Synthesis of 5,8,11-Dodecatriynoic Acid and Its Use in the Synthesis of Arachidonic Acid and Related Acids

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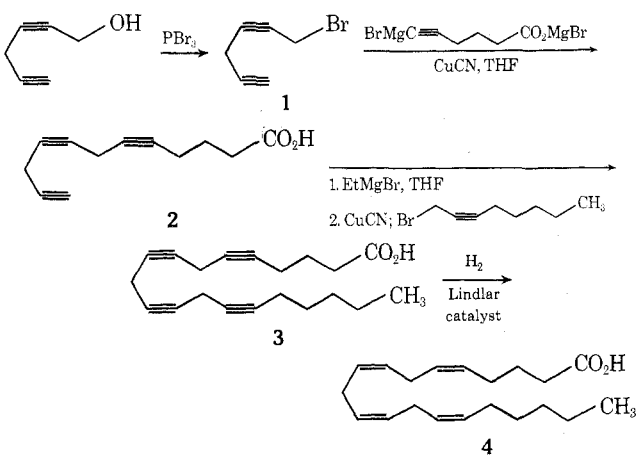
The preparation of 5,8,11-dodecatriynoic acid *via* a Grignard coupling of 1-bromo-2,5-hexadiyne and 5-hexynoic acid is described. The triynoic acid has been used as an intermediate in a new synthesis of arachidonic acid and of novel methyl-branched arachidonic acids.

The standard approach for the synthesis of arachidonic acid, *all-cis*-5,8,11,14-eicosatetraenoic acid (4), has been the preparation of long-chain polyacetylenic compounds followed by the selective hydrogenation of the acetylenic bonds to cis olefins. Two variations, among others, of this approach which have been adopted for the synthesis of arachidonic acid involve (a) the coupling of a C_{10} and a C_9 fragment[1] and (b) the coupling of a C_{14} and a C_6 fragment.[3-5]

We now wish to report a new synthesis of arachidonic acid which involves the coupling of a C_8 fragment with a novel C_{12} fragment, 5,8,11-dodecatriynoic acid (2).

The C_{12} acid **2** was prepared by bromination of 2,5-hexadiyn-1-ol[6] with phosphorus tribromide to give 1-bromo-2,5-hexadiyne (1) which was then coupled with 5-hexynoic acid in the presence of ethylmagnesium bromide and cuprous ion (see Scheme I). The acid **2** was then coupled with

Scheme I



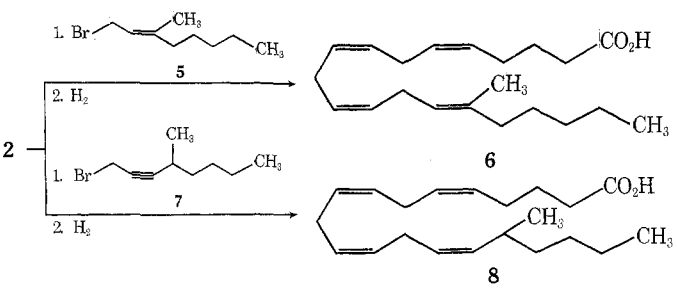
the C_8 fragment, 1-bromo-2-octyne,[7] under the same Grignard conditions, to give 5,8,11,14-eicosatetraynoic acid (3) which was selectively reduced to arachidonic acid (4), according to literature procedures.[3]

The triynoic acid **2**, which is very sensitive to air oxidation, was purified by low-temperature recrystallization and can be stored for several months at −40° when kept in an inert (argon) atmosphere.

The intermediate **2** has also been used for the preparation of 15-methyl- and 16-methylarachidonic acids, compounds **6** and **8** respectively, two novel alkyl-branched fatty acids (see Scheme II).

The allylic bromide **5** was prepared by bromination of *cis*- and *trans*-3-methyl-2-octen-1-ol[8] with phosphorus tribromide. The acetylenic bromide **7** was prepared by the hydroxyalkalation of 3-methyl-1-heptyne[9] followed by bromination with phosphorus tribromide.

