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SYNTHESES OF α- AND γ-TOCOPHEROLS SELECTIVELY LABELLED WITH DEUTERIUM[1]

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Summary

Four deuterium-substituted α-tocopherols (dideutero-*RRR*, nonadeutero-*ambo*, nonadeutero-*all-rac* and undecadeutero-*all-rac*) and a dideutero-*RRR*-γ-tocopherol have been synthesized for use in studies of the biokinetics, bioavailability and metabolism of vitamin E.

Key Words: deuterated α-tocopherol, deuterated γ-tocopherol, biokinetics, bioavailability

Introduction

In our studies of the biokinetics and bioavailabilities of different forms of vitamin E we have required various deuterium-substituted α-tocopherols for use both as sources of labelled vitamin E and as internal standards for gas chromatography-mass spectrometric analyses (1-8). The syntheses of some of these compounds have already been reported in an earlier paper (2). In this paper we report the syntheses of other deuterium substituted α-tocopherols with the natural, 2*R*,4'*R*,8'*R* (*RRR*) configuration, **1** (the most active form *in vivo* (9)), and the mixed configurations of the *ambo* (*RRR* + *SRR*) and the *all-racemic* (all eight possible stereoisomers) forms. We also report the first synthesis of a deuterium-substituted

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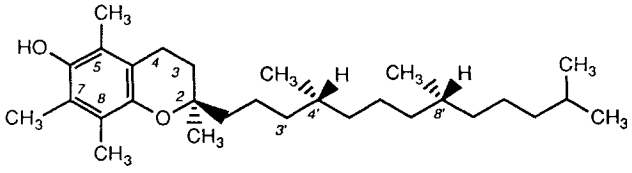
(dideutero) *RRR*-γ-tocopherol, a compound which differs from α-tocopherol in that it lacks the 5-methyl group.



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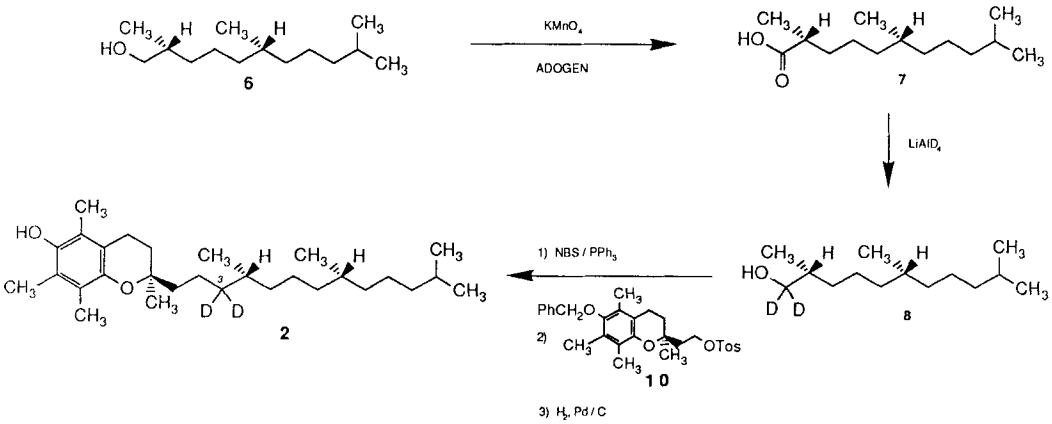
Discussion

The deuterium labelled tocopherols that have been prepared are: 2*R*,4'*R*,8'*R*-α-(3',3'-$^2H_2$)tocopherol (**2**), 2*RS*,4'*RS*,8'*RS*-α-(5,7,8-(C^2H_3)$_3$)tocopherol (**3a**), 2*RS*,4'*R*,8'*R*-α-(5,7,8-(C^2H_3)$_3$)tocopherol (**3b**), 2*RS*,4'*RS*,8'*RS*-α-(3,4-$^2H_2$-5,7,8-(C^2H_3)$_3$)tocopherol (**4**) and 2*R*,4'*R*,8'*R*-γ-(3,4-$^2H_2$)tocopherol (**5**) (Schemes 1-3).

