Synthesis and Antitumor Activity of Conjugates of Muramyldipeptide, Normuramyldipeptide, and Desmuramylpeptides with Acridine/Acridone Derivatives

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The synthesis of two groups (Chart 1, types A and B) of conjugates of MDP (muramyldipeptide) and nor-MDP (normuramyldipeptide) with acridine/acridone derivatives and the synthesis of analogues of desmuramylpeptides (Chart 1, types C and D) containing acridine/ acridone derivatives have been described. In type A conjugates, the hydroxyl group at C6 of the sugar moiety was acylated with acridine/acridone *N*-substituted *ω*-aminoalkanocarboxylic acids (Scheme 1), whereas the conjugates of type B (Table 2) and three analogues of type C or D (Scheme 2) have an amide bond formed between the carboxylic group of isoglutamine and the amine function of the respective acridine/acridone derivatives. The preliminary screening data indicate that the analogues of groups A, C, and D exhibit small cytotoxic activity, whereas several analogues of type B, **4b**, **4c**, **4e**, **4g**, **4h**, **4i**, and **4l,** exhibiting potent in vitro cytotoxic activity against a panel of human cell lines (Table 4), have been selected by the National Cancer Institute (NCI) Evaluation Committee for further testing. Analogues **4b** and **4h** were active in the in vivo hollow fiber assay (Table 5). Analogue **3a** shows an immunostimulating effect on the cytotoxic activity of the NK cells obtained from the spleen of healthy and Ab melanoma bearing animals.

Introduction

In the last 20 years intensive investigation of muramyldipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine, MDP) derivatives revealed their adjuvant activity and stimulation of nonspecific resistance against bacterial, viral, and parasite infections and anticancer properties.[1,2] They can also stimulate tumoricidal activity of macrophages[3,4] and monocytes.[5,6] Our studies have shown that the muramyldipeptide analogues increase the in vitro cytotoxic activity of NK cells, which are able to bind and kill neoplastic and virus-infected cells without prior immunization.[7,8] They also act synergistically with several medicines, such as antibiotics, cytostatics, and natural immunomodulators (trehalose dimycolate, cytokines, lipopolysaccharide, lipid A).[3,9,10] Low anticancer activity of MDP and nor-MDP may be considerably enhanced by certain chemical modifications. In early attempts to enhance its antitumor activity, acylation of MDP at the carbohydrate 6-position with some mycolic acids,[11] hydroxy fatty acids,[12] and quinonylalkanoic acids[13] was found to be successful. It showed that modification of the MDP molecule can be a chance leading to analogues of great pharmaceutical usefulness. The second group of compounds exploited in this project—derivatives of acridine and acridone—are known as strong anticancer agents. However, due to their high toxicity and poor solubility in water, they are seldom utilized in clinical therapy. In our opinion the strong immunostimulating activity of MDP and its synergistic effects in combination with acridine/acridone derivatives may not only strengthen their anticancer activities and improve their pharmacological properties, but also increase the self-defense of the patient organism. To realize this idea, we combined our experience in the synthesis of biologically active MDP analogues with the achievements of the team of A. Ledóchowski,[14] J. Konopa,[15−17] and others[18] in the investigation of the anticancer activity of acridine/acridone derivatives. Unfortunately, both Ledakrin[19] and Amsacrine,[20] the well-known antitumor drugs with an acridine skeleton, are not suitable for coupling to MDP as they are devoid of functional groups capable of forming a covalent bond with a sugar or peptide molecule. Acridine/acridone moieties exploited in the conjugates presented herein correspond to variously modified structures of the antitumor acridine/acridone derivatives.

Besides muramylpeptides, there are also desmuramylpeptides, similar natural peptides devoid of a

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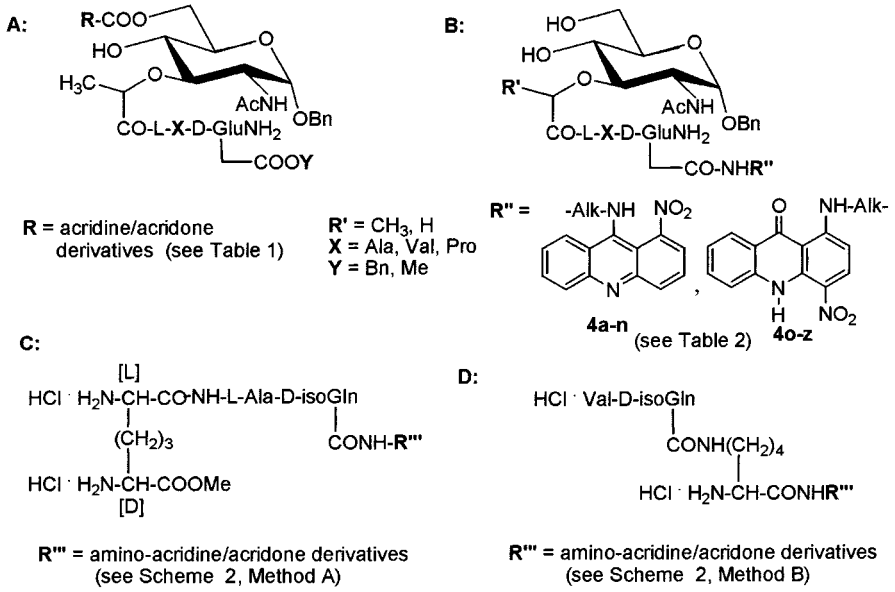
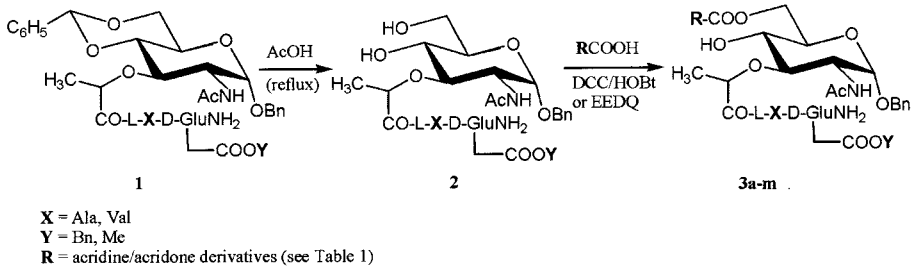
Chart 1



Scheme 1

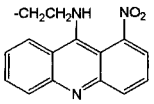
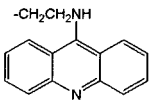
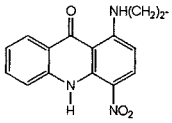
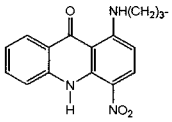
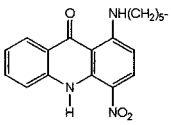
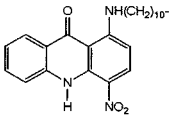
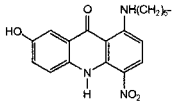
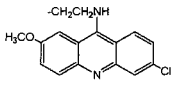
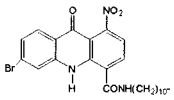
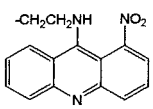
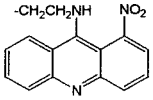
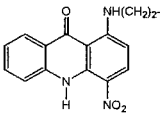
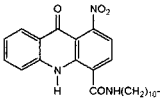


muramyl residue, and their analogues. Studies on some desmuramylpeptides containing *meso*-diaminopimelic acid (*meso*-A_2pm) or L-lysine showed that they are able to enhance the host defense ability against microbial infections; they also exhibit strong antiviral activity and remarkable antitumor potency. It was found that heptanoyl-γ-D-glutamyl-(L)-*meso*-2,6-diaminopimelyl-(L)-D-alanine (FK-565) alone and in combination with zidovudine (AZT) inhibited retroviral infections by Friend leukemia virus in mice. There were even suggestions that FK-565 could be used not only in cancer therapy in conjunction with cancer surgery or/and radiotherapy but also in the treatment of human acquired immunodeficiency syndrome (AIDS).[21] Thus, we expected that incorporation of acridine/acridone derivatives into MDP, nor-MDP, and desmuramylpeptide molecules could not only improve their pharmacological properties by increasing the lipophilicity of MDP analogues but also create an interesting new group of antitumor agents. In this paper we present summary data on the synthesis and both in vitro and in vivo (hollow fiber assay) biological evaluation of two series of MDP and nor-MDP analogues modified at position 6 of the carbohydrate moiety with acridine/acridone *N*-substituted ω-aminoalkanocarboxylic acids or at the C-terminal of the peptide residues by the formation of an amide bond between the isoglutamine carboxylic group and the amine derivatives of acridine/acridone. The synthesis of three analogues of desmuramylpeptides modified with a heterocyclic residue is also reported.

