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Improved Synthesis of Amino Acid and Dipeptide Chloromethyl Esters Using Bromochloromethane

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ABSTRACT

Peptide chloromethyl esters are important compounds in prodrug synthesis. A simple, mild and efficient method for the synthesis of chloromethyl esters of *N*-blocked amino acids and dipeptides using exclusively bromochloromethane is reported. These *N*-blocked amino acid and dipeptide chloromethyl esters react readily with the carboxylic acid group of aspirin and with the sulfonamido group of the antimalarial sulfamethazine, to give the corresponding prodrugs.

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RESEARCH REPORT

The search for suitable prodrugs as chemical drug delivery systems is a high-priority task to overcome serious problems related to drug solubility, oral absorption, toxicity and metabolic inactivation.^[1,2] Amino acids are promising carriers in prodrug design, as they can improve water solubility and, thus, oral absorption of the drug.^[3] Dipeptides have also received attention as potential drug carriers to improve oral absorption, targeted to the dipeptide transporters present in the gastrointestinal tract. In this context, peptide chloromethyl esters are suitable coupling agents, capable of reacting with several nucleophilic groups frequently found in drugs,^[4-6] such as the carboxylic acid functionality.

In 1979, Wheeler and co-workers described the synthesis of *N*-blocked amino acid chloromethyl esters [**1**] using triethylamine and chloriodomethane (Fig. 1).^[7] However, they obtained rather low global yields (9–25%) of [**1**], and had to face the difficulties of separating the desired ester from large amounts of the *gem*-diester [**2**], also formed as a by-product.^[7] Recent work from Tsujihara's group describes an alternative synthetic route to *N*-blocked amino acid chloromethyl esters [**1**] using the reagent chloromethyl chlorosulfate, with good to excellent yields (79–100%).^[8] Even though chloromethyl chlorosulfate is an excellent reagent for this reaction step, its synthesis occurs in modest yields (ca. 30%) and involves hazardous reagents (chlorosulfonic acid) and products.^[9]

We now wish to report an alternative method for the synthesis of *N*-blocked amino acid chloromethyl esters [**4**], based on the reaction between the corresponding *N*-blocked amino acid caesium salts [**3**] and bromochloromethane (Fig. 2 and Table 1). Moreover, we have successfully extended this method to the synthesis of *N*-blocked dipeptide

Figure 1. Synthetic route to chloromethyl esters by Wheeler and co-workers.^[7]

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Figure 2. Synthetic route to potential sulfamethazine and aspirin prodrugs: (i) preparation of *N*-blocked amino acid and dipeptide caesium salts; (ii) synthesis of *N*-blocked amino acid and dipeptide chloromethyl esters; (iii) derivatization of sulfamethazine and aspirin by reaction with amino acid and dipeptide chloromethyl esters.

chloromethyl esters [5] (Table 1). To our best knowledge, this is the first report on the synthesis of *N*-blocked dipeptide chloromethyl esters.

The method now described involves the previous preparation of the caesium salts of the *N*-protected amino acids and dipeptides, which were formed quantitatively from the corresponding *N*-protected amino acids and peptides. The use of caesium salts is known to improve the esterification rates and yields of amino acid chloromethylation when compared with the corresponding potassium, sodium, calcium or quaternary ammonium salts.^[10,11] The overall yields of [4] and [5] herein described range from 50 to 74% (Table 1), representing a significant improvement over the previous method using chloriodomethane as the chloromethylating agent.^[7] The formation of the *gem*-diester [2] was almost completely suppressed, and thus it was easily separated from [4] or [5] by column chromatography.

The applicability of chloromethyl esters [4] and [5] in prodrug synthesis was assessed by preparing sulfamethazine and aspirin derivatives (Fig. 2). Chloromethyl esters [4] and [5] react readily both with the carboxylic acid group of aspirin and the sulfonamido group of the antimalarial sulfamethazine, to give the corresponding prodrugs with quite reasonable yields (ca. 40%, Table 2). Interestingly, no cyclization of the *N*-blocked dipeptide chloromethyl esters [5] was observed in the presence of the weak base sulfamethazine.

Table 1. Chloromethyl esters [**1**] synthesized: spectroscopic and analytical data.

