In the Search for New Anticancer Drugs, XVI
Selective Protection and Deprotection of Primary Amino Groups in Spermine, Spermidine and Other Polyamines

George Sosnovsky* and Jan Lukszo

Chemistry Department, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201, USA

Z. Naturforsch. **41b**, 122–129 (1986); received July 30, 1985

Spermine, Spermidine, Polyamines, Nefkens Reagent, N-Ethoxycarbonylphthalimide

Spermidine, spermine and other polyamines **1–5** were selectively protected at the terminal primary amino functions without affecting the secondary amino groups using N-ethoxycarbonylphthalimide (**15**), the Nefkens' reagent. Three representative products, **17, 18** and **20,** readily underwent acylation at the secondary amino nitrogen to give the corresponding compounds **21–26.** Selective deprotection of two representative samples **22** and **25** at the primary amino function by hydrazinolysis yielded the corresponding derivatives **27** and **28** with free primary amino groups.

In summary, the application of Nefkens' reagent for the terminal protection of primary amino groups in various polyamines results in a simple, efficient and selective one-step procedure using commercially available reagents.

Introduction

In the past two decades considerable efforts have been expanded to the elucidation of structures and biological functions of various naturally occurring polyamines [1–4]. The elevated levels of polyamines in cancer patients have been correlated with the rate of proliferation of cancer cells [5]. On the basis of these results it was proposed [6] to use polyamines as cancer markers. Furthermore, attempts have been made to suppress the formation of polyamines by the administration of suitable inhibitors of ornithine decarboxylase which is responsible for the formation of putrescine which, in turn, is considered to be the key intermediate in the formation of other polyamines [7, 8]. With this approach, it was hoped to retard the proliferation of cancer cells and hence to develop a chemotherapy for certain cancers.

Another interesting approach was proposed [9] for the design of antineoplastic drugs by suitable derivatizations of the secondary amino nitrogens in such polyamines as spermidine (**1**) and spermine (**4**).

This approach was proposed on the basis of recent findings [10, 11] that the uptake and concentrations of polyamines in L1210 lymphoid leukemia cells are not affected by modifications of secondary nitrogens of polyamines while substitutions at the primary amino nitrogen have a decisive effect. The ability to selectively derivatize the secondary nitrogens in polyamines presents an opportunity to deliver antineoplastic moieties to the cancer cells [9, 10]. Consequently, the development of the synthetic methodologies leading to such a selective modification of the secondary amino nitrogen with either a preservation or restoration of the unblocked primary amino functins is of considerable interest.

At present, there are five methods available for a selective functionalization of polyamines. In the earliest method [12] N^4-tosyl-N^8-phthaloylspermidine (**6**) was used in order to introduce three different substituents into the spermidine molecule. Unfortunately, the eight-step sequence which is required to obtain the reagent **6,** combined with the subsequent stepwise derivatization make this method little attractive. The more recent reagent, N^4,N^8-di-*t*-butyloxycarbonylspermidine (**7**) was designed [13] prima-

$H_2N-(CH_2)_n-NH-(CH_2)_m-NH_2$

1: n = 3, m = 4

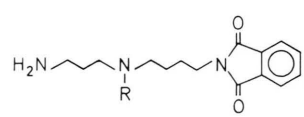
2: n = m = 3

3: n = m = 2

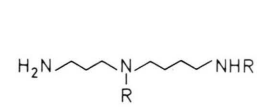
$H_2N-(CH_2)_n-NH-(CH_2)_m-NH-(CH_2)_n-NH_2$

4: n = 3, m = 4

5: n = m = 2



6: R = $-SO_2C_6H_4CH_3$

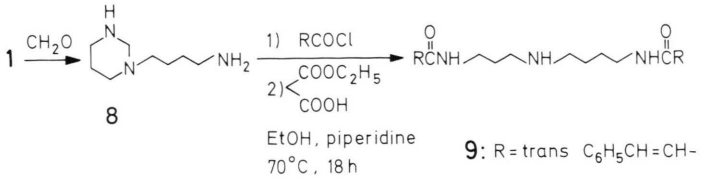


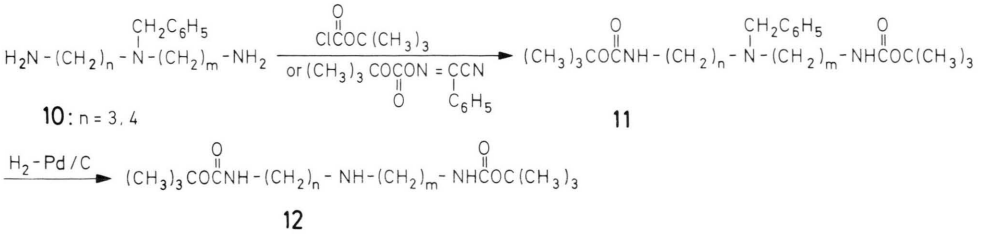
7: R = $-COOC(CH_3)_3$

* Reprint requests to Prof. Dr. G. Sosnovsky.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0340–5087/86/0100–0122/$ 01.00/0

