ACES Asian Chemical Editorial Society

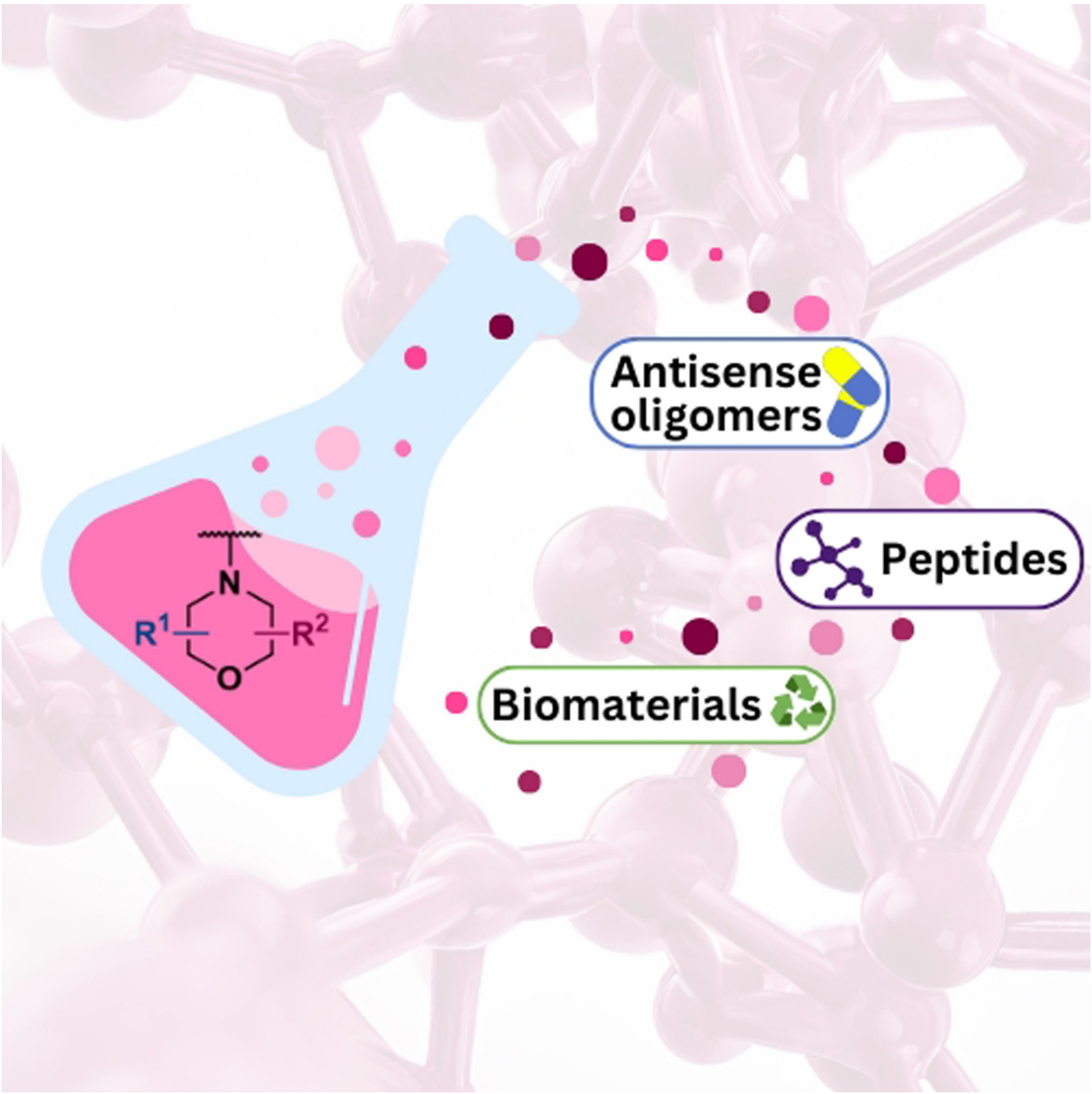
doi.org/10.1002/ajoc.202300540

ASIAN JOURNAL OF ORGANIC CHEMISTRY
Review

www.asianjoc.org

Morpholine Scaffolds' Preparation for Foldamers' Design and Construction

Nicola Schiaroli,[a] Camilla Loro,[a] Sara Colombo,[a] Gianluigi Broggini,[a] Marta Papis,*[a] and Francesca Foschi*[a]



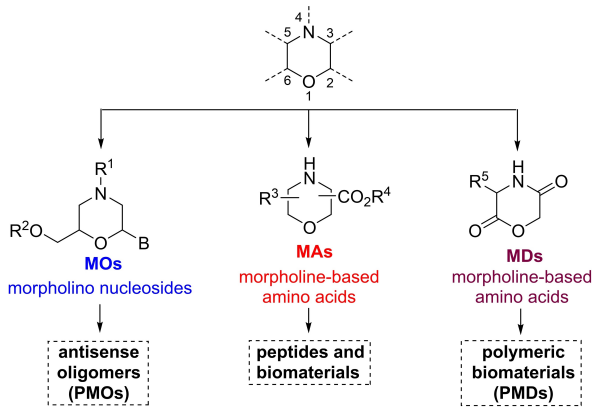
This review highlights the advances in the synthesis of morpholine building blocks useful for artificial oligomers synthesis and peptide foldamers or biopolymers. Both morpholino nucleosides and morpholine derived from amino acids have been considered focusing on the preparation of antisense strands, peptides and bio-based materials.

1. Introduction

The morpholine unit is a pivotal component of the pharmacophore of many drugs as small-molecules (active pharmaceutical ingredients – APIs)[1] or foldamers (oligonucleotides and peptides)[2] active in the treatment of diseases involved in gene expression.

Morpholine and its derivatives, in fact, promote specific interaction with a range of biological targets, modulating binding drug affinity, potency or selectivity. The morpholine skeleton is also able to increase the drug solubility enhancing the ability of the drug to pass the blood-brain barrier. These features depend on the presence of specific groups linked to the four carbon atoms and a nitrogen and oxygen atoms able to generate specific bonding interactions, ionization, and to offer a well-balanced lipophilic-hydrophilic profile.[3]

Considering that reviews concerning the synthesis of small bioactive morpholines have been recently published,[4] this review aims to give an outline of the advances in the synthesis of morpholine sub-units useful in foldamer chemistry. As depicted in Scheme 1, the review will be structured on the basis of the application of the morpholine monomer: morpholino nucleosides **MOs** precursors of oligonucleotides, morpholine derived from amino acids **MAs** as building blocks for peptide synthesis, and as monomers (morpholinine-2,5-diones, **MDs**) for the production of bio-medical and bio-degradable materials.



Scheme 1. Morpholine based oligomers: fields of application.

