Synthesis of a hybrid fullerene–trimethoxyindole–oligonucleotide conjugate

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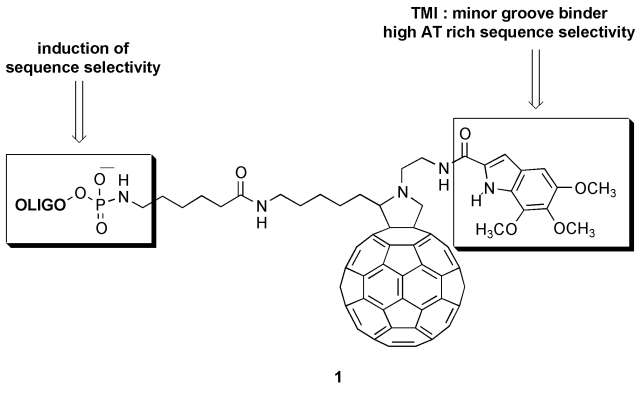
The synthesis of novel functionalized fullerene derivatives is reported: a DNA minor groove binder such as trimethoxyindole-2-carboxylate (TMI) and an oligonucleotide chain have been covalently linked to C_{60} with the aim of duplicating DNA interactions for increasing sequence selectivity.

Fullerene C_{60} and its organofunctionalized derivatives have recently become a topic of interest in bioorganic and medicinal chemistry. This class of compounds has shown high potential in a wide variety of biological activities, such as DNA photocleavage, HIV protease inhibition, neuroprotection and apoptosis.[1] In particular, the excited state properties of C_{60} may offer viable routes to novel pharmacological tools.[2]

One of the problems of photodynamic therapy is the addressed delivery of a photoactive agent to its target. That is why conjugates of fullerene with molecules possessing biological affinity to certain nucleic acids, proteins, cell types, organelles, *etc.*, might be of particular interest. Additionally, C_{60} itself might facilitate the interactions of certain biologically active molecules with lipophilic membranes of living cells and consequently improve cellular uptake due to its high hydrophobicity.

Recently, some conjugates between C_{60} and nucleic acid-specific agents (acridine,[3] netropsin[4] and complementary oligonucleotides[5,6]) have been reported, with the aim of better understanding the mechanism of action and to increase their cytotoxic properties and specificity. Coupling of fullerene to an intercalator or a minor groove binder already leads to higher affinity and specificity of the derivatives towards target DNA.[4] The next step envisages the improvement of the photocleavage efficiency of the conjugates, possibly by stabilizing their duplexes and triplexes and increasing their sequence selectivity.

In order to achieve these goals, we have designed derivative (**1**) of fulleropyrrolidine linked to trimethoxyindole (TMI) and



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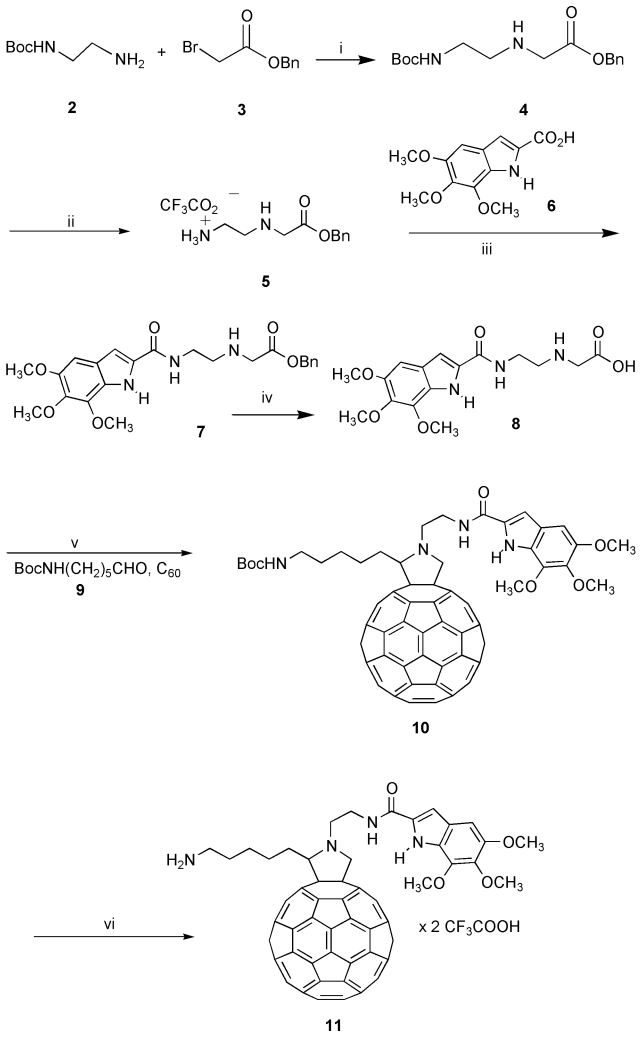
OLIGO = 5'-TTT-TC*T-TTT-C*C*C*-C*C*C*-T-3'
C* = Methylated Cytosine

