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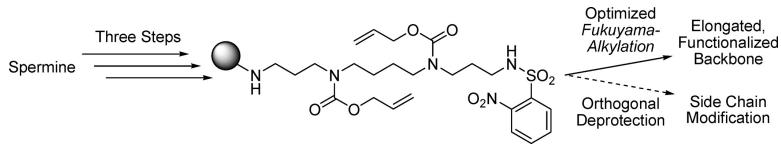

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Versatile Procedure for Asymmetric and Orthogonal Protection of Symmetric Polyamines and Its Advantages for Solid Phase Synthesis

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To date, many polyamine syntheses are carried out on solid phase to allow the generation of biologically active polyamine conjugates and libraries of natural product analogs. The synthesis of compounds and libraries, which derive from a symmetric polyamine building block such as spermine requires asymmetric and orthogonal protection of the symmetric polyamine. For this purpose we have established a novel Aloc- and Nosyl-protection group strategy, which displays several advantages. Solution phase synthesis and an easy workup reveals high yield of the asymmetrically and orthogonally protected polyamine. Asymmetric protection prevents cross-linking of the resin, and sequential deprotection can occur on highly acid and base labile resins without cleavage of the linker. Finally, it tolerates the elongation and modification of the symmetric polyamine backbone with several functional groups by conventional *Fukuyama-alkylation*. The suitability of this protection group strategy was shown by the first solid phase synthesis of the philanthotoxin-analog HO359b.

Many of the naturally occurring polyamine structures such as spermine and spermidine, which are ubiquitously present in all prokaryotic and eukaryotic cells, are based on simple aliphatic structures. Although their diversity is low, the cellular concentration within a millimolar range suggests a variety of important biological functions. (for reviews, see refs 1 and 2). Due to their protonation in the cellular environment, they can interact with many negatively charged molecule species, which comprise phosphate groups of membrane lipids, DNA and RNA, negatively charged amino acids, and more. They have been shown to condense DNA to stabilize its conformation or to prevent its degradation. Further, they are involved not only in the growth of normal tissue and wound healing processes but also in the proliferation of malignant tumors. Besides their function in promoting cell growth, some analogs also play an important role in cell death (apoptosis) by regulating gene expression making them good candidates for anticancer drugs. Other naturally occurring polyamines are potent immunosuppressants, antibiotics, and spider toxins.[2,3] Due to their cationic nature, polyamines were found to enhance the water solubility and the cellular uptake of many therapeutically active molecules and hence can serve as potent drug delivery agents. Therefore, current research on natural polyamines, their analogs, and also conjugates requires straightforward syntheses to gain novel mimics for therapeutic applications.[2]

In the search for therapeutically active compounds, several combinatorial approaches on solid phase have been undertaken, which exploit a variety of polyamine backbones.[4–8] However, many polyamine conjugates have either amphiphilic character or are highly sensitive to acids and bases. In some approaches, hydrophilic polyamine building blocks have been cleaved from a solid support and sequentially coupled to hydrophobic moieties to overcome the difficulties (for review, see ref 2). Therefore, novel strategies, which address the requirements mentioned above, have to be developed to enlarge the diversity of the libraries. Besides several reports on libraries based on unsymmetric polyamines such as spermidine, the synthesis of compounds and libraries that derive from symmetric polyamine building blocks such as spermine becomes interesting and often requires a sophisticated protection group strategy to allow a directed functionalization of selected amino groups.

In general, solid phase synthesis of polyamines and their conjugates can be pursued by two strategies.[9,10] The first strategy is based on the stepwise or modular elongation of the backbone with small building blocks. The other strategy comprises the coupling of larger partially protected polyamine building blocks to rapidly elongate the backbone. The preference for one of these methods depends on the functional properties of the building blocks. Finally, both require a combination of solution and solid phase methods.

Although the first strategy allows the introduction of more diversity in the aliphatic backbone between two amino groups and in the side chains, it is limited by the fact that every elongation step decreases the yield. Likewise, the smaller building blocks also have to be prepared in at least one solution phase step making the modular approach less favorable for the generation of longer backbones.

For the synthesis of longer backbones, it is more economical to prepare building blocks, which can be coupled to the support in one step. This method is especially favorable for

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commercially available backbones such as spermine. However, this requires an efficient protection of the primary and secondary amino groups in a solution phase synthesis step.[4,11–17] For high throughput library productions and large-scale SPS, large quantities of highly stable and partially or fully protected polyamine building blocks are required. They should be asymmetrically protected to exclude cross-linking reactions that would severely decrease yields and purities of the solid phase synthesis. The substitution should also reveal direct access to a variety of different polyamine structures without laborious conversions after coupling to solid support.

