

Novel convenient synthesis of biologically active esters of hydroxylamine

Maxim A. Khomutov · Swati Mandal · Janne Weisell ·
Neiha Saxena · Alina R. Simonian · Jouko Vepsäläinen ·
Rentala Madhubala · Sergey N. Kochetkov

Received: 10 August 2009 / Accepted: 16 September 2009 / Published online: 8 December 2009
© Springer-Verlag 2009

Abstract Alkylation of ethyl *N*-hydroxyacetimidate with readily available methanesulfonates of functionally substituted alcohols and subsequent deprotection of aminooxy group is a novel and convenient method to prepare functionally substituted esters of hydroxylamine with high overall yield. This approach is a good alternative to well-known reaction of *N*-hydroxyphthalimide with alcohols under the Mitsunobu conditions. The properties of ethoxyethylidene protection of aminooxy group on the contrary to that of *N*-alkoxyphthalimide group allow to perform a wide spectra of the transformations in the radical of *N*-protected hydroxylamine derivatives. This is essential for synthetic strategies consisting in the introduction of *N*-protected aminooxy group at one of the first steps of synthesis and subsequent transformations of the radical.

The inhibitory effect of one of the newly synthesized compound, 1-guanidinoxy-3-aminopropane (GAPA), was compared with that of well-known inhibitors of ornithine decarboxylase namely, α -difluoromethylornithine (DFMO) and 1-aminooxy-3-aminopropane (APA) on *Leishmania donovani*, a protozoan parasite that causes visceral

leishmaniasis. GAPA, on the contrary with APA and DFMO, in micromolar concentrations, inhibited the growth of both amastigotes and promastigotes of sodium antimony gluconate-resistant forms of *L. donovani*.

Keywords Hydroxylamines · Synthesis · Enzyme inhibitors · Leishmaniasis · Polyamines

Abbreviations

AdoMet	<i>S</i> -Adenosylmethionine
Agm	Agmatine (1-guanidino-4-aminobutane)
APA	1-Aminooxy-3-aminopropane
DFMO	α -Difluoromethylornithine
GAPA	1-Guanidinoxy-3-aminopropane
Put	Putrescine (1,4-diaminobutane)
Spd	Spermidine (1,8-diamino-4-azaoctane)
SAG	Sodium stibogluconate

Introduction

During last decade, *O*-substituted hydroxylamines have become increasingly important in enzymology and bioorganic chemistry. One of the main reasons of this is well-known spontaneous condensation of *O*-alkyl hydroxylamines with aldehydes and ketones taking place in water solution at physiological pH values and yielding stable oximes with near quantitative yields. This was the reason: (i) to use substrate-like *O*-substituted hydroxylamines as effective inhibitors of carbonyl-dependent enzymes of amino acids metabolism. The specificity and efficiency of the inhibition is well interpreted in terms of structural similarity of the oxime of the enzyme with the external aldimine—the first

M. A. Khomutov, S. Mandal, and J. Weisell equally contributed to this work.

M. A. Khomutov (✉) · A. R. Simonian · S. N. Kochetkov
Engelhardt Institute of Molecular Biology,
Russian Academy of Sciences, Moscow, Russia
e-mail: hommaximus@mail.ru

S. Mandal · N. Saxena · R. Madhubala
School of Life Sciences, Jawaharlal Nehru University,
New Delhi, India

J. Weisell · J. Vepsäläinen
Department of Biosciences, Biocenter Kuopio,
University of Kuopio, Kuopio, Finland

intermediate of these types of enzymatic transformations (Markovic-Housley et al. 1996; Capitani et al. 2003; Liu et al. 2004). Hydroxylamine-containing analogs of *S*-adenosylmethionine (AdoMet) and putrescine (1,4-diaminobutane) (Put) are nanomolar inhibitors of decarboxylases of AdoMet and Orn (Wallace and Fraser 2004; Khomutov 2002) and in vitro showed antitrypanosomal (Guo et al. 1995), antimalarial (Das Gupta et al. 2005), and antileishmanial activity (Heby et al. 2007). (ii) To use the reaction of the oxime formation, which occurs with almost complete functional group compatibility, as the key step of a convenient chemoselective ligation strategy to prepare different biologically important conjugates and semisynthetic biopolymers using hydroxylamine-containing peptides and oligonucleotides as building blocks (Peri et al. 1998; Chen et al. 2003; Salo et al. 1999). (iii) To use hydroxylamine-containing resins for the isolation of oligosaccharides (Shimaoka et al. 2007 and ref. within), and even to prepare surfaces being coated with nanolayers (Lee et al. 2004).

To solve the above and related applications, a lot of different *O*-substituted hydroxylamines are required, including compounds with different functional groups in the alkyl substituent. Classic methods of the preparation of the hydroxylamine esters start from corresponding alkyl halogenides, which react with a proper *N*-protected hydroxylamine derivative that after the removal of *N*-protection group results in required *O*-substituted hydroxylamines (Zeeh and Metzger 1971). However, a family of functionally substituted alcohols is much diverse, as compared with corresponding alkyl halides. The only preparative method to synthesize functionally substituted esters of hydroxylamines starting from alcohols is to use *N*-hydroxyphthalimide in Mitsunobu reaction (Hughes 1992; Maillard et al. 2005). This allows preparing different *O*-substituted hydroxylamines, including nucleoside (Salo et al. 1999), and carbohydrate (Rodriguez et al. 1998) derivatives, and also hydroxylamines bearing different reporter groups (Muddana and Peterson 2004). Unfortunately, the properties of *N*-alkoxyphthalimide group make it impossible to perform some of commonly used reactions of nucleophilic substitution to introduce functional group(s) in the side-chain of *N*-protected hydroxylamine ester. This makes phthaloxime protection useless for many of synthetic schemes, which involve introduction of *N*-hydroxyphthalimide at one of the first steps and subsequent “functionalization of the radical.” Recently, an alkylation of *N*-Boc-hydroxylamine with alkyl methanesulfonates of alcohols was published. In the case of nonsubstituted alcohols, the target hydroxylamines were prepared in good or moderate yields (Albrecht et al. 2006).