Scheme II



These two bromo compounds were individually coupled with **2** under the Grignard conditions described above to give polyacetylenic intermediates, which were extremely unstable and difficult to purify. The crude acetylenic products were treated with silver nitrate in aqueous ethanol to remove excess acid **2**, followed by recrystallization at −10° to give unstable, low-melting solids. The nmr spectra of these acetylenic acids were consistent with the assigned structures. These partially purified acids were immediately hydrogenated over Lindlar catalyst to the tetraenoic acids **6** and **8** which were easily purified by column chromatography.

The 15-methyl acid **6** was obtained as a mixture of cis–trans isomers at the C_{14} double bond. The original alcohol, 3-methyl-2-octen-1-ol,[8] was obtained as a 4:1 trans:cis isomeric mixture and the final tetraenoic acid **6** was assumed to have predominantly the trans configuration at the 14–15 double bond, although this point was not conclusively proven.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected. The ir spectra were recorded with either a Beckman IR-9 or a Perkin-Elmer 137 spectrophotometer. The nmr spectra were recorded with a Varian T60, A-60, or HA-100 instrument in deuteriochloroform. Absorption peaks are recorded in parts per million downfield from an internal standard (TMS). All reactions were carried out under argon. All Grignard reagents were prepared immediately before use and were standardized by titration.

1-Bromo-2,5-hexadiyne (1). To a solution of 10.9 g (116 mmol) of crude 2,5-hexadiyn-1-ol[6] in 175 ml of ether (cooled to 5°) was added, *via* syringe, 4.35 ml (12.4 g, 46 mmol) of phosphorus tribromide (PBr_3). The mixture was stirred at 3–10° for 6 hr. After pouring into ice water, the aqueous phase was extracted well with ether. The ether extracts were combined, washed with saturated bicarbonate, brine, dried (Na_2SO_4), concentrated, and distilled (cautiously) through a short-path column to yield 9.6 g (53%) of 1 as a colorless oil, bp 40–43° (0.3 mm). The compound darkens rapidly and should be used as soon as possible after distillation: ir (neat) 3240 (C≡CH) 1405, 1310, 1210 cm^{-1} (no absorption due to OH); nmr δ 2.12 (t, 1 H, C≡CH), 3.52 (q, 2 H, C≡CCH$_2$C≡C), 3.93 (t, 2 H, CH$_2$Br).

5,8,11-Dodecatriynoic Acid (2). To a solution of 10.98 g (98 mmol) of 5-hexynoic acid in 100 ml of THF, cooled to 5°, was

added dropwise 116 ml (196 mmol) of a 1.69 *M* solution of ethylmagnesium bromide in THF. The solution was allowed to warm to room temperature over 1 hr and 500 mg of cuprous cyanide (CuCN) was added. After stirring 20 min, a solution of 7.7 g (49 mmol) of 1 in 40 ml of THF was added dropwise over 30 min. The reaction was stirred for 5 hr at room temperature and an additional 500 mg of CuCN was added. After stirring for a total of 22 hr, the mixture was poured into 300 ml of 3 *N* H_2SO_4 and 100 g of ice. After extraction with ether, the organic phase was concentrated *in vacuo*. The residue was dissolved in ether and the ether solution washed with Versene[10] several times to remove copper, washed with water, dried ($MgSO_4$), and concentrated to yield a brown oil. The oil was dissolved in a mixture of ether and petroleum ether (bp 30–60°) and stored overnight at −40°. The crystalline product was recrystallized three times at −30° from ether–petroleum ether to yield 3.0 g (30%) of **2**, mp 51–53°. The triynoic acid was sensitive to air as judged by coloration and lowering of melting points. An analytical sample was prepared by recrystallization from hexane at −15° to give beige plates: mp 57–58°; ir (KBr) 3295, 3285 (C≡CH), 1710, 1690 (C=O), 920 cm^{-1}; nmr δ 1.80 (m, 2 H, $CH_2CH_2CH_2$), 2.07 (t, 1 H, C≡CH), 2.25 (dist. t, 2 H, C≡CCH_2CH_2), 2.47 (t, 2 H, CH_2C=O), 3.13 (m, 4 H, C≡CCH_2C≡CCH_2C≡C), 10.77 (s, 1 H, CO_2H).

Anal. Calcd for $C_{12}H_{12}O_2$: C, 76.58; H, 6.43. Found: C, 76.43; H, 6.61.

