance of a minor signal from tris(pyrrolidino)phosphine oxide, due to hydrolysis.

Table 3. Activation by Various Phosphorus Reagents (250 mM, $CDCl_3$)[a]

reagent	$t_{1/2}$	P=S (%)	CSNH (%)	yield (%)	remarks
PyBOP	41 min	8	71	68	turns yellow
BOP	91 min	5	12	57	yellow
PyNOP	<2 min	8	79	65	red
PyFOP	2 min	6	77	88	yellow
PyPOP	>6 h	0	0	nd	colorless
PyTOP	>6 h	91	0	22	yellow
NOP	<2 min	14	46	73	red, precipitation
PyBrOP	4 min	73	4	68	colorless
PyClOP	57 min	47	25	70	colorless
PyDOP	<2 min	0	0	65	ring opening
ENDPP	6 h	1	65	92	heavy precpn
BOP-Cl	30 min	1	71	43	yellow, precpn
Mukaiyama	<2 min	94	0	nd	yellow

[a] For explanations of columns, see Table 1.

The results of delayed addition of the amine in PyBOP activation of thioacetic acid are shown in Table 2 (preactivation). The low yield of thioamide and the development of dark colors, first red, later black, indicate that the thiono ester must be trapped as soon as it is formed.

Use of Different Phosphorus Reagents. The results of activation with the different PyBOP analogues are listed in Table 3. As can be seen, BOP reacted significantly slower than PyBOP, possibly because of the greater steric requirement of six methyl groups, relative to three "backtied" pyrrolidino groups. Otherwise, the $t_{1/2}$ values are more or less in accordance with the expected leaving group abilities of oxybenzotriazoles, etc. PyNOP, PyFOP, PyBOP, PyPOP, and PyTOP show properties in accordance with the strengths of the acids from which they are derived: 6-NO_2HOBt > 6-CF_3HOBt > HOBt > PfpOH > 2-MP. The bromo and especially the chloro derivatives deviates from this reacting slower than what might be expected according to acidity considerations.

The observed O/S-selectivities (Table 3, P=S values) seem to be correlated with the hardness/softness of the involved reaction center.[40] Accordingly, when the leaving atom is a soft sulfur atom (PyTOP) or a soft bromine atom (PyBrOP), the ambident nucleophilic monothiocarboxylate binds to phosphorus primarily by the soft sulfur atom (Scheme 1, route b), and when the leaving atom is a hard oxygen atom (PyBOP, etc.), the bonding takes place at the oxygen primarily. Furthermore, chlorine, which is considered borderline in the hard/soft classification, renders its derivative PyClOP borderline in O/S-selectivity (47% P=S).

From the above consideration, PyDOP constitutes a special case. Even though the reaction of PyDOP is fast, and the selectivity apparently optimal (0% P=S), thioamide is not formed. Since we find no DhbtOH in the product mixture, this is most likely a result of attack at the carbonyl of the Dhbt residue to give ring opening (Scheme 9). Two colored aromatic substances have been isolated from the reaction mixture, but their structures have not been solved. Ring-opening reactions have previously been observed during preparation of Dhbt esters.[41]

It should be noted that PyNOP, PyFOP, and NOP are much less soluble in organic solvents than the other

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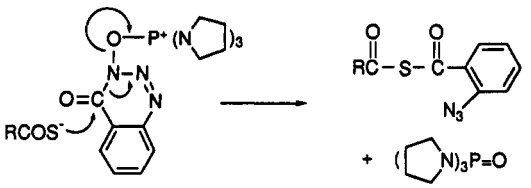
Scheme 9



analogues. However, they dissolve very quickly as activation proceeds. Both PyNOP and NOP gave red reaction mixtures, as expected, from the color of the 6-nitro-1-hydroxybenzotriazolate ion.

