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Tetrahedron Letters

Tetrahedron Letters 45 (2004) 3451–3454

Efficient preparation of carbohydrate- and related polyol-amphiphiles by hydrazone ligation

Cyrille Grandjean,[a,*] Valérie Santraine,[a] Nathalie Fardel,[a] Ange Polidori,[b] Bernard Pucci,[b] Hélène Gras-Masse[a,c] and Dominique Bonnet[a]

[a]*SEDAC-Therapeutics, Parc Eurasanté, Le Galénis, 85, rue Nelson Mandela, 59120 Loos, France*

[b]*Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels, Faculté des Sciences, Université d'Avignon, 33, rue Louis Pasteur, 84000 Avignon, France*

[c]*UMR CNRS 8525, Institut de Biologie de Lille, 1, rue du Pr Calmette, 59000 Lille, France*

Received 3 December 2003; revised 17 February 2004; accepted 3 March 2004

Abstract—The hydrazone ligation can be used to couple clustered-glycosides or glycomimetics functionalized with a hydrazino group with lipophilic glyoxylyl acid derivatives to give the corresponding amphiphiles in high yield and purity.

Glycolipids receive considerable attention for their involvement in a variety of physiological events[1] as well as their unique surfactant properties, which have led to numerous industrial applications.[2] Besides, carbohydrate amphiphiles are likely to bind membrane lectins thanks to their polar headgroups and thus to act as recognition patterns for selective targeting systems.[3] In this case, their lipid moiety is expected to provide a cluster effect through self-association[4] or allows their anchoring onto vesicles. Such amphiphiles also facilitate the development of models for in vitro interaction studies.[5] Many synthetic efforts have been devoted to the preparation of sophisticated polar headgroups or lipidic tails conferring on the amphiphile optimized binding properties or absence of detergency toward cell membranes, respectively.[4,6] However, the actual structures remain largely obtained through glycosylation,[2] N-acylation,[7] nucleophilic opening of lactones,[8] or reductive amination,[9] reactions not completely suitable when applied to costly materials. In this context, chemoselective ligation reactions would offer a valuable alternative for the preparation of carbohydrate amphiphiles.[10] These tools would allow the separate handling of the hydrophobic and hydrophilic moieties until the ultimate stage of the synthesis and their actual assembly in absence of a protecting group in high yield and purity, thus avoiding most of the troubles encountered during the purification of surfactants. Herein, we describe a flexible and highly efficient approach allowing a rapid and convenient access to amphiphilic structures.

To test the efficiency of such a strategy, we planned to couple known palmitoylated α-oxo aldehyde **1**[11] with the tetravalent quinoylated, mannosylated, and galactosylated hydrazino-L-lysinyl trees **2–4** through a hydrazone ligation (Fig. 1). Clusters **2** and **3** have been selected as potent macrophage mannose receptor ligands[12] whilst cluster **3**, although not optimized, might bind to asialoglycoprotein receptor.[3c,13]

Trees **2–4** were synthesized on a rink amide-Nle-AM-PS resin preloaded with a Fmoc-L-Lys(Mtt)-β-Ala dipeptide residue using the Fmoc/*tert*-butyl chemistry (Scheme 1).[14] Introduction of the hydrazino was secured using [*N,N′*-tri(*tert*-butyloxycarbonyl)hydrazino]acetic acid **5**,[15] after selective TFA removal of the 4-methyltrityl protective group[16] to give peptidyl resin **6**. Several deprotection steps with piperidine and couplings with Fmoc-L-Lys(Fmoc)-OH gave the tetravalent core **7**, which was further linked to either peracetylated acid derivatives **8**,[17] **9**, or **10**. Compounds **9** and **10** were obtained by catalytic ruthenium trichloride oxidation of the corresponding allyl tetra-*O*-acetyl-α-D-pyranosides[18] as depicted by Ghosh et al.[19] in about a 70% yield.[20]

Keywords: Carbohydrate; Amphiphile; Chemoselective ligation.

