31-41 and 62-85. The space inside the reaction and buffer zones was filled with water by overlaying a previously-equilibrated box of TIP3P water molecules. Water molecules within 2.6 Å of any protein atom were removed from the system. A constant dielectric function of 1 was employed, and a cutoff distance of 10 Å for nonbonded interactions was used for both the van der Waals and the electrostatic terms. The temperature was maintained at 300 ± 5 K by coupling all non-hydrogen atoms in the buffer zone to a Langevin bath. The SHAKE constraint algorithm was employed to keep bonds involving hydrogen atoms fixed at their equilibrium position. After an 8000-step equilibration of the water structure in the presence of the fixed protein and a 20000-step equilibration of water and protein structures, a second TIP3P water overlay was applied to fill voids in the solvent. The 100000-step dynamics simulation was carried out with data collection every 50 steps. For a dynamics trajectory of N frames the rms fluctuation (Δrms) of an atom is given by $\Delta\text{rms} = ((1/N)\sum_{i=1}|r - (r)_{av}|^2)^{1/2}$.

Acknowledgment. The authors are indebted to Dr. J. D'angelo of l'Hopital Maisonneuve Rosemont for expert technical assistance in performing platelet aggregation assays.

Determination of the Absolute Configuration of the Active Amlodipine Enantiomer as (-)-*S*: A Correction

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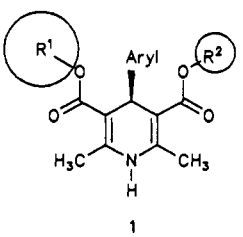
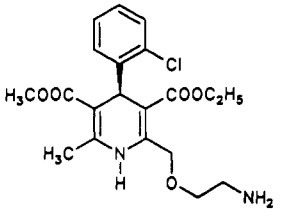
The active (-) enantiomer of amlodipine was originally reported to have *R* configuration. This does not concur with other 1,4-dihydropyridines with known absolute configuration. This configuration has now been determined by X-ray structural analysis using (1*S*)-camphanic acid and (*S*)-2-methoxy-2-phenylethanol as chiral probes. Both determinations gave the *S* configuration for the amlodipine (-) enantiomer with the greater Ca-antagonistic activity.

Ca-antagonistic 1,4-dihydropyridines of type 1 with asymmetric ester substitution are chiral, and the enantiomers naturally differ in their pharmacological activity. A number of derivatives of 1 with known absolute configuration demonstrate that the more active enantiomer is the one that—with the aryl residue represented as shown—has the larger ester on the left-hand side[1] (usually the *S* configuration).



1



R-2

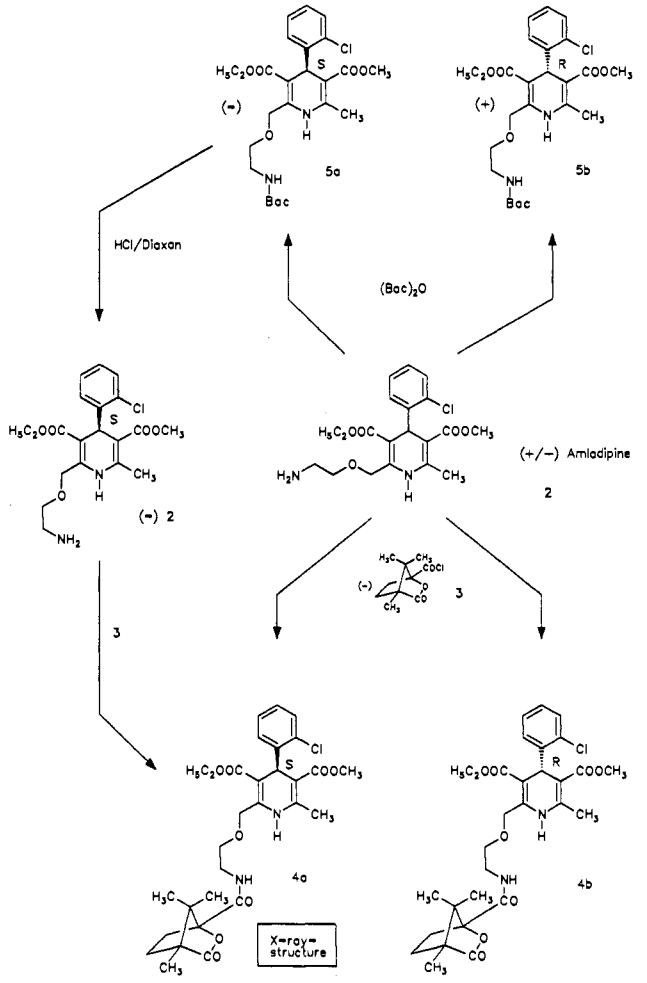
more potent enantiomer ($R^1 > R^2$)

