Structure, Biological Activity and Synthesis of Polyamine Analogues and Conjugates

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The structure and the biological significance of naturally occurring and synthetic polyamine analogues and conjugates are presented and the available methodologies for their synthesis are described. These methodologies involve either the selective functionalization of the amino functions, using suitable protecting groups or acylating agents, or fragment synthesis protocols. The latter employ suitable amino components and simple reactions, like Michael addition to α,β-unsaturated nitriles, alkylation of amines and sulfonamides, reductive alkylation and acylation followed by reduction of the thus-obtained amides, as key-reactions, or azides as key-intermediates, for the assembly of the polyamine skeleton. Syntheses performed in solution as well as in the solid phase are discussed.

Introduction

Polymethylene polyamines (PAs), either in their free polycationic form or conjugated to other biomolecules in a linear or cyclic fashion, occur naturally and show interesting biological activities. The determination of their structure and the need for a deeper understanding of their mode of action has led in recent years to the preparation of a vast array of synthetic PA analogues and conjugates in the search for the development of PA-based compounds with potential pharmaceutical or technical applications. A number of reviews have already appeared in the literature describing the biological activity of natural PAs and synthetic analogues, with particular emphasis in their use as anticancer agents and their involvement in the function of CNS.[1] In addition, in recent years it has been realized that the naturally occurring PAs and conjugates, as well as their synthetic analogues, are involved in a multitude of other important biological functions and show promise as antiparasitic agents, antimalarials, antidiarrhoeals, anti-HIV agents, metal chelators and gene delivery agents.

In the present Microreview, we will concentrate mainly on the available synthetic methods which allow the efficient preparation of linear PA analogues and conjugates, taking examples from the most recent literature. However, a short description of the structural variety of the most common natural PAs and conjugates, as well as their synthetic counterparts, and their biological significance will be also considered and actually forms the first two chapters of this work. Chapter 3 deals with the various synthetic protocols which have been developed for the synthesis of PA analogues and conjugates. These protocols are divided into

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George Karigiannis was born in Athens, Greece, in 1971. He studied chemistry at the University of Patras and completed his undergraduates studies in 1994. He then joined Prof. Papaioannou's research group and completed his PhD thesis in 1999. His research focused on the preparation of selectively N-mono- and -bisalkylated polyamines and polyamine alkaloids of the kukoamine A type, both in the liquid and the solid phase. He has been a visiting researcher in the Universities of Calabria and Napoli, Italy and Bergen, Norway through the ERASMUS/TMR student/researcher exchange programmes.

Dionissios Papaioannou was born in Patras, Greece, in 1952. He studied chemistry at the University of Patras from where he graduated in 1974. He carried out postgraduate studies at Imperial College of Science and Technology in London and completed his PhD thesis on syntheses using isonitriles under the supervision of Prof. D. H. R. Barton in 1997. In 1980 he joined the faculty of the Chemistry Department of the University of Patras as a lecturer. Since 1996 he is professor of Organic Chemistry. He has conducted postdoctoral work at Imperial College and the Chemistry Department of the University of Bergen, Norway on organochromium and organofluorine compounds with Dr. D. A. Widdowson and terpenyl carbohydrates with Prof. G. W. Francis, respectively. His main research activities have been focused on the development of methodologies for the efficient synthesis of peptides in liquid and on solid phase, the synthesis of amino acid derivatives suitable for use in peptide synthesis, the asymmetric synthesis of biologically interesting amino acids using suitable proteinogenic or other naturally occurring amino acids as chiral templates and the synthesis of alkaloids and terpenyl polyols and aminopolyols. Recently, his research interests lie in the area of the total synthesis of polyamine analogues and conjugates and chiral isodideoxynucleoside analogues using readily available amino acids as starting materials.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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Table 1. Abbreviations used in this Microreview

γAba	γ-aminobutyric acid	Ac	acetyl	Acbz	4-azidobenzyloxycarbonyl
Ad	adamantyl	βAla	β-alanine	All	allyl
Alloc	allyloxycarbonyl	Arg	arginine	Asn	asparagine
Asp	aspartic acid	Bn	benzyl	Boc	*tert*-butoxycarbonyl
Boc-ON	*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile	BOP	benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate	Bpoc	2-(4-biphenylyl)prop-2-yloxycarbonyl
Bz	benzoyl	CAD	cadaverine	CDI	carbonyldiimidazole
CNS	central nervous system	Cys	cysteine	DCC	*N,N'*-dicyclohexylcarbodiimide
DCM	dichloromethane	Dde	*N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl	DIBAH	diisobutylaluminium hydride
DIC	*N,N'*-diisopropylcarbodiimide	DIEA	diisopropylethylamine	Dmbz	2,3-dimethoxybenzoyl
DPPA	diphenylphosphoryl azide	DTT	dithiothreitol	EHM	ethyl hydrogen malonate
Etoc	ethoxycarbonyl	Fmoc	9-fluorenylmethoxycarbonyl	For	formyl
Glu	glutamic acid	Gly	glycine	GSPS	glutathione SPD synthase
HCA(s)	hydroxycinnamic acid(s)	cHp	cycloheptyl	HMPA	4-hydroxymethylphenoxyacetic acid
HOBt	1-hydroxybenzotriazole	HSPD	homospermidine	$HT(S)_2$	homotrypanothione
		HSPM	homospermine		
[L]	linker	Lys	lysine	Met	methionine
Mtr	*p*-methoxytrityl	Mts	mesitylenesulfonyl	Nde	*N*-1-(4-nitro-1,3-dioxoindan-2-ylidene)ethyl
NMDA	*N*-methyl D-aspartic acid	NSPD	norspermidine	$NT(S)_2$	nortrypanothione
		NSPM	norspermine		
Orn	ornithine	(P)	polymeric support	PA(s)	polyamine(s)
PCtr	2-chlorotrityl resin	Phth	phthalyl	Pip	piperidine
PLC	phospholipidase C	Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl	cPr	cyclopropyl
PUT	putrescine	Py	pyridine	PyS	pyridine-2-sulfonyl
SES	trimethylsilylethanesulfonyl	SPD	spermidine	SPM	spermine
		Spd		Spm	
SPS	solid-phase synthesis	Su	succinimide	TBDPS	*tert*-butyldiphenylsilyl
TCBoc	2,2,2-trichloro-*tert*-butoxycarbonyl	Tf	triflyl	Tfa	trifluoroacetyl
TFA	trifluoroacetic acid	TFE	trifluoroethanol	TMS	trimethylsilyl
TR	trypanothione reductase	Troc	2,2,2-trichloethoxycarbonyl	Trt	trityl (triphenylmethyl)
Ts	4-toluenesulfonyl	$T(S)_2$	oxidized trypanothione	$T(SH)_2$	reduced trypanothione
Tyr	tyrosine	Z	benzyloxycarbonyl	$Z(NO_2)$	4-nitrobenzyloxycarbonyl

those which simply involve the selective modification of the amino functions of PAs, and the fragment-synthesis-related protocols through which the PA skeleton is assembled with the desired length and number of differently functionalized nitrogen atoms using relatively simple amino building blocks. Finally, Chapter 4 focuses on the most recent syntheses of PA analogues and conjugates using solid supports to perform either the selective functionalization of the amino functions of PAs or the assembly of the PA skeleton. A list of abbreviations used throughout this Microreview can be found in Table 1.

1. Naturally Occurring Polyamines and Conjugates and Their Biological Significance

1.1 Biogenic Polyamines: Structure, Biosynthesis and Biological Activity

Polyamines, such as PUT (**1**), SPD (**2**) and SPM (**3**) (Figure 1) are natural products, found in micro-organisms, plants and animals, which are involved in a variety of important biological functions.[1] Biosynthetically, the 1,4-diaminobutane unit of polyamines originates from L-Orn or L-Arg, whereas the aminopropyl unit comes from L-Met. On the other hand, SPM and SPD are catabolized to PUT by N^1-acetylation followed by oxidation of the thus obtained N^1-acetylpolyamine. The intracellular concentration of PAs is regulated by changes in the activities of the biosynthetic and catabolic enzymes involved and the PA uptake and efflux systems.[2] The most important factors appear to be the regulation of the biosynthesis of PAs and their transport through the membranes.[3,4]

PAs interact strongly with nucleic acids and play an important role in their biosynthesis and metabolism. The interaction of PAs with molecules of DNA results in stabilization of their conformation. This stabilization occurs through the electrostatic attraction between the polycationic PA molecules and the negatively charged phosphate groups on the DNA molecules.[1b,5] For the same reason, PAs can form bridges with a molecule of DNA, or two different molecules, thus inducing structural changes in the DNA.[6] The DNA−PA interactions also account for the protection of DNA from denaturation which can be caused by heat, chemical reagents or radiation.[7] Furthermore, PAs cause specific modifications in certain specialized RNA molecules,[1b,7,8] stabilize ribonucleases[9] and stimulate the action of ribonucleases[1b] and ribozymes.[10] PAs affect the protein synthesis in several ways.[1d,5a,8,11−16] Furthermore, they are essential for normal growth and are even involved in the differentiation of mammalian cells.[1d,12a,17] The concentrations of PAs and the enzymes responsible for their biosynthesis are notably higher in fast multiplying animal tissues and increase rapidly when growth or differentiation is induced in the rest of the cells. PAs are directly responsible for the increase of the rate of the macromolecular synthesis which takes place during development or tumour growth.[1f,1h]

PAs are also involved in the modification of the NMDA receptor[18] and attempts have been made to exploit this interaction for the therapy or prevention of neurotoxicity, epilepsy and neurodegenerative diseases.[19] The modification of other than NMDA receptors and ion channels with PAs

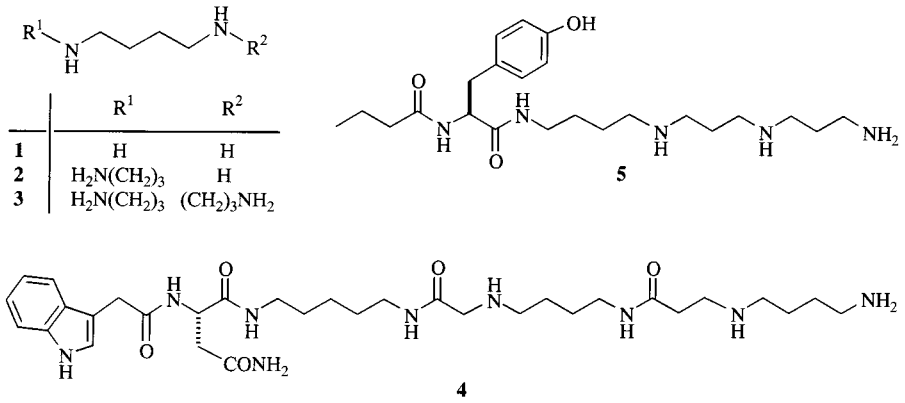


Figure 1. Biogenetic polyamines (**1**−**3**) and polyamine conjugates with amino acids (**4**, **5**)

has been also reported,[20] whereas Alzheimer's disease has been recently associated with a PA homeostasis disorder.[21] In addition, PAs interact with the phospolipids of biological membranes and inhibit their peroxidation.[22] This particular action of polyamines has a beneficial effect on the structure of biological membranes[23] and the defence of the organisms against tumour development.[24]

1.2 Polyamine Conjugates

A large number of biologically interesting PA conjugates have recently been isolated from natural sources and their biological action studied. There is a growing interest not only in the study of the mechanism of their action, but also in their total synthesis and possible commercial exploitation. PA conjugates may be classified as follows:

1.2.1 Polyamine Conjugates from Spiders and Wasps

A variety of PAs conjugated to amino acids, such as Tyr, Arg, Asn, βAla, Gly and Lys, have been isolated from the venom of spiders and wasps.[25,26] Nephilatoxin-643 (NPTX-643, **4**) and philanthotoxin-433 (PhTX-433, **5**) are examples of spider and wasp toxins, respectively.

The interest in these acylpolyamines arises from their ability to act as potent antagonists of the mammalian neuroexcitatory glutamic acid receptors; some of them also inhibit the nicotinic acetylcholine receptors. Furthermore, they have been used as tools for the understanding of excitatory amino acid neurotransmission.

