Synthesis and Evaluation of Cyclic Acetals of Serine Hydroxylamine for Amide-Forming KAHA Ligations

Simon Baldauf
Jeffrey W. Bode

Abstract The α-ketoacid–hydroxylamine (KAHA) ligation allows the coupling of unprotected peptide segments. The most widely used variant employs a 5-membered cyclic hydroxylamine that forms a homoserine ester as the primary ligation product. While very effective, monomers that give canonical amino acid residues are in high demand. In order to preserve the stability and reactivity of cyclic hydroxylamines, but form a canonical amino acid residue upon ligation, we sought to prepare cyclic derivatives of serine hydroxylamine. An evaluation of several cyclization strategies led to cyclobutanone ketals as the leading structures. The preparation, stability, and amide-forming ligation of these serine-derived ketals are described.

Key words ligation, hydroxylamines, acetals, amides, peptides

In 2006, we reported the α-ketoacid–hydroxylamine (KAHA) amide-forming ligation,1 which complemented the well-established native chemical ligation2 (NCL) for the chemical synthesis of proteins. These methods allow chemoselective couplings of unprotected peptide segments for the synthesis of peptides and proteins.3 Currently the most used hydroxylamine for KAHA ligations is 5-oxaproline, due to its high stability and chemoselective reactivity with α-ketoacids to form a homoserine residue at the ligation site.4 The primary product of this reaction is an ester, which rearranges to an amide under basic conditions (Scheme 1).5 In 2015, we developed a novel oxazetidine acid which gave serine at the ligation site, exclusively as the amide product.6 Unfortunately, the synthesis of this monomer is rather long and it is not stable in its unprotected form.

In this report, we describe the synthesis of a six-membered cyclic hydroxylamine, which directly yields a native serine residue at the ligation site (Scheme 1). Furthermore, we describe our efforts to incorporate these novel monomers into a peptide segment.

In designing an alternative, serine-forming ligation monomer we sought to identify a larger ring structure that we hoped would be more easily prepared. This consideration led us to consider cyclic variants of L-serine hydroxylamine, including cyclic carbonates, cyclic sulfonates, and cyclic acetals. To begin, L-serine was converted into the corresponding ethyl ester 1. TBS protection of the free alcohol and subsequent treatment with bromoacetonitrile gave 2. This was treated with a slightly modified protocol from Fukuyama to give the N-hydroxyl intermediate 3.7 Initially we envisioned selective Boc-protection of the nitrogen, but this was unsuccessful under various conditions.8 We instead elected to first protect the free hydroxyl group using TBSCl followed by N-protection with Fmoc-Cl to give 4. Although initial attempts to remove the TBS protecting group

scheme 1 KAHA ligation with 5-oxaproline and proposed cyclic serine hydroxylamine

Received: 21.11.2018
Accepted: 27.11.2018
Published online: 07.01.2019
License terms: 

SYNTHESIS 2019, 51, 1273–1283
using TBAF led mainly to decomposition, treatment of 4 with concentrated HCl gave clean conversion into diol 5 (Scheme 2).

Scheme 2 Synthesis of core structure 5

We explored the formation of the corresponding cyclic boronic esters, silanes, carbonates, sulfites, and phosphoric acid esters; however, we were not able to isolate the corresponding six-membered cyclic hydroxylamines. As an alternative, we considered cyclic acetals, as similar structures have proven to be well suited for KAHA ligation. For use in protein synthesis, however, these acid-labile functional groups would need to survive cleavage from the resin under standard TFA conditions. Prior to in-depth investigations of suitable acetals, we carried out preliminary tests to check that cyclic acetals of 5 could be formed and that Fmoc-removal was feasible. We treated compound 5 with 1,1-dimethoxyethylbenzene in the presence of p-toluenesulfonic acid to give 6 without problem. Removal of the Fmoc group under basic conditions also proceeded smoothly. With this promising result we sought to prepare and evaluate various cyclic acetals.

We initially targeted a methylene acetal, as these are known to be more resistant to acid cleavage than other structures. Unfortunately, all attempts to form the desired compound from 5 with various methylene sources using different catalysts or activation using Brønsted or Lewis acids did not yield the desired product. Despite this setback, we continued to synthesize various acetals and ketals by using either a catalytic amount of p-toluenesulfonic acid for aldehyde-derived compounds, or In(OTf)₃ for the ketone derivatives. Selected acetals and ketals prepared are shown in Table 1. However, when these compounds were tested for their stability under SPPS conditions [resin cleavage conditions, TFA/DODT/H₂O (95:2.5:2.5 v/v) for 2 h], only cyclobutanone-derivative 12 showed reasonable stability (Table 1).

Despite the poor acid stability, we tested these substrates for activity in KAHA ligation. For this purpose, the Fmoc protecting group was removed with piperidine and the monomers were allowed to react with simple α-ketoacid 13 to give amide 14 (Table 2). Commonly reported KAHA conditions use DMSO/H₂O at 60 °C, but a HFIP/AcOH mixture showed much better solubility and the reaction was observed to proceed at 45 °C. Therefore all ligation studies were carried out under these conditions. Almost all monomers tested showed good activity in the KAHA ligation. After a period of 12 hours, α-ketoacid 13 was consumed. The benzyl acetics were stable under the acidic ligation conditions, and electron-poor nitrobenzyl acetal 16 gave slightly better conversion compared to bromobenzyl acetal 15.