Unauthenticated
Download Date | 5/3/16 12:25 PM



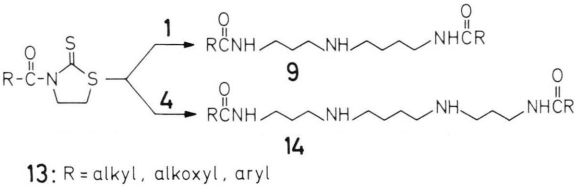


rily for the introduction of acyl groups at the N^1-position of spermidine, and, consequently, is of limited value for a general preparation of the desired terminally protected polyamine derivatives.

In the third method [14] the reaction of spermidine with formaldehyde produces 1-(4-aminobutyl)-hexahydropyrimidine (**8**) which on successive acylation and ring cleavage is transformed into terminally N-acylated polyamine derivatives (**9**). However, this procedure apparently cannot be adopted for homospermidine, and the ring cleavage conditions might be a limiting factor [9].

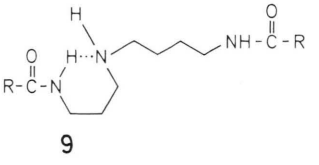
More recently, a comprehensive methodology was developed [9] for the N-acylation of terminal primary amino groups of triamines *via* their N-benzylated derivatives at the secondary nitrogen atom (**10**). The necessary N-benzylated compounds (**10**) are readily synthesized [15, 16] from the commercially available benzylamine and 3-(benzylamino)-propionitrile. Although this methodology is convenient and relatively simple, it nevertheless, requires a minimum of four steps to secure the desired, selectively protected polyamine derivatives **12.** Furthermore, an adaptation of this methodology to the protection of linear tetramines, such as spermine, has not been reported.

The most recently reported methodology [17] appears to obviate these problems. In this method, 1,3-thiazolidine-2-thione is used as the transfer agent for the protecting moieties. Thus, the reaction of 3-benzyloxycarbonyl-1,3-thiazolidine-2-thione (**13,** R = $C_6H_5CH_2O-$) with spermidine (**1**) gave the N^1,N^{10}-bis(benzyloxycarbonyl)spermidine (**9,** R = $C_6H_5CH_2O-$) in 69% yield [17], and the reaction of various 3-acyl-1,3-thiazolidine-2-thiones **13** with spermidine (**1**) and spermine (**4**) rendered the corresponding terminally N-acylated polyamine derivatives **9** and **14** [18–21].



13: R = alkyl, alkoxyl, aryl

It was suggested [19] that the high selectivity achieved with this methodology is attributable to hydrogen bonding involving the hydrogen of the amide moiety and the lone pair of electrons of the secondary amino group, thus decreasing the nucleophilicity of the secondary amino group.



However, the same hydrogen bond formation responsible for the good selectivity may also cause [17] difficulties during the subsequent acylation step of the secondary amino group in terminally protected polyamines.

Unauthenticated
Download Date | 5/3/16 12:25 PM

These difficulties were eventually resolved [17] by the use of either the phenyl-bis(2-thiono-1,3-thiazolidine-3-yl)phosphine oxide reagent which is commercially not available or triphenylphosphine/2,2′-dipyridyl-disulfide reagents [22–24] which resulted in lower yields of products.

In summary, all presently known methods suffer from some drawbacks. Ideally, a selective reagent for the protection of terminal primary amino groups should be (1) readily commercially available, (2) should be inexpensive, and (3) the protecting reaction should involve one step without involvement of the secondary amino group which would be available for further direct derivatization.

In our ongoing research on new anticancer drugs we have also been interested in derivatization of spermine and spermidine at secondary amino groups. Since the existing methodologies appeared to be unsatisfactory for our purposes which could involve sensitive moieties it was decided to search for a new approach for the protection of primary amino functions without affecting the secondary amino groups.

Results and Discussion

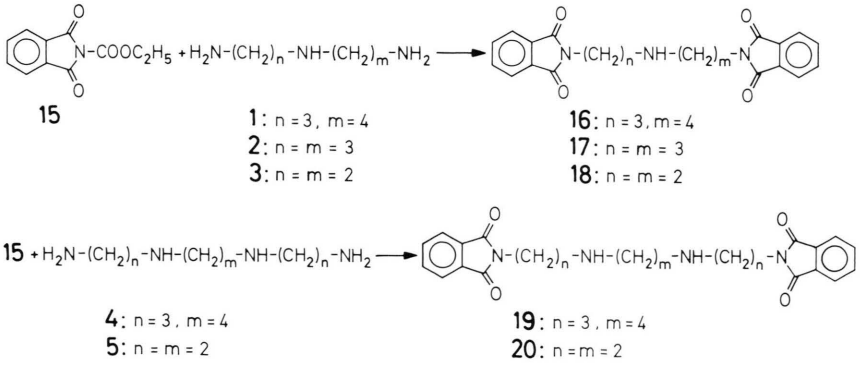
Now we have developed a convenient, selective, one-step method for the protection of the primary amino functions using N-ethoxycarbonylphthalimide (**15**) introduced by Nefkens [25] about 25 years ago. Although this reagent has been widely used for the N-protection of amino acids [25, 26], to the best of our knowledge, no application of this reagent for the selective protection of primary amino groups in polyamines has been reported. The to date reported diethylenetriamine and triethylenetetramine derivatives protected with the phthaloyl moiety at the terminal amino groups have been prepared using either some difficult to obtain intermediates or harsh experimental conditions [27, 28]. These methodologies were, therefore, of limited value in our studies.

