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CONVERSION OF SERINE AND THREONINE RESIDUES INTO α-ACYLOXY-, α-ALKYLTHIO-, AND α-HALOGENOGLYCINE MOIETIES: A NEW STRATEGY FOR THE MODIFICATION OF PEPTIDES

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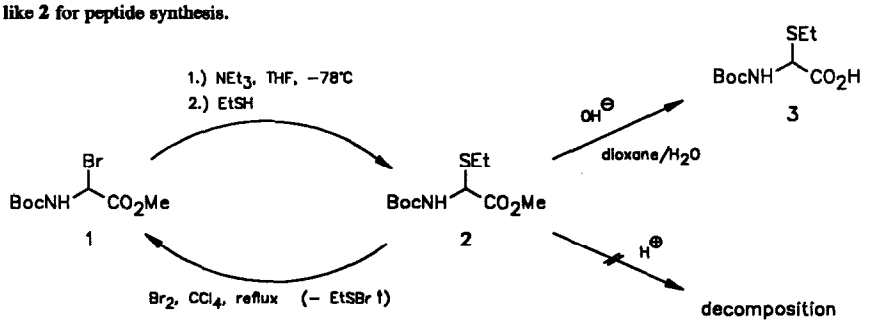
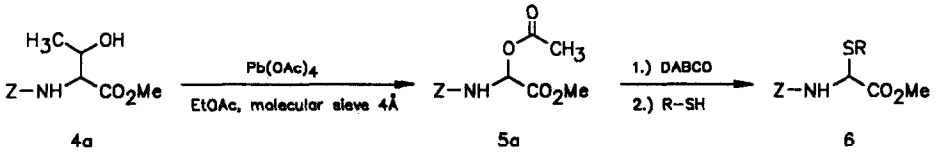
Summary: Selective modification of serine and threonine containing peptides is accomplished by oxidation with lead tetraacetate. Conversion of the derived α-acyloxyglycine derivatives into α-alkylthio- and α-halogenoglycine residues offers a novel synthetic route to modified glycyl peptides, enzyme inhibitors, and dehydropeptides.

The value of cationic glycine equivalents for the synthesis of α-heterosubstituted glycine derivatives, α-amino acids and dehydroamino acids is well documented.[1,2] The application of this principle for the modification of peptides has been confined to special cases, due to the difficulty of incorporating suitably activated glycine residues in peptide chains. Thus, direct bromination[3] and electrochemical oxidation[4] of glycyl peptides have been studied with limited success. Peptides containing C-terminal α-hydroxyglycyl residues were obtained by condensation of *N*-protected amino acid amides with glyoxylic acid derivatives,[5-7] and *N*-protected α-dialkoxyphosphorylglycinates have been incorporated in peptides and subsequently transformed into dehydroamino acid residues.[8] In the following we describe a novel approach to the modification of peptides by means of cationic glycine equivalents.

Initially, we tried to use α-heterosubstituted glycine derivatives directly for peptide synthesis. Therefore, racemic methyl *N*-Boc-α-ethylthioglycinate[9] **2** was prepared from the corresponding bromo derivative[10] **1** by treatment with ethanethiol and triethylamine in THF at -78°C. Since sulfide **2** is quantitatively reconverted into compound[11] **1** with bromine in CCl_4, the sulfides can be used as stable equivalents of the reactive bromo derivatives. Alkaline hydrolysis of **2** in aqueous dioxane was easily accomplished,[12] but all efforts to cleave the *N*-Boc protecting group even in the presence of an activated *N*-acylamino acid derivative led to rapid decomposition with the formation of glyoxylic acid derivatives.[5,13] This severely hampers the use of compounds like **2** for peptide synthesis.



A new strategy for the synthesis of peptides with α-substituted glycine residues is based on Oettmeier's[14] observation that *N*-acyl serine and threonine derivatives yield α-hydroxyglycines on treatment with lead tetraacetate in benzene . We found that under anhydrous conditions (molecular sieve 4Å, refluxing ethyl acetate) this reaction leads to α-acetoxy glycine derivatives. Thus, *N*-Z-threonine methylester **4a** afforded d,l-*N*-Z-α-acetoxyglycine methyl ester **5a** in nearly quantitative yield. Further examples for this conversion are given in Table I.[15]



