Epimerisation-free Peptide Formation from Carboxylic Acid Anhydrides and Azido Derivatives

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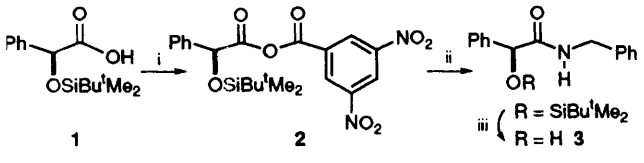
Conversion of *C*-terminal carboxy groups of *N*-protected α-amino acids to the corresponding 3,5-dinitrobenzoyl mixed anhydrides, followed by treatment with α-azido esters and trialkylphosphines, affords good yields of peptides, without appreciable formation of epimers even for reactions involving Phe–Val and Val–Val couplings and/or *N*-benzoyl α-amino acids.

We have been interested in developing new macrolactamisation procedures starting from ω-azido acids.[1] These approaches are potential alternative methods for the formation of any kind of amide bonds in which a precursor of the amino group (an azide) is used instead. As far as peptide-type compounds are concerned, we had assumed these methods would be restricted to the synthesis of natural products containing non-proteinogenic amino acids since, owing to the availability of the most common α-amino acids, there seemed to be no sense in converting them to and storing them as α-azido esters (in order to regenerate an amino-like derivative during the reaction). However, we have more recently realised that, under appropriate conditions, the reaction of carboxylic acids and azides is so simple, and proceeds with so little racemisation or epimerisation, that it may compete with standard peptide couplings when reluctant substrates are involved.

The idea came to us when, in connection with another work, we converted (*S*)-2-(*tert*-butyldimethylsilyloxy)phenylacetic acid **1** (optical purity 98.5%), into its 3,5-dinitrobenzoyl mixed anhydride **2**,[2] and treated it with benzyl azide and tributylphosphine. After deprotection we obtained *N*-benzylmandelamide **3**[3] with an optical purity >97% despite the fact that no special precautions were taken (Scheme 1).

It is known[4–6] that coupling of Z-Gly-L-Phe-OH with L-H-Val-OMe gives the expected tripeptide contaminated with significant percentages of epimer Z-Gly-D-Phe-L-Val-OMe unless either 3,3′-(chlorophosphoryl)bis(1,3-oxazolidin-2-one) [usually called *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)] and 1-hydroxybenzotriazole (HOBt),[4] or 2-trifluoroacetylthiopyridine and NaOBt,[5] or certain tetramethyluronium salts and HOBt[6] are used. For the sake of comparison, we activated the carboxy group of Z-Gly-L-Phe-OH (1.0 mmol) as a mixed anhydride by treatment with 3,5-dinitrobenzoyl chloride and Et_3N (1.0 mmol of each) in anhydrous THF at 0 °C for 2 h and, after filtering off the precipitate of $Et_3NH^+Cl^-$ under N_2 and removing the solvent (cold, *ca.* 1 Torr), the residue was treated with equimolar amounts of methyl (*R*)-2-azido-3-methylbutanoate (the azide analogue of methyl D-valinate, prepared from L-valine in four steps)[7] and Bu_3P (1.2 mmol) in anhydrous benzene (5 ml) at *ca.* 5 °C overnight. Standard workup† afforded a 87% overall yield of pure Z-Gly-L-Phe-D-Val-OMe (d.e. > 99%).‡,§



Scheme 1 *Reagents and conditions*: i, 3,5-dinitrobenzoyl chloride, Et_3N, THF, room temp., 2 h; ii, $PhCH_2N_3$ (1.0 equiv), Bu_3P (1.2 equiv), reflux, benzene, 1 h; iii, $Bu_4N^+F^-\cdot nH_2O$, THF, room temp. (70% overall yield).

Substrates showing a very high tendency to racemise such as the *N*-benzoyl-protected amino acids (Bz-AA_1-OH), which are seldom used in practice because of this well-known problem, were treated with either the D- or L-α-azido ester structurally related with methyl D- or L-valinate, respectively, following the same protocol. The results are summarised in Table 1.

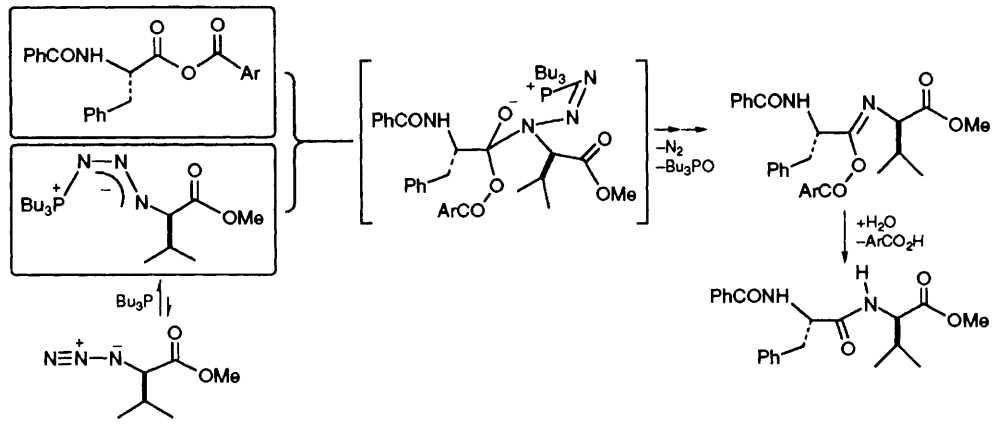
It can be seen that the less polar solvents afford the lower percentages of epimer(s), other factors being almost irrelevant.¶ Comparison of the above results with those reported for the preparation by standard procedures of Bz-Phe-Val-OMe (epimer percentages between 11 and 44%)[6] and Bz-Val-Val-OMe (epimer percentages between 14 and 62%, with one exception)[8] suggests that the present approach is indeed competitive. The exception (≤2%) concerns the case in which 7-aza-1-hydroxybenzotriazole (HOAt), the additive of choice very recently introduced by Carpino,[8] was used together with DCC in CH_2Cl_2 (after neutralisation of H-Val-OMe·HCl and extraction).

