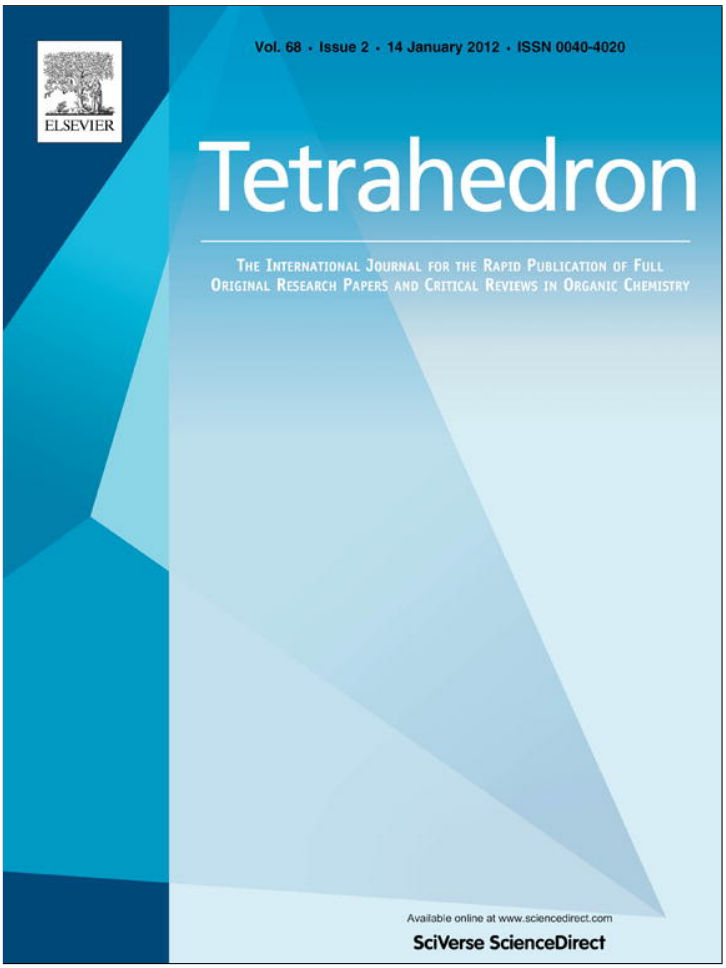

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Major increases of the reactivity and selectivity in aminoglycoside *O*-alkylation due to the presence of fluoride ions

Olivier Jackowski [a], Antoine Bussière [a], Cécile Vanhaverbeke [a], Isabelle Baussanne [a], Eric Peyrin [a], Marie-Paule Mingeot-Leclercq [b], Jean-Luc Décout [a,*]

[a] Université Joseph Fourier-Grenoble/CNRS, Département de Pharmacochimie Moléculaire UMR 5063, ICMG FR CNRS 2607, F-38041 Grenoble, France
[b] Université Catholique de Louvain, Louvain Drug Research Institute, LDRI, Unité de Pharmacologie Cellulaire et Moléculaire, FACM, Brussels, Belgium

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ABSTRACT

Major increases of the selectivity and/or reactivity in aminosugar and sugar *O*-alkylation were observed in the presence of tetrabutylammonium fluoride (TBAF) in comparison to TBAI under phase-transfer conditions or in solution. The presence of TBAF allowed the selective and rapid alkylation of the 6-hydroxyl function of neamine and efficient preparation of protected intermediates useful in synthesis and potent or potential antimicrobial *O*-alkylated derivatives of neamine and paromamine. In regard to the observed strong effects of TBAF, the alkylation and acylation of carbohydrates merit to be studied in the presence of TBAF under phase-transfer conditions.

1. Introduction

The aminoglycosides, such as neomycin B **1**, paromomycin **2**, kanamycin B **3** and amikacin **4** (Fig. 1) are pseudo-oligosaccharidic antibiotic drugs active against a broad range of microorganisms.[1] Two main classes of aminoglycosides can be defined in regard to the position of attachment of ring III to the 2-deoxystreptamine ring I (Fig. 1). Paromomycin **2** in which ring III is attached at the 5-position of ring I like in neomycin B **1** is an aminoglycoside of the neomycin class, whereas amikacin **4** in which ring III is linked at the 6-position of ring I like in kanamycin B **3** is an aminoglycoside of the kanamycin class.

At physiological pH, these polycations strongly bind to the bacterial 16S ribosomal RNA and then disturb bacterial protein synthesis.[1] The emergence of high level antibiotic resistances that involve enzymatic modifications of aminoglycosides, reduction of the intracellular concentration through surexpression of efflux pumps, alteration of the 16S ribosomal subunit target by

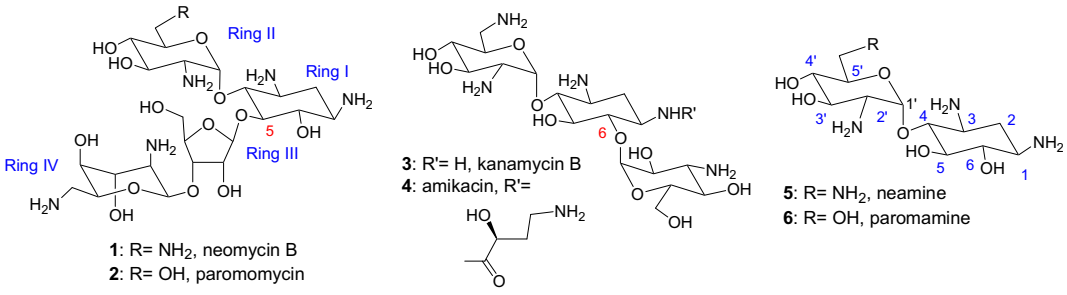

Fig. 1. Structure of antibiotic aminoglycosides and of neamine and paromamine.

* Corresponding author. Tel.: +33 4 76 63 53 17; fax: +33 4 76 63 52 98; e-mail address: Jean-Luc.Decout@ujf-grenoble.fr (J.-L. Décout).

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methylation of the aminoglycoside binding site[2] has led to a renaissance of interest in the search for antibiotic agents through chemical modifications of aminoglycosides.[1] Aminoglycosides, such as neomycin B (Fig. 1) also bind strongly to some HIV RNA sequences in vitro and have appeared to be promising as antiviral agents.[3] They showed other interesting properties since that lipophilic aminoglycoside derivatives were proved to be potent in DNA transfection in view of gene therapy[4] and found to allow, via binding to rRNA, the read-through of disease-causing nonsense mutations by the translation complex and therefore the synthesis of full-length active proteins.[5]

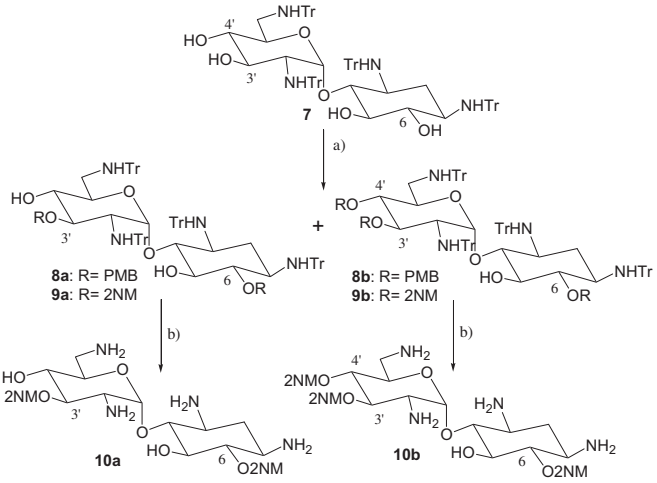
The neamine and paromamine cores incorporated in neomycin B and paromomycin (rings I and II, Fig. 1), respectively, are the main structural elements necessary for binding to the 16S subunit of ribosomal RNA.[6] Therefore, neamine **5**, which can be prepared by methanolysis of neomycin has appeared to be a very attractive building block in the search for antibiotic and antiviral agents and many works were developed to synthesise various derivatives mainly modified at the 5- or 6-position in regard to the parent classes of antibiotic aminoglycosides (neomycin class and kanamycin class, respectively).[1] Long procedures of protection and deprotection of the amine and hydroxyl functions have been generally used in order to alkylate neamine at position 6 for obtaining analogues of amikacin and kanamycins.[7]

In order to alkylate the hydroxyl functions of neamine and more complex aminoglycosides, such as neomycin B, Wong and coworkers inactivated the reactive amine functions by conversion to azido groups.[8a] In this fruitful approach[8b–d] used, for example, in the preparation of neomycin analogues and 5,5-neamine dimers, the main difficulties can be found in (i) the high cost of the triflic azide reagent necessary for the conversion of the amine to azide functions (excess of reagent and moderate yields), (ii) the low solubility of the starting azidosugars in apolar solvents and (iii) the lack of chromophore useful for easily monitoring the reaction progress (UV detection in TLC).

