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Synthesis of an Enlarged Library of Dynamic DNA Activators with Oxime, Disulfide and Hydrazone Bridges

Javier Montenegro,[a, b] Eun-Kyoung Bang,[a] Naomi Sakai,[a] and Stefan Matile*[a]

Abstract: Dynamic amphiphiles have a "bridge" between their charged head and their hydrophobic tails. The presence of dynamic covalent bonds is of interest for differential and biosensing applications as well as for rapid access to the libraries needed to screen for gene delivery or cellular uptake of siRNA. However, efforts to develop libraries have so far concentrated on hydrazone bridges to monocationic heads. Here, we report synthesis efforts to enlarge this focused library with oxime and disulfide bridges and dynamic amphiphiles with more than one positive charge. Evaluation in fluorogenic vesicles reveals best activation of DNA as ion transporters by dynamic amphiphiles with dendritic scaffolds, doubly charged heads and four tails. Moreover, oximes, contrary to hydrazones, remain active under acidic conditions. Linear elongation of dendritic headgroups seems to cause increasing detergent effects and should therefore be avoided.

Keywords: amphiphiles · disulfides · DNA · hydrazones · ion transporters

Introduction

Dynamic amphiphiles have a charged head, a hydrophobic tail, and a dynamic connector or "bridge" (Figure 1).[1–4] The dynamic covalent bonds of bridges, for example, hydrazones, disulfides or oximes, are weaker than covalent bonds but stronger than noncovalent interactions, such as hydrogen bonds.[5,6] Because of the dynamic nature of their bridges, dynamic amphiphiles can be formed, modified and destroyed, in situ, and they can transform during function, depending on conditions. The unique characteristics of dynamic amphiphiles are of interest in the context of polyion–counterion transport systems.[1–4,7–10] In general, the movement of polyions across lipid bilayer membranes is strongly influenced by their counterions. Hydrophilic counterions are referred to as inactivators because they produce hydrophilic polyion–counterion complexes that do not interact with the membrane. Amphiphilic counterions are activators because they give hydrophobic polyion–counterion complexes that can move across bulk and bilayer membranes and act as carriers of hydrophilic counterions. Dynamic amphiphiles with positively charged heads can thus activate DNA or RNA as cation transporters in lipid bilayer membranes.[8,9] Negatively charged dynamic amphiphiles can activate cell-penetrating peptides (CPPs, or protein transduction domains, PTDs)[7,10] as anion transporters.[11]

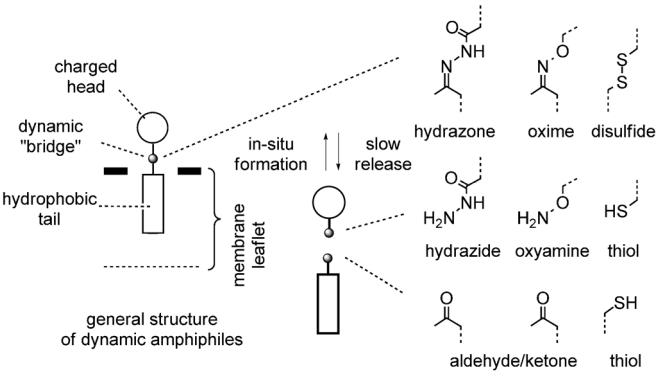

Figure 1. Dynamic amphiphiles are composed of charged heads and hydrophobic tails that are linked together by dynamic "bridges" that can form, change and break in situ in response to changing conditions. This report adds oximes and disulfides to the previously reported collection of hydrazone bridges.

In this context, dynamic amphiphiles offer several advantages. They were introduced originally to sense hydrophobic analytes with membrane-based sensing systems.[1,2] Early applications toward cholesterol biosensors focused on signal generation with cholesterol oxidase, covalent capture of the cholestenone product with an anionic hydrazide, and activation of CPPs with the obtained dynamic amphiphile.[2] However, attention soon shifted to the general challenge of differential sensing with membrane-based sensing systems.[1] Covalent capture of analytes with several different cationic

[a] Dr. J. Montenegro, Dr. E.-K. Bang, Dr. N. Sakai, Prof. S. Matile
Department of Organic Chemistry
University of Geneva, Geneva (Switzerland)
Fax: (+41) 22-379-3215
E-mail: stefan.matile@unige.ch
Homepage: www.unige.ch/sciences/chiorg/matile/

[b] Dr. J. Montenegro
Current address: Departamento de Química Orgánica
y Centro Singular de Investigación en Química Biológica
y Materiales Moleculares (CIQUS)
University of Santiago de Compostela
Santiago de Compostela (Spain)