Compound **2** (Scheme 1) was prepared by selective oxidation of **6**, 2*R*,6*R*-(+)-2,6,10-trimethylundecan-1-ol (10), via phase transfer catalysis. Adogen 464 was used to effect the solubilization of permanganate anion (11) to give 2*R*,6*R*-(+)-2,6,10-trimethylundecanoic acid (**7**). $LiAlD_4$ reduction gave the key dideutero alcohol **8**. Subsequently, the title compound **2** was obtained via the synthesis reported by Cohen et al (10), i.e. bromination of **8**, Fouquet-Schlosser (12) coupling of the bromide **9** with the tosylate **10** followed by hydrogenation.

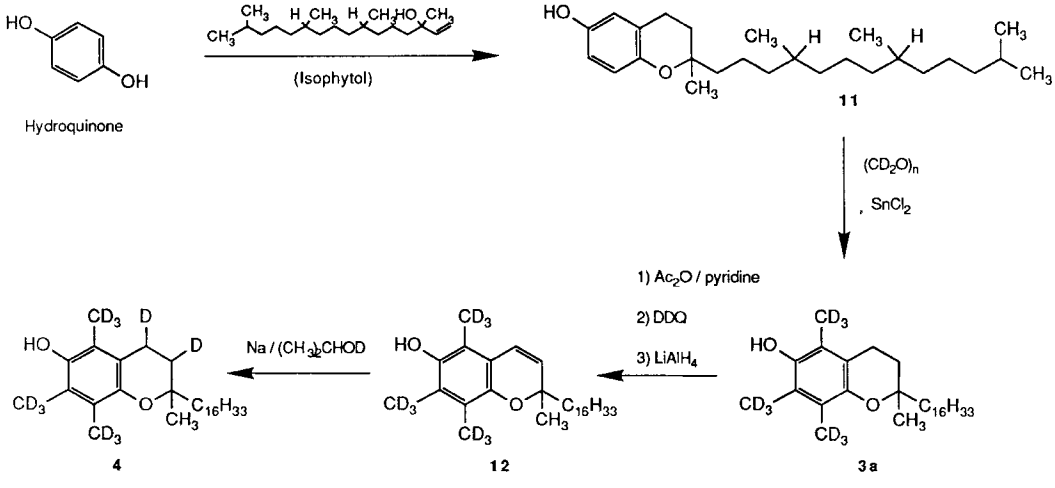
SCHEME 1



Compound **3a** (Scheme 2) was synthesized by stannous chloride-catalyzed deuteriomethylation with perdeutero-paraformaldehyde (13) of tocol **11**. For the synthesis of

11, i.e. condensation of hydroquinone with isophytol catalysed by formic acid, the method of Manalis et al (14) was utilized. We have optimized the yield of **11** (51% vs 4.5% (14)) by monitoring the reaction using thin-layer chromatography (T.L.C.) and purifying the crude product by flash column chromatography (15). Compound **3b** was synthesized in the same way as **3a** except that natural phytol was used in place of isophytol.

SCHEME 2



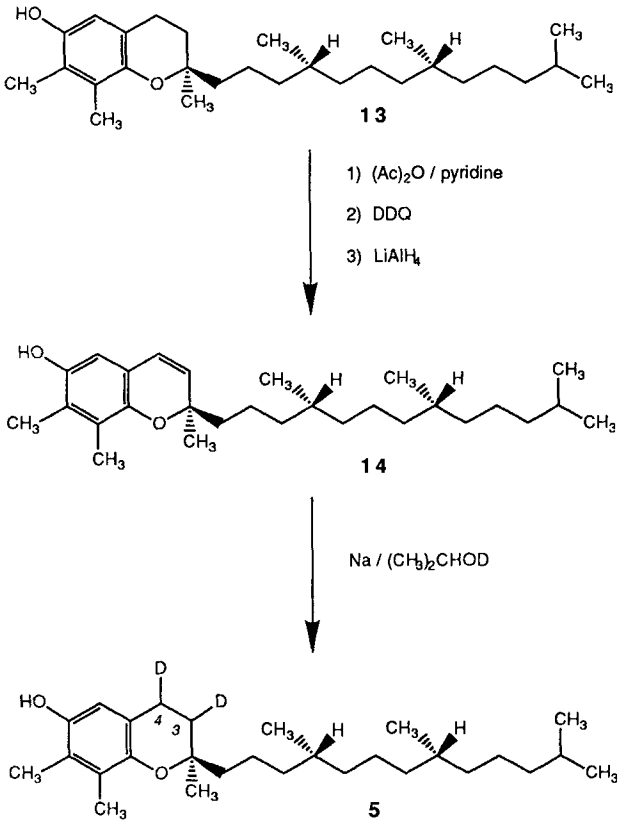
Compound **4** was obtained via DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) dehydrogenation (16) of **3a** and subsequent Bouveault-Blanc reduction of 3,4-dehydro-2*RS*,4'*RS*,8'*RS*-α-(5,7,8-(C^2H_3)$_3$)tocopherol (**12**) with sodium in 2-propan(ol-d). Similar treatment of 2*R*,4'*R*,8'*R*-γ-tocopherol (i.e., dehydrogenation followed by reduction) yielded compound **5**. (Scheme 3).

