

Efficient Synthesis of (S)-4-Phthalimido-1,3,4,5-tetrahydro-8-(2,6-dichlorobenzyloxy)-3-oxo-2H-2-benzazepin-2-acetic Acid (Pht-Hba(2,6-Cl₂-Bn)-Gly-OH)¹

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4-Amino-2-benzazepin-3-ones have proven very useful for studying the biologically active conformations of peptides. The synthesis of Pht-Aba-Xaa-OH by reaction of the corresponding 1,3-oxazolidin-5-one with trifluoromethanesulfonic acid (TFMSA) has been reported in the literature. However, when this procedure was applied to the preparation of Pht-Hba(Bn)-Gly-OH **8**, many byproducts were formed and the yield of the desired aminobenzazepinones **7** and **8** was very low. We report in this paper an efficient methodology for the synthesis of Pht-Hba(2,6-Cl₂-Bn)-Gly-OH **17** starting from the commercially available tyrosine. In our procedure, the dipeptide Pht-Tyr(2,6-Cl₂-Bn)-Gly-OH **15** is converted to the 1,3-oxazolidin-5-one **16** which then undergoes Friedel–Crafts cyclization in the presence of tin tetrachloride to afford the desired 4-phthalimido-1,3,4,5-tetrahydro-8-(2,6-dichlorobenzyloxy)-2-benzazepin-3-one **17** in excellent yield.

Introduction

Conformational restriction is a well-established strategy in the design of more selective and/or more potent peptides as enzyme inhibitors and agonists or antagonists at receptors.^{2,3} Lactams, which maintain a given dipeptidyl unit in the trans amide conformation and bias neighboring χ , ψ and ϕ angles, have proven particularly useful in a number of cases.^{4,5} Moreover, the χ angles in conjunction with the backbone angles define the position of the side-chain functional groups in space and thus must be regarded as of key importance in understanding the mode of action of peptides.^{6,7}

In our ongoing research on the study of the interaction of the antigen HEL(52–61),⁵² Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg⁶¹, with MHC class II I–A^k molecule and

the recognition of the peptide/I–A^k complex by T-cell receptors,^{8,9} we decided to replace the Tyr⁵³ residue by several conformationally constrained amino acids in order to determine the antigenically active conformation of this decapeptide.¹⁰ One such constrained amino acid was the lactam 4-amino-1,3,4,5-tetrahydro-8-hydroxy-2-benzazepin-3-one (Hba) in which the preferred conformations of the side chain are gauche(+) and trans, the gauche(–) conformation being excluded.¹¹ However, the synthesis of Hba has not been reported in the literature. In this paper, we describe an efficient methodology for the synthesis of Pht-Hba(2,6-Cl₂-Bn)-Gly-OH **17** which would be applicable to the preparation of other dipeptides containing the Hba motif.

Results and Discussion

Since glycine is the amino acid succeeding tyrosine in the sequence of HEL(52–61), we first tried to prepare the dipeptide Pht-Hba-Gly by the method described for the synthesis of the phenylalanine analogue Pht-Aba-Gly.^{11–14} Accordingly, Pht-Tyr(Bn)-OH **1** (Scheme 1) was converted to the acid chloride **2** using PCl₅ in benzene at

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(1) Abbreviations and definitions recommended by IUPAC–IUB Commission of Biochemical Nomenclature have been used. Other abbreviations used: Aba, 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one; BOP, benzotriazole-1-yloxytris(dimethylamino)phosphonium-hexafluorophosphate; 2,6-Cl₂-Bn, 2,6-dichlorobenzyloxy; EI, electrospray ionization; EMM, exact mass measurement; GITC, 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate; Hba, 4-amino-1,2,4,5-tetrahydro-8-hydroxy-2-benzazepin-3-one; HEL, hen egg lysozyme; HOBt, N-hydroxybenzotriazole; MHC, major histocompatibility complex; Pht, phthaloyl; TFA, trifluoroacetic acid; TFMSA, trifluoromethane sulfonic acid.

(2) Warshawsky, A. M.; Flynn, G. A.; Koehl, J. R.; Mehdi, S. *Bioorg. Med. Chem. Lett.* **1996**, 6, 957.

(3) Mosberg, H. I.; Lomize, A. I.; Wang, C.; Kroona, H.; Heyl, D. L. *J. Med. Chem.* **1994**, 37, 4371.

(4) Flynn, G. A.; Giroux, E. L.; Dage, R. *J. Am. Chem. Soc.* **1987**, 109, 9, 7914.

(5) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein R. *Science* **1980**, 210, 656.

(6) Hruby, V. J.; Li, G.; Haskell-Luevano, C. *Biopolymers* **1997**, 43, 219.

(7) Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, 55, 585.

