2-Chloro-4,6-dimethoxy-1,3,5-triazine. A New Coupling Reagent for Peptide Synthesis

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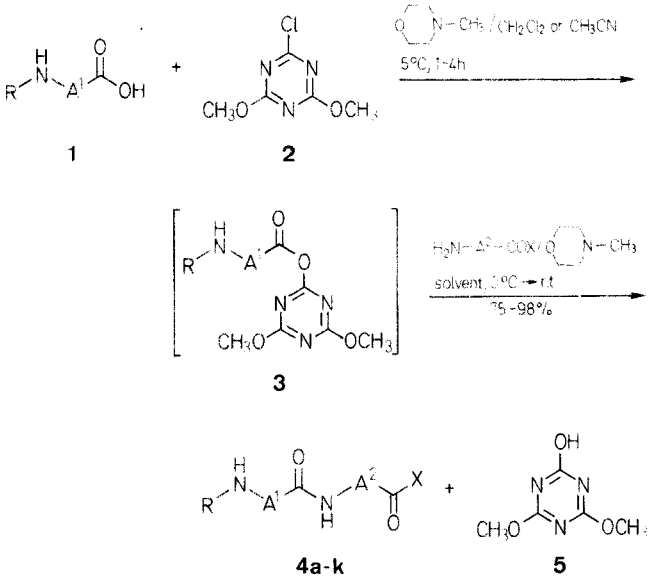
In order to facilitate the purification of peptides, the new coupling reagent 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) is proposed. Due to the weakly basic properties of the triazine ring, the side products and excess coupling reagent are easily removed by washing the crude reaction product with diluted acids. CDMT enables the synthesis of di-, tri-, and pentapeptides in 75–98% yield under mild conditions and without concomitant racemization. Moreover, the reagent diminishes the formation of side products during the incorporation of serine with an unprotected hydroxy group or of *N*-protected ω-nitroarginine into the peptide chain.

Although a large variety of coupling methods has been developed over the last years, further developments in the synthesis of more complex peptides require new reagents[1] in order to avoid the formation of undesired side products[2,3] and to facilitate purification. As a contribution in this field we describe here the use of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, **2**) for the peptide bond formation. The new condensing agent **2** is a crystalline compound, stable on storage, soluble in most of organic solvents, readilly accessible even in large amounts from commercially available and unexpensive cyanuric chloride.[4]

The crucial step of the presented approach is based on the reaction between carboxylic acids and CDMT, which gives entirely new products, the highly reactive 2-acyloxy-4,6-dimethoxy-1,3,5-triazines **3**[5] instead of the expected acyl chlorides.[6] According to preliminary experiments, compound **3** appear to be powerful acylating agent able to acylate alcohols, amines, and carboxylic acids to appropriate esters, amides, and acid anhydrides, respectively, in 60–98% yields.[7]

The preparation of peptides using CDMT proceeds as a sequence of two independent steps in a one-pot synthesis. In the first step, the *N*-protected amino acid or peptide **1** is activated by treatment with stoichiometric amounts of **2** and a base. In the second step, the peptide bond is formed after addition of a *C*-protected amino-acid or peptide directly to the preactivated mixture at −5°C, followed by reaction at room temperature for 14 h.



The best results in the activation stage were achieved by adding *N*-methylmorpholine dropwise to the solution of **1** and **2** in an appropriate inert solvent such as dichloromethane, acetonitrile, or tetrahydrofuran at −5°C. Monitoring of the reaction by TLC showed that formation of the reactive intermediate **3** was usually completed within 1–2 h, except for the sterically more hindered α-methylalanine in which case a reaction time of 3–4 h was required.

The pure protected peptides **4** were obtained after washing the reaction mixture with diluted acids and sodium hydrogen carbonate solution. This convenient and efficient purification is made possible by the weakly basic properties of the 1,3,5-triazine ring. Thus, simple extraction with acid solution simultaneously removes the co-product **5**, any side products, as well as unreacted coupling reagent **2**. It is worthy of note that certain structural features of the potential side products derived from **2**, if any, facilitate their detection by standard analytical methods such as ^{1}H-NMR spectrometry (sharp singlet of two methoxy groups at $\delta = 4.0–4.1$), element analysis (exceedingly high N content), and UV spectroscopy (strong absorption of the triazine ring at $\lambda = 205–207$ nm and 234–236 nm).

