Pergamon

Tetrahedron 54 (1998) 15879–15890

TETRAHEDRON

Combinatorial Chemistry of Natural Products: Solid Phase Synthesis of D- and L-Cycloserine Derivatives

Mikhail F. Gordeev,* Gary W. Luehr, Hon C. Hui, Eric M. Gordon, and Dinesh V. Patel

Versicor Inc., 34790 Ardentech Ct., Fremont, CA 94555, U.S.A.

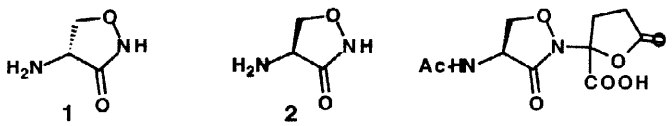
Received 23 September 1998; revised 29 September 1998; accepted 20 October 1998

Abstract: An efficient methodology for a solid phase synthesis of D- and L-cycloserine derivatives is described. Fmoc-D-cycloserine **4** and its L-enantiomer **5** prepared by a selective amine acylation of *bis*-silylated parent compounds are immobilized on Sasrin or 2-chlorotrityl linker resins using Mitsunobu-type reaction or direct tritylation, respectively. The resulting Fmoc-cycloserine resins **7, 10,** and **11** are deprotected with piperidine in DMF or DCM to generate immobilized cycloserine reagents with a primary amino group exposed for various synthetic transformations. An example of the parallel D-cycloserine library synthesis on a reaction plate is described.

Combinatorial chemistry has been well recognized as an important drug discovery tool. An ongoing trend is to integrate the combinatorial approach with fundamentals of medicinal chemistry and rational drug design. Popular synthetic targets include peptidomimetic scaffolds, mechanism- or structure-based pharmacophoric structures, *de novo* designed scaffolds, and natural product derivatives.[1] While historical precedence provides an ample number of drug examples derived from natural products, the latter has received relatively little attention from combinatorial chemists, mainly because of the degree of synthetic complexity associated with such molecules. Several reported sudies include solid phase syntheses (SPS) of carbohydrates,[2] *Rauvolfia* alkaloid derivatives,[3] epothilones[4] and taxoids,[5] and prostaglandin[6] derivatives. Indeed, combinatorial chemistry of natural products often presents serious difficulties, such as accessibility of natural product building blocks, chemical selectivity in a mulitifunctional molecular environment, stability of intermediates and target products under immobilization and cleavage conditions, etc. In this communication, we wish to report the first SPS of D- and L-cycloserine derivatives developed as part of our combinatorial chemistry aided drug discovery program.

D-Cycloserine [(*R*)-4-aminoisoxazolidine-3-one] **1** is a natural product isolated from fermentation broths of *Streptomyces orchidaceus, Streptomyces garyphalus*, and *Streptomyces lavendulus*.[7] The compound has received attention mainly as a broad spectrum antibiotic, which inhibits the bacterial cell wall biosynthesis *via*

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00997-1

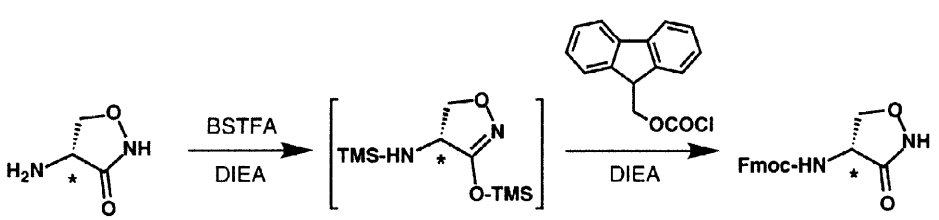


two mechanisms involving L-alanine racemase and D-alanyl-D-alanine synthetase inhibition.[8] Its enantiomeric L-cycloserine structure **2** is featured in the natural product lactivicin **3** isolated from *Empedobacter lactamgenus* and *Lysobacter albus* culture filtrates.[9] Lactivicin is the first non-β-lactam PBP-binding broad-spectrum antibacterial agent with a mode of action similar to that of β-lactams.[10] Despite these findings, a very limited number of both D- and L-cycloserine derivatives has been reported to date.[11] The aforementioned occurrence of antibacterial compounds in the D- and L-cycloserine series has prompted the present studies toward SPS of these natural product derivatives as part of our anti-infective drug discovery program.

Since both D- and L-cycloserine are commercially available compounds, "the scaffold decoration" strategy has been selected over "a total synthesis" *en route* to SPS of respective derivatives.[12] Thus, our study commenced with the design and synthesis of protected cycloserine building blocks compatible with immobilization, various synthetic transformations on a solid phase, and the release of products from a suitable polymeric support.

Due to the labile nature of the isoxazolidine-3-one heterocycle,[13] which can be also viewed as an activated amide by virtue of an electronegative oxygen substituent on the amide nitrogen,[14] the base-sensitive 9-fluorenylmethoxycarbonyl (Fmoc) group was chosen to protect the exocyclic amino group of D- and L-cycloserine as shown in Scheme 1.[15] Preliminary results indicated that mixtures of endo- and exocyclic N-acylated products were formed under Schotten-Baumann–type conditions (Fmoc-Cl or Fmoc-OSu acylating reagents in the presence of pyridine). After some experimentation, high yields of the desired products **4** and **5** were obtained on multigram scale when cycloserines **1** or **2** were silylated prior to acylation to protect *in situ* the reactive endocyclic nitrogen as *bis*-silylated intermediates **3** (Scheme 1).

