intensity) 292 (M - 2 × $C_5H_8O^+$) (8.7), 85 ($C_5H_9O^+$) (100).

(16*S*)-13-(1′-Hydroxyethyl)-8α-hydroxy-11-epicostus Lactone (13a). **16a** (5 mg) was dissolved in 25 mL of MeOH/HCl(aq) (0.05 N) (3:1) and allowed to stand for 72 h. The solution was neutralized with NaOH(aq) (0.1 N). Acetone was added. The salts were removed by filtration and the solvent was removed by distillation under reduced pressure. Purification by preparative TLC (three times in hexane/EtOAc, 7:1) yielded 2 mg of **13a** as a crystalline compound: mp 124–126 °C; ^1H NMR ($CDCl_3$) δ 3.88 (dd, 1 H, $J_{5,6} = J_{6,7} = 9.5$ Hz, C_6-H), 2.65 (m, 1 H, C_{11}-H), 2.32 (m, 1 H, C_7-H), 2.22–2.11 (m, 2 H, C_{13}-H).

(16*R*)-13-(1′-Hydroxyethyl)-8α-hydroxy-11-epicostus Lactone (13b). Deprotection of **16b** (5 mg) as described for **16a**, after purification by preparative TLC (three times in hexane/EtOAc, 7:1) gave 2 mg of **13b** as a crystalline compound: mp 122–124 °C; ^1H NMR ($CDCl_3$) δ 3.91 (dd, 1 H, $J_{5,6} = J_{6,7} = 9.5$ Hz, C_6-H), 2.75 (m, 1 H, C_{11}-H), 2.32 (m, 1 H, C_7-H), 2.22–2.11 (m, 2 H, C_{13}-H).

(16*S*)-8,16-Bis(2″-tetrahydropyranyl)-13-(1′-hydroxyethyl)-8α,11α-dihydroxycostus Lactone (17a) and (16*S*)-8,16-Bis(2″-tetrahydropyranyl)-13-(1′-hydroxyethyl)-8α,11β-dihydroxycostus Lactone (17b). A solution of **16a** (90 mg) in dry THF (4.5 mL) was dripped into a mixture containing 0.2 mL of diisopropylamine, 0.5 mL of a hexane solution of butyllithium (15%), and 1 mL of THF and stirred continuously for 30 min at −70 °C under a dry nitrogen atmosphere. After the mixture had been stirred for 40 min, it reached 0 °C and then dry oxygen was bubbled through for 30 min. It was carefully neutralized with HCl(aq) (1 N). The mixture was extracted with ethyl acetate, and after purification by CC (hexane/EtOAc, 9:1), it yielded 43 mg (48%) of **17a** and 12 mg (13%) of **17b**. **17a** was isolated as a colorless gum: IR (film) 3350, 1760, 1640, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.20 (s br, 1 H, C_{15}-H), 5.03 (s br, 1 H, C_{15}-H′), 4.97–4.75 (m, 4 H, C_{14}-H_2, $C_{2'}$-H, and $C_{2''}$-H), 4.56 (m, 1 H, C_{16}-H), 4.26–3.40 (m, 6 H, C_6-H, C_8-H, $C_{6'}$-H_2, and $C_{6''}$-H_2), 2.98–2.82 (m, 2 H, C_1-H and C_5-H), 2.70 (m, 1 H, C_7-H), 2.65 (m, 1 H, C_9-H), 2.55–2.40 (m, 2 H, C_3-H_2), 2.28 (dd, 1 H, $J_{8,9} = 8$ and $J_{9,9'} = 12$ Hz, C_9-H′), 2.10–1.37 (m, 14 H), 1.30–1.10 (m, 3 H, C_{16}-CH_3). **17b** was isolated as a colorless gum: IR (film) 3350, 1760, 1640, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.19 (s br, 1 H, C_{15}-H), 5.08 (s br, 1 H, C_{15}-H′), 5.01–4.84 (m, 4 H, C_{14}-H_2, $C_{2'}$-H, and $C_{2''}$-H), 4.56 (m, 1 H, C_{16}-H), 4.36 (dd, 1 H, $J_{5,6} = J_{6,7} = 10$ Hz, C_6-H), 4.05–3.37 (m, 5 H, C_8-H, $C_{6'}$-H_2, and $C_{6''}$-H_2), 2.95–2.82 (m, 2 H, C_1-H and C_5-H), 2.71 (dd, 1 H, $J_{8,9} = 5$ and $J_{9,9'} = 12$ Hz, C_9-H), 2.53–2.12 (m, 4 H, C_3-H_2, C_7-H, and C_9-H′), 2.12-1.40 (m, 14 H), 1.30–1.18 (m, 3 H, C_{16}-CH_3).

Subexpinnatin C (5) and 8α,11α-Dihydroxy-13-(1′-hydroxyethyl)-11-epicostus Lactone (18). **17a** (35 mg) was treated as described for the deprotection of **16a**, and 8 mg (40%) of **5** and 5 mg (25%) of **18** were obtained after purification by preparative TLC (six times in $HCCl_3$/*t*-BuOH, 19:1). **5** was isolated as a crystalline compound: mp 186–188 °C; IR (KBr) 3400, 1770, 1630; ^{13}C NMR ($CDCl_3$/MeOH-d_4 1:1) δ 178.9 (C-12), 151.6 (C-4), 145.1 (C-10), 115.0 (C-14), 110.4 (C-15), 79.7 (C-6), 76.5 (C-11), 68.0 (C-8), 64.4 (C-16), 55.5 (C-7), 53.3 (C-5), 44.5 (C-1)*, 44.1 (C-13)*, 32.3 (C-9), 30.5 (C-2)*, 30.2 (C-3)*, 24.3 (C-17). (Assignments denoted with asterisks may be interchanged.)

Acknowledgment. This work has been supported by the Comité Conjunto Hispano Norteamericano para el desarrollo científico y Tecnológico CCB-84 109/023. We thank the Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Málaga, for recording the 200-MHz NMR spectra, and Prof. Conrado Pascual Rigau for recording the ^{13}C NMR spectrum of subexpinnatin C.

Registry No. **5**, 99305-07-4; **11**, 81421-76-3; **12**, 108743-49-3; **13a**, 108743-50-6; **13b**, 108743-51-7; **14**, 108743-52-8; **15**, 108743-53-9; **16a**, 108743-54-0; **16b**, 108814-49-9; **17a**, 108743-55-1; **17b**, 108814-50-2; 18, 106533-22-6.

Synthesis of (*R*)-Serine-*2-d* and Its Conversion to the Broad Spectrum Antibiotic Fludalanine

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Received December 22, 1986

A novel and practical synthesis of (*R*)-serine-*2-d* that is stoichiometric in its use of deuterium is described. Isopropyl (*R,S*)-2-phenyl-2-oxazoline-4-carboxylate is metalated, deuteriated, resolved, and hydrolyzed to provide the optically pure (>99.8%) unnatural amino acid with >98% isotopic purity. Fluorination of the primary hydroxyl with SF_4 produces (*S*)-3-fluoroalanine-*2-d* (Fludalanine).

(*S*)-3-Fluoroalanine-*2-d* (Fludalanine, 1) in combination with the 2,4-pentanedione enamine of cycloserine sodium salt constitutes a novel, uniquely synergistic bacteriacidal antimicrobial (MK-641/642).[1] The introductions of deuterium and fluorine present unique problems which have been previously solved by the photofluorination of (*R*)-alanine-d^2 or the reductive amination of lithium fluoropyruvate using $NaBD_4$ followed by resolution.[3] With the advent of the requirement for large quantities of Fludalanine (1, MK-641) we had to address the practical and economical introduction of deuterium into an organic molecule. Neither of the original routes had addressed this critical issue, although the latter has been run on a multikilo scale.

In approaching the synthesis of 1 there are three prime criteria for a good route: (1) the specific introduction of

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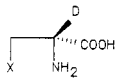

1 X = F FLUDALANINE -- MK 641

2 X = OH

a single deuteium; (2) formation of a carbon–fluorine bond; (3) establishment of the unnatural stereochemistry at C2. We now report a synthesis of Fludalanine which meets these criteria.

Noting Kollonitsch's conversion[4] of (*S*)-serine to (*R*)-3-fluoroalanine with sulfur tetrafluoride in anhydrous HF, we chose (*R*)-2-serine-*2-d* (**2**) as our target. This chiral, deuteriated amino acid would produce Fludalanine (**1**) upon fluorination. The challenge then became how to specifically introduce a single deuterium into a compound containing five exchangeable hydrogens and come out with the optically pure unnatural enantiomer **2**?