In regard to these difficulties, we have introduced trityl groups for protecting the amine functions in neamine (compound **7**, Scheme 1) in order to: (i) obtain intermediates highly soluble in apolar organic solvents, (ii) induce steric hindrance in the next *O*-alkylation steps and (iii) deprotect the alkylated derivatives obtained in mild acidic conditions (TFA).[9a] Using this approach, we have obtained in a nearly 50:50 mixture the *N*-tetratritylated 3′,6-diPMB (**8a**) and 3′,4′,6-triPMB (**8b**) neamine derivatives (Scheme 1), which were separated and selectively alkylated at the 4′- and/or 5-positions and then deprotected to lead for the first time to 4′-neamine derivatives, which bind strongly to the HIV-1 TAR RNA.[9a,b] **8b** was recently described as an intermediate in the preparation of a neamine thiol useful in dynamic combinatorial chemistry for the identification of ligands for a pre-mRNA sequence, which is involved in the onset of several tauopathies including dementia with Parkinsonism.[10]

From **8a** and **8b**, we conjugated peptide nucleic acids (PNA) at the 4′- or 5-position of the neamine core in order to target the HIV-1 TAR RNA through complementary base pairing.[9c,d] The PNA conjugates were able to strongly inhibit viral replication since the neamine core permits the cellular uptake, whereas the PNA alone was not taken up by the cells. We also showed that neamine derivatives carrying lipophilic chains at the 4′- or 5-position are efficient as gene vectors[9e] and in the separation of amino acid and nucleoside enantiomers by chiral ligand-exchange chromatography.[9f,g]

Amphiphilic aminoglycosides have also shown interesting antibacterial effects.[11] We used the previously developed route for the rapid preparation of amphiphilic 3′,6-(2-(naphthyl)methylene) (3′,6-di2NM) (**10a**) and 3′,4′,6-tri2NM (**10b**) neamines strongly active against wild-type and resistant strains of Gram (−) and/or Gram (+) bacteria (Scheme 1).[11h,i] These antibacterial compounds



Scheme 1. Previously reported alkylation of the tritylated derivative **7** with PMBCl and 2-naphthylmethylene bromide (2NMBr) in the presence of NaH in DMF.[9,11h] (a) RX, NaH, DMF, rt; (b) for **9a** and **9b**: TFA/DCM, anisole, 0 °C.

were obtained from compound **7** in two steps (Scheme 1) through alkylation with 2-methylnaphthalene bromide (2NMBr) in the presence of NaH in DMF leading mainly to an about 50:50 mixture of the tritylated 3′,6-2NM (**9a**) and 3′,4′,6-tri2NM (**9b**) derivatives, which were separated by chromatography on silica gel and deprotected with TFA.[11h]

In the search for bioactive aminoglycosides, the selective and rapid alkylation of aminoglycosides, such as neamine remains a challenge in regard to the number of carried hydroxyl groups. In the new approach of the neamine *O*-alkylation reported here, we were interested in the binding of fluoride ions to the hydroxyl functions of sugars in aprotic polar solvents described in the literature.

For example, the binding of KF to the OH functions of carbohydrates in DMSO-d_6 leads to breaking of intra- and intermolecular H-bonds as observed by ^{1}H and ^{13}C NMR spectrometry.[12a] TBAF improves the solubility of cellulose in DMSO and has a strong effect in its acetylation on the degree of substitution and the distribution of acetate groups.[12b,c]

The remarkable effects of organic and inorganic F^- as a base in synthesis have been reviewed.[13a] Such a basic character of fluorides in polar aprotic solvents has been used in a large range of reactions, such as alkylation, arylation, esterification, condensation and elimination…[13a–c] Fluoride ion assisted reactions are thought to occur via strong H-bonding of the fluoride ion (the electron donor) to the reactant protic molecule (the electron acceptor), with resulting enhancement of the nucleophilicity of the protic compound while at the same time the nucleophilicity of the fluoride is reduced.[13a] More recently, a complex in which F^- is coordinated by H-bonds to four *tert*-butanol molecules has been prepared from TBAF, crystallized and used as a highly effective fluorination agent.[14]

Here, we report on the study of the neamine alkylation in the presence of tetrabutylammonium fluoride (TBAF) in toluene. Toluene was chosen as an aprotic solvent in order to favour the H-bonding of fluorides to NH and OH groups of the neamine core that should modify the reactivity of the hydroxyl functions in the presence of a strong base. Under these conditions, the H-bonding of fluorides to OH groups should favour the deprotonation of particular hydroxyl functions and their reaction. Also, the H-bonding of fluorides to tritylated NH groups and/or to undeprotonated OH groups could induce strong conformational effects leading to a modulation of the reactivity. In the presence of aq NaOH under phase-transfer conditions (PTC) or in solution in the presence of NaH, the presence of TBAF in toluene should strongly increase the

reactivity of alkylation of carbohydrates, enhance their solubility and could improve the selectivity.

A remarkable shift in the selectivity and rate of *O*-alkylation of *N*-tetratritylated neamine **7** was observed under PTC and in solution and was used for a more efficient preparation of the tritylated key derivatives **8a** and **8b**. Analogues of the antibacterial neamine derivatives **10a** and **10b** and similar derivatives of paromamine **6** were also prepared.

We also show here that TBAF allows the straightforward preparation of 6-alkylated neamine derivatives, which was exploited in two different approaches: (i) the rapid access after deprotection to new potential antibacterial neamine derivative members of the kanamycin class of aminoglycosides agents and (ii) the selective preparation of potential antibacterial 3′,4′-dialkylated neamines[11h] from the tritylated 6-monoPMB neamine derivative obtained in good yield.

TBAF appeared to be a very interesting phase-transfer agent for the alkylation of neamine **5** and paromamine **6** and we also studied its effects in the benzylation of a glucose derivative.

2. Results and discussion

2.1. Alkylation of compound 7 with PMBCl in toluene and in the presence of TBAI or TBAF

In the search for a simple procedure to prepare in a large scale the 3′,6-diPMB (**8a**) and 3′,4′,6-triPMB (**8b**) neamine derivatives, which are key intermediates, the alkylation of **7** with *para*-methoxybenzyl chloride (PMBCl) in excess was studied under phase-transfer conditions (PTC) in the presence of aq NaOH at high concentration (30–50%) and TBAF or TBAI. The reactions were monitored by HPLC on C18 reversed phase.

At room temperature in the presence of 30% aq NaOH, TBAI (1.5 equiv) and PMBCl (3 equiv), the alkylation did not proceed (neither in the absence of TBAI) and appeared to be slow using 50% aq NaOH. In the presence of 50% aq NaOH at 50 °C, the conversion appeared to be limited by the decomposition of the TBA cation. However, using 30% aq NaOH, TBAI (1.5 equiv) and PMBCl (3 equiv) at 50 °C, the 3′,6-diPMB derivative **8a** (Scheme 2, method A) could be prepared in a good 72% yield (Table 1).

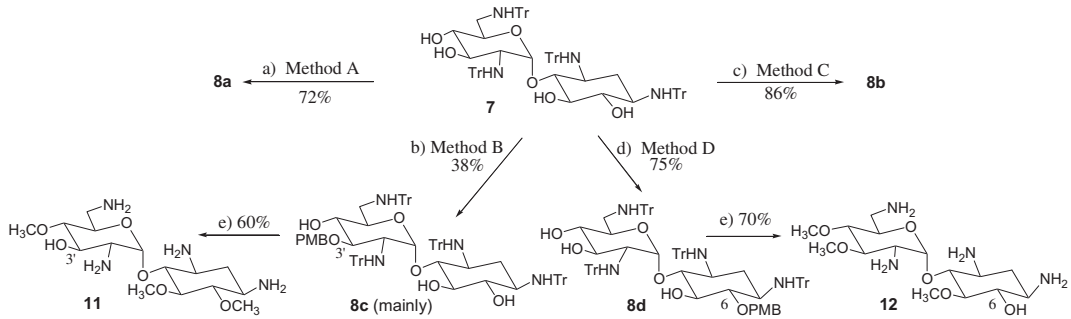
Table 1
Synthesis of the 3′,6-di- and 3′,4′,6-trialkylated neamine derivatives through PTC. Method A: TBAI (1.5 equiv), 30% aq NaOH/toluene, RX (3.6 equiv), 50 °C; method B: TBAF (1.5 equiv), 50% aq NaOH/toluene, RX (4 equiv), rt

Starting tritylated aminoglycoside	RX	Method A: 3′, 6-dialkylated compound, yield %	Method C: 3′,4′, 6-trialkylated compound, yield %
7	PMBCl	**8a**, 72 (+**8b**, 9)	**8b**, 86 (+**8a**, 6)
7	2NMBr	**9a**, 58 (+**9b**, 14)	**9b**, 55 (+**9a**, 28)
7	1-hexylBr	**13a**, 72 (+**13b**, 20)	**13b**, 60 (+**13a**, 20)
17	2NMBr	**18a**, 58 (+**18b**, 21)	**18b**, 57 (+**18a**, 30)

Remarkably, in the presence of TBAF (1.5 equiv) under PTC at room temperature (50% aq NaOH, 4 equiv PMBCl, method C), the alkylation occurred much more rapidly in comparison to the experiments conducted with TBAI (25 °C: $t_{1/2}$=2.5 min and 35 h, respectively; Fig. 2). The main product formed in the presence of an excess of PMBCl appeared to be the 3′,4′,6-tri-*O*-PMB derivative **8b**, which was isolated in a high 86% yield (Scheme 2, Table 1). It has been previously isolated in 32% yield using NaH/DMF.[9a] The yield was strongly increased in the presence of TBAF and the 3′,6-diPMB derivative **8a** was a minor product. The tetraPMB derivative appeared to be difficult to form in the presence of TBAF under PTC, whereas, in solution (NaH, DMF, excess PMBCl), it is rapidly formed. Probably, fluoride ions bind strongly to the 5-hydroxyl function in **8b** and prevent the corresponding alkylation.