1.2.2 Macrocyclic Alkaloids from Plants

Plants also contain a plethora of SPD and SPM conjugates (Figure 2). Representative examples are the macrolactam SPD alkaloids oncinotines, such as the oncinotine (**6**), and inandenines, such as the inandenine-12-one (**7**), and the SPM alkaloids pithecolobines (**8**).[27,28] Other examples of macrocyclic SPD alkaloids are the loesenerines, such as the (*R*)-loesenerine (**9**), and myricoidine alkaloids, which contain a thirteen-membered lactam ring made up of SPD and part of a C_{10} fatty acid.[29] Of similar structure is the alkaloid celacinnine (**10**) in which the ring is formed by SPD and a β-phenylpropionate unit.[30] A β-phenylpropionate unit is also present in the seventeen-membered ring of the SPM alkaloid (*S*)-verbasitrine (**11**)[31] and the protoverbine class of SPM alkaloids.[32] A seventeen-membered ring is also contained in the antibacterial and cytotoxic SPM alkaloids the budmunciamines.[33]

1.2.3 Linear Spermidine and Spermine Alkaloids from Plants

In addition to macrocyclic alkaloids, plants also contain a variety of linear PA alkaloids. A classic example is the SPD alkaloid meytenine (**12**, Figure 3).[30] A variety of other SPD alkaloids have been isolated and characterised from the reproductive organs of many higher plants which are actually SPD amides of HCAs, such as *p*-coumaric, ferulic, caffeic or sinapic acid. These alkaloids often contain one HCA unit, usually at the position N^4,[34] two HCA units at the positions N^1 and N^4, N^4 and N^8 or N^1 and N^8, e.g. the alkaloid **13**,[35] or three HCA units.[35] These units can be either the same or combinations of HCA units.[36] Recently, N^4-benzoylspermidine[37] and tenuilobine (**14**)[38] were isolated from the same source. Compound **14** is the first PA alkaloid containing both SPM and SPD, cross-conjugated through a long aliphatic chain.

Linear SPM alkaloids, such as kukoamines A (**15**) and B (**16**) (Figure 3) have been isolated from the extract of the root bark of the plant *Lycium chinense*.[39,40] These alkaloids contain two dihydrocaffeoyl moieties per molecule of SPM. Kukoamine A shows hypotensive properties and therefore is of interest from the medicinal point of view.

1.2.4 Linear Polyamine Alkaloids from Marine Organisms

It has recently been shown that secondary metabolites of the PA type from marine sponges have interesting biological properties. For example, the SPD conjugate pseudokeratidine (**17**, Figure 4) shows antifouling and antimicrobial activity[41,42] and is therefore of considerable commercial interest as a component of antifouling paints. Another example is the penaramides, an inseparable mixture of PA amides, which strongly inhibit the *N*-type Ca^{2+} channels.[43] SPD metabolites like **18**, isolated from a soft coral, exhibit cytotoxic properties.[44] Ptilomycalin A (**19**) is a complex marine

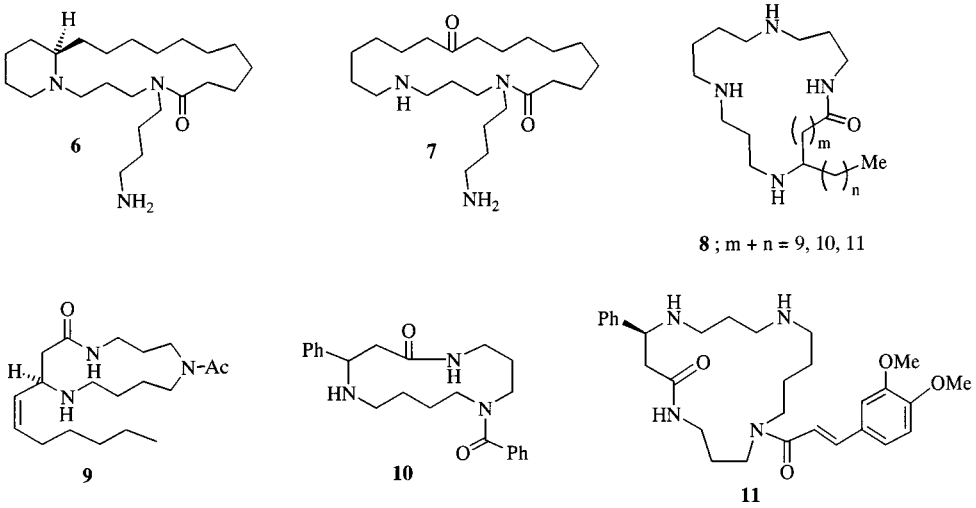


Figure 2. Representative examples of macrolactam SPD and SPM alkaloids

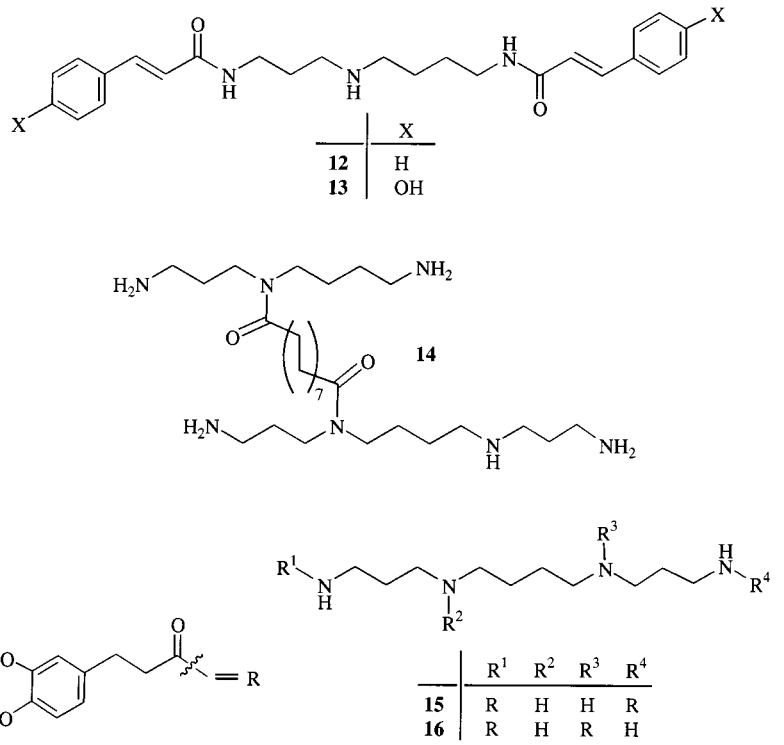


Figure 3. Representative examples of linear plant SPD and SPM alkaloids

SPD alkaloid which shows potent antiviral and antibiotic activity.[45]

1.2.5 Various Polyamine Conjugates

Trypanosomatid parasites such as *Leishmania* and *Trypanosoma cruzi*, which invade and infect the mammalian CNS, use endogenous antioxidant molecules to confront the oxidative stress from various active oxygen species. The SPD−glutathione conjugate $T(SH)_2$ is the sole antioxidant molecule of the parasites which is oxidized by oxygen reactive species with the formation of a disulfide bridge, giving rise to the cyclic conjugate $T(S)_2$. The survival of the parasite depends on the enzyme TR which reduces $T(S)_2$ back to $T(SH)_2$ (Scheme 1). The above mentioned alkaloid kukoamine A has been recently shown to be an inhibitor of TR.[46]

The SPD alkaloid ispidospermidine (**20**, Figure 5) is a natural inhibitor of the enzyme PLC, which affects the cellular growth, the differentiation and the multiplication of the cell.[47] Several PA natural products with interesting biological functions incorporate the guanidino function. Examples are the alkaloid hirudonine (**21**),[1a,48] the potent immunosuppressive drug (±)-15-deoxyspergualin (DSG)[49] and the novel octapeptides MS-681a-d, inhibitors of the myosin light-chain kinase, which incorporate α-alkyl-α-amino acids and a PA chain.[50]

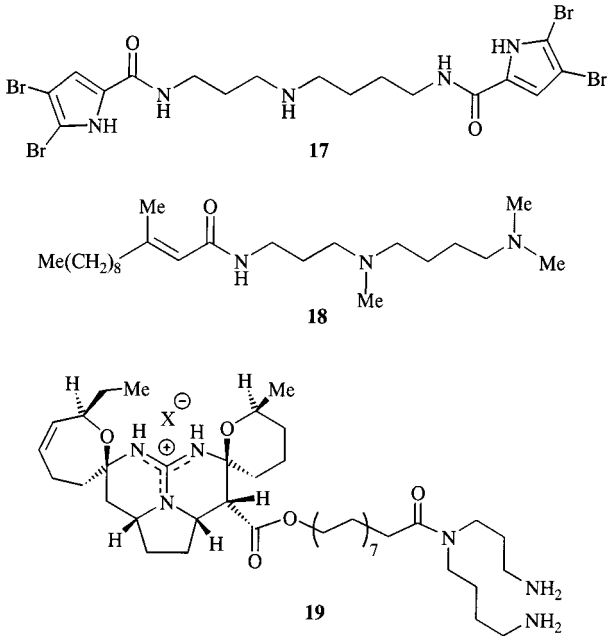
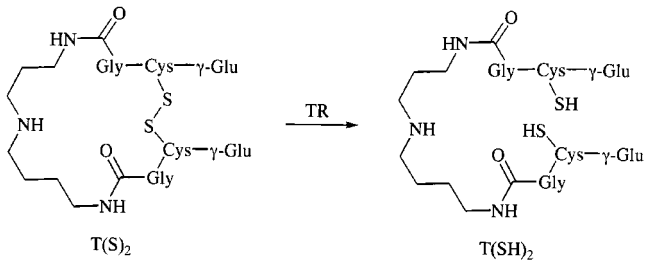


Figure 4. Examples of marine SPD alkaloids



Scheme 1. Trypanothione reductase mediated transformation of trypanothione to reduced trypanothione

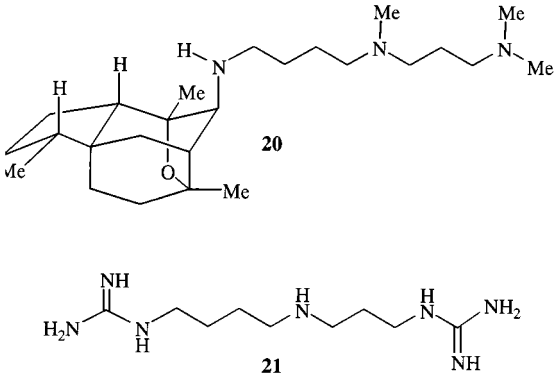


Figure 5. Biologically interesting SPD alkaloids

2. Synthetic Polyamine Analogues and Conjugates and Their Biological Significance

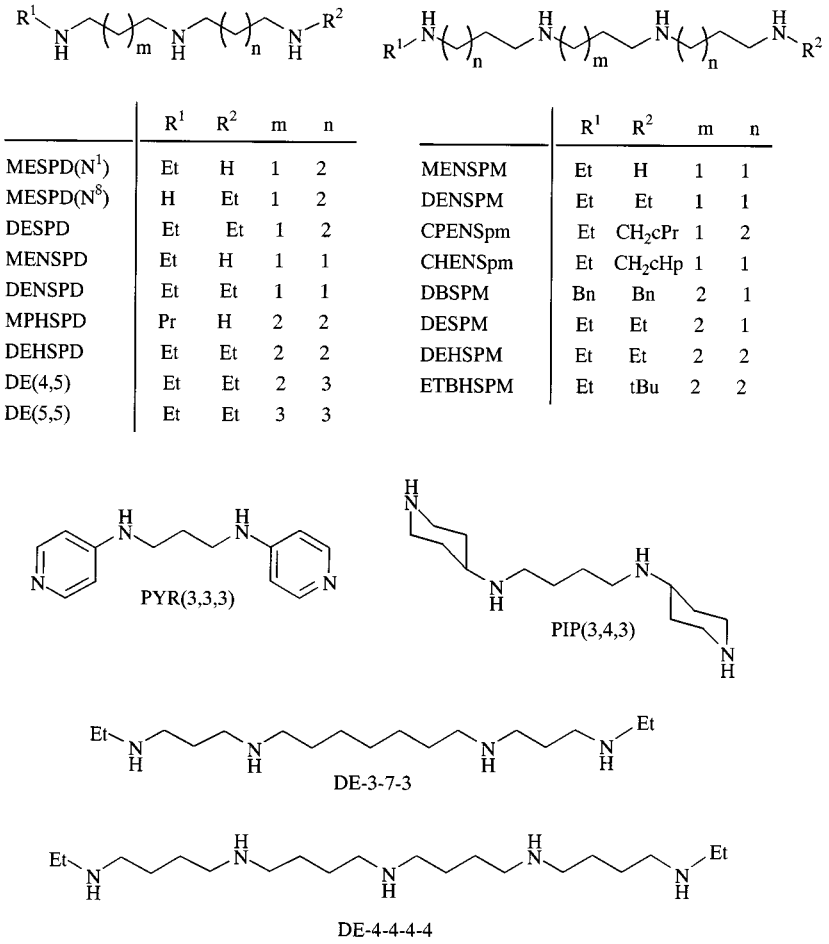
2.1 Linear Polyamine Analogues and Anticancer Activity

Due to the fact that in tumour cells there is a high concentration of PAs and a higher activity of the anabolic enzymes, inhibitors for all the enzymes involved in the biosynthesis of PAs have been developed for use as anticancer agents.[1f] The most interesting ones are the inhibitors of the L-Orn and *S*-Adenosyl-L-Met decarboxylases.[51–53] However, therapies based on such inhibitors, or particular combinations, have failed with human cancers possibly due to the uptake of exogenous PAs from foods.[54,55,1h] This problem is more pronounced with the cancerous cells which exhibit increased activity of the PA uptake system compared to healthy cells.[53,56]

In recent years, a series of PA analogues have been synthesized mainly by Bergeron et al., Edwards et al. and Woster et al., which partially mimic natural PA behaviour, do not support the cell growth and are metabolically stable. A selection of such analogues is presented in Figure 6. These analogues are designed on the basis of the structure of natural PAs and their interactions with DNA which, as mentioned above, result in well described changes in its conformation. Having similar structure with natural PAs, these analogues can efficiently use the transport system of the former to enter the cells, having at the same time better specificity for cancer than normal cells and low toxicity.