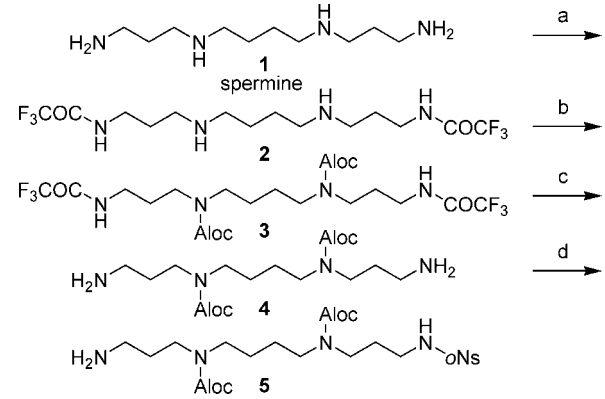
Here, we present the solution synthesis of such asymmetrically and orthogonally protected building blocks derived from symmetrical polyamine precursors comprising the combination of Aloc- and Nosyl-protection groups. After attachment to the solid support, this strategy exhibits advantages compared to other common protection group systems. It prevents cross-linking of the resin, and sequential deprotection can occur on highly acid and base labile resins without cleavage of the linker. Finally, it tolerates the elongation and modification of the symmetric polyamine backbone with several functional groups by conventional *Fukuyama-alkylation*.

So far, several examples can be found for the orthogonal protection of secondary and primary amines in solution phase as well as on solid phase. While secondary amines are mainly protected by Boc groups, Tfa, Dde, or Mmt groups usually serve for the temporary protection of primary amines.[18–23] As Boc groups are only removed under strong acidic conditions, their use precludes the synthesis of acid labile polyamine conjugates on highly acid labile trityl linkers. The permanently protected secondary amines will also be blocked during solid phase synthesis disabling further manipulation such as branching of the backbone.

The Aloc group is one of the most commonly used amino-protection groups, which particularly found its application in the synthesis of acid and base sensitive conjugates.[24–27] It is completely orthogonal to almost all other protection groups and can be removed under very mild conditions without affecting most common protecting groups and functionalities. During deprotection with palladium complex, the allyl moiety forms a complex with the Pd^0 species and is then trapped by a scavenger. The broad spectrum of the commercially available scavengers allows its use for a variety of immobilized substrates with different properties.

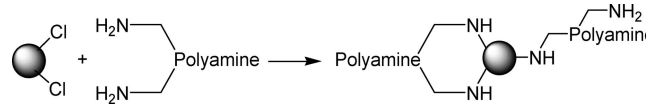
The first step in the development of a suitable asymmetric protection strategy was adapted from a published procedure, which used Boc as permanent protection group of the secondary amines and Tfa as temporary protection group for the primary amines. In our approach, Boc was replaced by Aloc as depicted in Scheme 1. Deprotection of the primary amines provided **4** in 99% yield in a one-pot reaction (Scheme 1).[22] The symmetric building block **4** was coupled to a conventional 2-chlorotrityl resin (Barlos resin; loading 1.3 mmol/g)[28–30] and an alkoxytrityl resin (Fukuyama resin; loading about 0.4 mmol/g)[31,32] by slowly adding the resin to a large excess (up to 50 equiv) of **4** according to previously published solid phase polyamine syntheses. However, further

Scheme 1. Synthesis of the Protected Polyamine Building Block **5**[a]



[a] Reagents and conditions: (a) $F_3C-COOEt$ (3.5 equiv), MeOH, −80 °C; (b) Aloc-Cl (5 equiv), Et_3N (5 equiv), 0 °C to rt; (c) $NaOH_{conc}$/H_2O (4:3), rt; (d) *o*Ns-Cl (0.6 equiv), collidine (1 equiv), rt.

Scheme 2. Cross-linking Reactions which Lead to Distinct Losses of Purity, Yield, and Resin Capacity

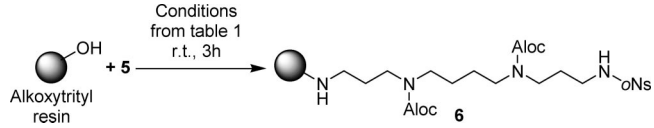


derivatization and cleavage from the resin revealed a large portion of nonderivatized educt **4**, due to cross-linking of the symmetric spermine building block, which seemed to be independent from the applied excess (Scheme 2).

