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Design, synthesis and antiproliferative properties of oligomers with chromophore units linked by amide backbones

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Abstract—The preparation of oligomers made up of several chromophore units as compounds with potential fluorescent and antiproliferative properties is described. Specifically, chromophore units with protected-amino groups and one carboxylic group are described, together with methods to assemble these units using peptide chemistry. Some of these compounds have antiproliferative activity.

Interest in new DNA-intercalating drugs has led to the development of protocols for the rapid synthesis of compounds carrying several chromophore units. In the previous paper we described the preparation of oligomers with phosphodiester bonds using the phosphoramidite method and solid-phase synthesis protocols. Here we describe the synthesis of oligomers carrying amide bonds.

For the synthesis of these polymers, three trifunctional scaffolds were chosen: 4-aminoproline, 2-aminoethylglycine, and ornithine. All three have two amino groups and one carboxylic acid function. This distribution allows both the growth of a polyamide skeleton and the incorporation of the DNA-intercalating unit on the other amino group if suitable amino-protecting groups are used. γ-Aminoproline backbones had been used previously for the synthesis of conformational constrained peptide nucleic acids.[1,2] Polyproline oligomers form a rigid backbone that is suitable for drug delivery.[3,4] The 2-aminoethylglycine backbone is more flexible and has been used for the design of peptide nucleic acids (PNA) DNA backbone mimetics.[5,6] The synthesis of several functionalized PNA oligomers containing anthracene, [7] flavin,[8] pyrene,[9] and naphthalimide[10] has led to compounds with improved DNA binding properties. Using similar procedures, ornithine has also been used as a DNA mimetic; deoxyribose has been replaced with a poly-ornithine chain.[11]

For the synthesis of 4-aminoproline polymers the backbone was grown on solid-phase, and then the intercalating agent was assembled on solid support. This strategy is more convenient for the rapid synthesis of large libraries, as it is unnecessary to construct each monomer with its intercalating agent. The synthesis protocols were based on a previous study.[12] Thus, the assembly of 4-aminoproline oligomers was carried out using the

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methylbenzhydrylamine (MBHA) resin applying an Fmoc/Boc hybrid strategy and using *trans*-γ-Fmoc-amino-α-Boc-L-proline [Boc-Amp(Fmoc)-OH] as a building block. The Fmoc group was used to protect the γ-amino group of each monomer, which thus facilitated the elongation of the backbone. Boc was the semi-permanent protecting group for the α-amino group through which the chromophore units were introduced. α-Amino derivatizations were carried out on solid-phase using standard Boc chemistry protocols. A glycine molecule was introduced as a spacer between this amino group and the chromophore moiety. In general, the Fmoc group was removed using 20% of piperidine in *N,N*-dimethylformamide (DMF) and Boc-Amp(Fmoc)-OH (5 equiv) was coupled to the resin using diisopropylcarbodiimide (DIPCDI, 5 equiv) and 1-hydroxybenzotriazole (HOBt, 5 equiv) as coupling reagents. Boc groups were removed using 40% trifluoroacetic acid (TFA) in dichloromethane (DCM), followed by the treatment of the resin with 5% diisopropylethylamine (DIEA) in DCM. Boc-Gly-OH (5 equiv) was coupled to the resin using DIPCDI (5 equiv) and HOBt (5 equiv) as coupling reagents. Carboxyl derivatives (**1a**–**g**, 3 equiv) were coupled to the resin using O-(benzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium tetrafluoroborate (TBTU, 3 equiv) and DIEA (6 equiv) as coupling reagents. Acetylations were carried out using acetic anhydride (Ac_2O, 5 equiv) and DIEA (5 equiv).

