A Racemization Test in Peptide Synthesis Using 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Chloride (DMT-MM)

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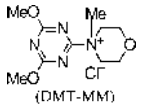
Racemization of the C-terminal amino acid (Ala) has been studied in various solvents during coupling between 4-methoxybenzyloxycarbonyl (Z(OMe))-Gly-L-Ala-OH and phenylalanine benzyl ester (H-Phe-OBzl) with 4-(4,6-dimethoxy-1,3,5-thiazin-2-yl)-4-methylmorpholinium chloride (DMT-MM). The reaction occurred without substantial racemization in AcOEt, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), CH_3CN, and 2-PrOH, while a slight racemization was observed in dimethyl sulfoxide (DMSO), EtOH, and MeOH. The extent of racemization may correlate with the polarity of the solvents.

Key words dehydrating condensation; racemization; peptide; amide

Recently, we introduced 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as a new dehydrating condensing agent.[1] Practical features of DMT-MM are as follows: 1) the reaction proceeds by a convenient one-step procedure in which DMT-MM is simply added to a mixture of acids and amines in a solvent; 2) no additives are required; 3) DMT-MM is a stable but not hygroscopic solid; 4) no irritating properties to the eye and nose, nor allergenic properties were observed in our laboratory; 5) the highly water-soluble co-products can be readily removed by washing with water; 6) DMT-MM can be prepared at a low cost from inexpensive cyanuric chloride. In addition, it is noteworthy that DMT-MM enables us to carry out the direct one-pot condensation of carboxylic acids and amines in protic solvents like methanol, ethanol, 2-propanol, or water, as well as in a variety of aprotic organic solvents.[2]

In order to evaluate the usefulness of DMT-MM in peptide synthesis, the degree of racemization with this reagent should be examined. We have already reported that no diastereomeric isomer resulting from racemization was detected in the coupling of *tert*-butoxycarbonyl (Boc)-L-Leu-OH and H-L-Phe-OMe with DMT-MM in both tetrahydrofuran (THF) and MeOH.[2] Taddei and co-workers recently found that DMT-MM is a useful alternative to benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) with regard to reaction yields and the purity of products in solid phase peptide synthesis using *N*-Fmoc amino acids.[3] In contrast to the coupling of amino acids with a urethane-type *N*-protecting group that are known to be almost free from racemization, the C-terminal amino acids of

Table 1. Racemization Test of DMT-MM in Various Solvents[a]



(DMT-MM)

Z(OMe)-Gly-L-Ala-OH + H-L-Phe-OBzl·HCl —(Et_3N)→ Z(OMe)-Gly-L-Ala-L-Phe-OBzl + Z(OMe)-Gly-D-Ala-L-Phe-OBzl

Entry	Coupling reagents	Solvent	Conditions	Ratio (%)[b]		Yield (%)[b]
				-L-Ala-	-D-Ala-	
1	DMT-MM	AcOEt	rt, 2.5 h	>99.9	<0.1	quant
2	DMT-MM	THF	rt, 3 h	>99.9	<0.1	quant
3	DMT-MM	DMF	rt, 2 h	>99.9	<0.1	quant
4	DMT-MM	CH_3CN	rt, 2 h	>99.9	<0.1	quant
5	DMT-MM	DMSO	rt, 2 h	97.7	2.3	97
6	DMT-MM	2-PrOH	rt, 20 min	99.3	0.7	quant
7	DMT-MM	EtOH	rt, 2 h	96.6	3.4	quant
8	DMT-MM	MeOH	rt, 5.5 h	96.7	3.3	96
9	DMT-MM/HOBt	MeOH	rt, 30 h	97.3	2.7	15
10	DMT-MM	Water	rt, 24 h	89.5	10.5	69
11[c]	DCC	THF	rt, overnight	76.8	23.2	—[d]
12	DCC	THF	rt, 24 h	80.1	19.9	92
13[c]	DCC/HOBt	AcOEt	rt, overnight	99.5	0.5	—[d]
14	DCC/HOBt	AcOEt	rt, 24 h	>99.9	<0.1	quant
15[c]	DPPA	AcOEt	rt, overnight	97.6	2.4	—[d]
16	DPPA	AcOEt	rt, 24 h	96.6	3.4	41

a) For abbreviations: DCC: 1,3-dicyclohexylcarbodiimide; DPPA: diphenylphosphorylazide. rt, room temperature. *b*) Determined by HPLC. *c*) Data cited from ref. 6. *d*) Yields are unkown.

