Methods for the Selective Modification of Spermidine and Its Homologues

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In recent years a great deal of attention has been focused on the polyamines spermidine, norspermidine, homospermidine, 1,4-diaminobutane (putrescine), and spermine (Figure 1). These studies have been directed largely at the biological properties of the polyamines probably because of the role they play in proliferative processes.[1-3] It was shown early on that the polyamine levels in dividing cells, e.g., cancer cells are much higher than in resting cells, although the precise reason for this remains somewhat of a mystery.[4] However, regardless of the reason for increased polyamine levels the phenomenon can be and has been exploited in chemotherapy.[5-8]

When polyamine biosynthesis (spermidine biosynthesis) is shut down in transformed cells by treatment with α-difluoromethylornithine (DFMO), the cells incorporate spermidine at an accelerated rate relative to untreated cells.[9-16] This encouraged a number of workers to consider the spermidine uptake apparatus as a means of delivering antineoplastic drugs to transformed cells.[17] In fact the uptake of MGBG (methylglyoxal bis(guanylhydrazone)), an antineoplastic, was shown to occur via the polyamine transport apparatus and was enhanced by pretreatment of the cells with DFMO.[18] The drug DFMO is a very effective inhibitor[2] of ornithine decarboxylase (ODC), an essential enzyme in polyamine biosynthesis. This compound thus depletes the spermidine level of the cells, which are forced to take in exogenous polyamine. The enhanced MGBG uptake is, however, somewhat surprising to the author as the molecule is structurally so different from spermidine, although workers have claimed otherwise.[18] Nevertheless, the spermidine uptake system as a means of delivering anticancer drugs to tumors still offers tremendous opportunity.

It is clear that the best place to fix an antineoplastic to the polyamine is at one of the nitrogens simply because of the synthetic facility. However, it was necessary to first ascertain the structural boundary conditions set on the polyamines and their derivatives by the polyamine uptake apparatus, i.e., to determine what kind of structural modifications on the polyamine are compatible with the uptake apparatus. This meant structure activity studies and therefore substantial synthetic modification of the polyamines.

Early Methods of Polyamine Modification

At the outset of these investigations the literature revealed only a limited number of methods available for the selective modification of putrescine, spermidine, norspermidine, homospermidine, and spermine. There are two major problems with functionalizing the polyamine nitrogens: (1) fixing the group or groups to the correct nitrogen or nitrogens; (2) effecting such modification in a way which is viable in terms of yield. With regards to the second issue, many investigators have accepted the low yields and separation problems associated with random functionalizations aimed at getting "some" of the right product. The reagents available for polyamine functionalization included a number of nitriles,[19] di-*tert*-butoxycarbonylated spermidine,[20] a cyclic urea,[21] and several hexahydropyrimidines.[21] The nitriles include those indicated in Table I.

Nitrile A with x values of 3 and 4 allows access to selectively N-functionalized norspermidine and spermidine, respectively, while nitrile B with $x = 4$ provides access to selectively N-functionalized spermine compounds. In the nitrile A norspermidine and spermidine precursors the N^1,N^4 and N^4,N^8 positions, respectively, remain free for functionalization. This means that these positions can be altered and the nitrile reduced to the corresponding amine, providing the appropriate norspermidine and spermidine compounds. However, several factors must be considered when evaluating these systems. In each nitrile A, both a primary and

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$H_2NCH_2CH_2CH_2CH_2NH_2$

Putrescine

$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$

Norspermidine

$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$

Spermidine

$H_2NCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$

Homospermidine

$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$

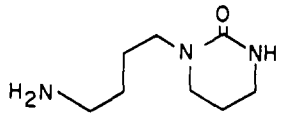
Spermine

Figure 1.

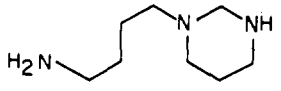
secondary amino nitrogen remain for functionalization. Regioselectivity therefore depends on reactivity differences between the primary and secondary nitrogens, a difference which can be difficult to exploit. It is unlikely that all acylating or alkylating agents can be utilized to cleanly anneal to either position. However it is not unreasonable to expect clean difunctionalization of the nitrogens and, in fact, this has been accomplished and has resulted in a second group of polyamine reagents for selective nitrogen functionalization.

The mononitrile with $x = 4$ was di-*tert*-butoxycarbonylated with BOC-ON [2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile] and the nitrile group then reduced in 70% yield to produce the free amine. The N^1 nitrogen of spermidine could then be functionalized and the *tert*-butoxycarbonyl groups could next be removed with trifluoroacetic acid. The same procedure could also be effected on any of the other mononitriles of Table I. Application of this procedure to the bis(nitriles) of Table I would allow for bis functionalization of the primary amines of spermine and its homologues. An additional series of reagents developed by Ganem[21] held the spermidine system masked as either a cyclic urea or as a hexahydropyrimidine (Figure 2). These cyclic systems offer several modes of N-functionalization. The urea can be N^8-functionalized (relative to spermidine) and the urea cleaved in base to the spermidine. The pyrimidine can be N^1,N^8-functionalized and opened with ethyl hydrogen malonate and piperidine or pyridine. Again, if functionalization reagents are available which discriminate between primary and secondary amines, N^1- or N^8-derivatives are available. These cyclic reagents have also been extended to spermine by simply coupling 2 mol of formalin with spermine to produce the corresponding bis(hexahydropyrimidine).

Because of our interest in the catecholamide siderophores and our interest in polyamine uptake, we initiated a program to develop a series of reagents for selectively functionalizing polyamines. The synthesis of these reagents was designed to permit selective modification of specific nitrogens on spermidine, norspermidine, and homospermidine backbones. The idea was to build on what was already available. The reagents were constructed so as not to depend on any differences in reactivity between nitrogen but simply on free versus blocked nitrogens.



CYCLIC UREA



HEXAHYDROPYRIMIDINE

Figure 2.

