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Mendeleev Commun., 2018, **28**, 479–481

Mendeleev
Communications

Acetylated derivatives of C-methylated analogues of spermidine: synthesis and interaction with N^1-acetylpolyamine oxidase

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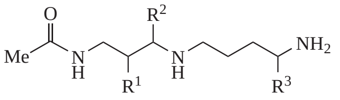
DOI: 10.1016/j.mencom.2018.09.008

The title compounds were prepared in high overall yields from properly protected (Boc, Cbz) 2-, 3- or 8-methyl-4-azaoctane-1,8-diamines. N^1-Acetyl-2-methyl- and N^1-acetyl-8-methylspermidines, but not N^1-acetyl-3-methylspermidine, were the substrates for N^1-acetylpolyamine oxidase.



R^1 = Me, R^2 = R^3 = H (N^1-Ac-2-MeSpd)
R^1 = R^3 = H, R^2 = Me (N^1-Ac-3-MeSpd)
R^1 = R^2 = H, R^3 = Me (N^1-Ac-8-MeSpd)

Biogenic polyamines spermine (Spm) and spermidine (Spd) are present in all mammalian cell types in μM–mM concentrations and are vital for their growth. High intracellular content of Spm and Spd *a priori* determines the diversity of their cellular functions, many of which have still not been investigated in details.[1]

Disturbances of the polyamine metabolism are associated with the development of many diseases, including malignant tumors (cancer cells have elevated level of polyamines), decreased immune response, acute pancreatitis, Snyder–Robinson's syndrome, Alzheimer's and Parkinson's diseases, and even type 2 diabetes.[2–4] Polyamines have also been recognized as important molecules in virus–host interactions.[5] The excess of polyamines might promote proliferation thus contributing to neoplastic transformations[6] and associate with various autoimmune diseases.[7] Respectively, intracellular polyamine levels should be tightly regulated to maintain cellular and organ functions but not to allow malignant transformation of the cell.

Investigation of the individual functions of Spm and Spd is one of the milestones of polyamine biochemistry. The partial interchangeability of Spm and Spd and the ease of their interconversion hamper these studies (Figure 1). Therefore, cells and microorganisms in which the genes encoding the enzymes of polyamine metabolism are deleted, or 'switched off', as well as transgenic animals are widely used to study the individual functions of Spm and Spd (see reviews[1,2,4,8]). The depletion of the intracellular polyamine pool and the use of functionally active metabolically stable mimetics of Spm and Spd for the reversal of the effect may complement well with the above approaches and may give an opportunity to discriminate cellular effects of Spm and Spd. C-Methylated derivatives of Spd (MeSpds) are the