Here, we report that the alkylation of ethyl *N*-hydroxyacetimidate with methanesulfonates of functionally substituted alcohols is an attractive alternative to both of the above methods and results in required *O*-substituted hydroxylamines in good or excellent yield. 1-Guanidinooxy-3-aminopropane (GAPA), obtained by this method, actively penetrated inside *L. donovani* and was very effective toward sodium stibogluconate (SAG) resistant forms of the parasite.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes with a “Manual Mel-Temp” apparatus (Barnstead International) and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance 500 DRX spectrometer using tetramethylsilane (TMS) in CDCl_3 or sodium trimethylsilylpropanesulfonate (TSP) in D_2O as internal standards. Chemical shifts are given in ppm, and *J* values are given in Hz. TLC was carried out on precoated Kieselgel 60 F254 plates, elution systems are indicated in the text. All reagents and solvents were used as received from manufacturers unless otherwise specified. 1-Butyl methanesulfonate (**1a**), 2-propyl methanesulfonate (**1b**), and cyclohexyl methanesulfonate (**1c**) were prepared by known method (Truce and Christensen 1968) and the rest of methanesulfonates (**1d–1g**) were prepared following the same method (Truce and Christensen 1968) and had the following characteristics:

(**1d**)—Yield 77%; mp 54–55°C (*i*-PrOH); R_f 0.41 (CHCl_3 –MeOH, 100:1). ^1H NMR (CDCl_3): δ 7.35–7.26 (5H, m); 5.09 (2H, s); 5.00 (1H, s); 4.27 (2H, t, *J* 5.8); 3.35–3.30 (2H, m); 2.97 (3H, s); 1.98–1.91 (2H, m). ^{13}C NMR (CDCl_3): δ 156.49, 136.45, 128.55, 128.19, 128.11, 67.26, 66.78, 37.36, 37.21, 29.52. Calculated, %: C 50.16; H 5.96; N 4.87. $\text{C}_{12}\text{H}_{17}\text{NO}_7\text{S}$. Found, %: C 50.09; H 5.98; N 4.91.

(**1e**)—yield quant.; R_f 0.23 (CHCl_3); ^1H NMR (CDCl_3): δ 4.23 (2H, t, *J* 6.5); 4.00 (2H, q, *J* 7.1); 3.88 (2H, t, *J* 6.5); 3.00 (3H, s); 1.92 (3H, s); 1.80–1.74 (2H, m); 1.67–1.61 (2H, m); 1.48–1.38 (4H, m); 1.27 (3H, t, *J* 7.1). ^{13}C NMR (CDCl_3): δ 162.19, 73.25, 70.04, 62.12, 37.39, 29.12, 28.69, 25.59, 25.34, 14.41, 13.62. Was used without isolation to prepare (**3e**).

(**1f**)—Yield 72.5%; mp 82.5–83°C (EtOAc–hexane), R_f 0.60 (CHCl_3 –MeOH, 98:2). ^1H NMR (CDCl_3): δ 4.37 (1H, bs); 4.28 (2H, t, *J* 6.1); 3.81 (1H, bs); 3.01 (3H, s); 1.95–1.72 (2H, m); 1.44 (9H, s); 1.20 (3H, d, *J* 6.7). ^{13}C NMR (CDCl_3): δ 155.38, 79.49, 67.29, 43.55, 37.34, 36.57,

28.38, 21.38. Calculated, %: C 44.93; H 7.92; N 5.24. $C_{10}H_{21}NO_5S$. Found, %: C 44.98; H 7.98; N 5.17.

(**1g**)—Yield 91.1%; bp 125–126°C/0.7 mmHg. 1H NMR ($CDCl_3$): δ 4.22 (2H, t, J 6.6); 3.53 (2H, t, J 6.5); 3.00 (3H, s); 1.81–1.75 (4H, m); 1.52–1.39 (4H, m). ^{13}C NMR ($CDCl_3$): δ 69.84, 44.82, 37.39, 32.31, 29.00, 26.27, 24.79. Found, %: C 39.25; H 6.97. $C_7H_{15}ClO_3S$. Calculated, %: C 39.16; H 7.04.

General method of ethyl *N*-hydroxyacetimidate alkylation with alkyl methanesulfonates

To a stirred 2 M solution of sodium salt of ethyl *N*-hydroxyacetimidate (ethyl *N*-hydroxyacetimidate from “Aldrich” was distilled before use, bp 62°C/12 mmHg) in MeOH or *i*-PrOH, an equivalent of the corresponding alkyl methanesulfonate was added slowly in small portions and stirring was continued at 20°C until the neutral pH of the reaction mixture. Precipitate was filtered off, filtrate was evaporated to dryness in vacuo, and the residue was poured into water and extracted with $CHCl_3$ (Et_2O was used in the case of **2a** and **2b**). Combined organic extracts were washed with 1 M $NaHCO_3$, H_2O , brine, dried ($MgSO_4$), and evaporated to dryness in vacuo, affording ethoxyethylidene derivatives (**2a**)–(**2g**):

(**2a**)—Reaction time 16 h; yield 78%; bp 91–91.5°C/47 mmHg; n_D^{20} 1.4178 (lit. (Nedospasov and Khomutov 1976): bp 64°C/14 mmHg, n_D^{16} 1.4216). 1H NMR ($CDCl_3$): δ 3.99 (2H, q, J 6.9); 3.86 (2H, t, J 6.5); 1.90 (3H, s); 1.62–1.55 (2H, m); 1.41–1.31 (2H, m); 1.25 (3H, t, J 6.9); 0.91 (3H, t).

(**2b**)—Reaction time 36 h; yield 65%; bp 74–74.5°C/72 mmHg; n_D^{20} 1.4096. 1H NMR ($CDCl_3$): δ 4.13–4.09 (1H, m); 4.01 (2H, q, J 7.1); 1.90 (3H, s); 1.27 (3H, t, J 7.1); 1.12 (6H, d, J 6.2). ^{13}C NMR ($CDCl_3$): δ 161.71, 74.45, 61.97, 21.51, 14.44, 13.66. Calculated, %: C 57.90; H 10.41; N 9.65. $C_7H_{15}NO_2$. Found, %: C 58.18; H 10.45; N 9.55.

(**2c**)—Reaction time 7 days; yield 57.6%; bp 94–96°C/12 mmHg; n_D^{20} 1.4512. 1H NMR ($CDCl_3$): δ 4.01 (2H, q, J 7.1); 3.86–3.80 (1H, m); 1.93 (3H, s); 1.91–1.89 (2H, m); 1.72–1.68 (2H, m); 1.54–1.49 (1H, m); 1.41–1.21 (5H, m); 1.26 (3H, t, J 7.1). ^{13}C NMR ($CDCl_3$): δ 161.89, 79.77, 61.99, 31.62, 25.98, 23.90, 14.46, 13.67. Calculated, %: C 64.83; H 10.34; N 7.56. $C_{10}H_{19}NO_2$. Found, %: C 64.88; H 10.44; N 7.62.