Table. Peptides **4** Prepared

	Peptide	Solvent for coupling	Yield[a] (%)	m.p. (°C)	Molecular Formula[b] or Lit. m.p. (°C)	IR (KBr) ν (cm^{-1})	^{1}H-NMR (solvent/TMS) δ, J(Hz)
4a	Z–Phe–Leu–OMe[c]	CH_2Cl_2	80	108–109	109–109.5[8]	3400; 3380; 1735; 1680; 1645; 1535	($CDCl_3$): 0.875 (d, 6H, J = 6); 1.40–1.75 (m, 3H); 3.05 (d, 2H, J = 7.2); 3.7 (s, 3H); 4.25–4.63 (m, 2H); 5.05 (s, 2H); 5.23–5.55 (m, 1H); 6.17–6.47 (m, 1H); 7.15 (s, 5H); 7.30 (s, 5H)
4b	Z–Phe–Ala–OBu^t[c]	CH_2Cl_2	75	104–105	$C_{24}H_{30}N_2O_5$ (426.5)	3400; 3320; 1735; 1710; 1680; 1650; 1540	($CDCl_3$): 1.25 (d, 3H, J = 7.1); 1.41 (s, 9H); 3.01 (d, 2H, J = 8); 4.10–4.57 (m, 2H); 4.95 (s, 2H); 5.40–5.65 (m, 1H); 6.32–6.70 (m, 1H); 7.03–7.20 (m, 10H)
4c	Boc–Ser(OH)–Val–OBzl[c]	CH_2Cl_2	82	53–55	$C_{20}H_{30}N_2O_6$ (394.5)	3350; 2990; 1725; 1690; 1660; 1545; 1520; 1390; 1370	(acetone-d_6): 0.84, 0.91 (2d, 6H, J = 6); 1.37 (s, 9H); 2.15 (m, 1H); 3.66–3.85 (m, 2H); 4.12–4.30 (m, 1H); 4.43, 4.54 (2d, 1H, J = 6); 5.17 (s, 2H); 6.12 (d, 1H, J = 7); 7.37 (s, 5H); 7.62 (br. m, 1H)
4d	Boc–Ser(Bzl)–Val–OBzl[c]	CH_2Cl_2	98	oil	$C_{27}H_{36}N_2O_6$ (484.6)	3300; 2980; 2930; 1730; 1720; 1710; 1670 broad; 1500; 1460; 1445	($CDCl_3$): 0.75, 0.85 (2d, 6H, J = 6); 1.43 (s, 9H); 3.40–4.72 (m, 4H); 4.50 (s, 2H); 5.13 (s, 2H); 5.45 (d, 1H, J = 7); 7.50 (s, 1H); 7.27 (s, 5H); 7.33 (s, 5H)
4e	Z–Ala–Ala–OMe[c]	CH_3CN	94	100–103	104–105[9]	3300; 1740; 1680; 1645; 1545; 1535	($CDCl_3$): 1.35 (d, 6H, J = 7); 3.73 (s, 3H); 4.25 (q, 1H, J = 7); 4.60 (q, 1H, J = 7); 5.11 (s, 2H); 5.48 (br. d, 1H, J = 7); 6.68 (br, 1H); 7.33 (s, 5H)
