Pergamon

Tetrahedron Letters 43 (2002) 4161–4165

TETRAHEDRON LETTERS

# New observations on peptide bond formation using CDMT

Christine E. Garrett,* Xinglong Jiang,* Kapa Prasad and Oljan Repič

*Process Research & Development, Novartis Institute for Biomedical Research, One Health Plaza, East Hanover, NJ 07936, USA*

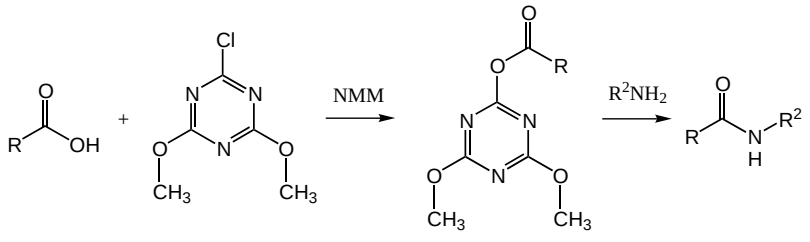
Received 2 April 2002; revised 16 April 2002; accepted 17 April 2002

**Abstract**—The optimized formation of the peptide bond by means of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) has been found to occur rapidly and essentially quantitatively in a one-pot, one-step procedure. This new method is effective for the coupling of a variety of reactive partners, including chiral amino acids (e.g. *N*-acetyl-L-leucine) without significant loss of configuration. Significant racemization was observed when the typical literature conditions were used, due to the formation of an azlactone intermediate which is configurationally unstable under the reaction conditions. A simpler, precipitative workup procedure is also disclosed in this report. 

Due to the need for a variety of diverse peptides, many coupling agents have been developed that facilitate formation of the peptide bond. The desired peptide often contains stereogenic centers and therefore retention of the optical purity of the compound is also of great importance for any method. The use of CDMT as a coupling agent has been investigated fairly extensively, and it demonstrates several advantages over other coupling agents. It is a stable, crystalline compound, with good solubility in organic solvents, and is commercially available in large quantities.[1] These qualities made CDMT attractive for process development in our laboratories. The present communication describes the results of these investigations.

The standard method for making amides using CDMT is to activate the acid using CDMT and a base such as *N*-methylmorpholine. This generates an active ester,[1,2] **1** (Scheme 1), which is subsequently reacted with the amine coupling partner in the same pot. The reaction typically proceeds to completion in a matter of 8–14 h.[1] This method is effective for the formation of a variety of compounds, including esters[3] and Weinreb amides.[4] Workup of the products is typically afforded by extraction with dilute acid, as the CDMT and its triazine by-products are typically weakly basic and easily removed extractively. Analytically pure material is generally isolated via column chromatography,[1] although the crude material is often pure enough to proceed with subsequent reactions.[4]

When we attempted to synthesize **5** (Scheme 2) via the standard two-step CDMT coupling conditions, we observed complete conversion of the amine (**4**) over about 22 h. Surprisingly, the intermediate observed after the pre-activation step was not the expected active ester, but an azlactone (**3**).[5] We theorized that the azlactone could be generated via the intermediacy of



