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2,4-Bis(octadecanoylamino)benzenesulfonic acid sodium salt as a novel scavenger receptor inhibitor with low molecular weight

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Abstract—In order to investigate the effect of the fixation of the orientations of the two long chains, three types of novel derivatives of scavenger receptor inhibitor **1** were synthesized, and their biological activities were evaluated. Among the novel derivatives, 2,4-bis(octadecanoylamino)benzenesulfonic acid sodium salt (**4d**) showed the most potent inhibitory activity against the incorporation of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetyl-LDL (DiI-acetyl-LDL) into macrophages. 2,5-Bis(octadecanoylamino)benzenesulfonic acid sodium salt (**4c**), a regioisomer of **4d**, did not exhibit as potent an inhibitory activity as **4d**, meaning that the substitution pattern of two long chains on the benzene ring must be important. Compound **4d** exhibited 10 times more potent inhibitory activity against the binding of ^{125}I-labeled acetyl-LDL to the surface of macrophages than compound **1**.

1. Introduction

The presence of form cells is one of characteristic features in the early stages of atherosclerosis. Numerous studies have demonstrated that the formation of foam cells begins with the attachment of circulating monocytes to the lumenal surface of endothelial cells. Then, the monocytes migrate into the subendothelial space, where they differentiate into macrophages. The resulting macrophages uptake lipoproteins, and then accumulate a large amount of cholesterol ester derived from the lipoproteins, which leads to the deposition of lipid droplets observed in form cells.[1–4]

It is generally accepted that the accumulation of cholesterol ester is caused by the uptake of modified low density lipoproteins (modified LDLs), not native low density lipoproteins (native LDLs). Scavenger receptors expressed on the surface of the macrophages recognize modified LDLs, and then incorporate them by endocytosis. The first scavenger receptors were identified by Kodama and co-workers in 1990 as receptors for acetyl-LDLs.[5,6] After that, several research groups[7–14] succeeded in the cloning of several types of scavenger receptors, which can recognize modified LDLs, and now the scavenger receptors discovered by Kodama and co-workers are called scavenger receptor class A.

Compounds which possess an inhibitory activity against the binding of modified LDLs to scavenger receptors (referred to as scavenger receptor inhibitors) should inhibit the accumulation of cholesterol ester, which should lead to the inhibition of the formation of foam cells. Therefore, such scavenger receptor inhibitors are expected to prevent the progress of atherosclerosis. Recently, Suzuki et al. generated class A scavenger receptor/apolipoprotein E double knockout mouse, and compared it with apolipoprotein E single knockout littermate.[15] As a result, the double knockout mouse developed 60% smaller atherosclerotic lesions than the single knockout mouse. This result supports our working hypothesis strongly.

Sulfatides, a mixture of glycolipids having two long chains (Fig. 1), is known to be an inhibitor against the binding of acetyl-LDLs to scavenger receptors.[16] In the previous paper, we reported the structure–activity relationships (SAR) of sulfatides.[17] In addition, we described novel scavenger receptor inhibitors, such as compound **1**, with synthetic facility.[17] In this paper, we report the synthesis of novel derivatives with two long alkyl chains whose orientations are fixed, and then describe their biological activities.

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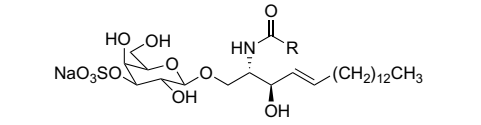
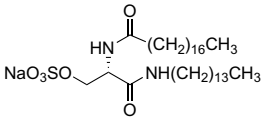


R = -$(CH_2)_{14}CH_3$, -$(CH_2)_{16}CH_3$, -$(CH_2)_{22}CH_3$, -$CHOH(CH_2)_{21}CH_3$, -$(CH_2)_{13}CH{=}CH(CH_2)_7CH_3$, -$CHOH(CH_2)_{12}CH{=}CH(CH_2)_7CH_3$, etc.