While α-deuteriation of amino acids is well-known,[5] there are very few reports of serine-*2-d* in the literature.[6] The most commonly used preparation is that of Miles and McPhie[6c] in which pyridoxal catalyzes the exchange of DL-serine's α-hydrogen during incubation in D_2O. While effective on the 1.0-g scale this procedure required >450 equiv of deuterium and gave the racemate. Kovacs[6d] has reported a nine-step conversion of (*RS*)-serine to *N*-CBZ-*O*-benzyl-(*S*)-(+)-serine-*2-d* (80–90% optical purity, 85% deuteriated) using >300 equiv of deuterium. Conceivably, this resolution-based method could yield (*R*)-serine-*2-d* (**2**). The only "stoichiometric" incorporation of deuterium is seen in the elegant work of Seebach[6e] wherein (*S*)-serine was deuteriated (78% D) with "self-reproduction" of chirality (91% optical purity). Unfortunately, this method requires unnatural (*R*)-serine as starting material for a synthesis of **2**. Other methods for the preparation of either (*R*)- or (*S*)-protioserine[7] offered little hope of a practical synthesis of **2**, thus, an alternate route had to be designed.

The need to eliminate serine's exchangeable hydrogens and concurrently enhance the acidity of its C2 hydrogen suggested the 2-oxazoline-4-carboxylate **3** as a serine surrogate. Early reports[8] of the (*S*)-2-phenyl-2-oxazoline-4-carboxylates derived from L-serine and the base-catalyzed racemization of the corresponding ethyl ester **3b** allow the logical conclusion that the hydrogen at C4 can be replaced by deuterium. If the oxazoline could be hydrolyzed without exchange, then the oxazoline-*4-d* **4** would yield racemic serine-*2-d*. In addition, a resolution of the enantiomeric oxazolines could be combined with racemization of the undesired *S* isomer to efficiently produce the optically pure target **2**.

The synthesis of Fludalanine (**1**) thus consists of five steps: (1) oxazoline formation; (2) deuteriation; (3) resolution; (4) hydrolysis; (5) fluorination (Scheme I).

Table I. % Deuteriation of Oxazoline

entry	base	deuterium quench[a,b]	% D
1	trityllithium	CH_3CO_2D[c]	96
2	trityllithium	(1) CH_3OD,[d] (2) CH_3CO_2D	95
3	Li HMDSA[f]	D_2SO_4[e]	55
4	Li HMDSA	CH_3CO_2D	67
5	Li HMDSA	CH_3CO_2D (rapid mixing)	80
6	Li HMDSA	(1) D_2O, (2) CH_3CO_2D	90
7	Li HMDSA	(1) CH_3OD, (2) CH_3CO_2D	95
8	Li HMDSA	(1) CH_3OD, (2) CH_3CO_2H	50

[a] 1.2–1.5 equiv of deuterium source used. [b] Double quenches were done sequentially. [c] 98% deuteriated. [d] 99.5% deuteriated. [e] 99% deuteriated, 96% in D_2O. [f] HMDSA = hexamethyldisilazide.

Results and Discussion

Oxazoline Formation. Although there are a number of good methods for oxazoline formaton[9] we chose to develop the classical condensation of an amino alcohol with an imidate[8b] into a convenient, high-yielding, one-pot procedure. Benzonitrile was converted to methyl benzimidate–HCl (**5**) with methanol–HCl, which without isolation, was treated with a serine ester hydrochloride salt and base in dichloromethane. In the case of isopropyl serinate, crystalline oxazoline **3c** was isolated in 72% yield (Scheme II). The scrupulous requirements of the subsequent deuteriation step demanded that impurities with exchangeable hydrogens by rigourously excluded (<1%). A troublesome contaminant proved to be benzamide, resulting from attack of chloride at the methyl group of imidate **5**. By control of reaction temperature (≤30 °C) and dichloromethane volume (0.5–1.0 mL per mL of methanol) benzonitrile could be converted (>97% by GC) to **5** with the formation of less than 2% benzamide. After reaction with isopropyl serinate the crystallization of oxazoline **3c** brought benzamide levels to ≤0.5%.

Deuteriation. Two methods were used to convert protiooxazolines **3** to their deuteriated analogous **4** in acceptable isotopic purity (>95%). In one, the acidic C4 hydrogen was abstracted by an organolithium base to produce the corresponding enolate which was quenched with a source of D^+ (CH_3COOD, CH_3OD, D_2SO_4, D_2O). Alternatively, statistical exchange could be effected by mixing oxazoline **3a** with 10 equiv of 99% CH_3OD in the presence of a catalytic amount of sodium methoxide, removing the now 90% CH_3OD and treating with another 10 equiv of 99% CH_3OD. This statistical method easily produced 98–99% isotopically pure **4a** and was forgiving of extraneous moisture. However, even with recycling of partially deuteriated CH_3OD it was impractical. Thus, the generation of the oxazoline anion and specific deuteriation of C4 using an equivalent of D^+ became the method of choice.

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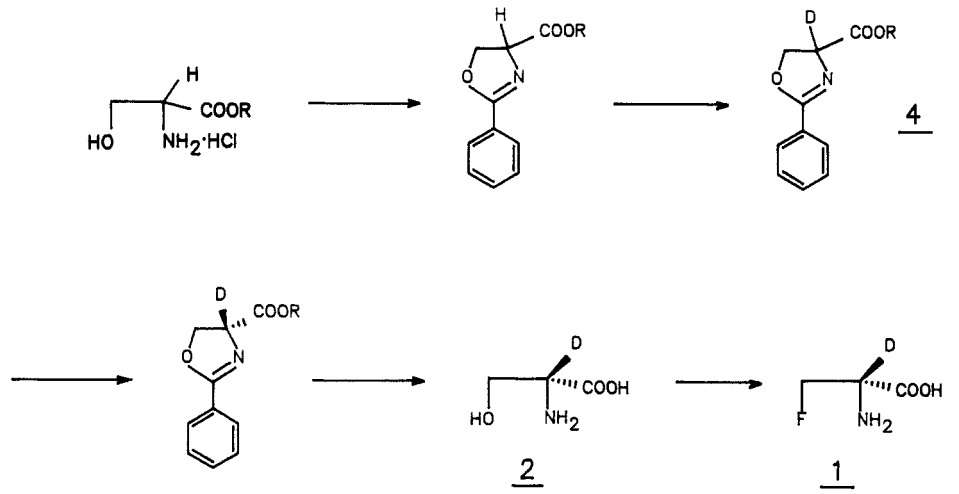
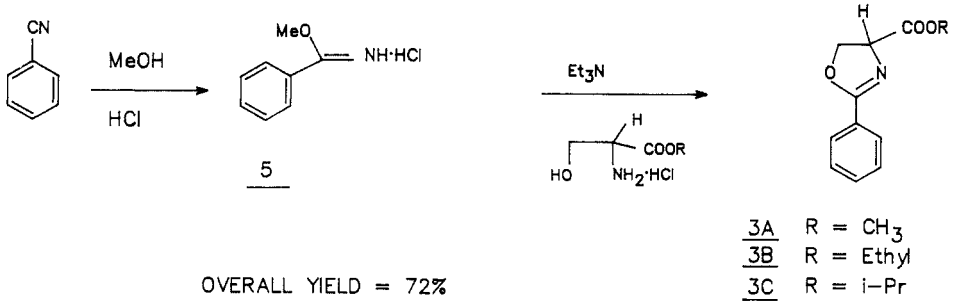
Scheme I



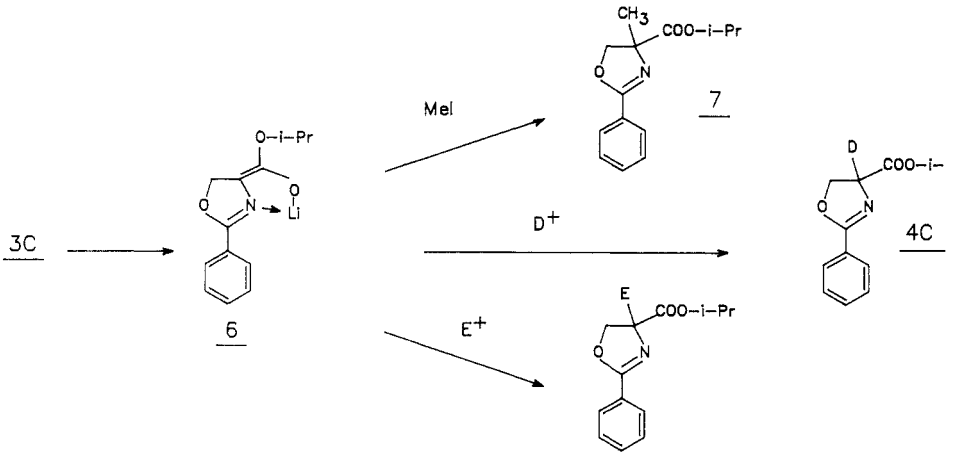
Scheme II



OVERALL YIELD = 72%

3A R = CH_3
3B R = Ethyl
3C R = i-Pr

Scheme III



Generation of enolate **6** at −78 °C with the lithium salt of 1,1,1,3,3,3-hexamethyldisilazane (LiHMDSA) was demonstrated by the addition of methyl iodide to produce the α-alkylated oxazoline **7** in >95% yield (Scheme III). The utility of this alkylation as a general method for the synthesis of α-substituted serines and threonines was further demonstrated by the use of electrophiles such as benzyl chloride, diphenyl disulfide, and benzaldehyde. Those results, however, are beyond the scope of this paper and for the threonine-derived oxazolines were independently reported by Seebach.[10]