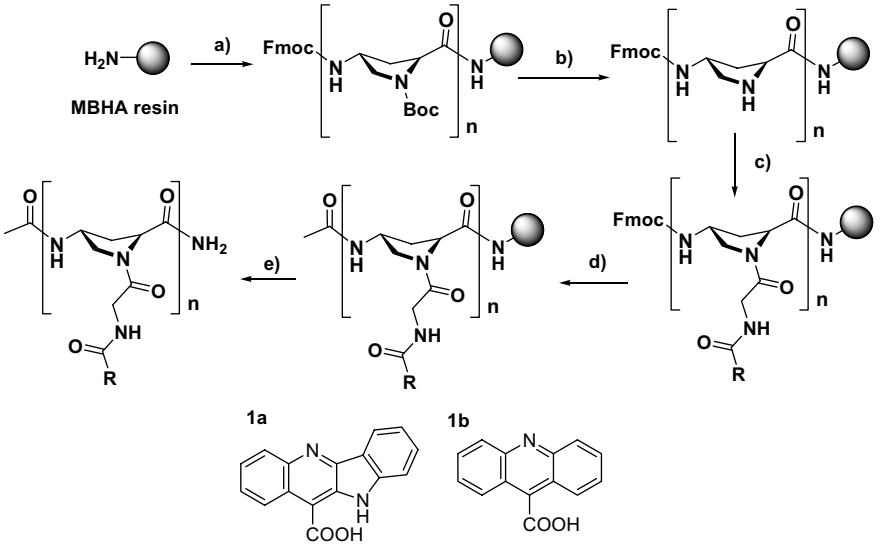
The solid-phase procedures depended on whether homopolymers or heteropolymers were the objects of synthesis, as described below.

Protocol I, for homopolymers (see Scheme 1). The γ-peptide backbone was synthesized first by repetitive couplings of Boc-Amp(Fmoc)-OH using Fmoc chemistry and, after the removal of Boc protecting groups, the spacer (Boc-Gly-OH) was introduced at the α-amino positions. After the removal of the Boc groups, carboxyl derivatives (**1a**, **1b**) were introduced through these glycines, forming the final homopolymers. A range of dimers and trimers (Table 1) were synthesized in this way.

Table 1. Oligomers prepared in this study

Compound	MS (expected)	MS (found)
Ac-Agr-Agr-NH_2	807.8	808.4
Ac-Qgr-Qgr-NH_2	885.9	886.5
Ac-Agr-Agr-Agr-NH_2	1182.2	1182.6
Ac-Qgr-Qgr-Qgr-NH_2	1299.3	1299.7
Ac-Agr-Qgr-Agr-NH_2	1221.3	1222.6
Ac-Qgr-Qgr-Agr-NH_2	1260.3	1260.2
Ac-Qgr-Agr-Agr-NH_2	1221.3	1222.6
Ac-Qgr-Agr-Qgr-NH_2	1260.3	1261.6
Ac-Agr-Agr-Qgr-NH_2	1221.3	1221.7
Ac-Agr-Qgr-Qgr-NH_2	1260.3	1260.6
Ac-Qgr-r-Agr-r-Pgr-NH_2	1555.6	1557.6
Ac-Pgr-r-Ogr-r-Pgr-NH_2	1559.6	1559.4
Ac-Agr-r-Pgr-r-Qgr-NH_2	1555.6	1557.1
Ac-Fgr-r-Pgr-r-Agr-NH_2	1517.6	1519.2
Ac-Qgr-r-r-Agr-r-r-Pgr-NH_2	1864.0	1865.8
Ac-Pgr-r-r-Ogr-r-r-Pgr-NH_2	1868.0	1868.6
Ac-Agr-r-r-Pgr-r-r-Qgr-NH_2	1864.0	1865.2
Ac-Fgr-r-r-Pgr-r-r-Agr-NH_2	1826.0	1827.7
Ac-Ago-Ago-Ago-NH_2	1188.3	1189.0
Ac-Qgo-Qgo-Qgo-NH_2	1305.4	1305.9
Ac-Ago-Qgo-Ago-NH_2	1227.3	1227.9
Ac-Ago-Qgo-Qgo-NH_2	1266.4	1266.9
Ac-Agp-Agp-NH-C_6H_{12}-OH	885.9	884.4
Ac-Agp-Agp-Agp-NH-C_6H_{12}-OH	1249.3	1246.4
Ac-Qgp-Qgp-NH-C_6H_{12}-OH	964.0	962.6

4-Aminoproline backbone: Agr (Acridine), Qgr (indoloquinoline), Fgr (fluorenyl), Pgr (2-phenylquinoline), Ogr (chromene); ornithine backbone: Ago (acridine), Qgo (indoloquinoline); 2-aminoethylglycine backbone: Agp (acridine), Qgp (indoloquinoline), Ac: acetyl; r: N^{α}-acetyl-γ-aminoproline.
C_6H_{12}: *n*-hexyl.