A major exception seemed to be amlodipine **2**, whose *R* enantiomer (*R*-**2**) was described as pharmacologically more active.[2] Basic substitution in the 2-position with the possibility of additional hydrogen bridges was postulated as an explanation for this behavior. However, findings that correlate better with the *S* configuration for the active (-) enantiomer of amlodipine led us to doubt whether the reported configuration was correct, particularly since the X-ray structure from which the *R* configuration had been inferred had not been published.[2] Accordingly, we repeated the investigation of the absolute configuration.

Correlation with (1*S*)-Camphanic Acid (Sheme I)

Using (-)-(1*S*)-camphanic acid chloride (**3**), racemic amlodipine (**2**) was converted into the diastereomeric

Scheme I



amides **4a** and **4b**, which are very easy to separate matographically. By crystallizing **4a** from DMF/water, single crystals suitable for X-ray structural analysis were obtained. The X-ray structure of **4a** gave the *S* configuration at the dihydropyridine carbon (Figure 1).

The agreement of the configuration of **4a** with the active (-) enantiomer of amlodipine ((-)-**2**) was shown by inde-

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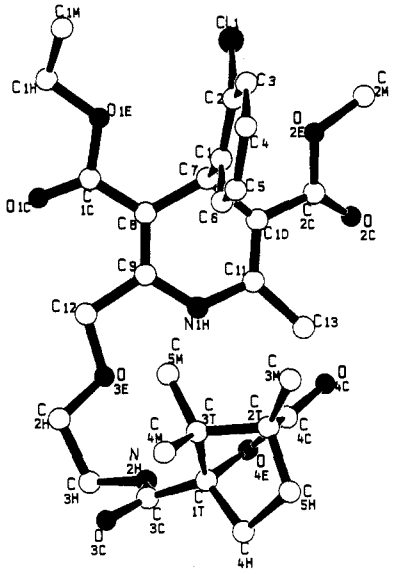

Figure 1. X-ray structure of 4a.

pendent synthesis: Amlodipine was converted into the (*tert*-butyloxy)carbonyl (Boc) derivative (5) and chromatographed on chiral columns, yielding enantiomerically pure (−)-5. Cleavage of the protective Boc group with HCl/dioxane gave (−)-2, the pharmacologically more active enantiomer,[3] whose maleate agreed with the literature values for optical rotation and melting point.[2]

Reaction with (1*S*)-camphanic acid chloride (3) gave the diastereomer 4a, and so the absolute configuration *S* in 4a corresponds to that of the active amlodipine enantiomer. As this is in conflict with the literature,[2] a further correlation was performed using the mandelic acid derivative (*S*)-2-methoxy-2-phenylethanol (7) employed in ref 2.

Correlation with (*S*)-2-Methoxy-2-phenylethanol (Scheme II)

Using the procedure described in ref 2, racemic dihydropyridinecarboxylic acid 6 was reacted with (−)-(*S*)-2-methoxy-2-phenylethanol (7) to give the diastereomers 8a and 8b. Chromatographic separation on silica gel yielded 8a (the more polar fraction) and 8b (the less polar fraction) in their pure forms. Although the physical data (^{1}H-NMR, $[\alpha]_D$, mp) were in agreement with ref 2, the melting point of 8b was more than 40 °C lower. By slow crystallization of 8a (identical to 50A in ref 2) from methanol, single crystals suitable X-ray structural analysis were obtained. The *S* configuration at the dihydropyridine carbon was obtained for 8a (Figure 2).

Transesterification with sodium alkoxide and reduction of the azide yielded (−)-amlodipine ((−)-2), whose identity was additionally confirmed after conversion to the camphanic acid amide 4a. This series, too, showed that the active (−) enantiomer of amlodipine has the *S* configuration.

Results and Discussion

Two independent X-ray structural analyses using (1*S*)-camphanic acid (Figure 1) and (*S*)-2-methoxy-2-phenylethanol (Figure 2) as chiral probes show that the pharmacologically more active (−) enantiomer of amlodipine has the *S* configuration.[4] Thus the rule that the more

(3) We thank S. Hebisch for confirming the pharmacological activity at the (−) enantiomer.
(4) The authors of ref 2 were informed in advance and agreed to our results recently.

