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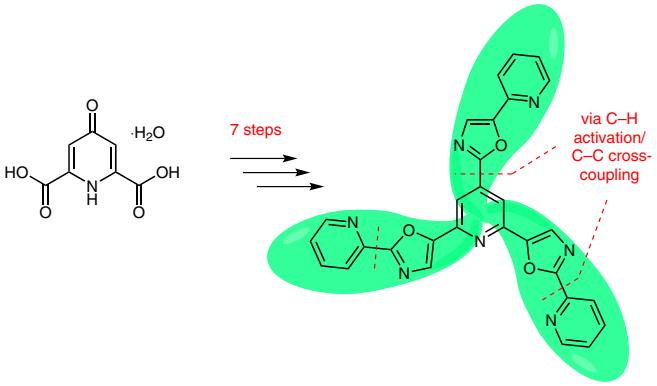
Synthesis of a 'Propeller-Like' Oligoheteroaryl with Alternating Pyridine and Oxazole Motifs

Savvas N. Georgiades*
Natalia Rizeq

Department of Chemistry, University of Cyprus, 1 Panepistimiou Ave., Aglandjia, Nicosia 2109, Cyprus
georgiades.savvas@ucy.ac.cy



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Abstract The molecular architecture of oligomeric pyridyl-oxazole compounds is key to determining their mode of interaction with G-quadruplex DNA structures, which is a family of prominent anticancer biomolecular targets. We report herein an efficient synthetic route that begins with chelidamic acid and affords, in just seven steps, an unusual 'propeller-like' pyridyl-oxazole architecture with alternating pyridine and oxazole rings, that has not been yet validated as a G-quadruplex binder. The synthesis employs Van Leusen chemistry for the construction of oxazole rings from aldehydes, and two Pd(II)/Cu(I)-mediated cross-coupling reactions involving C–H activation of oxazoles for the formation of C–C bonds between bromopyridine intermediates and oxazole fragments. This modular synthesis was designed to be amenable to the construction of analogues.

Key words anticancer, oligoheteroaryl, propeller architecture, C-H activation, G-quadruplex

Oligoheteroaryl compounds that incorporate in their structure several oxazole rings have become a focal point of recent synthetic efforts, inspired by the unique natural product telomestatin[1] (Figure 1 A, i). Telomestatin is a macrocycle of bacterial origin, made up of seven oxazoles and one thiazoline, all connected in a cyclic head-to-tail arrangement. The interest in this molecule derives from its ability to specifically bind the DNA G-quadruplex helix formed within the guanine-rich single-stranded sequence of the human telomeres (H-telo).[2] Telomestatin binding causes G-quadruplex stabilization, which stalls the elongation of the telomere by the enzyme telomerase, a process responsible for rendering cancer cells 'immortal'. Telomerase inhibition eventually triggers apoptosis. While the exact nature of telomestatin interaction with H-telo is not fully understood, it is believed that it involves π-π stacking of the macrocycle on the terminal planar surfaces of the quadruplex (the most exposed guanine tetrads),[2] probably reinforced by additional contacts of the oxazoles to other quadruplex binding elements (Figure 1 B, i).

Early synthetic examples of telomestatin analogues (e.g., Figure 1 A, ii), as well as synthetic precursors to telomestatin itself, have been peptide-like, in that they include some amide bonds as part of their macrocyclic periphery, and originate from amino acid building blocks.[3] More recently, focus has shifted to macrocyclic systems whose periphery combines both pyridine and oxazole

Savvas N. Georgiades (left) was born in Nicosia, Cyprus (1977). He obtained his B.Sc. in Chemistry from University of Cyprus (2001) and his Ph.D. in Organic Chemistry/Chemical Biology from Harvard University (2006). After postdoctoral appointments at the Scripps Research Institute-La Jolla, California (2006-2007) and Imperial College London (2007-2010), Dr. Georgiades worked for one year as Lecturer in Pharmacy and Chemistry at Kingston University (UK), before joining University of Cyprus as Lecturer in Organic and Medicinal Chemistry (2011). His research interests include: (a) Development of new DNA G-quadruplex binders as a basis for new anti-cancer treatments; (b) Synthesis and biological evaluation of small-molecule modulators of cellular signaling pathways, relevant to cancer and neurodegenerative diseases; (c) Development of novel synthetic methodologies.

