Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 3, pp. 291-294, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00011-X

SYNTHESIS OF (-)-3(*R*)-AMINO-4(*R*),5(*R*)-DIHYDROXY-1-CYCLOHEXENE-1-CARBOXYLIC ACID: THE 3(*R*)-AMINO ANALOGUE OF (-)-SHIKIMIC ACID

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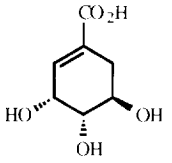
Abstract: (-)-3(*R*)-Amino-4(*R*),5(*R*)-dihydroxy-1-cyclohexene-1-carboxylic acid (the 3(*R*)-amino analogue of (-)-shikimic acid) has been synthesised from (-)-shikimic acid in seven steps.

The shikimate pathway is a biosynthetic pathway utilized by plants, fungi and micro-organisms for the synthesis of several essential aromatic metabolites including the three commonly occurring aromatic L-α-amino acids (Phe, Tyr, Trp) as well as the folate coenzymes and various isoprenoid quinones.[2,3] Through the course of evolution the enzymes that catalyse the transformations from acyclic C_3 and C_4 precursors to aromatics have become foreign to all higher species including mammals and the enzymology of the shikimate pathway has thus become the subject of intense research; compounds that inhibit the action of the enzymes of the shikimate pathway have been highlighted as materials with potential anti-fungal, bacteriocidal or herbicidal activity. Indeed the commercially important broad spectrum, post-emergence herbicide glyphosate[4] (marketed as Roundup®) is active against the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (5-EPS-3-P synthase, E.C. 2.5.1.19) and inhibits the transfer of an enolpyruvyl moiety to the 5-position of the shikimate nucleus.



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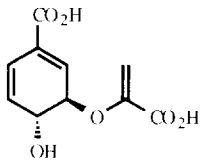

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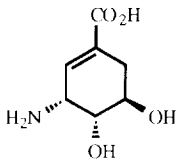

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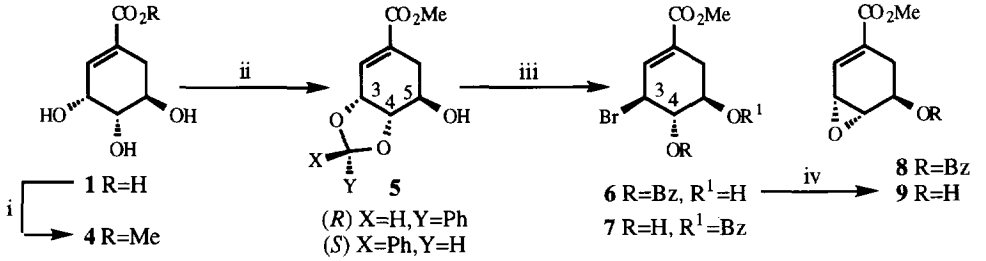
Our long standing interest in the enzymology of the shikimate pathway has led us to instigate a programme of research to develop routes to analogues of pathway intermediates, transition states analogues and related compounds directly from both (-)-shikimic acid **1** and (-)-chorismic acid **2** with particular emphasis being placed upon the synthesis of analogues of (-)-shikimic acid **1** itself. Recently several syntheses of specifically substituted analogues of acid **1** containing 3-hydroxymethyl,[5] 5-hydroxymethyl,[6] 6-fluoro[7-9] and 2-chloro[10] functionalities have been reported and have added weight to the idea that compounds closely resembling substrates or pathway intermediates may act as potent enzyme inhibitors.

