

A new stepwise synthesis of a family of propylamines derived from diatom silaffins and their activity in silicification†

Vadim V. Annenkov,^{*ab} Siddharth V. Patwardhan,^b David Belton,^b Elena N. Danilovtseva^a and Carole C. Perry^{*b}

Received (in Cambridge, UK) 10th November 2005, Accepted 9th February 2006

First published as an Advance Article on the web 24th February 2006

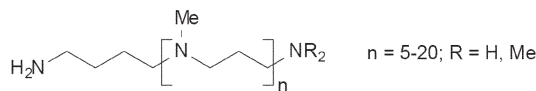
DOI: 10.1039/b515967a

A new method for the stepwise synthesis of propylamines containing fragments of *N*-methyl propylamine as found in diatom bioextracts is presented and their activity in silicic acid condensation is described.

Saturated speciality polyamines play important roles in various biological processes and have attracted the attention of synthetic chemists. A widely known naturally occurring polyamine is spermine (*N,N'*-bis(3-aminopropyl)-1,4-butanediamine), which had been isolated from sperm.¹ 3,3'-Methylimino-bis(*N*-methylpropylamine) (**N3**; Scheme 3) has been studied as a neurotoxic agent^{2,3} and as an inhibitor of ribonuclease activity.⁴ In the last decade, polyamines isolated from siliceous cell walls of diatoms have been investigated by biologists, biochemists and materials scientists.

Diatom biosilica polyamines have been found in the free state and as a part of complex proteins called silaffins in diatom biosilica.⁵ Silaffins and polyamines are proposed to play a significant role in diatom biosilicification producing diverse nano-structured silica valves.⁵ Diatom polyamines include repeated *N*-methylpropylamine (PA) fragments terminated with methylated or non-methylated nitrogen (Scheme 1). The propylamines have been shown to promote silica formation *in vitro* in a tetramethoxysilane (TMOS) based system, although the only experimental information available ascribed to a catalytic effect is obtained from how much precipitable silica is generated during a fixed time period.⁵ The interest in such compounds is apparent from their potential in the biomimetic design of nano-structured materials *via* "green chemistry" approaches.

In this communication, we present the successful synthesis of a series of linear methylated propylamines that are analogous to the



Scheme 1 Structure of polyamines isolated from diatoms.

^aLimnological Institute of Siberian Branch of Russian Academy of Sciences, 3, Ulan-Batorskaya St., P.O. Box 4199, Irkutsk, 664033, Russia. E-mail: annenkov@lin.irk.ru.; Fax: 3952 425405; Tel: 3952 428422

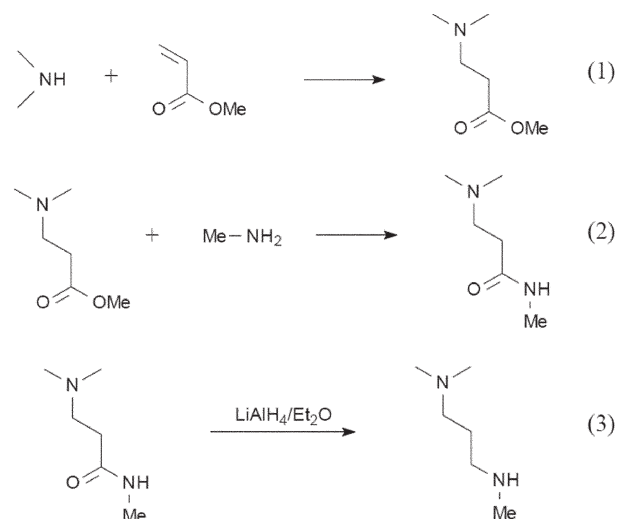
^bSchool of Biomedical & Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham, UK NG11 8NS. E-mail: Carole.Perry@ntu.ac.uk.; Fax: 0115 848 6636; Tel: 0115 848 6695

† Electronic supplementary information (ESI) available: detailed synthetic methods, NMR, MS and FTIR characterisation results and SEM of silica samples obtained in the presence of **N7**. See DOI: 10.1039/b515967a

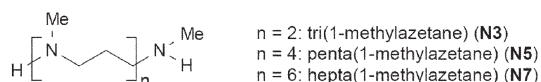
polyamine structural moieties isolated from several diatom species. The objective of this work is to establish a convenient universal method for propylamine synthesis capable of extending the *N*-methylpropylamine chain in a linear fashion. Furthermore, the activity of these PAs in silica formation *in vitro* is investigated. To our knowledge, this is the first investigation on the role of a series of synthetic methylated propylamines in silicification.