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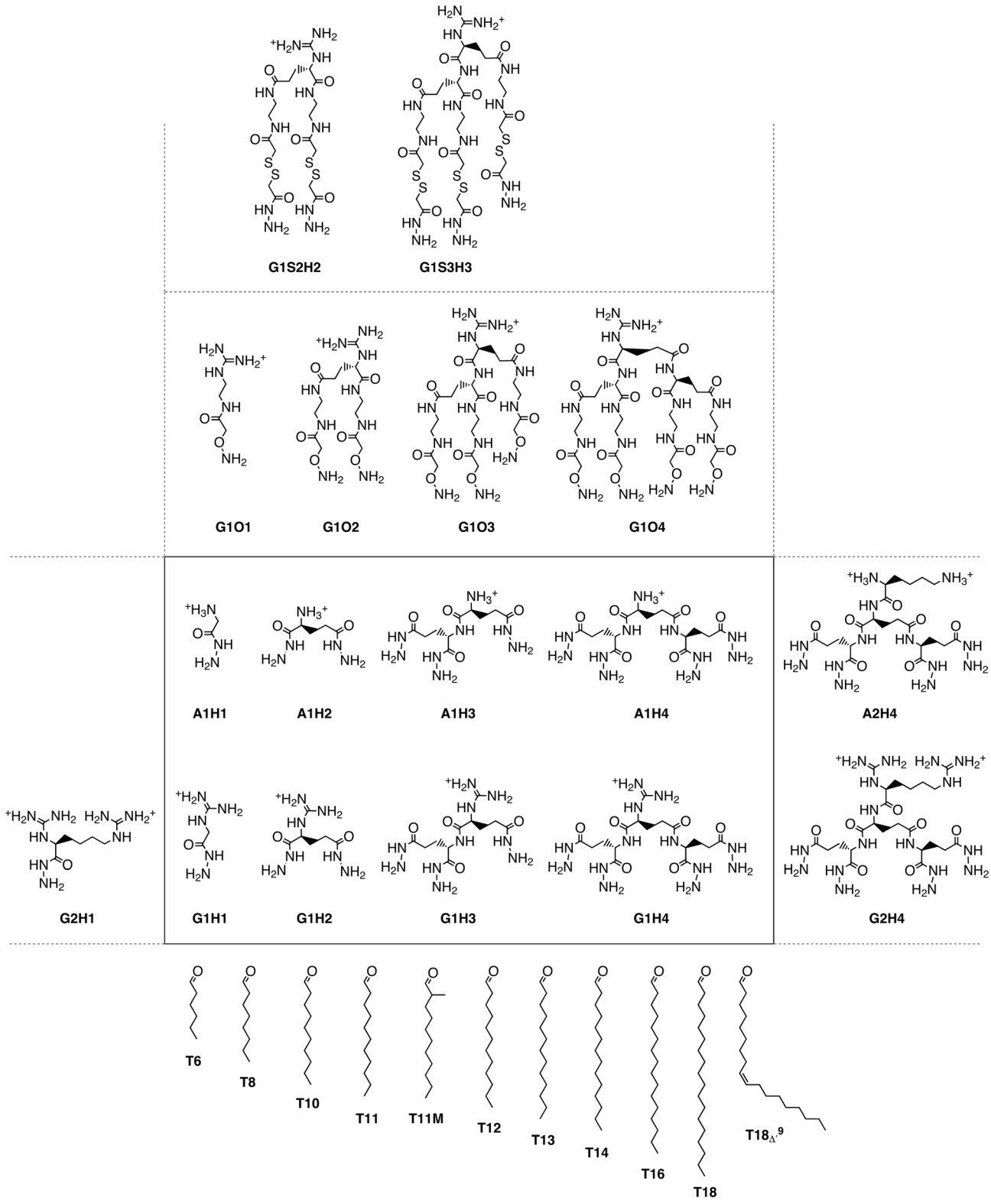


Figure 2. Library design for dynamic amphiphiles based on established heads (black box) and selected tails **T** with emphasis on oxime bridges, doubly bridged amphiphiles with disulfide and hydrazone bridges, and with doubly charged heads with as many or as few hydrazone bridges as possible.

hydrazides **A1H2**, **G1H2**, **A1H3** and **G1H3** gave the dynamic amphiphiles that activated DNA differently and thus could be used to generate patterns. Analysis of the obtained patterns by routine methods was found to discriminate odorants, including enantiomers, *cis–trans* isomers and single-atom homologues, as well as perfumes (Figure 2).[1]

For gene delivery or cellular uptake of siRNA,[4,9] dynamic amphiphiles are attractive because they provide facile access to libraries.[3] With rational design and predictions from model systems remaining problematic, the screening of large libraries appears to be a most promising approach to achieve progress in this field. So far, we have reported ten peptidic head-groups with one ammonium or guanidinium cation and one to six hydrazone tails (Figure 2).[1,3] Combined with 39 hydrophobic tails, this small collection has already given access to 390 dynamic amphiphiles without much effort spent on their synthesis. This focused library is currently being screened for siRNA uptake.[12] Here, we describe the expansion of this library toward the inclusion of oximes, disulfides as well as multiply charged head-groups. We report high activity for doubly charged amphiphiles and high stability of oximes under acidic conditions, whereas the linear elongation of dendritic heads needed to install oximes and disulfides leads to increasing interference from nonspecific detergent effects.