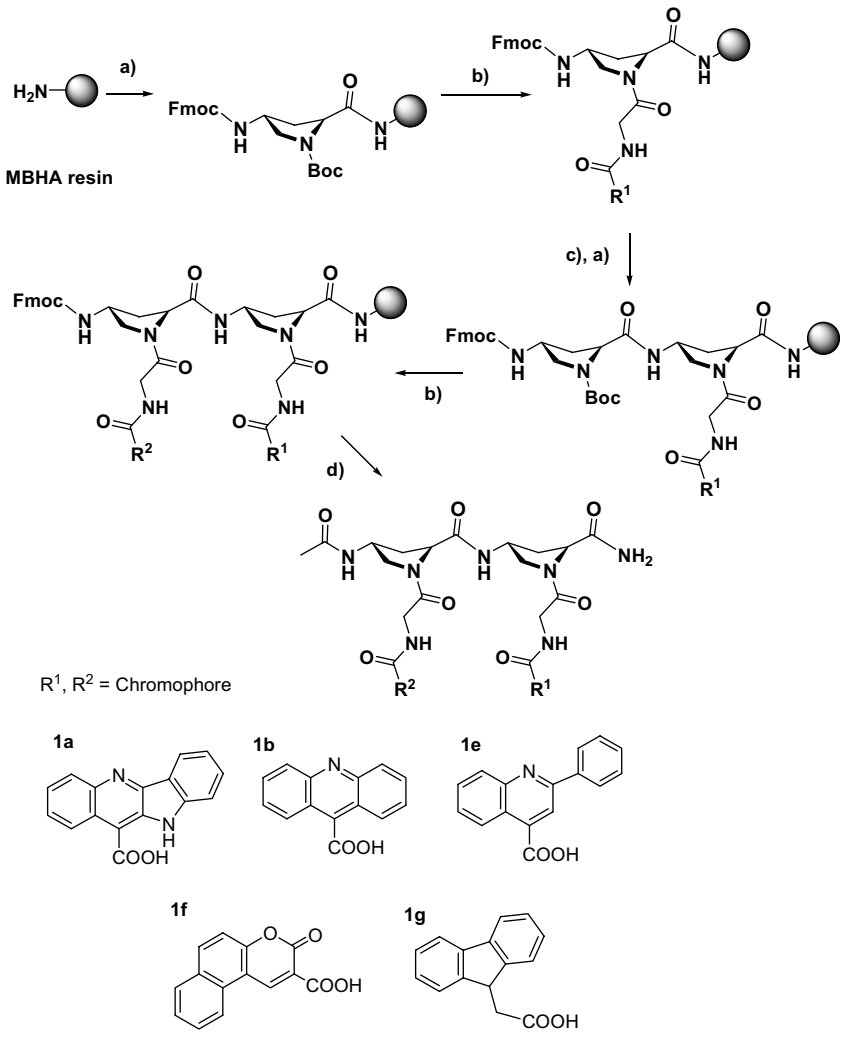
Scheme 1. Solid-phase synthesis of homo-oligomers. Reagents and conditions: (a) i—piperidine-DMF (2:3), 30 min; ii—(2*S*,4*R*)-Boc-Amp(Fmoc)-OH/DIPCDI/HOBt (3 equiv, 3 equiv, 3 equiv) in DMF, iii—repeat steps i and ii *n* times; (b) i—TFA-DCM (4:6), 30 min. ii—DIEA-DCM (1:9), 1 min. (c) i—Boc-Gly-OH/DIPCDI/HOBt (3 equiv, 3 equiv, 3 equiv) in DMF; ii—TFA-DCM (4:6), 30 min. iii—DIEA-DCM (1:9), 1 min. iv—R-COOH (**1a** or **1b**)/TBTU/DIEA (3 equiv, 3 equiv, 6 equiv) in DMF. (d) i—Piperidine-DMF (2:3), 30 min; ii—Ac_2O/DIEA (5 equiv, 10 equiv) in DMF, 2 h. (e) Anhydrous HF.