Chemistry

Synthesis of the Conjugates of MDP or nor-MDP with Acridine/Acridone *N*-Substituted ω-amino-alkanocarboxylic Acids (Chart 1, type A). The synthesis of the conjugates was carried out according to Scheme 1. The protected MDP **1** was prepared as described previously.[22] Heating of **1** in aqueous acetic acid caused deprotection of the C4 and C6 hydroxyl groups.[23] The partially protected MDP **2** was acylated with acridine/acridone derivatives in a mixture of pyridine/DMF by means of EEDQ or DCC and HOBt as coupling reagents. To obtain the product acylated selectively only at position C6, the reactions were carried out at low temperature (−10 °C). An excess (2-fold) of the acylating reagent was added to the reaction mixture in a few portions. Two procedures were applied to remove the benzyl group, the protection of the α-glycoside hydroxyl, from the products **3a**, **3b**, and **3d**. The deprotection method depended on the absence or presence of the nitro group in the molecule.[24] Thus, compound **3b** was hydrogenated on palladium black, whereas compounds **3a** and **3d** were treated with α-glycosidase (EC 3.2.1.20) to avoid reduction of the nitro group. Later, on the basis of the assay results, we observed that the removal of the benzyl protection of the hydroxyl group at C1 had practically no influence on the cytotoxic activity of these compounds, because the activities of the protected and unprotected forms were virtually the same. Acridine/acidone *N*-substituted

Table 1. Synthesized Protected Acridine/Acridone−MDP Conjugates **3a**−**m**[a]

compd	R	mp (°C)	X	Y	yield (%)	formula	method
3a		135-39	Ala	Bn	49	$C_{49}H_{55}N_7O_{14}$	DCC/HOBt
					52		EEDQ
3b		Oil	Ala	Bn	61	$C_{49}H_{56}N_6O_{12}$	DCC/HOBt
					65		EEDQ
3c		163-66	Ala	Bn	51	$C_{49}H_{55}N_7O_{15}$	DCC/HOBt
					55		EEDQ
3d		192-95	Ala	Bn	52	$C_{50}H_{57}N_7O_{15}$	DCC/HOBt
					55		EEDQ
3e		158-62	Ala	Bn	50	$C_{52}H_{61}N_7O_{15}$	DCC/HOBt
					49		EEDQ
3f		187-92	Ala	Bn	58	$C_{57}H_{71}N_7O_{15}$	EEDQ
3g		138-43	Ala	Bn	58	$C_{52}H_{61}N_7O_{16}$	DCC/HOBt
					53		EEDQ
3h		137-83	Ala	Bn	40	$C_{50}H_{57}N_6O_{13}Cl$	DCC/HOBt
3i		124-28	Ala	Bn	46	$C_{58}H_{70}N_6O_{16}Br$	EEDQ
3j		143-46	Ala	Me	48	$C_{43}H_{51}N_7O_{14}$	DCC/HOBt
3k		132-36	Val	Me	51	$C_{45}H_{55}N_7O_{14}$	EEDQ
3l		151-53	Val	Me	51	$C_{45}H_{55}N_7O_{15}$	DCC/HOBt
3m		110-14	Val	Me	60	$C_{54}H_{71}N_7O_{16}$	EEDQ

[a] The results of elemental analyses (C, H, N) of all compounds were within ±0.4% of the theoretical values.

ω-aminoalkanocarboxylic acids used for MDP and nor-MDP acylation were prepared according to the procedure published earlier.[19,24] The compounds **3a**, **3c**, **3e**, **3f**, **3g**, **3h**, **3i**, and **3m** have been described previously,[19,24] while **3b**, **3d**, **3j**, **3k**, and **3l** are new (Table 1).

Synthesis of the Conjugates of MDP and nor-MDP with Aminoacridine/Aminoacridone Derivatives (Chart 1, Type B). *N*-(1-*O*-Benzyl-*N*-acetylmuramyl/normuramyl)-L-amino acid-D-γ-isoglutaminyl)-9-(ω-aminoalkyl)amino-1-nitroacridines **4a**−**n** and the *N*-(1-*O*-benzyl-*N*-acetylmuramyl/normuramyl)-L-

Table 2. Aminoacridine (**4a**−**n**) and Aminoacridone (**4o**−**z**) Derivatives of MDP and nor-MDP[a] (See Chart 1, Type B)

compd	R′	X	alk	empirical formula	mp (°C)	yield (%)	method
4a	CH_3	Ala	$(CH_2)_3$	$C_{40}H_{52}N_8O_{12}$	178−182	52	EEDQ
4b	CH_3	Val	$(CH_2)_4$	$C_{45}H_{58}N_8O_{12}$	189−193	69	EEDQ
4c	H	Ala	$(CH_2)_3$	$C_{39}H_{49}N_8O_{12}$	119−120	47	mixed anhydride
4d	H	Pro	$(CH_2)_3$	$C_{43}H_{52}N_8O_{12}$	148−150	52	mixed anhydride
4e	CH_3	Ala	$(CH_2)_4$	$C_{43}H_{54}N_8O_{12}$	77−79	67	mixed anhydride
4f	CH_3	Ala	$(CH_2)_5$	$C_{44}H_{56}N_8O_{12}$	125−127	40	EEDQ
4g	H	Pro	$(CH_2)_5$	$C_{45}H_{56}N_8O_{12}$	161−163	48	EEDQ
4h	H	Ala	$(CH_2)_5$	$C_{43}H_{54}N_8O_{12}$	150−156	50	EEDQ
4i	H	Ala	$(CH_2)_4$	$C_{42}H_{52}N_8O_{12}$	98−101	58	DPPA
4j	H	Pro	$(CH_2)_4$	$C_{44}H_{54}N_8O_{12}$	105−107	60	DPPA
4k	CH_3	Val	$(CH_2)_5$	$C_{46}H_{60}N_8O_{12}$	136−138	55	DPPA
4l	CH_3	Ala	$(CH_2)_2NH(CH_2)_2$	$C_{43}H_{54}N_9O_{12}$	145−147	56	DPPA
4m	H	Ala	$(CH_2)_2NH(CH_2)_2$	$C_{42}H_{52}N_9O_{12}$	124−126	50	DPPA
4n	H	Pro	$(CH_2)_2NH(CH_2)_2$	$C_{44}H_{54}N_9O_{12}$	169−170	65	DPPA
4o	CH_3	Ala	$(CH_2)_2$	$C_{41}H_{50}N_8O_{13}$	192−194	49	EEDQ
4p	CH_3	Ala	$(CH_2)_3$	$C_{42}H_{52}N_8O_{13}$	165−167	52	EEDQ
4r	H	Ala	$(CH_2)_3$	$C_{41}H_{50}N_8O_{13}$	203−205	50	DPPA
4s	CH_3	Ala	$(CH_2)_5$	$C_{44}H_{56}N_8O_{13}$	153−156	51	EEDQ
4t	H	Ala	$(CH_2)_5$	$C_{43}H_{54}N_8O_{13}$	205−207	48	mixed anhydride
4u	H	Pro	$(CH_2)_5$	$C_{45}H_{56}N_8O_{13}$	185−187	54	mixed anhydride
4w	H	Ala	$(CH_2)_2$	$C_{40}H_{48}N_8O_{13}$	220−222	49	DPPA
4x	CH_3	Ala	$(CH_2)_4$	$C_{43}H_{54}N_8O_{13}$	198−200	49	DPPA
4y	H	Ala	$(CH_2)_4$	$C_{42}H_{52}N_8O_{13}$	174−176	58	DPPA
4z	CH_3	Val	$(CH_2)_5$	$C_{46}H_{60}N_8O_{13}$	235−237	55	DPPA

[a] Method of preparation elsewhere. The elemental analyses (C, H, N) of all compounds were within ±0.4% of the theoretical values.

amino acid-D-γ-isoglutaminyl)-1-(ω-aminoalkyl)-4-nitro-9(10*H*)-acridinones **4o**−**z** (Table 2) have been described previously.[25,26] Their cytotoxic and anticancer activities are presented now.

