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Solid-Phase Synthesis of 5-Noranagrelide Derivatives

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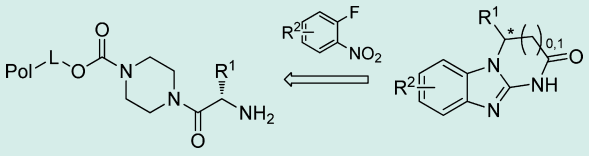
Supporting Information

ABSTRACT: Solid-phase synthesis of 1*H*-benzo[*d*]imidazo[1,2-*a*]imidazol-2(3*H*)-one derivatives employing Fmoc-*α*-amino acids and nitroaryl fluorides as key building blocks has been developed. The Fmoc-*α*-amino acids immobilized on Wang resin, equipped with a piperazine carbamate linker, were transformed to *o*-nitroanilines in two steps. After reduction of the nitro group, the corresponding *o*-phenylenediamines gave the 2-aminobenzimidazole scaffold by reaction either with cyanogen bromide or with Fmoc-NCS. Cleavage from the polymer support and further cyclization afforded the target compounds. The developed methodology represents a versatile and simple approach for the preparation of various corresponding 1*H*-benzo[*d*]imidazo[1,2-*a*]imidazol-2(3*H*)-ones from a large number of commercially available building blocks.



KEYWORDS: *anagrelide, Fmoc-amino acids, solid-phase synthesis, combinatorial chemistry*

INTRODUCTION

In our long-term research, we have been focused on the development of simple methodologies suitable for the preparation of pharmacologically promising substances. For such purposes, the solid-phase synthesis concept has been applied typically due to its significant advantages. In particular, the simple isolation of reaction intermediates allows for a rapid preparation of various target derivatives and easy access to combinatorial chemistry.[1]

Essential thrombocytosis is a chronic blood cancer characterized by the overproduction of platelets by megakaryocytes in the bone marrow. Anagrelide (Agrylid/Xagrid;[2,3] Figure 1) was the first platelet reducing agent that acts by

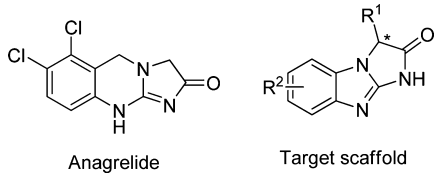


Figure 1. Anagrelide and a general structure of target compounds (5-noranagrelide scaffold).

retarding the maturation of megakaryocytes via the thrombopoietin receptor and reducing their chromosome number and cell diameter.[4] It also inhibits platelet aggregation as a result of the potent inhibition of cyclic adenosine monophosphate (cAMP) phosphodiesterase III activity.[5]

Along with the preparation of anagrelide scaffold containing compounds, some of its structural analogues have been reported in the literature. For instance, synthesis of 5-noranagrelide derivatives (Figure 1) has been described several times by the use of traditional solution-phase synthesis and 2-aminobenzimidazole as a key intermediate (Figure 2).[6−10]

In the past, biological properties of 5-noranagrelide derivatives have been studied very rarely. Only antifungal activity has been reported,[7] and similar tricyclic compounds have been patented as CRF receptor antagonists.[11] In order to get a deeper understanding of this lightly explored field, we focused on the development of the simple solid-phase synthesis of 5-noranagrelide analogues to access chemical libraries for a detailed biological assay.

In 2003, Kundu *et al.* described the high throughput synthesis of anagrelide derivatives based on solid-phase synthesis with the use of Fmoc-*α*-amino acids immobilized on Wang resin and *o*-nitrobenzaldehydes (Scheme 1).[12]

Inspired by Kundu *et al.*'s strategy, we proposed the use of *o*-nitrofluorobenzenes instead of *o*-nitrobenzaldehydes to access a high throughput synthesis of 5-noranagrelide derivatives. However, in such a case, the immobilization of Fmoc-amino acids via the ester bond used by Kundu *et al.* was not suitable, as it has been shown that a spontaneous cyclative cleavage occurs,[13] leading to the undesired release of reaction intermediates from the resin (Scheme 2).

