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The Synthesis of Azoxybacilin

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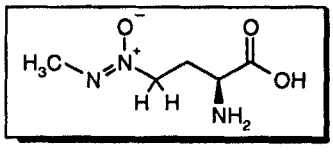
SUMMARY

(^{3}H)-Azoxybacilin, a new antifungal agent, was prepared in five steps. The label was introduced by reduction of 2-t-butoxycarbonylamino-succinic-acid-1-t-butylester-carbonic-acid-ethylester anhydride (2) with LiB^3H_4 generated from tritium gas via Li^3H.

Key Words: azoxybacilin, antifungal agent, tritium gas, lithium borotritide

INTRODUCTION

Azoxybacilin 1a (see below and in Scheme 2) is a new antifungal agent of microbial origin [1]. It inhibits methionine biosynthesis at the sulfur fixation step [2]. In order to establish an assay for high flux screening labeled azoxybacilin with high specific activity was required.
The synthesis of azoxybacilin 1a has been recently published [3]. It involves sodium borohydride reduction of the mixed carbonic acid anhydride 2, which is prepared in situ from α-*t*-butyl(N$^{\alpha}$(*t*-butyloxycarbonyl)-L-aspartic acid monoester 3.



Azoxybacilin

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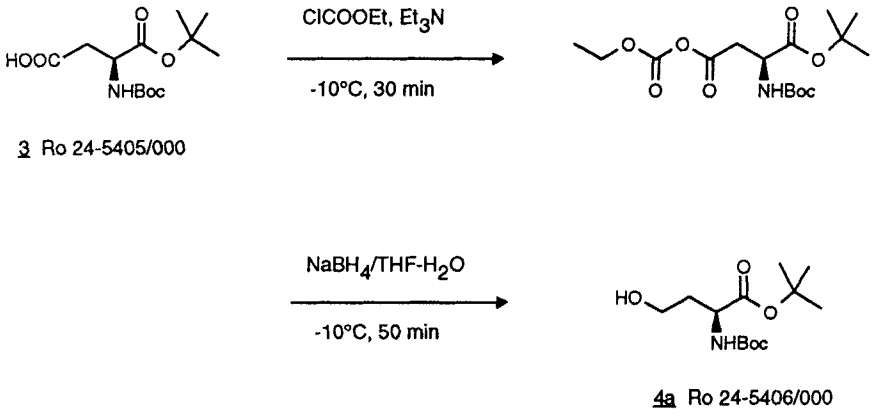