In our approach the one-step reaction of five representative polyamines **1–5,** including the naturally occurring spermidine (**1**) and spermine (**4**), with the Nefkens' reagent (**15**) resulted in the production of the corresponding terminally N-protected derivatives **21–25** in moderate to good yields of 53–86%.

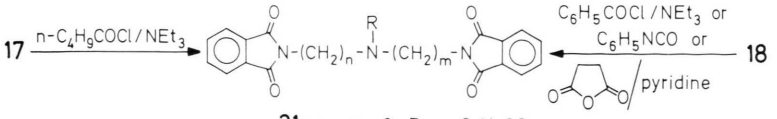
In practice, compounds **21–25** were conveniently synthesized in 45 min at room temperature by mixing the dichloromethane solutions of two molar equivalents of **15** with one molar equivalent of **1–5.** In order to ascertain the preferred reactivity of **15** with primary amino groups as compared to the secondary amino groups, three equivalents of **15** were interacted with one equivalent of **3** in chloroform at room temperature for 15 min and at 60 °C for 30 min. However, in spite of an excess of **15,** only one product (**18**) was isolated.

In all experiments the purification of the less soluble products in methanol was achieved by trituration with methanol, and the more soluble products were purified by recrystallization from methanol.

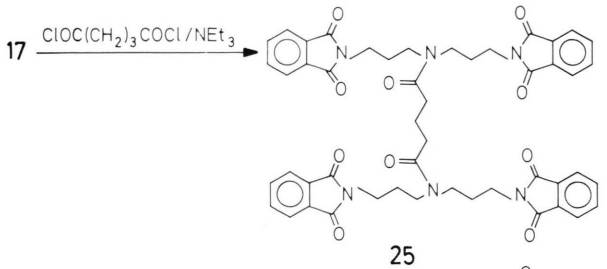
The melting point of compound **20** (m.p. = 153–155 °C) was significantly different from that (m.p. = 233–234 °C) found in the literature [18]. Since our comprehensive analytical data strongly support the proposed structure of **20** it is concluded that the previously reported [18] compound is not identical with our product (**20**).



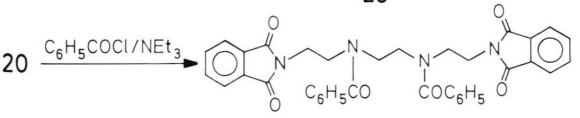
Unauthenticated
Download Date | 5/3/16 12:25 PM



21: n = m = 3, R = n-C_4H_9CO-
22: n = m = 2, R = C_6H_5CO-
23: n = m = 2, R = C_6H_5NHCO-
24: n = m = 2, R = $HOOC(CH_2)_2CO$-



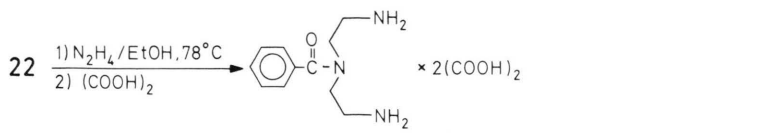
25



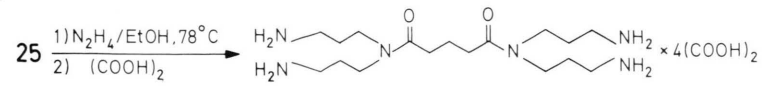
26

The terminally N-protected phthaloylated polyamines readily undergo acylation reactions at the secondary amino nitrogen. Thus, for example, the reaction of compounds **18** and **20** with benzoyl chloride in the presence of triethylamine gave derivatives **22** and **26** in 73% and 92% yield, respectively. Analogously, the reactions of **17** with valeryl and glutaryl chlorides gave **21** and **25** in 80% and 82% yield, respectively. Similarly, the acylation of **18** with succinic anhydride in the presence of pyridine readily produced the 2-carboxyethyl derivative **24** in 88% yield, and the reaction of **18** with phenylisocyanate resulted in the urea derivative **23** in 97% yield.

