Solid-Phase Synthesis of Diverse Spiroisoxazolinodiketopiperazines

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S *Supporting Information*

ABSTRACT: A convenient, efficient protocol to prepare diverse spiroisoxazolino-diketopiperazines via a parallel solid-supported synthesis was developed. The key steps are (1) a coupling reaction of an amino acid; (2) tosylation with concomitant β-elimination to form an α, β-unsaturated ester; (3) a 1,3-dipolar cycloaddition with an oxime to form isoxazoline rings; and (4) cyclic cleavage to release the product from the resin. All reaction steps and workup procedures were modified to allow the use of automated or semiautomated equipment. A 100-member demonstration library with two diversity sites was prepared in good purity and acceptable overall yields.

KEYWORDS: *solid-phase organic synthesis, spiroisoxazolino-diketopiperazine, 1,3-dipolar cycloaddition*
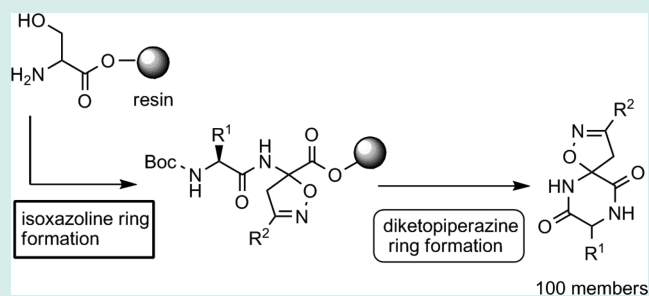
INTRODUCTION

Small molecule combinatorial chemistry has dramatically accelerated the development of biologically interesting molecules in chemical biology and drug discovery.[1] To allow the efficient preparation of small molecule libraries, solid-phase organic synthesis (SPOS)[1] has been developed for use with automated equipment, such as liquid handlers,[2] and techniques[3] such as the fluorous-tag approach and solid-phase extraction. These offer the opportunity for rapid synthesis of libraries of heterocyclic compounds for pharmaceutical and agrochemical discovery.[4]

Natural products and synthetic molecules that include a spirocyclic skeleton in their structures show a wide range of biological properties.[5−9] For example, spiroisoxazoline natural products exhibiting diverse antimicrobial, cytotoxic, and anti-inflammatory activities have been identified as a new class of novel alkaloids from marine sponges (Figure 1).[7] Additionally, spirodiketopiperazines have been reported with attractive bioactivities (Figure 1).[8] For example, aplaviroc is a potent CCR-5 antagonist for the treatment for HIV infection.[6,9]

Because of their broad-spectrum biological activities, the spirocyclic cores have been considered privileged scaffolds for drug design. Not surprisingly, many spirodiketopiperazine or spiroisoxazoline syntheses have been extensively studied.[5−9]

Our research interests include the design and synthesis of small heterocyclic libraries via a solid- or solution-phase combinatorial approach,[2,10,11] and in previous papers, we reported the development of a novel solution-phase synthetic route for the preparation of spiroisoxazolinohydantions and spirooxazolinoisoxazolines, by the direct fusion of an isoxazoline ring with a hydantoin ring and a oxazoline ring, respectively (Scheme 1).[2,10] To the best of our knowledge, though synthetic procedures for the simple spiro-diketopiperazines have been reported,[8,12,13] the direct combination of the diketopiperazine
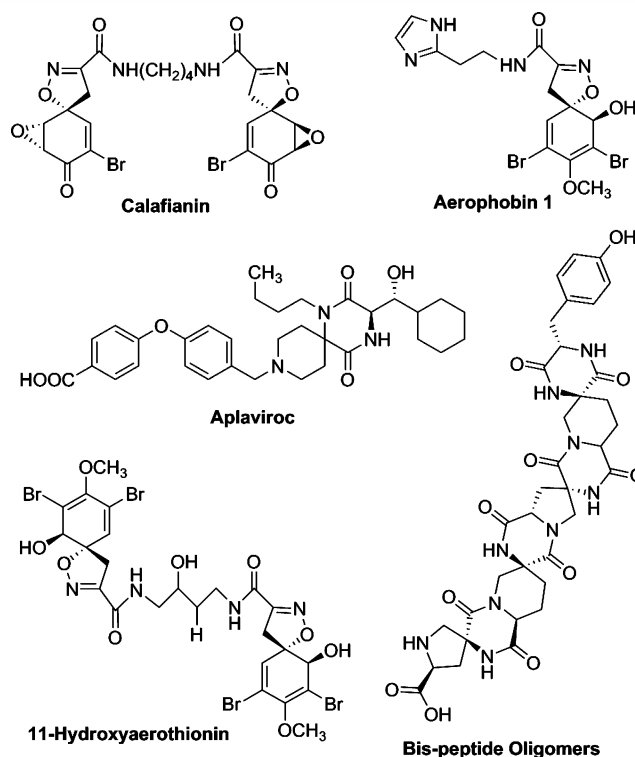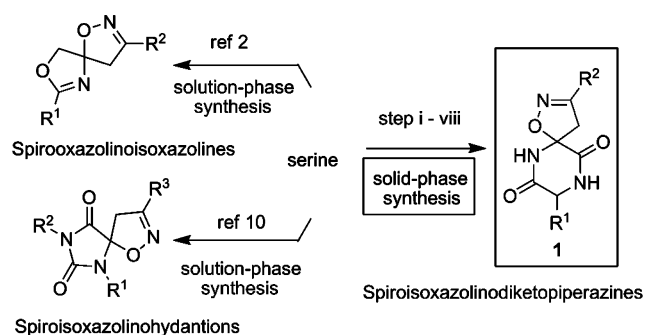

Figure 1. Examples of bioactive molecules containing spirooxazoline or spirodiketopiperazine scaffolds.

Scheme 1. General Synthetic Route for the Preparation of Isoxazoline-based Spiromolecules


and isoxazoline moieties to efficiently afford a spirocyclic skeleton on solid support has not been reported. In addition, preparation of 2,5-diketopiperazine scaffolds via isocyanide-based multicomponent reactions has also been extensively studied[13d,e] but this approach may not be suitable to our request because no isoxazoline building blocks are available and further chemical transformations are needed to construct an isoxazoline ring. Herein, we report an efficient, solid-phase parallel synthetic method (steps i−iv) under mild reaction conditions for combining these two interesting structural features within a single framework to form spiroisoxazolino-diketopiperazines $1\{1−10,1−10\}$ with two points of diversity (R^1 and R^2 in 1, see Scheme 1).[2,10]

■ RESULTS AND DISCUSSION

Model Synthetic Studies in the Solution Phase. Preliminary solution-phase experiments were undertaken to explore the feasible reaction conditions and to establish the required modifications for a practical SPOS. As shown in Scheme 2, N-Boc-O-TBDPS-DL-serine 2[10,14] was prepared from

Scheme 2. Solution Model Studies for the Preparation of Compound 6
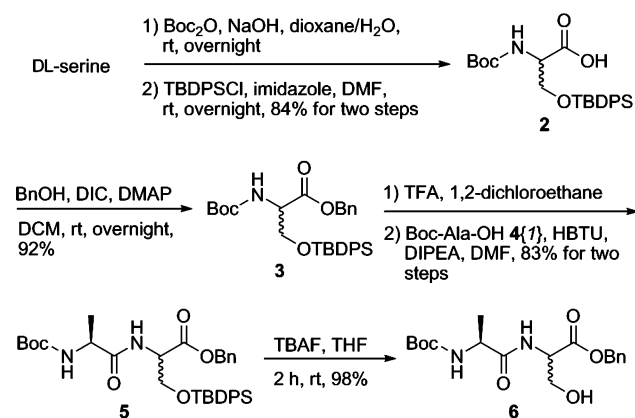
DL-serine in an 84% yield. Subsequent esterification of the protected serine 2 with benzyl alcohol under DIC-medicated coupling conditions gave the benzyl ester 3[15] in good yield (92%). Treatment of 3 with TFA to undergo N-Boc deprotection, followed by coupling with N-Boc amino acid $4\{1\}$ gave 5 in an overall 83% yield for the two steps.[10,16] After silane (TBDPS) deprotection under fluoride-based conditions for 2 h, alcohol 6 was smoothly obtained after purification by column chromatography. Notably, when TBAT (tetrabutylam-

monium triphenyldifluorosilicate) was used instead of TBAF (tetrabutylammonium fluoride) as a source of fluoride, a longer reaction time (24 h) was required to completely convert to 6.