* Corresponding author at present address: Laboratoire de Chimie Organique, Institut Pasteur, 25, rue du Docteur Roux, F75015 Paris, France. Tel.: +33-145688266; fax: +33-145688223; e-mail: cgrandje@pasteur.fr

0040-4039/$ - see front matter
doi:10.1016/j.tetlet.2004.03.011

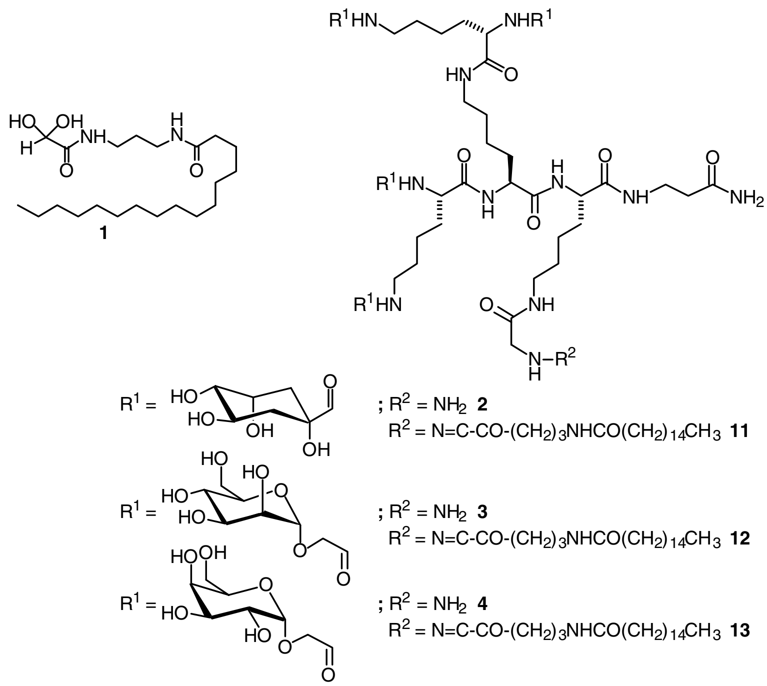
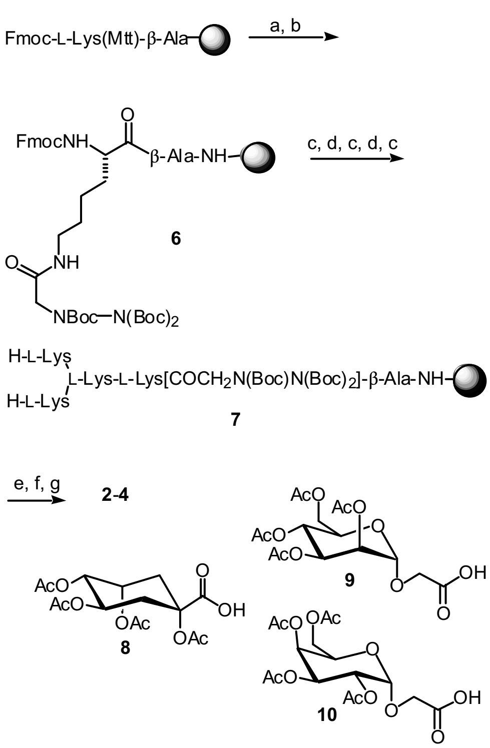


Figure 1. Lipophile and hydrophile partners used for the ligation and their corresponding hydrazones.



Scheme 1. Reagents and conditions: (a) TFA–CH_2Cl_2 1:99; (b) compound **5** 3 equiv, HBTU–HOBt–TEA, 2:2:6 equiv, DMF, 45 min; (c) piperidine–DMF 20:80; (d) Fmoc-L-Lys(Fmoc)-OH 4 equiv, HBTU–HOBt–TEA 4:4:12 equiv, DMF, 45 min; (e) compounds **8**, **9**, or **10** 4 equiv, HBTU–HOBt–TEA 4:4:12 equiv, DMF, 45 min; (f) NH_2–NH_2–DMF 10:90, 2 h, (2 times); (g) TFA–H_2O–*i*-Pr_3SiH 95:2.5:2.5, 0 °C, 1 h 30 min.

