A Facile Synthesis of (+)-L-Discadenine and its Deuterio Derivatives

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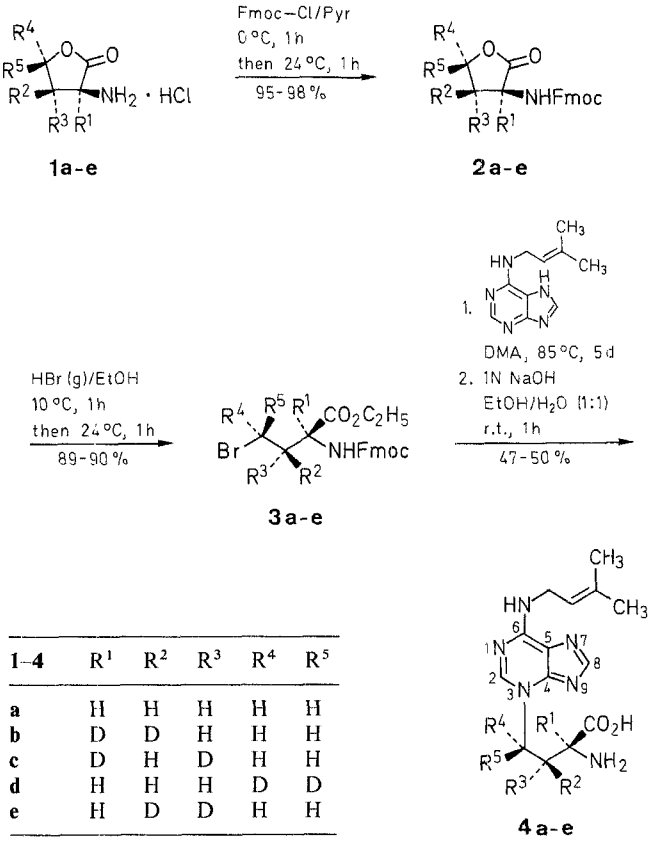
The synthesis of discadenine, 3-[(3*S*)-3-amino-3-carboxypropyl]-6-(3-methyl-2-butenyl)-3*H*-purine, and several of its deuteriated derivatives, which are useful in the study of the stereochemical mechanism of its biosynthesis, have been synthesized in three steps from the appropriate homoserine lactones via a route designed for maximal synthetic versatility. The yields of discadenine obtained from this new route represent a ten-fold increase over previous procedures.

Discadenine, 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenyl)-3*H*-purine (presumably having the *S*-configuration), is a potent self-germination inhibitor in certain cellular slime molds.[1] It has recently been demonstrated that discadenine is biosynthesized by the direct transfer of the *S*-3-amino-3-carboxypropyl moiety of *S*-adenosyl-L-methionine (SAM) to 6-(3-methyl-2-butenylamino)purine [AKA: N^6-(3,3-dimethylallylamino)purine] by discadenine "synthetase".[2,3] The direct donation of the 3-amino-3-carboxypropyl moiety of SAM, normally a methyl group donor for a variety of transmethylation reactions,[4] has been indicated in a number of other biosyntheses of modified nucleosides such as nucleoside Y in Yeast tRNAPhe,[5] 3-(3-amino-3-carboxypropyl)uridine,[6] and nicotianine[7] as well as in the biosyntheses of ACC[8] and A-2-C.[9]

(±)-Discadenine was first synthesized[10] by the alkylation of 6-(3-methyl-2-butenylamino)purine with ethyl (±)-2-phthalimido-4-bromobutanoate. A slight modification of this procedure has been applied to the synthesis of optically active (+)-L-discadenine from *S*-α-phthalimido-γ-butyrolactone as well as discadenine's deamino- and decarboxy derivatives but still in rather low overall yields [the overall yield of (+)-L-discadenine was ~ 4% based on L(+)-homoserine].[11] In connection with our studies on the biosynthesis of discadenine, we required a method for the preparation of standard samples of regio- and stereospecific deuterium-labelled discadenine. The method[11] described above would be rather inconvenient for the preparation of labelled discadenines since it requires at least a threefold molecular excess of alkylating agents, which is not always recoverable in our hands. We were, therefore, interested in a less tedious and higher-yielding synthetic procedure. An in depth literature survey revealed that there is no alternate method for the preparation of discadenines. We report here a short, facile, higher-yielding synthesis of discadenine which proves to be quite applicable to the synthesis of both discadenine and deuterium-labelled discadenines.