This is a major problem, which frequently occurs during coupling of polyamines with two terminal primary amino groups. It leads to a decrease of the resin loadings and to contamination of the final coupling product with the polyamine after cleavage from the resin. In most cases, the impurities are hard to separate and reduce the advantage of solid phase synthesis. Previously, resin cross-linking during further derivation of already-loaded polyamines was prevented by lowering the capacity of the resin. This was done by blocking about 50% of the linker binding sites.[33,34] However, there are only a few reports on cross-linking during the polyamine loading steps,[35] although decreased yields indicate similar problems.[17]

To avoid cross-linking, one of the primary amines was protected with an Ns group to ensure an unambiguous course of the reaction, which is more efficient than considerably lowering the resin capacity. Building block **4** was reacted with 2-nitrobenzenesulfonyl chloride (0.6 equiv) and collidine (1 equiv) in solution phase. The reaction yielded 71% of **5** based on the onset of *o*Ns-Cl and nonreacted **4** could be recovered for further reaction cycles (Scheme 1). A 0.6 equiv portion of *o*Ns-Cl was optimal with respect to avoid the formation of the bisnosylated byproduct. More than 0.6 equiv of *o*Ns-Cl increased the amount of the undesired bis-nosylated side product without increasing the amounts of the mononosylated product **5**. The overall yield for one reaction of 18.24 mmol spermine is 43%. However, it should be mentioned that more than 80% of the initially applied spermine can theoretically be converted to **5,** if the recovered 41% of **4** is reused in further reaction cycles. Building blocks

Table 1. Attachment of **5** to a Fukuyama-Trityl Resin



entry	base	solvent	excess (equiv)	loading (mmol/g)[a]
1		CH_2Cl_2	10	0.240
2	pyridine/CH_2Cl_2 (1:1)		10	
3	DMAP 0.2 equiv	pyridine	10	0.012
4	triethylamine 5 equiv	CH_2Cl_2	10	0.426
5	DiPEA 2.5 equiv	CH_2Cl_2	5	0.355
6	DMAP 1 equiv	CH_2Cl_2	5	0.351
7	pyridine 2 equiv	CH_2Cl_2	5	0.354
8	DiPEA 2.5 equiv	CH_2Cl_2	10	0.424

[a] Loadings were calculated by addition of 1,4-dioxane as a quantitative NMR standard.

Table 2. Aloc Deprotection with Different Scavengers[a]

scavenger	excess of scavenger (equiv)	amount of Pd $(PPh_3)_4$ in mol %	reaction time	deprotection in %[b]	allylamine formation in %[b]
pyrrolidine	10	50	20 h	100	33
	20	20	16 h	100	10
	20	5	21 h	27	0
	20	1	21 h	0	0
$PhSiH_3$	9	20	3 h	100	20
	10	5	1.5 h	100	12
	20	1	2 h	0	0
Me_3SiN_3	10	15	17 h	83	10
	8	10	17 h	68	1
dimedone	10	15	17 h	4	0
	8	10	17 h	29	0
	8	10	0.5 h	0	0
dimethylbarbituric acid	10	10	2 h/35 °C	99	<1

[a] If not indicated, the reactions were carried out at room temperature. [b] Amounts were calculated from the corresponding Aloc integrals in the ^1H NMR spectra of the crude product relative to those in **6**. The β-amino/carbamoyl−methylene groups of the spermine backbone between 1.3 and 2.1 ppm were set to be 8.0.

4 and **5** are both stable to aqueous solutions of pH values from 1 to 14, 15 M ammonia solution, and temperatures over 80 °C.