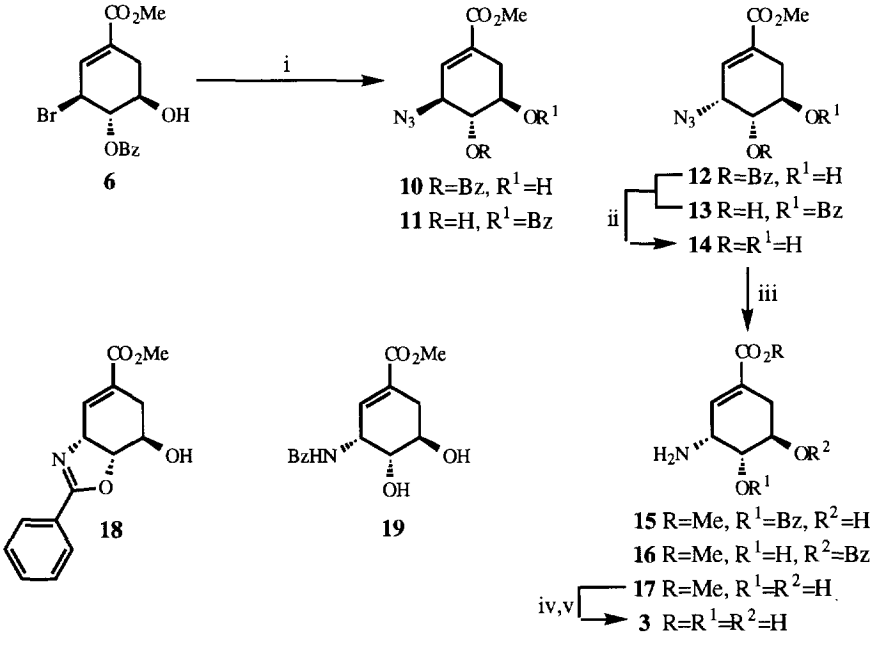
In this communication we wish to report on our studies concerned with the introduction of nitrogen functionality into the shikimate ring. We describe herein the first successful method for the introduction of nitrogen at C-3 of the shikimate nucleus and report the synthesis of the novel γ-amino acid 3(*R*)-amino-

4(*R*),5(*R*)-dihydroxy-1-cyclohexene-1-carboxylic acid **3** (the 3-amino analogue of **1**)[11] directly from (-)-shikimic acid **1** itself.



Scheme 1 *Reagents and conditions:* i, 1% HCl, MeOH, reflux; ii, $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$, THF, reflux; iii, NBS, CCl_4; iv, K_2CO_3, MeOH, THF, 40°C

(-)-Shikimic acid **1** was isolated from the ground seeds and carpels of star aniseed[12] according to a known procedure;[13] treatment of **1** with acidified methanol gave the known ester **4**[14] quantitatively. Selective 3,4-*cis*-diol protection of **4** was effected with various benzaldehyde equivalents under a variety of conditions; acid catalysed *trans*-acetalation using benzaldehyde dimethyl acetal in THF at reflux was found to be most consistent both in terms of yield and purity of acetals **5** (72%, *R*:*S* 3:2). The (*R*) and (*S*) isomers of acetal **5** were separable on silica but were used as a mixture in subsequent transformations. In NOE studies involving the **5**(*R*) isomer, irradiation of the benzylidene hydrogen gave an enhancement in both H-3 and H-4; a similar irradiation experiment for **5**(*S*) resulted in an enhancement in the signal corresponding to H-5.



Scheme 2 *Reagents and conditions:* i, NaN_3, MeOH, 40°C; ii, NaOMe, MeOH, 0°C; iii, PPh_3, H_2O, THF, reflux; iv, NaOH, H_2O; v, ion-exchange chromatography

Light induced radical bromination of **5** afforded a separable mixture of the allylic bromide **6** (62%) together with traces of the isomeric bromide **7** and epoxide **8**, (Scheme 1). That stereochemical inversion had occurred at C-3 upon formation of bromide **6** was apparent from coupling constant data in the ^{1}H nmr spectrum of **6** in $CDCl_3$. The *trans*-3,4-relationship in **6** resulted in a larger coupling constant between H-3 and H-4 ($J_{3,4}$ 7·5 Hz) when compared to those resulting from the 3,4-*cis*-sterochemical arrangements of **1**, **4** and **5** ($J_{3,4}$ 4-5 Hz). Bromide **6** was smoothly converted into the epoxy-ester **9** (82%) (a natural product from the fungus *Chalara microspora*)[15,16] when treated with basic methanol possibly *via* the intermediacy of **7** and **8**.