As shown in Scheme 3 and Table 1, a mixture of 7 and 8 (the elimination product) was obtained (entry 1, Table 1) when

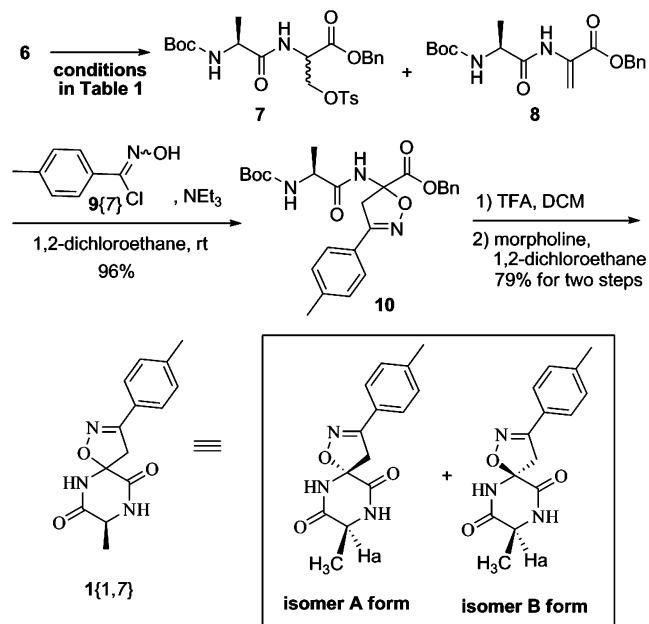## Scheme 3. Solution Model Studies of the Preparation of Compound 1{1,7}

Table 1. Optimization of Conditions for the Preparation of α,β-Unsaturated Ester 8

| entry	conditions (base/solvent/time)	yield (%) of 8 (7)[a]
1	TsCl, NEt$_3$/THF/overnight	66 (20)
2	TsCl, imidazole/THF/overnight	0 (>92)
3	TsCl, DBU/THF/2 h	78 (0)

[a]Isolated yield by silica-gel column chromatography.

Et$_3$N was used as the base for the tosylation of 6. Fortunately, 7 was obtained as the only product (>92%, entry 2, Table 1) by using imidazole as the base. Interestingly, one-pot conditions (entry 3, Table 1) to directly generate alkene 8 were found using DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as the base wherein 6 undergoes tosylation with concomitant with β-elimination (78%).[17] Treatment of alkene 8 with hydroximinoyl chloride $9\{7\}$[18] in the presence of Et$_3$N gave isoxazoline 10 (96%) via 1,3-dipolar reaction.[19] On the basis of preliminary literature studies,[2,10] we were not surprised to find that $8 \rightarrow 10$ proceeded with complete regioselectivity during 1,3-dipolar cycloaddition (only one of the regioisomer could be detected). After removal of the Boc group in 10 under acidic conditions, followed by treatment with morpholine to undergo cyclization,[20] the desired spiroisoxazolinodiketopiperazine $1\{1,7\}$ was obtained in an overall 79% yield for two steps. As expected, compound $1\{1,7\}$ contained two diastereoisomers with a ratio of around 1: 1−1.4: 1 as determined by NMR analysis.[21] Notably, this procedure also imitated the cyclization cleavage conditions to release the desired product from the solid support. Although these two isomers (A and B) were hard to separate by silica-gel column chromatography, small amounts of isomers A and B (see in Scheme 3) were fortunately obtained

after repeating the separation process several times. The ^{1}H NMR spectra of two isomers in $1\{1,7\}$ showed the methine proton (H_a) of the piperazinedione ring at δ 4.3 and 4.0 ppm, respectively, which indicated that the methine group in isomer A is oriented toward the proximal oxygen atom of the isoxazoline ring to result in the more downfield shift than that of isomer B.

Model Studies in Solid-Phase synthesis. With the reaction conditions and protocols developed in the above-mentioned solution phase synthesis in hand, we started to prepare the model product $1\{1,7\}$ on a solid support. As shown in Scheme 4, the synthetic work began with step i, esterification

**Scheme 4. Solid-Phase Model Studies of the Preparation of Spiroisoxazolino-diketopiperazine $1\{1,7\}$**
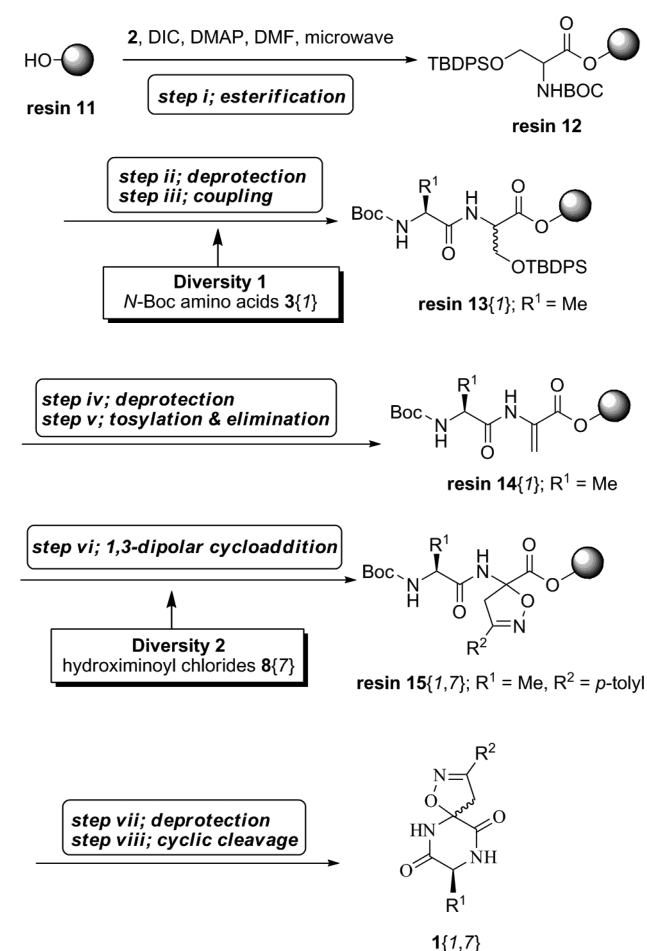
of the hydroxylmethyl resin **11** (hydroxylmethyl loading = 0.83 mmol/g)[22] with protected serine **2**. The reaction progress could be monitored by FTIR spectra, which showed appearance of the carboxylate absorption at ~1720 and ~1160 cm^{-1} and Si—O bond absorption at ~1111 cm^{-1}. Notably, although the reaction could be achieved at room temperature for 24 h, we realized this crucial step could be efficiently performed by using a microwave reactor to shorten the reaction time to 30 min.

The transformations including N-Boc deprotection (step ii) and the conjugation with N-Boc amino acids $4\{1\}$ (step iii) could be monitored by the Kaiser test and FTIR spectra. The TBAF-mediated O-deprotection of $13\{1\}$ (step iv), followed by tosylation and in situ elimination afforded resin $14\{1\}$ (step v). Step iv was amenable to FTIR monitoring (e.g., disappearance

of a diagnostic Si—O bond absorption at ~1111 cm^{-1}). Resin $14\{1\}$ was treated with fresh hydroximinoyl chloride $9\{7\}$ to give spiroisoxazoline resin $15\{1,7\}$ (step vi). The acid-mediated deprotection of resin $15\{1,7\}$ (step vii), followed by intra-molecular cyclization under basic conditions afforded the crude spiroisoxazolinodiketopiperazine $1\{1,7\}$.

Notably, when bases such as NH_3 and NEt_3 were used for the cleavage-cyclization step, the overall yields were not satisfactory (<15%) since their volatility and unpleasant odor made the operation unfriendly when we used a solid-phase synthesizer as our reactor. Next, morpholine was tested because of its higher boiling point (129 °C). Unfortunately, attempts to remove all the excess morpholine by the automated liquid–liquid extraction[2,23] module in acidic conditions were not successful and the yield dropped due to the repeated extraction process. Finally, we found an expedient way with assistance of solid-phase extraction technique (SPE) to remove the excess morpholine and impurities. Thus, the solid-phase synthetic conditions for the model compound $1\{1,7\}$ were successfully established.