In these analogues, changes of the basic skeleton of natural PAs concern substitution of the amino functions, homologation of the hydrocarbon chain and alteration of the number of nitrogen atoms in the chain. Thus, from structure-activity relationship studies of a large number of PA analogues,[57] which were carried out to identify those structural characteristics which determine the best cytotoxic ability of the PA analogues against tumor cells, it appears that this ability depends on the number of nitrogen atoms in the chain, the distance between the nitrogen atoms, the nature of the terminal alkyl substituents and the charge these molecules can bear in physiological pH. Weakly charged PA analogues cannot compete with natural PAs for transport through the cell membranes.[58] PAs bearing bulky alkyl substituents on the terminal nitrogen atoms are not efficiently transported, whereas the distance between the nitrogen functions appears to play an important role in their recognition by the PA uptake system.[58,59] Parallel work has been carried out in procaryotic cells.[11]

It is worth mentioning that different PA analogues are transported by different transport systems.[1f,60] PA analogues with Et or Bn groups at the terminal nitrogen atoms strongly inhibit the cell proliferation and thus constitute promising candidates for cancer treatment.[59][61] Bergeron et al. have shown that the diethylation of the terminal nitrogens of PA molecules is one of the most effective modifications of the natural PA skeleton towards cancer cell cytotoxicity.[58,62] The therapeutic effect of diethyl analogues appears not to be affected by the presence of exogenous PAs. The most promising analogues of this type, which are already under clinical trials, are N^1,N^{15}-Et_2-4,12-diaza-1,15-diaminopentadecane (DE-3-7-3),[57e] N^1,N^{11}-Et_2-4,8-diaza-1,11-undecane (DE-3-3-3 or DENSPM)[62] and N^1,N^{19}-Et_2-5,10,15-triaza-1,19-diaminononadecane (DE-4-4-4-4).[63] The cytotoxicity of PA analogues for cancer cells has been attributed to various mechanisms, such as the induction of the catabolic enzymes[1h,64] or the reduction in the activity of the anabolic enzymes.[57c,57d,65] Both mechanisms serve to reduce the PA content of the cell to lower levels than



	R^1	R^2	m	n
MESPD(N^1)	Et	H	1	2
MESPD(N^8)	H	Et	1	2
DESPD	Et	Et	1	2
MENSPD	Et	H	1	1
DENSPD	Et	Et	1	1
MPHSPD	Pr	H	2	2
DEHSPD	Et	Et	2	2
DE(4,5)	Et	Et	2	3
DE(5,5)	Et	Et	3	3

	R^1	R^2	m	n
MENSPM	Et	H	1	1
DENSPM	Et	Et	1	1
CPENSpm	Et	CH_2cPr	1	2
CHENSpm	Et	CH_2cHp	1	1
DBSPM	Bn	Bn	2	1
DESPM	Et	Et	2	1
DEHSPM	Et	Et	2	2
ETBHSPM	Et	tBu	2	2

Figure 6. Selected examples of synthetic *N*-alkylated and cyclic polyamine analogues

those needed for its survival. Other mechanisms simply involve the total accumulation of these analogues in the cell and their competition with natural polyamines for binding sites to macromolecules of vital importance to the cell,[66] inhibition of mitochondrial protein synthesis[12a,67] and interference with normal tubulin polymerization.[68]

2.2 Other Polyamine Analogues with Potential Anticancer Activity

A series of conformationally restricted SPD and SPM derivatives have been recently synthesized.[69] In these cyclopolyamines (e.g. **22** and **23**, Figure 7), the $N-C_4-N$ part of the natural PAs has been locked within a cycle of variable length. These analogues were designed to inhibit the uptake system of PAs. Methylene groups in the skeleton of SPD and SPM have been replaced with the isosteric oxygen atom. Oxa-isosters of PAs such as **24** and **25** have been used in studies of the cellular functions of PAs and the peculiarities of their biosynthesis and transport.[70] Compound **24** has been found to act as a competitive inhibitor of the enzyme SPD aminopropyl transferase, which is responsible for the biosynthesis of SPD.

Carboranyl analogues of natural PAs, such as **26** and **27** (Figure 7), incorporating ^{10}B atoms have also been prepared for possible use in boron neutron capture therapy.[71] Branched hexamines, such as **28**, were prepared to be used as non-leaving groups in anticancer platinum complexes.[72] It should be noted that other branched PAs, such as the tetramine **29** and the hexamine **30**, have been used in the preparation of synthetic iron chelators[73] and dendrimers,[74] respectively. PUT and SPD analogues incorporating Bn and Me or HS/BnS groups were synthesized and evaluated as regulators of the PA biosynthesis and transport systems.[75]

2.3 Polyamine Analogues Designed to Interact with DNA

The binding of polycationic amines to DNA through purely electrostatic interactions and hydrogen bonding leads to the stabilization of the double and triple helixes of DNA. The stability of the double helix increases with an increase in the ratio PA/phosphate groups and the number of the positively charged centres on the PA skeleton.[76] These interactions were thoroughly studied in order to allow for the selective design of anticancer drugs by the synthesis of a series of PA analogues.[77] These studies showed that there is no apparent correlation between DNA binding and cytotoxicity.

Recently, a variety of PA analogues interacting with DNA have been reported. For example, the N^1-(anthracene-9-carbonyl)-SPM (**31**, Figure 8) was developed to study the

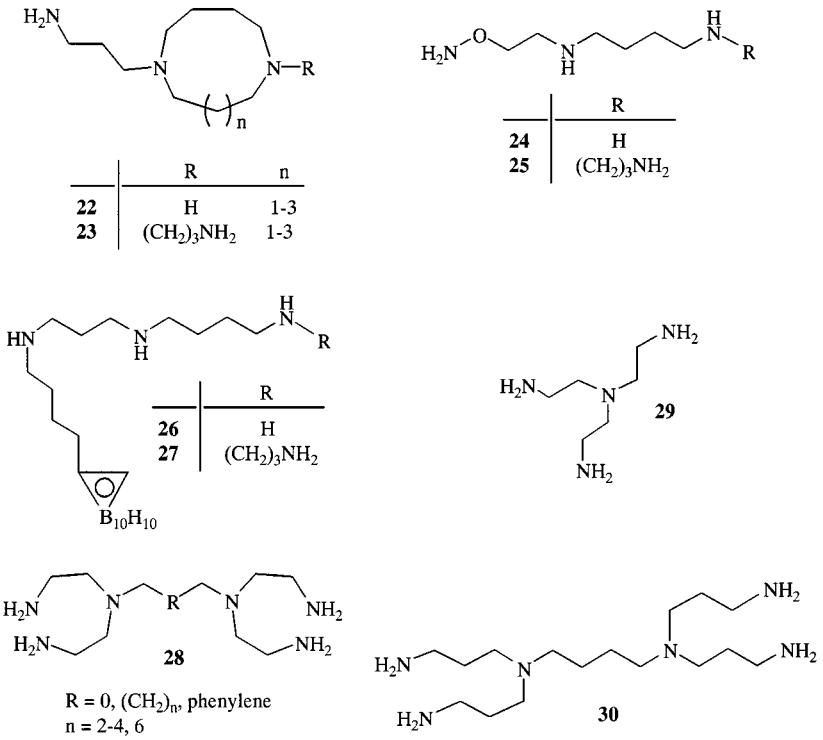


Figure 7. Synthetic conformationally restricted, branched and oxa-polyamine analogues

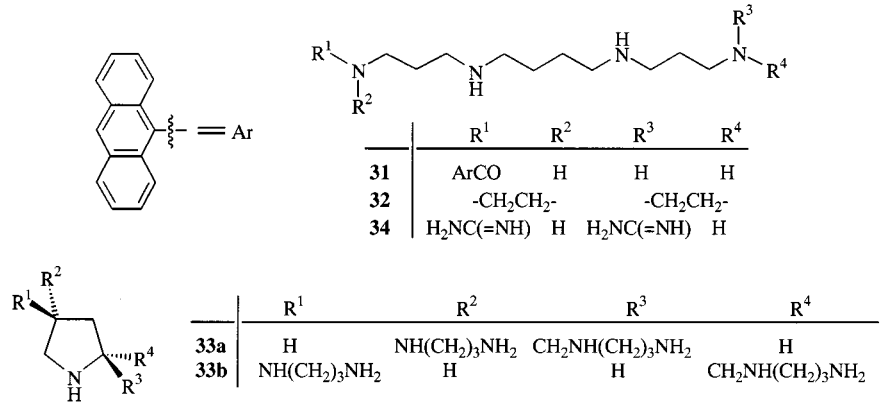


Figure 8. Synthetic DNA-binding polyamine analogues

multiple ways of binding of spermine to DNA.[78] Another interesting example is the N^1,N^{12}-bisaziridinyl-SPM (**32**), for which a cross-binding to DNA was observed along with a high cytotoxicity of tumour cells. The PA skeleton of this compound causess a high uptake by the cells and the aziridinyl moieties the cytotoxicity.[79] The chiral SPM analogues **33a** and **33b** are characterized by a rigidity in the N−C_4−N region of the natural PA. These analogues bind to the DNA double helix as strongly as SPM, but more strongly to the DNA triple helix.[80] Certain cyclic or branched PA complexes with Co^{III} bind to DNA and act as efficient catalysts for its hydrolysis.[81] The bisguanidyl SPD (**21**) and SPM (**34**) analogues (Figure 8) were synthesized with the expectation that substitution of the primary amino groups by guanidyl moieties would bring about considerably higher charges and therefore stronger binding to DNA.[82] Both analogues provided better duplex DNA stability than the parent compounds, whereas the analogue **34** stabilized DNA triplexes more selectively than the parent compound.

2.4 Polyamine Analogues Interacting with the NMDA Receptor

Taking into consideration the involvement of natural PAs in the function of the NMDA receptor, a series of analogues and homologues of SPM were developed and evaluated for their possible effect on the PA binding site of the receptor.[18b,83] This binding site can be a possible target for the development of drugs which, by acting as antagonists, will inhibit the death of neuron cells. Actually, many of the N^{α},N^{ω}-dialkylated SPM analogues and homologues which are shown in Figure 6 and initially developed as antineoplastics, appear to exhibit either agonist or antagonist activity, depending on their structure and concentration. Examples are DENSPM, DEHSPM, DMHSPM and

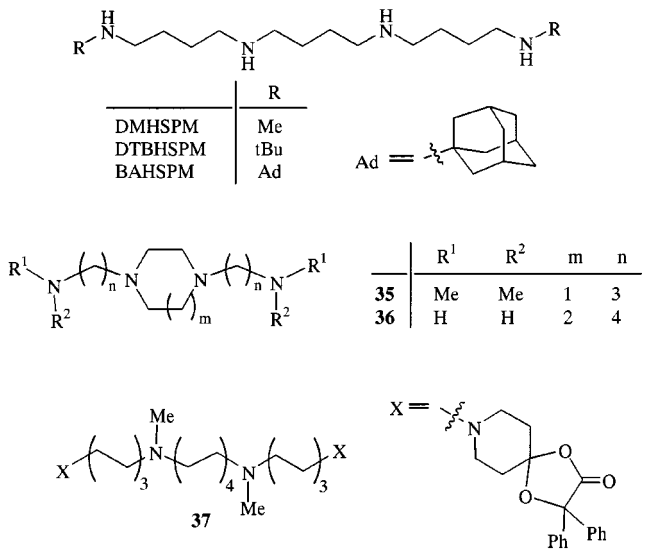


Figure 9. Synthetic polyamine analogues targeting the NMDA and muscrinic receptors

DTBHSPM, as well as the potent antagonist bisadamantyl derivative BAHSPM (Figure 9) and the conformationally restricted NSPM and SPM analogues PYR(3,3,3) and PIP(3,4,3) (Figure 6). As is true with their action as antineoplastics, the length of the PA skeleton, the charge and the substituents at the two terminal amino groups are important factors for the interaction of these analogues with the receptor and the regulation of its function.

Other PA analogues which have been synthesized to target the NMDA receptor are the tetramines **35** and **36** (Figure 9), incorporating piperazine and homopiperazine rings, respectively.[84] Polymethylenetetramines, such as **37**, have been developed to target the cholinergic muscarinic receptors.[85] Thus, polyamine **37** showed a high affinity for the muscarinic M_1 receptor subtype.

