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New approach to preparation of *N*-acylphosphoramido(thio)(seleno)ates

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This work is dedicated to Professor P. Mastalerz on the occasion of his 75th birthday

Abstract—*N*-[2-(X)-1,3,2-Oxathiaphospholane] derivatives (X = S, Se, O) of carboxamides were prepared and their DBU-assisted reaction with alcohols led to the corresponding *O*-alkyl-*N*-acylphosphoramido(thio)(seleno)ates. Their structures were confirmed by MS analysis and ^{1}H and ^{31}P NMR spectroscopy. Independently *N*-acylphosphoramidoselenoates were converted to *N*-acylphosphoramidates by treatment with *tert*-butylperoxytrimethylsilane. The oxathiaphospholane approach was also applied to the synthesis of derivatives having *N*-prolylphosphoramido(thio)(seleno)ate linkages on the 5′-OH group of AMP.

Numerous studies on the synthesis of *N*-acylphosphoramidates were prompted by their use as potential phosphorylating agents.[1] However, their accessibility has been limited by the low efficiency of synthetic procedures[2] and unexpected reactivity, such as $N \rightarrow O$ acyl migration.[3] Successful application of *N*-acylated oxazaphosphorinanes in the synthesis of enantiomerically pure anticancer drugs such as ifosfamide and bromofosfamide[4] demonstrated their synthetic utility. Furthermore, the discovery of natural products possessing the *N*-acylphosphoramidate motif[5] has attracted the interest of several research establishments to the search for the efficient methods for the synthesis of this class of compounds.[6]

Recently Richards and co-workers synthesized β-asparaginyladenylate as an inhibitor of asparagine synthethase,[7] while Sekine and co-workers in their elegant studies utilizing phosphoramidite chemistry described the synthesis of several aminoacyl-adenylates, where the oxygen atom of the mixed anhydride bond in aa-AMP was replaced by an NH-group.[8] Subsequently, the same group of researchers described the first synthesis of phosmidosine, a natural product having *N*-prolylphosphoramidate and *O*-methyl ester linkages on the 5′-*O*-phosphoryl residue of 8-oxoadenosine.[9] Very recently they also published the synthesis of a phosmidosine analogue having an *N*-prolylphosphoramidothioate linkage.[10] It has been suggested that phosmidosine and its related compounds can be considered as a novel class of anticancer drugs targeting cell cycle regulation.

As a continuation of our efforts towards further development of an oxathiaphospholane methodology beyond applications in the stereocontrolled synthesis of *P*-chiral analogues of oligonucleotides,[11] phosphorothioylated amino acids[12] and, independently, conjugates of amino acids with nucleoside-5′-*O*-phosphorothioates,[13] or nucleoside phosphorothioylated polyols mimicking the function of dinucleoside polyphosphates,[14] we present here a new route to *O*-alkyl-*N*-acylphosphoramido (thio)(seleno)ates. To our knowledge, *N*-acylphosphoramidoselenoates are not described in the literature.

N-Oxathiaphosphitylation of carboxamides was the initial step in the present approach towards the synthesis of compounds containing *N*-acylphosphoramido (thio)(seleno)ate linkages. Phenylacetamide (**1a**),

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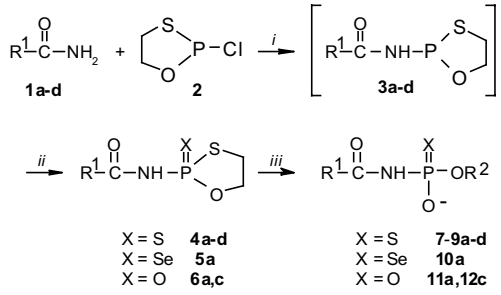


Scheme 1. Reagents: (i) Py, 12 h; or DIPEA, CH_2Cl_2 (ii) S_8 or Se or *t*-BuOOSiMe$_3$; (iii) R^2OH, DBU.

benzamide (**1b**), acetamide (**1c**) and nicotinamide (**1d**) have been used as representative carboxamides. Thus, **1a**–**d** were treated with 2-chloro-1,3,2-oxathiaphospholane (**2**) in pyridine. The resulting P^{III} intermediates **3a**–**d**, after treatment with elemental sulfur or selenium, gave compounds **4a**–**d** and **5a**, respectively (Scheme 1). The reactions were monitored by ^{31}P NMR and were complete within 12 h, and the final products, either **4a**–**d** or **5a**, were isolated by silica gel column chromatography in 40–93% yields. Their structures were confirmed by ^1H NMR, ^{31}P NMR and FAB-MS analyses (Table 1).