4f	Z–Aib–Cys(Bzl)–OMe	CH_2Cl_2	78	72–75	$C_{23}H_{28}N_2SO_5$ (444.55)	3300; 1745; 1725; 1690; 1655; 1525; 1450	($CDCl_3$): 1.50 (s, 6H); 2.86 (d, 2H, J = 7); 3.65 (s, 2H); 3.67 (s, 3H); 4.76 (t, 1H, J = 7); 5.05 (s, 2H); 5.45 (s, 1H); 7.20 (s, 5H); 7.25 (s, 5H); 7.56 (s, 1H)
4g	Z–Met–Aib–OMe	CH_2Cl_2	79	oil	$C_{18}H_{26}N_2SO_5$ (382.5)	3300; 1740; 1710; 1680; 1650	($CDCl_3$): 1.56 (s, 6H); 1.25–2.20 (m, 2H); 2.04 (s, 3H); 2.35–2.75 (m, 2H); 3.63 (s, 3H); 2.95–3.46 (m, 1H); 5.05 (s, 2H); 6.70 (d, 1H, J = 8); 7.31 (s, 5H); 7.69 (s, 1H)
4h	Boc–Arg(NO_2)–Ala–OMe[c]	CH_2Cl_2	83	foam	$C_{15}H_{28}N_6O_7$ (404.4)	3350; 1740; 1720; 1670; 1630; 1530	(CD_2Cl_2): 1.40 (d, 3H, J = 7 Hz); 1.41 (s, 9H); 1.60–2.00 (m, 4H); 2.05–2.50 (br. d, 1H); 3.23–3.53 (m, 2H); 3.75 (s, 3H); 4.20–4.60 (m, 2H); 5.75 (br. d, 1H, J = 7); 7.35 (br., 1H); 7.63 (br., 1H)
4i	Z–Tyr(Bu^t)–Aib–OMe	CH_2Cl_2	76	55–60	$C_{26}H_{34}N_2O_6$ (470.55)	3320; 1740; 1700; 1650; 1540	($CDCl_3$): 1.27 (s, 9H); 1.38 (s, 3H); 1.40 (s, 3H); 2.82–3.12 (m, 2H); 3.68 (s, 3H); 4.15–4.50 (m, 1H); 5.07 (s, 2H); 5.33–5.50 (m, 1H); 6.10–6.30 (m, 1H); 6.78–7.27 (m, 4H); 7.30 (s, 5H)
4j	Boc–Gly–Phe–Leu–OMe[c]	CH_2Cl_2	81	113–114	$C_{23}H_{35}N_3O_6$ (449.5) (oil[10])	3300; 3260; 1720; 1660; 1545; 1530	($CDCl_3$): 0.87 (d, 6H, J = 12.9); 1.42 (s, 9H); 1.33–1.63 (m, 3H); 3.04 (d, 2H, J = 7.2); 3.66 (s, 3H); 3.70, 3.76 (AB system, 2H, J = 8); 4.32–4.84 (m, 2H); 5.18–5.44 (m, 1H); 6.49–6.76 (m, 1H); 6.79–7.03 (m, 1H); 7.13 (s, 5H)
4k	Z–Tyr(Bu^t)–Aib–Gly–Phe–Leu–OMe	THF	76	92–93	$C_{43}H_{57}N_5O_9$ (788.0)	3320; 1750; 1670; 1540; 1520	($CDCl_3$): 0.88 (d, 6H, J = 6); 1.33 (s, 15H); 1.44–1.75 (m, 3H); 2.93 (d, 2H, J = 7); 3.04–3.46 (m, 2H); 3.61 (s, 3H); 4.06, 4.26 (AB system, 2H, J = 7.8); 4.42–4.95 (m, 1H); 5.06 (s, 2H); 5.95–6.20 (m, 1H); 6.57–6.75 (m, 1H); 6.75–7.20 (m, 4H); 7.25 (s, 5H); 7.35 (s, 5H)

