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A Protection Scheme for the Preparation of Acid Chlorides of Serine and Threonine

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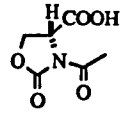
ABSTRACT: *N-acetyl oxazolone derivatives of serine and threonine are readily converted into reactive acid chlorides that condense efficiently with various C-protected aminoacids, even poorly nucleophilic ones.*

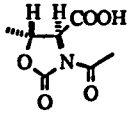
In connection with synthetic studies on luzopeptins[2] and congeners, a need arose to couple the C-terminus of serine with the inner N atom of the remarkable hydrazonoacid, PCA,[3] or one of its precursors. This seemingly trivial reaction was attempted under many standard peptide forming conditions,[3,4] and by the use of numerous permutations of blocking groups on the serine unit. In all cases, we observed only destruction of the serine and/or of the exceedingly sensitive PCA or of its forerunners. Evidently, the abnormally low nucleophilicity of the N atom destined to accept the serinyl group (much less nucleophilic than a secondary alcohol),[3] and the insufficient degree of electrophilicity of common activated forms of serine, were conspiring to undermine the feasibility of the desired operation. Analogous problems have been observed by other researchers in systems similar to ours.[5,6]

In light of earlier observations,[3,5,6] it became clear that an acyl chloride derivative of serine would have to be engaged in the desired acylation step. Known serinyl chlorides[7] proved to be unsuitable for our purpose. First, chlorides of (protected) aminoacids generally seem to work best in acylation reactions carried out in biphasic aqueous-organic media,[8] but with our receptors we observed no significant acylation under such conditions.[6b] Use of such acid chlorides in homogeneous organic solutions may be problematic.[8] In our case, β-elimination and polymerization of the resulting dehydroalanine, even under the influence of the mildly basic agents (N-methylmorpholine; 2,6-lutidine) customarily employed in acylation reactions, effectively competed with acylation of the poorly nucleophilic acceptors.

We wish to describe a protection scheme for serine and threonine that allows preparation of highly reactive, yet well-behaved, acid chloride derivatives. These condensed readily and efficiently with a variety of receptors, even notoriously unreactive ones. Crucial to the success of this effort was the initial conversion of the free aminoacids into derivatives **1** and **2**, wherein β-elimination is strongly retarded on stereoelectronic grounds.[9]



D-Serine ⟶ **1**

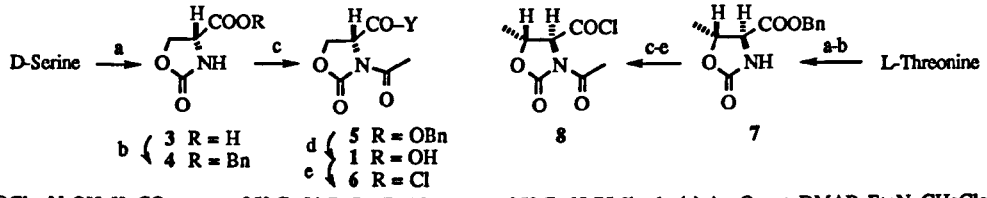


L-Threonine ⟶ **2**

An improved protocol for the preparation of the known[10] oxazolone derivative **3** of serine was developed as follows. Phosgene (CAUTION)[11] gas was bubbled through a water (150 ml) solution of serine (10g, 1 eq.; the D-antipode was employed in this case), NaOH (1 eq.) and K_2CO_3 (1.5 eq.), while the temperature of the mixture was maintained at 20-25° C. When the pH dropped to 5, bubbling was stopped, aspirator vacuum was briefly

applied (removal of phosgene),[11] and the the solution was concentrated *in vacuo*. The syrupy residue was acidified to pH = 2 with conc. HCl, then it was vacuum-concentrated to a thick mass. The residue was extracted with three 100-mL portions of methanol (removal of salts).[12] Vacuum concentration of the extracts left crude **3**, thick mass, which was advanced to the next step without further purification. A 0.5 M acetone solution of this material (some warming is necessary to effect dissolution of **3**) was treated with triethylamine (1.5 eq.) and benzyl bromide (1.2 eq.) and stirred at 25° C overnight. The white precipitate of $Et_3N{\cdot}HBr$ was filtered off and rinsed with ethyl acetate. The combined organic phases were concentrated, the residue was diluted with ethyl acetate (ppt. of more $Et_3N{\cdot}HBr$), the solution was filtered again, the filtrate was vacuum-concentrated, and the crude product was chromatographed (silica gel, gradient 40→60 % EtOAc/hexanes) to afford pure **4**, m.p. 80-80.5° C, $[\alpha]_D^{25} = +16.9°$ (c=0.115, CH_2Cl_2), in 65-75 % yield. The same procedure afforded the analogous L-threonine derivative **7**, thick syrup, in 74 % yield, $[\alpha]_D^{25} = +7.7°$ (c=0.49, CH_2Cl_2).