Since we have independently demonstrated that, at room temperature or below, the reaction of carboxylic anhydrides with simple azides and phosphines takes place *via* phosphatriazene intermediates,[9] the steps involved in the formation of Bz-L-Phe-D-Val-OMe may be assumed to be as shown in Scheme 2. The advantage of this approach lies in the absence of bases during the coupling reaction; in fact, neither amino groups are present nor tertiary amines must be added to prevent the

Table 1 Conversion of Bz-AA_1-OH to Bz-AA_1-AA_2-OMe

Entry	Bz derivative (AA_1)	Activation	Azido ester (AA_2)	Reaction conditions	Yield (%)	Epimer (%)	D.e. (%)
1	L-Phe	Et_3N, ArCOCl[a]	D-Val	Bu_3P, benzene, 5 °C, 15 h	87	1.3	97.4
2	L-Phe	Et_3N, ArCOCl	L-Val[b]	Bu_3P, benzene, 5 °C, 15 h	85	1.2	97.6
3	L-Phe	Et_3N, ArCOCl	D-Val	Bu_3P, toluene, 5 °C, 15 h	85	3.9	92.2
4	L-Phe	Et_3N, ArCOCl	D-Val	Bu_3P, CH_2Cl_2, 5 °C, 15 h	89	4.3	91.4
5	L-Phe	Et_3N, ArCOCl	D-Val	Bu_3P, THF,[c] 5 °C, 15 h	82	4.5	91.0
6	L-Phe	Et_3N, ArCOCl	D-Val	Bu_3P, DMF, 5 °C, 15 h	78	5.7	88.6
7	L-Phe	Et_3N, ArCOCl	D-Val	Bu_3P, benzene, 20 °C, 15 h	83	2.4	95.2
8	L-Phe	Et_3N, ArCOCl	D-Val	Me_3P, benzene, 5 °C, 15 h	87	0.9	98.2
9	L-Phe	$EtPr^i_2N$, ArCOCl	D-Val	Bu_3P, benzene, 5 °C, 15 h	83	1.0	98.0
10	L-Val	Et_3N, ArCOCl	L-Val	Bu_3P, benzene, 5 °C, 15 h	84	1.8	96.4

[a] 3,5-Dinitrobenzoyl chloride. [b] Methyl (*S*)-2-azido-3-methylbutanoate was prepared from D-valine in the same way as its enantiomer was obtained from L-valine (ref. 7). [c] After the activation step (in THF), the solvent was not removed; when the reaction was performed in one pot, without filtering off the $Et_3NH^+Cl^-$ precipitate, the epimer(s) percentage rose to 28%.



Scheme 2 Ar = 3,5-dinitrophenyl

protonation of the amino group of the remaining starting material (by HX produced during the reaction between RCOX + NH_2R, as occurs usually in the standard methods). Additional experiments confirmed how detrimental even a small excess of base would be: (*i*) when 1.5 mmol of Et_3N was used in entry 1 rather than 1.0 mmol, the dipeptide yield was similar but the d.e. fell to 42.6%; (*ii*) when Bz-L-Phe-D-Val-OMe was mixed with 1 mmol of Et_3N under the conditions of entry 1, epimerisation (presumably to LL-dipeptide) took place in 6.2%.

In summary, activation of a carboxy group as a mixed anhydride, as effected in some macrolactonisation methods,[10] is a suitable approach to peptide bond formation when α-azido esters and trialkylphosphines are used as counterparts: the attack of phosphatriazene intermediates to the mixed anhydride is regioselective (as no attack at the COAr moiety is detected), the reaction medium is essentially neutral (see Scheme 2), no special additives are needed, and it seems amenable to further improvements (*e.g.* by controlling the mixed anhydride formation step, where R_3N and $R_3NH^+Cl^-$ co-exist with the substrate most susceptible to undergo racemisation). The utility of this methodology is however limited at present to the cases mentioned in the introduction.

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Footnotes

† Evaporation of the solvent under reduced pressure, addition of AcOEt, washing (water), drying and concentration of the organic solution, followed by separation of the desired product from $Bu_3P{=}O$ by 'flash' column chromatography on silica gel (6:3:1 AcOEt–hexane–AcOH).

‡ As shown by HPLC (Spherisorb S3W SiO_2, 25 × 0.4 cm, 2:1 hexane–AcOEt, t_R = 9.6 min). Internal standards of its diastereoisomers (t_R = 8.4 min), independently prepared, confirmed this result.

§ Similarly, we obtained Boc-L-Val-D-Val-OMe from Boc-L-Val-OH and (*R*)-2-azido-3-methylbutanoate in 86% overall yield, without concomitant formation of epimer(s), as checked in the crude product by ^1H NMR (300 MHz).

¶ However, use of Me_3P (see entry 8) or Et_3P instead of Bu_3P simplifies the workup: the coproducts $Me_3P{=}O$ or $Et_3P{=}O$ are eliminated from the organic solution by washing with water, so that no column chromatography is required. When the experiments of entries 1 and 10 were repeated by adding 1.2 equiv. of DMAP after the activation step, the percentage of epimers rose to 26.4 and 15.5%, respectively; thus, the presence of nucleophilic bases is clearly detrimental, as it would be expected.

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