To overcome this problem, we suggested the use of a piperazine carbamate linker as recently reported by Krchňák and Neagoie.[14] The principle of this strategy centers around the immobilization of the Fmoc-amino acid via the amide bond, which does not undergo the intramolecular aminolysis

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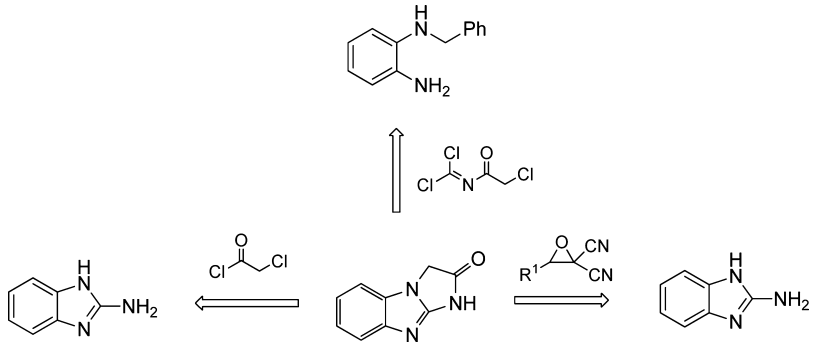
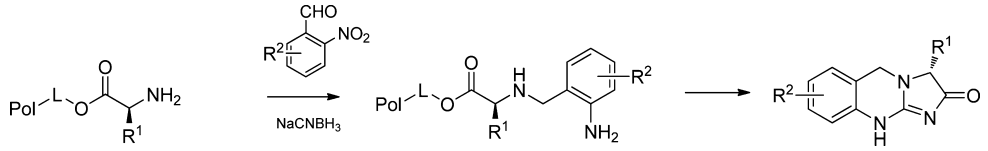
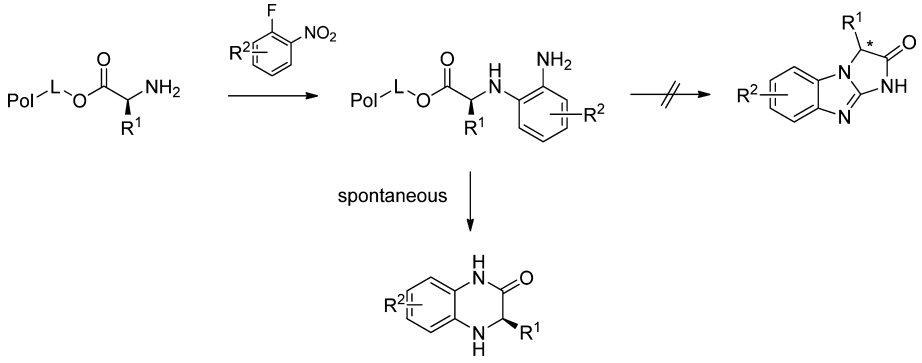


Figure 2. Some retrosynthetic approaches to 5-noranagrelide derivatives.

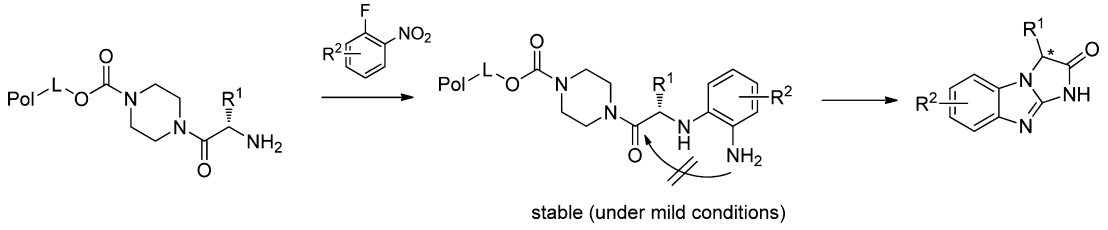
Scheme 1. Solid-Phase Synthesis of Anagrelide Derivatives by Kundu *et al.*



Scheme 2. Use of Wang Resin: Unapplicable Approach



Scheme 3. Use of Wang Resin and Carbamate Linker: Theoretically Applicable Approach



spontaneously but requires harsher conditions to proceed (Scheme 3).

