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trans -(9,10)-Dihydro-11-Aminoethanoanthracene-12-Carboxylic Acid (AMEAC), A New Synthon For ß-Peptides

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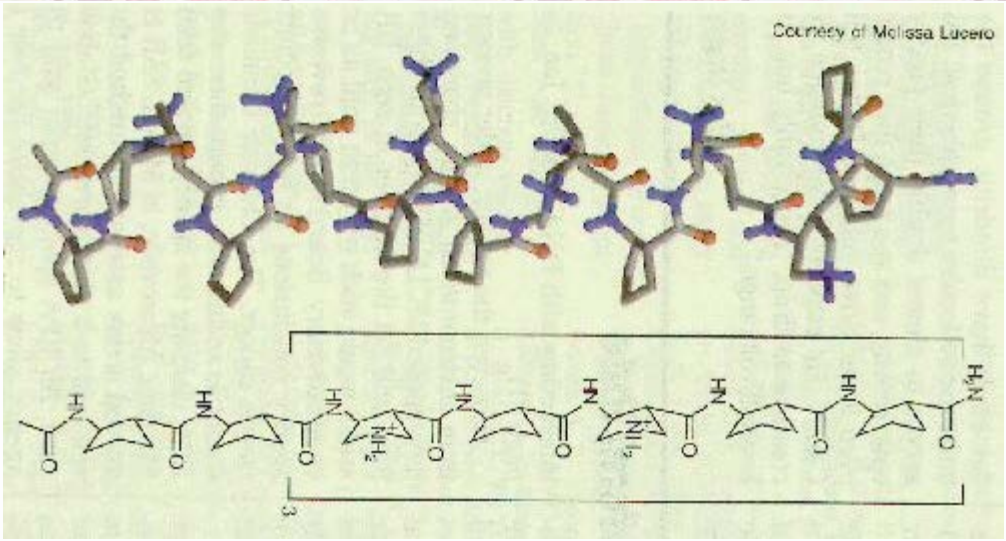
Contents:

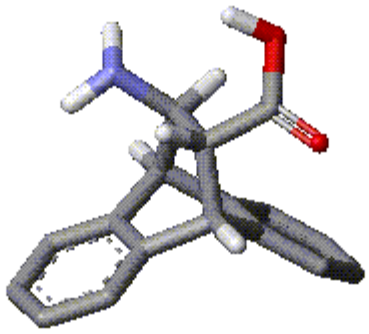
1. Introduction

ß-Amino acids and their peptides have most recently found intense interest in the research community [1].Oligomers ("foldamers") of unnatural amino acids have a wide range of potential applications. They may adopt compact, specific conformations. They could be used to form helices, turns and sheets and develop new types of tertiary structures. Certainly short peptides of this type have potential pharmaceutical applications.