Spermidines Protected at the Secondary Nitrogen

The first of the reagents[22,23] consisted of a group of secondary N-benzylated triamines (Figure 3). The synthesis was predicated on a series of equilibria which can be controlled by varying reaction conditions. In each case the starting material was benzylamine (I). In proceeding to the norspermidine reagent, benzylamine (I) can either be directly reacted with excess acrylonitrile under pressure to produce the bis(nitrile) II or can be reacted at room temperature with a mole of acrylonitrile to produce the mononitrile III which can be purified by distillation and reacted with a second mole of acrylonitrile to generate II.

The spermidine reagent can be prepared by alkylating the mononitrile III with 4-chlorobutyronitrile in butanol in the presence of sodium carbonate. This alkylation is associated with some retrograde cyanoethylation of the product dinitrile V resulting in the mononitrile VI. This mononitrile can then be alkylated with 4-chlorobutyronitrile to produce the dinitrile IV. However this symmetrical impurity amounts to a small percent of the products and can be removed later on in the synthesis by careful distillation. Finally, synthesis of the homospermidine reagent simply involves bis alkylation of benzylamine with 4-chlorobutyronitrile as before to produce the nitrile (IV). Each of the nitriles II, III, IV, and V is accessible in greater than 80% yield. All of these nitriles could be reduced to the corresponding amines VIII, IX, X, and XII with either lithium aluminum hydride or with W-2 Raney nickel in ethanol in the presence of sodium hydroxide. Although both reagents are practical we have found the Raney nickel procedure to be particularly effective because of its simplicity, high yield, and ease of product workup.[24] In an attempt to ascertain the general applicability of this catalyst, we evaluated a group of nitrile reductions (Table II).

In a model study utilizing *N*-(2-cyanoethyl)-*N*-(3-

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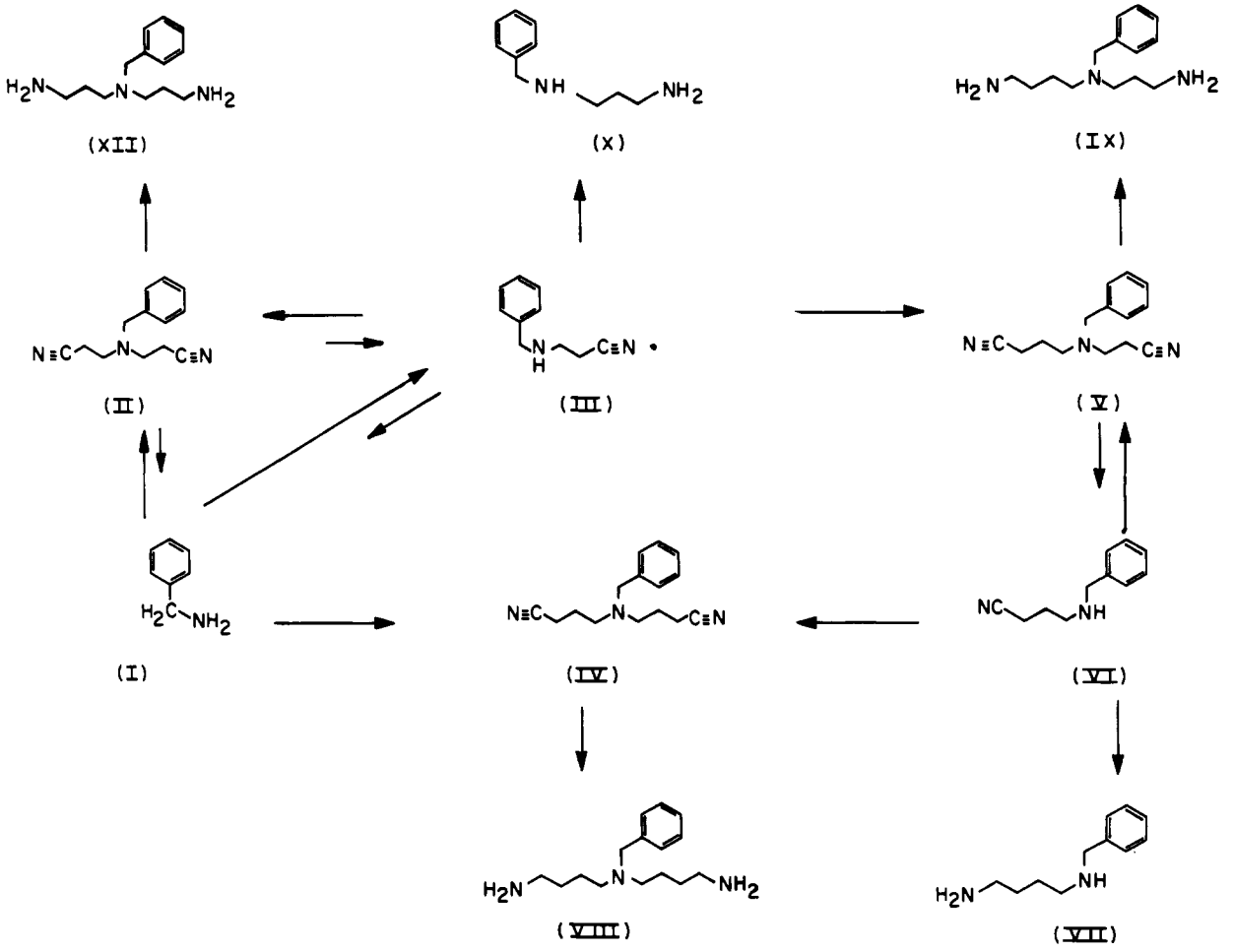

Figure 3.