4a 5a 6

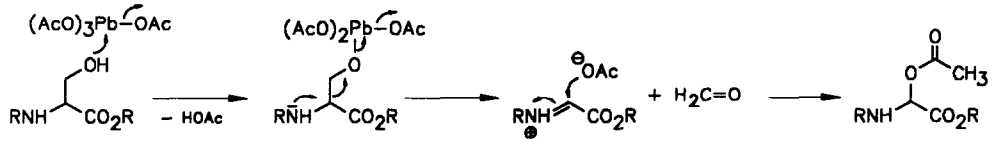
Table I: Oxidation of Seryl and Threonyl Peptides 4 with Lead Tetraacetate

Starting Peptide	α-Acetoxyglycine Derivative	Yield [%]	^{1}H-NMR Data (90 MHz, $CDCl_3$)
4a Z-Thr-OMe	5a Z-Gly(OAc)-OMe	95	2.03 (s, 3H), 3.71 (s, 3H), 5.10 (s, 2H), 6.26 (d, J=10.5 Hz, 1H), 6.50 (d, J=10.5 Hz, 1H), 7.30 (s, 5H)
4b Z-Phe-Ser-OMe	5b Z-Phe-Gly(OAc)-OMe	97	2.03 (s, 3H), 3.00 (m, 2H), 3.65 (s, 3H), 4.63 (m, 1H), 4.95 (s, 2H), 5.90[a] (d, J=9 Hz, 1H), 6.40 (d, J=9 Hz, 1H), 7.16 (s, 5H), 7.23 (s, 5H), 8.06 (d, J=9 Hz, 1H)
4d Boc-Phe-Ser-OMe	5d Boc-Phe-Gly(OAc)-OMe	89	1.35 (s, 9H), 2.03 (s, 3H), 3.05 (m, 2H), 3.71 (s, 3H), 4.42 (br, 1H), 5.36 (br, 1H), 6.40 (d, J=9 Hz, 1H), 7.20 (s, 5H), 7.83 (d, J=9 Hz, 1H)
4e Z-Ser-Gly-OEt	5e Z-Gly(OAc)-Gly-OEt	91	1.13 (t, J=7.5 Hz, 3H), 1.93 (s, 3H), 3.88 (d, J=6 Hz, 2H), 4.06 (q, J=7.5 Hz, 2H), 4.98 (s, 2H), 6.16-6.23 (m, 2H), 6.93-7.40 (m, 6H)
4f Z-Ser(OBz)-Ser-OMe	5f Z-Ser(OBz)-Gly(OAc)-OMe	97	2.03 (s, 3H), 3.36-4.03 (m, 2H), 3.70 (s, 3H), 4.16-4.46 (m, 1H), 4.48 (s, 2H), 5.05 (s, 2H), 5.56 (d, J=7 Hz, 1H), 6.35 (d, J=9 Hz, 1H), 7.25 (s, 5H), 7.28 (s, 5H), 7.71[a] (d, J=9 Hz, 1H)

[a] Signal doubling because of diastereomer formation

Interestingly, Z-Thr-Ser-OMe is smoothly converted into a mixture of the diastereomeric diacetates, whereas Z-Ser(OBz)-Ser-OMe yields only the monoacetates 5f. This permits the selective modification of different serine or threonine residues in the same peptide chain.

We assume that the first step in these reactions is an exchange of one of the acetate groups in $Pb(OAc)_4$ against the serine OH-group followed by oxidative fragmentation. The resulting acylimino intermediate adds acetate with the formation of the acetoxy compound, whereas the formaldehyde undergoes further oxidation to CO_2. A similar mechanism has recently been proposed for the degradation of C-terminal serine and threonine residues to peptide amides by ruthenium tetroxide.[16]



Treatment of the acetates 5 with thiols in the presence of diazabicyclo[2.2.2]octane (DABCO) affords the stable alkylthio derivatives 6 (Table II).[17-19] The latter can either be used for further elaboration of the peptide chain by hydrolysis of the ester group followed by peptide coupling or can be converted into the highly reactive α-chloro or α-bromoglycine derivatives[20] 7 by treatment with SO_2Cl_2[21] or Br_2,[11] respectively. The chlorination is accomplished in ethyl acetate within 15 minutes at 0°C, whereas the reaction with bromine requires 4 hours reflux in CCl_4. On addition of *tert.* amines, the halogenides 7 form reactive

