TOWARDS THE SYNTHESIS OF SCYPHOSTATIN

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Abstract - Stereocontrolled synthesis of the substituted cyclohexenone segment (2) of scyphostatin (1) is described starting from D-glucose.

Scyphostatin (1) is a novel neutral sphingomyelinase (N-Smase) inhibitor isolated\(^1\) during the search for N-Smase inhibitors from the fermentation broth of *Dascysphus mollissimus* SANK-13892. N-Smase inhibitors are useful in the regulation of ceramide level and thus are of immense need in the therapy of autoimmune diseases and inflammation.\(^2\) Scyphostatin (1) is a structurally unique natural product containing an unprecedented cyclohexenone epoxide moiety in which the tertiary chiral center at C-4 is linked to an n-propylamino alcohol and a lipophilic side chain[C(1′)-C(20′)]. The structure of scyphostatin (1) was determined by NMR and MS spectral studies. Chemical and spectroscopic analysis, coupled with modified Mosher’s method, provided stereo-chemical configuration of the cyclohexenone epoxide ring structure of scyphostatin. However, the initial report\(^1\) did not confirm the absolute configuration of the lipophilic side chain. Later Saito *et al.* has confirmed\(^3\) the absolute and relative configurations by degradation of 1 and chemical correlation to synthetic fragments. Hoye *et al.*\(^4\) have synthesized the lipophilic side chain and till date there is no single report on synthesis of polar cyclohexenone moiety. As part of our interest, we have embarked on developing a carbohydrate based strategy to prepare the suitably substituted cyclohexenone derivative (2) present in the scyphostatin (1). D-Glucose was converted\(^5\) into methyl 3-O-benzyl-4,6-benzylidene-α-D-glucopyranoside (3) whose oxidation at C-2 under
Swern conditions, gave the 2-ulose derivative (4) (Scheme 1). Subsequent C-allylation of 4 was studied under various conditions. However, we observed that 4 with allylmagnesium bromide in anhydrous ether/CH2Cl2 at –78 °C gave a 9:1 diastereomeric mixture of tertiary alcohols (5a) and (5b) which were separated by silica gel column chromatography. The major product was subjected to NOE studies which unequivocally proved the structure as 5a while the structure of the minor product (5b) was unambiguously assigned based on interesting chemical transformations.

![Scheme 1](image)

**Scheme 1:** a) (COCl)2, DMSO, Et3N, CH2Cl2, -78 °C, 89 %; b) allylmagnesium bromide, Et2O:CH2Cl2 (4:1), -78 °C, 68 %; c) NaH, BuBr, DMF, n-Bu4NI, 0 °C-rt, 97 %.

The derived O-benzyl derivative (6) was reduced with LiAlH4-AlCl3 to give rise to the 4-0-benzyl derivative (7) containing a primary hydroxy group at C-6. Treatment of 7 with Ph3P-I2-imidazole in toluene gave the 6-deoxy-6-iodo derivative (8) (Scheme 2). Catalytic osmylation of 8 in t-butanol-water gave a 4:1 mixture of diastereomeric products whose acetonide derivatives (9a/9b) could be separated by silica gel column chromatography. In accordance with Sharpless modifications, use of AD-MIX-α provided predominantly the diastereomer (9b) whose stereochemical assignments were based on hypothesis reported by Sharpless et al. Compound (9b) was treated with DBU in DMF at 100 °C for 12 h to give the 5,6-ene derivative (10). The Ferrier rearrangement of 10 in the presence of Hg(OAc)2 in aqueous acetone gave the carbocyclic derivative (11) whose dehydration in the presence of MsCl-Et3N-CH2Cl2 provided the α, β-unsaturated derivative (12) (Scheme 3). In order to rearrange 12, into the target structure (2), an approach based on Barton’s radical opening reaction of α,β-epoxythioimidazolide was investigated (Scheme 3).

Compound (12) was epoxidised with 30 % hydrogen peroxide-K2CO3 in THF-H2O to give 13 which was consequently reduced with NaBH4 in methanol to give the epoxy alcohol (14). In the 1H-NMR spectrum of 14, epoxy protons were clearly visible at 3.23 (doublet, J = 3.8 Hz) and 3.35 ppm (triplet, J = 3.8 Hz). Conversion of 14 into the thiocarbonate derivative (15) was accomplished with N,N'-thiocarbonyldiimidazole in
refluxing C₆H₆. The ¹H-NMR spectrum of 15 showed characteristic downfield shift of the proton carrying thiocarbonate group. Treatment of 15 with Bu₃SnH and cat. AIBN in refluxing benzene gave a mixture of products from which the major product was isolated (30 %) by silica gel chromatography. Subsequent oxidation with MnO₂ in refluxing CH₂Cl₂ provided the target cyclohexenone derivative (2). In the ¹H-NMR spectrum of 2, the characteristic signals due to olefinic protons were located at 5.96 (dd, J = 2.04, 10.39 Hz) and 6.85 ppm (dd, J = 1.89, 10.39 Hz). Rest of the spectral data was in conformity with the assigned structure (2).
In conclusion we have reported for the first time the stereocontrolled synthesis of a novel and highly substituted cyclohexenone ring system of scyphostatin.