[a] Yields refer to products recrystallized from EtOAc/petroleum ether or chromatographed on silica gel.
[b] Satisfactory microanalyses obtained: C ±0.30, H ±0.23, N ±0.22, S ±0.10.
[c] The optical purity of these compounds **4** was confirmed by GLC/MS analysis[14,15] 99.5% < L-enantiomeres of Ala, Val, Leu, Phe in hydrolysates were found. GLC column 2.0 mm × 2.5 m; stationary phase: *N*-lauroyl-Val-*t*-butylamide on Supelcoport; helium 20 ml/min; programmed temperature 90–140°C.

The following examples compiled in the Table demonstrate the efficiency of CDMT. They include an [Aib][2] [Leu][5] enkephalin analog obtained in high yield using CDMT at all synthetic stages. Generally, the proposed reagent **2** is efficient in the synthesis of dipeptides **4a–i** as well as in the fragment coupling procedure employed in the preparation of enkephalin **41**. Moreover, its efficiency is not diminished by steric hindrance of the two α-substituents in α-methylalanine.

Encouraging results were obtained in the synthesis of peptides bearing additional functional groups in the side chains of the amino-acid units. Protected functional groups such as hydroxy, mercapto, and guanidino groups were found to be affected neither in the activation nor coupling steps. Further, the unprotected serine hydroxy function did not interfere in the procedure mediated by **2**. Thus, even crude Boc-Ser-Val-OBz (**4c**) isolated from the reaction mixture was not contaminated with *O*-acylation side products. Another advantage of CDMT is demonstrated in the experiment with arginine dipeptide **4h**. It is known that intramolecular cyclization as a side reaction always accompanies the activation of *N*-protected ω-nitroarginine. Its extent depends on the activating agent and in a few cases it has even been found that side products preponderate in the reaction mixture.[11] Interestingly, in the coupling procedure mediated by CDMT the undesired side process was diminished and chromatographically homogeneous arginine dipeptide **4h** was obtained in 83% yield.

The racemization of amino acids in the course of the peptide synthesis by means of **2** was studied by using standard methods as well as by the estimation of the enantiomeric purity of **4**. Under the typical experimental conditions, no racemization was detected neither by the methods of Ref. 12 nor Ref. 13. The high optical purity of the peptides synthesized using CDMT was documented by determination of the enantiomeric composition of amino acids of hydrolyzates of **4**. It was evidenced by GLC on a Chirasil-Val capillary column[14,15] that the extent of racemization caused by hydrolytic degradation and by the synthetic procedure does not exceed 4.2% for peptides **4a–g** presented in the Table. More precise measurements of racemization were possible only for alanine, leucine, valine, and phenylalanine by the method described in the literature.[16] The analysis consists of several steps; 1. degradation of the peptide to amino acids in 6 normal deuterium chloride in D_2O; 2. formation of the volatile *N*-trifluoroacetylamino acid methyl esters; 3. GLC analysis[16] on a column packed with *N*-lauroyl-*L*-valine *tert*-butylamide[17] as a chiral stationary phase, and monitoring of the peaks of non-deuterated $CF_3CO-NH-CHR^+$ by mass spectrometry. This method allowed to deduct the racemization accompanying the hydrolytic degradation of peptides; it showed more than 99.5% optical purities for peptides **4a–e, 4h**, and **4j**.

All m.ps. were uncorrected. IR spectra were recorded on a Spectromom 2000 spectrometer. The 80 MHz ^{1}H-NMR spectra were run on a Tesla BS-487C. Enantiomeric purities of the amino-acids by GLC on a Chirasil-Val stationary phase (glass capillary column 0.3 mm × 21 m, programmed temperature 60–160°C, helium) were performed on a Chrom 5 chromatograph with computing integrator CI-100. GLC–MS analysis were performed on an LKB-2091 spectrometer equipped with a Pye-104 chromatograph. The amino-acids ratio was assayed on a Jeol ILC-6AH analyser.

Synthesis of Peptides 4 Using CDMT; Standard Procedure:

Activation: To a stirred solution of CDMT (**2**; 1.76 g, 10 mmol) and the *N*-protected amino acid (10.2 mmol) in CH_2Cl_2 (10 mL), *N*-methylmorpholine (1.12 mL, 10.2 mmol) is added dropwise at such a rate as to keep temperature at −5 to 0°C, and stirring is continued at 0°C for 1–4 h until all CDMT has been consumed.

Coupling: To the crude solution obtained as described above, a mixture of the appropriate amino acid ester hydrochloride (10 mmol) and *N*-methylmorpholine (1.10 mL, 10 mmol) in CH_2Cl_2 (5 mL) is added at −5 to 0°C. Stirring is continued at 0°C for 2 h, then the mixture is left at room temperature for 14 h. The solvent is evaporated and the residue is suspended in EtOAc (30 mL). This suspension is washed successively with H_2O (10 mL), 10% citric acid solution (10 mL), H_2O (10 mL), saturated $NaHCO_3$ solution (10 mL), and H_2O (10 mL). The organic layer is dried ($MgSO_4$), the solvent evaporated, and the residue recrystallised from EtOAc/petroleum ether.

Z–Ala–Ala–OMe (4e):

To a stirred and cooled solution of Z–Ala–OH (5.88 g, 25.5 mmol) and CDMT (**2**; 4.40 g, 25 mmol) in acetonitrile (75 mL), *N*-methylmorpholine (2.9 mL, 25.5 mmol) is added dropwise at such a rate as to keep the temperature at −5 to 0°C. A white precipitate is formed. The mixture is left at 0°C for 3 h, then a solution of alanine methyl ester hydrochloride (3.55 g, 25 mmol) and *N*-methylmorpholine (2.74 mL, 25 mmol) in acetonitrile (30 mL) is added at 0°C, and stirring is continued at 0°C for 3 h and at room temperature for 14 h. The slurry is then evaporated to dryness, and the solid residue dissolved in EtOAc (50 mL) + H_2O (50 mL). The organic layer is washed successively with H_2O (20 mL), 2 normal hydrochloric acid (20 mL), H_2O (20 mL), saturated $NaHCO_3$ solution (20 mL), and H_2O (20 mL), then dried ($MgSO_4$), and evaporated at reduced pressure. The solid residue is recrystallized from EtOAc/petroleum ether; yield: 7.25 g (94%); m.p. 100–103°C.