Table 1 Synthesized Monomers and Their Stability

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Stability to SPPS cleavage conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>not stable</td>
</tr>
<tr>
<td>7</td>
<td>not stable</td>
</tr>
<tr>
<td>8</td>
<td>not stable</td>
</tr>
<tr>
<td>9</td>
<td>not stable</td>
</tr>
<tr>
<td>10</td>
<td>not stable</td>
</tr>
<tr>
<td>11</td>
<td>not stable</td>
</tr>
<tr>
<td>12</td>
<td>stable</td>
</tr>
</tbody>
</table>

* Stability was tested by treatment with TFA/DODT/H₂O (95:2.5:2.5 v/v) for 2 h.
Cyclobutanone-derived acetal 18 was also stable during the ligation conditions and gave good conversion of the α-ketoacid. Only 17 did not perform KAHA ligation under HFIP/AcOH conditions because the deprotected monomer was not stable under the ligation conditions (Table 2).

This experiment also indicated that open, unsubstituted serine hydroxylamine does not react under these conditions and that the cyclic structure is essential. With these promising results, we sought to remove the ethyl ester in the presence of Fmoc and the acetal, for the purpose of applying this monomer in Fmoc-SPPS. Despite all our efforts, the various conditions only led to total decomposition or returned starting material without removing the Fmoc protecting group.

We instead introduced a benzyl ester at the beginning of the synthesis. All previously developed steps were compatible with the benzyl-protected starting material, and we were able to synthesize the benzyl ester analogue 19. Unfortunately, deprotection of the benzyl ester using Pd/C under a H2 atmosphere gave mainly decomposition and only traces of product was observed. We were pleased to see that exchanging Pd/C for Pd(OH)2 allowed a clean reaction.

Free acid 26 was coupled onto a short peptide segment, but proved not to be stable upon resin cleavage. Therefore we prepared and evaluated a number of other ketals from small rings. Although we were not successful in forming the product from oxetan-3-one, other cyclobutanone derivatives proved tractable. Brominated derivative 20 was readily formed and carboxylic esters 21 and 22 could be prepared from the corresponding acid followed by ester formation. The stability of compounds 19–22 was tested on the monomer and on a peptide segment (Table 3).

All benzyl-protected monomers were also tested for their performance in the KAHA ligation. Fmoc removal occurred smoothly in all cases and the deprotected monomer underwent ligation with α-ketoacid 13 in HFIP/AcOH (Table 4). The cyclobutanone ketal monomers could be converted into the corresponding acid by treatment with

**Table 2** KAHA Ligation with Selected Monomers

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Ligation yield after purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>64%</td>
</tr>
<tr>
<td>16</td>
<td>77%</td>
</tr>
<tr>
<td>17</td>
<td>no product observed</td>
</tr>
<tr>
<td>18</td>
<td>72%</td>
</tr>
</tbody>
</table>

\[16^b\] monomer 18\[c\]

**Table 3** Synthesized Monomers and Their Stability

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Stability to SPPS cleavage conditions</th>
<th>Stability to SPPS cleavage conditions on peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>stable</td>
<td>not stable</td>
</tr>
<tr>
<td>20</td>
<td>stable</td>
<td>stable</td>
</tr>
<tr>
<td>21</td>
<td>stable[b]</td>
<td>stable[b]</td>
</tr>
<tr>
<td>22</td>
<td>stable[b]</td>
<td>stable[b]</td>
</tr>
</tbody>
</table>

\[a\] KAHA ligation carried out at 30 mM at 45 °C in HFIP/AcOH with 1% H2O for 12 h.

\[b\] HPLC monitoring of the KAHA ligation of α-ketoacid 13 and hydroxylamine 16, and purified amide 14.

\[c\] HPLC monitoring of the KAHA ligation of α-ketoacid 13 and hydroxylamine 18, and purified amide 14.

**Figure**

T = 0 h

purified

T = 12 h

purified

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h

T = 12 h

T = 0 h
Pd(OH)$_2$ under H$_2$ atmosphere (Scheme 3) and all compounds tolerated the coupling conditions to introduce the monomer onto the N-terminus of a peptide segment. Since OMe ester 21 showed better conversion in the KAHA ligation, we selected this monomer for introduction onto a short peptide sequence.

### Table 4 KAHA Ligation with Selected Monomers$^a$

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Ligation yield after purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>79%</td>
</tr>
<tr>
<td>24</td>
<td>39%</td>
</tr>
<tr>
<td>25</td>
<td>86%</td>
</tr>
<tr>
<td>26</td>
<td>96%</td>
</tr>
<tr>
<td>27</td>
<td>90%</td>
</tr>
</tbody>
</table>

$^a$ KAHA ligation carried out at 30 mM at 45 °C in HFIP/AcOH with 1% H$_2$O for 12 h.

A short peptide segment with three amino acid residues, prepared by standard SPPS conditions on a Rink amide resin, was chosen as a model peptide. After deprotection of the terminal amine, the free acid of monomer 27 was coupled with HATU/NMM conditions to the resin and cleaved with TFA/DODT/H$_2$O (95:2.5:2.5 v/v) for 2 hours. TFA was removed at 40 °C under reduced pressure and the product was isolated by preparative HPLC. The Fmoc protecting group was removed with $N,N$-diethylamine and the KAHA ligation performed directly on the unprotected product without further purification using $\alpha$-ketoacid 13 in HFIP/AcOH. The free hydroxylamine 31 was observed by LCMS and was completely consumed within 12 hours (Scheme 4).

