SYNTHESIS OF [5-^{14}C]PENTOSTATIN, AN ANTILEUKEMIC AGENT AND POTENT ADENOSINE DEAMINASE INHIBITOR*

Peter W. K. Woo and Helen T. Lee

Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
2800 Plymouth Road
Ann Arbor, MI 48105, U.S.A.

SUMMARY

Reaction of triethyl ortho[^{14}C]formate (2) with 2-amino-1-(5-amino-1H-imidazol-4-yl)ethanone dihydrochloride (1) in the presence of molecular sieves 4A gave 6,7-dihydro[5-^{14}C]imidazo[4,5-d][1,3]diazepin-8(3H)-one hydrochloride monodimethyl sulfoxide (3) (radiochemical yield, 60%). The latter was persilylated with bis(trimethylsilyl)trifluroacetamide (4) and glycosylated with 2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (6) to give a mixture from which the 3-N-β-glycosylated product 8 was isolated by chromatography and crystallization (13%). Deprotective saponification with methanolic sodium methoxide and subsequent sodium borohydride reduction of the 8-keto function gave a (R,S)-mixture from which the desired (R)-isomer, [5-^{14}C]pentostatin (11), was isolated by preparative HPLC over a C18 column, desalting with Diaion-HP20, and subsequent crystallization (39%).

Key Words: Antileukemic agent, adenosine deaminase inhibitor, 6,7-dihydro[5-^{14}C]imidazo[4,5-d][1,3]diazepin-8(3H)-one, [5-^{14}C]pentostatin.