ACKNOWLEDGEMENT
SH thanks CSIR, New Delhi for the financial support in the form of Senior Research Fellowship.

REFERENCES

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\begin{align*}
5b & \xrightarrow{a,b} c & \xrightarrow{d} \\
\text{a)} & \text{NaH, BnBr, DMF, rt, 90\%;} & \text{b)} & \text{LiAlH}_4, \text{AlCl}_3, \text{Et}_2\text{O, CH}_2\text{Cl}_2, 15 \text{ min, 95\%;} & \text{c)} & (\text{COCl})_2, \text{DMSO, Et}_3\text{N, CH}_2\text{Cl}_2, -78^\circ\text{C, 80\%;} & \text{d)} & \text{Grubbs catalyst, C}_6\text{H}_5\text{Me, reflux, 3 days, 45\%.}
\end{align*}
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14. Structures of new compounds were established by $^1$H NMR, FABMS/HRMS or microanalysis. The $^1$H NMR spectral data of some key intermediates are given below:

5a (400 MHz, CDCl$_3$): 2.29 (dd, 1 H, J = 7.6, 15.3 Hz), 2.57 (dd, 1 H, J = 3.8, 15.3 Hz), 3.35 (s, 3 H), 3.65 (d, 1 H, J = 7.7 Hz), 3.83 (m, 2 H), 4.10 (m, 1 H), 4.32 (m, 1 H), 4.40 (s, 1 H), 4.65, 4.95 (ABq, 2 H, J = 15.6 Hz), 5.10 (m, 2 H), 5.60 (s, 1 H), 5.85 (m, 1 H), 7.30 (m, 10 H); 8 (200 MHz, CDCl$_3$): 2.27 (dd, 1 H, J = 9.0, 14.5 Hz), 3.09 (dd, 1 H, J = 4.5, 14.5 Hz), 3.27 (dd, 1 H, J = 6.8, 10.0 Hz), 3.38 (s, 3 H), 3.45 (m, 2 H), 3.75 (d, 2 H, J = 4.5 Hz), 4.52, 4.73 (ABq, 2 H, J = 10.2 Hz), 4.61, 4.79 (ABq, 2 H, J = 11.4 Hz), 4.65, 4.88 (ABq, 2 H, J = 10.9 Hz), 4.72 (s, 1 H), 5.11 (m, 2 H), 5.81 (m, 1 H), 7.30 (m, 15 H); 9 (200 MHz, CDCl$_3$): 1.29, 1.36 (2s, 6 H), 1.70 (dd, 1 H, J = 9.0, 15.9 Hz), 2.77 (dd, 1 H, J = 4.3, 15.9 Hz), 3.29 (dd, 1 H, J = 8.1, 11.3 Hz), 3.40 (s, 3 H), 3.47 (t, 3 H, J = 9.0 Hz), 3.75 (m, 2 H), 3.95 (dd, 1 H, J = 6.8 Hz), 4.20 (m, 1 H), 4.45 (s, 1 H), 4.63, 4.79 (ABq, 2 H, J = 11.5 Hz), 4.70, 4.90 (ABq, 2 H, J = 11.2 Hz), 4.75, 5.06 (ABq, 2 H, J = 11.5 Hz), 7.30 (m, 15 H); 12 (200 MHz, CDCl$_3$): 1.15, 1.31 (2s, 6 H), 2.04 (m, 1H), 2.18 (dd, 1 H, J = 2.3, 15.9 Hz), 3.25 (m, 1 H), 3.56 (m, 2 H), 3.88 (d, 1 H, J = 9.0 Hz), 4.54, 4.77 (ABq, 2 H, J = 10.5 Hz), 4.61, 5.00 (ABq, 2 H, J = 11.4 Hz), 4.67, 5.11 (ABq, 2 H, J = 11.4 Hz), 4.68 (d, 1 H, J = 9.0 Hz), 6.04 (d, 1 H, J = 9.0 Hz), 6.90 (d, 1 H, J = 9.0 Hz), 7.30 (m, 15 H); 2 (500 MHz, CDCl$_3$): 1.22, 1.30 (2s, 6 H), 2.05 (dd, 1 H, J = 5.0, 13.8 Hz), 2.69 (dd, 1 H, J = 7.5, 13.7 Hz), 3.53 (t, 1 H, J = 7.7 Hz), 3.98 (d, 1 H, J = 7.4 Hz), 4.00 (t, 1 H, J = 7.6 Hz), 4.14 (m, 1 H), 4.42, 4.60 (ABq, 2 H, J = 11.6 Hz), 4.78 (s, 2 H), 4.75, 5.13 (ABq, 2 H, J = 11.6 Hz), 4.77 (ddd, 1 H, J = 1.9, 2.1, 7.7 Hz), 5.96 (dd, 1 H, J = 2.1, 10.4 Hz), 6.85 (dd, 1H, J = 1.89, 10.4 Hz), 7.3 (m, 15 H).