Scheme 1



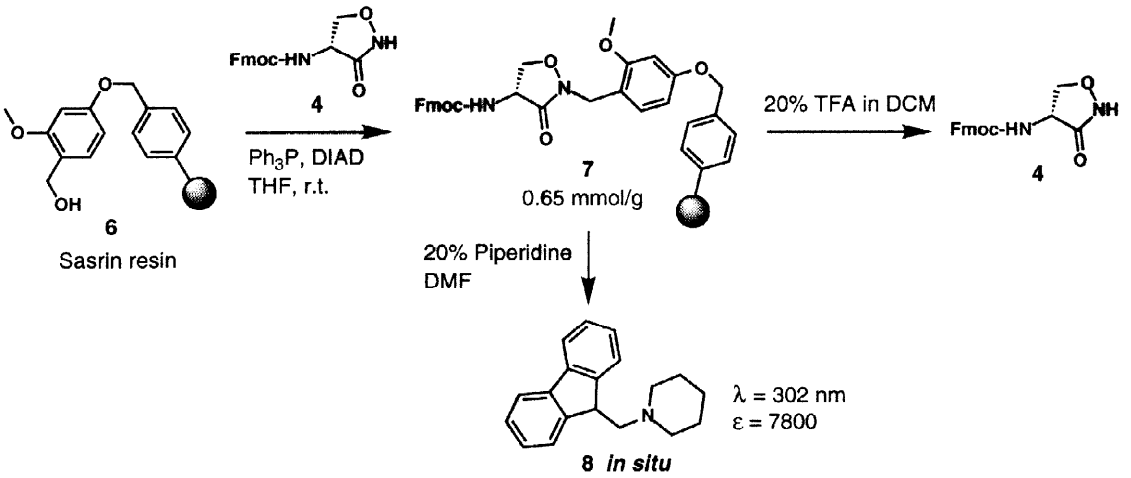
1 R-isomer
2 S-isomer

3

4 R-isomer $[\alpha]^{20}$ +18.8°
5 S-isomer $[\alpha]^{20}$ -18.9°

Next, conditions for immobilization and cleavage of cycloserines were studied using Fmoc-D-cycloserine **4** as a model compound. Superacid-labile Sasrin[16] and 2-chlorotrityl[17] linkers were selected to allow for a subsequent cleavage of the acid-sensitive cycloserine derivatives under mild acidolytic conditions. Thus, Fmoc-D-cycloserine **4** has been successfully immobilized on the alcohol Sasrin resin employing a Mitsunobu-type reaction with triphenylphosphine and diisopropylazodicarboxylate (DIAD) as shown in Scheme 2. High reaction efficiency is noteworthy: loading of the resulting Fmoc-D-cycloserine functionalized Sasrin resin has been determined by spectrophotometry of the *in situ*-generated piperidine–dibenzofulvene adduct **8** after Fmoc-group

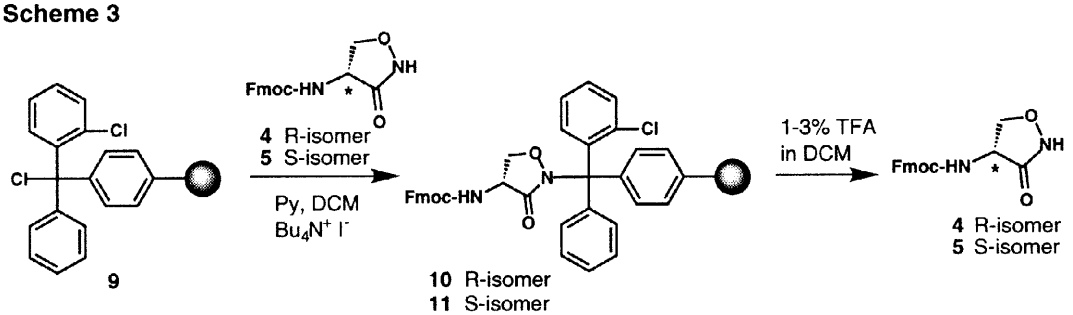
Scheme 2



deprotection[21] at 0.65 mmol/g (Scheme 2; yield of *ca.* 80% starting from the Sasrin resin). Finally, smooth cleavage of Fmoc-D-cycloserine **4** from the resin **7** was achieved with 20% TFA in DCM over *ca.* 2 h. It should be noted that incomplete cleavage with dilute TFA or for reduced reaction times was observed. Enhanced acidolytic stability of the resin **7** as compared to Sasrin esters which are typically cleavable with 1% TFA in DCM over 20-30 min reaction course[16] is in agreement with the alkylated amide structure for this intermediate **7**.[22] HPLC purity (220 nm) of the crude compound **4** thus obtained was 85%. Cleavage efficiency was approximately 94% as estimated spectrophotometrically after TFA removal followed by addition of 20% piperidine in DMF to the crude cleaved product **4** to generate the dibenzofulvene-piperidine adduct[21] (λ = 302 nm, ε = 7800; *cf.* Scheme 2).

Next, immobilization and cleavage of Fmoc-D-cycloserine **4** was studied with the more acid-labile 2-chlorotrityl linker functionalized polystyrene as shown in Scheme 3.[13] After some experimentation, we found that efficient immobilization of compound **4** on 2-chlorotrityl resin **10** could be achieved when the alkylation was performed in the presence of tetrabutylammonium iodide in a mixture of pyridine and DCM.

Scheme 3



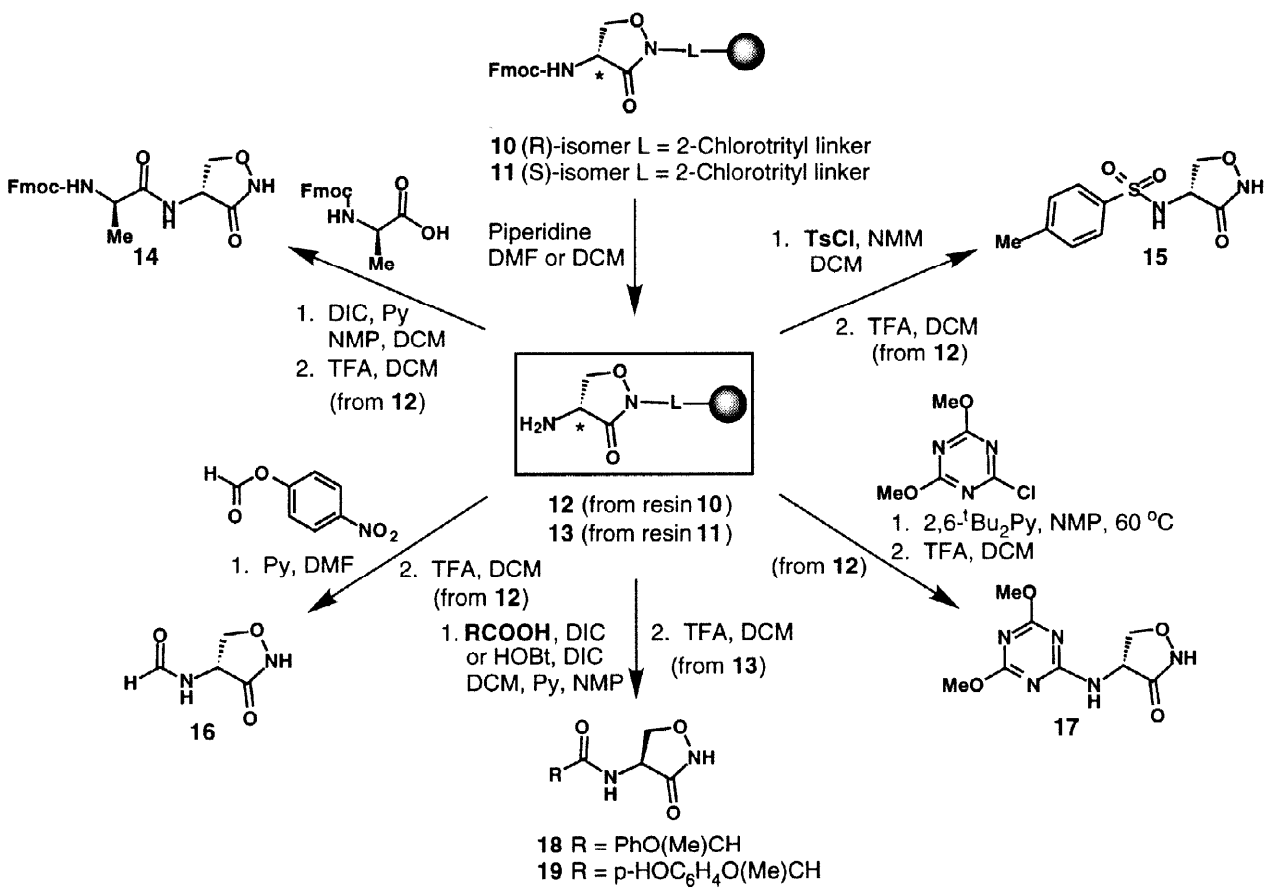
Loading of the tethered intermediate **10** thus obtained was estimated at *ca.* 0.59 mmol/g by spectrophotometry after Fmoc-deprotection (*ca.* 60% chemical efficiency). As expected, compound **4** could be easily cleaved from the respective 2-chlorotrityl linker resins **10** or **11** with dilute TFA solutions (1-3% TFA in DCM, 1-2 h). Thus, cleavage efficiency for the resin **10** using 1% TFA in DCM over 2 h has been estimated at *ca.* 94%, and HPLC purity (220 nm) of the crude product **4** was 91%. An analogous sequence of