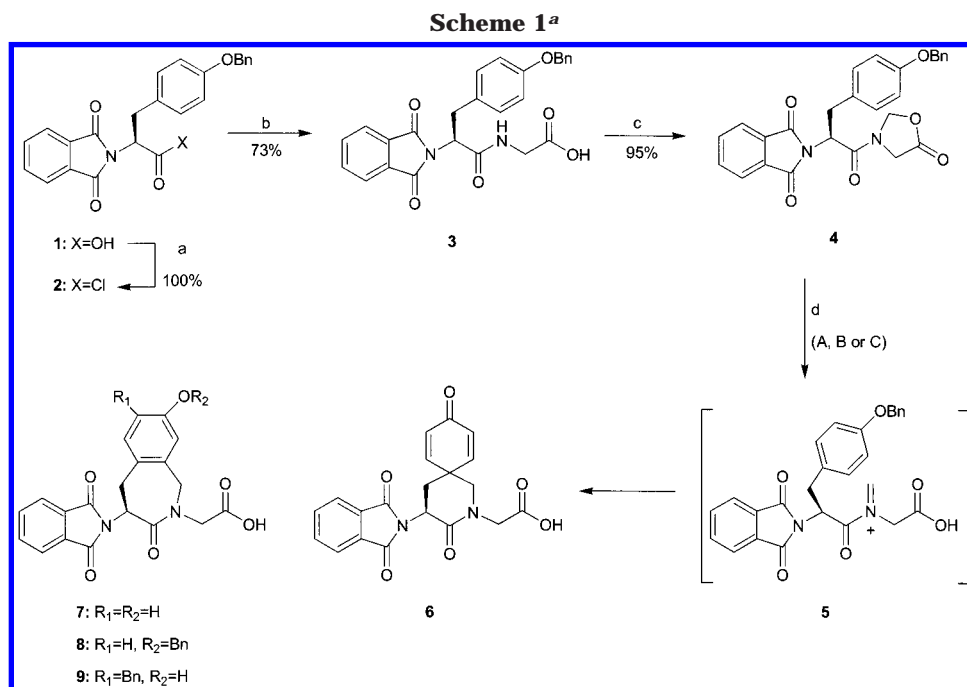
(8) Hernandez, J. F.; Cretin, F.; Lombard-Platet, S.; Salvi, J. P.; Walchshofer, N.; Gerlier, D.; Paris, J.; Rabourdin-Combe, C. *Peptides* **1994**, 15, 583.

(9) Ettouati, L.; Casimir, J. R.; Raynaud, I.; Trescol-Biemont, M.-C.; Carrupt, P.-A.; Gerlier, D.; Rabourdin-Combe, Ch.; Testa, B.; Paris, J. *Prot. Pept. Lett.* **1998**, 5, 221.

(10) Casimir J. R.; Trescol-Biemont, M.-C.; Tourwé, D.; Paris, J.; Ettouati, L.; Iterbeke, K.; Van Den Nest, W.; Gerlier, D.; Rabourdin-Combe, C. In *Peptides 1998, Proceedings of the 25th European Peptide Symposium*; Bajusz, S., Hudecz F., Eds.; Akadémiai Kiado: Budapest, 1999; p 654.

(11) Tourwé, D.; Verschueren, K.; Frycia, A.; Davis, P.; Porreca, F.; Hruby, V. J.; Toth, G.; Jaspers, H.; Verheyden, P.; Van Binst, G. *Biopolymers* **1996**, 38, 1.

(12) Flynn, G. A.; Burkholder, T. P.; Huber, E. W.; Bey, P. *Bioorg. Med. Chem. Lett.* **1991**, 6, 309.



^a (a) PCl₅, C₆H₆, 50–55 °C, 2.5 h; (b) Na₂CO₃·10H₂O, glycine, rt, 1 h; (c) (CH₂O)_n, *p*TsOH cat., reflux, 6 h; (d) Method A: CH₂Cl₂, TFMSA, rt, 24 h. Method B: CH₂Cl₂, SnCl₄, reflux, 4 h then rt, 20 h. Method C: CH₂Cl₂, SnCl₄, rt, 24 h.

50–55 °C,¹⁵ and then reacted with glycine under the Schotten–Baumann conditions¹² to afford the dipeptide **3** in 73% yield. No racemization was detected during this reaction, as demonstrated by GITC derivatization¹⁶ after phthaloyl deprotection.¹⁷ The dipeptide **3** was then converted into the oxazolidinone **4** in 95% yield by reaction with paraformaldehyde in the presence of catalytic amount of *p*-toluenesulfonic acid.¹³ Under acidic conditions and in the absence of nucleophiles, the oxazolidinone **4** yields an acyliminium ion **5** which would form the desired compound **8** by Friedel–Crafts cyclization. However, reaction of compound **4** with trifluoromethanesulfonic acid (TFMSA) in dichloromethane¹³ (method A, Scheme 1) afforded a mixture from which the spirodienone **6** (13%) and the 4-amino-2-benzazepin-3-ones **7** (7%) and **8** (3%) were isolated. As demonstrated by HPLC analysis of the crude before purification, no trace of the oxazolidinone **4** was detected in the mixture. The remaining sticky material consisted of several products which were difficult to characterize. The structure of spirodienone **6** was unambiguously assigned by the characteristic four ethylenic protons in the ¹H NMR spectrum as well as the chemical shift of 184 ppm in the ¹³C NMR and the absorption at 1663 cm^{−1} in the IR spectra, respectively, both of them typical for the dienone carbonyl.¹⁸ The yield of the aminobenzazepinone **7** or **8** might be improved by changing from TFMSA to a Lewis acid catalyst. Thus, when the oxazolidinone **4** was reacted

Table 1. Comparison of TFMSA with Tin Tetrachloride in the Synthesis of Aminobenzazepinones from Oxazolidinones **4 and **16****

entry	oxazolidinone	reaction conditions ^a	products formed (% yield) ^{b,c}				
			6	7	8	9	17
1	4	method A	13 (57)	7 (33)	3 (10)		
2	4	method B		50 (68)		23 (32)	
3	4	method C		(67)		(33)	
4	16	method D					84

^a Method A: TFMSA, CH₂Cl₂, rt, 24 h. Method B: SnCl₄, CH₂Cl₂, reflux, 4 h then rt, 20 h. Method C: SnCl₄ (1 M in CH₂Cl₂), rt, 24 h. Method D: SnCl₄, CH₂Cl₂, reflux, 3 h then rt, 24 h.

^b Yields of isolated products. ^c The relative percentages of compounds present in the crude were determined by HPLC and are given in parentheses.

with tin tetrachloride (method B, Scheme 1) the benzazepinone **7** was isolated in 50% yield, showing that tin tetrachloride is a better catalyst than TFMSA for the formation of Hba ring from oxazolidinone **4** (Table 1).