2.5 Polyamine Analogues Involved in Other Cellular Functions

Although the synthetic PA analogues have been studied mostly for their antineoplastic activity and their ability to stabilize the nucleic acids, these derivatives were recently examined for their activity on other biological systems as it is well-established that the natural PAs affect many other enzyme systems[86] and act as inhibitors of ion channels.[87] It has been shown that certain PA analogues can act, for example, as antiparasitic[88] and antimalarial[89] agents, and as antidiarrhoeals.[90] Thus, DEHSPM is already under clinical evaluation to control chronic diarrhoea, in particular associated with AIDS patients, whereas a new class of metabolically unstable, to avoid accumulation of cytotoxic metabolites, PA antidiarrhoeals (e.g. **38**, Figure 10) has been recently developed by Bergeron et al.[90]

Macrocyclic polyamines, such as the bicyclams IPS31[91] or JM 3100 (Figure 10)[92] or their complexes with metals, e.g. IPS32,[91] have been found effective against HIV-1 and HIV-2, acting as fusion inhibitors. Recently, novel macrocyclic PA-nucleoside conjugates and bicyclam-AZT conjugates have been synthesized and their anti-HIV properties evaluated.[93] It should be noted that the synthesis of cyclic derivatives of SPD and SPM has been reported by Kimura et al.,[94] whereas macrocyclic PAs bearing intelligent side chains, suitable for use in the selective uptake and transport of metal ions as well as for catalysis and enzyme models have been recently reviewed by Kimura.[95]

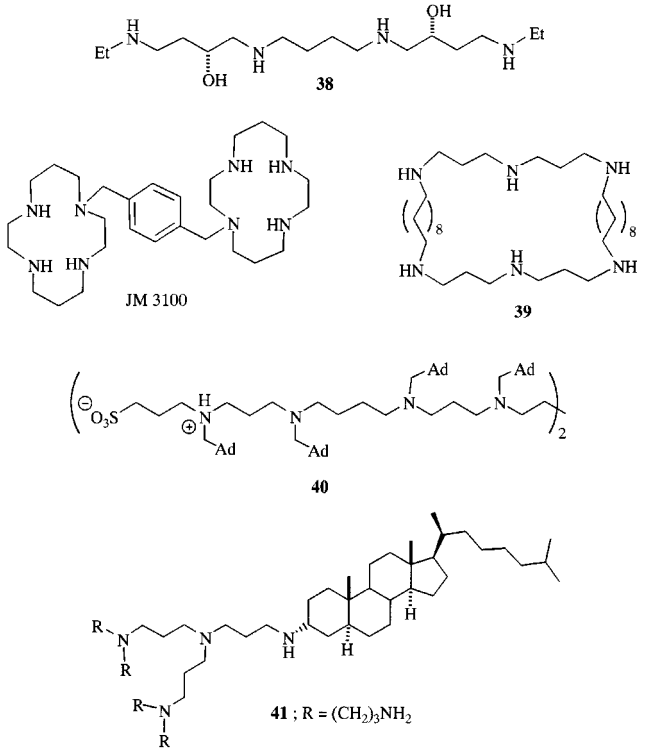


Figure 10. Synthetic polyamine analogues interacting with various cellular targets

The ability of various linear PA homologues and analogues to inhibit cell growth and induce haemoglobin production was recently studied in murine erythroleukaemia (MEL) cells. It was found that the more the nitrogen atoms in the chain the stronger haemoglobin production inducers were the PA homologues, e.g. pentamines were stronger inducers than tetramines. On the other hand, alkylation at the terminal nitrogen atoms resulted in weaker haemoglobin inducers and better cell growth inhibitors.[96] Taking into consideration that PA bolaphiles are stimulants of polymerase T7 and inducers of actin polymerization, the synthesis of a series of linear and macrocyclic bolaphiles (e.g. **39**, Figure 10) has been recently reported.[97] Linear PA analogues of suitable length bearing lipophilic side-chains, e.g. Ad groups (octamine **40**),[98] or polyamine dendrimers anchored on a hydrophobic moiety, e.g. a steroid (dendrimer **41**),[99] considerably facilitate transmembrane ion transport.

2.6 Synthetic Polyamine Conjugates

Anchoring of a PA on another organic or bioorganic molecule results in the formation of a PA conjugate. These conjugates, depending on the structure of the non-PA moiety, are designed to show improved biological activity in par-

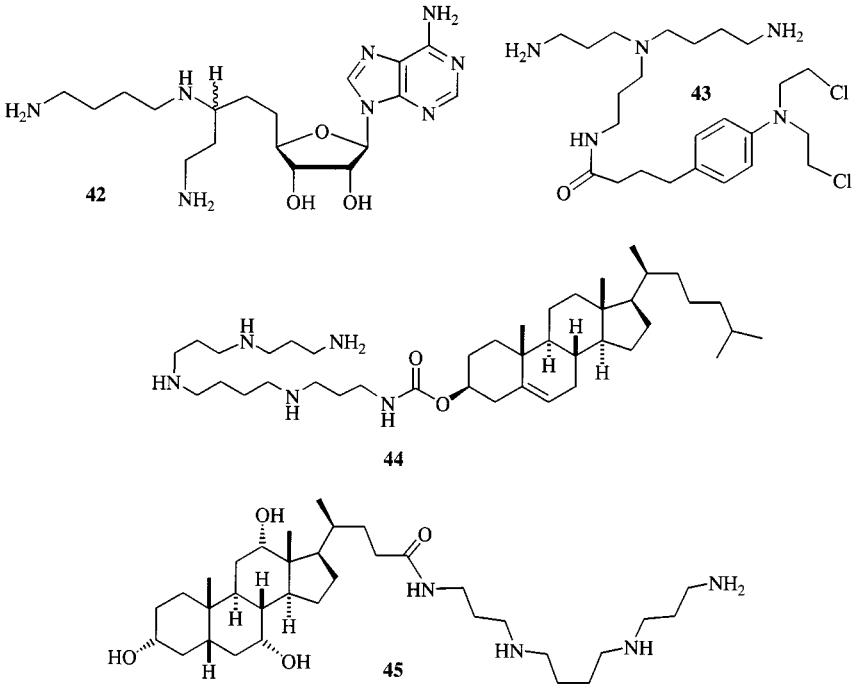


Figure 11. Examples of synthetic polyamine conjugates with nucleosides, cytoxic agents and steroids

ticular cellular targets, compared to the parent molecules, or combine the activities of the constituent molecules.

2.6.1 Conjugates with Nucleosides

A new class of inhibitors of the PA biosynthesis, namely conjugates of PAs with nucleosides, has been recently developed. For example, adenosyl-SPD (**42**, Figure 11) was found to be the most potent inhibitor of the anabolic enzyme SPD aminopropyl transferase.[100] Conjugates of macrocyclic polyamines with nucleosides, e.g. AZT, have been already referred in this Microreview.[93]

2.6.2 Conjugates with Cytotoxic Agents

Conjugation of PAs with known cytotoxic agents, such as chlorambucil, has led to PA conjugates of the type **43** (Figure 11).[101] These conjugates, being PA derivatives, can cross the membranes to enter the cell. Target of compounds like chlorambucil is the DNA. Chlorambucil is known to cross-link the two helixes of the DNA. This cross-linking was shown to be more effective with e.g. the conjugate **43** because of the strong binding of PAs to the DNA.

2.6.3 Conjugates with Steroids and Fatty Acids

Conjugates of PAs with cholesterol,[102] bile acids[103] or a long aliphatic chain[104] have been recently developed as potential agents for the introduction of polynucleic acids into cells. This methodology, known as gene delivery, may be useful in the correction of a variety of disorders in humans, such as cancer, inflammation and neurodegeneration. Representative examples are the PA conjugates **44** and **45** (Figure 11).

2.6.4 Conjugates with Amino Acids

A variety of analogues of the naturally occurring spider and wasp toxins of the PA amide type has been recently synthesized in order to study the relationship between their structure and their biological activity, namely the inhibition of ionotropic glutamate and nicotinic acetylcholine receptors.[105] These studies served to identify the key-structural features of these PA conjugates which are responsible for their biological action and even identify structural modification leading to even enhanced binding to the receptors. It was thus found that, as concerns the PA moiety of these conjugates, longer PA chains lead to increased affinity for the receptors.

The synthesis and biological evaluation of an array of analogues of the immunosuppressive agent DSG, with a wide range of modifications at the central hydroxyglycine moiety of the molecule, has been quite recently disclosed.[49]

2.6.5 Other Polyamine Conjugates

A variety of PA conjugates with HCA derivatives have been synthesized as potential inhibitors of the enzyme TR which, as mentioned above, is very important for the survival of trypanosomatid parasites. The design of these analogues was based on the known inhibitory activity on this enzyme of the naturally occurring SPM alkaloid kukoamine A.[46] The only other analogue which showed comparable activity to the lead compound was the corresponding SPD conjugate. Other PA analogues and conjugates have been recently developed as potential TR inhibitors. These involve SPD or SPM derivatives bearing simple aromatic side chains[106] or 2-aminodiphenylsulfide moieties,[107] or PAs conjugated to monoindolylmaleimides.[108]

Other PA analogues and conjugates have been designed to address other biochemical targets of the trypanosomes. Recent examples are the SPD conjugate **46** (Figure 12) targeting the enzyme GSPS,[109] the PA derivative **47** as inhib-

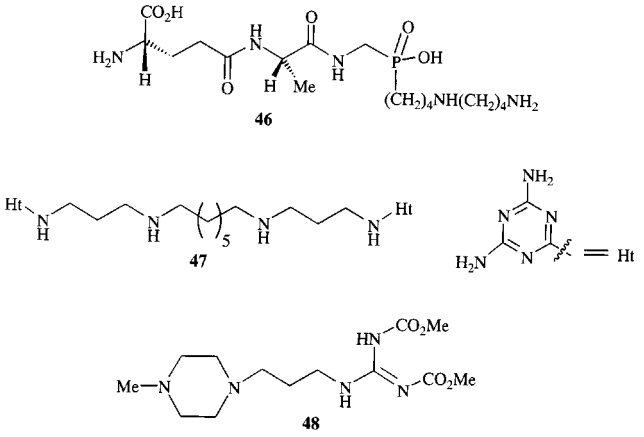


Figure 12. Synthetic polyamine conjugates addressing other than TR trypanosomatid targets

itor of the adenosine uptake via the P2 transporter[110] and the 1,3-diaminopropane analogue **48**, a leishmanicidal agent probably inhibiting the Arg transport.[111]

SPD and SPM conjugates with 4-nitrobenzoic acid have been developed as effective hypoxic cell sensitizers,[112] whereas SPD conjugates and N^1,N^8-dialkylated SPDs bearing polyhydroxylated benzoic acids have been shown to be excellent metal chelating agents.[113]

3. Synthetic Protocols for the Preparation of Polyamine Analogues and Conjugates

Various synthetic protocols have been devised which allow the preparation of PA analogues and conjugates selectively modified at their amino functions. These protocols involve either the selective functionalization/protection of the amino functions of PAs or the assembly of the PA skeleton from suitably protected amino building blocks.

3.1 Selective Functionalization of the Amino Functions of Linear Polyamines

3.1.1 Methods for the Direct Selective Functionalization of the Primary Amino Groups

These methods involve the reaction of PAs with reagents which selectively deliver protecting groups or acyl groups on the primary amino functions. Protecting groups which have been used for this purpose (in parentheses, the most often used reagents for their introduction) are for example the Phth (PhthN−CO_2Et),[114] the Z (Z-Cl or Z-CN),[115,117] the Boc (Boc-OSu),[116] the Tfa (Tfa-OEt),[118] the Trt (Trt-Cl),[72] the Mtr (Mtr-Cl)[119] and the Dde (Dde-OH)[120] groups. These protecting groups are introduced in the PAs in high yields using readily available reagents and can be removed under mild deprotection conditions, such as hydrazinolysis (Phth, Dde), acidolysis (Z, Boc, Trt, Mtr), catalytic hydrogenolysis (Z, Trt) and hydrolysis with weak bases (Tfa). Most useful synthetic intermediates prepared through this approach are the PA derivatives **49**−**54** (Figure 13).

Selective bis-acylation of PAs at the primary amino functions can be effected with a variety of activated derivatives of carboxylic acids.[115,121−127] For example, Murahashi et al. directly acylated SPD with *trans*-cinnamoyl cyanide to obtain an excellent yield of the alkaloid maytenine (**12**).[115] Acylimidazoles,[124] readily formed on reacting carboxylic acids with the commercially available coupling agent CDI, are the simplest to obtain activated derivatives whereas of special interest is the preparation of PA-HCA conjugates without the need for protecting the hydroxyl functions of the HCA components.[127] Regioselective bis-benzoylation of SPD, in the presence of an immobilized lipase and direct reductive bis-benzylation of symmetrical PAs have been recently reported by Jéso et al. [128] and by Van Arman et al.,[129] respectively.