To obtain 2-oxo-1,3,2-oxathiaphospholane derivatives **6a**,**c**, we carried out the reaction between carboxamides **1a**,**c** and **2** for 3 h in the presence of bis(*N*,*N*-diisopropyl)ethylamine in methylene chloride. The resulting P^{III} derivatives **3a**,**c** were treated at 0 °C with *tert*-butylperoxytrimethylsilane (*t*-BuOOSiMe$_3$).[15a] The ^{31}P NMR spectrum of the reaction mixture recorded after 2 h contained a peak at around 40 ppm, indicating the formation of **6a**,**c**. It is worth mentioning that the common oxidant *t*-BuOOH, which was successfully used for the oxidation of P^{III} oxathiaphospholane derivatives of nucleosides,[16] provided **6a**,**c** low yields accompanied by unidentified side products, as detected by ^{31}P NMR. Subsequently, compounds **4a**–**d**, **5a** and **6a**,**c**, after treatment with alcohols such as methanol, benzyl alcohol or 3-hydroxypropionitrile in the presence of DBU, provided the corresponding, *O*-alkyl-*N*-acylphosphoramidothioates **7**–**9**, *O*-alkyl-*N*-acylphosphoroselenoamidates **10a**, or *O*-alkyl-*N*-acylphosphoramidates **11a** and **12c**, respectively (Scheme 1). All compounds **7**–**12** were isolated from the reaction mixture by silica gel column chromatography and were characterized by ^{31}P NMR and FAB-MS analysis (Table 2). The yields of *N*-acylphosphoramidothioate and *N*-acylphosphorselenoamidate derivatives **7**–**10** are presented in Table 2 and reflect the efficiency of the last step as depicted in Scheme 1. However, in the case of **11a** and **12c** the overall yields are provided because crude compounds **6a**,**c** were converted into the final products without isolation of these intermediates. All attempts to purify **6a**,**c** were unsuccessful due to their instability on silica gel.

An alternative route leading to *N*-acylphosphoramidate utilized 2-seleno-1,3,2-oxathiaphospholane derivative **5a**. Its DBU-assisted reaction with methanol afforded

Table 1. The physicochemical characteristics of compounds **4**–**6** prepared via Scheme 1

Entry	Compound	R^1	X	^{31}P NMR (δ, ppm)[a]	FAB-MS (M−1) (m/z)	Yield (%)
1	**4a**	$PhCH_2$	S	89.3	273	72
2	**4b**	Ph	S	90.3	256	93
3	**4c**	CH_3	S	88.9	196	55
4	**4d**	C_5H_4N	S	89.9	259	42
5	**5a**	$PhCH_2$	Se	79.2[b]	320	40
6	**6a**	$PhCH_2$	O	42.5	—	75[c]
7	**6c**	CH_3	O	41.3	—	57[c]

[a] The spectra were measured on a 200 MHz spectrometer.
[b] The coupling constant $^1J_{PSe} = 910$ Hz.
[c] Based on ^{31}P NMR.

Table 2. The physicochemical characteristics of compounds **7**–**12** prepared via Scheme 1

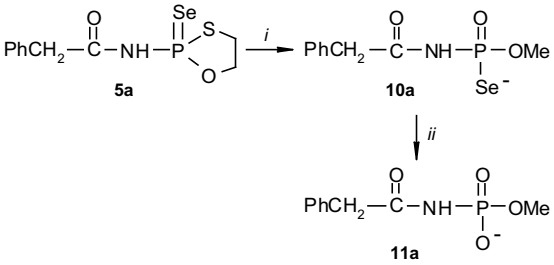
Entry	Substrate	Product						
		No.	X	R^1	R^2	^{31}P NMR (δ, ppm)[a]	FAB-MS (M−1) (m/z)	Yield (%)
1	**4a**	**7a**	S	$PhCH_2$	CH_3	48.6	244	92
2	**4b**	**7b**	S	Ph	CH_3	48.2	230	85
3	**4c**	**7c**	S	CH_3	CH_3	48.8	168	97
4	**4d**	**7d**	S	C_5H_4N	CH_3	48.7	231	97
5	**4a**	**8a**	S	$PhCH_2$	$PhCH_2$	48.3	320	96
6	**4b**	**8b**	S	Ph	$PhCH_2$	48.9	306	96
7	**4a**	**9a**	S	$PhCH_2$	CH_2CH_2CN	48.3	283	79
8	**4b**	**9b**	S	Ph	CH_2CH_2CN	48.6	269	81
9	**5a**	**10a**	Se	$PhCH_2$	CH_3	40.6[b]	290	70
10	**6a**	**11a**	O	$PhCH_2$	CH_3	−6.0	228	39
11	**6c**	**12c**	O	CH_3	$PhCH_2$	−7.2	228	20