1–4	R^1	R^2	R^3	R^4	R^5
a	H	H	H	H	H
b	D	D	H	H	H
c	D	H	D	H	H
d	H	H	H	D	D
e	H	D	D	H	H

The synthetic route we chose for the synthesis of discadenine is outlined in the Scheme.[16] The 9-fluorenylmethoxycarbonyl-L-homoserine lactone (**2a**) was prepared from L-homoserine lactone hydrochloride and 9-fluorenylmethyl chloroformate (Fmoc-chloride) in dry pyridine in 98% yield.[12] Treatment of **2a** with dry hydrogen bromide gas in absolute ethanol afforded the key intermediate **3a** in 90% yield. The 6-(3-methyl-2-butenylamino)purine was alkylated with 1.4 equivalents of the bromo Fmoc ethyl ester derivative **3a**, in the dark, in anhydrous *N,N*-dimethylacetamide (DMA) at 85°C for 5 days to give the protected discadenine, which was not isolated. The advantage of using the Fmoc protective group is that one may now simultaneously hydrolyze both the ethyl ester and Fmoc group with 1 normal sodium hydroxide (the reaction is carried out in the same vessel as the alkylation step without purifying the intermediate alkylation product). After recrystallization from aqueous ethanol, discadenine was obtained in 50% yield (overall yield 44% based on homoserine lactone hydrochloride). Labelled discadenines **4b–e** were similarly prepared in 41–44% overall yield, demonstrating the utility of this procedure for the preparation of labelled discadenine. The major problem with our procedure as well as those of past procedures is that it requires the alkylation of an ambident heterocyclic nucleophile, namely the alkylation at N-6 of the adenine system.[13] Although the overall yield of alkylated purine is acceptable, the desired product, discadenine, is contaminated with homoserine and some N^3-alkylation product, both of which are easily separable from the target molecule. The ratio of N^3:N^6 alkylation is ~ 4–5:1. The major advantage of our procedure in addition to

improvement in yields is the use of the Fmoc protective group which gives easily purified and handled solids and allows one to simultaneously hydrolyze both the ethyl ester and Fmoc group in one step.

Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on an IBM WP270SY spectrometer operating at 270 MHz without deuterium decoupling. Samples were prepared by dissolving the compound in D_2O or $CDCl_3$ and chemical shifts are reported downfield from DSS and TMS, respectively.

The 6-(3,3-dimethylallylamino)purine and 9-fluorenylmethyl chloroformate were purchased from the Sigma Chemical Co. and used without further purification. The syntheses of the regio- and stereospecific labelled homoserine lactone hydrochlorides have been described elsewhere.[14]

N-(9-Fluorenylmethoxycarbonyl)-L-homoserine Lactone (2a); Typical Procedure:

A solution of L-homoserine lactone hydrochloride (**1**; 200 mg, 1.46 mmol) in dry pyridine (5.0 mL) is cooled to 0 °C and 9-fluorenylmethyl chloroformate (377 mg, 1.46 mmol) is added slowly. The mixture is stirred at 0 °C for 1 h and at 24 °C for 1 h, then poured into ice water (100 mL). The solid is isolated by suction, washed with H_2O, and recrystallized from 95% EtOH; yield: 464 mg (98%); colorless crystals; mp 208–209 °C.

$C_{19}H_{17}NO_4$	calc.	C 70.57	H 5.30	N 4.33
(323.35)	found	70.48	5.30	4.29

^{1}H-NMR ($CDCl_3$): δ = 2.17, 2.78 (m, 2H, 3-CH_2); 4.21 (m, 2H, 2-H, 9'-H); 4.41 (m, 4H, 4-CH_2, ArCH_2OCO); 5.28 (s, 1H, NH); 7.27–7.76 (m, 8 H_{arom}).