Clustered-glycomimetics or glycosides **2–4** were finally obtained following deacetylation on the peptidyl resin via hydrazine in DMF treatment, acidic cleavage from the resin and RP-HPLC purification, in 10–15% overall yield. Deprotection of the hydroxy groups by sodium methoxide or hydrazine after cleavage from the resin did not improve the yields.

We next examined the preparation of the amphiphiles by the hydrazone ligation. Reaction of glyoxylyl derivative **1** with α-hydrazinoacetyl peptides was shown to proceed cleanly to give the corresponding lipopeptides when performed in a 95/5 2-methyl-propan-2-ol/water mixture and, interestingly, in absence of buffer.[11]

For the present study, we decided to attempt the ligation in a similar solvent mixture, the amount of water being

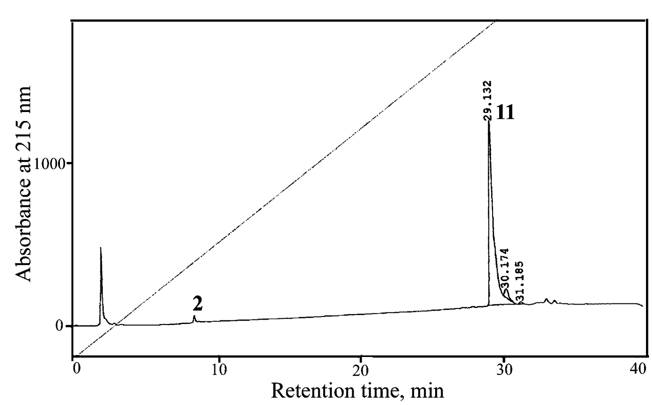


Figure 2. RP-HPLC profile of hydrazone ligation of **1** with tetra-quinoylated tree **2** after 5 h. Chromatographic conditions: C3 Zorbax column (5 mm, 4.6 × 250 mm). Buffer A: 0.05% aq TFA. Buffer B: 0.05% TFA in CH_3CN–H_2O (80:20). Gradient 0–100% B over 30 min, 100% B for 10 min. Flow rate 1 mL min^{-1}, 50 °C.

Table 1. Physicochemical data of amphiphiles **11–13**[a]

Compound	cmc (mol L^{-1})			a_s Size area of the polar head (Å^2)		
	4 °C	25 °C	37 °C	4 °C	25 °C	37 °C
11	2.8×10^{-5}	2.8×10^{-5}	3.7×10^{-5}	78	58	42
12		4.6×10^{-5}			41	
13		3.2×10^{-5}			62	

[a] The cmc were measured using a tensiometer Kruss K12 equipped with a LabDesk User Interface 2.01.2216. All measurements were triplicated, cmc results and size area of polar head were given $\pm 0.2 \times 10^{-5}$ and ± 2 Å^2, respectively.

somewhat increased to 20% to favor the solubilization of the quinoylated and glycosylated trees. Thus, reaction of derivatives **2**, **3**, or **4** with a slight excess of **1** (1.2 equiv), in 80/20 2-methyl-propan-2-ol/water at 30 °C for 5 h led to their corresponding amphiphilic hydrazones **11–13** in an almost quantitative yield (Figs. 1 and 2).[21] The high efficiency of the process and the absence of buffer allowed to overcome a tedious final purification. Thus, final constructs were simply isolated following a freeze-drying step and directly used for physicochemical characterization and biological assessment.

These novel palmitoylated clusters **11–13** exhibit surfactant properties as determined by surface tension measurements of aqueous solutions thereof (Table 1). Their critical micellar concentration are very low and in good agreement with the values usually obtained with nonionic surfactants bearing a hexadecylhydrocarbon chain. These results highlight the same ability of the compounds to self assemble in aqueous solution whatever the nature of the polar head is. However, we can note a slight but real evolution of these cmc according to the nature and the hydration power of the different glycoside derivatives. For **11**, the cmc slightly increases from 25 to 37 °C whenever the size of the polar head decreases with temperature: this later parameter can be correlated to the rate of hydratation of the polar head.