Natalia Rizeq (right) was born in Larnaca, Cyprus (1990). In 2012, she obtained her B.Sc. in Chemistry from Aristotle University of Thessaloniki (Greece). She is currently a M.Sc. student at the Department of Chemistry, University of Cyprus, carrying out her research on novel binders for DNA G-quadruplexes under the supervision of Dr. Savvas N. Georgiades.

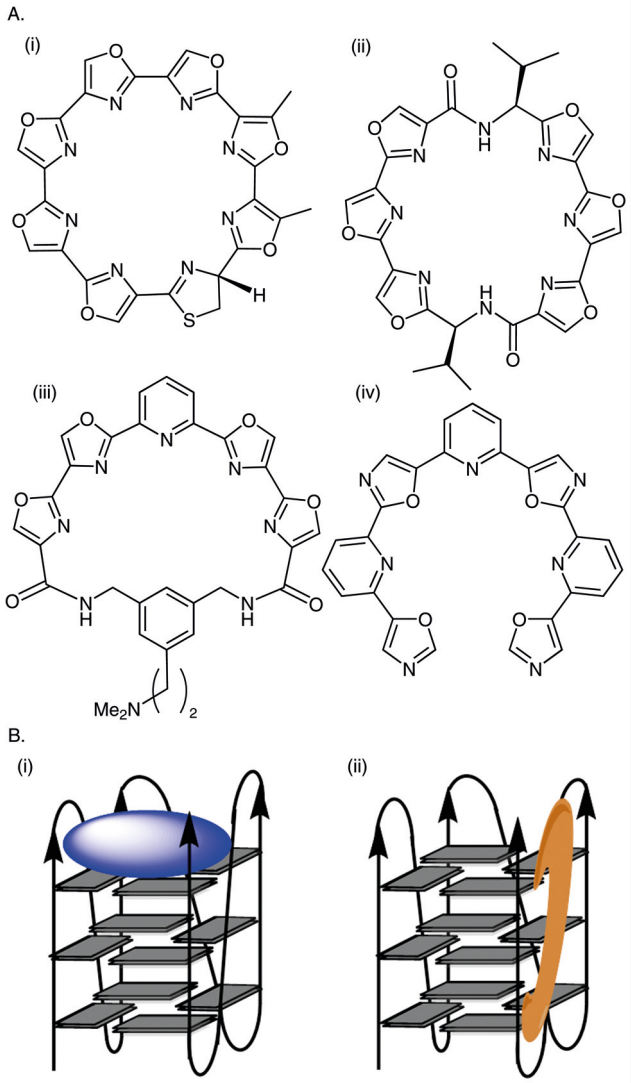


Figure 1 (A) Telomestatin (i) and examples of previously reported synthetic polyoxazoles (ii–iv), inspired from telomestatin. (B) Proposed mode of binding for macrocyclic telomestatin-like (blue) vs. non-cyclic (orange) pyridyl-oxazole ligands onto a DNA G-quadruplex (each gray rectangle represents one guanine base)

heteroaromatic rings (e.g., Figure 1 A, iii),[4] which facilitates synthesis and increases versatility when it comes to chemical functionalization. Biological evaluation of all these molecules suggests that they successfully mimic the binding of telomestatin to H-telo and hence are also considered potential anticancer lead structures.

Identifying promising new molecules that can distinguish between different G-quadruplexes remains a major challenge, especially in light of the fact that G-rich sequences with potential of folding into quadruplex are found not only in the telomeres, but all across the human genome, including several oncogene promoter regions.[5] Stabilization of G-quadruplexes in these regions by small molecules has been shown to down-regulate the expression of the corresponding oncogenes, which also acts in an anticancer capacity.[6]

Recognizing the potential of the pyridyl-oxazole moiety to serve as a privileged motif in the design of selective G-quadruplex binders, Hamon et al. went on to report on non-cyclic pyridyl-oxazole counterparts (e.g., Figure 1 A, iv), which were surprisingly found to discriminate between two different G-quadruplex folds of the human telomeric sequence.[7] Moreover, the same group described cationic pyridyl-oxadiazole systems that exhibit the opposite preference towards the same two variants of the H-telo quadruplex.[8] Such non-cyclic pyridyl-oxa(dia)zole compounds represent a different mode of binding (Figure 1 B, ii), which resembles groove binding in duplex DNA. This is consistent with the crescent-like shape of the molecules. Since the grooves differ significantly between diverse G-quadruplexes, they offer unique opportunities as sites of selective recognition by small molecules.