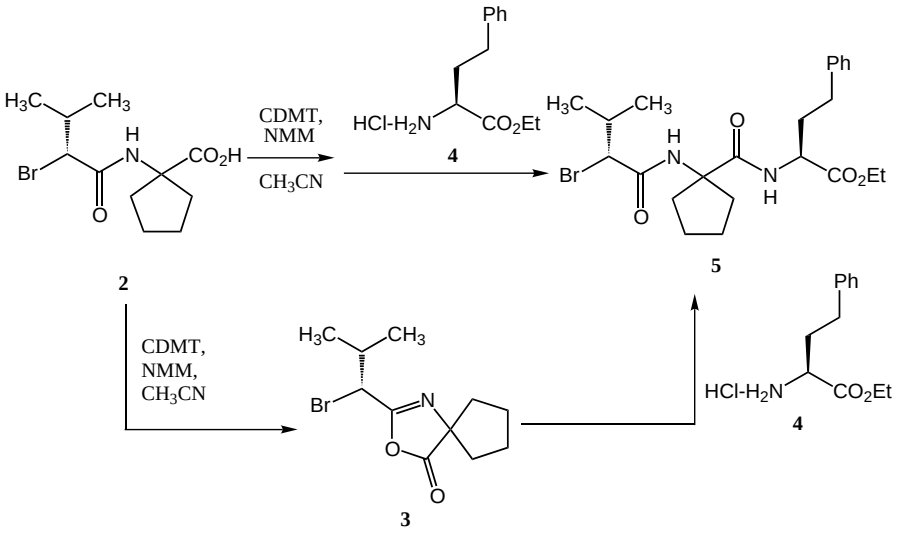

**1**

**Scheme 1.** Typical procedure for two-step peptide bond formation using CDMT.

*Keywords*: CDMT; amides; chiral amides.

* Corresponding authors. E-mail: christine.garrett@pharma.novartis.com; xinglong.jiang@pharma.novartis.com

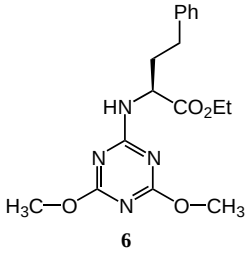
0040-4039/02/$ - see front matter 
PII: S0040-4039(02)00754-2




**Scheme 2.** Synthesis of **5** under standard CDMT coupling conditions.

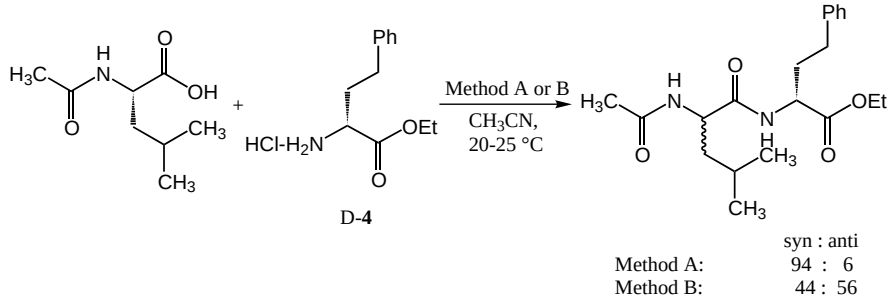
the desired active ester. In addition, we thought that the presence of the amine from the outset of the reaction might intercept the active ester as it is formed and provide for a faster and more direct route to the amide.

Therefore the coupling of **2** and **4** was attempted in one-step instead of two; the CDMT, **2**, **4**, and solvent were mixed together and then the NMM was added. We observed complete conversion of the amine in less than 1 h. A trace of **3** was still observed after all the amine (**4**) had reacted due to the fact that the amine is partially consumed by the formation of a by-product, **6**. The product was purified by extraction and used for the next step of the process. The product is essentially indistinguishable from that produced via the two-step process.




To determine if this one-step method really resulted in a significantly different pathway (i.e. active ester versus azlactone intermediate), we applied this one-step method to a chiral amino acid, e.g. *N*-acetyl-L-leucine, which is typically prone to racemization under standard coupling conditions. As shown (Fig. 1), the coupling of the acid with D-**4** under the one-step conditions generated a product with a diastereomeric ratio of 94:6. The product generated via the two-step method had a diastereomeric ratio of 44:56.[6]

While optimizing the reaction in Fig. 1 using the Surveyor parallel reactor system, we noted that there was a significant concentration, solvent and temperature effect. The reactions were found to generate less impurities when run at higher dilution (<0.10 M of **4**). This was adopted as the standard condition for concentration of the reaction. Retention of stereochemistry is more pronounced in the alkylacetate solvents (Table 1, compare entry 1 with entries 2–4), although there is little difference between the three alkylacetate solvents. Additionally, while there seems to be no effect of the temperature on the amount of racemization observed (compare entries 2, 5, and 6), fewer side-products are observed when the reaction is run at 25°C, and therefore a higher yield is possible.[7]




Method A = CDMT mixed with acid and amine and then NMM added.
Method B = CDMT mixed with acid and NMM, stirred for 1 h, then amine added.