The formation of compound **6** in method A (Scheme 1), however, prompted us to reinvestigate the mechanism of the cyclization reaction. Indeed, we reasoned that the aminobenzazepinone **7** might have been formed from **6** by the dienone–phenol rearrangement.^{19,20} In such a case, the 7-hydroxy-substituted isomer **10** (Scheme 2) would also be expected. However, when compound **6** was subjected to TFMSA in dichloromethane for 24 h (under the same acidic conditions used in method A, Scheme 1),¹³ we did not observe any rearrangement compound **7** or **10**. Moreover, the structure of **7** was confirmed by ¹H NMR analysis. 2D experiments allowed us to assign the spin system of the phenolic ring protons, and ROESY experiments showed the presence of NOEs between the isolated H(9) and the benzylic H(1) protons, as well as between H(6) and the H(5) protons which firmly establishes the position of the HO-substituent. These results

(13) de Laszlo, S. E.; Bush, B. L.; Doyle, J. J.; Greenlee, W. J.; Hangauer, D. G.; Halgren, T. A.; Lynch, R. J.; Schorn, T. W.; Siegl, P. K. S. *J. Med. Chem.* **1992**, *35*, 833.

(14) Tourwé, D.; Verschuere, K.; Van Binst G.; Davis P.; Porreca F.; Hraby, V. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1305.

(15) Almquist, R. G.; Olsen, C. M.; Uyeno, E. T.; Toll, L. *J. Med. Chem.* **1984**, *27*, 115.

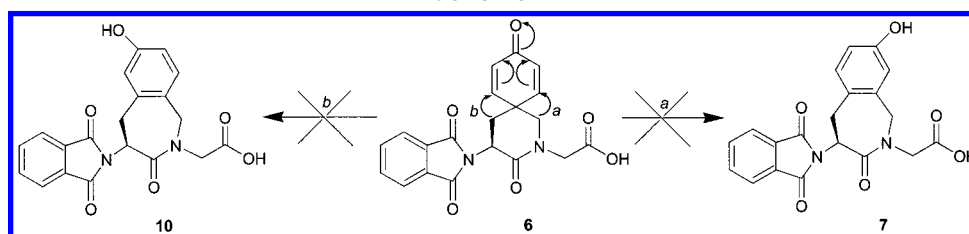
(16) Nimura, N.; Ogura, H.; Kinoshita, T. *J. Chromatogr.* **1980**, *202*, 375.

(17) Sheehan, J. C.; Chapman, D. W.; Roth, R. W. *J. Am. Chem. Soc.* **1952**, *74*, 3822.

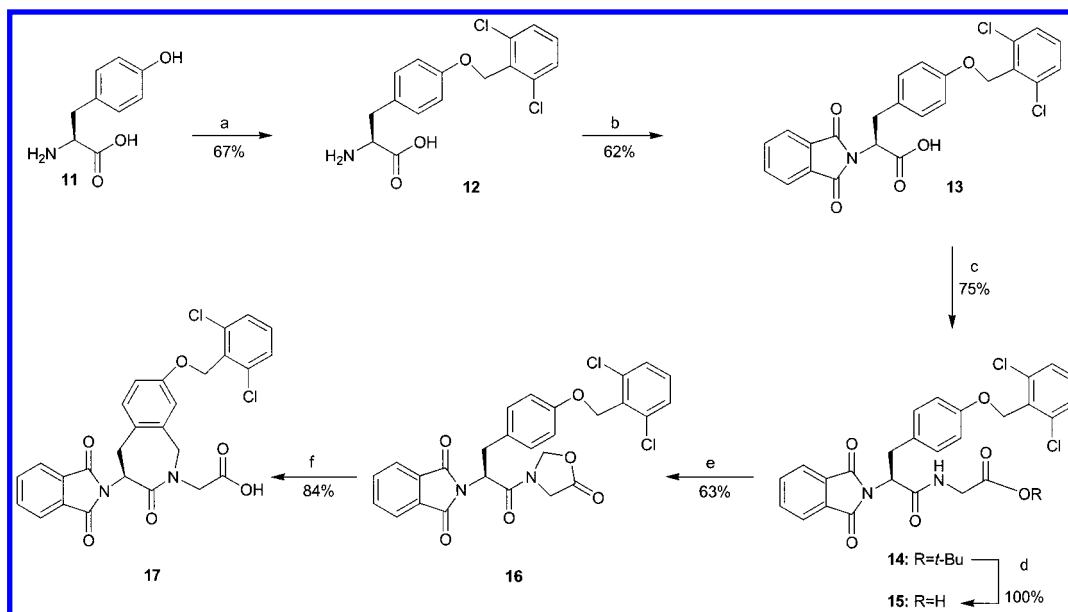
(18) Kita, Y.; Takada, T.; Gyoten, M.; Tohma, H.; Zenk, M. H.; Eichhorn, J. *J. Org. Chem.* **1996**, *61*, 5857.

(19) Vitullo, V. P.; Grossman, N. *J. Am. Chem. Soc.* **1972**, *94*, 3844.

(20) Vitullo, V. P. *J. Org. Chem.* **1968**, *34*, 224.

Scheme 2^a

^a Reaction conditions: CH₂Cl₂, TFMSA, rt, 24 h.

Scheme 3^a

^a (a) (i) 2 N NaOH, CuSO₄·5H₂O, 60 °C then rt, (ii) 2,6-Cl₂BnBr, 90 min; (b) 1,4-dioxane/H₂O, Et₃N, PhtO, reflux, Dean–Stark; (c) GlyOtBu·HCl, BOP/HOBT, Pr₂NEt, CH₂Cl₂; (d) CH₂Cl₂/TFA, reflux, 2 h; (e) (CH₂O)_n, *p*-TsOH cat., reflux, 6 h; (f) CH₂Cl₂, SnCl₄, reflux, 3 h then rt, 24 h.

indicate that the formation of the Hba ring does not occur via a dienone-phenol rearrangement of the spirodienone **6**, but rather through a Friedel–Crafts alkylation of the aromatic ring at the meta position with respect to the benzyloxy or hydroxyl group.