2. Structure of ß-peptides

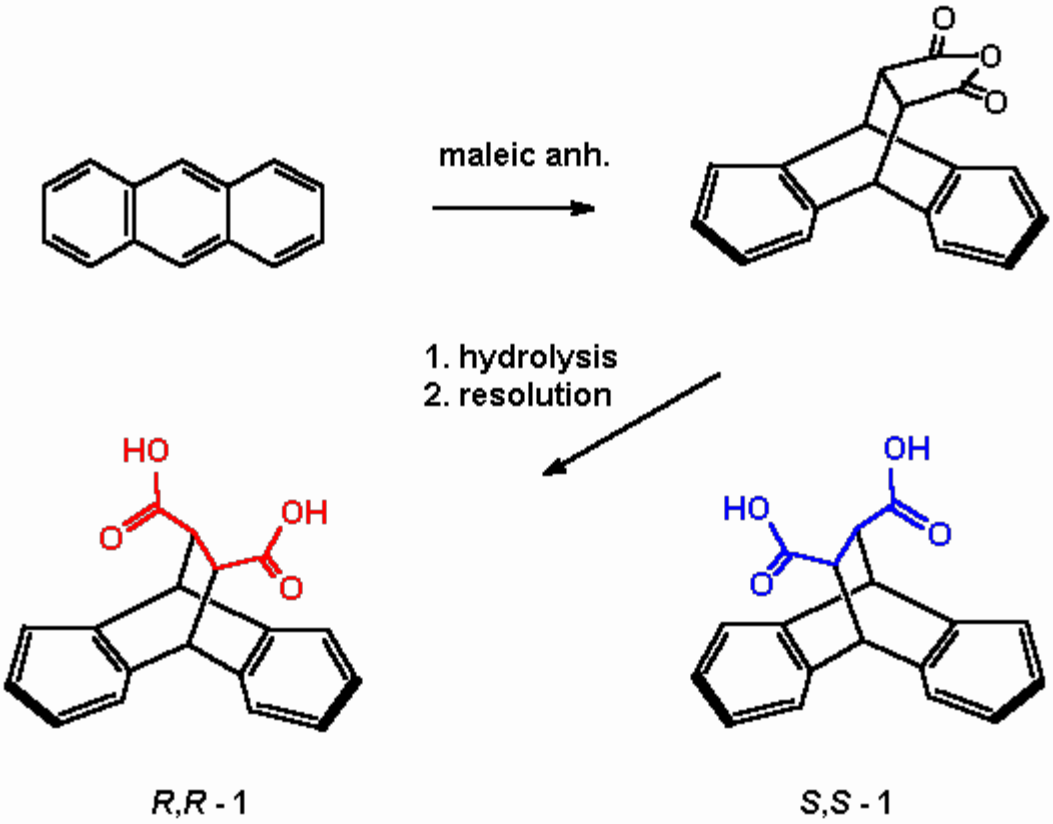


This is a reproduction of ß-17, developed by Sam H. Gellman and coworkers, a ß-peptide active against four species of bacteria, including vancomycin resistant Enterococcus faecium and methicillin resistant Staphylococcus aureus. (red=oxygen, blue=nitrogen and NH). It is composed of the ß-amino acids (R,R)-trans-2-aminopentanecarboxylic acid and (3,R,4,S)-trans-4-aminopyrrolidine-3-carboxylic acid. The peptide folds into a helix similar to that formed by the natural peptide antibiotics called magainins, -that is, it has the hydrophobic side chain on the one side of the helix and the cationic side chain on the other [2]. From the three dimensional structure it is obvious, that a peptide having replaced the aminocyclopentane carboxylate by a similar , but more rigid ß-amino acid type structure could form a helix with interesting properties. We think that two additional condensed aromatic rings as shown in our below depicted target compound could leave enough space to form a helical structure like Gellman´s ß-17 peptide.