transformations was performed using Fmoc-L-cycloserine **5** to afford the respective resin **11.** Essentially identical cleavage results were obtained for both enantiomeric materials **10** and **11**.

The two immobilization strategies employing a chemically more robust Sasrin-type linker (Scheme 2) and a highly acid-labile 2-chlorotrityl resin (Scheme 3) are complementary and can be applied as required by the desired connnection chemistries.[23] Notably, successful loading of Fmoc-D-cycloserine **4** on the alcohol linker using Mitsunobu reaction opens up access to N^2-alkylated cycloserine derivatives with potential for PBP binding properties (*cf.* with the antimicrobial compound lactivicin **3**). The 2-chlorotrityl linker resin has been chosen for subsequent library synthesis due to the particularly facile cleavage of the final products from this super-acid labile resin.

Having established a SP methodology for immobilization and cleavage of cycloserine derivatives, we next rehearsed a series of synthetic transformations using the new immobilized cycloserine reagents **10** and **11** as shown in Scheme 4. The transformations commence with Fmoc-deprotection on a solid support by treatment with piperidine in DMF or DCM. Interestingly, it has been observed that a freshly prepared solution of piperidine in DCM is best suited for the deprotection of 2-chlorotrityl resin–tethered cycloserines **10** and **11.** In these cases, incomplete Fmoc-deprotection persisted when 20% piperidine in DMF was employed under standard reaction conditions (30 min, r.t.). This fact may be accounted for by improved swelling characteristics of DCM in

Scheme 4

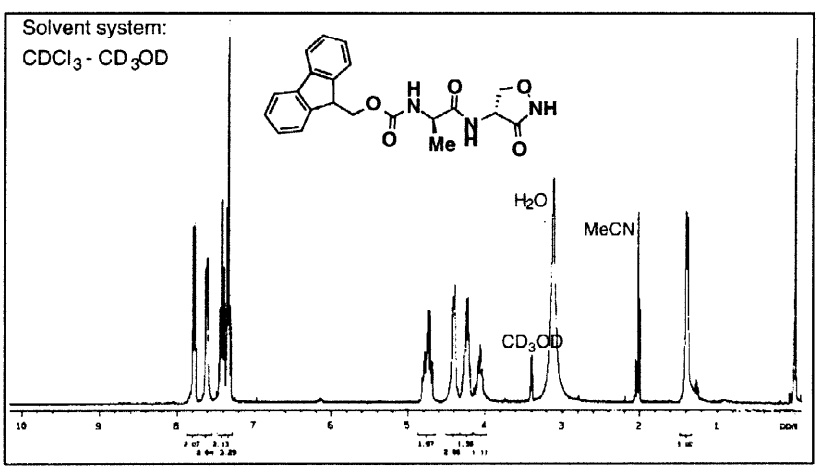


comparison with DMF resulting in better access of the deprotection reagent to internal reaction sites of the resin beads.

As clear from the Scheme 4, our synthetic strategy for preparation of diverse D- and L-cycloserine derivatives is based on endocyclic N immobilization of the cycloserine pharmacophore on polymeric supports with exposure of the primary amino group to selected chemical reactions. Thus, the linker serves to both immobilize the key cycloserine reagents on a resin, and to protect the sensitive cyclic hydroxamate ether functionality during chemical transformations. Several types of electrophilic capping reagents have been successfully employed to afford the respective D- and L-cycloserine derivatives **14-19**: carboxylic (**18**, **19**) and amino acid (**14**) anhydrides, sulfonyl chloride (**15**), activated formate (**16**), and an activated heterocyclic chloride (**17**). HPLC purity (220 nm) for most of the crude products was in a range of 80-90%, and the isolated yield for purified compounds was 50-80% (see Experimental).

A clean SP transformation employing immobilized D-cycloserine **10** is illustrated by the ^{1}H NMR spectrum for the crude product **14** cleaved from the 2-chlorotrityl resin shown in Fig. 1 below. In agreement with HPLC data, little or no racemization has been detected. Compounds **18** and **19** synthesized from racemic acid building blocks were isolated as mixtures of two diastereomers in each case (see Experimental).

Figure 1. ^{1}H NMR data for the crude product **14** cleaved from 2-chlorotrityl linker resin.

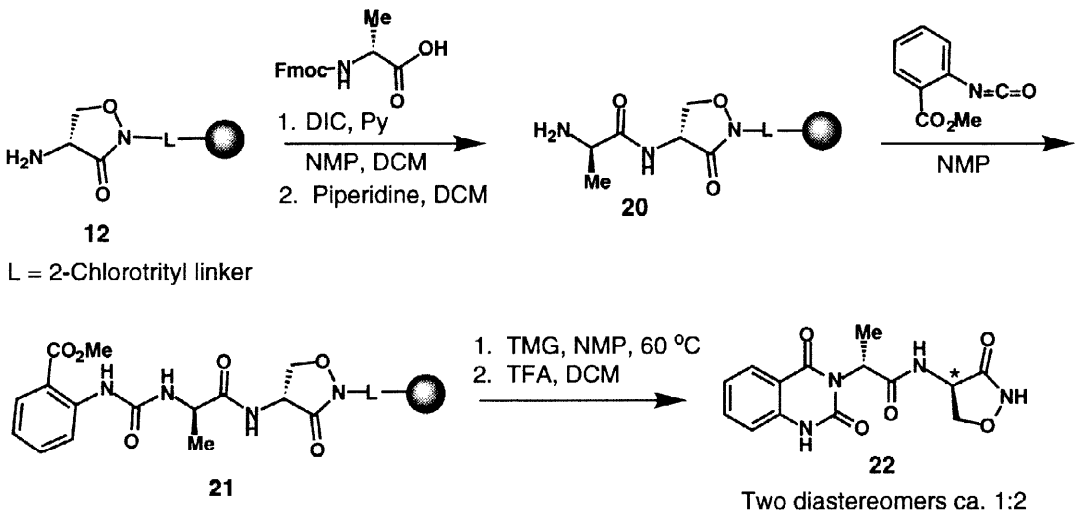


The methodology is general and can be extended to construction of peptidomimetic heterocyclic cycloserine derivatives exemplified by SPS of quinazolinedione derivatives shown in Scheme 5 below. In this case, the product **22** has been isolated after preparative TLC as a mixture of two diastereomers in (*ca.* 1:2).[24]