**Figure 1.** One-step versus two-step coupling.

**Table 1.** Optimization of conditions for one-step coupling with CDMT (Fig. 1)[7]

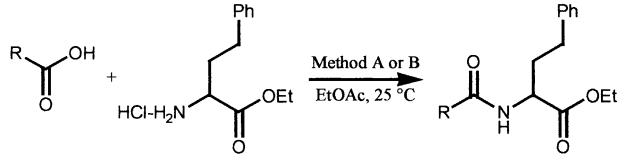
| Entry | Solvent | Temp. (°C) | Diastereomeric ratio |
|---|---|---|---|
| 1 | $CH_3CN$ | 15 | 92:8 |
| 2 | EtOAc | 15 | 98:2 |
| 3 | *i*-PrOAc | 35 | 99:1 |
| 4 | *n*-BuOAc | 35 | 99:1 |
| 5 | EtOAc | 35 | 98:2 |
| 6 | EtOAc | 25 | 99:1 |

A variety of amino acids were then coupled with D- or L-**4** under the optimized conditions[8] (See Table 2). For comparison, the diastereomer ratio achieved under the two-step conditions (Method B) is also shown. All of the amides generated by the one-step method (Method A) were isolated in >84% yield and with >97% purity. The retention of stereochemistry for the *N*-acetyl-L-leucine and *N*-acetyl-L-phenylalanine is significantly improved with the one-step conditions (compare entries 1 and 2; 3 and 4). The Boc-protected amino acid (entry 5) should not racemize under the conditions employed in either method. Compound **5** (entries 7 and 8) is generated under either method in comparable diastereomer ratio, but here the stereocenter is *exo* to the azlactone intermediate so racemization would be more difficult.

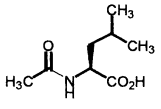
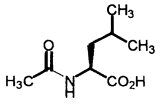
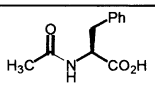
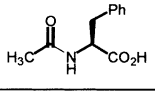
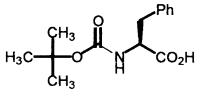
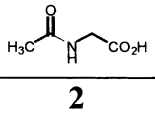
While optimizing the coupling of non-cyclizable substrates, we developed a very simple workup for this reaction.[9] When the one-step coupling of *trans*-cinnamic acid and 2-phenylethyleneamine was run in acetonitrile,[10] it was observed that the product could be precipitated out of the reaction solution simply by the addition of water. The product was isolated in 97% yield and was analytically pure. This one-step coupling method and workup[11] were then applied to the preparation of a number of amides,[12] as shown in Table 3.[13]

Both simple and sterically hindered primary and secondary amines, either aryl or alkyl, couple with acids effectively under these conditions providing good (entries 2, 3, 5, 7 and 8) to high (1 and 6) yields of amide. One notable exception is entry 4, which couples very slowly due to the presence of two sterically hindered groups in close proximity to the coupling site. α,β-Unsaturated acids couple effectively (entry 6) as

**Table 2.** Coupling of amino acids and **4**

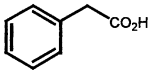
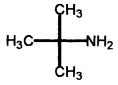
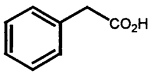
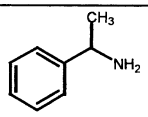
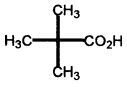
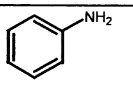
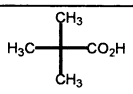
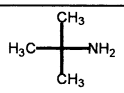
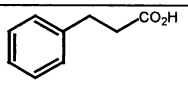
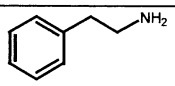
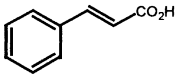
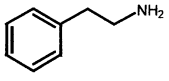
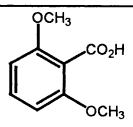
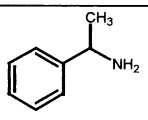
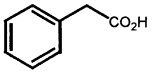
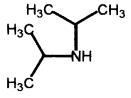
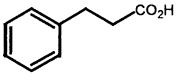
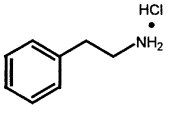
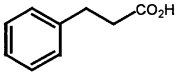
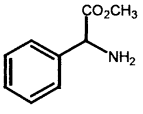
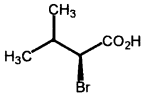
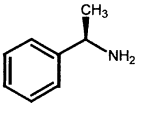
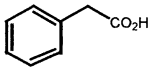
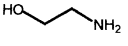