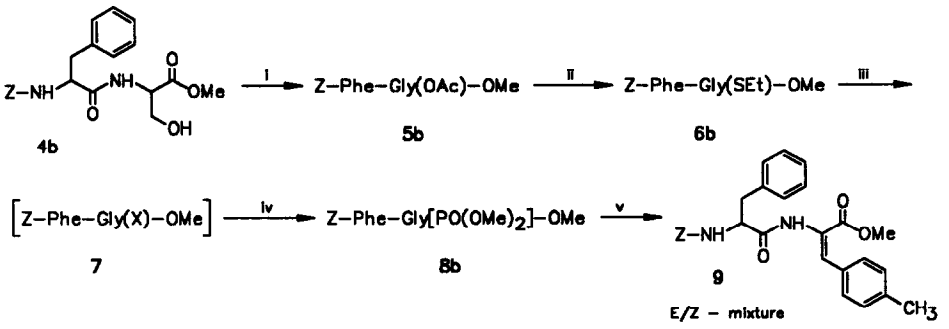
N-acylimine intermediates which add various nucleophiles and therefore possess a great potential for the synthesis of modified peptides. It should be mentioned, however, that these reactions in general lead to mixtures of diastereomers. Therefore one still has to investigate, if amino acids present in the peptide chain exert a directing effect on the nuclephile in certain cases.

Table II: Conversion of α-Acetoxyglycyl Peptides 5 into α-Alkyl(aryl)thioglycine Derivatives 6

α-Alkyl(aryl)thioglycine Derivative	Yield [%]	m.p. [°C]	^{1}H-NMR Data (90 MHz, $CDCl_3$):
6b Z-Phe-Gly(SEt)-OMe	90	138-139	1.20[a] (t, *J*=7.5 Hz, 3H), 2.56 (q, *J*=7.5 Hz, 2H), 3.03 (d, *J*=7.5 Hz, 2H), 3.70 (s, 3H), 4.53 (m, 1H), 5.05 (s, 2H), 5.36-5.56 (m, 2H), 6.95 (d, *J*=7.5 Hz, 1H), 7.20 (s, 5H), 7.28 (s, 5H)
6c Z-Phe-Gly(SPh)-OMe	75	103-106	2.98 (d, *J*=7.5 Hz, 2H), 3.63 (s, 3H), 4.43 (m, 1H), 5.03 (s, 2H), 5.25 (d, *J*=7.5 Hz, 1H), 5.61/5.66[a] (d, *J*=9 Hz, 1H), 6.80 (d, *J*=9 Hz, 1H), 7.03-7.46 (m, 15H)
6d BOC-Phe-Gly(SEt)-OMe	74	oil	1.23[a] (t, *J*=7.5 Hz, 3H), 1.38 (s, 9H), 2.51/2.61[a] (q, *J*=7.5 Hz, 2H), 3.05 (d, *J*=7.5 Hz, 2H), 3.71 (s, 3H), 4.35 (m, 1H), 5.00 (d, *J*=7.5 Hz, 1H), 5.45 (d, *J*=9 Hz, 1H), 6.83 (d, *J*=9 Hz, 1H), 7.20 (s, 5H)
6e Z-Gly(SEt)-Gly-OEt	83	110	1.06 (t, *J*=7.5 Hz, 3H), 1.26 (t, *J*=7.5 Hz, 3H), 2.61 (q, *J*=7.5 Hz, 2H), 3.96 (d, *J*=5 Hz, 2H), 4.13 (q, *J*=7.5 Hz, 2H), 5.05 (s, 2H), 5.35 (d, *J*=8 Hz, 1H), 6.03 (d, *J*=8 Hz, 1H), 6.86 (br, 1H), 7.21 (s, 5H)
6f Z-Ser(OBz)-Gly(SEt)-OMe	70	111-113	1.20 (t, *J*=7.5 Hz, 3H), 2.58 (q, *J*=7.5 Hz, 2H), 3.33-4.03 (m, 2H), 3.71 (s, 3H), 4.16-4.45 (m, 1H), 4.50 (s, 2H), 5.05 (s, 2H), 5.46 (d, *J*=9 Hz, 1H), 5.60 (d, *J*=9 Hz, 1H), 7.00-7.50 (m, 11H)