Finally, the methodology has been applied to the SPS of cycloserine libraries. As an example, Fmoc-protected D-cycloserine resin **10** was distributed into 80 wells of the reaction plate, deprotected, and coupled separately with 10 individual amino acids (Scheme 6; 1 column/8 wells of the plate per each amino acid). Resulting immobilized intermediates **23** were deprotected (piperidine in DCM), and each of the 8 plate rows was coupled in parallel fashion with one of 8 individual amino acids to produce the total of 80 discrete resins **24** (10 x 8 = 80) Double coupling with disopropylcarbodiimide–activated acids was employed to complete the

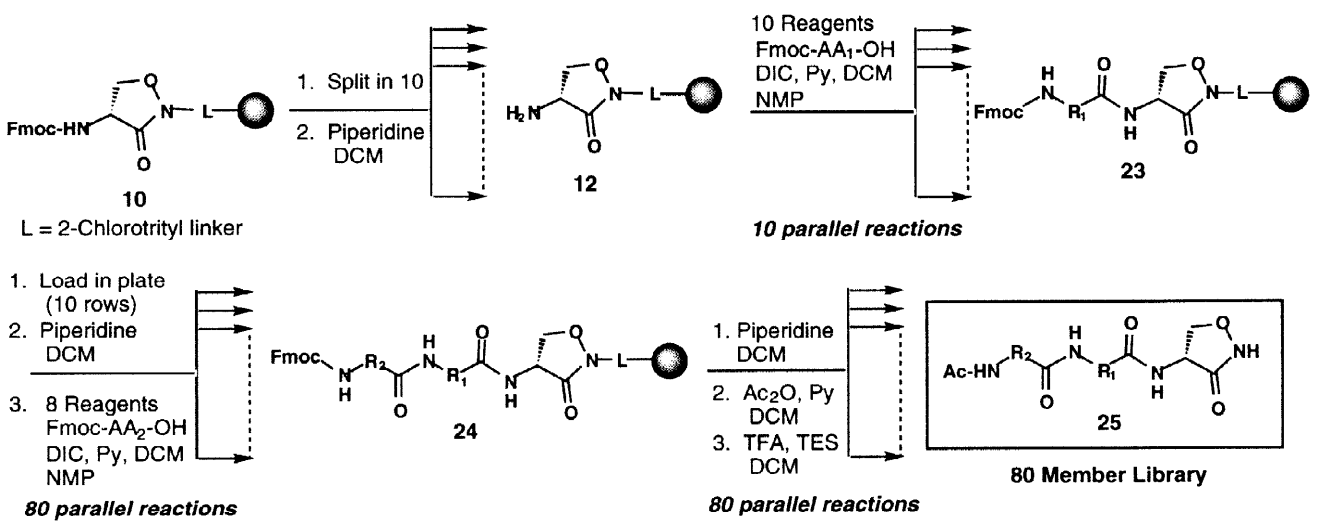
transformations (as judged by negative Kaiser test indicating the absence of the free amine). This was followed in the next round by Fmoc-deprotection with piperidine in DMF, capping with acetic anhydride, and TFA cleavage to generate a library of 80 dipeptidic D-cycloserine derivatives **25**. t-Butyl ester (for glutamic acid and serine derivatives) and Boc (for L-lysine) side chains were further deprotected by treatment with TFA in DCM containing triethylsilane (see also Experimental).

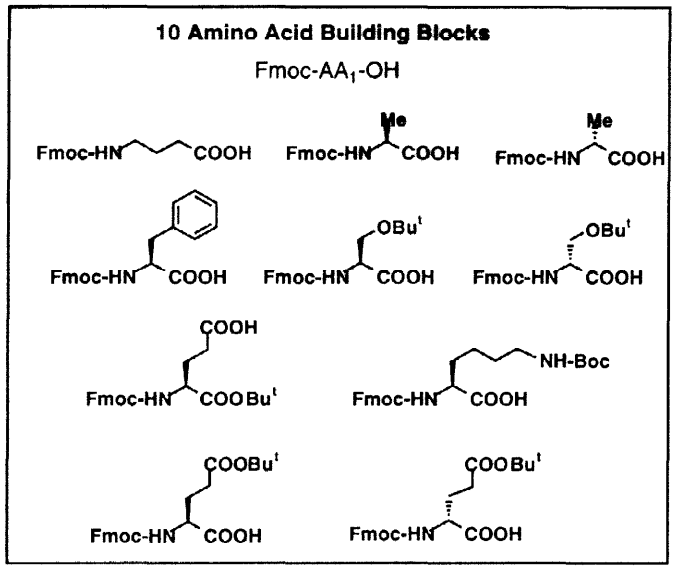
Scheme 5

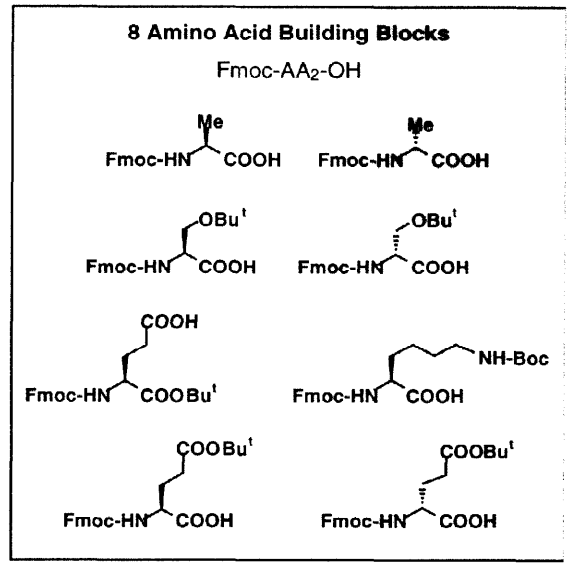


All library members were analyzed by ESI MS, and >80% of the compounds showed the expected molecular ions. Average HPLC purity for UV-active compounds (L-phenylalanine derivatives; 220 nm) was 76%, in agreement with ^{1}H NMR analysis for selected library members. This and other cycloserine libraries prepared using the new methodology are currently being screened for bacterial enzyme inhibitory and antimicrobial activity, and these results will be reported in due course.

