Carbohydrate Research 346 (2011) 678–684

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

The first ferrocene analogues of muramyldipeptide

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ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form 10 December 2010
Accepted 7 January 2011
Available online 15 January 2011

Keywords:
Ferrocene
1′-Aminoferrocene-1-carboxylic acid (Fca),
Muramyldipeptide (MDP)
β-Turn
Intramolecular hydrogen bond (IHB)

ABSTRACT

The two structurally interesting bioorganometallic analogues of muramyldipeptide (MDP) with potential immunomodulatory activity were synthesized starting from the O-protected *N*-acetylmuramic acid (MurNAc), L- or D-Ala and 1′-aminoferrocene-1-carboxylic acid (Fca). They were fully characterized by IR, ^{1}H NMR, ^{13}C NMR and CD spectroscopy as well as by FD mass spectrometry.

The bacterial cell wall is composed of peptidoglycan (PGN) build up of numerous glycan chains that are cross-linked by oligopeptides. Glycan chains contain alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) subunits, with the amino acids coupled to the MurNAc.[1,2] During bacterial growing, PGN undergo enzymatic degradation producing fragments named muropeptides that bear at least one MurNAc and one amino acid moieties. Both in vitro and in vivo experiments confirmed strong influence of muropeptides on the immune system. Namely, muropeptides can improve resistance to infection[3–5] by activating macrophages to generate oxygen radicals which are able to kill microbes.[6,7] Since muropeptides were recognized as the active components in Freund's complete adjuvant[8,9], researches were focused on identification of minimal structural component required for adjuvant activity. Ellouz et al. reported *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyldipeptide, MDP) (Fig. 1) as minimal adjuvant-active subunit of bacterial cell walls.[10] The conformational analysis of MDP using one- and two-dimensional ^{1}H NMR spectroscopy and molecular modeling confirmed the presence of one stable 10-membered β-turn resulting from the formation of intramolecular hydrogen bond (IHB) between the CO_{MurNAc} and the NH_{Ala}[11,12] (Fig. 1).

The proposed IHB involving CO_{Lac} and the NH_{iGln}[11] which would lead to second 10-membered β-turn was not corroborated by the NMR data.[12] In order to mimic the structure of the proposed second β-turn, D-iGln moiety was replaced with rigid proline subunit giving *N*-acetylmuramyl-L-alanyl-3-methoxycarbonyl-D-proline methyl ester (MAP) which was found to adopt an S-shaped structure similar to β-turn.[12]

In order to improve its immunostimulatory properties, the natural MDP has been modified to give numerous analogues which enable the establishment of structure–activity relationship[13–15] (SAR). The most important result of SAR points out that the activity of MDP analogues critically depends on the configuration of Ala and iGln moieties. While the role of Ala is well determined in β-turn formation through hydrogen bonding with CO_{MurNAc},[11,12] the conformation of iGln is less defined.[16] Since (i) the insight in three-dimensional structures of the moieties involved in biological events is crucial for understanding of their function[17] and (ii) differences in biological activity of a series of analogues are not only related to the strength of binding to the receptor but also to differences regarding transport and passage through membranes,[16] our concept was based on replacement of non-hydrogen bonded iGln with amino acid moiety which is supposed not only to be involved in hydrogen bonding providing 3D-structure, but to facilitate the transport through cell membrane, too.

According to our former researches, Fca arouse as a proper candidate due to its well-known turn-inducing role as well as its lipophilicity. Namely, unnatural organometallic amino acid Fca[18] was coupled with natural amino acids in solution as well as in the solid phase.[19–23] The structural analysis of prepared ferrocene peptides confirmed that Fca induces a turn structures with anti-parallel strands which is stabilized by IHB resulting in a hellical conformation of metallocene.

With particular regard to the presence of β-turn structures in MDP and in its sterically constrained analogue MAP, one can expect that replacement of Ala or iGln moieties with turn inducing

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doi:10.1016/j.carres.2011.01.006

