4-(4,6-Dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium Chloride (DMTMM): A Valuable Alternative to PyBOP for Solid Phase Peptide Synthesis

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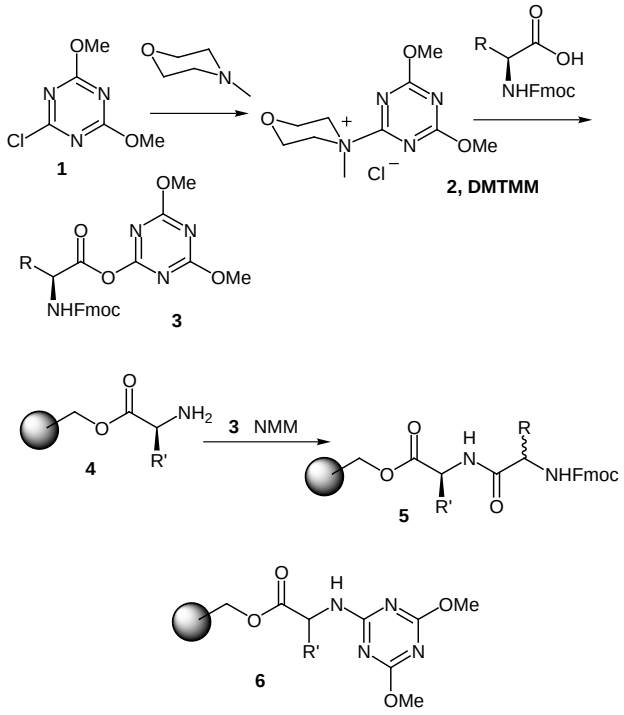
Abstract: The salt formed from 2-chloro-4,6-dimethoxy[1,3,5]triazine and *N*-methylmorpholine (DMTMM) is an effective coupling agent for solid phase peptide synthesis that can be used as economical alternative to PyBOP. Several oligopeptides were prepared on a Wang type resin using this reagent and the yields and purity of the products were always comparable with those obtained with PyBOP as the coupling agent.

Key words: coupling reagents, solid phase synthesis, peptides, triazines

The formation of new peptidic bonds has been developed following two different approaches. In the solution phase approach the carboxylic acid is generally transformed into an activated ester (e.g. p-nitrophenyl, pentafluorophenyl) or a mixed anhydride (using for example ethyl or isobutyl-chloroformate); then the intermediate compound is treated with the amine to give the desired amide.[1] The reagents required in this procedure are simple low cost molecules to minimise the formation of by-products and to allow bulky preparations. In the solid phase approach a reagent that forms a new amide bond from a carboxylic acid and an amine is required. The reagent employed must be effective, react rapidly and selectively, thus several complex (and expensive) molecules have been developed as coupling agents for solid phase peptide synthesis.[2] In this approach the presence of by-products is negligible and the amounts of product employed justify the use of expensive reagents.

Recently, 2-chloro-4,6-dimethoxy[1,3,5]triazine (CDMT) has been described as a versatile reagent for the selective formation of amides in solution phase.[3] Following our interest in the use of this product in organic synthesis,[4] we decided to explore the potential of CDMT as a reagent for solid phase peptide synthesis and we report here that 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium chloride (**2**) (the salt between CDMT and *N*-methylmorpholine, DMTMM) is an effective coupling agent that can be used as economical alternative to PyBOP in batch manual syntheses.

Our first approach was to try to extend the already established protocol for CDMT activation of carboxylic acids[5] to solid phase. CDMT, previously activated with *N*-methylmorpholine, was treated with *N*-Fmoc amino acid and the THF solution of the activated ester (**3** in Scheme 1) was then added to a NH_2-free amino acid (**4**) loaded on a Wang-type resin.



Scheme 1

The reaction in THF proceeded rapidly at room temperature as monitored by the ninhydrin test carried out on the beads.[6] The dipeptide **5** was cleaved from the resin and analysed or, alternatively, the *N*-Fmoc was deprotected and the sequence repeated with other amino acids to give the products reported in Table 1.

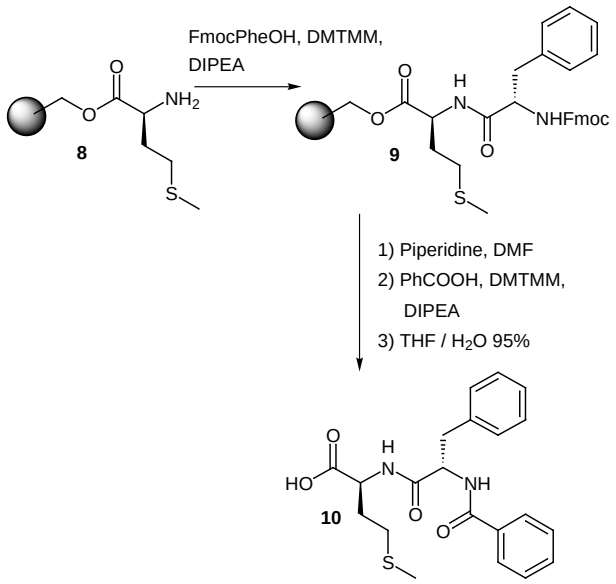
Table 1 Peptides obtained on solid phase using **2** and the activated ester protocol