Table I.

nitrile structure	methylene bridge size	yield of nitrile
(A) $H_2N(CH_2)_xNHCH_2CH_2CN$	x = 2, 3, 4, 5, 6, 9, 10	x = 2 (42), 3 (53), 4 (50), 5 (68), 6 (46), 9 (34), 10 (55)
(B) $NCCH_2CH_2NH(CH_2)_xNHCH_2CH_2CN$	x = 2, 3, 4, 5, 6, 9, 10, 12	x = 2 (25), 3 (57), 4 (50), 5 (93), 6 (15), 9 (98), 10 (99), 12 (97)

Table II.
Reduction of Nitriles 1 to Polyamines 2

nitrile	amine	yield, %
1a, $NC(CH_2)_2N(CH_2C_6H_5)(CH_2)_2CN$	**2a**, $H_2N(CH_2)_3N(CH_2C_6H_5)(CH_2)_3NH_2$	91
1b, $NC(CH_2)_2N(CH_2C_6H_5)(CH_2)_3CN$	**2b**, $H_2N(CH_2)_3N(CH_2C_6H_5)(CH_2)_4NH_2$	91
1c, $NC(CH_2)_3N(CH_2C_6H_5)(CH_2)_3CN$	**2c**, $H_2N(CH_2)_4N(CH_2C_6H_5)(CH_2)_4NH_2$	78
1d, $NC(CH_2)_2NH(CH_2)_2CN$	**2d**, $H_2N(CH_2)_3NH(CH_2)_3NH_2$	81
1e, $NC(CH_2)_2NH(CH_2)_4NH(CH_2)_2CN$	**2e**, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	76
1f, $NC(CH_2)_2N(CH_3)(CH_2)_3CN$	**2f**, $H_2N(CH_2)_3N(CH_3)(CH_2)_4NH_2$	71
1g, $C_6H_5CH_2NH(CH_2)_2CN$	**2g**, $C_6H_5CH_2NH(CH_2)_3NH_2$	90

cyanopropyl)benzylamine (**1b**) the ratio of nitrile to Raney nickel catalyst (w/w) was varied from 5:1 to 7:1 with the higher ratios requiring longer reaction times for complete reduction as expected. The concentration of sodium hydroxide was varied from 1 M to 5 M in 95% ethanol with similar results. However, substituting ammonium hydroxide for sodium hydroxide gave only a 17% yield of the bis(amine) from the bis(nitrile) under identical conditions. This is surprising in view of the widespread use of ammonia and amines in general to prevent the formation of secondary amines in high temperature, high pressure reductions of this type. Without any added base as cocatalyst, we found that only 33% of the nitrile is reduced and the reaction is not nearly as clean.

In an effort to determine if the sodium hydroxide was generating a more active form of Raney nickel, a mixture of Raney nickel and sodium hydroxide in 95% ethanol was shaken under hydrogen (40 psi). The catalyst was then filtered, washed free of sodium hydroxide, and put back under hydrogen (40 psi) along with *N*-(2-cyanoethyl)-*N*-(3-cyanopropyl)benzylamine (**1b**) in 95% ethanol. Only a low yield of bis(amine) **2b** was observed (33%), an amount comparable to the results with no added base and no pretreatment of the catalyst. Therefore, sodium hydroxide must play an active role in the reduction process and not simply preactivate the catalyst.

We have utilized the secondary N-benzylated reagents resulting from these reductions in the synthesis of a large number of terminally N-substituted triamines, both acylated and alkylated systems (Table III, R_2 =

Table III
Structures of SPD Derivatives $HN^1(R_1)CH_2CH_2CH_2N^4(R_2)CH_2CH_2CH_2CH_2N^8(R_3)H$

derivative	R_1	R_2	R_3	N^4 positively charged[a]	N^1 and N^8 positively charged[a]
SPD	H	H	H	yes	yes
N^4-methyl-SPD	H	CH_3	H	yes	yes
N^4-ethyl-SPD	H	CH_2CH_3	H	yes	yes
N^4-acetyl-SPD	H	Ac	H	no	yes
N^4-hexyl-SPD	H	$CH_2(CH_2)_4CH_3$	H	yes	yes
N^4-hexanoyl-SPD	H	$CO(CH_2)_4CH_3$	H	no	yes
N^4-benzyl-SPD	H	CH_2Ph	H	yes	yes
N^4-benzoyl-SPD	H	COPh	H	no	yes
N^1,N^8-diethyl-SPD	CH_2CH_3	H	CH_2CH_3	yes	yes
N^1,N^8-diacetyl-SPD	Ac	H	Ac	yes	no
N^1,N^8-dipropyl-SPD	$CH_2CH_2CH_3$	H	$CH_2CH_2CH_3$	yes	yes
N^1,N^8-dipropionyl-SPD	$COCH_2CH_3$	H	$COCH_2CH_3$	yes	no

[a] At physiological pH.

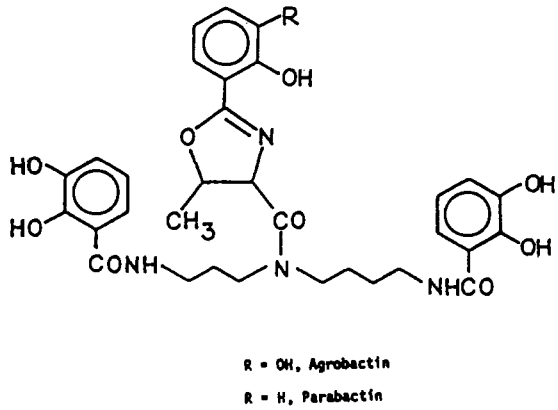

Figure 4.

H). The alkyl compounds were generated by reducing the corresponding acyl compound with lithium aluminum hydride. Initially once acylated the benzyl protecting group was removed by hydrogenolysis in methanol HCl over palladium chloride at atmospheric pressure.

We also utilized these reagents in the synthesis of a number of polyamine catecholamide iron chelators, including parabactin and agrobactin (Figure 4), isolated from microorganisms.[25-32] The synthesis in each case was initiated with the polyamine secondary N-benzylated reagent I, which was first terminally N-acylated with 2,3-dimethoxybenzoyl chloride and the product II isolated in 87% yield (Figure 5). The N-benzyl protecting group was again removed by hydrogenolysis over palladium at atmospheric pressure to afford III in 90% yield. This compound was next acylated at the free secondary nitrogen with L-*N*-*tert*-butoxycarbonylated threonine, activated as the *N*-hydroxysuccinimide ester, to produce IV in 84% yield.

