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# Peptide Bond Formation *via* an Intramolecular Rearrangement

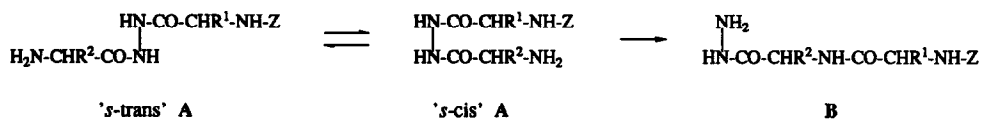
**Jac C.H.M. Wijkmans, Jacques H. van Boom,* and Wim Bloemhoff**

Gorlaeus Laboratories, University of Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

***Abstract:*** *Intramolecular peptide synthesis could be achieved using tetrahydrophthalazine (1) as template. Peptide bond formation proceeded via the N-acyl-N'-α-aminoacylhydrazine-rearrangement. The feasibility of the approach is illustrated by the synthesis of the protected tripeptide Z-Val-Gly-Ala-OMe (8).*

Peptide bond formation usually entails condensation of the free amino function with the activated carboxyl group of an incoming N-protected amino acid. This approach may be accompanied, due to overactivation, by racemization and other side-reactions. In order to circumvent these problems, Brenner[1] proposed in the early sixties the so-called 'low power approach' which combines fast coupling with low-level carboxyl activation. In this approach, the individual amino and carboxyl components are linked to a template and peptide bond formation occurs *via* intramolecular aminolysis.[2] In order to achieve this goal, Brenner *et al.* used hydrazine as template.[3,4] Thus, easily accessible di-acyl hydrazine **A** (Scheme 1) was shown to rearrange *via* an intramolecular nucleophilic attack of the free amino function on the neighbouring hydrazide linkage to give dipeptide hydrazide **B**. This so-called '*N*-acyl-*N'*-α-aminoacylhydrazine-rearrangement' is favoured by a six-membered transition state. Moreover, the use of hydrazine as template allows an iterated process with consequent formation of oligopeptides which, in contrast to the conventional chain elongation at the N-terminus, proceeds *via* extension at the C-terminus.

**Scheme 1**




'*s*-trans' **A** '*s*-cis' **A** **B**

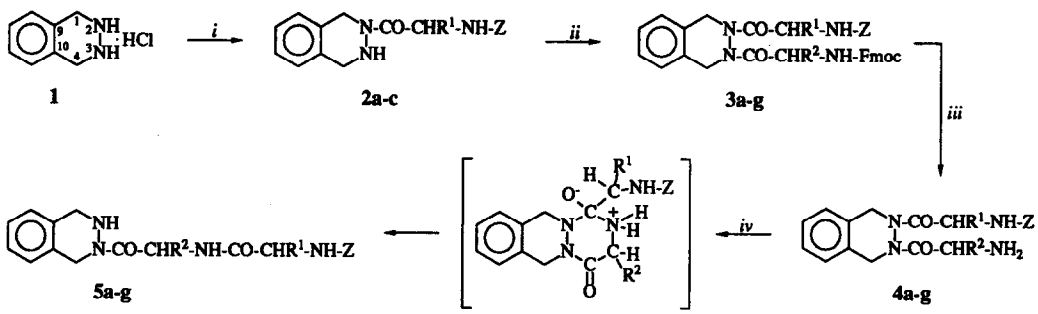
A prerequisite for smooth rearrangement is that the di-acyl compound **A** adopts the '*s*-cis conformation'. However, the presence of the energetically more favourable '*s*-trans conformation' will have an unfavourable effect on the rate of rearrangement.

We now report that the latter disadvantage can be overcome by using the conformationally rigid tetrahydrophthalazine (THPhth)[5] as template for intramolecular peptide synthesis. The viability of this approach will be exemplified by the synthesis of the protected tripeptide Z-Val-Gly-Ala-OMe (**8**).

The requisite HCl salt of THPhth (**1**), prepared according to a slight modification of the procedure

of Carpino,[6] was mono-acylated (Scheme 2) with Z-amino acids by *in situ* activation with benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate[7] (BOP) to yield THPhth derivatives **2a-c**. Condensation of the glycine, alanine, and valine derivatives **2a-c** (*i.e.* $R^1$ = H, Me, or *i*-Pr, respectively) could be effected (entries 1 - 4 in Table 1) using the Fmoc-amino acid chlorides[8] of glycine and alanine in the presence of *N,N*-diisopropylethylamine (DIEA) to afford the di-substituted derivatives **3a-d** in an excellent yield. In contrast, the DIEA-assisted condensation of hydrazide **2a-c** with the Fmoc-amino acid chlorides of (iso-)leucine and valine proceeded unsatisfactory. The latter phenomenon may be explained by the decreased reactivity of these Fmoc-amino acid chlorides and their base-catalyzed conversion into the corresponding less reactive oxazolones. Fortunately, it was found that acylation of **2a-c** with the Fmoc-amino acid chlorides of (iso-)leucine and valine (entries 5 - 7) could be realized using the non-nucleophilic base 2,6-di-*tert*-butylpyridine which minimizes oxazolone formation.[9]