5,8,11,14-Eicosatetraynoic Acid (3). To a solution of 3.95 g (21 mmol) of **2** in 30 ml of THF at 3° was added dropwise 37.2 ml (42 mmol) of a 1.13 *M* solution of ethylmagnesium bromide in THF. The mixture was stirred at room temperature for 1.5 hr and 150 mg of CuCN added. After stirring for 20 min, 2.65 g (14 mmol) of 1-bromo-2-octyne[7] was added and rinsed in with 5 ml of THF. The mixture was then stirred for 18 hr with an additional 150 mg of CuCN added after 6 hr. The product was isolated in the same manner as that described for **2**, and was recrystallized from isopropyl alcohol to give 2.70 g (64%) of **3**. An analytical sample was prepared by recrystallization from *i*-PrOH at −10°. The acid was obtained as beige plates: mp 80.5–82° (lit.[3b] mp 81–82°); ir ($CHCl_3$) 3400–2500 (broad OH), 1715; nmr δ 0.90 (dist. t, 3 H, CH_3), 1.38 (br m, 6 H, $(CH_2)_3CH_3$), 1.85 (m, 2 H, CH_2CH_2C=O), 2.03–2.37 (br m, 4 H, CH_2CH_2C≡C), 2.49 (t, 2 H, CH_2CO_2), 3.15 (m, 6 H, C≡CCH_2C≡C), 10.85 (s, 1 H, CO_2H).

Anal. Calcd for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 81.18; H, 8.25.

1-Bromo-3-methyl-*cis,trans*-2-octene (5). To a solution of 19.0 g (135 mmol) of 3-methyl-*cis,trans*-2-octen-1-ol[8] in 400 ml of ether, cooled to 5°, was added, *via* syringe, 9.8 ml (14.3 g, 53 mmol) of PBr_3. The reaction was stirred at 3–10° for 5 hr, and the product isolated following the procedure given for **1**. Distillation of the crude product yielded 19.6 g (71%) of **5** as a colorless oil: bp 50.5–51.5° (0.7 mm); ir (neat) 1605, 1460, 1375, 1205 cm^{-1} (no absorption due to OH); nmr δ 0.90 (dist. t, 3 H, CH_3CH_2), 1.30 (br m, 6 H, $CH_2CH_2CH_2$), 1.70 (d, 3 H, CH_3C=C, J = 2 Hz), 2.03 (dist. t, 2 H, CH_2C=C), 4.00 (d, 2 H, CH_2Br), 5.50 (t, 1 H, C=CH).

Anal. Calcd for $C_9H_{17}Br$: C, 52.70; H, 8.35. Found: C, 53.03; H, 8.30.

15-Methyl-14-*cis,trans*-eicosaen-5,8,11-triynoic Acid. To a solution of 12.4 g (66 mmol) of **2** in 120 ml of THF, cooled to 0°, was added dropwise 132 ml (132 mmol) of a 1.0 *M* solution of ethylmagnesium bromide in THF. The mixture was warmed to room temperature and stirred for 4.5 hr and then 400 mg of CuCN was added. After stirring 20 min, a solution of 9.02 g of **5** in 50 ml of THF was added dropwise. The mixture was then stirred for 17 hr, with an additional 400 mg of CuCN added after 6 hr. The reaction was worked up as described for **2** to give 20.5 g of crude product. The crude product was dissolved in 60 ml of absolute EtOH and added to a solution of 22 g of $AgNO_3$ dissolved in 25 ml of H_2O and 225 ml of absolute EtOH to precipitate the silver salt of **2**. The mixture was filtered through Hyflo and the filtrate was diluted with an equal volume of water and extracted twice with a 1:1 mixture of ether–pentane. The combined organic extracts were washed three times with water, once with brine, dried ($MgSO_4$), and concentrated to give 12.5 g of orange oil. The oil dissolved in pentane and recrystallized at −10°. The product was filtered quickly through a prechilled funnel and recrystallized from ether–pentane at 5° to give 3.8 g of a gummy yellow solid (low melting) which was dried under high vacuum. The product should be stored under argon at −40° to prevent decomposition. The product was too unstable to obtain a satisfactory elemental analysis: ir (neat) 3500–2400 (br OH), 1710 (C=O), 1410 cm^{-1}; nmr δ 0.89 (dist. t, 3 H, CH_3CH_2), 1.45 (br m, 6 H, $CH_2CH_2CH_2CH_3$), 1.60 (s, 3 H, CH_3C=C), 1.85–2.68 (m, 8 H, $CH_2CH_2CH_2CO_2$, CH_2C=C), 2.88 (d, 2 H, C=CHCH_2C≡C), 3.13 (t, 4 H, C≡CCH_2C≡CCH_2C≡C, J = 2 Hz), 5.02 (t, 1 H, olefin proton).