Synthesis of the Conjugates of Desmuramylpeptides with Aminoacridine/Aminoacridone Derivatives (Chart 1, Types C and D). The synthesis of the conjugate types C and D was carried out according to Scheme 2 by amide bond formation between the carboxylic group of isoglutamine (method A) or L-lysine (method B) and the amine group of acridine/acridone derivatives by means of the classical mixed anhydride method. Method A: *N*-Benzyloxycarbonyl-(D)-*tert*-butoxycarbonyl-(L)-*meso*-2,6-diaminopimelic-(D)-methyl ester **5** was synthesized according to the procedure described in previous papers.[27,28] Compound **5** was treated with TFA·H_2N-L-Ala-D-isoGln-OBn using isobutyl chloroformate and NMM in DMF to give tripeptide ester **7** in 85% yield. Removal of the Z protecting groups in **7** by catalytic hydrogenation over 10% palladium charcoal and Boc protection of the amino group at the D-center by treatment with di-*tert*-butyl dicarbonate gave compound **8**. Di-*tert*-butoxycarbonyl-(L)-*meso*-2,6-diaminopimelyl-(D)-methyl ester-(L)-L-alanyl-D-isoglutamine **8** was coupled to 9-[*N*-(β-aminoethyl)-β-aminoethyl]amino-1-nitroacridine **9a** or 1-(γ-aminopropyl)-amino-4-nitro-9(10*H*)-acridinone **9b** using the mixed anhydride method. The product was deprotected with hydrochloride in diethyl ether to give **10a,b** in 40−46% yield (Table 3). Method B: The starting material of *tert*-butoxycarbonyl-L-valyl-D-isoglutamines **11** and **12** was obtained according to standard procedures used in peptide chemistry. For the synthesis of compounds **14** and **15** the classical mixed anhydride method was chosen (Table 3).

The final products (all types) were purified using radial chromatography and preparative TLC. The amino acid composition of the conjugates was confirmed by TLC qualitative amino acid analysis, ^{1}H NMR (500 MHz) spectroscopy, and elemental analyses.

Biological Results and Discussion

The unusual biological activity of 1-nitroacridine derivatives is well documented. Ledakrin (WHO-recommended name Nitracrine), 1-nitro-9[(3′-dimethylamino)-propylamino]acridine, is an antitumor drug that was clinically used for several years some time ago.[29−31] The studies "made of action" [32] showed that Ledakrin is the latent form of the drug and requires metabolic activation before exhibition of any cytotoxic and antitumor activity. Activation results in covalent binding of the drug to DNA and other cellular macromolecules. It was suggested that these cross-links to DNA products play a crucial role in the development of the antitumor activity of the drug.[33,34] W. R. Wilson, W. Denny, et al.[35,36] suggested that nitro group reduction is the major route of Ledakrin metabolism in hypoxic cells, as the drug is selectively toxic to the AA8 cell line growing under hypoxic conditions. In this paper we describe the activity of conjugates of MDP, nor-MDP, and desmuramylpeptides with 1-nitroacridine/4-nitroacridone derivatives. We hoped that combining strong anticancer but toxic acridine/acridone compounds with muramylpeptides would improve their clinical properties. Although the results of the biological assays of the compounds are incomplete, some regularities can be noted: (1) the length of the hydrocarbon chain between muramyldipeptide and the acridine residue plays an important role; i.e., the longer the chain, the more active is the corresponding derivative; (2) compounds containing the acridine moiety are more active than the acridone derivatives; (3) the cytotoxic activity depends on the amino acid residue directly bonded to muramic acid; e.g., the derivative containing alanine is more active than the analogue with proline or valine; (4) among several substituents in the heterocyclic residue (−NO_2, −CH_3O, −Cl, −OH, and −Br) only 1- and 4-nitro derivatives exhibit considerable cytotoxic or anticancer activity; (5) higher activity was demonstrated by type B MDP and nor-MDP analogues **4a**−**n** modified with acridine/acri-

Scheme 2

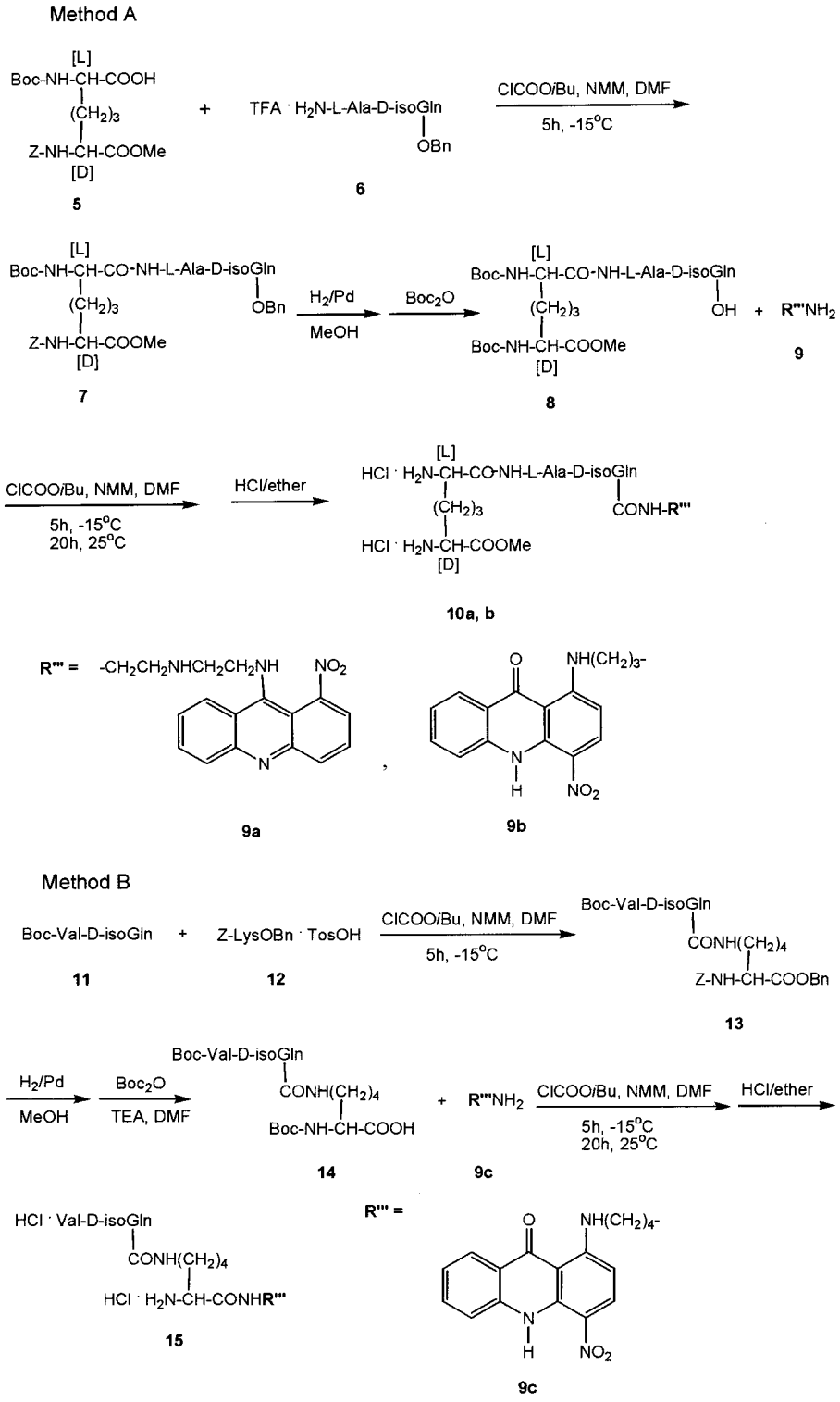


Table 3. Desmuramylpeptides **10a,b** and **15**[a] (See Chart 1, Types C and D)

compd	empirical formula	mp (°C)	yield (%)
10a	$C_{33}H_{46}N_{10}O_8 \cdot 2HCl$	133−136	41
10b	$C_{32}H_{43}N_9O_9 \cdot 2HCl$	117−119	46
15	$C_{32}H_{45}N_9O_7 \cdot 2HCl$	223−224	65