Library Synthesis. On the basis of our design, ten amino acids $4\{1-10\}$ including eight L-amino acids $4\{1\}$ and $4\{4-10\}$ (Figure 2) and ten hydroximinoyl chlorides $9\{1-10\}$ (Figure
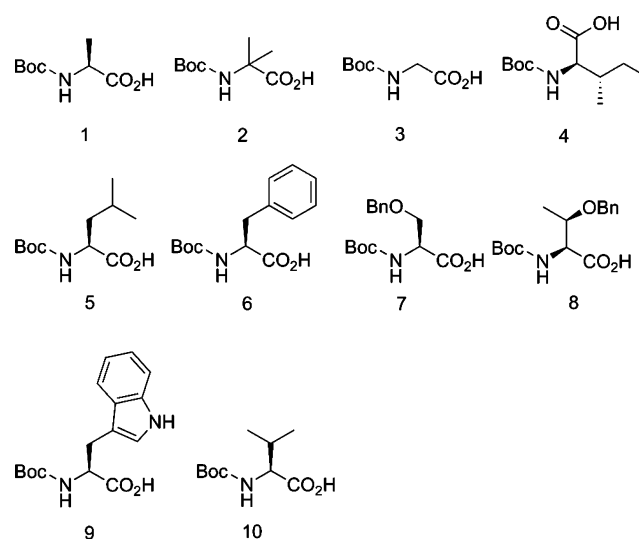
Figure 2. Set of amino acids $4\{1-10\}$ for the library.

3), freshly prepared using a solution-phase synthesizer, were utilized as the first and second diversity substituents, respectively. Following the same protocol developed in the model reaction (Scheme 4), one hundred desired molecules containing diastereoisomers were generated without further purification in an average yield of ~65% and the average yield was around 65%. This library was characterized by HPLC and mass (ESI) analysis with the purity of the crude products ranging from 70 to 99% (see in Table 2). The average purity was 94%, and more than with 80% of compounds had a purity of more than with 80%.

■ CONCLUSION

We have developed a practical and efficient, solid-phase parallel synthetic route for spiroisoxazolinodiketopiperazines with two diversity points. The key transformations in the synthesis were the preparation of the α,β-unsaturated ester **8** via tosylation and concomitant β-elimination, as well as the 1,3-dipolar cyclo-
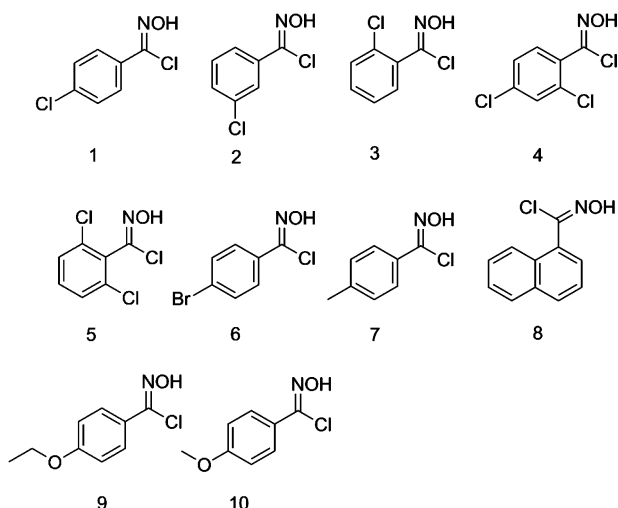
Figure 3. Set of hydroximinoyl chlorides **9**{*1−10*} for the library.

addition for isoxazoline formation and intramolecular diketo-piperazine formation to release our desired molecules from the resin. Equipment, such as a microwave reactor, solid- and solution-phase synthesizer, multichannel liquid handler, and vacuum centrifuge allowed the efficient preparation of a 100-membered demonstration library.

■ EXPERIMENTAL PROCEDURES

General Information. The hydroxymethyl resin (1% DVB, 100−200 mesh, loading = 0.83 mmol/g determined by analysis of the Fmoc loading on resins)[22] was purchased from ADVANCED CHEMTECH. Analytical HPLC spectra were recorded at 220 nm on a HITACHI L-2455 equipped with photodiode array detector, and a Zorbax column (XDB-C18, GP 50 × 2.1 mm, 3.5 μm) isocratic flow with 50% MeOH/50% H$_2$O over 10 min, flow rate = 0.2 mL/min. High resolution mass spectra were obtained by Bruker Daltonics BioTOF III. Microwave irradiation was carried by CEM focused microwave synthesis system. Solid-phase organic synthesis was carried out using FlexChem Blocks (96 well reactor with volume of 1.7 mL per well, which composed of polytetrafluoroethylene), FlexChem Rotating Oven (model 404) for stirring and heating, Vacuum manifold with collection tray for washing resin or collecting samples, and Hydra 96 Microdispenser for solvent or reagent dispensed. Multiple-functional liquid handler (Freedom EVO, TECAN) was utilized for extraction and separation.

Solvent evaporation was performed on Thermo Scientific Savant Explorer SpeedVac Concentrator Explorer 220. Mini-Block (24 position reactor from Mettler Toledo) with C18 cartridges was applied for solid-phase extraction.

Synthesis of *N*-Boc-*O*-TBDPS-DL-serine 2.[10,14] A solution of di-*tert*-butyl dicarbonate [(Boc)$_2$O, 2.84 g, 13.0 mmol] in 1,4-dioxane (11 mL) was added to the solution of DL-serine (1.14 g, 10.8 mmol) in 1 N NaOH$_{(aq)}$ (22 mL, 21.9 mmol) at 0 °C. After The reaction was stirred at rt for 8 h and then concentrated to remove 1,4-dioxane, acidified to pH 2−3, and extracted with EtOAc (15 mL × 3). The combined extracts were dried with MgSO$_4$ and concentrated to give *N*-Boc-DL-serine as a colorless oil. A mixture of *N*-Boc-DL-serine (1.79 g, 8.7 mmol) and imidazole (1.78 g, 26.1 mmol) was dissolved in DMF (15 mL), and added with TBDPSCl (2.88 g, 10.5 mmol) at 0 °C. The reaction was stirred at rt for 8 h and then poured into a mixture of H$_2$O (15 mL) and ether (30 mL). The organic layer was separated and the aqueous layer was extracted with ether. Combined organic layers were finally dried with MgSO$_4$, concentrated and recrystallized with hexanes/Et$_2$O to give product **2** (4.06 g, 9.1 mmol, 84%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.01 (s, 9H), 1.45 (s, 9H), 3.89 (dd, 1H, *J* = 10.1, 2.7 Hz), 4.10 (dd, 1H, *J* = 9.9, 2.0 Hz), 4.41−4.43 (br, 1H), 5.37 (d, 1H, *J* = 8.3 Hz), 7.34−7.43 (m, 6H), 7.59−7.61 (m, 4H); ^{13}C NMR (150 MHz, CDCl$_3$): δ 175.1, 155.5, 135.5 (×2), 132.8, 132.5, 129.9 (×2), 127.8 (×6), 80.2, 64.2, 55.2, 28.3 (×3), 26.7 (×3), 19.3; MS calcd for [C$_{24}$H$_{33}$NO$_5$Si + H]$^+$ 444.2, found 444.1.

Solution Phase Model Synthesis Studies. *Synthesis of Benzyl Ester of N-Boc-O-TBDPS-DL-serine: Benzyl 2-(tert-butoxycarbonylamino)-3-(tert-butyldiphenylsilyloxy)-propanoate 3.*[15] To a solution of benzyl alcohol (0.94 g, 8.7 mmol) in CH$_2$Cl$_2$/DMF 9:1 (10 mL), was added DMAP (0.21g, 1.7 mmol), and *N*-Boc-*O*-TBDPS-DL-serine **2** (3.86 g, 8.7 mmol), followed by DIC (2.21 g, 17.5 mmol) at rt. The mixture was subsequently irradiated in the microwaves cavity at 100 W with magnetic stirring (*T* = 44 °C) for 30 min. The reaction was cooled down and filtered. The clear liquid was concentrated under vacuum and purified by CC (EtOAc/hexanes = 1/4) to afford the desired ester **3** (4.16 g, 7.79 mmol, 89%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.00 (s, 9H), 1.44 (s, 9H), 3.87 (dd, 1H, *J* = 10.2, 2.8 Hz), 4.10 (dd, 1H, *J* = 10.2, 2.7 Hz), 4.42 (m, 1H), 5.17 (s, 2H), 5.43 (d, 1H, *J* = 8.8 Hz), 7.29−7.42 (m, 11H), 7.54−7.59 (m, 4H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 170.7, 155.4, 135.3 (×4), 135.2, 132.8, 132.7, 129.8 (×2), 128.6 (×3), 128.3 (×3), 127.7 (×3), 79.9, 67.2,

