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ARENESULPHONYLTRIAZOLIDES AS CONDENSING REAGENTS IN SOLID PHASE PEPTIDE SYNTHESIS

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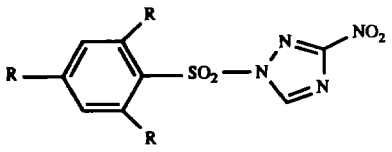
Results on the use of 1-arenesulphonyl-3-nitro-1,2,4-triazoles and N-methylimidazole or 4-morpholinopyridine-1-oxide as reagents for peptide bond formation on a solid support are reported.

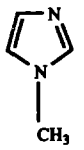
Arenesulphonyl-1,2,4-triazolides have been currently used in the synthesis of oligonucleotides to activate a phosphate diester group, which, by reaction with a hydroxyl group, yields a phosphate triester (1). The coupling time with these reagents can be considerably shortened by addition of certain catalysts such as N-methylimidazole (2, 3) or 4-substituted pyridine-1-oxides (4).

We have evaluated the use of 1-(mesitylenesulphonyl)-3-nitro-1,2,4-triazole (MSNT) and 1-(2,4,6-triisopropylbenzenesulphonyl)-3-nitro-1,2,4-triazole (TPSNT) in the presence of N-methylimidazole (NMI) or 4-morpholine pyridine-1-oxide (MPO) as coupling reagents for peptide bond formation on a solid support. For this study we have carried out the synthesis of the model peptide H-Leu-Ala-Gly-Val-OH on a p-alkoxybenzyl ester-type resin and Fmoc-amino acids.

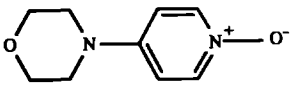


R=CH_3 MSNT

R=iPr TPSNT



NMI



MPO

The C-terminal amino acid was anchored *via* the preformed handle 2,4,5-trichlorophenyl-3'-4''-(Fmoc-valyloxymethyl)phenoxy propionate (5) to a *p*-methylbenzhydrylamine resin. The following amino acids were attached by a single five minutes-coupling under the conditions indicated in Table I. The Fmoc group was removed by three successive treatments of one minute with 50% piperidine in dimethylformamide (DMF), and the progress of the synthesis was evaluated by spectrophotometric quantification of the amount of N-(9--fluorenylmethyl)piperidine (Fmp) formed at each deprotection step (6). At the end of the synthesis the peptide-resin was treated with 70% trifluoroacetic acid in dichloromethane for 30 minutes, and the crude peptide was analysed by reversed phase HPLC (7).

Table I

Spectrophotometric quantification of Fmp after each coupling in different approaches of the synthesis of H-Leu-Ala-Gly-Val-OH

					A/A_0[a]			
	RELATIVE PROPORTION OF REAGENTS				NUMBER OF AA COUPLED ON THE RESIN			
METHOD[b]	FMOC-AA-OH	REACTIVE	CATALYST	FMOC-VAL-O-Ⓡ	1	2	3	4
I	5	5.5[c]	10[d]	1	1.00	0.78	0.69	0.56
II	5	5.5[c]	10[d]	1	1.00	0.98	0.91	0.94
III	5	5.5[e]	10[d]	1	1.00	1.03	0.90	0.98
IV	5	4.5[e]	10[f]	1	1.00	0.96	0.99	0.98

a) Ratio between the absorbance found after each deprotection and the initial value, A_0
b) The difference between method I and the others is the order of addition of the reagents (see text)
c) MSNT; d) NMI; e) TPSNT; f) MPO

The excess of catalyst was necessary in order to neutralize the sulphonic acid liberated after the coupling (8). The order of addition of the reagents was very important. In method I (Table I) coupling was carried out by first adding a solution of the Fmoc-amino acid in DMF to the resin, followed by addition of solutions of MSNT and NMI in DMF. The amount of Fmp formed at each deprotection step diminished along the synthesis, giving an indication of a decrease of reactive amines after each coupling (ninhydrin tests were negative after the incorporation of each amino acid). HPLC analysis of the crude peptide (7) showed the presence of peptide impurities with high UV absorption at a wavelength different from that of the amide bond. These results suggested that during the coupling step amino groups had been

blocked by sulphonylation, and this side reaction might be prevented by changing the order of addition of reagents.

Thus, in syntheses II, III and IV (Table I), the Fmoc-amino acid, MSNT and NMI were first stirred in the minimum amount of DMF, and, after one minute, this solution containing the preactivated amino acid was poured onto the polymeric support. The light fall of the Fmp absorbance along synthesis II together with HPLC analysis of the crude peptide confirmed that the undesired blocking reaction with a sulphonylating reagent had considerably decreased.

To try to completely eliminate this side reaction, MSNT was replaced by TPSNT, with the idea of directing the attack of the N-terminal amino to the activated carboxylic group instead of to the sulphonyl group now more sterically hindered by the presence of two isopropyl groups at positions 2 and 6. Syntheses with TPSNT were carried out both with NMI and MPO (III and IV in Table I) without appreciable blockage on the amino groups as suggested by Fmp absorbance and HPLC of the crude peptide.

The extent of racemisation was evaluated when the amino acid was activated with TPSNT and any of the two catalysts. Fmoc-L-valine was attached by any of the two methods (III or IV) to the same Fmoc-Val-resin used to carry out the syntheses of the model peptide. After cleavage with trifluoroacetic acid, the crude Fmoc-dipeptides were analysed by HPLC (10). The comparison with the chromatogram of a previously synthesised sample which contained the two diastereomers showed that less than 4% racemisation had taken place during the coupling with TPSNT and NMI, and that there is no appreciable racemisation in the activation of the amino acid with TPSNT and MPO (less than 0.1%). All these results prompt us to recommend method IV for peptide bond formation.

Finally, amino acid activation with TPSNT and MPO (method IV) was used to synthesise Leu-enkephalinamide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) on a (5--(4-Fmoc-aminomethyl-3,5-dimethoxyphenyl)valeryl)aminomethyl resin (PAL resin) (11). After cleavage of the peptide-resin bond with 70% trifluoroacetic acid the desired peptide was obtained with an overall synthesis yield of 70%, a satisfactory proportion of amino acids and 85-90% purity as shown by HPLC.

In conclusion, use of TPSNT and MPO can be considered as an alternative to dicyclohexylcarbodiimide for peptide bond formation on a solid matrix. The percentage of racemisation is virtually nil, but, as the reagent is relatively voluminous, we anticipate that the kinetics of the coupling may be lower than desired if activation is to be carried out on a hindered carboxyl group.

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7.- Chromatographic analysis was carried out on a ODS Vydac column (25x0.46 cm). Eluents: A: H_2O/0.045% trifluoroacetic acid; B: CH_3CN/0.035% trifluoroacetic acid. Gradient: 10-30% B, 10 min + 15 min 30% B. Detection wavelength: 220 nm. RT LAGV : 3.7 min.
8.- According to the mechanism proposed for the activation of phosphate diesters in oligonucleotide synthesis (see reference 9), after reaction of the amino group with any of the possible activated forms of the amino acid (mixed carboxylic-sulphonic anhydride, symmetrical anhydride or acyl-N-methylimidazolium) to yield the amide bond, an equivalent amount of arenesulphonic acid is liberated which has to be neutralised to prevent protonation of unreacted amino groups.
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10.-Chromatographic conditions used were the same as described in note 7, except that elution was carried out in an isocratic mode with 40% B for 15 min and detection at 300 nm.
RT: Fmoc-L-Val-L-Val-OH : 9.1 min; Fmoc-D-Val-L-Val-OH : 10.3 min.
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