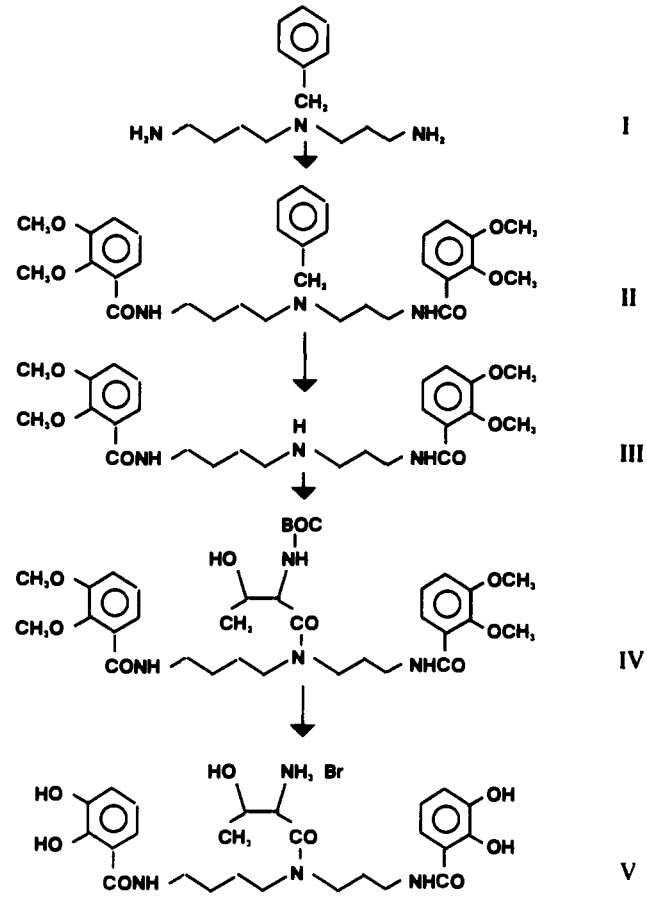

Figure 5.

The *tert*-butoxycarbonyl group was removed in 95% yield by brief exposure to trifluoroacetic acid and next the methoxy methyl groups removed by treatment with boron tribromide in methylene chloride resulting in V in 90% yield. At this point the option of generating either parabactin or agrobactin is available and both depend on the condensation of the appropriate ethyl imidate with the threonyl segment of the molecule. Stereospecific formation of the acid sensitive trans oxazoline ring of parabactin was achieved in high yield by heating V with ethyl 2-hydroxybenzimidate, which was derived from 2-cyanophenol and ethanolic hydrogen chloride (Pinner reaction). The condensation of V with ethyl 2,3-dihydroxybenzimidate, instead, would generate agrobactin. Unfortunately, 2,3-dihydroxybenzonitrile did not undergo addition of ethanol even upon

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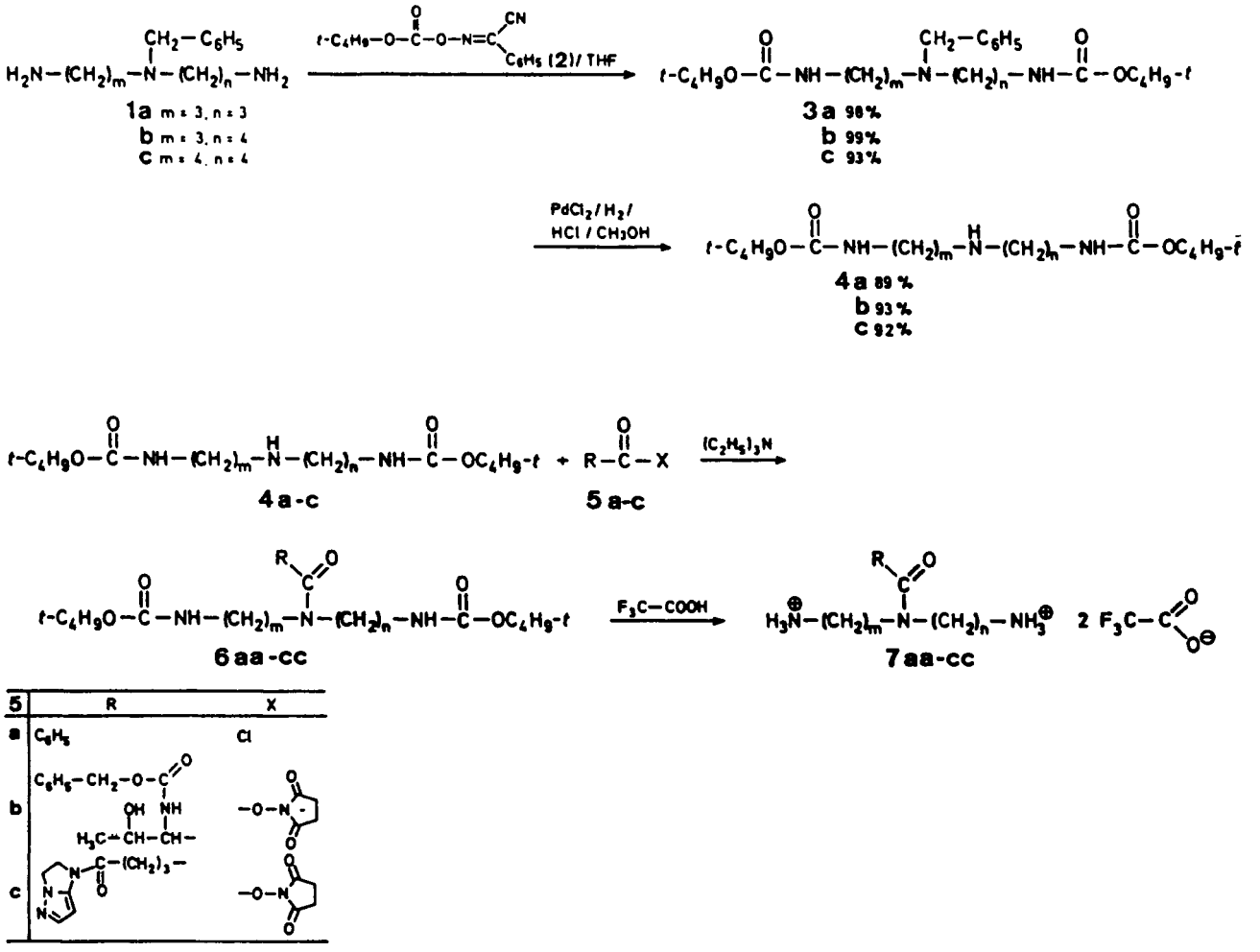

Figure 6.

heating in a high pressure reactor.