(**2d**)—Reaction time 12 h; yield quant.; R_f 0.78 ($CHCl_3$ –MeOH, 100:1); 1H NMR ($CDCl_3$): δ 7.37–7.27 (5H, m); 5.09 (3H, m); 3.99 (2H, q, J 7.2); 3.96 (2H, t, J 6.2); 3.35–3.27 (2H, m); 1.90 (3H, s); 1.88–1.79 (2H, m); 1.25 (3H, t, J 7.2). (**2d**) was used without isolation for the preparation of (**3d**).

(**2e**)—Reaction time 96 h; yield 70.5%; bp 121–122°C/1 mmHg; n_D^{20} 1.4480. 1H NMR ($CDCl_3$): δ 3.99 (4H, q,

J 7.2); 3.86 (4H, t, J 6.6); 1.91 (6H, s); 1.65–1.59 (4H, m); 1.39–1.36 (4H, m); 1.25 (6H, t, J 7.2). ^{13}C NMR ($CDCl_3$): δ 162.13, 73.54, 62.09, 28.09, 26.00, 14.41, 13.60. Calculated, %: C 58.31; H 9.79; N 9.71. $C_{14}H_{28}N_2O_4$. Found, %: C 58.54; H 9.80; N 9.73.

(**2f**)—Reaction time 48 h; yield 68.6%; self-crystallized oil, solid has mp 38°C. R_f 0.67 ($CHCl_3$ –MeOH, 98:2); 1H NMR ($CDCl_3$): δ 4.63 (1H, s); 4.03–3.93 (4H, m); 3.78 (1H, bs); 1.94 (3H, s); 1.84–1.70 (2H, m); 1.44 (9H, s); 1.26 (3H, t, J 7.1); 1.17 (3H, d, J 6.6). ^{13}C NMR ($CDCl_3$): δ 162.36, 155.34, 78.88, 70.63, 62.20, 44.70, 35.73, 28.44, 21.18, 14.39, 13.73. Calculated, %: C 56.91; H 9.55; N 10.21. $C_{13}H_{26}N_2O_4$. Found, %: C 57.06; H 9.69; N 10.05.

(**2g**)—Reaction time 96 h; yield 82%; bp 72–73°C/0.7 mmHg; n_D^{20} 1.4475. 1H NMR ($CDCl_3$): 4.00 (2H, q, J 7.2); 3.87 (2H, t, J 6.5); 3.52 (2H, t, J 6.9); 1.91 (3H, s); 1.81–1.74 (2H, m); 1.66–1.59 (2H, m); 1.50–1.34 (4H, m); 1.26 (3H, t, J 7.2). ^{13}C NMR ($CDCl_3$): δ 162.16, 73.35, 62.11, 45.03, 32.61, 28.76, 26.78, 25.45, 14.41, 13.61. Calculated, %: C 54.17; H 9.09; N 6.32. $C_{10}H_{20}ClNO_2$. Found, %: C 54.28; H 9.11; N 6.18.

General procedure for the removal of ethoxyethylidene protection of aminoxy group

To the solution of 1 equiv. of ethyl *N*-alkoxyacetimidate's (**2a**)–(**2e**), and (**2g**) in *i*-PrOH was added 2.5 equiv. of 37% aq. HCl and after 3–5 min at 20°C, the reaction mixture was evaporated to dryness in vacuo. The residue was co-evaporated twice with abs. *i*-PrOH and crystallized from *i*-PrOH– Et_2O that after drying in vacuo over P_2O_5 /KOH yielded compounds (**3a**)–(**3e**), and (**3g**):

(**3a**)—Yield 97%; mp 157°C (lit. Theilacker and Ebke 1956): mp 159–160°C).

(**3b**)—Yield 87%; mp 85–86°C (lit. Theilacker and Ebke 1956): mp 84.8°C).

(**3c**)—Yield 86%; mp 184–185°C, dec. (lit. Albrecht et al. 2006): mp 210–220°C, subl. at 176–178°C; (lit. Theilacker and Ebke 1956): mp 175°C. 1H NMR (D_2O): δ 4.09–4.00 (1H, m), 1.98–1.89 (2H, m), 1.75–1.66 (2H, m), 1.53–1.45 (1H, m), 1.43–1.14 (5H, m). ^{13}C NMR (D_2O): δ 86.13, 32.37, 27.25, 25.52. Calculated, %: C 47.53; H 9.31; N 9.24. $C_6H_{14}ClNO$. Found, %: C 47.38; H 9.49; N 9.17.

(**3d**)—Yield 91%; mp 188–189°C (lit. Lee et al. 1995): mp 191°C). 1H NMR (D_2O): 7.46–7.42 (5H, m); 5.11 (2H, s); 4.11 (2H, t, J 6.1); 3.23 (2H, t, J 6.5); 1.89–1.84 (2H, m). ^{13}C NMR ($CDCl_3$): δ 161.30, 139.34, 131.65, 131.24, 130.53, 75.66, 69.76, 39.67, 30.26.

(**3e**)—Yield 93%; mp 227°C, dec. (MeOH–*i*-PrOH). 1H NMR (D_2O): δ 4.02 (4H, t, J 6.4), 1.69–1.56 (4H, m), 1.41–1.30 (4H, m). ^{13}C NMR (D_2O): δ 78.22, 29.42, 27.05. Calculated, %: C 32.59; H 8.20; N 12.67. $C_6H_{18}Cl_2N_2O_2$. Found, %: C 32.72; H 8.31; N 12.54.

(3g)—Yield 87%; mp 127–128°C. ^1H NMR (D_2O): δ 4.05 (2H, t, J 6.5), 3.60 (2H, t, J 6.7), 1.79–1.71 (2H, m), 1.71–1.63 (2H, m), 1.48–1.34 (4H, m). ^{13}C NMR (D_2O): δ 78.24, 48.36, 34.33, 29.47, 28.35, 26.73. Calculated, %: C 38.31; H 8.04; N 7.45. $\text{C}_6\text{H}_{15}\text{Cl}_2\text{NO}$. Found, %: C 38.16; H 8.13; N 7.31.