Scheme 6







In conclusion, a new and efficient solid phase synthesis of D- and L-cycloserine natural products derivatives has been developed. The methodology is general and well suited for the construction of cycloserine pharmacophore libraries.

EXPERIMENTAL

General. All reagents were of the best grade available (Aldrich, Sigma, Bachem Biosciences, and Novabiochem) and used without further purification. 96-Well microtiter plates were from Polyfiltronics. ^1H NMR spectra (300 MHz) were recorded in CD_3OD as solvent unless noted. High resolution mass-spectra were obtained using the FAB technique. Analytical HPLC was performed using YMC C18 4.6 mm x 50 mm reverse phase column, gradient from 100% of 0.1% TFA in water to 100% of 0.1% TFA in MeCN over 6 min (flow rate 2 mL/min; detection at 220 nm). Preparative HPLC was performed using a 5 μm C18 (10 mm x 250 mm) reverse phase column. Preparative TLC was performed using 1mm Merck SiO_2 plates (20 x 20 cm).

General Procedure for Preparation of 4-(9-Fluorenylmethyloxycarbonyl)amino-isoxazolidine-3-ones (4) and (5). N,O-Bis(trimethylsilyl)acetamide (45.0 mL, 0.185 mol) was added in one portion under nitrogen atmosphere to a suspension of D- or L-cycloserine (7.50 g, 0.074 mol) in dry CD_2Cl_2 (70 mL). The mixture was stirred *ca.* 45 min, and pyridine (120 mL, 1.48 mol) was added and the mixture stirred another 15 minutes. N-(9-Fluorenylmethoxycarbonyloxy)succinimide (25.0 g, 0.074 mol) was added portionwise, and the mixture stirred overnight. Solvent was removed *in vacuo*, and residue dissolved in EtOAc (150 mL), washed with dilute aq. HCl (pH *ca.* 2, 3 x 50 mL), and brine. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. Resulting white solid was triturated with 10% EtOAc in hexanes, filtered and dried to afford the respective compound **4** or **5.**

4-(*R*)-(9-Fluorenylmethyloxycarbonyl)aminoisoxazolidine-3-one (4). Prepared from D-cycloserine (7.50 g, 0.074 mol) as described above in the General Procedure. Yield 22.0 g (92%). M.p. 146-148 °C. $[\alpha]^{20}$

+18.8° (c = 0.5, MeOH). ^{1}H NMR in $CDCl_3$: 4.11 (m, 1 H), 4.21 (m, 1 H), 4.43 (d, J = 7.0 Hz, 2 H), 4.66 (m, 1 H), 4.81 (m, 1 H), 5.43 (m, 1 H), 6.00-6.30 (br. s, 1 H), 7.26-7.43 (m, 4 H), 7.58 (d, J = 7.4 Hz, 2 H), 7.77 (d, J = 7.4 Hz, 2 H). MS (m/z): 325 $[M+H]^+$.

4-(*S*)-(9-Fluorenylmethyloxycarbonyl)aminoisoxazolidine-3-one (5). Prepared from L-cycloserine (5.00 g, 0.049 mmol) as described above in the General Procedure. Yield 15.8 g (99%). M.p. 144-146 °C. $[\alpha]^{20}$ -18.9° (c = 0.5, MeOH). ^{1}H NMR in $(CD_3)_2SO$: 3.97 (m, 1 H), 4.26 (m, 1 H), 4.35 (d, J = 6.6 Hz, 2 H), 4.45-4.62 (m, 2 H), 7.30-7.53 (m, 4 H), 7.71 (d, J = 7.5 Hz, 2 H), 7.76-7.94 (m, 3 H), 11.49 (s, 1 H). MS (m/z): 325 $[M+H]^+$.

Preparation of Sasrin Resin-Immobilized Fmoc-D-Cycloserine (7). Diisopropylazodicarboxylate (0.45 mL, 2.30 mmol) was added dropwise with stirring to Fmoc-D-Cycloserine **4** (0.745 g, 2.30 mmol) and triphenylphosphine (0.601 g, 2.30 mmol) in THF (7.5 mL) under nitrogen atmosphere at 0 °C. The mixture was allowed to warm to r.t., and stirred for 24 h. Resulted resin **7** was filtered, washed liberally with CH_2Cl_2, MeOH, and acetone, and dried *in vacuo*.

Preparation of 2-Chlorotrityl Resin–Immobilized Fmoc-Cycloserine Reagents (10) and (11). Fmoc-D-Cycloserine **4** or Fmoc-L-Cycloserine (0.502 g, 1.55 mmol), 2-chlorotritylchloride resin (0.50 g, 0.515 mmol), tetrabutylammonium iodide (0.114 g, 0.309 mmol) in DCM (40 ml) with and pyridine (40 ml) were agitated at room temperature for 48 h. The resulting resin **12** or **13** was filtered, washed liberally with CH_2Cl_2, MeOH, and acetone, and dried *in vacuo*.

4-(*R*)-[N-(9-Fluorenylmethyloxycarbonyl)-D-alanine]aminoisoxazolidine-3-one (15).

A. From 2-chlorotrityl resin (10). Fmoc-D-cycloserine resin **10** (0.100 g, 0.059 mmol) was deprotected with 20% piperidine in CH_2Cl_2 (2.0 mL, 1.5 h), resulting in amine resin **13** filtered, washed liberally with CH_2Cl_2 and MeOH, and dried *in vacuo*. Diisopropylcarbodiimide (0.055 mL, 0.354 mmol) was addded to Fmoc-L-Ala-OH (0.184 g, 0.59 mmol) in a solution of N-methylpyrrolidine-2-one–CH_2Cl_2 1:6 (2.0 mL), and the mixture stirred for 1 h. Pyridine (0.095 mL, 1.18 mmol) was added, and the mixture transferred to the resin **13**. The reaction mixture was agitated overnight at r.t., and the resulting was resin filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1 h. Acetonitrile (7 mL) was added, the supernatant evaporated *in vacuo*, and the crude product purified by preparative HPLC to afford compound **15** as a glassy solid (21.0 mg, 90%). R_t 4.4 min. ^{1}H NMR in $CDCl_3$: 1.34 (d, J = 7.0 Hz, 3 H), 4.01 (m, 1 H), 4.17 (m, 2 H), 4.35 (d, J = 6.9 Hz, 2 H), 4.60-4.68 (m, 2 H), 7.23-7.39 (m, 4 H), 7.54 (d, J = 7.4 Hz, 2 H), 7.72 (d, J = 7.4 Hz, 2 H). MS (m/z): 396 $[M+H]^+$.