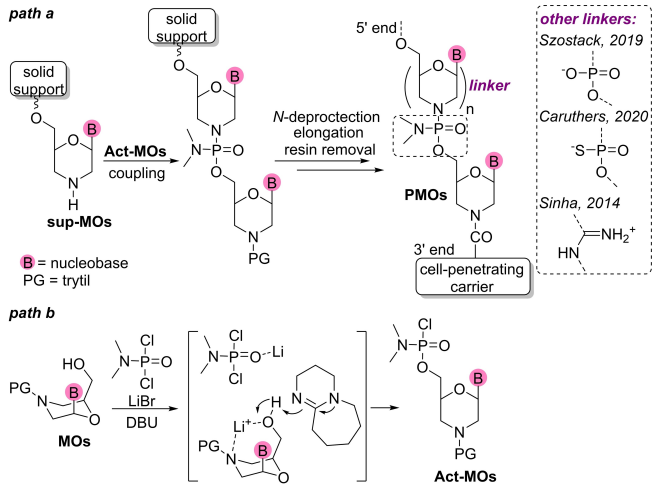
[a] *Dr. N. Schiaroli, Dr. C. Loro, Dr. S. Colombo, Prof. G. Broggini, Dr. M. Papis, Dr. F. Foschi*
DiSAT
Università degli Studi dell'Insubria
Via Valleggio, 9, Como, Italy
E-mail: francesca.foschi@uninsubria.it
mpapis@uninsubria.it

2. Antisense Field: Morpholines

2.1. Phosphorodiamidate Morpholino Oligonucleotides (PMOs): A Brief Introduction

PMOs[5] are one of the most promising classes of antisense agents. In **PMOs** a morpholine ring replaces the ribose sugar, and a neutral phosphorodiamidate tether replaces the natural phosphates linkage. **PMOs** showed drug-like properties such a high both efficacy in blocking translation and biological stability, together with a minimized toxicity. These properties make **PMOs** based therapies a straightforward alternative for a broad range of life-threatening diseases such Huntington's disease,[6] Ebola,[7] cancer,[8] and Duchenne muscular dystrophy (DMD). It is worth of note that four DMD **PMOs**-based drugs (eteplirsen, golodirsen, casimersen, viltolarsen) have been approved by the U.S. Food and Drug Administration (FDA) on the market.[9]

The standard protocol of **PMOs** synthesis involves the assembling of the solid supported morpholine subunit **sup-MOs** with the chlorophosphoramidate-activated monomer **Act-MOs** in the presence of a coupling agent (Scheme 2, path a).[10] **Act-MOs** could be prepared from *N*-protected **MOs** through chlorophosphoramidate chemistry under basic conditions. Furthermore, the low reactivity of the pentavalent phosphorus reagent has been enhanced by using lithium salts (Scheme 2, path b).[11] LiBr, indeed, is able to chelate the **MOs** nitrogen atom and the exocyclic oxygen atom increasing the OH acidity and reactivity under basic conditions. H-phosphonate chemistry was also exploited for the synthesis of standard **Act-MOs.**[12] After elongation, the final cleavage step furnished the target **PMOs** strand. Many modifications have been realized at the phosphoramidate intersubunit linkage (for linkers see box in Scheme 2). For example, recently Szostak developed a charged-phosphate backbone variant,[13] Caruthers research group described the synthesis of thiophosphoramidate morpholino oligomers,[14] while Sinha reported a guanidinium-linked morpholino pentamer.[15] It is worth noting that delivery of **PMOs** into tissues has been successfully realized by tethering the nitrogen atom at the 3′ end of **PMOs** with cell-penetrating carriers, such as dendrimers containing guanidine head groups (Vivo-Morpholinos, marketed by GeneTools – Sarepta Therapeutics Inc.)[16] or arginine-rich peptides (PPMOs, marketed by AVI Biopharma – Sarepta Therapeutics Inc.).[17]



Scheme 2. General protocol for the synthesis of **PMOs** morpholine-based oligomers.

2.2. Morpholino Nucleosides (MOs) Synthesis

The most used synthetic protocol for **MOs** preparations is disclosed by J. E. Summerton and D. D. Weller during the 1990s at Antivirals Inc. (now Sarepta Therapeutics Inc.). The process is formally a one-pot reaction with the nucleoside, in which the amine of nucleobase is protected, is reacted with an excess of sodium periodate to trig the oxidative ring opening of the sugar ring forming the key intermediate **1** (Scheme 3).[18] The latter, in turn, undergoes reductive amination promoted by ammonium biborate and sodium cyanoborohydride. The final protection of the morpholine nitrogen atom as *N*-trityl (Tr) allows the isolation of target chimera nucleoside **MOs**.

During the years, the efficiency of the process was thoroughly improved by Sinha research group (Scheme 4).[19] While maintaining the routine periodate cleavage/reductive amination reactions, the Sinha strategy foresees the protection of the heterocyclic base at a later stage to avoid the use of scarcely available corresponding amine-protected ribonucleosides. Unlike the previous protocol, this strategy also includes a protection/deprotection sequence of the 5′-OH residue of the starting nucleoside. This protocol allows to prevent side reactions (i.e. intramolecular cyclization or polymerization reactions, bis-aldimine or hemiacetal bond formation) increasing the yields of the target **MOs** and decreasing the complexity of the purification methods. In particular, the 5′-oxygen atom of the nucleoside has been protected as *tert*-butyldiphenylsilyl (TBDPS) ether prior to the one-pot nucleoside cleavage/morpholine ring formation step. *N*-Tr protection of the morpho-

Nicola Schiaroli was born in Senigallia (Italy) in 1989. He graduated in Industrial Chemistry at the University of Bologna where he received his Ph.D in Chemistry in 2019, under the supervision of Prof. Angelo Vaccari. He his currently a researcher at the Department of Science and High Technology (DiSAT) of the University of Insubria.

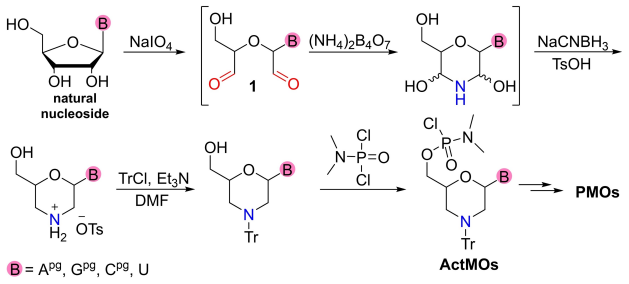
Camilla Loro, born in 1994, received her PhD in Organic Chemistry in April 2023 from Università degli Studi dell'Insubria (Italy). During her PhD, she did an internship at Sorbonne Université (France), joining the group of Prof. Giovanni Poli and Dr. Julie Oble. She is currently working in Prof. Broggini's research group as Postdoc. Her main interests are in transition-metal catalyzed C-H functionalization reactions and electrochemical approach for the synthesis of small molecules.

Sara Colombo, born in 1999, obtained her degree in Chemistry at the University of Insubria in 2023. Currently she is a PhD student in the research group of Prof. Broggini. Her main interests are in transition metal-catalyzed reactions to provide the functionalization of unactivated bonds for the synthesis of small molecules.

