Direct Synthesis of Polybenzylated Glutamic Acid Monoesters: Disambiguation of *N*,*N*-Dibenzylglutamic Acid α- and γ-Benzyl Esters

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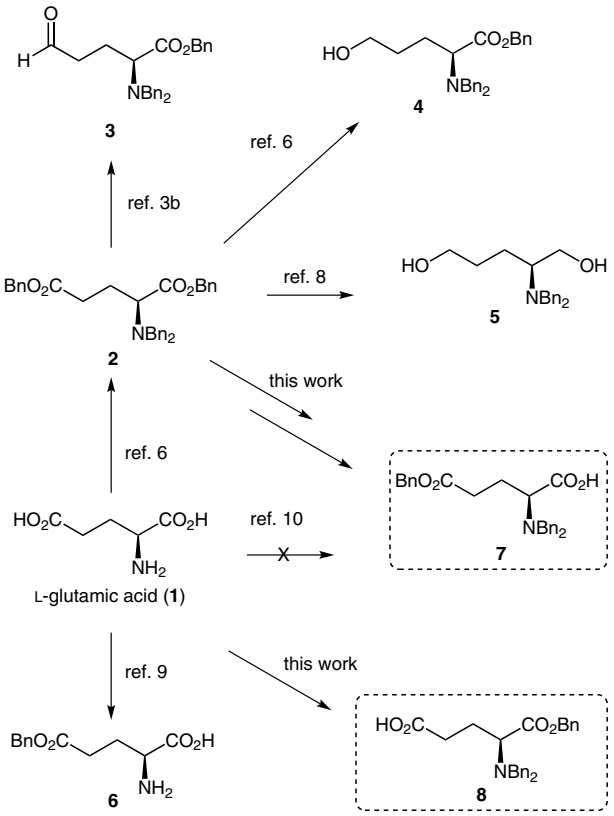
Abstract: In this study, we explored the regioselective benzylation of L-glutamic acid under substoichiometric amounts of the alkylating agent. Our results demonstrate unambiguously that *N*,*N*-dibenzylglutamic acid γ-benzyl ester was not obtained by direct benzylation of L-glutamic acid as reported by other authors. Instead, under such reaction conditions *N*,*N*-dibenzylglutamic acid α-benzyl ester was obtained.

Key words: amino acids, benzylation, disambiguation, protecting groups, regioselectivity

Amino acids represent a natural source of enantiomerically pure building blocks useful in organic synthesis. In this particular context, L-aspartic and L-glutamic acids are potentially valuable providing that the two carboxyl groups could be differentiated. Within our program directed at the discovery of biologically active sphingosine analogues,[1] we have developed general methodologies for the synthesis of saturated[2] and unsaturated[3] lipidic α-amino acids. The latter method is based on the regioselective ω-reduction of *N*,*N*-di-Boc-aspartic or glutamic dialkyl esters. The resulting semialdehydes have proven to be versatile intermediates in the synthesis of biologically relevant substances.[4]

In addition to Boc, other suitable amino protecting groups have been explored extensively, from which the benzyl group remains as highly useful in synthetic organic chemistry.[5] In the particular case of L-glutamic acid (**1**), methods have been reported to prepare diverse *N*,*N*-dibenzylamino acid derivatives (Scheme 1). Thus, the perbenzylation of L-glutamic acid (**1**) with excess of BnBr under basic conditions is a commonly used process to obtain derivative **2** in 61–96% yield.[6] The regioselective reduction of perbenzylated L-glutamic acid **2** affords the corresponding γ-aldehyde of *N*,*N*-dibenzylglutamic acid α-benzyl ester (**3**).[3b] Although in less extent, the latter has been used as intermediate for the preparation of biologically significant compounds.[7] Additionally, perbenzylated L-glutamic acid **2** served also as the precursor of γ-alcohol **4**[6] and diol **5**[8] (Scheme 1). Similarly, the γ-benzyl ester of L-glutamic acid **6** is prepared typically under acidic conditions in 84% yield.[9]



Scheme 1 Reported methods for the synthesis of (poly)benzylated L-glutamic acid derivatives

Recently, the use of *N*,*N*-dibenzylglutamic acid γ-benzyl ester (**7**) in the synthesis of schulzeines was reported.[10] However, a complete experimental procedure for its preparation and full characterization with relevant spectroscopic data were never reported in the literature. After a thorough search, we found that the preparation of compound **7** in 18% yield was described in a PhD thesis.[11] The low yield of the reaction together with the synthetic versatility of intermediate **7** encouraged us to study the process in more detail.

