New Activating and Coupling Agents for Formation of Amide and Peptide Bonds

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In electronegatively substituted sulfonates of *N*-hydroxysuccinimide (NHS) and similar compounds we have discovered a new class of potent, inexpensive reagents of wide applicability for the formation of different amide bonds. Reaction can be brought about either in two steps or, especially, directly by coupling in one step. The two-step procedure is believed to involve activation by transacylation of the carboxyl component, followed by aminolysis. Personal preferences have led us to investigate first the application of the new reagents in peptide synthesis. We have been especially interested to find conditions under which no racemization takes place. For simplicity we have chosen to carry out our first experiments with polymeric NHS.

NHS-esters of amino acids are useful reagents for the synthesis of peptides.[1] NHS has also been used to suppress racemization in peptide synthesis with *N*,*N*'-dicyclohexylcarbodiimide.[2] In this case an NHS ester is probably formed *in situ*. Polymeric NHS esters of amino acids have also been described and applied.[3]

In a recent paper Sahni *et al.* used the methanesulfonate and *p*-toluenesulfonate of copoly(ethylene-*N*-hydroxymaleimide) in a two-step procedure for the preparation of amides and simple dipeptides.[4] In our hands, however, only a trace of product was obtained when we tried to prepare the peptide Boc-Leu-Val-O^{t}Bu using the methanesulfonate or *p*-toluenesulfonate of cross-linked, macrolattice copoly(styrene-*N*-hydroxymaleimide),[5] indicating insufficient reactivity. We, therefore, prepared the *p*-nitrobenzenesulfonate of this resin, which was sufficiently reactive to be useful for the preparation of peptides in both one and two steps. We now report on reactivity and racemization as a function of the solvent and base used.

In our two-step procedure the *p*-nitrobenzenesulfonate derivative of the resin was first transacylated with equivalent amounts of Boc-Leu and base for 1 h in different solvents and then the reaction mixture was submitted to aminolysis with Val-O^{t}Bu in DMF for 5 h. After filtering off the resin and careful washing, the solution was evaporated to dryness. A small, constant aliquot was subjected to solvolysis in trifluoroacetic acid and then analyzed for its content of starting materials and diastereoisomeric products on an amino acid analyzer, essentially as described by Manning and Moore.[6] With DMF as solvent the following bases were investigated: Triethylamine, dicyclohexylamine, ethyldiisopropylamine, *N*-methylmorpholine, 2,4,6-trimethylpyridine and pyridine. Racemization decreased within this series and so did the reactivity. For triethylamine about 15 % dipeptide was present as D-Leu-L-Val, compared to about 8 % for dicyclohexylamine, a few per cent for the next two amines, below 1 % for the 2,4,6-trimethylpyridine and well below 0.1 % for pyridine. These results were obtained at room temperature, but in one experiment with *N*-methylmorpholine at 0 °C, the amount of DL-peptide was reduced by a factor of 3 – 4 to about 1 %. Reactivity was such that for the first three bases only traces of starting materials remained but was considered quite satisfactory even in the remaining cases. Addition of more than one equivalent of pyridine to increase the reaction rate was not successful and had the opposite effect. We found that the best results were obtained with less than 1 equivalent of pyridine. Pyridine as solvent in the transacylation step was consequently not ideal with respect to the reaction rate. Other solvents tried included dichloromethane, tetrahydrofuran and ethyl acetate, all of which were even less satisfactory than pyridine.

In our one-step procedure equivalent amounts of carboxyl component, amino component, pyridine and *p*-nitrobenzenesulfonate resin were mixed and reacted at room temperature for different periods of time and then the reaction mixture was worked up as above. Those experiments have so far indicated that after 30 min the reaction was about 90 % complete as compared to 2, 4, 7, 12 and 24 h.

We firmly believe in the potential of this new class of activating and coupling reagents for applications in addition to peptide synthesis. Monomeric sulfonates are easy and cheap to prepare and their reactivities can be varied easily according to different requirements. Polymeric reagents belonging to this class offer two further advantages: they can be regenerated and further tailor-made by variation of the polymer backbone to make them compatible with other reagents, as required. The typical capacity of our new reagents is 2 – 3 meq g^{-1} as compared with, for example, 4.8 meq g^{-1} for dicyclohexylcarbodiimide.

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