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Novel fully protected muramic acid: A facile synthesis and structural study

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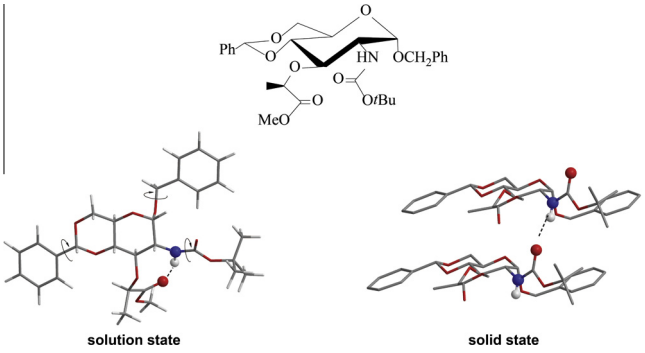
HIGHLIGHTS

- *N*-Ac-Mur-OMe is transformed into *N*-Boc-Mur-OMe.
- Eight membered NH···OC_{ester} intramolecular hydrogen bond is preserved in solution upon alteration of protecting group.
- Intermolecular hydrogen bonding occurs in the solid-state of *N*-Boc-Mur-OMe.

GRAPHICAL ABSTRACT



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ABSTRACT

Synthesis and structural characterisation of novel fully protected muramic acid **2** (*N*-Boc-Mur-OMe, Mur = muramic acid) has been reported. *N*-Ac-Mur-OMe (**1**) prepared starting from commercially available *N*-acetylglucosamine, was treated with di-*tert*-butyl dicarbonate (Boc_2O) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) in tetrahydrofuran. The intermediate mixed imide *N*-Ac-*N*-Boc-Mur-OMe was converted to *N*-Boc-Mur-OMe (**2**) upon *in situ* treatment with hydrazine hydrate in methanol. The structural analysis of **2**, performed by IR and NMR spectroscopic methods and X-ray crystallography, was augmented by computational calculations including molecular and density functional theory studies (DFT) using M06/6-31G(d) computational model. The spectroscopic and DFT data obtained for novel Boc-protected **2** were compared with corresponding experimental values of its previously described Ac-protected analogue **1** in order to examine if the replacement of the protecting groups influences the conformational properties.

1. Introduction

Muropeptides are degradation products of peptidoglycans that contain muramic acid (Mur) coupled to amino acids. They are known as biologically active compounds, e.g. they exhibit increased immunoadjuvant activity [1].

The structural properties of bioorganometallic muropeptides **I** [*N*-Ac-Mur-Ala-Fca; Fca = 1′-aminoferrocene-1-carboxylic acid)] and **II** [*N*-Ac-Mur-NH-Fn-R; Fn = 1,1′-ferrocenylene, R = H (**IIa**), COOMe (**IIb**), NHAc (**IIc**)] have already been investigated in our group [2,3] (Fig. 1). The detailed structural studies in solution confirmed strong influence of ferrocene moiety on conformational properties of **I** and **II**. Moreover, the cyclic voltammograms of **II** featured a one-electron oxidation for the ferrocene/ferrocenium redox couple. Furthermore, Fca-containing peptides with Ala were

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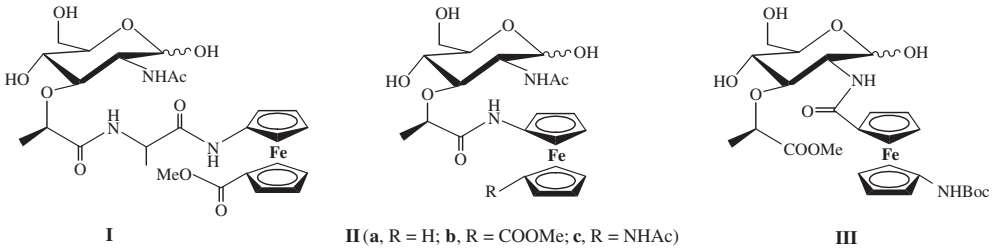
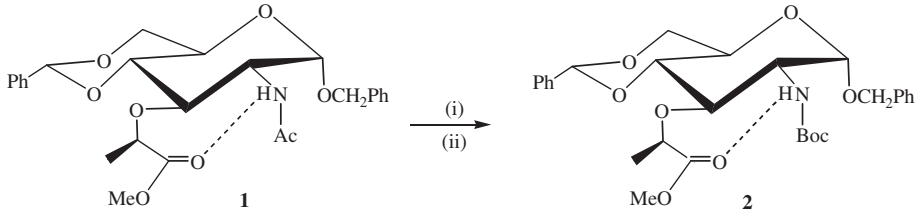


Fig. 1. Ferrocene conjugates with muramic acid **I–III**.



Scheme 1. Transformation of *N*-Ac-Mur-OMe (**1**) to Boc-protected analogue **2**. Intramolecular hydrogen bonds are shown by dashed lines.

found to exhibit quite different conformational properties depending on their primary structures [4]. Taking these findings into account, our future research will be aimed to investigate conformational and electrochemical properties of muropeptides **III** (Fca-Mur) which contain an exchanged sequence of amino acids relative to **IIb** (Fig. 1). Since the design of bioorganometallics **III** is based on coupling of *N*-protected Fca with *C*-protected Mur, there is a demand to provide a suitable *N*-protected muramic acid. Taken into consideration that the cleavage of *N*-acetyl protecting group of *N*-Ac-Mur-OMe (**1**) requires harsh conditions (refluxing in HCl [5]) incompatible with other sensitive functionalities, its conversion [6,7] into *N*-Boc-Mur-OMe (**2**) that requires milder cleavage conditions (TFA/CH_2Cl_2) [5] is mandatory.