Peptide	Yield[%][a]	Purity[%] HPLC
H-Phe-Val-Gly-OH	92	85
H-Phe-Val-Pro-Phe-OH	73	80
H-Phe-Leu-Met-Gly-OH	81	95
H-Phe-Sar-Leu-Gly-OH	69	80
H-Phe-Val-Leu-Leu-Gly-OH	89	85

[a] Yields of crude product isolated after cleavage (TFA/CH_2Cl_2 1/4) from the beads

Although the peptides were obtained in satisfactory yields, the HPLC analysis of the cleaved sequences showed the formation of 5-15% of diastereoisomers. This trouble was due to the time needed to prepare the activated ester in THF solution (at least 2 h, NMR analysis).[7] During this period a partial racemisation of the *N*-Fmoc activated ester may occur although this fact was never observed before in the formation of amides and peptides in solution phase.[3b,3e] To overcome this problem we tried to add CDMT to a mixture of the resin bound amino acid **4** and the *N*-Fmoc amino acid in the presence of NMM. Unfortunately using this approach we obtained mainly the link of the dimethoxytriazinyl moiety to the resin (**6** in Scheme 1).

The next step was to isolate the *N*-methylmorpholinium salt of CDMT (**2,** DMTMM) and use it directly as a coupling agent. During the development of the work described here, Kunishima and coll. described the isolation of salt **2** and its use as an efficent condensing agent for the preparation of esters and amides.[8] DMTMM was obtained as a white solid directly from a cooled THF solution of CDMT and NMM after filtration and drying under vacuum.[8]



Scheme 2

Addition of **2** to a mixture of the resin bound amino acid **4** and a *N*-Fmoc amino acid in the presence of DIPEA gave rise to a rapid formation of the desired peptide in good yield.[9] To verify the possibility of racemisation during the procedure we prepared the peptide **10** using alternatively L-Fmoc-PheOH and DL-Fmoc-PheOH and compared the corresponding HPLC profiles. The amount of the undesired diastereoisomer was always under the detection limit of the technique employed. An analogous HPLC profile was observed with compound **10** prepared using PyPOB.[10]

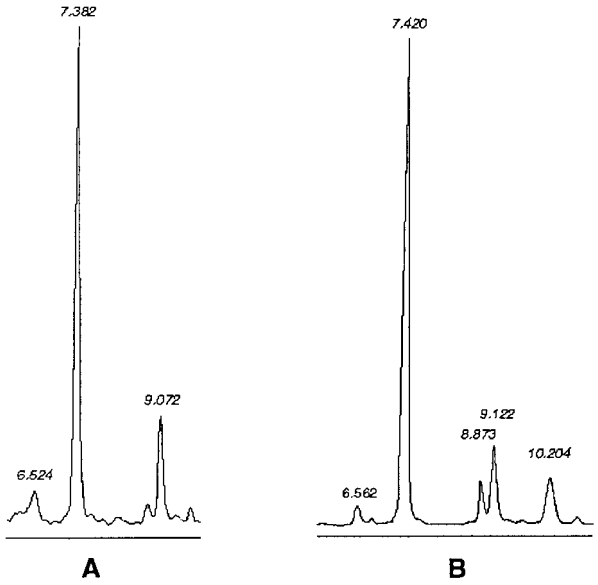
In the effort to find the best reaction conditions, we carried out the preparation of **10** using different solvents and amounts of bases and found that the best results were obtained using *N*-methyl-2-pyrrolidinone (NMP) as the solvent, 4 equiv of the *N*-Fmoc amino acid with respect to the resin, 3 equiv of **2** and 6 equiv of DIPEA as additional base. Under these conditions the coupling was complete after 2-3 min (ninhydrin test)[6] and the yields (after cleavage) were always higher than 90%.

To verify the potential of reagent **2** we prepared the peptides reported in Table 2 using our protocol and the classical protocol with PyBOP[11] obtaining always comparable results in terms of yields and purity of the final products. In these cases we chose to prepare *N*-protected peptides as *N*-Fmoc, *N*-benzoyl (Bz) or *N*-4,6-dimethoxytriazin-2-yl (DMT) derivatives to get a better resolution in the HPLC analysis. In any case we observed the formation of variable amounts of deletion compounds as showed in the HPLC enclosed (Figure).

Table 2 Peptides prepared on solid phase with **2** as the coupling reagent.