Scheme II

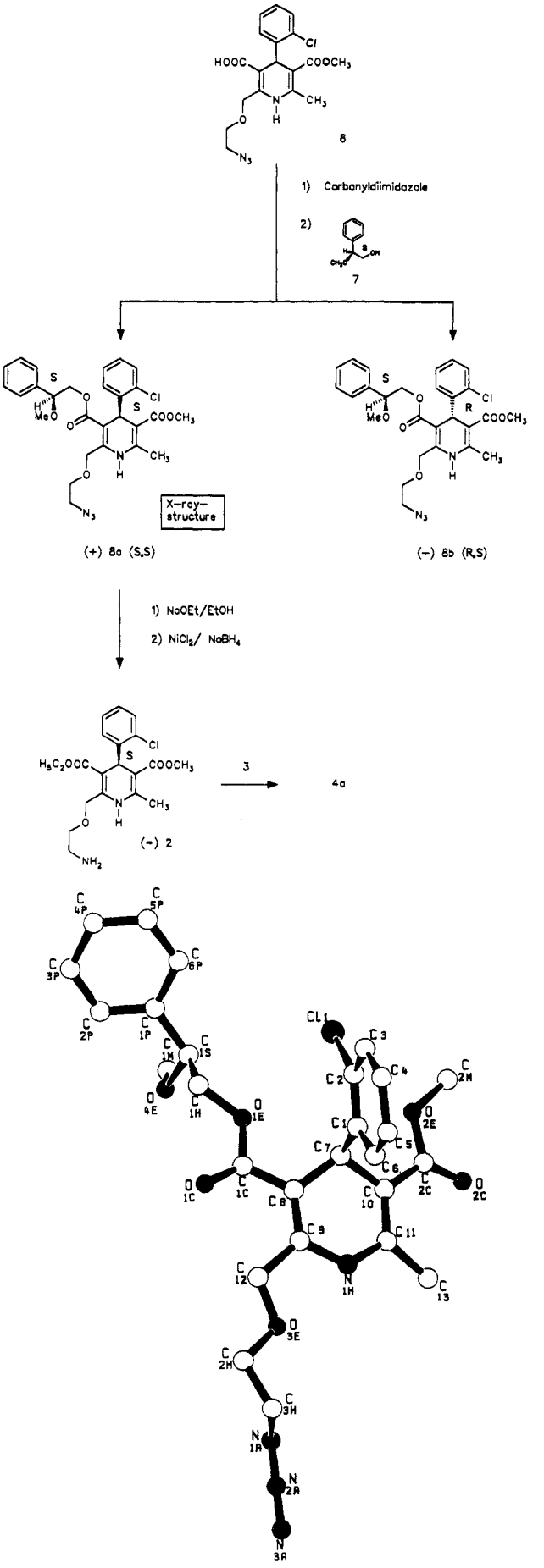

Figure 2. X-ray structure of 8a.

Table I. Crystal Structure Data

	4a	8a
a, Å	11.744 (3)	7.699 (2)
b, Å	9.013 (2)	11.842 (6)
c, Å	14.347 (3)	29.224 (4)
β, deg	99.48 (3)	
v, Å^3	1500 (4)	2664 (2)
space group	$P2_1$	$P2_12_12_1$
formula	$C_{30}H_{37}ClN_2O_8$	$C_{27}H_{29}ClN_4O_6$
formula weight	589.09	541.01
Z	2	4
d (calcd) g/cm^3	1.303	1.349
range, deg	$1.5 < 0 < 72$	$1.5 < 0 < 70$
range h	$-14 \le h \le +14$	$0 \le h \le 9$
range k	$0 \le k \le 11$	$0 \le k \le 14$
range l	$0 \le l \le 17$	$0 \le l \le 35$
trans[a] (min)	0.8036	0.8944
trans (max)	0.9981	0.9983
NREFL[b]	3175	2889
NUNI[c]	3136	2889
NOBS[d]	2677	2330
NV[e]	389	416
R[f]	0.087	0.057 (0.065)
R_w	0.108	0.076 (0.084)
S	3.39	2.46 (2.73)

[a] Trans: relative transmission coefficient from absorption corrections. [b] NREFL: total number of reflections collected. [c] NUNI: number of unique reflections. [d] NOBS: number of observed reflections with $I < 3\sigma(I)$ used in refinment. [e] NV: number of refined variables. [f] R: values in parentheses are refined with enantiomer coordinates.

active enantiomer of 1,4-dihydropyridines of type 1 has the larger residue R_1 on the *left* side[1] applies to amlodipine, too.

