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Synthesis of peptide C-terminal derivatives using the transfer active ester condensation technique

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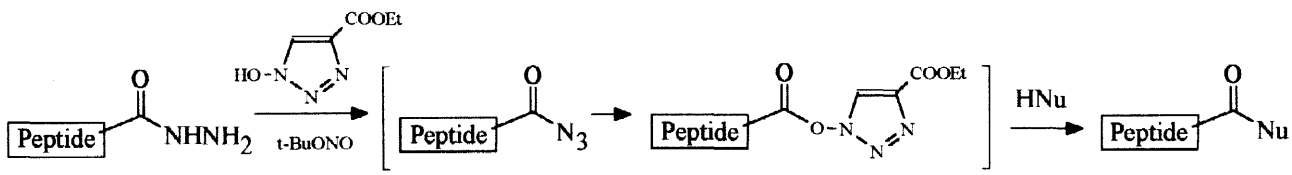
Abstract: Peptide C-terminal derivatives: ethyl ester, trifluoroethyl ester, benzylthio ester, N-hydroxyl amide, N-ureido amide and N-methyl-N-methoxy amide are synthesised using transfer active ester condensation technique.

Keywords: Peptides; Active esters; Peptide C-terminal derivatives.

Peptide C-terminal derivatives (PCD) are of interest due to their potentially important biological activities and as intermediates in peptide modification. For example, the mystixins are peptide amides with anti-inflammatory activity [1]. A series of peptide C-terminal methyl esters have been reported with anti-HIV activity [2] The peptide aldehyde, GYKI-14116, is a highly active and selective anticoagulant [3]. Peptide thioesters have been used in chemical ligation applied to the synthesis of proteins such as the tethered dimer of HIV-1 protease [4], turkey ovomucoid third domain [5] and cMyc-max, a transcription factor-related protein [6].

Some resins or linkers have been developed for the solid phase synthesis of PCD such as Sieber amide resin and HMPB-MBHA resin for peptide amides or N-alkyl amides [7, 8] whereas the dibenzocycloheptadienyl linker has been designed for peptide hydrazide and various PCD [9]. Peptide aldehydes have been synthesised with solution or solid phase techniques [3, 10, 11, 12, 13]. However, synthesis of diverse PCD from a peptide C-terminal intermediate is not trivial in peptide chemistry because of the number of linker systems required.

Prior work in Edinburgh has developed a method termed transfer active ester condensation (TAEC) for peptide segment coupling [14]. Under TAEC conditions, a peptide active ester of ethyl 1-hydroxyl-1H-1,2,3-triazole-4-carboxylate (HOCt) can be formed from a peptide hydrazide, *via* the corresponding azide intermediate, which can then react with an amine segment to give the coupling product with higher yield than the classical azide method.



HNu = NH_2OH, $NH_2NHCONH_2$, $NH(OCH_3)CH_3$, HOEt, $HOCH_2CF_3$, $HSCH_2C_6H_5$

Figure 1. Synthesis of peptide C-terminal derivatives using TAEC

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In the present paper, we report an extension of the use of TAEC in which the peptide C-terminal active ester of HOCt reacts with various nucleophilic reagents to produce the corresponding PCD (Figure 1).

The peptide hydrazide (ubiquitin$_{67\text{-}76}$NHNH$_2$), synthesised using the Fmoc solid phase peptide synthesis strategy on an ABI 430A synthesiser with the dibenzocycloheptadienyl linker, was transformed into the active ester at room temperature in 0.5 hour, then excess nucleophilic reagent and DIEA were added. After reacting for 4 hours, the peptide C-terminal derivatives were separated by HPLC and identified by amino acid analysis as well as high resolution mass spectra (Table 1).

Table 1. PCD synthesised with TAEC method

Nucleophile	Peptide C-terminal derivative	Molecular formula	Molecular weight (Da) Found	Requires
HOEt	A). Fmoc.LHLVLRLRGG.OEt	$C_{68}H_{107}N_{18}O_{13}$	1383.82172	1383.82650
$HOCH_2CF_3$	B). Fmoc.LHLVLRLRGG.OCH_2CF_3	$C_{68}F_3H_{105}N_{18}O_{13}$	1438.80202	1438.80606
NH_2OH	C). Fmoc.LHLVLRLRGG.NHOH	$C_{66}H_{104}N_{19}O_{13}$	1370.80612	1370.80411
$NH_2NHCONH_2$	D). Fmoc.LHLVLRLRGG.$NHNHCONH_2$	$C_{67}H_{106}N_{21}O_{13}$	1412.82554	1412.82790
$NH(OCH_3)CH_3$	E). Fmoc.LHLVLRLRGG.$N(OCH_3)CH_3$	$C_{68}H_{108}N_{19}O_{13}$	1398.83279	1398.83740
$HSCH_2C_6H_5$	F). Fmoc.LHLVLRLRGG.$SCH_2C_6H_5$	$C_{73}H_{109}N_{18}O_{12}S$	1461.81237	1461.81237

It has been found recently that C-terminal trifluoroethyl esters such as B are extremely efficient in the enzymatic catalysed peptide synthesis [15,16]. Since N-methyl-N-methoxy amides have been reported to be a key intermediate in synthesis of α-amino aldehydes [17,18], product E is a valuable precursor for further synthesis of the corresponding peptide aldehyde. Product F can be used as a building block in chemical ligation for the synthesis of proteins.

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