2-Amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylic Acid (FlAib), a Completely Rigidified, Fluoren-9-one-Based α-Amino Acid

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Dedicated to Prof. *Dieter Seebach* on the occasion of his 75th birthday

The synthesis, optical resolution, determination of absolute configuration and conformational preference, and spectroscopic characteristics of terminally protected (blocked) derivatives and short peptides of 2-amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylic acid (FlAib), a novel, rigid, chiral, cyclized $C^{\alpha,\alpha}$-disubstituted glycine are described.

Introduction. – $C_i^{\alpha} \rightarrow C_i^{\alpha}$-cyclized, $C^{\alpha,\alpha}$-disubstituted glycines [1] are members of a class of sterically restricted α-amino acids that promote formation of β-turns [2] and 3_{10}-/α-helical structures [3] when incorporated into peptides. In particular, 2-aminoindane-2-carboxylic acid (Aic; *Fig. 1*) has been used as a Phe-constrained analog for the synthesis of biologically active peptides with limited conformational flexibility [4]. We have previously synthesized α-amino acids containing the Aic motif for use as a fluorescence marker (antAib) [5] or as a photoaffinity label (BpAib) [6].

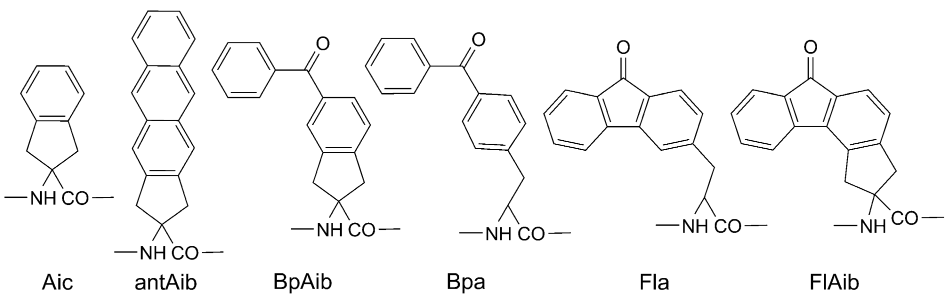
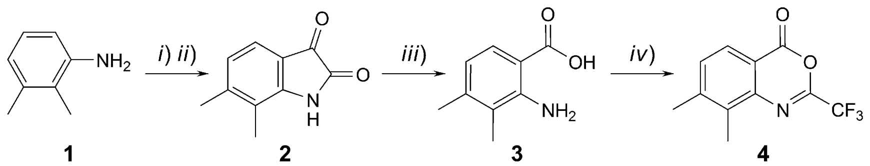


Fig. 1. *Chemical structures of the Aic, antAib, BpAib, Bpa, Fla, and FlAib α-amino acid residues*

Photoreactive α-amino acids with a benzophenone side chain, such as the widely exploited 4-benzoylphenylalanine (Bpa), have been employed as photoprobes for the covalent modification of enzymes and receptors in protein-mapping studies [7]. However, results of photocross-linking experiments need to take into account the marked flexibility of the Bpa side chain, which allows reaction with the amino acid side chain of a residue up to 10-Å distant [8]. To counteract this effect, *Chorev* and co-workers have prepared the more constrained, fluorenone-based Fla residue, and demonstrated its ability to behave as a photoactive cross-linker when introduced into a parathyroid hormone analog [9]. The increased restrictions characterizing the chemical structure of 2-amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylic acid (FlAib), described in this article, which belongs to the above mentioned cyclized $C^{\alpha,\alpha}$-disubstituted glycine class of α-amino acids, completely locks the side-chain fluoren-9-one CO group into a fixed position and orientation relative to the peptide main chain. The photophysical properties of fluoren-9-one and its derivatives have been studied in depth, due to their sensitivity to solvent changes, making them interesting candidates as microenvironment reporters [10].

Results and Discussion. – A key intermediate for the formation of the dimethylfluorenone core is the dimethylanthranilic acid **3** (*Scheme 1*). Two methods have been described in the literature for the synthesis of **3**: either by nitration and subsequent reduction of 3,4-dimethylbenzoic acid (leading to a mixture of regioisomers) [11] or *via* oxidative opening of dimethylisatin **2** [12]. We chose to pursue the second route to avoid the potentially difficult separation of the regioisomers. Compound **3** was obtained in 55% yield from 2,3-dimethylaniline (**1**), *via* **2**, over three steps. Protection of the amino function of **3** with trifluoroacetic anhydride ($(CF_3CO)_2O$) at room temperature resulted in the exclusive formation of the benzoxazinone **4** in 84% yield, as determined from its ^{13}C-NMR spectrum which displays only one CO peak. This finding is in contrast to that observed when anthranilic acid is treated with $(CF_3CO)_2O$ under the same conditions, where the free carboxylic acid was recovered [13]. Benzoxazinone **4** is stable to atmospheric moisture, unlike the related, non-methylated compounds described in [14].

Scheme 1. *Synthesis of Benzoxazinone* **4**

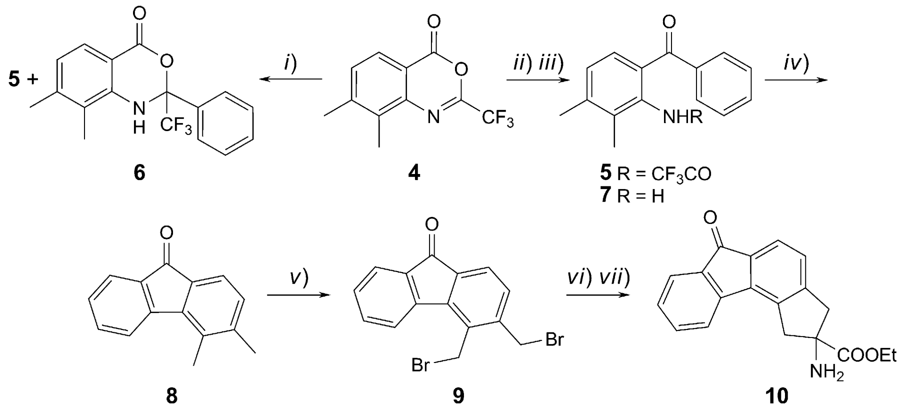


i) Chloral hydrate (=2,2,2-trichloroethane-1,1-diol), $Na_2SO_4 \cdot 5\,H_2O$, $(NH_3OH)_2SO_4$, 1M aq. HCl; 45–75°. *ii*) $MeSO_3H$; 75°. *iii*) 5% aq. NaOH, 35% aq. H_2O_2; 0°. *iv*) $(CF_3CO)_2O$; r.t.

A first attempt to obtain the 2-amino-3,4-dimethylbenzophenone **5** from the benzoxazinone **4** by *Friedel–Crafts* acylation of benzene gave a mixture of the desired product in low yield accompanied by the 1,2-dihydrobenzoxazinone **6**[1]) (*Scheme 2*).

[1]) For related compounds, see [15].