Table 2. Yields and Purities of the Spiroisoxazolinodiketopiperazine Library[a,b,c]

R^1 =	1	2	3	4	5	6	7	8	9	10
1	42 (90)	36 (99)	37 (99)	76 (99)	37 (92)	68 (99)	65 (91)	77 (99)	58 (91)	63 (99)
2	77 (89)	32 (99)	43 (99)	43 (91)	32 (79)	47 (96)	39 (95)	77 (99)	47 (88)	44 (99)
3	84 (99)	50 (81)	37 (89)	67 (92)	55 (94)	69 (96)	70 (91)	66 (99)	54 (99)	57 (99)
4	91 (98)	41 (89)	59 (99)	18 (99)	36 (84)	66 (85)	43 (81)	61 (99)	46 (98)	60 (99)
5	93 (99)	81 (99)	45 (99)	86 (99)	48 (99)	67 (96)	74 (93)	70 (90)	55 (99)	50 (99)
6	42 (99)	32 (99)	34 (99)	66 (92)	31 (78)	40 (75)	37 (83)	60 (94)	56 (93)	56 (99)
7	40 (99)	55 (99)	49 (80)	66 (99)	53 (99)	77 (99)	49 (99)	88 (88)	43 (99)	37 (99)
8	50 (96)	52 (90)	49 (81)	86 (99)	55 (88)	54 (82)	57 (99)	81 (77)	58 (97)	53 (99)
9	62 (95)	35 (97)	46 (99)	74 (98)	53 (83)	50 (83)	70 (83)	93 (91)	71 (99)	45 (99)
10	61 (99)	46 (99)	46 (70)	65 (99)	64 (99)	41 (99)	72 (98)	96 (90)	59 (99)	46 (99)

[a]Crude yields after overall synthesis. [b]Average purity determined by HPLC and mass (ESI) analysis of the material at 220 nm. [c]Purities shown in parentheses.

64.6, 55.6, 28.3 (×3), 26.7 (×3), 19.2. MS calcd for $[C_{31}H_{39}NO_5Si + H]^+$ 534.2, found 534.1.

Synthesis of Benzyl Ester of Boc-Ala-DL-serine: (S)-Benzyl 2-(2-(tert-butoxycarbonylamino)propanamido)-3-(tert-butyldiphenylsilyloxy)propanoate 5.[10,16] A solution of TFA/CH_2Cl_2 (1/1, 17 mL) was added to the solution of N-Boc-O-TBDPS-DL-serine benzoic ester 3 (3.13 g, 5.8 mmol) in 1,2-dichloroethane (6 mL) at 0 °C. The solution was stirred for 10 min and then warmed up to rt. After 2 h, the reaction was concentrated to give the amine intermediate as colorless oil without further purification. A mixture of the amine intermediate (2.92 g, 5.3 mmol), Boc-Ala-OH 4{1} (3.02 g, 15.9 mmol), and HBTU (6.06 g, 15.9 mmol) was dissolved in DMF (40 mL) in the presence of DIPEA (3.5 mL, 21.1 mmol) and the reaction was stirred for overnight. The reaction was extracted by Et_2O (20 mL × 3) and water. The combined organic layer was dried ($MgSO_4$), concentrated, and purify by CC (EtOAc/hexanes = 1/4) to give Boc-Ala-DL-serine benzoic ester 5 (2.93 g, 4.8 mmol, 83% over two steps). ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.02 (s, 9H), 1.34 (d, 3H, J = 8.9 Hz), 1.44 (s, 9H), 3.88 (dd, 1H, J = 10.3, 2.9 Hz), 4.16 (br, 1H), 4.71 (br, 1H), 5.17 (s, 2H), 7.29–7.42 (m, 11H), 7.54–7.59 (m, 4H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 172.3, 169.9, 155.2, 135.4 (×4), 135.0, 132.5, 132.4, 129.8 (×2), 128.5 (×3), 128.3 (×3), 127.7 (×3), 79.8, 67.3, 64.0, 54.1, 49.9, 28.2 (×3), 26.6 (×3), 19.1, 18.5. MS calcd for $[C_{34}H_{44}N_2O_6Si + H]^+$ 605.3, found 605.3.

Synthesis of Benzyl Ester of Boc-Ala-DL-serin-2-ene: (S)-Benzyl 2-[2-(tert-Butoxycarbonylamino)propanamido]-acrylate 8.[17] The solution of Boc-Ala-DL-serine benzoic ester 5 (1.41 g, 2.3 mmol) in THF (10 mL) was stirred with TBAF (1 M solution in THF, 3.5 mL) at ambient temperature for 4 h. The mixture was concentrated and purify by CC (EtOAc/hexanes =1/1) to give alcohol 6 (0.82 g, 2.2 mmol, 98%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.33 (d, 3H, J = 7.1 Hz), 1.40 (s, 9H), 3.95 (br, 2H), 4.15 (br, 1H), 4.71 (q, 1H, J = 3.7 Hz), 5.18 (s, 2H), 7.15 (m, 1H), 7.29–7.35 (m, 4H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 172.9, 170.2, 155.8, 135.1, 128.6 (×2), 128.5, 128.1 (×2), 80.6, 67.46, 62.6, 54.9, 50.5, 28.2 (×3), 18.0. HRMS calcd for $[C_{18}H_{26}N_2O_6 + H]^+$ 367.1864, found 367.1860. A mixture of alcohol 6 (0.82 g, 2.2 mmol), TsCl (0.65 g, 3.4 mmol) and DBU (1 mL, 7.2 mmol) was dissolved in THF (8 mL) at 0 °C and then the reaction was stirred at rt for 2 h. The solution was washed by H_2O (10 mL × 3), dried ($MgSO_4$), concentrated, and purified by CC (EtOAc/hexanes = 1/4) to afford the desired α,β-unsaturated benzoic ester 8 (0.63 g, 1.8 mmol, 78%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.36 (d, 3H, J = 7.1 Hz), 1.41 (s, 9H), 5.15 (br, 1H), 5.23 (s, 2H), 5.92 (s, 1H), 6.58 (s, 1H), 7.30–7.34 (m, 4H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 171.6, 163.6, 155.4, 135.0, 130.8, 128.6 (×2), 128.4, 128.1 (×2), 109.4, 80.3, 67.6, 50.9, 28.2 (×3), 17.8. HRMS calcd for $[C_{18}H_{24}N_2O_5 + Na]^+$ 371.1577, found 371.1576.

Synthesis of Substituted Isoxazoline Esters: Benzyl 5-[(S)-2-(tert-Butoxycarbonylamino)propanamido]-3-p-tolyl-4,5-dihydroisoxazole-5-carboxylate 10.[2,10,19] Triethyl amine (0.2 mL, 1.4 mmol) was slowly added to the mixture of α,β-unsaturated benzoic ester 8 (0.51 g, 1.4 mmol) and hydroximinoyl chloride 9{7} (0.74 g, 4.3 mmol) in 1,2-dichloroethane (10 mL) at 0 °C. The reaction was stirred at rt for overnight and then the solution was extracted by H_2O (10 mL × 3), dried ($MgSO_4$), and concetrated. The crude product was purified by CC (EtOAc/hexanes =1/4) to yield the mixture

of diastereomers of substituted isoxazoline ester 10 as colorless oil (0.68 g, 1.4 mmol, 96%, a 7:3 (trans/cis) ratio based on NMR analysis). ^{1}H NMR (600 MHz, CDCl$_3$, mixture of diastereomers): δ 1.24 (d, 3H, J = 7.1 Hz), 1.37 (s, 9H), 2.33 (s, 3H), 3.75 and 3.79 (d, 0.3H and 0.7H, J = 17.4 Hz), 4.03 and 4.05 (d, 0.7H and 0.3H, J = 17.2 Hz), 4.22 (br, 0.7H), 5.13 (br, 0.3H), 5.23 (s, 2H), 7.14 (m, 2H), 7.29 (m, 5H), 7.50 (m, 2H), 7.80 (br, 1H), 7.94 (br, 1H). ^{13}C NMR (150 MHz, CDCl$_3$, mixture of diastereomers): δ 172.8, 166.8, 156.8, 155.4, 140.8, 134.5, 129.3 (×2), 128.4 (×2), 128.1 (×3), 126.9 (×2), 125.2 and 128.4, 91.9, 80.1 and 68.4, 68.4 and 49.7, 44.0, 36.5 and 23.3, 28.2 (×3), 21.3, 17.6. HRMS calcd for $[C_{26}H_{31}N_3O_6 + H]^+$ 482.2286, found 482.2271.