3.1.2 Methods for the Indirect Functionalization of the Primary Amino Functions

Reaction of PAs with sulfonyl chlorides, e.g. TsCl or MtsCl, followed by alkylation with alkyl halides in the presence of NaH, leads to dialkylated polysulfonamides, e.g. **55** (Figure 13). From these intermediates, the corresponding PA analogues, e.g. **56**, are obtained on treatment with Na in liquid NH_3 or acidolysis with HBr. Best results are obtained with the Mts group and primary alkyl halides, whereas secondary and tertiary alkyl groups can be only introduced through fragment syntheses.[57a,130] Alternatively, the Boc group can be used in the place of the sulfonyl groups and then deprotection is effected under milder reaction conditions, e.g. HCl in MeOH.[57e]

Ragnarsson et al.[131] used the intermediate **57** to obtain the SPD derivatives **51** and **58** whereas Bergeron et al. [58,133] used the intermediates **59** and **60** to obtain the PA derivatives **51** and **61** and the SPM analogue **62** (Figure 13). The Boc groups were delivered using the reagents Boc_2O or Boc-ON, whereas the Bn and Z(NO_2) groups were removed by catalytic hydrogenolysis. The terminal alkyl groups in **62** were introduced through acylation, followed by BH_3-mediated amide bonds reduction.

Complexes of linear tetramines with metal (M = Cr, Mo, W) carbonyls, such as **63** (Figure 14), were reductively alkylated by des Abbayes et al. to obtain a series of monoalkylated and symmetrically or unsymmetrically ω,ω'-dialkylated PAs (**64**), after oxidative decomplexation with air.[132]

3.1.3 Methods for the Selective Functionalization of Secondary Amino Functions

The methods previously described, which allow the selective protection/modification of the primary amino functions of PAs, can give access to a variety of derivatives of PAs functionalized at the secondary amino functions, through their reaction with electrophiles, followed by primary amino groups deprotection, if necessary. One such recent example can be found in the synthesis of the branched alkaloid tenuilobine (**14**) by Hesse et al.,[38] which involved

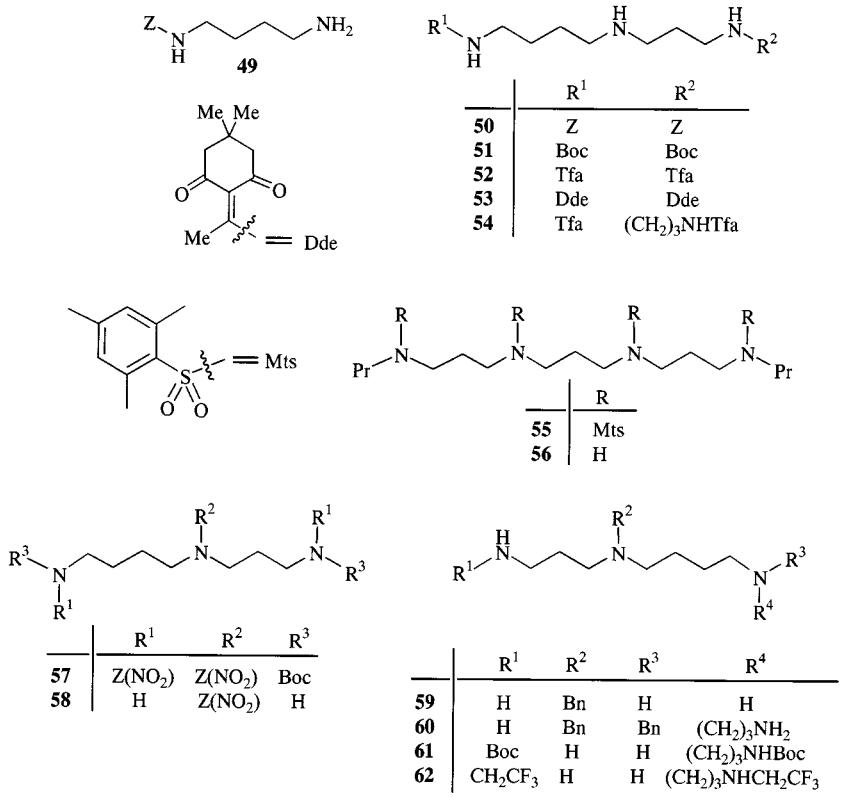


Figure 13. Synthetically interesting *N*-functionalized polyamines

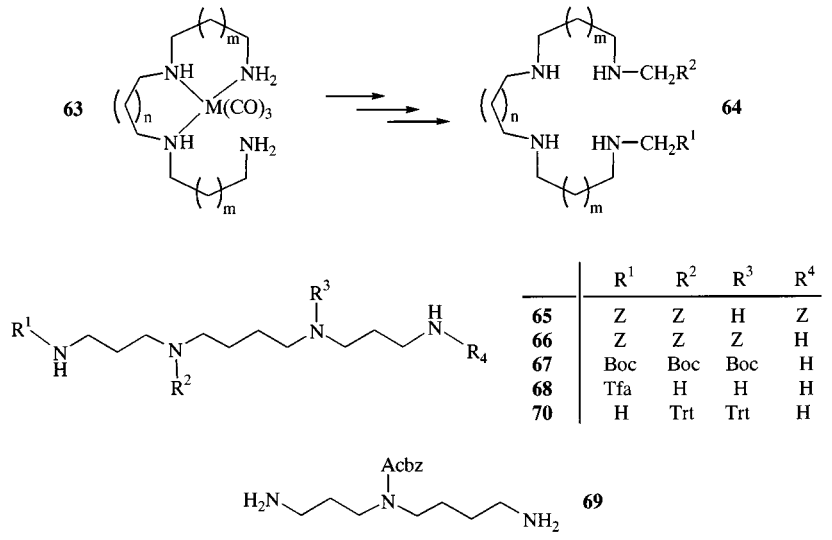
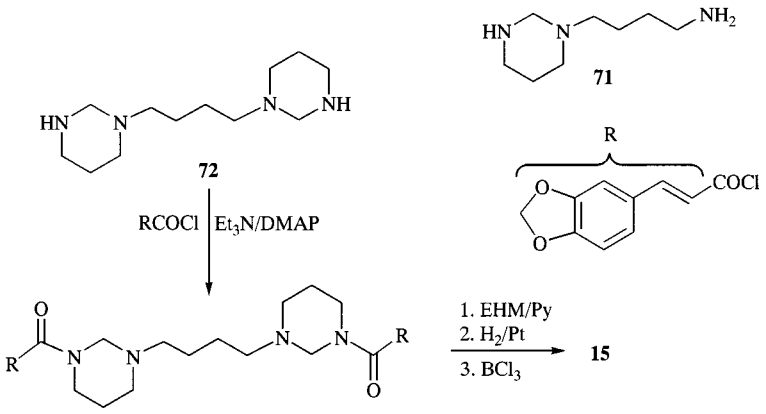


Figure 14. Selectively *N*-functionalized polyamines obtained through indirect synthetic protocols

the use of N^1,N^8-Z_2-SPD (**50**) and N^1,N^4,N^{12}-Z_3-SPM (**65**) (Figure 14) for the assembly of the alkaloid skeleton. Blagbrough et al. prepared N^1,N^4,N^9-Z_3(**66**)- and N^1,N^4,N^9-Boc_3(**67**)-SPM, in moderate yields after chromatographic purification,[102d,104] from N^1-Tfa-SPM (**68**) obtained as the major product in mixture with N^1,N^8-Tfa_2-SPM (**54**). These derivatives were shown to be suitable intermediates in the preparation of SPM-lipid conjugates.

With a few exceptions, protection of the secondary amino functions of PAs is usually effected through indirect methods. We have already seen one such example in the preparation of the SPD derivative **58**. Another example can be found in the synthesis of hirudonine by Golding et al., with the use of the Tfa group for the temporary protection of the primary amino functions.[48] This allowed the preparation of the interesting synthetic intermediate N^4-Acbz-SPD (**69**) (Figure 14), which in turn can be used for the indirect modification of the primary amino functions of SPD. The Acbz protecting group is removed through reduction with DTT in the presence of Et_3N. Similar methodology was recently applied to the synthesis of N^4,N^9-Trt_2-SPM (**70**) starting with N^1,N^{12}-Tfa_2-SPM (**54**).[134]



Scheme 2. Hexahydropyrimidine polyamine derivatives as key intermediates in the synthesis of N^{α},N^{ω}-diacylated PAs-like kukoamine A (**15**)

A direct method for the protection of the secondary amino groups has been already described above and uses metal complexes of PAs. However, the classical and most often used method for the protection of the secondary amino groups in PAs, bearing the *N*-monosubstituted 1,3-diamine moiety like SPD and SPM, has been developed by Ganem et al. and involves their reaction with either formaldehyde or other aldehydes and in particular benzaldehyde.[30,135] Reaction of SPD or SPM with formaldehyde leads to the corresponding mono- and bishexahydropyrimidine derivatives **71** and **72** (Scheme 2), respectively. Derivative **71** is a very interesting intermediate since on reaction with reagents which selectively deliver acyl groups at a primary amino function can lead to either N^8-functionalized or protected SPDs. The latter can find use in the synthesis of N^1-functionalized SPDs. Alternatively, both amino functions can be acylated to provide access to N^1,N^8-functionalized SPDs. In either case, the methylene protective group can be readily removed under Knoevenagel condensation conditions with the use of EHM and Py.

This methodology is exemplified with the efficient syntheses of the marine alkaloid pseudoceratidine (**17**) and analogues[42] and the plant alkaloid kukoamine A (Scheme 2)[135a] by Ganem et al. N^4,N^9-Bisfunctionalized SPMs were readily obtained by Hesse et al. on reacting the bishexahydropyrimidine from SPM and benzaldehyde with suitable acyl chlorides.[135b]

3.2 Fragment Syntheses of Polyamine Analogues and Conjugates

This type of syntheses offers greater flexibility in the design and preparation of a variety of PA analogues and conjugates. The PA skeleton is assembled at the appropriate length and desired number of differently protected nitrogen functions with the formation of new C−N bond(s) by coupling suitable amino components. These syntheses are classified below according to the type of the key-reaction used to create the new C−N bond(s).

3.2.1 Michael Addition of Amino Components to Acrylonitrile and Crotononitrile

Michael addition of diamines to acrylonitrile is one of the oldest and very frequently used methods to extent a diaminoalkane chain by one or two aminopropyl moieties. It was initially developed to allow the preparation of SPD and SPM molecules with variations in the tetramethylene portion of the natural molecules.[136] Thus, reaction of *a,ω*-diaminoalkanes with one or two molecules of acrylonitrile, followed by catalytic hydrogenation of the nitrile group(s) using Ni or PtO_2 as catalyst or $LiAlH_4$ provides ease access

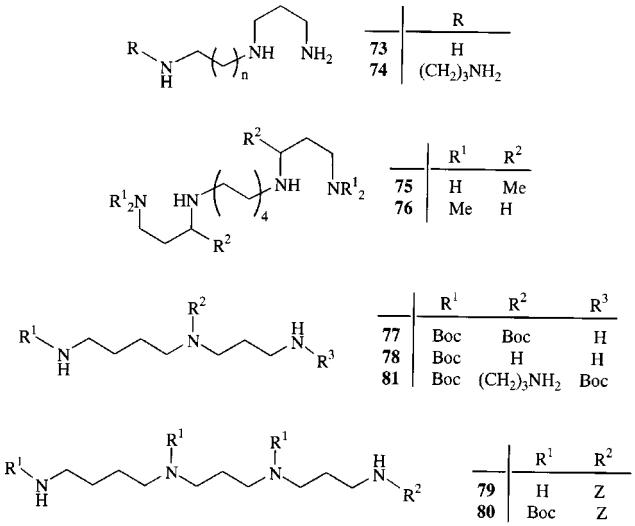


Figure 15. Polyamine analogues and important synthetic intermediates obtained through the Michael addition approach

to homologues of SPD (**73**) and SPM (**74**) (Figure 15). Edward et al. used crotononitrile as the Michael acceptor to obtain *C*-methylated analogues of PAs, e.g. **75**.[57e] When catalytic hydrogenation of suitable dimethylaminonitriles takes place in the presence of a *a,ω*-diaminoalkane, PA analogues like **76** can be readily produced.[57e]

Using the acrylonitrile approach, Quick et al.[137] and Hesse et al.[138] independently prepared N^4,N^8-Boc_2-SPD (**77**) (Figure 15). This compound together with N^8-Boc-SPD (**78**), also prepared by Hesse et al., are interesting intermediates in the synthesis of N^1-monoacylated and N^1,N^4-diacylated natural SPD conjugates,[137,138] whereas compound **77** has been recently applied by Nakanishi et al. to the synthesis of philanthotoxin analogues.[105a] Extension of intermediate **77** by an aminopropyl unit using the same methodology, allowed Nakanishi et al. to obtain the 4-3-3