N3 (Scheme 3) is the longest synthetic methylated propylamine to have been synthesised previously. It can be prepared by the reaction of methylamine with 3-(*N*-methylamino)propionitrile in the presence of hydrogen and a hydrogenation catalyst.⁶ However, this reaction gives rise to a mixture of amines that needs to be separated to yield **N3** and does not allow the controlled synthesis of long-chain polyamines. Other non-methylated polyamines containing four and six nitrogen atoms have been obtained by reaction of trimethylene dibromide with trimethylenediamine or dipropylenetriamine.^{7,8} The reaction is complicated and a mixture of polymeric and branched products are synthesised requiring complicated separations. As the amine chain is increased, the likelihood of branching also increases as the relative concentration of end amino-groups decreases. Moreover, in the case of long-chain amines it will be practically impossible to separate a target linear product from polymeric and branched admixtures.

The proposed approach to target polyamines consists of repetitions of the following reactions: (1) condensation of *NH*-amine with methyl acrylate, (2) substitution of the resulting ester with methylamine and (3) reduction of the amide with lithium



Scheme 2 The consecutive stages of the polyamines synthesis.



Scheme 3 Structure of the obtained polyamines.

aluminium hydride (Scheme 2, reactions 1–3 respectively).[‡] We have obtained polyamines with 3, 5 and 7 nitrogen atoms starting from methylamine as in reaction (1). These compounds can be considered as derivatives of the cyclic monomer 1-methylazetane (Scheme 3). The propylamines were characterised by proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR) in a variety of solvents and by Fourier Transform Infrared Spectroscopy (FTIR).[†] Potentiometric titration (see below) and mass spectrometry data[†] are in good agreement with the molecular mass of the polyamines.

It has been demonstrated previously that small (bio)molecules (e.g. diamines, ethyleneamines, amino acids, etc.) “regulate” silicification.^{8–15} Propylamines isolated from diatom species have also shown promising prospects in generating tailored silicas.⁶ It is of great interest to us to understand how the synthetic propylamines interact with silicic acid. The effect of N3, N5 and N7 on orthosilicic acid condensation was studied using a silicon-catecholate complex as the silica precursor. To a supersaturated solution of silicic acid (30 mM), the propylamines were added maintaining the Si : N ratio to unity and the pH was maintained at neutral without addition of external buffers.

In the presence of all PAs under consideration, rapid precipitation was observed in <1 minute. N.B. the concentration of silicic acid used in this study (30 mM) is significantly lower than that used by others (100 mM)⁵ and hence rapid precipitation of silica is unexpected. The loss of silicic acid was monitored by the colorimetric molybdc blue assay and data are presented in Fig. 1. It is evident that the precipitation was accompanied by a rapid loss in silicic acid (from initial 30 mM to *ca.* 15 mM) when PAs were introduced into the system, thus suggesting a specific action of propylamines on silica formation. Although several other small- and poly-amines have been previously shown to promote silicic acid condensation and/or aggregation,^{9,11} a rapid drop in silicic acid concentration (in <60 seconds) as reported here has not been reported previously, even when higher silicic acid concentrations were used (~100 mM).¹⁶

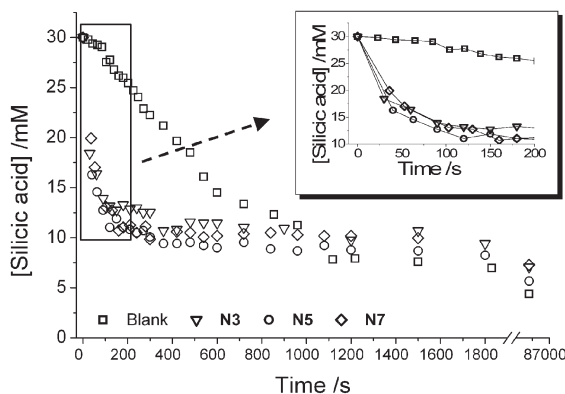


Fig. 1 Silicic acid condensation with or without added propylamines. Insert shows the initial stages of condensation.