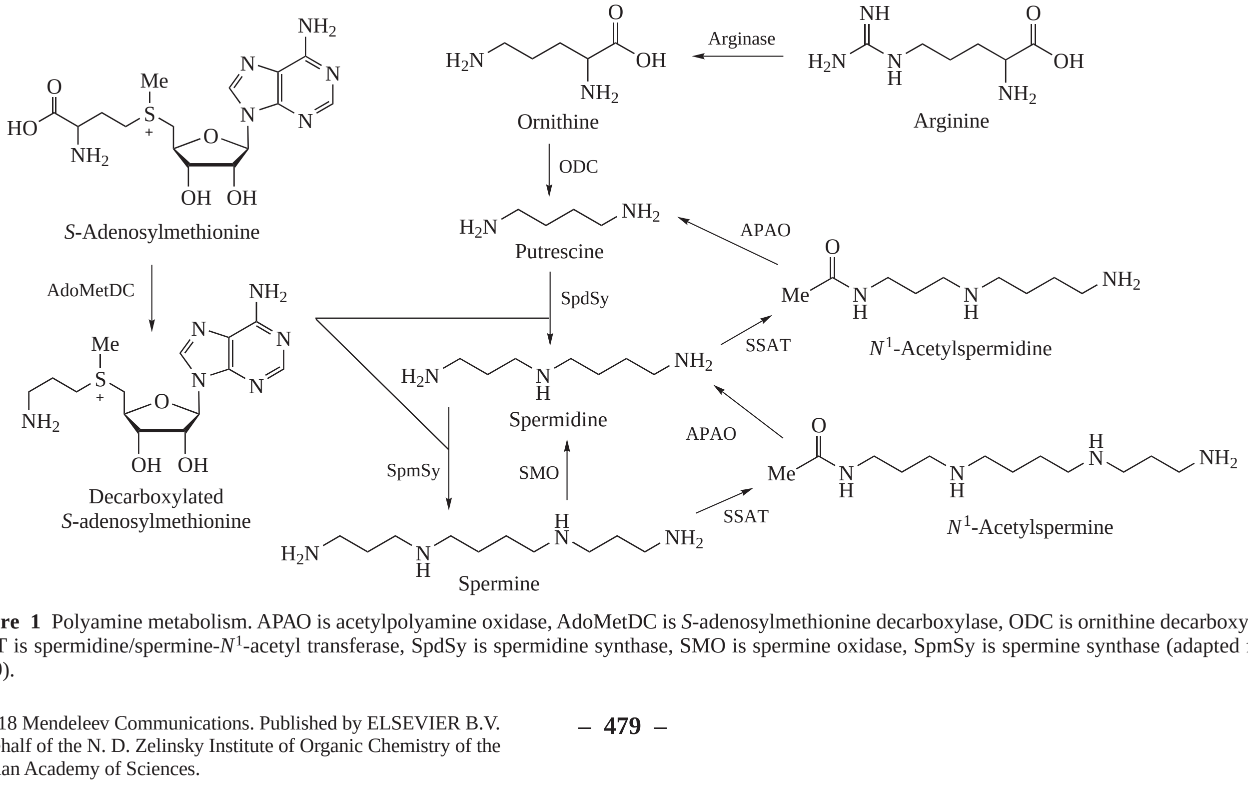
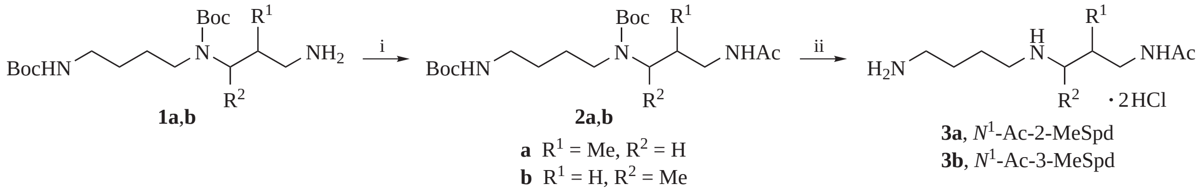


Figure 1 Polyamine metabolism. APAO is acetylpolyamine oxidase, AdoMetDC is *S*-adenosylmethionine decarboxylase, ODC is ornithine decarboxylase, SSAT is spermidine/spermine-N^1-acetyl transferase, SpdSy is spermidine synthase, SMO is spermine oxidase, SpmSy is spermine synthase (adapted from ref. 9).



1a,**b**

2a,**b**

a R^1 = Me, R^2 = H
b R^1 = H, R^2 = Me

3a, N^1-Ac-2-MeSpd
3b, N^1-Ac-3-MeSpd

Scheme 1 *Reagents and conditions*: i, AcCl, CH_2Cl_2, Et_3N; ii, HCl, MeOH.

best source of polyamine analogues possessing the required set of properties.