By decreasing the amounts of PMBCl (2.5 equiv) and TBAF (1 equiv), it was possible to isolate in 75% yield the 6-monoPMB derivative **8d** (Scheme 2, method D), which was characterized through its 3′,4′,5-trimethylation and then deprotection to afford compound **12**. The HPLC analysis in the course of the alkylation showed that **8d** reacts to lead to **8b** but the corresponding reaction rate appeared to be slower than the rate of the first 6-monoalkylation step (kinetic profiles B in Fig. 2). Thus, remarkably, it was possible to prepare rapidly in good yield the 6-monoPMB derivative **8d**.

Therefore, the nature of the TBA halide affects strongly the course of the alkylation (Scheme 2): (i) in the presence of TBAI, the 3′-monoPMB (**8c**) and 3′,6′-diPMB (**8a**) derivatives can be prepared and the reaction is slow (preparation at 50 °C), (ii) in the presence



Scheme 2. Alkylation of the tritylated neamine derivative **7** under phase-transfer conditions in the presence of TBAI or TBAF. (a) Method A: TBAI (1.5 equiv), 30% aq NaOH/toluene, PMBCl (3.6 equiv), 50 °C, 24 h, 72%; (b) method B: TBAI (1.5 equiv), 30% aq NaOH/toluene, PMBCl (2 equiv), 50 °C, 4 days, 38%; (c) method C: TBAF (1.5 equiv), 50% aq NaOH/toluene, PMBCl (4 equiv), rt, 4 h, 86%; (d) method D: TBAF (1 equiv), 50% aq NaOH/toluene, PMBCl (2.5 equiv), rt, 1 h, 75%; (e) two steps: (i) NaH (6 equiv), MeI (3.5 equiv), DMF, rt, 2 h; (ii) TFA/CH_2Cl_2, anisole, rt; **11**: 60% and **12**: 70%.

Either in the presence of 30% and 50% aq NaOH, the 3′,4′,6-triPMB derivative **8b** was detected as a minor product (kinetic profiles A in Fig. 2). By decreasing the amount of PMBCl, it was possible to isolate the 3′-monoPMB derivative **8c** in 38% yield (Scheme 2, method B). HPLC analysis showed that the 3′-monoPMB product can be converted to the 3′,6-diPMB derivative **8a** by adding more PMBCl. The structure of **8c** was determined through NMR experiments with compound **11** obtained through 4′,5,6-tri-*O*-methylation (NaH/DMF, CH_3I) and deprotection with TFA (Scheme 2).

of TBAF, the 6-monoPMB (**8d**) and the 3′,4′,6-triPMB (**8b**) derivatives can be prepared in high yields and (iii) the presence of fluorides induces the drastic increase of the reaction rate in comparison to iodides especially the rate of the first monoalkylation step (Fig. 2).

The reactions of **7** with PMBCl were also studied by HPLC in toluene using NaH as a base in the presence of TBAF or TBAI and in the absence of TBA halide (Supplementary data). The rate and the selectivity of the reactions in toluene were compared to those

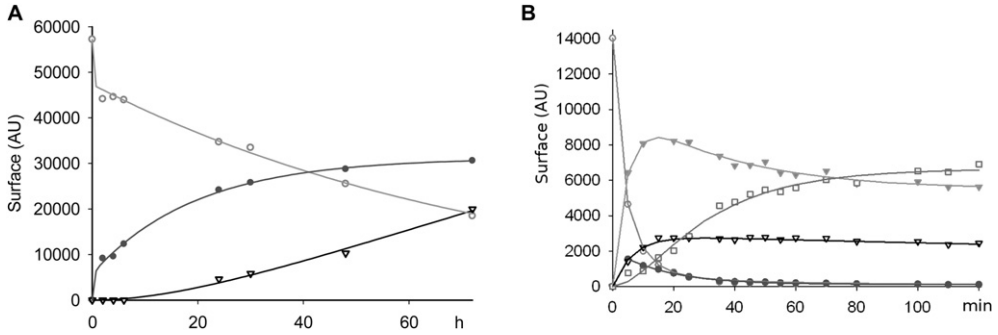
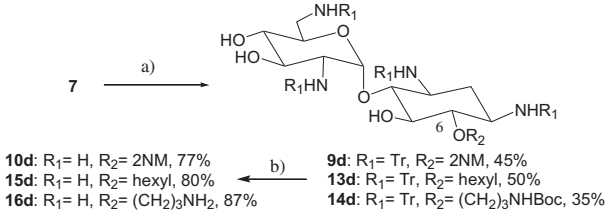


Fig. 2. Kinetic profiles of alkylation of compound **7** with PMBCl (4 equiv) at 25 °C under phase-transfer conditions (PTC) with 50% aq NaOH/toluene. Surfaces of peaks in arbitrary units (254 nm) as a function of time: **7** (○), 3′-monoPMB **8c** (●), 6-monoPMB **8d** (▼), 3′,6-diPMB **8a** (▽), 3′,4′,6-triPMB **8b** (□).A: TBAI (1.5 equiv)B; TBAF (1.5 equiv).

observed in DMF. In DMF, in the absence or in the presence of TBAI or TBAF, the reactions appeared to be rapid and unselective. A mixture of 6-mono- (**8c**), 3′,6-di- (**8a**), 3′,4′,6-tri-PMB (**8b**) and also tetraPMB derivatives was rapidly formed. In toluene, the results obtained were similar to those obtained under PTC with 50% aq NaOH: (i) no reaction in the absence of TBA halide, (ii) in the presence of TBA halide, slower reactions than in DMF and much stronger increase of the reaction rate in the presence of TBAF in comparison to TBAI, especially of the monoalkylation step with the same selectivity leading mainly to **8b** with TBAF and **8a** with TBAI.

2.2. Preparation of 6-monoalkylated neamine derivatives analogues of kanamycin B

Previously, we have reported the obtention of tritylated 6-monoalkylated neamine derivatives in low yields (10–20%) through reaction of **7** with NaH in DMF/THF (50:50), for example, the 6-mono2NM neamine derivative **9d** (Scheme 3, method D) has been obtained in 12% yield.[11h]



Scheme 3. Alkylation of **7** under phase-transfer conditions in the presence of TBAF and preparation of kanamycin analogues. (a) Method D: TBAF, 50% aq NaOH/toluene, RX, rt; (b) TFA/CH_2Cl_2, anisole.

In regard to the selective obtention of the tritylated 6-monoPMB derivative **8d** in 75% yield from **7**, **9d** was also prepared from **7** and 2NMBr under PTC in the presence of TBAF and was isolated in 43% yield.

It appears to be possible to alkylate selectively **7** in toluene under PTC at room temperature in the presence of TBAF (1.5 equiv) with the non-benzylic alkylating agents 1-bromohexane and *N*-(*tert*-butoxycarbonyl)-3-bromopropylamine. The new 6-alkylated neamine derivatives **13d** and **14d** (Scheme 3) were obtained in 50 and 35% yields, respectively. The reaction appeared to be totally regioselective, it was limited by an unexplained stop in the conversion leading to a mixture of only the starting derivative **7** and the 6-alkylated product, which were easily separated. Addition of TBAF and/or alkylbromide and/or increase of the temperature were inefficient to improve the conversion ratios.

The tritylated 6-monoalkylated products **13d** and **14d** were treated with TFA/anisole for obtaining compounds **15d** and **16d** in 80% and 87% yields, respectively (Scheme 3). This method constitutes a very short route for preparing 6-alkylated neamine derivatives in the search for antibacterial agents as analogues of the natural antibiotic aminoglycosides kanamycins.[7] Evaluated against Gram (+) and Gram (−) bacteria, the 6-(3-aminopropyl) neamine derivative **16d** showed an interesting minimum inhibitory concentration (MIC) of 4 μg/mL against wild-type *Staphylococcus aureus* ATCC 25923.