N ^o	Compound ^a	Yield (%)	¹ H-NMR (CDCl ₃) δ_{H} /ppm ^b	¹³ C-NMR (CDCl ₃) δ_{C} /ppm ^b	Microanalysis ^c (% element)	Molecular weight (Da) ^{c,d}
1a	BocGlyOCH ₂ Cl (yellowish waxy oil)	49	5.75 (2H, s), 5.19 (1H, t), 3.99 (2H, d), 1.46 (9H, s)	168.9, 155.7, 80.4, 69.0, 42.4, 28.0	Found: C, 43.12; H, 6.28; N, 6.28; Cl, 15.88. Expected: C, 42.96; H, 6.31; N, 6.26; Cl, 15.85.	224 (223.7)
1a	ZGlyOCH ₂ Cl (colorless oil)	65	7.33 (5H, m), 5.69 (2H, s), 5.55 (1H, t), 5.10 (2H, s), 3.99 (2H, d)	168.6, 156.4, 136.0, 128.6, 128.3, 128.1, 69.0, 67.20, 42.6	—	258 (257.7)
1c	BocAlaOCH ₂ Cl (white solid, m.p. 36–38°C)	74	5.85 (1H, d), 5.65 (1H, d), 5.16 (1H, d), 4.36 (1H, m), 1.45 (9H, s), 1.42 (3H, d)	171.6, 155.0, 80.1, 69.0, 49.0, 28.2, 17.7	Found: C, 45.26; H, 6.90; N, 5.75; Cl, 14.82. Expected: C, 45.48; H, 6.78; N, 5.89; Cl, 14.92.	238 (237.7)
1d	ZAlaOCH ₂ Cl (colorless oil)	74	7.34 (5H, m), 5.81 (1H, d), 5.64 (1H, d), 5.38 (1H, d), 5.13 (1H, d), 5.09 (1H, d), 4.43 (1H, m), 1.43 (3H, d)	171.4, 155.6, 136.1, 128.6, 128.3, 128.1, 69.1, 67.1, 49.5, 17.9	Found: C, 53.18; H, 5.04; N, 5.14; Cl, 13.11. Expected: C, 53.05; H, 5.19; N, 5.16; Cl, 13.05.	272 (271.7)
1e	ZPheOCH ₂ Cl (white solid m.p. 51–53°C)	52	7.34 (5H, m), 7.28, 7.14 (5H, m), 5.80 (1H, d), 5.63 (1H, d), 5.22 (1H, d), 5.08 (2H, s), 4.69 (1H, m), 3.12 (2H, m)	170.0, 155.6, 136.0, 134.9, 129.4, 128.8, 128.6, 128.1, 127.4, 69.1, 67.2, 54.5, 45.1, 37.6	—	348.58 (347.79)
1f	BocGlyGlyOCH ₂ Cl (whitish and pasty oil)	50	7.28 (1H, m), 5.74 (2H, s), 5.41 (1H, m), 4.14 (2H, d), 4.06 (2H, d), 1.46 (9H, s)	170.2, 168.3, 156.2, 80.4, 69.1, 44.1, 41.0, 28.3	—	281.57 (280.71)

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1g	ZGlyGlyOCH ₂ Cl (colorless oil)	54	7.35 (5H, m), 6.76 (1H, m), 5.72 (2H, d), 5.56 (1H, t), 5.13 (2H, s), 4.11 (2H, d), 3.93 (2H, d)	169.6, 168.2, 136.0, 128.6, 128.4, 128.2, 69.1, 67.4, 44.4, 41.1	—	315.61 (314.72)
1h	BocPheGlyOCH ₂ Cl (white crystals, m.p. 90–91°C)	31	7.25 (5H, m), 6.83 (1H, m), 5.72 (2H, dd), 5.17 (1H, d), 4.47 (1H, m), 4.10 (1H, dd), 4.01 (1H, dd), 3.14 (1H, dd), 3.02 (1H, dd), 1.38 (9H, s)	172.1, 168.0, 155.6, 136.5, 129.3, 128.6, 127.0, 80.4, 69.0, 55.5, 41.1, 38.2, 28.2	Found: C, 54.03; H, 6.08; N, 7.31; Cl, 9.55. Expected (with ½ H ₂ O): C, 53.76; H, 6.37; N, 7.37; Cl, 9.33.	—

^aAmino acid residues are represented by the three letter code according to Ref.^[12]; Boc, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl (*N*^z-protecting groups).