**Scheme 2**




**Conditions:** (*i*) Z-NH-CHR$^1$-COOH, BOP, DIEA, $CH_2Cl_2$, 30 min, 86-88%; (*ii*) Fmoc-NH-CHR$^2$-COCl, DIEA, $CH_2Cl_2$; (*iii*) 40% aq. $HN(CH_3)_2$/THF (1/2, v/v), 1 h; (*iv*) 1.5% AcOH/THF

**Table 1** Relevant data on the synthesis of THPhth derivatives **3**[10] and the rearranged products **5**[11]

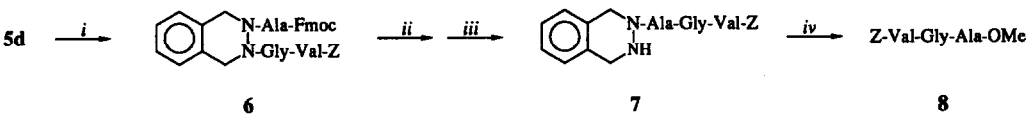
| Entry | 2 | $R^1$ | 3 | $R^2$ | Time (min) | Yield (%) | 5 | Time (min) | Yield (%) |
|---|---|---|---|---|---|---|---|---|---|
| *1* | **2a** | H | **3a** | H | < 5 | 85 | **5a** | 25 | 81 |
| *2* | **2a** | H | **3b** | Me | < 5 | 97 | **5b** | 20 | 85 |
| *3* | **2b** | Me | **3c** | H | < 5 | 83 | **5c** | 45 | 79 |
| *4* | **2c** | *i*-Pr | **3d** | H | < 5 | 86 | **5d** | 9 h (2 h)[c] | 73 (70)[c] |
| *5* | **2a** | H | **3e** | *i*-Pr | 15[a] | 98[a] | **5e** | 10 | 70 |
| *6* | **2b** | Me | **3f** | *i*-Bu | 5[a] (30)[b] | 97[a] (81)[b] | **5f** | 20 | 90 |
| *7* | **2c** | *i*-Pr | **3g** | *s*-Bu | 1.5 h[a] (16 h)[b] | 90[a] (83)[b] | **5g** | 3.5 h | 69 |

[a] In the presence of 2,6-di-*tert*-butylpyridine [b] Without addition of base [c] Conducted at 40 °C

In the next step, the Fmoc-protective group of di-acylated THPhth derivatives **3a-g** was removed by treatment with *N,N*-dimethylamine to provide the amino derivatives **4a-g**. Isomerization of the resulting derivatives **4a-g** could be effected, without prior separation from the dibenzofulvene-dimethylamine adduct,[12] by the catalytic action of acetic acid[13] to furnish the rearranged products **5a-g** (see Table 1) in a yield of 69% to 90%. In most cases, the rearrangement process was complete within 1 h at 20 °C as gauged by TLC analysis. In this respect it is interesting to note that comparable hydrazine derivatives **A** rearranged much slower or not at all.[3] The observed enhanced reaction rates of the THPhth compounds **4a-g** are in agreement with the expectation that fixation of the '*s*-cis conformation' of hydrazide **A** using a cyclic hydrazine accelerates the rearrangement process. The relatively slow rearrangement displayed by valine residues **4d** and **4g** (entries 4 and 7) may be rationalized on steric grounds.

The potential usefulness of the rearrangement procedure is nicely demonstrated by the synthesis of the protected tripeptide **8** (Scheme 3). Thus, the anchored dipeptide derivative **5d** was effectively coupled with Fmoc-Ala-Cl to afford the di-acylated THPhth derivative **6**. Fmoc-deprotection of **6** followed by acetic acid-catalyzed rearrangement yielded the mono-acylated THPhth derivative **7**. Release of the tripeptide from the template by oxidative treatment of **7** with *N*-bromosuccinimide[14] in the presence of MeOH resulted in the isolation Z-Val-Gly-Ala-OMe (**8**)[15] in 57% overall yield from **5d**.

**Scheme 3**




**6** **7** **8**

**Conditions:** (*i*) Fmoc-Ala-Cl, DIEA, $CH_2Cl_2$, 5 min, 94%; (*ii*) 40% aq. $HN(CH_3)_2$/THF (1/2, v/v), 1 h; (*iii*) 1.5% AcOH/THF, 35 min, 91%; (*iv*) NBS, MeOH/$CH_2Cl_2$ (1/1, v/v), 2 h, 67%

In conclusion, the results presented in this paper clearly show that the THPhth-template approach may open the way for the future preparation of peptides by an intramolecular peptide bond forming process. The scope and limitations of this method will be published in due course.