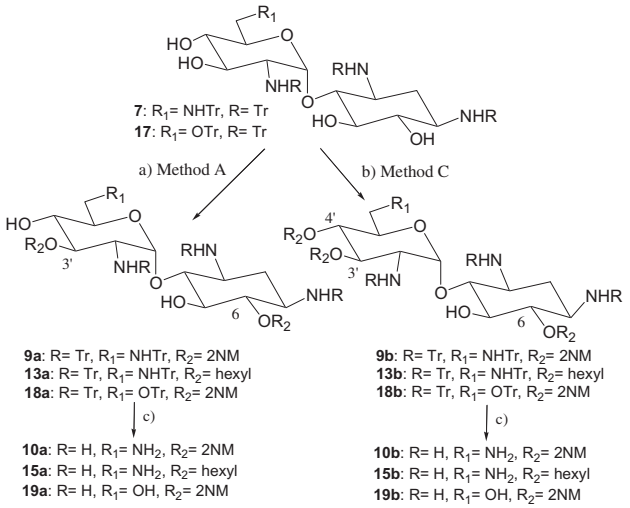
2.3. Alkylation of compound 7 with PMBCl and TBA hydrogen sulfate ($TBAHSO_4$) in toluene under PTC

TBA hydrogen sulfate is used as a phase-transfer agent[17a,b] for instance in the alkylation of sugars[17b] and therefore we compared by HPLC the rates of the *para*-methoxybenzylation of the tetratritylated neamine derivative **7** under PTC at room temperature with $TBAHSO_4$ and TBAF (1.5 equiv of phase-transfer agent, 4 equiv PMBCl). The rate of the first alkylation step appeared to be much lower with $TBAHSO_4$ than with TBAF (in the disappearance of **7** at 25 °C $t_{1/2} \approx 6$ h and 3 min, respectively). First, the 3′-monoPMB derivative **8c** was mainly formed like with TBAI but less selectively due to the concomitant formation of the 6-isomer **8d** and 3′,6-diPMB derivative **8a**. However with $TBAHSO_4$, after the monoalkylation step, which appeared to be the rate limiting step, the 3′,4′,6-triPMB derivative **8b** was mainly formed at room temperature after 24 h and was isolated (50% yield). This result confirms the great interest of TBAF for preparing the 6-monoPMB derivative **8d** and to strongly increase the rate of the alkylation leading to the 3′,4′,6-triPMB derivative **8b**.

2.4. Preparation of new 3′,6-di- and 3′,4′,6-tri-alkylated neamine and paromamine derivatives in toluene in the presence of TBAF or TBAI

In regard to the antimicrobial effects of **10a** and **10b**,[11h,i] their synthesis was achieved under PTC. In the presence of TBAF, the tritylated 3′,4′,6-2NM neamine intermediate **9b** previously obtained in 38% yield[11h] was prepared in 55% yield from **7** and 2NMBr (Scheme 4, method C, Table 1). The 3′,6-di2NM derivative **9a** was also isolated in 28% yield. Here, alkylation with 2NMBr more reactive than PMBCl is less selective. In addition, a side reaction of 2NM bromide with TBAF leading to 2NM fluoride was observed by ^{1}H NMR under the conditions used. In the presence of TBAI, **9a** and **9b** were isolated in 58% and 14% yields, respectively (Scheme 4, method A, Table 1).

The reaction of **7** with 1-bromohexane in the presence of TBAI at 50 °C under PTC (30% aq NaOH/toluene) led to the 3′,6-dihexyl derivative **13a** in 68% yield and to the 3′,4′,6-trihexyl derivative **13b** in 20% yield (Scheme 4, method A, Table 1). As expected with TBAF under PTC at room temperature (50% aq NaOH), the 3′,4′,6-



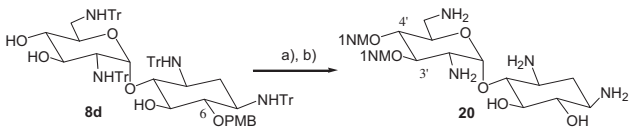
Scheme 4. Synthesis of the 3′,6-di- and 3′,4′,6-trialkylated neamine derivatives through PTC. (a) Method A: TBAI (1.5 equiv), 30% aq NaOH/toluene, R_2X (3.6 equiv), 50 °C, 24 h; (b) method C: TBAF (1.5 equiv), 50% aq NaOH/toluene, R_2X (4 equiv), rt, 24 h; (c) TFA/CH_2Cl_2, anisole.

trihexyl derivative **13b** was obtained in 60% yield with isolation of **13a** in 20%. The deprotection of **13a** and **13b** in TFA led to **15a** and **15b**, respectively.

This methodology was applied to the *N*-tetratritylated derivative of paromamine **17** (Scheme 4, Table 1), which was treated with 2NMBr under PTC. In the presence of TBAF at room temperature, alkylation of **17** led to the tritylated 3′,6-di2NM (**18a**, 30%) and 3′,4′,6-tri2NM (**18b**, 57%) derivatives, which gave separately after treatment with TFA **19a** and **19b**. In the presence of TBAI at 50 °C, **18a** and **18b** were obtained in 58 and 21% yields, respectively.

2.5. An access to 3′,4′-dialkylated neamine derivatives

In another illustration of the methodology described here, the tritylated 6-monoPMB derivative **8d** was alkylated with 1NM chloride under PTC in the presence of TBAF to lead to the 3′,4′-di1NM-6-PMB derivative (61%, Scheme 5). After deprotection in TFA, the 3′,4′-di1NM derivative **20** was obtained in good 60% yield in regard to its sensitivity to acidic conditions. The antimicrobial activity of **20** against *S. aureus* strains will be interesting to compare to the strong antibacterial effects observed with its 3′,4′-di2NM isomer obtained previously in 51% yield through the careful treatment with TFA of the 3′,4′,6-tri2NM derivative **10b**.[11h]



Scheme 5. Preparation of the 3′,4′-di1NM neamine derivative **20** from the 6-monoPMB neamine derivative **8d** under PTC with TBAF as a phase-transfer agent. (a) TBAF (2 equiv), 50% aq NaOH/toluene, 1NMCl (3 equiv), rt, 5 h, 61%; (b) TFA/CH_2Cl_2, anisole, 0 °C, 60%.

2.6. Study by NMR spectrometry of the binding of compound 7 to fluorides

In order to confirm that the observed strong effects of TBAF on the rate and selectivity of alkylation of compound **7** are related to the H-binding of F^- to the neamine core, we investigated by NMR spectrometry the expected interactions between fluoride ions and compound **7**.

The NMR study was performed under phase-transfer conditions with 50% aq NaOH/toluene-d_8/TBAF, in the absence of the alkylating agent at the concentrations and [TBAF]/[**7**] molar ratio used in the performed synthesis (method C). The toluene phase was analyzed after decantation.

In the presence of **7**, the signal corresponding to the TBA cations were detected in toluene-d_8 by ^{1}H NMR but were not observed in the absence of **7**. The presence of the corresponding fluoride ions in the toluene phase could not be directly observed by ^{19}F NMR. However, their presence was confirmed after extraction of the toluene phase with water and analysis by ^{19}F NMR of the resulting aqueous phase (signal at −120 ppm). The fluoride ions in toluene in equilibria with 50% aq NaOH appeared difficult to be detected by ^{19}F NMR spectrometry neither in the presence nor in the absence of **7** (very weak and large signals).

Clearly, these experiments demonstrate that the presence of fluorides and TBA^+ in toluene is related to the presence of compound **7** and suggest the strong TBAF-**7** affinity.

To get more information about the aminoglycoside-TBAF binding, titrations were performed in toluene by ^{19}F and ^{1}H NMR spectrometry.

The titration of **7** by TBAF in toluene-d_8 showed the progressive appearance of three ^{19}F NMR signals, a broad signal at around 120 ppm and two narrow minor signals at nearly −126 and −74 ppm, the latter being very weak (Fig. 3). The intensity of the two main signals increased with the TBAF concentration and the broad signal appeared to be progressively and strongly shifted downfield from −121 to −116 ppm with the increase of the TBAF/**7** molar ratio from 0.2 to 2, respectively. This broad signal was assigned to the free fluorides according to the broad signal detected at nearly −114 ppm for TBAF alone in toluene-d_8.