Displacement of bromide in **6** with azide ion afforded a separable mixture of three isomeric azides (ν_{max} 2100 cm^{-1}) **11** (8%), **12** (48%) and **13** (13%) (Scheme 2); interestingly none of the fourth possible isomeric azide **10** could be isolated. Staudinger reduction[17] (triphenylphosphine/water) of the major α-azide **12** ($J_{3,4}$ 4 Hz) proceeded smoothly in THF at 64°C. The resulting amine **15** proved to be unisolable because of rapid accompanying condensation and migration processes involving the internal 4-benzoate functionality; dihydrooxazole **18** and benzamide **19** were isolated in 42% and 37% yield respectively. Compounds akin to **18** have recently been highlighted in the synthesis of potent antiviral agents that are active against influenza.[18] In stark contrast, the isomeric α-azide **13** ($J_{3,4}$ 4 Hz) reduced cleanly under identical conditions to afford amine **16** (82%). Attempted saponification of **16** (NaOH, H_2O, 0°C) resulted in substrate aromatization; the extreme efficacy of the aromatization process was presumed to be a direct result of the presence of the 5-benzoate functionality in **16**.

Controlled removal of the benzoyl functionalities of **12** and **13** prior to azide reduction was seen as the key to the solution of these problems. Thus a mixture of azides **12** and **13** was deprotected with methoxide ion at 0°C to afford the α-azidodiol **14** (84%). Reaction temperature proved crucial to the success of this procedure; similar reactions performed at or near to room temperature invariably afforded mostly aromatized products. Azidodiol **14** reduced cleanly (PPh_3/H_2O) in THF at 64°C to yield the amino ester **17** (80%). Saponification of **17** gave the laevorotatory amino acid **3** (84%) after ion-exchange chromatography [Amberlite IR-120(H)].

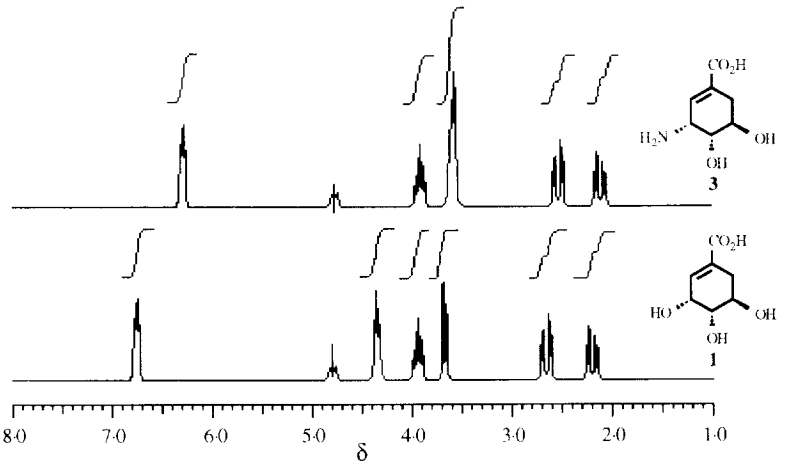
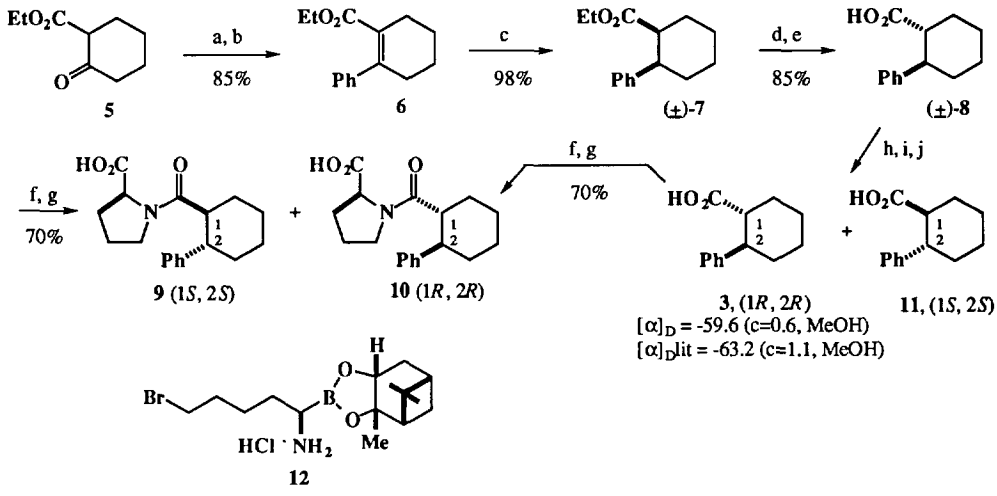