[a] The structures of compounds **10a**, **10b**, and **15** were confirmed by their spectral data (^{1}H NMR) and by elemental analysis.

done derivatives in the peptide part; (6) conjugates containing desmuramylpeptides and acridine/acridone derivatives **(10a**, **10b**, **15)** are totally inactive. The last two remarks are evidence that the cytotoxicity of the presented analogues is due to not only the presence of the acridine residue but also the presence of the MDP moiety.

a. Evaluation of Cytotoxicity. All the final compounds have been sent for cytotoxicity testing to the National Cancer Institute (NC; Bethesda, MD). Most of them have already been checked in the screening system based on 60 human tumor lines.[37−39] The data from this assay are in the form of three histograms for

Table 4. Cytotoxicity of Selected Conjugates in the NCI Cell Line Panel[a]

compd	MID log LC_{50} (M)	Δ	sensitive cell lines[b]
3a	−4.42	0.85	COLO 205, HCC-2998, HCT-116, SK-MEL-2
3g	−4.23	1.0	COLO 205, HCT-116, KM20L2
3m	−4.25	0.91	HL-60(TB), HCC-2998, KM12, OVCAR-3, CAKI-1
4b	−4.17	1.06	M14, MDA-MB-435
4c	−4.97	2.35	HCT-116, SK-MEL-5, 786-O
4e	−5.49	1.73	COLO 205, HCC-2998
4g	−5.51	1.70	COLO 205, HCC-2998, RXF-393, MDA-MB-231/ATCC, MDA-MB-435
4h	−5.82	2.18	COLO 205, HCC-2998, HT29, KM12, MALME3M, SK-MEL-2, UACC-62
4i	−4.26	1.09	HCC-2998
4l	−4.56	1.90	NCIH23, SK-MEL-5

[a] MID = the calculated mean panel LC_{50} concentration (M). LC_{50} = half-lethal concentration. Δ is the number of log units by which the Δ of the most sensitive lines of the panel differs from the corresponding MID. The individual Δ values are calculated by subtracting each log LC_{50} from the panel mean. [b] Sensitive cell lines correspond to Δ values reported in the table.

each compound which represent the concentration of the drug required for 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI), and 50% cell kill (LC_{50}). This method of presentation allows the activity of a given drug against specific tumor cell lines to be quickly ascertained. The results of the assay for the 10 most potent compounds are presented in Table 4 as midvalue LC_{50} (MID log LC_{50}). The MID values are based on a calculation of the average LC_{50} for all of the cell lines tested in which LC_{50} values below and above the test range (10^{-4} to 10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. In Table 4 are also presented Δ values, which express the difference in sensitivity of the most susceptible line and the average value. Computer simulation suggested that values of $\Delta > 1$ (response parameter at least 10 times greater than the average, logarithmic scale) are statistically significant. The results obtained from the National Cancer Institute (NCI) screen showed differentiation of activity on the GI_{50} and LC_{50} levels. The selected compounds were of high activity on the LC_{50} level but low activity on the TGI and GI_{50} levels (the latter data not shown in Table 4). These data suggest that the selected compounds are cytotoxic but not cytostatic. The screen revealed a consistent fingerprint of highly sensitive colon, melanoma, renal, lung, and breast cancer cell lines. The analogues **4b**, **4c**, **4e**, **4g**, **4h**, **4i**, and **4l**, by virtue of their activity and the subpanel disease selectivity, were selected by the NCI Biological Evaluation Committee for further testing in in vivo hollow fiber assay.

b. Hollow Fiber Assay for Preliminary in Vivo Testing.[40] In the assay human tumor cells are cultivated in hollow fibers, which are implanted in mice. Some mice are treated with tested compounds at various concentrations, whereas the control mice receive only the compound diluent. The fiber cultures are collected on the day following the last day of treatment, and the effect of anticancer activity of the tested compounds is calculated on the basis of the net increase in the cancer cell mass. Each compound is tested against a minimum of 12 human cancer cell lines: lung (NCI-H23, NCI-H522), breast (MDA-MB-231, MDA-MB-435), colon (SW-620, COLO-205), melanoma (LOX, UACC-62), ovary (OVCAR-3, OVCAR-5), and gliomas (U251, SF-295). The data of the experiments are reported as % T/C (percent treated/control calculated by dividing the median treated tumor weight by the median tumor weight on each observation day and multiplying by 100) for each of the two compound doses against each of the cell lines with separate values calculated for the intraperitoneal (ip) and subcutaneous (sc) samples. Compounds are selected for further in vivo testing in standard subcutaneous xenograft models on the basis of several hollow fiber assay criteria. These include (1) a % T/C of 50 or less in 10 of the 48 possible test combinations, (2) activity at a distance (intraperitoneal drug/subcutaneous culture) in a minimum of 4 of the 24 possible combinations, and (3) a net cell kill of one or more cell lines in either implant site. To simplify evaluation, a point system has been adopted which allows rapid viewing of the activity of a given compound. For this, a value of 2 is assigned for each compound dose which results in a 50% or greater reduction in viable cell mass. The intraperitoneal and subcutaneous samples are scored separately so that criteria 1 and 2 can be evaluated. Compounds with a combined ip + sc score of ≥20, an sc score of ≥8, or a net cell kill of one or more cell lines are referred for xenograft testing. In Table 5 are presented ip + sc values, sc values, and a net cell kill of one or more cell line compounds. On the basis of the in vivo hollow fiber assay results, three compounds, **4b**, **4e**, and **4h**, were selected by the NCI Biological Evaluation Committee for evaluations in subcutaneous human tumor xenograft assays.

Table 5. Results of the in Vivo Hollow Fiber Assay[a]

compd	ip score	sc score	ip + sc	cell kill activity
4b	32	2	34	Y
4c	2	2	4	N
4e	4	12	16	N[b]
4g	14	0	14	N
4h	16	8	24	Y
4i	4	2	6	N
4l	2	4	6	N

[a] ip = intraperitoneal implants, sc = subcutaneous implants, Y = yes, and N = no. [b] Although the compound did not show activity in the hollow fiber assay, it was directed to investigations on xenograft assays.

c. In Vivo Influence of Compound 3a on the Activity of the NK Cells in Hamsters Bearing Ab Bomirski Melanoma.[41] These investigations were carried out in the Department of Histology and Immunology, Medical University of Gdańsk, Poland.