1-Aminooxy-3-[*N*-(*tert*-butoxycarbonyl)]aminobutane (**3f'**)

To the solution of (**2f**) (0.85 g, 3.1 mmol) in MeOH (6 ml) was added with stirring 0.5 M H_2SO_4 (4.65 ml) at 20°C and after 5 min at 20°C the reaction mixture was cooled to +4°C and pooled into cooled (+4°C) mixture of MeOH (1 ml) and 25% NH_4OH (1 ml). The resulted solution was evaporated practically to dryness in vacuo, diluted with 2 M Na_2CO_3 , extracted with CHCl_3 (3×2 ml), washed with H_2O , brine and dried (MgSO_4). The solvent was removed in vacuo, the residue dried over P_2O_5 at 1 mmHg that afforded 0.6 g (95%) of compound (**3f'**) as a vaxi oil, which slowly solidified on standing. R_f 0.23 (CHCl_3 –MeOH, 98:2); ^1H NMR (CDCl_3): δ 5.40 (2H, s); 4.55 (1H, bs); 3.79–3.69 (2H + 1H, m); 1.76–1.65 (2H, m); 1.44 (9H, s); 1.15 (3H, d, J 6.6). ^{13}C NMR (CDCl_3): δ 155.38, 79.02, 72.95, 44.24, 35.62, 28.45, 21.16. Calculated, %: C 52.92; H 9.87; N 13.71. $\text{C}_9\text{H}_{20}\text{N}_2\text{O}_3$. Found, %: C 52.79; H 9.91; N 13.62.

1-Aminooxy-3-aminobutane dihydrochloride (**3f''**)

A solution of (**2f**) (90 mg, 0.3 mmol) in EtOH (2 ml) was added 37% HCl (0.35 ml) and after 16 h at 20°C the reaction mixture was evaporated to dryness in vacuo and the residue was dried over $\text{P}_2\text{O}_5/\text{KOH}$ in vacuo. Resulted solid was treated with abs. EtOH, filtered off and dried over P_2O_5 in vacuo to afford 45 mg (85%) of target (**3f**), mp 157–159°C (lit. (Keinänen et al. 1994): mp 156–158°C); R_f 0.12 (dioxane—25% NH_4OH , 9:1). ^1H NMR (D_2O): δ 4.26–4.18 (2H, m); 3.57–3.53 (1H, m); 2.14–2.07 (2H, m); 1.35 (3H, d, J 6.5). ^{13}C NMR (CDCl_3): δ 74.61, 48.41, 34.96, 20.43.

3-[*N,N'*-di-(*tert*-Butoxycarbonyl)]guanidinoxy-1-(benzyloxycarbonyl)aminopropane (**4**)

A solution of (**3d**) (0.32 g, 1.4 mmol), *N,N'*-di-Boc-*N''*-triflylguanidine (0.49 g, 1.26 mmol) and Et_3N (0.2 ml, 1.4 mmol) in dry CHCl_3 (6 ml) was incubated for 24 h at 37°C. Reaction mixture was diluted with CHCl_3 (6 ml), washed with 10% citric acid, H_2O , 1 M NaHCO_3 , H_2O , brine and dried (MgSO_4). The solvent was removed in vacuo, the residue dried over P_2O_5 at 1 mmHg that afforded 0.49 g (88%) of compound (**4**) as a vaxi oil. R_f 0.52

(CHCl_3 –MeOH, 4:1). ^1H NMR (CDCl_3): δ 9.00 (1H, s); 7.70 (1H, s); 7.34–7.26 (5H, m); 5.59 (1H, bs); 5.07 (2H, s); 4.07 (2H, t, J 5.6); 3.31–3.25 (2H, m); 1.87–1.80 (2H, m); 1.46 (9H, s); 1.44 (9H, s). ^{13}C NMR (CDCl_3): δ 156.59, 151.85, 149.72, 140.85, 136.92, 128.33, 127.76, 83.26, 81.37, 71.13, 66.25, 37.93, 29.61, 28.09, 28.00.

3-Guanidinoxy-1-aminopropane dihydrobromide (GAPA) (**5**)

To a solution of (**4**) (0.49 g, 1.1 mmol) in glacial AcOH (5 ml) a 32% HBr/AcOH (3 ml) was added and after 2 h at 20°C to the reaction mixture was added AcOH– Et_2O , 1:1 (8 ml), the precipitated material was filtered off, washed with Et_2O and dried in vacuo over KOH. Recrystallisation from abs. EtOH gave 0.26 g (70%) of the compound (**5**). R_f 0.25 (dioxane—25% NH_4OH , 7:3); mp 172–173°C, dec. ^1H NMR (D_2O): δ 4.06 (2H, t, J 6.2); 3.14 (2H, t, J 7.5); 2.15–2.00 (2H, m). ^{13}C NMR (D_2O): δ 161.54, 77.07, 39.67, 28.08. Calculated, %: C 16.34, H 4.80, N 19.06. $\text{C}_4\text{H}_{14}\text{Br}_2\text{N}_4\text{O}$. Found, %: C 16.48, H 4.77, N 19.20.

Biochemistry

Growth media and antibiotics were purchased from Sigma (St. Louis, MO) and fetal bovine serum (FBS) from Gibco/BRL (Life Technologies Scotland, United Kingdom).

Parasite and culture condition

Promastigotes of Indian *Leishmania donovani* clone GE1 (MHOM/IN/80/GE1F8R) (Bhattacharyya et al. 2001), *L. donovani* strain MHOM/IN/80/AG83 and three untyped clonal strains S-1, R-1, and R-2 were isolated from patients with visceral leishmaniasis and were routinely cultured at 22°C in M199 medium with Hanks' salts including 25 mM HEPES buffer (Sigma, USA) supplemented with 10% heat inactivated FBS and 0.13 mg/ml penicillin and streptomycin. Clinical isolate S-1 was sensitive to sodium antimony gluconate (SAG), whereas the three SAG-resistant isolates were GE1-R, R-1, and R-2. The SAG-resistant isolates were maintained in the absence of drug pressure in vitro (Singh et al. 2007).

Drug susceptibility assay

The effect of GAPA on the growth of the promastigotes was determined in 96-wells microtiter plates. Briefly, 1×10^6 parasites in 0.2 ml of modified M199 medium with 10% FBS were placed in each well and incubated with various concentrations of the drug. Two wells in which cells were permitted to grow in the absence of drug were maintained in parallel as controls. After 72 h of incubation

under normal growth condition, cell densities were determined by the Neubauer hemacytometer. The concentrations of GAPA, which inhibited the growth of the cells by 50%, were determined. Two or more independent experiments in triplicate were performed for the determination of sensitivity to each drug.

DNA constructs and transfection

An episomal *Leishmania* expression vector (pGL- α NEO α LUC) containing luciferase encoding DNA and neomycin phosphotransferase selectable marker (Roy et al. 2000) was used in the present study. Twenty micrograms of the construct was transfected into promastigotes of *L. donovani* field isolates by electroporation in 2 mm gap cuvettes, at 450 V, 500 μ F (BTX Electro Cell Manipulator 600). Transfectants were selected for resistance to G418 (50 μ g/ml) as described earlier (Papadopolou et al. 1992).

