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Review Article

ANALYTICAL METHODS FOR DETERMINATION OF ANTICANCER DRUGS FROM MARINE SOURCES

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ABSTRACT

The purpose of this review article was to study different types of anticancer drugs which are Aplidine, Bryostatin, Ecteinascidin-743 and Dolastatin, derived from the marine sources and are determined by using various analytical methods like HPLC,GC,LC-MS,GC-MS,SOLID PHASE EXTRACTION, NMR, MASS SPECTROPHOTOMETRYMS/MS LC/MS/MS LC/UV X-ray crystallography. The result of the given marine products should meet their predetermined Specifications.

Keywords: Aplidine, Bryostatin, Ecteinascidin-743, Dolastatin.

INTRODUCTION

What Is Cancer?[1]

Cancer refers to a group of illnesses that result from cells in the body growing abnormally. These cells divide and produce new cells in an uncontrolled way that can spread throughout the body and cause damage to essential organs. Tumors are masses (or lumps) that can develop as abnormal cells accumulate. Not all tumors are cancer. Benign(non-cancerous or nonmalignant) tumors do not spread toother parts of the body and are rarely life-threatening.

There are four main types of cancer:

1. Carcinomas – cancers of the organs
2. Sarcomas – cancers of the muscles, bone, cartilage, and connective tissue
3. Lymphomas – cancers of the lymphatic system
4. Leukemias – cancers of the blood-making system.

Marine Sources[2]

The world's oceans, covering more than 70% of the earth's surface, represent an enormous resource for the discovery of potential chemotherapeutic agents and the marine environment has been increasingly explored as a source of novel bioactive agents. The marine environment has proved to be a prolific source of structurally novel bioactive agents, and several have advanced to clinical development as potential anticancer agents. The chemical and biological diversity of the marine environment is immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs. An exciting "marine pipeline" of new anticancer clinical and preclinical agents has emerged from intense efforts over the past decade to more effectively explore the rich chemical diversity offered by marine life .The marine ecosystem is more and more acknowledged as a source of potential anticancer agents.

TYPES OF ANTI CANCER DRUGS FROM MARINE SOURCES[3]		
Development Drug Name	**Source Organism (type)**	**Collection Region**
Aplidine	Aplidium albicans (tunicate)	Mediterranean sea
Bryostatin 1	Bugula neritina (bryozoan)	Gulf of California
Dolastatin 10	Dolabella auricularia (mollusk)	Indian Ocean
Ecteinascidin 743	Ecteinascidia turbinata (tunicate)	Caribbean Sea
Kahalahide F	Elysia rubefescens (mollusk)	Hawaii
Halichondrin B analog	Lissodendoryx species (sponge)	New Zealand
Hemiasterlin analog	Cymbastella species (sponge)	Papua New Guinea
Isogranulatimide	Didemnum granulatum(tunicate)	Brazil

A) APLIDINE

Molecular formula: $C_{57}H_{87}N_7O_{15}$

IUPAC/name
11-hydroxy-20-isobutyl-15-isopropyl-3-(4-methoxybenzyl)-2,6,17-trimethyl-1,4,8,13,16,18,21-heptaoxodocosahydro-1H-pyrrolo[2,1-f(2S)-N((2R)1(((7R,11S,15S,17R,20S,25aS)-10-((S)-sec butyl)][1,15,4,7,10,20]dioxatetraazacyclotricosin-7-yl)amino)-4-methyl-1-oxopentan-2-yl)-Nmethyl-1-(2-oxopropanoyl)pyrrolidine-2-carboxamide.

1. Bioanalysis of aplidine, a new marine antitumoral depsipeptide, in plasma by high-performance liquid chromatography after derivatization with *trans*-4′-hydrazino-2-stilbazole[4]
A sensitive bio-analytical assay in plasma of the depsipeptide aplidine is reported, based on reversed-phase liquid chromatography and fluorescence detection of the *trans*-4′-hydrazino-2-stilbazole (4′H_2S) derivative of the analyte. Aplidine is isolated from the matrix by solid-phase extraction on an octadecyl modified silica stationary phase. After evaporation of the acetone eluate, a derivatization with 4′H_2S is performed in a water–acetonitrile mixture at pH 4. The reaction mixture is injected directly into the chromatograph and the analyte is quantified by fluorescence detection at 410 and 560 nm for excitation and emission, respectively. The method has been validated in the 2–100 ng/ml-range, 2 ng/ml being the lower limit of quantification. Precision and accuracy both meet the current requirements for a bioanalytical assay. The identity of the 4′H_2S reaction products of aplidine have been confirmed by mass spectrometric analysis.