The selective deblocking of primary amino groups without affecting the secondary amino functions can be achieved either by the hydrazinolysis [29, 30] or aminolysis [31] with a 40% aqueous methylamine solution. Whereas the use of the latter reagent was unsuccessful, the hydrazinolysis of two selected samples **22** and **25** in boiling ethanol afforded products **27** and **28** which were isolated as their oxalic acid salts in 86% and 67% yield, respectively. All products were characterized by microanalysis and IR, ^{1}H NMR, ^{13}C NMR and mass spectrometries (Table I). The purity control of all products was also monitored by the TLC analysis.



27



28

Unauthenticated
Download Date | 5/3/16 12:25 PM

Table I. N^{α},N^{ω}-Bis(phthaloyl)polyamines **16–20.**

Compound	Yield [%]	m.p. (lit.m.p.) [°C]	Molecular formula[a]	M.S.[b] *m/e*	IR (KBr) νmax [cm^{-1}]	^{1}H NMR ($CDCl_3$) [δ ppm]	^{13}C NMR ($CDCl_3$)[c] [δ ppm]
16	75	143–145	$C_{23}H_{23}N_3O_4$ 405.43	406(100) 407(26)	1026, 1362, 1394, 1437, 1466, 1718, 1769, 2932, 3327	1.05–1.85 (m, 6H); 2.40 (t, 4H); 3.22–3.60 (m, 4H); 6.90–7.30 (m, 8H)	26.37; 27.36; 28.90; 35.93; 37.82; 46.90; 49.32
17	86	134–135	$C_{22}H_{21}N_3O_4$ 391.41	392(100) 393(25) 374(12)	1047, 1396, 1440, 1716, 1772, 2938, 3330, 3361	1.38–1.95 (m, 5H); 2.43 (t, 4H); 3.45 (t, 4H); 6.90–7.35 (m, 8H)	28.98; 36.00; 46.95
18	82	178–180 (178–180) [27]	$C_{20}H_{17}N_3O_4$ 363.36	364(100) 365(24) 346(11)	1047, 1372, 1395, 1435, 1466, 1713, 1770, 2946, 3323	2.70 (t, 4H); 3.47 (t, 4H); 7.07 (b.s., 8H)	37.59; 47.18
19	61	91–93	$C_{26}H_{30}N_4O_4$ 462.53	463(100) 464(30) 445(53)	1343, 1384, 1439, 1467, 1707, 1767, 2942	1.16–2.13 (m, 10H); 2.20–2.63 (m, 8H); 3.45 (t, 4H); 6.97–7.43 (m, 8H)	27.94; 29.01; 36.02; 46.97; 49.82
20	66	153–155 (233–234) [28]	$C_{22}H_{22}N_4O_4$ 406.43	407(100) 408(26) 389(23)	1019, 1035, 1132, 1383, 1395, 1439, 1466, 1714, 1769, 3322	2.42–2.85 (m, 8H); 3.47 (t, 4H); 6.93–7.33 (m, 8H)	37.87; 47.65; 48.79

[a] The microanalyses were in good agreement with the calculated values: C ± 0.34, H ± 0.10, N ± 0.30;
[b] by chemical ionization using methane as reactant gas. Therefore, for Mol. wt. $M^+ + 1$ values are reported. Numbers in the brackets represent relative intensities of the peaks;
[c] only shifts of methylene ($-CH_2-$) carbon atoms are presented.

In conclusion, the use of Nefkens' reagent for the protection of primary amino groups in polyamines without affecting the secondary amino groups presents a simple and effective one-step procedure based on readily available and inexpensive commercial starting materials.

Experimental

Materials

All reagents were of the finest quality available commercially. Triethylamine was kept over potassium hydroxide pellets. N-Ethoxycarbonylphthalimide (the Nefkens' reagent) was purchased from Fluka AG.

Analytical procedures

Melting points were determined on a Thomas Hoover melting point apparatus, model 6406-K with a calibrated thermometer. The IR spectra were recorded either on a Perkin-Elmer Spectrophotometer, model 735B or on a Nicolet-10MX FTIR Spectrophotometer. ^{1}H NMR analyses were performed on a Varian 60 MHz NMR Spectrometer, model EM-360L. ^{13}C NMR spectra were obtained on a Varian CFT-20 Spectrometer using an 8K data table, TMS standard, D_2O lock, and a sweep width of 4000 Hz. Mass spectra in the chemical ionization (CI) and/or electron impact (EI) modes were recorded on a Hewlett-Packard Mass Spectrometer, model 5985GS. Microanalyses were obtained on a Perkin-Elmer 240C Elemental Analyzer. TLC analyses were performed on silica gel $60F_{254}$ precoated sheets (EM Reagents), layer thickness 0.2 mm with visualization using UV light and/or iodine chamber.