Lactone **2b**: (2*S*,3*R*)-[2,3-$^2H_2$]; yield: 95.6% from (2*S*,3*R*)-[2,3-$^2H_2$] homoserine lactone hydrobromide **1b**; mp 207–208 °C.

^{1}H-NMR ($CDCl_3$): δ = 2.77 (br s, 1H, 3-C^2H_RH$_S$-); 4.22 (m, 1H, 9'-H); 4.42 (m, 4H, 4-CH_2, ArCH_2OCO); 5.26 (s, 1H, NH); 7.27–7.76 (m, 8 H_{arom}).

Lactone **2c**: (2*S*,3*S*)-[2,3-$^2H_2$]; yield: 97% from (2*S*,3*S*)-[2,3-$^2H_2$] homoserine lactone hydrobromide **1c**; mp 207–209 °C.

^{1}H-NMR ($CDCl_3$): δ = 2.18 (t, 1*H*, *J* = 7.0 Hz, 3-C^2H_SH$_R$-); 4.24 (m, 1H, 9'-H); 4.42 (m, 4H; 4-CH_2, ArCH_2OCO); 5.28 (s, 1H, NH); 7.27–7.76 (m, 8 H_{arom}).

Lactone **2d**: (2*S*)-[4,4-$^2H_2$]; yield: 95% from (2*S*)-[4.4-$^2H_2$]homoserine lactone hydrobromide **1d**; mp 208–209 °C.

^{1}H-NMR ($CDCl_3$): δ = 2.17, 2.77 (m, 2H, 3-CH_2); 4.21 (m, 2H, 2-H, 9'-H); 4.42 (m, 2H, ArCH_2OCO); 5.34 (s, 1H, NH); 7.27–7.76 (m, 8 H_{arom}).

Lactone **2e**: (2*S*,*R*)-[3,3-$^2H_2$]; yield: 96% from (2*S*,*R*)-[3,3-$^2H_2$]homoserine lactone hydrobromide **1e**; mp 208–209 °C.

^{1}H-NMR ($CDCl_3$): δ = 4.22 (m, 2H; 2-H, 9'-H); 4.42 (m, 4H, 4-CH_2, ArCH_2OCO); 5.29 (s, 1H, NH); 7.27–7.76 (m, 8 H_{arom}).

Ethyl (2*S*)-4-Bromo-2-(9-fluorenylmethoxycarbonylamino)butanoate (3a); Typical Procedure:

Dry HBr gas is vigorously passed through a stirred solution of Fmoc lactone **2a** (400 mg, 1.24 mmol) in absolute EtOH (6.0 mL) at 10 °C for 1 h. The mixture is then stirred at 24 °C for 1 h. Excess HBr and EtOH are removed on a rotary evaporator at 40 °C under diminished pressure (water aspirator). The residue is poured into ice water (~100 mL). The resultant light yellow solid is isolated by suction, washed with H_2O, dried in a vacuum dessicator over KOH for 24 h, and recrystallized from hexane; yield: 482 mg (90%) of pure **3a**; mp 82–83 °C.

$C_{21}H_{22}BrNO_4$	calc.	C 58.34	H 5.13	N 3.24
(432.3)	found	58.50	5.15	3.12

^{1}H-NMR ($CDCl_3$): δ = 1.28 (t, 3H, *J* = 7.0 Hz, CH_3); 2.20, 2.42 (m, 2H, 3-CH_2); 3.38 (t, 2H, *J* = 7.0 Hz, 4-CH_2); 4.19 (m, 4H; 2-H, 9'-H, CH_2CH$_3$); 4.43 (d, 2H, *J* = 7.0 Hz, ArCH_2OCO); 5.33 (d, 1H, *J* = 8 Hz, NH); 7.30–7.75 (m, 8 H_{arom}).

Ester **3b**: (2*S*, 3*R*)-[2,3-$^2H_2$]; yield: 89% from **2b**; mp 81–82 °C.