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peptides are susceptible to racemization during coupling *via* activation of the carboxyl group.[4] In this note, we report the racemization test of DMT-MM in tripeptide synthesis as a fragment coupling model.[5]

According to the method reported by Kiso and coworkers,[6] condensation of 4-methoxybenzyloxycarbonyl (Z(OMe))-Gly-L-Ala-OH and H-L-Phe-OBzl with DMT-MM was examined in various solvents. The diastereomeric purity of the products [Z(OMe)-Gly-L-Ala-L-Phe-OBzl *vs.* Z(OMe)-Gly-D-Ala-L-Phe-OBzl] was analyzed by HPLC, and the results are summarized in Table 1. The reproducibility of the results in the literature was confirmed by conducting reactions using other coupling reagents under the same conditions (Entries 11—16). Most of solvents, except for water, can be employed for the coupling without serious racemization, even though DMT-MM is insoluble in less-polar solvents. No racemization was observed in AcOEt, THF, *N*,*N*-dimethylformamide (DMF), or CH_3CN; slight racemization occurred in dimethyl sulfoxide (DMSO), MeOH, and EtOH. The extent of racemization seemed to increase with increasing solvent polarity. By comparison of dielectric constants, DMSO (ε_r=47), which is the most polar among aprotic solvents examined here, caused 2.3% racemization. Among protic solvents, the extent of racemization could be reduced by conducting the reaction in 2-PrOH whose dielectric constant (ε_r=18) was smaller than that of EtOH (ε_r=24) or MeOH (ε_r=33). Although *N*-hydroxyl compounds such as 1-hydroxybenzotriazole (HOBt) and *N*-hydroxysuccinimide are known to be effective additives for preventing racemization during coupling using carbodiimides, only the addition of HOBt to the reaction conducted in MeOH caused a significant decrease in product yield.

In summary, DMT-MM is a good condensing agent available for peptide fragment coupling as well as for conventional solid phase peptide synthesis. Although most solvents can be used, a less-polar solvent is recommended to prevent racemization in peptide fragment coupling.

Experimental

HPLC analysis was performed on a nacalai tesque COSMOSIL $5C_{18}$-ARII (4.6×150 mm) using a UV detector at 280 nm.

DMT-MM was prepared according to our procedure described previously.[1] Z(OMe)-Gly-L-Ala-OH was prepared according to the literature.[7] H-Gly-L-Ala-OH and H-L-Phe-OBzl·HCl were purchased from Kokusan Chemical Co., Ltd., and used without further purification. Other solvents and chemicals were used as received.

Typical Procedure for Racemization Test To a stirred solution of Z(OMe)-Gly-L-Ala-OH (25 mg, 0.081 mmol), H-Phe-OBzl·HCl (20.6 mg, 0.071 mmol), and Et_3N (8.1 mg, 0.081 mmol) in CH_3CN (1 ml) was added DMT-MM (22.3 mg, 0.081 mmol) dissolved in CH_3CN (1.5 ml) at room temperature. The mixture was stirred at room temperature until H-Phe-OBzl·HCl disappeared (2 h). The resulting mixture was poured into 10% citric acid and extracted with AcOEt. The organic layer was washed successively with $NaHCO_3$ and brine, dried, and concentrated. The product was quantified by HPLC (65% MeOH, 1 ml/min, internal standard: biphenyl).

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5) Kaminski reported that peptide syntheses using CDMT, which involved the formation of acyloxytriazines, which should be the same type of intermediates in our reaction, proceeded with less than 0.5% racemization in aprotic solvents. See: Kaminski Z. J., *Synthesis*, **1987**, 917—920.
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