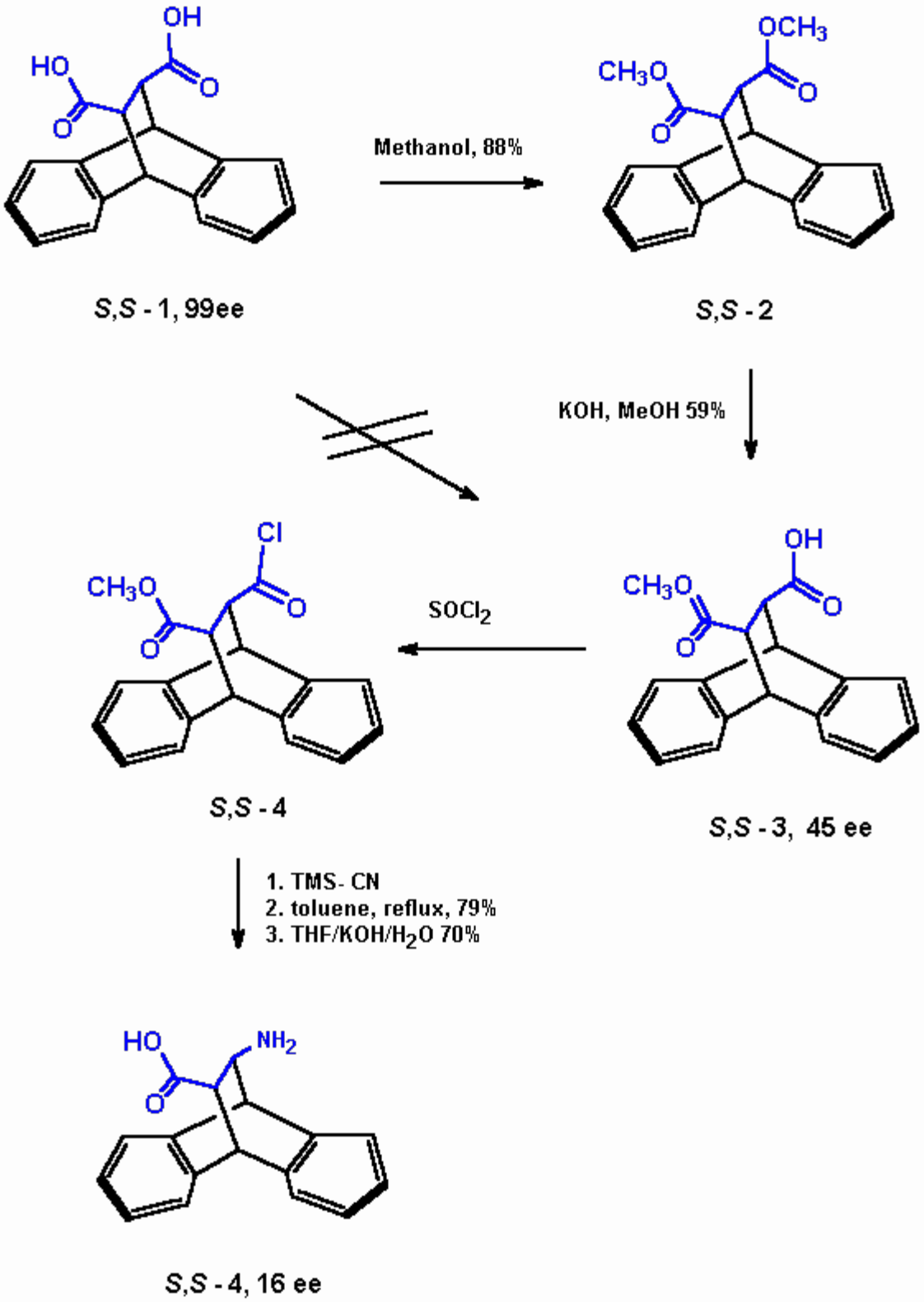
aminoethanoanthracene carboxylate AMEAC

3. Synthesis

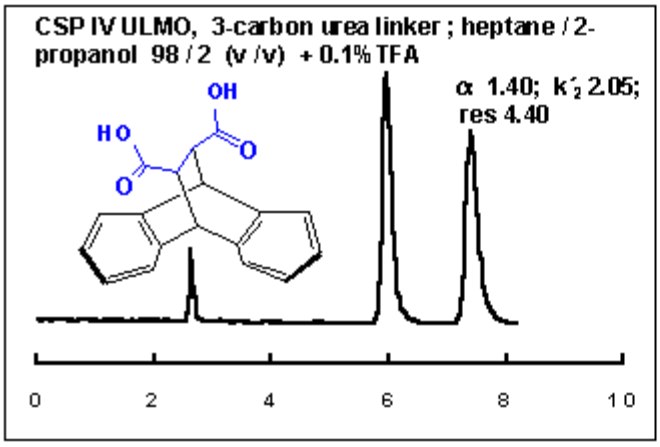


Starting from anthracene and maleic anhydride, Diels Alder cycloaddition , hydrolysis and inversion quantitatively leads to the well known [3] *trans* -9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid **1** (EADC, Scheme 1). This C2-symmetric acid can be easily separated into the enantiomers [3, 6]. **1** has been used previously to prepare the 11,12-diamino analogue, which has found use as catalyst [4] for asymmetric allylic alkylations and as benzoylated variation for an enantioselective synthesis of carbanucleosides [5]. It has also been used by us to prepare a Pirkle-Type chiral stationary phase which especially well separated enantiomers of aryl substituted lactones and cyclic carbamates [6]