Macrophage infection and intracellular amastigote drug susceptibility assay

Stationary phase *Leishmania* promastigotes expressing the luciferase gene (pGL- α NEO α LUC) were used to infect J774A.1 macrophages. Macrophage cell line J774A.1 (American Type Culture Collection) was maintained at 37°C in RPMI-1640 medium (Sigma) containing 10% heat inactivated FBS as described previously (Kapoor et al. 2001). Briefly, J774A.1 murine macrophages (1×10^5 cells/250 μ l/per well) were infected with 1×10^6 promastigotes in M199 media with 10% FBS (Roy et al. 2000). After 3 h, the non-internalized parasites were removed by washing and drug was added at different concentrations. After 5 days of drug exposure, plates containing adherent macrophages were washed and luciferase activity was determined (Roy et al. 2000). The 50% inhibitory concentration (IC₅₀) was determined from the graph representing different concentrations of drug plotted against relative light units (RLU) produced by luciferase expressing parasites.

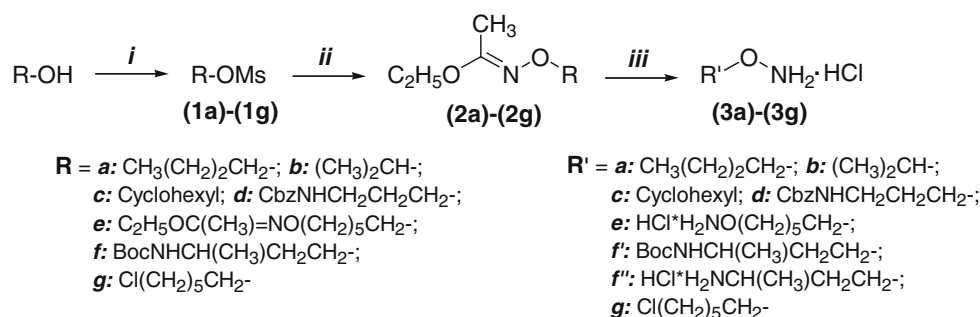
Results and discussion

Chemistry

Ethyl *N*-hydroxyacetimidate is not so widely used to prepare *O*-substituted hydroxylamines as *N*-hydroxyphthalimide. However, ethyl *N*-hydroxyacetimidate easily and with high yields adds to activated double bond according to Michael and easily alkylated with alkyl halogenides (Khomutov et al. 1996 and ref. within). Ethoxyethylidene protection group is complementary to a wide set of

nucleophilic substitution in *N*-alkoxy group and besides it is resistant toward some oxidizing and reducing agents (Khomutov et al. 1996 and ref. within), while phthaloxime group, which is typically removed by hydrazine, or even aliphatic amine treatment (Maillard et al. 2005), is not stable even under standard conditions of preparation of thiols from alkyl bromides (Bauer and Suresh 1963). The main advantage of ethoxyethylidene protection group is extremely mild conditions of its removal—normally 1 equiv. of strong acid and 1 equiv. of water within couple of min at 20°C is enough to get required *O*-substituted hydroxylamine with practically quantitative yield. The removal conditions are so mild that it is possible to deprotect aminoxy group in the presence of *N*-Boc-amino group (Simonian et al. 2006). All above make ethyl *N*-hydroxyacetimidate the best *N*-protected hydroxylamine derivative, in the case synthetic strategy consists in the introduction of the protected aminoxy group at one of the first steps of synthesis and subsequent “functionalization” of the radical. However, the alkylation of ethyl *N*-hydroxyacetimidate with easily available methanesulfonates of functionally substituted alcohols, which are more available as compared with alkyl halogenides, remains unknown.

To begin the study we compared the rates of the alkylation of Na-salt of ethyl *N*-hydroxyacetimidate with 1-butyl- and 2-propyl methanesulfonates with that of 1-bromobutane. The reaction of 1-butyl methanesulfonate (**1a**) with Na-salt of ethyl *N*-hydroxyacetimidate (2 M final concentration of each) in MeOH was slightly exothermic and practically completed within 5–6 h, while after 16 h the pH of reaction mixture became neutral (Scheme 1). 2-Propyl methanesulfonate (**1b**) was less reactive under the above conditions and after 12 h at 20°C, the reaction mixture was still basic, while after 36 h the reaction completed. The yields of pure (**2a**) and (**2b**) were only 78 and 65%, respectively. In the first case, it was decreased due to enough laborious separation of (**2a**) from the traces of nonreacted ethyl *N*-hydroxyacetimidate having close bp, while (**2b**) turned to be enough volatile like methyl and ethyl esters of ethyl *N*-hydroxyacetimidate. The reaction mixture containing 2 M of 1-bromobutane and 2 M of Na-salt of ethyl *N*-hydroxyacetimidate in MeOH remained alkaline even after 96 h at 20°C and heating was required to get (**2a**) in a good yield. Hence, even 2-propyl methanesulfonate turned to be more reactive toward ethyl *N*-hydroxyacetimidate as compared with that of 1-bromobutane. As expected, cyclohexyl methanesulfonate (**1c**) was much less reactive as compared with (**1b**). In this case, the yield of (**2c**) was only 58% after 7 days at 20°C, but it was much better than 36%, being obtained with *N*-Boc-hydroxylamine and (**1c**) under the conditions outlined in (Albrecht et al. 2006). One of the main advantages of



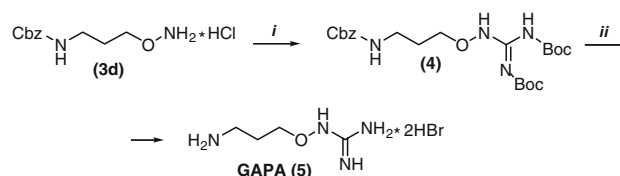
Scheme 1 Synthesis of *O*-substituted hydroxylamines by the alkylation of ethyl *N*-hydroxyacetimidate with alkyl methanesulfonates and subsequent deprotection of aminooxy group. i—MsCl/C₆H₆/

Et₃N; ii—C₂H₅OC(CH₃)=NONa/*i*-PrOH, or MeOH/20°C; iii—HCl/H₂O for **3a–3e**, **3f'**, and **3g**; 0.75 mol. equiv. H₂SO₄/H₂O/MeOH for **3f''**

ethoxyethylidene protection group is the possibility to remove it under mild conditions with practically quantitative yield. This made possible to obtain the required (**3a**)–(**3c**) with the overall yields of 76, 57, and 50%, as calculated for ethyl *N*-hydroxyacetimidate, that is much better as compared with the overall yields of 64, 40, and 20%, for the same (**3a**)–(**3c**) being prepared from *N*-Boc-hydroxylamine and mesylates (**1a**)–(**1c**) under earlier published protocol (Albrecht et al. 2006).