In this study, we investigated the benzylation reaction of L-glutamic acid and the unexpected results obtained when we tried to repeat the procedure reported for the synthesis of **7**.

A direct comparison of the ^{1}H NMR spectra of compounds **2** and **3** was reported earlier by us,[3b] and the ^{1}H NMR spectrum for compound **7** was described by

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Akkarasamiyo[11] that showed marked differences in the signals of the benzyl ester protons PhCH_2O (Figure 1). Indeed, the ^{1}H NMR spectrum reported for **7** showed the appearance of the α-ester derivative and not the γ-ester analogue. The same typical ^{1}H NMR signal was found for the reported intermediate in the synthesis of schulzeines.[10]

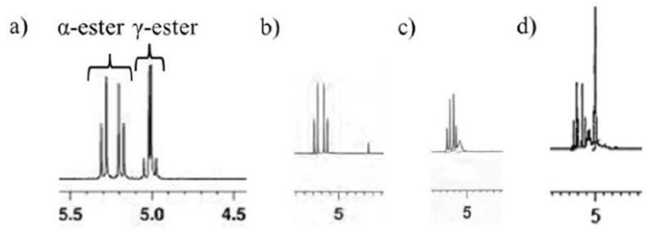
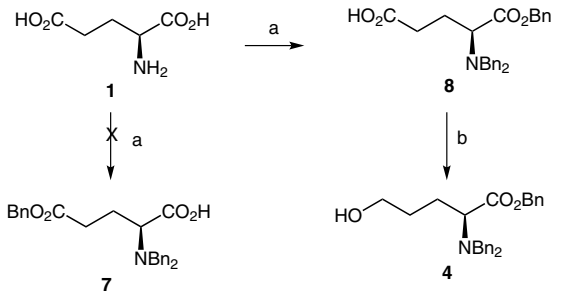


Figure 1 ^{1}H NMR signals of benzylic protons (PhCH_2O) in L-glutamic acid benzyl esters. (a) **2**; (b) compound **43** in ref. 11; (c) compound **11** in ref. 10a (Supporting Information); d) compound **13** in ref. 10b (Supporting Information).

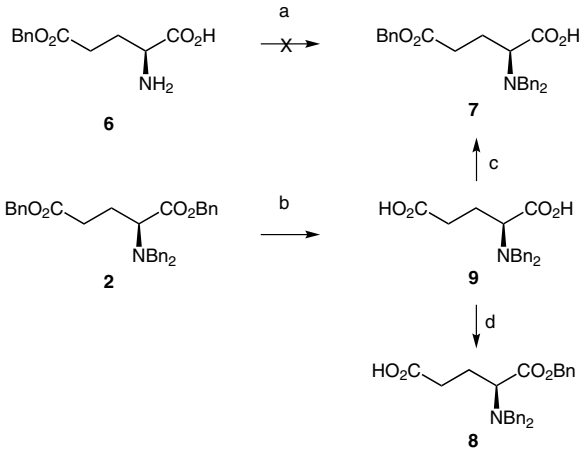
At this point, we suspected that the compound resulting from the experimental conditions described by Akkarasamiyo was *N*,*N*-dibenzylglutamic acid α-benzyl ester (**8**). This subtle difference was unnoticed for the authors since the *N*,*N*-dibenzylglutamic acid monobenzyl ester was transformed into a 3-amino-2,6-piperidinedione intermediate. The reaction conditions are leading to the synthesis, and the undoubtedly identification of compounds **7** and **8** will be discussed below.

As shown in Scheme 2, when we repeated the benzylation of L-glutamic acid (**1**) under the experimental conditions described by Akkarasamiyo,[10] *N*,*N*-dibenzylglutamic acid α-benzyl ester (**8**) was obtained in 20% yield (Table 1, entry 1). As a byproduct of the reaction, the perbenzylated derivative **2** was isolated in 1% yield. More important, compound **7** was not obtained. Subsequent selective reduction of the carboxylic acid group of **8** using standard methods allowed us to obtain compound **4**. The spectroscopic data was in full agreement with that reported in the literature. Consequently, the compound synthesized using the reported protocol is the α-benzyl ester **8** and not the γ-benzyl ester **7**.



