# Synthesis and Cytotoxic Activity of Conjugates of Muramyl and Normuramyl Dipeptides with Batracylin Derivatives

Krystyna Dzierzbicka,*,† Piotr Trzonkowski,‡ Przemysław Sewerynek,§ and Andrzej Myśliwski‡

*Department of Organic Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-952 Gdansk, Poland, and Department of Histology and Immunology and Intercollegiate Faculty of Biotechnology, Medical University of Gdansk, Debinki 1 Street, 80-210 Gdansk, Poland*

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The synthesis of MDP (muramyl dipeptide) or nor-MDP (normuramyl dipeptide) conjugates modified at the peptide part with batracylin (BAT) or batracylin derivatives is described. Batracylin was synthesized by our modified method (Scheme 3). The synthesis of BAT via this modified route now appears to be feasible on a multigram scale. Preliminary screening data obtained at the National Cancer Institute (NCI, Bethesda, MD) have revealed that the conjugates did not expose any cytotoxic activity even at $10^{-4}$−$10^{-8}$ M or $\mu$g/mL. During tests performed at Medical University of Gdansk, Poland, two analogues **11c** and **11e** reduced the proliferation of Ab melanoma cells in vitro compared with batracylin alone (Table 2, Figure 1).

## Introduction

MDP (*N*-acetylmuramyl-L-alanyl-D-isoglutamine), the minimal structure of the *Mycobacterium* cell wall, retains most of its biological activity, in particular the adjuvant activity stimulates nonspecific resistance to microbial infections, exhibits some residual anticancer and antiviral potency, and also acts synergistically with many drugs including antibiotics and anticancer agents.[1,2] Immunoactive properties of muramyl peptides depend on the stimulation or inhibition of the biosynthesis of many cytokines.[3] As we have already shown, MDP and some of its analogues are able to increase the anticancer activity of compounds possessing acridine rings.[4] On the other hand, batracylin, 8-aminoisoindolo-[1,2-*b*]quinazolin-1,2-(10*H*)-one (BAT), displays antitumor activity in vivo against murine leukemia P-388 and colon adenocarcinoma 38 in mice sublines with acquired resistance to adriamycin, cisplatin, and methotrexate.[5,6] Oral administration of batracylin is effective against other murine solid tumors including pancreatic ductal adenocarcinoma, colon adenocarcinoma no. 51, and hepatoma 129.[7] BAT is inactive against B16 melanoma, CD8F1 mammary carcinoma, L1210 leukemia, Lewis lung carcinoma, and human MX-1 mammary xenograft.[5] Recently, the chemotherapeutic mechanism of BAT was shown to differ from that leading to genotoxicity.[8,9] BAT acts as a topoisomerase II inhibitor and induces unscheduled DNA synthesis (UDS) of nonproliferating cells. Susceptibility to BAT toxicity was species-dependent.[10,11] The greater sensitivity of the rat has been associated with a high plasma concentration of the *N*-acetyl metabolite of BAT. The authors suggested that the presence of the metabolite may be associated with its acute toxicity and initiation of the genotoxic response in nonproliferating cells. The role of acetylation in the genotoxicity of the BAT was evaluated in *Salmonella typhimurium* strains expressing various levels of *N*- and *O*-acetyltransferase activity. The results demonstrate that the mutagenicity of BAT is directly related to *N*-acetyltransferase activity.[12] These data suggest that the genotoxic effects of BAT require the free amine while the antitumor effects are independent of the amino group. The limitations associated with the chemotherapeutic potential of BAT, along with the large dose levels required for anticancer activity and high toxicity, especially in rats, therefore turn our attention to the synthesis of its analogues.[13−15] Up to now, several BAT analogues have been synthesized with modifications in positions C8 (Cl, Br, $NO_2$, $CH_3$, $NH_2$) and C7 (Cl).[16] Recently, three patents on different types of BAT analogues were published.[17−19] BAT and its analogues are poorly soluble in water. To increase their solubility in water, they were acylated with amino acids, dipeptide, and tripeptide[17] or coupled to sugar moieties.[18]

Continuing our program of syntheses of MDP (muramyl dipeptide) and nor-MDP (normuramyl dipeptide) conjugates with anticancer active compounds,[20−23] we present syntheses of MDP and nor-MDP analogues that are modified at the C-terminus of the peptide residue by the formation of an amide bond between the isoglutamine carboxylic group and the amine group of the respective BAT or *N*-(*N*$^n$-amino acid)-BAT derivatives. In this paper we describe a modified method of batracylin (BAT) synthesis (Scheme 3). The synthesized conjugates have been submitted to the National Cancer Institute (NCI, Bethesda, MD) and the Medical University of Gdansk, Poland, for testing of cytotoxic activity.

## Chemistry

Conjugates of MDP and nor-MDP with BAT or BAT derivatives [*N*-(*N*$^n$-amino acid)-BAT] were synthesized according to Scheme 1. The protected MDP or nor-MDP **1** were synthesized as described previously.[24,25] These substrates were subjected to a cautious hydrolysis at

* To whom correspondence should be addressed. Telephone: (48 58) 347-27-36. Fax: (48 58) 347-26-94., E-mail: kd@chem.pg.gda.pl.
† Gdansk University of Technology.
‡ Department of Histology and Immunology, Medical University of Gdansk.
§ Intercollegiate Faculty of Biotechnology, Medical University of Gdansk.

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