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Suppression of Formation of *N,N′*-Dicyclohexylurea Derivatives During *DCC*-Activation of Proline-Containing Dipeptides

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Summary. The syntheses of dipeptide esters containing a *C*-terminal *L*-proline moiety using carbodiimides as coupling reagents strongly depend on the choice of appropriate conditions. Thus, the use of *DCC* prefers the formation of the undesirable *N,N′*-dicyclohexylurea derivative **3** as a consequence of a CO → N-shift in the *O*-acyl isourea intermediate instead of the desired dipeptide ester **4**. In our hands, only *DIC* was able to yield the desired product exclusively.

Keywords. Carbodiimide; Dicyclohexylurea; Ester synthesis.

Introduction

Since the first report on the utilization of *N,N′*-dicyclohexylcarbodiimide (*DCC*) as a reagent that can effect the formation of peptide bonds in 1955 [1], *DCC* is the most frequently used coupling reagent of the carbodiimide type. In contradiction to this impressive fact, there are several shortcomings of the *DCC* method. The *N,N′*-dicyclohexylurea by-product, while indeed insoluble in most organic solvents (except alcohols) and thus removable by filtration, is not entirely insoluble and therefore it frequently contaminates the product. A more disturbing side reaction is the intramolecular CO → N-shift in the *O*-acyl isourea intermediate yielding an *N*-acylurea derivative as by-product.

Herein, we report on the formation of *N,N′*-dicyclohexylurea derivatives as by-products during the activation of proline-containing dipeptides and on successfull variations of the reaction conditions which result (1) in an increased selectivity in

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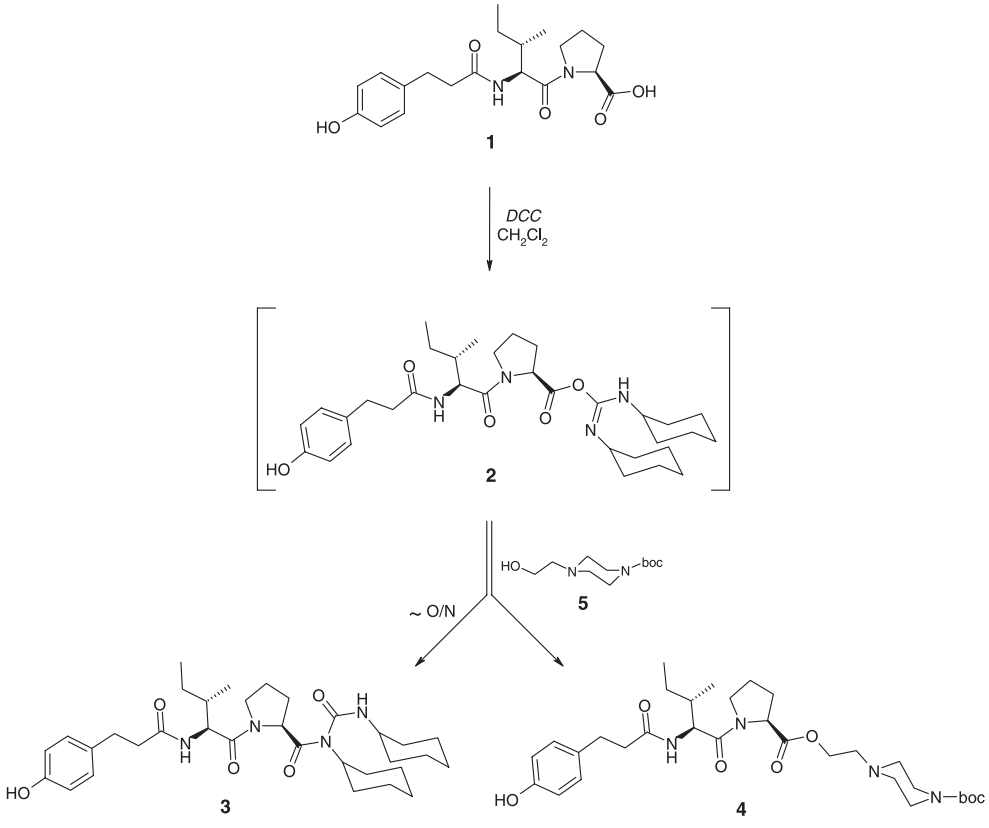
favour of the desired product, and (2) in a complete suppression of the formation of undesirable urea derivatives.