Scheme 2 *Reagents and conditions*: (a) ref. 10 [BnCl (3.5 equiv), K_2CO_3, KOH, MeOH–H_2O (1:1), Δ, overnight], 20%; (b) BH_3–SMe_2, THF, 0 °C, 4 h, 86%.

In order to synthesize compound **7**, we envisioned a strategy based on the widely known process to prepare derivative **6** (Scheme 3). Our first attempt to prepare **7** was the benzylation of **6** under basic conditions. However, the desired compound could not be obtained. Alternatively, compound **2** was treated under saponification conditions to give diacid **9** in high yield. Selective benzylation of **9** under acidic conditions led to the expected compound **7** in 70% yield. This time the ^{1}H NMR spectrum was consistent, as shown in Figure 2.



Scheme 3 *Reagents and conditions*: (a) BnCl (2.5 equiv), K_2CO_3, KOH, MeOH–H_2O (1:1), Δ, overnight; (b) NaOH, MeOH, Δ, 20 h, 90%; (c) CH_3SO_3H, BnOH, toluene, Δ, 5 h, 70%; (d) BnBr (1.1 equiv), K_2CO_3, KOH, MeOH–H_2O (1:1), 70 °C, 5 h, 25%.

Interestingly, we found that upon treatment of **9** under the conditions reported by Akkarasamiyo, but using instead 1.1 equivalents of benzyl bromide, the α-benzyl ester **8** was obtained in 25% yield (Scheme 3). This intriguing result, due to the similarity with that obtained by the direct benzylation of L-glutamic acid (**1**) with 3.5 equivalents of benzyl bromide, together with the low yield obtained led us to explore the influence of the reaction conditions.

The reaction conditions for the perbenzylation of **1** described in the literature uses indistinctly benzyl chloride or benzyl bromide as alkylating agents and NaOH or KOH as base (together with K_2CO_3). In addition, some methods described the use of MeOH as cosolvent. As additional factors in our study, we selected time and temperature. With these premises we ran a set of reactions, and the results are given in Table 1.

We speculated that under the aforementioned perbenzylation conditions the partial hydrolysis of **2** might occur. This might be an explanation for the low yields obtained by Akkarasamiyo. Thus, initially, we studied the benzylation process at shorter reaction times (5 h) and lower temperature (70 °C). The results (Table 1, entries 2 and 3) showed that lower reaction times and temperature benefit the process since compounds **8** and **2** are obtained in slightly higher yields, while compound **7** is obtained as minor compound. Under these conditions, the base KOH seems to increase the overall yield while the halide BnBr