RESULTS AND DISCUSSION

To test the suggested synthetic approach, Fmoc-L-Ala-OH and 3-fluoro-4-nitrobenzotrifluoride were selected as the representative building blocks. The general synthetic pathway leading to target compounds is shown in Scheme 4. Wang resin was equipped with piperazine carbamate linker **1** which was then acylated with the Fmoc-amino acid to give intermediate **2**. Cleavage of the Fmoc protective group with piperidine afforded the intermediate **3**{*1*}. The subsequent reaction with 3-fluoro-4-nitrobenzotrifluoride led to the formation of nitroaniline derivative **4**{*1,1*}. The nitro group was further reduced to amine **5**{*1,1*}, employing sodium dithionite as the reducing agent and tetrabutylammonium hydrogen sulfate as the phase-transfer catalyst as recently reported in the literature.[15] Reaction of intermediate **5**{*1,1*} with cyanogen bromide gave the corresponding 2-aminobenzimidazole intermediate **7**{*1,1*}. After cleavage of intermediate **8**{*1,1*} from the polymer support, the final cyclization was achieved by heating at 80 °C in DMSO for 3 days. The model compound **9**{*1,1*} was obtained in good crude purity (62%, calculated from LC-UV traces) and an overall yield of 58% (quantified after preparative HPLC purification). Increasing the temperature (up to 150 °C) in order to shorten the reaction time led to the formation of multiple side products, lowering the efficiency of the cyclization step. Also, microwave heating of the cleaved material as well as microwave-assisted cyclative cleavage of the final compound from the resin **7**{*1,1*} were tested, but the crude purity was significantly lower in each case.

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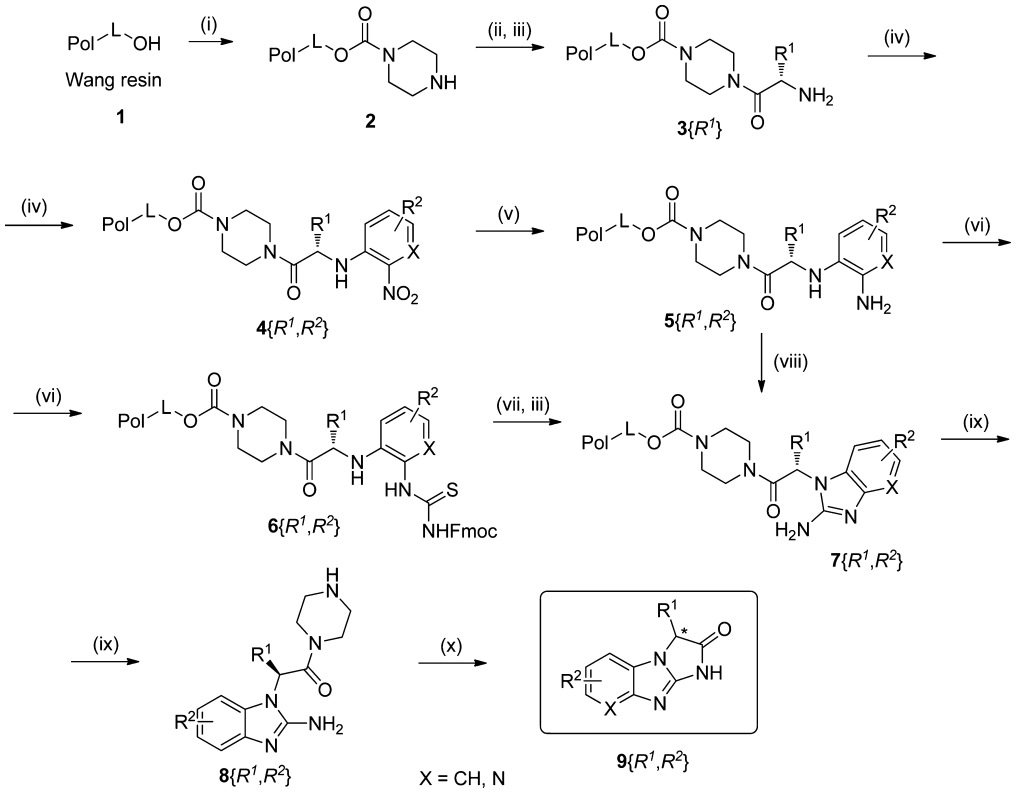
Scheme 4. Synthetic Pathway Leading to Target Compounds[a]



[a]Reagents and conditions: (i) (a) CDI, pyridine, DCM, 2 h; (b) piperazine, DCM, overnight. (ii) Fmoc-α-amino acid, DIC, HOBt, DCM/DMF, rt, overnight. (iii) piperidine, DMF, rt, 30 min. (iv) nitroaryl fluoride, DIPEA, 1,4-dioxane, 80 °C, overnight. (v) sodium dithionite, potassium carbonate, TBAHS, DCM/water, rt, 2 h. (vi) Only for intermediates $\mathbf{5}\{R_1,3\}$: Fmoc-NCS, THF, rt, 30 min. (vii) Only for intermediates $\mathbf{6}\{R_1,3\}$: DIC, DMF, rt, overnight. (viii) BrCN, DCM, 45 °C, overnight. (ix) TFA, DCM, rt, 30 min. (x) DMSO, 80 °C, 3 days.

