

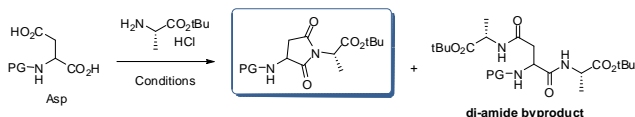
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Introduction

Due to their importance in many biological functions, bioactive peptides are interesting starting points in drug discovery. The incorporation of a cycle in a peptidic sequence is a common approach to constrain peptides into their bioactive conformation. The preparation of imides by cyclisation of aspartic or glutamic carboxylic acid side chains with an amide backbone is scarcely described^{1,2}. Moreover, the C-N bond formation on aminosuccinimide or aminoglutaramide derivatives has never been reported to our knowledge. This poster will outline the different strategies devised and applied to overcome reactivity, selectivity and epimerisation issues in the synthesis of aminosuccinimide and aminoglutaramide containing peptides.

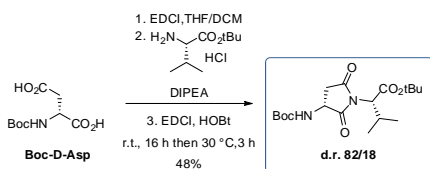
1. Preparation of imide by cyclisation of *N*-protected aspartic acid



Entry	Di-acid	Solvent	Conditions	Y (%)	d.r. (NMR)
1	Cbz-D-Asp	DCM	1. DIPEA (1.5 eq.), EDCI (1.25 eq.), 2 h 2. Ala-OtBu, DIPEA (1.5 eq.), 1.5 h 3. EDCI (1.25 eq.), HOBt (1.1 eq.), 16 h	17	50/50
2	Boc-D-Asp	DCM	1. EDCI (1.25 eq.), 20 min 2. Ala-OtBu, DIPEA (2.0 eq.), 2 h 3. EDCI (1.3 eq.), HOBt (1.5 eq.), 16 h	58	>95 / 5
3	Boc-L-Asp	DCM	1. Ala-OtBu, HOBt (2.2 eq.), EDCI (1.2 eq.), DIPEA (3.0 eq.), 0.5 h 2. EDCI (1.3 eq.), 4 h	9	>95 / 5
4	Boc-L-Asp	DCM	1. EDCI (1.25 eq.), 20 min 2. Ala-OtBu, DIPEA (1.0 eq.), 2 h 3. EDCI (1.3 eq.), HOBt (1.5 eq.), 16 h	12	>95 / 5
5	Boc-L-Asp	DCM	1. EDCI (1.1 eq.), 16 h 2. Ala-OtBu, DIPEA (1.0 eq.), 4 h 3. EDCI (1.5 eq.), HOBt (1.5 eq.), DIPEA (1.0 eq.), 16 h	48	~90 / 10
6	Boc-L-Asp	THF	1. DCC (1.05 eq.), 16 h 2. Ala-OtBu, DIPEA (1.0 eq.), 4 h 3. DCC (1.5 eq.), HOBt (1.5 eq.), DIPEA (1.0 eq.), 16 h	84	>95 / 5

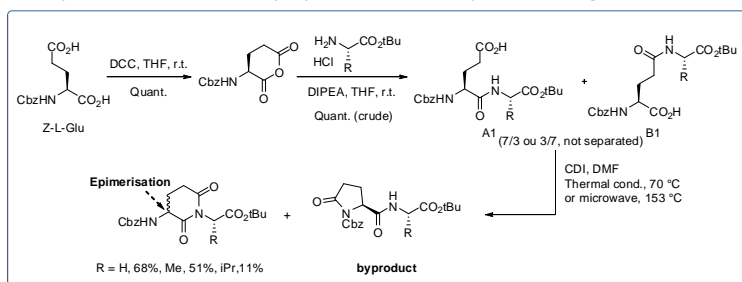
- Low yield of cyclisation and complete racemisation were observed with Cbz-D-Asp whereas a good yield of cyclisation and very low epimerisation were achieved with Boc-D-Asp.
- Sequential addition of the reactants allowed us to avoid di-amide formation.
- The use of the combination of DCC, THF and DIPEA led to high yields of product with limited epimerisation.