Branched pyridyl-oxazoles, in which all the branches are oxazole-pyridine oligomers, have not been previously reported or evaluated for binding to G-quadruplex DNA. In the current communication, we describe the development of a versatile and efficient synthetic method that allows easy access to pyridyl-oxazoles of propeller-like architecture. The synthesis has been exemplified with the preparation of a model compound (Figure 2). This type of structure deviates from the classical crescent-like one, in that it introduces a third branching point on the central pyridine moiety. With appropriate selection of a substituted pyridyl early precursor, three oxazoles can occupy positions 2, 4, and 6 of the central pyridine ring and offer sites for further outward extension. This type of molecule is designed to have rotational flexibility at the aryl–aryl connections and the ability to randomly test different modes of binding before settling for a preferred one, depending on which G-quadruplex it is called to interact with and which branches it uses. In case it acts as groove binder, the third branch could, in principle, allow contacts with more flexible domains of the quadruplexes (e.g., loop domains), which are located away from the grooves and which have so far remained unexploited.

Our synthesis began from commercially available chelidamic acid (**1**), which offers three positions for modification around its heterocyclic ring (Scheme 1). The 2- and 6-carboxylate positions were both modified as ethyl esters to afford **2**[9] in excellent yield (98%), by using thionyl chloride in EtOH.[10] The esters served as protecting groups in the early stages and could be converted into desirable functionalities later in the synthesis.

The 4-hydroxy group of **2** was subsequently replaced by bromide in high yield (92%), upon treatment with PBr_5 (neat) at 95 °C,[10] to furnish compound **3**.[11] The bromide was