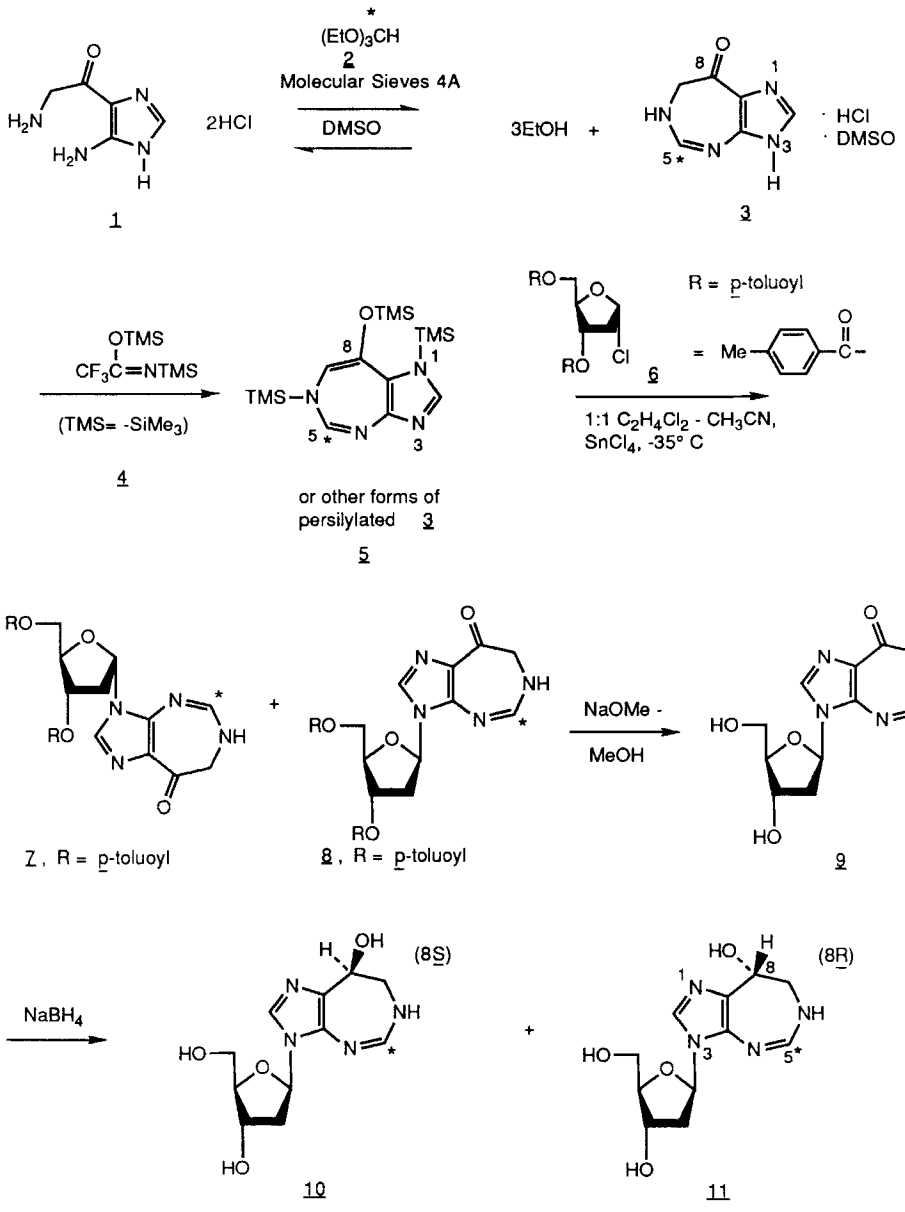
INTRODUCTION

Pentostatin (11),[1] a selective and highly potent inhibitor of the enzyme adenosine deaminase (ADA), originally derived from fermentation by Dion, Ryder, Woo, et al.[2] of these laboratories, has generated considerable interest as an agent in anticancer and antiviral chemotherapy.[3-9] It has shown efficacy as a possible codrug for enhancing the anticancer and antiviral activities of various adenine nucleosides, by inhibiting the deaminative inactivation of these

* This paper is dedicated to Professor Kenneth L. Rinehart, Jr., in honor of his 60th birthday.

0362–4803/90/040445–10$05.00

Received October 20, 1989



therapeutic agents.[3a,4,5] More importantly, as an inhibitor of cellular ADA essential for the growth and function of lymphocytes, both normal as well as malignant, it has been found effective in the treatment of a variety of lymphoid malignancies.[6,7] Indeed, pentostatin is potentially the agent of choice against hairy cell leukemia.[8,9]

Various tritium or C-14 labeled pentostatins have been reported previously. The former include [^{3}H(G)]pentostatin by tritium exchange[10a] and [8-^{3}H]pentostatin by chemical modifications.[10b] The latter have been obtained through biosynthesis with various C-14 labeled precursors, such as [^{14}C(U)]adenine, [^{14}C(U)]adenosine,[11a] [1-^{14}C]ribose (which labeled the 7 and 1' positions), and [3-^{14}C]serine (which labeled the 2, 5, and 5' positions).[11b] We report herein the synthesis of [5-^{14}C]pentostatin, undertaken to provide, at reasonable cost, a C-14 labeled form of the drug for pharmacokinetics and metabolism studies.

RESULTS AND DISCUSSION

The synthesis (1 to 11) was an adaptation of the chemistry developed by Baker, Showalter, Chan, and Putt,[12] which theoretically should allow selective labeling at several positions of 11. Presently, the label was introduced to C-5 by reaction of triethyl ortho[^{14}C]formate (2) with 2-amino-1-(5-amino-1H-imidazol-4-yl)ethanone dihydrochloride (1; prepared in six steps from 4-methyl-5-nitro-1H-imidazole). Reagent 2, available commercially, could be prepared according to Oae, et al.;[13] it is reportedly unstable in neat form and therefore must be used as soon after preparation as possible.

A major problem in this key labeling step (1 to 3) is the reversibility of the reaction and hence the possible need to use a large excess of the reagent triethyl orthoformate, which would render the reaction impractical for labeled synthesis. Thus, according to published procedure, a fivefold excess of triethyl orthoformate was used to react with 1, giving unlabeled 3 in 80% yield. The problem was overcome and the highly expensive labeled reagent conserved by the use of molecular sieves 4A, which drove the reaction equilibrium to the product side by removal of the by-product ethanol. Fracture of molecular sieves was minimized by rotating the reaction mixture instead of stirring. Thus product 3 containing about 30% of powdered molecular sieves was isolated in a radiochemical yield of 60%, which could have been higher with refinements in reaction time and work-up conditions.

The yield in the overall synthesis was limited by the yield in the glycosylation steps (3 to 8). Showalter et al.,[12] in their extensive process improvement studies, achieved a 30% yield of unlabeled 8. In the present study, the combined yield of 7 and 8 was 46% in an initial 2.56 mmol run but dropped to only 26% in the scale-up 8.56 mmol run using some changes of conditions. Crystallization of the chromatographically purified mixture of 7 and 8, which had identical Rf values, gave the desired purified 8 in a combined 13% radiochemical yield from the two runs, which obviously did not represent the optimum possible.