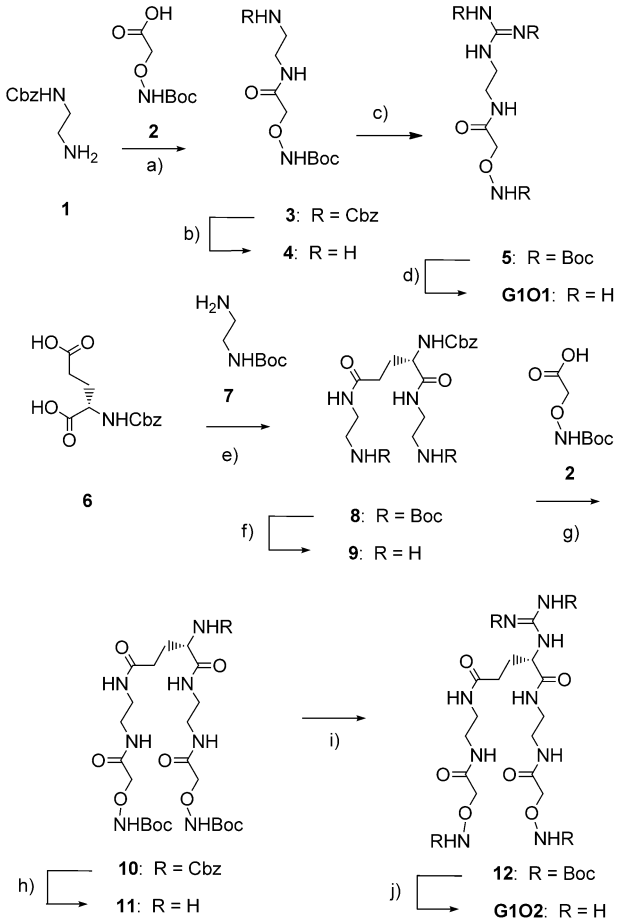
Results and Discussion

Design: The head-groups so far available for the construction of libraries of cationic amphiphiles contain an ammonium (**A**) or a guanidinium (**G**) cation and one (**A1H1**, **G1H1**) to six (**A1H6**, **G1H6**, not shown) hydrazone bridges.[1,3] DNA activation was always better with guanidinium than with ammonium cations. This known difference is attributed to the high pK_a of guanidinium cations (i.e., permanent charges),[7] the in-plane directionality of their hydrogen bonds, and the delocalization of their positive charge to possibly support arene-templated ion pairing. Activities increased with the number of tails until **G1H3**.[1] With **G1H4** and particularly **G1H6**, satisfactory to high activities were observed with shorter tails, whereas longer tails did not function well, presumably because the polyion–counterion complexes obtained with calf-thymus (ct) DNA became too hydrophobic.[3]

To expand our library based on these trends, four possibilities were considered. Substitution of the hydrazone bridges with the more stable, acid resistant oxime bridges[6] should reveal the impact of different stability of dynamic amphiphiles on DNA activation, sensing and cellular uptake. For these reasons, we decided to develop the oxime series with one guanidinium head and up to four tails, that is, from **G1O1** to **G1O4** (Figure 2). Dynamic amphiphiles with disulfide and hydrazone bridges should respond to both acid and reduction.[5] We decided to explore these doubly bridged amphiphiles first with the most informative combination of one guanidinium head and two (**G1S2H2**) to three tails (**G1S3H3**).

Multiply charged head-groups are very attractive because, driven by their need to minimize intramolecular charge repulsion, their ability to form inert intermolecular ion pairs should be exceptional (as long as their acidity is weak enough to avoid charge reduction by deprotonation).[7,13] Increasing the number of charges in the head-group should be most effective with four tails, in which case singly charged **G1H4** starts to lose activity because of being too hydrophobic. Guanidinium (**G2H4**) and ammonium cations (**A2H4**) were selected to modulate the proximity effects originating from intramolecular charge repulsion.[7] Single tail, multiply charged amphiphiles at the other end of the established structural space, that is, **G2H1** and higher homologues, will be of interest to handle unusual tails of exceptional hydrophobicity. **G2H1** and the **GnH1** family will be described elsewhere once all matching tails are made and studied.[12]

Synthesis: **G1O1** was readily synthesized from monoprotected ethylenediamine (**1**) and the protected alkoxyamine of glycolic acid (**2**; Scheme 1; see the Supporting Information). Standard peptide coupling conditions with TBTU as activating agent gave amide **3**, which was deprotected chemoselectively by hydrogenolysis. Guanidinylation of amine **4** with



Scheme 1. Synthesis of **G1O1** and **G1O2**: a) TBTU, DIEA, DCM, room temperature, 54%; b) Pd/C, H_2, MeOH, 1 M HCl in Et_2O, room temperature, 87%; c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 67%; d) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 71%; e) TBTU, DIEA, DCM, room temperature, 66%; f) Et_3SiH, TFA/DCM 1:1, room temperature, quantitative; g) TBTU, DIEA, DMF, room temperature, 52%; h) Pd/C, 1 M HCl in Et_2O, H_2, MeOH, room temperature, 89%; i) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 65%; j) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 72%.

N,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine gave the protected target molecule **5**, which was converted into **G1O1** under acidic conditions.

Multiaminoxy head-groups were all synthesized from glutamate. The synthesis of the simplest member of this family, **G1O2**, was carried out from amine-protected glutamate **6** (Scheme 1). Amide formation with partially Boc-protected ethylenediamine **7** gave diamide **8**. Boc removal with acid gave diamine **9**, which was treated with the aminoxy glycolate **2**. Tetraamide **10** was chemoselectively deprotected, and the resulting amine **11** was guanidinylated. Deprotection of the obtained product **12** gave the desired **G1O2**.