Boc–Arg(NO_2)–Ala–OMe (4h):

The coupling of Boc–Arg(NO_2)OH · THF (4.78 g, 12.2 mmol) with alanine methyl ester hydrochloride (2.10 g, 15 mmol) is performed according to the standard procedure. The crude product is poured onto Kieselgel 60 G, Merck in a short column (15 × 5 cm), and eluted with $CHCl_3$/MeOH (1:1), then EtOAc/MeOH (1:1). After evaporation of solvent, dipeptide **4h** is isolated as a foam; yield: 4.3 g (83%).

Z–Tyr(*t*-Bu)–Aib–OH:

A suspension of Z–Tyr(*t*-Bu)–Aib–OMe (**4i**, 9.41 g, 20 mmol) in isopropanol/H_2O (30 mL + 5 mL) is stirred with aqueous 2 normal NaOH (21 mL) for 12 h at room temperature. The resultant clear solution is diluted with H_2O (40 mL), evaporated to the half of its volume, and extracted with Et_2O (1 × 20 mL). The aqueous layer is cooled to 0°C, and acidified to pH 3 with 10% citric acid solution. The white precipitate is isolated by suction, washed with H_2O, dried in a desiccator over P_2O_5, and recrystallized from EtOAc/petroleum ether; yield: 7.85 g (86%); m.p. 138–139°C; $[\alpha]_D^{20}$: −12.6° (c = 1, DMF); R_f: 0.65 ($CHCl_3$/i-PrOH/AcOH 45:5:1).

$C_{25}H_{32}N_2O_6$	calc.	C 65.67	H 7.06	N 6.14
(456.5)	found	65.63	7.03	6.07

IR (KBr): ν = 3400, 3280, 1700, 1630, 1500 cm^{-1}.

H–Gly–Phe–Leu–OMe · HCl

The protected tripeptide Boc–Gly–Phe–Leu–OMe (2.25 g, 5 mmol) is dissolved in a 5 normal solution of dry HCl in EtOAc (25 mL) and this solution is left at room temperature for 20 min. The solvent is evaporated and the oily residue is triturated with Et_2O for crystallization. The crystalline product isolated by suction, washed with Et_2O, and dried; yield: 2.20 g (~100%); m.p. 128–129°C; R_f: 0.58 (BuOH/AcOH/pyridine/H_2O 15:3:10:12).

Z–Tyr–(*t*-Bu)–Aib–Gly–Phe–Leu–OMe (4k):

To a cooled and stirred solution of Z–Tyr(*t*-Bu)–Aib–OH (2.28 g, 5 mmol) and CDMT (**2**; 0.875 g, 5 mmol) in THF (20 mL), *N*-methylmorpholine (0.505 g, 5 mmol) is added at 0°C. The mixture is stirred at 0°C for 4 h, then a suspension of H–Gly–Phe–Leu–OMe · HCl (2.20 g, 5 mmol) and *N*-methylmorpholine (0.505 g, 5 mmol) in THF (20 mL) is added at 0°C. Stirring is continued for 12 h at 0°C and for 24 h at room temperature. After evaporation of solvent, the residue is dissolved in EtOAc (30 mL) + H_2O (30 mL). The organic layer is washed successively with 10% citric acid solution (10 mL), saturated $NaHCO_3$ solution (10 mL), H_2O (10 mL), and saturated NaCl solution (10 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The solid residue is recrystallized from EtOAc/petroleum ether; yield: 3.00 g (76%); m.p. 92–93°C; $[\alpha]_D^{20}$: −25.0 (c = 1, DMF); R_f: 0.46 ($CHCl_3$/i-PrOH, 9:1).

Amino Acid Analysis: Gly 1.03; Aib 1.10; Leu 1.02; Tyr 0.94; Phe 1.00.

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