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Synthesis of 1-thio-phytosphingolipid analogs by microwave promoted reactions of thiols and aziridine derivatives

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ABSTRACT

A practical and versatile method for the synthesis of 1-thio-phytosphingolipid analogs through regioselective nucleophilic ring-opening reactions of phytosphingosine aziridine derivatives with thiols is described. The reactions were carried out with *N*-acylaziridines and a variety of thiol compounds. Microwave irradiation highly improved the yield of the ring-opening reaction and the intermediate *N*-acyl adducts were converted into 1-*S*-phytosphingolipid analogs, such as phytoceramide and phytosphingosine derivatives.

Sphingolipids are essential biomolecules for the physiological cell function. In addition to its structural role in cell membranes, these lipids have also crucial functions in signal transduction and cell regulation.[1–3] Glycosphingolipids having a phytoceramide scaffold have been the object of attention since the discovery of their immunostimulant activity.[4] Particularly α-galactosylceramides, such as the natural agelasphins, the synthetic KRN7000, and other synthesized thioglycloside analogs have been studied for their effect on NKT cell activation.[5–7] As a consequence of the important biological roles of sphingolipids, the synthesis of their analogs and the development of chemical inhibitors of sphingolipid enzymes are the object of current interest.[8–11]

In this context, we are interested in the development of a synthetic methodology for the obtention of 1-thio-phytosphingolipid analog libraries at milligram scale to find active modulators of sphingolipid metabolism. To achieve this aim, we envisaged the preparation of thiosphingolipid analogs through nucleophilic ring-opening reactions of an aziridine sphingolipid with thiols. The main advantage of this strategy would be its high versatility, allowing the obtention of different structural analogs from a common aziridine precursor by simply changing the sulfur nucleophile.

It was previously described that the synthesis of *N*-nosylaziridine derivatives from phytosphingosine hydrochloride **1**, is useful for obtaining 1,2-diaminophytosphingolipids by reaction with nitrogen nucleophiles.[12] This sulfonyl group conferred a high reactivity to aziridine and it was easily deprotected by the reaction with thiolates.[13] Although some examples of ring-opening reactions of *N*-nosylaziridines with thiolates have been described,[13] it was considered that the ring-opening reactions of *N*-nosyl aziridines with thiols might not be of general application for the preparation of thio-phytosphingolipid analogs. Therefore, a different nitrogen activating group should be introduced in aziridine **2** to obtain the desired thioether sphingolipids. Different nitrogen substituents have been reported to activate aziridines for thiol opening. Since in our case most of the desired modifications in the sphingolipid framework contain *N*-acyl groups, it was considered that *N*-acylaziridines would be an optimal solution because this would directly lead to ceramides avoiding the formation and cleavage steps of other groups and increasing the synthetic efficiency. Moreover we thought that this approach could be expanded to the synthesis of phytosphingosine derivatives having a free amino group if a benzyl carbamate was used in the aziridine intermediate as amino protecting group.

The described synthesis of aziridine **2**[12] from phytosphingosine hydrochloride **1** started with the protection of the amino functionality of **1** as an azide group with trifluoromethanesulfonyl azide (TfN_3) to afford azido-phytosphingosine **3**, followed by the selective protection of the primary alcohol with *t*-butyldiphenylsilyl chloride (TBDPSCl) to get diol **4** in direct analogy with the reported synthesis of D-*erythro*-sphingosine and D-*ribo*-phytosphingosine from phytosphingosine.[14] Then, conversion of the 3,4-vicinal diol **4** into its cyclic isopropylidene acetal in the presence of 2,2-dimethoxypropane (DMP) and *p*-toluenesulfonic acid (pTSA) to give **5**