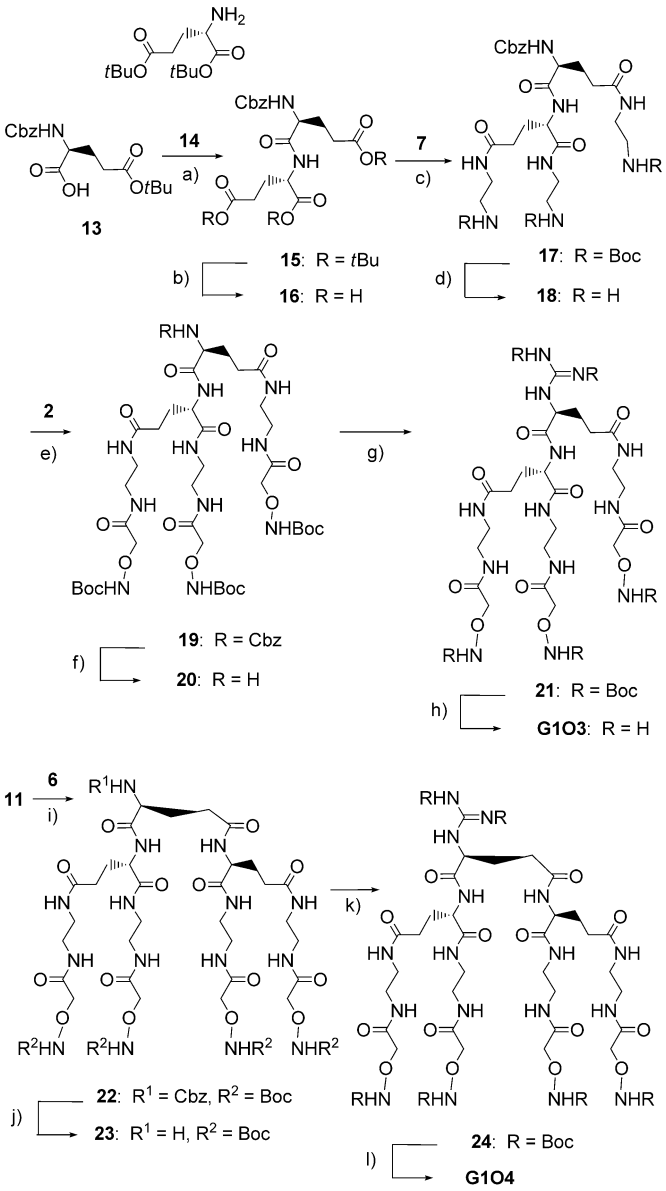
The minimalist peptide dendron[14] **G1O3** was prepared from glutamate derivatives **13** and **14** (Scheme 2). Selective deprotection of the coupling product **15** gave triacid **16**, which was treated with amine **7** to give triamide **17**. Boc removal prepared for the attachment of the aminoxy glycolate **2** to amine **18**. Selective deprotection of **19** by hydrogenolysis gave amine **20** in quantitative yield. Guanidinylation afforded **21**, which was deprotected to give the target molecule **G1O3**.

G1O4, the most complex peptide dendron of the series, could be prepared from intermediate **11**. Coupling to glutamate **6** afforded **22** with the desired four-tail scaffold. Selective deprotection followed by guanidinylation of **23** and final deprotection of **24** gave the desired target molecule.

The less demanding disulfide hydrazide **G1S2H2** was synthesized from glutamate **25** (Scheme 3). Coupling with monoprotected ethylenediamine **1** gave diamide **26**, which was deprotected selectively under acidic conditions. The obtained amine **27** was guanidinylated. Selective deprotection of diamide **28** by hydrogenolysis gave diamine **29**. To introduce the disulfide bridges, diacid **30** was partially coupled with the Boc-protected hydrazine **31**. The resulting acid **32** was treated with diamine **29**, and product **33** was fully deprotected to give the target molecule, **G1S2H2**.

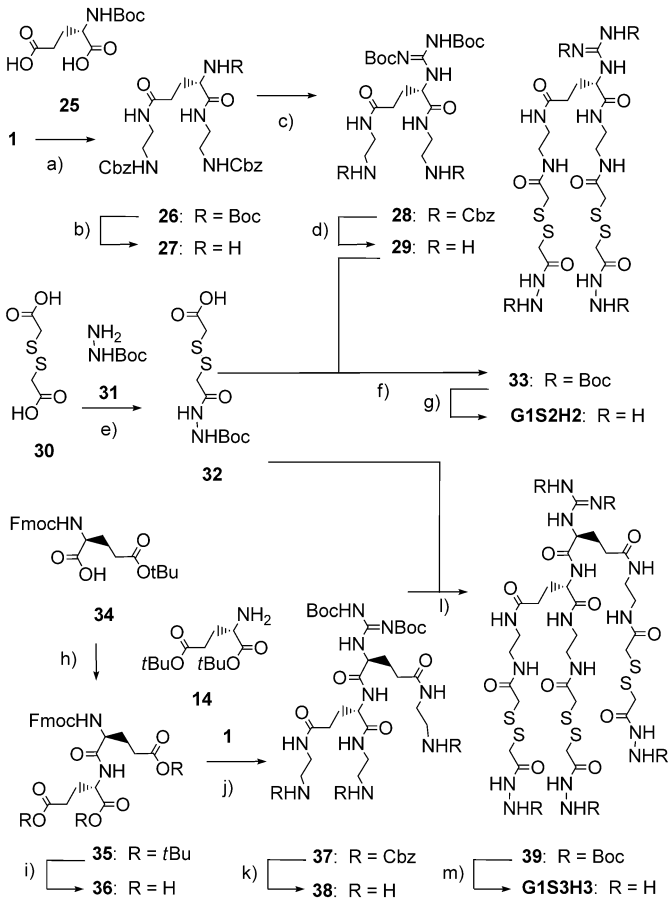
The higher homologue **G1S3H3** was synthesized analogously (Scheme 3). The glutamates **14** and **34** were coupled to give the branched minidendron **35**. Deprotection and "umpolung" of triacid **36** with monoprotected ethylenediamine (**1**) followed by deprotection and guanidinylation gave dendron **37**. To introduce disulfides and hydrazides together, the three amines were liberated chemoselectively for the amide coupling of **38** with acid **32**. Deprotection of the obtained product **39** gave the desired trihydrazide, **G1S3H3**.

