Synthesis of *N*-Succinyl-L,L-Diaminopimelic Acid Mimetics *Via* Selective Protection

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Abstract: The search for potential inhibitors that target so far unexplored bacterial enzyme mono-N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE) has stimulated a development of methodology for quick and efficient preparation of mono-N-acylated 2,6-diaminopimelic acid (DAP) derivatives bearing the different carboxyl groups or lipophilic moieties on their amino group.

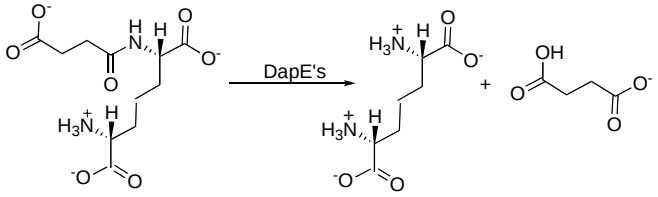
Keywords: Bacterial enzyme, N-succinyl-L,L-diaminopimelic acid (SDAP), synthesis of SDAP analogues, selective protection.

INTRODUCTION

Bacterial infections are a significant and growing medical problem around the World [1] in part, because an increasing number of disease-causing microbes have become resistant to existing antibiotics [2-5]. The World Health Organization (WHO) has reported that level of antibiotics resistance is increasing at an alarming rate (resistance towards tetracyclines has increased from 0% in 1948 to 98% in 1998). Today, 1500 people die each hour from an infectious disease, half of these children under five years of age [4]. These findings have stimulated a sustained search for new potent antimicrobial agents against drug resistant bacterial strains [5,6].

Based on bacterial genetic information, the *meso*-diaminopimelate (mDAP)/lysine biosynthetic pathway offers several potential anti-bacterial targets that have yet to be explored [7-9]. One of the products of this pathway, lysine, is required in protein synthesis and is also used in the peptidoglycan layer of Gram-positive bacterial cell walls. A second product, *meso*-diaminopimelic acid (mDAP) is an essential component of the peptidoglycan cell wall in Gram-negative bacteria, providing a link between polysaccharide strands. It has been shown that deletion of the gene encoding for one of the enzymes in the (mDAP)/lysine biosynthetic pathway, the dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE; EC 3.5.1.18), is lethal to *Helicobacter pylori* and *Mycobacterium smegmatis* [10,11]. Even in the presence of lysine supplemented media *H. pylori* was unable to grow. Therefore, DapE's are essential for cell growth and proliferation and are part of a biosynthetic pathway that is the only source for lysine in bacteria. Since there are no similar biosynthetic pathways in mammals, inhibitors that target one or more of the enzymes in the (mDAP)/lysine biosynthetic pathway are hypothesized to exhibit selective toxicity against only bacteria, providing a previously undescribed class of antimicrobial agents [7,12].

DapE's catalyze the hydrolysis of *N*-succinyl-L,L-diaminopimelic acid (SDAP) forming L,L-diaminopimelic acid and succinate "Scheme (**1**)". With an intention to discover and develop new antimicrobial agents that target DapE enzyme, we focused our effort to the synthesis of potential DapE inhibitors based on the substrate SDAP.



Scheme 1.

The most eligible structural alternation of SDAP from enzyme mechanistic and synthetic standpoints (*i.e.* those potentially inducing inhibitory properties) is the succinate moiety. Altering the structure of the *N*-linked succinate moiety will likely inhibit the enzymes ability to cleave the adjacent amide bond. Therefore, we have prepared compounds bearing different *N*-linked acyl side chains terminated with (i) a carboxyl group or (ii) a lipophilic moiety.