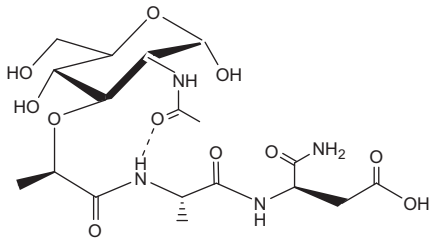
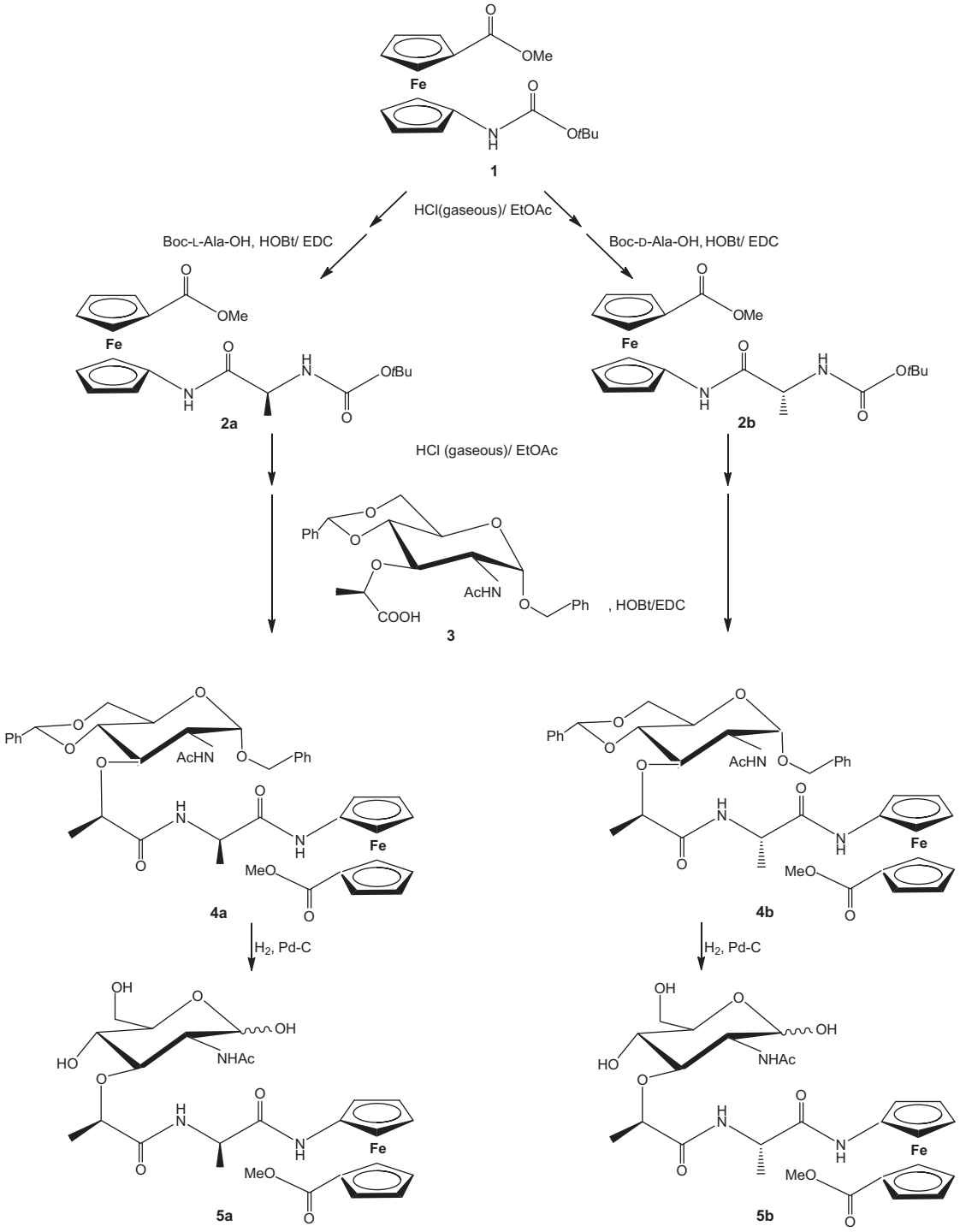


Figure 1. Ten-membered β-turn in MDP.

Fca should generate interesting structural motifs in the resulting analogues.

Furthermore, it is well known that derivatization of established biologically active molecules (tamoxifen, chloroquine) with ferrocene (ferrocifen,[24–26] ferroquine[27]) not only enhanced but also restored their biological activity due to ferrocene unique properties (small size, lipophilicity, easy chemical modifications, redox properties). Consequently, the replacement of muramic, Ala or iGln moieties of MDP with Fca is expected to improve immunostimulatory activity of MDP analogues.



Scheme 1. Synthesis of ferrocene analogues of MDP.