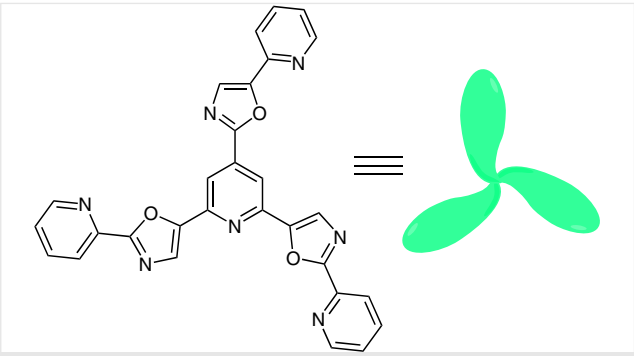


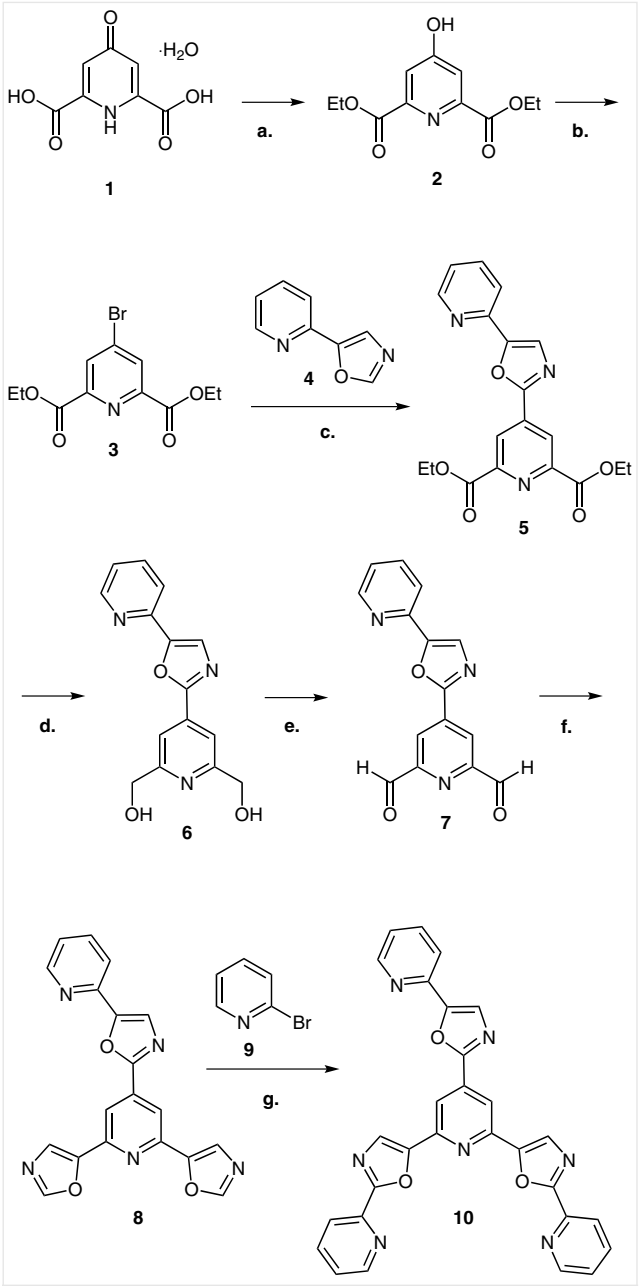
Figure 2 Structure of the propeller-like pyridyl-oxazole compound synthesized in this study

needed to serve as a handle for carrying out a C–C coupling reaction, which allowed us to introduce the third branching point.

Pyridyl-oxazole **4**,[12] which was prepared in-house from 2-pyridine carboxaldehyde in 98% yield as described in the supporting information, was introduced onto the 4-position of the pyridine ring of compound **3**, in a transformation that involved a palladium/copper cocatalyzed C–H activation of the oxazole. The reaction, which afforded intermediate **5**[13] in 51% yield after chromatographic purification, was a modification of previous protocols describing C–C cross-couplings of 5-aryloxazoles directly on aryl bromides,[14] and made use of the reaction conditions employed by Hamon et al.[7] in their assembly of crescent-like pyridyl-oxazoles. Specifically, the cross-coupling was carried out in 1,4-dioxane, with $Pd(OAc)_2$ and CuI as cocatalysts, Cs_2CO_3 as base, and $P(Cy)_3{\cdot}HBF_4$ as an additive. In our case, the bromopyridine involved (**3**) is more substituted and the success of the reaction demonstrates that it is tolerant to ester functionalities. However, its usefulness in constructing bis-aryl pyridyl-oxazole systems is somewhat limited by the formation of undesired side-products, which were not fully characterized. Attempts to replace 1,4-dioxane with higher boiling point solvents, such as DMF and NMP, typically led to lower yields and problematic isolation of the desired product.

A stepwise reduction-reoxidation approach was applied on **5** to obtain a dialdehyde. Our initial efforts for direct conversion of **5** into a dialdehyde by using sterically hindered aluminum reagents, such as DIBAL-H and Red-Al, only led to incomplete reductions and mixtures of products, which may be attributed to the tendency of the substrate to coordinate to the metal. Therefore, we turned to a method that employs $NaBH_4$ in anhydrous MeOH[15] for the full reduction of diester **5** to the diol. Diol **6**[16] was obtained clean after simple aqueous workup, without any need for chromatographic purification, in 76% yield.

Diol **6** was subsequently oxidized to dialdehyde **7**[17] by using hypervalent iodine reagent 2-iodoxybenzoic acid (IBX) as the oxidant, in anhydrous DMSO.[18] Freshly prepared batches of IBX[19] behaved in our hands more reproducibly than SeO_2 or MnO_2, the performance of which varied significantly between batches, giving dialdehyde **7** in 56% yield.