**4**

| Entry | Acid | Amine Config. | Method* | Diaster. Ratio |
|---|---|---|---|---|
| 1 |  | D | A | 99:1 |
| 2 |  | D | B | 44:56 |
| 3 |  | L | A | 98:2 |
| 4 |  | L | B | 34:66 |
| 5 |  | D | A | 100:0 |
| 6 |  | D | A | ---- |
| 7 | **2** | L | A | 99:1 |
| 8 | **2** | L | B | 99:1 |

* Method A = one-step, Method B = 2 step

**Table 3.** Amides prepared by a one-step coupling (in $CH_3CN$) with precipitative workup

| Entry | Acid | Amine | Time (h) | % Yld. |
|---|---|---|---|---|
| 1 |  |  | 1 | 96 |
| 2 |  |  | 2 | 94 |
| 3 |  |  | 4 | 87 |
| 4 |  |  | 48 | 30 |
| 5 |  |  | 2 | 92 |
| 6 |  |  | 1.5 | 97 |
| 7 |  |  | 2 | 92 |
| 8 |  |  | 1 | 96 |
| 9 |  |  | 3 | 80 |
| 10 |  |  | 3 | 90 |
| 11 |  |  | 2 | 92 |
| 12 |  |  | 1.5 | 35 |

well as amine hydrochloride salts (compare entries 9 and 5), although the salts require additional NMM to neutralize the HCl.[11] The coupling reaction where both partners are chiral (entry 11) also proceeds quite well, with retention of configuration at both stereogenic sites. Interestingly, when the amine contains both a hydroxyl and an amine functional group (entry 12), no ester by-product is observed demonstrating that the amine couples exclusively and there is no competition between the two functional groups. The reaction proceeds to completion, but the low yield here is attributed to the solubility of the product in $CH_3CN$/water.

In conclusion, we have demonstrated that a one-pot, one-step procedure for the coupling of acids and amines proceeds effectively with CDMT as the coupling agent. Chiral amino acids with unhindered *N*-substituents can be effectively coupled without loss of configuration. Additionally, when the product is a non-water soluble solid, the reaction can be run in acetoni-

trile and many of the products isolated simply by adding water and recovering the precipitate. The product is typically isolated in >98% purity, and recovery of the product is comparable to the percent conversion achieved in the reaction. This new coupling method and workup therefore represents a much simpler, and in some cases faster, method for the CDMT mediated coupling reaction.

## Acknowledgements

We thank Mr. Guy Yowell (Novartis) for the mass spectral analyses.