Scheme 2. *Synthesis of H-FlAib-OMe* **10**



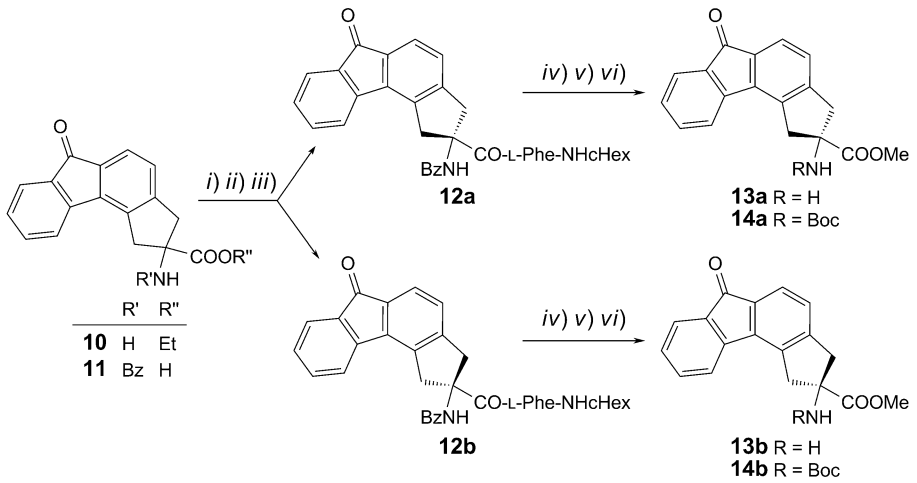
i) $AlCl_3$, benzene; 0–90°. *ii*) PhMgBr, THF; –15°. *iii*) $LiOH \cdot H_2O$, THF/MeOH/H_2O 2:2:1; 70°. *iv*) $NaNO_2$, 2M aq. HCl; 0° then 70°. *v*) *N*-Bromosuccinimide (NBS), benzoyl peroxide, CCl_4; 90°. *vi*) Ethyl isocyanoacetate, 18-crown-6, K_2CO_3, MeCN; r.t. *vii*) 10M aq. HCl, CH_2Cl_2, EtOH; r.t.

An alternative route to aminobenzophenones, proposed by *Walsh* [16], using the inverse addition of a *Grignard* reagent to an excess of benzoxazinone at low temperature, was studied next. Addition of PhMgBr to **4** at –15° was successful, giving the trifluoroacetamide **5** in 69% yield. The amide function was hydrolyzed smoothly in the presence of LiOH to afford the free amine **7** in 94% yield. Diazotization, followed by *Pschorr* cyclization under heating in aqueous dilute HCl [17], gave the 3,4-dimethylfluoren-9-one core structure **8** in 58% yield (addition of Cu salts did not improve this result). Bromination of the two Me groups of **8** afforded the 3,4-bis(bromomethyl)fluoren-9-one (**9**). Bis(alkylation) of ethyl isocyanoacetate in refluxing MeCN in the presence of K_2CO_3 and tetrabutylammonium hydrogen sulfate (Bu_4NHSO_4; TBAHS) to form the cyclic disubstituted α-amino acid, as described by *Kotha* and *Brahmachary* [18], and previously successful in our hands in similar cases, gave only a mixture of dark colored tars, when **9** was used as electrophile. However, alkylation under the conditions used to form the similar amino acid Fla (room temperature and 18-crown-6 as phase-transfer agent) [9] avoided decomposition of the fluorenone moiety. Racemic H-FlAib-OEt **10** was isolated in 53% yield after acidic hydrolysis of the intermediate isonitrile.

Optical resolution of FlAib was attempted using H-(*S*)-Phe-NHcHex (NHcHex, cyclohexylamino) as the chiral auxiliary [19] which had proved successful in the case of the related amino acid BpAib [6]. N^{α}-Benzoyl (N^{α}-Bz) protection, saponification of the ester function, and coupling with H-(*S*)-Phe-NHcHex in the presence of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) [20] gave dipeptide diastereomers **12a**/**12b** (*Scheme 3*), which could be resolved by crystallization and chromatography.

A single crystal of one of these N^{α}-Bz diastereoisomers was subjected to X-ray-diffraction analysis (see below). The two enantiomers of H-FlAib-OMe **13a**/**13b** were obtained from the separated diastereoisomers by acid hydrolysis of the amide

Scheme 3. *Optical Resolution of FlAib*



i) Bz_2O, MeCN; r.t. *ii*) NaOH, THF/MeOH/H_2O; 60°. *iii*) H-(*S*)-Phe-NHcHex, HATU, EtN^iPr_2 (DIEA); THF; r.t. *iv*) 10M HCl, dioxane; 100°. *v*) $SOCl_2$; MeOH; r.t. *vi*) Boc_2O, MeCN; r.t.

functions, followed by esterification of the resulting α-amino acids in the presence of $SOCl_2$. Reaction of each enantiomer with *tert*-butyldicarbonate (Boc_2O) gave the corresponding N^α-Boc-protected α-amino esters **14a**/**14b**.

The 3D structure of Bz-(*R*)-FlAib-(*S*)-Phe-NHcHex (**12b**) is illustrated in *Fig. 2* with atom numbering. The known (*S*)-configuration of the Phe residue allowed us to unambiguously establish the configuration of FlAib as (*R*). Moreover, the N^α-blocked dipeptide alkylamide **12a** adopts an intramolecularly H-bonded β-turn conformation [2], clearly generated by the presence of the $C^{\alpha,\alpha}$-disubstituted glycine FlAib. This finding is not surprising, because such a folded conformation is that expected for short peptides containing at least one residue of this class of conformationally restricted α-amino acids, either $C^{\alpha,\alpha}$-dimethylated (α-aminoisobutyric acid, Aib) [1][3][21] or $C^{\alpha,\alpha}$-cyclized, *e.g.*, the related 1-aminocyclopentane-1-carboxylic acid, Ac_5c [1][22][23].

The molecule is folded into a type-I β-turn [2], stabilized by an C=O···H–N intramolecular H-bond between the NH group of the C-terminal cyclohexylamino moiety and the benzoyl O-atom. The NT···O0 and HT···O0 separations are 3.040(10) Å and 2.18 Å, respectively, and the NT–HT···O0 angle is 164°. The backbone torsion angles adopted by the FlAib(1) and Phe(2) residues are: $\phi_1 = -54.5(10)°$, $\psi_1 = -42.3(10)°$, and $\phi_2 = -96.9(10)°$, $\psi_2 = 14.0(12)°$, respectively. Interestingly, the (*R*)-configured FlAib residue is right-handed helical [24], *i.e.*, its screw sense is the same as that of C^α-trisubstituted (protein) amino acids of (*S*)-configuration.

The angle between normals to the average planes of the N-terminal Bz and the FlAib fluorenone rings is 74.0(2)°, whereas that between the latter and the Phe aromatic ring is 82.9(2)°. The cyclopentene ring which connects the C^α-atom of FlAib to the fluorenone moiety, with puckering parameters [25] $\theta_2 = 0.196(8)$ Å and $\varphi_2 = 197.6(19)°$ (relative to the atom sequence C1A–C1B1–C101–C106–-C1B2), is close