Experimental Section

(1) Crystallographic Studies. Crystal data and some details of the structure refinements are given in Table I. Data collection were performed at room temperature with graphite monochromated Cu Kα (λ = 1.5418 Å) radiation on an Enraf/Nonius CAD4 diffractometer in the W-20 scanning mode. Absorption corrections[5] were based on ψ scans. The structures were solved by direct[6] and difference Fourier methods. Full matrix least-squares refinement was carried out with anisotropic temperature factors for the non-H atoms on the basis of all observed reflections, using the SDP software package.[7] The weighting factor was $w = 4F_o^2[\sigma^2(I) + (0.04F_o^2)^2]^{-1}$. Empirical correction for secondary extinction was applied: F_c, corr $= F_c/(1 + gI)$. In the case of **4a** only seven H atoms, located from a difference map, were included in the refinement. In the case of **8a** 20 H atoms were calculated geometrically. A following difference map did reveal the positions of two methyl H atoms. All the H atoms were included in the refinement with isotropic temperature factors. Thereafter, in the case of **8a**, four H atoms were eliminated because of improper positions and great temperature factors. Final difference maps contained no significant features.

(2) Chemistry. All melting points are uncorrected and were determined using a Tottoli (Büchi 535) apparatus. All structures were confirmed by NMR spectroscopy and some by microanalysis. NMR spectra were recorded in the solvents specified, using AM 250 (Bruker) apparatus. The specific rotation was recorded using a 241MC apparatus (Perkin-Elmer).

(A) 2-[[2-(Camphanoylamino)ethoxy]methyl]-4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine (4a/b). 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine (**2**, 4.1 g, 10 mmol) was dissolved in 80 mL of methylene chloride, treated successively with 1 mL of pyridine and (1*S*)-(-)-camphanic acid chloride (**3**, 2.2 g, 10 mmol, Aldrich), stirred for 2 h at room temperature, washed with 1 M citric acid, dried, and concentrated by evaporation. The foam was crystallized with ether and recrystallized once from methanol. A 1:1 diastereomer mixture of **4a** and **4b** (3.4 g, 58%) was obtained: mp 158–159 °C; $[\alpha]^{20}_D$ = -19.7° (c = 1.06, $CHCl_3$). Anal. ($C_{30}H_{37}ClN_2O_8$), C, H, N, O, Cl.

Separation of Diastereomers. The mixture **4a/4b** was separated both analytically and preparatively on a Chiralcel OD column (Baker). The retention times were 5.3 min (**4a**) and 8.1 min (**4b**) (25-cm column, 10-μm Chiralcel OD, 1 mL/min *n*-heptane/ethanol 7:3).

4a: mp 154–155 °C (ether); $[\alpha]^{20}_D$ -32.4° (c = 0.96, MeOH), -34.8° (c = 0.98, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.9 (s, 3 H), 1.15 (2 s, 6 H), 1.2 (t, J = 7 Hz, 3 H), 1.65–2.1 (m, 3 H), 2.4 (s, 3 H), 2.5–2.65 (m, 1 H), 3.6 (s, 3 H), 3.55–3.7 (m, 4 H), 4.0–4.1 (m, 2 H), 4.65 and 4.75 (2 d, J = 15 Hz, each 1 H), 5.4 (s, 1 H), 6.8 (t, NH), 7.0–7.4 (m, 4 H and NH) ppm. Anal. ($C_{30}H_{37}ClN_2O_8$) C, H, N, O, Cl.

4b: amorphous substance; $[\alpha]^{20}_D$ +6.1° (c = 0.93, MeOH), -4.2° (c = 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) somewhat smaller shift difference of the geminal hydrogen atoms at 4.7 ppm, otherwise very similar to **4a**. Anal. ($C_{30}H_{37}ClN_2O_8$) C, H, N, O, Cl.

