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Natural α-amino acid based synthesis of morpholin-2-ones, prospective monomers for new-generation polymeric lipofectants

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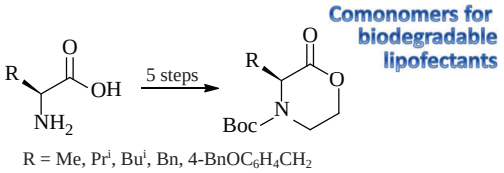
***N*-Boc-protected morpholin-2-ones have been synthesized with a high to moderate yields from the natural L-amino acids (Ala, Val, Leu, Phe, Tyr). These compounds can find application in the development of biodegradable and biocompatible polymeric vehicles for DNA and RNA delivery.**



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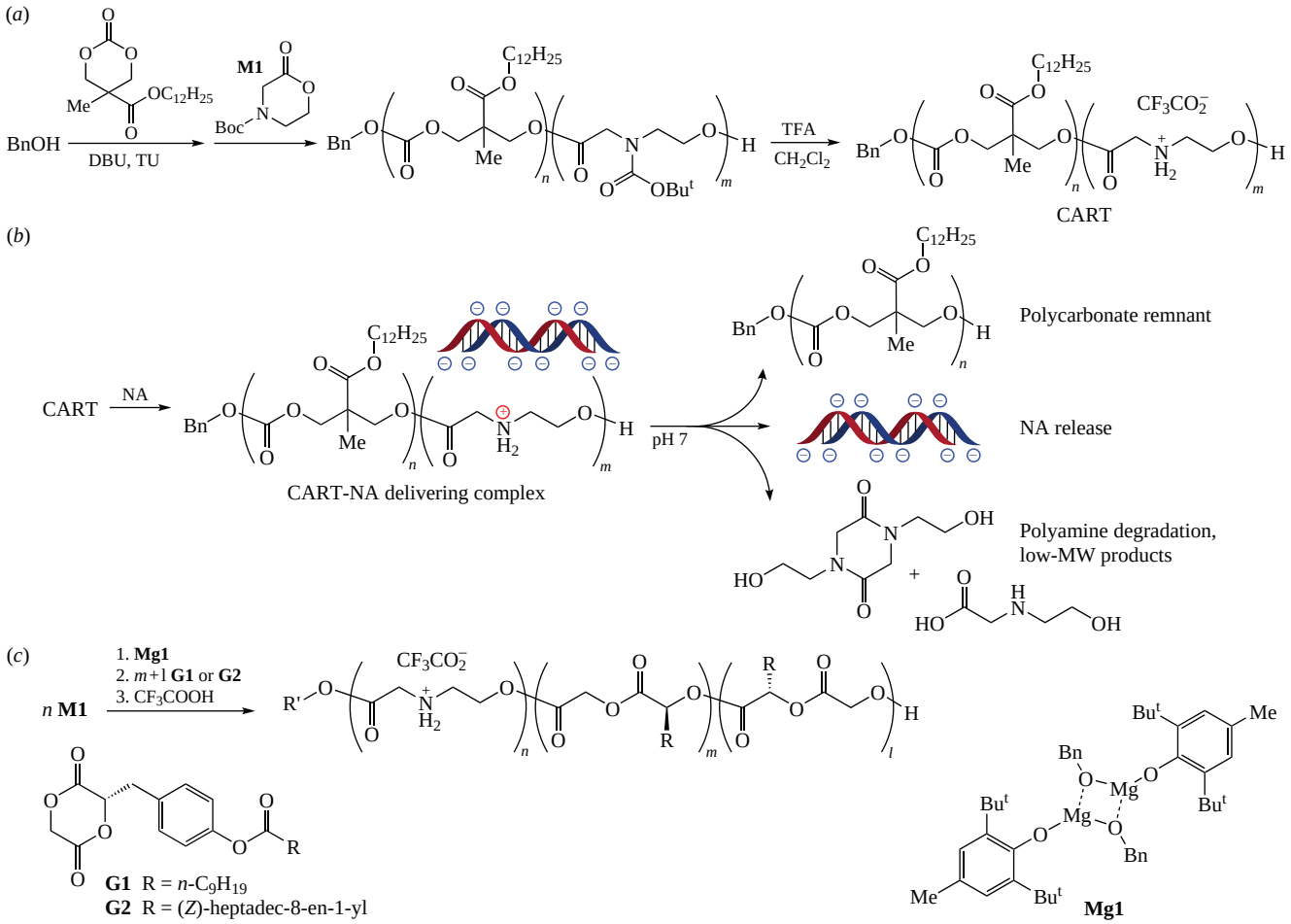
Genome manupulations represent prospective approach to the treatment of infectious diseases, cancers, genetic or immune disorders.[1] The most efficient chemical methods of such manipulations are based on transfection, the non-viral NA delivery into the eukaryotic cells, using ionisable lipid nanoparticles (LNPs), *i.e.* lipofection.[2,3] The common approach to LNPs is based on self-assembling of multicomponent mixtures containing lipophilic components, amphiphilic (poly)cations (*e.g.* polyamines), and NAs.[1,4] This common approach is unable to provide the constancy of LNP composition and properties (in particular, the thermal stability) that, however, did not prevent similar formulations from successful commercialization by an example of RNA vaccine for SARS-CoV-2.[5]