For the last three investigated activation reagents the results are shown in Table 3. ENDPP (8, Scheme 6), used without preactivation, gave good selectivity and moderate thioamide yield–despite its very slow reaction. BOP-Cl, used with the usual 30-min preactivation,[31] gave fast reaction, good selectivity, and good thioamide/amide ratio, but the overall yield was low. The Mukaiyama reagent (7, Scheme 6), used without preactivation, afforded no thioamide, only triphenylphosphine sulfide. Since the leaving atom in this in situ formed activation reagent is probably sulfur, this observation is in accordance with the above discussion of hard/soft reaction centers.

Conclusion

Thioamides can be formed by activation of monothio acids with phosphorus-containing coupling reagents, probably by thiono esters as intermediates. The coformation of oxoamides has not been completely avoided, and care must be taken in choosing the optimal reagent. In the present study [(6-nitrobenzotriazol-1-yl)oxy]tris-(pyrrolidino)phosphonium hexafluorophosphate, nicknamed PyNOP, and its 6-(trifluoromethyl) analogue (PyFOP) have been found to give thioamide/amide ratios of approximately 4:1. Lipophilic solvents ($CHCl_3$ or CH_2Cl_2), maximal concentrations, and use of 2 equiv of DIEA are other requirements for optimal performance.

Experimental Section

General Methods. Melting points are reported uncorrected. All NMR-monitored reactions were performed in 5-mm NMR tubes, previously dried at 120 °C overnight. All transfers of reagents and solvents were done in an atmosphere of N_2. TLC was done on silica F_{264} sheets (Merck), and spots were visualized by use of an UV lamp, iodine, and/or Jungnickels phosphorus detecting reagent.[42] HPLC was done on a C-18 column with water/acetonitrile (85;15) as eluent. ^1H NMR spectra were obtained at 250 MHz, $^{13}C\{^1H\}$ NMR spectra at 62 MHz, and $^{31}P\{^1H\}$ NMR spectra at 101 MHz. Unless otherwise specified, spectra were measured in $CDCl_3$ solutions at 295 K. For ^1H and ^{13}C NMR spectra, chemical shifts are reported with respect to internal TMS. For ^{31}P NMR spectra, 85% phosphoric acid was used as an external reference. Coupling constants J are expressed in Hz. As most reactions were run at 250 mM concentration (or higher), ^{31}P NMR spectra could be measured in good quality by use of a single scan with a 90° pulse.[43] This procedure was followed in order to eliminate problems with the different relaxation times of the relevant phosphorus nuclei. Also, when quantitative measurements on reaction mixtures were desired, ^1H decoupling of ^{31}P NMR spectra was done in inverse gated mode. This ensured elimination of NOE's. Half-lives for the activation steps of the reactions were measured by following the disappearance of the phosphonium signal, relative to the appearance of signals from phosphine oxide and sulfide, with ^{31}P NMR. Likewise, the distributions between phosphorus oxide and phosphorus sulfide were measured by the use of ^{31}P NMR. The product distributions between amide 9 and thioamide 10 were measured by integrating the ^1H NMR signals from the methyl groups of these acetamides. The total yield of the two amides was measured after 18 h by comparing the above integrals with that of an internal standard, acetanilide. The signals from 9, 10, and acetanilide were well separated at 1.94, 2.51, and 2.19 ppm.

Materials. All solvents were dried by standard methods and purged with N_2. CH_3COSH (Aldrich) was redistilled in a N_2 atmosphere. Iodine/thiosulfate titration evaluated the purity of the resulting material as 98.3%. DIEA (Aldrich) was distilled from ninhydrin and from KOH. Cyclohexylamine (Fluka) was distilled from KOH. Other chemicals were used as obtained from the following sources: acetanilide, DhbtOH, PfpOH, TEA, P_2S_5, Ph_3P, and 2,2'-dipyridyl disulfide from Aldrich. Tris(pyrrolidino)phosphine oxide, HMPA, S_8, BOP-Cl, KPF_6 and 2-MP from Fluka; PCl_3 and pyrrolidine from Riedel-de-Haën; $POCl_3$ from Heraeus. PyBOP, BOP, and PyBroP from Novabiochem-Calbiochem. ENDPP was kindly provided by Dr. Carola Griehl, Martin Luther King University, Halle, Germany. 6-Nitro-1-hydroxybenzotriazole[44] and 6-(trifluoromethyl)-1-hydroxybenzotriazole[45] were prepared as described in the literature.