an oligonucleotide chain simultaneously. The rational design of this derivative was based on a synergistic effect of two different linkers such as TMI and oligonucleotide. The TMI unit is characteristic of a class of natural antibiotics named duocarmycins, possessing antitumor activity in the picomolar range, in part due to high affinity and selectivity of the TMI group for the minor groove in AT-rich sequences of DNA.[7] The effect of the oligonucleotide chain could be considered both to induce high sequence-selectivity and to increase water solubility, one of the main problems of C_{60} derivatives for biological application.

The designed compound **1** was prepared following the general synthetic strategy summarized in Schemes 1–2, according to the known procedure for the synthesis of fulleropyrrolidines, based on the 1,3-dipolar cycloaddition of azomethine ylides to C_{60}.[8,9]



Scheme 1 *Reagents*: i, dioxane, 0 °C, then rt 18 h; ii, TFA, CH_2Cl_2, rt, 20 min; iii, NMM, HOBT, EDC, CH_2Cl_2; iv, Pd/C 10%, H_2, MeOH; v, toluene, reflux, 50 min; vi, TFA, CH_2Cl_2, rt, 1 h.

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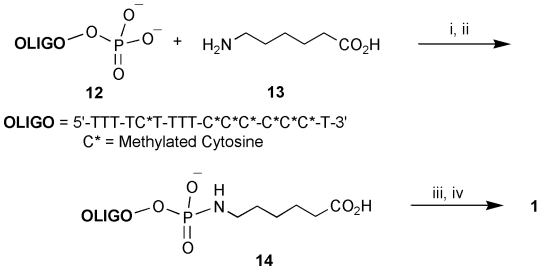

Scheme 2 *Reagents*: i, PPh_3, DMSO, DMAP, Py_2S_2; ii, DMSO; iii, PPh_3, DMSO, Py_2S_2; iv, Et_3N, DMSO, **11**.

The appropriate *O*-benzylaminoacid **4** was prepared by alkylation of the mono protected (Boc) ethylenediamine **2** with benzyl α-bromoacetate **3** (Scheme 1).

Deprotection of the amino functionality by TFA in **8** led to amine **5**, which was coupled to trimethoxyindole-2-carboxylic acid **6**[10] to afford amino ester **7**. The latter was deprotected at the carboxylic function by catalytic hydrogenation and the resulting acid **8** was allowed to react with C_{60} and *N*-Boc-6-aminohexanal **9** to yield the desired multifunctional fulleropyrrolidine **10**.† The latter compound was in turn deprotected to obtain the desired salt **11** by treatment with TFA (Scheme 1).

The designed product **1** was synthesized following a general synthetic strategy for the preparation of oligonucleotide conjugates previously reported[11–13] and summarized in Scheme 2, but the new procedure contained several modifications. Originally the reaction was initiated by activation of the oligonucleotide terminal phosphate by means of Mukaiyama reagents, triphenylphosphine–dipyridyl-2,2′-disulfide in the presence of DMAP.[14] However, it appeared that interaction of the activated phosphate with the amino group of the fullerene derivative did not lead to satisfactory yields of conjugation. Thus, it was necessary to use 6-aminocaproic acid as a spacer between the two moieties.