2. Pharmaceutical Development of a Parenteral Lyophilized Formulation of the Novel Antitumor Agent Aplidine[5]
Aplidine is a naturally occurring cyclic depsipeptide isolated from the Mediterranean tunicate *Aplidium albicans*. Aplidine displays promising *in vitro*and *in vivo* antitumor activities against various solid human tumor xenografts and is therefore developed now for clinical testing. The aim of this study was to develop a stable parenteral pharmaceutical dosage form for clinical Phase I testing. Aplidine raw material was characterized by using several chromatographic and spectrometric techniques. These experiments showed that aplidine exists as two isomers. A stability-indicating HPLC assay was developed. Solubility testing showed that aplidine exhibits very poor aqueous solubility. Because solubilized aplidine showed substantial degradation under heat and light stress testing conditions, it was decided to develop a lyophilized dosage form. Freezedrying was carried out with a 500 µg/mL solution of aplidine in 40% (v/v) tert-butanol in Water for Injection (WfI) containing 25 mg/mL D-mannitol as a bulking agent. Differential scanning calorimetry was applied to determine the optimal freeze-drying cycle parameters.

3. HPLC-UV Method development and impurity profiling of the marine anticancer agent aplidine in raw drug substance and pharmaceutical dosage form[6]
The development and validation of a reversed-phase high performance liquid chromatographic (RP-HPLC) method with ultra-violet (UV) detection for the quantification and purity determination of aplidine in raw drug substance and pharmaceutical dosage form is described. Using this method, the aplidine rotamers present as a consequence of cis-trans isomerism of the peptide bond between the proline and pyruvoyl moieties in the molecule, elute as one single peak. Linear calibration curves in the range of 12.5–300 µg/mL of aplidine with correlation coefficients > 0.999 were obtained. Major impurities were identified as didemnin-A, acetyldidemnin A, and noraplidine using liquid chromatography-mass spectrometry (LC-MS). No significant differences in chromatographic profile between aplidine raw drug substance and its pharmaceutical dosage form were found.

4. Structure elucidation of aplidine metabolites formed *in vitro* by human liver microsomes using triple quadrupole mass spectrometry[7]
The cyclic depsipeptide aplidine is a new anti-cancer drug of marine origin. Four metabolites of this compound were found after incubation with pooled human microsomes using gradient high-performance liquid chromatography with

ultraviolet detection. After chromatographic isolation, the metabolites have been identified using nano-electrospray triple quadrupole mass spectrometry. A highly specific sodium-ion interaction with the cyclic structure opens the depsipeptide ring, and cleavage of the amino acid residues gives sequence information when activated by collision-induced dissociation in the second quadrupole. The aplidine molecule could undergo the following metabolic reactions: hydroxylation at the isopropyl group (metabolites apli-h 1 and apli-h 2); C-dealkylation at the N(Me)-leucine group (metabolite apli-da); hydroxylation at the isopropyl group and C-dealkylation at the N(Me)-leucine group (metabolite apli-da/h), and C-demethylation at the threonine group (metabolite apli-dm). The identification of these metabolites formed *in vitro* may greatly aid the elucidation of the metabolic pathways of aplidine in humans.