The dicationic **G2H4** was readily accessible from intermediate **40** of the previously reported syntheses of **A1H4** (Scheme 4). Attachment of lysine **41** and liberation of the Cbz-protected amines in **42** gave diamine **43**. Guanidinylation and complete deprotection of product **44** gave the doubly charged target molecule **G2H4**. The diamine analogue **A2H4** was obtained from the same starting material (see the Supporting Information). The synthesis of **G2H1** will be described in the context of efforts to introduce more unusual tails from the materials sciences, including fullerenes, mesogens or fluorophiles, with multiply charged head-groups.

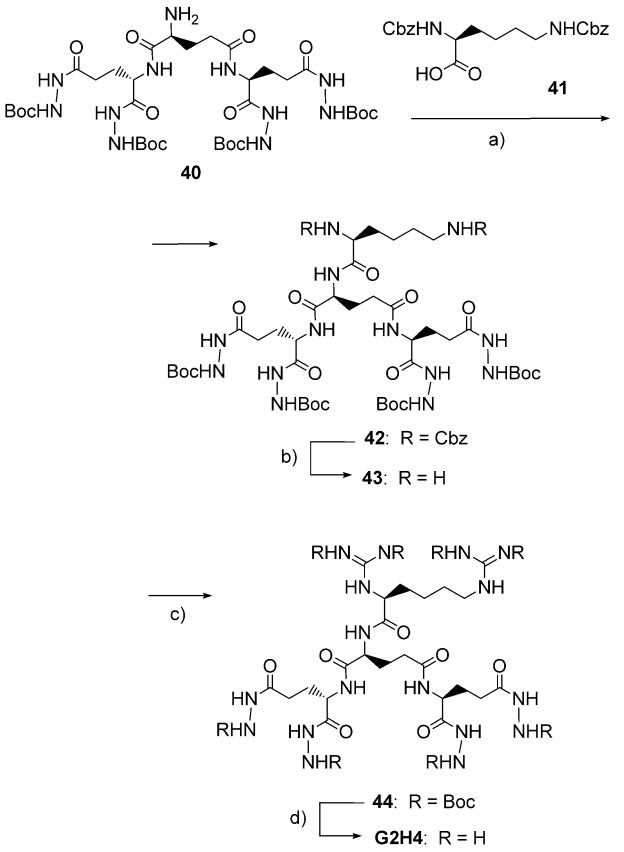


Scheme 2. Synthesis of **G1O3** and **G1O4**: a) TBTU, DIEA, DCM, room temperature, 79%; b) Et_3SiH, TFA/DCM 1:1, room temperature, 79%; c) TBTU, DIEA, DMF, room temperature, 61%; d) Et_3SiH, TFA/DCM 1:1, room temperature, 88%; e) TBTU, DIEA, DMF, room temperature, 59%; f) Pd/C, 1 M HCl in Et_2O, H_2, MeOH, room temperature, quantitative; g) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 66%; h) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 63%; i) TBTU, DIEA, DMF, room temperature, 49%; j) Pd/C, H_2, MeOH, 1 M HCl in Et_2O, room temperature, 93%; k) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 46%; l) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 63%.

Evaluation: The new head-groups were evaluated with linear alkyl tails of different length from six carbons in **T6** to eighteen carbons in **T18** plus the branched 2-methyl undecyl tail **T11M** and the kinked oleoyl tail $\mathbf{T18_{\Delta}^{9}}$ (Figure 2). Hydrazones and oximes were produced in situ by incubation for 1 h in DMSO at 60 °C. Product formation was confirmed



Scheme 3. Synthesis of **G1S2H2** and **G1S3H3**: a) TBTU, DIEA, DCM, room temperature, 72%; b) Et_3SiH, TFA/DCM 1:1, room temperature, 81%; c) *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 47%; d) Pd/C, H_2, MeOH, 1 M HCl in Et_2O, room temperature, quantitative; e) EDC, DIEA, DCM, room temperature, 15%; f) TBTU, DIEA, DMF, room temperature, 64%; g) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 85%; h) TBTU, DIEA, DCM, room temperature, 70%; i) Et_3SiH, TFA/DCM 1:1, room temperature, 86%; j) 1) TBTU, DIEA, **1**, DMF, room temperature, 2) DMF/DMSO/piperidine 8:2:3, room temperature, 3) *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, MeOH, room temperature, 30% (3 steps); k) Pd/C, 1 M HCl in Et_2O, H_2, MeOH, room temperature, quantitative; l) TBTU, DIEA, **32**, DCM, room temperature, 61%; m) 1 M HCl in H_2O, MeOH, 70 °C, 5 min, 67%.