3 Ro 24-5405/000

4a Ro 24-5406/000

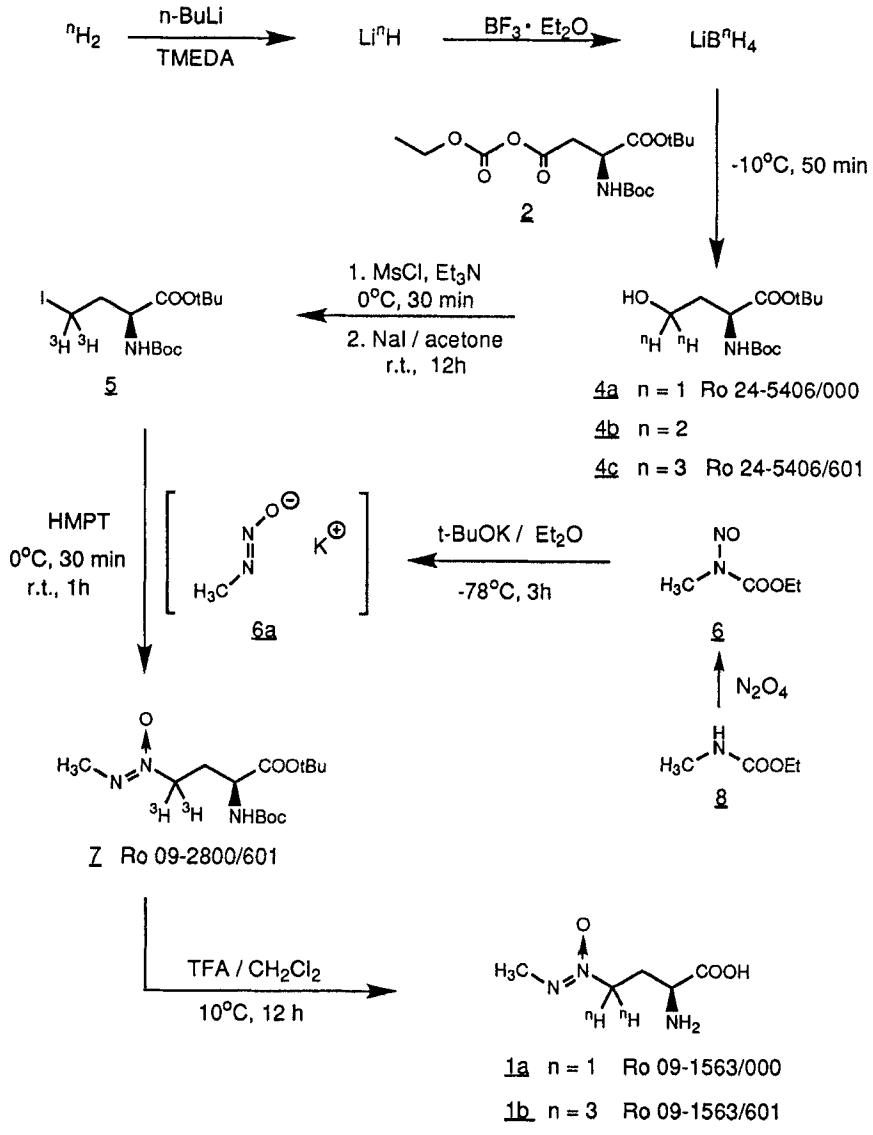
Scheme 1

Since the highest specific activity was desired, we resorted to the methodology of H.Andres [4] using carrier free lithium borotritide as the reducing agent. In this report we describe the synthesis of (^{3}H)-azoxybacilin employing this powerful methodology.

RESULTS AND DISCUSSION

An initial reduction experiment revealed that the formation of alcohol 4a proceeded as well if lithium- instead of sodium borohydride was used. This fact allowed us to follow the labeling strategy outlined in Scheme 2. Two preliminary experiments to reduce the mixed anhydride 2 with $LiBD_4$ indicated that 78-88% of the bis-deutero-species were formed according to mass spectrometric analysis. The main experiment, starting from tritium gas and 390µmoles of n-butyl lithium, afforded 32µmoles of pure alcohol 4c after chromatography. A fraction of this sample (18.5µmoles) was converted into iodide 5 using a high excess of reagents. The radiochemical yield amounted to 64%. Regio- and stereoselective alkylation of the diazotate 6a generated from N-nitroso-N-methylcarbamate 6 afforded BOC-protected azoxybacilin 7 in 74% radiochemical yield. N-nitroso-methylcarbamate 6 was prepared by treatment of N-methylcarbamate 8 with nitrogen tetroxide [5].

The specific activity of 7 was 57.32Ci/mmole based on liquid scintillation counting and HPLC-quantitation with UV-detection. The determination by mass spectrometry resulted in a value of 51.4 ± 5 Ci/mmole. ^{3}H-NMR revealed a multiplet at 4.25 ppm compatible with structure 7. A fraction of 7 (4.37µmoles) was deprotected by treatment with trifluoro-acetic acid. Purification by HPLC afforded azoxybacilin 1b in 80% radiochemical yield.



Scheme 2

EXPERIMENTAL

General

The tritiation apparatus was purchased from *Radiumchemie AG*, 9053 Teufen [6]. The triitium gas trap employed had already been used previously and therefore the tritium gas might have

been diluted to some extent. The mass spectrometry was performed on a API-III Sciex *Perkin Elmer* instrument using the ion spray (negative mode ionization).
Radiochemical samples were counted in a *Berthold* BF 5020 liquid scintillation counter using *Safetron*-150 as scintillation cocktail. Counting efficiency was determined by adding ^{3}H-hexadecane standard (*Amersham Radiochemicals*) to each sample.
The radiochemical purity was determined on a TLC-linear analyzer LB284 from *Berthold AG*.
Thin layer chromatography was performed on silica gel 60F_{254} plates (*Merck* # 1.05715).
For preparative chromatography a 2/1 (w/w) mixture of silicagel 60 (40-63µm) (*Merck* # 1.09385) and silica gel 60H (*Merck* # 7736) was used.

