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MONO-ESTERIFICATION OF N-PROTECTED DI-ACIDS ASPARTIC AND GLUTAMIC BY CHLOROFORMATE ACTIVATION.

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Summary : Mono-esters of N-protected di-acids aspartic and glutamic are prepared by a one-pot activation with alkyl chloroformates or isopropenyl chloroformate and an additionnal alcohol. This process involves the intermediate internal anhydride formation.

With respect to the mono-esterification of N-protected aspartic **1** or glutamic diacid **2**, the most suitable reaction already described was a multi-steps procedure that involved the partially regioselective opening of the preformed internal anhydride **3** or **4**, leading predominantly to the α-isomer **7α** or **8α** [1]. The internal anhydride was mostly prepared from acetic anhydride [2] or carbodiimide [3] activation of the N-protected di-acid. The use of chloroformates for di-acids activation has not been investigated so far, although activation of N-protected amino acids with chloroformates has been extensively studied [4], including esterification reactions [5]. We report in this paper the one-pot preparation of mono-esters of N-protected aspartic and glutamic acids, by alkyl-chloroformate activation. In this process, the ester formed corresponds to the chloroformate used and is limited by the accessibility of the reagent [5]. We demonstrated in a previous communication, the efficiency of isopropenyl chloroformate activation for the esterification of N-protected aminoacids by alcohols [6]. We show herein that the isopropenyl chloroformate activation in the presence of an alcohol also prouved to be suitable for mono-esterification of di-acids.

The mono-esterification was obtained by adding one equivalent of the chloroformate to the di-acid **1** or **2** neutralized with two equivalents of base (pyridine, triethylamine with or without a catalytic amount of DMAP) in dichloromethane solution, at 0° C. The mono-esterification occured directly from alkyl-chloroformates (method A) or with an additional equivalent of alcohol in the case of IPCF activation (method B). The mono-esters **7** or **8** were purified by aqueous basic extraction of the methylene chloride solution after aqueous acidic washing : the mixture of mono-esters was then extracted from acidified

Table : Mono-esterification of N-Z-Asp and N-Z-Glu.

entry	compound	ClCOOR	ROH	base	time	α / β or γ	isolated ester (lit.)	yield	mp °C	$[\alpha]_D$ c 1, EtOH
	di-acid									
1	Z-Asp	R = Me	none	Pyr	24h	85/15	ZAsp(OH)OMe,Dcha (a)[7]	62%	159-160	+5.0
2	—	IPCF	MeOH	Pyr	18h	80/20	—	60%	159-160	+4.9
3	—	IPCF	MeOH	TEA	2h	75/25	—	56%	—	
4	—	IPCF	MeOH	TEA/DMAP	2h	78/22	—	60%	—	
5	—	R = Bzl	none	TEA	1h	80/20	ZAsp(OH)OBzl (b)[7]	58%	85-86	-17.0
6	—	IPCF	BzlOH	TEA	2h	76/24	—	57%	85-86	-17.3
7	—	IPCF	cetyl.	TEA	2h	*	ZAsp(OH)Ocetyl	64%	62-63	-11.4
8	—	IPCF	tBuOH	TEA/DMAP		70/30	ZAsp(OH)OtBu,Dcha (c)[8]			
9	Z-Glu	R = Me	none	TEA	2h	67/33	ZGlu(OH)OMe,Dcha (d)[7]	41%	175-176	-11.2
10	—	IPCF	MeOH	TEA	2h	66/34	—	40%	174-175	-10.9
11	—	R = Me	none	TEA/DMAP	1h	10/90	ZGlu(OMe)OH,Dcha (e)[7]	63%	152-153	+9.2
12	—	IPCF	MeOH	TEA/DMAP	2h	12/88	—	60%	151-152	+9.4
13	—	IPCF	cetyl.	TEA/DMAP	2h	*	ZGlu(Ocetyl)OH	68%	58-59	-6.5
	anhydride									
14	Z-Asp	none	MeOH	TEA	2h	80/20				
15	—	none	MeOH	TEA/DMAP	1h	74/26				
16	Z-Glu	none	MeOH	TEA	2h	85/15				
17	—	none	MeOH	TEA/DMAP	2h	16/84				

* non evaluated.

Data references : (a) mp 159-160 °C, $[\alpha]_D$ +4.9 (c 1 MeOH) ; (b) mp 84 °C, $[\alpha]_D$ -9.3 (c 1.2 AcOH) ; (c) mp 102-104 °C, $[\alpha]_D$ -5.0 (c 2 95% AcOH) ; (d) mp 171-173 °C, $[\alpha]_D$ -10.6 (c 1.53 MeOH) ; (e) mp 149-151 °C, $[\alpha]_D$ +10.1 (c 1 EtOH).