With this in mind, the development of single-component amphiphilic lipofectants with given composition becomes attractive. Synthetic lipofectants could be able to bind with NAs, to prevent genetic material inactivation by physiologic media, to penetrate cell membranes, and to release NAs into the cell interior. Biocompatibility and the absence of toxicity are also obvious requirements for prospective lipofectants. 'Living' coordination ring-opening polymerization (ROP) of cyclic esters seems to be a convenient chemical solution to provide desired balance between lipophilic and polyamine parts of lipofectant.[6] Ideally it would be better to design the structure of similar amphiphilic block copolymer in such a manner that it would provide NA delivery into cell and NA release inside the cell, with a formation of non-toxic low-MW products of the lipofectant degradation.

With these requirements in mind, the biggest problems arise when developing degradable polycationic part of the lipofectant, for example, the most synthetically available poly(ethylene imine)s are hydrolytically stable and toxic.[6] In early studies of Lim *et al.*[7,8] and de Gracia Lux *et al*.,[9] the introduction of amino group to the polyester backbone was proposed; it was modified in such a way that polyesters became capable of degradation at physiological pH *via* intramolecular ammonolysis of the ester fragments (see Online Supplementary Materials, Scheme S1). Recently, Waymouth *et al*. have designed block copolymers of lipophilic polycarbonate[10] and *N*-Boc-protected morpholin-2-one **M1**;[11] after deprotection, these copolymers formed efficient macromolecular vehicles for NA delivery, named 'Charge Altering Releasable Transporters' [CARTs, Scheme 1(*a*)].[12–14] The chemical structure of CARTs ensures high transfection efficiency, and at pH ~7, NA-CART complex undergoes fast intracellular self-immolative degradation of the cationic poly(2-oxyethylglycine) blocks. As a result, low-MW diketopiperazine and (2-hydroxyethyl)glycine are formed, NA is released, but lipophilic homopolymer block is remained in a medium [Scheme 1(*b*); more detailed mechanism is presented in Scheme S2]. In our recent studies, we developed glycolide-based lipophilic block for CARTs, that is completely biocompatible being derived from glycolic acid, metabolic precursor of L-tyrosine, and natural fatty acids [Scheme 1(*c*)].[15]

Monomer **M1** represents derivative of glycine, and the further development of morpholin-2-one based CARTs may involve other natural α-amino acids for the synthesis of biocompatible substituted analogs of **M1**. However, only a few examples of the synthesis of 3-substituted morpholin-2-ones are known from the literature [Scheme 2(*a*)]. In particular, L-valine derivative was prepared from hydroxy acid with 66% yield,[16] the yield of L-phenylalanine derivative was only 23%,[17] L-ornithine and L-lysine based morpholinones were synthesized with 56 and 47% yields, respectively.[18] A wide range of *N*-benzyl substituted morpholin-2-ones was obtained with high yields from α-amino acids,[17] however, as was shown by Waymouth *et al*., even *N*-benzylmorpholin-2-one is inactive in ROP.[11]

In the present study, we set out to develop the common synthetic approach to *N*-Boc-protected morpholin-2-ones based on natural L-α-amino acids. In view of the results of Waymouth *et al*.,[18] we concluded that the yield-limiting step of the synthesis of morpholin-2-ones is an alkylation of α-amino acid esters. To avoid prolonged reflux in the presence of base with the risk of epimerization of α-amino acid, we preferred to use reductive amination of aldehyde $BnOCH_2CHO$[19,20] with α-amino acid