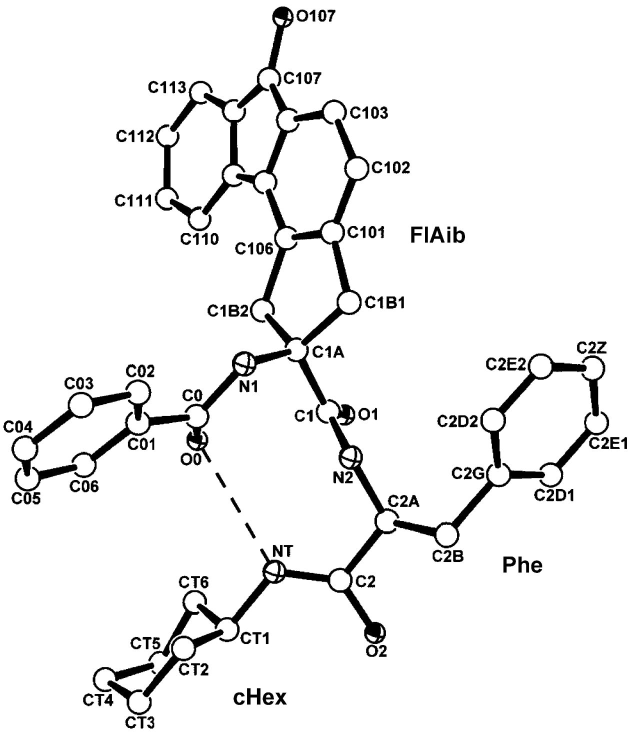


Fig. 2. *X-Ray-diffraction structure of the* N$^{\alpha}$*-blocked dipeptide alkylamide Bz-(*R*)-FlAib-(*S*)-Phe-NHcHex with atom numbering*. The H-atoms and the co-crystallized AcOEt molecule are omitted for clarity. The intramolecular C=O ··· H–N H-bond is indicated by a dashed line.

to the $^2T_1$ (*twist*) disposition. The cyclohexane ring of the C-terminal NHcHex moiety adopts a chair conformation ($Q_T = 0.562(17)$ Å, $\theta_2 = 173.7(18)°$, φ_2 undefined), with the amino substituent in the equatorial disposition. In the packing mode the N1–H group is H-bonded to a ($x-y$, $x-1$, $z+1/6$) symmetry equivalent of O1, thus connecting molecules that wrap around the sixfold screw axis. The N2–H group is H-bonded, within the same asymmetric unit, to the CO O-atom of the co-crystallized AcOEt molecule.

Finally, it is worth mentioning that the conformational preference exhibited by FlAib in this work is strictly comparable to that already published for BpAib [6].

The electronic properties of the fluoren-9-one chromophore are of great interest [9][10]. To spectroscopically characterize this novel amino acid, we used the model compound Boc-(*S*)-FlAib-OMe. As expected for a fluoren-9-one derivative, the near-UV absorption spectrum in CH_2Cl_2 solution (*Fig. 3*, bottom) exhibits a few, strong and well-resolved peaks (vibrational fine structure) at 290, 302 (maximum), 322, and 335 nm, originating from different $\pi \rightarrow \pi^*$ electronic transitions [10], followed by a very weak and broad band at *ca*. 375 nm. This spectral region represents an optimal window for photoexciting peptide molecules. The electronic circular dichroism (ECD) spectrum is also shown in *Fig. 3* (top). All above mentioned near-UV transitions are

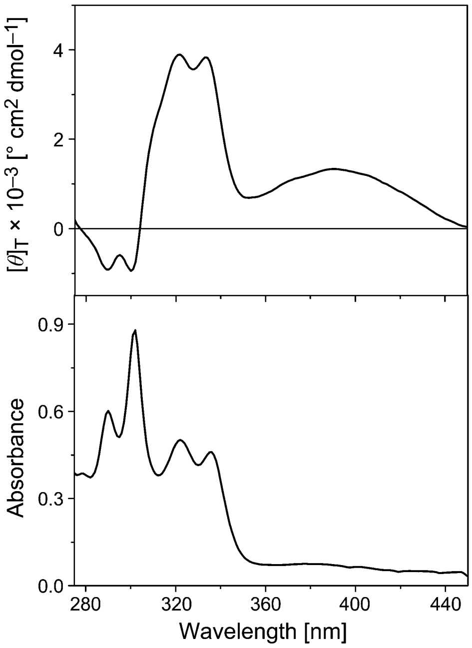


Fig. 3. *Near-UV* (bottom) *and ECD* (top) *absorption spectrum of Boc-(*S*)-FlAib-OMe in CH_2Cl_2 solution* (concentration 0.2 mM)

optically active, displaying negative *Cotton* effects at 290 and 302 nm, and positive *Cotton* effects at 322, 335, and 390 nm.

The fluorescence emission spectra of Boc-(*S*)-FlAib-OMe in four solvents of different polarity are shown in *Fig. 4*. The excitation was accomplished at 335 nm, where a relative maximum of absorption is observed (*Fig. 3*). Excitation at different wavelengths, corresponding to absorption maxima (*e.g.* 322 and 302 nm), did not modify the fluorescence properties of the amino acid derivative. Interestingly, a remarkable blue-shift is observed for the maximum emission on going from polar (CH_2Cl_2, 504 nm; DMF, 498 nm) to apolar (toluene, 486 nm; cyclohexane, 485 nm) solvents. This phenomenon, similar to that already reported for fluoren-9-one [10], makes FlAib an effective polarity probe of local peptide/protein environments.

To further characterize the spectroscopic properties of FlAib, we recorded a time-resolved electron paramagnetic resonance (TR-EPR) spectrum of its photoexcited state. *Fig. 5* shows the TR-EPR spectrum of a frozen benzene solution of Boc-(*S*)-FlAib-OMe. As the conventional B_0 field modulation was not applied, the positive curve is due to absorption, while the negative curve is due to the emission of microwaves. Fitting of the experimental curve allowed us to obtain the following spectral parameters: $D = -106.0$ mT, $E = 7.2 \pm 0.5$ mT, g factor (assumed isotropic) = 2.0020 ± 0.0005, anisotropic population rates $p_x, = 0.01$, $p_y, = 0.75$, $p_z = 1.00$. Overall, this TR-EPR spectrum of the $\pi \rightarrow \pi^*$ *triplet* state of Boc-(*S*)-FlAib-OMe is quite similar to that reported for fluoren-9-one [26].

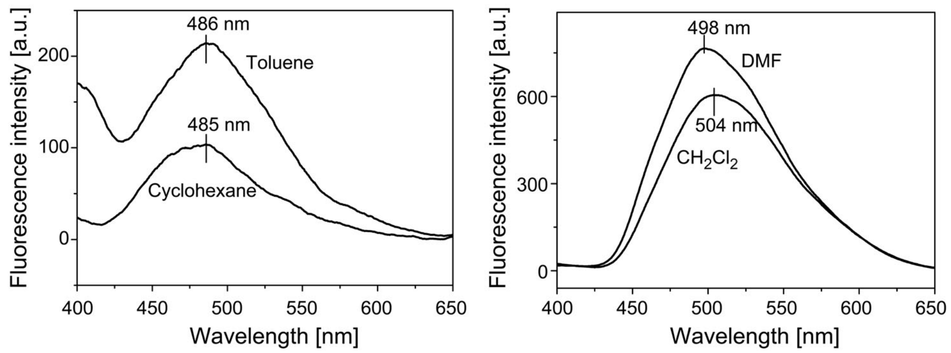


Fig. 4. *Fluorescence spectra of Boc-(*S*)-FlAib-OMe in toluene and cyclohexane* (left), *and in DMF and CH_2Cl_2* (right). λ_{exc}, 335 nm; concentration, 0.01 mM.