B. From resin (7). Following the procedure described above for reaction with resin **10** (except that 20% TFA in CH_2Cl_2 was employed in cleavage step), Fmoc-D-cycloserine resin **7** (0.100 g, 0.065 mmol) yielded 17.0 mg (65%) of the product **15**.

4-(*R*)-(*p*-Toluenesulfonamido)isoxazolidine-3-one (16). Fmoc-D-cycloserine resin **10** (0.100 g, 0.059 mmol) was deprotected as described above for preparation of compound **15**. Tosyl chloride (0.078 g, 0.41 mmol) in 10% N-methylmorpholine in CH_2Cl_2 (2.0 mL) was added to the amine resin **13**, and the mixture agitated for 3 h. The resulting resin was filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1 h. Acetonitrile (7 mL) was added, supernatant evaporated *in vacuo*, and the crude product purified by preparative TLC (eluent

CH_2Cl_2–MeOH 7:1) to afford compound **16** as a glassy solid (6.8 mg, 45%). R_t 3.3 min. ^1H NMR in $CDCl_3$: 2.45 (s, 3 H), 4.04-4.25 (m, 2 H), 4.69 (m, 1 H), 5.53 (br. s, 1 H), 7.35 (d, J = 8.1 Hz, 2 H), 7.78 (d, J = 8.1 Hz, 2 H). MS (m/z): 257 $[M+H]^+$.

4-(*R*)-Formamidoisoxazolidine-3-one (17). Fmoc-D-cycloserine resin **10** (0.200 g, 0.118 mmol) has been deprotected as described above for preparation of the compound **15**. Solution of *para*-nitrophenyl formate (0.118 g, 0.708 mmol) in 10% Pyridine in DMF (4.0 mL) was added to the amine resin **13**, and the mixture agitated at r.t. for 16 h. Resulted resin was filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid in CH_2Cl_2 (4.0 mL) was added, and the mixture agitated for 1 h. Acetonitrile (10 mL) was added, supernatant evaporated *in vacuo*, and the crude product was purified by preparative TLC (eluent CH_2Cl_2–MeOH 6:1) to afford the compound **17** as a white solid (7.7 mg, 50%). ^1H NMR: 4.09 (m, 1 H), 4.64 (m, 1 H), 4.95 (m, 1 H), 8.18 (s, 1 H). MS (m/z): 131 $[M+H]^+$.

4-(*R*)-(4,5-Dimethoxytriazine-2-yl)aminoisoxazolidine-3-one (18). Fmoc-D-cycloserine resin **10** (0.100 g, 0.059 mmol) has been deprotected as described above for preparation of the compound **15**. Solution of 2-chloro-4,5-dimethoxytriazine (0.103 g, 0.59 mmol) in N-methylpyrrolidine-2-one (1 mL) and 2,6-di(t-butyl)pyridine (0.25 mL, 1.5 mmol) was added to the amine resin **13**, and the mixture agitated for 11 h at 60 °C. The resulting resin was filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1 h. Acetonitrile (7 mL) was added, supernatant evaporated *in vacuo*, and the crude product purified by preparative TLC (eluent CH_2Cl_2–MeOH 7:1) to afford compound **18** as a white solid (7.7 mg, 54%). R_t 2.8 min. ^1H NMR: 3.94 (s, 3 H), 3.96 (s, 3 H), 4.19 (m, 1 H), 4.79 (m, 1 H), 5.01 (m, 1 H). MS (m/z): 242 $[M+H]^+$.

4-(*S*)-[(2-Phenoxypropionyl)amino]isoxazolidine-3-one (19). Fmoc-L-cycloserine resin **11** (0.100 g, 0.059 mmol) was deprotected with 20% piperidine in CH_2Cl_2 (2.0 mL, 1.5 h), and the resulting amine resin **14** was filtered, washed liberally with CH_2Cl_2 and MeOH, and dried *in vacuo*. Diisopropylcarbodiimide (0.055 mL, 0.354 mmol) was addded to 2-phenoxypropionic acid (0.098 g, 0.59 mmol) in a solution of dimethylformamide–CH_2Cl_2 3:1 (2.0 mL), and the mixture was stirred for 2 h. Pyridine (0.095 mL, 1.18 mmol) was added, and the mixture transferred to the resin **14**. The reaction mixture was agitated overnight at r.t., and the resulting resin filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid and 2% triisopropylsilane in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1.5 h. Supernatant was evaporated *in vacuo* and the crude product purified by preparative HPLC to afford the compound **19** as a white solid (7.4 mg, 50%; mixture of two diastereomers 1:1). R_t 2.8 min. ^1H NMR: 1.54 (d, J = 6.6 Hz, 3 H, diastereomer A), 1.56 (d, J = 6.0 Hz, 3 H, diastereomer B), 4.03 (m, 1 H, diastereomer A or B), 4.17 (m, 1 H, diastereomer B or A), 4.57 (m, 1 H), 4.74 (m, 1 H), 4.94 (m, 1 H), 6.90-7.00 (m, 2 H), 7.25-7.30 (m, 2 H). MS (m/z): 251 $[M+H]^+$.

4-(*S*)-[2-(4-Hydroxyphenoxypropionyl]amino]isoxazolidine-3-one (20). *t*-Butyl-dimethylsilyl chloride (6.21 g, 0.041 mol) in THF (10 mL) was added dropwise with stirring at 0 °C to a solution of 2-(4-hydroxyphenoxy)propionic acid (2.50 g, 0.014 mol) and triethylamine (5.74 mL, 0.041 mol) in THF (50 mL). The mixture was allowed to warm to r.t. and stirred overnight. Water (100 mL) was added, and the mixture extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried ($MgSO_4$), and solvent removed *in vacuo*. MeOH and water 1.5:1 (90mL) were added, followed by K_2CO_3 (6.06 g, 0.0438 mol), and the mixture