Figure: ^{1}H Nmr spectra of **3** and **1** measured in D_2O at 250 MHz

As part of our thrombin inhibitor program, we have used this X-ray crystal structure information to guide the design of ring-constrained inhibitors.[5] Specifically, we sought to maximize the edge-to-face aromatic interaction with Trp215 observed in the S3 pocket. We have designed rigidized mimics for the 3-arylpropionic acid P3 residue which, when incorporated into the boropeptide, rely on a cyclohexane or pyrrolidine template to hold an aromatic ring near the proline residue in approximately the same spatial orientation as it occupies in the X-ray crystal structure of the **1**:thrombin complex (Figure 1). The diequatorially-substituted (1*R*, 2*R*)-2-phenylcyclohexane carboxylic acid **3** appeared to allow excellent phenyl ring overlap when its minimized conformation was compared with the bound conformation of the P3 residue of **1**. Similarly, the (3*S*, 4*R*)-4-arylpyrrolidine-3-carboxylic acids **4** also showed good overlap between the aromatic rings, although it appeared to be less favorable due to the flatter conformation and different bond angles of the pyrrolidine ring. Potential advantages of this template relate to its greater flexibility and to the structural diversity which can be introduced onto the pyrrolidine nitrogen, both of which were expected to influence its conformation and possibly compensate for what initially looked to be a less favorable overlap between the aromatic rings. In each series it was also of interest to prepare inhibitors containing the enantiomers of each template as a test of our binding hypothesis.

The synthesis of the cyclohexane-based inhibitors begins as outlined in Scheme 1. Suzuki coupling[6] of phenylboric acid with the enol triflate derived from the β-ketoester **5** afforded the unsaturated ester **6**.[7] Hydrogenation over 10% Pd/C catalyst gave the saturated *cis*-ester (±)-**7**, which underwent essentially complete epimerization and subsequent ester saponification to afford the racemic *trans*-2-phenylcyclohexane carboxylic acid (±)-**8**. Coupling of this racemate with proline methyl ester hydrochloride, followed by saponification, gave the expected 1:1 mixture of diastereomers **9** and **10**.

Scheme 1



Reagents: (a) NaH, $PhN(SO_2CF_3)_2$, THF/DMSO, 0° C to 25° C (b) $PhB(OH)_2$, 3 mol% $Pd(PPh_3)_4$, 2M aq Na_2CO_3, C_6H_6/EtOH, 80° C (c) H_2 (1 atm), 10% Pd/C, EtOH (d) NaOEt, EtOH, reflux (e) KOH, MeOH/H_2O, reflux (f) Pro-OMe hydrochloride, DCC, HOBT, Et_3N, THF (g) LiOH · H_2O, THF/H_2O (h) (*R*)-α-phenylethylamine, MeOH (i) filter solid (j) H_3O^+