The examination showed that derivative **3a** had an immunostimulating effect on the cytotoxic activity of the NK cells obtained from the spleen of healthy and Ab melanoma bearing animals. The effect was much more expressed in the melanoma bearing animals than in the healthy ones (Figure 1). In the previous in vitro inves-

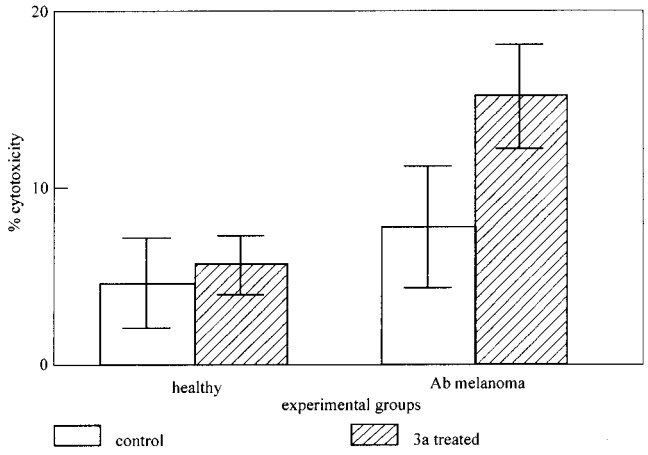


Figure 1. Cytotoxic activity of NK cells obtained from spleen (19 days of tumor growth) (**3a** treated in vivo).

tigation[8] it was found that MDP itself could stimulate NK activity; however, in complex with the acridine derivative the stimulation of **3a** was more effective. Therefore, we assume that a stimulatory effect of the conjugate **3a** depends on both the MDP moiety and the presence of the acridine residue. The mechanism of action of the MDP on NK cells is not known, but a possible role of macrophages can be excluded because adherent cells (macrophages) were removed in the in vitro examination.[8] Also, it is difficult to explain why the stimulatory effect which was observed in the examination in vivo was more marked in the tumor bearing animals than in the control ones. Anyway, it is an advantageous effect from a medical point of view.

Experimental Section

Melting points were determined with a Kofler-block apparatus and are uncorrected. ^{1}H NMR spectra were measured in DMSO solutions with Bruker AM-500 and Varian 500 NMR spectrometers. Preparative column chromatography and radial chromatography were performed on silica gel (Kieselgel 60, 100−200 mesh) in solvent systems specified in the text. All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Merck F_{254} silica gel precoated plates. The following solvent systems (by volume) were used for TLC development: (A) *n*-BuOH−H_2O−AcOH (4:1:1), (B) $CHCl_3$–MeOH (9:1), (C) $CHCl_3$–MeOH (4:1), and (D) the organic layer of a *n*-BuOH−acetone−H_2O (3:2:1) mixture diluted with MeOH (1:10). Elemental analyses were performed by the Laboratory of Elemental Analysis, University of Gdańsk. Qualitative amino acid analyses of the hydrolyzates of the compounds were accomplished on TLC plates.[42]

The following abbreviations also apply: Bn (benzyl), DCC (*N*,*N*′-dicyclohexylcarbodiimide), DMF (dimethylformamide), EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline), HOBt (1-hydroxybenzotriazole hydrate), Me (methyl), NMM (*N*-methylmorpholine), TEA (triethylamine), TFA (trifluoroacetic acid). Benzyl 1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-amino acid-D-isoglutaminate **1** was prepared according to the procedures described previously.[22,42] Benzyloxycarbonyl-(D)-*N*-*tert*-butoxycarbonyl-(L)-*meso*-2,6-diaminopimelic-(D)-methyl ester (**5**) was prepared as described.[27,28] The physical data are in agreement with those given in ref 28.

The general procedure for the synthesis of compounds 3a−m (Scheme 1) was published previously.[24] The new compounds **3b**, **3d**, **3j**, **3k**, and **3l** were prepared according to the same procedure as described for **3a**, **3c**, **3e**, **3f**, **3g**, **3h**, **3i**, and **3m**[24,43] (Table 1).

Benzyl 1-*O*-benzyl-6-*O*-[*N*-(9-acridinyl)-*β*-alanyl]-*N*-acetylmuramyl-L-alanyl-D-isoglutaminate (3b): ^{1}H NMR (DMSO) δ 1.20 (d, J = 7 Hz, 3H, CH_3CH), 1.25 (d, J = 6.7 Hz, 3H, CH_3-Ala), 1.65−1.7 (m, 1H, βCH-isoGln), 1.76 (s, 3H, AcN), 1.95−2.03 (m, 1H, βCH-isoGln), 2.2 (m, 2H, γCH_2-isoGln), 2.34 (t, J = 7.2 Hz, 2H, NHCH$_2$$CH_2$), 2.54 (t, J = 6.9 Hz, 2H, NHCH_2CH$_2$), 3.45−3.9 (m, 6H, H-2,3,4,5,6, sugar moiety), 4.12−4.31 (m, 3H, CH$_3$*C*HCO, NH*C*HCO, NH*CH*CONH$_2$), 4.52 and 4.75 (2d, J = 12.5 Hz, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 4.76 (d, J = 3.4 Hz, 1H, H-1), 5.06 (s, 2H, $CO_2CH_2C_6H_5$), 7.08 (t, J = 7.1 Hz, 2H, C2−H, C7−H), 7.12 and 7.59 (2s, 2H, CONH$_2$), 7.27−7.34 (m, 10H, 2C$_6$H$_5$), 7.28 (t, J = 7.3 Hz, 2H, C3−H, C6−H), 7.32 (t, J = 6.8 Hz, 2H, C4−H, C5−H), 8.15 (br s, 2H, C1−H, C8−H), 10.14 (s, 1H, N10−H). Anal. ($C_{49}H_{56}N_6O_{12}$) C, H, N.

Benzyl 1-*O*-benzyl-6-*O*-[*N*-(4-nitro-9(10*H*)-acridinon-1-yl)-*γ*-aminopropanoyl]-*N*-acetylmuramyl-L-alanyl-D-isoglutaminate (3d): ^{1}H NMR (DMSO) δ 1.20 (d, J = 7 Hz, 3H, CH$_3$CH), 1.24 (d, J = 6.7 Hz, 3H, CH$_3$-Ala), 1.65−1.7 (m, 1H, βCH-isoGln), 1.77 (s, 3H, AcN), 1.95−2.03 (m, 1H, βCH-isoGln), 2.1 (m, 2H, γCH$_2$-isoGln), 2.15−2.24 (m, 2H, CH$_2$$CH_2$-CH$_2$), 2.34 (t, J = 7.4 Hz, 2H, NHCH$_2$CH$_2$$CH_2$), 2.54−2.56 (m, 2H, NH$CH_2CH_2CH_2$), 3.45−3.85 (m, 6H, H-2,3,4,5,6, sugar moiety), 4.10−4.32 (m, 3H, CH$_3$*C*HCO, NH*C*HCO, NH*CH*-CONH$_2$), 4.54 and 4.74 (2d, J = 12.5 Hz, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 4.76 (d, J = 3.4 Hz, 1H, H-1), 5.06 (s, 2H, $CO_2CH_2C_6H_5$), 6.68 (d, J = 9.9 Hz, 1H, C2−H), 7.27−7.33 (m, 10H, 2C$_6$H$_5$), 7.40 (t, J = 7.4 Hz, 1H, C7−H), 7.08 and 7.57 (2s, 2H, CONH$_2$), 7.78 (t, J = 7.3 Hz, 1H, C6−H), 7.92 (d, J = 7.2 Hz, 1H, C5−H), 8.21 (d, J = 8 Hz, 1H, C8−H), 8.36 (d, J = 9.8 Hz, 1H, C3−H), 11.91 (t, J = 5 Hz, 1H, *NH*CH$_2$), 12.38 (s, 1H, N10−H). Anal. ($C_{50}H_{57}N_7O_{15}$) C, H, N.

Methyl 1-*O*-benzyl-6-*O*-[*N*-(1-nitro-9-acridinyl)-*β*-alanyl]-*N*-acetylmuramyl-L-alanyl-D-isoglutaminate (3j): ^{1}H NMR (DMSO) δ 1.20 (d, J = 7 Hz, 3H, CH_3CH), 1.25 (d, J = 6.7 Hz, 3H, CH$_3$-Ala), 1.66−1.72 (m, 1H, βCH-isoGln), 1.76 (s, 3H, AcN), 1.95−2.03 (m, 1H, βCH-isoGln), 2.14 (m, 2H, γCH$_2$-isoGln), 2.34 (t, J = 7.2 Hz, 2H, NHCH$_2$$CH_2$), 2.54 (t, J = 6.9 Hz 2H, NHCH_2CH$_2$), 3.45−3.9 (m, 6H, H-2,3,4,5,6, sugar moiety), 3.54 (s, 3H, CO_2CH_3), 4.12−4.31(m, 3H, CH$_3$*C*HCO, NH*C*HCO, NH*CH*CONH$_2$), 4.53 and 4.74 (2d, J = 12.5 Hz, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 4.76 (d, J = 3.4 Hz, 1H, H-1), 7.08 (t, J = 7.2 Hz, 1H, C7−H), 7.12 and 7.59 (2s, 2H, CONH$_2$), 7.26 (d, J = 7.4 Hz, 1H, C2−H), 7.27−7.34 (m, 10H, 2C$_6$H$_5$), 7.29−7.40 (m, 2H, C4−H, C5−H), 7.49 (t, J = 7.9 Hz, 2H, C3−H, C6−H), 7.85 (d, J = 7.2 Hz, 1H, C8−H), 10.7 (s, 1H, N10−H). The spectrum shows the tautomeric imine form in the acridine structure. Anal. ($C_{43}H_{51}N_7O_{14}$) C, H, N.