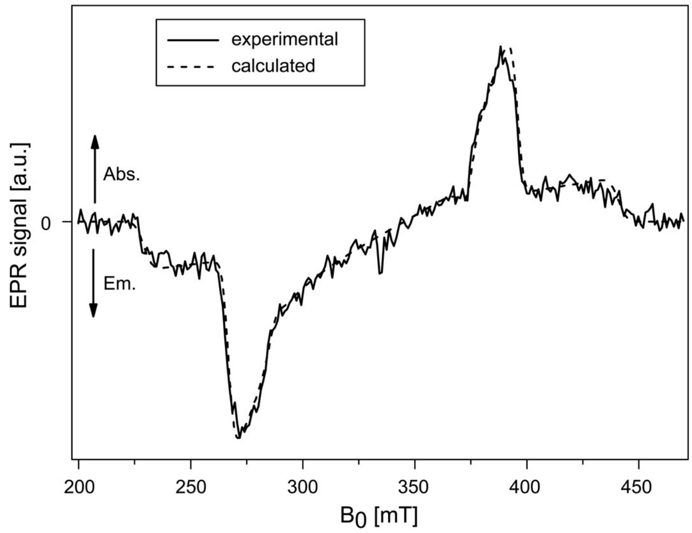


Fig. 5. *TR-EPR Spectrum of Boc-(*S*)-FlAib-OMe in a frozen benzene solution* (concentration, 1 mM; *T*, 125 K), *recorded with a 0.6-µs delay after the laser pulse at 355 nm* (see text for parameters; Abs., absorption; Em., emission).

Conclusions. – The highly constrained, racemic α-amino acid derivative H-FlAib-OEt (**10**) was prepared in three steps and 19% yield from the 2-amino-3,4-dimethylbenzophenone **7** by *Pschorr* cyclization, bromination of the Me groups and bis(alkylation) of ethyl isocyanoacetate. Resolution of the FlAib amino acid was achieved by synthesizing the Bz-FlAib-(*S*)-Phe-NHcHex diastereoisomers which were separated by crystallization and chromatography. Acid hydrolysis of each diastereoisomer, esterification and *N*-(*tert*-butoxy)carbonylation afforded the enantiomerically pure Boc-FlAib-OMe derivatives **14a** and **14b**.

An X-ray diffraction analysis of one diastereoisomer of Bz-FlAib-(*S*)-Phe-NHcHex allowed us to assign the FlAib absolute configuration ((*R*)) and provided information on the β-turn forming propensity of this completely rigidified $C^{\alpha,\alpha}$-disubstituted glycyl residue. The spectroscopic properties (UV absorption, fluorescence, ECD, and triplet state EPR) of FlAib were also investigated. In particular, the emission spectrum of FlAib maintains the remarkable solvent dependence typical of fluoren-9-one [10]. Therefore, this novel, chiral, rigidified α-amino acid is a promising microenvironment sensor. Moreover, this fluoren-9-one-based residue might also play a role in the emerging field of polymer light-emitting diodes where polyfluorene molecules with a central keto defect are extensively investigated [27]. Results on photocross-linking experiments using FlAib-containing peptides will be reported in due course.

Experimental Part

General. Abbreviations: FlAib, 2-Amino-1,2,3,6-tetrahydro-6-oxo-cyclopenta[*c*]fluorene-2-carboxylic acid; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate. Anal. TLC and prep. FC: silica gel *F 254* plates and silica gel *60* (SiO_2; 0.040 ± 0.063 mm; *Merck*), resp.; UV fluorescence detection (254 nm) or ninhydrin development. M.p.: *Tottoli* apparatus (*Büchi*); uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter (1-dm cell) at r.t. UV Spectra: *Perkin-Elmer UV/Vis/NIR Lambda 19* spectrophotometer. Fluorescence spectra: *Perkin Elmer model MPF-66* spectrofluorimeter. CD Spectra: *Jasco model J-715* spectropolarimeter; a fused quartz cell of 10.0 mm path length; the values expressed in terms of $[\theta]_T$, the total molar ellipticity (deg × cm^2 × $dmol^{-1}$). IR Spectra: *Nicolet iS 10* (*SMART iTR diamond ATR*) spectrophotometer; λ in cm^{-1}. NMR Spectra: *Bruker Avance-300* spectrometer at 300.13 (^{1}H) and 75.77 MHz (^{13}C); δ in ppm rel. to the signal of the solvent (^{1}H: δ (residual $CHCl_3$) = 7.27 ppm; ^{13}C: δ ($CDCl_3$) = 77.23 ppm), *J* in Hz. MS: *Waters Xevo Q-TOF.* Elemental analyses: C.N.R.S. Service of Microanalyses, Gif-sur-Yvette, France.

*7,8-Dimethyl-2-(trifluoromethyl)-4*H*-3,1-benzooxazin-4-one* (**4**). *2-Amino-3,4-dimethylbenzoic acid* (**3**; 1 g, 6.06 mmol) was added slowly in portions (over 15 min.) to $(CF_3CO)_2O$ (2.4 ml). The resulting soln. was stirred at r.t. for 1 h then cooled on an ice bath. H_2O (7 ml) was added, and the resulting precipitate was filtered and dried under vacuum. The solid was purified by CC (CH_2Cl_2/cyclohexane 8 : 2) to give **4** (1.34 g, 84%). White solid. M.p. 113 – 115°. R_f (CH_2Cl_2/cyclohexane 8 : 2) 0.93. IR: 2920*w*, 1775*s*, 1771*s*, 1598*m*, 1344*s*, 1215*s*, 1144*m*, 1120*s*, 1045*m*, 841*m*, 781*m*, 766*m*, 704*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.01 (*d*, *J* = 7.9, 1 arom. H); 7.47 (*d*, *J* = 8.0, 1 arom. H); 2.52 (*s*, Me); 2.46 (*s*, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 157.6 (CO); 148.0; 146.3 (*q*, ^2J(C,F) = 42.5); 142.3; 136.4; 132.4; 126.4; 116.6 (*q*, ^1J(C,F) = 281.1); 115.8 (ArC); 21.4; 13.4 (Me). ESI-MS: 298.2 ($[M + Na + MeOH]^+$). HR-ESI-MS: 244.0585 ($[M + H]^+$, $C_{11}H_9F_3NO_2^+$; calc. 244.0585). Anal. calc. for $C_{11}H_8F_3NO_2$ (243.18): C 54.33, H 3.32, N 5.76; found: C 54.21, H 3.31, N 5.77.

N*-(6-Benzoyl-2,3-dimethylphenyl)-2,2,2-trifluoroacetamide* (**5**) *and 7,8-Dimethyl-2-phenyl-2-(trifluoromethyl)-1,2-dihydro-4*H*-3,1-benzooxazin-4-one* (**6**). *Method A:* The benzoxazinone **4** (500 mg, 1.79 mmol) was dissolved in benzene (4 ml) and the soln. was cooled on an ice bath. $AlCl_3$ (720 mg, 5.37 mmol) was added. The mixture was heated at 90° for 3 h. The mixture was cooled on an ice bath, and ice cold 0.5M aq. HCl was added. The mixture was extracted twice with CH_2Cl_2. The combined extracts were washed with H_2O and then with sat. aq. $NaHCO_3$ soln. The org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue obtained was purified by CC (pentane/AcOEt 95 : 5) to give **5** (65 mg, 16%) and **6** (162 mg, 40%).