The phosphorylated oligonucleotide (16-mer) **12** was activated at its terminal phosphate, precipitated by lithium perchlorate in acetone according to the method described by Knorre *et al.*[11] and attached to the ε-amino group of 6-aminocaproic acid **13** in water in the presence of triethylamine to afford the carboxylic acid derivative of oligonucleotide **14** in quantitative yield. Coupling of **14** with fullerene derivative **11** was performed in a similar way, by activation of the carboxylic group with Mukaiyama reagents in organic media to obtain the desired conjugate **1**. Purification of **1** was performed by electrophoresis in 1% agarose–0.1% triton X-100 gel using tris-acetate buffer, followed by excision of the colored band and electroelution, or digestion of agarose with β-agarase.[5,6]

In summary, we have described the synthesis of the bis-functionalized fulleropyrrolidine **1** containing two different linkers capable of modulating and inducing high sequence selectivity. Results on duplex and triplex helicex DNA formation and the synthesis of an enlarged series of compounds for structure–activity relationship studies will be reported in due course.

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Notes and references

† *Analytical and spectroscopic data* of derivative **10**: $C_{86}H_{40}N_4O_6$ (MW 1225.30), yield: 19%. ^{1}H-NMR: δ 9.27 (br s, 1H), 7.30 (br s, 1H), 6.93 (s, 1H), 6.75 (s, 1H), 4.98 (d, J = 10.3 Hz, 1H), 4.52 (m, 1H), 4.37 (t, J = 5.9 Hz, 1H), 4.30 (d, J = 10.5 Hz 1H), 4.08–3.95 (m, 4H), 4.05 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.25 (m, 1H), 3.11 (m, 2H), 2.45 (m, 2H), 1.89 (m, 2H), 1.49 (m, 2H), 1.43 (s, 9H). ^{13}C-NMR: δ 166.9, 156.4, 154.4, 153.2, 150.0, 147.1, 146.3, 146.2, 146.1, 146.0, 145.9, 145.5, 145.2, 145.1, 144.6, 144.4, 144.3, 143.0, 142.6, 142.2, 142.1, 142.0, 141.9, 141.7, 140.2, 139.7, 136.7, 135.8, 135.6, 135.4, 130.6, 123.3, 97.1, 71.2, 67.4, 65.4, 61.1, 56.2, 44.1, 41.1, 31.0, 29.9, 28.5. ES-MS (THF–MeOH 1:1): m/z 1225 (MH^+). UV–VIS (cyclohexane) λ_{max}: nm 254, 312, 431, 703. IR (KBr, DRIFT) cm^{-1}: 3330, 2935, 2850, 1690, 1645, 1245, 1169, 526.

1 T. Da Ros and M. Prato, *Chem. Commun.*, 1999, 663.
2 D. Guldi and M. Prato, *Acc. Chem. Res.*, 2000, **33**, 695.
3 Y. N. Yamakoshi, T. Yagami, S. Sueyoshi and N. Miyata, *J. Org. Chem.*, 1996, **61**, 7236.
4 E. Nakamura, H. Tokuyama, S. Yamago, T. Shiraki and Y. Sugiura, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2143.
5 A. Boutorine, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2462.
6 Y.-Z. An, C.-H. B. Chen, J. L. Anderson, D. S. Sigman, C. S. Foote and Y. Rubin, *Tetrahedron*, 1996, **52**, 5179.
7 D. L. Boger and D. S. Johnson, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1438.
8 M. Maggini, G. Scorrano and M. Prato, *J. Am. Chem. Soc.*, 1993, **115**, 9798.
9 M. Prato and M. Maggini, *Acc. Chem. Res.*, 1998, **31**, 519.
10 D. Boger, T. Ishizaki, H. Zarrinmayeh, P. Kitos and O. Suntornwat, *J. Org. Chem.*, 1990, **55**, 4499.
11 D. G. Knorre, P. V. Alekseyev, Y. V. Gerassimova, V. N. Silnikov, G. A. Maksakova and T. S. Godovikova, *Nucleosides, Nucleotides*, 1998, **17**, 397.
12 T. S. Godovikova, V. F. Zarytova, T. V. Maltseva and L. M. Khalimskaya, *Bioorg. Khim.*, 1989, **15**, 1246.
13 A. S. Boutorine, T. Le Doan, J. P. Battioni, D. Mansuy, D. Dupré and C. Hélène, *Bioconj. Chem.*, 1990, **1**, 350.
14 T. Mukaiyama, R. Matsueda and M. Suzuki, *Tetrahedron Lett.*, 1970, **22**, 1901.