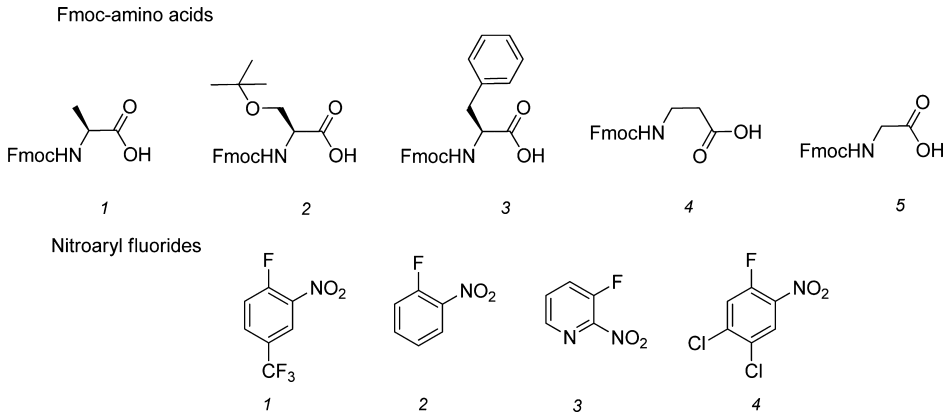


Figure 3. Building blocks used for the verification of a synthetic route.

To evaluate limitation and scopes of the developed methodology, various building blocks with different electronic and steric properties were tested. For such purposes, we selected four different nitroaryl fluorides and five Fmoc-amino acids with different substitutions of the side chain (Figure 3).

When testing the selected nitroaryl fluorides in the reaction sequence, we found out that intermediates $\mathbf{5}\{R^1,3\}$ prepared from 3-fluoro-2-nitropyridine did not furnish the desired compounds $\mathbf{7}\{R^1,3\}$ with use of the cyanogen bromide method. After the reaction, a mixture of unknown compounds was detected. For this reason, we synthesized such intermediates in two steps via the corresponding Fmoc-thioureas $\mathbf{6}\{R^1,3\}$ (see Scheme 4, steps vi and vii). In the case of Fmoc-amino acids, the building blocks **1**−**3** (Figure 3) afforded the final compounds $\mathbf{9}\{R^1,R^2\}$ in good overal crude purity. The only exception appeared in the case of Fmoc-Ser(O*t*Bu)-OH: The standard cleavage procedure (TFA/DCM, rt, 30 min) led to partial cleavage of the *t*Bu-protective group, and two intermediates, $\mathbf{8}\{2,R^2\}$ and $\mathbf{8}\{2a,R^2\}$, were obtained. After the cyclization step, the final compounds $\mathbf{9}\{2,R^2\}$ and $\mathbf{9}\{2a,R^2\}$ were detected. However, the expected compounds were also accompanied by compounds $\mathbf{9}\{2b,R^2\}$, resulting from a thermal elimination of water (Scheme 5). Only with the use of this procedure was it possible to isolate some compounds, $\mathbf{10}\{2,R^2\}$,