In the case of anion deuteriation the choice of the organolithium base–deuterium source combination proved critical. Typical lithium dialkylamide bases (LDA, LiHMDSA, etc.) produce a mole of secondary amine upon proton abstraction. The exchangeable N–H is capable of entering and diluting the deuterium pool so that the relative rates of anion deuteriation and R_2NH/D^+ exchange become important.[11] The obvious answer is a base that produces no exchangeable hydrogen after deprotonation. The choice of base, however, was complicated by a temperature dependent β-elimination of anion **6** to the benzamido acrylate **8** above −50 °C. This potent Michael acceptor can rapidly react with another molecule of **6** to form the dimeric species **9** (Scheme IV). The successful base

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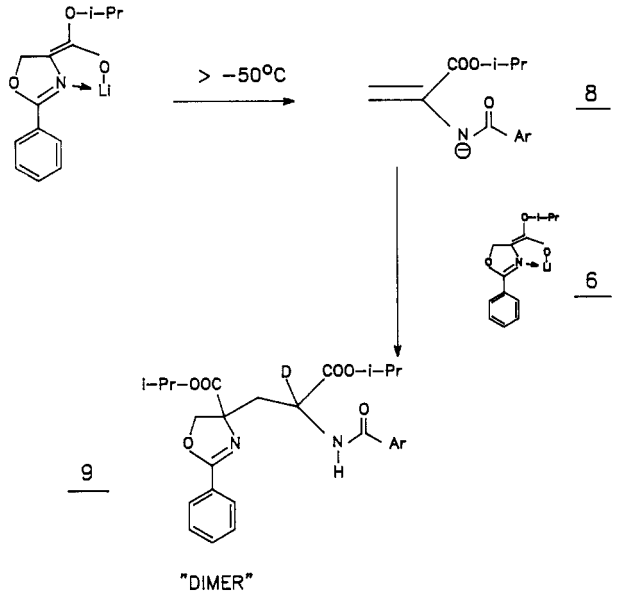
(11) In the case of lithium diisopropylamide Seebach has found (via X-ray) that diisopropylamine can be intricately involved with the enolate in a nonmonomeric complex. In such a situation the return of a proton from diisopropylamine may effectively compete with the deuterium quench. Cf.: (a) Laube, T.; Dunitz, J. D.; Seebach, D. *Helv. Chim. Acta* **1985**, *68*, 1373. (b) Amstutz, R.; Schweizer, W. B.; Seebach, D.; Dunitz, J. D. *Helv. Chim. Acta* **1981**, *64*, 2617. (c) Seebach, D.; Amstutz, R.; Dunitz, J. D. *Helv. Chim. Acta* **1981**, *64*, 2622. (d) Seebach, D.; Amstutz, R.; Laube, T.; Schweizer, W. B.; Dunitz, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 5403. (e) Bauer, W.; Seebach, D. *Helv. Chim. Acta* **1984**, *67*, 1972.

Scheme IV



must be capable of rapid proton abstraction below −50 °C. (Sodium and potassium hydrides gave only β-elimination products.) (Triphenylmethyl)lithium (tritylllithium) proved to be effective as a nonnucleophilic, nonexchanging base useable at temperatures below −50 °C, producing deuteriooxazoline **4c** in 90% yield (>95% deuterio). Table I indicates the percent deuteriation obtained in **4** by using both exchanging and nonexchanging bases.

As shown in Table I (entries 1 and 2), tritylllithium produces >95% deuteriated oxazoline **4c** with either CH_3COOD or CH_3OD quenches. The competition between deuteriation and N–H/D^+ exchange can be clearly seen in the reactions using lithium hexamethyldisilazane (Table I, entries 3–8). Here two factors come into play: (1) the acidity of the deuterium source correlates with the rate of N–H/D^+ exchange; the stronger the acid the more rapidly the amine exchanges ($D_2SO_4 > CH_3CO_2D > D_2O$, CH_3OD); (2) the solubility of the quenching agent at ≤−65 °C and the rate of mixing of a heterogeneous system can effect the extent of deuteriation. Entries 4 and 5 show an increase in deuterium incorporation with more rapid stirring. These CH_3CO_2D quenches were partially heterogeneous and it is reasonable to assume that hetero- vs. homogeneous rate differences [$k_{\text{anion deuteriation (homo)}} > k_{\text{exchange (homo)}}$, while $k_{\text{anion deuteriation (hetero)}} \gg k_{\text{exchange (hetero)}}$] are responsible for the effect of mixing.

The less acidic deuterium sources (D_2O, CH_3OD) clearly favored deuteriation over N–H/D^+ exchange (entries 6 and 7). However, due to the acidity of the deuterium at C4 the concurrently formed ^-OD or $^-OCH_3$ was capable of scrambling the label if a proton source (e.g., H_2O) was introduced. Thus, an additional mole of acetic acid-*d* was needed to neutralize the bases before workup. When the alkoxide is neutralized with *protio*acetic acid (entry 8) the oxazoline formed reflected the isotopic dilution of the deuterium pool (50%). As expected, with tritylllithium the CH_3OD quench also had to be followed with CH_3COOD to achieve acceptable deuterium levels (entry 2).

An investigation of other bases and conditions is summarized in Table II. High levels of deuterium incorporation were achieved with dimsyllithium, pentylsodium, phenylsodium, and tritylsodium (entries 6–9). However, none of these bases rivaled tritylllithium in yield, convenience, and/or deuterium incorporation. The use of lithium diisopropylamide (LDA) with either a dual quench (CH_3OD followed by CH_3CO_2D, entry 3) or in situ removal

Table II. Deprotonation and Deuteriation of Oxazoline

entry	base	% deuterium	% yield
1	LDA	50–60	90
2	LiTMP	30–40	90
3	LDA[a]	94	92
4	LDA/*n*-BuLi	90	70
5	Ph_3CLi	95–97	87–90
6	$CH_3S(O)CH_2Li$	90	70
7	n-$C_5H_{11}Na$	93	61
8	PhNa	94	73
9	Ph_3CNa	94	80

[a] Quench with CH_3OD followed by CH_3CO_2D.

of the diisopropylamine proton (entry 4)[12] proved effective but, again, not as efficient as tritylllithium. A host of organolithium reagents (*n*-butyl-, *sec*-butyl-, *tert*-butyl-, phenyl-, (diphenylmethyl)-, fluorenyl-, and indenyllithium) led to nucleophilic attack at the ester carbonyl of **3C** to form ketonic products in addition to C-4 proton abstraction. A large number of bases (NaH, KH, Grignards, 2-lithio-*n*,*n*-dimethylaniline) were not reactive enough to deprotonate **3C** below −50 °C and at higher temperatures produced the β-elimination products **8** and **9**.

It is apparent from Tables I and II that the only deuterium efficient method was anion formation with a nonexchanging base (tritylllithium) followed by a CH_3COOD quench. It will be seen later that the presence of triphenylmethane in the crude deuteriated oxazoline **4C** was at worst a minor annoyance, being easily separated in the next step.

Resolution. The deuteriooxazoline **4C** provides an excellent point in the synthesis to establish the correct "D" stereochemistry for the subsequent amino acids serine **2** and fluoroalanine **1**. The same properties that permit anion formation at C4 for deuteriation will allow racemization of the undesired *S* enantiomer. We envisioned a resolution which would first yield optically pure *R* oxazoline **4C** and then recycle the *S* isomer after racemization.

The weak basicity of the oxazoline nitrogen severely limited the choice of readily available chiral acids capable of forming stable diastereomeric salts. Only the relatively strong sulfonic acids were appropriate. While D-10-camphorsulfonic acid (CSA) readily reacted with racemic oxazoline **4C** to provide a crystalline camphorsulfonate, the product proved to be racemic upon breaking of the salt. *d*-α-Bromocamphor-π-sulfonic acid (BCSA), however, cleanly effected resolution of the *R* and *S* oxazolines.