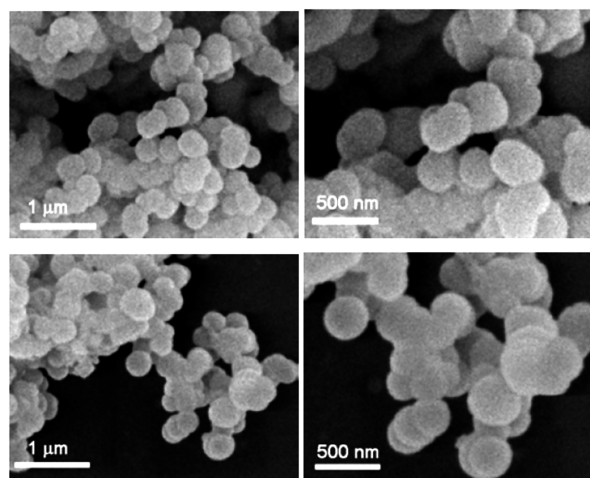


Fig. 2 Silica prepared in the presence of N3 (top) and N5 collected in 6 and 1 min respectively.

The precipitates were collected from as early as after one minute to seven days, washed and analysed by Scanning Electron Microscopy (SEM); representative data for N3 and N5 is presented in Fig. 2 (see ESI[†] for N7 data). The formation of spherical silica particles (~250–400 nm) was observed in the presence of all the PAs studied herein. Elemental analysis using Energy Dispersive Spectroscopy (EDS) revealed that the particles were composed of silicon and oxygen and hence eliminates the possibility that the precipitates are propylamine complexes (data not shown). Nitrogen gas adsorption carried out on selected samples showed that the silica particles possessed unusually low surface areas (<10 m² g^{−1}) and negligible pore volume (<0.01 cc g^{−1}) with most of the pores being below 5 Å in radius. In contrast, the earliest silica samples that could be isolated from the blank system (at half gel time = 18 h) were found to be porous with high surface area (~700 m² g^{−1}), high pore radius (~20 Å) and high pore volume (~0.3 cc g^{−1}).

To our knowledge, the rapid precipitation of non-porous silica particles from “pure” orthosilicic acid solutions has not been reported previously. It is noted that “dense” silica particles have been previously prepared when tetramethoxysilane (TMOS) was used as the silica precursor.^{13,17} However, we have shown previously that TMOS produces a mixture of monomer and oligomers upon prehydrolysis (with only 20% free silicic acid)¹² which produces non-porous silicas in the presence of additives such as peptides, synthetic polymers and small molecules (e.g. ethyleneamines).¹³ Hence our results are significant as we demonstrate the synthesis of dense silica from “pure” orthosilicic acid.

In order to understand how the propylamines might be interacting with the silica species present in solution, a preliminary investigation using modelling¹⁸ and potentiometric titrations on the protonated and unprotonated propylamine species under our experimental pH conditions was undertaken. Potentiometric data (Fig. 3) shows two inflections on the curves, one of them corresponds to full neutralisation of the amines and the other is near the point of protonation for alternate nitrogen atoms. This means that there are 25–30% amine groups which are unprotonated at pH 7. This is also confirmed from theoretical calculations

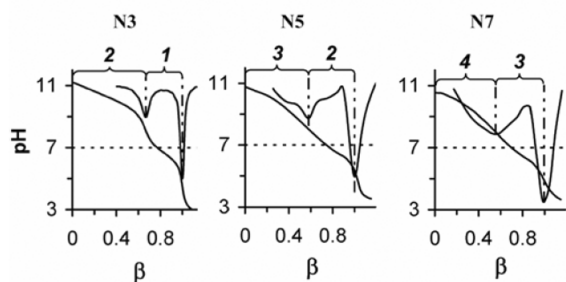


Fig. 3 Potentiometry titration curves for new propylamines (β = neutralization degree).