An alternate approach to the imidate ester was devised, based on O-alkylation of amides[32,33]. It was necessary to prevent alkylation of the catechol hydroxyls of 2,3-dihydroxybenzamide. The benzyl protecting group was chosen, because it can be removed catalytically under mild, neutral conditions.[34]

Aminolysis of 2,3-bis(benzyloxy)benzoyl chloride, available by a known route from 2,3-dihydroxybenzaldehyde,[35] furnished the corresponding amide in 92% yield. Selective amide O-alkylation with triethyloxonium hexafluorophosphate (1.1 equiv) in CH_2Cl_2, followed by basification[33] provided the protected imidate ester in 81% yield.

Oxazoline-forming condensation of V with the above imidate, followed by debenzylation, would afford agrobactin. However, we decided that debenzylation to the less hindered imidate would allow a more convergent total synthesis of agrobactin. Hydrogenolysis (10% Pd–C, 1 atm) was accomplished in 73% yield. It is important to note that benzyl cleavage was preferential to imidate reduction. Finally, heating the debenzylated imidate with V in refluxing methanol followed by purification on Sephadex LH-20 (20% ethanol–benzene) afforded agrobactin in 61% yield. The 300-MHz NMR spectrum was identical with that reported in the literature for naturally occurring agrobactin.[36]

Utilizing the protected polyamines, we were able to prepare a large number of parabactin and agrobactin analogues in high yield. These polyamine reagents clearly provided excellent opportunities for selective N-terminal polyamine modification.

Bis Primary Modified Spermidines

We now required a second group of reagents which would allow us to selectively access the secondary nitrogen of spermidine, homospermidine, and norspermidine. This would make it possible to assess the importance of this nitrogen in polyamine uptake and also to synthesize the agrobactin and parabactin A series of siderophores. These polyamine reagents were easily synthesized (Figure 6) from the corresponding secondary N-benzyl compounds by reaction with BOC-ON followed by hydrogenolysis.[37] The overall yield is in excess of 85%.

These reagents again allowed us to synthesize a large number of secondary N-substituted polyamines (Table III) R_1R_3 = H by first acylating the secondary nitrogen followed by brief exposure of the product to trifluoroacetic acid. The resulting amides could then be reduced with lithium aluminum hydride to generate the corre-

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Table IV
Biological Properties of SPD Derivatives

derivative	summary of biological effects of the various SPD derivatives: inhibition of SPD uptake (K_i < 0.1 mM)	growth inhibition (IC_{50} < 0.1 mM)	substitution for SPD (at 10 μM)[a]	suppression of ornithine decarboxylase activity (>50% at 10 μM)	polyamine depletion (at IC_{50} < 0.1 mM)[b]
SPD	+	−	+	+	−
N^4-methyl-SPD	+	−	+	−	*b*
N^4-ethyl-SPD	+	−	+	−	*b*
N^4-acetyl-SPD	±	−	+	−	*b*
N^4-hexyl-SPD	+	+	−	−	−
N^4-hexanoyl-SPD	−	−	−	−	*b*
N^4-benzyl-SPD	+	−	−	−	*b*
N^4-benzoyl-SPD	−	−	−	−	*b*
N^1,N^8-diethyl-SPD	+	+	−	+	+
N^1,N^8-diacetyl-SPD	−	−	−	−	*b*
N^1,N^8-dipropyl-SPD	+	+	−	+	+
N^1,N^8-dipropionyl-SPD	−	−	−	−	*b*

[a] As determined by prevention of DFMO cytostasis at >50% of control cell growth. [b] IC_{50}, > 0.1 mM.

sponding alkyl compounds. As described early on in the text, the purpose of these systems was to help determine what structural restrictions the polyamine uptake apparatus of L1210 cells (mouse leukemia cells) set on the triamines. This was determined by measuring how effectively the polyamine derivatives compete with spermidine for uptake,[17] K_i values (Table IV).

Several things are clear from Table IV. First, of all except for N^4-acetylspermidine, N-acylated spermidines do not compete well for spermidine uptake. However any primary amino or secondary amino alkylated polyamines compete favorably for spermidine uptake, implying that when considering spermidine as an antineoplastic vector, the antineoplastic should not be annealed to the nitrogens in the form of an acyl moiety.

We also utilized these reagents to synthesize the microbial siderophore agrobactin A[30] and its homologues (Figure 7). Unlike the synthesis of parabactin, however, we began from the "inside" and worked to the primary amino functions. The reagent I was first reacted with the *N*-hydroxysuccinimide activated ester of *tert*-butoxycarbonylated threonine to produce compound II in 97% yield. Compound II is next briefly exposed to trifluoroacetic acid resulting in quantitative removal of all three *tert*-butoxycarbonyl protecting groups. The resulting intermediate III was finally reacted with the *N*-hydroxysuccinimide ester of 2,3-dihydroxybenzoic acid to produce the final product agrobactin A in 75% yield.

Both the bis-BOC reagents and the secondary *N*-benzyl reagents are of course restricted in terms of their utility. The former is applicable to selective secondary N-functionalization while the latter is applicable to selective primary amino difunctionalization.