stirred for 2 h. MeOH was removed *in vacuo*, EtOAc (25 mL) added, and the mixture acidified to pH *ca.* 6 with 2N HCl. The aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to afford the crude 2-[4-(t-butyl)dimethylsilyloxy]phenoxy]propanoic acid (3.37 g, 83%), which was used directly in the next step. The crude silylated acid (0.175 g, 0.590 mmol) was preactivated for 1 h with diisopropylcarbodiimide (0.092 mL, 0.590 mmol) and 1-hydroxybenzotriazole (0.0797 g, 0.590 mmol) in N-methylpyrrolidine-2-one–CH_2Cl_2 3:1 (2.0 mL). Pyridine (0.048 mL, 0.59 mmol) was added, and the mixture agitated with the amine resin **11** prepared from Fmoc-L-cycloserine resin (0.100 g, 0.059 mmol as described above for the synthesis of compound **19**. The resulting resin was filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid and 2% triisopropylsilane in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1.5 h. The supernatant was evaporated *in vacuo* and 1M tetrabutylammonium fluroride in THF (1.0 mL, *ca.* 1 mmol) added. The mixture was stirred at r.t. for 2 h, the solvent removed *in vacuo*, and the crude product purified by preparative HPLC to afford the compound **21** as a white solid (9.0 mg, 57%; mixture of two diastereomers in a ratio *ca.* 1:1; a partial separation had been achieved during HPLC purification). R_t 2.0 min. Diastereomer A: ^{1}H NMR: 1.51 (d, J = 6.9 Hz, 3 H), 4.04 (m, 1 H), 4.58 (m, 2 H), 4.97 (m, 1 H), 6.70 (d, J = 6.6 Hz, 2 H), 6.82 (d, J = 6.6 Hz, 2 H). MS (m/z): 267 $[M+H]^+$. Diastereomer B: ^{1}H NMR: 1.49 (d, J = 6.6 Hz, 3 H), 4.14 (m, 1 H), 4.60 (m, 2 H), 4.92 (m, 1 H), 6.70 (d, J = 6.6 Hz, 2 H), 6.82 (d, J = 6.6 Hz, 2 H). MS (m/z): 267 $[M+H]^+$.

3-[1-(*R*)-(Isoxazolidine-3-one-4-yl)aminocarbonyl]ethylquinazoline-2,4-dione (22). Fmoc-D-cycloserine resin **10** (0.100 g, 0.059 mmol) was deprotected and then coupled with Fmoc-D-Ala-OH (0.184 g, 0.59 mmol) as described above for the preparation of compound **15**. The resulting resin **21** was deprotected with 20% piperidine in CH_2Cl_2 (2.0 mL, 45 min), washed liberally with MeOH and CH_2Cl_2, and dried *in vacuo*. Solution of *ortho*-methoxycarbonylphenyl isocyanate (0.105 g, 0.59 mmol) in N-methylpyrrolidine-2-one (2 mL) was added, and the mixture agitated overnight (until negative Kaiser test). The resulting urea resin **22** was washed liberally with MeOH and CH_2Cl_2, and dried *in vacuo*. 5% Tetramethylguanidine in N-methylpyrrolidine-2-one (2 mL) was added, and the mixture agitated at 60 ^{0}C for 5 h. to the amine resin **13**, and the mixture agitated for 11 h at 60 °C. The derivatized resin was filtered, washed with CH_2Cl_2 and MeOH, and dried *in vacuo*. A solution of 3% trifluroacetic acid in CH_2Cl_2 (2.0 mL) was added, and the mixture agitated for 1 h. Acetonitrile (7 mL) was added, the supernatant evaporated *in vacuo*, and the crude product purified by preparative TLC (eluent CH_2Cl_2–MeOH 6:1) to afford compound **22** as a glassy solid (6.6 mg, 35%). R_t 3.0 min. ^{1}H NMR: 1.56 (d, J = 7.0 Hz, 3 H, major isomer), 1.63 (d, J = 7.0 Hz, 3 H, minor isomer), 3.77 (m, 1 H, major isomer), 3.92 (m, 1 H, minor isomer), 4.44 (m, 1 H, major isomer), 4.45 (m, 1 H, minor isomer), 4.70-4.87 (m, 1 H), 5.57 (m, 1 H), 7.10-7.25 (m, 2 H), 7.64 (m, 1 H), 8.04 (m, 1 H). MS (m/z): 319 $[M+H]^+$.

Preparation of D-Cycloserine Library (25). Fmoc-D-Cycloserine resin **10** (4.80 g, 3.36 mmol) was suspended in dimethylformamide–CH_2Cl_2 (*ca.* 5:1), and the suspension distributed into 80 wells of the reaction plate (*ca.* 0.06 g, 0.04 mmol of the immobilized reagent per well). 20% Piperidine in CH_2Cl_2 was added (1 mL in each well), and the plate agitated using an orbital shaker for 1.5 h. The reagent was drained, and resin **12** washed liberally with CH_2Cl_2, MeOH, and acetone. Ten selected acids Fmoc-AA_1-OH (3.20 mmol of each reagent, *ca.* 10 equivalents) were separately dissolved in dimethylformamide–CH_2Cl_2 (3:1, 7 mL). Diisopropylcarbodiimide (0.30 mL, 1.92 mmol) and pyridine (0.5 mL) were added to each amino acid, and the

mixtures agitated for 2 h. The resulting solutions of amino acid anhydrides were separated from precipitated urea using disposable filter syringes, and individually distributed (*ca.* 1.1-1.2 mL per well) into 8 respective wells of the reaction plate (1 column for each reagent). The plate was agitated 48 h, washed liberally with dimethylformamide, CH_2Cl_2, MeOH, and acetone, and dried *in vacuo*. Coupling was repeated using fresh, activated acid solutions for wells which tested positive for the presence of the free amine (ninhydrine test). The plate was washed liberally with dimethylformamide, CH_2Cl_2, MeOH, and capped with a mixture of acetic anhydride–pyridine–CH_2Cl_2 (1:1.5:3, 1 mL in each well) for 2 h. The plate was washed as above, and deprotected with 20% piperidine in CH_2Cl_2 (1 mL in each well, 1.5 h). The reagent was drained, and resin washed liberally with CH_2Cl_2, MeOH, and acetone. Next, coupling was repeated as described above, except that 8 amino acid Fmoc-AA_2-OH were employed, and each preactivated reagent individually delivered in 10 respective wells of the plate (1 row for each acid). The plate was washed liberally with CH_2Cl_2, MeOH, and acetone, and dried *in vacuo*. Coupling was repeated with the same set of preactivated reagents Fmoc-AA_2-OH, resins **24** deprotected with 20% piperidine in CH_2Cl_2, and capped with acetic anhydride as described above. The plate was mounted on top of the 96-well microtiter plate, and resins cleaved with 2% triethylsilane in 3% TFA in CH_2Cl_2 added (*ca.* 6 portions of 0.2 mL added in each well over 1.5 h). Solvent was removed at r.t. *in vacuo* using GeneVac. 2% Triethylsilane in 40% TFA in CH_2Cl_2 (*ca.* 0.5 mL) was added to each of the wells containing side-chain protected amino acids, the plate was kept at r.t. for 1.5 h, and the solvent removed *in vacuo* to afford the library **25**. Typical analytical data for representative products are provided below. The crude compounds were analyzed directly from the receptacle polypropylene plate, and ^{1}H NMR signals for minor impurities and residual solvent have been omitted.

N-Acetyl-L-Ala-L-Ala-D-Cycloserine. ^{1}H NMR: 1.35 (d, J = 7 Hz, 3 H), 1.38 (d, J = 6.9 Hz, 3 H), 2.05 (s, 3 H), 4.00-4.15 (m, 2 H), 4.20-4.48 (m, 2 H), 4.58 (m, 1 H). MS (m/z): 287 $[M+H]^+$.