**Acknowledgement**

We wish to thank Mr. A.C. Hoek for his preliminary research on this subject and Mr. A.W.M. Lefeber for recording the 300 MHz NMR spectra.

**References and Notes**

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5. Abbreviations: BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate; DIEA, *N,N*-diisopropylethylamine; Fmoc, 9-fluorenylmethoxycarbonyl; NBS, *N*-bromosuccinimide; THPhth, tetrahydrophthalazine; Z, benzyloxycarbonyl
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10. *General procedure for acylations with Fmoc-amino acid chloride*: To a solution of the appropriate monoacylated THPhth **2** (0.3 mmol) in $CH_2Cl_2$ (3 mL) is added Fmoc-NH-CHR$^2$-COCl (0.45 mmol) and DIEA (78 µL, 0.45 mmol) or 2,6-di-*tert*-butylpyridine (100 µL, 0.45 mmol). After completion of the reaction (TLC analysis), the mixture is diluted with ethyl acetate (100 mL), washed with water (2 x 30 mL), M $KHSO_4$ (2 x 30 mL), water (2 x 30 mL), dried ($MgSO_4$), and concentrated. The residue is purified by silica gel column chromatography to yield **3**. Unambiguous characterization of compound **3** by $^1H$ and $^{13}C$ NMR was hampered due to the existence of several stable conformers. The latter phenomenon was also endorsed by TLC analysis. Pertinent structural information was obtained by electrospray mass spectroscopy.
11. *General procedure for the synthesis of* **5**: A solution of **3** (0.15 mmol) in THF (2.0 mL) is treated with 40% aq. *N,N*-dimethylamine (1.0 mL). After 1 h, the mixture is concentrated and coevaporated with toluene (3 x 5 mL) at room temperature. A solution of the residue in 1.5% AcOH/THF (3.0 mL) is stirred until TLC analysis ($CH_2Cl_2$/MeOH, 19/1, v/v) shows complete disappearance of amine **4**. Evaporation of the solvent with toluene (3 x 5 mL) followed by precipitation or crystallization from $CH_2Cl_2$/light petroleum (b.p. 40-60 °C) affords **5**. Selected analytical data: **5d**: $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.4 - 7.1 (m, 9H, H-arom.), 6.82 (bs, 1H, NH Gly), 5.48 (bd, 1H, NH Val, $J_{H\alpha NH}$ 8.6 Hz), 5.10 (AB, 2H, $CH_2$ Z), 4.75 (s, 2H, H-1), 4.37 (d, 2H, Hα Gly), 4.2 - 4.1 (m, 1H, Hα Val), 4.05 (d, 2H, H-4, $J_{3,4}$ 7.7 Hz), 3.81 (t, 1H, H-3, $J_{3,4}$ 7.7 Hz), 2.2 - 2.1 (m, 1H, Cβ Val), 0.98, 0.93 (2 x d, each 3H, Hγ Val, $J_{\beta,\gamma}$ 6.9 Hz). $^{13}C$ NMR (50.1 MHz, $CDCl_3$): δ 171.2, 169.9 (C=O Gly, Val), 156.3 (C=O Z), 136.3 ($C_q$ Z), 132.8, 130.4 (C-9, C-10), 128.5 - 126.0 (CH-arom.), 66.9 ($CH_2$ Z), 60.2 (Cα Val), 49.1, 43.0, 41.7 (C-1, C-4, Cα Gly), 31.3 (Cβ Val), 19.2, 17.6 (Cγ Val).
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15. The spectroscopic data were in full agreement with the tripeptide derivative synthesized by classical methods.[16] **8**: electrospray MS (*m/z*): 394 $[M+H]^+$, 416 $[M+Na]^+$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.4 - 7.3 (m, 5H, H-arom.), 7.1 (m, 2H, NH Ala, Val), 5.61 (d, 1H, NH Gly, $J_{H\alpha NH}$ 8.4 Hz), 5.08 (AB, 2H, $CH_2$ Z), 4.6 (m, 1H, Hα Ala), 4.2 - 3.9 (m, 3H, Hα Gly, Val), 3.71 (s, 3H, $OCH_3$), 2.1 (m, 1H, Hβ Val), 1.39 (d, 3H, Hβ Ala, $J_{\alpha,\beta}$ 7.2 Hz), 0.97, 0.94 (2 x d, each 3H, Hγ Val, $J_{\beta,\gamma}$ 6.8 Hz). $^{13}C$ NMR (50.1 MHz, $CDCl_3$): δ 173.2, 172.0, 168.4 (C=O Ala, Gly, Val), 156.6 (C=O Z), 136.0 ($C_q$ Z), 128.5, 128.2, 128.0 (CH-arom.), 67.1 ($CH_2$ Z), 60.8 (Cα Val), 52.5 ($OCH_3$), 48.2 (Cα Ala), 43.0 (Cα Gly), 30.8 (Cβ Val), 19.2, 18.0 (Cβ Ala, Cγ Val).
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