Model Study for the Synthesis of Spiroisoxazolinodiketo-piperazine 1{1,7}.[21] A solution of TFA/1,2-dichloroethane (1/1, 2 mL) was added to the solution of 10 (0.32 g, 0.67 mmol) in 1,2-dichloroethane (2 mL) at 0 °C and then the reaction was stirred at rt for 2 h. After removal of TFA, a solution of morpholine/1,2-dichloroethane (1/1, 0.12 mL) was added to the reaction mixture in 1,2-dichloroethane (5 mL) at rt. After 8 h, additional 1,2-dichloroethane (10 mL) was added to the reaction solution. The mixture was extracted by H_2O (10 mL × 3), dried ($MgSO_4$), and concentrated. The crude sample was purified by CC (EtOAc/hexanes = 4/1) to yield 1{1,7}[18] (0.09 g, 0.33 mmol, 79%, a 2:1 trans/cis ratio based on NMR analysis. Small amounts of isomer A (trans form) and B (cis form) forms were purified by repeating column chromatography.

trans-8-Methyl-3-(4-methylphenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione: Isomer A (trans Form) of 1{1,7}. ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.45 (d, 3H, J = 7.0 Hz), 2.39 (s, 3H), 3.43 (d, 1H, J = 17.6 Hz), 4.27 (d, 1H, J = 17.6 Hz), 4.32 (q, 1H, J = 7.0 Hz), 4.59 (br, NH), 7.27 (d, 2H, J = 8.0 Hz), 7.60 (d, 2H, J = 8.0 Hz). ^{13}C NMR (150 MHz, CDCl$_3$): δ 173.2, 165.9, 158.2, 142.5, 130.7 (×2), 128.1 (×2), 127.3, 93.5, 51.8, 43.3, 21.6, 17.4. HRMS calcd for $[C_{14}H_{15}N_3O_3 + H]^+$ 274.1186, found 274.1133.

cis-8-Methyl-3-(4-methylphenyl)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione: Isomer B (cis Form) of 1{1,7}.[21] ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.61 (d, 3H, J = 7.0 Hz), 2.39 (s, 3H), 3.42 (d, 1H, J = 17.7 Hz), 4.06 (q, 1H, J = 7.1 Hz), 4.27 (d, 1H, J = 17.7 Hz), 4.59 (br, NH), 7.27 (d, 2H J = 8.1 Hz), 7.60 (d, 2H J = 8.0 Hz). ^{13}C NMR (150 MHz, CDCl$_3$): δ 173.3, 165.1, 158.1, 142.5, 130.7 (× 2), 128.1 (× 2), 127.3, 92.9, 53.7, 43.8, 22.6, 21.6; HRMS calcd for $[C_{14}H_{15}N_3O_3 + H]^+$ 274.1186, found 274.1157.

Synthesis of the Spiroisoxazolinodiketopiperazine Library. *General Procedure for Step i.* Resin 11 (1% DVB, loading = 0.83 mmol/g, 5.0 g), preswollen in CH_2Cl_2/DMF (9/1, 5 mL) for 10 min, was reacted with a mixture of N-Boc-O-TBDPS-DL-serine 1 (13.6 g, 30.7 mmol), DIC (3.2 mL, 20.4 mmol), and a catalytic amount of DMAP (0.25 g, 2.0 mmol) in CH_2Cl_2/DMF (9/1, 10 mL) at rt. The reaction mixture was subsequently irradiated in the microwaves cavity at 100 W with magnetic stirring (T = 44 °C) for 30 min. The resin was collected, washed with MeOH (10 mL), DCM (10 mL), DMF (10 mL), THF/H_2O (10 mL), THF (10 mL), DCM (10 mL), MeOH (10 mL), and Et_2O (10 mL) and then dried by vacuum oven (40 °C) overnight to give resin 12 as yellow beads: IR (single bead reflectance) 1716, 1492, 1160, 1112, 822 cm^{-1}.

General Procedures for Steps ii and iii. Resin 12 (50 mg per well) was loaded to the reaction block by a trap-door loader first and then the resin, preswollen by 1,2-dichloroethane (500 μL/well) for 10 min, was reacted with a solution of TFA/1,2-

dichloroethane =1/1 (300 μL/well). The reactor was rotated at rt for 2 h and the resin was washed with MeOH (10 mL), DCM (10 mL), DMF (10 mL), THF/H$_2$O (10 mL), THF (10 mL), DCM (10 mL), MeOH (10 mL), and Et$_2$O (10 mL) to give N-Boc-deprotected serine resin. The resin, preswollen by DMF (500 μL/well) for 10 min, was reacted with a solution of Boc-amino acid **4**{*1−10*} (0.13 g/well, 0.42−0.74 mmol/well), HBTU (0.12 g/well, 0.31 mmol/well) and DIPEA (0.07 mL/well, 0.41 mmol/well) in DMF (300 μL/well) at rt for 2 h. The resin was collected by filtration, washed with MeOH (5 mL), DCM (5 mL), DMF (5 mL), THF/H$_2$O (5 mL), DCM (5 mL), MeOH (5 mL), and Et$_2$O (5 mL). The resin was dried in vacuum oven (40 °C) overnight to give Boc-amino serine resin **13**{*1−10*} as yellow beads: IR (single bead reflectance) 1685, 1492, 1165, 1112, 823 cm^{-1}.

General Procedures for Steps iv and v. Resin **13**{*1−10*}, preswollen by THF (500 μL/well) for 10 min, was reacted with TBAF (1 M solution in THF, 150 μL/well) at rt for 2 h. The resin was collected by filtration, washed by MeOH (5 mL), DCM (5 mL), DMF (5 mL), THF/H$_2$O (5 mL), THF (5 mL), DCM (5 mL), MeOH (5 mL), and Et$_2$O (5 mL). A mixture of this intermediate resin and TsCl (0.03 g/well, 0.15 mmol/well) in THF (200 μL/well) in the presence of DBU (40 μL/well) was rotated at rt for 2 h. The resin was collected, washed with MeOH (5 mL), DCM (5 mL), DMF (5 mL), THF/H$_2$O (5 mL), DCM (5 mL), MeOH (5 mL), and Et$_2$O (5 mL) and dried in vacuum oven (40 °C) overnight to afford resin **14**{*1−10*} as yellow beads: IR (single bead reflectance) 1690, 1492, 1452, 1164 cm^{-1}.

General Procedure for Step vi. A mixture of resin **14**{*1−10*}, preswollen by 1,2-dichloroethane (500 μL/well) for 10 min, and hydroximinoyl chloride **9**{*1−10*} (0.08 g/well, 0.40−0.59 mmol/well) in 1,2-dichloroethane (500 μL/well) in the presence of Et$_3$N (15 μL/well, 0.10 mmol/well) was rotated at rt for overnight. The resin was collected, washed with MeOH (5 mL), DCM (5 mL), DMF (5 mL), THF/H$_2$O (5 mL), THF (5 mL), DCM (5 mL), MeOH (5 mL), and Et$_2$O (5 mL), and then dried in vacuum oven (40 °C) overnight to give resin **15**{*1−10,1−10*} as yellow beads: IR (single bead reflectance of the representative **15**{*1,7*}) 1748, 1681, 1492, 1452, 1162 cm^{-1}.

