Spinosyn G: Proof of Structure by Semisynthesis

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Natural products that contain the tricyclic core

ikarugamycin

(-)-spinosyn A, R = H
a.k.a. (-)-A83543A, (-)-lepicidin A
(-)-spinosyn D, R = Me

capsimycin

FR182877

hexacyclinic acid
• Spinosyn A isolated in 1990 from the fermentation broth of the soil microbe *Saccharopolyspora spinosa*.

• Spinosad (Tracer®, Naturalyte®, SpinTor® and Conserve®) is an environmentally benign insecticide marketed by Dow-Elanco for crop protection. Spinosad is marketed to the retail market for the control of fire ants.

• Manufactured by fermentation and consists of a 90:10 mixture of spinosyn A and spinosyn D.

• 24 spinosyns have been characterized.

• Based on tentative assignments, Spinosyn G is the only compound with a sugar of the L-configuration (L-ossamine).

• Is the assignment of spinosyn G correct?
Proposed Mechanism for the formation of Spinosyn from the PKS Product

Biosynthesis of Forosamine


(-)-spinosyn A
Roush’s Synthesis of (-)-Spinosyn A


CHO
RhamO
Br
O
Me
OPMB
O
Et
O
P
O
OEt
OEt
O
O
Me
OPMB
Et
Br
RhamO
H
H
i-Pr2NEt, LiCl, CH3CN (1 mM)
23 °C, 19 h, 75%
E/Z = >95:5
ds = 73:12:9:6

Me3P (8 eq)
t-amyl alcohol (0.005M)
23 °C, 6 h, quantitative
26:27:C(3)-epi-26 = 88:7:5

1) tris(trimethylsilyl)3SiH, AIBN, dioxane, 80 °C, 1.5 h
2) DDQ, CH2Cl2/pH 7 buffer
0 °C, 3 h, 73% (2-steps)

(-)-spinosyn A pseudoaglycon

Completion of the Synthesis

1) guanidinium nitrate, NaOMe, MeOH, CH₂Cl₂, 95%
2) SnCl₂, PhSH, Et₃N, THF, 92%
3) CH₂O, NaBH₃CN, MeOH, HOAc, NaOAc, 87%

1) thiocarbonyldiimidazole, DMAP, toluene
2) Bu₃SnH (25 eq), AIBN, dioxane, 35%

23 steps (longest linear)
31 steps total
3% overall yield

(-)-spinosyn A

Mergott, D. J.; Frank, S. A.; Roush, W. R. PNAS, 101, 11955-11959.
Evans’ Synthesis of (+)-Spinosyn A

Evans’ Synthesis of (+)-Spinosyn A

1) AcOH, aq THF, rt
2) TBS-Cl, imidazole, CH$_2$Cl$_2$, rt
3) (CICO)$_2$, DMSO, Et$_3$N, CH$_2$Cl$_2$, 90\% (3 steps)
4) Et$_3$SiH, 5\% Pd/CaCO$_3$/PbO acetone, rt

Martin Sulfurane
CH$_2$Cl$_2$, 0 °C
81\% (3 steps)

Paquette’s Synthesis of Spinosyn A

Which of the Structures is Spinosyn G?

(-)-spinosyn A

\[ \text{α-D-forosamine} \]

\[ \text{β-L-ossamine} \]

\[ \text{β-D-ossamine} \]

19 (5'' epi-spinosyn A)

3 (5'' epi-spinosyn A)

4 (4'' epi-spinosyn A)

RhamO...
Synthesis of β-D-ossamine Spinosyn

(-)-spinosyn A

1) MeSO₂Cl, (i-Pr)₂NEt, CH₂Cl₂, 0°C
2) NaN₃, DMF, 120 °C

1) SnCl₂, MeOH, rt
2) MeI (excess), MeCN, reflux
3) m-xylene, reflux

RhamO

O

Et

Me

Me

Cl

F

N⁺

N⁺

2 BF₄⁻

F-TEDA
(Selectfluor™)
Synthesis of the Ossamines

1) H₂, 45 psi, Pd(OH)₂/C, EtOH, rt
2) formalin, MeOH, reflux, 1 h, then NaBH₄

TROC-Cl, K₂CO₃, toluene reflux

Major

Minor

Tom Graham @ Wipf Group 13 6/6/2005
α-L-Ossamine and β-L-Ossamine

1) PPTS, BF₃•OEt₂, 1,2-dichloroethane
2) Zn, 1 M NH₄OAc THF-MeOH, rt 80%, 2:1 eq/ax
3) MeI (2.5 equiv), DIPEA, THF, rt 49% combined
Conclusions:

- The structure of Spinosyn G has been unambiguously assigned and contains the L-ossamine residue.
- The result has important implications for understanding the biosynthesis of the spinosyn class of molecules.