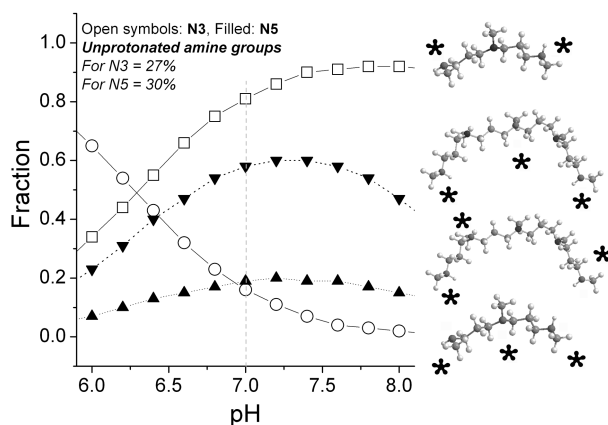


Fig. 4 Estimated speciation of N3 and N5 (only species present at significant fractions are shown). Models of propylamines are shown on the right of each curve. Charged nitrogen atoms are marked with asterisks.

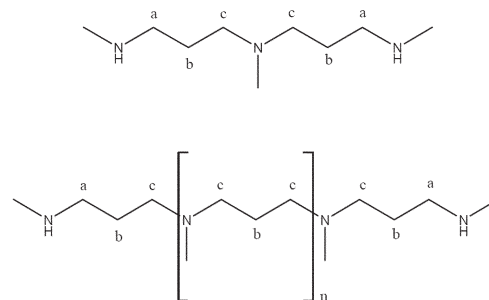
(Fig. 4). For example, Fig. 4 also shows the estimated speciation of N3 and N5 and respective molecular models. It appears that the rapid formation of dense silicas in the presence of PA arises from the fact that PAs are partially protonated at neutral pH with labile protons, thus enabling PAs to act as Brønsted acids, similar to the proposed activity for ethylamines.¹³ Future work is being undertaken in order to investigate in more details the role(s) of propylamines of a variety of architectures in silica formation and the data will be presented in due course. It is also possible to synthesise polyamines with an even number of nitrogen atoms from *N,N'*-dimethyl-1,3-propanediamine which is commercially available or can be obtained using a 1 : 1 ratio of reagents as in reaction (1). Sequential realisation of reactions (1)–(3) with various amines and acrylates opens up the way to a wide range of polyamines that will be evaluated in due course.

This work was supported by Presidium of the Russian Academy of Sciences (project # 10.3), The Royal Society short visit grant (for VVA), AFOSR and EU.

Notes and references

† Synthetic procedures are described briefly below, see ESI† for details on the synthetic procedures and characterisation of PAs. Condensation of NH-amines with methyl acrylate [reaction (1)] was carried out in ethanol solution (15% of the corresponding amine) at 1 : 2.1 amine : acrylate ratio. In the case of methylamine the reaction mixtures stayed at r.t. for 6 days

and for N3 and N5 amines were boiled for 6–10 hours. Reaction (2) was also performed in ethanol using a 50% excess of methylamine under ester groups, at r.t. during 3–5 days. The completions of reactions (1) and (2) were checked by FTIR spectroscopy by disappearance of NH band (3300 cm^{-1}) and ester C=O band (1740 cm^{-1}) respectively.