Preparation of N^{α},N^{ω}-bis(phthaloyl)polyamines **16–20**

General Procedure: A solution of N-ethoxycarbonylphthalimide (**15**) (2.19 g, 10.0 mmol) in chloroform (10 ml) was added rapidly at 20 °C to a solution of an appropriate polyamine **1–5** (5.0 mmol) in chloroform (10 ml). The resultant clear solution was kept at 20–25 °C for 45 min. Removal of the solvent on a rotating evaporator at

Unauthenticated
Download Date | 5/3/16 12:25 PM

30 °C/20 torr gave the crude products either as solids or oils. Analytically pure products **17** and **18** were obtained by trituration of the crude solids with boiling methanol (10 ml). Analytically pure **16, 19** and **20** were obtained by crystallization of the oily crude products from methanol. The analytical data for the new compounds are presented in Table I.

Reaction of diethylenetriamine (**3**)
with three equivalents of the Nefkens' reagent

A solution of diethylenetriamine (**3,** 0.52 g, 5.0 mmol) in chloroform (10 ml) was added rapidly to a stirred solution of the Nefkens' reagent (**15,** 3.28 g, 15.0 mmol) in chloroform (15 ml). The reaction mixture was stirred for 15 min at 25 °C and monitored by TLC (chloroform:methanol = 10:1, v/v) revealing basically two components **18** and the unreacted Nefkens' reagent. The reaction mixture was subsequently boiled with reflux (60 °C) for 30 min. At the end of this period it was found by TLC analysis that the mixture contained mainly **18** and the unreacted Nefkens' reagent, plus some minor impurities.

The reaction mixture was concentrated at 30 °C/20 torr to a volume of 15 ml. Boiling methanol (50 ml) was then added, and the resultant solution was kept overnight in a refrigerator at 0 °C. The precipitated product was collected by filtration and washed with cold (0 °C) methanol (2×3 ml) to give 1.15 g (63%) of a pure compound, m.p. 178–180 °C. This product was identical in all respects with an authentic sample of **18** (Table I).

Preparation of N^1,N^9-bis(phthaloyl)-N^5-valeroyl-1,5,9-triazanonane (**21**)

To a solution of **17** (1.17 g, 3.0 mmol) and triethylamine (0.50 g, 5.0 mmol) in dichloromethane (10 ml) was added dropwise, at −20 °C, a solution of valeroyl chloride (0.39 g, 3.2 mmol) in dichloromethane. The reaction mixture was stirred at 20 °C for 12 h. After dilution with 25 ml of dichloromethane the reaction mixture was washed with a 5% aqueous solution of citric acid (2×25 ml), water (25 ml) and a 2% aqueous sodium carbonate solution (2×25 ml). The organic layer was dried with anhydrous magnesium sulfate and filtered. Removal of the solvent on a rotating evaporator at 30 °C/20 torr afforded a colorless, oily residue which crystallized slowly. Trituration of this material with a mixture of *t*-butyl methyl ether and *n*-hexane (1:1, v/v) followed by filtration gave 1.18 g (82%) of pure **21,** m.p. 82–84 °C.

MS (CI): *m/e* = 476 ($M^+ + 1$, 100%), 477 ($M^+ + 2$, 32%). – IR (KBr): ν = 710, 1010, 1345, 1381, 1433, 1615, 1670, 1751, 2890 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 0.82 (t, 3H), 1.00–2.37 (m, 10H), 3.03–3.77 (m, 8H), 7.40 ppm (s, 8H). – ^{13}C NMR ($CDCl_3$): δ = 13.79, 22.48, 27.16, 27.46, 28.35, 32.92, 35.58, 35.85, 43.50, 45.59, 123.22, 132.06, 133.89, 168.18, 172.87 ppm.

$C_{27}H_{29}N_3O_5$ (475.52)
Calcd C 68.19 H 6.15 N 8.84,
Found C 67.86 H 6.13 N 8.80.

Preparation of N^1,N^7-bis(phthaloyl)-N^4-benzoyl-1,4,7-triazaheptane (**22**)

To a solution of **18** (1.09 g, 3.0 mmol) and triethylamine (0.40 g, 4.0 mmol) in dichloromethane (25 ml) was added dropwise at 20 °C a solution of benzoyl chloride (0.46 g, 3.3 mmol) in dichloromethane (5 ml). After 2 h of stirring at 20–25 °C the reaction mixture was washed with 1N hydrochloric acid (2×50 ml), water (50 ml) and a 2% aqueous sodium carbonate solution. The organic layer was dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 30 °C/20 torr gave the crude product **22.** Crystallization of **22** from 90% aqueous methanol resulted in 1.02 g (73%) of pure **22,** m.p. 135–137 °C.

MS (CI): *m/e* = 468 ($M^+ + 1$, 100%), 496 ($M^+ + 28$, 20%). – IR (KBr): ν = 1100, 1356, 1393, 1426, 1632, 1710, 1769, 2946, 3054 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 3.15–3.87 (m, 8H); 6.38–6.80 (m, 5H); 7.20 ppm (b.s., 8H). – ^{13}C NMR ($CDCl_3$): δ = 35.77 ppm (signal related to polyamine carbon atoms).