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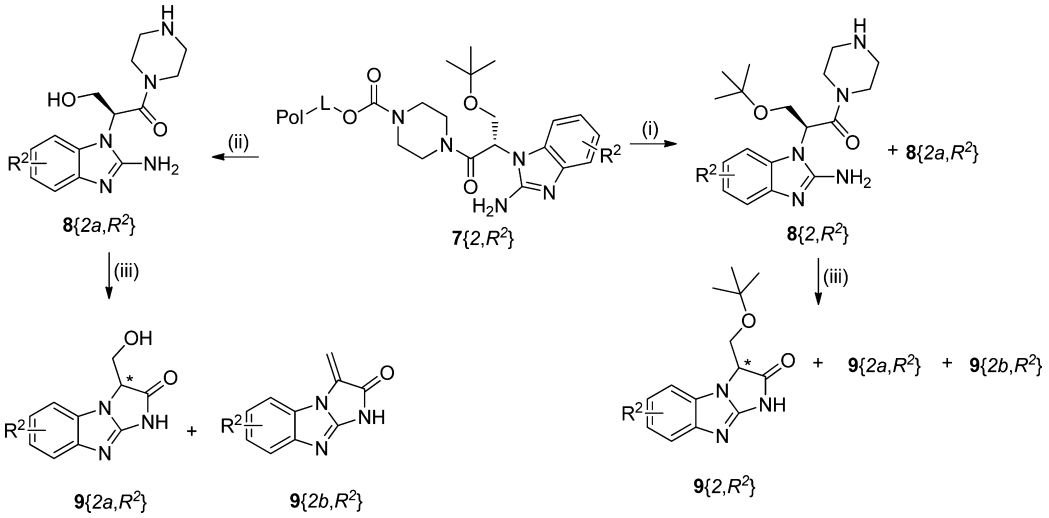
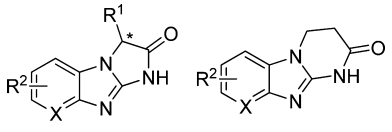
Scheme 5. Cyclization of Intermediates Prepared from Fmoc-Ser(O*t*Bu)-OH[a]



[a]Reagents and conditions: (i) TFA, DCM, rt, 30 min; (ii) TFA, DCM, rt, 120 min; (iii) DMSO, 80 °C, 3 days.

with semipreparative HPLC (in limited yields, see Table 1). When the longer treatment (2 h) with TFA was used and

Table 1. List of Prepared Compounds



9{R^1,R^2} 10{4,R^2}

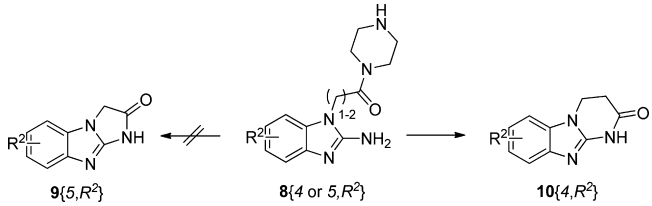
compound	R^1	R^2	X	crude purity[a] (%)	yield[b] (%)	final purity[c] (%)
9{1,1}	Me	7-CF_3	CH	62	58	92
9{1,2}	Me	H	CH	73	51	98
9{1,3}	Me	H	N	64	54	93
9{1,4}	Me	6,7-Cl	CH	78	52	91
9{2,1}	CH_2OtBu	7-CF_3	CH	52	10	88
9{2,4}	CH_2OtBu	6,7-Cl	CH	56	14	97
9{2a,1}	CH_2OH	7-CF_3	CH	70	30	87
9{2a,4}	CH_2OH	6,7-Cl	CH	58	25	82
9{2b,2}	CH_2=	H	CH	67	30	88
9{3,1}	CH_2Ph	7-CF_3	CH	63	42	98
9{3,2}	CH_2Ph	H	CH	65	42	98
9{3,3}	CH_2Ph	H	N	66	65	92
9{3,4}	CH_2Ph	6,7-Cl	CH	75	49	93
10{4,1}		7-CF_3	CH	61	71	96
10{4,2}		H	CH	72	57	98
10{4,3}		H	N	65	59	93
10{4,4}		6,7-Cl	CH	58	66	98

[a]Crude purity calculated from LC-UV traces after the cleavage from the resin. [b]Overall yields calculated from NMR after preparative HPLC purification. [c]Calculated from LC-UV traces after preparative HPLC purification.

followed by the cyclization step, just two products, 9{2a,R^2} and 9{2b,R^2}, were obtained. Their ratio was influenced by the substitution of the benzene ring (R^2): in the case of unsubstituted and pyridine derivatives, we observed complete elimination leading to single products 9{2b,2} and 9{2b,3}, while in the case of trifluoromethyl and dichloro substitution, we obtained a mixture of compounds 9{2a,1}/9{2b,1} and 9{2a,4}/9{2b,4} in a ratio of 30:70. The separation of individual compounds was successful in some cases with semipreparative HPLC. The structure of the methylene byproduct was finally confirmed by NMR analysis of compound 9{2b,2}.