The major drawback of the synthesis of Pht-Hba-Gly **7** from oxazolidinone **4** and tin tetrachloride (method B, Scheme 1), however, is the formation of nonnegligible amount (23%, Table 1) of the 7-benzylated compound **9** resulting from the Fries rearrangement.²¹

In an attempt to improve the yield of this aminobenzazepinone **7**, we replaced the benzyl group by the 2,6-dichlorobenzyl protection which is less prone to Fries rearrangement and more stable toward acidolysis than the benzyl group. Accordingly, *L*-tyrosine **11** was converted to *L*-Tyr(2,6-Cl₂-Bn)-OH **12**, using 2,6-dichlorobenzylbromide and copper sulfate,²² then allowed to react with phthalic anhydride in the presence of Et₃N to afford Pht-Tyr(2,6-Cl₂-Bn)-OH **13** in good yield as shown in Scheme 3.²³ The dipeptide **15** was obtained in 75% yield from compound **13** after reaction with *tert*-butylglycine hydrochloride followed by deprotection with tri-

fluoroacetic acid (TFA). This dipeptide **15** was converted to the oxazolidinone **16** which was then reacted with tin tetrachloride as shown (Scheme 3). To our satisfaction, the aminobenzazepinone **17** was isolated in 84% yield (Table 1), showing not only that the undesired Fries rearrangement product can be avoided by replacing the benzyl group by its 2,6-dichloro derivative but also that, unlike the benzyl group, the 2,6-dichlorobenzyl derivative is resistant to cleavage by tin tetrachloride. This is very advantageous since after phthaloyl deprotection and subsequent protection of the primary amino group by a Boc group the protected dipeptide **17** is more convenient than analogue **7** for incorporation into peptides sequences. Moreover, the fact that the aminobenzazepinone **17** could be obtained from oxazolidinone **16** without loss of the 2,6-dichlorobenzyl protection provides further evidence that the formation of the Hba ring does not occur via the dienone–phenol rearrangement of a spirodienone.

Conclusion

In conclusion, we have disclosed in this paper an efficient methodology for the preparation of Pht-Hba(2,6-Cl₂-Bn)-Gly-OH. Our procedure could be efficiently applied to the preparation of other dipeptides of type Pht-Hba(2,6-Cl₂-Bn)-Xaa-OH or Pht-Aba-Xaa-OH and therefore would be very useful to the synthesis of peptide

(21) Erickson, B. W.; Merrifield, R. B. *J. Am. Chem. Soc.* **1973**, *95*, 3750.

(22) Bodanszky, M.; Bodanszky, A. In *The Practice of Peptide Synthesis*; Hafner, K., Ed.; Springer-Verlag: Berlin, 1984; p 62.

(23) Wünsch E.; Wendlberger G.; Jentsch J. *Chem. Ber.* **1964**, *97*, 8.

analogues designed to interact with relevant biological targets.¹⁰ We have already demonstrated the use of the phthaloyl-protected dipeptide mimic in peptide synthesis on a 4-methylbenzhydrylamine resin.²⁴ The phthaloyl group can also be easily replaced by a Boc-group for use in the Boc-peptide synthesis protocol,^{10,17} where the 2,6-dichlorobenzyl group is a standard protection for tyrosine.²¹

Experimental Section

General Methods. Melting points were measured on a hot stage apparatus and were not corrected. IR spectra were carried out on a FT-IR spectrometer. Optical rotations were determined at rt (22 °C) on a digital polarimeter. Mass spectra (positive mode) were recorded using electrospray ionization (EI) or a linear MALDI-TOF instrument using α -cyano-4-hydroxycinnamic acid as matrix. Exact mass measurements (EMM) were recorded at low resolution using electrospray ionization and a quadrupole mass spectrometer with PEG-Na or PEG-Me as standard.²⁵ 1D NMR spectra were recorded in CDCl₃ or DMSO-*d*₆ on a 250 or 500 MHz spectrometer. Preparative HPLC separations were performed with a RP-C₁₈ column (15–20 μ m, 2.5 \times 15 cm) at a flow rate of 13 mL/min with UV detection at λ = 215 nm by using a linear gradient of A (0.1% TFA in H₂O) and B (0.1% TFA in MeCN/H₂O, 80:20).

(S)-N²-Phthaloyl-O-benzyltyrosine Chloride (2).¹⁵ A solution of N²-Pht-Tyr(Bn)-OH **1** (1.01 g, 2.51 mmol) and PCl₅ (0.83 g, 4 mmol) in dry benzene (10 mL) was stirred at 50–55 °C for 90 min, and then the solvent was eliminated under reduced pressure. The residue was redissolved in dry benzene and evaporated. This last operation was repeated twice to yield a white solid (1.05 g, 100%): ¹H NMR (500 MHz, CDCl₃) δ 3.49 (dd, *J* = 10.8, 14.3 Hz, 1H), 3.58 (dd, *J* = 5.2, 14.3 Hz, 1H), 4.94 (s, 2H), 5.27 (dd, *J* = 5.2, 10.8 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.31 (m, 5H), 7.73 (m, 2H), 7.82 (m, 2H).