After this positive result, we expanded to a larger peptide fragment consisting of 50 amino acid residues. Although the Fmoc group could be removed cleanly, with a peak-to-peak conversion by HPLC, the acetal protecting group unfortunately did not survive the two-step procedure of basic Fmoc-removal conditions and acidic purification. The obtained product was 36, the free hydroxylamine of serine at the N-terminus (Scheme 5).
The results were the same regardless of the choice of base used to remove the Fmoc protecting group, including basic aqueous solutions. To date, we were not able to overcome this problem and longer peptides containing the cyclic ketal serine hydroxylamines cannot be isolated. A search for a more stable acetal protecting group is currently under investigation.

In conclusion, we devised a synthetic route to new hydroxylamine monomers that yield serine residues upon KAHA ligation. We found that substituted cyclobutane ketals are stable under acidic conditions and are excellent ligation partners. Further investigations are necessary to apply this monomer on larger peptides, however this strategy provides a promising new approach to KAHA ligation that forms canonical amino acid residues at the ligation site.

Fmoc-amino acids with suitable side-chain protecting groups, HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) were purchased from Peptides International (Louisville, KY, USA) and ChemImpex (Wood Dale, IL, USA). Solvents for flash chromatography (EOAc, hexanes, MeOH) were of technical grade and distilled prior to use. HPLC grade MeCN containing 0.1% (v/v) TFA. Analytical HPLC was performed on Shiseido Capcell Pak PM (5 μm, 20 mm i.d. × 250 mm) or Vydac 248MS C18 (10 μm, 22 mm i.d. × 250 mm) columns at a flow rate of 1 mL/min. Preparative HPLC was performed on Shiseido Capcell Pak PM (5 μm, 20 mm i.d. × 250 mm) or Vydac 248MS C18 (10 μm, 22 mm i.d. × 250 mm) columns at a flow rate of 10 mL/min.

**Ethyl (1-{tert-Butyldimethylsilyl})-N-(cyanomethyl)-l-serinate (2)**

IR (neat); 2954, 2929, 2857, 1733, 1471, 1251, 1195 cm−1.

1H NMR (400 MHz, CDCl3): δ = 4.19 (q, J = 7.1 Hz, 2 H), 3.92–3.84 (m, 2 H), 3.80–3.59 (m, 2 H), 3.48 (dt, J = 8.2, 4.4 Hz, 1 H), 2.28 (q, J = 7.0 Hz, 1 H), 1.27 (t, J = 7.2 Hz, 3 H), 0.85 (s, 9 H), 0.03 (d, J = 5.7 Hz, 6 H).

13C NMR (101 MHz, CDCl3): δ = 171.54 (CO), 117.73 (CN), 64.41 (CH2), 61.61 (CH), 61.39 (CH), 36.07 (CH2), 25.82 (3 CH3), 18.27 (C), 14.29 (CH3), -5.41 (CH3), -5.53 (CH3).


**Ethyl (1-{tert-Butyldimethylsilyl})-N-hydroxy-l-serinate (3)**

IR (neat); 2953, 2929, 2857, 1737, 1471, 1252, 1107 cm−1.

1H NMR (400 MHz, CDCl3): δ = 6.59 (br s, 1 H), 5.83 (br s, 1 H), 4.21 (qd, J = 7.2, 0.8 Hz, 2 H), 3.88 (qd, J = 10.2, 4.8 Hz, 2 H), 3.74 (dd, J = 5.6, 4.0 Hz, 1 H), 1.27 (t, J = 7.1 Hz, 3 H), 0.85 (s, 9 H), 0.03 (d, J = 3.3 Hz, 6 H).

13C NMR (101 MHz, CDCl3): δ = 171.21 (CO), 66.83 (CH), 61.21 (CH), 61.08 (CH2), 25.85 (3 CH3), 18.29 (C), 14.31 (CH3), -5.46 (CH3), -5.53 (CH3).


**Ethyl N-(1-{tert-Butyldimethylsilyl})-O-(1-{tert-Butyldimethylsilyl})-N-{[9H-fluoren-9-ylmethoxy]carbonyl}-l-serinate (4)**

IR (neat); 2953, 2928, 2856, 1738, 1463, 1249, 1095 cm−1.

1H NMR (400 MHz, CDCl3): δ = 7.79 (dt, J = 7.6, 0.9 Hz, 2 H), 7.69 (dt, J = 7.5, 1.0 Hz, 2 H), 7.42 (tt, J = 7.5, 0.8 Hz, 2 H), 7.32 (tt, J = 7.4, 1.1 Hz, 2 H), 4.72 (dd, J = 7.9, 6.3 Hz, 1 H), 4.55–4.39 (m, 2 H), 4.31 (t, J = 7.1 Hz, 1 H), 4.21 (qd, J = 7.1, 3.2 Hz, 2 H), 4.10 (dd, J = 7.2, 1.8 Hz, 2 H), 1.28 (t, J = 7.1 Hz, 3 H), 0.96 (s, 9 H), 0.91 (s, 9 H), 0.21 (d, J = 22.0 Hz, 6 H), 0.09 (s, 6 H).