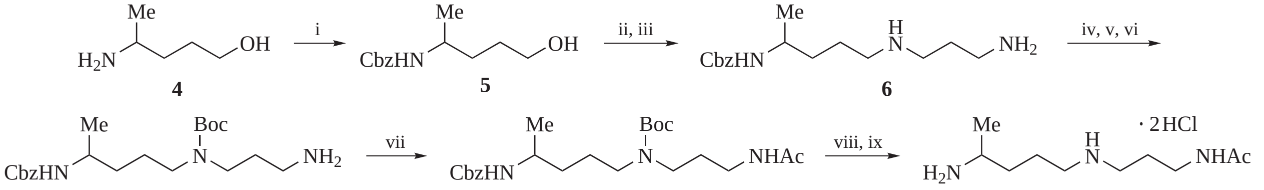
The biochemical properties of MeSpds depend on the position of methyl group in the carbon chain. Thus, 2-MeSpd and 8-MeSpd are the substrates of spermidine/spermine-N^1-acetyltransferase (SSAT), while 1-MeSpd and 3-MeSpd are not acetylated.[10] In contrast, 1-MeSpd, 2-MeSpd and 8-MeSpd are substrates of spermine synthase (SpmSy), while 3-MeSpd is not.[10]

Since N^1-Ac-1-MeSpd is the substrate of N^1-acetylpolyamine oxidase (APAO),[11] the catabolic stability of 1-MeSpd is due only to the impossibility of its N^1-acetylation. The interaction of N^1-acetylated derivatives of other C-methylated analogues of Spd with APAO has not been studied yet. Therefore, we report herein on syntheses of three such new compounds and investigation of their interaction with human recombinant APAO.

Usually to prepare terminally substituted analogues of Spm and Spd, their orthogonally-protected derivatives are used (see reviews[12–14]). Some other alternative approaches, including CuI-catalyzed arylation of non-protected polyamines,[15] were also reported. In the present study to access mono-N^1-acetylated derivatives of 2-, 3- and 8-MeSpds, we used orthogonally protected precursors. The starting bis-Boc-triamines **1a**,**b**[10] were acetylated (Scheme 1), and then Boc-groups were removed to give the target N^1-Ac-2-MeSpd **3a** and N^1-Ac-3-MeSpd **3b** in 72 and 67% overall yields, respectively.[†]

In the synthesis of isomeric N^1-Ac-8-MeSpd **3c** (Scheme 2), its backbone was built up from commercially available amino alcohol **4** which was converted into *N*-Cbz derivative **5**. Compound **5** was O-mesylated, and this methanesulfonate was reacted with excess of 1,3-diaminopropane in THF to give triamine **6**. This step was carried out at 0–20 °C, which reduced the number of byproducts difficult to separate and enabled good (70%) yield. Orthogonally protected triamine **7** was obtained in one-pot from substrate **6** in 73% yield using salicylic aldehyde for temporary protection of primary amino group followed by placement of Boc-group at the secondary NH group, and subsequent removal of salicylidene protection at neutral pH with $MeONH_2$-base (see Scheme 2). Subsequent acetylation and stepwise removal of Cbz and Boc protective groups afforded target **3c** in nine steps in 18% overall yield calculating from **4**.[‡]

Investigation of the interaction of compounds **3a–c** with human recombinant APAO clearly demonstrated that the substrate properties of these compounds are determined by the position of the methyl substituent at polyamine backbone. Thus, **3a** and



4

5

6

7

8

3c, N^1-Ac-8-MeSpd

Scheme 2 *Reagents and conditions*: i, CbzCl, H_2O, $NaHCO_3$; ii, MsCl, CH_2Cl_2, Et_3N; iii, $H_2N(CH_2)_3NH_2$, THF, 0–20 °C; iv, 2-HOC_6H_4CHO, THF; v, Boc_2O, THF; vi, $MeONH_2$, THF; vii, AcCl, Et_3N, THF; viii, HCl, EtOH; ix, H_2/Pd, AcOH, MeOH.