Method B. The benzoxazinone **4** (3.5 g, 14.4 mmol) was dissolved in THF (35 ml) under Ar. The mixture was cooled to – 15°, and a 1.8M soln. of PhMgBr in Et_2O (6 ml) was added dropwise over 2 h. The resulting mixture was stirred at r.t. for 90 min, then diluted with Et_2O. The mixture was cooled on an ice bath and acidified by the dropwise addition of 2M aq. HCl. The mixture was washed with 0.5M aq. HCl then with H_2O. The org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue obtained was purified by CC (pentane/AcOEt 95 : 5) to give **5** (2.39 g, 69%).

Data of **5**. White solid. M.p. 146 – 148°. R_f (CH_2Cl_2/cyclohexane 8 : 2) 0.69. IR: 3205*w*, 3057*w*, 2882*w*, 1718*s*, 1647*s*, 1607*m*, 1448*m*, 1290*m*, 1206*m*, 1167*m*, 1148*s*, 992*w*, 908*w*, 828*m*, 740*m*, 710*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 10.01 (*s*, NH); 7.78 – 7.79 (*m*, 2 arom. H); 7.58 – 7.63 (*m*, 1 arom. H); 7.44 – 7.50 (*m*, 2 arom. H); 7.28 (*d*, *J* = 7.9, 1 arom. H); 7.18 (*d*, *J* = 7.9, 1 arom. H); 2.40 (*s*, Me); 2.17 (*s*, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 197.9 (CO); 155.7 (*q*, ^2J(C,F) = 38.1, OCN); 143.8; 137.6; 135.3; 133.5; 132.9; 130.7; 130.2; 129.7; 128.7; 128.3 (ArC); 116.2 (*q*, ^1J(C,F) = 294.6, CF_3); 21.4; 15.6 (Me). HR-ESI-MS: 322.1060 ($[M + H]^+$, $C_{17}H_{15}F_3NO_2^+$; calc. 322.1055). ESI-MS: 344.1 ($[M + Na]^+$), 665.1 ($[2\,M + Na]^+$). Anal. calc. for $C_{17}H_{14}F_3NO_2$ (321.30): C 63.55, H 4.39, N 4.36; found: C 63.51, H 4.44, N 4.35.

Data of **6**. White solid. M.p. 130 – 132°. R_f (pentane/AcOEt 95 : 5) 0.27. IR: 3266*w*, 2916*w*, 1700*s*, 1605*s*, 1509*m*, 1285*m*, 1234*m*, 1188*m* 1089*w*, 976*m*, 757*m*, 719*m*, 698*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.64 – 7.67 (*m*, 3 arom. H); 7.36 – 7.39 (*m*, 3 arom. H); 6.85 (*d*, *J* = 8.1, 1 arom. H); 4.68 (*s*, NH); 2.31 (*s*, Me); 2.28 (*s*, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 161.4 (CO); 145.3; 141.1; 133.9; 130.4 (ArC); 129.6 (*q*, $^1J_{CF}$ = 279.3, CF_3); 128.7; 127.4; 125.6; 124.9; 114.1 (ArC); 89.6 (*q*, $^2J_{CF}$ = 32.7, C–N); 20.9; 12.6 (Me). ESI-MS: 344.1 ($[M + Na]^+$), 665.1 ($[2\,M + Na]^+$). HR-ESI-MS: ($[M + H]^+$, $C_{17}H_{15}F_3NO_2^+$; calc. 322.1055) 322.1053. Anal. calc. for $C_{17}H_{14}F_3NO_2$ (321.30): C 63.55, H 4.39, N 4.36; found: C 63.58, H 4.44, N 4.33.

(2-Amino-3,4-dimethylphenyl)(phenyl)methanone (**7**). Compound **5** (1.118 g, 3.48 mmol) was dissolved in THF/MeOH/H_2O 20 : 20 : 10 ml, and LiOH · H_2O (2.19 g, 52.24 mmol) was added to the soln. The mixture was stirred at 70° for two d. Volatiles were removed under reduced pressure, and the residue was taken up in CH_2Cl_2 and H_2O. The phases were separated, and the aq. phase was extracted twice with CH_2Cl_2. The combined org. phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue obtained was recrystallized from cyclohexane/Et_2O to give **7** (735 mg, 94%). Yellow needles. M.p. 105 – 107°. R_f (CH_2Cl_2/cyclohexane 8 : 2) 0.37. IR: 3467*w*, 3315*w*, 2920*w*, 2357*w*, 1585*s*, 1575*s*, 1538*m*, 1444*w*, 1406*m*, 1376*m*, 1317*m*, 1241*m*, 1173*m*, 1094*m*, 977*w*, 881*w*, 833*w*, 759*s*, 715*m*, 704*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.44 – 7.47 (*m*, 2 arom. H); 7.25 – 7.38 (*m*, 3 arom. H); 7.09 (*d*, *J* = 7.7, 1 arom. H); 6.31 (*d*, *J* = 8.1, 1 arom. H); 2.17 (*s*, Me); 1.97 (*s*, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 199.5 (CO); 149.8; 143.2; 140.9; 132.4; 131.0; 129.3; 128.3; 121.4; 117.9; 116.3 (ArC); 21.5; 12.7 (Me). ESI-MS: 226.2 ($[M + H]^+$). HR-ESI-MS: 226.1232 ($[M + H]^+$, $C_{15}H_{16}NO^+$; calc. 226.1232). Anal.calc. for $C_{15}H_{15}NO$ (225.29): C 79.97, H 6.71, N 6.22; found:C 79.91, H 6.69, N 6.23.

*3,4-Dimethyl-9*H*-fluoren-9-one* (**8**). Compound **7** (2.42 g, 10.75 mmol) was dissolved in 2M aq HCl (25 ml), and the soln. was cooled on an ice bath. A soln. of $NaNO_2$ (1.48 g, 21.5 mmol) in H_2O (25 ml) was added. The soln. was stirred at 0° for 1 h, then heated at 70° for 3 h. The mixture was allowed to cool, and diluted with CH_2Cl_2 and sat. aq. NaCl soln. The phases were separated, and the aq. phase was extracted with CH_2Cl_2. The combined org. phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/cyclohexane 1 : 1) to give **8** (1.29 g, 58%). Yellow solid. M.p. 114 – 116°. R_f (CH_2Cl_2/cyclohexane 1 : 1) 0.19. IR: 3064*w*, 2969*w*, 2927*w*, 1700*s*, 1606*m*, 1596*m*, 1577*m*, 1444*w*, 1304*m*, 1228*m*, 1173*w*, 961*m*, 833*m*, 774*m*, 739*m*, 718*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.65 – 7.71 (*m*, 2 arom. H); 7.41 – 7.49 (*m*, 2 arom. H); 7.24 – 7.29 (*m*, 1 arom. H); 7.09 (*d*, *J* = 7.5, 1 arom. H); 2.50 (*s*, Me); 2.36 (*s*, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 194.3 (CO); 145.8; 145.4; 142.8; 135.5; 134.7; 133.2; 133.1; 130.6; 128.5; 124.5; 123.9; 122.0 (ArC); 21.2; 16.1 (Me). ESI-MS: 231.2 ($[M + Na]^+$), 439.2 ($[2\,M + Na]^+$). HR-ESI-MS: ($[M + H]^+$, $C_{15}H_{13}O^+$; calc. 209.0958) 209.0966.