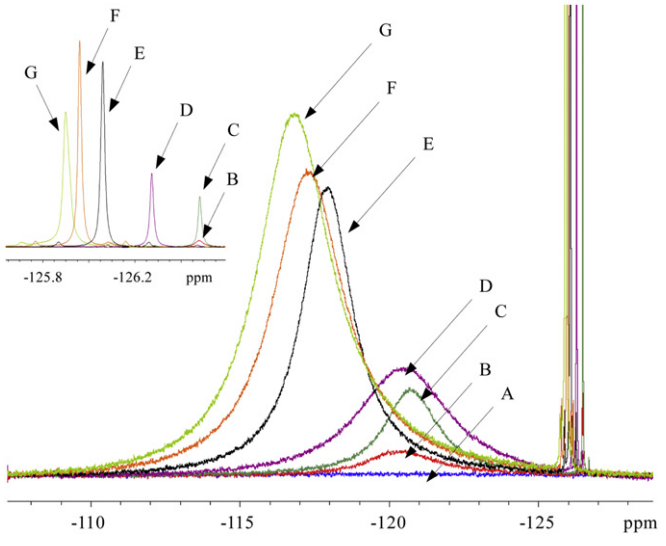


Fig. 3. ^{19}F NMR spectra (282 MHz, 28 °C) of **7** (A) at a concentration of 77 mM in toluene-d_8 and upon titration by a 0.386 M TBAF solution in toluene-d_8, at the molar ratios ([TBAF]/[**7**]) of 0.2 (B), 0.5 (C), 1 (D), 1.25 (E), 1.5 (F) and 2 (G). Inset: enlargement of the region between −126.6 and −125.6 ppm.

With the increase of TBAF/**7** ratio, the major sharp signal increased in intensity and was progressively weakly shifted downfield (Fig. 3). It was assigned to the fluoride ions bound to **7**. The titration of TBAF by **7** in toluene-d_8 led to the same conclusion. In regard to the shape of the signals and their varying positions, it appeared difficult to quantify the fluoride binding to **7**.

The interactions between F^- and **7** were also confirmed upon the titration by ^{1}H NMR spectrometry (Figs. 4 and 5). Most of proton signals of **7** were strongly shifted by fluoride addition. The larger

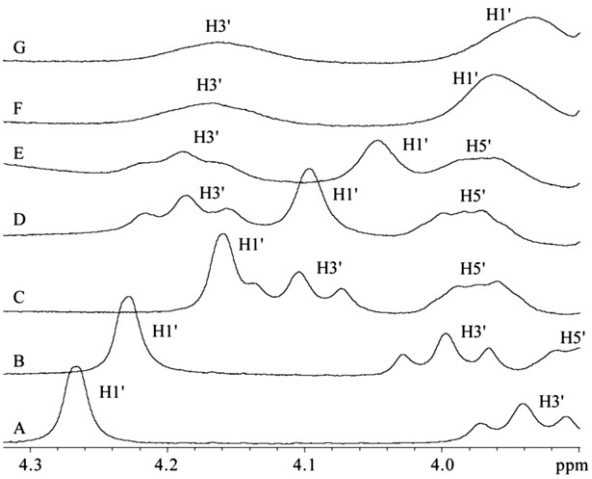


Fig. 4. ^{1}H NMR spectra (300 MHz, 28 °C) of **7** (A) at a concentration of 77 mM in toluene-d_8 and upon titration by a 0.386 M TBAF solution in toluene-d_8, at the molar ratios ([TBAF]/[**7**]) of 0.2 (B), 0.5 (C), 1 (D), 1.25 (E), 1.5 (F) and 2 (G). Only the region from 3.8 to 4.3 ppm is represented.

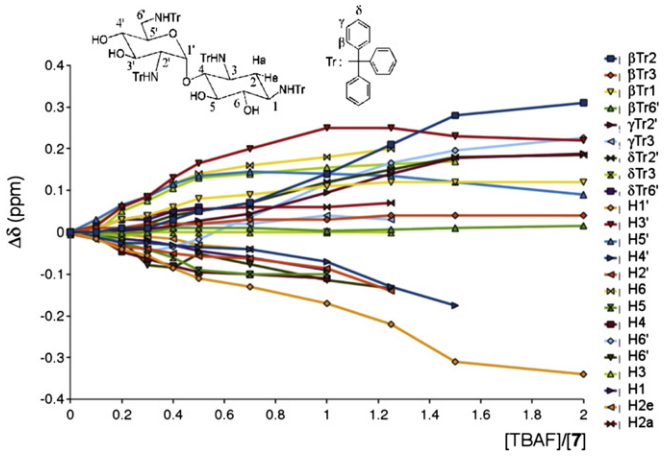


Fig. 5. Variations of the ^{1}H NMR chemical shifts of **7** at a concentration of 77 mM in toluene-d_8 upon titration by a 0.386 M TBAF solution in toluene-d_8. For each proton, the difference ($\Delta\delta$) between the chemical shifts in presence and absence of TBAF is displayed as a function of the molar ratios ([TBAF]/[**7**]).

downfield shifts were observed for H3, H6, H3′, H5′ and for the trityl protons attached to the 2′-nitrogen atom, whereas upfield shifts were detected for H1′, H4 and H5.

These experiments suggest that in toluene fluoride ions bind strongly to the neamine core probably through the formation of hydrogen bonds with the hydroxyl and the amine functions. However, the corresponding signals of the hydroxyl functions could not be identified in the complex ^{1}H NMR spectra observed.

2.7. Alkylation of a monosaccharide in toluene in the presence of TBAI or TBAF

The 6-*O*-tritylated methylglucopyranoside **21** (Fig. 6) was prepared for alkylation under PTC (50% aq NaOH/toluene) in the presence of TBAF or TBAI. Compound **21** appeared to be insoluble in toluene and remarkably the addition of TBAF (3 equiv) allowed dissolution (Fig. 6). The dissolution of **21** appeared to be complete with 3 equiv of TBAF that corresponds to one fluoride ion for one hydroxyl group. In the presence of benzyl bromide (4 equiv) at room temperature, a rapid and complete benzylation was observed to lead to the tribenzyl derivative **22** (Fig. 6) in 70% yield. The rate of benzylation appeared to be strongly increased by TBAF addition in

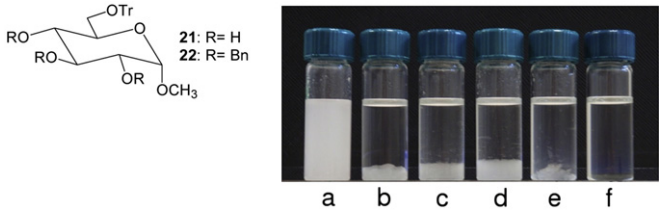


Fig. 6. Dissolution of **21** (75.7 mmol) during progressive addition of TBAF in toluene. (a): starting mixture; from (b) to (f): mixtures of 21 and TBAF at ([TBAF]/[**21**]) molar ratios of 0.5, 1, 1.5, 2.5 and 3, respectively.

comparison to TBAI (Supplementary data) in the presence of which **21** appeared to be weakly soluble (3 equiv). Any regioselectivity was observed as well as in the *para*-methoxybenzylation of **21**.

The observed stoichiometric solubilization of **21** confirms the strong binding of fluorides to the hydroxyl groups of carbohydrates in toluene.

2.8. First results obtained with another aminoglycoside: *O*-alkylation of ribostamycin 23 (see Supplementary data)

In order to *O*-alkylate ribostamycin **23** made of rings I, II and III of neomycin B **1** (Fig. 7), pentatritylribostamycin **25** was prepared by tritylation of the free base. The alkylation of **25** with 1NMCl (6 equiv) under PTC led mainly after detritylation with TFA to an *O*-tetra1NM derivative in the presence of TBAF, whereas in the presence of TBAI a mono1NM derivative **24** was isolated. The 1NM group in the latter compound appeared to be carried by the 2″-oxygen atom of the ribose ring. In regard to the lack of antibacterial activity of **24**, we did not continue in this approach. These preliminary experiments again point out the increased reactivity of the hydroxyl groups of aminoglycosides in the presence of TBAF in comparison to TBAI.

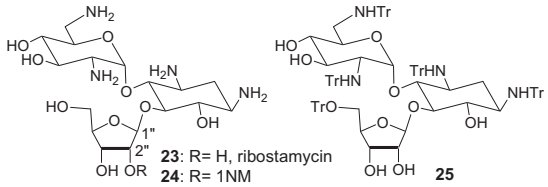


Fig. 7. Structure of ribostamycin and of the corresponding prepared derivatives.

3. Conclusion

To the best of our knowledge, here, we report for the first time on the major increases of the selectivity and/or reactivity of carbohydrates in the presence of fluorides associated to a strong base in an apolar aprotic solvent. The corresponding methodology involving TBAF in toluene and aq NaOH under phase-transfer conditions or NaH offers prospects of selectivity in the modification of carbohydrates and polyols through a simple procedure. It provides selectivity and rapidity for preparing aminoglycosides of medicinal interest especially the 3′,6-diPMB (**8a**) and 3′,4′,6-triPMB (**8b**) neamine derivatives, which are key intermediates in the preparation of 4′-, 5- and 4′,5-neamine derivatives. This procedure also allows the selective preparation of the tritylated 6-monoPMB neamine derivative **8d** useful for the preparation of potential antibacterial 3′,4′-neamine derivatives, such as **20**. Using PTC in toluene and TBAF as a phase-transfer agent, 6-monoalkylated neamine derivatives, which can be seen as analogues of the antibiotic aminoglycosides kanamycins, were rapidly and selectively prepared.