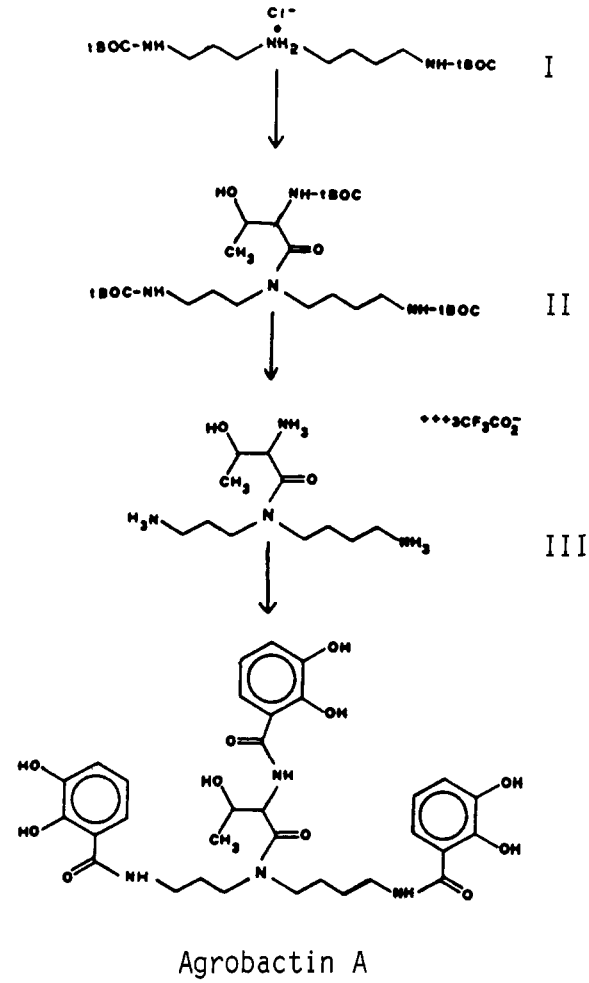


Figure 7.

Spermidines Protected with Three Different Groups

At this point in our polyamine program it was necessary to fix one, two, or three different groups to the triamine nitrogens singularly and in combination. This meant consideration of a reagent which could essentially allow for fixing any number of functional groups to polyamines in any order necessary. We solved this problem by developing a group of reagents with three separate amino protecting groups each quantitatively removed under different conditions and each accessible in high yield (Figure 8). Although the reagent N^4-tosyl-N^8-phthaloylspermidine designed by Eugster[38] was already available for fixing three different substituents to the spermidine backbone, the eight steps required for synthesis and the rather harsh conditions for protective group removal do not make it particularly attractive. The generation of our spermidine reagent proceeds in five facile high-yield steps, starting from the commercially available 3-(benzylamino)propionitrile. Furthermore, and most important, the homo- and norspermidine tris-protected reagents can also be syn-

(38) Walchli-Schaer, E.; Eugster, C. H. *Helv. Chim. Acta* **1978**, *61*, 928.

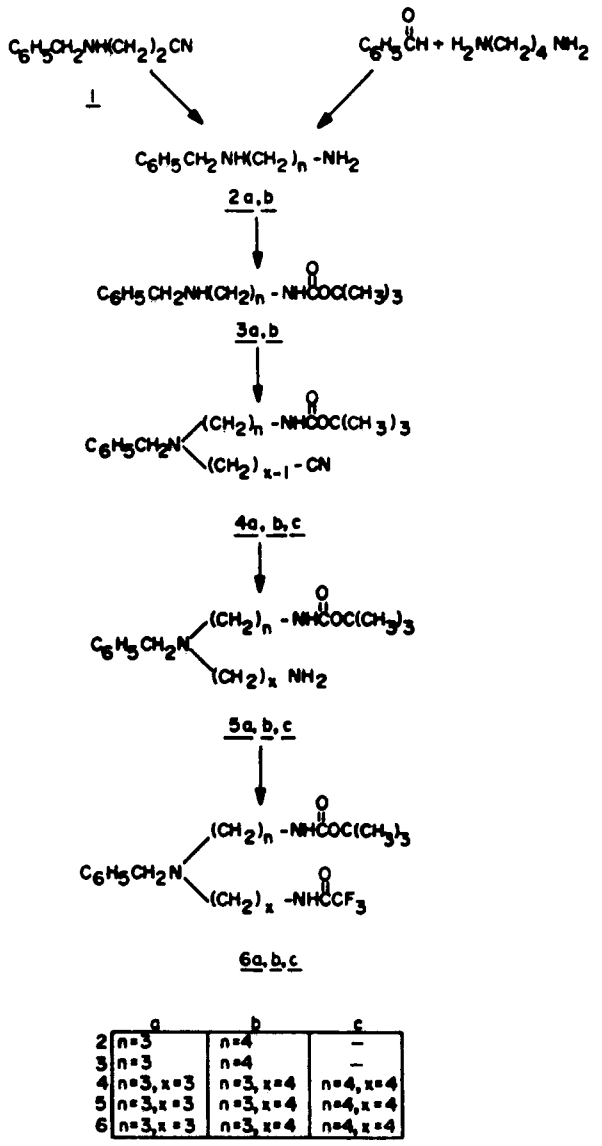


Figure 8.

thesized and used similarly.

Earlier we determined that debenzylation of benzylspermidine required only mild hydrogenolysis conditions, and removal of the *t*-BOC groups only required brief exposure to trifluoroacetic acid. These conditions, of course, dictated that the third protecting group be stable to acid and hydrogenolysis and we thus chose a base-labile protecting group for the "last" nitrogen. The *N*-trifluoroacetyl group fulfills these requirements as it is easily removed by refluxing the corresponding amide with methanolic sodium carbonate.[39]

The synthesis of the polyamine reagents[40] begins with the appropriate N-benzyl protected amine, Figure 8. Thus nitrile 1 (prepared previously in high yield)[22] was conveniently reduced to diamine 2 (n = 3) by utilizing a Raney nickel catalyst recently reported for moderate-scale reductions of this type[24]. Unfortunately, the analogous diamine 2 (n = 4) was not readily available and attempts to monoalkylate benzylamine met with limited success. The commercial availability of 1,4-diaminobutane (putrescine) prompted attempts to selectively functionalize only one of the amino groups.

(39) Newnon, H. J. *J. Org. Chem.* **1965**, *30*, 1287.
(40) Bergeron, R. J.; Garlich, J. R.; Stolowich, N. J. *J. Org. Chem.* **1984**, *49*, 2997.