Scheme 4. Synthesis of **G2H4**: a) TBTU, DIEA, DCM, room temperature, 4 h, 80%; b) $Pd(OH)_2$/C, H_2, MeOH, room temperature, 3 h, quantitative; c) *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN/H_2O 10:1, 55 °C, 12 h, 52%; d) 1 M HCl in Et_2O, DCM, 70 °C, 24 h, 61%.

in all cases by ESI MS (Tables S1 in the Supporting Information).

The activation of DNA as cation transporters by the new dynamic amphiphiles was evaluated under routine conditions.[1,3,8,13,15] In brief, egg yolk phosphatidylcholine large unilamellar vesicles (EYPC-LUVs) are loaded with the anionic 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) and the cationic *p*-xylene-bis-pyridinium bromide (DPX, Figure 3b). The addition of dynamic amphiphiles can then destroy the vesicles, converting the bilayer membranes into mixed micelles. In the HPTS/DPX assay, such detergent effects can be monitored as fluorescence recovery in the absence of DNA because release of the entrapped probes increases the average distance between fluorophores and quenchers. Alternatively, the dynamic amphiphiles can form membrane-active polyion–counterion complexes with DNA. Export of the DPX quenchers[8] or HPTS fluorophores by these polyion–counterion complexes is then reported as fluorescence recovery (Figure 3b).

In a typical experiment, the dynamic amphiphile was added first to the EYPC-LUVs⊃HPTS/DPX. Fluorescence recovery at this point demonstrates that the dynamic amphiphile is active also in the absence of DNA, and presumably acts as a detergent to destroy the lipid bilayer. In the absence of detergent effects, variation of the incubation time before addition of the DNA can be of use to determine the stability of the dynamic amphiphile under experimental conditions (see below). ctDNA was then added as representative polyanion transporter, and the increase in activity with increasing amphiphile concentration was recorded in dose-response curves (ctDNA without counterion activator is inactive, Figure S51a in the Supporting Information).[16] Dose-response curves were subjected to Hill analysis, and Y_{MAX}, which is the maximal accessible fractional activity under these conditions, the EC_{50}, which is the effective activator concentration needed to reach 50% of Y_{MAX}, and the Hill coefficient n were obtained (Figure S51b in the Supporting Information).

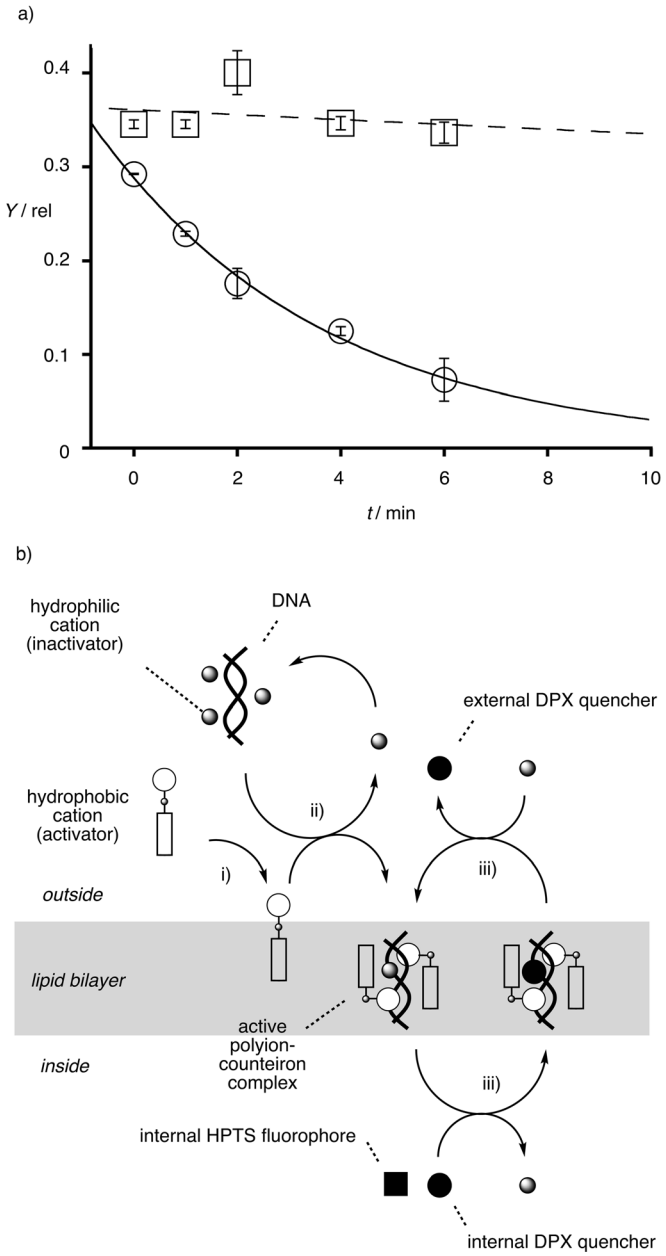


Figure 3. a) Dependence of the fractional activity Y of DNA as ion transporters in fluorogenic vesicles in the presence of **G1H1T18** (○) and **G1O1T18** (□) on the time of incubation of the amphiphiles at pH 5.5 prior to DNA addition. At pH 7.4, both amphiphiles act independently of incubation time. b) Design of vesicle experiments: i) addition of dynamic amphiphiles as counterion activators to vesicles with internal reporter ions (e.g., DPX, HPTS) possibly causes membrane destruction (detergent effects: D in Table 1; data not shown), or ii) the formation of membrane-active polyion–counterion complexes with DNA for iii) the export of the DPX quenchers and fluorescence recovery of the internal HPTS fluorophores.