[a] The spectra were measured on a 200 MHz spectrometer.
[b] The coupling constant $^1J_{PSe} = 778$ Hz.

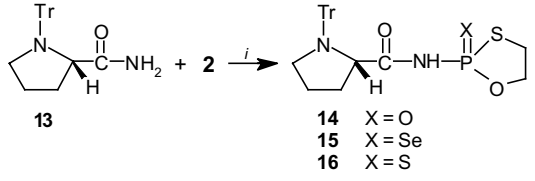
N-acylphosphoramidoselenoate **10a**, which was oxidized in situ to **11a** in 42% overall yield by treatment with *t*-BuOOSiMe$_3$ (Scheme 2).[15b]

These results prompted us to synthesize nucleotides containing an acylphosphoramidate linkage. Prolylamido-AMP[7] and prolylamido-AMPS became our target molecules. *N*-Trityl protected prolinamide (**13**)[8] was used as the substrate because the 2-thiono-1,3,2-oxathiaphospholane derivative of unprotected prolinamide underwent an intramolecular cyclization in the presence of DBU (data not shown). Thus, **13** was allowed to react with **2** in pyridine, and the resulting P^{III} intermediate was oxidized in situ by treatment with *t*-BuOOSiMe$_3$ (Scheme 3). However, examination of the reaction mixture by ^{31}P NMR revealed that the oxidation leading to **14** was inefficient and the desired product was accompanied by several unidentified side products. As the 2-oxo-1,3,2-oxathiaphospholane derivatives are relatively unstable in the presence of silica gel, an attempt to prepare prolylamido-AMP via the oxidation of *N*-phosphoramidoselenoate derivatives, as already described for the synthesis of **10a**, was undertaken.

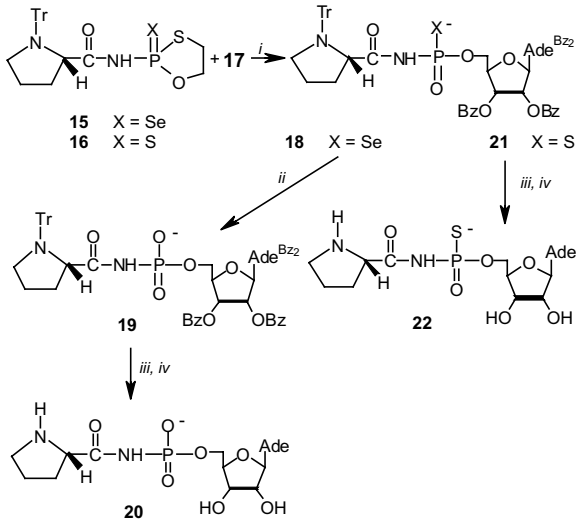
Thus, reaction between *N*-Tr-prolinamide (**13**) and **2** performed in the presence of black selenium gave, after 1 h, the expected product **15** (Scheme 3), which was isolated by column chromatography in 72% yield.[17] No side products were observed in this case, albeit extending the reaction time led to decomposition, as evidenced by ^{31}P NMR. The condensation of *N*-(*N*-Tr-L-prolinamido)-2-seleno-1,3,2-oxathiaphospholane (**15**) with N^6, N^6,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine (**17**) was carried out in the presence of DBU and the product **18** (Scheme 4), was obtained as a mixture of diastereoisomers.[18] Then **18** was oxidized in situ by treatment with 4 equiv of *t*-BuOOSiMe$_3$. The reaction was monitored by ^{31}P NMR and gave signals at: 42.8 and 41.7 ppm corresponding to diastereomers at the phosphoroselenoate function, and appearance of a resonance at ca. −5 ppm, characteristic



Scheme 2. Reagents and conditions: (i) MeOH, DBU, CH_3CN, 3 h; (ii) 2 equiv *t*-BuOOSiMe$_3$, 12 h.