It was not possible to directly convert intermediates of the type **3** to acid chlorides with $(COCl)_2$, as the reagent also condensed with the oxazolone NH group. Reaction of **3** with $SOCl_2$ did produce an unstable acid chloride, but this fragile material decomposed readily even under mildly basic conditions. On the other hand, N-blocking of **3** was troublesome, due to the presence of a free COOH. These technical difficulties necessitated the preparation of benzyl esters **4** and **7** prior to subsequent operations.



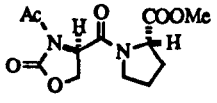
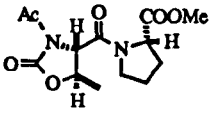
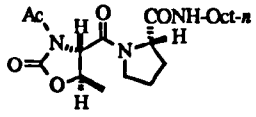
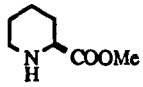
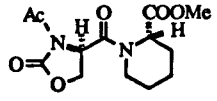
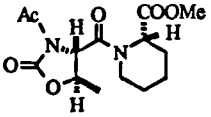
(a) $COCl_2$, NaOH, K_2CO_3, water, 25° C; (b) BnBr, Et_3N, acetone, 25° C; 65-75 % a-b; (c) Ac_2O, cat. DMAP, Et_3N, CH_2Cl_2, 0° C, 98 %; (d) 10 % Pd(C), MeOH, 25° C, 98 %; (e) $(COCl)_2$, CH_2Cl_2, cat. DMF (text), 25° C.

Acetimide **5** was obtained upon reaction of **4** with Ac_2O / cat. DMAP / Et_2N in CH_2Cl_2, at 0° C for 1 h. The imide underwent smooth hydrogenolysis to **1**, thick syrup, $[\alpha]_D^{25} = +74.7°$ (c=0.22, CH_2Cl_2), which formed the expected acid chloride **6** upon treatment with 2 eq. $(COCl)_2$ in CH_2Cl_2, at room temperature, in the presence of a catalytic amount of DMF.[13] Evaporation of the solvent left a dark syrupy residue of crude acid chloride. In a like fashion, chloride **8** was made from L-threonine.

The acid chlorides, reactive materials that are not readily purified, were not thoroughly characterized at this stage. However, they efficiently condensed with various receptors. Representative coupling examples are shown in Table 1. Noteworthy is the facile merger of **6** and **8** with pipecolinate esters, which are notoriously unreactive under standard peptide-forming conditions. We note that the diminished yields of entries **9** and **10** are attributable to the a combination of water solubility and significant polarity, which caused problems during aqueous workup and chromatography. By contrast, the considerably more lipophilic n-octylamide derivative **11** was much easier to handle and purify, and it was obtained in much higher yield. Water solubility and polarity problems were greatly reduced in the pipecolinic ester series. Compounds **12** and **13** were thus obtained in excellent chromatographed yield.

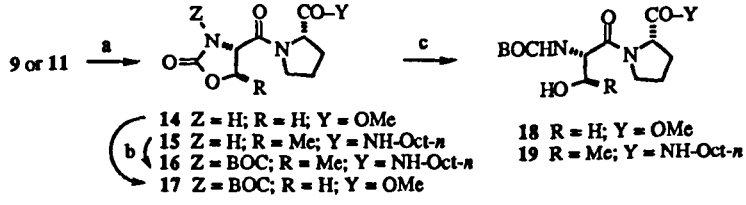
Proton and ^{13}C NMR spectra of **9 - 13** revealed that threonine derivatives exist substantially as a single rotamer, while serine derivatives appear as a pair of slowly interconverting rotamers. These seem to arise through retarded rotation about the peptide bond (tertiary amide), and not through rotation of the oxazolone N-acetyl group, as rotamers were not evident in the NMR spectra of compounds **1, 5-6** and **8**. In **9**, the ratio of rotamers was roughly 1.2:1; in **12**, 1.5:1. ^1H and ^{13}C spectra of **9 - 13** showed no evidence that diastereomers of the the expected product had formed, suggesting that negligible, if any, racemization had occurred.

Table 1: Representative coupling reactions of acid chlorides 6 and 8

chloride	receptor	product[a]	entry	$[\alpha]_D^{25}$ [b]	% yield[c]
6			9	-102.3° [d]	70
8	"		10	-36.6° [e]	72
8			11	-46.3° [f]	90
6			12	-51.1° [g]	91
8	"		13	-50.7° [h]	95

[a]Typical coupling procedure: a solution of crude acid chloride (1.3 eq.) in CH_2Cl_2 was added slowly to a CH_2Cl_2 solution of receptor (1 eq.) and collidine (2.3 eq.) at 0° C. After stirring at 0° C for 2 hrs, the cooling bath was removed and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between CH_2Cl_2 and dilute aq. HCl. The organic layer was further washed with water and vacuum concentrated. The residue was chromatographed. All products except 13 were obtained as thick, hygroscopic syrups that did not crystallize. [b]All rotations were measured in CH_2Cl_2 solutions. [c]Chromatographed (silica gel; ethyl acetate/hexanes mixtures) [d] c=0.110; [e] c=0.046; [f] c=0.130; [g] c=0.041; [h] c=0.019; m.p. 142.5-143.0° C.