Results and Discussion

In the course of our continuing studies towards the synthesis of cyanopeptide-derived inhibitors of trypsin-like serine proteases [2–4], **1** was synthesised as a common intermediate scaffold of diverse target molecules [5]. Thus, **1** was activated *in situ* by means of *DCC* to the corresponding *O*-acylisourea **2** in order to react with nucleophiles like alcohols or amines (*e.g.* **5**, Scheme 1) to esters **4** or amides.

DCC turned out to be not suitable for catalyzing the coupling reaction effectively, but this statement depends on the reaction conditions. Using the 1-hydroxybenzotriazole (HO*Bt*)/*DMF* protocol, *DCC* exclusively allows the formation of **3** (Table 1) whose proline moiety is not provided with sufficient carbonyl reactivity to react with the alcohol – in contrast to **2**. When compared to the esterification with **5** to give **4**, the CO → N-rearrangement to **3** is the exclusive reaction that takes place. Compared to the desired product **4**, urea **3** possesses excellent crystallization properties.

The use of *DCC*/dichloromethane results in a little satisfying mixture of **3** and **4**. Separation and purification of this mixture by column chromatography requires a high degree of experience and talent.



Scheme 1

Table 1. Influence of reaction conditions on product formation

Conditions	**3** %*	**4** %*
DCC/CH_2Cl_2	19	29
DIC/CH_2Cl_2	0	55
DCC/HO*Bt*/*DMF*	35	0

* Yield related to **1**

The combination of *N*,*N*′-diisopropylcarbodiimide (*DIC*)/dichloromethane in our hands was the most successful variation. The desired product **4** was the only product formed with a yield of 55% and purification by chromatography worked without any problems. The formation of an analogous *N*,*N*′-diisopropylurea derivative was not detectable.

In conclusion, we have shown that the formation of esters of peptide derivatives containing a *C*-terminal *L*-proline moiety using carbodiimides as coupling reagents strongly depends on the choice of appropriate reaction conditions. Predictions concerning the course of the reaction and the suppression of formation of by-products still maintain difficult.

Experimental

General: Melting points are not corrected. IR spectra (KBr): IR spectrometer Perkin-Elmer 1600 series FTIR. NMR spectra: Bruker DPX 300 (300 MHz), solvent: $CDCl_3$, internal standard: *TMS*. Elemental analyses: Perkin-Elmer Elemental Analyzer 2400 CHN, all compounds gave satisfactory elemental analyses. Chromatography: cc: Merck silica gel 60 (0.063–0.200 mm). Optical rotation ($[\alpha]_D$): Polartronic D (Schmidt Haensch GmbH).

Abbreviations of amino acids follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature [6]. Other abbreviations: *Boc: tert.*-Butyloxycarbonyl, *DCC*: *N*,*N*′-dicyclohexylcarbodiimide, *DIC*: *N*,*N*′-diisopropylcarbodiimide, *Et*O*Ac*: ethyl acetate, *PE*: petroleum ether.

1-[N-[3-(4-Hydroxyphenyl)propionyl]-L-isoleucyl-L-prolyl]-1,3-dicyclohexylurea (**3**, $C_{33}H_{50}N_4O_5$)