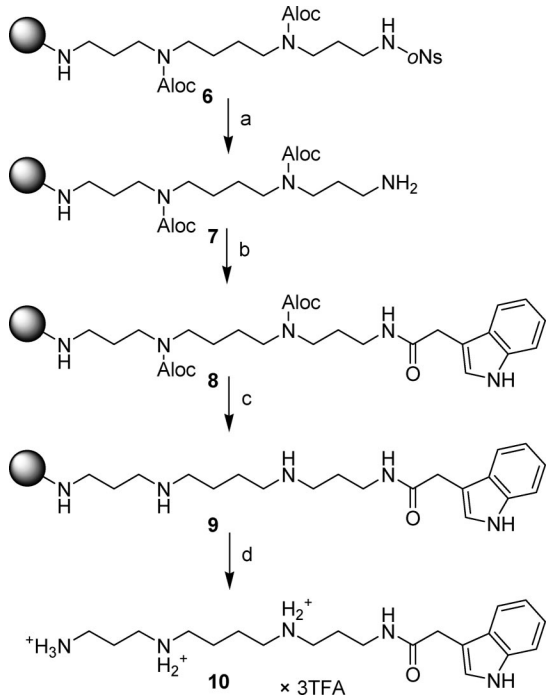
This stability of the Aloc and Nosyl groups to aqueous acidic conditions greatly facilitates the workup procedure. While the bis-protonated form of **4** and collidine could be extracted with half concentrated HCl, the mono-protonated form of **5** completely remained in the organic phase. After removal of the base from the combined aqueous layers in high vacuum, **4** was recovered in high purity. This acidic treatment is the key step in this workup procedure and is not compatible to protection with Boc or Mmt groups, which are sensitive to aqueous solutions with a pH below 5. Hydrolized nosylchloride was removed by quickly washing the organic layers with NaOH (0.5 M). The resulting crude mixture primarily contains **5** and traces of the bis-nosylated side product, which can easily be separated by filtration over a short bed of silica gel. This bis-nosylated side product is also an interesting building block for polyamine synthesis. The easy workup allows the efficient large-scale preparation of useful starting materials for polyamine solid phase chemistry. The whole procedure should be transferable to other symmetric polyamines for example norspermidine.

To find the optimal conditions for the attachment of the building block to a trityl resin with respect to an easy recovery of **5**, we investigated the use of different bases, solvents, and excesses of building block **5**. A 100 mg portion of an alkoxytrityl resin was activated with $SOCl_2$ and subsequently reacted under the following conditions (Table 1).

A 10 equiv portion of building block **5** and 2.5 equiv DiPEA in dichloromethane provided the best loadings. The addition of a base seemed to be necessary to get feasible loadings within a short time. The choice of the base was of minor importance, although it should be mentioned that a large excess of triethylamine and pyridine partially blocked the linker. Longer reaction times than 3 h did not produce significantly higher loadings. After loading of the resin, excess of building block **5** could easily be recovered by the removal of solvent and Hünig's base in vacuo and could be reused in further reactions.

During deprotection of the Aloc groups, the formation of the more stable allylamines was constantly observed (Table 2). This was probably due to the close proximity of the liberated amines, which could themselves act as allyl scavengers during deprotection. Higher amounts of the Pd catalyst as well as decreasing levels of scavenger enforced the problem, while in the vice versa situation the deprotection was incomplete even after long reaction times. Basic scavengers, hydride donors, and azides did not sufficiently suppress the allylamine formation. Indeed, dimedone reprotonated the deprotected amine to some extend and thereby avoided this problem but resulted in only poor deprotection even after 17 h.

Scheme 3. Synthesis of Philanthotoxin Analog HO359b[a]



[a] Reagents and conditions: (a) β-mercaptoethanol (10 equiv), DBU (5 equiv), DMF, rt (3×); (b) indole-acetic acid (5 equiv), DCC (5 equiv), HOBt (5 equiv), DMF; (c) dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 35°C; (d) TFA (5%)/CH_2Cl_2.

Usually, Rh- or Ni-catalysts are used to remove allylamines. Due to their costs and the fact that heavy metal complexes are hard to separate from the resin, their usage did not display an elegant solution of the problem. It was reported that allylamines can be removed at a slightly raised temperature in presence of Pd catalyst using *N,N′*-dimethylbarbituric acid as scavenger (Table 2).[36] Reaction with 10 equiv of scavenger and catalyst loading of 10 mol % for 2 h at 35 °C resulted in full deprotection without allylamine formation. The *o*Ns protection remains unaffected under those conditions.

To prove the versatility of this protection strategy in polyamine SPS, **6** was used for the solid phase synthesis of philanthotoxin analog HO359b (Scheme 3), which so far has only been synthesized in solution phase.[37,38] *o*Ns was removed from **6** with β-mercaptoethanol/DBU. 2-Indolacetic acid was coupled to the free primary amine by peptide bond formation. After Aloc deprotection and cleavage from the resin, the product was obtained in 90% yield and 93% purity demonstrating the suitability of the solid phase strategy.