(B) 2-[[2-[(*tert*-Butoxycarbonyl)amino]ethoxy]methyl]-4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine (5). While cooling with ice, a solution of **2** maleate (0.46 g, 1 mmol) in 4 mL of THF/1 mL of H_2O was treated with potassium carbonate (0.22 g, 1.6 mmol) and with di-*tert*-butyl pyrocarbonate (0.24 g, 1.1 mmol). After 4 h the usual workup procedure was employed, yielding 0.5 g (100%) of **5** as an oil: ^{1}H-NMR ($CDCl_3$) δ 1.2 (t, J = 7 Hz, 3 H), 1.45 (s, 9 H), 2.4 (s, 3 H), 3.4 (m, 2 H), 3.6 (s, 3 H), 4.05 (m, 2 H), 4.65 and 4.75 (2 d, J = 15 Hz, each 1 H), 4.8 (s, NH), 5.4 (s, 1 H), 7.0–7.4 (m, 4 H), 7.2 (s, NH) ppm. The enantiomers were separated on a Chiralcel OD column (Baker) with *n*-heptane/ethanol (98:2), yielding enantiomerically pure (-)-**5** as an oil (1st peak): $[\alpha]^{20}_D$ = -23.5° (c = 1.06, MeOH).

(C) (-)-(*S*)-2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine [(-)-2]. A solution of (-)-**5** (130 mg, 0.26 mmol) in 3 mL of acetonitrile was treated with 0.2 mL of 6.7 M HCl in dioxane and stirred for 1 h. After addition of ethyl acetate and water and alkalization with ammonia, the phases were separated and the organic phase was dried, concentrated by evaporation, and flash-chromatographed with CH_2Cl_2/MeOH on silica gel, yielding 68 mg (64%) (-)-**2** as an oil: $[\alpha]^{20}_D$ = -19.4° (c = 0.45, MeOH). **(-)-2 Maleate.** By adding 1 equiv of maleic acid the maleate was prepared as in ref 2: mp 56–58 °C (lit. mp 58 °C); $[\alpha]^{20}_D$ -26.8° (c = 0.91, MeOH) (lit. value -26.2°, c = 1.16, MeOH).

Camphanic Acid Amide (4a). Analogously to procedure A, (-)-**2** was reacted with (1*S*)-(-)-camphanic acid chloride (**3**). Mp and HPLC showed that the product was identical to **4a**.

(D) 2-[(2-Azidoethoxy)methyl]-4-(2-chlorophenyl)-5-(methoxycarbonyl)-3-[(2(*S*)-methoxy-2-phenethoxy)carbonyl]-6-methyl-1,4-dihydropyridine (8a and 8b). Using the same procedure as in ref 2, the diastereomer mixture **8a,b** was prepared and separated with methylene chloride/ethyl acetate mixture on silica gel.

8a: the more polar fraction; mp 105–106 °C (lit. mp 104–106 °C); $[\alpha]^{20}_D$ +47.8° (c = 0.92, DMF) (lit. $[\alpha]^{25}_D$ = +41.2° (solvent?)). ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3 H), 3.25 (s, 3 H), 3.5 (m, 2 H), 3.65 (s, 3 H), 3.7 (m, 2 H), 3.9–4.0 (m, 1 H), 4.3 (m, 1 H), 4.4 (m, 1 H), 4.6 and 4.75 (2 d, J = 17 Hz, each 1 H), 5.45 (s, 1 H), 7.0–7.4 (m, 9 H and NH) ppm. Anal. ($C_{27}H_{29}ClN_4O_6$) C, H, N, Cl.

8b: the less polar fraction; mp 96–98 °C (lit. mp 139–140 °C); $[\alpha]^{20}_D$ = -13.9° (c = 0.87, DMF) (lit. $[\alpha]^{25}_D$ = -14.9° (solvent?)); ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3 H), 3.1 (s, 3 H), 3.5 (m, 2 H), 3.65 (s, 3 H), 3.7 (m, 2 H), 3.9–4.0 (m, 1 H), 4.3–4.45 (m, 2 H), 4.7 and 4.75 (2 d, J = 17 Hz, each 1 H), 5.45 (s, 1 H), 7.0–7.4 (m, 9 H and NH) ppm.

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(7) Enraf-Nonius: Structure Determination Package, version 1.2.0. Enraf-Nonius, Delft, 1985.