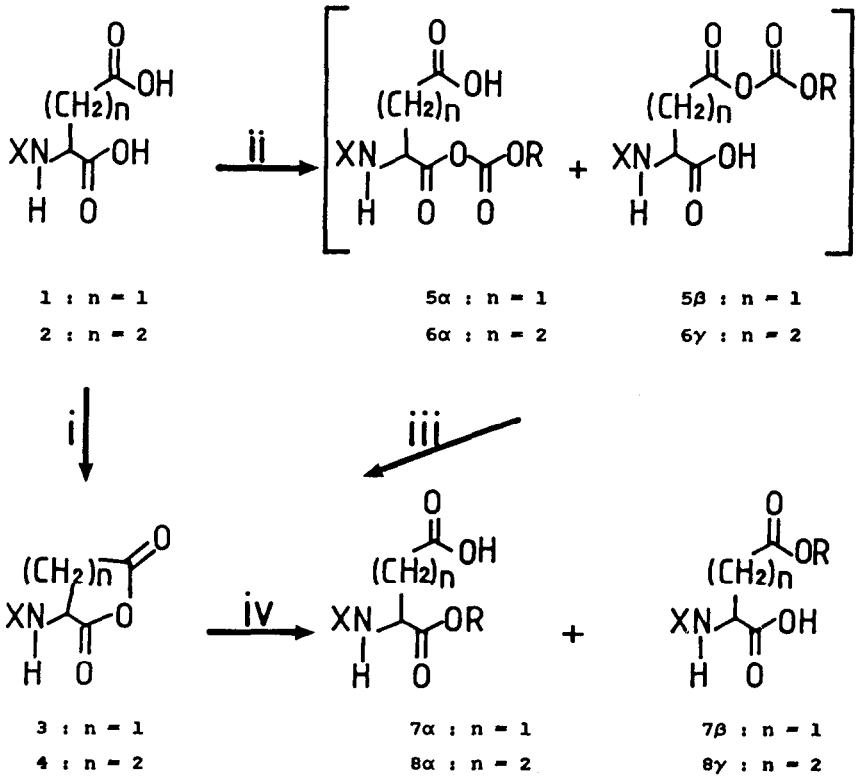
aqueous solution. The isomeric ratio was quantified by n.m.r..

The α-isomer of aspartic ester **7** was isolated, either as its di-cyclohexylamine salt precipitated from the crude mixture, or by column chromatography. The α and γ-isomers of glutamic esters **8** were separated on a silicagel column. The results of these experiments are listed in the table.

The following points emerged from these data : first, both methods (A and B) were comparable in terms of reaction time, total and relative yields of mono-esters formed (table, compare entries 1 and 2 ; 5 and 6 ; 9 and 10 ; 11 and 12). Moreover, the use of isopropenyl chloroformate was of particular interest when an unusual ester has to be prepared, thus preventing the preparation of the corresponding chloroformate. To illustrate this point, IPCF activation has been successfully used for the preparation of mono-esters with lipophilic properties (table, entries 7 and 13) as well as mono tertiobutyl ester (table, entry 8). Secondly, addition of DMAP largely favoured the mono-esterification of glutamic acid to the γ-position (table, entries 11, 12 and 13) whereas the general regioselectivity leading to the (7α) -ester was observed for the aspartic acid in the same conditions (entries 4 and 8).

The activation of the N-protected di-acids with chloroformates was supposed to provide mono-esterification *via* the internal anhydride formation.

Scheme : *Mono-esterification of N-protected Asp and Glu.*



1 : n = 1 ; 2 : n = 2

5α : n = 1 ; 6α : n = 2 ; 5β : n = 1 ; 6γ : n = 2

3 : n = 1 ; 4 : n = 2

7α : n = 1 ; 8α : n = 2 ; 7β : n = 1 ; 8γ : n = 2

i) DCC or Ac_2O ; ii) ClCOOR, base ; iii) $-CO_2$, - ROH (R = alkyl) or - acetone (R = isopropenyl) ; iv) ROH.

The internal anhydride arises from decomposition of either or both mixed anhydrides **5α** and **5β** (respectively **6α** and **6γ**) which could not be isolated in our reaction conditions. The alcohol liberated reacted with the so formed internal anhydride. However, when isopropenyl chloroformate was used, the mixed anhydrides decomposed with liberation of acetone instead of alcohol ; thus addition of an alcohol led to the expected corresponding esters **7** or **8**.

To support this pathway, we have been able to isolate internal anhydride **3** from N-Z-aspartic acid, in moderated yield, when activation with IPCF was performed in the absence of alcohol. To ascertain the formation of this intermediate **3** or **4** we reinvestigated the reactivity of the internal anhydride towards alcohol in our experimental conditions. We prepared respectively the internal anhydride of N-Z-glutamic acid and N-Z-aspartic acid by the usual DCC activation. We examinated by n.m.r. the ratio of **7α** or **8α** *versus* **7β** or **8γ** mono-ester formed when this internal anhydride was opened in the presence of methanol, according to the nature of the base added (table, entries 14, 15, 16 and 17). The observed regioselectivity in these conditions was identical to the one obtained when N-protected Asp or Glu was reacted with methyl chloroformate as well as with IPCF and methanol (table, entries 3, 4, 9, 10, 11 and 12) ; we noticed the high proportion of Glu-γ-ester **8γ** formed when the anhydride **3** was open in the presence of DMAP (table, entry 17), as well as by direct chloroformate activation with DMAP catalysis (table, entries 11, 12 and 13) ; these results support also the internal anhydride formation in this latter process.

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