In conclusion, TBAF can be advantageously used as a phase-transfer agent for the alkylation in toluene at room temperature of aminosugars and sugars, which bind to fluoride ions through H-bonds. Experiments performed in the *para*-methoxylation of the tetratritylated neamine derivative **7** under PTC showed that TBAF can be replaced by the TBA hydroxide/NaF mixture cheaper than TBAF.

4. Experimental section

4.1. General procedures

General: ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a BRUKER AVANCE 400 spectrometer using the residual solvent signal as internal standard. LRMS were achieved with a NERMAG spectrometer for the FAB, DCI and EI techniques, with an AUTOFLEX BRUKER spectrometer for the MALDI and with a ZQ WATERS for the ESI. HRMS were obtained from the Mass Spectrometry Service, CRMPO, at the University of Rennes I, France, using a MICROMASS ZABSPEC-TOF spectrometer and a VARIAN MAT311 spectrometer. Melting points were determined with a BUCHI 510 apparatus. Thin layer chromatographies were performed on silica gel (Alugram Sil G/UV254) or Alumina gel (Alugram Alox N/UV254) from Macherey–Nagel and spots were detected either by UV-absorption, by using a cerium-molybdate stain or by charring with ninhydrin. Columns chromatography were performed on alumina gel (MP Ecochrom Biomedicals, Act II-III acc. to Brockman), on silica gel (MN Kieselgel 60, 0.063–0.2 mm/70–230 mesh, Macherey–Nagel) or on C18 reversed phase (Macherey–Nagel polygoprep 60-50C18). All starting materials were obtained from suppliers and used without further purification unless otherwise noted. DMF was distilled in the presence of CaH_2, THF over sodium with benzophenone and stored under argon atmosphere prior to use. Compound **7** was synthezised as previously described.[9a]

*4.1.1. Method A: general procedure used for the 3′,6-di-O-alkylation of the tetra-N-tritylated neamine derivative **7**.* To a solution of compound **7** (1 g, 1 equiv) in toluene (30 mL) were added TBAI (1.5 equiv), the halide (3 equiv) and an aqueous solution of NaOH (30% w/w, 15 mL). The resulting mixture was heated at 50 °C and stirred vigorously. After 5 h, another portion of halide (0.6 equiv) was added to the reacting mixture. After 24 h, the organic solution was extracted, diluted with ethyl acetate and washed twice with an aq saturated ammonium chloride solution before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The dialkylated product was purified by chromatography on alumina or silica gel neutralized with triethylamine eluting with a gradient of toluene/ethyl acetate or cyclohexane/ethyl acetate.

*4.1.2. Method B: general procedure used for the 3′-mono-O-alkylation of the tetra-N-tritylated neamine derivative **7**.* To a solution of compound **7** (1 g, 1 equiv) in toluene (30 mL) were added TBAI (1.5 equiv), the halide (1.2 equiv) and an aqueous solution of NaOH (30% w/w, 15 mL). The resulting mixture was heated at 50 °C and stirred vigorously. Two other portions of halide (2×0.4 equiv) were added to the reacting mixture after 1 day and then 3 days. After 4 days, the organic solution was extracted, diluted with ethyl acetate and washed twice with an aq saturated ammonium chloride solution before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The monoalkylated product was purified by chromatography on alumina or silica gel neutralized with triethylamine eluting with a gradient of toluene/ethyl acetate or cyclohexane/ethyl acetate.

*4.1.3. Method C: general procedure used for the 3′,4′,6-tri-O-alkylation of the tetra-N-tritylated neamine derivative **7**.* To a solution of compound **7** (1 g, 1 equiv) in toluene (30 mL) were added $TBAF\cdot 3H_2O$ (1.5 equiv), the halide (4 equiv) and an aqueous solution of NaOH (50% w/w, 15 mL). The resulting mixture was stirred vigorously for 4–24 h at room temperature. The organic solution was extracted, diluted with ethyl acetate, then washed twice with an aq saturated ammonium chloride solution before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The trialkylated product was purified by chromatography on alumina or silica gel neutralized with triethylamine eluting with a gradient of toluene/ethyl acetate or cyclohexane/ethyl acetate.

*4.1.4. Method D: general procedure used for the 6-mono-O-alkylation of the tetra-N-tritylated neamine derivative **7**.* To a solution of compound **7** (1 g, 1 equiv) in toluene (30 mL) were added $TBAF\cdot 3H_2O$ (1 equiv), the halide (1.2–2.5 equiv) and an aqueous solution of NaOH (50% w/w, 15 mL). The resulting mixture was stirred vigorously for 1–24 h at room temperature (25 °C). The organic solution was extracted, diluted with ethyl acetate and washed twice with an aq saturated ammonium chloride solution before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The monoalkylated product was purified by chromatography on alumina or silica gel neutralized with triethylamine eluting with a gradient of toluene/ethyl acetate or cyclohexane/ethyl acetate.

4.1.5. Method E: general procedure for the deprotection of the tetra-N-tritylated neamine derivative. The protected compound was dissolved at 0 °C or at room temperature in CH_2Cl_2/TFA (1/1, v/v) in the presence of anisole (0.1 mL/mL). After 2 h stirring, the solvents were evaporated under reduced pressure. H_2O and Et_2O were added and the aqueous phase was washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. The compound was eluted with a H_2O/MeOH gradient and obtained pure as the TFA salt.

4.2. Synthesis and characterization

*4.2.1. 3′,6-Di-O-(4-methoxybenzyl)-tetra-N-trityl neamine derivative (**8a**).* Compound **8a** was obtained from **7** (1 g) and 4-methoxybenzyl chloride with 72% yield (854 mg) following the method A and after a purification by chromatography on silica gel with a gradient of toluene/ethyl acetate (v/v: 100/0 to 90/10) with triethylamine (0.2%) as eluent. The NMR and mass spectra were in agreement with those obtained for **8a** previously described.[9a]

*4.2.2. 3′-Mono-O-(4-methoxybenzyl)-tetra-N-trityl neamine derivative (**8c**).* Compound **8c** was obtained from **7** (1 g) and 4-methoxybenzyl chloride with 38% yield (416 mg) following the method B and after a purification by chromatography on silica gel eluting with a gradient of toluene/ethyl acetate (v/v: 100/0 to 50/50) with triethylamine (0.2%). HRMS (ESI^+): $[M+H]^+$ *m*/*z* calculated 1411.6888, found 1411.6906, $[M+Na]^+$ *m*/*z* calculated 1433.6707, found 1433.6722. The identification was done via the synthesis of **11** due to the difficulty of interpretation of the NMR spectra of **8c**.

*4.2.3. 4′,5,6-Tri-O-methyl-neamine derivative (**11**).* To a solution of compound **8c** (230 mg, 0.16 mmol) in dry DMF (5 mL) under argon were added sodium hydride (60%, 6 equiv) and after 30 min stirring at room temperature, iodomethane (2.3 equiv). The resulting mixture was stirred for 2 h at room temperature before the addition of ethanol (5 mL). The solution was concentrated under reduced pressure. The crude product was diluted with ethyl acetate and then washed with water and brine before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The trimethylated compound obtained was enough pure to be deprotected

directly following the method E. After purification on a C18 reversed phase column eluting with water, the compound was obtained as trifluoroacetic acid salt. Compound **11**: 60% (80 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 5.71 (d, 1H), 4.23–4.10 (m, 3H), 3.65 (s, 3H), 3.62 (s, 3H), 3.56 (s, 3H), 3.51–3.17 (m, 8H), 2.45 (m, 1H), 1.97 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.8, 163.5, 163.2 and 162.8 (CO TFA), 122.6, 119.7, 116.8 and 113.9 (CF3), 94.7, 86.2, 84.1, 81.1, 77.1, 72.2, 69.0, 61.4, 60.9, 60.5, 54.6, 50.7, 50.3, 41.2, 29.7; HRMS (ESI+): $[M+H]^+$ *m*/*z* calculated 365.2400, found 365.2403, $[M+Na]^+$ *m*/*z* calculated 387.2220, found 387.2207.

*4.2.4. 3′,4′,6-Tri-O-(4-methoxybenzyl)-tetra-N-trityl neamine derivative (**8b**).* Compound **8b** was obtained from **7** (1 g) and 4-methoxybenzyl chloride with 86% yield (1.10 g) following the method C and after a purification on silica gel with a gradient of toluene/ethyl acetate (v/v: 100/0 to 95/5) and triethylamine (0.2%). The NMR and mass spectra were in agreement with those obtained for **8b** previously described.[9a]

*4.2.5. 6-Mono-O-(4-methoxybenzyl)-tetra-N-trityl neamine derivative (**8d**).* The compound **8d** was obtained from **7** (2 g) and 4-methoxybenzyl chloride with 75% yield (1.64 g) following the method D for 2 h. Yet, the crude product was not purified by chromatography, instead, the yellow solid gum was dissolved in CH_2Cl_2 and the solvent removed under reduced pressure, then the solid was triturated in Et_2O, filtrated, washed with H_2O and dried to give the pure compound as a white powder in 86% yield. HRMS (ESI^+): $[M+H]^+$ *m*/*z* calculated 1411.6888, found 1411.6898, $[M+Na]^+$ *m*/*z* calculated 1433.6707, found 1433.6689. The identification was done via the synthesis of **12** due to the difficulty of interpretation of the NMR spectra of **8d**.