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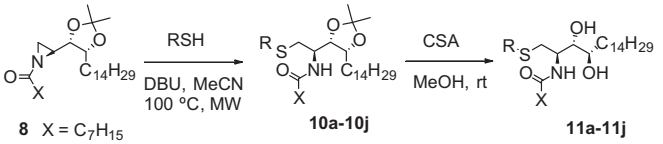
and the subsequent deprotection of the primary alcohol with tetrabutylammonium fluoride (TBAF) afforded intermediate **6**.[15] Transformation of the alcohol function of **6** in a good leaving group after mesylation reaction in the presence of methanesulfonyl chloride (MsCl) reached aziridine precursor **7**, which afforded aziridine derivative **2** after Staudinger reduction of **7** with in situ intramolecular cyclization.[12]

Then, the *N*-acylaziridines were prepared from known aziridine **2**,[12] by the reaction with octanoyl chloride or benzylchloroformate to give aziridines **8** and **9**, respectively (Scheme 1). Ring-opening reactions of **8** and **9** with mercaptans followed by diol deprotection, would lead to the thio-phytosphingolipid analogs.

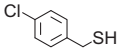
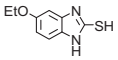
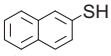
Literature reports for nucleophilic opening of acylaziridines with thiols are known but limited.[16–19] We decided to explore the reaction of thiophenol and aziridine **8**, according to the procedure[16] reported by Galonic et al. for the conjugation of thiols with *N*-acylaziridine-containing peptides. When aziridine **8** was reacted at room temperature with thiophenol (1.2 mol-equiv) in the presence of DBU (0.1 mol-equiv) in MeCN, only partial consumption of the starting aziridine **8** was observed after 12 h of reaction. It was necessary to increase the amount of DBU (1 mol-equiv) and to warm the mixture to 85 °C for 3 days to afford the ring-opened product in a 55% yield. Attempts to optimize the reaction by modifications on the relative amount of reagents, solvent, or other parameters were unsuccessful. However, when the reaction was performed under microwave irradiation (MW), its rate was impressively improved over the thermal reaction. The reaction time reduced from 4 days to 5 min and the yield in **10a** increased from 55% to 90% (Table 1, entry 1). In all cases, the nucleophilic attack was giving exclusively the product resulting from reaction at the less hindered aziridine carbon. Under MW conditions, the acylated products **10a–10j** could be smoothly obtained from starting aziridines **8** and **9** and different thiols.[20] Moreover, the acidic cleavage of isopropylidene acetal in direct analogy with previously reported diol deprotections in phytoceramide synthetic intermediates[21] gave the final thio-phytoceramide analogs **11a–11j** (Table 1) in two steps from aziridine derivatives.[22]

The reactivity of aziridines **8** and **9** (Table 1, entries 1 and 8) with thiophenol was similar, giving the corresponding adducts in high yields with short reaction times. A variety of thiols can be used including aliphatic and aromatic ones. When *t*-butyl mercaptan (Table 1, entry 7) was used the corresponding thiosphingolipid **10g** was obtained in a good yield, this requiring an additional closed reaction flask inside the microwave vessel to avoid thiol losses due to its volatility. Other thiols such as 4-chlorobenzylthiol (Table 1, entry 3), 2-naphthalenethiol, (Table 1, entry 9) and octyl mercaptan

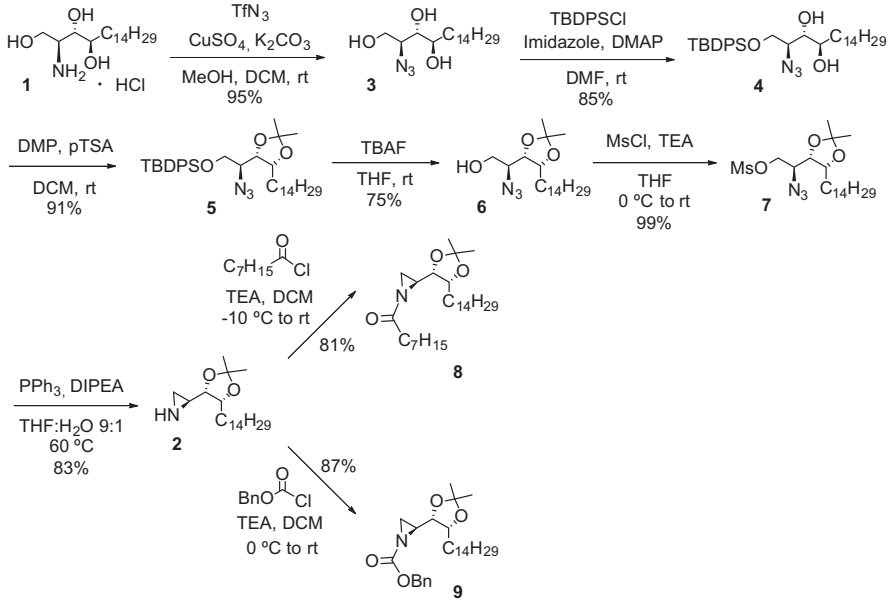
Table 1
Ring-opening reaction of *N*-acylaziridines with thiols under microwave irradiation for the synthesis of phytoceramides