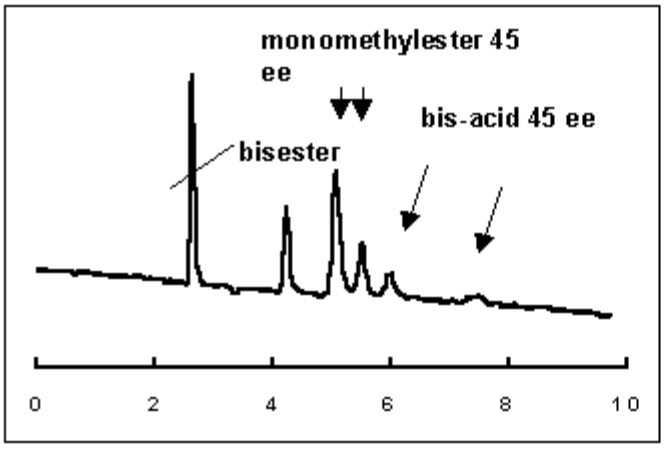
From racemic **1** we have prepared the racemic title compound in three steps without major problems (Scheme 2). Synthesis of the pure *(R,R)* and *(S,S)*-enantiomers proved to be more difficult than expected since partly racemisation occured in the step of alkaline hydrolysis to monoester **3** and also after or during Curtius degradation to the desired amino acid **4**. (see Chapter HPLC-analysis below).

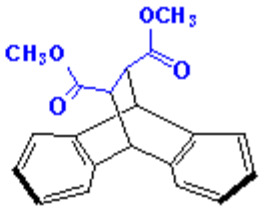


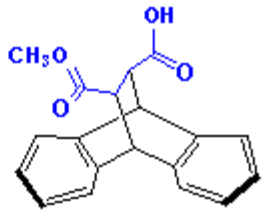
4. HPLC Analysis of Enantiomers



HPLC-analysis of EADC, above analyzed as racemate, and below from S,S-EADC-dimethylester hydrolysis the partly racemised mixture of monoester (note that the formed small amount of acid has still 47 ee). The bisester is not resolved.