^{1}H-NMR ($CDCl_3$): δ = 1.28 (t, 3H, *J* = 7.0 Hz, CH_3), 2.20 (br s, 1H, 3-C^2H_RH$_S$); 3.37 (d, 2H, *J* = 7.0 Hz, 4-CH_2; 4.21 (m, 3H, 9'-H, CH_2CH_3); 4.43 (d, 2H, *J* = 7 Hz, ArCH_2OCO); 5.32 (s, 1H, NH); 7.30–7.75 (m, 8 H_{arom}).

Ester **3c**: (2*S*, 3*S*)-[2,3-$^2H_2$]; yield: 90% from **2c**; mp 81–83 °C.

^{1}H-NMR ($CDCl_3$): δ = 1.28 (t, 3H, *J* = 7.0 Hz, CH_3); 2.40 (br s, 1H, 3-^2H_SH$_R$); 3.37 (d, 2H, *J* = 7.0 Hz, 4-CH_2); 4.21 (m, 3H, 9'-H, CH_2CH$_3$); 4.43 (d, 2H, *J* = 7.0 Hz, ArCH_2OCO); 5.33 (s, 1H, NH); 7.30–7.75 (m, 8 H_{arom}).

Ester **3d**: (2*S*)-[4,4-$^2H_2$]; yield: 90% from **2d**; mp 82–83 °C.

^{1}H-NMR ($CDCl_3$): δ = 1.28 (t, 3H, *J* = 7.0 Hz, CH_3); 2.20, 2.42 (m, 2H, 3-CH_2); 4.22 (m, 4H, 2-H, 9'-H, CH_2CH$_3$); 4.43 (d, 2H, *J* = 7.0 Hz, ArCH_2OCO); 5.34 (d, 1H, *J* = 8.0 Hz, NH); 7.30–7.75 (m, 8 H_{arom}).

Ester **3e**: (2*S*,*R*)-[3,3-$^2H_2$]; yield: 89.7% from **2e**; mp 82–83 °C.

^{1}H-NMR ($CDCl_3$): δ = 1.28 (t, 3H, *J* = 7.0 Hz, CH_3); 3.37 (s, 2H, 4-CH_2); 4.22 (m, 4H, 2-H, 9'-H, CH_2CH_3); 4.43 (d, 2H, *J* = 7.0 Hz, ArCH_2OCO); 5.34 (d, 1H, *J* = 7.0 Hz, NH); 7.30–7.75 (m, 8 H_{arom}).

3-[(3*S*)-3-Amino-3-carboxypropyl]-6-(3-methyl-2-butenylamino)-3*H*-purine[15] (4a); Typical Procedure:

To a solution of 6-(3-methyl-2-butenylamino)purine 93.5 mg, 0.46 mmol) in dry DMA (3.0 mL) is added the bromo derivative **3a** (200 mg, 0.46 mmol) all in one portion. The flask is wrapped with aluminum foil. The mixture is heated at 85 °C, with stirring, for 3 days after which time additional **3a** (100 mg, 0.23 mmol) is added and stirring at 85 °C is continued for 2 days. The mixture is cooled, the DMA is removed under vacuum and 1:1 aqueous-ethanolic 1 N NaOH (3 mL) is added to the residue. The mixture is stirred at room temperature for 1 h, EtOH is removed on a rotary evaporator (40 °C), and the aqueous solution is extracted with EtOAc (3 × 50 mL) to remove non-acidic impurities. The pH of the solution is slowly adjusted, with cooling and stirring, to 6 with 3 N HCl/H_2O. The crystalline precipitate is isolated by suction, washed with ice-cold H_2O, dried, and recrystallized from EtOH; yield of **4a**: 105 mg (50%, based on homoserine derivative **3a**); mp 193–195 °C (Lit.[10,11] mp 193–195 °C); $[\alpha]_D^{26}$ + 28° (*c* = 1, 0.1 N HCl) (Lit.[11] $[\alpha]_D^{23}$ + 27.3 (*c* = 0.7, 0.1 N HCl).

^{1}H-NMR (D_2O/DCl): δ = 1.77 [s, 6H, C(CH_3)$_2$]; 2.62 [m, 2H, N^3-(2'-CH_2)]; 4.10 [t, 1H, *J* = 7 Hz, N^3-(3'-CH)]; 4.29 (d, 2H, *J* = 7.0 Hz, N^6-CH_2); 4.71 [m, 2H, N^3-(1'-CH_2)]; 5.40 [t, 1H, *J* = 7.0 Hz, CH =C(CH_3)$_2$]; 8.41 (s, 1H, 8-H); 8.65 (s, 1H, 2-H). [N^3 and N^6 correspond to N^3 and N^6 in adenine).