The aim of the study presented in this paper is to acquire additional insights about the novel compound *N*-Boc-Mur-OMe (**2**). The synthesis of the *N*-Boc-Mur-OMe (**2**) is reported, the spectroscopic and structural properties in solution and solid phase are determined and further supplemented with the computational studies (DFT). Since conformational analysis of correlated *N*-Ac-Mur-OMe (**1**) in solution indicated the presence of a strong 8-membered $NH_{Ac}\cdots OC_{ester}$ *intramolecular hydrogen bond* (IHB) in equilibrium with open forms [3] (Scheme 1), the focus of herein presented research is to explore whether the replacement of Ac-protecting group with more sterically demanding Boc-group will influence the conformational properties of the resulted *N*-Boc-Mur-OMe (**2**).

2. Experimental and theoretical methods

2.1. General procedure

Most of the syntheses were carried out under argon. The CH_2Cl_2 used for synthesis and FT-IR was dried (P_2O_5), distilled over CaH_2, and stored over molecular sieves (4 Å). *N*-Acetyl-D-glucosamine (Aldrich), the starting compound for preparation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(metoxycarbonyl)-ethyl]-α-D-glucopyranoside (**1**, *N*-Ac-Mur-OMe), was used as received [8]. All solvents were dried according to general procedures for purification of solvents, unless indicated otherwise. Product was purified by preparative thin layer chromatography on silica gel (Merck, Kieselgel 60 HF_{254}) by using EtOAc/hexane mixture. Melting point was determined by using a Büchi apparatus. Infrared spectra were recorded as CH_2Cl_2 solutions between NaCl windows or as KBr disks by using a Bomem MB 100 mid FTIR spectrometer. The ^1H and ^{13}C NMR spectra were recorded on BrukerAvance 600 MHz in $CDCl_3$ with Me_4Si as internal standard. Spectral assignment was carried out by using standard 2D-spectroscopy.

2.2. Synthesis of benzyl 2-tert-butoxycarbonyl-4,6-O-benzylidene-2-deoxy-3-O-[(R)-1-(metoxycarbonyl)ethyl]-α-D-glucopyranoside (2, N-Boc-Mur-OMe)

The conversion of *N*-Ac-Mur-OMe (**1**) [8] to *N*-Boc-Mur-OMe (**2**) was performed following the procedure for amide to carbamate transformation [7]. *N*-Ac-Mur-OMe (**1**) (1 g, 2.05 mmol), prepared starting from *N*-acetyl-D-glucosamine [8], was dissolved in freshly distilled THF (10 ml) and di-*tert*-butyl dicarbonate $(Boc)_2O$ (0.3 g, 1.37 mmol) and DMAP (58.2 mg, 0.48 mmol) were added. After ½ h stirring at room temperature, the second portion of $(Boc)_2O$ (0.6 g, 2.74 mmol) was added and the stirring was continued at 50 °C for 1 h. Upon addition of third portion of $(Boc)_2O$ (0.6 g, 2.74 mmol), the reaction mixture was refluxed for 3 h and stirred at room temperature for 36 h until no increasing of imide intermediate[1] had been detected by TLC analysis (hexane:ethylacetate = 3:1). The obtained crude imide was diluted with 20 ml of MeOH and *in situ* treated with hydrazine hydrate (0.25 ml, 7.96 mmol). After 24 h of stirring at room temperature, the solvents were removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 10% aqueous solution of citric acid and brine, dried over Na_2SO_4 and evaporated *in vacuo*. After TLC-purification (hexane:ethylacetate = 3:1) the white crystals of pure *N*-Boc-Mur-OMe **2** (0.8 g,

[1] Small amount of intermediate was worked-up as it was described above and subjected to NMR analysis in order to improve the proposed imide structure: ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.49–7.29 (m, 10H, CH_{Ph}), 5.55 (s, 1H, $CH_{benzylidene}$), 4.91(s, 1H, H-1), 4.81(brs, 1H, H-2), 4.65 (d, 1H, *J* = 12.5 Hz, OCH_{2a}-Ph), 4.50 (d, 1H, *J* = 12.0 Hz, OCH_{2b}-Ph), 4.31 (q, 1H,*J* = 6.7 Hz, CH_{Lac}), 4.15 (dd, 1H, *J* = 4.9 Hz, *J* = 9.8 Hz, H-3), 3.87–3.83 (m, 1H, H-4), 3.74–3.67 (m, 2H,H-6b,H-6a), 3.64 (s, 3H, OCH_3),3.58 (pt, 1H, H-5), 2.36 (s, 3H, $COCH_3$), 1.40 (s, 9H, $(C(CH_3)_3)$, 1.37 (d, 3H, *J* = 6.8 Hz, $CH_{3\text{-}Lac}$).