The HPLC apparatus consisted of a Anacomp 220 controller, T-114 pump, Uvikon 720 LC detector and a plotter 800 from *Kontron* AG, Zurich.

Deuterium gas was purchased from Eurisotop, *Radiumchemie* AG (99.8% enrichment).

α-*t*-Butyl-N$^{\alpha}$-(*t*-butyloxycarbonyl)-L-aspartic acid monoester (3) was obtained from *Bachem, Feinchemikalien* AG, 4416 Bubendorf.
Prior to use borontrifluoride-ethyletherate (BF_3•Et_2O) was distilled under reduced pressure (15 mbar) from calcium hydride. Tetrahydrofuran (THF) was distilled from $LiAlH_4$.
N,N,N',N'-Tetramethyl-ethylenediamine (TMEDA) (Fluka) was distilled from potassium hydroxide. Anhydrous dichloromethane 99%+ was purchased from *Aldrich*.

Mixed carbonic acid anhydride 2:

α-*t*-Butyl(N$^{\alpha}$-(*t*-butyloxycarbonyl)-L-aspartic acid monoester 3 (170 mg, 0.588 mmole) was dissolved in toluene and this solution was evaporated to remove any moisture. After drying in vacuo the residue was dissolved in 1.96 ml of THF. After cooling to -10°C 90µl of triethlamine and 62µl of ethyl chloroformate were added. The reaction mixture was stirred for 30 min. at -10°C and the precipitate was filtered off under argon and washed with about 1 ml of THF. The filtrate containing the mixed carbonic acid anhydride 2 (0.588 mmole in 1.2 ml of THF) was stored under argon at 5°C and used within two days.

t-Butyl-(S)-2-[(*t*-butoxycarbonyl)amino]-4-hydroxy-4,4-$^3H_2$-butanoate 4c

A 10ml two-necked flask was attached to the tritiation apparatus and rinsed with argon. 250µl of 1.56M n-BuLi (0.39 mmoles) and 65µl of TMEDA was added and the side neck was sealed with a 10mm three layer septum from *Hamilton AG*, CH-7402 Bonaduz. The solution was degassed by repetitive (three times) freezing, evacuating and thawing using liquid nitrogen as coolant. Then tritium gas was introduced (initial pressure 478mbar) and the solution was stirred for 3h, while the tritium pressure decreased to 389mbar. A white precipitate of Li^{3}H was observed. The reaction mixture was cooled to -190°C and excess tritium gas was reabsorbed onto

the uranium bed. The volatile components, including ^{3}H-n-butane, were lyophilized into another flask and the vacuum was relieved using dry nitrogen. 440µl of THF was syringed via the septum whereupon Li^{3}H dissolved almost completely. The solution was cooled to -15°C and BF_3·Et_2O (36µl, 280µmoles) was added. After stirring for 15 min. at room temperature a THF solution of the mixed carbonic acid anhydride 2 (147µmoles in 500µl) was added at -10°C and the solution kept stirring for 50 min. at this temperature. The reaction mixture was cooled to -190°C and 0.29 ml of 1N HCl was added to decompose excess LiB^{3}H$_4$.The flask was evacuated and upon thawing bubble formation indicated tritium gas evolution. The reaction mixture was again frozen and the tritium gas transferred back into the uranium trap. The volatile components were lyophilized off and 500µl of THF/H_2O 5/1 (v/v) was added. After brief stirring and cooling to -190°C residual tritium gas was again transferred onto the uranium trap. The volatile components were again lyophilized off and 500µl of THF/H_2O 5/1 (v/v) was added. The reaction flask was disconnected from the tritiation apparatus and the reaction mixture was transferred into a 10ml separatory funnel. The flask was rinsed with 4 ml of ice water and the desired alcohol 4 was extracted with 2x3ml of ethyl acetate. The organic phase was dried over Na_2SO_4 and diluted to 50 ml. The total activity of crude 4c was 2.56 Ci and the radiochemical purity was 71% by TLC [n-hexane/EtOAc 2/1 (v/v)].