Materials and Methods

2*R*,4'*R*,8'*R*-γ-tocopherol and 2*R*,6*R*-(+)-2,6,10-trimethylundecan-1-ol were generous gifts from Eastman Chemicals and Hoffmann-La Roche respectively. Isophytol was purchased from ICN Biochemicals. Adogen 464, hydroquinone, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and 2-propan(ol-d) were purchased from Aldrich. Lithium aluminum deuteride, perdeutero-formaldehyde and DCl (35% in D_2O) were obtained from Merck, Sharp and Dohme. Commercial stannous chloride was made anhydrous by treatment with acetic anhydride,filtering, washing with ether and drying under high vacuum for 24 hr.

Thin layer chromatography (T.L.C.) was performed on silica gel (60F-254) BDH plates and developed with ethyl acetate/hexane. Spots were visualized using a phosphomolybdic acid spray (3.5% in ethanol) followed by heating at 80°C. Column chromatographic purification followed the "flash" method (15) using Merck grade 60 silica gel

SCHEME 3



(230-400 mesh, 60Å) from Aldrich. Unless otherwise noted, reactions were carried out under a nitrogen atmosphere. Normal work-up involved three extractions into the solvent specified. The organic extracts were combined, washed with water, $NaHCO_3$ solution or dilute HCl as required, and saturated brine, dried over Na_2SO_4, filtered, and concentrated at 30°C on a rotary evaporator. The residue was further dried to constant weight under high vacuum. All yields refer to isolated yields obtained after a final purification by column chromatography using ethyl acetate in hexane as the eluting solvent.

Mass spectra were measured on a Hewlett-Packard 5970A Mass Selective Detector using an HP-Ultra 1 fused silica capillary gas chromatographic column (10 m x 0.2 mm i.d., OV-101 type, cross-linked bonded phase).

^{1}H NMR spectra were recorded on a Varian EM 360A 60 MHz instrument. Compounds **2, 3** and **4** were homogeneous on T.L.C. (12% ethyl acetate/hexane) with a reference sample of 2*R*,4'*R*,8'*R*-α-tocopherol, R_f = 0.59. Compound **5** was homogeneous on T.L.C. with a reference sample of 2*R*,4'*R*,8'*R*-γ-tocopherol, R_f = 0.43.

Experimental

2*R*,6*R*-(+)-2,6,10-Trimethylundecanoic acid (7).

Compound **6** (10) (10 g; 43.8 mmol) was dissolved in CH_2Cl_2 (410 ml), water (275 ml) and acetic acid (13.8 ml). Adogen 464 (3.1 ml) was then added to the above well stirred heterogeneous solution, followed by the portionwise addition of $KMnO_4$ (13.7 g). The reaction was stirred 18 hr at 25°C after which time T.L.C. analysis (25% ethyl acetate/hexane) showed no **6** remaining. Sodium bisulfite was then added, in portions, until the brown color disappeared. The CH_2Cl_2 phase was separated, the aqueous phase saturated with solid NaCl and normal work up in ether gave the crude acid **7** (14.6 g). Purification by column chromatography (15% ethyl acetate/hexane) gave **7** as the pure acid (9.8 g; 90%). ^{1}H NMR ($CDCl_3$, Me_4Si (int)), δ 0.7 - 1.9 (m, 26H, 4(CH_3), 6 (CH_2), 2(CH)), 2.0 - 2.7 (m, 1H, CHCOOH), 11.5 (s, 1H, COO**H**). GC-MS, m/z (rel. intensity), 228 ($M^{+\bullet}$, 1), 195 (1), 152 (39), 74 (100).