Methyl 1-*O*-benzyl-6-*O*-[*N*-(1-nitro-9-acridinyl)-*β*-alanyl]-*N*-acetylmuramyl-L-valyl-D-isoglutaminate (3k): ^{1}H NMR (DMSO) δ 0.88 and 0.89 (2d, J = 6.3 Hz, J = 6.3 Hz, 6H, 2CH$_3$), 1.19 (d, J = 6.7 Hz, 3H, CH_3CH), 1.69−1.71 (m, 1H, βCH-isoGln), 1.77 (s, 3H, AcN), 1.93−2.03 (m, 1H, βCH-isoGln), 2.01 (m, 1H, CH$_3$*C*HCH$_3$), 2.12 (m, 2H, γCH_2-isoGln), 2.32 (t, J = 7.1 Hz, 2H, NHCH$_2$$CH_2$), 2.56 (t, J = 6.8 Hz, 2H, NHCH_2CH$_2$), 3.45−3.9 (m, 6H, H-2,3,4,5,6, sugar moiety), 3.52 (s, 3H, CO_2CH_3), 4.12−4.31 (m, 3H, CH$_3$*C*HCO, NH*C*HCO, NH*CH*CONH$_2$), 4.53 and 4.74 (2d, J = 12.5 Hz, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 4.76 (d, J = 3.4 Hz, 1H, H-1), 7.08 (d, J = 7.2 Hz, 1H, C7−H), 7.12 and 7.59 (2s, 2H, CONH$_2$), 7.26 (d, J = 7.4 Hz, 1H, C2−H), 7.26 (d, J = 7.4 Hz, 1H, C2−H), 7.27−7.34 (m, 10H, 2C$_6$H$_5$), 7.29−7.40 (m, 2H, C4−H, C5−H), 7.49 (t, J = 7.9 Hz, 2H, C3−H, C6−H), 7.85 (d, J = 7.2 Hz, 1H, C8−H), 10.72 (s, 1H, N10−H). The spectrum shows the tautomeric imine form in the acridine structure. Anal. ($C_{45}H_{55}N_7O_{14}$) C, H, N.

Methyl 1-*O*-benzyl-6-*O*-[*N*-(4-nitro-9(10*H*)-acridinon-1-yl)-*β*-alanyl]-*N*-acetylmuramyl-L-valyl-D-isoglutaminate (3l): ^{1}H NMR (DMSO) δ 0.87 and 0.89 (2d, J = 6.3 Hz, J = 6.3 Hz, 6H, 2CH$_3$), 1.19 (d, J = 6.7 Hz, 3H, CH_3CH), 1.69−1.72 (m, 1H, βCH-isoGln), 1.77 (s, 3H, AcN), 1.93−2.03 (m, 1H, βCH-isoGln), 2.01 (m, 1H, CH$_3$*C*HCH$_3$), 2.12 (m, 2H, γCH$_2$-isoGln), 2.34 (t, J = 7.2 Hz, 2H, NHCH$_2$$CH_2$), 2.52 (t, J = 6.9 Hz, 2H, NHCH_2CH$_2$), 3.45−3.90 (m, 6H, H-2,3,4,5,6, sugar moiety), 3.54 (s, 3H, $COOCH_3$), 4.05 (t, J = 7.5 Hz, 1H, NH*C*HCO), 4.10−4.32 (m, 2H, NH*C*HCO, NH*CH*CONH$_2$), 4.45

and 4.68 (2d, J = 12.5 Hz, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 4.76 (d, J = 3.5 Hz, 1H, H-1), 6.68 (d, J = 9.9 Hz, 1H, C2−H), 7.24−7.3 (m, 5H, C_6H_5), 7.40 (t, J = 7.4 Hz, 1H, C7−H), 7.08 and 7.57 (2s, 2H, $CONH_2$), 7.78 (t, J = 7.3 Hz, 1H, C6−H), 7.92 (d, J = 7.2 Hz, 1H, C5−H), 8.21(d, J = 8 Hz, 1H, C8−H), 8.38 (d, J = 9.9 Hz, 1H, C3−H), 11.91 (t, J = 5 Hz, 1H, $NHCH_2$), 12.38 (s, 1H, N10−H). Anal. ($C_{45}H_{55}N_7O_{15}$) C, H, N.

The general procedure for the synthesis of compounds 4a−z (Table 2) was published previously.[25,26] The structures of the compounds were confirmed by their spectral data (^{1}H NMR)[44] and by elemental analyses.[26]

***N*-Benzyloxycarbonyl-(D)-*N*-*tert*-butoxycarbonyl-(L)-*meso*-2,6-diaminopimelyl-(D)-methyl ester-(L)-L-alanyl-D-isoglutamine Benzyl Ester (7).** To a solution of 3.3 g (7.5 mmol) of selectively protected diaminopimelic acid **5** in 24 mL of acetonitrile cooled to −15 °C were added 0.84 mL (7.5 mmol) of NMM and 0.96 mL (7.5 mmol) of isobutyl chloroformate. Three minutes later a cold solution of 3.6 g (11.2 mmol) of TFA·NH_2-Ala-D-isoGln-OBn in 15 mL of DMF and 1.25 mL (11.2 mmol) of NMM in 5 mL of DMF were added. The mixture was stirred for 5 h at −15 °C, and then after addition of 4.7 mL of 2.5 M $KHCO_3$, stirring was continued at 0 °C for 30 min. The reaction mixture was diluted with 30 mL of ethyl acetate, and the acetate solution was washed with the following cooled liquids, solution of potassium hydrogen sulfate (10%), water, solution of potassium hydrogen carbonate (10%), water, and saturated sodium chloride solution, and then dried over magnesium sulfate. After that ethyl acetate was removed with a vacuum rotary evaporator, and the raw product was crystallized from ethyl acetate. As a result 4.4 g (85%) of compound **7** was obtained: mp 150−153 °C; R_f = 0.62 (in solvent system B); ^{1}H NMR (DMSO) δ 1.2 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.35 (s, 9H, $(CH_3)_3$), 1.4−1.8 (m 6H, $(CH_2)_3$), 1.8−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 3.6 (s, 3H, $COOCH_3$), 3.85, 3.95, and 4.2 (4m, 4H, αCH-Ala, αCH-isoGln, 2CH-A_2pm), 5.01 (s, 4H, $2CH_2C_6H_5$), 6.9 (d, J = 10 Hz, 1H, Boc*NH*), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.35 (m, 10H, C_6H_5), 7.7 (d, J = 6.8 Hz, 1H, NH-Ala), 8.0 (d, J = 8 Hz, 1H, NH-isoGln).