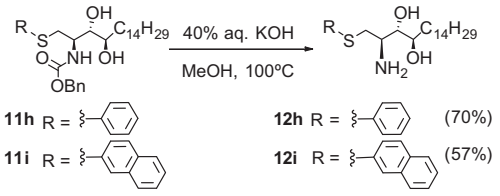
8 X = C_7H_{15}
9 X = OBn
10a-10j
11a-11j

Entry	R-SH	Aziridine	Time (min)	Yield[a] (%)	Yield[b] (%)
1		**8**	5	**10a**: 90	**11a**: 60
2		**8**	80	**10b**: 66	**11b**: 81
3		**8**	20	**10c**: 60	**11c**: 75
4		**8**	60	**10d**: 87	**11d**: –
5		**8**	50	**10e**: 53	**11e**: 95
6		**8**	50	**10f**: 56	**11f**: 98
7		**8**	5	**10g**: 80	**11g**: 68
8		**9**	5	**10h**: 87	**11h**: 90
9		**9**	15	**10i**: 75	**11i**: 77
10		**9**	20	**10j**: 82[c]	**11j**: 97

[a] Isolated yields of ring-opening reactions.
[b] Isolated yields of diol deprotection reactions.
[c] 1.6 mol-equiv of RSH were used.



Scheme 1. Synthesis of aziridine derivative **2** from phytosphingosine hydrochloride **1** and acylation reactions with octanoyl chloride and benzylchloroformate.



Scheme 2. Carbamate cleavage of diol **11h** and **11i.**

(Table 1, entry 10) needed longer reaction times than thiophenol and produced **10c**, **10i**, and **10j**, respectively in moderate to high yields. Other mercaptans such as 2-mercaptobenzyl alcohol (Table 1, entry 2), 5-ethoxy-2-mercaptobenzimidazole (Table 1, entry 4), furfuryl mercaptan, (Table 1, entry 5) and cyclohexanethiol (Table 1, entry 6) required even longer reaction times for starting aziridine consumption. In general, extended reaction times were detrimental for reaction yields.

The final diol deprotection in acidic MeOH was achieved in moderate to high yields, except for compound **11d** that decomposed to a mixture of products under these conditions.

It is noteworthy that diols **11h, 11i** afforded thio-phytosphingosines **12h, 12i** (Scheme 2)[23] by the cleavage of carbamate group in the presence of 40% aq KOH in MeOH, according to a related literature procedure.[24] Catalytic hydrogenolysis was not effective for the benzylcarbamate cleavage, presumably due to catalyst poisoning by the sulfur functionality. The N-acylation of these sphingosine derivatives represents an alternative way to obtain thio-ceramides increasing the versatility of the aziridine route toward 1-*S*-phytosphingolipids.

In summary, we synthesized 1-thio-phytosphingolipid analogs in moderate to good yields by means of regioselective nucleophilic ring-opening reactions of acylated aziridine derivatives **8** and **9** followed by acetal deprotection. This synthetic methodology led us to obtain phytosphingosine analogs by benzylcarbamate cleavage, which would afford other ceramide analogs by subsequent acylation of the resulting amino group. This approach is a short and flexible route for the synthesis of a variety of 1-thio-phytosphingolipids by changing the thiol in the ring-opening step or the acylating agent in the acylation of phytosphingosine analogs.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.066. These data include MOL files and InChiKeys of the most important compounds described in this article.