PyClOP, Chlorotris(pyrrolidino)phosphonium Hexafluorophosphate (1e). Tris(pyrrolidino)phosphine oxide (12.8 g, 50.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 30 min to an ice-cooled solution of $POCl_3$ (7.67 g, 50.0 mmol) in CH_2Cl_2 (20 mL) and left at 0 °C for 30 min. The solvent was removed *in vacuo* without heating, leaving a brown oil. Water (100 mL) containing TEA (17.5 mL, 125 mmol) was added, and more water was added until the oil was largely dissolved (~200 mL). The resulting mixture was washed with ether. When a solution of KPF_6 (9.20 g, 50.0 mmol) in water (100 mL) was added, the crude product precipitated. It was recrystallized from acetone/ether and the product obtained as a white powder (12.8 g, 61%): mp 151–52 °C (lit.[46] mp 150–51 °C); ^1H NMR δ 3.37 (m, CH_2N, 12H), 2.05 (m, CH_2, 12H); ^{13}C NMR δ 48.5 (d, J = 4.5), 26.2 (d, J = 9.9); ^{31}P NMR δ 37.1 (s, P^+, 1P), –143.5 (hept, PF_6^-, 1P); FAB-MS m/z 276/278 (M^+), 697 ($2M^+$, PF_6^-). Anal. Calcd for $C_{12}H_{24}ClF_6N_3OP_2$: C, 34.18; H, 5.74; N, 9.96. Found: C, 34.08; H, 5.80; N, 9.87.

PyNOP, [(6-Nitrobenzotriazol-1-yl)oxy]tris(pyrrolidino)phosphonium Hexafluorophosphate (1b). PyClOP (3.62 g, 7.78 mmol) and 6-nitro-1-hydroxybenzotriazole (1.40 g, 7.78 mmol) were suspended in CH_2Cl_2 (10 mL), and TEA (1.08 mL, 7.78 mmol) was added. The red color of the 6-nitro-1-hydroxybenzotriazolate ion faded within 1 h. Ether (100 mL) was added, and the precipitate was collected on a filter, washed with cold water (3 × 50 mL), and recrystallized from acetone/ether to give an off-white powder (2.98 g, 68%): mp 160–64 °C dec: ^1H NMR δ 8.44–8.31 (m, ArH, 3H), 3.43 (m, CH_2N, 12H), 2.00 (m, CH_2, 12H); ^{13}C NMR δ 145.0, 122.5, 120.9, 104.8, 48.6 (d), 26.1 (d); ^{31}P NMR δ 32.6 (s, P^+, 1P), –143.9 (hept, PF_6^-, 1P); FAB-MS m/z 420 (M^+); R_f (acetone) 0.63, and a yellow tail from decomposition.

PyFOP, [[6-(Trifluoromethyl)benzotriazol-1-yl]oxy]tris(pyrrolidino)phosphonium Hexafluorophosphate (1c). Prepared as described for 1b above from PyClOP (0.420 g, 1.00 mmol), 6-(trifluoromethyl)-1-hydroxybenzotriazole (0.203 g, 1.00 mmol), and TEA (0.138 mL, 1.00 mmol). Isolated as a white powder (0.540 g, 92%): mp 176–80 °C dec; ^1H NMR δ 8.28 (d, ArH, 1H), 7.86 (s, ArH, 1H), 7.79 (d, ArH, 1H), 3.43 (m, CH_2N, 12H), 1.98 (m, CH_2, 12H); ^{13}C NMR δ 144.1, 122.8, 127.2, 122.8 (d), 122.4, 106.0 (d) 48.6 (d), 26.2 (d); ^{31}P NMR δ 33.0 (s, P^+, 1P), –143.9 (hept, PF_6^-, 1P); FAB-MS m/z 443 (M^+). Anal. Calcd for $C_{19}H_{27}F_9N_6OP_2$: C, 38.78; H, 4.63; N, 14.28. Found: C, 38.43; H, 4.65; N, 13.85.