Acid **4b**: N^3-(2'*S*, 3'*S*)-[2',3'-$^2H_2$]; yield: 47% from **3b**; mp 192–194 °C.

^{1}H-NMR (D_2O/DCl): δ = 1.77 [s, 6H, C(CH_3)$_2$]; 2.59 [t, 1H, *J* = 7.0 Hz, N^3-(2'-C^2H_SH$_R$)]; 4.29 (d, 2H, *J* = 7.0 Hz, N^6-CH_2); 4.71 [m, 2H, N^3-(1'-CH_2)]; 5.40 [t, 1H, *J* = 7.0 Hz, CH =C(CH_3)$_2$]; 8.41 (s, 1H, 8-H); 8.65 (s, 1H, 2-H).

Acid **4c**: N^3-(2'*R*, 3'*S*)-[2',3'-$^2H_2$]; yield: 50% from **3c**; mp 191–193 °C.

^{1}H-NMR (D_2O/DCl): δ = 1.77 [s, 6H, C(CH_3)$_2$]; 2.65 [t, 1H, *J* = 7.0 Hz, N^3-(2'-C^2H_RH$_S$)]; 4.29 (d, 2H, *J* = 7.0 Hz, N^6-CH_2); 4.72 [m, 2H, N^3-(1'-CH_2)]; 5.40 [t, 1H, *J* = 7.0 Hz, CH =C(CH_3)$_2$]; 8.42 (s, 1H, 8-H); 8.65 (s, 1H, 2-H).

Acid **4d**: N^3-(3'*S*)-[1',1'-$^2H_2$]; yield: 48% from **3d**; mp 193–195 °C.

^{1}H-NMR (D_2O/DCl): δ = 1.77 [s, 6H, C(CH_3)$_2$]; 2.62 [m, 2H, N^3-(2'-CH_2)]; 4.14 (t, 1H, *J* = 7.0 Hz, N^3-(3'-CH)]; 4.29 (d, 2H, *J* = 7 Hz, N^6-CH_2); 5.40 [t, 1H, *J* = 7.0 Hz, CH =C(CH_3)$_2$]; 8.42 (s, 1H, 8-H); 8.65 (s, 1H, 2-H).

Acid **4e**: N^3-(3'*S*,*R*)-[2',2'-$^2H_2$]; yield: 49% from **3e**; mp 193–195 °C.

^{1}H-NMR (D_2O/DCl): δ = 1.77 [s, 6H, C(CH_3)$_2$]; 4.12 [s, 1H, N^3-(3'-CH)]; 4.29 (d, 2H, *J* = 7 Hz, N^6-CH_2); 4.71 [q, 2H, *J* = AB quartet, N^3-(1'-CH_2)]; 5.40 [t, 1H, *J* = 7.0 Hz, CH =C(CH_3)$_2$]; 8.42 (s, 1H, 8-H); 8.65 (s, 1H, 2-H).

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(15) There is a numbering change in the amino acid in going from the various homoserine derivatives to the side chain of discadenine. In particular the α-carbon goes from C-2 in homoserine to N^3-(3′-C), the γ-carbon from C-4 to N^3-(1′-C), and the β-carbon from C-3 to N^3-(2′-C). There is also a change in configuration at the C-β carbon in the synthesis, from the homoserine derivatives to the discadenine derivative, due to a priority change in going from a Br atom to an N atom.
The *CA* name for 4a is (*S*)-α-amino-6-[(3-methyl-2-butenyl)amino]-3*H*-purine-3-butanoic acid.
(16) The Scheme depicts two S_N2-type reactions (i.e. inversion of configuration): one, the bromination of the Fmoc homoserine and the other, the alkylation of the substituted purine ring. These are only speculative and are drawn as such, only to facilitate the following of the movement of the labels through the various transformations in the Scheme.