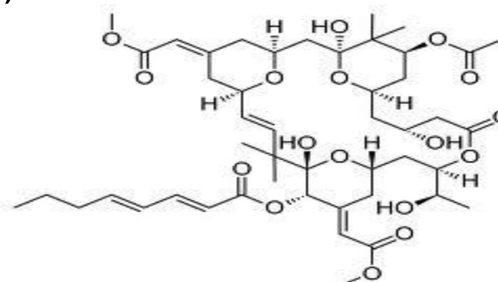
5. Degradation kinetics of aplidine, a new marine antitumoural cyclic peptide, in aqueous solution[8]

The degradation kinetics of aplidine were investigated using reversed-phase high-performance liquid chromatography combined with UV detection. Aplidine consists of at least two isomers that undergo interconversion at a low rate. Influences of pH, temperature, buffer ions and ionic strength on the degradation kinetics were studied. The log k_{obs}–pH profile can be divided into three parts, a proton, a solvent and a hydroxyl-catalysed section. The stability-indicating properties of the used analysis technique as well as the identities of the main degradation products were checked using gradient liquid chromatography and mass spectrometric detection. The overall degradation rate constant as a function of the temperature under acidic and alkaline conditions obeys the Arrhenius equation. Results from gradient LC–MS indicated that hydrolysis of the ester groups present in the ring structure was the main degradation route. There is no difference in degradation rate constants for the individual isomers.

6. Conformational Analysis of Dehydrodidemnin B (Aplidine) by NMR Spectroscopy and Molecular Mechanics/Dynamics Calculations[9]

These are sufficiently long-lived so as to allow their resolution by HPLC. NMR spectroscopy shows that this phenomenon is a consequence of restricted rotation about the Pyr-Pro[8] terminal amide bond of the molecule's side chain. The same technique also indicates that the overall three-dimensional structures of both the cis and trans isomers of DDB are similar despite the conformational change. Molecular dynamics simulations with different implicit and explicit solvent models show that the ensembles of three-dimensional structures produced are indeed similar for both the cis and trans isomers. These studies also show that hydrogen bonding patterns in both isomers are alike and that each one is stabilized by a hydrogen bond between the pyruvyl unit at the terminus of the molecule's side chain and the Thr[6] residue situated at the junction betwen the macrocycle and the molecule's side chain. Nevertheless, each conformational isomer forms this hydrogen bond using a different pyruvyl carbonyl group: CO^2 in the case of the cis isomer and CO^1 in the case of the trans isomer.

B) BRYOSTATIN



Molecular Formula: $C_{47}H_{68}O_{17}$

Chemical Name:

(1*S*,3*S*,5*Z*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*-,21*R*,23*R*,25*S*)-25-(Acetyloxy)-1,11,21-trihydroxy-17-[(1*R*)-1-hydroxyethyl]-5,13-*bis*(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.13,7.111,15]nonacos-8-en-12-yl 2,4-octadienoic acid ester

1. Separation of the Bryostatin Derivatives By High Performance LiquidChromatography[10]

The Separation of Bryostatin derivatives (26-acetate, 26-metabromobenzoate, 26-ketone, and 13→ 30 epoxide) by high performance liquid chromatography (HPLC) using normal phase and reverse phase systems was accomplished. Using *n*-hexane/EtOAc/MeOH/H_2O (26:5:1:0.01) as mobile phase for normal phase HPLC and acetonitrile (CH_3CN)/H_2O system for reverse phase HPLC provided good separation of bryostatin derivatives. In addition, contributions of functional group for eluate order were discussed.

2. Fluorescent high-performance liquid chromatography assay for lipophilic alcohols[11]

A new ultrasensitive fluorescent derivatization procedure for chromatographic analysis of primary, secondary, and nonpolar tertiary alcohols is described. The procedure uses Bodipy FL in basic dichloromethane solution with Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide) to form highly fluorescent ester derivatives that can be separated by silica normal-phase high-performance liquid chromatography (HPLC). Rhodamine WT and Oregon green 488 were also useful derivatization reagents. The detection limit for detection of cholesterol and bryostatin by Bodipy FL was less than 1 fmol. The reaction conditions are gentle enough that low concentrations of unstable alcohols such as bryostatin 1 can be measured.