N-Acetyl-D-Glu-L-Phe-D-Cycloserine. ^{1}H NMR: 1.57-1.84 (m, 2 H), 1.85-2.05 (m, 2 H), 2.03 (s, 3 H), 2.85 (m, 2 H), 4.02 (m, 1 H), 4.15 (m, 1 H), 4.52 (m, 1 H), 4,68 (m, 1 H), 5.00 (m, 1 H), 7.18-7.30 (m, 5 H). MS (m/z): 421 $[M+H]^+$.

N-Acetyl-D-Ser-L-Ser-D-Cycloserine. ^{1}H NMR: 2.05 (s, 3 H), 3.80-3.90 (m, 4 H), 4.10 (m, 1 H), 5.00 (m, 1 H). MS (m/z): 319 $[M+H]^+$.

REFERENCES

1. See, *e.g.*, reviews: (a) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385; (b) Gordon, E. M. *Current Opinion in Biotechnology.* **1995**, *6*, 624; (c) Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*; 144; (d) Patel, D. V.; Gordon, E. M. *Drug Discovery Today.* **1996**, 134; (e) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555; (f) Hermkens, P. P. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron.* **1996**, *52*, 4527; (g) Patel, D. V.; Gordon, E. M. *Drug Discovery Today.* **1996**, *1*, 134; (g) Gordeev, M. F.; Patel, D. V. In: *Combinatorial Chemistry and Molecular Diversity in Drug Discovery.* Eds. E. M. Gordon and J. F. Kerwin. **1998**, in press.
2. For an early account, see: (a) Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135. The topic has been recently reviewed in ref. (b) Sofia, M. J. In: *Combinatorial Chemistry and Molecular Diversity in Drug Discovery.* Eds. E. M. Gordon and J. F. Kerwin. **1998**, in press.
3. Atuegbu, A.; Maclean, D.; Nguyen, C.; Gordon, E. M.; Jacobs, J. W. *Bioorg. Med. Chem.* **1996**, *4*, 1097.

4. Nicolaou, K. C.; Winssinger, N.; **Pastor, J.**; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; **Yang, Z.**; Li, T.; Giannakakou, P.; Hamel, E. *Nature (London)* . **1997,** *387,* 268.
5. Xiao, X.-Y.; Parandoosh, Z.; Nova, M. P. *J. Org. Chem.* **1997**, *62,* 6029.
6. Chen, S.; Janda, K. D. W. *J. Am. Chem. Soc.* **1997**, *119*, 8724.
7. De Amici, M.; De Micheli, C. D.; Cateni, F.; Carrea, G.; Ottolina, G. *Tetrahedron: Asymmetry.* **1993, *4,*** 1073.
8. (a) Strominger, J. L.; Threnn, R. H.; Scott, S.S. *J. Am. Chem. Soc.* **1959**, *81,* 3803; (b) Ito, E.; Stromiger, J. L. *J. Biol. Chem.* **1962**, *237*, 2696; (c) Neuhaus, F. C.; Lynch, J. L. Biochemistry, **1964**, *3*, 471; (d) Gale, I. F.; Cuncliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. In: *The Molecular Basis of Antibiotic Action.* John Wiley & Sons, **1981**, p.65.
9. Nozaki, Y.; Katayama, N.; Ono, H.; Tsubotani, S.; Harada, S.; Okazaki, H.; Nakao, Y. *Nature (London)*, **1987**, *325,* 179; *Critical Rev. Biotech.* **1992**, *12*, 225.
10. Nozaki, Y.; Katayama, N.; Harada, S.; Ono, H.; Okazaki, H. *J. Antibiot. (Tokyo)*, **1989**, *42,* 84.
11. SciFinder search in CAS data base (1965 - present) has produced *ca.* 171 and 4246 hits for topics "synthesis of cycloserines" and "synthesis of β-lactams", respectively.
12. Herein, we employ the terms "a scaffold decoration" and "a total synthesis" to describe two different combichem strategies. In the former, the key scaffold building block is produced in solution, immobilized, and then derivatized on a solid phase. In the second approach, the scaffold itself is constructed *via* SPS starting from an appropriate immobilized precursor reagent.
13. Payne, R. A.; Stammer, C. H. *J. Org. Chem.* **1968**, *33*, 2421. Notably, the authors also described a solution phase N-tritylation of the D-cycloserine *ortho*-salicylimine.
14. Baldwin, J. E.; Ng, S. C.; Pratt, A. J. *Tetrahedron Lett.* **1987**, *28*, 4319.
15. Attempts to employ several other amine protection groups, such as benzaldimine and *ortho*-salicylimine functionalities were less satisfactory.
16. Polystyrene-based 2-methoxy-4-alkoxybenzyl alcohol resin: Mergler, M.; Tanner, R.; Gosteli, J.; Grogg, P. *Terahedron Lett.* **1988**, *29*, 4005.
17. Barlos, K, et al. *Tetrahedron Lett.* **1989**, *30*, 3947.
18. Interestingly, while both base-mediated solution phase alkylation of cycloserine derivatives[19] and related solution Mitsunobu reactions with N-acidic compounds[20] have been well documented, no Mitsunobu-type transformation of cycloserine derivatives has been reported.
19. (a) Payne, R. A.; Stammer, C. H. *J. Org. Chem.* **1968**, 33, 2421; (b) Baldwin, J. E.; Ng, S. C.; Pratt, A. J. *Tetrahedron Lett.* **1987**, *28*, 4319.
20. See, *e.g.* a review: Mitsunobu, O. *Synthesis* **1981**, 1.
21. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37,* 3404.
22. *Cf.* a facile cleavage of Sasrin esters[16] with that for acidolytically more stable PAL amide analogs derived from 2,6-dimethoxy-4-alkoxybenzylamine resin: Albericio, F.; Barany, G. *Int. Pept. Protein Res.* **1987**, *30*, 206.
23. A preference for certain types of polymeric supports in specific chemical transformations is well documented: see, *e.g.* (a) Gordeev, M. F.; Patel, D. V.; Gordon, E. M. *J. Org. Chem.* **1997**, *62*, 8177; (b) Czarnik, A. *Biotech. Bioeng. (Combin. Chem.).* **1998**, *61*, 77.
24. A partial epimerization of the D-cycloserine fragment is in agreement with ^{1}H NMR data for the compound **23** revealing two sets of signals for each of the two CH_2O resonances (AB part of the ABX cycloserine system), whereas a single multiplet for the α-proton of D-Ala fragment observed (δ 5.57 ppm). Interestingly, little or no racemization has been detected in a similar base-mediated heterocyclization with a dipeptidic analog of the urea **22**: Gordeev, M. F.; Hui. H. C.; Gordon, E. M.; Patel, D. V. *Tetrahedron Lett.* **1997**, *38*, 1729. Absolute configuration for the two diastereomers **23** was not determined.