Scheme 1 Synthetic route for the preparation of a model propeller-like tris-oxazole substituted pyridyl compound **10**, from chelidamic acid (**1**). *Reagents and conditions*: (a) EtOH, $SOCl_2$, 0 °C, then r.t. for 18 h, then reflux for 2 h, 98%; (b) PBr_5 (neat), 95 °C, 3.5 h, then $CHCl_3$, EtOH, 0 °C, 3 h, 92%; (c) 1,4-dioxane, $Pd(OAc)_2$, CuI, Cs_2CO_3, $P(Cy)_3{\cdot}HBF_4$, 130 °C, 24 h, 51%; (d) MeOH, $NaBH_4$, 0 °C, then r.t. for 18 h, 76%; (e) DMSO, IBX, r.t., 10 h, 56% (f) MeOH, TosMIC, K_2CO_3, reflux, 4 h, 77%; (g) 1,4-dioxane, $Pd(OAc)_2$, CuI, Cs_2CO_3, $P(Cy)_3{\cdot}HBF_4$, 130 °C, 24 h, 54%.

A 1,3-dipolar cycloaddition carried out on the two aldehyde moieties of **7** by the reagent tosylmethyl isocyanide (TosMIC), upon its deprotonation by K_2CO_3 in MeOH heated to reflux (Van Leusen reaction),[20] assured an efficient conversion of **7** into the tris-oxazole system **8**[21] (77% yield). Tris-oxazole adducts with this pattern of oxazole connectivity to a central pyridine ring represent a new family of compounds and could prove to be useful synthetic intermediates in many applications, including biologicals and materials.

In our case, the two terminal oxazoles allowed for yet another C–C cross-coupling step, under the same conditions as the previous one,[7] but this time with 2-bromopyridine (**9**) as the coupling partner, to introduce one additional pyridine ring on each of the two termini and extend the respective branches. The use of excess 2-bromopyridine (3 equiv) was key to driving the valuable intermediate **8** to the corresponding bis-pyridinylated product **10**[22] (54% yield). We anticipate that the same reaction can be applied to modified/substituted 2-bromopyridines and could lead to even more extended oligomers with the same architecture, some of which would make good candidates for targeting G-quadruplexes.

In summary, we have described an expedient method leading to the generation of a model propeller-like pyridyl-oxazole compound **10**, with three branches and alternating pyridine and oxazole rings in each branch. This is a novel architecture with potential interest for anticancer research. Several analogues of model compound **10** are being prepared in our laboratory by variations of this method, and their evaluation for G-quadruplex binding is underway and will be reported in due course.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1379549.