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20. Typical procedure for microwave-enhanced nucleophilic ring-opening reaction of acylated aziridine **9** with thiophenol to afford **10h**: In a 10 mL vessel, a solution of aziridine derivative **9** (24.9 mg, 0.053 mmol) and thiophenol (350 μL, 0.066 mmol) in MeCN (500 μL) was prepared. After, DBU (9 μL, 0.060 mmol) was added and the mixture was stirred for 3 min under nitrogen. The vessel was sealed with a septum and placed into the microwave cavity. The microwave source was then turned on. Constant microwave irradiation (150 W, 689.5 kPa, 100 °C) as well as simultaneous air-cooling were used during the entire reaction time. The evolution of the reaction was monitored by TLC and total consumption of the starting aziridine was achieved after irradiating for 5 min. After cooling the reaction mixture to room temperature, the solvent was removed under reduced pressure to yield the crude product. *Flash* chromatographic purification (silica gel, hexane-ethyl acetate 9:1) afforded adduct **10h** as a colorless oil (26.9 mg, 87%). $[\alpha]_D^{20}$ −18.7 (*c* 1.06, $CHCl_3$). IR (film): ν = 3360, 2920, 2852, 1692, 1525, 1237, 1220, 1039 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.5 Hz, 2H), 7.32–7.23 (m, 5H), 7.22–7.15 (m, 2H), 7.12 (t, *J* = 7.2 Hz, 1H), 5.03 (d, A part of a AB system, J_{AB} = 12.2 Hz, 1H), 4.96 (d, B part of a AB system, J_{AB} = 12.2 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.08–3.98 (m, 2H), 3.97–3.87 (m, 1H), 3.22 (dd, A part of a AB system, J_{AB} = 13.9 Hz, *J* = 3.0 Hz, 1H), 3.09 (dd, B part of a AB system, J_{AB} = 13.9 Hz, *J* = 6.8 Hz, 1H), 1.43–1.28 (m, 3H), 1.35 (s, 3H), 1.28–1.14 (m, 26H), 0.82 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.7 (C=O), 136.4 (C), 136.0 (C), 130.7 (CH), 129.1 (CH), 128.6 (CH), 128.3 (CH), 128.1 (CH), 126.8 (CH), 108.2 (C), 77.9 (CH), 77.8 (CH), 67.0 (CH_2), 50.8 (CH), 37.5 (CH_2), 32.1 (CH_2), 29.9 (CH_2), 29.8 (2CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 27.8 (CH_3), 26.7 (CH_2), 25.5 (CH_3), 22.8 (CH_2), 14.3 (CH_3). HRMS calculated for $C_{35}H_{54}NO_4S$: 584.3774 $[M+H]^+$; found: 584.3752. Data for compounds **10a**–**10j** reported in Table 1 are collected in the Supplementary data.
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22. Typical procedure for diol deprotection reaction of ring-opened product **10h** to afford **11h**: To a solution of ring-opened product **10h** (61.7 mg, 0.11 mmol) in MeOH (10 mL), was added CSA (52.0 mg, 0.22 mmol). The mixture was stirred at room temperature overnight. Then, the solvent was removed under reduced pressure to give a crude that was purified by *flash* chromatography (silica gel, hexane–ethyl acetate 7:3) and pure diol 11 h was isolated