*4.2.6. 3′,4′,5,-Tri-O-methyl-neamine derivative (**12**).* To a solution of compound **8d** (250 mg, 1 equiv) in dry DMF (5 mL) under argon were added sodium hydride (60%, 6 equiv) and after 30 min stirring at room temperature, iodomethane (2.3 equiv). The resulting mixture was stirred for 2 h at room temperature before the addition of ethanol (5 mL). The solution was concentrated under reduced pressure. The crude product was diluted with ethyl acetate and then washed with water and brine before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The trimethylated compound obtained was enough pure to be deprotected directly following the method E. After purification on a C18 reversed phase column with water, the compound was obtained as the trifluoroacetic acid salt, which was converted to the chlorhydrate salt with an ion exchange resin. **12**: 70% (102 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 5.76 (d, 1H), 4.32 (dd, 1H), 4.21 (m, 1H), 4.08 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.59 (s, 3H), 3.66–3.57 (m, 2H), 3.50 (dd, 1H), 3.43 (dd, 1H), 3.40–3.22 (m, 4H), 2.41 (ddd, 1H), 2.05 (ddd, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 94.5, 86.2, 81.6, 78.3, 75.7, 74.8, 71.4, 60.9, 60.7, 60.6, 53.8, 51.4, 50.4, 41.2, 29.5; HRMS (ESI^+): $[M+H]^+$ *m*/*z* calculated 365.2400, found 365.2398, $[M+Na]^+$ *m*/*z* calculated 387.2220, found 387.2211.

*4.2.7. 3′,6-Di-O-[(2-naphthyl)methyl] neamine derivative (**10a**).* Compound **10a** was prepared following the method A from **7** (250 mg) and 2-(bromomethyl)naphthalene. The protected derivative **9a** was obtained with 58% yield (177 mg). The deprotection was achieved following the method E to give **10a** with 70% yield (83.2 mg). The NMR and mass spectra were in agreement with those obtained for **10a** previously described.[11h]

*4.2.8. 3′,4′,6-Tri-O-[(2-naphthyl)methyl] neamine derivative (**10b**).* Compound **10b** was synthesised following the method C from **7** (250 mg) and 2-(bromomethyl)naphthalene. The protected derivative **9b** was obtained with 55% yield (182 mg). The deprotection was achieved following the method E to give **10b** with 65% yield (83 mg).The NMR and mass spectra were in agreement with those obtained for **10b** previously described.[11h]

*4.2.9. 6-Mono-O-naphthyl neamine derivative (**10c**).* Compound **10c** was synthesised following the method C from **7** (250 mg) and 2-(bromomethyl)naphthalene (1.2 equiv) for 1.5 h. The protected derivative **9c** was obtained with 45% yield (134 mg). The deprotection of **9c** (94 mg) was achieved following the method E to give **10c** with 77% yield (38 mg). The NMR and mass spectra were in agreement with those obtained for **10c** previously described.[11h]

*4.2.10. 3′,6-Di-O-hexyl neamine derivative (**15a**).* Compound **15a** was obtained following the method A from **7** (200 mg) and 1-bromohexane (3 equiv) and after the addition of another portion of halide (3 equiv) and stirring for 16 h more. Protected derivative **13a** was obtained with 72% yield (163 mg). The deprotection of **13a** (285 mg) was achieved following the method E. Compound **15a**: 57% (106 mg), ^{1}H NMR (400 MHz, CD3OD) δ 5.94 (d, *J*=3.6 Hz, 1H), 4.07–3.89 (m, 4H), 3.84 (dd, *J*=8.5, 10.3 Hz, 1H), 3.75–3.62 (m, 3H), 3.45–3.37 (m, 3H), 3.33–3.19 (m, 3H), 3.10 (dd, *J*=8.7, 13.3 Hz, 1H), 2.44 (m, *J*=4.1, 12.3 Hz, 1H), 2.00 (m, *J*=12.5 Hz, 1H), 1.72–1.61 (m, 4H), 1.34 (br s, 12H), 0.91 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 97.0, 82.4, 79.0, 77.7, 77.6, 75.2, 74.8, 73.5, 71.8, 54.6, 50.7, 50.2, 41.8, 33.0, 31.2, 31.0, 30.1, 26.8, 26.7, 23.8, 14.5 (2 CH_3); HRMS (ESI+): $[M+H]^+$ *m*/*z* calculated 491.3809, found 491.3815, $[M+Na]^+$ *m*/*z* calculated 513.3628, found 513.3631.

*4.2.11. 3′,4′,6-Tri-O-hexyl neamine derivative (**15b**).* Compound **13b** was synthesised following the method C from **7** (250 mg) and 1-bromohexane (4 equiv) for 4 h. Protected derivative **13b** was obtained with 60% yield (182 mg). The deprotection of **13b** (40 mg) was achieved following the method E. Compound **15b**: 52% (14 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 5.89 (d, *J*=3.4 Hz, 1H), 4.14 (m, *J*=9.2, 2.4 Hz, 1H), 4.03–3.58 (m, 9H), 3.44–3.15 (m, 7H), 2.43 (m, *J*=4.0, 12.4 Hz, 1H), 1.99 (dd, *J*=12.4 Hz, 1H), 1.75–1.58 (m, 6H), 1.36 (br s, 18H.), 0.94 (m, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 96.9, 82.5, 81.0, 79.9, 77.9, 77.7, 75.2, 74.8, 74.5, 71.3, 54.7, 50.9, 50.1, 41.6, 33.1 ($3CH_2$), 31.5, 31.2, 31.1, 30.4, 27.0, 26.9, 26.7, 23.8 (3 CH_2), 14.5 (3 CH_3); HRMS (ESI^+): $[M+H]^+$ *m*/*z* calculated 575.4748, found 575.4748, $[M+Na]^+$ *m*/*z* calculated 597.4567, found 597.4566, $[M+K]^+$ *m*/*z* calculated 613.4306., found 613.4311.

*4.2.12. 6-Mono-O-hexyl neamine derivative (**15d**).* Compound **13d** was synthesised following the method D from **7** (250 mg) and 1-bromohexane (1.5 equiv). After 6 h, another portion of halide (1.5 equiv) was added and the mixture was stirred 16 h more. Protected derivative **13d** was obtained with 50% yield (134 mg). The deprotection of **13d** (94 mg) was achieved following the method E to lead to **15d**: 80% (38 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 5.94 (d, *J*=3.8 Hz, 1H), 4.07–3.88 (m, 4H), 3.72 (dd, *J*=9.2 Hz, 1H), 3.65 (m, 1H), 3.48–3.38 (m, 2H), 3.37–3.19 (m, 4H), 3.11 (dd, *J*=8.4, 13.3 Hz, 1H), 2.46 (m, *J*=4.1, 12.4 Hz, 1H), 2.01 (m, *J*=12.5 Hz, 1H), 1.72–1.62 (m, 2H), 1.32 (br s, 6H), 0.91 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 97.3, 82.3, 79.1, 77.6, 75.8, 73.3, 71.1, 69.6, 55.5, 50.6, 50.1, 42.0, 33.0, 31.0, 29.8, 26.6, 23.7, 14.4; HRMS (ESI^+): $[M+H]^+$ *m*/*z* calculated 407.2864, found 407.2861, $[M+Na]^+$ *m*/*z* calculated 429.2684, found 429.2683, $[M+K]^+$ *m*/*z* calculated 445.2423, found 445.2418.

*4.2.13. 6-Mono-O-(3-amino)propyl neamine derivative (**16d**).* Compound **14d** was synthesised following the method D from **7** (250 mg) and 1-bromo-*N*-Boc-propylamine (1.5 equiv). After 2 h, another portion of halide (1.5 equiv) was added and the mixture was stirred 16 h more. The protected derivative **14d** was obtained with 35% yield (96 mg). The deprotection of **14d** (91 mg) was achieved following the method E to lead to **16d**: 87% (55 mg), ^{1}H NMR (400 MHz, CD_3OD)

δ 6.01 (d, J=3.7 Hz, 1H), 4.04–3.89 (m, 4H), 3.87–3.74 (m, 2H), 3.46–3.33 (m, 4H), 3.32–3.21 (m, 3H), 3.15–3.05 (m, 3H), 2.43 (m, J=4.2, 12.5 Hz, 1H), 2.10–1.87 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 97.1, 83.2, 79.1, 77.5, 73.4, 71.6, 71.1, 70.1, 55.6, 50.6, 50.3, 42.1, 38.8, 30.5, 29.0; HRMS (ESI+): $[M+H]^+$ *m/z* calculated 380.2504, found 380.2503, $[M+Na]^+$ *m/z* calculated 402.2323, found 402.2323.