RESULTS

In order to provide an efficient and reliable synthetic approach for the preparation of large series of unsymmetrical diaminopimelic acid (DAP) derivatives with *N*-linked acyl side chains, a synthetic method was sought that (i) did not require large amounts of expensive starting materials, (ii) gave the desired product after a minimum number of steps, and (iii) did not demand complicated purification procedures of the product as previously reported [13-16]. The first attempts were performed with *RS,RS*-2,6-diaminopimelic acid with an intention to re-synthesize the most successful DAP derivatives from dapE inhibitory assay in optical pure form

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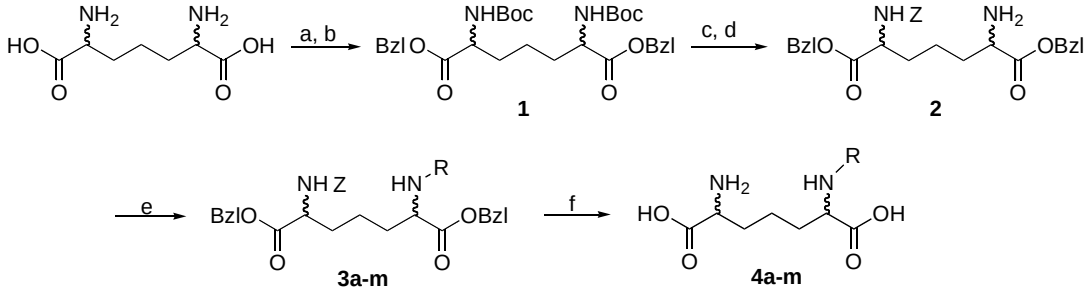
later or to resolve them. In our hands, the direct N-acylation of *RS*,*RS*-2,6-diaminopimelic acid did not give good yields, mostly due to poor solubility of the starting material in a reaction mixture [17]. Therefore, the synthetic methods were developed based on the condensation of different anhydrides or acids with suitably protected monoamine **2** "Scheme (**2**)" and monoamine **6** "Scheme (**3**)".

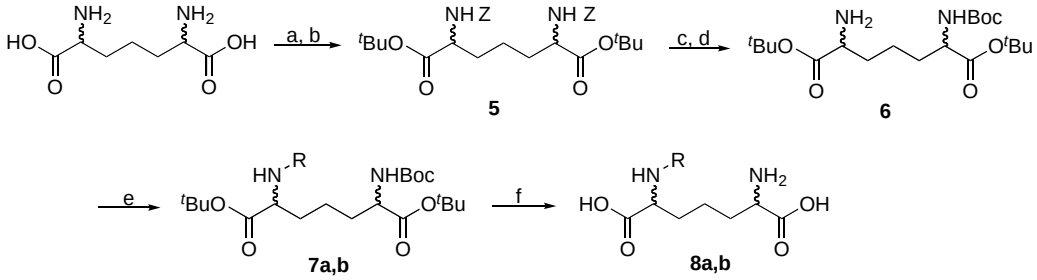
The first key intermediate **2** was synthesized by first protecting the free amino groups with Boc (tert-butyloxycarbonyl) followed by esterification of the carboxyls using *N*,*N´*-diisopropyl-*O*-benzylisourea in DMF (N,N-dimethylformamide) under mild conditions, affording the benzyl ester **1**. The Boc groups were removed by acid catalyzed hydrolysis with TFA. Treatment of the raw product with one equivalent of Z-Cl and TEA in THF provided the mono-protected intermediate **2.** ^{1}H NMR (600 MHz, DMSO-d_6): 1.37 (2H, m, CH_2); 1.43 and 1.56 (2H, m, CH_2); 1.62 and 1.68 (2H, m, CH_2); 3.31 (1H, m, N-CH-CO); 4.04 (1H, m, N-CH-CO); 5.03-5.11 (6H, m, 3x O-CH_2-); 7.30-7.36 (15H, m, 3x C_6H_5); ^{13}C NMR (150.9 MHz, DMSO-d_6): two diastereoisomers – some carbon signals are doubled, 21.96 and 21.99 (CH_2); 30.60 and 30.64 (CH_2); 34.18 (CH_2); 54.00 and 54.07 (NCH<); 54.18 and 54.19 (N-CH<); 65.68, 65.70 and 66.06 (3x O-CH_2); 127.90, 127.92, 128.02, 128.15, 128.20, 128.52 and 128.59 (aromatic –CH=, 3x C_6H_5); 136.13, 136.36 and 137.08 (aromatic >C=, 3x C_6H_5); 156.38 (N-CO-O); 172.43, 172.45, 175.72 and 175.73 (2x C-CO-O). HRMS (ESI) calcd for $C_{29}H_{33}N_2O_6$ $[M+H]^+$ 505.2333; found: 505.2336.