Di-*tert*-butoxycarbonyl-(L)-*meso*-2,6-diaminopimelyl-(D)-methyl ester-(L)-L-alanyl-D-isoglutamine (8). The solution of protected tripeptide **7** (3.18 g, 4.3 mmol) in 30 mL of methanol was hydrogenated in the presence of 0.15 g of 10% Pd/C for 4 h. After removal of the catalyst from the reaction mixture by filtration, the solvent was evaporated. The residue was dissolved in 11 mL of DMF, and 0.58 mL (4.3 mmol) of TEA and 1.1 g (4.73 mmol) of $(Boc)_2O$ were added. The mixture was stirred for 24 h at room temperature. Then the reaction mixture was cooled, acidified with 1 N HCl to pH 2−3, and extracted with ethyl acetate (3 × 30 mL). The acetate solution was washed with water and saturated sodium chloride solution and dried over magnesium sulfate, and the solvent was evaporated. The product was crystallized from ethyl acetate−hexane mixture to give 2.2 g (81%) of compound **8**: mp 179−181 °C; R_f = 0.76 (solvent system B); ^{1}H NMR (DMSO) δ 1.2 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.35 (s, 18H, $(CH_3)_3$), 1.4−1.8 (m 6H, $(CH_2)_3$), 1.4−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 3.6 (s, 3H, $COOCH_3$), 3.85, 3.95, and 4.2 (4m, 4H, αCH-Ala, αCH-isoGln, 2CH-A_2pm), 6.9 (2d, J = 10 Hz, 2H, Boc*NH*), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.7 (d, J = 6.8 Hz, 1H, NH-Ala), 8.0 (d, J = 8 Hz, 1H, NH-isoGln), 10.05 (s, 1H, COOH).

General Procedure for the Synthesis of Compounds 10a,b. To a solution of 0.06 g (0.09 mmol) of protected tripeptide **8** in 2 mL of DMF cooled to −15 °C were added 0.01 mL (0.09 mmol) of NMM and 0.12 mL (0.09 mmol) of isobutyl chloroformate. Three minutes later a cold solution containing (0.09 mmol) of aminoacridine/aminoacridone derivative **9** in 2 mL of DMF was added. The reaction mixture was stirred at −10 °C for 5 h and then at room temperature for 24 h. The solvent was evaporated with a vacuum rotary evaporator. The product was poured into an ethereal solution of hydrogen chloride and left for 1 h at 0 °C. Then the solution was decanted, and the residue was washed with ethyl ether several times. The crude product was purified using preparative TLC in solvent system A. The yields and melting points of **10a** and **10b** are given in Table 3.

9-[*N*-[(L)-*meso*-2,6-Diaminopimelyl-(D)-methyl ester-(L)-L-alanyl-D-isoglutaminyl(*β*-aminoethyl-*β*-aminoethyl)]-amino]-1-nitroacridine dihydrochloride (10a): yield 0.028 g (41%); mp 133−136 °C; ^{1}H NMR (DMSO) δ 1.2 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.4−1.8 (m, 6H, $(CH_2)_3$), 1.5 (m, 1H, CH_2NHCH_2), 1.4−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 2.95 and 3.15 (2m, 4H, CH_2NHCH_2), 3.4 and 3.5 (2m, 4H, $COOCH_2CH_2NHCH_2CH_2$), 3.62 (s, 3H, $COOCH_3$), 3.85, 3.95, and 4.2 (4m, αCH-Ala, αCH-isoGln, 2CH-A_2pm), 6.2 (br, 2H, $2NH_3^+$-A_2pm), 6.75 (t, J = 7.4 Hz, 1H, C3−H), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.12−7.45 (m, 4H, C4,5,6,7−H), 7.5 (d, J = 7.2 Hz, 1H, C8−H), 7.6 (d, J = 8.4 Hz, 1H, γNH-isoGln), 7.7 (d, J = 6.8 Hz, 1H, NH-Ala), 7.85 (d, J = 7.8 Hz, 1H, C2−H), 8.2 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.8 (s, 1H, N10−H). Anal. ($C_{33}H_{46}N_{10}O_8$. 2HCl) C, H, N.

1-[*N*-[(L)-meso-2,6-Diaminopimelyl-(D)-methyl ester-(L)-L-alanyl-D-isoglutaminyl(*γ*-aminopropanoyl)]amino]-4-nitro-9(10*H*)-acridinone dihydrochloride (10b): yield 0.032 g (46%); mp 117−119 °C; ^{1}H NMR (DMSO) δ 1.2 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.4−1.8 (m, 6H, $(CH_2)_3$), 1.4−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 1.6 (m, 2H, CH_2(2)-Pr), 2.3 (m, 2H, γCH_2-isoGln), 3.1 (m, 2H, CH_2(1)-Pr), 3.45 (m, 2H, CH_2-(3)-Pr), 3.6 (s, 3H, $COOCH_3$), 3.85, 3.95, and 4.2 (4m, αCH-Ala, αCH-isoGln, 2CH-A_2pm), 6.3 (br, 2H, $2NH_3^+$-A_2pm), 6.7 (d, J = 9.8 Hz, 1H, C2−H), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.35 (m, 1H, C7−H), 7.7 (d, J = 6.8 Hz, 1H, NH-Ala), 7.75 (m, 1H, C6−H), 7.9 (d, J = 8.3 Hz, 1H, γNH-isoGln), 7.95 (d, J = 5.4 Hz, 1H, C5−H), 8.0 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.2 (d, J = 6.8 Hz, 1H, C8−H), 8.35 (d, J = 9.8 Hz, 1H, C3−H), 11.8 (t, 1H, Pr-NH-Acr), 12.4 (s, 1H, N10−H). Anal. ($C_{32}H_{43}N_9O_9$·2HCl) C, H, N.

***N*-*tert*-Butoxycarbonyl-L-valyl-D-isoglutaminyl(*N*α-benzyloxycarbonyl)-L-lysine Benzyl Ester (13).** To a solution containing 0.31 g (0.85 mmol) of Boc-L-Val-D-isoGln-OH (**11**) in 2 mL of DMF cooled to −15 °C were added 0.1 mL (0.85 mmol) of NMM and 0.11 mL (0.85 mmol) of isobutyl chloroformate were. Three minutes later a cooled solution of 0.45 g (0.85 mmol) of TsOH·L-Lys(*N*-Z)-OBn and a mixture of 0.096 mL (0.85 mmol) of NMM in 2 mL of DMF were added. The reaction was stirred for 5 h at −15 °C, then 0.5 mL of 2.5 M $KHCO_3$ was added, and stirring was continued at 0 °C for 30 min. Then the reaction mixture was poured into 200 mL of saturated aqueous sodium chloride solution, and the solid was filtered off and recrystallized from ethanol−hexane to give **13**: 0.51 g (87%) yield; mp 184−86 °C, R_f = 0.68 (solvent system B); ^{1}H NMR (DMSO) δ 0.9 and 0.92 (2d, J = 7.3 Hz, J = 7.3 Hz, 6H, CH_3-Val), 1.35 (s, 9H, $(CH_3)_3$), 1.7 (m, 1H, CH-Val), 1.7−1.9 (m, 6H, $(CH_2)_3$-Lys), 1.8−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 3.2 (m, 2H, ϵCH_2-Lys), 3.9, 3.95, and 4.0 (3m, 3H, αCH-Val, αCH-isoGln, αCH-Lys), 5.05 and 5.1 (2s, 4H, $2CH_2C_6H_5$), 6.9 (d, J = 10 Hz, 1H, Boc*NH*), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.35 (m, 10H, C_6H_5), 7.65 (d, J = 8.5 Hz, 1H, NH-Lys), 7.9 (d, J = 8 Hz, 1H, NH-isoGln), 8.0 (t, J = 9 Hz, 1H, NH-Lys).