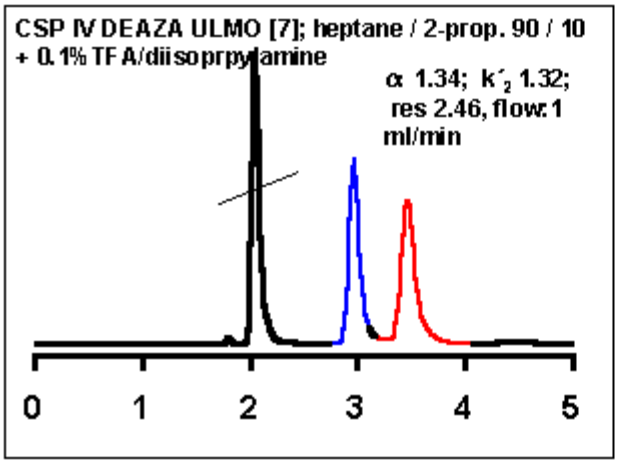


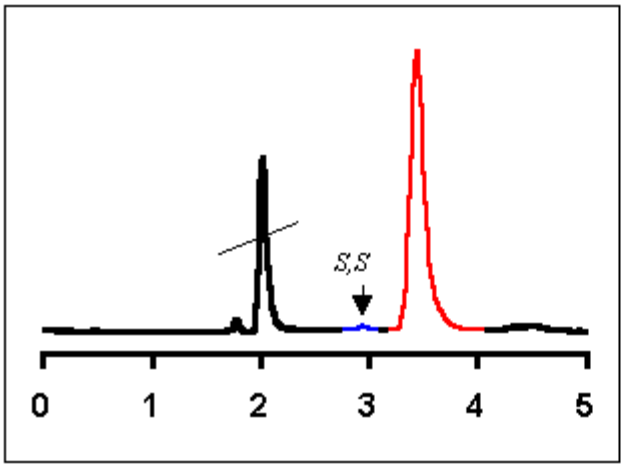


As mentioned above, starting from enantiomer *S,S* -**1**, we found significant racemization (45ee) after HPLC analysis of the monomethylester **3**. From previous work we used an optimized chiral stationary phase (CSP) which is based on a ULMO analogue, but contains a short urea linker to silica [6]. Conditions: 25°C; flow 1.0 ml/min; mobile phase n-heptane/2-propanol= 98/2; 0.1% TFA. Under those conditions the separation factor of diacid 1 (resp.monoester 3) was 1.42 (1.17), k'_2 2.08 (1.27) and resolution 3.96 (1.90). The enantiomers of the bisester appeared as singlet(k´0.74), but that was well separated from the other four signals. Despite the loss of enantiomeric purity, it is important that one can easily deduct that the first eluting enantiomer is the *(S,S)*-monoester, exactly as it has been observed with the acid.

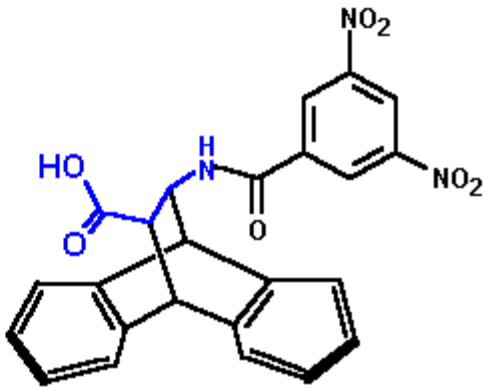
Analysis of the enantiomers of AMEAC could not be directly achieved on our Pirkle columns. However, derivatization with dinitrobenzoylchloride (DNB-Cl) proved to be easy and separation of DNB-AMEAC was achieved using a deaza ULMO CSP published by us as CSP IV in [7]. This CSP has previously been shown by us to separate DNB-derivatives[8]. Using this method, we found that also the Curtius degradation lead to a further loss of enantiomerical purity. The analysis showed only ee 16. This was somewhat disappointing, but, as mentioned above, the absulute configuration of AMEAC could now be easily deduced from the chromatogram since the starting material had *(S,S)*-configuration and certainly that remained the major component.

A semi-preparative HPLC separation loading 0.25 mg DNB-derivative onto the analytical column yielded 0.1 mg enantiopure products with 99.5 for the first and 99ee for the second peak , DNB-*(R,R)*-AMEAC). The chromatogram below shows the racemate and the pure *(R,R)*-derivative.



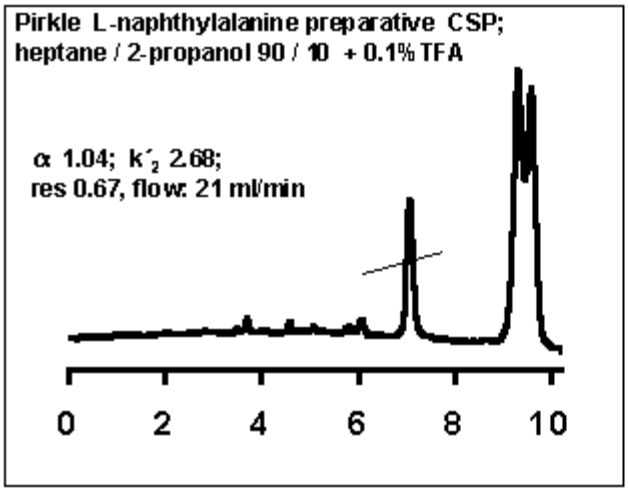


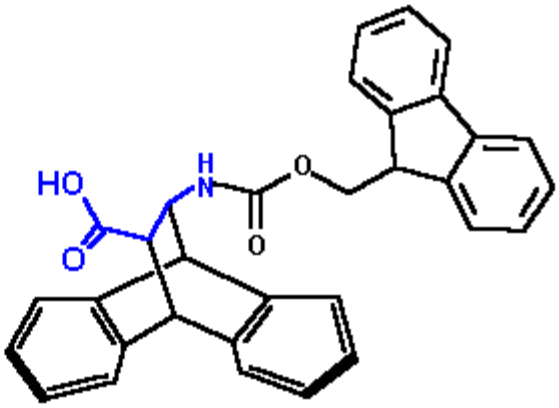
HPLC-analysis of AMEAC as 3,5-dinitrobenoylamide **5**. Above analyzed as racemate, and below as the purified (*R,R*)-enantiomer (99ee) after semipreparative chromatography on the deaza-ULMO CSP [7].