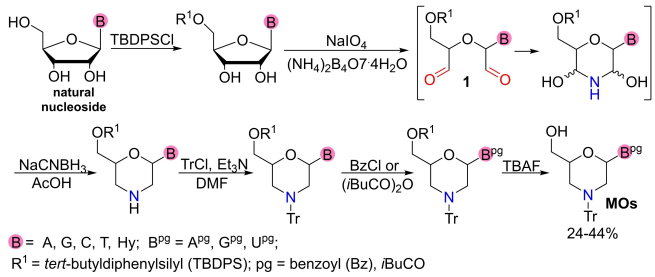
Marta Papis, born in 1997, obtained her degree in Chemistry at the University of Insubria in 2021. Currently she is a PhD student in the research group of Prof. Broggini. She did an internship at the University of Hamburg, joining the group of Prof. Chris Meier, and a research period at the pharmaceutical industry Olon S.p.A. in Milan. Her main interests are in the synthesis of nucleoside analogues, exploiting transition-metal catalyzed reactions.

Francesca Foschi was born in Milan (Italy). She received her BS degree in Industrial Chemistry from the University of the Study of Milan in 2007 and her Ph.D. in Industrial Chemistry 2010 at University of the Study of Milan. Her research focuses on the development of catalytic or depolymerization processes. She is currently a researcher in Industrial chemistry at the University of the Study of Insubria (Dipartimento di Scienza e Alta Tecnologia – DiSAT).

Gianluigi Broggini obtained his PhD degree from the University of Milano in 1993. He then worked at the same University as a postdoctoral fellow. After some years as assistant lecturer at the University of Insubria, he was appointed Full Professor at the same University. His research is focused on the development of new methodologies for the synthesis of heterocyclic compounds by transition metal-catalyzed reactions, mainly involving C-H functionalization reactions.



Scheme 3. Summerton standard procedure for the conversion of ribonucleosides to **MOs** compounds.

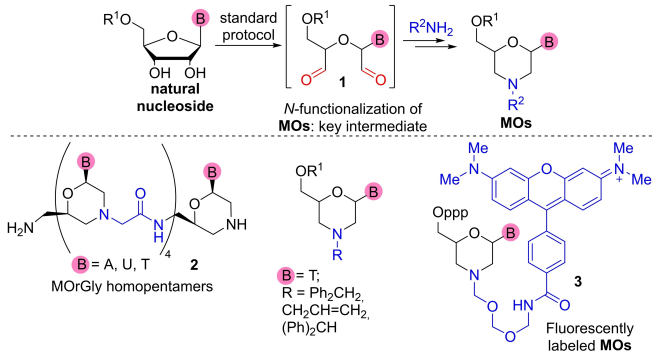


Scheme 4. Sinha **MOs** optimization of the standard procedure.

line nitrogen atom followed by *O*-silyl deprotection reaction affords the final **MOs**.

It should be noted that Micklefield research group observed that, while the use of equimolar amounts of nucleoside and nitrogen source furnished low yields of products, reaction conducted in the presence of an excess of nucleoside (and thus an excess of **1**) furnished the target **MOs** in higher yields. Micklefield assumed that the low yields reported by Summerton were ascribable to the instability of the di-aldehyde **1**, easily subjected to decomposition or over-oxidation.[20]

Using the standard protocol depicted in Scheme 3, intermediates **1** were used as electrophile in the presence of several nitrogen nucleophiles (*i. e.* carboxymethylamine, hydroxylamine, and various alkylamines; representative examples are shown in Scheme 5).[21] Due to their unique properties, the morpholino glycine oligomers (MorGly) **2** are promising oligonucleotide mimics in antisense therapy[21a] and the inhibition activity of

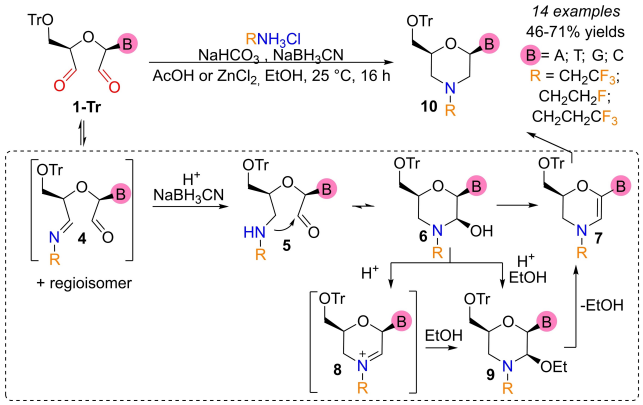


Scheme 5. Preparation of exotic *N*-substituted **MOs**.

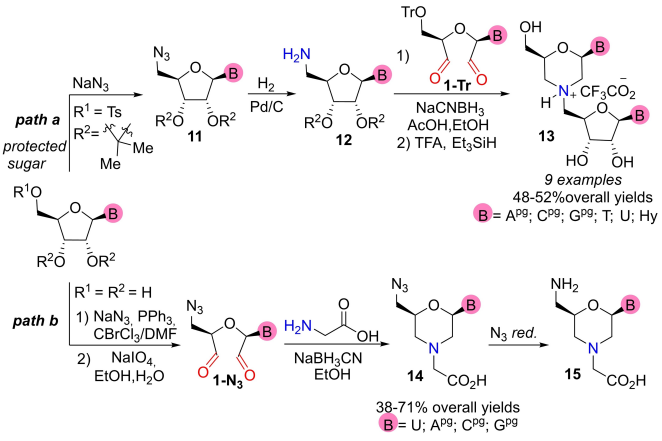
new fluorescently labeled morpholino nucleoside derivatives **3** was investigated by Silnikov research group.[21c]

Furthermore, recently Borbàs reported the synthesis of *N*-fluoroalkylated MOs compounds **10** (Scheme 6).[22] *O*-Tr protected dialdehydes **1-Tr** were subjected to reductive amination-cyclization using a series of fluorinated alkyl amines. Target **10** could be formed from imine **4** (or its regioisomer) through the hemiaminal **6** by cyclization of amino aldehyde **5**. Products **10** arise from a formal dehydration of **6** and subsequent reduction of the enamine **7**. The conversion of **6** into **7** could involve ethanol elimination from **9**, in turn generated directly from **6** or from the intermediate **8**. During the optimization process, the authors were able to detect and characterize by-products **6**, **7** and **9**. Efficient *O*-deprotection was accomplished using trifluoroacetic acid (TFA) and triethylsilane; the application of a one-pot ring closing/*O*-deprotection protocol starting from **1-Tr** did not affect the reaction yields.

In 2021 Borbás developed the synthesis of morpholino nucleoside dimers **13** using dialdehyde **1-Tr** and 5′-amino nucleosides **12** as the key building blocks for the coupling step (Scheme 7, path a).[23] Compound **12** was prepared starting from natural nucleosides in a three-step procedure: *O*-tosylation of the primary hydroxy group, tosylate exchange with azide affording **11**, and reduction of the azido residue by catalytic



Scheme 6. Preparation of *N*-fluoroalkylated **MOs 10**.



Scheme 7. Synthesis of morpholine-ribonucleosides dinucleotides **13** (path a) and 2′-aminomethyl **MOs 15** (path b).