$C_{27}H_{21}N_3O_5$ (467.46)
Calcd C 69.37 H 4.53 N 8.99,
Found C 69.71 H 4.74 N 9.04.

Preparation of N^1,N^7-bis(phthaloyl)-N^4-phenylaminocarbonyl-1,4,7-triazaheptane (**23**)

Phenyl isocyanate (0.25 g, 2.1 mmol) was added in one portion to a boiling solution of **18** (0.73 g, 2.0 mmol) in dichloromethane (25 ml). After boiling with reflux for 30 min the reaction mixture was concentrated on a rotating evaporator at 30 °C/20 torr. Trituration of the solid residue with hot methanol (10 ml) followed by filtration, and washing of the solid with methanol (5 ml) afforded 0.94 g (97%) of the pure **23,** m.p. 244–246 °C (dec). An analytical sample was prepared by crystallization of **23** from a mixture of dimethylformamide and *n*-hexane.

MS (CI): *m/e* = 483 ($M^+ + 1$, 57%), 364 ($M^+ - 118$, 100%). – IR (KBr): ν = 870, 1007, 1067, 1115, 1169, 1244, 1395, 1540, 1599, 1695, 1769, 2944,

Unauthenticated
Download Date | 5/3/16 12:25 PM

3069 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 1.34 (b.s., 1H), 3.66 (t, 4H), 3.95 (t, 4H), 6.89–7.92 ppm (m, 13H). – ^{13}C NMR ($CDCl_3$): δ = 36.09, 45.25, 119.64, 122.70, 123.48, 128.59, 132.06, 134.10, 139.29, 154.88, 168.39 ppm.

$C_{27}H_{22}N_4O_5$ (482.48)

Calcd	C 67.21	H 4.60	N 11.61,
Found	C 67.44	H 4.75	N 11.73.

Preparation of N^1,N^7-bis(phthaloyl)-N^4-(2-carboxyethyl)-1,4,7-triazaheptane (**24**)

To a stirred solution of **18** (4.00 g, 11.0 mmol) and pyridine (2 ml) in chloroform (60 ml), was added, in one portion, powdered succinic anhydride (1.21 g, 12.0 mmol). After stirring of the reaction mixture for 6 h at 20 °C the resultant clear solution was washed with 4 N aqueous hydrochloric acid (2×50 ml) and water (50 ml). The organic layer was separated, dried with anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 40 °C/20 torr gave the crude produce **24** in the form of a thick oil. Crystallization from methanol gave 4.50 g (88%) of pure **24,** m.p. 177–178 °C.

MS (EI): *m/e* = 364 (M^+ – 99, 13%), 101 (100%). – IR (KBr): ν = 1105, 1150, 1265, 1415, 1650, 1775, 2920 cm^{-1}. – ^1H NMR (NaOH/D_2O): δ = 2.30–3.00 (m, 4H), 3.42–3.86 (m, 8H), 7.15–7.57 ppm (m, 8H). – ^{13}C NMR ($CDCl_3$): δ = 27.08, 28.91, 35.19, 44.03, 45.10, 122.87, 123.13, 131.37, 131.61, 133.58, 133.91, 167.56, 167.87, 172.27, 175.89 ppm.

$C_{24}H_{21}N_3O_7$ (463.43)

Calcd	C 62.19	H 9.07	N 4.57,
Found	C 62.56	H 9.37	N 4.33.

N,N,N^1,N^1-[Tetra(3-phthalimidopropyl)]glutaryl diamide (**25**)

To a solution of **17** (3.80 g, 9.7 mmol) and triethylamine (1.50 ml, 10.6 mmol) in dichloromethane (30 ml) was added dropwise a solution of glutaryl chloride (0.78 g, 4.6 mmol) in dichloromethane (6 ml) at –30 °C. The reaction mixture was first stirred for 30 min at –5 °C to –10 °C and subsequently for 18 h at 20 °C. The reaction mixture was then washed with a 5% aqueous solution of citric acid (2×25 ml) and a 2% aqueous solution of sodium bicarbonate (50 ml). The organic layer was dried with anhydrous magnesium sulfate, and filtered. Removal of the solvent on a rotating evaporator at 30 °C/20 torr gave 3.24 g (80%) of pure **25** in the form of an amorphous solid, m.p. 65–76 °C.

The mass spectrum could not be recorded because the sample did not vaporize.

IR (KBr): ν = 1035, 1188, 1385, 1397, 1437, 1467, 1640, 1711, 2940 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 1.66–2.50 (m, 14H), 3.07–3.80 (m, 16H), 7.40 ppm (s, 16H). – ^{13}C NMR ($CDCl_3$): δ = 20.74, 26.90, 28.04, 32.02, 35.41, 35.67, 43.31, 45.44, 123.02, 131.96, 133.69, 167.99, 172.20 ppm.