Further, we investigated the suitability of the protection strategy to other reaction types. The most common synthetic strategies used for SPS of polyamines are S_N2-displacement,[34,39,40] Fukuyama-alkylation,[31,41–44] reduction of amides,[45–51] and reductive amination.[52–55] All of these methods display certain limits for their applicability on solid phase. S_N2-displacement easily leads to overalkylation and is therefore limited to the synthesis of tertiary amines, while reduction of amides and reductive amination are selective but require strong reducing agents like BH_3 and $Na(CN)BH_3$, making them inappropriate for the use with several functional groups as well as with protecting groups such as the Aloc group. Fukuyama-alkylation avoids those drawbacks. It is selective for the generation of secondary amines, and all steps can be carried out under mild conditions making the reaction interesting for the functionalization of polyamine libraries.

The primary amine is converted to a nitrophenyl- or dinitrophenylsulfonamide (*o*-/*p*-Ns or *d*Ns) group. The enhanced nucleophilicity of the masked amine leads to distinctly higher rates in halogen displacement or Mitsunobu reactions even in the presence of weak bases. Secondary amines are protected allowing the differentiation between primary and secondary amines in one step. Particular efforts have been made to optimize the Fukuyama−Mitsunobu alkylation.[42,43] However, after the first two alkylation steps, yields rapidly decrease in each further reaction cycle. Thus, an SPS of longer polyamines exclusively by this approach seems to be unfavorable. Due to the sensitivity of Mitsunobu conditions to air and humidity, we focused on the more robust reaction with halides. A broad range of halides is commercially available or can easily be obtained from the corresponding alcohols by reaction with PPh_3, iodine, and imidazole.

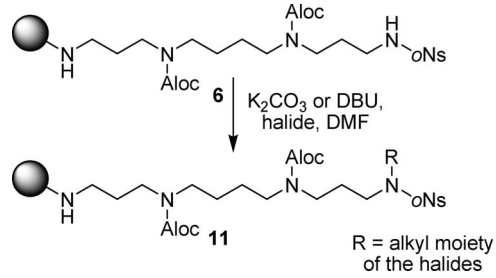
There were only a few examples of a conventional Fukuyama-alkylation with halides on solid phase.[56,57] It was used in the synthesis of spider toxins by Fukuyama and co-workers.[31,32] Previously, Stromgaard et al. investigated the use of different solvents, leaving groups, bases, temperatures, and numbers of reaction cycles.[56] It was reported that the choice of the base was of major importance. Strong bases like MTBD and DBU gave the best results with nearly quantitative conversion after three reaction cycles with MTBD being slightly superior to DBU.

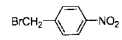
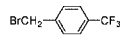
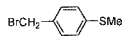
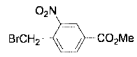
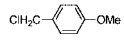
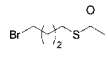
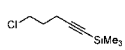
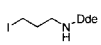
We investigated the reaction with differently functionalized halides under two sets of conditions (Table 3). The first was based on the original Fukuyama conditions (electrophile (20 equiv), K_2CO_3 (20 equiv), 60 °C or room temperature, 18 h), which were milder but, due to the weak base, resulted in lower deprotonation and hence lower displacement rates. As the originally applied 20 equiv could be critical for the use of valuable and expensive halides, we tested the reaction with 13 equiv. The second set of conditions (electrophile (6 equiv), DBU (6 equiv), rt, 3–5 h) gave higher rates but provoked side reactions due to the stronger basicity of DBU.

Table 3 can be summarized as follows: Benzyl- and allylhalides require DBU as a base. In the presence of K_2CO_3, the conversion was low in each case. Benzyl bromide shows the best result, while electron-rich benzyl compounds are superior to those that are electron-deficient (entries 1–10). Finally, entry 6 revealed no alkylation product according to Fukuyama. Instead, the formed Fukuyama product showed deprotonation at the benzyl position of the electron-deficient ring system. Subsequent nucleophilic addition to the Nosyl group and SO_2 elimination resulted in **12** (Figure 1).