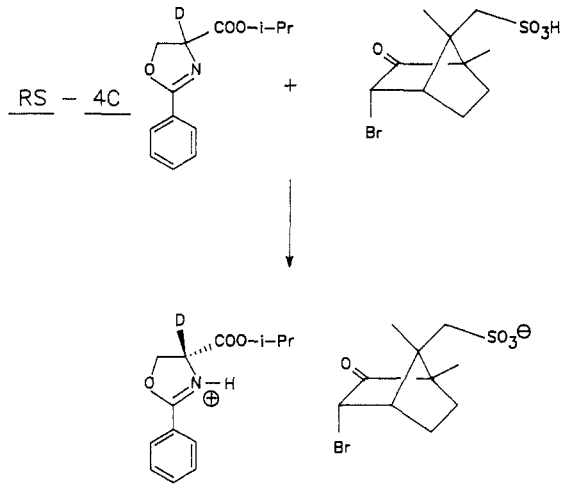
Treatment of racemic **4C** in dry CH_3CN with 0.5 mol of BCSA in CH_3CN produced a suspension of the desired *R* oxazoline bromocamphorsulfonate **10**, with the undesired *S* enantiomer in the mother liquor. Isolation of the *R* oxazoline BCSA **10** by filtration was followed by breaking of the salt (aqueous $NaHCO_3$ or NH_4OH) to give the optically pure (>99.8%) (*R*)-(−)-isopropyl 4-deuterio-2-phenyl-2-oxazoline-4-carboxylate [(*R*)-**4C**] in 80% yield (Scheme V).

In order to determine whether the resolution was kinetic or thermodynamic in nature a 1:1 mixture of the *R* and *S* oxazoline BCSA salts (from racemic oxazoline plus 1.0 mol of BCSA) was suspended in acetonitrile and treated with 1.0 mol of free racemic oxazoline **4C**. After being stirred for 4 h at 25 °C the mixture was filtered and the precipitated salts isolated. Analysis showed only the *R* oxazoline. The resolution is presumably thermodynamic, dependent upon solubility differences between the salts

(12) While deuterium incorporation in this case can be optimized, double the amount of CH_3CO_2D is required: 1 equiv for enolate **6** and 1 equiv for the regenerated LDA.

Scheme V

RESOLUTION



DIASTEREOMERIC SALT CRYSTALLIZES IN 42% YIELD (84% OF THEORY)

of the *R* and *S* oxazolines. In light of this result the pure salts were made and their solubilities determined. The *S* oxazoline BCSA was three times as soluble in CH_3CN (70 °C) as the *R* isomer (67 vs. 22 mg/mL). No other solvent offered as large a difference combined with sufficient solubility to work in reasonable volumes.

Development of the resolution was greatly facilitated by the use of the Pirkle L-phenylglycine chiral stationary phase HPLC column.[13] The ample π character of **4C** provides the necessary π–π interactions to achieve base line separation of the *R* and *S* enantiomers. Figure 1 shows a typical separation of the racemate and the high purity (>99%) of the resolved *R* enantiomer.

As mentioned earlier the use of tritylllithium in the deuteriation leaves a mole of triphenylmethane in the crude deuteriated oxazoline **4C**. In the resolution we chose to treat the triphenylmethane as a nonpolar organic "solvent" and ignore it. Resolution proceeded normally in the presence of as much as 2.5 mol of Ph_3CH with the only change being an increase in CH_3CN volumes necessary to dissolve the crude product.

It should be noted that the resolutions of the racemic protio- and deuteriooxazolines **3C** and **4C** were virtually identical, providing facile routes to optically pure protio- and deuterio-(*R*)-serines.

Racemization. Using the chiral HPLC assay the racemization of the *S* oxazoline was studied, first with protio (*S*)-**3C** and eventually with the deuteriated (*S*)-**4C**. In acetonitrile amine bases such as triethylamine, Dabco, and DBU were effective in racemizing the chiral center at C4. However, removal of the amine was necessary before the now racemic oxazoline could be resolved. Polymer-bound tertiary amines (e.g., IRA 47) made removal simple but were extremely difficult to dry. The presence of resin-trapped-H_2O in the racemization inevitably led to scrambling of the deuterium. Anhydrous K_2CO_3 proved to be ideal. A catalytic amount (4 mol %) suspended in acetonitrile completely racemized the *S* isomer after 8 h at 80 °C. After cooling to 25 °C the carbonate was removed by

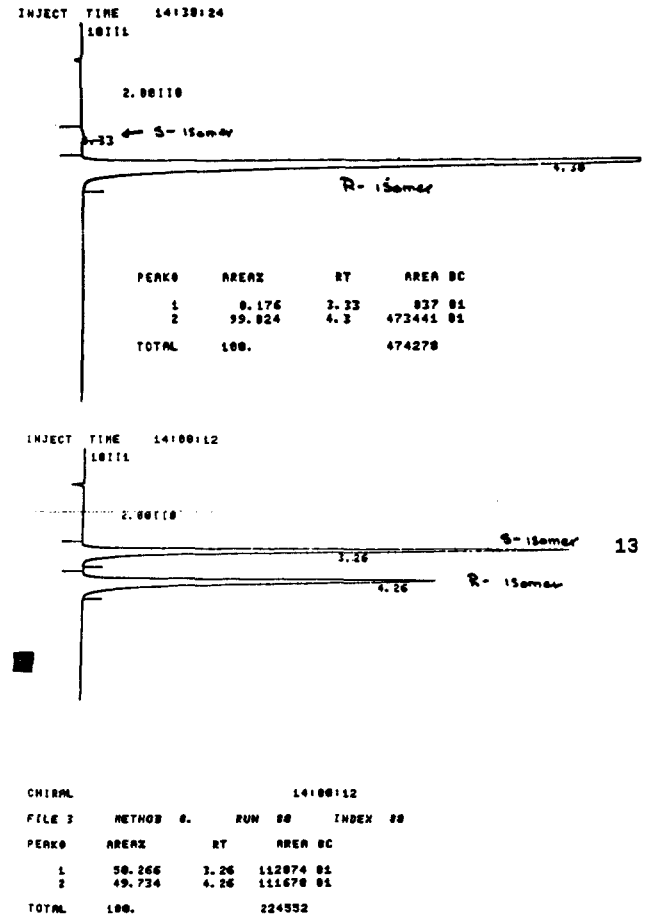


Figure 1.

filtration, and the resulting acetonitrile solution was treated with 0.5 mol of *d*-BCSA to complete the recycle (Scheme VI). In the case of deuteriooxzaoline (*S*)-**4C** the same method was used paying special attention to the amount of extraneous water. By predrying the K_2CO_3/CH_3CN suspension (reflux through a Soxhlet extractor filled with 3-Å sieves) before introduction of the *S* oxazoline only minimal losses in the isotopic purity were incurred. The use of a single solvent (CH_3CN) for racemization and the subsequent resolution permits an efficient recycle sequence for the undesired *S* isomer which rivals an asymmetric synthesis.[6c]

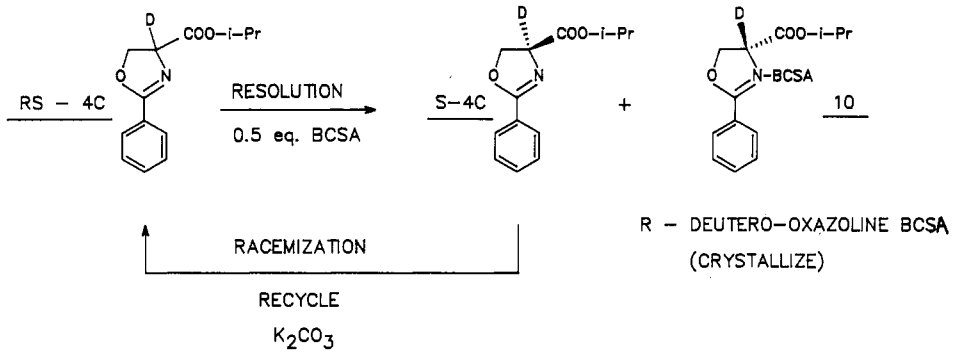
Hydrolysis. *R* Oxazoline-4-*d* (*R*)-**4C** is converted to (*R*)-serine-2-*d* by acid hydrolysis.[14] The crystalline bromocamphorsulfonate salt **10** is broken with aqueous ammonia. The oxazoline free base is extracted into toluene and then refluxed with 6 N HCl. NMR studies indicate that the mode of hydrolysis is initial protonation on nitrogen with ring opening to give isopropyl *O*-benzoylserinate–HCl, followed by isopropyl ester hydrolysis to *O*-benzoylserine–HCl (Scheme VII). Finally, cleavage of the *O*-benzoyl group produces (*R*)-serine-2-*d* (**2**) plus benzoic acid. After separation of benzoic acid and pH adjustment the zwitterionic amino acid is isolated in 92% yield.

(13) Chiral HPLC assays were run on a Pirkle L-phenylglycine column (regis Chemical Co., Merton Grove, IL). Pirkle, W. H.; Finn, J. M.; Schreiner, J. L.; Hamper, B. C. *J. Am. Chem. Soc.* **1981**, *103*, 3964.