^b¹H NMR signal chemical shifts are followed by the corresponding number of protons and multiplicity, in parenthesis. Spectra were recorded on a Brüker AMX (300 MHz) at the Chemistry Department of the University of Aveiro (Portugal).

^cGenerally, elementary microanalysis and high resolution mass spectrometry analyses were done in alternative to each other.

^donly molecular ion peaks are provided, with expected molecular weights in parenthesis; low resolution analyses were done in our laboratory using a Varian Saturn II GC/MS instrument (FAB ionization/Ion trap MS); high resolution analyses were done by MALDI-TOF MS, using an anthracene matrix; MALDI spectra were recorded on a Finnigan MAT Lasermat equipment at the Serveis Científico-Tècnics from the University of Barcelona (Spain).

Table 2. Sulfamethazine and aspirin derivatives^a synthesized: analytical and spectroscopic data.

Compound	Global yield (%)	δ_{H} /ppm ^b	^{13}C -NMR (CDCl ₃) δ_{C} /ppm ^b	Microanalysis ^c (% element)	Molecular weight (Da) ^{c,d}
(white solid, mp. 72-73 °C)	35	7.90 (2H, d), 6.62 (3H, m), 6.41 (2H, s), 5.10 (1H, t), 4.30 (2H, s), 3.89 (2H, d), 2.32 (6H, s), 1.43 (9H, s)	169.6, 167.7, 156.6, 155.6, 151.3, 131.9, 127.1, 115.5, 112.9, 80.0, 70.7, 65.8, 42.3, 28.3, 23.6	Found: C, 51.47 H; 5.95; N, 14.12. Expected: C, 51.60; H, 5.85; N, 15.04.	—
(white solid, mp. 56-59 °C)	41	7.90, 6.62 (2H+2H+1H, d+d+s), 6.40 (1H, d), 6.36 (1H, d), 5.13 (1H, d), 4.27 (3H, br), 2.31 (6H, s), 1.43 (9H, s), 1.31 (3H, d)	172.5, 167.6, 156.6, 155.0, 151.2, 131.9, 127.2, 115.4, 112.9, 79.8, 70.9, 49.2, 28.3, 23.6, 18.8	—	480.52 (479.55)
(white solid, mp. 86-88 °C)	32	7.90 (2H, d), 7.26 (5H, m), 6.67 (2H, d), 6.61 (1H, s), 6.49 (1H, m), 6.37 (2H, s), 5.01 (1H, m), 4.36 (1H, m), 3.94 (1H, dd), 3.87 (1H, dd), 3.16 (1H, dd), 2.97 (2H, br s), 2.29 (6H, s), 1.35 (9H, s)	172.1, 168.0, 167.7, 156.6, 155.6, 151.3, 136.5, 132.0, 129.3, 128.6, 127.1, 127.0, 115.3, 112.8, 80.4, 70.8, 55.5, 41.1, 38.2, 28.2, 23.6	—	613.44 (612.70)

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(yellowish waxy oil)	43	8.08 (1H, d), 7.62 (1H, t), 7.57 (1H, t), 7.37 (1H, d), 5.99 (2H, s), 5.06 (1H, s), 3.97 (2H, d), 2.36 (3H, s), 1.45 (9H, s)	170.1, 168.9, 155.7, 152.6, 139.3, 134.8, 132.2, 126.1, 124.0, 122.2, 80.4, 69.0, 42.2, 28.2, 21.0	—	365.23 (364.30)
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^aApplication of amino acid and dipeptide chloromethyl esters in prodrug synthesis.^b¹H NMR signal chemical shifts are followed by the corresponding number of protons and multiplicity, in parenthesis. Spectra were recorded on a Brüker AMX (300 MHz) at the Chemistry Department of the University of Aveiro (Portugal).^cMicroanalysis and high resolution mass spectrometry were done in alternative to each other.^dHigh resolution analyses were done by MALDI-TOF MS, using a 2,5-dihydroxybenzoic acid matrix; spectra were recorded on a Finnigan MAT Lasermat spectrometer at the Serveis Científico-Tècnics from the University of Barcelona (Spain).