*3,4-Bis(bromomethyl)-9*H*-fluoren-9-one* (**9**). Compound **8** (1.292 g, 6.21 mmol) was dissolved in CCl_4 (30 ml). The mixture was placed under Ar, and NBS (2.16 g, 12.11 mmol) and benzoyl peroxide (Bz_2O_2; 60 mg) were added. The mixture was heated at reflux for 2 h. The mixture was filtered through *Celite*, and the filtrate was concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/cyclohexane 1 : 1) to give **9** (1.43 g, 63%). Yellow solid. M.p. 178 – 180°. R_f (CH_2Cl_2/cyclohexane 8 : 2) 0.80. IR: 3034*w*, 2357*w*, 1709*s*, 1695*m*, 1605*m*, 1423*w*, 1303*m*, 1197*m*, 1088*m*, 984*w*, 874*m*, 847*m*, 751*m*, 700*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.79 – 7.82 (*m*, 1 arom. H); 7.70 – 7.73 (*m*, 1 arom. H); 7.55 – 7.62 (*m*, 2 arom. H); 7.31 – 7.39 (*m*, 1 arom. H); 7.32 (*d*, *J* = 7.5, 1 arom. H); 4.90 (*s*, CH_2); 4.62 (*s*, CH_2). ^{13}C-NMR (77 MHz, $CDCl_3$): 192.7 (CO); 144.1; 143.8; 143.4; 135.9; 135.6; 135.0; 132.5; 132.0;

129.8; 125.0; 125.0; 124.7 (ArC); 29.3; 25.9 (CH_2). ESI-MS: 389 ([$M+Na]^+$), 754.8 ([2 $M+Na]^+$). HR-ESI-MS: 364.9182 ([$M+H]^+$, $C_{15}H_{11}Br_2O^+$; calc. 364.9177).

*Ethyl 2-Amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylate* (**10**). Compound **9** (1.22 g, 3.34 mmol) was dissolved in MeCN (150 ml). The mixture was placed under Ar, and 18-crown-6 (87 mg, 0.33 mmol) and K_2CO_3 (4.61 g, 33.4 mmol) were added. Ethyl isocyanoacetate (0.37 ml, 3.34 mmol) was added, and the mixture was stirred at r.t. for 24h. The mixture was filtered through *Celite*, and the filtrate was concentrated under reduced pressure. The residue obtained was taken up in CH_2Cl_2 (30 ml) and EtOH (30 ml), and the mixture was cooled on an ice bath. Conc. aq. HCl (36% *w*/*v*, 1.3 ml) was added, and the mixture was stirred at 0° for 1 h, then at r.t. for 3 h. The mixture was diluted with CH_2Cl_2 and extracted four times with 0.5M aq. HCl. The pH of the extracts was adjusted to 7 by addition of $NaHCO_3$. This mixture was extracted three times with CH_2Cl_2. The combined org. extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/MeOH 95 : 5) to give **10** (546 mg, 53%). Yellow solid. M.p. 143–145°. R_f (CH_2Cl_2/MeOH 95 : 5) 0.45. IR: 3349*w*, 3288*w*, 3057*w*, 2980*w*, 2897*w*, 2357*w*, 1718*s*, 1700*s*, 1597*s*, 1471*m*, 1429*m*, 1364*m*, 1299*m*, 1220*s*, 1166*m*, 1102*w*, 1025*m*, 973*m*; 888*m*, 868*m*, 775*s*, 744*s*, 726*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.48–7.51 (*m*, 1 arom. H); 7.37 (*d*, $J=7.5$, 1 arom. H); 7.23–7.33 (*m*, 2 arom. H); 7.10–7.15 (*m*, 1 arom. H); 6.98 (*d*, $J=7.5$, 1 arom. H); 4.13 (*q*, $J=7.1$, CH_2); 3.53 (*d*, $J=16.1$, 1 H of CH_2); 3.40 (*d*, $J=16.9$, 1 H of CH_2); 3.04 (*d*, $J=16.4$, 1 H of CH_2); 2.77 (*d*, $J=16.9$, 1 H of CH_2); 1.19 (*t*, $J=7.1$, Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 193.8 (CO); 176.3 (CO); 149.7; 144.49; 140.75; 135.34; 134.9; 134.7; 133.7; 128.9; 125.0; 124.5; 123.6; 122.4 (ArC); 65.4 (C); 61.9 (OCH_2); 46.0; 44.6 (CH_2); 14.4 (Me). ESI-MS: 308.2 ([$M+H]^+$), 330.2 ([$M+Na]^+$), 637.2 ([2 $M+Na]^+$); 944.6 ([3 $M+Na]^+$). HR-ESI-MS: 308.1289 ([$M+H]^+$, $C_{19}H_{18}NO_3^+$; calc. 308.1287).

*2-(Benzoylamino)-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylic Acid* (**11**). Compound **10** (546 mg, 1.78 mmol) was dissolved in MeCN (30 ml). Bz_2O (1 g, 4.45 mmol) was added, and the mixture was stirred at r.t. for 18 h. The mixture was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2, and washed twice with 2M aq. NaOH soln. and then with sat. aq. NaCl soln. The combined org. extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/^{i}PrOH 98 : 2). The residue obtained was dissolved in THF/MeOH/H_2O 5 : 2 : 1, and NaOH (170 mg, 4.27 mmol) was added. The mixture was heated at 60° for 1 h. The mixture was allowed to cool, diluted with H_2O, and volatiles were removed under reduced pressure. The resulting soln. was cooled on an ice bath and acidified by addition of 2M aq. HCl. The mixture was extracted three times with AcOEt. The combined org. extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/MeOH 90 : 10) to give **11** (540 mg, 79%). Yellow solid. M.p. 102–105°. R_f (CH_2Cl_2/MeOH 90 : 10) 0.29. IR: 3338*w*, 3254*w*, 3049*w*, 2923*w*, 2581*w*, 2349*w*, 1734*m*, 1707*s*, 1696*s*, 1648*m*, 1599*m*, 1576*m*, 1525*m*, 1486*m*, 1429*m*, 1294*m*, 1221*m*, 1170*m*, 969*w*, 881*w*, 769*m*, 727*s*, 693*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.84–7.88 (*m*, 2 arom. H); 7.41–7.56 (*m*, 7 arom. H); 7.26–7.31 (*m*, 1 arom. H); 7.16 (*d*, $J=7.5$, 1 arom. H); 3.91 (*d*, $J=16.9$, 1 H of CH_2); 3.65–3.77 (*m*, CH_2); 3.52 (*d*, $J=17.5$, 1 H of CH_2). ^{13}C-NMR (77 MHz, $CDCl_3$): 196.1 (CO); 177.2 (CO); 177.6 (CO); 152.0; 146.2; 142.1; 137.4; 136.9; 136.6; 136.1; 135.2; 133.6; 130.8; 130.3; 129.4; 126.5; 125.8; 125.0; 124.6 (ArC); 68.5 (C); 45.0; 43.5 (CH_2). HR-ESI-MS: 384.1232 ([$M+H]^+$, $C_{24}H_{18}NO_4^+$; calc. 384.1236).

*Bz-(*S*)-FlAib-Phe-NHcHex (=(2*S*)-2-(Benzoylamino)-*N*-(*N*-cyclohexylphenylalanyl)-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxamide*; **12a**) *and Bz-(*R*)-FlAib-Phe-NHChex (=(2*R*)-2-(Benzoylamino)-*N*-(*N*-cyclohexylphenylalanyl)-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxamide*; **12b**). Compound **11** (502 mg, 1.31 mmol) was dissolved in THF (4 ml). The soln. was cooled on an ice bath, and L-phenylalanine cyclohexylamide (444 mg, 1.57 mmol) was added. HATU (597 mg, 1.57 mmol) and DIEA (0.8 ml) were added, and the mixture was stirred at r.t. for 5 d. The mixture was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2, and washed twice with 0.5M aq. HCl, then with H_2O and finally with sat. aq. $NaHCO_3$ soln. The org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue obtained was recrystallized from AcOEt/cyclohexane to give **12b** (237 mg) as yellow needles. The mother liquor was concentrated under reduced pressure, and the residue was purified by CC (AcOEt/cyclohexane 1 : 1) to give **12a** (269 mg, 34%) and **12b** (45 mg; total recovered, 269 mg, 35%).