*4.2.14. 1,3,2′,6′-Tetra-N-trityl paromamine derivative (**17**).* Compound **17** was obtained under the conditions used previously for the preparation of **7**.[9a] A solution of paromamine tetrahydrochloride **6**[15] (2.5 g, 1 equiv) in DMF/triethylamine (60:8 mL) under argon atmosphere was stirred at room temperature for 1 h. Then a solution of trityl chloride (13.6 g, 48.6 mmol) in DMF/triethylamine (130:8 mL) and DMAP (0.2 equiv) were added. After 4 h at room temperature, dichloromethane (100 mL) was added. The resulting solution was washed with water (2×100 mL), dried over Na_2SO_4 and evaporated. The residue was precipitated in pentane and then chromatographed on alumina gel eluting with toluene/ethyl acetate (v/v: 100/0 to 50/50) to obtain the tetratritylated derivative **17** with 77% yield (7.7 g). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3–143.2 (CPh), 129.7–125.9 (CHPh), 99.8 (CH), 86.7 (CH), 76.9 (CH), 75.6 (CH), 71.4 (C), 70.9 (CH), 69.7 (C), 65.9 (CH_2), 57.8 (CH), 54.3 (CH), 52.8 (CH), 21.3 (CH_2); LRMS (MALDI, DHB) *m/z*: 1332 $[M+K]^+$, 1316 $[M+Na]^+$, 1292 $[M+H]^+$, 1089 $[M-Tr+K]^+$, 1073 $[M-Tr+H]^+$, 1047 $[M-Tr+H]^+$; HRMS (ESI^+): $[M+Na]^+$ *m/z* calculated 1314.5967, found 1314.5976.

*4.2.15. 3′,6-Di-O-[(2-naphthyl)methyl] paromamine derivative (**19a**).* Compound **19a** was synthesised following the method A from **17** (1 g) and 2-(bromomethyl)naphthalene (3 equiv). Protected derivative **18a** was obtained with 58% yield (705 mg). The deprotection was achieved following the procedure E. Compound **19a**: 44% (187 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 7.93–7.83 (m, 8H), 7.60–7.55 (m, 2H), 7.51–7.43 (m, 4H), 5.55 (d, J=3.5 Hz, 1H), 5.24 (d, J=11.1 Hz, 1H), 5.00–4.83 (m, J=11.2 Hz, 3H), 4.04–3.94 (m, 3H), 3.89–3.85 (m, 2H), 3.71 (dd, J=9.0, 11.7 Hz, 1H), 3.70–3.60 (m, 2H), 3.56–3.47 (m, 2H), 3.37 (m, 1H), 2.49 (m, 1H), 1.87 (m, J=12.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 136.9–134.6 (6C-naphthyl), 129.1–127.1 (14CH-naphthyl), 98.7, 84.1, 81.3, 78.4, 77.0, 76.9, 76.1, 76.0, 71.9, 61.9, 54.7, 50.6, 50.4, 30.1; HRMS (ESI^+): $[M+H]^+$ *m/z* calculated 604.3017, found 604.3013, $[M+Na]^+$ *m/z* calculated 626.2837, found 626.2830.

*4.2.16. 3′,4′,6-Tri-O-[(2-naphthyl)methyl] paromamine derivative (**19b**).* Compound **19b** was synthesised following the method C from **17** (1 g) and 2-(bromomethyl)naphthalene (4 equiv). Protected derivative **18b** was obtained with 57% yield (755 mg). The deprotection was achieved following the method E. Compound **19b**: 32% (153 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 7.93–7.30 (m, 21H), 5.60 (d, J=3.2 Hz, 1H), 5.24 (d, J=11.1 Hz, 1H), 5.05–4.82 (m, 5H), 4.18–4.13 (m, 2H), 3.92–3.80 (m, 4H), 3.72–3.60 (m, 3H), 3.48 (m, 1H), 3.37 (m, 1H), 2.48 (m, J=4.1, 12.4 Hz, 1H), 1.85 (m, J=12.7 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 136.8–134.6 (9C-naphthyl), 129.4–126.7 (21CH-naphthyl), 98.0, 83.4, 81.5, 78.5, 78.1, 77.3, 76.6, 76.2, 75.9, 75.6, 61.4, 54.5, 50.6, 50.5, 30.1; HRMS (ESI^+): $[M+H]^+$ *m/z* calculated 744.3643, found 744.3639, $[M+Na]^+$ *m/z* calculated 766.3463, found 766.3449.

*4.2.17. 3′,4′-Di-O-[(1-naphthyl)methyl] neamine derivative (**20**).* To a solution of **8d** (1 g) in toluene (30 mL) were added TBAF·$3H_2O$ (2 equiv), 1-(chloromethyl)naphthalene (3 equiv) and an aqueous solution of NaOH (50% w/w, 15 mL). The resulting mixture was stirred vigorously for 5 h at room temperature. The organic solution was extracted, diluted with ethyl acetate then washed twice with an ammonium chloride aqueous saturated solution before being dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The desired tetratritylated product was purified rapidly by chromatography on alumina with a gradient of cyclohexane/toluene to give a pale yellow gum in 61% yield (730 mg). The deprotection was achieved following the procedure E. Compound **20**: 60% (274 mg), ^{1}H NMR (400 MHz, CD_3OD) δ 7.88–7.60 (m, 7H), 7.45–7.20 (m, 7H), 6.01 (d, J=3.5 Hz, 1H), 5.40 (d, J=12.4 Hz, 1H), 5.20 (d, J=12.4 Hz, 1H), 5.02 (d, J=12.0 Hz, 1H), 4.90 (m br, 1H and solvent), 4.44 (dd, J=7.8, 9.4 Hz, 1H), 4.28 (m, J=3.0, 8.4, 9.8 Hz, 1H), 4.06 (dd, J=9.3, 10.1 Hz, 1H), 3.68–3.57 (m, 3H), 3.53–3.42 (m, 2H), 3.21 (m, J=4.3, 10.3, 12.5 Hz, 1H), 2.98 (m, 2H), 2.48 (m, J=4.2, 12.4 Hz, 1H), 2.02 (dd, J=12.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 135.2–132.2 (6C-naphtyl), 130.0–124.1 (14CH-naphtyl), 96.3, 79.6, 79.2, 77.9, 77.3, 74.4, 74.0, 73.6, 71.6, 54.4, 51.6, 50.3, 41.5, 29.9; HRMS (ESI^+): $[M+Na]^+$ *m/z* calculated 625.3002, found 625.3002, $[M+K]^+$ *m/z* calculated 641.2741, found 641.2744; HPLC: method C.

*4.2.18. Methyl-6-O-trityl-2,3,4-tri-O-benzyL-α-D-glucopyranoside derivative (**22**)*[16a]. Compound **22** was prepared with 70% yield following method B from **21**[16] (1 g) and benzyl bromide (718 mg). The NMR and mass spectra were in agreement with those obtained for **22** previously described.

4.3. Kinetic studies by HPLC

Kinetic studies were performed by HPLC with an Agilent 1100 series HPLC machine with a diode array detector using a C18 reversed phase column (Nucleosil C18 column, Macherey–Nagel, 5 μm particle size, 250×4.6 mm) and MeOH as a mobile phase.

4.4. NMR study of fluoride binding to compound 7

In the NMR study of phase transfer experiments, TBAF·$3H_2O$ (12.2 mg, 38.7 mmol) were dissolved in toluene (1 mL) and 50% aq NaOH (0.5 mL) in the presence or in the absence of **7** (33.3 mg, 25.8 mmol). The mixture was stirred at room temperature for 2 h. The toluene phase was extracted with 1 mL of distilled water. 500 μL of this aqueous phase were introduced in an NMR tube together with 50 μL of D_2O.

For the titration of **7** by TBAF, a 500 μL solution of **7** was prepared at 77 mM in toluene-d_8. The **7**/TBAF binding was studied by direct addition of aliquots of a concentrated solution of TBAF in toluene-d_8 (typically 0.386 M) to the solution of **7**.

For the titration of TBAF by **7**, a 500 μL solution of TBAF·$3H_2O$ was prepared at 39 mM in toluene-d_8. The **7**/TBAF binding was studied by direct addition of aliquots of a concentrated solution of **7** in toluene-d_8 (typically 0.157 M) to the NMR TBAF sample.

NMR spectra were recorded at 28 °C, on Bruker Avance 300 and 400 MHz spectrometers. To follow the proton chemical shift variations in **7** upon binding to fluoride, chemical shifts of **7** in the absence and in the presence of TBAF at a ([TBAF]/[**7**]) molar ratio of 1 were assigned using COSY, HMQC, HMBC experiments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.10.102.

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