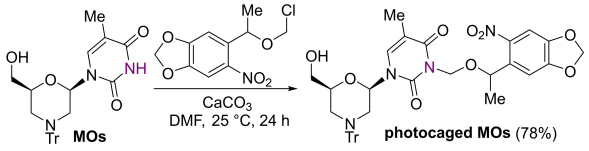
hydrogenation. The reductive amino-cyclization of **1-Tr** with **12** was accomplished under standard acidic conditions using $NaCNBH_3$ as the reducing agent. After concomitant deprotection of the alkoxy groups and quaternarization of the morpholine nitrogen atom, cationic dimers **13** (9 examples) were obtained. Conversion of the primary hydroxy residue of dimers **13** into amine followed by reaction with **1-Tr** allowed the construction of short charged-modified **PMOs** derivatives. The quaternary ammonium salt in **13** could facilitate cellular uptake. A related process toward 6-amino-**MOs** **15** was disclosed by Abramova and Kasakin (Scheme 7, path b).[24] The synthesis of the **1-N_3** intermediate was realized in a one pot procedure through a Mitsunobu reaction using unprotected nucleosides as starting materials for the insertion of N_3, followed by the oxidative ring-opening. After ring-closing reductive amination of **1-N_3** with glycine as the nucleophile, intermediate **14** was obtained then transformed into **15**. This class of nucleoside analogs could be used to synthesize peptide mimics by using solid phase peptide synthesis (SPPS).[25]

The research group of Borbás and Herczegh reported the synthesis of constrained morpholine-containing tricyclic structures, a family of nucleosides named by authors *tricyclanos* (**TOs**) in analogy with the Summerton's morpholinos **MOs** (Scheme 8).[26] The **TOs** have been prepared via a one-pot procedure involving the oxidative cleavage of *O*-Tr nucleosides followed by cyclo-condensation of intermediate **1-Tr** with the amino triol compound **16**. The reaction took place with complete stereoselectivity, probably through a series of consecutive nuclephilic additions controlled by the configurations of the two stereogenic centers in **1-Tr**. In addition, the removal of water produced during the process with molecular sieves promotes the exclusive formation of the kinetic product. Rapid deprotection of the *O*-Tr group in **TOs** was realized under mild reaction conditions in the presence of a Lewis acid ($ZnCl_2$), a reducing quenching agent (triethylsilane) in 1,1,1,3,3,3-hexafluoroisopropanol.

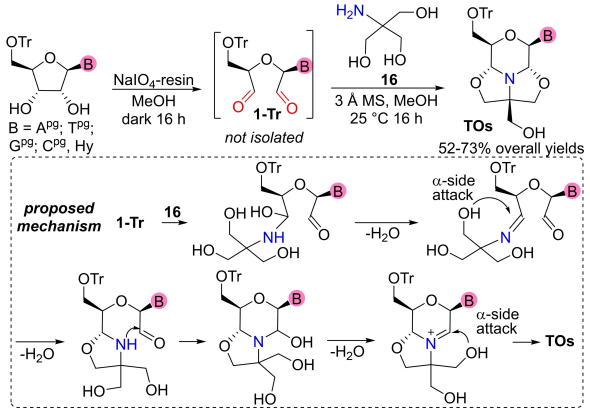
MOs containing appropriately functionalized nucleobases can be effective tools for spatio-temporal control of light-activated gene expression in multiple contexts. Functionalization by nucleophilic substitution of the NH residue in thymidine-**MOs** with photosensitive probes furnished photocaged monomers which were entered in light-activable **PMO** strands (Scheme 9).[27]

Concerning the structure modification, Sinha developed a straightforward protocol for the halogenation of the C-5 position of pyrimidines and C-8 position of purines in preformed **MOs** (Scheme 10).[28] Functionalization of these positions did not interfere with Watson-Crick base pair formation and could allow further nucleoside modification involving palladium-catalyzed cross-coupling reactions to attach labelling residues.[29] Iodination of the uridine-**MOs** compound under oxidative conditions furnished product **17** which was used in a range of cross-coupling reactions (Scheme 10, path a). alkynyl-substituted compounds **18** were synthesized in good yields through Sonogashira coupling reactions. Similarly, arylation of iodouridine **17** with aryl boronic acids were successfully realized.[30] A similar approach was disclosed by Abramova. In this case, the process involves the iodination of the nucleoside before construction of the morpholine moiety (Scheme 10, path b).[31] more in detail, periodate-promoted oxidation of **19** allowed the selective formation of **20** through Schiff base formation and reduction at controlled pH (5–6). Conversely, the use of $NaBH_3CN$ under more acidic conditions provided **20** together with the de-halogenated pyrimidine-**MOs** compound. An alternative strategy which involves iodination of cytidine in presence of ceric (IV) ammonium nitrate (CAN) was also disclosed by the authors.

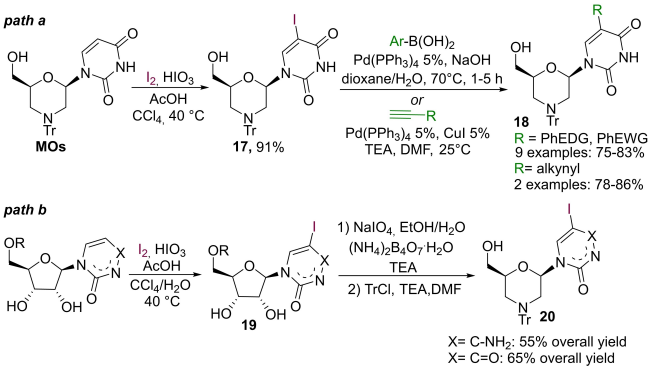
In 2022, our group reported a convergent strategy to access nucleobase-modified **MOs**.[32] The method involves the late-stage nucleic base installation to a pre-formed morpholine ring, which is the common precursor of target nucleoside analogs (Scheme 11). The morpholine acetal **22** was easily synthesized via oxirane ring opening of (*R*)-glycidol by *N*-nosyl amino-acetaldehyde **21** as nucleophile, followed by a *O*-benzoylation/ring-closure tandem reaction sequence. Functionalization of **22**



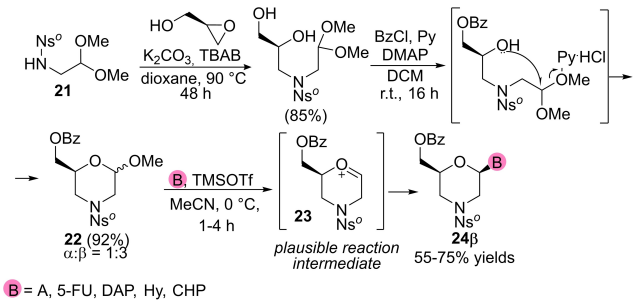
Scheme 9. Nucleobase-functionalized **MOs** synthesis.



Scheme 8. Preparation of tricyclic constrained **TOs** analogs.



Scheme 10. MOs containing functionalized nucleobase.