Data of **12a**. Yellow solid. M.p. 139 – 141°. R_f (AcOEt/cyclohexane 6 : 4) 0.51. $[\alpha]^{25}_{589} = +170$ ($c = 1.0$, CH_2Cl_2). IR: 3304*w*, 3060*w*, 2923*w*, 2851*w*, 2364*w*, 1706*m*, 1640*s*, 1520*s*, 1486*s*, 1447*m*, 1294*m*, 1221*m*, 971*m*, 885*m*, 737*s*, 693*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.01 (*s*, 1 arom. H); 7.85 – 7.88 (*m*, 2 arom. H); 7.47 – 7.52 (*m*, 1 arom. H); 7.32 – 7.41 (*m*, 4 arom. H); 7.08 – 7.18 (*m*, 7 arom. H, NH); 6.92 (*d*, *J* = 7.5, 1 arom. H); 6.80 (*d*, *J* = 7.5, 1 arom. H); 6.63 (*d*, *J* = 8.3, NH); 6.53 (*d*, *J* = 8.1, NH); 4.73 (*dd*, *J* = 7.3, 15.4, H–C(α)(Phe)); 3.81 (*d*, *J* = 18.1, 1 H of CH_2); 3.67 – 3.71 (*m*, H–C); 3.23 – 3.40 (*m*, CH_2); 3.15 – 3.19 (*m*, $CH_2(\beta)$(Phe)); 1.57 – 1.75 (*m*, 3 CH_2); 1.06 – 1.18 (*m*, 2 CH_2). ^{13}C-NMR (77 MHz, $CDCl_3$): 194.3 (CO); 172.0; 169.7; 168.4 (CO); 149.9; 144.1; 140.4; 137.2; 134.9; 134.8; 134.1; 133.7; 133.1; 132.8; 129.6; 129.3; 128.9; 128.9; 127.8; 127.2; 125.3; 124.6; 123.8; 122.7 (ArC); 68.3 (C); 54.6 (C); 48.9 (CH); 42.9; 42.4; 37.9; 33.2; 33.0; 25.9; 25.3 (CH_2). HR-ESI-MS: 612.2866 ($[M+H]^+$, $C_{39}H_{38}N_3O_4^+$; calc. 612.2862).

Data of **12b**. Yellow solid. M.p. 125 – 128°. R_f (AcOEt/cyclohexane 6 : 4) 0.41. $[\alpha]^{25}_{589} = -75$ ($c = 1.0$, CH_2Cl_2). IR: 3311*w*, 3057*w*, 2927*w*, 2844*w*, 2364*w*, 1700*m*, 1638*s*, 1522*s*, 1486*s*, 1447*m*, 1293*m*, 1229*m*, 972*m*, 893*m*, 736*s*, 694*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.77 – 7.83 (*m*, 3 arom. H); 7.49 – 7.54 (*m*, 1 arom. H); 7.37 – 7.43 (*m*, 3 arom. H); 7.25 – 7.30 (*m*, 1 arom. H); 7.08 – 7.21 (*m*, 8 arom. H, NH); 6.92 (*d*, *J* = 7.5, 1 arom. H); 6.75 (*d*, *J* = 7.5, NH); 6.64 (*d*, *J* = 7.5, NH); 4.71 (*dd*, *J* = 6.5, 13.7, H–C(α)(Phe)); 3.88 (*d*, *J* = 17.1, 1 H of CH_2); 3.68 (*m*, H–C); 3.45 (*d*, *J* = 16.9, 1 H of CH_2); 3.35 (*d*, *J* = 17.7, 1 H of CH_2); 3.25 (*d*, *J* = 17.7, 1 H of CH_2); 3.15 (*m*, $CH_2(\beta)$(Phe)); 1.74 (*m*, 5 H of CH_2); 1.18 (*m*, 5 H of CH_2). ^{13}C-NMR (77 MHz, $CDCl_3$): 194.1 (CO); 172.2; 169.7; 168.5 (CO); 148.3; 144.1; 140.7; 137.0; 135.2; 134.9; 134.7; 133.9; 133.1; 132.7; 129.5; 129.2; 128.9; 128.9; 127.7; 127.3; 124.9; 124.4; 123.7; 122.9 (ArC); 68.0 (C); 54.7 (C); 48.8 (CH); 43.8; 41.5; 37.8; 33.2; 33.0; 25.9; 25.3 (CH_2). HR-ESI-MS: 612.2869 ($[M+H]^+$, $C_{39}H_{38}N_3O_4^+$; calc. 612.2862).

*Methyl (2*S*)-2-Amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylate* (**13a**). Compound **12a** (125 mg, 0.20mmol) was dissolved in a mixture of dioxane (5 ml) and conc. aq. HCl (36% (*w*/*v*), 5 ml) and heated at 110° for 42 h. The mixture was allowed to cool, and concentrated under reduced pressure. Toluene was added to the residue, and the mixture was concentrated again. The residue was taken up in MeOH (7 ml), and the mixture was cooled on an ice bath. $SOCl_2$ (0.2 ml) was added dropwise. The mixture was left to stir at r.t. for 6 d. The mixture was concentrated under reduced pressure. Toluene was added to the residue, and the mixture was concentrated again. The residue was taken up in AcOEt and washed with a sat. aq. soln. of $NaHCO_3$. The org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by CC (CH_2Cl_2/MeOH 95 : 5) to give **13a** (36 mg, 61%). Yellow oil. R_f (CH_2Cl_2/MeOH 95 : 5) 0.31. $[\alpha]^{25}_{589} = +16$ ($c = 0.28$, MeOH). IR: 3372*w*, 2954*w*, 2923*w*, 2844*w*, 2353*w*, 1730*m*, 1704*s*, 1588*s*, 1429*m*, 1387*m*, 1293*w*, 1199*m*, 1047*m*, 971*m*, 869*m*, 770*m*, 735*s*, 674*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.62 (*d*, *J* = 7.3, 1 arom. H); 7.49 (*d*, *J* = 7.3, 1 arom. H); 7.35 – 7.45 (*m*, 2 arom. H); 7.22 – 7.27 (*m*, 1 arom. H); 7.10 (*d*, *J* = 7.5, 1 arom. H); 3.80 (*s*, MeO); 3.65 (*d*, *J* = 16.4, 1 H of CH_2); 3.52 (*d*, *J* = 16.7, 1 H of CH_2); 3.14 (*d*, *J* = 16.4, 1 H of CH_2); 2.91 (*d*, *J* = 16.7, 1 H of CH_2). ^{13}C-NMR (77 MHz, $CDCl_3$): 193.5 (CO); 176.4 (CO); 149.3; 144.1; 140.4; 134.9; 134.6; 134.4; 133.4; 128.7; 124.7; 124.2; 123.4; 122.1 (ArC); 65.1 (C); 52.7 (Me); 45.7; 44.3 (CH_2). HR-ESI-MS: 294.1130 ($[M+H]^+$, $C_{18}H_{16}NO_3^+$; calc. 294.1130).

