Stereoselective synthesis of stable isotopomers of *meso*-2,6-diaminopimelic acid

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Introduction

Due to heavy use of antibiotics in our modern society, bacteria developed many resistance mechanisms. It is only a matter of time before the latest discovered antibiotic becomes obsolete[1,2,3]. In that regard scientists continuously need to find new targets to design new antibiotics.

meso-2,6-Diaminopimelic acid or *m*-A_2pm, is a diamino diacid only found in bacterial peptidoglycan. It is essential to a large proportion of bacterial species, mostly Gram negative bacteria, allowing reticulation between glycan strands (Figure 1).

Bacteria can not survive without peptidoglycan. This macromolecule has a structural role and allows them to resist to their high inner osmotic pressure. Therefore peptidoglycan metabolism is a target of choice in antibiotics development.

In this work, we synthesized stable isotopomers ($^{13}C_1$ and $^2H_2$) of *m*-A_2pm to provide tools that will help biologists to study peptidoglycan metabolism.

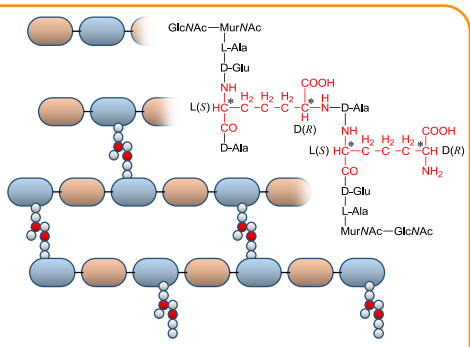

Figure 1 | Peptidoglycan structure from *Escherichia coli*, *m*-A_2pm highlighted in red.

Results

1. Strategy

The key intermediate of our strategy is pictured in Figure 2. This family of compounds can be obtained by cross metathesis (CM) between vinylglycine (Vgl) and allylglycine (Agl) synthons[4].

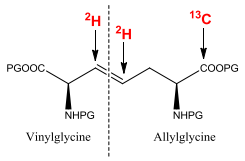

Figure 2 | Key intermediate.

Deuterium can be introduced in the synthesis after the CM step by catalytic reduction of the alkene bond. The cheapest way to introduce carbon-13 is to synthesize a ^{13}C-Agl from glycine-1-^{13}C (154€/g).

2. Allylglycine synthons synthesis

(*S*)-Allylglycine synthons **4a** and **4b** were prepared by alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester **1** (a Schiff base, commercially available) using chiral phase transfer catalysis (Figure 3). The cinchonidine based catalyst **2** was synthesized in our lab[5].

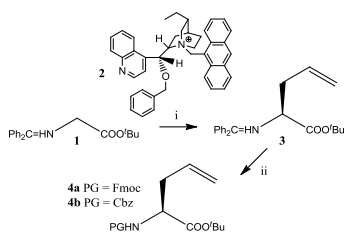

Figure 3 | ^{12}C-Allylglycines syntheses. i) CH_2=CH-CH_2-Br, KOH (50%wt, aq), toluene, CH_2Cl_2, -20°C. ii) citric acid (10%wt, aq), THF ; **4a** : Fmoc-OSu, THF, H_2O (67%, 3 steps) ; **4b** : Cbz-Cl, Na_2CO_3 (10%wt, aq), toluene, 0 °C (53%, 3 steps).

Enantiomeric excess are very satisfying (**4a** 97% ee, **4b** 90% ee, measured on chiral Daicel OD-H HPLC column). Once ^{13}C-Schiff base **6** prepared from glycine-1-^{13}C **5** (Figure 4), the same alkylation process was applied to yield protected ^{13}C-Agl **8**. Vinylglycine synthon **9** (Figure 5) was also synthesized in our lab[6] (65%, 3 steps, ee = 98%).

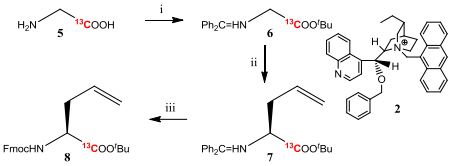

Figure 4 | ^{13}C-Allylglycine synthesis. i) Cbz-Cl, NaOH (aq), toluene, 0 °C → rt ; ^{t}BuOH, DCC, DMAP, CH_2Cl_2 ; H_2 (6 bar), Pd/C (10%wt, dry), HCl, MeOH ; Ph_2C=NH, CH_2Cl_2, $MgSO_4$. ii) CH_2=CH-CH_2-Br, KOH (50%wt, aq), toluene, CH_2Cl_2, -20 °C. iii) citric acid (10%wt, aq), THF ; Fmoc-OSu, THF, H_2O (51%, 7 steps).