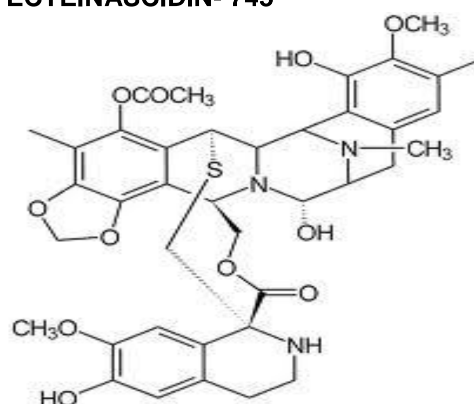
3) Identification of bryostatins in Bugula neritina extracts by high performance liquid chromatography and Q-Tof mass spectrometry[12]

A method with tandem high performance liquid chromatography (HPLC) and Q-Tof mass spectrometry (MS) has been established for the qualitative assay of bryostatins in Bugula neritina extracts. Ten bryostatins were well separated on a Polaris C18-5 column (4.6 mm i.d. x 200 mm, 5 microm) with methanol and water (80:20, v/v) as mobile phase. The bryostatins were qualitatively detected with the mass spectrometric detection. Ten bryostatins were found in the Bugula neritina collected from Gulf of Dayawan (Shenzhen, China). Mass spectra of the peaks led to the identification of nine known bryostatins that were bryostatins 4, 5, 6(9), 7, 8, 10, 16, 17, 18. Bryostatins 4, 5, 8, 10 could be further verified by the retention times of the corresponding standards. The two trace constituents, bryostatin 7 and 17, were for the first time found in Bugula neritina inhabiting in China Sea.It is interesting that the result indicated the existence of a possibly new bryostatin in the mixture. The work above provides a quick and accurate assay method for the qualitative identification of the bryostatins in Bugula neritina extracts.

4) High-performance liquid chromatographic assay for the novel antitumor drug, bryostatin-1, incorporating a serum extraction technique[13]

An HPLC assay incorporating a solid-phase extraction technique has been devised for bryostatin-1. Quantitation of bryostatin was found to be linear over the concentration range 0.012-25 microg/ml (0.2-25 ng on column) and was found to have a limit of detection of 0.2 ng on column, with a correlation coefficient of 0.9999. Following extraction of bryostatin over a range of concentrations from horse serum (0.012-25 microg/ml) and human serum (0.01-0.32 microg/ml) using a 100-mg C18 solid-phase extraction cartridge, extraction efficiencies consistently greater than 90% were obtained for extraction from horse serum and varied between 57 and 85% from human serum. However, on extending this work to blood samples from patients undergoing therapy with bryostatin-1, the drug was not detectable even at the maximum dose given, demonstrating the rapid loss of this agent from peripheral circulation.

C) ECTEINASCIDIN- 743



Molecular formula: $C_{39}H_{43}N_3O_{11}S$

IUPAC:

(1'*R*,6*R*,6a*R*,7*R*,13*S*,14*S*,16*R*)-6',8,14-trihydroxy-7',9-dimethoxy-4,10,23-trimethyl-19-oxo-3',4',6,7,12,13,14,16-octahydrospiro[6,16-(epithiopropano-oxymethano)-7,13-imino-6a*H*-1,3-dioxolo[7,8]isoquino[3,2-*b*][3]benzazocine-20,1'(2'*H*)-isoquinolin]-5-yl acetate.

1) Search for metabolites of ecteinascidin 743, a novel, marine-derived, anti-cancer agent, in man[14]

Ecteinascidin 743 (ET-743) is a potent anti-tumoral agent of a marine origin. In the present study, a qualitative chromatographic discovery of metabolites of ET-743 in man is reported. ET-743 and its demethylated analog ET-729 were incubated at 37°C in the presence of enzyme systems, pooled human microsomes, pooled human plasma and uridine 5′-diphosphoglucuronyltransferase, respectively, in appropriate media. Reaction products were investigated chromatographically using photodiode array and ion spray-mass spectrometric detection (LC-MS). The main reaction products in microsomal incubations of ET-743 resulted

from a remarkable breakdown of the molecule. In plasma the drugs were deacetylated, and the transferase did actually yield a glucuronide of both ET-743 and ET-729. In contrast, screening of urine, plasma and bile, collected from patients treated with ET-743 at the highest dose levels, using a sensitive LC-MS assay, did not result in detection of ET-729 and metabolites which were generated *in vitro*. The urinary excretion of ET-743 in man was lower than 0.7% of the administered dose for a 24-h infusion.