[†] 1-Amino-4-*tert*-butoxycarbonyl-8-*tert*-butoxycarbonylamino-2-methyl-4-azaoctane **1a** and 1-amino-4-*tert*-butoxycarbonyl-8-*tert*-butoxycarbonylamino-3-methyl-4-azaoctane **1b** were obtained as reported.[10] All other reagents of the highest grade were available from Sigma and Acros Organics and used as purchased unless otherwise noted. TLC was performed on Merck Kieselgel 60 F_{254} plates; the compounds were visualized with a UV lamp (Cole Parmer 9815), using color reaction with ninhydrine, or with bromophenol blue to develop Boc-derivatives. Merck silica gel (Kieselgel, 40–63 μm) was used for column chromatography. Melting points were determined in open glass capillaries on a Mel-Temp 3.0 apparatus (Laboratory Devices Inc.). High-resolution electrospray mass spectra were obtained on Applied Biosystems/MDS Sciex QSTAR XL spectrometer using ESI technique.

(N^1*-Acetyl)methylspermidines* **3a**,**b**. A solution of AcCl (6 mmol) in dry CH_2Cl_2 (6 ml) was added dropwise to a stirred and cooled (0 °C) solution of bis-Boc-triamines **1a**,**b** (5.5 mmol) and Et_3N (7 mmol) in dry CH_2Cl_2 (15 ml), and the mixture was stirred for 30 min at 0 °C. Then MeOH (1 ml) was added, the mixture was stirred for 10 min, and washed subsequently with 1 M $NaHCO_3$, H_2O, 10% citric acid, H_2O, brine and dried over $MgSO_4$. To thus obtained acetylated derivatives **2a**,**b** in MeOH (16 ml), 9.4 M HCl/MeOH (4 ml) was added and after 1 h keeping at 20 °C the reaction mixture was concentrated *in vacuo* at 20 °C and co-evaporated *in vacuo* with MeOH (2×5 ml). The residue was dissolved in minimal volume of MeOH, poured into Et_2O and this was kept at –20 °C for 4 h. Compound **3a** was recrystallized from EtOH/Pr^{i}OH, while dihydrochloride **3b** turned to be oil.

1-Acetylamino-8-amino-2-methyl-4-azaoctane dihydrochloride **3a**: mp 141–142 °C, R_f 0.42 (dioxane–NH_4OH, 7 : 3). ^1H NMR (500 MHz, D_2O) δ: 1.01 (d, 3H, CH*Me*, *J* 6.8 Hz), 1.70–1.82 [m, 4H, $(CH_2)_2CH_2NH_2$], 2.04 (s, 3H, Ac), 2.11–2.17 (m, 1H, C*H*Me), 2.91 (d, 2H, NHC*H*$_2$CH, *J* 7.2 Hz), 3.02–3.16 (m, 5H, AcNHC*H*$_2$ + CH_2CH_2NH + CH_2NH_2), 3.29–3.34 (m, 1H, AcNHC*H*$_2$). ^{13}C NMR (125 MHz, D_2O) δ: 15.2, 22.2, 23.2, 24.4, 31.5, 39.3, 42.1, 47.9, 50.9, 175.6. HRMS (ESI), *m*/*z*: 202.1921 [M + H$^+$] (calc. for $C_{10}H_{23}N_3O$, *m*/*z*: 202.1914).

1-Acetylamino-8-amino-3-methyl-4-azaoctane dihydrochloride **3b**: waxy oil, R_f 0.38 (dioxane–NH_4OH, 7 : 3). ^1H NMR (500 MHz, D_2O) δ: 1.26 (d, 3H, CH*Me*, *J* 6.5 Hz), 1.74–1.83 [m, 5H, CHC*H*$_2$, $CH_2(CH_2)_2CH_2$], 1.96–2.00 (m, 1H, CHC*H*$_2$), 2.03 (s, 3H, Ac), 3.07–3.42 (m, 7H, H_2NCH_2 + + CH_2NH + NHC*H* + CH_2NHAc). ^{13}C NMR (125 MHz, D_2O) δ: 18.2, 24.7, 25.9, 26.9, 35.3, 38.3, 41.7, 46.9, 55.2, 177.5. HRMS (ESI), *m*/*z*: 202.1920 [M + H$^+$] (calc. for $C_{10}H_{23}N_3O$, *m*/*z*: 202.1914).