13C NMR (101 MHz, CDCl3): δ = 168.68 (CO), 159.38 (CO), 143.89 (C), 143.82 (C), 141.44 (C), 141.42 (C), 127.87 (CH), 127.86 (CH), 127.21 (2 CH), 125.49 (CH2), 125.43 (CH), 121.00 (CH), 120.09 (CH), 68.36 (CH2), 66.93 (CH), 61.45 (CH2), 59.85 (CH2), 47.13 (CH), 26.09 (3 CH3), 26.00 (3 CH3), 18.41 (2 CH), 14.32 (CH3), -4.70 (CH3), -4.73 (CH3), -5.21 (CH3), -5.29 (CH3).

HRMS (ESI): m/z [M + H]+ calcd for C39H33NO6Si2: 600.3171; found: 600.3174.


IR (neat); 2956, 1730, 1700, 1450, 1312, 1111, 1047 cm−1.
Benzyl N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N-hydroxy-l-serinate (benzyl-5)

IR (neat): 3674, 2969, 2900, 1738, 1708, 1450, 1262, 1106, 1048 cm⁻¹.

1H NMR (400 MHz, CDCl₃): δ = 7.73 (dd, J = 7.6, 2.1, 0.9 Hz, 2 H), 7.53 (t, J = 8.3 Hz, 2 H), 7.37 (t, J = 7.5, 3.3, 0.8 Hz, 2 H), 7.35–7.17 (m, 7 H), 5.18 (s, 2 H), 4.88 (br s, 1 H), 4.77–4.34 (m, 2 H), 4.22–4.07 (m, 3 H), 3.13 (br s, 1 H); 1 H not observed.

13C NMR (101 MHz, CDCl₃): δ = 168.65 (CO), 158.95 (CO), 143.39 (2 C), 141.37 (2 C), 134.97 (2 C), 128.76 (2 CH), 128.65 (2 CH), 128.22 (2 CH), 127.99 (2 CH)², 127.97 (2 CH), 127.31 (2 CH)², 127.29 (2 CH), 125.21 (2 CH)², 125.16 (2 CH), 120.14 (2 CH), 69.16 (CH₂), 67.70 (CH₃), 64.31 (CH), 59.96 (CH₆), 46.91 (CH); * signals of rotamers.


Ketal Formation with In(OT₂): General Procedure

In(O₂)₃ (0.30 equiv) was added to a solution of S (1.00 equiv) and ketone (5.00 equiv) in CH₂Cl₂ (0.15 M) at 0 °C. The reaction was allowed to warm up to r.t. and was stirred overnight. The solution was diluted with CH₂Cl₂ and 10% citric acid solution was added. The phases were separated and the aqueous layer was extracted with CH₂Cl₂ (2 ×). The combined organic layers were washed with sat. aq NaHCO₃ solution and brine and dried (Na₂SO₄). The drying agent was removed by filtration and the solvent was evaporated. The residue was purified by flash chromatography.

3-Ethyl-2-(9H-Fluoren-9-ylmethyl) (3S)-6-Methyl-6-phenyl-1,5,2-dioazaspiro[3.5]nonane-6,7-dicarboxylate (6)

IR (neat): 2987, 2900, 1713, 1449, 1304, 1098, 1065 cm⁻¹.

1H NMR (500 MHz, CDCl₃): δ = 7.78 (d, J = 7.6, 7.0, 2 H), 7.69–7.62 (m, 2 H), 7.69–7.66 (m, 1 H), 4.49–4.35 (m, 2 H), 4.19 (J = 7.0 Hz, 1 H), 4.14–4.07 (m, 2 H), 0.93 (s, 9 H), 0.88 (s, 9 H), 0.20 (s, 3 H), 0.15 (s, 3 H), 0.07 (s, 3 H), 0.06 (s, 3 H).

13C NMR (126 MHz, CDCl₃): δ = 168.07 (CO), 156.52 (CO), 144.05 (C), 143.43 (C), 141.57 (C), 141.46 (C), 140.67 (CH₂), 128.82 (CH), 128.30 (2 CH), 127.97 (2 CH)², 127.92 (2 CH), 127.25 (2 CH)², 127.20 (2 CH), 125.66 (CH), 125.35 (2 CH), 125.24 (2 CH)², 121.0 (2 CH), 120.10 (2 CH), 103.89 (C), 68.23 (CH₂), 62.16 (CH₃), 59.91 (CH₃), 57.53 (CH), 47.14 (CH), 21.58 (CH₃), 14.22 (CH₃); * signals of rotamers/diastereomers.

HRMS (MALDI): m/z [M + H]⁺ calc for C₃₃H₄₄N₂O₉Si: 747.1911; found: 747.1907.

7-Ethyl 6-(9H-Fluoren-9-ylmethyl) (S)-5,9-Dioxa-6-azaspiro[3.5]monaoane-6,7-dicarboxylate (12)

IR (neat): 3674, 2987, 2901, 1714, 1450, 1408, 1313, 1058 cm⁻¹.

1H NMR (500 MHz, CDCl₃): δ = 7.35–7.21 (m, 7 H), 7.29–7.22 (m, 2 H), 7.14–7.09 (m, 1 H), 6.79 (s, 1 H), 5.82–5.65 (m, 1 H), 4.13 (J = 7.0 Hz, 1 H), 4.09 (J = 6.6 Hz, 1 H), 4.00–3.95 (m, 2 H), 3.89–3.84 (m, 1 H), 0.97 (s, 3 H), 0.89 (s, 3 H), 0.20 (s, 3 H), 0.11 (s, 3 H), 0.06 (s, 3 H).