The results for **T11M**, 2-methylundecanal, an ambergris odorant from kumquat peel oil used in perfumes, soaps and detergents, are summarized in Table 1 because they illustrate the identified trends very well. All dose-response curves and complete transport data for all head–tail combinations can be found in the Supporting Information (Figure S52 and Table S2). The best results were obtained for **G2H4** (Table 1, entry 7). The $Y_{MAX}=84.5(\pm9.4)\,\%$ was exceptional for this series. This high Y_{MAX} indicates that transport occurs in nearly all vesicles, excluding significant interference from inactivating processes, such as precipitation, irreversible partitioning, disappearance in the membrane core, amphiphile hydrolysis, and so on. At the same time, the $EC_{50}=1.4(\pm0.1)$ μM was very low. This is one of the best EC_{50} values ever seen for polyion–counterion complexes with satisfactory $Y_{MAX}>50\,\%$. Compared to **G1H2** with the same head/tail ratio,[1] **G2H4** was 14-times more active (Table 1, entries 7 and 9). This significant improvement was not achieved at the cost of reduced Y_{MAX}. Quite the contrary, the maximal fractional activities also increased from $Y_{MAX}=59.9(\pm2.4)\,\%$ for **G1H2** to $Y_{MAX}=84.5(\pm9.4)\,\%$ for **G2H4**. High Y_{MAX} with four **T11M** tails was also remarkable considering the strong decrease to unsatisfactory $Y_{MAX}=26.8(\pm0.3)\,\%$ reported for the three-tail **G1H3** (Table 1, entry 11).

Table 1. DNA activation data for dynamic amphiphiles with **T11M** tails.[a]

Cpd[b]	m/z[c]	Y_{MAX} [%][d]	EC_{50} [μM][e]	n[f]
G1O1	343	55.4±1.6	16.6±0.2	14.1±2.0
G1O2	753	D	D	D
G1O3	1163	46.5±5.2	3.1±0.7	2.5±1.5
G1O4	1574	22.5±1.0	1.4±0.1	4.6±2.1
G1S2H2	963	D	D	D
G1S3H3	1478	D	D	D
G2H4	1339	84.5±9.4	1.4±0.1	3.4±0.8
A2H4	1226	39.0±5.3	2.1±0.2	3.7±1.5
G1H2[g]	550	59.9±2.4	19.6±0.9	3.2±0.3
A1H2[g]	509	66.2±2.4	46.6±1.1	3.3±0.2
G1H3[g]	860	26.8±0.3	10.4±0.7	6.2±0.2
A1H3[g]	818	18.9±1.4	35.7±2.4	3.9±0.2

[a] From transport experiments in fluorogenic vesicles; see Figure S52 in the Supporting Information; D = detergent effects. Complete data for all tails can be found in the Supporting Information; [b] compounds, see Figure 2; [c] $[M+H]^+$ peak in the mass spectra of hydrazones and oximes formed with **T11M**; [d] maximal fractional activity of DNA as ion transporters at saturation with counterion activators; from Hill analysis; [e] effective counterion concentration needed to reach 50% of Y_{MAX}; from Hill analysis; [f] Hill coefficient; [g] from ref. [1].

The analogue **A2H4** with two ammonium heads and four **T11M** tails gave similarly outstanding $EC_{50}=2.1(\pm0.2)$ μM (Table 1, entry 8). However, this 22-fold improvement in effective concentration compared to **A1H2** came at the cost of a decreasing maximal fractional activity from $Y_{MAX}=66.2(\pm2.4)\,\%$ to less satisfactory $Y_{MAX}=39.0(\pm5.3)\,\%$ (Table 1, entries 8 and 10). This important decrease is contrary to the increase found with two guanidinium headgroups. It suggested that the proximity effects to strengthen ion pairing in weakly acidic guanidinium but not in more acidic ammonium cations are essential to reach perfect activity of dynamic amphiphiles, that is, minimal EC_{50} at maximal Y_{MAX}.

In the oxime series, decreasing EC_{50} with increasing number of tails coincided with decreasing Y_{MAX} (Table 1, en-

tries 1–4). Whereas the $EC_{50}=1.4(\pm0.1)$ μM of **G1O4** was as outstanding as that of **G2H4**, the $Y_{MAX}=22.1(\pm1.0)$% was insufficient and indicated marginal activity that is overshadowed by presumably much precipitation or irreversible disappearance in the bilayer core of the dynamic amphiphiles **G1O4T11M** as well as their DNA complexes (Table 1, entry 4).

The difference in stability of oxime and hydrazone bridges under acidic conditions was explored with **G1O1T18** and **G1H1T18**. With use of the same assay in fluorogenic vesicles at pH 5.5 instead of the usually maintained pH 7.4, the incubation time before addition of DNA and detection of activity was varied. With increasing incubation time, the ability of **G1H1T18** to activate DNA as ion transporters decreased rapidly (Figure 3a, ○). Exponential curve fitting suggested that the half-life of the hydrazone in **G1H1T18** is about 3 min at pH 5.5. The ability of **G1O1T18** to activate DNA as ion transporters did not decrease significantly during incubation at pH 5.5 (Figure 3a, □). The clear contrast between the stable **G1O1T18** and the unstable **G1H1T18** was in agreement with the expected lifetimes of oximes and hydrazones under acidic conditions.[6] Prolonged incubation of both amphiphiles at pH 7.4 did not reduce their activity significantly.