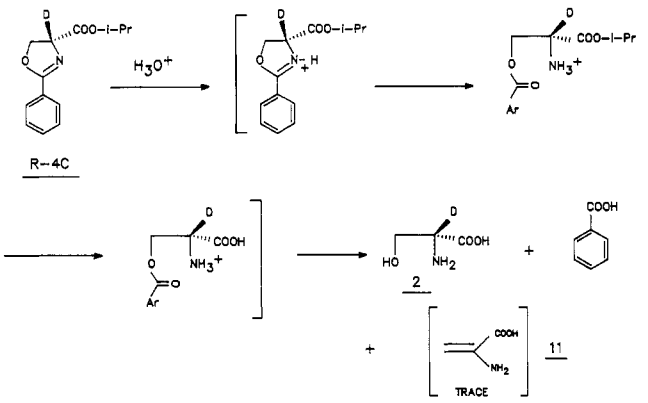
(14) Among α-amino acids, serine and threonine are unique in their "optical" stability toward aqueous acid. At 110 °C in 6 N HCl there is no detectable racemization. Liardon, R.; Jost, R. *Int. J. Pept. Protein Res.* **1981**, *18*, 500.

(15) (a) Gerstenberger, M. R. C.; Haas, A. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 647. (b) Schlosser, M. *Tetrahedron* **1978**, *34*, 3. (c) Olah, G. A.; Welch, J. T.; Vankar, Y. D.; Nujima, M.; Kerekes, I.; Olah, J. H. *J. Org. Chem.* **1979**, *44*, 3872. (d) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574.

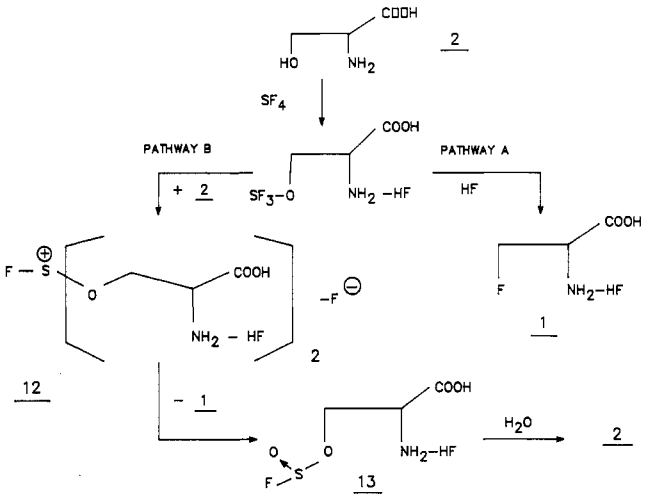
Scheme VI



Scheme VII



Scheme VIII



An isotopic enrichment occurs during the hydrolysis. Generally, 95% deuteriated oxazoline produces >98% deuteriated serine. This is, presumably, an H/D kinetic isotope effect in the side reaction which produces dehydroalanine **11** via elimination.

Fluorination. Of the variety of methods[14] for conversion of a primary hydroxyl to an alkyl fluoride only the use of sulfur tetrafluoride is suitable for serine fluorination.[4] The use of liquid HF as solvent provides both protection for the amine (via protonation) and a homogeneous medium. Investigation of this reaction under the reported conditions showed as much as 40% "unreacted" serine, even with large excesses of SF_4.[4] In situ ^{19}F and ^{13}C NMR studies[16] elucidated a pathway (B) involving the coupling of two serines to one SF_4 to produce **12** (Scheme VIII). With time **12** decomposed to form equal amounts of fluoroalanine **1** and fluorosulfite **13**. Upon aqueous workup the fluorosulfite **13** hydrolyzes back to serine **2**. While pathway B was competitive with the desired fluorination (pathway A) at high concentration (e.g., 5 M), dilution (≤0.5 M) favored pathway A producing 99:1 ratios of fluoroalanine to serine.[17]

Conclusion. 2-Phenyl-2-oxazoline-4-carboxylate **3** has proved to be an ideal serine surrogate. While protecting the amine and hydroxy functions it has enhanced the acidity of the C4 hydrogen to permit stoichiometric deuteriation. In addition, the oxazoline has provided a site for stereochemical control via resolution and subsequent racemization of the unwanted enantiomer. The result is the first synthesis of (*R*)-serine-2-*d* (**2**), which produces this unnatural amino acid in >99.8% optical purity with >98% isotopic purity. Fluorination of **2** results in its direct conversion to >99% (*S*)-3-fluoroalanine-2-*d* (Fludalanine, 1), culminating the most direct and only deuterium efficient route known to this important antimicrobial agent.

Experimental Section

Proton NMR spectra were measured on a Bruker WM250 and are relative to Me_4Si as an internal standard (δ 0.0). Carbon-13 spectra were obtained on a Varian XL-100 or CFT-20 instrument. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer Model 241 polarimeter using a 1-d cell. Amino acid analyses were carried out on a Spinco-Beckman automatic amino acid analyzer. Mass spectra were obtained on a Finnigan 4500 quadrapole mass spectrometer. The isotopic purity of 3-fluoroalanine-2-*d* at C2 and C3 was determined by comparison of the intensity ratio *I m/e* 134:135:136 (M – 117 = M – COO-$SiMe_3$) with that of *I m/e* 218:219 (M – CH_2F, –CHDF, –CD_2F) using unlabeled 3-fluoroalanine as a reference. Chiral GC assays of amino acids were run on the corresponding isopropyl ester–*N*-trifluoroacetamides by using an (*S*)-valine-α-polyethyl acrylamide (PEA) column (50 m, 0.25 mm i.d.). Chiral HPLC analyses were run on a Pirkle covalent L-phenylglycine column (25 cm × 4.6 mm i.d.) eluted with 5% isopropyl alcohol in hexane. Karl Fischer (KF) determinations (H_2O content) were done on a Photovolt Aquatest IV titrator. Microanalyses were obtained on a Control Equipment Model 240X elemental analyzer.

(±)-Isopropyl Serinate. To a 12-L three-neck flask equipped with a mechanical stirrer, gas inlet tube, and a condenser fitted with a drying tube was added 9000 mL of isopropyl alcohol, followed by 1000 g (9.52 mol) of *dl*-serine. Into the stirred slurry, initially at ambient temperature, HCl gas was bubbled at a rate of ~3.2 mol/h for 3.5 h (total addition ≃11.2 mol). The HCl addition is exothermic and is used to bring the reaction mixture to mild reflux. Upon completion of the HCl addition the condenser was removed, the gas inlet tube was raised above the surface of the mixture and closed off, and then 3000 mL of isopropyl alcohol–H_2O was removed by distillation at atmospheric pressure. The resulting clear, colorless solution was assayed for unreacted serine by TLC (SiO_2; 70:30 isopropyl alcohol/concen-

(16) Douglas, A. W.; Reider, P. J. *Tetrahedron Lett.* **1984**, *25*, 2851.
(17) An excess of SF_4 (1.5 equiv) was necessary for complete conversion of **2** to **1**; although this resulted in some acyl fluoride formation, the aqueous workup rapidly cleaved acyl fluoride back to fluoroalanine **1**. Isolated yield of **1** was >80%.

trated NH_4OH; ninhydrin visualization; serine R_f = 0.29, violet; isopropyl serinate R_f = 0.8, orange). Upon completion of the reaction the solution was cooled to ~60 °C, and 6.5 L of isopropyl acetate were added as rapidly as was convenient. The mixture was then cooled to 20 °C and aged for 3 h. The precipitated isopropyl serinate hydrochloride was filtered, washed with 5 L of 5:1 isopropyl acetate/isopropyl alcohol and 5 L of isopropyl acetate, and then vacuum-dried at 50 °C for 12 h: yield, 1.607 kg (92%); mp 144–148 °C; Cl^- 19.8% (titration), H_2O 0.3 mg/g. Anal. Calcd $C_6H_{14}ClNO_3$: C, 39.20; H, 7.68; N, 7.63; Cl, 19.3. Found: C, 39.21; H, 7.64; N, 7.68; Cl, 19.08.