Oxazolone cleavage was readily accomplished through the Kunieda procedure.[14] As shown below, N-deacetylation was best conducted through reaction of 9 or 11 with pyrrolidine in MeCN. Free oxazolones of the type 14-15 were extremely polar; therefore they were not purified or extensively characterized. N-Derivatization with BOC_2O/cat. DMAP provided 17, m.p. 177.0-177.5 (effervescence; recr. EtOH),[15] and 16, thick oil, $[\alpha]_D^{25} = -62.7°$ (c=0.063, CH_2Cl_2), in 85 % and 72 % yield, respectively. Cleavage proper was effected with Cs_2CO_3 in MeOH to form N-BOC dipeptides 18,[16] oil, 77%, $[\alpha]_D^{25} = -35.1°$ (c=0.078, CH_2Cl_2), and 19, oil, 79 %, $[\alpha]_D^{25} = -57.5°$ (c=0.057, CH_2Cl_2). NMR spectra of 18 and 19 (^1H and ^{13}C) showed no evidence of



(a) Pyrrolidine (1.3 eq.), MeCN, 25° C, 12 hrs, then remove solvents and advance crude material to next step; (b) BOC_2O (1.1 eq.), cat. DMAP, Et_3N (1.2 eq.), CH_2Cl_2, 0° C, 30 min. 85 % (17) and 72% (16) a-b; (c) MeOH, Cs_2CO_3, 25° C, 3 hrs, 77% (18) and 79 % (19) chromatographed.

erosion of stereochemical integrity of the aminoacid units; moreover, they revealed that **18** exists as a 4:1 mixture of rotamers, while in **19** the rotamer ratio is ca. 15:1.

The methodology described in this Letter has already proven to be successful for the attachment of a serine fragment to a PCA precursor. Details of the application of the present protocols to the luzopeptin problem will be described in due course.

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2. Luzopeptins: Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Am. Chem. Soc.*, **1981**, *103*, 1241; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.*, **1981**, *103*, 1243.
3. Ciufolini, M. A.; Xi. N. *J. Chem. Soc., Chem. Commun.* **1994**, 1867.
4. It is conceivable that more recent technology for peptide synthesis based on the use of N-FMOC-acid fluorides as acylating agents (Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9561; Wenschuh, H.; Beyermann, M.; Krause, E.; Brudel, M; Winter, R.; Schümann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1994**, *59*, 3275) and/or on the use of HOAt as a promoter (Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4367) may resolve at least some of the problems we encountered, but we have not explored the application of these newer methods to our system.
5. Cf. (a) Rebert, N. W. *Dissertation*, Utah State University, 1987; (b) Schmidt, U.; Riedl, B. *Synthesis* **1993**, 809.
6. (a) For an example requiring activation of the acid chloride by AgCN in a system resembling ours see: Hale, K. J.; Delisser, V. M.; Yeh, L. K.; Peak, S. A.; Manaviazar, S.; Bhatia, G. S. *Tetrahedron Lett.* **1994**, *35*, 7685. (b) These investigators describe problems very similar to the ones we observed.
7. Cf. Coppola, G. M.; Schuster, H. F. *Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids*; John Wiley & Sons: New York, NY, 1987; ch. 5. See especially pp. 132-133 and references cited therein.
8. Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732, and references cited therein.
9. In Baldwin's parlance, β-elimination would be a retrograde 5-endo-trig process: Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734; see also March, J. *Advanced Organic Chemistry*; John Wiley & Sons: New York, NY, 1992; 4th. ed.; p. 212 ff.
10. Kaneko, T; Takeuchi, I.; Inui, T. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 974.
11. **CAUTION**: extremely hazardous gas. This reaction must be carried out in an efficient hood, with suitable provision for trapping / venting excess reagent that escapes from the reaction vessel.
12. Repeated extraction (7-8 times) of the residue with hot ethyl acetate provides essentially pure **3**, m.p. 114-115° C (lit.[8] 116-117° C).
13. Cf. (a) Wissner, A.; Grudzinskas, C. V. *J. Org. Chem.* **1978**, *43*, 3972; (b) Fujisawa, T.; Sato, T. *Org. Synth. Coll. Vol. VIII*, **1993**, 498.
14. Ishizuka, T.; Kunieda T. *Tetrahedron Lett.* **1987**, *28*, 4185.
15. Because of extremely unfavorable solubility properties, the rotation of **17** could not be measured.
16. Compound **17** is poorly soluble in MeOH. Fortunately, a suspension of **17** in MeOH was fully satisfactory for the Kunieda reaction, completion of which was visually signaled by the mixture becoming homogeneous.

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