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(9) Data for compound **2**: ^{1}H NMR ($CDCl_3$): δ = 1.41 (t, *J* = 7.1 Hz, 6 H), 4.46 (q, *J* = 7.1 Hz, 4 H), 7.35 (s, 2 H). ^{13}C NMR ($CDCl_3$ + trace DMSO-d_6): δ = 13.6, 61.6, 115.6, 148.1, 163.9, 167.1. MS (ESI): *m*/*z* = 238.08 (calcd 238.07 [M – H]$^-$), 261.06 (calcd 261.06, [M + Na – H]$^-$).
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(11) Data for compound **3**: ^{1}H NMR ($CDCl_3$): δ = 1.45 (t, *J* = 7.2 Hz, 6 H), 4.49 (q, *J* = 7.2 Hz, 4 H), 8.42 (s, 2 H). ^{13}C NMR ($CDCl_3$): δ = 14.1, 62.7, 131.0, 134.9, 149.5, 163.5. MS (ESI): *m*/*z* = 302.01 (calcd 302.00, [M + H]$^+$).
(12) Data for compound **4**: ^{1}H NMR ($CDCl_3$): δ = 7.13 (t, *J* =7.0 Hz, 1 H), 7.55 (d, *J* = 7.0 Hz, 1 H), 7.61 (s, 1 H), 7.65 (t, *J* = 7.0 Hz, 1 H), 7.89 (s, 1 H), 8.53 (d, *J* = 7.0 Hz, 1 H). ^{13}C NMR ($CDCl_3$): δ = 119.0, 122.7, 124.5, 136.6, 146.7, 149.5, 150.7, 150.8. MS (ESI): *m*/*z* = 147.06 (calcd 147.06, [M + H]$^+$).
(13) Data for compound **5**: ^{1}H NMR ($CDCl_3$): δ = 1.46 (t, *J* = 7.2 Hz, 6 H), 4.51 (q, *J* = 7.2 Hz, 4 H), 7.29 (td, J_1 = 5.5 Hz, J_2=2.8 Hz, 1 H), 7.80–7.82 (m, 2 H, overlapping), 7.90 (s, 1 H), 8.65 (dt, J_1 = 4.7 Hz, J_2 = 1.5 Hz, 1 H), 8.87 (s, 2 H). ^{13}C NMR ($CDCl_3$): δ = 14.1, 62.5, 119.7, 123.6, 123.8, 127.6, 136.5, 137.0, 146.2, 149.6, 150.0, 152.5, 158.0, 164.0. MS (ESI): *m*/*z* = 368.12 (calcd 368.13, [M + H]$^+$).
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(16) Data for compound **6**: ^{1}H NMR (DMSO-d_6): δ = 4.63 (d, *J* = 6.1 Hz, 4 H), 5.63 (t, *J* = 6.1 Hz, 2 H), 7.44 (t, *J* = 5.8 Hz, 1 H), 7.96–7.98 (m, 4 H, overlapping), 8.06 (s, 1 H), 8.69 (d, *J* = 5.0 Hz, 1 H). ^{13}C NMR (DMSO-d_6): δ = 64.1, 114.2, 119.9, 123.9, 127.4, 134.4, 137.6, 146.1, 150.1, 151.5, 159.7, 162.8. MS (ESI): *m*/*z* = 282.10 (calcd 282.09, [M – H]$^-$).
(17) Data for compound **7**: ^{1}H NMR ($CDCl_3$): δ = 7.30 (t, *J* = 5.6 Hz, 1 H), 7.80–7.84 (m, 2 H, overlapping), 7.91 (s, 1 H), 8.66 (d, *J* = 4.7 Hz, 1 H), 8.76 (s, 2 H), 10.20 (s, 2 H). ^{13}C NMR ($CDCl_3$): δ =

120.3, 121.5, 124.1, 128.4, 136.7, 138.0, 145.6, 149.7, 152.0, 153.9, 158.1, 191.6. MS (ESI): *m*/*z* = 280.06 (calcd 280.07, [M + H]$^+$).

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(21) Data for compound **8**: ^{1}H NMR ($CDCl_3$): δ = 7.31 (t, *J* = 5.6 Hz, 1 H), 7.83–7.87 (m, 4 H, overlapping), 7.93 (s, 1 H), 8.05 (s, 2 H), 8.26 (s, 2 H), 8.69 (d, *J* = 4.5 Hz, 1 H). ^{13}C NMR ($CDCl_3$ + trace CD_3OD): δ = 114.0, 114.8, 119.9, 123.6, 126.3, 127.5, 131.4, 136.1, 137.2, 146.5, 148.3, 150.1, 152.2, 158.9. MS (ESI): *m*/*z* = 358.10 (calcd 358.10, [M + H]$^+$).

(22) Data for compound **10**: ^{1}H NMR ($CDCl_3$): δ = 7.33 (dd, J_1 =7.9, J_2 = 4.9 Hz, 1 H), 7.45 (dd, J_1 = 7.9, J_2 = 4.9 Hz, 2 H), 7.85–7.95 (m, 3 H, overlaid), 7.95 (d, *J* = 6.5 Hz, 1 H), 7.96 (s, 1 H), 8.08 (s, 2 H), 8.30 (d, *J* = 7.9 Hz, 2 H), 8.49 (s, 2 H), 8.72 (d, *J* = 4.9 Hz, 1 H), 8.83 (d, *J* = 4.1 Hz, 2 H). ^{13}C NMR ($CDCl_3$): δ = 115.1, 120.0, 122.7, 123.6, 125.1, 127.6, 128.8, 136.2, 137.0, 137.2, 145.8, 146.7, 148.3, 149.0, 150.2, 151.2, 152.5, 159.1, 161.2. MS (ESI): *m*/*z* = 512.14 (calcd 512.15, [M + H]$^+$).