(S)-N²-Phthaloyl-O-benzyltyrosinylglycine (3). A solution of N²-Pht-Tyr(Bn)-Cl **2** (1.05 g, 2.5 mmol) in dry acetone (27.8 mL) was added slowly (1 h) to a solution of glycine (0.45 g, 6.0 mmol) and Na₂CO₃·10H₂O (1.41 g, 4.92 mmol) in 40.7 mL of the mixture acetone/water (7:4). After removal of acetone in vacuo, the mixture was diluted with water (25 mL) and acidified with 10 N HCl until pH 1–2. The resulting aqueous solution was extracted with EtOAc (3 \times 50 mL), and the combined organic phases were concentrated to 50 mL. The latter solution was extracted with 6% NaHCO₃ solution (3 \times 30 mL), and the combined aqueous phases acidified to pH 1 with 10 N HCl then extracted with EtOAc (3 \times 100 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo to give a residue which was recrystallized from EtOH/water (7:3) to yield a white solid (0.84 g, 73%): mp 152 °C; [α]_D –139.2 (*c* 1.0, MeOH); IR (KBr) ν 3500–2548, 3490, 3375, 3033, 1774, 1714, 1675, 1612 cm^{–1}; ¹H NMR (250 MHz, DMSO-*d*₆) δ 3.30 (d, *J* = 14.1 Hz, 1H), 3.45 (dd, *J* = 4.8, 14.1 Hz, 1H), 3.66 (dd, *J* = 5.5, 17.3 Hz, 1H), 3.86 (dd, *J* = 6.0, 7.3 Hz, 1H), 4.95 (s, 2H), 4.97 (m, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 7.29 (m, 5H), 7.80 (s, 4H), 8.49 (m, 1H); MS (EI) *m/z* 459 (M + 1, 100), 460 (28), 481 (M + 23, 8), 917 (45), 918 (25); EMM calcd for C₂₆H₂₂N₂O₆ *m/z* 459.1556 (MH⁺), found 459.1550.

3-[2(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-3-(4-benzoyloxyphenyl)propanoyl]-1,3-oxazolidin-5-one (4). A suspension of the dipeptide **3** (6.46 g, 14.1 mmol), *p*-toluenesulfonic acid (0.31 g, 1.63 mmol), and paraformaldehyde (5.46 g, 13.8 equiv) in dry benzene (200 mL) was refluxed for 6 h with azeotropic removal of water (3.2 g of paraformaldehyde

was added at intervals of 1.5 h). The benzene was eliminated under reduced pressure, and the residue dissolved in 400 mL of CHCl₃. The solution was washed with aqueous 8% NaHCO₃ (2 \times 250 mL) and water (1 \times 200 mL), dried with MgSO₄, and concentrated in vacuo to yield a pale-yellow solid (6.3 g, 95%): mp 87–89 °C; [α]_D –130.7 (*c* 1.1, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δ 3.40 (dd, *J* = 8.7, 14.1 Hz, 1H), 3.59 (dd, *J* = 7.2 Hz, 14.1 Hz, 1H), 3.93 (m, 2H), 4.96 (m, 2H), 4.97 (m, 1H), 5.41 (d, *J* = 28.4 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 2H), 7.30 (m, 5H), 7.71 (m, 2H), 7.79 (m, 2H); ¹³C NMR (62.5 MHz, CDCl₃) δ 33.9 (CH₂), 43.9 (CH₂), 54.1 (CH), 70.0 (CH₂), 115.2 (CH), 123.8 (CH), 127.4 (CH), 128.0 (C), 128.4 (C), 128.6 (CH), 130.3 (CH), 131.2 (C), 134.6 (CH), 136.9 (C), 158.0 (C), 166.3 (C), 167.4 (C), 168.7 (C); MS (EI) *m/z* 471 (M + 1, 100), 472 (30), 473 (20), 493 (M + 23, 26), 494 (11); EMM calcd for C₂₇H₂₂N₂O₆ *m/z* 471.1556 (MH⁺), found 471.1606.

Synthesis of Spirodienone (6) and Aminobenzazepinones (7–9). Method A (Scheme 1). A solution of oxazolidinone **4** (3.24 g, 6.9 mmol) in CH₂Cl₂ (11 mL) under nitrogen atmosphere was cooled to 0 °C, then TFMSA (10 mL) was rapidly added. After 30 min, the cooling bath was removed and stirring continued at room temperature for 24 h before dilution of the mixture with CH₂Cl₂ (70 mL). The flask was cooled to 0 °C, and then water (70 mL) was slowly added. The two layers were separated, and then the aqueous layer was extracted with a 6% solution of NaHCO₃ (3 \times 140 mL). The combined aqueous phase was acidified to pH 2 with 6 N HCl and then extracted with CH₂Cl₂ (3 \times 250 mL). The combined organic phase was dried with MgSO₄ and then concentrated under reduced pressure to give a brown residue that was purified by flash chromatography (SiO₂, EtOAc/MeOH, 1:1) to yield 0.875 g of a brown solid. RP-HPLC purification of 100 mg of this mixture afforded three white solids: **6** (39 mg, 13%), **7** (21 mg, 7%) and **8** (11 mg, 3%). **Method B (Scheme 1).** A solution of SnCl₄ (0.6 mL, 5.13 mmol) in CH₂Cl₂ (10 mL) was added dropwise (over 10 min) to a stirred solution of oxazolidinone **4** (0.91 g, 1.936 mmol) in dichloromethane (20 mL). The solution was refluxed for 4 h, and then stirring was continued at room temperature for 20 h. The mixture was cooled in an ice bath and then slowly hydrolyzed with 0.5 N HCl (30 mL) over a period of 15 min. The two layers were separated. The solid remaining in the flask was dissolved in EtOAc (50 mL) and the solution washed with water (2 \times 20 mL). The combined organic phase was concentrated in vacuo to give a light yellow solid that was dissolved in EtOAc (15 mL) and precipitated by slow addition of cyclohexane (45 mL). After being cooled to 0 °C, the solid was collected by filtration and dried in vacuo to give 0.58 g of a white solid consisting only of the two aminobenzazepinones **7** and **9**. A 50 mg portion of this mixture was purified by RP-HPLC to afford 31 mg (50%) of **7** and 18 mg (23%) of **9**. **Method C (Scheme 1).** A solution of oxazolidinone **4** (0.33 g, 0.7 mmol) in 10 mL of a molar solution of SnCl₄ in CH₂Cl₂ was stirred at room temperature for 24 h. The reaction was stopped by hydrolysis with 1 N HCl (10 mL), concentrated in vacuo, and then analyzed by HPLC (Table 1).