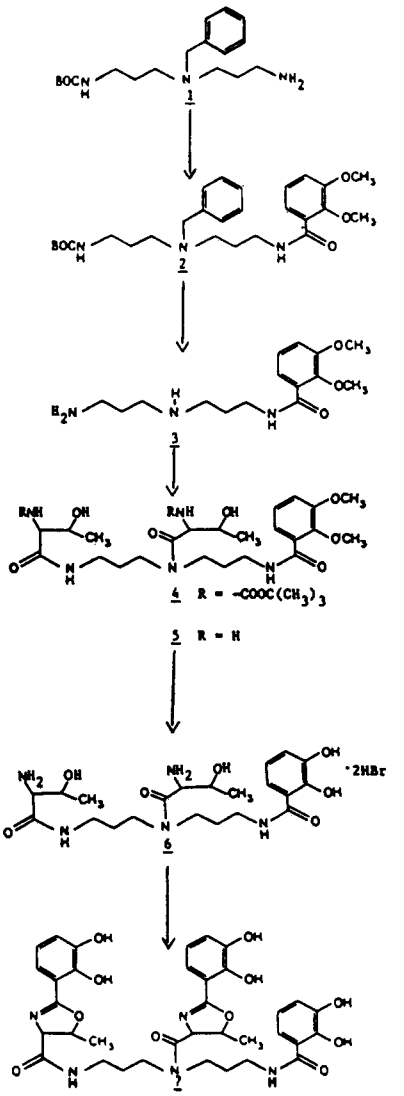


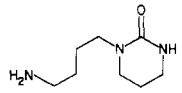
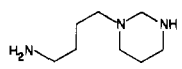
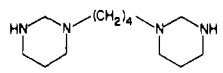
Figure 9.

However, 1,4-diaminobutane tends to react at both amino groups to give difunctionalization, even when the starting diamine is present in large excess relative to the functionalizing agent. For example, acylation of 1,4-diaminobutane (10 mmol) with benzoyl chloride (2 mmol) gave a 95% yield of the bisacylated product. Additionally, treatment of putrescine (90.7 mmol) with benzaldehyde (22.7 mmol) gave a 67% yield of the corresponding bis(imine). This reactivity of putrescine can be circumvented by the use of acidic conditions to give monofunctionalization as in the preparation of *N*-acetyl-1,4-diaminobutane from 1,4-diaminobutane and acetic anhydride in glacial acetic acid.[41] Successful monobenzylation of putrescine with benzaldehyde was effected under reductive amination conditions (formic acid) in high yields (81% distilled). Additionally, the excess amount of 1,4-diaminobutane required for monofunctionalization in this step is readily recovered (70% after distillation), making the procedure cost effective.

The diamines **2** are further protected by reacting them with 1 equiv of 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON). Although both of the amino groups can be protected by using this reag-

(41) Tabor, H.; Tabor, C. W.; DeMeis, L. *Methods Enzymol.* **1971**, *17B*, 829.

Table V.

reagent	position of functionalization	condition required for protective group removal	reagent access, % yield	ref
$H_2N(CH_2)_xNHCH_2CH_2CN$ x = 2, 3, 4, 5, 6, 9, 10	$RNH(CH_2)_xNRCH_2CH_2CH_2NH_2$ x = 2, 3, 4, 5, 6, 9, 10	catalytic redn, hydride redn	steps 1, 50	19
$NCCH_2CH_2NH$-$(CH_2)_xNHCH_2CH_2CN$ x = 2, 3, 4, 5, 6, 9, 10	$H_2NCH_2CH_2CH_2NR(CH_2)_xNRCH_2CH_2CH_2NH_2$ x = 2, 3, 4, 5, 6, 9, 10	catalytic redn, hydride redn	steps 1, 82	19
	$HNRCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$	basic hydrolysis	steps 2, 95	21
	$HNRCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NHR$	ethyl hydrogen malonate in piperidine	steps 1, 87	21
	$HNR(CH_2)_3NH(CH_2)_4NH(CH_2)_3NHR$	ethyl hydrogen malonate in piperidine	steps 1, 95	21
$BOCNH(CH_2)_4N(BOC)$-$(CH_2)_3NH_2$	$H_2N(CH_2)_4NH(CH_2)_3NHR$	F_3CCO_2H	steps 2, 49	20
$H_2N(CH_2)_aN(CH_2Ph)$-$(CH_2)_bNH_2$ (a, b) = (3, 3), (3, 4), (4, 4)	$RNH(CH_2)_aNH(CH_2)_bNHR$ (a, b) = (3, 3), (3, 4), (4, 4)	Pd/H_2	steps 3, 72	22, 23
$BOCNH(CH_2)_aNH(CH_2)_b$-$NHBOC$ (a, b) = (3, 3), (3, 4), (4, 4)	$H_2N(CH_2)_aNR(CH_2)_bNH_2$ (a, b) = (3, 3), (3, 4), (4, 4)	F_3CCO_2H	steps 2, 88	37
$CF_3CONH(CH_2)_aN$-$(CH_2Ph)(CH_2)_bNHt$-BOC (a, b) = (3, 3), (3, 4), (4, 4)	$RNH(CH_2)_aNH(CH_2)_bNH_2$		steps 5, 60	40
	$H_2N(CH_2)_aNR(CH_2)_bNH_2$ $H_2N(CH_2)_aNH(CH_2)_bNHR$ $R^1NH(CH_2)_aNR(CH_2)_bNH_2$	CF_3CO, K_2CO_3/CH_3OH; BOC, F_3CCO_2H; $PhCH_2$, Pd/H_2		
	$R^1NH(CH_2)_aNH(CH_2)_bNHR$ $H_2N(CH_2)_aNR(CH)_bNHR$ $H_2N(CH_2)_aNR(CH_2)_bNHR^1$ $RNH(CH_2)_aNR^1(CH_2)_bNH_2$ $RNH(CH_2)_aNH(CH_2)_bNHR^1$ $RNH(CH_2)_aNR^1(CH_2)_bNHR^{11}$ $RNH(CH_2)_aNR^{11}(CH_2)_bNHR^1$ $R^1NH(CH_2)_aNR(CH_2)_bNHR^{11}$ $R^{11}NH(CH_2)_aNR(CH_2)_bNHR^1$ $R^{11}NH(CH_2)_aNR^1(CH_2)_bNHR$ $R^1NH(CH_2)_aNR^{11}(CH_2)_bNHR$				

ent,[20] we found that with 1 equiv of BOC-ON at 0 °C, regioselective acylation occurs quantitatively at the primary amine site of **2**. The resulting products **3** of this reaction were purified by vacuum distillation at less than 0.5 mmHg as at higher pressures the high temperature required promotes thermal decomposition of the BOC protecting group.