Most aliphatic halides produced low conversion rates with DBU (entries 11–20), especially when the substrates were susceptible to β-elimination (entry 14). In the presence of carbonate, the conversions were distinctly higher. However, raising the temperature was necessary to achieve higher conversion rates after one reaction cycle. While bromides and iodides reacted equally well (entries 11–16, 19, and 20),

Table 3. Conversion of Halides in the Fukuyama-Alkylation Reaction



Entry	Halide	Base	Reaction time	Temperature	Conversion in %[a]
1		K_2CO_3	18 h	rt	20
		DBU	18 h	rt	98
		DBU	8 h	rt	100
		DBU	3 h	rt	97
2		K_2CO_3	10 h	60°C	30
		DBU	18 h	rt	80
		DBU	8 h	rt	59
3		DBU	30 h	rt	93
		DBU	5 h	rt	78
		DBU	3 h	rt	66
4		DBU	5 h	rt	95
		DBU	3 h	rt	84
5		DBU	5 h	rt	87
		DBU	3 h	rt	95
6		DBU	3 h	rt	37
7		DBU	3 h	rt	86
8		DBU	3 h	rt	97
9		DBU	3 h	rt	91
10		DBU	3 h	rt	92
11		K_2CO_3	18 h	rt	89
		DBU	3 h	rt	38
12		K_2CO_3	18 h	60°C	57
		K_2CO_3	18 h	rt	68
		DBU	3 h	rt	45
13		K_2CO_3	8 h	60°C	93
14		K_2CO_3	8 h	60°C	97
		K_2CO_3	18 h	rt	48
		DBU	3 h	rt	2
15		K_2CO_3	18 h	rt	44
		DBU	3 h	rt	20
16		K_2CO_3	18 h	rt	75
		DBU	3 h	rt	22
17		K_2CO_3	18 h	60°C	47
		K_2CO_3	18 h	rt	6
		DBU	3 h	rt	1
		DBU	3 h	rt	1
18		K_2CO_3	18 h	rt	< 5
		DBU	3 h	rt	< 5
		DBU	3 h	rt	0
19		K_2CO_3	18 h	rt	86
		DBU	3 h	rt	35
20		K_2CO_3	18 h	60°C	98
		DBU	3 h	rt	3

[a] After cleavage from the resin, the conversions were determined by the RP-HPLC peak integration of the product peak in comparison to remaining educt and side products (UV-detection at 254 nm).

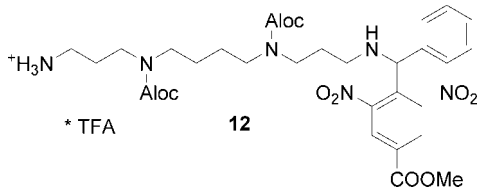


Figure 1. Isolated product in entry 6.

chlorides showed only minimal conversions either after long reaction times or after addition of NaI (1 equiv) (entries 17 and 18). Moreover, higher temperatures could slightly improve the conversions and multiple reaction cycles could maybe raise them to nearly quantitative.

As expected, the purities in the reaction with the symmetric dibromide (entry 12) are diminished by the formation of a side product.[58] This is due to the cross-linking reaction of both halides with two immobilized sulfonamides. Although this reaction was carried out with 20 equiv of the bromide, the amount of side product was still significant. In entries 15 and 19, the products were obtained as the hydrolyzed compounds. As no ether-formation was found, hydrolysis of epoxide and acetal exclusively happened during cleavage from the resin and purification of the crude product.

In summary, we have shown an economical procedure for the large-scale preparation of asymmetrically and orthogonally protected polyamine building blocks starting from their symmetric precursor. The method in combination with an easy workup procedure allows the large-scale preparation of polyamine building blocks and the easy recovery of the intermediate for further reaction cycles. Laborious column chromatography is avoided.

We further demonstrated the suitability of such polyamine building blocks for SPS. The building block **5** could be coupled to the solid support avoiding cross-linking of the resin and could be recovered for further reaction cycles. Problems that occurred during Aloc deprotection were successfully solved and the philanthotoxin analog HO359b was synthesized in high yield and purity to demonstrate orthogonality and equality to other protection group systems. For further derivatization and elongation of the backbone, Fukuyama-alkylation was identified to be the preferred method. Its compatibility with the Aloc protection strategy and a broad range of functional groups was shown. We could show that benzyl and allyl halides need DBU as a base, while aliphatic halides prefer carbonate. Variation of fundamental reaction parameters gave an insight into substrate specifity and the achievement of high conversion rates for each substrate class.

Abbreviations. SPS, solid phase synthesis; SP, solid phase; Tfa, trifluoroacetyl; Dde, 2-acetyl-5,5-dimethyl-1,3-dioxocyclohexyl; Mmt, monomethoxytrityl; Aloc, allyloxycarbonyl; *o*Ns, *ortho*-nitrobenzylsulfonamide; DiPEA, diisopropylethylamine; MTBD, 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene.

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Supporting Information Available. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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