13C NMR (126 MHz, CDCl₃): δ = 168.06 (CO), 156.43 (CO), 144.05 (2 C)², 143.37 (2 C), 141.50 (2 CO), 141.43 (2 CH), 127.98 (2 CH)², 127.93 (2 CH), 127.25 (2 CH)², 127.12 (2 CH), 125.58 (2 CH)², 125.18 (2 CH), 120.15 (2 CH)², 120.13 (2 CH), 104.99 (C), 68.50 (CH₃), 62.34 (CH₄), 60.27 (CH₂), 57.22 (CH₃), 47.03 (CH₂), 32.70 (CH₃), 30.37 (CH), 26.08 (CH₃), 11.59 (CH); * signals of rotamers/diastereomers.
7-Benzyl 6-(9H-Fluoren-9-ylmethyl) (S)-5,9-Dioxo-6-azaspiro[3.5]nonane-6,7-dicarboxylic (19)

IR (neat): 3674, 2987, 2900, 1746, 1716, 1451, 1407, 1260, 1098, 1065 cm⁻¹.

1H NMR (500 MHz, CDCl₃): δ = 7.77 (dd, J = 7.6, 1.1 Hz, 2 H), 7.75–7.54 (m, 2 H), 7.47–7.21 (m, 9 H), 5.25 (s, 2 H), 4.56 (dd, J = 10.4, 6.7 Hz, 2 H), 4.40–4.31 (m, 2 H), 4.22 (t, J = 7.4 Hz, 1 H), 4.17–4.00 (m, 1 H), 2.50–2.19 (m, 4 H), 1.99–1.78 (m, 2 H).

13C NMR (126 MHz, CDCl₃): δ = 168.05 (CH₂), 156.45 (CO), 144.47 (2 C*), 144.05 (2 C'), 143.34 (2 C), 141.66 (2 C'), 141.49 (2 C'), 141.41 (2 C'), 135.22 (C), 128.63 (2 CH), 128.44 (CH), 128.09 (2 CH), 127.97 (2 CH*), 127.91 (2 CH*), 127.73 (2 CH), 127.24 (2 CH*), 127.11 (2 CH), 125.60 (2 CH*), 125.18 (2 CH), 124.84 (2 CH), 120.20 (2 CH*), 120.13 (2 CH*), 120.10 (2 CH), 105.02 (C), 68.47 (CH₃), 68.77 (CH₂), 60.30 (CH₂), 57.34 (CH), 46.96 (CH), 32.72 (CH₂), 30.41 (CH₃), 11.60 (CH); * signals of rotamers/diastereomers.


7-Benzyl 6-(9H-Fluoren-9-ylmethyl) (S)-2-Bromo-5,9-dioxo-6-azaspiro[3.5]nonane-6,7-dicarboxylic (20)

IR (neat): 3674, 2987, 2900, 1732, 1406, 1393, 1275, 1065 cm⁻¹.

1H NMR (400 MHz, CDCl₃): δ = 7.76 (ddd, J = 8.4, 2.7, 1.0 Hz, 2 H), 7.55 (dd, J = 33.2, 7.5 Hz, 2 H), 7.43–7.22 (m, 9 H), 5.19 (s, 2 H), 4.62–4.32 (m, 4 H), 4.24–4.04 (m, 2 H), 3.90 (m, 6 H), 3.30–3.27 (m, 2 H), 2.70–2.46 (m, 2 H).

13C NMR (101 MHz, CDCl₃): δ = 167.56 (CO), 156.01 (CO), 143.81 (2 C*), 143.15 (2 C), 141.53 (2 C'), 141.44 (2 C'), 135.04 (C), 128.65 (2 CH), 128.52 (CH), 128.12 (2 CH), 128.03 (2 CH*), 128.01 (2 CH), 127.26 (2 CH*), 127.19 (2 CH), 125.19 (2 CH*), 124.94 (2 CH), 120.20 (2 CH*), 120.12 (2 CH), 103.37 (C), 67.97 (CH₃), 65.98 (CH₂), 60.86 (CH₂), 57.26 (CH), 47.07 (CH), 45.50 (CH₂), 43.97 (CH), 31.60 (CH); * signals of rotamers/diastereomers.


(S)-7-(Benzyloxy carbonyl)-6-[9H-fluoren-9-ylmethyl]carbonetyl]-5,9-dioxo-6-azaspiro[3.5]nonane-2-carboxylic Acid (21a)

IR (neat): 3674, 2987, 2900, 1730, 1450, 1406, 1393, 1275, 1065 cm⁻¹.

1H NMR (400 MHz, CDCl₃): δ = 10.67 (br s, 1 H), 7.83–7.73 (m, 2 H), 7.72–7.52 (m, 2 H), 7.48–7.21 (m, 9 H), 5.25 (s, 2 H), 4.72–4.15 (m, 5 H), 4.11–3.91 (m, 1 H), 3.09–2.95 (m, 1 H), 2.84–2.83 (m, 4 H).