First functionally substituted alcohols of this study were 6-chloro-1-hexanol and 6-(1-ethoxyethylideneamino-oxy)-1-hexanol, the last was prepared by alkylation of Na-salt of ethyl *N*-hydroxyacetimidate with 6-chloro-1-hexanol in boiling alcohol. Corresponding mesylates (**1g**) and (**1e**), the last was used without isolation, smoothly alkylated Na-salt of ethyl *N*-hydroxyacetimidate in MeOH and required ethoxyethylidene derivatives (**2g**) and (**2e**) were isolated by distillation with the yields of 82 and 71%, respectively (Scheme 1). Mild acidic hydrolysis resulted in dihydrochloride (**3g**) and hydrochloride (**3e**) with the yields of 93 and 87%, respectively. The conditions of the removal of the ethoxyethylidene protection of the aminooxy group are so that, that it turned possible to deprotect aminooxy group of (**2f**) selectively using slight excess of H₂SO₄ in water–MeOH mixture and (**3f'**) was finally obtained as a base with 95% yield. Simultaneous removal of ethoxyethylidene and *N*-Boc protections gave dihydrochloride (**3f''**) in 85% yield (Scheme 1).

N-(Benzyloxycarbonyl)-3-amino-1-propanol was selected as another example of functionally substituted alcohol (Scheme 1). Corresponding methanesulfonate (**1d**) was prepared with an excellent yield and the alkylation was performed by adding small portions of solid (**1d**) to a stirred 2 M solution of Na-salt of ethyl *N*-hydroxyacetimidate in *i*-PrOH at 20°C. The reaction practically completed after 4–5 h, while after 12 h starting (**1d**) could not be detected by TLC. Intermediate ethoxyethylidene derivative (**2d**) was without isolation treated with 2–3 equiv. of 37% aq. HCl for 3–5 min at 20°C, that after recrystallisation



Scheme 2 Synthesis of 1-guanidinoxy-3-aminopropane (GAPA). i—Tf-Boc₂-Guanidine/Et₃N/CH₂Cl₂; ii—HBr/AcOH

afforded target (**3d**) with the yield of 91% (two steps). Thus, obtained 1-aminooxy-3-[*N*-(benzyloxycarbonyl)amino]propane was reacted (Scheme 2) with an equivalent of *N,N'*-di-Boc-*N''*-triflylguanidine at 37°C for 24 h, that gave protected GAPA (**4**) with practically quantitative yield. Subsequent removal of *N*-Boc- and *N*-Cbz-groups with HBr/AcOH afforded target GAPA (**5**) with the yield of 61% (two steps).

Above data clearly proves that the alkylation of ethyl *N*-hydroxyacetimidate with alkyl methanesulfonates is a convenient approach to prepare functionally substituted esters of hydroxylamine with excellent or high yields.

Biochemistry

A protozoan parasite *L. donovani* is the causative agent of visceral leishmaniasis. The pentavalent antimonials SAG (Fig. 1) and meglumine antimonate, the first line of drugs for visceral and cutaneous leishmaniasis and resistance to these drug is one of the major problem in the field. This increase in resistance has led to the need for more selective and efficacious drugs. As polyamine biosynthetic pathway is essential for growth and survival of *L. donovani*, inhibition of this pathway is an important leishmanicidal strategy (Heby et al. 2007). α -Difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, is used to treat late stage of African sleeping sickness caused by the protozoan parasite *Trypanosoma brucei gambiense* (Burri and Brun 2003), and it is effective against other genera of protozoan parasites including *Plasmodium* species (Bitonti et al. 1989), *Giardia* (Gillin et al. 1984), and *Leishmania*

Fig. 1 Structures of 1-guanidinoxy-3-aminopropane (GAPA), 1-aminooxy-3-aminopropane (APA), and sodium stibogluconate (SAG)

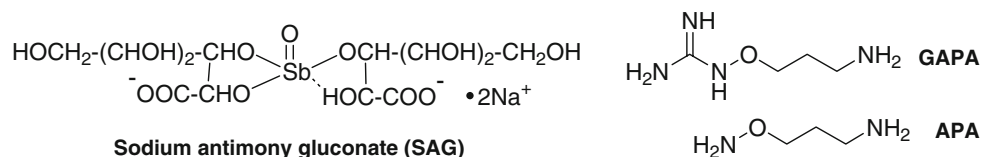


Table 1 Comparative analysis of the effect of sodium antimony gluconate (SAG), APA, and GAPA on the SAG-sensitive and SAG-resistant clinical isolates

Isolates	IC ₅₀ (μM)					
	Promastigotes			Intracellular amastigotes		
	SAG ^a	APA ^b	GAPA ^c	SAG ^a	APA ^b	GAPA ^d
WT	57 ± 5.0	42 ± 8.0	36 ± 7.0	15.0 ± 6.0	5 ± 2.0	9 ± 1.0
S-1	47 ± 5.6 (1)	19 ± 0.7	10 ± 4.2	13.0 ± 1.5 (1)	10 ± 2.5	9 ± 0.7
R-1	128 ± 3.3 (2.7)	>400	8 ± 1.4	62.0 ± 3.4 (5)	>200	18 ± 2.0
R-2	68 ± 4.2 (1.4)	46 ± 2.8	78 ± 1.4	23.0 ± 1.4 (1.8)	>200	22 ± 3.0
GE1	164 ± 5.1 (3.4)	>400	8 ± 1.4	>95.0 (> 7.6)	>200	3 ± 0.7

^a Data for IC₅₀ values for SAG are adopted from (Singh et al. 2007)

^b Data for IC₅₀ values for APA are adopted from (Singh et al. 2007)

^c IC₅₀s were determined in promastigotes after 72 h of drug addition

^d IC₅₀s were determined from the graph representing concentration of drug plotted against RLU produced by luciferase expressing parasites. The stationary phase *L. donovani* isolates transfected with pGL- α Neo α Luc were used to infect J774A.1 macrophages as described in “Materials and methods” section. After 5 days of drug exposure, luciferase activity was determined. IC₅₀ are given as means ± standard deviations of at least three independent determinations. Fold change in the IC₅₀ compared to S-1 is given in parenthesis

(Kaur et al. 1986). 1-Aminooxy-3-aminopropane (APA) (Fig. 1) being another very effective inhibitor of ODC (K_I toward recombinant *L. donovani* ODC is 1 nM (Singh et al. 2007) inhibits the growth of amastigotes and promastigotes of wild type *L. donovani* at 5 and 45 μM, respectively (Singh et al. 2007). However, both DFMO and APA passively penetrate inside cells and parasites.