2*R*,6*R*-(+)-2,6,10-Trimethylundecan(1,1-$^2H_2$)ol (8) and 2*R*, 4'*R*, 8'*R*-α-(3',3'-$^2H_2$)tocopherol (2).

Compound **7**, (9.8 g, 46.4 mmol), was reduced with $LiAlD_4$, (2.7 g, 65.8 mmol), in ether (108 ml). Work up with ether gave 8.5 g (94.4%) of **8**. ^{1}H NMR ($CDCl_3$, Me_4Si (int)), δ 0.7 - 1.7 (m, 27H, 4(CH_3), 6(CH_2), 3(CH)), absence of triplet at 3.5 (CH_2-OH). The synthesis was continued according to the procedure of Cohen et al. (10) to give **2**. ^{1}H NMR ($CDCl_3$, Me_4Si (int)) δ 0.7 - 2.0 (m, 36H, phytyl tail, 2-CH_3 and Ar-CH_2-CH_2), 2.1 (broad s, 9H, 3(Ar-CH_3)), 2.6 (t, 2H, Ar-CH_2-CH_2, J = 9Hz). GC-MS, m/z (rel. intensity), 432 ($M^{+\bullet}$, 97), 430 (0.4), 165 (100).

2-Methyl-2-(4,8,12-trimethytridecyl)-6-chromanol (tocol 11).

Hydroquinone (5.36 g; 48 mmol), isophytol (14.4 g; 48 mmol), HCOOH (98%; 73 ml), and

benzene (73 ml) were refluxed for 4 hr. T.L.C. analysis (10% ethyl acetate/hexane) showed no starting hydroquinone. The organic layer was separated and the aqueous layer was extracted twice with ether. Normal work up and purification by column chromatography (5% ethyl acetate/hexane) afforded 9.6 g (51%) of pure tocol **11** as a pale yellow oil. ^{1}H NMR ($CDCl_3$, Me_4Si (int)), δ 0.7-2.0 (m, 38H, phytyl tail, 2-C**H**$_3$ and Ar-CH_2-C**H**$_2$), 2.6 (t, 2H, Ar-C**H**$_2$-CH_2, J = 9Hz), 5.0 (s, 1H, O**H**), 6.5 (m, 3H, 5,7,8-Ar**H**). GC-MS, m/z (rel. intensity), 388 ($M^{+\bullet}$, 100), 163 (7), 123 (85).

2*RS*,4'*RS*,8'*RS*-α-(5,7,8-(C^2H_3)$_3$)Tocopherol (3a).

The synthesis followed that of Urano et al (13). Minor modifications improved the yield from 44% (13) to 74%. Tocol **11** (9.6 g; 24.7 mmol), was dissolved in anhydrous isopropyl ether (960 ml). To this solution were added anhydrous $SnCl_2$ (129.3 g; 0.6 mol) , DCl (35% in D_2O; 443 ml) and $(CD_2O)_n$ (5.38 g). The heterogeneous solution was refluxed 2.5 hr, after which time no **11** could be detected by T.L.C. (12% ethyl acetate/hexane). The reaction mixture was poured onto ice, the organic phase was separated and the aqueous phase re-extracted into ether. Work up and purification by column chromatography (3% ethyl acetate/hexane) gave pure **3a** (8.0 g; 74%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, Me_4Si (int)) δ 0.7-2.0 (m, 38H, phytyl tail, 2-C**H**$_3$ and Ar-CH_2-C**H**$_2$), absence of 5,7,8-ArC**H**$_3$ at 2.1, 2.6 (broad t, 2H, Ar-C**H**$_2$-CH_2, J = 10Hz), 4.1 (s, 1H, O**H**). GC-MS, m/z (rel. intensity), 439 ($M^{+\bullet}$, 78), 438 (13), 437 (1), 174 (100).

2*RS*,4'*R*,8'*R*-α-(5,7,8-(C^2H_3)$_3$)Tocopherol (3b).