General Procedures for Steps vii and viii. A mixture of resin **15**{*1−10,1−10*}, preswollen by 1,2-dichloroethane (500 μL/well) for 10 min, and a solution of TFA/1,2-dichloroethane = 1/1 (300 μL/well) was rotated at rt. After 2 h, the resin was collected and washed with MeOH (5 mL), DCM (5 mL), DMF (5 mL), THF/H$_2$O (5 mL), THF (5 mL), DCM (5 mL), MeOH (5 mL), and Et$_2$O (5 mL). A mixture of this intermediate resin and a solution of morpholine/1,2-dichloroethane = 1/1 (60 μL/well) was rotated at rt for overnight. The reaction mixture was filtered and washed with DMF (200 μL/well ×3), MeOH (200 μL/well ×2). The filtrate was concentrated and the pH value of the mixture was adjusted to ∼6 with 0.3 mL 1 N HCl$_{(aq)}$. The residue, dissolved in MeOH/H$_2$O (1:4, 0.3 mL), was loaded onto the top of a solid-phase exchange cartridge (2 g, C18 cartridge; 1.4 × 2.3 cm), and eluted with MeOH/H$_2$O (1:4, 10 mL; first fraction) to remove morpholine salt and impurities, followed by eluting with MeOH/H$_2$O (4:1, 10 mL; second fraction) to directly collect into a sample vial and dried by SpeedVac to give desired compound **1**{*1−10,1−10*} without further purification.

8-Methyl-3-(2,6-dichlorophenyl)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{1,5}: crude yield = 93%; diastereomeric ratio (dr value) = 3/5; ^{1}H NMR (600 MHz, methanol-

d_4, mixture of isomers) δ 1.46 and 1.63 (d, 1.1H and 1.9H, J = 7.0 Hz), 3.22 and 3.35 (br, 0.4H and 0.6H), 4.07 and 4.36 (q, 0.6H and 0.4H, J = 7.0 Hz), 4.34 (br, 1H), 7.46 (m, 1H), 7.52 (m, 2H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of diastereomers) δ 184.1 and 179.8, 173.6 and 164.9, 155.1, 136.5, 133.3, 129.7 (×3), 128.8, 94.2 and 93.6, 53.8 and 51.7, 45.2 and 44.6, 22.5 and 17.0; HRMS m/z calcd for [C$_{13}$H$_{11}$Cl$_2$N$_3$O$_3$ + Na]$^+$ 350.0170, found 350.0182; crude purity =99%; t_R = 1.65 min.

8-Methyl-3-(4-methylphenyl)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{1,7}: crude yield =40%; diastereomeric ratio (dr value) = 9/5; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 1.45 and 1.61 (d, 1.9H and 1.1H, J = 7.1 Hz), 2.39 (s, 3H), 3.42 and 3.43 (d, 0.4H and 0.6H J = 17.7 Hz), 4.06 (q, 0.4H, J = 7.1 Hz), 4.34 (br, 1H), 4.36 (q, 0.6H, J = 7.1 Hz), 4.59 (br, NH), 7.46 (m, 1H), 7.52 (m, 2H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of diastereomers) δ 173.3 and 173.1, 165.9 and 165.1, 158.2 and 158.1, 142.5, 130.7 (×2), 128.1 (×2), 127.3, 93.5 and 92.9, 53.7 and 51.8, 43.8 and 43.3, 22.5 and 21.6, 21.6 and 17.4; HRMS m/z calcd for [C$_{14}$H$_{15}$N$_3$O$_3$ + H]$^+$ 274.1186, found 274.1133; crude purity = 99%; t_R = 1.66 min.

8,8′-Dimethyl-3-(2-chlorophenyl)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{2,3}: crude yield =50%; ^{1}H NMR (600 MHz, acetone-d_6,) δ 1.51 (s, 3H), 1.63 (s, 3H), 3.54 (d, 1H, J = 17.8 Hz), 4.55 (d, 1H, J = 17.8 Hz), 7.43−7.56 (m, 3H), 7.71−7.73 (m, 1H), 7.88 (br s, 1H), 8.66 (br s, 1H); ^{13}C NMR (150 MHz, acetone-d_6) δ 173.7, 163.4, 156.4, 133.3, 132.3, 132.0, 131.6, 129.3, 128.2, 93.7, 57.9, 45.5, 26.9; HRMS m/z calcd for [C$_{14}$H$_{14}$ClN$_3$O$_3$ + Na]$^+$ 330.0616, found 330.0661; crude purity =81%; t_R = 2.67 min.

8,8′-Dimethyl-3-(2,6-dichlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{2,5}: crude yield = 81%; ^{1}H NMR (600 MHz, acetone-d_6) δ 1.51 (s, 3H), 1.64 (s, 3H), 3.44 (d, 1H, J = 18.0 Hz), 4.41 (d, 1H, J = 18.0 Hz), 7.56 (m, 3H), 7.85 (br s, 1H), 8.50 (br, 1H); ^{13}C NMR (150 MHz, acetone-d_6) δ 173.8, 163.4, 156.4, 135.9, 133.0, 129.4 (×3), 128.8 and 127.1, 93.9, 57.9, 45.1, 26.9; HRMS m/z calcd for [C$_{14}$H$_{13}$Cl$_2$N$_3$O$_3$ + H]$^+$ 342.0407, found 342.0403; crude purity = 99%; t_R = 1.81 min.

8,8′-Dimethyl-3-(4-methylphenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{2,7}: crude yield =55%; ^{1}H NMR (600 MHz, acetone-d_6) δ 1.50 (s, 3H), 1.62 (s, 3H), 2.37 (s, 3H), 3.48 (d, 1H, J = 17.5 Hz), 4.29 (d, 1H, J = 17.5 Hz), 7.28 (d, 2H, J = 6.2 Hz), 7.61 (d, 2H, J = 6.2 Hz), 7.84 (br s, 1H), 8.54 (br s, 1H); ^{13}C NMR (150 MHz, acetone-d_6) δ 173.7, 163.7, 156.9, 141.5, 130.3 (×2), 127.7 (×2), 127.4, 93.3, 57.9, 43.4, 21.5; HRMS m/z calcd for [C$_{15}$H$_{17}$N$_3$O$_3$ + H]$^+$ 288.1343, found 288.1355; crude purity =99%; t_R = 1.83 min.

8,8′-Dimethyl-3-(1-naphthalene)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{2,8}: crude yield = 52%; ^{1}H NMR (600 MHz, methanol-d_4) δ 1.46 (s, 3H), 1.62 (s, 3H), 3.56 (d, 1H, J = 17.5 Hz), 4.46 (d, 1H, J = 17.5 Hz), 7.50−8.00 (m, 6H), 8.84−8.85 (m, 1H); ^{13}C NMR (150 MHz, methanol-d_4) δ 175.6, 165.4, 158.5, 135.7, 132.4, 132.0, 129.9, 129.7, 128.6, 127.9, 127.6, 126.9, 126.2, 92.4, 58.3, 46.7, 30.4, 26.9; HRMS m/z calcd for [C$_{18}$H$_{17}$N$_3$O$_3$ + H]$^+$ 324.1343, found 324.1367; crude purity =90%; t_R = 1.53 min.

8-(1-Methylpropyl)-3-(4-chlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{4,1}: crude yield =76%; diastereomeric ratio (dr value) = 1/3; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.95 and 0.99 (t, 0.8H and 2.2H J = 7.5 Hz), 1.08 (d, 3H, J = 7.0 Hz), 1.33 and 1.71 (dq,

1.5H and 0.5H J = 1.7, 7.5 Hz), 2.05 and 2.14 (dqt, 0.75H and 0.25H, J = 5.4, 7.0, 1.7 Hz), 3.47 (d, 1H, J = 18.0 Hz), 3.88 and 4.19 (d, 0.75H and 0.25H, J = 5.4 Hz), 4.44 and 4.47 (d, 0.25H and 0.75H, J = 18.0 Hz), 7.40−7.71 (m, 4H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 171.0, 165.6, 157.3, 134.1, 132.8, 132.2, 129.3, 128.5, 93.4 and 93.1, 62.4 and 60.9, 47.1 and 46.1, 41.5 and 39.3, 26.0 and 25.6, 15.9 and 15.4, 12.6 and 12.0; HRMS m/z calcd for $[C_{16}H_{18}ClN_3O_3 + H]^+$ 336.1109, found 336.1105; crude purity =99%; t_R = 2.39 min.