Yield of the target compounds was near 100%. Reduction of the amides [reaction (3)] was done by dropwise addition of the amide to LiAlH_4 suspension in diethyl ether (2.1 moles of LiAlH_4 per amide group) with 50–70% yield. The non-quantitative yield at this stage is connected with association of the resulting amine with lithium and aluminium hydroxides. One would hope that the yield would be enhanced in future optimization of the procedure. The obtained polyamines are colourless liquids, b.p.: 65°C , 142°C and 185°C at 0.2 mm Hg for N3, N5 and N7 respectively. FTIR (film, cm^{-1}): 3292–3296, 2939–2945, 2788, 2839, 1450–1465, 1373, 1315, 1150, 1122, 1068, 733–740. N3: ^1HMR , 5% in CDCl_3 , 1.63 ppm (5^H , 4H, $2 \times \text{CH}_2$ (b)), 2.15 (1^H , 3H (NMe)), 2.35 (3^H , 4H, $2 \times \text{CH}_2$ (c)), 2.40 (1^H , 6H, NHCH_3), 2.58 (3^H , 4H, (a)). ESI-MS +ve ion. 174.3 ($[\text{M} + \text{H}]^+$). N5 ($n = 2$): ^1HMR , 5% in CDCl_3 , 1.62 ppm (m^H , 8H (b)), 2.14 (1^H , 9H (NMe)), 2.28–2.35 (m^H , 12H (c)), 2.40 (1^H , 6H (NHMe)), 2.58 (m^H , 4H (a)). ESI-MS +ve ion. 316.4 ($[\text{M} + \text{H}]^+$), 245.4 ($[\text{M} + \text{H}]^+ - \text{C}_3\text{H}_5\text{NHMe}$), 174.3 ($[\text{M} + \text{H}]^+ - \text{C}_3\text{H}_5\text{N(Me)C}_3\text{H}_6\text{NHMe}$).

N7 ($n = 4$): $^1\text{H-NMR}$, 5% in CDCl_3 , 1.65 ppm (m^H , 12H (b)), 2.20 (1^H , 15H (NMe)), 2.30–2.40 (m^H , 20H (c)), 2.42 (1^H , 6H (NHMe)), 2.62 (3^H , 4H (a)).

ESI-MS +ve ion. 458.5 ($[\text{M} + \text{H}]^+$), 387.5 ($[\text{M} + \text{H}]^+ - \text{C}_3\text{H}_5\text{NHMe}$), 316.3 ($[\text{M} + \text{H}]^+ - \text{C}_3\text{H}_5\text{NMeC}_3\text{H}_6\text{NHMe}$).

- S. S. Cohen, *A guide to the polyamines*, Oxford University Press, New York, 1998.
- D. F. Brown, J. P. McGuirk, S. P. Larsen and S. D. Minter, *Physiol. Behav.*, 1991, **49**, 41.
- S. Levine, R. Sowinski and D. Nochlin, *Brain Res.*, 1982, **242**, 219.
- T. P. Karpetsky, K. K. Shriver and C. C. Levy, *Biochem. J.*, 1981, **193**, 325.
- M. Sumper and N. Kroger, *J. Mater. Chem.*, 2004, **14**, 2059–2065.
- J. A. Marsella and W. E. Starner, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 921.
- G. S. Whitby, N. Wellman, V. W. Floutz and H. L. Stephens, *Ind. Eng. Chem.*, 1950, **42**, 452.
- F. Noll, M. Sumper and N. Hampp, *Nano Lett.*, 2002, **2**, 91–95.
- T. Mizutani, H. Nagase, N. Fujiwara and H. Ogoshi, *Bull. Chem. Soc. Jpn.*, 1998, **71**, 2017–2022.
- L. Sudheendra and A. R. Raju, *Mater. Res. Bull.*, 2002, **37**, 151.
- T. Coradin, O. Durupthy and J. Livage, *Langmuir*, 2002, **18**, 2331–2336.
- D. Belton, G. Paine, S. V. Patwardhan and C. C. Perry, *J. Mater. Chem.*, 2004, **14**, 2231–2241.
- D. Belton, S. V. Patwardhan and C. C. Perry, *J. Mater. Chem.*, 2005, **15**, 4629–4638.
- D. Belton, S. V. Patwardhan and C. C. Perry, *Chem. Commun.*, 2005, 3475–3477.
- K. M. Roth, Y. Zhou, W. Yang and D. E. Morse, *J. Am. Chem. Soc.*, 2005, **127**, 325–330.
- S. V. Patwardhan, S. J. Clarson and C. C. Perry, *Chem. Commun.*, 2005, **9**, 1113–1121.
- D. Belton, S. V. Patwardhan and C. C. Perry, unpublished data.
- S. W. Karickhoff and L. A. Carreira, *SPARC On-Line Calculator*, 2005.