(±)-Isopropyl 2-Phenyl-2-oxazoline-4-carboxylate (3C). Into a 12-L three-neck flask equipped with a mechanical stirrer, gas inlet tube, and a condenser were added 890 mL (8.71 mol) of benzonitrile, 423 mL (10.46 mol) of methanol, and 215 mL of methylene chloride. Into the stirred solution, initially at ambient temperature, was bubbled HCl gas at a rate of ~3.6 mol/h for 2.75 h (total addition ~365 g, 10 mol). During the addition of HCl an internal temperature of 20–25 °C was maintained. Approximately 2 h into the HCl addition solid imidate–HCl began to precipitate. At this time an additional 215 mL of CH_2Cl_2 was added to facilitate stirring. The mixture was stirred at 20–25 °C until the GC assay indicated <3% residual benzonitrile.[18] CH_2Cl_2 (1000 mL) was added to the thick suspension, and then it was distilled off at ≤20 °C (vacuum) in order to remove any residual HCl. This operation was repeated a second time to ensure complete removal of free HCl. The methyl benzimidate suspension was diluted with 4500 mL of CH_2Cl_2, and then isopropyl serinate HCl (1600 g, 8.71 mol) was added. While an internal temperature of 20–25 °C was maintained (with cooling) 1254 mL (9.0 mol) of triethylamine was added over 30 min. The white suspension was stirred at 20–25 °C for about 8 h.[19] Upon completion of the reaction the suspension was diluted with 5.0 L of CH_2Cl_2 and washed with 6.0 L of H_2O. After separation of the layers the CH_2Cl_2 was washed with an additional 6.0 L of H_2O. The organic layer was then dried over Na_2SO_4 (200 g) until the water content was ≤1.5 mg/mL. The dried methylene chloride solution was vacuum concentrated at or below 25 °C to a small stirrable volume. To this solution was added 4.0 L of hexane and the mixture was concentrated with the temperature kept between 30 and 50 °C. The oxazoline was then dissolved in 16.0 L of hot hexane (60 °C). The solution was treated with 80 g of Darco G-60, cooled to 30–35 °C, and filtered. The Darco was washed with 2 L of ~35 °C hexane, and the filtrates were combined. The volume of the hexane solution was adjusted to ~11 L by vacuum distillation. The inherent cooling was used to lower the temperature to 10–15 °C. This temperature was maintained until the final volume of 11.0 L was reached. At this point the temperature was lowered with external cooling to −5 °C and held for 3 h to allow complete crystallization. The cold suspension was filtered, washed with 2.0 L of <0 °C hexane, and vacuum-dried at ≤24 °C. The mother liquors were concentrated in vacuo to a volume of ~2.0 L. Cooling to −5 °C produced a second crop which was filtered and washed with 300 mL of <0 °C hexane. Yield: first crop, 65%; second crop, 8%. After melting points were checked the crops were combined to yield 1460 g (6.76 mol) of (±)-isopropyl 2-phenyl-2-oxazoline-4-carboxylate (**3C**): mp 40–42 °C; titration ($HClO_4$) >97%. Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48, N, 6.00. Found: C, 66.57; H, 6.45; N, 6.01. ^1H NMR ($CDCl_3$) δ.

(Triphenylmethyl)lithium. A 12-L three-neck flask was equipped with a mechanical stirrer, a 2-L pressure-equalizing addition funnel, a nitrogen inlet, and a thermometer. The entire system was dried with a heat gun under a stream of dry nitrogen. After the system had cooled to room temperature, triphenylmethane (733 g, 3.00 mol) was added under a positive N_2 stream. Tetrahydrofuran[20] (3.0 L) was added and the triphenylmethane dissolved. The reaction mixture was cooled in an ice/water bath to an internal temperature of 10 °C. *n*-Butyllithium [1.733 L of 1.54 M (2.67 mol)] was transferred to the addition funnel by a cannula. The *n*-butyllithium was added over 1.5 h such that the internal temperature remained at 10–15 °C. On completion of the addition, the addition funnel was flushed with a minimal amount of THF and the reaction mixture stirred at 15 °C (internal temperature) for 1.5 h. The reaction was then cooled to −70 °C (internal temperature) in a dry ice/acetone bath. The red solution was used in the next experiment.

Isopropyl (*R*,*S*)-2-Phenyl-2-oxazoline-*4*-*d*-4-carboxylate (*RS*-4C). *It is imperative that this reaction be carried out with the exclusion of water and oxygen.* A solution of the oxazoline **3c** (500 g, 2.4 mol) in 500 mL of THF was prepared (KF = 136 μg/mL). To the (triphenylmethyl)lithium solution prepared in the previous experimental, at −70 °C, was added the oxazoline solution in THF, at a rate to maintain the internal temperature below −60 °C. This required 30 min. On completion of the addition, the addition funnel was rinsed with a minimal amount of dry THF and the reaction mixture stirred at −60 to −70 °C for 0.5 h. Acetic acid-*O*-*d* (196 g, 3.21 moles) was transferred to the addition funnel by a cannula and added to the reaction mixture over 15 min, with the internal temperature kept below −60 °C. On completion of the addition, the reaction mixture was warmed to room temperature with stirring. The reaction mixture was diluted with 5 L of ethyl acetate and transferred to an extractor. Water (5 L) was added and the system stirred for 15 min, and the layers were separated. The aqueous phase was washed with an additional 3 L of ethyl acetate. The combined organics (ca. 16 L) were dried with 1.1 kg of granular, anhydrous sodium sulfate. The ethyl acetate solution was filtered and the sodium sulfate washed with 2 L of ethyl acetate. The organic solution was concentrated to ca. 2 L ($T \leq 25$ °C) (solution A). Acetonitrile (3 L) was added. The reaction mixture was agaiin concentrated to 2 L keeping the internal temperature ca. 20 °C. Following concentration acetonitrile was added (ca. 3 L) to a final volume of 5 L. This solution (KF = 360 μg/mL) was carried into the resolution to produce **10**. Alternately, the organic concentrate (solution A, above) could be stripped to a solid mixture of (*RS*)-**4C** and triphenylmethane. The pure racemic deuteriated (96%) oxazoline [(*RS*)-**4C**] was isolated by Kugelrohr distillation [110–120 °C (0.05 mm)]. The distillate was triturated with hexane, filtered, and vacuum-dried (mp 42–43 °C, yield 91%): ^1H NMR ($CDCl_3$) δ 1.30 (3 H, d, J = 6.3 Hz), 1.31 (3 H, d, J = 6.3 Hz), 4.6 (2 H, center of AB, ^2J = 8.6 Hz), 5.11 (1 H, septet, J = 6.3 Hz), 7.4 (2 H, m, meta), 7.5 (1 H, m, para), 8.0 (2 H, m, ortho).

***d*-α-Bromocamphorsulfonic Acid.** A 1-L Dowex 40 × 4 resin column was prepared and placed on the H^+ cycle. A solution of 350 g of ammonium bromocamphorsulfonate ($[\alpha]^{25}_D$ +84.5°) in 3 L of H_2O was prepared. The solution was passed through a 1-L column of Dowex 50 × 4 resin on the H^+ cycle at a rate of 25 mL/min. The first 500 mL of solution was neutral and was discarded. The next 6 L, which were acidic (pH 1 to 3), were collected, concentrated at $T \leq 55$ °C to about 600 mL, and flushed with 6 × 1 L of acetonitrile to a final KF of 3–4 mg/mL. The solution was adjusted to a volume of 1800 mL with dry acetonitrile and stored in a closed vessel under N_2.[21]

Isopropyl (*R*)-2-Phenyloxazolinium-*4*-*d*-4-carboxylate *d*-α-Bromocamphor-π-sulfonate (10). Isopropyl (*R*,*S*)-4-

(18) The reaction is monitored by the disappearance of benzonitrile relative to an internal standard. Capillary GC: DB1-column, 15 m; flow (helium), 160 mm/s; temperature program, 125 °C for 2 min, increase at 10 °C/min to 175 °C, hold 5 min; injector (split ratio 150:1) temperature = 250 °C; detector (FID) temperature = 250 °C; benzonitrile, t_r 2.41 min; imino ether, t_r 3.46 min; benzamide, t_r 4.89 min; dodecane (standard) t_r 3.98 min. An aliquot of the CH_2Cl_2 supernatant was removed, diluted (25x) with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$. The CH_2Cl_2 layer was injected (1–2 μL).

(19) After the mixture was stirred for 8 h the CH_2Cl_2 supernatant was assayed by GC. The area % ratio of oxazoline to imino ether was used to determine the reaction end point. A 0.5-mL aliquot was removed, diluted to 10 mL with CH_2Cl_2, and then washed with 5 mL of H_2O. The CH_2Cl_2 layer (1.0 μL) was injected onto a capillary GC DB1-column, 15 m: flow (helium), 160 mm/s; temperature program, 150 °C for 2.5 min, increase at 20 °C/min until 200 °C, hold for 8 min; injector (split ratio 150:1) temperature = 250 °C; detector temperature (FID) = 250 °C; components, benzonitrile (t_r 2.26 min), imino ether (t_r 2.76 min), dodecane (standard) (t_r 3.00 min), benzamide (t_r 3.55 min), oxazoline (t_r 7.19 min); methylene chloride = 1.8 min – integration inhibited. The reaction is considerrd complete when the ratio (area %) of oxazoline/imino ether is >30:1.

(20) The THF was previously dried over 4-Å molecular sieves to a KF of 26 μg/mL. It was transferred by cannula from the reagent bottle, fitted with a septum cap, under N_2 pressure. The KF of the resulting solution was 42 μg of H_2O/mL solution.