15-Methyl-5,8,11-*cis*-14-*cis,trans*-eicosatetraenoic Acid (6). To a solution of 870 mg (2.7 mmol) of 15-methyl-14-*cis,trans*-eicosaen-5,8,11-triynoic acid in 25 ml of absolute EtOH was added 300 mg of Lindlar catalyst followed by 0.1 ml of quinoline. Immediately, the mixture was reduced under a slight positive pressure of hydrogen. The reaction took up 213 ml of H_2 in 7500 sec; theoretical uptake (corrected), 206 ml. The mixture was filtered through hyflo and concentrated *in vacuo*. The residue was dissolved in ether, washed with cold 1 *N* HCl, brine, dried ($MgSO_4$), and concentrated to give 827 mg of crude product as a gold colored oil. Chromatography on a 21 × 1.3 cm column of silica gel, using ether in hexane (the percentage of ether was gradually increased from 0 to 16%) as eluent, gave 689 mg (79%) of **6** as a lightly colored oil. A small amount was rechromatographed to give the analytical sample: ir (neat) 3550–2550 (br OH), 1710 (C=O) cm^{-1}; nmr δ 0.87 (dist. t, 3 H, CH_2CH_3), 1.26 (br m, 6 H, $CH_2CH_2CH_2CH_3$), 1.60 (s, 3 H, CH_3C=C), 1.69 (t, 2 H, $CH_2CH_2CO_2$), 2.02 (m, 4 H, CH_2C=C, C=CHCH_2CH_2), 2.35 (t, 2 H, CH_2CO_2), 2.81 (br m, 6 H, 3 × C=CCH_2C=C), 5.09 (t, 1 H, CH_3C=CH), 5.35 (m, 6 H, 3 × CH=CH), 10.5 (br s, 1 H, CO_2H).

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 78.81; H, 10.51.

4-Methyl-2-octyn-1-ol. To a solution of 28.g (254 mmol) of 3-methyl-1-heptyne[9] in 1 l. of THF, cooled to 0°, was added dropwise 145 ml (230 mmol) of a 1.6 *M* solution of *n*-butyllithium in hexane. After stirring for 45 min, 10.5 g (350 mmol) of *p*-formaldehyde (dried over P_2O_5) was added. The reaction was stirred for 1 hr at room temperature, heated to 45–50° for 3 hr, cooled, and concentrated *in vacuo* to a volume of 300 ml. The concentrated solution was poured into 1200 ml of saturated NH_4Cl solution and extracted with ether. The ether extract was washed with saturated NH_4Cl, dried ($MgSO_4$), and concentrated. Distillation of the crude product gave 17.6 g (50%) of 4-methyl-2-octyn-1-ol as a colorless oil: bp 59–62° (0.3 mm); ir ($CHCl_3$) 3615 (free OH), 3700–3250 (bonded OH), 2240 (C≡C), 1460, 1380 cm^{-1}; nmr δ 0.91 (dist. t, 3 H, CH_2CH_3), 1.15 (d, 3 H, $CHCH_3$), 1.43 (br m, 6 H, $CH_2CH_2CH_2$), 2.07 (br s, 1 H, OH), 2.18–2.60 (br m, 1 H, CHC≡C), 4.76 (s, 2 H, CH_2OH).

Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 76.23; 76.38; H, 11.51, 11.50.

1-Bromo-4-methyl-2-octyne (7). To a solution of 17.6 g (127 mmol) of 4-methyl-2-octyn-1-ol in 200 ml of ether, cooled to 5°, was added, *via* syringe, 4.7 ml (13.8 g, 51 mmol) of PBr_3. After stirring at 3–10° for 5.5 hr, the product was isolated following the procedure given for the isolation of **1**. Distillation of the crude product gave 13.73 g (57%) of **7**, as a colorless oil: bp 45–46° (0.35 mm); ir (neat) 2240 (C≡C), 1460, 1210, 610 (CBr) cm^{-1} (no absorption due to OH); nmr δ 0.91 (dist. t, 3 H, CH_3), 1.13 (d, 3 H, CH_3CH), 1.40 (br m, 6 H, $CH_2CH_2CH_2$), 2.42 (br m, 1 H, CH), 3.93 (s, 2 H, CH_2Br).