Deprotection of 8 was conveniently carried out using sodium methoxide in methanol to give 9. Reduction of 9 with sodium borohydride was carried out both at room temperature and at -7°C. However, no significant improvement in stereoselectivity was observed by using the lower temperature, the ratio of 10/11 being approximately 46/54. The two components were then separated cleanly by HPLC of the mixture, in portions, using a C18 preparative column.

A convenient and efficient procedure was developed for desalting the purified 11 from HPLC eluates. The compound in a concentrated solution was preferentially absorbed onto Diaion-HP20[15] and eluted with 57% aqueous methanol. The recovery was 90 to 95%. This procedure contributed significantly to the high yield realized, 39% (radiochemical), in the conversion of 8 to the single crystalline 8R isomer, [^{14}C]pentostatin (11), of over 99% purity.

EXPERIMENTAL

Triethyl ortho[^{14}C]formate was purchased from Chemsyn Science Laboratories (Lenexa, Kansas). Radioactivity was determined in a Packard Tri-Carb 4530 liquid scintillation spectrometer using Beckman Ready-Solv MP as the counting medium. TLC plates, E. Merck silica gel 60 F-254, were scanned on a Berthold LB2832 automatic TLC linear analyzer. Column chromatography was performed with E. Merck silica gel, 230-400 mesh.

2-Deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (6). The procedure of Bhat[14] was followed with minor modifications. Thus, 2-deoxy-D-ribose was converted, through methyl furanosides formation and acylation, to an

anomeric mixture of methyl 2-deoxy-3,5-di-O-p-toluoyl-α(β)-D-erythro-pentofuranosides (6, Cl = OMe; and, Cl = H, 1-H = OMe); purification by crystallization and chromatography gave the crystalline, higher-Rf anomer (22%) and a mixture containing both anomers (61%) (Rf in hexane:ether-1.4:1, 0.43, 0.36). The latter mixture of anomers was then converted to crystalline 6 (76%-82%), mp 112.5-115°C, which was kept under argon at -20°C to minimize decompositions.

6,7-Dihydro[5-^{14}C]imidazo[4,5-d][1,3]diazepin-8(3H)-one Hydrochloride Monodimethyl Sulfoxide (3). A mixture of 4.57 g (21.4 mmoles) of 1, finely ground and dried, in 121 ml of DMSO (dimethyl sulfoxide; previously dried with molecular sieves 4A), was degassed at 0.5 torr for about ten minutes. Molecular sieves 4A (previously dried for four hours at 350°C) were added, and the stoppered flask was rotated by securely attaching it to a rotating evaporator for two hours. Triethyl ortho[^{14}C]formate (2) (1.846 g, 12.3 mmoles, 700 mCi) was added, and the reaction was rotated for one hour, at which time NMR indicated the presence of 3 (δ8.23, s; 8.07, s; 4.33, s), a trace amount of ethyl formate (δ4.15, q), and the absence of triethyl orthoformate (δ5.14, s). Unlabeled triethyl orthoformate (1.39 g, 9.37 mmoles) was added, and the reaction mixture again rotated. Within two hours after the addition of the unlabeled reagent, solid 1 appeared to have mostly dissolved and product 3 had started to precipitate. After rotating continuously for ten hours, then occasionally during two days, the reaction mixture, which had turned very dark, was transferred by positive argon pressure through bent glass tubing to a medium-porosity sintered glass funnel under an atmosphere of nitrogen, care being taken to avoid transfer of the beads of molecular sieves. The solid product was washed with a little dried DMSO, then with ether to wash off the largely immiscible DMSO. The solid was then dried at 0.1 torr at 65°C for seven hours to give about 4.4 g of crude [^{14}C] 3. The product, though contaminated by molecular sieves (mostly in finely powdered form), was otherwise homogeneous according to NMR. It was stored under dried argon at -20°C.

Based on the 1445 mg of molecular sieves recovered in the subsequent reaction of 3 to 5, the specific activity of 37 mCi/mmol for 8 synthesized without isotopic dilution, and mol wt of 264.74 for unlabeled 3, the actual

weight of labeled 3 recovered was estimated to be 2.96 g, chemical yield, 52%, and radiochemical yield, 59%.