as a white solid (53.9 mg, 90%). $[\alpha]_D^{20}$ −31.1 (c 1.43, $CHCl_3$). IR (film): ν = 3439, 3338, 3066, 3037, 2958, 2914, 2847, 1689, 1527, 1342–1244 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.30 (m, 7H_{ar}), 7.26 (t, *J* = 7.5 Hz, 2H_{ar}), 7.19 (t, *J* = 7.3 Hz, 1H_{ar}), 5.27 (d, *J* = 7.5 Hz, 1H (NH)), 5.06 (s, 2H (CH_2–O)), 4.00–3.92 (m, 1H (CH–N)), 3.71–3.63 (m, 2H (CH–O (3) and CH–O (4)), 3.38 (dd, A part of a AB system, J_{AB} = 13.6 Hz, *J* = 4.0 Hz, 1H (CH_2–S)), 3.27 (dd, B part of a AB system, J_{AB} = 13.6 Hz, *J* = 7.8 Hz, 1H (CH_2–S)), 1.78 (br s, 2H (2OH)), 1.65–1.55 (m, 1H), 1.51–1.42 (m, 1H), 1.41–1.35 (m, 1H), 1.35–1.18 (m, 23H), 0.88 (t, *J* = 6.9 Hz, 3H (CH_3)). ^{13}C NMR (101 MHz, CD_3OD) δ 158.4 (C=O), 138.4 (C_{ar}), 138.2 (C_{ar}), 130.5 (CH_{ar}), 129.9 (CH_{ar}), 129.4 (CH_{ar}), 128.9 (CH_{ar}), 128.7 (CH_{ar}), 126.9 (CH_{ar}), 77.6 (CH–O), 73.2 (CH–O), 67.4 (CH_2–O), 53.9 (CH–N), 35.8 (CH_2–S), 33.6 (CH_2), 33.1 (CH_2), 30.8 (3CH_2), 30.5 (CH_2), 26.8 (CH_2), 23.7 (CH_2), 14.4 (CH_3). HRMS calculated for $C_{32}H_{49}NO_4NaS$: 566.3280 $[M+Na]^+$; found: 566.3270. mp: 107–109 °C. Data for compounds **11a**–**11j** reported in Table 1 are collected in the Supplementary data.
23. Typical procedure for carbamate cleavage of product **11h** to afford aminodiol **12h**: To a solution of carbamate **11h** (19.1 mg, 0.035 mmol) in MeOH (500 μL), was added 40% aq KOH (240 μL, 0.18 mmol). The mixture was heated to 100 °C to give complete conversion after 24 h. After, the solvent was removed in vacuo to give crude, which was purified by *flash* chromatography (silica gel, hexane–ethyl acetate 2:8 + 1% aq NH_3) to afford aminodiol **12h** as a colorless oil (10.1 mg, 70%). $[\alpha]_D^{20}$D −29.2 (c 0.98, $CHCl_3$). IR (film): ν = 3351, 3276, 3060, 2920, 2850, 1736, 1584, 1468 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 7.43 (d, *J* = 7.5 Hz, 2H_{ar}), 7.30 (t, *J* = 7.7 Hz, 2H_{ar}), 7.19 (t, *J* = 7.4 Hz, 1H_{ar}), 3.55–3.47 (m, 2H (CH–O (4) and A part of a CH_2–S AB system)), 3.42 (dd, *J* = 8.3, 4.5 Hz, 1H (CH–O (3)), 3.21–3.14 (m, 1H (CH–N)), 2.84 (dd, B part of a AB system, J_{AB} = 13.9 Hz, *J* = 10.4 Hz, 1H (CH_2–S)), 1.78–1.71 (m, 1H), 1.60–1.50 (m, 1H), 1.38–1.27 (m, 24H), 0.90 (t, *J* = 6.9 Hz, 3H (CH_3)). ^{13}C NMR (101 MHz, CD_3OD) δ 136.8 (C_{ar}), 130.4 (CH_{ar}), 130.1 (CH_{ar}), 127.3 (CH_{ar}), 76.5 (CH–O (3)), 73.9 (CH–O (4)), 53.3 (CH–N), 36.4 (CH_2–S), 35.3 (CH_2), 33.1 (CH_2), 30.9 (CH_2), 30.8 (2CH_2), 30.5 (CH_2), 26.5 (CH_2), 23.8 (CH_2), 14.5 (CH_3). HRMS calculated for $C_{24}H_{44}NO_2S$: 410.3093 $[M+H]^+$; found: 410.3087. Data for compound **12i** reported in Scheme 2 are collected in the Supplementary data.
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