5. Fmoc- and Boc derivatives

In order to provide building blocks for peptide synthesis, Fmoc and Boc derivatives of AMEAC were prepared using standard conditions. Both crystalline products could be isolated in good yield and the enantiomers separated on chiral stationary phases. Chiral recognition of the Fmoc-derivative on a commercial preparative p-basic Pirkle naphthyl-CSP (250x20) was sufficient to separate 100 mg of the substance in three runs.





Semipreparative HPLC-separation of AMEAC as Fmoc-derivative. Column dimension (REGIS, Morton Grove, USA): 250 x 21.1 5 micron silica. Knauer (Germany) WellChrom K-1001 with 50 ml pump-head, Hewlett-Packard 1050 variable wavelength detector with preparative cell.

6. Conclusion

We have demonstrated, that Diels-Alder products of anthracene and maleic acid (and fumaric acid derivatives [10]) can be rearranged to give chiral ß-amino acids having a rigid aromatic backbone. The synthesis is streightforward and the yield is acceptable. Separation of enantiomers of AMEAC or the Boc-and Fmoc derivatives is an alternative to the direct synthesis from a enantiopure precursor since partly racemization is there at least a problem which would require careful optimization.
As analytical tools a set of Pirkle type chiral stationary phases were found to be optimal, to analyse directly the enantiomers of the precursors as well as the acyl- and carbamoyl derivatives.

7. Experimental

All compounds and solvents were commercially available and used without further purification. HPLC runs were performed at 25 °C and usually monitored at 254 nm. Solvents used for mobile phases were of HPLC grade (MERCK, Darmstadt, Germany)

Instrumentation
HPLC measurements were performed using a Hewlett-Packard series HP1050 instrument (consisting of a pumping system, a multiple wavelength detector and an autosampler) and the HPChemstation software. NMR experiments were done on a Bruker 360 MHz instrument in $CDCl_3$ as the solvent.
Semipreparative HPLC-separation of AMEAC as Fmoc-derivative was performed with a Knauer Pump (Germany) WellChrom Maxi Star K-1001 equipped with 50ml pump-head, a Hewlett-Packard 1050 variable wavelength detector with preparative cell.
Column : L-Naphthylalanin (REGIS, Morton Grove,Ill,USA); dimension 250 x 21.1mm; 5 micron silica.

LC/MS:
For LC/MS investigation of AMEAC **4** and 3,5-dinitrobenzoyl-AMEAC a Hewlett-Packard 1100 combined with an HP LC/Mass specific detector fitted with an APCI ion source was used.
Chromatografic Conditions:

FIA mode (no column); Mobile phase: 75% acetonitrile, 20% water/ acetonitrile (9:1), 5% methanol
Flow rate: 1ml/min; Injection volume: 5ml.
MS Conditions:
Source: APCI; Ion mode: positive and negative; Vcap:3000V (positive), 3000V(negative); Nebulizer: 60psig; Drying gas flow: 5l/min; Drying gas temp.: 350°C; Vaporizer temp.: 350°C; Corona Current: 5mA (pos.), 20mA (neg.); Peakwidth: 0.1min; Time filter: off; Fragmentor: 50V, 100V, 150V, 200V.;