$C_{49}H_{46}N_6O_{10}$ (878.91)

Calcd	C 66.96	H 5.28	N 9.56,
Found	C 66.59	H 5.11	N 9.25.

Preparation of N^1,N^{10}-bis(phthaloyl)-N^4,N^7-bis-benzoyl-1,4,7,10-tetraazadecane (**26**)

To a solution of **20** (0.61 g, 1.5 mmol) and triethylamine (0.40 g, 4.0 mmol) in dichloromethane (20 ml) was added dropwise a solution of benzoyl chloride (0.44 g, 3.1 mmol) in dichloromethane (5 ml) at 15–20 °C. After stirring for 2 h the reaction mixture was washed with 2 N hydrochloric acid (2×25 ml), water (25 ml) and a 2% sodium bicarbonate solution (50 ml). The organic layer was dried with anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 30 °C/20 torr gave the crude product **26** in the form of a white solid. Crystallization from 80% aqueous methanol gave 0.85 g (92%) of pure **26,** m.p. 208–210 °C.

MS (CI): *m/e* = 615 (M^+ + 1, 36%), 391 (M^+ – 223, 80%), 149 (100%). – IR (KBr): ν = 725, 1110, 1252, 1360, 1394, 1429, 1630, 1716, 2930, 3060 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 3.28–3.99 (m, 12H), 7.03–7.83 ppm (m, 18H). – ^{13}C NMR ($CDCl_3$): δ = 35.66, 46.60, 123.35, 126.49, 128.42, 129.42, 131.92, 134.07, 168.40, 170.45 ppm.

$C_{36}H_{30}N_4O_6$ (614.63)

Calcd	C 70.34	H 4.92	N 9.12,
Found	C 69.97	H 4.86	N 8.87.

Preparation of N^4-benzoyl-1,4,7-triazaheptane dioxalate (**27**)

To a boiling solution of **22** (0.663 g, 1.42 mmol) in anhydrous ethanol (10 ml) was added, in one portion, a solution of anhydrous hydrazine (0.095 g, 2.98 mmol) in anhydrous ethanol (2 ml). The reaction mixture was maintained at reflux for 3 h, followed by removal of the solvent on a rotating evaporator at 40 °C/20 torr. The semi-solid residue was dissolved in water (15 ml), and the solution was acidified with acetic acid (1 ml). The precipitated 2,3-dihydro-1,4-phthalazinedione was collected by filtration and the filtrate concentrated on a rotating evaporator at 60 °C/20 torr. The oily residue was dissolved in ethanol (10 ml), and the solution mixed with a solution of oxalic acid dihydrate (0.447 g,

Unauthenticated
Download Date | 5/3/16 12:25 PM

3.55 mmol, 25% excess) in ethanol (25 ml). The instantly formed white precipitate was collected by filtration and washed with ethanol (2×5 ml) and ethyl ether (2×5 ml). Recrystallization of the crude product (0.595 g) from water gave 0.472 g (86%) of pure **27,** m.p. 214–216 °C (dec.).

MS (CI): m/e = 208 ($M^+ + 1$, 100% – calculated for free amine), 190 ($M^+ - 17$, 40%). – IR (KBr): ν = 1230, 1296, 1391, 1460, 1482, 1664, 1707, 1721, 2840, 3070 cm^{-1}. – ^1H NMR (D_2O/NaOH): δ = 2.50 (t, 4H), 3.16 (t, 4H), 6.75–7.26 ppm (m, 5H). – ^{13}C NMR (D_2O/NaOH): δ = 39.41, 40.09, 47.44, 50.65, 127.13, 128.88, 132.21, 133.66, 170.68 ppm.

$C_{15}H_{21}N_3O_9 \cdot 1/2\ H_2O$ (387.34)
Calcd C 45.45 H 5.60 N 10.50,
Found C 45.54 H 5.52 N 10.60.

Preparation of N,N,N^1,N^1-tetra(3-aminopropyl)-glutaryl diamide tetraoxalate (**28**)

A solution of hydrazine hydrate (100%, 0.210 g, 4.2 mmol) in anhydrous ethanol (3 ml) was added rapidly to a boiling suspension of **25** (0.880 g, 1.0 mmol) in anhydrous ethanol (17 ml). The stirred reaction mixture was boiled with reflux for 4 h. After removal of the solvent on a rotating evaporator at 40 °C/20 torr, the residue was dissolved in water (50 ml) and acidified with oxalic acid dihydrate (0.63 g, 5.0 mmol). The white precipitate of 2,3-dihydro-1,4-phthalazinedione was colleted by filtration and washed with hot water (2×10 ml). Concentration of the combined washings and filtrate on a rotating evaporator at 60 °C/20 torr gave the crude **28.** Crystallization from aqueous methanol afforded 0.485 g (67%) of pure **28,** m.p. 182–184 °C (dec.). Mass spectrum could not be recorded because the sample did not vaporize.