Peptide	Prepared using **2**		Prepared using PyBOP	
	yields[%][a]	purity[%](HPLC)	yields[%][a]	purity[%](HPLC)
Bz-Phe-Met-OH (**11**)	92	92	90	89
Bz-Phe-Leu-Gly-OH (**12**)	90	91	90	90
Fmoc-Ala-Ser-Phe-Met-OH (**13**)	86	89	80	88
Fmoc-Phe-Pro-Asp-Met-OH (**15**)	91	90	90	91
DMT-Leu-Met-Phe-Met-OH (**16**)	87	85	89	88
DMT-Ala-Pro-Ile-Met-Leu- -Phe-Phe-Met-OH (**17**)	70	88	62	85

a) Yields of crude product isolated after cleavage (TFA/CH_2Cl_2 1/4) from the beads



Comparison of the HPLC profiles of product **16** prepared using 2 (**A**) and using PyBOP (**B**)

Figure

It is noteworthy to see that serine containing peptides can be prepared without protection at the OH group (ES/MS spectrum of **13**: 677.6; $C_{35}H_{40}N_4O_8S$ requires 676.26) and that also proline can be easily coupled with high yields. Comparing the results obtained using DMTMM with those obtained using PyBOP, we are convinced that **2** is a convenient and economical alternative to PyBOP[12] (or to other similar coupling agents) for batch manual solid phase peptide synthesis on Wang resins following the Fmoc protocol.

Typical experimental procedure: DMT-Ala-Pro-Ile-Met-Leu-Phe-Phe-Met-OH (17):

The synthesis was carried out in a manual 20 mL reactor equipped with a sintered glass and using a nitrogen flow for agitation and filtration. The 1% cross-linked polystyrene resin (Wang -type) preloaded with Fmoc-Met (0.2 g of 0.52 mmol/g loaded beads, 0.1 mmol) was placed in the reactor and swollen for 2 hrs with *N*-methylpyrrolidinone (NMP) (5 mL). The Fmoc group was removed by treatment with 25% piperidine in DMF (5 mL 1 x 6 min, 1 x 9 min) (positive Kaiser's test)[6] and the resin was rinsed with DMF (6 x 2 min, 5 ml). The resin was treated with NMP (5 mL) and Fmoc-phenylalanine (0.16 g, 0.4 mmol) was added to the suspension followed by DMTMM (0.08 g, 0.3 mmol) and DIPEA (75 µL, 0.6 mmol). After 5 min (negative Kaiser's test)[6] the resin was rinsed with DMSO (2 x 2 min, 5 ml) and NMP (2 x 2min, 5 ml). The cycle of Fmoc deprotection and coupling was repeated adding to the growing peptides the following amino acids: Fmoc-Phe-OH (0.16 g, 0.4 mmol), Fmoc-Leu-OH (0.15 g, 0.4 mmol), Fmoc-Met-OH (0.16 g, 0.4 mmol), Fmoc-Ile-OH (0.15 g, 0.4 mmol), Fmoc-Pro-OH (0.14 g, 0.4 mmol) (chloranil test),[13] Fmoc-Ala-OH (0.13 g, 0.4 mmol). After removal of the terminal Fmoc group and rinsing of the resin with DMF, CDMT (0.07 g, 0.4 mmol) was added to the suspension of the resin in NMP (5 mL), followed by the NMM (70 µL, 6 equiv). After 30 min (negative Kaiser's test)[6] the resin was rinsed with DMSO (2 x 2 min, 5 mL), NMP (2 x 2min, 5 mL), CH_2Cl_2 (2 x 2min, 5 mL), and Et_2O (2 x 2min, 5 mL), and dried under a nitrogen stream. The resin was then suspended in a 20% solution of TFA in CH_2Cl_2 (10 mL) for 2 h. The resin was filtered off and the filtrate was evaporated under vacuum to give the DMT-protected octamer **17** (78 mg, 70%). ES/MS m/z: Found 1108.7 ($C_{53}H_{76}N_{11}O_{11}S$ requires 1107.38).

Acknowledgement

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(9) For an analogous behaviour with isobutyl chloroformate see: Shieh, W-C.; Carlson, J.A.; Shore, M.E. *Tetrahedron Lett.* **1999**, *40*, 7167.
(10) HPLC conditions: Column C-18-Lichrospher 100, 5µm, 250 x 4 mm, eluent MeCN/$H_2$0 (0.1% TFA) fron 80/20 to 20/80 in 20 min. Detector UV lamp, λ 254 nm, flow 1 mL / min.
(11) We followed the protocol described on the Novabiochem Catalogue & Peptide Synthesis Handbook, 1999, Synthesis Notes, S69.
(12) The price of PyBOP is 11.9 Euro per mmol, whereas the cost of **2** (purchasing CDMT and NMM and considering a yield of 90%) is 1.5 Euro per mmol. Prices from Aldrich Catalogue 1999-2000, Italian Lira. Preparing CDMT from trichlorotriazine and MeOH the cost is even lower.
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