Treatment of **2** with various anhydrides furnished *N*-acylated compounds **3a**-**3k**, while in the preparation of **3l** and **3m**, the free carboxylic acid was coupled to **2** using Py-BOP as a condensation agent (Table **1**). Above intermediates were easily purified by flash chromatography on silica gel (40-63 μm, Fluka) using elution with linear gradient of ethanol in chloroform. Finally, all of the precursors were fully deprotected by hydrogenolysis under mild conditions, followed by purification on reverse-phase HPLC providing pure target compounds **4a**-**4m** (Table **1**).

For target compounds containing double bond in the *N*-linked side chain, different protection groups were employed "Scheme (**3**)". First, both amino groups of *RS*,*RS*-2,6-diaminopimelic acid were protected by reaction with Z-Cl. Heating of the resulting intermediate with two equivalents of *N*, *N´*-diisopropyl-*O*-*tert*-butylisourea afforded the fully protected compound **5**. The Z protecting groups were removed by hydrogenolysis and reaction of the amine with one equivalent of Boc_2O furnished the desired intermediate **6.** ^{1}H NMR (600 MHz, $CDCl_3$): 1.44 (9H, bs, t-Bu); 1.46 (18H, s, 2x t-Bu); 1.45 (2H, m, CH_2); 1.53 and 1.70 (2H, m, CH_2); 1.63 and 1.80 (2H, m, CH_2); 3.29 (1H, dd, J = 7.4 and 5.4, N-CH-CO); 4.17 (1H, m, N-CH-CO); 5.05 (1H, bd, J = 8.0, NH); ^{13}C NMR (150.9 MHz, CDCl3): two diastereoisomers – some carbon signals are doubled, 21.26 and 21.35 (CH_2); 27.97 (3x CH_3, t-Bu); 28.02 (3x CH_3, t-Bu); 28.30 (3x CH_3, t-Bu); 32.61 and 32.67 (CH_2); 34.58 and 34.60 (CH_2); 53.72 and 53.77 (N-CH<); 54.76 and 54.78 (N-CH<); 79.55 (>C<, t-Bu); 80.94 (>C<, t-Bu); 81.76 (>C<, t-Bu); 155.34 (NH-

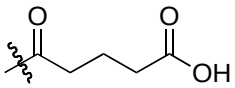
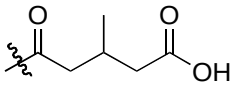
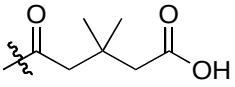
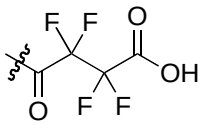
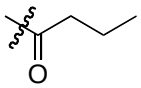
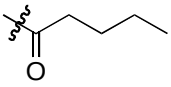
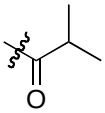
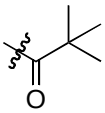
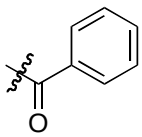
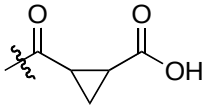
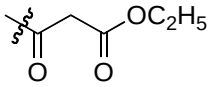


Scheme 2. Reagents, conditions, and yields: (a) Boc_2O, Na_2CO_3, water, dioxane, 0 °C, 1 h then at rt overnight (94%); (b) *N*,*N´*-diisopropyl-*O*-benzylisourea, benzene, DMF, 80°C, 8 h (83%); (c) TFA, dichloromethane, water, 1 h at rt; (d) benzyl chloroformate, TEA, THF, 0°C 1 h then at rt overnight (37% over two steps); (e) Method A: anhydride, chloroform, 50°C 4 h, (yields see Table **1**), Method B: carboxylic acid, PyBOP, TEA, dichloromethane, rt overnight, (yields see Table **1**); (f) 10% Pd/H_2, methanol, rt overnight, RP-HPLC.