Sulfatides



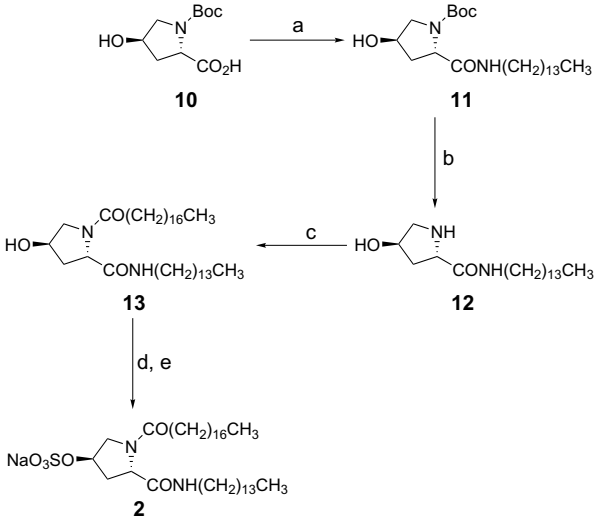
1

Figure 1. Structures of sulfatides and compound **1**.

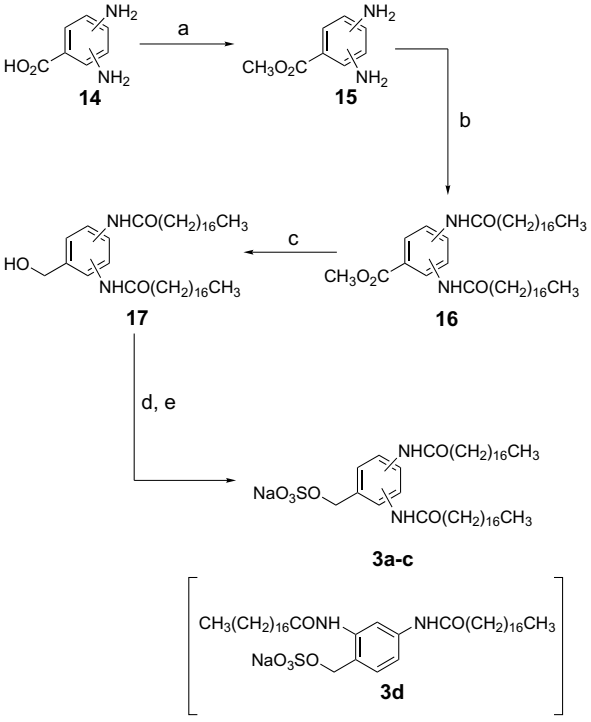
2. Chemistry

The synthetic route for the *trans*-4-hydroxy-L-proloine derivative **2** is shown in Scheme 1. To begin with, condensation of *trans*-*N*-Boc-4-hydroxy-L-proline **10** with tetradecylamine gave *N*-Boc-4-hydroxyprolinamide **11**. The Boc group of compound **11** was removed by use of trifluoroacetic acid (TFA) to give amine **12**. Acylation of **12** with octadecanoylchloride yielded *N*-octadecanoyl-4-hydroxyprolinamide **13**. Finally, compound **13** was sulfated with chlorosulfuric acid in pyridine, followed by treatment with sodium carbonate to give the target compound **2**.

Benzyl sulfate-type derivatives (**3a**–**c**) were prepared as outlined in Scheme 2. First of all, diaminobenzoic acids **14** were esterified under an acidic condition to give the corresponding methyl diaminobenzoates **15**. Acylation of the two amino groups of compounds **15** afforded compounds **16**. The ester moieties of compounds **16** were reduced by sodium borohydride in the presence of methanol in THF to give benzyl alcohols **17**. Finally, sulfation of compounds **17** with chlorosulfuric acid in pyridine and subsequent treatment with sodium carbonate yielded sulfate ester sodium salts **3a**,**b**, and **3c**. The attempt to synthesize the 2,4-disubstituted derivative **3d** failed because of the unstability of the final compound.