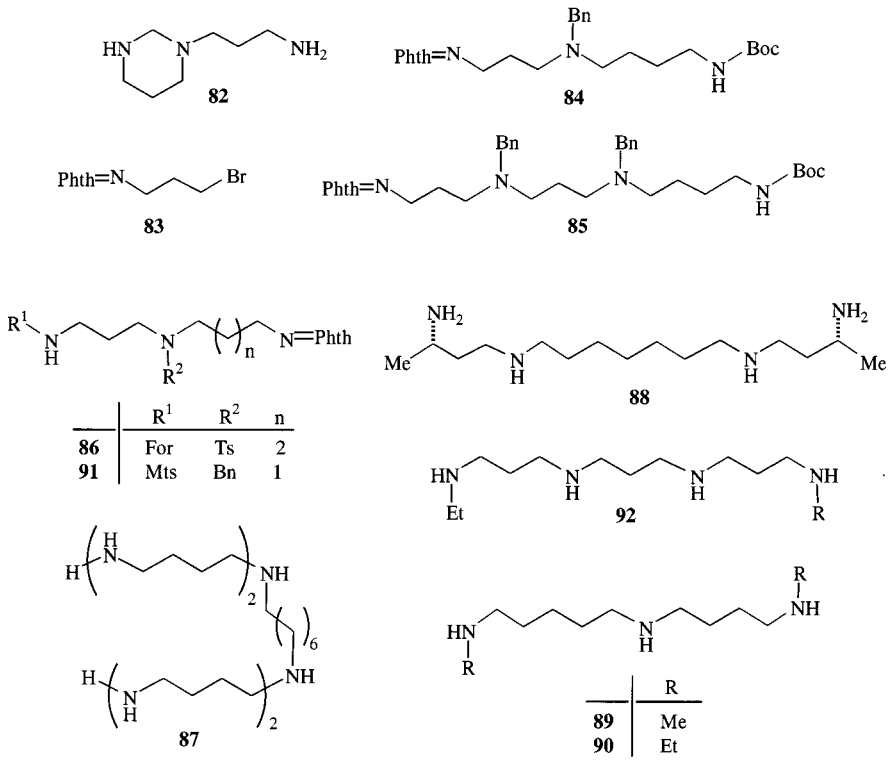


Figure 16. Interesting polyamine intermediates and final products obtained through the *N*-alkylation protocol

tetramine derivatives **79** and **80**. These compounds are suitable intermediates in the synthesis of philanthotoxin-433 and analogues.[139] The acrylonitrile approach was also used by Cullis et al. to obtain the branched PA derivative **81**, as a key-intermediate in the synthesis of SPD-chlorambucil conjugates.[101c] Other recent applications of this approach are the preparation of steroid−PA dendrimer conjugates by Matile et al.,[99] PAs bearing melamine moieties by Gilbert et al.,[110] and tetramines bearing same or different alkyl groups at the *N*-termini, like CHENSpm (Figure 9) by Woster et al.[88] Acrylonitrile or ω-bromonitriles have been recently used by Hesse et al. to produce homologues and isomers of naturally occurring dicumaroyl-SPMs.[140]

3.2.2 Reaction of Amino Components with Alkylating Agents

Direct *N*-alkylation of amino components has been used to prepare PA analogues and conjugates. Among the recent examples, the following may be mentioned. α′,ω′-Dibromoalkanes have been used by Edwards et al. to alkylate either α,ω-diaminoalkanes or Boc-protected 1,3-diaminopropanes *en route* to the development of novel antimalarial agents.[89] The polyamine skeleton of spirotramine was assembled by Melchliorre et al. using as a key-step the alkylation of *N*,*N*′-dimethyl-1,8-diaminooctane.[85]

Linear polyamine bolaphiles were readily obtained by Bradley et al. through direct alkylation of the NSPD derivative **82** (Figure 16) with a variety of linear α,ω-ditosylates or dimesylates, followed by hydrolysis. On the other hand, the macrocyclic PA bolaphile **39** was also prepared through alkylation of a fully tosylated suitable precursor, followed by detosylation.[97] In addition to ω-bromonitriles, which have been quite recently used by Hesse et al. as Cn−N building blocks to prepare polyamine homologues and conjugates,[140] the *N*-(3-bromopropyl)phthalimide (**83**) has been employed by Samejima et al. as an aminopropyl donor, for example in the preparation of ^{15}N-enriched SPD[141] and by Saccomano and Volkmann et al. in the total synthesis of argiotoxins.[142] In the latter example, bromide **83** was used in particular to prepare the orthogonally protected SPD derivative **84** and the 3-3-4 PA derivative **85**.

Direct alkylation of suitable aminooxy precursors has been used by Khomutov et al. to prepare hydroxylamine analogues of PAs.[70a] In addition to alkyl halides and sulfonates, oxiranes have been also used to alkylate suitable diamines. Thus, Bergeron et al. bisalkylated *N*,*N*′-Bn_2-PUT with (*S*)-epichlorohydrin as a key-reaction *en route* to the development of metabolically labile antidiarrhoeals.[90]

3.2.3 Alkylation of Sulfonamides

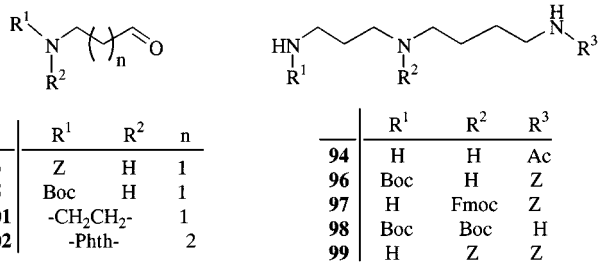
Alkylation of sulfonamides is also a very frequent and useful method to create new C−N bonds on the way to constructing PA molecules. The Ts group was initially used for this purpose but has been recently replaced by the Mts group. We have already described an example in which the Ts group was used in the preparation of the macrocyclic polyamine **39**. Other similar recent examples involve the bisalkylation with α,ω-dibromoalkanes of *N*,*N*′-Ts_2-PUT, by Hosseini et al.,[69] and of *N*,*N*′-Ts_2-diamines, by Krakowiak et al.,[143] as a key-reaction to obtain cyclo-SPDs and SPMs and pertosylated polyamines, respectively. The interesting synthetic SPD intermediate **86** (Figure 16), bearing the

Phth, Ts and For protecting groups, was prepared by Iwata et al., using as a key-step the alkylation of a suitable tosylamide.[144] Edwards et al. also used the Ts group, in combination with the Boc group, for the assembly of the skeleton of PAs, e.g. the 4-4-7-4-4 hexamine **87** and the Tf group for the preparation of chiral tetramines (e.g. **88**), using the Mitsunobu reaction.[145] The Mitsunobu reaction was employed to alkylate the sufficiently acidic intermediates TsNHBoc and triflylamides with suitable alcohols.

The Mts group has been extensively used by Bergeron et al. to assemble a wide variety of PA analogues, e.g. the SPD homologues DM(4,5) (**89**) and DE(4,5) (**90**), for structure-activity relationship studies with the aid of ω-bromonitriles as $C_{\omega+1}$-N synthons.[18,58,130a,130b] Woster et al. prepared the NSPD derivative **91** bearing the Mts group and used this as a key-intermediate in the synthesis of an array of unsymmetrically bisalkylated NSPM analogues (**92**).[146] Combinations of the Mts and Phth groups, and the Pmc and Bpoc groups, were employed by Kong Thoo Lin et al. to prepare SPD and SPM oxa-analogues and homologues, e.g. **25**.[70c] The Pmc and Bpoc groups are removed under milder deprotection conditions than their corresponding Mts and Boc groups and therefore are expected to be useful with sensitive substrates and provide cleaner final products.

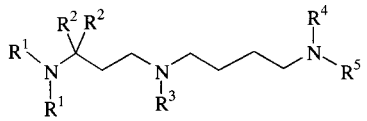
3.2.4 Reductive Alkylation

This type of synthesis involves the condensation of suitable *N*-protected ω-aminoaldehydes with amines, followed by reduction of the thus obtained imines. Golding et al. reductively coupled Ac-PUT and the aminoaldehyde **93** *en route* to the preparation of naturally occurring acylpolyamines, e.g. N^8-Ac-SPD (**94**).[147] Similarly, Haemers et al. condensed the aminoaldehyde **95** with Z-PUT in the presence of $NaBH_3(CN)$ to obtain the SPD derivative **96** (Figure 17). This compound was then used to obtain the diprotected SPD derivatives **97**–**99**,[148] which are versatile intermediates in the synthesis of SPD conjugates.



	R^1	R^2	n
93	Z	H	1
95	Boc	H	1
101	$-CH_2CH_2-$		1
102	-Phth-		2

	R^1	R^2	R^3
94	H	H	Ac
96	Boc	H	Z
97	H	Fmoc	Z
98	Boc	Boc	H
99	H	Z	Z



	R^1	R^2	R^3	R^4	R^5
100	H	Me	H	H	H
103	Me	H	Me	-Phth-	

Figure 17. Key intermediates and final products in the reductive alkylation approach

Other examples include the preparation of PYR(4,4,4) by Bergeron et al.,[58] the synthesis by Ganem et al. of nonmetabolizable derivatives of SPM and SPD, such as **100**,[149] and the assembly of the highly potent cytotoxic DNA cross-linking agent **32** by Callery et al. through reductive alkylation of PUT with the aldehyde **101**.[79] Recently, reductive alkylation has been frequently used in the assembly of the PA skeleton of complex PA conjugates, for example the hispidospermin (**20**) and spider and wasp toxins. Thus, condensation of aminoaldehyde (**102**) (Figure 17) with the commercially available diamine $Me_2N(CH_2)_3NHMe$, followed by reduction with $NaBH_3(CN)$, gave the SPD derivative **103** which was used by Danishefsky et al. to complete the first stereospecific total synthesis of hispidospermin.[47] The assembly of the PA skeleton of philanthotoxin analogues by Nakanishi et al. involved the reductive alkylation of the aminoaldehyde **93** with SPM as a key-step.[105b,105c] Blagbrough et al. also employed reductive alkylation to assemble the PA skeleton of spider and wasp toxins.[150]

3.2.5 Acylation of Amines with Carboxylic Acid Derivatives followed by Reduction

This approach involves the assembly of the polyamine skeleton by acylation of suitable amino components with activated carboxylic acids and reduction of the thus-obtained amides to the corresponding amines. The most widely used reducing agents are $LiAlH_4$ and B_2H_6. In some cases, DIBAH, AlH_3 or Ra-Ni-mediated desulfurization of the corresponding thioamide has been used to obtain cleaner reductions. Thus, Nordlander et al.[151] synthesized *N*-alkylated SPD analogues, such as **104** (Figure 18), using the amino acids βAla and γAba to provide the $N-C_3-N-C_4$ skeleton, whereas Bergeron et al. obtained the SPM analogue **105** with the acylation of $Et_2N(CH_2)_3NH_2$ with succinyl chloride as the key-reaction.[57b] Using the amide approach, Skolnick et al. synthesized the piperazine and homopiperazine tetramines **106** and **107**[84] and Blagbrough et al. assembled the skeleton of spider toxin FTX (**108**) by coupling N^1,N^4-Z_2-NSPD with Z_3-L-Arg.[152]

The amide approach was also used by Hicks et al. to obtain the projected anticancer drug N^1,N^{11}-Et_2-[6-^{14}C]NSPM,[153] by Dubowehik et al. to assemble the polyamine skeleton of the lipophilic PA **40**[98] and by Ikuina et al. to produce the PA moiety **109** (Figure 18) of the myosin light chain kinase inhibitors MS-681a-d, from the coupling of N^4,N^8-Boc_2-SPD with Boc-L-Phe in the presence of DPPA.[50]

Papaioannou et al. developed a simple and versatile synthetic protocol which allows the preparation of a variety of SPD and SPM analogues and conjugates. This involves the use of the amino acids βAla and γAba to furnish the required $N-C_3$ and $N-C_4$ building blocks, the triphenylmethyl (Trt) and/or the Fmoc group for the primary amino function protection and the Bn group for the protection of the secondary and, in some cases, even the primary amino function. Detritylation is effected with $TsOH\cdot H_2O$ in refluxing *i*PrOH, 20% TFA in DCM or with catalytic hydro-