13C NMR (101 MHz, CDCl₃): δ = 179.80 (CO*), 179.74 (CO), 167.74 (CO*), 167.67 (CO), 156.57 (CO*), 156.20 (CO), 143.80 (2 C*), 143.74 (2 C*), 143.32 (2 C'), 143.14 (2 C), 141.43 (2 C'), 141.34 (2 C'), 141.29 (2 C'), 141.22 (2 C), 135.03 (C*), 134.99 (C*), 128.55 (2 CH), 128.42 (CH*), 128.40 (CH), 128.02 (2 CH*), 128.01 (2 CH), 127.95 (2 CH*), 127.98 (2 CH*), 127.73 (2 CH*), 127.87 (2 CH*), 127.83 (2 CH*), 127.24 (2 CH*), 127.19 (2 CH*), 127.10 (2 CH), 125.52 (2 CH*), 125.31 (2 CH*), 124.25 (2 CH*), 124.95 (2 CH*), 120.10 (2 CH*), 120.08 (2 CH*), 119.96 (2 CH*), 101.56 (C), 68.25 (CH₂), 67.90 (CH₃), 67.87 (CH₂), 60.53 (CH₂*), 60.42 (CH₂), 57.44 (CH), 46.91 (CH*), 46.72 (CH), 36.34 (CH*), 36.15 (CH*), 34.65 (CH*), 34.21 (CH), 28.84 (C), 28.71 (CH); * signals of rotamers/diastereomers.


HPLC (Shiseido Capcell Pak UG80 C18 column (4.6 × 250 mm), heated to 60 °C, 10 to 90% CH₃CN with 0.1% TFA in 20 min): tₑₖ = 24.8 min.

Segment 33

HPLC (Shiseido Capcell Pak UG80 C18 column (4.6 × 250 mm), heated to 60 °C, 10 to 90% CH_{2}Cl_{2} with 0.1% TFA in 20 min): t_{R} = 24.9 min.

**Segment 34**

HRMS (ESI): m/z [M]+ calcd for C_{384}H_{43}N_{7}O_{8}S_{2}: 6249.1231; found: 6249.0065. HPLC (Shiseido Capcell Pak UG80 C18 column (4.6 × 250 mm), heated to 60 °C, 10 to 90% CH_{2}CN with 0.1% TFA in 20 min): t_{R} = 24.5 min.

**3-Ethyl 2-(9H-Fluoren-9-ylmethyl) (3S)-6-[(Phenylsulfonyl)methyl]-1,5,2-dioxazinane-2,3-dicarboxylate (9)**

IR (neat): 2978, 1376, 1447, 1306, 1147, 1116, 1065 cm⁻¹. 1H NMR (500 MHz, CDCl_{3}): δ = 8.11–7.24 (m, 13 H), 5.05 (dd, J = 20.8, 6.6, 6.1 Hz, 1 H), 4.50 (dd, J = 14.1, 10.4, 7.3 Hz, 1 H), 4.45–4.29 (m, 2 H), 4.28–4.19 (m, 2 H), 4.17–4.00 (m, 0.5 H), 3.95 (dd, J = 10.1, 4.2 Hz, 0.5 H), 3.54 (dt, J = 14.5, 7.4 Hz, 1 H), 3.39 (dt, J = 14.3, 4.2 Hz, 1 H), 3.31 (d, J = 6.1 Hz, 2 H), 2.79 (s, 2 H), 2.71 (s, 2 H), 2.42 (s, 2 H), 2.30–2.00 (m, 4 H), 2.00–1.50 (m, 4 H). 13C NMR (126 MHz, CDCl_{3}): δ = 167.26 (CO), 157.70 (CO), 143.93 (C), 143.70 (C)*, 141.42 (C), 141.36 (C), 139.67 (C), 139.48 (C), 134.18 (CH), 129.47 (2 CH), 128.15 (2 CH), 128.03 (CH), 127.89 (CH), 127.26 (2 CH), 125.62 (CH), 125.42 (CH), 120.08 (2 CH), 98.92 (CH)*, 97.72 (CH), 68.79 (CH_{2})*, 68.72 (CH_{2}), 62.22 (CH_{2})*, 61.19 (CH_{3}), 56.14 (CH_{2}), 55.78 (CH_{2}), 47.30 (CH), 14.27 (CH_{3}); * signals of rotamers/diastereomers.

HRMS (ESI): m/z [M]⁺ calcd for C_{284}H_{433}N_{69}O_{85}S_{2}: 6249.1231; found: 6234.1122; 6249.1231; found: 6249.1231.

**3-Ethyl 2-(9H-Fluoren-9-ylmethyl) (3S)-6-(4-Nitrophenyl)-1,5,2-dioxazinane-2,3-dicarboxylate (10)**

IR (neat): 3674, 2986, 2901, 1724, 1524, 1450, 1349, 1242, 1080 cm⁻¹. 1H NMR (500 MHz, CDCl_{3}): δ = 7.82–7.21 (m, 2 H), 7.81–7.52 (m, 6 H), 7.45–7.27 (m, 4 H), 5.67 (s, 1 H), 4.74 (s, 2 H), 4.59 (dd, J = 10.6, 7.2 Hz, 1 H), 4.28 (dq, J = 14.2, 6.9 Hz, 3 H), 4.19–4.04 (m, 2 H), 1.25 (t, J = 7.1 Hz, 3 H). 13C NMR (126 MHz, CDCl_{3}): δ = 167.55 (CO), 156.33 (CO), 148.85 (CNO), 143.90 (2 C)*, 141.09 (2 C)*, 141.44 (C), 141.00 (2 C), 128.07 (2 CH)*, 128.04 (2 CH), 127.77 (2 CH), 127.24 (2 CH), 125.24 (2 CH)*, 125.29 (2 CH), 123.63 (2 CH), 120.21 (2 CH)*, 120.17 (2 CH), 101.98 (CH), 68.23 (CH_{2}), 66.17 (CH), 62.51 (CH), 57.34 (CH), 47.19 (CH), 14.26 (CH_{3}); * signals of rotamers/diastereomers.