(21) This solution is stable for at least 30 days at 5 °C. Base titration showed it to be 0.5 M in acid.

deuterio-2-phenyl-2-oxazoline-4-carboxylate–acetonitrile solution for the above experiment: ca. 5 L (0.37 M in oxazoline, 1.85 mol also contains triphenylmethane, KF = 360 μg/mL). d-α-Bromocamphor-π-sulfonic acid, 0.5 M in acetonitrile: 1.86 L (0.93 mol, KF = 3–4 mg/mL). The acetonitrile solution of the oxazoline (RS)-**4C** under N_2 was placed in a three-neck, 12-L flask equipped with a mechanical stirrer, an addition funnel with a drying tube, and a thermometer, and cooled with a water bath. An aliquot of the solution was removed for perchloric acid titration and HPLC assay.[22] The acetonitrile solution of bromocamphorsulfonic acid (1.86 L, 0.5 M, KF = 3–4 mg/mL) was added over 15 min, with an internal temperature of 20–30 °C maintained. The reaction was stirred for 2 h after the completion of the addition until precipitation was complete. The salt was filtered, washed with 2 L of acetonitrile, and dried, in vacuo, overnight at 35 °C to yield 350 g (0.641 mol, 60% of theory based on **3C**) of the R oxazoline d-bromocamphorsulfonate **10**:[23] $[\alpha]^{25}_D$ +52.7°: mp 193–196 °C.

Isopropyl (R)-2-Phenyloxazolinium-4-d-4-carboxylate d-α-Bromocamphor-π-sulfonate (10) by Resolution of Isolated (RS)-4C. A solution of **4c** (18.83 g, 80.75 mmol) in dry acetonitrile (160 mL) was made up in a 500-mL, three-neck flask equipped with a mechanical stirrer and dropping funnel. A solution of d-α-bromocamphorsulfonic acid in acetonitrile (93.5 mL of a 0.475 M solution, 44.4 mol) was added over 30 min. After the mixture was stirred for 2 h at room temperature, the resulting white slurry was filtered and the precipitated salt washed with acetonitrile (100 mL). The product was vacuum dried overnight to yield 17.9 g (81.4% of theory) of **10**: mp 193–195 °C.

Isopropyl (R)-2-Phenyloxazoline-4-d-4-carboxylate [(R)-4C] by Reversal of Bromocamphorsulfonic Acid Salt (10). The oxazoline salt **10** (17.9 g, 32.8 mmol) was vigorously mixed with CH_2Cl_2 (350 mL) and saturated aqueous $NaHCO_3$ (200 mL). The layers were separated and the organic layer washed with aqueous $NaHCO_3$ (100 mL) and brine (100 mL), and then dried over $MgSO_4$. After removal of $MgSO_4$, the filtrate was concentrated to a solid, which was slurried in hexane and filtered. Vacuum drying gave 7.5 g (97%) of crystalline oxazoline (R)-**4C**: mp 55–57.5 °C; $[\alpha]^{25}_D$ −136° (c 1, isopropyl alcohol).

(R)-Serine-2-d. To a 2-L separatory funnel was added 110 g (202 mmol) of R oxazoline bromocamphorsulfonic acid salt **10**, 200 mL of toluene, and 200 mL of 2 N aqueous NH_4OH. The mixture was thoroughly mixed, and the layers were separated. The lower (aqueous) layer was then reextracted with 200 mL of toluene. Removal of the aqueous layer was followed by combining both toluene extracts in the separatory funnel.[24] The combined toluene extracts were washed with 200 mL of H_2O and then placed in a 2-L three-neck flask, equipped with a mechanical stirrer, vacuum distillation head, and a stopcock. The volume was reduced by distilling (vacuum) 200 mL of toluene. To the resulting toluene solution of the free R oxazoline was added 250 mL of 6 N HCl. After replacing the distillation head with a condenser the two-phase mixture was heated to reflux for 2.5 h and then cooled to ~25 °C. The reaction mixture was transferred to a 1000-mL separatory funnel, and the layers were separated. The aqueous layer was concentrated to a volume of 50 mL while an internal temperature was maintained at ≤50 °C (vacuum). H_2O (200 mL) was then added and the concentration to 50 mL repeated. When titration (HCl, CO_2H, $NH_3^+Cl^-$, Cl^-) indicated ≤50% excess HCl, the aqueous solution of serine–HCl was diluted to a volume of 100 mL with H_2O and the pH adjusted to 5.1 by the addition of Et_3N (~40–50 mL). The internal temperature was maintained below 20 °C by external cooling during the pH adjustment. When the aqueous solution was at pH 5.1 the product was crystallized by the addition of 500 mL of isopropyl alcohol. The suspension was aged at ~10 °C for 1 h and then filtered. The cake was washed with isopropyl alcohol (200 mL) and hexane (250 mL) and then dried under vacuum. Isolated yield 19.7 g (92%): NMR (18% DCl/D_2O) δ 4.12 (center of AB quartet, ^2J = 12.5 Hz); chiral GC, >99.8% (R)-serine; Spinco (97%); mp 219–220 °C dec; HPLC (>98%); KF (<0.3%); LOD (<0.1%); mass spectrum (>95% deuteriated); $[\alpha]^{25}_D$ −13.4° (c 1, 6 N HCl); $[\alpha]^{25}_{405}$ +125.5° (c 1, $CuSO_4$/NaOAc/HOAc[25]); $[\alpha]^{25}_{365}$ +205° (c 1, $CuSO_4$/NaOAc/HOAc).[25]

MK-641: (S)-3-Fluoroalanine-2-d (1). Into a 1000-mL stainless steel cylinder equipped with a stainless steel sparge (set 1 in. above bottom) and a needle valve was placed 25.0 g (236 mmol) of (R)-2-deuterioserine. The system was sealed, evacuated, and cooled to −40 °C. Liquid HF (500 mL) was introduced through the sparge and the system agitated to ensure dissolution of the serine. SF_4 (gas) [40 g, 370 mmol] was then rapidly introduced subsurface through the sparge at a rate of ~2 g/s. The inlet valve on the sparge was then closed and the mixture allowed to warm to 0 °C. When the reaction mixture reached 0 °C (approximately 3 h) the outlet valve was carefully opened and the HF vented to a KOH scrubber. The evaporation will cool the mixture to a point where HF venting slows down even with the outlet valve wide open. At this time a gentle flow of nitrogen was introduced through the sparge to aid in HF removal. After the nitrogen flow had been running for 1.0 h the system was sealed. A mixture of H_2SO_4 (8 mL) and H_2O (4 mL) was then blown, under pressure, into the vessel, subsurface. At this point the outlet valve was carefully vented, and HF removal was continued. The HF was removed until the volume became small but stirrable. CH_2Cl_2 (250 mL) was then introduced into the reaction vessel through the sparge by applying vacuum to the outlet valve. CH_2Cl_2 (200 mL) was distilled at reduced pressure to ensure removal of any residual HF. The resulting suspension of MK-0641 sulfate in 50 mL of CH_2Cl_2 was treated with 150 mL of H_2O, and vacuum distillation was resumed to remove the remaining CH_2Cl_2. The resulting aqueous solution was then treated with Et_3N (25 g, 247 mmol), while an internal temperature was maintained below 20 °C, until the pH reached 5.2. Ethanol (450 mL of 95%) was added to complete the crystallization. The resulting suspension was cooled to 5 °C and aged for 1 h. The MK-0641 was isolated by filtration, and the cake was washed with 95% ethanol (200 mL) and vacuum-dried: yield, 20.4 g (80%); LC 99.3% MK-0641; deuterium ≥98%; GC (optical purity) >99.7%. Anal. Calcd for $C_3H_5DFNO_2$: C, 33.34; H, 5.60; N, 12.96. Found: C, 33.34; H, 5.61; N, 12.96. Titration: 100.7%; $[\alpha]^{25}_{365}$ 305.4°;[25] $[\alpha]^{25}_{405}$ 211.1°;[25] ^1H NMR (D_2O) δ 4.87 (center of the AB of an ABX pattern where X = F, J_{HF} = 47 Hz, $^2J_{AB}$ = 10.8 Hz); ^{13}C NMR [D_2O, referenced to external dioxane (67.4 ppm)] δ 55.5 (C_2, $^1J_{CD} \sim {}^2J_{CF}$ = 21 Hz), 82.9 (C_3, $^1J_{CF}$ = 169 Hz), 171.2 (C_1, $^3J_{CF}$ = 6.4 Hz).

Methyl (S)-Serinate Hydrochloride. (S)-Serine (99.7 g, 0.949 mol) was suspended in sieve-dried methanol (1000 mL, 791 g, 24.7 mol) in a 2-L three-neck flask equipped with a mechanical stirrer and gas inlet tube. HCl gas was bubbled into the slurry until a solution was obtained. The solution was allowed to cool to room temperature and stand for 40 h, at which time the solvent was removed in vacuo. The resulting solid was triturated with ether (300 mL), filtered, washed with ether (2 × 100 mL), and vacuum-dried (50 °C) to yield 146 g (98%) of methyl (S)-serinate hydrochloride [mp 159–162 °C (lit. mp 163–165 °C [*Beilsteins Handboch der Organische Chemie*; Springer-Verlag: Heidelberg, Vol. 4, p 506])].