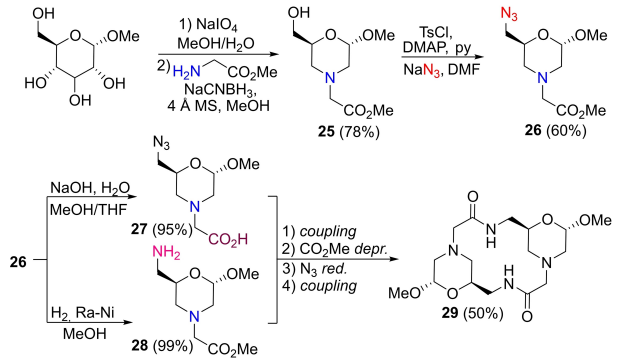
Scheme 11. Alternative synthesis of **MOs** starting from enantiopure glycidol.

was realized using unprotected nucleobases such as adenine (A), hypoxanthine (Hy), 2,6-diaminopurine (DAP), 5-fluorouracil (5-FU) and 6-chloropurine (CHP) under Lewis acid-promoted glycosylation reaction conditions. Since the anomeric configuration of **22** did not influence the stereochemistry of **24**, the nucleobase insertion most likely proceeds via an intermediate oxocarbenium ion **23**. The obtained **24α/β** mixture could depend on the absence of coordinating substituents in the C-5 position of **24**. The reported strategy gives access to optically pure compounds whose stereochemistry is closely related to the configuration of the starting epoxide source.

This proof-of-principle indicates that 2-morpholine acetals could represent versatile precursors of modified **MOs** building blocks.

3. Peptide and Biomaterial Fields: Morpholine Derived Amino Acids

The possibility to convert 2-morpholine acetal compounds to modified **MOs** structures was recognized by Wuest and co-workers who synthesized cyclic dimeric methyl morpholinosides **29** (Scheme 12).[33] The authors reported an application of the Summerton protocol in which, α-D-glucopyranoside and glycine were subjected to the classical $NaIO_4$-mediated oxidation/reductive amination process to access **25**. Introduction of an azido group gave **26**, whose hydrolysis under basic aqueous conditions provided carboxylic acid **27**, while catalytic hydrogenation of the azide residue generated amine **28**. Synthesis of the target **29** was achieved by amide coupling and macrolactamization between **27** and **28**. Unfortunately, any attempt to realize the nucleobase installation on **29** were unfruitful.

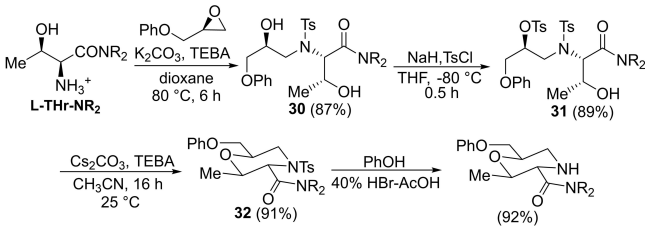


Scheme 12. Preparation of cyclic dimeric methyl morpholinosides **29**.

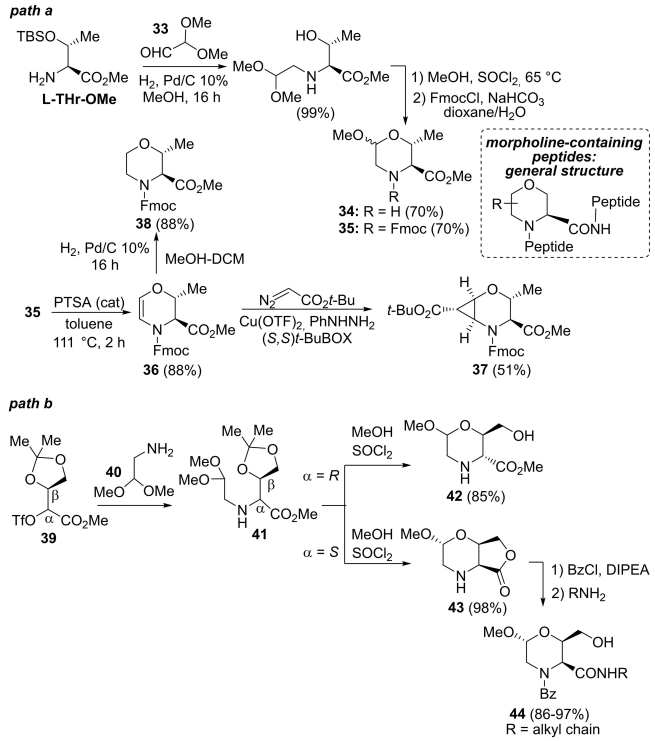
29 is formally a cyclic- dipeptide. It is worth of note that 2-morpholine acetals bearing a carboxylic residue, as well as morpholine carboxylic acids, have found wide applications in the synthesis of peptidomimetics as stabilizers or inductors of specific conformations and turns.[34] Concerning morpholine carboxylic acids, an interesting synthetic approach was reported by Penso research group (Scheme 13).[35] The ring-opening of optically pure oxiranes by threonine under solid-liquid phase transfer catalysis (SL-PTC) conditions furnished the diol derivative **30**. This latter was subjected to a regioselective mono-*O*-sulfonylation process which is controlled by the different stereo, electronic, and coordination properties of the two secondary hydroxylic groups providing compound **31**. Cyclization to target morpholine **32** proceeds under basic SL-PTC.

Guarna and Trabocchi research group has made a significant contribution to the development of a diversity-oriented synthesis (DOS) of constrained morpholine amino acids for peptidomimetic and medicinal applications.[36] In particular, morpholine carboxylic acids were realized starting from *O*-protected α-hydroxy amino acids such as serine[37] or threonine.[38] As depicted in Scheme 14, path a, reductive amination of acetal **33** by threonine, followed by acid-promoted *O*-desilylation and intramolecular trans-acetalization reactions furnished the 2-methoxy-morpholine **34**. This latter, in turn, after *N*-Fluorenylmethoxycarbonyl (Fmoc) protection to give **35**, could be converted into the enantiopure unsaturated morpholine **36** by p-Toluenesulfonic acid (PTSA) and 4 Å molecular sieves in refluxing toluene. The double bond represents a smart reactive site for further modification. For example, stereoselective Cu(I)-catalyzed cyclopropanation in the presence of a bisoxazolines (BOX) chiral ligand was realized in a diastereoselective manner leading to **37**. The double bond in **36** was also hydrogenated furnishing **38**. It is worth noting that all the reactions proceed without racemization and morpholine **34**–**38** were used to prepare model peptides on solid phase synthesis.[39]