8-(1-Methylpropyl)-3-(2,6-dichlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{4,5}: crude yield = 86%; diastereomeric ratio (dr value) = 1/2; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.97 and 1.00 (t, 1H and 2H J = 7.5 Hz), 1.08 and 1.09 (d, 1H and 2H, J = 6.9 Hz), 1.31 and 1.73 (dq, 1.3H and 0.7H, J = 1.7, 6.9 Hz), 2.07 and 2.15 (dqt, 0.7H and 0.3H, J = 6.2, 6.9, 1.7 Hz), 3.31 (d, 1H, J = 18.1 Hz), 3.83 (d, 1H, J = 6.2 Hz), 4.24 and 4.31 (d, 0.3H and 0.7H, J = 18.1 Hz), 7.45−7.51 (m, 3H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 171.4, 165.5, 155.1, 136.5, 133.3, 129.7 (×3), 128.8, 93.4, 62.5 and 60.9, 46.3 and 45.4, 41.6 and 39.0, 26.2 and 25.7, 15.9 and 15.4, 12.6 and 11.9; HRMS m/z calcd for $[C_{16}H_{17}Cl_2N_3O_3 + H]^+$ 370.0720, found 370.0724; crude purity = 99%; t_R = 2.18 min.

8-(1-Methylpropyl)-3-(4-bromophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{4,6}: crude yield =66%; diastereomeric ratio (dr value) = 2/3; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.95 and 0.98 (t, 1.2H and 1.8H, J = 7.4 Hz), 1.08 (d, 3H, J = 6.8 Hz), 1.31 and 1.70 (dq, 1.2H and 0.8H, J = 1.7, 7.4 Hz), 2.04 and 2.13 (dqt, 0.6H and 0.4H, J = 5.1, 6.8, 1.7 Hz), 3.42 (d, 1H J = 17.7 Hz), 3.89 and 4.22 (d, 0.6H and 0.4H, J = 5.1 Hz), 4.19 and 4.24 (d, 0.4H and 0.6H, J = 17.7 Hz), 7.63 (s, 4H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 170.9, 165.6, 157.1, 133.3 (× 2), 130.3 and 129.6, 129.8 (× 2), 129.4 and 125.9, 93.0, 62.3 and 61.0, 44.7 and 43.7, 41.5 and 39.4, 26.0 and 25.6, 15.9 and 15.4, 12.6 and 12.0; HRMS m/z calcd for $[C_{16}H_{18}BrN_3O_3 + H]^+$ 380.0604, found 380.0615; crude purity = 92%; t_R = 2.51 min.

8-(1-Methylpropyl)-3-(4-ethoxyphenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{4,9}: crude yield = 74%; diastereomeric ratio (dr value) = 2/5; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.95 and 0.98 (t, 0.9H and 2.1H, J = 6.0 Hz), 1.08 (d, 3H, J = 7.0 Hz), 1.31 and 1.70 (dq, 1.4H and 0.6H, J = 1.7, 6.0 Hz), 1.41 and 1.46 (t, 2.1H and 0.9H, J = 7.0 Hz), 2.03 and 2.05 (dqt, 0.7H and 0.3H, J = 5.3, 7.0, 1.7 Hz), 3.41 (d, 1H, J = 17.7 Hz), 3.87 (d, 0.7H, J = 5.3 Hz), 4.09 (q, 2H, J = 7.0 Hz), 4.17−4.24 (m, 1.3H), 6.97 (d, 2H, J = 8.7 Hz), 7.63 (d, 2H, J = 8.7 Hz); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 171.1, 165.9, 162.2, 157.6, 129.8 (×2), 122.2, 116.0 (×2), 92.8 and 92.4, 66.1 and 64.9, 62.3 and 60.9, 45.2 and 44.2, 41.5 and 39.3, 26.0 and 25.6, 15.9 and 15.2, 12.6 and 12.0; HRMS m/z calcd for $[C_{18}H_{23}N_3O_4 + H]^+$ 346.1761, found 346.1748; crude purity = 98%; t_R = 2.13 min.

8-(2-Methylpropyl)-3-(2-chlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{5,3}: crude yield =55%; diastereomeric ratio (dr value) = 1/2; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.96 and 1.00 (d, 1H and 2H, J = 6.0 Hz), 0.99 and 1.03 (d, 1H and 2H, J = 6.0 Hz), 1.78 (m, 1H), 1.91 (m, 2H), 3.45 (d, 1H, J = 17.9 Hz), 4.00 (dd, 1H, J = 8.7, 4.5 Hz), 4.52 (d, 1H, J = 17.9 Hz), 7.39−7.71 (m, 4H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 173.2 and 172.6, 165.8 and 165.1, 157.5, 134.1, 132.8, 132.2,

132.0, 129.3, 128.5, 93.7 and 93.3, 56.3 and 54.6, 47.0, 46.0, 25.4, 23.7, 22.0; HRMS m/z calcd for $[C_{16}H_{18}ClN_3O_3 + H]^+$ 336.1109, found 336.1102; crude purity = 94%; t_R = 2.09 min.

8-(2-Methylpropyl)-3-(4-methoxyphenyl)-1-oxa-2,6,9-tria-za-spiro[4.5]dec-2-ene-7,10-dione 1{5,10}: crude yield = 64%; diastereomeric ratio (dr value) = 1/2; ^{1}H NMR (600 MHz, Methanol−d_4, mixture of isomers) δ 0.95 and 0.99 (d, 1H and 2H, J = 6.4 Hz), 0.98 and 1.02 (d, 1H and 2H, J = 6.4 Hz), 1.77 (m, 1H), 1.91 (m, 2H), 3.41 (d, 1H, J = 17.7 Hz), 4.00 (dd, 1H, J = 9.3, 4.9 Hz), 4.27 (d, 1H, J = 17.7 Hz), 7.00 (d, 2H, J = 8.8 Hz), 7.66 (d, 2H, J = 8.8 Hz); ^{13}C NMR (150 MHz, Methanol−d_4, mixture of isomers) δ 173.2, 165.4, 163.3, 157.8, 129.8 (× 2), 122.4, 115.5 (× 2), 92.7, 56.3 and 56.0, 54.6, 47.0 and 43.8, 44.0 and 41.5, 25.6 and 25.4, 23.7 and 23.4, 22.6 and 22.0; HRMS m/z calcd for $[C_{17}H_{21}N_3O_4 + H]^+$ 332.1605, found 332.1612; crude purity =99%; t_R = 1.95 min.

8-(Benzyl)-3-(4-methylphenyl)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{6,7}: crude yield = 77%; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 2.39 (s, 3H), 3.23 (m, 2H), 3.43 (d, 1H, J = 17.0 Hz), 4.17 (m, 1H), 4.27 (d, 1H, J = 17.0 Hz), 7.28−7.61 (m, 9H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 172.0, 165.4, 158.1, 142.5, 137.6, 130.9 (×2), 130.7 (×2), 129.9 (×2), 128.4, 128.1 (×2), 127.3, 92.6, 59.5, 44.1, 43.4, 21.6; HRMS m/z calcd for $[C_{20}H_{19}N_3O_3 + H]^+$ 350.1499, found 350.1516; crude purity = 98.9%; t_R = 2.53 min.

8-(O-Benzylmethoxyl)-3-(3-chlorophenyl)-1-oxa-2,6,9-tria-za-spiro[4.5]dec-2-ene-7,10-dione 1{7,2}: crude yield = 39%; diastereomeric ratio (dr value) = 1/1; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 3.18 and 3.44 (d, 0.5H and 0.5H, J = 17.8 Hz), 3.77−3.81 (m, 1H), 3.85 and 4.25 (d, 0.5H and 0.5H, J = 17.8 Hz), 3.88−3.99 (m, 1H), 4.23−4.30 (m, 1H), 7.35−7.45 (m, 9H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 169.6 and 169, 166.9 and 165.4, 156.9 and 156.7, 139.33 and 139.23, 136.1 and 136.0, 132.2 and 132.1, 131.7, 129.7, 129.6, 129.3, 129.0, 128.9, 127.8, 126.5, 93.3 and 93.2, 75.0 and 74.5, 73.2 and 71.6, 58.5 and 57.5, 45.3 and 44.4; HRMS m/z calcd for $[C_{20}H_{18}ClN_3O_4 + H]^+$ 400.1059, found 400.1080; crude purity =95%; t_R = 2.69 min.