Cyclization of intermediates 8{5,R^2}, originated from Fmoc-Gly-OH, totally failed, and only the starting material was recovered (Scheme 6). Increasing the temperature or the

Scheme 6. Cyclization of Intermediates Prepared from Fmoc-Gly-OH and Fmoc-*β*-Ala-OH



reaction time led only to decomposition and the formation of numerous unknown products. The same results were obtained for all of the nitroaryl fluorides that were tested. On the other hand, the use of Fmoc-*β*-Ala-OH gave the desired derivatives of 3,4-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-2(10*H*)-ones 9{4,R^2} for each combination. A list of all prepared derivatives is displayed in Table 1.

Finally, we have been interested in the resulting stereochemistry of final compounds 9{R^1,R^2}. To evaluate the effect of the cyclization step on the single stereocenter leading to potential racemization, we synthesized two more compounds. Product 9^D{1,1} was prepared from Fmoc-D-Ala-OH, and compound 9^{DL}{1,1} was synthesized from an equimolar mixture of Fmoc-D-Ala-OH and Fmoc-L-Ala-OH. The three compounds 9{1,1}, 9^{DL}{1,1}, and 9^D{1,1} were subjected to capillary electrophoresis analysis by direct chiral separation employing sulfobuthyl ether *β*-cyclodextrin as a chiral selector. The results indicate that the racemization occurred during the synthesis of 9{1,1} and 9^D{1,1}, although in both cases the pure starting enantiomers were used (see Figure 4).

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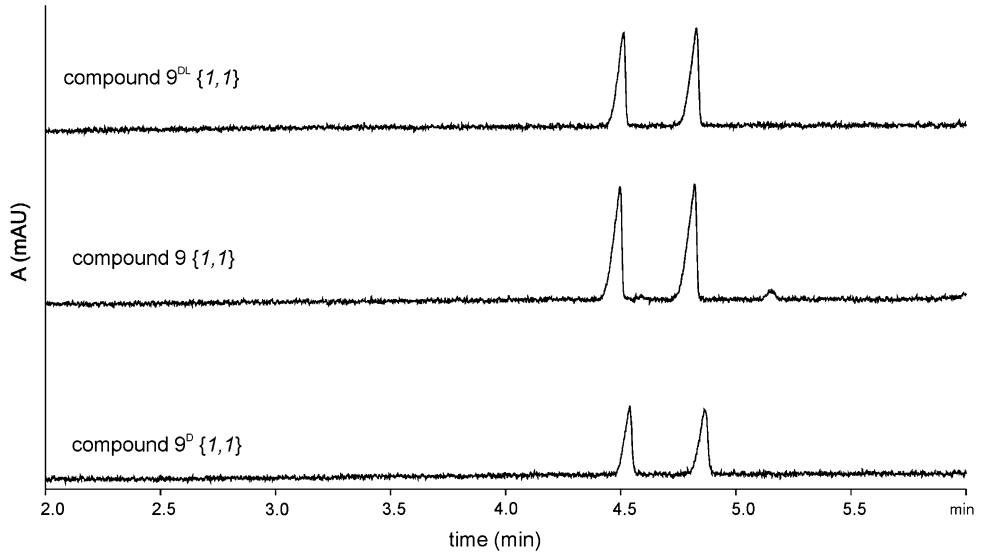

Figure 4. Electropherograms of enantioseparation of compounds $\mathbf{9}^{DL}\{1,1\}$, $\mathbf{9}\{1,1\}$, and $\mathbf{9}^{D}\{1,1\}$. Conditions: 100 mM sodium phosphate with 1.0% of sulfobuthyl ether β-cyclodextrin, $U = -15$ kV, $\lambda = 280$ nm, sample injection 50 mbar/5s.

In conclusion, we have developed an efficient method for the solid-phase synthesis of 5-noranagrelide derivatives with the use of Wang resin and the recently described piperazine carbamate linker. Various building blocks were tested, and 17 model compounds were prepared and characterized. The target compounds were isolated in very good overall yields. Despite observing a few limitations, this method can be applied for the preparation of chemical libraries with the use of combinatorial solid-phase organic synthesis due to the simplicity of the synthetic route and the commercial availability of larger numbers of building blocks (nitroaryl fluorides, Fmoc-amino acids).

■ ASSOCIATED CONTENT

Supporting Information

Details of experimental synthetic and analytical procedures and spectroscopic data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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