PyDOP, [(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]tris(pyrrolidino)phosphonium Hexafluorophosphate (1f). Prepared as described for 1b above from PyClOP (1.05 g, 2.50 mmol), DhbtOH (0.408 g, 2.50 mmol), and TEA (0.347 mL, 2.50 mmol). The yellow color of the $DhbtO^-$ ion disappeared after

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approximately 3 h. The product was isolated as a white powder (1.12 g, 94%): mp 170–75 °C dec; ^{1}H NMR δ 8.39 (d, J = 7.93, ArH, 1H), 8.30 (d, J = 8.10, ArH, 1H), 8.15 (t, J = 7.07, ArH, 1H), 7.99 (t, J = 7.75, ArH, 1H), 3.45 (m, CH_2N, 12H), 1.97 (m, CH_2, 12H); ^{13}C NMR δ 150.7, 143.5, 126.7, 124.2, 129.8, 125.8, 121.5, 48.2 (d), 26.2 (d); ^{31}P NMR δ 31.6 (s, P^+, 1P), −143.7 (hept, PF_6^-, 1P); FAB-MS 403 (M^+), 951 ($2M^+$, PF_6^-). Anal. Calcd for $C_{19}H_{28}F_6N_6O_2P_2$: C, 41.61, H, 5.15; N, 15.32. Found: C, 41.22; H, 5.15; N, 14.99.

PyPOP [(Pentafluorophenyl)oxy]tris(pyrrolidino)-phosphonium Hexafluorophosphate (1g). Prepared as described for **1b** above from PyClOP (1.05 g, 2.50 mmol), PfpOH (0.460 g, 2.50 mmol), and TEA (0.347 mL, 2.50 mmol). White powder (1.30 g, 92%): mp 146–47 °C dec; ^{1}H NMR δ 3.35 (m, CH_2N, 12H), 1.99 (m, CH_2, 12H); ^{13}C NMR δ 143.1 (m), 142.0 (m), 140.3 (m), 129.1 (m), 127.9 (m), 126.3 (m), 124.3 (m), 48.0 (d, J = 4.5), 26.2 (d, J = 9.0); ^{31}P NMR δ 26.4 (s, P^+, 1P), −143.5 (hept., PF_6^-, 1P); FAB-MS m/z 424 (M^+) 993 ($2M^+$, PF_6^-). Anal. Calcd for $C_{18}H_{24}F_{11}N_3OP_2$: C, 37.97; H, 4.25; N, 7.38. Found: C, 37.96; H, 4.24; N, 7.50.

PyTOP, (Pyridyl-2-thio)tris(pyrrolidino)phosphonium Hexafluorophosphate (1h). 2,2′-Dipyridyl disulfide (3.31 g, 15.0 mmol) was dissolved in ether (10 mL) and cooled with ice. Tris(pyrrolidino)phosphine (11) (3.62 g, 15.0 mmol) in ether (10 mL) was added dropwise over 30 min to the above, stirred solution, and the product mixture left for another 30 min. A moderate increase in temperature was observed, and a yellow oil separated. The mixture was poured into ice-cold water (50 mL), the organic phase removed, and a solution of KPF_6 (2.76 g, 15.0 mmol) in water (20 mL) added. A precipitate was formed, which soon turned into a yellow oil. The water was removed by decantation. After 2 days at 5 °C, the oil had solidified. The cake was crushed, and the yellow crystals (3.50 g, 47%) were washed with water, mp 60 °C. Attempts at recrystallization always resulted in the product separating as an oil: ^{1}H NMR δ 8.53 (d, ArH, 1H), 7.79 (t, ArH, 1H), 7.54 (d, ArH, 1H), 7.36 (t, ArH, 1H), 3.28 (m, CH_2N, 12H), 1.88 (m, CH_2, 12H); ^{13}C NMR δ 150.6, 148.7 (d), 128.7, 128.2 (d), 124.2, 48.3 (d), 26.1 (d); ^{31}P NMR δ 44.3 (s, P^+, 1P), −143.7 (hept, PF_6^-, 1P); FAB-MS 351 (M^+), 847 ($2M^+$, PF_6^-). Anal. Calcd for $C_{17}H_{28}F_6N_4P_2S$: C, 41.13; H, 5.67; N, 11.29. Found: C, 41.04; H, 5.70, N, 11.25.