Synthesis:

trans (9,10)-dihydro-11-aminoethanoanthracene-12- carboxylic acid (AMEAC) **4**.
10g (32.5mmol)monomethylester**3** is refluxed with 3.5ml thionyl chloride in 70 ml benzene. After good evaporation the residue is dissolved in toluene and 6.4ml trimethylsilylazide and 50mg 4-pyrrolidinopyridine are added. After 16h reflux the cooled solution is dissolved in a small amount of THF and 45ml 2N KOH are added. Two phases separate and so much water is added, that the solution becomes homogeneous. The mixture is stiired for 16h, the solution is extracted with two portions of 40 ml ether and the aqueous phase is neutralized with conz. HCl to a pH 6.3. Yield 4.0 g (46%)
Fp 242 $C_{17}H_{15}NO_2$ Calc C 76.96 H 5.70 N 5.28 found C 76.3 H 5.63 N 5.40 LC/MS m/e 266 (pos, M+H) and 264(neg, M-H). 500 MHz Bruker ^{1}H-NMR: (dmso-d_6) 2.12 dd; 3.50dd; 4.20, d, 2.7 Hz; 4.55, d, 2.4 Hz; 7-7.40 aromat.

trans -N-3,5-Dinitrobenzoyl (9,10)-dihydro-11-aminoethanoanthracene-12-carboxylic acid (DNB-AMEAC) **5**.
2mg AMEAC **4** (7.5 micromol) and 20 microliter H_2O in an 1.5 ml microcentifuge tube are treated ten times with 1 microliter portions of a stock solution (3.5 mg 3,5-dinitrobenzoylchloride in 10 microliter dichloromethane) and 2 microliter of a stock solution sodium carbonate (3.18 mg in 20 microliter water. Each addition is followed by 1 min ultrasound mixing.
After complete addition and further mixing for 1 min the suspension is acidified after 5 min with 1 drop trifluoroacetic acid. 25 microliter dioxan is added, and after mixing the product is extracted with 100 microliter diethylether. The ether solution is evaporised, the residue is taken up in mobile phase und subjected to HPLC-analysis.
A preparative run using 200 mg amino acid yielded 190 mg (55%) **5**
$C_{24}H_{17}N_3O_7$ (459.41) Mp 236; Calc. C 62.75 H 3.73 N 9.15 found C 63.00 H 3.83 N 9.01
LC/MS m/e 460 (pos, M+H) and 458(neg, M-H)

trans -N-Fluorenoxymethylcarbonyl-(9,10)-dihydro-11-aminoethanoanthracene-12-carboxylic acid (Fmoc-AMEAC) **6**
To a ice cold solution of 1.06 g (4 mmol) AMEAC **4** in 10 ml dioxan and 7.5 ml 10% sodium carbonate/water is slowly added a solution of 1.25g (3.89 mmol) Fmoc N-hydroxysuccinimidate in 15 ml dioxane. After stirring for 2h at RT and addition of some water to dissolve a precipitate the solution is extracted with ether (3x 20 ml). The aqueous layer is acidified with HCl to pH 1.5. After extraction with ethyl acetate the solution is dried ($MgSO_4$)and the solvent evaporated at 30° C. The product is recrystallised from ethanol. Yield 1.1 g (56%). **6**
$C_{32}H_{25}NO_4$ (487.55) Calc. C 78.83 H 5.17 N 2.87 found C 78.00 H 5.10 N 3.00

trans -N-tert-butoxycarbonyl-(9,10)-dihydro-11-aminoethanoanthracene-12-carboxylic acid (Boc-AMEAC) **7**
To 0.246 g (1 mmol) AMEAC **4** in a mixture of 0.7 ml dioxan and 1.1 ml 1N NaOH is added 240mg BOC-anhydride. After stirring for 16h the mixture is extracted with 2x 1mL pentane and the aqueous layer is acidified with $KHSO_4$. After extraction with ethyl acetate the solution is dried ($MgSO_4$)and the solvent evaporated at 30° C. The product is purified with dry flash chromatography (toluene:acetone 5:1) Yield 200mg **7**(55%).
$C_{22}H_{23}NO_4$ (365.42) Calc. C 72.31 H 6.34 N 3.83 found C 72.00 H 6.50 N 3.70 .
LC/MS m/e 460 (pos, M+H) and 458(neg, M-H).

8. Acknowledgement

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9. References

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