3-[2-Deoxy-3,5-di-O-p-toluoyl-D-erythro-pentofuranosyl]-6,7-dihydro-[5-^{14}C]imidazo[4,5-d][1,3]diazepin-8(3H)-one (8). To a suspension of 1006 mg of 3 (101 mci, 2.56 mmoles) and 8.0 ml of acetonitrile (Aldrich Sureseal, dried with molecular sieves 4A) in a 25-ml round bottom flask filled with argon was added 3.07 g (11.0 moles) of N,N-bis(trimethylsilyl)-trifluoroacetamide (4) (Aldrich Sureseal) and 943 mg (11.9 mmoles) of pyridine (Aldrich Sureseal, dried with molecular sieves 4A). After stirring magnetically for two days, the mixture was filtered through a Kimax 15-ml medium sintered glass filter under nitrogen into a 100-ml three-neck flask equipped with a 0.5-inch magnetic bar and filled with argon. The filtered solid, consisting of molecular sieves, was washed with a solution of 366 mg of 4 in 3 ml of acetonitrile, the washing being filtered into the three-neck flask. The total weight of molecular sieves after drying was 322 mg. The flask was connected to a vacuum line fitted with a trap cooled in liquid nitrogen. The stirred solution was concentrated under static vacuum up to 33°C, then under dynamic vacuum while the temperature was raised to 60°C and heated at this temperature at 0.15 mm Hg for 17 hours. The flask was cooled and filled with argon. The residual solid was warmed and stirred with 5 ml of dried acetonitrile, and the spattered solid was largely rinsed down from the side of the flask by the condensing vapor. The solvent was then evaporated and the residue subjected to high vacuum at 60°C as before. The coevaporation and drying were repeated two more times to give persilylated 3.

The flask was then quickly fitted with a mechanical stirrer and septa, and 19 ml of acetonitrile was added. At a bath temperature of -40°C, 1.091 g (4.19 mmoles) of anhydrous tin(IV) chloride was added with stirring during 20 minutes. The bath temperature was lowered to -45°C, and a solution of the chlorosugar 6 in 19 ml of dichloroethane (Aldrich Sureseal, dried with molecular sieves 4A) was added with stirring over 20 minutes. The reaction was stirred vigorously while the bath temperature was raised to -35°C over one hour. The bath temperature was then lowered to -48°C, and 48 ml of a saturated solution of sodium bicarbonate was added, and the mixture was

stirred for 50 minutes. The mixture was then filtered through a pad of Celite and the solid washed with 34 ml of ethyl acetate. The aqueous layer was extracted three more times with 12 ml portions of ethyl acetate. The combined extracts were dried with sodium sulfate overnight and evaporated *in vacuo* to 1.379 g of residue which was kept at -20°C. The total radioactivity was 76 mCi (75%); TLC (8% methanol in chloroform) showed Rf 0.67 (7 and 8; 61% of radioactivity), Rf 0 (14.5%), and Rf 0.96 (23.3%). The combined radiochemical yield of both α- and β- anomers in the crude reaction product, by calculation, is thus 46%.

Similarly, 8.56 mmoles (351 mCi) of 3 was converted to the persilylated derivative 5 with 40.3 mmoles of pyridine and 35.8 mmoles of 4, with five coevaporations during four days. The persilylated product 5 thus obtained was treated with 13.1 mmoles of tin(IV) chloride and 8.56 mmoles of chlorosugar 6, the latter being added in two minutes at a bath temperature of -50 to -58°C. The recovered radioactivity in the crude product was 59%, and the combined yield of the mixture of 7 and 8 in the crude product was 26%.

The combined products from the above two runs were chromatographed over 42 g of silica gel. Compounds 7 and 8 were coeluted with ethyl acetate containing increasing amounts of methanol. The residue from the appropriate fractions (last 75 ml of effluent during elution with 200 ml of 2% methanol, followed by all of 300 ml of 4%, then 100 ml of 6%) was treated with methylene chloride, and some 7 which crystallized was removed by filtration. The filtrate was evaporated to a gel, then heated at 63°C with 16 ml of 50% ethyl acetate-methanol, whereupon crystallization occurred. After about 30 to 45 minutes at room temperature the crystals were filtered and dried to give 1.06 g of 8 containing 4.8% of 7, specific activity 69.4 μCi/mg, or 37.1 mCi/mmole. Two more recrystallizations gave 796.3 mg of purified 8 still containing 1.8% of 7. The two compounds were differentiated by NMR ($CDCl_3$, H-1' at δ6.558, 6.530 for 7, δ6.512, 6.472, 6.437 for 8) and HPLC (Phenomenex Ultramex 5, C8, 0.05M $(NH_4)_2HPO_4$ at pH7.35:MeOH:MeCN-35:32.5:32.5, 1.25 ml/min; retention time, 6.30 min for 7, 7.80 min for 8).