HRMS (ESI): m/z [M + Na]⁺ calcd for C_{284}H_{434}N_{69}O_{85}S_{2}: 6257.1425; found: 5727.1425.

**3-Ethyl 2-(9H-Fluoren-9-ylmethyl) (3S)-6-(4-Iodo-1,5,2-dioxazinane-2,3-dicarboxylate (11)**

IR (neat): 3674, 2986, 2901, 1724, 1450, 1355, 1176, 1057 cm⁻¹. 1H NMR (500 MHz, CDCl_{3}): δ = 7.86–7.61 (m, 4 H), 7.47–7.31 (m, 4 H), 5.43 (s, 1 H), 4.72–4.61 (m, 2 H), 4.57–4.31 (m, 2 H), 4.28 (q, J = 7.1 Hz, 2 H), 4.14–4.04 (m, 1 H), 3.88 (dd, J = 44.5, 9.6, 7.1 Hz, 2 H), 1.37–1.24 (m, 6 H), 1 H not observed. 13C NMR (126 MHz, CDCl_{3}): δ = 167.46 (CO), 156.01 (CO), 143.85 (2 C)*, 143.31 (2 C), 141.50 (2 C)*, 141.44 (C), 141.00 (2 C), 128.07 (2 CH)*, 128.04 (2 CH), 127.77 (2 CH), 127.24 (2 CH), 125.24 (2 CH)*, 125.29 (2 CH), 123.63 (2 CH), 120.21 (2 CH)*, 120.17 (2 CH), 101.98 (CH), 68.23 (CH), 66.17 (CH), 62.51 (CH), 57.34 (CH), 47.19 (CH), 14.26 (CH_{3}); * signals of rotamers/diastereomers. 1C not observed.

HRMS (ESI): m/z [M + Na]⁺ calcd for C_{284}H_{434}N_{69}O_{85}S_{2}: 645.1969; found: 445.1966.

**Fmoc Deprotection of Acid-Protected Monomer; General Procedure**

Piperidine (5.00 equiv) was added dropwise to Fmoc-protected monomer (1.00 equiv) in CH_{2}Cl_{2} (0.025 M). The reaction was stirred for 1 h at r.t. The solution was diluted with CH_{2}Cl_{2} and sat. aq NH_{4}Cl solution was added. The phases were separated and the aqueous layer was extracted with CH_{2}Cl_{2} (2 ×). The combined organic layers were...
washed with brine and dried (Na₂SO₄). The drying agent was removed by filtration and the solvent was evaporated. The residue was purified by flash chromatography.

**Ethyl (3S)-6-(4-Bromophenyl)-1,5,2-dioxazinan-3-carboxylate (15)**

IR (neat): 3674, 2987, 2900, 1732, 1393, 1210, 1043 cm⁻¹.

**1H NMR (500 MHz, CDCl₃):** δ = 7.50–7.45 (m, 2 H), 7.32–7.28 (m, 2 H), 6.86 (br s, 1 H), 5.80 (s, 1 H), 4.66 (dd, J = 11.4, 1.4 Hz, 1 H), 4.38–4.26 (m, 3 H), 3.52 (dd, J = 3.2, 1.4 Hz, 1 H), 1.34 (t, J = 7.1 Hz, 3 H).

**13C NMR (126 MHz, CDCl₃):** δ = 170.61 (CO), 135.50 (C), 131.53 (2 CH), 128.30 (2 CH), 123.58 (C), 110.47 (CH), 62.92 (CH₂)*, 62.69 (CH₂), 62.63 (CH₂)*, 62.46 (CH₂)*, 61.97 (CH₃)*, 61.89 (CH₅), 57.65 (CH)*, 57.53 (CH), 14.40 (CH₃), 11.85 (CH₃).


**Ethyl (3S)-5-(4-Nitrophenyl)-1,5,2-dioxazinan-3-carboxylate (16)**

IR (neat): 3674, 2986, 2901, 1734, 1522, 1348, 1214, 1044 cm⁻¹.

**1H NMR (500 MHz, CDCl₃):** δ = 7.44–7.33 (m, 5 H), 6.23 (s, 1 H), 5.33–5.23 (m, 2 H), 4.33–4.13 (m, 2 H), 4.10 (dd, J = 11.5, 3.4 Hz, 1 H), 3.68 (dt, J = 7.7, 3.9 Hz, 1 H), 3.37–2.57 (m, 4 H).

**13C NMR (126 MHz, CDCl₃):** δ = 169.55 (CO)*, 169.45 (CO), 135.23 (CH), 128.80 (2 CH), 128.71 (CH), 128.41 (2 CH)*, 128.39 (2 CH), 100.62 (CH), 67.55 (CH₃), 60.88 (CH₃), 58.44 (CH)*, 58.41 (CH), 45.95 (CH₅), 45.78 (CH₃), 44.90 (CH₃)*, 44.57 (CH*), 32.98 (CH*), 32.75 (CH); *signals of rotamers/diastereomers.


**Ethyl (S)-5,9-Dioxa-6-azaspiro[3.5]nonane-7-carboxylate (26)**

IR (neat): 3674, 3296, 2987, 2900, 1738, 1454, 1282, 1198, 1065 cm⁻¹.