Cyanoethylation of **3a** with acrylonitrile gave the nitrile **4a** in quantitative yield. Alkylation of **3a** and **3b** with 4-chlorobutyronitrile gave the analogous nitriles **4b** and **4c**, respectively, also in high yields (95%). None of the nitriles required further purification and were subsequently reduced with Raney nickel as described earlier. The smooth, high-yield (91–100% crude yield) reductions allowed the amines **5a–c** to be acylated without further purification.

The intermediates **5a**, **5b**, or **5c** can also be easily converted to the corresponding secondary N-benzylated triamines described previously (Figure 3) by brief exposure to trifluoroacetic acid. This has the advantage of overcoming the problems associated with removing small amounts of contaminating N^5-benzylhomospermidine found in the N^4-benzylspermidine. Finally, reacting **5a**, **5b**, or **5c** with BOC-ON followed by hydrogenolysis in methanolic HCl leads to the earlier described bis(BOC) reagents.

One note of interest is the inertness of the carbamate and the *N*-benzyl moieties to the nitrile reduction conditions employed, both of which can be cleaved with some hydrogenation procedures. Finally, acylation with trifluoroacetic anhydride gave the desired triprotected spermidine and analogues (**6a–c**, respectively), again in good yield (91–96%).

We next applied the new reagents to the synthesis of vibriobactin **7** (Figure 9) a microbial siderophore isolated from *Vibrio cholerae*.[31] This iron ligand has been identified as *N*-[3-(2,3-dihydroxybenzamido)propyl]-1,3-bis[2-(2,3-dihydroxyphenyl)-*trans*-5-methyl-2-oxazoline-4-carboxamido]propane and has been given the trivial name vibriobactin. Unlike the spermidine siderophores, vibriobactin has no symmetry with respect to the terminal acyl groups. The disposition of the acyl groups thus presents a new synthetic challenge. A 2-(2,3-dihydroxyphenyl)-*trans*-5-methyl-2-oxazoline-4-carboxamido group is fixed to both a primary and a secondary amine nitrogen. The unsymmetrical structure sets the boundary conditions for the reaction scheme. It suggests that one should either begin with

a mono primary amino protected norspermidine or a secondary and primary amine protected norspermidine. The monoprotected triamine would allow for initial fixing of the threonyl groups while the diprotected triamine would allow for initial attachment of the 2,3-dihydroxybenzoyl group. Our polyamine reagents previously described allow for either approach. We chose the latter route in order to avoid the problems associated with acylation of the intermediate bis(threonyl) or bis(oxazoline) compounds. Of course, one could begin with norspermidine itself and hope for selective monoacylation of its primary amino nitrogen. It is probably unreasonable to hope that such a procedure would proceed in acceptable yield.

The synthesis begins with N^4-benzyl-N^1-(*tert*-butoxycarbonyl)norspermidine (1), one of the precursors to the above reagents. This amine was acylated with 2,3-dimethoxybenzoyl chloride in the presence of triethylamine to give the trisubstituted norspermidine **2** in quantitative yield (Figure 9). The functionalized norspermidine was then deprotected in a stepwise fashion to give the monoacylated norspermidine **3**. The order of deprotection is unimportant: the *tert*-butoxycarbonyl group was removed by brief exposure to trifluoroacetic acid and the N^4-benzyl group removed by hydrogenolysis over palladium chloride in methanolic HCl to give the dihydrochloride salt of the monoacylated norspermidine **3** in 93% overal yield. The salt was converted to the free amine by exposure to aqueous base and then bisacylated in DMF, using the activated ester of L-*N*-(*tert*-butoxycarbonyl)threonine to give the unsymmetrically substituted norspermidine **4** in quantitative yield. As expected on brief exposure of compound **4** to trifluoroacetic acid, the *t*-BOC protecting group collapsed to carbon dioxide, isobutylene, and N^1,N^4-bis[L-threonyl]-N^7-(2,3-dimethoxybenzoyl)-norspermidine (**5**) in excellent yield (93%). Exhaustive demethylation employing BBr_3 in methylene chloride afforded the dihydrobromide salt of the completely deprotected norspermidine **6**, in moderate yield (63%). The final step required cyclization of each of the threonyl groups to an imidate to give the proper bis(oxazoline) configuration. This was readily accomplished by exposure of **6** to an excess of ethyl 2,3-dihydroxybenzimidate in refluxing methanol to give vibriobactin (**7**) (58%). The product was identical with the naturally occurring compound in its 300-MHz ^1H NMR. Our methodology is of course applicable to both the homospermidine and spermidine analogues of vibriobactin.

This type of stepwise deprotection–functionalization works equally well for the other two triprotected spermidine analogues **6b** and **6c**, thus allowing for the first time easy access to selectively functionalized spermidine and its homologues. Additionally, these reagents provide access to large quantities of mono- and bisprotected putrescine (**2b** and **3b**, respectively, Figure 8) which can be envisioned in a number of ways as leading to multiprotected spermine derivatives. Work is now in progress in this area. Finally, we point out that each triprotected reagent is stable and may be stored for prolonged periods.

Conclusion

Although there is now a large number of reagents available (Table V) for the selective functionalization of the polyamine nitrogens, the most versatile are those described in the last section of this account. These systems offer the laboratory worker access to all of our reagents previously described. With these new reagents we can essentially complete our study of the structural boundary conditions set by the L1210 cell polyamine uptake apparatus on polyamine transport. Finally, as the protecting groups can be removed in any order we desire, one could add and change the nature of these groups relative to the conditions required for removal, thus providing access to an infinite number of selectively protected triamines.