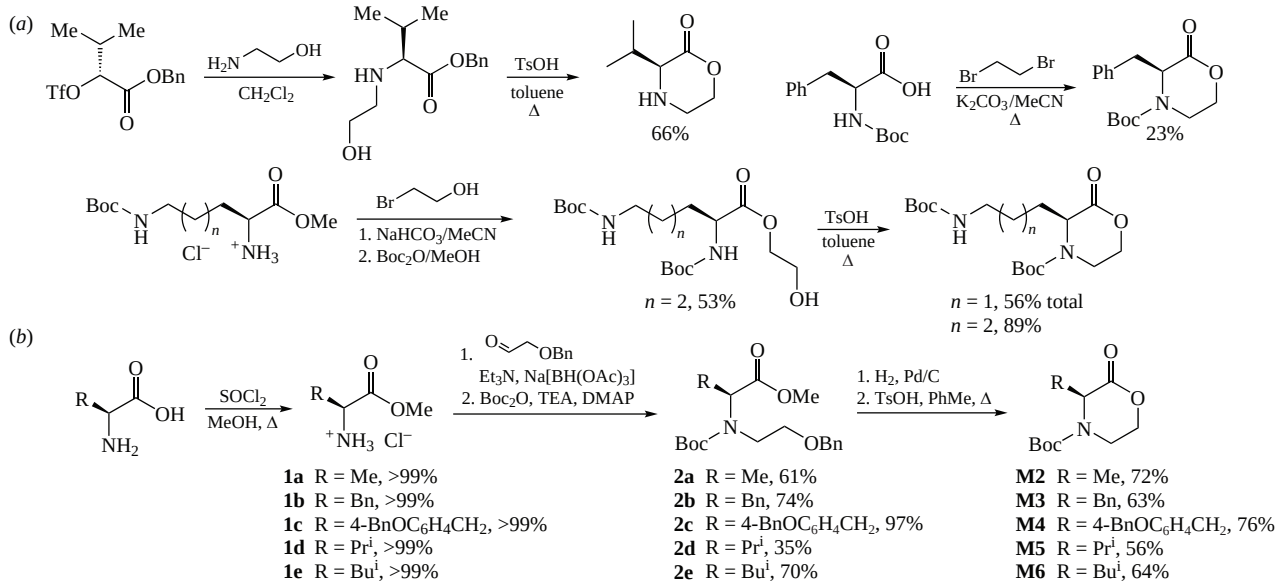


Scheme 1

esters, followed by *N*-Boc protection, catalytic hydrogenation and acid-catalyzed cyclization [Scheme 2(*b*)].

In our experiments, hydrochlorides of methyl esters of L-alanine, L-phenylalanine, *O*-benzyl-protected L-tyrosine,[21] L-valine and L-leucine were prepared in near quantitative yields and used as starting materials. At the stage of reductive amination of $BnOCH_2CHO$ with subsequent *N*-Boc protection, the lowest yield of the product (35%) was observed for sterically hindered L-valine derivative. At the final stage, the yields of substituted morpholin-2-ones **M2**–**M6** amounted to 56–84%. One should note the high chemoselectivity of the hydrogenolysis of **2e** that left benzyl protection of the phenolic group. Also note that column or flash chromatography is needed for purification of **2a**–**e** since negligible impurities in $BnOCH_2CHO$, prepared using the Swern oxidation, inhibit catalytic hydrogenation at a later stage.

Coordination ROP has high requirements for the purity of the monomers used. In our study, substituted morpholin-2-ones were purified using recrystallization from Et_2O and high-vacuum sublimation (**M1**–**M4**) or molecular distillation (**M5**, **M6**). NMR



Scheme 2

analysis of **M2**–**M6** was complicated by the broadening of the spectral lines caused by slow rotation around exocyclic C–N 'amide' bonds (see Figures S14, S16, S19, S21 and S23 in the Online Supplementary Materials). However, as can be seen in Figure S17 by an example of **M3**, a modest increase in the temperature of registration of the spectrum to 55 °C is sufficient for reliable identification of morpholin-2-ones.

In that way, we developed simple and efficient methods for the synthesis of substituted morpholin-2-ones **M2**–**M6**, their purification and characterization. These compounds can be used in the development of next-generation CARTs for NA delivery with regulated lipophilicity, and these studies are now being conducted in our laboratory.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.01.009.

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