Recently, we made an attempt to transform APA into actively transported form, converting terminal H₂NO-group into H₂NC(=NH)NHO-group (Singh et al. 2008). Thus, designed GAPA (Fig. 1) is a structural analog of Agm, but not a Put as parent APA is. Terminal guanidinoxy group of GAPA has the pK_a 6.71 that makes GAPA to be enough protonated at physiological pH in order to mimic charge distribution of Put. GAPA turned to be not very effective inhibitor of ODC, having K_I of only 60 μM toward recombinant *L. donovani* ODC (Singh et al. 2008). However, in vitro GAPA inhibited the growth of wild type *L. donovani* amastigotes and promastigotes at 9 and 36 μM, respectively (Singh et al. 2008), i.e., was as effective as APA. The relationship between K_I of ODC and activity toward amastigotes may be considered as an indication on the active transport of GAPA inside the parasite.

Cellular effects of GAPA, i.e., the decrease of Put and spermidine (1,8-diamino-4-azaoctane) (Spd) levels in

L. donovani and the ability of exogenous Put and Spd to reverse the growth of the parasite clearly indicate that the cellular target of GAPA is closely related to metabolism of polyamines (Singh et al. 2008).

Effect of GAPA on promastigotes and amastigotes of antimony susceptible/resistant clinical isolates

In the present study the efficacy of GAPA was analyzed on the promastigotes of field isolates from SAG-responsive and SAG-unresponsive patients. The IC₅₀ values of the promastigotes of wild type *L. donovani* and SAG-sensitive isolate S-1 to GAPA were 36 ± 7.0 and 10 ± 4.2 μM, respectively (Table 1). The promastigotes of SAG-unresponsive field isolates R-1, R-2, and GE1 had IC₅₀ values of 8 ± 1.4, 78 ± 1.4, and 8 ± 1.4 μM, respectively, with GAPA (Table 1). The sensitivity of these clinical isolates to GAPA was also tested in intracellular amastigotes using amastigote-macrophage model. GAPA was shown to effectively inhibit growth of amastigotes of wild type and S-1 isolate coming from SAG-responsive patients with an IC₅₀ value of 9 ± 1.0 and 9 ± 0.7 μM, respectively (Table 1). The amastigotes of the SAG-resistant isolates R-1, R-2, and GE1 had IC₅₀s of 18 ± 2.0, 22 ± 3.0, and 3 ± 0.7 μM, respectively, when treated with GAPA (Table 1). Amastigotes of GE1 strain, which was most

resistant to SAG, was most sensitive to GAPA when compared to all the other isolates.

Comparison has also been made between the effect of GAPA on these SAG-sensitive and SAG-resistant isolates and the effect of APA. As depicted in Table 1, the concentration of APA as high as 200 μ M did not inhibit SAG-resistant amastigotes, R-1, R-2, and GE1, while among promastigotes only R-2 was sensitive to APA.

ODC activity as well as Put and Spd levels were reported to be higher in the SAG-resistant isolates compared to the sensitive isolates (Singh et al. 2007). To show whether GAPA promotes its effect on SAG-sensitive and SAG-resistant cells by altering their polyamine concentrations, Put and Spd contents were measured. Effect of GAPA on the intracellular polyamine levels in promastigotes of SAG-sensitive and SAG-resistant isolates showed that GAPA (40 μ M) inhibited polyamine levels in both cases. Inhibition of Put was ~ 1.3 -, ~ 2.7 -, ~ 2.4 -fold, respectively, in R1, R2, and GE1 compared to the corresponding untreated control values (data not shown). However, no significant change was observed in the Spd levels in the resistant isolates when compared to the corresponding untreated control values (data not shown).

Hence, both in wild type and SAG-resistant forms of *L. donovani* the cellular target(s) of GAPA are the system of polyamine metabolism and polyamine-responsible cellular processes. However, the mechanism of GAPA's action cannot be described only in terms of intracellular transformation of the inhibitor into APA, which might take place in macrophages possessing agmatinase-like activity (Sastre et al. 1998), because APA is not active against SAG-resistant forms of *L. donovani*. It is more likely that the mechanism has a complex nature and includes the inhibition of ODC with GAPA "as such"; the decrease of Put level might also contribute into growth inhibition effect; and finally GAPA itself might target some important metabolic pathway, being different from the conversion of ornithine into Put.

Acknowledgments This work was supported by the Russian Foundation for Basic Research (project nos. 09-04-01272, 08-04-91317, and 08-04-91777), the program Molecular and Cell Biology of the Presidium of the Russian Academy of Sciences, grant from the Academy of Finland (project no. 124185), grant from the Council of Scientific and Industrial Research (CSIR), Government of India, and joint Indo-Russian DST-RFBR grant (project no. RUSP-883).