## References

1. Kamiński, Z. J. *Synthesis* **1987**, *10*, 917–920.
2. Kamiński, Z. J.; Paneth, P.; Rudziński, J. *J. Org. Chem.* **1998**, *63*, 4248–4255.
3. Kamińska, J. E.; Kamiński, Z. J.; Góra, J. *Synthesis* **1999**, *4*, 593–596.
4. De Luca, L.; Giacomelli, G.; Taddei, M. *J. Org. Chem.* **2001**, *66*, 2534–2537.
5. Compound **3** was prepared by mixing the acid, **2** (24.3 g, 83.2 mmol), with CDMT (21.3 g, 121.3 mmol) in ethyl acetate (400 mL). The slurry was cooled to ca. 15°C and NMM (22.0 mL, 200.1 mmol) was added over about 2 min. The slurry was then warmed to room temperature and stirred. Analysis showed that in less than 10 min, 95% of the acid had reacted. The solution was allowed to stir overnight. The solids were filtered from the solution, and the organic product liquor was washed with 2×250 mL of saturated $NaHCO_3$, 2×250 mL of water, and 1×250 mL of brine. The organic layer was dried over $MgSO_4$, filtered and the solvents removed. The oil was diluted with 150 mL of heptane and the resulting solids were removed by filtration. The product liquor was again stripped resulting in 22.5 g (98.7% yield) of a light-yellow oil: $^1$H NMR ($CDCl_3$): $\delta$ 1.06 (d, $J=6.6$ Hz, 3H), 1.18 (d, $J=6.6$ Hz, 3H), 1.8–2.1 (m, 8H), 2.31 (sextet, $J=6.6$ Hz, 1H), 4.28 (d, $J=8.5$ Hz, 1H), about 1% of an impurity was noted in the spectrum; $^{13}$C NMR ($CDCl_3$): $\delta$ 20.1, 20.5, 25.8, 32.4, 37.9, 38.2, 50.3, 74.5, 161.2, 181.0; MS exact mass, 273.0364; found 274.0218 (M+H). Anal. calcd for $C_{11}H_{16}BrNO_2$: C, 48.19; H, 5.88; Br, 29.15; N, 5.11; O, 11.67. Found: C, 48.65; H, 6.01; Br, 28.96; N, 5.44; O, 10.94%.
6. Although the absolute stereochemistry has not yet been confirmed, it is postulated that the major diastereomer in the one-step reaction is that which has retained the inherent stereochemistry of both coupling partners. Therefore, it appears that racemization of the acid component (via the intermediacy of the azlactone) dominates in the two-step reaction.
7. The reactions described in Table 1 were performed with 0.07 M **4**, 1.1 equiv. of *N*-acetyl-L-leucine, 1.2 equiv. of CDMT, and 3.5 equiv. of NMM for 2 h.
8. General experimental conditions for coupling of simply protected amino acids and an amine via the one-step coupling procedure: The acid (1.8 mmol, 1.1 equiv.), CDMT (1.9 mmol, 1.2 equiv.), and amine (1.6 mmol, 1.0 equiv.) were charged to a kettle. To this mixture of solids was added ethyl acetate (23 mL). The slurry was mixed and NMM (5.8 mmol, 3.6 equiv.) was added over ca. 1 min. The resulting slurry was stirred for 1–2 h and worked-up either extractively or via precipitation (see procedure below). Extractive workup consisted of washing the reaction solution with 2×12 mL of 1N HCl, 12 mL of water, and 12 mL of brine. The product liquor was dried over $MgSO_4$, filtered, and the solvents removed on a rotovap. The resulting material could then be subjected to the subsequent reaction steps.
9. There is a significant difference in the solubilities of the diastereomers of the amides prepared in Table 2. The amides shown in entries 1–5 were able to be isolated by the precipitative method. See Ref. 12 for details.
10. The reaction can be run in a variety of solvents (e.g. THF, methanol, or EtOAc) with $\geq$90% conversion over a 2 h time period. The reaction in water is slower and proceeds to 82% conversion in 2 h.
11. General experimental conditions for one-step coupling in acetonitrile and precipitative workup: The acid (13.8 mmol, 1.0 equiv.), amine (13.8 mmol, 1.0 equiv.), and CDMT (14.5 mmol, 1.1 equiv.) were mixed together. To the mixture was added acetonitrile (15 mL). The slurry was stirred and NMM (20.7 mmol, 1.5 equiv. if amine is free base; 34.5 mmol, 2.5 equiv. if amine is HCl salt) added over about 10 min. The slurry was stirred for 1–2 h. Water (50 mL) was added to the mixture, which initially dissolved the solids and then quickly generated a thick slurry as the product precipitated. The slurry was stirred for 1 h at room temperature, and the solids were isolated by filtration and washing with water (2×10 mL). The product was isolated in >97% purity by this method. This isolation method may also be used for substrates generated in solvents other than acetonitrile, e.g. the products of the simply-substituted amino acids (Table 2). The reaction mixture from these reactions was stripped to remove the ethyl acetate and some of the NMM. The residue was taken up in 4 mL of acetonitrile and warmed to 40°C to help dissolve the solids. To this thin slurry was added water (4 mL) and the slurry warmed to 40°C again. The slurry was stirred while an additional 8 mL of water was added. This quickly generated a thick slurry which was stirred for 1.5 h before filtration. The products were isolated in >84% yield and >97% purity.
12. Weinreb amides were prepared via the one-step method but were difficult to purify completely, as the products were typically oils and could not be precipitated using this method.
13. All products isolated in Table 3 were of high (>97%) purity.