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N-Methyl N-Carboxyanhydride: An Unexpected By-product When Coupling Boc-N-methyl Amino Acids

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Key Words: Coupling reagent; PyBroP; PyCloP; N-methyl amino acid; N-carboxyanhydride.

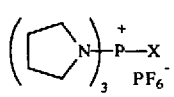
Abstract: *Activation of Boc-amino acids and Boc-N-methyl amino acids leads to the corresponding NCA. This side reaction explains the low yields obtained when coupling N-methyl amino acids. It was not observed with the Z- or Fmoc-protective groups.*

PyBroP **1** and PyCloP **2** reagents have proved to be efficient for coupling Z-protected N-methyl amino acids,[1] as we showed for example in the synthesis of the pentapeptide part of Dolastatin 15.[2] We have now established (Table) that, like BOP-Cl[3], these reagents are ineffective for coupling Boc-amino acids. This led us to elucidate the activation mechanism with reagents **1** and **2**.

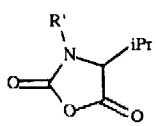
Table: % yield in coupling reactions

	Peptides[4]	1	2
3	Z-Val-MeVal-OMe	90	96
4	Z-MeVal-MeVal-OMe	87	84
5	Boc-Val-MeVal-OMe	44	58
6	Boc-MeVal-MeVal-OMe	33	39
7	Fmoc-Val-MeVal-OMe	84	-
8	Fmoc-MeVal-MeVal-OMe	83	75

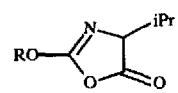
1.5 eq N-protected acid and reagent, 1 eq amino acid ester hydrochloride, 4 eq DIEA in CH_2Cl_2, 3 h at RT.



X = Br: PyBroP **1**
X = Cl: PyCloP **2**

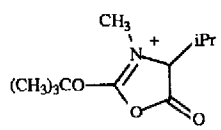


R' = H **10**
R' = Me **11**



R = tBu, $PhCH_2$, fluorenylmethyl

9



12

^{1}H NMR and IR studies of the reaction mixture obtained by reaction of Z-, Fmoc- or Boc-Val-OH with PyCloP/DIEA exhibited the formation of symmetrical anhydride[5] and of oxazol-5(*4H*)-one (oxazolone) **9**[5]. In the case of Boc-Val-OH, the N-carboxyanhydride (NCA) **10**[11] appeared after reaction for 90 min. The formation of this compound explains the low yield of the dipeptide **5**. On the other hand, NCA was not detected when Z- or Fmoc-Val-OH were activated, even after several hours.

Concerning the N-methyl amino acids, the activation of Z- or Fmoc-MeVal-OH with PyCloP/DIEA yielded the corresponding symmetrical anhydrides[12]. During the course of the coupling reaction, these intermediate were easily aminolyzed, providing good yields (peptides **4** and **8**). However, Boc-MeVal-OH led immediatly to a 1/1 mixture of the symmetrical anhydride and N-methyl-NCA **11**[13]. The formation of the latter product accounts for the low yield of the peptide **6**.

The NCA **10** arises from oxazolone **9** (R = ^{t}Bu); this type of reaction has already been reported.[9,14,15] Using the same reasoning, one could asume that N-methyl-NCA **11** was formed from the oxazolonium **12**. Similar ions have been obtained by activation of N-benzoyl N-methyl amino acids with DCC.[16] The intermediates **9** (R = ^{t}Bu) and **12** could easily lose the *t*-butyl cation: this was supported by the detection of *t*-butyl chloride and isobutene in the reaction medium. Thus, the easy loss of the *t*-butyl group could explain the particular behavior of the Boc-protected amino acids.

The formation of NCA seems to be a general phenomenon. We observed it with Boc-Leu-OH, Boc-Ala-OH, Boc-MeLeu-OH and Boc-MeAla-OH, but not with Boc-Pro-OH. Furthermore, we verified that, in the case of Boc-MeVal-OH, PyBroP reacts like PyCloP. Finally, compound **11** was also detected by activating Boc-MeVal-OH with other coupling reagents, namely, DCC and PyBOP®.

It should be noted that the slower the coupling reaction, the larger the amount of NCA formed and the lower the yield of the peptide. This is particularly the case when the C-protected amino acid is N-methylated.[1]

Based on these results, coupling of Boc-N-methyl amino acids should be avoided. We are currently using a Fmoc strategy for solid phase peptide synthesis.

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4. New compounds (**5-8**) have physical data consistent with proposed structures.
5. Authentic samples of symmetrical anhydrides were obtained with DCC using standard protocoles.[6-8] ^{1}H NMR of Z-, Fmoc-, and Boc-Val oxazolones have already been described.[9,10]
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11. **10** was also isolated from the reaction (16 h) of Boc-Val-OH with PyCloP/DIEA and purified by chromatography on silica gel. Its physical data were consistent with those previously described.[9]
12. Samples of these anhydrides were obtained with DCC. They gave IR and ^{1}H NMR spectra consistent with the expected structures.
13. Me-NCA **11** was also obtained by reaction of Boc-MeVal-OH with phosgene/DIEA. ^{1}H NMR ($CDCl_3$): δ ppm 0.97 (d, 7.0 Hz, 3H), 1.17 (d, 7.0 Hz, 3H), 2.26 (d.hept., 3.4, 7.0 Hz, 1H), 2.97 (s, 3H), 3.99 (d, 3.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ ppm 15.99 and 17.08 (CH_3), 28.46 (C_β), 28.64 (N-CH_3), 66.05 (C_α), 152.17 and 167.49 (C=O). IR ($CHCl_3$) ν: 1845, 1780 cm^{-1}.
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