Scheme 3. Reagents: (i) *t*-BuOOSiMe$_3$ or Se or S_8.



Scheme 4. Reagents and conditions: (i) DBU, CH_3CN, 12 h; (ii) 4 equiv *t*-BuOOSiMe, CH_2Cl_2, 12 h; (iii) NH_4OH; (iv) 50% CF_3CO_2H, CH_2Cl_2, 1 h.

for a phosphoramidate function. The structure of compound **19** was confirmed by FAB mass spectrometry after its isolation in 42% yield. Finally, removal of the benzoyl protecting groups of **19** with aqueous ammonia followed by treatment with 50% trifluoroacetic acid (removal of the trityl group) provided the prolylamido-AMP (**20**) in 52% yield. The structure of isolated product **20** was confirmed by ^{1}H and ^{31}P NMR analyses and MALDI-TOF mass spectrometry.[19]

Prolylamido-AMPS was obtained via a two-step process. *N*-Tr-prolinamide **13** was reacted with **2** in the presence of elemental sulfur providing the oxathiaphospholane derivative **16** in 68% yield.[20] Subsequent DBU activated ring-opening condensation of **16** with **17** gave compound **21** (Scheme 4). Removal of the benzoyl and trityl protecting groups afforded prolylamido-AMPS (**22**) in 47% yield.[21]

In summary, we have developed a new approach to the preparation of *O*-alkyl-*N*-acylphosphoramidates and *O*-alkyl-*N*-acylphosphoramidothioates that is based on oxathiaphospholane chemistry. Our efficient and facile preparation gives *N*-[2-oxo(seleno)(thiono)-1,3,2-oxathiaphospholane] derivatives of carboxamides (**4**–**6**, **15**, **16**), that were used for DBU-assisted condensation with various alcohols providing products with an *N*-acylphosphoramido(thio)(seleno)ate linkage. Furthermore we have demonstrated that *N*-acylphosphoramidoselenoates can be converted to their parent *N*-acylphosphoramidates by treatment with *t*-BuOOSiMe$_3$. Although *N*-acylphosphoramidates could be obtained directly from *N*-(2-oxo-1,3,2-oxathiaphospholane) derivatives (Table 2, entries 10 and 11), the synthesis of oxo derivatives was not always efficient as in the case of **14**. Therefore, an alternative route via seleno derivatives **5** and **15**, was elaborated. To our best knowledge *N*-acylphosphoramidoselenoates have not yet been described in the literature. These compounds after isolation from the reaction mixture can be stored under vacuum for

months and they can be considered either as intermediates for the corresponding phosphoramidates or by virtue of *P*-chirality, *N*-acylphosphoramidoselenoates can serve as new stereochemical tools in mechanistic and biological studies. It is worth mentioning that the role of selenium in biological systems is of increasing interest.[22] Besides its biological functions, there are numerous physical, spectroscopic and biological studies of proteins and other macromolecules, which are facilitated by the incorporation of a selenium atom.[23]

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17. Data for **15**: ^{31}P NMR (81 MHz, $CDCl_3$): δ 77.5, 77.2 ppm, $^1J_{PSe}$ 907 Hz; FAB-MS m/z 543 (M+1), 541 (M−1).
18. Data for **18**: ^{31}P NMR (81 MHz, CD_3OD): δ 42.8, 41.7 ppm, $^1J_{PSe}$ 792 Hz; MALDI-MS m/z 1164 (M−1).
19. Data for **20**: ^{1}H NMR (200 MHz, D_2O): δ 8.49 (s, 1H), 8.26 (s, 1H), 6.12 (d, 1H, $J = 5.3$ Hz), 4.51–4.47 (m, 2H), 4.37 (m, 2H), 4.15 (m, 2H), 3.36–3.29 (m, 2H), 2.41–2.32 (m, 2H), 2.00–1.89 (m, 2H); ^{31}P NMR (81 MHz, D_2O): δ −5.4 ppm; FAB-MS m/z 444 (M+1), 442 (M−1).
20. Data for **16**: ^{31}P NMR (81 MHz, $CDCl_3$): δ 88.3, 88.1 ppm; FAB-MS m/z 495.5 (M+1), 493.2 (M−1).
21. Data for **22**: ^{31}P NMR (81 MHz, CD_3OD): δ 47.9, 47.4 ppm; FAB-MS m/z 458 (M−1).
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