(E) Conversion of (+)-8a to (−)-2. Ten milliliters of ethanol (anhydrous) was treated with sodium hydride (30 mg, 1 mmol) and stirred for 5 min, and then **8a** (108 mg, 0.2 mmol) was added. After reflux for 1 h, the solution was poured into water, extracted with methylene chloride, and concentrated by evaporation. The residue was dissolved in 3.5 mL of a 6% ethanolic nickel chloride solution and treated dropwise with an ethanolic sodium borohydride solution at room temperature until the black coloration persisted. After rotary evaporation the residue was taken up in chloroform, washed once with concentrated ammonia solution, and concentrated by evaporation. The product was 90 mg of a viscous oil consisting of (−)-2 which contained about 20% of the corresponding diethyl ester. The oil was reacted with (1*S*)-(−)-camphanic acid chloride (3), analogously to procedure A. Mp (recrystallization from ether) and HPLC showed that the product was identical to **4a**.

Acknowledgment. We thank Drs. P. Schmitt and Ch. Wünsche for the spectroscopic data, Drs. J. Lenfers and V. Muschalek for chromatographic separation, and U. Appel, S. Borgmann, and P. Hilker for preparative work. We are grateful to C. Lettner for producing the manuscript.

Supplementary Material Available: Tables of bond distances, bond angles, torsion angles, positional parameters, and general displacement parameter expressions (11 pages). Ordering information is given on any current masthead page.

Synthesis and Functional Evaluation of a Peptide Derivative of 1-β-D-Arabinofuranosylcytosine

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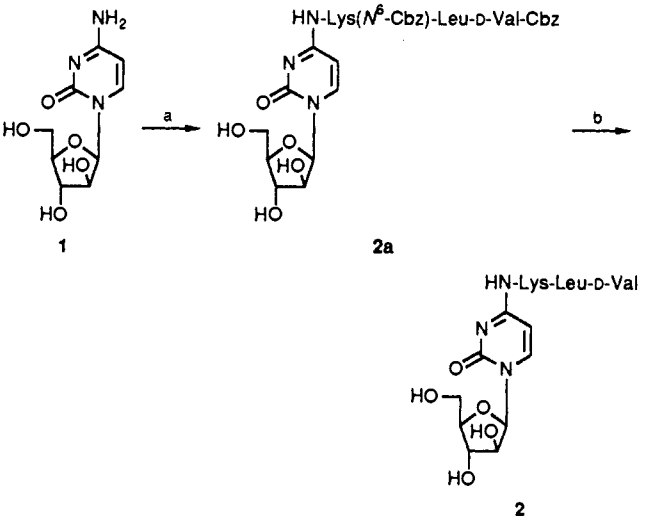
Received March 18, 1992

We have synthesized a peptidyl prodrug derivative of 1-β-D-arabinofuranosylcytosine (1) designed to be a selective substrate of plasmin. D-Val-Leu-Lys-*ara*-C (2) was obtained by coupling the protected peptide Cbz-D-Val-Leu-(N^6-Cbz)Lys-OH and *ara*-C (1) by a water-soluble carbodiimide (EDCI), followed by the removal of the Cbz groups by using catalytic hydrogenolysis over Pd/C. The kinetic constant of hydrolysis of 2 in the presence of plasmin demonstrated effective release of 1. The amino group of 1, which is sensitive to the removal by cytidine deaminase, is protected in 2 by the formation of the amide bond resulting in a prolonged half-life of 2 in biological milieu. The antiproliferative efficiency of 2 against L1210 leukemic cells was significantly higher than that of 1. The activity of 2 was abolished in the presence of serine proteinase inhibitor, (4-amidinopheny)methanesulfonyl fluoride. These data indicate that 2 is a prodrug form of 1 in systems generating plasmin.

The cytotoxic S-phase-specific antimetabolite *ara*-C[1,2] (1) is the most frequently used compound for the treatment of patients with acute myelogenous leukemias. Its application for treating patients, however, has a few disadvantages. Firstly, its specificity is low, since the compound may readily affect normal and healthy cells causing many unwanted toxic side effects. Secondly, the amino group of the cytosine in the drug is sensitive to deaminases, thus it can be easily removed leading to the inactivation of the drug by formation of an ineffective uridine derivative.[3]

A number of analogues of 1 have been prepared, most of them being less toxic than 1. An early attempt was the synthesis of the 2,2'-anhydro analogue of 1, which is resistant to cytidine deaminase and is slowly hydrolyzed releasing the active drug.[4] Other prodrugs of 1 include a large number of derivatives monosubstituted in either 5' or the 3' position of the arabinose or disubstituted in both of these positions or at the N-4 position of the cytosine ring.[5-7] These derivatives are particularly inter-

Scheme I[a]



[a] (a) DMSO, EDCI, Cbz-D-Val-Leu-Lys(N^6-Cbz); (b) Pd/C, H_2.

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