Protocol II, for heteropolymers (see Scheme 2). In this procedure, the fluorophore was introduced sequentially after each aminoproline coupling. Thus, Boc-Amp(Fmoc)-OH was attached to the MBHA resin, the Boc protecting group was removed and the spacer (Boc-Gly-OH) was coupled. After the removal of the Boc group the fluorophore was introduced using the coupling agents mentioned below. This cycle gave the first aminoproline functionalized with the first fluorophore. Then, the Fmoc group was removed, and the sequence was repeated, obtaining an aminoproline dimer with two different fluorophores. Repetitions of these steps allowed the synthesis of several dimers and trimers (Table 1).

Protocol III. This protocol was applied for those polymers that did not have monomer at all the α-amino proline positions. These compounds were treated as heteropolymers. They were synthesized using protocol II (see Scheme 2), with the difference that those α-amino positions of the proline that did not have a fluorophore were acetylated. Thus, the α-amino positions that were maintained without monomer were acetylated after the Boc group removal.

All γ-aminoproline oligomers were finally treated with 20% piperidine solution to remove the *N*-terminal Fmoc group, acetylated at this position, and treated with anhydrous HF to obtain the desired products. Products were finally purified by a reverse-phase semi-preparative HPLC (Fig. 1).

Ornithine polymers (Table 1) were synthesized following the same synthetic strategies used in the synthesis of γ-aminoproline oligomers, just replacing the Amp scaffold by Boc-L-Orn(Fmoc)-OH. Backbone elongation was carried out through the δ-amino function using Fmoc chemistry and monomers were introduced in the α-amino group using Boc chemistry and using glycine as a spacer.



Scheme 2. Example of the solid-phase synthesis of a heterodimer. Reagents and conditions: (a) (2*S*,4*R*)-Boc-Amp(Fmoc)-OH/DIPCDI/HOBt (3 equiv, 3 equiv, 3 equiv) in DMF. (b) i—TFA-DCM (4:6), 30 min. ii—DIEA-DCM (1:9), 1 min; iii—Boc-Gly-OH/DIPCDI/HOBt (3 equiv, 3 equiv, 3 equiv) in DMF; iv—TFA-DCM (4:6), 30 min. v— DIEA-DCM (1:9), 1 min. vi—R_n-COOH (**1a** or **1b** or **1e** or **1f** or **1g**)/TBTU/DIEA (3 equiv, 3 equiv, 6 equiv) in DMF. (c) Piperidine-DMF (2:3), 30 min. (d) i—Piperidine-DMF (2:3), 30 min; ii—Ac_2O/DIEA (5 equiv, 10 equiv) in DMF, 2 h; iii—anhydrous HF.

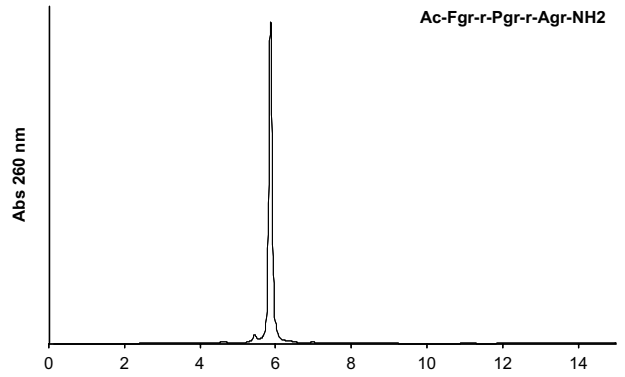
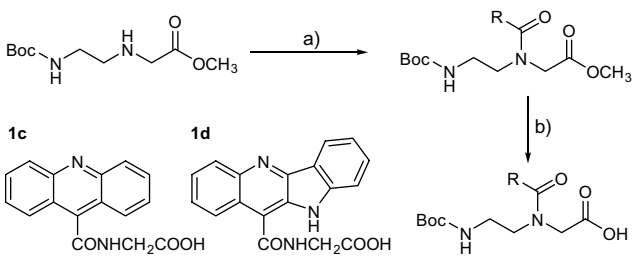


Figure 1. HPLC chromatogram of trimer Ac-Fgr-r-Pgr-r-Agr-NH_2.