[5-^{14}C]Pentostatin (11). To a mixture of 400 mg (0.746 mmole) of the

keto-β-nucleoside 8 and 3.0 ml of anhydrous methanol in a 25-ml flask filled with argon was added, with stirring, a solution of 86 mg of sodium methoxide in 4 ml of methanol. After two hours the reaction was complete (TLC, 10% methanol in ethyl acetate). After another hour the solution was saturated with carbon dioxide and evaporated in vacuo. The resulting residue was triturated with ether to remove methyl benzoate and filtered to give 348.5 mg of solid containing 9 and inorganic salts. A solution of the solid in 2.3 g of water and 0.85 g of methanol was stirred at -10°C, treated with 16.6 mg of sodium borohydride added in portions with swirling, and allowed to warm up to 0°C. After several hours and the addition of another 1.3 mg of sodium borohydride, the solution was allowed to warm up and stand for one hour at room temperature, then saturated with carbon dioxide. HPLC (Alltech Econosil C-18, 4.6 mm x 250 mm, 0.005M $(NH_4)_2HPO_4$ at pH 7.9:MeOH (88:12), 2.0 ml/min, 282 mμ) showed the presence of 10 and 11 in ratio of 45/55 (retention time, 5.92 and 8.76 min, respectively--highly sensitive to minor changes in conditions).

The solution was clarified through 0.45-μ HPLC filters, then divided into five portions for individual fractionation by preparative HPLC (Alltech Econosil C18, 4.6 mm x 250 mm, joint in series with a 22 mm x 250 mm column as precolumn, 4 ml/min). Compounds 10 and 11 were eluted at 32 min and 41 min, respectively. However, the front portion of the 11 peak contained a hidden minor unknown impurity X, detectable by analytical HPLC. A total of 6.05 mci of 11 containing <1% of X and 5.96 mCi containing no detectable X was obtained.

The fraction of 11 containing X was concentrated to 0.5 g and added to a column of 4.0 g of Diaion-HP20.[15] After collecting about 33 ml of aqueous eluate, the column was eluted with 50 ml of 56% aqueous methanol to give 5.5 mCi of activity (91% recovery). The solution was concentrated and freeze-dried to 47.1 mg of solid and crystallized from 600 mg of methanol. The mother liquor contained 3.7% of a high-Rf radioactive impurity, which was present in 0.8% before crystallization (TLC, chloroform:methanol-1:2).

Similarly, the fraction of 11 containing no X was concentrated to 1.8 ml and desalted with the same Diaion-HP20 column as above to give, without

recrystallization, 5.86 mCi of labeled pentostatin (98% recovery).

A solution containing 100 mg of unlabeled pentostatin was freeze-dried, and the residue was treated with 0.8 g of hot methanol to give crystals, m.p. 212-214°C (dec.; starting to soften at 200°C). NMR showed the presence of trace amounts of methanol; elemental analyses correspond to a formula of $C_{11}H_{16}N_4O_4 . 0.1CH_3OH$.

The two crops of desalted, purified 11 from above, 11.1 mCi, together with 34.6 mg of the freshly crystallized unlabeled pentostatin, were combined and freeze-dried to 120 mg of residue. The crystals which formed upon trituration with 1.0 ml of methanol were filtered, washed with a small amount of methanol, and dried in high vacuum to 110.6 mg of pure labeled pentostatin (10.4 mCi, 94 μCi/mg, radiochemical purity by HPLC 99.6%, chemical purity by HPLC 100%, radiochemical purity by TLC 99.4%).

The overall radiochemical yield of pure product, based on the labeled triethyl orthoformate (2) used, was 3.17%.

ACKNOWLEDGMENTS

The authors thank Prof. David C. Baker, University of Alabama, and Dr. Che C. Huang, Dr. Hollis Showalter, Ms. Annette Travalent, Mr. James L. Hicks, and Dr. James C. French, Parke-Davis Pharmaceutical Research, for helpful advice and discussions.