Methyl Benzimidate Hydrochloride (5). In a 1-L three-neck flask equipped with a gas inlet tube and mechanical stirrer were placed benzonitrile (66.4 mL, 0.65 mol), methanol (31.6 mL, 0.78 mol), and methylene chloride (16 mL). HCl gas (~36 g, 1 mol) was bubbled into the solution while a temperature of 15–25 °C was maintained. After 3 h an additional 16 mL of CH_2Cl_2 was added to the thick suspension. After a total of 5 h the solvents

(22) The aliquot was diluted 1:5 with acetonitrile and titrated. The previously diluted oxazoline–CH_3CN solution was rediluted 100× with hexane and assayed by HPLC using a Waters Microporasil column: eluant 90% hexane–10% ethyl acetate; 3 mL/min; 254 nm; t_r 7.1 min. The charge of bromocamphorsulfonic acid was 0.5 equiv on the basis of the HPLC assay.

(23) At this point 1 g of salt was broken by partitioning between 1 M NH_3 (aqueous) and CH_2Cl_2. The organic layer was dried with sodium sulfate and taken to dryness. The oxazoline was assayed for deuterium content by NMR (250 MHz) and for optical purity on a L-phenylglycine Pirkle column, 5% isopropyl alcohol in hexane, 2 mL/min, 254 nm, 0.5 mg/mL. D content = 95.4%; R/S ratio = 99.5/0.5.

(24) The aqueous layer contained the bromocamphorsulfonic acid as its NH_4 salt. After removal of dissolved NH_3 in vacuo, this solution was ready for recycling on Dowex 50 × 4.

(25) Optical rotations at 365 and 405 nm were measured in copper sulfate/acetic acid/sodium acetate buffer solutions (4.0 g anhydrous NaOAc, 10.0 mL glacial HOAc, 13.5 g of $CuSO_4$, diluted to 100 mL with H_2O). For comparison, L-serine (natural) showed $[\alpha]^{25}_{405}$ −126.1° (c 1), $[\alpha]^{25}_{365}$ −203.7° (c 1).

and any excess HCl were removed in vacuo, and the solid residue was treated with ether (850 mL). After the mixture was stirred for 30 min at room temperature the crystalline imidate **5** was filtered, washed with ether, and vacuum-dried at 25 °C to yield 100.2 g (90%). GC assay on the corresponding imino ether[18] indicated 99.2% purity (0.2% benzonitrile, 0.4% benzamide).

Methyl (*S*)-2-Phenyl-2-oxazoline-4-carboxylate (3A). In a 250-mL three-neck flask under N_2 were placed methylbenzimidate hydrochloride (**5**) (11.6 g 74.5 mmol), methyl (*S*)-serinate hydrochloride (12.8, 74.5 mmol), and CH_2Cl_2 (150 mL). The stirred mixture was treated with Et_3N (10.4 mL, 74.5 mmol) and stirred overnight at ambient temperature. The resulting suspension was diluted with CH_2Cl_2 (150 mL) and washed with H_2O (3 × 50 mL). The organic layer was dried ($MgSO_4$) and vacuum concentrated to an oil, which was Kugelrohr distilled [95 °C (0.05 mm)] to produce **3a** as a low-melting solid (9.74 g, 65%).

Methyl (*R,S*)-2-Phenyl-2-oxazoline-4-*d*-4-carboxylate [(*RS*)-4A]. (By Statistical Exchange.) Oxazoline **3A** (6.5 g, 31.7 mmol) was dissolved in 99% CH_3OD (20 mL) at 15–20 °C and treated with 50 mg of sodium methoxide. After the mixture was stirred for 2 h the partially exchanged CH_3OD was removed by vacuum distillation and replaced with fresh 99% CH_3OD (20 mL). After 2 h the $NaOCH_3$ was quenched by adding CH_3CO_2D (1.0 mL) and the mixture concentrated to an oil. The residue was Kugelrohr distilled [105 °C (0.05 mm)] to produce 6.1 g (94%) of >98% deuteriated, racemic oxazoline **4A**: ^1H NMR ($CDCl_3$) δ 3.81 (3 H, S), 4.6 (2 H, center of AB, ^2J = 9 Hz), 7.4 (2 H, m, meta), 7.5 (1 H, m, para), 7.98 (2 H, m, ortho).

(*RS*)-Serine-2-*d*. Deuteriooxazoline **4a** (6.1 g, 29.6 mmol) was refluxed for 2.5 h in 15 mL of 6 N HCl. After cooling to room temperature the crystalline benzoic acid was removed by washing with CH_2Cl_2 (2 × 25 mL). The aqueous layer was vacuum concentrated to a small volume, treated with H_2O (25 mL), and concentrated to ca. 8 mL. The resulting solution was diluted to 15 mL with H_2O and the pH adjusted to 5.1 with Et_3N. The free amino acid was crystallized by adding 75 mL of isopropyl alcohol. The product was filtered, washed with isopropyl alcohol (25 mL) and hexane (25 mL), and vacuum-dried to yield 2.83 g (90%) of >99% deuteriated, racemic serine (mp 233–234 °C), HPLC purity ≥98%.[26]

Registry No. **1**, 35523-45-6; **2**, 103292-62-2; **3a**, 78715-83-0; **3c**, 103292-60-0; (±)-**4a**, 103292-63-3; (±)-**4c**, 103292-61-1; (*R*)-**4c**, 103365-57-7; **5**, 5873-90-5; **10**, 103420-05-9; H-DL-Ser-OH, 302-84-1; H-DL-Ser-OPr-*i*·HCl, 103292-59-7; PhCN, 100-47-0; Ph_3CH, 519-73-3; Ph_3CLi, 733-90-4; H-L-Ser-OH, 56-45-1; H-L-Ser-OMe·HCl, 5680-80-8; DL-Serine-2-*d*, 53170-89-1; (+)-ammonium bromocamphorsulfonate, 14575-84-9; (+)-α-bromocamphorsulfonic acid, 5344-58-1.

(26) Amino acid HPLC analysis was run on an E. Merck RP-18 column under ion-pairing conditions: elution with aqueous sodium heptanesulfonate adjusted to pH 2.2; 210 nm; 1.5 mL/min. Serine, t_r 5.0 min; fluoralanine, t_r 4.1 min.

Three New Diterpene Isonitriles from a Palauan Sponge of the Genus *Halichondria*

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Received January 26, 1987

Three new diterpenes (3*S**,4*R**,7*S**,8*R**,11*S**,12*R**,13*S**)-7-isocyano-1-cycloamphilectene (**1**), (1*S**,3*S**,4*R**,7*S**,8*R**,13*R**)-7-isocyano-11-cycloamphilectene (**2**), and 8-isocyano-10,14-amphilectadiene (**3**) were isolated from the Palauan sponge *Halichondria sp.* The major metabolite of this sponge, (3*S**,4*R**,7*S**,8*S**,11*S**,13*R**)-8-isocyano-1(12)-cycloamphilectene (**4**), had previously been isolated from a sponge of the genus *Amphimedon* (ex. *Adocia*) and had been assigned an incorrect structure **5**. The structures of **1**, **2**, and **7**, the formamide derived from isonitrile **4**, were all determined by X-ray analysis.

Isonitriles are rare in nature, yet they are frequently found in sponges of the order Halichondrida[1] and in the dorsal mantle of nudibranchs that eat Halichondrid sponges.[2] Diterpene isonitriles have been found in *Amphimedon sp.* (ex. *Adocia sp.*),[3] *Hymeniacidon amphilecta*,[4] and *Acanthella sp.*[5] The Palauan sponge *Halichondria sp.* has now been found to contain four diterpene isonitriles, one which had physical and spectral properties identical with a compound previously reported by Kazlauskas et al.[3b] In this paper, we report the structural elucidation of 7-isocyano-1-cycloamphilectene (**1**), 7-isocyano-11-cycloamphilectene (**2**), 8-isocyano-10,14-amphilectadiene (**3**), and 8-isocyano-1(12)-cycloamphilectene (**4**), a compound that was previously assigned the structure 8-isocyano-11-cycloamphilectene (**5**). 7-Isocyano-1-cycloamphilectene (**1**) and 7-isocyano-11-cycloamphilectene (**2**) are unique among the diterpene isonitriles because they have cis-fused ring junctions.

Sequential solvent extraction of the freeze-dried sponge yielded a hexane extract that inhibited the growth of the microorganisms *Staphylococcus aureus* and *Bacillus subtilis*. Two antimicrobial fractions from flash chromatography on silica gel were further purified by HPLC on Partisil to obtain 7-isocyano-1-cycloamphilectene (**1**; 0.028% dry weight), 7-isocyano-11-cycloamphilectene (**2**; 0.018% dry weight), 8-isocyano-10,14-amphilectadiene (**3**; 0.006% dry weight), and 8-isocyano-1(12)-cyclo-

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