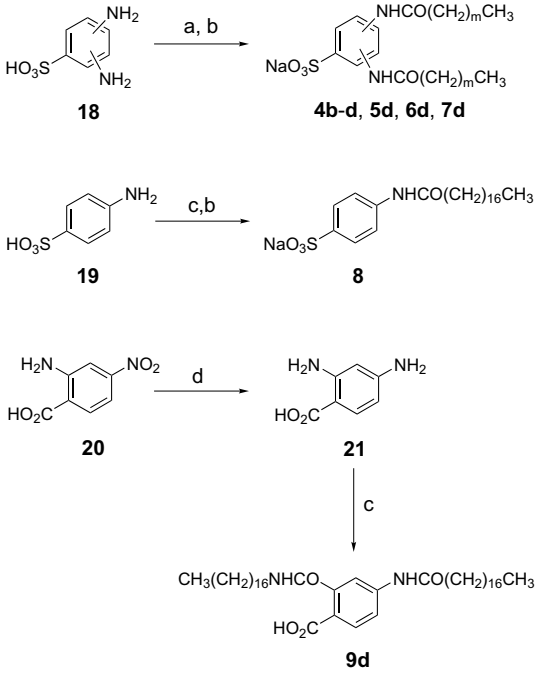


Scheme 1. Reagents: (a) $H_2N(CH_2)_{13}CH_3$, EDCI, HOBt; (b) TFA; (c) $ClCO(CH_2)_{16}CH_3$, *i*-Pr_2EtN; (d) $ClSO_3H$, pyridine; (e) Na_2CO_3.



Scheme 2. Reagents: (a) concd H_2SO_4/CH_3OH; (b) $ClCO(CH_2)_{16}CH_3$, *i*-Pr_2EtN; (c) $NaBH_4$, MeOH; (d) $ClSO_3H$, pyridine; (e) Na_2CO_3.

Scheme 3 shows the synthetic routes for benzenesulfonic acid-type derivatives and a related compound. Diami-



Scheme 3. Reagents: (a) $ClCO(CH_2)_mCH_3$, *i*-Pr_2EtN; (b) Na_2CO_3; (c) $ClCO(CH_2)_{16}CH_3$, *i*-Pr_2EtN; (d) Pd–C, $NaBH_4$, H_2.

nobenzenesulfonic acids **18** or 4-aminobenzenesulfonic acid **19** was acylated with appropriate acid chloride, then treated with sodium carbonate to afford bis(acylamino)benzenesulfonic acid sodium salts **4b–d**, **5d**, **6d**, **7d**, or mono(acylamino)benzenesulfonic acid sodium salt **8**. In the case of benzoic acid **9d**, first of all, 2-amino-4-nitrobenzoic acid **20** was hydrogenated in the presence of palladium–carbon and sodium borohydride, then the obtained 2,4-diaminobenzoic acid **21** was converted into the target compound **9d**.

3. Results and discussion

In the previous paper, we reported that we had synthesized novel derivatives of sulfatides and established the following SAR: (1) sulfate group was the most favorable among investigated functional groups, (2) the galactose moiety can be deleted or replaced with another structure without a large decrease in the inhibitory activity, (3) β-(*N*-acylamino)alcohol moiety of sulfatides can be replaced with another structure, (4) the existence of two long alkyl chains is essential for potent inhibitory activity. In addition, the synthesis of novel derivatives of sulfatides led to the discovery of novel scavenger receptor inhibitors, such as compound **1**.

Compound **1** has two long alkyl chains, which can be located in all directions. We were interested in fixing the orientations of the two long chains, and we designed compounds **2**, **3a–d**, and **4b–d** (see Fig. 2), and synthesized them. In order to monitor the inhibitory activities of the synthesized compounds against acetyl-LDL binding to scavenger receptors, all the compounds were tested for an inhibitory activity against the incorporation of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetyl-LDL (DiI-acetyl-LDL) into macrophages at the concentration of 100 μM at 37 °C.[18] When a tested compound showed an inhibitory activity of more than 50%, the compound was also tested in lower concentrations. The obtained results are summarized in Tables 1 and 2.

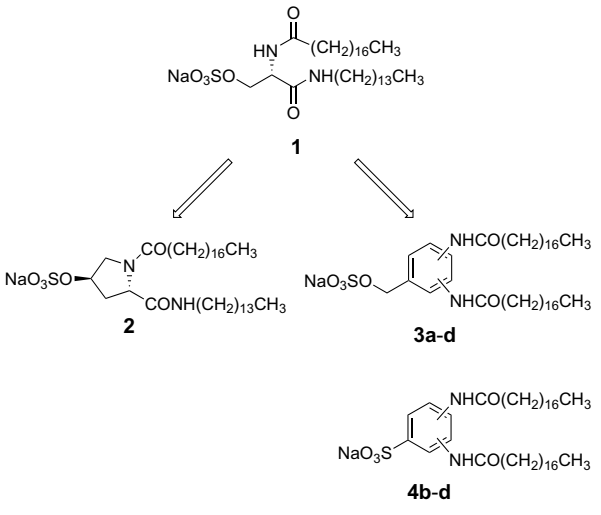


Figure 2. Design of novel derivatives of compound **1**.