***N*-*tert*-Butoxycarbonyl-L-valyl-D-isoglutaminyl(*N*α-*tert*-butoxycarbonyl)-L-lysine (14).** The protected tripeptide **13** (0.36 g, 0.52 mmol) was hydrogenated in the presence of 0.02 g of 10% Pd/C in 35 mL of methanol for 3 h. The reaction mixture was filtered and the solvent evaporated. The residue was dissolved in 2 mL of DMF, and 0.06 mL (0.5 mmol) of TEA and 0.12 g (0.6 mmol) of $(Boc)_2O$ were added. The mixture was stirred for 24 h at room temperature. The reaction was next cooled to 0 °C, acidified with 1 N HCl to pH 2−3, and extracted with ethyl acetate (3 × 30 mL). The acetate solution was washed with water and saturated sodium chloride solution and dried with magnesium sulfate, and the solvent was evaporated. The product was crystallized from the ethyl acetate−hexane mixture to give **14**: 0.198 g (67%) yield; mp 145−147 °C, R_f = 0.87 (solvent system D); ^{1}H NMR (DMSO) δ 0.9 and 0.92 (2d, J = 7.3 Hz, J = 7.3 Hz, 6H, CH_3-Val), 1.35 (s, 18H, $(CH_3)_3$), 1.4−1.9 (m, 6H, $(CH_2)_3$-Lys), 1.7 (m, 1H, CH-

Val), 1.7−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 3.2 (m, 2H, ϵCH_2-Lys), 3.9, 3.95, and 4.1 (3m, 3H, αCH-Val, αCH-isoGln, αCH-Lys), 6.7 and 6.95 (2d, J = 10 Hz, 2H, Boc*NH*), 7.1 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.6 (d, J = 8.5 Hz, 1H, NH-Lys), 7.94 (d, J = 8 Hz, 1H, NH-isoGln), 10.1 (s, 1H, COOH).

1-[*N*-[L-Valyl-D-isoglutaminyl-L-lysyl(δ-aminobutanoyl)]amino]-4-nitro-9(10*H*)-acridinone Dihydrochloride (15). The protected tripeptide **14** (0.06 g, 0.11 mmol) was dissolved in 2 mL of DMF and cooled to −15 °C. Then 0.01 mL (0.09 mmol) of NMM and 0.12 mL (0.09 mmol) of isobutyl chloroformate were added. Three minutes later the cooled solution containing 0.09 mmol of 1-[*N*-(δ-aminobutanoyl)amino]-4-nitro-9(10*H*)-acridinone (**9c**) in 2 mL of DMF was added. The reaction mixture was stirred at −10 °C for 5 h and then at room temperature for 24 h. After evaporation of the solvent the crude product was purified using preparative TLC in solvent system B to afford Boc-L-Val-D-isoGln-ϵ-*N*-Lys-CONH-Acr: mp 204−7 °C; yield 0.061 g (66%); R_f = 0.51 (solvent system B); qualitative amino acid analysis of the hydrolyzates (solvent system D), R_f(Glu) = 0.17, R_f(Val) = 0.39, R_f(Lys) = 0.40, R_f(Acr) = 0.62. The obtained Boc-Val-D-isoGln-ϵ-*N*-Lys-CONH-Acr was poured into a saturated solution of hydrogen chloride in diethyl ether and left for 1 h at 0 °C. Then the solution was decanted, and the residue was washed several times with ethyl ether. The product 2HCl·Val-D-isoGln-ϵ-*N*-Lys-CONH-Acr (**15**) was obtained: yield 0.039 g (65%); mp 223−224 °C; ^{1}H NMR (DMSO) δ 0.95 and 0.97 (2d, J = 7.3 Hz, J = 7.3 Hz, 6H, CH_3-Val), 1.4−1.95 (m, 6H, $(CH_2)_3$-Lys), 1.55−1.7 (2m, 4H, CH_2(2,3)-But), 1.7 (m, 1H, βCH-Val), 1.8−1.9 and 1.95−2.05 (2m, 2H, βCH_2-isoGln), 2.3 (m, 2H, γCH_2-isoGln), 3.1−3.25 (2m, 4H, CH_2(1)-But, ϵCH_2-Lys), 3.45 (m, 2H, CH_2(4)-But), 3.9, 3.95, and 4.0 (3m, 3H, αCH-Val, αCH-isoGln, αCH-Lys), 6.45 (br, 6H, NH_3^+-Lys, NH_3^+-Val), 6.7 (d, J = 9.8 Hz, C2−H), 7.15 and 7.3 (2s, 2H, $CONH_2$-isoGln), 7.35 (d, J = 6.8 Hz, 1H, C7−H), 7.75 (t, J = 7.3 Hz, 1H, C6−H), 7.92 (d, J = 8.2 Hz, 1H, ϵNH-Lys), 7.95 (d, J = 5 Hz, 1H, C5−H), 8.05 (d, J = 8 Hz, H, NH-isoGln), 8.25 (d, J = 9 Hz, 1H, Lys-NH-But), 8.25 (d, J = 6.8 Hz, 1H, C8−H), 8.35 (d, J = 10 Hz, 1H, C3−H), 11.8 (tr, J = 5 Hz, 1H, But-NH-Acr), 12.45 (s, 1H, N10−H). Anal. ($C_{32}H_{45}N_9O_7$·2HCl) C, H, N.

NCI in Vitro Cytotoxicity Assays. NCI uses the sulforhodamine B assay for assessing the cytotoxicity of test agents in their panel of 60 cell lines.[37−39] Briefly, the cell lines were inoculated into a series of 96-well microtiter plates, with varied seeding densities depending on the growth characteristics of particular cell lines. Following a 24 h drug-free incubation, test agents were added routinely at five 10-fold dilutions with a maximum concentration of 10^{-4} M. After 2 days of drug exposure, the change in protein stain optical density allowed the inhibition of cell growth to be analyzed.

In Vivo Influence on the Activity of the NK Cells in Hamsters Bearing Ab Bomirski Melanoma.[41] The derivative **3a** was dissolved in PBS/DMAC (dimethyl acetamide), 30 μL of DMAC/100 μL of PBS for sample.

Animals. Male Syrian golden hamsters (*Mesocricetus auratus* Watherhouse) at 3−4 months of age, weight about 100−130 g, were used in the study. They were obtained from a commercial breeder and maintained in the animal facility on a standard diet and water ad libitum. The Bomirski melanoma variant Ab was maintained by serial passage in hamsters using the suspension method of tumor implantation. Tumor cells were implanted by subcutaneous injection at 25 mg of viable tumor tissue suspended in 0.5 mL of 0.9% NaCl on the right side of the animals at the start of the experiments.

Effector Cells. On the 19th day of inoculation, the animals of all groups were sacrificed in ether anesthesia. The blood was collected by heard puncture, and then the sera were isolated and stored at −70 °C. Spleen mononuclear cells used as effector cells to determine the NK cytotoxic avtivity were isolated from the healthy and Bomirski melanoma[45] bearing hamsters. The cells were obtained by mechanically crushing the spleen in phosphate saline (PBS). The suspension was centrifuged on Lymphoprep gradient at 800*g* for 15 min, washed three times with PBS, and suspended at 1×10^6 cells/mL in RPMI 1640 medium containing 5% FCS (fetal calf serum). The adherent cells were removed by incubation of the spleen cells suspended in the medium on the plastic plate at 37 °C in 5% CO_2 for 2 h. Nonadherent cells were removed, counted, and suspended in the same medium at a concentration established by a pilot study. Using the histochemical reaction for the nonspecific esterase characteristic for monocytes, confirmation of removing adherent cells was obtained.

Target Cells. K562-human erythroleukemic cells were used as the target for the NK cytotoxic test with effector NK hamster cells. The line was kept in RPMI 1640 (Gibco) medium supplemented with FSC to give a concentration of 5%, 100 μg of streptomycin, 100 UI/mL of pencillin, and 2 mmol of L-glutamine at 37 °C in a 5% CO_2 atmosphere.

Assessment of the NK Cell Activity. A pellet of target cells (K562) was labeled with 100 μCi of Na_2CrO_4 (^{51}Cr) at 37 °C in an atmosphere containing 5% CO_2 for 1 h, then washed with RPMI medium supplemented with 5% FCS, and suspended in the same medium at 1×10^5 cells/mL. Samples were run in triplicate. Then thousands of these cells were used for each sample. The target cells were mixed with effector cells at a 25:1 ratio. The samples were incubated in 5% CO_2 at 37 °C for 4 h and then centrifuged at 900 rpm for 10 min, and 0.135 μL of supernatant was removed and counted for γ radioactivity in an LKB γ counter. The following formula was used to calculate the percent of chromium release (% cytotoxicity):

$$\%\ \text{cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

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