For the solid-phase synthesis of 2-aminoethylglycine backbones, the scaffolds containing several intercalating agents were prepared in solution (see Scheme 3). These aminoethylglycine derivatives were prepared from the *N*-Boc-2-aminoethylglycine methyl ester, which was reacted with the carboxyl derivatives of acridine (**1c**) and indoloquinoline (**1d**) using DIPCDI and hidroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HOOBt) as coupling reagents, yielding compounds **2c**–**d**. Hydrolysis of the resulting compounds with aq NaOH in dioxane (1:1) gave the desired derivatives **3c**–**d**. Oligomers with these *N*-aminoethylglycine scaffolds were then synthesized on polyethyleneglycol-polystyrene supports with the 6-aminohexylsuccinyl linker.[13] Standard solid-phase peptide synthesis protocols were used. The synthesis cycle consisted of two reactions, removal of the Boc group using 50% TFA in DCM and 5% cresol as a scavenger, followed by the coupling of the appropriate monomer (5 molar excess) using [*O*-(7-azabenzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 4.5 molar excess) and DIEA (15 molar excess) as coupling reagents. After the assembly of the sequence, the resulting supports were treated with conc. aqueous ammonia for 2 h at 55 °C to yield the desired unprotected oligomers (Table 1).

The excitation and emission wavelengths of the new compounds were measured (see Supporting information). Optimal excitation wavelengths ranged from 248 to 360 nm and the corresponding emission wavelengths ranged from 396 to 505 nm. The indoloquinoline (Qgr) derivatives with γ-aminoproline backbone had the higher emission wavelengths.



Scheme 3. Synthesis of 2-aminoethylglycine derivatives. Reagents: (a) R-COOH (**1c** or **1d**)/DIPCDI/HOOBt/*N*-ethylmorpholine, (b) NaOH (water/ethanol).

Table 2. Antiproliferative activity

Compound	IC_{50} (μM) Jurkat clon E6-1	IC_{50} (μM) GLC-4S	IC_{50} (μM) NIH-3T3
1c	n.a.	n.a.	n.a.
1c, methyl ester	n.a.	n.a.	n.a.
1d	12	n.a.	n.a.
1d, methyl ester	8	n.a.	n.a.
Ac-Agr-Agr-NH_2	149	135	n.a.
Ac-Qgr-Qgr-NH_2	37	54	n.a.
Ac-Agr-Qgn-NH_2	92	125	376
Ac-Qgr-Agr-NH_2	120	165	n.a.
Ac-Agr-Agr-Agr-NH_2	n.a.	134	n.a.
Ac-Qgr-Qgr-Qgr-NH_2	130	n.a.	n.a.
Ac-Agr-Agr-Qgr-NH_2	86	174	426
Ac-Agr-Qgr-Agr-NH_2	87	91	n.a.
Ac-Qgr-Agr-Agr-NH_2	129	169	357
Ac-Qgr-Agr-Qgr-NH_2	112	82	263
Ac-Agr-Qgr-Qgr-NH_2	103	151	227

n.a., not active; n.d, not determined.

The antiproliferative activity of the new compounds having the aminoprolyl backbones were analyzed on two human cancer cell lines, Jurkat clon E6-1 and GLC-4S, and one mouse fibroblast cell line, NIH-3T3. MTT assay was performed to determine IC_{50} values. Results are shown in Table 2. All compounds had a moderate antiproliferative activity in cancer cell lines (IC_{50} 37–170 μM), whereas IC_{50} values for NIH-3T3 were very high (IC_{50} > 227 μM), suggesting a low toxicity to non-tumoral cells. As comparison the carboxyl derivative **1c** and its methyl ester were not active in these conditions whereas the carboxyl derivative **1d** and its methyl ester were only active on Jurkat clon E6-1 cell lines.

In summary, we report an efficient solid-phase synthesis of several oligomers carrying fused heterocyclic derivatives linked through amide bonds. γ-Aminoproline, 2-aminoethylglycine, and ornithine backbones were studied. All oligomers were obtained rapidly and in high yields, suitable for the preparation of a wide range of these derivatives. Preliminary antiproliferative assays showed moderate but selective growth-inhibitory properties. The methods described here can be used in the search for more active compounds and for the introduction of fluorescent compounds into synthetic peptides.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.045.

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