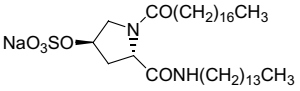
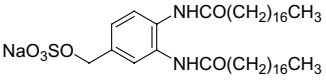
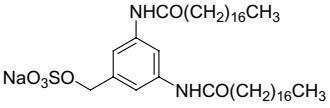
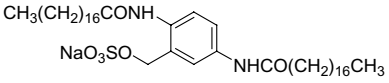
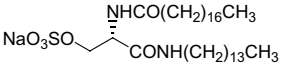
The 4-hydroxyproline derivative **2** was a weaker inhibitor than compound **1**, though compound **2** has the same two long chains as compound **1**. Among the benzyl sulfate derivatives **3a–c**, the 3,5-disubstituted benzyl derivative **3b** showed the most potent inhibitory activity against the DiI-acetyl-LDL incorporation. The 2,5-disubstituted benzyl derivative **3c** was slightly less potent than **3b**. The inhibitory activity of the 3,4-disubstituted benzyl derivative **3a** was less than 10% at 100 μM. These results might mean that the substitution pattern of two octadecanoylamino moieties on the ring is related to the potency. That is to say, when the substitution pattern of two octadecanoylamino moieties is so-called *meta*, a comparatively strong inhibitory activity might be obtained, and when the pattern is *ortho*, a comparatively weak inhibitory activity might be obtained. The two long chains of compound **2**, as well as those of compound **3a**, are substituted next to each other on the ring. The reason why compound **2** was a weak inhibitor could be explained by the undesirable substitution pattern of the two long chains.

Next, we were interested in the inhibitory activities of the benzenesulfonic acid-type derivatives, which were thought to be more stable than the benzyl sulfate-type derivatives. The potency of the 3,5-disubstituted derivative **4b** was almost equal to that of the corresponding benzyl sulfate derivative **3b**. Interestingly, also in the benzenesulfonic acid series, the *para*-substitution derivative **4c** was not as potent as the *meta*-substitution derivative **4b**, which was consistent with the result observed in the benzyl sulfate series. The 2,4-disubstituted derivative **4d**[19] with a *meta*-substitution pattern exhibited the strongest activity in the benzenesulfonic acid series.

Then, substitution pattern was fixed to 2,4-disubstitution, and the effect of the length of two acyl moieties was investigated. The tetradecanoyl derivative **6d** and the docosadecanoyl derivative **7d** were slightly less potent than **4d**, and the decanoyl derivative **5d** was inactive at 100 μM. In order to clarify whether both of the two octadecanoylamino moieties of **4d** are required for the excellent potency, the inhibitory activity of the 4-monosubstituted derivative **8** was tested. Compound **8** exhibited a very weak activity of less than 10% at 100 μM. This result indicates that both of the two long chains are necessary for the potent inhibitory activity. The carboxylic acid derivative **9d** was much less potent than the sulfonic acid derivative **4d**, meaning that sulfonic acid is a more preferable functional group than carboxylic acid.

Finally, the inhibitory activity of compound **4d** against the binding of ^{125}I-labeled acetyl-LDL to scavenger receptor was tested,[20] and compared with those of compound **1** and sulfatides. As apparent from Table 3, compound **4d** showed 10 times stronger ^{125}I-acetyl-LDL binding inhibitory activity ($IC_{50} = 2.0\,\mu M$) than compound **1**. The potency of compound **4d** was thought

Table 1. Effects of the 4-hydroxylproline derivative **2** and benzyl sulfates **3a–c** on the incorporation of DiI-acetyl-LDL into macrophages

Compound	Structure	Inhibitory activity against the incorporation of DiI-acetyl-LDL (%)[a]		
		100 μM	30 μM	10 μM
2		23.7(1)	NT[b]	NT[b]
3a		<10(1)	NT[b]	NT[b]
3b		63.0(3)	40.4(2)	NT[b]
3c		40.8(1)	NT[b]	NT[b]
1		61.4(5)	43.8(4)	20.5(2)

[a] The number on the left side of parentheses represents %inhibition at each concentration. The number inside parentheses represents the number of experiment.
[b] NT: not tested.