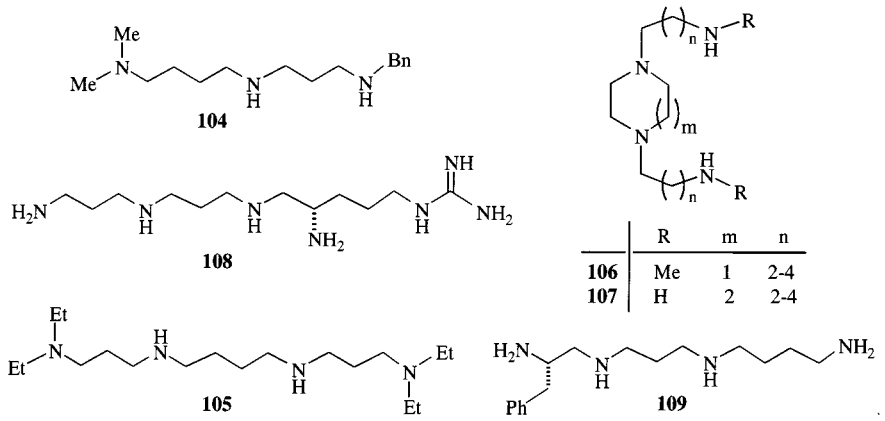
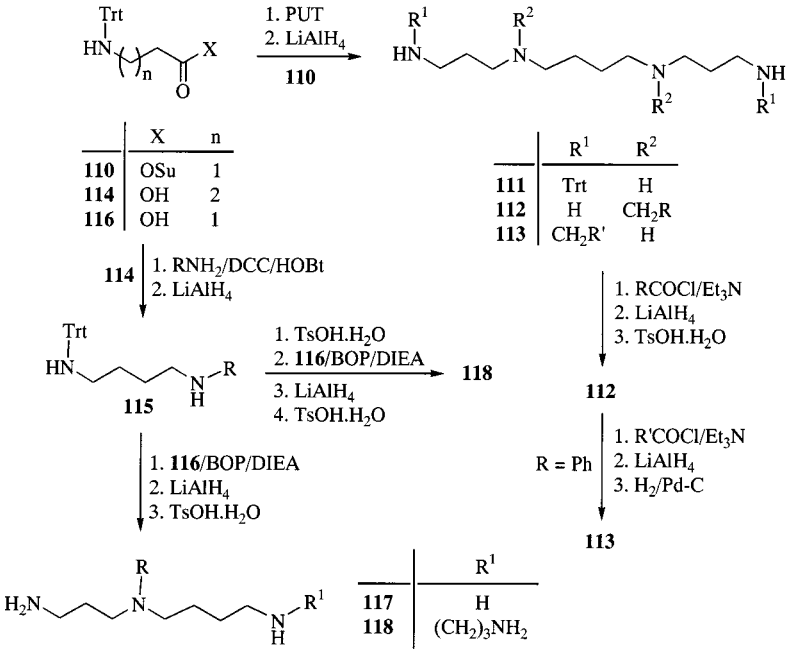


Figure 18. Polyamine analogues obtained through the amide protocol

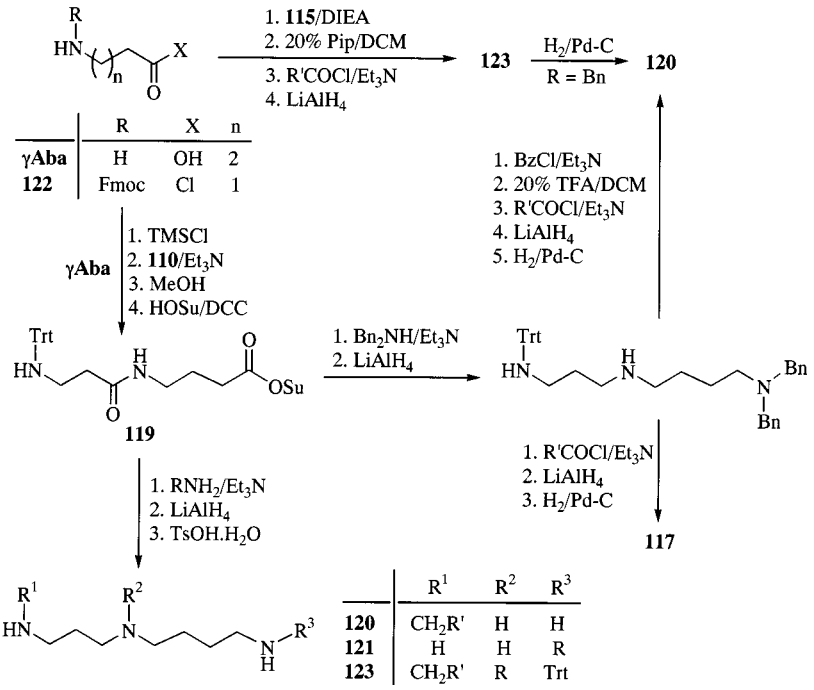
genolysis. The latter is also used to effect the removal of the Bn groups. Thus, coupling of the readily available Trt-βAla, in the form of either the isolated Trt-βAla-OSu (**110**) or in-situ-generated succinimidyl or benzotriazolyl "active" esters with PUT, considered as a N−C_4−N building block, afforded N^1,N^{12}-Trt_2-SPM (**111**) (Scheme 3) after $LiAlH_4$ reduction. This compound was used to prepare the N^4,N^9-diacyl- and -dialkyl-(**112**)-SPMs or N^1,N^{12}-diacyl- and -dialkyl-(**113**)-SPMs *via* the intermediate N^4,N^9-Bn_2-SPM (**60**). Alternatively, the also readily obtained Trt-γAba (**114**) was coupled to amines in the presence of DCC and HOBt and the obtained amides reduced with $LiAlH_4$ to give the N−C_4−N building blocks **115** for the assembly of SPD and SPM analogues. Thus, coupling of **115** with Trt-βAla-OH (**116**), followed by $LiAlH_4$ reduction and detritylation provides N^4-alkyl-SPDs (**117**). When coupling was performed after detritylation and the bisamides thus formed reduced with $LiAlH_4$, the N^4-alkyl-SPMs (**118**) were obtained after detritylation.[154]

Furthermore, Mamos and Papaioannou et al. reacted Trt-βAla-OSu with H-γAba-OTMS, generated in situ, to obtain the dipeptide Trt-βAla-γAba-OH, which was then converted into the corresponding isolable "active" ester Trt-βAla-γAba-OSu (**119**). This ester was used, in turn, to generate the N^1-(**120**), N^4-(**117**) and N^8-(**121**) alkylated SPDs according to Scheme 4.[155] On the other hand, the PUT derivatives **115** can be coupled to the readily available Fmoc-βAla-Cl (**122**) to give the corresponding amide which, upon routine deprotection with 20% Pip in DCM, followed by acylation and $LiAlH_4$ reduction, led to the N^1,N^4-bisalkylated SPD derivatives **123** with the same or different alkyl groups.[154] When R = Bn, catalytic hydrogenolysis removes both the Trt and Bn groups and this constitutes an alternative route to N^1-alkyl-SPDs (**120**).

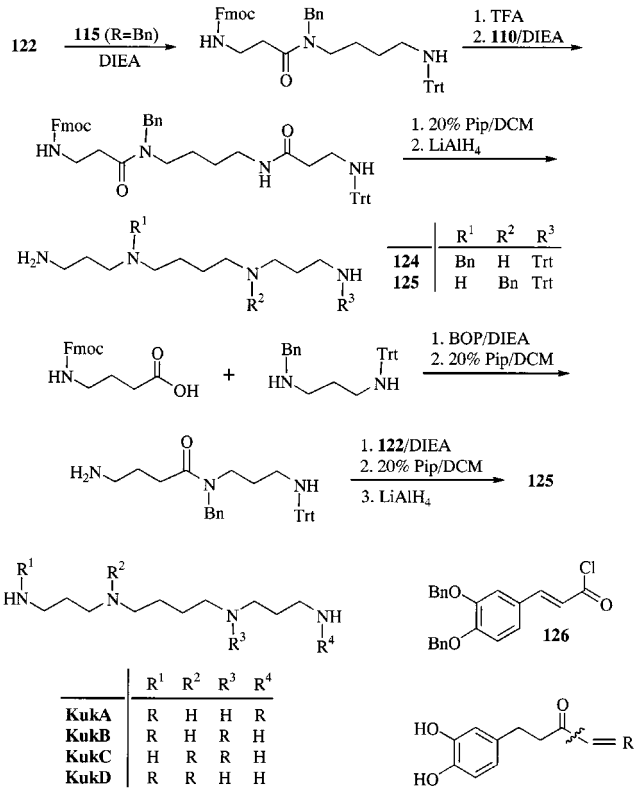
The novel SPM derivatives N^1-Trt-N^9-Bn-SPM (**124**) and N^1-Trt-N^4-Bn-SPM (**125**), also prepared by Papaioannou et al. using the methodology outlined in Scheme 5, are valuable intermediates for the preparation of N^1,N^9- and N^1,N^4-



Scheme 3. Synthesis of polyamine analogues using *N*-tritylated amino acids



Scheme 4. Fragment synthesis of selectively *N*-alkylated spermidine analogues using isolable "active" esters



Scheme 5. Preparation of key intermediates suitable for use in the total synthesis of kukoamine alkaloids

diacyl- and -dialkyl-SPMs. Indeed, these intermediates, as well as intermediates **60** and **111**, were used to prepare all four isomers of kukoamine alkaloids. Thus, their acylation with acyl chloride **126**, followed by simultaneous catalytic hydrogenation of the double bonds and hydrogenolysis of all protecting groups, gave kukoamines A−D (KukA-D).[156]

3.2.6 Methods Involving Azides as Intermediates

Suitably substituted alkyl azides have been used both as reacting entities and as latent amino functionalities in the fragment synthesis of PAs and conjugates. Thus, Vaultier et al. reported the synthesis of azides **127** (Figure 19), through the reaction of ω-azidoalkanamines with ω'-haloalkylboron dichlorides, and used these versatile intermediates to obtain *N*-alkylated SPM (**128**) and SPD (**129** and **130**) homologues. Azides were also used by the same authors as latent amino functionalities, for example in the synthesis of the SPD analogues **131** and **132**,[157] and by Moriwake et al. in another interesting synthesis of kukoamine A, involving the $TMSN_3$-mediated opening of a suitably substituted 5,6-dihydro-4*H*-1,3-oxazine.[158]

Golding et al. used the Mitsunobu reaction to obtain the azide **133** (Figure 19) and showed that this compound is a suitable N−C_4−N intermediate in another synthesis of N^1,N^8-Z_2-SPD (**50**).[159] An interesting finding of that work was that the Z amino protecting group can be directly converted into a methyl group upon reduction with diborane. Miyashita et al. have recently developed the efficient *azide strategy* for the total synthesis of spider toxins.[26,160] This strategy involves the use of suitable azides as latent amino functionalities in the assembly of the PA skeleton. For example, for the synthesis of the toxin NPTX-643 (**4**) the azides **134**−**136** were employed.

3.2.7 Combinations of Fragment Synthesis Protocols Leading to Selectively Protected Polyamines

Several examples of the use of combinations of the most important fragment synthesis protocols to prepare PA de-

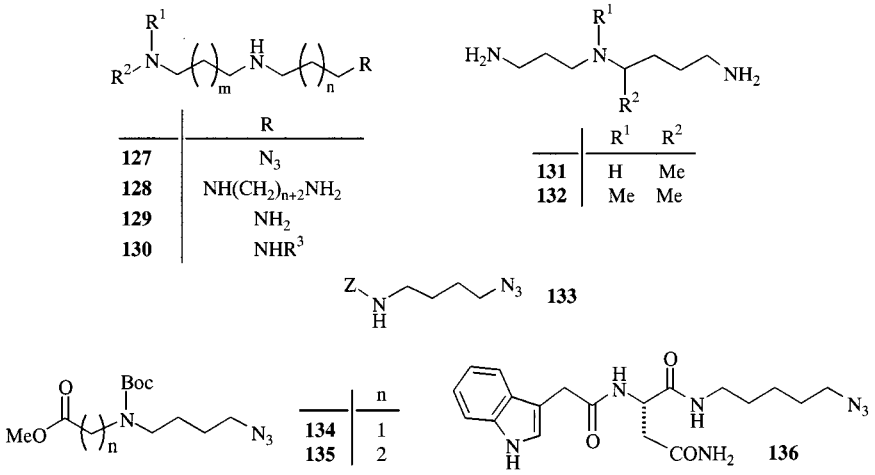


Figure 19. Azides as intermediates in the synthesis of polyamine analogues and conjugates

rivatives have been reported and can be found in the literature provided in the previous sections. In this section, we will describe methods to prepare PA derivatives bearing selectively removed amino protecting groups, using either combinations of fragment synthesis protocols or, in some cases, the alkylation methodology. These derivatives allow in turn the selective functionalization of amino functions and the efficient preparation of PA conjugates. For example, Bergeron et al. reported the preparation of the triprotected SPD (**137**)[161] and the tetraprotected SPM (**138**)[162] derivatives bearing the orthogonally removed Tfa, Boc, Bn and TCBoc protecting groups, and used the former to obtain the tri-acylated SPD derivative **139** (Figure 20).

Using fragment synthesis protocols, Hesse et al. have developed a series of very interesting polyamine derivatives, selectively protected at their amino functions. These include the SPD (**140**) and SPM (**141**) derivatives[163] and the penta-protected thermopentamine derivatives **142**–**144** (Figure 20),[164–166] which contain five orthogonally removable protecting groups. Trigo et al.[167] and Genêt et al.[168] have developed methodologies to obtain triamines selectively protected at two and three of the amino functions. The former research group used the protecting groups of the urethane type Boc, Z, Troc and Etoc, whereas the latter used combinations of three different protecting groups from the groups Boc, All, Alloc, Z and Troc. Examples are the SPD derivatives **145**, **146** and **147** and the NSPD derivative **148**.