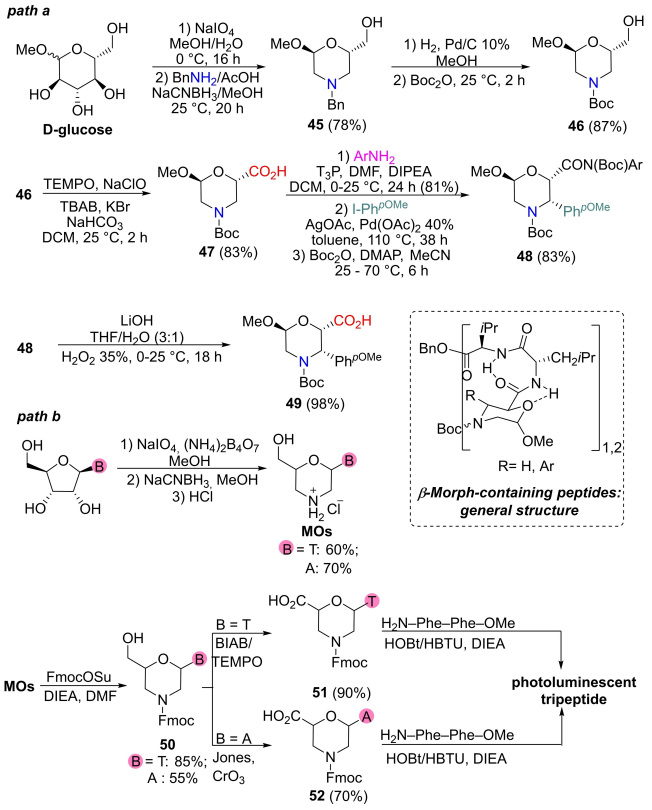
Skeletal diversity of morpholine carboxylic acid was improved by the authors using the activated sugar **39** (Scheme 14, path b).[40] The latter was subjected to a S_N2 reaction using the amino acetal **40** as the nucleophile. The behavior of the cyclization step strictly depends on the stereochemical configuration of the starting sugar. In fact, methanolysis under acidic



Scheme 13. Preparation of morpholine-α-carboxamides.



Scheme 14. Synthesis of morpholino-β-amino acids: Guarna DOS strategy.

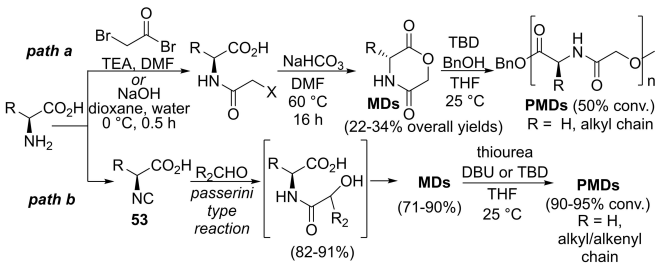


Scheme 15. Synthesis of morpholine-β-amino acids for peptide and opto-electronic applications.

conditions of **41**, bearing *R,R* configuration at α and β stereocenters, afforded morpholine **42** as the sole reaction product. On the other hand, a methanolysis/ring-closure tandem reaction sequence is triggered when (*S,R*)-**41** is employed and this divergent behavior is plausibly due to steric effects. Finally, bicyclic lactone **43** was converted in the carboxy-morpholine **44** after *N*-benzoylation under standard reaction conditions followed by amidation with a range of alkylamines. It is worth noting that morpholines **42** and **44** could represent a precursor of a new class of decorated **MOs**.

A complementary approach toward the preparation of non-natural morpholine amino acids for peptide applications was reported by Gelmi and co-workers via a two-step oxidative cleavage/reductive amination of α-D-glucopyranose compounds and benzylamine as the nitrogen source (Scheme 15, path a).[41] Morpholine **45** is accessible in good yields with this protocol. Its conversion into the target morpholine-β-amino acid (β-Morph) **47** was carried out after formation of morpholine **46** by a one pot nitrogen deprotection/Boc-protection reaction and a subsequent one-pot phase-transfer oxidation procedure of the alcohol group. Further functionalization at the C-3 position of **47** providing **48** was accomplished through a Pd-catalyzed stereoselective C(sp^3)-H arylation process. The hydrolysys of **48** afforded acid **49**. Experimental and computational data indicates that insertion of **47** or **49** in small peptide sequences induced an inverse γ-turn: while **47** gave an equilibrium between α- and PPII Helixes in a hexamer sequence,[41a] **49** favors the PPII Helix due to the C-3 aryl groups stabilizing the trans-rotamer of tertiary amide bond.[41c] The same research group has also expanded the family of β-Morph to compounds bearing a nucleobase on the C-2 position of the morpholine ring (Scheme 15, path b).[42] In this case, standard **MOs** were prepared following the classical Summerton procedure and were isolated in good yields as crystallizable hydrochloride salts. Fmoc protection of the morpholine nitrogen atom gave the protected **50**. Alcohol oxidation in the presence of 2,2,6,6-Tetramethylpiperidinyloxy (TEMPO) and (Diacetoxyiodo)benzene (BIAB) as the co-oxidant to the carboxylic acid **51** was realized for the thymine-**MO** compound, while Jones conditions were used for the oxidation of the adenine derivative. Scaffold **51** was used for the preparation of morpholine-based peptide oligomers for DNA binding,[43] and both **51** and **52** were used for the preparation of β-Morph-Phe-Phe tripeptides (Phe = phenylalanine).[43] This latter can self-assemble in sub-micrometric aggregates having both fluorescence and phosphorescence properties. Photoluminescent features are given by the β-Morph sub-unit while, the self-assembly behavior is due to the presence of the Phe-Phe residue. These bioinspired materials could find applications in both biotechnology and optoelectronic fields.

Another morpholine motif (morpholino-2,5-dione derivatives **MDs**) derived from amino acids, is gaining great attention in the field of nontoxic and biodegradable materials useful for biomedical applications. This class of polymers - Polydepsipeptides (**PDPs**) – has high thermal stability as well as good mechanical properties and are alternating copolymers of α-hydroxy acids and α-amino acids. **PDPs** have been synthesized



Scheme 16. Biodegradable material field: synthesis of **MDs**.

via ring-opening polymerization (ROP) of a range of *C*-substituted **MDs** subunits.[44] The most current polymerization method, involved the use of an organic super base, such as 1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU) as the reaction catalyst. Furthermore, organic base catalyzed system in the presence of urea compounds as co-catalysts could inhibit the deprotonation of NH residue in **MDs** increasing the selective formation of linear **PDPs** and avoiding the formation of cyclic oligomers.[45] **MDs** could be easily synthesized in good yields in a two-step procedure, reacting amino acid compounds with 2-bromoacetyl bromide under basic conditions (Scheme 16, path a).[46] Another appealing procedure was reported by Li in 2019 starting from isocyanides **53** and aldehydes via a Passerini type reaction (Scheme 16, path b).[47] It is worth noting that the presence of an alkenyl pendant group on the side chain of **MDs** allows further functionalization.

4. Conclusion & Outlook

In conclusion, this review provides an overview of the synthesis of morpholine moieties that find application in the field of artificial oligomers synthesis: from bio-inspired foldamers to biomaterials of new generation. Furthermore, the synthesis of 2 different classes of morpholine frameworks have been considered: morpholine nucleosides, as building block to prepare the antisense oligomers, and morpholine derived amino acids, as sub-unit for the development of peptide strands and biomaterials of new generation.