2) Simple and sensitive liquid chromatographic quantitative analysis of the novel marine anticancer drug Yondelis™ (ET-743, trabectedin) in human plasma using column switching and tandem mass spectrometric detection[15]

The development of a simple and sensitive assay for the quantitative analysis of the marine anticancer agent Yondelis (ET-743, trabectedin) in human plasma using liquid chromatography (LC) with column switching and tandem mass spectrometric (MS/MS) detection is described. After protein precipitation with methanol, diluted extracts were injected on to a small LC column (10 × 3.0 mm i.d.) for on-line concentration and further clean-up of the sample. Next, the analyte and deuterated internal standard were back-flushed on to an analytical column for separation and subsequent detection in an API 2000 triple-quadrupole mass spectrometer. The lower limit of quantitation was 0.05 ng mL^{-1} using 100 µl of plasma with a linear dynamic range up to 2.5 ng ml^{-1}. Validation of the method was performed according to the most recent FDA guidelines for bioanalytical method validation. The time needed for off-line sample preparation has been reduced 10-fold compared with an existing LC/MS/MS method for ET-743 in human plasma, employing a labor-intensive solid-phase extraction procedure for sample pretreatment.

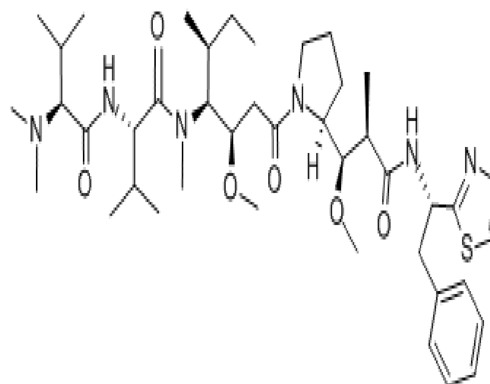
3) Quantitative determination of Ecteinascidin 743 in human plasma by miniaturized high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry[16]

Method was developed for the bio-analysis of Ecteinascidin 743 (ET-743) using miniaturized liquid chromatography (LC) coupled to an electrospray ionization sample inlet (TurboIonSpray) and two quadrupole mass analyzers (LC/ESI-MS/MS). Solid-phase extraction was used as a sample pretreatment procedure. Ecteinascidin 743 is a very potent anticancer compound and is administered in µg m^{-2}dosages, which demands special requirements in terms of sensitivity for the analytical method supporting clinical pharmacokinetic studies. Using conventional LC/UV, a lower limit of quantitation (LLQ) of 1 ng ml^{-1} plasma was reached using a 500 µl sample volume, but LC/ESI-MS/MSpermitted an LLQ of 10 pg ml^{-1}. The latter method was found to be accurate and precise, and provided a broad linear concentration range of 0.010–2.50 ng ml^{-1}.

4) Analysis of Ecteinascidin 743, a new potent marine-derived anticancer drug, in human plasma by high-performance liquid chromatography in combination with solid-phase extraction[16]

A reversed-phase high-performance liquid chromatographic method has been developed and validated for the quantification of the novel anticancer drug Ecteinascidin 743 in human plasma. The sample pretreatment of the plasma samples involved a solid-phase extraction (SPE) on cyano columns. Propyl-*p*-hydroxybenzoate was added after the sample pretreatment to correct for variability in injection volumes. The separation was performed on a Zorbax SB-C_{18} column (75×4.6 mm I.D., particle size 3.5 µm) with acetonitrile–25 m*M* phosphate buffer, pH 5.0 (70:30, v/v) as the mobile phase. The flow-rate was 1.0 ml/min and the eluent was monitored at 210 nm. The accuracies and precisions of the assay fall within ±15% for all quality control samples and within ±20% for the lower limit of quantitation, which was 1.0 ng/ml using 500 µl of plasma. The overall recovery of the sample pretreatment procedure for Ecteinascidin 743 was 87.0±5.9%. The drug was found to be stable in human plasma at −30°C for at least 2 months. At room temperature Ecteinascidin 743 was stable in human plasma for 5 h at most.

D) DOLASTATIN-10



Molecular Formula: C42H68N6O6S
IUPAC
3-methylbutanoyl]amino]-N-[3-methoxy-1-[2-[1-Name: 2-[[2-(dimethylamino)-methoxy-2-methyl-3-oxo-3-[[2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N,3-dimethylbutanamide.