[a] Signal doubling because of diastereomer formation

The reaction of the halogenides 7 with $P(OMe)_3$ affords dialkoxyphosphoryl derivatives[8,22] 8 which can be transformed into dehydropeptides under Horner-Wittig conditions.[8,23] The reaction sequence from sulfides 6 to phosphonates 8 can be carried out in one pot with an overall yield around 75%. Especially attractive is the fact that only gaseous side products are formed during this conversion. The sequence is illustrated by the transformation of Z-Phe-Ser-OMe 4b into dehydropeptide 9.



i: $Pb(OAc)_4$/EtOAc/molecular sieve 4Å, ii:1) DABCO/THF/-78°C, 2) EtSH, iii: SO_2Cl_2/EtOAc/0°C, 15 min. or Br_2/CCl_4 reflux, 4h, iv: $P(OMe)_3$/EtOAc/reflux, 1h, v: NaOMe/MeOH/p-tolylaldehyde/r.t.

Alternately, the acetates **5** can be converted directly into the phosphonates **8** by treatment with $P(OMe)_3/TiCl_4$[24,25] (CH_2Cl_2, 0°C -> r.t.). The facile conversion of seryl and threonyl residues into cationic glycine equivalents in preformed peptide chains offers interesting possibilities for the synthesis of modified glycyl peptides, enzyme inhibitors, and dehydropeptides, which are under active investigation.

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9. Boc-Gly(SEt)-OMe (**2**): oil, yield 82%. - ^{1}H-NMR (90 MHz, $CDCl_3$): δ 1.30 (t, *J*=7.5 Hz, 3H), 1.48 (s, 9H), 2.70 (q, *J*=7.5 Hz, 2H), 3.76 (s, 3H), 5.30 (d, *J*=9 Hz, 1H), 5.56 (d, *J*=9 Hz, 1H).
10. Boc-Gly(Br)-OMe (**1**): oil, yield 90%. - ^{1}H-NMR (90 MHz, $CDCl_3$): δ 1.44 (s, 9H), 3.79 (s, 3H), 5.98 (d, *J*=10.5 Hz, 1H), 6.28 (d, *J*=10.5 Hz, 1H).
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12. Boc-Gly(SEt)-OH (**3**): m.p. 79°C, yield 94%. - ^{1}H-NMR (90 MHz, $CDCl_3$): δ 1.28 (t, *J*=7.5 Hz, 3H), 1.46 (s, 9H), 2.71 (q, *J*=7.5 Hz, 2H), 5.08 (br., 1H), 5.38 (br., 1H), 10.10 (br., 1H)
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15. *General procedure for the oxidation of serine and threonine residues with $Pb(OAc)_4$:*
To a stirred solution of the serine or threonine containing peptide **4** (10 mmol) in dry EtOAc (100 ml), 8 g of molecular sieve 4Å and 15 mmol of $Pb(OAc)_4$, are added under an argon atmosphere. The reaction mixture is then heated under reflux for 1.5 - 3 hours. After cooling and filtration over Celite, the organic layer is stirred with 100 ml of aqueous 20% citric acid for 10 minutes. The organic layer is then separated, washed with brine, dried over $MgSO_4$ and evaporated in vacuo. The crude products can be directly converted into the α-alkylthio compounds **5**.
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19. *General procedure for the conversion of α-acyloxy compounds **5** into α-alkylthio compounds **6**:*
To a stirred, cooled (-78°C) mixture of α-acyloxy compound **5** (5 mmol) and a thiol (5 mmol) in dry THF (50 ml) under argon, a solution of diazabicyclo[2.2.2]octane (DABCO) (10 mmol) in THF, is added via syringe. After 2 h of stirring at -78°C the mixture is allowed to warm to r.t. overnight and quenched with 20% aqueous citric acid. Extractive workup with EtOAc, drying of the organic layer over $MgSO_4$ and evaporation of the solvent in vacuo leads to the products **6** which are further purified by flash chromatography on silica gel (eluent: petroleum ether/ethyl acetate).
20. Z-Phe-Gly(Br)-OMe (**7**): oil, yield 94%. - ^{1}H-NMR (90 MHz, $CDCl_3$): δ 3.05 (d, *J*=7.5 Hz, 2H), 3.70[a] (s, 3H), 4.61 (m, 1H), 5.01 (s, 2H), 5.66 (m, 1H), 6.63/6.41[a] (d, *J*=9 Hz, 1H), 7.16 (s, 5H), 7.23 (s, 5H), 7.70 (br, 1H).
([a]) Signal doubling because of diastereomer formation)
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