Colourless crystals, mp: 79–81°C; yield: 19% (*DCC*/CH_2Cl_2), 35% (*DCC*/HO*Bt*/CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.79$ (d, $J = 7.2$ Hz, *Ile*-CH-C$\underline{H}_3$), 0.94 (t, $J = 7.4$ Hz, *Ile*-CH_2-C$\underline{H}_3$), 1.00–2.30 (m, *Pro*-β-C$\underline{H}_2$-γ-C$\underline{H}_2$, *Ile*-β-H, *Ile*-C$\underline{H}_2$-CH_3, 2x C_6H_{11}), 2.32–2.45 (m, *Ph*-CH_2-C$\underline{H}_2$), 2.80–2.87 (m, *Ph*-C$\underline{H}_2$-CH_2), 3.60–3.70 (m, *Pro*-N-C$\underline{H}_2$), 3.80–3.90 (m, *Pro*-N-C$\underline{H}_2$), 4.54–4.64 (m, *Pro*-α-H), 4.78–4.83 (m, *Ile*-α-H), 6.07 (d, NH), 6.73 (d, $J = 8.4$ Hz, $H_{arom.}$), 6.98 (d, $J = 8.4$ Hz, H_{arom}), 7.71 (d, $J = 7.3$ Hz, NH_{urea}) ppm; IR (KBr): 3323, 3040, 2931, 2854, 1708, 1657, 1626, 1516, 1450, 1381, 1342, 1296, 1258, 1227, 1162, 1081, 1050, 987, 893, 831, 642, 534 cm^{-1}; $[\alpha]_D^{23} = +25.66$ ($c = 2$, *Me*OH).

N-[3-(4-Hydroxyphenyl)propionyl]-L-isoleucyl-L-proline 2-[4-(tert.-butyloxycarbonyl)-piperazin-1-yl]ethyl ester (**4**, $C_{31}H_{48}N_4O_7$)

Typical procedure (*DCC*/CH_2Cl_2 and *DCC*/HO*Bt*/CH_2Cl_2 methods were carried out in an analogous way): A solution of 0.56 g of *DIC* (4.40 mmol) in 30 cm^3 of CH_2Cl_2 was added dropwise to a stirred,

ice-cooled mixture of 1.65 g of **1** (4.40 mmol) in 120 cm^3 of CH_2Cl_2 over a period of 20 min and was stirred for one additional hour. Compound **5** (1.11 g, 4.84 mmol) in 70 cm^3 of CH_2Cl_2 was added dropwise over a period of 2 h. While stirring over night, the mixture was allowed to warm up to room temperature. The solvent was evaporated under reduced pressure and the resulting residue was purified by chromatography (silica gel, eluent: CH_2Cl_2:*Et*O*Ac*:*PE*:*Me*OH 10:10:10:1). Yield: 1.42 g (55%) of a colourless substance, mp: 60°C; ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, $J = 7.3$ Hz, *Ile*-CH-C$\underline{H}_3$), 0.95 (d, $J = 6.8$ Hz, *Ile*-CH_2-C$\underline{H}_3$), 1.00–1.15 (m, *Ile*-C$\underline{H}_2$-CH_3), 1.30–1.45 (m, *Ile*-C$\underline{H}_2$-CH_3), 1.46 (s, *t*-butyl), 1.60–1.75 (m, *Ile*-β-H), 1.85–2.25 (m, *Pro*-β-CH_2-γ-CH_2), 2.30–2.50 (m, $OCH_2C\underline{H}_2N$ + $N(C\underline{H}_2CH_2)_2$N-*Boc*), 2.58–2.64 (m, *Ph*-CH_2-C$\underline{H}_2$), 2.79–2.85 (m, *Ph*-C$\underline{H}_2$-CH_2), 3.36–3.44 (m, $N(CH_2C\underline{H}_2)_2$N-*Boc*), 3.50–3.60 (m, *Pro*-N-C$\underline{H}_2$), 3.80–3.90 (m, *Pro*-N-C$\underline{H}_2$), 4.24 (t, $J = 5.9$ Hz, OC$\underline{H}_2$), 4.41 (dd, $J = 8.3$, 3.5 Hz, *Pro*-α-H), 4.82 (dd, $J = 9.0$, 5.2 Hz, *Ile*-α-H), 6.14 (d, $J = 9.1$ Hz, NH), 6.73 (d, $J = 8.6$ Hz, $H_{arom.}$), 6.98 (d, $J = 8.6$ Hz, $H_{arom.}$) ppm; IR (KBr): 3293, 2970, 2934, 2876, 1748, 1698, 1628, 1516, 1453, 1366, 1245, 1171, 1130, 1004, 864, 830, 770 534 cm^{-1}; $[\alpha]_D^{23} = -32.83$ ($c = 2$, *Me*OH).

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