2-[4(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-1,2,4,5-tetrahydro-2-azin-3-one-6-spiro-1'-cyclohexa-2',5'-dien-4'-one]acetic acid (6): yield 39 mg (13%, method A); mp 157 °C; [α]_D –106.0 (*c* 0.7, AcOH); IR (KBr) ν 3500–2400, 3048, 1778, 1715, 1663 (CO spirodienone), 1619 cm^{–1}; ¹H NMR (250 MHz, DMSO-*d*₆) δ 1.98 (dd, *J* = 7.1, 12.6 Hz, 1H), 2.77 (m, 1H), 3.40 (d, *J* = 12.4 Hz, 1H), 3.86 (d, *J* = 12.4 Hz, 1H), 3.98 (d, *J* = 17.1 Hz, 1H), 4.17 (d, *J* = 17.1 Hz, 1H), 5.01 (dd, *J* = 7.0, 7.1 Hz, 1H), 6.27 (d, *J* = 9.9 Hz, 1H), 6.33 (d, *J* = 10.2 Hz, 1H), 7.19 (d, *J* = 9.9 Hz, 1H), 7.64 (d, *J* = 10.2 Hz, 1H), 7.89 (s, 4H); ¹³C NMR (62.5 MHz, DMSO-*d*₆) δ 34.3 (CH₂), 46.4 (CH), 48.6 (CH₂), 54.1 (CH₂), 123.2 (CH), 128.8 (CH), 129.0 (CH), 131.1 (C), 134.7 (CH), 149.6 (CH), 151.8 (CH), 165.4 (C), 166.9 (C), 169.7 (C), 184.3 (C, CO spirodienone); MS (EI) *m/z* 381 (M + 1, 100), 382 (22), 383 (15), 403 (M + 23, 50), 404 (11); EMM calcd for C₂₀H₁₆N₂O₆ *m/z* 381.1087 (MH⁺), found 381.1377.

4(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-1,3,4,5-tetrahydro-8-hydroxy-3-oxo-2H-2-benzazepin-2-acetic acid

(24) Tourwé, D.; Nikiforovich, G. V.; Iterbeke, K. In *Peptides 1996, Proceedings of the 24th European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower: Kingswinford, 1998; p 835.

(25) Tyler, A. N.; Clayton, E.; Green, B. N. *Anal. Chem.* **1996**, *68*, 3561.

(7): yield 21 mg (7%, method A), 31 mg (50%, method B); mp 175 °C; $[\alpha]_D -2.0$ (*c* 1.0, AcOH); IR (KBr) ν 3516, 3500–2400, 3156, 1765, 1740, 1692, 1656, 1600 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 3.14 (dd, *J* = 4.2, 15.5 Hz, 1H), 3.89 (m, 1H), 4.00 (d, *J* = 16.9 Hz, 1H), 4.21 (d, *J* = 16.9 Hz, 1H), 4.47 (d, *J* = 16.0 Hz, 1H), 4.80 (d, *J* = 16.0 Hz, 1H), 5.23 (m, 1H), 6.67 (d, *J* = 8.1 Hz, 1H), 6.71 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 7.89 (m, 4H), 9.35 (s, 1H); MS (EI) *m/z* 381 (*M* + 1, 100), 382 (22), 400 (38), 403 (*M* + 23, 30); EMM calcd for C₂₀H₁₆N₂O₆ *m/z* 381.1087 (MH⁺), found 381.1191.

4(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-1,3,4,5-tetrahydro-8-benzoyloxy-3-oxo-2H-2-benzazepin-2-acetic acid (8): yield 11 mg (3%, method A); IR (KBr) ν 3500–2400, 3153, 1767, 1738, 1602 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 2.71 (m, 1H), 3.05 (m, 1H), 3.60 (s, 2H), 3.90 (d, *J* = 12.1 Hz, 1H), 3.80–4.42 (m, 2H), 4.34 (d, *J* = 17.2 Hz, 1H), 5.01 (dd, *J* = 6.9, 12.9 Hz, 1H), 6.25 (s, 1H), 6.50 (d, *J* = 10.5 Hz, 1H), 7.11 (d, *J* = 10.5 Hz, 1H), 7.25 (m, 5H), 7.71 (m, 2H), 7.81 (m, 2H); MS (EI) *m/z* 471 (*M* + 1).

4(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-1,3,4,5-tetrahydro-7-benzyl-8-hydroxy-3-oxo-2H-2-benzazepin-2-acetic acid (9): yield 18 mg (23%, method B); mp 161 °C; $[\alpha]_D -2.0$ (*c* 1.0, AcOH); IR (KBr) ν 3396, 3500–2400, 3027, 1774, 1715, 1654, 1618 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 3.08 (dd, *J* = 4.2, 15.9 Hz, 1H), 3.85 (s, 2H), 3.87 (m, 1H), 4.01 (d, *J* = 17.3 Hz, 1H), 4.21 (d, *J* = 17.3 Hz, 1H), 4.42 (d, *J* = 16.1 Hz, 1H), 4.79 (d, *J* = 16.1 Hz, 1H), 5.21 (dd, *J* = 4.2, 12.5 Hz, 1H), 6.72 (s, 1H), 6.90 (s, 4H), 7.15 (m, 1H), 7.23 (m, 4H), 7.89 (m, 4H), 9.39 (s, 1H); MS (EI) *m/z* 471 (*M* + 1); EMM calcd for C₂₇H₂₂N₂O₆ *m/z* 471.1556 (MH⁺), found 471.1552.