EXPERIMENTAL SECTION

Synthesis of the *N*-Blocked Amino Acid/Dipeptide Caesium Salts

The appropriate *N*-blocked amino acid or dipeptide (5 mmol) was suspended in ethanol/water 7:3 v/v (25 mL) with magnetic stirring. The electrode of a pH-meter was dipped into the solution and aqueous caesium carbonate (1 M) was added dropwise until pH 6.5 was reached. The solvents were then evaporated under low pressure and the resulting white caesium salt was allowed to dry in vacuum.

Synthesis of the *N*-Blocked Amino Acid/Dipeptide Chloromethyl Esters

Bromochloromethane (35 mL) was slowly added to a solution of the appropriate *N*-blocked amino acid/dipeptide caesium salt (5 mmol) in dry *N,N'*-dimethylformamide (DMF, 15 mL), and the reaction was allowed to proceed in the dark at room temperature. After 20 h, the caesium bromide formed was removed by suction filtration and filtrate was evaporated to dryness. The resulting mixture presented three components (thin layer chromatography, TLC), by increasing order of R_f , caesium bromide, the *gem*-diester by-product and the main product. This main product was isolated by low pressure liquid chromatography (LPLC) on silica, using dichloromethane (DCM)/ethyl ether or DCM/acetone (in varying proportions) as eluents. Solid compounds were recrystallized from 1:1 ethyl ether/petroleum ether (40–60°) prior to analysis.

The identity and purity of the isolated chloromethyl esters were checked by ^1H and ^{13}C NMR, FAB/Ion Trap or MALDI-TOF MS and/or elemental analysis.

Synthesis of the *N*-Blocked Amino Acid/Dipeptide Sulfamethazine Derivatives

The sodium salt of sulfamethazine (1 mmol) was slowly added to a solution of the appropriate *N*-blocked amino acid/dipeptide chloromethyl ester (1 mmol) in dry DMF (4 mL). The reaction was allowed to proceed under constant stirring at room temperature, in the dark. After two days, the mixture was evaporated to dryness and the residue was redissolved in acetone (2 mL) upon heating. The solution was stored at 4°C overnight

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to promote the precipitation of sodium chloride, which was then removed by filtration under reduced pressure. The filtrate was evaporated to dryness and the resulting mixture revealed the presence of five to seven components (TLC), the main of which was isolated by LPLC on silica, using DCM/ethyl ether 1:1 as eluent. Isolated compounds were recrystallized from 1:1 ethyl ether/petroleum ether (40–60°) prior to analysis.

The structure and purity of the isolated compounds were confirmed by ^1H and ^{13}C NMR, MALDI-TOF MS and/or elemental analysis.

Synthesis of the *N*-Blocked Amino Acid Acetylsalicylic Acid (“Aspirin”) Derivatives

The *N*-blocked amino acid chloromethyl ester (1 mmol) was dissolved in dry DMF (2 mL) and acetylsalicylic acid (1 mmol) was added. Triethylamine (1 mmol) was then added dropwise. The reaction was allowed to proceed under constant stirring at room temperature, in the dark. After three days, the triethylammonium chloride formed was removed by vacuum filtration and the filtrate was evaporated to dryness. The residue was redissolved in acetone (2 mL) upon heating and left to stand at 4°C overnight for additional precipitation of the triethylammonium salt. After a new cycle of salt filtration and filtrate evaporation to dryness, the mixture was submitted to LPLC on silica, with ethyl acetate as eluent. The target product was isolated and characterized by ^1H and ^{13}C NMR and by MALDI-TOF MS.

ABBREVIATIONS

Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; DMF, *N,N'*-dimethylformamide; FAB-MS, fast atom bombardment mass spectrometry; LPLC, low pressure liquid chromatography; MALDI-TOF MS, matrix-assisted laser desorption-time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; PG, protecting group; TLC, thin layer chromatography; Z, benzyloxycarbonyl.

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