3. Cross-metatheses and PG removal

Protecting groups (PG) of **10** were chosen to suit future solid phase peptide synthesis. In this case, CM yields were very satisfying. Unfortunately the Fmoc removal proved to be the most challenging step of these syntheses.

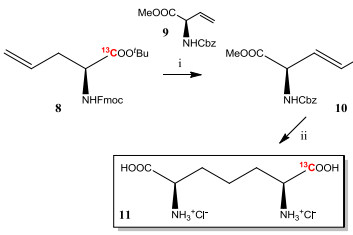

Figure 5 | ^{13}C-*m*-A_2pm synthesis. i) Grubbs 2nd gen. cat. 10 mol%, CH_2Cl_2, reflux, N_2 (yield = 66%). ii) HCl (2 M, aq), 1,4-dioxane, reflux ; H_2 (6 bar), Pd, HCl, MeOH (35%, 2 steps).

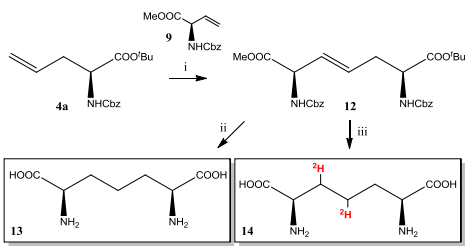

Figure 6 | *m*-A_2pm and $^2H_2$-*m*-A_2pm syntheses. i) Grubbs 2nd gen. cat. 20 mol%, CH_2Cl_2, reflux, N_2 (yield = 35%). ii) HCl (2 M, aq), 1,4-dioxane, reflux ; H_2 (6 bar), Pd, MeOH, H_2O (95%, 2 steps). iii) HCl (2N, aq), 1,4-dioxane, reflux ; $^2H_2$ (6 bar), Pd, MeOH, H_2O (95%, 2 steps).

To avoid difficult PG removal, we specifically synthesized CM product **12** which can easily lead to **13** and **14**. A simple HCl-dioxane treatment of **12** allowed the esters cleavage. Then choice of H_2 or $^2H_2$ in the last catalytic reduction step permitted production of *m*-A_2pm and its modified counterpart $^2H_2$-*m*-A_2pm (Figure 6).

3. Analyses

All molecules were analyzed in mass spectrometry, ^{1}H and ^{13}C NMR. Some evidences of labelling are pictured in the following spectra (Figure 7).

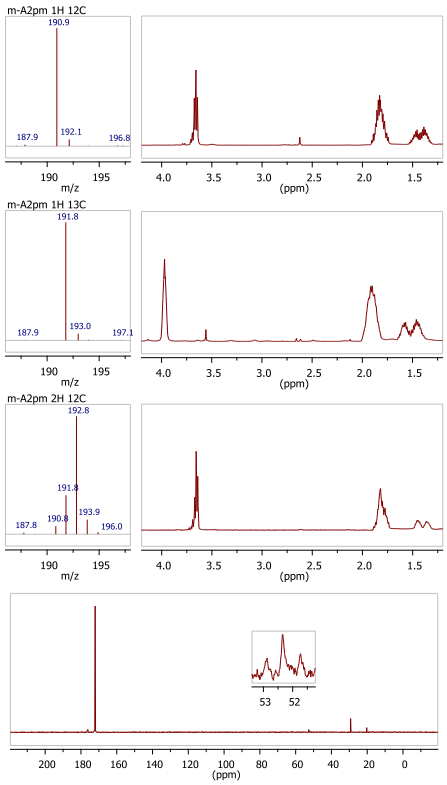

Figure 7 | First column : ESI-MS spectra [M+H$^+$]. Second column : ^{1}H NMR. Bottom : ^{13}C NMR spectrum of ^{13}C-*m*-A_2pm.

Conclusion

In this study, we prepared 3 different isotopomers of *m*-A_2pm by cross metathesis : natural *m*-A_2pm (18%, 6 steps), $^2H_2$-*m*-A_2pm (18%, 6 steps) and ^{13}C-*m*-A_2pm (12%, 10 steps). This last synthesis still needs some optimization in the last step. These compounds will be analyzed in HPLC to determine their diastereomeric excesses but no evidence of racemization was observed in NMR and HPLC analyses. Shortly, we will use a similar strategy to produce tritiated $^3H_2$-*m*-A_2pm.

References

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