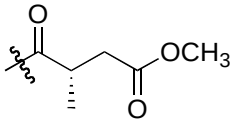
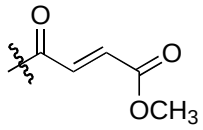
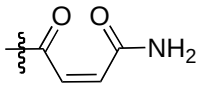


Scheme 3. Reagents, conditions, and yields: (a) benzyl chloroformate, Na_2CO_3, water, and dioxane, 0°C, 1h then at rt overnight (64%); (b) *N*,*N´*-diisopropyl-*O*-*tert*-butylisourea, dioxane, 80°C, 8 h (48%); (c) 10% Pd/H_2, methanol, rt overnight (90%); (d) Boc_2O, TEA, dioxane, 0°C, 1 h then at rt overnight (30%); (e) Method B: carboxylic acid, PyBOP, TEA, dichloromethane, rt overnight, (yields see Table **2**); (f) TFA, dichloromethane, water, 1 h rt, RP-HPLC.

Table 1. ***N*-Acylation of Intermediates 2 and 6 with Yields and Analytical Data of the Protected Intermediates 3a-3m, 7a, 7b and Final Products 4a- 4m and 8a, 8b**[a]

Method Intermediate Yield	R	ESI MS[b] Calc/found (m/z)		HPLC[c] in min
A **2** (73%)		**3a** $C_{34}H_{38}N_2O_9$ 618.35/641.25 $[M+Na]^+$	**4a** $C_{12}H_{20}N_2O_6$ 288.30 (289.13) $[M+H]^+$	**4a** 5.21 d
A **2** (71%)		**3b** $C_{35}H_{40}N_2O_9$ 632.34/655.24 $[M+Na]^+$	**4b** $C_{13}H_{22}N_2O_7$ 318.33 (319,14) $[M+H]^+$	**4b** 4.55 e
A **2** (86%)		**3c** $C_{36}H_{43}N_2O_9$ 646.29/647.18 $[M+H]^+$	**4c** $C_{14}H_{24}N_2O_7$ 332.16 (333.11) $[M+H]^+$	**4c** 6.69 e
A **2** (87%)		**3d** $C_{33}F_4H_{32}N_2O_9$ 676.29/699.19 $[M+Na]^+$	**4d** $C_{11}H_{14}F_4N_2O_7$ 362.24 (363.07) $[M+H]^+$	**4d** 5.12 e
A **2** (82%)		**3e** $C_{33}H_{39}N_2O_7$ 574.27/575.36 $[M+H]^+$	**4e** $C_{11}H_{20}N_2O_5$ 260.29 (261.14) $[M+H]^+$	**4e** 7.51 d
A **2** (87%)		**3f** $C_{34}H_{40}N_2O_7$ 588.28/589.11 $[M+H]^+$	**4f** $C_{12}H_{22}N_2O_5$ 274.32 (275.15) $[M+H]^+$	**4f** 6.89 f
A **2** (85%)		**3g** $C_{31}H_{34}N_2O_7$ 546.24/547.19 $[M+H]^+$	**4g** $C_9H_{16}N_2O_5$ 232.24 (233.11) $[M+H]^+$	**4g** 3.11 d
A **2** (90%)		**3h** $C_{33}H_{38}N_2O_7$ 574.26/575.22 $[M+H]^+$	**4h** $C_{11}H_{20}N_2O_5$ 260.29 (261.14) $[M+H]^+$	**4h** 8.14 d
A **2** (91%)		**3i** $C_{34}H_{40}N_2O_7$ 588.28/589.08 $[M+H]^+$	**4i** $C_{12}H_{22}N_2O_5$ 274.32 (275.15) $[M+H]^+$	**4i** 7.81 f
A **2** (88%)		**3j** $C_{36}H_{36}N_2O_7$ 608.25/609.15 $[M+H]^+$	**4j** $C_{14}H_{18}N_2O_5$ 294.31 (295.15) $[M+H]^+$	**4j** 6.55 f
A **2** (18 %)		**3k** $C_{34}H_{36}N_2O_9$ 616.24/617.14 $[M+H]^+$	**4k** $C_{12}H_{18}N_2O_7$ 302.29 (303.11) $[M+H]^+$	**4k** 4.33 d
B **2** (80%)		**3l** $C_{34}H_{38}N_2O_9$ 618.26/619.21 $[M+H]^+$	**4l** $C_{12}H_{20}N_2O_7$ 304.30 (305.13) $[M+H]^+$	**4l** 3.73 f

(Table 1) contd…..