Acknowledgements

The authors gratefully acknowledge the Università degli Studi dell'Insubria, for financial support.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: amino acids · morpholinos · nitrogen heterocycles · nucleobases · oligonucleotides

[1] Morpholine structures as: a) HIV protease inhibitors: J. A. Mccauley, C. Beaulieu, D. J. Bennett, C. J. Bungard, S. Crane, T. J. Greshock, M. K. Holloway, S. Lessard, D. Mckay, C. Molinaro, O. Moradei, M. V. Sivalenka, V. Linh Truong, S. Tummanapalli, P. D. Williams (MERCK SHARP & DOHME), WO2015134366 A1, **2015**; antimicrobial agents: b) E. Carlson, H. Hansen, L. Hawkins, S. Ishizaka, M. Mackey, S. Schiller, C. Ogawa, H. Davis (EISAI R&D MAN CO LTD), US20150105370 A1, **2015**; obesity and diabetes diseases: c) N. Kouyama, A. Yukimasa, Y. Nishiura, Y. Tamura (SHIONOGI & CO), WO2012020725A1, **2012**; sexual dysfunction: d) C. M. N Allerton, A. D. Baxter, A. S. Cook, D. Hepworth, K.-F. Wong (PFIZER LTD), WO2004052372A1, **2004**; pain experience: e) M. A. Ackley (PFIZER LTD), WO2008087512A1, Jul 24, 2008.

[2] a) J. A. Kulkarni, D. Witzigmann, S. B. Thomson, S. Chen, B. R. Leavitt, Pieter R. Cullis, Roy van der Meel, *Nat. Nanotechnol.* **2021**, *630*, 630–643; b) M. Egli, M. Manoharan, *Nucleic Acids Res.* **2023**, *51*, 2529–2573.

[3] A. P. Kourounakis, D. Xanthopoulos, A. Tzara, *Med. Res. Rev.* **2020**, *40*, 709–752.

[4] a) see ref [3], V. A. Palchykov, V. A. Chebanov, *Chem. Heterocycl. Compd.* **2019**, *55*, 324–332; b) A. Tzara, D. Xanthopoulos, A. P. Kourounakis, *ChemMedChem* **2020**, *15*, 392–403; c) A. P. Kourounakis, D. Xanthopoulos, A. Tzara, *Med. Res. Rev.* **2020**, *40*, 709–752; d) F. Arshad, M. F. Khan, W. Akhtar, M. M. Alam, *Eur. J. Med. Chem.* **2019**, *167*, 324–356.

[5] J. E. Summerton, D. D. Weller, (ANTI GENE DEV GROUP), US5034506, **1991**.

[6] X. Sun, L. O. Marque, Z. Cordner, J. L. Pruitt, M. Bhat, P. P. Li, G. Kannan, E. E. Ladenheim, T. H. Moran, R. L. Margolis, D. D. Rudnicki, *Hum. Mol. Genet.* **2014**, *23*, 6302–6317.

[7] A. E. Heald, P. L. Iversen, J. B. Saoud, P. Sazani, J. S. Charleston, T. Axtelle, M. Wong, W. B. Smith, A. Vutikullird, E. Kaye, *Antimicrob. Agents Chemother.* **2015**, *6*, e02344-14 .

[8] F. Arshad, M. Faraz Khan, W. Akhtar, M. Mumtaz Alam, L. Mohan Nainwal, S. Kumar Kaushik, M. Akhter, S. Parvez, S. Misbahul Hasan, M. Shaquiquzzaman, *Eur. J. Med. Chem.* **2019**, *167*, 324–356.

[9] For recent reviews concerning the advances in oligomers drug delivery see: a) J. A. Kulkarni, D. Witzigmann, S. B. Thomson, S. Chen, B. R. Leavitt, P. R. Cullis, R. Van der Meel *Nat. Nanotechnol.* **2021**, *16*, 630–643; b) C. Sun, L. Shen, Z. Zhang, X. Xie, *Genes* **2020**, *11*, 837; c) D. Duan, N. Goemans, S. Takeda, E. Mercuri, A. Aartsma-Rus, *Nat. Rev. Dis. Primers* **2021**, *1*, 13.

[10] a) J. E. Summerton, D. D. Weller, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 187–195; b) J. Kundu, A. Ghosh, U. Ghosh, A. Das, D. Nagar, S. Pattanayak, A. Ghose, S. Sinha, *J. Org. Chem.* **2022**, *87*, 9466–9478; c) J. Bhadra, S. Pattanayak, S. Sinha, *Curr. Protoc. Nucleic Acid Chem.* **2015**, *62*, 4–65; d) J. Kundu, U. Ghosh, A. Ghosh, S. Pattanayak, A. Das, S. Sinha, *Curr. Protoc.* **2023**, *3*, e686.

[11] a) See refs 8; b) S. Pattanayak, S. Sinha, *Tetrahedron Lett.* **2012**, *53*, 6714–6717; c) S. Jeong Min, N. Young Kim, H. Young Chae, K. Ho Chun, *Bull. Korean Chem. Soc.* **2020**, *41*, 252–253; d) C. Li, A. J. Callahan, M. D. Simon, K. A. Totaro, A. J. Mijalis, K.-S. Phadke, G. Zhang, N. Hartrampf, C. K. Schissel, M. Zhou, H. Zong, G. J. Hanson, A. Loas, N. L. B. Pohl, D. E. Verhoeven, B. L. Pentelute, *Nature* **2021**, *12*, 4396.

[12] J. Bhadra, J. Kundu, K. C. Ghosh, S. Sinha, *Tetrahedron Lett.* **2015**, *56*, 4565–4568.

[13] W. Zhang, A. Pal, A. Ricardo, J. W. Szostak, *J. Am. Chem. Soc.* **2019**, *141*, 12159–12166.

[14] a) H. K. Langner, K. Jastrzebska, M. H. Caruthers, *J. Am. Chem. Soc.* **2020**, *142*, 16240; b) B. T. Lea, S. Paulc, K. Jastrzebskad, H. Langere, M. H. Caruthers, R. N. Veedu, *PNAS* **2022**, *119*, e2207956119.

[15] S. Pattanayak, H. Khatra, S. Saha, S. Sinha, *RSC Adv.* **2014**, *4*, 1951–1954.

[16] a) https://www.gene-tools.com; b) https://www.sarepta.com; c) P. A. Morcos, Y. Li, S. Jiang, *BioTechniques* **2008**, *45*, 613–623.

[17] J. D. Moulton, S. Jiang, *Molecules* **2009**, *14*, 1304–1323.