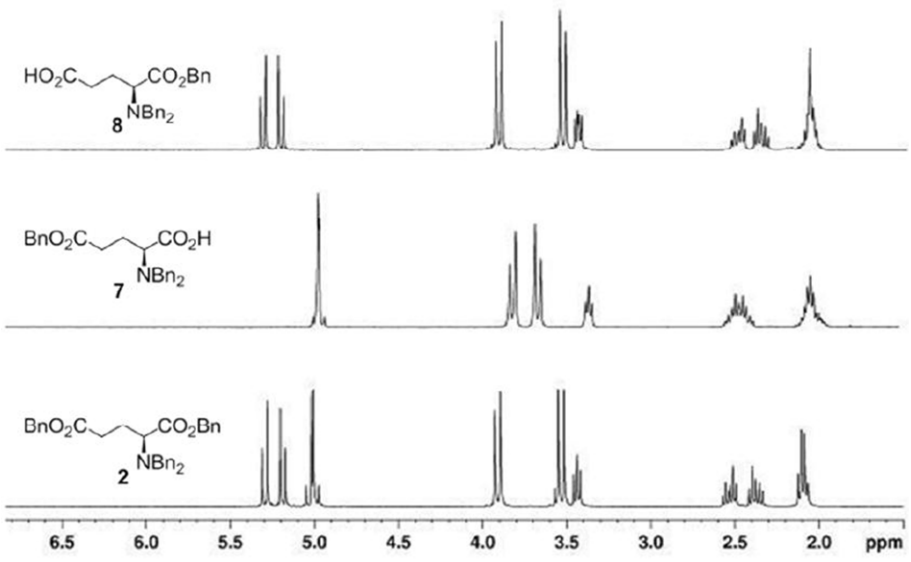


Figure 2 Partial ^{1}H NMR spectra of compounds **2**, **7**, and **8**

favors the formation of **2**. However, at longer reaction times the effect on the outcome of the reaction was independent of the base or halide used (Table 1, entries 7–10). The reduction of the amount of alkylating agent (Table 1, entry 4) is concomitant with a decrease in yield. Another point of attention is the solvent. When reducing the percentage of MeOH in the mixture (Table 1, entries 5–11) no improvement in yield is obtained, regardless of the other reaction conditions. Similarly, a MeOH–H_2O (3:1) mixture (Table 1, entries 12 and 13) produced **8** in low yields at low reaction times. To our surprise, when we increased the reaction time (Table 1, entry 14), the benzylation of **1** to give **8** proceeded in 73% yield giving *N*,*N*-dibenzylglutamic acid α-methyl ester as side product of

Table 1 Effect of the Reaction Conditions in the Polybenzylation of L-Glutamic Acid (**1**)

Entry	Reaction conditions[a]						Yield (%)		
	MeOH (%)	Time (h)	Temp (°C)	BnX (equiv)	X	Base (2.3 equiv)	**8**	**2**	**7**
1[b]	50	overnight	reflux	3.5	Cl	NaOH	20	1	n.d.[c]
2	50	5	70	3.5	Cl	KOH	25	7	1
3	50	5	70	3.5	Br	KOH	30	20	4
4	50	5	70	3	Cl	NaOH	12	2	n.d.
5	25	2	50	3	Br	KOH	n.d.	n.d.	n.d.
6	25	2	reflux	4	Br	KOH	19	26	1
7	25	12	80	3.25	Br	NaOH	12	19	n.d.
8	25	12	80	3.25	Cl	NaOH	12	14	n.d.
9	25	12	80	3.25	Cl	KOH	17	16	n.d.
10	25	12	80	3.25	Br	KOH	13	19	n.d.
11	0	overnight	70	3	Cl	KOH	9	13	n.d.
12	75	2	reflux	3	Br	KOH	14	10	n.d.
13	75	2	50	4	Br	KOH	23	10	7
14[d]	75	8	reflux	4	Br	KOH	73	n.d.	1

[a] **1** (1 equiv), K_2CO_3 (2.3 equiv), solvent (H_2O–MeOH for 0.23 M).
[b] Reaction conditions of ref. 11.
[c] n.d. = not detected.
[d] The α-methyl ester was obtained in 10% yield as a byproduct.

the process (10% yield). For processes where the α-ester should be transformed and thus transesterification is not an inconvenience, the yield adds up to 83%.

In summary, we have described a methodology to obtain both *N*,*N*-dibenzylglutamic acid monobenzyl esters **7** and **8** from commercially available L-glutamic acid (**1**). Both compounds can be differentiated in terms of their ^{1}H NMR spectra. The unprecedented compound **7** is reported and characterized for the first time. The preferred method to obtain **7** from L-glutamic acid is the three-step process reported herein. We explored the reaction conditions leading to the formation of **8**. A not optimized 73% yield was achieved plus 10% yield of the *N*,*N*-dibenzylglutamic acid α-methyl ester.