Table 2. Effects of benzenesulfonic acids and related compounds on the incorporation of DiI-acetyl-LDL into macrophages

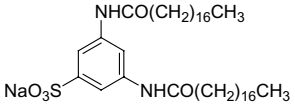
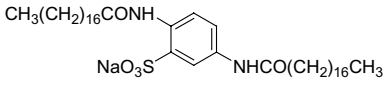
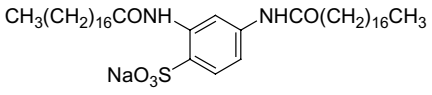
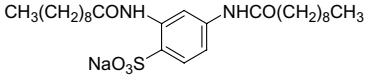
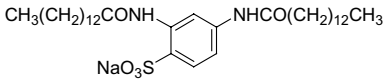
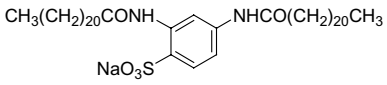
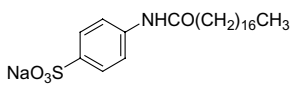
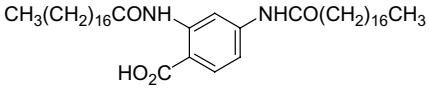
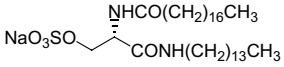
Compound	Structure	Inhibitory activity against the incorporation of DiI-acetyl-LDL (%)[a]		
		100 μM	30 μM	10 μM
4b		71.2(3)	46.2(3)	18.5(2)
4c		32.1(1)	NT[b]	NT[b]
4d		84.6(3)	72.5(3)	57.0(2)
5d		<10(1)	NT[b]	NT[b]
6d		66.6(1)	48.0(1)	NT[b]
7d		85.7(1)	48.0(1)	NT[b]
8		<10(1)	NT[b]	NT[b]

Table 2 (*continued*)

Compound	Structure	Inhibitory activity against the incorporation of DiI-acetyl-LDL (%)[a]		
		100 μM	30 μM	10 μM
9d		<10(1)	NT[b]	NT[b]
1		61.4(5)	43.8(4)	20.5(2)

[a] The number on the left side of parentheses represents %inhibition at each concentration. The number inside parentheses represents the number of experiment.
[b] NT: not tested.

Table 3. Effects of compounds **4d**, **1**, and sulfatides on the binding of ^{125}I-acetyl-LDL to macrophages

Compound	Inhibitory activity against the binding of ^{125}I-acetyl-LDL, IC_{50}[a]
4d	2.0 μM
1	41.8% at 30 μM (2)[b,c]
Sulfatides	2.6 μg/mL

[a] IC_{50} represents the concentration required 50% inhibition against the binding of ^{125}I-acetyl-LDL.
[b] The number inside parentheses represents the number of experiment at each concentration.
[c] 81.1% at 100 μM (2), and 6.6% at 10 μM (2).

to be almost the same as that of sulfatides ($IC_{50} = 2.6$ μg/mL). Compound **4d** is being evaluated in an in vivo model of atherosclerosis. The results will be presented in a subsequent paper.

4. Conclusion

In conclusion, we succeeded in the discovery of novel scavenger receptor inhibitor **4d** whose inhibitory activity against ^{125}I-acetyl-LDL binding was 10 times more potent than that of compound **1**. The substitution pattern of two long acylamino chains on the benzene ring was considered to be associated with the inhibitory activity. The obtained information would be very useful for the design of effective scavenger receptor inhibitors.