(S)-O-(2,6-Dichlorobenzyl)tyrosine (12). A solution of CuSO₄·5H₂O (7.03 g, 28.16 mmol) in 20 mL of water was added to a stirred solution of L-tyrosine **11** (10 g, 55.2 mmol) in 56 mL of 2 N NaOH (2.03 equiv). The mixture was heated to 60 °C and then allowed to cool to room temperature. MeOH (194 mL), 2 N NaOH (8 mL), and 2,6-dichlorobenzyl bromide (14.2 g, 58.9 mmol) were added. After being stirred at room temperature for 5 h, the mixture was filtered, and then the residue was successively washed with MeOH (26 mL) and water (100 mL then 15 mL). The residue was triturated in 1 N HCl (7 × 27.5 mL) and washed with water (3 × 100 mL) and 1 N NH₄OH (7 × 27.5 mL). After further washings with acetone (2 × 25 mL), water (2 × 25 mL), and Et₂O (2 × 25 mL), the product was dried in a vacuum to afford a white solid (12.6 g, 67%): mp 215–216 °C; $[\alpha]_D -17.4$ (*c* 1.0, MeOH); ¹H NMR (250 MHz, DMSO-*d*₆) δ 3.03 (d, *J* = 6.2 Hz, 2H), 4.11 (t, *J* = 6.2 Hz, 1H), 5.16 (s, 2H), 6.99 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 8.6 Hz, 2H), 7.39–7.55 (m, 3H), 8.29 (br s, 2H), 11.24 (br s, 1H); ¹³C NMR (62.5 MHz, DMSO-*d*₆) δ 35.1 (CH₂), 54.8 (CH), 65.3 (CH₂), 114.5 (CH), 128.2 (CH), 128.7 (CH), 130.7 (CH), 131.6 (C), 136.8 (C), 158.0 (C), 170.2 (C); MS (MALDI-TOF) *m/z* 340 (*M* + 1), 341, 342, 379, 380; EMM calcd for C₁₆H₁₅Cl₂NO₃ *m/z* 340.0507 (MH⁺), found 340.0653.

(S)-N^α-Phthaloyl-O-(2,6-dichlorobenzyl)tyrosine (13). A solution H₂N-Tyr(2,6-Cl₂-Bn)-OH **12** (12.58 g, 37.1 mmol) and phthalic anhydride (5.51 g, 37.2 mmol) in 312 mL of 1,4-dioxane was stirred at room temperature for 30 min. Et₃N (10.2 mL in 39 mL of water) was added. After being stirred at room temperature for 90 min, the mixture was diluted with 1,4-dioxane (390 mL) and Et₃N (10.2 mL), and then the mixture was gently heated with azeotropic removal of water (Dean–Stark). The excess dioxane was eliminated by evaporation then, the residue was dissolved in EtOAc (350 mL). The solution was washed with 1N HCl (3 × 150 mL), dried with MgSO₄, filtered then, concentrated in vacuo. The crude was finally purified by flash chromatography (EtOAc/hexane, 1:1) to afford the desired product as white solid (10.81 g, 62%): mp 159 °C; $[\alpha]_D +6.3$ (1.2, MeOH); ¹H NMR (250 MHz, DMSO-*d*₆) δ 3.05 (m, 2H), 4.59 (m, 1H), 5.21 (s, 2H), 7.01 (d, *J* = 8.6 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.35 (m, 1H), 7.44–7.61 (m, 4H), 7.74 (m, 1H), 8.68 (d, *J* = 8.0 Hz, 1H), 12.74 (br s, 1H); ¹³C NMR (62.5 MHz, DMSO-*d*₆) δ 35.7 (CH₂), 53.9 (CH),

64.8 (CH₂), 114.2 (CH), 127.6 (CH), 128.7 (CH), 128.9 (CH), 129.3 (CH), 130.1 (CH), 130.3 (C), 130.7 (CH), 131.4 (CH), 131.7 (CH), 135.9 (C), 137.3 (C), 157.0 (C), 168.0 (C), 172.7 (C).

(S)-N^α-Phthaloyl-O-2,6-dichlorobenzyltyrosinylglycine tert-Butyl Ester (14). A solution of Pht-Tyr(2,6-Cl₂-Bn)-OH **13** (3.9 g, 6.78 mmol), Gly-OtBu-HCl (1.25 g, 7.46 mmol), BOP (3.3 g, 7.45 mmol), and HOBT (1.14 g, 7.45 mmol) in 75 mL of CH₂Cl₂ and *i*-Pr₂NEt (5.9 mL, 5 equiv) was stirred at room temperature for 5 h. The mixture was diluted with 75 mL of CH₂Cl₂ and then successively washed with 1 N HCl (2 × 100 mL), saturated NaHCO₃ (2 × 100 mL), brine (100 mL), and water (100 mL). The organic phase was dried with Na₂SO₄, filtered, and concentrated in vacuo to afford a white solid (3.0 g, 100%): mp 66 °C; $[\alpha]_D -113.4$ (*c* 0.7, MeOH); ¹H NMR (250 MHz, CDCl₃) δ 1.47 (s, 9H), 3.66 (m, 2H), 3.97 (d, *J* = 4.9 Hz, 2H), 5.16 (s, 2H), 5.17 (m, 1H), 6.76–6.88 (m, 3H), 7.10–7.36 (m, 4H), 7.69–7.83 (m, 4H); ¹³C NMR (62.5 MHz, CDCl₃) δ 28.0 (CH₃), 34.0 (CH₂), 42.4 (CH₂), 55.9 (CH), 55.4 (CH), 82.5 (C), 115.4 (CH), 123.6 (CH), 128.4 (CH), 129.3 (C), 129.9 (CH), 130.4 (CH), 131.5 (C), 132.2 (C), 134.3 (CH), 136.9 (C), 157.9 (C), 168.0 (C), 168.6 (C); MS (MALDI-TOF) *m/z* 605 (*M* + 23), 606, 607, 608, 609; EMM calcd for C₂₄H₁₇Cl₂N₂O₅ *m/z* 583.1403 (MH⁺), found 583.1551.