REFERENCES

1. Also known as co-vidarabine and deoxycoformycin.

2. (a) Ryder A., Dion H.W., Woo P.W., and Howells J.D. - US Patent #3,923,785 (1975). (b) Woo P.W., Dion H.W., Lange S.M., Dahl L.F., and Durham L.J. - J. Heterocycl. Chem. 11: 641 (1974). (c) Dion H.W., Woo P.W.K., and Ryder A. - Ann. NY Acad. Sci. 284: 21 (1977)

3. (a) Glazer R.I. - Cancer Chemother. Pharmacol. 4: 227 (1980). (b) Seto S., Carrera C.J., Wasson D.B., and Carson D.A. - J. Immunol. 136: 2839 (1986)

4. "Cancer Treatment Symposia, Vol. 2, Proc. Conf. 2'-Deoxycoformycin: Current Status and Future Directions, Bethesda, 13 Apr. 1983," Wittes R.E. et al, Ed., National Institute of Health, Bethesda, p.1 (1984)

5. (a) Yu A., Mendelsohn J., and Matsumoto S.S. - Adv. Exp. Med. Biol. 195A: 491 (1986). (b) Major P.P., Agarwal R.P., and Kufe D.W. - Cancer Chemother. Pharmacol. 10: 125 (1983). (c) Allen L.B., Vanderslice L.K., Fingal C.M., McCright F.H., Harris E.F., and Cook P.D. - Antiviral Res. 2: 203 (1982). (d) Shannon W.M. and Schabel F.M.,Jr. - Pharmacol. Ther. 11: 263 (1980)

6. ADA is known to play an important role in lymphocytes in preventing the abnormal intracellular accumulation of deoxyadenosine triphosphate, which inhibits DNA synthesis in dividing lymphocytes and strand repair in mature lymphocytes, and of S-adenosylhomocysteine, which inhibits the methylation process critical for cell growth.[3]

7. (a) O'Dwyer P.J., Wagner B., Leyland-Jones B., Wittes R.E., Cheson B.D., and Hoth D.F. - Ann. Intern. Med. 108: 733 (1988). (b) Spiers A.S.D. - Blood Rev. 1: 106 (1987). (c) Lofters W., Campbell M., Gibbs W.N., and Cheson B.D. - Cancer 60: 2605 (1987)

8. (a) Drugs of the Future 14: 711 (1989). (b) Kraut E.H., Bouroncle B.A., and Grever M.R. - J. Clin. Oncol. 7: 168 (1989). (c) Lembersky B.C., Ratain M.J., Westbrook C., and Golomb H.M. - Am. J. Hematol. 27: 60 (1988). (d) Ho A.D., Kuse R., Prummer O., Porzsolt F., and Hunstein W. - Klin Wochenschr 65: 975 (1987)

9. The precise mechanism of the highly effective action of pentostatin in hairy cell leukemia, a chronic leukemia of B lymphocyte lineage, has not been determined with certainty, and a more definitive understanding may well lead to further advances in the treatment of other lymphoid malignancies.[8]

10. (a) Borondy P.E., Chang T., Maschewske E., and Glazko A.J. - Ann. NY Acad. Sci. 284: 9 (1977). (b) Putt S.R., Hartman J.D., Showalter H.D.H., Kepler J.A., and Taylor G. - J. Labelled Compd. Radiopharm. 18: 925 (1981)

11. (a) Hanvey J.C., Hardman J.K., Suhadolnik R.J., and Baker D.C.- Biochemistry 23: 904 (1984). (b) Hanvey J.C., Hawkins E.S., Tunac J.B., Dechter J.J., Baker D.C., Suhadolnik R.J. - Biochemistry 26: 5636 (1987).

12. (a) Chan E., Putt S.R., Showalter H.D.H., and Baker D.C. - J. Org. Chem. 47: 3457 (1982). (b) Showalter H.D.H., and Putt S.R. - Tetrahedron Lett. 22: 3155 (1981)

13. Oae S., Tagaki W., and Ohno A. - Tetrahedron 20: 417 (1964)

14. (a) Bhat C.C. - Synth. Proced. Nucleic. Acid. Chem. 1: 521 (1968). (b) Hoffer M. - Chem. Ber. 93: 2777 (1960). (c) Nuhn P., Zschunke A., Heller D., and Wagner G.-Pharmazie 24: 237 (1969).

15. A non-ionic polystyrene-type macroporous adsorbent manufactured by Mitsubishi Chemical Industries Ltd., Japan, and distributed by Nippon Rensui Co., Tokyo, Japan. Cf. Voser W. and Weiss K. - J. Chromatogr. 201: 287 (1980).