NOP, [(6-Nitrobenzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium Hexafluorophosphate (5b). HMPA (0.715 g, 4.00 mmol) in CH_2Cl_2 (2 mL) was added dropwise over 30 min to an ice-cooled solution of $POCl_3$ (0.615 g, 4.00 mmol) in CH_2Cl_2 (2 mL). After addition of TEA (0.560 mL, 4.00 mmol), a mixture of 6-nitro-1-hydroxybenzotriazole (0.720 g, 4.00 mmol) and TEA (0.560 mL, 4.00 mmol) in CH_2Cl_2 (2 mL) was prepared (dark red) and added to the solution. Upon stirring for 30 min the color had faded, and the mixture was poured into ice–water buffered with TEA (0.835 mL, 6.00 mmol). The organic phase was quickly extracted with water and the aqueous solution washed with ether. A solution of KPF_6 (0.735 g, 4.00 mmol) in water was quickly added to the aqueous solution and the precipitate collected and recrystallized from acetone/ether to yield off-white crystals (0.70 g, 36%): mp 179–82 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.86–8.16 (m, ArH, 3H), 2.89 (m, CH_3, 18H); ^{13}C NMR (DMSO-d_6) δ 146.2, 144.8, 127.1, 120.5, 118.9, 107.3, 36.4 (d, J = 4.5); ^{31}P NMR (DMSO-d_6) δ 44.8 (s, P^+, 1P), −143.9 (hept, PF_6^-, 1P); FAB-MS m/z 342 (M^+).

***N*-Cyclohexylacetamide (9).** Cyclohexylamine (22.9 mL, 200 mmol) was dissolved in water (40 mL) in a flask fitted with a reflux condenser and a device for mechanical stirring. Acetic anhydride (18.9 mL, 200 mmol) was added dropwise, with mechanical stirring. Then, solid $Na_2CO_3 \cdot 10H_2O$ (28 g) and 4 M NaOH solution (40 mL) were added, and the mixture was refluxed for 3 h. After the mixture was cooled in an ice bath, the solid was collected on a filter and washed with cold water. The resulting material was recrystallized from water to yield white crystals (8.19 g, 29%): mp 107–8 °C (lit.[47] 107–107.5 °C, lit.[48] mp 108–09 °C), ^{1}H NMR δ 5.53 (s, NH, 1H), 3.72 (m, CHN, 1H), 1.94 (s, CH_3, 3H), 1.89–1.10 (m, 10H); ^{13}C NMR δ 169.1, 48.2, 33.1, 25.5, 24.8, 23.5. Anal. Calcd for $C_8H_{15}NO$: C, 68.05; H, 10.70; N, 9.92. Found: C, 68.15; H, 10.90; N, 10.07.