4. Solid-Phase Synthesis of Polyamine Analogues and Conjugates

The need for the facile regioselective *N*-functionalization of the amino functions of the PAs, the facilitation of the

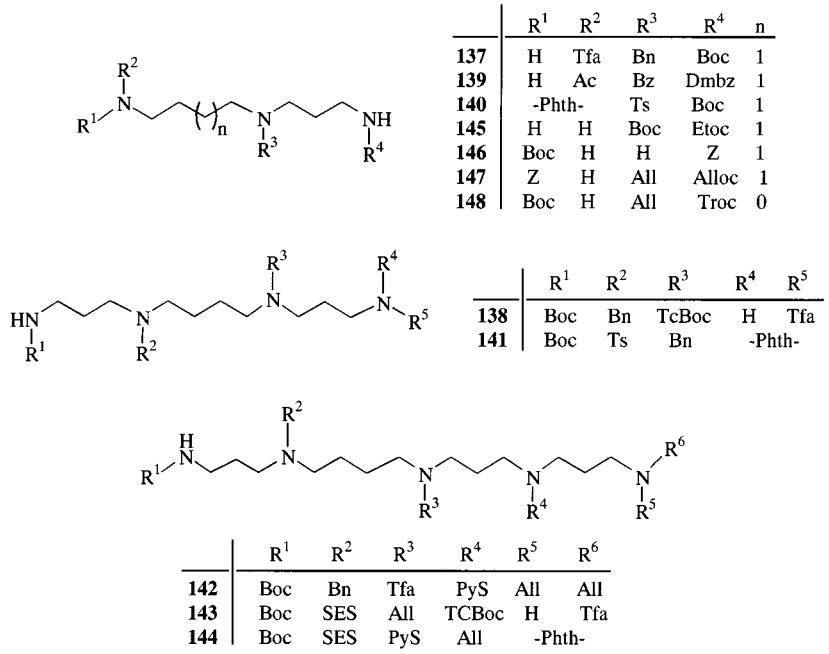


Figure 20. Synthetically interesting polyamine derivatives bearing selectively removable protecting groups

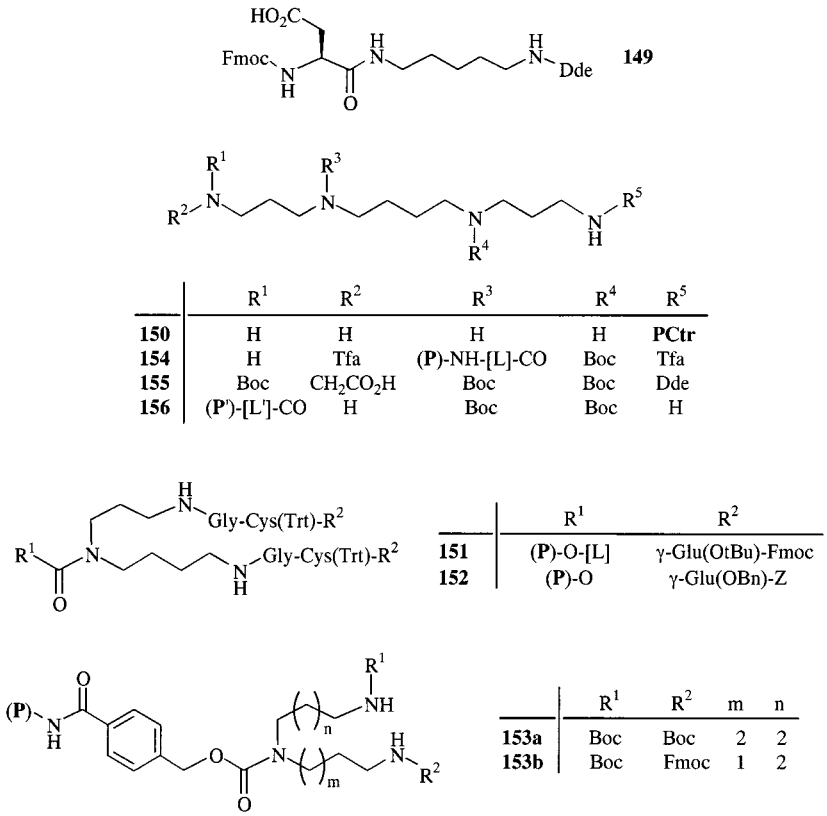


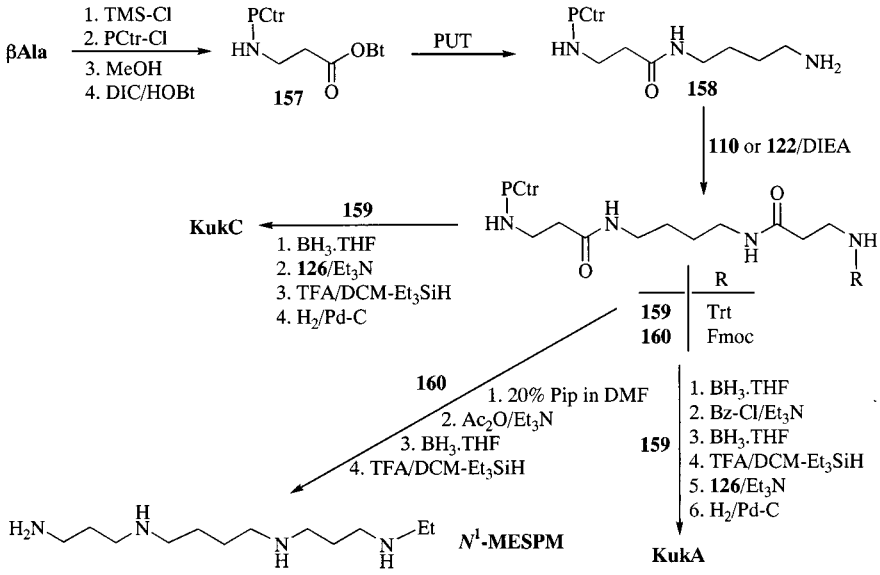
Figure 21. Examples of polyamine analogues and conjugates anchored on polymeric supports

manipulation of these highly polar molecules and their conjugates and, especially, the need for the development of combinatorial libraries of biologically interesting PA analogues and conjugates, gave an impetus in the use of solid supports to the synthesis of molecules of this kind. The SPS protocols developed so far can be divided into the same two categories already employed in this Microreview to classify the corresponding solution-phase syntheses.

4.1 Selective Functionalization of Amino Functions of Polyamines Using Solid Supports

Bycroft et al. reported the first examples of applying SPS techniques to the synthesis of PA conjugates, namely the natural spider toxins nephilatoxins (NPTX).[169] Thus, the CAD derivative **149** (Figure 21) was anchored on an Fmoc-UltraSyn C or Fmoc-PAL-PEG/PS amide resin and then the amino protecting groups were selectively removed to allow for the stepwise assembly of the NPTX skeleton from both ends. Furthermore, they attached SPM to the PCtr resin and used the thus-obtained PCTr-SPM (**150**) to synthesize the potent philanthotoxin analogue PhTX-343.[170] This group also reported an efficient synthesis of $T(S)_2$ using SPS techniques and the Dde or its analogue Nde group for primary amino group protection.[120] Thus, the N^1,N^8-Dde_2-SPD was attached to a NovaSyn® TGA support, derivatized with the HMPA linker, to give the corresponding polymeric SPD derivative, which was then used to obtain the fully protected polymeric $T(SH)_2$ (**151**). From **151**, $T(S)_2$ was readily obtained through sequential *N*-deprotection and simultaneous TFA-mediated removal of the side-chain protecting groups and detachment from the resin, followed by aerial oxidation. It should be noted that the first SPS of $T(S)_2$ was reported by Sergheraert et al. who used the novel derivative N^1,N^8-$TBDPS_2$-SPD to attach SPD to a functionalized Merrifield resin.[171] From the thus-obtained polymeric SPD derivative, the fully protected polymeric $T(SH)_2$ derivative **152** was assembled. However, simultaneous deprotection and cleavage from the resin to obtain $T(SH)_2$ required the use of anhydrous HF, a more destructive and less-convenient acid to handle.

Bradley et al. have developed a variety of PA linkers which, when attached on an aminomethylated resin to produce polymeric PA derivatives like **153** (Figure 21), are suitable for use in the SPS of $T(S)_2$, $NT(S)_2$ and $HT(S)_2$, open-chain or cyclic $T(S)_2$ analogues and combinatorial libraries of PA oligopeptides.[172] Furthermore, they have recently attached SPM derivatives on an aminomethylated resin and showed that the resulting polymeric SPM reagents, e.g. **154**, can be used for the preparation of SPM conjugates, such as kukoamine A.[173] Byk et al. exploited the extremely mild acidic conditions, namely treatment with TFE, required to detach the PCtr resin from a carboxy function to develop a series of linear (**155**), branched and cyclic unsymmetrically functionalized PAs, suitable for use in the preparation of combinatorial libraries.[174] Uriac et al. used functionalized Wang resins, e.g. **156**, to prepare a series of N^1-mono-



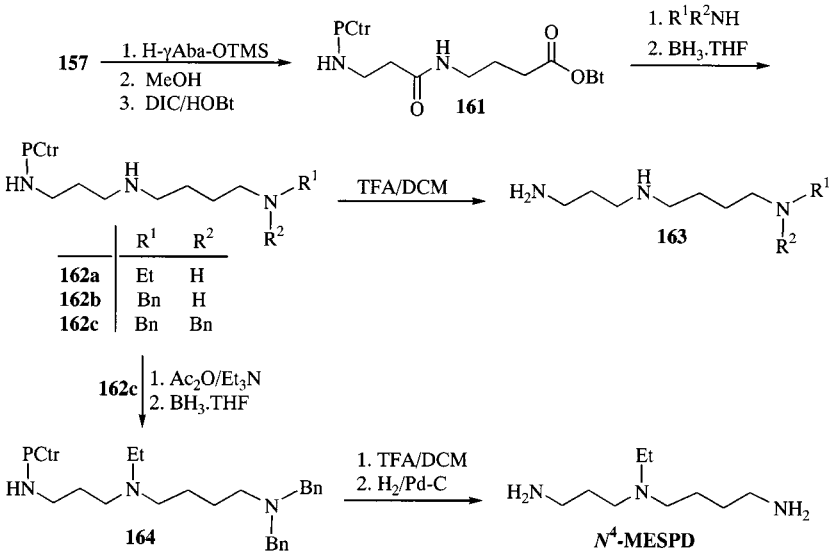
Scheme 6. Synthesis of polyamine analogues and conjugates using the PCTr resin for *N*-protection and the amide approach

functionalized PUT and SPM derivatives and evaluated their biological activity in various systems.[175]

4.2 Fragment Solid-Phase Synthesis of Polyamine Analogues and Conjugates

Papaioannou et al. attached βAla to the PCtr resin and activated the thus-obtained PCtr-βAla-OH with HOBt and DIC. "Active" ester **157** was then reacted with an excess of PUT to obtain the polymeric amide **158**, which, upon acylation with either Trt-βAla-OSu or Fmoc-βAla-Cl, provided the bisamides **159** and **160**, respectively (Scheme 6). The bisamide **159** was reduced with BH_3·THF to the corresponding SPM derivative, which can be used in the preparation of KukC or bisbenzoylated and then reduced also with BH_3·THF to give N^1-PCtr-N^4,N^9-Bn_2-N^{12}-Trt-SPM. Detachment from the resin with 20% TFA in DCM, in the presence of Et_3SiH, provided the N^4,N^9-Bn_2-SPM, which is an intermediate in the synthesis of KukA.[156] On the other hand, Fmoc deprotection of bisamide **160** with Pip, followed by acetylation, reduction with BH_3·THF and detachment from the resin, led to N^1-MESPM.[155]

Furthermore, the one-pot reaction of "active" ester **157** with the in-situ-generated H-γAba-OTMS, followed by activation with HOBt/DIC gave the key-intermediate **161** (Scheme 7). Acylation of primary and secondary amines with **161** and BH_3-mediated reduction of the thus-obtained bisamides, provided the polymeric SPD derivatives **162**. Detachment from the resin using 20% TFA in DCM finally led to N^8-substituted SPD analogues (**163**). Alternatively, the SPD derivative **162c** can be acylated, e.g. with Ac_2O, and the obtained amide further reduced with BH_3·THF to give the fully protected SPD derivative **164**. Detachment from the resin, followed by catalytic hydrogenolysis, resulted in the preparation of N^4-MESPD.[155]



Scheme 7. Synthesis of selectively *N*-alkylated spermidines using polymeric benzotriazolyl ester

These methodologies are suitable for the preparation of combinatorial libraries of PA analogues and conjugates, simply by varying the amino acids, the amines and the acylating agents necessary for the assembly of the polyamine skeleton and side-chains.

5. Conclusion

Naturally occurring PAs and PA analogues and conjugates are characterized by an amazing structural diversity and exhibit a variety of interesting biological activities. Structure-activity relationship studies of biologically active molecules bearing a PA chain have already allowed an understanding of their mode of action and the development of potent analogues with important medicinal applications. A variety of synthetic methodologies, involving partial or total syntheses, have already been developed which allow the efficient preparation of even the most complex PA conjugates. Recently, related solid-phase techniques have been established which allow the efficient preparation of biologically interesting PA conjugates and combinatorial libraries. These techniques will undoubtedly be widely used in the near future to speed up the process of identifying the most potent synthetic PA analogues and conjugates and thus facilitate the development of PA-based pharmaceuticals or compounds with agrochemical or technical interest.

Acknowledgments

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