8-(O-Benzylmethoxyl)-3-(4-ethoxyphenyl)-1-oxa-2,6,9-tri-aza-spiro[4.5]dec-2-ene-7,10-dione 1{7,9}: crude yield = 70%; diastereomeric ratio (dr value) = 1/2; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 1.41 and 1.46 (t, 1H and 2H, J = 6.9 Hz), 3.16 and 3.41 (d, 0.7H and 0.3H, J = 17.8 Hz), 3.76−3.81 (m, 1H), 3.83 and 4.21 (d, 0.7H and 0.3H, J = 17.8 Hz), 3.76−3.81 (m, 1H), 4.09 and 4.17 (dq, 0.7H and 1.3H J = 2.3, 6.9 Hz), 4.18−3.30 (m, 2H), 7.09−7.75 (m, 9H); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 157.8 and 156.8, 139.3, 134.4 and 133.6, 129.7 (×2), 129.6 (×2), 129.5 (×2), 129.0, 12.9, 128.3, 128.2, 115.9, 114.5, 93.0, 75.0 and 74.5, 73.2 and 71.5, 66.1 and 64.9, 58.5 and 57.5, 45.5 and 44.7; HRMS m/z calcd for $[C_{22}H_{23}N_3O_5 + H]^+$ 410.1710, found 410.1731; crude purity = 83%; t_R = 2.34 min.

8-(O-Benzylmethoxyl)-3-(4-mthoxyphenyl)-1-oxa-2,6,9-tri-aza-spiro[4.5]dec-2-ene-7,10-dione 1{7,10}: crude yield =72%; diastereomeric ratio (dr value) = 1/1; ^{1}H NMR (600 MHz, Methanol−d_4, mixture of isomers) δ 3.24 and 3.43 (d, 0.5H and 0.5H, J = 17.7 Hz), 3.77−3.82 (m, 1H), 3.84 (s, 3H), 3.91 and 4.26 (d, 0.5H and 0.5H, J = 17.7 Hz), 3.89−3.98 (m, 1H), 4.23−4.30 (m, 1H), 4.56−4.64 (m, 2H), 6.95−7.67 (m, 9H); ^{13}C NMR (150 MHz, Methanol−d_4, mixture of isomers) δ 169.7 and 169.2, 167.1 and 165.7, 163.3, 157.7 and 157.4, 139.3, 129.7 (× 2), 129.5 (× 2), 129.2 and 128.9, 129.0 (×

2), 122.4 and 122.3, 115.4 (× 2), 92.7 and 92.6, 74.9 and 74.5, 73.3 and 71.4, 58.5 and 57.4, 56.0, 45.8 and 44.9; HRMS m/z calcd for $[C_{21}H_{21}N_3O_5 + H]^+$ 396.1554, found 396.1580; crude purity =98%; t_R = 1.70 min.

8-(1-Methyl-O-benzylmethoxyl)-3-(2,6-dichlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{8,5}. crude yield = 70%; diastereomeric ratio (dr value) = 1/1; ^{1}H NMR (600 MHz, acetone-d_6, mixture of isomers) δ 1.33 and 1.34 (d, 1.5H and 1.5H, J = 6.8 Hz), 3.44 and 3.46 (d, 0.5H and 0.5H, J = 18.1 Hz), 3.93−4.16 (m, 1.5H), 4.36 and 4.54 (d, 0.5H and 0.5H, J = 18.1 Hz), 4.51−4.52 (m, 0.5H), 4.62−4.72 (m, 2H), 7.23−7.57 (m, 8H), 7.74 and 8.62 (br s, 0.5H and 0.5H), 8.01 and 8.68 (br s, 0.5H and 0.5H); ^{13}C NMR (150 MHz, acetone-d_6, mixture of isomers) δ 168.5 and 167.3, 163.8, 154.0, 139.8 and 139.6, 135.9, 133.0, 129.4 (×3), 129.1 and 129.0 (×1.5 and ×1.5), 128.6 (×2), 128.3 and 128.2, 93.4 and 93.1, 78.2 and 77.1, 72.4 and 71.4, 63.0 and 60.0, 46.5 and 45.8, 17.4 and 15.5; HRMS m/z calcd for $[C_{21}H_{19}Cl_2N_3O_4 + H]^+$ 448.0825, found 448.0844; crude purity = 90%; t_R = 2.31 min.

8-(Indole-3-methyl)-3-(2,4-dichlorophenyl)-1-oxa-2,6,9-tri-aza-spiro[4.5]dec-2-ene-7,10-dione 1{9,4}: crude yield = 46%; diastereomeric ratio (dr value) = 2/3; ^{1}H NMR (600 MHz, acetone-d_6, mixture of isomers) δ 2.81 and 3.58 (d, 0.4H and 0.6H, J = 17.9 Hz), 3.79 and 4.57 (d, 0.4H and 0.6H, J = 17.9 Hz), 3.41 and 4.56 (m, 0.6H and 0.4H), 3.39 and 3.49 (m, 0.4H and 0.6H), 3.39 and 4.24 (m, 0.6H and 0.4H), 7.00−7.79 (m, 8H), 8.51 and 8.80 (br s, 0.8H and 1.2H), 10.21 and 10.27 (br s, 0.6H and 0.4H); ^{13}C NMR (150 MHz, acetone-d_6, mixture of isomers) δ 170.5 and 169.1, 164.3 and 163.1, 155.8 and 155.2, 137.8 and 137.6, 136.9 and 136.8, 134.3 and 134.1, 133.1 and 132.9, 131.2 and 131.1, 128.8 and 128.7, 128.6 and 128.5, 128.2 and 128.1, 125.9 and 125.1, 122.4 and 122.3, 120.0 and 119.8, 119.8 and 119.3, 112.3, 110.5 and 109.4, 93.4 and 93.3, 58.9 and 56.6, 45.5 and 45.4, 33.4; HRMS m/z calcd for $[C_{21}H_{16}Cl_2N_4O_3 + Na]^+$ 465.0492, found 465.0516; crude purity = 98%; t_R = 2.45 min.

8-(Isopropyl)-3-(2,4-dichlorophenyl)-1-oxa-2,6,9-triaza-spiro[4.5]dec-2-ene-7,10-dione 1{10,4}: crude yield = 60%; diastereomeric ratio (dr value) = 1/2; ^{1}H NMR (600 MHz, methanol-d_4, mixture of isomers) δ 0.96 and 1.07 (d, 1H and 2H, J = 7.1 Hz), 1.096 and 1.10 (d, 2H and 1H, J = 7.1 Hz), 2.31 and 2.45 (m, 0.7H and 0.3H), 3.46 and 3.47 (d, 0.7H and 0.3H), 3.81 and 4.14 (d, 0.7H and 0.3H), 4.40 and 4.45 (d, 0.3H and 0.7H), 7.44 (d, 1H J = 8.4 Hz), 7.61 (s, 1H), 7.71 (d, 1H J = 8.4 Hz); ^{13}C NMR (150 MHz, methanol-d_4, mixture of isomers) δ 171.1, 156.4, 138.0, 134.9, 133.2, 131.7, 128.9 (×2), 128.2, 93.2, 63.2 and 61.4, 46.8 and 46.0, 34.8 and 32.3, 19.7 and 18.6, 18.3 and 16.7; HRMS m/z calcd for $[C_{15}H_{15}Cl_2N_3O_3 + H]^+$ 356.0563, found 356.0609; crude purity = 99%; t_R = 2.58 min.

8-(Isopropyl)-3-(1-naphthalene)-1-oxa-2,6,9-triaza-spiro-[4.5]dec-2-ene-7,10-dione 1{10,8}: . crude yield = 53%; diastereomeric ratio (dr value) = 2/3; ^{1}H NMR (600 MHz, acetone-d_6, mixture of isomers) δ 1.01 and 1.13 (d, 1.2H and 1.8H, J = 5.2 Hz), 1.13 and 1.18 (d, 1.8H and 1.2H, J = 5.2 Hz), 2.38 and 2.54 (m, 0.6H and 0.4H), 3.68 (d, 1H, J = 17.3 Hz), 3.87 and 4.24 (br s, 0.6H and 0.4H), 4.55 (d, 1H, J = 17.3 Hz), 7.59−8.04 (m, 7H), 8.79 (br s, 1H), 8.95 (br s, 1H); ^{13}C NMR (150 MHz, acetone-d_6, mixture of isomers) δ 169.9 and 169.3, 165.0 and 164.2, 157.8 and 157.7, 135.0 and 131.4, 131.94 and 131.89, 129.6, 129.54, 129.48, 128.3, 127.80, 127.75, 127.3, 126.8, 126.0, 91.9 and 91.5, 62.9 and 60.8, 47.0 and 45.8, 34.3, 19.6 and 18.5, 18.4 and 16.7; HRMS m/z calcd for

$[C_{19}H_{19}N_3O_3 + H]^+$ 338.1499, found 338.1525; crude purity =99%; t_R = 2.27 min.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for representative library members and characterization for this library. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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