References and notes

1. Stary, H. C.; Chandler, A. B.; Glagov, S.; Guyton, J. R.; Insull, W.; Rosenfeld, M. E.; Schaffer, S. A.; Schwartz, C. J.; Wagner, W. D.; Wissler, R. W. *Arterioscler. Thromb.* **1994**, *14*, 840.
2. Gerrity, R. G. *Am. J. Pathol.* **1981**, *103*, 181.
3. Stary, H. C. *Atherosclerosis* **1987**, *64*, 91.
4. Schwartz, C. J.; Valente, A. J.; Sprague, E. A.; Kelley, J. L.; Cayatte, A. J.; Mowery, J. *Circulation* **1992**, *86*(Suppl III), 117.
5. Kodama, T.; Freeman, M.; Rohrer, L.; Zabrecky, J.; Matsudaira, P.; Krieger, M. *Nature* **1990**, *343*, 531.
6. Rohrer, L.; Freeman, M.; Kodama, T.; Penman, M.; Krieger, M. *Nature* **1990**, *343*, 570.
7. Gough, P. J.; Greaves, D. R.; Gordon, S. *J. Lipid Res.* **1998**, *39*, 531.
8. Endemann, G.; Stanton, L. W.; Madden, K. S.; Bryant, C. M.; Wite, R. T.; Protter, A. A. *J. Biol. Chem.* **1993**, *268*, 11811.
9. Elomaa, O.; Kangas, M.; Sahberg, C.; Tuukkanen, J.; Sormunrn, R.; Liakka, A.; Thesleff, I.; Kraal, G.; Tryggvason, K. *Cell* **1995**, *80*, 603.
10. Acton, S. L.; Scherer, P. E.; Lodish, H. F.; Krieger, M. *J. Biol. Chem.* **1994**, *269*, 21003.
11. Ramprasad, M. P.; Fischer, W.; Witztum, J. L.; Sambrano, G. R.; Quehenberger, O.; Steinberg, D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9580.
12. Pearson, A.; Lux, A.; Krieger, M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4056.
13. Sawamura, T.; Kume, N.; Aoyama, T.; Moriwaki, H.; Hoshikawa, H.; Aiba, Y.; Tanaka, T.; Miwa, S.; Katsura, Y.; Kita, T.; Masaki, T. *Nature* **1997**, *385*, 73.
14. Adachi, H.; Tsujimoto, M.; Arai, H.; Inoue, K. *J. Boil. Chem.* **1997**, *272*, 31217.
15. Suzuki, H.; Kurihara, Y.; Takeya, M.; Kumada, N.; Kataoka, M.; Jishage, K.; Ueda, O.; Sakaguchi, H.; Higashi, T.; Suzuki, T.; Takashima, Y.; Kawabe, Y.; Cynshi, O.; Wada, Y.; Honda, M.; Kurihara, H.; Aburatani, H.; Doi, T.; Matsumoto, A.; Azuma, S.; Noda, T.; Toyoda, Y.; Itakura, H.; Yazaki, Y.; Horiuchi, S.; Takahashi, K.; Kruijt, J. K.; van Berkel, T. J. C.; Steinbrecher, U. P.; Ishibashi, S.; Maeda, N.; Gordon, S.; Kodama, T. *Nature* **1997**, *386*, 292.
16. Brown, M. S.; Basu, S. K.; Falck, J. R.; Ho, Y. K.; Goldstein, J. L. *J. Supramol. Struct.* **1980**, *13*, 67.
17. Yoshiizumi, K.; Nakajima, F.; Dobashi, R.; Nishimura, N.; Ikeda, S. *Bioorg. Med. Chem.* **2002**, *10*, 2445.
18. The details of the method are described in Ref. 17.
19. ^{1}H NMR (250 MHz, DMSO-d_6) δ: 0.84 (t, $J = 6.5$ Hz, 6H), 1.24 (s, 56H), 1.40–1.70 (m, 4H), 2.24 (t, $J = 6.3$ Hz, 2H), 2.27 (t, $J = 6.2$ Hz, 2H), 7.44 (dd, $J = 1.8$, 8.7 Hz, 1H), 7.52 (d, $J = 8.7$ Hz, 1H), 8.39 (d, $J = 1.8$ Hz, 1H), 9.94 (s, 1H), 10.42 (s, 1H). Anal. Calcd for $C_{42}H_{75}N_2NaO_5S{\cdot}0.5H_2O$: C, 67.07; H, 10.18; N, 3.72; found: C, 67.02; H, 10.09; N, 3.84.
20. The details of the method are described in Ref. 17.