Method Intermediate Yield	R	ESI MS[b] Calc/found (m/z)		HPLC[c] in min
B **2** (91%)		**3m** $C_{35}H_{40}N_2O_9$ 632.27/633.11 $[M+H]^+$	**4m** $C_{13}H_{22}N_2O_7$ 318.33 (319.21) $[M+H]^+$	**4m** 4.41 f
B **6** (71 %)		**7a** $C_{25}H_{42}N_2O_9$ 514.29/515.10 $[M+H]^+$	**8a** $C_{12}H_{18}N_2O_7$ 302.29 (303.11) $[M+H]^+$	**8a** 8.98 f
B **6** (65 %)		**7b** $C_{24}H_{41}N_3NaO_8$ 499.37/522.27 $[M+Na]^+$	**8b** $C_{11}H_{17}N_3O_6$ 287.27 (288.11) $[M+H]^+$	**8b** 5.06 f

[a] All of DAP derivatives had a correct C,H,N elemental analysis in the range of 0.3%. [b] Determined with an ESI technique using Agilent 5975B MSD equipment (Agilent Technologies, Santa Clara, Ca, USA). [c] In the analytical RP HPLC a TSP instrument with an SP 8800 pump, an SP 4290 integrator, TSP Spectra 100 UV detector at 220 nm and 5μm Supelco 15 x 0.4 cm column (Supelco, Bellefonte, PA, USA) with flow 1ml/min were used. An isocratic analysis with 0.05% TFA/aq (d), 2.5% CH_3CN in 0.05% TFA/aq (e) and 5% CH_3CN in 0.05% TFA/aq (f) was applied. For preparative RP HPLC using the same instruments, the 10 μm Vydac 25 x 1 cm column (Grace Davison Discovery Sciences, Hesperia, CA, USA) with flow 3 ml/min and a gradient of 5%-50% ACN in 0.05% TFA, 120 min at 230 nm was applied.

CO-O); 171.89 (O-CO-); 175.23 and 175.30 (O-CO-). HRMS (ESI) calcd for $C_{20}H_{38}N_2O_6$ $[M+H]^+$ 403.2803; found: 403.2803.

The PyBOP-mediated reaction of **6** with two fumaric or maleic acid derivatives afforded **7a** and **7b,** which were purified by column chromatography similarly to derivatives **3a-3m.** Full deprotection was accomplished by hydrolysis with TFA providing target compounds **8a** and **8b** (Table **1**).

CONCLUSIONS

We developed a simple methodology for quick and efficient preparation of mono-*N*-acylated 2,6-diaminopimelic acid derivatives providing a suitable route for the large scale preparation of series of DAP compounds. The unambiguous acylation of unsymmetrically protected key intermediates **2** or **6,** afforded **3a-3m** and **7a**, **7b** as the protected derivatives of the target compounds **4a- 4m** and **8a, 8b** in moderate to high yields. Moreover, these target compounds were obtained in high purity after only a one-step deprotection process under mild conditions (hydrogenolysis or acidic conditions).

ABBREVIATIONS

Boc = *tert*-Butyloxycarbonyl

Bzl = Benzyl

DAP = 2,6-Diaminopimelic acid

DapE = Mono-N-succinyl-L,L-diaminopimelic acid desuccinylase

DMF = *N,N*-dimethylformamide

ESIMS = Electro spray ionization mass spectrometry

HRMS = High resolution mass spectrometry

PyBOP = (Benzotriazol-1-yloxy)-tris(pyrrolidino) phosphonium hexafluorophosphate

RP HPLC = Reverse phase high performance liquid chromatography

TEA = Triethylamine

TFA = Trifluoroacetic acid

THF = Tetrahydrofurane

Z = Benzyloxycarbonyl

ACKNOWLEDGEMENTS

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