(S)-N^α-Phthaloyl-O-2,6-dichlorobenzyltyrosinylglycine (15). TFA (10 mL) was added to a solution of Pht-Tyr(2,6-Cl₂-Bn)-Gly-OtBu **14** (0.65 g, 1.115 mmol) in 20 mL of CH₂Cl₂, and then the mixture was refluxed for 2 h. After evaporation of the CH₂Cl₂ and TFA, water (50 mL) was added then product was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water (50 mL), dried with Na₂SO₄, filtered, and evaporated to afford a white solid (0.59 g, 100%): mp 146–149 °C; $[\alpha]_D -115.7$ (*c* 1.5, MeOH); ¹H NMR (250 MHz, CDCl₃) δ 3.45 (m, 2H), 4.00 (m, 2H), 5.05 (s, 2H), 5.15 (m, 1H), 6.75 (d, *J* = 8.2 Hz, 2H), 7.03 (d, *J* = 8.1 Hz, 2H), 7.07–7.22 (m, 2H), 7.39 (m, 1H), 7.57–7.64 (m, 4H), 10.54 (s, 1H); ¹³C NMR (62.5 MHz, CDCl₃) δ 33.8 (CH₂), 41.6 (CH₂), 55.4 (CH), 65.4 (CH), 115.4 (CH), 123.6 (CH), 128.4 (CH), 129.1 (C), 130.0 (CH), 130.4 (CH), 131.3 (C), 132.1 (C), 134.4 (CH), 136.9 (C), 157.9 (C), 168.2 (C), 170.2 (C), 172.8 (C); MS (MALDI-TOF) *m/z* 527 (*M* + 1), 528, 529, 549 (*M* + 23), 550, 551, 552, 553, 565, 572; EMM calcd for C₂₆H₂₀Cl₂N₂O₆ *m/z* 527.0777 (MH⁺), found 527.0796.

3-[2(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-3-(4-(2,6-dichlorobenzoyloxyphenyl)propanoyl)-5-oxazolidinone (16). Prepared from **15** (1.42 g, 2.69 mmol) using the procedure described for the synthesis of **4**. Oxazolidinone **16** was obtained as a pale yellow solid (0.91 g, 63%): mp 84 °C; $[\alpha]_D -114.2$ (*c* 0.9, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δ 3.47 (dd, *J* = 8.7, 14.2 Hz, 1H), 3.64 (dd, *J* = 7.2, 14.2 Hz, 1H), 4.09 (m, 2H), 5.02 (s, 2H), 5.02 (dd, *J* = 7.2, 8.7 Hz, 1H), 5.21 (s, 2H), 5.60 (d, *J* = 28.4 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.21–7.39 (m, 3H), 7.74–7.96 (m, 4H); ¹³C NMR (62.5 MHz, CDCl₃) δ 33.9 (CH₂), 43.9 (CH₂), 53.9 (CH), 65.4 (CH₂), 115.4 (CH), 123.8 (CH), 128.5 (CH), 128.7 (C), 130.0 (C), 130.3 (CH), 130.4 (CH), 131.1 (C), 132.1 (C), 134.6 (CH), 136.9 (C), 158.1 (C), 167.4 (C), 168.7 (C); MS (MALDI-TOF) *m/z* 539 (*M* + 1), 541, 542, 543, 544, 561 (*M* + 23), 562, 563, 564, 565; EMM calcd for C₂₇H₂₀Cl₂N₂O₆ *m/z* 539.0777 (MH⁺), found 539.0819.

4(S)-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-1,3,4,5-tetrahydro-8-(2,6-dichlorobenzoyloxy)-3-oxo-2H-2-benzazepine-2-acetic Acid (17). Method D (Scheme 3). To a solution of oxazolidinone **16** (0.87 g, 1.61 mmol) in 20 mL of freshly distilled CH₂Cl₂ was added SnCl₄ (1 mL in 10 mL of CH₂Cl₂) over 10 min. The mixture was refluxed for 3 h, and then stirring was continued at rt for 24 h. After elimination of the solvent and SnCl₄ under reduced pressure, the residue was dissolved in acetonitrile and water and then evaporated again. The crude was then purified by flash chromatography (CH₂Cl₂/acetone/MeOH, 85:10:5) to afford the aminobenzazepinone **17** as a white solid (0.731 g, 84%): mp 177–179 °C; $[\alpha]_D -40.9$ (*c* 1.1, AcOH); ¹H NMR (250 MHz, DMSO-*d*₆) δ 3.27 (dd, *J* = 4.5, 15.9 Hz, 1H), 3.77 (d, *J* = 16.7 Hz, 1H), 3.99 (dd, *J* = 12.2, 15.9 Hz, 1H), 4.22 (d, *J* = 16.7 Hz, 1H), 4.41 (d, *J* =

16.3 Hz, 1H), 4.96 (d, $J = 16.3$ Hz, 1H), 5.22 (s, 2H), 5.41 (dd, $J = 4.5, 12.2$ Hz, 1H), 6.97 (d, $J = 8.4$ Hz, 1H), 7.04 (s, 1H), 7.18 (d, $J = 8.4$ Hz, 1H), 7.43–7.59 (m, 3H), 7.90 (s, 4H); ^{13}C NMR (62.5 MHz, DMSO- d_6) δ 32.2 (CH₂), 51.4 (CH₂), 52.0 (CH), 55.8 (CH₂), 64.8 (CH₂), 113.7 (CH), 114.8 (CH), 123.1 (CH), 128.7 (CH), 130.8 (CH), 131.3 (CH), 131.4 (C), 131.6 (C), 134.6 (CH), 136.0 (C), 136.5 (C), 156.7 (C), 167.4 (C), 168.2 (C); MS (MALDI-TOF) m/z 539 (M + 1), 540, 541, 561 (M +

23), 563; EMM calcd for C₂₇H₂₀Cl₂N₂O₆ m/z 539.0777 (MH⁺), found 539.0786.

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