In summary, we have presented the efficient synthesis of novel carbohydrate and glycomimetic amphiphiles by means of a chemoselective hydrazone ligation reaction. This strategy might be easily extended to a large variety of aldehydes derived from fatty alcohols and to complex aminooxy-oligosaccharides[22] or to other chemoselective ligation reactions to give a rapid, convenient, and flexible entry to various amphiphilic molecules.

Acknowledgements

We thank Gérard Montagne and Hervé Drobecq for NMR and MALDI-TOF experiments (Institut de Biologie de Lille, France).

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20. Physical data for **9**: $[\alpha]_D = +55$ (c 1, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 2.00, 2.06, 2.11, 2.16 ($4 \times 3H$, 4s, $4CH_3$), 4.09–4.36 (3H, m, H-5, and 2H-6), 4.24 (1H, d, J 14.6 Hz, H-7), 4.33 (1H, d, J 14.6 Hz, H-7′), 4.95 (1H, d, J 1.4 Hz, H-1), 5.27–5.40 (3H, m, H-2, H-3, and H-4); δ_C (75 MHz, $CDCl_3$): 21.1 and 21.3 ($4CH_3$), 62.8 (C-6), 64.5 (C-7), 66.3 (C-4), 69.2, 69.3, and 69.9 (C-2, C-3, and C-5), 98.3 (C-1), 170.2, 170.5, 170.8, and 171.4 ($4CO_2Me$), 173.6 (COOH); m/z (MALDI-TOF) 429.0 $(M+Na)^+$. Anal. Calcd for $C_{16}H_{22}O_{12}\cdot 0.5H_2O$: C, 46.27; H, 5.58; O, 48.15. Found: C, 46.27; H, 5.25. Compound **10**: $[\alpha]_D = +175$ (c 0.4, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 2.00, 2.06, 2.11, 2.16 ($4 \times 3H$, 4s, $4CH_3$), 4.10 (2H, d, J 6.9 Hz, 2H-6), 4.25 (1H, d, J 17.3 Hz, H-7), 4.32 (1H, d, J 17.3 Hz, H-7′), 4.36 (1H, br t, J 6.9 Hz H-5), 5.15 (1H, dd, J 3.7 and 10.8 Hz, H-2), 5.23 (1H, d, J 3.7 Hz, H-1), 5.41 (1H, dd, J 3.2 and 10.8 Hz, H-3), 5.48 (1H, m, H-4); δ_C (75 MHz, $CDCl_3$): 21.0 ($4CH_3$), 62.0 (C-6), 64.3 (C-7), 67.3, and 67.5 (C-3 and C-5), 68.0 (C-2), 68.3 (C-4), 96.7 (C-1), 170.4, 170.7, 171.0, and 171.2 ($4CO_2Me$), 174.1 (COOH); m/z (MALDI-TOF) 429.0 $(M+Na)^+$. Anal. Calcd for $C_{16}H_{22}O_{12}\cdot 0.5H_2O$: C, 46.27; H, 5.58; O, 48.15. Found: C, 46.19; H, 5.44.

21. α-Oxo-aldehyde **1** (12 mg, 1.2 equiv), dissolved in a mixture of $H_2O/^tBuOH$ (20:80) (21 mL), was reacted with **2**, **3**, or **4** (1 equiv) at 30 °C for 5 h. Completion of the reaction was monitored by RP-HPLC on a C3 Zorbax column. The reaction mixture was then diluted with water and freeze-dried to give amphiphile **11**, **12**, or **13** as a white powder, which were used without further purification. m/z (positive MALDI-TOF-MS): Compound **11**, 1720.3 $(M+H)^+$; HR-MS (FAB): calcd $(M+Na^+ = C_{78}H_{138}N_{14}O_{28}Na^+)$, 1741.9703; found $(M+Na)^+$, m/z 1741.9757. Compound **12**, 1941 $(M+K)^+$, 1925 $(M+Na)^+$, 1903 $(M+H)^+$. Compound **13**, 1942 $(M+K)^+$, 1926 $(M+Na)^+$, 1903 $(M+H)^+$.

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