Anal. Calcd for $C_9H_{15}Br$: C, 53.22; H, 7.44. Found: C, 52.97; H, 7.34.

16-Methyl-5,8,11,14-eicosatetraynoic Acid. To a solution of 11.2 g (59.5 mmol) of **2** in 110 ml of THF, cooled to 0°, was added 120 ml (119 mmol) of a 0.99 *M* solution of ethylmagnesium bromide in THF. After warming to room temperature, the mixture was stirred for 4 hr, and then 350 mg of CuCN was added. After stirring 20 min, a solution of 6.91 g (34 mmol) of **7** in 45 ml of THF was added dropwise. The mixture was stirred for 17 hr with an additional 350 mg of CuCN added after 6 hr. The reaction was then worked up in the manner described for **2** to give 17.7 g of crude product, which was treated with 20 g of $AgNO_3$ as described above for the 15-methylenetriynoic acid. The crude product which was obtained was recrystallized from ether–pentane to give 4.6 g (44%) of 16-methyl-5,8,11,14-eicosatetraynoic acid, as a low-melting, unstable solid. The product was hydrogenated as soon as possible after isolation to avoid decomposition: ir (neat) 3500–2550 (br OH), 1710 (C=O), 1400 cm^{-1}; nmr δ 0.91 (dist. t, 3 H, CH_2CH_3), 1.13 (d, 3 H, CH_3CH), 1.40 (br m, 6 H, $CH_2CH_2CH_2$), 1.87 (q, 2 H, $CH_2CH_2CO_2$), 2.07–2.70 (m, 5 H, $CH_2CH_2CH_2CO_2$, CHC≡C), 10.97 (s, 1 H, OH).

16-Methyl-5,8,11,14-eicosatetraenoic Acid (8). To a solution of 670 mg (2.16 mmol) of 16-methyl-5,8,11,14-eicosatetraynoic acid in 15 ml of absolute EtOH was added 650 mg of Lindlar catalyst and 0.20 ml of quinoline. Immediately, the mixture was reduced under a slight positive pressure of hydrogen. The reaction took up 188 ml of hydrogen in 6800 sec; theoretical uptake (cor-

rected), 230 ml. Following the procedure given for the purification of **6**, 340 mg (53%) of **8** was obtained as a lightly colored oil, after chromatography. A small amount was rechromatographed to give the analytical sample: ir (neat) 3450–2450 (broad OH), 1690 (C=O) cm^{-1}; nmr δ 0.88 (dist. t, 3 H, CH_2CH_3), 0.94 (d, 3 H, $CHCH_3$), 1.27 (br m, 6 H, 3 × CH_2), 1.72 (q, 2 H, $CH_2CH_2CO_2$), 2.12 (t, 2 H, $C{=}CCH_2CH_2$), 2.20 (br s, 1 H, CHC≡C), 2.38 (t, 2 H, CH_2CO_2), 2.82 (br m, 6 H, 3 × $C{=}CCH_2C{=}C$), 5.06–5.46 (m, 8 H, 4 × CH=CH), 11.15 (br s, 1 H, CO_2H).

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.09; H, 10.96.

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Registry No.—**1**, 20334-69-4; **2**, 53292-96-9; **3**, 1191-85-1; **4**, 506-32-1; *cis*-**5**, 53369-62-3; *trans*-**5**, 53292-97-0; 14-*cis*-**6**, 53292-98-1; 14-*trans*-**6**, 53368-42-6; **7**, 53292-99-2; **8**, 53319-93-0; 2,5-hexadiyn-1-ol, 28255-99-4; phosphorus tribromide, 7789-60-8; 5-hexynoic acid, 53293-00-8; ethyl bromide, 74-96-4; 3-methyl-*cis*-2-octen-1-ol, 30804-78-5; 3-methyl-*trans*-2-octen-1-ol, 30804-71-8; 15-methyl-14-*cis*-eicosaen-5,8,11-triynoic acid, 53293-01-9; 15-methyl-14-*trans*-eicosaen-5,8,11-triynoic acid, 53293-02-0; 4-methyl-2-octyn-1-ol, 53369-63-4; 3-methyl-1-heptyne, 53293-03-1; 16-methyl-5,8,11,14-eicosatetraynoic acid, 53293-04-2.