Column chromatography on 8g of silica gel with n-hexane/EtOAc 2/1 (v/v) afforded 1.56 Ci of 4c with 97.6%- and 0.28 Ci of 4c with 90% radiochemical purity. The byproduct ^{3}H-n-butane was diluted with 15 ml of n-hexane. This solution (total activity 2.85 Ci) was divided in 3 portions, sealed in ampoules and stored together with additional waste from this reduction step (1.46 Ci) for later delivery to a radioactive waste disposal facility.

t-Butyl-(S)-[(*t*-butoxycarbonyl)amino]-4-iodo-4,4-($^3H_2$)-butanoate 5:

Part of the solution of the previous step (785 mCi of 4c with 97.6%- and 280 mCi of 4c with 90% radiochemical purity) was evaporated to a small volume and filtered through Millex SR 0.5µm. The filtrate was evaporated to dryness and the radioactive material was redissolved in acetonitrile. After evaporation to dryness 0.3 ml of dichloromethane and 40µl of triethylamine was added. After cooling to 0°C 19µl (244µmoles) of methanesulfonyl chloride was added. Stirring for 30 min. at 0°C was followed by addition of ice water, extraction with ether in two 10 ml separatory funnels and drying the organic phase over anhydrous Na_2SO_4. The radiochemical purity of the mesylate was 95% determined by TLC (n-hexane/EtOAc 2/1 (v/v)).

The mesylate was dissolved in 0.3 ml of acetone. Then 100 mg (667µmoles) of sodium iodide was added and the reaction mixture was stirred overnight under a slight stream of argon. Extractive work-up with EtOAc and drying the organic phase over anhydrous Na_2SO_4 provided 933 mCi of crude iodide 5. Column chromatography on 6g of silica gel with n-hexane/EtOAc 5l1 (v/v) afforded 680 mCi of iodide 5 in 99% radiochemical purity (determined by TLC: n-hexane/EtOAc 5/1 (v/v)).

t-Butyl-(S)-[(t-butoxycarbonyl)amino]-4-[(Z)-methyl-NNO-azoxy]-4,4-$^3H_2$-butanoate 7:

N-nitroso-N-methylcarbamate 6 (15µl, 129µmol) was added dropwise at - 78°C to a stirred suspension of potassium *t*-butoxide (14.7 mg , 131µmoles) in 0.35 ml of absolute diethyl ether. After stirring for 4 h at - 78°C iodide 5 (680mCi, 12 µmoles), dissolved in 250µl of HMPT, was added within 3 min. The reaction mixture was stirred for 30 min. at 0°C and 1 h at room temperature. Work-up by addition of ice water, extraction with diethyl ether and drying the organic phase over anhydrous $MgSO_4$ provided 540 mCi of crude 7 in 88% radiochemical purity (TLC n-hexane/EtOAc 4/1 (v/v).Column chromatography on 6g of silica gel with n-hexane-EtOAc 4/1 (v/v) afforded 501 mCi of product 7 with 99.28% radiochemical purity.

Mass spectrum(ISP-mode): 344.4 (MNa^+), 322.4 (MH^+), 266.3 (MH^+-isobutylene), 210.3 (MH^+-2x isobutylene)

^{3}H-NMR: 4.25 ppm (multiplet);

HPLC: column: Waters Nova-Pak silica 60A 4µm 3.9 x 150 mm (cartridge),
mobile phase: n-heptane/*t*-butylmethyl ether 4/1 (v/v)
flow rate: 1ml/min.,
detection: UV λ = 240 nm,
retention time: 5.37 min.
specific activity 57.32 Ci/mmole

^{3}H-Azoxybacilin 1b:

BOC-protected ^{3}H-azoxybacilin 7 (250 mCi, 4.36µmoles) was dissolved in 0.3 ml of dichloromethane and 0.15 ml of trifluoroacetic acid. After stirring overnight at 10°C 0.5 ml of methanol was added and the solution was lyophilized. The residue was dissolved in 0.5 ml of p-dioxane and the solution was again lyophilized. Purification by HPLC on a Merck Supersphere 100 RP-18 endcapped column 4 x 244 mm (cartridge), with water as the mobile phase at a flow rate of 0.5 ml/min. and UV-detection at 230 nm afforded 201 mCi (3.5µmoles) of pure azoxybacilin 1b with 99% radiochemical purity (determined by TLC using n-butanol/HOAc/H_2O) 4/1/2 (v/v/v) as eluant).

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