References

- Albrecht S, Defoin A, Tarnus C (2006) Simple preparation of *O*-substituted hydroxylamines from alcohols. *Synthesis* 1635–1638
- Bauer L, Suresh KS (1963) *S*-[ω -(Aminooxy)alkyl]isothiuronium salts, ω, ω' -bis(aminooxy)alkanes and related compounds. *J Org Chem* 28:1604–1608
- Bhattacharyya A, Roy M, Mandal A, Duttagupta S (2001) Effect of metal ions and other antileishmanial drugs on stibionate-resistant *Leishmania donovani* promastigotes of Indian origin. *Curr Sci* 81:1470–1473
- Bitonti AJ, Dumont JA, Bush TL et al (1989) Bis(benzyl) polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (*Plasmodium falciparum*) in vitro and in combination with alpha-difluoromethylornithine cure murine malaria. *Proc Natl Acad Sci USA* 86:651–655
- Burri C, Brun R (2003) Eflornithine for the treatment of human African trypanosomiasis. *Parasitol Res* 90:S49–S52
- Capitani G, Eliot AC, Gut H et al (2003) Structure of 1-aminocyclopropane-1-carboxylate synthase in complex with an amino-oxy analogue of the substrate: implications for substrate binding. *Biochim Biophys Acta* 1647:55–60
- Chen J, Zeng W, Offord R, Rose K (2003) A novel method for the rational construction of well-defined immunogens: the use of oxidation to conjugate cholera toxic B subunit to peptide-polyoxime complex. *Bioconjug Chem* 14:614–618
- Das Gupta R, Krause-Ihle T, Bergmann B et al (2005) 3-Aminooxy-1-aminopropane and derivatives have an antiproliferative effect on cultured *Plasmodium falciparum* by decreasing intracellular polyamine concentrations. *Antimicrob Agents Chemother* 49:2857–2864
- Gilllin FD, Reiner DS, McCann PP (1984) Inhibition of growth of *Giardia lamblia* by difluoromethylornithine, a specific inhibitor of polyamine biosynthesis. *J Protozool* 31:161–163
- Guo J, Wu YQ, Rattendi D, Bacchi CJ, Woster PM (1995) *S*-(5'-Deoxy-5'-adenosyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene (AdoMao): an irreversible inhibitor of *S*-adenosyl-methionine decarboxylase with potent in vitro antitrypanosomal activity. *J Med Chem* 38(10):1770–1777
- Heby O, Persson L, Rentala M (2007) Targeting the polyamine biosynthetic enzymes: a promising approach to therapy of African sleeping sickness, Chagas' disease, and leishmaniasis. *Amino Acids* 33(2):359–366
- Hughes DL (1992) In: *Organic Reactions*. N.-Y.: John Wiley & Sons Inc. 42:337–636
- Kapoor P, Raj VS, Saxena S, Balaraman S, Madhubala R (2001) Effect of *Leishmania donovani* lipophosphoglycan on ornithine decarboxylase activity in macrophages. *J Parasitol* 87:1071–1076
- Kaur K, Emmett K, McCann PP, Sjoerdsma A, Ullman B (1986) Effects of DL-alpha-difluoromethylornithine on *Leishmania donovani* promastigotes. *J Protozool* 33:518–521
- Keinänen TA, Hyvönen T, Pankaskie MC, Vepsäläinen JJ, Eloranta TO (1994) Derivatives of 1-aminoxy-3-aminopropane as polyamine antimetabolites: stability and effects on BHK21/C13 cells. *J Biochem* 116:1056–1062
- Khomutov AR (2002) Inhibition of enzymes of polyamine biosynthesis by substrate-like *O*-substituted hydroxylamines. *Biochemistry (Moscow)* 67:1403–1412
- Khomutov AR, Vepsäläinen JJ, Shvetsov AS et al (1996) Synthesis of hydroxylamine analogues of polyamines. *Tetrahedron* 52:13751–13766
- Lee YB, Park MH, Folk JE (1995) Diamine and triamine analogs and derivatives as inhibitors of deoxyhypusine synthase: synthesis and biological activity. *J Med Chem* 38:3053–3061
- Lee DC, Chang BJ, Yu L et al (2004) Polymer cushions functionalized with lipid molecules. *Langmuir* 20:11297–11300
- Liu W, Peterson PE, Carter RJ, Zhou X, Langston JA, Fisher AJ, Toney MD (2004) Crystal structures of unbound and aminooxyacetate-bound *Escherichia coli* γ -aminobutyrate aminotransferase. *Biochemistry* 43:10896–10905
- Maillard LT, Benohoud M, Durand P, Badet B (2005) A new supported reagent for the parallel synthesis of primary and

- O-alkyl hydroxylamines through a base-catalyzed Mitsunobu reaction. *J Org Chem* 70:6303–6312
- Markovic-Housley Z, Schirmer T, Hohenester E et al (1996) Crystal structure and solution studies of oxime adducts of mitochondrial aspartate aminotransferase. *Eur J Biochem* 236:1025–1032
- Muddana SS, Peterson BR (2004) Facile synthesis of CIDs: Biotinylated estrone oximes efficiently heterodimerize estrogen receptor and streptavidin proteins in yeast three hybrid systems. *Org Lett* 6:1409–1412
- Nedospasov AA, Khomutov RM (1976) Synthesis and some properties of aminoalkylcelluloses. *Izv Akad Nauk SSSR Ser Khim* 1136–1141
- Papadopoulou B, Roy G, Ouellette M (1992) A novel antifolate resistance gene on the amplified H circle of *Leishmania*. *EMBO J* 11:3601–3608
- Peri F, Dumy P, Mutter M (1998) Chemo- and stereoselective glycosylation of hydroxylamino derivatives: a versatile approach to glycoconjugates. *Tetrahedron* 54:12269–12278
- Rodriguez EC, Marcaurelle LA, Bertozzi CR (1998) Aminoxy-hydrazide, and thiosemicarbazide-functionalized saccharides: versatile reagents for glycoconjugate synthesis. *J Org Chem* 63:7134–7135
- Roy G, Dumas C, Sereno D et al (2000) Episomal and stable expression of the luciferase reporter gene for quantifying *Leishmania* spp Infections in macrophages and animal models. *Mol Biochem Parasitol* 110:195–206
- Salo H, Hakala H, Prakash TP et al (1999) Aminoxy functionalized oligonucleotides: preparation on-support derivatization and postsynthetic attachment to polymer support. *Bioconj Chem* 10:815–823
- Sastre M, Galea E, Feinstein D, Reis DJ, Regunathan S (1998) Metabolism of agmatine in macrophages: modulation by lipopolysaccharide and inhibitory cytokines. *Biochem J* 330:1405–1409
- Shimaoka H, Kuramoto H, Furukawa J et al (2007) One pot solid-phase glycoblotting and probing by transoximization for high-throughput glycomics and glycoproteomics. *Chem Eur J* 13:1664–1673
- Simonian AR, Vepsäläinen JJ, Khomutov AR (2006) Aminoxy analogues of spermine and their monoacetyl derivatives. *Bioorg Khim* 32:643–650
- Singh S, Mukherjee A, Khomutov AR, Persson L, Heby O, Chatterjee M, Madhubala R (2007) Antileishmanial effect of 3-aminoxy-1-aminopropane is due to polyamine depletion. *Antimicrob Agents Chemother* 51:528–534
- Singh S, Jhingran A, Sharma A et al (2008) Novel agmatine analogue, γ -guanidinooxypropylamine (GAPA) efficiently inhibits proliferation of *Leishmania donovani* by depletion of intracellular polyamine levels. *Biochem Biophys Res Commun* 375:168–172
- Theilacker W, Ebke K (1956) Darstellung von O-alkyl-hydroxylaminen. *Angew Chem* 68:303
- Truce WE, Christensen LW (1968) Mass spectral of alkyl methane-sulfonates. *J Org Chem* 33:2261–2266
- Wallace HM, Fraser AV (2004) Inhibitors of polyamine metabolism. *Amino Acids* 26(4):353–365
- Zeeh B, Metzger H (1971) Methoden zur Herstellung und Umwandlung von Hydroxylaminen. In: Müller E (ed) Houben-Weyl, Methoden der Organischen Chemie, vol X-1. Thieme Verlag, Stuttgart, pp 1097–1279