References and Notes

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(10) The versene solution was prepared by mixing 100 g of Versene 100 (obtained from Fisher Scientific Co.), 500 ml of H_2O, and 20 ml of 37% hydrochloric acid.

Synthesis of Di- and Tripeptides Containing 4-Aminocyclohexanecarboxylic Acid

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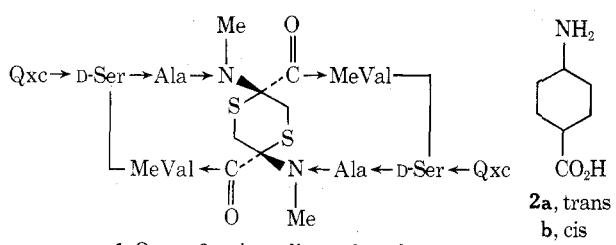
Studies of selected coupling methods for attachment of amino acid derivatives to *cis*- and *trans*-4-aminocyclohexanecarboxylic acid have shown diethylphosphoryl cyanide to be an effective coupling reagent. *N-tert*-Butyloxycarbonyl-*trans*-4-aminocyclohexanecarboxylic acid (**3a**) was converted, using diethylphosphoryl cyanide, to dipeptide **4a** by condensation with L-valine methyl ester. Dipeptide **4a** was transformed by deprotection and coupling with *N-tert*-butyloxycarbonyl-L-alanine to tripeptide **6a**. Similar transformations were effected using the *N-tert*-butyloxycarbonyl derivative **3b** of *cis*-4-aminocyclohexanecarboxylic acid. Other coupling procedures investigated were the carbodiimide, *p*-nitrophenyl active ester, and symmetrical anhydride methods; these methods were less satisfactory for effecting coupling to the above cyclohexaneamino acids.

The quinomycins[1] are a group of depsipeptide antibiotics that possess a depsipeptide lactone system interconnected by a 1,4-dithiane ring as shown for echinomycin (**1**). Our interest in the synthesis of quinomycin model systems that have a cyclohexane ring substituted for the dithiane moiety has resulted in an investigation of methods for attachment of amino acid derivatives to the simple model 4-aminocyclohexanecarboxylic acid (**2**).



2a, trans
b, cis

1, Qxc = 2-quinoxalinecarbonyl

Interest in the preparation of peptide derivatives of 1-aminocyclopentane- and 1-aminocyclohexanecarboxylic acids was prompted by the reported[2] cytotoxic activity of the former substance. Amino acid derivatives were attached to the above cycloalkylamino acids by application of the acid chloride,[3a] carbodiimide,[3a-c] symmetrical anhydride,[3d] and oxazolone[3d] methods. Amino acids also have been attached to cyclohexylamine by use of active esters.[4]

In this study, *trans*-4-aminocyclohexanecarboxylic acid (**2a**)[5] was chosen as an appropriate model, since in the quinomycins the dithiane amino acid moiety has, in the 2,5 positions, amino and carboxyl groups in a trans relationship; studies were made also on the corresponding cis isomer **2b**. Conversion of **2** to the *N-tert*-butyloxycarbonyl derivative **3** was effected by standard procedures.[6] Initial attempts to couple glycine ethyl ester or L-alanine methyl ester to **3** using *N,N'*-dicyclohexylcarbodiimide[7] with or without added 1-hydroxybenzotriazole[8] were not successful. Similar failures employing the carbodiimide method in coupling reactions with cycloalkylamino acids have been observed.[3d,9]

Diethylphosphoryl cyanide recently has been shown[9] to be an effective coupling agent in peptide synthesis. Of significance, condensation of cyclohexylamine with benzoic acid was reported to give *N*-cyclohexylbenzamide in good yield using diethylphosphoryl cyanide, while none of the desired amide was obtained by the carbodiimide method.

Treatment of *N-tert*-butyloxycarbonyl-*trans*-4-aminocyclohexanecarboxylic acid **3a** with L-valine methyl ester and diethylphosphoryl cyanide in dimethylformamide gave