IR (KBr): ν = 1228, 1280, 1385, 1404, 1559, 1637, 1703, 1720, 2150–3050 cm^{-1}. – ^1H NMR (D_2O): δ = 2.10–2.82 (m, 14H), 3.20–3.77 ppm (m, 16H). – ^{13}C NMR (D_2O): δ = 21.78, 23.83, 25.67, 35.01, 36.16, 36.70, 44.64, 45.40, 165.33, 176.43 ppm.

$C_{25}H_{46}N_6O_{18}$ (718.68)
Calcd C 41.78 H 6.45 N 11.69,
Found C 41.46 H 6.07 N 11.50.

These studies were conducted pursuant to a contract with the National Foundation for Cancer Research.

[1] Polyamines in Normal and Neoplastic Growth, D. H. Russell (ed.), Raven Press, New York 1973.
[2] S. S. Cohen, Introduction to Polyamines, Prentice Hall, Englewood Cliffs, New Jersey 1971.
[3] O. Heby, L. J. Marton, L. Zardi, D. H. Russell, and R. Baserga, Exp. Cell. Res. **90 (1),** 8 (1975); C. A. **82,** 70773w (1975).
[4] O. Heby, L. J. Marton, C. B. Wilson, and J. W. Gray, Eur. J. Cancer **13,** 1009 (1977).
[5] J. Janne, H. Poso, and A. Raina, Biochim. Biophys. Acta **473,** 241 (1977).
[6] Contemporary Biomedicine, Human Cancer Markers, S. Sell and B. Wahren (eds), Humana Press, Clifton, New Jersey 1980.
[7] N. E. Newton and M. M. Abdel-Monem, J. Med. Chem. **20,** 249 (1977).
[8] A. Corti, C. Dave, H. G. Williams-Ashman, E. Mihich, and A. Schenone, Biochem. J. **139,** 351 (1974).
[9] R. J. Bergeron, N. J. Stolowich, and C. W. Porter, Synthesis **1982,** 689.
[10] C. W. Porter, R. J. Bergeron, and N. J. Stolowich, Cancer Res. **42,** 4072 (1982).
[11] C. W. Porter and R. J. Bergeron, Science **219,** 1083 (1983).
[12] E. Walchi-Schaer and C. H. Eugster, Helv. Chim. Acta **61,** 928 (1978).
[13] M. Humora and J. Quick, J. Org. Chem. **44,** 1166 (1979).
[14] J. S. McManis and B. Ganem, J. Org. Chem. **45,** 2042 (1980).
[15] R. J. Bergeron, P. S. Burton, K. A. McGovern, and S. J. Kline, Synthesis **1981,** 732.
[16] R. J. Bergeron and J. R. Garlich, Synthesis **1984,** 782.
[17] Y. Nagao, T. Miyasaka, Y. Hagiwara, and E. Fujita, J. Chem. Soc. Perkin Trans. I **1984,** 183.
[18] Y. Nagao, K. Seno, K. Kawabata, T. Miyasaka, S. Takao, and E. Fujita, Tetrahedron Lett. **21,** 841 (1980).
[19] Y. Nagao, K. Seno, T. Miyasaka, and E. Fujita, Chemistry Lett. **1980,** 159.
[20] Y. Nagao, T. Miyasaka, K. Seno, and E. Fujita, Heterocycles **15,** 1037 (1981).
[21] Y. Nagao and E. Fujita, Heterocycles **17,** 537 (1982).
[22] T. Mukaiyama, R. Matsueda, and M. Suzuki, Tetrahedron Lett. **1970,** 1901.
[23] T. Mukaiyama, Angew. Chem., Int. Ed. Engl. **15,** 94 (1976).
[24] S. Kobayashi, T. Iimori, T. Izawa, and M. Ohno, J. Am. Chem. Soc. **103,** 2406 (1981).
[25] G. H. L. Nefkens, Nature **185,** 309 (1960).
[26] G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, Rec. Trav. Chim. **79,** 688 (1960).
[27] C. H. Paik, D. E. Herman, W. C. Eckelman, and R. C. Reba, J. Radioanal. Chem. **57,** 553 (1980).
[28] E. Witek and A. Lis, Pol. Pat. 113845 (1982); C. A. **98,** 71923 m (1983).
[29] H. R. Ing and R. H. F. Manske, J. Chem. Soc. **1926,** 2348.
[30] E. Wunsch, Methodische Voraussetzungen. Blockierung und Schutz der α-Amino-Function, Houben-Weyl 15/1, pp. 48–314, E. Müller (ed.), Georg Thieme Verlag, Stuttgart (1974).
[31] S. Wolfe and S. K. Hasan, Can. J. Chem. **48,** 3572 (1970).

Unauthenticated
Download Date | 5/3/16 12:25 PM