**1H NMR (500 MHz, CDCl₃):** δ = 7.74–7.33 (m, 5 H), 6.23 (s, 1 H), 5.33–5.23 (m, 2 H), 4.33–4.13 (m, 2 H), 4.10 (dd, J = 11.5, 3.4 Hz, 1 H), 3.68 (dt, J = 7.7, 3.9 Hz, 1 H), 3.37–2.57 (m, 4 H).

**13C NMR (126 MHz, CDCl₃):** δ = 169.55 (CO)*, 169.45 (CO), 135.23 (C), 128.80 (2 CH), 128.71 (CH), 128.41 (2 CH)*, 128.39 (2 CH), 100.62 (C), 67.55 (CH₃), 60.88 (CH₃), 58.44 (CH)*, 58.41 (CH), 45.95 (CH₅), 45.78 (CH₃), 44.90 (CH₃)*, 44.57 (CH*), 32.98 (CH*), 32.75 (CH); * signals of rotamers/diastereomers.


**Benzyl Deprotection; General Procedure**

Pd(OH)₂-charcoal (20% on carbon wetted with ca. 50% water) (1.00 m/m) was added to a solution of benzyl-protected acid (1.00 equiv) in CH₂Cl₂/EtOH (1:4) at 0 °C. The mixture was stirred under H₂ atmosphere for 1 h while gradually warming up to r.t. The mixture was filtered through a plug of celite and the solvent was evaporated.

**Benzyl (S)-2-Bromo-5,9-dioxo-6-azaspiro[3.5]nonane-7-carboxylate (25)**

IR (neat): 3674, 3296, 2987, 2900, 1738, 1454, 1282, 1198, 1065 cm⁻¹.

**1H NMR (500 MHz, CDCl₃):** δ = 7.74–7.33 (m, 5 H), 6.23 (s, 1 H), 5.33–5.23 (m, 2 H), 4.33–4.13 (m, 2 H), 4.10 (dd, J = 11.5, 3.4 Hz, 1 H), 3.68 (dt, J = 7.7, 3.9 Hz, 1 H), 3.37–2.57 (m, 4 H).

**13C NMR (126 MHz, CDCl₃):** δ = 169.55 (CO)*, 169.45 (CO), 135.23 (C), 128.80 (2 CH), 128.71 (CH), 128.41 (2 CH)*, 128.39 (2 CH), 100.62 (C), 67.55 (CH₃), 60.88 (CH₃), 58.44 (CH)*, 58.41 (CH), 45.95 (CH₅), 45.78 (CH₃), 44.90 (CH₃)*, 44.57 (CH*), 32.98 (CH*), 32.75 (CH); * signals of rotamers/diastereomers.


S. Baldauf, J. W. Bode

Synthesis

CH\textsuperscript{3+}, 127.99 (2 CH), 127.95 (CH), 127.39 (2 CH\textsuperscript{3+}), 127.37 (2 CH), 127.19 (2 CH\textsuperscript{3+}), 127.06 (2 CH\textsuperscript{2+}), 127.04 (2 CH), 125.61 (2 CH\textsuperscript{3+}), 125.32 (2 CH\textsuperscript{3+}), 125.02 (2 CH\textsuperscript{2+}), 125.02 (2 CH), 120.16 (2 CH\textsuperscript{2+}), 120.09 (2 CH), 101.88 (C\textsuperscript{3+}), 101.65 (C), 68.97 (C\textsuperscript{3+}), 68.34 (CH\textsuperscript{2+}), 60.52 (CH\textsuperscript{2+}), 60.42 (CH\textsuperscript{2}), 53.20 (CH\textsuperscript{2}), 47.06 (CH\textsuperscript{2}), 46.89 (CH\textsuperscript{2}), 36.54 (CH\textsuperscript{2}), 36.37 (CH\textsuperscript{2}), 34.60 (CH\textsuperscript{2}), 34.20 (CH\textsuperscript{2}), 28.79 (CH), 28.67 (C); * signals of rotamers/diastereomers.

HRMS (MALDI/ESI): m/z [M + Na\textsuperscript{+}] calcd for C\textsubscript{23}H\textsubscript{25}NNaO\textsubscript{8}: 518.1783; found: 516.1316.

Ethyl N-[4-(Nitrophenyl)propanoyl]-L-serinate (14)

IR (neat): 3893, 2928, 1740, 1643, 1454, 1313, 1191 cm\textsuperscript{–1}.
HRMS (MALDI/ESI): m/z [M + Na\textsuperscript{+}] calcd for C\textsubscript{14}H\textsubscript{18}NO\textsubscript{6}: 333.1057; found: 333.1061.

Benzyl N-[3-(4-Nitrophenyl)propanoyl]-L-serinate (14)

IR (neat): 3893, 2928, 1737, 1643, 1515, 1454, 1313, 1191 cm\textsuperscript{–1}.
HRMS (MALDI/ESI): m/z [M + Na\textsuperscript{+}] calcd for C\textsubscript{19}H\textsubscript{20}NO\textsubscript{6}: 395.1214.1057; found: 395.1218.

Funding Information
This work was supported by the Swiss National Science Foundation (169451).

Acknowledgment
We thank the MS and NMR services of the Laboratorium für Organische Chemie at ETH Zürich for analyses and Haewon Song for correcting the manuscript.

Supporting Information
Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1611635.
References

(6) Pusterla, I.; Bode, J. W. Nat. Chem. 2015, 7, 668.