*Methyl (2*R*)-2-Amino-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylate* (**13b**). Compound **12b** (125 mg, 0.20 mmol) was treated in the same way as described above to give **13b** (43 mg, 72%). $[\alpha]^{25}_{589} = -17$ ($c = 0.33$, MeOH). HR-ESI-MS: 294.1131 ($[M+H]^+$, $C_{18}H_{16}NO_3^+$; calc. 294.1130).

*Methyl (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1,2,3,6-tetrahydro-6-oxocyclopenta[*c*]fluorene-2-carboxylate* (**14a**). Compound **13a** (39 mg, 0.13 mmol) was dissolved in MeCN (3 ml), and Boc_2O (40 mg, 0.18 mmol) was added. The mixture was stirred at r.t. for 7 d. The mixture was concentrated under reduced pressure. The residue was purified by CC (AcOEt/cyclohexane 3 : 7) to give **14a** (41 mg, 80%). Yellow foam. R_f (AcOEt/cyclohexane 4 : 6) 0.51. $[\alpha]^{25}_{589} = +59$ ($c = 0.23$, CH_2Cl_2). IR: 3315*m*, 2977*w*, 2927*m*, 2844*w*, 2361*w*, 1734*s*, 1700*s*, 1675*s*, 1525*s*, 1429*m*, 1366*m*, 1290*m*, 1225*m*, 1160*s*, 1048*m*, 1019*m*, 968*m*, 836*m*, 736*s*, 677*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.65 (*d*, *J* = 7.2, 1 arom. H); 7.52 (*d*, *J* = 7.4, 1 arom. H); 7.40 – 7.47 (*m*, 2 arom. H); 7.30 (*d*, *J* = 7.3, 1 arom. H); 7.11 (*d*, *J* = 7.4, 1 arom. H); 5.30 (*s*, NH); 3.80 – 3.84 (*m*, Me, 1 H of CH_2); 3.49 – 3.61 (*m*, CH_2); 3.27 (*d*, *J* = 17.4, 1 H of CH_2); 1.44 (*s*, 3 Me). ^{13}C-NMR (77 MHz, $CDCl_3$): 193.5 (CO); 173.7 (CO); 155.0 (CO); 148.5; 144.0; 140.3; 134.7; 134.5; 133.6; 128.8; 124.5; 124.3; 123.5; 122.3 (ArC); 80.7 (C); 65.9 (C); 52.9 (Me); 43.8; 41.9 (CH_2); 28.2 (Me). HR-ESI-MS: 394.1653 ($[M+H]^+$, $C_{23}H_{24}NO_5^+$; calc. 394.1654).

*Methyl (2*R*)-2-[(*tert*-Butoxycarbonyl)amino]-6-oxo-1,2,3,6-tetrahydrocyclopenta[*c*]fluorene-2-carboxylate* (**14b**). Compound **13b** (54 mg, 0.18 mmol) was treated in the same way as described above to give **14b** (53 mg, 75%). $[\alpha]^{25}_{589} = -56$ ($c = 0.21$, CH_2Cl_2). HR-ESI-MS: 394.1652 ($[M+H]^+$, $C_{23}H_{24}NO_5^+$; calc. 394.1654).

EPR Measurements. An EPR quartz tube (4 mm o.d.) containing a 1 mM soln. of Boc-(*S*)-FlAib-OMe was connected to a vacuum line and sealed, after several 'freeze-pump-thaw' cycles, to avoid any O_2 presence. Time-resolved EPR spectra (TR-EPR) were acquired with a *cw Bruker ER200D X-band* spectrometer, equipped with a temp.-control unit working with liquid N_2. The sample was photoexcited inside the microwave cavity of the spectrometer by using short UV pulses produced by the 3rd harmonic of a *Quantel Brilliant Nd:YAG* laser (λ, 355 nm, 5-ns duration, 50-Hz repetition rate). Data were then acquired according to published procedures [28].

X-Ray Diffraction. Crystals, in the shape of thin needles, were grown from an AcOEt/cyclohexane mixture by slow evaporation. A crystal, *ca.* $0.30 \times 0.05 \times 0.05$ mm^3 in size, was glued on the tip of a glass fiber and coated with paratone. X-Ray diffraction data were collected at 180° K with an *Agilent Technologies Gemini E four-circle kappa* diffractometer equipped with a 92-mm EOS CCD detector, using graphite monochromated CuK_α radiation (λ 1.54178 Å). Data collection and reduction were performed with the CrysAlisPro software (version 1.171.33.52; *Agilent Technologies*). A semi-empirical absorption correction, based on the multi-scan technique using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm, was applied.

The structure was solved by direct methods of the SIR 2002 program [29]. The asymmetric unit is composed of one peptide molecule and one co-crystallized AcOEt molecule. The choice of the space group $P6_1$, rather than its enantiomorph $P6_3$, was based on the known (*S*)-configuration of the Phe residue used in racemization-free procedures for the synthesis of the *N*-acylated dipeptide alkylamide. Refinement was carried out by full-matrix least-squares procedures on F^2, using all data, by application of the SHELXL-97 program [30]. All aromatic rings were constrained to the idealized geometry. Restraints were applied to the anisotropic displacement parameters of all atoms, to approach isotropic behavior. The H-atoms were calculated at idealized positions and refined using a riding model. Relevant crystallographic data are compiled in the *Table*. Overall, a number of crystallographic parameters suffer from the far from optimal crystal size and quality. We are confident, however, that the basic conformational features of the molecule, as discussed in this work, are unambiguously established. CCDC-887940 contains the supplementary crystallographic data for this paper. These data can be obtained from *The Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

Table. *Crystal Data and Structure Refinement for Bz-(*R*)-FlAib-(*S*)-Phe-NH-cHex Ethyl Acetate Solvate*

Identification code		mc166f	$F(000)$	2232
Empirical formula		$C_{43}H_{45}N_3O_6$	Crystal size	$0.30 \times 0.05 \times 0.05$ mm^3
Formula weight		699.82	θ Range for data collection	3.57 to 61.36°
Temp.		180(2) K	Index ranges	$-13 \leq h \leq 17$, $-19 \leq k \leq 18$,
Wavelength		1.54178 Å		$-25 \leq l \leq 16$
Crystal system		Hexagonal	Reflections collected	11121
Space group		$P6_1$	Independent reflections	4543 ($R_{int} = 0.0806$)
Unit cell dimensions	a	17.1665(10) Å	Completeness to $\theta = 61.36°$	99.0%
	b	17.1665(10) Å	Absorption correction	Semi-empirical from equivalents
	c	22.3956(10) Å	Max. and min. transmission	1.00000 and 0.53482
	α	90°	Refinement method	Full-matrix least-squares on F^2
	β	90°	Data/restraints/parameters	4543/313/414
	γ	120°	Goodness-of-fit on F^2	1.329
Volume		5715.5(5) Å^3	Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.1078$, $wR_2 = 0.3118$
Z		6	R indices (all data)	$R_1 = 0.1330$, $wR_2 = 0.3303$
Density (calculated)		1.220 Mg/m^3	Absolute structure parameter	$-0.1(8)$
Absorption coefficient		0.655 mm^{-1}	Largest diff. peak and hole	0.678 and -0.409 e Å^{-3}

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