1) Quantitation of dolastatin-10 using HPLC/electrospray ionization mass spectrometry[17]

A highly sensitive and specific assay for the quantitation of the anticancer agent dolastatin-10 (DOL-10) in human plasma is described. The method was based on the use of electrospray ionization-high-performance liquid chromatography/mass spectrometry (ESP-LC/MS). The analytical procedure involved extraction of plasma samples containing DOL-10 and the internal standard (DOL-15) with n-butyl chloride, which was then evaporated under nitrogen. The residue was dissolved in 50 microl mobile phase and 10 microl was subjected to ESP-LC/MS analysis using a C18 microbore column. A linear gradient using water/acetonitrile was used to keep the retention times of the analytes of interest under 5 min. The method exhibited a linear range from 0.005 to 50 ng/ml with a lower limit of quantitation (LLQ) at 0.005 ng/ml. Absolute recoveries of extracted samples in the 85-90% range were obtained. The method's accuracy (< or =5% relative error) and precision (< or =10% CV) were well within industry standards. The analytical procedure was applied to extract DOL-10 metabolites from samples obtained following incubation of the drug with an activated S9 rat liver preparation. Two metabolic products were detected and were tentatively identified as a N-demethyl-DOL-10 and hydroxy-DOL-10. Structural assignments were made based on the fragmentation patterns obtained using the electrospray source to produce collision-induced dissociation (CID). The method was also applied to the measurement of DOL-10 in the plasma of patients treated with this drug. Preliminary investigation of the pharmacokinetics suggested that drug distribution and elimination may be best described by a three-compartment model with t1/2alpha = 0.087 h, t1/2beta = 0.69 h and t1/2gamma = 8.0 h. Plasma clearance was 3.7 l/h per m^2.

2) Procedures for the Analyses of Dolastatins 10 and 15 by High Performance Liquid Chromatography[18]

A series of HPLC procedures were evaluated for assessing the purity of dolastatin 10 (1) and dolastatin 15 (2) samples. Interestingly two readily interconvertible (ambient temperature) dolastatin 10 (1) conformers were detected using a potassium dihydrogen phosphate buffered solvent (methanol-water) with a C8 reversed-phase column. A solvent system composed of acetonitrile-2-propanol-water containing sodium 1-hexanesulfonate was found especially useful for evaluating the purity of dolastatin 10 and 15 specimens. Useful HPLC procedures were also found for detecting diastereomeric isomers in the key dolastatin 10 synthetic intermediate using β-cyclodextrin in 3:2 methanol-water.

3) Pitiprolamide, a proline-rich dolastatin 16 analogue from the marine cyanobacterium Lyngbya majuscula from Guam[19]

An unusual cyclic depsipeptide, pitiprolamide (1), was isolated from the marine cyanobacterium Lyngbya majuscula collected at Piti Bomb Holes, Guam. The structure was deduced using NMR, MS, X-ray crystallography, and enantioselective HPLC-MS techniques. Remarkably, proline represents half of the residues forming pitiprolamide (1). Other distinctive features include a 4-phenylvaline (dolaphenvaline, Dpv) moiety initially found in dolastatin 16 and the rare 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha) unit condensed in a unique sequence in one single molecule. Pitiprolamide (1) showed weak cytotoxic activity against HCT116 colon and MCF7 breast cancer cell lines, as well as weak antibacterial activities against Mycobacterium tuberculosis and Bacillus cereus.

CONCLUSION

These are the different types of anticancer drugs derived from the marine sources and are determined by using various analytical methods like HPLC,GC,LC-MS,GC-MS,SOLID PHASE EXTRACTION, NMR, MASS SPECTROPHOTOMETRY. Aplidine is determined by HPLC, MS, SOLID-PHASE EXTRACTION, bryostatin is determined by HPLC,MS,ecteinascidin-743 by LC-MS, MS/MS, LC/MS/MS, LC/UV, dolastatin by ESP-LC/MS, HPLC, NMR, X-ray crystallography. All given products were meet their predetermined specifications.

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