2. Preparation of Boc-D-Asp analog with Valine residue



- Cyclisation with a Valine residue proved to be more difficult. The increase in temperature to complete the cyclisation reaction could account for a higher degree of epimerisation.

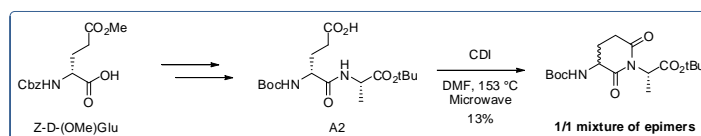
3. Synthesis of imide by cyclisation of *N*-protected glutamic acid



- As for Cbz-D-Asp, full epimerisation was observed and harsher conditions were needed to achieve cyclisation.
- The regioselectivity determined by the relative bulk of side chains of the flanking residue led to C5 byproduct. Its proportion increases with the bulkiness of the amino acid residue (Glycine < Alanine < Valine).

References : 1. M. Angels Estiarte and al. *Tetrahedron*, **1999**, *55*, 3073-3075
2. Nazar Flaih and al. *Synlett*, **2000**, *6*, 896-898.

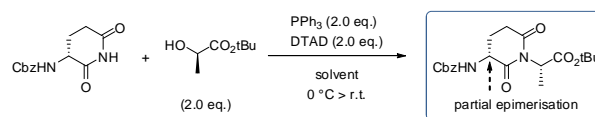
The hypothesis envisaged would involve a favored epimerisation on isomer B. So, a second approach to the 2,6-piperidinedione analogue was investigated starting from Z-D-(OMe)Glu, by activation of CO₂H group of the lateral chain and use of a Boc protecting group.



Unfortunately, the cyclisation reaction gave a low yield of a 1/1 epimeric mixture.

4. C-N bond formation on *N*-Cbz-L-2,6-piperidinedione

4.1 Via Mitsunobu reaction

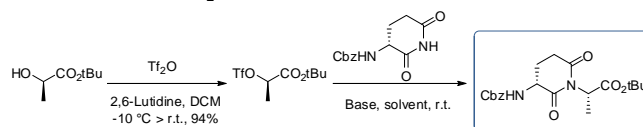


Entry	Solvent	[c]	Time (h)	Y (%)	d.r. ^(a)
1	Toluene	0.10 M	1	72	71/29
2	THF	0.12 M	22	67	54/46
3	DCM	0.19 M	3.5	78	56/44
4	Toluene/ DCM (4 / 1)	0.15 M	24	44	73/27 to 96/4

(a) Determined by chiral HPLC

- The results showed that the Mitsunobu approach lacks reproducibility.
- Depending on the reaction conditions, mixture of epimers with variable ratios were obtained.

4.2 Via triflate SN₂ substitution



Entry	Base	Solvent	Time (h)	Y (%)	d.r. ^(a)
1	K ₂ CO ₃ (1.3 eq.)	MeCN	18	99	86 / 14
2	K ₂ CO ₃ (1.05 eq.)	MeCN	24	53 ^(b)	98 / 2
3	KHCO ₃ (1.05 eq.)	MeCN	48	No conversion	-
4	K ₂ CO ₃ (1.05 eq.)	DCM	22	69 % conversion A lot of impurities formed	-
5	K ₂ CO ₃ (1.3 eq.)	MeCN	30	46	98 / 2
6	K ₂ CO ₃ (1.3 eq.)	MeCN	17	47 (+ 15% acid form)	96 / 4

(a) Determined by chiral HPLC (b) Reaction conducted at 0 °C then at r.t.

- The degree of epimerisation could be controlled and maintained < 5%.
- Partial hydrolysis of tBu ester was observed during work-up.

Conclusion

We have developed three different strategies to overcome selectivity and epimerisation issues in the synthesis of aminosuccinimide and aminoglutaramide containing peptides. The screening of various conditions for the cyclisation led to improved overall yield and reproducibility of the cyclisation reaction. The direct substitution of enantiopure triflate was successfully achieved on the glutaramide moiety with control of the racemisation of the stereocenters.