N-Cyclohexylthioacetamide (10). P_4S_{10} (6.67 g, 15.0 mmol) and Na_2CO_3 (1.59 g, 15.0 mmol) were added to THF (80 mL) and the mixture stirred for 20 min, leaving a greenish yellow solution. Amide **9** (2.82 g, 20.0 mmol) was added and the solution stirred for 2 h. At this point, TLC demonstrated the disappearance of starting material **9**. The solution was poured into a mixture of ethyl acetate (75 mL) and 5% Na_3PO_4 (75 mL). The organic phase was separated and washed with 5% Na_3PO_4 (75 mL) and water (75 mL). The solvent was removed in vacuo and the resulting material recrystallized from water. The product separated as an oil, which, however, soon solidified as a white material (2.29 g, 65%): mp 78 °C (lit.[49] mp 75–78 °C), ^{1}H NMR δ 7.24 (s, NH, 1H), 4.30 (m, CHN, 1H), 2.51 (s, CH_3, 3H), 2.05–1.10 (m, 10H); ^{13}C NMR δ 198.8, 54.6, 34.6, 31.5, 25.4, 24.6. Anal. Calcd for $C_8H_{15}NS$: C, 61.11; H, 9.62; N, 8.91; S, 20.37. Found: C, 61.04; H, 9.69; N, 8.85; S, 20.50.

Tris(pyrrolidino)phosphine (11). PCl_3 (13.7 g, 100 mmol) was mixed with ether (50 mL) and carefully added dropwise to an ice-cooled solution of pyrrolidine (43.5 g, 612 mmol) in ether (75 mL). The reaction was very exothermic. The mixture was stirred for 2 h and filtered and the ether removed in vacuo. The residue was distilled in vacuo, and a thick oil (12.31 g, 51%) was collected at 135–36 °C (1.5 mmHg) (lit.[50] bp 103–04 °C (0.1 mmHg)).

Tris(pyrrolidino)phosphine Sulfide (12). Solid sulfur (0.653 g, 20.4 mmol) was added slowly in small portions to a flask containing stirred, ice-cooled tris(pyrrolidino)phosphine (4.83 g, 20.0 mmol). The reaction was very exothermic. Attempts to collect the product by distillation *in vacuo* resulted in the formation of a black tar. A small part of the crude product was instead purified by chromatography on a silica gel column eluted with CH_2Cl_2. Removal of the solvent gave clear crystals: mp 33–34 °C; ^{1}H NMR δ 3.17 (m, CH_2N, 12H), 1.83 (m, CH_2, 12H); ^{13}C NMR δ 47.24 (d, J = 3.6), 26.35 (d, J = 9.0); ^{31}P NMR δ 66.2. Anal. Calcd for $C_{12}H_{24}N_3PS$: C, 52.73; H, 8.85; N, 15.37. Found: C, 52.39; H, 8.87; N, 14.94.

Model Experiments Monitored by NMR. Typical Procedure. PyBOP (130 mg, 250 μmol) was dissolved in $CDCl_3$ (1.00 mL) in a 5-mm NMR tube. DIEA (85.6 μL, 500 μmol) and cyclohexylamine (28.7 μL, 250 μmol) were added, and the solution was well mixed before CH_3COSH (17.9 μL, 250 μmol) was added. The reaction was monitored by the use of ^{31}P NMR. After 18 h, acetanilide (33.8 mg, 250 μmol) in $CDCl_3$ was added and the ratio of amide/thioamide, as well as their total yield, found by integrating the methyl groups in the ^{1}H NMR spectrum.

When other solvents were employed, these were removed in vacuo after 18 h. The residue was dissolved in $CDCl_3$ and the standard added.

^{31}P NMR chemical shift values for relevant compounds, which are not noted above: PyBOP, δ 31.9; PyBrOP, δ 28.6; BOP, δ 45.4; tris(pyrrolidino)phosphine oxide, δ 14.9; HMPA, δ 25.6; hexamethylphosphoricthioamide, δ 83.5; ENDPP, δ 7.5; diphenyl phosphate, δ 6.3; BOP-Cl, δ 2.0; BOP-OH, δ −0.1; BOP-SH, δ 36.2; Ph_3P, δ −1.3; Ph_3PO, δ 32.9; Ph_3PS, δ 43.8.

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