[‡] *1-Amino-8-benzyloxycarbonylamino-4-*tert*-butoxycarbonyl-4-azanonane* **7**. Analogously to the published procedure for unbranched homologue,[11] amino alcohol **4** (2.06 g, 20 mmol) was N-Cbz-protected to afford product **5** (3.55 g, 79%): mp 58–58.5 °C (EtOAc–hexane), R_f 0.21 (CH_2Cl_2–MeOH, 97 : 3). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.15 (d, 3H, Me, *J* 7.5 Hz), 1.46–1.64 [m, 4H, $(CH_2)_2CH_2OH$], 1.83 (s, 1H, OH), 3.64 (t, 2H, CH_2OH, *J* 6.1 Hz), 3.70–3.81 (m, 1H, MeC*H*), 4.67 (br. s, 1H, N*H*Cbz), 5.08 (s, 2H, CH_2Ph), 7.28–7.37 (m, 5H, Ph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 21.6, 29.3, 33.9, 47.3, 62.9, 66.9, 128.38, 128.40 (2C), 128.8 (2C), 137.0, 156.3. HRMS (ESI), *m*/*z*: 238.1444 [M + H$^+$] (calc. for $C_{13}H_{19}NO_3$, *m*/*z*: 238.1443).

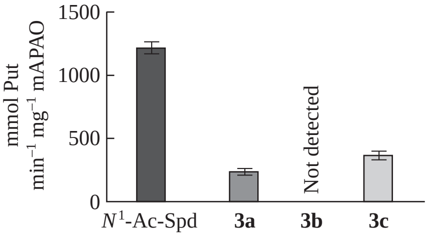


Figure 2 N^1-Ac-MeSpds **3a**–**c** (1 mM) as substrates of human recombinant N^1-acetylpolyamine oxidase. Assay conditions were as described earlier[10] and the activity was measured twice in triplicates.

3c were the substrates of APAO and **3b** did not show any substrate properties at all (Figure 2). Apparently, the methyl group in α-position to the secondary amino group sterically hinders the cleavage of the hydrogen being required for the formation of Schiff base, the first intermediate of APAO reaction. Hence, the catabolic stability of **3b** at the pathways of polyamine metabolism is determined by its inability to serve as a substrate of SSAT, and also because **3b** lacks substrate properties in APAO reaction.

Synthesis of the compounds was supported by the Russian Science Foundation (grant no. 17-74-20049) and the Program of fundamental research for state academies for 2013–2020 years (no. 01201363818); enzymatic experiments by the Academy of Finland (grant nos. 292574 and 315487).

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Received: 20th March 2018; Com. 18/5516

Compound **5** (3.25 g, 13.7 mmol) was converted to its *O*-mesylate (*cf.* ref. 11), R_f 0.58 (CH_2Cl_2–MeOH, 97 : 3), and the mesylate without isolation was reacted with 1,3-diaminopropane (7.4 g, 110 mmol) in THF (20 ml) first for 16 h at 0 °C and then for 24 h at 20 °C. The mixture was concentrated (1 Torr), the residue was taken into 2 M NaOH (15 ml), extracted with CH_2Cl_2 (3×10 ml), washed with brine (10 ml) and dried ($MgSO_4$). Purification on a silica gel (elution with dioxane–NH_4OH, 95 : 5) gave triamine **6** (2.7 g, 67% based on **5**) as a viscous oil: R_f 0.14 (dioxane–NH_4OH, 95 : 5). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.14 (d, 3H, Me, *J* 6.6 Hz), 1.25 (br. s, 3H, NH, NH_2), 1.44–1.55 [m, 4H, $CH(CH_2)_2$], 1.57–1.65 (m, 2H, $CH_2CH_2NH_2$), 2.56–2.66 (m, 4H, CH_2NHCH_2), 2.74 (t, 2H, CH_2NH_2, *J* 6.8 Hz), 3.62–3.77 (m, 1H, C*H*Me), 5.04 (br. s, 1H, N*H*Cbz), 5.08 (s, 2H, CH_2Ph), 7.29–7.35 (m, 5H, Ph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 21.47, 26.67, 34.13, 35.14, 40.90, 47.32, 48.19, 50.11, 66.75, 128.33, 128.37, 128.80, 137.10, 156.18. HRMS (ESI), *m*/*z*: 294.2179 [M + H$^+$] (calc. for $C_{16}H_{27}N_3O_2$, *m*/*z*: 294.2176).