This compound was prepared in an identical way to that of **3a** using natural phytol instead of isophytol to prepare the 2-*ambo* (2*RS*,4'*R*,8'*R*) analogue of **11**.

2*RS*,4'*RS*,8'*RS*-α-(3,4-$^2H_2$-5,7,8-(C^2H_3)$_3$)Tocopherol (4).

Treatment of the acetate of **3a** with DDQ, as described below for the conversion of **13** to **14**, provided 3,4-dehydro-2*RS*,4'*RS*,8'*RS*-α-(5,7,8-(C^2H_3)$_3$)tocopherol, **12**. GC-MS, m/z (rel. intensity), 437 ($M^{+\bullet}$, 3), 212 (100). **12** was reduced with sodium in 2-propan(ol-d) as described below for the conversion of **14** to **5** to give the title compound, **4**. GC-MS, m/z (rel. intensity), 441 ($M^{+\bullet}$, 70), 440 (11), 439 (1), 175 (100). ^{1}H NMR ($CDCl_3$, Me_4Si (int)) δ 0.7-2.0 (m, 37H, phytyl tail, 2-C**H**$_3$ and Ar-CHD-C**H**D), absence of 5,7,8-ArC**H**$_3$ at 2.1, 2.5 (m, 1H, Ar-C**H**D-CHD), 4.1 (s, 1H, O**H**).

3,4-Dehydro-2*R*,4'*R*,8'*R*-γ-Tocopherol (14).

2*R*, 4'*R*, 8'*R*-γ-tocopherol, **13** (5.4 g; 13.0 mmol), was dissolved in pyridine and cooled to 0°C. Acetic anhydride (18.2 ml) was added dropwise and the solution stirred at 25°C for 1 hr. T.L.C. analysis (5% ethyl acetate/hexane) indicated none of the starting material, **13**, was left. The solution was poured onto ice and stirred 10 min. Work up with ether gave 6.2 g of crude and column chromatography (3% ethyl acetate/hexane) gave 4.9 g (83%) of pure 2*R*,4'*R*,8'*R*-γ-tocopheryl acetate. ^{1}H NMR ($CDCl_3$, Me_4Si (int)), δ 0.7-1.9 (m, 38H, phytyl tail, Ar-CH_2-C**H**$_2$ and 2-C**H**$_3$), 2.0 and 2.1 (2s, 6H, 7,8-ArC**H**$_3$), 2.3 (s, 3H, C**H**$_3$-CO), 2.7 (t, 2H, Ar-C**H**$_2$-CH_2, J=9Hz), 6.5 (s, 1H, 5-Ar**H**). This compound (4.9 g; 10.6 mmol) was dissolved in toluene (109 ml) and purged with nitrogen. The solution was heated to 90°C and a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 5.2 g; 22.9 mmol) in toluene (110 ml) was added dropwise, in four portions, over a period of 2 hr. After this time the resultant mixture was refluxed 18 hr. The dark brown mixture was subsequently cooled to 0°C, filtered and the filtrate evaporated. Treatment with 200 ml 5% ethyl acetate/hexane, filtration and evaporation of the filtrate afforded crude 3,4-dehydro-2R,4'R,8'R-γ-tocopheryl acetate. Column chromatography (2% ethyl acetate/hexane) afforded 4.1 g (83.6%) of pure material. ^{1}H NMR ($CDCl_3$, Me_4Si (int)), δ 0.7-1.7 (m, 36H, phytyl tail and 2-C**H**$_3$), 2.0 and 2.1 (2s, 6H, 7,8-ArC**H**$_3$), 2.3 (s, 3H, O-CO-C**H**$_3$), 5.5 and 6.2 (2d, 2H, Ar-C**H**=C**H**, J = 10Hz), 6.4 (s, 1H, 5-Ar**H**). GC-MS m/z (rel. intensity), 456 ($M^{+\bullet}$,2), 231(100), 189(37). Reduction of this dehydro-γ-tocopheryl acetate (4.1 g; 8.9 mmol) with $LiAlH_4$ (1.0 g; 27 mmol) in ether (126 ml) gave pure **14** (3.7 g; 100%) after purification by column chromatography (2% ethyl acetate/hexane). GC-MS, m/z (rel. intensity), 414 ($M^{+\bullet}$, 3), 189 (100).