(2*S*)-5-(Benzyloxy)-2-(dibenzylamino)-5-oxopentanoic Acid (7)
Methane sulfonic acid (3.6 mmol, 0.2 mL) was added dropwise to a suspension of **9** (1.0 g, 3 mmol) and benzyl alcohol (4.8 mmol, 0.5 mL) in dry toluene (30 mL). The resulting mixture was heated at reflux in a Dean–Stark system for 5 h, after which time it was allowed to cool to r.t. Then, the solvent was evaporated under reduced pressure. The residue was diluted with H_2O (30 mL) and extracted with Et_2O (2 × 20 mL). The combined organic extracts were washed with brine (30 mL) and dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexanes–EtOAc, 20:50) to give 1.3 g of **7** (70% yield) as pale yellow oil. $[\alpha]^{25}_D$ –31.3 (*c* 0.98, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.11–2.21 (m, 2 H), 2.53–2.66 (m, 2 H), 3.47 (t, *J* = 7.0 Hz, 1 H), 3.78 (d, *J* = 13.4 Hz, 2 H), 3.91 (d, *J* = 13.4 Hz, 2 H), 5.08 (AB system, 2 H) 7.29–7.42 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9, 31.1, 54.5, 60.2, 66.3, 127.6, 128.3, 128.6, 129.2, 135.9, 138.2, 172.9, 176.5 ppm. IR: ν_{max} = 3362.1–2328.4, 1729.2, 1257.4, 1162.4 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{26}H_{27}NO_4$ $[M + H]^+$: 418.2018; found: 418.2002.

(4*S*)-5-(Benzyloxy)-4-(dibenzylamino)-5-oxopentanoic Acid (8)
To a solution of L-glutamic acid (1.0 g, 6.8 mmol), K_2CO_3 (2.2 g, 15.6 mmol), and KOH (0.87 g, 15.6 mmol) in MeOH–H_2O (1:1, 30 mL) was slowly added benzyl bromide (4.08 g, 23.8 mmol) at r.t. The mixture was stirred at 70 °C for 5 h. After being cooled to 0 °C, it was neutralized (pH 7) with a 5% solution of HCl. Then, the mixture was extracted with CH_2Cl_2 (2 × 50 mL), and the organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexanes–EtOAc, 90:10 to 70:30) to give 700 mg of **8** (25% yield) as a colorless oil. $[\alpha]^{25}_D$ –72.7 (*c* 1.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.00–2.14 (m, 2 H), 2.33–2.40 (m, 1 H), 2.46–2.54 (m, 1 H), 3.45 (dd, *J* = 6.7, 9.5 Hz, 1 H), 3.54 (d, *J* = 14.3 Hz, 2 H), 3.94 (d, *J* = 14.3 Hz, 2 H), 5.26 (AB system, 2 H), 7.22–7.48 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.9, 30.4, 54.5, 59.6, 66.2, 127.2, 128.3, 128.4, 128.6, 128.6, 128.4, 135.9, 138.9, 172.0, 178.79 ppm. IR: ν_{max} = 3450.3–2500.2, 1729.7, 1711.1, 1247.8, 1160.7 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{26}H_{27}NO_4$ $[M + Na]^+$: 440.1838; found: 440.1846.

(2*S*)-2-(Dibenzylamino)pentanedioic Acid (9)
Perbenzylated L-glutamic acid (**2**, 10 g, 19.7 mmol) was dissolved in MeOH (70 mL). A freshly prepared, ice-cold aq solution of 2.4 M NaOH (22 mL, 50 mmol) was added to the solution, and the resulting mixture was heated at reflux for 20 h. The reaction mixture was then allowed to cool to r.t., and the solvent was evaporated under reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 to remove the benzyl alcohol formed in the hydrolysis. The basic aqueous layer was acidified (pH 3–4) with concentrated HCl, and a precipitate was formed. The water layer was extracted with EtOAc (3 × 50 mL). The organic extracts were combined, and the solvent was removed under reduced pressure. The solid residue was recrystallized from MeOH to give **9** as a white solid (5.8 g, 90% yield): mp 228–229 °C; $[\alpha]^{25}_D$ –71.3 (*c* 1.03, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.84–1.90 (m, 2 H), 2.13–2.21 (m, 1 H), 2.29–2.36 (m, 1 H), 3.12–3.16 (m, 1 H), 3.59 (d, *J* = 14.2, 2 H), 3.80 (d, *J* = 14.2, 2 H), 7.20–7.32 (m, 10 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 24.2, 30.6, 50.0, 59.86, 126.3, 128.2, 128,4, 139.6, 173.6, 174.05 ppm. IR: ν_{max} = 3340.3–2750.4, 2672.3–2172.5, 1729.3, 1612.6, 1452.6, 1245.5 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{19}H_{20}NO_4$ $[M + 2Na]^+$ 372.1188; found: 372.1180.

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