G1O2T11M and all other amphiphiles obtained from **G1O2**, except for the shorter **G1O2T8** and **G1O2T10**, were detergents, and showed membrane activity already in the absence of DNA (Table 1 and Table S2 in the Supporting Information). Nearly identical detergent effects were observed with the doubly bridged **G1S2H2**, whereas the original **G1H2** was not affected (Table 1 and Table S2 in the Supporting Information). Detergent effects were even worse with **G1S3H3**, whereas the homologous **G1O3** and **G1H3** acted reliably as activators of DNA as ion transporters (Table 1 and Table S2 in the Supporting Information). These trends suggested that not the nature of the bridges but the linear elongations of the original dendrons account for detergent effects.

Conclusion

The objective of this study was to expand a collection of dynamic amphiphiles based on observed trends and innovative chemistry. For this purpose, the existing hydrazone (**H**) bridges were complemented by oxime (**O**) and disulfide (**S**) bridges. Secondly, head-groups with two rather than one positive charge and amphiphiles with two rather than one bridge connecting head and tails were designed, synthesized and evaluated.

Lessons learned with singly charged hydrazone amphiphiles suggested the preparation of doubly charged amphiphiles with four tails. The obtained **G2H4** peptide dendrons with two guanidinium cations and four reactive hydrazides gave, when coupled in situ with hydrophobic aldehyde tails, the best dynamic activators of DNA as ion transporter in fluorogenic vesicles known so far, and low effective concentrations (EC_{50}) were reached without losses in maximal activity, Y_{MAX}, at saturation. Less satisfactory performances were obtained with diammonium analogues **A2H4**. They confirmed that proximity effects to minimize intramolecular charge repulsion by maximized intermolecular ion pairing account for the excellent activity of **G2H4**.[13]

Oxime (**O**) bridges were introduced in singly charged amphiphiles with one to four tails, and disulfide (**S**) bridges in singly charged amphiphiles with two or three tails. Compared to hydrazone bridges, oxime bridges provided the expected insensitivity to acidic conditions. However, the increasingly long linear elongations that had to be introduced between the dendritic heads and the bridges resulted in increasing detergent effects, particularly for the doubly bridged **G1S2H2** and **G1S3H3**. These detergent effects render the dynamic amphiphiles less useful for sensing applications in fluorogenic vesicles but do not exclude activity with regard to cellular uptake.

With eight new members added to the ten original head-groups and 39 tails tested so far, the expanded library provides formal access to 702 dynamic amphiphiles, and increasing the number of meaningful tails beyond 39 is absolutely unproblematic. Current efforts focus on the import of more unusual tails such as fullerenes, mesogenes or fluorophiles from the materials sciences to increase the probability to discover unexpected hits, and the growing library is being screened for gene and siRNA delivery. Preliminary results are encouraging, and confirm the construction and screening of expanded libraries of dynamic amphiphiles as a meaningful approach, because the current hits are: 1) competitive with the current delivery agents on the market, 2) unpredictable from model studies in vesicles, and 3) unpredictable from the literature.[12a] These results will be reported in due course.

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[13] Although ion pairs between multiply charged partners are stabilized thermodynamically but not kinetically (i.e., ion exchange remains fast), attention to intracellular release of oligonucleotides from multiply charged amphiphiles is indicated. However, this powerful proximity effect has been exploited successfully for gene delivery with cationic polymers, including CPP mimics (see ref. [10]), and preliminary results suggest that **G2H4** delivers siRNA most efficiently (unpublished).

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[16] Addition of DNA to membrane-active amphiphiles can either reduce or increase the velocity of fluorescence recovery, or have no influence at all. The formation of polyion–counterion complexes of cationic detergents with DNA should reduce their intrinsic activity. Preserved or increased activity thus suggests that this loss is overcompensated by the activity of polyion–counterion complexes, or that the formation of these complexes is hindered during membrane destruction.

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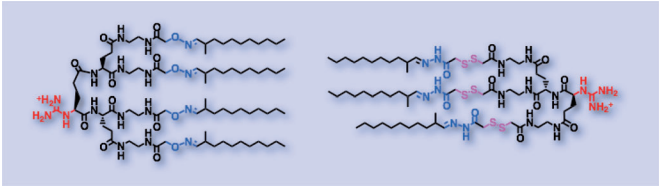

Head on: Design, synthesis and evaluation of dynamic amphiphiles with singly or doubly charged peptide dendrons as head-groups, hydrazones, oximes and disulfides as bridges, and with fragrant tails are reported. Amphiphiles with two charges and four tails (see figure) are identified as the most powerful activators of DNA as ion transporters and thus as the most promising family to screen for gene and siRNA delivery.

Amphiphiles

*J. Montenegro, E.-K. Bang, N. Sakai, S. Matile** ..... ▮▮▮▮–▮▮▮▮

Synthesis of an Enlarged Library of Dynamic DNA Activators with Oxime, Disulfide and Hydrazone Bridges