2*R*,4'*R*,8'*R*-γ-(3,4-$^2H_2$)Tocopherol (5)

Compound **14** (1 g; 2.4 mmol) was dissolved in 2-propan(ol-d) (30 ml) and heated to 60°C. Sodium (4g; 0.17 g atoms) was added quickly and the mixture refluxed for 15 min., after which time more 2-propan(ol-d) (30 ml) was added. The thick mixture was refluxed until all the sodium had appeared to go into solution. The mixture was allowed to stand under nitrogen at 25°C for 12 hr. After this time GC-MS analysis indicated 15% of the starting material, **14**, was left. In order to ensure complete solution of the sodium, more 2-propan(ol-d) (30 ml) was added with efficient stirring. Work-up was effected by *carefully* pouring the dark green mixture onto ice. Three-fold extraction into ether and purification by column

chromatography (5% ethyl acetate/hexane) gave 0.7 g of a mixture comprising 85% **5** and 15% of the starting material, **14**, as determined by GC-MS analysis. This mixture (0.7 g) was dissolved in 2-propan(ol-d) (10 ml), heated to 60°C and sodium (2.5 g) was quickly added. Treatment and purification of the reaction mixture as described above gave **5** (0.58 g; 68%). GC-MS analysis indicated **5** was 100% pure. GC-MS, m/z (rel. intensity), 418 ($M^{+\bullet}$, 77), 416 (0.5), 193 (22), 152 (100). ^1H NMR ($CDCl_3$, Me_4Si (int)) δ 0.7-2.0 (m, 37H, phytyl tail, 2-C**H**$_3$ and Ar-CHD-C**H**D), 2.1 (broad s, 6H, 7,8-ArC**H**$_3$), 2.6 (m, 1H, Ar-C**H**D-CHD),4.2 (s, 1H, O**H**), 6.3 (s, 1H, 5-Ar**H**).

Acknowledgments

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References

1. Ingold, K.U., Burton, G.W., Foster, D.O., Hughes, L., Lindsay, D.A. and Webb, A. - Lipids **22**: 163 (1987).
2. Ingold, K.U., Hughes, L., Slaby, M. and Burton, G.W. - J. Labelled Compd. Radiopharm. **24**: 817 (1987).
3. Cheng, S.C., Burton, G.W., Ingold, K.U. and Foster, D.O. - Lipids **22**: 469 (1987).
4. Traber, M.G., Ingold, K.U., Burton, G.W. and Kayden, H.J. - Lipids **23**: 791 (1988).
5. Burton, G.W., Ingold, K.U., Foster, D.O., Cheng, S.C., Webb, A., Hughes, L. and Lusztyk, E. - Lipids **23**: 834 (1988).
6. Traber, M.G., Sokol, R.J., Burton, G.W., Ingold, K.U., Papas, A.M., Huffaker, J.E. and Kayden, H.J. - J. Clin. Invest. **85**: 397 (1990).
7. Traber, M.G., Burton, G.W., Ingold, K.U. and Kayden, H.J. - J. Lipid Res. **31** (In press) (1990).
8. Burton, G.W., Wronska, U., Stone, L., Foster, D.O. and Ingold, K.U. - Lipids **25**: (In press) (1990).
9. Wieser, H. and Vecchi, M. - Inter. J. Vit. Nutr. Res. **52**: 351 (1982).
10. Cohen, N., Eichel, W.F., Lopresti, R.J., Neukom, C. and Saucy, G. - J. Org. Chem. **41**: 3505 (1976).

11. Lee, D.G.- Oxidation in Organic Chemistry. (Part D), Academic Press, New York: 193 (1982).
12. Fouquet, G. and Schlosser, M. - Angew. Chem. Int. Ed. Engl. **13**: 82 (1974).
13. Urano, S., Hattori, Y., Yamanoi, S. and Matsuo, M. - Chem. Pharm. Bull. **28**:1992 (1980).
14. Mamalis, P., McHale, D., Green, J. and Marcinkiewicz, S. - J. Chem. Soc. 1850 (1958).
15. Still, W. C., Kahn, M. and Mitra, A. - J. Org. Chem. **43**: 2923 (1978).
16. Mayer, H. and Isler, O. - Methods in Enzymology **18**: 328 (1971).