A solution of Cbz-triamine **6** (2.6 g, 8.87 mmol) and salicylaldehyde (1.12 g, 9.2 mmol) in THF (10 ml) was kept for 30 min at 20 °C followed by addition of Boc_2O (2.0 g, 9.2 mmol) in THF (5 ml), and this was kept at 20 °C for 2 h. Reagent $MeONH_2$ (as the base, 0.94 g, 20 mmol) in THF (5 ml) was then added, the colourless solution was concentrated *in vacuo* and the residue was purified on silica gel (elution with dioxane–NH_4OH, 98 : 2) to afford Boc-Cbz-triamine **7** (2.52 g, 73% based on **6**) as a viscous oil: R_f 0.36 (dioxane–NH_4OH, 95 : 5). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.14 (d, 3H, CH*Me*, *J* 6.7 Hz), 1.34–1.45 (m, 11H, CMe_3 + + $CH_2CH_2NH_2$), 1.47–1.66 [m, 4H, $CH(CH_2)_2$], 1.69 (s, 2H, NH_2), 2.65 (t, 2H, CH_2NH_2, *J* 6.7 Hz), 3.05–3.28 [m, 4H, $CH_2N(Boc)CH_2$], 3.66–3.78 (m, 1H, C*H*Me), 4.68 (br. s, 1H, N*H*Cbz), 5.06 (s, 2H, CH_2Ph), 7.27–7.36 (m, 5H, Ph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 21.6, 25.3, 28.8, 32.0, 32.8, 34.7, 39.6, 44.3, 47.0, 66.8, 79.7, 128.32, 128.34 (2C), 128.8 (2C), 137.0, 156.0, 156.1. HRMS (ESI), *m*/*z*: 394.2704 [M + H$^+$] (calc. for $C_{21}H_{35}N_3O_4$, *m*/*z*: 394.2700).

Further N^1-acetylation and Boc-deprotection was performed analogously to the syntheses of compounds **3a**,**b**. Removal of Cbz group after Boc-deprotection in substrate **8** (H_2, Pd-black) gave product **3c** (as a dihydrochloride) which was recrystallized from Pr^{i}OH. The purity of thus obtained compounds **3a**–**c** was ⩾ 99% (data not shown) based on analytical HPLC used according to the published method.[16]

1-Acetylamino-8-amino-4-azanonane dihydrochloride **3c**: mp 144–146 °C, R_f 0.44 (dioxane–NH_4OH, 7 : 3). ^{1}H NMR (500 MHz, D_2O) δ: 1.32 (d, 3H, CH*Me*, *J* 6.6 Hz), 1.69–1.61 (m, 1H, $CHCH_2$), 1.72–1.82 [m, 3H, $CHCH_2$ + $CH(CH_2)_2$], 1.87–1.93 (m, 2H, CH_2CH_2NHAc), 2.02 (s, 3H, Ac), 3.04–3.10 (m, 4H, CH_2NCH_2), 3.29 (t, 2H, CH_2NHAc, *J* 6.6 Hz), 3.37–3.44 (m, 1H, H_2NCH). ^{13}C NMR (125 MHz, D_2O) δ: 20.18, 24.65, 24.82, 28.52, 33.77, 38.89, 47.91, 49.95, 50.12, 177.59. HRMS (ESI), *m*/*z*: 202.1914 [M + H$^+$] (calc. for $C_{10}H_{23}N_3O$, *m*/*z*: 202.1914).