[18] For a review concerning the original synthesis of MOs see, a) J. E. Summerton, D. D. Weller, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 187–195; b) see refs. 8a,b.
[19] a) S. Pattanayak, S. Paul, B. Nandi, S. Sinha *Nucleosides Nucleotides Nucleic Acids* **2012**, *31*, 763–782; b) N. Zhang, C. Tan, P. Cai, Y. Jiang, P. Zhang, Y. Zhao, *Tetrahedron Lett.* **2008**, *49*, 3570–3573; c) see refs. 8b–e.
[20] N. M. Bell, R. Wong, J. Micklefield, *Chem. Eur. J.* **2010**, *16*, 2026–2030.
[21] a) Y. V. Tarasenko, T. V. Abramova, V. I. Mamatuk, V. N. Silnikov, *Nucleosides Nucleotides Nucleic Acids* **2016**, *35*, 32–42; b) T. V. Abramova, S. S. Belov, Y. V. Tarasenko, V. N. Silnikov, *Beilstein J. Org. Chem.* **2014**, *10*, 1151–1158; c) M. D. Nekrasov, E. R. Lukyanenko, A. V. Kurkin, *Nucleosides Nucleotides Nucleic Acids* **2020**, *39*, 1223–1245.
[22] N. Debreczeni, J. Hotzi, M. Bege, M. Lovas, E. Mező, I. Bereczki, P. Herczegh, L. Kiss, A. Borbás, *Chem. Eur. J.* **2023**, *29*, e202203248.
[23] N. Debreczeni, M. Bege, M. Herczeg, I. Bereczki, G. Batta, P. Herczegh, A. Borbás, *Org. Biomol. Chem.* **2021**, *19*, 8711–8721.
[24] M. F. Kasakin, T. V. Abramova, V. N. Silnikov, *Russ. J. Bioorg. Chem.* **2011**, *37*, 752–757.
[25] T. V Abramova, S. S Belov, Y. V Tarasenko, V. N. Silnikov, *Beilstein J. Org. Chem.* **2014**, *10*, 1151–1158.
[26] M. Kicsák, A. Mándi, S. Varga, M. Herczeg, G. Batta, A. Bényei, A. Borbás, P. Herczegh, *Org. Biomol. Chem.* **2018**, *16*, 393–401.
[27] A. Deiters, R. A. Garner, H. Lusic, J. M. Govan, M. Dush, N. M. Nascone-Yoder, J. A. Yoder *J. Am. Chem. Soc.* **2010**, *132*, 15644–15650.
[28] For a recent publication concerning the synthesis of Nucleobase-Functionalized **MOs** see: B. Nandi, S. Pattanayak, S. Paul, J. Kundu, S. Sinha in *Methods in Molecular Biology* (Ed. N. Shank, Humana New York, **2019**, p. 107).
[29] a) S. Paul, S. Pattanayak, S. Sinha, *Tetrahedron Lett.* **2014**, *55*, 1072–1076; b) S. Paul, B. Nandi, S. Pattanayak, S. Sinha, *Tetrahedron Lett.* **2012**, *53*, 4179–4183; c) S. Paul, S. Jana, J. Bhadraa, S. Sinha *Chem. Commun.* **2013**, *49*, 11278–11280; d) B. Nandi, S. Pattanayak, S. Paul, S. Sinha, *Eur. J. Org. Chem.* **2013**, 1271–1286; e) B. Nandi, H. Khatra, P. P. Khan, J. Bhadra, S. Pattanayak, S. Sinha, *ChemistrySelect* **2017**, *2*, 5059–5067.
[30] A. Das, A. Ghosh, S. Sinha, *Org. Biomol. Chem.* **2023**, *21*, 1242–1253.
[31] a) T. V. Abramova, L. S. Koroleva, V. N. Silnikov, *Mendeleev Commun.* **2019**, *29*, 169–171; b) I. P. Vohtancev, Y. V. Sherstyuk, V. N. Silnikov, T. V. Abramova, *Org. Prep. Proced. Int.* **2018**, *50*, 332–342.
[32] M. Papis, C. Loro, M. Penso, G. Broggini, F. Foschi, *Org. Chem. Front.* **2022**, *9*, 2949–2954.
[33] C. R. Kinzie, A. D. Steele, S. M. Pasciolla, W. M. Wuest, *Tetrahedron Lett.* **2014**, *55*, 4966–4968.
[34] For recent reviews about non-natural amino acids and peptidomimics see: a) T. G. Castro, M. Melle-Franco, C. E. A. Sousa, A. Cavaco-Paulo, J. C. Marcos, *Biomol. Eng.* **2023**, *13*, 981–1006; b) R. Bucci, F. Foschi, C. Loro, E. Erba, M. L. Gelmi, S. Pellegrino, *Eur. J. Org. Chem.* **2021**, 2887–2900.
[35] F. Foschi, D. Albanese, I. Pecnikaj, A. Tagliabue, M Penso, *Org. Lett.* **2017**, *19*, 70–73.
[36] For a recent review concerning Diversity-Oriented Synthesis of Morpholine Peptidomimetics see: E. Lenci, R. Innocenti, G. Menchi, A. Trabocchi, *Front. Chem.* **2018**, *6*, 522.
[37] F. Sladojevich, A. Trabocchi, A. Guarna, *J. Org. Chem.* **2007**, *72*, 4254–4257.
[38] a) F. Sladojevich, A. Trabocchi, A. Guarna, *Org. Biomol. Chem.* **2008**, *6*, 3328–3336; b) L. Ciofi, M. Morvillo, F. Sladojevich, A. Guarna, A. Trabocchi *Tetrahedron Lett.* **2010**, *51*, 6282–6285.
[39] F. Sladojevich, A. Guarna, E. Trabocchi, *Org. Biomol. Chem.* **2010**, *7*, 916–924.
[40] C. Lalli, A. Trabocchi, F. Sladojevich, G. Menchi, A. Guarna, *Chem. Eur. J.* **2009**, *15*, 7871–7875.
[41] a) R. Bucci, A. Contini, F. Clerici, S. Pellegrino, M. L. Gelmi, *Org. Chem. Front.* **2019**, *6*, 972–982; b) M. Penso, F. Foschi, S. Pellegrino, A. Testa, M. L. Gelmi, *J. Org. Chem.* **2012**, *77*, 3454–3461; c) F. Vaghi, R. Bucci, F. Clerici, A. Contini, M. L. Gelmi, *Org. Lett.* **2021**, *15*, 6197–6202.
[42] R. Bucci, A. Bossi, E. Erba, F. Vaghi, A. Saha, S. Yuran, D. Maggioni, M. L. Gelmi, M. Reches, S. Pellegrino, *Sci. Rep.* **2020**, *10*, 19331.
[43] A. Contini, E. Erba, V. Bondavalli, A. Barbiroli, M. L. Gelmi, A. Romanelli, *Biochem. Biophys. Res. Commun.* **2021**, *549*, 8–13.
[44] For a review concerning the development of polydepsipeptides see: Y. Feng, J. Lu, M. Behl, A. Lendlein, *Macromol. Biosci.* **2010**, *10*, 1008–1021.
[45] a) T. F. Burton, J. Pinaud, N. Pétry, F. Lamaty, O. Giani, *ACS Macro Lett.* **2021**, *10*, 1454–1459; b) T. F. Burton, J. Pinaud, O. Giani, *Macromolecules* **2020**, *53*, 6598–6607.
[46] M. Dirauf, D. Bandelli, C. Weber, H. Görls, M. Gottschaldt, U. S. Schubert, *Macromol. Rapid Commun.* **2018**, 1800433.
[47] C.-X. Shi, Y.-T. Guo, Y.-H. Wu, Z.-Y. Li, Y.-Z. Wang, F.-S. Du, Z.-C. Li, *Macromolecules* **2019**, *52*, 4260–4269.

Manuscript received: October 13, 2023
Revised manuscript received: November 30, 2023
Accepted manuscript online: December 7, 2023
Version of record online: December 21, 2023